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Wodtke, R.; Hauser, C.; Ruiz-Gómez, G.; Jäckel, E.; Bauer, D.; Lohse, M.; Wong, A.; Pufe, J.; Ludwig, F.-A.; Fischer, S.; Hauser, S.; Greif, D.; Pisabarro, M. T.; Pietzsch, J.; Pietsch, M.; Löser, R.;

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## $\mathbf{N}^{\mathbf{k}}$-Acryloyllysine piperazides as irreversible inhibitors of transglutaminase 2 synthesis, structure-activity relationships and pharmacokinetic profiling ${ }^{\ddagger}$

Robert Wodtke ${ }^{\text {a,e,t, }}$, Christoph Hauser ${ }^{\text {b }}$, Gloria Ruiz-Gómez ${ }^{\text {c }}$, Elisabeth Jäckel ${ }^{\text {a,e },}$ David Bauer ${ }^{\text {a,f }}$, Martin Lohse ${ }^{\text {a,e }}$, Alan Wong ${ }^{\mathrm{a}}$, Johanna Pufe ${ }^{\mathrm{a}}$, Friedrich-Alexander Ludwig ${ }^{\text {d }}$, Steffen Fischer ${ }^{\text {d }}$, Sandra Hauser ${ }^{\text {a }}$, Dieter Greife, M. Teresa Pisabarroc ${ }^{\text {c }}$, Jens Pietzsch ${ }^{\text {a,f, }, ~ M a r k u s ~ P i e t s c h ~}{ }^{\text {b,* }}$, Reik Löser ${ }^{\text {a,f,* }}$

[a] Helmholtz-Zentrum Dresden-Rossendorf, Institut für Radiopharmazeutische Krebsforschung, Bautzner Landstraße 400, 01328 Dresden, Germany
[b] Zentrum für Pharmakologie, Medizinische Fakultät, Universität zu Köln, Gleueler Straße 24, 50931 Köln, Germany
[c] Structural Bioinformatics, BIOTEC, TU Dresden, Tatzberg 47-51, 01307 Dresden, Germany
[d] Helmholtz-Zentrum Dresden-Rossendorf, Institut für Radiopharmazeutische Krebsforschung, Forschungsstelle Leipzig, Permoserstraße 15, 04318 Leipzig, Germany
[e] Fakultät Natur- und Umweltwissenschaften, Hochschule Zittau/Görlitz, Theodor-Körner-Allee 16, 02763 Zittau, Germany
[f] Fakultät Chemie und Lebensmittelchemie, Technische Universität Dresden, Mommsenstraße 4, 01062 Dresden, Germany

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#### Abstract

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Transglutaminase 2 (TGase 2)-catalysed transamidation represents an important posttranslational mechanism for protein modification with implications in physiological and pathophysiological conditions including fibrotic and neoplastic processes. Consequently, this enzyme is considered a promising target for the diagnosis and therapy of these diseases. In this study, we report on the synthesis and kinetic characterisation of $N^{\kappa}$-acryloyllysine piperazides as irreversible inhibitors of TGase 2. Systematic structural modifications on 54 new compounds were performed with a major focus on fluorine-bearing substituents due to the potential of such compounds to serve as radiotracer candidates for positron emission tomography. The determined inhibitory activities ranged from $100-10000 \mathrm{M}^{-1} \mathrm{~s}^{-1}$, which resulted in comprehensive structure-activity relationships. Structure-activity correlations using various substituent parameters accompanied by covalent docking studies provide an advanced understanding of the molecular recognition for this inhibitor class within the active site of TGase 2. Selectivity profiling of selected compounds for other transglutaminases demonstrated an excellent selectivity towards transglutaminase 2. Furthermore, an initial pharmacokinetic profiling of selected inhibitors was performed including the assessment of potential membrane permeability and liver microsomal stability.


## Introduction

Transglutaminases have been discovered 60 years ago in guinea pig liver. ${ }^{1}$ Even though initially identified in mammals, their occurrence has been confirmed for all kingdoms of eukaryotic organisms including plants and fungi. ${ }^{2-4} \mathrm{In}$ addition, transglutaminases are found in bacteria, which indicates the fundamental importance of these enzymes for biological processes.

The human transglutaminases constitute a family of nine homologues, which comprises the transglutaminases 1-7, the blood coagulation factor XIIla and the catalytically inactive erythrocyte protein band 4.2. All human family members represent multidomain proteins consisting of a $\beta$-sandwich, the central $\alpha / \beta$ transamidase domain which harbours the acyl transferase active site and exhibits similarity to papain-like cysteine proteases, and two Cterminal $\beta$-barrels. ${ }^{5}$

Transglutaminase 2 (TGase 2), which is also referred to as tissue transglutaminase, represents the most intensively studied family member. Its acyltransferase site is constituted by the residues Cys277, His335, Asp358 and Trp241 (numbering according to the human enzyme), which directly participate in catalysis. The central $\alpha / \beta$ transamidase domain catalyses the $\gamma$-glutamyl transfer between protein-bound glutamine residues and a variety of primary amines. In particular, protein-bound lysine residues, low-molecular weight polyamines, e.g. cadaverine, spermidine and spermine, and biogenic monoamines can comprise the latter category of substrates. Beside its eponymous acyl transferase activity, TGase 2 can bind to fibronectin and exhibits GTPase activity, functions which have been ascribed to the N -terminal $\beta$-sandwich and the C-terminal $\beta$-barrel domains, respectively. Due to its multidomain structure the enzyme exists in two distinguished conformational states: a stretched open one and a compact closed conformation. In the closed state, which is induced and stabilised by GTP, the C-terminal $\beta$-barrels block the access to the active site of the transamidase domain. The open conformation, in which TGase 2 is fully active with regards to its acyl transferase activity, is induced and stabilised by $\mathrm{Ca}^{2+}$ ions. ${ }^{6-7}$

TGase 2 is ubiquitously expressed and is mainly located in the cytoplasm under physiological conditions. This implies that under these conditions the enzyme is mainly inactive due to the high levels of GTP and GDP and the low concentration of $\mathrm{Ca}^{2+}$ ions in the cytosol. Under certain circumstances, TGase 2 can occur in other cell organelles such as the nucleus and
mitochondria and can be released to the extracellular milieu. Despite higher levels of $\mathrm{Ca}^{2+}$ and low concentrations of GTP and GDP, the enzyme is largely inactive in that latter compartment as well. Activation can occur under the influence of certain pathogenic stimuli, such as local injury, ${ }^{8}$ pro-inflammatory mediators or hypoxia. ${ }^{9-10}$ Extracellular inactivity of the enzyme has been mainly attributed to the formation of a vicinal disulphide bridge between Cys370 and Cys371, which renders TGase 2 inactive regarding acyl transfer catalysis. Thioredoxincatalysed reduction of this disulphide bond can activate TGase 2, which is stimulated by inflammatory signals such as the cytokine interferon- $\gamma .{ }^{11}$

Even though strictly regulated and largely supressed under physiological conditions, increased acyl transferase activity by TGase 2 has been shown to contribute to the pathogenesis of several diseases such as such as celiac disease, ${ }^{12-14}$ neurodegenerative disorders, ${ }^{15-16}$ diseases related to fibrotic processes, ${ }^{17-20}$ and cancer. ${ }^{21-23}$ While the extent of $\gamma$-glutamyl transfer catalysis clearly correlates to the disease state for kidney fibrosis, ${ }^{17}$ the function of TGase 2 in neoplastic diseases is less clear. However, it becomes more and more apparent that TGase 2 is a key player for the progression of several kinds of cancer, including breast carcinoma ${ }^{24}$, renal cell carcinoma ${ }^{25}$ and malignant melanoma ${ }^{26}$. In this context, the elevated levels of TGase 2 expression are directly correlated with poor prognostic indicators for survival, e.g. metastatic phenotype and drug resistance of the cancer cells. Moreover, recent studies identified TGase 2 as a survival factor for cancer stem cells from different tumour entities, ${ }^{27-31}$ even though its detailed function at the molecular level is not completely understood. Direct evidence for the involvement of extracellular TGase 2-catalysed acyl transfer in tumour progression has been obtained by studying the enzyme's function in pancreatic ductal adenocarcinoma. According to this work, the expression of TGase 2 is highly increased in the malignant pancreatic tissue and immunohistochemical staining for the enzyme is accompanied by $N^{\varepsilon}$-( $\gamma$-glutamyl)-lysine isopeptide cross-links in the extracellular matrix and basement membrane. Furthermore, investigations at the cellular level have demonstrated the efficient crosslinking of collagen by TGase 2 secreted from cancer cells, which stimulates fibroblasts for further matrix deposition. In turn, proliferation of tumour cells through mechanical stimuli is elicited. In line with these results, siRNA-mediated knockdown of TGase 2 led to the attenuation of tumour growth in an orthotopic xenograft mouse model of pancreatic cancer. ${ }^{32}$ Similar results were obtained for the invasive ductal carcinoma-type of breast cancer. ${ }^{33}$ Besides extracellular crosslinking, the posttranslational modification of potentially oncogenic intracellular proteins, such as $I_{\kappa} B \alpha,{ }^{34-35}$ phospholipase $\mathrm{A}_{2}{ }^{36}$ and aconitase $2,{ }^{37}$ by TGase 2mediated transamidation is discussed in the context of tumour progression. Moreover, the release of ammonia through TGase 2-catalysed hydrolytic deamidation of protein-bound
glutamine residues was identified as a potential mechanism for counteracting the acidification of the tumour microenvironment associated with increased aerobic glycolysis of tumour cells. ${ }^{38}$ Further tumour-promoting functions of TGase 2 independent of its acyl transferase activity have been attributed to its capability of acting as intracellular GTP-binding protein ${ }^{39}$ and its role as extracellular adapter protein, ${ }^{40}$ both of which involve the closed conformation of the enzyme. ${ }^{7}$

In consequence of its obvious involvement in the progression of different tumours and further pathogenic processes, TGase 2 represents an interesting target for pharmacological inhibition to treat these diseases. Furthermore, considering the remaining open questions with regards to its detailed functions - particularly in cancer - compounds that can be employed as molecular probes for imaging this enzyme in vivo are highly desirable and their development receives increasing interest. ${ }^{41-42}$ In addition, appropriate imaging agents which allow for quantitative assessment of target occupancy and dose selection will support the clinical translation of inhibitors. Based on its quantitative character and its sensitivity, positronemission tomography (PET) is often considered as the most advantageous imaging modality for such purposes. ${ }^{43-47}$

Based on the motivation to develop ${ }^{18}$ F-labelled radiotracers for PET studies, the present study focusses on the synthesis and kinetic characterisation of fluorinated irreversible inhibitors for targeting TGase 2. Irreversible inhibitors ranging from small molecules to peptidic compounds with different electrophilic warheads, which form a covalent bond with the active cysteine residue, represent the most intensively studied class of TGase 2 inhibitors. ${ }^{48-49}$ Radiotracers derived from such irreversibly acting inhibitors appear to be favourable for imaging purposes due to the absent elimination from the target tissue, which potentially results in a high signal/noise ratio as demonstrated for PET tracers targeting monoamine oxidase $B$ and fatty acid amide hydrolase. ${ }^{50-51}$ Despite directed against the $\alpha / \beta$ transamidase domain, irreversible TGase 2 inhibitors have been shown to attenuate tumour proliferation even when acyl transferase-independent functions are mainly involved. ${ }^{52-53}$ This finding is attributed to the presence of a dynamic equilibrium between the open and closed conformations of TGase 2. ${ }^{54}$ An important subclass of irreversible inhibitors is represented by Michael acceptors, which react with the active cysteine residue according to a 1,4-addition. In this context, Signorini et al. observed the inhibition of the transamidase activity of TGase 2 from erythrocytes by acrylamide. ${ }^{55}$ Later, Marrano et al. incorporated the acrylamide moiety as electrophilic warhead in different diaminocarboxylic acid derivatives and identified $N^{\kappa}$-acryloyllysines as potent inhibitors of TGase 2. ${ }^{56-57}$

Based on these results, Wityak et al. investigated structure-activity relationships (SARs), which led to the identification of the phenylacetyl and arylpiperazine moieties as favourable substituents at the $\alpha$-amino and carboxyl group of lysine, respectively. ${ }^{58}$ The most potent compound of this study is compound $\mathbf{6 a}$, which exhibits an excellent selectivity for TGase 2 within the transglutaminase family. Furthermore, this compound is amenable to structural modifications with fluorine, particularly via its phenylacetyl and pyridylpiperazine moieties (see Figure 1). Therefore, 6a was chosen as lead compound for the development of potential fluorinated radiotracers in the present study.


Figure 1. Course of development within acrylamide-based TGase 2 inhibitors and selected fluorinated derivatives envisaged in this study

In addition to fluorination, further structural modifications were considered to uncover novel SARs. On the one hand, this will support radiotracer design by combining reactivity-conferring and fluorinated moieties. On the other hand, SAR studies supported by in silico molecular docking could give insights into the molecular basis for recognition of these inhibitors. To assess the inhibitory potential of the synthesised $N^{\varepsilon}$-acryloyllysines, two TGase 2 activity assays recently developed by us were used. ${ }^{59-60}$ Furthermore, covalent docking studies together with structure-activity relationships concluded from the kinetic data shed light on the binding mode of the $N^{\varepsilon}$-acryloyllysines. Regarding the suitability of compounds as potential radiotracers, both their reactivity towards the desired molecular target and their pharmacokinetic properties are important. Therefore, an initial in vitro pharmacokinetic characterisation of the novel $\mathrm{N}^{\mathrm{E}}$-acryloyllysines was carried out. These studies included the assessment of the compound's potential membrane permeability, their stability towards oxidative metabolism and their potential unspecific binding, using appropriate analytical assays and computational methods.

## Results and Discussion

## Synthetic strategies for the $\boldsymbol{N}^{\boldsymbol{k}}$-acryloyllysine piperazides

## Synthesis of $N^{a}$-Boc- $N^{\kappa}$-acryloyllysine

Structural modifications of lead compound 6a were primarily focused on the introduction of fluorine at different sites, but also other functional groups were considered to uncover SARs. According to retrosynthetic considerations, compound $\mathbf{6 a}$ can be fragmented into three main parts: the central $N^{\varepsilon}$-acryloyl-L-lysine, the N -terminal phenyl acetic acid and the C -terminal 6 -methylpyridin-2-ylpiperazine. For chemical modifications, the C - and N -terminal moieties seem to be most appropriate. Based on the principles of amino acid and peptide chemistry, incorporation of the phenylacetyl group at the $\alpha$-amino group should be conducted after incorporation of the piperazinyl moiety at the $\alpha$-carboxyl group (Scheme 1 ). Furthermore, $N^{\varepsilon}$ acryloylation was accomplished as first step, which allows for a modular synthetic approach. As the positions for structural modfications are at two different moieties of the inhibitors, the inhibitory potential can be finally further increased by combination of advantageous substituents. It should be mentioned that the synthetic strategy for the inhibitors in this study basically corresponds to the published method, but it varies in the conditions for distinct steps. ${ }^{58,61}$


Scheme 1. General synthesis steps for the $N^{\alpha}$-acyl- $\boldsymbol{N}^{k}$-acryloyllysine piperazides using the example of reference compound 6a

Reagents and conditions: a) TEA, $\mathrm{CH}_{3} \mathrm{OH}, 2 \mathrm{~h}$; b) $n-\mathrm{BuOH}, 130^{\circ} \mathrm{C}, 5 \mathrm{~d}$; c) PyBOP, DIPEA, THF, 5 h ; d) $\mathrm{TFA} / \mathrm{CH}_{2} \mathrm{Cl}_{2}(1 / 1, \mathrm{v} / \mathrm{v}), 2 \mathrm{~h}$; e) phenylacetyl chloride, TEA, $\mathrm{CH}_{2} \mathrm{Cl}_{2}, 5 \mathrm{~h}$.

For the synthesis of $N^{\alpha}$-Boc- $N^{\varepsilon}$-acryloyllysine, $N^{\varepsilon}$-acryloylation was performed by the use of $N$ acryloxysuccinimide (1a), which was synthesised from acryloyl chloride and N -
hydroxysuccinimide according to published methods. ${ }^{62-64}$ In this context, $N$ acryloxysuccinimide appeared to be advantageous over acryloyl chloride, as the latter one led to the formation of the condensation product of $N^{a}$-Boc- $N^{\varepsilon}$-acryloyllysine and $N^{a}$-Boc-lysine as side product. The high solubility of the desired product in water caused challenges during processing and purification of the reaction mixture. Therefore, initially the crude product was used for the subsequent coupling reaction with the piperazine building blocks. Residual N hydroxysuccinimide and triethylamine should not have detrimental effects on that reaction. However, ongoing attempts to purify the crude product (without processing) revealed a method for column chromatography which uses $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ with gradually increasing content of isopropanol (from 5 to $8 \%$ ) containing a constant portion of acetic acid (2\%) as eluent. To avoid formation of acetamide during the subsequent coupling reaction, residual acetic acid was removed by repeated azeotropic evaporations of the product in the presence of toluene. ${ }^{65}$ Compound 2a was obtained in $50 \%$ yield by this method.

## Syntheses of the piperazine building blocks

Most of the piperazine building blocks used in this study were synthesised by the reaction of piperazine or Boc-piperazine with the respective pyridine, benzoic acid and sulfonic acid derivatives (Scheme 2). For compounds 3a and 3b, the thermal substitution at 2-chloropicoline (in $n$-butanol ${ }^{66}$ ) and 3 -bromo-6-nitropyridine (in acetonitrile ${ }^{67}$ ), respectively, with piperazine was used without the addition of a further base. Due to the high reactivity of 2,6difluoropyridine, the procedure of Prante et al. was adopted for the synthesis of Boc-3d, which uses Boc-piperazine instead of piperazine, triethylamine as base and DMF instead of $n$-butanol as solvent. ${ }^{68}$ A series of pyridyl-substituted piperazines (Boc-3i-Boc-3n) was obtained by Buchwald-Hartwig amination of Boc-piperazine with the respective bromo-substituted pyridine derivatives, ${ }^{69}$ with the exception of the 6 -nitropyridin- 2 -yl derivative, which was synthesised by the use of 2-chloro-6-nitropyridine. The coupling reactions furnished the desired piperazine building blocks in good yields (40-76\%) after purification by column chromatography. 2-Bromo6 -tert-butylpyridine ( $\mathbf{B r}-3 \mathbf{n}$ ), which was needed for the synthesis of Boc-3n, was accessible by a novel $\mathrm{Cu}(\mathrm{I}$-catalysed coupling reaction of tertiary Grignard reagents with azacyclic electrophiles. ${ }^{70-71}$ The other pyridine derivatives were commercially available. A structural variation, which was not intended arose during the reaction of Boc-piperazine with 2-chloro-6trichloromethylpyridine under the conditions of a Buchwald-Hartwig amination. Instead of the intended 6 -trichloromethylpyridyl moiety, the incorporation of the 6-chloropicolinoyl moiety occurred, which was confirmed by NMR and ESI-MS analyses (compound Boc-3h). The substitution of the ring chlorine is known, ${ }^{72}$ however, even under different conditions ( $\mathrm{NaOH} / \mathrm{TEA}$ as bases and $\mathrm{CH}_{3} \mathrm{CN} / \mathrm{DMF} / \mathrm{CH}_{3} \mathrm{OH}$ as solvents), the formation of the picolinic acid
derivative was exclusively observed. Such reaction products have been identified for the reaction of $\beta$-trichloromethylpyridines with nucleophiles as well as for the hydrolysis of 2-chloro-6-trichloromethylpyridine. ${ }^{72-73}$

For the selective mono-acylation with the 4-fluorobenzoyl- and 4-nitrobenzoyl groups, Bocpiperazine was reacted with the respective benzoyl chlorides. After processing of the reaction mixtures (especially washing with 1 M HCl ), a purification step for both compounds (Boc-3e and Boc-3f) could be omitted. The incorporation of the dansyl moiety was managed according to the procedure by Sashuk et al. using piperazine and dansyl chloride without the need for purification (3c). ${ }^{74}$ The synthesis of the 4 -fluorobenzyl-substituted piperazine Boc-3g was realised by reductive amination between Boc-piperazine and 4-fluorobenzaldehyde using sodium triacetoxyborohydride as reducing agent. ${ }^{75}$ After purification by column chromatography, this piperazine derivative was obtained in moderate yield ( $42 \%$ ).

Some variations in the piperazine building blocks were achieved by direct modification of pyridyl-substituted piperazines (Scheme 2). In this context, the fluoroethoxy group was introduced via nucleophilic aromatic substitution of 1-Boc-4-(6-fluoropyridin-2-yl)piperazine and 2-fluoroethanol. ${ }^{76}$ Worth of note, the fluoroethoxy-substituted product Boc-3o could not be separated from its fluoro-substituted educt Boc-3d, neither by RP-HPLC nor by conventional normal-phase column chromatography. Analysis of the crude product by NMR revealed a proportion of $40 \%$ of the product in the mixture. As the extent of conversion could not be increased by different conditions ( $\mathrm{CaH}_{2}$ instead of NaH and longer reaction time), subsequent Boc deprotection of the crude product was performed followed by purification via RP-HPLC. The commercially available 1-Boc-4-(6-bromopyridin-2-yl)piperazine served as starting material for two further piperazine building blocks. The 1-Boc-6-iodopyridin-2ylpiperazine (Boc-3p) was obtained quantitatively via cooper-catalysed „aromatic Finkelstein reaction" with Nal. ${ }^{77}$ For the synthesis of 1-Boc-6-phenylpyridin-2-ylpiperazine (Boc-3q, 59\%), a Suzuki coupling with phenylboronic acid in aqueous environment was selected, according to the procedure for the Suzuki coupling of $\beta$-chloroacroleins with boronic acid described by Hesse and Kirsch. ${ }^{78}$

Starting from the final inhibitor bearing a picolinic acid methyl ester moiety (compound 8f), two further structural variations were obtained. Hydrolysis of the ester bond furnished the respective picolinic acid derivative $\mathbf{8 e}$, which was subsequently used for the conversion to the primary amide $\mathbf{8 g}$.










Scheme 2. Synthetic methods for the preparation of the piperazine building blocks 3
Boc removal from Boc-piperazine derivatives Boc-3d-Boc-3q to yield $\mathbf{3 d}$-3q was performed using a mixture of TFA/CH2Cl $\mathrm{Cl}_{2}$ as outlined in the main text and in the Chemistry Section in Supporting Information. Compounds $\mathbf{3 r} \mathbf{- 3 x}$ are not shown in the scheme as they are commercially available. Reagents and conditions: $\mathbf{a}_{1}$ ) 2 chloropicoline, $n$-butanol, $130^{\circ} \mathrm{C}, 5 \mathrm{~d}$; $\mathbf{a}_{2}$ ) 3-bromo-6-nitropyridine, $\mathrm{CH}_{3} \mathrm{CN}, 95^{\circ} \mathrm{C}, 6 \mathrm{~h}$; $\mathbf{a}_{3}$ ) dansyl chloride, $\left.\mathrm{CH}_{2} \mathrm{Cl}_{2}, 15 \mathrm{~min} ; \mathbf{b}_{1}\right)$ 2,6-difluoropyridine, TEA, DMF, $150^{\circ} \mathrm{C}, 24 \mathrm{~h}$; $\mathbf{b}_{2}$ ) 4-fluorobenzoyl chloride, TEA, $\mathrm{CH}_{2} \mathrm{Cl}_{2}$, 2 h ; $\mathrm{b}_{3}$ ) 4-nitrobenzoyl chloride, TEA, THF, 2 h ; $\mathrm{b}_{4}$ ) 4-fluorobenzaldehyde, sodium triacetoxyborohydride, THF, $\mathrm{N}_{2}, 5 \mathrm{~h} ; \mathbf{b}_{5}$ ) nitrapyrin, $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, xantphos, $\mathrm{Pd}_{2}(\mathrm{dba})_{3}, \mathrm{Ar}, 70^{\circ} \mathrm{C}, 24 \mathrm{~h} ; \mathbf{c}$ ) respective bromopyridine derivative, $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, xantphos, $\mathrm{Pd}_{2}(\mathrm{dba})_{3}, \mathrm{THF}, \mathrm{Ar}, 70^{\circ} \mathrm{C}, 5-12 \mathrm{~h}$; d) Cul, tert-butylmagnesium chloride, THF, $\mathrm{Ar},<0^{\circ} \mathrm{C} \rightarrow$ $30^{\circ} \mathrm{C}, 3 \mathrm{~h} ; \mathbf{e}$ ) fluoroethanol, $\mathrm{NaH}, \mathrm{DMSO}, \mathrm{Ar}, 100^{\circ} \mathrm{C}, 2 \mathrm{~d} ; \mathbf{f}$ ) CuI, NaI, TMEDA, 1,4-dioxane, $\mathrm{Ar}, 100^{\circ} \mathrm{C}, 44 \mathrm{~h} ; \mathbf{g}$ ) phenylboronic acid, $\mathrm{Pd}(\mathrm{OAc})_{2},\left(\mathrm{C}_{4} \mathrm{H}_{9}\right) 4 \mathrm{NBr}, \mathrm{K}_{2} \mathrm{CO}_{3}, \mathrm{H}_{2} \mathrm{O} / \mathrm{THF}(5: 2, \mathrm{v} / \mathrm{v}), 100^{\circ} \mathrm{C}, 6 \mathrm{~h}$.

In the case of the Boc-protected piperazine derivatives, the Boc group had to be removed prior to coupling with $N^{a}$-Boc- $N^{\varepsilon}$-acryloyllysine. To this end, the Boc-piperazine derivatives were
treated with a mixture of TFA/CH2Cl $\mathrm{Cl}_{2}(1: 1, \mathrm{v} / \mathrm{v})$ for 2 h . To avoid excessive use of base for the amide coupling reactions, the resulting TFA salts were neutralised with aqueous NaOH and extracted into the organic phase to obtain the free secondary amines. This procedure was omitted for the picolinic acid methyl ester derivative Boc-3I due to its susceptibility to basic hydrolysis.

## Amide coupling between $N^{a}$-Boc- $N^{\varepsilon}$-acryloyllysine and the piperazine building blocks

The formation of the amide bond was realised using PyBOP as a coupling agent in the presence of DIPEA as base. All coupling products (compounds 4) were purified by column chromatography. The conversion of the crude product of $N^{a}$-Boc- $N^{\varepsilon}$-acryloyllysine resulted in only slightly diminished yields compared to the purified product ( $20-90 \%$ and $50-90 \%$ ), which justifies the initial approach omitting the purification after $N^{\varepsilon}$-acryloylation.

## Boc removal and functionalisation at the $\alpha$-amino group

The removal of the Boc group from the coupling products was conducted as described for the Boc-protected piperazine building blocks (TFA/CH2Cl2 (1:1, v/v), 2 h ). However, the neutralisation of the TFA salts (compounds 5) was not accomplished. Therefore, additional equivalents of base had to be considered for the next reaction step. The last step to the final inhibitors (compounds 6-21) comprised the functionalisation at the $\alpha-a m i n o$ group. The incorporation of the different substituents was achieved by three different ways: reaction of activated carboxylic acid derivatives or analogues, PyBOP-mediated reaction with carboxylic acids and reductive amination.

In the course of the last reaction step several side reactions were observed. When possible, the respective side products ( $\mathbf{6 c}, \mathbf{1 6 a - d}, \mathbf{1 4 n} \mathbf{- q}$ ) were separated and characterised by NMR and ESI-MS. The results are discussed in the Supporting Information (Discussion S1).

## Transglutaminase assays

## Fluorimetric assay method

For the characterisation of the $N^{\varepsilon}$-acryloyllysines towards inhibition of hTGase 2 (human transglutaminase 2), a continuous fluorimetric assay method was used, which was recently developed by our group. ${ }^{59}$ This assay is based on the measurement of an increase in fluorescence over time due to the TGase 2-catalysed hydrolysis of the water-soluble fluorogenic acyl donor Z-Glu(HMC)-Gly-OH resulting in the formation of HMC and Z-Glu-GlyOH . Due to significant spontaneous hydrolysis of Z-Glu(HMC)-Gly-OH at higher pH values, kinetic characterisation of inhibitors was performed at pH 6.5 using six different concentrations of inhibitor. Analysis of the progress curves was done under the formalism of slow-binding inhibition providing finally values for the performance constants $k_{\text {inact }} / K_{1} .{ }^{59}$ In case of hyperbolically decreasing initial velocities with increasing inhibitor concentration, the dissociation constants ( $K_{\mathrm{i}}$ ) of the non-covalent enzyme-inhibitor complex were also derived.

In addition to the characterisation of the desired drug-target interaction, assessment of specificity and selectivity of drug candidates is fundamental. As the familiy of transglutaminases consists of eight enzymatically active proteins, the development of selective hTGase 2 inhibitors is challenging. To address the issue of selectivity, assay methods for the characterisation of transglutaminases beside hTGase 2 are necessary. For the characterisation of the selectivity (based on $k_{\text {inact }} / K_{1}$ values) of irreversible inhibitors with 3-bromo-4,5-dihydroisoxazol warhead, Klöck et al. ${ }^{79}$ utilised the well-known GDH-coupled assay method $^{80}$ with appropriate small glutamine-containing peptides for the respective transglutaminases (Z-QG-OH for hTGase 2 and mTGase 2, Z-QS-OH for hTGase 1 and hTGase 3, H-QEQVSPLSLLK-OH for hfXIIIa). Inspired by the results of this study, we hypothesised that the fluorogenic acyl donor Z-Glu(HMC)-Gly-OH, which is a good substrate for gpTGase 2 (guinea pig transglutaminase 2) and hTGase $2,{ }^{59}$ could be also used to characterise further TGases. Thus, the enzymatic hydrolysis of Z-Glu(HMC)-Gly-OH by mTGase 2 (murine transglutaminase 2), hTGase 1, hTGase 3, hTGase 6 and hfXIIla was investigated at pH 6.5 . Worth of note, acyl donor Z-Glu(HMC)-Gly-OH is also a suitable substrate for all other TGases studied (Figure 2 and Table 1). While the kinetic parameter towards mTGase 2 are comparable to gpTGase 2 and hTGase 2, the substrate properties towards hTGase 1, hTGase 3, hTGase 6 and hfXIIIa are significantly diminished. The performance constants ( $K_{\text {cat }} / K_{m}$ ) for these TGases are reduced to $1 / 18$ or less compared to hTGase 2. Considering the off-target TGases, Z-Glu(HMC)-Gly-OH exhibits the most favourable properties towards hTGase 6, followed by hTGase 1, hfXIIla and hTGase 3. In a recent study, Akbar et al. ${ }^{81}$ kinetically characterised the well-known TGase 2 substrate Z-

Glu( $p \mathrm{NP}$ )-Gly-OH ( $k_{\text {cat }} / K_{\mathrm{M}}=11833 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ for hTGase 2) towards hTGase 1 and hTGase 6 at $\mathrm{pH}=6.5$. Similar to its coumarinylester analogue, this compound is also a suitable substrate for these TGases even though the performance constants are significantly reduced compared to hTGase 2. In contrast to Z-Glu(HMC)-Gly-OH, Z-Glu(pNP)-Gly-OH is better tolerated by $\mathrm{hTGase} 1\left(k_{\text {cat }} / K_{\mathrm{M}}=1333 \mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$ than by hTGase $6\left(k_{\text {cat }} / K_{\mathrm{M}}=500 \mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$, which might indicate differences in the recognition of these substrates within the active sites of the different TGases.


Figure 2. Enzymatic hydrolysis of Z-Glu(HMC)-Gly-OH by different TGases at pH 6.5
Plots of vototal $=\mathrm{f}([\mathbf{Z}-\mathrm{Glu}(\mathbf{H M C})-\mathrm{Gly}-\mathbf{O H}])$ for gpTGase 2, hTGase 2 and mTGase 2 and plots of vocorr=f([Z-Glu(HMC)-Gly-OH]) for hTGase 1, hTGase 3, hTGase 6 and hFXIIla including nonlinear regressions according to equation VI (Experimental Section). Data shown are mean values $\pm$ SEM of three separate experiments, each performed in duplicate. When not apparent, error bars are smaller than the symbols. Plots for gpTGase 2 and hTGase 2 have already been shown in a previous study. ${ }^{59}$ Conditions: $\mathrm{pH} 6.5,3{ }^{\circ} \mathrm{C}, 5 \%$ DMSO, $500 \mu \mathrm{M}$ TCEP, $3 \mu \mathrm{~g} / \mathrm{mL}$ TGase.

Table 1. Kinetic parameters for the enzymatic hydrolysis of acyl donor Z-Glu(HMC)-Gly-OH by different TGases at pH 6.5 and $30^{\circ} \mathrm{C}$

| Enzym | regression analysis |  |  | numerical integration |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $K_{\text {m }}(\mu \mathrm{M})$ | $k_{\text {cat }}\left(\mathbf{s}^{-1}\right)$ | $\boldsymbol{K}_{\text {cat }} / K_{\mathrm{m}}\left(\mathbf{M}^{-1} \mathrm{~s}^{-1}\right)$ | $K_{\text {m }}(\boldsymbol{\mu} \mathrm{M})$ | $\boldsymbol{k}_{\text {cat }}\left(\mathbf{s}^{-1}\right)$ | $\boldsymbol{K}_{\text {cat }} / K_{\text {m }}\left(\mathbf{M}^{-1} \mathbf{s}^{-1}\right)$ |
| gpTGase $\mathbf{2}^{*}$ | 2.53 (0.18) | 0.33 (0.01) | 130000 | 2.67 (0.20) | 0.34 (0.01) | 129000 |
| hTGase $\mathbf{2}^{*}$ | 6.60 (1.06) | 0.32 (0.02) | 48500 | 5.78 (1.03) | 0.32 (0.02) | 57600 |
| mTGase 2 | 6.10 (0.26) | 0.29 (0.01) | 47500 | 5.55 (0.34) | 0.28 (0.01) | 50500 |
| hTGase 1 | $\begin{aligned} & 183(39) \\ & 226^{\#} \end{aligned}$ | $\begin{aligned} & 0.17 \text { (0.02) } \\ & 0.19^{*} \end{aligned}$ | $\begin{aligned} & 929 \\ & 841^{\#} \end{aligned}$ | 190 (52) | 0.18 (0.03) | 947 |
| hTGase 3 | $\begin{aligned} & 202(25) \\ & 506^{*} \end{aligned}$ | $\begin{aligned} & 0.07(0.01) \\ & 0.06^{\neq} \end{aligned}$ | $\begin{aligned} & 347 \\ & 119^{*} \end{aligned}$ | - | - | - |
| hTGase 6 | 46.1 (2.7) | 0.13 (0.01) | 2820 | 44.1 (2.4) | 0.14 (0.01) | 3170 |
| hFXIIIa | $498(148)$ | $\begin{aligned} & 0.34(0.08) \\ & 0.48^{\ddagger} \end{aligned}$ | $\begin{aligned} & 683 \\ & 622^{\#} \end{aligned}$ | 441 (104) | 0.33 (0.07) | 748 |

For details on the calculation of the kinetic parameters, see Experimental Section. Data shown are mean values ( $\pm$ SEM) of three separate experiments, each performed in duplicate. Active concentrations of mTGase 2, hTGase 1, hTGase 3, hTGase 6 and hFXIIla were calculated from Zedira's activity data. *Kinetic data for gpTGase 2 and hTGase 2 have previously been published. ${ }^{59}$ \#Data obtained by the method of Cornish-Bowden and Eisenthal. 82

The same data sets were also analysed by numerical regression ${ }^{59}$ providing kinetic parameters that are in good agreement to those of the classical regression analyses (Table 1). However, no fit traces could be obtained for the data of hTGase 3. The high proportion of spontaneous reaction of $\mathbf{Z}-\mathrm{Glu}(\mathrm{HMC})$-Gly- $\mathbf{O H}^{59}$ might be problematic for the accurate analysis of the data for this enzyme by numerical integration.

Due to the low rates of substrate conversion by hTGase 1, hTGase 3, hTGase 6 and hfXIIla, the progression curves for each substrate concentration are linear. Therefore, acyl donor Z-Glu(HMC)-Gly-OH can be applied for the kinetic characterisation of irreversible inhibitors within its solubility limit ( $<250 \mu \mathrm{M}$ ) towards these TGases, even though the enzymes are not saturated by the substrate.

## Fluorescence anisotropy assay

Another assay method, which was recently described by our group, ${ }^{60}$ is based on the measurement of an increase in fluorescence anisotropy (FA) during the TGase 2-catalysed transamidation of a fluorophore-conjugated cadaverine derivative with DMC. Out of the three investigated cadaverine derivatives towards gpTGase 2-catalysed transamidation, rhodamine B-isonipecotyl-cadaverine (R-I-Cad) exhibits the most favourable substrate properties. With respect to the potential usefulness of this assay method for characterising cellular TGase 2's transamidation activity, the characterisation of the substrate pair R-I-Cad and DMC towards hTGase 2 was envisaged. Furthermore, the characterisation of selected
inhibitors by this assay method was sought to compare the results with those of the fluorimetric assay method. This enabled the evaluation of the inhibitory properties of the $N^{\kappa}$-acryloyllysines at different pH values ( 6.5 versus 8.0 ) as well as towards different enzymatic activities (hydrolase versus transamidase activity).

Initial investigations on the dependence between the activity and concentration of hTGase 2 provided similiar results compared to gpTGase 2 as linearity predominates over a wide range of enzyme concentrations ( $0.5-5 \mu \mathrm{~g} / \mathrm{mL}$, Figure S1 in Supporting Information). The results obtained by varying the concentration of one substrate in the presence of the other substrate at a fixed concentration are shown in Table 2 and Figure S2 in Supporting Information. Compared to gpTGase 2, a similar kinetic behaviour of both substrates can be observed towards hTGase 2, especially with regards to the maximum velocities. However, the $K_{\mathrm{m}}$ value of DMC towards hTGase 2 is significantly reduced ( $2.78 \mu \mathrm{M}$ ) compared to gpTGase 2 $(27.5 \mu \mathrm{M})$. Concerning the characterisation of irreversible inhibitors, the potential of the FA assay method was also recently demonstrated. ${ }^{60}$ Due to the high rate of hTGase 2-catalysed incorporation of R-I-Cad in DMC it was decided to reduce the enzyme concentration from $5 \mu \mathrm{~g} / \mathrm{mL}$ to $2 \mu \mathrm{~g} / \mathrm{mL}$ for inhibitor characterisation. Compared to gpTGase 2, the time for preincubation of enzyme and inhibitor had to be reduced for hTGase 2 from 30 to 5 min due to the significantly higher reaction rate of most of the $N^{\kappa}$-acryloyllysines towards the human enzyme (see below).

Table 2. Kinetic parameters for the substrate pairs DMC/F-Cad towards gpTGase 2 and DMC/R-I-Cad towards gpTGase 2 and hTGase 2

| Enzym |  | $\boldsymbol{K}_{\mathbf{m}}(\boldsymbol{\mu M})$ | $\boldsymbol{V}_{\max }\left(\mathrm{mA} \mathrm{s}^{-1}\right)$ | $\boldsymbol{K}_{\mathbf{i}}(\boldsymbol{\mu M})$ |
| :--- | :--- | :--- | :--- | :---: |
| gpTGase 2 | DMC | $3.05(0.44)$ | $0.027(0.002)$ | - |
|  | F-Cad | $2.22(1.40) \times 10^{-4}$ | $0.018(0.001)$ | $9.15(3.11)$ |
| gpTGase 2 | DMC | $27.5(6.4)$ | $0.176(0.028)$ | $135(32)$ |
|  | R-I-Cad | $2.42(0.69) \times 10^{-4}$ | $0.113(0.013)$ | $7.14(2.20)$ |
| hTGase 2 | DMC | $2.78(0.10)$ | $0.132(0.001)$ | $174(16)$ |
|  | R-I-Cad | $3.43(0.02) \times 10^{-4}$ | $0.121(0.002)$ | $6.81(0.45)$ |

For details on the calculation of the kinetic parameters, see Experimental Section. Data shown are mean values ( $\pm$ SEM) of three (gpTGase 2) or two (hTGase 2) experiments, each performed in duplicate (hTGase 2) or triplicate (gpTGase 2). Kinetic data for gpTGase 2 have previously been published. ${ }^{60}$

Taken together, two reliable assay methods based on fluorescence intensity and fluorescence anisotropy were established for inhibitor characterisation, which facilitate investigations of the inhibitory behaviour at different pH values towards different enzymatic activities as well as towards different TGase enzymes. Due to the known similarity between gpTGase 2 and
hTGase 2, which has often justified the use of gpTGase 2 as cost-efficient alternative to the human variant in the past, ${ }^{83}$ there was a special interest on the behaviour of the $N^{\text {E}}$ acryloyllysines towards both enzymes. Even though the settings of both assay methods were basically identical for these TGases, it should be mentioned that for gpTGase 2 fluoresceinlabelled cadaverine ( F -Cad) was chosen as acyl acceptor for the measurements by the FA assay method while R-I-Cad was used for experiments on hTGase 2 (kinetic plots and data are also shown for the purpose of comparison in Table 2 and Supporting Information). This difference in the composition of the assay mixture should, however, be unproblematic, as F-Cad and R-I-Cad were shown to provide reasonably comparable results for the characterisation of irreversible inhibitors. ${ }^{60}$

To confirm the irreversibility of the inhibition of gpTGase 2 and hTGase 2 by the $N^{\text {E}}$ acryloyllysines, a jump-dilution experiment according to Copeland ${ }^{84}$ was exemplarily performed for the inhibition of gpTGase 2 by lead compound 6a. As expected, no enzymatic activity has been restored after 1:100 dilution of a pre-incubated mixture of enzyme and inhibitor (both 100-fold concentrated) into the assay mixture, which clearly confirms the irreversible inhibition of TGase 2 by $N^{\varepsilon}$-acryloyllysines (Figure S3 in Supporting Information).

In order to determine the active enzyme concentrations, the FA-based assay has recently been shown to be a reliable and fast method for active-site titration of gpTGase 2 by using iodoacetamide in a concentration range stoichiometric to the enzyme. ${ }^{60}$ However, when TCEP instead of DTT was used as antioxidant for hTGase 2, the active-site titration with iodoacetamide led to false results for that enzyme due to the known reactivity of the inhibitor with TCEP. ${ }^{85}$ Therefore, one of the most potent $N^{\varepsilon}$-acryloyllysines in this study, compound 8d (see below), was applied for the active-site titration hTGase 2. By using a preincubation time of 40 min , the concentration of catalytically active hTGase 2 could be determined (with R-I-Cad and DMC as substrates; Figure S4 in Supporting Information). Furthermore, a comparable result was obtained for hTGase 2 with the fluorimetric assay following the hydrolysis of Z-Glu(HMC)-Gly-OH in the presence of $\mathbf{8 d}$ after a preincubation period of 40 min (Figure S4).

## Molecular docking studies

Computational methods that are available for the modelling of covalent enzyme-inhibitor complexes were considerably improved over the recent years. ${ }^{86}$ Within this study, covalent docking was carried out to predict the binding mode of the synthesised irreversible covalent inhibitors towards hTGase 2 and rationalise the differences in enzymatic activity which were experimentally observed at atomic detail. The crystal structure of the open conformation of hTGase 2 in complex with a peptide-like irreversible inhibitor developed by Khosla's group was
used for this purpose (PDB ID 2Q3Z). ${ }^{87}$ In this structure, two hydrophobic pockets are well defined. The entrance to pocket 1 through the catalytic site is flanked by Lys176 and Met252 and its tunnel-like shape involves the residues Gln169, Ile178, Trp180, and and the backbone of Trp254. Pocket 2 is deeper and comprises Asn302, Ala304, Leu312, Ile313, Phe316, Met330, Ile331 and Leu420. In the crystal structure, there is no electron density for residues 307-308 and 319-327, which are located proximal to the catalytic site. Due to the potential role of these missed residues on defining proximal binding sites for the newly developed inhibitors, they were modelled before performing the docking studies (see Experimental Section, Figure S5 in Supporting Information). A similar approach was previously undertaken by Badarau et al. with good results regarding the predictions. ${ }^{88}$ The energy-refined 3D molecular model of hTGase 2 in the open conformation suggested an additional pocket 3 proximal to the pocket 2 flanked by Arg317 and Asn308 (Figure 3A), whose existence has been proposed before even though no experimental or computational proof was provided. ${ }^{49}$ Covalent docking studies were first performed with the lead compound 6a. Binding poses were predicted either with the phenylacetyl group or the pyridylpiperazinyl moiety occupying the hydrophobic pocket 2 . The first scenario with the phenylacetyl in pocket 2 (Figure 3A) has revealed three H bonds involving the hTGase 2 residues Trp241, Ile331 and Asn333. In this case, the methyl group of the pyridylpiperazinyl moiety was forming van-der-Waals contacts with Arg317. In the second scenario with the pyridylpiperazinyl moiety located in pocket 2 (Figure S 6 in Supporting Information), multiple binding modes ${ }^{89}$ were predicted in which the phenyl group could either be oriented towards Arg317, Phe320 or Phe334. The latter binding mode was more favourable than the other two, being stabilised by three H bonds (Trp241, Cys277 and Asn333). Hence, the hTGase 2-6a complex might be represented by a dynamic ensemble of multiple binding poses with the phenylacetyl group occupying pocket 2 or interacting with Phe334. Residues Arg317 and Phe320 might act as intermediate anchor points for the dynamic interconversion between the two binding modes.


Figure 3. Molecular modelling of the interaction of hTGase 2 with covalent inhibitors
The enzyme is shown in gray surface with relevant residues coloured by atom type and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds and cation- $\pi$ interactions are depicted by black and green dashed lines, respectively. A) 6a (green), B) $\mathbf{7 e}$ (yellow, iodine atom highligted in magenta surface) superimposed with 6a (methyl substitution in pyridine ring highlighted in green surface), C) $\mathbf{7 f}$ (orange) and $\mathbf{D}$ ) $7 \mathbf{i}$ (light orange). Figure generated in Maestro (Schrödinger). ${ }^{90}$

## Structure-activity relationships for human TGase 2

## Enantiomeric purity of the $N^{\varepsilon}$-acryloyllysine piperazides

Concerning the enantiomeric purity of the inhibitors, the amide coupling between the lysine main-chain carbonyl and the piperazine building blocks is the most crucial step. However, due to the low electron withdrawing effect of the lysine side chain as well as the protection of the $\alpha$-amino group as urethane, enantiomerisation is strongly hindered. To further support the conservation of the configuration at the $\mathrm{C}_{\alpha}$ atom, the moderately strong base DIPEA and the moderately polar solvent THF were used. ${ }^{91}$ Finally, the enantiomeric purity of the inhibitors was analysed exemplarily for compound 6a by CD spectroscopy and HPLC using a chiral stationary phase (chiral HPLC). In addition to the appropriate analytical method, both enantiomers are needed. Therefore, compound $\mathbf{6} \mathbf{b}$ was synthesised starting from Boc-D-lysine. The CD spectra
confirmed that both compounds are enantiomers (Figure S7 in Supporting Information). Analysis of mixtures of $\mathbf{6 a}$ with increasing amounts of $\mathbf{6 b}$ by chiral HPLC revealed that the enantiomeric purity is greater than 98\% (Figure S8 in Supporting Information). Therefore, the stereochemical integrity was not affected during the synthesis of the compounds. As the procedure for the amide coupling was similarly performed for all compounds, the enantiomeric purity of $6 \mathbf{a}$ can be considered as representative for all inhibitors.

## Influence of configuration at $C^{a}$

Even though $N^{k}$-acryloyllysines are known for a long time to act as potent inhibitors of TGase $2,{ }^{56}$ the influence of the configuration at $C^{\alpha}$ on their inhibitory behaviour has not been investigated so far. The focus on the L-enantiomer is probably a result from studies on substrates for gpTGase 2 where peptides containing L-glutamine exhibit a significantly higher substrate potential than peptides with D-glutamine. ${ }^{92}$ However, to confirm this assumption the D-enantiomer (6b) of compound $\mathbf{6 a}$ was kinetically characterised towards hTGase 2 (Table 3). The change of the configuration leads to a marked decrease in the inhibitory potential. The ratio of the inactivation constants of both enantiomers $\left(\left(k_{\text {inact }} / K_{1}\right) s /\left(k_{\text {inact }} / K_{1}\right)_{\mathrm{R}}\right.$, eudismic ratio) is 24. Consequently, this verifies the focus on the L-configuration in the past and justifies the synthesis of all inhibitors in this work starting from Boc-L-lysine.

Table 3. Influence of the configuration at $\mathrm{C}_{\alpha}$ on the inhibition of hTGase 2

| compound |  <br> configuration |  $k_{\text {inact }} / K_{\mathrm{l}}\left(\mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$ | $K_{\text {i }}(\mu \mathrm{M})$ |
| :---: | :---: | :---: | :---: |
| 6a ${ }^{\text {\# }}$ | $S$ | 4880 (20) | 5.73 (0.77) |
| 6b | $R$ | 206 (8) | - |
| Data shown are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. \#Kinetic data for compound 6 a have previously published. ${ }^{59}$ |  |  |  |

## Substitution at position 6 of pyridine-2-y/piperazines

As discovered by Wityak et al., the methyl group in position 6 of the pyridine ring significantly increases the inhibitory potential compared to the unsubstituted pyridine (as investigated for compounds 40 and $\mathbf{4 p}$ by Wityak et al., which differ from $7 a$ and $6 a$ in the way that the phenylacetyl group is replaced by a Z group as $N^{a}$-substituent)..$^{58}$ Therefore, we initially
focussed on the systematic variation of the substituent in this position. The kinetic data of the respective series of analogues are shown in Table 4.

Table 4. Influence of the substituents in position 6 of the pyridin-2-yl moiety on the inhibition of hTGase 2

| compound |  <br> R |  | $K_{\text {i }}(\boldsymbol{\mu M})$ |
| :---: | :---: | :---: | :---: |
| 6a* | $\mathrm{CH}_{3}$ | 4880 (20) | 5.73 (0.77) |
| 7a | H | 2980 (80) | 8.42 (1.29) |
| 7b | F | 3850 (240) | 6.28 (0.33) |
| 7c | Cl | 4910 (160) | 4.43 (0.25) |
| 7d | Br | 5200 (180) | 5.65 (0.69) |
| 7 e | 1 | 7350 (200) | 2.92 (0.02) |
| 7 f | tert-butyl | 6140 (140) | 3.50 (0.05) |
| 7 g | phenyl | 4560 (220) | 7.76 (2.43) |
| 7h | 2-fluoroethoxy | 3020 (160) | 7.32 (0.36) |
| 7i | $\mathrm{NO}_{2}$ | 10200 (100) | 2.26 (0.10) |

In accordance with the results by Wityak et al., the unsubstituted pyridine derivative 7a ( $k_{\text {inact }} / K_{1}=2980 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) is less potent than the reference compound 6a, even though the phenylacetyl group is present at the $\alpha$-amino group instead of the $Z$ group. ${ }^{58}$ Incorporation of fluorine in compound $\mathbf{7 b}$ ( $k_{\text {inact }} / K_{l}=3850 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) leads to a slight increase of the inactivation constant compared to $\mathbf{7 a}$, however, the level of $\mathbf{6 a}$ is not restored. Subsequent substitution by the other halogen atoms $\mathrm{Cl}, \mathrm{Br}$ and I (compounds 7c, 7d and 7e) further increases the inhibitory potential with the iodine derivative ( $k_{\text {inact }} / K_{1}=7350 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) being even more potent than the reference compound 6a. This trend indicates that the inactivation constant increases with the size of the substituents. Concerning quantitiative structure-activity relationships, there are parameters available describing the sterical demand of a substituent. These substituent parameters include the Taft parameter $E_{s}{ }^{93-95}$ and the differential van-der-Waals radius $v$ (Charton values ${ }^{96-97}$ ). As shown in Figure 4, there are linear relationships between these substituent parameters and the logarithmically transformed inactivation constants ( $\mathrm{R}^{2} \approx 0.9$ ). In this context, the potential bioisosteric behaviour of Cl and $\mathrm{CH}_{3}$ based on their similar sterical demand has already been observed in other SAR studies, both at $\mathrm{sp}^{2}$ and $\mathrm{sp}^{3}$ carbon atoms. ${ }^{98-}$
${ }^{100}$ For example, 3-aminopyrazinone-derived thrombin inhibitors with 4-chloropyridin-2-yl and 4-methylpyridin-2-yl moieties show similar $K_{\mathrm{i}}$ values for the inhibition of thrombin. ${ }^{99}$



Figure 4. Relationship between the size of the substituent in position 6 of the pyridin-2-yl moiety and the inactivation constants of the inhibitors 6a and 7a-e

> Plots of $\lg \left(k_{\text {inact }} / K_{\mathrm{I}}\right)=\mathrm{f}(v)(\mathrm{left})$ and $\lg \left(k_{\text {inact }} / K_{\mathrm{I}}\right)=\mathrm{f}(\mathrm{Es})($ right $)$ using the mean values of the inactivation constants of compounds 6 a and $7 \mathrm{a}-\mathrm{e}($ Table 4$)$ and the following values for $v\left(\AA \AA^{97,101-102: 0.00(H), 0.27(\mathrm{~F}), 0.52\left(\mathrm{CH}_{3}\right),}\right.$ $0.55(\mathrm{Cl}), 0.65(\mathrm{Br})$ and $0.78(\mathrm{I})$ and $\mathrm{Es}^{101-102}: 0.00(\mathrm{H}),-0.46(\mathrm{~F}),-1.24\left(\mathrm{CH}_{3}\right),-0.97(\mathrm{CI}),-1.16(\mathrm{Br})$ and -1.40 (I). Regression analysis was performed by linear regression; $n$ denotes to the amount of data points, s to the standard deviation of the regression equation and $\mathrm{R}^{2}$ to the coefficient of determination. ${ }^{103-104}$

In addition to steric parameters, the presence of the different substituents strongly influences the $\mathrm{p} K_{\mathrm{a}}$ values of the respective pyridinium ions (Table 13). However, a linear relation to the logarithmic transformed inactivation constants can not be derived (Figure S9 Supporting Information), which underlines the importance of the sterical demand for the inhibitory potential. Therefore, there might be a hydrophobic pocket within the catalytic centre which could be occupied by the substituents in position 6 of the pyridine ring allowing a more favourable binding of larger substituents. This is further supported by the obtained dissociation constants, $K_{\mathrm{i}}$, of the reversible enzyme-inhibitor complex (Table 4). These values decrease with increasing size of the substituents illustrating the contribution of the substituents to noncovalent interactions.

Interestingly, covalent docking studies indeed predicted a common recognition region for the functionalised fragment pyridine-2-yl in $\mathbf{7 a} \mathbf{- e}$ and $\mathbf{7 i}$ around pocket 3 flanked by the side chains of Arg317 and Asn308, which resembles the binding mode found for $\mathbf{6 a}$ (Figure 3B).

To assess the limits of the size of substituents in position 6, substituents with even greater sterical demand than iodine were introduced. As a result of the incorporation of the tert-butyl $\left(v=1.24 \AA, E_{s}=-2.78\right)^{97,102}$ and phenyl groups ( $E_{s}=-3.82$; value is refered to the width $)^{102}$, the inhibitory potential of 7 f ( $k_{\text {inact }} / K_{\mathrm{l}}=6140 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) und 7 g ( $k_{\text {inact }} / K_{\mathrm{l}}=4560 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) decreases compared to $\mathbf{7 e}$. The decrease is even more pronounced for compound $\mathbf{7 h}$, although the 2 fluoroethoxy might be of similar size as iodine ( $v($ ethoxy $)=0.48 \AA, v(F)=0.27 \AA)^{97}$, but the asymmetric character is obviously less advantageous.

In contrast to the pyridine-2-yl derivatives 6a and 7a-e, covalent docking results suggested a different binding mode for $7 \mathrm{f}-\mathrm{h}$ in which the pyridine-2-yl moiety functionalised with the bulky tert-butyl, phenyl and 2-fluoroethoxy groups, respectively, occupy pocket 2 by making contacts with the protein residues Ile313, Phe316, lle331 and Leu420, and the phenylacetyl moiety being either solvent-exposed or engaged in contacts with Lys176 or Phe334, which resembles the alternative binding mode predicted for $\mathbf{6 a}$ (Figure 3C and Figure S6).

As ${ }^{18} \mathrm{~F}$-labelled pyridines are accessible via the respective nitropyridine-derived precursors, ${ }^{105-}$ ${ }^{106}$ compound $7 \mathbf{i}$ was synthesised as a potential precursor for radiolabelling and characterised. Considering the SARs so far, compound $7 \mathbf{7 i}$ ( $k_{\text {inact }} / K_{1}=10200 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) surprisingly exhibits the highest inhibitory potential within this series of analogues, which is twice as high as the inhibitory potential of reference compound $\mathbf{6 a}$. Concomitantly to the increase of the inactivation constant, the $K_{\mathrm{i}}$ value decreases from $5.73 \mu \mathrm{M}$ for $\mathbf{6 a}$ to $2.26 \mu \mathrm{M}$ for $\mathbf{7 i}$ indicating that the increased inhibitory potential is driven by more favourable non-covalent interactions. As the sterical demand of the nitro group ( $E_{s=-2.52)^{102}}$ is similar to that of the tert-butyl group, the reason for the increased inhibitory potential might be independent of the size. However, the nitro group could participate in polar interactions, particularly as hydrogen bond acceptor. Using the Eyring equation and the Van't Hoff isotherm the relation between the equilibrium constant of a reaction and the free enthalpy of a transition state can be expressed with equation I.

$$
\begin{equation*}
\ln \mathrm{k}=\ln \left(\frac{\kappa \mathrm{k}_{\mathrm{B}} \mathrm{~T}}{\mathrm{~h}}\right)-\frac{\Delta \mathrm{G}_{0}^{\neq}}{\mathrm{RT}} \tag{I}
\end{equation*}
$$

| k...rate constant | $\kappa$ к...transmission coefficient |
| :---: | :---: |
| $\mathrm{k}_{\mathrm{B}} . .$. Boltzmann's-constant | R...gas constant |
| T...temperature (303,15 K) |  |
| h...PLANCK`s constant |  |
| $\mathrm{K}^{\ldots}$...equilibrium constant of transition state |  |
| $\Delta \mathrm{G}_{0}^{\neq . . . f r e e ~ e n t h a l p y ~ o f ~ t r a n ~}$ | sition state |

Accordingly, to quantify the contribution of the nitro group to lowering of the free enthalpy of the transition state, the difference of the $K_{\text {inact }} / K_{I}$ values of $7 \mathbf{i}$ and 7 a can be used (equation II).

$$
\begin{equation*}
\Delta \Delta \mathrm{G}_{0}^{\neq}=-\mathrm{RT} \ln \frac{\left(k_{\text {inact }} / K_{\mathrm{I}}\right)_{7 \mathrm{i}}}{\left(k_{\text {inact }} / K_{\mathrm{I}}\right)_{7 \mathrm{a}}} \tag{II}
\end{equation*}
$$

This results in an energy gain of $3.1 \mathrm{~kJ} / \mathrm{mol}(0.74 \mathrm{kcal} / \mathrm{mol})$ due to the presence of the nitro group compared to H . A similar value ( $3.31 \mathrm{~kJ} / \mathrm{mol}, 0.79 \mathrm{kcal} / \mathrm{mol}$ ) is obtained by the use of the $K_{\mathrm{i}}$ instead of the $K_{\text {inact }} / K_{l}$ values. Typical energy gains of H bonds are in between 4 to $12 \mathrm{~kJ} / \mathrm{mol}$ and for ionic interactions up to $20 \mathrm{~kJ} / \mathrm{mol},{ }^{107}$ but strongly depend in both cases on desolvation effects accompanied by the effect of enthalpy-entropy compensation. ${ }^{108-111}$ In view of a possible H bond, the nitro group generally represents a weak H bond acceptor $\left(\mathrm{pK}\left(-\mathrm{NO}_{2} \mathrm{H}^{+}\right)=-\right.$ 11 , Ref. ${ }^{112}$ ). However, the potential of aromatic nitro groups is higher than that of aliphatic ones. ${ }^{113}$ Covalent docking results on $7 \mathbf{i}$ are supporting this assumption. The nitro group establishes side-on interactions with the guanidine group of Arg317 (Figure 3D). Interestingly, this charged residue located proximal to the catalytic site constitutes an exclusive feature of hTGase 2 (and also gpTGase 2) in comparison to all other human transglutaminases, which might confer certain selectivity towards hTGase 2 to those covalent inhibitors forming polar contacts with the side chain of Arg317 (see Figure S10 in Supporting Information).

## Substitution at position 6 of pyridine-3-ylpiperazines

In addition to the favourable influence of the methyl group in meta position to the piperazine ring, Wityak et al. discovered also an increase of the inhibitory potential by incorporation of a trifluoromethyl group in para position to the piperazine ring (compounds 40 and $\mathbf{4 s}$ in Ref. ${ }^{58}$, with the $Z$ group as $N^{a}$-substituent and a pyridine-2-ylpiperazine moiety in $\mathbf{4 s}$ ). These results as well as the SARs from 2,6-disubstituted pyridine rings led to the idea of investigating substituents in position 6 of the pyridine-3-ylpiperazine in more detail (Table 5). The influence
of the ring position of the pyridine nitrogen atom, which was moved from ortho to meta position in this series, was separately investigated as discussed below.

Surprisingly, inhibitor 8a ( $k_{\text {inact }} / K_{\mathrm{l}}=6520 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) bearing the unsubstituted pyridine-3-yl group exhibits a two times higher inhibitory potential than its regioisomer $7 \mathbf{a}$ and is even more potent than reference compound $\mathbf{6 a}$. Because the pyridine nitrogen in $\mathbf{8 a}$ has a similar spatial orientation as the nitro group in 7i, polar interactions might again be responsible for the increase in the inhibitory potential. The low $K_{\mathrm{i}}$ value of $2.60 \mu \mathrm{M}$ is in accordance with this hypothesis. However, the interactions of the pyridine nitrogen atom are obviously less favourable than those of the nitro group as the inhibitory potential of $8 \mathbf{a}$ is still lower than that of 7i. In agreement with these experimental observations, the binding mode predicted for $\mathbf{8 a}$ enables interactions in which the nitrogen of the pyridine-3-yl group acts as H bond acceptor towards Arg317 in pocket 3 in a similar manner as the nitro group of $7 \mathbf{i}$ but showing a different orientation of the piperazine group. Although both derivatives were also supported by additional H bonds with Trp241 and Asn333, 7i was forming one additional favourable interaction with Gln276 (Figure 3D and Figure 5A).


Figure 5. Molecular modelling of the interaction of hTGase 2 with covalent inhibitors.
The enzyme is shown in gray surface with relevant residues coloured by atom type and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds are depicted by black dashed lines. A) 8a (green) and 10 (light orange) superimposed, B) 8d. Figure generated in Maestro (Schrödinger). ${ }^{90}$

The incorporation of F and $\mathrm{CF}_{3}$ in position 6, especially with regards to potential radioligands, considerably diminishes the inhibitory potential of $\mathbf{8 b}$ and $\mathbf{8 c}$ in comparison to $\mathbf{8 a}$ (Table 5). While the trifluoromethyl derivative $\mathbf{8 c}$ is equipotent to the pyridine-2-yl derivative $\mathbf{7 a}$, the fluoropyridyl derivative $\mathbf{8 b}$ is even less tolerated by hTGase 2. A sterical hindrance by the substituents can therefore be excluded. The reason for the reduced inhibitory potencies might be the dramatically diminished electron density at the pyridine nitrogen due to the strong
electron withdrawing effect of F and $\mathrm{CF}_{3}$. This is reflected by the calculated $\mathrm{p} K_{\mathrm{a}}$ values of the respective pyridinium ions $\left(\mathrm{cp}_{\mathrm{a}}(\mathbf{8 a})=7.0 ; \mathrm{cp} K_{\mathrm{a}}(\mathbf{8 b})=1.3 ; \mathrm{cp}_{\mathrm{a}}(\mathbf{8} \mathbf{c})=2.3\right.$, see Table 13 and Table S 1 in Supporting Information). In turn, this lowers the suitability of the pyridine nitrogen as H bond acceptor. Even though the nitro group further decreases the electron density at the pyridine nitrogen ( $\mathrm{cp} K_{\mathrm{a}}=-2.15$ ), the inhibitory potential of compound $8 \mathbf{d}\left(k_{\text {inact }} / K_{l}=8460 \mathrm{M}^{-1} \mathrm{~s}^{-1}\right.$ ) is higher than that of compound $\mathbf{8 a}$. Consequently, the nitro group is able to compensate for the interactions of the pyridine nitrogen and can even increase the inhibitory potential. In contrast, the substituents F and $\mathrm{CF}_{3}$ are weak H bond acceptors ${ }^{48}$ and have therefore no compensatory function. The slightly higher inhibitory potential of $\mathbf{7 i}$ compared to $\mathbf{8 d}$ might indicate a more favourable orientation of the nitro group in meta position to the piperazine ring compared to the para position, in case they would interact with identical amino acid residues on the hTGase 2 protein. The predictions from covalent docking studies indicated side-on interactions of the nitro group and the pyridine nitrogen of $\mathbf{8 d}$ with Arg317. However, 8d showed one H bond less with the protein than $\mathbf{7 i}$, which would explain the lower inhibitory potency for 8d (Figure 5B).

Table 5. Influence of the substituents in position 6 of the pyridin-3-yl moiety on the inhibition of hTGase 2


Subsequently, to further characterise the supposed polar interactions of the nitro group, the three picolinic acid derivatives $\mathbf{8 e}, \mathbf{8 f}$ and $\mathbf{8 g}$ were synthesised. The isoelectronic substitution of the nitro group by the carboxylate group in compound 8 e reduced the inactivation constant to $1 / 5$ of the value of $\mathbf{8 d}$. Notably, both methyl ester $\mathbf{8 f}$ and primary amide $\mathbf{8 g}$ are more potent
than $\mathbf{8 e}$ resulting in inactivation constants similar to that of the $\mathrm{CF}_{3}$ derivative $\mathbf{8 c}$. Comparable to the F and $\mathrm{CF}_{3}$ substituents, the $\mathrm{COO}, \mathrm{COOCH}_{3}$ and $\mathrm{CONH}_{2}$ groups lower the electron density at the pyridine nitrogen ( $\mathrm{cp} K_{\mathrm{a}}$ values for the respective pyridinium ions 6.4 ( 8 e ), 3.4 (8f) and $4.3(8 \mathbf{g})$ ). Although the potential of these groups for polar interactions can be rated higher than that of $\mathrm{F}, \mathrm{CF}_{3}$ and $\mathrm{NO}_{2}$, they are obviously not able to compensate for the interactions of the pyridine nitrogen. ${ }^{113-116}$

The supposed polar interactions of the substituents in position 6 should be crucially influenced by their electron withdrawing character. To analyse this relation quantitatively, Hammett sigma constants can be used. ${ }^{117-118}$ Indeed, as shown in Figure 6, the logarithmically transformed inactivation constants of the inhibitors increase with the intensity of the electron withdrawing character of the substituents as defined by their $\sigma_{\mathrm{p}}$ values ( $\mathrm{R}^{2}=0.76$ ). In this context, the unsubstituted pyridine-3-yl derivative 8a deviates from that relation as the pyridine nitrogen of this compound exerts strong interactions to the enzyme, which is not the case for the other inhibitors according to the docking predictions. As a specific characteristic in this series of analogues, the pyridine ring represents a push-pull system due to the para arrangement of the piperazine ring (+M effect) and the electron withdrawing substituents. A more appropriate parameter for the substituents in such systems is refered to as $\sigma_{\mathrm{p}}$, which also considers occurrent resonance effects. ${ }^{19-120}$ For the inhibitors $\mathbf{8 c} \mathbf{c} \mathbf{8 g}$, there is a linear relation $\left(R^{2}=0.99\right)$ between the $\sigma_{\mathrm{p}}{ }^{-}$and the logarithmically transformed inactivation constants (Figure 6).

The deviation of the derivative $\mathbf{8 b}$ from that correlation in addition to compound $\mathbf{8 a}$ might originate from the limited interaction potential of fluorine due to its smaller size compared to the other substituents. The significantly improved correlation for the substituents $\mathrm{CF}_{3}, \mathrm{NO}_{2}$, $\mathrm{COO}, \mathrm{COOCH}_{3}$ and $\mathrm{CONH}_{2}$ using their $\sigma_{\mathrm{p}}{ }^{-}$constants confirms the influence of the electron donating piperazine ring. The increased propensity of the nitro group for polar interactions in such push-pull systems, especially as H bond acceptor, is known. ${ }^{113,121}$ Nevertheless, the distinct predominance of $\mathrm{NO}_{2}$ over $\mathrm{COO}^{-}, \mathrm{CONH}_{2}$ and $\mathrm{COOCH}_{3}$ is surprising and might indicate a more complex relation. A similar tendency between these substituents to act as H bond acceptors was discovered by Tan et al. for structure-activity relationships of dual cyclooxygenase $2 / 5$-lipoxygenase inhibitors. ${ }^{122}$ However, in case of these inhibitors the substituents were bound to a phenyl group.



Figure 6. Relationship between the electronic properties ( $\sigma_{\mathrm{p}}$ und $\sigma_{\mathrm{p}}{ }^{\text {- }}$ ) of the substituents in position 6 of the pyridin- 3 -yl moiety and the inactivation constants of the inhibitors $8 \mathrm{a}-\mathrm{g}$

Plots of $\lg \left(k_{\text {inact }} / K_{\mathrm{I}}\right)=\mathrm{f}\left(\sigma_{\mathrm{p}}\right)(\mathrm{left})$ and $\lg \left(k_{\text {inact }} / K_{\mathrm{I}}\right)=\mathrm{f}\left(\sigma_{\mathrm{p}}^{-}\right)$(right) using the mean values of the inactivation constants of compounds $8 \mathrm{a}-\mathbf{8 g}$ (Table 5) and the following values for $\sigma_{\mathrm{p}}^{19,123:} 0.00(\mathrm{H}), 0.06(\mathrm{~F}), 0.00\left(\mathrm{COO}^{-}\right), 0.36$ $\left(\mathrm{CONH}_{2}\right), 0.45\left(\mathrm{COOCH}_{3}\right), 0.54\left(\mathrm{CF}_{3}\right)$ und $0.78\left(\mathrm{NO}_{2}\right)$ and $\sigma_{p}{ }^{-119}: 0.00(\mathrm{H}),-0.03(\mathrm{~F}), 0.31\left(\mathrm{COO}^{-}\right), 0.61$ $\left(\mathrm{CONH}_{2}\right), 0.64\left(\mathrm{COOCH}_{3}\right), 0.65\left(\mathrm{CF}_{3}\right)$ und $1.27\left(\mathrm{NO}_{2}\right)$. Regression analysis was performed by linear regression. Data points in red were not considered for regression analysis. See text for the respective explanation.

## Influence of the pyridine nitrogen

Previous SARs in this study indicated that the pyridine nitrogen influences the inhibitory potential in addition to the influence of the substituents at the pyridine ring. To further investigate this assumption, a series of phenylpiperazines and pyridylpiperazines (9-12) were synthesised and kinetically characterised (Table 6).

Table 6. Influence of the pyridine nitrogen on the inhibition of hTGase 2

| compound | R |  | $K_{\mathrm{i}}(\boldsymbol{\mu M})$ |
| :---: | :---: | :---: | :---: |
| 9 |  | 2970 (80) | - |
| $7 a^{a}$ |  | 2980 (80) | 8.42 (1.29) |
| $8 a^{a}$ |  | 6520 (40) | 2.60 (0.22) |
| 10 |  | 10500 (100) | 2.55 (0.08) |
| $6 \mathbf{a}^{\mathbf{a}}$ |  | 4880 (20) | 5.73 (0.77) |
| 11 |  | $4050 \text { (40) }$ | 7.55 (0.79) |
| $8 d^{\text {a }}$ |  | 8460 (710) | 2.45 (0.09) |
| 12 |  | 7700 (130) | 2.52 (0.08) |
| Data shown are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. \#Kinetic data for these compounds are already listed in Table 3, Table 4 and Table 5. |  |  |  |

Firstly, it was realised that the pyridine nitrogen in ortho position to the piperazine ring exerts no interactions to the enzyme as the pyridine-2-yl derivatives $\mathbf{7 a}$ and $\mathbf{6 a}$ exhibit simililar inactivation constants as their deaza analogues 9 and 11, respectively. Within the series of unsubstituted pyridine derivatives, the shift of the pyridine nitrogen from ortho to meta leads to a two-fold increase of the inactivation constants. A further doubling of the inhibitory potential is achieved by shifting the nitrogen to the para position ( $k_{\text {inact }} / K_{1}=10500 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) with the resulting compound 10 being equipotent to compound $\mathbf{7 i}$.

Due to the high $\mathrm{p} K_{\mathrm{a}}$ value of the respective pyridinium ion in 10 ( $\mathrm{cp} K_{\mathrm{a}}=10.7$, Table 13), the pyridine nitrogen might be mainly protonated at pH 6.5 ( pH value applied in the fluorimetric assay). In turn, this means that the $\mathrm{NH}^{+}$group functions as an H bond donor instead of an H bond acceptor as in the case of the nitro group. Consequently, compounds 10 and $\mathbf{7 i}$ are probably interacting with different residues within the binding site of hTGase 2. Covalent docking studies suggested two different binding modes for $\mathbf{1 0}$, resembling previous findings. In the top-ranked pose, Asp306 could act as H bond acceptor towards the pyridine $\mathrm{NH}^{+}$when the phenylacetyl group is located in pocket 2 (Figure 5A). Alternatively, the pyridine $\mathrm{NH}^{+}$could form an H bond with the backbone of Phe316, while the phenylacetyl moiety is engaged in cation- $\pi$ interactions with Lys176 (Figure S11 in Supporting Information).

The compensatory function of the nitro group in compound $8 \mathbf{d}$ concerning the diminished interaction potential of the pyridine nitrogen was also confirmed as $8 \mathbf{d}$ and its deaza analogue 12 exhibit similar inactivation constants ( $8460 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ and $7700 \mathrm{M}^{-1} \mathrm{~s}^{-1}$, respectively) and showed similar binding modes.

## Benz(o)yl and sulfonyl substituents at piperazine

With respect to the synthesis of potential radiotracer candidates, the piperazine ring enables the incorporation of prosthetic groups containing fluorine, e.g. the 4-fluorobenzoyl group via N -succinimidyl-4-[ $\left.{ }^{18} \mathrm{~F}\right]$ fluorobenzoate ${ }^{124-125}$ or the 4-fluorobenzyl group via reductive alkylation with $4-\left[{ }^{18}\right.$ F]fluorobenzaldehyde. ${ }^{126}$ Accordingly, the influence of benz(o)yl substituents on the inhibitory potential was investigated (Table 7).

The exchange of the 6-methylpyridin-2-yl moiety in reference compound 6 a by a 4fluorobenzoyl group in 13a leads to a reduction of the $k_{\text {inact }} / K_{1}$ value from 4880 to $1620 \mathrm{M}^{-1} \mathrm{~s}^{-1}$. Noteworthy, this difference in the inactivation constant is similar to that between $\mathbf{6 a}$ and $\mathbf{8 b}$ even though the structural variations differ significantly, which indicates a certain tolerance of hTGase 2 towards different aromatic groups at the piperazine ring. Due to the favourable impact of the nitro group attached to the pyridine ring on the inhibitory potential, the 4nitrobenzoyl derivative 13b was synthesised. Furthermore, this compound could act as a precursor for ${ }^{18}$ F-fluorinations. ${ }^{127}$ The kinetic characterisation revealed a twofold higher inhibitory potential of 13b compared to its fluorine analogue 13a, which demonstrates that the positive effect of the nitro group does also exist when it is attached to structurally different moieties. However, the potential polar interactions are obviously less favoured compared to $\mathbf{7 i}$ and 8d. In line with these observations, covalent docking results predicted mainly two different binding modes for 13b. Thus, the nitro group could establish salt bridge or/and $\pi$-cation interactions with Arg317 instead of side-on H bonds, as observed for $\mathbf{7 i}$ and $\mathbf{8 d}$, and the
phenylacetyl group might participate in $\pi-\pi$ interactions with Trp278. Alternatively, the carbonyl oxygen of the 4-nitrobenzoyl group could make contacts with Arg317, while the phenylacetyl group could be located in pocket 2 (Figure S 12 in Supporting Information). The lower number of H bonds between 13b and hTGase 2 observed for these binding modes in comparison to those obtained for $\mathbf{7 i}$ and $\mathbf{8 d}$ is also in good agreement with the lower inhibitory capacity of 13b.

Table 7. Influence of benz(o)yl and sulfonyl substituents at the piperazine ring on the inhibition of hTGase 2

| compound | R |  | $K_{\mathrm{i}}(\boldsymbol{\mu} \mathrm{M})$ |
| :---: | :---: | :---: | :---: |
| 13a |  | 1620 (190) | 13.4 (2.7) |
| 13b |  | $2950 \text { (470) }$ | 7.71 (0.19) |
| 13c |  | 3030 (120) | 7.60 (0.07) |
| 13d |  | $1340 \text { (80) }$ | - |
| 13e |  | $1980 \text { (70) }$ | - |
| Data shown are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. |  |  |  |

Formal reduction of the benzoyl-piperazine amide bond in 13a led to the tertiary amine 13c, which exhibits a twofold higher inhibitory potential than its amidic parent. Compound 13c might benefit from increased conformational flexibility, which facilitates an advantageous orientation of the 4 -fluorophenyl group within the binding site. The 6-chloropicolinic acid derivative 13d belongs also to this group of compounds. Its $k_{\text {inact }} / K_{1}$ value is slightly lower than that of the 4fluorobenzoyl derivative 13a. Therefore, as the interaction potential of the pyridine nitrogen might be negligible ( $\mathrm{cp} K_{a}$ value of 0.8 for the respective pyridinium ion, Table S 1 in Supporting Information), the meta substitution by chlorine at the benzoyl group appears to be less well tolerated than the para substitution by fluorine.

Due to the good compatibility of the dansyl group as fluorescent moiety in TGase 2 inhibitors enabling diverse in vitro experiments, ${ }^{88,128}$ the dansyl group was also installed at the piperazine ring. Although this fluorophore has a significantly greater steric demand than the 6-methylpyridin-2-yl moiety of reference compound 6a, the inhibitory potential of 13e declines only to $\mathbf{4 0 \%}$ of that from $\mathbf{6 a}$. Therefore, compound $\mathbf{1 3}$ e is even more potent than 13a and 13d. Interestingly, covalent docking results predicted a good tolerance for the dansyl group in pocket 2 while the phenylacetyl moiety would make contacts with Tyr245 and Met252 (Figure S13 in Supporting Information). This tolerance towards sterically demanding substituents at the piperazine ring was already demonstrated by Wityak et al., as compounds containing a 2 naphthyl or pyridine-2-yl moiety at the piperazine ring exhibit similar inhibitory capacity (compounds $\mathbf{4 n}$ and $\mathbf{4 o}$ in Ref. ${ }^{58}$, with the $Z$ group as $N^{a}$-substituent). Furthermore, Akbar et al. recently reported an extensive SAR study for $N^{\alpha}$-Z- $N^{-}$-acryloyllysine piperazides with a primary focus on different sterically demanding acyl and sulfonyl groups at the piperazine ring. ${ }^{81}$ Worth of note, all structural modifications led to comparably potent hTGase 2 inhibitors with inactivation constants ranging from $582 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ to $1883 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ (measured at $\mathrm{pH}=6.5$ using Z-Glu( $p N P$ )-Gly-OH as substrate). These results clearly emphasise the tolerance of hTGase 2 towards structurally different substituents at the piperazine ring.

Variations of the $N^{a}$ substituent
As mentioned above, systematic structural variations were also performed at the $\alpha$-amino group of the lysine moiety (Table 8).

Table 8. Influence of different $N^{\alpha}$-substituents on the inhibition of hTGase 2

| cpd. | R |  <br> $\boldsymbol{k}_{\text {inact }} / K_{\mathbf{l}}\left(\mathbf{M}^{-1} \mathbf{s}^{-1}\right)$ |  <br> cpd. | R | $\boldsymbol{K}_{\text {inact }} / K_{\mathbf{I}}\left(\mathbf{M}^{-1} \mathbf{S}^{-1}\right)$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 14a |  | 676 (61) | $14 h$ |  | 278 (25) |
| 14b\# |  | 3480 (270) | $14 i$ |  | 79 (1) |
| 14c |  | 1680 (63) | $14 j$ |  | 113 (10) |
| 14d |  | 232 (14) | $14 k$ |  | 423 (22) |
| 14 e |  | 362 (39) | $14 I$ | I | 349 (21) |
| 14 f |  | 95 (12) | 14m |  | 127 (11) |
| 14 g |  | 287 (13) |  |  |  |
| Data shown are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. ${ }^{\#} K_{\mathrm{i}}=5.56$ (0.02) $\mu \mathrm{M}$ |  |  |  |  |  |

Pyridyl and thienyl groups are often employed as bioisoteric replacements for phenyl groups, ${ }^{129-130}$ which is due to the similar steric and electronic properties of $-\mathrm{CH}=$ and $-\mathrm{N}=$ as well as $-\mathrm{CH}=\mathrm{CH}$ - and $-\mathrm{S}-$, respectively. ${ }^{131-132}$ However, exchange of the phenylacetyl group by a pyridin-2-ylacetyl moiety results in a dramatic reduction of the inactivation constant for compound $\mathbf{1 4 a}$ to $13 \%$ of the value for reference compound $\mathbf{6 a}$. The other two regioisomeric pyridylacetyl derivatives were also synthesised, however, their kinetic characterisation had to be omitted due to the observed macrocyclisation between the respective pyridine nitrogens and the acrylamide group (Discussion S1 in Supporting Information). A significantly higher reactivity towards hTGase 2 is achieved by the two regioisomeric thienylacetyl derivatives $\mathbf{1 4 b}$ ( $k_{\text {inact }} / K_{l}=3480 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) and $\mathbf{1 4 c}\left(k_{\text {inact }} / K_{l}=1680 \mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$, which exhibit 68 and $33 \%$ of the activity of the phenylacetyl derivative 6a, respectively. As benzene, pyridine and thiophene are similar
in their sterical demand, the size of the different aromatic systems might not be causative for the different inhibitory potential. In contrast to their similar size, their logP values differ significantly indicating a possible reason for the observed differences in reactivity. Indeed, the relationship between the respective Hansch's $\pi$ values and the logarithmically transformed inactivation constants reveals a trend to higher inhibitory potential with increasing hydrophobicity of the substituents (Figure S14 in Supporting Information). This indicates that the aromatic rings might occupy a shared hydrophobic pocket, which simultaneously accounts for the differences in reactivity of the isomeric thienylacetyl derivatives. In agreement with this finding, docking studies indicate that the binding modes of $\mathbf{6 a}, \mathbf{1 4 b}$ and $\mathbf{1 4 c}$ are similar. However, it was observed that the phenyl- and 3-thienylacetyl groups of 6a and 14c were occupying pocket 2 more efficiently than the 2-thienylacetyl moiety in 14b, which lost van-derWaals contacts with Leu420. Nevertheless, the pyridyl group in 14b establishes van-der-Waals interactions with Arg317, while this contact is not formed with 14c, which might explain the different inhibitory potencies of the two regioisomers (Figure S15 in Supporting Information). Alternative binding modes were predicted exhibting the pyridine ring positioned in pocket 2 and the phenyl- and thienylacetyl groups making contacts with Phe334 and Met252 or Phe320. Under these circumstances, the phenylacetyl group of $\mathbf{6 a}$ fills the available space in pocket 2 better than the thienylacetyl derivatives. The electron density at the sulphur atom is slightly increased compared to the carbon atoms and this might be less advantageous when the sulphur atom extends deeper into the hydrophobic pocket. An even higher hydrophobicity is exhibited by the cyclohexylacetyl group, however, the formal saturation of the phenyl group also increases the sterical demand. This is probably the reason for the dramatically reduced inhibitory potential of $\mathbf{1 4 d}$ to $5 \%$ of the value of reference compound $\mathbf{6 a}$.

The further structural modifications performed at the $\alpha$-amino group comprise sulfonyl, carbamoyl, thiocarbamoyl and benz(o)yl groups, which all resulted in compounds with a significantly reduced inhibitory potential ( $k_{\text {inact }} / K_{l}<500 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) compared to lead compound $\mathbf{6 a}$ (for a more detailed discussion see Discussion S2 in Supporting Information). Consequently, rigidisation and shortening of the substituent at the $\alpha$-amino group is detrimental for the inhibitory potential and the most favourable interactions are still exerted by the phenylacetyl group. In this regard, the binding modes predicted for this series of compounds appeared to be diverse. The newly incorporated functionalities could be disposed either along pocket 2 in a non-optimal manner, mostly only via interactions with Phe316, or making contacts with Phe320 or Phe334. The pyridine ring substituted with a methyl group in position 6 could be positioned either in pocket 2 forming van-der-Waals contacts with Ala304 at the surface of pocket 2 or being located in the proximity of Arg317.

None of the phenylacetyl substitutions at the $\alpha$-amino group resulted in a more potent inhibitor than reference compound $\mathbf{6 a}$. Consequently, a series of compounds was envisaged to investigate the influence of substituents at the phenyl group of the phenylacetyl moiety (Table 9).

For this purpose, a series of analogues varying in position 4 of the phenyl group was taken into focus. Incorporation of a methyl group, as done in compound $15 a\left(k_{\text {inact }} / K_{1}=2940 \mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$, reduced the inhibitory potential to almost $50 \%$ of the value of $6 \mathbf{a}$, whereas the presence of the halogen atoms F ( $\mathbf{1 5 b} \mathbf{)}, \mathrm{Cl}(\mathbf{1 5 c}), \mathrm{Br}(\mathbf{1 5 d})$ and $\mathrm{I}(\mathbf{1 5 e})$ is even worse tolerated. Compared to the analogous substitution at position 6 of the pyridine ring (compounds 6a, 7a-7e), a dependence of the inhibitory potential on the size of the substituents cannot be derived (Figure S16 in Supporting Information). Similarily, $\pi$-values as parameters for the hydrophobicity of the substituents are not suitable to describe the observed trend within compounds 6a and 15a15e (Figure S17 in Supporting Information). However, with respect to the different inactivation constants of the thienylacetyl derivatives $\mathbf{1 4 b}$ and $\mathbf{1 4 c}$, the electronic properties of the substituents might be pivotal for the observed trend of the inhibitory potential. The relationship between Hammett's $\sigma_{p}$ values and the logarithmically transformed inactivation constants (Figure 7) reveals a linear increase of the inhibitory potential for 15a-15e with increasing electron withdrawing effect of the substituents. Clearly, the hydrogen atom lies well above the regression line, which indicates that substitution of the 4-position at the phenylacetyl group could change the binding mode. This conclusion is supported by the results obtained from covalent docking. The 4 -methylphenyl ring of compound $\mathbf{1 5 a}$ is predicted to form cation $-\pi$ interactions with the guanidino group of Arg317, while the 6-methylpyridyl moiety is positioned in pocket 2 (Figure 8 A ). This docking result is in line with the striking correlation between $\lg \left(K_{\text {inact }} / K_{1}\right)$ and $\sigma_{p}$ shown in Figure 7, as the observed substituent effects resemble those that have been found for the cation- $\pi$ interaction in supramolecular complexes between the tetramethylammonium ion and uranyl-salophen receptors bearing substituted benzyloxy arms. ${ }^{133-134}$ Docking results for the 4-halophenyl derivatives 15b-e indicate similar interactions with Arg317 as predicted for 15a. In turn, the obtained docking results explain the deviation of 6a from the correlation in Figure 7 as the presence of substituents in the para position of the phenyl ring obviously induces a different binding mode compared to the unsubstituted derivative (phenylacetyl moiety preferably goes to pocket 2; Figure 3A), which is associated with the loss of one H bond. One H bond is also lost in alternative docking poses predicted for the enzyme-inhibitor complexes derived from the 15 series, in which the substituted phenylacetyl group is placed in the hydrophobic pocket 2 (Figure 8B). In general, the loss of
one H bond for the $\mathbf{1 5}$ series of compounds with respect to $\mathbf{6 a}$ in both observed binding modes is consistent with their lower inhibitory capacity.

Table 9. Influence of substituents at the phenylacetyl moiety on the inhibition of hTGase 2

| compound | R |  | $K_{\text {i }}(\boldsymbol{\mu M})$ |
| :---: | :---: | :---: | :---: |
| $6{ }^{\text {* }}$ | H | 4880 (20) | 5.73 (0.77) |
| 15a | $4-\mathrm{CH}_{3}$ | 2940 (90) |  |
| 15b | 4-F | 1530 (50) | - |
| 15c | $4-\mathrm{Cl}$ | 1050 (90) | - |
| 15d | $4-\mathrm{Br}$ | 1130 (40) | - |
| 15e | 4-1 | 1080 (10) | - |
| $15 f$ | 2-F | 3770 (30) | 4.49 (0.12) |
| 15 g | 3-F | 2000 (170) | - |
| Data shown are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. \#Kinetic data for $\mathbf{6 a}$ are already listed in Table 3. |  |  |  |




Figure 7. Relationship between the electronic properties ( $\sigma_{p}$ ) of the substituents in position 4 of the phenylacetyl moiety and the inactivation constants of the inhibitors 6a and 15a-e

Plots of $\lg \left(k_{\text {inact }} / K_{\mathrm{I}}\right)=\mathrm{f}\left(\sigma_{\mathrm{p}}\right)$ using the mean values of the inactivation constants of compounds 6a and 15a-e (Table 9 ) and the following values for $\sigma_{\mathrm{p}}{ }^{119}: 0.00(\mathrm{H}),-0.17\left(\mathrm{CH}_{3}\right), 0.06(\mathrm{~F}), 0.23(\mathrm{Cl}), 0.23(\mathrm{Br}), 0.18$ (I). Regression analysis was performed by linear regression. Compound $\mathbf{6 a}$ (data point in red) was not considered for regression analysis. See text for the respective explanation.


Figure 8. Different binding modes of the interaction of hTGase 2 with 15a (green) and 15e (yellow)
hTGase 2 is shown in gray surface with relevant residues coloured by atom type and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds and salt bridges are depicted by black and magenta dashed lines, respectively. Figure generated in Maestro (Schrödinger). ${ }^{90}$

To investigate the dependence of the inhibitory potential on the position of substituents at the phenyl group, compounds $\mathbf{1 5 f}$ and $\mathbf{1 5 g}$ bearing a 2 -fluorophenylacetyl and 3fluorophenylacetyl moiety, respectively, were synthesised. According to their kinetic characterisation, substitution by fluorine in position 2 and 3 is better tolerated than in position

4; however, the 2-fluorophenylacetyl derivative 15 f is even twice as potent than the 3fluorophenylacetyl derivative $\mathbf{1 5 g}$. Noteworthy, a similar trend was observed by Wityak et al. for $\mathrm{CF}_{3}$ instead of F at the phenyl group in $N^{a}$-Z- $N^{\varepsilon}$-acryloyllysine ( $\mathrm{IC}_{50}$ values of $3.5 \mu \mathrm{M}$ (2$\left.\mathrm{CF}_{3}\right), 10 \mu \mathrm{M}\left(3-\mathrm{CF}_{3}\right)$ and $\left.33 \mu \mathrm{M}\left(4-\mathrm{CF}_{3}\right)\right)^{58}$

## Combination of selected pyridylpiperazinyl and $N^{a}$ substituents

In addition to structural modifications, which were either performed at the piperazine ring or the $\alpha$-amino group, compounds with variations at both sites were also envisaged to study whether the different moieties contribute additively to the inhibitory potential (Table 10).

In this context, compound 16c bearing a 6-phenylpyridin-2-yl and a trifluoroacetyl group represents rather an accidentally obtained product as the trilfuoroacetylation at the $\alpha$-amino group was a side reaction during the phenylacetylation (Discussion S1 in Supporting Information). However, compared to the phenylacetyl analogue $\mathbf{7 g}$ ( $K_{\text {inact }} / K_{\mathrm{l}}=4560 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ), the presence of the trifluoroacetyl group ( $k_{\text {inact }} / K_{\mathrm{l}}=1600 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) is detrimental for the inhibitory potential but better tolerated than most of the other variations at the $\alpha$-amino group (Table 8).

To investigate the favourable effect of the 6-nitropyridin-3-yl moiety (present in $8 \mathbf{d}$ ), this group also combined with three other substituents at the $\alpha$-amino group (compounds 17a-c). The inhibitory potentials of these inhibitors increase compared to their 6-methylpyridin-2-yl analogues by factors of 1.1-3.2 for the inactivation constants (Figure S18 in Supporting Information). The propensity of the nitro group to engage in non-covalent interactions is also reflected by the systematic variations of the initial velocities $v_{i}$ observed for 17a-c, which accounts for the determination of their $K_{\mathrm{i}}$ values in contrast to compounds $\mathbf{1 4 g}, \mathbf{1 4 k}$ and $\mathbf{1 5 b}$. A similar result was obtained using the 6 -nitropyridin- 2 -yl moiety (present in $\mathbf{7 i}$ ). Compound 18 bearing this pyridyl group and a 2 -fluorophenylacetyl group at $N^{a}$ is two times more potent than its 6 -methylpyridin-2-yl derivative $\mathbf{1 5 f}$. Consequently, the detrimental effect of the $N^{a}$ substituent is partially compensated by the nitropyridyl moiety, independently of the kind of substituent.

Further inhibitors obtained by combination of different pyridylpiperazinyl and $N^{a}$ substituents are included in Figure S19 in Supporting Information.

Table 10. Inhibition of hTGase 2 by double modified $\boldsymbol{N}^{\boldsymbol{k}}$-acryloyllysines
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Reactivity of $N^{\varepsilon}$-acryloyllysines towards different enzymatic activities and different pH values
Concerning the issue of assay comparability as well as dependence of inhibitory activity on pH value, selected $N^{\kappa}$-acryloyllysines were screened with both assay methods towards both enzymes. The relationships between values of $k_{\text {inact }} / K_{\mathrm{l}}$ and IC $\mathrm{IC}_{50}$ for both enzymes are shown in Figure 9. Worth of note, an apparent linear correlation can be obtained for both enzyme species even though the two assay methods use different pH values ( 6.5 versus 8.0 ) and measure different enzymatic activities (hydrolase versus transamidase activity). Consequently, the same trend in inhibitory activity is retained under both assay conditions (whereas the absolute reactivity might be different) and - eventually even more interesting - the inhibitory behaviour towards enzymatic hydrolysis and transamidation are essentially comparable. However, compound $\mathbf{7 b}$ significantly deviates from the correlation for the human enzyme as it has higher inhibitory potential at pH 8.0 (or lower potential at pH 6.5 ) compared to the expected value based on the tendency for the other compounds.


Figure 9. Relationship between $\mathrm{IC}_{50}$ values from FA assay ( pH 8.0 ) and $\boldsymbol{k}_{\text {inact }} / K_{\mathbf{1}}$ values from fluorimetric assay ( pH 6.5 )

Plots of $\lg \left(I C_{50}\right)=f\left(\lg \left(K_{\text {inact }} K_{I}\right)\right)$ for $g p T G a s e 2$ (inhibitors $\mathbf{6 a}, \mathbf{6 b}, \mathbf{7 b}, \mathbf{7 i}, \mathbf{8 d}, \mathbf{1 4 h}$ and $\left.\mathbf{1 5 e}\right)$ (left) and hTGase 2 (6a, 7a-i and 8d) (right) using selected inhibitors. The coefficients of determination resulting from the regression analyses by linear regression are shown. Data point in red (7b) (right) was not considered for regression analysis. See text for the respective explanation.

## $N^{\varepsilon}$-propionyllysine piperazides as reversible inhibitors

According to the two-step inhibition mechanism of TGase 2 by the $N^{\varepsilon}$-acryloyllysine piperazides, which was concluded based on the observed systematic reduction of the initial velocities $v_{i}$ in the substrate conversion curves with increasing inhibitor concentration, there is a non-covalent enzyme inhibitor complex prior to the final covalent complex. Consequently, inhibition of the activity of hTGase 2 should also be observed for the case that the acrylamide warhead is replaced by a similar group which does not form a covalent bond with the active cysteine residue. To prove this hypothesis, the respective $\Lambda^{\kappa}$-propionyl analogues 21a and 21b (Figure 10) of lead compound 6a and the nitropyridyl derivative 8d, respectively, were synthesised and kinetically characterised. Moreover, an analogue of a potential radiotracer which does not form a covalent bond with hTGase 2 could act as a control compound for PET studies to get information on the envisaged target binding of the radiotracer in vivo.

The formal saturation of the C-C double bond within the acryloyl group leads to an inert group, but simultaneously the respective propionyl group exhibits a slightly greater sterical demand, which might influence the interactions with the enzyme. As expected, the enzymatic conversion of Z-Glu(HMC)-Gly-OH in the presence of 21a or 21b yielded linear progression curves where the slopes decrease with increasing inhibitor concentrations (Figure S20 in Supporting Information). Analysis of the recorded data on the basis of a competitive inhibition mechanism provided $K_{\mathrm{i}}$ values of 64.3 and $15 \mu \mathrm{M}$ for 21a and 21b, respectively. These values are significantly higher than those for $\mathbf{6 a}(5.73 \mu \mathrm{M})$ and $\mathbf{8 d}(2.45 \mu \mathrm{M})$, which might not only result
from the different sterical demand of the $N^{\varepsilon}$-substituents but also from the fact that the $K_{\mathrm{i}}$ values determined for 6a and 8d are kinetically defined, whereas those of 21a and 21b represent equilibrium constants.


Figure 10. Structures of the reversible inhibitors 21a and 21b

## Reactivity of $\boldsymbol{N}^{\boldsymbol{E}}$-acryloyllysines towards guinea pig TGase 2 in comparison to their reactivity towards human TGase 2

In the course of the kinetic characterisation of the $N^{\varepsilon}$-acryloyllysines towards hTGase 2 we were also interested in their reactivity towards gpTGase 2, which was used for a long time as cost-efficient alternative to the human enzyme. ${ }^{83}$ In this context, a recent study demonstrated that several substrates and inhibitors exhibit similar kinetic parameters towards both enzymes. ${ }^{83}$ However, as shown in our previous study, the reactivity of $\mathbf{6 a}$ by means of $k_{\text {inact }} / K_{1}$ values towards hTGase 2 is by a factor of $\approx 7$ higher when compared with gpTGase 2 , which indicates a difference in the binding of $\mathbf{6 a}$ within the catalytic centres of both isoforms. In addition to reference compound $\mathbf{6 a}$, its D -enantiomer $\mathbf{6 b}$ was also characterised towards gpTGase 2 using the fluorimetric assay. Similar to the human isoform, the L-configuration is preferred by gpTGase 2 as compound $\mathbf{6 b}$ ( $K_{\text {inact }} / K_{l}=133 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ) exhibits a lower inhibitory potential than $\mathbf{6 a}$ ( $k_{\text {inact }} / K_{l}=740 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ). However, as the inhibitory activity of $\mathbf{6 a}$ (eutomer) towards gpTGase 2 is lower compared to hTGase 2, the eudismic ratio is also lower ( 24 and 5 for hTGase 2 and gpTGase 2, respectively).

In accordance to the lower reactivity of $\mathbf{6 a}$, the inactivation constants $k_{\text {inact }} / K_{1}$ of compounds $\mathbf{7 b}$, $7 \mathbf{i}$ and $8 \mathbf{d}$ are reduced towards gpTGase 2 by factors of $\approx 5-7$. This difference in reactivity was further proven by determination of $\mathrm{IC}_{50}$ values using the fluorescence anisotropy assay for the compound series $\mathbf{7}$ as well as compounds $\mathbf{6 a}$ and $\mathbf{8 d}$ towards both enzymes (Table 11). Even though the pre-incubation period for gpTGase 2 was six times longer than for hTGase 2 ( 30 min versus 5 min ), the $\mathrm{IC}_{50}$ values are similar or even lower for the human enzyme. Concerning SARs for the gpTGase 2, the nitropyridyl derivatives $\mathbf{7 i}$ and $\mathbf{8 d}$ are again
significantly more potent than the lead compound $\mathbf{6 a}$ as reflected by their high inactivation constants and low $\mathrm{IC}_{50}$ values. Generally, this indicates similar interactions of these compounds with gpTGase 2 and hTGase 2. However, the productivity of binding is considerably higher at the humane enzyme. Apart from that analogy, the trend within the series of compounds 7 (see $\mathrm{IC}_{50}$ values) is less distinct for gpTGase 2 compared to hTGase 2. Substitutions in position 6 of the pyridine-2-yl moiety are obviously well tolerated but no explicit relation could be derived.

Table 11. Reactivity of selected $\boldsymbol{N}^{\boldsymbol{k}}$-acryloyllysines towards hTGase 2 and gpTGase 2

| cpd. | $k_{\text {inact }} / K_{\mathbf{l}}\left(\mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$ |  | $\mathrm{IC}_{50}(\mu \mathrm{M})$ |  |
| :---: | :---: | :---: | :---: | :---: |
|  | hTGase 2 | gpTGase 2 | hTGase 2 | gpTGase 2 |
| 6a | 4880 | 740 | 0.31 | 0.27 |
| 6b | 206 | 133 | - | 0.79 |
| 7a | 2980 | - | 0.34 | 0.31 |
| 7b | 3850 | 771 | 0.11 | 0.20 |
| 7c | 4910 | - | 0.18 | 0.16 |
| 7d | 5200 | - | 0.22 | 0.20 |
| 7e | 7350 | - | 0.14 | - |
| 7f | 6140 | - | 0.17 | 0.17 |
| 7 g | 4560 | - | 0.20 | 0.15 |
| 7h | 3020 | - | 0.39 | 0.27 |
| 7i | 10200 | 1630 | 0.10 | 0.12 |
| 8d | 8460 | 1370 | 0.09 | 0.15 |
| 14h | 278 | 259 | - | 0.46 |
| 15e | 1080 | 1010 | - | 0.16 |

For details on the calculation of the kinetic parameters, see Experimental Section. Data shown for hTGase 2 and gpTGase 2 are mean values of at least two separate experiments, each performed in duplicate.

In contrast to these results, compounds 14h (4-fluorophenylcarbamoyl group at $N^{a}$ ) and 15e (4-iodophenylacetyl group at $N^{a}$ ) exhibit similar inactivation constants towards both enzymes. Interestingly, $\mathbf{1 5 e}$ is even more potent than $\mathbf{6 a}$ towards gpTGase 2 indicating other structural requirements for the substituents at the $\alpha$-amino group. A model of gpTGase 2 was built to investigate the binding mode of $\mathbf{6 a}$ and 15 e and shed light on the molecular basis for recognition that could rationalise their inhibitory activities towards this enzyme with respect to hTGase 2. First, the comparison of the active sites modelled for hTGase 2 and gpTGase 2 suggested differences in pocket 2 and pocket 3 (Figure S21 in Supporting Information). Pocket 2 appears slightly more closed in hTGase 2 because of the disposition of Phe320, which corresponds to Ser320 in gpTGase 2, and leave Met330 more exposed. On the other hand, pocket 3 seems to be broader in the guinea pig homologue. These structural differences could affect the inhibitory capacity of the developed inhibitors on these two orthologous enzymes.

Further covalent docking of $\mathbf{6 a}$ on the gpTGase 2 revealed a lack of important interactions with the protein compared to the human enzyme, which is consistent with the kinetic data. The pyridyl moiety was positioned in pocket 2, the phenylacetyl was oriented towards Arg317 and only one H bond with Trp241 was predicted. In contrast, the predicted binding mode for 15e is similar for both orthologues, which is reflected by similar inhibitory pontencies towards gpTGase 2 and hTGase 2 (Figure S22 in Supporting Information).

## Selectivity and initial pharmacokinetic profiling of selected inhibitors

In addition to the kinetic characterisation towards hTGase 2 and gpTGase 2, selected inhibitors were subjected to selectivity and pharmacokinetic analyses to obtain more information with regards to their suitability for in vivo application. For selectivity studies, compounds 7b and $\mathbf{7 i}$ were chosen as they represent one of the most potent fluorinated inhibitors and one of the most potent inhibitors in this study in general, respectively. The kinetic characterisation towards hTGase 1, hTGase 3, hTGase 6 and hfXIIla using the fluorogenic acyl donor Z-Glu(HMC)-Gly-OH revealed an excellent selectivity of both compounds for hTGase 2 with selectivity factors between 275 and 1925 (Table 12). Therefore, the substitution of the methyl group by fluorine or a nitro group does not seem to influence the reactivity towards these human TGase-isoforms, even though the given selectivity for reference compound 6a by Wityak et al. was not re-assessed. ${ }^{58}$ Due to the use of mouse models for preclinical evaluations of biological active compounds, especially radiotracers, the inhibitory potentials of $\mathbf{6 a}, \mathbf{7 b}$ and $\mathbf{7 i}$ were also determined towards mTGase 2. In accordance to the similar substrate properties of Z-Glu(HMC)-Gly-OH towards human and mTGase 2, the $k_{\text {inact }} / K_{1}$ values of these three inhibitors are similar between the two enzyme isoforms, which indicates a high similarity within their active sites.

To demonstrate the reactivity of the $N^{\kappa}$-acryloyllysines towards TGase 2 in a biological matrix, the inhibitory potencies of $\mathbf{6 a}, \mathbf{7 b}$ and $\mathbf{7 i}$ were characterised in cell lysates from A375 human melanoma cells. These cells exhibit a significant expression of hTGase 2 as determined by Western blot analyses (Figure S23 in Supporting Information). hTGase 2 activity in cell lysates was measured by using the fluorescence anisotropy assay with DMC and R-I-Cad as substrates. Notably, the increase in the fluorescence anisotropy originates exclusively from the hTGase 2-catalysed incorporation of R-I-Cad into DMC as no signal increase can be detected in the presence of the selective inhibitors $\mathbf{6 a}, \mathbf{7 b}$ and $\mathbf{7 i}$ or in the absence of DMC (Figure S24 and Figure S25 in Supporting Information). Furthermore, Iysates from MeWo cells, which show virtually no expression of hTGase 2, did not lead to any measurable signal either (Figure S24). The inhibitory activity of the three $N^{\varepsilon}$-acryloyllysines was quantified by the determination of $\mathrm{IC}_{50}$
values. As shown in Table 12, the inhibitors largely retain the submicromolar activity as observed for the isolated target enzyme. Compound 7 i is the most potent inhibitor $(0.40 \mu \mathrm{M})$, even though the $\mathrm{IC}_{50}$ values in cell lysates (see Figure S 25 for the respective curves) are considerably higher compared to the values obtained for the recombinant enzyme. It should be mentioned that due to the rather artificial assay conditions ( 3 mM CaCl$)_{2}$ ) applied in these experiments, the detected hTGase 2 activity corresponds to the amount of activatable hTGase 2 and does not reflect hTGase 2 activity in intact cells. Nevertheless, such assay methods provide evidence for target reactivity in a biologically more relevant assay setting for the respective inhibitors. ${ }^{135}$

Table 12. Selectvity and cellular lysate activity of inhibitors 6a, 7b and 7i

$$
k_{\text {inact }} / K_{1}\left(M^{-1} \mathrm{~s}^{-1}\right)^{a}
$$



| $\mathbf{6 a}$ | 4880 | 3420 | n. $\boldsymbol{d}$. | n. $d$. | n. $d$. | n. $d$. | 0.31 | 0.79 |
| :--- | ---: | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 7b | 3850 | 3990 | 9 | $n . d$. | $14^{d}$ | 2 | 0.11 | 1.10 |
| 7i | 10200 | 8900 | 23 | $<13$ | $13^{d}$ | 13 | 0.10 | 0.40 |

For details on the calculation of the kinetic parameters, see Experimental Section. ${ }^{\text {a The }}$, following concentrations of acyl donor Z-Glu(HMC)-Gly-OH were used: $25 \mu \mathrm{M}$ (gpTGase 2), $30 \mu \mathrm{M}$ (hTGase), $35 \mu \mathrm{M}$ (mTGase 2), $40 \mu \mathrm{M}$ (hTGase 1, hTGase 3, hTGase 6, hFXIIIa). Data shown for hTGase 2 and mTGase 2 are mean values of two separate experiments, each performed in duplicate. Data shown for hTGase 1, hTGase 3, hTGase 6 and hfXIIIa are mean values of one experiment, which was performed in duplicate. bhTGase 2 was preincubated with inhibitor for 5 min , DMC $(30 \mu \mathrm{M})$ and R-I-Cad $(0.81 \mu \mathrm{M})$ were used as substrates. ${ }^{\text {c Same conditions as described under b }}$ with a protein concentration of the lysate of $2 \mathrm{~g} / \mathrm{l}$ (corresponds to a TGase 2 activity of approx. $1 \mu \mathrm{~g} / \mathrm{mL}$ ). IC 50 values shown are mean values of two to three experiments, each performed in duplicate. ${ }^{\text {d }}$ Calculated value based on the kobs value at $[I]=200 \mu \mathrm{M}$. n. d. denotes to not determined.

One of the biggest challenges during drug development is often faced by the gastrointestinal absorption of the drug after oral administration. Even though this barrier is not relevant for potential radiotracers, the knowledge about their cell and tissue penetration is essential, which for most of the small molecules takes place via passive diffusion. ${ }^{136}$ The physicochemical parameters influencing the passive diffusion of molecules include acid-base character, lipophilicity, solubility, and membrane permeability. To obtain these parameters, appropriate experimental as well as computational methods exist. ${ }^{137}$

To characterise the $N^{\varepsilon}$-acryloyllysines regarding their membrane permeability, the PAMPA (parallel artificial membrane permeability assay) method was used, which allows for the screening of a library of compounds. ${ }^{136,138}$ Using this method, the passive diffusion of compounds through an artificial membrane can be characterised. The PAMPA method was performed in a 96 -well plate format with subsequent spectrophotometric determination of the compound concentrations in donor and acceptor wells. A mixture of 1,2-dioleoyl-sn-glycero-3phosphocholine (DOPC, 10\%, w/v) and cholesterol ( $5 \%$, w/v) in dodecane was used as artificial membrane. ${ }^{136,139-140}$ Finally, to quantify the membrane permeability, the effective
permeability $\mathrm{P}_{\mathrm{e}}$ (in $\mathrm{nm} / \mathrm{s}$ ) was calculated for each compound considering the respective membrane retention $\mathrm{R}_{\mathrm{M}} .{ }^{141}$ Initially, the validity of the PAMPA method and the differentiable range of $P_{e}$ values was characterised using reference compounds of known membrane permeability. To this end, hydrochlorothiazide (poorly permeable), metoprolol (moderately permeable) and verapamil (highly permeable) were chosen ${ }^{142}$ and the expected tendency of their $P_{e}$ values was confirmed (Table 13, range from 0.6 to $520 \mathrm{~nm} / \mathrm{s}$ ). However, it should be mentioned that the PAMPA method as performed here does not consider the phenomenon of unstirred water layers (UWLs), which are adjacent to both sides of the membrane. ${ }^{143-145}$ These UWLs particularly influence the permeation rate of highly permeable compounds where the permeation through the UWLs becomes rate-limiting, which results in an upper limit for $\mathrm{P}_{\mathrm{e}} .{ }^{146}$ Therefore, structure-property relationships within a group of well permeable compounds cannot be derived.

Similar to the reference compounds, the $P_{e}$ values for the $N^{\varepsilon}$-acryloyllysines range from 3 to $220 \mathrm{~nm} / \mathrm{s}$ revealing significant differences in the permeation rates. The majority of compounds appears to be well permeable exhibiting $P_{e}$ values between $100-220 \mathrm{~nm} / \mathrm{s}$. The narrow range of $\mathrm{P}_{\mathrm{e}}$ values might reflect the aforementioned limitations for the characterisation of well permeable compounds due to the present UWLs.

Considering the $\mathrm{P}_{\mathrm{e}}$ values of hydrochlorothiazide and metoprolol, a series of compounds with poor or moderate permeability was identified. This is of particular importance with respect to the selective targeting of intra- and extracellular TGase 2, especially by radiotracers in vivo. The picolinic acid derivatives $\mathbf{8 e}$ and $\mathbf{8 g}$ and the pyridine-4-yl derivative 10 can be classified as poorly permeable. The low $\mathrm{P}_{\mathrm{e}}$ value of $\mathbf{1 0}(5.1 \mathrm{~nm} / \mathrm{s})$ might originate from the high $p K_{\mathrm{a}}$ value of the respective pyridinium ion ( $\mathrm{cp} K_{\mathrm{a}}=10.7$, Table 13) resulting in a positively charged molecule at pH 7.5 . Accordingly, due to the lower $\mathrm{p} K_{\mathrm{a}}$ value of the pyridinium ion, the regioisomeric pyridine-3-yl derivative $8 \mathbf{a}\left(\mathrm{cp}_{\mathrm{a}}=7.0\right.$, Table S 1 ) exhibits a higher $\mathrm{P}_{\mathrm{e}}$ value than compound 10 ( $12.9 \mathrm{~nm} / \mathrm{s}$ ). However, the highest $\mathrm{P}_{\mathrm{e}}$ value within the regioisomeric pyridine derivatives was determined for the pyridine-2-yl derivative $7 \mathrm{a}(61.3 \mathrm{~nm} / \mathrm{s})$ even though the $\mathrm{p} K_{\mathrm{a}}$ value of the pyridinium ion ( $\mathrm{cp} K_{\mathrm{a}}=8.4$, Table S1) lies between that of $\mathbf{1 0}$ and $\mathbf{8 a}$. In accordance with this result, compounds bearing a 6-methylpyridine-2-yl moiety exhibit high $P_{e}$ values, which was not expected based on the $\mathrm{p} K_{\mathrm{a}}$ value of the pyridinium ion ( $\mathrm{cp} K_{\mathrm{a}}=9.1$, Table S1). In this context, Chen et al. ${ }^{147}$ recently showed within their mechanistic study on the permability of meta-substitued pyridines that not membrane partitioning but rather aqueous desolvation of these compounds dictates the permeation rate. Consequently, an unfavourable solvation of the pyridine-2-yl moieties could be the reason for the relatively high permeation rates of the respective $N^{\varepsilon}$-acryloyllysines.

Noteworthy, the group of compounds with moderate permeability comprises amongst others the 6-nitropyridin-3-yl derivatives $\mathbf{8 d}$ and $\mathbf{1 7 a - 1 7 c}$, while the 6 -nitropyridine-2-yl derivatives ( $\mathbf{7 i}$ and 18) exhibit significantly higher $P_{e}$ values. The difference in the permeation rates for the nitropyridine derivatives might originate from the aforementioned push-pull effect within the 6-nitropyridine-3-yl substituents, which increases the polar character of these compounds.

During the calculation of the $P_{e}$ values, the membrane retention $R_{M}$ (portion of compound which remains in the membrane) can be derived. This parameter is given in Table 13 as molar fraction (\%) of the applied amount of substance. With respect to the development of radiotracers, high non-specific binding of compounds is often a problem and a common reason for the failure of promising candidates. ${ }^{148-149}$ Therefore, an in vitro method which can predict the potential degree of non-specific binding is highly desirable. In this context, Jiang et al. observed that the chromatographic hydrophobicity index values (CHI IAM, determined at an immobilised artifical membrane) correlate well with data from in vitro non-specific binding obtained by equilibrium dialysis $\left(R^{2}=0.79\right) .{ }^{148}$ Due to the similiar origin, $R_{M}$ values should contain similar information as $\mathrm{CHI} \mathrm{IAM}_{7.4}$ values. To this end, for a series of compounds including all fluorinated inhibitors, the $\mathrm{CHI}^{2} \mathrm{IAM}_{7.4}$ values were measured and compared to the $R_{M}$ values (Table 13). Indeed, a similar trend between both parameters can be observed. Whereas most of the compounds exhibit $\mathrm{R}_{M}$ values of $<30 \%$, some of them show signifcantly higher values including the 6-tert-butyl-pyridine-2-yl derivative $7 f\left(\mathrm{R}_{\mathrm{M}}=69 \%\right)$ and the 6 -phenyl-pyridine-2-yl derivative 7 g ( $\mathrm{R}_{\mathrm{M}}=64 \%$ ). Accordingly, the $\mathrm{CHI} \mathrm{IAM}_{7.4}$ values for most of the compounds are around 30 , whereas 7 f and 7 g exhibit values of $\approx 39$.

In addition to the calculation of $\log \mathrm{D}_{7.4}$ values, these parameters were also experimentally determined for several fluorinated inhibitors by using a novel ${ }^{19} \mathrm{~F}$-NMR-based method developed by Linclau et al. ${ }^{150}$ This method is basically a variation of the classical shake-flask method in which the proportion of the fluorinated compound in the octanol and aqueous phase were measured by ${ }^{19} \mathrm{~F}$-NMR spectroscopy with trifluoroethanol as internal standard. As seen in Table 13, the tendency for the calculated $\log \mathrm{D}_{7.4}$ values is in accordance with the experimental values. However, the absolute experimental values are 0.32 to 1.21 log units higher than the calculated values, clearly demonstrating the limits of computational methods.

As mentioned above, solubility is a further parameter which co-determines their passive diffusion as it characterises the concentration a compound can reach in a distinct solution. ${ }^{151}$ For compounds $\mathbf{7 b}$ and $\mathbf{7 i}$, the thermodynamic solubility in PBS $\left(\mathrm{pH} 7.4\right.$ and $22^{\circ} \mathrm{C}$ over 26 h and 39 h , respectively) was measured revealing values of 645 and $452 \mu \mathrm{M}$, respectively. These values will guide in vitro and in vivo experiments in which the addition of organic co-
solvents to increase the solubility is more limited than in experiments using recombinant enzymes.

Table 13. Determined and calculated physicochemical parameters of selected $\boldsymbol{N}^{\boldsymbol{E}}$ acryloyllysines

| cpd. | cp $K_{\text {a }}{ }^{\text {a }}$ | $\boldsymbol{\operatorname { l o g }} \mathrm{D}_{7.4}$ | $\operatorname{clog}^{\text {D.4 }}$ | $\mathrm{Pe}_{\mathrm{e}(7.5)}(\mathrm{nm} / \mathrm{s})$ | RM (\%) | CHI IAM ${ }_{7.4}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6a | 9.1 | - | -0.36 | 87.7 (3.9) | -1 | 29.4 |
| 7b | 2.8 | 2.10 | 1.27 | 133 (19) | 14 | 31.8 |
| 7f | 8.9 | - | 2.77 | 219 (28) | 69 | 39.5 |
| 7 g | 7.6 | - | 2.79 | 219 (57) | 64 | 39.0 |
| 7i | 0.6 | - | 1.02 | 91.6 (8.1) | 10 | 30.7 |
| 8b | 1.3 | 1.66 | 1.05 | 35.4 (3.2) | 4 | 26.8 |
| 8c | 2.3 | 2.13 | 1.81 | 89.8 (3.1) | 11 | 31.8 |
| 10 | 10.7 | - | -0.37 | 5.1 (0.8) | 31 | 25.4 |
| 13a | - | 1.60 | 1.18 | 30.5 (5.2) | -3 | 25.0 |
| 13c | $6.2^{\text {b }}$ | 2.65 | 1.98 | 103 (13) | 12 | 32.0 |
| 13 e | $4.1^{\text {c }}$ | - | 3.08 | 76.8 (4.8) | 38 | 37.7 |
| 14h | 9.1 | 0.47 | -0.32 | 125 (31) | 11 | 34.3 |
| 14k | 9.1 | 0.28 | -0.10 | 127 (27) | 16 | 31.4 |
| 15b | 9.1 | 0.36 | -0.32 | 114 (7.0) | 10 | 32.4 |
| 15f | 9.1 | - | -0.32 | 114 (9.0) | 21 | 30.1 |
| 15 g | 9.1 | - | -0.32 | 125 (3.2) | 24 | 30.9 |
| 17b | -2.5 | 1.85 | 1.04 | 19.7 (3.1) | 9 | 28.3 |
| 17c | -2.5 | 2.21 | 1.17 | 40.8 (5.7) | 12 | 28.5 |
| 18 | 0.6 | 2.25 | 1.04 | 87.4 (5.4) | 12 | 30.7 |

Fluorinated compounds are coloured in blue. $\mathrm{P}_{\mathrm{e}(7.5)}$ values for the reference compounds are as follows: hydrochlorothiazide ( $0.6 \pm 0.1$ ), metoprolol ( $40.4 \pm 5.1$ ), verapamil ( $520 \pm 80$ ). Further information to the calculated parameter $\mathrm{cp}_{\mathrm{a}}, \operatorname{clog} \mathrm{D}_{7.4}$ and the experimentally determined parameter $\log _{7.4}, \mathrm{P}_{\mathrm{e}(.5)}$, RM and $\mathrm{CHI} \mathrm{IAM}_{7.4}$ can be found within the Experimental Section and in Table S1 of the Supporting Information. alf not otherwise stated, the $\mathrm{cp}_{\mathrm{a}} K_{\mathrm{a}}$ value corresponds to the pKa value of the respective pyridinium ion. ${ }^{\text {b }}$ Value corresponds to the piperazinium ion. ${ }^{\text {c }}$ Value corresponds to the dimethylammonio group of the dansyl moiety.

Evidence towards the susceptibility of compounds against oxidative in vivo metabolism can be obtained by investigating their stability in the presence of liver microsomes. ${ }^{152}$ Data from such studies for the lead compound $\mathbf{6 a}$ reported by Wityak et al. suggest that it is insufficiently stable against oxidative metabolism. ${ }^{58}$ To get more detailed information, $\mathbf{6 a}$ and a series of selected inhibitors, mainly fluorinated compounds, were tested towards their stability in mouse liver microsomes. Therefore, compounds were incubated with mouse liver microsomes in the presence of NADPH and the resulting mixtures were analysed after 30 and 60 min by HPLCUV analysis. Finally, the portion of residual intact inhibitor was determined and is graphically depicted in Figure 11. As control measurements, each compound was incubated with mouse liver microsomes in the absence of NADPH. In accordance to Wityak et al., $\mathbf{6 a}$ is metabolised relatively fast with $62 \%$ of original compound remaining after $60 \mathrm{~min} .{ }^{58}$ The substitution of the methyl group by hydrogen or fluorine in compounds $\mathbf{7 a}$ and $\mathbf{7 b}$, respectively, does not
significantly alter the metabolic degradation rate. In contrast, compound 10 containing a pyridyl moiety with a para orientation of pyridine nitrogen and piperazine ring is virtually fully stable over 60 min . At a first glance, this allows for the conclusion that the site for oxidation reactions is mainly the respective pyridine-2-yl ring. However, compound 13a in which the pyridine ring is substituted by a 4 -fluorobenzoyl is more stable than the lead compound $\mathbf{6 a}$ ( $85 \%$ intact compound after 60 min ) but less stable than compound 10. These observations taken together with enhanced-product ion (EPI) mass spectra of compound 7b (Figure S26 in Supporting Information) suggest the pyridylpiperazine moiety as major site of metabolic transformation, even though it cannot be stated clearly whether the pyridine or the piperazine ring is more prone to hydroxylation. Data on the metabolism of established piperazine-containing drugs, such as the tricyclic neuroleptic clozapine, indicate that the piperazine ring undergoes CYP(cytochrome P450)-mediated semiaminal formation, which can give rise to reactive iminium ions. ${ }^{153-154}$ Generally, hydroxylation catalysed by CYP monooxygenases at $\mathrm{sp}^{3}$ hybridised carbon atoms adjacent to nitrogen is strongly dependent on the electron-donating capacity of the latter atom. ${ }^{155}$ Hence, electron-deficient pyridine rings should attenuate the propensity for metabolic hydroxylation at potential aromatic sites and - via mesomeric effects involving the piperazine nitrogen - also at aliphatic sites. Accordingly, substitution of the 6-methylpyridine-2-yl ring in 6a by the pyridine-4-yl ring in 10 increases the stability of the pyridylpiperazine moiety towards oxidative metabolism. A possible explanation for that could be the aforementioned push-pull effect due to the para orientation of the piperazine ring and pyridine nitrogen, which might lead to a strong shift of the electron density to the pyridine nitrogen. This hypothesis is further supported as compounds $17 \mathrm{~b}, 17 \mathrm{c}$ and 8 c bearing 6 -nitropyridine-3-yl and 6-trifluoropyridine-3-yl moieties, respectively, exhibit a comparable stability to $\mathbf{1 0}$.

Even though potential metabolically labile moieties were identified, it should be mentioned that for most of the compounds no significant further metabolisation occurs between 30 and 60 min. This might indicate a potential inhibition of the CYP enzymes by the original compounds or formed metabolites, which could lead to an underestimation of the overall stability of the compounds.


CPd

Figure 11. Stability of selected inhibitors in mouse liver microsomes
Determined fractions of peak areas of original compound at 30 and 60 min were normalised to the fraction of peak area of original compound determined after incubation for 60 min without NADPH, microsomal protein and substrates. Data shown are mean values ( $\pm$ SD) of one experiment which was performed in duplicate.

## Conclusions

Based on the $N^{\varepsilon}$-acryloyllysine piperazide core structure, we synthesised more than 50 new inhibitors by varying either the substituent at the $\alpha$-amino group or at the piperazine ring or both. Their kinetic characterisation, which considered both hTGase 2-catalysed hydrolysis and transamidation, allowed for the derivation of comprehensive structure-activity relationships. The observed inhibitory potencies based on inactivation constants $k_{\text {inact }} / K_{1}$ comprised two orders of magnitude ranging from 100 to $10000 \mathrm{M}^{-1} \mathrm{~s}^{-1}$. Activity data generated by systematic structural variations allowed for correlation analyses considering different substituent parameters. Furthermore, to facilitate the interpretation of the SAR data, covalent docking studies were conducted using an hTGase 2 molecular model based on a recent crystal structure of this protein in the open conformation and completed by modelling of a flexible loop
close to the catalytic centre. The predicted binding modes of the inhibitors within the active site of hTGase 2 are in agreement with experimentally obtained kinetic data. The obtained docking results in combination with SAR data led to the identification of amino acid residues that are crucial for inhibitor binding and shed light on the principles of molecular recognition for the chemotype of acryloyllysine-derived hTGase 2 inhibitors. The rationale established in our studies indicate that $N^{\xi}$-acryloyllysine piperazides are characterised by multiple binding modes that are sensitive to subtle structural changes rather than a strictly defined targeting of subsites by distinct moieties. These results will direct future design of inhibitors for hTGase 2.

Evaluation of selected potent analogues against other human transglutaminases has revealed an excellent selectivity profile in favour to hTGase 2. In addition to their interaction with the target enzyme, compounds were profiled with regards to their membrane permeability using the PAMPA method, which supports prediction of the pharmacokinetic behavior. Furthermore, selected inhibitors were assessed concerning their stability against oxidative microsomal degradation. Considering the determined inhibitory potencies in combination with these pharmacokinetic in vitro data and inhibitor characterisation in complex biological matrix (cell lysates) provides valuable hints for the development of radiotracers for TGase 2 imaging. Some promising candidates for labelling with fluorine-18 were already identified within this study (i.e. compounds 7b, 8c, 13c, 17b, 18).

## Experimental Section

## Fluorimetric assay ${ }^{59}$

All measurements were conducted at $30^{\circ} \mathrm{C}$ over 900 s (interval of 20 s ) with a Synergy 4 MultiMode Microplate Reader (BioTek Instruments, Software Gen 5, Winooski, VT, USA,) and black 96- well BRANDplates with transparent bottoms (BRAND, Wertheim, Germany). Fluorescence was detected in bottom read mode. To detect released HMC, a combination of optical filters adjusted to $365 / 40 \mathrm{~nm}$ and $465 / 40 \mathrm{~nm}$ as ranges of wavelengths for excitation and emission, respectively, were used. Measurements at pH 6.5 were conducted with a sensitivity of 45 . The assay mixture $(200 \mu \mathrm{~L})$ contained aqueous solution ( $190 \mu \mathrm{~L}$ ) and DMSO ( $5 \%, \mathrm{v} / \mathrm{v}, 10 \mu \mathrm{~L}$ ). The following two buffer systems were used: assay buffer ( 100 mM MES, $3 \mathrm{mM} \mathrm{CaCl} 2,50 \mu \mathrm{M}$ EDTA, adjusted to pH 6.5 with 1 M NaOH ) and enzyme buffer ( 100 mM MES , 3 mM CaCl , 10 mM TCEP, $20 \%(\mathrm{v} / \mathrm{v})$ glycerol). The buffers were stored at $0^{\circ} \mathrm{C}$ for periods of up to two weeks and freshly prepared after that period. The concentrations of the enzyme stock solutions were $0.5 \mathrm{mg} / \mathrm{mL}$ or $1 \mathrm{mg} / \mathrm{mL}$. All regression analyses were done with GraphPad Prism (version 5.02, GraphPad Software, San Diego, CA, USA). To provide values of means and SEMs, the
corresponding regression analyses were separately performed for each experiment, and the obtained fit values were collected and statistically analysed. Analysis by numerical integration was done as described previously by our group. ${ }^{59}$ The kinetic characterisation of Z-Glu(HMC)-Gly-OH (including its synthesis) towards enzymatic hydrolysis by hTGase 2 and gpTGase 2 and that of inhibitor 6a towards hTGase 2 and gpTGase 2 using acyl donor Z-Glu(HMC)-GlyOH were also conducted within the aforementioned study. All TGase isoforms (gpTGase 2 (T006), hTGase 2 (T022), mTGase 2(T040), hTGase 1 (T009), hTGase 3 (T013), hTGase 6 (T021), hFXIIIa (T070) and the inhibitors Z006 and Z013 were purchased from Zedira ${ }^{\circledR}$ (Darmstadt, Germany).

## Characterisation of Z-GIu(HMC)-Gly-OH towards enzymatic hydrolysis by different TGases

For investigations of enzyme-catalysed hydrolysis reactions of acyl donor Z-Glu(HMC)-Gly$\mathbf{O H}$, six or eight different concentrations of Z-Glu(HMC)-Gly-OH were used (three separate experiments, each performed in duplicate). The corresponding stock solutions were prepared in DMSO. DMSO ( $5 \mu \mathrm{~L}$ ) and the acyl donor stock solution ( $5 \mu \mathrm{~L}$ ) were added to assay buffer $(180 \mu \mathrm{~L})$. The reactions were initiated by addition of TGase stock solution ( $10 \mu \mathrm{~L}, 60 \mu \mathrm{~g} / \mathrm{mL}$ for each TGase isoform, TGase 3 was preincubated for 30 min at $30^{\circ} \mathrm{C}$ (Figure S27 in Supporting Information)). For measurements of the spontaneous reactions, the solution of the respective TGase isoform was replaced by enzyme buffer. The recorded time courses of type (RFU$\left.R F U_{0}\right)=f(t)$ for the enzymatic conversion were analysed either by nonlinear (equation III) or linear regression to the experimental data over the first 300 s , depending on the shape of the curve. For the case of nonlinear regression, the first derivative of equation III at $\mathrm{t}=0$ (equation IV) afforded the initial slopes, which are equal to the values of $\mathrm{v}_{\text {ototal }}$ (units of RFU/s).

$$
\begin{equation*}
\mathrm{RFU}-\mathrm{RFU}_{0}=\text { Plateau } *\left(1-\mathrm{e}^{-k * t}\right) \tag{III}
\end{equation*}
$$

$R F U_{0}: R F U_{t=0} \quad$ Plateau: $\mathrm{RFU}_{\mathrm{t} \rightarrow \infty}$
$k$. rate constant to reach the plateau

$$
\begin{equation*}
\mathrm{v}_{\text {0total }}=k * \text { Plateau } \tag{IV}
\end{equation*}
$$

For analysis of the spontaneous reactions, the recorded time courses of type (RFU-RFU ${ }_{0}$ ) $=f(t)$ were analysed by linear regression to the experimental data over the first 300 s . The respective slopes are equal to the values of $v_{0 c o n t r o l}$ (units of RFU/s).

All fluorescence rates (RFU/s) were converted into molar rates ( $\mu \mathrm{M} / \mathrm{min}$ ) by dividing by the corresponding fluorescence coefficients. ${ }^{59}$ Subsequently, the two sets of initial rates
( $\mathrm{V}_{\text {ototal }}=\mathrm{f}([\mathbf{Z}-\mathrm{Glu}(\mathrm{HMC})-\mathrm{Gly}-\mathrm{OH}])$ and $\left.\mathrm{v}_{0 \text { control }}=\mathrm{f}([\mathbf{Z}-\mathrm{Glu}(\mathrm{HMC})-\mathrm{Gly}-\mathrm{OH}])\right)$ were globally analysed by use of the model of total and nonspecific binding as implemented in GraphPad Prism to determine the kinetic parameters for the enzymatic conversion. Accordingly, the following rule was defined (equation V ):

$$
\begin{equation*}
\mathrm{v}_{\text {ototal }}=\mathrm{v}_{0 \text { corr }}+\mathrm{v}_{\text {0control }} \tag{V}
\end{equation*}
$$

where $\mathrm{v}_{\text {ocorr }}$ represents the rates for the enzymatic conversions. Within this model, the portion of $\mathrm{V}_{\text {ocorr }} \mathrm{f}([\mathrm{Z}-\mathrm{Glu}(\mathrm{HMC})-\mathrm{Gly}-\mathrm{OH}])$ and $\mathrm{V}_{\text {ocontrol }}=\mathrm{f}([\mathrm{Z}-\mathrm{Glu}(\mathrm{HMC})-\mathrm{Gly}-\mathrm{OH}])$ were analysed by nonlinear regression by use of equation VI (Michaelis-Menten equation) and linear regression ( $\mathrm{V}_{\text {ocontrol }}=K_{\text {obs }}{ }^{*}[$ Z-Glu(HMC)-Gly-OH]), respectively.

$$
\begin{equation*}
\mathrm{v}_{0 \text { corr }}=\frac{\mathrm{v}_{\max } *[\mathrm{~S}]}{K_{\mathrm{m}}+[\mathrm{S}]} \tag{VI}
\end{equation*}
$$

Because of the negligible spontaneous reaction of compound Z-Glu(HMC)-Gly-OH within the range of concentrations for mTGase 2 , plots of $\mathrm{v}_{\text {ototal }} \mathrm{f}([\mathrm{Z}-\mathrm{Glu}(\mathrm{HMC})-\mathrm{Gly}-\mathrm{OH}])$ were directly analysed by nonlinear regression to the data by use of equation VI. The evaluation of the data sets for hTGase 1, hTGase 3 and hFXIIla were also performed by the method of CornishBowden and Eisenthal. ${ }^{82}$

## Characterisation of irreversible inhibitors

For the characterisation of irreversible inhibitors towards gpTGase 2, hTGase 2 and mTGase 2 at pH 6.5 , six or eight different concentrations of the inhibitors were used (two separate experiments, each performed in duplicate). In contrast to this, four different concentrations were investigated (one experiment, each concentration in duplicate) for the inhibition of hTGase 1, hTGase 3, hTGase 6 and hFXIIIa. Compound Z-Glu(HMC)-Gly-OH was chosen as acyl donor. The appropriate stock solutions were prepared in DMSO. Stock solutions of inhibitor ( $5 \mu \mathrm{~L}$ ) and Z-Glu(HMC)-Gly-OH ( $5 \mu \mathrm{~L}$, 1 or 1.2 mM for gpTGase 2, 1.2 or 1.4 mM for hTGase 2, 1.4 mM for mTGase 2, 1.6 mM for hTGase 1, hTGase 3, hTGase 6 and hFXIIla) were added to assay buffer ( $180 \mu \mathrm{~L}$ ). The reactions were initiated upon addition of the respective TGase stock solution ( $10 \mu \mathrm{~L}, 60 \mu \mathrm{~g} / \mathrm{mL}$ for hTGase 2, mTGase 2, gpTGase 2, hTGase 6 and $120 \mu \mathrm{~g} / \mathrm{mL}$ for hTGase 1, hTGase 3, hFXIIla). The recorded time courses of type ( $R F U-R F U_{0}$ ) $=f(t)$ were analysed by nonlinear regression to the experimental data over the entire measurement period ( 900 s ) by use of equation VII:

$$
\begin{equation*}
\mathrm{RFU}-\mathrm{RFU}_{0}=\mathrm{v}_{\mathrm{S}} * \mathrm{t}+\frac{\left(\mathrm{V}_{\mathrm{i}}-\mathrm{V}_{\mathrm{S}}\right) *\left(1-\mathrm{e}^{-k_{\mathrm{obs}} * \mathrm{t}}\right)}{k_{\mathrm{obs}}} \tag{VII}
\end{equation*}
$$

vs: steady state velocity
$\mathrm{v}_{\mathrm{i}}$ initial velocity
$k_{\text {obs }}=$ pseudo-first-order rate constant for the transition of $\mathrm{v}_{\mathrm{i}}$ into $\mathrm{v}_{\mathrm{s}}$

For the case, that the initial velocities do not vary significantly with increasing concentrations of inhibitor, the plots of $k_{\text {obs }}=\mathrm{f}([1])$ were analysed by linear regression. To obtain the value of $K_{\text {inact }} / K_{\mathrm{l}}$, the corresponding slope ( $K_{\text {obs }} /[\mathrm{l}]=K_{\text {inact }} / K_{\mathrm{l}}$ ) was multiplied by ( $1+[Z-\mathrm{Glu}(H M C)$-Gly$\mathbf{O H}] / K_{\mathrm{m}}$ ) (except for hTGase 3 and hFXIIIa, where $k_{\text {obs }} /[I]$ reflects $k_{\text {inact }} / K_{l}$ as [Z-Glu(HMC)-Gly$\left.\mathrm{OH}] / K_{\mathrm{m}} \ll 1\right)$. For the case, that the initial velocities vary significantly with increasing concentrations of inhibitor, the double reciprocal plots $1 / k_{\text {obs }}=\mathrm{f}(1 /[I])$ were analysed by linear regression according to equation IX. The obtained values $k_{\mathrm{obs}} /[\mathrm{I}]$ were also converted into $K_{\text {inact }} / K_{\mathrm{l}}$ by multiplication by ( $1+[\mathbf{Z}-\mathrm{Glu}(\mathrm{HMC})$-Gly-OH $] / K_{\mathrm{m}}$ ). The respective value of $K_{\mathrm{i}}$ was calculated by analysis of the plot $\mathrm{v}_{\mathrm{i}} \mathrm{f}([\mathrm{II})$ by equation X and equation XI.

$$
\begin{align*}
& k_{\mathrm{obs}}=\frac{k_{\text {inact }} *[\mathrm{I}]}{K_{\mathrm{I}}^{\prime}+[\mathrm{I}]}  \tag{VIII}\\
& \frac{1}{k_{\text {obs }}}=\frac{K_{\mathrm{I}}^{\prime}}{k_{\text {inact }}} * \frac{1}{[1]}+\frac{1}{k_{\text {inact }}}  \tag{IX}\\
& \mathrm{V}_{\mathrm{i}}=\frac{\mathrm{V}_{0}}{1+\frac{[I]}{K_{\mathrm{i}}^{\prime}}}+\mathrm{v}_{\text {ocontrol }}  \tag{X}\\
& K_{\mathrm{i}}=\frac{K_{\mathrm{i}}^{\prime}}{1+\frac{[\mathrm{Z}-\mathrm{Glu}(\mathrm{HC})-\mathrm{Gly}-\mathrm{OH}]}{K_{\mathrm{m}}}} \tag{XI}
\end{align*}
$$

## Characterisation of $N^{\varepsilon}$-propionyllysines

For the characterisation of the $N^{\varepsilon}$-propionyllysines 21a and 21b towards hTGase 2 at pH 6.5 , the enzymatic hydrolysis of acyl donor Z-Glu(HMC)-Gly-OH ( $35 \mu \mathrm{M}$ ) was followed in the presence of six different concentrations of the inhibitors ( $0,10,20,40,80$ and $100 \mu \mathrm{M}$; one to two separate experiments, each performed in duplicate). The appropriate stock solutions were prepared in DMSO. Stock solutions of inhibitor ( $5 \mu \mathrm{~L}$ ) and Z-Glu(HMC)-Gly-OH ( $5 \mu \mathrm{~L}, 1.4 \mathrm{mM}$ ) were added to assay buffer ( $180 \mu \mathrm{~L}$ ). The reactions were initiated upon addition of the hTGase 2 stock solution ( $10 \mu \mathrm{~L}, 60 \mu \mathrm{~g} / \mathrm{mL}$ ). The recorded time courses of type (RFU$\left.R F U_{0}\right)=f(\mathrm{t})$ were analysed by linear regression to the experimental data over $180 \mathrm{~s} . K_{\mathrm{i}}$ values were determined as described for the irreversible inhibitors.

## Active site titration using 8d and Z-GIu(HMC)-Gly-OH

For the active site titration at pH 6.5 , the enzymatic hydrolysis of acyl donor Z-Glu(HMC)-Gly$\mathbf{O H}(30 \mu \mathrm{M})$ was followed after preincubation of hTGase 2 with inhibitor $\mathbf{8 d}(5,10,15,20,25$, 60, 80 and 100 nM ; four separate experiments, each performed in duplicate). The appropriate stock solutions for Z-Glu(HMC)-Gly-OH and 8d were prepared in DMSO and 1\% DMSO/assay buffer, respectively. Stock solutions of inhibitor $\mathbf{8 d}(10 \mu \mathrm{~L})$ and hTGase $2(10 \mu \mathrm{~L}, 60 \mu \mathrm{~g} / \mathrm{mL})$ were added to assay buffer ( $10 \mu \mathrm{~L}$ ) and the mixture was incubated for 40 min at $30^{\circ} \mathrm{C}$. Afterwards, assay buffer $(160 \mu \mathrm{~L})$ and $\mathrm{DMSO}(5 \mu \mathrm{~L})$ were added. The reactions were initiated upon addition of Z-Glu(HMC)-Gly-OH ( $5 \mu \mathrm{~L}, 1.2 \mathrm{mM}$ ). The recorded time courses of type $\left(R F U-R F U_{0}\right)=f(t)$ were analysed by linear regression to the experimental data over 300 s . Within the obtained plots of $\mathrm{v}_{\text {ototal }}=\mathrm{f}([8 \mathbf{d}])$, linear regressions were separately performed for data below and above the applied enzyme concentration ( 38.5 nM ) and the active enzyme concentration was determined as x value of the intersection point of the two lines.

## Fluorescence anisotropy assay ${ }^{60}$

All measurements were conducted at $30^{\circ} \mathrm{C}$ over 900 s (interval of 30 s ) using Synergy 2 and Synergy 4 Multi-Mode Microplate Readers (BioTek Instruments, Software Gen 5, Winooski, VT, USA) and black 96- well BRANDplates with F-bottom wells (BRAND, Wertheim, Germany). Experiments were done at excitation wavelengths of 485 nm (F-Cad) and 540 nm (R-I-Cad) and emission wavelengths of 528 nm (F-Cad) and 620 nm (R-I-Cad), respectively. The FA (r) was calculated by the Gen 5 software from the measured parallel and perpendicular fluorescence intensities ( $l_{\|}$and $I_{\perp}$, respectively) according to the equation XII

$$
\begin{equation*}
r=\frac{I_{\|}-G \times I_{\perp}}{I_{\|}+2 G \times I_{\perp}} \tag{XII}
\end{equation*}
$$

using a G factor of 0.87 (preset value). ${ }^{60,156}$ All further data analyses including calculation of rates by linear regression of the FA over time, curve fitting, and statistics were conducted with GraphPad Prism (version 5.02or version 5.04, GraphPad Software, San Diego, CA, USA). The assay mixture $(200 \mu \mathrm{~L})$ contained aqueous solution $(190 \mu \mathrm{~L})$ and DMSO $(5 \%, \mathrm{v} / \mathrm{v}, 10 \mu \mathrm{~L})$. The following four buffer systems were used: assay buffer A ( 100 mM MOPS, $3 \mathrm{mM} \mathrm{CaCl} \mathrm{Cl}_{2}, 50 \mu \mathrm{M}$ EDTA, adjusted to pH 8.0 with 1 M NaOH ), assay buffer B (as A but $6 \mathrm{mM} \mathrm{CaCl} \mathrm{m}_{2}$ ), enzyme buffer for hTGase 2 ( 100 mM MOPS, 3 mM CaCl , 10 mM TCEP, $20 \%$ ( $\mathrm{v} / \mathrm{v}$ ) glycerol) and enzyme buffer for gpTGase 2 ( 100 mM MOPS, $3 \mathrm{mM} \mathrm{CaCl} 2,10 \mathrm{mM}$ DTT, $20 \%$ ( $\mathrm{v} / \mathrm{v}$ ) glycerol). The buffers were stored at $0^{\circ} \mathrm{C}$ for periods of up to two weeks and freshly prepared after that period. The concentrations of the enzyme stock solutions were $0.5 \mathrm{mg} / \mathrm{mL}$ or $1 \mathrm{mg} / \mathrm{mL}$. To
provide values of means and SEMs, the corresponding regression analyses were separately accomplished for each experiment, and the obtained fit values were collected and statistically analysed. The kinetic characterisation of the substrate pair F-Cad and DMC and of inhibitor 6a towards gpTGase 2 was already performed in a previous study of our group. ${ }^{60}$

## Characterisation of substrate pair R-I-Cad and DMC towards hTGase 2

For the kinetic characterisation of DMC in the presence of a fixed concentration of R-I-Cad $(0,81 \mu \mathrm{M})$, eight different concentrations of DMC $(0.3-300 \mu \mathrm{M})$ were used (two separate experiments, each performed in duplicate). The corresponding stock solutions of DMC and R-I-Cad were prepared in assay buffer A and DMSO, respectively. DMC ( $50 \mu \mathrm{~L}$ ), R-I-Cad ( $5 \mu \mathrm{~L}$ ) and DMSO $(5 \mu \mathrm{~L})$ were added to assay buffer $\mathrm{A}(130 \mu \mathrm{~L})$ and the mixture was preincubated for 30 min at $30^{\circ} \mathrm{C}$. The reactions were initiated by addition of hTGase 2 stock solution ( $10 \mu \mathrm{~L}$, $100 \mu \mathrm{~g} / \mathrm{mL}$ ) or enzyme buffer. The recorded time courses of type $\mathrm{FA}=\mathrm{f}(\mathrm{t})$ were analysed by linear regression to the experimental data over $900 \mathrm{~s} . K_{\mathrm{m}}, K_{\mathrm{i}}$ and $\mathrm{V}_{\text {max }}$ were calculated according to the equation of substrate inhibition (XIII). ${ }^{157}$

$$
\begin{equation*}
\mathrm{v}=\frac{\mathrm{V}_{\mathrm{max}} *[\mathrm{~S}]}{K_{\mathrm{m}}+[\mathrm{S}] *\left(1+\frac{[\mathrm{S}]}{K_{\mathrm{i}}}\right)} \tag{XIII}
\end{equation*}
$$

For the kinetic characterisation of R-I-Cad in the presence of a fixed concentration of DMC $(30 \mu \mathrm{M})$, nine different concentrations of R-I-Cad (0.000162-4.06 $\mu \mathrm{M}$ ) were used (two or three seperate experiments, each performed in duplicate). DMC ( $50 \mu \mathrm{~L}$ ), R-I-Cad ( $5 \mu \mathrm{~L}$ ) and DMSO $(5 \mu \mathrm{~L})$ were added to assay buffer $\mathrm{A}(130 \mu \mathrm{~L})$ and the mixture was preincubated for 30 min at $30^{\circ} \mathrm{C}$. The reactions were initiated by addition of hTGase 2 stock solution ( $10 \mu \mathrm{~L}, 100 \mu \mathrm{~g} / \mathrm{mL}$ ) or enzyme buffer. Data analysis was done as described above.

To investigate the dependence of the enzyme activity on hTGase 2 concentration, $30 \mu \mathrm{M}$ DMC, $0.81 \mu \mathrm{M}$ R-I-Cad, and seven different concentrations of hTGase $2(0-5 \mu \mathrm{~g} / \mathrm{mL})$ were used. R-I-Cad $(5 \mu \mathrm{~L})$, DMSO $(5 \mu \mathrm{~L})$ and hTGase $2(10 \mu \mathrm{~L})$ or enzyme buffer ( $10 \mu \mathrm{~L}$ ) were added to assay buffer $\mathrm{A}(130 \mu \mathrm{~L})$ and the mixture was preincubated for 30 min at $30^{\circ} \mathrm{C}$. The reactions were initiated by addition of DMC. The recorded time courses of type FA=f(t) were analysed by linear regression to the experimental data over 420 s .

## Characterisation of irreversible inhibitors towards hTGase 2 and gpTGase 2

For the characterisation of irreversible inhibitors towards hTGase 2 and gpTGase 2 at a fixed preincubation time, 7 and 10 different concentrations of the inhibitors were used, respectively (two to four separate experiments, each performed in duplicate). The appropriate stock solutions of the inhibitors were prepared in DMSO. For hTGase 2, DMC (30 $\mu \mathrm{M}$ ) and R-I-Cad
$(0.81 \mu \mathrm{M})$ were chosen as acyl donor and acyl acceptor, respectively, whereas for gpTGase 2, F-Cad $(0.81 \mu \mathrm{M})$ was chosen as acyl acceptor and DMC was used in a concentration of $10 \mu \mathrm{M}$. Inhibitor ( $5 \mu \mathrm{~L}$ ), R-I-Cad/F-Cad ( $5 \mu \mathrm{~L}$ ) and TGase $2(10 \mu \mathrm{~L}, 40 \mu \mathrm{~g} / \mathrm{mL}$ for hTGase 2 and $100 \mu \mathrm{~g} / \mathrm{mL}$ for gpTGase 2) were added to assay buffer A ( $130 \mu \mathrm{~L}$ ) and the mixture was incubated for 5 min (hTGase 2) or 30 min (gpTGase 2) at $30^{\circ} \mathrm{C}$. The reactions were initiated by addition of DMC $(50 \mu \mathrm{~L})$. The recorded time courses of type FA=f(t) were analysed by linear regression to the experimental data over 900 s . The inhibitor concentration, [I], causing $50 \%$ inhibition, IC50, and the Hill slope, $\mathrm{n}_{\mathrm{H}}$, were calculated according to equation XIV

$$
\begin{equation*}
\text { rate }=\text { Bottom }+\frac{(\text { Top }- \text { Bottom }) \times[I]^{\mathrm{nH}}}{[I]^{\mathrm{nH}}+I \mathrm{IC}_{50}{ }^{\mathrm{nH}}} \tag{XIV}
\end{equation*}
$$

with Bottom and Top representing the lower and upper plateaus of the sigmoid dose-response curve, respectively.

Active site titration using 8d and the substrate pair R-I-Cad and DMC
For the active site titration at pH 8.0 , the hTGase 2-catalysed reaction between R-I-Cad $(0.81 \mu \mathrm{M})$ and DMC ( $30 \mu \mathrm{M}$ ) was followed after preincubation ( 40 min ) of hTGase 2 with inhibitor 8d at eight different concentrations (10, 20, 30, 40, 50, 60, 120, 240 nM ; three separate experiments, each performed in duplicate). Reaction rates of concentrations below and above the expected enzyme concentrations were subjected to a linear reagression with the abscissa value of the intersection point of the two lines resulting in the active concentration of the enzyme.

## Confirmation of irreversible binding of $\mathbf{6}$ a to gpTGase 2

To confirm the irreversible mode of action of $\mathbf{6 a}$ towards gpTGase 2, a jump dilution experiment according to Copeland ${ }^{84}$ was performed. Here, $500 \mu \mathrm{~g} / \mathrm{mL}$ TGase 2 ( 100 x the assay concentration) and $30 \mu \mathrm{M}$ inhibitor $\mathbf{6 a}\left(\approx 100 \times \mathrm{IC}_{50}\right)$ were preincubated for 30 min before being diluted 1:100 into assay buffer and F-Cad $(0.81 \mu \mathrm{M})$, with the reaction being subsequently started by the addition of DMC ( $10 \mu \mathrm{M}$ ). As a control, the inhibition of $5 \mu \mathrm{~g} / \mathrm{mL}$ TGase 2 ( 1 x ) by $300 \mathrm{nM} 6 \mathbf{a}\left(\approx 1 \times \mathrm{IC}_{50}\right)$ was investigated as described. The remaining activities are given as percentage values (mean values $\pm$ SEM of three separate experiments, each performed in triplicate) relative to the reaction rates in absence of inhibitor.

## Determination of TGase 2 activity in cell lysates from A375 and MeWo cells

The human metastatic melanoma cell lines A375 and MeWo were obtained from the ATCC. They were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 vol-\% fetal calf serum (FCS) and $1 \mathrm{U} / \mathrm{mL}$ penicillin/streptomycin (P/S, all reagents from

Biochrom, Berlin, Germany) at $37^{\circ} \mathrm{C}$ in a humidified atmosphere with $5 \% \mathrm{CO}_{2}$. Cells were monthly tested to be mycolasma negative with Venor ${ }^{\circledR}$ GeM Mycoplasma Detection Kit (Minerva Biolabs, Berlin, Germany). Cell pellets were collected by detaching cells, grown in a $75 \mathrm{~cm}^{2}$ culture flask (Greiner Bio-One, Kremsmünster, Austria), with 2 mM EDTA in PBS, afterwards cell suspension was centrifuged ( $3 \mathrm{~min}, 300 \times \mathrm{g}$ ) and the pellet was washed with PBS twice. For cell lysis, pellets were resuspended in $50 \mu \mathrm{~L}$ modified RIPA buffer ( 150 mM $\mathrm{NaCl}, 50 \mathrm{mM}$ Tris pH 8.0, $1 \mu \mathrm{~g} / \mathrm{mL}$ Leupeptin, 1 mM PMSF, $5 \mathrm{mM} \mathrm{NaF}, 1 \mathrm{mM} \mathrm{NaVO}{ }_{4}, 1 \mathrm{mM}$ DTT), incubated on ice for 30 min and applied to ultrasound. Subsequently, cell lysates were spinned for 15 min at $4^{\circ} \mathrm{C}$ at $15000 \times g$ and the supernatant was transferred into a fresh tube. The protein content was determined with a BCA protein assay (ThermoFisher, Waltham, MA, USA) according to the manufacturer's protocol. Lysates were stored at $-60^{\circ} \mathrm{C}$.

For determination of TGase 2 activity in cell lysates (2 g protein/l), DMC ( $30 \mu \mathrm{M}$ ) and R-I-Cad $(0.81 \mu \mathrm{M})$ were chosen as acyl donor and acyl acceptor, respectively, and the volume of the assay mixture was reduced to $100 \mu \mathrm{~L}$ compared to measurements using the recombinant enzymes. For control measurements, inhibitor $\mathbf{6 a}(10 \mu \mathrm{M})$ was used or DMC was omitted. Assay buffer B ( $20 \mu \mathrm{~L}$ ) and DMSO or inhibitor $6 \mathbf{a}(2.5 \mu \mathrm{~L})$ were added to the solution of cell lysate ( $50 \mu \mathrm{~L}$ ) and the mixture was incubated for 10 min at $30^{\circ} \mathrm{C}$. The reactions were initiated upon addition of R-I-Cad ( $2.5 \mu \mathrm{~L}$ ) and DMC ( $25 \mu \mathrm{~L}$ ). The recorded time courses of type FA=f(t) were analysed by linear regression to the experimental data over 900 s .

## Characterisation of compounds 6a, 7b and 7i towards A375 cell lysates

For the characterisation of irreversible inhibitors towards A375 cell lysates at a fixed preincubation time, 7 different concentrations of the inhibitors were used (two to three separate experiments, each performed in duplicate). Assay buffer B ( $20 \mu \mathrm{~L}$ ), inhibitor ( $2.5 \mu \mathrm{~L}$ ), and R-ICad $(2.5 \mu \mathrm{~L})$ were added to the solution of cell lysate $(50 \mu \mathrm{~L})$ and the mixture was incubated for 5 min at $30^{\circ} \mathrm{C}$. The reactions were initiated by addition of DMC $(25 \mu \mathrm{~L})$. The recorded time courses of type $F A=f(t)$ were analysed by linear regression to the experimental data over 900 s . The inhibitor concentration, [I], causing $50 \%$ inhibition, IC50, was calculated as described above.

## Molecular modelling

## Comparative modelling

The 3D structure of gpTGase 2 was modelled taking the crystal structure of the homologous hTGase 2 (PDB ID 2Q3Z, 2.0 Å) as template. ${ }^{87}$ The covalently bound irreversible inhibitor was removed. Both orthologues share $83 \%$ sequence identity and $91 \%$ sequence similarity. The
programme Modeller as implemented in Discovery Studio (Accelrys, San Diego, CA, USA) ${ }^{158}$ was used for the modeling. The missed loops of hTGase 2 were also modelled using this programme.

## Molecular docking

Protein preparation: Previous to the docking studies, the modelled structures of hTGase 2 and gpTGase 2 were prepared with Protein Preparation Wizard from Schrödinger ${ }^{159}$ (Schrödinger, New York, NY, USA) keeping His335 in its protonated state.

Ligand preparation: Ligands were prepared with the LigPrep ${ }^{160}$ in Maestro suite. ${ }^{90}$ Epik ${ }^{161-162}$ was used to generate ionization state at $\mathrm{pH} 7.0 \pm 2.0$. The OPLS2005 and OPLS3 force fields were used. ${ }^{163}$

Covalent docking: Covalent docking studies were carried out with Glide by using the workflow CovDock v1.2. ${ }^{164}$ The grid box was set up around residues Trp241, Ile313 and Asn333 with an inner and outer boxes of $10 \AA \times 10 \AA \times 10 \AA$ and $30 \AA \times 30 \AA \times 30 \AA$, respectively. The sulphur of Cys277 was defined as the site to form a covalent bond to the acrylate group of the designed inhibitors. The OPLS2005 and OPLS3 force fields were used. ${ }^{163}$ Docking results were ranked according to their Prime energy and MM-GBSA ${ }^{165}$ binding energies.

## PAMPA method ${ }^{136,139-140}$

## Buffer: <br> Membrane forming solution:

Reference compounds:

96-well plates:

50 mM HEPES, adjusted to pH 7.5 with 1 M NaOH
1,2-dioleoyl-sn-glycero-3-phosphocholine ( 25 mg , final $10 \%, \mathrm{w} / \mathrm{v}$ ) and cholesterol ( 1.25 mg , final $5 \%$, w/v) in ndodecane $(250 \mu \mathrm{~L})$, the mixture was sonificated for 20 min at $37^{\circ} \mathrm{C}$ to obtain a clear solution
hydrochlorothiazide (not permeable), metoprolol (as metoprolol tartrate, moderate permeable) and verapamil (as ( $\pm$ )-verapamil hydrochloride, well permeable)

MultiScreen ${ }^{\circledR} 96$-well Transport Receiver Plate, not sterile (Merck, Cat. No. MATRNPS50; used as donor plates)

MultiScreen-IP, $0.45 \mu \mathrm{~m}$, transparent, not sterile (Merck, Cat. No. MAIPNTR10; used as acceptor plates)

UV-Star ${ }^{\circledR}$ Microplate, 96 Well, F-Bottom (Chimney Well), $\mu$ Clear $^{\circledR}$ (Greiner Bio-One, Item No. 655801, used for measurements of UV absorption)

The determination of the effective permeability $P_{e}$ was conducted in triplicate. Stock solutions of the reference compounds and TGase 2 inhibitors were prepared in DMSO ( $0.4-4 \mathrm{mM}$ ). $70 \mu \mathrm{~L}$ of these stock solutions were diluted with $1330 \mu \mathrm{~L}$ buffer ( $5 \%$ DMSO, $\mathrm{v} / \mathrm{v}, 20-200 \mu \mathrm{M}$, depending on solubility). $300 \mu \mathrm{~L}$ of these solutions were added into the wells of the donor plate and $150 \mu \mathrm{~L}$ into the wells of the UV-Star ${ }^{\circledR}$ Microplate. $7 \mu \mathrm{~L}$ of the preheated membrane forming solution were carefully added on the filter of the acceptor plate. Then, the acceptor plate was inserted into the donor plate. Subsequently, $200 \mu \mathrm{~L}$ of a mixture of HEPES buffer and DMSO $(5 \%, \mathrm{v} / \mathrm{v})$ were added into the wells of the acceptor plate. The incubation of the plates was conducted in a sealed, wet chamber for 6 or 24 h at $37^{\circ} \mathrm{C}$. After that time, $150 \mu \mathrm{~L}$ out of the wells of the donor and acceptor plate were transferred into the UV-Star ${ }^{\circledR}$ Microplate. To determine the amount of compound in the donor and acceptor wells, the absorption spectra were recorded between 200 and 700 nm using a Synergy 4 Multi-Mode Microplate Reader (BioTek Instruments). These absorption spectra were corrected for the absorption of the HEPES/DMSO ( $5 \%$, $\mathrm{v} / \mathrm{v}$ ) mixture and, subsequently, the maximum of absorption was determined between 260 and 700 nm . Finally, equation XV (bi-directional permeation model with consideration of mass retention in the membrane) ${ }^{141}$ was used for the calculation of the effective permeability $\mathrm{P}_{\mathrm{e}}$.

$$
\begin{align*}
& c_{A}(t)=\frac{M-m}{V_{D}+V_{A}}+\left(c_{A}(0)-\frac{M-m}{V_{D}+V_{A}}\right) * e^{-P_{e} * S *\left(\frac{1}{V_{A}}+\frac{1}{V_{D}}\right) * t} \\
& c_{D}=c_{0} * \frac{A_{D}-A_{\text {buffer }}}{A_{0}-A_{\text {buffer }}} \\
& c_{A}=c_{0} * \frac{A_{A}-A_{\text {buffer }}}{A_{0}-A_{\text {buffer }}} \\
& P_{e}=-\ln \left[\frac{c_{A}(t)-\frac{M-m}{V_{D}+V_{A}}}{c_{0}-\frac{M-m}{V_{D}+V_{A}}}\right] * \frac{1}{S *\left(\frac{1}{V_{D}}+\frac{1}{V_{A}}\right) * t} \tag{XV}
\end{align*}
$$

| S: area of membrane $\left(0,24 \mathrm{~cm}^{2}\right)$ | t: permeation time |
| :--- | :--- |
| M: total amount of compound (mol) | $\mathrm{m}:$ amount of compound lost to membrane (mol) |
| $c_{0}$ : initial concentration of compound | CD: concentration of compount in donor well |

$c_{A}(t)$ : concentration of compound in acceptor well at time $t$
$V_{D}$ : volume of donor well ( $300 \mu \mathrm{l}$ )
$A_{D}$ : absorption of donor well
$A_{0}$ : absorption of the initial concentration
$\mathrm{V}_{\mathrm{A}}$ : volume of acceptor well ( $200 \mu \mathrm{l}$ )
$A_{A}$ : absorption of acceptor well
$A_{\text {buffer: }}$ absorption of HEPES/DMSO (5\%, v/v)

## Determination of $\log \mathrm{D}_{7.4}$ of fluorinated inhibitors by ${ }^{19} \mathrm{~F}$-NMR

According to Linclau et al. ${ }^{150}$
The test compound (at least 5 mg ) was dissolved in $800 \mu \mathrm{~L}$-octanol and $800 \mu \mathrm{IPBS}(0.01 \mathrm{M}$ sodium phosphate $\mathrm{pH} 7.4, \mathrm{NaCl}$; each phase was saturated with the other by shaking the neat media vigorously in a separating funnel) in a 2 mL Eppendorf tube. Trifluoroethanol (TFE, preferably equimolar to test compound, at least $0.5 \mu \mathrm{~L}$ ) and the mixture was vortexed until the test compound was completely dissolved and then shaken in a thermomixer at 1200 rpm at $25^{\circ} \mathrm{C}$ for 2 h . If necessary, the mixture centrifuged at 15000 rpm at $25^{\circ} \mathrm{C}$ for $10 \mathrm{~min} .500 \mu \mathrm{~L}$ of each layer were transferred via syringe to separate NMR tubes; transfer of the aqueous phase needs special caution according to Linclau et al. ${ }^{150}$ Acetone- $d_{6}(100 \mu \mathrm{~L})$ was added to each NMR tube and carefully mixed with the phase contents. The ${ }^{19} \mathrm{~F}$ NMR spectra were recorded for each layer, the relaxation delay times were 30 s and 60 s for octanol and aqueous phase, respectively, as suggested by Linclau et al. ${ }^{150}$ The acquisition times were adjusted in order to assure sufficient $\mathrm{S} / \mathrm{N}$ ratios, typically between 200 and 800 scans. The peak areas (AUC) for the ${ }^{19} \mathrm{~F}$ signals of trifluoroethanol and the ${ }^{19} \mathrm{~F}$ signal of the test compound were determined using the integration function implemented in the MestReNova programme, and the area of the trifluoroethanol peak was set to 100 . Finally, the $\log \mathrm{D}_{7.4}$ value was calculated using equation XVI.

$$
\begin{equation*}
\log \mathrm{D}_{7.4}=\log \mathrm{D}_{7.4(\mathrm{TFE})}+\log \left(\frac{\rho_{\mathrm{oct}}}{\rho_{\mathrm{aq}}}\right) \tag{XVI}
\end{equation*}
$$

with $\rho=A U C$ (test compound)/ AUC(TFE)
$\log \mathrm{D}_{7.4}(\mathrm{TFE})=\log \mathrm{P}(\mathrm{TFE})=+0.36$

## HPLC-based determination of chromatographic hydrophobicity indizes (CHI) at an immobilised artificial membrane (IAM)

According to Valko et al. ${ }^{166}$

For analysis of the CHI-IAM values, an analytical HPLC system from Agilent (1100 Series, Santa Clara, CA, USA) was used. A Regis IAM PC DD2 column ( $10 \times 4.6 \mathrm{~cm}$ ) was used as
stationary phase. A binary gradient of $\mathrm{NH}_{4} \mathrm{OAc}$ buffer ( $\mathrm{pH}=7.4$, solvent A ) and $\mathrm{CH}_{3} \mathrm{CN}$ (solvent B) was used at a flow rate of $1 \mathrm{mg} / \mathrm{mL}$. The programme for elution was as follows: 0-9 min gradient from $100 \%$ A to $100 \%$ B, $9-9.5$ min $100 \%$ B, $9.5-10.5$ min gradient back to $100 \%$ A. The wavelength for detection was 254 nm . To obtain a calibration curve where the gradient retention times of the inhibitors can be converted to CHI-IAM values, a set of reference compounds (benzoic acid, acetanilide, acetophenone, 1,4-dinitrobenzene, anisole, propiophenone, valerophenone and octaphenone) ${ }^{166}$ of known CHI -IAM values was analysed by the system above. For HPLC analysis, the inhibitors were dissolved in a mixture of $\mathrm{CH}_{3} \mathrm{CN} /$ water $70 / 30$ and their $\mathrm{CHI}-\mathrm{IAM}$ values were finally calculated using the calibration curve.

## Determination of the thermodynamic solubility of compounds 7 b and 7 i

The thermodynamic solubility was determined as recently described by Badarau et al. ${ }^{88}$ To the respective inhibitor ( $1-1.5 \mathrm{mg}$ ) in a 2 mL vial was added PBS ( 0.01 M sodium phosphate pH $7.4, \mathrm{NaCl}$ ) to theoretically obtain a $1 \mathrm{mg} / \mathrm{mL}$ solution. The suspension was vigorously stirred. After 20 h , the suspension was centrifuged at 4000 g for $10 \mathrm{~min} .100 \mu \mathrm{~L}$ of the supernatant were transferred to a vial containing a PVDF membrane (pore size $0.22 \mu \mathrm{M}$, Ultrafree filtration systems from Roth ${ }^{\circledR}$, Karlsruhe, Germany) and were filtered by centrifugation at 4000 g for 2 min . $10 \mu \mathrm{~L}$ of the filtrate was transferred to a sample vial for DAD-LC-MS analysis and $80 \mu \mathrm{~L}$ water and $10 \mu \mathrm{~L}$ DMSO were added. $5 \mu \mathrm{~L}$ of that solution were injected into the system. A 4 mM stock solution of the respective inhibitor was prepared in DMSO, which was then diluted to eight solutions between 100 and $2000 \mu \mathrm{M}$ with DMSO. Each solution including the stock solution was then diluted with water ( $1: 9, \mathrm{v} / \mathrm{v}$ ) to obtain a final range of concentration between 10 and $400 \mu \mathrm{M} .5 \mu \mathrm{~L}$ of each solution were analysed by DAD-LC-MS analysis. A calibration curve was obtained for each inhibitor by determination of the peak areas (at $\lambda=305 \mathrm{~nm}$ and $\lambda=405 \mathrm{~nm}$ for $\mathbf{7 b}$ and $\mathbf{7 i}$, respectively). Using these calibration curves, the concentration of the inhibitors in PBS could be calculated considering the additional dilution (1:9) of the PBS solution. According to this procedure, the suspension in PBS was also analysed at later time points until the concentration did not further increase indicating the maximum thermodynamic solubility. Mass spectrometry was only used to ensure that the observed UV peak corresponds to the compound of interest.

## Stability in liver microsomes

For microsome experiments the following instruments were used: BioShake iQ (QUANTIFOIL Instruments, Jena, Germany) and Centrifuge 5424 (Eppendorf, Hamburg, Germany), UltiMate

3000 UHPLC System (Thermo Scientific, Germering, Germany) including a DAD detector (DAD-3000RS) coupled to an MSQ Plus Single Quadrupole Mass Spectrometer (Thermo Scientific, Austin, Texas, USA), Agilent 1260 Infinity Quaternary LC system (Agilent Technologies, Böblingen, Germany) coupled with a QTRAP 5500 hybrid linear ion-trap triple quadrupole mass spectrometer (AB SCIEX, Concord, Ontario, Canada).

NADPH (nicotinamide adenine dinucleotide phosphate) and testosterone were purchased from Sigma-Aldrich (Steinheim, Germany). GIBCO mouse liver microsomes (MLM, $20 \mathrm{mg} / \mathrm{mL}$ ) were purchased from Life Technologies (Darmstadt, Germany). Dulbecco's phosphate buffered saline (PBS) (without $\mathrm{Ca}^{2+}, \mathrm{Mg}^{2+}$ ) was purchased from Biochrom (Berlin, Germany).

Incubations had a final volume of $250 \mu \mathrm{~L}$ and were performed in duplicate in PBS (pH 7.4) as follows, with final concentrations as stated in brackets (according to Ludwig et al. ${ }^{167}$ ). PBS, MLM $(1 \mathrm{mg} / \mathrm{mL})$ and inhibitors ( $100 \mu \mathrm{M}$ ) were mixed and preincubated at $37^{\circ} \mathrm{C}$ for 5 min . Analogously preincubated NADPH ( 2 mM ) was added and mixtures were shaken gently at $37^{\circ} \mathrm{C}$. After 30 and 60 min , respectively, $100 \mu \mathrm{~L}$ were taken and added to $100 \mu \mathrm{~L}$ cold acetonitrile $\left(-20^{\circ} \mathrm{C}\right)$, followed by vigorous shaking ( 30 s ), cooling at $-20^{\circ} \mathrm{C}(0.5 \mathrm{~h})$, and centrigutation at $14000 \mathrm{rpm}(10 \mathrm{~min})$. Supernatants were filtered with Phenex-RC 4 mm Syringe Filters $0.2 \mu \mathrm{~m}$ (Phenomenex, Aschaffenburg, Germany), diluted with water and stored at $4^{\circ} \mathrm{C}$ until analysed by HPLC-UV. As positive control testosterone was used as substrate and incubated at appropriate concentration, similarly to the protocol described above, to give complete conversion confirmed by HPLC. Furthermore, incubations without NADPH, microsomal protein, and substrates were analysed as negative controls. Microsomal stabilities were calculated from relative peak areas after HPLC-UV analyses on a ReproSil-Pur 120 C18-AQ-column, $125 \mathrm{~mm} \times 3 \mathrm{~mm}, 3 \mu \mathrm{~m}$ (Dr. Maisch GmbH, Ammerbuch, Germany) at $40^{\circ} \mathrm{C}$. The solvent system consisted of eluent A: $0.1 \%$ acetic acid, and eluent B: water/acetonitrile 20/80 ( $\mathrm{v} / \mathrm{v}$ ), containing $0.1 \%$ acetic acid. Gradient elution (\% acetonitrile) at a flow rate of $0.7 \mathrm{~mL} / \mathrm{min}$ with UV detection at 235 nm (bandwidth 20 nm ), unless otherwise stated, for incubation samples from a) 6a, 7a, 10, 15f: 0-1.5 min, 10\%; 1.5-10 min, 10-35\%; 10-13 min, $80 \%$; 13$17 \mathrm{~min}, 10 \%$ (detection at 284 nm for 10), b) 7b, 7i, 8c, 13a, 13c: 0-1.5 min, 20\%; 1.5-10 min, $20-60 \%$; $10-13 \mathrm{~min}, 80 \%$; $13-17 \mathrm{~min}, 20 \%$ (detection at 270 nm for $\mathbf{8 c}$ ), and c) 17b, 17c: $\mathbf{0}$ $1.5 \mathrm{~min}, 20 \% ; 1.5-10 \mathrm{~min}, 20-50 \%$; $10-13 \mathrm{~min}, 80 \% ; 13-17 \mathrm{~min}, 20 \%$. Conditions for subsequent analyses using the MSQ Plus single quadrupol mass spectrometer were: probe temperature $500^{\circ} \mathrm{C}$, needle voltage 3 V , cone voltage 75 V . For further structural elucidation of metabolites, LC conditions were used as described above and enhanced product ion (EPI) and $\mathrm{MS}^{3}$ spectra were recorded on the QTRAP 5500 hybrid linear ion-trap triple quadrupole
mass spectrometer. Both mass spectrometers were operated in positive electrospray ionisation mode.

## Associated Content

## Supporting Information

All synthetic methods and analytical data (NMR, ESI-MS) of the compounds as well as additional Figures and Discussions (as mentioned in the text) are included in the Supporting Information.

## Author Information

## Corresponding Author

*E-mail: markus.pietsch@uk-koeln.de; r.loeser@hzdr.de

## Notes

The authors declare no competing financial interest.

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## Abbreviations Used

hFXIIla, human Factor XIIla; gpTGase 2, transglutaminase from guinea pig liver; hTGase, human transglutaminase; mTGase 2, murine transglutaminase 2; SAR, structure-activity relationship; HMC, 7-hydroxy-4-methylcoumarin; $p N P$, $p$-nitrophenylate; PET, positron emission tomography; FA, fluorescence anisotropy; DMC, N,N-dimethylcasein; R-I-Cad, rhodamine B-isonipectoyl-cadaverine; CD, circular dichroism; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; CYP, cytochrome P450; PAMPA, parallel artificial membrane permeability assay

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## Supporting Information

## $\mathbf{N}^{\boldsymbol{\varepsilon}}$-Acryloyllysine piperazides as irreversible inhibitors of transglutaminase 2 synthesis, structure-activity relationships and pharmacokinetic profiling

Robert Wodtke ${ }^{\mathrm{a}, \mathrm{e}, \mathrm{f}}$, Christoph Hauser ${ }^{\mathrm{b}}$, Gloria Ruiz-Gómez ${ }^{\mathrm{c}}$, Elisabeth Jäckel ${ }^{\text {a,e },}$ David Bauer ${ }^{\text {a,f }, ~ M a r t i n ~ L o h s e ~}{ }^{\text {ae, }}$, Alan Wong ${ }^{\text {a }}$, Johanna Pufe ${ }^{\text {a }}$, Friedrich-Alexander Ludwig ${ }^{\text {d }}$, Steffen Fischer ${ }^{\text {d }}$, Sandra Hauser ${ }^{\text {a }}$, Dieter Greife ${ }^{e}$, 

[a] Helmholtz-Zentrum Dresden-Rossendorf, Institut für Radiopharmazeutische Krebsforschung, Bautzner Landstraße 400, 01328 Dresden, Germany
[b] Zentrum für Pharmakologie, Medizinische Fakultät, Universität zu Köln, Gleueler Straße 24, 50931 Köln, Germany
[c] Structural Bioinformatics, BIOTEC, TU Dresden, Tatzberg 47-51, 01307 Dresden, Germany
[d] Helmholtz-Zentrum Dresden-Rossendorf, Institut für Radiopharmazeutische Krebsforschung, Forschungsstelle Leipzig, Permoserstraße 15, 04318 Leipzig, Germany
[e] Fakultät Natur- und Umweltwissenschaften, Hochschule Zittau/Görlitz, Theodor-Körner-Allee 16, 02763 Zittau, Germany
[f] Fakultät Chemie und Lebensmittelchemie, Technische Universität Dresden, Mommsenstraße 4, 01062 Dresden, Germany

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## Discussion S1: Side and degradation products of distinct final inhibitors

## Compound 6c



This side product arose during initial attempts to functionalise at the $\alpha$-amino group of 5 a using 1.5 equiv. of phenylacetyl chloride. The formation of $\mathbf{6 c}$ and its structure was confirmed by analytical RP-HPLC and by ESI-MS and NMR spectroscopical analyses. Compared to the ${ }^{1} \mathrm{H}$ NMR spectrum of compound 6a, an additional signal at around 5 ppm is visible in the spectrum of $\mathbf{6 c}$ (integration of 1 ), which corresponds to the benzylic methane proton. Furthermore, the majority of signals appear in duplicate which indicates a mixture of diastereomers due to the newly formed stereocentre. According to the formation of $\mathbf{6 c}$, subsequent phenylacetylations were performed using 1 equiv. of phenylacetyl chloride.

Compound $\mathbf{6 c}(9.2 \mathrm{mg}, 8 \%)$ was obtained as an oily solid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\boldsymbol{d}_{6}$ ): $\boldsymbol{\delta}=8.79-8.73$ (ps-t, ${ }^{3}=7.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.09-7.99 (m, 1H, $\mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.70-7.58 (m, 1H, H-4 of pyridine), 7.367.17 (m, 8H, H-2,3,5,6 2×phenyl), 7.15-7.04 (m, 2H, H-4 2×phenyl), 6.90-6.73 (m, 1H, H-3 of pyridine), 6.67 (ps-t, ${ }^{3} \mathrm{~J}=6.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), $6.23-6.11$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $6.07-$ $5.98(\mathrm{~m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}), 5.56-5.48(\mathrm{~m}, 1 \mathrm{H}, \mathrm{CH} H), 5.08-5.04$ ( $\mathrm{m}, 1 \mathrm{H}$, benzylic CH group), 4.82$4.68\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.88-3.22\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, benzylic $\mathrm{CH}_{2}$ group), 3.15-2.96 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 2.40-2.37 (m,3H, $\mathrm{CH}_{3}$ ), 1.74-1.08 (m, 6H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); MS (ESI+): m/z calculated for $\mathrm{C}_{35} \mathrm{H}_{42} \mathrm{~N}_{5} \mathrm{O}_{4}: 596.32[\mathrm{M}+\mathrm{H}]^{+}$, found: 596.2.

## Compounds 16a-d


$\mathrm{R}^{1}=\operatorname{Br}(16 a)$
tert-butyl (16b)
phenyl (16c)
2-fluoroethoxy (16d)

These side products arose during the phenylacetylation of compounds $\mathbf{5 n}, \mathbf{5 0}, \mathbf{5 q}$ and $\mathbf{5 r}$. They were formed most likely due to the intermediate activation of residual TFA by phenylacetyl chloride as mixed anhydride. Because of the identical retention times of 16a-d and their respective phenylacetyl derivatives 7d and 7f-h using analytical and preparative RP-HPLC, purification of 7d and 7f-h had to be performed by convential normal-phase column chromatography. In the case of $\mathbf{7 g}$, the trifluoroacetylated side product $\mathbf{1 6 c}$ could be isolated in sufficient amounts for analytical and kinetic analyses. Analytical data are only shown for compound 16c.

The crude product of 16c was purified via column chromatography (isocratic ethyl acetate). The product-containing fractions were combined, evaporated and dried by lyophilisation (from $0.1 \%$ TFA/water) to afford $\mathbf{1 6 c}(7.9 \mathrm{mg}, 13 \%)$ as a white solid. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): ~ \delta=8.03-7.97$ (m, 2H, H-2,6 of phenyl), 7.61 (dd, ${ }^{3} \mathrm{~J}=8.3,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.56 (d, ${ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $7.48-7.42$ (m, 2H, H-3,5 of phenyl), $7.42-7.36$ (m, 1H, H-4 of phenyl), 7.19 (d, ${ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.64 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.26 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz}$, ${ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 6.07 (dd, ${ }^{3} \mathrm{~J}=17.0,10.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.70-5.64 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH} H$ ), 4.99-4.92 (m, 1H, CaH), 3.91-3.59 (m, 8H, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.39-3.30 (m, 2H, C $\mathrm{C}_{\varepsilon}$ ), 1.94-1.82 (m, 1H, C ${ }_{\beta} H \mathrm{H}$ ), 1.80-1.52 (m, 3H, $\mathrm{C}_{\beta} \mathrm{HH}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.46-1.37 (m, 2H, C $\mathrm{C}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}^{\left(\mathrm{CDCl}_{3}\right): ~} \delta=168.74,165.88,155.39,139.04$ ( $\mathrm{C}-4$ of pyridine), $130.85\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 129.16$ ( $\mathrm{C}-4$ of phenyl), 128.73 ( $\mathrm{C}-3,5$ of phenyl), 127.02 ( $\mathrm{C}-2,6$ of phenyl), $126.64\left(\mathrm{CH}=\mathrm{CH}_{2}\right.$ ), 111.11 ( $\mathrm{C}-5$ of pyridine), 106.21 ( $\mathrm{C}-3$ of pyridine), 49.55 $\left(\mathrm{C}_{\alpha}\right), 45.66\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.44\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), $42.22\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.93$ $\left(\mathrm{C}_{\varepsilon}\right), 32.46\left(\mathrm{C}_{\beta}\right), 28.97\left(\mathrm{C}_{\delta}\right), 22.06\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{C}_{\text {quarternary }}$ are not visible; ${ }^{19} \mathrm{~F}$-NMR $\left(\mathrm{CDCl}_{3}\right): \delta=-75.77$ (s, Trifluoracetyl and TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{31} \mathrm{~F}_{3} \mathrm{~N}_{5} \mathrm{O}_{3}$ : $518.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 518.2.

## Compounds 14n and 140



Both compounds arose by decomposition of compounds 14 i and 14 j during storage (as solids) at $4^{\circ} \mathrm{C}$. Purification by preparative RP-HPLC afforded 6-methylpyridine-2-ylpiperazine and the respective phenylthiohydantoin derivatives $\mathbf{1 4 n}$ and 140. This decomposition resembles an Edman degradation. ${ }^{1-2}$ Analytical data are only shown for compound 140.

Compound $140(6.6 \mathrm{mg})$ was obtained as a white solid. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): \delta=7.92$ (broad s, $1 \mathrm{H}, \mathrm{NaH}$ ), 7.33-7.24 (m, 2H, H-2,6 of fluorophenyl), 7.23-7.12 (m, 2H, H-3,5 of fluorophenyl), $6.35\left(\mathrm{~d},{ }^{3}=17.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 6.10\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.0,10.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right), 5.83-5.68(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 4.34-4.27 (m, 1H, CaH), 3.56-3.44 (m, 1H, C $\mathrm{C}_{\varepsilon} H \mathrm{H}$ ), 3.40-3.26 (m, 1H, $\mathrm{C}_{\varepsilon} \mathrm{HH}$ ), 2.22-1.86 (m, 2H, $\left.\mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.70-1.60\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.58-1.45\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}$-NMR $\left(\mathrm{CDCl}_{3}\right): \delta=130.31$ ( $\mathrm{d},{ }^{3} \mathrm{~J}=9.0 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), $130.13\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 128.11\left(\mathrm{CH}=\mathrm{CH}_{2}\right)$ 116.39 ( $\mathrm{d},{ }^{2} \mathrm{~J}=23.1 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorophenyl), $59.67\left(\mathrm{C}_{\alpha}\right), 38.76\left(\mathrm{C}_{\varepsilon}\right), 30.51\left(\mathrm{C}_{\beta}\right), 29.10\left(\mathrm{C}_{\delta}\right)$, $21.17\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-1,4$ of fluorophenyl, $2 \times \mathrm{CO}$ and CS are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ : $\delta=-111.47--111.57$ ( m , fluorophenyl); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{16} \mathrm{H}_{19} \mathrm{FN}_{3} \mathrm{O}_{2} \mathrm{~S}$ : 336.12 $[\mathrm{M}+\mathrm{H}]^{+}$, found: 336.3.

## Compounds 14p and 14q




After introduction of the regioisomeric pyridylacetyl residues at compound $\mathbf{5 a}$, further side products were observed. Whereas for the pyridine-2-yl derivative 14a the desired product was exclusively formed, compounds $\mathbf{1 4 p}$ and $\mathbf{1 4 q}$ exist in an equilibrium with the respective macrocycles (determined by NMR analyses), which were probably formed by intramolecular aza-Michael addition. For both mixtures, the equilibrium is located on the side of the macrocycle (e.g. for compound $\mathbf{1 4 q} \mathbf{1 6 \%}$ open form and $84 \%$ macrocycle) as determined by integration of the respective $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ signals from the ${ }^{1} \mathrm{H}$-NMR spectra. The reasons for the missing macrocyclisation in 14a might be the higher sterical shielding of the pyridine nitrogen adjacent to the acetyl residue as well as the higher conformational strain of the respective macrocycle. Aza-Michael additions with aromatic azacycles as nucleophiles are known; however, such reactions usually require catalysis even in the case of highly reactive Michael acceptors such as enones and enales. ${ }^{3}$ Accordingly, the driving force for the observed macrocyclisation might result from the small spatial distance of both reaction partners, and thus, from a high inherent tendency for cyclisation. Interestingly, macrocyclisations between the acrylamide group and the pyridine rings in compounds $\mathbf{7 a}, \mathbf{8 a}$ and 10 have not been observed. Analytical data are only shown for compound 14q.

Compound $\mathbf{1 4 q}$ ( $27 \mathrm{mg}, 64 \%$ ) was obtained as a yellow oil. Based on the integration of the $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ signals, $\mathbf{1 6 \%}$ of $\mathbf{1 4 q}$ exists in the open form and $84 \%$ as the macrocycle. If not otherwise stated, the NMR data below are primarily listed for the macrocycle. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ): $\delta=8.95\left(\mathrm{~d},{ }^{3} \mathrm{~J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6\right.$ of pyridine), 8.81-8.60 (m, $\left.1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.13-8.02\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right)$, 7.97 ( $\mathrm{d},{ }^{3} \mathrm{~J}=6.4 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5$ of pyridine), 7.63-7.49 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-4$ of methylpyridine), 6.82-6.67 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-3$ of methylpyridine), 6.66-6.53 (m, $1 \mathrm{H}, \mathrm{H}-5$ of methylpyridine), 6.18 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, \mathrm{CH}=\mathrm{CH}_{2}$ of open form), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, \mathrm{C}=\mathrm{CHH}$ of open form), $5.57-$ 5.52 ( $\mathrm{m}, \mathrm{C}=\mathrm{CHH}$ of open form), 4.80-4.65 (m, 3H, CaH, C $\mathrm{C}_{2}$ ), 3.96-3.83 (m, 2H, CH $2^{-}$
pyridine), 3.72-3.39 ( $\mathrm{m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.14-3.04 ( $\mathrm{m}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ of open form), 3.04$2.91\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{8} \mathrm{H}_{2}\right), 2.84\left(\mathrm{t},{ }^{3} \mathrm{~J}=6.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{N}-\mathrm{C}_{\beta} \mathrm{H}_{2}\right.$ ), $2.33\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.71-1.58(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{C}_{\beta} \mathrm{H} H$ ), 1.57-1.45 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right)$, 1.43-1.15 (m, 4H, C $\mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ): $\delta=169.78\left(\mathrm{C}_{\alpha} \mathrm{CO}\right), 168.28\left(\mathrm{~N}_{\varepsilon} \mathrm{CO}\right), 166.90\left(\mathrm{~N}_{\mathrm{a}} \mathrm{CO}\right), 158.14$ ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=} 35.7 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 155.88 (C-1 of pyridine), 144.43 (C-2,6 of pyridine), 139.35, 131.81, 131.60, 128.12 (C-3,5 of pyridine), $112.64\left(\mathrm{C}-5\right.$ of methylpyridine), $56.57\left(\mathrm{C}_{\beta} \mathrm{H}_{2}\right), 48.71\left(\mathrm{C}_{\alpha} \mathrm{H}\right), 44.66\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.09\left(\mathrm{CH}_{2}\right.$-pyridine), $38.38\left(\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 35.37\left(\mathrm{C}_{\alpha} \mathrm{H}_{2}\right), 31.23\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.56$ $\left(\mathrm{C}_{\gamma}\right)$, signals for $3 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{3}, 1 \times \mathrm{C}_{\text {Methylpyridine }}$ are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ): $\delta=-74.64$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{35} \mathrm{~N}_{6} \mathrm{O}_{3}: 479.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 479.4.

## Discussion S2: Kinetic characterisation of inhibitors with sulfonyl, carbamoyl, thiocarbamoyl and benz(o)yl residues at the $\alpha$-amino group

In addition to the aromatic moiety of the phenylacetyl group, the carboxamide group was planned to be substituted. One alternative functionality is represented by the sulfonamide group, which exhibits a lower rotation barrier around the S-N bound because of the missing double bond character, ${ }^{4-5}$ among other structural differences in comparison to carboxamides. This could be beneficial for the orientation of the respective phenylmethanesulfonyl residue. However, the inhibitory potential of compound $\mathbf{1 4 e}\left(k_{\text {inact }} / K_{1}=362 \mathrm{M}^{-1} \mathrm{~s}^{-1}\right)$ is surprisingly low and indicates that certain structural features of sulfonamides ${ }^{5-7}$ are not well tolerated in the binding site. Consequently, the dansyl derivative $\mathbf{1 4 f}$ exhibits also a low reactivity towards hTGase 2 ( $k_{\text {inact }} / K_{1}=95 \mathrm{M}^{-1} \mathrm{~s}^{-1}$ ). Therefore, the dansyl derivative 13 e , which is discussed in the main text, is 20 times more potent than $\mathbf{1 4 f}$.

In addition, the carboxamide group was replaced by urea and thiourea functionalities. Again, both modifications lead to a strong reduction of the inactivation constants with carbamoyl residues (in $\mathbf{1 4 g}$ and $\mathbf{1 4 h}$ with $k_{\text {inact }} / K_{\mathrm{I}}$ values of 287 and $278 \mathrm{M}^{-1} \mathrm{~s}^{-1}$, respectively) being better tolerated than thiocarbamoyl residues (in 14i and $\mathbf{1 4 j}$ with $k_{\text {inact }} / K_{l}$ values of 79 and $113 \mathrm{M}^{-1} \mathrm{~s}^{-1}$, respectively). The presence of both functional groups leads to a rigidisation, which might impede the orientation of the aromatic rings into the binding site.

In an analogoues manner to the piperazine ring, the $\alpha$-amino group enables ${ }^{18} \mathrm{~F}$-fluorination via incorporation of prosthetic groups containing fluorine-18. In contrast to the 4-fluorobenzoyl and 4 -fluorobenzyl groups at the piperazine ring, these residues are badly tolerated the $\alpha$ amino group as reflected by the inhibitory potentials of compounds $\mathbf{1 4 k}$ and $\mathbf{1 4 m}$. This is in accordance to the obtained SARs so far as the truncation by the methylene group leads to a limited rotation of the 4 -fluorophenyl group as well as a less efficient occupation of the respective pocket. Although the 4-fluorobenzyl group exhibits a higher flexibility than the 4fluorobenzoyl group, the inactivation constant of compound 14m is even lower than that of $\mathbf{1 4 k}$. Therefore, it can be concluded that positive charge associated with the secondary amine function of $\mathbf{1 4 m}$ imparts repulsive interactions within the binding site of hTGase 2.

In addition to the labelling with fluorine-18, incorporations of the radioisotopes of iodine can be envisaged. With this regard, indirect labelling with iodine can be achieved using the prosthetic labelling reagent $N$-succinimidyl-3-[t]iodobenzoate. ${ }^{8}$ Kinetic characterisation of the respective
non-radioactive reference compound $\mathbf{1 4 I}$ revealed a similar reactivity as the 4 -fluorobenzoyl derivative 14k, which was expected based on the observed SARs so far.

Figure S1: Dependence of enzyme activity on hTGase 2 concentration using the fluorescence polarisation assay


Plot of rate $=\mathrm{f}([\mathrm{hTGase} 2])$ for the reaction of $\mathrm{DMC}(30 \mu \mathrm{M})$ with R-I-Cad $(0.81 \mu \mathrm{M})$ at pH 8.0 . Data shown are mean values ( $\pm$ SEM) of three separate experiments, each performed in duplicate. Analysis by linear regression gave a slope (mean value $\pm$ SEM) of $0.0303 \pm 0.0026$ $\mathrm{mA} \mathrm{mL} \mathrm{s} \mathrm{s}^{-1} \mathrm{~g}^{-1}$.

Figure S2: TGase 2-catalysed incorporation of F-Cad and R-I-Cad in DMC at pH 8.0


A: Plots of rate versus substrate concentration for the reaction of DMC with F-Cad ( $0.81 \mu \mathrm{M}$, red symbols) or R-I-Cad ( $0.81 \mu \mathrm{M}$, grey symbols) in the presence (circles) and absence (squares) of gpTGase 2 and for the reaction of DMC with R-I-Cad ( $0.81 \mu \mathrm{M}$, black symbols) in the presence (circles) and absence (squares) of hTGase 2. B: Plots of rate versus substrate concentration for the reaction of F-Cad with DMC ( $5 \mu \mathrm{M}$, red symbols) or R-I-Cad with DMC ( $30 \mu \mathrm{M}$, grey symbols) in the presence (circles) and absence (squares) of gpTGase 2 and for the reaction of R-I-Cad with DMC ( $30 \mu \mathrm{M}$, black symbols) in the presence (circles) and absence (squares) of hTGase 2. Data shown in $\mathbf{A}$ and $\mathbf{B}$ are mean values ( $\pm$ SEM) of three (gpTGase 2) or two (hTGase 2) experiments, each performed in duplicate (hTGase 2) or triplicate (gpTGase 2). When not apparent, error bars are smaller than the symbols. Conditions: pH 8.0, $30^{\circ} \mathrm{C}, 5 \% \mathrm{DMSO}, 500 \mu \mathrm{M} \mathrm{DTT}$ (gpTGase 2) or $500 \mu \mathrm{M}$ TCEP (hTGase 2), $5 \mu \mathrm{~g} / \mathrm{mL}$ TGase 2. Plots for gpTGase 2 have already been shown in a previous study ${ }^{9}$.

## Figure S3: Confirmation of irreversibility



Inhibition of the gpTGase 2-catalysed reaction of DMC ( $10 \mu \mathrm{M}$ ) and F-Cad ( $0.81 \mu \mathrm{M}$ ) by compound 6a. Prior to the addition of DMC to start the reaction, either TGase $2(5 \mu \mathrm{~g} / \mathrm{mL}=1 \mathrm{x}$ TGase 2) was preincubated ( 30 min ) with assay buffer, F -Cad and inhibitor ( $300 \mathrm{nM} \approx 1 \mathrm{x} \mathrm{IC}_{50}$ ) or TGase $2\left(500 \mu \mathrm{~g} / \mathrm{mL}=100 \mathrm{x}\right.$ TGase 2) was preincubated with inhibitor ( $30 \mu \mathrm{M} \approx 100 \mathrm{x}$ IC $\mathrm{C}_{50}$ ) for 30 min and then diluted ( $1: 100$, v/v) into assay buffer and F-Cad. Values given (mean values $\pm$ SEM of three separate experiments, each performed in triplicate) are reaction rates in presence of inhibitor relative to the respective rate in absence of inhibitor. * $\mathrm{p} \leq 0.05$ unpaired Student's t test

Figure S4: Active site titration using inhibitor 8d


Plots of rate versus concentration of 8d after preincubation of hTGase 2 with inhibitor for 40 min and determination of residual enzymatic activity by A: FA assay, $\mathrm{pH}=8.0,30^{\circ} \mathrm{C}, 5 \%$ DMSO, $500 \mu \mathrm{M}$ TCEP, $30 \mu \mathrm{M}$ DMC, $0.81 \mu \mathrm{M}$ R-I-Cad, $5 \mu \mathrm{~g} / \mathrm{mL}$ ( $\mathrm{E}_{\text {total }}=64.1 \mathrm{nM}$ ) hTGase 2 and B: fluorimetric assay, $\mathrm{pH}=6.5,30^{\circ} \mathrm{C}, 5 \% \mathrm{DMSO}, 500 \mu \mathrm{M}$ TCEP, $30 \mu \mathrm{M}$ Z-Glu(HMC)-Gly-OH, $3 \mu \mathrm{~g} / \mathrm{mL}$ ( $\mathrm{E}_{\text {total }}=38.5 \mathrm{nM}$ ) hTGase 2. Linear regressions were separately performed for data below (black) and above (red) the respective enzyme concentrations. The abscissa value of the intersection point of the lines corresponds to the active enzyme concentration, which is given along with the respective activity factor in each diagram. Both assay methods provided activity factors which are not significantly different from each other (unpaired Student's $t$ test, $P \leq 0.05$ ). Data shown in $\mathbf{A}$ and $\mathbf{B}$ are mean values ( $\pm$ SEM) of three to four separate experiments, each performed in duplicate.

Figure S5: Modelling of missing residues in the crystal structure of hTGase 2

A) hTGase 2 (PDB ID 2Q3Z, 2.0 Å). B) hTGase 2 with modelled residues 307-308 and 319327. hTGase 2 is shown in gray surface, catalytic Cys277 is highlighted in green and numbered, and modelled residues are highlighted in yellow and numbered. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S6: Molecular modelling of the interaction of hTGase 2 with 6a

hTGase 2 is shown in gray surface with relevant residues coloured by atom type and $\mathbf{6 a}$ is shown in sticks and coloured by atom type. Intermolecular H bonds and $\pi-\pi$ interactions are depicted by black and cyan dashed lines, respectively. Different binding modes of 6a are highlighted as gradient of green colour. Figure generated in Maestro (Schrödinger). ${ }^{10}$

## Figure S7: CD spectroscopy of compounds 6a and 6b

Stock solutions of the enantiomers $\mathbf{6 a}$ and $\mathbf{6} \mathbf{b}$ were prepared in a concentration of 0.25 or $0.5 \mathrm{mg} / \mathrm{mL}$ in ethanol. The CD spectra were measured in a quartz cuvette with an optical path length of 1 mm (Starna, USA) using a J-810 spectropolarimeter (Jasco, Japan). The conditions of the measurements were as follows: a spectral region of $180-260 \mathrm{~nm}$ or $210-400 \mathrm{~nm}$, a scanning speed of $20 \mathrm{~nm} / \mathrm{min}$, a response time of 8 s , a resolution of 1 nm , a bandwidth of 1 nm and a sensitivity of 100 mdeg. The final spectrum was obtained as an average of 5 accumulations. The spectra were corrected for a baseline by subtracting the spectra of the corresponding enantiomer-free solution. The ECD measurements were conducted at room temperature.

A) UV absorption and CD spectra between 180 and 260 nm of $\mathbf{6 a}$ and $\mathbf{6 b}$ at a concentration of $0.25 \mathrm{mg} / \mathrm{mL}$. B) UV absorption and CD spectra between 210 and 400 nm of $\mathbf{6 a}$ and $\mathbf{6 b}$ at a concentration of $0.5 \mathrm{mg} / \mathrm{mL}$.

## Figure S8:HPLC analysis of compounds 6 a and 6 b using a chiral stationary phase

HPLC for determining the enantiomeric composition of compounds $\mathbf{6 a}$ and $\mathbf{6 b}$ was carried out with a system consisting of a Merck Hitachi L7100 gradient pump combined with a Jasco DG2080 four-line degasser with UV detection by a Merck Hitachi L7450 diode array detector. The system was operated with D-700 HSM software and use of a Merck Hitachi D7000 interface. A Chiralpak ${ }^{\circledR}$ IA column ( $250 \times 4.6 \mathrm{~mm}, 5 \mu \mathrm{~m}$ ) served as stationary phase. Separation of the enatiomers was achieved by an isocratic solvent mixture of $5 \%$ hexane $/ \mathrm{CH}_{3} \mathrm{CN}(\mathrm{v} / \mathrm{v})$ at a flow rate of $1 \mathrm{~mL} / \mathrm{min}$.


Figure S9: Relationship between the $\mathbf{c p} K_{a}$ values of the respective pyridinium ions and the inactivation constants of the inhibitors 6a and 7a-e


Plots of $\lg \left(K_{\text {nact }} / K_{1}\right)=\mathrm{f}\left(\mathrm{p} K_{\mathrm{a}}\right)$ using the mean values of the inactivation constants (Table 4 within the main article) and the following $\mathrm{cp} K_{\mathrm{a}}$ values (Table S1): $8.4(\mathrm{H})$, $2.8(\mathrm{~F}), 9.1\left(\mathrm{CH}_{3}\right), 4.8(\mathrm{Cl})$, $4.0(\mathrm{Br})$ und 5.0 (I). The regression analysis was performed by linear regression.

Figure S10: Sequence alignment of TGase family members


Sequence alignment of hTGase 2, gpTGase 2, hTGase 1, hTGase 3, hTGase 6 and hfXIIIa. Arg317 of hTGase 2 and the corresponding residues at the same position for the family members are highlighted by the red frame. Figure generated in Discovery Studio (Accelrys). ${ }^{11}$

Sequence identity and similarity for members of the TGase family.

|  | hTGase 2 | gpsTGase <br> 2 | hTGase 1 | hTGase 3 | hTGase 6 | hfXIIIa |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| hTGase 2 |  | 91 | 52 | 57 | 58 | 51 |
| gpTGase 2 | 83 |  | 52 | 57 | 57 | 50 |
| hTGase 1 | 36 | 35 |  | 44 | 45 | 53 |
| hTGase 3 | 38 | 39 | 29 |  | 66 | 51 |
| hTGase 6 | 42 | 40 | 31 | 51 |  | 50 |
| hfXIIIa | 37 | 36 | 38 | 33 | 34 |  |

Sequence identity (\%) and similarity (\%) are shown in yellow and green, respectively.

Figure S11: Alternative binding mode for the interaction of hTGase 2 with 10

hTGase 2 is shown in gray surface with relevant residues colored by atom type, and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds and cation- $\pi$ interactions are depicted by black and green dashed lines, respectively. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S12: Binding modes of the interaction for hTGase 2 with 13b

hTGase 2 is shown in gray surface with relevant residues colored by atom type, and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds, salt bridges and $\pi-\pi$ interactions are depicted by black, magenta and cyan dashed lines, respectively. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S13: Molecular modelling of the interaction of hTGase 2 with 13 e

hTGase 2 is shown in gray surface with relevant residues colored by atom type, and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds are depicted by black dashed lines. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S14: Relationship between the hydrophobicity of the bioisosteric substituents phenylacetyl, 2-thienylacetyl and 2pyridylacetyl and the inactivation constants of the inhibitors 6a and 14a-b



Plots of $\lg \left(k_{\text {inact }} / K_{1}\right)=\mathrm{f}(\pi)$ using the mean values of the inactivation constants (Table 3 and Table 8 within the main article) and the following values for $\pi^{12}$ : 0.5 (2-pyridyl), 1.61 (2-thienyl), 2.15 (phenyl). The regression analysis was performed by linear regression.

Figure S15: Molecular modelling of the interaction of hTGase 2 with 14b (A and C) and 14c (B and D)

hTGase 2 is shown in gray surface with relevant residues colored by atom type, and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds, cation $-\pi$ and $\pi-\pi$ interactions are depicted by black, green and cyan dashed lines, respectively. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S16: Relationship between the size of the substituent in position 4 of the phenylacetyl moiety and the inactivation constants of the inhibitors 6a and 15a-e



Plots of $\lg \left(K_{\text {inact }} / K_{1}\right)=\mathrm{f}(v)$ using the mean values of the inactivation constants (Table 9 within the main article) and the following values for $v(\AA)^{13-15}: 0.00(\mathrm{H}), 0.27(\mathrm{~F}), 0.52\left(\mathrm{CH}_{3}\right), 0.55(\mathrm{Cl}), 0.65$ $(\mathrm{Br})$ und 0.78 ( I$)$. The regression analysis was performed by linear regression.

Figure S17: Relationship between the hydrophobicity of the substituents in position 4 of the phenylacetyl moiety and the inactivation constants of the inhibitors 6a, 15a-e


Plots of $\lg \left(k_{\text {inact }} / K_{1}\right)=\mathrm{f}(\pi)$ using the mean values of the inactivation constants (Table 9 within the main article) and the following values for $\pi^{16}: 0.00(\mathrm{H}), 0.14(\mathrm{~F}), 0.55\left(\mathrm{CH}_{3}\right), 0.71(\mathrm{Cl}), 0.86(\mathrm{Br})$ und 1.12 (I). The regression analysis was performed by linear regression.

Figure S18: Influence of the 6-nitropyridin-3-yl moiety compared to the 6-methylpyridine-2-yl moiety on the inhibition of hTGase 2


Data shown are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. When not apparent, error bars are too small for the representation. For calculation of factors $f$, mean values of the inactivation constants were used.

Figure S19: Further $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyllysines obtained by combination of different pyridylpiperazinyl and $N^{\alpha}$ residues


Figure S20: Kinetic characterisation of $\boldsymbol{N}^{\boldsymbol{E}}$-propionyllysines 21a and 21b

A) Typical time courses of the hTGase 2-catalysed hydrolysis of Z-Glu(HMC)-Gly-OH ( $35 \mu \mathrm{M}$, $\approx 5.3 \times K_{\mathrm{m}}$ ) in the presence of different concentrations of propionylamide 21b ( $0 \mu \mathrm{M}(\mathrm{O}), 10 \mu \mathrm{M}$ $(\triangle), 20 \mu \mathrm{M}(+), 40 \mu \mathrm{M}(\times), 80 \mu \mathrm{M}(\diamond)$ and $100 \mu \mathrm{M}(\nabla))$. B) Secondary plot of initial rates versus concentration of $\mathbf{2 1 b}$ with nonlinear regression to the data for determination of $K_{i}^{c}$ (assuming competitive inhibition). Data shown in $\mathbf{B}$ are mean values ( $\pm$ SEM) of two separate experiments, each performed in duplicate. Conditions: $\mathrm{pH}=6.5,30^{\circ} \mathrm{C}, 5 \% \mathrm{DMSO}, 500 \mu \mathrm{M}$ TCEP, $3 \mu \mathrm{~g} / \mathrm{ml} \mathrm{hTGase} 2$.

Figure S21: Molecular modelling of hTGase $2(A)$ and gpTGase 2 (B)

hTGase 2 and gpTGase 2 are shown in gray surface with relevant residues colored and numbered. Pocket two is highlighted in yellow in both. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S22: Molecular modelling of the interaction of gpTGase 2 with $6 \mathrm{a}(\mathrm{A})$ and 15e (B)

gpTGase 2 is shown in gray surface with relevant residues colored by atom type, and inhibitors are shown in sticks and colored by atom type. Intermolecular H bonds and $\pi-\pi$ interactions are depicted by black and cyan dashed lines, respectively. Figure generated in Maestro (Schrödinger). ${ }^{10}$

Figure S23: Expression of TGase 2 in A375 and MeWo cells


TGase 2 expression of melanoma cell lines A375 and MeWo determined by Western Blotting. Cell cultivation, cell lysis, SDS-PAGE and Western blotting were performed as described previously ${ }^{17-18}$ and the following antibodies were used: primary antibody anti-TGase 2 (monoclonal mouse, ab2386 from Abcam, dilution 1:500) and secondary antibody anti-mouse IgG POD (polyclonal rabbit, A9044 from Sigma-Aldrich, dilution 1:2000).

Figure S24: Determination of hTGase 2-activity in cell lysates


B

|  | A375 | A375 <br> $\mathbf{+ 6 a}$ | A375 <br> - DMC | MeWo | MeWo <br> $\mathbf{+ 6 a}$ | MeWo <br> -DMC |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{v}(\mathrm{mA} / \mathrm{s}) * 10^{3}$ | 20.71 | 1.42 | 3.31 | 2.26 | 1.94 | 2.26 |
|  | $(0.44)$ | $(1.36)$ | $(0.69)$ | $(0.64)$ | $(0.16)$ | $(0.21)$ |

A) Plots of FA over time for the reaction of R-I-Cad $(0.81 \mu \mathrm{M})$ with DMC $(30 \mu \mathrm{M})$ in the presence of lysates ( 2 g protein/l) from A375 and MeWo cells. To ensure that the FA increase over time mainly originate from the hTGase 2-catalysed reaction of R-I-Cad with DMC, control measurements in the presence of the selective inhibitor $\mathbf{6 a}(10 \mu \mathrm{M}, 10 \mathrm{~min}$ precincubation time of inhibitor and lysate) and in the absence of DMC were performed. Conditions: $\mathrm{pH}=8.0,30^{\circ} \mathrm{C}$, $3 \mathrm{mM} \mathrm{CaCl}, 5 \%$ DMSO. B) Summary of rates for FA increase under different conditions for lysates from A375 and MeWo cells. Data shown in B are mean values ( $\pm$ SD) of two separate experiments, each performed in duplicate (or single measurement for -DMC).

Figure S25: Determination of $\mathrm{IC}_{50}$ values of selected inhibitors towards hTGase $\mathbf{2}$ using A375 cell lysates


Plots of rate versus inhibitor concentration for the reaction of DMC $(30 \mu \mathrm{M})$ with R-I-Cad $(0.81 \mu \mathrm{M})$ using lysates from A375 cells (2 g protein/l). The inhibitor concentration, [I], causing $50 \%$ inhibition, $\mathrm{IC}_{50}$, and the Hill slope, $\mathrm{n}_{\mathrm{H}}$, were calculated according to the equation

$$
\text { rate }=\text { Bottom }+\frac{(\text { Top }- \text { Bottom }) \times[I]^{\mathrm{nH}}}{[I]^{\mathrm{nH}}+\mathrm{IC}_{50}{ }^{\mathrm{nH}}}
$$

with Bottom and Top representing the lower and upper plateaus of the sigmoid dose-response curve, respectively. Data shown are mean values ( $\pm$ SEM) of two to three separate experiments, each performed in duplicate. Conditions: $\mathrm{pH}=8.0,30^{\circ} \mathrm{C}, 3 \mathrm{mM} \mathrm{CaCl} 2,5 \% \mathrm{DMSO}^{2}$.

Figure S26: Analysis of potential metabolites of compound 7b

m/z 482.3

Enhanced-product ion (EPI) mass spectra of original compound 7b


Analytical RP-HPLC chromatogram ( $@ 235 \mathrm{~nm}$ ) of the mixture obtained after incubation of compound $\mathbf{7 b}$ with mouse liver microsomes for 60 min . Potential structures of metabolites (M1-M6, see below) were identified by their EPI mass spectra considering the EPI mass spectra of $\mathbf{7 b}$.


M2


M1, M3


M4*


M5*, M6

Structures of metabolites M1-M6 ( $\mathrm{m} / \mathrm{z} 498.3\left[\mathrm{M}_{7 \mathrm{~b}}+\mathrm{O}+\mathrm{H}\right]^{+}$) formed by MLM incubation of 7b, *assignment not completely proven.

Figure S27:Influence of preincubation time on the activity of hTGase 3


Plots of RFU $\mathrm{f}(\mathrm{t})$ for the enzymatic hydrolysis ( pH 6.5 and $30^{\circ} \mathrm{C}$ in the presence of $5 \%$ DMSO and $500 \mu \mathrm{M}$ TCEP) of Z-Glu(HMC)-Gly-OH ( $80 \mu \mathrm{M}$ ) by hTGase $3(3 \mu \mathrm{~g} / \mathrm{mL})$ after different preincubation times of enzyme at $30^{\circ} \mathrm{C}$. Linear progress curves are obtained after 30 min of preincubation, while shorter times of preincubation result in hysteretic curve progressions (probably because of the increasing activity of hTGase 3 with time).

## Table S1:Summary of the determined and calculated physico-

 chemical parameters of the $\boldsymbol{N}^{\mathrm{E}}$-acryloyllysines| cpd. | cp $K^{\text {a }}{ }^{\text {a,b }}$ | $\operatorname{clog}{ }^{\text {a }}$ | $\boldsymbol{\operatorname { l o g }} \mathrm{D}_{7.4}{ }^{\text {c }}$ | $\operatorname{clog}_{7.4}{ }^{\text {a }}$ | $\mathbf{P e}_{\mathbf{e}(7.5)}(\mathrm{nm} / \mathrm{s})^{d}$ | $\mathrm{R}_{\mathrm{M}}(\%)^{e}$ | CHI $\mathrm{IAM}_{7.4}{ }^{\text {t }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6a | 9.1 | 1.37 | - | -0.36 | 87.7 (3.9) | -1 | 29.4 |
| 6b | 9.1 | 1.37 | - | -0.36 | 101 (7.0) | 3 | - |
| 7a | 8.4 | 1.15 | - | 0.05 | 61.3 (8.2) | 2 | - |
| 7b | 2.8 | 1.27 | 2.10 | 1.27 | 133 (19) | 14 | 31.8 |
| 7c | 4.8 | 1.91 | - | 1.91 | 152 (10) | 29 | - |
| 7d | 4.0 | 2.03 | - | 2.03 | 125 (22) | 34 | - |
| 7e | 5.0 | 2.20 | - | 2.20 | 231 (36) | 48 | - |
| 7f | 8.9 | 2.77 | - | 1.31 | 219 (28) | 69 | 39.5 |
| 7 g | 7.6 | 2.79 | - | 2.33 | 219 (57) | 64 | 39.0 |
| 7h | 6.7 | 1.62 | - | 1.54 | 117 (21) | 24 | - |
| 7i | 0.6 | 1.02 | - | 1.02 | 91.6 (8.1) | 10 | 30.7 |
| 8a | 7.0 | 1.17 | - | 1.13 | 12.9 (0.9) | -7 | - |
| 8b | 1.3 | 1.05 | 1.66 | 1.05 | 35.4 (3.2) | 4 | 26.8 |
| 8c | 2.3 | 1.81 | 2.13 | 1.81 | 89.8 (3.1) | 11 | 31.8 |
| 8d | -2.5 | 1.09 | - | 1.09 | 17.8 (2.1) | -14 | - |
| 8 e | 6.4 (1.5) ${ }^{g}$ | 1.18 | - | -1.55 | 3.1 (0.2) | -8 | - |
| 8 f | 3.4 | 1.22 | - | 1.22 | 10.7 (1.3) | 2 | - |
| 8 g | 4.3 | 0.51 | - | 0.51 | 3.2 (0.9) | -7 | - |
| 9 | $3.0{ }^{\text {h }}$ | 1.87 | - | 1.87 | 98.8 (17.5) | -2 | - |
| 10 | 10.7 | 1.15 | - | -0.37 | 5.1 (0.8) | 31 | 25.4 |
| 11 | $3.2{ }^{\text {h }}$ | 2.29 | - | 2.29 | 168 (32) | 6 | - |
| 12 | $1.5^{h}$ | 1.88 | - | 1.88 | 128 (20) | 22 | - |
| 13a | - | 1.18 | 1.60 | 1.18 | 30.5 (5.2) | -3 | 25.0 |
| 13b | - | 0.99 | - | 0.99 | 28.1 (4.4) | 1 | - |
| 13c | $6.2{ }^{\text {i }}$ | 1.99 | 2.65 | 1.98 | 103 (13.4) | 12 | 32.0 |
| 13d | 0.8 | 1.12 | - | 1.12 | 39.9 (23.3) | -15 | - |
| 13e | $4.1^{j}$ | 3.08 | - | 3.08 | 76.8 (4.8) | 38 | 37.7 |
| 14a | 9.1 | 0.68 | - | -1.05 | 39.7 (11.5) | 10 | - |
| 14b | 9.1 | 1.41 | - | -0.32 | 99.7 (13.2) | 14 | - |
| 14c | 9.1 | 1.41 | - | -0.32 | 125 (31) | 11 | - |
| 14d | 9.1 | 2.44 | - | 0.71 | 172 (20) | 24 | - |
| 14e | 9.1 | 1.40 | - | -0.36 | 150 (20) | 25 | - |
| 14 f | 9.1 | 3.11 | - | 1.34 | 156 (18) | 66 | - |
| 14 g | 9.1 | 1.42 | - | -0.26 | 163 (14) | 32 | - |
| 14h | 9.1 | 1.51 | 0.47 | -0.17 | 141 (17) | 39 | 34.3 |
| 14k | 9.1 | 1.65 | 0.28 | -0.10 | 127 (27) | 16 | 31.4 |
| 141 | 9.1 | 2.11 | - | 0.36 | 69.8 (3.2) | 51 | - |
| 14m | 9.1 (7.8) ${ }^{\text {k }}$ | 2.05 | - | -0.04 | 71.9 (8.5) | 44 | - |
| 15a | 9.1 | 1.87 | - | 0.14 | 252 (47)* | 5 | - |
| 15b | 9.1 | 1.41 | 0.36 | -0.32 | 114 (7.0) | 10 | 32.4 |
| 15c | 9.1 | 2.11 | - | 0.38 | 123 (5.0)* | 8 | - |
| 15d | 9.1 | 2.02 | - | 0.29 | 142 (25)* | 25 | - |
| 15e | 9.1 | 2.26 | - | 0.53 | 81.1 (2.3) | 34 | - |
| $15 f$ | 9.1 | 1.41 | - | -0.32 | 114 (9.0) | 21 | 30.1 |
| 15 g | 9.1 | 1.41 | - | -0.32 | 125 (32) | 24 | 30.9 |
| 16c | 7.6 | 2.27 | - | 2.22 | 111 (23) | 75 | - |


| cpd. | $\mathbf{c p} K_{\text {a }}{ }^{\text {a,b }}$ | $\operatorname{clog} \mathrm{P}^{\text {a }}$ | $\log \mathrm{D}_{7.4}{ }^{\text {c }}$ | $\operatorname{clog}^{\mathrm{D}_{7.4}{ }^{\text {a }}}$ | $\mathbf{P e}_{\mathrm{e}(7.5)}(\mathrm{nm} / \mathbf{s})^{d}$ | RM (\%) ${ }^{\text {e }}$ | CHI IAM ${ }_{7.4}{ }^{\text {f }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 17a | -2.5 | 0.96 | - | 0.96 | 11.3 (3.7) | 17 | - |
| 17b | -2.5 | 1.04 | 1.04 | 1.04 | 19.7 (3.1) | 9 | 28.3 |
| 17c | -2.5 | 1.17 | 1.17 | 1.17 | 40.8 (5.7) | 12 | 28.5 |
| 18 | 0.6 | 1.04 | - | 1.04 | 87.4 (5.4) | 12 | 30.7 |
| 19 | 10.7 | 1.79 | - | 0.27 | 11.6 (0.8) | 21 | - |
| 20a | 2.8 | 1.25 | - | 1.25 | 172 (15) | 56 | - |
| 20b | 1.3 | 1.01 | - | 1.01 | 63.4 (4.2) | 5 | - |
| 21a | 9.1 | 1.78 | - | 0.05 | 98.4 (33.3) | 7 | - |
| 21b | -2.5 | 1.24 | - | 1.24 | 16.7 (1.1) | 12 | - |

Fluorinated inhibitors are coloured in blue. ${ }^{a}{ }^{\mathrm{Cp}} \mathrm{K}_{\mathrm{a}}, ~ c \log \mathrm{P}$ and $\operatorname{clog}_{7.4}$ values were calculated using Advance Chemistry Development (ACD/Lab) Software V14.0.0 (© 1994-2016 ACD/Labs). clogP values were calculated as „Consensus log ${ }^{19}$ and the $\mathrm{cp} K_{a}$ values were calculated bsed on the „Classic" algorithm ${ }^{20}$. Calculation of the clog $\mathrm{D}_{7,4}$ values in turn is based on the obtained clog P and $\mathrm{cp} K_{a}$ values ${ }^{21}$. If not otherwise stated, the $\mathrm{cp} K_{\mathrm{a}}$ value corresponds to the pKa value of the respective pyridinium ion. "Data shown are values of one determination. ${ }^{d}$ Data shown are mean values ( $\pm$ SD) of one experiment which was performed in triplicate or mean values ( $\pm$ SEM) of 2-4 separate experiments, each performed in triplicate. ${ }^{e}$ Data shown are mean values. ${ }^{\text {f }}$ Data shown are values of one determination. ${ }^{9}$ Value in brackets corresponds to the carboxyl group. ${ }^{h}$ Value corresponds to the anilinium ion. Value corresponds to the piperazinium ion. Value corresponds to the dimethylammonio group of the dansyl moiety. K Values in brackets corresponds to the $\alpha$-ammonio group.

## Chemistry

## General

All commercial reagents and solvents were used without further purification unless otherwise specified. Melting points were determined on a Galen III Boetius apparatus from Cambridge Instruments. Nuclear magnetic resonance spectra were recorded on an Agilent Technologies 400 MR spectrometer ( 400 MHz for ${ }^{1} \mathrm{H}, 101 \mathrm{MHz}$ for ${ }^{13} \mathrm{C}$ and 376 MHz for ${ }^{19} \mathrm{~F}$ ). Spectra were processed by using the programme MestreNova (version 6.1.1-6384). ${ }^{22}$ NMR chemical shifts were referenced to the residual solvent resonances relative to tetramethylsilane (TMS; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ ) and trichlorofluoromethane $\left(\mathrm{CFCl}_{3} ;{ }^{19} \mathrm{~F}\right)$. Mass spectra (ESI) were obtained on a Waters Xevo TQ-S mass spectrometer driven by the Mass Lynx software.

## Chromatography

Thin-layer chromatography (TLC) was performed on Merck silica gel F-254 aluminium plates with visualisation under UV ( 254 nm ) and/or staining with a $0.1 \%(\mathrm{w} / \mathrm{V})$ ninhydrin solution in ethanol. Preparative column chromatography was carried out on Merck silica gel (mesh size 230-400 ASTM) with solvent mixtures as specified for the particular compounds. For purification of the compounds, analytical and preparative HPLC were performed on a Varian Prepstar system equipped with a UV detector (Prostar, Varian). Two Microsorb C18 60-8 columns (Varian Dynamax $250 \times 4.6 \mathrm{~mm}$ and $250 \times 21.4 \mathrm{~mm}$ ) were used as the stationary phases for analytical and preparative RP-HPLC, respectively. A binary gradient system of $0.1 \% \mathrm{CF}_{3} \mathrm{COOH} /$ water (solvent A) and $0.1 \% \mathrm{CF}_{3} \mathrm{COOH} / \mathrm{CH}_{3} \mathrm{CN}$ (solvent B) at a flow rate of $1 \mathrm{~mL} / \mathrm{min}$ or $10 \mathrm{~mL} / \mathrm{min}$ served as the eluent. With regards to analytical RP-HPLC, the programme for elution of the compounds was a gradient from low to high percentage of solvent B with a slope of $2 \%$ per min. An appropriate range of concentrations were used for each specific compound. With regards to preparative RP-HPLC, similar conditions for the gradient elution of the compounds were applied as for analytical RP-HPLC but with a slope of $1 \%$ per min. HPLC for determining the enantiomeric composition of compounds $\mathbf{6 a}$ and $\mathbf{6 b}$ was carried out with a system consisting of a Merck Hitachi L7100 gradient pump combined with a Jasco DG2080 four-line degasser with UV detection by a Merck Hitachi L7450 diode array detector. The system was operated with D-700 HSM software and use of a Merck Hitachi D7000 interface. A Chiralpak ${ }^{\circledR}$ IA column ( $250 \times 4.6 \mathrm{~mm}, 5 \mu \mathrm{~m}$ ) served as stationary phase. Separation of the enatiomers was achieved by an isocratic solvent mixture of $5 \%$ hexane $/ \mathrm{CH}_{3} \mathrm{CN}$ at a flow rate of $1 \mathrm{~mL} / \mathrm{min}$.

## General synthetic procedures

## GP I: General procedure for Benzoylpiperazines

A solution of the respective benzoyl chloride ( $1.53 \mathrm{mmol}, 0.95$ eq.) in the given solvent ( 3 mL ) was added dropwise to a solution of 1-tert-butoxycarbonylpiperazine ( $300 \mathrm{mg}, 1.61 \mathrm{mmol}$, 1 eq.) and TEA ( $450 \mu \mathrm{~L}, 3.22 \mathrm{mmol}, 2 \mathrm{eq}$.) in the same solvent. The reaction mixture was stirred for 2 h . Subsequently, the solvent was removed in vacuo and the residue was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(10 \mathrm{~mL})$. The solution was washed with $1 \mathrm{M} \mathrm{HCl}(2 \times 5 \mathrm{~mL}), 0.1 \mathrm{M} \mathrm{NaOH}(2 \times 5 \mathrm{~mL})$ and brine ( $1 \times 5 \mathrm{~mL}$ ). The organic phase was dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated to obtain the desired products in sufficient purity for further reactions.

## GP II: General procedure for Buchwald-Hartwig amination

The procedure is based on the protocol of Yin and Buchwald. ${ }^{23}$ To a solution of 1-tertbutoxycarbonylpiperazine ( $500 \mathrm{mg}, 2.68 \mathrm{mmol}, 1$ eq.) in dry THF ( 10 mL ) under Ar atmosphere was added $\mathrm{Cs}_{2} \mathrm{CO}_{3}(2.13 \mathrm{~g}, 8.04 \mathrm{mmol}, 3 \mathrm{eq}$.). The resulting suspension was stirred for 1 min followed by the addition of the respective halogen-substituted pyridine derivative ( $2.68 \mathrm{mmol}, 1 \mathrm{eq}$.), Xantphos ( $93.2 \mathrm{mg}, 0.16 \mathrm{mmol}, 0.06 \mathrm{eq}$.) and $\mathrm{Pd}_{2}(\mathrm{dba})_{3}$ ( $49.1 \mathrm{mg}, 0.05 \mathrm{mmol}, 0.02$ eq.) in the given order. The reaction mixture was stirred at $70^{\circ} \mathrm{C}$ under Ar atmosphere over night. Afterwards, the solvent was removed in vacuo and the residue dissolved in ethyl acetate was washed with 0.03 M sodium diethyldithiocarbamate ( $5 \times 15 \mathrm{~mL}$ ) and brine ( $1 \times 15 \mathrm{~mL}$ ). The organic phase was filtered through celite, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. The crude product was purified by column chromatography with the respective solvent mixtures given for each compound.

## GP III: General procedure for Boc removal from piperazine derivatives

The Boc-protected piperazine building block was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(10 \mathrm{~mL})$. Subsequently, TFA ( 10 mL ) was added slowly under stirring. The reaction mixture was stirred for 3 h . The volatile components were removed in an $\mathrm{N}_{2}$ stream. The residue was dissolved in water ( 2 mL ) and the pH of the solution was adjusted to 14 with 4 M NaOH . The alkaline solution was extracted with $\mathrm{CH}_{2} \mathrm{Cl}_{2}(5 \times 10 \mathrm{~mL})$. The organic phases were combined, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated to afford the respective free amine.

Due to the possible hydrolysis of the methyl ester group, alkaline extraction for compound 31 was omitted.

Before alkaline extraction, compound $\mathbf{3 0}$ was purified by preparative RP-HPLC. The productcontaining fractions were combined and dried by lyophilisation.

## GP IV: General procedure for acylation of N-hydroxysuccinimide

The general procedure is based on the method for the synthesis of $N$-acryloxysuccinimide (1a) described by Bergbreiter et al. ${ }^{24}$. To a suspension of $N$-hydroxysuccinimide ( $5.0 \mathrm{~g}, 43.0 \mathrm{mmol}$, 1 eq.) in $\mathrm{CHCl}_{3}(30 \mathrm{~mL})$ was added TEA ( $6.66 \mathrm{~mL}, 48.0 \mathrm{mmol}, 1.1$ eq.). The resulting solution was cooled to $0^{\circ} \mathrm{C}$ in an ice bath and subsequently the respective acyl chloride $(43.0 \mathrm{mmol}$, 1 eq.) was added dropwise over 10 min . The reaction mixture was stirred at $0^{\circ} \mathrm{C}$ for 10 min and then at room temperature for 1 h . Afterwards, the reaction mixture was washed with water $(1 \times 15 \mathrm{~mL})$ and brine $(1 \times 15 \mathrm{~mL})$ and the organic phase was dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$. Further specific instructions are given below for the respective N -hydroxysuccinimide esters.

## GP V: General procedure for $N^{\varepsilon}$-acylation

To a solution of $N^{a}$-Boc-lysine ( $1.0 \mathrm{~g}, 4.06 \mathrm{mmol}, 1 \mathrm{eq}$. ) and TEA ( $1.69 \mathrm{~mL}, 12.18 \mathrm{mmol}, 3 \mathrm{eq}$.) in methanol ( 20 mL ) was added the respective $N$-hydroxysuccinimide ester ( 687 mg , $4.06 \mathrm{mmol}, 1$ eq.) in small portions. The mixture was stirred for 2 h followed by removal of the solvent in vacuo.

## GP VI: General procedure for amide coupling

To a solution of the respective $N^{\alpha}$-Boc- $N^{\varepsilon}$-acyllysine ( $0.50 \mathrm{mmol}, 1$ eq.) and DIPEA ( $174 \mu \mathrm{~L}$, $1.00 \mathrm{mmol}, 2$ eq.) in THF ( 3 mL ) was added the respective piperazine building block ( 0.50 mmol , 1 eq.). Subsequently, PyBOP ( $312 \mathrm{mg}, 0.60 \mathrm{mmol}, 1.2$ eq.) was added and the reaction mixture was stirred for 3 h . The solvent was removed in vacuo and the residue was dissolved in ethyl acetate ( 10 mL ). The organic phase was washed with saturated $\mathrm{NaHCO}_{3}$ $(2 \times 5 \mathrm{~mL})$ and brine ( 5 mL ), followed by drying over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporation.

GP VII: General procedure for Boc removal from the lysine piperazides
The $N^{a}$-Boc- $N^{\varepsilon}$-acyllysine piperazide was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(5 \mathrm{~mL})$. Subsequently, TFA ( 5 mL ) was added slowly under stirring. The reaction mixture was stirred for 3 h . The volatile components were removed in an $\mathrm{N}_{2}$ stream. The residue was dissolved in a mixture of wateracetonitrile 3:1 ( 2 mL ) and the solution was lyophilised. The yields were quantitative.

GP VIII: General procedure for coupling of activated carboxylic acid derivatives
To a solution of the respective free amine ( $0.19 \mathrm{mmol}, 1 \mathrm{eq}$.) and TEA ( $106 \mu \mathrm{~L}, 0.76 \mathrm{mmol}$, 4 eq.) in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ ( 4 mL ) was added the respective activated carboxylic acid derivative ( $0.19 \mathrm{mmol}, 1$ eq.). The reaction mixture was stirred for 3 h . Afterwards, $\mathrm{CH}_{2} \mathrm{Cl}_{2}(10 \mathrm{~mL})$ was added and the organic phase was washed with saturated $\mathrm{NaHCO}_{3}(1 \times 5 \mathrm{~mL})$ and brine ( $1 \times 5 \mathrm{~mL}$ ), dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. The crude product was purified by preparative

RP-HPLC. The product-containing fractions were combined and lyophilised to afford the respective final inhibitors.

GP IX: General procedure for coupling of carboxylic acids
To a solution of the respective free amine ( $0.063 \mathrm{mmol}, 1$ eq.) and DIPEA ( $33 \mu \mathrm{~L}, 0.188 \mathrm{mmol}$, 3 eq.) in DMF ( 2 mL ) was added a solution of the respective carboxylic acid ( 0.063 mmol , 1 eq.) and DIPEA ( $11 \mu \mathrm{~L}, 0.063 \mathrm{mmol}, 1$ eq.) in DMF ( 1 mL ). After 1 min of stirring PyBOP ( $32.5 \mathrm{mg}, 0.063 \mathrm{mmol}, 1$ eq.) was added. The reaction mixture was stirred for 4 h . The solvent was removed in vacuo and the residue was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(10 \mathrm{~mL})$. The organic phase was washed with saturated $\mathrm{NaHCO}_{3}(6 \mathrm{~mL})$ and brine ( 6 mL ), dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. The crude product was purified by preparative RP-HPLC. The product-containing fractions were combined and lyophilised to afford the respective final inhibitors.

## N -Acyloxysuccinimide building blocks 1

## N -Acryloxysuccinimide (1a) ${ }^{24-25}$



The synthesis was accomplished according to GP IV using acryloyl chloride ( 43.0 mmol ). After drying over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ the organic phase was concentrated to approx. 20 mL of $\mathrm{CHCl}_{3}$ in vacuo. The solution was cooled to $0^{\circ} \mathrm{C}$ in an ice bath, followed by the addition of hexane ( 10 mL ) and ethyl acetate ( 2 mL ). The mixture was stirred at $0^{\circ} \mathrm{C}$ for 45 min . The emerging precipitate was filtered off and washed with mixtures of ethyl acetate and hexane ( $1 \times 10 \mathrm{~mL} 4: 1,1 \times 10 \mathrm{~mL} 9: 1$ ) and hexane $(1 \times 10 \mathrm{~mL})$. To the filtrate was added hexane and the emerging precipitate was filtered off again and washed as described above. The solids were combined and after drying under oil pump vacuum compound $\mathbf{1 a}(6.03 \mathrm{~g}, 83 \%)$ was obtained as an off-white solid. $R_{\mathrm{f}} 0.62$ (ethyl acetate-acetic acid 99:1); mp: 67-69 ${ }^{\circ} \mathrm{C}\left(\mathrm{lit} .{ }^{25}\right) 67-69{ }^{\circ} \mathrm{C}$ ); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=6.70(\mathrm{dd}$, ${ }^{3} \mathrm{~J}=17.3 \mathrm{~Hz},{ }^{2} \mathrm{~J}=0.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 6.33 (dd, ${ }^{3} \mathrm{~J}=17.3 \mathrm{~Hz},{ }^{3} \mathrm{~J}=10.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.17 (dd, ${ }^{3} \mathrm{~J}=10.7 \mathrm{~Hz},{ }^{2} \mathrm{~J}=0.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $2.86\left(\mathrm{~s}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=169.14$ $(2 \times \mathrm{CO}), 161.18(\mathrm{COO}), 136.31\left(\mathrm{CH}_{2}=\mathrm{C}\right), 123.11\left(\mathrm{CH}_{2}=\mathrm{C}\right), 25.77\left(2 \times \mathrm{CH}_{2}\right),{ }^{1} \mathrm{H}-$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature. ${ }^{24-25}$
$N$-Propionyloxysuccinimide (1b) ${ }^{26-27}$


The synthesis was accomplished according to GP IV using propionyl chloride ( 17.4 mmol ). After drying over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ the organic phase was evaporated to afford a colourless oil which solidified during storage in the refrigerator. After drying under oil pump vacuum compound 1b ( $1.91 \mathrm{~g}, 64 \%$ ) was obtained as a white solid. $R_{\mathrm{f}} 0.48$ (ethyl acetate-petroleum ether $1: 1$ ); mp: $43-45{ }^{\circ} \mathrm{C}$ (lit. ${ }^{26} 44-46{ }^{\circ} \mathrm{C}$ ); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=2.84\left(\mathrm{~s}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of pyrrolidine), $2.65\left(\mathrm{q},{ }^{3} \mathrm{~J}=7.5\right.$ $\mathrm{Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}$ of propionyl), $1.28\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ ); ${ }^{1} \mathrm{H}-\mathrm{NMR}$ data are in agreement with those reported in literature. ${ }^{27}$

## $N^{\alpha}$-Boc- $N^{k}$-acyllysines 2

$\boldsymbol{N}^{\alpha}$-Boc- $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine (2a) ${ }^{28}$


The synthesis was accomplished according to GP V using $N^{a}$-Boc-L-lysine ( 4.06 mmol ) and $N$ acryloxysuccinimide. The crude product was purified via column chromatography (gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-isopropanol-acetic acid 95:5+2 to 92:8+2). The product-containing fractions were combined and evaporated. Residual acetic acid was removed by repeated addition and evaporation of toluene ( $3 \times 10 \mathrm{~mL}$, boiling point of the azeotropic mixture acetic acid-toluene (mole fractions $0.375 / 0.625$ ) at $100.7^{\circ} \mathrm{C}$ ) ${ }^{29}$. After drying under oil pump vacuum compound $\mathbf{2 a}$ ( $609 \mathrm{mg}, 50 \%$ ) was obtained as a colourless oil. $R_{\mathrm{f}} 0.12\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-isopropanol-acetic acid 95:5+2); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=6.29\left(\mathrm{~d},{ }^{3} \mathrm{~J}=16.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 6.18-6.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right.$, $\mathrm{N}_{\varepsilon} \mathrm{H}$ ), $5.65\left(\mathrm{~d},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 5.33-5.24\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 4.28\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.39-3.30$ ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.94-1.80 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.80-1.66 (m, 1H, $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.64-1.52 (m, 2H, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.50-1.38 (m, 11H, $\left.3 \times \mathrm{CH}_{3}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=175.67(\mathrm{COOH}), 166.38\left(\mathrm{CON}_{\varepsilon}\right), 156.11$ $\left(\mathrm{CON}_{\mathrm{a}}\right), 130.74\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 127.02\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 80.40$ (quart. C of Boc), $53.25\left(\mathrm{C}_{\alpha}\right), 39.36\left(\mathrm{C}_{\varepsilon}\right)$, $32.05\left(\mathrm{C}_{\beta}\right), 28.96\left(\mathrm{C}_{\bar{\delta}}\right), 28.48\left(3 \times \mathrm{CH}_{3}\right), 22.53\left(\mathrm{C}_{\gamma}\right)$; $\mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{14} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{NaO}_{5}$ : $323.16[\mathrm{M}+\mathrm{Na}]^{+}$, found: 323.1.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {E }}$-Acryloyl-D-lysine (2b)



The synthesis was accomplished according to GP V using $N^{a}$-Boc-D-lysine ( 0.81 mmol ) and $N$-acryloxysuccinimide. The crude product was directly used for the next step.

## $N^{\alpha}$-Boc- $N^{k}$-Propionyl-L-Iysine (2c) ${ }^{30}$



The synthesis was accomplished according to GP V using $N^{a}$-Boc-L-lysine ( 1.46 mmol ) and $N$ propionyloxysuccinimide. The crude product was purified via column chromatography $\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}-\right.$ methanol-acetic acid 95:5+2). The product-containing fractions were combined and evaporated. Residual acetic acid was removed by repeated addition and evaporation of toluene ( $3 \times 10 \mathrm{~mL}$ ). After drying under oil pump vacuum compound 2c ( $418 \mathrm{mg}, 95 \%$ ) was obtained as a reddish oil. $R_{\mathrm{f}} 0.23\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol-acetic acid 95:5+2); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=5.73$ (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.27 (broad s, $1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 4.28 (broad s, $1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.26 (broad s, $2 \mathrm{H}, \mathrm{C}_{8} \mathrm{H}_{2}$ ), 2.26-2.16 (m, 2H, $\mathrm{CH}_{2}-\mathrm{CH}_{3}$ ), 1.87 (broad s, $1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}$ ), 1.74 (broad s, $1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.60-1.49 (m, 2H, C ${ }_{\delta} \mathrm{H}_{2}$ ), $1.45\left(\mathrm{~s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}\right), 1.15\left(\mathrm{t}, 3 \mathrm{H},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, \mathrm{CH}_{2} \mathrm{CH}_{3}\right)$; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=175.04(\mathrm{COOH}), 174.81\left(\mathrm{CON}_{\varepsilon}\right), 156.14\left(\mathrm{CON}_{\mathrm{a}}\right), 80.36$ (quart. C of Boc), $53.41\left(\mathrm{C}_{\alpha}\right) 39.19\left(\mathrm{C}_{\varepsilon}\right), 31.97\left(\mathrm{C}_{\beta}\right), 29.89\left(\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$, $29.16\left(\mathrm{C}_{\bar{\delta}}\right), 28.49\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.41$ $\left(\mathrm{C}_{\mathrm{Y}}\right), 10.07\left(\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: m/z calculated for $\mathrm{C}_{14} \mathrm{H}_{26} \mathrm{~N}_{2} \mathrm{NaO}_{5}: 325.17[\mathrm{M}+\mathrm{Na}]^{+}$, found: 325.1.

## Piperazine building blocks 3

## 1-(6-Methylpyridine-2-yl)piperazine (3a) ${ }^{31}$



The synthesis was accomplished according to Pavia et al. ${ }^{31}$ 2-Chloro-6-methylpyridine ( $2.12 \mathrm{~mL}, 19.4 \mathrm{mmol}, 3 \mathrm{eq}$.) was added to a solution of piperazine ( $5.0 \mathrm{~g}, 58 \mathrm{mmol}, 1 \mathrm{eq}$.) in 1butanol ( 20 mL ). The mixture was stirred at $130^{\circ} \mathrm{C}$ for 5 d . Afterwards, the solvent was removed in vacuo. The crude product was purified via column chromatography (gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 9:1 to 3:7). The product-containing fractions were combined and evaporated to afford $\mathbf{3 a}(2.05 \mathrm{~g}, 20 \%)$ as a red brown oil. $R_{\mathrm{f}} 0.05\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}-\right.$ methanol $\left.9: 1\right)$; ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=7.38\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.4,7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.52\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.43\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-3$ ), 3.62-3.56 ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{H}-2,6$ of piperazine), 3.09-3.05 ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{H}-3,5$ of piperazine), 2.39 ( $\mathrm{s}, 3 \mathrm{H}, \mathrm{CH}_{3}$ ), signal for NH is not visible; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=159.20,157.04,137.96$ (C-4), 113.23 ( $\mathrm{C}-5$ ), 103.90 ( $\mathrm{C}-3$ ), 45.65 (C-2,6 of piperazine), 45.41 ( $\mathrm{C}-3,5$ of piperazine), 24.70 $\left(\mathrm{CH}_{3}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{10} \mathrm{H}_{16} \mathrm{~N}_{3}$ : $178.13[\mathrm{M}+\mathrm{H}]^{+}$, found: 178.2 .

As side product 1,4-Bis(6-methylpyridin-2-yl)piperazine was isolated ( 3.6 mg , buff solid). $R_{\mathrm{f}}$ $0.00\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}-\right.$ methanol 9:1); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.40\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.4,7.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-4\right), 6.52(\mathrm{~d}$, ${ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3$ ), $6.48\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-5\right)$, $3.67\left(\mathrm{~s}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right), 2.42\left(\mathrm{~s}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{3}\right)$; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=159.24,156.94,137.97(2 \times \mathrm{C}-4), 112.99(2 \times \mathrm{C}-5)$, $104.00(2 \times \mathrm{C}-3)$, $45.31\left(4 \times \mathrm{CH}_{2}\right), 24.67\left(2 \times \mathrm{CH}_{3}\right)$.

## 1-(6-Nitropyridin-2-yl)piperazine (3b) ${ }^{32}$



The synthesis was accomplished according to Swanson et al. ${ }^{32}$. A suspension of 3-bromo-6nitropyridine ( $2.0 \mathrm{~g}, 9.85 \mathrm{mmol}, 1 \mathrm{eq}$.) in acetonitrile ( 5 mL ) was added to a suspension of piperazine ( $2.29 \mathrm{~g}, 26.6 \mathrm{mmol}, 2.7 \mathrm{eq}$.) in acetonitrile ( 15 mL ). The mixture was stirred at $95^{\circ} \mathrm{C}$ for 6 h . During this time, the suspension cleared off and subsequently a white precipitate was formed. Afterwards, the solvent was removed in vacuo and the orange residue was dissolved
in ethyl acetate ( 40 mL ). The solution was washed with $1 \mathrm{M} \mathrm{NaOH}(1 \times 60 \mathrm{~mL})$. Then, the organic phase was separated and the aequous phase was washed mit ethyl acetate $(4 \times 40 \mathrm{~mL})$. The combined organic layers were dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and the solvent was removed in vacuo. The crude product was purified via column chromatography $\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol- $\mathrm{N}, \mathrm{N}$ dimethylethylamine 9.5:0.5+0.1). The product-containing fractions were combined and evaporated to afford 3b ( $1.79 \mathrm{~g}, 87 \%$ ) as a yellow solid. $R_{\mathrm{f}} 0.30\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol $-\mathrm{N}, \mathrm{N}$ dimethylethylamine $8: 2+0.1$ ); mp: $138-142^{\circ} \mathrm{C}$; ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.16\left(\mathrm{~d},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-5$ ), 8.13 ( $\mathrm{d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 7.19 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $3.44-3.39$ ( m , $4 \mathrm{H}, \mathrm{H}-2,6$ of piperazine), $3.08-3.03\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{H}-3,5\right.$ of piperazine), $1.68(\mathrm{~s}, 1 \mathrm{H}, \mathrm{NH}),{ }^{1} \mathrm{H}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{32}{ }^{13} \mathbf{C}$-NMR $\left(\mathrm{CDCl}_{3}\right) \delta=150.32,147.88$, 133.89 (C-2), 120.58, 119.86, 47.89 (C-2,6 of piperazine), 45.70 (C-3.5 of piperazine); MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{9} \mathrm{H}_{13} \mathrm{~N}_{4} \mathrm{O}_{2}$ : $209.10\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 209.2.

Dansylpiperazine (3c) ${ }^{33-34}$


The synthesis was accomplished according to Sashuk et al. ${ }^{33}$. Dansylchloride ( 250 mg , $0.93 \mathrm{mmol}, 1$ eq.) and piperazine ( $479 \mathrm{mg}, 3.71 \mathrm{mmol}, 6$ eq.) were dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(30 \mathrm{~mL})$ and the mixture was stirred for 15 min . Afterwards, the reaction mixture was washed with satured $\mathrm{NaHCO}_{3}(3 \times 20 \mathrm{~mL})$ and the organic phase was separated, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated to obtain compound $\mathbf{3 c}$ ( $172 \mathrm{mg}, 58 \%$ ) as a light green solid in sufficient purity for further reactions. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.56\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of dansyl), 8.43 ( d , ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 8.19 (dd, ${ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 7.56-7.50 (m, $2 \mathrm{H}, 2 \times \mathrm{H}$ of dansyl), 7.19-7.17 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-6$ of dansyl), 3.19-3.12 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 2.90-2.85 (m, $10 \mathrm{H}, 2 \times \mathrm{CH}_{3}, 2 \times \mathrm{CH}_{2}$ ), 1.61 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{NH}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ): $\delta=151.83$ (C-5 of dansyl), 132.77 (quart. C of dansyl), 130.77 (CH of dansyl), 130.75 ( $\left.\mathrm{CH}_{\text {dansyl }}\right), 130.67$ (quart. C of dansyl), 130.22 (quart. C of dansyl), 128.08 (CH of dansyl), 123.27 (CH of dansyl), 119.98 (CH of dansyl), 115.35 (C-6 of dansyl), $46.59\left(2 \times \mathrm{CH}_{2}\right), 45.65\left(2 \times \mathrm{CH}_{3}\right), 45.58\left(2 \times \mathrm{CH}_{2}\right)$, NMR data are in agreement with those reported in literature, ${ }^{33-34} \mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{16} \mathrm{H}_{22} \mathrm{~N}_{3} \mathrm{O}_{2} \mathrm{~S}$ : $320.14[\mathrm{M}+\mathrm{H}]^{+}$, found: 320.1.

## 4-tert-Butoxycarbonyl-1-(6-fluoropyridin-2-yl)piperazine (Boc-3d) ${ }^{35}$



The synthesis was accomplished according to Prante et al. ${ }^{35}$. 2,6 Difluoropyridine ( $394 \mu \mathrm{~L}$, $4.35 \mathrm{mmol}, 1$ eq.) was added to a solution of 1 -tert-butoxycarbonylpiperazine $(0.81 \mathrm{~g}$, $4.34 \mathrm{mmol}, 1$ eq.) and TEA ( $903 \mu \mathrm{~L}, 6.52 \mathrm{mmol}, 1.5 \mathrm{eq}$.) in DMF ( 3 mL ). The mixture was stirred at $150{ }^{\circ} \mathrm{C}$ for 24 h . Subsequently, saturated $\mathrm{NaHCO}_{3}(10 \mathrm{~mL})$ was added to the reaction mixture and the aequous phase was extracted with ethyl acetate ( $6 \times 20 \mathrm{~mL}$ ). The organic layer was dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and the solvent was removed in vacuo. The crude product was purified via column chromatography $\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate $\left.95: 5\right)$. The product-containing fractions were combined and evaporated to afford Boc-3d ( $550 \mathrm{mg}, 45 \%$ ) as a yellow oil. $R_{\mathrm{f}} 0.44\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}-\right.$ ethyl acetate 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.54$ (ps-q, $\left.\mathrm{J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.41$ (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=8.2 \mathrm{~Hz} \text {, }}$ ${ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.19 ( $\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=7.7 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), $3.52\left(\mathrm{~s}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ ), $1.48\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right),{ }^{1} \mathrm{H}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{35}{ }^{13} \mathrm{C}$ NMR ( $\mathrm{CDCl}_{3}$ ) $\delta=162.91$ ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=236.3 \mathrm{~Hz}, \mathrm{C}-6$ ), 158.23 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=16.0 \mathrm{~Hz}, \mathrm{C}-2$ ), 154.90 (C=O of Boc), 142.18 (d, $\left.{ }^{3} J_{C, F}=8.3 \mathrm{~Hz}, \mathrm{C}-4\right), 103.10\left({ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=} 3.6 \mathrm{~Hz}, \mathrm{C}-3\right), 96.77$ (d, $\left.{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=37.2 \mathrm{~Hz}, \mathrm{C}-5\right), 80.24$ (quart. C of Boc), $45.04\left(4 \times \mathrm{CH}_{2}\right), 28.57\left(3 \times \mathrm{CH}_{3}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=-68.41$ (d, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}=8.3 \mathrm{~Hz}) ; \mathrm{MS}(E S I+): ~ m / z ~ c a l c u l a t e d ~ f o r ~} \mathrm{C}_{14} \mathrm{H}_{20} \mathrm{FN}_{3} \mathrm{NaO}_{2}: 304.14[\mathrm{M}+\mathrm{Na}]^{+}$, found: 304.2.

## 1-(6-Fluoropyridin-2-yl)piperazine (3d) ${ }^{35}$



Compound 3d (332 mg, orange red, highly fluid oil) was synthesised according to GP III using compound Boc-3d. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.52$ (ps-q, ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.40 (dd, $\left.{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=}=8.2 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right), 6.16\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=7.7,{ }^{3} \mathrm{~J}_{\mathrm{H}, F}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 3.55-3.46$ ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{H}-2,6$ of piperazine), $3.00-2.92$ ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{H}-3,5$ of piperazine), signal for NH is not visible, ${ }^{1} \mathrm{H}$-NMR data are in agreement with those reported in literature, ${ }^{35}{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=162.93$ ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=235.6 \mathrm{~Hz}, \mathrm{C}-6$ ), 158.71 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=15.8 \mathrm{~Hz}, \mathrm{C}-2$ ), $141.96\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.3 \mathrm{~Hz}\right.$, C-4), 102.77 ( $d,{ }^{4} J_{\mathrm{C}, \mathrm{F}}=4.0 \mathrm{~Hz}, \mathrm{C}-3$ ), 96.17 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=37.5 \mathrm{~Hz}, \mathrm{C}-5$ ), 46.08 (C-2,6 of
piperazine), 45.81 ( $\mathrm{C}-3,5$ of piperazine); ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ : $\delta=-68.54$ (broad s); MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{9} \mathrm{H}_{13} \mathrm{FN}_{3}$ : $182.11[\mathrm{M}+\mathrm{H}]^{+}$, found: 182.2.

## 4-tert-Butoxycarbonyl-1-(4-fluorobenzoyl)piperazine (Boc-3e) ${ }^{36}$



Compound Boc-3e ( $390 \mathrm{mg}, 83 \%$ ) was synthesised according to GP I using 4-fluorobenzoyl chloride and $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ as solvent and was obtained as a white solid. $R_{\mathrm{f}} 0.08\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate
 $\left.{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=}{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=8.6 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 3.85-3.25\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right), 1.47\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ $\left(\mathrm{CDCl}_{3}\right) \delta=169.84(\mathrm{CO}), 163.67\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=250.4 \mathrm{~Hz}, \mathrm{C}-4\right), 154.68(\mathrm{COO}), 131.59\left(\mathrm{~d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.5\right.$ $\mathrm{Hz}, \mathrm{C}-1$ ), 129.55 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.5 \mathrm{~Hz}, \mathrm{C}-2,6$ ), 115.85 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.8 \mathrm{~Hz}, \mathrm{C}-3,5$ ), 80.57 (quart. C of Boc), $28.52\left(3 \times \mathrm{CH}_{3}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): \delta=-$ 109.79-109.95 (m); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{16} \mathrm{H}_{2} \mathrm{FN}_{2} \mathrm{NaO}_{3}$ : $331.14[\mathrm{M}+\mathrm{Na}]^{+}$, found: 330.9 .

## 1-(4-Fluorobenzoyl)piperazine (3e) ${ }^{37}$



Compound 3 e ( 244 mg , white solid) was synthesised according to GP III using compound Boc-3e. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.44-7.38(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6), 7.13-7.05(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5), 3.87-3.28$ ( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ ), $3.03-2.74\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 1.77(\mathrm{~s}, 1 \mathrm{H}, \mathrm{NH}) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=169.65(\mathrm{CO})$, 163.51 ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=249.6 \mathrm{~Hz}, \mathrm{C}-4$ ), 132.01 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.5 \mathrm{~Hz}, \mathrm{C}-1$ ), 129.48 ( $\mathrm{d},{ }^{3}{ }^{\mathrm{J}} \mathrm{C}, \mathrm{F}=8.6 \mathrm{~Hz}, \mathrm{C}-$ 2,6 ), 115.71 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.8 \mathrm{~Hz}, \mathrm{C}-3,5$ ), signals for $4 \times \mathrm{CH}_{2}$ are not visible, ${ }^{1} \mathrm{H}-$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{37}{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-110.43-110.52(\mathrm{~m})$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{11} \mathrm{H}_{14} \mathrm{FN} \mathrm{N}_{2} \mathrm{O}: 209.11[\mathrm{M}+\mathrm{H}]^{+}$, found: 209.2.

## 4-tert-Butoxycarbonyl-1-(4-nitrobenzoyl)piperazine (Boc-3f) ${ }^{38}$



Compound Boc-3f ( 320 mg , 62\%) was synthesised according to GP I using 4-nitrobenzoyl chloride and THF as solvent and was obtained as a yellow solid. $R_{\mathrm{f}} 0.16\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.29\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 7.57\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6\right), 3.84-$ $3.25\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), $1.47\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc$) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=168.35$ (CO), 154.57 (COO), 148.65, 141.67, 128.23 (C-2,6), 124.15 (C-3,5), 80.78 (quart. C of Boc), $47.53\left(\mathrm{CH}_{2}\right)$, $42.27\left(\mathrm{CH}_{2}\right), 28.49\left(3 \times \mathrm{CH}_{3}\right)$, signals for $2 \times \mathrm{CH}_{2}$ of piperazine are not visible; MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{16} \mathrm{H}_{21} \mathrm{~N}_{3} \mathrm{NaO}_{5}: 358.14[\mathrm{M}+\mathrm{H}]^{+}$, found: 358.1.

1-(4-Nitrobenzoyl)piperazine (3f) ${ }^{37}$


Compound $\mathbf{3 f}$ ( 204 mg , rose solid) was synthesised according to GP III using compound Boc3f. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.31-8.25(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5), 7.59-7.54(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6), 3.85-3.70(\mathrm{~m}$, 2H, CH2 ), 3.41-3.27 (m, 2H, CH2), 3.03-2.90 (m, 2H, CH2 $)$, 2.89-2.77 (m, 2H, CH ${ }_{2}$ ), 1.67 (s, $1 \mathrm{H}, \mathrm{NH}) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=168.16(\mathrm{CO}), 148.50,142.21,128.18(\mathrm{C}-2,6), 124.05(\mathrm{C}-3,5)$, $48.99\left(\mathrm{CH}_{2}\right), 46.63\left(\mathrm{CH}_{2}\right), 46.01\left(\mathrm{CH}_{2}\right), 43.50\left(\mathrm{CH}_{2}\right),{ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{37} \mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{11} \mathrm{H}_{14} \mathrm{~N}_{3} \mathrm{O}_{3}: 236.10[\mathrm{M}+\mathrm{H}]^{+}$, found: 236.2.

## 4-tert-Butoxycarbonyl-1-(4-fluorobenzyl)piperazine (Boc-3g) ${ }^{39}$



The synthesis was accomplished according to the general procedure for reductive alkylation described by Abdel-Magid and Mehrman. ${ }^{40}$ To a solution of 1-tert-butoxycarbonylpiperazine ( $500 \mathrm{mg}, 2.68 \mathrm{mmol}, 1 \mathrm{eq}$.) in THF ( 10 mL ) under $\mathrm{N}_{2}$ atmosphere was added 4fluorobenzaldehyde ( $283 \mu \mathrm{~L}, 2.68 \mathrm{mmol}, 1$ eq.) and sodium triacetoxyborohydride ( 795 mg ,
$3.75 \mathrm{mmol}, 1.4$ eq.). The reaction mixture was stirred for 5 h . Subsequently, the solvent was removed, the residue was dissolved in $3 \mathrm{M} \mathrm{NaOH}(10 \mathrm{~mL})$ and the solution was extracted with ethyl acetate ( $3 \times 20 \mathrm{~mL}$ ). The organic phases were combined, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. The crude product was purified by column chromatography (gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}-$ methanol 99.5:0.5 to $97.5: 2.5$ ). The product-containing fractions were combined and evaporated to afford Boc-3g ( $334 \mathrm{mg}, 42 \%$ ) as a light yellow liquid. $R_{\mathrm{f}} 0.11\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 98:2); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.30-7.25(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6), 7.00\left(\mathrm{t},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 3.47(\mathrm{~s}$, $2 \mathrm{H}, \mathrm{CH}_{2}$-fluorophenyl), $3.45-3.39\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), $2.41-2.34\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), $1.45\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=162.24\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=245.1 \mathrm{~Hz}, \mathrm{C}-4\right), 154.91$
 79.76 (quart. C of Boc), $62.35\left(\mathrm{CH}_{2}\right.$-Fluorphenyl), $52.93\left(\mathrm{CH}_{2}\right.$ of piperazine), $28.58\left(3 \times \mathrm{CH}_{3}\right)$, signals for $3 \times \mathrm{CH}_{2}$ of piperazine are not visible, ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{39}{ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ): $\delta=-115.50-116.06$ ( m ); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{16} \mathrm{H}_{24} \mathrm{FN}_{2} \mathrm{O}_{2}$ : $295.18[\mathrm{M}+\mathrm{H}]^{+}$, found: 295.2.

## 1-(4-Fluorobenzyl)piperazine (3g) ${ }^{39}$



Compound $\mathbf{3 g}$ ( 211 mg , oange oil) was synthesised according to GP III using compound Boc3g. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.30-7.25(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6), 7.02-6.96(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5), 3.45\left(\mathrm{~s}, 2 \mathrm{H}, \mathrm{CH}_{2}-\right.$ fluorophenyl), 2.92-2.86 (m, $4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), 2.47-2.34 (m, $4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), 1.83 (s, 1H, NH); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=162.14$ ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=244.8 \mathrm{~Hz}, \mathrm{C}-4$ ), 133.90 ( d , ${ }^{4} J_{\mathrm{C}, \mathrm{F}}=3.1 \mathrm{~Hz}, \mathrm{C}-1$ ), 130.76 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.9 \mathrm{~Hz}, \mathrm{C}-2,6$ ), 115.12 ( $\mathrm{d},{ }^{2}{ }^{\mathrm{J}} \mathrm{C}, \mathrm{F}=21.2 \mathrm{~Hz}, \mathrm{C}-3,5$ ), 62.95 $\left(\mathrm{CH}_{2}\right.$-fluorophenyl), $54.38\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine $), 46.13\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine $),{ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$ NMR data are in agreement with those reported in literature; ${ }^{39}{ }^{19} \mathrm{~F}$-NMR $\left(\mathrm{CDCl}_{3}\right) \delta=-115.99-$ -116.05 (m); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{11} \mathrm{H}_{16} \mathrm{FN}_{2}: 195.13[\mathrm{M}+\mathrm{H}]^{+}$, found: 195.2.

## 4-tert-Butoxycarbonyl-1-(6-chloropicolinoyl)piperazine (Boc-3h)



Compound Boc-3h (135 mg, 19\%, brown oil) was synthesised according to GP II using 2-chloro-6-trichloromethylpyridine (nitrapyrin). Solvent for column chromatography: gradient
from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-ethyl acetate $9: 1$ to $8: 2 ; R_{\mathrm{f}} 0.12\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate $\left.9: 1\right) ;{ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=7.80-7.75(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4), 7.62\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right), 7.40\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz},{ }^{4} \mathrm{~J}=0.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\right.$ 5), 3.78-3.72 (m, 2H, CH2), 3.63-3.46 (m, 8H, $3 \times \mathrm{CH}_{2}$ ), $1.47\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right)$; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=166.04$ (CO Amid), 154.73 (CON of Boc), 154.01, 150.16, 139.92 (C-4), 125.69 (C-5), 122.90 (C-3), 80.48 (quart. C of Boc), $47.28\left(2 \times \mathrm{CH}_{2}\right), 42.62\left(2 \times \mathrm{CH}_{2}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right)$; MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{15} \mathrm{H}_{20} \mathrm{ClN}_{3} \mathrm{O}_{3}: 348.11\left[\mathrm{M}\left({ }^{35} \mathrm{Cl}\right)+\mathrm{Na}\right]^{+}$, found: 348.1.

## 1-(6-Chloropicolinoyl)piperazine (3h)



Compound $\mathbf{3 h}$ ( 70 mg , yellow oil) was synthesised according to GP III using compound Boc3h. NMR data were recorded prior to basic extraction ( $\mathbf{3 h} \times 2$ TFA). ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.98$ (s, 2H, NH ${ }_{2}{ }^{+}$), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 7.69-7.65(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5), 3.88-3.80\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{CH}_{2}\right)$, 3.69-3.61 (m, 2H, CH2), 3.27-3.09 (m, 4H, 2×CH2); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=165.01$ (CO), 153.06, 148.95, 141.08 (C-4), $125.68\left(\mathrm{CH}\right.$ of pyridine), $122.67\left(\mathrm{CH}\right.$ of pyridine), $43.51\left(\mathrm{CH}_{2}\right)$, $42.78\left(\mathrm{CH}_{2}\right)$, $42.45\left(\mathrm{CH}_{2}\right), 38.59\left(\mathrm{CH}_{2}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-79.79\left(\mathrm{~s}, \mathrm{CF}_{3}\right.$ of TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{10} \mathrm{H}_{13} \mathrm{ClN}_{3} \mathrm{O}$ : $226.07[\mathrm{M}+\mathrm{H}]^{+}$, found: 226.0.

## 4-tert-Butoxycarbonyl-1-pyridin-3-ylpiperazine (Boc-3i) ${ }^{41}$



Compound Boc-3i (263 mg, 69\%, yellow solid) was synthesised according to GP II using 3bromopyridine. Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 98:2 to 92:8; $R_{\mathrm{f}} 0.58\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 92:8); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.32-8.30(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-2), 8.14-8.11$ (dd, ${ }^{3} \mathrm{~J}=3.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6$ ), 7.19-7.16 (m, 2H, H-4,5), 3.61-3.58 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 3.18-3.14 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), $1.48\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right),{ }^{1} \mathrm{H}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{41}{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): \delta=154.76$ (CON), 147.08 (C-3), 141.31 (C-6), 139.13 (C-2), $123.69(\mathrm{CH}), 123.14(\mathrm{CH}), 80.24$ (quart. C of Boc), $48.84\left(4 \times \mathrm{CH}_{2}\right), 28.56\left(3 \times \mathrm{CH}_{3}\right)$; MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{14} \mathrm{H}_{22} \mathrm{~N}_{3} \mathrm{O}_{2}$ : $264.17[\mathrm{M}+\mathrm{H}]^{+}$, found: 264.1.

## 1-Pyridin-3-ylpiperazine (3i) ${ }^{42}$



Compound $\mathbf{3 i}$ ( 97 mg , orange oil) was synthesised according to GP III using compound Boc3i. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.31$ (dd, ${ }^{4} \mathrm{~J}=2.6,1.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), $8.10\left(\mathrm{dd},{ }^{3} \mathrm{~J}=4.0 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.9 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-6$ ), 7.20-7.13 (m, 2H, H-4/5), 3.21-3.15 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 3.07-3.02 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 1.73 (broad s, 1H, NH); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=147.54(\mathrm{C}-3), 140.89(\mathrm{C}-6), 138.83(\mathrm{C}-2), 123.59(\mathrm{CH})$, $122.51(\mathrm{CH}), 49.80\left(2 \times \mathrm{CH}_{2}\right), 46.09\left(2 \times \mathrm{CH}_{2}\right),{ }^{1} \mathrm{H}-$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{42} \mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{9} \mathrm{H}_{14} \mathrm{~N}_{3}$ : $164.12[\mathrm{M}+\mathrm{H}]^{+}$, found: 164.1

## 4-tert-Butoxycarbonyl-1-(6-fluoropyridin-3-yl)piperazine (Boc-3j)



Compound Boc-3j ( $391 \mathrm{mg}, 64 \%$, light yellow oil) was synthesised according to GP II using 3-bromo-6-fluoropyridine. Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-ethyl acetate $95: 5$ to $75: 25 ; R_{\mathrm{f}} 0.18\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate $\left.95: 5\right)$; ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ : $\delta=7.80$ (dd, $\left.{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=2.9 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=1.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 7.35$ (ddd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, F}=6.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=3.2 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{H}-4$ ), 6.84 (ddd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=}=8.8 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=3.6 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=0.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), $3.62-3.56$ (m, 4H, $2 \times \mathrm{CH}_{2}$ ), $3.11-3.05\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 1.48\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=158.34$ (d, $\left.{ }^{1} J_{\mathrm{C}, \mathrm{F}}=233.4 \mathrm{~Hz}, \mathrm{C}-6\right), 154.71$ (CO), 145.56 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=4.2 \mathrm{~Hz}, \mathrm{C}-3$ ), 135.67 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=15.0 \mathrm{~Hz}$, C-2), 130.27 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.5 \mathrm{~Hz}, \mathrm{C}-4$ ), 109.48 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=39.3 \mathrm{~Hz}, \mathrm{C}-5$ ), 80.33 (quart. C of Boc), $50.07\left(4 \times \mathrm{CH}_{2}\right), 28.56\left(3 \times \mathrm{CH}_{3}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-78.30(\mathrm{~s}) ; \mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{14} \mathrm{H}_{21} \mathrm{FN}_{3} \mathrm{O}_{2}$ : $282.16[\mathrm{M}+\mathrm{H}]^{+}$, found: 282.3.

## 1-(6-Fluoropyridin-3-yl)piperazine (3j)



Compound $3 \mathbf{j}$ ( 213 mg , brown oil) was synthesised according to GP III using compound Boc-3j. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.79$ (dd, ${ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=3.0 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=1.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 7.34 (ddd, $\left.{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=6.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=3.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.82\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.8 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, F}=3.4 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-5)$, 3.13-3.02 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ ); signal for NH is not visible; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=158.03(\mathrm{~d}$, $\left.{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=232.4 \mathrm{~Hz}, \mathrm{C}-6\right), 146.04\left(\mathrm{~d},{ }^{4}{ }_{\mathrm{C}, \mathrm{F},}=4.2 \mathrm{~Hz}, \mathrm{C}-3\right.$ ), 135.12 ( $\mathrm{d},{ }^{3}{ }_{\mathrm{C}, \mathrm{F}}=14.9 \mathrm{~Hz}, \mathrm{C}-2$ ), 129.60 (d, ${ }^{3} J_{\mathrm{C}, \mathrm{F}}=7.4 \mathrm{~Hz}, \mathrm{C}-4$ ), 109.30 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=39.2 \mathrm{~Hz}, \mathrm{C}-5$ ), 50.58 (C-2,6), 45.85 (C-3,5); ${ }^{19} \mathrm{~F}$ NMR ( $\mathrm{CDCl}_{3}$ ) $\delta=-79.25$ (s); MS (ESI+): m/z calculated for $\mathrm{C}_{9} \mathrm{H}_{13} \mathrm{FN}_{3}$ : $182.11[\mathrm{M}+\mathrm{H}]^{+}$, found: 182.2.

## 4-tert-Butoxycarbonyl-1-(6-trifluoromethylpyridin-3-yl)piperazine (Boc-3k) ${ }^{43}$



Compound Boc-3k ( $467 \mathrm{mg}, 53 \%$, white solid) was synthesised according to GP II using 3-bromo-6-trifluoromethylpyridine. Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ ethyl acetate 100:0 to 66:33; $R_{\mathrm{f}} 0.19\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.34$ (d, $\left.{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 7.53\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 7.20\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right)$, $3.64-3.58\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 3.32-3.26\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 1.49\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{1} \mathrm{H}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{43}{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=154.67$ (CO), 148.36 (C3), 138.39 ( $\mathrm{q},{ }^{2}{ }^{2} \mathrm{C}, \mathrm{F}=35.1 \mathrm{~Hz}, \mathrm{C}-6$ ), 137.81 (C-2), 122.19 (psd, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=272.4 \mathrm{~Hz}, \mathrm{CF}_{3}$ ), 121.43 (C-4), 121.03 ( $\mathrm{q},{ }^{3} \mathrm{~J}_{\mathrm{c}, \mathrm{F}=2} .6 \mathrm{~Hz}, \mathrm{C}-5$ ), 80.50 (quart. C of Boc), $47.60\left(2 \times \mathrm{CH}_{2}\right), 43.17\left(2 \times \mathrm{CH}_{2}\right)$, $28.55\left(3 \times \mathrm{CH}_{3}\right) ;{ }^{19} \mathrm{~F}$-NMR $\left(\mathrm{CDCl}_{3}\right)$ : $\delta=-66.80(\mathrm{~s}) ; \mathrm{MS}(\mathrm{ESI})$ : m/z calculated for $\mathrm{C}_{15} \mathrm{H}_{21} \mathrm{~F}_{3} \mathrm{~N}_{3} \mathrm{O}_{2}$ : $332.16[\mathrm{M}+\mathrm{H}]^{+}$, found: 332.2 .

## 1-(6-Trifluoromethylpyridin-3-yl)piperazine (3k) ${ }^{43}$



Compound $3 \mathbf{k}$ ( 275 mg , light yellow solid) was synthesised according to GP III using compound Boc-3k. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.34\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 7.51\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-5$ ), 7.18 (dd, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $3.31-3.26$ ( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ ), 3.08-3.02 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 1.73 (s, NH); ${ }^{1} \mathrm{H}-\mathrm{NMR}$ data are in agreement with those reported in literature; ${ }^{43}{ }^{13} \mathrm{C}$ NMR ( $\mathrm{CDCl}_{3}$ ): $\delta=148.80(\mathrm{C}-3), 137.84\left(\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.0 \mathrm{~Hz}, \mathrm{C}-6\right), 137.51(\mathrm{C}-2), 122.28(\mathrm{q}$, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=273.9 \mathrm{~Hz}, \mathrm{CF}_{3}$ ), $120.96\left(\mathrm{q},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=2.8 \mathrm{~Hz}\right), 120.90(\mathrm{C}-4), 48.51\left(2 \times \mathrm{CH}_{2}\right), 45.78\left(2 \times \mathrm{CH}_{2}\right)$; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): \delta=-66.74(\mathrm{~s})$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{10} \mathrm{H}_{13} \mathrm{~F}_{3} \mathrm{~N}_{3}: 232.11[\mathrm{M}+\mathrm{H}]^{+}$, found: 232.2.

## 4-tert-Butoxycarbonyl-1-(6-methoxycarbonylpyridin-3-yl)piperazine (Boc-3I)



Compound Boc-3I ( $347 \mathrm{mg}, 40 \%$, light yellow solid) was synthesised according to GP II using methyl-5-bromopicolinate. Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-ethyl acetate 100:0 to 66:33; $R_{\mathrm{f}} 0.07\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-ethyl acetate 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.34$ (d, ${ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 8.01 (d, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 7.15 (dd, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 3.96 (s, 3H, CH3O), 3.64-3.57 (m, 4H, 2×CH2), 3.38-3.31 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 1.48 (s, $9 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=165.83\left(\mathrm{COOCH}_{3}\right), 154.65(\mathrm{NCOO}), 148.60(\mathrm{C}-3), 137.78(\mathrm{C}-6), 137.06$ (C-2), 126.25 (C-5), 120.46 (C-4), 80.49 (quart. C of Boc), $52.62\left(\mathrm{CH}_{3} \mathrm{O}\right), 47.13\left(2 \times \mathrm{CH}_{2}\right)$, $43.04\left(2 \times \mathrm{CH}_{2}\right), 28.54\left(3 \times \mathrm{CH}_{3}\right)$; MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{16} \mathrm{H}_{24} \mathrm{~N}_{3} \mathrm{O}_{4}: 322.18[\mathrm{M}+\mathrm{H}]^{+}$, found: 322.2.

## 1-(6-Methoxycarbonylpyridin-3-yl)piperazinex2TFA (3I)



Compound $\mathbf{3 I}$ ( 475 mg , yellow solid) was synthesised according to GP III using compound Boc-3I. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right): \delta=8.87$ (broad s, $2 \mathrm{H}, \mathrm{NH}_{2}{ }^{+}$), 8.44 ( $\mathrm{d},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 7.93 (d, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 7.44 (dd, ${ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $3.82\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 3.63-$ $3.56\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 3.29-3.22\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right): \delta=164.97(\mathrm{CO}), 158.27$
 $120.53(\mathrm{C}-4), 51.92\left(\mathrm{CH}_{3}\right), 43.57\left(2 \times \mathrm{CH}_{2}\right), 42.32\left(2 \times \mathrm{CH}_{2}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right): \delta=-74.73(\mathrm{~s}$, $\mathrm{CF}_{3}$ of TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{11} \mathrm{H}_{16} \mathrm{~N}_{3} \mathrm{O}_{2}$ : $222.12[\mathrm{M}+\mathrm{H}]^{+}$, found: 222.2.

## 4-tert-Butoxycarbonyl-1-(6-nitropyridin-2-yl)piperazine (Boc-3m)



Compound Boc-3m (329 mg, 40\%, yellow solid) was synthesised according to GP II using 2-chloro-6-nitropyridine. Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-ethyl acetate 100:0 to 66:33; $R_{\mathrm{f}} 0.10\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right) ;{ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.72\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.4,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 7.47$ ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.91 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), $3.69-3.61\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ ), $3.60-3.51$ ( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ ), $1.49\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=157.79,155.96,154.83,140.49(\mathrm{C}-$ 4), 111.94 (C-5), $106.00(\mathrm{C}-3), 80.41$ (quart. C of Boc), $44.75\left(\mathrm{CH}_{2}\right), 28.55\left(3 \times \mathrm{CH}_{3}\right)$, signals for $3 \times \mathrm{CH}_{2}$ of piperazine are not visible; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{16} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{NaO}_{4}: 331.14$ [ $\mathrm{M}+\mathrm{Na}]^{+}$, found: 331.2.

## 1-(6-Nitropyridin-2-yl)piperazine (3m)



Compound $\mathbf{3 m}$ ( 167 mg , orange solid) was synthesised according to GP III using compound Boc-3m. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.69\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.5,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 7.43\left(\mathrm{~d},{ }^{3}{ }^{\mathrm{J}}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right)$, $6.89\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right), 3.65-3.60\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 3.02-2.95\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 1.63(\mathrm{~s}$, 1H, NH); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): \delta=158.16,156.06,140.22$ (C-4), 111.75 (C-5), 105.51 (C-3), 46.10-45.72 (4×CH2); MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{9} \mathrm{H}_{13} \mathrm{~N}_{4} \mathrm{O}_{2}$ : $209.10[\mathrm{M}+\mathrm{H}]^{+}$, found: 209.2.

## 2-Bromo-6-tert-butylpyridine (Br-3n) ${ }^{44}$



The synthesis was accomplished according to Hintermann et al. ${ }^{45}$ and Henrion et al. ${ }^{46}$. A mixture of 2,6-dibromopyridine ( $311 \mathrm{mg}, 1.31 \mathrm{mmol}, 1 \mathrm{eq}$. ) and Cul ( $10 \mathrm{mg}, 0.05 \mathrm{mmol}$, 0.04 eq.) in dry THF ( 4 mL ) was cooled to $0^{\circ} \mathrm{C}$ in an ice bath under Ar atmosphere. tertButylmagnesium chloride ( 1 M in THF, $1.97 \mathrm{~mL}, 1.97 \mathrm{mmol}, 1.5 \mathrm{eq}$.) was added dropwise over 30 min via a syringe through a septum. Afterwards, the ice bath was removed and the reaction mixture was allowed to warm up to room temperature. The reaction was stopped after an overall time of 3 h . After removal of the solvent in vacuo, the residue was suspended in saturated $\mathrm{NaHCO}_{3}(30 \mathrm{~mL})$ and extracted with $\mathrm{CH}_{2} \mathrm{Cl}_{2}(5 \times 5 \mathrm{~mL})$. The combined organic phases were washed with brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. The crude product was purified via column chromatography (gradient from petroleum ether-ethyl acetate 100:0 to 95:5). The product-containing fractions were combined and evaporated to afford $\mathrm{Br}-\mathbf{3 n}$ ( $181 \mathrm{mg}, 64 \%$ ) as a yellow liquid. $R_{\mathrm{f}} 0.80$ (petroleum ether-ethyl acetate $95: 5$ ); ${ }^{1} \mathrm{H}-\mathrm{NMR}$ $\left(\mathrm{CDCl}_{3}\right) \delta=7.44\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 7.28-7.23(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5), 1.34\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-$ NMR ( $\mathrm{CDCl}_{3}$ ) $\delta=171.38$ (C-6), $141.33(\mathrm{C}-2), 138.60(\mathrm{C}-4), 125.09(\mathrm{C}-3), 117.93(\mathrm{C}-5), 37.76$ (quart. C of tert-butyl), $30.13\left(3 \times \mathrm{CH}_{3}\right)$; NMR data are in agreement with those reported in literature; $\left.{ }^{44} \mathrm{MS}(\mathrm{ESI})^{+}\right)$: m/z calculated for $\mathrm{C}_{19} \mathrm{H}_{13} \mathrm{BrN}: 214.02\left[\mathrm{M}\left({ }^{79} \mathrm{Br}\right)+\mathrm{H}\right]^{+}$, found: 214.0.

## 4-tert-Butoxycarbonyl-1-(6-tert-butylpyridin-2-yl)piperazine (Boc-3n)



Compound Boc-3n (115 mg, 70\%, orange oil) was synthesised according to GP II using 2-bromo-6-tert-butylpyridine ( $\mathbf{B r}-\mathbf{3 n}$ ). Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}-$ methanol 100:0 to 98.5:1.5; $R_{\mathrm{f}} 0.52\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 99:1); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right): \delta=7.43(\mathrm{dd}$, ${ }^{3} \mathrm{~J}=8.3,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $6.68\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.45\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 3.54 (s, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ ), $1.48\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc ), $1.30\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of tert-butyl); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ : $\delta=167.83$ (C-6), 158.25 (C-2), 155.03(C, CON), 137.94 (C-4), 108.88 (C-5), $104.08(\mathrm{C}-3)$, 79.94 (quart. C of Boc), $45.39\left(4 \times \mathrm{CH}_{2}\right), 37.60$ (quart. C of tert-butyl), $30.22\left(3 \times \mathrm{CH}_{3}\right.$ of tertbutyl), $28.61\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$)$.

## 1-(6-tert-Butylpyridin-2-yl)piperazine (3n) ${ }^{31}$



Compound $3 \mathbf{n}$ ( 70 mg , colourless oil) was synthesised according to GP III using compound Boc-3n. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.42\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.3,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.66\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right)$, $6.43\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right), 3.56-3.47\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 3.05-2.93\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right), 1.71$ (broad $\mathrm{s}, 1 \mathrm{H}, \mathrm{NH}$ ), 1.31 (s, $9 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=167.56$ (C-6), 158.58 (C-2), 137.53 (C4), 108.28 (C-5), 103.47 (C-3), 46.34 (C-2,6 of piperazine), 45.93 (C-3,5 of piperazine), 37.42 (quart. C of tert-butyl), $30.06\left(3 \times \mathrm{CH}_{3}\right) ; \mathrm{MS}\left(E S I^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{13} \mathrm{H}_{2} \mathrm{~N}_{3}: 220.18[\mathrm{M}+\mathrm{H}]^{+}$, found: 220.2.

## 4-tert-Butoxycarbonyl-1-(6-(2-fluoroethoxy)pyridin-2-yl)piperazine (Boc-3o)



The synthesis was accomplished according to the general procedure for the preparation of fluoropyridyl ethers described by Debien et al. ${ }^{47}$. Sodium hydride ( $107 \mathrm{mg}, 4.44 \mathrm{mmol}, 5 \mathrm{eq}$.) and 2-fluoroethanol ( $259 \mu \mathrm{~L}, 4.44 \mathrm{mmol}, 5 \mathrm{eq}$.) were dissolved in DMSO ( 4 mL ) under Ar atmosphere. After 20 min of stirring, 4-tert-butoxycarbonyl-1-(6-fluoropyridin-2-yl)piperazine (Boc-3d, $250 \mathrm{mg}, 0.89 \mathrm{mmol}, 1 \mathrm{eq}$. ) was added and the reaction mixture was stirred at $100^{\circ} \mathrm{C}$ for 2 d . Afterwards, water ( 25 mL ) and $\mathrm{CH}_{2} \mathrm{Cl}_{2}(15 \mathrm{~mL})$ were added to the reaction mixture, the organic phase was separated and the aequous phase was repeatedly extracted with $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ $(5 \times 5 \mathrm{~mL})$. The combined organic phases were washed with brine $(1 \times 10 \mathrm{~mL})$, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. Due to similiar retention factors and retention times in TLC and RPHPLC analyses, the product was purified after removal of the Boc group (see compound 30). The crude product ( 265 mg ) contained compounds Boc-3o and Boc-3d in a ratio of 1:0.75 based on NMR integration. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.55\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=16.6 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.1 \mathrm{~Hz}, 0.75 \mathrm{H}\right.$, $\mathrm{H}-5$ pyridine ${ }_{\text {Boc-3d }}$ ), $7.43\left(\mathrm{t},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ pyridine ${ }_{\text {вос-30 }}$ ), $6.42\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.2 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.5\right.$ $\mathrm{Hz}, 0.75 \mathrm{H}, \mathrm{H}-4$ pyridine $_{\text {Boc-3d }}$ ), 6.23-6.12 ( $\mathrm{m}, 2+0.75 \mathrm{H}, \mathrm{H}-3 / 5$ pyridine $_{\text {вoc-30 }}, \mathrm{H}-3$ pyridine $_{\text {Boc- }}$ 3d), 4.81-4.65 (dm, $\left.{ }^{2} J_{\mathrm{H}, \mathrm{F}}=48.0 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right), 4.58-4.45\left(\mathrm{dm}, 2 \mathrm{H},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=28.0 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\right.$
 ${ }^{19}$ F-NMR ( $\mathrm{CDCl}_{3}$ ) $\delta=-68.37-$-68.45 ( $\mathrm{m}, \mathrm{F}_{\text {Boc-3d/Boc-30 }}$ ); MS (ESI $)$ : m/z calculated for $\mathrm{C}_{16} \mathrm{H}_{25} \mathrm{FN}_{3} \mathrm{O}_{2}$ : $326.19[\mathrm{M}+\mathrm{H}]^{+}$, found: 326.1.

## 1-(6-(2-Fluoroethoxy)pyridin-2-yl)piperazine (30)



Compound 30 ( $52 \mathrm{mg}, \mathbf{3 7 \%}$ starting from compound Boc-3d, yellow oil) was synthesised according to GP III using compound Boc-3o. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=7.45\left(\mathrm{t},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\right.$ 4), 6.30 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.05 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), $4.80-4.62$ ( $\mathrm{dm},{ }^{2} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=48.0 \mathrm{~Hz}$, $\left.2 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right)$, 4.49-4.36 (dm, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=32.0 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}$ ), 3.39-3.34 (m, $4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), $2.86-2.71$ ( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ): $\delta=161.43(\mathrm{C}-6)$,
157.99 (C-2), 140.50 (C-4), 98.49 (C-3), 97.43 (C-5), 82.18 (d, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=165.8 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}$ ), $64.09\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=19.0 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right), 45.53\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine $), 45.20\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine $)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{11} \mathrm{H}_{17} \mathrm{FN}_{3} \mathrm{O}: 226.14\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 226.1.

## 4-tert-Butoxycarbonyl-1-(6-iodopyridin-2-yl)piperazine (Boc-3p)



The synthesis was accomplished according to the general procedure for an aromatic Finkelstein reaction described by Klapars and Buchwald ${ }^{48}$. Under Ar atmosphere, TMEDA ( $17.2 \mu \mathrm{~L}, 0.11 \mathrm{mmol}, 0.2 \mathrm{eq}$.) was added to a suspension of 4-tert-butoxycarbonyl-1-(6-bromopyridin-2-yl)piperazine ( $200 \mathrm{mg}, 0.53 \mathrm{mmol}, 1$ eq.), Cul ( $12 \mathrm{mg}, 0.06 \mathrm{mmol}, 0.1 \mathrm{eq}$.) and $\mathrm{NaI}(351 \mathrm{mg}, 2.34 \mathrm{mmol}, 4$ eq.) in 1,4-dioxane ( 1 mL ). The reaction mixture was stirred at $100^{\circ} \mathrm{C}$ for 44 h . Afterwards, water ( 5 mL ) was added and the mixture was extracted with ethyl acetate $(3 \times 10 \mathrm{~mL})$. The organic phases were combined and dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$. Evaporation afforded Boc-3p ( $237 \mathrm{mg},>100 \%$, contains residual ethyl acetate) as a brown waxy solid. $R_{\mathrm{f}}$ 0.51 (petroleum ether-ethyl acetate 8:2); mp: $108-113^{\circ} \mathrm{C}$; ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.08-7.00(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{H}-4,5$ ), $6.52\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), $3.55-3.48\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right), 1.48\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-$ NMR ( $\mathrm{CDCl}_{3}$ ) $\delta=159.06(\mathrm{C}-2), 154.90(\mathrm{CO}), 138.84(\mathrm{C}-4), 123.86(\mathrm{C}-5), 116.37(\mathrm{C}-6)$, $105.54(\mathrm{C}-3), 80.20$ (quart. C of Boc ), $44.87\left(4 \times \mathrm{CH}_{2}\right), 28.57\left(3 \times \mathrm{CH}_{3}\right)$; MS (ESI $\left.{ }^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{14} \mathrm{H}_{21} 1 \mathrm{~N}_{3} \mathrm{O}_{2}$ : $390.07[\mathrm{M}+\mathrm{H}]^{+}$, found: 389.9.

## 1-(6-lodopyridin-2-yl)piperazine (3p) ${ }^{49}$



Compound 3 p ( 81 mg , dark yellow, waxy solid) was synthesised according to GP III using compound Boc-3p. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.06-6.97$ (m, 2H, H-4,5), 6.51 (dd, ${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}$, ${ }^{4} \mathrm{~J}=0.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 3.50-3.45 (m,4H,H-2,6 of piperazine), 2.97-2.92 (m, 4H, H-3,5 of piperazine); signal for NH is not visible; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=159.49$ ( $\mathrm{C}-2$ ), 138.70 (C-4), 123.41 (C-5), $116.46(\mathrm{C}-6), 105.31(\mathrm{C}-3), 46.12\left(2 \times \mathrm{CH}_{2}\right), 45.95\left(2 \times \mathrm{CH}_{2}\right),{ }^{1} \mathrm{H}-\mathrm{and}{ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature ${ }^{49}$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{9} \mathrm{H}_{13} \mathrm{IN}_{3}$ : $290.01\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 290.0.

## 4-tert-Butoxycarbonyl-1-(6-phenylpyridin-2-yl)piperazine (Boc-3q)



The synthesis was accomplished according to the procedure for Suzuki coupling reactions with $\beta$-chloroacroleins in aequous media described by Hesse and Kirsch. ${ }^{50} \mathrm{~A}$ suspension of 4 -tert-butoxycarbonyl-1-(6-bromopyridin-2-yl)piperazine ( $250 \mathrm{mg}, 0.73 \mathrm{mmol}, 1$ eq.), phenylboronic acid ( $98 \mathrm{mg}, 0.80 \mathrm{mmol}, 1.1 \mathrm{eq}$.), $\mathrm{Pd}(\mathrm{OAc})_{2}(3.3 \mathrm{mg}, 0.015 \mathrm{mmol}, 0.02 \mathrm{eq}$.$) , tetra- n-$ butylammonium chloride ( $235 \mathrm{mg}, 0.73 \mathrm{mmol}, 1$ eq.) and $\mathrm{K}_{2} \mathrm{CO}_{3}(252 \mathrm{mg}, 1.80 \mathrm{mmol}, 2.3 \mathrm{eq}$.) in water ( 5 mL ) was stirred at $100^{\circ} \mathrm{C}$ for 6 h . The solvent was removed in vacuo and the residue was dissolved in ethyl acetate ( 10 mL ), followed by washing with saturated $\mathrm{NaHCO}_{3}$ $(2 \times 5 \mathrm{~mL})$ and brine $(5 \mathrm{~mL})$. The organic phase was dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and the solvent was removed in vacuo. The crude product was purified by column chromatography (gradient from petroleum ether-ethyl acetate $95: 5$ to 80:20). The product-containing fractions were combined and evaporated to afford Boc-3q ( $147 \mathrm{mg}, 59 \%$ ) as a light yellow solid. $R_{\mathrm{f}} 0.40$ (petroleum ether-ethyl acetate $90: 10$ ); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.05-7.98(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2 / 6$ of phenyl), 7.57 (dd, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.46-7.41 (m, 2H, H-3/5 of phenyl), 7.40-7.35 (m, $1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 7.13 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.61 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 3.69-3.54 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ ), $1.50\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=159.02,155.40$, 155.02 (C-2, C-6 of pyridine, CON), 139.86 (C-1 of phenyl), 138.45 (C-4 of pyridine), 128.80 (C-4 of phenyl), 128.63 (C-3/5 of phenyl), 126.89 (C-2/6 of phenyl), 110.20 (C-5 of pyridine), 105.78 (C-3 of pyridine), 80.04 (quart. C of Boc), $45.25\left(4 \times \mathrm{CH}_{2}\right)$, $28.61\left(3 \times \mathrm{CH}_{3}\right)$; MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{20} \mathrm{H}_{26} \mathrm{~N}_{3} \mathrm{O}_{2}$ : $340.20[\mathrm{M}+\mathrm{H}]^{+}$, found: 340.2.

## 1-(6-Phenylpyridin-2-yl)piperazine (3q)



Compound $\mathbf{3 q}$ ( 81 mg , yellow oil) was synthesised according to GP III using compound Boc3q. ${ }^{1} \mathrm{H}$-NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.07-8.00(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6$ of phenyl), 7.66-7.57 (m, 1H, H-4 of pyridine), 7.49-7.42 (m, 2H, H-3,5 of phenyl), 7.41-7.36 (m, 1H, H-4 of phenyl), 7.24-7.19 (m, $1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.84-6.74 (m, 1H, H-3 of pyridine), 3.57-3.49 (m, 4H, $2 \times \mathrm{CH}_{2}$ ), 2.91-2.80
( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ ), signal for NH is not visible; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=158.78$ (C-2 of pyridine), 153.69 (C-6 of pyridine), 139.19 (C-1 of phenyl), 138.45 (C-4 of pyridine), 128.59 (C-4 of phenyl), 128.50 (C-3,5 of phenyl), 126.25 (C-2,6 of phenyl), 108.97 (C-5 of pyridine), 105.81 (C-3 of pyridine), $45.22\left(2 \times \mathrm{CH}_{2}\right), 45.10\left(2 \times \mathrm{CH}_{2}\right) ; \mathrm{MS}\left(\mathrm{ESI}^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{15} \mathrm{H}_{18} \mathrm{~N}_{3}$ : $240.15[\mathrm{M}+\mathrm{H}]^{+}$, found: 240.1.

## 1-(6-Bromoyridin-2-yl)piperazine (3r) ${ }^{49,35}$



Compound 3 r ( 98 mg , yellow oil) was synthesised according to GP III using 4-tert-Butoxycarbonyl-1-(6-bromopyridin-2-yl)piperazine. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \quad \delta=7.27$ (dd, ${ }^{3} \mathrm{~J}=8.4$, $7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4), 6.73\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 3.52-3.48(m, $4 \mathrm{H}, \mathrm{H}-2,6$ ), 2.98-2.93 (m, 4H, H-3,5); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=159.57$ (C-2), 140.37 (C-6), 139.56 (C-4), $116.07(\mathrm{C}-5), 104.85(\mathrm{C}-3), 46.16\left(2 \times \mathrm{CH}_{2}\right), 45.94\left(2 \times \mathrm{CH}_{2}\right),{ }^{1} \mathrm{H}-$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data are in agreement with those reported in literature; $49,35 \mathrm{MS}$ (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{9} \mathrm{H}_{13} \mathrm{Br} \mathrm{N}_{3}: 242.03\left[\mathrm{M}\left({ }^{79} \mathrm{Br}\right)+\mathrm{H}\right]^{+}$, found: 242.1.

## $\boldsymbol{N}^{\text {a }}$-Boc- $\boldsymbol{N}^{\text {E}}$-acyllysine piperazides 4

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{k}$-Acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide (4a)



Compound 4a ( $118 \mathrm{mg}, 46 \%$, colourless oil) was synthesised according to GP VI using compounds $2 \mathbf{a}$ and $\mathbf{3 a}$ ( 0.56 mmol$)$. Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 85:15; $R_{\mathrm{f}} 0.35$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.40$ (dd, ${ }^{3} \mathrm{~J}=8.3$, $7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $6.55\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.45\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 6.27 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.85 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.52\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), 4.66-4.59 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.83-3.47 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.42-3.25 (m,2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ of pyridine), 1.79-1.53 (m, 4H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), $1.49-1.41\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\mathrm{C}_{\mathrm{r}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.81$ (CON), $165.74\left(\mathrm{CON}_{\varepsilon}\right), 158.75,157.14,155.90$, 138.11 (C-4), $131.03\left(\mathrm{CH}_{2}=\mathrm{C}\right), 126.36\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $113.62(\mathrm{C}-5), 104.09(\mathrm{C}-3), 79.93$ (quart. C of Boc), $49.82\left(\mathrm{C}_{\alpha}\right)$, $45.58\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.44\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.40\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.01\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.37\left(\mathrm{C}_{\varepsilon}\right), 33.50\left(\mathrm{C}_{\beta}\right), 28.96\left(\mathrm{C}_{\delta}\right), 28.54\left(3 \times \mathrm{CH}_{3}\right.$ of $\mathrm{Boc})$, $24.67\left(\mathrm{CH}_{3}\right.$ of pyridine), $22.60\left(\mathrm{C}_{\mathrm{y}}\right)$; MS (ESI $)$ : $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{24} \mathrm{H}_{38} \mathrm{~N}_{5} \mathrm{O}_{4}: 460.29$ $[\mathrm{M}+\mathrm{H}]^{+}$, found: 460.3.

Product contains significant amounts of tris(pyrrolidinophosphine) oxide which was not removed by column chromatography. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=3.19-3.14\left(\mathrm{~m}, 6 \times \mathrm{CH}_{2} \mathrm{~N}\right)$, 1.84-1.79 ( $\mathrm{m}, 6 \times \mathrm{CH}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=46.45\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{p}}=4.5 \mathrm{~Hz}, 6 \times \mathrm{CH}_{2} \mathrm{~N}\right.$ ), $26.58\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{c}, \mathrm{P}=8.0 \mathrm{~Hz} \text {, }}\right.$ $6 \times \mathrm{CH}_{2}$ ).

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {}}$-Acryloyl-L-Iysine-4-(6-nitropyridin-3-yl)piperazide (4b)



Compound 4b ( $161 \mathrm{mg}, 27 \%$, yellow oil) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 b}(1.22 \mathrm{mmol})$. Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to $85: 15$; $R_{\mathrm{f}} 0.06$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.20(\mathrm{~d}$, ${ }^{3} \mathrm{~J}=9.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 8.15 (d, ${ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 7.24 (dd, ${ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.24 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.08 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.75 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.38 (d, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}$, $\left.\mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.64-4.56\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.97-3.44\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.44-3.25(m,2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.79-1.55 (m, 4H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.47-1.38 (m, 11H, $3 \times \mathrm{CH}_{3}$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}$-NMR $\left(\mathrm{CDCl}_{3}\right) \delta=171.19(\mathrm{CON}), 165.75\left(\mathrm{CON}_{\varepsilon}\right), 155.82\left(\mathrm{COON}_{\alpha}\right), 149.61,148.57,134.23,130.97$ $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 126.54\left(\mathrm{CH}_{2}=\mathrm{C}\right), 121.39,119.84,80.17$ (quart. C of Boc), $49.85\left(\mathrm{C}_{\mathrm{a}}\right), 47.01\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.65\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.71\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.41\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $39.04\left(\mathrm{C}_{\varepsilon}\right), 33.02\left(\mathrm{C}_{\beta}\right), 29.15\left(\mathrm{C}_{\delta}\right), 28.50\left(3 \times \mathrm{CH}_{3}\right.$ of Boc), $22.49\left(\mathrm{C}_{\gamma}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{~N}_{6} \mathrm{O}_{4}$ : $391.21[\mathrm{M}-\mathrm{Boc}+\mathrm{H}]^{+}$, found: 391.3 .

## $\boldsymbol{N}^{\text {a }}$-Boc- $\boldsymbol{N}^{\text {E }}$-Acryloyl-L-lysine-4-dansylpiperazide (4c)



Compound $\mathbf{4 c}$ ( $42 \mathrm{mg}, 27 \%$, yellow-green solid) was synthesised according to GP VI using compounds $2 \mathbf{a}$ and $3 \mathbf{c}(0.26 \mathrm{mmol})$. The crude product was purified by preparative RP-HPLC; ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.64\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of dansyl), $8.55\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of dansyl), $8.26\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of dansyl), 7.67-7.59 (m, 2H, $2 \times \mathrm{H}$ of dansyl), $7.38\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}\right.$,

H-6 of dansyl), 6.26 (dd, ${ }^{3} \mathrm{~J}=16.9 \mathrm{~Hz},{ }^{2} \mathrm{~J}=0.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH}_{2}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.3 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), $6.00\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 5.66$ (dd, ${ }^{3} \mathrm{~J}=10.3 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH}_{2}$ ), $5.38\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}$ ), 4.51-4.42 (m, 1H, $\mathrm{C}_{a} \mathrm{H}$ ), 3.83 (broad s, $1 \mathrm{H}, \mathrm{H}$ of piperazine), 3.63 (broad s, $1 \mathrm{H}, \mathrm{H}$ of piperazine), $3.50\left(\mathrm{~s}, 2 \mathrm{H}, 2 \times \mathrm{H}\right.$ of piperazine), 3.39-3.04 ( $\mathrm{m}, 12 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}, 2 \times \mathrm{CH}_{2}$ of piperazine, $2 \times \mathrm{CH}_{3}$ of dansyl), 1.62-1.24 (m, 15H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}, 3 \times \mathrm{CH}_{3}$ of Boc ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ ( $\mathrm{CDCl}_{3}$ ) $\delta=171.00(\mathrm{CCON}), 166.49\left(\mathrm{CON}_{\varepsilon}\right), 155.86\left(\mathrm{CON}_{\alpha}\right), 132.96$ (quart. C of dansyl), $131.37(\mathrm{CH}$ of dansyl), $130.49\left(\mathrm{CH}_{2}=C\right), 130.46$ (quart. C of dansyl), 130.04 ( CH of dansyl), 128.17 ( CH of dansyl), $127.23\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.61(\mathrm{CH}$ of dansyl), 122.13 ( CH of dansyl), 116.55 (C-6 of dansyl), 80.33 (quart. C of Boc$), 49.75\left(\mathrm{C}_{\alpha}\right), 46.02\left(2 \times \mathrm{CH}_{3}\right.$ of dansyl), $45.79\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.45\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.36\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.91\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.40\left(\mathrm{C}_{\varepsilon}\right)$, $32.99\left(\mathrm{C}_{\beta}\right), 28.76\left(\mathrm{C}_{\bar{\delta}}\right), 28.43\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.47\left(\mathrm{C}_{\mathrm{Y}}\right)$, signals for $\mathrm{C}-5$ and $1 \times \mathrm{C}_{\text {quart. }}$ of dansyl are not visible; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{30} \mathrm{H}_{44} \mathrm{~N}_{5} \mathrm{O}_{6} \mathrm{~S}: 602.30[\mathrm{M}+\mathrm{H}]^{+}$, found: 602.3.

## $\boldsymbol{N}^{\boldsymbol{a}}$-Boc- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-Acryloyl-L-lysine-4-(6-fluoropyridin-2-yl)piperazide (4d)



Compound 4d ( $205 \mathrm{mg}, 35 \%$, brown oil) was synthesised according to GP VI using compounds 2a and 3d ( 1.22 mmol ). Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to $85: 15$; $R_{\mathrm{f}} 0.19$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.57$ (ps-q, $\mathrm{J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $6.43\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.2 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, F}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 6.26 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz}$, ${ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.23 ( $\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=7.9 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.09 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0$, $10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.80 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.46\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.68-4.53\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.84-3.47\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.44-3.25 (m, 2H, C ${ }_{\varepsilon} \mathrm{H}$ ), 1.76-1.52 (m, 4H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.47-1.39 (m, 11H, $3 \times \mathrm{CH}_{3}$ of $\left.\mathrm{Boc}, \mathrm{C}_{\mathrm{y}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.94(\mathrm{CON}), 165.74\left(\mathrm{CON}_{\varepsilon}\right), 162.87(\mathrm{~d}$, $\left.{ }^{1} J_{\mathrm{C}, \mathrm{F}}=236.9 \mathrm{~Hz}, \quad \mathrm{C}-6\right), 157.95\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=15.5 \mathrm{~Hz}, \mathrm{C}-2\right), 155.86\left(\mathrm{COON}_{\mathrm{a}}\right), 142.32$ (d, $\left.{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.4 \mathrm{~Hz}, \mathrm{C}-4\right), 131.00\left(\mathrm{CH}_{2}=C\right), 126.42\left(\mathrm{CH}_{2}=\mathrm{C}\right), 103.04\left(\mathrm{~d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=4.1 \mathrm{~Hz}, \mathrm{C}-3\right), 97.19(\mathrm{~d}$, $\left.{ }^{2} J_{\mathrm{C}, \mathrm{F}}=37.2 \mathrm{~Hz}, \mathrm{C}-5\right), 80.00$ (quart. C of Boc), $49.84\left(\mathrm{C}_{\alpha}\right), 45.14\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), 44.93 $\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.75\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $39.28\left(\mathrm{C}_{\varepsilon}\right), 33.36\left(\mathrm{C}_{\beta}\right), 29.02\left(\mathrm{C}_{\bar{\delta}}\right), 28.52\left(3 \times \mathrm{CH}_{3}\right.$
of Boc), $22.55\left(\mathrm{C}_{\mathrm{Y}}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-68.27\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{HF}}=8.6 \mathrm{~Hz}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{35} \mathrm{FN}_{5} \mathrm{O}_{4}$ : $464.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 464.3.
$\boldsymbol{N}^{\boldsymbol{a}}$-Boc- $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(4-fluorobenzoyl)piperazide (4e)


Compound 4 e ( $31 \mathrm{mg}, 13 \%$, white solid) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 e}(0.50 \mathrm{mmol})$. Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 90:10; $R_{\mathrm{f}} 0.07$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.48-7.40$ ( m , 2H, H-2,6), 7.16-7.09 (m, 2H, H-3,5), 6.26 (dd, ${ }^{3}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 6.08 (dd, $\left.{ }^{3} J=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right), 5.77-5.69\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 5.63\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{C}=\mathrm{CHH}$ ), $5.39\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), 4.63-4.51(m,1H, CaH), 3.87-3.44(m,8H,4×CH20of piperazine), 3.43-3.25 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.50 (m, 4H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.47-1.36 (m, 11H, C $\mathrm{H}_{2}$, $3 \times \mathrm{CH}_{3}$ of Boc ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=171.11,169.86,165.72,163.82$ (d, $\left.{ }^{1} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=250.8 \mathrm{~Hz}, \mathrm{C}-4\right)$, 155.81 (COO), 131.15 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=} 3.5 \mathrm{~Hz}, \mathrm{C}-1$ ), 130.96 ( $\mathrm{CH}_{2}=C$ ), $129.68\left(\mathrm{~d},{ }^{3}{ }_{\mathrm{C}, ~} \mathrm{~F}=8.6 \mathrm{~Hz}, \mathrm{C}-2,6\right)$, $126.53\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.97\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.9 \mathrm{~Hz}, \mathrm{C}-3,5\right), 80.11$ (quart. C of Boc), $49.84\left(\mathrm{C}_{\mathrm{a}}\right), 45.67$ $\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), $42.29\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), $39.15\left(\mathrm{C}_{\varepsilon}\right), 33.15\left(\mathrm{C}_{\beta}\right), 29.11\left(\mathrm{C}_{\delta}\right), 28.51$ $\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$)$, $22.51\left(\mathrm{C}_{\gamma}\right)$; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-109.32--109.44(\mathrm{~m}) ; \mathrm{MS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{35} \mathrm{FN}_{4} \mathrm{NaO}_{5}$ : $513.25\left[\mathrm{M}+\mathrm{Na}^{+}\right.$, found: 513.3.
$N^{\text {a }}$-Boc- $\boldsymbol{N}^{\text {k }}$-Acryloyl-L-Iysine-4-(4-nitrobenzoyl)piperazide (4f)


Compound $\mathbf{4 f}$ ( $60 \mathrm{mg}, \mathbf{2 3 \%}$, white solid) was synthesised according to GP VI using compounds 2a and $\mathbf{3 f}$ ( 0.50 mmol ). Solvent for column chromatography: gradient from ethyl acetate-
methanol 100:0 to 90:10; $R_{\mathrm{f}} 0.24$ (ethyl acetate-methanol 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.31$ (d, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3,5$ ), 7.61 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2,6$ ), $6.31-6.18$ (m, 1H, C=CHH), 6.07 (dd, ${ }^{3} \mathrm{~J}=16.8,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.71 (broad s, $\left.1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 5.65-5.60(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}), 5.36(\mathrm{~d}$, $\left.{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 4.57\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 4.03-3.24\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right)$, 1.76$1.51\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right), 1.49-1.35\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of $\left.\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right)$, diffuse signals due to the amide bonds on both sides of the piperazine ring; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=171.20,168.39,165.74$, 155.79, 148.81, 141.26, $130.94\left(\mathrm{CH}_{2}=C\right)$, $128.35(\mathrm{C}-2,6), 126.58\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $124.22(\mathrm{C}-3.5)$, 80.17 (quart. C of Boc), $49.85\left(\mathrm{C}_{\alpha}\right), 39.06\left(\mathrm{C}_{\varepsilon}\right), 33.01\left(\mathrm{C}_{\beta}\right), 29.16\left(\mathrm{C}_{\delta}\right), 28.50\left(3 \times \mathrm{CH}_{3}\right.$ of Boc), $22.45\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine are not visible; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{35} \mathrm{~N}_{5} \mathrm{NaO}_{7}: 540.24[\mathrm{M}+\mathrm{Na}]^{+}$, found: 540.2.

## $N^{\text {a }}$-Boc- $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(4-fluorobenzyl)piperazide (4g)



Compound $\mathbf{4 g}$ ( $53 \mathrm{mg}, 22 \%$, colourless, waxy solid) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 g}$ ( 0.50 mmol$)$. Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 90:10; $R_{\mathrm{f}} 0.21$ (ethyl acetate-methanol 96:4); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=7.31-7.24(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6), 7.01\left(\mathrm{t},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 6.27\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $5.90-5.82\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 5.62$ (dd, $\left.{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 5.51\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.62-4.50\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right)$, 3.73-3.42 (m, 6H, $2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-fluorophenyl), 3.42-3.22 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 2.53$2.36\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), 1.72-1.48(m, $\left.4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.48-1.34\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.53,165.75,162.31\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=245.4 \mathrm{~Hz}, \mathrm{C}-4\right), 155.90$ (COO), $131.03\left(\mathrm{CH}_{2}=\mathrm{C}\right), 130.75\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=6.0 \mathrm{~Hz}, \mathrm{C}-2,6\right), 126.38\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.37(\mathrm{~d}$, $\left.{ }^{2} J_{\mathrm{C}, \mathrm{F}}=21.5 \mathrm{~Hz}, \mathrm{C}-3,5\right), 79.87$ (quart. C of Boc), $62.04\left(\mathrm{CH}_{2}\right.$-fluorophenyl), $53.04\left(\mathrm{CH}_{2}\right.$ of piperazine), $52.66\left(\mathrm{CH}_{2}\right.$ of piperazine), $49.68\left(\mathrm{C}_{\mathrm{a}}\right), 45.59\left(\mathrm{CH}_{2}\right.$ of piperazine $), 42.17\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.38\left(\mathrm{C}_{\varepsilon}\right)$, $33.48\left(\mathrm{C}_{\beta}\right), 28.89\left(\mathrm{C}_{\delta}\right), 28.52\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$)$, $22.54\left(\mathrm{C}_{\gamma}\right)$, signal for $\mathrm{C}-$ 1 is not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-115.18--115.64(\mathrm{~m})$; $\mathrm{MS}\left(E I^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{38} \mathrm{FN}_{4} \mathrm{O}_{4}$ : $477.29[\mathrm{M}+\mathrm{H}]^{+}$, found: 477.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\mathrm{E}}$-Acryloyl-L-Iysine-4-(6-chloropicolinoyl)piperazide (4h)



Compound 4 h ( $28 \mathrm{mg}, 18 \%$, yellow oil) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 h}(0.30 \mathrm{mmol})$. Solvent for column chromatography: $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 95:5; $R_{\mathrm{f}} 0.28$ $\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.80\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 7.67\left(\mathrm{pst},{ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{H}-3$ ), 7.42 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.34-6.19 (m, 1H, CH=CHH), 6.15-6.02 (m, 1H, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 5.77 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), $5.69-5.58(\mathrm{~m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}), 5.41\left(\mathrm{~d},{ }^{3}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right)$, $4.56\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right)$, 4.01-3.52 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.43-3.24 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.78-1.49 ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\boldsymbol{0}} \mathrm{H}_{2}$ ), 1.42 ( $\mathrm{s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), diffuse signals (partly in duplicate) due to the amide bonds on both sides of the piperazine ring; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=165.72$, 155.82, 153.57, $140.04(\mathrm{C}-4), 130.98\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 126.49\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 125.95(\mathrm{C}-5)$, 123.24/123.10 (C-3), 80.09 (quart. C of Boc ), $49.84\left(\mathrm{C}_{\alpha}\right), 47.44 / 47.13\left(\mathrm{CH}_{2}\right.$ of piperazine), 45.87/45.32 $\left(\mathrm{CH}_{2}\right.$ of piperazine), 42.86/42.61 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.50 / 41.95\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.24\left(\mathrm{C}_{\varepsilon}\right), 33.22\left(\mathrm{C}_{\beta}\right), 29.07\left(\mathrm{C}_{\delta}\right), 28.52\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.56\left(\mathrm{C}_{\mathrm{\gamma}}\right)$, signals for $3 \times \mathrm{C}_{\text {quart. }}$ are not visible; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{24} \mathrm{H}_{35} \mathrm{CIN}_{5} \mathrm{O}_{5}: 508.23\left[\mathrm{M}\left({ }^{35} \mathrm{Cl}\right)+\mathrm{H}\right]^{+}$, found: 508.1.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {E }}$-Acryloyl-L-Iysine-4-(pyridin-3-yl)piperazide (4i)



Compound $\mathbf{4 i}$ ( $109 \mathrm{mg}, 41 \%$, brown oil) was synthesised according to GP VI using compounds 2a and $\mathbf{3 i}$ ( 0.59 mmol ). Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 95:5 to 91:9; $R_{\mathrm{f}} 0.59\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 91:9); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.31$ (s, $\left.1 \mathrm{H}, \mathrm{H}-2\right), 8.18-8.08$
( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-6$ ), $7.25-7.22$ ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{H}-4,5$ ), $6.25\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right.$ ), 6.10 (dd, ${ }^{3} \mathrm{~J}=17.0,{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.87 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right), 5.45\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.66-4.57\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.93-3.62(\mathrm{~m}$, $4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), 3.44-3.09 (m, $6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{8} \mathrm{H}_{2}$ ), 1.76-1.66 (m, 1 H , $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.65-1.53 (m, $3 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.53-1.37 ( $\mathrm{s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ of $\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ ( $\mathrm{CDCl}_{3}$ ) $\delta=170.86$ (CCON), $165.81\left(\mathrm{CON}_{\varepsilon}\right), 155.86\left(\mathrm{CON}_{\mathrm{a}}\right), 146.82(\mathrm{C}-3), 140.86(\mathrm{C}-6), 138.39$ (C-2), $131.03\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 126.42\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 124.05(\mathrm{CH}$ of pyridine), $123.70(\mathrm{CH}$ of pyridine), 80.01 (quart. C of Boc ), $49.80\left(\mathrm{C}_{\alpha}\right), 48.99\left(\mathrm{CH}_{2}\right.$ of piperazine), $48.63\left(\mathrm{CH}_{2}\right.$ of piperazine), 45.32 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.89\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.27\left(\mathrm{C}_{\varepsilon}\right), 33.34\left(\mathrm{C}_{\beta}\right), 29.00\left(\mathrm{C}_{\delta}\right), 28.52\left(3 \times \mathrm{CH}_{3}\right.$ of Boc), $22.56\left(\mathrm{C}_{\mathrm{Y}}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{4}: 446.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 446.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {E }}$-Acryloyl-L-Iysine-4-(6-fluoropyridin-3-yl)piperazide (4j)



Compound $\mathbf{4 j}$ ( $189 \mathrm{mg}, 34 \%$, yellow oil) was synthesised according to GP VI using compounds 2a and $\mathbf{3 j}$ ( 1.22 mmol ). Solvent for column chromatography: gradient from ethyl acetatemethanol 100:0 to $85: 15$; $R_{\mathrm{f}} 0.13$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.82$ ( $\mathrm{dd},{ }^{4}{ }^{\mathrm{H}} \mathrm{H}, \mathrm{H}=2.9 \mathrm{~Hz}$, $\left.{ }^{5} J_{\mathrm{H}, \mathrm{H}=}=1.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 7.37$ (ddd, $\left.{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=9.0 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=6.7 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.86$ (dd, ${ }^{3}{ }_{\mathrm{H}, \mathrm{H}}=8.9 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=3.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.26 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.08 (dd, ${ }^{3}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.77 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.44\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.65-4.57\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.91-3.60\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.43-3.24 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 3.20-3.06 (m, $4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), 1.77-1.53 ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), $1.48-1.39\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of $\left.\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}^{2}\left(\mathrm{CDCl}_{3}\right) \delta=170.80$ (CON), $165.71\left(\mathrm{CON}_{\varepsilon}\right), 158.48$ (d, $\left.{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=}=233.7 \mathrm{~Hz}, \mathrm{C}-6\right), 155.85\left(\mathrm{COON}_{\alpha}\right), 145.24$ (d, $\left.{ }^{4} J_{C, F}=4.3 \mathrm{~Hz}, \mathrm{C}-3\right), 135.85\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=14.9 \mathrm{~Hz}, \mathrm{C}-2\right), 131.01\left(\mathrm{CH}_{2}=C\right), 130.29\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.6 \mathrm{~Hz}\right.$, $\mathrm{C}-4), 126.45\left(\mathrm{CH}_{2}=\mathrm{C}\right), 109.57\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=39.4 \mathrm{~Hz}, \mathrm{C}-5\right), 80.03$ (quart. C of Boc), 50.31 ( $\mathrm{C}_{\mathrm{a}}$ ), $49.97\left(\mathrm{CH}_{2}\right.$ of piperzaine), $49.77\left(\mathrm{CH}_{2}\right.$ of piperzaine), $45.45\left(\mathrm{CH}_{2}\right.$ of piperzaine), $41.98\left(\mathrm{CH}_{2}\right.$ of piperzaine), $39.24\left(\mathrm{C}_{\varepsilon}\right), 33.34\left(\mathrm{C}_{\beta}\right)$, $29.07\left(\mathrm{C}_{\delta}\right)$, $28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc), $22.55\left(\mathrm{C}_{\gamma}\right)$; ${ }^{19} \mathrm{~F}-{ }^{2} \mathrm{NMR}$ $\left(\mathrm{CDCl}_{3}\right) \delta=-77.65(\mathrm{~s}) ; \mathrm{MS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{23} \mathrm{H}_{35} \mathrm{FN}_{5} \mathrm{O}_{4}: 464.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 464.1.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\boldsymbol{E}}$-Acryloyl-L-lysine-4-(6-trifluoromethylpyridin-3-yl)piperazide (4k)



Compound $\mathbf{4 k}$ ( $61 \mathrm{mg}, \mathbf{2 4 \%}$, colourless oil) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 k}(0.50 \mathrm{mmol})$. Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to $90: 10 ; R_{\mathrm{f}} 0.13$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.35$ (d, ${ }^{4} \mathrm{~J}=2.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 7.55 (d, ${ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 7.22 (dd, ${ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.25 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.08 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.75 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.40 (d, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}$, $\left.\mathrm{N}_{\mathrm{a}} \mathrm{H}\right)$, 4.66-4.57 (m, 1H, $\mathrm{C}_{a} \mathrm{H}$ ), 3.96-3.64 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), 3.44-3.25 (m, 6H, $2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.79-1.53 (m, 4H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{8} \mathrm{H}_{2}$ ), 1.49-1.39 (m, 11H, $3 \times \mathrm{CH}_{3}$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.97,165.74,155.84(\mathrm{COO}), 148.01(\mathrm{C}-3), 138.88\left(\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.3\right.$ $\mathrm{Hz}, \mathrm{C}-6), 137.93(\mathrm{C}-2), 130.98\left(\mathrm{CH}_{2}=C\right)$, $126.51\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 122.11 ( $\mathrm{q},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=272.7 \mathrm{~Hz}, \mathrm{CF}_{3}$ ), 121.68 (C-4), 121.08 ( $\mathrm{q},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=2.7 \mathrm{~Hz}, \mathrm{C}-5$ ), 80.11 (quart. C of Boc), $49.80\left(\mathrm{C}_{\alpha}\right), 47.94\left(\mathrm{CH}_{2}\right.$ of piperazine), $47.58\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.04\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.64\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $39.16\left(\mathrm{C}_{\varepsilon}\right), 33.20\left(\mathrm{C}_{\beta}\right), 29.11\left(\mathrm{C}_{\delta}\right), 28.52\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.53\left(\mathrm{C}_{\gamma}\right)$; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-$ 66.88 (s); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{24} \mathrm{H}_{35} \mathrm{~F}_{3} \mathrm{~N}_{5} \mathrm{O}_{4}: 514.26$ [ $\left.\mathrm{M}+\mathrm{H}\right]^{+}$, found: 514.3.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{k}$-Acryloyl-L-lysine-4-(6-methoxycarbonylpyridin-3-yl)piperazide (4I)



Compound $\mathbf{4 I}$ ( 159 mg , colourless oil) was synthesised according to GP VI using compounds 2a and $\mathbf{3 I}$ ( 0.70 mmol ). Solvent for column chromatography: gradient from ethyl acetatemethanol 100:0 to 90:10; $R_{\mathrm{f}} 0.20$ (ethyl acetate-methanol 90:10); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.35(\mathrm{~d}$,
$\left.{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 8.03\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 7.18\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right)$, 6.25 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.86 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.41 (d, ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}$, $\left.\mathrm{N}_{\mathrm{a}} \mathrm{H}\right)$, 4.64-4.56 (m, 1H, $\left.\mathrm{C}_{a} \mathrm{H}\right), 3.99-3.65\left(\mathrm{~m}, 7 \mathrm{H}, \mathrm{CH}_{3} \mathrm{O}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.50-3.25 (m, $6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right)$, 1.76-1.53 (m, $\left.4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.48-1.37\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.99,165.77,165.74,155.83$ (NCOO), 148.26 (C-3), 138.29 (C-6), $137.20(\mathrm{C}-2), 130.98\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $126.47\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $126.26(\mathrm{C}-5)$, 120.71 ( $\mathrm{C}-4$ ), 80.09 (quart. C of Boc), $52.68\left(\mathrm{CH}_{3} \mathrm{O}\right), 49.80\left(\mathrm{C}_{a}\right), 47.46\left(\mathrm{CH}_{2}\right.$ of piperazine), $47.08\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.97\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.60\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.14\left(\mathrm{C}_{\varepsilon}\right), 33.19\left(\mathrm{C}_{\beta}\right), 29.08$ $\left(\mathrm{C}_{\delta}\right), 28.50\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.53\left(\mathrm{C}_{\gamma}\right)$; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{38} \mathrm{~N}_{5} \mathrm{O}_{6}$ : 504.28 $[\mathrm{M}+\mathrm{H}]^{+}$, found: 504.2.

Product contains significant amounts of tris(pyrrolidinophosphine) oxide which was not removed by column chromatography. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=3.20-3.11\left(\mathrm{~m}, 6 \times \mathrm{CH}_{2} \mathrm{~N}\right), 1.87-1.77$ (m, $6 \times \mathrm{CH}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=46.44\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{P}}=4.5 \mathrm{~Hz}, 6 \times \mathrm{CH}_{2} \mathrm{~N}\right.$ ), $26.57\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{c}, \mathrm{P}}=8.0 \mathrm{~Hz}\right.$, $6 \times \mathrm{CH}_{2}$ ).

## $N^{\text {a }}$-Boc- $\boldsymbol{N}^{\text {® }}$-Acryloyl-L-Iysine-4-(6-nitropyridin-2-yl)piperazide (4m)



Compound 4 m ( $146 \mathrm{mg}, 72 \%$, yellow solid) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 m}$ ( 0.41 mmol ). Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 95:5 to 92:8; $R_{\mathrm{f}} 0.27\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.76$ (dd, ${ }^{3} \mathrm{~J}=8.3,7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 7.51 (d, ${ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.94 (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.26 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.80 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.44\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 4.66-$ 4.57 (m, 1H, CaH), 3.88-3.59 (m, 8H, 4×CH2 of piperazine), 3.43-3.25 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.78$1.52\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right), 1.49-1.39\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of $\left.\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=171.08$ (CON), $165.75\left(\mathrm{CON}_{\varepsilon}\right), 157.54,155.92,155.85,140.72(\mathrm{C}-4), 130.98\left(\mathrm{CH}_{2}=C\right), 126.49$ $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112.01(\mathrm{C}-3), 106.50(\mathrm{C}-5), 80.06$ (quart. C of Boc ), $49.88\left(\mathrm{C}_{\alpha}\right), 45.06\left(\mathrm{CH}_{2}\right.$ of
piperazine), $44.88\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.76\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.69\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $39.22\left(\mathrm{C}_{\varepsilon}\right), 33.29\left(\mathrm{C}_{\beta}\right), 29.07\left(\mathrm{C}_{\delta}\right)$, $28.52\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.56\left(\mathrm{C}_{\gamma}\right)$; MS (ESI $\left.{ }^{+}\right)$: m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{34} \mathrm{~N}_{6} \mathrm{NaO}_{6}$ : $513.24[\mathrm{M}+\mathrm{Na}]^{+}$, found: 513.3.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(6-tert-butylpyridin-2-yl)piperazide (4n)



Compound $\mathbf{4 n}$ ( $45 \mathrm{mg}, 28 \%$, brown oil) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 n}$ ( 0.32 mmol ). Solvent for column chromatography: gradient from ethyl acetatepetroleum ether 77:33 to 100:0; $R_{\mathrm{f}} 0.33$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.45$ (dd, ${ }^{3} \mathrm{~J}=8.2$, $7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.72 (d, ${ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.47 (d, ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.27 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.84 (broad $\mathrm{s}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2,{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), $5.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), 4.69$4.57\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.89-3.21\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.75-1,65\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right)$, 1.64-1.52 (m, $3 \mathrm{H} ; \mathrm{C}_{\beta} \mathrm{HH}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.50-1.40 (m, $11 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ of $\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), $1.31\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of tert-butyl); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.78$ (CCON), 167.95, $165.73\left(\mathrm{CON}_{\varepsilon}\right), 157.88,155.90$ $\left(\mathrm{CON}_{\mathrm{a}}\right), 138.06(\mathrm{C}-4), 131.05\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 126.35\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 109.30(\mathrm{C}-5), 104.13(\mathrm{C}-3), 79.92$ (quart. C of Boc), $49.81\left(\mathrm{C}_{\mathrm{a}}\right), 45.56\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.39\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), 41.95 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.39\left(\mathrm{C}_{\varepsilon}\right), 37.62$ (quart. C of tert-butyl), $33.51\left(\mathrm{C}_{\beta}\right), 30.22\left(3 \times \mathrm{CH}_{3}\right.$ of tertbutyl), $28.96\left(\mathrm{C}_{\bar{\delta}}\right), 28.54\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.62\left(\mathrm{C}_{\mathrm{\gamma}}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{44} \mathrm{~N}_{5} \mathrm{O}_{4}$ : $502.34[\mathrm{M}+\mathrm{H}]^{+}$, found: 502.2.


Compound 40 ( $26 \mathrm{mg}, 22 \%$, colourless oil) was synthesised according to GP VI using compounds 2a and $\mathbf{3 0}$ ( 0.23 mmol$)$. Solvent for column chromatography: gradient from ethyl acetate-petroleum ether 77:33 to 100:0; $R_{\mathrm{f}} 0.36$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.45$ ( t , $\left.{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.26$ (dd, $\left.{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 6.21-6.17(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5)$, 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.82 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ) $5.62\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}\right.$, ${ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH} H$ ), $5.48\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.74\left(\mathrm{dm},{ }^{2} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=47.5 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}-\right.$ $\left.\mathrm{CH}_{2}-\mathrm{F}\right)$, 4.66-4.58 (m, 1H, $\mathrm{C}_{a} \mathrm{H}$ ), $4.52\left(\mathrm{dm},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=28.5 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{z} \mathrm{CH}_{2}-\mathrm{F}\right), 3.84-3.25(\mathrm{~m}, 10 \mathrm{H}$, $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.89-1.50 (m, $4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.50-1.37 (m, 11H, $3 \times \mathrm{CH}_{3}$ of Boc, $\left.\mathrm{C}_{\mathrm{Y}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.86(\mathrm{CCON}), 165.75\left(\mathrm{CON}_{\varepsilon}\right), 162.26(\mathrm{C}-6), 157.62(\mathrm{C}-2)$, $155.89\left(\mathrm{CON}_{\mathrm{a}}\right), 140.72(\mathrm{CH}, \mathrm{C}-4), 131.01\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 126.42\left(\mathrm{CH}_{2}=\mathrm{CH}\right)$, $99.95(\mathrm{CH}$ of pyridine), 98.94 ( CH of pyridine), 82.23 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=169.4 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}$ ), 79.99 (quart. C of Boc ), $64.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=20.4 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right), 49.82\left(\mathrm{C}_{\alpha}\right), 45.51\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.29\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.24\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.86\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.34\left(\mathrm{C}_{\varepsilon}\right), 33.42\left(\mathrm{C}_{\beta}\right)$, $29.01\left(\mathrm{C}_{\delta}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.60\left(\mathrm{C}_{\mathrm{y}}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-75.76-75.82(\mathrm{~m})$; MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{39} \mathrm{FN}_{5} \mathrm{O}_{5}$ : $508.29\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 508.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{k}$-Acryloyl-L-lysine-4-(6-iodopyridin-2-yl)piperazide (4p)



Compound 4 p ( $28 \mathrm{mg}, 11 \%$, light yellow oil) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 p}$ ( 0.26 mmol ). Solvent for column chromatography: gradient from ethyl
acetate-methanol 100:0 to 85:15; $R_{\mathrm{f}} 0.31$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.22 (dd, ${ }^{3} \mathrm{~J}=7.42,8.32 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 7.06 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.94 ( d , ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $6.83\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 6.18 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 ( dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.41-4.32 (m, 1H, $\left.\mathrm{C}_{\alpha} \mathrm{H}\right), 3.69-3.37\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.16-3.05 (m, $\left.2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}\right)$, 1.61-1.21 (m, 15H, $3 \times \mathrm{CH}_{3}$ of Boc, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.48$ (CON), $164.39\left(\mathrm{CON}_{\varepsilon}\right), 158.63,155.28,139.47(\mathrm{C}-4), 131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right), 123.04$ (C-5), 116.21 (C-6), $106.06(\mathrm{C}-3), 77.98$ (quart. C of Boc ), $50.03\left(\mathrm{C}_{a}\right), 44.52\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.22\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.14\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.08\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.25\left(\mathrm{C}_{\varepsilon}\right), 31.03\left(\mathrm{C}_{\beta}\right)$, $28.81\left(\mathrm{C}_{\delta}\right), 28.19\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.73\left(\mathrm{C}_{\gamma}\right)$; MS $\left(\mathrm{ESI}^{+}\right)$: m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{35} I \mathrm{~N}_{5} \mathrm{O}_{4}$ : $572.17[\mathrm{M}+\mathrm{H}]^{+}$, found: 572.1.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-Acryloyl-L-lysine-4-(6-phenylpyridin-2-yl)piperazide (4q)



Compound $\mathbf{4 q}$ ( $54 \mathrm{mg}, 32 \%$, yellow oil) was synthesised according to GP VI using compounds 2a and $\mathbf{3 q}$ ( 0.32 mmol ). Solvent for column chromatography: ethyl acetate; $R_{\mathrm{f}} 0.30$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.02-7.97(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6), 7.59\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.4,7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), $7.48-7.36$ ( $\mathrm{m}, 3 \mathrm{H}, \mathrm{H}-3,4,5$ of phenyl), 7.17 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.62 (d, ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.27 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz} 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.84 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CHH}$ ), $5.51\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.68-4.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.88-3.57\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.43-3.26 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.76-1,52 (m, $\left.4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right), 1.45\left(\mathrm{~s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of $\left.\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=171.30\left(\mathrm{C}_{\alpha} \mathrm{CON}\right), 165.73\left(\mathrm{CON}_{\varepsilon}\right), 158.65,155.90155 .48$, 139.69 (C-1 of phenyl), 138.61 ( $\mathrm{C}-4$ of pyridine), $131.02\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 128.91$ ( $\mathrm{C}-4$ of phenyl), 128.68 (C-3,5 of phenyl), 126.88 ( $\mathrm{C}-2,6$ of phenyl), $126.40\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 110.65$ ( $\mathrm{C}-5$ of pyridine), 105.85 (C-3 of pyridine), 79.95 (quart. C of Boc), $49.83\left(\mathrm{Ca}_{\mathrm{a}}\right), 45.42\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), $45.30\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.00\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.37\left(\mathrm{C}_{\varepsilon}\right), 33.50\left(\mathrm{C}_{\beta}\right), 28.98\left(\mathrm{C}_{\delta}\right), 28.54$ $\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.61\left(\mathrm{C}_{\mathrm{y}}\right)$; $\mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right)$: m/z calculated for $\mathrm{C}_{29} \mathrm{H}_{40} \mathrm{~N}_{5} \mathrm{O}_{4}$ : $522.31\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 522.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(6-bromopyridin-2-yl)piperazide (4r)



Compound $4 \mathbf{r}$ ( $62 \mathrm{mg}, 31 \%$, colourless solid) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and $\mathbf{3 r}$ ( 0.38 mmol ). Solvent for column chromatography: ethyl acetate; $R_{\mathrm{f}} 0.28$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.33$ (dd, $\left.{ }^{3} \mathrm{~J}=7.6,8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.81\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-5)$, 6.53 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.26 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.80 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{C}=\mathrm{CHH}$ ), $5.46\left(\mathrm{~d},{ }^{3}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), $4.65-4.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.85-3.46\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.43-3.24 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.76-1.65 (m, 4H, C ${ }_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.48-1.38 (m, 11H, $3 \times \mathrm{CH}_{3}$ of $\left.\mathrm{Boc}, \mathrm{C}_{\curlyvee} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.94(\mathrm{CON}), 165.74\left(\mathrm{CON}_{\varepsilon}\right), 158.81,155.86$, 140.39, 139.86, $131.00\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $126.44\left(\mathrm{CH}_{2}=\mathrm{C}\right)$,, $117.09(\mathrm{C}-5), 105.18(\mathrm{C}-3), 80.00$ (quart. C of Boc), $49.83\left(\mathrm{C}_{\alpha}\right), 45.16\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.09\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.91\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.77\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.29\left(\mathrm{C}_{\varepsilon}\right)$, $33.39\left(\mathrm{C}_{\beta}\right), 29.02\left(\mathrm{C}_{\delta}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of $\mathrm{Boc})$, $22.57\left(\mathrm{C}_{\mathrm{y}}\right)$; MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{35} \mathrm{BrN}_{5} \mathrm{O}_{4}$ : $524.19\left[\mathrm{M}\left({ }^{79} \mathrm{Br}\right)+\mathrm{H}\right]^{+}$, found: 524.1.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{k}$-Acryloyl-L-Iysine-4-(pyridin-2-yl)piperazide (4s)



Compound $4 \mathbf{s}$ ( $44 \mathrm{mg}, 20 \%$, light yellow oil) was synthesised according to GP VI using compounds 2a and commercially available 1-(pyridin-2-yl)piperazine ( 0.50 mmol ). Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 85:15; $R_{\mathrm{f}} 0.20$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.22-8.18(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-6), 7.54-7.48(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4), 6.70-6.64$
(m, 2H, H-3,5), 6.26 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 5.84 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.49 (d, $\left.{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, \mathrm{~N}_{\mathrm{a}} \mathrm{H}\right), 4.66-4.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.83-3.48\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.42-3.26 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.77-1.52 (m, 4H, $\left.\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.48-1.37\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-$ NMR ( $\mathrm{CDCl}_{3}$ ) $\delta=170.85(\mathrm{CON}), 165.73\left(\mathrm{CON}_{\varepsilon}\right), 159.06,155.88,148.14(\mathrm{C}-6), 137.88(\mathrm{C}-4)$, $131.03\left(\mathrm{CH}_{2}=\mathrm{C}\right), 126.38\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $114.24(\mathrm{C}-5), 107.46(\mathrm{C}-3), 79.95$ (quart. C of Boc), 49.82 $\left(\mathrm{C}_{\alpha}\right)$, $45.49\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.35\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.29\left(\mathrm{CH}_{2}\right.$ of piperazine), 41.94 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.33\left(\mathrm{C}_{\varepsilon}\right), 33.45\left(\mathrm{C}_{\beta}\right), 28.99\left(\mathrm{C}_{\bar{\delta}}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc), $22.57\left(\mathrm{C}_{\gamma}\right)$; MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{23} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{4}: 446.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 446.2 .
$N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {E }}$-Acryloyl-L-Iysine-4-(pyridin-4-yl)piperazide (4t)


Compound $\mathbf{4 t}$ ( $62 \mathrm{mg}, 28 \%$, brown oil) was synthesised according to GP VI using compounds 2a and commercially available 1-(pyridin-4-yl)piperazine ( 0.50 mmol ). Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol $85: 15$ to $75: 25 ; R_{\mathrm{f}} 0.13\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 87:13); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.31$ (dd, $\left.{ }^{3}=5.1 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.5 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6\right), 6.68$ (dd, ${ }^{3} \mathrm{~J}=5.1 \mathrm{~Hz}$, $\left.{ }^{4} J=1.6 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 6.25$ ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH} H$ ), 6.08 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0$, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.83 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CHH}$ ), 5.41 (d, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 4.60 (td, ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz},{ }^{3} \mathrm{~J}=4.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.90-3.60 ( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), 3.49-3.23 ( $\mathrm{m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1,65 (m, 1 H , $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.64-1.52 (m, 3H, $\mathrm{C}_{\beta} \mathrm{HH}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), $1.43\left(\mathrm{~s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of $\left.\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=171.05(\mathrm{CCON}), 165.73\left(\mathrm{CON}_{\varepsilon}\right), 155.83\left(\mathrm{CON}_{\mathrm{a}}\right), 154.91(\mathrm{C}-4), 149.57(\mathrm{C}-2,6), 130.99$ $\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 126.46\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 108.60(\mathrm{C}-3,5), 80.08$ (quart. C of Boc), $49.83\left(\mathrm{C}_{\alpha}\right), 46.20\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.81\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.81\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.51\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.14\left(\mathrm{C}_{\varepsilon}\right), 33.16\left(\mathrm{C}_{\beta}\right), 29.10\left(\mathrm{C}_{\delta}\right), 28.51\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.52\left(\mathrm{C}_{\gamma}\right)$; MS ( ESI$)^{+}$): m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{4}: 446.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 446.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {}}$-Acryloyl-L-Iysine-4-phenylpiperazide (4u)



Compound $4 \mathbf{u}$ ( $90 \mathrm{mg}, 40 \%$, yellowish solid) was synthesised according to GP VI using compounds $2 \mathbf{a}$ and commercially available 1-phenylpiperazine ( 0.50 mmol ). Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 97:3 to 95:5; $R_{\mathrm{f}} 0.58\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}-\right.$ methanol 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.31-7.24$ (m, 2H, H-2,6), 6.96-6.91 (m, 3H, H-3,4,5), 6.27 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH} H$ ), 6.09 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.85 (broad s, 1H, $\mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.49 (d, ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $4.62\left(\mathrm{td},{ }^{3} \mathrm{~J}=8.5,4.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right.$ ), 3.89-3.49 ( $\mathrm{m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), 3.43-3.10 (m, $6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.76-1,52 (m, $4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), $1.44\left(\mathrm{~s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\left.\mathrm{C}_{\mathrm{r}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.68(\mathrm{CCON}), 165.73\left(\mathrm{CON}_{\varepsilon}\right), 155.88\left(\mathrm{CON}_{\mathrm{a}}\right), 150.90(\mathrm{C}-1)$, $131.03\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 129.35(\mathrm{C}-2,6), 126.38\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 120.86(\mathrm{C}-4), 116.87(\mathrm{C}-3,5), 79.94$ (quart. C of Boc), 49.92, 49.76, 49.55, $45.63\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.20\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.34\left(\mathrm{C}_{\varepsilon}\right), 33.46\left(\mathrm{C}_{\beta}\right), 28.98\left(\mathrm{C}_{\bar{\delta}}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.59\left(\mathrm{C}_{\gamma}\right)$; MS (ESI $\left.{ }^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{24} \mathrm{H}_{3} \mathrm{~N}_{4} \mathrm{O}_{4}: 445.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 445.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\kappa}$-Acryloyl-L-lysine-4-(6-chloropyridin-2-yl)piperazide (4v)



Compound $4 v$ ( $71 \mathrm{mg}, 20 \%$, yellowish oil) was synthesised according to GP VI using compounds 2a and commercially available 1-(6-chloropyridin-2-yl)piperazine ( 0.76 mmol ). Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 85:15; $R_{\mathfrak{f}}$ 0.33 (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.44\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.3,7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.66\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}\right.$,
$1 \mathrm{H}, \mathrm{H}-5$ ), $6.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 6.26 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 6.09 (dd, ${ }^{3}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.80 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.46\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 4.65-4.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.82-3.49\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.42-3.26 (m, 2H, C $\varepsilon_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.53 (m, 4H, C ${ }_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.48-1.38 (m, 11H, $3 \times \mathrm{CH}_{3}$ of $\left.\mathrm{Boc}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.93(\mathrm{CON}), 165.72\left(\mathrm{CON}_{\varepsilon}\right), 158.74,155.86$, 149.75 (C-6), $140.12(\mathrm{C}-4), 131.01\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $126.42\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $113.22(\mathrm{C}-5)$, $104.88(\mathrm{C}-3)$, 80.00 (quart. C of Boc ), $49.84\left(\mathrm{C}_{\alpha}\right), 45.17\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.11\left(\mathrm{CH}_{2}\right.$ of piperazine), 44.93 ( $\mathrm{CH}_{2}$ of piperazine), $41.78\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.29\left(\mathrm{C}_{\varepsilon}\right), 33.40\left(\mathrm{C}_{\beta}\right), 29.02\left(\mathrm{C}_{\delta}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc ), $22.57\left(\mathrm{C}_{\mathrm{Y}}\right)$; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{35} \mathrm{CIN}_{5} \mathrm{O}_{4}: 480.24\left[\mathrm{M}\left({ }^{35} \mathrm{CI}\right)+\mathrm{H}\right]^{+}$, found: 480.1.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {k }}$-Acryloyl-L-Iysine-4-(3-methylphenyl)piperazide (4w)



Compound 4w ( $45 \mathrm{mg}, 25 \%$, white solid) was synthesised according to GP VI using compounds $\mathbf{2 a}$ and 1-(3-methylphenyl)piperazine ( 0.40 mmol ). Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 90:10; $R_{\mathrm{f}} 0.37$ (ethyl acetatemethanol 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.18\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.80-6.71$ ( $\mathrm{m}, 3 \mathrm{H}, \mathrm{H}-2,4,6$ ), 6.27 (dd, ${ }^{3}=17.0 \mathrm{~Hz},{ }^{2}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz}, 10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.85 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\left.\mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.66-4.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.88-3.59\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.42-3.25(m,2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 3.24-3.11 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), $2.33\left(\mathrm{~s}, \mathrm{CH}_{3}\right)$, 1.75-1.52 (m, $4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.49-1.39 (m, 11H, $3 \times \mathrm{CH}_{3}$ of $\left.\mathrm{Boc}, \mathrm{C}_{7} \mathrm{H}_{2}\right)$; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=165.74,155.90,131.01$ $\left(\mathrm{CH}_{2}=C\right), 129.29(\mathrm{C}-5), 126.42\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 79.96 (quart. C of Boc), $49.74\left(\mathrm{C}_{a}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), $45.59\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.11\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.35\left(\mathrm{C}_{\varepsilon}\right), 33.47\left(\mathrm{C}_{\beta}\right), 28.97$ $\left(\mathrm{C}_{\delta}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$)$, $22.59\left(\mathrm{C}_{\gamma}\right), 21.87\left(\mathrm{CH}_{3}\right)$, signals for $\mathrm{C}-1,2,3,4,6$ and $1 \times \mathrm{CO}$ are not visible; $\mathrm{MS}\left(E S I^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{39} \mathrm{~N}_{4} \mathrm{O}_{4}: 459.30[\mathrm{M}+\mathrm{H}]^{+}$, found: 459.3 .

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\text {² }}$-Acryloyl-L-Iysine-4-(4-nitrophenyl)piperazide (4x)



Compound $\mathbf{4 x}$ ( $66 \mathrm{mg}, 27 \%$, yellow oil) was synthesised according to GP VI using compounds 2a and 1-(4-nitrophenyl)piperazine ( 0.50 mmol ). Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to 90:10; $R_{\mathrm{f}} 0.20$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.19-$ 8.13 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{H}-3,5$ ), 6.87-6.81 (m, 2H, H-2,6), 6.25 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.08 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.75 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 5.40\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 4.65-4.57\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.93-3.65(\mathrm{~m}$, $4 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine), $3.57-3.25\left(\mathrm{~m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.78-1.68(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.66-1.53 (m, 3H, $\left.\mathrm{C}_{\beta} H \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.48-1.38\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ $\left(\mathrm{CDCl}_{3}\right) \delta=171.09,165.72,155.84,154.47,139.43,130.98\left(\mathrm{CH}_{2}=\mathrm{C}\right), 126.50\left(\mathrm{CH}_{2}=\mathrm{C}\right), 126.10$ (C-3,5), 113.27 (C-2,6), 80.11 (quart. C of Boc), $49.85\left(\mathrm{C}_{\alpha}\right), 47.35\left(\mathrm{CH}_{2}\right.$ of piperazine), 46.98 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.88\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.61\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.13\left(\mathrm{C}_{\varepsilon}\right), 33.16$ $\left(\mathrm{C}_{\beta}\right), 29.12\left(\mathrm{C}_{\delta}\right), 28.52\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.52\left(\mathrm{C}_{\gamma}\right) ; \mathrm{MS}(\mathrm{ESI})$ : $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{24} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{6}$ : $490.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 490.2 .

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\varepsilon}$-Acryloyl-D-lysine-4-(6-methylpyridin-2-yl)piperazide (4y)



Compound 4y (127 mg, 34\%, brown oil) was synthesised according to GP VI using compounds $\mathbf{2 b}$ and $\mathbf{3 a}$ ( 0.81 mmol$)$. Solvent for column chromatography: gradient from ethyl acetate-methanol 100:0 to $85: 15 ; R_{\mathrm{f}} 0.35$ (ethyl acetate); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.40$ (dd, ${ }^{3} \mathrm{~J}=8.3$, $7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.55 (d, ${ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.45 (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.27 (dd,
${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 6.09 (dd, ${ }^{3} \mathrm{~J}=17.0,10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.84 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.61 (dd, ${ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), 4.66-4.59 (m, 1H, Car $)$, 3.86-3.45 (m, 8H, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.43-3.23 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ of pyridine), 1.76-1.53 (m, 4H, $\left.\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right), 1.48-1.39\left(\mathrm{~m}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=170.79(\mathrm{CON}), 165.72\left(\mathrm{CON}_{\varepsilon}\right), 158.75,157.14,155.89$, $138.10(\mathrm{C}-4), 131.04\left(\mathrm{CH}_{2}=\mathrm{C}\right), 126.35\left(\mathrm{CH}_{2}=\mathrm{C}\right), 113.62(\mathrm{C}-5), 104.09(\mathrm{C}-3), 79.93$ (quart. C of Boc), $49.81\left(\mathrm{C}_{a}\right), 45.58\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.43\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.40\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.01\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.38\left(\mathrm{C}_{\varepsilon}\right), 33.52\left(\mathrm{C}_{\beta}\right), 28.97\left(\mathrm{C}_{\delta}\right), 28.54\left(3 \times \mathrm{CH}_{3}\right.$ of Boc), $24.67\left(\mathrm{CH}_{3}\right.$ of pyridine), $22.60\left(\mathrm{C}_{\mathrm{y}}\right)$; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right)$: m/z calculated for $\mathrm{C}_{24} \mathrm{H}_{38} \mathrm{~N}_{5} \mathrm{O}_{4}: 460.29[\mathrm{M}+\mathrm{H}]^{+}$, found: 460.3 .

## $N^{\alpha}$-Boc- $N^{\varepsilon}$-Propionyl-L-Iysine-4-(6-methylpyridin-2-yl)piperazide (4z)



Compound $\mathbf{4 z}$ ( $77 \mathrm{mg}, 50 \%$, yellow oil) was synthesised according to GP VI using compounds 2c and $\mathbf{3 a}$ ( 0.33 mmol ). Solvent for column chromatography: gradient from $\mathrm{CH}_{2} \mathrm{Cl}_{2}$-methanol 97:3 to 95:5; $R_{\mathrm{f}} 0.47\left(\mathrm{CH}_{2} \mathrm{Cl}_{2}\right.$-methanol 95:5); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.41\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right)$, 6.55 (d, ${ }^{3}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.45 (d, ${ }^{3}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 5.60 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 5.48 ( d , $\left.{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right)$, 4.68-4.57 (m, $\left.1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.83-3.47\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.33$3.19\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{8} \mathrm{H}_{2}\right), 2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ of pyridine), $2.19\left(\mathrm{q},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 2 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 1.75-1.65$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.63-1.48 (m, $3 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.48-1.34 (m, $11 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), 1.15 $\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 3 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=173.92\left(\mathrm{CON}_{\varepsilon}\right), 170.82(\mathrm{CCON}), 158.74$, 157.14, $155.85\left(\mathrm{CON}_{\mathrm{a}}\right), 138.10(\mathrm{C}-4), 113.63(\mathrm{C}-5), 104.09(\mathrm{C}-3), 79.88$ (quart. C of Boc), $49.86\left(\mathrm{C}_{\alpha}\right), 45.60\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.43\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine $), 41.99\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $39.31\left(\mathrm{C}_{\varepsilon}\right), 33.51\left(\mathrm{C}_{\beta}\right), 29.89\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 29.20\left(\mathrm{C}_{\bar{\delta}}\right), 28.53\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 24.68\left(-\mathrm{CH}_{3}\right), 22.61$ $\left(\mathrm{C}_{\mathrm{Y}}\right), 10.09\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$; MS (ESI+ $): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{24} \mathrm{H}_{40} \mathrm{~N}_{5} \mathrm{O}_{4}: 462.31[\mathrm{M}+\mathrm{H}]^{+}$, found: 462.2.

## $N^{\alpha}$-Boc- $\boldsymbol{N}^{\varepsilon}$-Propionyl-L-lysine-4-(6-nitropyridin-3-yl)piperazide (4aa)



Compound 4aa ( $141 \mathrm{mg}, 87 \%$, yellow oil) was synthesised according to GP VI using compounds $\mathbf{2 c}$ and $\mathbf{3 b}$ ( 0.33 mmol ). Solvent for column chromatography: gradient from ethyl acetate-methanol 95:5 to 91:9; $R_{\mathrm{f}} 0.43$ (ethyl acetate-methanol 91:9); ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.20$ (d, ${ }^{3}=9.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 8.15 (d, ${ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 7.26-7.22 (m, 1H, H-4), 5.54 (broad s, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), $5.36\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 4.67-4.51\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 4.01-3.66\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.63-3.38 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), $3.36-3.12\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right)$, $2.18\left(\mathrm{q},{ }^{3} \mathrm{~J}=7.6\right.$ $\mathrm{Hz}, 2 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}$ ), $1.72\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.65-1.47\left(\mathrm{~m}, 3 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.43\left(\mathrm{~s}, 11 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of Boc, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), $1.14\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 3 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=173.98\left(\mathrm{CON}_{\varepsilon}\right)$, 171.22 (CCON), 155.81 (CON ${ }_{\alpha}$ ), 149.62, 148.58, 134.24 (C-2), 121.39 (C-4), 119.84 (C-5 Pyridin), 80.13 (quart. C of Boc$), 49.88\left(\mathrm{C}_{\mathrm{a}}\right), 47.04\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.66\left(\mathrm{CH}_{2}\right.$ of piperazine), 44.70 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.41\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.96\left(\mathrm{C}_{\varepsilon}\right), 33.01\left(\mathrm{C}_{\beta}\right)$, $29.89\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$, 29.34 $\left(\mathrm{C}_{\delta}\right), 28.50\left(3 \times \mathrm{CH}_{3}\right.$ of Boc$), 22.49\left(\mathrm{C}_{\gamma}\right), 10.08\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$; MS (ESI+): m/z calculated for $\mathrm{C}_{23} \mathrm{H}_{37} \mathrm{~N}_{6} \mathrm{O}_{6}$ : $493.28\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 493.2.

## $\boldsymbol{N}^{\text {E-Acyllysine piperazides (5) }}$

## $N^{\kappa}$-Acryloyl-L-Iysine-4-(6-methylpyridin-2-yl)piperazide×2TFA (5a)



Compound $\mathbf{5 a}$ ( 149 mg , amber oil) was synthesised according to GP VII using compound $\mathbf{4 a}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.12\left(\mathrm{~d},{ }^{3} \mathrm{~J}=3.8 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.07\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}\right), 7.64$ (ps-t, $\left.{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.85\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 6.67 (d, ${ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 ( dd , $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.48-4.38\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.76-3.40\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.26-2.99 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.38\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.75-1.65\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.53-1.18$ ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.38$ (CON), $164.51\left(\mathrm{CON}_{\varepsilon}\right), 158.17$ ( q , ${ }^{2} J_{\mathrm{C}, \mathrm{F}}=35.8 \mathrm{~Hz}, \mathrm{CO}$ TFA), $131.78\left(\mathrm{CH}_{2}=C\right)$, $124.88\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.79$ (psd, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=292.6 \mathrm{~Hz}, \mathrm{CF}_{3}$ TFA), $112.84(\mathrm{C}-5), 49.48\left(\mathrm{C}_{\alpha}\right), 45.20\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.73\left(\mathrm{CH}_{2}\right.$ of piperazine), 44.15 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.26\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.97\left(\mathrm{C}_{\varepsilon}\right), 30.02\left(\mathrm{C}_{\beta}\right), 28.70\left(\mathrm{C}_{\bar{\delta}}\right), 21.24\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-2,3,4,6$ and $\mathrm{CH}_{3}$ are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ): $\delta=-74.69$ (s, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{19} \mathrm{H}_{30} \mathrm{~N}_{5} \mathrm{O}_{2}: 360.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 360.2.

## $\mathbf{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(6-nitropyridin-3-yl)piperazide $\times 2$ TFA (5b)



Compound $\mathbf{5 b}$ ( 165 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 b}$. ${ }^{1} \mathrm{H}-$ NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.29\left(\mathrm{~d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ ), $8.20\left(\mathrm{~d},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ ), 8.11 ( d , ${ }^{3} \mathrm{~J}=3.7 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}$), $8.06\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}\right), 7.52\left(\mathrm{dd},{ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right)$, 6.17 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.01 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53
(dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.49-4.39 (m, 1H, CaH), 3.77-3.45 (m, 8H, 4×CH2 of piperazine), 3.22-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.76-1.63 (m, 1H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.52-1.28 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ): $\delta=-74.28$ (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{~N}_{6} \mathrm{O}_{4}$ : $391.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 391.3.

## $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-Acryloyl-L-lysine-4-dansylpiperazide $\times 2$ TFA (5c)



- 2TFA

Compound $5 \mathbf{5 c}$ ( 35 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 c}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.55\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of dansyl), $8.30\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of dansyl), 8.16 (dd, ${ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.97$ (broad d, ${ }^{3} \mathrm{~J}=4.8 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}$), 7.69 (dd, ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz},{ }^{2} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 7.63 (dd, ${ }^{3} \mathrm{~J}=8.7,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 7.28 (d, ${ }^{3} \mathrm{~J}=7.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 6.16 (dd, ${ }^{3} \mathrm{~J}=17.1$, $10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 4.32-4.22\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.83-3.75(\mathrm{~m}, 1 \mathrm{H}, \mathrm{CH}$ of piperazine), 3.673.56 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{CH}$ of piperazine), 3.49-3.25 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), 3.10-3.01 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 2.95-2.87 (m, 2H, $\mathrm{CH}_{2}$ of piperazine), $2.83\left(\mathrm{~s}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{3}\right) 1.63-1.53\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right)$, 1.43-1.31 (m, 2H, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.31-1.15 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.37,164.47$, 157.97 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=}=34.0 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 151.45 (quart. C of dansyl), $132.23,131.78\left(\mathrm{CH}=\mathrm{CH}_{2}\right)$, 130.57 (CH of dansyl), 130.31 (CH of dansyl), 129.60 (quart. C of dansyl), 129.19 (quart. C of dansyl), $128.38\left(\mathrm{CH}\right.$ of dansyl), $124.88\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 123.74(\mathrm{CH}$ of dansyl), $118.80(\mathrm{CH}$ of dansyl), $115.39\left(\mathrm{CH}\right.$ of dansyl), $49.41\left(\mathrm{C}_{\alpha}\right), 45.41\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.16\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.05\left(2 \times \mathrm{CH}_{3}\right)$, $44.52\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $41.29\left(\mathrm{CH}_{2}\right.$ of piperazine $), 37.90\left(\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right)$, $29.85\left(\mathrm{C}_{\beta} \mathrm{H}_{2}\right), 28.60\left(\mathrm{C}_{\delta} \mathrm{H}_{2}\right), 21.22\left(\mathrm{C}_{\gamma} \mathrm{H}_{2}\right)$, signal for $1 \times \mathrm{C}_{\text {quart. }}$ of dansyl is not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.24$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{25} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{4} \mathrm{~S}: 502.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 501.9.

## $N^{\text {-Acryloyl-L-lysine-4-(6-fluoropyridin-2-yl)piperazide×2TFA (5d) }}$



Compound 5d (257 mg, yellow oil) was synthesised according to GP VII using compound 4d. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.11\left(\mathrm{~d},{ }^{3} \mathrm{~J}=3.9 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.06\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}\right), 7.72$ (ps-q, ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.73 (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.3 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.33 (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=7.7 \mathrm{~Hz}$, ${ }^{3} J_{\mathrm{H}, \mathrm{F}}=2.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.17 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.52 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.47-4.38(m,1H, $\mathrm{C}_{a} \mathrm{H}$ ), 3.74-3.39 (m, 8H, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.20-3.04 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74-1.63 (m, 2H, $\left.\mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.51-1.20\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=167.34(\mathrm{CON}), 164.49\left(\mathrm{CON}_{\varepsilon}\right)$, 161.96 ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=233.3 \mathrm{~Hz}, \mathrm{C}-6$ ), 158.18 ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.8 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 157.56 (d, $\left.{ }^{3} J_{\mathrm{C}, \mathrm{F}}=16.0 \mathrm{~Hz}, \mathrm{C}-2\right), 142.81\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.1 \mathrm{~Hz}, \mathrm{C}-4\right), 131.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.81$ (psd, ${ }^{1}{ }^{\mathrm{J}, \mathrm{F}}=293.1 \mathrm{~Hz}, \mathrm{CF}_{3}$ of TFA), 103.72 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.8 \mathrm{~Hz}, \mathrm{C}-3$ ), 95.87 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=36.9 \mathrm{~Hz}$, $\mathrm{C}-5), 49.49\left(\mathrm{C}_{\alpha}\right), 44.48\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.21\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.02\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.24\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.96\left(\mathrm{C}_{\varepsilon}\right), 30.03\left(\mathrm{C}_{\beta}\right), 28.67\left(\mathrm{C}_{\delta}\right), 21.23\left(\mathrm{C}_{\gamma}\right)$; ${ }^{13} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-68.81--68.87(m, F-6),-74.68$ (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{FN}_{5} \mathrm{O}_{2}$ : $364.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 364.3.

## $N^{\text {E-Acryloyl-L-lysine-4-(4-fluorobenzoyl)piperazide×TFA (5e) }}$



Compound $\mathbf{5 e}$ ( 32 mg , brown solid) was synthesised according to GP VII using compound $\mathbf{4 e}$.
${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.15-8.02\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.52\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.7 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=5.5 \mathrm{~Hz}, 2 \mathrm{H}\right.$, $\mathrm{H}-2,6), 7.31\left(\mathrm{t},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}={ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=8.9 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 6.19\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right), 6.05$
(dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.57 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.44$4.29\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.95-3.30\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.20-3.03(m,2H, C $\mathrm{C}_{2}$ ), 1.731.61 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.49-1.20 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-$ NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=168.32$, 167.36, 164.51, $131.78\left(\mathrm{CH}_{2}=\mathrm{C}\right), 129.67\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=}=8.6 \mathrm{~Hz}, \mathrm{C}-2,6\right), 124.88\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.44$ ( d , $\left.{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.8 \mathrm{~Hz}, \mathrm{C}-3,5\right), 49.51\left(\mathrm{C}_{\alpha}\right), 37.92\left(\mathrm{C}_{\varepsilon}\right), 29.96\left(\mathrm{C}_{\beta}\right), 28.64\left(\mathrm{C}_{\delta}\right), 21.21\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine and $\mathrm{C}-1,4$ are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-73.81(\mathrm{~s}$, TFA), -110.76--110.88 (m, F-4); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{20} \mathrm{H}_{28} \mathrm{FN}_{4} \mathrm{O}_{3}: 391.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 391.3.

## $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(4-nitrobenzoyl)piperazide×TFA (5f)



Compound $\mathbf{5 f}$ ( 66 mg , light brown solid) was synthesised according to GP VII using compound 4f. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.32\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right), 8.16-8.03\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 7.72$ (d, ${ }^{3}=8.2 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ ), 6.25-6.13 (m, 1H, CH=CH2), 6.11-5.98 (m, 1H, C=CHH), 5.61-5.51 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.48-4.27 (m, 1H, $\mathrm{C}_{a} \mathrm{H}$ ), 3.80-3.41 (m, 6H, $3 \times \mathrm{CH}_{2}$ of piperazine), 3.39-3.22 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{CH}_{2}$ of Piperazine), 3.20-3.03 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.59 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.51-1.20 (m, $4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{6} \mathrm{H}_{2}$ ), diffuse signals (partly in duplicate) due to the amide bonds on both sides of the piperazine ring; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.41,167.31,164.53,158.11$ ( $\mathrm{q},{ }^{2}{ }^{\mathrm{J}} \mathrm{c}, \mathrm{F}=35.3 \mathrm{~Hz}$, CO of TFA), 147.93, 141.78, $131.80\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.40(\mathrm{C}-2,6), 124.90\left(\mathrm{CH}_{2}=\mathrm{C}\right), 123.81(\mathrm{C}-$ $3,5), 49.50\left(\mathrm{C}_{\alpha}\right), 37.94\left(\mathrm{C}_{\varepsilon}\right), 29.97\left(\mathrm{C}_{\beta}\right), 28.66\left(\mathrm{C}_{\delta}\right), 21.22\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.58$ ( $\mathrm{s}, \mathrm{TFA}$ ); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{20} \mathrm{H}_{28} \mathrm{~N}_{5} \mathrm{O}_{5}$ : $418.21\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 418.3.

## $\boldsymbol{N}^{\text {-Acryloyl-L-Iysine-4-(4-fluorobenzyl)piperazide×2TFA (5g) }}$



Compound $\mathbf{5 g}$ ( 66 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 g}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.19-8.06\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.58-7.49 (m, 2H, H-2,6), 7.33 ( t , ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5$ ), 6.20 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.06 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.58 (dd, ${ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.41-4.25(m,1H, $\left.\mathrm{C}_{\alpha} \mathrm{H}\right), 3.18-3.07\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.74-1.59\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.50-1.20\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine and $\mathrm{CH}_{2}$-fluorobenzyl are not visible; ${ }^{13} \mathrm{C}$-NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=164.61,158.08\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=32.8 \mathrm{~Hz}, \mathrm{CO}\right.$ of TFA), 133.43 (pss, C-2,6), $131.82\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.98$ $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.80\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=20.9 \mathrm{~Hz}, \mathrm{C}-3,5\right), 49.44\left(\mathrm{C}_{\alpha}\right), 37.90\left(\mathrm{C}_{\varepsilon}\right), 28.71\left(\mathrm{C}_{\delta}\right), 21.29\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-fluorobenzyl, $\mathrm{C}-1,2,4,6, \mathrm{C}_{\beta}, 1 \times \mathrm{CO}$ are not visible; ${ }^{19} \mathrm{~F}$ NMR (DMSO- $d_{6}$ ) $\delta=-74.04$ (s, TFA), -111.91--112.04 (m, F-4); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{20} \mathrm{H}_{30} \mathrm{FN}_{4} \mathrm{O}_{2}$ : $377.23[\mathrm{M}+\mathrm{H}]^{+}$, found: 377.2.

## $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(6-chloropicolinoyl)piperazide×2TFA (5h)



Compound $\mathbf{5 h}$ ( 35 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 h}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.14-7.99\left(\mathrm{~m}, 5 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}, \mathrm{N}_{\varepsilon} \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), 7.69-7.61 (m, 2H, H3,5 of pyridine), 6.23-6.14 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.10-6.01 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), $5.60-5.53(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CHH}), 4.46-4.29\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.81-3.27\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.20-3.05(m, $\left.2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.73-1.61\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.55-1.17\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right)$, diffuse signals (partly in duplicate) due to the amide bonds on both sides of the piperazine ring; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ )
$\delta=-73.68$ (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{19} \mathrm{H}_{27} \mathrm{ClN}_{5} \mathrm{O}_{3}: 408.18\left[\mathrm{M}\left({ }^{35} \mathrm{Cl}\right)+\mathrm{H}\right]^{+}$, found: 408.0.

## $N^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(pyridin-3-yl)piperazide×2TFA (5i)



Compound $\mathbf{5 i}$ ( 144 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 i}$.
${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.47\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ ), $8.23\left(\mathrm{~d},{ }^{3} \mathrm{~J}=5.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6\right), 8.20-8.03$ ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{NH}_{3}{ }^{+} / \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.99 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 7.78 (dd, ${ }^{3} \mathrm{~J}=8.8,5.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.17 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.00 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.53 (dd, $\left.{ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 4.50-4.40\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.81-3.54(\mathrm{~m}, 4 \mathrm{H}$, $2 \times \mathrm{CH}_{2}$ of piperazine), 3.54-3.28 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), 3.23-3.07 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.78$1.62\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.52-1.29\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\gamma}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.38\left(\mathrm{C}_{\alpha} \mathrm{CON}\right)$, $164.50\left(\mathrm{CON}_{\varepsilon}\right), 158.10\left(\mathrm{q},{ }^{2}{ }^{2} \mathrm{C}, \mathrm{F}=34.6 \mathrm{~Hz}, \mathrm{CO}\right.$ of TFA), $147.61(\mathrm{C}-3), 131.78\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 124.87$ $\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 49.41\left(\mathrm{C}_{\alpha}\right), 46.63\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.17\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.92\left(\mathrm{CH}_{2}\right.$ of piperazine), $40.96\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.95\left(\mathrm{C}_{\varepsilon}\right)$, $30.04\left(\mathrm{C}_{\beta}\right), 28.70\left(\mathrm{C}_{\delta}\right)$, $21.20\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-2,4,5,6$ of are not visible; $\mathrm{MS}\left(E S I^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{18} \mathrm{H}_{28} \mathrm{~N}_{5} \mathrm{O}_{2}: 346.22[\mathrm{M}+\mathrm{H}]^{+}$, found: 346.2 .

## $\boldsymbol{N}^{\text {E-Acryloyl-L-lysine-4-(6-fluoropyridin-3-yl)piperazide×2TFA (5j) }}$



Compound $\mathbf{5 j}$ ( 270 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 j}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.12$ (broad s, $3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}$), $8.06\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}\right.$ ), 7.87 (broad s,

${ }^{3} J_{H, H}=8.9 \mathrm{~Hz},{ }^{3} J_{\mathrm{H}, \mathrm{F}}=3.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.17 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.49-4.37 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.78-3.53 (m,4H,2×CH2 of piperazine), 3.28-3.03 (m, $6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.62 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.52-1.20 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=167.19(C O N), 164.50\left(\mathrm{CON}_{\varepsilon}\right), 158.16$ ( $\mathrm{q},{ }^{2}{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.9 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 157.03 ( d , $\left.{ }^{1} J_{\mathrm{C}, \mathrm{F}}=228.9 \mathrm{~Hz}, \mathrm{C}-6\right), 145.15\left(\mathrm{~d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.9 \mathrm{~Hz}, \mathrm{C}-3\right.$ ), 134.15 ( $\mathrm{d},{ }^{3}{ }_{\mathrm{C}, \mathrm{F}}=15.3 \mathrm{~Hz}, \mathrm{C}-2$ ), 131.79 ( $\mathrm{CH}_{2}=C$ ), 129.82 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.3 \mathrm{~Hz}, \mathrm{C}-4$ ), $124.86\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 109.14 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=39.6 \mathrm{~Hz}, \mathrm{C}-5$ Pyridin), $49.40\left(\mathrm{C}_{\alpha}\right)$, $48.74\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $48.27\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $44.48\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.32\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.98\left(\mathrm{C}_{\varepsilon}\right), 30.10\left(\mathrm{C}_{\beta}\right), 28.70\left(\mathrm{C}_{\delta}\right), 21.25\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.69$ (s, TFA), -79.89 (broad $s, F-6$ ); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{FN}_{5} \mathrm{O}_{2}$ : $364.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 364.1.

## $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-Iysine-4-(6-trifluoromethylpyridin-3-yl)piperazide×2TFA (5k)



Compound $\mathbf{5 k}$ ( 76 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 k}$.
${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.47\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 8.11\left(\mathrm{~d},{ }^{3} \mathrm{~J}=4.0 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.06(\mathrm{t}$, ${ }^{3} \mathrm{~J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), $7.69\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 7.48\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right)$, $6.17\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right), 6.01\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), 5.52 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.49-4.40(m,1H, CaH), 3.79-3.56(m,4H,2×CH2 of piperazine), $3.53-3.30\left(\mathrm{~m}, 4 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.21-3.04 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.64 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.51-1.21 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.32,164.48,158.06$ ( $\mathrm{q},{ }^{2}{ }^{2} \mathrm{C}, \mathrm{F}=35.2 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 147.86 (C-3), 137.03 (C-2), 135.53 ( $\mathrm{q},{ }^{2}{ }^{\mathrm{J}} \mathrm{C}, \mathrm{F}=34.3 \mathrm{~Hz}, \mathrm{C}-6$ ), $131.77\left(\mathrm{CH}_{2}=C\right), 124.85\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $121.05-120.91(\mathrm{C}-4,5), 49.42\left(\mathrm{C}_{\alpha}\right), 46.56\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.06\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.06\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.08\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $37.96\left(\mathrm{C}_{\varepsilon}\right), 30.05\left(\mathrm{C}_{\beta}\right), 28.69\left(\mathrm{C}_{\delta}\right), 21.24\left(\mathrm{C}_{\gamma}\right)$, signal for $\mathrm{CF}_{3}$ at pyridine is not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-64.92\left(\mathrm{~s}, \mathrm{CF}_{3}\right.$ of pyridine), -74.54 ( $\mathrm{s}, \mathrm{TFA}$ ); MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{19} \mathrm{H}_{27} \mathrm{~F}_{3} \mathrm{~N}_{5} \mathrm{O}_{2}$ : $414.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 414.2.

## $\boldsymbol{N}^{\text {E-Acryloyl-L-Iysine-4-(6-methoxycarbonylpyridin-3-yl)piperazide×2TFA (5I) }}$



Compound $5 \mathbf{I I}$ ( 228 mg , brown oil) was synthesised according to GP VII using compound $\mathbf{4 I}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.41\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 8.11\left(\mathrm{~d},{ }^{3} \mathrm{~J}=4.0 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.05$ (t, ${ }^{3} \mathrm{~J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.91 (d, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 7.39 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.17 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.01 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.52 (dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.49-4.39 (m, 1H, C ${ }_{a} \mathrm{H}$ ), $3.81\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ ), 3.77$3.33\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 1.49-1.20 (m,4H, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right)$, signals for $\mathrm{C}_{\beta} \mathrm{H}_{2}$ and $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ interfere with the signals from tris(pyrrolidinophosphine) oxide and pyrrolidine; ${ }^{13} \mathrm{C}$-NMR (DMSO- $d_{6}$ ) $\delta=167.35,165.00,164.49,158.12$ ( $\mathrm{q},{ }^{2}{ }^{\mathrm{J}} \mathrm{C}, \mathrm{F}=35.5 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 147.81 (C-3), 136.32, 136.19, $131.77\left(\mathrm{CH}_{2}=C\right), 125.73,124.87,119.91(\mathrm{C}-4), 51.79\left(\mathrm{CH}_{3}\right), 49.43\left(\mathrm{C}_{\mathrm{a}}\right), 46.25$ $\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.73\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.06\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.13\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.96\left(\mathrm{C}_{\varepsilon}\right), 30.04\left(\mathrm{C}_{\beta}\right), 28.69\left(\mathrm{C}_{\delta}\right), 21.25\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.60(\mathrm{~s}$, TFA); MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{20} \mathrm{H}_{30} \mathrm{~N}_{5} \mathrm{O}_{4}: 404.23[\mathrm{M}+\mathrm{H}]^{+}$, found: 404.3.

Product contains significant amounts of tris(pyrrolidinophosphine) oxide: ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right)$ $\delta=3.05-2.95\left(\mathrm{~m}, 6 \times \mathrm{CH}_{2} \mathrm{~N}\right), 1.79-1.65\left(\mathrm{~m}, 6 \times \mathrm{CH}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=45.85\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{p}}=4.1 \mathrm{~Hz}\right.$, $6 \times \mathrm{CH}_{2} \mathrm{~N}$ ), $25.88\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{p}}=7.7 \mathrm{~Hz}, 6 \times \mathrm{CH}_{2}\right.$ ) and pyrrolidine: ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=3.18-3.06(\mathrm{~m}$, $\left.2 \times \mathrm{CH}_{2} \mathrm{~N}\right), 1.86-1.81\left(\mathrm{~m}, 2 \times \mathrm{CH}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=44.89\left(2 \times \mathrm{CH}_{2} \mathrm{~N}\right), 23.61\left(2 \times \mathrm{CH}_{2}\right)$.

## $\boldsymbol{N}^{\text {E-Acryloyl-L-lysine-4-(6-nitropyridin-2-yl)piperazide×2TFA (5m) }}$



Compound $5 \mathbf{m}$ ( 108 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 m}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.12\left(\mathrm{~d},{ }^{3} \mathrm{~J}=4.0 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right.$), $8.07\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.94 (dd, $\left.{ }^{3} \mathrm{~J}=8.5,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 7.52\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 7.34\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right), 6.17$ (dd, ${ }^{3} J=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.01 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.52 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.47-4.38\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.79-3.52\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.20-3.04 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.64 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.51-1.20 (m, $\left.4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right)$; ${ }^{13}$ C-NMR (DMSO- $d_{6}$ ) $\delta=167.40,164.50,158.09$ ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=35.1 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 157.19, 155.22, $141.15(\mathrm{C}-4), 131.77\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $124.85\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $113.27(\mathrm{C}-3), 105.83(\mathrm{C}-5), 49.51\left(\mathrm{C}_{\alpha}\right)$, $44.31\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $44.16\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $43.90\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.23\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.96\left(\mathrm{C}_{\varepsilon}\right), 30.03\left(\mathrm{C}_{\beta}\right), 28.68\left(\mathrm{C}_{\bar{\delta}}\right), 21.25\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=-74.56(\mathrm{~s}$, TFA); MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{~N}_{6} \mathrm{O}_{4}: 391.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 391.3.

## $\boldsymbol{N}^{\text {E }}$-Acryloyl-L-Iysine-4-(6-tert-butylpyridin-2-yl)piperazide×2TFA (5n)



Compound $\mathbf{5 n}$ ( 61 mg , orange oil) was synthesised according to GP VII using compound $\mathbf{4 n}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.13-8.00\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.51 (dd, $\left.{ }^{3}=8.3,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.72$ ( $d^{3}{ }^{3}=7.5 \mathrm{~Hz}, 1 \mathrm{H}$ ), $6.67\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}\right), 6.17$ (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.52 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.47$4.38\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.75-3.39\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.19-3.05(m,2H, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.74-1.63$
(m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.50-1.14 (m, 4H, $\mathrm{C}_{8} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), 1.26 (s, $9 \mathrm{H}, 3 \times \mathrm{CH}_{3}$ tert-butyl); ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO$\left.d_{6}\right) \delta=-73.76$ (s, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{22} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{2}: 402.29[\mathrm{M}+\mathrm{H}]^{+}$, found: 402.2.

## $\boldsymbol{N}^{\boldsymbol{E}}$-Acryloyl-L-lysine-4-(6-(2-fluoroethoxy)pyridin-2-yl)piperazide×2TFA (50)



Compound $\mathbf{5 0}$ ( 16 mg , colourless solid) was synthesised according to GP VII using compound 40. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.18-8.09\left(\mathrm{~m}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.07\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{8} \mathrm{H}\right), 7.51(\mathrm{t}$, $\left.{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.39\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of pyridine), 6.24-6.10(m,2H,CH=CH, H of pyridine), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.52 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.2 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}), 4.72\left(\mathrm{dm},{ }^{2} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=48.0 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right), 4.45\left(\mathrm{dm},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=28.0 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}\right.$ $\left.\mathrm{CH}_{2}-\mathrm{F}\right)$, 4.45-4.41 (m, 1H, $\mathrm{C}_{\mathrm{a}} \mathrm{H}$ ), 3.81-3.36 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.24-3.02 (m, 2 H , $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.84-1.60 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.58-1.16 (m, 4H, $\mathrm{C}_{6} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=167.29$ ( $\mathrm{C}_{\alpha} \mathrm{CON}$ ), $164.51\left(\mathrm{CON}_{\varepsilon}\right), 161.53(\mathrm{C}-6), 157.25(\mathrm{C}-2), 140.81(\mathrm{C}-4), 131.79\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 124.83$ $\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 98.99\left(\mathrm{CH}\right.$ of pyridine), 98.25 ( CH of pyridine), $82.15\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=165.8 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}{ }^{-}\right.$ F), $64.24\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=19.1 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right) .49 .49\left(\mathrm{C}_{\mathrm{a}}\right), 44.72\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.34\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.28\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.32\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.98\left(\mathrm{C}_{\varepsilon}\right), 30.05\left(\mathrm{C}_{\beta}\right), 28.67$ ( $\mathrm{C}_{\delta}$ ), $21.26\left(\mathrm{C}_{\mathrm{\gamma}}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=-74.51$ ( $\mathrm{s}, \mathrm{TFA}$ ) ; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{20} \mathrm{H}_{31} \mathrm{FN}_{5} \mathrm{O}_{3}$ : $408.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 408.0.

## $N^{\boldsymbol{E}}$-Acryloyl-L-lysine-4-(6-iodopyridin-2-yl)piperazide $\times 2$ TFA (5p)



Compound 5p (49 mg, yellow, waxy solid) was synthesised according to GP VII using compound 4p. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.11\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.06\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}\right), 7.24$ (dd,
${ }^{3} \mathrm{~J}=8.4,7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), $7.08\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, \mathrm{H}-5\right), 6.86\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), 6.17 ( dd , ${ }^{3} J=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1,{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.42 (broad s, $1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), $3.95-3.37\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.20-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74-1.65 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.50-1.19 (m,4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$, $\mathrm{C}_{\overline{5}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.31$ (CON), $164.50\left(\mathrm{CON}_{\varepsilon}\right), 158.53(\mathrm{C}-2), 157.97$ (psd,
 $116.20(\mathrm{C}-6), 106.19(\mathrm{C}-3), 49.50\left(\mathrm{C}_{\alpha}\right), 44.39\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.23\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.95\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.27\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.97\left(\mathrm{C}_{\varepsilon}\right), 30.03\left(\mathrm{C}_{\beta}\right), 28.67\left(\mathrm{C}_{\bar{\delta}}\right), 21.25$ $\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.29(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}(E S I+): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{~N}_{5} \mathrm{O}_{2}$ : $472.12[\mathrm{M}+\mathrm{H}]^{+}$, found: 472.0.

## $N^{\boldsymbol{E}}$-Acryloyl-L-lysine-4-(6-phenylpyridin-2-yl)piperazide $\times 2$ TFA (5q)



Compound $5 \mathbf{q}$ ( 70 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 q}$. Analytical data (NMR, MS) of this compound were not recorded.

## $\boldsymbol{N}^{\text {E-Acryloyl-L-lysine-4-(6-bromopyridin-2-yl)piperazide×2TFA (5r) }}$



Compound $5 \mathbf{5 r}$ ( 41 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 r}$. ${ }^{1} \mathrm{H}$ NMR (DMSO- $d_{6}$ ) $\delta=8.15-8.08\left(\mathrm{~m}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.58 \mathrm{~Hz}, \mathrm{~N}_{\varepsilon} \mathrm{H}\right.$ ), $7.49\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.4\right.$, $7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4), 6.86$ (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), $6.85\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right) 6.17$ (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.50-4.14\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.71-3.40\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of
piperazine), 3.19-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74-1.66 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.50-1.22 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$, $\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=167.32$ (CON), $164.50\left(\mathrm{CON}_{\varepsilon}\right), 158.53$ (C-2), 158.09 ( q , ${ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.7 \mathrm{~Hz}, \mathrm{CO}$ of TFA), $140.54(\mathrm{C}-4), 139.11(\mathrm{C}-6), 131.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.87\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $115.92(\mathrm{C}-5), 105.91(\mathrm{C}-3), 49.50\left(\mathrm{C}_{\alpha}\right), 44.43\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.22\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.97\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.26\left(\mathrm{CH}_{2}\right.$ of piperazine $), 37.96\left(\mathrm{C}_{\varepsilon}\right), 30.03\left(\mathrm{C}_{\beta}\right), 28.67\left(\mathrm{C}_{\bar{\delta}}\right), 21.24$ $\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.64$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{BrN}_{5} \mathrm{O}_{2}$ : $424.13\left[\mathrm{M}\left({ }^{(99} \mathrm{Br}\right)+\mathrm{H}\right]^{+}$, found: 424.0.

## $\boldsymbol{N}^{\text {E-Acryloyl-L-lysine-4-(pyridin-2-yl)piperazide×2TFA (5s) }}$



Compound $5 \mathbf{5}$ ( 89 mg , light brown oil) was synthesised according to GP VII using compound 4s. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.18-8.09\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}, \mathrm{H}-6\right), 8.06\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.74-7.67$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-4$ ), 7.02 (d, ${ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.78 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=5.6,6.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.17 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $4.46-4.41\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.75-3.46\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.19-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74-1.65 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.49-1.21 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$, $\mathrm{C}_{\overline{\mathrm{H}}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=167.38(\mathrm{CON}), 164.51\left(\mathrm{CON}_{\varepsilon}\right), 158.08\left(\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=34.9 \mathrm{~Hz}\right.$, CO of TFA), $144.79(\mathrm{C}-6), 139.29(\mathrm{C}-4), 131.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.88\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 116.05 (psd, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=293.0 \mathrm{~Hz}, \mathrm{CF}_{3}$ of TFA), $113.40(\mathrm{C}-5)$, $108.71(\mathrm{C}-3), 49.48\left(\mathrm{C}_{\alpha}\right), 44.91\left(\mathrm{CH}_{2}\right.$ Piperazin), $44.43\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $44.12\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $41.23\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.96\left(\mathrm{C}_{\varepsilon}\right)$, $30.01\left(\mathrm{C}_{\beta}\right)$, $28.69\left(\mathrm{C}_{\delta}\right)$, $21.23\left(\mathrm{C}_{\gamma}\right)$, signal for $\mathrm{C}-2$ is not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $\left.d_{6}\right) \delta=-74.49$ (s, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{18} \mathrm{H}_{28} \mathrm{~N}_{5} \mathrm{O}_{2}: 346.22[\mathrm{M}+\mathrm{H}]^{+}$, found: 346.2.

## $N^{\text {-Acryloyl-L-lysine-4-(pyridin-4-yl)piperazide×2TFA (5t) }}$



Compound $5 \mathbf{t}$ ( 60 mg , brown oil) was synthesised according to GP VII using compound $\mathbf{4 t}$. ${ }^{1} \mathrm{H}$ NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.31\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6\right), 8.24-8.12\left(\mathrm{~m}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.09\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.21 (d, ${ }^{3} \mathrm{~J}=7.7 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5$ ), 6.26-6.13 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.10-5.94 (m, 1H, $\mathrm{CH}=\mathrm{CHH}$ ), 5.62-5.48 (m, 1H, CH=CHH), 4.50-4.35 (m, 1H, CaH), 3.95-3.55 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.22-2.91 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.84-1.56 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.50-1.17 (m, 4H, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right)$; ${ }^{13} \mathrm{C}-$ NMR (DMSO- $d_{6}$ ) $\delta=168.15\left(\mathrm{C}_{a} \mathrm{CON}\right.$ ), $164.96\left(\mathrm{CON}_{\varepsilon}\right), 158.47$ ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=33.6 \mathrm{~Hz}$, CO of TFA), $157.10(\mathrm{C}-1), 140.35(\mathrm{C}-3,5), 132.22\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 125.34\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 108.02(\mathrm{C}-2,6)$, $49.89\left(\mathrm{C}_{\alpha}\right), 45.85\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.28\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.82\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.39\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.35\left(\mathrm{C}_{\varepsilon}\right), 30.36\left(\mathrm{C}_{\beta}\right), 29.14\left(\mathrm{C}_{\delta}\right), 21.66\left(\mathrm{C}_{\vee}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=-74.22$ (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{18} \mathrm{H}_{28} \mathrm{~N}_{5} \mathrm{O}_{2}: 346.22[\mathrm{M}+\mathrm{H}]^{+}$, found: 346.2.

## $\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-phenylpiperazide $\times 2$ TFA (5u)



Compound $\mathbf{5 u}$ ( 60 mg , orange crystals) was synthesised according to GP VII using compound 4u. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.14\left(\mathrm{~d},{ }^{3} \mathrm{~J}=3.6 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right.$), $8.08\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.30-$ 7.17 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{H}-3,5$ ), 7.04-6.95 (m, 2H, H-2,6), 6.86-6.80 (m, 1H, H-4), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $6.03\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right.$ ), 5.53 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right)$, 4.49-4.36 (m, 1H, CaH), 3.80-3.53 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), 3.31-2.98 (m, $6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.82-1.61 (m, $2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.52-1.10 (m, $4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}$, $\left.\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=167.21\left(\mathrm{C}_{\alpha} \mathrm{CON}\right), 164.58\left(\mathrm{CON}_{\varepsilon}\right), 158.46\left(\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=36.6 \mathrm{~Hz}, \mathrm{CO}\right.$
of TFA), $150.49(\mathrm{C}-1), 131.85\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 129.07(\mathrm{C}-3,5), 124.88\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 119.74(\mathrm{C}-4)$, 116.09 (C-2,6), $115.69\left(\mathrm{q},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=292.4 \mathrm{~Hz}, \mathrm{CF}_{3}\right.$ of TFA), $49.49\left(\mathrm{C}_{\alpha}\right), 48.82\left(\mathrm{CH}_{2}\right.$ of piperazine), $48.36\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.71\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.58\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.05\left(\mathrm{C}_{\varepsilon}\right)$, $30.14\left(\mathrm{C}_{\beta}\right), 28.73\left(\mathrm{C}_{\delta}\right), 21.32\left(\mathrm{C}_{\mathrm{\gamma}}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.95(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}\left(E S I^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{19} \mathrm{H}_{29} \mathrm{~N}_{4} \mathrm{O}_{2}$ : $345.23[\mathrm{M}+\mathrm{H}]^{+}$, found: 345.2.

## $\boldsymbol{N}^{\text {E }}$-Acryloyl-L-lysine-4-(6-chloropyridin-2-yl)piperazide $\times 2$ TFA (5v)



Compound $5 \mathbf{v}$ (107 mg, yellow oil) was synthesised according to GP VII using compound $\mathbf{4 v}$.
${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.17-8.08\left(\mathrm{~m}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.06\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.60\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.4\right.$, $7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ ), 6.83 (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ ), 6.72 (d, ${ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ ), 6.17 (dd, ${ }^{3} J=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} J=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.53 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.47-4.37(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C} \alpha \mathrm{H}), 3.73-3.41\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.20-3.04 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74-1.65 (m, $2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.51-1.22 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.33(\mathrm{CON}), 164.50\left(\mathrm{CON}_{\varepsilon}\right), 158.43(\mathrm{C}-2), 158.15(\mathrm{q}$, ${ }^{2} J_{\mathrm{C}, \mathrm{F}}=36.3 \mathrm{~Hz}, \mathrm{CO}$ of TFA), $148.10(\mathrm{C}-6), 140.75(\mathrm{C}-4), 131.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.87\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 115.68 (psd, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=293.0 \mathrm{~Hz}, \mathrm{CF}_{3}$ of TFA), $112.06(\mathrm{C}-5), 105.66(\mathrm{C}-3), 49.50\left(\mathrm{C}_{\alpha}\right), 44.45\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.23\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.99\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.27\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $37.97\left(\mathrm{C}_{\varepsilon}\right), 30.03\left(\mathrm{C}_{\beta}\right), 28.67\left(\mathrm{C}_{\delta}\right), 21.24\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.77(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}$ $\left(E S I^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{18} \mathrm{H}_{27} \mathrm{CIN}_{5} \mathrm{O}_{2}: 380.18\left[\mathrm{M}\left({ }^{35} \mathrm{CI}\right)+\mathrm{H}\right]^{+}$, found: 380.1.

## $N^{\text {E-Acryloyl-L-lysine-4-(3-methylphenyl)piperazide×2TFA (5w) }}$



Compound 5 w ( 54 mg , yellow oil) was synthesised according to GP VII using compound 4 w . ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.15-8.02\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.12\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.82-6.73$ ( $\mathrm{m}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of phenyl), 6.65 (d, ${ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.54\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{C}=\mathrm{CHH}$ ), 4.48-4.37 (m, 1H, $\mathrm{C}_{a} \mathrm{H}$ ), 3.76-3.47 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), $3.25-3.00(\mathrm{~m}, 6 \mathrm{H}$, $2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.26\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.75-1.61\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.52-1.21(\mathrm{~m}, 4 \mathrm{H}$, $\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.11,164.50,158.03$ ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.0 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 150.57, 138.10, $131.78\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $128.83(\mathrm{C}-5), 124.87\left(\mathrm{CH}_{2}=\mathrm{C}\right), 120.39(\mathrm{C}$ of phenyl), 116.63 (C of phenyl), 113.18 (C of phenyl), $49.44\left(\mathrm{C}_{\alpha}\right), 48.74\left(\mathrm{CH}_{2}\right.$ of piperazine), $48.31\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.69\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.58\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.00\left(\mathrm{C}_{\varepsilon}\right), 30.07\left(\mathrm{C}_{\beta}\right), 28.68$ $\left(\mathrm{C}_{\delta}\right), 21.36\left(\mathrm{CH}_{3}\right), 21.26\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.46(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{20} \mathrm{H}_{31} \mathrm{~N}_{4} \mathrm{O}_{2}: 359.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 359.3.

## $N^{\boldsymbol{k}}$-Acryloyl-L-lysine-4-(4-nitrophenyl)piperazide×2TFA (5x)



Compound $\mathbf{5 x}$ ( 73 mg , brown oil) was synthesised according to GP VII using compound $\mathbf{4 x}$. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.15-8.03\left(\mathrm{~m}, 6 \mathrm{H}, \mathrm{H}-3,5, \mathrm{NH}_{3}{ }^{+}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.09-7.03$ (m, 2H, H-2,6), 6.17 (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3}=17.1 \mathrm{~Hz},{ }^{2}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.47-4.40\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.77-3.42\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.19-3.04 (m, 2H, C $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74-1.65 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.50-1.21 (m, 4H, C $\mathrm{C}_{2} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ );
${ }^{13}$ C-NMR (DMSO- $d_{6}$ ) $\delta=167.45,164.50$, 158.03 ( $q$, ${ }^{2}{ }^{J}{ }_{\mathrm{C}, \mathrm{F}}=34.5 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 154.27, 137.25, $131.76\left(\mathrm{CH}_{2}=\mathrm{C}\right), 125.69(\mathrm{C}-3,5), 124.89\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112.76(\mathrm{C}-2,6), 49.46\left(\mathrm{C}_{\alpha}\right), 46.20\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.64\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.92\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.20\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $37.95\left(\mathrm{C}_{\varepsilon}\right), 29.99\left(\mathrm{C}_{\beta}\right), 28.69\left(\mathrm{C}_{\delta}\right), 21.25\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.43(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}$ (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{19} \mathrm{H}_{28} \mathrm{~N}_{5} \mathrm{O}_{4}: 390.21[\mathrm{M}+\mathrm{H}]^{+}$, found: 390.2.
$\boldsymbol{N}^{\boldsymbol{k}}$-Acryloyl-D-Iysine-4-(6-methylpyridin-2-yl)piperazide $\times 2$ TFA (5y)


Compound $5 \mathbf{5}$ ( 196 mg , yellow oil) was synthesised according to GP VII using compound $\mathbf{4 y}$.
${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.13\left(\mathrm{~d},{ }^{3} \mathrm{~J}=4.5 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 8.07\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}\right), 7.66$ (ps-t, $\left.{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.87\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ ), $6.68\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.18$ (dd, ${ }^{3} J=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.49-4.38(m,1H, CaH), 3.77-3.41(m,8H,4×CH2 of piperazine), 3.22-3.01 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.75-1.64\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.54-1.19$ ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=167.40(\mathrm{CON}), 164.52\left(\mathrm{CON}_{\varepsilon}\right), 158.18$ (q, ${ }^{2} J_{\mathrm{C}, \mathrm{F}}=35.9 \mathrm{~Hz}, \mathrm{CO}$ TFA), $131.79\left(\mathrm{CH}_{2}=C\right)$, $124.88\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.77\left(\mathrm{psd},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=291.8 \mathrm{~Hz}, \mathrm{CF}_{3}\right.$ of TFA), $112.86(\mathrm{C}-5), 49.48\left(\mathrm{C}_{\alpha}\right), 45.27\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.79\left(\mathrm{CH}_{2}\right.$ of piperazine), 44.13 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.24\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.97\left(\mathrm{C}_{\varepsilon}\right), 30.02\left(\mathrm{C}_{\beta}\right), 28.70\left(\mathrm{C}_{\bar{\delta}}\right), 21.24\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-2,3,4,6$ and $\mathrm{CH}_{3}$ are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ): $\delta=-74.72$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{19} \mathrm{H}_{30} \mathrm{~N}_{5} \mathrm{O}_{2}: 360.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 360.2.

## $\boldsymbol{N}^{\varepsilon}$-Propionyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×2TFA (5z)



Compound $5 \mathbf{z}$ ( 103 mg , colourless oil) was synthesised according to GP VII using compound 4z. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.11\left(\mathrm{~d},{ }^{3} \mathrm{~J}=3.7 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{NH}_{3}{ }^{+}\right), 7.70\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.55(\mathrm{t}$, $\left.{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 6.75\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}\right), 6.62\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}\right), 4.46-4.37\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right)$, 3.78-3.40 (m, 8H, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.12-2.96 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.35\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right)$, $2.03(\mathrm{q}$, $\left.{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 2 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 1.78-1.63\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.46-1.19\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right), 0.96(\mathrm{t}$, $\left.{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 3 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=172.69\left(\mathrm{CON}_{\varepsilon}\right), 167.30\left(\mathrm{C}_{a} \mathrm{CON}\right), 158.01$ ( q , ${ }^{3} J_{\mathrm{C}, \mathrm{F}}=34.3 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 112.73 (CH of pyridine), $49.52\left(\mathrm{C}_{\mathrm{a}}\right), 44.96\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.52\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $44.31\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.35\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.85\left(\mathrm{C}_{\varepsilon}\right)$, $30.03\left(\mathrm{C}_{\beta}\right), 28.81\left(\mathrm{C}_{\bar{\delta}}\right), 28.49\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 23.59\left(-\mathrm{CH}_{3}\right), 21.20\left(\mathrm{C}_{\mathrm{\gamma}}\right), 9.94\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$, signals for $4 \times C$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ): $\delta=-74.35$ ( s , TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{19} \mathrm{H}_{32} \mathrm{~N}_{5} \mathrm{O}_{2}: 362.26[\mathrm{M}+\mathrm{H}]^{+}$, found: 362.1.

## $\boldsymbol{N}^{\text {E-Propionyl-L-Iysine-4-(6-nitropyridin-3-yl)piperazide (5aa) }}$



Compound 5aa (yellow oil) was synthesised according to GP VII using compound 4aa. After removal of volatile components in a $\mathrm{N}_{2}$ stream the residue was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(20 \mathrm{~mL})$ and the organic phase was washed with $\mathrm{NaHCO}_{3}(2 \times 10 \mathrm{~mL})$. Then, the solvent was removed in vacuo and the residue was dissolved in a mixture of water-acetonitrile 3:1 ( 2 mL ) and the solution was lyophilised. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.26$ ( $\mathrm{d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ ), 8.18 ( d , $\left.{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 7.71\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.49\left(\mathrm{dd},{ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right)$,
3.79-3.46 (m, 9H, CaH, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.08-2.92 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.03\left(\mathrm{q},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 2 \mathrm{H}\right.$, $\left.-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 1.57-1.47\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.45-1.19\left(\mathrm{~m}, 5 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}^{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 0.96\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}\right.$, $3 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}$ ), signal for $\mathrm{Na}_{\mathrm{a}} \mathrm{H}_{2}$ is not visible; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=173.27,172.59\left(\mathrm{C}_{a} \mathrm{CON}\right.$, $\left.\mathrm{CON}_{\varepsilon}\right), 157.75$ ( $\mathrm{q},{ }^{2}{ }^{\mathrm{J}, \mathrm{F}} \mathrm{F}=30.5 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 149.48 (C-6), 146.84 (C-3), 133.51 (C-2), 120.62 (C-4), 119.80 (C-5), 117.36 ( $\mathrm{q},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=301.1 \mathrm{~Hz}, \mathrm{CF}_{3}$ of TFA), $49.95\left(\mathrm{C}_{a}\right), 46.07\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.63\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.63\left(\mathrm{CH}_{2}\right.$ of piperazine $), 40.66\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.24\left(\mathrm{C}_{\varepsilon}\right), 34.27\left(\mathrm{C}_{\beta}\right), 29.14\left(\mathrm{C}_{\bar{\delta}}\right), 28.49\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 22.52\left(\mathrm{C}_{\gamma}\right)$, $9.99\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right)$; MS (ESI $\left.{ }^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{18} \mathrm{H}_{29} \mathrm{~N}_{6} \mathrm{O}_{4}$ : $393.22[\mathrm{M}+\mathrm{H}]^{+}$, found: 393.1.

## Final inhibitors 6-21

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\kappa}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (6a) ${ }^{28}$



Compound 6a ( $26 \mathrm{mg}, 23 \%$, white solid) was synthesised according to GP VIII using compound $5 \mathrm{a}(0.17 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.40$ ( d , ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}$ ), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.70$ (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.32-7.22 (m, 4H, 4×H of phenyl), 7.21-7.13 (m, 1H, H of phenyl), 6.89 (broad s, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.70 ( $\mathrm{d},{ }^{3} \mathrm{~J}=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.19 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.74-4.66 (m, 1H, $\left.\mathrm{C}_{a} \mathrm{H}\right), 3.76-3.38\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.14-3.02 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.41\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.71-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.59-1.47\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right)$, 1.46-1.35 (m, 2H, C ${ }_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.31-1.17 (m, 2H, C $\mathrm{C}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.16$, 169.79, $164.42\left(\mathrm{CON}_{\varepsilon}\right), 136.35\left(\mathrm{C}-1\right.$ of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.96(2 \times \mathrm{C}$ of phenyl), $128.13(2 \times \mathrm{C}$ of phenyl), $126.29\left(\mathrm{C}-4\right.$ of phenyl), $124.76\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112.72\left(\mathrm{C}-5\right.$ of phenyl), $48.16\left(\mathrm{C}_{\alpha}\right), 45.45$ ( $\mathrm{CH}_{2}$ of piperazine), $45.06\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.05\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.93\left(\mathrm{CH}_{2}\right.$-phenyl), $40.90\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.27\left(\mathrm{C}_{\varepsilon}\right)$, $31.28\left(\mathrm{C}_{\beta}\right)$, $28.82\left(\mathrm{C}_{\delta}\right)$, $22.56\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-2,3,4,6$ of pyridine and $\mathrm{CH}_{3}$ are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.57$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{3}: 478.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 478.1.

## $N^{\alpha}$-Phenylacetyl- $N^{k}$-acryloyl-D-Iysine-4-(6-methylpyridin-2-yl)piperazide×TFA (6b)



Compound 6b ( $109 \mathrm{mg}, 70 \%$, yellow oil) was synthesised according to GP VIII using compound $5 y$ ( 0.28 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.40(\mathrm{~d}$, $\left.{ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.79\left(\mathrm{ps}-\mathrm{t},{ }^{3} \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), $7.31-7.23\left(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}\right.$ of phenyl), $7.22-7.15\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}\right.$ of phenyl), $6.98\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-3$ of pyridine), 6.75 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.19 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.54\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{C}=\mathrm{CHH}$ ), 4.74-4.66 (m, 1H, $\left.\mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.76-3.40\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-Phenyl), $3.13-3.02\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 2.45\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.70-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.59-1.47(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.47-1.34 (m, 2H, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.33-1.17 (m, 2H, Cy $\mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=170.25$, 169.83, $164.43\left(\mathrm{CON}_{\varepsilon}\right), 158.34,158.00,136.35\left(\mathrm{C}-1\right.$ of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.97(2 \times \mathrm{C}$ of phenyl), $128.14\left(2 \times \mathrm{C}\right.$ of phenyl), 126.30 ( $\mathrm{C}-4$ of phenyl), $124.78\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112.83(\mathrm{C}-5$ of pyridine), $48.18\left(\mathrm{C}_{\alpha}\right), 45.68\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.29\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.90\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.93\left(\mathrm{CH}_{2}\right.$-phenyl), $40.78\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.27\left(\mathrm{C}_{\varepsilon}\right), 31.25\left(\mathrm{C}_{\beta}\right), 28.84\left(\mathrm{C}_{\delta}\right)$, $22.56\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-3,4$ of pyridine and $\mathrm{CH}_{3}$ are not visible; ${ }^{19} \mathrm{~F}$-NMR $\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.86$ (s, TFA); MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{3}: 478.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 478.1.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(pyridin-2-yl)piperazide×TFA (7a)



Compound 7a (22 mg, 83\%, colourless oil) was synthesised according to GP VIII using compound 5 s ( 0.05 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.39$ (d,
${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.08 (dd, ${ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6$ of pyridine), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.86-7.79 (m, 1H, H-4 of pyridine), 7.30-7.09 (m, 6H,5×H of phenyl, H-3 of pyridine), $6.87-6.82\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.74-4.67 (m, $\left.1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.76-3.37\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.11-3.04 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.73-1.59 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.58-1.47 (m, 1H, $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.46-1.34 (m, 2H, C $\mathrm{C}_{2}$ ), 1.34-1.14 (m, 2H, C ${ }_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.23,169.81,164.42\left(\mathrm{CON}_{\varepsilon}\right), 136.34$ (C-1 of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.96(2 \times \mathrm{C}$ of phenyl), $128.13(2 \times \mathrm{C}$ of phenyl), 126.29 (C-4 of phenyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 113.08\left(\mathrm{C}-5\right.$ of pyridine), $48.17\left(\mathrm{C}_{a}\right), 45.15\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.72\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.85\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.92\left(\mathrm{CH}_{2}\right.$-phenyl), 40.74 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.26\left(\mathrm{C}_{\varepsilon}\right), 31.26\left(\mathrm{C}_{\beta}\right), 28.83\left(\mathrm{C}_{\delta}\right)$, $22.55\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.65$ ( s , TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{3}$ : $464.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 464.2.

## $N^{a}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-Iysine-4-(6-fluoropyridin-2-yl)piperazide×TFA (7b)



Compound 7b ( $57 \mathrm{mg}, 45 \%$, yellow solid) was synthesised according to GP VIII using compound 5d ( 0.21 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.38$ (d, $\left.{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.69(\mathrm{ps}-\mathrm{q}, \mathrm{J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.32-7.22$ ( $\mathrm{m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), $7.22-7.13$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}$ of phenyl), 6.66 (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=8.2 \mathrm{~Hz} \text {, }}$ ${ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), $6.30\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=7.7 \mathrm{~Hz},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $4.75-4.65\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.68-3.24\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), $3.13-3.01\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.70-1.59\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.59-1.47$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.47-1.34 (m, 2H, $\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.31-1.19 (m, 2H, C $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=170.00,169.74,164.40\left(\mathrm{CON}_{\varepsilon}\right), 161.94$ ( $\mathrm{d},{ }^{1}{ }^{\mathrm{J}} \mathrm{C}, \mathrm{F}=233.2 \mathrm{~Hz}, \mathrm{C}-6$ of pyridine), 157.64 ( d , ${ }^{3} J_{\mathrm{C}, \mathrm{F}}=15.7 \mathrm{~Hz}, \mathrm{C}-2$ of pyridine), 142.68 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.2 \mathrm{~Hz}, \mathrm{C}-4$ of pyridine), 136.36 (C-1 of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.95(2 \times \mathrm{C}$ of phenyl), 128.12 ( $2 \times \mathrm{C}$ of phenyl), 126.28 ( $\mathrm{C}-4$ of phenyl), $124.73\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 103.59 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.9 \mathrm{~Hz}, \mathrm{C}-3$ of pyridine), $95.64\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=37.0 \mathrm{~Hz}\right.$,

C-5 of pyridine), $48.12\left(\mathrm{C}_{\mathrm{a}}\right), 44.56\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.25\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.10\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$-phenyl), $41.01\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.27\left(\mathrm{C}_{\varepsilon}\right), 31.35\left(\mathrm{C}_{\beta}\right), 28.80$ ( $\mathrm{C}_{\bar{\delta}}$ ), $22.56\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-68.80-68.88(\mathrm{~m}, \mathrm{~F}-6),-74.70(\mathrm{~s}, \mathrm{TFA}) ;$ MS (ESI $)$ : $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{FN}_{5} \mathrm{O}_{3}$ : $482.26[\mathrm{M}+\mathrm{H}]^{+}$, found: 482.1 .

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\text {en }}$-acryloyl-L-lysine-4-(6-chloropyridin-2-yl)piperazide×TFA (7c)



Compound 7c ( $57 \mathrm{mg}, 50 \%$, light yellow oil) was synthesised according to GP VIII using compound $5 \mathbf{v}(0.19 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.38$ ( d , ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.57\left(\mathrm{dd},{ }^{3} \mathrm{~J}=7.51,8.38 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), $7.30-7.24(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), $7.21-7.15(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 6.76 (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.69 (d, ${ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} \mathrm{H}\right), 4.74-4.67\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.66-3.44\left(\mathrm{~m}, 8 \mathrm{H}, 3 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.43-3.28 (m, 2H, CH $\mathrm{CH}_{2}$ of piperazine), 3.11-3.04 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.70-1.59 (m, $\left.1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H} H\right), 1.59-1.47\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.34\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{0} \mathrm{H}_{2}\right), 1.30-1.19\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-$ NMR (DMSO- $d_{6}$ ) $\delta=169.99,169.74,164.40\left(\mathrm{CON}_{\varepsilon}\right), 158.49$ (C-2 of pyridine), 148.06 (C-6 of pyridine), 140.64 ( $\mathrm{C}-4$ of pyridine), 136.36 ( $\mathrm{C}-1$ of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.95(2 \times \mathrm{C}$ of phenyl), 128.12 ( $2 \times \mathrm{C}$ of phenyl), 126.29 ( $\mathrm{C}-4$ of phenyl), $124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right), 111.83(\mathrm{C}-5$ of pyridine), 105.51 ( $\mathrm{C}-3$ of pyridine), $48.13\left(\mathrm{C}_{\alpha}\right), 44.51\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.26\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.06\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}-\right.$ Phenyl $), 41.03\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.27$ $\left(\mathrm{C}_{\varepsilon}\right), 31.35\left(\mathrm{C}_{\beta}\right), 28.80\left(\mathrm{C}_{\bar{\delta}}\right), 22.57\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.82(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}\left(E S I^{+}\right):$ $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{CIN}_{5} \mathrm{O}_{3}$ : $498.23\left[\mathrm{M}\left({ }^{35} \mathrm{CI}\right)+\mathrm{H}\right]^{+}$, found: 498.0.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\text {E }}$-acryloyl-L-Iysine-4-(6-bromopyridin-2-yl)piperazidexTFA (7d)



Compound 7d (16 mg, 28\%, colourless solid) was synthesised according to GP VIII using compound 5 r ( 0.09 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.38$ ( d , $\left.{ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.46\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.1,7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine $)$, 7.31-7.22 (m, 4H, H-2,3,5,6 of phenyl), 7.21-7.13 (m, 1H, H-4 of phenyl), 6.82 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{H}$ of pyridine), 6.79 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.0 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}), 4.74-4.66\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.67-3.25\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.13-3.03 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.71-1.58 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right), 1.58-1.46\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.46-1.34(\mathrm{~m}, 2 \mathrm{H}$, $\mathrm{C}_{\mathrm{\delta}} \mathrm{H}_{2}$ ), 1.33-1.19 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=169.98,169.73,164.39\left(\mathrm{C}_{a} \mathrm{CON}\right.$, $\mathrm{CON}_{\alpha}, \mathrm{CON}_{\varepsilon}$ ), 158.59 ( $\mathrm{C}-2$ of pyridine), 140.43 (C-4 of pyridine), 139.08 (C-6 of pyridine), $136.35\left(\mathrm{C}-1\right.$ phenyl), $131.84\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.94(2 \times \mathrm{CH}$ of phenyl), $128.12(2 \mathrm{CH}$ of phenyl), 126.28 ( $\mathrm{C}-4$ of phenyl), $124.73\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.69(\mathrm{CH}$ of pyridine), 105.76 ( CH of pyridine), $48.12\left(\mathrm{C}_{\alpha}\right), 44.49\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.25\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.06\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $41.94\left(\mathrm{CH}_{2}\right.$-phenyl), $41.02\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.27\left(\mathrm{C}_{\varepsilon}\right), 31.35\left(\mathrm{C}_{\beta}\right), 28.79\left(\mathrm{C}_{\delta}\right), 22.57\left(\mathrm{C}_{\gamma}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{BrN}_{5} \mathrm{O}_{3}$ : $542.18\left[\mathrm{M}\left({ }^{79} \mathrm{Br}\right)+\mathrm{H}\right]^{+}$, found: 542.2.

## $N^{a}$-Phenylacetyl- $N^{\varepsilon}$-acryloyl-L-lysine-4-(6-iodopyridin-2-yl)piperazide×TFA (7e)



Compound 7 e ( $15 \mathrm{mg}, 50 \%$, yellow solid) was synthesised according to GP VIII using compound 5 p ( 0.04 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.37$ ( d ,
${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.32-7.14(\mathrm{~m}, 6 \mathrm{H}, 5 \times \mathrm{H}$ of phenyl, $\mathrm{H}-4$ of pyridine), 7.05 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.79 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.18 (dd, ${ }^{3} J=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $4.74-4.65\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.64-3.24\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), $3.13-3.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.69-1.59\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.58-1.46$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.46-1.35 (m, 2H, $\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.32-1.20 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=169.96,169.74,164.40\left(\mathrm{CON}_{\varepsilon}\right), 158.59$ ( $\mathrm{C}-2$ of pyridine), 139.45 ( $\mathrm{C}-4$ of pyridine), 136.36 ( $\mathrm{C}-1$ of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.95(2 \times \mathrm{C}$ of phenyl), $128.13(2 \times \mathrm{C}$ of phenyl), 126.29 ( $\mathrm{C}-4$ of phenyl), $124.75\left(\mathrm{CH}_{2}=\mathrm{C}\right), 123.05$ ( $\mathrm{C}-5$ of pyridine), 116.19 ( $\mathrm{C}-6$ of pyridine), 106.04 (C-3 of pyridine), $48.13\left(\mathrm{C}_{\alpha}\right), 44.46\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.26\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.04\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.96\left(\mathrm{CH}_{2}\right.$-phenyl), $41.04\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right), 31.36\left(\mathrm{C}_{\beta}\right), 28.80$ ( $\mathrm{C}_{\bar{\delta}}$ ), $22.58\left(\mathrm{C}_{\gamma}\right)$; MS ( $\mathrm{ESI}{ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{~N}_{5} \mathrm{O}_{3}: 590.16[\mathrm{M}+\mathrm{H}]^{+}$, found: 590.0.
$N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-tert-butylpyridin-2-yl)piperazide (7f)


Compound 7 f ( $16 \mathrm{mg}, 27 \%$, colourless solid) was synthesised according to GP VIII using compound $\mathbf{5 n}(0.09 \mathrm{mmol})$ and phenylacetyl chloride. To separate compound $\mathbf{7 f}$ from its $N^{a}$ trifluoroacetyl analogue, which could not be separated by RP-HPLC, the product was finally purified by column chromatography (ethyl acetate). ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.60$ (dd, ${ }^{3} \mathrm{~J}=8.3$, $7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.38-7.22\left(\mathrm{~m}, 5 \mathrm{H}, \mathrm{H}-2,3,4,5,6\right.$ of phenyl), $6.80\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$, H of pyridine), $6.69\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 6.60\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of pyridine), 6.33-6.26 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), $6.26\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right.$ ), $6.08\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.0,10.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), $5.62\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.3 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right), 4.93\left(\mathrm{td},{ }^{3} \mathrm{~J}=8.4,4.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right)$, 3.89-3.43 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), $3.60\left(\mathrm{~s}, 2 \mathrm{H}, \mathrm{CH}_{2}\right.$-phenyl), $3.35-3.22\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{8} \mathrm{H}_{2}\right)$, 1.75-1.50 (m, $4 \mathrm{H} \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), $1.36\left(\mathrm{~s}, 9 \mathrm{H}, 3 \times \mathrm{CH}_{3}\right.$ of tert-butyl), 1.35-1.21 (m, $2 \mathrm{H}, \mathrm{C}_{\mathrm{Y}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}$ NMR $\left(\mathrm{CDCl}_{3}\right) \delta=171.82,170.41,166.57,166.19,156.93,140.37$ (C-4 of pyridine), 134.37, $130.68\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 129.45(2 \times \mathrm{CH}$ of phenyl), $129.16(2 \times \mathrm{CH}$ of phenyl), $127.67(\mathrm{C}-4$ of phenyl), $126.84\left(\mathrm{CH}_{2}=\mathrm{CH}\right), 110.14(\mathrm{CH}$ of pyridine $), 106.39(\mathrm{CH}$ of pyridine $), 48.65\left(\mathrm{C}_{\alpha}\right), 46.59\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.21\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.14\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.64\left(\mathrm{CH}_{2}\right.$-phenyl), 41.76
$\left(\mathrm{CH}_{2}\right.$ of piperazine), $39.36\left(\mathrm{C}_{\varepsilon}\right)$, 37.32 (quart. C of tert-butyl), $32.79\left(\mathrm{C}_{\beta}\right)$, $29.85\left(3 \times \mathrm{CH}_{3}\right.$ tertbutyl), $28.32\left(\mathrm{C}_{\bar{\delta}}\right), 22.34\left(\mathrm{C}_{\curlyvee}\right)$; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{30} \mathrm{H}_{42} \mathrm{~N}_{5} \mathrm{O}_{3}: 520.33[\mathrm{M}+\mathrm{H}]^{+}$, found: 520.4.
$N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-phenylpyridin-2-yl)piperazide ( 7 g )


Compound 7 g ( $23 \mathrm{mg}, 34 \%$, white solid) was synthesised according to GP VIII using compound $5 \mathbf{q}(0.10 \mathrm{mmol})$ and phenylacetyl chloride. To separate compound $\mathbf{7 g}$ from its $\mathrm{N}^{a}$ trifluoroacetyl analogue 16c, which could not be separated by RP-HPLC, the product was finally purified by column chromatography (ethyl acetate). ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.92$ (dd, ${ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.4 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ of phenyl), 7.67 (dd, ${ }^{3} \mathrm{~J}=8.3,7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.497.38 (m, 3H, H-3,4,5 of phenyl), 7.38-7.32 (m, 2H, H-2,6 of benzyl), 7.31-7.24 (m, 3H, H-3,4,5 of benzyl), 7.15 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.76 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 6.68 ( d , ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), $6.26\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.2 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 6.06$ (dd, ${ }^{3} J=17.0,10.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.62 (dd, ${ }^{3} \mathrm{~J}=10.3 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.96 (td, $\left.{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz},{ }^{4} \mathrm{~J}=4.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.89-3.62\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), $3.61\left(\mathrm{~s}, 2 \mathrm{H}, \mathrm{CH}_{2}-\right.$ penzyl), 3.36-3.21 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.75-1.45 (m, 4H, $\left.\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.39-1.28\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-$ NMR $\left(\mathrm{CDCl}_{3}\right) \delta=171.88,170.40,166.59,157.66,154.81,139.86$ (C-4 of pyridine), 137.96, 134.35, $130.61\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 129.54$ (C-4 phenyl), 129.44 ( $2 \times \mathrm{C}$ of benzyl), 129.16 ( $2 \times \mathrm{C}$ of benzyl), 128.78 (C-3,5 of phenyl), 127.67 (C-4 of benzyl), 127.20 (C-2,6 of phenyl), 126.92 $\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 111.38\left(\mathrm{C}-5\right.$ of pyridine), $106.81\left(\mathrm{C}-3\right.$ of pyridine), $48.66\left(\mathrm{C}_{\mathrm{a}}\right), 45.87\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.68\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.25\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.63\left(\mathrm{CH}_{2}\right.$-phenyl), 41.95 ( $\mathrm{CH}_{2}$ of piperazine), $39.36\left(\mathrm{C}_{\varepsilon}\right), 32.80\left(\mathrm{C}_{\beta}\right)$, $28.32\left(\mathrm{C}_{\bar{\delta}}\right), 22.32\left(\mathrm{C}_{\gamma}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{32} \mathrm{H}_{38} \mathrm{~N}_{5} \mathrm{O}_{3}: 540.30[\mathrm{M}+\mathrm{H}]^{+}$, found: 540.3.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\text {E }}$-acryloyl-L-Iysine-4-(6-(2-fluoroethoxy)pyridin-2-yl)piperazide×TFA

 (7h)

Compound $\mathbf{7 h}$ ( $7 \mathrm{mg}, 42 \%$, white solid) was synthesised according to GP VIII using compound $50(0.025 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=7.45\left(\mathrm{t},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), $7.38-7.24$ ( $\mathrm{m}, 5 \mathrm{H}, \mathrm{H}-2,3,4,5,6$ of phenyl), 6.81 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, \mathrm{~N}_{a} \mathrm{H}$ ), 6.27 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 6.26-6.17 (m,3H$, \mathrm{H}-3,5$ of pyridine, $\mathrm{N}_{\varepsilon} \mathrm{H}$ ), 6.07 (dd, ${ }^{3} J=17.0,10.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 5.66 (dd, ${ }^{3} \mathrm{~J}=10.4 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.96 (td, $\left.{ }^{3} \mathrm{~J}=8.7,4.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 4.73\left(\mathrm{dm},{ }^{2} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=48.0 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right), 4.51\left(\mathrm{dm},{ }^{3} J_{\mathrm{H}, \mathrm{F}}=28.0 \mathrm{~Hz}\right.$, $2 \mathrm{H}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}$ ), 3.81-3.40 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), $3.62\left(\mathrm{~s}, 2 \mathrm{H}, \mathrm{CH}_{2}\right.$-phenyl), 3.37-3.20 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.79-1.43 (m, 4H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.39-1.22 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-{ }^{-N M R ~\left(\mathrm{CDCl}_{3}\right) ~}$ $\delta=172.32,170.27,166.90,162.26,157.40,140.82$ (C-4 of pyridine), 134.07, $130.36\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $129.45\left(2 \times \mathrm{CH}\right.$ of phenyl), $129.23\left(2 \times \mathrm{CH}\right.$ of phenyl), 127.81 ( $\mathrm{C}-4$ of phenyl), $127.37\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 100.16 (CH of pyridine), 99.06 ( CH of pyridine), 82.18 ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{c}, \mathrm{F}=}=169.4 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}$ ), 64.58 (d, $\left.{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=20.5 \mathrm{~Hz}, \mathrm{CH}_{2}-\mathrm{CH}_{2}-\mathrm{F}\right), 48.69\left(\mathrm{C}_{\alpha}\right), 45.43\left(\mathrm{CH}_{2}\right.$ of piperazine), $54.37\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.19\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.57\left(\mathrm{CH}_{2}\right.$-phenyl), $42.08\left(\mathrm{CH}_{2}\right.$ of piperazine $), 39.51$ $\left(\mathrm{C}_{\varepsilon}\right), 32.86\left(\mathrm{C}_{\beta}\right), 28.25\left(\mathrm{C}_{\delta}\right), 22.35\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=-75.90(\mathrm{~s}) ; \mathrm{MS}\left(\mathrm{ESI}^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{28} \mathrm{H}_{37} \mathrm{FN}_{5} \mathrm{O}_{4}$ : $526.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 526.3.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-Iysine-4-(6-nitropyridin-2-yl)piperazide×TFA (7i)



Compound $7 \mathbf{7 i}$ (18 mg, 59\%, yellow solid) was synthesised according to GP VIII using compound 5 m ( 0.05 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.39$ ( d , $\left.{ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.91\left(\mathrm{dd},{ }^{3} \mathrm{~J}=8.5,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), $7.49\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), $7.30-7.23(\mathrm{~m}, 5 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl, $\mathrm{H}-3$ of pyridine), $7.20-7.14\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}\right.$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.53\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), 4.75-4.67 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}$ ), 3.69-3.40 (m, 10H, $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.12-3.04 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $1.72-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H-1.59-1.48\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.47-1.34\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right), 1.32-1.20(\mathrm{~m}\right.$, $2 \mathrm{H}, \mathrm{C}_{7} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=170.08,169.76,164.39,157.22,155.22,141.02$ (C-4 of pyridine), $136.36,131.85\left(\mathrm{CH}_{2}=C\right), 128.95(2 \times \mathrm{C}$ of phenyl), $128.12(2 \times \mathrm{C}$ of phenyl), 126.28 ( $\mathrm{C}-4$ of phenyl), $124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right), 113.12$ ( $\mathrm{C}-3$ of pyridine), 105.61 ( $\mathrm{C}-5$ of pyridine), 48.16 $\left(\mathrm{C}_{\alpha}\right), 44.38\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.20\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.97\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.95$ $\left(\mathrm{CH}_{2}\right.$-phenyl), $40.99\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right), 31.34\left(\mathrm{C}_{\beta}\right), 28.81\left(\mathrm{C}_{\delta}\right), 22.58\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-$ NMR (DMSO- $d_{6}$ ) $\delta=-75.72$ (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{~N}_{6} \mathrm{O}_{5}: 509.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 509.3.

## $N^{a}$-Phenylacetyl- $N^{\varepsilon}$-acryloyl-L-lysine-4-(pyridin-3-yl)piperazide×TFA (8a)



Compound $\mathbf{8 a}$ ( $49 \mathrm{mg}, 42 \%$, colourless solid) was synthesised according to GP VIII using compound $5 \mathbf{i}(0.20 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathbf{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.43-8.39(\mathrm{~m}, 2 \mathrm{H}$,
$\mathrm{N}_{\mathrm{a}} \mathrm{H}, \mathrm{H}-2$ of pyridine), 8.22 ( $\mathrm{d},{ }^{3} \mathrm{~J}=5.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6$ of pyridine), 8.08-7.94 (m, 2H, $\mathrm{N}, \mathrm{H}, \mathrm{H}$ of pyridine), 7.88-7.76 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}$ of pyridine), 7.29-7.22 ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{H}-2,3,5,6$ of phenyl), 7.20-7.11 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} J=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.75-4.64 ( $\mathrm{m}, 1 \mathrm{H}$, $\mathrm{C}_{a} \mathrm{H}$ ), 3.81-3.15 ( $\mathrm{m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.11-3.05 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.74$1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.60-1.48\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.46-1.34\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\bar{\circ}} \mathrm{H}_{2}\right), 1.34-1.13(\mathrm{~m}, 2 \mathrm{H}$, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.07\left(\mathrm{C}_{\alpha} \mathrm{CON}\right), 169.78\left(\mathrm{CON}_{\alpha}\right), 164.41\left(\mathrm{CON}_{\varepsilon}\right), 158.15(\mathrm{q}$, ${ }^{2} J_{\mathrm{C}, \mathrm{F}}=35.6 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 147.87 (C-3 of pyridine), 136.36 (C-1 of phenyl), $131.85\left(\mathrm{CH}=\mathrm{CH}_{2}\right)$, 130.70 (C-6 of pyridine), 128.95 ( $2 \times \mathrm{CH}$ of phenyl), 128.74 (CH of pyridine), $128.13(2 \times \mathrm{CH}$ of phenyl), 128.05 (C-2 of pyridine), 126.83 ( CH of pyridine), 126.28 (C-4 of phenyl), 124.75 $\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 48.06\left(\mathrm{C}_{\alpha}\right), 46.48\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.09\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.93\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$-phenyl), $40.68\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.26\left(\mathrm{C}_{\varepsilon}\right), 31.27\left(\mathrm{C}_{\beta}\right), 28.84\left(\mathrm{C}_{\delta}\right)$, $22.57\left(\mathrm{C}_{\mathrm{Y}}\right)$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{3}: 464.27$ [ $\left.\mathrm{M}+\mathrm{H}\right]^{+}$, found: 464.4.
$N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{k}$-acryloyl-L-lysine-4-(6-fluoropyridin-3-yl)piperazide×TFA (8b)


Compound 8b ( $61 \mathrm{mg}, 51 \%$, brownish oil) was synthesised according to GP VIII using compound $5 \mathbf{j}$ ( 0.20 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ ( $\mathrm{DMSO}-d_{6}$ ) $\delta=8.39$ ( d , ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.80\left(\mathrm{dd},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=2.9 \mathrm{~Hz},{ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=1.7 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-2$ of pyridine), 7.57 (ddd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=9.1 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=7.1 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=3.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.30-7.23$ ( $\mathrm{m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), $7.20-7.12\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}\right.$ of phenyl), 7.04 (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=9.0 \mathrm{~Hz} \text {, }}$ ${ }^{3} J_{H, F}=3.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.54\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), 4.75-4.66 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}$ ), 3.72-3.40(m,6H,2×CH2 of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.18-2.84 (m, $6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.71-1.59 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.58-1.46 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.33(\mathrm{~m}, 2 \mathrm{H}$, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.33-1.17 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=169.75,169.71,164.39\left(\mathrm{CON}_{\varepsilon}\right)$, 156.92 ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=228.5 \mathrm{~Hz}, \mathrm{C}-6$ of pyridine), 145.26 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.9 \mathrm{~Hz}, \mathrm{C}-3$ of pyridine), 136.35 (C-1 of phenyl), 134.02 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=15.3 \mathrm{~Hz}, \mathrm{C}-2$ of pyridine), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 129.63(\mathrm{~d}$, ${ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.2 \mathrm{~Hz}, \mathrm{C}-4$ of pyridine), 128.94 ( $2 \times \mathrm{C}$ of phenyl), 128.12 ( $2 \times \mathrm{C}$ of phenyl), 126.28 (C-4
of phenyl), $124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 109.04 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=39.6 \mathrm{~Hz}, \mathrm{C}-5$ of pyridine), $48.77\left(\mathrm{CH}_{2}\right.$ of piperazine), $48.37\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $47.98\left(\mathrm{C}_{\alpha}\right), 44.50\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $41.97\left(\mathrm{CH}_{2}-\right.$ phenyl), $41.10\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.29\left(\mathrm{C}_{\varepsilon}\right), 31.37\left(\mathrm{C}_{\beta}\right), 28.81\left(\mathrm{C}_{\bar{\delta}}\right), 22.59\left(\mathrm{C}_{\gamma}\right)$; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-80.13(\mathrm{~s}, \mathrm{~F}-6),-74.58(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{FN}_{5} \mathrm{O}_{3}$ : $482.26[\mathrm{M}+\mathrm{H}]^{+}$, found: 482.1.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-trifluoromethylpyridin-3-yl)piperazide×TFA

 (8c)

Compound 8c ( $58 \mathrm{mg}, 80 \%$, yellow solid) was synthesised according to GP VIII using compound $5 \mathbf{k}$ ( 0.11 mmol ) and phenylacetyl chloride. ${ }^{1} \mathbf{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.44-8.36$ ( m , $2 \mathrm{H}, \mathrm{NaH}, \mathrm{H}-2$ of pyridine), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.66\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), 7.40 (dd, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.29-7.21$ ( $\mathrm{m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), 7.207.11 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} J=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.75-4.66(m,1H, $\mathrm{C}_{\mathrm{a}} \mathrm{H}$ ), 3.74-3.31 (m, $8 \mathrm{H}, 3 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.29-3.12 (m, $2 \mathrm{H}, \mathrm{CH}_{2}$ of piperazine), 3.12-3.03 (m, 2H, C $\mathrm{C}_{2}$ ), 1.73-1.60 (m, 1H, C $\mathrm{C}_{\beta} H H$ ), 1.60-1.46 (m, 1H, C ${ }_{\beta} H H$ ), 1.46-1.33 (m, 2H, C ${ }_{6} \mathrm{H}_{2}$ ), 1.32-1.18 (m, 2H, C $\mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=169.92$, 169.74, 164.39, 158.21 ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=37.5 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 147.94 ( $\mathrm{C}-3$ of pyridine), 136.91 (C-2 of pyridine), 136.35 ( $\mathrm{C}-1$ of phenyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.94(2 \times \mathrm{C}$ of phenyl), $128.12(2 \times \mathrm{C}$ of phenyl), 126.28 ( $\mathrm{C}-4$ of phenyl), $124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 122.36 ( $\mathrm{q},{ }^{1} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=272.2 \mathrm{~Hz}, \mathrm{CF}_{3}$ of pyridine), 120.91 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=2.2 \mathrm{~Hz}, \mathrm{C}-5$ of pyridine), 120.77 (C-4 of pyridine), $48.04\left(\mathrm{C}_{\mathrm{a}}\right), 46.54\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.17\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.12\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.96\left(\mathrm{CH}_{2}\right.$-phenyl), 40.85 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right), 31.33\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.59\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-$ 64.89 ( $\mathrm{s}, \mathrm{CF}_{3}$ of pyridine), -74.99 (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{33} \mathrm{~F}_{3} \mathrm{~N}_{5} \mathrm{O}_{3}: 532.25$ $[\mathrm{M}+\mathrm{H}]^{+}$, found: 532.2.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(6-nitropyridin-3-yl)piperazide×TFA (8d)



Compound 8d (59 mg, 70\%, orange solid) was synthesised according to GP VIII using compound 5b ( 0.16 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.41$ ( d , ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.22\left(\mathrm{~d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), 8.17 (d, ${ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.44 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{2} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.30-7.24\left(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}\right.$ of phenyl), $7.22-7.12\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}\right.$ of phenyl), $6.18\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{C}=\mathrm{CHH})$, 4.74-4.66 (m, 1H, $\left.\mathrm{C}_{a} \mathrm{H}\right), 3.78-3.26\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$ of phenyl), 3.12-3.01 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.73-1.59 (m, $1 \mathrm{H}, \mathrm{C}_{\beta} H H$ ), 1.59-1.47 (m, $1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.47-1.33 (m, 2H, C ${ }_{\delta} \mathrm{H}_{2}$ ), 1.33-1.18 (m, 2H, C $\mathrm{C}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.10,169.78$, $164.40\left(\mathrm{CON}_{\varepsilon}\right), 149.43,146.86,136.34$ (C-1 of phenyl), 133.52 ( $\mathrm{C}-2$ of pyridine), 131.85 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.95(2 \times \mathrm{C}$ of phenyl), $128.13(2 \times \mathrm{C}$ of phenyl), $126.30(\mathrm{C}-4$ of phenyl), 124.76 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 120.63$ ( $\mathrm{C}-4$ of pyridine), $119.77\left(\mathrm{C}-5\right.$ of pyridine), $48.09\left(\mathrm{C}_{\alpha}\right), 45.94\left(\mathrm{CH}_{2}\right.$ of piperazine), 45.58 ( $\mathrm{CH}_{2}$ of piperazine), $43.88\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$ of phenyl), 40.76 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right), 31.28\left(\mathrm{C}_{\beta}\right), 28.83\left(\mathrm{C}_{\delta}\right), 22.59\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}$-NMR (DMSO- $\left.d_{6}\right) \delta=-$ 74.86 (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{~N}_{6} \mathrm{O}_{5}$ : $509.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 509.1.

## $N^{\alpha}$-Phenylacetyl- $N^{\varepsilon}$-acryloyl-L-lysine-4-(6-carboxypyridin-3-yl)piperazide×TFA (8e)



Compound $8 \mathbf{8 f}$ ( $54 \mathrm{mg}, 0.085 \mathrm{mmol}, 1 \mathrm{eq}$.) was dissolved in a mixture of THF-methanol ( 4 mL , $3: 1$ ). To this solution 1 M NaOH ( $0.21 \mathrm{~mL}, 0.21 \mathrm{mmol}, 2.5 \mathrm{eq}$.) was added and the reaction
mixture was stirred for 20 h . Subsequently, 1 M NaOH ( $0.043 \mathrm{~mL}, 0.043 \mathrm{mmol}, 0.5 \mathrm{eq}$.) was added again and the reaction mixture was stirred for another 24 h . Finally, $1 \mathrm{M} \mathrm{HCl}(0.255 \mathrm{ml}$, $0.255 \mathrm{mmol}, 3$ eq.) was added and the solvent was removed in vacuo. The crude product was purified by preparative RP-HPLC. The product-containing fractions were combined and lyophilised to afford compound $\mathbf{8 e}(37 \mathrm{mg}, 70 \%)$ as a light yellow solid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.40\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), $8.32\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.94 (d, ${ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 7.44 (dd, ${ }^{3} \mathrm{~J}=9.0 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.30-7.22$ (m, $4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), $7.21-7.12$ (m, $1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.76-4.65\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.75-3.17\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.13-3.03 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.72-1.60 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.59-1.47 (m, $1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.46-1.35 (m, 2H, C $\mathrm{C}_{6} \mathrm{H}_{2}$ ), 1.31-1.19 (m, 2H, C $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=170.00$, 169.76, 165.02, 164.40, 158.19 ( ${ }^{2}{ }^{2}{ }^{\mathrm{J}, \mathrm{F}} \mathrm{F}=37.4 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 148.12 (C-3 of pyridine), 136.35 (C-1 phenyl), 134.53 ( $\mathrm{C}-2$ of pyridine), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.95(2 \times \mathrm{C}$ of phenyl), $128.13(2 \times \mathrm{C}$ of phenyl), 126.30 ( $\mathrm{C}-4$ of phenyl), 125.91 ( $\mathrm{C}-5$ of pyridine), $124.76\left(\mathrm{CH}_{2}=\mathrm{C}\right), 121.19$ ( $\mathrm{C}-4$ of pyridine), $48.06\left(\mathrm{C}_{\alpha}\right), 46.29\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.93\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.05\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$-phenyl), $40.84\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right)$, $31.31\left(\mathrm{C}_{\beta}\right), 28.83\left(\mathrm{C}_{\delta}\right)$, $22.59\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=-74.96$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{5}$ : $508.26[\mathrm{M}+\mathrm{H}]^{+}$, found: 508.3.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-methoxycarbonylpyridin-3-yl)piperazide×TFA

 (8f)

Compound $\mathbf{8 f}$ ( $63 \mathrm{mg}, 47 \%$, yellow oil) was synthesised according to GP VIII using compound $5 \mathbf{I}(0.21 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.40\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right)$, $8.34\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.90\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-5$ of pyridine), 7.33 (dd, ${ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.28-7.23(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), 7.19-7.13 (m, 1H, H-4 of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.75-
$4.66\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.81\left(\mathrm{~s}, \mathrm{CH}_{3}\right), 3.72-3.15\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}-$ phenyl), 3.123.03 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.72-1.60 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.58-1.46 (m, 1H, $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.46-1.34 (m, 2H, $\mathrm{C}_{8} \mathrm{H}_{2}$ ), 1.30-1.18 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=169.96,169.75,164.90$, 164.39, 158.18 ( $\mathrm{q},{ }^{2}{ }^{2} \mathrm{C}, \mathrm{F}=36.9 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 147.93 (C-3 of pyridine), 136.35, 136.00, 135.70, 131.85 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.94(2 \times \mathrm{C}$ of phenyl), 128.20 ( $2 \times \mathrm{C}$ of phenyl), 126.29 (C-4 of phenyl), 125.76 (C5 of pyridine), $124.75\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $119.87\left(\mathrm{C}-4\right.$ of pyridine), $51.80\left(\mathrm{CH}_{3}\right), 48.05\left(\mathrm{C}_{\alpha}\right), 46.22\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.84\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.09\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$-phenyl), 40.87 ( $\mathrm{CH}_{2}$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right), 31.32\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.59\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}$-NMR (DMSO- $\left.d_{6}\right) \delta=-$ 74.92 (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{28} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{5}: 522.27$ [M+H] ${ }^{+}$, found: 522.3.
$N$-Phenylacetylpyrrolidine ( 2.3 mg , white solid) was isolated as side product.
${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=7.32-7.18\left(\mathrm{~m}, 5 \mathrm{H}, 5 \times \mathrm{H}\right.$ of phenyl), $3.61\left(\mathrm{~s}, 2 \mathrm{H}, \mathrm{CH}_{2} \mathrm{CO}\right), 3.45(\mathrm{t}$, $\left.{ }^{3} \mathrm{~J}=6.8 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2} \mathrm{~N}\right), 3.28\left(\mathrm{t},{ }^{3} \mathrm{~J}=6.8 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{CH}_{2} \mathrm{~N}\right), 1.91-1.81\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{CH}_{2}\right), 1.80-1.70(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{CH}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=168.46(\mathrm{CO}), 135.72$ (C-1 of phenyl), 129.21 ( $2 \times \mathrm{C}$ of phenyl), $128.14\left(2 \times \mathrm{C}\right.$ of phenyl), 126.22 ( $\mathrm{C}-4$ of phenyl), $46.23\left(\mathrm{CH}_{2} \mathrm{~N}\right), 45.44\left(\mathrm{CH}_{2} \mathrm{~N}\right), 41.00$ $\left(\mathrm{CH}_{2} \mathrm{CO}\right), 25.62\left(\mathrm{CH}_{2}\right), 23.92\left(\mathrm{CH}_{2}\right)$; MS $\left(\mathrm{ESI}^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{12} \mathrm{H}_{16} \mathrm{NO}: 190.12[\mathrm{M}+\mathrm{H}]^{+}$, found: 190.1.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{k}$-acryloyl-L-lysine-4-(6-carbamoylpyridin-3-yl)piperazide $\times$ TFA ( 8 g )



A solution of compound $8 \mathbf{e}(20 \mathrm{mg}, 0.03 \mathrm{mmol}, 1$ eq.) and NMM ( $8.9 \mu \mathrm{~L}, 0.08 \mathrm{mmol}, 2.5 \mathrm{eq}$.) in THF ( 2 mL ) was cooled to $-30^{\circ} \mathrm{C}$ by a mixture of isopropanol and liquid nitrogen. Subsequently, isobutyl chloroformate ( $4.2 \mu \mathrm{~L}, 0.03 \mathrm{mmol}, 1$ eq.) and aqueous $\mathrm{NH}_{3}(\omega=25 \%$, $12.1 \mu \mathrm{~L}, 0.16 \mathrm{mmol}, 5 \mathrm{eq}$.) was added. Cooling was stopped and the reaction mixture was stirred until the temperature reached $4^{\circ} \mathrm{C}$ (approx. 2 h ). To completely convert the acyl isobutyl carbonate to the desired amide, aequous $\mathrm{NH}_{3}(\omega=25 \%, 12.1 \mu \mathrm{~L}, 0.16 \mathrm{mmol}, 5$ eq.) was added again and the reaction mixture was heated to $66^{\circ} \mathrm{C}$ for 30 min . The solvent was removed in vacuo and the residue was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$. The organic phase was washed with $\mathrm{NaHCO}_{3}$ $(3 \times 6 \mathrm{~mL})$ and brine $(1 \times 6 \mathrm{~mL})$, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and the solvent was removed in vacuo. The
crude product was purified by preparative RP-HPLC.The product-containing fractions were combined and lyophilised to afford compound $\mathbf{8 g}(5.4 \mathrm{mg}, 27 \%)$ as a light yellow solid. ${ }^{1} \mathrm{H}$ NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.40\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.25\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), 8.03 (t, ${ }^{3} \mathrm{~J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.88 (d, ${ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 7.84 , (s, $1 \mathrm{H}, \mathrm{NHH}$ ), 7.40 (dd, ${ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}=2.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.37(\mathrm{~s}, 1 \mathrm{H}, \mathrm{NHH}), 7.29-7.22(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), $7.20-7.12$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.76-4.66 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.74-3.03 (m, 12H, $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.71-1.60 (m, 1 H , $\left.\mathrm{C}_{\beta} \mathrm{HH}\right), 1.59-1.47\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.34\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.31-1.18\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=169.90,169.74,165.88,164.39,147.78$ (C-3 of pyridine), 136.35 (C-1 phenyl), 134.93 (C-2 of pyridine), $131.85\left(\mathrm{CH}_{2}=C\right), 128.94(2 \times \mathrm{C}$ of phenyl), $128.12(2 \times \mathrm{C}$ of phenyl), 126.29 ( $\mathrm{C}-4$ of phenyl), $124.75\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 122.53 ( $\mathrm{C}-5$ of pyridine), 121.37 ( $\mathrm{C}-4$ of pyridine), $48.03\left(\mathrm{C}_{\alpha}\right), 46.79\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.41\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.19\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$-phenyl), $40.92\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.28\left(\mathrm{C}_{\varepsilon}\right), 31.34\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.59\left(\mathrm{C}_{\gamma}\right)$; ${ }^{19}$ F-NMR (DMSO- $d_{6}$ ) $\delta=-74.75$ (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{~N}_{6} \mathrm{O}_{4}$ : 507.27 $[\mathrm{M}+\mathrm{H}]^{+}$, found: 507.3.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-Iysine-4-phenylpiperazide×TFA (9)



Compound 9 ( $63 \mathrm{mg}, 47 \%$, yellow oil) was synthesised according to GP VIII using compound $5 u(0.21 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.40\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.34\left(\mathrm{~d},{ }^{4} \mathrm{~J}=2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.90\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 7.33 (dd, ${ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.28-7.23$ ( $\mathrm{m}, 4 \mathrm{H}$, $4 \times \mathrm{H}$ of phenyl), 7.19-7.13 (m, $1 \mathrm{H}, \mathrm{H}-4$ of phenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.53\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), 4.75-4.66 (m, 1H, CaH), $3.81\left(\mathrm{~s}, \mathrm{CH}_{3}\right), 3.72-3.15\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), $3.12-3.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.72-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H} H\right), 1.58-1.46\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.34(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.30-1.18 (m, 2H, C $\mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=169.96,169.75,164.90$, 164.39, 158.18 ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=36.9 \mathrm{~Hz}$, CO of TFA), 147.93 (C-3 of pyridine), 136.35, 136.00, 135.70, 131.85
$\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.94(2 \times \mathrm{C}$ of phenyl), 128.20 ( $2 \times \mathrm{C}$ of phenyl), 126.29 ( $\mathrm{C}-4$ of phenyl), 125.76 ( $\mathrm{C}-$ 5 of pyridine), $124.75\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $119.87(\mathrm{C}-4$ of pyridine $)$, $51.80\left(\mathrm{CH}_{3}\right), 48.05\left(\mathrm{C}_{\alpha}\right), 46.22\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.84\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.09\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.95\left(\mathrm{CH}_{2}\right.$-phenyl), 40.87 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right)$, $31.32\left(\mathrm{C}_{\beta}\right)$, $28.82\left(\mathrm{C}_{\delta}\right), 22.59\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}$-NMR (DMSO- $\left.d_{6}\right) \delta=-$ 74.92 (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{28} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{5}: 522.27$ [M+H] ${ }^{+}$, found: 522.3.

## $N^{\alpha}$-Phenylacetyl- $N^{\kappa}$-acryloyl-L-lysine-4-(pyridin-4-yl)piperazidexTFA (10)



Compound 10 ( $10 \mathrm{mg}, 16 \%$, colourless solid) was synthesised according to GP VIII using compound 5 t ( 0.10 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.42$ ( d , ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.28\left(\mathrm{~d},{ }^{3} \mathrm{~J}=6.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6\right.$ of pyridine), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right)$, 7.31-7.23 (m, 4H, H-2,3,5,6 of phenyl), 7.21-7.17 (m, 1H, H-4 of phenyl), 7.15 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.7 \mathrm{~Hz}$, $2 \mathrm{H}, \mathrm{H}-3,5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.71-4.65 (m, 1H, $\mathrm{C}_{a} \mathrm{H}$ ), 3.80-3.52 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.51-3.40 (m, 2H, CH2-phenyl), 3.15-3.02 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.72-1.60 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right)$, 1.59-1.49 (m, 1H, $\mathrm{C}_{\beta} H H$ ), 1.47-1.33 (m, 2H, C $\mathrm{C}_{2} \mathrm{H}_{2}$, 1.33$1.19\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.42,169.85,164.41\left(\mathrm{C}_{\alpha} \mathrm{CON}, \mathrm{CON}_{\alpha}, \mathrm{CON}_{\varepsilon}\right)$, 156.59 (C-4 of pyridine), 139.77 ( $\mathrm{C}-3,5$ of pyridine), 136.32 ( $\mathrm{C}-1$ of phenyl), $131.84\left(\mathrm{CH}=\mathrm{CH}_{2}\right.$ ), $128.95\left(2 \times \mathrm{CH}\right.$ of phenyl), $128.13\left(2 \times \mathrm{CH}\right.$ of phenyl), 126.31 ( $\mathrm{C}-4$ of phenyl), $124.78\left(\mathrm{CH}=\mathrm{CH}_{2}\right)$, 107.44 (C-2,6 of pyridine), $48.20\left(\mathrm{C}_{\alpha}\right), 41.90\left(\mathrm{CH}_{2}\right.$-phenyl), $38.24\left(\mathrm{C}_{\varepsilon}\right), 31.13\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right)$, $22.55\left(\mathrm{C}_{\mathrm{y}}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=-74.36(\mathrm{~s}$, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{3}: 464.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 464.2.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(3-methylphenyl)piperazide×TFA (11)



Compound 11 ( $31 \mathrm{mg}, 74 \%$, light yellow solid) was synthesised according to GP VIII using compound 5 w ( 0.09 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.38$ ( d , ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.31-7.23(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), $7.23-$ $7.15\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of phenyl), $7.10\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of methylphenyl), 6.77-6.69(m,2H, $2 \times \mathrm{H}$ of methylphenyl), $6.64\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}\right.$ of methylphenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $6.04\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), $5.54\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}), 4.74-4.66\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.72-3.40\left(\mathrm{~m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.18-2.89 ( $\mathrm{m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.25\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.71-1.59\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right)$, 1.58-1.46 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.34\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.32-1.19\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=169.72,169.70,164.40,150.55,138.04,136.36,131.85\left(\mathrm{CH}_{2}=C\right), 128.94(2 \times \mathrm{C}$ of phenyl), 128.77 ( $\mathrm{C}-5$ of methylphenyl), 128.12 ( $2 \times \mathrm{C}$ of phenyl), 126.29 ( $\mathrm{C}-4$ of phenyl), $124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right), 120.35,116.62,113.18,48.80\left(\mathrm{CH}_{2}\right.$ of piperazine $), 48.57\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $48.02\left(\mathrm{C}_{\alpha}\right), 44.67\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.95\left(\mathrm{CH}_{2}\right.$-phenyl), $41.32\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.29$ $\left(\mathrm{C}_{\varepsilon}\right), 31.40\left(\mathrm{C}_{\beta}\right), 28.79\left(\mathrm{C}_{\delta}\right), 22.59\left(\mathrm{C}_{\gamma}\right), 21.35\left(\mathrm{CH}_{3}\right)$; MS (ESI+): m/z calculated for $\mathrm{C}_{28} \mathrm{H}_{3} \mathrm{~N}_{4} \mathrm{O}_{3}$ : $477.29[\mathrm{M}+\mathrm{H}]^{+}$, found: 477.3.

## $N^{\alpha}$-Phenylacetyl- $N^{\varepsilon}$-acryloyl-L-lysine-4-(4-nitrophenyl)piperazide×TFA (12)



Compound 12 ( $20 \mathrm{mg}, 57 \%$, yellow solid) was synthesised according to GP VIII using compound $5 \mathbf{x}(0.07 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.40$ ( d ,
$\left.{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right), 8.10-8.05\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5\right.$ of nitrophenyl), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right)$, 7.31-7.23 ( $\mathrm{m}, 4 \mathrm{H}, 4 \times \mathrm{H}$ of phenyl), 7.22-7.14 (m, 1H, H-4 of phenyl), 7.02-6.95 (m, 2H, $\mathrm{H}-2,6$ of nitrophenyl), 6.18 (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $6.03\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{C}=\mathrm{CH} H$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.74-4.66 (m, 1H, CaH), 3.74-3.26 ( $\mathrm{m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.12-3.03 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.72-1.60 (m, 1H, $\mathrm{C}_{\beta} \mathrm{H} H$ ), 1.59-1.47 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.35\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.31-1.19\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=170.09,169.77,164.39,154.33,137.05,136.34,131.84\left(\mathrm{CH}_{2}=C\right), 128.94(2 \times \mathrm{C}$ of phenyl), 128.13 ( $2 \times$ C of phenyl), 126.31 ( $\mathrm{C}-4$ of phenyl), 125.66 ( $\mathrm{C}-3,5$ of nitrophenyl), $124.76\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112.59\left(\mathrm{C}-2,6\right.$ of nitrophenyl), $48.12\left(\mathrm{C}_{\mathrm{a}}\right), 46.17\left(\mathrm{CH}_{2}\right.$ of piperazine), 45.78 ( $\mathrm{CH}_{2}$ of piperazine), $43.97\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.94\left(\mathrm{CH}_{2}\right.$-phenyl), $40.92\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.27\left(\mathrm{C}_{\varepsilon}\right), 31.29\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.58\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-73.45(\mathrm{~s}, \mathrm{TFA}) ;$ MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{27} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{5}: 508.26[\mathrm{M}+\mathrm{H}]^{+}$, found: 530.2.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-Iysine-4-(4-fluorobenzoyl)piperazide×TFA (13a)



Compound 13a ( $16 \mathrm{mg}, 53 \%$, yellow oil) was synthesised according to GP VIII using compound 5 e $(0.06 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.37$ ( d ,
 2,6 of fluorobenzoyl), $7.32-7.13$ (m, $7 \mathrm{H}, 5 \times \mathrm{H}$ of phenyl, $\mathrm{H}-3,5$ of fluorobenzoyl), 6.19 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.55 (dd, $\left.{ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.65\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.50-3.38\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{CH}_{2}-\right.$ phenyl), 3.123.03 (m, 2H, C $\varepsilon_{\varepsilon} \mathrm{H}_{2}$ ), 1.69-1.57 (m, 1H, C ${ }_{\beta} H \mathrm{H}$ ), 1.57-1.45 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.45-1.33 (m, 2H, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.31-1.16 (m, 2H, C $\mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.07,169.74,168.25,164.40$, 136.34 (C-1 of phenyl), 131.97 (d, ${ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.4 \mathrm{~Hz}, \mathrm{C}-1$ of fluorobenzoyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 129.62 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorobenzoyl), 128.95 ( $2 \times \mathrm{C}$ of phenyl), 128.12 ( $2 \times \mathrm{C}$ of phenyl), 126.31 (C-4 of phenyl), 124.75 ( $\mathrm{CH}_{2}=\mathrm{C}$ ), 115.39 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.5 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorobenzoyl), $48.16\left(\mathrm{C}_{\alpha}\right), 41.93\left(\mathrm{CH}_{2}\right.$-phenyl), $38.25\left(\mathrm{C}_{\varepsilon}\right), 31.28\left(\mathrm{C}_{\beta}\right), 28.79\left(\mathrm{C}_{\delta}\right), 22.54\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine are not visible; $\mathrm{MS}\left(E S I^{+}\right)$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{28} \mathrm{H}_{34} \mathrm{FN}_{4} \mathrm{O}_{4}: 509.26\left[\mathrm{M}+\mathrm{H}^{+}\right.$, found: 509.3.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(4-nitrobenzoyl)piperazide×TFA (13b)



Compound 13b (22 mg, 73\%, white solid) was synthesised according to GP VIII using compound $5 \mathbf{f}(0.06 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.43-8.35(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.30\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.8 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right.$ of nitrobenzoyl), $8.04\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.73-7.64(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-$ 2,6 of nitrobenzoyl), 7.32-7.16 (m,5H,5×H of phenyl), 6.25-6.12 (m, 1H, CH=CH2), 6.10-5.99 (m, 1H, C=CHH), 5.60-5.51 (m, 1H, C=CHH), 4.76-4.56 (m, 1H, CaH), 3.74-3.13 (m, 8H, $4 \times \mathrm{CH}_{2}$ of piperazine), $3.12-3.00\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.69-1.57\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.57-1.45(\mathrm{~m}, 1 \mathrm{H}$, $\left.\mathrm{C}_{\beta} \mathrm{H} H\right)$, 1.45-1.32 (m, 2H, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.32-1.14 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), diffuse signals (partly in duplicate) due to the amide bonds on both sides of the piperazine ring; ${ }^{13} \mathrm{C}$-NMR (DMSO- $d_{6}$ ) $\delta=170.11$, 169.77, 167.24, 164.42, 147.85, 141.93, 136.34, $131.85\left(\mathrm{CH}_{2}=C\right), 128.97(2 \times$ C Pheny $)$, 128.35 (C-2,6 of nitrobenzoyl), 128.14 ( $2 \times \mathrm{C}$ of phenyl), 126.33 ( $\mathrm{C}-4$ of phenyl), $124.79\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 123.78 (C-3,5 of nitrobenzoyl), $48.16\left(\mathrm{C}_{\alpha}\right), 41.94\left(\mathrm{CH}_{2}\right.$-phenyl), $38.26\left(\mathrm{C}_{\varepsilon}\right), 31.27\left(\mathrm{C}_{\beta}\right), 28.80$ $\left(\mathrm{C}_{\delta}\right)$, $22.56\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine are not visible; $\mathrm{MS}\left(\mathrm{ESI}^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{28} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{6}$ : $536.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 536.3.

## $N^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{\boldsymbol{k}}$-acryloyl-L-lysine-4-(4-fluorobenzyl)piperazide×TFA (13c)



Compound 13c (12 mg, 42\%, white solid) was synthesised according to GP VIII using compound 5 g ( 0.05 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.39$ (d, $\left.{ }^{3} \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.12-7.99\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.59-7.48$ (m, 2H, H-2,6 of fluorophenyl), 7.427.09 (m, $7 \mathrm{H}, 5 \times \mathrm{H}$ of phenyl , $\mathrm{H}-3,5$ of fluorophenyl), 6.20 (dd, ${ }^{3} \mathrm{~J}=17.1,9.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ),
6.06 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.60-5.54(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}), 4.70-4.58(\mathrm{~m}, 1 \mathrm{H}$, $\left.\mathrm{C}_{a} \mathrm{H}\right), 4.50-4.10\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{CH}_{2}\right), 3.53-3.19\left(\mathrm{~m}, 8 \mathrm{H}, \mathrm{CH}_{2}\right.$-phenyl, $\left.3 \times \mathrm{CH}_{2}\right), 3.17-2.73\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right.$, $\left.\mathrm{CH}_{2}\right)$, 1.69-1.46 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.46-1.34 (m, 2H, C $\mathrm{C}_{2}$ ), 1.34-1.12 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}^{2}$ (DMSO- $d_{6}$ ) $\delta=170.27,169.93,164.48,136.28$ (C-1 of phenyl), 133.61 ( $d,{ }^{3}{ }^{J} \mathrm{C}, \mathrm{F}=6.6 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), $131.86\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.96(2 \times \mathrm{C}$ of phenyl), $128.16(2 \times \mathrm{C}$ of phenyl), $126.34(\mathrm{C}-$ 4 of phenyl), $124.81\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.79\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=22.1 \mathrm{~Hz}, \mathrm{C}-3,5\right.$ fluorophenyl), $58.11\left(\mathrm{CH}_{2}\right)$, $50.40\left(\mathrm{CH}_{2}\right)$, $48.10\left(\mathrm{C}_{\alpha}\right), 41.85\left(\mathrm{CH}_{2}\right.$-phenyl), $38.19\left(\mathrm{C}_{\varepsilon}\right), 31.11\left(\mathrm{C}_{\beta}\right), 28.80\left(\mathrm{C}_{\delta}\right), 22.49\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-1,4$ fluorophenyl and $3 \times \mathrm{CH}_{2}$ are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-73.92$ (s, TFA), -111.46--111.63 (m, F-4); MS (ESI + ): m/z calculated for $\mathrm{C}_{28} \mathrm{H}_{36} \mathrm{FN}_{4} \mathrm{O}_{3}: 495.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 495.4.

## $N^{\alpha}$-Phenylacetyl- $N^{\text {E }}$-acryloyl-L-lysine-4-(6-chloropicolinoyl)piperazidexTFA (13d)



Compound 13d (15 mg, 49\%, colourless solid) was synthesised according to GP VIII using compound $5 \mathrm{~h}(0.05 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=7.80(\mathrm{t}$, ${ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.66 (dd, ${ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 7.42 (d, ${ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 7.39-7.28 (m, 3H; $3 \times \mathrm{H}$ of phenyl), 7.28-7.23 (m, 2H, $2 \times \mathrm{H}$ of phenyl), 6.66 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 6.34-6.21 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 6.17-5.99 (m, 2H, $\mathrm{CH}=\mathrm{CH}_{2}, \mathrm{~N}_{\varepsilon} \mathrm{H}$ ), $5.63\left(\mathrm{~d},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right.$ ), 4.99-4.86 (m, $1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.98-3.51 (m, 10 $\mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.35-3.19 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.73-1.47 (m, $4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.37-1.23 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), diffuse signals (partly in duplicate) due to the amide bonds on both sides of the piperazine ring; ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=171.76,170.53 / 170.47,166.45$, $166.05 / 165.96,153.31,150.15,140.07$ (C-4 of pyridine), 134.32, $130.61\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 129.42$ ( $2 \times \mathrm{CH}$ of phenyl), 129.18 ( $2 \times \mathrm{CH}$ of phenyl), 127.70 (C-4 of phenyl), $126.96\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 126.06$ ( $\mathrm{C}-3$ of pyridine), 123.23/123.16 (C-5 of pyridine), $48.61\left(\mathrm{C}_{a}\right), 47.27 / 47.02\left(\mathrm{CH}_{2}\right.$ of piperazine), 45.85/45.28 ( $\mathrm{CH}_{2}$ of piperazine), $43.65\left(\mathrm{CH}_{2}\right.$-phenyl), 42.76/42.56/42.53/42.01 $\left(2 \times \mathrm{CH}_{2}\right.$ of piperazine), $39.25\left(\mathrm{C}_{\varepsilon}\right), 32.71\left(\mathrm{C}_{\beta}\right), 28.43\left(\mathrm{C}_{\delta}\right), 22.25\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $\left.\mathrm{d}_{6}\right) \delta=-75.82(\mathrm{~s}$, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{27} \mathrm{H}_{33} \mathrm{CIN}_{5} \mathrm{O}_{4}: 526.22\left[\mathrm{M}\left({ }^{35} \mathrm{CI}\right)+\mathrm{H}\right]^{+}$, found: 526.3.

## $\boldsymbol{N}^{\alpha}$-Phenylacetyl- $\boldsymbol{N}^{k}$-acryloyl-L-Iysine-4-dansylpiperazide×TFA (13e)



Compound $\mathbf{1 3 e}$ ( $19 \mathrm{mg}, 54 \%$, yellow-green solid) was synthesised according to GP VIII using compound 5 c ( 0.05 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.54$ ( d , ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 8.28 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}$ of dansyl, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.12 (dd, ${ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}$, ${ }^{2} J=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), $7.99\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.67 (dd, ${ }^{3} \mathrm{~J}=8.5,7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 7.62 (dd, ${ }^{3} \mathrm{~J}=8.6,7.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 7.28 (d, ${ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 7.247.12 ( $\mathrm{m}, 5 \mathrm{H}, \mathrm{H}-2,3,4,5,6$ phenyl), 6.16 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 5.53 (dd, ${ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.61$4.50\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.68-3.10\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.06-2.97 ( $\mathrm{m}, 2 \mathrm{H}$, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 2.83 ( $\mathrm{s}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{3}$ ), 1.60-1.09 (m, 6H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=169.92,169.66,164.36,151.28$ (quart. C of dansyl), 142.67 (quart. C of dansyl), 136.20, $131.84\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 130.40$ ( CH of dansyl), 130.09 ( CH of dansyl), 129.59 (quart. C of dansyl), 129.15 (quart. C of dansyl), 128.84 ( $2 \times \mathrm{CH}$ of phenyl), 128.27 (CH of dansyl), $128.06(2 \times \mathrm{CH}$ of phenyl), $126.25\left(\mathrm{C}-4\right.$ of phenyl), $124.72\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 123.71(\mathrm{CH}$ of dansyl), $118.92(\mathrm{CH}$ of dansyl), $115.38\left(\mathrm{CH}\right.$ of dansyl), $48.06\left(\mathrm{C}_{a} \mathrm{H}\right), 45.44\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.18\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.05\left(2 \times \mathrm{CH}_{3}\right)$, $44.53\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.80\left(\mathrm{CH}_{2}\right.$-phenyl), $41.12\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.21\left(\mathrm{C}_{\varepsilon}\right), 31.10\left(\mathrm{C}_{\beta}\right), 28.72\left(\mathrm{C}_{\delta}\right), 22.47\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.48(\mathrm{~s}$, TFA); MS (ESI $)$ : m/z calculated for $\mathrm{C}_{33} \mathrm{H}_{42} \mathrm{~N}_{5} \mathrm{O}_{5} \mathrm{~S}: 620.29[\mathrm{M}+\mathrm{H}]^{+}$, found: 620.4.

## $N^{\alpha}$-2-Pyridylacetyl- $N^{\text {ºn }}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×2TFA (14a)



Compound $\mathbf{1 4 a}$ ( $27 \mathrm{mg}, 64 \%$, yellow oil) was synthesised according to GP IX using compound $5 \mathrm{a}(0.06 \mathrm{mmol})$ and 2-pyridylacetic acid chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.68\left(\mathrm{~d},{ }^{3} \mathrm{~J}=4.6 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{H}-6$ of pyridine), 8.62 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.16 (t, ${ }^{3} \mathrm{~J}=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.67 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), $7.64-7.59(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4$ of methylpyridine), $6.91-6.83\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3\right.$ of methylpyridine), $6.69\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right)$, $6.64\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 6.18\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right.$ ), 6.04 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} J=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 ( dd, ${ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.81-4.66(m,1H, $\mathrm{C}_{\alpha} \mathrm{H}$ ), 3.94-3.83 (m, 2H, CH $\mathrm{CH}_{2}$-pyridine), 3.74-3.43 ( $\mathrm{m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.16-3.07 ( m , $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.74-1.62\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.62-1.50\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.49-1.37$ ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{6} \mathrm{H}_{2}$ ), 1.37-1.21 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=169.94,167.36,164.44$, $158.11\left(\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=35.8 \mathrm{~Hz}, \mathrm{CO}\right.$ of TFA), $131.83\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $124.81\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 112.71, $48.51\left(\mathrm{C}_{\alpha}\right)$, $38.27\left(\mathrm{C}_{\varepsilon}\right), 31.27\left(\mathrm{C}_{\beta}\right), 28.85\left(\mathrm{C}_{\delta}\right), 22.53\left(\mathrm{C}_{\gamma}\right)$, signals for $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{3}, \mathrm{C}-2,3,4,6$ of methylpyridine/pyridine and $1 \times \mathrm{C}-5$ are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.69$ (s, TFA); MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{35} \mathrm{~N}_{6} \mathrm{O}_{3}: 479.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 479.3.

## $N^{\alpha}$-2-Thienylacetyl- $\boldsymbol{N}^{k}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14b)



Compound 14b ( 21 mg , 59\%, white solid) was synthesised according to GP VIII using compound $5 \mathbf{5 a}(0.06 \mathrm{mmol})$ and 2-thienylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.43$ ( d , ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}$ ), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=4.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.72 (borad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.33
(dd, ${ }^{3} \mathrm{~J}=5.0 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of thiophene), $6.97-6.87$ ( $\mathrm{m}, 3 \mathrm{H}, \mathrm{H}-3,4$ of thiophene, $\mathrm{H}-3$ of pyridine), 6.75-6.66 (m, 1H, H-5 of pyridine), 6.18 (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 ( dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.77-4.67 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}$ ), 3.78-3.43 (m, $10 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-thiophene), 3.15-3.02 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.42\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.74-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H} H\right), 1.59-1.48\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right)$, 1.48$1.35\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.34-1.20\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.04,168.78,164.41$, $137.53,131.85\left(\mathrm{CH}_{2}=C\right), 126.51(\mathrm{CH}$ of thiophene $), 126.05(\mathrm{CH}$ of thiophene $), 124.78\left(\mathrm{CH}_{2}=\mathrm{C}\right.$, $\mathrm{C}-5$ of thiophene), 112.74 (C-5 of pyridine), $48.24\left(\mathrm{C}_{\mathrm{a}}\right), 45.51\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.11\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.06\left(\mathrm{CH}_{2}\right.$ of piperazine $), 40.87\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.26\left(\mathrm{C}_{\varepsilon}\right)$, $36.12\left(\mathrm{CH}_{2}-\right.$ thiophene), $31.28\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.52\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.70$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{25} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{3} \mathrm{~S}: 484.24$ $[\mathrm{M}+\mathrm{H}]^{+}$, found: 484.2.

## $N^{\alpha}$-3-Thienylacetyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14c)



Compound 14c ( $21 \mathrm{mg}, 60 \%$, yellow oil) was synthesised according to GP IX using compound $5 \mathrm{a}(0.06 \mathrm{mmol})$ and 3-thienylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.35\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{a} \mathrm{H}\right)$, 8.05 (t, ${ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.75 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.43 (dd, ${ }^{3} \mathrm{~J}=4.9 \mathrm{~Hz}$, ${ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of thiophene), 7.24 (dd, ${ }^{4} \mathrm{~J}=2.9,1.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ of thiophene), 7.02 (dd, ${ }^{3} \mathrm{~J}=4.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of thiophene), 6.95 (broad s, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.73 (d, ${ }^{3} \mathrm{~J}=7.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.19 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), $5.54\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H\right), 4.76-4.66$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.76-3.42 (m, 10H, $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-thiophene), 3.15-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.43\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.71-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.60-1.48\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.47-1.34(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.33-1.20 (m, 2H, $\mathrm{C}_{7} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.23,169.44,164.42,158.13$ ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=36.4 \mathrm{~Hz}, \mathrm{CO}$ of TFA), $136.05,131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.64$ (C-4 of thiophene), 125.61 ( $\mathrm{C}-5$ of thiophene), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 122.13(\mathrm{C}-5$ of thiophene), 112.78 ( $\mathrm{C}-5$ of pyridine), $48.19\left(\mathrm{C}_{a} \mathrm{H}\right)$, $45.58\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.21\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.96\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $40.84\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.27\left(\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 36.67\left(\mathrm{CH}_{2}\right.$-thiophene), $31.24\left(\mathrm{C}_{\beta}\right), 28.84\left(\mathrm{C}_{\delta}\right), 22.56$
$\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=-74.77$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{25} \mathrm{H}_{34} \mathrm{~N}_{5} \mathrm{O}_{3} \mathrm{~S}: 484.24$ [M+H] ${ }^{+}$, found: 484.2.

## $N^{\alpha}$-Cyclohexylacetyl- $\boldsymbol{N}^{k}$-acryloyl-L-Iysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14d)



Compound 14d (13 mg, 54\%, yellow solid) was synthesised according to GP VIII using compound $5 \mathbf{5 a}(0.04 \mathrm{mmol})$ and cyclohexylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.11-8.03$ ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.68 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 6.89 (broad s, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.69 (broad s, $1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.75-4.66 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}$ ), 3.78-3.43 (m, 8H, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.15-3.04 (m, 2H, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 2.41(\mathrm{~s}, 3 \mathrm{H}$, $\mathrm{CH}_{3}$ ), 2.06-1.92 (m, 2H, CH $\mathrm{C}_{2}$-cyclohexyl), 1.71-0.81 (m, 17H, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}, 5 \times \mathrm{CH}_{2}$ of cyclohexyl, $1 \times \mathrm{CH}$ of cyclohexyl); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=171.08,170.32,164.42,158.04$ (q, ${ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=34.9 \mathrm{~Hz}, \mathrm{CO}$ of TFA), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.79\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112.70$ (C-5 of pyridine), 47.93 $\left(\mathrm{C}_{\alpha}\right), 45.53\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.08\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.12\left(\mathrm{CH}_{2}\right.$ of piperazine $), 42.84$ ( $\mathrm{CH}_{2}$-cyclohexyl), $40.93\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.29\left(\mathrm{C}_{\varepsilon}\right), 34.72\left(\mathrm{CH}\right.$ of cyclohexyl), $32.55\left(\mathrm{CH}_{2}\right.$ of cyclohexyl), $32.42\left(\mathrm{CH}_{2}\right.$ of cyclohexyl), $31.15\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 25.86\left(\mathrm{CH}_{2}\right.$ of cyclohexyl), $25.64\left(\mathrm{CH}_{2}\right.$ of cyclohexyl), $25.61\left(\mathrm{CH}_{2}\right.$ of cyclohexyl), $22.67\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.52$ (s, TFA); MS (ESI ${ }^{+}$): $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{27} \mathrm{H}_{42} \mathrm{~N}_{5} \mathrm{O}_{3}$ : $484.33[\mathrm{M}+\mathrm{H}]^{+}$, found: 484.4.


Compound $\mathbf{1 4 e}$ ( $8 \mathrm{mg}, 32 \%$, yellow solid) was synthesised according to GP VIII using compound 5 a ( 0.04 mmol ) and phenylmethanesulfonyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.05$ ( t , ${ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.65 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.41-7.29(\mathrm{~m}, 6 \mathrm{H}, 5 \times \mathrm{H}$ of phenyl, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 6.85 (broad s, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.68 (broad s, $1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz}$, ${ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.36-4.24 (m, 2H, CH 2 -phenyl), 4.24-4.15 (m, 1H, C $\mathrm{C}_{a} \mathrm{H}$ ), 3.70-3.45 ( $\mathrm{m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), $3.14-3.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right.$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.63-1.19(\mathrm{~m}, 6 \mathrm{H}$, $\mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.09,164.42,158.00\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{c}, \mathrm{F}}=34.7 \mathrm{~Hz}, \mathrm{CO}\right.$ of TFA), $131.83\left(\mathrm{CH}_{2}=C\right), 130.84(2 \times \mathrm{C}$ of phenyl), 130.13 ( $\mathrm{C}-1$ of phenyl), $128.17(2 \times \mathrm{C}$ of phenyl), 127.94 ( $\mathrm{C}-4$ of phenyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 112.69 ( $\mathrm{C}-5$ of pyridine), $58.54\left(\mathrm{CH}_{2}-\right.$ phenyl), $52.10\left(\mathrm{C}_{\alpha}\right), 45.21\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.92\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.18\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.04\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.24\left(\mathrm{C}_{\varepsilon}\right), 32.28\left(\mathrm{C}_{\beta}\right), 28.79\left(\mathrm{C}_{\delta}\right), 22.32\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{99} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.47$ (s, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{36} \mathrm{~N}_{5} \mathrm{O}_{4}: 514.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 514.2.

## $N^{\alpha}$-Dansyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14f)



Compound $\mathbf{1 4 f}$ ( $67 \mathrm{mg}, 49 \%$, yellow solid) was synthesised according to GP VIII using compound $5 \mathbf{5 a}(0.19 \mathrm{mmol})$ and dansyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.41\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$,

H of dansyl), 8.33 (d, ${ }^{3} \mathrm{~J}=8.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 8.25 (d, ${ }^{3} \mathrm{~J}=8.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.13 (dd, ${ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), $7.95\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.69 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.60-7.54\left(\mathrm{~m}, 2 \mathrm{H}, 2 \times \mathrm{H}\right.$ of dansyl), 7.24 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of dansyl), 6.83 (broad s, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.70 (d, ${ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.15 (dd, ${ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.01 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.52 (dd, ${ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.19-4.09\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.56-3.10\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 2.93-2.82 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.79\left(\mathrm{~s}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{3}\right.$ of dansyl), $2.41\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.52-1.34(\mathrm{~m}, 2 \mathrm{H}$, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.26-0.96 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=169.20(\mathrm{CON}), 164.33\left(\mathrm{CON}_{\varepsilon}\right)$, 158.29, 157.93, 150.93 (quart. C of dansyl), 136.32 (quart. C of dansyl), $131.81\left(\mathrm{CH}_{2}=C\right)$, 129.43, 129.14 (quart. C of dansyl), 128.83 (quart. $C$ of dansyl), $128.32,127.68,124.75$ $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 123.38,119.63,115.11,112.78(\mathrm{C}-5$ of pyridine $), 51.75\left(\mathrm{C}_{\alpha}\right), 45.21\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.05\left(2 \times \mathrm{CH}_{3}\right.$ of dansyl), $44.73\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.89\left(\mathrm{CH}_{2}\right.$ of piperazine), $40.65\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.09\left(\mathrm{C}_{\varepsilon}\right), 31.70\left(\mathrm{C}_{\beta}\right), 28.41\left(\mathrm{C}_{\delta}\right), 22.20\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-3,4$ of pyridine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=-74.74$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{31} \mathrm{H}_{41} \mathrm{~N}_{6} \mathrm{O}_{4} \mathrm{~S}: 593.29[\mathrm{M}+\mathrm{H}]^{+}$, found: 593.1.

## $\boldsymbol{N}^{\alpha}$-Phenylcarbamoyl- $\boldsymbol{N}^{\boldsymbol{k}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14g)



Compound $\mathbf{1 4 g}$ ( $46 \mathrm{mg}, 40 \%$, white solid) was synthesised according to GP VIII using compound 5 a $(0.19 \mathrm{mmol})$ and phenyl isocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.68(\mathrm{~s}, 1 \mathrm{H}, \mathrm{NH}-$ phenyl), $8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.67 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.36 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}$, $2 \mathrm{H}, \mathrm{H}-2,6$ of phenyl), 7.21 ( $\mathrm{t},{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5$ of phenyl), 6.89 (ps-t, ${ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-4$ of phenyl, $\mathrm{H}-3$ of pyridine), $6.68\left(\mathrm{~d},{ }^{3} \mathrm{~J}=6.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), $6.50\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $6.03\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), 5.52 (dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $4.77-4.69\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\alpha} \mathrm{H}\right), 3.82-3.50(\mathrm{~m}, 8 \mathrm{H}$, $4 \times \mathrm{CH}_{2}$ of piperazine), 3.15-3.06 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.72-1.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right)$, $1.56-1.26\left(\mathrm{~m}, 5 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.86(\mathrm{CON}), 164.42\left(\mathrm{CON}_{\varepsilon}\right)$, $154.66\left(\mathrm{CON}_{\alpha}\right), 140.23\left(\mathrm{C}-1\right.$ of phenyl), $131.84\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.67(\mathrm{C}-3,5$ of phenyl), 124.76 ( $\mathrm{CH}_{2}=\mathrm{C}$ ), 121.16 ( $\mathrm{C}-4$ of phenyl), 117.51 ( $\mathrm{C}-2,6$ of phenyl), 112.68 ( $\mathrm{C}-5$ of pyridine), 48.41
$\left(\mathrm{C}_{\alpha}\right), 45.41\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.98\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.21\left(\mathrm{CH}_{2}\right.$ of piperazine), 40.94 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.30\left(\mathrm{C}_{\varepsilon}\right)$, $32.27\left(\mathrm{C}_{\beta}\right)$, $28.87\left(\mathrm{C}_{\delta}\right)$, $22.31\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}, 2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.49$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{35} \mathrm{~N}_{6} \mathrm{O}_{3}: 479.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 479.1.
$N^{\alpha}$-4-Fluorophenylcarbamoyl- $\boldsymbol{N}^{\mathrm{E}}$-acryloyl-L-Iysine-4-(6-methylpyridin-2yl)piperazide $\times$ TFA (14h)


Compound 14 h ( $71 \mathrm{mg}, 87 \%$, white solid) was synthesised according to GP VIII using compound $5 \mathrm{a}(0.13 \mathrm{mmol})$ and 4-fluorophenyl isocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.72(\mathrm{~s}, 1 \mathrm{H}$, NH -phenyl), $8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.75 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.41-7.33 (m, $2 \mathrm{H}, \mathrm{H}-2,6$ of fluorophenyl), 7.05 (t, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}={ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=8.9 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5$ of fluorophenyl), 6.97 (s, 1H, $\mathrm{H}-3$ of pyridine), $6.73\left(\mathrm{~d},{ }^{3} \mathrm{~J}=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), $6.49\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 6.18$ (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.80-4.67\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.84-3.54\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.17-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.44\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.70-1.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.58-1.19$ ( $\mathrm{m}, 5 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=170.91$ (CON), $164.43\left(\mathrm{CON}_{\varepsilon}\right)$, 158.29, 157.93, 156.91 ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=237.3 \mathrm{~Hz}, \mathrm{C}-4}$ of fluorophenyl), 154.71 ( $\mathrm{CON}_{\mathrm{a}}$ ), 136.59 (d, ${ }^{4} J_{\mathrm{C}, \mathrm{F}}=2.2 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), $131.84\left(\mathrm{CH}_{2}=\mathrm{C}\right), 124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 119.09\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.6 \mathrm{~Hz}\right.$, $\mathrm{C}-2,6$ of fluorophenyl), 115.14 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=22.1 \mathrm{~Hz}, \mathrm{C}-3,5$ of phenyl), 112.78 ( $\mathrm{C}-5$ of pyridine), $48.47\left(\mathrm{C}_{\alpha}\right), 45.63\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.19\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.03\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $40.84\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.29\left(\mathrm{C}_{\varepsilon}\right), 32.20\left(\mathrm{C}_{\beta}\right)$, $28.88\left(\mathrm{C}_{\delta}\right), 22.29\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right)$ $\delta=-74.77$ (s, TFA), -122.37--122.46 (m, F-4); MS (ESI + ): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{34} \mathrm{FN}_{6} \mathrm{O}_{3}$ : $497.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 497.1.

## $N^{\alpha}$-Phenylthiocarbamoyl- $N^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA

(14i)


Compound 14i ( $72 \mathrm{mg}, 61 \%$, colourless oil) was synthesised according to GP VIII using compound 5 a $(0.19 \mathrm{mmol})$ and phenyl isothiocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=10.57(\mathrm{~s}, 1 \mathrm{H}$, NH-phenyl), 8.78 (broad s, 1H, NH-phenyl), $8.09\left(\mathrm{t},{ }^{3} \mathrm{~J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), $7.55-7.42(\mathrm{~m}, 4 \mathrm{H}$, $\mathrm{H}-4$ of pyridine, $3 \times \mathrm{H}$ of phenyl), $7.29-7.24\left(\mathrm{~m}, 2 \mathrm{H}, 2 \times \mathrm{H}\right.$ of phenyl), $6.73\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-3$ of pyridine), 6.62 (d, ${ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.21 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 6.07 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ) $5.57\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{C}=\mathrm{CHH}), 4.77-4.69\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.82-3.50\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.15-3.06(m, $\left.2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.89-1.78\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.78-1.68\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.52-1.35$ (m, 4H, C ${ }_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=182.38$ (CS), 174.27 (CON), $164.47\left(\mathrm{CON}_{\varepsilon}\right)$, 158.00 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=34.5 \mathrm{~Hz}, \mathrm{CO}$ of TFA), $157.33,155.48,138.56$ (C-4 of pyridine), 133.40 (C-1 of phenyl), $131.85\left(\mathrm{CH}_{2}=C\right), 128.79(2 \times \mathrm{C}$ of phenyl), $128.68(2 \times \mathrm{C}$ of phenyl), $128.55(\mathrm{C}-4$ of phenyl), $124.79\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 113.24 ( $\mathrm{C}-5$ of pyridine), $104.69\left(\mathrm{C}-3\right.$ of pyridine), $59.20\left(\mathrm{C}_{\alpha}\right), 42.53$ $\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.44\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.85\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.81\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.29\left(\mathrm{C}_{\varepsilon}\right), 30.48\left(\mathrm{C}_{\beta}\right), 28.70\left(\mathrm{C}_{\delta}\right), 23.92\left(\mathrm{CH}_{3}\right), 21.48\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.33$ (s, TFA); MS (ESI ${ }^{+}$: m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{35} \mathrm{~N}_{6} \mathrm{O}_{2} \mathrm{~S}: 495.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 495.1.
$N^{\alpha}$-4-Fluorophenylthiocarbamoyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-
yl)piperazide×TFA (14j)


Compound 14j ( $66 \mathrm{mg}, 79 \%$, white solid) was synthesised according to GP VIII using compound $5 \mathbf{a}(0.13 \mathrm{mmol})$ and 4 -fluorophenyl isothiocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=10.60(\mathrm{~s}$, $1 \mathrm{H}, \mathrm{NH}$-phenyl), 8.80 (s, $1 \mathrm{H}, \mathrm{NH}$-phenyl), 8.09 ( $\mathrm{t},{ }^{3} \mathrm{~J}=5.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.55 (dd, ${ }^{3} \mathrm{~J}=8.4$, $7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ Pyridin), $7.34-7.30\left(\mathrm{~m}, 4 \mathrm{H}, 4 \times \mathrm{H}\right.$ of fluorophenyl), $6.76\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, \mathrm{H}-3\right.$ of pyridine), 6.64 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.3,1 \mathrm{H}, \mathrm{H}-5$ of pyridine), $6.20\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}\right.$ ), 6.07 ( dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.57 (dd, ${ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.43-4.39 (m, 1H, Ca H ), 3.74-3.67 (m, 4H, $2 \times \mathrm{CH}_{2}$ of piperazine), $3.24-3.09\left(\mathrm{~m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.34\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.89-1.68\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.58-1.19\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right.$, $\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=182.29$ (CS of fluorophenyl), 174.24 (CON), $164.48\left(\mathrm{CON}_{\varepsilon}\right)$, 161.70 ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=245.4 \mathrm{~Hz}, \mathrm{C}-4$ of fluorophenyl), $158.29,157.94,138.90$ (C-4 of pyridine), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 131.01$ ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=9.0 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), $129.63\left(\mathrm{~d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.0 \mathrm{~Hz}, \mathrm{C}-1\right.$ of fluorophenyl), $124.81\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.61$ ( $\mathrm{d},{ }^{2}{ }^{\mathrm{J}} \mathrm{C}, \mathrm{F}=22.8 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorophenyl), 113.28 ( $\mathrm{C}-5$ of pyridine), 105.01 ( $\mathrm{C}-3$ of pyridine), $59.23\left(\mathrm{C}_{a}\right), 42.50\left(\mathrm{CH}_{2}\right.$ of piperazine), $42.41\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.96\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.92\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.29\left(\mathrm{C}_{\varepsilon}\right), 30.45\left(\mathrm{C}_{\beta}\right)$, $28.70\left(\mathrm{C}_{\bar{\delta}}\right), 23.65\left(\mathrm{CH}_{3}\right), 21.53\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.61(\mathrm{~s}, \mathrm{TFA}),-113.07--113.18$ (m, F-4); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{34} \mathrm{FN}_{6} \mathrm{O}_{2} \mathrm{~S}: 513.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 513.1.

## $N^{a}$-4-Fluorobenzoyl- $\boldsymbol{N}^{\text {E }}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14k)



Compound 14k ( $91 \mathrm{mg}, 71 \%$, rose oil) was synthesised according to GP VIII using compound $5 \mathrm{a}(0.21 \mathrm{mmol})$ and 4-fluorobenzoyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=8.67\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.07\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.97 ( $\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.9 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=5.5 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ of fluorobenzoyl), 7.75 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.29\left(\mathrm{t},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}={ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=8.9 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5\right.$ of fluorobenzoyl), 6.98 (breites $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.73 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $6.03\left(\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right.$ ), 5.54 (dd, ${ }^{3}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.93-4.85 (m, CaH), 3.86-3.52 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.18-3.05 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.43\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.78-1.68\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.52-1.29$ (m, 4H, $\mathrm{C}_{\mathrm{r}} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=170.31$ (CON), 165.18, $164.38\left(\mathrm{CON}_{\varepsilon}\right), 163.95$ (d, ${ }^{2} J_{\mathrm{C}, \mathrm{F}}=248.7 \mathrm{~Hz}, \mathrm{C}-4$ of fluorobenzoyl), 158.29, 157.93, $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 130.30(\mathrm{~d}$, ${ }^{4} J_{\mathrm{C}, \mathrm{F}}=2.9 \mathrm{~Hz}, \mathrm{C}-1$ of fluorobenzoyl), 130.18 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=9.0 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorobenzoyl), 124.77 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.14$ ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.7 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorobenzoyl), 112.74 (C-5 of pyridine), 49.30 $\left(\mathrm{C}_{a}\right), 45.65\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.25\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.04\left(\mathrm{CH}_{2}\right.$ of piperazine $), 40.93$ $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.26\left(\mathrm{C}_{\varepsilon}\right), 30.73\left(\mathrm{C}_{\beta}\right), 28.90\left(\mathrm{C}_{\delta}\right), 22.93\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-3,4$ of pyridine are not visible; ${ }^{19}$ F-NMR (DMSO- $d_{6}$ ) $\delta=-72.55$ (s, TFA), -107.00--107.14(m, F-4); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{FN}_{5} \mathrm{O}_{3}$ : $482.26[\mathrm{M}+\mathrm{H}]^{+}$, found: 482.2.

## $\boldsymbol{N}^{\alpha}$-3-lodobenzoyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-Iysine-4-(6-methylpyridin-2-yl)piperazide×TFA (14I)



Compound 141 ( $60 \mathrm{mg}, 40 \%$, yellow solid) was synthesised according to GP IX using compound $5 \mathbf{a}(0.21 \mathrm{mmol})$ and 3 -iodobenzoic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.77\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.26\left(\mathrm{t},{ }^{4} \mathrm{~J}=1.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of iodobenzoyl), $8.07\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.92-7.90$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-4$ of iodobenzoyl), $7.90-7.88$ (m, 1H, $\mathrm{H}-6$ of iodobenzoyl), 7.71 (borad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.28\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, \mathrm{H}-5\right.$ of iodobenzoyl), 6.94 (breites $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.71 (d, ${ }^{3} \mathrm{~J}=6.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.94-4.85 $\left(\mathrm{m}, \mathrm{C}_{a} \mathrm{H}\right), 3.83-3.48\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.17-3.06(m,2H, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right)$, $2.42\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right)$, 1.78-1.67 (m, 2H, C ${ }_{\beta} \mathrm{H}_{2}$ ), 1.53-1.27 (m, 4H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.18$ (CON), 164.65, 164.44, 158.59, 158.24, 157.88, 139.90 (C-4 of iodobenzoyl), 135.81 (C-2 of iodobenzoyl), $131.85\left(\mathrm{CH}_{2}=\mathrm{C}\right), 130.44$ ( $\mathrm{C}-5$ of iodobenzoyl), 127.01 ( $\mathrm{C}-6$ of iodobenzoyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $112.70\left(\mathrm{C}-5\right.$ of pyridine), 99.69, $94.58\left(\mathrm{C}-3\right.$ of iodobenzoyl), $49.32\left(\mathrm{C}_{\alpha}\right)$, $45.54\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.16\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $44.09\left(\mathrm{CH}_{2}\right.$ of piperazine), $40.98\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.25\left(\mathrm{C}_{\varepsilon}\right)$, $30.68\left(\mathrm{C}_{\beta}\right)$, $28.86\left(\mathrm{C}_{\delta}\right)$, $22.91\left(\mathrm{C}_{\gamma}\right)$, signal for $\mathrm{CH}_{3}$ is not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.63(\mathrm{~s}, \mathrm{TFA})$; MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{IN}_{5} \mathrm{O}_{3}$ : $590.16[\mathrm{M}+\mathrm{H}]^{+}$, found: 590.2.

## $N^{\alpha}$-4-Fluorobenzyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×2TFA (14m)



The synthesis was accomplished according to the general procedure for reductive alkylation described by Abdel-Magid and Mehrman ${ }^{40}$. To a solution of compound 5a 80.9 mg , $0.14 \mathrm{mmol}, 1 \mathrm{eq}$.) in THF ( 10 mL ) under $\mathrm{N}_{2}$ atmosphere were added TEA ( $48 \mu \mathrm{~L}, 0.345 \mathrm{mmol}$, 2.5 eq.) and 4 -fluorobenzaldehyde ( $13.8 \mu \mathrm{~L}, 0.13 \mathrm{mmol}, 0.95$ eq.) followey by the addition of sodium triacetoxyborohydride ( $40.9 \mathrm{mg}, 0.19 \mathrm{mmol}, 1.4 \mathrm{eq}$.). The reaction mixture was stirred for 24 h . 4-Fluorobenzaldehyde ( $13.9 \mu \mathrm{~L}, 0.13 \mathrm{mmol}, 0.95 \mathrm{eq}$.) was added again and stirring was continued for 24 h . Subsequently, the solvent was removed in vacuo, the residue was dissolved in $3 \mathrm{M} \mathrm{NaOH}(10 \mathrm{~mL})$ and extracted with ethyl acetate $(3 \times 20 \mathrm{~mL})$. The organic phases were combined, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and evaporated. The crude product was purified by preparative RP-HPLC. The product-containing fractions were combined and lyophilised to afford compound $\mathbf{1 4 m}(9 \mathrm{mg}, 9 \%)$ as a light yellow solid. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=9.28(\mathrm{~s}, 1 \mathrm{H}$, NH ), $9.12(\mathrm{~s}, 1 \mathrm{H}, \mathrm{NH}), 8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.58-7.46(\mathrm{~m}, 3 \mathrm{H}, \mathrm{H}-2,6$ of fluorophenyl, $\mathrm{H}-4$ of pyridine), $7.32-7.25\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5\right.$ of fluorophenyl), $6.73\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ of pyridine), 6.61 (d, ${ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.16 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.00 ( dd, ${ }^{3} J=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.51 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.52-4.43 (s, 1H, Cat), 4.15-4.00 (m, 2H, CH2-fluorophenyl), 3.67-3.46 (m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.20-3.03 (m, 2H, C $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.35\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.87-1.71\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right), 1.48-1.20$ ( $\mathrm{m}, 4 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=165.98,164.50,158.00\left(\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=34.1 \mathrm{~Hz}, \mathrm{CO}\right.$ of TFA), 132.74 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.7 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), $131.72\left(\mathrm{CH}_{2}=\mathrm{C}\right), 127.52$ (s), 124.89 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.49$ ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.7 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorophenyl), 112.70 (C-5 of pyridine), 55.76 $\left(\mathrm{C}_{a}\right)$, $48.69\left(\mathrm{CH}_{2}\right.$-fluorophenyl), $44.73\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.43\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.33$ $\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.48\left(\mathrm{CH}_{2}\right.$ of piperazine), $37.90\left(\mathrm{C}_{\varepsilon}\right)$, $29.43\left(\mathrm{C}_{\beta}\right)$, $28.88\left(\mathrm{C}_{\delta}\right)$, $21.17\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{C}-1,4$ of fluorophenyl, $\mathrm{C}-2,3,4,6$ of pyridine and $\mathrm{CH}_{3}$ are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.31$ ( $\mathrm{s}, \mathrm{TFA}$ ), $-122.44--112.56$ ( $\mathrm{m}, \mathrm{F}-4$ ); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{35} \mathrm{FN}_{5} \mathrm{O}_{2}$ : $468.28[\mathrm{M}+\mathrm{H}]^{+}$, found: 468.3.

## $N^{\alpha}$-4-Methylphenylacetyl- $\boldsymbol{N}^{\text {E }}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide $\times$ TFA

 (15a)

Compound 15a ( $15 \mathrm{mg}, 24 \%$, white solid) was synthesised according to GP IX using compound $5 \mathbf{5 a}(0.10 \mathrm{mmol})$ and 4-methylphenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.34$ ( d , ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.63 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.14 (d, ${ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of methylphenyl), 7.07 (d, ${ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of methylphenyl), 6.79 (broad s, 1H, H-3 of pyridine), 6.66 (d, ${ }^{3} \mathrm{~J}=5.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.73-4.65\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.72-3.30\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-methylphenyl), $3.13-3.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 2.38\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ of pyridine), $2.22\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right.$ of phenyl), 1.71-1.59 (m, 1H, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.58-1.47 (m, 1H, $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.46-1.35 (m, 2H, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.32$1.20\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.07,169.92,164.40,135.27,133.25,131.85$ $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.81\left(2 \times \mathrm{C}\right.$ of methylphenyl), $128.68\left(2 \times \mathrm{C}\right.$ of methylphenyl), $124.74\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 112.63 ( $\mathrm{C}-5$ of pyridine), $48.08\left(\mathrm{C}_{a}\right), 45.23\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.83\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.14\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.56\left(\mathrm{CH}_{2}\right.$-methylphenyl), $40.98\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.28\left(\mathrm{C}_{\varepsilon}\right)$, $31.30\left(\mathrm{C}_{\beta}\right), 28.81\left(\mathrm{C}_{\delta}\right), 22.56\left(\mathrm{C}_{\gamma}\right), 20.57\left(\mathrm{CH}_{3}\right.$-phenyl), signals for $\mathrm{C}-2,3,4,6$ and $\mathrm{CH}_{3}$ of pyridine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.43$ ( $\mathrm{s}, \mathrm{TFA}$ ); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{28} \mathrm{H}_{38} \mathrm{~N}_{5} \mathrm{O}_{3}$ : $492.30[\mathrm{M}+\mathrm{H}]^{+}$, found: 492.3.

## $N^{\alpha}$-4-Fluorophenylacetyl- $N^{\text {E}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA

(15b)


Compound 15b (104 mg, 58\%, rosy solid) was synthesised according to GP IX using compound $5 \mathbf{a}$ ( 0.29 mmol ) and 4 -fluorophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.41$ ( d , ${ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.75 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.28 (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=8.7 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=5.6 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ of fluorophenyl), $7.10\left(\mathrm{t},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}={ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=8.9 \mathrm{~Hz}, 2 \mathrm{H}\right.$, $\mathrm{H}-3,5$ of fluorophenyl), 6.94 (broad s, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.73 (d, ${ }^{3} \mathrm{~J}=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} J=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{C}=\mathrm{CH} H$ ), 5.54 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.0 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.75-4.64 (m, CaH), 3.78-3.38 (m, $10 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-fluorophenyl), $3.13-3.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 2.43\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right)$, $1.70-1.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H H\right), 1.58-1.46\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.33\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.31-1.17(\mathrm{~m}$, $\left.2 \mathrm{H}, \mathrm{C}_{\mathrm{r}} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.18,169.72,164.42\left(\mathrm{CON}_{\varepsilon}\right), 160.97\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=242.1 \mathrm{~Hz}\right.$, C-4 of fluorophenyl), $158.26,157.90,132.49$ ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.0 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), 131.85 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 130.75$ ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 114.84 (d, ${ }^{2} J_{\mathrm{C}, \mathrm{F}}=21.1 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorophenyl), 112.78 (C-5 of pyridine), 99.34 (C-3 of pyridine), 48.18 $\left(\mathrm{C}_{\alpha}\right), 45.56\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.17\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.95\left(\mathrm{CH}_{2}\right.$ of piperazine), 40.94 ( $\mathrm{CH}_{2}$-phenyl), $40.84\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.26\left(\mathrm{C}_{\varepsilon}\right), 31.25\left(\mathrm{C}_{\beta}\right), 28.83\left(\mathrm{C}_{\delta}\right), 22.55\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-4$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $\mathrm{d}_{6}$ ) $\delta=-74.75$ (s, TFA), -116.76--116.96 (m, F-4); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{FN}_{5} \mathrm{O}_{3}: 496.27$ [M+H] ${ }^{+}$, found: 496.3.

## $N^{\alpha}$-4-Chlorophenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide $\times$ TFA

 (15c)

Compound 15c (18 mg, 29\%, white solid) was synthesised according to GP IX using compound $5 \mathbf{a}(0.10 \mathrm{mmol})$ and 4 -chlorophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.44$ ( d , $\left.{ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.73\left(\mathrm{t},{ }^{3} \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine $)$, 7.37-7.31 (m, 2H, $2 \times \mathrm{H}$ of chlorophenyl), $7.30-7.24(\mathrm{~m}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of chlorophenyl), 6.92 ( d , ${ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.72 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.73-4.66\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.69-3.40\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-chlorophenyl), 3.12-3.04 (m, 2H, C $\varepsilon_{\varepsilon} \mathrm{H}_{2}$ ), $2.42\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.71-1.60\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right)$, 1.59-1.47 (m, 1H, C ${ }_{\beta} H \mathrm{H}$ ), 1.46-1.35 (m, 1H, C $\mathrm{H}_{2}$ ), 1.32-1.19 (m, 2H, C $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=170.17,169.49,164.47,135.36,131.85\left(\mathrm{CH}_{2}=C\right), 131.10,130.84(2 \times \mathrm{C}$ of chlorophenyl), 128.10 ( $2 \times \mathrm{C}$ of chlorophenyl), $124.83\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 112.80 ( $\mathrm{C}-5$ of pyridine), 48.24 $\left(\mathrm{C}_{\alpha}\right), 45.55\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.17\left(\mathrm{CH}_{2}\right.$ of piperazine $), 44.00\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.11$ $\left(\mathrm{CH}_{2}\right.$-chlorophenyl), $40.87\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right), 31.25\left(\mathrm{C}_{\beta}\right), 28.84\left(\mathrm{C}_{\delta}\right), 22.58\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=-74.67$ (s, TFA); MS (ESI $)$ : m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{CIN}_{5} \mathrm{O}_{3}: 512.24\left[\mathrm{M}\left({ }^{35} \mathrm{CI}\right)+\mathrm{H}\right]^{+}$, found: 512.2.

## $N^{\alpha}$-4-Bromophenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide $\times$ TFA

 (15d)

Compound 15d (34 mg, 51\%, white solid) was synthesised according to GP IX using compound 5 a ( 0.10 mmol ) and 4-bromophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.44$ ( d , ${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.73 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.47 (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of bromophenyl), 7.21 (d, ${ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of bromophenyl), 6.93 (broad $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.72 (d, ${ }^{3} \mathrm{~J}=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $5.54\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{C}=\mathrm{CH} H), 4.74-4.66\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), 3.77-3.38\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}-$ bromophenyl), 3.13-3.04 (m, 2H, C $\varepsilon_{\varepsilon} \mathrm{H}_{2}$ ), $2.43\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.71-1.59\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.59-$ $1.48\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.46-1.35\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right), 1.32-1.18\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=170.13,169.37,164.42,158.12$ ( $\mathrm{q},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=36.3 \mathrm{~Hz}, \mathrm{CO}$ of TFA), 135.77 (C-1 of bromophenyl), $131.84\left(\mathrm{CH}_{2}=C\right), 131.21(2 \times \mathrm{C}$ of bromophenyl), $131.00(2 \times \mathrm{C}$ of bromophenyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 119.53\left(\mathrm{C}-4\right.$ of bromophenyl), $112.78\left(\mathrm{C}-5\right.$ of pyridine), $48.21\left(\mathrm{C}_{\alpha}\right), 45.54$ $\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.14\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.97\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $41.15\left(\mathrm{CH}_{2}-\right.$ bromophenyl), $40.86\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.25\left(\mathrm{C}_{\varepsilon}\right), 31.24\left(\mathrm{C}_{\beta}\right), 28.83\left(\mathrm{C}_{\delta}\right), 22.56\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-2,3,4,6$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.76$ (s, TFA); MS (ESI ${ }^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{BrN}_{5} \mathrm{O}_{3}: 556.19\left[\mathrm{M}\left({ }^{79} \mathrm{Br}\right)+\mathrm{H}\right]^{+}$, found: 556.2.

## $N^{\alpha}$-4-lodophenylacetyl- $N^{*}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA (X)



Compound 15e ( $49 \mathrm{mg}, 32 \%$, light yellow solid) was synthesised according to GP IX using compound $5 \mathbf{a}$ ( 0.21 mmol ) and 4-iodophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=8.42$ ( d , ${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.69 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.64 ( d , ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-3,5$ of iodophenyl), 7.07 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ of iodophenyl), 6.87 (broad $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.69 ( $\mathrm{d},{ }^{3} \mathrm{~J}=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1$, $10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz}$, $\left.{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.74-4.64\left(\mathrm{~m}, \mathrm{C}_{a} \mathrm{H}\right), 3.73-3.36\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}-$ iodophenyl), 3.15-3.04 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.41\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right), 1.70-1.59\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right), 1.58-1.47$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.47-1.33 (m, 2H, $\mathrm{C}_{\bar{\circ}} \mathrm{H}_{2}$ ), 1.33-1.17 (m, 2H, C $\mathrm{C}_{\gamma} \mathrm{H}_{2}$; ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=170.07,169.35,164.41,157.35,152.70,136.88$ (C-3.5 of iodophenyl), 136.15 (C-1 of iodophenyl), $131.84\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, $131.40\left(\mathrm{C}-2,6\right.$ of iodophenyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 112,71(\mathrm{C}-5$ of pyridine), 92.15 (C-4 of iodophenyl), $48.20\left(\mathrm{C}_{\alpha}\right), 45.77\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.90\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.14\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.29\left(\mathrm{CH}_{2}\right.$-iodophenyl), $40.90\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.26\left(\mathrm{C}_{\varepsilon}\right), 31.26\left(\mathrm{C}_{\beta}\right), 28.83\left(\mathrm{C}_{\delta}\right), 22.57\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-3,4$ of pyridine are not visible; ${ }^{19} \mathrm{~F}$-NMR (DMSO- $d_{6}$ ) $\delta=-74.52$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{IN}_{5} \mathrm{O}_{3}$ : $604.18[\mathrm{M}+\mathrm{H}]^{+}$, found: 604.2.

## $N^{\alpha}$-2-Fluorophenylacetyl- $N^{*}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA

 (15f)

Compound $\mathbf{1 5 f}$ ( $13 \mathrm{mg}, \mathbf{3 5 \%}$, colourless solid) was synthesised according to GP IX using compound $5 \mathbf{a}$ ( 0.06 mmol ) and 2-fluorophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.42$ ( d , ${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.06 (t, ${ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.67 (broad s, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.35$7.20(\mathrm{~m}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of fluorophenyl), 7.17-7.08 (m, $2 \mathrm{H}, 2 \times \mathrm{H}$ of fluorophenyl), 6.87 (broad s, 1 H , $\mathrm{H}-3$ of pyridine), 6.68 (d, ${ }^{3} \mathrm{~J}=6.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), 6.19 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 6.04 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH} H$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.78-4.67 (m, 1H, CaH), 3.82-3.34 (m, 10H, $4 \times \mathrm{CH}_{2}$ of piperazine $/ \mathrm{CH}_{2}-$ fluorophenyl), 3.18-3.02 (m, 2H, C $\mathrm{C}_{2}$ ), $2.40\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right)$, 1.72-1.60 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right), 1.60-1.49$ (m, 1H, $\mathrm{C}_{\beta} H \mathrm{H}$ ), 1.49-1.36 (m, 2H, $\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}$ ), 1.35-1.19 (m, 2H, C $\mathrm{C}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=170.10(\mathrm{CON}), 168.78\left(\mathrm{C}_{\alpha} \mathrm{CON}\right)$, $164.43\left(\mathrm{CON}_{\varepsilon}\right), 160.54\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=244.3 \mathrm{~Hz}, \mathrm{C}-2\right.$ of fluorophenyl), 158.04 (psd, ${ }^{2}{ }_{\mathrm{C}, \mathrm{F}, \mathrm{F}}=35.4 \mathrm{~Hz}, \mathrm{C}-1$ of TFA), $131.86\left(\mathrm{CH}_{2}=C\right), 131.72$ (d, ${ }^{3} J_{\mathrm{C}, \mathrm{F}}=4.5 \mathrm{~Hz}, \mathrm{CH}$ of fluorophenyl), 128.55 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.1 \mathrm{~Hz}, \mathrm{CH}$ of fluorophenyl), 124.78 ( $\mathrm{CH}_{2}=\mathrm{C}$ ), 124.10 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.5 \mathrm{~Hz}, \mathrm{C}-5$ of fluorophenyl), 123.25 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=15.9 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), 114.95 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.7 \mathrm{~Hz}$, $\mathrm{C}-3$ of fluorophenyl), 112.70, $48.29\left(\mathrm{C}_{\alpha}\right), 45.40\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.01\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.13\left(\mathrm{CH}_{2}\right.$ of piperazine), $40.95\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.29\left(\mathrm{C}_{\varepsilon}\right), 34.88\left(\mathrm{CH}_{2}\right.$-fluorophenyl), $31.27\left(\mathrm{C}_{\beta}\right), 28.85\left(\mathrm{C}_{\delta}\right), 22.56\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and C-2,3,4,6 of pyridine are not visible; ${ }^{19}$ F-NMR (DMSO- $d_{6}$ ) $\delta=-74.48$ (s, TFA), $-117.26--117.36$ ( $\mathrm{m}, \mathrm{F}-2$ ); $\mathrm{MS}\left(\mathrm{ESI}{ }^{+}\right)$: m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{FN}_{5} \mathrm{O}_{3}: 496.27$ [M+H] ${ }^{+}$, found: 496.4.

## $N^{\alpha}$-3-Fluorophenylacetyl- $\boldsymbol{N}^{\mathbf{z}}$-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide×TFA

 (15g)

Compound $\mathbf{1 5 g}$ ( $14 \mathrm{mg}, 38 \%$, colourless solid) was synthesised according to GP IX using compound $5 \mathbf{5 a}(0.06 \mathrm{mmol})$ and 3 -fluorophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.45$ (d, ${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.66-7.58 (m, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.34-7.28 ( $\mathrm{m}, \mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of fluorophenyl), 7.13-7.06 (m, 2H, H-2,6 of fluorophenyl), 7.05-6.95 (m, 1H, $\mathrm{H}-4$ of fluorophenyl), 6.87-6.76 (m, $1 \mathrm{H}, \mathrm{H}-3$ of pyridine), $6.66\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), 6.18 (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), 4.77-4.65 (m, 1H, $\left.\mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.72-3.34$ ( $\mathrm{m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-fluorophenyl), 3.19-3.03 (m, $2 \mathrm{H}, \mathrm{C}_{8} \mathrm{H}_{2}$ ), $2.38\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{CH}_{3}\right)$, 1.71-1.60 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right), 1.59-1.48\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}\right)$, 1.47-1.33 (m, 2H, $\left.\mathrm{C}_{\bar{\delta}} \mathrm{H}_{2}\right), 1.31-1.13(\mathrm{~m}, 2 \mathrm{H}$, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.05,169.26,164.40\left(\mathrm{C}_{\alpha} \mathrm{CON}, \mathrm{CON}_{\alpha}, \mathrm{CON}_{\varepsilon}\right), 161.95(\mathrm{~d}$, ${ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=242.9 \mathrm{~Hz}, \mathrm{C}-3$ of fluorophenyl), 139.09 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.8 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), 131.83 $\left(\mathrm{CH}_{2}=C\right), 129.95$ ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.5 \mathrm{~Hz}, \mathrm{C}-5$ of fluorophenyl), 125.11 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=2.6 \mathrm{~Hz}, \mathrm{C}-6$ of fluorophenyl), $124.77\left(\mathrm{CH}_{2}=\mathrm{C}\right), 115.69\left(\mathrm{~d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.3 \mathrm{~Hz}, \mathrm{C}-2\right.$ of fluorophenyl), 113.11 (d, ${ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=20.7 \mathrm{~Hz}, \mathrm{C}-4$ of fluorophenyl), 112.66 (C-5 of pyridine), $48.21\left(\mathrm{C}_{\mathrm{a}}\right), 45.29\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.89\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.14\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.47\left(\mathrm{CH}_{2}\right.$-fluorophenyl), $40.97\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.25\left(\mathrm{C}_{\varepsilon}\right), 31.26\left(\mathrm{C}_{\beta}\right), 28.80\left(\mathrm{C}_{\bar{\delta}}\right), 22.56\left(\mathrm{C}_{\gamma}\right)$, signals for $\mathrm{CH}_{3}$ and $\mathrm{C}-$ 3,4 are not visible; ${ }^{19}$ F-NMR (DMSO- $d_{6}$ ) $\delta=-74.30$ ( s , TFA), -113.86--113.96 (m, F-3); MS ( $\mathrm{ESI} \mathrm{I}^{+}$: $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{27} \mathrm{H}_{35} \mathrm{FN}_{5} \mathrm{O}_{3}: 496.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 496.4.

## $N^{\alpha}$-Phenylcarbamoyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(6-nitropyridin-3-yl)piperazidexTFA (17a)



Compound 17a ( $72 \mathrm{mg}, 86 \%$, yellow solid) was synthesised according to GP VIII using compound 5b ( 0.16 mmol ) and phenyl isocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.66$ ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{NH}-$ phenyl), 8.26 (d, ${ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ of pyridine), 8.18 ( $\mathrm{d},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), $8.05\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.48 (dd, ${ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{2} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.36 (dd, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.1 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ of phenyl), $7.26-7.14$ (m, 2H, H-3,5 of phenyl), 6.92-6.87 (m, 1H, H-4 of phenyl), $6.50\left(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right.$ ), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.76-4.69 (m, 1H, C C H), 3.82-3.51 ( $\mathrm{m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.16-3.07 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.72-1.60 (m, 1H, $\mathrm{C}_{\beta} H H$ ), 1.57-1.39 (m, 3H, $\mathrm{C}_{\beta} H \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.39-1.26 (m, 2H, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}\right) ;{ }^{13} \mathrm{C}-$ NMR (DMSO- $\left.d_{6}\right) \delta=170.89(C O N), 164.41\left(\mathrm{CON}_{\varepsilon}\right), 154.65\left(\mathrm{CON}_{\alpha}\right), 149.43,146.85,140.21$, 133.52, $131.83\left(\mathrm{CH}_{2}=C\right)$, $128.66\left(\mathrm{C}-3,5\right.$ of phenyl), $124.76\left(\mathrm{CH}_{2}=\mathrm{C}\right), 121.16,120.63,119.80$, 117.52 (C-2,6 of phenyl), $48.38\left(\mathrm{C}_{\alpha}\right), 46.03\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.59\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.98\left(\mathrm{CH}_{2}\right.$ of piperazine), $40.75\left(\mathrm{CH}_{2}\right.$ of piperazine $), 38.29\left(\mathrm{C}_{\varepsilon}\right), 32.25\left(\mathrm{C}_{\beta}\right), 28.87\left(\mathrm{C}_{\delta}\right), 22.32$ $\left.\left(\mathrm{C}_{7}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.82(\mathrm{~s}, \mathrm{TFA}) ; \mathrm{MS}(\mathrm{ESI})^{+}\right): \mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{32} \mathrm{~N}_{7} \mathrm{O}_{5}: 510.25$ $[\mathrm{M}+\mathrm{H}]^{+}$, found: 510.1.

## $N^{a}$-4-Fluorophenylacetyl- $\boldsymbol{N}^{\varepsilon}$-acryloyl-L-lysine-4-(6-nitropyridin-3-yl)piperazide×TFA

 (17b)

Compound 17b ( $15 \mathrm{mg}, 38 \%$, yellow solid) was synthesised according to GP IX using compound $5 \mathbf{5 b}(0.06 \mathrm{mmol})$ and 4 -fluorophenyl acetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.42$ ( d , $\left.{ }^{3} \mathrm{~J}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}\right), 8.23\left(\mathrm{~d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), $8.17\left(\mathrm{~d},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), $8.03\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.45\left(\mathrm{dd},{ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of pyridine), $7.32-7.25(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-2,6$ of fluorophenyl), 7.12-7.05 (m, 2H, H-3,5 of fluorophenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.54 (dd, $\left.{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}\right), 4.74-4.66\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.76-3.29\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-fluorophenyl), $3.12-3.03\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.72-1.59\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}\right), 1.59-1.47$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}$ ), 1.46-1.34 (m, 2H, $\mathrm{C}_{\bar{\circ}} \mathrm{H}_{2}$ ), 1.31-1.18 (m, 2H, C $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=170.08,169.68,164.39,160.96$ ( $\mathrm{d},{ }^{1}{ }_{\mathrm{C}, \mathrm{F}}=241.9 \mathrm{~Hz}, \mathrm{C}-4$ of fluorophenyl), 149.43, 146.87, 133.51 ( $\mathrm{C}-2$ of pyridine), 132.49 ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.0 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), $131.84\left(\mathrm{CH}_{2}=\mathrm{C}\right)$, 130.74 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.9 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), $124.75\left(\mathrm{CH}_{2}=\mathrm{C}\right.$ ), 120.62 ( $\mathrm{C}-4$ of pyridine), 119.75 (C-5 of pyridine), 114.84 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=21.1 \mathrm{~Hz}, \mathrm{C}-3.5$ of fluorophenyl), $48.10\left(\mathrm{C}_{\mathrm{a}}\right), 45.96$ $\left(\mathrm{CH}_{2}\right.$ of piperazine $), 45.58\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.89\left(\mathrm{CH}_{2}\right.$ of piperazine $), 40.95\left(\mathrm{CH}_{2}-\right.$ fluorophenyl), $40.76\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.26\left(\mathrm{C}_{\varepsilon}\right), 31.26\left(\mathrm{C}_{\beta}\right), 28.82\left(\mathrm{C}_{\delta}\right), 22.57\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-$ NMR (DMSO- $d_{6}$ ) $\delta=-74.70$ (s, TFA), -116.79--116.88 (m, F-4); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{32} \mathrm{FN}_{6} \mathrm{O}_{5}: 527.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 527.2.

## $N^{\alpha}$-4-Fluorobenzoyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-nitropyridin-3-yl)piperazide×TFA (17c)



Compound 17c ( $22 \mathrm{mg}, 55 \%$, yellow solid) was synthesised according to GP VIII using compound 5b ( 0.06 mmol ) and 4-fluorobenzoyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.67$ (d, ${ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.26\left(\mathrm{~d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), $8.17\left(\mathrm{~d},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), 8.06 (t, ${ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), $8.00-7.94$ (m, $2 \mathrm{H}, \mathrm{H}-2,6$ of fluorophenyl), 7.48 (dd, ${ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.33-7.25(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5$ of fluorophenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.53 ( dd , ${ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 4.94-4.84 (m, 1H, CaH), 3.87-3.46(m, $8 \mathrm{H}, 4 \times \mathrm{CH}_{2}$ of piperazine), 3.16-3.07 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.79-1.67 (m, 2H, $\mathrm{C}_{\beta} \mathrm{H}_{2}$ ), 1.51-1.28 (m, 4H, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}\right)$; ${ }^{13}$ C-NMR (DMSO- $d_{6}$ ) $\delta=170.27,165.12,164.43,163.93\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=248.8 \mathrm{~Hz}, \mathrm{C}-4\right.$ of fluorophenyl), 149.45, 146.83, 133.51 ( $\mathrm{C}-2$ of pyridine), $131.84\left(\mathrm{CH}_{2}=\mathrm{C}\right), 130.31$ ( d , ${ }^{4} J_{\mathrm{C}, \mathrm{F}}=2.9 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), 130.18 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=9.0 \mathrm{~Hz}, \mathrm{C}-2,6$ of fluorophenyl), 124.76 $\left(\mathrm{CH}_{2}=\mathrm{C}\right), 120.59$ ( $\mathrm{C}-4$ o pyridine), 119.79 ( $\mathrm{C}-5$ of pyridine), 115.12 ( $\mathrm{d},{ }^{2}{ }^{\mathrm{J}} \mathrm{J}, \mathrm{F}=21.7 \mathrm{~Hz}, \mathrm{C}-3,5$ of fluorophenyl), $49.26\left(\mathrm{C}_{a}\right), 46.03\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.63\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.99\left(\mathrm{CH}_{2}\right.$ of piperazine), $40.85\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.27\left(\mathrm{C}_{\varepsilon}\right), 30.75\left(\mathrm{C}_{\beta}\right), 28.89\left(\mathrm{C}_{\delta}\right), 22.95\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-$ NMR (DMSO- $d_{6}$ ) $\delta=-74.70(\mathrm{~s}, \mathrm{TFA}),-109.28\left(\mathrm{tt},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=8.8 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=5.5 \mathrm{~Hz}, \mathrm{~F}-4\right)$; MS (ESI $\left.{ }^{+}\right):$ $\mathrm{m} / \mathrm{z}$ calculated for $\mathrm{C}_{25} \mathrm{H}_{30} \mathrm{FN}_{6} \mathrm{O}_{5}: 513.23[\mathrm{M}+\mathrm{H}]^{+}$, found: 513.2.

## $\boldsymbol{N}^{\alpha}$-2-Fluorophenylacetyl- $\boldsymbol{N}^{\mathbf{E}}$-acryloyl-L-lysine-4-(6-nitropyridin-2-yl)piperazide×TFA (18)



Compound 18 ( $27 \mathrm{mg}, 86 \%$, yellow solid) was synthesised according to GP IX using compound 5 m ( 0.05 mmol ) and 2-fluorophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.41$ ( d , ${ }^{3} \mathrm{~J}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.91 (dd, ${ }^{3} \mathrm{~J}=8.5,7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), $7.49\left(\mathrm{~d},{ }^{3} \mathrm{~J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), $7.36-7.21(\mathrm{~m}, 3 \mathrm{H}, \mathrm{H}-3$ of pyridine, $2 \times \mathrm{CH}$ of fluorophenyl), 7.16-7.06 (m, 2H, $2 \times \mathrm{CH}$ of fluorophenyl), 6.18 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{CH}=\mathrm{CH}_{2}$ ), 6.03 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}$ ), $5.53\left(\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH})$, 4.77-4.69 (m, $1 \mathrm{H}, \mathrm{C}, \mathrm{H})$, 3.72-3.48 (m, 10H, $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2^{-}}$ fluorophenyl), 3.14-3.05 ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.73-1.61 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{HH}$ ), 1.60-1.50 (m, $1 \mathrm{H}, \mathrm{C}_{\beta} H \mathrm{H}$ ), 1.48-1.37 (m, 2H, $\mathrm{C}_{6} \mathrm{H}_{2}$ ), 1.33-1.23 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=170.04,168.74$, $164.40\left(\mathrm{C}_{\alpha} \mathrm{CON}, \mathrm{CON}_{\alpha}, \mathrm{CON}_{\varepsilon}\right), 160.52$ ( $\mathrm{d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=}=244.2 \mathrm{~Hz}, \mathrm{C}-2$ of fluorophenyl), 157.23, 155.22, 141.03 (C-4 of pyridine), $131.85\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 131.71$ ( $\mathrm{d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=4.5 \mathrm{~Hz}, \mathrm{CH}$ of fluorophenyl), 128.51 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.1 \mathrm{~Hz}, \mathrm{CH}$ of fluorophenyl), $124.73\left(\mathrm{CH}=\mathrm{CH}_{2}\right), 124.08$ ( d , ${ }^{3} \mathrm{~J}=3.5 \mathrm{~Hz}, \mathrm{CH}$ of fluorophenyl), 123.24 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=16.0 \mathrm{~Hz}, \mathrm{C}-1$ of fluorophenyl), 114.92 ( d , ${ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}=} 21.7 \mathrm{~Hz}, \mathrm{C}-3$ of fluorophenyl), 113.12 (C-3 of pyridine), 105.61 (C-5 of pyridine), 48.30 $\left(\mathrm{C}_{a} \mathrm{H}\right), 44.42\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.23\left(\mathrm{CH}_{2}\right.$ of piperazine $), 43.99\left(\mathrm{CH}_{2}\right.$ of piperazine), 41.01 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.28\left(\mathrm{C}_{\varepsilon}\right)$, $34.87\left(\mathrm{CH}_{2}\right.$-fluorophenyl), $31.31\left(\mathrm{C}_{\beta}\right)$, $28.82\left(\mathrm{C}_{\delta}\right), 22.56\left(\mathrm{C}_{\gamma}\right)$; ${ }^{19}$ F-NMR (DMSO- $d_{6}$ ) $\delta=-74.84$ (s, TFA), -117.25--117.36 (m, F-2); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{32} \mathrm{FN}_{6} \mathrm{O}_{5}$ : $527.24[\mathrm{M}+\mathrm{H}]^{+}$, found: 527.3.

## $N^{\alpha}$-2-Iodophenylacetyl- $N^{k}$-acryloyl-L-lysine-4-(pyridin-4-yl)piperazidexTFA (19)



Compound 19 ( $64 \mathrm{mg}, 63 \%$, colourless oil) was synthesised according to GP IX using compound $5 \mathbf{t}(0.14 \mathrm{mmol})$ and 2 -iodophenylacetic acid. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}\right) \delta=8.31\left(\mathrm{~d},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}\right.$, $2 \mathrm{H}, \mathrm{H}-2,6$ of pyridine), 7.86 (d, ${ }^{3} \mathrm{~J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}$ of iodophenyl), $7.40-7.30(\mathrm{~m}, 2 \mathrm{H}, 2 \times \mathrm{H}$ of iodophenyl), $7.05-6.98$ (m, 1H, H of iodophenyl), 6.88 (broad s, 2H, H-3,5 of pyridine), 6.56 ( $d,{ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $6.24\left(\mathrm{~d},{ }^{3} \mathrm{~J}=16.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH} H\right.$ ), 6.16-6.03 (m, $2 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), $5.66\left(\mathrm{~d},{ }^{3} \mathrm{~J}=10.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CHH}\right.$ ), 4.93 (broad s, $1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}$ ), $4.06-3.58\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-iodophenyl), 3.46-3.19 (m, 2H, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.80-1.21 (m, $6 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}, \mathrm{C}_{\gamma} \mathrm{H}_{2}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{33} \mathrm{~N}_{5} \mathrm{O}_{3}: 590.16[\mathrm{M}+\mathrm{H}]^{+}$, found: 590.3.

## $N^{\alpha}$-Phenylcarbamoyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-fluoropyridin-2-yl)piperazide×TFA (20a)



Compound 20a (49 mg, 49\%, white solid) was synthesised according to GP VIII using compound 5 d ( 0.21 mmol ) and phenyl isocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $d_{6}$ ) $\delta=8.68$ (s, NH-phenyl), 8.66 (s, 1H, NH-phenyl) 8.04 (t, ${ }^{3} \mathrm{~J}=5.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}$ ), 7.70 (ps-q, J=8.3 Hz, $1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.45 (dd, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz},{ }^{2} \mathrm{~J}=1.1 \mathrm{~Hz}, \mathrm{H}-2,6$ of phenyl), 7.36 (dd, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz},{ }^{4} \mathrm{~J}=1.1 \mathrm{~Hz}$, $\mathrm{H}-2,6$ of phenyl), $7.30-7.25$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-3,5$ of phenyl), $7.24-7.17$ ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{H}-3,5$ of phenyl), 6.99-6.94 (m, H-4 of phenyl), 6.92-6.86 (m, H-4 of phenyl), 6.70 (dd, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}=8.3 \mathrm{~Hz} \text {, }}$ ${ }^{5} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=2.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3$ of pyridine), 6.49 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 6.31 ( $\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=7.7 \mathrm{~Hz}$, ${ }^{3} J_{\mathrm{H}, \mathrm{F}}=2.7 \mathrm{~Hz}, \mathrm{H}-5$ of pyridine), 6.18 (dd, ${ }^{3}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz}$, ${ }^{2} J=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), 5.51 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CH}$ ), 4.76-4.69(m,1H,
$\left.\mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.73-3.42\left(\mathrm{~m}, 8 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine), 3.15-3.04(m,2H, $\left.\mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 1.71-1.58(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{C}_{\beta} \mathrm{HH}$ ), 1.56-1.37 (m,3H, $\mathrm{C}_{\beta} H \mathrm{H}_{\mathrm{H}}, \mathrm{C}_{\bar{\gamma}} \mathrm{H}_{2}$ ), 1.39-1.19 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), signals for $\mathrm{H}-2,3,4,5,6$ and NH -phenyl appear in duplicate, probably due to potential atropisomers which originate from the hindered rotation around the N-phenyl bond ${ }^{51-52}$; ${ }^{13} \mathrm{C}$-NMR (DMSO- $d_{6}$ ) $\delta=170.73$ (CON), $164.40\left(\mathrm{CON}_{\varepsilon}\right), 161.96\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=233.4 \mathrm{~Hz}, \mathrm{C}-6\right.$ of pyridine), $157.65\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=15.8 \mathrm{~Hz}, \mathrm{C}-2\right.$ of pyridine), $154.63\left(\mathrm{CON}_{\alpha}\right), 142.71$ ( $\mathrm{d},{ }^{4}{ }^{4} \mathrm{C}, \mathrm{F}=8.2 \mathrm{~Hz}, \mathrm{C}-3$ of pyridine), 140.24 ( $\mathrm{C}-1$ of phenyl), 139.69 ( $\mathrm{C}-1$ of phenyl), $131.83\left(\mathrm{CH}_{2}=\mathrm{C}\right), 128.70$ ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=8.4 \mathrm{~Hz}, \mathrm{C}-4$ of pyridine), 128.67 ( $\mathrm{C}-3,5$ of phenyl), $124.72\left(\mathrm{CH}_{2}=\mathrm{C}\right), 121.76,121.13$ ( $\mathrm{C}-4$ of phenyl), 118.14 ( $\mathrm{C}-2,6$ of phenyl), 117.50, 103.60 ( $\mathrm{d}, \mathrm{J}=3.8 \mathrm{~Hz}$ ), 95.65 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=37 \mathrm{~Hz}, \mathrm{C}-5$ of pyridine), $48.40\left(\mathrm{C}_{\mathrm{a}}\right), 44.61\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.36\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.14\left(\mathrm{CH}_{2}\right.$ of piperazine $), 41.00\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, $38.29\left(\mathrm{C}_{\varepsilon}\right), 32.33\left(\mathrm{C}_{\beta}\right), 28.84\left(\mathrm{C}_{\bar{\delta}}\right), 22.28\left(\mathrm{C}_{\gamma}\right) ;{ }^{9} \mathrm{~F}$-NMR (DMSO- $\left.\mathrm{d}_{6}\right) \delta=-68.86\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=9.0 \mathrm{~Hz}\right.$, F-6), -74.61 (s, TFA); MS (ESI + ): m/z calculated for $\mathrm{C}_{25} \mathrm{H}_{32} \mathrm{FN}_{6} \mathrm{O}_{3}: 483.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 483.1.

## $N^{\alpha}$-Phenylcarbamoyl- $\boldsymbol{N}^{\boldsymbol{\varepsilon}}$-acryloyl-L-lysine-4-(6-fluoropyridin-3-yl)piperazide $\times$ TFA (20b)



Compound 20b (79 mg, 67\%, yellow solid) was synthesised according to GP VIII using compound $5 \mathbf{j}(0.20 \mathrm{mmol})$ and phenyl isocyanate. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.67(\mathrm{~s}, 1 \mathrm{H}, \mathrm{NH}-$ phenyl), $8.04\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right), 7.85\left(\mathrm{dd},{ }^{4}{ }_{\mathrm{J}}^{\mathrm{H}, \mathrm{H}} \mathrm{H}=2.8 \mathrm{~Hz},{ }^{5}{ }_{\mathrm{H}, \mathrm{H}}=1.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right.$ of pyridine), 7.63 (ddd, ${ }^{3} J_{\mathrm{H}, \mathrm{H}}=9.1 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, F}=7.1 \mathrm{~Hz},{ }^{4} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=3.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.36 (dd, ${ }^{3} \mathrm{~J}=8.6 \mathrm{~Hz}$, ${ }^{4} J=1.0 \mathrm{~Hz}, 2 \mathrm{H}, \mathrm{H}-2,6$ of phenyl), $7.26-7.17\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-3,5\right.$ of phenyl), $7.05\left(\mathrm{dd},{ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{H}}=9.0 \mathrm{~Hz}\right.$, ${ }^{3} \mathrm{~J}_{\mathrm{H}, \mathrm{F}}=3.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), $6.89\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right.$ of phenyl), 6.48 ( $\mathrm{d},{ }^{3} \mathrm{~J}=8.4 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 6.17 (dd, ${ }^{3} \mathrm{~J}=17.1,10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{CH}=\mathrm{CH}_{2}$ ), 6.02 (dd, ${ }^{3} \mathrm{~J}=17.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}$, $\mathrm{C}=\mathrm{CHH}$ ), 5.52 ( $\mathrm{dd},{ }^{3} \mathrm{~J}=10.1 \mathrm{~Hz},{ }^{2} \mathrm{~J}=2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}=\mathrm{CHH}$ ), $4.77-4.68\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C}_{\mathrm{a}} \mathrm{H}\right), 3.99-3.45$ ( $\mathrm{m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), $3.23-3.05\left(\mathrm{~m}, 6 \mathrm{H}, 2 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), 1.71-1.57 (m, 1H, $\mathrm{C}_{\beta} H H$ ), 1.56-1.38 (m, 3H, $\mathrm{C}_{\beta} H \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.37-1.26 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ); ${ }^{13} \mathrm{C}-$ NMR (DMSO- $d_{6}$ ) $\delta=170.55(\mathrm{CON})$, $164.41\left(\mathrm{CON}_{\varepsilon}\right), 156.94\left(\mathrm{~d},{ }^{1} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=228.5 \mathrm{~Hz}, \mathrm{C}-6\right.$ of pyridine), $154.63\left(\mathrm{CON}_{\mathrm{a}}\right), 145.30\left(\mathrm{~d},{ }^{4} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=3.9 \mathrm{~Hz}, \mathrm{C}-3\right.$ of pyridine), 140.23 ( $\mathrm{C}-1$ of phenyl), 134.08 ( $\mathrm{d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=15.2 \mathrm{~Hz}, \mathrm{C}-2$ of pyridine), $131.84\left(\mathrm{CH}_{2}=C\right), 129.69\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=7.4 \mathrm{~Hz}, \mathrm{C}-4\right.$ of pyridine), 128.67 ( $\mathrm{C}-3,5$ of phenyl), $124.75\left(\mathrm{CH}_{2}=\mathrm{C}\right), 121.15$ ( $\mathrm{C}-4$ of phenyl), 117.51 ( $\mathrm{C}-2,6$
of phenyl), 109.09 ( $\mathrm{d},{ }^{2} \mathrm{~J}_{\mathrm{C}, \mathrm{F}}=39.6 \mathrm{~Hz}, \mathrm{C}-5$ of pyridine), $48.90\left(\mathrm{CH}_{2}\right.$ of piperazine), $48.41\left(\mathrm{CH}_{2}\right.$ $\mathrm{CH}_{2}$ of piperazine), $48.31\left(\mathrm{C}_{a}\right), 44.62\left(\mathrm{CH}_{2} \mathrm{CH}_{2}\right.$ of piperazine), $41.10\left(\mathrm{CH}_{2} \mathrm{CH}_{2}\right.$ of piperazine), $38.31\left(\mathrm{C}_{\varepsilon}\right), 32.36\left(\mathrm{C}_{\beta}\right), 28.86\left(\mathrm{C}_{\delta}\right), 22.32\left(\mathrm{C}_{\gamma}\right) ;{ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-\mathrm{d}_{6}\right) \delta=-80.10(\mathrm{~s}, \mathrm{~F}-6),-74.48$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{25} \mathrm{H}_{32} \mathrm{FN}_{6} \mathrm{O}_{3}: 483.25[\mathrm{M}+\mathrm{H}]^{+}$, found: 483.1.

## $N^{\alpha}$-Phenylacetyl- $N^{\varepsilon}$-propionyl-L-Iysine-4-(6-methylpyridin-2-yl)piperazide $\times$ TFA (21a)



Compound 21a ( $5 \mathrm{mg}, 4 \%$, colourless solid) was synthesised according to GP VIII using compound $5 \mathbf{z}(0.17 \mathrm{mmol})$ and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=7.80(\mathrm{t}$, ${ }^{3} \mathrm{~J}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.37-7.20 (m, 4H, H-2,3,5,6 of phenyl), $6.98\left(\mathrm{~d},{ }^{3} \mathrm{~J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), $6.82\left(\mathrm{~d},{ }^{3} \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right.$ of pyridine), 6.71 ( $\mathrm{d},{ }^{3} \mathrm{~J}=7.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5$ of pyridine), $6.60(\mathrm{~s}$, $\left.1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right)$, 4.93-4.78 (m, $\left.1 \mathrm{H}, \mathrm{C}_{a} \mathrm{H}\right), ~ 4.04-3.49\left(\mathrm{~m}, 10 \mathrm{H}, 4 \times \mathrm{CH}_{2}\right.$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.26$3.10\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}\right), 2.60\left(\mathrm{~s}, 3 \mathrm{H},-\mathrm{CH}_{3}\right), 2.26-2.14\left(\mathrm{~m}, 2 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 1.77-1.42\left(\mathrm{~m}, 4 \mathrm{H}, \mathrm{C}_{\beta} \mathrm{H}_{2}\right.$, $\mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.38-1.23 (m, 2H, $\mathrm{C}_{\gamma} \mathrm{H}_{2}$ ), $1.11\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.3 \mathrm{~Hz}, 3 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right.$ ); ${ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=175.65,171.67,170.90\left(\mathrm{C}_{\alpha} \mathrm{CON}, \mathrm{CON}_{\alpha}, \mathrm{CON}_{\varepsilon}\right.$ ), 153.59, 150.82, 144.46 (C-4 of pyridine), 134.68, 129.35 ( $2 \times \mathrm{CH}$ of phenyl), 128.96 ( $2 \times \mathrm{CH}$ of phenyl), 127.42 ( $\mathrm{C}-4$ of phenyl), 114.17 (C5 of pyridine), 109.32 (C-3 of pyridine), $48.89\left(\mathrm{C}_{\alpha}\right), 46.90\left(\mathrm{CH}_{2}\right.$ of piperazine), $46.61\left(\mathrm{CH}_{2}\right.$ of piperazine), $44.31\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.31\left(\mathrm{CH}_{2}\right.$-phenyl), $41.17\left(\mathrm{CH}_{2}\right.$ of piperazine $)$, 39.14 $\left(\mathrm{C}_{\varepsilon}\right), 32.13\left(\mathrm{C}_{\beta}\right), 29.44\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 28.67\left(\mathrm{C}_{\delta}\right), 22.33\left(\mathrm{C}_{\gamma}\right), 19.50\left(-\mathrm{CH}_{3}\right), 10.11\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right) ;{ }^{19} \mathrm{~F}-$ NMR (DMSO- $d_{6}$ ) $\delta=-75.89\left(\mathrm{~s}\right.$, TFA); MS (ESI $\left.{ }^{+}\right)$: m/z calculated for $\mathrm{C}_{27} \mathrm{H}_{38} \mathrm{~N}_{5} \mathrm{O}_{3}: 483.30[\mathrm{M}+\mathrm{H}]^{+}$, found: 480.3.

## $N^{\alpha}$-Phenylacetyl- $N^{\varepsilon}$-propionyl-L-Iysine-4-(6-nitropyridin-3-yl)piperazide×TFA (21b)



Compound 21b ( $75 \mathrm{mg}, 41 \%$, yellow solid) was synthesised according to GP VIII using compound 5aa ( 0.28 mmol ) and phenylacetyl chloride. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=8.40$ (d, ${ }^{3} \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\mathrm{a}} \mathrm{H}$ ), 8.22 ( $\mathrm{d},{ }^{4} \mathrm{~J}=3.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ of pyridine), $8.17\left(\mathrm{~d},{ }^{3} \mathrm{~J}=9.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right.$ of pyridine), $7.67\left(\mathrm{t},{ }^{3} \mathrm{~J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{N}_{\varepsilon} \mathrm{H}\right.$ ), 7.44 (dd, ${ }^{3} \mathrm{~J}=9.3 \mathrm{~Hz},{ }^{4} \mathrm{~J}=3.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4$ of pyridine), 7.28-7.24 (m, 4H, H-2,3,5,6 of phenyl), 7.19-7.14 (m, 1H, H-4 of phenyl), 4.73-4.65 (m, 1H, $\mathrm{C}_{\mathrm{a}} \mathrm{H}$ ), 3.76-3.28 (m, 10H, $4 \times \mathrm{CH}_{2}$ of piperazine, $\mathrm{CH}_{2}$-phenyl), 3.02-2.92 (m, $2 \mathrm{H}, \mathrm{C}_{\varepsilon} \mathrm{H}_{2}$ ), $2.03(\mathrm{q}$, ${ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 2 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}$ ), 1.71-1.59 (m, 1H, $\left.\mathrm{C}_{\beta} H \mathrm{H}\right)$, 1.58-1.45 (m, 1H, $\left.\mathrm{C}_{\beta} \mathrm{HH}\right), 1.41-1.30(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{C}_{\delta} \mathrm{H}_{2}$ ), 1.29-1.17 (m, 2H, $\left.\mathrm{C}_{\gamma} \mathrm{H}_{2}\right), 0.96\left(\mathrm{t},{ }^{3} \mathrm{~J}=7.6 \mathrm{~Hz}, 3 \mathrm{H},-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ (DMSO- $\mathrm{d}_{6}$ ) $\delta=172.58,170.11,169.77\left(\mathrm{C}_{\alpha} \mathrm{CON}, \mathrm{CON}_{\alpha}, \mathrm{CON}_{\varepsilon}\right.$ ), 149.42 (C-6 of pyridine), 146.86 (C-3 of pyridine), 136.35 (C-1 of phenyl), $128.94(2 \times \mathrm{CH}$ of phenyl), $128.12(2 \times \mathrm{CH}$ of phenyl), 126.29 (C-4 of phenyl), 120.63 (C-4 of pyridine), 119.77 (C-5 of pyridine), $48.11\left(\mathrm{C}_{\mathrm{a}}\right), 45.95\left(\mathrm{CH}_{2}\right.$ of piperazine), $45.60\left(\mathrm{CH}_{2}\right.$ of piperazine), $43.87\left(\mathrm{CH}_{2}\right.$ of piperazine), $41.94\left(\mathrm{CH}_{2}\right.$-phenyl), 40.77 $\left(\mathrm{CH}_{2}\right.$ of piperazine), $38.14\left(\mathrm{C}_{\varepsilon}\right), 31.29\left(\mathrm{C}_{\beta}\right), 28.96\left(\mathrm{C}_{\delta}\right), 28.48\left(-\mathrm{CH}_{2}-\mathrm{CH}_{3}\right), 22.53\left(\mathrm{C}_{\gamma}\right), 9.97(-$ $\mathrm{CH}_{2}-\mathrm{CH}_{3}$ ); ${ }^{19} \mathrm{~F}-\mathrm{NMR}\left(\mathrm{DMSO}-d_{6}\right) \delta=-74.73$ (s, TFA); MS (ESI ${ }^{+}$): m/z calculated for $\mathrm{C}_{26} \mathrm{H}_{35} \mathrm{~N}_{6} \mathrm{O}_{5}$ : $511.27[\mathrm{M}+\mathrm{H}]^{+}$, found: 511.2 .

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