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ARTICLE

Copper-free Technetium-99m-click-labelling using strained cyclononyne derivatives

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Click chemistry, and in particular copper-free click reactions, have gained growing interest for radiolabeling purposes in the field of radiopharmaceutical sciences. $[^{99m}Tc][Tc(CO)_3(H_2O)_3]^+$ works as excellent starting complex for the radiolabeling of biomolecules under mild conditions. A new chelator, investigated for the copper-free strain-promoted cycloaddition (SPAAC), was synthesised containing the 2,2'-dipicolylamine (DPA) moiety for the ^{99m}Tc-tricarbonyl core and compared with a chelator based on activated esters for conventional radiolabeling. For the copper-free click labeling procedure, a DPA containing 4,8-diazacyclononyne moiety was prepared from a sulfonyl-modified diamide (four steps, 64% yield) ensued by the Nicholas reaction with butyne-1,3-diol. The ^{99m}Tc-DPA-DACN-complex was prepared with a radiochemical conversion (RCC) of 89% after 30 min. The following SPAAC reaction with an azide-functionalised PSMA molecule was performed within 4 hours to obtain the PSMA (prostate-specific membrane antigen) targeting ^{99m}Tc-complex with 79% RCC and without side products. For comparison, a second DPA-chelator based on a tetrafluorophenyl (TFP) ester was prepared (three steps, 64% yield) and was successfully radiolabeled with $[[^{99m}Tc]Tc(CO)_3(H_2O)_3]^+$ in 89% RCC after 20 min and >99% radiochemical purity after separation using an RP18 cartridge. The subsequent conjugation of an amine-functionalised PSMA targeting molecule was performed with 23% RCC after 150 min. Two other unknown side products were observed indicating hydrolysis of the TFP ester during the labeling. All nonradioactive $Re(CO)_3$ complexes were synthesised from $(Et_4N)_2[ReBr_3(CO)_3]$ (91% yield for the ^{nat}Re-DPA-TFP ester, 76% yield for the ^{nat}Re-DPA-DACN) and characterised to confirm the identity of the ^{99m}Tc-complexes.

Introduction

The demand for new, innovative and facile strategies for the design of new radiotracers continues unabated. Especially the development of target-specific radiotracers, that requires the radiolabelling biomolecules, such as peptides, proteins, and antibodies, faces synthetic challenges. Click chemistry, as it was first described by Sharpless and co-workers in 2001[1] and honoured with the Nobel prize for Chemistry in 2022, has emerged as a powerful tool in almost all fields of modern chemistry, including bioconjugation, materials science, and drug discovery.[2,3] The wide applicability of click chemistry has influenced the field of radiopharmacy[4,5] and established these reactions as a significant and indispensable tool in the design and syntheses of radiopharmaceuticals.[6-9]

Considering the general composition of target-specific radiometal-based radiotracers, the chelator for the respective radiometal is connected to a biologically or pharmaceutically active molecule to target the biological tissue of choice. However, the bioconjugation between both molecule parts is often characterised by time-consuming multi-step syntheses and low efficiencies due to cross-reactivity with other functional groups. Click chemistry enables the rapid and facile conjugation of chelators to biomolecules with high selectivity and, over the past decade, it has been frequently and efficiently used to prepare radiolabelled imaging agents.[10,11] The most prominent click reaction is the Huisgen 1,3-dipolar cycloaddition of alkynes and azides.[12] The Cu(I)-catalysed version (CuAAC) leads to the selective and efficient synthesis of 1,4-disubstituted 1,2,3-triazoles under mild reaction conditions and has been found access mostly for the radiolabelling with fluorine-18 and less for radiometals.[13] However, metal-catalysed click reactions can be problematic or even harmful for biological studies owing to the toxicity of copper[14] when it comes to radiolabelling biomolecules. Moreover, the metal catalyst can interfere in the complexation of radiometals[15] with the chelator and it can be partly bound by the targeting biomolecule. Metal-free click reactions like the Diels-Alder cycloaddition the strain-promoted azide-alkyne cycloaddition (SPAAC),[16] and the Staudinger ligation,[17] are therefore valuable alternatives in the design of radiopharmaceuticals.

The bottleneck for the application of SPAAC in radiopharmacy is often the strained alkyne compound, which consists of a

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† Electronic Supplementary Information (ESI) available: NMR spectra of compounds, radiolabelling methods and chromatograms. See DOI: 10.1039/x0xx00000x

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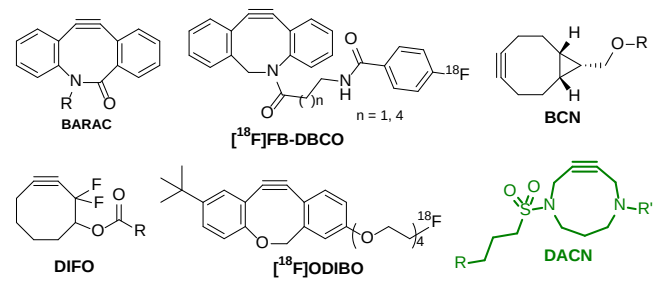
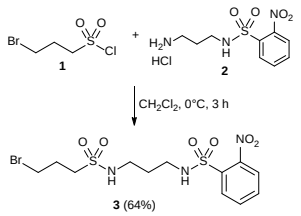


Scheme 1. Different cycloalkyne derivatives used for radiolabelling and new DACN derivative (green) developed in this work.

functionalised cyclooctyne or cyclononyne moiety with high lipophilicity due to additional benzene rings like in DBCO and/or a reduced stability as found with BCN, which makes them unfavourable even for radiolabelling purposes under physiological conditions. Additionally, the alteration of the final radiotracer is often enormous due to the additional high molar mass of the respective click building block. To overcome these obstacles, functionalised diazacyclononyne (DACN) derivatives were developed, which are more hydrophilic and allow a double functionalization at the heteroatoms to connect, e.g., a bio(macro)molecule, a fluorescent dye or a chelating system for a later labelling with radiometals.

Technetium-99m is still most prominent in radiopharmacy as it is referred to as the 'workhorse' of nuclear medicine due to its ideal physical characteristics (γ-emitter, E_γ = 141 keV, $t_{1/2}$ = 6.01 h).[18] A diverse redox chemistry with oxidation states from −I to +VII is found for technetium. [19,20] Classically, $[^{99m}Tc]TcO_4^-$ comprises Tc in the oxidation state +7 and has to be reduced to a lower oxidation state to be conjugated to biomolecules or other carriers with suitable reducing agents, like $SnCl_2{\cdot}H_2O$ or $NaBH_4$. A variety of technetium cores have been used, such as the $[Tc{=}O]^{3+}$, $[Tc{\equiv}N]^{+2}$, $[Tc(CO)_3]^+$, and Tc-HYNIC cores.[21,22] Especially the ^{99m}Tc-tricarbonyl core[23] with Tc in the oxidation state +1 has gained a special interest due to its facile one-pot synthesis, water solubility, and the rapid ligand exchange of the water ligands with various suitable ligands and bear the possibility of a kit formulation.[24] Several approaches with the ^{99m}Tc-tricarbonyl core and click chemistry are known using the tetrazine click,[25,26] the click-to-chelate approach[27] or the Cu-catalysed click version.[28-30] One example is known to the best of our knowledge using SPAAC for ^{99m}Tc-radiolabeling.[31] Here, our aim is to synthesise a new and convenient click system on the basis of DACN as alkyne basis connected with the DPA chelator for the ^{99m}Tc-tricarbonyl for an easy and Cu-free click-labelling procedure. This approach is compared with the DPA-chelator, which contains an activated ester allowing a conventional amine labelling. For this purpose, two biologically active molecules with PSMA-binding moiety either with azide or with N-terminal amine functional group were prepared.



Scheme 2. Synthesis of the 1,3-functionalised propanediamine **3** from sulfonyl chloride **1** and nosylated propanediamine **2**.

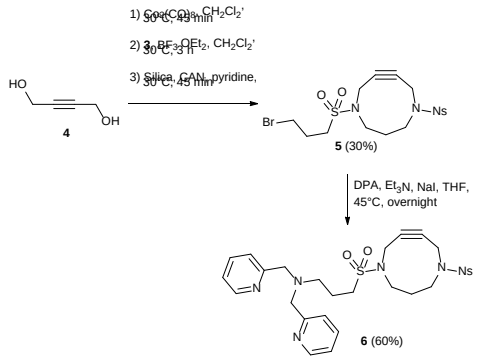
Results and discussion

Synthesis of the new DACN chelator

Both new tridentate chelators are based on the 2,2'-dipicolylamine (DPA) moiety, which is proven to be an effective ligand for the stable coordination of ^{99m}Tc in the oxidation state +1.[32-34] The design of the novel chelator for the Cu-free click-labelling is based on the diazacyclononyne (DACN) heterocycle. It was recently introduced for SPAAC by Kawasaki *et al.*[35,36] and is easily accessible via a convenient one-pot double Nicholas reaction. To make the DACN derivatives suitable for radiolabelling with the ^{99m}Tc-tricrbonyl core, they have to be equipped with the DPA chelator. The functionalised bis-sulfonylated diamide **3**, that acts as bis-nucleophile in the Nicholas reaction, is used for the cyclisation. It was synthesised from 3-bromopropylsulfonyl chloride (**1**)[37] and *N*-(6-aminopropyl)-2-nitrobenzenesulfon-amide (**2**)[36] as colorless crystals with a yield of 64% (Scheme 2).

With the dinucleophile **3** in hand, the DACN **5** was prepared by the reaction with butyne-1,3-diol (**4**) in a three-step one-pot double Nicholas reaction using $Co_2(CO)_8$ and $BF_3{\cdot}OEt$ (Scheme 3). After the formation of the nine-membered cyclic cobalt complex, silica gel and ceric ammonium nitrate (CAN) were added to remove the cobalt moiety by oxidation and pyridine to remove borane residues. The DACN **5** was isolated with a yield of 30% as colourless crystals after purification by column chromatography and allows a further functionalisation either at the aliphatic bromine by a nucleophilic substitution or directly at the second nitrogen atom of the DACN ring after removal of the nosyl group. Finally, the DPA-DACN **6** was prepared by connecting the dipicolylamine moiety to the DACN **7** via the propylsulfonyl linker in a Finkelstein reaction at 45 °C overnight (Scheme 3) in 60% yield.

To compare the SPAAC with conventional radiolabelling using activated esters for primary amines, a second building block **10** was prepared comprising the tetrafluorophenyl (TFP) ester as activated carboxylic function (Scheme 4). The DPA-TFP ester **10**



Scheme 3. One-pot double Nicholas reaction to yield DACN **7** and synthesis of chelator DPA-DACN **8** by reaction with of 2,2'-dipicolylamine.

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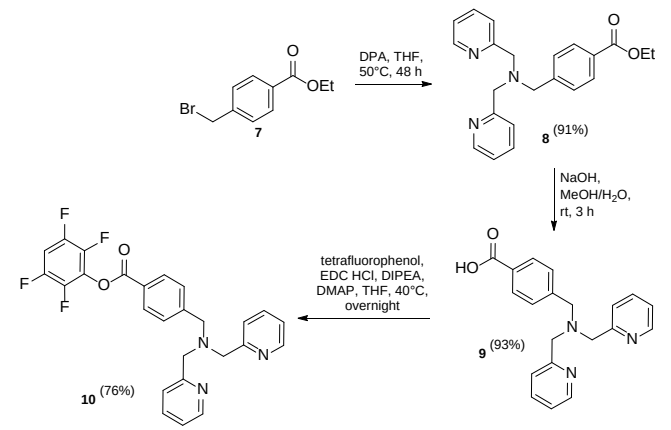
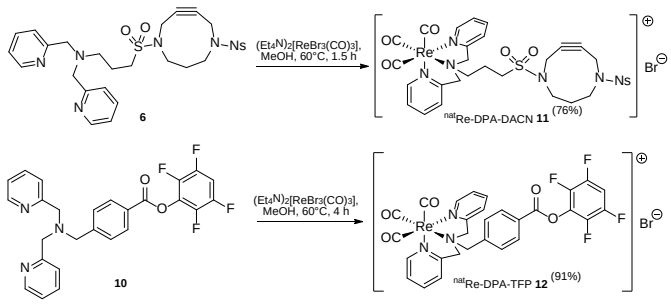


Scheme 4. Synthesis of chelator **10** starting from ethyl 4-(bromomethyl)benzoate (**7**).

was synthesised in three steps starting with the reaction of 2,2'-dipicolylamine with ethyl 4-(bromomethyl)benzoate (**7**), which was carried out at 50°C over 48 h. Ester **8** was isolated with a yield of 91% as an orange oil after purification and then saponified by treatment with sodium hydroxide at 40°C in methanol/water (v/v 2/1) for 1 h. Compound **9** was obtained as greenish crystals in 93% yield after acidification and extraction with ethyl acetate. The subsequent Steglich esterification of **9** with 2,3,5,6-tetrafluorophenol was carried out at 40°C overnight under the presence of EDC•HCl and DMAP. TFP ester **10** was obtained with 76% yield as yellow oil.

The syntheses of the nonradioactive rhenium reference complexes **11** and **12** were carried out using the tribromo-tricarbonylrhenium(I) complex $(Et_4N)_2[ReBr_3(CO)_3]$[38] at 60 °C in methanol (Scheme 5). The consumption of the chelators **6** and **10** was monitored by TLC. After work-up, the crude products were dissolved in dichloromethane and precipitated by adding n-hexane to give the complexes as yellow crystals with yields of 76% for ^{nat}Re-DPA-DACN **11** and 91% for ^{nat}Re-DPA-TFP ester **12**. The successful formation of the rhenium complexes **11** and **12** was verified inter alia by ^{1}H NMR spectroscopy. One distinct indication is provided by the methylene groups connected to the pyridine rings of the DPA moiety. In both free chelators **6** and **9**, the symmetry and free single bond rotation result in the formation of one singlet (δ = 3.81 ppm for **6** and 3.85 ppm for **9**) comprising four protons. However, in the rhenium complexes **11** and **12**, the DPA moiety is fixed and cannot rotate freely resulting in magnetically unequal equatorial and axial protons at the methylene groups, which form two doublets (δ = 4.61/



Scheme 5. Synthesis of the nonradioactive reference rhenium(I) complexes **11** and **12** with chelators DPA-DACN **6** and DPA-TFP **10**, respectively.

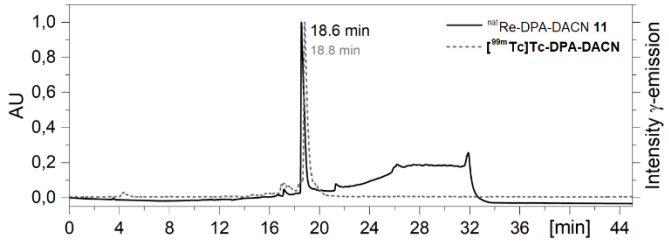


Figure 1. HPLC chromatogram of ^{nat}Re-DPA-DACN **11** (t_R = 18.6 min, black line) superimposed with radio-HPLC chromatogram of **[^{99m}Tc]Tc-DPA-DACN** (t_R = 18.8 min, grey dashed line) using optimised labelling conditions.

6.15 ppm with ^2J = 16.3 Hz for **11** and 4.75/6.12 ppm with ^2J = 16.2 Hz for **12**).

Preparation of the ^{99m}Tc-Radiolabeling building blocks

For the radiolabelling using the ^{99m}Tc-tricabonyl approach, *fac*-[^{99m}Tc][Tc(CO)$_3$(H$_2$O)$_3$]$^+$ as starting complex was prepared with the tricarbonyl kit as published[39,34] with a RCC of >99% by adding [^{99m}Tc]TcO$_4^-$ (1 mL, 1–1.5 GBq) eluted from a ^{99}Mo/^{99m}Tc generator with 0.9% saline to the kit followed by heating to 100 °C for 20 min. *fac*-[[^{99m}Tc]Tc(CO)$_3$(H$_2$O)$_3$]$^+$ with t_R = 13.7 min was used without purification for all ensuing radiolabeling procedures (see SI).

The preparation of the click-labeling building block **[^{99m}Tc]Tc-DPA-DACN** was tested at four different temperatures (25°C, 40°C, 75°C, 100°C). *fac*-[[^{99m}Tc]Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was neutralized with 1 M MES buffer (pH 5.5) prior to the addition of ligand **6**. The reactions were heated to the respective temperature for 30 min, and the reaction progress was analyzed with radio-HPLC finding a new peak at t_R = 18.8 min, which is related to **[^{99m}Tc]Tc-DPA-DACN**. The RCC was lowest at rt with 21%, followed by 100°C with 78% and 40°C with 83%. The starting complex *fac*-[^{99m}Tc][Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was still present in both batches at rt and 40 °C. An unknown by-product (t_R = 17.1 min) was formed independent of the reaction conditions, but most of it was formed at 100 °C. It can therefore be concluded that low temperatures like 25°C are not enough to fully convert *fac*-[^{99m}Tc][Tc(CO)$_3$(H$_2$O)$_3$]$^+$ to the desired product, while high temperatures like 100°C result in an increased formation of the by-product. The highest RCC with 89% combined with a

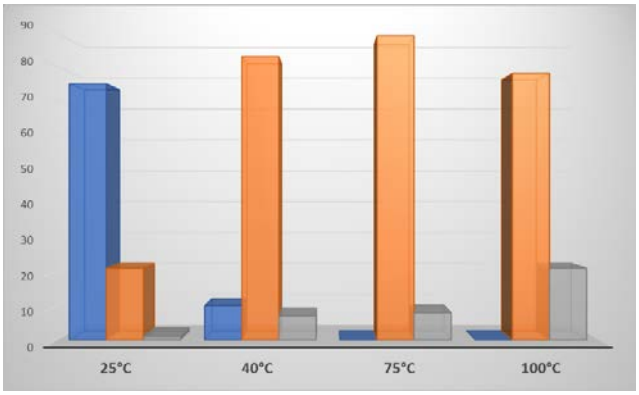


Figure 2. Radiochemical conversion of [^{99m}Tc][Tc(CO)$_3$(H$_2$O)$_3$]$^+$ (blue) with ligand **6** to give **[^{99m}Tc]Tc-DPA-DACN** (orange) and a by-product (grey) at different temperatures in MES buffer at pH 5.5 and a labelling time of 30 min determined by radio-HPLC.

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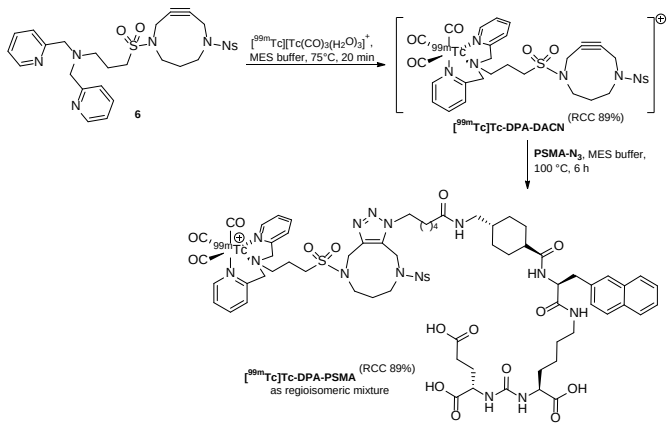


Scheme 6. Preparation of the radiolabelling building block **[^{99m}Tc]Tc-DPA-DACN** and click-radiolabelling of **PSMA-N$_3$** to obtain **[^{99m}Tc]Tc-DPA-PSMA**.

suppression of by-products was observed at 75°C, which was chosen as optimal for further radiolabeling approaches with DPA-DACN ligand **6** (Scheme 6 and Figure 2). The obtained radiolabelled complex **[^{99m}Tc]Tc-DPA-DACN** (t_R = 18.8 min) was verified by comparing the nonradioactive rhenium complex ^{nat}Re-DPA-DACN **11** (t_R = 18.6 min) using radio-HPLC (Figure 1). **[^{99m}Tc]Tc-DPA-DACN** was immediately used for the copper-free click reaction without further purification. For this purpose, the azide-functionalised PSMA derivative **PSMA-N$_3$** with binding motif according to PSMA-617 was prepared (see SI). The click-labelling with **[^{99m}Tc]Tc-DPA-DACN** was tested at three different temperatures. At 40°C and 75°C, hardly any product was observed after 120 min with RCCs of 4% each. However, a considerable conversion of **[^{99m}Tc]Tc-DPA-DACN** to the ^{99m}Tc-PSMA derivative was observed at 100°C. The reaction progress was monitored over a time span of 6 h. The RCC to **[^{99m}Tc]Tc-DPA-PSMA** was 59% after 2 h, increased to 79% and 86% after 4 respective 5 h. Almost quantitative conversion of the starting material **[^{99m}Tc]Tc-DPA-DACN** was detected after 6 h with a RCC of 89% (Figure 3). The identity of **[^{99m}Tc]Tc-DPA-PSMA** (t_R = 18.0 min) was verified by radio-HPLC (Figure 4) with the isostructural nonradioactive rhenium reference compound **^{nat}Re-DPA-PSMA** (t_R = 17.6 min; see SI for details).

To compare the newly developed Cu-free click-labelling with conventional labelling, **PSMA-NH$_2$** as biomolecule with amine

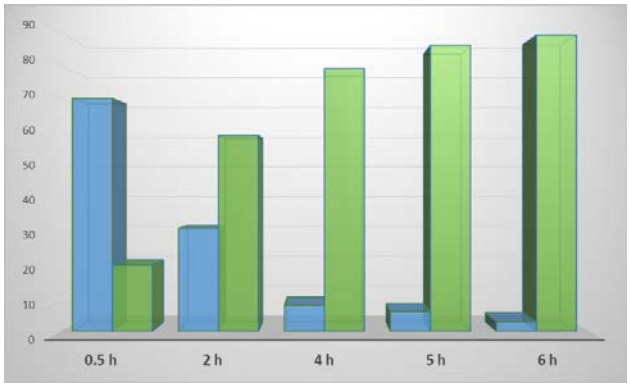


Figure 3. Radiochemical conversion of **[^{99m}Tc]Tc-DPA-DACN** (blue) with **PSMA-N$_3$** to give **[^{99m}Tc]Tc-DPA-PSMA** (green) at different time points in MES buffer at pH 5.5 and a labelling temperature of 100°C determined by radio-HPLC.

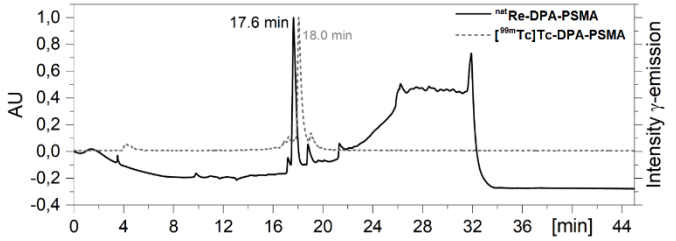
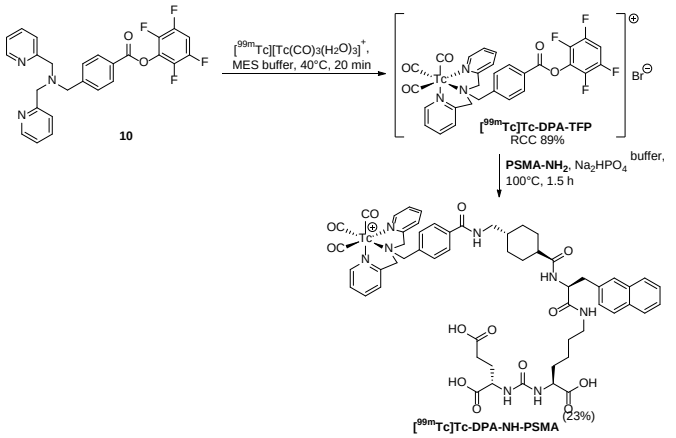


Figure 4. HPLC chromatogram of reference **^{nat}Re-DPA-PSMA** (t_R = 17.6 min, black line) superimposed with radio-HPLC chromatogram of **[^{99m}Tc]Tc-DPA-PSMA** (t_R = 18.0 min, grey dashed line) using optimised labelling conditions.



Scheme 7. Preparation of the radiolabelling building block **[^{99m}Tc]Tc-DPA-TFP** and conventional radiolabelling of **PSMA-NH$_2$** to obtain **[^{99m}Tc]Tc-DPA-NH-PSMA**.

function (see SI for details) and the activated TFP ester **10** as ligand was used to prepare the second radiolabelling building block **[^{99m}Tc]Tc-DPA-TFP**. Here the neutral pH was important for the stability of chelator **10** and the resulting **[^{99m}Tc]Tc-DPA-TFP** to avoid hydrolysis of the ester function. Thus, the labelling was performed at a pH of 5.5. The highest RCC was obtained by neutralizing the *fac*-[^{99m}Tc][Tc(CO)$_3$(H$_2$O)$_3$]$^+$ complex solution with 1 M MES buffer and heating the mixture only to 40 °C for 20 min after the addition of the chelator **10** (Scheme 7). **[^{99m}Tc]Tc-DPA-TFP** was necessary to be purified via a RP 18 cartridge with the eluents water, 0.9% saline, 50% ethanol, and 100% ethanol and was obtained with a RCY of 89% and a radiochemical purity >99% in ethanol with the optimised radiolabeling conditions. The identity of **[^{99m}Tc]Tc-DPA-TFP** was verified (Figure 5) by comparing the retention times (t_R = 20.3 min) with the nonradioactive isostructural rhenium reference complex **12** (t_R = 19.8 min).

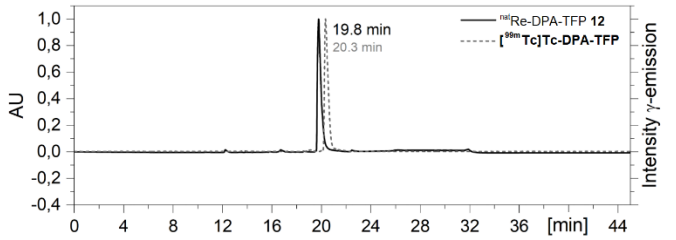


Figure 5. HPLC chromatogram of ^{nat}Re-DPA-TFP **12** (UV, t_R = 19.8 min, black line) superimposed with radio-HPLC chromatogram of **[^{99m}Tc]Tc-DPA-TFP** (t_R = 20.3 min, grey dashed line) using optimised labelling conditions.

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The subsequent radiolabelling of **PSMA-NH₂** (see SI for details), a PSMA targeting motif according to PSMA-617 containing a primary amine function, was tested at three different temperatures (25°C, 40°C, 100°C). The ethanol phase from the cartridge separation with **[^{99m}Tc]Tc-DPA-TFP** was concentrated. 0.1 M Na_2HPO_4 (pH 8.5) and 300 µg of **PSMA-NH₂** dissolved in ethanol were added and the reaction progress was monitored after different time points by HPLC analysis.

At 25°C, almost no conversion of **[^{99m}Tc]Tc-DPA-TFP** was observed after 30 min. After 150 min, three new peaks were formed at t_R = 16.3 min, 17.2 min and 18.5 min, indicating the formation of three new radiolabeled compounds (see SI). However, the majority of **[^{99m}Tc]Tc-DPA-TFP** was still present (t_R = 20.3 min, 59%). Similar results were achieved at 40°C but with a faster conversion of **[^{99m}Tc]Tc-DPA-TFP** and a faster formation of the three new radiolabeled compounds. **[^{99m}Tc]Tc-DPA-NH-PSMA** was formed with RCCs of 6% and 13% at 25°C and 40 °C, respectively. In contrast to the obtained results, the reaction of **[^{99m}Tc]Tc-DPA-TFP** with **PSMA-NH₂** was done with 21% RCC at 100 °C after 30 min, but with the same two additional by-products (analyzed by radio-HPLC, see Figure 6) indicating the hydrolysis/degradation of **[^{99m}Tc]Tc-DPA-TFP**. A higher conversion to the desired product was not found at this temperature and longer labeling times. The identity of the two other compounds could not be full-on clarified yet. The identity of the desired **[^{99m}Tc]Tc-DPA-NH-PSMA** (t_R = 16.3 min) was verified with the nonradioactive rhenium reference complex **^{nat}Re-DPA-NH-PSMA** (t_R = 15.7 min).

Comparison of click and conventional labelling

The transfer of the DACN-click reaction to radiolabelling conditions was successful due to the easy access to the respective DACN compound and the functionalisation for 99mTc-complexation. The click-labelling build block is stable under certain conditions like high temperatures. However, the actual click-labelling with the new DACN-building block **[^{99m}Tc]Tc-DPA-DACN** and the biomolecule **PSMA-N₃** was too slow in labelling kinetics at low to moderate temperatures indicating that the strained alkyne is not reactive enough for the cycloaddition under mild conditions. However, **[^{99m}Tc]Tc-DPA-DACN** was not mandatory to be purified and only the desired ^{99m}Tc-labelled PSMA derivative **[^{99m}Tc]Tc-DPA-PSMA** was obtained as single product after click-radiolabelling. In contrast, the conventional TFP ester-containing building block **[^{99m}Tc]Tc-DPA-TFP** led to degradation under aqueous conditions even at low temperatures and had to be purified as well as the radiolabelled product **[^{99m}Tc]Tc-DPA-NH-PSMA**.

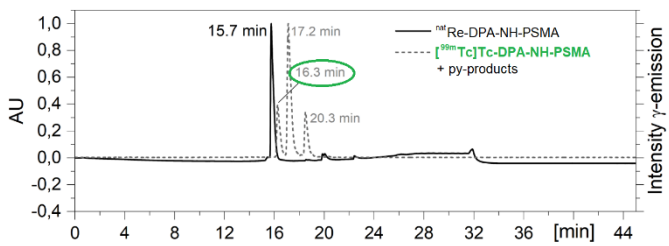

Figure 6. HPLC chromatogram of rhenium complex **^{nat}Re-DPA-NH-PSMA** (UV, t_R = 15.7 min, black line) and superimposition with radio-HPLC chromatogram of the labelling reaction of **PSMA-NH₂** to give **[^{99m}Tc]Tc-DPA-PSMA** (t_R = 16.3 min, gray dashed line) and two by-products (t_R = 17.2 min and t_R = 20.3 min) at 100 °C after 30 min.

The observed labelling time is too long to be adapted for short-lived radionuclides like ^{11}C ($t_{1/2}$ = 20.4 min) and ^{68}Ga ($t_{1/2}$ = 67.6 min), but it is on the borderline for radionuclides like ^{18}F ($t_{1/2}$ = 109.8 min). The long reaction time was also found for the preparation of the nonradioactive reference **^{nat}Re-DPA-PSMA**. Three days at 40 °C are required for a quantitative conversion of the starting materials of the click reaction between the **^{nat}Re-DPA-DACN** and the **PSMA-N₃**.

In contrast, the conventional building block **[^{99m}Tc]Tc-DPA-TFP** was successfully prepared, but under observance of the pH. Prior to the radiolabelling with **PSMA-NH₂**, a purification step was necessary, and by-products were formed during the labelling procedure presumably coming from hydrolysis of **[^{99m}Tc]Tc-DPA-TFP**, which is common for activated esters like TFP or succinimide, but leading to a rather low radiochemical conversion to the desired **[^{99m}Tc]Tc-DPA-NH-PSMA** of only 21% with the additional need of purification.

Experimental

Materials and methods

Reagents and solvents were purchased from commercial suppliers and used without further purification if not stated otherwise. 2,2'-Dipicolylamine was purified by distillation prior to the syntheses. $Co_2(CO)_8$ leads to slow decomposition and was stored in the fridge. 3-Bromopropanesulfonyl chloride (**1**),[37] *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide hydrochloride (**2**)[36] and the PSMA targeting compounds **PSMA-N₃** and **PSMA-NH₂**[40] were prepared according to the literature. NMR spectra of all synthesised compounds were carried out on an Agilent DD2 400 MHz NMR or Agilent DD2 600 MHz NMR spectrometer with ProbeOne at room temperature. Chemical shifts are given in parts per million (ppm) and coupling constants *J* in Hz. ^{1}H and ^{13}C spectra were internally referenced using the signal of the deuterated solvent. Mass spectra were recorded on an Advion Expression compact mass spectrometer using electrospray ionization. TLC analyses for reaction control were performed on Merck Silica Gel 60 F_{254} TLC plates and visualised using UV light or with the following TLC stains: iodine, ninhydrin or Hanessian's stain. Analytical HPLC was performed on VWR Hitachi system (column: Agilent Zorbax 300SB-C18, 100 x 4.6mm) with solvent A: water + 0.1% TFA, solvent B: MeCN + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 90/10 → 5/95 in 14 min. Analytical radio-HPLC was carried out using the Perkin-Elmer FLEXAR™ LC system (Flexar FX-20) with a Phenomenex C12 column (Phenomenex Jupiter™ 4 µm Proteo 90 Å, 4.6 x 250 mm) with solvent A: water + 0.1% TFA, solvent B: MeCN + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 95/5 → 5/95 in 20 min. Chromatographic flash purification was performed using the Biotage Isolera Four and Biotage Selekt systems and silica gel cartridges (SNAP KP-Sil or Sfär Silica HC: 5 g, 10 g or 25 g). Preparative HPLC analysis was performed on a reversed phase HPLC system Knauer Azura with Zorbax 300SB-

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C18 semi-preparative column and MeCN + 0.1% TFA/water + 0.1% TFA as eluent. The tricarbonyl kits were prepared in-house as published elsewhere.[34,39]

***N*-(3-((3-bromopropyl)sulfonamido)propyl)-2-nitrobenzene-sulfonamide 3.** Et_3N (4.45 g, 44.0 mmol, 5.00 eq.) was added to a suspension of *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide hydrochloride (**2**, 2.60 g, 8.8 mmol, 1.00 eq.) in anhydrous DCM (50 mL) at 0 °C. The mixture was stirred at that temperature for 30 min. A solution of 3-bromopropanesulfonyl chloride (**1**, 2.92 g, 13.2 mmol, 1.50 eq.) in DCM (20 mL) was added slowly to keep the inner temperature under 4°C. The resulting mixture was stirred at 0°C for 4 h and quenched with 1 M HCl. The mixture was extracted with DCM (3 x 20 mL), and the combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. Purification was done via automated flash column chromatography ($CHCl_3$→$CHCl_3$/EtOAc 1/3) to obtain compound **5** as colorless solid (2.13 g, 64%). Mp 103–109 °C; R_f = 0.30 ($CHCl_3$/MeOH 19/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15–8.10 (m, 1H, Ar-H), 7.90–7.85 (m, 1H, Ar-H), 7.78–7.73 (m, 2H, Ar-H), 5.69 (t, ^3J = 6.5 Hz, 1H, NH), 4.86 (t, ^3J = 6.5 Hz, 1H, NH), 3.53 (t, ^3J = 6.3 Hz, 2H, CH_2), 3.32–3.16 (m, 6H, CH_2), 2.36 (p, ^3J = 6.2 Hz, 2H, CH_2), 1.81 (p, ^3J = 6.2 Hz, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 148.2 (C_{Ar}), 134.0 (CH_{Ar}), 133.6 (C_{Ar}), 133.1 (CH_{Ar}), 131.1 (CH_{Ar}), 125.7 (CH_{Ar}), 51.2, 40.3, 39.8, 31.1, 31.0, 27.1 (6 x CH_2); MS (ESI+) *m/z*: 445 $[M+H]^+$; $C_{12}H_{18}BrN_3O_6S_2$ (444.32).

***N*-(3-Bromopropanesulfonyl)-*N'*-nosyl-4,8-diazacyclononyne 5.** 2-Butyne-1,4-diol (**4**, 279 mg, 3.20 mmol, 1.20 eq.) was dissolved in anhydrous DCM (110 mL), $Co_2(CO)_8$ (1.15 g, 3.40 mmol, 1.25 eq.) was added at 30 °C and the mixture was stirred at that temperature for 45 min. Compound **3** (1.20 g, 2.70 mmol, 1.00 eq.) dissolved in anhydrous DCM (270 mL) and $BF_3{\cdot}OEt_2$ (1.33 mL, 10.8 mmol, 4.00 eq.) were added in three portions every 15 min. After the mixture was stirred at 30°C for 3 h, silica gel (23 g) and CAN (4.44 g, 8.10 mmol, 3.00 eq.) were added in three portions every 15 min. Pyridine (653 µL, 641 mg, 8.10 mmol, 3.00 eq.) was added after 1 h and the mixture was stirred at 30°C for further 45 min. The solvent was removed under reduced pressure. The crude product was purified via column chromatography ($CHCl_3$→$CHCl_3$/EtOAc 9/1). Compound **5** was obtained as colorless crystals (397 mg, 30%). Mp 149–154°C; R_f = 0.43 ($CHCl_3$/EtOAc 3/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (dd, ^3J = 7.3 Hz, ^4J = 2.0 Hz, 1H, Ar-H), 7.77–7.69 (m, 2H, Ar-H), 7.67–7.63 (m, 1H, Ar-H), 4.11 (s, 2H, NCH_2), 4.00 (s, 2H, NCH_2), 3.57–3.44 (m, 6H, CH_2), 3.12 (t, ^3J = 7.3 Hz, 2H, CH_2), 2.36 (p, ^3J = 6.4 Hz, 2H, CH_2), 2.23–2.07 (m, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 148.4 (C_{Ar}), 134.2 (CH_{Ar}), 131.9 (CH_{Ar}), 131.8 (C_{Ar}), 131.2 (CH_{Ar}), 124.5 (CH_{Ar}), 88.5 (C≡), 88.3 (C≡), 47.7, 45.3, 45.1, 41.2, 40.4, 32.3, 31.2, 26.5 (8 x CH_2); MS (ESI+) *m/z*: 496 $[M+H]^+$; $C_{16}H_{20}BrN_3O_6S_2$ (494.38).

***N*-(3-(Bis(pyridin-2-ylmethyl)amino)propanesulfonyl)-*N'*-nosyl-4,8-diazacyclononyne 6.** Compound **5** (62 mg, 0.13 mmol, 1.10 eq.), 2,2'-dipicolylamine (23 mg, 0.11 mmol, 1.00 eq.), Et_3N (15 mg, 0.15 mmol, 1.30 eq.) and KI (15 mg) were dissolved in anhydrous THF (6 mL). The mixture was stirred at 45°C for overnight. After the solvent was removed under reduced pressure, the crude product was purified via automated flash column chromatography ($CHCl_3$→EtOAc→EtOAc/EtOH 9/1) to give compound **6** as yellow oil (42 mg, 60%). R_f = 0.24 ($CHCl_3$/MeOH 19/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.55–8.49 (m, 2H, Py-H), 8.01–7.94 (m, 1H, Ar-H), 7.76–7.59 (m, 5H, Ar-H), 7.40 (d, ^3J = 7.7 Hz, 2H, Py-H), 7.20–7.12 (m, 2H, Py-H), 4.08 (t, ^5J = 2.4 Hz, 2H, CH_2), 3.92 (t, ^5J = 2.4 Hz, 2H, CH_2), 3.81 (s, 4H, CH_2Ar), 3.51–3.43 (m, 2H, CH_2), 3.43–3.36 (m, 2H, CH_2), 3.06–2.91 (m, 2H, CH_2), 2.68 (t, ^3J = 6.7 Hz, 2H, CH_2), 2.16–2.05 (m, 2H, CH_2), 2.01–1.90 (m, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.1 (C_{Ar}), 149.3 (CH_{Py}), 148.4 (C_{Ar}), 136.6 (CH_{Ar}), 134.1 (CH_{Ar}), 131.9 (CH_{Ar}), 131.7 (C_{Ar}), 131.2 (CH_{Ar}), 124.5 (CH_{Ar}), 123.3 (CH_{Py}), 122.4 (CH_{Py}), 88.7 (C≡), 88.2 (C≡), 60.4 (CH_2Ar), 52.4, 48.5, 45.2, 44.9, 41.2, 40.4, 32.3, 21.4 (8 x CH_2); MS (ESI+) *m/z*: 613 $[M+H]^+$; $C_{28}H_{32}N_6O_6S_2$ (612.72); Analytical HPLC: t_R = 9.0 min, radio-HPLC (UV trace): t_R = 14.6 min.

4-(Bis(pyridin-2-ylmethyl)amino)methyl)benzoic acid ethyl ester 8. 2,2'-Dipicolylamine (350 mg, 1.75 mmol, 1.20 eq.) was added to a solution of ethyl 4-(bromomethyl)benzoate (**7**, 355 mg, 1.46 mmol, 1.00 eq.) and Et_3N (193 mg, 1.90 mmol, 1.30 eq.) in anhydrous THF (6 mL) and the resulting mixture was stirred at 50 °C for two days. Afterwards, the solvent was removed, saturated hydrogen carbonate solution (20 mL) was added, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified via automated flash column chromatography (EtOAc→EtOAc/EtOH 9/1) to give compound **8** as orange oil (427 mg, 81%). R_f = 0.51 ($CHCl_3$/MeOH 9/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (ddd, ^3J = 4.9 Hz, ^4J = 1.8, 0.9 Hz, 1H, CH_{Py}),7.99 (d, ^3J = 8.5 Hz, 2H, CH_{Ar}), 7.68 (td, ^3J = 7.6, 1.8 Hz, 2H, CH_{Py}), 7.57 (d, ^3J = 7.8 Hz, 2H, CH_{Py}), 7.50 (d, ^3J = 8.6 Hz, 2H, CH_{Ar}), 7.17 (ddd, ^3J = 7.5, 4.9 Hz, ^4J = 1.3 Hz, 2H, CH_{Py}), 4.35 (q, ^3J = 7.1 Hz, 2H, OCH_2), 3.87 (s, 4H, $ArCH_2$), 3.80 (s, 2H, NCH_2), 1.37 (t, ^3J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 166.6 (C=O), 158.9 (C_{Py}), 149.0 (CH_{Py}), 144.0 (C_{Ar}), 136.9 (CH_{Py}), 129.8 (CH_{Ar}), 129.7 (C_{Ar}), 129.0 (CH_{Ar}), 123.3 (CH_{Py}), 122.4 (CH_{Py}), 61.0 (OCH_2), 59.9 ($ArCH_2$), 58.3 (NCH_2), 14.5 (CH_3); MS (ESI+) *m/z*: 362 $[M+H]^+$; $C_{22}H_{23}N_3O_2$ (361.45).

4-(Bis(pyridin-2-ylmethyl)amino)methyl)benzoic acid 9. NaOH (111 mg, 2.77 mmol, 5.00 eq.) was added to a solution of compound **8** (200 mg, 0.55 mmol, 1.00 eq.) in MeOH/H_2O (9 mL, v/v 2/1) and the resulting mixture was stirred at 40°C for 3 h. Afterwards, the solvent was removed, and the pH was adjusted to pH = 6 with aq. HCl (5%). The aqueous layer was extracted with EtOAc (3 x 10 mL). After phase separation, the organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give compound **9** as greenish crystals (170 mg, 93%). R_f = 0.23 ($CHCl_3$/MeOH 9/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.21 (s, 1H, OH), 8.63 (d, ^3J = 4.9 Hz, 2H, CH_{Py}), 8.02 (d, ^3J = 8.0 Hz, 2H, CH_{Ar}), 7.71 (td, ^3J = 7.7 Hz,

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^4J = 1.9 Hz, 2H, CH_{Py}), 7.62 (d, ^3J = 7.8 Hz, 2H, CH_{Ar}), 7.47 (d, ^3J = 8.2 Hz, 2H, CH_{Py}), 7.21 (ddd, ^3J = 7.4, 5.0 Hz, ^4J = 1.3 Hz, 2H, CH_{Py}), 3.90 (s, 4H, $ArCH_2$), 3.79 (s, 2H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ =169.4 (C=O), 158.8 (C_{Py}), 148.6 (CH_{Py}), 142.9 (C_{Ar}), 137.4 (CH_{Py}), 130.3 (C_{Ar}), 130.2 (CH_{Ar}), 129.2 (CH_{Py}), 123.7 (CH_{Ar}), 122.6 (CH_{Py}), 59.2 ($ArCH_2$), 58.2 (NCH_2); MS (ESI+) *m/z*: 334 $[M+H]^+$; $C_{20}H_{19}N_3O_2$ (333.39).

4-(Bis(pyridin-2-ylmethyl)amino)methyl)benzoic acid 2,3,5,6-tetrafluorophenylester 10. Compound **9** (156 mg, 4.6 mmol, 1.00 eq.), 2,3,5,6-tetrafluorophenol (93 mg, 5.6 mmol, 1.2 eq.), EDC·HCl (135 mg, 7.0 mmol, 1.50 eq.), DMAP (40 mg, cat.) and DIPEA (91 mg, 7.0 mmol, 1.50 eq.) were dissolved in anhydrous THF (15 mL) and the mixture was stirred at 40 °C overnight. The solvent was removed under reduced pressure, and DCM (10 mL) was added. The organic layer was washed with water (2 x 5 mL), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. Purification was done via automated flash column chromatography ($CHCl_3$→$CHCl_3$/EtOAc 1/9) to give compound **10** as yellow oil (168 mg, 76%). R_f = 0.10 ($CHCl_3$/EtOAc 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (d, ^3J = 4.9 Hz, 2H, CH_{Py}), 8.14 (d, ^3J = 8.3 Hz, 2H, CH_{Ar}), 7.68 (td, ^3J = 7.6 Hz, ^4J = 1.8 Hz, 2H, CH_{Py}), 7.60 (d, ^3J = 8.2 Hz, 2H, CH_{Ar}), 7.56 (d, ^3J = 7.9 Hz, 2H, CH_{Py}), 7.17 (ddd, ^3J = 7.5, 4.9 Hz, ^4J = 1.2 Hz, 2H, CH_{Py}), 7.07–6.97 (m, 1H, CH_{TFP}), 3.85 (s, 4H, Ar-CH_2), 3.81 (s, 2H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.6 (C=O), 159.1 (C_{Py}), 149.1 (CH_{Py}), 146.8 (C_{Ar}), 136.8 (CH_{Py}), 130.9 (CH_{Ar}), 129.3 (CH_{Ar}), 126.0 (C_{Ar}), 123.2 (CH_{Py}), 122.4 (CH_{Py}), 103.6 (F-C_q), 103.3 (F-C_q), 103.1 (CH_{TFP}), 60.1 ($ArCH_2$), 58.3 (NCH_2); MS (ESI+) *m/z*: 482 $[M+H]^+$; $C_{26}H_{19}F_4N_3O_2$ (481.45); Analytical HPLC: t_R = 9.6 min, radio-HPLC (UV trace): t_R = 15.4 min.

$[Re(CO)_3$8]Br (13)

$(Et_4N)_2[Re(CO)_3Br_3]$ (52 mg, 0.06 mmol, 1.00 eq.) and compound **8** (41 mg, 0.06 mmol, 1.00 eq.) were dissolved in anhydrous MeOH (4 mL). After the mixture was stirred for 1.5 h at 60°C, the solvent was removed and DCM (5 mL) was added. The organic phase was washed with water (2 x 5 mL), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was dissolved in DCM (3 mL) and was precipitated with n-hexane to afford **13** as yellow crystals (45 mg, 76%). R_f = 0.85 (RP18, MeCN/H_2O 10/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.66 (d, ^3J = 5.6 Hz, 2H, CH_{Py}), 8.05–7.98 (m, 1H, CH_{Ar}), 7.91 (d, ^3J = 7.9 Hz, 2H, CH_{Py}), 7.83 (t, ^3J = 7.7 Hz, 2H, CH_{Py}), 7.77–7.71 (m, 2H, CH_{Py}), 7.68–7.62 (m, 1H, CH_{Ar}), 7.22 (t, ^3J = 6.6 Hz, 2H, CH_{Py}), 6.15 (d, ^2J = 16.3 Hz, 2H, $ArCH_2$), 4.61 (d, ^2J = 16.3 Hz, 2H, $ArCH_2$), 4.18 (s, 2H, CH_2), 4.13 (s, 2H, CH_2), 4.09–3.99 (m, 2H, CH_2), 3.62–3.49 (m, 4H, CH_2), 3.29 (t, ^3J = 7.0 Hz, 2H, CH_2), 2.66 (p, ^3J = 8.3 Hz, 2H, CH_2), 2.18–2.11 (m, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 160.8 (C_{Py}), 150.4 (CH_{Py}), 148.1 (C_{Ar}), 140.2 (CH_{Ar}), 132.8 (2 x CH_{Ar}), 130.8 (CH_{Ar}), 124.8 (CH_{Ar}), 124.3 (CH_{Ar}), 125.3 (5''''-CH), 87.8 (1-/2-C_q), 88.5 (1-/2-C_q), 68.5 (CH_2), 66.9 (1'''a-/1'''b-CH_2), 66.5 (1'''a-/1'''b-CH_2), 47.9 (3'-CH_2), 45.3 (CH_2), 41.0 (3-/9-CH_2), 40.7,(3-/9-CH_2), 45.4 (CH_2), 31.7 (2'-CH_2), 20.5 (6-CH_2); MS (ESI+): *m/z* =881 $[M-Br]^+$ (^{185}Re), 883 $[M-Br]^+$ (^{187}Re); $C_{31}H_{32}BrN_6O_9ReS_2$ (962.86). Analytical HPLC: t_R = 11.9 min, radio-HPLC (UV trace): t_R = 18.6 min.

$[Re(CO)_3$10]Br (14)

$(Et_4N)_2[Re(CO)_3Br_3]$ (112 mg, 0.15 mmol, 1.00 eq.) and compound **10** (70 mg, 0.15 mmol, 1.00 eq.) were dissolved in anhydrous MeOH (4 mL) and the mixture was stirred for 4 h at 60 °C. Afterwards, the solvent was removed, and DCM (5 mL) was added. The organic phase was washed with water (5 mL), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was dissolved in DCM (3 mL) and compound **12** was precipitated with *n*-hexane as colourless solid (102 mg, 91%); R_f = 0.73 (RP18, MeCN/H_2O 10/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.66 (d, ^3J = 5.6 Hz, 2H, CH_{Py}), 8.34 (d, ^3J = 8.0 Hz, 2H, CH_{Ar}), 8.12 (d, ^3J = 7.9 Hz, 2H, CH_{Py}), 7.98 (d, ^3J = 8.0 Hz, 2H, CH_{Ar}), 7.85 (t, ^3J = 7.8 Hz, 2H, CH_{Py}), 7.22 (t, ^3J = 6.7 Hz, 2H, CH_{Py}), 7.05 (m, 1H, CH_{TFP}), 6.12 (d, ^2J = 16.2 Hz, 2H, $ArCH_2$), 5.01 (s, 2H, NCH_2), 4.75 (d, ^2J = 16.2 Hz, 2H, $ArCH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.0 (C=O), 160.7 (C_{Py}), 150.9 (CH_{Py}), 147.7 (F-C_q), 146.3 (F-C_q), 143.9 (C_{Ar}), 140.9 (CH_{Py}), 138.2 (C_{Ar}), 134.2 (CH_{Ar}), 132.1 (CH_{Ar}), 129.2 (CH_{Py}), 129.0 (F-C_q), 125.6 (CH_{Py}), 103.6 (CH_{TFP}), 73.3 (NCH_2), 71.1 ($ArCH_2$), 71.1 ($ArCH_2$); ^{19}F NMR (376 MHz, $CDCl_3$): δ = -138.8 (2F, ArF), -152.3 (2F, ArF); MS (ESI +) *m/z*: 750 $[M-Br]^+$ (^{185}Re), 752 $[M-Br]^+$ (^{187}Re). $C_{29}H_{19}BrF_4N_3O_5Re$ (831.59). Radio-HPLC (UV trace): t_R = 19.8 min.

Conclusions

Two novel radiolabelling building blocks **[^{99m}Tc]Tc-DPA-DACN** for Cu-free click-lablelling and **[^{99m}Tc]Tc-DPA-TFP** for conventional amine-labelling containing the DPA chelator were prepared and successfully used for ^{99m}Tc-radiolabeling of two PSMA derivatives. A new basic skeleton based on the DACN molecules was chosen for Cu-free strain-promoted click radiolabelling. Advantageously, DACNs bear the possibility for an easy preparation using the double Nicholas reaction and the convenient functionalisation, e.g. with chelators like DPA or fluorescent dyes for optical imaging due to the both nitrogen atoms in the DACN.

^{99m}Tc-Radiolabeling of the DPA-DACN ligand **6** was achieved with *fac*-$[^{99m}Tc][Tc(CO)_3(H_2O)_3]^+$ within 30 min at 100 °C and a high RCC of 89%. The following click reaction with the azide-functionalised PSMA conjugate **PSMA-N$_3$** yielded the ^{99m}Tc-labelled click product **^{99m}Tc-DACN-PSMA** solely with an RCC of 79% at 100 °C after four hours.

The TFP ester **10** was labelled with *fac*-$[^{99m}Tc][Tc(CO)_3(H_2O)_3]^+$ at 40 °C for 30 min allowing a conventional labelling of, e.g. primary amines. The optimised radiolabelling conditions yielded the **^{99m}Tc-DPA-TFP** with an RCC of 89% and an RCP above 99% after cartridge purification. The ensuing labelling of **PSMA-NH$_2$** containing a primary amine function was accomplished with 21% RCY at 100 °C after 30 min, but with two additional by-products.

We have shown that the strain-promoted click-labelling was successful. The DACN has high potential for further applications due to the easy access and the possibilities to functionalise the DACN skeleton. Further experiments are required to improve

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the radiolabelling conditions of the strain-promoted click-labelling, e.g., by modifying the structure of the heterocyclic ring for a higher reactivity, so that lower temperatures and shorter reaction times of the SPAAC reaction could be achieved.

Conflicts of interest

There are no conflicts to declare.

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