Copper-free Click Labeling with the ^{99m}Tc-Tricarbonyl-Core Using DACN Derivatives

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Introduction

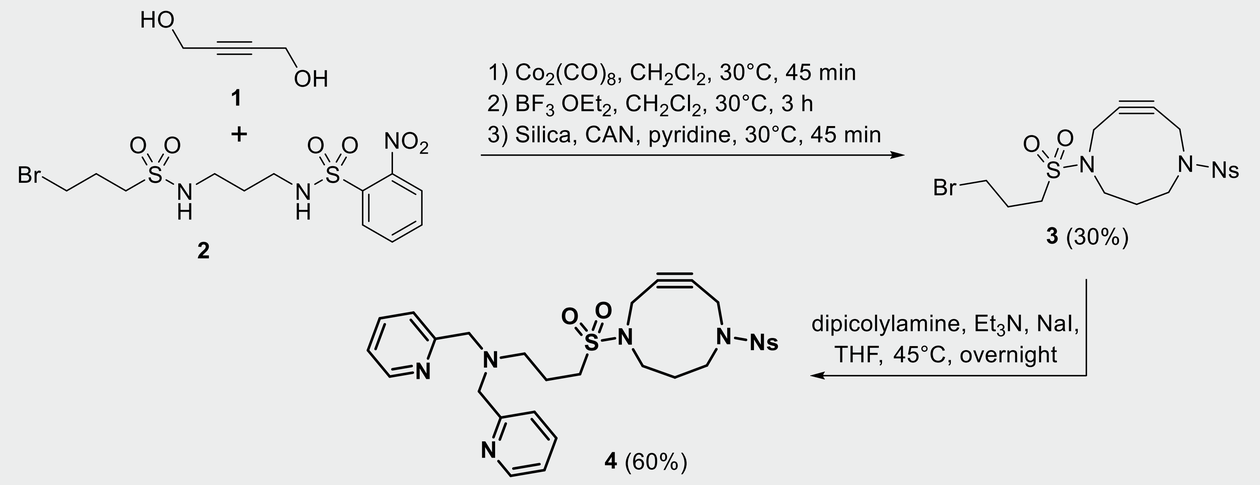
Diazacyclononyne (DACN) derivatives belong to the class of diazamacrocycles containing a strained alkyne bond. Therefore, DACNs are predestinated starting materials to be applied in copper-free strain-promoted click labeling with azide-functionalized bio(macro)molecules. The basic diazamacrocyclic scaffold is easy to prepare via a double Nicholas reaction. The following functionalization of the DACN skeleton is convenient by the use of modified sulfonamides or by the alkylation of the secondary amines provided by the molecule.

To make the DACN derivatives suitable for radiolabeling with technetium-99m, a dipicolylamine (DPA) derivative was prepared allowing the use of the ^{99m}Tc-tricarbonyl core. For this purpose, the DPA ligand is connected to the DACN macrocycle allowing the Cu-free click labeling. For the proof of concept, a PSMA-binding derivative based on PSMA-617 was chosen for final radiolabeling containing a terminal azide function.

For the click labeling procedure, the functionalized DACN macrocycle was prepared from a sulfonyl-modified diamide (4 steps) which was reacted with butyne-1,3-diol under Nicholas conditions. Finally, the DPA moiety was connected to the DACN via the propylsulfonyl linker. Both derivatives were reacted with $(Et_4N)_2[ReBr_3(CO)_3]$ in methanol to obtain the reference complex ^{nat}Re-DPA-DACN **5**.

The DPA-DACN derivative **4** was radiolabeled using the tricarbonyl kit ($[^{99m}Tc][Tc(CO)_3]^+$) at 40°C for 30 min in MES buffer (pH 5.5) yielding the ^{99m}Tc-DPA-DACN complex with 83% RCC. The following click reaction with the azide-functionalized PSMA derivative was performed at 100°C leading to 86% RCC after 5 hours.

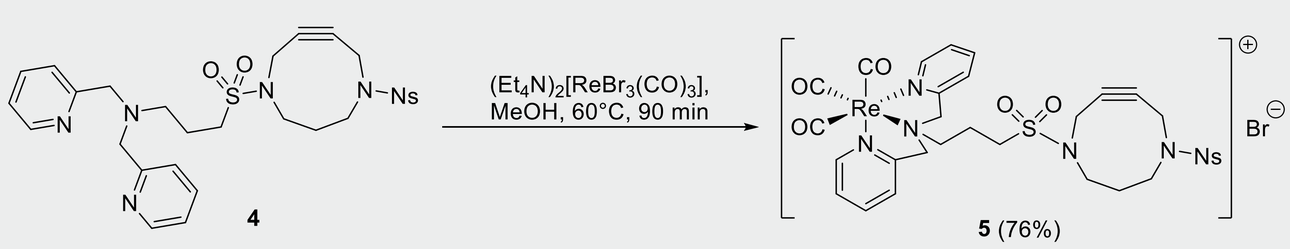
Synthesis of the DACN Chelator and Re-Complex



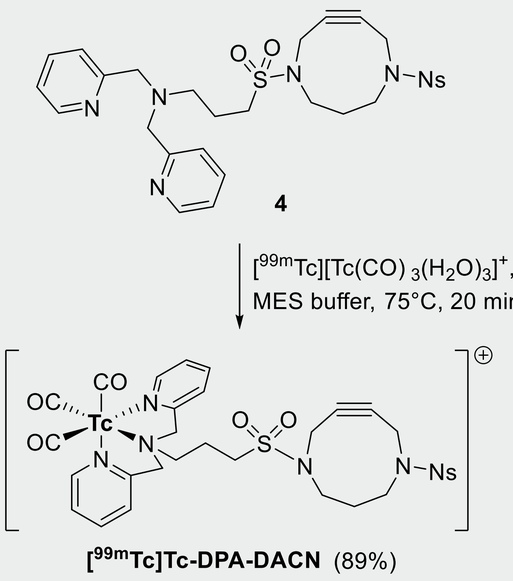
DACNs ready for the connection of DPA-ligand were prepared by the double Nicholas reaction using butyne-1,3-diol (**1**) and the functionalized bis-sulfonamide **2**. The first step involved the preparation of the Co-complex, which is necessary for the cyclization, followed by the nucleophilic attack of the sulfonamides of **2** to force the ring-closure to **3** under Lewis-acidic conditions. The last step involved the cleavage of the Co-complex with CAN to re-establish the triple bond yielding DACN **3** in 30% yield over three steps.

The dipicolylamine unit was introduced next by reacting dipicolylamine with **3** in a nucleophilic substitution reaction. The final building block **4** was obtained in 60% yield ready for radiolabeling with $[^{99m}Tc][Tc(CO)_3]^+$.

To prove the radiolabeling with ^{99m}Tc, the respective nonradioactive rhenium complex **5** was prepared as reference using DPA-ligand **4**, which was reacted with $(Et_4N)_2[ReBr_3(CO)_3]$ in methanol for 90 min (analyt. HPLC: t_R = 18.6 min) .

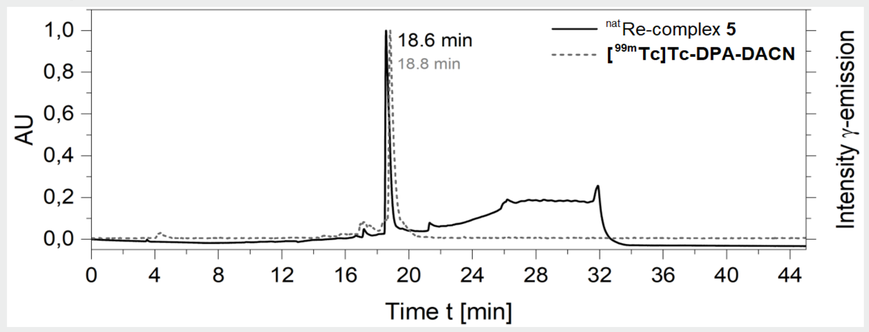


Preparation of the ^{99m}Tc-DACN Click Building Block



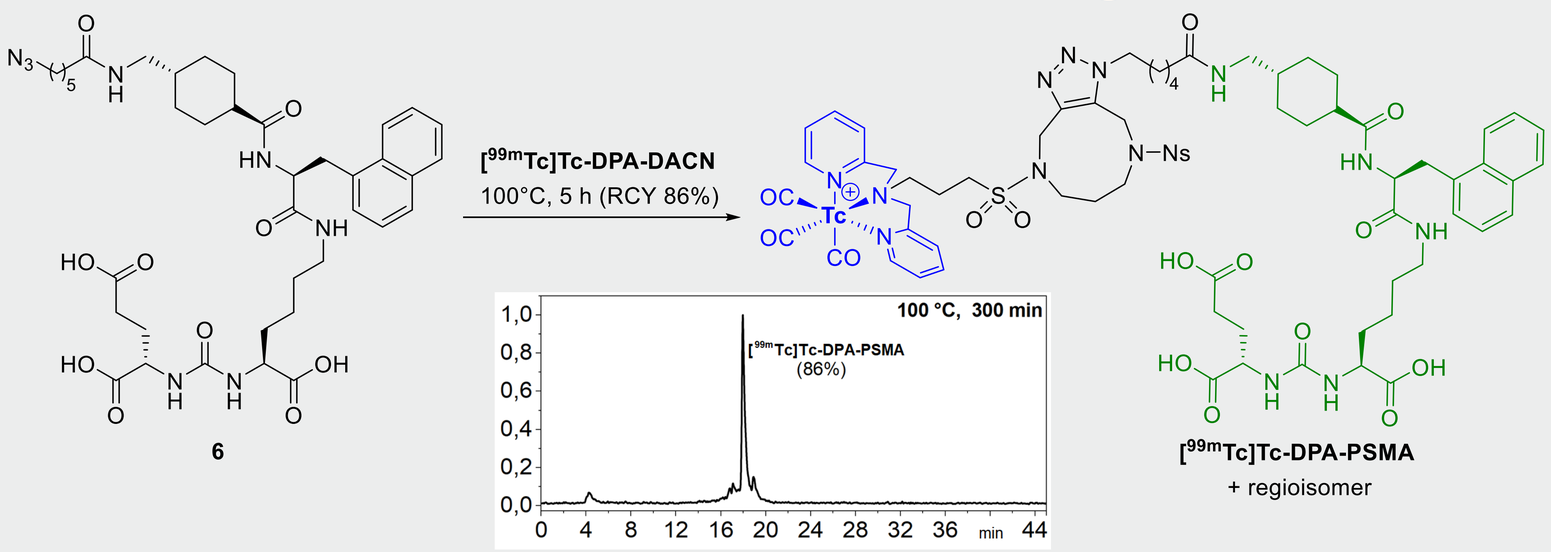
The $[^{99m}Tc]Tc(CO)_3$ core has gained high attraction for the convenient introduction of ^{99m}Tc into bio(macro)-molecules. The starting complex $[^{99m}Tc][Tc(CO)_3(H_2O)_3]^+$ (t_R = 13.7 min) was prepared using the tricarbonyl kit and was used without purification.

The preparation of the click-labeling building block **[^{99m}Tc]Tc-DPA-DACN** was tested at four different temperatures (rt, 40°C, 75°C, 100°C). Prior to the addition of ligand **4** (300 µg in 600 µL ethanol/water 1/1), *fac*-$[^{99m}Tc][Tc(CO)_3(H_2O)_3]^+$ was neutralized with 1 M MES buffer (pH 5.5). 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. The RCC was lowest at rt with 21%, followed by 40°C with 83% and 100°C with 78%. The starting complex $[^{99m}Tc][Tc(CO)_3(H_2O)_3]^+$ was still present in both batches at rt and 40°C. The highest RCC with 89% was observed at 75°C with full conversion, which was chosen as optimal for further radiolabeling approaches with the DPA-DACN ligand **4**. The obtained radiolabeled complex **[^{99m}Tc]Tc-DPA-DACN** was successfully verified using the nonradioactive rhenium complex **5**.



Cu-free Click-Radiolabeling

The Cu-free click-labeling was accomplished using an azide-functionalized PSMA-binder based on the binding motif found in PSMA-617. **[^{99m}Tc]Tc-DPA-DACN** was used without further purification. The reaction was tested at different temperatures (40°C, 75°C, 100°C) and different time points (30 min, 2 h, 4 h, 6 h). Unfortunately, the progress of the click-labeling was rather slow at 40°C and 75°C (RCC: 4% after 120 min). However, a considerable conversion of **[^{99m}Tc]Tc-DPA-DACN** with **6** to **[^{99m}Tc]Tc-PSMA-DACN** was observed at 100°C. The RCC to the ^{99m}Tc-PSMA derivative was 59% at 2 h and increased 86% after 5 h. The identity of the radiolabeled PSMA derivative was verified by the rhenium reference (t_R = 17.6 min and t_R = 18.0 min for **[^{99m}Tc]Tc-PSMA-DACN**).



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