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Bioconjugation

Propargyl-Substituted Thiocarbamoylbenzamidines of Technetium and Rhenium: Steps towards Bioconjugation with Use of Click Chemistry

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Abstract: A new propargyl-substituted thiocarbamoylbenzamidine has been synthesized. The compound acts as a tetradentate ligand and forms stable complexes with $\{Re^{V}O\}^{3+}$ and $\{Tc^{V}O\}^{3+}$ cores. Click couplings of the resulting complexes with benzylazide lead to prototype triazole derivatives, which were analyzed by NMR and IR spectroscopy and mass spectrometry, as well as by X-ray diffraction. A similar coupling procedure has been applied to an azido-modified angiotensin-II peptide, which gives the desired rhenium(V) bioconjugate in good yield. The ease of this coupling and the stability of the conjugate recommend such tetradentate thiocarbamoylbenzamidines also for clinically relevant bioconjugates with ^{99m}Tc.

Introduction

The metastable technetium nuclide ^{99m}Tc has been the most used radioisotope for diagnostic applications in nuclear medicine in the past decades.[1–7] Its favorable nuclear properties (virtually pure γ-emitter, E_{γ} = 143 keV, $t_{1/2}$ = 6.01 h) and the ready availability from $^{99}Mo/^{99m}Tc$ generators make it to the workhorse for most routine applications. $^{99m}TcO_4^-$ solutions from such generators normally contain the metal at an approximately nanomolar level, which is advantageous for clinical application, but causes considerable problems during the development of new Tc radiopharmaceuticals because of the lack of reliable structural information.

Therefore, structural studies on technetium compounds are commonly performed with the long-lived isotope ^{99}Tc (weak β^--emitter, E_{max} = 294 keV, $t_{1/2}$ = 2.11×10^5 a) or its heavier homolog rhenium. The element rhenium itself has two isotopes with strong β^--emissions (^{186}Re and ^{188}Re), which make them interesting for applications in nuclear medical therapy.[8–10]

One goal of current radiochemical research is the search for suitable chelating agents for radiometals used in nuclear medicine, which build stable complexes and can be attached to biomolecules under mild conditions without affecting their biological activity. Ligand systems which are not restricted to technetium and also form stable complexes with other radioactive metal ions having potential for either imaging or therapeutic applications are of particular interest.[11]

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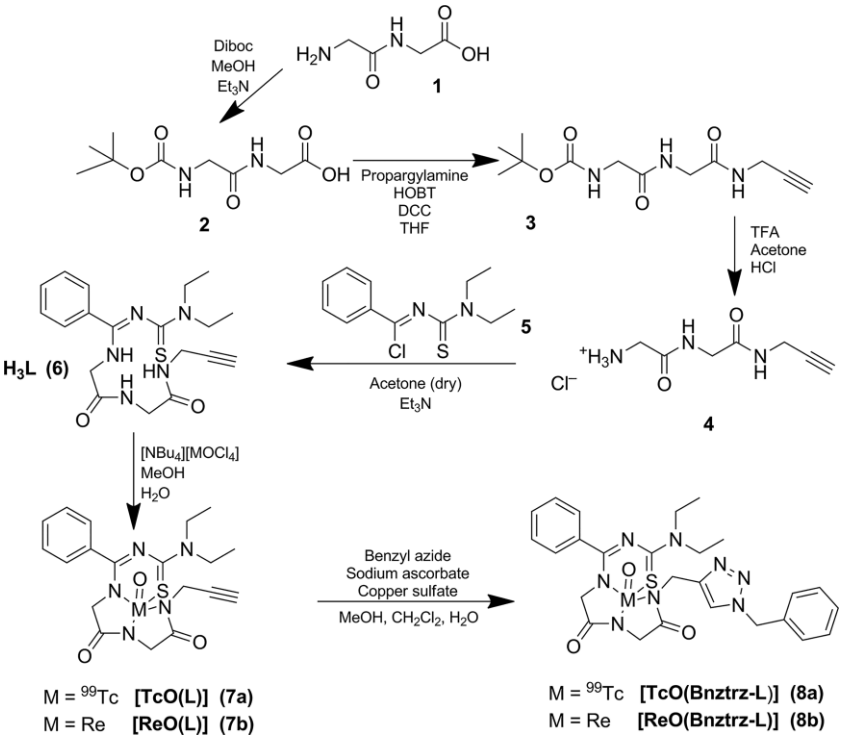
Thiocarbamoylbenzamidines are excellent chelating agents, and they form stable complexes with many metal ions.[12] The development of tri-, tetra-, and pentadentate derivatives makes them also interesting for the complexation of rhenium and technetium, since they are suitable candidates for the stabilization of their $\{M^{V}O\}^{3+}$ or $\{M^{V}N\}^{2+}$ cores.[13–19] Recently, we reported on some representatives of such ligands with orthogonal anchor groups in their periphery for bioconjugation and the corresponding Re and Tc complexes.[20,21] The coupling approach applied for these examples was the classical peptide coupling. Another promising coupling method for bioconjugation is provided by Click chemistry,[22] bearing in mind that azide derivatives of many biomolecules of interest are available or can be synthesized by well-established procedures.

In the present paper, we discuss an approach to bioconjugates using propargyl-substituted, tetradentate thiocarbamoylbenzamidines as chelating agents for rhenium and technetium with use of Click chemistry.

Results and Discussion

The synthesis of a suitable propargyl-substituted thiocarbamoyl benzamidine was performed in the multiple-step synthesis shown in Scheme 1. The key step is the coupling of propargylaminoglycylglycine **4** to thiocarbamoylbenzimide chloride **5**. In order to avoid undesired cyclization reactions, which we have recently observed during the attempted synthesis of a related ligand system,[23] this reaction was scheduled as the last step of the ligand synthesis.

Propargylaminodiglycine hydrochloride (**4**) can be obtained through a three-step synthesis. First, the amine function of diglycine (**1**) was protected with di-*tert*-butyldicarbonate (diBoc). The protected dipeptide **2** was activated with hydroxybenzotriazole (HOBT) and dicyclohexylcarbodiimide (DCC) in order to attach propargylamine. Finally, **4** was obtained after treatment



Scheme 1. Synthesis route.

of protected dipeptide **3** with trifluoroacetic acid and HCl. Benzimidoyl chloride **5** was prepared by a reaction of bis(*N*,*N*-diethyl-*N*′-benzoylthioureato)nickel(II) with $SOCl_2$ following a standard procedure.[13,24] Then it was coupled to **4** in dry acetone with the addition of NEt_3 as a supporting base. The resulting proligand H_3L (**6**) was obtained a pale yellow solid.

The ^1H NMR spectrum of H_3L (**6**) in $CDCl_3$ shows one multiplet at 1.22 ppm and two quartets at 3.62 and 3.90 ppm, which are all assigned to the diethyl residues of the molecule due to a hindered rotation around the related C–N bonds. This is not unusual for such ligands and has been described before.[13–21] The terminal alkyne proton leads to a triplet with a coupling constant of J = 2.4 Hz due to a long-range coupling with the methylene group of the propargyl function. This group itself displays a doublet of a doublet with coupling constants of 5.4 and 2.4 Hz at 4.02 ppm. Both methylene groups of the glycine units lead to doublets at 3.97 and 4.12 ppm. The hydrogen atoms of the NH groups lead to three triplets at 6.46, 6.93, and 7.59 ppm. These signals merge to form a broad singlet depending on the water content of the solvent $CDCl_3$. All aromatic signals appear between 7.38 and 7.49 ppm.

A strong absorption band at 3271 cm^{-1} in the IR spectrum of compound **6** can be assigned to the C–H stretch of the alkyne moiety. Two strong absorptions at 1670 and 1645 cm^{-1} are attributed to the carbonyl groups. The ESI^+ mass spectrum shows the expected molecular ion peak $[M + H]^+$ at m/z = 388.1802.

Reactions of **6** with $(NBu_4)[TcOCl_4]$ or $(NBu_4)[ReOCl_4]$ in methanol result in the formation of orange-red solutions. After the addition of 3–5 drops of water and keeping the solutions overnight in a freezer at –20 °C, orange-red single crystals of the compositions [MO(L)] (M = Tc, Re) were obtained.

Both complexes crystallize in the monoclinic space group $P2_1/n$. The metal atoms are five-coordinate with the tetradentate ligands L^{3-} as the basal planes and the oxido ligands as the apexes of square pyramids. An ellipsoid representation of the molecular structure of [TcO(L)] is shown in Figure 1. The structure of the corresponding rhenium complex is virtually identical and is therefore not shown. An ellipsoid plot of this compound can be found in Figure S1 in the Supporting Information. Selected bond lengths and angles of both complexes,

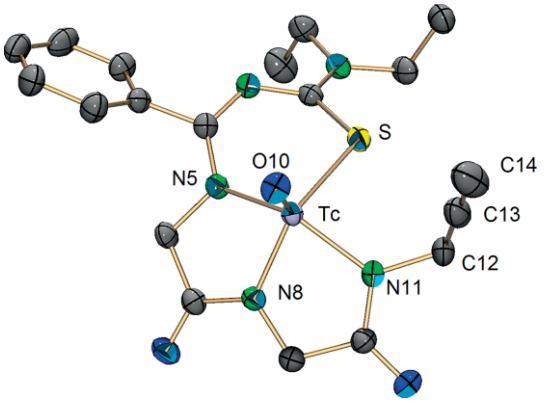


Figure 1. Ellipsoid representation of the molecular structure of [TcO(L)] (**7a**). Hydrogen atoms are omitted for clarity. Thermal ellipsoids represent 50 % probability.

are compared in Table 1. The labeling scheme of the technetium complex (Figure 1) has also been used for its rhenium analog.

Table 1. Selected bond lengths [Å] and angles [°] in [TcO(L)] (**7a**) and [ReO(L)] (**7b**).

7a		**7b**	
Tc–O10	1.654(2)	Re–O10	1.674(4)
Tc–S	2.307(1)	Re–S	2.301(2)
Tc–N5	2.017(2)	Re–N5	2.021(4)
Tc–N8	1.956(2)	Re–N8	1.982(4)
Tc–N11	2.025(2)	Re–N11	2.026(5)
N11–C12	1.479(3)	N11–C12	1.481(6)
C12–C13	1.455(4)	C12–C13	1.460(9)
C13–C14	1.173(4)	C13–C14	1.18(2)
O10–Tc–S	108.84(7)	O10–Re–S	108.4(2)
S–Tc–N5	91.31(6)	S–Re–N5	92.0(2)
N5–Tc–N8	78.78(8)	N5–Re–N11	78.2(2)
N8–Tc–N11	78.06(8)	N8–Re–N11	77.8(2)
N11–C12–C13	112.0(2)	N11–C12–C13	111.1(5)
C12–C13–C14	179.5(3)	C12–C13–C14	179.8(8)

The M–O10 bond lengths of 1.654(2) and 1.674(4) Å are in the typical range of metal-oxygen double bonds. The Tc–S and Re–S bond lengths are almost identical [2.307(2) and 2.301(2) Å]. The same holds true for the respective M–N bond lengths. The propargyl groups are almost perfectly linear and form an angle of approximately 112° with the mean planes of the tetradentate ligands.

Although the molecular structures of both complexes are very similar, their ^{1}H NMR spectra show some substantial differences. The alkyne hydrogen atoms display triplets with coupling constants of J = 2.45 Hz for both [TcO(L)] and [ReO(L)] at 2.27 ppm. These patterns are caused by long-range couplings with the adjacent methylene group of the propargyl function. This methylene group leads to two doublets of doublets at 5.34 and 4.86 ppm (coupling constants each J = 2.45 Hz) in the spectrum of [ReO(L)]. In the spectrum of [TcO(L)], the equivalent signals can be found at 5.10 and 4.75 ppm. All hydrogen atoms of the methylene groups are diastereotopic in both complexes. This leads to a further splitting of the methylene signals due to geminal couplings, and consequently complicated signal patterns in the range between 3.7 and 5.4 ppm are observed, which are different in the spectra of the analogous technetium and rhenium complexes.

The IR spectra of the studied [MO(L)] complexes are, as expected, very similar. They show $\nu_{M=O}$ stretches at 972 (Tc compound) and 983 cm^{-1} (Re complex). Sharp bands at 3261 cm^{-1} reflect the presence of the alkyne C–H bonds. Each two intense bands at 1664, 1635 cm^{-1} (Tc complex) and 1672, 1643 cm^{-1} (Re compound) are attributed to the peptide C=O bonds. The ESI$^+$ mass spectrum of [ReO(L)] shows the expected molecular ion peak [M + H]$^+$ at m/z = 588.1069. That of the technetium compound has not been recorded for radiation safety reasons.

In order to get information about the robustness of the new complexes in ongoing reactions, the propargyl-substituted technetium complex **7a** as well as its rhenium analogue **7b** have been used for model Click reactions with benzylazide. Unlike classical Click reactions, which are commonly carried out with small amounts of copper sulfate (ca. 1 mol-%) and sodium ascorbate (up to 5 mol-%), the reaction has been performed with equivalent amounts of the Cu^{2+} ions and an excess of ascorbic acid. Although such conditions might cause problems during the purification of the products, they have been chosen since (i) they should accelerate the reactions and (ii) keeping in mind, that for prospective reactions with ^{99m}Tc (and the nanomolar concentrations of this nuclide in real ^{99}Mo/^{99m}Tc generator eluates) a large excess of copper ions cannot be avoided. With equivalent amounts of Cu^{2+} ions also the role of these ions as competitors in the complex formation with H_3L could be tested, and the possible formation of copper complexes with the tetradentate ligand should be investigated..

The reactions between the [MO(L)] complexes **7a** and **7b** and benzylazide have been performed in mixtures of dichloromethane, methanol, and water (1:3:1 v/v/v). This ensured solubility of all components and a homogenous reaction. After stirring for 5 h at room temperature, dichloromethane was evaporated, which resulted in the precipitation of the orange-red coupling products [TcO(Bnztrz-L)] (**8a**) and [ReO(Bnztrz-L)] (**8b**). No evidence was found for the formation of Cu^{II} complexes with the tetradentate ligand. The completion of the reaction was confirmed by ^{1}H NMR analysis of the crude products.

Single crystals suitable for the X-ray analyses were obtained by recrystallization from toluene or acetone/toluene mixtures. The molecular structure of technetium complex **8a** is shown in Figure 2. The structure of the analogous rhenium compound is identical and therefore only shown in the Supporting Information. Selected bond lengths and angles of both compounds are given in Table 2.

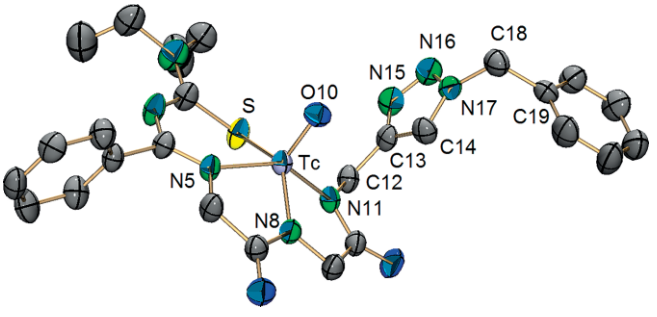


Figure 2. Molecular structure of **8a**. Hydrogen atoms are omitted for clarity. Thermal ellipsoids represent 50 % probability.

Similar to the structures of the starting materials **7a** and **7b**, the molecular structures of the products of the Click couplings possess five-coordinate metal atoms. The coordination spheres can best be described as square pyramids with the oxido ligands as their apexes. The coupling reactions in the peripheries of the tetradentate ligands do not change the coordination environment of the metal cores in comparison to the starting materials. All bond lengths and angles in the coordination spheres of the metal atoms remain practically unchanged after the formation of the triazole rings.

A good indication for the progress of the coupling reaction is the disappearance of the triplet signals at about 2.27 ppm displayed by the alkyne protons in the ^{1}H NMR spectra. Instead, new singlets, which are assigned to the two protons of the methylene groups of the benzyl residues, appear in the spectra

Table 2. Selected bond lengths [Å] and angles [°] in [TcO(Bnztrz-L)] (**8a**) and [ReO(Bnztrz-L)] (**8b**).

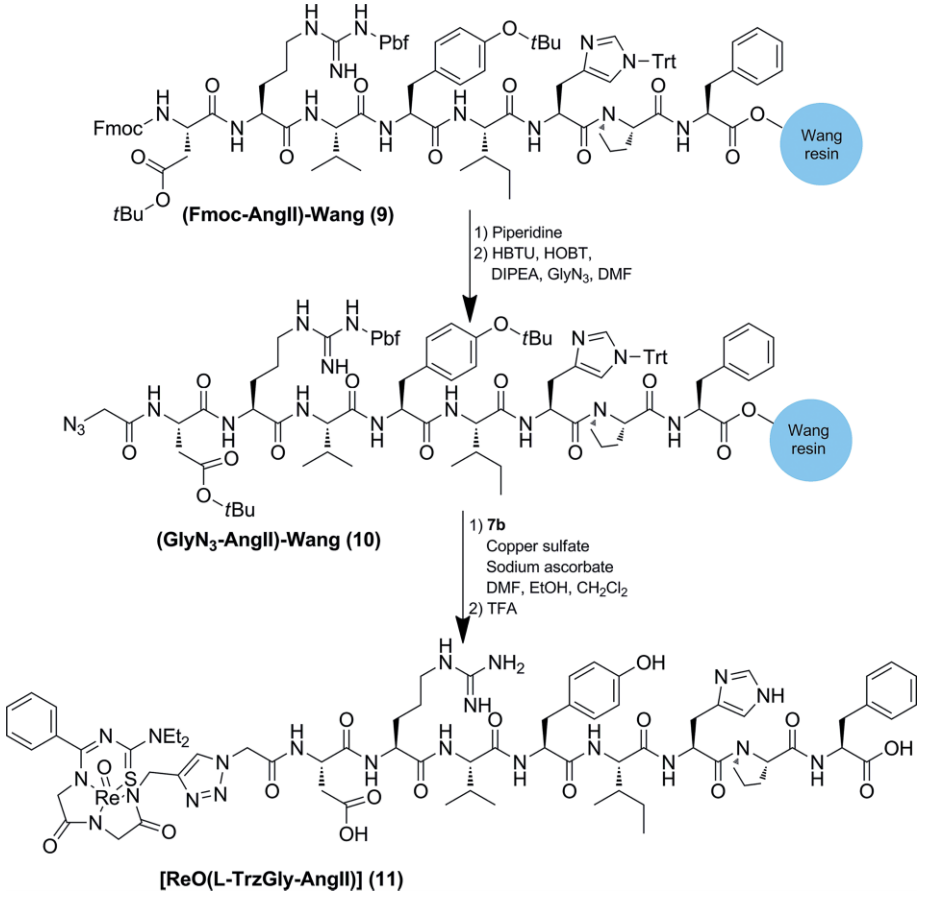
8a		**8b**	
Tc–O10	1.651(3)	Re–O10	1.671(5)
Tc–S	2.306(1)	Re–S	2.305(2)
Tc–N5	2.050(3)	Re–N5	2.056(5)
Tc–N8	1.942(3)	Re–N8	1.945(7)
Tc–N11	2.014(3)	Re–N11	2.015(5)
N11–C12	1.463(5)	N11–C12	1.470(9)
C12–C13	1.492(5)	C12–C13	1.50(2)
C13–C14	1.357(5)	C13–C14	1.36(2)
C13–N15	1.356(5)	C13–N15	1.37(2)
N15–N16	1.321(5)	N15–N16	1.33(1)
C14–N17	1.357(5)	C14–N17	1.365(9)
N16–N17	1.345(4)	N16–N17	1.33(2)
N17–C18	1.472(5)	N17–C18	1.47(2)
C18–C19	1.518(6)	C18–C19	1.51(2)
O10–Tc–S	111.45(9)	O10–Re–S	110.9(2)
S–Tc–N5	89.54(8)	S–Re–N5	90.3(2)
N5–Tc–N8	79.3(1)	N5–Re–N11	79.4(2)
N8–Tc–N11	78.9(1)	N8–Re–N11	78.4(2)
N11–C12–C13	113.0(3)	N11–C12–C13	113.1(6)
C12–C13–C14	130.0(4)	C12–C13–C14	129.4(7)
N17–C18–C19	112.5(3)	N17–C18–C19	113.0(7)

of **8a** and **8b** at about 5.45 ppm. The protons of carbon atoms C12 lead to two doublets at 5.30 and 5.60 ppm in the spectrum of **8a** and at 5.39 and 5.88 ppm in that of the rhenium complex. All other methylene signals appear as expected between 4.2 and 4.8 ppm in the spectra of both compounds. The signals of the aromatic protons of the triazole rings overlap with the other aromatic signals giving complex multiplets.

The IR spectra of **8a** and **8b** are expectedly very similar. They show $\nu_{Tc=O}$ and $\nu_{Re=O}$ bands at 981 and 993 cm^{-1}, respectively. Each two sharp absorptions at 1672 and 1651 cm^{-1} in the spectrum of the technetium complex and at 1678 and 1658 cm^{-1} in that of **8b** originate from by the carbonyl stretches. The triazole rings display sharp absorption bands at 1417, 1354, and 1328 cm^{-1} in the spectra of both compounds. The ESI^+ mass spectrum of the rhenium complex shows the molecular ion peak $[M + H]^+$ at m/z = 721.1716.

The successful coupling of **7a** and **7b** to benzylazide stimulated us to attempt conjugation with a small peptide. For this experiment, we prepared an azido-substituted angiotensin-II derivative by a conventional solid-phase peptide synthesis, $GlyN_3$-AngII-Wang (Scheme 2). Angiotensin-II is a peptide hormone consisting of eight amino acids (H_2N-Asp–Arg–Val–Tyr–Ile–His–Pro–Phe-COOH). This peptide plays an important role in the regulation of blood pressure and water balance in the body and has an effect on the whole cardiovascular system. Primarily, angiotensin-II causes constriction of blood vessels.[25–27]

The coupling reaction was intended as a proof-of-principle experiment and performed in the solid phase. Wang resin was used as a solid carrier with the peptides still having protected



Scheme 2. Synthesis of the angiotensin-II derivatives.

amino acid side chains. This ensures that the functional groups of the peptide do not interfere with the coupling procedure, for example by undesired side-reactions with the Cu^{2+} ions. Deprotection and cleavage from the resin were performed in subsequent steps.

The identity of the coupling product between the azido-substituted angiotensin-II derivative and [ReO(L)] (**7b**) was analyzed with ESI mass spectrometry of the crude product. Signals for the molecular ion $[M + H]^+$ at m/z = 1716.6488 and the ion $[M + H]^{2+}$ at m/z = 858.8291 are found in the positive-mode ESI spectrum. The detection of doubly charged ions is not uncommon for the mass spectra of peptides. The corresponding signal $[M - H]^-$ was found at m/z = 1714.6336 in the ESI^- spectrum. The corresponding molecular ion regions of both mass spectra are shown in Figure 3 together with the calculated isotope distributions.

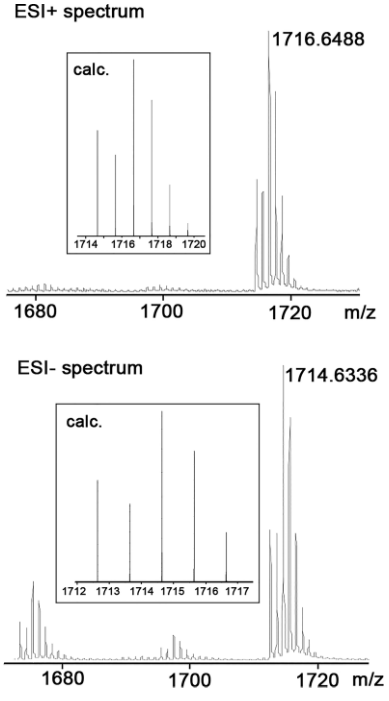


Figure 3. Molecular-ion regions of the ESI^+ and ESI^- mass spectra of the rhenium bioconjugate.

Conclusions

Tetradentate thiocarbamoylbenzamidines, which have an alkynyl group attached to their periphery, are suitable chelating agents for the $\{Tc^{V}O\}^{3+}$ and $\{Re^{V}O\}^{3+}$ cores and can be used for precomplexation of the metals before bioconjugation. If a propargylic group is used, bioconjugation though copper-catalyzed Click chemistry is possible. A disadvantage of this approach is the fact that interfering functional groups of the biomolecule need to be protected. One way to avoid this inconvenience is the use of cyclooctyne moieties instead of propargyl moieties as a source of the alkyne groups,[28] so that a copper-free Click reaction can be performed. This would be of special importance for reactions with ^{99m}Tc, since time can be saved, and more convenient reaction conditions can be chosen. Another opportunity for the synthesis of suitable ^{99m}Tc derivatives is the use of transmetalation reactions starting from nonradioactive rhenium compounds or preferably from copper complexes. Related studies are currently under investigation in our research group.

Experimental Section

Materials: All chemicals used in this study were reagent grade and used without further purification. Diethylaminobenzimidoyl chloride **5**,[24] $(NBu_4)[ReOCl_4]$,[29] $(NBu_4)[TcOCl_4]$,[30] benzylazide,[31] and azidoacetic acid ($GlyN_3$)[32] were prepared by published methods. Solvents were dried with use of standard procedures. Peptides were synthesized by using modifications of standard procedures of solid phase peptide synthesis.[33]

Physical Measurements: Infrared spectra were recorded from KBr pellets with a Shimadzu FTIR-spectrometer between 400 and 4000 cm^{-1}. ESI mass spectra were recorded with an Agilent 6210 ESI-TOF (Agilent Technologies). All MS results are given in the form: *m/z*, assignment. Elemental analyses of carbon, hydrogen, nitrogen, and sulfur were performed with a Heraeus Vario EL elemental analyzer. The technetium content was determined with a Beckman LS6500 liquid szintillation counter. NMR spectra were recorded with a JEOL 400 MHz multinuclear spectrometer.

Radiation Protection: The radioisotope ^{99}Tc was handled following standard radiation protection procedures in a laboratory approved for the work with long-lived beta emitters.

***N*-Boc-Glycylglycine (2):** Diglycine (6.55 g, 50 mmol) and diBoc (15 g, 50 mmol) were suspended in methanol (50 mL). Triethylamine (5.05 g, 50 mmol) was added, and the reaction mixture was heated to reflux for 4 h. After cooling to room temperature, the resulting solution was filtered and reduced to a volume of about 10 mL with a rotary evaporator. The resulting solution was added under stirring to diethylether (200 mL). An oily substance, which separated from this solution, was isolated, washed several times with diethylether, and dried under vacuum for a period of several days. Finally, a hard wax was formed. Yield: 11.01 g (47.5 mmol, 95 %). $C_9H_{16}N_2O_5$ (232.24): calcd. C 46.55, H 6.94, N 12.06; found C 50.91, H 10.08, N 11.75. IR (KBr): $\tilde{\nu}$ = 3325 (s), 2976 (s), 2937 (s), 2802 (w), 2756 (w), 2738 (m), 2677 (s), 2492 (s), 1699 (s), 1668 (s), 1606 (w), 1531 (s), 1394 (s), 1367 (s), 1280 (w), 1251 (s), 1170 (s), 1033 (m), 947 (m), 864 (m), 678 (m) cm^{-1}. ^1H NMR ([D_6]DMSO): δ = 1.38 (s, 9 H, -CH_3), 3.53 (d, J = 6.1 Hz, 2 H, -CH_2-), 3.61 (d, J = 5.2 Hz, 2 H, -CH_2-), 7.05 (t, J = 6.1 Hz, 1 H, -CO–NH-), 7.77 (t, J = 5.2 Hz, 1 H, -CO–NH-) ppm. $ESI\text{-}TOF^+$ (*m/z*): calcd. $[M + Na]^+$ 255.0951; found 255.0999. $ESI\text{-}TOF^-$ (*m/z*): calcd. $[M - H]^-$ 231.0986; found: 231.0859.

***N*-Propargylamino Glycylglycine (3):** Compound **2** (6.25 g, 26 mmol), propargylamine (2.22 g, 40 mmol), HOBT (5.40 g, 40 mmol), and DCC (8.24 g, 40 mmol) were dissolved in THF (50 mL). The reaction mixture was stirred for 6 h at room temperature. The resulting precipitate was filtered off, and the remaining solvents were evaporated with a rotary evaporator until an oily residue was obtained. The residue was dissolved in warm acetone (50 mL), and left overnight in a refrigerator for crystallization. The obtained crystalline compound was filtered off, washed with cold acetone, and dried. Yield: 2.31 g (8.6 mol, 33 %). $C_{12}H_{19}N_3O_4$ (269.30): calcd. C 53.52, H 7.11, N 15.60; found C 59.37, H 7.75, N 15.98. IR (KBr): $\tilde{\nu}$ = 3327 (s), 3180 (w), 3122 (w), 3034 (m), 2927 (s), 2850 (s), 2791 (w), 2657 (w), 1625 (s), 1573 (s), 1537 (m), 1462 (w), 1435 (m), 1346 (w),

1311 (s), 1271 (m), 1244 (s), 1186 (w), 1157 (w), 1087 (s), 1086 (w), 1045 (m), 966 (w), 893 (s), 842 (w), 802 (w), 732 (w), 640 (s) cm^{-1}. ^1H NMR ([D_6]DMSO): δ = 1.38 (s, 9 H, -CH_3), 3.11 (t, J = 2.1 Hz, 1 H, -CCH), 3.57 (d, J = 5.8 Hz, 2 H, -CH_2-), 3.69 (d, J = 5.6 Hz, 2 H, -CH_2-), 3.86 (dd, J = 5.3, 2.3 Hz, 2 H, -CH_2-), 7.03 (t, J = 5.7 Hz, 1 H, -CO–NH-), 8.08 (t, J = 5.3 Hz, 1 H, -CO–NH-), 8.24 (t, J = 5.0 Hz, 1 H, -CO–NH-) ppm. ESI-TOF$^+$ (*m/z*): calcd. [M + Na]$^+$ 292.1268; found [M + Na]$^+$ 292.1272, [M + K]$^+$ 308.1010.

Propargylamidoglycylglycine Hydrochloride (4): Compound **3** (2.22 g, 8.2 mmol) was dissolved in trifluoracetic acid (30 mL). The solution was stirred for 4 h at room temperature, and then the solvent was slowly evaporated under a flow of argon until an oily residue was obtained. The residue was isolated and redissolved in acetone (50 mL). HCl (37 %, 2.5 mL) was added dropwise to this solution. The resulting precipitate was filtered off, washed with acetone, and dried. Yield: 1.34 g (6.6 mmol, 80 %). $C_7H_{12}ClN_3O_2$ (205.64): calcd. C 40.88, H 5.88, N 20.43; found C 40.51, H 6.56, N 21.77. IR (KBr): $\tilde{\nu}$ = 3246 (s), 3045 (s), 2931 (s), 2850 (w), 2798 (w), 2713 (w), 2623 (w), 2121 (w), 2005 (w), 1693 (s), 1660 (s), 1602 (w), 1558 (m), 1527 (s), 1496 (s), 1429 (w), 1404 (m), 1342 (s), 1311 (w), 1290 (w), 1259 (s), 1139 (w), 1116 (w), 1087 (m), 1006 (m), 933 (m), 900 (m), 889 (w), 867 (w), 738 (m), 705 (m), 675 (s) cm^{-1}. . ^1H NMR ([D_6]DMSO): δ = 1.31 (t, J = 2.5 Hz, 1 H, -CCH), 3.59 (q, J = 5.5 Hz, 2 H, -CH_2-), 3.77 (d, J = 5.9 Hz, 2 H, -CH_2-), 3.85 (dd, J = 5.5, 2.5 Hz, 2 H, -CH_2-), 8.25 (s, br, 3 H, -NH_3^+), 8.54 (t, J = 5.5 Hz, 1 H, -CO–NH-), 8.79 (t, J = 5.8 Hz, 1 H, -CO–NH-) ppm. ESI-TOF$^+$ (*m/z*): calcd. [M]$^+$ 170.0924; found 170.0932.

H_3L (6): Compound **4** (556 mg, 2.7 mmol) was suspended in dry acetone (50 mL). Triethylamine (1.01 g, 10 mmol) and solid diethylaminobenzimidoyl chloride **5** (685 mg, 2.7 mmol) were subsequently added to this suspension. The reaction mixture was stirred for 5 h at room temperature and filtered. The resulting clear solution was reduced to a volume of about 5 mL and finally added dropwise under vigorous stirring to diethylether (50 mL). The formed precipitate was filtered off, washed with diethylether, and dried. Yield: 667 mg (1.72 mmol, 63.8 %). $C_{19}H_{25}N_5O_2S$ (387.50): calcd. C 58.89, H 6.50, N 18.07, S 8.27; found C 58.59, H 7.34, N 18.09, S 8.28. IR (KBr): $\tilde{\nu}$ = 3321 (s), 3271 (s), 3064 (m), 3034 (w), 2976 (s), 2931 (s), 2872 (w), 1670 (s), 1645 (s), 1622 (s), 1597 (m), 1550 (s), 1535 (m), 1519 (w), 1490 (s), 1458 (w), 1419 (m), 1375 (w), 1355 (w), 1338 (m), 1309 (m), 1269 (m), 1240 (s), 1143 (w), 1124 (m), 1093 (m), 1074 (w), 1049 (w), 1022 (m), 993 (w), 889 (m), 779 (m), 702 (s), 682 (s), 648 (m) cm^{-1}. ^1H NMR ($CDCl_3$:): δ = 1.18–1.26 (m, 6 H, -CH_3), 2.20 (t, J = 2.2 Hz, 1 H, -CCH), 3.62 (q, J = 6.8 Hz, 2 H, -CH_2-), 3.91 (q, J = 6.8 Hz, 2 H, -CH_2-), 3.97 (d, J = 5.7 Hz, 2 H, -CH_2-), 4.02 (dd, J = 5.4, 2.4, Hz, 2 H, -CH_2-) 4.13 (d, J = 5.1 Hz, 2 H, -CH_2-), 6.46 (s, br., 1 H, -NH-) 6.93 (s, br., 1 H, -CO–NH-), 7.38–7.49 (m, 5 H, Ph) 7.59 (s, br., 1 H, -CO–NH-) ppm. ESI-TOF$^+$ (*m/z*): calcd. [M + H]$^+$ 388.1802; found [M + H]$^+$ 388.1802, [M + Na]$^+$ 410.1627, [M + K]$^+$ 426.1361.

[TcO(L)] (7a): [NBu_4][$TcOCl_4$] (192 mg, 0.49 mmol) was dissolved in methanol (5 mL). A solution of **6** (224 mg, 0.57 mmol) in methanol (5 mL) was added, and the reaction mixture was stirred for 15 min at room temperature. After the addition of five drops of water, the solution was filtered and left overnight at –20 °C in the freezer. Orange-red crystals were formed. They were filtered off, washed with cold methanol and water, and dried. Yield: 142 mg (0.3 mmol, 62 %). $C_{19}H_{22}N_5O_3STc$ (499.38): calcd. Tc 19.8; found Tc 19.4. IR (KBr): $\tilde{\nu}$ = 3442 (s), 3261 (s), 2978 (w), 2937 (w), 1664 (s), 1635 (s), 1529 (s), 1508 (s), 1448 (m), 1433 (m), 1415 (m), 1394 (m), 1382 (w), 1334 (s), 1315 (m), 1288 (w), 1064 (m), 1008 (w), 972 (s), 775 (m), 698 (s), 690 (s), 669 (m) cm^{-1}. ^1H NMR ($CDCl_3$:): δ = 1.25 (t, J = 7.1 Hz, 3 H, -CH_3), 1.45 (t, J = 7.1 Hz, 3 H, -CH_3), 2.27 (t, J = 2.4 Hz, 1 H, -CCH), 3.77–4.10 (m, 4 H, -CH_2-), 4.34 (d, J = 17.7 Hz, 1 H, -CH_2-), 4.42 (d, J = 18.6 Hz, 1 H, -CH_2-) 4.50 (d, J = 18.6 Hz, 1 H, -CH_2-), 4.74 (d, J = 17.8 Hz, 1 H, -CH_2-) 4.75 (dd, J = 17.6, 2.4 Hz, 1 H, -CH_2-), 5.10 (dd, J = 17.6, 2.5 Hz, 1 H, -CH_2-) 7.38–7.41 (m, 2 H, *o*-Ph) 7.48–7.52 (m, 3 H, *m*-*p*-Ph) ppm.

[ReO(L)] (7b): [NBu_4][$ReOCl_4$] (290 mg, 0.5 mmol) was dissolved in methanol (5 mL). A solution of **6** (215 mg, 0.55 mmol) in methanol (5 mL) was added. The reaction mixture was stirred for 15 min at room temperature. Five drops of water were added, and the resulting solution was filtered and kept overnight in the freezer at –20 °C. Orange-red crystals were formed. They were filtered off, washed with cold methanol and water, and dried. Yield: 214 mg (0.36 mmol, 73 %). $C_{19}H_{22}N_5O_3ReS$ (586.67): calcd. C 38.90, H 3.78, N 11.94, S 5.47; found C 38.88, H 3.63, N 11.76, S 5.36. IR (KBr): $\tilde{\nu}$ = 3442 (s), 3261 (s), 2978 (w), 2935 (m), 1672 (s), 1643 (s), 1531 (s), 1514 (s), 1435 (w), 1417 (w), 1381 (m), 1334 (s), 1317 (m), 1290 (w), 1230 (w), 1064 (m), 983 (s), 937 (w), 883 (w), 827 (w), 775 (m), 688 (s), 671 (m) cm^{-1}. ^1H NMR ($CDCl_3$:): δ = 1.29 (t, J = 7.1 Hz, 3 H, -CH_3), 1.45 (t, J = 7.2 Hz, 3 H, -CH_3), 2.27 (t, J = 2.5 Hz, 1 H, -CCH), 3.80–4.11 (m, 4 H, -CH_2-), 4.40 (d, J = 18.7 Hz, 1 H, -CH_2-), 4.49 (d, J = 18.7 Hz, 1 H, -CH_2-), 4.55 (d, J = 17.7 Hz, 1 H, -CH_2-), 4.73 (d, J = 17.7 Hz, 1 H, -CH_2-), 4.86 (dd, J = 17.4, 2.5 Hz, 1 H, -CH_2-), 5.34 (dd, J = 17.4, 2.5 Hz, 1 H, -CH_2-), 7.42–7.55 (m, 5 H, Ph) ppm. ESI-TOF$^+$ (*m/z*): calcd. [M + H]$^+$ 588.1074; found 588.1069.

[TcO(Bnztrz-L)] (8a): Method 1: Compound **7a** (25 mg, 0.05 mmol) was dissolved in ethanol (10 mL). Subsequently, benzylazide (78 % in diethyl ether, 58 mg, 0.35 mmol), copper sulfate pentahydrate (1.24 mg, 0.005 mmol) in water (100 µL), and a solution of sodium ascorbate (2.47 mg, 0.012 mmol) in water (100 µL) were added. The reaction mixture was stirred for 8 h at room temperature and left for slow evaporation of the solvents at room temperature over a period of several days. The obtained residue was dissolved in a mixture of acetone/toluene (1:1 v/v, 2 mL). Crystals of the product could be obtained by slow evaporation of this solution. The crystals were filtered off, washed with cold toluene, and dried. Yield: 15 mg (0.023 mmol, 46 %). **Method 2: 7a** (50 mg, 0.1 mmol) was dissolved in methanol (3 mL). Subsequently, benzylazide (78 % in diethyl ether, 130 mg, 0.8 mmol), solid copper sulfate pentahydrate (24.8 mg, 0.1 mmol), and sodium ascorbate (39.6 mg, 0.2 mmol) were added to the reaction mixture. dichloromethane (1 mL), water (1 mL), and methanol (3 mL) were added, so that all components dissolved and a clear solution without phase separations was obtained. The solution was then stirred for 5 h at room temperature. The solvents were reduced to a volume of about 3 mL under reduced pressure. The obtained precipitate was filtered off, washed with cold methanol and water, and dried. Single crystals of the product were obtained from slow evaporation of a solution of the complex in toluene. Yield: 36 mg (0.057 mmol, 57 %). $C_{26}H_{29}N_8O_3STc$ (632.53): calcd. Tc 15.6; found Tc 15.4. IR (KBr): $\tilde{\nu}$ = 3441 (s), 3136 (m), 3064 (w), 2976 (m), 2935 (m), 2906 (m), 1672 (s), 1651 (s), 1508 (s), 1417 (s), 1355 (s), 1328 (m), 1294 (m), 1255 (w), 1211 (w), 1145 (w), 1130 (w), 1072 (m), 1049 (m), 1012 (m), 981 (s), 869 (m), 823 (w), 775 (w), 729 (w), 700 (w), 669 (w) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.23 (t, J = 7.1 Hz, 3 H, -CH_3), 1.41 (t, J = 7.1 Hz, 3 H, -CH_3), 3.71–4.10 (m, 4 H, -CH_2-), 4.31 (d, J = 17.7 Hz, 1 H, -CH_2-), 4.39 (d, J = 18.6 Hz, 1 H, -CH_2-), 4.48 (d, J = 18.6 Hz, 1 H, -CH_2-), 4.70 (d, J = 17.8 Hz, 1 H, -CH_2-), 5.31 (d, J = 15.5 Hz, 1 H, -CH_2-), 5.46 (s, 2 H, -CH_2-), 5.60 (d, J = 15.5 Hz, 1 H, -CH_2-), 7.21–7.49 (m, 11 H, H_{arom}) ppm.

[ReO(Bnztrz-L)] (8b): Compound **7b** (58 mg, 0.1 mmol) was suspended in methanol (3 mL). Subsequently, benzylazide (78 % in diethyl ether, 130 mg, 0.8 mmol), solid copper sulfate pentahydrate

(24.8 mg, 0.1 mmol), and sodium ascorbate (39.6 mg, 0.2 mmol) were added to the reaction mixture. Finally, dichloromethane (1 mL), water (1 mL), and methanol (3 mL) were added, so that all components dissolved and a clear solution without phase separations was obtained. The solution was stirred for 5 h at room temperature, and dichloromethane was evaporated under reduced pressure. Water (50 mL) was added to the reaction mixture, which was then extracted with dichloromethane (3 × 50 mL). The combined organic phases were dried with magnesium sulfate, filtered, and reduced to a volume of about 5 mL. This solution was added under vigorous stirring to *n*-hexane (50 mL). The obtained precipitate was filtered off, washed with *n*-hexane, and dried. The resulting solid was dissolved in a mixture of acetone/toluene (1:1, 2 mL). Good-quality crystals of the product, suitable for X-ray analysis, were obtained by slow evaporation of this solution at room temperature. The crystals were filtered off, washed with cold toluene, and dried. Yield: 37 mg (0.051 mmol, 51 %). $C_{26}H_{29}N_8O_3ReS$ (719.83): calcd. C 43.38, H 4.06, N 15.57, S 4.45; found C 43.43, H 4.08, N 15.47, S 5.91. IR (KBr): $\tilde{\nu}$ = 3442 (s), 3138 (m), 3064 (w), 3978 (m), 3935 (m), 3912 (m), 1678 (s), 1658 (s), 1516 (s), 1417 (s), 1354 (s), 1328 (m), 1296 (m), 1132 (w), 1051 (w), 993 (s), 871 (w) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.25 (t, J = 7.1 Hz, 3 H, -CH_3), 1.40 (t, J = 7.1 Hz, 3 H, -CH_3), 3.77–4.10 (m, 4 H, -CH_2-), 4.38 (d, J = 18.7 Hz, 1 H, -CH_2-), 4.46 (d, J = 18.8 Hz, 1 H, -CH_2-), 4.51 (d, J = 17.7 Hz, 1 H, -CH_2-), 4.69 (d, J = 17.7 Hz, 1 H, -CH_2-), 5.39 (d, J = 15.2 Hz, 1 H, -CH_2-), 5.45 (s, 2 H, -CH_2-), 5.88 (d, J = 15.2 Hz, 1 H, -CH_2-), 7.20–7.51 (m, 11 H, H_{arom}) ppm. ESI-TOF$^+$ (*m*/*z*): calcd. $[M + H]^+$ 721.1714; found $[M + H]^+$ 721.1716, $[M + Na]^+$ 743.1540, $[M + K]^+$ 759.1273.

Fmoc-AngII-Wang (9) and AngII: Fmoc-Phe-Wang resin (711 mg, 0.4 mmol) was shaken for 30 min in DMF (5 mL). After filtration, the resin was suspended in piperidine (2 mL) and shaken for further 30 min. The resin was filtered off and washed several times with DMF. The resin was then treated with a solution of HOBT (217 mg, 1.6 mmol), DIPEA (207 mg, 1.6 mmol), HBTU (606 mg, 1.6 mmol), and Fmoc–Pro-OH (509 mg, 1.6 mmol) in DMF (5 mL) over a period of 3 h. After filtration, the resin was washed several times with DMF and treated again with piperidine (2 mL) for 30 min. The same procedure was repeated with all other amino acid derivatives: Fmoc–Pro-OH (539 mg, 1.6 mmol), Fmoc–His(trt)-OH (992 mg, 1.6 mmol), Fmoc–Ile-OH (565 mg, 1.6 mmol), Fmoc–Tyr(*t*Bu)-OH (755 mg, 1.6 mmol), Fmoc–Val-OH (543 mg, 1.6 mmol), Fmoc–Arg(Pbf)-OH (779 mg, 1.6 mmol), Fmoc–Asp(O*t*Bu)-OH (657 mg, 1.6 mmol). The still protected and resin-bound peptide was dried and used in this form for further reactions. For the characterization of the product, the resin-bound peptide (10 mg) was treated with piperidine (2 mL) for 1 h, washed several times with DMF and dichloromethane, and cleaved from the resin and the protecting groups by treatment with a mixture of trifuoroacetic acid/dichloromethane (95:5, v/v, 1 mL) over a period of 3 h. The resin was filtered off, and the residue was concentrated and analyzed by ESI mass spectrometry. Yield of Fmoc-AngII-Wang: 1.11 g (resin-bound and protected). Mass spectra of the unprotected peptide AngII: ESI-TOF$^+$ (*m*/*z*): calcd. $[M + H]^+$ 1046.5418; found 1046.5429. ESI-TOF$^-$ (*m*/*z*): calcd. $[M - H]^-$ 1044.5272; found 1044.5478.

($GlyN_3$-AngII)-Wang (10) and $GlyN_3$-AngII: (Fmoc-AngII)-Wang (200 mg, about 0.03 mmol) was suspended in DMF (5 mL) and shaken for 30 min. After filtration, the resin was treated with piperidine (5 mL) over a period of 1 h and washed several times with DMF. The resin was then treated with a solution of HOBT (108 mg, 0.8 mmol), HBTU (302 mg, 0.8 mmol), DIPEA (103 mg, 0.8 mmol), and $GlyN_3$•$2Et_2O$ (200 mg, 0.8 mmol) in DMF (2 mL) over a period of 4 h. The resulting resin was washed several times with DMF and dichloromethane, and dried. The product ($GlyN_3$-AngII)-Wang was used in this form for further reactions. For the characterization of the product, part of the product (10 mg) was treated with a mixture of trifluoroacetic acid/dichloromethane (95:5, v/v, 1 mL) for 3 h. The resin was filtered off, and the residue was concentrated and analyzed by ESI mass spectrometry. Yield of ($GlyN_3$-AngII)-Wang: 187 mg (resin-bound and protected). Mass spectra of the unprotected peptide $GlyN_3$-AngII: ESI-TOF$^+$ (*m*/*z*): calcd. $[M + H]^+$ 1129.5543; found 1129.5544. ESI-TOF$^-$ (*m*/*z*): calcd. $[M - H]^-$ 1127.5387; found 1127.5402.

[ReO(L-TrzGly-AngII)] (11): ($GlyN_3$-AngII)-Wang (20 mg) was suspended in DMF (5 mL) and shaken for 30 min. After filtration, the resin was treated with a solution of [ReO(L)] (58 mg, 0.1 mmol),

Table 3. Crystal data and details of the structure determinations.

	[ReO(L)]	[TcO(L)]	[ReO(Bnztrz-L)]	[TcO(Bnztrz-L)]
Formula	$C_{19}H_{22}N_5O_3ReS$	$C_{19}H_{22}N_5O_3STc$	$C_{26}H_{29}N_8O_3ReS$	$C_{26}H_{29}N_8O_3STc$
M_w [g mol^{-1}]	586.69	498.48	719.83	631.63
Crystal system	monoclinic	monoclinic	orthorhombic	orthorhombic
a [Å]	12.853(1)	12.832(2)	14.789(1)	14.856(2)
b [Å]	9.661(1)	9.683(1)	14.737(1)	14.732(1)
c [Å]	16.617(2)	16.592(2)	25.113(2)	25.092(2)
α [°]	90	90	90	90
β [°]	91.58(1)	91.22(1)	90	90
γ [°]	90	90	90	90
V [Å^3]	2062.6(4)	2061.1(5)	5473.3(7)	5491.6(9)
Space group	$P2_1/n$	$P2_1/n$	*Pbcn*	*Pbcn*
Z	4	4	8	8
$\varrho_{calcd.}$ [g cm^{-3}]	1.889	1.606	1.747	1.528
μ [mm^{-1}]	6.023	0.831	4.561	0.644
Absorption correction	integration	none	integration	integration
T_{min}/T_{max}	0.4858 / 0.8215	–	0.5373/0.7422	0.7280/0.9353
No. of reflections	15645	14288	45955	29023
No. of independent	5558	5498	5364	7396
No. parameters	264	264	354	348
R_1/wR_2	0.0619/0.0822	0.0476/0.0672	0.1019/0.0701	0.1069/0.0943
GOF	0.854	0.995	0.802	1.542
CCDC nos.	CCDC-1499406	CCDC-1499405	CCDC-1499408	CCDC-1499407

copper sulfate pentahydrate (24.8 mg, 0.1 mmol), and sodium ascorbate (39.6 mg, 0.2 mmol) in DMF/ethanol/water mixture (1:1:1, v/v/v, 3 mL) over a period of 3 h. The resin was filtered off and washed several times with water, DMF, ethanol, and dichloromethane. For cleavage of the bioconjugate the resin was treated with a mixture of trifluoroacetic acid/dichloromethane (95:5, v/v, 1 mL) for 3 h, filtered off, and washed several times with dichloromethane. The washing solution was added to the trifluoroacetic acid solution, and the solvents were removed under reduced pressure. The residue was analyzed by ESI mass spectrometry without further purification. Yield (crude product): 17 mg (ca. 0.01 mmol). ESI-TOF$^+$ (*m*/*z*): calcd. [M + H]$^+$ 1716.6544; found [M + H]$^+$ 1716.6488, [(M + 2 H)/2]$^+$ 858.8291. ESI-TOF$^-$ (*m*/*z*): calcd. [M – H]$^-$: 1714.6387; found 1714.6336.

X-ray Crystallography: The intensities for the X-ray determinations were recorded with a STOE IPDS 2T with Mo K_α radiation (λ = 0.71073 Å). Standard procedures were applied for data reduction and absorption correction. Structure solution and refinement were performed with SHELXS and SHELXL.[34] Hydrogen atom positions were calculated for idealized positions and treated with the "riding model" option of SHELXL. More details on data collections and structure calculations are summarized in Table 3. Additional information on the structure determinations has been deposited with the Cambridge Crystallographic Data Centre (Cambridge, UK).

CCDC 1499406 (for ReO(L)]), 1499405 (for [TcO(L)]), 1499408 (for [ReO(Bnztrz-L)]), and 1499407 (for [TcO(Bnztrz-L)]) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Keywords: Technetium · Rhenium · Click chemistry · Bioconjugation · Structure determination

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Bioconjugation

Ligands with propargyl-substituted thiocarbamoylbenzamidines form stable complexes with $\{^{99}TcO\}^{3+}$ and $\{ReO\}^{3+}$ cores. Prototype Click coupling products with benzylazide confirm the suitability of such units for orthogonal couplings. Successful conjugation of the rhenium compound with the peptide angiotensin-II was performed and represents a proof-of-principle solution for ongoing experiments with ^{99m}Tc.

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