"CLICK TO CHELATE": SYNTHESIS AND INSTALLATION OF METAL CHELATORS INTO BIOMOLECULES IN A SINGLE STEP

Thomas L. Mindt[1], Harriet Struthers[2], Luc Brans[3], Todor Anguelov[2], Christian Schweinsberg[2], Veronique Maes[3], Dirk Tourwé[3], Roger Schibli[1]

[1] Dept. of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland

[2] Center for Radiopharmaceutical Science ETH-PSI-USZ, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland

[3] Faculty of Sciences Vrije Universiteit Brussels 1050, Brussels, Belgium

* Corresponding author:
Roger Schibli
Dept. of Chemistry and Applied Biosciences of the ETH Zürich, 8093 Zürich, Switzerland
Phone: +41-56-310 2837; fax: +41-56-310 2849; e-mail: roger.schibli@pharma.ethz.ch

Table of Contents

1 Synthesis of triazole ligand systems

1.1 General methods

Melting points were taken on a Büchi-535 apparatus and are uncorrected. Infrared spectra were recorded on either a Jasco FT/IR-6200 ATR-IR or a Perkin Elmer Spectrum BX II FT-IR, with a Pike MIRacle(TM) ATR accessory. Nuclear magnetic resonance spectra were recorded with a Bruker 400 MHz or 300 MHz Varian Gemini 2000 spectrometer with the corresponding solvent signals as an internal standard. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (0.00 ppm). Values of the coupling constant, *J*, are given in Hertz (Hz); the following abbreviations are used in the experimental section for the description of ^{1}H-NMR spectra: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), broad singlet (bs). The chemical shifts of complex multiplets are given as the range of their occurrence. Further assignment of NMR-signals was achieved using D_2O-exchange experiments and two-dimensional NMR experiments when appropriate (COSY, NOESY, HMQC and HMBC). Low resolution mass spectra (LR-MS) were recorded with a Micromass Quattro micro^{TM} API LC-ESI or an LCT Premier ESI-TOF from Waters, using either the negative or positive ionization mode. High resolution mass spectra (HR-MS) were recorded with a Bruker FTMS 4.7T BioAPEXII (ESI) or an Ionspec Ultima FTMS 4.7T (MALDI). Optical rotation values were measured in a 0.5 mL cell using a Jasco P-1020 polarimeter.

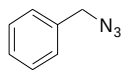
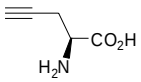
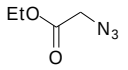
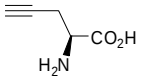
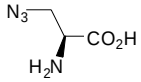
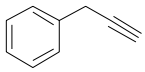
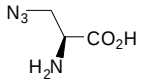
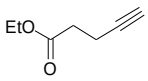
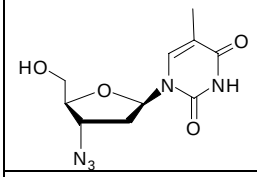
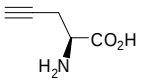
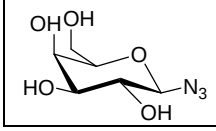
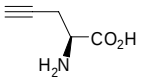
$[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared using the Isolink^{TM}-kit (Mallinkrodt-Tyco, Petten, the Netherlands). $[Na][^{99m}TcO_4]$ was eluted from a $^{99}Mo/^{99m}Tc$-generator (Mallinckrodt-Tyco, Petten) with a 0.9% saline solution. Commercial Boc-propargylglycine dicyclohexylamine salt (Aldrich) was dissolved in ethyl acetate and washed with citric acid (1M) and brine. The organic extract was dried over Na_2SO_4 and evaporated under reduced pressure to yield the free acid for click reactions. Sep-Pak® columns (Waters) were washed with methanol and water prior to use. $[Re(Br)_3(CO)_3][Et_4N]_2$, pentanoic acid ethyl ester and L-azido-alanine (**2**) were prepared according to literature procedures[1] QuadraPure-IDA^{TM} metal scavenging resin (Aldrich) was washed well with methanol prior to use. All other commercially available reagents and solvents were used as supplied unless stated otherwise.
Reactions were monitored by thin layer chromatography (TLC, performed on EM Science 0.25 mm thick, pre-coated silica gel 60 F-254 glass supported plates) or HPLC. HPLC was performed on a Merck-Hitachi L-7000 system equipped with an L-7400 tunable absorption detector and a Berthold LB 506 B radiometric detector. Analytical HPLC was performed with either an XTerra® column (MSC18, 5µm, 4.6 x 150 mm, Waters) or a Nucleosil® 5 C18 column (5µm, 4.6 x 250 mm, Macherey-Nagel). An XBridge^{TM} column (Prep C18, 5µm, 10 x 150 mm, Waters) was used for semi-preparative HPLC. HPLC solvent system **I**: Aqueous 0.05 M triethylammonium phosphate buffer, pH 2.25 (solvent A), methanol (solvent B). The HPLC system started with 95% A, with a linear gradient to 20% A and 80% B over 15 min, followed by 5 min of 100% A, with a flow rate of 1 ml/min. System **II**: Aqueous 0.05 M triethylammonium phosphate buffer, pH 2.25 (solvent A), methanol (solvent B). 0 min, 95% A, 1 mL/min; 3 min 95% A, 1 mL/min; 6 min, 75% A, 1 mL/min; 9 min, 67% A, 1 mL/min; 20 min, 0% A, 1 mL/min; 22 min, 0% A, 2 mL/min; 25 min 95% A, 2 mL/min; 30 min, 95% A, 1 mL/min. System **III**: 90% water, 10% acetonitrile, 0.1% trifluoroacetic acid (solvent A), acetonitrile (solvent B). 0 min, 95% A, 1 mL/min; 12 min, 10% A, 1 mL/min; 15 min, 10% A, 1 mL/min; 19 min, 95% A, 1 mL/min.

1.2 Synthesis of triazole ligands with unprotected click substrates

All investigated click reactions proceeded quantitatively with unprotected substrates (determined by HPLC or ^{1}H-NMR, see Table 1 below). For ease of purification and chemical characterization of some of the compounds they were synthesized in 2-3 steps via protected click substrates (see Section 1.3).

[1] R. Alberto, A. Egli, U. Abram, K. Hegetschweiler, V. Gramlich, P.A. Schubiger, *J. Chem. Soc. Dalton. Trans.* **1994**, 2815; S.B. Rosenblum, T. Huynh, A. Afonso, H.R. Davis Jr., *Tetrahedron* **2000**, *56*, 5735; A.J. Link, M. K.S Vink, D.A. Tirrell, *J. Am. Chem. Soc*. **2004**, *126*, 10598.

Table 1: Conversion of click substrates after 45 min at 75 °C: [a)] 25 mM in D_2O[2], conversion of substrates determined by ^{1}H-NMR; [b)] click products, which precipitated from the reaction mixture were dissolved for NMR measurements by adding a few drops of 10% DCl and gently heating; [c)] 0.2 mM in water, conversion of substrates determined by HPLC.

Azide substrate	Alkyne substrate	Product (% conversion)
 **3a**	 **1**	**5** (>95 %[a,b])
 **3b**	 **1**	**6** (>95 %[a])
 **2**	 **4a**	**7** (>95 %[a,b])
 **2**	 **4b**	**8** (>95 %[a])
	 **1**	**10** (>95%[c])
	 **1**	**11** (>95 %[a])

[2] Reactions in D_2O yielded triazole products bearing a Deuterium atom at the triazole heterocycle. This is presumably a result of proteolysis of the click intermediates (Rodionov, V. O.; Fokin V.V.; Finn F. G. *Angew. Chem. Int. Ed.* **2005**, *44*, 2210-2215).

Selected example of click product formation with unprotected substrates

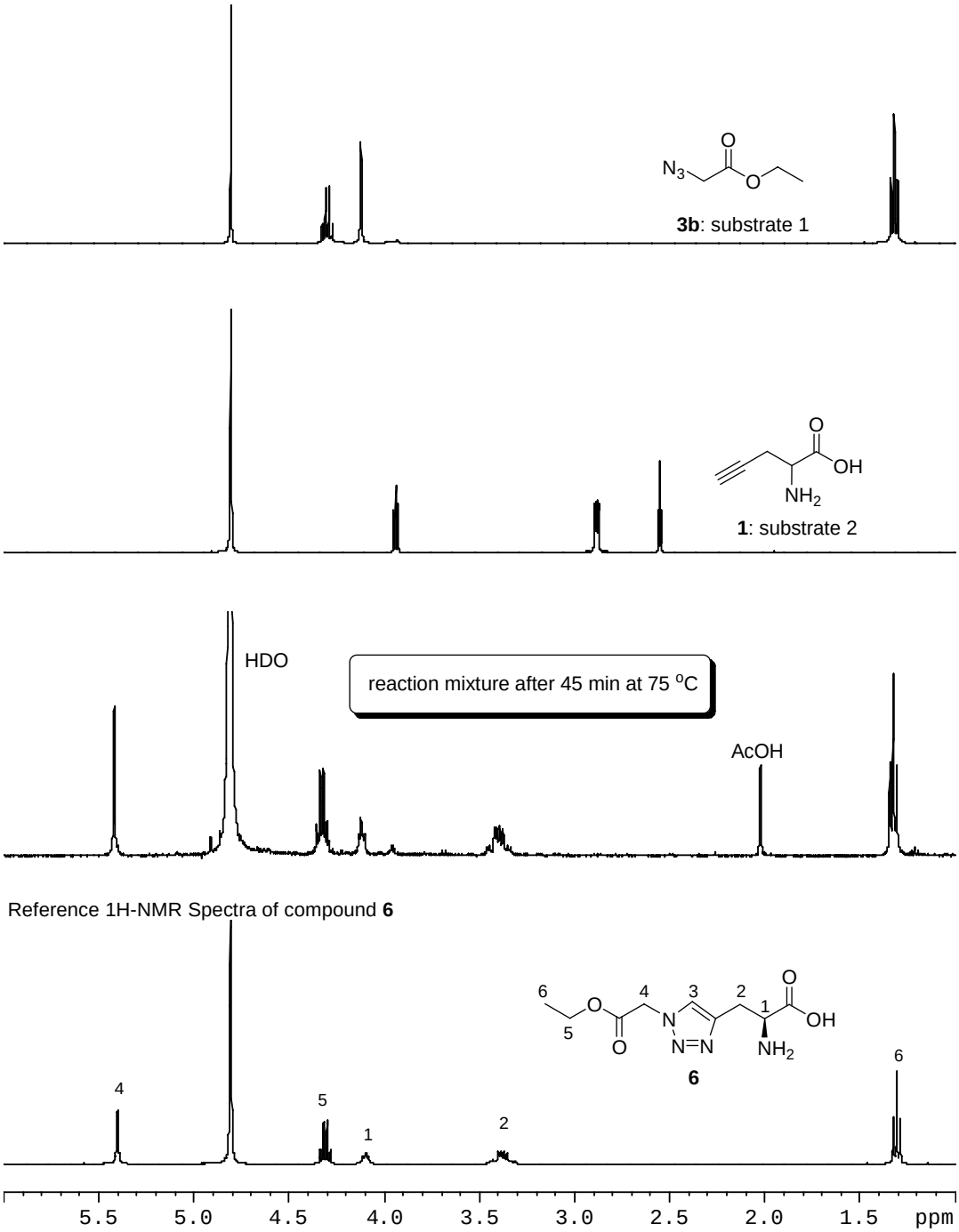
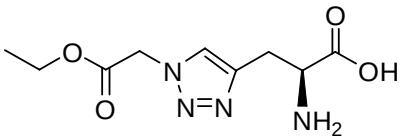
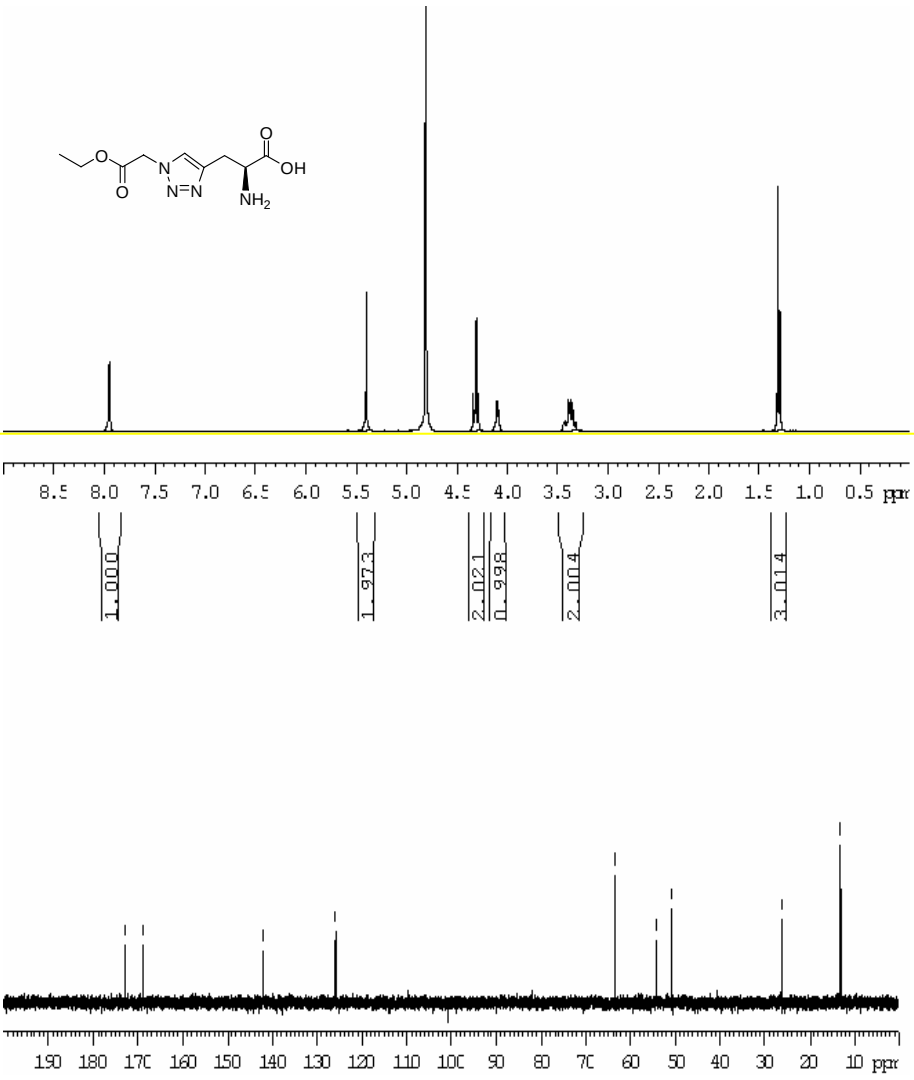


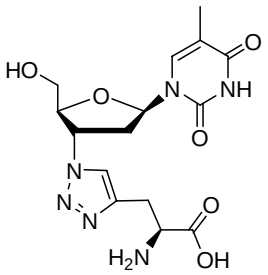
Figure 1. [1]H-NMR spectrum showing formation of compound **6** from L-propargyl glycine **1** and azide **3b**.



Compound 6. Azidoacetic acid ethyl ester (129 mg, 1.0 mmol), L-propargyl glycine (113 mg, 1.0 mmol), copper (II) acetate (18 mg, 0.1 mmol) and sodium ascorbate (40 mg, 0.2 mmol) were mixed in *t*-butanol / water (1:1; 6.0 mL) and stirred at rt overnight. QuadraPure-IDA® resin (0.2 g) was added and the mixture was gently shaken at rt for 2 h during which the blue color of the solution faded. The resulting brown solution was decanted and added drop wise to ethanol (100 mL). Filtration at 0 °C yielded compound **6** as a white powder (220 mg, 91%): mp 272-274 °C; IR (neat) ν 3126, 2980, 2909, 1745, 1577, 1491, 14.09, 1220, 1197, 1061 cm^{-1}; ^{1}H-NMR (D_2O) δ 7.95 (s, 1H), 5.40 (s, 2H), 4.31 (q, 2H, *J* = 7.2 Hz), 4.10 (t, 1H, *J* = 6.4 Hz), 3.39 (dd, 1H, *J* = 15.7 and 4.9 Hz), 3.36 (dd, 1H, *J* = 15.7 and 7.1 Hz), 1.31 (t, 3H, *J* = 7.2 Hz) ppm; ^{13}C-NMR (D_2O) δ 173.1, 169.0, 142.1, 125.9, 63.4, 54.3, 51.0, 26.3, 13.2 ppm; HiRes-ESI-MS: $[M+H]^+$ = 243.109 (calc. for $C_9H_{15}N_4O_4$: 243.109); elemental analysis (calculated %-values in parenthesis) C 44.51 (44.63), H 5.70 (5.83), N 22.88 (23.13), O 26.52 (26.42); $[\alpha]_D^{20}$ = -10.5 (c=1.0 in H_2O).

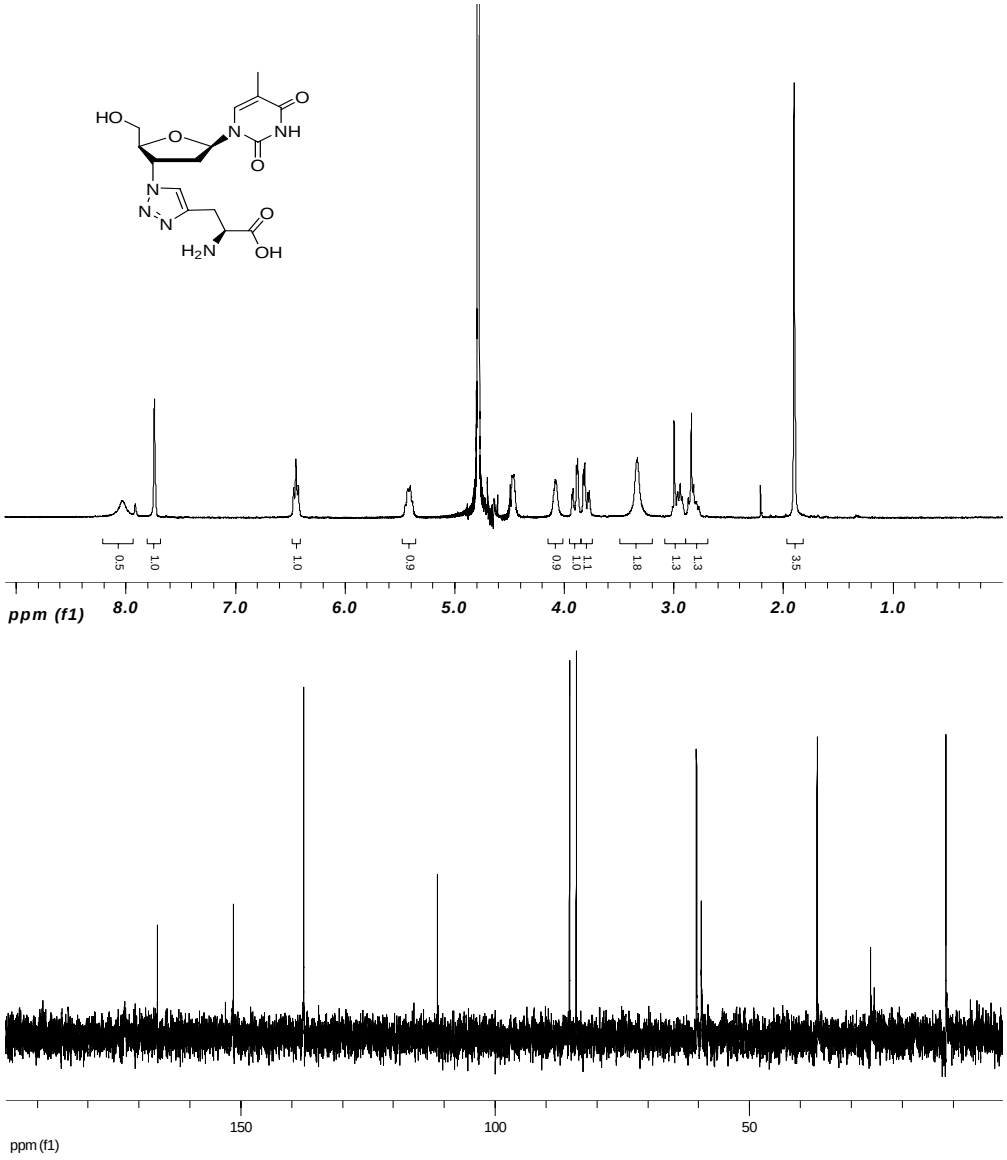
^{1}H- and ^{13}C-NMR of compound 6, D_2O

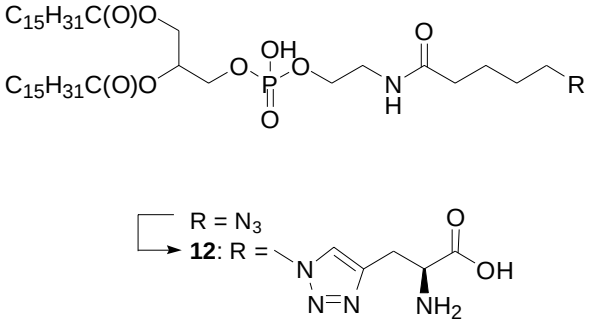




Compound 10. 3-Azidothymidine (30.0 mg, 0.112 mmol), L-propargyl glycine (13.0 mg, 0.115 mmol), copper (II) acetate (0.8 mg, 0.004 mmol) and sodium ascorbate (2.2 mg, 0.011 mmol) were mixed in *t*-butanol / water (1:1; 0.5 mL) and stirred at rt overnight. Water (0.5 ml) and QuadraPure-IDA® resin (50 mg) were added and the mixture was gently shaken at 50°C for 2 h during which time the blue color of the solution disappeared. The solution was added drop wise to methanol (5 mL). Filtration of the resulting suspension yielded compound **11** (34.8 mg, 82 %) as a white solid: mp >190 °C (decomp.); IR (neat) ν 3535, 3426, 3136, 3018, 2990, 2930, 2880, 2815, 2750, 1690, 1665, 1652, 1628, 1598, 1538, 1473, 1410, 1388, 1345, 1306, 1272, 1255, 1235, 1214, 1150, 1093, 1047, 1020, 1000, 955, 929, 902, 882, 857, 785, 761, 669, 614 cm^{-1}; ^{1}H-NMR (D_2O) δ 8.03 (s, 1H), 7.74 (s, 1H), 6.45 (t, *J* = 6.5 Hz, 1H), 5.48 – 5.36 (dd, 1H), 4.79 - 4.77 (m, 1H), 4.50 - 4.43 (m, 1H), 4.08 (s, 1H), 3.90 (dd, *J* = 12.6 and 3.0 Hz, 1H), 3.80 (dd, *J* = 12.6 and 4.2 Hz, 1H), 3.33 (s, 1H), 2.96 (dd, *J* = 14.6, 7.5 and 4.5 Hz, 1H), 2.82 (ddd, 1H, *J* = 14.6, 8.6 and 6.1 Hz), 1.90 (s, 3H) ppm; ^{13}C-NMR (D_2O) δ 185.2, 166.4, 151.4, 137.6, 111.3, 99.9, 85.4, 84.1, 60.4, 59.5, 36.7, 26.2, 21.8, 11.4 ppm; HR-MS: $[M-H]^-$ = 379.1370 (calc. for $C_{15}H_{19}N_6O_6$: 379.1372).

^{1}H- and ^{13}C-NMR of compound 10, D_2O





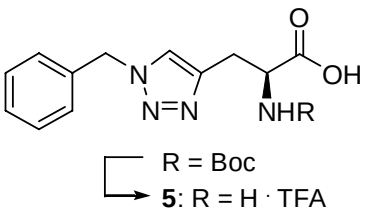
Azido-phospholipid intermediate: 5-Azido-pentanoicacid-NHS-ester [3] (48 mg, 0.2 mmol) was dissolved in DMF (1.0 mL) and a solution of 1,2-dipalmitoyl-R/S-glycero-3-phosphoethanolamine (124 mg, 0.18 mmol) and triethylamine (50 µL, 36 mg, 0.36 mmol) in $CHCl_3$ / MeOH (2:1; 9.0 mL) was added. The resulting mixture was stirred at rt overnight and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with mixtures of CH_2Cl_2/MeOH (10:1→4:1) to afford the azido phospholipid intermediate as a white wax (125 mg, 85 %): mp 80-120 °C; IR (neat) ν 3385, 2919, 2855, 2096 (N_3), 1735, 1642, 1459, 1237, 1108, 1066 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.55-5.50 (bs, 2H), 4.25-3.30 (m, 7H), 3.27 (t, 2H, *J* = 6.5 Hz), 2.50-2.20 (m, 7H), 1.75-1.50 (m, 7H), 1.23 (s, 50H), 0.86 (t, 6H, *J* = 7.0 Hz) ppm; ^{31}P-NMR ($CDCl_3$) δ -2.47 ppm; LRLC-MS: $[M+H]^+$ = 817.60 (calc. for $C_{42}H_{81}N_4O_9P$: 817.11).

Compound 12. Azido-phospholipid (100mg, 0.12 mmol), propargyl glycine (14 mg, 0.12 mmol), copper (II) acetate (5 mg, 0.02 mmol) and sodium ascorbate (10 mg, 0.05 mmol) were mixed in *t*-butanol / water (1:1; 1.5 mL) and stirred at 50 °C for 8 hours. The resulting green solution was filtered and added to acetonitrile (100 mL). Filtration at 0 °C gave a green solid which was dissolved in hot THF (30 mL) and filtered through Celite. Treatment of the blue-green solution with QuadraPure-IDA® resin (0.5 g) at rt for 4 days resulted in a pale yellow solution. Filtration through Celite® and concentration under reduced pressure yielded compound **12** as a colorless oil (65 mg, 60 %): IR (neat) ν 3299, 2923, 2855, 1735, 1652, 1054 cm^{-1}; ^{31}P-NMR ($CDCl_3$): δ -2.29 ppm; LRLC-MS: $[M+H]^+$ = 930.60 (calc. for $C_{42}H_{81}N_4O_9P$: 930,23).

[3] T.S, Seo, Z. Li, H. Ruparel, J. Ju, *J. Org. Chem.* **2003**, *68*, 609.

1.3 Synthesis of triazole ligands using Boc-protected substrates

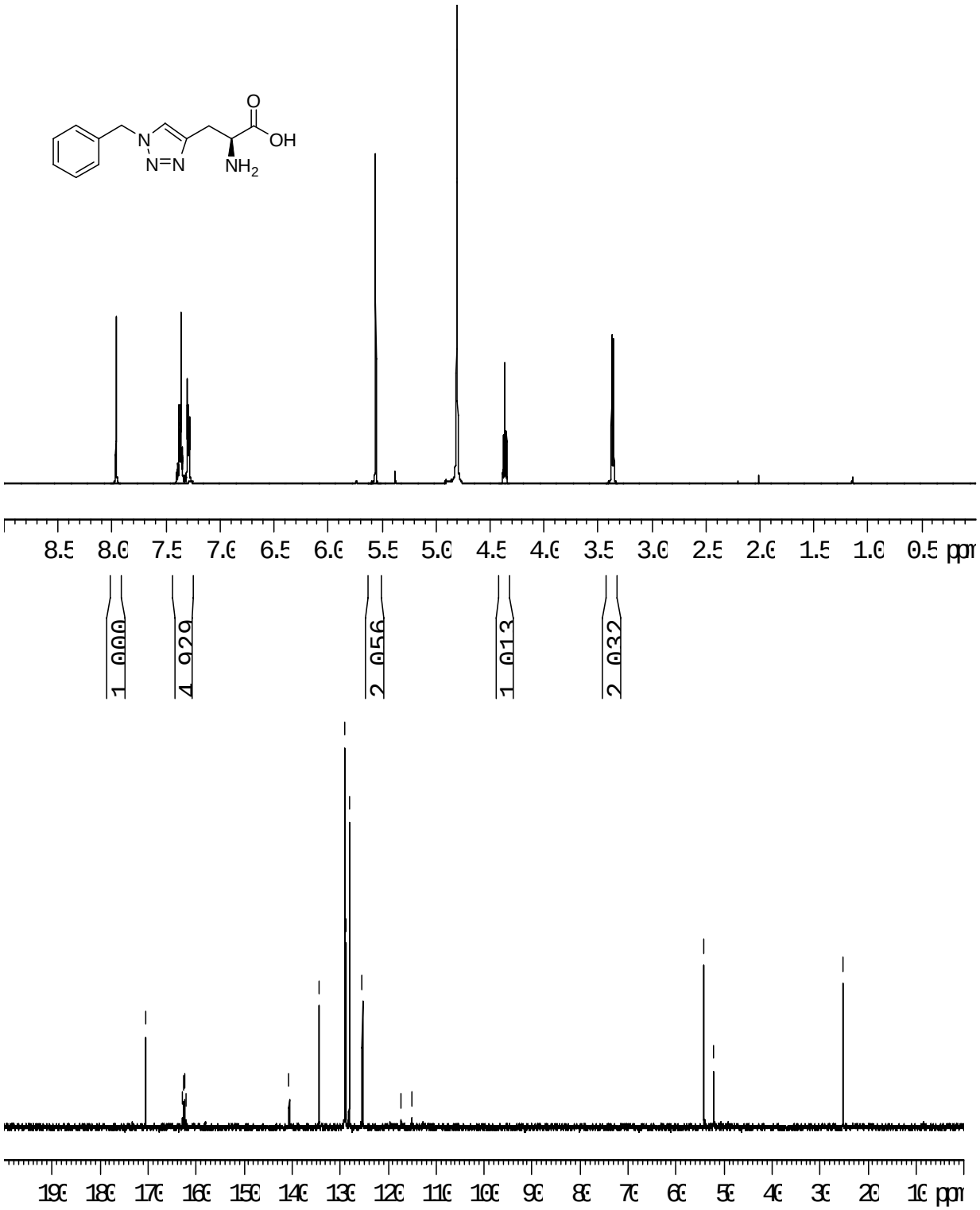
Compounds **5, 7, 8** and **11** were prepared using either N^{α}-Boc-propargyl-L-glycine or N^{α}-Boc-azido-L-alanine**,** to facilitate isolation of the triazole ligands. Unlike the water soluble deprotected triazoles, Boc-protected intermediates could be readily isolated by extraction und purified by flash chromatography on silica gel.

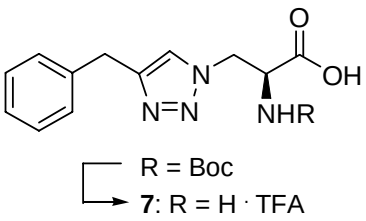


N(α)-Boc-5. Benzylazide (53 mg, 0.4 mmol), N(α)-Boc-L-propargylglycine (85 mg, 0.4 mmol) copper (II) acetate (7 mg, 0.04 mmol) and sodium ascorbate (16 mg, 0.08 mmol) were mixed in *t*-butanol / water (1:1; 3.0 mL) and stirred at rt overnight. The resulting green solution was diluted with ethyl acetate (5 mL) and washed with brine (2 x 5 mL). The aqueous solutions were extracted with ethyl acetate (2 x 5 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel with mixtures of CH_2Cl_2 / MeOH (4:1→2:1) to afford the Boc-protected intermediate as a pale yellow solid (86 mg, 62%): mp >170 °C (decomp.); IR (neat) ν 3359, 2977, 2927, 1691, 1562, 1402, 1051 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.74 (s, 1H), 7.36-7.28 (m, 5H), 5.55 (s, 2H), 4.30 (bs, 1H), 3.27-3.01 (m, 2H), 1.36 (s, 9H) ppm; ^{13}C-NMR (CD_3OD) δ 157.6, 136.8, 130.0, 129.5, 129.0, 124.8 (broad), 80.4, 62.7, 54.9, 30.2, 28.7 ppm (one carbon (carbonyl) not observed); LRLC-MS: $[M+H]^+$ = 347.05 (calc. for $C_{17}H_{22}N_4O_4$: 346.38); $[\alpha]_D^{20}$ = +11.0 (c=0.9 in $CHCl_3$).

Compound 5. N(α)-Boc-5 (65 mg, 0.19 mmol) was dissolved in CH_2Cl_2 / TFA (2:1; 2.0 mL) and stirred at rt overnight. Concentration under reduced pressure followed by repeated dissolving of the residue in MeOH and evaporation under reduced pressure provided compound **5** as a hygroscopic, white solid (68 mg, 98%): mp >195 °C (decomp.); IR (neat) ν 3130, 2930, 2859, 1674, 1592, 1198, 1137, 718 cm^{-1}; ^{1}H-NMR (D_2O containing 0.5% DCl) δ 7.96 (s, 1H), 7.40-7.25 (m, 5H), 5.56 (s, 2H), 4.36 (t, 1H, *J* = 6.2 Hz), 3.37 (d, 2H, *J* = 6.2 Hz) ppm; ^{13}C-NMR (D_2O containing 0.5% DCl) δ 170.4, 162.5 (q, *J* = 36.0 Hz, TFA), 140.6, 134.4, 129.1, 128.8, 128.1, 125.4, 116.1 (q, *J* = 291.8 Hz, TFA), 54.2, 52.2, 25.3 ppm; LRLC-MS: $[M+H]^+$ = 247.06 (calc. for $C_{12}H_{14}N_4O_2$: 246.11); HR-MALDI-MS $[M+H]^+$ = 247.1185 (calc. for $C_{12}H_{14}N_4O_2$: 246.11); elemental analysis (calculated %-values for $C_{12}H_{14}N_4O_2(C_2HF_3O_2)_{0.2}$ in parenthesis) C 55.65 (55.35), H 5.69 (5.32), N 21.17 (20.82).

^{1}H- and ^{13}C-NMR of compound 5, 0.5% DCl

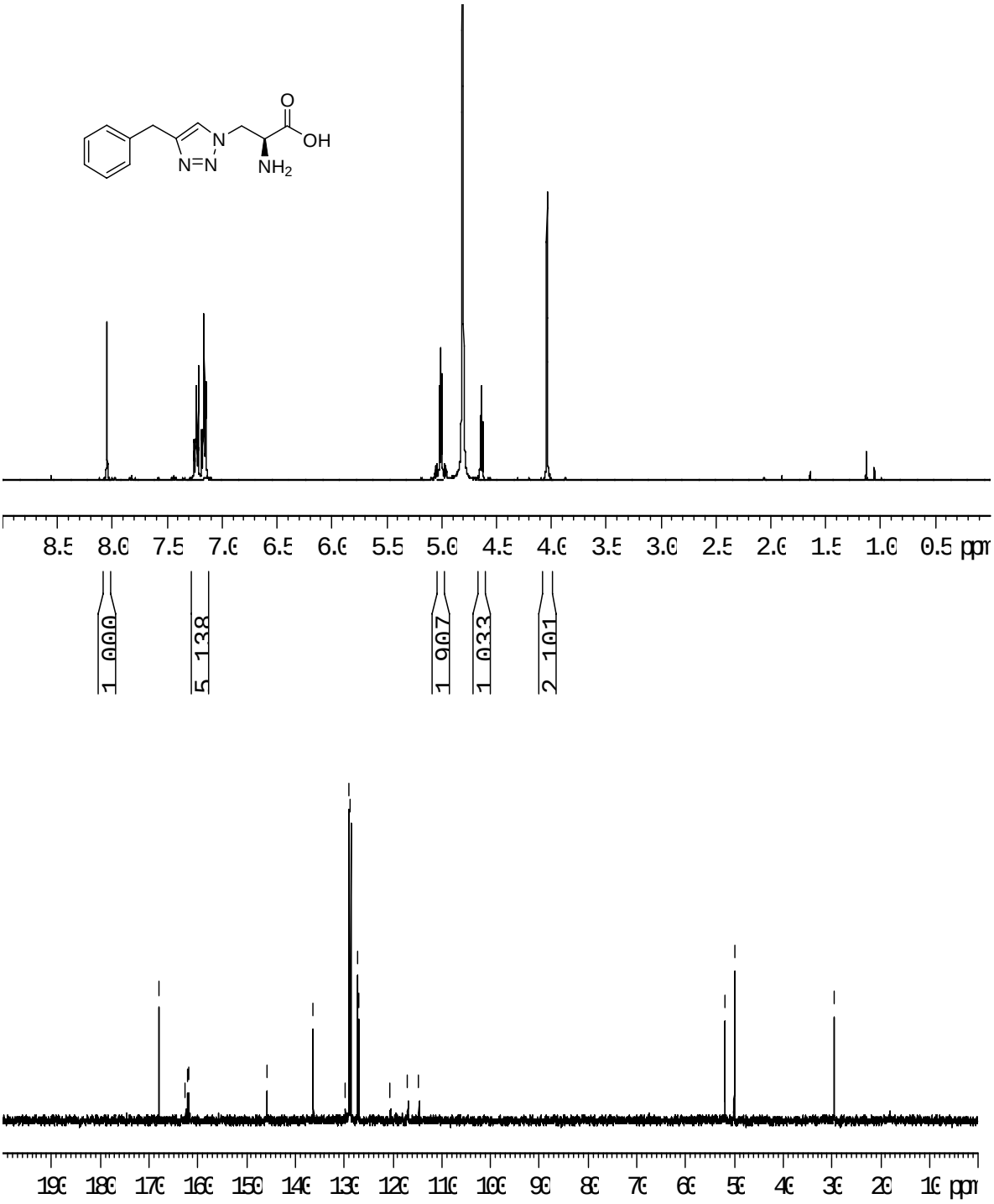


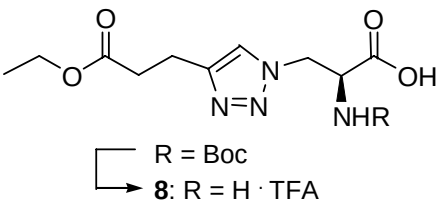


N(α)-Boc-7. 3-Phenyl-1-propyne (93 µL, 87 mg, 0.75 mmol), N(α)-Boc-L-azidoalanine (173 mg, 0.75 mmol) copper (II) acetate (14 mg, 0.08 mmol) and sodium ascorbate (30 mg, 0.15 mmol) were mixed in *t*-butanol / water (1:1; 6.0 mL) and stirred at rt overnight. The resulting green solution was diluted with ethyl acetate (10 mL) and washed with brine (2 x 10 mL). The aqueous solutions were extracted with ethyl acetate (2 x 5 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel with mixtures of CH_2Cl_2 / MeOH (4:1→2:1) to afford the Boc protected intermediate as a pale yellow solid (177 mg, 68%): mp >190 °C (decomp.); IR (neat) ν 3206, 2980, 1688, 1602, 1368, 1190, 1151, 1066 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.59 (s, 1H), 7.35-7.25 (m, 5H), 4.91-4.79 (m, partly covered by HDO signal, 1H, *J* = 4.2 Hz), 4.62 (dd, 1H, *J* = 13.6 and 7.1 Hz), 4.38 (dd, 1H, *J* = 7.1 and 4.2 Hz), 4.00 (s, 2H), 1.33 (s, 9H) ppm; ^{13}C-NMR (CD_3OD) δ 157.5, 140.3, 131.2, 129.7, 129.6, 127.5, 125.1 (broad), 101.5, 80.6, 52.9, 32.6, 28.7 ppm. (One carbon (carbonyl) not observed); LRLC-MS: $[M+H]^+$ = 347.02 (calc. for $C_{17}H_{22}N_4O_4$: 346.38); $[\alpha]_D^{20}$ = +24.7 (c=0.9 in $CHCl_3$).

Compound 7. N(α)-Boc-7 (113 mg, 0.33 mmol) was deprotected in CH_2Cl_2 / TFA (2:1; 3.0 mL) by the procedure described for compound **5** to give a hygroscopic, white solid (**7**, 110 mg, 93%): mp >220 °C (decomp.); IR (neat) ν 3363, 2977, 1710, 1674, 1198, 721 cm^{-1}; ^{1}H-NMR (D_2O containing 0.5% DCl) δ 8.05 (s, 1H), 7.29-7.13 (m, 5H), 5.03 (dd, 1H, *J* = 15.3 and 5.4 Hz), 4.99 (dd, 1H, *J* = 15.3 and 4.5 Hz), 4.64 (dd, 1H, *J* = 5.4 and 4.5 Hz) 4.04 (s, 2H) ppm; ^{13}C-NMR (D_2O containing 0.5% DCl) δ 167.9, 162.1 (q, *J* = 37.0 Hz, TFA), 145.8, 129.0, 128.7, 127.3, 127.0, 115.8 (q, *J* = 289.8 Hz, TFA), 51.9, 50.0, 29.5 ppm; LRLC-MS: $[M+H]^+$ = 247.06 (calc. for $C_{12}H_{14}N_4O_2$: 246.27); HR-MALDI-MS 247.1185 = $[M+H]^+$ (calc. for $C_{12}H_{14}N_4O_2$: 246.11); elemental analysis (calculated %-values for $C_{12}H_{14}N_4O_2(C_2HF_3O_2)_{0.08}$ in parenthesis) C 57.59 (57.19), H 5.73 (5.56), N 22.12 (21.94).

^{1}H- and ^{13}C-NMR of compound 7, 0.5% DCl

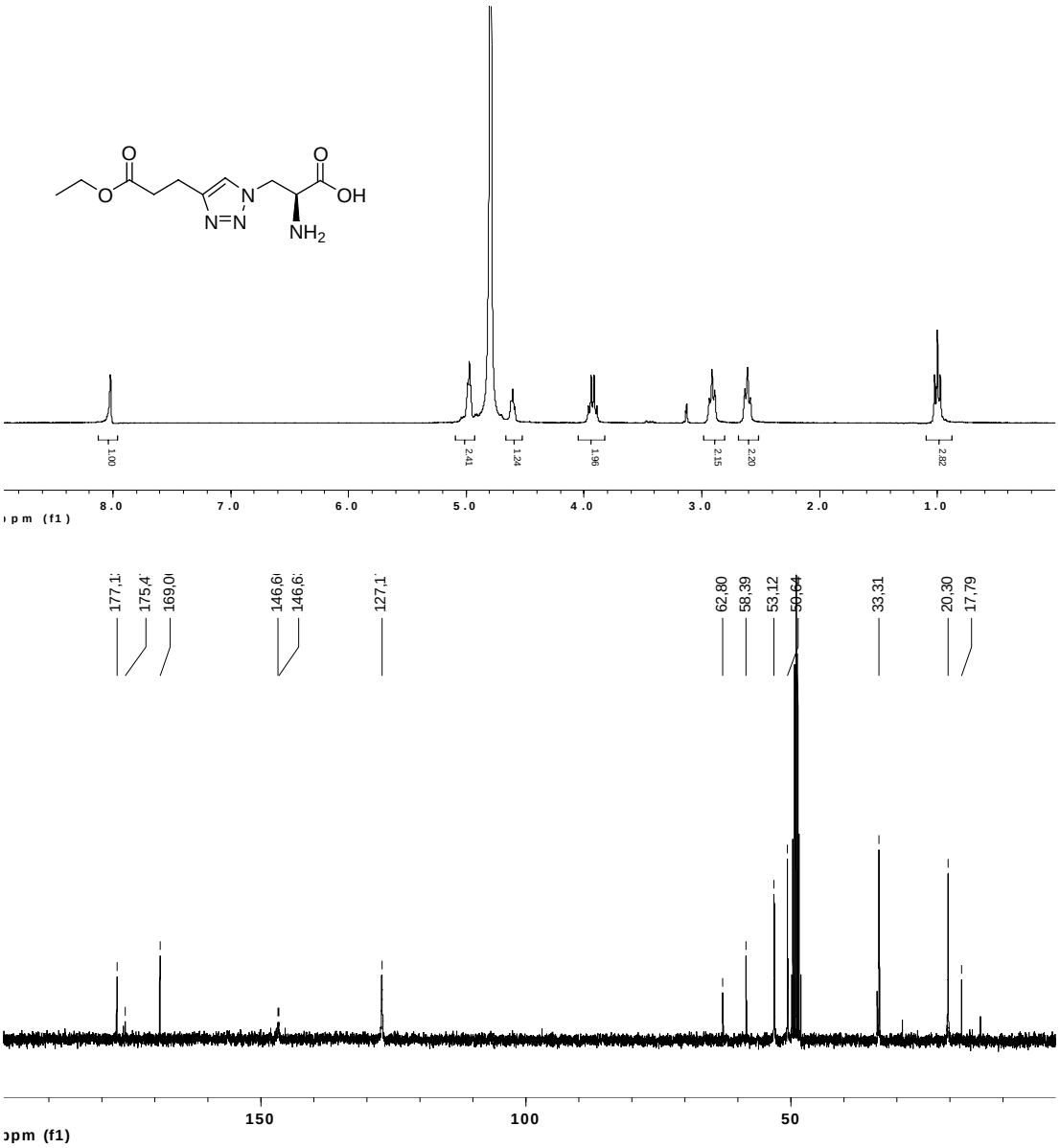


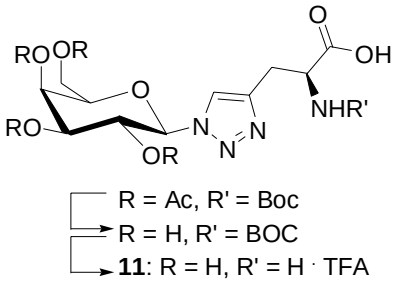


N(α)-Boc-8. Pent-4-ynoic acid ethyl ester (**4b**, 202 mg, 1.60 mmol), N(α)-Boc-L-azidoalanine (369 mg, 1.60 mmol) copper (II) acetate (29 mg, 0.16 mmol) and sodium ascorbate (63 mg, 0.32 mmol) were mixed in *t*-butanol / water (1:1; 12 mL) and stirred at rt overnight. The resulting green solution was diluted with ethyl acetate (10 mL) and washed with brine (2 x 10 mL). The aqueous solutions were extracted with ethyl acetate (2 x 10 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel with mixtures of CH_2Cl_2 / MeOH (5:1→2:1) to afford the Boc-protected intermediate as a pale yellow oil (233 mg, 41%): IR (neat) ν 3382, 2984, 1694, 1381, 1233, 1192, 1155, 1062, 951 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.71 (s, 1H), 4.88-4.82 (m, 1H), 469-4.59 (m, 2H), 4.12 (q, 2H, *J* = 7.1 Hz), 2.98 (t, 2H, *J* = 7.3 Hz), 2.69 (t, 2H, *J* = 7.3 Hz), 1.40 (s, 9H), 1.23 (t, 3H, *J* = 7.1 Hz) ppm; ^{13}C-NMR (CD_3OD) δ 174.1, 157.5, 124.3 (broad), 123.4, 119.2, 80.7, 61.7, 52.6, 34.5, 28.7, 21.8, 14.5 ppm (one carbon (carbonyl) not observed); LR-ES 357.10 = $[M+H]^+$ (calc. $C_{15}H_{24}N_4O_6$: 356.17); $[\alpha]_D^{20}$ = +8.9 (c=1.0 in MeOH).

Compound 8. N(α)-Boc-8 (200 mg, 0.56 mmol) was dissolved in CH_2Cl_2 / TFA (9:1; 10 mL) and stirred at rt overnight. After concentration under reduced pressure followed by repeated dissolving of the residue in MeOH and evaporation under reduced pressure, compound **8** precipitated as a white solid (62 mg, 43%): mp >200 °C (decomp.); IR (neat) ν 3062, 1728, 1622, 1580, 1483, 1438, 1402, 1323, 1164, 1054, 865 cm^{-1}; ^{1}H-NMR (D_2O/DCl) δ 8.01 (s, 1H), 4.99 (t, 2H, *J* = 4.9 Hz), 4.62 (t, 1H, *J* = 4.9 Hz), 3.96 (q, 2H, *J* = 7.1 Hz), 2.94 (t, 2H, *J* = 7.0 Hz), 2.64 (t, 2H, *J* = 7.0 Hz), 1.04 (t, 3H, *J* = 7.1 Hz) ppm; ^{13}C-NMR (D_2O/DCl/CD_3OD) δ 177.2, 175.5, 169.0, 146.6 (broad), 127.2, 62.8, 58.4, 53.1, 33.3, 20.3, 17.8 ppm; HR-MS 257.1244 = $[M+H]^+$ (calc. for $C_{10}H_{17}N_4O_4$: 257.1244); $[\alpha]_D^{20}$ = + 4.1 (c=1.0 in HCl/H_2O).

^{1}H- and ^{13}C-NMR of compound 8, 1% DCl



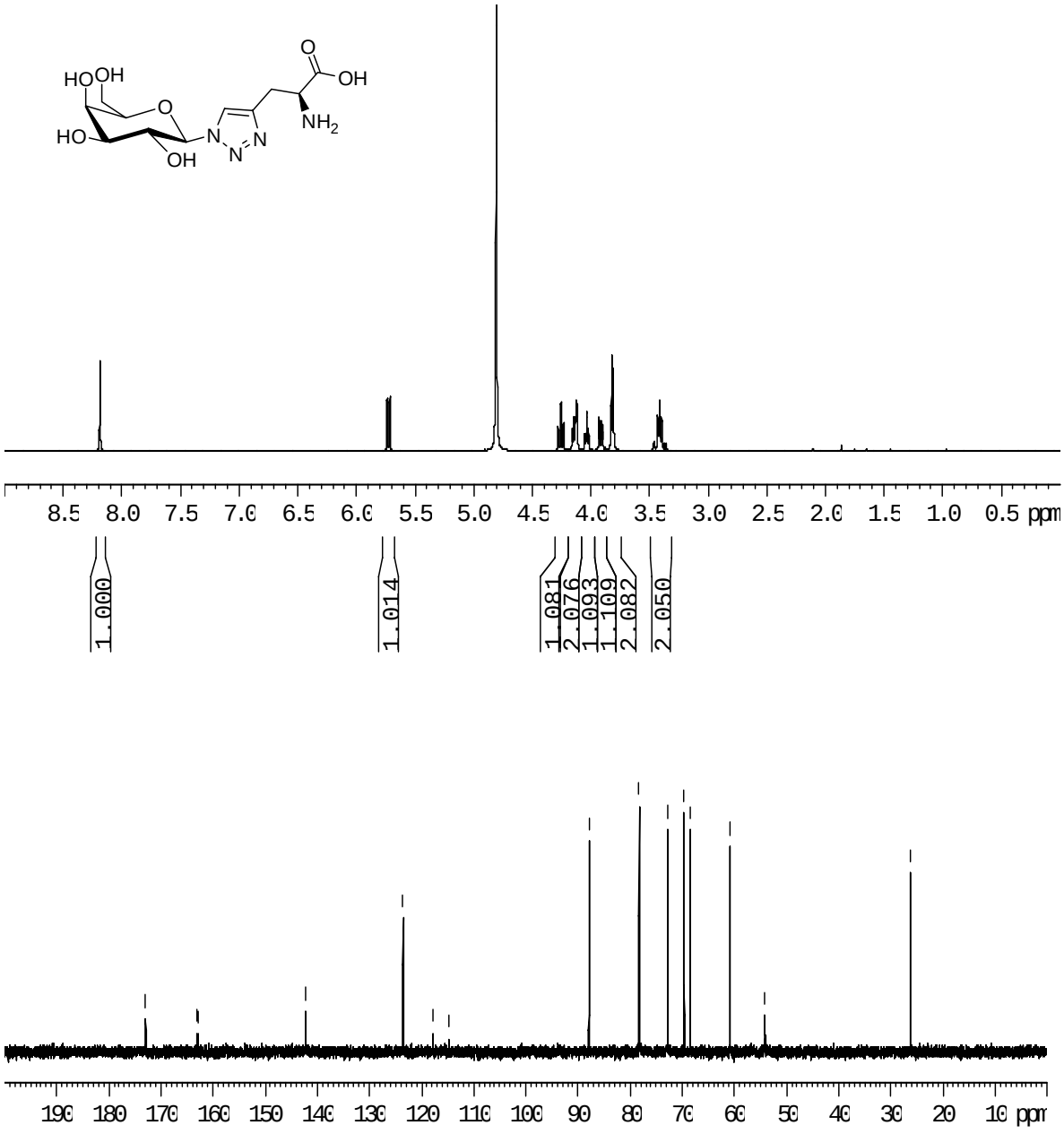


N(α)-Boc-11-tetraacetate. 1-Azido-1-deoxy-*β*-D-galactopyranoside tetraacetate (187 mg, 0.5 mmol), N(α)-Boc-L-propargylglycine (106 mg, 0.5 mmol) copper (II) acetate (9 mg, 0.05 mmol) and sodium ascorbate (20 mg, 0.10 mmol) were mixed in *t*-butanol / water (1:1; 4.0 mL) and stirred at rt overnight. The resulting green solution was diluted with ethyl acetate (10 mL) and washed with brine (2 x 10 mL). The aqueous solutions were extracted with ethyl acetate (2 x 5 mL) . The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel with mixtures of CH_2Cl_2 / MeOH (10:1→4:1) to afford N(α)-Boc-12-tetraacetate as a white solid (217 mg, 74%): mp >190 °C (decomp.);IR (neat) ν 3406, 2977, 2934, 1752, 1684, 1588, 1395, 1366, 1215, 1254 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.95 (s, 1H), 6.06 (d, 1H, *J* = 9.2 Hz), 5.65 (t, 1H, *J* = 9.8 Hz), 5.55 (d, 1H, *J* = 2.7 Hz), 5.41 (dd, 1H, *J* = 10.3 and 3.4 Hz), 4.46 (t, 1H, *J* = 6.5 Hz), 4.30- 4.15 (m, 2H), 4.12 (dd, 1H, *J* = 11.4 and 6.9 Hz), 3.38-3.25 (m, partly covered by CD_3OD signal, 1H, *J* = 5.0 Hz), 3.15 (dd, 1H, *J* = 14.8 and 6.6 Hz), 2.21 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.86 (s, 3H), 1.42 (s, 9H) ppm; ^{13}C-NMR (CD_3OD) δ 172.0, 171.9, 171.3, 170.6, 158.3, 146.5, 123.4, 87.0, 80.4, 75.0, 72.4, 69.6, 68.7, 62.6, 49.0, 29.6, 28.8, 20.6, 20.5, 20.4, 20.2 ppm (one quaternary carbon is not visible); LRLC-MS: $[M+H]^+$ = 587.12 (calc. for $C_{24}H_{34}N_4O_{13}$: 586.55).

N(α)-Boc-11. Tetraacetate (152 mg, 0.26 mmol) was dissolved in methanol (2.0 mL) and a catalytic amount of sodium methoxide (1.4 mg, 0.03 mmol) was added. The solution was stirred at rt overnight and then concentrated under reduced pressure to yield N(α)-Boc-12 as a white solid (107 mg, 98%): mp >110 °C (decomp.); IR (neat) ν 3345, 2980, 2930, 1681, 1592, 1398, 1162, 1090, 1054, 886 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.99 (s, 1H), 5.54 (d, 1H, *J* = 8.9 Hz), 4.27 (bs, 1H), 4.17 (t, 1H, *J* = 9.1 Hz), 4.09 (bs, 1H), 3.90-3.18 (m, 4H), 3.29-3.07 (m, 2H), 1.29 (s, 9H) ppm; ^{13}C-NMR (CD_3OD) δ 179.4, 157.8, 145.5, 123.4, 90.4, 80.7, 79.9, 75.3, 71.6, 70.5, 62.5, 56.6, 29.9, 28.9 ppm; LRLC-MS: $[M+H]^+$ = 419.06 (calc. for $C_{16}H_{26}N_4O_9$: 418.40).

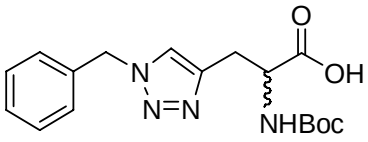
Compound 11. N(α)-Boc-11 (113 mg, 0.33 mmol) was deprotected in CH_2Cl_2 / TFA (2:1; 3.0 mL) by the procedure described for compound **5** to give as an off-white solid (**11**, 80 mg, 96%): mp >145 °C (decomp.); IR (neat) ν 3298, 2919, 1670, 1438, 1198, 1134, 1093, 1065, 725 cm^{-1}; ^{1}H-NMR (D_2O) δ 8.08 (s, 1H), 5.62 (d, 1H, *J* = 9.2 Hz), 4.15 (t, 1H, *J* = 9.6 Hz), 4.08-4.00 (m, 2H), 3.93 (t, 1H, *J* = 5.9 Hz), 3.81 (dd, 1H, *J* = 9.8 and 3.3 Hz), 3.71 (d ,2H, *J* = 6.0 Hz), 3.34 (dd, 1H, *J* = 15.7 and 5.2 Hz), 3.29 (dd, 1H, *J* = 15.7 and 6.8 Hz) ppm; ^{13}C-NMR (D_2O) δ 172.9, 163.0 (q, *J* = 35.6 Hz), 142.2, 123.6, 116.3 (q, *J* = 291.7 Hz), 87.9, 78.3, 72.9, 69.7, 68.5, 60.8, 54.2, 26.3 ppm; LRLC-MS: $[M+H]^+$ = 319.03 (calc. for $C_{11}H_{18}N_4O_7$: 318.28); $[\alpha]_D^{20}$ = +5.5 (c=4.2 in MeOH).

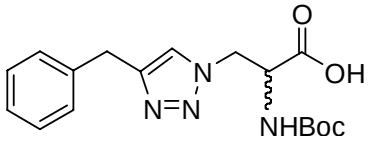
^{1}H- and ^{13}C-NMR of compound 11, D_2O



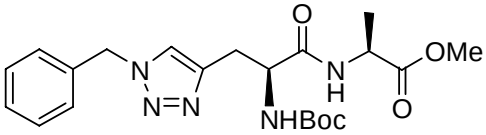
*1.4 ee-Determination of click compounds **5** and **7** by NMR-spectroscopy*

For the preparation of racemic material for NMR-comparison, commercial D and L isomers of N(α)-Boc-diaminopropionic acid were mixed in equal amounts and the mixture was converted to N(α)-Boc-D/L-azidoalanine according to literature procedure.[4] Likewise, commercial D and L isomers of N(α)-Boc-propargylglycine were mixed in equal amounts. Both racemic mixtures were converted to the corresponding triazole products *rac*-N(α)-Boc-**5** and *rac*-N(α)-Boc-**7** by the procedures described for the chiral compounds **5** and **7** and in similar yields. With the exception of the optical rotation [α], the obtained racemic products were identical in all respects to the chiral triazoles N(α)-Boc-**5** and N(α)-Boc-**7**.

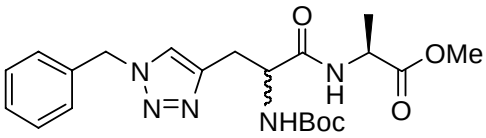




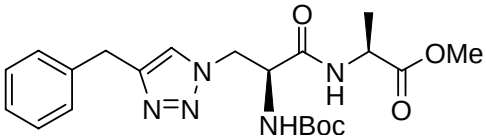
General procedure for the coupling of intermediates with H-Ala-OMe hydrochloride. The Boc-protected intermediate (35 mg, 0.1 mmol) was dissolved in DMF (2.0 mL) and H-Ala-OMe hydrochloride (14 mg, 0.1 mmol), triethylamine (42 µL, 30 mg, 0.3 mmol) and HBTU (38 mg, 0.1 mmol) were added. After stirring at rt overnight, the reaction mixture was concentrated under reduced pressure and the residue purified by flash chromatography on silica gel with CH_2Cl_2 / MeOH (50:1).



L-N(α)-Boc-5-Ala-OMe. Yellow oil (35 mg, 82 %): IR (neat) ν 3302, 2973, 1738, 1705, 1663, 1516, 1212, 1162, 1051, 725 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.35-7.19 (m, 6H), 7.1 (bs, 1H), 5.90 (broad d, 1H, *J* = 6.9 Hz), 5.45 (d, 1H, *J* = 15.2 Hz), 5.41 (d, 1H, *J* = 15.2 Hz), 4.55-4.31 (m, 1H), 4.40 (quint., 1H, *J* = 7.2 Hz), 3.66 (s, 3H), 3.24 (dd, 1H, *J* = 15.1 and 4.8 Hz), 3.05 (dd, 1H, *J* = 15.1 and 5.5 Hz), 1.38 (s, 9H), 1.15 (d, 3H, *J* = 7.2 Hz) ppm; ^{13}C-NMR ($CDCl_3$) δ 172.8, 170.7, 155.7, 143.9, 134.5, 129.1, 128.8, 128.1, 122.5, 80.3, 54.2, 53.7, 52.4, 48.1, 28.3, 27.8, 18.1 ppm; HiResMALDI: $[M+H]^+$ = 432.224 (calc. for $C_{21}H_{30}N_5O_5$: 432.225); $[\alpha]_D^{20}$ = -12.6 (c=0.8 in MeOH).

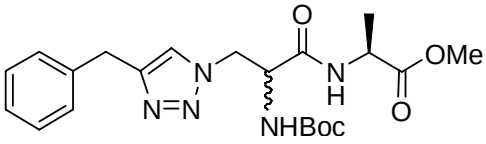


***rac*-N(α)-Boc-5-Ala-OMe.** Yellow oil (37 mg, 86 %): IR (neat) ν 3309, 2980, 1738, 1706, 1663, 1498, 1455, 1366, 1212, 1158, 1054 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.36-7.2 (m, 6H), 7.15 and 6.92 (each bs, each 0.5H), 5.89 and 5.80 (each bs, each 0.5H), 5.53-5.33 (m, 2H), 4.50-4.32 (m, 2H), 3.65 and 3.64 (each s, each 1.5H), 3.28-3.18 (m, 1H), 3.05 and 3.02 (each t, each 0.5H, *J* = 5.4 and 5.2 Hz in the order given), 1.39 and 1.38 (each s, each 4.5H), 1.28 and 1.22 (each t, each 1.5H, each *J* = 7.2 Hz) ppm; ^{13}C-NMR ($CDCl_3$) δ 172.9, 172.8, 170.8, 170.7, 155.8, 155.7, 143.9, 143.7, 134.6, 134.5, 129.1, 129.06, 128.8, 128.76, 128.71, 128.1, 122.7, 122.5, 80.4, 80.3, 54.14, 54.11, 54.0, 53.7, 52.4, 52.3, 48.1, 28.3, 27.9, 18.2, 17.9 ppm; LRLC-MS: $[M+H]^+$ = 432.14 (calc. for $C_{21}H_{29}N_5O_5$: 431.49); $[\alpha]_D^{20}$ = +3.0 (c=1.0 in MeOH).



[4] A. J. Link, M. K. S. Vink, D. A. Tirrell, *J. Am. Chem. Soc.* **2004**, *126*, 10598.

L-N(α)-Boc-7-Ala-OMe. White solid (38 mg, 88%): mp 139-140 ^{o}C; IR (neat) ν 3309, 2977, 1738, 1713, 1663, 1516, 1495, 1452, 1366, 1215, 1162, 1047, 729 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.25-7.10 (m, 6H), 7.05 (broad d, 1H, *J* = 5.8 Hz), 5.74 (broad d, 1H, *J* = 7.8 Hz), 4.78-4.67 (m, 1H), 4.61-4.48 (m, 2H), 4.34 (m (quintet), 1H, *J* = 7.2 Hz), 3.97 (s, 2H), 3.64 (s, 3H), 1.36 (s, 9H), 1.12 (d, 3H, *J* = 7.2 Hz) ppm; ^{13}C-NMR ($CDCl_3$) δ 172.7, 168.6, 155.6, 147.8, 138.9, 128.9, 128.8, 126.7, 123.6, 81.2, 54.5, 52.7, 51.0, 48.4, 32.3, 28.4, 18.2 ppm; LRLC-MS: $[M+H]^+$ = 432.15 (calc. for $C_{21}H_{29}N_5O_5$: 431.49); $[\alpha]_D^{20}$ = -21.7 (c=1.1 in MeOH).



***rac*-N(α)-Boc-7-Ala-OMe.** Yellow oil (21 mg, 47%): IR (neat) ν 3310, 2980, 1742, 1665, 1527, 1455, 1367, 1323, 1210, 1323, 1210, 1162, 1054, 1027, 913, 855, 728, 698, 637 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.26-7.14 (m, 6H), 7.02 (d, 0.5H, *J* = 5.9 Hz), 6.91 (d, 0.5H, *J* = 6.4 Hz), 5.74 (d, 0.5H, *J* = 7.3 Hz), 5.67 (d, 0.5H, *J* = 7.3 Hz), 4.79-4.73 (m, 1H), 4.58-4.53 (m, 2H), 4.43-4.32 (m (overlapping quintets), 1H, *J* = 7.1 and 7.3 Hz for both), 4.00 (s, 1H), 3.99 (s, 1H), 3.66 (s, 1.5H), 3.65 (s, 1.5H), 1.39 (s, 4.5H), 1.38 (s, 4.5H), 1.29 (d, 1.5 H, *J* = 7.1 Hz), 1.13 (d, 1.5 H, *J* = 7.1 Hz) ppm; ^{13}C-NMR ($CDCl_3$) δ 172.7, 168.63, 168.56, 155.8, 139.0, 138.9, 130.7, 128.9, 128.8, 128.6, 126.80, 126.75, 123.6, 81.4, 54.5, 52.8, 52.7, 51.0, 50.7, 48.5, 48.4, 41.0, 32.3, 29.9, 28.4, 18.3, 18.1 ppm; TOF-ES-MS 432.13 = $[M+H]^+$ (calc. for $C_{21}H_{29}N_5O_5$: 431.22); $[\alpha]_D^{20}$ = -7.7 (c=1.0 in $CDCl_3$).

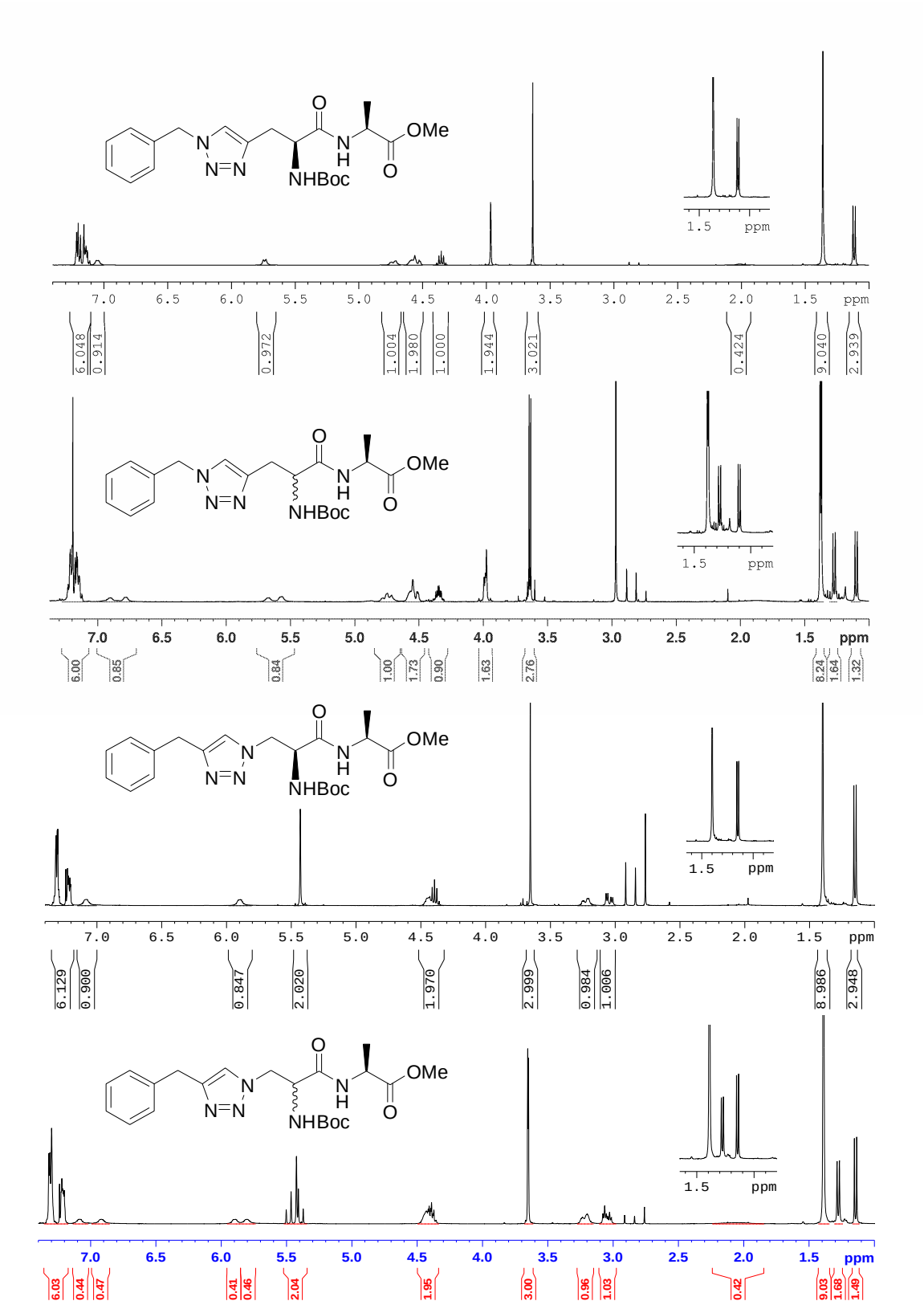


Figure 2. ^{1}H-NMR comparison of racemic versus chiral isomers of click products

*1.5 Syntheses on solid support (peptide derivative **9**)*

General Methods

The Fmoc-protected L-amino acids used in solid-phase peptide synthesis (SPPS), Fmoc-Nle-OH, Fmoc-Cha-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Val-OH, Fmoc-Dap-OH, Fmoc-Pra-OH as well as diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), thioanisole and trifluoroacetic acid were purchased from Fluka (Bornem, Belgium). The Rink amide resin and Fmoc-Ala-OH were obtained from NovaBiochem (Läufelfingen, Switzerland). Piperidine was obtained from Aldrich (Bornem, Belgium) and DMF, diisopropylethylamine and ethanedithiol from Acros (Geel, Belgium).

Analytical HPLC:

- system = Waters Breeze, Waters 1525 pump
- column type = reverse phase C18 column (Discovery®BIO SUPELCO Wide Pore C_{18} column , 25cm x 4.6 mm, 5µm)
- gradient = linear gradient, from 3% CH_3CN in H_2O to 100% CH_3CN in H_2O (containing 0.1% TFA) in 20 min
- detection = UV detection, 215 nm – Waters 2487
- flow rate = 1ml/min

Purification of peptides:

- system = semipreparative high-performance liquid chromatography system (Gilson) Gilson 322 pump
- column type = reverse phase C18 column (Discovery®BIO SUPELCO Wide Pore C_{18} column , 25cm x 2.21 cm, 5µm)
- gradient = linear gradient, from 3% CH_3CN in H_2O to 80% CH_3CN in H_2O (containing 0.1% TFA) in 20 min
- detection = UV detection, 215 nm – Gilson UV/VIS-156
- flow rate = 20ml/min

MS:

- VG Quatro II spectrometer (electrospray ionisation, cone voltage 70V)
- MassLynx2.22 software for data analysis

LCMS:

- MS: see above
- HPLC:
    - HPLC Waters system, Waters 600E pump
    - RP column (Discovery®BIO SUPELCO Wide Pore C_{18} column , 25cm x 4.6 mm, 5µm)
    - Flow rate = 1ml/min
    - Detection: UV (Waters 2487), 215 nm

TLC:

Glass-silica-coated plates with F_{254} indicator from Merck (Darmstadt, Germany), using EtOAc/*n*-BuOH/AcOH/H_2O (1:1:1:1) as eluent. The plates were treated with a permanganate solution ($KMnO_4$ (3 g), K_2CO_3 (20 g), 5 % aqueous NaOH (5 ml) and H_2O (300 ml)) to reveal the spots.

Boc-β-N_3Ala-OH and azido acetic acid were synthesised according to literature procedures[5]

Compound 9

SPPS was performed on a Rink amide polystyrene resin. Fluorenylmethyloxycarbonyl main-chain protected amino acids were used. The Fmoc deprotection was performed in a mixture of 20 % piperidine in DMF (2 x 10 min). After filtration and washing of the resin, the couplings were performed by using 3 equiv of protected amino acid and DIC (3 equiv) in the presence of HOBt (3 equiv). The completeness of the couplings was checked with the ninhydrin test.

[5] (a) J. T. Lundquist, J. C. Pelletier, *Org. Lett.* **2001**, *5*, 781. (b) L. A. Banaszynski, C. W. Liu, T. J. Wandless, *J. Am. Chem. Soc.* **2005**, *13,* 4715.

After coupling and deprotection of Fmoc-βAla-OH, azido-acetic acid was coupled using the same protocol. After filtration and washing of the resin (with DMF, iPrOH, DMF), 0.2 eq Cu(I)Br, 2 eq DIPEA and 2 eq Fmoc-L-Pra-OH in DMF were added to the resin [6]. The click reaction was left shaking overnight at room temperature, after which the Fmoc protection was removed. The peptide was cleaved from the resin with 10 % thioanisole/ethanedithiol (7:3) in TFA. After 3 h the resin was removed by filtration and the filtrate was added dropwise to dry, cold ether to precipitate the product. HPLC purification of the peptide gave an overall yield of 38 %. (TLC, R_f = 0.58). Analytical data of the compound are presented in Table 2.

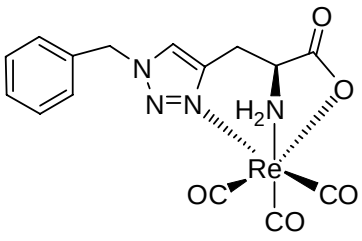
Table 2: Analytical data for compounds **9**.

Compd	% purity	MW calcd m/z	ES-MS [M+H$^+$]	HPLC t_R (min)
9	> 90%	1228.6 [M+2H$^+$]/2 = 615.3	1229.2 not present	13.4

Click reaction on solid support. HPLC analysis of peptide samples cleaved before and after the click reactions indicated quantitative conversion without formation of detectable amounts of side products.

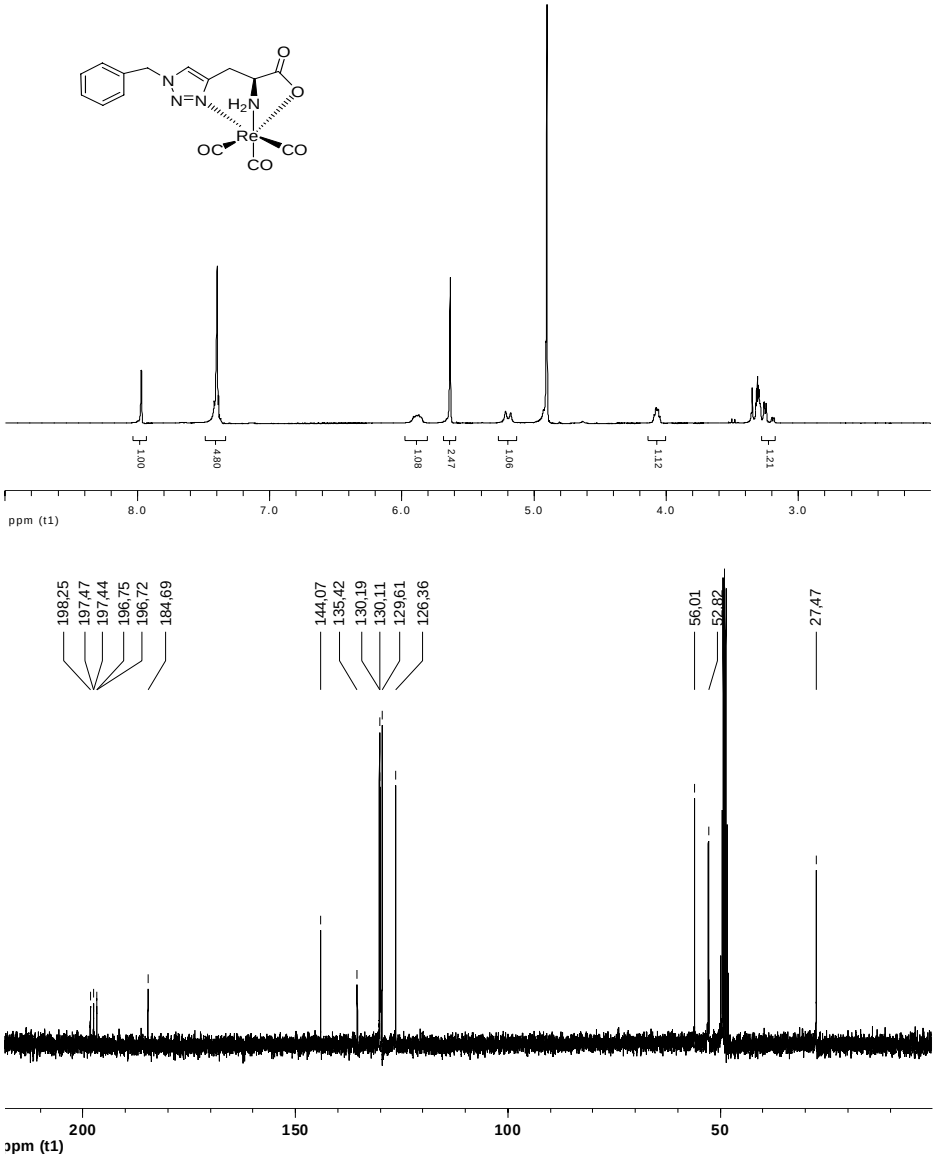
[6] C. W. Tornoe, C. Christensen, M. Meldalm, *J. Org. Chem.* **2002**, *9*, 3057.

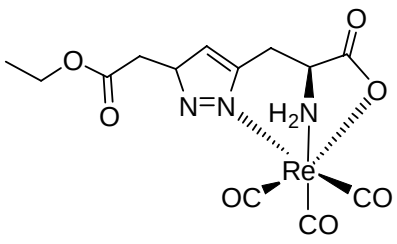
2 Synthesis of Re-complexes



[Re(CO)$_3$(5)]. Ligand **5** (9.5 mg, 0.04 mmol) and [Re(CO)$_3$Br$_3$][NEt$_4$]$_2$ (27 mg, 0.04 mmol) were added to a 1:1 mixture of methanol and water (4 mL) and stirred at 65 °C. The reaction was followed by HPLC. After 2 hours all of the starting material had been consumed. The solvent was removed under reduced pressure, and the residue redissolved in water. The product was purified with a Sep-Pak column (H_2O/methanol ratio 1:0, 3:1, 2:1, 1:1, 1:2, 1:3, 0:1). The product was eluted with a 1:2 ratio of water to methanol. The fractions containing the product were combined and the solvent removed under reduced pressure to give [Re(CO)$_3$(5)] as a white powder (15 mg, 82%): IR (neat) ν 2923, 2022, 1902, 1867, 1633, 1074, 734 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.97 (s, 1H), 7.49-7.33 (m, 5H), 5.88 (dd, 1H, J = 5.8 and 11.2), 5.64 (s, 2H), 5.20 (d, 1H, J = 11.2), 4.14-4.04 (m, 1H), 3.36-3.29 (m, 1H, obscured by solvent signal), 3.22 (dd, 1H, J = 4.0, 17.7) ppm; ^{13}C-NMR (CD_3OD) δ 198.3, 197.5, 196.7, 184.7, 144.1, 135.4, 130.2, 130.1, 129.6, 126.4, 56.0, 52.7, 27.5 ppm; LR-MS 516.94 = $[M+H]^+$ (calc. For $C_{15}H_{13}N_4O_5Re$ 516.04); HR-MS 515.0370 = $[M-H]^-$ (calc. for $C_{15}H_{12}N_4O_5Re$ 515.0371).

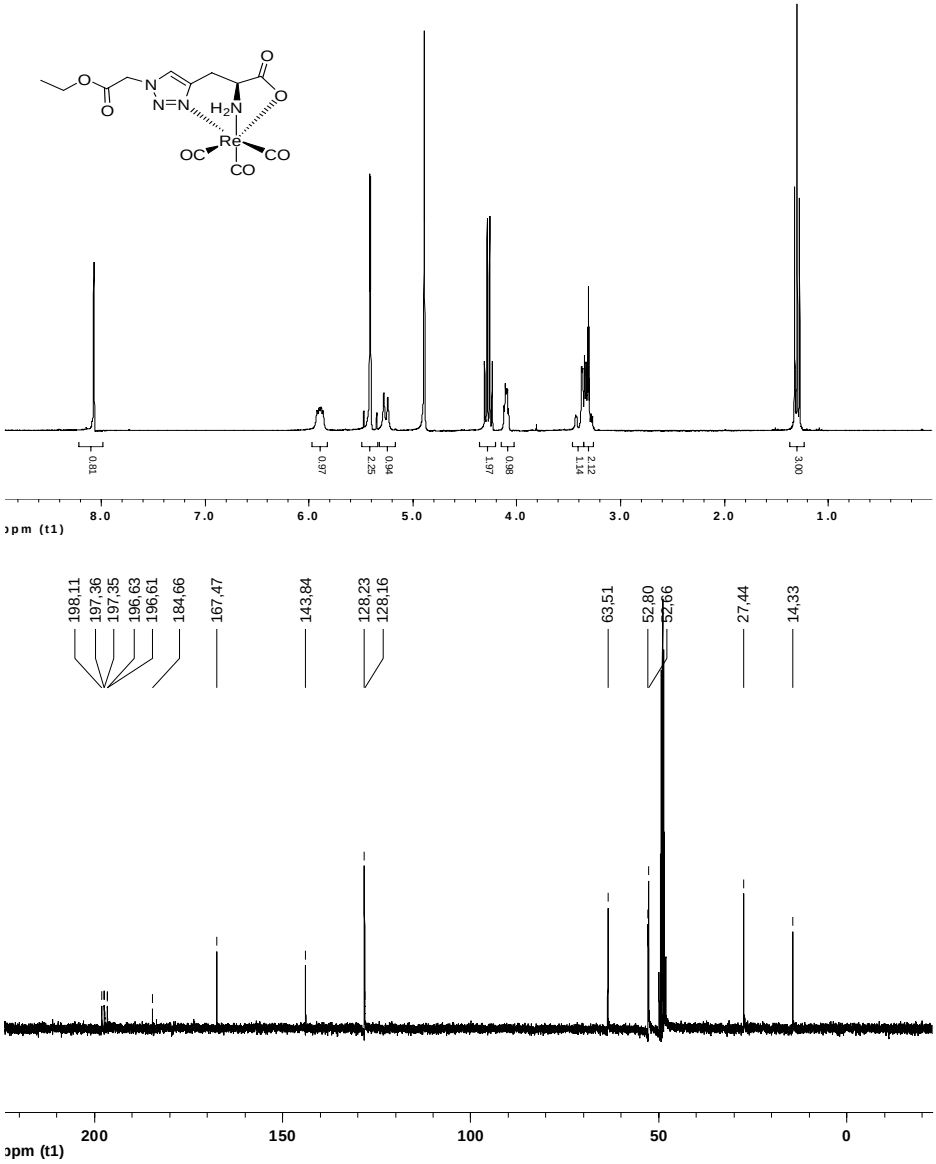
^{1}H- and ^{13}C-NMR of [Re(CO)$_3$(5)], CD_3OD

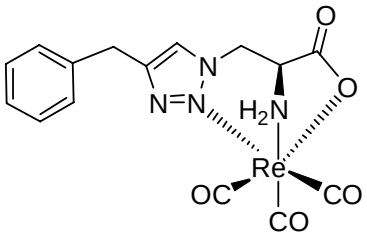




[Re(CO)$_3$(6)]. Ligand **6** (12 mg, 0.05 mmol) was dissolved in 5 mL ethanol. [Re(CO)$_3$Br$_3$][NEt$_4$]$_2$ (37 mg, 0.05 mmol) was added and the mixture was stirred at 50 °C. The reaction was followed by HPLC. After 2 hours all of the starting material had been consumed. The solvent was removed under reduced pressure, and the residue redissolved in water. The product was purified with a Sep-Pak column (H$_2$O/methanol ratio 1:0, 3:1, 2:1, 1:1, 1:2, 1:3, 0:1). The product was eluted with a 1:1 ratio of water to methanol. The fractions containing the product were combined and the solvent removed under reduced pressure to give [Re(CO)$_3$(6)] as a white powder (19 mg, 77%): IR (neat) ν 2360, 2337, 2025, 1871, 1748, 1636, 1376, 1220, 656 cm^{-1}; ^{1}H-NMR (CD$_3$OD) δ 8.07 (s, 1H), 5.89 (dd, 1H, *J* = 5.8 and 11.2 Hz), 5.44 (d, 1H, *J* = 11.2 Hz), 5.38 (d, 1H, *J* = 17.5 Hz), 5.26 (d, 1H, *J* = 17.5 Hz), 4.27 (q, 2H, *J* = 7.1 Hz), 4.15-4.03 (m, 1H), 3.40 (dd, 1H, *J* = 2.6 and 17.6 Hz), 3.35-3.26 (m, 1H), 1.30 (t, 3H, *J* = 7.1 Hz) ppm; ^{13}C-NMR (CD$_3$OD) δ 198.1, 197.4, 196.7, 184.7, 167.5, 143.9, 128.2, 63.5, 52.8, 52.7, 27.4, 14.3 ppm; TOF-ES-MS: 512.97 = [M+H]$^+$ (calc. for C$_{12}$H$_{13}$N$_4$O$_7$Re: 512.03); HR-ESI-MS 511.0272 = [M-H]$^-$ (calc. for C$_{12}$H$_{12}$N$_4$O$_7$Re: 511.0269); elemental analysis (calculated %-values in parenthesis) C 28.13 (28.18), H 2.77 (2.56), N 10.95 (10.95).

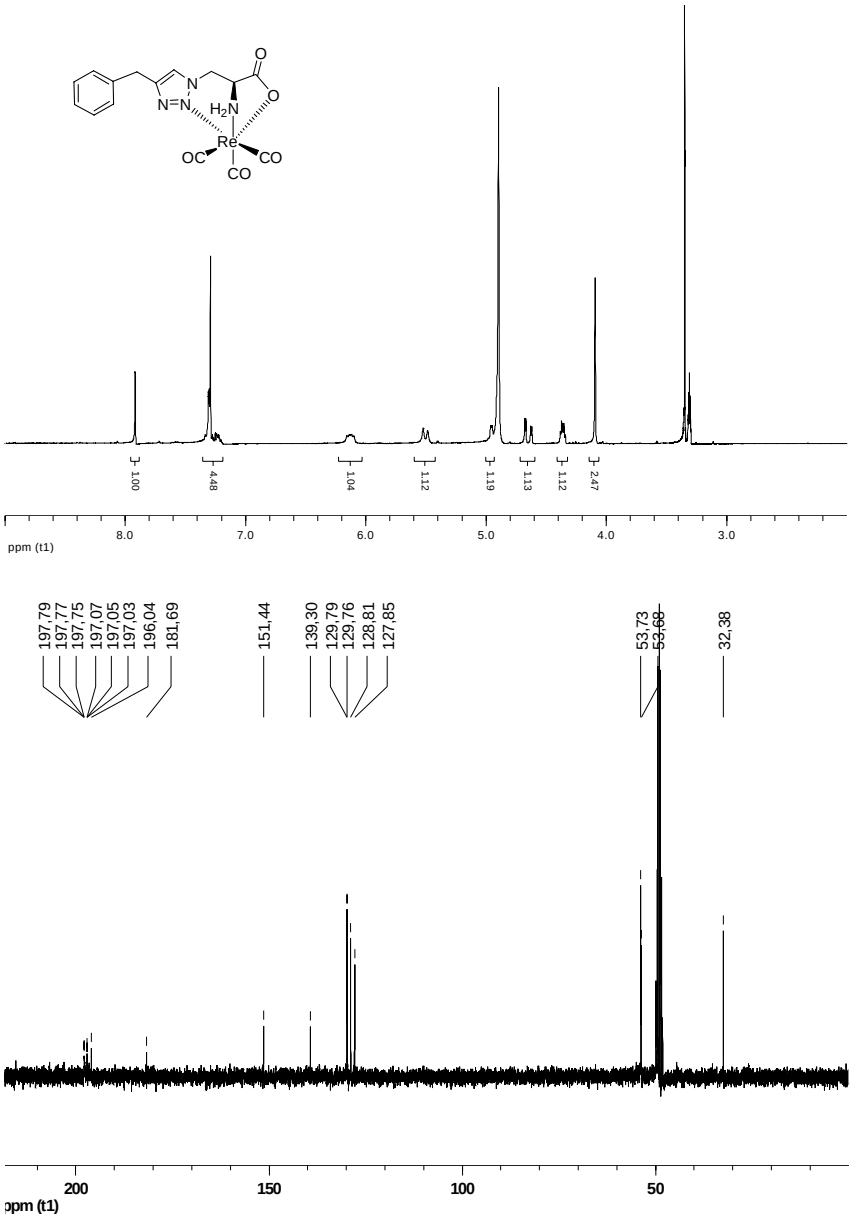
^{1}H- and ^{13}C-NMR of [Re(CO)$_3$(6)], CD$_3$OD

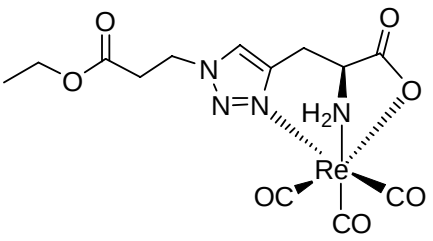




[Re(CO)$_3$(7)]. Ligand **7** (19.6 mg, 0.08 mmol) and [Re(CO)$_3$Br$_3$][NEt$_4$]$_2$ (59 mg, 0.08 mmol) were added to a 1:1 mixture of methanol and water (8 mL) and stirred at 65 °C. The reaction was followed by HPLC. After 4 hours all of the starting material had been consumed. The solvent was removed under reduced pressure, and the residue re-dissolved in water. The product was purified with a Sep-Pak column (H_2O/methanol ratio 1:0, 3:1, 2:1, 1:1, 1:2, 1:3, 0:1). The product was eluted with a 1:2 ratio of water to methanol. The fractions containing the product were combined and the solvent removed under reduced pressure to give [Re(CO)$_3$(**7**)] as a pale yellow powder (22 mg, 55%): IR (neat) ν 2025, 1876, 1643, 1370, 1147, 729 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.92 (s, 1H), 7.34-7.19 (m, 5H), 6.13 (dd, 1H, *J* = 6.0 and 11.1 Hz), 5.50 (d, 1H, *J* = 11.1 Hz), 5.00-4.79 (m, 1H, obscured by H_2O signal), 4.65 (dd, 1H, *J* = 2.9 and 14.9 Hz), 4.41-4.32 (m, 1H, *J* = 2.9 and 6.0 Hz), 4.09 (s, 2H) ppm; ^{13}C-NMR (CD_3OD) δ 197.8, 197.1, 196.1, 181.7, 151.5, 139.3, 129.81, 129.77, 128.8, 127.9, 53.73, 53.68, 32.4 ppm; TOF-ES-MS 517.06 = $[M+H]^+$ (calc. for $C_{15}H_{13}N_4O_5Re$: 516.04); HR-ESI-MS 515.0376 = $[M-H]^-$ (calc. for $C_{15}H_{12}N_4O_5Re$: 515.0371).

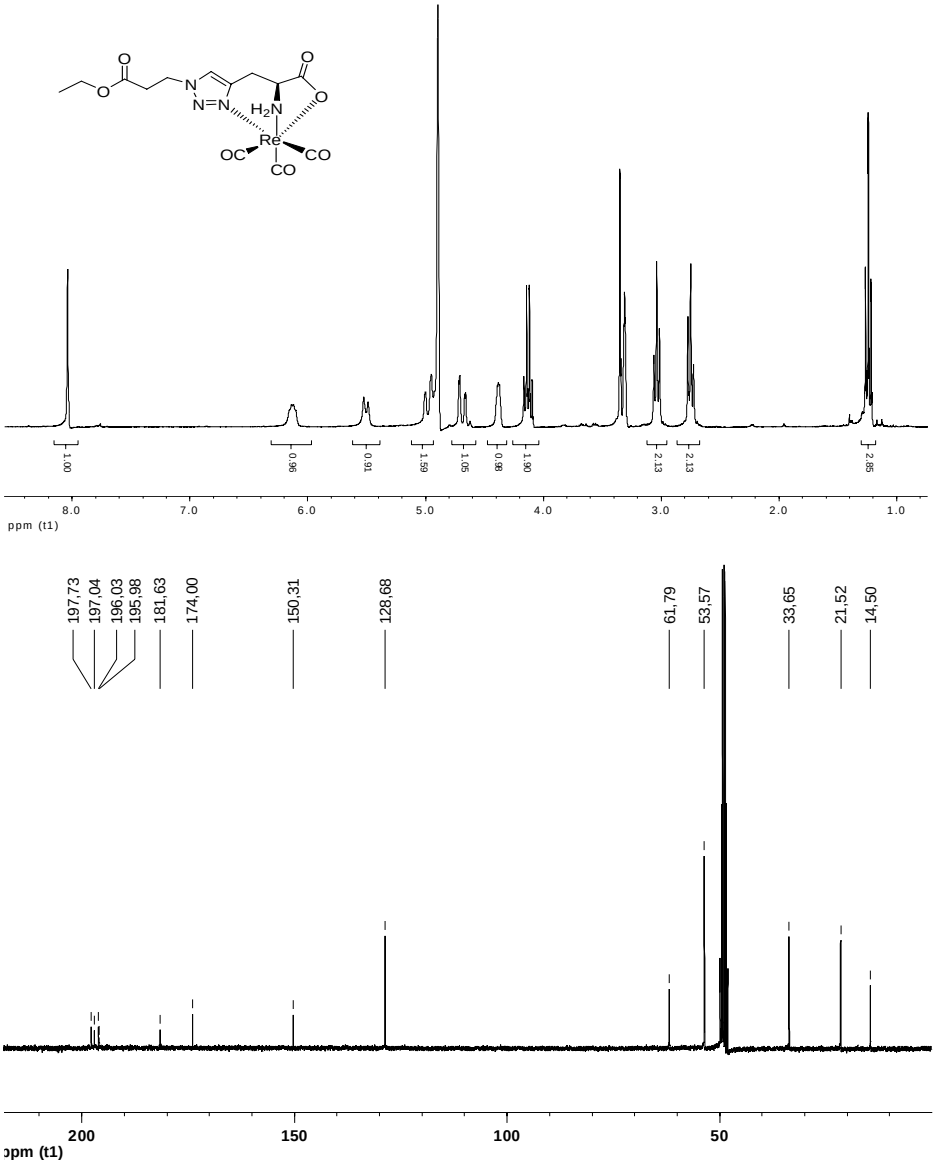
^{1}H- and ^{13}C-NMR of [Re(CO)$_3$(7)], CD_3OD

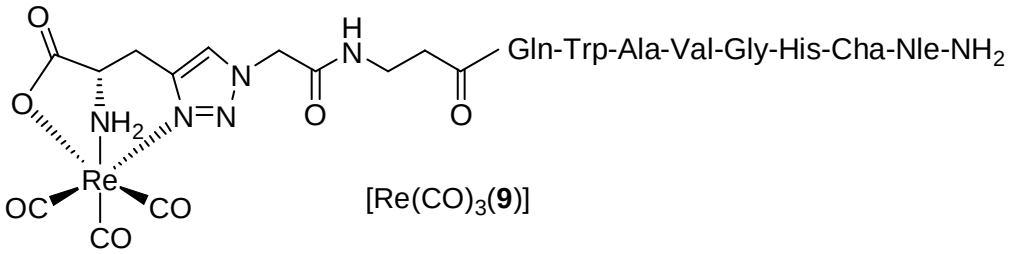




[Re(CO)$_3$(8)]. Ligand **8** (15 mg, 0.06 mmol) was dissolved in 6 mL ethanol. $[Re(CO)_3Br_3][NEt_4]_2$ (45 mg, 0.06 mmol) was added and the mixture was stirred at 50 °C. The reaction was followed by HPLC. After 1 hour all of the starting material had been consumed. The solvent was removed under reduced pressure, and the residue redissolved in water. The product was purified with a Sep-Pak column (H_2O/methanol ratio 1:0, 3:1, 2:1, 1:1, 1:2, 1:3, 0:1). The product was eluted with a 1:2 ratio of water to methanol. The fractions containing the product were combined and the solvent removed under reduced pressure to give [Re(CO)$_3$(**8**)] as a pale yellow powder (20 mg, 64%): mp >220 °C; IR (neat) ν 2024, 1881, 1716, 1643, 1445, 1375, 1348, 1158, 1034, 910, 836, 654 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 8.04 (s, 1H), 6.13 (dd, 1H, *J* = 5.1 and 10.3 Hz), 5.51 (d, 1H, *J* = 10.3 Hz), 4.97 (d, 1H, *J* = 14.9 Hz), 4.69 (dd, 1H, *J* =2.4 and 14.9 Hz), 4.41-4.35 (m, 1H), 4.13 (q, 2H, *J* =7.2 Hz), 3.04 (t, 2H, *J* = 7.1 Hz), 2.75 (t, 2H, *J* = 7.1 Hz), 1.24 (t, 3H, *J* = 7.2 Hz) ppm; ^{13}C-NMR (CD_3OD) δ 197.8, 197.1, 196.0, 181.6, 174.0, 150.3, 128.7, 61.8, 53.6, 53.6, 33.7, 21.5, 14.5 ppm; TOF-ES-MS 527.04 = $[M+H]^+$ (calc. for $C_{13}H_{15}N_4O_7Re$ 526.05).

^{1}H- and ^{13}C-NMR of [Re(CO)$_3$(8)], CD_3OD

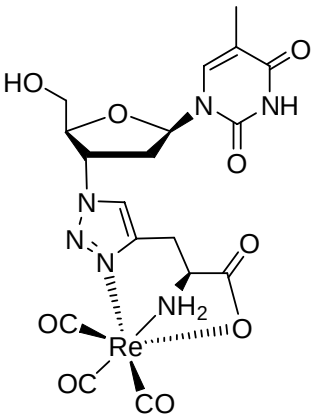




[Re(CO)$_3$(9)]. An aqueous solution of bombesin derivative **9** (10^{-3} M, 100 μL) was mixed with $[Re(Br)_3(CO)_3][Et_4N]_2$ (10^{-3} M in water, 200 μL). The solution was heated to 100 °C for 60 min. HPLC analysis of the reaction mixture revealed complete consumption of the starting material. MS of the product confirmed formation of the complex. The data are presented in Table 3.

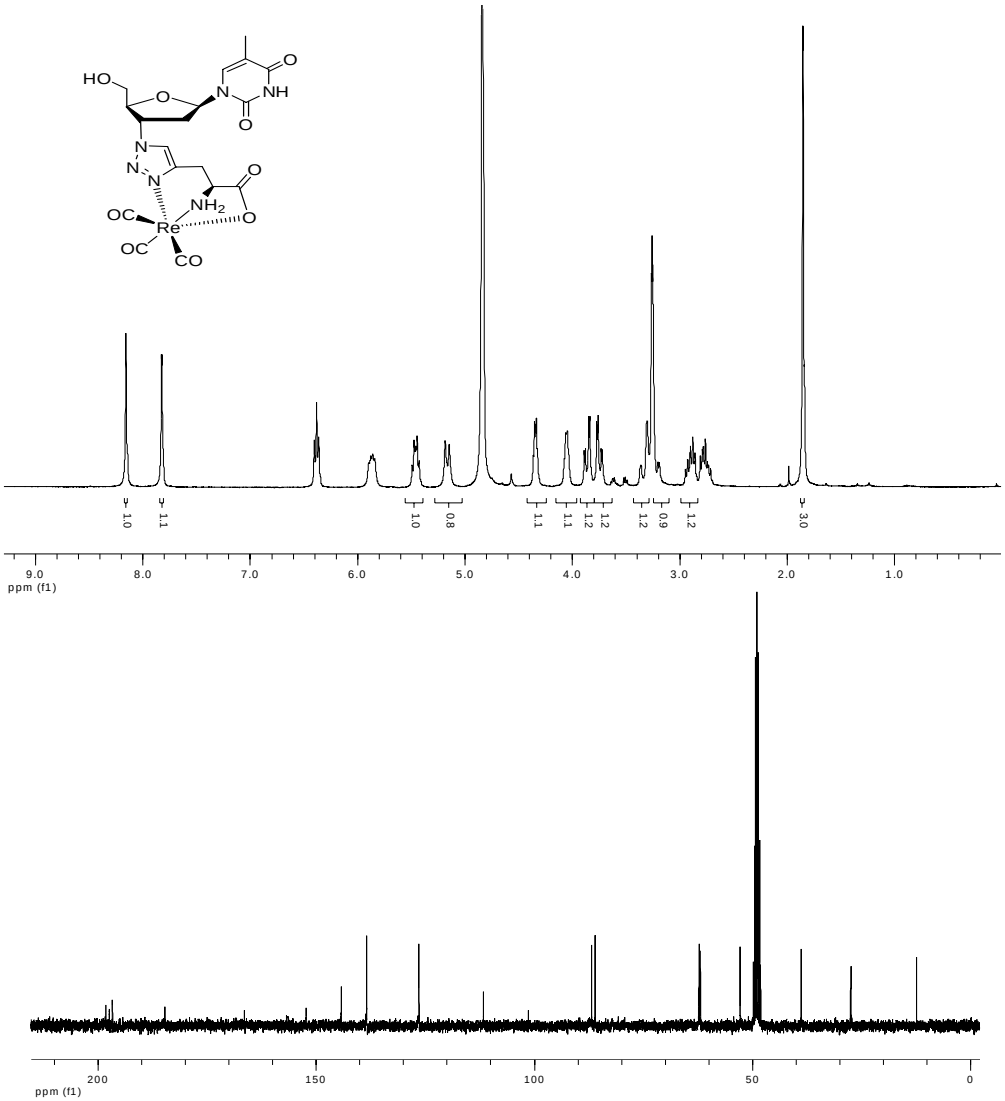
Table 3. MS data of [Re(CO)$_3$(**9**)] and [Re(CO)$_3$(**10**)]

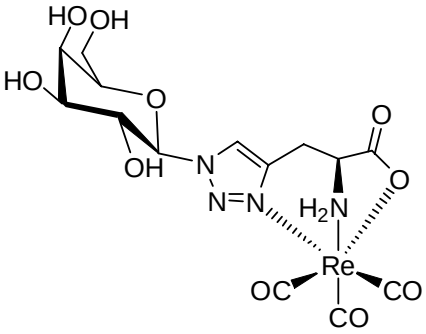
Compound	MW calcd. m/z	ESI-MS [M+H$^+$]
[Re(CO)$_3$(**9**)]	1497.8 [M+2H]/2 = 749.9	1499.6 [M+2H]/2 = 750.1



[Re(CO)$_3$(10)]. Compound **10** (15.0 mg, 0.039 mmol) and [Re(Br)$_3$(CO)$_3$][Et$_4$N]$_2$ (30.0 mg, 0.039 mmol) were mixed in methanol / water (1:2; 1.5 mL) and stirred at 80 °C for 1.5 hours. The reaction mixture was diluted with water (3.5 ml) and loaded on a 5 g RP-C18 SepPak® column. Elution with mixtures of water / acetonitrile (10→35% acetonitrile) and subsequent evaporation under reduced pressure yielded [Re(CO)$_3$(**10**)] (20.4 mg, 81%) as a white solid: IR (neat) ν 3384, 2948, 2831, 2412, 2324, 2022, 1879, 1627, 1474, 1433, 1373, 1274, 1152, 1095, 1076, 1021, 970, 900, 819, 768, 654, 636 cm^{-1}; ^{1}H-NMR (CD$_3$OD) δ 8.16 (s, 1H), 7.83 (d, 1H, *J* = 1.1 Hz), 6.38 (t, 1H, *J* = 6.4 Hz), 5.87 (m, 1H, *J* = 5.4 Hz), 5.46 (dt, 1H, *J* = 8.5 and 5.6 Hz), 5.17 (d, 1H, *J* = 11.2 Hz), 4.34 (dt, 1H, *J* = 5.8 and 3.0 Hz), 4.05 (q, 1H, *J* = 4.0 Hz), 3.86 (dd, 1H, *J* = 12.2 and 3.0 Hz), 3.75 (dd, 1H, *J* = 12.2 and 3.2 Hz), 3.33 (dd, 1H, *J* = 17.3 and 2.4 Hz), 3.23 (dd, 1H, *J* = 17.3 and 4.1 Hz), 2.90 (ddd, 1H, *J* = 14.1, 6.7 and 6.2 Hz), 2.76 (ddd, 1H, *J* = 14.1, 8.6 and 6.0 Hz), 1.85 (d, 3H, *J* = 0.9 Hz) ppm; ^{13}C-NMR (CD$_3$OD) δ 198.2, 197.4, 196.7, 184.6, 166.5, 156.7, 156.4, 152.3, 144.3, 138.4, 126.5, 111.7, 101.5, 86.9, 86.1, 62.3, 62.0, 52.9, 38.9, 27.6, 12.5 ppm; HR-MS: [M-H]$^-$ = 649.0688 (calc. for $C_{18}H_{18}N_6O_9Re$: 649.0698).

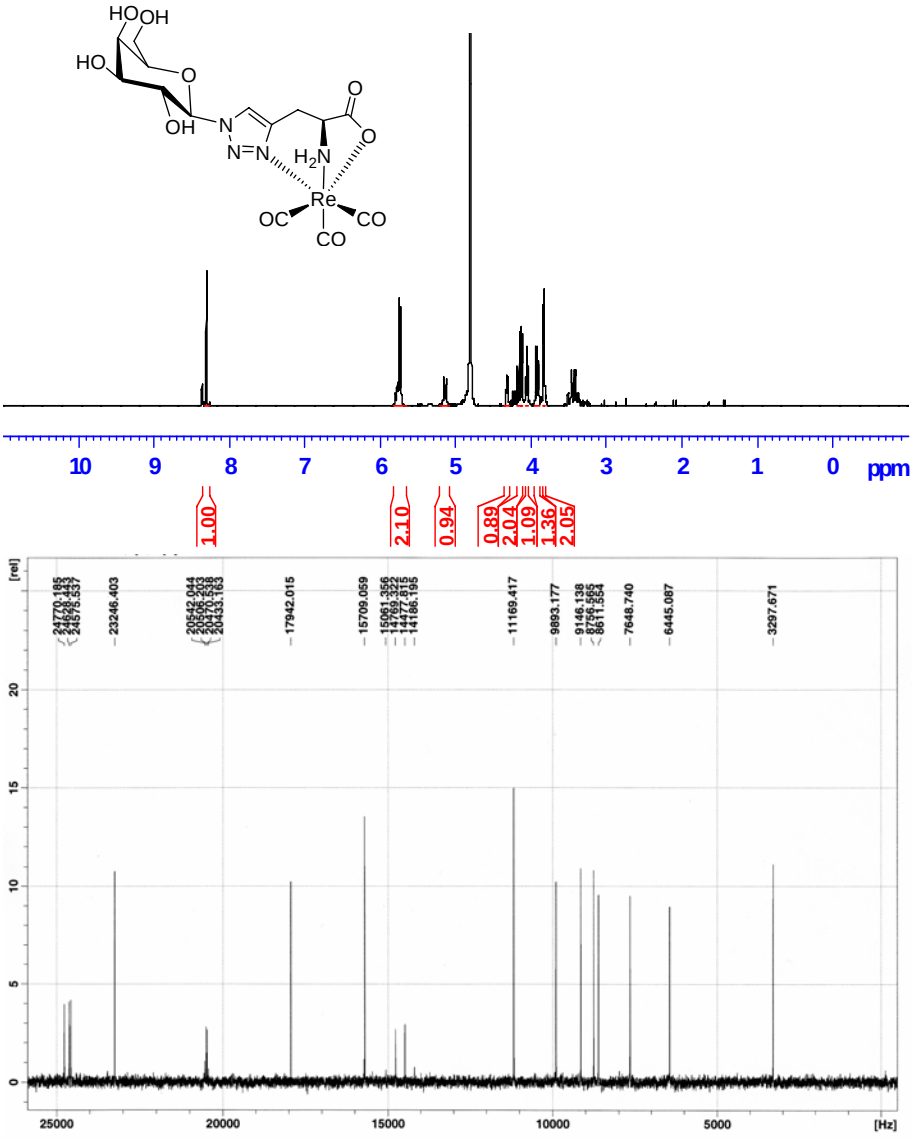
^{1}H- and ^{13}C-NMR of [Re(CO)$_3$(10)], CD$_3$OD





[Re(CO)$_3$(11)]. Carbohydrate ligand **11** (22 mg, 0.05 mmol) and [Re(Br)$_3$(CO)$_3$][Et$_4$N]$_2$ (39 mg, 0.05 mmol) were dissolved in water (3 mL) and the pH was adjusted to pH 7-8 with an aqueous solution of Et$_4$NOH (10%, 3 drops). The resulting solution was stirred at 50 °C for 3 h. Concentration under reduced pressure followed by HPLC purification of the residue yielded [Re(CO)$_3$(**11**)] as a white solid (17 mg, 58%): mp >195 °C (decomp.); IR (neat) ν 3274, 3157, 2027, 1889, 1671, 1631, 1391, 1202, 1140, 1086, 1046 cm^{-1}; ^{1}H-NMR (D$_2$O) δ 8.31 (s, 1H), 5.82-5.70 (m, 1H), 5.76 (d, 1H, *J* = 9.1 Hz), 5.15 (d, 1H, *J* = 12.0 Hz), 4.35-4.28 (m, 1H), 4.16 (t, 1H, *J* = 9.7 Hz), 4.12 (d, 1H, *J* = 3.2 Hz), 4.05 (t, 1H, *J* = 6.0 Hz), 3.91 (dd, 1H, *J* = 9.7 and 3.3 Hz), 3.87-3.80 (m, 2H), 3.49 (dd, 1H, *J* = 18.2 and 2.4 Hz), 3.45 (dd, 1H, *J* = 28.2 and 4.4 Hz) ppm; ^{13}C-NMR (D$_2$O) δ 197.0, 195.8, 195.4, 184.9, 142.7, 124.9, 88.8, 78.7, 72.7, 69.6, 68.5, 60.8, 51.3, 26.2 ppm; HR-MS: [M+Na]$^+$ = 611.0397 (calc. for $C_{14}H_{17}N_4O_{10}ReNa$: 611.040).

^{1}H- and ^{13}C-NMR of [Re(CO)$_3$(11)], D$_2$O



3 Structural assignment of [Re(CO)$_3$(5-7)] by NMR-spectroscopy

NMR signals of the metal chelator in the rhenium complexes exhibit a low field shift compared to the corresponding signals of the free ligand. We were able to record ^{1}H-NMR spectra of compound 6 and the corresponding complex [Re(CO)$_3$(**6**)] in the same solvent (D_2O/DCl), although comparisons for other complexes were made difficult by the low water solubility of the rhenium complexes, and the low solubility of the ligands in common organic solvents.

For all complexes signals are observed for the NH_2 protons even in protic solvents, as a result of a large decrease in the rate of H/D exchange. There are two distinct NH signals, with well defined coupling to each other, and in the case of one signal, to the α-proton of the amino acid. Both NH signals disappear after 24 hours in CD_3OD.

Finally as evidence for the suggested structures, the ^{1}H-NMR spectra of [Re(CO)$_3$(**5**)] and [Re(CO)$_3$(**7**)] are compared with the spectrum of [Re(CO)$_3$(Me-His)].

Comparison of the ^{1}H spectra of ligand 6 and the complex [Re(CO)$_3$(6)] in 1% DCl

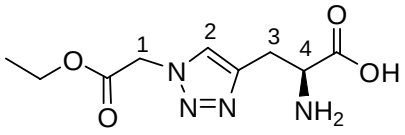
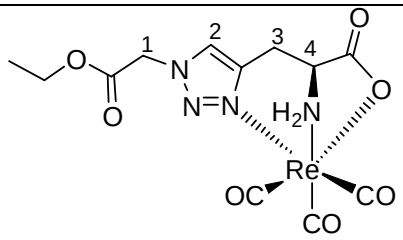
	
Ligand **6**	[Re(CO)$_3$(**6**)]

Table 4. Chemical shifts of protons in the ^{1}H spectra of ligand 6 and the complex [Re(CO)$_3$(**6**)]

	Ligand **6**		[Re(CO)$_3$(**6**)]	
Protons	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
1	4.72		5.16	
2	7.49		7.83	
3	2.80	6.4	3.15	
4	3.76	6.4	4.02	
NH	-		4.88	11.8
NH	-		5.49	6.0, 11.8

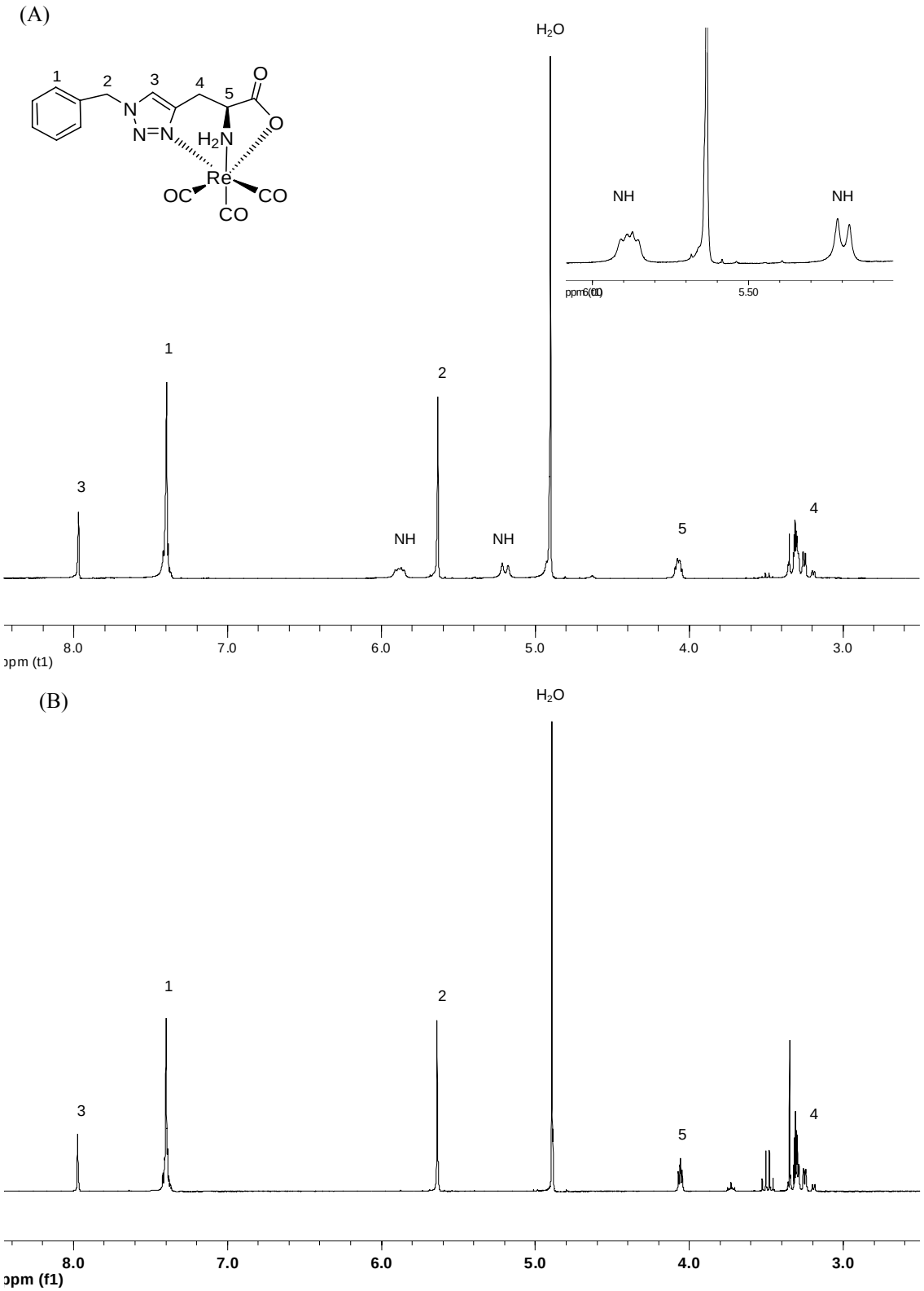


Figure 3. ^{1}H NMR spectra of [Re(CO)$_3$(**5**)] after 15 minutes (A), and after 24 hours (B) in CD_3OD

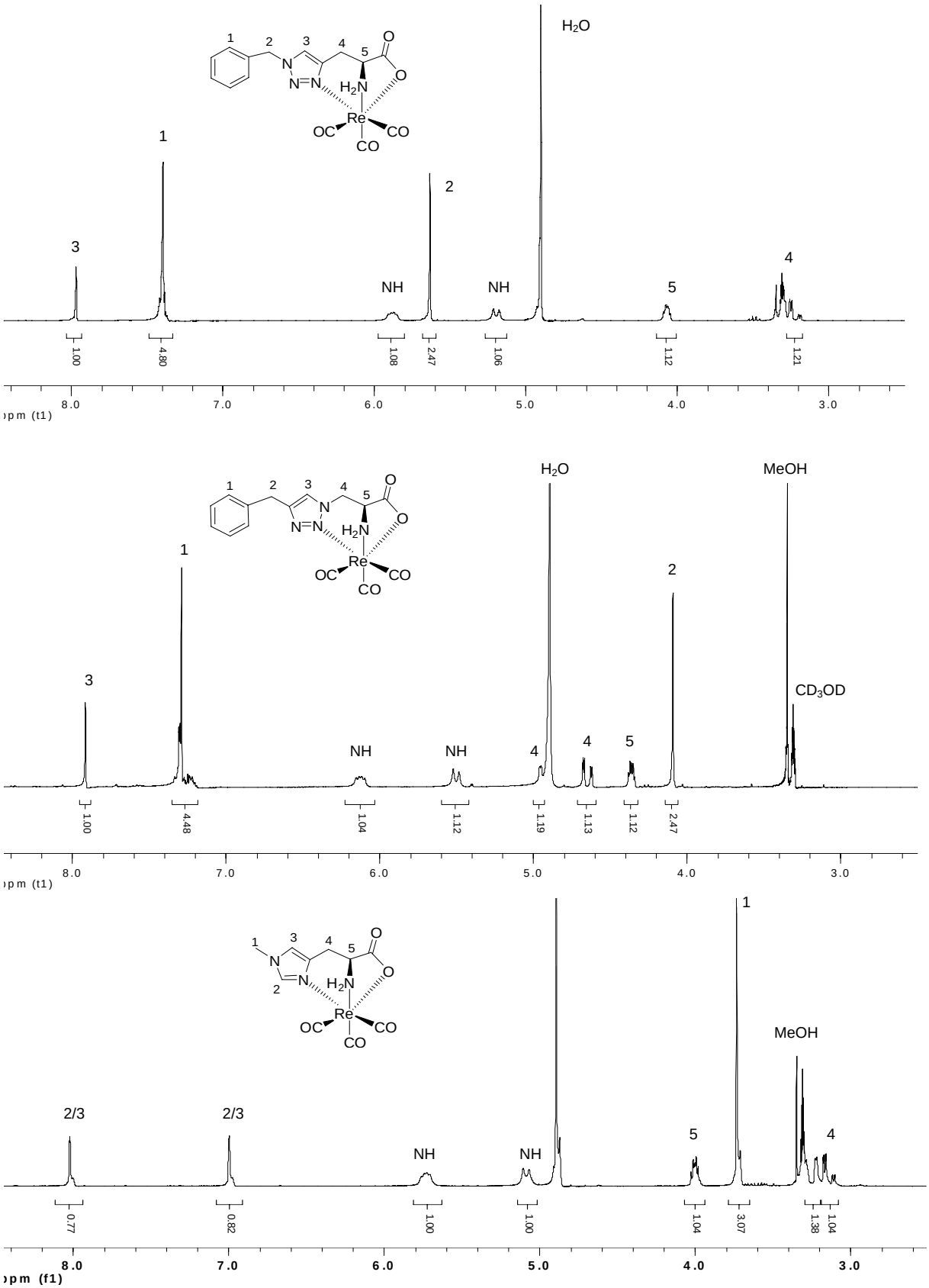
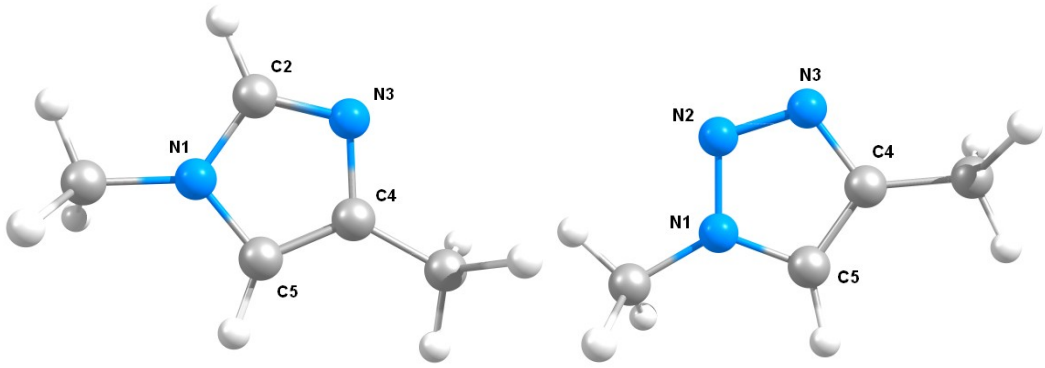


Figure 4. ^{1}H-NMR spectra of [Re(CO)$_3$(**5**)], [Re(CO)$_3$(**7**)] and [Re(CO)$_3$(Me-His)]

4 Density Functional Theory Calculations

All calculations were conducted using density functional theory (DFT) as implemented in the Gaussian 03, Revision C.02 suite of *ab initio* quantum chemistry programs.[7] Geometry optimizations and vibrational frequency calculations were performed using the restricted B3LYP exchange and correlation functionals and either the double-ζ 6-31G(d) or triple-ζ 6-311+G(d) basis sets for all atoms. Normal SCF and geometry convergence criteria were used and no symmetry constraints were imposed. Harmonic frequency analysis based on analytical second derivates was used to characterize the optimized geometries as local minima.



(a) (b)

Figure 5**:** B3LYP/6-31G(d) optimized geometries and atom numbering scheme for (a) 1,4-dimethylimidazole and (b)1,4-dimethyltriazole.

Figure 5 shows the B3LYP/6-31G(d) optimized geometries and atom numbering scheme for 1,4-dimethylimidazole and 1,4-dimethyltriazole, used as models for the histidine and click based ligands respectively. The Cartesian coordinates for the optimized structures are given in Table 5 below. Mulliken population analysis and Natural population analysis charges on the atoms of the imidazole and triazole rings are given in Table 6.

[7] R. C. Gaussian 03, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A.; Gaussian, Inc., Wallingford CT, 2004.

Table 5. Cartesian coordinates for the optimized structures of 1,4-dimethylimidazole and 1,4-dimethyltriazole.

1,4-Dimethylimidazole				1,4-Dimethyltriazole			
B3LYP/6-31G(d)				**B3LYP/6-31G(d)**			
7	1.088731000	-0.090134000	-0.000104000	7	-1.062618000	0.084371000	0.000000000
6	0.608147000	1.188692000	-0.000038000	7	-0.643424000	-1.200536000	0.000000000
7	-0.705946000	1.233387000	0.000026000	7	0.661155000	-1.188628000	0.000000000
6	-1.117087000	-0.085824000	0.000001000	6	1.105173000	0.105509000	0.000000000
6	-0.020716000	-0.916196000	-0.000060000	6	0.000000000	0.930740000	0.000000000
1	1.272352000	2.044191000	0.000004000	1	-0.111268000	2.004848000	0.000000000
1	0.073909000	-1.992662000	-0.000084000	6	2.562250000	0.445378000	0.000000000
6	-2.568004000	-0.455011000	0.000056000	1	3.062194000	0.030207000	0.882291000
1	-3.078395000	-0.049540000	0.881840000	1	3.062194000	0.030207000	-0.882291000
1	-3.078533000	-0.049042000	-0.881478000	1	2.713897000	1.529073000	0.000000000
1	-2.699123000	-1.542052000	-0.000305000	6	-2.482197000	0.384928000	0.000000000
6	2.479898000	-0.500940000	0.000071000	1	-2.756916000	0.954591000	0.893167000
1	2.712620000	-1.093401000	0.891564000	1	-2.756916000	0.954591000	-0.893167000
1	2.712312000	-1.094887000	-0.890454000	1	-3.010326000	-0.569293000	0.000000000
1	3.111933000	0.390286000	-0.000722000				
B3LYP/6-311+G(d)				**B3LYP/6-311+G(d)**			
7	1.088086000	-0.088825000	-0.000067000	7	-1.061640000	0.081473000	0.000000000
6	0.608145000	1.187965000	-0.000014000	7	-0.640121000	-1.198739000	0.000000000
7	-0.704518000	1.228401000	0.000039000	7	0.661537000	-1.184883000	0.000000000
6	-1.117727000	-0.087708000	0.000003000	6	1.105613000	0.106002000	0.000000000
6	-0.020650000	-0.914969000	-0.000046000	6	-0.000126000	0.927395000	0.000000000
1	1.266820000	2.045334000	-0.000020000	1	-0.111987000	1.999836000	0.000000000
1	0.073574000	-1.990027000	-0.000089000	6	2.559957000	0.445873000	0.000000000
6	-2.566207000	-0.455095000	0.000022000	1	3.058781000	0.032127000	0.880238000
1	-3.074085000	-0.049633000	0.879955000	1	3.058781000	0.032127000	-0.880238000
1	-3.074196000	-0.049229000	-0.879659000	1	2.713682000	1.526845000	0.000000000
1	-2.700944000	-1.539265000	-0.000218000	6	-2.481135000	0.384988000	0.000000000
6	2.479515000	-0.501277000	0.000089000	1	-2.753071000	0.953188000	0.891450000
1	2.710901000	-1.091608000	0.889791000	1	-2.753071000	0.953188000	-0.891450000
1	2.710631000	-1.092968000	-0.888773000	1	-3.013999000	-0.563435000	0.000000000
1	3.112753000	0.385770000	-0.000697000				

From the atomic charge analysis using different two basis sets and the two different methods (MPA and NPA) it can been seen that Mulliken charges are highly sensitive to the basis set used whereas the Natural Population charges vary less between the two calculations.

Table 6**:** Mulliken population analysis (MPA) and natural population analysis (NPA) charges on the atoms of the imidazole and triazole rings. The atomic numbering is shown in Figure 5.

	Imidazole				Triazole			
	B3LYP/6-31G(d)		B3LYP/6-311+G(d)		B3LYP/6-31G(d)		B3LYP/6-311+G(d)	
Atom	MPA	NPA	MPA	NPA	MPA	NPA	MPA	NPA
N1	-0.365	-0.399	0.283	-0.412	-0.218	-0.198	0.409	-0.208
N2	-	-	-	-	-0.083	-0.071	-0.098	-0.078
C2	0.196	0.187	-0.042	0.217	-	-	-	-
N3	-0.474	-0.502	-0.125	-0.511	-0.327	-0.268	-0.188	-0.277
C4	0.232	0.106	0.517	0.107	0.259	0.083	0.442	0.086
C5	-0.028	-0.106	-0.468	-0.083	-0.014	-0.086	-0.545	-0.060

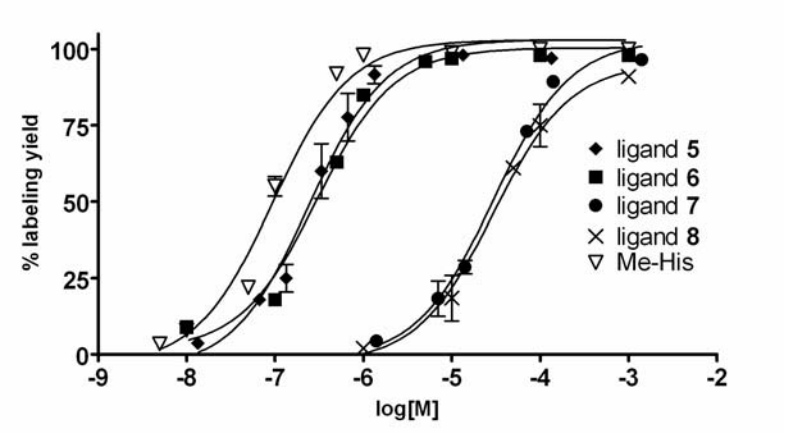
5 Synthesis of ^{99m}Tc-complexes

5.1 General two-step procedure

Step 1: The precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared according to the literature.[8] Briefly, 1 mL $[^{99m}TcO_4]^-$ in 0.9% NaCl was added to the IsoLink™ kit (Mallinckrodt-Tyco, Petten, Holland) via the septum. The reaction was heated for 20 min at 100°C. The solution was cooled and neutralized (pH = 7.2) with 1 M phosphate buffer pH = 7.4 and 1 M HCl (1:1 mixture).
Step 2: 50 µL of a stock solution (10^{-2} M to 10^{-7} M in physiological phosphate buffer pH = 7.4) of the relevant ligand **5-8** was added to a solution of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (100 µL; ~1 GBq/mL). Phosphate buffered saline (PBS pH 7.4, 350 µL) was added to adjust the final concentration. The reaction was heated for 50 min at 100°C. Radiolabeling yields were determined via HPLC. Complexes were analyzed via HPLC and the identity confirmed by comparison with the UV trace of the corresponding Re-complexes. This is common practice with Tc-99m complexes on the n.c.a. level.

Table 7: EC_{50} values (best-fit values) for ligands **5-8** and N^{ε}-methyl histidine.

Ligand	**5**	**6**	**7**	**8**	**N^{ε}-Me-His**
$LogEC_{50}$	-6.61	-6.52	-4.528	-4.529	-7.024
EC_{50}	2.45E-07	3.02E-07	2.97E-05	2.96E-05	9.47E-08
Std. Error $LogEC_{50}$	0.08825	0.104	0.08478	0.1174	0.0636
R^2	0.9579	0.9842	0.9804	0.9774	0.983
Number of X values	24	26	27	26	26
Number of Y replicates	3	3	3	3	3



[8] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *Journal of the American Chemical Society* **2001**, *123*, 3135.

5.2 One-step procedures

Procedure A:
In situ preparation and radiolabeling of compounds **5, 7, 10** and **11.**
The following reagents were combined in a 10 mL vial: 40 µL of a 10^{-2} M aqueous solution of copper (II) acetate, Na-ascorbate (80 µL in H_2O, 10^{-2} M), propargyl glycine or azido-alanine (100 µL in H_2O, 10^{-3} M) and 200 µL of benzyl azide, 3-phenyl propylene, 3'-azidothymidine or 1-azido-1-deoxy-β-D-galactopyranoside (10^{-3} M in H_2O). The reactions were heated at 50°C for 30 min. 100 µL of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (pH = 7.4) was added to the pale yellow solutions. The reactions were incubated for 1 h at 100°C and monitored via HPLC (Figure 14). Yield: 84-92 %.

Procedure B:
For clinical applications it is desirable for ^{99m}Tc-labeled products to be obtained in a single step directly from $[^{99m}TcO_4]^-$ as eluted from the $^{99}Mo/^{99m}Tc$ generator. We were able to show that this can also be accomplished with the new click ligands by applying a different one-pot procedure.
0.9 mL $[^{99m}TcO_4]^-$ in 0.9% NaCl was added to the IsoLink™ kit (Mallinckrodt-Tyco) via the septum. 0.1 mL of stock solution (10^{-4}-10^{-3} M in saline) of the relevant ligand (**10** or **11**) was added. The reactions were heated for 60 min at 100°C and then cooled to room temperature. The solution was cooled and neutralized (pH = 7.2) with 1 M phosphate buffer pH = 7.4 and 1 M HCl (1:1 mixture). Reactions were analyzed via HPLC.

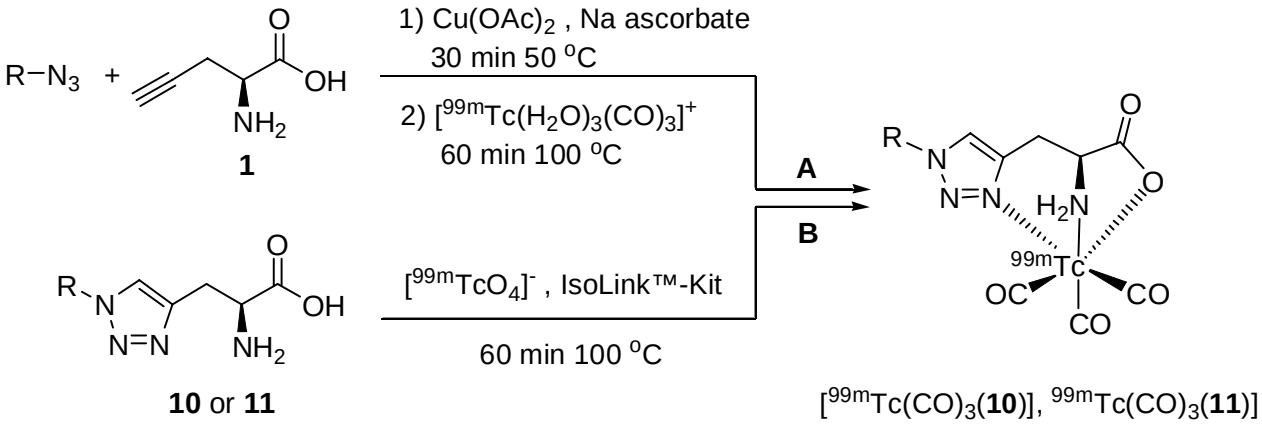


Figure 6: One-pot procedures **A** and **B** for the preparation of ^{99m}Tc complexes.

6 In vitro and in vivo studies of ^{99m}Tc-labeled bombesin derivatives

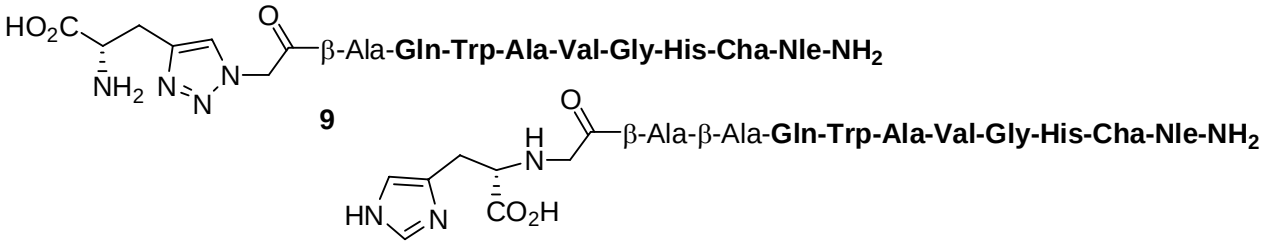


Figure 7: Amino acid sequence of bombesin derivatives included in these studies (receptor binding sequence in bold).

In vitro characterizazion: The human prostate adenocarcinoma cell line PC-3 was purchased from the European Collection of Cell Culture (ECACC; Salisbury, England). Cells were maintained in DMEM GLUTAMAX-I supplemented with 10% FCS, 100 IU/ml penicillin G sodium, 100 µg/ml streptomycin sulphate, 0.25 µg/ml amphotericin B. The cell culture was incubated at 37°C in an atmosphere containing 7.5% CO_2. The cells were subcultured weekly after detaching them with trypsin/EDTA (0.25%).

Inhibition studies (IC_{50}): PC-3 cells at confluence were placed in 48-well plates (1.5 x 10^5/well). Cells were incubated in triplicate for 1 h at 37°C in a special binding buffer (0.2 ml final volume per well) including protease inhibitors (50 mM HEPES, 125 mM NaCl, 7.5 mM KCl, 5.5 mM $MgCl_2$, 1 mM EGTA, 5 g/l BSA, 2 mg/l chymostatin, 100 mg/l soybean trypsin inhibitor, 50 mg/l bacitracin) with 150000-250000 cpm of the corresponding [^{99m}Tc(CO)$_3$(BBN)] complex per well and increasing concentrations of the different unlabeled BBN analogs (0-30000 nM). After incubation cells were washed twice with cold PBS and solubilized with 400µl (2x) of 1 N NaOH at 37°C and the final suspension measured in a γ-counter. IC_{50} values were calculated by non-linear regression analysis using GraphPad Prism™. Experiments were performed twice in triplicate.

Saturation studies (K_D): C-3 cells were prepared as described above and were incubated in triplicate with increasing concentrations (0.001-1 nM) of the ^{99m}Tc-bombesin analogs for 1 h at 37°C in the binding buffer already described. The total concentrations of technetium (^{99}Tc + ^{99m}Tc) were estimated according to Bauer and Pabst.[9] After incubation cells were washed twice with cold PBS and solubilized with 400µl (2x) of 1 N NaOH at 37°C. The bound radioactivity was measured in a γ-counter. Non-specific binding was determined under the same conditions by co-incubation of ^{99m}Tc-bombesin analogs and 1µM unlabeled BBN. Experiments were performed two to four times in triplicate.

Table 8: IC_{50} and K_D values of bombesin analogs.

	n	IC_{50} [nM]*	n	K_D [nM]**
BBN-N$^{\alpha}$AcHis	2	5.10 ± 1.75	2	0.19 ± 0.12
9	2	1.96 ± 1.55	3	0.19 ± 0.06

***** non-radiolabeled peptides

****** ^{99m}Tc-labeled peptides

[9] R. Bauer, H. Pabst, *Eur. J. Nucl. Med.* **1982**, *7*, 35.

In vivo characterization: The bombesin derivative has a β-Ala spacer between the binding sequence and the chelator/chelate has been shown previously to improve the pharmacokinetic profile of the radioconjugates.[10] N^{α}-Ac-His is currently being used in our ongoing peptide projects as a tridentate chelator for radiolabeling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ and $[^{188}Re(OH_2)_3(CO)_3]^+$.[11]

All animal experiments were conducted in compliance with the Swiss animal protection laws and with the ethical principles and guidelines for scientific animal trials established by the Swiss Academy of Medical Sciences and the Swiss Academy of Natural Sciences. Studies were performed with 6 to 8 weeks old female CD-1 nu/nu mice (20-25 g), purchased from Charles River Laboratories (Sulzfeld, Germany). On the day of the assay 3 groups of 3 mice each received the radiolabeled BBN analog (3.7 MBq/mouse) intravenously into the tail vein. To determine in vivo non-specific uptake another group of 3 mice received 100 μg of unlabeled Bombesin, co-injected with the radiolabeled BBN analog.

The first three groups of mice were sacrificed 0.5, 1.5 or 5 h[12] after injection by cervical dislocation and dissected. Samples of blood and tissues (heart, lung, spleen, kidneys, stomach, pancreas, ileum, colon, liver, muscle and bone) were removed, wet weighed and the amount of radioactivity was determined with a γ-counter. Results are expressed as a percentage of the injected dose per gram of tissue (% i.d./g).

Table 9 shows the tissue distribution of radioactivity 1.5 h (unblocked and blocked) and 24 h post injection in female CD-1 mice. The conjugate exhibited a rapid clearance from the blood pool and most tissues. The highest non-specific uptake was found in the kidneys and the liver. A significant uptake of radioactivity was observed in the GRP receptor-bearing pancreas and colon. The specificity of the uptake was confirmed by the receptor blocking study which led to an almost complete loss of radioactivity uptake in the pancreas and colon. The differences in uptake in other organs were not significant.

Table 9: Biodistribution (% I.D./g) of $[^{99m}Tc(CO)_3(\mathbf{N^{\alpha}AcHis\text{-}BBN})]$ and $[^{99m}Tc(CO)_3(\mathbf{9})]$ in CD-1 mice 1.5 h post injection of the radiotracers. Animals received the labeled analog by i.v. administration. Data, expressed as a percentage of the injected dose per gram of tissue, are mean ± SD (n = 3).

Organ	N^{α}AcHis-BBN	N^{α}AcHis-BBN (blocked)*	**9**	**9** (blocked)*
Blood	0.13 ± 0.01	0.35 ± 0.14	0.17 ± 0.01	0.19 ± 0.08
Heart	0.05 ± 0.01	0.14 ± 0.06	0.08 ± 0.02	0.16 ± 0.08
Kidneys	0.67 ± 0.04	1.36 ± 0.60	0.99 ± 0.11	0.84 ± 0.40
Liver	0.52 ± 0.13	1.66 ± 0.02	1.05 ± 0.17	1.59 ± 0.31
Pancreas	8.31 ± 3.36	3.73 ± 0.52	12.76 ± 2.91	0.80 ± 0.27
Stomach	0.68 ± 0.08	0.60 ± 0.09	0.89 ± 0.26	0.28 ± 0.10
Colon	5.80 ± 1.52	0.80 ± 0.01	2.87 ± 1.01	0.40 ± 0.15
Muscle	0.03 ± 0.02	0.60 ± 0.77	0.08 ± 0.07	0.37 ± 0.38
Bone	0.10 ± 0.05	0.24 ± 0.01	0.10 ± 0.03	0.20 ± 0.21

***** Co-injection of 100 μg unlabeled bombesin

[10] C. J. Smith, H. Gali, G. L. Sieckman, C. Higginbotham, W. A. Volkert, T. J. Hoffman, *Bioconjugate Chem.* **2003**, *14*, 93.

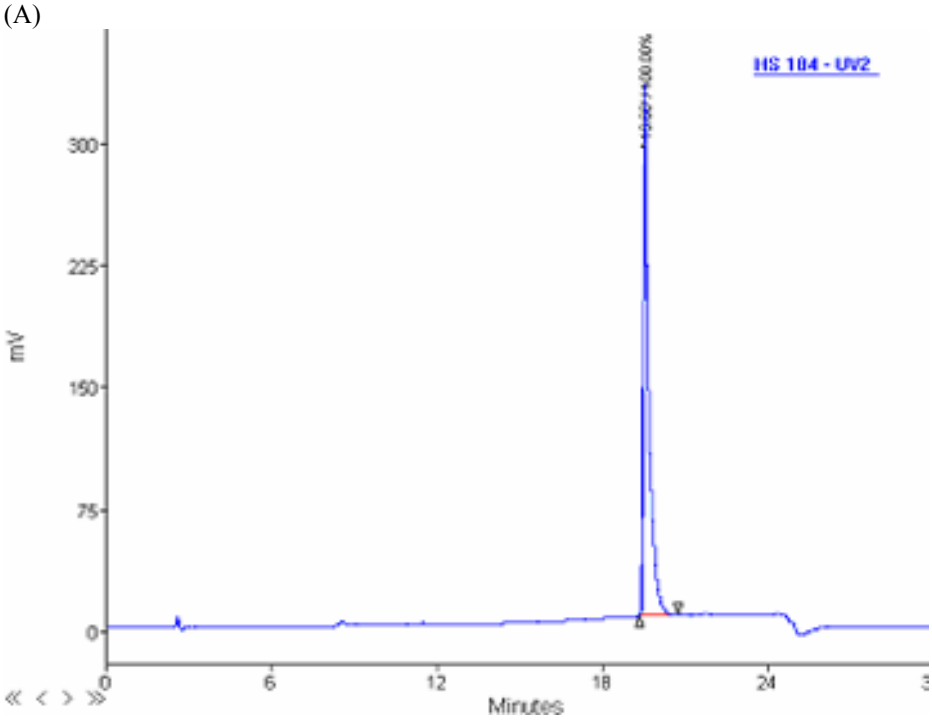
[11] (a) R. La Bella, E. Garcia-Garayoa, M. Bahler, P. Blauenstein, R. Schibli, P. Conrath, D. Tourwe, P. A. Schubiger, *Bioconjugate Chemistry* **2002**, *13*, 599. (b) E. Garcia-Garayoa, P. Blauenstein, M. Bruehlmeier, A. Blanc, K. Iterbeke, P. Conrath, D. Tourwe, P. A. Schubiger, *Journal of Nuclear Medicine* **2002**, *43*, 374. (c) A. Egli, R. Alberto, L. Tannahill, R. Schibli, U. Abram, A. Schaffland, R. Waibel, D. Tourwe, L. Jeannin, K. Iterbeke, P. A. Schubiger, *Journal of Nuclear Medicine* **1999**, *40*, 1913.

[12] Data for 0.5 h and 5 h will be published elsewhere.

7 HPLC analysis of ^{99m}Tc- and Re-complexes

The serial arrangement of the detectors (UV and radiometric detector) is responsible for the observed small differences of the retention time of Re- and ^{99m}Tc-complexes.

(A)



(B)

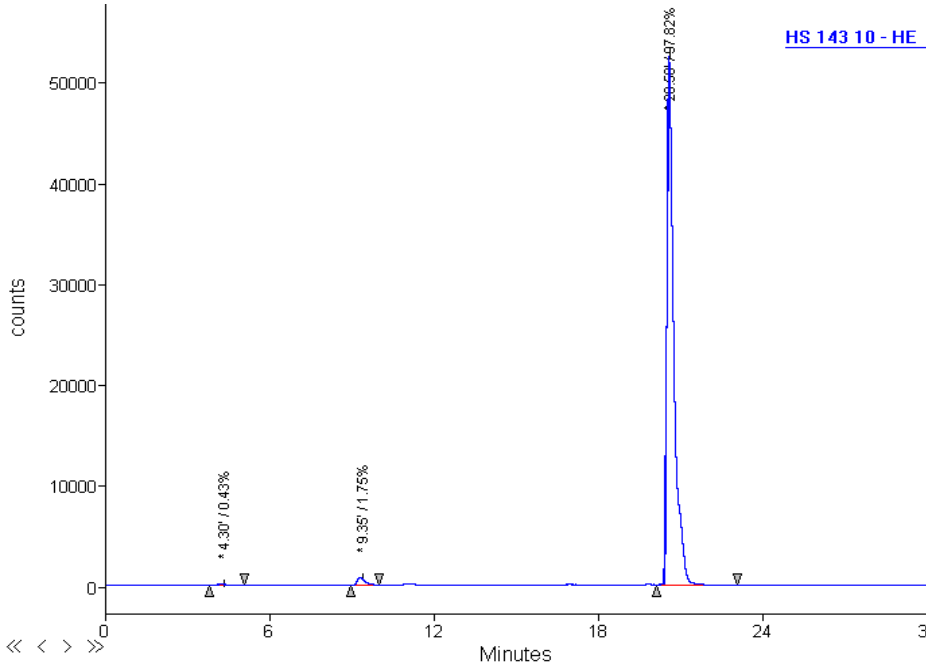
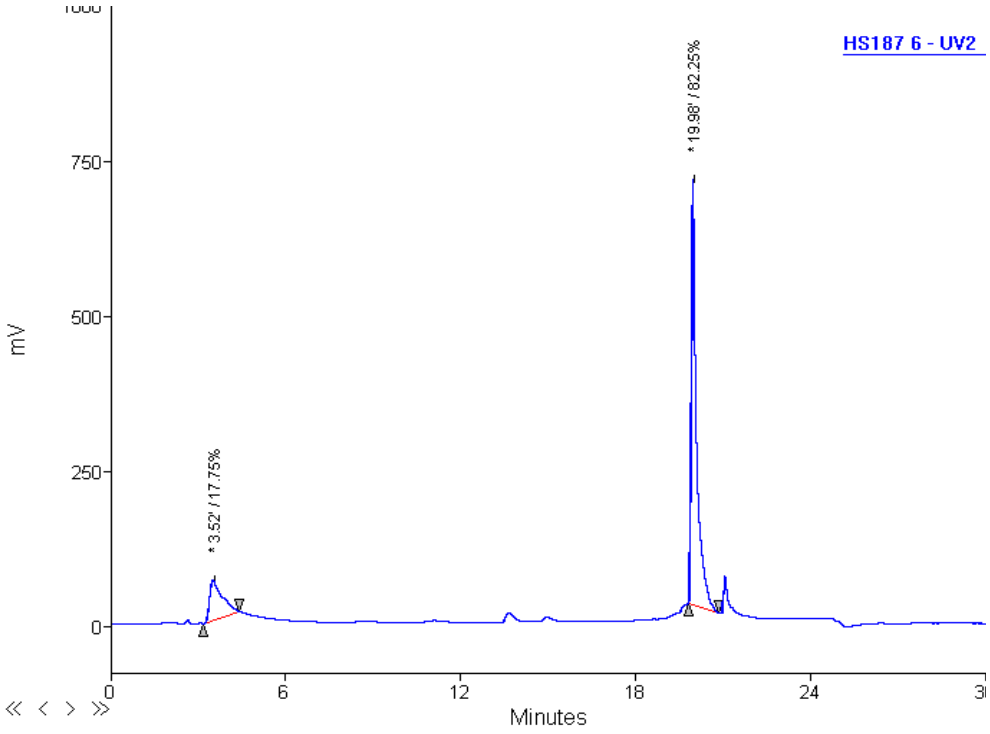


Figure 8: (A) HPLC trace of the purified complex [Re(CO)$_3$(**5**)] recorded at a wavelength of 254 nm. (B) HPLC trace (gamma trace) of [^{99m}Tc(CO)$_3$(**5**)]. Ligand concentration 10^{-5} M. HPLC parameters: Solvent system II; Nucleosil column.

(A)



(B)

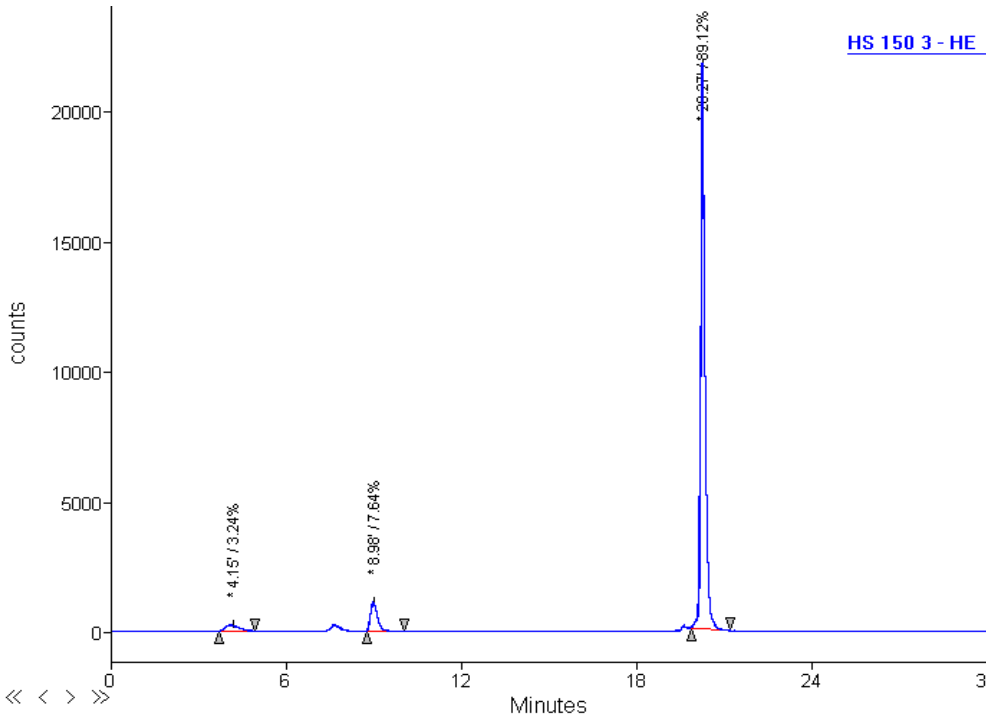
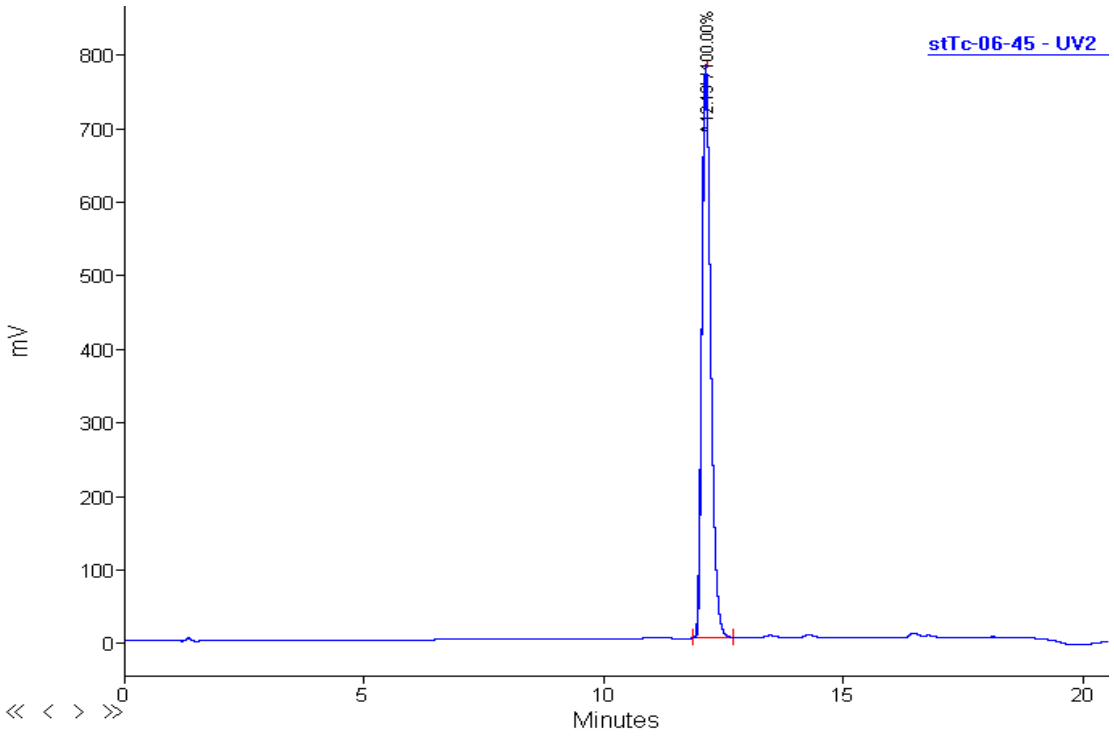


Figure 9: (A) HPLC trace of the complex [Re(CO)$_3$(**7**)] recorded at a wavelength of 254 nm. Signal at 3.5 min corresponds to [Re(CO)$_3$(H$_2$O)$_3$]$^+$. (B) HPLC trace (gamma trace) of [^{99m}Tc(CO)$_3$(**7**)]. Ligand concentration 10^{-4} M. HPLC parameters: Solvent system II; Nucleosil column.

A



B

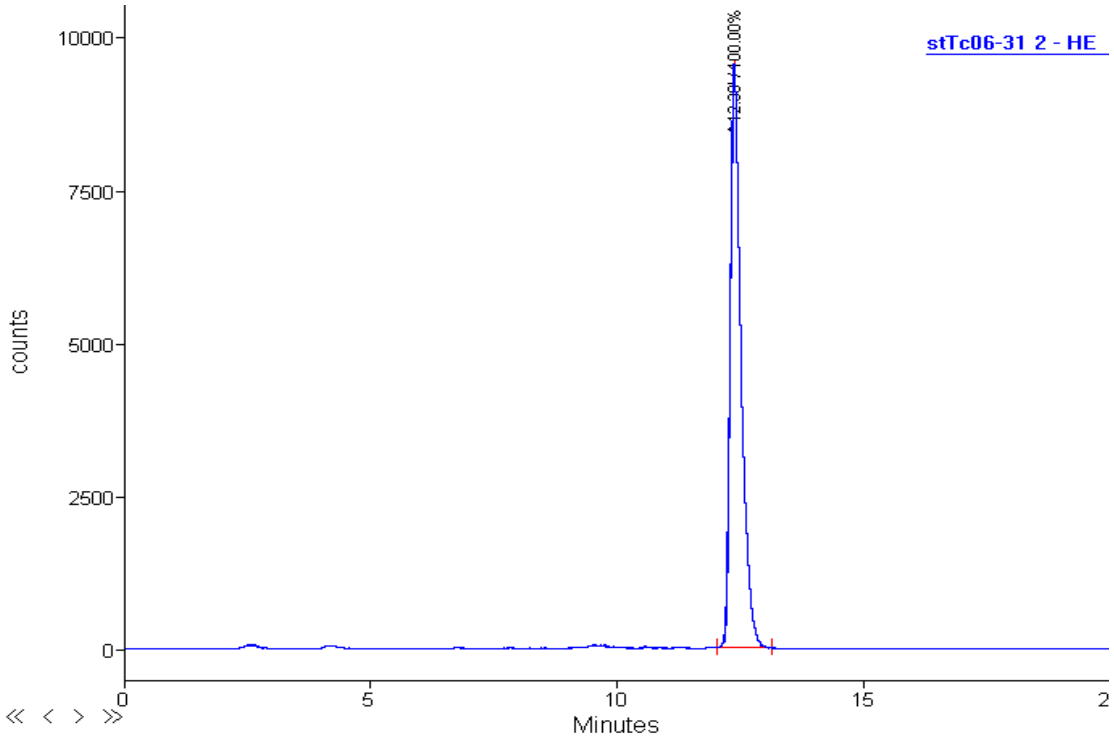


Figure 10: (A) HPLC trace of the purified complex [$Re(CO)_3$(**10**)] recorded at a wavelength of 254 nm. (B) HPLC trace (gamma trace) of [$^{99m}Tc(CO)_3$(**10**)]. Ligand concentration 10^{-5} M. HPLC parameters: Solvent system I; XTerra column.

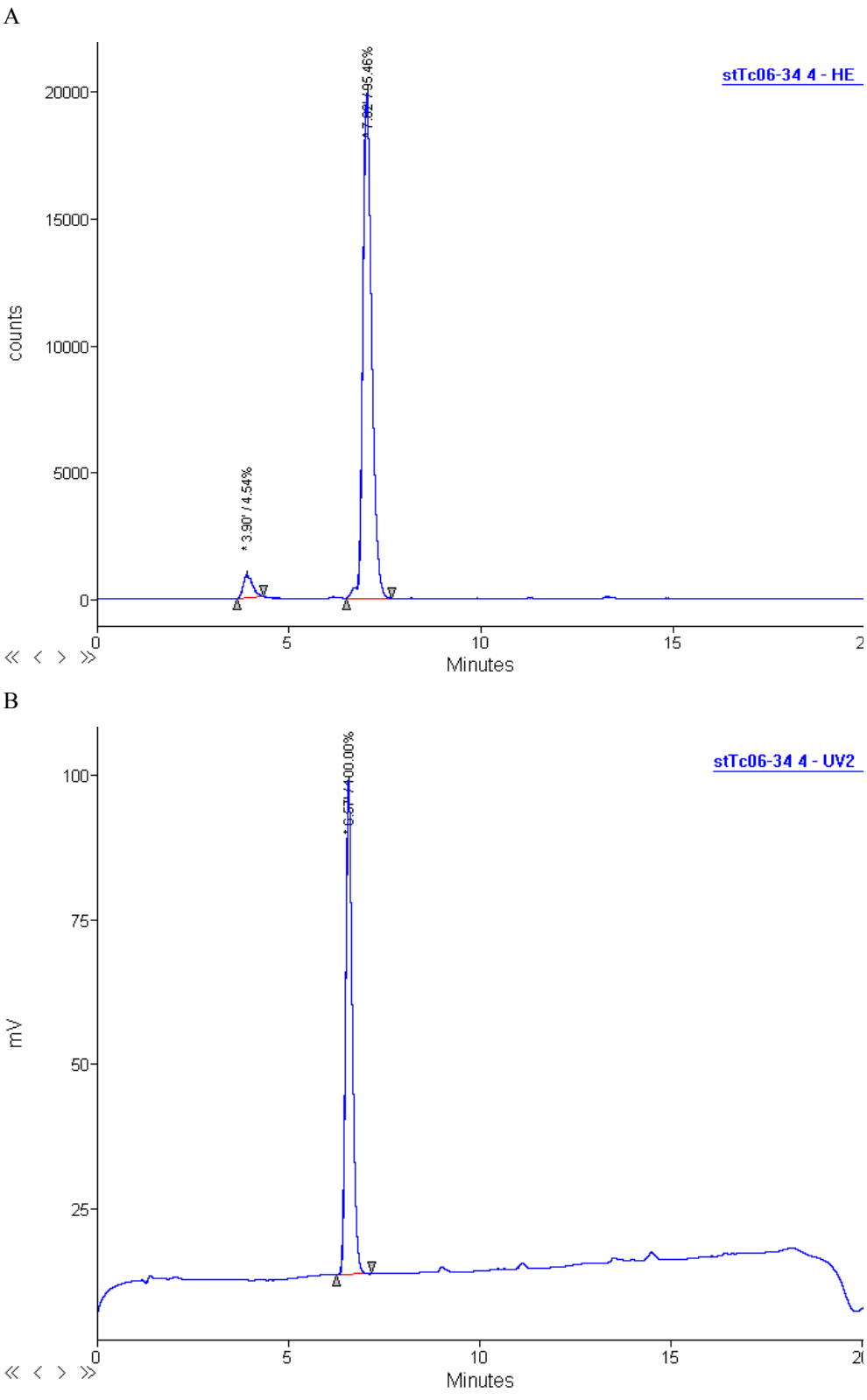


Figure 11: (A) HPLC trace of the purified complex [$Re(CO)_3$(**11**)] recorded at a wavelength of 254 nm. (B) HPLC trace (gamma trace) of [$^{99m}Tc(CO)_3$(**11**)]. Ligand concentration 10^{-5} M. HPLC parameters: Solvent system I; XTerra column.

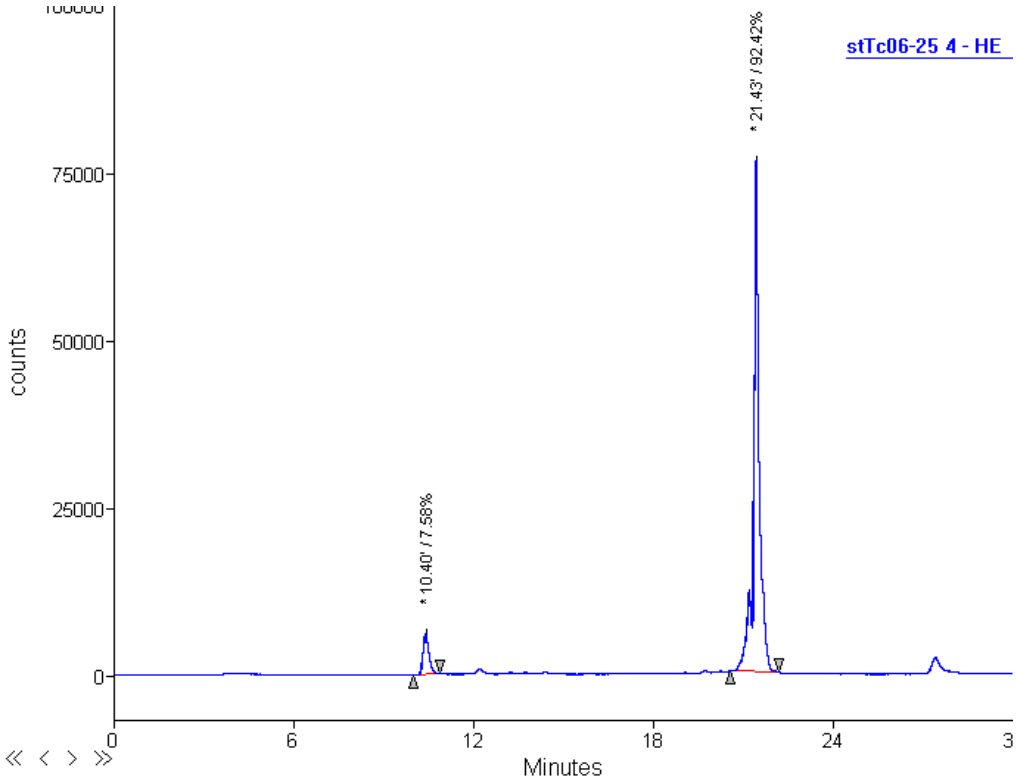


Figure 12: HPLC traces (gamma traces) of the radioactive labeling reaction of compound **9** with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ at a ligand concentration of 10^{-5} M. HPLC parameters: Solvent system I; Nucleosil C-18 column.

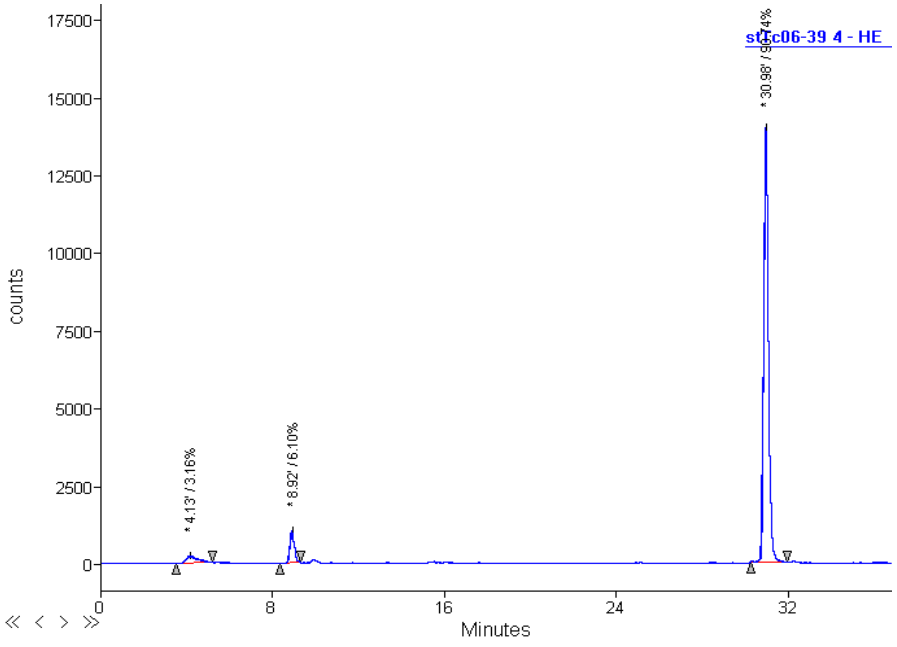


Figure 13: HPLC trace (gamma trace) of $[^{99m}Tc(CO)_3(\mathbf{12})]$. Ligand concentration 10^{-4} M. HPLC parameters: Solvent system I; Nucleosil C-18 column. Signal around 4 min corresponds to $[^{99m}Tc(OH_2)_3(CO)_3]^+$, signal around 10 min corresponds to $[^{99m}TcO_4]^-$.

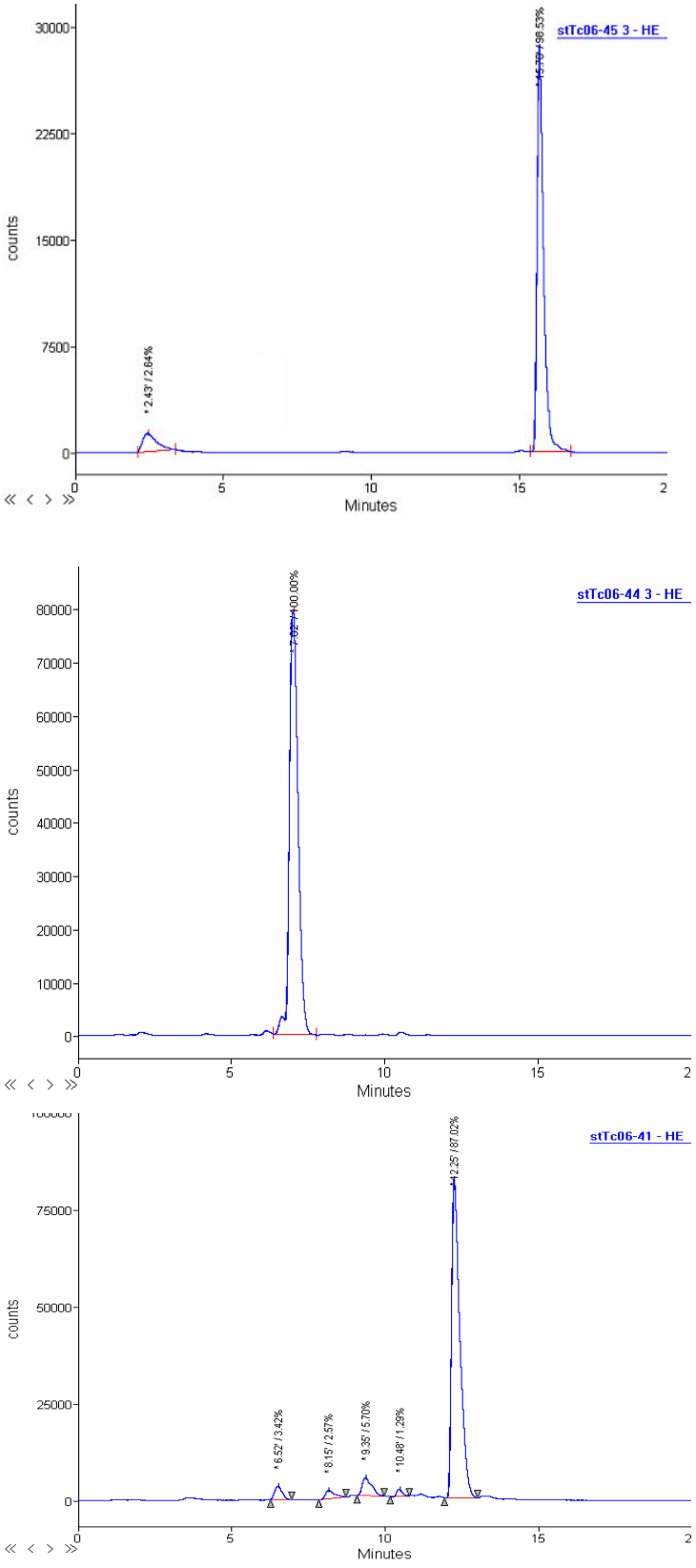


Figure 14: HPLC traces (gamma trace) of the Tc-99m complexes [^{99m}Tc(CO)$_3$(**5**)], [^{99m}Tc(CO)$_3$(**11**)] and [^{99m}Tc(CO)$_3$(**10**)] prepared via the one-pot procedure A described in Section 5.2. HPLC parameters: Solvent system II; XTerra column.