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# Molecular Assembly of Multifunctional $^{99m}$Tc Radiopharmaceuticals Using "Clickable" Amino Acid Derivatives

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Synthetic strategies that enable the efficient and selective combination of different biologically active entities hold great promise for the development of multifunctional hybrid conjugates useful for biochemical and medical applications. Starting from side-chain-functionalized N(α)-propargyl lysine derivatives, conjugates containing a $^{99m}$Tc-based imaging probe for SPECT and two different moieties (e.g., tumor-targeting vectors, pharmacological modifiers, affinity tags, or second imaging probes) can be assembled using the Cu$^{I}$-catalyzed alkyne–azide cycloaddition in efficient one-pot protocols. This strategy was successfully applied to the preparation of a $^{99m}$Tc-labeled conjugate comprising a tumor-targeting peptide sequence (bombesin(7–14)) and a low-molecular-weight albumin binder, a pharmacological modifier that prolongs the blood circulation time of the conjugate. Evaluation of the conjugate in vitro and in vivo provided promising results for its use as an imaging agent for the visualization of tumors positive for the gastrin-releasing peptide receptor. The methodology presented herein provides an attractive synthetic tool for the preparation of multifunctional $^{99m}$Tc-based radiopharmaceuticals with significant potential for a multitude of applications.

## Introduction

The radiopharmaceutical sciences are engaged in the development of radioactively labeled compounds for applications in nuclear medicine as imaging probes (employing diagnostic γ- and $\beta^+$-emitting isotopes) or radiotherapeutics (using emitters of α or $\beta^-$ particles or Auger electrons).[1,2] The majority of radioisotopes with decay properties suitable for diagnosis and particularly therapy are transition metals or elements that possess metallic character. As a consequence, bifunctional chelating agents (BFCA) are required for the stable complexation of the radionuclide and its attachment to targeted (bio)molecules (vectors) of interest. Besides efficient methodologies for the selective conjugation of radionuclide chelates to vectors, it would be desirable to have synthetic procedures at hand that facilitate the simultaneous introduction of pharmacological modifiers, which optimize the biological characteristics of a radioconjugate. Examples of other moieties of interest include affinity tags (e.g., biotin for pre-targeting approaches) and therapeutic agents (for the development of theranostics). Alternatively, the introduction of different imaging probes would yield dual-mode imaging agents, the development of which is of significant contemporary interest.[3–5] While the potential of multifunctional radioconjugates is intriguing, their preparation by conventional synthetic approaches is a challenging task. Therefore, new and efficient synthetic strategies, such as click chemistry, for the controlled assembly of multifunctional radionuclide-containing conjugates are of paramount importance.

We have shown that the Cu$^{I}$-catalyzed alkyne–azide cycloaddition (CuAAC),[6,7] a transformation known as a click reaction,[8] can be used for the efficient labeling of (bio)molecules with $^{99m}$Tc. By reacting appropriate alkyne pro-chelators with azide-functionalized derivatives of (bio)molecules, the synthesis of the ligand system and its selective conjugation can be accomplished simultaneously in a single step without protecting groups.[9] The conjugates obtained are functionalized with a tridentate ligand system containing a 1,4-disubstituted 1,2,3-triazole that can be radiolabeled directly in situ with the organometallic core $[^{99m}Tc(CO)_3(H_2O)_3]^+$.[10] Given the efficiency of this one-pot, two-step protocol, we termed this strategy "click-to-chelate". In the meantime we have demonstrated that this

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strategy can be applied to the preparation of a variety of $^{99m}$Tc-based single photon emission computed tomography (SPECT) probes suitable for applications in vitro and in vivo.[11–13] We now report an extension of our click chemistry strategy. The use of readily available N(α)-propargyl amino acid derivatives as click substrates not only offers a convenient and efficient method for labeling targeted (bio)molecules with $[^{99m}Tc(CO)_3]^+$ (e.g., R = H, Figure 1), it also provides a means for the concurrent introduction of a second entity of interest (R = various entities attached by functionalization of the side chain, Figure 1). The possibility of combining two different moieties with biological function and a chelating system in a single step is an attractive feature for the development of metallic radiotracers and opens up numerous potential applications.

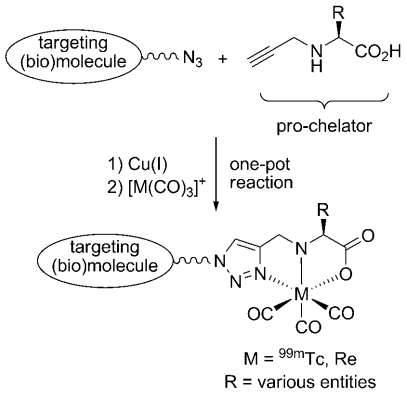
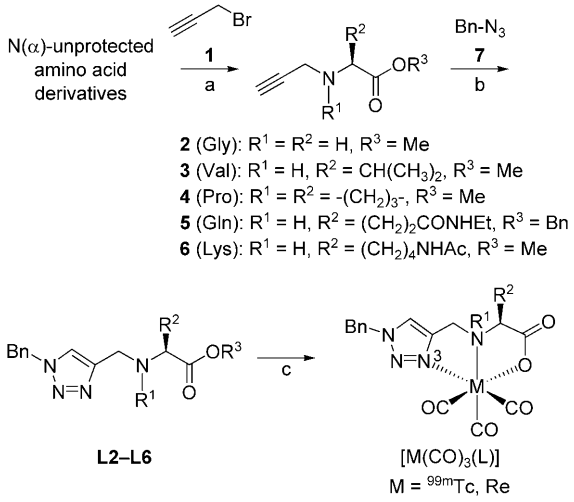



**Figure 1.** N(α)-propargyl derivatives of amino acids as CuAAC substrates for the one-pot assembly of multifunctional $^{99m}$Tc radioconjugates. Squiggled lines represent different spacers and R (residue) stands for amino acid specific side chains to which different entities can be conjugated.

## Results and Discussion

### Chemical syntheses

Amino acids represent a structurally diverse pool of building blocks for the development of ligand systems for complexation of the tricarbonyl core $[M(CO)_3]^+$ (M = $^{99m}$Tc, Re). For example, chelators derived from histidine, cysteine, lysine, or synthetic analogues have all been reported in this context.[9,14,15] We recognized that N(α)-propargyl-functionalized amino acids are readily available precursors for the "click-to-chelate" strategy (Scheme 1).[12,13] To verify their general utility for the preparation of $^{99m}$Tc-based radiotracers, we studied a series of N(α)-propargyl amino acid derivatives. Thus, reaction of propargyl bromide (**1**) with HGlyOMe, HValOMe, HProOMe, HGln(Et)OBn, and HLys(Ac)OMe efficiently provided N(α)-alkylated propargyl derivatives **2**–**6**.[16,17] Subsequent CuAAC with model compound benzyl azide (**7**) in aqueous media at room temperature yielded 1,2,3-triazole-containing ligands **L2**–**L6** in a single step and high yields.[12] Reaction of the tridentate chelators with $[Re(CO)_3Br_3][Et_4N]_2$[18] in alcohol or water according to previously described procedures resulted in hydrolysis of the ester functionalities and gave complexes $[Re(CO_3)(L)]$ (L = **L2**–**L6**) as single products (HPLC).[12] All rhenium complexes were fully




**Scheme 1.** Synthesis of N(α)-propargyl amino acid derived tridentate chelating systems and their complexes with $[M(CO)_3]^+$ (M = $^{99m}$Tc, Re). a) For GlyOMe, ValOMe and ProOMe: MeOH, $K_2CO_3$, RT; for Gln(Et)OBn and Lys(Ac)OMe: THF, DIPEA, 50 °C, 1–2 days; b) for **2**, **3**, **6**: *t*BuOH/$H_2O$ (1:1), 20 mol% $Cu(OAc)_2$, 40 mol% sodium ascorbate, RT, 18 h; for **4**, **5**: *t*BuOH/$H_2O$ (1:1), excess Cu(0), RT, 20 h; c) for M = Re: $[Re(CO)_3Br_3][Et_4N]_2$, alcohol/$H_2O$, 50–75 °C, 0.5–4 h; for M = $^{99m}$Tc: $[^{99m}Tc(CO)_3(H_2O)_3]^+$, PBS pH ~7, 100 °C, 30 min.

characterized (NMR, IR, MS). In the case of L = **L4**, **L5**, and **L6**, crystals of the corresponding rhenium complexes suitable for X-ray analysis could be obtained. Figure 2 shows the ORTEP plots of the three complexes, confirming the coordination by the N(3) atom of the 1,2,3-triazole heterocycle, and the N(α) and carboxylate of the amino acid as depicted in Scheme 1. All amino acid derived chelators could also be readily radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ in aqueous phosphate buffer at 100 °C and pH ~7. Quantitative formation of radionuclide complexes was achieved in all cases at ligand concentrations in the micromolar range ($10^{-5}$–$10^{-6}$ M), which again demonstrates the excellent efficiency of 1,2,3-triazole-containing tridentate chelating systems for complexation of the $^{99m}$Tc–tricarbonyl core. Identity of the $[^{99m}Tc(CO)_3(L)]$ (L = **L2**–**L6**) complexes was confirmed in each case by comparison of the γ-HPLC with the UV trace of the corresponding rhenium analogues, a procedure which is common practice with $^{99m}$Tc complexes on a no carrier added (n.c.a.) level.

While all investigated N(α)-1,2,3-triazolyl amino acid derivatives were shown to be efficient chelators for $[M(CO)_3]^+$, only those with a functionalizable side chain group (Lys, Gln/Glu) could be considered for an extended "click-to-chelate" approach. In an initial series, we investigated the utility of protected glutamic acid derivatives. However, we observed that intermediates obtained from protected HGlu(*t*Bu)OMe according to Scheme 1 spontaneously undergo intramolecular lactamization (data not shown). We therefore focused on Lys derivatives and investigated the chemistry required for the synthesis of a common click precursor to which different entities can be selectively conjugated via the N(ε) amine (Scheme 2). BocLysOMe **8** was converted into trifluoroacetate **9**, and the Boc group was cleaved using TFA. N(α) alkylation of intermedi-

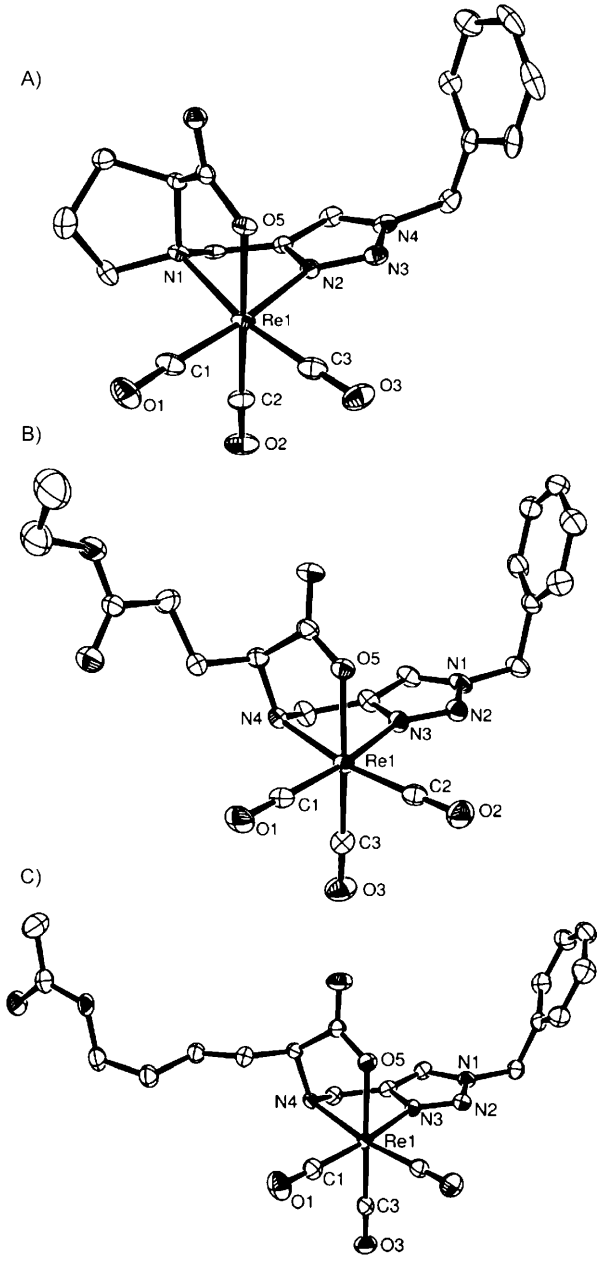
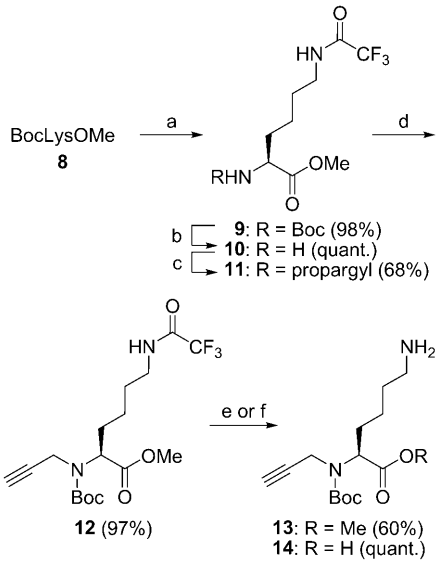



**Figure 2.** ORTEP-3[19] representations of the complexes A) $[Re(CO)_3(\mathbf{L4})]$ with thermal ellipsoids shown at 20% probability, and B) $[Re(CO)_3(\mathbf{L5})]$ and C) $[Re(CO)_3(\mathbf{L6})]$ with thermal ellipsoids shown at 50% probability. Only one out of seven molecules in the asymmetric unit of $[Re(CO)_3(\mathbf{L4})]$ is shown. Hydrogen atoms and solvent molecules are omitted for clarity. Selected bond lengths [Å] and angles [°]: $[Re(CO)_3(\mathbf{L4})]$ Re(1)–C(3) 1.890(9), Re(1)–C(2) 1.920(8), Re(1)–C(1) 1.920(8), Re(1)–O(5) 2.110(4), Re(1)–N(2) 2.142(5), Re(1)–N(1) 2.250(5), C(3)–Re(1)–C(2) 87.1(3), C(3)–Re(1)–C(1) 88.6(3), C(2)–Re(1)–C(1) 88.1(3), C(2)–Re(1)–O(5) 176.8(2), C(3)–Re(1)–N(1) 170.0(3), C(1)–Re(1)–N(2) 173.4(3). $[Re(CO)_3(\mathbf{L5})]$ Re(1)–C(1) 1.907(4), Re(1)–C(3) 1.912(4), Re(1)–C(2) 1.919(4), Re(1)–O(5) 2.137(3), Re(1)–N(3) 2.156(3), Re(1)–N(4) 2.228(3); C(1)–Re(1)–C(3) 86.32(17), C(1)–Re(1)–C(2) 92.87(16), C(3)–Re(1)–C(2) 86.72(17), C(3)–Re(1)–O(5) 174.20(13), C(1)–Re(1)–N(3) 171.59(14), C(2)–Re(1)–N(4) 170.16(13). $[Re(CO)_3(\mathbf{L6})]$ Re(1)–C(3) 1.888(3), Re(1)–C(2) 1.912(3), Re(1)–C(1) 1.925(3), Re(1)–O(5) 2.1373(19), Re(1)–N(3) 2.157(2), Re(1)–N(4) 2.243(2); C(3)–Re(1)–C(2) 88.29(12), C(3)–Re(1)–C(1) 87.00(13), C(2)–Re(1)–C(1) 88.12(13), C(3)–Re(1)–O(5) 173.46(11), C(1)–Re(1)–N(3) 171.44(10), C(2)–Re(1)–N(4) 171.92(10).

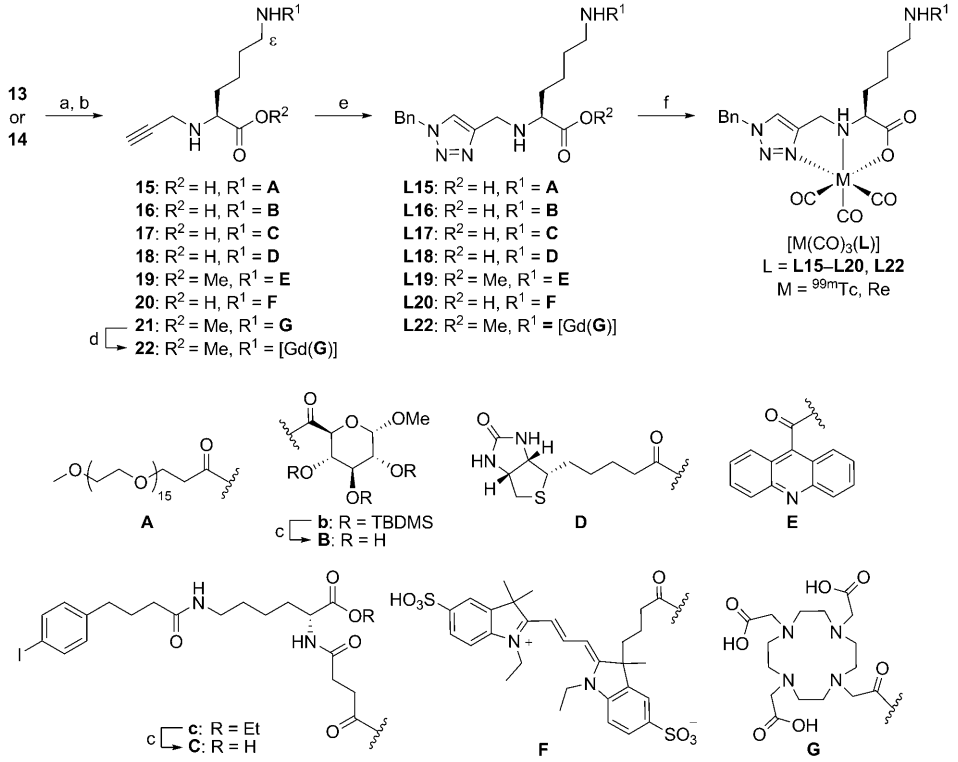



**Scheme 2.** Synthesis of a Lys-derived, common alkyne precursor for the extended "click-to-chelate" approach. a) trifluoroacetic acid anhydride, THF, DIPEA, RT, 5 h; b) $CH_2Cl_2$/TFA (3:1), RT, 18 h; c) propargyl bromide (**1**), THF, DIPEA, RT, 2 days; d) $Boc_2O$, THF, DIPEA, 55 °C, 2 days; e) for **13**: MeOH/$NH_4HCO_{3(aq)}$ 1 M (1:1), 55 °C, 3 days; f) for **14**: MeOH/$K_2CO_{3(aq)}$ 0.5 M (1:1), 75 °C, 3 h.

ate **10** with propargyl bromide (**1**) followed by reaction of the resulting secondary amine **11** with Boc anhydride yielded compound **12**. Installation of the N(α)-Boc group became necessary, because attempted direct alkylation/acylation of the primary N(ε)-amine in the presence of the N(α) secondary amino group of Lys derivatives yielded complex mixtures of isomeric products (data not shown). Selective removal of the N(ε)-trifluoroacetate in the presence of the N(α)-Boc group was achieved under carefully optimized reaction conditions.[20] Thus, treatment of a solution of intermediate **12** in methanol with $NH_4HCO_3$ provided methyl ester **13**, whereas the use of $K_2CO_3$ gave intermediate **14**. Compounds **13** and **14**, each prepared in five steps and good overall yield, were equally suitable as a common alkyne precursor for our studies.

Conjugation of various commercial or synthesized carboxylic acid derivatives to the N(ε) amino group of precursors **13** or **14** by standard amide coupling protocols was straightforward (Scheme 3; see Experimental Section and the Supporting Information for details). After successful coupling, the N(α)-Boc group of the Lys moiety of intermediates was removed in quantitative yield by treatment with TFA. In the case of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) derivative **21**, this also resulted in the cleavage of the *tert*-butyl ester groups. For compounds **16** and **17**, an additional deprotection step was required to remove TBDMS and the ethyl ester group, respectively. Thus, starting from either **13** or **14**, alkyne substrates **15**–**22**, ready for use in click conjugations, were obtained in 2–3 synthetic steps and in good yields (60–90%). The alkyne derivatives described herein are already interesting for the modification of (bio)molecules by CuAAC.




**Scheme 3.** Synthesis of N(ε)-functionalized, N(α)-propargyl Lys click precursors, CuAAC thereof with model compound benzyl azide (**7**) and formation of the corresponding $^{99m}$Tc and Re complexes. a) For **15**: **14**, NHS-m-dPEG$_{15}$, DMSO, RT, 24 h; for **16**: **14**, 1-*O*-methyl-β-D-glucuronic acid-2,3,4-tris-TBDMS ether,[16] HBTU, DIPEA, DMF, RT, 2 h; for **17**: **13**, (*R*)-2-succinylamido-6-[4-(4-iodophenyl)butanamido]hexanoic acid ethyl ester,[16] HBTU, DIPEA, DMF, RT, 2 h; for **18**: **14**, biotin–NHS ester, DMSO, RT, 20 h; for **19**: **13**, acridine-9-carboxylic acid, HOBT, DCC, DMF, 80 °C, 18 h; for **20**: **14**, DY547–NHS ester, DMSO, RT, 18 h; for **21**: **13**, DOTA(*t*Bu)$_3$–NHS ester, $CH_2Cl_2$, reflux, 2 h; b) $CH_2Cl_2$/TFA (4:1, 25 mL mmol$^{-1}$), RT, 18 h; c) for **16**: TBAF, THF, RT, 18 h; for **17**: MeOH, aqueous NaOH, RT, 18 h; d) $GdCl_3$, $H_2O$, 90 °C, 1.5 h; e) for **15**, **16**, **18**, **20**: benzyl azide (**7**), *t*BuOH/$H_2O$ (1:1), excess Cu(0), RT, 20 h; for **17**, **19**, **22**: benzyl azide (**7**), *t*BuOH/$H_2O$ (1:1), 20 mol% $Cu(OAc)_2$, 40 mol% sodium ascorbate, RT, 18 h; f) for M = Re: $[Re(CO)_3Br_3][Et_4N]_2$, alcohol or $H_2O$, 60 °C, 0.5–4 h; for M = $^{99m}$Tc: $[^{99m}Tc(CO)_3(H_2O)_3]^+$, PBS pH ~7, 100 °C, 30 min.

However, only when labeled with $^{99m}$Tc is the in vivo tracking and quantification of the conjugate possible.

Compounds **15**–**17** contain various pharmacological modifiers. It has been shown that PEGylation or glycosylation of peptide-based radiotracers can improve their characteristics in vivo.[21–23] Thus, derivatives **15** ($PEG_{15}$) and **16** (glucuronic acid) could be generally useful for the attachment of polar modifiers to $^{99m}Tc(CO)_3$-based radiopharmaceuticals. Substructure **17** contains a recently published example of a small organic albumin-binding molecule (**C**) useful for prolonging the circulation time of conjugates in the bloodstream (see below).[24,25] Derivatives **18** and **19** carry biotin, an affinity tag applicable for sequential bioconjugations (e.g., pre-targeting approaches[26]) and acridine, a DNA intercalator, respectively. Application of the latter to radiopharmaceuticals has been suggested for the delivery of short-range Auger-electron-emitting radioisotopes to the cell nucleus to enhance their therapeutic efficacy.[27,28] Finally, alkyne precursors **20** and **21** functionalized with an organic dye (DY547) or the macrocyclic chelator DOTA, respectively, represent examples of substrates for the development of dual-modality imaging agents. Conjugates derived from precursor **20** could be used for SPECT and optical (fluorescence) imaging whereas those obtained from DOTA derivative **21** would enable combination with other metals, such as paramagnetic gadolinium, yielding a potential MRI/SPECT probe. The described approach for the preparation of functionalized alkyne pro-chelators for bioconjugation by CuAAC is not limited to the examples discussed above, but is also applicable to virtually any biochemical and chemical entity of interest as well as combinations thereof.

With the exception of the chelating system DOTA, the various moieties conjugated to the N(α)-propargyl Lys precursors **13** and **14** did not interfere with the CuAAC and the subsequent chelation step with $[M(CO)_3]^+$. Thus, compounds **15**–**20** were subjected to CuAAC with benzyl azide (**7**) under reaction conditions similar to those described above (Scheme 1). In all cases, ligand systems **L15**–**L20** were obtained in good yields. Compound **21** was not a suitable substrate for the copper-catalyzed cycloaddition because the copper catalyst was sequestered by the macrocyclic chelator DOTA.[29] However, CuAAC of the pre-formed[30,31] $Gd^{3+}$ complex **22** provided the desired product **L22** cleanly, with quantitative conversion of the substrates.[32] Metal labeling of the ligands **L15**, **L20**, and **L22** with $[M(CO)_3]^+$ (M = $^{99m}$Tc, Re) was performed according to the procedure described for the amino acid model compounds. Complexes $[^{99m}Tc(CO)_3(L)]$ (L = **L15**–**L20** and **L22**) were obtained as single products (HPLC), the identities of which were again confirmed by HPLC comparison with the corresponding fully characterized rhenium analogues $[Re(CO)_3(L)]$ (L = **L15**–**L19** and **L22**),[16] with the exception of $[Re(CO)_3(\mathbf{L20})]^+$, which was available only in very small amounts. In addition, we were able to show that our previously reported one-pot, two-step protocol[9,13] can be applied not only to simple alkyne precursors of ligand systems **L2**–**L6** but also to functionalized propargyl substrates **15**, **18**, and **20** (Figure 3). For example, treatment of aliquots of the crude reaction solutions obtained from the CuAAC of benzyl azide (**7**) and alkynes **15**, **18**, or **20** with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ yielded final radioconjugates $[^{99m}Tc(CO)_3(L)]$ (L = **L15**, **L18**, **L20**) directly in high purity (> 90%), identical to those obtained with pre-synthesized and purified ligands. These examples represent unprecedentedly effi-

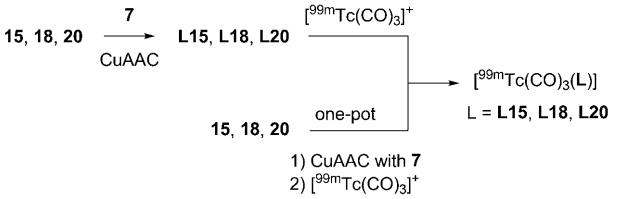



**Figure 3.** One-pot, two-step procedure (bottom) yields $^{99m}$Tc-labeled conjugates, which are identical to those prepared by multistep synthesis involving isolation and purification of intermediates (top).

cient examples of the combination a radionuclide chelate with two different moieties without the use of protecting groups.

### In vitro and in vivo evaluation

We previously reported a series of $^{99m}$Tc-labeled derivatives of bombesin (BBS),[22,33] a regulatory peptide that targets the gastrin-releasing peptide (GRP) receptor, which is overexpressed in various tumor types such as prostate, breast, and small-cell lung cancer.[34] Like other radiolabeled regulatory peptides potentially useful as imaging probes for the detection of tumors and metastasis,[35] radiotracers derived from BBS or its binding sequence BBS(7–14) are rapidly degraded both extracellularly and intracellularly by peptidases. The fast clearance of radiolabeled peptides from the blood pool is generally appreciated for diagnostic purposes. However, it also limits tumor uptake, and thus may represent a potential limitation for their use as therapeutic radiopharmaceuticals. Common strategies to extend the serum half-life of peptide-based radiotracers in vivo include PEGylation, the formation of multimers or structural modifications for stabilization against enzymatic degradation.[23,36,37] Alternative approaches make use of albumin-binding molecules.[24,38] By noncovalent interaction with serum albumin, this class of compounds has been shown to prolong the in vivo circulatory half-life of conjugated probes (e.g., fluorophores and MRI contrast agents) as well as antibody fragments.[24,25] We reasoned that the fusion of a low-molecular-weight albumin binder ($K_d$: micromolar range) to BBS(7–14) radioconjugates would result in a prolonged circulation time in the blood without compromising the targeting capacity of the high-affinity peptide ($K_d$: nanomolar range). In addition, association of the radioconjugate to albumin could provide some protection against enzymatic degradation.

Stabilized BBS(7–14) derivative **23**, containing a "clickable" azide functionality attached to its N terminus, was prepared by standard solid-phase peptide synthesis (SPPS) employing Fmoc strategies in analogy to reported procedures (Figure 4).[12,33] Alkyne **17** equipped with the albumin binding molecule **C** (Scheme 3) was conjugated to azidopeptide **23** by CuAAC in a mixture of water and ethanol at 60 °C to yield BBS(7–14) derivative **L24**. Reference compound N$^{\alpha}$HisAc-BBS(7–14), identical in all respects but lacking the albumin binding moiety and functionalized with the established N(α)-HisAc chelating system, was prepared by SPPS.[39] Both peptides were readily radiolabeled with $[^{99m}Tc(CO)_3]^+$ in PBS (pH 6.5) for 1 h at 75 °C. The

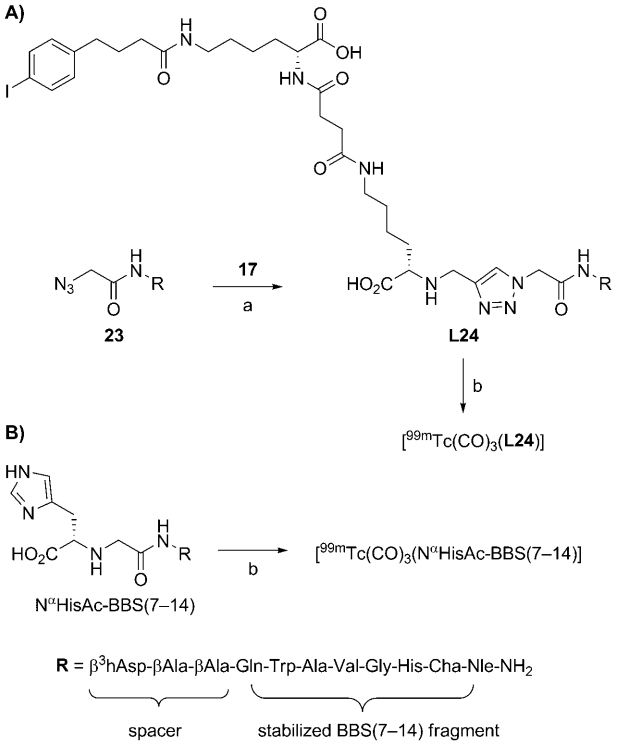



**Figure 4.** Syntheses of a radiolabeled BBS(7–14) derivative A) functionalized with an albumin binding moiety by click chemistry and B) reference compound N$^{\alpha}$HisAc-BBS(7–14). a) EtOH/$H_2O$ (1:1), 10 mol% $Cu(OAc)_2$, 20 mol% sodium ascorbate, 60 °C, 4 h; b) 0.1 mM peptide in PBS (pH 6.5), $[^{99m}Tc(CO)_3]^+$ (200—500 MBq), 1 h at 75 °C. (Cha = Cyclohexylalanine, Nle = Norleucine).

formation of a single radioactive species was confirmed in each case by γ-HPLC (radiochemical yield and purity > 90%).

$[^{99m}Tc(CO)_3$(**L24**)] was assessed in vitro for its binding to albumin and GRP-receptor-overexpressing PC3 cells.[16] In brief, incubation of $[^{99m}Tc(CO)_3$(**L24**)] in mouse blood serum at 37 °C for 15 min and analysis of samples by size-exclusion chromatography indicated quantitative binding of the radioconjugate to mouse serum albumin. Binding of $[^{99m}Tc(CO)_3$(**L24**)] to intact PC3 cells was also examined. Approximately 6% of total radioactivity added was found bound to the cells after 1 h at 37 °C. The binding could be blocked by the presence of excess BBS(7–14) (1 μM), hence proving receptor specificity.

To study the effect of the albumin binder conjugated to radiolabeled BBS derivatives in vivo, time-dependant biodistribution studies were performed with $[^{99m}Tc(CO)_3$(**L24**)] and reference compound $[^{99m}Tc(CO)_3$(N$^{\alpha}$HisAc-BBS(7–14))] in nude mice bearing PC3 tumor xenografts.[16] Figure 5 shows selected data of the biodistribution of the two radiotracers at 1 and 24 h post injection (p.i.). Clearly, blood clearance was much slower for $[^{99m}Tc(CO)_3$(**L24**)] (14 and 6% of injected dose per gram ($ID\,g^{-1}$) after 1 and 24 h p.i., respectively) than for $[^{99m}Tc(CO)_3$(N$^{\alpha}$HisAc-BBS(7–14))] (0.23% at 1 h p.i., and complete clearance after 24 h). The longer circulation time of $[^{99m}Tc(CO)_3$(**L24**)] is likely the result of its binding to serum albumin, which is also consistent with the higher uptake observed in most organs and tissue 24 h p.i., in particular in the

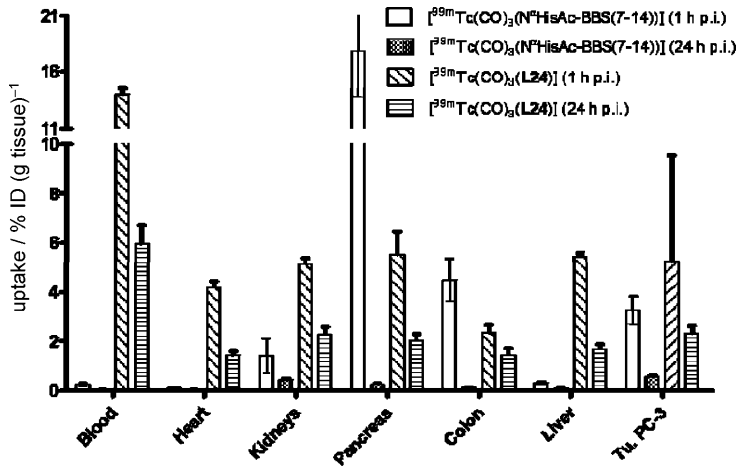



**Figure 5.** Comparison of the biodistribution of [$^{99m}$Tc(CO)$_3$(**L24**)] and [$^{99m}$Tc(CO)$_3$(N$^{\alpha}$HisAc-BBS(7–14))] in nude mice bearing PC3 xenografts 1 h and 24 h p.i. ($n = 3$–4).

well-perfused heart, spleen, lung, liver, and kidney.[40] Initially, reference compound [$^{99m}$Tc(CO)$_3$(N$^{\alpha}$HisAc-BBS(7–14))] was taken up to a higher extent by GRP-receptor-positive tumors, colon, and pancreas (1 h p.i.); however, accumulation of radioactivity was significantly higher for [$^{99m}$Tc(CO)$_3$(**L24**)] after 24 h. Biodistribution studies performed after co-injection of natural bombesin (0.1 mg per mouse; blocking experiments) revealed a significant attenuation of the radioactivity in receptor-positive colon and pancreas, while the uptake in non-targeted tissue and organs (e.g., blood, liver, and kidney) remained unchanged. These results confirm the specific, GRP-receptor-mediated uptake of the radiotracer (see the Supporting Information for details).

While the attachment of a low-molecular-weight albumin binder to radiolabeled BBS derivatives resulted in the desired prolongation of the circulation time of the conjugate in vivo, the tumor-to-background ratio is not yet suitable for nuclear imaging and therapy due to high background radioactivity. We speculate that the interaction of the radioconjugate with albumin may be too strong, or alternatively, may restrict the accessibility of the BBS moiety, both of which could influence the binding to GRP receptors. Alternatively, the radiopeptide bound to the albumin could be partly enzymatically degraded; this could explain the observed high level of radioactivity circulating in the blood after 24 h without an increase in uptake by tumor tissue. Investigations employing analogues of the albumin binder moiety **C** (Scheme 3) of lower affinity,[24] the use of extended linkers between the different functional moieties of the radioconjugate, and applications to other tumor-seeking vectors are currently ongoing.

## Conclusions

In summary, we have shown that CuAAC can be used to combine a (radio)metal chelating system, a targeting (bio)molecule, and various secondary entities in a single step. Starting from azide-functionalized (bio)molecules and suitable alkyne prochelators derived from a common N(α)-propargyl Lys derivative, targeted radionuclide conjugates containing pharmacological modifiers, affinity tags, or a second imaging probe can be assembled in a few synthetic steps with high yields using convenient and efficient one-pot procedures. The extended "click-to-chelate" approach was successfully applied to the preparation of a novel $^{99m}$Tc-labeled bombesin derivative equipped with a low-molecular-weight albumin-binding molecule to extend the biological half-life of the radioconjugate. Evaluation of the compound in vitro and in vivo provided encouraging results in terms of prolonged circulation time in the bloodstream as well as receptor-specific targeting of GRP-receptor positive tumors. The methodology presented herein provides an attractive synthetic tool for the preparation of multifunctional $^{99m}$Tc-based radiopharmaceuticals with high potential for a multitude of applications.

## Experimental Section

Only key intermediates and final products are described here. For detailed information on experimental procedures, including general methods, equipment, solid-phase peptide synthesis (SPPS), in vitro and in vivo experiments, and analytical data for all compounds ($^1$H and $^{13}$C NMR spectra, HPLC chromatograms of $^{nat}$Re and $^{99m}$Tc complexes), see the Supporting Information; CIF files of X-ray structures are available at the CCDC (see below).

**CAUTION:** $^{99m}$Tc is a γ-emitter (140 keV) with a half-life of 6.01 h. All reactions involving $^{99m}$Tc were performed in a laboratory approved for the handling of radioisotopes, and appropriate safety procedures were followed at all times to prevent contamination.

### Chemical syntheses

**General procedure A: Synthesis of N(α)-propargyl amino acid derivatives.** *Method A1:* Amino acid substrates were dissolved in MeOH (4 mL mmol$^{-1}$) and $K_2CO_3$ (3 equiv), and propargyl bromide (**1**; 80% in toluene, 2 equiv) was added. The mixture was stirred at RT for 2 days, filtered and concentrated under reduced pressure. The crude products were purified by flash chromatography on silica gel. *Method A2:* Amino acid substrates were dissolved in THF (15 mL mmol$^{-1}$) and DIPEA (2.5 equiv), and propargyl bromide (**1**; 80% in toluene, 2 equiv) was added. The mixture was stirred at 50 °C for 1–2 days and concentrated under reduced pressure. The crude products were purified by flash chromatography on silica gel.

**General procedure B: CuAAC of alkynes with benzyl azide (7).** *Method B1:* N(α)-propargyl amino acid derivatives were dissolved in *t*BuOH/$H_2O$ (1:1, 20–50 mL mmol$^{-1}$) and $Cu(OAc)_2$ (0.1–0,2 equiv), sodium ascorbate (0.2–0.4 equiv) and benzyl azide (**7**, 1.1 equiv) were added. The mixture was stirred at RT for 18 h, diluted with brine and extracted with EtOAc. The organic extracts were washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude products were purified by semi-preparative HPLC or flash chromatography on silica gel. *Method B2:* N(α)-propargyl amino acid derivatives were dissolved in *t*BuOH/$H_2O$ (1:1, 50–75 mL mmol$^{-1}$) and excess metallic Cu(0) (~40 mg mmol$^{-1}$) and benzyl azide (**7**, 1.1 equiv) were added. The mixture was stirred at RT for 20 h, filtered and concentrated under reduced pressure. The

crude products were purified by semi-preparative HPLC or flash chromatography on silica gel.

**General procedure C: Removal of Boc protecting groups.** Where appropriate, the Boc group was cleaved by stirring intermediates in $CH_2Cl_2$/TFA (4:1, ~50 mL mmol$^{-1}$) at RT for 18 h. Removal of the volatile components under reduced pressure yielded the corresponding amine products quantitatively as their TFA salts.

**General procedure D: Synthesis of rhenium complexes.** $[Re(CO)_3Br_3][Et_4N]_2$ was prepared according to published procedures. 1,2,3-Triazoles were dissolved in water, alcohol or mixtures thereof (1:1; ~80 mL mmol$^{-1}$) and $[Re(CO)_3Br_3][Et_4N]_2$ (1 equiv) was added. The solution was adjusted to pH ~7 by the addition of dilute aqueous $Et_4NOH$ (~0.5%). The mixture was then stirred at 50–75 °C until HPLC analysis indicated complete conversion of the substrates (0.5–4 h). The solution was concentrated under reduced pressure, and the crude products were purified by semi-preparative HPLC or by using $C_{18}$ Sep-Pak® columns and mixtures of water and methanol (0→50% MeOH). Occasionally, carbonyl ligands of the complexes exhibited weak signals in the $^{13}C$ NMR spectra. However, the presence of *fac*-$Re(CO)_3$ could be confirmed in each case by its characteristic and strong IR absorptions.[18]

**General procedure F: Synthesis of $^{99m}$Tc complexes.** $[Na][^{99m}TcO_4]$ was eluted from a $^{99}Mo/^{99m}Tc$ generator (Mallinckrodt-Tyco, Petten, The Netherlands) with a 0.9% saline solution. The precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared according to published procedures.[41] In brief, 1 mL $[^{99m}TcO_4]^-$ in 0.9% NaCl was added to the IsoLink™ kit (Mallinckrodt-Tyco, Petten, The Netherlands) via the septum. The reaction was heated for 20 min at 100 °C. The solution was cooled and the adjusted to pH ~7 with a 1:1 mixture of 1 M phosphate buffer (pH 7.4) and 1 M HCl. Aliquots of stock solutions of the 1,2,3-triazole ligand systems in water (50 μL, $10^{-3}$–$10^{-5}$ M) were added to a solution of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (100 μL; ~1 GBq mL$^{-1}$) and phosphate-buffered saline (PBS; 350 μL, pH 7.2) was added to adjust the final concentration of the chelator ($10^{-4}$–$10^{-6}$ M). The reaction mixture was heated at 100 °C for 30 min and radiolabeling yields were determined by HPLC.

**One-pot synthesis of $^{99m}$Tc complexes.** Aliquots (50 μL) of the filtered solution of crude ligands (**L15**, **L18**, **L20**; ~15 mM in *t*BuOH/$H_2O$ 1:1) were diluted with PBS (350 μL, pH 7) and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (100 μL; ~1 GBq mL$^{-1}$) was added. The reaction mixture was heated at 100 °C for 30 min and product formation was confirmed by γ-HPLC.

**Solid-phase peptide synthesis (SPPS).** The synthesis of peptides **23** and N$^{\alpha}$HisAc-BBS(7–14) was carried out on solid phase on a Rink amide polystyrene resin (0.60 mmol g$^{-1}$) using standard Fmoc strategy and procedures previously described.[12,16] After cleavage from the resin and deprotection, the peptides were purified by preparative HPLC and analyzed by LC–MS.

**Peptide $^{99m}$Tc labeling.** Solutions of peptides **L24** and N$^{\alpha}$HisAc-BBS(7–14) (30–50 μL; 1 mM in PBS, pH ~6.5) were mixed with 500 μL of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ solution and heated at 75 °C for 1 h, after which the radiolabeled products were analyzed and purified prior to in vitro and in vivo experiments by HPLC.

## Characterization of compounds

**N(α)-propargyl GlyOMe (2).**[13] As per general procedure A1 from HGlyOMe (HCl salt; 250 mg, 2 mmol) and purification by flash chromatography with EtOAc/hexane (1:4). Yellow oil (235 mg, 72%).

**N(α)-propargyl ValOMe (3).** As per general procedure A1 from HValOMe (HCl salt; 336 mg, 2.0 mmol) and purification by flash chromatography with $CH_2Cl_2$/MeOH (25:1). Yellow oil (223 mg, 67%): IR (neat): $\tilde{\nu}$ = 3311, 2958, 1727, 1465, 1201, 1144, 994 cm$^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 3.69 (s, 3H), 3.40 and 3.31 (each dd, each 1H, $J$ = 16.7 and 2.6 Hz), 3.14 (d, 1H, $J$ = 5.8 Hz), 2.15 (t, 1H, $J$ = 2.6 Hz), 1.96–1.85 (m, 1H), 1.70 (bs, 1H), 0.91 and 0.89 (each d, each 3H, $J$ = 6.7 Hz); $^{13}C$ NMR ($CDCl_3$): $\delta$ = 175.2, 81.8, 71.6, 66.2, 51.7, 37.7, 31.8, 19.3, 18.6 ppm; HRMS: $[M+Na]^+$ = 192.0966 (calcd for $C_9H_{15}NO_2Na$: 192.1001).

**N(α)-propargyl ProOMe (4).** As per general procedure A1 from HProOMe (HCl salt; 331 mg, 2.0 mmol) and purification by flash chromatography with EtOAc/hexane (3:1). Yellow oil (250 mg, 75%): IR (neat): $\tilde{\nu}$ = 3282, 2953, 2818, 1741, 1436, 1278, 1200, 1173, 1130, 905, 669 cm$^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 3.70 (s, 3H), 3.57 (t, 2H, $J$ = 2.5 Hz), 3.41 (dd, 1H, $J$ = 8.8 and 6.6 Hz), 3.06–3.00 (m, 1H), 2.73–2.67 (m, 1H), 2.17 (t, 1H, $J$ = 2.5 Hz), 2.16–2.07 (m, 1H), 2.00–1.84 (m, 2H), 1.83–1.73 (m, 1H); $^{13}C$ NMR ($CDCl_3$): $\delta$ = 174.3, 78.6, 73.4, 62.8, 52.5, 52.2, 41.4, 29.8, 23.5 ppm; HRMS: $[M+H]^+$ = 168.1019 (calcd for $C_9H_{14}NO_2$: 168.1025).

**N(α)-propargyl Gln(Et)OBn (5).** As per general procedure A2 from HGln(Et)OBn[16] ((90 mg, 0.34 mmol)) and purification by flash chromatography with $CH_2Cl_2$/MeOH (30:1). Brown oil (56 mg, 54%): IR (neat): $\tilde{\nu}$ = 3293, 2920, 1729, 1643, 1453, 1253, 1168, 964, 733, 697 cm$^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 7.38–7.27 (m, 5H), 5.68 (bs, 1H), 5.16 and 5.12 (each d, each 1H, $J$ = 12.0 Hz), 3.47–3.42 (m, 1H), 3.42 and 3.33 (each dd, each 1H, $J$ = 16.8 and 2.3 Hz), 3.21 (quint., 2H, $J$ = 7.4 Hz), 2.26–2.15 (m, 2H), 2.12 (t, 1H, $J$ = 2.3 Hz), 1.92–1.80 (m, 3H), 1.07 (t, 3H, $J$ = 7.3 Hz); $^{13}C$ NMR ($CDCl_3$): $\delta$ = 174.4, 172.1, 135.7, 128.8, 128.6, 128.5, 81.7, 72.0, 66.9, 59.5, 37.0, 34.5, 32.8, 28.8, 15.0 ppm; HRMS: $[M+Na]^+$ = 325.1521 (calcd for $C_{17}H_{22}N_2O_3Na$: 325.1528).

**N(α)-propargyl Lys(Ac)OMe (6).** As per general procedure A2 from HLys(Ac)OMe[16] (TFA salt; 144 mg, 0.44 mmol) and purification by flash chromatography with $CH_2Cl_2$/MeOH (15:1). Yellow oil (100 mg, 73%): IR (neat): $\tilde{\nu}$ = 3285, 2941, 1731, 1645, 1546, 1433, 1367, 1288, 1198, 1171, 1139, 990 cm$^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 6.20 (bs, 1H), 3.59 (s, 3H), 3.32–3.19 (m, 3H), 3.07 (q, 2H, $J$ = 6.8 Hz), 2.11 (t, 1H, $J$ = 2.2 Hz), 1.82 (s, 3H), 1.73 (bs, 1H), 1.60–1.43 (m, 2H), 1.42–1.33 (m, 2H), 1.31–1.20 (m, 2H); $^{13}C$ NMR ($CDCl_3$): $\delta$ = 175.1, 170.2, 81.3, 71.7, 59.7, 51.8, 39.2, 36.8, 32.7, 29.1, 23.1, 22.9 ppm; HRMS: $[M+Na]^+$ = 263.1365 (calcd for $C_{12}H_{20}N_2O_3Na$: 263.1372).

**Ligand L3.** As per general procedure B1 from N(α)-propargyl ValOMe (**3**; 40 mg, 0.24 mmol) and purification by flash chromatography with EtOAc/hexane (3:1). Colorless oil (52 mg, 71%): IR (neat): $\tilde{\nu}$ = 2955, 1731, 1459, 1198, 1047, 721 cm$^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 7.32–7.26 (m, 3H), 7.20–7.17 (m, 2H), 5.43 (s, 2H), 3.85 and 3.66 (each d, each 1H, $J$ = 13.7 Hz), 3.61 (s, 3H), 2.98 (d, 2H, $J$ = 5.8 Hz), 1.88–1.80 (m, 2H), 0.83 and 0.81 (each d, each 3H, $J$ = 6.2 Hz); $^1H$ NMR ($CDCl_3$): $\delta$ = 175.4, 147.5, 134.9, 129.2, 128.8, 128.2, 127.8, 66.8, 54.2, 51.6, 44.0, 31.7, 19.3, 18.7 ppm; HRMS: $[M+Na]^+$ = 325.1633 (calcd for $C_{16}H_{22}N_4O_2Na$: 325.1640).

**Ligand L4.** As per general procedure B1 from N(α)-propargyl ProOMe (**4**; 170 mg, 1.0 mmol) and purification by flash chromatography with EtOAc/EtOH (25:1). Brown oil (285 mg, 95%): IR (neat): $\tilde{\nu}$ = 2955, 2826, 1735, 1455, 1433, 1205, 1186, 1126, 1047, 725 cm$^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 7.36 (s, 1H), 7.32–7.26 (m, 3H), 7.21–7.18 (m, 2H), 5.45 and 5.40 (each d, each 1H, $J$ = 14.9 Hz), 3.91 and 3.73 (each d, each 1H, $J$ = 13.6 Hz), 3.56 (s, 3H), 3.22 (dd, 1H $J$ = 8.9 and 6.3 Hz), 3.06–3.00 (m, 1H), 2.47–2.41 (m, 1H), 2.09–1.99 (m, 1H), 1.91–1.64 (m, 3H); $^{13}C$ NMR ($CDCl_3$): $\delta$ = 174.5, 145.1, 134.7,

129.1, 128.7, 128.1, 122.6, 64.9, 54.1, 53.4, 51.8, 48.8, 23.0, 14.2 ppm; HRMS: $[M+H]^+ = 323.1477$ (calcd for $C_{16}H_{20}N_4O_2Na$: 323.1484).

**Ligand L5.** As per general procedure B2 from N(α)-propargyl Gln(Et)OBn (**5**; 25 mg, 0.08 mmol) and purification by flash chromatography with $CH_2Cl_2$/MeOH (25:1). White solid (25 mg, 71%): mp 79–81 °C; IR (neat): $\tilde{\nu} = 3301, 2970, 2934, 1731, 1652, 1543, 1453, 1213, 1167, 1050, 724, 699$ cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta = 7.42–7.25$ (m, 11H), 5.88 (bs, 1H), 5.49 (s, 2H), 5.18 and 5.15 (each d, each 2H, $J = 12.2$ Hz), 3.94 and 3.74 (each d, each 1H, $J = 13.6$ Hz), 3.36 (dd, 1H, $J = 8.0$ and 5.2 Hz), 3.26–3.14 (m, 2H), 2.31–2.13 (m, 2H), 2.11–2.01 (m, 1H), 1.97 (bs, 1H), 1.93–1.83 (m, 1H), 1.07 (t, 3H, $J = 7.3$ Hz); $^{13}$C NMR ($CDCl_3$) δ174.6, 172.2, 147.0, 135.8, 134.8, 129.3, 128.9, 128.8, 128.6, 128.5, 128.3, 121.8, 66.9, 60.2, 54.3, 43.2, 34.4, 33.0, 29.0, 15.0 ppm; HRMS: $[M+H]^+ = 436.2343$ (calcd for $C_{24}H_{30}N_5O_3$: 436.2349).

**Ligand L6.** As per general procedure B2 from N(α)-propargyl Lys(Ac)OMe (**6**; 80 mg, 0.3 mmol) and purification by flash chromatography with $CH_2Cl_2$/MeOH (15:1). Yellow oil (100 mg, 81%): IR (neat): $\tilde{\nu} = 3294, 2932, 1731, 1652, 1548, 1453, 1433, 1286, 1200, 1169, 1137, 1047, 719$ cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta = 7.41–7.35$ (m, 4H), 7.31–7.26 (m, 2H), 5.72 (bs, 1H), 5.52 (s, 2H), 3.92 and 3.76 (each d, each 1H, $J = 14.0$ Hz), 3.71 (s, 3H), 3.31 (t, 1H, $J = 6.4$ Hz), 3.22 (q, 2H, $J = 6.5$ Hz), 1.97 (s, 3H), 1.93 (bs, 1H), 1.74–1.55 (m, 2H), 1.52–1.43 (m, 2H), 1.42–1.32 (m, 2H); $^{13}$C NMR ($CDCl_3$): $\delta = 175.4$, 170.1, 146.9, 134.7, 129.1, 128.7, 128.1, 121.6, 60.5, 54.1, 51.8, 43.2, 39.3, 32.7, 29.1, 23.3, 22.9 ppm; HRMS: $[M+H]^+ = 374.2188$ (calcd for $C_{19}H_{28}N_5O_3$: 374.2192).

**[Re(CO)$_3$(L3)].** As per general procedure D from ligand **L3** (7 mg, 0.03 mmol) in ethanol and purification by Sep-Pak® (1 g). White solid (20 mg, 83%; purity according to HPLC > 95%): mp > 240 °C (dec.); $[\alpha]_D^{20} = +21.3$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 2963, 2019$ and 1892 (strong), 1630, 1047, 1022, 932 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH): $\delta =$ 8.10 (s, 1H), 7.43–7.34 (m, 5H), 6.42 (bs, 1H), 5.71 and 5.67 (each d, each 1H, $J = 14.9$ Hz), 4.84–4.80 (shoulder of HDO signal, m, 1H), 4.29–4.21 (m, 2H), 2.34–2.26 (m, 1H), 1.16 and 1.11 (each d, each 3H, each $J = 7.1$ Hz); $^{13}$C NMR ([D$_4$]MeOH): $\delta = 196.9$, 195.4, 195.2, 184.5, 148.0, 134.0, 128.9, 128.7, 128.8, 123.0, 70.6, 55.0, 53.2, 31.6, 17.8, 17.2 ppm; HRMS: $[M+Na]^+ = 581.0814$ (calcd for $C_{18}H_{19}N_4O_5ReNa$: 581.0811).

**[Re(CO)$_3$(L4)].** As per general procedure D from ligand **L4** (28 mg, 0.1 mmol) in ethanol and purification by Sep-Pak® (1 g). White solid (30 mg, 55%; purity according to HPLC > 95%): mp > 240 °C (dec.); $[\alpha]_D^{20} = +8.2$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 2955, 2021$ and 1877 (strong), 1638, 1355 cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta = 7.72$ (s, 1H), 7.44–7.36 (m, 5H), 5.75 and 5.58 (each d, each 1H, $J = 15.2$ Hz), 4.14 and 4.09 (each d, each 1H, $J = 14.7$ Hz), 3.99–3.95 (m, 1H), 3.41–3.30 (m, 1H), 3.27 (dd, 1H, $J = 10.6$ and 6.6 Hz), 2.48–2.40 (m, 1H), 2.35–2.26 (m, 1H), 2.21–2.15 (m, 1H), 2.10–2.03 (m, 1H); $^{13}$C NMR ($CDCl_3$): $\delta = 197.5$, 196.7, 195.5, 183.3, 146.8, 133.3, 129.6, 129.5, 128.8, 123.5, 71.0, 68.3, 60.6, 56.3, 31.8, 24.7 ppm; HRMS: $[M+Na]^+ = 579.0652$ (calcd for $C_{18}H_{20}N_4O_5ReNa$: 579.0654). Crystals suitable for X-ray analysis were obtained by slow diffusion of hexane into a solution of [Re(CO)$_3$(**L4**)] in THF.

**[Re(CO)$_3$(L5)].** As per general procedure D from ligand **L5** (15 mg, 0.03 mmol) in ethanol/water (5:1) and purification by Sep-Pak® (2 g). White solid (13 mg, 62%; purity according to HPLC > 94%): mp > 200 °C (dec.); $[\alpha]_D^{20} = +15.9$ ($c = 0.5$ in MeOH); IR (neat): $\tilde{\nu} =$ 3306, 2925, 2013 and 1889 (strong), 1640, 1452, 1353, 1045 cm$^{-1}$; $^1$H NMR (MeOD; recorded after amide proton has exchanged): $\delta =$ 8.09 (s, 1H), 7.42–7.34 (m, 5H), 5.68 (s, 2H), 4.33 and 4.24 (each d, each 1H, $J = 15.7$ Hz), 3.30–3.25 (m, 1H; overlapping with MeOD signal; confirmed by COSY), 3.25–3.18 (m, 2H), 3.17–3.14 (m, 1H), 2.53–2.41 (m, 2H), 1.19–2.02 (m, 2H), 1.12 (t, 3H, $J = 7.3$ Hz); $^{13}$C NMR (MeOD): $\delta = 198.3$, 196.8, 196.6, 185.8, 174.8, 149.8, 135.5, 130.4, 130.2, 129.6, 124.4, 67.7, 56.5, 53.7, 35.5, 33.7, 29.8, 14.8 ppm; HRMS: $[M+Na]^+ = 638.1019$ (calcd for $C_{20}H_{22}N_5O_6ReNa$: 638.1025). Crystals suitable for X-ray analysis were obtained by slow evaporation of a solution of [Re(CO)$_3$(**L5**)] in $CH_2Cl_2$.

**[Re(CO)$_3$(L6)].** As per general procedure D from ligand **L6** (25 mg, 0.07 mmol) in methanol/water (1:1) and purification by trituration in water (twice). White solid (35 mg, 83%; purity according to HPLC > 95%): mp 138–142 °C; $[\alpha]_D^{20} = +19.8$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 3165, 2939, 2022$ and 1885 (strong), 1631, 1454, 1366, 722 cm$^{-1}$; $^1$H NMR (MeOD): $\delta = 8.10$ (s, 1H), 7.45–7.32 (m, 5H), 6.63 (bd, 1H, $J = 5.0$ Hz; exchanges with MeOD after 18 h), 5.67 (s, 2H), 4.35–4.19 (m, 2H; becomes 2 doublets (4.30 and 4.22, each 1H, $J =$ 15.0 Hz) after completed H/D exchange), 3.31–3.24 (m, 2H), 3.13 (t, 1H, $J = 6.4$ Hz), 1.94 (s, 3H), 1.91–1.82 (m, 2H), 1.60–1.52 (m, 4H); $^{13}$C NMR (MeOD): $\delta = 198.5$, 196.8, 195.6, 186.3, 173.5, 149.6, 135.5, 130.5, 130.2, 129.6, 124.5, 67.7, 56.5, 53.8, 40.2, 33.9, 30.2, 24.5, 22.6 ppm; HRMS: $[M+Na]^+ = 652.1181$ (calcd for $C_{21}H_{24}N_5O_6ReNa$: 652.1182). Crystals suitable for X-ray analysis were obtained by slow diffusion of $CH_2Cl_2$ into a solution of [Re(CO)$_3$(**L6**)] in MeOH.

**Intermediate 9.** To a solution of BocLysOMe·AcOH (**8**; 942 mg, 68%, 2.0 mmol) in THF (15 mL) and DIPEA (1.4 mL, 8.0 mmol) was added dropwise at 0 °C trifluoroacetic acid (TFA) anhydride (834 μL, 6.0 mmol). The solution was warmed to RT and stirred for 5 h. The mixture was diluted with water and extracted with EtOAc. The organic layers were washed with water and then combined, dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica gel with EtOAc/hexane (1:1.5) gave product **9** as a yellow oil (0.7 g, 98%): $[\alpha]_D^{20} = -15.2$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 3324, 2943, 1706, 1518, 1367, 1206, 1161, 720$ cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta = 6.67$ (bs, 1H), 5.08 (bd, 1H, $J = 8.5$ Hz), 4.30–4.21 (m, 1H), 3.71 (s, 3H), 3.33 (q, 2H, $J =$ 6.7 Hz), 1.85–1.74 (m, 1H), 1.67–1.55 (m, 3H), 1.45–1.31 (m, 2H), 1.41 (s, 9H); $^{13}$C NMR ($CDCl_3$): $\delta = 173.3$, 157.6 (q, $J = 38.3$ Hz), 155.7, 116.1 (q, $J = 287.3$ Hz), 80.3, 53.2, 52.6, 39.8, 32.8, 28.5, 28.4, 22.7 ppm; HRMS: $[M+Na]^+ = 379.1453$ (calcd for $C_{14}H_{23}N_2O_5F_3Na$: 379.1457).

**Intermediate 10.** As per general procedure C from intermediate **9** (680 mg, 1.9 mmol). Yellow oil (700 mg, quantitative): $[\alpha]_D^{20} = +10.4$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 3088, 2952, 1701, 1670, 1557, 1441, 1183, 1156, 1132, 722$ cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH with TMS): $\delta = 4.00$ (t, 1H, $J = 6.8$ Hz), 3.80 (s, 3H), 3.30–3.25 (m, 2H, obscured by the MeOD signal), 1.99–1.85 (m, 2H), 1.59 (quint., 2H, $J = 7.5$ Hz), 1.52–1.32 (m, 2H); $^{13}$C NMR ([D$_4$]MeOH with TMS): $\delta = 170.9$, 161.6 and 159.2 (each q, each $J = 37.0$ Hz), 117.6 and 116.6 (each q, each $J =$ 287.0 Hz), 53.8, 53.6, 40.1, 31.1, 29.3, 23.1 ppm; HRMS: $[M+H]^+ =$ 257.1110 (calcd for $C_9H_{16}N_2O_3F_3$: 257.1113).

**Intermediate 11.** As per general procedure A2 from intermediate **10** (TFA salt; 667 mg, 1.8 mmol) at RT for 2 days and purification by flash chromatography with EtOAc/hexane (1:1). Yellow oil (360 mg, 68%): $[\alpha]_D^{20} = -10.0$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 3306, 2943, 1706, 1554, 1437, 1201, 1172, 1156, 722$ cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta = 6.56$ (bs, 1H), 3.72 (s, 3H), 3.46–3.31 (m, 5H), 2.18 (t, 1H, $J = 2.5$ Hz), 1.74 (bs, 1H), 1.72–1.64 (m, 1H), 1.61–1.54 (m, 3H), 1.46–1.36 (m, 2H); $^{13}$C NMR ($CDCl_3$): $\delta = 175.3$, 157.5 (q, $J = 38.2$ Hz), 116.1 (q, $J = 287.9$ Hz), 81.4, 72.1, 59.9, 52.2, 39.8, 37.2, 32.5, 28.6, 22.9 ppm; HRMS: $[M+H]^+ = 295.1263$ (calcd for $C_{12}H_{18}N_2O_3F_3$: 295.1270).

**Intermediate 12.** Intermediate **11** (295 mg, 1.0 mmol) was dissolved in THF (10 mL) and DIPEA (420 μL, 2.5 mmol) and $BOC_2O$ (327 mg, 1.5 mmol) was added. The solution was stirred at 55 °C for 2 days and concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica gel with EtOAc/hexane (1:2) gave compound **12** as a yellow oil (382 mg, 97%): $[\alpha]_D^{20} = -34.1$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 3306, 2949, 1706, 1554, 1441, 1364, 1206, 1161, 866$ cm$^{-1}$; $^1$H NMR ([D$_6$]DMSO, 75 °C): $\delta = 9.11$ (bs, 1H), 4.36 (bs, 1H), 4.02 (dd, 1H, $J = 18.3$ and 2.8 Hz), 3.97 (bd, 1H, $J = 18.3$ Hz), 3.65 (s, 3H), 3.20 (t, 2H, $J = 6.9$ Hz), 2.95 (t, 1H, $J = 2.5$ Hz), 1.98–1.89 (m, 1H), 1.86–1.78 (m, 1H), 1.61–1.49 (m, 2H), 1.41 (s, 9H), 1.42–1.32 (m, 2H); $^{13}$C NMR ([D$_6$]DMSO, 75 °C): $\delta = 170.9, 156.9$ (q, $J = 36.2$ Hz), 153.8, 115.7 (q, $J = 289.5$ Hz), 79.9, 79.8, 72.9, 58.2, 51.3, 38.7, 35.0, 28.5, 27.52, 27.47, 22.7 ppm; HRMS: $[M+Na]^+ = 417.1596$ (calcd for $C_{17}H_{25}N_2O_5F_3Na$: 417.1613).

**Precursor 13.** Intermediate **12** (150 mg, 0.35 mmol) was dissolved in MeOH (0.5 mL) and $NH_4HCO_3$ (5 mL; 1 M in $H_2O$/MeOH 1:1) were added. The solution was stirred at 55 °C for 3 days, diluted with brine and extracted with EtOAc. The organic phases were washed once with brine and then combined, dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel at ambient pressure with $CH_2Cl_2$/MeOH (10:1, containing 0.5% $Et_3N$) to yield product **13** as a colorless oil (68 mg, 60%): $[\alpha]_D^{20} = -25.3$ ($c = 1$ in MeOH); IR (neat): $\tilde{\nu} = 3270, 2925, 1742, 1695, 1364, 1247, 1163, 772$ cm$^{-1}$; $^1$H NMR ([D$_6$]DMSO with one drop $D_2O$, 75 °C; despite the elevated temperature there are still multiple signals observed): $\delta = 4.36$ (bs, 1H), 4.05–3.95 (m, 2H), 3.65 (s, 3H), 3.07–2.86 (several multiplets, total 3H), 2.59–2.54 (m, 2H), 1.95–1.74 (several multiplets, total 4H), 1.42 and 1.40 (each s (intensity ~1:2), total 9H), 1.35–1.25 (m, 2H); $^1$H NMR ([D$_6$]DMSO with one drop $D_2O$, 75 °C): $\delta = 171.0, 170.9, 80.0, 79.7, 72.9, 72.0, 58.3, 51.2, 40.8, 35.0, 32.1, 28.7, 28.5, 27.6, 27.5, 22.8$ ppm; HRMS: $[M+H]^+ = 299.1966$ (calcd for $C_{15}H_{27}N_2O_4$: 299.1971).

**Precursor 14.** Intermediate **12** (660 mg, 1.67 mmol) was dissolved in MeOH (10 mL) and aqueous $K_2CO_3$ (0.5 M, 10 mL) was added. The mixture was stirred at 75 °C for 3 h after which TLC indicated completed conversion of the substrate. The solution was adjusted to pH 6 by addition of aqueous TFA (1 M) and the volatile components were removed under reduced pressure. The residue was stirred several times in EtOH, centrifuged (3 min, 16000 rpm) and the decanted solutions were concentrated under reduced pressure to yield the TFA salt of product **14** as a white solid (540 mg, quantitative): $[\alpha]_D^{20} = -30.4$ ($c = 0.5$ in MeOH); IR (neat): $\tilde{\nu} = 3401, 3297, 2973, 1672, 1581, 1387, 1203, 1167, 1136, 719$ cm$^{-1}$; $^1$H NMR ([D$_6$]DMSO with one drop $D_2O$, 75 °C): $\delta = 4.13$ (bs, 1H), 4.02 and 3.89 (each dd, each 1H, $J = 18.1$ and 3.0 Hz), 2.71 (t, 1H, $J = 2.5$ Hz), 2.66 (t, 2H, $J = 6.6$ Hz), 1.93–1.85 (m, 1H), 1.63–1.53 (m, 1H), 1.53–1.45 (m, 2H), 1.39 (s, 9H), 1.37–1.29 (m, 2H); $^{13}$C NMR ([D$_6$]DMSO with one drop $D_2O$, 75 °C): $\delta = 173.7, 158.4$ (q, $J = 37.5$ Hz), 155.2, 117.2 (q, $J = 303$ Hz), 82.7, 82.3, 82.2, 78.7, 61.0, 33.7, 30.2, 29.3, 28.1, 23.7 ppm; HRMS: $[M+H]^+ = 285.1812$ (calcd for $C_{14}H_{25}N_2O_4$: 285.1814).

**Compound 15.** Precursor **14** (TFA salt; 40 mg, 0.10 mmol) was suspended in dry DMSO (MS 4 Å, 2 mL) and NHS-m-dPEG$_{15}$ (*BioConnect*, 86 mg, 0.10 mmol) was added. The resulting solution was stirred at RT for 24 h and quenched by addition of water (1 mL). Purification of the mixture by semi-preparative HPLC (5%→80% $CH_3CN$ within 14 min, rest: 0.1% aqueous TFA; flow: 3.5 mL min$^{-1}$) yielded N(α)-Boc protected intermediate as a colorless oil (60 mg, 58%; purity according to HPLC > 95%): HRMS $[M+Na]^+ =$ 1053.5944 (calcd for $C_{48}H_{90}N_2O_{21}Na$: 1053.5934). The intermediate was deprotected according to procedure C to yield compound **15** as a colorless oil (60 mg, quantitative; purity according to HPLC > 95%): HRMS-ESI: $[M+Na]^+ = 953.5400$ (calcd for $C_{43}H_{82}N_2O_{19}Na$: 953.5409); NMR spectra were inconclusive presumably because of micelle formation.

**Compound 16.** TBDMS-protected glucuronic acid[16] (100 mg, 0.18 mmol) was dissolved in DMF (3 mL) and HBTU (65 mg, 0.17 mmol) and DIPEA (65 μL, 0.36 mmol) were added. The solution was stirred at RT for 20 min and then added dropwise at RT to a suspension of precursor **14** (TFA salt; 72 mg, 0.18 mmol) in DMF (3 mL) containing DIPEA (140 μL, 0.72 mmol). The mixture was stirred at RT for 2 h and the resulting solution was diluted with aqueous citric acid (1 M, pH 3–4) and extracted with EtOAc. The organic phases were washed with aqueous citric acid (0.1 M) and brine and then combined, dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica gel with $CH_2Cl_2$/MeOH gave the fully protected intermediate as a white solidified foam (94 mg, 64%): HRMS-ESI $[M+Na]^+ = 839.4709$ (calcd for $C_{39}H_{76}N_2NaO_{10}Si_3$: 839.4705). The intermediate was deprotected in two steps. 1) Removal of the TBDMS ether groups: TBAF (124 mg, 3.3 equiv) in THF (3 mL) at RT for 18 h followed by purification using Sep-Pak® (5 g; 0→50% MeOH, rest: water). White amorphous solid (38 mg, 78%): HRMS $[M+Na]^+ = 497.2115$ (calcd for $C_{21}H_{34}N_2NaO_{10}$: 497.2111). 2) Removal of the N(α)-Boc group according to general procedure C yielded TFA salt of compound **16** as colorless oil (39 mg, quantitative): IR (neat): $\tilde{\nu} = 3298, 2949, 2864, 1734, 1660, 1554, 1443, 1190, 1144, 1083, 1026, 723$ cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH): $\delta = 4.24$ (d, 1H, $J = 7.5$ Hz), 4.10 (dd, 1H, $J = 5.0$ and 6.5 Hz), 4.04 and 3.99 (each dd, each 1H, each $J = 2.6$ and 16.6 Hz), 3.71 (d, 1H, $J = 9.4$ Hz), 3.54 (s, 3H), 3.47 (t, 1H, $J = 9.2$ Hz), 3.42 (t, 1H, $J = 9.0$ Hz), 3.30–3.23 (m, 3H), 3.22 (t, 1H, $J = 8.2$ Hz), 2.09–1.92 (m, 2H), 1.65–1.57 (m, 2H), 1.56–1.38 (m, 2H); $^{13}$C NMR ([D$_4$]MeOH): $\delta = 172.1, 171.0, 105.8, 79.8, 77.7, 76.5, 74.7, 74.4, 73.7, 60.0, 57.8, 39.5, 36.6, 30.0, 29.9, 23.0$ ppm (signals of the TFA counter ion are not listed); HRMS $[M+Na]^+ = 397.1586$ (calcd for $C_{16}H_{26}N_2NaO_8$: 397.1587).

**Compound 17.** (*R*)-2-succinylamido-6-(4-(4-iodophenyl)butanamido)-hexanoic acid ethyl ester[16] (25 mg, 0.045 mmol) was dissolved in DMF (2 mL) and HBTU (17 mg, 0.045 mmol) and DIPEA (16 μL, 0.09 mmol) were added. The solution was stirred at RT for 20 min and then added dropwise at RT to a solution of precursor **13** (14 mg, 0.045 mmol) in DMF (2 mL) containing DIPEA (16 μL, 0.09 mmol). The mixture was stirred at RT for 2 h and the resulting solution was diluted with aqueous citric acid (1 M) and extracted with EtOAc. The organic phases were washed with aqueous citric acid (0.1 M) and brine and then combined, dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica gel with $CH_2Cl_2$/MeOH gave the protected intermediate as a colorless oil (30 mg, 81%): HRMS-ESI $[M+Na]^+ = 849.2906$ (calcd for $C_{37}H_{55}IN_4NaO_9$: 849.2911). The intermediate was deprotected in two steps. 1) Removal of the N(α)-Boc group according to general procedure C gave a yellow oil (25 mg, quantitative): HRMS $[M+Na]^+ = 749.2393$ (calcd for $C_{32}H_{47}IN_4NaO_7$: 749.2382). 2) Saponification of the ethyl ester by stirring the isolated compound in MeOH (1 mL) and aqueous NaOH 1 M (1 mL) at RT for 18 h. The pH was adjusted to pH 4–5 by addition of aqueous HCl 1 M and concentrated under reduced pressure. The residue was stirred several times in EtOH (2 mL), centrifuged (3 min, 13000 rpm) and the solution was decanted and concentrated under reduced pressure to yield compound **17** as a yellow oil (25 mg, quantitative): IR (neat): $\tilde{\nu} = 3278, 2931, 2861, 1723, 1634, 1460, 1217, 1005, 735$ cm$^{-1}$; $^1$H NMR (MeOD): $\delta = 7.61$

and 6.99 (each d, each 2H, $J=8.2$ Hz), 4.35 (dd, 1H, $J=9.0$ and 4.9 Hz), 4.05–3.94 (m, 3H), 3.22 (t, 1H, $J=2.5$ Hz), 3.21–3.13 (m, 4H), 2.61–2.53 (m, 4H), 2.50–2.44 (m, 2H), 2.20 (t, 2H, $J=7.8$ Hz), 2.03–1.86 (m, 5H), 1.77–1.66 (m, 1H), 1.61–1.41 (m, 6H), 1.45–1.36 (m, 2H); $^{13}$C NMR (MeOD): $\delta=175.9$, 175.7, 175.0, 174.8, 171.7, 143.0, 138.7, 132.0, 91.6, 79.6, 74.7, 60.9, 53.8, 40.2, 39.8, 36.7, 36.6, 35.9, 32.4, 32.3, 32.2, 30.2, 30.1, 30.0, 28.7, 24.4, 23.1 ppm; HRMS $[M+Na]^+=707.1902$ (calcd for $C_{29}H_{41}IN_4NaO_7$: 707.1912).

**Compound 18.** Precursor **14** (TFA salt; 40 mg, 0.1 mmol) was suspended in dry DMSO (MS 4 Å, 2 mL) and biotin–NHS ester (34 mg, 0.1 mmol) was added. The resulting mixture was stirred at RT for 20 h and quenched by addition of water (1 mL). Purification of the mixture by semi-preparative HPLC (5%→95% $CH_3CN$ within 15 min, rest: 0.1% aqueous TFA; flow: 3.5 mL min$^{-1}$) yielded the N($\alpha$)-Boc protected intermediate as a white solid (40 mg, 78%, purity according to HPLC > 95%): HRMS $[M+Na]^+=533.2402$ (calcd for $C_{24}H_{38}N_4NaO_6S$: 533.2410). The intermediate was deprotected according to procedure C to yield compound **18** as a colorless oil (41 mg, quantitative; purity according HPLC > 95%): IR (neat): $\tilde{\nu}=3293$, 2929, 1672, 1642, 1561, 1461, 1190, 1136, 720 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH with TMS): $\delta=4.50$ (dd, 1H, $J=4.5$ and 7.4 Hz), 4.31 (dd, 1H, $J=4.6$ and 7.4 Hz), 4.10 (t, 1H, $J=6.1$ Hz), 4.04 and 3.99 (each dd, each 1H, each $J=2.0$ and 17.2 Hz), 3.25–3.18 (m, 4H), 2.93 (dd, 1H, $J=4.9$ and 12.7 Hz), 2.71 (d, 1H, $J=12.7$ Hz), 2.21 (t, 2H, $J=7.3$ Hz), 2.06–1.96 (m, 2H), 1.78–1.35 (m, 10H); $^{13}$C NMR ([D$_4$]MeOH with TMS): $\delta=176.4$, 171.0, 166.3, 79.8, 74.4, 63.6, 61.8, 60.1, 57.1, 41.2, 39.8, 36.9, 36.6, 30.1, 30.0, 29.9, 29.6, 27.0, 23.2 ppm (signal of TFA not observed); HRMS $[M+Na]^+=433.1878$ (calcd for $C_{19}H_{30}N_4NaO_4S$: 433.1885).

**Compound 19.** Acridine-9-carboxylic acid (67 mg, 0.3 mmol), precursor **13** (90 mg, 0.3 mmol) and HOBT (45 mg, 0.33 mmol) were dissolved in DMF (4 mL) and DCC (68 mg, 0.33 mmol) was added. The reaction mixture was stirred at 80 °C for 18 h, diluted with water and extracted with $CH_2Cl_2$. The organic phases were washed with water and then combined, dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica gel with mixtures of EtOAc/hexane gave the N($\alpha$)-Boc protected intermediate as a yellow oil (106 mg, 70%; purity according to HPLC > 95%): HRMS-ESI $[M+H]^+=504.2502$ (calcd for $C_{29}H_{34}N_3O_5$: 504.2498). The intermediate was dissolved in TFA/$CH_2Cl_2$ (1:4, 5 mL) and kept at RT overnight. The solution was diluted with $CH_2Cl_2$ and washed with aqueous NaOH (0.1 M). The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure to yield compound **19** as a yellow oil (80 mg, 94%): IR (neat): $\tilde{\nu}=3260$, 2929, 1730, 1640, 1545, 1436, 1212, 752 cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta=8.04$ (d, 2H, $J=9.0$ Hz), 7.90 (d, 2H, $J=8.7$ Hz), 7.67 (t, 2H, $J=8.1$ Hz), 7.45 (t, 2H, $J=7.5$ Hz), 7.03 (bs, 1H; exchanges with $D_2O$), 3.71 (s, 3H), 3.71–3.65 (m, 2H), 3.45 (t, 1H, $J=6.8$ Hz), 3.42 and 3.35 (each dd, each 1H, each $J=2.4$ and 16.8 Hz), 2.18 (t, 1H, $J=2.4$ Hz), 1.88–1.77 (m, 4H; one proton exchanges with $D_2O$), 1.75–1.68 (m, 1H), 1.65–1.54 (m, 2H); $^{13}$C NMR ($CDCl_3$): $\delta=175.1$, 167.0, 148.3, 141.1, 130.3, 129.4, 126.7, 125.3, 122.0, 81.3, 71.8, 59.8, 51.9, 40.0, 36.9, 32.7, 29.5, 23.2 ppm; HRMS $[M+H]^+=404.1976$ (calcd for $C_{24}H_{26}N_3O_3$: 404.1974).

**Compound 20.** Precursor **14** (TFA salt; 0.54 mg, 1.35 μmol) was dissolved in DMSO (100 μL) and DIPEA (5.5 μmol) and DY547-NHS (1.0 mg, 1.35 μmol) were added. The solution was stirred at RT in the dark for 18 h and then diluted with water (0.4 mL). Purification of the mixture by analytical HPLC (5%→90% $CH_3CN$ within 13 min, rest: 0.1% aqueous TFA; flow 1 mL min$^{-1}$) gave the N($\alpha$)-Boc intermediate as a red solid (~0.6 mg, 50%; purity according to HPLC > 95%): HRMS-ESI $[M]^+=883.3609$ (calcd for $C_{44}H_{59}N_4O_{11}S_2$: 883.3616). The intermediate was deprotected according to procedure C to yield compound **20** as a red solid (50% yield for 2 steps as determined by UV/Vis: $\lambda=557$ nm, $d=1$ cm, $\varepsilon=150\,000$ M$^{-1}$cm$^{-1}$; purity according to HPLC ≥ 95%): HRMS $[M]^+=783.3104$ (calcd for $C_{39}H_{51}N_4O_9S_2$ 783.3092).

**Compound 21.** A solution of precursor **13** (45 mg, 0.15 mmol), DOTA($t$Bu)$_3$-NHS ester (100 mg, 0.15 mmol) and DIPEA (52 μL, 0.3 mmol) in $CH_2Cl_2$ (5 mL) was stirred at reflux for 2 h. The solution was diluted with $CH_2Cl_2$ and washed twice with aqueous citric acid (0.1 M). The organic extracts were dried over $Na_2SO_4$ and concentrated under reduced pressure to yield the fully protected intermediate as a solidified white foam (110 mg, 86%): LR-MS $[M+Na]^+=876.2$ (calcd for $C_{43}H_{76}N_6NaO_{11}$: 876.1). The intermediate was deprotected according to procedure C to yield compound **21** as a yellow oil (penta-TFA salt, 90 mg, quantitative): IR (neat): $\tilde{\nu}=3093$, 2861, 1730, 1665, 1190, 1136, 839 cm$^{-1}$; $^1$H NMR (MeOD): $\delta=4.17$ (dd, 1H, $J=7.3$ and 4.6 Hz), 4.10–3.65 (several broad signals, 8H), 4.07 and 3.99 (each d, 1H, $J=15.3$ Hz), 3.87 (s, 3H), 3.50–3.15 (bs, 18H), 3.24 (t, 1H, $J=2.1$ Hz), 2.09–1.89 (m, 2H), 1.63–1.52 (m, 2H), 1.43–1.31 (m, 2H) ($^{13}$C NMR is not conclusive due to similar chemical shifts of signals of the DOTA moiety); HRMS $[M+H]^+=607.3059$ (calcd for $C_{26}H_{44}N_6NaO_9$: 607.3067).

**Compound 22.** Compound **21** (penta-TFA salt; 34 mg, 0.03 mmol) was dissolved in water (4 mL) and $GdCl_3$ (hexahydrate; 11 mg, 0.03 mmol) was added. The solution was stirred at 90 °C for 1.5 h. Purification by semi-preparative HPLC (isocratic 5% $CH_3CN$, rest: 0.1% aqueous TFA; flow: 3 mL min$^{-1}$) gave the TFA salt of Gd-complex **22** as a white solid (15 mg, 60%; purity according to HPLC > 95%): HRMS $[M+H]^+=740.2246$ (calcd for $C_{26}H_{42}GdN_6O_9$: 740.2254).

**Ligand L15.** As per general procedure B2 from compound **15** (19 mg, 0.02 mmol). The mixture was filtered through Celite and treated with QuadraPure™; IDA resin (~0.25 g) for 18 h. Filtration and removal of volatile components under reduced pressure yielded 1,2,3-triazole X as a yellow oil (18 mg, 84%, purity according to HPLC > 90%): HRMS $[M+H]^+=1064.6226$ (calcd for $C_{50}H_{90}N_5O_{19}$: 1064.6230).

**Ligand L16.** As per general procedure B2 from compound **16** (TFA salt; 24 mg, 0.05 mmol) and purification by semi-preparative HPLC (5%→90% $CH_3CN$ within 14 min, rest: 0.1% aqueous TFA; flow: 3 mL min$^{-1}$). White solid (50 mg, 60%; purity according to HPLC > 95%): mp > 150 °C (dec.); IR (neat): $\tilde{\nu}=3318$, 2929, 2964, 1646, 1554, 1455, 1192, 1127, 1057, 1027, 719 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH): $\delta=8.14$ (s, 1H), 7.42–7.32 (m, 5H), 5.64 (s, 2H), 4.41 and 4.38 (each d, each 1H, $J=14.8$ Hz), 4.27 (d, 1H, $J=7.7$ Hz), 4.01 (t, 1H, $J=5.8$ Hz), 3.72 (d, 1H, $J=9.3$ Hz), 3.53 (s, 3H), 3.48 (t, 1H, $J=8.6$ Hz), 3.43 (t, 1H, $J=8.7$ Hz), 3.29–3.23 (m, 3H), 2.08–1.93 (m, 2H), 1.63–1.54 (m, 2H), 1.53–1.46 (m, 1H), 1.45–1.35 (m, 1H); $^{13}$C NMR ([D$_4$]MeOH): $\delta=172.0$, 171.4, 139.7, 136.4, 130.3, 130.0, 129.4, 127.1, 105.6, 77.5, 76.5, 74.6, 73.5, 60.6, 57.9, 55.3, 41.9, 39.6, 30.0, 29.9, 23.0 ppm; HRMS $[M+H]^+=508.2404$ (calcd for $C_{23}H_{34}N_5O_8$: 508.2407).

**Ligand L17.** As per general procedure B2 from compound **17** (35 mg, 0.05 mmol) and purification using a C-18 Sep-Pak® column and mixtures of water and methanol (0% → 100% MeOH). White solid (20 mg, 50%; purity according to HPLC > 95%): IR (neat): $\tilde{\nu}=$ 3302, 2929, 2860, 1737, 1634, 1599, 1549, 1484, 1456, 1395, 1381, 1355, 1231, 1203, 1173, 1122, 1053, 1027, 1007, 823, 792, 719, 693, 665 cm$^{-1}$; $^1$H NMR ([D$_6$]DMSO): $\delta=8.06$ (s, 1H), 8.03 (d, 1H, $J=$ 7.7 Hz), 7.73 (dd, 2H, $J=9.8$, 5.3 Hz), 7.62 (d, 2H, $J=8.3$ Hz), 7.41–7.27 (m, 5H), 7.00 (d, 2H, $J=8.3$ Hz), 5.59 (s, 2H), 4.11 (m, 1H), 3.93

(d, 1H, $J$=13.9 Hz), 3.80 (d, 1H, $J$=13.9 Hz), 3.16 (t, 2H, $J$=6.3 Hz), 2.97 (m, 4H), 2.48 (m, 2H, obscured by DMSO signal), 2.35 (t, 2H, $J$=7.8 Hz), 2.26 (t, 2H, $J$=7.8 Hz), 2.04 (t, 2H, $J$=7.4 Hz), 1.75 (m, 2H, $J$=7.4 Hz), 1.70–1.46 (m, 4H), 1.42–1.20 (m, 8H); $^{13}$C NMR ([D$_6$]DMSO): $\delta$=173.8, 173.2, 171.5 (two C=O), 171.0, 143.7, 141.6, 137.0, 136.0, 130.8, 128.7, 128.1, 127.9, 123.7, 91.2, 60.4, 52.8, 51.9, 41.8, 39.6, 38.3, 38.2, 34.7, 34.0, 31.3, 30.9, 30.8, 30.7, 28.9, 28.7, 26.7, 22.8, 22.6 ppm; HRMS $[M+H]^+$=818.2698 (calcd for $C_{36}H_{49}IN_7O_7$: 818.2738).

**Ligand L18.** As per general procedure B2 from compound **18** (26 mg, 0.05 mmol) and purification by semi-preparative HPLC (5%→95% $CH_3CN$ within 15 min, rest: 0.1% aqueous TFA; flow: 3.5 mL min$^{-1}$). White solid (18 mg, 66%): mp 145–148 °C; IR (neat): $\tilde{\nu}$=3320, 2929, 1665, 1638, 1457, 1190, 1129, 715 cm$^{-1}$; $^1$H NMR ([D$_6$]DMSO with one drop $D_2O$): $\delta$=8.21 (s, 1H), 7.41–7.29 (m, 5H), 5.62 (s, 2H), 4.31 dd, 1H, $J$=4.9 and 7.8 Hz), 4.26 (s, 2H), 4.13 (dd, 1H, $J$=4.4 and 7.8 Hz), 3.93 (dd, 1H, $J$=4.8 and 6.6 Hz), 3.12–3.05 (m, 1H), 2.99 (t, 2H, $J$=6.1 Hz), 2.80 (dd, 1H, $J$=4.9 and 12.5 Hz), 2.57 (d, 1H, $J$=12.5 Hz), 2.03 (t, 2H, $J$=7.1 Hz), 1.92–1.76 (m, 2H), 1.65–1.55 (m, 1H), 1.52–1.15 (m, 10H); $^{13}$C NMR ([D$_6$]DMSO with one drop $D_2O$): $\delta$=172.4, 169.8, 162.9, 138.0, 135.5, 128.8, 128.3, 128.0, 125.7, 61.0, 59.1, 58.2, 55.3, 53.0, 40.0, 39.7, 37.8, 35.1, 28.5, 28.2, 27.9, 25.2, 21.6 ppm (some signals are obscured by the solvent peak but confirmed by HSQC); HRMS $[M+H]^+$=544.2707 (calcd for $C_{26}H_{38}N_7O_4S$: 544.2706).

**Ligand L19.** As per general procedure B1 from compound **19** (40 mg, 0.1 mmol) and purification by flash chromatography with $CH_2Cl_2$/MeOH (25:1). Yellow oil (50 mg, 93%): IR (neat): $\tilde{\nu}$=3260, 3061, 2938, 1730, 1649, 1543, 1515, 1457, 1439, 1203, 758 cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$=8.07 (d, 2H, $J$=9.3 Hz), 7.93 (d, 2H, $J$=7.7 Hz), 7.68 (t, 2H, $J$=8.3 Hz), 7.46 (t, 2H, $J$=7.6 Hz), 7.34–7.25 (m, 3H), 7.27 (s, 1H), 7.21–7.15 (m, 2H), 7.05 (bs, 1H), 5.38 and 5.35 (each d, each 1H, $J$=16.8 Hz), 3.84 (d, 1H, $J$=14.0 Hz), 3.73–3.62 (m, 3H), 3.68 (s, 3H), 3.34 (t, 1H, $J$=6.3 Hz), 2.00 (bs, 1H), 1.84–1.66 (m, 4H), 1.62–1.51 (m, 2H); $^{13}$C NMR ($CDCl_3$): $\delta$=175.3, 167.0, 148.4, 146.8, 141.2, 134.6, 130.3, 129.4, 129.0, 128.7, 128.0, 126.7, 125.3, 122.1, 121.5, 60.5, 54.0, 51.9, 43.1, 39.9, 32.7, 29.3, 23.2 ppm; HRMS $[M+H]^+$=537.2613 (calcd for $C_{31}H_{33}N_6O_3$: 537.2614).

**Ligand L20.** As per general procedure B2 from compound **19** (0.49 mg, 0.6 μmol) and purification by analytical HPLC (5%→90% $CH_3CN$ within 13 min, rest: 0.1% aqueous TFA; flow 1 mL min$^{-1}$). Red solid (50% yield as determined by UV/Vis: $\lambda$=557 nm, $d$=1 cm, $\varepsilon$=150000 M$^{-1}$ cm$^{-1}$; purity according to HPLC ≥ 95%): HRMS-ESI $[M]^+$=916.3737 (calcd for $C_{46}H_{58}N_7O_9S_2$: 916.3732).

**Ligand L22.** As per general procedure B1 from compound **22** (TFA salt; 13 mg, 0.015 mmol) and purification by semi-preparative HPLC (5%→95% $CH_3CN$ within 14 min, rest: 0.1% aqueous TFA; flow: 3 mL min$^{-1}$). White solid (68%; purity according to HPLC > 95%): mp 110–115 °C; IR (neat): $\tilde{\nu}$=2957, 2870, 1744, 1674, 1622, 1196, 1137, 718 cm$^{-1}$; HRMS $[M+H]^+$=873.2899 (calcd for $C_{33}H_{49}GdN_9O_9$: 873.2894).

**Peptide 23.** Prepared by SPPS. White solid (7.4 mg, 40%; purity > 95%): LC–MS: $[M]^+$=1316, 659 (calcd for $C_{60}H_{89}N_{19}O_{15}$: 1316.467).

**Peptide L24.** 50 μL of peptide **23** (10 mM in EtOH), 62.5 μL of compound **17** (10 mM in 1:1 EtOH/$H_2O$), 12.5 μL of $Cu(OAc)_2$ (10 mM in $H_2O$) and 25 μL of sodium ascorbate (10 mM n in $H_2O$) were stirred at 60 °C for 4 h. Formation of the product was monitored by analytical HPLC and the reaction mixture analyzed by MS: $[M+2H]^{2+}$=1001.62 (calcd for $C_{89}H_{130}IN_{23}O_{22}$: 2001.03). The solution was radiolabeled in situ without further purification in analogy to previously reported procedures.[9,13]

**Peptide N$^{\alpha}$HisAc-BBS(7–14).** Prepared by SPPS. White solid (7.8 mg, 34%; purity > 95%): LC–MS: $[M]^+$=1428, 715 (calcd for $C_{66}H_{97}N_{19}O_{17}$: 1428.594).

**[Re(CO)$_3$(L15)].** As per general procedure D from ligand **L15** (11 mg, 0.01 mmol) in MeOH/$H_2O$ (1:1) and purification by semi-preparative HPLC (5→95% $CH_3CN$ within 14 min, rest: 0.1% aqueous TFA; flow: 3 mL min$^{-1}$). Colorless oil (8 mg, 60%, purity according to HPLC > 95%): $[\alpha]_D^{20}$=+23.6 ($c$=0.2 in MeOH); IR (neat): $\tilde{\nu}$=2870, 2023 and 1887 (strong), 1638, 1450, 1346, 1102, 946 cm$^{-1}$; HRMS $[M+Na]^+$=1356.5382 (calcd for $C_{53}H_{88}N_5O_{22}NaRe$: 1356.5376).

**[Re(CO)$_3$(L16)].** As per general procedure D from ligand **L16** (25 mg, 0.05 mmol) in MeOH/$H_2O$ (1:1) and purification by semi-preparative HPLC (5%→90% $CH_3CN$ within 13 min, rest: 0.1% aqueous TFA; flow 3 mL min$^{-1}$). White amorphous solid (29 mg, 75%; purity according to HPLC > 95%): mp > 150 °C (dec.); $[\alpha]_D^{20}$=−12.0 ($c$=1 in MeOH); IR (neat): $\tilde{\nu}$=3281, 2932, 2026 and 1905 (strong), 1670, 1441, 1198, 1141, 1030, 723 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH): $\delta$=8.10 (s, 1H), 7.44–7.34 (m, 5H), 5.68 (s, 2H), 4.31–4.20 (m, 3H), 3.71 (d, 1H, $J$=9.8 Hz), 3.53 (s, 3H), 3.49 (t, 1H, $J$=9.8 Hz), 3.42–3.32 (m, 1H), 3.39 (t, 1H, $J$=9.6 Hz), 3.27–3.19 (m, 2H), 3.11 (t, 1H, $J$=6.8 Hz), 1.92–1.84 (m, 2H), 1.65–1.53 (m, 4H); $^{13}$C NMR ([D$_4$]MeOH): $\delta$=198.5, 197.0, 196.6, 186.3, 172.2, 149.7, 135.6, 130.4, 130.3, 129.7, 124.4, 105.8, 77.7, 76.6, 74.7, 73.7, 67.8, 57.8, 56.5, 53.8, 39.6, 33.8, 30.2, 24.4 ppm; HRMS $[M+Na]^+$=800.1553 (calcd for $C_{26}H_{32}N_5O_{11}ReNa$: 800.1554).

**[Re(CO)$_3$(L17)].** As per general procedure D from ligand **L17** (10 mg, 0.01 mmol) in MeOH/$H_2O$ (1:1). The complex was purified using a Sep-Pak column and eluting with a mixture of MeOH (80%) and water. White solid (5 mg, 38%; purity according to HPLC > 95%): IR (neat): $\tilde{\nu}$=3286, 2933, 2020 and 1897 (strong), 1639, 1545, 1202, 1144, 723 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH): $\delta$=7.60 (d, 2H, $J$=8.3 Hz), 7.44–7.31 (m, 6H), 6.99 (d, 2H, $J$=8.3 Hz), 5.67 (d, 2H $J$=8.7 Hz,), 4.34–4.20 (m, 2H), 3.20–3.13 (m, 3H), 2.58 (t, 2H), 2.52–2.48 (m, 3H), 2.18 (t, $J$=7.5 Hz, 2H), 1.95–1.78 (m, 4H), 1.75–1.64 (m, 2H), 1.59–1.29 (m, 12H); $^{13}$C NMR ([D$_4$]MeOH): $\delta$=174.4, 173.3, 149.1, 148.2, 141.5, 137.2, 134.0, 130.41, 128.9, 128.7, 128.1, 128.1, 122.9, 122.5, 90.3, 66.2, 62.1, 61.4, 55.0, 54.9, 38.7, 38.5, 35.1, 35.0, 34.3, 31.5, 31.0, 30.9, 30.8, 28.8, 28.7, 28.5, 28.3, 27.1, 26.0, 23.0, 23.0, 22.8, 22.3, 21.7 ppm. HRMS-ESI $[M+H]^+$=1088.2061 (calcd for $C_{39}H_{48}IN_7O_{10}Re$: 1088.2065).

**[Re(CO)$_3$(L18)].** As per general procedure D from ligand **L18** (22 mg, 0.04 mmol) in MeOH/$H_2O$ (1:1) and purification by semi-preparative HPLC (15%→95% $CH_3CN$ within 15 min, rest: 0.1% aqueous TFA; flow 3 mL min$^{-1}$). White solid (20 mg, 62%; purity according to HPLC > 95%): mp 165–168 °C; $[\alpha]_D^{20}$=+31.5 ($c$=1 in MeOH); IR (neat): $\tilde{\nu}$=2938, 2018 and 1889 (strong), 1631, 1439 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH): $\delta$=8.10 (s, 1H), 7.44–7.34 (m, 5H), 5.68 (s, 2H), 4.48 (dd, 1H, $J$=7.8 and 4.8 Hz), 4.32–4.20 (m, 3H), 3.28–3.13 (m, 3H), 3.11 (t, 1H, $J$=6.7 Hz), 2.92 (dd, 1H, $J$=12.6 and 5.0 Hz), 2.71 (d, 1H, $J$=12.6 Hz), 2.21 (t, 2H, $J$=7.2 Hz), 1.92–1.84 (m, 2H), 1.78–1.59 (m, 4H), 1.58–1.53 (m, 4H), 1.48–1.39 (m, 2H); $^{13}$C NMR ([D$_4$]MeOH): $\delta$=198.6, 197.0, 196.7, 186.1, 176.4, 166.3, 149.7, 135.6, 130.4, 130.2, 129.6, 124.4, 67.7, 63.5, 61.8, 57.1, 56.5, 53.8, 41.2, 40.0, 37.0, 33.9, 30.3, 29.9, 29.6, 27.0, 24.5 ppm; HRMS $[M+H]^+$=836.1843 (calcd for $C_{29}H_{36}N_7NaO_7ReS$: 836.1852).

**[Re(CO)$_3$(L19)].** As per general procedure D from ligand **L19** (16 mg, 0.03 mmol) in MeOH/$H_2O$ (1:1). Adjustment of the pH of

the reaction mixture to pH 8 by addition of aqueous NaOH (0.1 M) resulted in precipitation of the product. Centrifugation (2 min, 13200 rpm, 16.1 *g*), decanting the solution and drying of the solid provided [Re(CO)$_3$(**L19**)] as a yellow powder (18 mg, 75%; purity according to HPLC > 95%; $^1$H NMR indicates the presence of 2% (*w*/*w*) $Et_4NBr$): mp > 250 °C (dec.); $[\alpha]_D^{20} = +25.5$ (*c* = 1.0 in MeOH); IR (neat): $\tilde{\nu}$ = 2939, 2018 and 1894 (strong), 1642, 1260, 756 cm$^{-1}$; $^1$H NMR ([D$_4$]MeOH with one drop of 1% DCl; recorded after complete H/D exchange): $\delta$ = 8.38 (d, 2H, *J* = 8.0 Hz), 8.35–8.28 (m, 4H), 8.14 (s, 1H), 8.02–7.98 (m, 2H), 7.43–7.35 (m, 5H), 5.71 and 5.68 (each d, each 1H, each *J* = 15.4 Hz), 4.36 (d, 1H, *J* = 16.1 Hz), 4.26 (dd, 1H, *J* = 1.0 and 16.1 Hz), 3.75 (t, 2H, *J* = 6.9 Hz), 3.22 (t, 1H, *J* = 6.7 Hz), 2.04–1.99 (m, 2H), 1.93–1.85 (m, 2H), 1.84–1.68 (m, 2H); $^{13}$C NMR ([D$_4$]MeOH with one drop of 1% DCl): $\delta$ = 198.6, 196.8, 196.6, 186.1, 166.4, 153.4, 149.6, 142.6, 138.7, 135.5, 130.4, 130.2, 129.6, 128.3, 128.2, 124.5, 124.1, 122.1, 67.6, 56.5, 53.8, 41.1, 33.9, 30.1, 24.7 ppm; HRMS $[M+H]^+$ = 793. 1776 (calcd for $C_{33}H_{30}N_6O_6Re$: 793.1784).

**[Re(CO)$_3$(L20)].** As per general procedure D from ligand **L20** (~0.1 mg, 0.1 μmol) in EtOH/H$_2$O (1:1) and purification by analytical HPLC (5%→90% $CH_3CN$ within 13 min, rest: 0.1% aqueous TFA; flow 1 mL min$^{-1}$) yielded a red solution of Re complex X (HPLC: quantitative reaction; purity > 95%): IR (neat): $\tilde{\nu}$ = 3415, 2920, 2023 and 1909 (strong), 1681, 1441, 1199, 1136, 1031, 722 cm$^{-1}$; HRMS $[M]^+$ = 1186.3050 (calcd for $C_{49}H_{57}N_7O_{12}ReS_2$: 1186.3059).

**[Re(CO)$_3$(L22)].** As per general procedure D from ligand **L22** (5 mg, 5 μmol) in water and purification by semi-preparative HPLC (5%→95% $CH_3CN$ within 14 min, rest: 0.1% aqueous TFA; flow: 3 mL min$^{-1}$). White solid (3 mg, 54%; purity according to HPLC > 95%): mp > 200 °C (dec.); $[\alpha]_D^{20} = +12.3$ (*c* = 0.3 in MeOH); IR (neat): $\tilde{\nu}$ = 3249, 2938, 2020 and 1901 (strong), 1623, 1403, 1198, 1140, 1083, 717 cm$^{-1}$; HRMS $[M+H]^+$ = 1127.2033 (calcd for $C_{35}H_{46}GdN_9O_{12}Re$: 1129.2064).

### Crystal data

Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using MoK$_\alpha$ radiation ($\lambda$ = 0.7107 Å) that was graphite-monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fiber and immediately transferred to the diffractometer. The program suite CrysAlis$^{Pro}$ was used for data collection, semi-empirical absorption correction and data reduction.[42] The structures of [Re(CO)$_3$(**L6**)] and [Re(CO)$_3$(**L5**)] were solved with direct methods using SIR97[43] whereas the one of [Re(CO)$_3$(**L4**)] was solved with the help of SIR2004.[44] All structures were refined by full-matrix least-squares methods on $F^2$ with SHELXL-97[45] and checked for higher symmetry using the program Platon.[46] Crystal data and structure refinement can be found in table S3 of the Supporting Information. In addition, CCDC-781592, -781593, and -781594 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk. The structure of [Re(CO)$_3$(**L4**)] contains seven complexes, two and a half molecules of THF as well as half a molecule of water in the asymmetric unit. Six out of the seven complexes overlay almost perfectly if the phenyl groups are omitted. Only in one case is the whole benzyl group oriented differently.

### In vitro Experiments

**Mouse serum albumin binding.** Evaluation of the binding of radiolabeled peptides to mouse albumin was performed after incubation of the radiolabeled conjugate (~100 kBq) with 500 μL of mouse serum (Sigma–Aldrich) for 15 min at 37 °C. Samples were analyzed by FPLC (column: Superose 12, Pharmacia Biotech) using PBS as eluent at a flow rate of 0.5 mL min$^{-1}$.

**Binding to PC-3 cells.** The binding to GRP receptors was tested in intact PC-3 cells. Cells were placed in 6-well plates one day prior to the assay ($10^6$ cells per well). Cells were incubated in triplicate with the radiolabeled conjugate (20 kBq) for 1 h at 37 °C. Then the cells were washed twice with PBS to remove unbound radiotracers and recovered with NaOH (1 M). Bound radioactivity was measured in a γ counter. Nonspecific binding was determined in the presence of 1 μM natural bombesin.

### Biodistribution studies

Animal experiments were conducted in compliance with the Swiss animal protection laws and the ethical principles and guidelines for scientific animal trials of the Swiss Academy of Medical Sciences and the Swiss Academy of Natural Sciences. Female CD-1 *nu*/*nu* mice (6–8-week-old) were injected s.c. with $8\times10^6$ cells in 150 μL culture medium without supplements. Three weeks after tumor implantation the mice were injected i.v. with ~1 MBq of the radiolabeled peptides (3–4 animals per group). At different p.i. times (1 and 24 h), the animals were sacrificed by cervical dislocation. The organs and blood were collected and weighed. The radioactivity was measured in a γ counter. To determine specificity of the in vivo uptake, one group of mice received a co-injection of 100 μg of unlabeled natural BBS and the radiolabeled analogue and sacrificed 1.5 h p.i. Results are presented as percentage of injected dose per gram of tissue (% ID g$^{-1}$).

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