DOI: 10.1002/cmdc.200800418

A Click Approach to Structurally Diverse Conjugates Containing a Central Di-1,2,3-triazole Metal Chelate

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The selective and efficient synthesis of novel tridentate metal chelating systems containing two 1,4-disubstituted 1,2,3-triazole heterocycles obtained via the copper(I)-catalyzed cycloaddition of alkynes and azides (click reaction) is described. The constructs are shown to be efficient ligand systems for the chelation of *fac*-$[M(CO)_3(H_2O)_3]^+$ ($M = {}^{99m}Tc$, Re) yielding well-defined and stable complexes. The organometallic ^{99m}Tc conjugates are suitable for application as diagnostic radiotracers for single photon emission computed tomography (SPECT) as demonstrated in vivo with a fragment of the tumor-targeting bombesin peptide functionalized with a di-1,2,3-triazole chelator and radiolabeled with $[^{99m}Tc(CO)_3]^+$. Starting from readily available dialkyne precursors, the central chelating systems are formed as the conjugates are assembled by click reaction with azide-functionalized entities. Depending on the nature of the azide substrates employed (e.g. lipophilic or hydrophilic residues) pharmacologically relevant characteristics of the final metal conjugate such as hydrophilicity or overall charge can be readily modulated. The procedures described also enable the facile introduction of other probes into the metal conjugate, providing access to potential multimodal imaging agents.

Introduction

The preparation of conjugates made up of multiple components, such as biologically relevant molecules, pharmacological modifiers, diagnostic probes, therapeutic agents or a combination thereof, by chemical methods is often a challenging task. For instance, the attachment of (radio)metal chelates to (bio)-molecules for diagnostic (imaging) or therapeutic applications can be complicated by the multifunctional character of both the metal chelating system and the (bio)molecule. Potential cross reactivity and/or incompatibility of the various functional groups present frequently require the extensive use of protection/deprotection reaction sequences in order to obtain the desired metal conjugates in a selective and controllable manner. As a consequence, new and efficient synthetic strategies are needed in order to overcome these drawbacks.

The copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC)[1,2] has become a prime example of click chemistry[3] because of its extraordinary efficiency, selectivity, compatibility with a wide range of functional groups, and the benign reaction conditions. Not surprisingly, the attractive features of this click reaction have inspired numerous applications across different scientific disciplines.[4] The majority of reported applications of CuAAC use the 1,4-disubstituted 1,2,3-triazole formed as a stable linker for the connection of two chemical/biological entities.[4] By contrast, the potential of the heterocycle itself has received less attention so far.[5] For example, while the CuAAC has been successfully implemented for the attachment of ligand systems or metal complexes thereof to various molecules and materials via a triazole linker, only recently has there been an interest in employing this click reaction to design chelating systems in which the 1,2,3-triazole forms an integral part of the complex.

First reported as copper(I)-stabilizing ligands,[6] chelators containing a 1,2,3-triazole[7] obtained by click chemistry have since been employed in transition metal catalysis[8–11] and in the development of metal responsive fluorophores[12–14] and platinum complexes[15] that exhibit anticancer activity in vitro. We have shown that such ligand systems can be used for the chelation of $[M(CO)_3(H_2O)_3]^+$ ($M = {}^{99m}Tc$, Re),[16] a prominent organometallic precursor for the preparation of ^{99m}Tc-based diagnostic radiotracers for single photon emission computed tomography (SPECT).[17] Because the synthesis of the chelating system and its attachment to a (bio)molecule are achieved simultaneously in a single step, we call this strategy "click-to-chelate". Metal complex formation is highly efficient provided that coordination of the 1,2,3-triazole occurs via the N(3) (rather than N(2) or N(1)) of the heterocycle, as we and others have observed.[15,16] In addition, mono-1,2,3-triazole chelators

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200800418.

have been shown to be suitable for applications in vitro and in vivo.[15,16,18,19]

Examples of chelators containing multiple 1,2,3-triazoles and their complexes with different metals have also been described.[7,20–25] However, these ligand systems have not yet been applied to the functionalization of a (bio)molecule with a (radio)metal. Herein, we report the preparation of novel tridentate di-1,2,3-triazole ligand systems, their complexes with $[M(CO)_3]^+$ (M = ^{99m}Tc, Re) and their use as bi- or trifunctional chelating agents for the assembly of structurally diverse metal conjugates useful for diagnostic and potentially therapeutic applications.[26]

Results and Discussion

The advantages of using the CuAAC for the assembly of conjugates and the synthesis of metal chelators are well documented in the literature. We recognized that a combination of the two approaches could provide convenient access to multicomponent conjugates that contain a central di-1,2,3-triazole metal chelate (Figure 1). If the dialkyne moiety is linked to a (bio)molecule (e.g. R^1), the strategy described herein is complementary to our previously reported "click-to-chelate" approach,[16] which

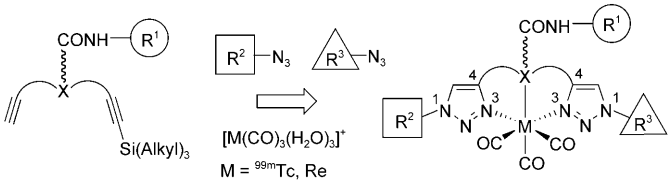
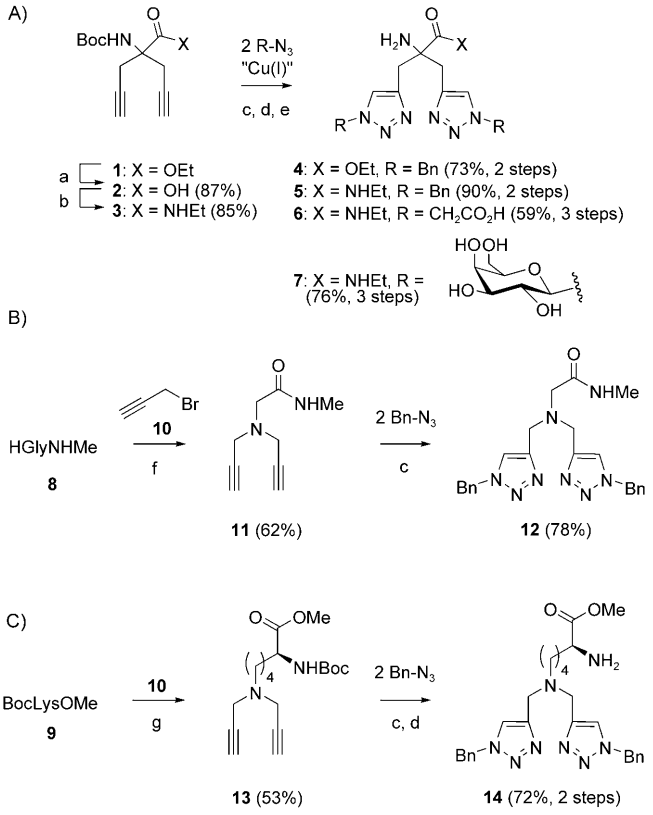


Figure 1. Click assembly of conjugates and simultaneous formation of tridentate di-1,2,3-triazole metal chelating systems (X, coordination site (e.g. a heteroatom); R^1–R^3, various substituents.



Scheme 1. Synthesis of symmetrical di-1,2,3-triazole chelators derived from A) *C*(α)-bis(propargyl)glycine; B) *N*(α)-bis(propargyl)glycine **11**; C) *N*(ε)-bis-(propargyl) lysine **13**. *Reagents and conditions*: a) 1 M NaOH/MeOH (1:1), RT; b) $EtNH_3Cl$, Et_3N, HBTU, DMF, 0 °C→RT; c) 2.5 equiv azide, $Cu(OAc)_2$ (20 mol%), sodium ascorbate (40 mol%), H_2O/*t*BuOH (1:1), RT (20–48 h) or 50–80 °C (2–5 h); d) CH_2Cl_2/TFA (3:1), RT; e) for **6**: 1 M NaOH/MeOH (1:1), RT; for **7**: cat NaOMe, MeOH, RT; f) 2.5 equiv **10**, K_2CO_3, MeOH, RT; g) 3 equiv **10**, pyridine, THF, 50 °C.

employs azide-functionalized (bio)molecules in the synthesis and attachment of mono-1,2,3-triazole ligand systems. In principle, any azide-functionalized entity of interest could be attached to the dialkyne-derived chelator and thus, the final metal conjugate. In the context of the development of radiopharmaceuticals or imaging probes in general, the introduction of targeting (bio)molecules, pharmacological modifiers, secondary imaging probes, affinity tags, or therapeutic agents into the metal conjugate would be of particular interest.

Synthesis of di-1,2,3-triazole ligand systems

Initially, we investigated the synthesis of di-1,2,3-triazole ligand systems derived from *C*(α)-bis(propargyl)glycine **2**[27] (Scheme 1 A). Initial studies were performed with ethyl ester derivative **1**, however, we soon learned that having an ester functionality in compound **4** is not ideal because of ester hydrolysis and/or transesterification side reactions occurring during the (radio)metal labeling step (see below). Thus, carboxylic acid ester **1** was converted to ethyl amide **3** providing a stable compound for subsequent metal complexation and, at the same time, mimicking the conjugation of the chelator or its precursor, respectively, via an amide linkage to, for example, a biomolecule. In this study, three commercial azides were used for the click reaction with dialkynes. While the reaction with benzyl azide or azidoacetic acid ethyl ester (followed by hydrolysis) provides chelators of different hydrophilicity, 1-azido-1-deoxy-β-D-galactopyranoside tetraacetate was chosen as an azide substrate because 1) glycosylation of peptide-based radiotracers has been shown to improve their pharmacological characteristics[28,29] and 2) carbohydrate–metal complexes represent interesting conjugates that may be used as tools for studying protein interactions.[30]

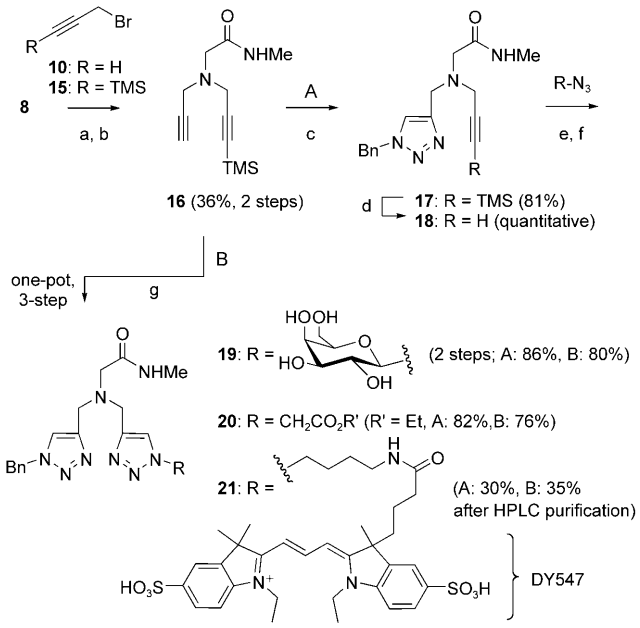
Even though the CuAAC proceeds efficiently in the presence of other functional groups,[31] protected azides and dialkynes were employed in this study in order to facilitate isolation and characterization of intermediates and products. Reaction of dialkyne **1** or **3** with excess of the corresponding azide was carried out in aqueous media (H_2O/*t*BuOH) in the presence of catalytic amounts of in situ generated Cu^I ($Cu(OAc)_2$/sodium ascorbate) according to the procedure initially described by the Sharpless group.[1] While the double click reactions proceeded only slowly at room temperature, complete conversion of the dialkyne substrate was usually achieved at elevated tempera-

tures (50–80 °C) within a few hours.[32] Both reaction conditions yielded symmetrical di-1,2,3-triazole chelators **4**–**7** in good overall yields after the removal of the remaining protecting groups.

In order to avoid the multistep synthesis required for the preparation of *C*(α)-bis(propargyl)glycine **2**, we sought alternative precursors from which suitable dialkyne substrates can readily be obtained. Based on our previous experience,[18] we postulated that *N*-bis(propargyl) amines derived from amino acids would be ideal candidates for our purposes. Thus, dialkynes **11** and **13** were synthesized in one step and with satisfying yields by alkylation of HGlyNHMe (**8**) and BocLysOMe (**9**), respectively, with propargyl bromide (**10**) (Scheme 1 B/C).[33] Subsequent double click reaction with benzyl azide as described above gave, after an additional deprotection step in the case of the Lys derivative, di-1,2,3-triazole chelators **12** and **14** in good yields.

Side-chain-modified amino acid derivatives like **13** and **14** offer an opportunity for insertion into the backbone of peptides. In fact, lysine derivatives (single amino acid chelators) equipped with a chelating system via the nitrogen N(ε) have been reported for the incorporation of $[M(CO)_3]^+$ (M = ^{99m}Tc, Re) into peptides by solid phase synthesis.[34] The *N*(ε)-propargyl Lys derivatives described herein are distinguished from the reported system in that they not only offer the possibility to introduce a chelator into the peptide selectively even after deprotection and cleavage from the resin, but they could also be used for the simultaneous conjugation of various entities by click reaction with appropriate azide substrates.

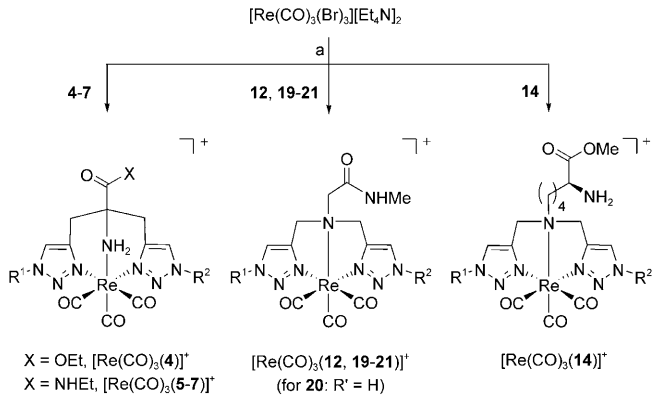
The reaction of dialkynes with commercial or otherwise readily available azide substrates already offers efficient access to structurally diverse conjugates that contain a di-1,2,3-triazole metal chelating system. However, application of the recently reported "sequential click strategy"[35,36] would allow the use of two different azide substrates, and therefore increase the number of possible combinations. We thus prepared compound **16** with both a terminal and trialkylsilyl-protected alkyne by the stepwise alkylation of HGlyNHMe (**8**) with propargyl bromide (**10**) and the TMS-protected analogue **15**, respectively (Scheme 2). Attempted reaction of compound **16** with benzyl azide in aqueous media using $Cu(OAc)_2$/sodium ascorbate resulted in the loss of the TMS protecting group, and a mixture of mono- and di-1,2,3-triazoles was obtained. Following a procedure described by Montagnat et al.,[35] the use of copper powder as the source of Cu^I catalyst prevented the desilylation and the desired mono-1,2,3-triazole product **17** was isolated in high yields. Removal of the TMS protecting group under basic conditions[37] furnished alkyne **18**, which was subjected to the second click reaction with either protected 1-azido-galactopyranoside, azidoacetic acid ethyl ester or an azide derivative of the fluorophore DY547 (a CY3 analogue[38]). The observed instability of the TMS protecting group in intermediate **16** could be exploited for the development of an efficient one-pot, three-step procedure. Thus, reaction of dialkyne **16** with benzyl azide at room temperature using Cu(0) followed by addition of the second azide substrate and catalytic amounts of $Cu(OAc)_2$/sodium ascorbate yielded the asymmetric di-1,2,3-triazole directly. Either of the two procedures described provided, after an additional deprotection step in the case of derivative **19**, products **19**–**21** in good yields.



Scheme 2. Synthesis of asymmetric di-1,2,3-triazole chelators by sequential CuAAC with A) isolation of intermediates or by B) one-pot procedures. *Reagents and conditions*: a) **10**, DIPEA, MeOH, RT; b) **15**, DIPEA, CH_3CN, RT; c) benzyl azide, excess Cu(0), H_2O/*t*BuOH (1:1), RT; d) cat K_2CO_3, MeOH, RT; e) 1.1 equiv azide, $Cu(OAc)_2$ (20 mol%), sodium ascorbate (40 mol%), H_2O/*t*BuOH (1:1), RT ; f) for **19**: cat NaOMe, MeOH, RT; g) one-pot procedure: H_2O/*t*BuOH (1:1); 1) 1 equiv benzyl azide, excess Cu(0), RT; 2) 1 equiv of second azide, $Cu(OAc)_2$ (20 mol%), sodium ascorbate (40 mol%), RT.

Metal labeling of di-1,2,3-triazole chelators

With a set of di-1,2,3-triazoles in hand, we next investigated their use as tridentate ligand systems for the complexation of *fac*-$[M(CO)_3]^+$ (M = ^{99m}Tc, Re). Like the tridentate di-1,2,3-triazole chelators obtained from *C*(α)-bis(propargyl)glycine (Scheme 1 A), those derived from bis(propargyl) amines[39] (Schemes 1 B, 1 C and 2) provide two coordination sites via the preferred nitrogen N(3) of the heterocycle, but form 5-membered ring chelates when coordinated to the metal (Scheme 3). Nonradioactive labeling was performed with the precursor $[Re(CO)_3(Br)_3][Et_4N]_2$[40] in water or alcohol following previously described procedures.[16,18] Interestingly, HPLC analysis of reaction mixtures obtained under neutral reaction conditions (pH ~ 7) indicated the formation of two products, both of which corresponded to the expected cationic rhenium complexes according to mass spectrometric analysis of the isolated samples. However, decreasing or increasing the pH yielded one Re species as the major component in all cases.[38] The pH-dependant formation of distinguishable species of Re complexes is presumably the result of different protonation states of the ligand systems that contain multiple amine functionalities. In the following, metal labeling experiments with di-1,2,3-triazoles were performed at pH ~ 4–6. Under these conditions,



Scheme 3. Synthesis and proposed structures of cationic $Re(CO)_3$ complexes of di-1,2,3-triazole ligands. *Reagents and conditions*: a) For **4**: EtOH, 50 °C; for **5**–**7**, **12**, **14**, **19**–**21**: H_2O, H_2O/EtOH or H_2O/MeOH (1:1), 65–80 °C; pH 4–5. The counter ion of the cationic complexes is a bromide ion derived from the Re precursor. Yields of complexes obtained range from 60–90% depending on the purification method (HPLC or reversed phase chromatography).

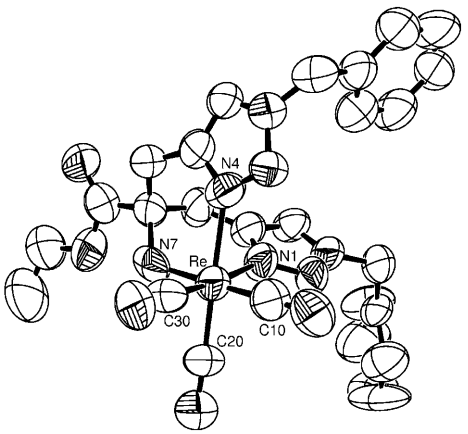
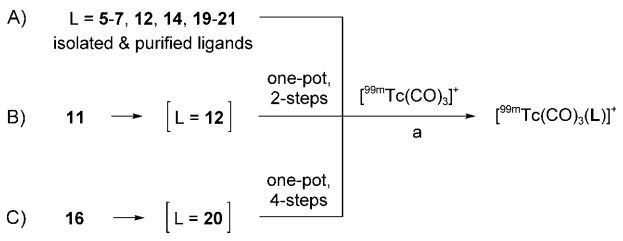


Figure 2. ORTEP-3 representation of cationic $[Re(CO)_3(\mathbf{4})]^+$ with thermal ellipsoids shown at 50% probability. Hydrogen atoms, the bromide counter ion and solvent molecules (THF) are omitted for clarity. Selected bond lengths [Å] and angles [°]: Re-N(1) 2.19(1), Re-N(4) 2.18(1), Re-N(7) 2.16(1), Re-C(10) 1.76(1), Re-C(20) 1.85(1), Re-C(30) 1.82(2), N(7)-Re-N(1) 79.3(5), N(1)-Re-C(10) 96.6(7), C(10)-Re-C(30) 86.2(8), C(30)-Re-N(7) 97.9(6), N(7)-Re-N(4) 88.4(5), N(4)-Re-C(10) 91.2(7), C(10)-Re-C(20) 90.2(7), C(20)-Re-N(7) 90.0(6), N(4)-Re-N(1) 78.0(5), N(1)-Re-C(20) 98.5(6), C(20)-Re-C(30) 86.3(7), C(30)-Re-N(4) 97.2(6).

rhenium complexes $[Re(CO)_3(\mathbf{L})]^+$ (**L** = **4**–**7**, **12**, **14**, **19**–**21**) were obtained as a single product (>90%). Conveniently, metal labeling of ligand **20** in aqueous media resulted in the simultaneous hydrolysis of the ester functionality and carboxylic acid derivative $[Re(CO)_3(\mathbf{20})]^+$ was obtained directly. Ester derivative $[Re(CO)_3(\mathbf{4})]^+$ could be obtained by reaction in ethanol whereas metal labeling of ligand **4** in aqueous media resulted in the formation of a mixture of products due to partial hydrolysis of the carboxylic ester functionality (data not shown). On the other hand, no cleavage of the methyl ester was observed with ligand **14**, probably because the amino acid side chain acts as a spacer and slows down metal-assisted ester hydrolysis. All Re complexes, except $[Re(CO)_3(\mathbf{21})]^+$ that was available only in very small amounts, were fully characterized by spectroscopic methods (NMR, MS, IR).[38,41] In case of ethyl ester $[Re(CO)_3(\mathbf{4})]^+$, crystals suitable for X-ray analysis were obtained. Figure 2 shows an ORTREP plot of the complex, which confirms coordination of the metal via the two N(3) atoms of the 1,2,3-triazole heterocycle and the N(α) of the glycine precursor.

All di-1,2,3-triazole compounds could also be readily radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ in aqueous phosphate buffer at 80–100 °C and pH ~5.5–6.5 (Scheme 4A). Quantitative formation of complexes with the organometallic ^{99m}Tc tricarbonyl precursor was achieved at ligand concentrations in the micromolar range (10^{-4}–10^{-6} M), which demonstrates the efficiency of the new tridentate chelating systems. Identity of the $[^{99m}Tc(CO)_3(\mathbf{L})]^+$ (**L** = **5**–**7**, **12**, **14**, **19**–**21**) was confirmed in each case by comparison of the γ-HPLC trace with the UV trace of the corresponding rhenium complexes, a procedure which is common practice with ^{99m}Tc complexes on a n.c.a. (no carrier added) level.[38] Noteworthy, compound $[^{99m}Tc(CO)_3(\mathbf{21})]^+$ containing an organic dye represents an example of a conjugate potentially useful for SPECT and fluorescence imaging. The recognition that many biomedical imaging modalities provide complementary information has recently stimulated great interest in the development of both probes suitable for multimodal imaging and instrumentation merging different imaging techniques (SPECT, PET, CT, MRI, optical).[42] The concept of employing azide- (or alkyne-) functionalized probes or precursors thereof for the assembly of chelators suitable for the stable complexation of a (radio)metal might be applicable to the synthesis of a variety of multimodal imaging agents as well as conjugates combining an imaging probe and a therapeutic agent.

The stepwise synthesis of multicomponent conjugates containing a (radio)metal chelate may be considered relatively efficient by current standards. However, we have previously shown that the synthesis of mono-1,2,3-triazole chelators by CuAAC and radiometal labeling with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ can be achieved by efficient one-pot procedures.[16,18] We were pleased to find that this approach could also be applied to the novel di-1,2,3-triazole ligand systems, therefore simplifying further the synthetic procedure (Scheme 4B and C). For example, heating of an aqueous solution of dialkyne **11** and benzyl azide in the presence of Cu^I catalyst for 20 min followed by the addition of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and continuous heating for 30 min provided $[^{99m}Tc(CO)_3(\mathbf{12})]^+$ in high purity (>90%) and identical with all respects to the product obtained from isolat-

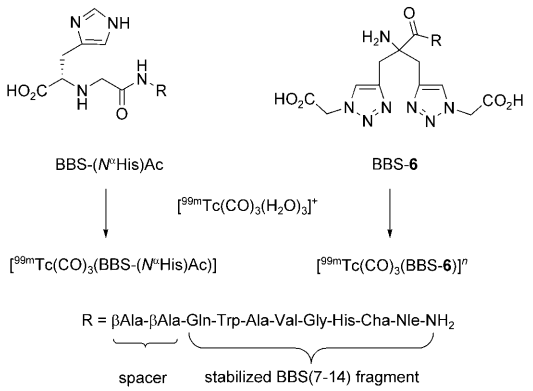


Scheme 4. A) Radiolabeling of di-1,2,3-triazole chelators with $[^{99m}Tc(CO)_3(H_2O)_3]^+$; B) and C) one-pot procedures yielding ^{99m}Tc tricarbonyl complexes from dialkyne precursors directly. *Reagents and conditions*: a) $[^{99m}Tc(CO)_3(H_2O)_3]^+$, PBS buffer pH 5.5, 80–100 °C, 20–30 min.

ed and pre-purified precursors **12**.[38] Similarly, starting from dialkyne **16**, the final radiometal complex $[^{99m}Tc(CO)_3(\mathbf{20})]^+$ could be obtained in a total of four synthetic steps and high purity without isolation and purification of any of the intermediates described above.

In vitro and in vivo evaluation of di-1,2,3-triazole ligand systems

We previously reported the functionalization of a stabilized, gastrin-releasing peptide (GRP) receptor-targeting bombesin (BBS) peptide fragment with a mono-1,2,3-triazole chelating system, its radiometal labeling with $[^{99m}Tc(CO)_3]^+$ and in vivo evaluation.[16] This study and other recent results reported by our group[19] have demonstrated the viability of "clicked" chelators for the development of tumor-targeting radiotracers. In order to assess the novel di-1,2,3-triazole-containing ligand systems in this context, we set out to attach them to the same BBS peptide fragment, radiolabel the conjugate with $[^{99m}Tc(CO)_3]^+$ and compare it in vitro and in vivo with a previously reported BBS analogue with sequence homology but equipped with an (N^{α}His)Ac chelate[43] (Scheme 5). Notably,



Scheme 5. Structures of bombesin derivatives equipped with a (N^{α}His)Ac chelator or a di-1,2,3-triazole ligand system and their radiolabeling with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ for evaluation in vitro and in vivo (Cha = Cyclohexylalanine, Nle = Norleucine). The overall charge (n) of $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]^n$ is expected to be positive ($n = +1$) at acidic pH and negative ($n = -1$) under physiological conditions due to deprotonation of the appending carboxylic acid functionalities.

chelation of the $^{99m}Tc^{I}$ tricarbonyl precursor with (N^{α}His)Ac yields a neutral complex, whereas use of di-1,2,3-triazole ligands gives rise to cationic products (Scheme 3). It is known that positively charged small peptidic radiotracers tend to exhibit unfavorable high accumulation and retention in radiation sensitive kidneys.[44,45] In order to avoid this potential drawback, we selected di-1,2,3-triazole ligand system **6** for attachment to the BBS fragment and proof of principle studies. We reasoned that the two appending carboxylic acid functionalities are deprotonated at physiological pH and thus would not only compensate for the cationic character of the chelate, but also result in an overall negative charge of the peptide conjugate. We have recently shown that the latter can have a positive effect on the pharmacological characteristics of BBS-derived radiotracers in comparison to neutral analogues.[46]

Dialkyne **2** was coupled on solid support to the N terminus of the BBS derivative using standard coupling conditions and clicked with azidoacetic acid[47] in DMF in the presence of CuBr and DIPEA according to the procedure described by the Meldal group.[2] Deprotection and cleavage from the resin provided the desired peptide derivative BBS-**6** after HPLC purification.[38] Labeling of BBS-**6** with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ in phosphate buffer at pH 6.5 yielded complex $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]^+$ efficiently as a single product (HPLC). Compound $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)] was prepared as previously described.[43]

In vitro experiments with GRP overexpressing PC-3 cells revealed similarly high receptor affinity for both radiolabeled compounds ($K_d = 0.19 \pm 0.12$ nM for $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)] and 0.35 ± 0.09 nM for $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]$). Time-dependant biodistribution studies were performed in nude mice bearing PC-3 tumor xenografts. Figure 3 shows a representative comparison of the two radiotracers at 1.5 h p. i.[38] On average, $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]$ showed a similar biodistribution pattern and pharmacokinetic profile as the previously reported compound $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)].[43] Clearance of the radiotracer from the blood was fast and highly specific uptake was observed in the GRP receptor-positive colon, pancreas and tumors while retention in nontargeted organs and tissues was generally low. Biodistributions performed after co-injection of natural bombesin (blocking experiments) revealed a significant attenuation of the radioactivity in tumors, colon and pancreas, therefore confirming specific, GRP receptor-mediated uptake of the radiotracer. Thus, the click-modified peptide retained its full biological affinity and specificity. Accumulation of $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]$ in the colon and pancreas was in the same range as that of $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)], whereas tumor uptake was slightly improved at all time points investigated. Compared to $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)], $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]$ ac-

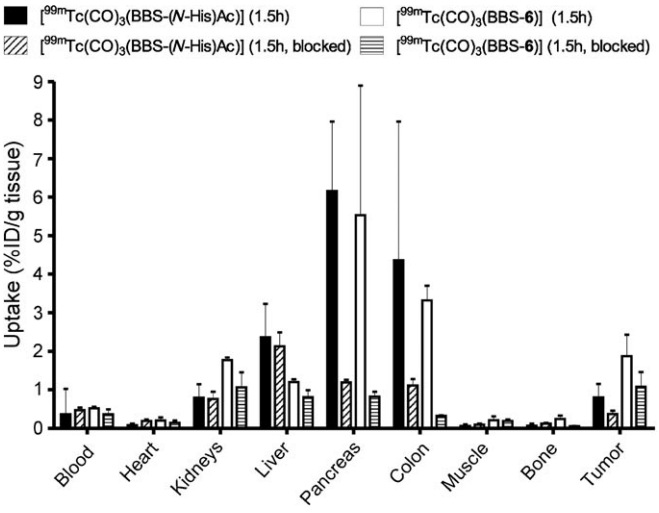


Figure 3. Comparison of the biodistribution of $[^{99m}Tc(CO)_3(\mathbf{BBS\text{-}6})]$ and $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)] in nude mice with PC-3 tumor xenografts (1.5 h p.i. of 3.7 MBq per mouse i.v., $n = 3$). Blocking experiments were conducted by co-injection of natural bombesin (0.1 mg per mouse). Data of $[^{99m}Tc(CO)_3$(BBS-(N^{α}His)Ac)] are taken from reference [43].

cumulated less in the liver but showed increased kidney uptake at early time points p. i. However, kidney-associated activity was rapidly cleared. These findings indicate preferential renal excretion as is expected for the less lipophilic (negatively charged at physiological pH) [^{99m}Tc(CO)$_3$(BBS-**6**)] and are in agreement with results we obtained with a ^{99m}Tc–BBS derivative containing a negative charge (additional Glu residue).[46] In contrast, we have shown that the introduction of a single positive charge (Lys residue) into the spacer unit of [^{99m}Tc(CO)$_3$(BBS-(N^{α}His)Ac)] resulted in a high (up to tenfold increased) and persistent kidney uptake.[46] Thus, the observed low renal uptake and retention of [^{99m}Tc(CO)$_3$(BBS-**6**)] suggests that the two appending carboxylic acid functionalities act as modulators, which successfully masked the cationic character of the di-1,2,3-triazole-containing chelate and led to a favorable biodistribution of the radiotracer.

Conclusions

In summary, we have shown that application of click chemistry provides efficient synthetic access to structurally diverse conjugates containing a tridentate, di-1,2,3-triazole ligand system suitable for the stable complexation of [M(CO)$_3$]$^+$ (M = ^{99m}Tc, Re). Starting from appropriate dialkyne precursors, the central metal chelator is formed as the conjugate is assembled by click reactions with azides. So far, metal conjugates containing up to three different entities attached to the chelator could be obtained selectively and efficiently. Use of different azide substrates yields metal conjugates of variable pharmacologically relevant characteristics such as hydrophilicity or overall charge. The potential of the novel chelators for the development of SPECT tracers was demonstrated by the in vivo evaluation of a tumor-targeting bombesin peptide fragment functionalized and radiolabeled with ^{99m}Tc via a di-1,2,3-triazole chelator. The procedures described also enable the facile introduction of other probes (e.g. fluorophores) into the metal conjugate, an attractive feature that could find application for the development of multimodal imaging agents. Further studies investigating the use (and combination) of various azide- and dialkyne-functionalized moieties, including targeted (bio)molecules, pharmacological modifiers, therapeutic agents and probes suitable for imaging modalities other than SPECT are ongoing.

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 and in vitro and in vivo experiments, and analytical data for all compounds (^{1}H and ^{13}C NMR spectra and HPLC chromatograms of Re and ^{99m}Tc complexes) see the Supporting Information.

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.

General procedures A–C: Cycloaddition of alkynes and azides

Procedure A: The alkyne precursor was dissolved in *t*BuOH/H$_2$O (1:1, ~30 mL mmol^{-1}) and the corresponding azide substrate (2.5 equiv), Cu(OAc)$_2$ (0.2 equiv) and sodium ascorbate (0.4 equiv) were added. The resulting mixture was stirred either at RT for 20–48 h, or at 50–80 °C for 2–5 h, until TLC indicated complete conversion of the dialkyne substrate. The mixture was diluted with EtOAc and washed with aq NH$_4$OH (0.2%) and brine. The aqueous phases were extracted with EtOAc (×2). The combined organic extracts were dried (Na$_2$SO$_4$) and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel with CH$_2$Cl$_2$/MeOH.

Procedure B: Same as procedure A but with 1.1 equivalents of azide.

Procedure C (*one-pot procedure*): TMS-protected dialkyne **16** was dissolved in *t*BuOH/H$_2$O (1:1, ~30 mL mmol^{-1}) and benzyl azide (1 equiv) and excess copper powder (50 mg/0.1 mmol) was added. The resulting suspension was stirred at RT for 18 h after which the corresponding second azide substrate (1.2 equiv), Cu(OAc)$_2$ (0.2 equiv) and sodium ascorbate (0.4 equiv) were added. Stirring of the mixture was continued at RT for 18 h, or at 50 °C for 5 h. Work-up and purification were performed as described above (for yields see Scheme 2).

General procedure D: Removal of Boc protecting groups

Where appropriate, the Boc group was cleaved by stirring intermediates in CH$_2$Cl$_2$/TFA (4:1, ~50 mL mmol^{-1}) at RT for 20 h. Removal of the volatile components under reduced pressure yielded the corresponding amine products as their TFA-salts.

General procedure E: Synthesis of rhenium complexes

[Re(Br)$_3$(CO)$_3$][Et$_4$N]$_2$ was prepared according to literature procedures.[40] Di-1,2,3-triazoles were dissolved in water, alcohol or mixtures thereof (1:1, ~80 mL mmol^{-1}) and [Re(CO)$_3$(Br)$_3$][Et$_4$N]$_2$ (1 equiv) was added. The mixtures (pH ~4) were stirred at 50–80 °C until HPLC analysis indicated completed conversion of the di-1,2,3-triazole substrate (0.5–5 h). The resulting solutions were concentrated in vacuo and the crude products were purified either by semi-preparative HPLC or reversed phase chromatography using C-18 Sep-Pak® columns and mixtures of water and MeOH (0→50% MeOH). Occasionally, carbonyl ligands of the complexes gave 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.[40]

General procedure F: Synthesis of ^{99m}Tc complexes

[^{99m}TcO$_4$][Na] was eluted from a ^{99}Mo/^{99m}Tc generator (Mallinckrodt-Tyco, Petten, The Netherlands) with a 0.9% saline solution. The precursor [^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ was prepared according to the literature.[48] Briefly, a solution of [^{99m}TcO$_4$]$^-$ (1 mL) in 0.9% aq 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 pH adjusted to 6.5 with 1 M phosphate buffer pH 7.4 and 1 M HCl (1:1 mixture). Aliquots of stock solutions of the di-1,2,3-triazole ligands in water (50 μL, 10^{-3}–10^{-5} M) were added to a solution of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ (100 μL; ~1 GBq mL^{-1}) and phosphate buffered saline (PBS, 350 μL, pH 5.5) was added to adjust the final concentration of the chelator (10^{-4}–

10^{-6} M). The reaction mixture was heated to 100 °C for 30 min and radiolabeling yields were determined by HPLC.

One-pot procedure: Stock solutions of dialkyne **11** (1 mM), copper(II) acetate (0.01 M) and sodium ascorbate (0.02 M) were prepared in water and a benzyl azide solution (0.01 M) in a 1:1 mixture of water and MeOH. The click reaction was performed on a 150 μL scale with 1 equiv of the dialkyne, 2.5 equiv of benzyl azide, 0.2 equiv of sodium ascorbate and 0.1 equiv of copper(II) acetate. The reaction mixture was heated at 80 °C for 20 min, after which time product formation was confirmed by mass spectroscopy and HPLC. The crude reaction solution was diluted with PBS (pH 5.5, 300 μL) and the precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (50 μL, ~0.5 GBq mL^{-1}) was added. The reaction mixture was heated again at 100 °C for 30 min before product formation was confirmed by γ HPLC. The same radiolabeling procedure was applied to the filtered crude reaction mixture of product **20**, which was obtained by the one-pot, 3-step procedure described above.

Peptide labeling: BBS-**6** was labeled with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ in PBS at pH ~6.5 as previously described for the derivative BBS-(N^{α}His)Ac.[44] In brief, 500 μL of the ^{99m}Tc solution were mixed with 15–30 μL of a 1 mM peptide solution and heated to 75 °C for 1 h after which the product was analyzed and purified by HPLC.

Compound characterization

Compound 4. As per procedure A with compound **1** and benzyl azide followed by procedure D. White solid (324 mg, 73% for 2 steps); mp 117–119 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta = 7.44$ (s, 2H), 7.39–7.26 (m, 10H), 5.57 and 5.53 (each d, each 2H, $J = 15.0$ Hz), 3.91 (q, 2H, $J = 7.2$ Hz), 3.23 and 2.98 (each d, each 2H, $J = 14.6$ Hz), 0.99 ppm (t, 3H, 7.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 176.0$, 144.3, 137.0, 130.1, 129.7, 129.2, 125.2, 62.7, 62.5, 55.0, 36.4, 14.4 ppm; IR (neat) $\tilde{\nu} = 3370$, 3132, 2982, 1727, 1453, 1215, 1044, 720 cm^{-1}; HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{24}H_{27}N_7O_2Na$: 468.2124, found: 468.2127.

Compound 5. As per procedure A with compound **3** and benzyl azide followed by procedure D. White solid (40 mg, 90% for 2 steps): mp 165–167 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta = 7.82$ (s, 2H), 7.38–7.28 (m, 10H), 5.59 and 5.55 (each d, each 2H, $J = 15.2$ Hz), 3.44 and 3.34 (each d, each 2H, $J = 15.7$ Hz), 3.06 (q, 2H, $J = 7.4$ Hz), 0.86 ppm (t, 3H, $J = 7.4$ Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 169.5$, 141.8, 136.7, 130.2, 129.8, 129.4, 125.6, 64.5, 55.1, 36.0, 32.4, 14.7 ppm; IR (neat) $\tilde{\nu} = 3074$, 2984, 1663, 1552, 1498, 1459, 1201, 1179, 1131, 1054, 792, 720 cm^{-1}; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{29}N_8O$: 445.24643, found: 445.2460.

Compound 6. The ethyl ester of compound **6** was obtained from dialkyne **3** and azidoacetic acid ethyl ester by procedure A followed by procedure D. The intermediate (35 mg, 0.08 mmol) was dissolved in aq NaOH/MeOH (1 M, 1:1, 3 mL) and stirred at RT overnight. The pH was adjusted to pH 5–6 by addition of aq HCl (1 M) and the solution was concentrated in vacuo. The residue was taken up in EtOH, centrifuged (5 min, 3000 rpm) and the solution was decanted; this procedure was repeated 5 times. The EtOH solutions were concentrated in vacuo to yield compound **6** as a white solid (30 mg, 59% for 3 steps): mp > 250 °C (decomposition); ^{1}H NMR (400 MHz, CD_3OD) $\delta = 7.83$ (s, 2H), 4.99 (s, 4H), 3.47 and 3.35 (each d, each 2H, $J = 14.8$ Hz), 3.28 (q, 2H, $J = 7.7$ Hz), 1.13 ppm (t, 3H, $J = 7.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 173.2$, 171.5, 141.5, 127.1, 63.9, 54.4, 36.1, 33.3, 14.8 ppm; IR (neat) $\tilde{\nu} = 3396$, 2995, 1620, 1392, 1310, 1140, 1059, 812 cm^{-1}; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{14}H_{21}N_8O_5$: 381.16349, found: 381.16298.

Compound 7. The acetate-protected derivative of compound **7** was obtained from dialkyne **3** and 1-azido-1-deoxy-β-D-galactopyranoside tetraacetate by procedure A followed by procedure D. The intermediate (200 mg, 0.19 mmol) was dissolved in MeOH (8 mL) and NaOMe (2 mg, 0.04 mmol) was added. The resulting solution was stirred at RT over night. The pH was adjusted to pH 7 by addition of aq HCl (0.1 M) and the solution was concentrated in vacuo. The residue was taken up in EtOH, centrifuged (5 min, 10000 rpm) and the solution was decanted; this process was repeated 3 times. The EtOH solutions were concentrated in vacuo to yield compound **7** as a white, amorphous solid (95 mg, 85%): ^{1}H NMR (400 MHz, CD_3OD) $\delta = 8.51$ (br s, 1H), 8.02 and 7.99 (each s, each 1H), 5.55 and 5.53 (each d, each 1H, $J = 3.9$ Hz), 4.18–4.11 (m, 2H with apparent $J = 3.5$ Hz), 4.0 and 3.99 (each bs, each 1H), 3.86–3.81 (m, 2H), 3.76–3.72 (m, 4H), 3.71 and 3.69 (each d, each 1H, $J = 3.0$ Hz), 3.36–3.31 (m, 2H; overlapping with solvent signal but confirmed by HSQC), 3.18–2.97 (m, 4H), 0.99 ppm (t, 3H, $J = 7.3$ Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 170.1$, 144.0, 124.2, 90.3, 80.0, 75.4, 75.3, 71.6, 70.5, 62.5, 36.0, 35.6, 14.9 ppm; IR (neat) $\tilde{\nu} = 3387$, 2979, 1615, 1549, 1389, 1310, 1145, 1061, 817 cm^{-1}; HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{22}H_{36}N_8O_{11}Na$: 611.24012, found: 611.23986.

Compound 11. K_2CO_3 (1.10 g, 8.0 mmol) and propargyl bromide (80% in toluene; 540 μL, 5.0 mmol) were added to a solution of HGlyNHMe·HCl (**8**, 249 mg, 2.0 mmol) in MeOH (8 mL). The mixture was stirred at RT for 2 days, filtered and concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel with EtOAc/hexane (3:1→4:1) yielded compound **11** as a yellow oil (199 mg, 62%): ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 6.99$ (br s, 1H), 3.43 (d, 2H, $J = 2.4$ Hz), 3.21 (s, 2H), 2.82 (d, 3H, $J = 5.0$ Hz), 2.42 ppm (t, 1H, $J = 2.4$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.5$, 78.3, 73.8, 57.0, 43.4, 26.0 ppm; IR (neat) $\tilde{\nu} = 3288$, 2930, 2830, 1660, 1534, 1417, 1122, 650 cm^{-1}; HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_9H_{11}N_2O$: 163.0871, found: 163.0835.

Compound 12. As per procedure A with compound **11** and benzyl azide. White solid (100 mg, 78%, 1 step): mp 158–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.55$ (br s, 1H), 7.49 (s, 2H), 7.43–7.32 (m, 6H), 7.30–7.25 (m, 4H), 5.52 (s, 4H), 3.77 (s, 4H), 3.13 (s, 2H), 2.78 ppm (d, 3H, $J = 4.9$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 171.2$, 144.3, 134.8, 129.4, 129.0, 128.2, 123.2, 57.6, 54.4, 48.8, 26.0 ppm; IR (neat) $\tilde{\nu} = 3663$, 2972, 2898, 1660, 1530, 1444, 1402, 1219, 1126, 1051, 718 cm^{-1}; HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{23}H_{26}N_8ONa$: 453.2127, found: 453.2120.

Compound 13. Pyridine (250 μL, 3.0 mmol) and propargyl bromide (80% in toluene; 324 μL, 3.0 mmol) were added to a solution of BocLysOMe acetate (320 mg, 1.0 mmol) in THF (5 mL). The mixture was stirred at 50 °C for 20 hrs, filtered and concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel with EtOAc/hexane (1:2) yielded compound **13** as a yellow oil (177 mg, 53%): $[\alpha]_D^{20} = -12.3$ ($c = 0.5$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 5.00$ (d, 1H, $J = 8.0$ Hz), 4.30–4.21 (m, 1H), 3.71 (s, 3H), 3.41 (d, 2H, $J = 2.8$ Hz), 2.51 (t, 2H, $J = 6.9$ Hz), 2.20 (t, 1H, $J = 2.8$ Hz), 1.84–1.74 (m, 1H), 1.66–1.57 (m, 1H), 1.51-.131 (m, 4H), 1.41 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 173.6$, 155.6, 80.2, 78.8, 73.4, 52.7, 52.4, 42.3, 32.7, 28.5, 27.1, 23.2 ppm; IR (neat) $\tilde{\nu} = 3289$, 2930, 1742, 1710, 1511, 1366, 1162, 1049, 1020 cm^{-1}; HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{18}H_{28}N_2O_4Na$: 359.1947, found: 359.1941.

Compound 14. As per procedure A with compound **11** and benzyl azide followed by procedure D. Pale yellow oil (24 mg, 72% for 2 steps): $[\alpha]_D^{20} = +8.5$ ($c = 0.5$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD)

$\delta = 8.24$ (s, 2H), 7.41–7.33 (m, 10H), 5.65 (s, 4H), 4.45 (br s, 4H), 4.08 (t, 1H, $J = 6.2$ Hz), 3.84 (s, 3H), 3.19–3.13 (m, 2H), 2.04–1.87 (m, 4H), 1.59–1.39 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 171.3$, 138.7, 136.5, 130.3, 130.0, 129.5, 55.4, 55.3, 53.9, 53.7, 49.3, 47.6, 31.3, 23.6 20.2 ppm (some signals are very weak or obscured by the solvent signal but all were confirmed by HSQC and HMBC); IR (neat) $\tilde{\nu} = 3425$, 2923, 1668, 1460, 1202, 1146, 846, 798 cm^{-1}; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{27}H_{35}N_8O_2$: 502.2883, found: 503.2875.

Compound 16. DIPEA (1.3 mL, 7.5 mmol) and propargylbromide (80% in toluene; 400 μL, 3.6 mmol) were added to a solution of HGlyNHMe (375 mg, 3.0 mmol) in MeOH (25 mL). The resulting solution was stirred at RT for 3 days and then concentrated in vacuo. The residue was taken up in THF, filtered and concentrated in vacuo. Purification of the crude product by chromatography on silica gel with CH_2Cl_2/MeOH (20:1→15:1) yielded propargyl-GlyNHMe as a yellow oil (180 mg, 48%). The intermediate (100 mg, 0.8 mmol) was dissolved in CH_3CN (5 mL) and DIPEA (280 μL, 1.6 mmol) and 3-bromo-1-(trimethylsilyl)-1-propyne (157 μL, 1.0 mmol) were added. The resulting solution was stirred at RT over night and concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel with CH_2Cl_2/MeOH (20:1) yielded compound **16** as a yellow oil (142 mg, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.01$ (br s, 1H), 3.41 (s, 2H), 3.39 (d, 2H, $J = 2.4$ Hz), 3.19 (s, 2H), 2.81 (d, 3H, $J = 5.2$ Hz), 2.22 (t, 1H, $J = 2.4$ Hz), 0.13 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.7$, 100.1, 90.9, 78.6, 73.7, 57.0, 44.5, 43.4, 26.0, 0.1 ppm; IR (neat) $\tilde{\nu} = 3293$, 2957, 2167, 1663, 1531, 1409, 1245, 1113, 986, 844, 760 cm^{-1}; HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{12}H_{20}N_2OSiNa$: 259.1243, found: 259.1238.

Compound 18. Dialkyne **16** (71 mg, 0.3 mmol) was dissolved in *t*BuOH/H_2O (1:1, 4 mL) and benzyl azide (94%; 47 mg, 0.33 mmol) and metallic Cu(0) (powder; 50 mg, excess) were added. The suspension was stirred at RT for 24 h. The suspension was filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with EtOAc to yield protected alkyne **17** as a white solid (90 mg, 81%). Intermediate **17** (50 mg, 0.13 mmol) was dissolved in MeOH (3 mL) and K_2CO_3 (2 mg, 0.01 mmol) was added. The solution was stirred at RT for 2 h. The pH was adjusted to pH 7 by addition of aq HCl (1 M) and the solution was cleared by filtration through celite© and concentrated in vacuo. The residue was taken up in water and extracted with EtOAc (×3). The organic phases were washed once with water and then combined, dried ($MgSO_4$) and concentrated in vacuo to yield compound **18** as a white solid (40 mg, quantitative): mp 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.31$–7.23 (m, 4H), 7.20–7.12 (m, 3H), 5.41 (s, 2H), 3.69 (s, 2H), 3.25 (d, 2H, $J = 2.7$ Hz), 3.11 (s, 2H), 2.68 (d, 3H, $J = 5.1$ Hz), 2.14 ppm (t, 1H, $J = 2.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.9$, 144.4, 134.7, 129.4, 129.0, 128.2, 122.8, 78.1, 74.0, 57.3, 54.4, 48.8, 43.6, 25.9 ppm; IR (neat) $\tilde{\nu} = 3293$, 2934, 1660, 1534, 1452, 1328, 1126, 1050, 719 cm^{-1}; HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{16}H_{19}N_5ONa$: 320.1487, found: 320.1478.

Compound 19. The acetate-protected derivative of compound **19** was obtained from compound **18** and 1-azido-1-deoxy-β-D-galactopyranoside tetraacetate by procedure A. The intermediate (45 mg, 0.07 mmol) was dissolved in MeOH (3 mL) and NaOMe (0.4 mg, 0.01 mmol) was added. The resulting solution was stirred at RT for 18 h. The pH of the solution was adjusted to pH 7 by addition of aq HCl (0.1 M) and concentrated in vacuo to yield compound **19** as a yellow oil (35 mg, 86% for 2 steps): $[\alpha]_D^{20} = +15.2$ ($c = 0.5$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD) $\delta = 8.32$ and 8.08 (each s, each 1H), 7.40–7.30 (m, 5H), 563–5.58 (m, 1H), 5.61 (s, 2H), 4.19 (t, 1H, $J = 8.4$ Hz), 4.10–3.96 (m, 4H), 3.87 (t, 1H, $J = 6.0$ Hz), 3.79–3.71 (m, 3H), 3.33 (br s, 1H), 2.68 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 171.8$, 143.5, 143.2, 136.8, 130.2, 129.8, 129.3, 126.6, 125.5, 90.3, 80.1, 75.4, 71.6, 70.6, 62.6, 57.1, 55.2, 26.3 ppm (some signals are obscured by the solvent signal but confirmed by HSQC); IR (neat) $\tilde{\nu} = 3356$, 2939, 1644, 1545, 1455, 1442, 1330, 1226, 1122, 1090, 1057, 889, 719 cm^{-1}; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{31}N_8O_6$: 503.2367, found: 503.2365.

Compound 20. As per procedure A with compound **19** and azidoacetic acid ethyl ester. White solid (28 mg, 82% for 1 step): mp 107–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.72$ (s, 1H), 7.59–7.52 (m, 1H), 7.48 (s, 1H), 7.41–7.33 (m, 3H), 7.29–7.24 (m, 2H), 5.52 (s, 2H), 5.14 (s, 2H), 4.26 (q, 2H, $J = 7.2$ Hz), 3.81 and 3.77 (each s, each 2H), 3.16 (s, 2H), 2.79 (d, 3H, $J = 5.5$ Hz), 1.30 ppm (t, 3H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 171.7$, 166.4, 144.2, 144.1, 134.7, 129.3, 129.0, 128.2, 124.7, 123.2, 62.6, 57.5, 54.3, 51.0, 48.7, 48.6, 26.0, 14.2 ppm; IR (neat) $\tilde{\nu} = 3365$, 2934, 1746, 1658, 1531, 1219, 1052, 723 cm^{-1}; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{20}H_{27}N_8O_3$: 427.2206, found: 427.2204.

Compound 21. Alkyne **19** (0.3 mg, 1.0 μmol) was reacted with the azide derivative of the fluorophore DY547,[38] $Cu(OAc)_2$ (0.2 mol%) and sodium ascorbate (0.4 mol%) in water at RT in the dark (20 h). Purification of the crude product by HPLC (using the same gradient as described for analytical HPLC) gave compound **21** as a red solid (0.35 μmol, 30% yield for two 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-ESI *m/z* $[M]^+$ calcd for $C_{50}H_{64}N_{11}O_{11}S_2$: 1010.4375, found: 1010.4374.

Complex $[Re(CO)_3(4)]^+$. As per general procedure E in EtOH, 2 h at 50 °C, purified by Sep-Pak©. White solid (49 mg, 62%; purity according to HPLC > 95%): mp 128–132 °C; ^{1}H NMR (400 MHz, CD_3OD, recorded immediately) $\delta = 7.85$ (s, 2H), 7.42–7.36 (m, 6H), 7.31–7.26 (m, 4H), 6.24 (br s, 2H, exchanges with MeOD after 3 days), 5.63 and 5.54 (each d, each 2H, $J = 14.7$ Hz), 3.61 (q, 2H, $J = 6.8$ Hz), 3.41 and 3.26 (each d, each 2H, $J = 17.5$ Hz; partly overlapping with MeOD signal), 1.19 ppm (t, 3H, 6.8 Hz); ^{13}C NMR (100 MHz, [D_8]THF) $\delta = 196.8$ and 194.0 (~3 CO), 173.4, 143.8, 136.0, 129.8, 129.7, 129.4, 127.7, 63.6, 57.8, 55.1, 32.1, 14.7 ppm; IR (neat) $\tilde{\nu} = 2996$, 2360, 2027 and 1902 (strong),1734, 1269, 728 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{27}H_{27}N_7O_5Re$: 716.1631, found: 716.1628.

Crystals for x-ray diffraction were grown by diffusion of hexane into a solution of the complex in THF.

Complex $[Re(CO)_3(5)]^+$. As per general procedure E in MeOH, 2 h at 60 °C, purified by semi-preparative HPLC (isocratic, 1:1, CH_3CN/aq 0.1% TFA). White solid (18 mg, 64%; purity according to HPLC > 95%): mp 188–191 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta = 7.83$ (s, 2H), 7.41–7.36 (m, 6H), 7.30–7.25 (m, 4H), 5.88 (br s, 2H, exchanges with MeOD after 3 days), 5.50 and 5.45 (each d, each 2H, $J = 14.5$ Hz), 3.40–3.20 (m, 4H; obscured by MeOD signal), 3.19 (d, 2H, $J = 16.5$ Hz), 1.18 ppm (t, 3H, 7.3 Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 196.6$ and 193.9 (~3 CO), 174.1, 144.5, 135.4, 130.4, 130.3, 129.7, 126.7, 57.9, 56.1, 36.5, 32.5, 14.7 ppm; IR (neat) $\tilde{\nu} = 3143$, 2945, 2031 and 1909 (strong), 1667, 1202, 1174, 1131, 720 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{27}H_{28}N_8O_4Re$: 715.1791, found: 715.1794.

Complex $[Re(CO)_3(6)]^+$. As per general procedure E in EtOH/water (2:1), 5 h at 65 °C, purified by semi-preparative HPLC (CH_3CN/aq 0.1% TFA, 70→20% within 10 min). White solid (20 mg, 60%; purity according to HPLC > 95%): mp > 220 °C (decomposition); ^{1}H NMR (400 MHz, CD_3OD) $\delta = 7.89$ (s, 2H), 5.95 (br s, 2H), 5.25 and

5.20 (each d, each 2H, $J=14.8$ Hz), 3.49 (d, 2H, $J=17.0$ Hz), 3.35 (q, 2H, $J=7.4$ Hz), 3.25 (d, 2H, $J=17.0$ Hz), 1.22 ppm (t, 3H, $J=7.4$ Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta=196.7$ and 193,7 (~3 CO), 174.1, 169.1, 144.3, 128.4, 58.4, 52.8, 36.5, 32.4, 14.7 ppm; IR (neat) $\tilde{\nu}=3233$, 3147, 2980, 2031, and 1911 (strong), 1737, 1660, 1552, 1194, 1147 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{14}H_{20}N_8O_8Re$: 651.09616, found: 651.0959.

Complex $[Re(CO)_3(7)]^+$. As per general procedure E in water, 5 h at 80 °C, purified by semi-preparative HPLC (isocratic, 1.8:8.2, CH_3CN/aq 0.1% TFA). White solid (10 mg, 60%; purity according to HPLC >95%): mp >180 °C (decomposition); ^{1}H NMR (400 MHz, CD_3OD) $\delta=8.17$ and 8.16 (each s, each 1H), 5.94 (br s, 2H; NH_2, completed D/H exchange after 4 weeks), 5.55 and 5.52 (each d, each 1H, $J=9.0$ Hz), 3.98–3.89 (m, 4H), 3.86–3.80 (m, 2H), 3.78–3.70 (m, 4H), 3.67 and 3.64 (each t, each 1H, $J=3.2$ Hz), 3.52–3.44 (m, 2H), 3.35 (q, 2H, $J=7.2$ Hz), 3.30–3.20 (overlap with signal of MeOD; m, 2H), 1.22 ppm (t, 3H, $J=7.8$ Hz); ^{13}C NMR (100 MHz, CD_3OD) $\delta=196.3$ and 196.7 (~3CO), 174.1, 144.4, 144.3, 125.5, 125.2, 90.9, 90.8, 80.6, 80.5, 75.2, 75.15, 71.8, 71.5, 70.5, 70.4, 62.8, 62.4, 57.7, 36.6, 32.8, 32.5, 14.8 ppm; IR (neat) $\tilde{\nu}=3326$, 2936, 2031 and 1903 (strong), 1675, 1618, 1200, 1135, 1094 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{25}H_{36}N_8O_{14}$: 859.19085, found 859.19076.

Complex $[Re(CO)_3(12)]^+$. As per general procedure E in EtOH, 30 min at 50 °C. The crude product was triturated twice in water to yield complex $[Re(CO)_3(\mathbf{12})]^+$ as a white solid (25 mg, 89%; purity according to HPLC >90%): mp 130–135 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta=7.99$ (s, 2H), 7.43–7.37 (m, 6H), 7.35–7.22 (m, 4H), 5.56 and 5.52 (each d, each 2H, $J=15.2$ Hz), 4.83 and 4.64 (each d, each 2H, $J=16.3$ Hz), 4.56 (s, 2H), 2.77 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) $\delta=196.5$, 195.2, 193.7, 170.1, 151.2, 135.3, 130.5, 130.4, 129.7, 124.2, 69.7, 60.7, 56.6, 26.2 ppm; IR (neat) $\tilde{\nu}=3442$, 2943, 2036 and 1913 (strong), 1663, 1457, 719 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{26}H_{26}N_8O_4Re$: 701.1635, found: 701.1626.

Complex $[Re(CO)_3(14)]^+$. As per general procedure E in MeOH, 30 min at 55 °C, purified by Sep-Pak©. White solid (20 mg, 83%; purity according to HPLC >95%): mp 83–86 °C; $[\alpha]_D^{20}=+10.3$ ($c=0.4$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD) $\delta=8.00$ (s, 2H), 7.42–7.37 (m, 6H), 7.33–7.28 (m, 4H), 5.54 and 5.50 (each d, each 2H, $J=14.5$ Hz), 4.49 (s, 4H), 4.10 (t, 1H, $J=6.2$ Hz), 3.85 (s, 3H), 3.86–3.79 (m, 2H), 2.11–1.92 (m, 4H), 1.64–1.45 (m, 2H) pm; ^{13}C NMR (100 MHz, CD_3OD) $\delta=197.0$, 194.0, 171.3, 150.9, 135.3, 130.5, 130.4, 129.7, 124.2, 70.8, 59.5, 56.6, 53.9, 53.8, 31.5, 26.1, 23.5 ppm; IR (neat) $\tilde{\nu}=3415$, 2952, 2031 and 1914 (strong) 1748, 1681, 1199, 1176, 1133, 720 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{30}H_{34}N_8O_5Re$: 773.2210, found: 773.2207.

Complex $[Re(CO)_3(19)]^+$. As per general procedure E in MeOH/water (2:1), 1 h at 60 °C, purified by semi-preparative HPLC (isocratic, 3:7, CH_3CN/0.1% aq TFA). White solid (23 mg, 68%): mp 98–104 °C (decomposition); $[\alpha]_D^{20}=+37.2$ ($c=0.5$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD) $\delta=8.32$ and 8.31 (each s, each 1H), 8.00 and 7.96 (each s, each 1H), 7.43–7.27 (m, 5H), 5.61–5.50 (m, 3H), 4.85–4.78 (m, 1H, obscured by solvent signal; confirmed by NOE and HSQC), 4.78–4.65 (m, 3H), 4.54 (s, 2H), 4.10–4.00 (m, 2H), 3.92–3.87 (m, 1H), 3.78–3.70 (m, 3H), 2.79 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) $\delta=193.2$, 196.2, 193.8, 193.6, 170.18, 170.16, 161.1, 160.7, 151.07, 151.05, 151.0, 150.8, 135.0, 134.9, 130.5, 130.4, 129.7, 129.6, 124.4, 124.3, 123.4, 123.0, 91.2, 91.0, 80.3, 80.2, 75.0, 74.8, 71.5, 71.2, 70.2, 70.1, 69.33, 69.29, 62.3, 62.1, 61.0, 60.8, 60.7, 56.6, 26.4 ppm; IR (neat) $\tilde{\nu}=3342$, 2952, 2040 and 1920 (strong), 1674, 1201, 1133, 1095, 722 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{25}H_{30}N_8O_9Re$: 773.1693, found: 773.1702.

Complex $[Re(CO)_3(20)]^+$ (R′=H). As per general procedure E in MeOH, 1 h at reflux, purified by semi-preparative HPLC (isocratic, 4:6, CH_3CN/aq 0.1% TFA). White solid (19 mg, 62%, purity according to HPLC >95%): mp 138–142 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta=8.02$ and 7.91 (each s, each 1H), 7.42–7.36 (m, 3H), 7.33–7.28 (m, 2H), 5.58 and 5.54 (each d, each 1H, $J=14.6$ Hz), 5.26 (s, 2H), 4.93 and 4.85 (each d, each 1H, $J=16.4$ Hz), 4.67 and 4.63 (each d, each 1H, $J=4.8$ Hz), 5.49 (br s, 2H), 2.79 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) $\delta=196.6$, 196.5, 193.7, 170.2, 168.9, 151.2, 150.7, 135.2, 130.5, 130.4, 129.8, 125.9, 124.1, 69.8, 60.6, 60.5, 56.6, 53.1, 26.2 ppm; IR (neat) $\tilde{\nu}=2036$ and 1921 (strong), 1669, 1194, 1138, 719 cm^{-1}; HRMS-ESI *m/z* $[M-H+Na]^+$ calcd for $C_{21}H_{21}N_8O_6ReNa$: 691.1039, found: 691.1043.

Complex $[Re(CO)_3(21)]^+$. As per general procedure E in MeOH/water (1:1), 30 min at 60 °C, purified by analytical HPLC (using the same gradient as described for analytical HPLC). Red solid (65% yield as determined by UV/vis: $\lambda=557$ nm, $d=1$ cm, $\varepsilon=150'000$ $M^{-1}cm^{-1}$): purity according to HPLC ≥95%; IR (neat) $\tilde{\nu}=3442$, 2036 and 1932 (strong), 1683, 1558, 1436, 1199, 1136, 1031, 722 cm^{-1}; HRMS-ESI *m/z* $[M]^+$ calcd for $C_{53}H_{63}N_{11}O_{11}ReS_2$: 1280.3707, found: 1280.3704.

Solid phase peptide synthesis (SPPS)

Peptide synthesis was carried out on solid phase on a Rink amide polystyrene resin (0.60 mmol g^{-1}) using Fmoc peptide strategy as previously described.[43] Dialkyne **2** (3 equiv) was coupled to βAlaβAlaGln(Trt)Trp(Boc)AlaValGlyHis(Trt)ChaNle on solid support using standard coupling protocols (3.0 equiv of DIC and HOBt in DMF, 2 h at RT). After washing the resin, the copper(I)-catalyzed cycloaddition with azidoacetic acid[48] (4 equiv), Cu^IBr (1.5 equiv) and DIPEA (4.0 equiv) was performed overnight in DMF according to the procedure described by Meldal and co-workers.[2] The resin was then washed with DMF, *i*PrOH and Et_2O (×3) and dried under reduced pressure. The peptide was cleaved from the resin by adding the dry resin to a mixture of 10% TA/EDT (7:3) in TFA (10 mL g^{-1} of resin). After 3 h, the resin was removed by filtration and the filtrate was added dropwise to dry, cold Et_2O. The precipitated peptide was isolated by centrifugation and the Et_2O was decanted. Cold Et_2O was added two more times to the peptide and it was decanted again after centrifugation. After this isolation process, the peptide was purified by preparative HPLC to yield BBS-**6** as a white solid (28% overall yield; purity according to HPLC >99%): LRLC-MS (ESI) *m/z* $[M+H]^+$ calcd for $C_{65}H_{95}N_{22}O_{16}$: 1439.7, found: 1439.7.

Crystal data for $[Re(CO)_3(4)]Br$

Monoclinic, space group $P2_1/n$, $a=10.759(5)$ Å, $b=10.732(5)$ Å, $c=33.878(5)$ Å, $\beta=92.630°$, $V=8908(5)$ Å^3, $Z=4$. Data collection for $C_{35}H_{43}BrN_7O_7Re$: STOE (IPDS2T), Mo Kα radiation ($\alpha=0.71073$), $T=200$ K, 23537 reflections collected, 6801 unique, 3110 with $I>2\sigma$, $\mu=4.186$ mm^{-1}, 455 parameters, numerical absorption correction. Structure solution and refinement: SHELXS97, SHELXL97.[49] R1=0.0785, wR2=0,1572, GooF=0.958.

Full details of the crystal structure have been deposited with the Cambridge Crystallographic Data Centre under the deposition number CCDC 711913.

In vitro receptor binding assay

PC-3 cells at confluence in 48-well plates were incubated for 1 h at 37 °C in binding buffer (50 mM HEPES, 125 mM NaCl, 7.5 mM KCl, 5.5 mM $MgCl\cdot 6H_2O$, 1 mM EGTA, 5 g L^{-1} BSA, 2 mg L^{-1} Chymostatin,

100 mg L^{-1} Soybean Trypsin inhibitor, 50 mg L^{-1} bacitracin, pH 7.4) with increasing concentrations of the ^{99m}Tc-labeled BBS analogues (0.01–2 nM). Nonspecific binding was determined by co-incubation with 1 μM natural BBS. After incubation the cells were washed twice with cold PBS and recovered with 1 N NaOH (2×400 μL). The radioactivity was measured in a NaI-γ-counter. Experiments were performed 2–3 times in triplicate.

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 8×10^6 cells in 150 μL culture medium without supplements. Three weeks after tumor implantation the mice were injected i.v. with 3.7 MBq of the radiolabeled peptides. At different p. i. times (0.5, 1.5 and 5 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}).

Acknowledgements

This work was funded in part by Oncosuisse (grant number KLS 02040-02-2007) and by the Fund for Scientific Research, (grant number G.0036.04,) Flanders (Belgium). L.B. is a fellow of the "Institute for the Promotion of Innovation through Science and Technology in Flanders" (IWT Vlaanderen). We thank Dr. Bernhard Pfeiffer (ETH Zurich) for NMR measurements, Dr. Cristina Müller and Alain Blanc (PSI) for technical assistance and Covidien, Petten (The Netherlands) for financial support.

Keywords: click chemistry · di-1,2,3-triazoles · bombesin · imaging agents · technetium

[1] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
[2] C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[3] C. Kolb Hartmuth, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128–1137.
[4] M. Meldal, C. W. Tornoe, *Chem. Rev.* **2008**, *108*, 2952–3015, and references therein.
[5] Few examples are reported. For a review on the use of 1,2,3-triazoles as peptide mimetics see: Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, *36*, 1674–1689.
[6] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853–2855.
[7] For a review of 1,2,3-triazole metal ligands not obtained by CuAAC see: D. S. Moore, S. D. Robinson, *Adv. Inorg. Chem.* **1988**, *32*, 171–239.
[8] D. Liu, W. Gao, Q. Dai, X. Zhang, *Org. Lett.* **2005**, *7*, 4907–4910.
[9] Q. Dai, W. Gao, D. Liu, L. M. Kapes, X. Zhang, *J. Org. Chem.* **2006**, *71*, 3928–3934.
[10] R. J. Detz, S. Arevalo Heras, R. De Gelder, P. W. N. M. Van Leeuwen, H. Hiemstra, J. N. H. Reek, J. H. Van Maarseveen, *Org. Lett.* **2006**, *8*, 3227–3230.
[11] S.-I. Fukuzawa, H. Oki, M. Hosaka, J. Sugasawa, S. Kikuchi, *Org. Lett.* **2007**, *9*, 5557–5560.
[12] S. Huang, R. J. Clark, L. Zhu, *Org. Lett.* **2007**, *9*, 4999–5002.
[13] O. David, S. Maisonneuve, J. Xie, *Tetrahedron Lett.* **2007**, *48*, 6527–6530.
[14] D. Schweinfurth, K. I. Hardcastle, U. H. F. Bunz, *Chem. Commun.* **2008**, 2203–2205.
[15] A. Maisonial, P. Serafin, M. Traikia, E. Debiton, V. Thery, D. J. Aitken, P. Lemoine, B. Viossat, A. Gautier, *Eur. J. Inorg. Chem.* **2008**, 298–305.
[16] T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwe, R. Schibli, *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.
[17] R. Alberto, R. Schibli, A. Egli, A. P. Schubiger, U. Abram, T. A. Kaden, *J. Am. Chem. Soc.* **1998**, *120*, 7987–7988.
[18] H. Struthers, B. Spingler, T. L. Mindt, R. Schibli, *Chem. Eur. J.* **2008**, *14*, 6173–6183.
[19] T. L. Mindt, C. Mueller, M. Melis, M. de Jong, R. Schibli, *Bioconjugate Chem.* **2008**, *19*, 1689–1695.
[20] Y. Li, J. C. Huffman, A. H. Flood, *Chem. Commun.* **2007**, 2692–2694.
[21] R. M. Meudtner, M. Ostermeier, R. Goddard, C. Limberg, S. Hecht, *Chem. Eur. J.* **2007**, *13*, 9834–9840.
[22] J. T. Fletcher, B. J. Bumgarner, N. D. Engels, D. A. Skoglund, *Organometallics* **2008**, *27*, 5430–5433.
[23] C. Camp, S. Dorbes, C. Picard, E. Benoist, *Tetrahedron Lett.* **2008**, *49*, 1979–1983.
[24] T. Ziegler, C. Hermann, *Tetrahedron Lett.* **2008**, *49*, 2166–2169.
[25] U. Monkowius, S. Ritter, B. Koenig, M. Zabel, H. Yersin, *Eur. J. Inorg. Chem.* **2007**, 4597–4606.
[26] For preliminary data on di-1,2,3-triazole compounds and their complexes with $[^{99m}Tc(CO)3]^+$ see: *Seventh International Symposium on Technetium in Chemistry and Nuclear Medicine* **2006**, Bressanone (Italy).
[27] S. Kotha, E. Brahmachary, *Bioorg. Med. Chem.* **2002**, *10*, 2291–2295.
[28] P. Antunes, M. Ginj, M. A. Walter, J. Chen, J.-C. Reubi, H. R. Maecke, *Bioconjugate Chem.* **2007**, *18*, 84–92.
[29] C. Schweinsberg, V. Maes, L. Brans, P. Bläuenstein, D. Tourwé, P. A. Schubiger, R. Schibli, E. García Garayoa, *Bioconjugate Chem.* **2008**, *19*, 2432–2439..
[30] a) R. Roy, J. M. Kim, *Tetrahedron* **2003**, *59*, 3881–3893; b) S. Orlandi, R. Annunziata, M. Benaglia, F. Cozzi, L. Manzoni, *Tetrahedron* **2005**, *61*, 10048–10060 and references therein.
[31] Few side reactions of the CuAAC are reported, see: a) T. L. Mindt, R. Schibli, *J. Org. Chem.* **2007**, *72*, 10247–10250 and ref.[4] and references therein.
[32] We did not observe a stepwise reaction of the two alkynes as reported by T. Ziegler and C. Hermann for double click reactions of aromatic *ortho*-bispropargyl amides in organic solvents employing $(EtO)_3PCuI$ as catalyst (reference [24]).
[33] Several procedures for the N(α)-alkylation of amino acids with propargyl bromide are described in the literature. However, in our experience none of these protocols is universally applicable.
[34] a) K. A. Stephenson, J. Zubieta, S. R. Banerjee, M. K. Levadala, L. Taggart, L. Ryan, N. McFarlane, D. R. Boreham, K. P. Maresca, J. W. Babich, J. F. Valliant, *Bioconjugate Chem.* **2004**, *15*, 128–136; b) P. Schaffer, J. A. Gleave, J. A. Lemon, L. C. Reid, L. K. K. Pacey, T. H. Farncombe, D. R. Boreham, J. Zubieta, J. W. Babich, L. C. Doering, J. F. Valliant, *Nucl. Med. Biol.* **2008**, *35*, 159–169.
[35] O. D. Montagnat, G. Lessene, A. B. Hughes, *Tetrahedron Lett.* **2006**, *47*, 6971–6974.
[36] V. Aucagne, D. A. Leigh, *Org. Lett.* **2006**, *8*, 4505–4507.
[37] Removal of a trialkylsilyl protecting group to liberate the terminal alkyne can also be accomplished using TBAF or Ag^I salts (see references [21], [35] and [36]).
[38] See Supporting Information for details.
[39] Related di-1,2,3-triazoles derived from bispropargyl amines have been investigated as copper stabilizing ligands in catalysis (reference [6]).
[40] R. Alberto, A. Egli, U. Abram, K. Hegetschweiler, V. Gramlich, P. A. Schubiger, *J. Chem. Soc. Dalton Trans.* **1994**, 2815–2820.
[41] NMR analysis (^1H, ^{13}C, H/D exchange experiments, COSY, HSQC, HMBC) of all rhenium complexes are consistent with the structures depicted in Scheme 3. Only in the case of $[Re(CO)_3(\mathbf{7})]^+$ and $[Re(CO)_3(\mathbf{19})]^+$ did we observe multiple NMR signals (in different solvents and pH) even though HPLC and MS indicate the presence of a single compound. We tentatively ascribe this observation to the possible formation of isomeric products as the result of the carbohydrate residues.

[42] See for example: a) R. G. Blasberg, *Nucl. Med. Biol.* **2003**, *30*, 879–888; b) J. M. Park, S. S. Gambhir, *Proc. IEEE* **2005**, *93*, 771–783; c) T. Koullourou, L. S. Natrajan, H. Bhavsar, S. J. A. Pope, J. Feng, J. Narvainen, R. Shaw, E. Scales, R. Kauppinen, A. M. Kenwright, S. Faulkner, *J. Am. Chem. Soc.* **2008**, *130*, 2178–2179; and references therein.
[43] E. García Garayoa, D. Rüegg, P. Blauenstein, M. Zwimpfer, I. U. Khan, V. Maes, A. Blanc, A. G. Beck-Sickinger, D. A. Tourwé, P. A. Schubiger, *Nucl. Med. Biol.* **2007**, *34*, 17–28.
[44] H. Akizawa, Y. Arano, M. Mifune, A. Iwado, Y. Saito, T. Mukai, T. Uehara, M. Ono, Y. Fujioka, K. Ogawa, Y. Kiso, H. Saji, *Nucl. Med. Biol.* **2001**, *28*, 761–768.
[45] S. Froidevaux, M. Calame-Christe, H. Tanner, A. N. Eberle, *J. Nucl. Med.* **2005**, *46*, 887–895.
[46] E. García Garayoa, C. Schweinsberg, V. Maes, L. Brans, P. Bläuenstein, D. A. Tourwe, R. Schibli, P. A. Schubiger, *Bioconjugate Chem.* **2008**, 19, 2409–2416.
[47] L. A. Banaszynski, C. W. Liu, T. J. Wandless, *J. Am. Chem. Soc.* **2005**, *127*, 4715–4721.
[48] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.
[49] a) G. M. Sheldrick, *Acta Crystallogr. Sect. A.* **1990**, *46*, 467–473; b) G. M. Sheldrick, *SHELX97–A Programme Package for the Solution and Refinement of Crystal Structures* **1997**, University of Göttingen (Germany).

Received: December 5, 2008
Published online on February 23, 2009

