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Synthesis of Novel 1,4,7,10-Tetraazacyclodecane-1,4,7,10-Tetraacetic Acid (DOTA) Derivatives for Chemoselective Attachment to Unprotected Polyfunctionalized Compounds

Sebastian Knör,[a] Armin Modlinger,[a] Thorsten Poethko,[b] Margret Schottelius,[b] Hans-Jürgen Wester,[b] and Horst Kessler*[a]

In memoriam of Professor Miklos Bodanszky, a hero of peptide chemistry

Abstract: A convenient synthesis of novel bifunctional poly(amino carboxylate) chelating agents allowing chemoselective attachment to highly functionalized biomolecules is described. Based on the well known chelator 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA), we synthesized novel bifunctional chelating agents bearing additional functional groups by alkylating 1,4,7,10-tetraazacyclododecane (cyclen) with one equivalent of *para*-functionalized alkyl 2-bromophenyl-acetate and three equivalents of *tert*-butyl 2-bromoacetate. The resulting compounds, which contain an additional carbonyl or alkyne functionality, allow site specific labeling of appropriately functionalized unprotected biomolecules in a rapid manner via click reactions. This was demonstrated by the attachment of our new DOTA derivatives to the somatostatin analogue Tyr^3-octreotate by chemoselective oxime ligation and Cu^I-catalyzed azide–alkyne cycloaddition. Initial biodistribution studies in mice with the radiometalated compound demonstrated the applicability of the described DOTA conjugation.

Keywords: antitumor agents · click reactions · isotopic labeling · oxime ligation

Introduction

For non-covalent binding of radionuclides, bifunctional chelating agents (BFCAs) are used to connect radioactive markers and a targeting molecule. Among these BFCAs, the 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA) is a well-studied macrocyclic complex ligand which has been shown to form extremely stable complexes of both divalent and trivalent metals.[1,2] Radiopharmaceuticals containing this ligand as metal chelator have found widespread use in therapy and diagnostic imaging.[3,4]

[a] S. Knör, Dr. A. Modlinger, Prof. Dr. H. Kessler
Department Chemie, Lehrstuhl für Organische Chemie II
Technische Universität München, Lichtenbergstrasse 4
85747 Garching (Germany)
Fax: (+49) 89-298-13210
E-mail: horst.kessler@ch.tum.de

[b] Dr. T. Poethko, Dr. M. Schottelius, Prof. Dr. H.-J. Wester
Department of Nuclear Medicine
Nuklearmedizinische Klinik und Poliklinik
Klinikum rechts der Isar, Technische Universität München
Ismaningerstrasse 22, 81675 München (Germany)

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DOTA-modified peptides are generally synthesized either in solution[5] or on solid support attaching the DOTA residue to a free amine of the resin bound peptide. For this, unprotected[6] or more conveniently, protected DOTA derivatives are used to overcome side reactions by polyactivation of the four carboxylic groups of DOTA. Therefore, a number of DOTA derivatives were developed allowing selective formation of monoconjugates. For example, the triprotected and commercially available DOTA-tris(*tert*-butyl) ester,[7] the corresponding benzyl protected analogues DOTA-tris(benzyl) ester,[8] the isothiocyanate functionalized *p*-NCS-Bz-DOTA[9] or DOTAGA(tBu)$_4$,[10] which contains an additional unprotected carboxylic group, are widely used BFCAs. In other approaches, derivatized amino acids containing a DOTA moiety in the side chain were used[11] or the DOTA moiety was synthesized stepwise on the *N*-terminus of a resin bound peptide.[12] The main limitation of all these methods is the introduction of the DOTA residue into the bioconjugate via an electrophilic reaction. This procedure tolerates no other *N*- or *S*-nucleophilic groups for a selective reaction.

Chemoselective approaches allowing a site-selective functionalization of polyfunctionalized compounds remain in

demand. Such a method would be a powerful tool for the synthesis of DOTA-linked radiopharmaceuticals enabling new synthetic strategies and the synthesis of complex structures. For example, we recently developed multimeric aminooxy-functionalized RGD derivatives[13] which were ^{18}F-labeled via oxime ligation using 4-[^{18}F]fluorobenzaldehyde.[14] A carbonyl functionalized DOTA derivative would allow a selective conjugation with DOTA and subsequent labeling with various radiometals. Very recently, Lin and co-workers have demonstrated a site-specific protein modification through Cu^{I}-catalyzed 1,2,3-triazole formation.[15] Applying this methodology and using an alkyne functionalized DOTA derivative would enable site specific DOTA labeling of proteins. In a recent publication, Hovinen reported the synthesis of an aminooxy-functionalized chelate by derivatization of the tris *tert*-butyl ester of DOTA which was conjugated with naltrexone and 2-deoxy-D-ribose.[16] However, the reported procedure requires expensive starting material and the applicability to polyfunctionalized compounds such as peptides remains unclear.

Thus, our goal was to design novel DOTA derivatives enabling the chemoselective attachment in presence of a wide range of functional groups. Thereby, the focus was on structures readily accessible in few synthetic steps from cheap commercial materials to make our method convenient and even a general alternative to the widely used DOTA-tris-(*tert*-butyl) ester. Furthermore, the accessibility of the correspondingly polyfunctionalized compounds was an important aspect in our consideration. Click reactions,[17] in particular the oxime ligation[18,19] as well as the Cu^{I}-catalyzed azide–alkyne cycloaddition[20,21] are well-studied and powerful reactions for chemoselective couplings which fulfil all requirements for our purpose. The oxime ligation denotes the highly selective reaction between an aminooxy component and aldehydes or methyl ketones[22] under formation of an oxime bond, which is known to be stable both in vitro and in vivo.[23] The reaction was shown to tolerate every free amino acid side chain except an *N*-terminal cysteine and found widespread use, for example, in the synthesis of template-assembled synthetic proteins,[24,25] radioactive labeled peptide conjugates,[23,26] cyclic peptides[27] and protein analogues.[28,29] The Cu^{I}-catalyzed azide–alkyne cycloaddition is a catalyzed variant of the chemoselective Huisgen 1,3-dipolar cycloaddition[30–33] of an azide and an alkyne for the formation of a triazole which has found application in various developments in medicinal chemistry.[34–39] To provide chemists with different methodologies for the synthesis of DOTA conjugates, we focused on appropriate DOTA derivatives for both reaction types described above.

Thus, in this report we present the synthesis of 2-[1-(1,4,7,10-tetraazacyclodecane)-4,7,10-tris(*tert*-butylacetate)]-(4-acetylphenyl) acetic acid *tert*-butyl ester (**1**) as carbonyl component for oxime ligations with aminooxy-functionalized compounds and 2-[1-(1,4,7,10-tetraazacyclodecane)-4,7,10-tris(*tert*-butylacetate)]acetic acid methyl ester (**2**) for Cu^{I}-catalyzed azide–alkyne cycloadditions with azide functionalized compounds (Figure 1). Both compounds can be easily synthesized by established synthetic methods. To prove their applicability, Tyr^3-octreotate, a somatostatin (sst) analogue which is a well known targeting molecule for tumor diagnostics and endoradiotherapeutic purposes,[40–45] was chosen as a highly functionalized model compound providing a broad range of functionalities. Both classes of derivatives proved to react selectively with appropriately modified unprotected Tyr^3-octreotate. In addition, one of the radioligands was labeled with ^{68}Ga and an initial biodistribution study in AR42J tumor bearing nude mice was performed proving applicability of the modified linkage.

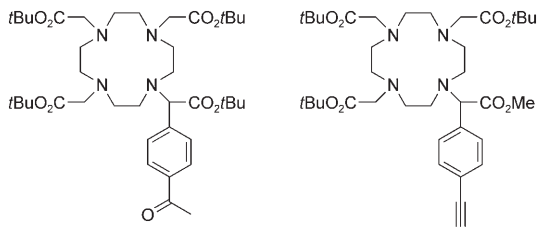

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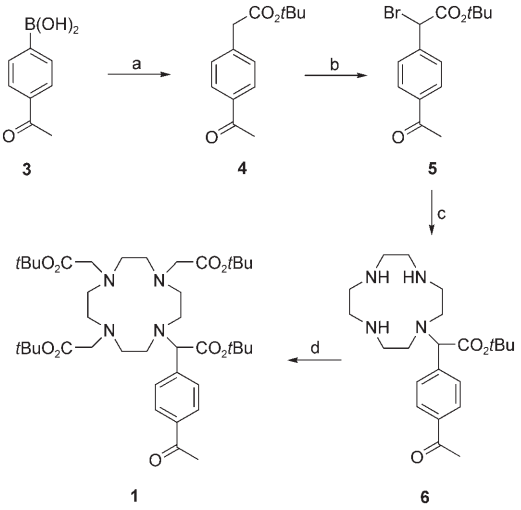
Figure 1. Structures of 4-acetylphenyl-DOTA derivative **1** and ethynylphenyl-DOTA derivative **2**.

Results

Synthesis of a carbonyl-substituted DOTA derivative for chemoselective attachment to aminooxy-functionalized peptides via oxime ligation: Planning our synthetic strategy, we decided to attach the carbonyl functionality to the DOTA moiety. The aminooxy group can be readily implemented in peptides as a aminooxy-functionalized building block (e.g. (Boc-aminooxy)acetic acid, Boc-*O*-(Fmoc-amino)-serine (Boc-Ams(Fmoc)-OH) and 3-(Boc-aminooxyacetamido)-2-Fmoc-aminopropionic acid (Fmoc-Dpr(Boc-Aoa)-OH)). As carbonyl functionality, a methyl ketone was preferred over an aldehyde due to its significant higher stability. This procedure avoids additional protection and deprotection steps, which would have been essential when working with an aldehyde, and makes the final compound storable for longer periods.

Our synthesis started with 4-acetylphenylboronic acid (**3**) which was reacted with *tert*-butyl 2-bromoacetate in a Suzuki-type cross coupling reaction.[46] The resulting 2-(4-acetylphenyl) substituted acetate **4** was obtained in 76% yield (Scheme 1). The α-bromination using *N*-bromosuccinimide (NBS) in presence of catalytic amounts of bromine and initiation with light gave the α-bromo ester **5** in high yield (88%). The latter compound was slowly added to a 1.2-fold excess of cyclen in DMF in the presence of K_2CO_3 to afford the monoalkylated cyclen-adduct **6** (75% yield). Subsequent peralkylation with *tert*-butyl 2-bromoacetate then gave the desired tetraester **1** (72% yield).

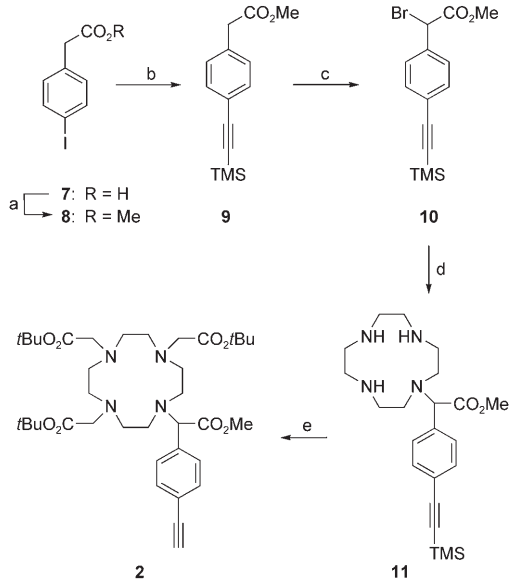


Scheme 1. Synthesis of carbonyl-substituted DOTA derivative **1**. a) $BrCH_2CO_2tBu$, $Pd(OAc)_2/P(o\text{-}Tol)_3$, K_2CO_3, THF/H_2O, RT, 18 h; 76%; b) NBS, Br_2, *hν*, CCl_4, 60°C, 1 h; 88%; c) cyclen, K_2CO_3, DMF, RT, 10 h; 75%; d) $BrCH_2CO_2tBu$, K_2CO_3, DMF, RT, 4 h; 72%.

Synthesis of alkyne-substituted DOTA derivatives for chemoselective attachment to azido functionalized peptides via Cu^I-catalyzed azide–alkyne cycloaddition: To design the appropriate DOTA derivative for attachment to peptides via Cu^I-catalyzed azide–alkyne cycloaddition,[17] we decided to introduce the alkyne functionality to the DOTA derivative, as azido functionalized peptides are readily accessible, for example, by introduction of azido acids[47–49] or by diazo transfer on the solid phase.[50] After our positive results with the synthesis of the carbonyl-substituted derivative, we again chose 2-bromo-2-phenylacetic acid as the core residue for the implementation of the alkyne, providing an analogous synthetic route as described above.

The synthesis was started from commercially available 4-iodophenylacetic acid (**7**). The free acid was protected as methyl ester **8** by treating with thionyl chloride in methanol in almost quantitative yield (93%) and high purity.

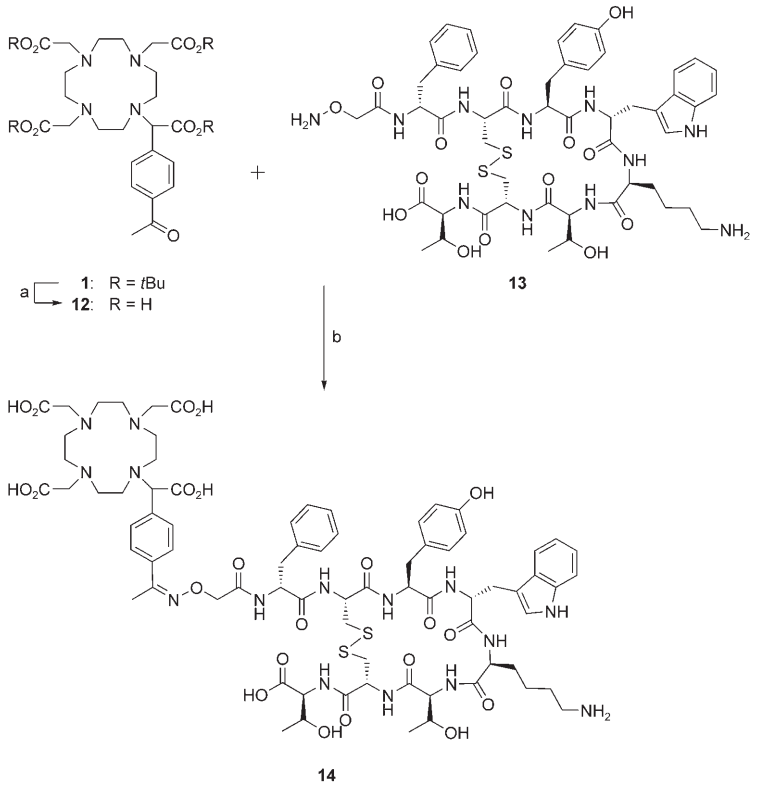
Subsequently, **8** was coupled with trimethylsilyl (TMS) acetylene in a Sonogashira reaction using $[Pd(PPh_3)_4]$/CuI as a catalyst affording the 4-alkynylphenylacetate **9** in 93% yield (Scheme 2).[51,52] The reaction was easy to handle and proceeded cleanly. However, the α-bromination of **9** with NBS failed with both, $Br_2/h\nu$ and 2,2′-azobisisobutyronitrile (AIBN) as initiators. This is most likely due to side reactions caused by the presence of a triple bond. Therefore, we circumvented this problem by introducing the bromide in an electrophilic manner. For this purpose, we transformed ester **9** into the boron enolate in an analogous manner as described by Evans et al.,[53] treating subsequently with lithium diisopropyl amide (LDA) and Bu_2BOTf. After addition of *N*-bromosuccinimide as the electrophilic brominating reagent, the α-bromo ester **10** was isolated in rather poor yield. In an attempt to further optimize the reaction, we found that the conversion proceeds cleanly if the lithium enolate of **9**, obtained by deprotonation with LDA, was directly reacted with NBS. In this manner, **10** could be obtained in 47% yield together with unreacted **9** which was recovered in 49% yield. This result is explained by the much higher acidity of the α-bromo ester **10** to **9** leading to a rapid deprotonation of the formed product with one equivalent of the lithium enolate of **9** during the reaction. However, based on recovered starting material **9**, which could be readily separated by flash chromatography, the yield was almost quantitative. The monoalkylation of cyclen with α-bromo ester **10** was performed in an analogous way as described above to yield **11**. After peralkylation with *tert*-butyl 2-bromoacetate and cleavage of the TMS group using tetrabutylammonium fluoride (TBAF), the tetraester **2** was obtained in 85% yield over two steps.



Scheme 2. Synthesis of alkyne-functionalized DOTA derivatives. a) $SOCl_2$, MeOH, 0°C → RT, 1 h; 93%; b) HC≡C-TMS, $[Pd(PPh_3)_4]$/CuI, NEt_3, CH_3CN, 0°C → RT, 3 h; 93%; c) 1) LDA, THF, −78°C, 1 h; 2) NBS, THF, −78°C → RT, 18 h; 47% (92% related to recovered **14**); d) cyclen, K_2CO_3, DMF, RT, 10 h; 73%; e) 1) $BrCH_2CO_2tBu$, K_2CO_3, DMF, RT, 4 h; 2) TBAF ; THF, RT, 15 min; 85%.

With these novel functionalized chelators in hand, we scrutinized chemoselective attachment to *N*-terminally aminooxy and azide-functionalized Tyr^3-octreotates **13** and **18**.

Chemoselective synthesis of the DOTA-Tyr^3-octreotate conjugate 14 via oxime ligation using the DOTA ketone derivative 1: In our initial experiments, the *tert*-butyl protected DOTA ketone **1** was directly used for the oxime ligation. However, the oxime bond of the resulting conjugate was unstable under the strong acidic conditions required for deprotection of the *tert*-butyl groups. Therefore the procedure was switched and **1** was deprotected in situ prior to the ligation. A quantitative cleavage was realized treating with 10 N aqueous HCl in dioxane 50:50 (Scheme 3). Alternative methods applying formic acid,[54] 50% trifluoroacetic acid



Scheme 3. Chemoselective oxime ligation reaction of DOTA derivative **1** and aminooxy functionalized Tyr^3-octreotate **13**. a) 10 N HCl/dioxane 1:1, RT, 18 h; b) CH_3CN/H_2O 1:1 (HPLC grade), pH 4 (TFA, HPLC grade), RT, 18 h; 73 % (two steps).

(TFA)/water or a mixture of TFA, triisopropylsilane (TIPS) and water (95:2.5:2.5)[55]—a standard deprotection mixture used in Fmoc peptide chemistry—failed. The free chelator **12** thus obtained reacted cleanly with equimolar amounts of aminooxy-functionalized Tyr^3-octreotate **13** in an acetonitrile/water mixture at pH 4 (TFA) to give the desired conjugate **14** with high purity, as proven by HPLC analysis (Figure 2).

It should be emphasized that any reaction where free aminooxy functionalities occur, demand high solvent purities (HPLC grade) in order to prevent side reactions with carbonyl functionalized impurities. The final product was further purified by preparative HPLC to give **14** in 73 % yield and 97 % purity.

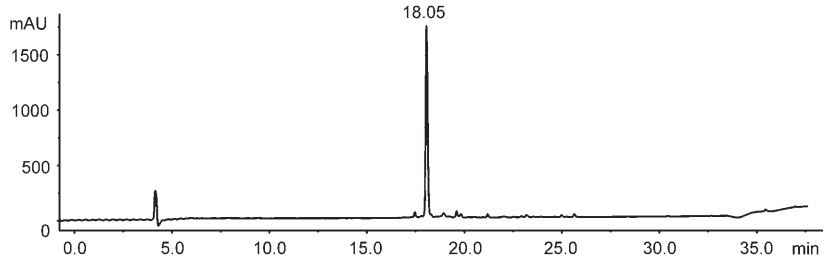
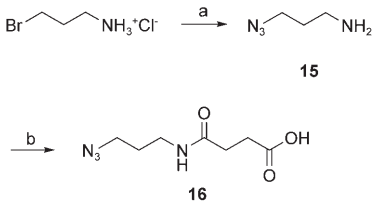


Figure 2. HPLC spectrum (UV 220 nm) of crude DOTA conjugate **14** obtained by oxime ligation.

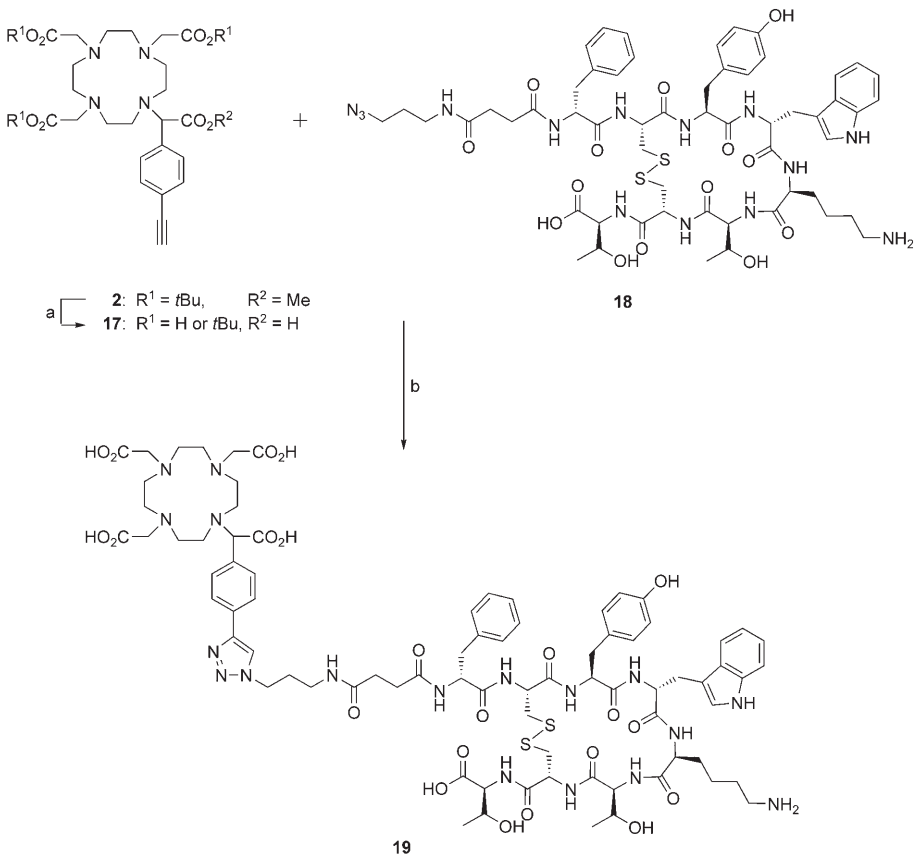
Chemoselective synthesis of the DOTA-Tyr^3-octreotate conjugate 19 via Cu^I-catalyzed azide–alkyne cycloaddition using DOTA alkyne derivative 2: With respect to the azide functionalization of Tyr^3-octreotate, we chose a simple *N*-terminal elongation with 3-(3-azidopropylcarbamoyl) propanoic acid (**16**) on the solid support. Since in several biological active peptides it is of great importance to use a spacer between BFCA and the targeting moiety and based on earlier experience in our group, **16** was the linker of choice. The two-step synthesis of **16** starting from 1-bromo-3-aminopropane is cheap, high yielding, easy to scale up and does not require any chromatography (Scheme 4). Compound **16** was attached to Tyr^3-octreotate following standard peptide coupling protocols to obtain the azido functionalized Tyr^3-octreotate **18** (see Supporting Information).

For the azide–alkyne cycloaddition, a one-pot procedure was worked out: Prior to the cycloaddition, methyl ester **2** was saponified in situ using lithium hydroxide in THF/water.



Scheme 4. Synthesis of 3-(3-azidopropylcarbamoyl)propanoic acid (**16**). a) NaN_3, H_2O, 80 °C, 24 h; 84 %; b) succinic anhydride, NEt_3, acetone, RT, 15 h; 71 %.

Surprisingly, this resulted in a partial cleavage of *tert*-butyl groups as well, which, however, had no impact on the subsequent procedure. The resulting mixture was further reacted with the azido functionalized Tyr^3-octreotate **18** using Cu/$CuSO_4$ as catalyst system (Scheme 5). After evaporation, the crude mixture was directly



Scheme 5. Chemoselective Cu^{I}-catalyzed azide–alkyne cycloaddition of DOTA derivative **17** and azido functionalized Tyr^3-octreotate **18**. a) LiOH, THF/H_2O, RT, 18 h; b) 1) Cu/$CuSO_4$, THF/H_2O, RT, 18 h; 2) TFA/TIPS/H_2O 95:5:5, RT, 2 h; 3) Na_2S, THF/H_2O, RT; 4) DMSO, NH_3, CH_3CN/H_2O, RT, 24 h; 37% (five steps).

deprotected by adding TFA/TIPS/water 95:2.5:2.5[55] to afford the crude product **19** with high purity, as shown by HPLC analysis (Figure 3). ESI mass spectroscopy showed that **19** was obtained as a copper complex. Thus, the copper ions were removed from the solution and from the chelator by precipitation with sodium sulfide to give the pure free conjugate. During the four-step procedure, no side reactions with functional groups were observed, except of a reductive opening of the intramolecular disulfide bond in the last step which was easily reformed in quantitative yield by treating with DMSO in acetonitrile/water for 24 h. After HPLC purification, **19** was obtained in 37% yield and 97% purity from **18** (five steps).

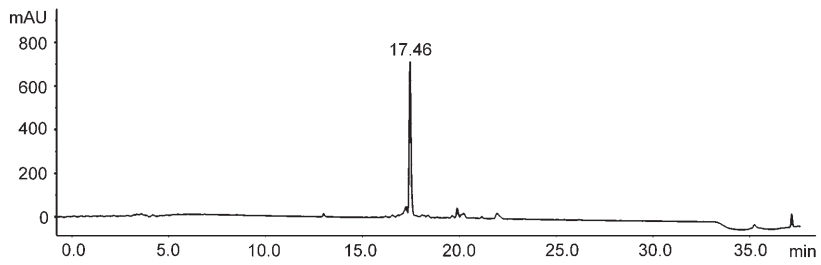


Figure 3. HPLC spectrum (UV 220 nm) of crude DOTA conjugate **19** obtained after click reaction and subsequent deprotection (step 2, Scheme 5).

In order to check our procedure, we repeated the reaction with the azido functionalized, but non-cysteine containing sample peptide 3-(3-azidopropylcarbamoyl)propanoyl-Tyr-Glu-Trp-Lys (**20**, see Supporting Information). As expected, the reaction sequence proceeded without side reactions. Deprotection of the *tert*-butyl esters gave the crude product with high purity (see Supporting Information). After HPLC purification the corresponding conjugate **21** was obtained in 51% yield (four steps).

Biodistribution studies: The DOTA–Tyr^3-octreotate conjugate **14** was chosen for biodistribution studies to verify the applicability of the new DOTA derivatives. ^{68}Ga labeling was performed using a $^{68}Ge/^{68}Ga$ generator to give [^{68}Ga]**14** with specific activity of 570 Ci mmol^{-1} at the time point of injection into mice. [^{68}Ga]**14** was obtained in 55.7% radiochemical yield and 91.4% radiochemical purity. The biodistribution data for [^{68}Ga]**14** in AR42J tumor bearing nude mice 30 and 60 min p.i. are displayed in Figure 4.

At both time points, blood concentration of the radioligand was comparably high (2.37 ± 0.35 and 1.71 ± 0.17% iD g^{-1} at 30 and 60 min p.i., respectively). While kidney accumulation rapidly decreased within the observation period (20.0 ± 2.4 to 11.9 ± 1.9% iD g^{-1}), indicating renal clearance of [^{68}Ga]**14**, the tracer accumulation in the other excretion organs, that is, liver and intestine, decreased only slowly over time, probably due to nonspecific accumulation that is not associated with excretion. In the sst-expressing tissues, a strong divergence between the tumor and the other sst-positive organs was observed. While tracer accumulation in pancreas, adrenals and stomach significantly decreased between 30 and 60 min p.i., tumor accumulation remained almost constant within this period (7.73 ± 1.52 and 7.46 ± 1.30 at 30 and 60 min p.i., respectively). This and the overall decrease in non-specific tracer accumulation in the other organs lead to

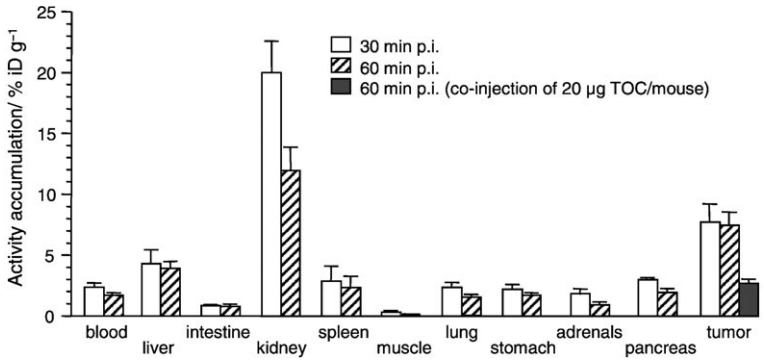


Figure 4. Biodistribution of [^{68}Ga]**14** in AR42 J tumor bearing nude mice 30 and 60 min p.i. ($n=5$; $n=3$ for the competition study). Data are given in % injected dose per gram tissue (% iD g^{-1}) and are means ± SD.

increasing tumor/organ ratios between 30 and 60 min p.i. (see Supporting Information). That the tumor accumulation is mainly receptor mediated was demonstrated in a competition study (60 min p.i.) by co-injection of an excess of unlabeled competitor (20 µg Tyr^3-octreotate per mouse). Under these conditions, tumor accumulation was reduced 2.68 ± 0.36 % iD g^{-1}.

Discussion

DOTA and its derivatives emerged as an important class of chelators for imaging technologies in medicine due to their ability to form very stable complexes with a variety of di- and trivalent metal ions. For the convenient synthesis of chelator conjugated targeting biomolecules, different suitable prochelators bearing orthogonally protected carboxy groups have been described. However, so far there is only one report published in the literature on the synthesis of prochelators enabling connections other than through amine or carboxyl functionalities within the targeting molecule.[16] Our approach in developing BFCAs which allow chemoselective attachment via click reaction resulted in the synthesis of novel chelators **1** and **2**.

For the synthesis of both compounds, a monoalkylation of cyclen had to be accomplished to obtain **6** and **11**. In the literature, there are several examples of similar monoalkylations where high yields of monoalkylated product were achieved by applying cyclen in great excess (e.g. 2 equiv[7] or 5 equiv[8]) and where the yields are calculated based on the amount of alkylating agents. As a result of cyclen being the most expensive reagent in the sequence, in our synthesis we used almost equimolar amounts of the alkylating agent and obtained satisfying yields of 75 and 73 % for compounds **6** and **11**. Although the crude product could be directly further alkylated, purification of this intermediate was carried out via flash chromatography at this stage, as it was easier to separate the monoalkylated product from higher alkylated products and unreacted cyclen, rather than separation after peralkylation, where the resulting compounds only differ in the nature of the alkyl substituent. The syntheses of the α-bromo esters **5** and **10** were straightforward and the products were obtained in good yields.

For the Cu^{I}-catalyzed azide–alkyne cycloaddition[17,20,34–39] we used 1.2 equivalents of copper sulfate together with of an excess of copper metal, which leads to in situ generation of the catalytically active Cu^{I} species. Although there are reports[56] where only 1 mol % of Cu^{II} salts are used, we had to use an excess, given the fact that one equivalent of the Cu^{II} is immediately complexed with the DOTA residue. However, in the literature there are a plethora of different procedures[56] and for our purposes, optimization of the catalyst loading was not essential as dissolved copper ions could be precipitated and filtered off by addition of sodium sulfide. Using Tyr^3-octreotate as a model compound, the conjugation by azide–alkyne cycloaddition was shown to be applicable with a broad range of functionalities. As the only side reaction, an opening of disulfide bridge was found. However, this causes no problem, as the reduced DOTA conjugate is smoothly recyclized in quantitative yield. Nevertheless, this side reaction should be kept in mind when planning to synthesize a disulfide bridged conjugate. Of course, it can be avoided if the linear precursor peptide is used for conjugation and the disulfide bridge is formed afterwards. Hence, our alkyne derivatized chelator offers a promising alternative for attachment of BFCAs in a highly chemoselective manner as demonstrated by reaction with model peptides **18** and **20**. During the reviewing process we became aware of an alternative approach for DOTA conjugation via azide–alkyne cycloaddition.[57]

The oxime ligation of our new methyl ketone functionalized chelator **12** with unprotected *N*-terminally aminooxy-functionalized Tyr^3-octreotate proceeded smoothly and without by-products, thereby adding another example of the chemoselective condensation of a methyl ketone and a hydroxylamine. Hence, we have successfully shown the chemoselective attachment of a novel bench stable DOTA derivative suitable for the ligation with aminooxy-functionalized biomolecules. In comparison to the recently presented aminooxy-functionalized DOTA derivatives,[16] our inverse approach allows the introduction of our chelator at any position in a peptide sequence, since various appropriate aminooxy functionalized building blocks for SPPS are commercially available (e.g. Boc-Ams(Fmoc)-OH and Fmoc-Dpr(Boc-Aoa)-OH).

As a first proof of principle, we used the ^{68}Ga-labeled derivative of the oxime linked DOTA conjugate **14** in an in vivo experiment. AR42J tumor bearing nude mice were treated with ^{68}Ga labeled **14** per mouse and investigated for tumor accumulation at 30 and 60 min p.i. The results exhibit

good contrast and high accumulation in the tumor (see Figure 4).

Conclusion

The novel alkyne- and keto-functionalized DOTA derivatives described in this article allow a facile and chemoselective conjugation with polyfunctionalized compounds. With regard to the synthesis of our new modified chelators, we have developed economical straightforward procedures avoiding complicated protection group chemistry considering the final application of the BFCA. This allows easy access to labeled compounds in few synthetic steps. Both the alkyne as well as the methyl ketone functionalized DOTA derivatives proved to react selectively in the corresponding conjugation with *N*-terminally modified Tyr^3-octreotate. Furthermore, the pharmacokinetics of [^{68}Ga]**14** in AR42J tumor bearing nude mice demonstrate the suitability of the chemoselective BFC conjugation approach for the synthesis of new radiometalated peptide ligands for diagnostic and therapeutic in vivo applications.

Experimental Section

General methods: Tritylchloride polystyrol (TCP) resin (0.94 mmol g^{-1}) was purchased from PepChem (Tübingen, Germany). Coupling reagents and amino acid derivatives were purchased from Novabiochem, Neosystem, and IRIS Biotech GmbH, Merck Biosciences, Perseptive Biosystems GmbH and Neosystem. Dry solvents were purchased from Fluka. All other reagents and solvents were purchased from Merck, Aldrich and Fluka and were used as received. Standard syringe techniques were applied for transferring dry solvents. Thin-layer chromatography (TLC) was performed on aluminium-backed plates Merck silica gel 60 F_{254}. Compounds were visualized by UV absorption at 254 nm or coloration with cerium ammonium molybdate (CAM). Flash chromatography was performed on silica gel 60 (Merck, 230–400 mesh) (ca. 50 g for 1 g of material to be separated) with the indicated eluent. Solvents for chromatography were distilled prior to use. Chromatographic elution solvent systems are reported as volume/volume ratios. RP-HPLC analyses was performed using an Omnicrom YMC column (4.6 mm × 250 mm, 5 μm C_{18}, 1 mL min^{-1}) and detection at 254 nm for non-peptidic compounds and 220 nm for peptidic compounds. The eluent was a linear gradient from water (0.1 % TFA) to acetonitrile (0.1 % TFA) over 30 minutes. The retention time (t_R) of the analytical RP-HPLC is given in minutes with the gradient in percentage of acetonitrile. NMR: Bruker AC-250, AV-360, AV-500 and DMX500. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature. Spectra were calibrated to their respective solvent signals ($CDCl_3$: ^{1}H 7.26 ppm, ^{13}C 77.0 ppm; [D_4]MeOH: ^{1}H 3.31 ppm, ^{13}C 49.05 ppm;). Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J values) are given in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s, single; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; b, broad. MS: Finnigan MAT 8200 (EI), Finnigan LCQ (ESI). Peptide sequence analysis was performed on a Bruker Ultraflex TOF/TOF.

***tert*-Butyl 2-(4-acetylphenyl)acetate (4)**: A solution of 4-acetylphenylboronic acid (**3**) (9.84 g, 60.0 mmol, 1.3 equiv) in THF/H_2O (160 mL/2.2 mL) was added dropwise under argon over 1 h to a suspension of *tert*-butyl 2-bromoacetate (6.80 mL, 46.4 mmol, 1.0 equiv), $Pd(OAc)_2$ (336 mg, 1.50 mmol, 3 mol %), P(*o*-tolyl)$_3$ (1.36 g, 4.46 mmol, 10 mol %) and K_2CO_3 (34.6 g, 0.25 mmol, 5.4 equiv) in THF (160 mL). After stirring for 18 h at room temperature, the mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc (250 mL) and subsequently washed with saturated aqueous NH_4Cl (150 mL), saturated aqueous $NaHCO_3$ (150 mL) and brine. After drying over $MgSO_4$, the organic layer was filtered through silica gel and concentrated. Flash chromatography on silica gel (EtOAc/hexane 1:8) yielded **4** (8.30 g, 76 %) as a pale yellow solid. R_f = 0.27 (EtOAc/hexane 1:4); m.p. 50–52 °C; ^{1}H NMR (360 MHz, $CDCl_3$): δ = 7.92 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 3.58 (s, 2H), 2.59 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 197.7, 170.0, 140.1, 135.8, 129.4 (2C), 128.5 (2C), 81.2, 42.6, 28.0, 26.5; HRMS (EI): m/z: calcd for $C_{14}H_{18}O_3$: 234.12559; found 234.12532 [M]$^+$.

(*R*/*S*)-*tert*-Butyl 2-(4-acetylphenyl)-2-bromoacetate (5): *N*-Bromosuccinimide (NBS) (2.58 g, 14.5 mmol, 1.2 equiv) and Br_2 (2 drops) were added to a solution of **4** (2.84 g, 12.1 mmol, 1.0 equiv) in dry CCl_4 (250 mL). The mixture was heated to reflux, illuminated with a 500 W halogen lamp for 10 min and stirred for further 50 min under reflux. After cooling to room temperature, the reaction solution was filtered, the solvent concentrated and the crude product purified by flash chromatography on silica gel (EtOAc/hexane 1:10) to give **5** (3.34 g, 88 %) as a pale yellow solid. R_f = 0.27 (EtOAc/hexane 1:4); m.p. 54–56 °C; ^{1}H NMR (360 MHz, $CDCl_3$): δ = 7.93 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.3 Hz, 2H), 5.27 (s, 1H), 2.59 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 197.2, 166.6, 141.1, 137.3, 128.8 (2C), 128.6 (2C), 83.5, 47.1, 27.6, 26.6; MS (EI): m/z (%): 314 (<1) [M(^{81}Br)]$^+$, 312 (<1) [M(^{79}Br)]$^+$, 241 (10) [M-(^{81}Br)−O*t*Bu]$^+$, 239 (8) [M(^{79}Br)−O*t*Bu]$^+$; HRMS (EI): m/z: calcd for $C_{10}H_8{}^{81}BrO_2$: 240.96872; found 240.96833 [M(^{81}Br)−O*t*Bu]$^+$, 238.97074 [M(^{79}Br)−O*t*Bu]$^+$.

(*R*/*S*)-*tert*-Butyl 2-[1-(1,4,7,10-tetraazacyclodecane)]-2-(4-acetylphenyl)-acetate (6): A solution of **5** (500 mg, 1.60 mmol, 1.0 equiv) in DMF (50 mL) was added dropwise at room temperature over 10 h to a suspension of 1,4,7,10-tetraazacyclodecane (331 mg, 1.92 mmol, 1.2 equiv) and K_2CO_3 (552 mg, 3.99 mmol, 2.5 equiv) in DMF (20 mL). The mixture was filtered and concentrated under reduced pressure. Flash chromatography on silica gel (MeOH/$CHCl_3$ 1:7→7:1, 1 % NEt_3) yielded **6** (488 mg, 75 %) as a pale yellow solid. R_f = 0.10 (MeOH/$CHCl_3$ 3:1, 1 % NEt_3); m.p. 33–36 °C; ^{1}H NMR (360 MHz, $CDCl_3$): δ = 7.91 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 4.62 (s, 1H), 2.90–2.67 (m, 11H), 2.64–2.44 (m, 10H), 1.47 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 197.4, 170.7, 142.3, 136.4, 129.3, 128.3, 81.9, 67.8, 49.3, 47.7, 45.9, 45.8, 28.1, 26.5; HRMS (EI): m/z: calcd for $C_{22}H_{36}N_4O_3$: 404.27875; found 404.27951 [M]$^+$.

(*R*/*S*)-*tert*-butyl 2-[1-(1,4,7,10-tetraazacyclodecane)-4,7,10-tris(*tert*-butyl-acetate)]-2-(4-acetylphenyl)acetate (1): A solution of *tert*-butyl 2-bromoacetate (0.84 mL, 5.71 mmol, 3.3 equiv) in DMF (20 mL) was added at room temperature over 30 min to a suspension of **6** (700 mg, 1.73 mmol, 1.0 equiv) and K_2CO_3 (1.08 mg, 7.79 mmol, 4.5 equiv) in DMF (50 mL). After stirring for 4 h, the mixture was filtrated and concentrated under reduced pressure. Flash chromatography on silica gel (MeOH/$CHCl_3$ 9:1, 1 % TEA) yielded **1** (930 mg, 72 %) as a pale yellow solid. 99 % purity; RP-HPLC (10→100 %) t_R = 22.0 min; R_f = 0.83 (MeOH/$CHCl_3$ 3:1, 1 % NEt_3); m.p. 70–71 °C; ^{1}H NMR (360 MHz, $CDCl_3$): δ = 7.94 (d, J = 8.3 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H), 4.66 (s, 1H), 3.60 (d, J = 17.3 Hz, 1H), 3.45 (d, J = 17.6 Hz, 1H), 3.38 (d, J = 17.5 Hz, 1H), 3.22–3.05 (m, 3H), 2.98–2.70 (m, 6H), 2.61 (s, 3H), 2.61–2.48 (m, 2H), 2.46–2.38 (m, 1H), 2.36–2.24 (m, 2H), 2.22–2.04 (m, 5H), 1.49 (s, 9H), 1.48 (s, 9H), 1.46 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 197.6, 173.4, 173.1, 173.0, 172.9, 137.2, 136.6, 130.3 (2C), 128.1 (2C), 82.9, 82.2, 82.1, 82.0, 65.4, 56.0, 55.8, 55.5, 52.7, 52.4, 52.1, 48.5, 48.1, 48.0, 47.9, 44.5, 27.9 (3C), 27.8 (6C), 27.7 (3C), 26.6; MS (EI): m/z (%): 746.0 (9) [M]$^+$, 645.1 (47) [M−CO_2*t*Bu]$^+$; HRMS (EI): m/z: calcd for $C_{35}H_{57}N_4O_7$: 645.42273; found 645.42257 [M−CO_2*t*Bu]$^+$.

Methyl 2-(4-(2-(trimethylsilyl)ethynyl)phenyl)acetate (9): [$Pd(PPh_3)_4$] (3.6 g, 3.15 mmol, 0.07 equiv) and CuI (6.00 g, 31.5 mmol, 0.7 equiv) was added under argon at 0 °C to a solution of methyl (4-iodophenyl)acetate (**8**) (12.8 g, 45 mmol, 1.0 equiv), trimethylsilylacetylene (9.30 mL, 67.5 mmol, 1.5 equiv) and triethylamine (TEA) (14.9 mL, 108 mmol, 2.4 equiv) in dry CH_3CN (120 mL). After stirring for 30 min at 0 °C and 3 h at room temperature, the mixture was filtered through a short

column of silica gel using EtOAc/hexane 1:1 as eluent. The solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel (EtOAc/hexane 1:80→1:20) to yield **9** (10.3 g, 93%) as pale yellow crystals. R_f=0.27 (EtOAc/hexane 1:10); m.p. 55–58 °C; ^{1}H NMR (360 MHz, $CDCl_3$): δ=7.42 (d, J=8.4 Hz, 2H), 7.21 (d, J=8.5 Hz, 2H), 3.68 (s, 3H), 3.61 (s, 2H), 0.25 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ=171.4, 134.3, 132.0 (2C), 129.1 (2C), 122.0, 104.7, 94.2, 52.0, 41.0, −0.0 (3C); HRMS (EI): m/z: calcd for $C_{14}H_{18}O_2Si$: 246.10761; found 246.10744 $[M]^+$.

(*R*/*S*)-Methyl 2-bromo-2-(4-(2-(trimethylsilyl)ethynyl)phenyl)acetate (10): Lithium diisopropylamide (2 M solution in THF/*n*-heptane/ethylbenzene, 5.04 mL, 10.1 mmol, 1.2 equiv) was added at −78 °C to a solution of **9** (2.07 g, 8.40 mmol, 1.0 equiv) in dry THF (20 mL) and the solution stirred for 1 h. After this time, a suspension of NBS (1.79 g, 10.1 mmol, 1.2 equiv) in dry THF (20 mL) was added and the mixture warmed to room temperature over 18 h. The solvent was removed under reduced pressure, the residue suspended in CCl_4 (30 mL), filtered and evaporated. Purification by flash chromatography on silica gel (gradient EtOAc/hexane 1:80→1:20, 1% NEt_3) gave **10** (1.28 g, 47%; 92% related to recovered **9** (1.01 g, 49%)). R_f=0.42 (EtOAc/hexane, 1:10); m.p. 82–84 °C; ^{1}H NMR (360 MHz, $CDCl_3$): δ=7.47 (d, J=8.7 Hz, 2H), 7.44 (d, J=8.7 Hz, 2H), 5.32 (s, 1H), 3.77 (s, 3H), 0.25 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ=168.3, 135.7, 132.2 (2C), 128.5 (2C), 124.2, 104.1, 95.8, 53.3, 45.8, 45.8, −0.1 (3C); HRMS (EI): m/z: calcd for $C_{14}H_{17}{}^{81}BrO_2Si$: 326.01608; found 326.01622 $[M(^{81}Br)]^+$.

(*R*/*S*)-Methyl 2-[1-(1,4,7,10-tetraazacyclodecane)]-2-(4-(2-(trimethylsilyl)-ethynyl)-phenyl)acetate (11): A solution of **10** (316 mg, 0.97 mmol, 1.0 equiv) in DMF (50 mL) was added dropwise at room temperature over 10 h to a suspension of 1,4,7,10-tetraazacyclodecane (cyclen) (200 mg, 1.16 mmol, 1.2 equiv) and K_2CO_3 (160 mg, 1.16 mmol, 1.2 equiv) in DMF (10 mL). The mixture was filtered and concentrated under reduced pressure. Flash chromatography on silica gel (MeOH/$CHCl_3$ 1:1→9:1, 0.5% NEt_3) yielded **11** (295 mg, 73%) as a pale yellow solid. R_f= 0.10 (MeOH/$CHCl_3$ 9:1, 0.5% NEt_3); m.p. 70–75 °C; ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ=7.49 (d, J=8.2 Hz, 2H), 7.30 (d, J=8.3 Hz, 2H), 4.90 (s, 1H), 3.78 (s, 3H), 3.21–3.01 (m, 6H), 3.01–2.82 (m, 8H), 2.71–2.62 (m, 2H), 0.23 (s, 9H); ^{13}C NMR (125 MHz, [D$_4$]MeOH): δ=174.2, 135.6, 133.2 (2C), 130.8 (2C), 124.9, 105.4, 95.9, 67.2, 53.0, 48.4 (2C), 47.0 (2C), 44.9 (2C), 44.4 (2C), −0.0 (3C); MS (ESI): m/z: calcd for $C_{22}H_{36}N_4O_2Si$: 416.3; found 417.4 $[M+H]^+$, 439.4 $[M+Na]^+$.

(*R*/*S*)-Methyl 2-[1-(1,4,7,10-tetraazacyclodecane)-4,7,10-tris(*tert*-butylacetate)]-2-(4-ethynyl)phenyl)acetate (2): A solution of *tert*-butyl 2-bromoacetate (615 μL, 4.19 mmol, 3.3 equiv) in DMF (20 mL) was added at room temperature over 30 min to a suspension of **11** (530 mg, 1.27 mmol, 1.0 equiv) and K_2CO_3 (634 mg, 4.57 mmol, 3.6 equiv) in DMF (50 mL). After stirring for 4 h, the mixture was filtered, concentrated under reduced pressure and the residue dissolved in THF (20 mL). Then, tetrabutylammonium fluoride (TBAF) (481 mg, 1.52 mmol, 1.2 equiv) was added, and after stirring for 15 min, the solvent was removed and the crude product purified by flash chromatography on silica gel (MeOH/$CHCl_3$ 1:10, 1% NEt_3) to yield **2** (508 mg, 85%) as a pale yellow solid. 99% purity; RP-HPLC (10→100%) t_R=20.0 min; R_f=0.28 (MeOH/$CHCl_3$ 1:9, 1% NEt_3); m.p. 63–68 °C; ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ=7.48 (d, J=8.2 Hz, 2H), 7.20 (d, J=8.0 Hz, 2H), 4.83 (s, 1H), 3.75 (d, J=17.2 Hz, 1H), 3.74 (s, 3H), 3.55 (s, 1H), 3.54 (d, J=17.5 Hz, 1H), 3.51 (d, J=17.6 Hz, 1H), 3.26–3.21 (m, 1H), 3.18–3.05 (m, 4H), 2.99–2.92 (m, 3H), 2.89 (d, J=17.6 Hz, 1H), 2.87 (d, J=17.6 Hz, 1H), 2.74–2.63 (m, 2H), 2.33 (d, J=11.5 Hz, 1H), 2.28–2.12 (m, 4H), 2.12–2.05 (m, 2H), 1.54 (s, 9H), 1.52 (s, 18H); ^{13}C NMR (125 MHz, [D$_4$]MeOH): δ=176.3, 175.4, 175.1, 174.7, 134.1, 132.8, 131.7, 123.8, 83.8, 83.5, 83.1, 79.7, 66.4, 59.61, 59.59, 59.57, 57.1, 56.8, 56.7, 54.2, 53.9, 53.7, 53.2, 45.9, 28.5, 28.4, 28.3, 24.8; HRMS (EI): m/z: calcd for $C_{37}H_{58}N_4O_8$: 686.42546; found 686.42532 $[M]^+$.

Chemoselective oxime ligation—Synthesis of DOTA-Tyr^3-octreotate derivative 14: Compound **1** (3.3 mg, 4.5 μmol, 1.0 equiv) was deprotected in 10 N aqueous HCl in dioxane 50:50 (2 mL) for 18 h after which the solvent was removed under reduced pressure. The residue was dissolved in CH_3CN/H_2O 1:1 (0.2 mL, HPLC grade) at pH 4 (TFA, HPLC grade) and **13** (6.1 mg, 4.5 μmol, 1.0 equiv) was added. After stirring for 18 h, the solvent was concentrated and the crude product was directly purified by semipreparative RP-HPLC (20→50%, 30 min) to yield **14** (6.1 mg, 73%) as a colorless powder after lyophilization. 97% purity; RP-HPLC (10→60%); t_R=18.1 min; MS (ESI): m/z: calcd for $C_{75}H_{99}N_{15}O_{22}S_2$: 1625.7; found 1626.6 $[M+H]^+$, 1664.6 $[M+K]^+$.

Chemoselective click reaction—Synthesis of DOTA-Tyr^3-octreotate derivative 19: A solution of LiOH (0.33 mg, 14 μmol, 3.4 equiv) in H_2O (30 μL) was added at room temperature to a solution of **2** (3.1 mg, 4.1 μmol, 1.0 equiv) in THF (0.2 mL) and the mixture stirred for 18 h. Subsequently, H_2O (0.2 mL), peptide **18** (5.5 mg, 4.1 μmol, 1.0 equiv), 0.1 M aqueous $CuSO_4$ (49 μL, 4.9 μmol, 1.2 equiv) and copper powder (10 mg) were added and the mixture stirred for 18 h. After this time, the copper powder was filtered off, the solvent removed under reduced pressure and the *tert*-butyl esters were cleaved by treating with a mixture of TFA/TIPS/H_2O 95:5:5 (1 mL) for 2 h. The solvent was again removed and the residue was taken up THF/H_2O 1:1 (1 mL) to precipitate the copper salts by addition of $Na_2S\cdot 9H_2O$ (12 mg, 49 μmol, 12.0 equiv). The mixture was filtrated and the crude product directly purified by semipreparative RP-HPLC (20→50%, 30 min) to yield linear **19** (3.0 mg, 37%) as a colorless powder after lyophilization. The linear peptide was recyclized in quantitative yield by stirring in CH_3CN/H_2O/DMSO 1:1:0.2 (4 mL) at pH 8 (25% NH_3 solution) for 24 h. After evaporation and lyophilization from CH_3CN/H_2O 1:2 (10 mL, pH 1–3 (TFA)), **19** (3.0 mg, 37% from **18**) was isolated as a white powder. 97% purity; RP-HPLC (10→60%) t_R=16.1 min; MS (ESI): m/z: calcd for $C_{80}H_{106}N_{18}O_{22}S_2$: 1734.7; found 868.8 $[(M+2H)/2]^+$, 1735.5 $[M+H]^+$.

Animal experiments: Due to its high sst_2-somatostatin receptor expression, the rat pancreatic tumor cell line AR42J was used as a tumor model.[58] To establish tumor growth, cells were detached from the surface of the culture flasks using 1 mM EDTA in PBS, centrifuged and resuspended in serum-free culture medium (RPMI-1640, Biochrom, Berlin, Germany). Concentration of the cell suspension was 3.7×10^6 cells per 100 μL serum. Nude mice (female, 6–8 weeks) were injected 100 μL of the cell suspension subcutaneously into the flank. Ten days after tumor transplantation all mice showed solid palpable tumor masses (tumor weight 0.7–1.4 g) and were used for the experiments. For biodistribution studies, mice were intravenously injected 38 μCi [^{68}Ga]**14** (corresponding to 0.15 μg of peptide) in 100 μL PBS into the tail vein. Non-specific tissue accumulation of the radioligand was determined by coinjection of an excess of cold competitor (20 μg Tyr^3-octreotide per mouse). At different time points after radioligand injection (30 and 60 min p.i.) mice (n=5 per time point; n=3 for blocking study at 60 min p.i.) were sacrificed and dissected. The organs of interest were removed, weighed and counted in a γ counter (Wallach, Turku, Finland). Data are expressed as percent injected dose per gram tissue (% iD g^{-1}).

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