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DOTA-tris(OPp ester) as a bifunctional prochelator for the preparation of DOTA–peptide conjugates

Mazen Jamous, Uwe Haberkorn, Walter Mier *

Department of Nuclear Medicine, University Hospital Heidelberg, INF 400, 69120 Heidelberg, Germany

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ABSTRACT

Peptides containing the chelator DOTA have gained importance for molecular imaging and therapy with radionuclides. However, all synthons described for the convergent solid phase synthesis of DOTA–peptide conjugates show windows of stability that are too narrow to allow a clean and convergent deprotection process. The synthesis of the new prochelator DOTA-tris(OPp ester) starting from cyclen is reported. Using this prochelator for the synthesis of several DOTA peptide conjugates revealed that its cleavage—in contrast to the cleavage of DOTA-tris(tBu ester) conjugates—does not require an extended deprotection time, and therefore results in clean and homogenous products.

The side effects limit the efficiency of chemotherapeutic agents. As the targeting of tumors offers the potential to reduce this systemic toxicity, efforts have been undertaken to develop drugs that specifically accumulate in tumors. Several chelate-peptide conjugates such as DOTATOC (DOTA-D-Phe[1]-Tyr[3]-octreotide) have been shown to ideally fulfill this task by selectively transporting metallic radionuclides to tumors. Due to its capability to stably complex many radioisotopes of clinical interest, DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid) is considered as the gold standard of chelators for diagnostic and therapeutic applications. This necessitates in turn the development of efficient synthetic strategies for the preparation of DOTA-coupled radiolabelling precursors. Improved methodologies are required for the synthesis of DOTA-conjugated peptides—in particular methodologies that are convergent to the increasing demands set by the biopharmaceuticals accessible by solid phase peptide synthesis.

DOTA was first synthesized by Stetter and Frank.[1] It efficiently complexes ^{111}In, ^{67}Ga for SPECT,[2] Gd^{3+} for MRI,[3] ^{68}Ga and ^{64}Cu for PET,[4] Eu^{3+} and Tb^{3+} for optical imaging[5] as well as ^{90}Y and ^{177}Lu for radiotherapy.[6] Several different species of DOTA-based bifunctional chelators have been described for attaching DOTA to biomolecules: protected DOTA forms[7] that are deprotected after the coupling using coupling reagents, active DOTA esters[8] offering a reactivity comparable to BOP- and HBTU-style reagents[9] and DOTA-derivatives with a coupling moiety that was introduced into the macrocycle[10] or at the α-position of one carboxylate arm.[11] The total solid-phase synthesis of the DOTA chelator on a peptidyl resin[12] offers an alternative to these synthesis precursors. Currently, DOTA-tris-*tert*-butyl ester is the preferred monoreactive DOTA analog used for the solid phase synthesis of peptide derivatives. While the *tert*-butyl esters on glutamic acid are readily cleaved within the standard TFA peptide cleavage protocol, severe problems are encountered when deprotecting the DOTA tert-butyl protecting groups under the standard cleavage conditions with trifluoroacetic acid (TFA). The standard deprotection conditions[13] lead to incomplete deprotection and the harsh deprotection conditions required for complete deprotection lead to peptide degradation.

The cleavage of the tBu protecting groups of DOTA-tris (tBu ester) is known to be sluggish.[14] In the solid phase peptide synthesis process it is best performed by the successive treatment with TFA/radical scavenger cocktails followed by reaction with neat TFA. In the case of DOTA-tris(tBu ester) incomplete deprotection of the tBu groups often leads to significantly reduced yields and as consequence to complicated purification steps. Several attempts have been made to synthesize DOTA with protecting groups that can be removed under mild conditions, such as allyl esters, which can be deprotected by a Pd catalyst,[15] methyl esters, hydrolyzed with aqueous NaOH,[16] a method that was recently optimized[17] and benzyl esters, which can be deprotected by catalytic hydrogenolysis.[18] However, as these methods are either complicated or not convergent to the solid phase peptide synthesis process, these derivatives have not yet found widespread application. The aim of this work was to prepare a protecting group for DOTA-based prochelators that is labile under the deprotection conditions where Fmoc groups are stable and convergently cleaved under the cleavage conditions of the amino acid protecting groups of the peptide.

* Corresponding author. Tel.: +49 6221 567720; fax: +49 6221 565473.
E-mail address: walter.mier@med.uni-heidelberg.de (W. Mier).

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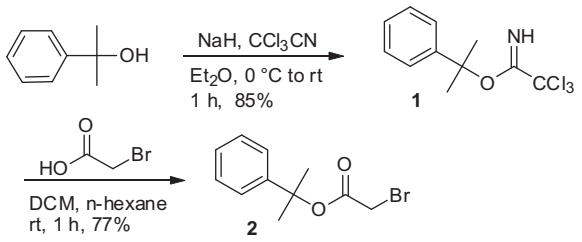
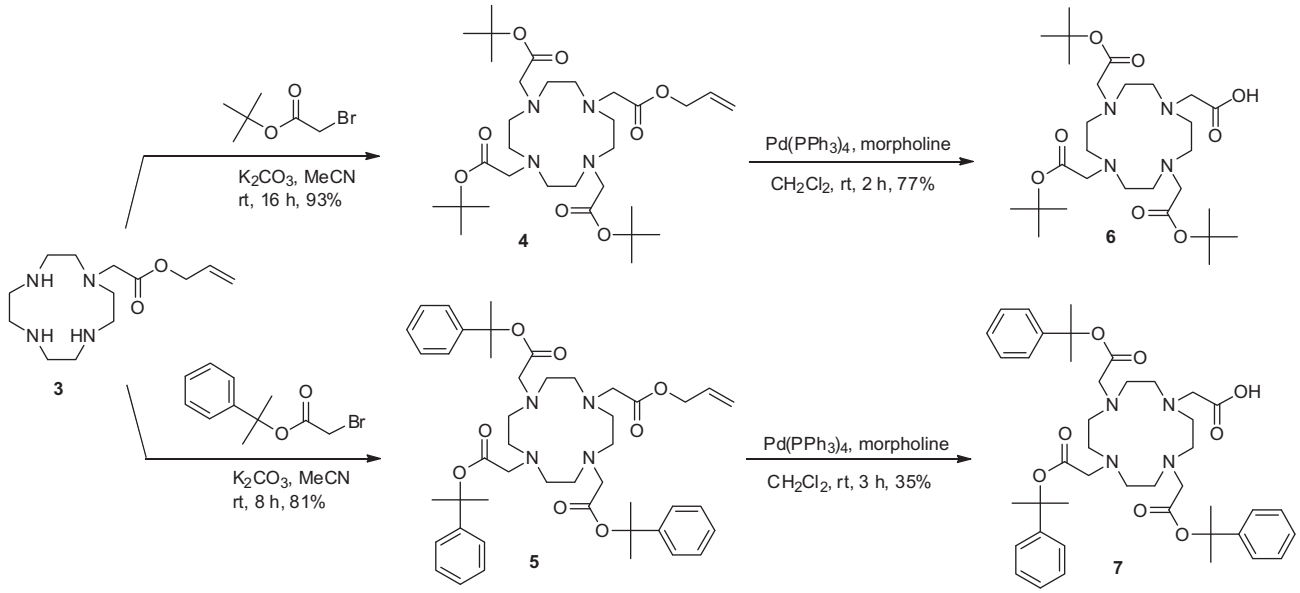


Scheme 1. Synthesis of bromoacetic acid 1-methyl-1-phenylethyl ester.

The use of esters derived from 1-methyl-1-phenylethanol as protecting groups was explored by Blotny and Taschner.[19] OPp esters are significantly more sensitive toward acid than tBu esters. Deprotection occurs with 2% trifluoroacetic acid in DCM. These conditions do not affect tBu or Boc groups.[20] In addition, OPp esters show excellent stability under the removal conditions applied for Fmoc or Alloc groups.[21] It has therefore been employed in Fmoc-strategy of peptide synthesis for example as the synthon Fmoc-Asp(1-methyl-1-phenylethyl ester)-OH.

The preparation of bromoacetic acid 1-methyl-1-phenylethyl ester (**2**) was performed successfully from the intermediate 1-methyl-1-phenylethyltrichloroacetimidate (**1**) (Scheme 1). The esterification of bromoacetic acid with **1** in DCM yielded **2** in 77% yield. The efficiency of this reaction can be explained by the fact that alkyltrichloroacetimidates are more reactive than the compounds previously used for the formation of bulky aliphatic esters-including alkenes in the presence of strong acid or acid anhydrides or acids and DCC and the corresponding aliphatic alcohol.[22] We envisaged that 1-methyl-1-phenylethyltrichloroacetimidate would be a more convenient source of the 1-methyl-1-phenylethyl cation in the preparation of 1-methyl-1-phenylethyl esters. The 1-methyl-1-phenylethyltrichloroacetimidate required was prepared as described by Fabrice et al.[23] and Wessel at al.[24]



Scheme 2. Synthesis of DOTA-tris(tBu ester) and DOTA-tris(OPp ester).

Table 1
Analytical data (^{1}H, ^{13}C NMR[a] and ESI-MS[b])

Compound	^{1}H NMR (500 MHz, DMSO-d_6)	^{1}C NMR (125 MHz, DMSO-d_6)	ESI-MS (m/z) for
2	δ = 7.41–7.39 (m, 2H), 7.37–7.34 (m, 2H), 7.30–7.26 (m, 1H), 3.80 (s, 2H), 1.82 (s, 6H)	δ = 165.5, 144.8, 128.3, 127.3, 124.2, 83.9, 28.3, 27.2	
4	δ = 5.94–5.86 (m, 1H), 5.32–5.19 (m, 2H), 4.59–4.57 (m, 2H), 3.73–3.73 (m, 2H), 3.44–2.84 (m, 22H), 1.41–1.40 (m, 27H)	δ = 173.5, 173.2, 173.1, 132.6, 118.9, 81.8, 81.7, 65.6, 55.7, 55.5, 54.8, 51.2, 50.6, 50.2, 28.2, 27.9	$C_{31}H_{56}N_4O_8$ [M], $[M+H]^+$ (calculated): 613.4160 (613.4176)
5	δ = 7.32–7.17 (m, 15H), 5.84–5.71 (m, 1H), 5.24–5.16 (m, 2H), 4.42–4.29 (m, 2H), 3.39–3.32 (m, 2H), 3.31–2.69 (m, 22H), 1.58 (s, 12H), 1.54 (s, 6H)	δ = 172.5, 172.4, 172.3, 145.9, 145.8, 132.7, 128.5, 128.4, 127.2, 124.4, 124.3, 118.6, 83.1, 65.4, 65.3, 55.6, 54.5, 28.9, 28.7	$C_{46}H_{62}N_4O_8$ [M], $[M+H]^+$ (calculated): 799.4642 (799.4646)
6	δ = 4.10–3.48 (m, 8H), 3.45–2.89 (m, 16H), 1.45 (s, 9H), 1.38 (s, 18H)	δ = 158.7, 158.3, 81.9, 54.7, 54.3, 53.9, 53.9, 51, 48.9, 28.2	$C_{28}H_{52}N_4O_8$ [M], $[M+H]^+$ (calculated): 573.3854 (573.3863)
7	δ = 7.35–7.26 (m, 12H), 7.24–7.15 (m, 3H), 3.59–3.41 (m, 2H), 3.38–3.24 (m, 6H), 3.06–2.57 (m, 14H), 1.69–1.61 (m, 18H)	δ = 177.7, 176.1, 174.9, 150.9, 150.8, 133.8, 133.3, 132, 131.9, 129.3, 129.1, 87.6, 87.4, 61, 60.8, 60.4, 58, 56.9, 54.7, 52.2, 33.9, 33.8	$C_{43}H_{58}N_4O_8$ [M], $[M+H]^+$ (calculated): 759.4344 (759.4333)
8	δ = 7.38–7.33 (m, 6H), 7.33–7.29 (m, 6H), 7.26–7.19 (m, 7H), 7.16–7.12 (m, 1H), 4.65–4.58 (m, 1H), 3.8–3.46 (m, 8H), 3.13–3.09 (m, 2H), 3.05–2.72 (m, 16H), 1.74 (s, 18H)	δ = 172.5, 158.6, 158.3, 146, 145.9, 138.2, 129.6, 128.8, 128.6, 128.4, 127.4, 127.3, 126.7, 124.7, 124.6, 83.1, 55.2, 54.3, 54.3, 52.2, 52.1, 51.3, 49.1, 38.4, 29, 28.9	$C_{52}H_{68}N_6O_8$ [M], $[M+H]^+$ (calculated): 905.5182 (905.5176)
9	δ = 7.26–7.22 (m, 4H), 7.19–7.15 (m, 1H), 4.61–4.57 (m, 1H), 3.91–3.38 (m, 8H), 3.13–2.76 (m, 18H), 1.45 (s, 9H), 1.43 (s, 18H)	δ = 172.5, 158.6, 158.3, 138.1, 129.7, 128.5, 126.7, 81.7, 55.2, 54.9, 54.2, 51.7, 49.1, 38.5, 28.2, 28.1	$C_{37}H_{62}N_6O_8$ [M], $[M+H]^+$ (calculated): 719.5245 (719.4707)

[a] Proton and carbon NMR spectra were recorded on a Varian Mercury Plus 500 MHz spectrometer at 25 °C.
[b] A mass spectrometer supporting orbitrap technology (Exactive, Thermo Fisher Scientific) was used to analyze the compounds and the peptides synthesized.

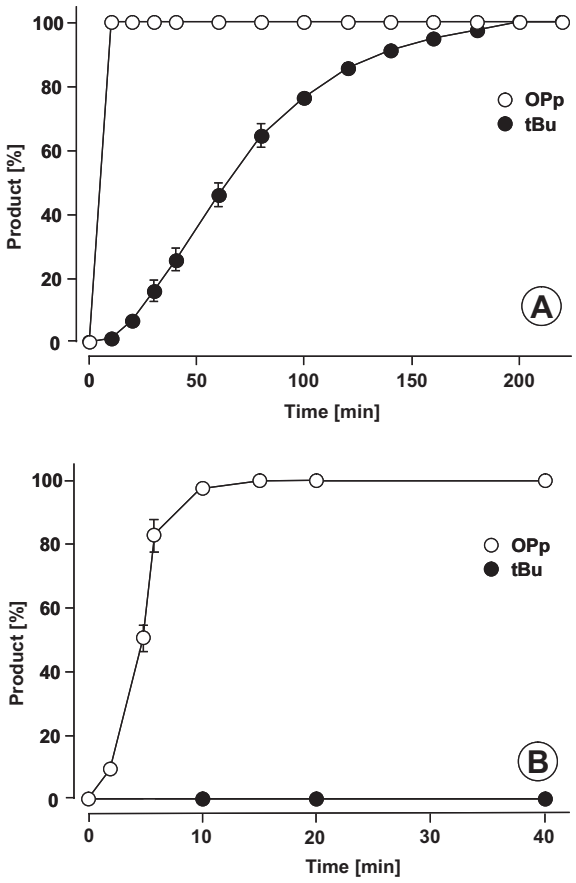


Figure 1. Cleavage kinetics of ○ = the OPp groups on DOTA-tris(OPp ester)-Phe-NH_2 and ● = the tBu groups on DOTA-tris(tBu ester)-Phe-NH_2 by: (A) TFA/H_2O/TIS = 95:2.5:2.5 and (B) TFA/TIS/DCM = 2:2:96.

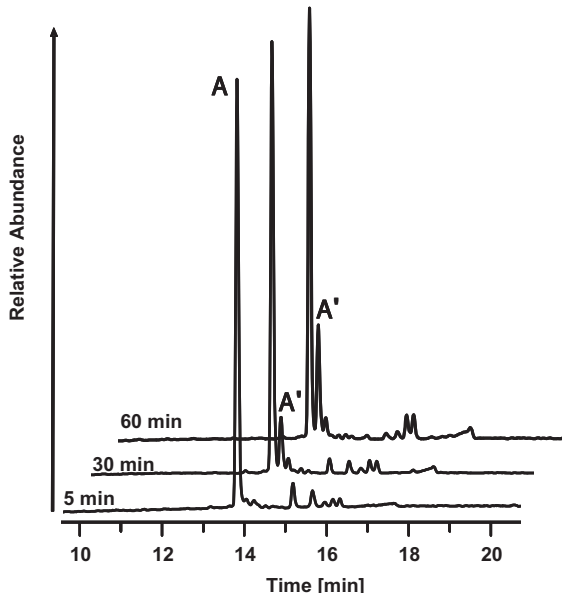


Figure 2. HPLC-MS analysis of crude DOTA–TATE (A) synthesized with the prochelator DOTA-tris(OPp ester) after the pre-activation times indicated in the figure. Increased pre-activation times of the prochelator lead to a decreased coupling efficiency and thus an increased amount of TATE (A′). Elution gradient: 0–100% MeCN + 0.1% TFA in 30 min.

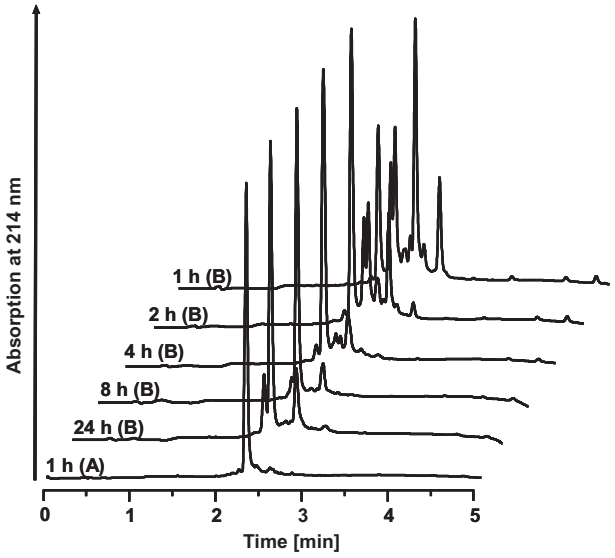


Figure 3. The RP-HPLC-chromatograms of crude DOTA-TATE synthesized with prochelator: (A) DOTA-tris(OPp ester) after a cleavage time of 1 h; (B) DOTA-tris(tBu ester) after a deprotection time of 1 h, 2 h, 4 h, 8 h, and 24 h. Deprotection conditions: TFA/H_2O/TIS = 95:2.5:2.5. Elution gradient: 0–100% MeCN + 0.1% TFA in 5 min.

by reacting 1-methyl-1-phenylethanol with trichloroacetonitrile in the presence of a catalytic amount of sodium hydride. The esterification of bromoacetic acid with 1-methyl-1-phenylethyl-trichloroacetimidate did not require any acidic catalyst. In contrast, in all the examples using allyl and benzyl-type trichloroacetimidates,[25] a catalyst (BF_3–Et_2O or methanesulfonic acid) was required for esterification.[20]

The syntheses of DOTA-tris(tBu ester) (**6**) and DOTA-tris (OPp ester) (**7**) started from commercially available cyclen (Scheme 2). The synthesis of the prochelators followed the procedure described earlier.[7a,b] 1,4,7,10-Tetraazacyclododecane-1-yl-acetic acid allyl ester (**3**) was synthesized as described.[26] N-alkylation of **3** with 4 equivalents either of bromoacetic acid 1-tert-butyl ester or of bromoacetic acid 1-methyl-1-phenylethyl ester (**2**) under basic conditions in acetonitrile yielded **4** (93%) or **5** (81%). Subsequent deprotection of the allyl protecting group by the palladium catalyst tetrakis(triphenylphosphine)palladium in the presence of morpholine followed by chromatographic purification yielded the prochelator **6** in 77% and **7** in 35%, which were further characterized by using ESI-MS and NMR (Table 1). To obtain high yields the product requires rapid chromatographic purification to avoid fragmentation of the protective groups. Furthermore, high temperatures during the reaction of the product 1,4,7,10-Tetraazacyclododecane-1-yl-acetic acid allyl ester (**3**) lead to intramolecular ring formation.

For the determination of the cleavage kinetics of the OPp and tBu protecting groups the model DOTA-tris(OPp ester)-Phe-NH_2 (**8**) and DOTA-tris(tBu ester)-Phe-NH_2 (**9**) were synthesized and treated with two different concentrations of TFA. For the synthesis, the prochelator was preactivated with HATU and DIPEA in DMF and added to H-Phe-NH_2. After a coupling time of 30 min at room temperature, the DMF was evaporated and the crude mixture was purified by HPLC (yield 64% to 83%) and characterized by ESI-MS and NMR (Table 1). Under the standard TFA peptide cleavage conditions, the deprotection of the OPp group from DOTA-tris (OPp ester)-Phe-NH_2 was found to be efficient, but very slow: at least 3 h were required to completely remove the tBu groups (Fig. 1A). The kinetic studies showed that the acid-labile protecting group OPp is readily cleaved with 2% TFA/DCM (Fig. 1B).

To apply the new prochelator in comparison to DOTA-tris(tBu ester), Tyr^3-octreotate (TATE), Tyr^3-octreotide (TOC), and the

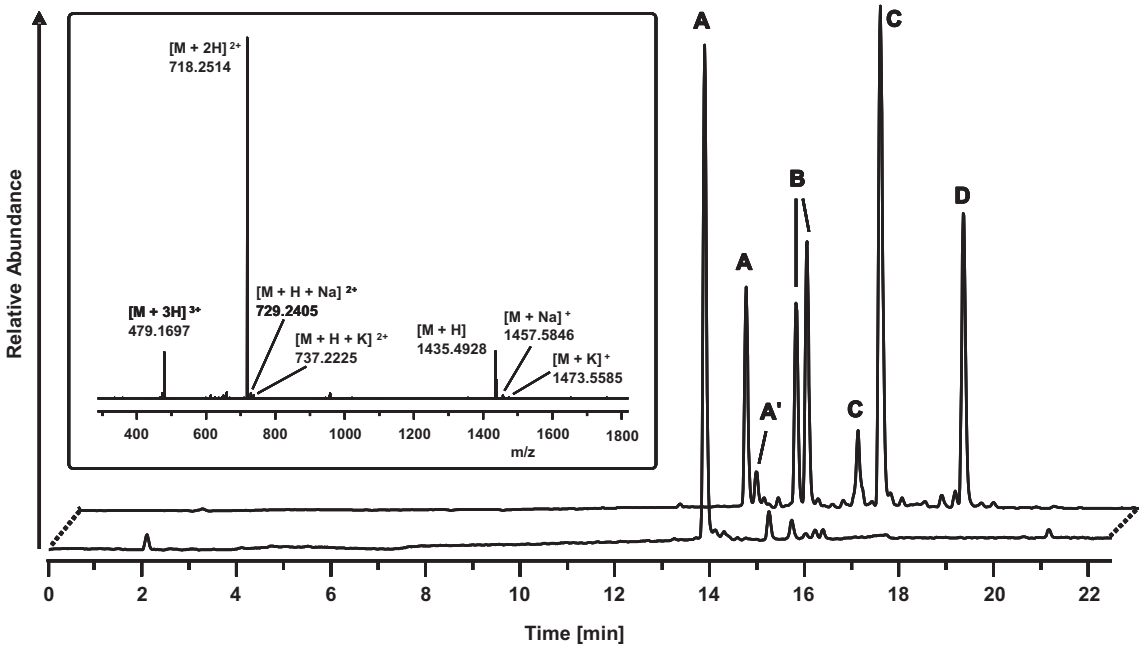


Figure 4. Mass spectrometric analysis of the deprotection reaction with TFA/H_2O/TIS (95:2.5:2.5) after a reaction time of 1 h to yield DOTA-TATE (**A**) synthesized with either DOTA-tris(OPp ester) (lower trace) or DOTA-tris(tBu ester) (upper trace). The deprotection of the peptide obtained with DOTA-tris(tBu ester) shows a significant amount of TATE (A′) and partially protected compounds with one (B), two (C), and three (D) tBu protection groups. Prolonged reaction of this compounds leads to the formation of side products as demonstrated in Fig. 3. Elution gradient: 0–100% MeCN + 0.1% TFA in 30 min. Insert: Mass spectrum of the product peak (**A**) (13.9 min) revealing the homogeneity of the product peak.

Table 2
Results of the synthesis of DOTA–TATE

Product	Chelator used	Purity (%)	Yield (%)
TATE[a]	No Chelator	>99.5	36
DOTA–TATE[b]	**6**	>99.5	22
DOTA–TATE[a]	**7**	>99.5	30

[a] Deprotection time = 1 h.
[b] Deprotection time = 3.5 h.

peptide Val-Lys-Asp-Gly-Tyr-Ile-amide were synthesized on a solid support. The syntheses were performed according to published procedures[27] using the Fmoc-strategy on either a Fmoc-Thr-(tBu)-Wang resin, an *O-t*-butylthreoninol 2-chlorotrityl resin, or a Rink amide MBHA resin, respectively. Batches of the resins were coupled with DOTA-tris(tBu ester) or DOTA-tris(OPp ester). As these two prochelators are bulky, their coupling efficiency has to be carefully controlled. Using the tetramethyluronium-type coupling reagent HATU in DMF quantitative conjugation yields could be achieved. However, the timing of the addition of the highly reactive active esters of the two prochelators DOTA-tris(tBu ester) and DOTA-tris(OPp ester) generated with HATU in situ to the resin was found to be crucial. A pre-activation time of 5 min was found to be sufficient prior to the addition to the resin, as the efficiency of reactive esters generated gradually decreased (Fig. 2).

The crude product of DOTA-TATE (A) synthesized with either DOTA-tris(OPp ester) (**7**) or DOTA-tris(tBu ester) (**6**) with the standard TFA SPPS deprotection conditions was characterized by analytical reversed-phase HPLC (Fig. 3) and LC-ESI-MS (Fig. 4).[28] Analysis of RP-HPLC-chromatogram and ESI-MS shows the advantage of the new prochelator DOTA-tris(OPp ester) in comparison to DOTA-tris(tBu ester) in the synthesis of DOTA-TATE (**A**). Using DOTA-tris(OPp ester) the product could be obtained with high homogeneity already after one hour (Fig. 4). The results (Fig. 4) show the extremely slow cleavage of the tBu-ester groups. As a consequence, peptides containing a tBu ester protected DOTA moiety require very long deprotection times for completeness. This results in the formation of side products as shown in Fig. 3. The numbers given in Table 2 were determined after a cleavage time of 3.5 h. This prolonged reaction time is required to achieve an acceptable product to side product ratio of DOTA–TATE when synthesized with tBu ester protecting groups. As building blocks containing protecting groups that can be cleaved under mild conditions have to be carefully checked for the robustness of their application, the stability of this new prochelator was studied. In neat DMF, no sign of degradation was seen at room temperature, when incubated at 37 °C, 98.8% and at 60 °C 89.8% of the intact precursor remained after 24 h.

In conclusion, both prochelators DOTA-tris(tBu ester) and DOTA-tris(OPp ester) can be quantitatively conjugated to the peptides tested. However, the ease of deprotection of the prochelator DOTA-tris(OPp ester) under standard deprotection conditions revealed that this compound is of advantage for the synthesis of DOTA–peptide conjugates.

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28. All DOTA–peptide conjugates were characterized on the basis of their analytical data (ESI-MS). The data are as follows: ESI-MS (m/z) for DOTA–TATE $C_{65}H_{90}N_{14}O_{19}S_2$ [M], $[M+H]^+$ (calculated): 1435.6003 (1435.6026). ESI-MS (m/z) for DOTA-TOC $C_{65}H_{92}N_{14}O_{18}S_2$ [M], $[M+H]^+$ (calculated): 1421.6241 (1421.6233). ESI-MS (m/z) for DOTA-VKDGYI-NH_2 $C_{48}H_{78}N_{12}O_{16}$ [M], $[M+H]^+$ (calculated): 1079.5732 (1079.5731).