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

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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.

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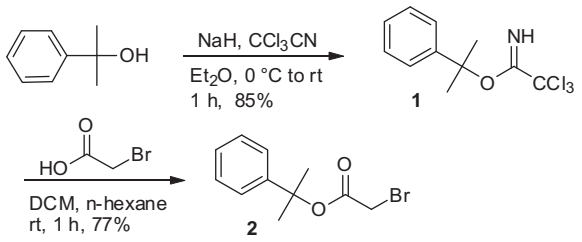
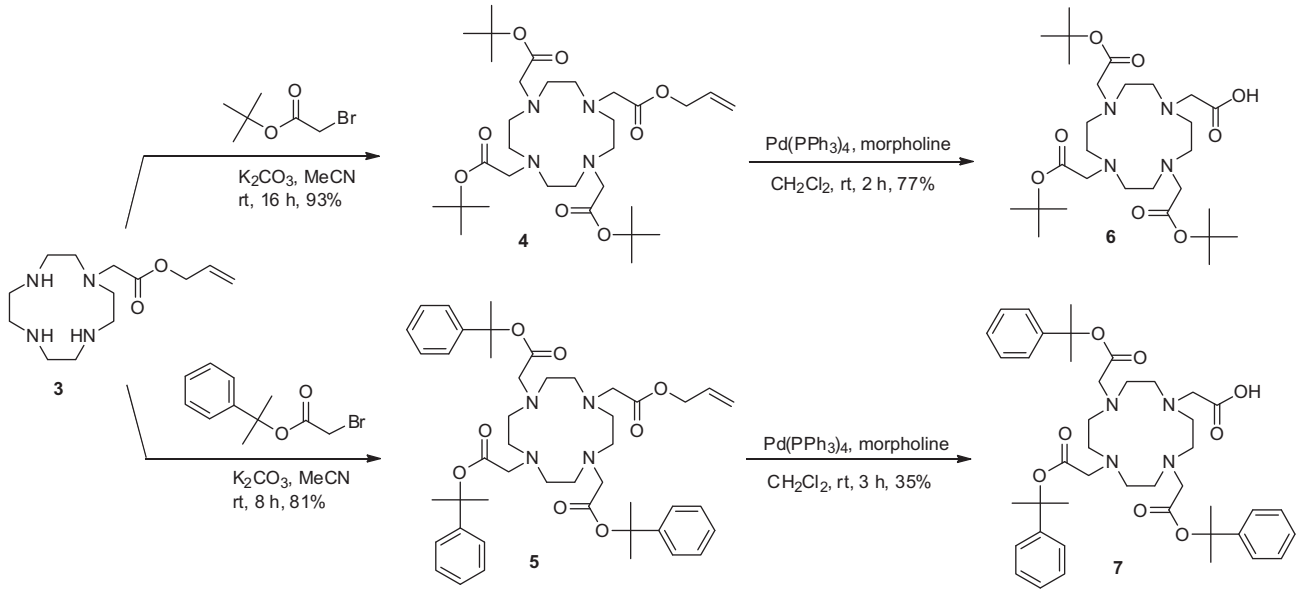


Scheme 1. Synthesis of bromoacetic acid 1-methyl-1-phenylethyl ester.

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.