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Synthesis of Two Novel Mixed Bifunctional Chelating Agents: DO2AP(tBu)$_4$ and DO3AP(tBu)$_4$

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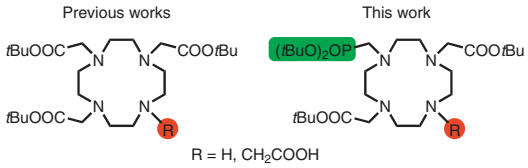

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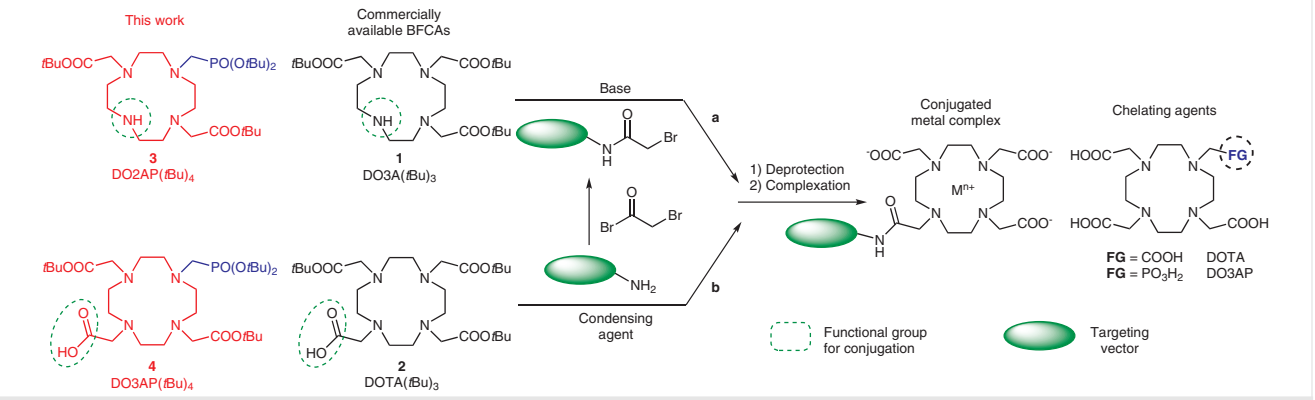
Abstract A convenient synthesis of two novel macrocyclic bifunctional chelating agents (BFCAs), formally derived from the well-known ligands DO3A and DOTA by selective replacement of one carboxymethyl side arm with a phosphonomethyl residue, is reported.

Key words bifunctional chelating agents, DO3A, DOTA, phosphonic group, protecting group

Bifunctional chelating agents (BFCAs) are small molecules containing a metal chelating unit and a reactive functional group.[1] They find widespread applications in chemistry, biochemistry and medicine because they can be easily conjugated to biomolecules, such as peptides[2] or antibodies,[3] and then loaded with metals or radiometals to obtain diagnostic, therapeutic or theranostic agents, which are extensively employed nowadays in research, preclinical and clinical studies.[4]

Many popular and widely applied BFCAs are based on polyaminopolycarboxylic acids due to their versatility and ability to coordinate a broad variety of metal ions, generating stable complexes.[5] High thermodynamic and kinetic stabilities of metal complexes are usually achieved with macrocyclic chelating agents owing to the preorganized structure of these ligands. The typical example of this class is DOTA (Scheme 1), which has been exploited extensively for the preparation of metal complexes to be used in vivo.[6,7]

The macrocyclic derivatives DO3A tri-*tert*-butyl ester **1**[8] and DOTA(tBu)$_3$ **2**[9] (Scheme 1), both based on the 1,4,7,10-tetrazacyclododecane ('cyclen'), are among the most used BFCAs. Both are commercially available and can be easily prepared through relatively simple procedures.[8–10] Compound **1** has been used in solid-phase synthesis by derivatization of the peptide on the resin with bromoacetyl bromide (Scheme 1, route a), which is then reacted with the BFCA.[11] Compound **2** can be directly conjugated to molecules of interest bearing an amino group (e.g. a protected peptide) by means of a suitable coupling agent.[12] Moreover,



Scheme 1 Macrocyclic BFCAs: conjugation strategies, commercially available examples, and synthesized compounds

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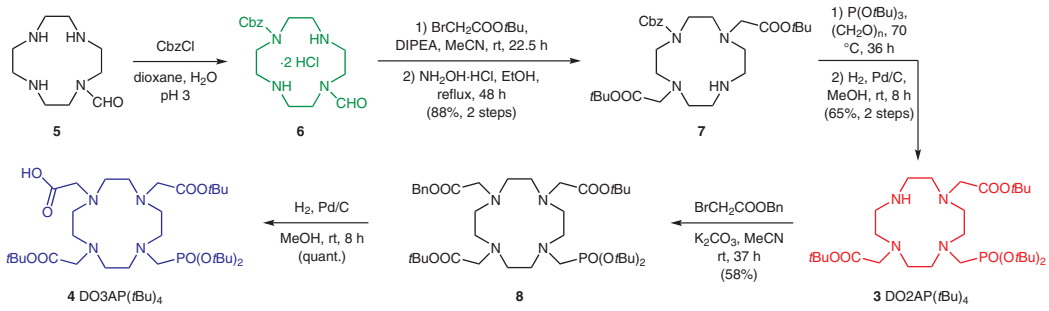

Scheme 2 Synthesis of monophosphonic BFCAs DO2AP(tBu)$_4$ **3** and DO3AP(tBu)$_4$ **4**

compound **2** has been used extensively in the solid-phase synthesis of DOTA-functionalized peptides (Scheme 1, route b).[13]

We have been interested and involved for several years in the design and synthesis of novel BFCAs, ranging from linear DTPA to mesocyclic AAZTA[14] and to macrocyclic DOTA or HP-DO3A chelating agents.[14,15]

Here, we report the synthesis of the novel bifunctional chelating agents **3** and **4** (Scheme 2), differing from **1** and **2** by the replacement of a carboxylic moiety with a phosphonic group. The phosphonic group is commonly used as a (bio)isostere of carboxylic acid in medicinal chemistry.[16] At physiological pH values the phosphonate tetrahedral deprotonated form (PO_3^{2-}) offers multiple coordination modes, leading to stabilities of the metal complexes comparable to those of the carboxylic counterpart or even higher.[6] DO3AP (Scheme 1) forms complexes with lanthanide (Ln^{3+}) with thermodynamic stabilities higher than those of the corresponding Ln^{3+}-DOTA chelates.[17] Moreover, the residual anionic charges left after complexation of the dianionic phosphonate group provide a beneficial effect on the solvation of the corresponding chelate, due to the establishment of an extended hydrogen-bond network. This is extremely important in Gd^{3+} complexes, which are currently used as MRI contrast agents, and is clearly demonstrated in the comparison of Gd^{3+}-DO3AP, with the carboxylic congener Gd^{3+}-DOTA.[18] The former, by virtue of the higher hydration of the paramagnetic complex, shows an improved relaxivity (i.e. contrast efficiency), pointing out the importance of this specific isosteric substitution of metal-coordinating groups.

N-Formyl-cyclen **5** was selected as the starting material for the synthesis of the new monophosphonic derivatives **3** and **4**, since it can be easily prepared in two steps from cyclen in almost quantitative yield.[19] Compound **5** was regioselectively protected at the 7-position by reaction with benzylchloroformate in 1,4-dioxane/water at pH 3.[20] The regioselective protection relies on a modification of the protocol originally proposed by Kovacs and Sherry,[21] and exploits a pH-controlled procedure for the selective functionalization of polyazamacrocycles. The orthogonally heterodiprotected derivative **6** was the key intermediate for the synthesis of both BFCAs. Alkylation of the secondary amines with *tert*-butyl bromoacetate in the presence of *N*,*N*-diisopropylethylamine (DIPEA) as the base, followed by selective removal of the formyl protective group by refluxing in ethanol in the presence of hydroxylamine hydrochloride, led to the advanced synthon **7** in 88% yield. The key phosphonic moiety was introduced by applying a modified Kabachnik–Fields reaction;[22] namely, treating **7** with tri-*tert*-butyl phosphite and paraformaldehyde at 70 °C for 36 hours.[23] The resulting protected mixed phosphonic-carboxylic ester was not isolated due to observed instability. The Cbz protective group was directly and smoothly removed by hydrogenolysis (Pd/C in methanol), to obtain the monophosphonic DO2AP tetra-*tert*-butyl ester **3** in 65% yield over two steps.[24]

The second BFCA bears an additional side arm containing the free carboxylic acid acting as the remote reactive functional group. The side arm is introduced by alkylation of the secondary amine **3** with benzyl bromoacetate in acetonitrile at room temperature in the presence of K_2CO_3 as the base. Selective removal of the benzyl ester by catalytic hydrogenolysis completes the preparation of the desired bifunctional chelating agent **4** (DO3AP(tBu)$_4$, Scheme 2).[25]

The preparation of both BFCAs was performed on a gram scale, testifying to the robustness of this concise synthetic approach and allowing suitable amounts to be obtained for further applications.[26,27]

In summary, a concise entry to two macrocyclic bifunctional chelating agents, formally derived from the widely used DOTA/DO3A ligands, has been described in this work. The synthesis of the two new BFCAs starts from a readily available mono-protected cyclen and involves simple steps, requiring common and inexpensive reagents and exploiting a recently reported and very effective protection method.[20] Future development of this work will deal with the conjugation of the two new BFCAs to molecular vectors and evaluation of the corresponding conjugated metal complexes for diagnostic or therapeutic purposes.[26]

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1707893.

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(24) **Di-*tert*-butyl 4-[[Di(*tert*-butoxy)phosphoryl]methyl]-1,4,7,10-tetrazacyclododecane-1,7-diacetate (3):** A mixture of **7** (13.9 g, 26 mmol, 1 equiv), tri-*tert*-butyl phosphite (7.6 g, 28.6 mmol, 1.1 equiv) and paraformaldehyde (0.9 g, 30 mmol, 1.15 equiv) was stirred and heated at 70 °C. After 16 h, additional tri-*tert*-butyl phosphite (1 g, 3.8 mmol, 0.15 equiv) and paraformaldehyde (0.1 g, 3.3 mmol, 0.13 equiv) were added and the mixture was heated for a further 20 h. The mixture was evaporated under vacuum at 80 °C to eliminate volatile by-products. The oily residue was dissolved in methanol (145 mL), 5% Pd/C (2.6 g) was added and the mixture was stirred under hydrogen atmosphere at room temperature for 8 h. The mixture was filtered through a Millipore FT 0.45 µm filter, evaporated, and the residue was purified by flash chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH = 9:1) to give compound **3** (10.3 g, 65% over two steps) as a brown oil. IR (neat): 2977, 2927, 1730, 1368, 1223, 1152, 973, 917, 849, 692 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 333 K): δ = 3.02–2.91 (m, 5 H), 2.71–2.38 (m, 18 H), 1.09 (s, 18 H), 1.04 (s, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 333 K): δ = 169.8 (C), 82.2 (d, *J* = 9.8 Hz, C), 80.6 (C), 57.5 (CH_2), 51.2 (CH_2), 50.9 (CH_2), 48.9 (CH_2), 46.8 (CH_2), 45.3 (d, *J* = 145 Hz, CH_2), 29.9 (d, *J* = 3.7 Hz, CH_3), 27.5 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$, 333 K): δ = 18.1 (s) ppm. MS (ESI^+): *m*/*z* (%) = 607.24 (100) $[M + H]^+$, 551.20 (21), 495.16 (35), 439.15 (34), 383.18 (20). HRMS (ESI^+): *m*/*z* calcd for $C_{29}H_{59}N_4O_7P$: 606.41214; found: 607.41886 (100) $[M + H]^+$, 629.40094 (46) $[M + Na]^+$.

(25) **7-[[Di(*t*-butoxy)phosphoryl]methyl]-1,4,7,10-tetrazacyclododecane-1,4,10-triacetic acid 4,10-di-*t*-butyl ester (4):** A mixture of compound **8** (1.38 g, 1.83 mmol), 10% Pd/C (0.28 g) and MeOH (10 mL) was stirred under hydrogen atmosphere at room temperature for 10 h. The mixture was filtered through Celite and evaporated to give compound **4** (1.20 g, 99%) as a pale-yellow oil. IR (neat): 3381, 2975, 1725, 1650, 1367, 1151, 1070, 976, 567, 433 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, 313 K): δ = 3.79–2.82 (m, 24 H), 1.52–1.48 (m, 36 H) ppm. ^{13}C NMR (75 MHz, CD_3OD, 298 K): δ = 170.9 (C), 169.1 (C), 82.7 (C), 79.9 (d, *J* = 8.1 Hz, C), 57.4 (CH_2), 54.2 (CH_2), 52.4 (d, *J* = 140.6 Hz, CH_2), 52.3 (CH_2), 51.8 (6 CH_2), 30.1 (d, *J* = 3.8 Hz, CH_3), 27.6 (CH_3) ppm. ^{31}P NMR (121 MHz, CD_3OD, 313 K): δ = 5.85 (s) ppm. MS (ESI^+): *m*/*z* (%) = 703.2 (36) $[M + K]^+$, 687.4 (100) $[M + Na]^+$, 630.9 (12). HRMS (ESI^+): *m*/*z* calcd for $C_{31}H_{61}N_4O_9P$: 664.41762; found: 665.42279 (100) $[M + H]^+$, 687.40452 (55) $[M + Na]^+$.

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