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A Convenient Synthesis of Novel Bifunctional Prochelators for Coupling to Bioactive Peptides for Radiometal Labelling

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Abstract—New DOTA-based bifunctional prochelators, e.g., 1-(1-carboxy-3-carbo*tert*butoxypropyl)-4,7,10-(carbo*tert*butoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAGA(tBu)$_4$), (**6d**) for a broad application in the modification of biomolecules with metal ions were prepared. The five-step synthesis of **6d** has an overall yield of about 20%. The coupling of **6d** to a bioactive peptide on solid-phase was exemplified with use of a CCK-B (cholecystokinin) analogue.

DOTA (1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10 tetraazacyclododecane) and its derivatives constitute an important class of chelators for biomedical applications as they accomodate very stably a variety of di- and trivalent metal ions. Gd(DOTA)[1] is an important MRI (Magnetic Resonance Imaging) contrast agent and as bifunctional versions DOTA is used in radioimmunotherapy.[2]

An emerging area is the use of chelator conjugated bioactive peptides for labeling with radiometals in different fields of diagnostic and therapeutic nuclear oncology.[3] For their convenient and high yield synthesis prochelators (compounds which become chelators upon deprotection) are necessary which are compatible with the solid and solution phase peptide synthethic procedures.

We describe herein the synthetic steps towards bifunctional orthogonally protected prochelators for coupling to the N-terminus of bioactive peptides or other useful amino functions in biomedical applications. The DOTA-derived chelator should provide four intact carboxylic acid functions besides the macrocyclic tetraazacyclododecane ring for a stable and efficient binding of metal ions and a function for biomolecule coupling.

The strategy included the synthesis of an orthogonally protected bromo-alkyl-dicarboxylic acid diester for the monoalkylation of cyclen (1,4,7,10-tetraazacyclododecane). High yield monoalkylation of cyclen was demonstrated before.[3–5] The synthesis of **6** ($n=1,2$) is a five-step procedure starting from the commercially available aspartic (**1b**) or glutamic acid-4-(5) benzyl ester (**1d**) (Scheme 1) using a method analogeous to Holmberg[6] followed by *tert*-butylation using *tert*-butyl-trichloroacetimidate (TBTA) as reagent.[7,8]

The monoalkylation of cyclen, the crucial step, showed strongly differing yields depending on the bromo-alkyl-dicarboxylic acid diester (**3a–d**) used (Table 1). In earlier studies our strategy was to use metals as protecting groups.[9] In that work we attempted to introduce succinic acid-di-*tert*-butylester (**3c**) and found yields below 5% for the monoalkylation with the elimination product fumaric acid-di-*tert*-butylester as the main product. Interestingly the corresponding diphenylmethyl diester (**3a**) gave high monoalkylation yields and negligible elimination. With the homologous 2-bromoglutaric-1-*tert*butyl-5-benzylester (**3d**), no elimination product was found, obviously because no conjugated π-system could be formed. The remaining nitrogens were alkylated by use of three equivalents of bromoacetic acid-*tert*-butyl ester in $CHCl_3/K_2CO_3$. Deprotection of the benzyl ester group was performed with H_2/Pd/C (Scheme 2).

The overall yield of 1-(1-carboxy-3-carbo*tert*butoxypropyl)-4,7,10-(carbo*tert*butoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAGA(*t*Bu)$_4$) (**6d**) over five-steps was about 20%[10] and of 1-(1-carboxy-2-carbo*tert*butoxyethyl)-4,7,10-(carbo*tert*butoxymethyl)-1,4,7,10-tetraaza-cyclododecane (DOTASA(*t*Bu)$_4$) (**6b**) only about 2%. The convenient use of **6d** is exemplified by its coupling to the CCK-B analogue D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (**7**) attached to Rink-amide resin using

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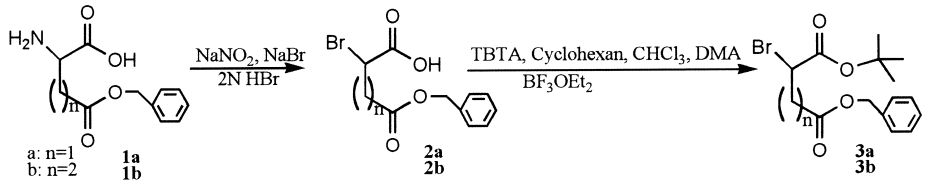
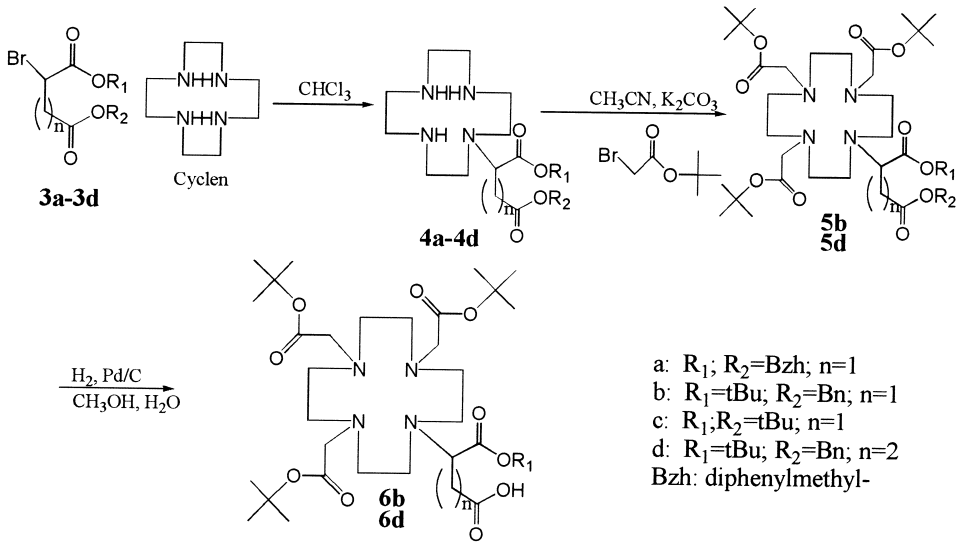
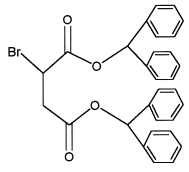
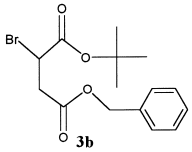
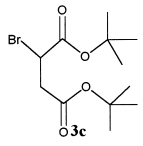
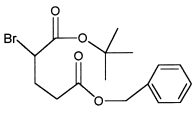


Scheme 1. Synthesis of α-bromosuccinic acid-1-*tert*butylester-4-benzyl ester (**3b**) and α-bromoglutaric acid-1-*tert*butyl ester-5-benzyl ester (**3d**).



Scheme 2. Synthesis of DOTASA(tBu)$_4$ (**6b**) and DOTAGA(tBu)$_4$ (**6d**).

Table 1. Mono-alkylation yields of cyclen with different bromo-dicarboxylic acid diester

Alkyl agent	Yield
 **3a**	79%
 **3b**	21%
 **3c**	< 5%
 **3d**	83%

HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluoro-phosphate) as coupling reagent. After deprotection, (18h, rt, TFA:phenol:thioanisol: water 85:5:5:5) DOTAGA-**7** was obtained in high yield[11] and showed superior properties in comparison to other radiolabelled CCK-B analogues.

We conclude that the new prochelator **6d** has widespread utility in the field of metallo-radiopeptides, other radiolabeled biomolecules and for the synthesis of Gd^{3+} based MRI contrast agents.[9] DOTAGA will allow to label with different radiometals for both diagnostic (^{111}In, $^{67/68}$Ga) and internal radiotherapeutic applications (^{90}Y, ^{177}Lu).

Acknowledgements

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References and Notes

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5. Kruper, W. J.; Rudolf, P. R.; Langhoff, C. A. *J. Org. Chem.* **1993**, *58*, 3869.
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7. Armstrong, A.; Brackenridge, I.; Jackson, R. R. W.; Kirk, J. M. *Tetrahedron Lett.* **1988**, *29*, 2483.
8. Typical procedure for the reaction of **1** to **2**: To a solution of 6 g (25.9 mmol) L-glutamic acid-5-benzylester (**1d**) and 9.1 g (88.5 mmol) sodium bromide in 45 mL aqueous 1N hydrobromic acid (46 mmol) cooled to 0 °C was added portionwise 3.175 g (46 mmol) sodium nitrite. After stirring for 2 h at 0 °C 2.25 mL, concd sulfuric acid was added followed by diethylether. The water phase was extracted three times with diethylether. The combined organic phases were extracted four times with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by chromatography (silica gel 60; hexane:EtOAc 3:1 to 2:1) and obtained as a yellow oil in a yield of 4.8 g (63%). ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$): 10.1 (1H, COOH); 7.3 (m, 5H, Ar); 5.15 (s, 2 H, CH_2-Ph); 4.4 (dd, $^3J = 5.7$, 1H, CHBr); 2.6 (t, $^3J = 6.8$, 2H, CH_2-COOBzl); 2.5–2.2 (m, 2H, CHBr-CH_2-CH_2); ^{13}C NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 174.5 (COOH); 171.9 (COOBzl); 135.5 (CH_2C(Ar)); 128.6, 128.4, 128.3 (C(Ar)); 66.8 (O-CH_2-Ar); 44.1 (HCBr); 31.4 (HCBr-CH_2); 29.4 (CH_2COOBzl; EI–MS *m/z* (intensity): 302, 300 (12, $[M]^+$); 91 (100, $[Bzl]^+$). Reaction of **2** to **3**: To a solution of 4.8 g (15.9 mmol) **2d** in 20 mL $CHCl_3$ a solution of 6.26 mL (34.1 mmol) TBTA (*tert*-butyltrichloroacetimidate) in 20 mL cyclohexane was added dropwise over 20 min. During the addition a white precipitate formed, which was dissolved by the addition of 3.5 mL of dimethylacetamide (DMA) followed by 320 µl boron trifluoride ethyl etherate as catalyst. The reaction mixture was stirred for 3 days at rt. The mixture was concentrated and the remaining DMA phase was extracted three times with 30 mL hexane. The hexane phase was evaporated and the residue chromatographed over silica gel 60 (Hexane:EtOAc 20:1 later 9:1) affording 3.5 g (61%) of a colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$): 7.4 (m, 5H, Ar); 5.15 (s, 2H, CH_2-Ph); 4.35 (dd, 1H, CHBr); 2.6 (td, 2H, CH_2-COOBzl); 2.5–2.2 (m, 2H, CHBr-CH_2-CH_2); 1.5 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 172.4 (COOBzl); 168.7 (COOtBu); 136.1 (CH_2C(Ar)); 129.0, 128.8, 128.7 (C(Ar)); 83.1 (C(CH_3)$_3$); 67.0 (O-CH_2-Ar); 47.1 (HCBr); 32.0 (HCBr-CH_2); 30.1 (CH_2 COOBzl); 28.1 (C(CH_3)$_3$); EI–MS *m/z* (intensity): 302, 300 (18, $[M\text{-}C_4H_9]^+$); 57 (100, $[C_4H_9]^+$).
9. André, J. P.; Tóth, É.; Fischer, H.; Seelig, A.; Mäcke, H. R.; Merbach, A. E. *Chem. Eur. J.* **1999**, *5*, 2977.
10. General procedure of the monoalkylation of cyclen: A solution of 870 mg (2.44 mmol) α-bromoglutaric acid-1-*tert*-butylester-5-benzylester (**3d**) in $CHCl_3$ was added dropwise over a period of 1 h to a solution of 885 mg (4.9 mmol) cyclen in 4 mL $CHCl_3$. The mixture was stirred for 2 days at room temperature and concentrated to a brown oil. The crude product was purified by column chromatography (silica gel 60; ethanol/NH_3 95:5), yield 920 mg (83%) of a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$): 7.35 (m, 5H, Ar); 5.1 (s, 2H, CH_2-Ph); 3.25 (dd, 1H, CHBr); 2.9–2.5 (m, 18H, NCH_2, CH_2 COOBzl); 2.2–1.85 (m, 2H, CHN-CH_2-CH_2); 1.45 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 173.1 (COOBzl); 171.5 (COOtBu); 135.8 (CH_2C(Ar)); 128.5, 128.3, 128.2 (C(Ar)); 81.4 (C(CH_3)$_3$); 66.2 (O-CH_2-Ar); 63.5 (HCNCH_2); 48.8, 48.0, 46.5, 45.6 (NCH_2CH_2N); 30.6 (CH_2COOBzl); 28.2 (C(CH_3)$_3$); 24.5 (HCN-CH_2); EI–MS m/z: (intensity): 449.3 (56, $[M+H]^+$); 245.8 (100, $[M + CH_3CN + 2H]^{++}$). Synthesis of 1-(1-carbobenzyloxy-3-carbo*tert*butoxypropyl)-4,7,10-(carbo*tert*butoxymethyl)-1,4,7,10-tetraazacyclododecane (**5d**): A suspension of 1.1 g (5.6 mmol) bromoacetic acid-*tert*-butylester, 1.02 g (2.27 mmol) 1-(1-carbobenzyloxy-3-carbo*tert*butoxypropyl)-1,4,7,10-tetraazacyclododecane (**4d**), and 2.63 g (19.1 mmol) of dry potassium carbonate in 10 mL dry acetonitrile was stirred for 18 h at rt and was filtrated afterwards over Celite and evaporated to dryness. The crude product was purified by column chromatographie (silica gel 60; CH_2Cl_2:EtOH 9:1 followed by EtOH:NH_3 95:5) yield 1.3 g (73%) of a yellow oil (**5d**). ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$): 7.35 (m, 5H, Ar); 5.1 (s, 2H, CH_2-Ph); 3.6–1.9 (m, 27H, CHN, NCH_2, CH_2COOBzl, CHN-CH_2-CH_2, $CH_2COOC(CH_3)_3$); 1.45 (s, 36H, C(CH_3)$_3$); ^{13}C NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 174.6 (COOBzl); 172.9, 172.8, 172.6 (COOtBu); 135.6 (CH_2C(Ar)); 128.5, 128.3, 128.2 (C(Ar)); 82.4, 81.8, 81.8 (C(CH_3)$_3$); 66.3 (O-CH_2-Ar); 55.8, 55.7, 55.4, 52.6, 52.3, 50.3, 48.5, 48.1, 47.1, 44.3 (13C, HCN CH_2, NCH_2CH_2N, CH_2COOtBu, CH_2COOBzl); (NCHCH_2 CH_2); 28.0, 28.0, 27.8, 27.6 (C(CH_3)$_3$); EI–MS *m/z* (intensity): 813.6 (22, $[M + Na]^+$); 791.6 (38, $[M + H]^+$); 396.5 (100, $[M + 2H]^{++}$). Synthesis of DOTAGA(tBu)$_4$ (**6d**): 600 mg (0.76 mmol) **5d** was dissolved in methanol, and 30 mg Pd/C suspended in 1 mL H_2O was added. The mixture was hydrogenated for 2 days, filtrated over Celite and evaporated to dryness. The crude product was chromatographed on silica gel 60 (EtOH:NH_3 95:5) to obtain 470 mg (84.6%) of a white solid (**6d**). ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$): 6.5 (br, 1H, COOH); 3.6–2.0 (m, 27H, CHN, NCH_2, CH_2COOH, CHN-CH_2-CH_2, $CH_2COOC(CH_3)_3$); 1.45 (s, 36H, C(CH_3)$_3$); ^{13}C NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 175.2 (COOH); 175.0, 172.9, 172.8, 172.6 (COOtBu); 82.4, 82.1, 81.9 (C(CH_3)$_3$); 55.8, 60.1 (NCHCOOtBu); 55.9, 55.8, 55.6, 52.7, 52.6, 52.5, 48.6, 48.5, 48.2, 47.1, 44.3 (12C, NCH_2CH_2N, CH_2COOtBu, CH_2CO OH); 33.4 ($NCHCH_2CH_2$); 27.9, 27.8 (C(CH_3)$_3$); EI–MS *m/z* (intensity): 723.5 (27, $[M + Na]^+$); 701.5 (68, $[M + H]^+$); 351.4 (100, $[M + 2H]^{++}$).
11. Data of DOTAGA-CCK-B analogue (DOTAGA-**7**): Yield: 12.7 mg, HPLC purity >95%, (+) EI–MS *m/z* (intensity): 1486.1 (48, $[M + H]^+$); 743.7 (60, $[M + 2H]^{++}$); (−) EI–MS *m/z* (intensity): 1484.0 (28, $[M + H]^-$); 741.8 (90, $[M + 2H]^-$)