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A facile synthesis of α-amino-DOTA as a versatile molecular imaging probe

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Abstract—An amino group has been introduced into one ligand of DOTA that can couple to peptidyl carboxylates by coupling α-brominated glycine to DO3A-^{t}Bu (1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid, *tert*-butylester)). α-Amino-DOTA was coupled to the carboxylate backbone terminus of a peptide to demonstrate the utility for derivatization.
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Macrocyclic metal chelates using DOTA are often administered to in vivo patients and animal models to create or enhance contrast in biomedical molecular imaging studies. Examples include paramagnetic Gd^{3+}-DOTA for magnetic resonance imaging (MRI),[1] radioactive ^{111}In-DOTA for SPECT imaging,[2] and radioactive ^{64}Cu-DOTA for PET imaging.[3] More recently, metal-DOTA chelates have been conjugated to peptides to affect the pharmacokinetics of the metal-DOTA imaging agent within in vivo systems, which can be used to gain additional information about biological processes at the molecular level. A variety of peptidyl ligands[4] have been employed for these molecular imaging studies, including peptides that bind to specific cell surface receptors,[5] peptides that penetrate cell membranes,[6] peptides that nonspecifically interact with the extracellular matrix,[7] and very large peptide homopolymers that drastically alter renal clearance rates.[8]

To synthesize these peptidyl imaging agents, the carboxylates of DOTA have been conjugated to the amines of peptides, including the N-terminus, the side chain of lysine, and unnatural amino acid derivatives such as p-NH_2-phenylalanine.[9] Other DOTA derivatives have been devised for conjugation to peptide amino groups, such as succinimide DOTA derivatives[10] and isothiocyanate DOTA derivatives.[11] However, coupling DOTA only to peptide amines can limit synthesis methodologies.[12] Also, modification of a peptide N-terminus or side chain amino group can compromise the utility of the peptide for molecular imaging applications, because peptide–biomolecule interactions critically depend on the (native) peptide amino acid sequence.

To address these limitations, a facile methodology is required for conjugating DOTA to peptide carboxylates, especially the C-terminus of a peptide. We have developed new α-amino-DOTA derivatives to conjugate directly to the C-terminus of a peptide and used two different glycine templates (BOC and CBZ protected) to accommodate future applications via orthogonal protection strategies. BOC protection is more advantageous in solution phase synthesis, and CBZ protection has advantages in orthogonal synthesis and more complex structures. We have also chelated lanthanide ions with these DOTA derivatives. Finally, we have confirmed that α-amino-DOTA-Gd^{3+} has good T_1 MR relaxivity to demonstrate that these DOTA derivatives may serve as molecular imaging agents.

Two different synthetic pathways for the α-bromination of glycine templates are shown in Scheme 1. The compound **2**[13] was obtained by following previously reported methods by use of *N*-bromosuccinimide and filtered UV radiation (254 nm). The bromination showed high efficiency in purity as determined by NMR.[14] The compounds **5–7**[15] were synthesized according to a previously reported synthetic pathway[16] and acquired with overall yield of 70% as determined by weight.

The compound **9**[17] was synthesized from **2** and **8** and purified with a silica column. To continue further

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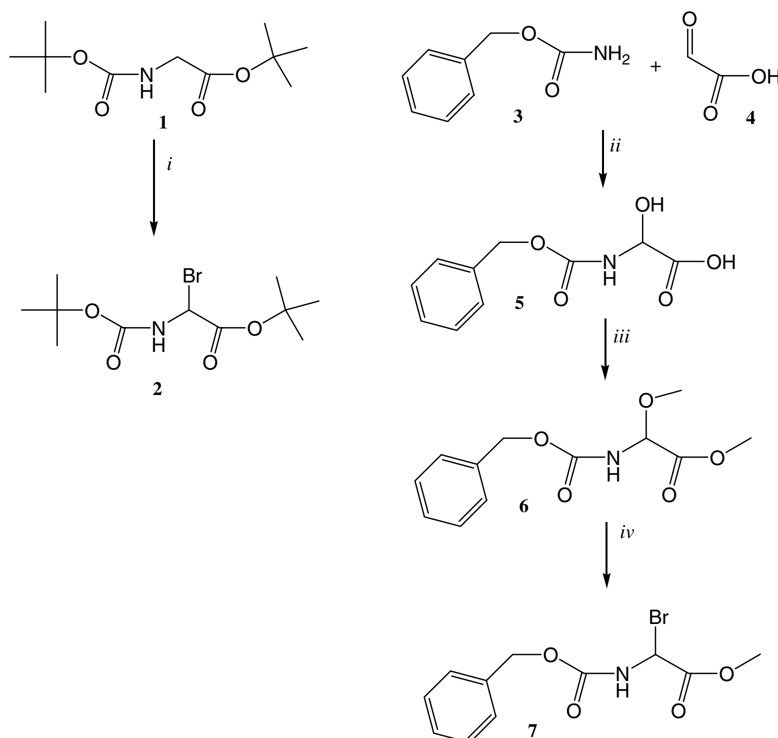


Scheme 1. Synthesis of α-brominated glycine templates. Reagents and conditions: (i) *N*-bromosuccinimide, UV (254 nm, filtered), CCl_4, 25 °C, 1.5 h, 97%; (ii) diethyl ether, rt, 6 days, 96%; (iii) MeOH, H_2SO_4, rt, 2 days, 95%; (iv) PBr_3, CCl_4, rt, 7 days, 75%.

experiments, the protecting groups of **9** were completely removed with a cleavage cocktail (95% TFA/2.5% water/2.5% thioanisole) for 30 min. The solution was concentrated in vacuo and precipitated with ice-cooled diethyl ether and purified with an Amberlite column, yielding **11**[18] as a white solid (Scheme 2).

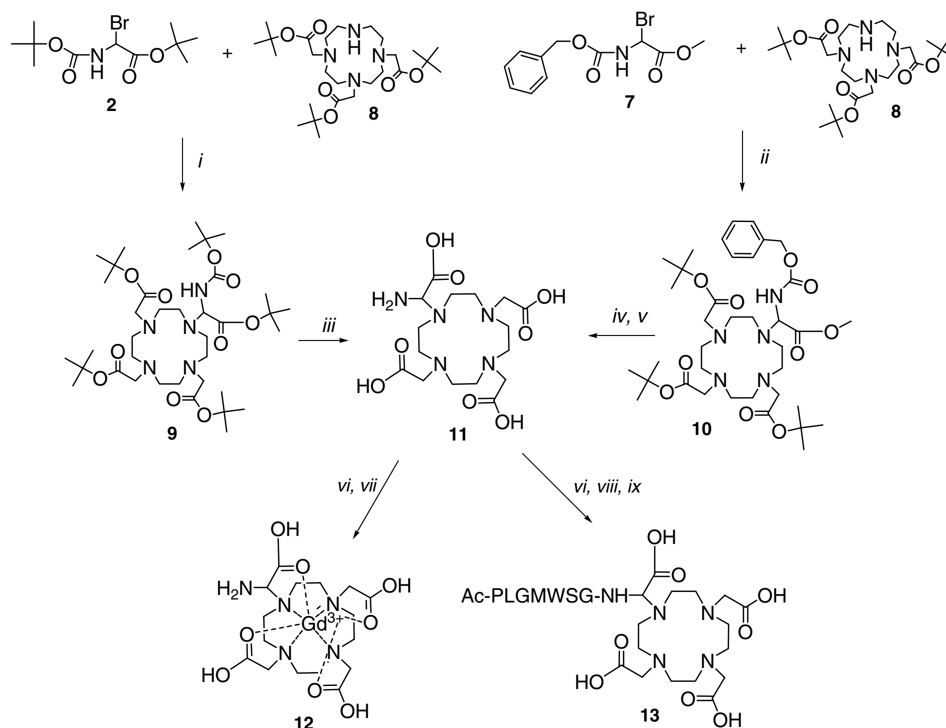
To prepare **10**,[19] the compound **7** was coupled to **8** using exhaustive alkylation conditions and purified with a silica column. To remove the CBZ group by hydrogenolysis, **10** was dissolved in 10 mL of absolute ethanol, and 1,4-hexadiene (0.94 mL, 10 mmol) and 10% Pd/C (0.55 g, 10 mmol) were added to the solution. The remaining methyl and *tert*-butyl protecting groups were removed by hydrolysis using a 1 N-NaOH solution. The aqueous solution was lyophilized after purification with an Amberlite column, yielding a slightly yellowish solid of **11**.[18]

Due to the bulky protecting groups of **2** and **7**, the reaction yields were expected to be lower than conventional exhaustive alkylation reactions,[12] but the reactions showed sufficiently high yields to be used in subsequent synthesis steps without further purification. This demonstrates the facile nature of the synthesis methodology. The α-bromo glycine templates were produced as racemates so that (*R*)- and (*S*)-types were formed at approximately a 1 to 1 ratio, as evidenced by doublets at 5.20 and 5.46 ppm in the NMR spectrum. After the α-bromo glycine templates were coupled to **8**, the diastereomers in **9** and **10** were also determined to be in the same ratio as the α-bromo glycine templates.

To demonstrate the coupling of **11** with the C-terminus of a peptide, a peptide was synthesized with a Wang resin (Fluka, 0.75 mmol/g), HBTU and HOBt coupling agents, and standard Fmoc chemistry protocols using an Applied Biosystems 433 A peptide synthesizer.[20] The synthesized peptide sequence was selected to be a substrate for the MMP-2 enzyme.[21] The MMP-2 targeting peptide sequence was Ac-Pro-Leu-Gly-Met-Trp-Ser-Gly (Ac-PLG-MWSG) and the interaction of the peptide-α-amino-DOTA complex with MMP-2 enzyme will be the focus of a future report. The peptide was cleaved from the resin with a 95% TFA/2.5% water/2.5% thioanisole cocktail for 30 min.[22] The peptide was purified by crystallization in dichloromethane/diethyl ether and an Amberlite column (yield 90% by weight) and characterized with a MALDI mass spectrometer (*m*/*z*: 811.90 (calcd 811.91) $[M+Na]^+$).

To show the versatile application of coupling **11** to the C-terminus of a peptide to synthesize peptidyl-DOTA structure **13**, the synthesized peptide (120 mg, 0.15 mmol) was dissolved in NMP (5 mL) with 63 mg of HBTU and 26 mg of HOBt and stirred for 40 min to activate the carboxyl group of the peptide. Compound **11** (80 mg, 0.15 mmol) and TEA (110 μL, 0.75 mmol) in 1 mL of NMP was added slowly at room temperature. The reaction mixture was stirred for 1 h. After removal of half of the solvents under reduced pressure, the product **13** was precipitated by addition of diethyl ether (100 mL). The obtained crude product was purified with an Amberlite column, yielding the product as a white solid (yield 71% by weight) and characterized with a MALDI-MASS spectrometer (*m*/*z*: 1190.3 (calcd 1189.54) $[M+H]^+$).

To verify that **11** can serve as a molecular imaging contrast agent, the T_1 relaxivity of **12** was measured to



Scheme 2. Synthesis of α-amino-DOTA derivatives and coupling to a peptide backbone carboxylate group. Reagents and conditions: (i) K_2CO_3 (6 equiv), acetonitrile, 70 °C, 6 h, 95%; (ii) K_2CO_3 (6 equiv), acetonitrile, 70 °C, 6 h, 90%; (iii) 95%TFA/2.5%water/2.5%thioanisole, 30 min, 95%; (iv) 1,4-cyclohexadiene/10% Pd–C, EtOH; (v) 1 N-NaOH; (vi) Amberlite column; 71%; (vii) pH, 60 °C, $GdCl_3$ (150 μL of 0.4 mM solution), 48 h; (viii) Ac-PLGMWSG-OH (1 equiv), HBTU (1.1 equiv), HOBt (1.1 equiv), TEA (5 equiv), NMP, rt, 1 h; (ix) dialysis (MWCO-100); total yield 85% by weight.

assess the efficiency of the chelate to alter T_1-weighted MR image contrast. Compound **11** (25.2 mg, 0.06 mmol) was dissolved in water at pH 6 and 60 °C, and $GdCl_3$ (150 μL of 0.4 mM solution) was added to this solution and stirred for 1 h. The pH was adjusted to 8 using 1 N-NaOH and stirred for 48 h. The chelation was monitored using a standard Arsenazo III color test.[23] T_1 measurements of **12** were conducted at 18 °C and pH 7.1, with samples ranging from 5 to 50 mM. The T_1 inversion-recovery experiment was conducted with a 600 MHz NMR spectrometer. The measured relaxivity of 4.83 $mM^{-1}\,s^{-1}$ compared very favorably to the 3.83 $mM^{-1}\,s^{-1}$ relaxivity of Gd^{3+}-DOTA that is used in clinical MRI studies.[24]

This report has demonstrated the preparation and application of newly synthesized chelators for lanthanides, **11**–**13**. Also, compound **11** was synthesized from two different glycine templates to accommodate future solution phase and solid phase synthesis methods. The incorporation of an amino group facilitated the conjugation of **11** to the C-terminus of a peptide backbone with high efficiency and relatively high yield. Coupling of **11** to peptide carboxylates greatly expands peptide–DOTA synthesis strategies by complementing standard methods that couple DOTA to peptide amines. Furthermore, the number of amino groups incorporated into DOTA can be controlled from 1 to 4 by the selection of the macrocyclic starting material, such as cyclen (1,4,7,10-tetraazacyclododecane), DO1A-^{t}Bu, DO2A-^{t}Bu, or DO3A-^{t}Bu.

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13. *N*-Bromosuccinimide (1.78 g, 10 mmol) was added to a solution of **1** (2.31 g, 10 mmol) dissolved in dry carbon tetrachloride (50 mL). The mixture was irradiated with a 254 nm filtered UV lamp at 25 °C for 1.5 h (3.00 g, yield 97% by weight, with purity confirmed using NMR). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.50 (s, 9H), 5.90 (d, 1H), 6.20 (d, 1H); ^{13}C (125 MHz, DMSO-d_6) δ 171.01, 156.29, 79.51, 63.72, 28.53; MS-ESI *m*/*z*: 310.02 (calcd 309.06) $[M+H]^+$.
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15. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 3H), 5.22 (s, 2H), 6.20 and 6.45 (split, 1H), 7.55 (s, 5H); ^{13}C (125 MHz, DMSO-d_6) δ 170.71, 156.07, 137.37, 129.03, 128.57, 128.53, 73.78, 66.25; MS-ESI *m*/*z*: 304.07 (calcd 302.99) $[M+H]^+$.
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17. Compound **2** (0.62 g, 2.0 mmol) in dry acetonitrile (100 mL) was stirred with **8** (1.1 g, 2.1 mmol) in the presence of K_2CO_3 (1.66 g, 12 mmol, 6 equiv). The reaction mixture was heated to 70 °C for 6 h. After removal of the undissolved solids by filtration, the solution was concentrated in vacuo. The product was purified with silica column using ethylacetate as an eluent, yielding the product as a powdery solid (yield 95% by weight, with purity confirmed using NMR). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 45H), 2.76 (t, 16H), 3.40 (s, 6H), 5.20 and 5.46 (split, 1H); ^{13}C (125 MHz, DMSO-d_6) δ 171.45, 171.43, 171.37, 80.78, 80.65, 77.61, 57.45, 51.96, 46.93, 28.46, 28.30; MS-ESI *m*/*z*: 744.44 (calcd 743.50) $[M+H]^+$.
18. Yield 90% by weight, with purity confirmed using NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.0 (s, 2H), 2.80 (t, 16H), 3.60 (s, 6H), 5.20 and 5.46 (split, 1H); ^{13}C (125 MHz, DMSO-d_6) δ 173.44, 172.87, 78.52, 53.33, 52.14, 47.93; MS-ESI *m*/*z*: 459.31 (calcd 458.17) $[M+K]^+$.
19. Compound **7** (0.6 g, 2 mmol) was coupled to **8** (1.1 g, 2.1 mmol) using the exhaustive alkylation conditions and the product was purified with silica column using ethyl acetate as an eluent (yield 90% by weight, with purity confirmed using NMR). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 27H), 2.78 (t, 16H), 3.40 (s, 6H), 3.80 (s, 3H), 5.20 and 5.46 (split, 1H), 7.20 (s, 5H); ^{13}C (125 MHz, DMSO-d_6) δ 171.43, 170.44, 170.10, 156.53, 136.71, 129.68, 128.22, 127.64, 79.06, 78.85, 78.63, 69.72, 65.51, 64.81, 57.96, 56.20, 55.75, 55.56, 51.89, 51.37, 49.76, 49.31, 48.35, 47.72, 47.39, 45.80, 27.75; MS-ESI *m*/*z*: 736.34 (calcd 735.44) $[M+H]^+$.
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