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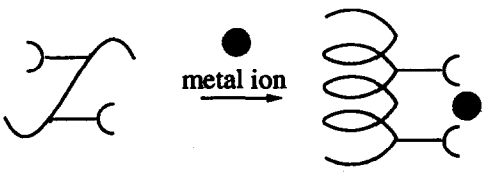
Metal Chelating Amino Acids in the Design of Peptides and Proteins. Synthesis of N$^{\alpha}$-Fmoc/Bu^{t} Protected Amino Acids Incorporating Aminodiacetic Acid Moiety.

Wieslaw M. Kazmierski *
Department of Pharmacognosy, University of Rhode Island, Kingston, RI 02881,USA

Abstract: *The synthesis of Fmoc/Bu^{t} protected amino acid chelators **14, 15, 16** and **24** is described. With respect to their Boc/Bzl derivatives, the title compounds offer synthetic advantage: peptide Ac-Ada(1)-Ala$_3$-Ada(1)-Ala$_4$-Glu-Lys-NH$_2$ was assembled by Solid PPS in 74.2% yield.*

Non-eukaryotic amino acids which exert defined conformational constraints are key to the *de novo* design of peptides and proteins.[1,2] Chelating arms, such as 2,2'-bipyridyl,[3,4] and EDTA-like side chain bearing amino acids,[5,6] are capable of metal chelation, thus forcing the backbone of otherwise flexible peptide to attain an α-helical conformation (Figure I).

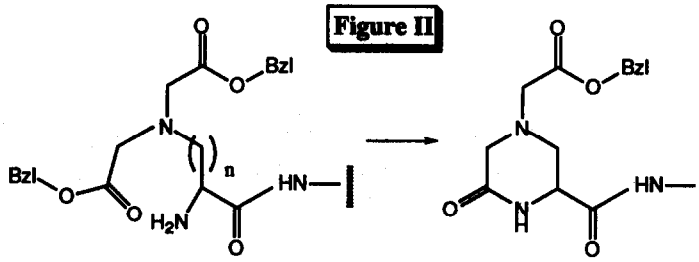
Figure I



Another distinct application of chelating amino acids is in the vector (protein) based delivery of different metals (including radionuclides) to a variety of targets,[7,8] such as DNA.[9] While synthetic routes to N$^{\alpha}$-Boc/Bzl derivatives of aminodiacetic acid were recently published,[5,6] their practical utility remains limited due to several severe side reactions observed for these derivatives.[5] Specifically, a spontaneous lactam formation for Ada(1) is observed (Figure II), capping the N-terminal amine and terminating the peptide chain.[5]

Additionally, a frequent N-terminal amine capping by TFA (trapped in the polymer matrix) during the peptide coupling cycle was reported in peptides incorporating Ada(3) and Ada(4), resulting in peptide chain termination as well.[5] To overcome these side reactions, Ruan et al.[5] had to resort to a laborious solution synthesis of the protected Ada(n) dipeptides, that were consequently used in the solid phase synthesis.

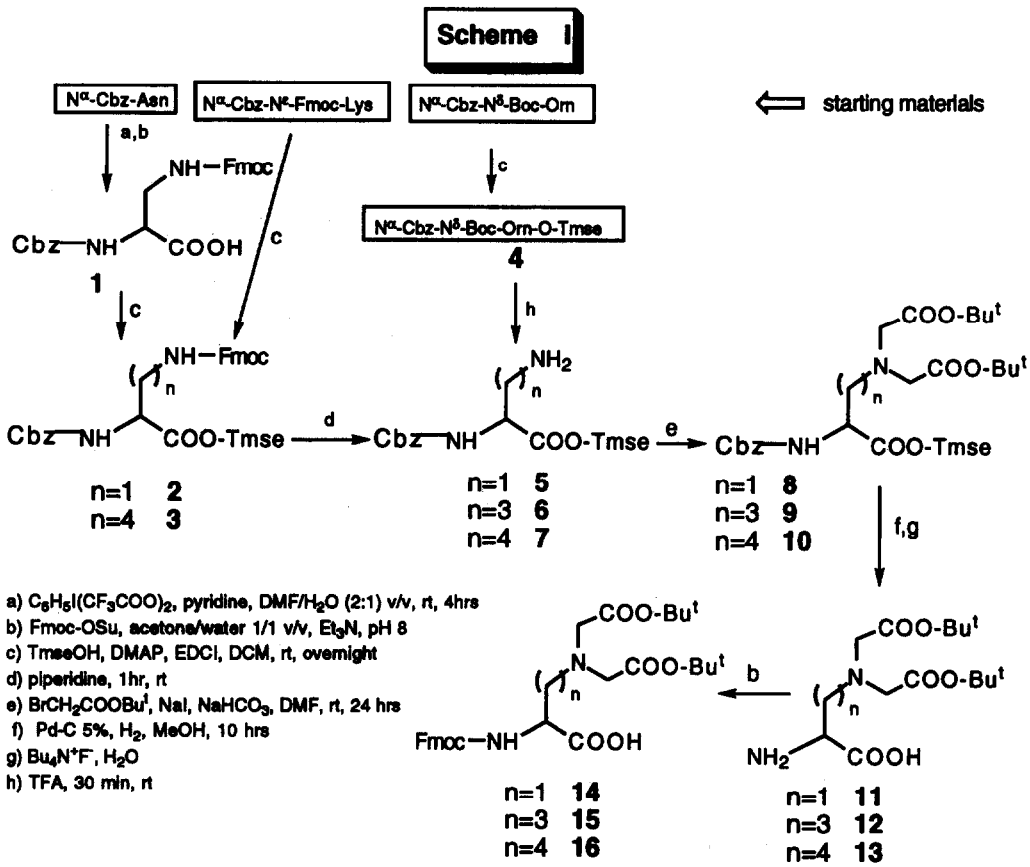
Figure II



Ada(n) n=1

We reasoned that these side reactions would best be prevented by employing the Fmoc/Bu^t chemistry. The current research interest in chelating amino acids precipitated by their possible applications,[4,5,6,7,8] prompted us to design and execute several short schemes leading to four N$^\alpha$-Fmoc/Bu^t protected amino acids **14, 15, 16,** (Scheme I) and **24** (Scheme II).

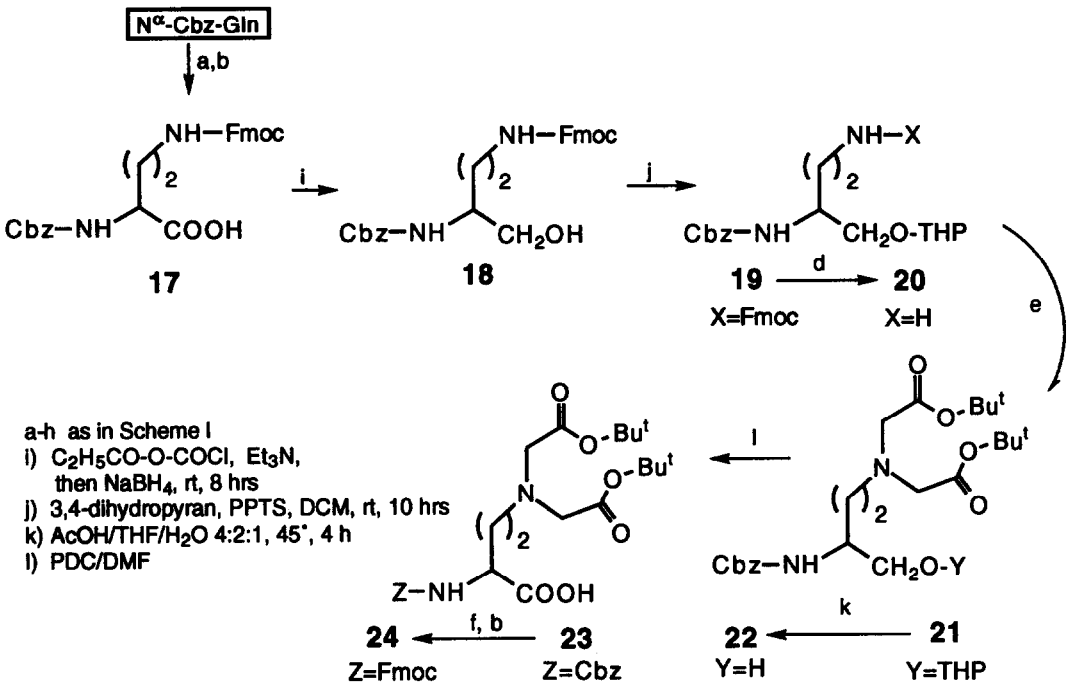
Scheme I



Briefly, protected diamino acids **5, 6 , 7** and **18** were obtained either directly from commercially available N$^\alpha$-Cbz-N$^\delta$(Boc)Orn and N$^\alpha$-Cbz-N$^\varepsilon$(Fmoc)Lys or synthesized by amide degradation from

N^{α}-Cbz-Asn and N^{α}-Cbz-Gln. The O-Tmse derivative of the N^{α}-Cbz diaminobutyric acid (n=2) spontaneously cyclized during the synthesis (not shown). Thus **17** was converted to an alcohol **18**, protected as THP ether and γ-Fmoc protecting group removed. In all the cases, key alkylation was accomplished with $BrCH_2COO\text{-}CMe_3$ in presence of NaI resulting in **8**, **9**, **10** and **21**. Final amino acids **14**, **15**, **16** were obtained by simple and high yield deprotection/protection reactions (Scheme I), while **24** in addition required prior pyridinium dichromate oxidation of **22** (Scheme II).

Scheme II



To determine a practical utility of the Fmoc/Bu^t protected aminodiacetic amino acid derivatives, the test peptide Ac-Ada(1)-Ala-Ala-Ala-Ada(1)-Ala-Ala-Ala-Ala-Glu-Lys-NH_2,[5] was completely assembled using automated Solid PPS with a cumulative yield of 74.2%. The N^{α}-Fmoc/Bu^t protection scheme employed by **14**, **15**, **16** and **24** eliminates the threat of a preliminary peptide capping by the TFA. In addition, Bu^t side chain protection makes **14** stable to spontaneous lactamization during the peptide synthesis (Figure II). Therefore, these amino acids can be conveniently used in an automated Solid PPS without the need of prior dipeptide formation.[5] We were able to synthesize gram quantities of each **14**, **15**, **16** and **24** with cumulative yields of 14-26%.[10]

Abbreviations: DMF, dimethylformamide; TEA, triethylamine; DCM, dichloromethane; PPTS, pyridinium p-toluene sulfonate; EDCI, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide; THF, tetrahydrofurane; DMAP, 4-dimethylaminopyridine; TFA, trifluoroacetic acid; Tmse,

trimethylsilylethyl; Ada(n) abbreviates aminodiacetic amino acid derivative,[6] where (n) denotes the number of methylenes between Ada moiety and α-carbon in the amino acid.

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10. Correct MS, ^{1}H, ^{13}C NMR and elemental analyses for all the compounds were obtained.

Example of synthesis of **14**. N^{α}-Cbz-N^{β}-Fmoc-Apa (**1**). 3.0 g (11.27 mM) of N^{α}-Cbz-Asn was dissolved in 50 ml of 2/1 (v/v) DMF/water, followed by addition of 6.02 g (14.0 mM) of Bis(trifluoroacetyl)iodobenzene. After 30 min, 1.5 ml of pyridine was added and reaction allowed for 4.5 hrs. The solution was concentrated, dissolved in 50 ml of 1:1, v/v acetone/water, added 4.21 g (50.0 mM) of $NaHCO_3$, ice cooled and added 4.70 g (14.0 mM) of Fmoc-OSu, and stirred for 3 hrs. The pH was maintained at ≈ 8.5 with 10% aqueous sodium carbonate. Solvents were concentrated and the residue crystallized from hot ethyl acetate/hexane (2.19 g of **1**, yield 69.3%). N^{α}-Cbz-N^{β}-Fmoc-Apa-OTmse (**2**). 1.83 g (3.97 mM) of **1** was dissolved in 15 ml of DCM, followed by 1.435 g (7.485 mM) of EDCI and 0.662g (5.60 mM) of 2-trimethylsilylethanol. After stirring for 13 hrs and silica gel separation with 1:3 v/v ethyl acetate/hexane, the yield of **2** was 1.80 g (80.84%). N^{α}-Cbz-N^{β}-(bis-Bu^{t}-acetate)-Apa-OTmse (**8**). 1.70 g of **2** was dissolved in 10 ml of piperidine and stirred for 80 min at rt and concentrated *in vacuo*, then dissolved in 10.0 ml of DMF followed by addition of 0.408 g of NaI (2.72 mM), 8.49 g (43.52 mM) of Bu^{t}-bromoacetate, 3.656 g (43.52 mM) of sodium bicarbonate, and stirred at rt for 24 hrs. The oily residue obtained after the brine workup was further purified on a silica gel column yielding 0.66 g (cumulative yield 42.8% relative to **2**) of **8**. N^{α}-Fmoc-N^{β}-(bis-Bu^{t}-acetate)-Apa (**14**): 0.53 g (0.936 mM) of **8** was dissolved in 6 ml of THF and added 13.11 ml of 1M tetrabutylammonium fluoride in THF. After stirring for 3 minutes, the solution was cooled to 0°C, diluted with 30 ml of water and concentrated in vacuo. The residue was worked up resulting in 0.41 g of a brown oil (yield 94.0%). Next, 0.35 g of that residue dissolved in 5 ml of methanol was catalytically hydrogenated in Parr apparatus using 100 mg of 5% Pd on calcium carbonate for 24 hrs. Filtration and concentration yielded 0.24 g of yellow oil N^{β}-(bis-Bu^{t}-acetate)-Apa **11**. The ice cooled solution of 0.24 g of **11** in 2.5 ml of 1:1 (v/v) acetone/water was pH≈8 adjusted with 0.252 g (3.0 mM) of sodium bicarbonate, followed by 0.337 g (1.0 mM) of FmocOSu. Reaction was carried out for 17 hrs, and solvents evaporated in vacuo. Crude **14**, was further purified on a silica gel using first 1:3 (v/v) ethyl acetate / hexane and then 1% acetic acid in 1:3 (v/v) ethyl acetate / hexane. Yield 0.33 g of oily **14**, 14.7% (cumulative). ^{1}H NMR ($CDCl_3$): 10.65 (broad s, COOH), 7.72 (2H, Fmoc, d, J=7.50), 7.61 (2H, Fmoc, d, J=7.30), 7.36 (2H, Fmoc, t, J=7.05), 7.27 (2H, Fmoc, t, J=7.40), 6.57 (1H, NH, d, J=5.61), 4.28 (m, 4H), 3.38 (pseudo q, 4H, NCH_2CO), 3.33 (dd, 1H, J=6.00, 13.80), 2.93 (dd, 1H, J=8.40, 13.50), 1.46 (s, 18H, CMe_3). ^{13}H NMR ($CDCl_3$): 173.51 (COO), 170.57 (COO), 156.27 (CON), 143.67 (Car), 141.18 (Car), 127.59 (CHar), 126.99 (CHar), 125.14 (CHar), 119.84 (CHar), 82.33 (CMe_3), 67.15 (CH_2), 56.69 (CH_2), 56.36 (CH_2), 51.98 (CH), 47.03 (CH), 27.98 (CMe_3). FAB-MS: 555 (M+1), calculated 554.306.

* Current address: *Wellcome Research Laboratories, Research Triangle Park, NC 27709, USA*

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