Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 5767–5770

Preparation of new monomers aza-β^3-aminoacids for solid-phase syntheses of aza-β^3-peptides

Olivier Busnel and Michèle Baudy-Floc'h*

Groupe 'Ciblage et Auto-Assemblages Fonctionnels', UMR CNRS 6226, Institut de Chimie, Université de Rennes I, 263 Av. du Général Leclerc, F-35042 Rennes Cedex, France

Received 4 May 2007; revised 3 June 2007; accepted 18 June 2007
Available online 22 June 2007

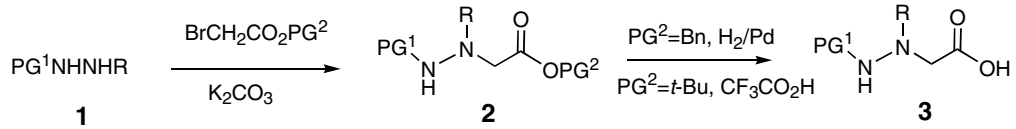
Abstract—The preparation of new N^{β}-Fmoc-protected aza-β^3-amino acids (aza-β^3-aa) with proteinogenic side chains as well as their N^{β}-Fmoc, N^{β}-Cbz or N^{β}-Boc aza-β^3-amino esters (from Pro, Asn, Asp, Glu, Gln) by successive nucleophilic substitutions will be described.

In recent years, there has been considerable interest in the design and synthesis of non-natural oligomers that form secondary structures and present enhanced metabolic stability, bioavailability and biological absorption.[1–7] In this class of peptidomimetics, peptides consisting exclusively or including aza-β^3-amino acid have emerged as a promising new class of compounds that form N–N turns and present potentially useful biological properties.[8,9]

For our ongoing projects on the synthesis of aza-β^3-peptides, we need to have ample access to the aza-β^3-amino acid building blocks with proteinogenic side chains and N^{β}-Fmoc protection. With the exceptions of aza-β^3-Lys, aza-β^3-Tyr, aza-β^3-Arg only Fmoc-protected aza-β^3-amino acids with nonfunctionalized and achiral side chains have been published.[10] We described here, in detail, the preparation of five new aza-β^3-amino acid derivatives, with the side chains of aspartic, glutamic, proline, asparagine and glutamine.

We have previously reported two methods to prepare aza-β^3-amino acids, the first one consisting in a nucleophilic substitution of benzyl or *t*-Bu bromoacetate by N^{α}-substituted-N^{β}-protected hydrazines **1** (Scheme 1).[11] Then, the required monomers **3** were obtained by deprotection of the carboxy protecting group of esters **2** in satisfactory to good yields. Nevertheless, nucleophilic substitution of bromo acetate by N-substituted Fmoc hydrazine proceeds in low yield (36–50%). Therefore, an alternative approach, in one step, to obtain aza-β^3-amino acid has been described, which relies on reductive amination of glyoxylic acid and N^{β}-Fmoc protected-N^{α}-substituted hydrazine **1** (Scheme 2).[12]

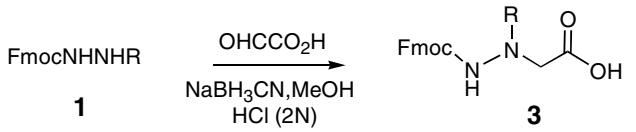
N-Substituted Fmoc hydrazines **1** were prepared according to literature procedures by reduction of Fmoc hydrazones, derived from the reaction of Fmoc carbazate with either aldehyde or ketone.[13,14] To introduce side chains such as aspartic, glutamic, proline and asparagine, the corresponding aldehydes were not available



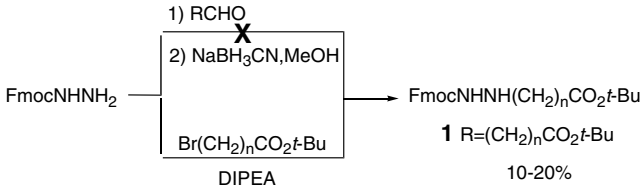
Scheme 1. Synthesis of N^{β}-protected-aza-β^3-amino acids.

Keywords: Aza-β^3-amino acid; Fmoc protecting group; Peptidomimetics/nucleophilic substitution.
* Corresponding author. Fax: +33 223236933; e-mail: Michele.Baudy-Floch@univ-rennes1.fr

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.06.082



Scheme 2. Synthesis of Fmoc-aza-β^3-amino acid.



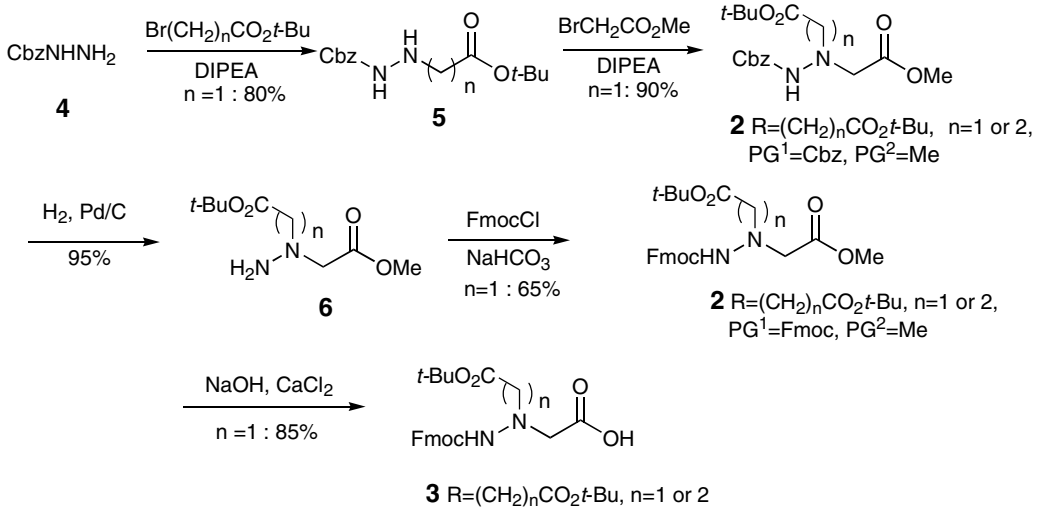
Scheme 3. Synthesis of N^{β}-substituted Fmoc hydrazines.

or did not lead to hydrazones (Scheme 3). In the literature the introduction of aspartic acid was achieved by reduction of the condensation product from glyoxylic acid and 9-fluorenylmethyl carbazate. The acylhydrazone was then reduced with 5 fold excess of cyanoborohydride and the resulting acid was then protected as *t*-butyl ester using *t*-butyl trichloroacetimidate in an overall yield of 20%.[15] Nucleophilic substitution of the corresponding bromo acetate or propionate with Fmoc carbazate would be an alternative to introduce side chains. Unfortunately, this reaction proceeds in somewhat lower yield (20%) than the corresponding Boc or Cbz carbazates (80%).[16]

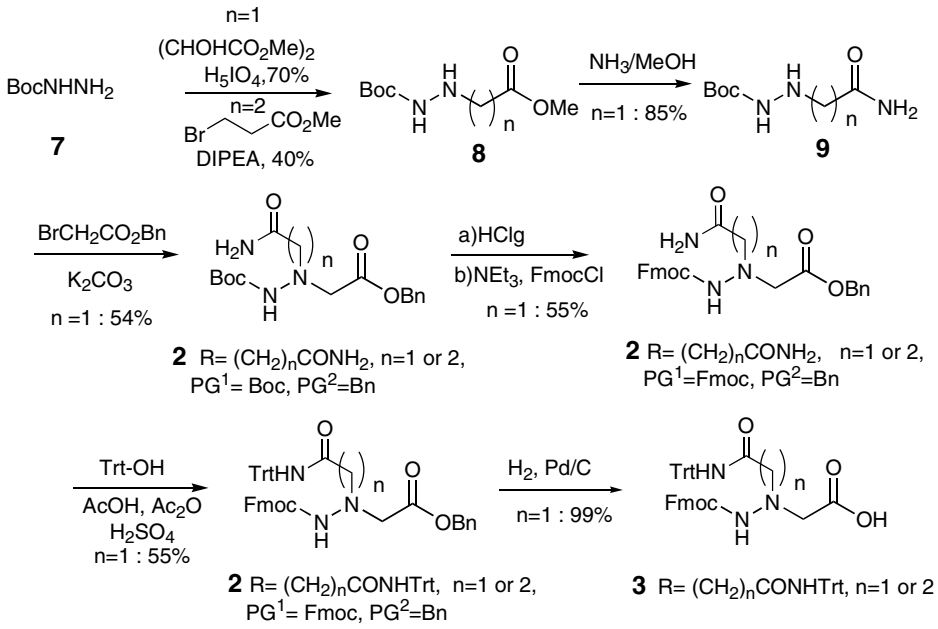
Therefore, to overcome this problem Cbz-aza-β^3-aspartic OMe **2** ($n = 1$, $R = CH_2CO_2t$-Bu, $PG^1 =$ Cbz, $PG^2 =$ Me) and Cbz-aza-β^3-glutamic OMe **2** ($n = 2$, $R = (CH_2)_2CO_2t$-Bu, $PG^1 =$ Cbz, $PG^2 =$ Me) were first prepared by two successive nucleophilic substitutions.[16] After hydrogenolysis of **2** in the presence of Pd/C to remove the benzyl group,[17] treatment of **6** with FmocCl in the presence of $NaHCO_3$ afforded *N*-Fmoc protected aza-β^3-aspartic **2** ($n = 1$, $R = CH_2CO_2t$-Bu, $PG^1 =$ Fmoc, $PG^2 =$ Me) or glutamic methyl ester **2** ($n = 2$, $R = (CH_2)_2CO_2t$-Bu, $PG^1 =$ Fmoc, $PG^2 =$ Me). Then saponification of the corresponding C-terminal methyl ester, catalyzed by $CaCl_2$ to suppress Fmoc cleavage under basic conditions,[18,19] leads to the corresponding *N*-Fmoc protected aza-β^3-aspartic **3** ($n = 1$, $R = (CH^2)_2$-CO_2t-Bu) in an overall yield of 40% or glutamic acid **3** ($n = 2$, $R = (CH_2)_2CO_2t$-Bu) in an overall yield of 20% (Scheme 4).

Asparagine or glutamine side chains could be introduced by aminolysis of ester group. First of all, Boc-aza-β^3-Gly-OMe **8** ($n = 1$) was made in 70% yield by catalytic hydrogenation of the condensation product from glyoxylic methyl ester obtained in situ by oxidation of dimethyl L-tartrate. Analogue **8** ($n = 2$) was obtained by nucleophilic substitution of methyl bromopropionate in 40% yield. Introduction of asparagine and glutamine chains was achieved by aminolysis of the ester group of **8**.[20] Then nucleophilic substitution of benzyl bromoacetate by hydrazine **9** leads, respectively, to Boc-aza-β^3-Asn-OBn **2** ($n = 1$, $R = CH_2CONH_2$, $PG^1 =$ Boc, $PG^2 =$ Bn) and Boc-aza-β^3-Gln-OBn **2** ($n = 2$, $R = CH_2CONH_2$, $PG^1 =$ Boc, $PG^2 =$ Bn). After deprotection of hydrazine function with HCl_g, the N^{β}-Fmoc derivatives **2** ($R = CH_2CONH_2$, $PG^1 =$ Fmoc, $PG^2 =$ Bn) and **2** ($R = (CH_2)_2CONH_2$, $PG^1 =$ Fmoc, $PG^2 =$ Bn) are preferably obtained by reaction of Fmoc-Cl and NEt_3 (Scheme 5).

The synthesis of peptides containing asparagine or glutamine with unprotected side-chains is known to be fraught with different types of problems. Reaction of the activated α-carboxyl group of asparagine or glutamine with β-carboxamide function leads to nitrile and succinimide derivatives. This is often the cause of incomplete or low coupling with the amino acid and of by-product formation.[21] The trityl group seems to be a good protecting group for carboxamide functions of asparagine and glutamine as tritylated carboxamide could have a cleavage rate similar to the usual *t*-butyl-type protecting group for peptides. The carboxamide



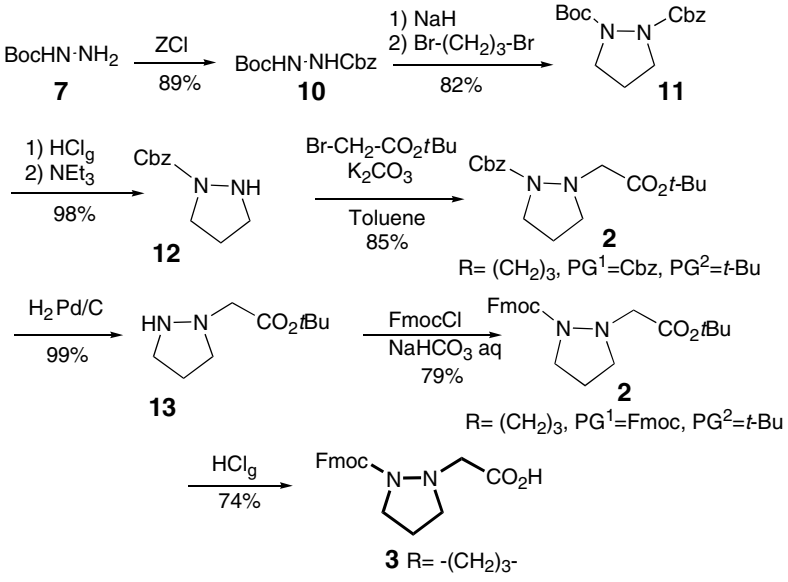
Scheme 4. Synthesis of Fmoc-aza-β^3-Asp-OH ($n = 1$) and Fmoc-aza-β^3-Glu-OH ($n = 2$).



Scheme 5. Synthesis of Fmoc-aza-β^3-Asn-OH ($n = 1$) Fmoc-aza-β^3-Gln-OH ($n = 2$).

functions of asparagine and glutamine analogues were tritylated by an acid-catalyzed reaction with triphenylmethanol and acetic anhydride in glacial acetic acid at 60 °C. Catalytic hydrogenation of ester **2** ($R = (CH_2)_2CONHTrt$, $PG^1 = Fmoc$, $PG^2 = Bn$) finally gives Fmoc-aza-β^3-Asn(Trt)-OH **3** ($n = 1$, $R = CH_2CONHTrt$) in 30% overall yield and Fmoc-aza-β^3-Gln(Trt)-OH **3** ($n = 2$, $R = (CH_2)_2CONHTrt$) in 10% overall yield (Scheme 5).

Proline is the amino acid with the highest propensity to occur in turn structures in proteins, because of the constraints of pyrrolidine ring formation. *tert*-Butoxycarbonylhydrazine **7** was converted to 1-*tert*-butoxycarbonyl-2-carboxybenzyloxyhydrazine **11** by treatment with carboxybenzyloxy chloride. Treatment of the orthogonally protected hydrazine with sodium hydride in DMF followed by reaction with 1,3-dibromopropane afforded the protected five-membered cyclic hydrazine **12**.[22] After deprotection of the Boc group using trifluoro acetic acid or HCl_g, nucleophilic substitution of *tert*-butyl bromoacetate affords Cbz-aza-β^3-Pro-O*t*Bu **2** ($R = (CH_2)_3$, $PG^1 = Cbz$, $PG^2 = t$-Bu). After reductive deprotection of the Cbz group using 10% Pd/C as a catalyst in methanol, the N^{β}-Fmoc derivative **2**, $R = (CH_2)_3$, $PG^1 = Fmoc$, $PG^2 = t$-Bu, is obtained by nucleophilic substitution of Fmoc-Cl by **13**. Finally deprotection of the Boc group using HCl_g, leads to proline analogue Fmoc-aza-β^3-Pro-OH **3**, $R = (CH_2)_3$, $PG^1 = Fmoc$, in 35% overall yield (Scheme 6).



Scheme 6. Synthesis of N^{β}-Fmoc-aza-β^3-Pro-OH.

In conclusion, we described herein the preparation of five new N^{β}-Fmoc-aza-β^3-amino acids with four heteroatomic side chains by successive nucleophilic substitutions. The solid-phase synthesis of oligomers or hybrid peptides incorporating these analogues will be published later.

References and notes

1. Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699–1720.
2. Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180.
3. Seebach, D.; Schaeffer, L.; Gessier, F.; Bindschädler, P.; Jäger, C.; Josien, D.; Kopp, S.; Lelais, G.; Mahajan, Y. R.; Micuch, P.; Sebesta, R.; Schweizer, B. W. *Helv. Chim. Acta* **2003**, *86*, 1852–1861.
4. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500.
5. Kirshenbaum, K.; Zukermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530–535.
6. Liskamp, R. M. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 633–636.
7. Quibell, M.; Turnell, W. G.; Johnson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2843–2849.
8. Cheguillaume, A.; Salaun, A.; Sinbandhit, S.; Potel, M.; Gall, P.; Baudy-Floc'h, M.; Le Grel, P. *J. Org. Chem.* **2001**, *66*, 4923–4929.
9. Baudy-Floc'h, M.; Muller, S.; Busnel, O. 23 Décembre **2004**, CNRS WO 2004/111086.
10. Busnel, O.; Bi, L.; Dali, H.; Cheguillaume, A.; Chevance, S.; Bondon, A.; Muller, S.; Baudy-Floc'h, M. *J. Org. Chem.* **2005**, *26*, 10701.
11. Cheguillaume, A.; Doubli-Bounoua, I.; Baudy-Floc'h, M.; Le Grel, P. *Synlett* **2000**, 331–334.
12. Busnel, O.; Bi, L.; Baudy-Floc'h, M. *Tetrahedron Lett.* **2005**, *46*, 7073.
13. Dutta, A. S.; Morley, J. S. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1712.
14. Ragnarsson, U. *Chem. Soc. Rev.* **2001**, *30*, 205.
15. Boeglin, D.; Lubell, W. D. *J. Comb. Chem.* **2005**, *7*, 864–878.
16. *General procedure for nucleophilic substitution of Cbz-aza-β^3-Gly-OtBu* **5** ($n = 1$). To a solution of **5** ($n = 1$) (5.12 g, 18.3 mmol) in toluene (40 mL), were added DIPEA (2.39 g, 1 equiv) and methyl-bromoacetate (5.6 g, 2 equiv). The mixture was stirred at 75 °C for 4 days, cooled at room temperature and filtrated. The filtrate was concentrated under vacuum and the resulting crude oil was purified by chromatography on silica gel (ethyl acetate/methylene chloride 7:93) to give **6** as a yellow oil (5.80 g, 90%). ^{1}H NMR ($CDCl_3$) δ ppm: 1.47 (s, 9H, CH_3), 3.72 (s, 5H, CH_2+CH_3), 3.82 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 7.10 (br, 1H, NH), 7.29–7.40 (m, 5H, CH_{ar}). ^{13}C NMR ($CDCl_3$)δ ppm: 169.92 CO_2Me, 168.80 CO_2tBu, 155.21 NHCO, 136.16 C_{ar}, 128.29 128.14 127.97 CH_{ar}, 81.61 $C(CH_3)$, 66.62 OCH_2, 57.71 NCH_2, 57.04 NCH_2, 51.61 OCH_3, 27.87 CH_3. HRMS (ESI) m/z calculated for $C_{17}H_{24}N_2O_6Na$ $[M+Na]^+$: 375.1532; found, 375.1526 (2 ppm).
17. *General procedure for catalytic hydrogenolysis of* **6** ($n = 1$). Compound **6** (3.21 g, 7.5 mmol) was dissolved in methanol (30 mL) with 10% Pd/C (250 mg) for 12 h. The palladium was removed by filtration over Celite and the resulting solution was concentrated under vacuum to afford **7** as a yellow oil (1.46 g, 95%). ^{1}H NMR ($CDCl_3$) δ ppm: 1.49 (s, 9H, CH_3), 3.70 (s, 2H, CH_2), 3.76 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 5.01 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ ppm: 170.78 CO_2Me, 169.53 CO_2tBu, 81.46 $C(CH_3)$, 60.00 NCH_2, 59.22 NCH_2, 51.51 OCH_3, 27.94 CH_3. HRMS (ESI) m/z calculated for $C_9H_{20}N_2O_4$ $[M+H]^+$: 219.1345; found, 219.1359 (6 ppm).
18. *General procedure for hydrolysis of* **2** (2.19 g, 5 mmol) in methanol (77 mL) was added a solution of NaOH (0.24 g, 1.2 equiv) in aqueous $CaCl_2$ 0.8 M (9.77 g in 33 mL of water). The mixture was stirred at room temperature for 7 h. Methanol was removed by evaporation and the resulting solution was acidified with HCl 2 N and extracted twice with methylene chloride (40 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated. The crude oil was purified by chromatography on silica gel (ethyl acetate/methylene chloride 1:1 and ether/methanol 1:1) to give **3** as a moss (1.81 g, 85%). ^{1}H NMR ($CDCl_3$) δ ppm: 1.51 (s, 9H, CH_3), 3.63 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 4.23 (t, 1H, $J = 6.3$ Hz, CH), 4.56 (d, 2H, $J = 6.3$ Hz, CH_2), 7.29–7.80 (m, 8H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ ppm: 171.80 CO_2H, 169.46 CO, 157.19 CO_{Fmoc}, 143.43, 141.32 C_{ar}, 127.89, 127.19, 125.07, 120.08 CH_{ar}, 82.99 $C(CH_3)$, 67.68 CH_{2Fmoc}, 59.25, 58.60 NCH_2, 47.05 CH_{Fmoc}, 28.10 CH_3. HRMS (ESI) m/z calculated for $C_{23}H_{25}N_2O_6Na_2$ $[M-H+2Na]^+$: 471.1508; found, 471.1509 (0 ppm).
19. Pascal, R.; Sola, R. *Tetrahedron Lett.* **1998**, *39*, 5031–5034.
20. *General procedure for aminolysis* **9** ($n = 2$). The ester **9** (10.5 g, 43 mmol) was dissolved into a solution of methanol with ammoniac 6 N (20 mL). The solution was stirred at room temperature for 3 days and concentrated under vacuum. The crude oil was precipitated in ether (10 mL) to afford **10** as a white powder (8.92 g, 85%): mp = 105 °C. ^{1}H NMR ($CDCl_3$) δ ppm: 1.48 (s, 9H, CH_3), 2.41 (t, 2H, $J = 5.9$ Hz, CH_2), 3.14 (t, 2H, $J = 5.9$ Hz, CH_2), 5.60 (br, 1H, NH), 6.24 (s, 1H, NH amide), 7.06 (s, 1H, NH amide). ^{13}C NMR ($CDCl_3$) δ ppm: 173.93, 156.07, 79.79, 47.04, 32.83, 27.32. HRMS (ESI) m/z calculated for $C_8H_{17}N_3O_3K$ $[M+K]^+$: 242.0907; found, 242.0909 (1 ppm).
21. Sieber, P.; Riniker, B. *Tetrahedron Lett.* **1991**, *32*(6), 739.
22. Toya, T.; Yamaguchi, K.; Endo, Y. *Bioorg. Med. Chem.* **2002**, *10*, 953–962.