Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 7073–7075

Synthesis of Fmoc-protected aza-β^3-amino acids via reductive amination of glyoxylic acid

Olivier Busnel, Lanrong Bi and Michèle Baudy-Floc'h*

Groupe 'Ciblage et Auto-Assemblages Fonctionnels', UMR 6510 CNRS, Institut de Chimie, Université de Rennes I, 263 Av. du Général Leclerc, F-35042 Rennes Cedex, France

Received 26 May 2005; revised 28 July 2005; accepted 29 July 2005
Available online 26 August 2005

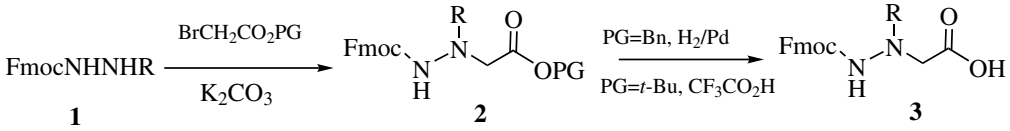
Abstract—The reductive amination of glyoxylic acid, with a protected Fmoc hydrazine, has been developed as a simple and efficient method for the preparation of Fmoc-aza-β^3-amino acid residues (aza-β^3-aa). Anchoring on resin of these residues will be described as well as the synthesis of hybrid peptide.

Solid-phase synthesis (SPS) is one of the most promising methods in automated synthesis, which allows rapid access to peptides. However, peptides are not ideal candidates for pharmaceutical development as their bioavailabilities are not high, mainly due to proteolytic degradation. Due to their enhanced metabolic stability, bioavailability and biological absorption, peptidomimetics have been the focus of research interests.[1–4]

As part of our research program aimed at obtaining new peptide analogues with potentially useful biological properties, we have been developing a synthetic strategy for aza-β^3-peptides.[5] The synthesis of the required building blocks is as important as the construction of the oligomeric structure. Aza-β^3-amino acids are a particularly attractive class of amino acid analogues since they can be incorporated in peptide syntheses leading to the formation of supported hybrid peptides or oligomers with a defined sequence.[6] The Fmoc (fluorenylmethyloxycarbonyl)/*tert*-butyl strategy is attractive to achieve these new analogues, since Fmoc groups can easily be removed under mild basic conditions.

We have previously reported a method to prepare aza-β^3-amino esters starting from N^{α}-substituted-N^{β}-protected hydrazines and esters of bromoacetate (Scheme 1).[7] Then, the required monomers **3** were obtained by deprotection of the carboxy protecting group.

For the synthesis of functionalized monomers, the most commonly used acid-labile protections, reported for solid-phase synthesis using the Fmoc strategy, are Boc (*tert*-butyloxy carbonyl), *t*-Bu or Trt (trityl) groups. Due to the acid-lability of these groups, a protecting group for the carboxylic function requiring acid deprotection could not be used. Also, the use of a benzylic protecting group was restricted due to the poor nucleophilicity of substituted Fmoc-hydrazines against benzyl bromoacetate as well as partial cleavage of the Fmoc protecting group during the catalytic hydrogenation step. Therefore, an alternative approach to obtain Fmoc-aza-β^3-amino acid was studied. This approach relied on reductive amination of glyoxylic acid and N^{β}-Fmoc protected-N^{α}-substituted hydrazine **1**, itself prepared by reductive amination of Fmoc carbazate

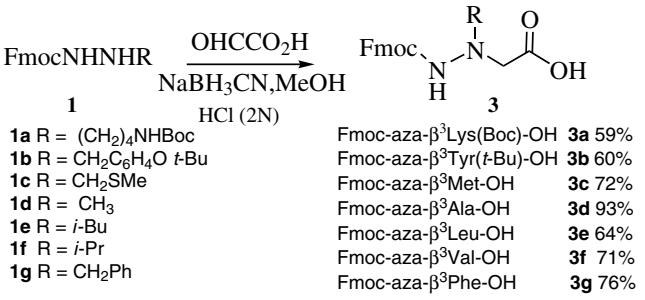


1 2 3

Scheme 1. Synthesis of unfunctionalized Fmoc-aza-β^3-amino acid.

Keywords: Aza-β^3-amino acid; Fmoc protecting group; Peptidomimetics; Loading resin; Reductive amination.
* Corresponding author. Fax: +33 2232 36933; e-mail: michele.baudy-floch@univ-rennes1.fr

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.07.160



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

with the corresponding aldehyde, as previously described[8] (Scheme 2).

This convenient reductive amination allows a simplification of the synthesis of the required Fmoc-aza-β^3-amino acids. Thus, by employing glyoxylic acid these Fmoc-aza-β^3-amino acids are accessible in one step from Fmoc substituted hydrazine **1** in good yields (59–93%).

For example, Fmoc-aza-β^3-Lys (Boc)-OH was prepared starting from the commercially available 4-aminobutylaldehyde diethyl acetal.[9] The required aldehyde obtained from Boc-protected diethyl acetal exists in equilibrium with N^{α}-Boc-pyrrolidin-2-ol. Reaction between Fmoc hydrazine and the mixture in DCM (dichloromethane) gave the hydrazone as a mixture of geometrical isomers in 90% yield. Reduction of this crude mixture with sodium cyanoborohydride gave the required *N*,*N*′-disubstituted hydrazine **1a** in 56% yield and subsequent reductive amination of glyoxylic acid led to the final product Fmoc-aza-β^3-Lys(Boc)-OH **3a** in 59% yield (Scheme 3).[10]

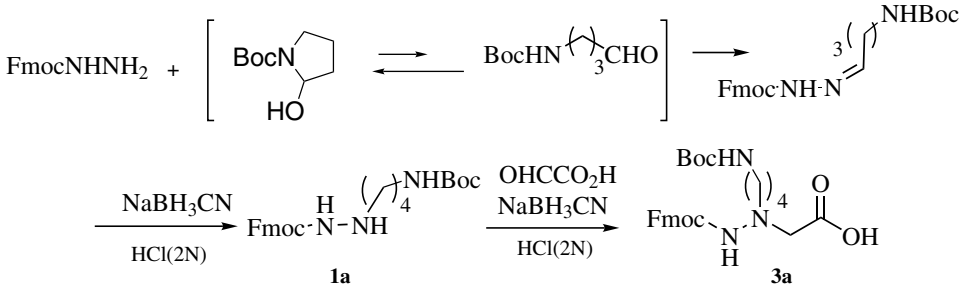
To use the Fmoc-aza-β^3-amino acids in the preparation of oligomers, attaching the prepared monomers on resin is necessary. Anchoring the first monomer Fmoc-aza-β^3-aa-OH could be achieved by esterification of the Fmoc-aza-β^3-aa-OH (5 equiv with respect to the resin loading) to the hydroxyl group of the resin with activation by DIC (diisopropylcarbodiimide) at room temperature in DMF (dimethylformamide) for 2 h. Completion of the coupling was confirmed by a TNBS (2,4,6-trinitro-benzenesulfonic acid) test and the loading was determined by Fmoc cleavage from a resin simply by measuring the UV absorption of the dibenzofulvene–piperidine adduct, which is formed after cleavage.[11,12]

RGD mimetic (H-Aza-β^3 Val-Arg-Aza-β^3Asp-Gly-Aza-β^3Phe-OH) was synthesized as an example by coupling aza β^3-amino acids or α-amino acids on the preloaded resin. Deprotection cycles, cleavage from the resin and removal of the protecting groups are identical to those for peptides.[13]

In conclusion, we have shown that Fmoc-aza-β^3-amino acids, with chemically diverse side chains, can be conveniently prepared by reductive amination of glyoxylic acid. Moreover, these monomers could easily be anchored to a resin, allowing the preparation of oligomers or mixed peptides on solid-phase support.

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. Kirshenbaum, K.; Zukermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530–535.
4. Liskamp, R. M. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 633–636.
5. Cheguillaume, A.; Salaun, A.; Sinbandhit, S.; Potel, M.; Gall, P.; Baudy-Floc'h, M.; Le Grel, P. *J. Org. Chem.* **2001**, *66*, 4923–4929.
6. Baudy-Floc'h, M.; Muller, S.; Busnel, O. 23 Décembre 2004, CNRS WO 2004/111086.
7. Cheguillaume, A.; Doubli-Bounoua, I.; Baudy-Floc'h, M.; Le Grel, P. *Synlett* **2000**, 331–334.
8. Cheguillaume, A.; Lehardy, F.; Bouget, K.; Baudy-Floc'h, M.; Le Grel, P. *J. Org. Chem.* **1999**, *64*, 2924–2927.
9. Quibell, M.; Turnell, W. G.; Johnson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2843–2849.
10. General procedure for reductive amination of **1a**: To a stirred solution of **(1a)** (3.00 g, 7.02 mmol) in DCM/MeOH (15/30 mL), glyoxylic acid monohydrate (0.78 g, 1.2 equiv) was added. $NaBH_3CN$ (0.62 g, 1.5 equiv) was added fractionally into the above mixture, which was maintained under stirring for 0.5 h, then the pH was adjusted to 1 over 10 min and finally increased to 5 with solid $NaHCO_3$. The mixture was filtered, concentrated, taken up with EtOAc (30 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated to give a crude foam, which was purified by chromatography on silica gel (ether/MeOH/AcOH 95/5/0.25, fractions washed with water to remove AcOH) to give 2.00 g (59%) of **(3a)** as a colourless oil; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, CH_3), 1.40–1.60 (m, 4H, CH_2), 2.89 (m, 2H, CH_2), 3.09 (m, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.21 (br t, $J = 5.9$ Hz, 1H, CH), 4.46 (br d, $J = 5.9$ Hz, 2H,



Scheme 3. Synthesis of Fmoc-aza-β^3-Lys(Boc)-OH.

CH_2), 4.87 (br s, 1H, NH), 7.04 (br s, 1H, NH), 7.29–7.80 (m, 8H, Ar), 9.72 (br s, 1H, CO_2H). ^{13}C NMR ($CDCl_3$) δ 173.0, 157.3, 156.1, 143.6, 141.3, 127.8, 127.1, 125.0, 120.0, 79.3, 67.0, 58.4, 56.5, 47.2, 40.2, 28.4, 27.2, 24.3. HRMS (ESI) *m/z* calcd for $C_{26}H_{33}N_3O_6Na$ $[M+Na]^+$: 506.22671, found: 506.2276 (2 ppm).

11. Fmoc-aza-β^3-Phe-OH was first loaded onto the resin. All manipulations were carried out under N_2. Wang resin (1 g, 200–400 mesh, 0.68 mmol/g) was swelled in DMF (10 mL) for 30 min. Fmoc-aza-β^3-Phe-OH (1.37 g, 4 equiv) was dissolved in dry DCM (10 mL). DIC (5 equiv) was added to the solution, which was stirred for 20 min at 0 °C under N_2. The DCM was removed by evaporation and the residue was dissolved in the minimum of DMF (3 mL) and added to the resin with 0.1 equiv DMAP (8 mg). The mixture was mixed for 2 h by N_2 bubling. A resin sample was removed to determine the level of Fmoc-aza-β^3-Phe-OH attachment by Fmoc cleavage. The dry Fmoc-aza-β^3-Phe-OH resin was weighed (3–5 mg) into each of two UV cells, 20% of piperidine in DMF (3 mL) was added and the solution was agitated for 3 min. The absorbance of the UV absorption of the dibenzofulvene–piperidine adduct gave the estimated loading of the Fmoc-aza-β^3-Phe-OH monomer, which was 0.60 mmol/g.
12. Hancock, W. S.; Battersby, J. E. *Anal. Biochem.* **1976**, *71*, 260–264.
13. The peptide was prepared from 1 g of resin loaded with the Fmoc-aza-β^3-Phe-OH (0.60 mmol/g) in a manual synthesizer using nitrogen bubbling. The resin was swelled with DMF for 15 min. After the first Fmoc cleavage with 20% piperidine in DMF during 20 min, each coupling for 2 h was done with 4 equiv (2.4 mmol) of Fmoc amino acid or Fmoc-aza-β^3 amino acid in solution 0.5 M of DIC in DMF (4.8 mL) with 4.8 mL of solution 0.5 M of HOBt in DMF. After the last Fmoc cleavage, the anchored hybrid peptide was cleaved from the resin by treatment with TFA/TIS/water (95/2.5/2.5) for 2 h. The mixture was then filtered and the resin was washed thoroughly with TFA and then with DCM. The total filtrate was concentrated in vacuo and then cold ether was added to precipitate the peptide. The precipitated peptide was collected by filtration through a sintered glass funnel. The crude product was purified by reverse phased preparative HPLC (XTerra® RP18 19×300 10 μm). The HPLC fraction was freeze-dried to give the target hybrid peptide as a white fluffy solid. (95 mg, 40%). HRMS (ESI) *m/z* calcd for $C_{26}H_{44}N_{11}O_8$ $[M+H]^+$: 638.3374, found: 638.3377 (0 ppm).