Tetrahedron Letters 54 (2013) 775–778

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fmoc-aza-β^3-Lys-OAllyl and Fmoc-aza-β^3-Asp-OAllyl for on-resin head-to-tail cyclization of aza-β^3-peptides

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ARTICLE INFO

Article history:
Received 14 September 2012
Revised 8 November 2012
Accepted 12 November 2012
Available online 2 December 2012

Keywords:
Aza-β^3-amino acids
Allyl protection
Lysine analog
Aspartique analog
Cyclic pseudopeptides

ABSTRACT

A rapid and efficient Fmoc/*t*-Bu solid-phase peptide synthesis (SPPS) of cyclic aza-β^3-peptide analogs by allyl strategy is described. Our synthetic approach includes the synthesis of two new aza-β^3-amino acids, Fmoc-aza-β^3-Lys-OAllyl and Fmoc-aza-β^3-Asp-OAllyl, then the resin attachment of the first aza-β^3-amino acid via its side chain, successful use of combination of three orthogonal removable protecting groups, stepwise solid-phase synthesis of linear peptide analog, and on-resin head-to-tail cyclization.

Solid-phase peptide synthesis (SPPS) is one of the most promising methods in automated synthesis, which allows rapid access to peptides. However, peptides are not ideal candidates for many avenues of pharmaceutical development. The main reason peptides were not used more extensively as pharmaceuticals is that 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 over the past several years.[1–7]

Cyclic aza-β^3-peptides have been shown as lead compounds for the development of new antimicrobial agents against fungi, bacteria.[8–10] Therefore total SPPS of these important products represents an important step toward complete exploitation of their therapeutic potential. Poorly chosen places for ring closure can lead to slow cyclization rates, thus facilitating side reactions such as dimerization, oligomerization, and/or epimerization of the C-terminal residue. Traditional method to prepare cyclic peptides[11–14] and therefore cyclic aza-β^3-peptides involves SPPS of the selectively protected linear precursor and cyclization in solution under high dilution conditions.[8–10,15] As an attractive alternative, cyclization could be performed while peptides still remain anchored to the resin, the on-resin method has been more efficient to prepare cyclic peptides than the solution-phase approach. The principle of on-resin cyclization is based on anchoring the peptide to the resin through a side chain and applying orthogonal protecting groups for ring closure. It possesses advantages, such as time saving, less side reactions, and easy purification. In Fmoc/*t*-Bu strategy, the allyl protecting group is used as another orthogonal protection strategy.[16–26] The allyl group is generally removed by treatment of the protected resin with tetrakis (triphenyl-phosphine) palladium (0) [$Pd(PPh_3)_4$] in different mixtures of solvents and in the presence of nucleophiles such as morpholine, *N*-methylaniline, or phenyl silane ($PhSiH_3$) the role of which is to scavenge the allyl group released during the deprotection.[27–30]

As part of our research program aimed at developing new peptide analogs with potentially useful biological properties, we have developed a synthesis strategy for aza-β^3-peptides.[31–36] Here we described our study on developing a rapid and efficient on-resin synthesis of cyclic aza-β^3-peptides fully compatible with standard Fmoc/*t*-Bu chemistry in order to establish a general synthetic route to these analogs.

Fmoc-aza-β^{3}Lys-OAllyl 4 (R′ = $(CH_2)_4NH_2$) and Fmoc-aza-β^{3}Asp-OAllyl **4** (R′ = CH_2CO_2H) may be useful building blocks for the synthesis of cyclic aza-β^3-peptides involving side-chain attachment and on-resin head-to-tail cyclization (Scheme 1).

N-substituted Fmoc hydrazine **1** (R = $(CH_2)_4NHBoc$) was prepared according to previous results by reduction of Fmoc hydrazone, derived from the reaction of Fmoc carbazate with aldehyde,[31,37–39] while N-substituted Fmoc hydrazine **1** (**R′** = CH_2CO_2t-Bu) was obtained via nucleophilic substitution of *t*-butyl bromo acetate by Fmoc carbazate.[40] Then nucleophilic substitution of allyl bromo acetate **2** with Fmoc hydrazine **1** in the presence of K_2CO_3 and NaI led respectively after four days at 80 °C to Fmoc-aza-β^3-Lys(Boc)-OAllyl **3** (R = $(CH_2)_4NHBoc$) in 60% yield or to Fmoc-aza-β^3-Asp(*t*-Bu)-OAllyl **3** (R = CH_2CO_2t-Bu) in

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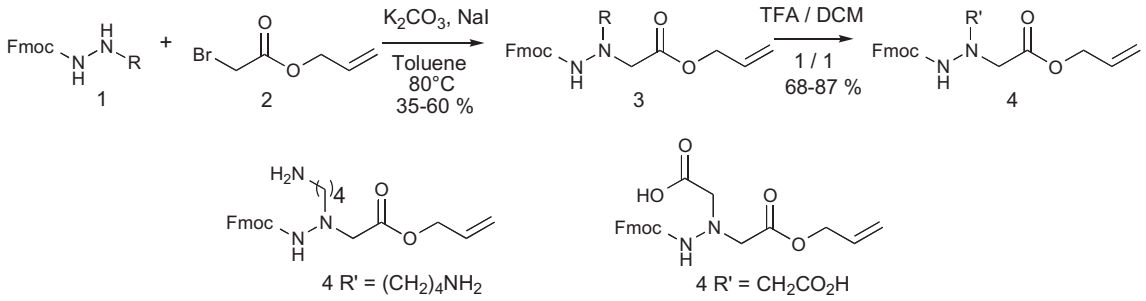
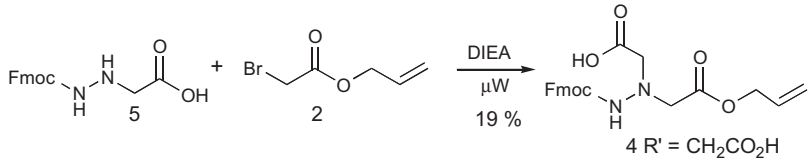
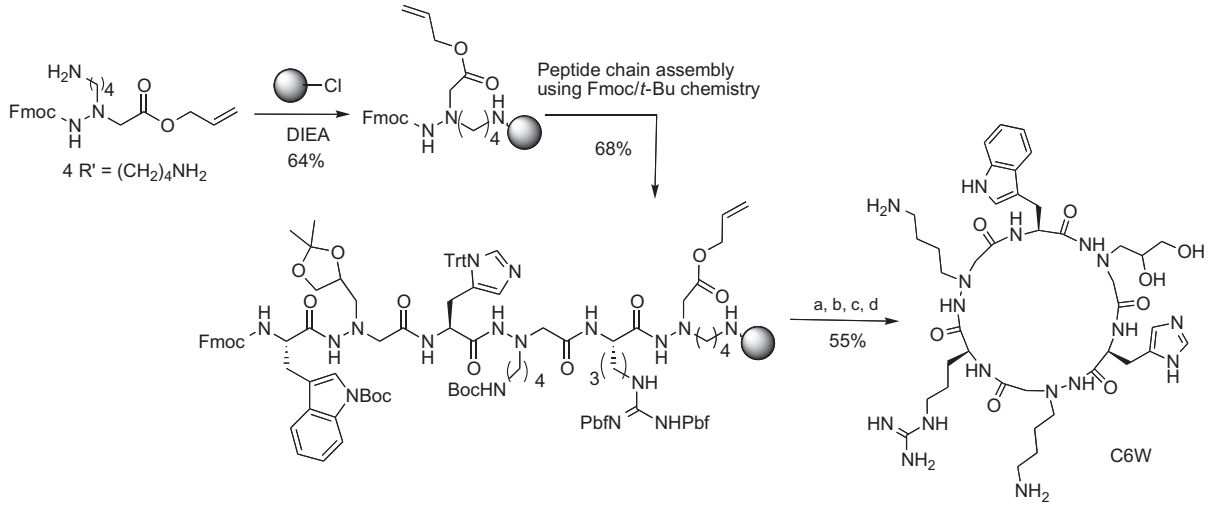
http://dx.doi.org/10.1016/j.tetlet.2012.11.113



Scheme 1. Synthesis of Fmoc-aza-β^3-Lys-OAllyl and Fmoc-aza-β^3-Asp-OAllyl.



Scheme 2. Synthesis of Fmoc-aza-β^3-Asp-OAllyl by μwaves activation.



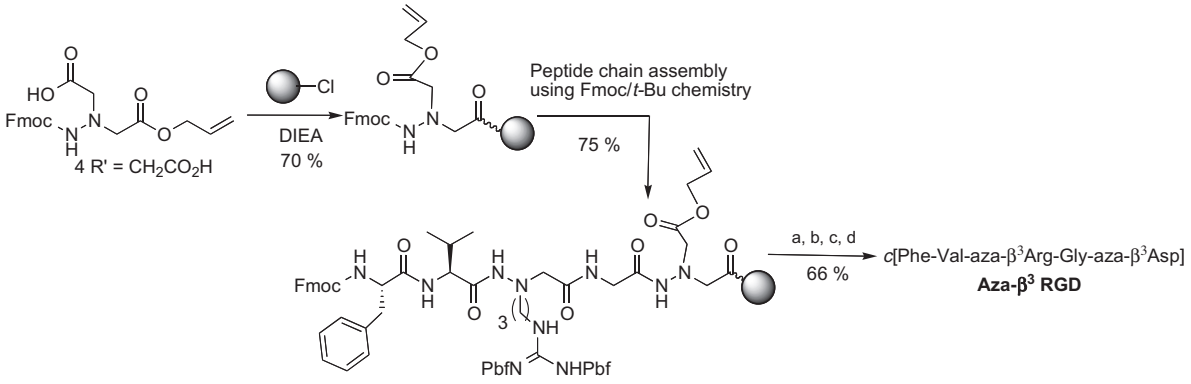
Scheme 3. Synthesis of **C6W**: (a) $Pd(Ph_3P)_4/PhSiH_3$ in DCM, 30 min twice; (b) 20% piperidine in DMF; (c) PyBOP/HOBt/DIEA in DMF; (d) TFA/TIS/H_20.

35% yield.[41] Selective deprotection with trifluoro acetic acid (TFA) of Fmoc-aza-β^3-Lys(Boc)-OAllyl **3** (R = $(CH_2)_4NHBoc$) or Fmoc-aza-β^3-Asp(*t*-Bu)-OAllyl **3** (R = CH_2CO_2t-Bu) afforded respectively Fmoc-aza-β^3-Lys-OAllyl **4** (R′ = $(CH_2)_4NH_2$) in 68% yield or Fmoc-aza-β^3-Asp-OAllyl **4** (R′ = CH_2CO_2H) in 87% yield (Scheme 2).[42]

As the nucleophilic substitution proceeds in somewhat lower yield (35% for Fmoc-aza-β^3-Asp(*t*-Bu)-OAllyl **3** R = CH_2CO_2t-Bu), we tried another way to get Fmoc-aza-β^3-Asp-OAllyl **4** (R′ = CH_2CO_2H). Starting from Fmoc-aza-β^3-Gly-OH **5** already described,[31] we realized a nucleophilic substitution of allyl bromo acetate **2** in the presence of DIPEA. Even if the reaction was realized on microwave activation (15 W, 2 h), the reaction proceeded in 19% yield, such a low yield of nucleophilic substitution by Fmoc carbazate has already been observed previously. The Fmoc protecting group must deactivate this reaction as we did not observe such low yield with Boc carbazate.[37]

Fmoc-aza-β^3-Lys-OAllyl, and Fmoc- aza-β^3-Asp-OAllyl could be loaded with a substitution level of 0.99 mmol/g and 1.05 mmol/g respectively. The yield of 64–68% was due to the non activation of the CTC resin before loading.[43] Commercially available Chloro Trityl Chloride resin (CTC) (1 mmol, 1.54 mmol/g) was swollen in DCM for 30 min and washed several times with dry DCM. Fmoc-aza-β^3-aa-OAllyl (1.2 equiv) was dissolved in dry DCM (10 mL) and transferred to the resin bed. Following the addition of DIPEA (4 equiv) via syringe, the mixture was gently agitated for 4 h at room temperature. After draining, the resin was washed several times with DMF and then with DCM. A capping step was proceeded by addition of a mixture of DCM/MeOH/DIPEA (17/2/1). Then the resin was washed with DCM (2 × 10 mL) and dried overnight in a desiccator at room temperature. The substitution level was determined by UV spectroscopy.[44]

To demonstrate the feasibility of the on-resin cyclization of aza-β^3-peptides, we carried out the synthesis of a de novo cyclohexapseudopeptide **C6W** (Scheme 3) and a cyclic aza-β^3RGD analog (Scheme 4). The side-chain immobilized Fmoc-aa-(CTC resin)-OAllyl prepared above were used in the Fmoc/tBu-based synthesis. The primary sequence was assembled using a microwave synthesizer utilizing a single coupling, TBTU/DIEA activation protocol, a four-fold excess of each amino acid residue, and conventional protecting groups. The removal of the allyl protection was carried out



Scheme 4. Synthesis of **aza-β³-RGD**: (a) $Pd(Ph_3P)_4$/$PhSiH_3$ in DCM, 30 min twice; (b) 20% piperidine in DMF; (c) PyBOP/HOBt/DIEA in DMF; (d) TFA/TIS/H_2O.

according to the method by Albericio and co-workers.[22] Two 30 min treatments at room temperature with a catalytic amount of $Pd(PPh_3)_4$ (0.1 equiv) and an excess of allyl acceptor phenylsilane (24 equiv) in DCM resulted in clean removal of the allyl protecting group. To control the allyl deprotection a small amount of resin was cleaved to afford the linear pseudopeptide *N*-Fmoc protected and the mass was checked by MALDI-TOF MS. The resin-bound pseudopeptide, after the *N*-ter Fmoc deprotection, was used in the on-resin cyclization with 4 equiv of PyBOP/HOBt and 10 equiv of DIEA in DMF overnight at room temperature (Schemes 2 and 3). The cyclic product was then deprotected and cleaved from the resin support using a mixture of TFA/TIS/H2O (95/2.5/2.5, v/v) for 3 h at room temperature. After precipitation by addition of cold ether and filtration, the final product of each reaction was purified by rp-HPLC and its sequence as well as its purity (>95%) was checked by MALDI-TOF MS for **C6W** (calcd $[M+H]^+$: 912.528; found $[M+H]^+$: 912.752) and for the **aza-β³-RGD** by HRMS electrospray (calcd $[M+H]^+$: 605.3160; found $[M+H]^+$: 605.3160 (0 ppm)). Based on the initial loading, an overall yield of the on-resin synthesis was calculated respectively at 23.9% and 34.7%.

In summary, we have demonstrated that Fmoc-aza-β³Lys-OAllyl and Fmoc-aza-β³Asp-OAllyl could be easily prepared and conveniently used as a tether point for solid-phase linkage. With the synthesis of two cyclo-aza-β³-peptides **C6W** and **aza-β³-RGD** we demonstrated the feasibility of our synthetic approach that includes resin attachment of the first aza-β³-amino acid via its side chain, successful use of combination of three orthogonal removable protecting groups, stepwise Fmoc solid-phase synthesis of a linear precursor aza-β³-peptide, and on-resin head-to-tail cyclization.

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41. *General procedure for the synthesis of Fmoc-aza-β³-aa-OAllyl* **3***:* In a round-bottom flask containing hydrazine **1** or **5** (1 equiv) in toluene (100 mL) were added K_2CO_3 (0.8 equiv), NaI (2 equiv) and the allyl bromoacetate **2** (2 equiv). The mixture was stirred 4 days at 80 °C. The solvent was then removed under vacuum. Water and ethyl acetate were added to the mixture, and the organic layer was washed with water and brine, dried on Na_2SO_4, and evaporated to afford the crude product. Purification by CombiFlash chromatography (Petroleum ether/ethyl acetate 55/45) led to a colorless oil. *Fmoc-aza-β³-Lys(Boc)-OAllyl* **3** R = $(CH_2)_4NHBoc$ (60% yield). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 1.33 (s, 9H), 1.40–1.42 (m, 4H), 2.81 (m, 2H), 3.01 (m, 2H), 3.64 (s, 2H), 4.12 (t, *J* = 6.5 Hz,1H), 4.36 (d, *J* = 6.5 Hz, 2H), 4.54 (d, *J* = 7.0 Hz, 2H), 4.69 (br, 1H), 5.17–5.28 (m, 2H), 5.76–5.89 (m, 1H), 6.78 (br, 1H), 7.18–7.33 (m, 4H), 7.50 (d, *J* = 6.7 Hz, 2H), 7.67 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 170.4, 156.1, 155.9, 143.8, 141.3, 131.5, 127.7, 127.0, 125.1, 120.0, 119.1, 78.9, 66.7, 65.4, 57.5, 56.1, 47.2, 40.2, 28.4, 27.3, 24.6. HRMS (ESI) calcd for $C_{29}H_{37}N_3O_6Na$ $[M+Na]^+$: 546.25746; found 546.2575 (0 ppm). *Fmoc-aza-β³-Asp(t-Bu)-OAllyl* **3** R = CH_2CO_2t-Bu (35% yield): ^1H NMR (300 MHz, $CDCl_3$) δ

ppm: 1.38 (s, 9H), 3.62 (s, 2H), 3.76 (s, 2H), 4.13 (t, J = 7.1 Hz, 1H), 4.31 (d, J = 7.1 Hz, 2H), 4.53 (dt, J = 5.8 Hz, 2H), 5.12–5.25 (m, 2H), 5.74–5.88 (m, 1H), 7.06 (br, 1H), 7.17–7.31 (m, 4H), 7.49 (m, 2H), 7.65 (d, J = 7.4 Hz, 2H).^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 169.3, 169.0, 155.3, 143.8, 141.3, 131.6, 127.7, 127.1, 125.2, 120.0, 118.9, 82.2, 67.1, 65.7, 57.9, 57.3, 47.1, 28.1. HRMS (ESI) calcd for $C_{26}H_{31}N_2O_6Na$ $[M+Na]^+$: 489.19961; found 489.1996 (0 ppm).

42. *General procedure for the synthesis of Fmoc-aza-β^3-aa-OAllyl* ***4****:* Monomer **3** (2 mmol) was dissolved in TFA/DCM (10 mL/10 mL) and the reaction was stirred at room temperature during 5 h. The solvent was removed under vacuum, and the crude product was purified by CombiFlash (CH_2Cl_2/MeOH: 90/10) to afford an orange oil. *Fmoc-aza-β^3-Lys-OAllyl* ***4*** R = $(CH_2)_4NH_2$ (68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.41 (m, 2H), 1.71 (m, 2H), 2.80 (m, 2H), 2.92 (m, 2H), 3.61 (s, 2H), 4.07 (t, J = 6.3 Hz, 1H), 4.33 (d, J = 6.3 Hz, 2H), 4.50 (d, J = 5.6 Hz, 2H), 5.13–5.23 (m, 2H), 5.72–5.82 (m, 1H), 6.99 (br, 1H), 7.18–7.32 (m, 4H), 7.45 (d, J = 7.2 Hz, 2H,), 7.62 (d, J = 7.3 Hz, 2H), 10.03 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 170.0, 157.0, 143.4$_r$, 141.3, 131.2, 127.8, 127.1, 124.9, 120.0, 119.1, 67.1, 65.8, 57.2, 56.2, 47.1, 39.9, 26.2, 24.2. HRMS (ESI) calcd for $C_{24}H_{30}N_3O_4$ $[M+H]^+$: 424.22308; found, 424.2230 (0 ppm). *Fmoc-aza-β^3-Asp-OAllyl* ***4*** R = CH_2CO_2H: (87% yield) ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.66–3.76 (m, 4H), 4.13 (t, J = 7.1 Hz, 1H), 4.34 (d, J = 7.1 Hz, 2H,), 4.53 (d, J = 5.9 Hz, 2H), 5.16–5.30 (m, 2H), 5.76–5.90 (m, 1H), 7.13 (br, 1H), 7.20–7.36 (m, 4H), 7.47 (d, J = 7.3 Hz, 2H), 7.68 (d, J = 7.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 171.1, 169.9, 158.5, 143.2, 141.4, 131.0, 127.9, 127.2, 124.9, 120.1, 119.7, 67.9, 66.2, 59.7, 58.0, 47.0. HRMS (EI+) calcd for $C_{22}H_{23}N_2O_6$ $[M+H]^+$: 411.15506; found, 411.1551 (0 ppm).
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