Efficient Synthesis of Fmoc-Protected Azido Amino Acids

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Abstract: The efficient two-step synthesis of Fmoc-protected L-azidoalanine and L-azidohomoalanine from readily available Fmoc-protected asparagine and glutamine, respectively, is reported. The synthetic route proceeds in good yield, requires no extra purification steps, and can be carried out on gram scale. The resulting azido amino acids are of sufficient purity for solid-phase peptide synthesis, as demonstrated in the synthesis of a model pentapeptide.

Key words: azides, amino acids, diazo transfer, Hofmann rearrangement, Fmoc solid-phase peptide synthesis

Non-natural amino acids bearing azide groups are useful building blocks in synthetic peptides and engineered proteins, providing a bioorthogonal handle to which additional functionality can be installed via Staudinger ligation[1] or Cu-catalyzed azide–alkyne cycloaddition reactions.[2] A diverse range of applications has been developed for azido amino acids based on these bioconjugation methods. For example, Davis and coworkers have used L-azidohomoalanine (Aha) as a site for attaching glycans onto a LacZ reporter enzyme scaffold,[3] achieving site-selective glycosylation in a similar manner to post-translational modification of proteins. The groups of Bertozzi and Tirrell have exploited the ability of *E. coli* to substitute Aha for methionine during translation,[4] observing the presentation of azide groups on the cell surface of *E. coli* cells.[5]

Azido amino acids such as L-azidoalanine [Ala(N_3)] have also been utilized in the synthesis of peptidomimetics, including triazole-based macrocycles[6] and antifreeze glycopeptides analogues[7] via the Cu-catalyzed cycloaddition reaction. Ala(N_3) has also been incorporated into modified leucine zipper peptide sequences which gain tertiary helical structure upon being constrained by macrocyclization with a bisalkyne functionalized linker.[8] The properties of the azide group itself can also be utilized as an IR probe to monitor protein folding and aggregation.[9]

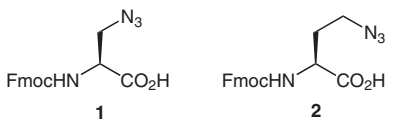

Figure 1 Fmoc-protected derivatives of L-azidoalanine [Fmoc-Ala(N_3)-OH, **1**] and L-azidohomoalanine (Fmoc-Aha-OH, **2**)

In our efforts to create azide-functionalized peptides on solid phase using standard Fmoc chemistry, we looked to develop an efficient synthesis for Fmoc-protected derivatives of L-azidoalanine [Fmoc-Ala(N_3)-OH, **1**] and L-azidohomoalanine (Fmoc-Aha-OH, **2**; Figure 1). As each coupling step in solid-phase peptide synthesis requires several equivalents of amino acid to ensure reaction completion, we aimed to find a route that would be short, high yielding, and scalable, starting from simple and readily available compounds.

There are several existing literature procedures for the synthesis of Fmoc azido amino acids **1** and **2** (Table 1). Fmoc-Aha-OH (**2**) has exclusively been synthesized from the Boc-protected azido amino acid by removal of the Boc protecting group and subsequent reprotection of the amine with Fmoc.[3,10] The analogous route to Fmoc-Ala(N_3)-OH (**1**) has also been reported.[8] This synthetic strategy is related to the well-established methods for synthesizing unprotected L-Aha via Boc-protected intermediates.[11]

In search of a more direct and atom-efficient route, the nucleophilic substitution of Fmoc-protected serine was explored, similar to the synthesis of the unprotected azido amino acids reported by Roth and Thomas.[11c,d] However, our attempts to obtain the desired azido amino acid from Fmoc-Ser-OH by mesylation and substitution with sodium azide were unsuccessful, consistent with the difficulties previously reported by Panda and Rao when attempting the reaction under either mesylation–substitution or Mitsunobu conditions.[12]

Table 1 Summary of Literature Routes[a]

Synthetic approach	Product	Starting material	Number of steps (overall yield)
Boc replacement	**1**	Boc-Dap-OH	3 (<50%)[8]
	2	homoserine	8 (<13%)[10]
	2	Boc-Dab-OH	4 (not reported)[3]
nucleophilic substitution	**1**	Fmoc-Ser-OH	3 (63%)[12,13]
diazo transfer with triflyl azide	**1**	Fmoc-Dap-OH	1 (72–88%)[6,7,9]
Hofmann/imidazole diazo transfer	**1**	Fmoc-Asn-OH	2 (49–60%)[b]
	2	Fmoc-Gln-OH	2 (46–55%)[b]

[a] Dap = 2,3-diaminopropionic acid; Dab = 2,4-diaminobutyric acid.
[b] This work.

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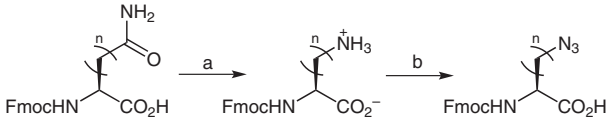
Under these conditions, elimination to form the α,β-unsaturated compound is exclusively observed. Whilst masking the free carboxylate has been shown to resolve this problem,[12,13] it was decided not to introduce a new set of protecting groups in our synthesis. Furthermore, the starting material for accessing **2** via this route would be the less available Fmoc-protected homoserine.

Consequently, a copper-catalyzed diazo transfer strategy from amine precursors was adopted, as diazo transfer using triflyl azide has previously been employed in the synthesis of **1**.[6,7,9] However, to avoid safety issues associated with using a potentially explosive diazo transfer reagent on gram scale, it was decided to utilize imidazole-1-sulfonyl azide to effect the diazo transfer in a safer and more practical manner. This reagent can be synthesized on large scale and is stable to long-term storage.[14] It was also decided to synthesize the amine precursors Fmoc-Dap-OH (**5**) and Fmoc-Dab-OH (**6**) from Fmoc-protected asparagine (**3**) and glutamine (**4**, Scheme 1). Both **3** and **4** are standard, readily available protected amino acids that are used in solid-phase peptide synthesis.



3 (n = 1) **4** (n = 2) — **5** (n = 1) 79–80% **6** (n = 2) 71–74% — **1** (n = 1) 62–75% **2** (n = 2) 65–74%

Scheme 1 *Reagents and conditions*: a) [Bis(trifluoroacetoxy)iodo]benzene, DMF–H_2O (2:1), pyridine, 14 h; b) imidazole-1-sulfonyl azide hydrochloride, K_2CO_3, H_2O–MeOH–CH_2Cl_2 (1:1:1), pH 9, 18 h.

Using [bis(trifluoroacetoxy)iodo]benzene and pyridine in a Hofmann rearrangement based on literature procedures,[15] **3** and **4** were converted to **5** and **6** in good yields of 79–80% and 71–74%, respectively. This step can be done on multigram scale, with the pure product easily isolated by filtration.

From the amino compounds **5** and **6**, initial attempts using the original literature conditions[14] for the diazo transfer reagent with K_2CO_3 in MeOH resulted in significant Fmoc deprotection due to the basic conditions of the reaction. Changing to a biphasic solvent mixture of H_2O, MeOH, and CH_2Cl_2 adjusted to pH 9 with K_2CO_3 gave the desired azido amino acids in yields of 62–75% and 65–74% for **1** and **2**, respectively.[6] Notably, both compounds were found to be >98% pure after workup, thus not requiring column chromatography.

To test the azido amino acids in Fmoc solid-phase peptide synthesis, the pentapeptide Ac-Ala(N_3)-Ala-Arg-Ala-Aha-NH_2 was synthesized on Rink amide resin (Figure 2). The couplings proceeded smoothly with three equivalents of each azido amino acid, and after cleavage from the resin, the crude product gave the correct mass by mass spectrometry and a single peak on HPLC (see Supporting Information), with no significant byproducts or evidence of diastereomers due to epimerization.

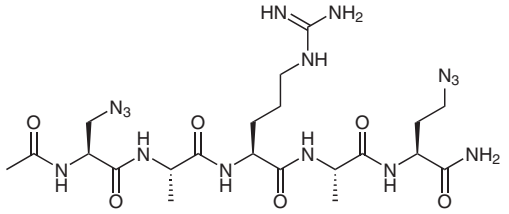


Figure 2 Model pentapeptide Ac-Ala(N_3)-Ala-Arg-Ala-Aha-NH_2 synthesized using Fmoc solid-phase peptide synthesis

In summary, the Hofmann rearrangement/diazo transfer strategy is a simple and efficient route for synthesizing the important protected amino acids Fmoc-Ala(N_3)-OH (**1**) and Fmoc-Aha-OH (**2**). Starting from readily available materials, the procedure can easily be scaled up, and pure products can be obtained without the need for column chromatography or recrystallization. The ready availability of these azido building blocks should have an impact on numerous applications in chemical biology[16] and diversity-oriented synthesis.[17]

Fmoc-Asn-OH (**3**, 3.30 g, 9.31 mmol) was added to a solution of [bis(trifluoroacetoxy)iodo]benzene (6.05 g, 14.1 mmol) in DMF–H_2O (2:1, 66 mL). After 15 min, pyridine (1.60 mL, 19.9 mmol) was added, and the mixture was stirred at r.t. for 14 h. The solvent was removed under reduced pressure, and the oily residue was dissolved in H_2O (50 mL). Concentrated HCl (1 mL) was added, and the acidified solution was washed with Et_2O (4 × 30 mL). The aqueous phase was adjusted to pH 6 with 2 M NaOH solution, and the resulting precipitate was filtered, washed with H_2O (5 × 30 mL), ice-cold EtOH (10 mL), and Et_2O (10 × 10 mL) and dried in vacuo to give Fmoc-Dap-OH (**5**, 2.44g, 80%) as a beige powder. IR (ATR): 3297, 3037, 1724, 1686, 1631, 1575, 1533, 1491, 1448, 1409, 1324, 1248, 1068, 735 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 7.89 (d, *J* = 7.4 Hz, 2 H, ArH), 7.70 (d, *J* = 5.5 Hz, 2 H, ArH), 7.42 (t, *J* = 7.2 Hz, 2 H, ArH), 7.33 (t, *J* = 7.0 Hz, 2 H, ArH), 6.78 (d, *J* = 4.6 Hz, 1 H, CONH), 4.37–4.18 (m, 3 H, Fmoc-CH and CH_2), 3.71 (m, 1 H, α-CH), 3.01 (dd, *J* = 10.6, 4.1 Hz, 1 H, one of β-H), 2.78 (t, *J* = 10.5 Hz, 1 H, one of β-H). HRMS (ES^+): *m/z* $[M + H]^+$ calcd for $C_{18}H_{19}N_2O_4$: 327.1345; found: 327.1348. $[\alpha]_D^{27}$ +27.7 (*c* 0.41, DMSO).

Fmoc-Dap-OH (**5**, 1.01 g, 3.11 mmol) was dissolved in a biphasic mixture of H_2O (15 mL), MeOH (30 mL), and CH_2Cl_2 (25 mL). $CuSO_4 \cdot 5H_2O$ (5.0 mg, 0.020 mmol) and imidazole-1-sulfonyl azide hydrochloride (2.02 g, 9.62 mmol) were added, and the mixture was adjusted to pH 9 with aq K_2CO_3 solution. After stirring vigorously for 18 h, the reaction mixture was diluted with CH_2Cl_2 (30 mL), and the aqueous phase was isolated. The organic phase was extracted with sat. $NaHCO_3$ (2 × 50 mL). The combined aqueous extracts were washed with Et_2O (2 × 50 mL), acidified to pH 2 with concentrated HCl, and extracted with Et_2O (3 × 60 mL). The organic extracts were dried over $MgSO_4$ and concentrated in vacuo to give Fmoc-Ala(N_3)-OH (**1**, 820 mg, 75%) as a beige amorphous solid. IR (ATR): 3303, 2103, 1690, 1536, 1446, 1341, 1259, 1088, 737 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.5 Hz, 2 H, ArH), 7.59 (d, *J* = 6.2 Hz, 2 H, ArH), 7.40 (t, *J* = 7.5 Hz, 2 H, ArH), 7.31 (tt, *J* = 7.5, 1.1 Hz, 2 H, ArH), 5.65 (d, *J* = 7.6 Hz, 1 H, CONH), 4.57 (m, 1 H, α-CH), 4.49–4.37 (m, 2 H, Fmoc-CH_2), 4.23 (t, *J* = 6.7 Hz, Fmoc-CH), 3.80 (m, 2 H, β-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (COOH), 156.2 (CONH), 143.6 (2 × C_QAr), 141.4 (2 × CHAr), 127.9 (2 × CHAr), 127.2 (2 × CHAr), 125.1 (2 × CHAr), 120.1 (2 × CHAr), 67.6 (Fmoc-CH_2), 53.9 (α-CH), 52.4 (β-CH_2), 47.1 (Fmoc-CH). HRMS (ES^+): *m/z* $[M + H]^+$ calcd

for $C_{18}H_{17}N_4O_4$: 353.1250; found: 341.1236. $[\alpha]_D^{27}$ –10.3 (*c* 1.0, DMF).

Fmoc-Dab-OH (**6**) was prepared in a similar manner to **5** from Fmoc-Gln-OH (**2**, 3.14 g, 8.52 mmol), [bis(trifluoroacetoxy)-iodo]benzene (6.01 g, 14.0 mmol) and pyridine (1.60 mL, 19.9 mmol) to give the product (2.16 g, 74%) as a white powder. IR (ATR): 2954, 1699, 1649, 1531, 1403, 1261, 1077, 760, 736 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 7.89 (d, *J* = 7.4 Hz, 2 H, ArH), 7.67 (d, *J* = 7.1 Hz, 2 H, ArH), 7.42 (t, *J* = 7.4 Hz, 2 H, ArH), 7.33 (t, *J* = 7.3 Hz, 2 H, ArH), 6.63 (d, *J* = 3.4 Hz, 1 H, CONH), 4.34–4.17 (m, 3 H, Fmoc-CH and CH_2), 3.62 (m, 1 H, α-CH), 2.99–2.80 (m, 2 H, β-H), 1.95–1.84 (m, 1 H, one of γ-H), 1.78–1.65 (m, 1 H, one of γ-H). HRMS (ES^+): *m/z* $[M + H]^+$ calcd for $C_{19}H_{21}N_2O_4$: 341.1501; found: 341.1492. $[\alpha]_D^{27}$ +22.0 (*c* 1.0, DMSO).

Fmoc-Aha-OH (**2**) was prepared in a similar manner to **1** from Fmoc-Dab-OH (**6**, 1.03 g, 3.01 mmol), $CuSO_4 \cdot 5H_2O$ (5.0 mg, 0.020 mmol) and imidazole-1-sulfonyl azide hydrochloride (2.01 g, 9.59 mmol) to give the product (815 mg, 74%) as a white amorphous solid. IR (ATR): 3329, 2106, 1693, 1535, 1445, 1242, 734 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, *J* = 7.3 Hz, 2 H, ArH), 7.59 (d, *J* = 7.3 Hz, 2 H, ArH), 7.41 (t, *J* = 7.3 Hz, 2 H, ArH), 7.32 (tt, *J* = 7.4, 1.1 Hz, 2 H, ArH), 5.41 (d, *J* = 7.0 Hz, 1 H, CONH), 4.56–4.39 (m, 3 H, α-CH and Fmoc-CH_2), 4.23 (t, *J* = 6.6 Hz, 1 H, Fmoc-CH), 3.43 (m, 2 H, γ-CH_2), 2.19 (m, 1 H, one of β-CH_2), 2.02 (m, 1 H, one of β-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.0 (COOH), 156.3 (CONH), 143.6 (2 × C_QAr), 141.4 (2 × C_QAr), 127.9 (2 × CHAr), 127.2 (2 × CHAr), 125.1 (2 × CHAr), 120.1 (2 × CHAr), 67.3 (Fmoc-CH_2), 51.7 (α-CH), 47.7 (γ-CH_2), 47.2 (Fmoc-CH), 31.3 (β-CH_2). HRMS (ES^+): *m/z* $[M + H]^+$ calcd for $C_{19}H_{19}N_4O_4$: 367.1406; found: 367.1424. $[\alpha]_D^{27}$ –14.4 (*c* 1.0, MeOH, lit.[10] $[\alpha]_D$ –11.5).

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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