Synthesis of *N*-Fmoc-*O*-(*N*′-Boc-*N*′-methyl)-aminohomoserine, an Amino Acid for the Facile Preparation of Neoglycopeptides†

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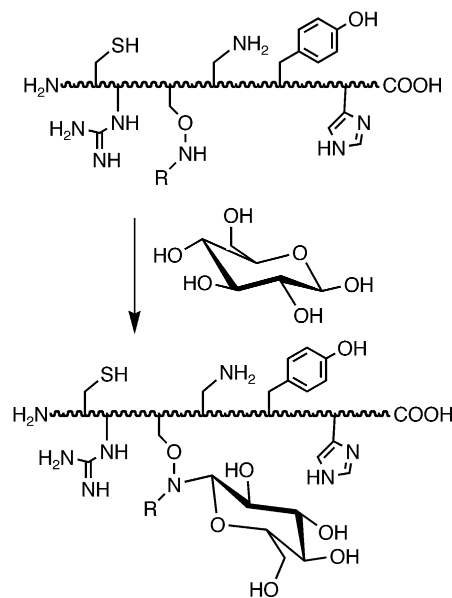
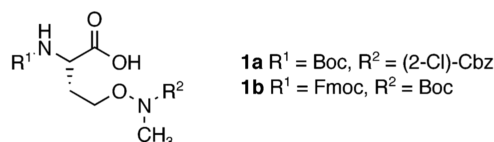
Abstract: The synthesis of *N*-Fmoc-*O*-(*N*′-Boc-*N*′-methyl)-aminohomoserine in 35% overall yield from L-homoserine is described. This amino acid can be efficiently incorporated into peptides using Fmoc-chemistry-based solid-phase peptide synthesis, and the resulting peptides can be chemoselectively glycosylated at the aminooxy side chains to generate neoglycopeptides. The synthesis of this derivative greatly expands the availability of a previously developed neoglycopeptide synthesis strategy.

The chemoselective reaction of aminooxy-derivatized peptides with reducing sugars has proven an attractive method for the site-specific glycosylation of peptides.[1-3] This general strategy for neoglycopeptide synthesis allows the coupling of a given peptide with a variety of sugars and therefore facilitates the formation of combinatorial neoglycopeptide arrays (Scheme 1). Recently, we[2] and others[3] have developed aminooxy amino acid derivatives designed to create neoglycopeptides that are much closer to their natural counterparts. The key features of these amino acids are that they contain short side chains and incorporate *N*-alkylation of the aminooxy moiety to ensure cyclic sugar conformations. Our previously reported derivative, **1a**, has been efficiently incorporated into peptides using Boc-chemistry based solid-phase peptide synthesis (SPPS) procedures, and the resulting peptides were efficiently glycosylated.

Because an estimated 60% of all peptide research laboratories employ 9-fluorenylmethoxycarbonyl (Fmoc) based SPPS,[4] we have now synthesized aminooxy amino acid **1b** in protected form suitable for Fmoc-chemistry based SPPS to make our approach available to a broader community. Two routes were considered for making **1b** based on our synthesis of **1a**, which proceeded in a few, mild, and high-yielding steps. Initial protection of homoserine with the Fmoc group was considered and pursued as the most expedient route to **1b**; however, several issues were envisioned as potential drawbacks to this approach. For one, the reported yield for *N*-Fmoc-homoserine was low (49%).[5] Additionally, *N*-Fmoc amino acid derivatives often exhibit poor solubility. Most importantly, the Fmoc carbamate might prove unstable to the basic conditions of the hydroxylamine oxyanion substitution reaction used for side chain elaboration. As a result, initial protection of homoserine with the allyloxycarbonyl (Alloc) group and its conversion to an Fmoc group in the last step of the synthesis was pursued as an alternative route to avoid such difficulties.

SCHEME 1. General Neoglycopeptide Synthesis





In the direct approach, L-homoserine was converted to its *N*-Fmoc, allyl ester derivative **2a** by treatment with Fmoc-hydroxy succinimide (Fmoc-OSu) and $NaHCO_3$ followed by allyl bromide (Scheme 2). Because **2a** could not be conveniently purified from the *O*-allylhydroxysuccinimide byproduct, the mixture was reacted directly with MsCl in CH_2Cl_2 followed by LiBr in acetone to produce bromide **3a** in 37% overall yield from L-homoserine. The next step was the critical nucleophilic displacement of the bromide with the anion of *tert*-butyl *N*-methyl-*N*-hydroxycarbamate.[6] In the event, our fears of the instability of the Fmoc carbamate were realized. The reaction proved unsuccessful, and from the complex product

† In memory of Professor Henry Rapoport.

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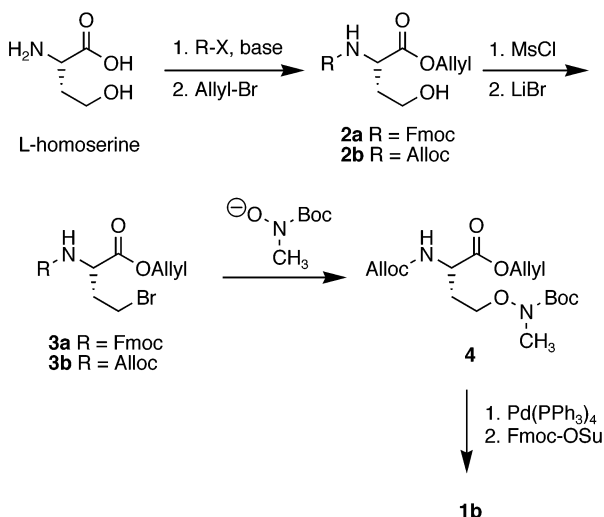
SCHEME 2



mixture a substantial amount of dibenzofulvene from elimination of the Fmoc group was recovered.

By contrast, the indirect, Alloc approach proved successful (Scheme 2). Treatment of L-homoserine with allyl chloroformate and $NaHCO_3$ followed by allyl bromide yielded the *N*-Alloc, allyl ester **2b**. Despite experimenting with a variety of reaction conditions, we were unable to produce **2b** without also forming 5–8% of *N*-Alloc-homoserine lactone. This byproduct proved inseparable from **2b** in all the chromatography conditions attempted. Because all other byproducts could be removed by simple extractive workup procedures, we avoided chromatography at this stage and proceeded with the impure **2b**. The **2b**/lactone mixture was reacted with MsCl in CH_2Cl_2 followed by LiBr in acetone to produce pure bromide **3b** in 56% overall yield from L-homoserine. At this point the lactone, which was unreactive in the bromination conditions, was easily separated by chromatography from **3b** and characterized by NMR. Unlike with **3a**, nucleophilic displacement of the bromine of **3b** with the anion (generated by NaH) of *tert*-butyl *N*-methyl-*N*-hydroxycarbamate proceeded cleanly to afford **4** in 81% yield. At this point, what remained was to convert the Alloc to Fmoc and deprotect the carboxylic acid. We removed the Alloc and the allyl ester simultaneously with catalytic $Pd(PPh_3)_4$. After trying a variety of nonbasic allyl scavengers, including dimedone[7] and formic acid,[8] we found pyrrolidine[9] to be the only additive that effected the deprotection cleanly and rapidly. Cognizant that residual pyrrolidine would stymie our subsequent Fmoc protection, we developed a method to ensure its removal without using chromatography or aqueous extractions, both of which would be extremely difficult with the deprotected intermediate. Because residual pyrrolidine might be complexed as the pyrrolidinium salt of the carboxylic acid, our solution was to treat the reaction mixture with 100 mol % of $NaHCO_3$ to ensure the free-base form of all the amines, remove the solvents, and heat the residue under vacuum at 50 °C overnight. This procedure effectively removed the remaining pyrrolidine and the majority of the *N*-allylpyrrolidine. The remaining residue was treated directly with Fmoc-OSu to provide 78% of **1b**. The overall yield of **1b** from L-homoserine was 35%.

We then verified the efficacy of **1b** by using it to synthesize the peptide $H_2N-FKAZSK-NH_2$, where Z = *O*-(*N*-methyl)-aminohomoserine, by standard Fmoc-chemistry-based SPPS.[10] We chose this model sequence because it had been synthesized by Boc-chemistry-based SPPS previously and because its glycosylated derivative is easily separable from the parent peptide.[2] After the peptide was deprotected and cleaved from the resin, HPLC analysis of the crude peptide product showed a single major peak (>90%), which corresponded to the expected mass by electrospray ionization MS. A purified sample of the peptide was treated with D-glucose (0.1 M NaOAc, pH 4.0, 45 °C) to verify the availability of the *N*-methyl-aminooxy side chain. Glycosylation proceeded in a manner consistent with that reported for the peptide synthesized by Boc-chemistry-based SPPS,[2] and the peptide underwent 72% conversion to its glycosylated derivative in 4 h without the formation of any side products. Importantly, the retention times for the parent and glycosylated peptides made by the Fmoc-chemistry-based SPPS were identical with those made previously by Boc-chemistry-based SPPS.

We have demonstrated a practical synthesis of *N*-Fmoc-*O*-(*N*′-Boc-*N*′-methyl)-aminohomoserine from L-homoserine. This amino acid derivative is efficiently incorporated into peptides using Fmoc-chemistry-based SPPS, and the resulting peptides can be glycosylated chemoselectively at the *N*-methyl-aminooxy side chains. Because a growing majority of peptide syntheses are performed using Fmoc-based chemistry, this new derivative greatly expands the ability of others to take advantage of our method for the synthesis of neoglycopeptides.

Experimental Section

Chromatographic separations were performed using silica gel (230–400 mesh). Organic solutions were dried with Na_2SO_4, and solvents were removed using standard rotary evaporation under reduced pressure. Products were dried under high vacuum. Commercial reagents were used in all cases without further purification. Spectral characterizations of **1b** were performed at elevated temperatures because of the presence of distinct rotational isomers under most conditions.

SPPS was performed using a Rink amide resin using standard Fmoc-chemistry-based procedures[10] with the following variations. The amino acids were activated with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and $NEt(i\text{-}Pr)_2$ in DMF. All commercial amino acids were used in a 10-fold excess to the resin loading and allowed to react for 10 min. **1b** was used in a 2.5-fold excess and allowed to react for 25 min. Fmoc deprotections were carried out using 20% piperidine in DMF. Resin washings between steps were performed with a continuous flow of DMF for 1 min. Final

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deprotection and resin cleavage was accomplished by treatment with 95:2.5:2.5 TFA:H_2O:triisopropylsilane.

Allyl 2-(*N*-Allyloxycarbonyl)-amino-4-bromobutanoate (3b). L-homoserine (1.01 g, 8.49 mmol) and Na_2CO_3 (899 mg, 8.49 mmol) were dissolved in H_2O (10 mL) and CH_3CN (5 mL) and treated with allyl chloroformate (897 μL, 8.49 mmol). The mixture was stirred for 21 h, and the solvents were removed. The resulting residue was dissolved in DMF (20 mL), treated with allyl bromide (807 μL, 9.34 mmol) and $NaHCO_3$ (713 mg, 8.49 mmol), and stirred for 65 h. The solvent was removed, and the residue was dissolved in EtOAc. This organic layer was washed with H_2O (50 mL), sat. $NaHCO_3$ (3 × 50 mL), H_2O (50 mL), 0.1 M $KHSO_4$ (2 × 50 mL), and brine (50 mL) and then dried. Removal of the solvent left a residue that corresponded to >90% pure **2b** by ^{1}H NMR.

The residue was dissolved in CH_2Cl_2 (30 mL), treated with MsCl (514 μL, 6.64 mmol) and NEt_3 (998 μL, 7.20 mmol), and stirred for 45 min. LiBr (4.81 g, 55.4 mmol) and acetone (30 mL) were added, and the mixture was stirred for an additional 17 h. The solvents were removed, and the residue was redissolved in EtOAc (125 mL) and washed with H_2O (2 × 75 mL), sat. $NaHCO_3$ (75 mL), and brine (75 mL). After drying and removal of the solvent, the residue was chromatographed (EtOAc: hexanes, 20:80) to yield **3b** (1.47 g, 4.79 mmol, 56%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.91 (m, 2H), 5.51 (d, J = 7.8 Hz, 1H), 5.38−5.21 (m, 4H), 4.66 (dd, J = 1.1, 5.9 Hz, 2H), 4.59 (d, J = 5.5 Hz, 2H), 4.52 (m, 1H), 3.45 (t, J = 7.1 Hz, 2H), 2.45 (m, 1H), 2.26 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4, 156.0, 132.6, 131.4, 119.3, 118.1, 66.5, 66.1, 53.0, 35.6, 28.3. Anal. Calcd for $C_{11}H_{16}BrNO_4$: C, 43.15; H, 5.27; N, 4.58. Found: C, 43.06; H, 5.43; N, 4.52.

Allyl 2-(*N*-Allyloxycarbonyl)-amino-4-(*O*-[*N*-*tert*-butoxycarbonyl-*N*-methyl]-amino)-hydroxybutanoate (4). *tert*-Butyl *N*-methyl-*N*-hydroxycarbamate (904 mg, 6.15 mmol) was dissolved in anhydrous DMF (5 mL), treated with NaH (236 mg, 60% dispersion in mineral oil, 5.90 mmol), and stirred for 1 h under N_2. The mixture was cooled to 0 °C, treated with a solution of **3b** (1.51 g, 4.92 mmol) in DMF (10 mL), and stirred at 0 °C for an additional 3 h. The solvents were removed, and the residue was dissolved in EtOAc (150 mL) and poured into a separatory funnel. The organic layer was washed with 0.1 M NaOH (5 × 50 mL), H_2O (50 mL), 0.1 M $KHSO_4$ (2 × 50 mL), and brine (50 mL) and then dried. After removal of the solvent, the residue was chromatographed (EtOAc:hexanes, 30:70) to yield **4** (1.48 g, 3.98 mmol, 81%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.29 (d, J = 7.6 Hz, 1H), 5.90 (m, 2H), 5.32 (m, 2H), 5.21 (dd, J = 10.4, 17.3 Hz, 2H), 4.64 (d, J = 5.7 Hz, 2H), 4.57 (d, J = 5.3 Hz, 2H), 4.51 (m, 1H), 3.95 (m, 2H), 3.08 (s, 3H), 2.18 (m, 1H), 2.09 (m, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6, 156.8, 156.0, 132.7, 131.6, 118.5, 117.3, 81.5, 70.2, 65.7, 65.5, 51.8, 36.4, 30.0, 28.1. Anal. Calcd for $C_{17}H_{28}N_2O_7$: C, 54.83; H, 7.58; N, 7.52. Found: C, 54.50; H, 7.74; N, 7.26.

***O*-[*N*-*tert*-Butoxycarbonyl-*N*-methyl]-amino-*N*-(9-fluorenylmethoxycarbonyl)-homoserine (1b).** A solution of **4** (464 mg, 1.25 mmol), PPh_3 (16 mg, 0.063 mmol), $Pd(PPh_3)_4$ (3.6 mg, 0.031 mmol), and pyrrolidine (313 μL, 3.75 mmol) in CH_2Cl_2 was stirred for 40 min. The solvent was evaporated, and the residue was redissolved in CH_3CN (10 mL). A solution of $NaHCO_3$ (105 mg, 1.25 mmol) in H_2O (5 mL) was added and the mixture was stirred for 30 min. The solvents were evaporated, and the resulting mixture was dried overnight under vacuum at 50 °C. The resulting solids were dissolved in DMF (30 mL) and H_2O (30 mL), treated with $NaHCO_3$ (210 mg, 2.5 mmol) and Fmoc-OSu (464 mg, 1.37 mmol), and stirred for 24 h. The solvents were removed, and the residue was dissolved in EtOAc (150 mL) and washed with 0.1 M $KHSO_4$ (4 × 50 mL), H_2O (4 × 50 mL), and brine (100 mL). After drying and removal of the solvent, the residue was chromatographed (acetone:CH_2Cl_2:AcOH, 5:95: 0.5 to 10:90:0.5) and then purified by size exclusion chromatography (LH-20, CH_2Cl_2) to yield **1b** (456 mg, 0.969 mmol, 78%) as a glassy solid: ^{1}H NMR (CD_3CN, 55 °C, 400 MHz) δ 7.80 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.30 (dt, J = 1.2, 7.5 Hz, 2H), 6.44 (br s, 1H), 4.34 (m, 3H), 4.21 (t, J = 6.8 Hz, 1H), 3.88 (m, 2H), 3.03 (s, 3H), 2.07 (m, 1H), 1.94 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (CD_3OD, 55 °C, 100 MHz) δ 175.5, 158.6, 145.4, 145.3, 142.6, 128.8, 128.2, 126.3, 121.0, 82.9, 71.7, 68.1, 53.0, 48.5, 36.9, 31.3, 28.7. Anal. Calcd for $C_{25}H_{30}N_2O_7$: C, 63.82; H, 6.43; N, 5.95. Found: C, 63.46; H, 6.65; N, 6.17.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra for **2b**, **3b**, **4**, and **1b**; experimental procedure and data for *tert*-butyl *N*-methyl-*N*-hydroxycarbamate. This material is available free of charge via the Internet at http://pubs.acs.org.

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