An Efficient Synthetic Route to *N*-Glycosylamino Acids Using *N*$^{\alpha}$-Fmoc-Asp/Glu-5-oxazolidinone as Internal Protection

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Abstract: A new facile synthetic route to prepare *N*-glycosylamino acids in fewer steps is reported. 5-Oxazolidinone served as an effective protecting moiety for *N*$^{\alpha}$-Fmoc-Asp/Glu and after glycosylation, the ring opening resulted in free α-carboxylic acid, which can be directly used to extend the chain to obtain *N*-glycopeptides. Both the minimum number of steps as well as circumvention of orthogonal protection strategy leads to a cost-effective route for their synthesis. This protocol can be easily scaled up to prepare N-glycosylated Asn/Gln acids in large quantities in fairly good yields.

Key words: 5-oxazolidinone, glycosyl amine, protecting group, *N*-glycosylamino acid, coupling agent, building-block strategy

N-Linked and O-linked glycoproteins and glycopeptides are of significant biological interest due to their widespread role in cell recognition, cell adhesion and tumor metastasis.[1] Glycosylation of peptides has been shown to increase proteolytic stability and promote blood–brain barrier permeability.[2] Glycosylation also enhances solubility[3–5] and may contribute to the stabilization of peptide structures. Recent developments in the area of glycopeptide synthesis have been reviewed.[6] It is therefore desirable that a convenient and high yielding procedure be available for the routine synthesis of *N*-glycopeptides.

In the literature, the commonly employed approach for the synthesis of glycosylated amino acid makes use of *N*$^{\alpha}$-Fmoc-Asp-O*t*-Bu as the key intermediate with β-carboxyl group being activated using pentafluorophenyl ester or acid chloride or in situ activation with Dhbt followed by coupling with glycosylamine to obtain *N*$^{\alpha}$-Fmoc-Asn(sugar)-O*t*-Bu. The resulting N-glycosylated amino acid has been utilized as a building block for chain extension after deprotecting *tert*-butyl group ester group with TFA followed by activation of α-carboxylic group.[7] However, the glycosydic bond is labile to the acidic conditions employed in the process of *tert*-butyl deprotection and the molecule is prone to decomposition. This naturally results in formation of more contaminants leading to laborious procedure for purification of the final targets.[8] A similar problem is encountered in the solid-phase peptide synthesis, where TFA is used to cleave glycopeptides from Wang resin.[9]

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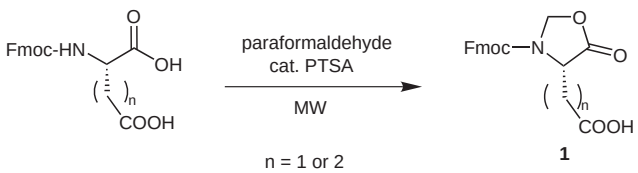
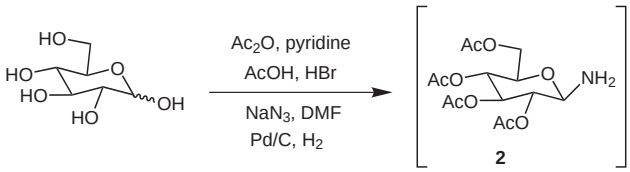
Alternatively, the use of allyl ester was also exploited which involves the deprotection of allyl group using palladium(0) or Wilkinson catalyst [$(PPh_3)_3Rh(I)Cl$].[10,11] An equally important aspect of this kind of orthogonal strategy is the generation of the free β/γ-carboxyl of Fmoc-Asp/Glu-O*t*-Bu/Allyl/Me after deprotecting the concerned protecting group of the side chain, and its preparation, which itself is a multistep process.

Consequently, it is desirable to develop both mild and efficient protection and easy deprotection strategy to construct N-glycosylated amino acids in a facile way. In this context, we envisaged the usage of 5-oxazolidinones as superior to the corresponding orthogonal protecting groups[12] because (1) they are capable of providing complete protection for both N$^{\alpha}$- and carboxylic functions; (2) they are easy to prepare; (3) the β/γ-carboxyl functions for direct coupling are readily available; and (4) mild conditions are sufficient for their cleavage. Further, Fmoc-Asp/Glu-5-oxazolidinone can be obtained in about 20% higher yields than the corresponding Boc analogues.

The *N*$^{\alpha}$-Fmoc-Asp/Glu-OH were converted into their 5-oxazolidinones using paraformaldehyde and a catalytic amount of *p*-toluenesulfonic acid in toluene under microwave irradiation in 95% yield (Scheme 1).[13] In these *N*$^{\alpha}$-Fmoc-Asp/Glu-5-oxazolidinones, the β- or γ-carboxylic acid moiety is now readily available for chemoselective transformation.[14] The carbohydrate partner of the glycopeptide was synthesized using the literature procedure (Scheme 2).[15,16] The azido functionality was introduced using penta-*O*-acetyl glucosyl pyranose via its 1-bromo compound and then reduced to obtain the required glycosyl amine.



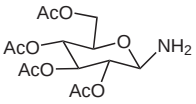
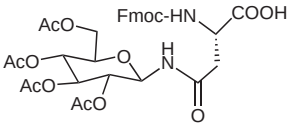
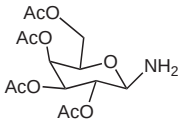
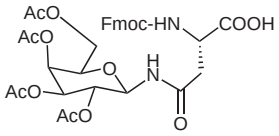
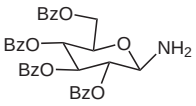
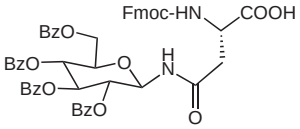
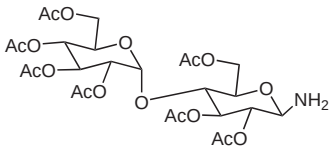
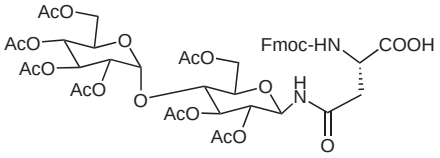
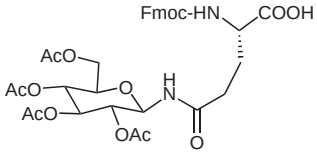
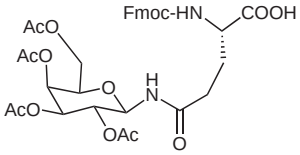
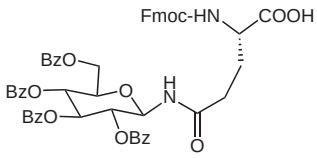
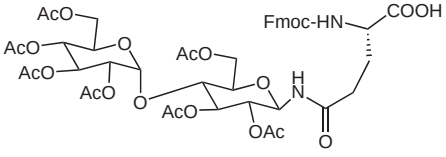
Scheme 1

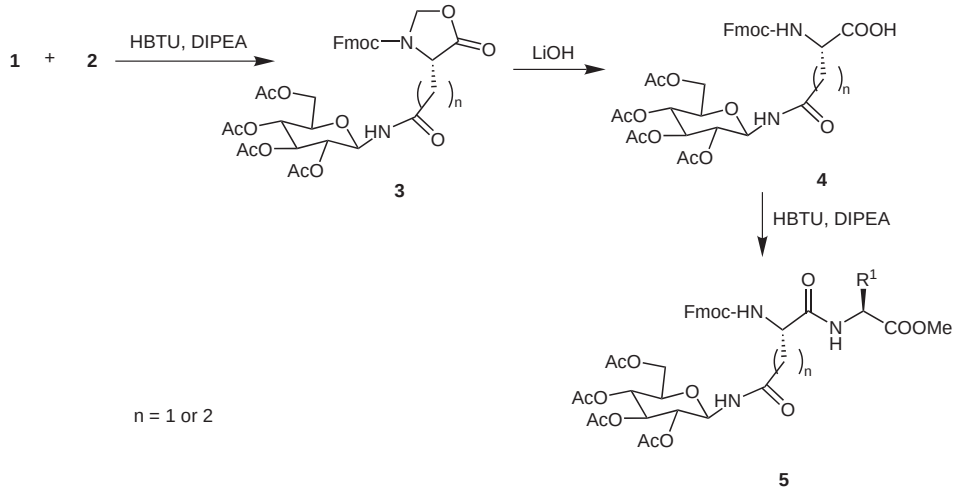


Scheme 2

The glucosylamines were then coupled with the β- or γ-carboxylic acid moiety of N^{α}-Fmoc-Asp/Glu-5-oxazolidinone. The coupling between the glycosyl amine and N^{α}-Fmoc-Asp/Glu-5-oxazolidinone was carried out with different coupling agents. Fmoc-Asp/Glu-5-oxazolidinones were converted into pentafluorophenyl ester, ONSu ester, acid chloride and were also directly coupled using HBTU/DIPEA. The best results were obtained using HBTU/DIPEA to furnish the compound **3** in 90% yield.[17] As an extension of this methodology, several other glycosylamines were prepared and coupled to the N^{α}-Fmoc-Asp/Glu-5-oxazolidinones and in all cases these corresponding glycosylated 5-oxazolidinones were obtained in 88–90% yield. Finally, the compound **3** was hydrolyzed back to its acid by treating with LiOH (1 equiv, 1 N aq solution) in tetrahydrofuran for 30 minutes. These results

Table 1 Synthesis of N^{α}-Fmoc-glycosylated Amino Acids

Entry	Amine	Product	Yield (%)	Mass (Calcd/Obsd)
1	 **2a**	 **4a**	84	707.2064/707.2080
2	 **2b**	 **4b**	82	707.2064/707.2134
3	 **2c**	 **4c**	88	955.26/955.2
4	 **2d**	 **4d**	75	995.29/995.4
5	**2a**	 **4e**	78	721.2221/721.2413
6	**2b**	 **4f**	80	721.2221/721.2455
7	**2c**	 **4g**	83	969.28/969.3
8	**2d**	 **4h**	77	1009.30/1009.4



Scheme 3

are summarized in Table 1. Initial studies on hydrolysis of 5-oxazolidinone using aqueous LiOH in methanol resulted in partial deprotection of Fmoc group. Finally, the same reaction was carried in tetrahydrofuran as a solvent which gave pure product. The site-selective cleavage of 5-oxazolidinone ring can be attributed to higher lability of 5-oxazolidinone ring towards base-catalyzed hydrolysis in the presence of Fmoc or acetyl groups (Scheme 3).[18] The presence of free acid group in **3** enabled the extension on C-terminus by coupling with amino acid ester using HBTU/DIPEA that resulted in the formation of *N*-glycopeptides (Table 2). The glycosylated 5-oxazolidinones derivatives and their amino acids were analyzed by IR, ^{1}H NMR, mass and their purities confirmed using RP-HPLC. The HPLC profile of glycosylated 5-oxazolidinones **3a** and **3b** contained peak at t_R = 22.45 and 21.53 minutes whereas the glycoysylated acids **4a** and **4e** showed sharp peaks at t_R values 19.24 and 17.72 minutes, respectively. All the HPLC profiles were recorded using Eclipse XDB-C18 column 2.5 with the gradient elution method as water–acetonirile (10–90%) in 30 minutes.

In conclusion, we have described in this letter a facile approach to the synthesis of N^{α}-Fmoc-Asn/Gln-glycosylated amino acids. The N^{α}-Fmoc-Asn/Gln-glycosylated amino acids can be used directly in the synthesis of glycopeptides by the building-block strategy. We have adopted N^{α}-Fmoc-5-oxazolidinone moiety for protection of α-amino and carboxylic group as an alternative to the conventional protection and deprotection strategy for Asp/Glu. Furthermore, all four steps, namely preparation of Fmoc-Asp/Glu, 5-oxazolidinone, glycosylation, and ring opening, are high-yielding reactions. Both the intermediates as well as the final products are crystalline solids. The entire protocol is completely devoid of the use of any acid treatment. As all the reagents used for protection and deprotection are simple, the overall strategy leads to N-glycosylated Asn/Gln by way of a less expensive route. The scale-up of this protocol is easy.

Table 2 Synthesis of N^{α}-Fmoc-glycosylated Amino Acids

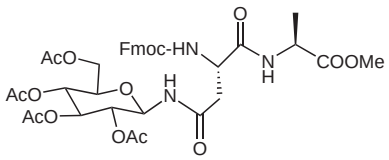
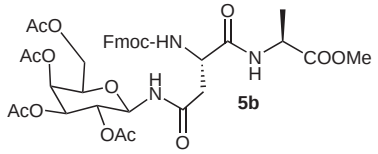
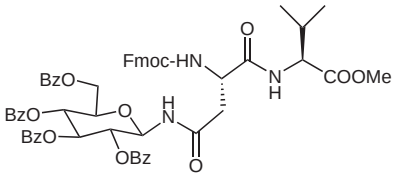
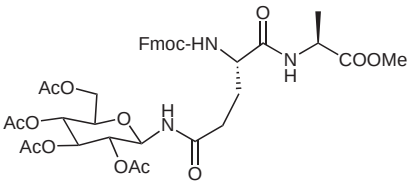
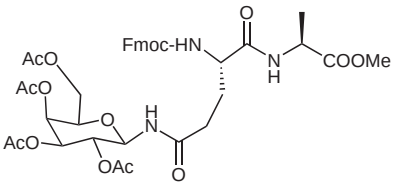
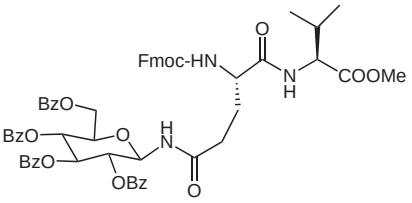
Entry	Product	Yield (%)	Mass (Calcd/Obsd)
1	 **5a**	89	792.2592/792.2514
2	 **5b**	90	792.2592/792.2552

Table 2 Synthesis of N^{α}-Fmoc-glycosylated Amino Acids (continued)

Entry	Product	Yield (%)	Mass (Calcd/Obsd)
3	 **5c**	78	1068.3531/1068.78
4	 **5d**	86	806.2748/806.2864
5	 **5e**	88	806.2748/806.2912
6	 **5f**	81	1082.3687/1082.46

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(11) (a) Likhosherstov, L.; Novikova, O.; Derveitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1. (b) Corey, E. J.; Suggs, J. W. *J. Org. Chem.* **1973**, *38*, 3234.
(12) For similar applications of oxazolidinones in the synthesis of 2,3-diaminopropionic acid (2,3-Dap) and 2,4-diaminobutanoic acid (2,4-Dab). See: (a) Teng, H.; He, Y.; Wu, L.; Su, J.; Feng, X.; Qui, G.; Liang, S.; Hu, X. *Synlett* **2006**, 877. (b) Ramanarao, R. V.; Tantry, S. J.; Sureshbabu, V. V. *Synth. Commun.* **2006**, *36*, 2912. (c) Teng, H.; Jiang, Z.; Wu, L.; Su, J.; Feng, X.; Qiu, G.; Liang, S.; Hu, X. *Synth. Commun.* **2006**, *36*, 3803.
(13) For an efficient synthesis of Fmoc-amino acid-5-oxazolidinones employing a rapid protocol under microwave irradiation, see: (a) Tantry, S. J.; Kantharaju; Sureshbabu, V. V. *Tetrahedron Lett.* **2002**, *43*, 9461. (b) For further optimized reaction conditions using MeCN as a solvent, see: Govender, T.; Arvidsson, P. I. *Tetrahedron Lett.* **2006**, *47*, 1691.
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(17) **Typical Procedure for the Synthesis of Glycosylated *N*$^{\alpha}$-Fmoc-Asp/Glu-5-oxazolidinones**

To a solution of Fmoc-Asp/Glu-5-oxazolidinone (10 mmol) in 10 mL of THF was added 1-amino β-glucose (10 mmol) in THF (40 mL), HBTU (10 mmol), and DIPEA (11 mmol), and stirred at r.t. for about 30 min. The solvent was evaporated under reduced pressure and the residue was diluted with H_2O (15 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with 10% citric acid (3 × 10 mL), 5% Na_2CO_3 solution (3 × 15 mL), brine, and dried over anhyd Na_2SO_4. The solution was concentrated and purified by column chromatography using EtOAc–hexane (2:8).

Selected Spectral Data

Compound Fmoc-Asn(2,3,4,6-tetra-*O*-acetyl-β-D-gluco-pyranosyl)oxazolidinone (**3a**: n = 1): white solid. IR (KBr): ν_{max} = 1700, 1735, 1801 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.03–2.13 (4 s, 12 H), 2.81 (m, 2 H), 3.80 (m, 1 H), 4.03 (t, 1 H), 4.24 (m, 2 H), 4.42–4.52 (m, 2 H), 4.98 (t, 1 H), 5.05 (t, 1 H), 5.11 (s, 2 H), 5.21–5.35 (m, 2 H), 5.92 (br d, 1 H), 7. 33 (t, 2 H), 7.41 (t, 2 H), 7.52 (d, 2 H), 7.77 (d, 2 H). HRMS (ES): *m/z* calcd for $C_{34}H_{36}N_2NaO_{14}$ [M + Na]$^+$: 719.2064; found: 719.2081.

Compound Fmoc-Gln(2,3,4,6-tetra-*O*-acetyl-β-D-gluco-pyranosyl)oxazolidinone (**3b**: n = 2): white solid. IR (KBr): ν_{max} = 1698, 1730, 1800 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.86 (m, 2 H), 2.03–2.13 (4 s, 12 H), 2.22 (m, 2 H), 3.80 (m, 1 H), 4.03 (d, 1 H), 4.21–4.32 (m, 3 H), 4.48 (m, 2 H), 4.88 (t, 1 H), 5.06 (t, 1 H), 5.12 (s, 2 H), 5.27 (m, 2 H), 5.50 (br d, 1 H), 7. 33 (t, 2 H), 7.41 (t, 2 H), 7.52 (d, 2 H), 7.77 (d, 2 H). HRMS (ES): *m/z* calcd for $C_{35}H_{38}N_2NaO_{14}$ [M + Na]$^+$: 733.2221; found: 733.2254.

(18) **Typical Procedure for the Preparation of Glycosylated *N*$^{\alpha}$-Fmoc-Asn/Gln-OH**

To the glycosylated *N*$^{\alpha}$-Fmoc-Asn/Gln-5-oxazolidinone (10 mmol) dissolved in THF was added 1 N LiOH solution (1 equiv) and stirred at r.t. for 30 min. The reaction was monitored by TLC. After the completion of reaction, the reaction mixture was acidified with 10% citric acid solution and extracted with EtOAc. The organic layer was washed with brine and dried over anhyd Na_2SO_4. The solvent was removed in vacuo, and the resulted residue was purified by column chromatography using $CHCl_3$, MeOH and AcOH (40:2:1).

Compound **4a**: white solid. IR (KBr): ν_{max} = 1705, 1733 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.03–2.13 (4 s, 12 H), 2.81 (m, 2 H), 3.80 (m, 1 H), 4.03 (t, 1 H), 4.24 (m, 2 H), 4.42–4.52 (m, 2 H), 4.98 (t, 1 H), 5.05 (t, 1 H), 5.21–5.35 (m, 2 H), 5.92 (br d, 1 H), 7. 33 (t, 2 H), 7.41 (t, 2 H), 7.52 (d, 2 H), 7.77 (d, 2 H). HRMS (ES): *m/z* calcd for $C_{33}H_{36}N_2NaO_{14}$ [M + Na]$^+$: 707.2064; found: 707.2080.

Compound **4e**: white solid. IR (KBr): ν_{max} = 1698, 1732 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.86 (m, 2 H), 2.03–2.13 (4 s, 12 H), 2.22 (m, 2 H), 3.80 (m, 1 H), 4.03 (d, 1 H), 4.21–4.32 (m, 3 H), 4.48 (m, 2 H), 4.88 (t, 1 H), 5.06 (t, 1 H), 5.27 (2 H, m), 5.50 (br d, 1 H), 7. 33 (t, 2 H), 7.41 (t, 2 H), 7.52 (d, 2 H), 7.77 (d, 2 H). HRMS (ES): *m/z* calcd for $C_{34}H_{38}N_2NaO_{14}$ [M + Na]$^+$: 721.2221; found: 721.2413.