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Synthesis of Ureido-Linked Glycosylated Amino Acids from *N*^z-Fmoc-Asp/Glu-5-oxazolidinones and Their Application to Neoglycopeptide Synthesis

Vommina V. Sureshbabu, Rao Venkataramanarao, Shankar A. Naik,
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Abstract: A simple route for the synthesis of ureido-linked glycosylated amino acids has been described. The key step involves the reaction of isocyanates derived from *N*^z-Fmoc-Asp/Glu-5-oxazolidinones **1** with glycosyl amines followed by hydrolysis. The resulting ureido-linked glycosylated amino acids have been incorporated into peptides. The overall procedure is simple, high-yielding, and involves fewer steps.

Keywords: Curtius rearrangement, *N*^z-Fmoc-Asp/Glu-5-oxazolidinones, neoglycopeptides, ureido-linked glycosylated amino acids

INTRODUCTION

Glycopeptides play decisive roles in biomolecular functions such as cell–cell adhesion, recognition and signaling.^[1,2] The quantitative synthesis of natural glycopeptides is challenging because of the complex nature of native samples, extensive branching, exhaustive protection and deprotection steps, and difficulties involved in carbohydrate coupling reactions.^[3] Hence, synthesizing small molecules of glycopeptide mimics as substitutes

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for the native compounds in biological studies has been emphasized. Synthesis of urea-linked glycopeptides has gained importance because of the biological applications of this class of neoglycopeptides. Therefore, we turned our attention toward development of an efficient synthesis of ureido-linked glycosylated amino acids.

Ichikawa et al. reported the synthesis of urea-linked neoglycopeptides by coupling 1-sugar isocyanates with protected 1,3-diamino propionic acid^[4] (Fig. 1a). Their method involves in situ generation of sugar isocyanates through a multistep procedure and usage of diamino propionic acid, which is expensive or prepared through another lengthy protocol.^[5] On the other hand, Burger et al. synthesized these compounds through the reaction of isocyanates of hexafluoro acetone (HFA)-protected α -hydroxy acids with sugar amines.^[6] This protocol is useful particularly for N^2 -methyl amino acids,^[7] α -hydroxy acids,^[8] and α -mercapto acids.^[9] However, it cannot be extended to obtain isocyanates of HFA-protected activated α -amino acids because of intramolecular trapping of the generated ω isocyanates by the $-\text{NH}$ of the oxazolidinone ring.^[10] Hence while following the approach as in Fig. 1 (b and c), a protocol devoid of internal urea formation is required. Thus, we envisaged an alternate route of generating an isocyanate moiety from N -Fmoc 5-oxazolidinones of aspartic and glutamic acids and coupling it to a sugar amine (Fig. 1, present approach). This approach is advantageous as it

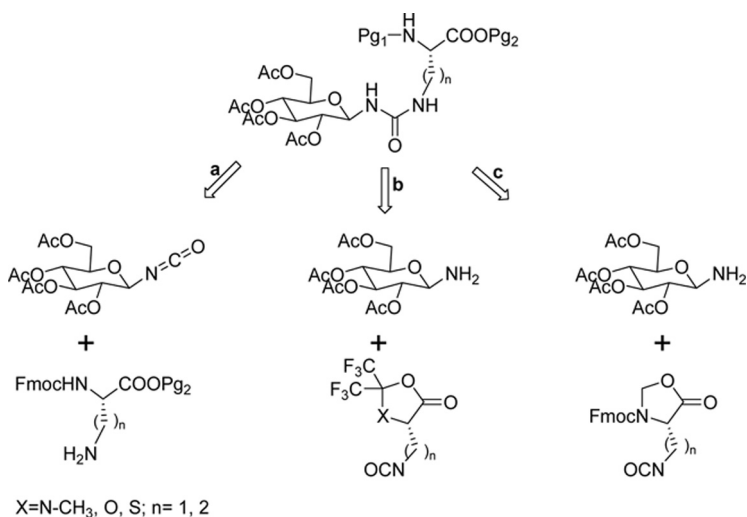
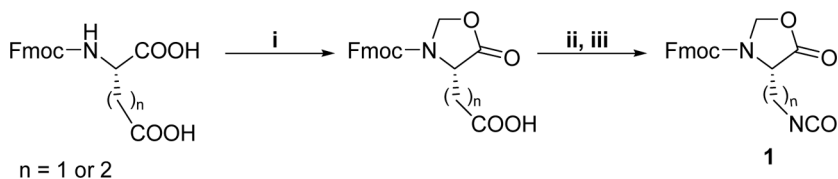


Figure 1. Protocols for ureido-linked glycosylated amino acid synthesis: (a) Ichikawa's approach, (b) Burger's approach, (c) present approach.



Scheme 1. Synthesis of N^α -Fmoc-Asp/Glu-5-oxazolidinone isocyanate: (i) Fmoc-Asp/Glu-oxazolidinone, paraformaldehyde, PTSA, MW; (ii) N-methylmorpholine (NMM), isobutylchloroformate, aq. NaN_3 ; and (iii) MW, 2 min.

involves isocyanates of Fmoc-Asp/Glu-derived 5-oxazolidinones and sugar amines whose preparation is simple, inexpensive, and high-yielding and involves fewer steps.

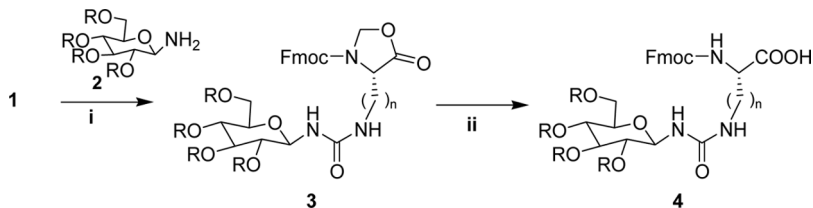
RESULTS AND DISCUSSION

N^α -Fmoc-Asp/Glu-5-oxazolidinones were prepared in high yields following the reported protocols.^[11,12] The yields of these oxazolidinones obtained after column purification were higher by 20% than the corresponding Boc counterparts, thus adding to the advantage of the Fmoc protection strategy. They were then converted to the corresponding acyl azides by the reaction of mixed anhydride with sodium azide and subsequently to isocyanates via Curtius rearrangement upon heating or exposure to microwave irradiation for 1–2 min (Scheme 1). The resulting isocyanates **1** were isolated as solid powders and fully characterized. 1-Amino-2,3,4,6-O-protected- β -D-sugars **2** were prepared following reported protocols.^[13] In all these cases, the β anomers were obtained in an anomeric excess of 98:2, which was evident from their NMR spectra.

The isocyanates **1** were then reacted with **2** in presence of diisopropylethyl amine (DIEA) to obtain the ureido-linked glycosylated amino acid-5-oxazolidinones **3** in good yields. Finally, ureido bonds containing glycosylated amino acids (Scheme 2) were obtained through LiOH-mediated hydrolysis. The compounds **3** were treated with aqueous 1N LiOH solution in THF for 20 min to give corresponding N^α -Fmoc-protected glycosylated amino acids **4** in 70–90% yields (Table 1).

It is noteworthy that the conditions employed selectively hydrolyzed the oxazolidinone ring without any deprotection of either the Fmoc group of amino acid residue or the acetyl or the benzoyl group of the sugar component.

Although the isocyanates **1** made are stable enough at 0 °C for a reasonable period of time, it is desirable to have shelf-stable compounds as key intermediates. Thus the isocyanate **1** was converted to active



Scheme 2. Synthesis of ureido-linked glycosylated amino acids: (i) reagents **1**, **2**, N-methylmorpholine, THF; (ii) 1N LiOH, THF. $n = 1$ or 2 ; R = Ac, Bz.

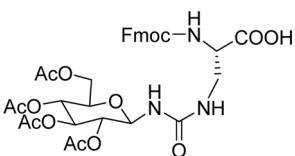
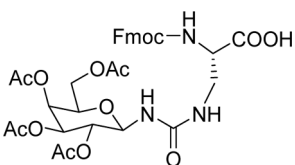
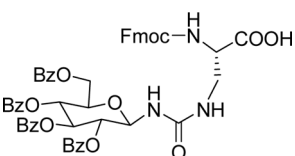
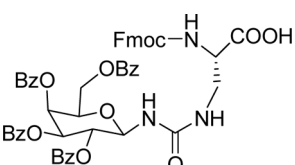
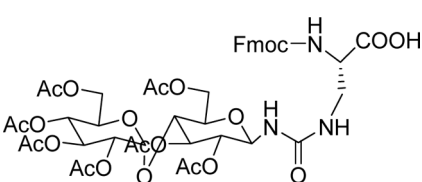
pentafluorophenyl carbamates **5** by reacting with pentafluorophenol in the presence of NMM (Scheme 3). These precursoric carbamates retain the reactivity of isocyanates but show greater stability. The carbamates were found to be stable even up to several months or a year and hence could be stored with extended shelf life. On reaction of **5** with **2**, the ureido-linked glycopeptides were readily obtained. The side product pentafluorophenol can be removed completely during crystallization using ethyl acetate–hexane (2:8). Further, the insertion of ureido-linked glycosylated amino acids into the peptide sequences was done in a straightforward way. Direct coupling of the compounds **4** with amino acid methyl esters using *N,N*-dicyclohexyl carbodiimide-*N*-hydroxy benzotriazole (DCC-HOBt) as peptide coupling agent yielded dipeptidic neoglycopeptides **6** (Scheme 4). The yields in all cases were satisfactory.

For completeness, we undertook the synthesis of the model neoglycopeptide **8** by the solid-phase method. The synthesis was accomplished using commercial Fmoc-Val-Wang resin, and stepwise assembly of the peptide was carried out. Thus, the Fmoc-protected urea bond containing glycosylated aspartic acid was incorporated using HBTU/HOBt in the presence of DIEA, and the peptide was cleaved from the acyl resin using TFA containing phenol/water/thioanisole/1,2-ethanedithiol. The final peptide **8** (Fig. 2) was obtained in 98% purity with 75% yield and was characterized using $^1\text{H-NMR}$, $^{13}\text{C NMR}$, and MALDI mass spectra.

CONCLUSION

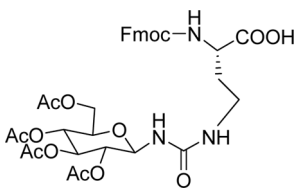
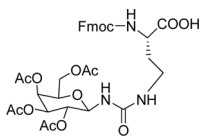
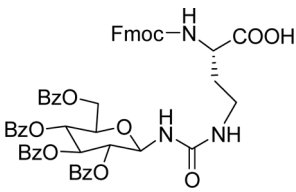
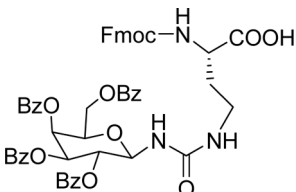
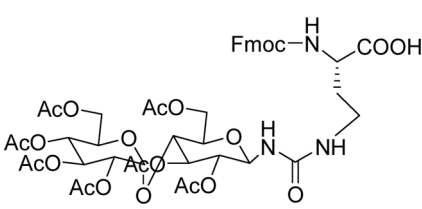
In conclusion, we have developed a simple and rapid approach for the synthesis of ureido-linked glycosylated amino acids employing isocyanates of *N*^z-Fmoc-Asp/Glu-5-oxazolidinones as key and stable intermediates. The sugar amines were prepared using a common literature procedure and coupled to the isocyanates to obtain the target ureas. In parallel, the isocyanates were converted into stable active

Table 1. Synthesis of urea-linked glycosylated amino acids

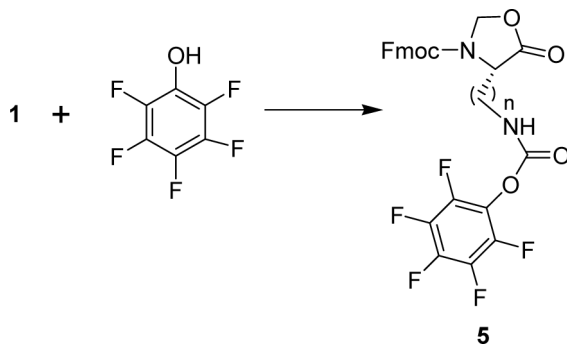
Entry	Product	Mp (°C)	Yield (%)	Mass [M + Na] (calcd.)
1		123	82	722.2173 ^a (722.2120)
2		115	83	722.2173 ^a (722.2132)
3		127	86	914.4 ^b (914.1)
4		113	90	914.4 ^b (914.2)
5		105	71	1175.3 ^b (1175.8)

(Continued)

Table 1. Continued

Entry	Product	Mp (°C)	Yield (%)	Mass [M + Na] (calcd.)
6		99	79	736.2336 ^a (736.2308)
7		135	84	736.2336 ^a (713.2310)
8		102	87	928.4 ^b (928.1)
9		129	89	928.4 ^b (928.2)
10		107	72	1189.3 ^b (1189.1)

^aHRMS.^bES-MS.



Scheme 3. Synthesis of N^z -Fmoc-Asp/Glu-5-oxazolidinone pentafluorophenyl carbamate **5**: (i) **1**, pentafluorophenol, *N*-methylmorpholine, THF. $n = 1$ or 2 .

pentafluorophenyl carbamates and were employed to generate the same ureido-linked glycosylated amino acids. The glycosylated oxazolidinones were then hydrolyzed to free acids. The overall procedure is efficient as it involves fewer steps, is simple to execute, and results in good yields of products. The utility of such ureido-linked glycosylated amino acids to synthesize neoglycopeptides both by solid phase and in solution has also been demonstrated.

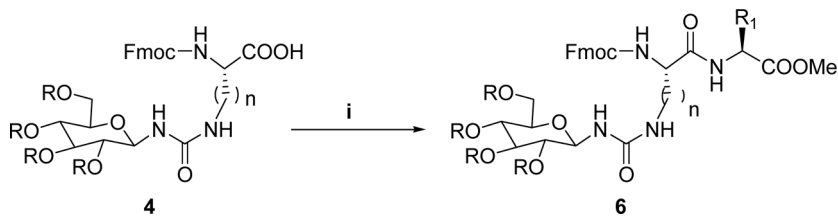
EXPERIMENTAL

All solvents were freshly distilled before use. Amino acids were used as received from Sigma-Aldrich Company. Melting points were determined on a Buchi model 150 melting-point apparatus in open capillaries and are uncorrected. IR spectra were recorded on a Nicolet model impact 400D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). ^1H NMR spectra were recorded on a Bruker AMX 400-MHz spectrometer. Mass spectra were recorded on MALDI-TOF (Kratos) mass spectrometer. Unless or otherwise mentioned, all amino acids used have the L-configuration. Thin-layer chromatography (TLC) was carried out using the precoated silica-gel G₂₅₄ plates.

Synthesis of N^z -Fmoc-Asp/Glu-oxazolidinone Isocyanate **1**:

Typical Procedure

To a solution of N^z -Fmoc-Asp/Glu-oxazolidinone (1 mmol) in 10 mL of dry THF, *N*-methylmorpholine (1.1 mmol) and isobutylchloroformate (1.1 mmol) were added and stirred for 5 min at 0°C in an ice-salt bath.



Scheme 4. Synthesis of neoglycopeptides using ureido-linked glycosylated amino acids: (i) reagent **4**, DCC, HOBt, amino acid ester, HCl, N-methylmorpholine, DCM. **6a**) $n = 1$, $R_1 = \text{CH}(\text{CH}_3)_2$; **6b**) $n = 1$, $R_1 = \text{CH}_3$; **6c**) $n = 1$, $R_1 = \text{CH}_3$; **6d**) $n = 2$, $R_1 = \text{CH}(\text{CH}_3)_2$; **6e**) $n = 2$, $R_1 = \text{CH}_3$; **6f**) $n = 2$, $R_1 = \text{CH}_3$; $R = \text{Ac}$ or Bz .

Sodium azide (1.5 mmol) in water (1 mL) was added to the solution and stirred at the same temperature for about 15 min. After the reaction was complete, the THF was evaporated and extracted with ethyl acetate (15 mL). The organic layer was washed with dilute citric acid, 10% Na_2CO_3 , and brine; dried over anhydrous Na_2SO_4 ; and evaporated in vacuo. The resulting azide was dissolved in toluene (10 mL) and exposed to microwave irradiation for 2 min. After the reaction was complete, solvent was removed in vacuo to get the isocyanate.

Urea-Linked Glycosylated Fmoc-Asp/Glu-oxazolidinone Derivatives: Procedure for the Synthesis of **3**

To the solution containing N^{α} -Fmoc-Asp/Glu-oxazolidinone isocyanate (1 mmol) in dry DCM (10 mL), glycosyl amine and N-methylmorpholine (1.1 mmol) were added at 0°C and stirred overnight. After the completion of the reaction, the residue was washed with citric acid and brine

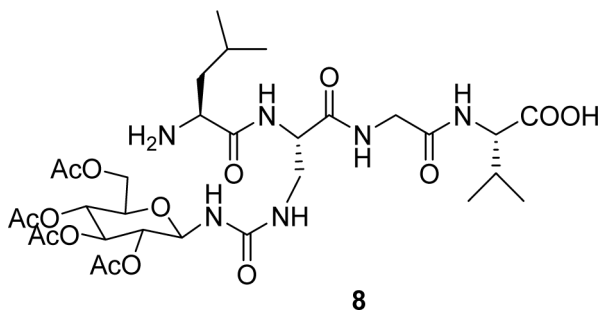


Figure 2. A model ureido-linked glycopeptide synthesized by the solid-phase approach.

and dried over anhydrous Na_2SO_4 . The solvent was evaporated under reduced pressure to get the desired compound.

Ureido-Linked Amino Acids: Procedure for the Synthesis of 4 (Table 1)

Solution of LiOH (1 N, 2 mmol) was added in one portion to a solution of 3 (1 mmol) in THF (10 mL) and stirred for 1 h. The resulting solution was acidified with 10% HCl (10 mL) and extracted with EtOAc (2×10 mL). The combined organic extract was washed with brine and dried (Na_2SO_4). The solvent was removed in vacuo to leave the crude product as a colorless solid, which was column purified (CHCl_3 –methanol–acetic acid; 40:2:1).

Data

Fmoc-Asp- β -[NH-CO-NH-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl)]-OH (Entry 1)

IR (KBr) 1251, 1707, 1740 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.99, 2.02, 2.04, 2.06 (4 s, 12H), 3.72 (m, 2H), 3.81 (m, 1H), 4.12 (dd, $J=7.1$ Hz, 1H), 4.19 (t, $J=6.9$ Hz, 1H), 4.21 (t, $J=7.1$ Hz, 1H), 4.26 (dd, $J=8.4$ Hz, 1H), 4.55 (d, $J=6.7$ Hz, 2H), 4.65 (d, $J=7.1$ Hz, 1H), 4.93 (dd, $J=8.2$ Hz, 1H), 5.06 (t, $J=9.6$ Hz, 1H), 5.17 (t, $J=9.8$ Hz, 1H), 5.57 (d, $J=6.2$ Hz, 1H), 6.58 (br, 1H), 6.72 (br, 1H), 7.25–7.75 (m, 8H).

Fmoc-Asp- β -[NH-CO-NH-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)]-OH (Entry 2)

IR (KBr): 1250, 1707, 1740 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.00, 2.02, 2.05, 2.07 (4 s, 12H), 3.74 (m, 2H), 3.80 (m, 1H), 4.14 (dd, $J=7.1$ Hz, 1H), 4.22 (t, $J=6.9$ Hz, 1H), 4.26 (t, $J=7.1$ Hz, 1H), 4.28 (dd, $J=8.4$ Hz, 1H), 4.56 (d, $J=6.7$ Hz, 2H), 4.65 (d, $J=7.1$ Hz, 1H), 4.90 (dd, $J=8.1$ Hz, 1H), 5.08 (t, $J=9.5$ Hz, 1H), 5.15 (t, $J=9.8$ Hz, 1H), 5.60 (d, $J=6.2$ Hz, 1H), 6.56 (br, 1H), 6.70 (br, 1H), 7.20–7.75 (m, 8H).

Fmoc-Asp- β -[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl- β -D-glucopyranosyl)]-OH (Entry 3)

IR (KBr): 1251, 1703, 1740 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.99, 2.01, 2.03, 2.06 (4 s, 12H), 3.73 (m, 2H), 3.82 (m, 1H), 4.13 (dd, $J=7.1$ Hz,

Hz, 1H), 4.19 (t, $J=6.8$ Hz, 1H), 4.21 (t, $J=7.2$ Hz, 1H), 4.30 (dd, $J=8.4$ Hz, 1H), 4.56 (d, $J=6.5$ Hz, 2H), 4.65 (d, $J=7.3$ Hz, 1H), 4.90 (dd, $J=8.2$ Hz, 1H), 5.08 (t, $J=9.6$ Hz, 1H), 5.15 (t, $J=9.8$ Hz, 1H), 5.60 (d, $J=6.1$ Hz, 1H), 6.59 (br, 1H), 6.71 (br, 1H), 7.10–7.70 (m, 28H).

Fmoc-Asp- β -[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)]-OH (Entry 4)

IR (KBr): 1251, 1702, 1745 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.99, 2.01, 2.03, 2.05 (4s, 12H), 3.73 (m, 2H), 3.82 (m, 1H), 4.13 (dd, $J=7.1$ Hz, 1H), 4.19 (t, $J=6.8$ Hz, 1H), 4.21 (t, $J=7.2$ Hz, 1H), 4.30 (dd, $J=8.4$ Hz, 1H), 4.56 (d, $J=6.5$ Hz, 2H), 4.65 (d, $J=7.3$ Hz, 1H), 4.90 (dd, $J=8.2$ Hz, 1H), 5.08 (t, $J=9.6$ Hz, 1H), 5.15 (t, $J=9.8$ Hz, 1H), 5.60 (d, $J=6.1$ Hz, 1H), 6.59 (br, 1H), 6.71 (br, 1H), 7.15–7.70 (m, 28H).

Fmoc-Asp- β -[NH-CO-NH-(2,2',3,3',4',6,6'-hepta-O-acetyl- β -D-maltosyl)]-OH (Entry 5)

IR (KBr): 1257, 1715, 1735 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.03–2.13 (s, 21H), 2.61 (d, $J=4.5$ Hz, 1H), 3.30 (d, $J=9.0$ Hz, 1H), 4.13 (t, $J=7.1$ Hz, 1H), 4.24 (d, $J=7.0$ Hz, 2H), 4.28 (m, 1H), 4.48 (dd, $J=7.1$ Hz, 1H), 4.63 (dd, $J=6.9$ Hz, 1H), 4.66 (d, $J=7.0$ Hz, 1H), 5.50 (t, $J=9.6$ Hz, 1H), 5.71 (t, $J=9.1$ Hz, 1H), 5.92 (t, $J=7.1$ Hz, 1H), 6.58 (br, 1H), 6.72 (br, 1H), 7.20–7.80 (m, 8H).

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl)]-OH (Entry 6)

IR (KBr): 1222, 1698, 1749 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.65–1.75 (m, 2H), 2.00–2.07 (4s, 12H), 2.95–3.12 (br d, 2H), 3.15–3.25 (m, 2H), 4.12 (m, 1H), 4.20 (t, $J=7.0$ Hz, 1H), 4.29 (m, 1H), 4.43 (m, 2H), 4.45 (q, $J=7.1$ Hz, 1H), 4.62 (q, $J=7.0$ Hz, 1H), 4.99 (d, $J=7.1$ Hz, 1H), 5.50 (t, $J=9.5$ Hz, 1H), 5.71 (t, $J=9.0$ Hz, 1H), 5.93 (t, $J=7.2$ Hz, 1H), 6.55 (br, 1H), 6.69 (br, 1H), 7.33–7.77 (m, 8H).

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)]-OH (Entry 7)

IR (KBr): 1222, 1701, 1750 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.70–1.78 (br d, 2H), 1.99–2.06 (4s, 12H), 2.92–3.10 (br d, 2H), 3.12–3.20

(m, 2H), 4.13 (m, 1H), 4.21 (t, $J=7.1$ Hz, 1H), 4.30 (m, 1H), 4.43 (m, 2H), 4.45 (q, $J=6.9$ Hz, 1H), 4.60 (q, $J=7.0$ Hz, 1H), 4.97 (d, $J=7.2$ Hz, 1H), 5.51 (t, $J=9.6$ Hz, 1H), 5.70 (t, $J=9.0$ Hz, 1H), 5.93 (t, $J=7.1$ Hz, 1H), 6.62 (br, 1H), 7.30–7.78 (m, 8H).

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl- β -D-glucopyranosyl)]-OH (Entry 8)

IR (KBr): 1222, 1698, 1749, 1800 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.72–1.78 (br d, 2H), 1.99–2.08 (4s, 12H), 2.92 (m, 2H), 3.82 (m, 1H), 4.12 (dd, $J=7.1$ Hz, 1H), 4.19 (t, $J=6.8$ Hz, 1H), 4.21 (t, $J=7.2$ Hz, 1H), 4.30 (dd, $J=8.4$ Hz, 1H), 4.56 (d, $J=6.5$ Hz, 2H), 4.65 (d, $J=7.3$ Hz, 1H), 4.90 (dd, $J=8.2$ Hz, 1H), 5.08 (t, $J=9.6$ Hz, 1H), 5.15 (t, $J=9.8$ Hz, 1H), 5.60 (d, $J=6.1$ Hz, 1H), 6.59 (br, 1H), 6.71 (br, 1H), 7.10–7.70 (m, 28H).

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)]-OH (Entry 9)

IR (KBr): 1222, 1700, 1745 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.72–1.76 (br d, 2H), 1.98–2.05 (4s, 12H), 3.73 (m, 2H), 3.82 (m, 1H), 4.13 (dd, $J=7.1$ Hz, 1H), 4.19 (t, $J=6.8$ Hz, 1H), 4.20 (t, $J=7.1$ Hz, 1H), 4.31 (dd, $J=8.5$ Hz, 1H), 4.56 (d, $J=6.2$ Hz, 2H), 4.66 (d, $J=7.3$ Hz, 1H), 4.91 (dd, $J=8.1$ Hz, 1H), 5.09 (t, $J=9.5$ Hz, 1H), 5.14 (t, $J=9.8$ Hz, 1H), 5.62 (d, $J=6.1$ Hz, 1H), 6.58 (br, 1H), 6.70 (br, 1H), 7.15–7.70 (m, 28H).

Fmoc-Glu- γ -[NH-CO-NH-(2,2',3,3',4',6,6'-hepta-O-acetyl- β -D-maltosyl)]-OH (Entry 10)

IR (KBr): 1254, 1698, 1742 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 1.70–1.76 (br d, 2H), 2.03–2.13 (s, 21H), 2.61 (d, $J=4.5$ Hz, 1H), 3.30 (d, $J=9.0$ Hz, 1H), 4.12 (t, $J=7.1$ Hz, 1H), 4.20 (t, $J=7.1$ Hz, 1H), 4.24 (d, $J=7.0$ Hz, 2H), 4.28 (m, 1H), 4.48 (dd, $J=7.1$ Hz, 1H), 4.63 (dd, $J=6.9$ Hz, 1H), 4.66 (d, $J=7.0$ Hz, 1H), 5.50 (t, $J=9.6$ Hz, 1H), 5.71 (t, $J=9.1$ Hz, 1H), 5.92 (t, $J=7.1$ Hz, 1H), 6.58 (br, 1H), 6.72 (br, 1H), 7.20–7.80 (m, 8H).

Synthesis of Neoglycopeptides: General Procedure for Synthesis of **6** (Scheme 4)

To a stirred solution of N^z -Fmoc-urea-linked glycosyl amino acid (1 mmol) in dry DCM (50 mL), DCC (1.2 mmol) and HOBT (1.2 mmol)

were added at 0 °C and stirred for 10 min. A solution of amino acid methyl ester (1.5 mmol) neutralized with NMM (2 mmol) in CH₂Cl₂ was added to the reaction mixture and stirred for 4 h. Precipitated DCU was filtered out, and the filtrate was acidified using dil. HCl (20 mL). The resulting organic layer was washed with 10% Na₂CO₃ and brine, then dried over anhydrous Na₂SO₄. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography using EtOAc–hexane (3:7) as eluant to afford the final product as a white solid.

Data

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)]-val-OMe (**6a**)

Mp 126–128 °C; Yield: 82%; IR (KBr): 1700, 1740 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.93 (d, *J* = 6.6 Hz, 6H), 1.84 (m, 1H), 2.00–2.06 (s, 12H), 2.70 (br s, 1H), 3.29 (br s, 1H), 3.70 (s, 3H), 4.11 (m, 1H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.49 (dd, *J* = 6.9 Hz, 1H), 4.61 (dd, *J* = 7.1 Hz, 1H), 5.01 (d, *J* = 9.5 Hz, 1H), 5.51 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.26–7.95 (m, 8H); HRMS (ES): *m/z* calcd. for C₃₉H₄₈N₄NaO₁₅, [M + Na]⁺: 835.8162; found: 835.7024.

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)]-Ala-OMe (**6b**)

Mp 126–128 °C; yield: 82%; IR (KBr): 1251, 1702, 1745 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.16 (d, *J* = 7.9 Hz, 3H), 2.70 (br s, 1H), 3.70 (s, 3H), 4.12 (m, 1H), 4.20 (t, *J* = 7.1 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.50 (d, *J* = 6.9 Hz, 1H), 4.61 (d, *J* = 7.1 Hz, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.70 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.23–8.07 (m, 28H); ESI MS: *m/z* calcd. for C₅₇H₅₂N₄NaO₁₅, [M + Na]⁺: 1055.3; found: 1055.5.

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)]-Ala-OMe (**6c**)

Mp 122–124 °C; yield: 82%; IR (KBr): 1251, 1700, 1740 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.16 (d, *J* = 7.9 Hz, 3H), 2.70 (br s, 1H), 3.70 (s, 3H), 4.12 (m, 1H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.50 (d, *J* = 6.9 Hz, 1H), 4.61 (d, *J* = 7.1 Hz, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.50

(t, $J=9.6$ Hz, 1H), 5.70 (t, $J=9.0$ Hz, 1H), 5.93 (t, $J=4.3$ Hz, 1H), 7.23–8.07 (m, 28H); ESI MS: m/z calcd. for $C_{57}H_{52}N_4NaO_{15}$, $[M + Na]^+$: 1055.3; found: 1055.8.

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl)] Val-Ome (**6d**)

Mp 152–154 °C; yield: 85%; IR (KBr): 1222, 1700, 1749 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 0.93 (t, $J=6.6$ Hz, 6H), 1.84 (m, 1H), 2.00–2.06 (s, 12H), 1.90–2.01 (br d, 2H), 2.95–3.12 (br d, 2H), 3.70 (s, 3H), 4.12 (m, 1H), 4.48 (m, 1H), 4.62 (m, 1H), 4.99 (d, $J=9.0$ Hz, 1H), 5.50 (t, $J=9.6$ Hz, 1H), 5.71 (t, $J=9.0$ Hz, 1H), 5.93 (t, $J=4.3$ Hz, 1H), 7.28–7.77 (m, 8H); ESI MS: m/z calcd. for $C_{40}H_{50}N_4NaO_{15}$, $[M + Na]^+$: 849.84; found: 849.4.

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl- β -D-glucopyranosyl)]-Ala-OMe (**6e**)

Mp 110–112 °C; yield: 85%; IR (KBr): 1222, 1698, 1750 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, $J=7.9$ Hz, 3H), 1.90–2.01 (br d, 2H), 2.95–3.12 (br d, 2H), 3.69 (s, 3H), 4.11 (m, 1H), 4.47 (m, 1H), 4.62 (m, 1H), 5.00 (d, $J=9.0$ Hz, 1H), 5.50 (t, $J=9.6$ Hz, 1H), 5.71 (t, $J=9.0$ Hz, 1H), 5.93 (t, $J=4.3$ Hz, 1H), 7.23–8.07 (m, 28H); ESI MS: m/z calcd. for $C_{58}H_{54}N_4NaO_{15}$, $[M + Na]^+$: 1069.3; found: 1069.7.

Fmoc-Glu- γ -[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)]-Ala-OMe (**6f**)

Mp 164–166 °C; yield: 85%; IR (KBr): 1222, 1700, 1750 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, $J=7.9$ Hz, 3H), 1.90–2.01 (br d, 2H), 2.95–3.12 (br d, 2H), 3.69 (s, 3H), 4.11 (m, 1H), 4.47 (m, 1H), 4.62 (m, 1H), 5.00 (d, $J=9.0$ Hz, 1H), 5.50 (t, $J=9.5$ Hz, 1H), 5.71 (t, $J=9.0$ Hz, 1H), 5.93 (t, $J=4.3$ Hz, 1H), 7.23–8.07 (m, 28H); ESI MS: m/z calcd. for $C_{58}H_{54}N_4NaO_{15}$, $[M + Na]^+$: 1069.3, found: 1069.3.

Peptide Data

H-Leu-Asp[ψ (NH-CO-NH)]-Gly-Val-OH

1H NMR (400 MHz, $CDCl_3$) δ 0.93 (m, 12H), 1.3–1.35 (m, 2H), 1.63–1.72 (m, 2H), 2.02–2.10 (s, 12H), 3.67 (m, 2H), 4.12 (m, 2H), 4.34 (m, 2H),

4.51–4.78 (m, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 17.2, 20.1, 20.2, 24.3, 30.8, 38.7, 39.2, 43.5, 53.8, 54.0, 63.0, 64.2, 67.0, 68.6, 70.8, 91.2, 152.3, 166.3, 166.7, 167.1, 170.2, 171.0 MALDI-TOF: anal. calcd. for $\text{C}_{31}\text{H}_{50}\text{N}_6\text{O}_{15}$ $[\text{M} + 1]^+$: 747. 2.

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