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Homoserine as an Aspartic Acid Precursor for Synthesis of Proteoglycan Glycopeptide Containing Aspartic Acid and a Sulfated Glycan Chain

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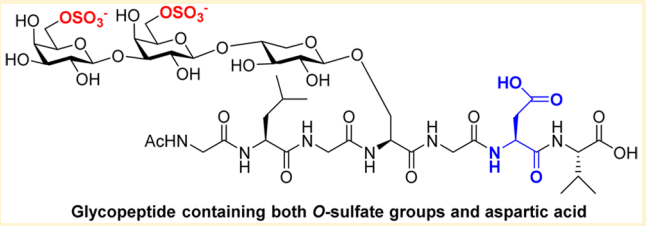
Supporting Information

ABSTRACT: Among many hurdles in synthesizing proteoglycan glycopeptides, one challenge is the incorporation of aspartic acid in the peptide backbone and acid sensitive *O*-sulfated glycan chains. To overcome this, a new strategy was developed utilizing homoserine as an aspartic acid precursor. The conversion of homoserine to aspartic acid in the glycopeptide was successfully accomplished by late stage oxidation using (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO) and bis(acetoxy)iodobenzene (BAIB). This is the first time that a glycopeptide containing aspartic acid and an *O*-sulfated glycan was synthesized.



Glycopeptide containing both *O*-sulfate groups and aspartic acid

Proteoglycans (PGs) are macromolecular glycoproteins that contain a core protein bearing one or more glycosaminoglycan (GAG) chains. The GAG chains are attached to a serine (Ser) residue in the core protein through a tetrasaccharide linkage region (GlcA-β-1,3-Gal-β-1,3-Gal-β-1,4-Xyl), where both the GAG chains and the linkage glycan can contain multiple sulfates.[1,2] PGs play important roles in many biological processes including cell proliferation, differentiation, wound repair, and tissue remodeling.[3−6] As naturally existing PGs are highly heterogeneous in glycan structures, to better understand their structure−activity relationships, synthetic methods enabling access to well-defined PG glycoproteins or glycopeptides are urgently needed.

Although glycosaminoglycan oligosaccharides[7−12] and glycopeptides with the linkage region[7,13−21] have been synthesized, synthetic strategies toward sulfated glycopeptides are underdeveloped. Recently, we have initiated a program targeting the synthesis of the highly complex PG glycopeptides.[7,21] One significant challenge encountered is that *O*-sulfates are highly sensitive to acids. As a result, common amino acid side chain protective groups such as ^{t}butyl (^{t}Bu) ester and trityl cannot be utilized in order to avoid sulfate cleavage during acid promoted deprotection.

The PG amino acid sequences in regions adjacent to the glycosylated serine are known to be rich in acidic amino acid residues including both aspartic acids (Asp) and glutamic acids, usually within a few residues from the serine.[22] During chemical synthesis of the glycopeptide, the side chain carboxylic acids must be protected. Benzyl esters were explored first as the side chain protective group, which was successful in the synthesis of glutamic acid containing glycopeptides.[7] However, when benzyl ester protected Asp was used, cyclic aspartimide was formed due to nucleophilic attack of the benzyl ester by the nitrogen atom in the preceding amide in the backbone (Figure 1). The aspartimide could not be converted back to Asp in high yields.[23] Although the generation of aspartimide in the *N*-glycan glycopeptide synthesis could be suppressed by using pseudoproline on Ser or threonine (Thr) within the tripeptide sequence Asn-Xaa-Ser/Thr,[24,25] it is not applicable to PG synthesis due to the need for strong acidic condition to deprotect the pseudoproline as well as the infrequencies of Asn-Xaa-Ser/Thr sequences in PG. We have also tested allyl ester as a protective group for Asp, which gave similar outcomes as the benzyl ester. The backbone amide preceding the Asp was protected next using a methoxy benzyl group. However, while this completely prevented aspartimide formation, the much reduced reaction rate in peptide formation using the methoxy benzyl protected amino acid and difficulty in deprotection rendered the backbone protection approach undesirable. To overcome the difficulties encountered and provide access to glycopeptides containing both sulfated glycans and Asp, herein we report a new strategy utilizing homoserine as the Asp

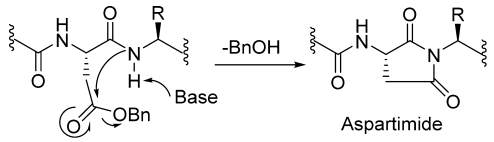


Figure 1. Benzyl ester protected Asp forms undesired aspartimide side products during glycopeptide synthesis.

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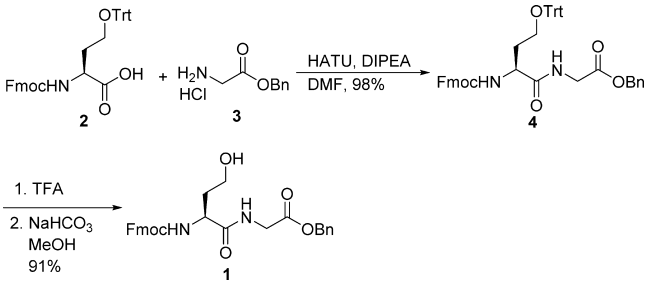
precursor and its late stage transformation to Asp for sulfated glycopeptide synthesis.

To establish a mild and efficient protocol to convert homoserine to Asp in a peptide, we first synthesized a homoserine dipeptide **1** (Scheme 1). Trityl protected Fmoc homoserine **2** was coupled with glycine benzyl ester **3** using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate (HATU) to give the dipeptide **4**. This was followed by trityl removal leading to free primary alcohol containing dipeptide **1** in an excellent overall yield.

Scheme 1. Synthesis of Dipeptide 1

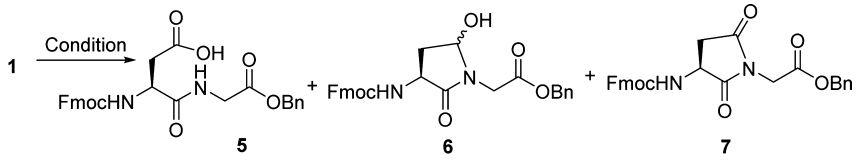


The oxidation of **1** was tested first under the Parikh–Doering conditions[26] with SO_3·pyridine and DMSO (Table 1, entry 1). No desired carboxylic acid **5** or aldehyde intermediate was obtained. Instead, the γ-hydroxy lactam **6** was isolated in 71% yield. Pyridinium dichromate (PDC) oxidation was examined next. When dipeptide **1** was exposed to 3 equiv of PDC in dimethylformamide (DMF),[27] 26% of the desired product **5** was produced along with **6** (13%) and aspartimide **7** (28%) (entry 2). Imide formation from oxidation of a primary alcohol in the presence of a neighboring primary amide has been reported before.[28] Ruthenium tetroxide *in situ* generated from $RuCl_3$ and $NaIO_4$[29] has been used to convert homoserine derivatives to Asp in moderate yields.[30,31] In our hands, this condition afforded the desired product in ∼40% yield with both catalytic and stoichiometric amounts of $RuCl_3$ used (entries 3–4). Another mild oxidation condition, i.e., (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)/bis(acetoxy)iodobenzene (BAIB),[32−34] was investigated next. With a catalytic amount of TEMPO (0.2 equiv), the full consumption of alcohol **1** was achieved in only 1.5 h leading to compound **5** in 57% yield (entry 5). When the amounts of TEMPO in the reaction increased to 0.5, 0.9, or 1.2 equiv, the yields of product **5** were 61%, 70%, and 81% respectively, while the yields of hydroxy lactam **6** decreased correspondingly with about 17% of aspartimide **7** (entries 6–8). Addition of more than 1.2 equiv of TEMPO did not further improve the yield of **5**.

The conversion of homoserine to Asp presumably goes through an aldehyde intermediate. The aldehyde can be hydrated to form a germinal diol, which can be subsequently transformed to the desired carboxylic acid in one pot (Figure 2, pathway a). However, a competing reaction is the nucleophilic attack of the aldehyde by the backbone amide preceding the homoserine resulting in the formation of the undesired five membered γ-hydroxy lactam **6** (Figure 2, pathway b). The Parikh–Doering oxidation primarily led to the carbonyl product, thus resulting in the predominant formation of γ-hydroxy lactam (Table 1, entry 1). The increase of the yield of carboxylic acid **5** with higher amounts of TEMPO (entries 5–8) is presumably due to more rapid oxidation of the germinal diol to the carboxylic acid.

The aspartimide **7** could be formed either through dehydration of carboxylic acid **5** or oxidation of the γ-hydroxy lactam **6**. When the reaction time for TEMPO/BAIB oxidation was shortened to 0.5 h (entry 9), the aspartimide **7** was not detected and compound **6** was increased to 19% yield, while the yield for carboxylic acid **5** experienced little change. This indicated that the aspartimide **7** was most likely derived from the hydroxy lactam **6** (Figure 2). It has been shown that addition of an acidic additive, i.e., 1-hydroxybenzotriazole (HOBt), to a peptide coupling reaction could reduce the formation of aspartimide.[23,35,36] However, when HOBt was added to our oxidation reaction, the yield of the desired carboxylic acid **5** was reduced to 35% along with 58% hydroxy lactam **6** (Table 1, entry 10). Other acidic conditions such as

Table 1. Establishment of a Suitable Condition for Oxidation of Homoserine in Dipeptide 1[a]



entry	condition	*t* (h)	yield[e] 5	6	7
1	DMSO, SO_3·Py (5 equiv)	5	–	71%	–
2	PDC (3 equiv), DMF	20	26%	13%	28%
3	$RuCl_3$ (0.2 equiv), $NaIO_4$ (3 equiv)[b]	3	40%	36%	–
4	$RuCl_3$ (1.2 equiv), $NaIO_4$ (3 equiv)[b]	3	44%	6%	–
5	TEMPO (0.2 equiv), BAIB (2.5 equiv)[c]	1.5	57%	19%	18%
6	TEMPO (0.5 equiv), BAIB (2.5 equiv)[c]	1.5	61%	10%	18%
7	TEMPO (0.9 equiv), BAIB (2.5 equiv)[c]	1.5	70%	6%	17%
8	TEMPO (1.2 equiv), BAIB (2.5 equiv)[c]	1.5	81%	3%	15%
9	TEMPO (1.2 equiv), BAIB (2.5 equiv)[c]	0.5	80%	19%	–
10	TEMPO (1.2 equiv), BAIB (2.5 equiv)[c,d]	0.5	35%	58%	–

[a]All reactions were performed at room temperature. [b]Solvent is $CH_3CN/CCl_4/H_2O$ = 2/2/3. [c]Solvent is dichloromethane (DCM)/^{t}BuOH/H_2O = 4/4/1. [d]HOBt (1 equiv) is additive. [e]Isolated yield.

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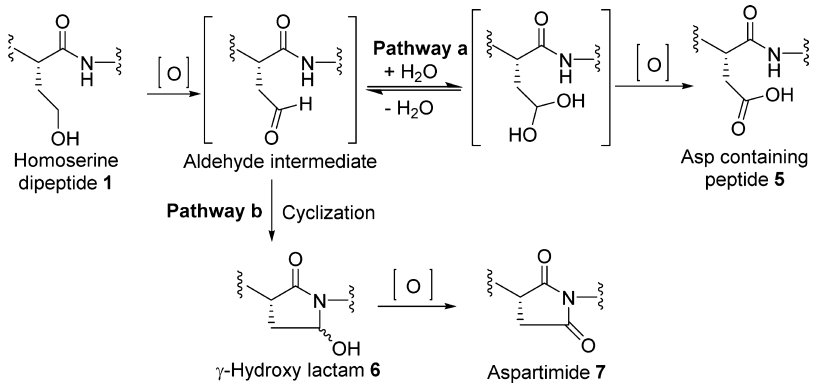
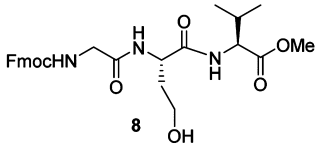
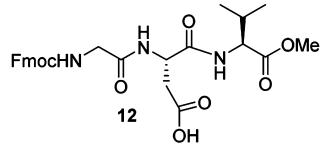
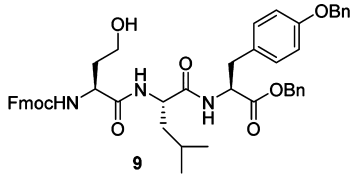
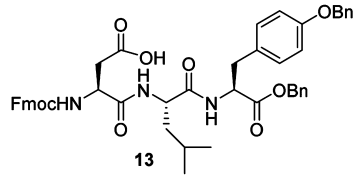
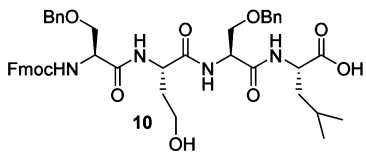
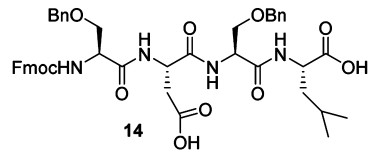
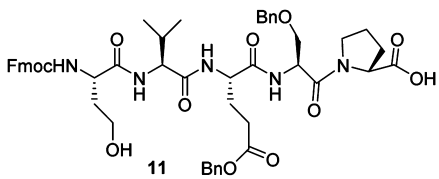
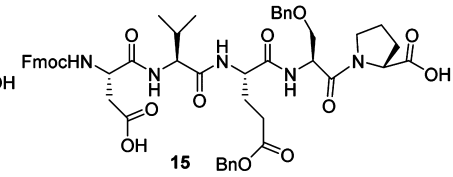


Figure 2. Proposed intermediates and mechanism for homoserine oxidation.

Table 2. Multiple Homoserine Containing Oligopeptides Can Be Converted to the Corresponding Asp Peptides in Good Yields[a]

entry	Substrate	Product	Yield[b]
1			78%
2			80%
3			67%
4			58%

[a]Reaction conditions: alcohol (0.05 mmol), TEMPO (1.2 equiv), BAIB (2.4 equiv), 1.2 mL of DCM/^{t}BuOH/H_2O = 4/4/1, room temperature, 45 min. [b]Isolated yield.

addition of acetic acid and an acidic buffer were also investigated, which did not lead to better yields of **5** compared to that without the acid.

With the oxidation protocol established, longer peptides were examined as substrates. When tripeptides **8** and **9** were subjected to the TEMPO/BAIB condition, the reactions proceeded smoothly producing carboxylic acid products in good yields (Table 2, entries 1 and 2). The *p*-benzyloxy phenyl moiety in **9** was not affected by the oxidation. Homoserine containing oligo-peptides **10** and **11** were successfully synthesized in good yields using Fmoc based solid phase peptide synthesis. The tetrapeptide **10** and pentapeptide **11** were also converted to the desired products **12** and **13** (entries 3 and 4). These results encouraged us to proceed to the synthesis of glycopeptides.

Glycopeptide **16** without *O*-sulfate groups was synthesized starting from the trisaccharide serine module **17**[7,21] (Scheme 2). Trisaccharide **17** was exposed to piperidine for Fmoc removal followed by chemoselective coupling with homoserine containing tripeptide **18** to afford the homoserine glycopeptide **19**. During the course of coupling, it was observed that the free primary hydroxyl group in **18** did not interfere with the amide bond formation. Subsequently, glycopeptide **19** was oxidized with our TEMPO/BAIB condition. The oxidation was chemoselective, which did not affect the two free secondary alcohols in the terminal galactosyl unit of the peptide. Lastly,

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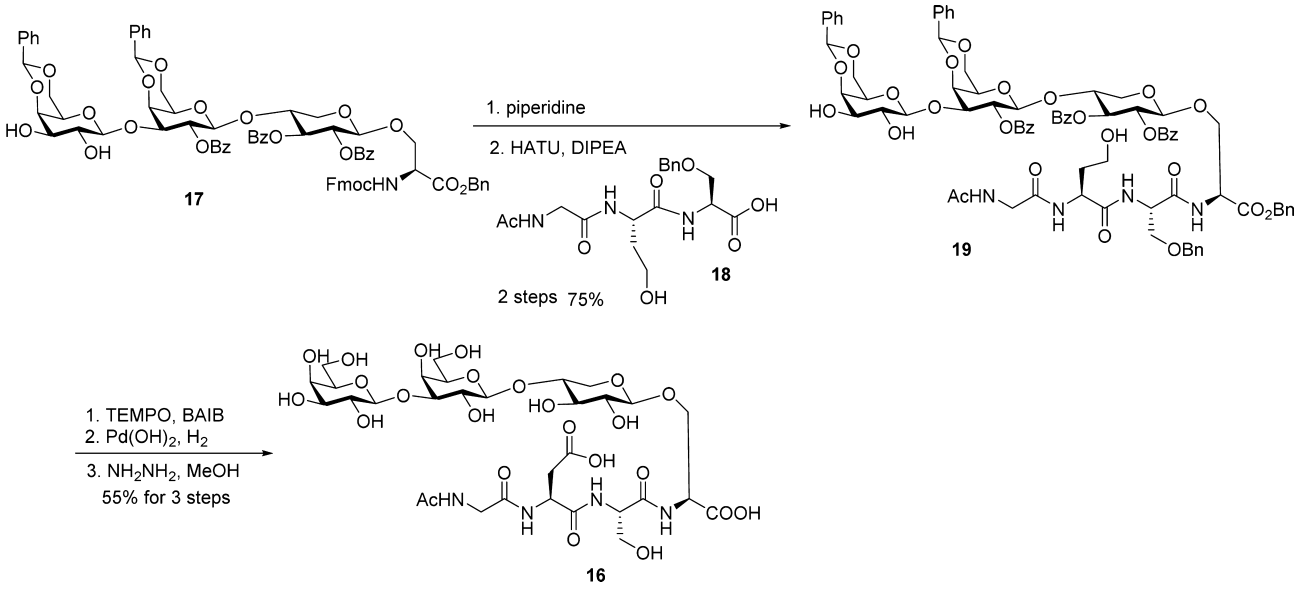
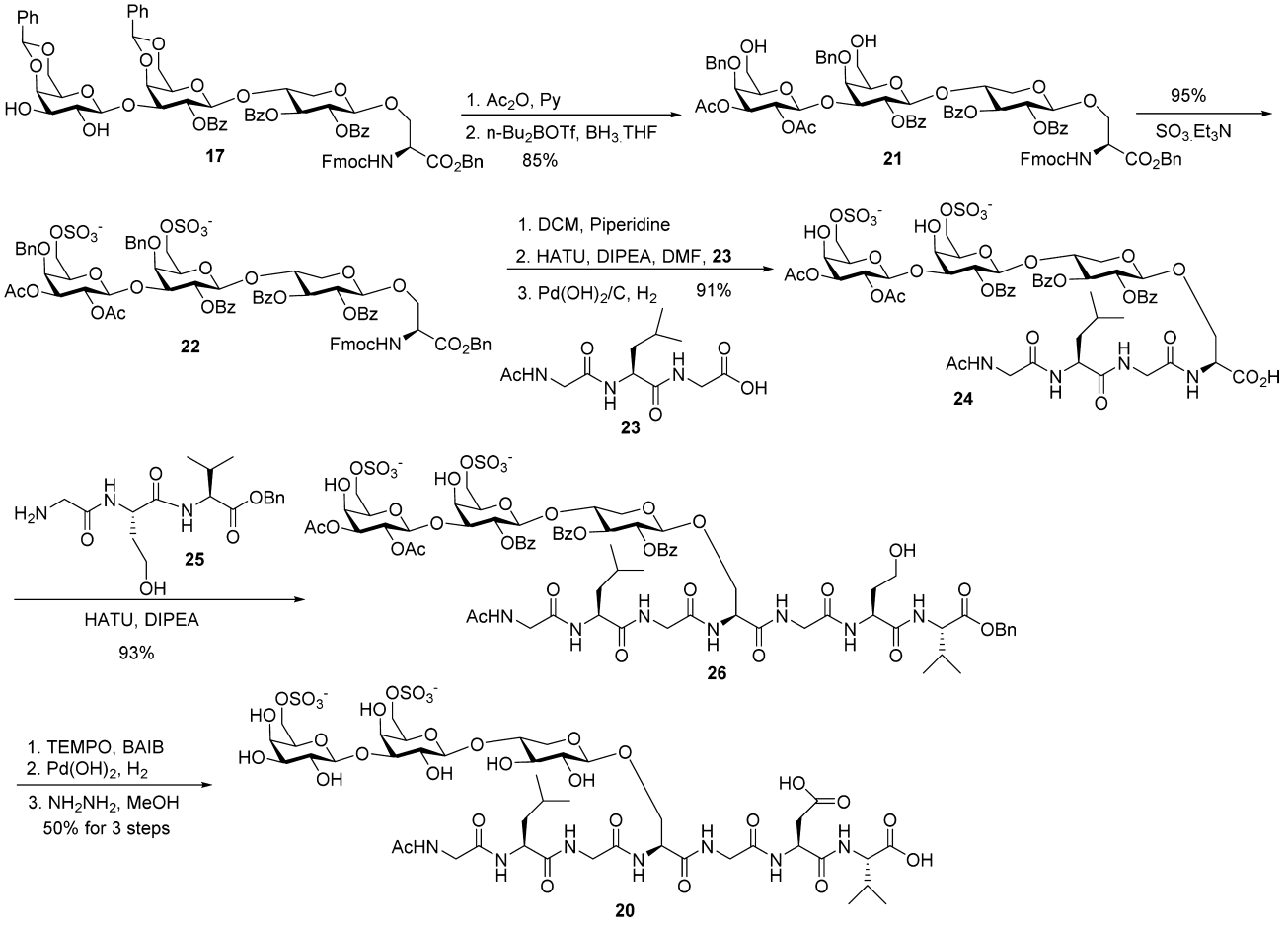
Scheme 2. Synthesis of Glycopeptide 16 Using the Homoserine Strategy



Scheme 3. Synthesis of *O*-Sulfated Glycopeptide 24 Using the Homoserine Strategy



catalytic hydrogenation and Bz removal by hydrazinolysis afforded the fully deprotected glycopeptide **16** in 55% yield over three steps.

In PGs, the linkage region can be sulfated at *O*-4 or *O*-6 of the galactose moieties.[37,38] While syntheses of sulfated glycopeptides have been reported,[7,14,21,39−44] none of these contained Asp due to the aforementioned challenges. Therefore, we selected perlecan glycopeptide **20** as a representative PG target with an Asp in the backbone.[45] The glycosyl serine **17** was transformed to diol **21** by acetylation and selective opening of the benzylidines (Scheme 3). Sulfation of the free hydroxyl groups in **21** proceeded smoothly to generate

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compound **22** in 95% yield. The sulfated trisaccharide serine was used as a cassette[46] to assemble the glycopeptide. Fmoc removal from **22** followed by HATU promoted coupling with tripeptide **23** and hydrogenolysis to cleave the benzyl ester and ethers produced glycopeptide **24** in 91% yield over three steps. The homoserine containing tripeptide **25** was then coupled with **24** to give glycopeptide **26**. TEMPO/BAIB oxidation followed by hydrogenation and deacylation produced the desired perlecan glycopeptide **20** in 50% yield over the three steps. The *O*-sulfate groups and the secondary alcohols in **26** were stable in the oxidation reaction.

In conclusion, side chain protective groups such as ^{t}Bu and Bn esters on Asp are incompatible with *O*-sulfated glycopeptide synthesis. To address this challenge, we established a new method using homoserine as a precursor of Asp. The homoserine can be transformed to Asp by a mild TEMPO/BAIB oxidation condition following (glyco)peptide assembly in good yields. During the oxidation reaction, the generation of γ-hydroxyl lactams was minimized with a stoichiometric amount of TEMPO. Using this new homoserine strategy, a perlecan heptapeptide bearing a sulfated linkage region was successfully prepared. This is the first time that an *O*-sulfated glycopeptide containing Asp in a peptide sequence was synthesized. This study has laid the foundation for synthetic access to a wide range of PG glycopeptides.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were carried out under nitrogen with anhydrous solvents in flame-dried glassware, unless otherwise noted. Glycosylation reactions were performed in the presence of 4 Å molecular sieves, which were flame-dried right before the reaction under high vacuum. Glycosylation solvents were dried using a solvent purification system and used directly without further drying. The chemicals used were reagent grade as supplied except where noted. Compounds were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (230−400 Mesh). NMR spectra were referenced using residual $CHCl_3$ (δ ^{1}H NMR 7.26 ppm) and $CDCl_3$ (δ ^{13}C NMR 77.0 ppm). Peak and coupling constant assignments are based on ^{1}H NMR, ^{1}H−^{1}H gCOSY and (or) ^{1}H−^{13}C gHMQC, and ^{1}H−^{13}C gHMBC experiments. HRMS (ESI) was performed on a TOF-MS spectrometer.

General Procedure for Oxidation of the Homoserine Moiety. TEMPO (1.2 equiv) and BAIB (2.4 equiv) were added into the solution of the alcohol (0.05 M) in DCM/^{t}BuOH/H_2O (4/4/1). The reaction was stirred at room temperature for 45 min until TLC indicated the completion of reaction. The solvents were removed *in vacuo* (with toluene 3×), and the residue was washed by small portions of Et_2O (3×) in the same flask. The resulting mixture was purified by silica gel flash chromatography or high performance liquid chromatography (HPLC).

Solid-Phase Peptide Synthesis with the Fmoc-Strategy. Peptides were synthesized following the standard solid phase Fmoc protocols.[7] The following Fmoc amino acids from Chem-impex were employed: Fmoc-Glu(OBn)-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-Ser(OBn)−OH, Fmoc-Val-OH, and Fmoc-homoSerine-(OTrt)−OH. The coupling reagents were 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluoro-phosphate (HATU), and 1-hydroxy-7-azabenzotriazole (HOAt) with *N*,*N*-diisopropylethylamine (DIPEA) as the base. After each coupling, the Fmoc group was removed with a mixture of *N*,*N*-dimethyl-formamide (DMF)/piperidine (4/1 v/v). Upon completion of the synthesis on a 0.05 mmol scale, the peptide bearing resin was treated with trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (Tips) (95:2.5:2.5) for 3 × 10 min to yield peptidyl acids in good yield. After solvent removal, the crude peptidyl acids were exposed to saturated $NaHCO_3$ in MeOH/DCM (1:1) at room temperature to remove the partially formed TFA esters. After thin-layer chromatography (TLC) indicated the reaction was complete, the product was precipitated by Et_2O and directly used in the next step.

General Procedure for Peptide Synthesis in Solution Phase. Carboxylic acid (1.1 equiv) and the free amine (1 equiv) were dissolved in DMF (0.125 M). After addition of HATU (1.1 equiv) and DIPEA (3 equiv), the reaction was stirred at room temperature for 1 h or until TLC indicated the reaction was complete. The reaction mixture was extracted by ethyl acetate and washed with 10% HCl, $NaHCO_3$ (sat.), and brine. The organic phase was concentrated, and the mixture was purified by silica gel column chromatography (hexanes−ethyl acetate) to afford the desired product.

General Procedure for Fmoc Removal. The coupling product was dissolved in piperidine/DCM (1/4, 0.4 mmol coupling product in 5 mL), and the reaction was stirred at room temperature for 15 min. TLC indicated the completion of reaction. Then the mixture was extracted with ethyl acetate and washed with water (twice) and brine. After removal of the organic solvent, the mixture was purified by silica gel column chromatography (MeOH−DCM) to afford the coupling product.

General Procedure for Trt Removal. The peptides with Trt protected homoserine was dissolved in TFA (0.1 mmol in 2 mL), and the reaction was stirred at room temperature until TLC indicated that the starting material disappeared. TFA was removed *in vacuo* (twice with toluene), and the residue was dissolved in MeOH/DCM (1:1, 0.05 M), followed by addition of solid $NaHCO_3$. The reaction was stirred at room temperature until TLC indicated the reaction was complete. Then the mixture was filtered by cotton, and the filtrate was concentrated in vacuum. Silica gel column chromatography afforded the alcohol.

Dipeptide ***4***. Compound **4** was synthesized from commercially available **2** and **3** following the general procedure for peptide synthesis in 98% yield (eluent: hexanes/ethyl acetate = 2/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79−7.75 (m, 2 H), 7.52 (t, J = 7.0 Hz, 2 H), 7.43−7.31 (m, 13 H), 7.29−7.25 (m, 8 H), 7.22−7.19 (m, 3 H), 6.61 (bs, 1 H), 6.06 (d, J = 3.5 Hz, 1 H), 5.17−5.12 (m, 2 H), 4.43−4.37 (m, 3 H), 4.21 (t, J = 7.0 Hz, 1 H), 3.92−3.85 (m, 2 H), 3.32−3.26 (m, 2 H), 2.15−2.03 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.9, 171.5, 169.1, 146.8, 143.8, 143.5, 141.3, 135.1, 128.7, 128.6 (2 C), 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.2 (2 C), 127.1, 125.1, 125.0, 120.0, 87.4, 67.4, 67.3, 67.2, 60.7, 47.1, 41.3, 31.9. HRMS: $C_{47}H_{42}N_2O_6$ $[M + NH_4]^+$ calcd: 748.3387; obsd: 748.3381.

Dipeptide ***1***. Following the general procedure for Trt removal, compound **1** was obtained in 91% yield from dipeptide **4** (eluent: hexanes/ethyl acetate = 1/1). ^{1}H NMR (500 MHz, CD_3OD): δ 7.75 (d, J = 7.5 Hz, 2 H), 7.66, (t, J = 7.5 Hz, 2 H), 7.39−7.28 (m, 9 H), 5.14 (s, 2 H), 4.38−4.28 (m, 3 H), 4.21 (t, J = 7.0 Hz, 1 H), 4.04 (d, J = 17.5 Hz, 1 H), 3.96 (d, J = 17.5 Hz, 1 H), 3.64−3.62 (m, 2 H), 2.05−1.98 (m, 1 H), 1.85−1.78 (m, 1 H). ^{13}C NMR (125 MHz, CD_3OD): δ 174.0, 169.5, 157.0, 143.9, 143.8, 135.7, 128.1, 127.9, 127.4, 126.7, 124.8 (2 C), 119.5, 66.6, 66.5, 57.9, 52.3, 47.0, 40.7, 34.3. HRMS: $C_{28}H_{28}N_2O_6$ $[M + Na]^+$ calcd: 511.1845; obsd: 511.1842.

Dipeptide ***5***. Following the general procedure for homoserine oxidation, dipeptide **5** was obtained in 81% yield from **1** (eluent: DCM/MeOH = 8/1). ^{1}H NMR (500 MHz, CD_3OD): δ 7.78 (d, J = 7.5 Hz, 2 H), 7.65−7.63, (m, 2 H), 7.38−7.27 (m, 9 H), 5.13 (s, 2 H), 4.62−4.59 (m, 1 H), 4.35−4.31 (m, 2 H), 4.20−4.18 (m, 1 H), 4.00 (s, 2 H), 2.77 (dd, J = 5.5, 16.5 Hz, 1 H), 2.65 (dd, J = 7.0, 16.5 Hz, 1 H). ^{13}C NMR (125 MHz, CD_3OD): δ 177.1, 173.7, 169.7, 157.0, 143.8, 143.7, 141.1, 135.7, 128.1, 127.9, 127.4, 126.8, 124.9, 124.8, 119.5, 66.8, 66.5, 52.2, 46.9, 40.8, 38.5. HRMS: $C_{28}H_{26}N_2O_7$ $[M + Na]^+$ calcd: 525.1638; obsd: 525.1633.

γ-Hydroxy Lactam ***6*** *(The two diastereomers R/S were separated by silica gel chromatography).* ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, J = 7.5 Hz, 2 H), 7.60 (d, J = 7.5 Hz, 2 H), 7.43−7.20 (m, 9 H), 5.42−5.41 (m, 1 H), 5.25−5.24 (m, 1 H), 5.18 (s, 2 H), 4.63−4.59 (m, 1 H), 4.44−4.36 (m, 3 H), 4.23 (t, J = 7.0 Hz, 1 H), 4.10−4.07 (m, 1 H), 2.64−2.59 (m, 1 H), 2.26−2.21 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.1, 169.9, 156.2, 143.7, 141.3, 134.7, 133.0, 129.7,

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128.7 (2 C), 128.5, 128.4, 128.3, 127.7, 128.1, 125.1, 112.0, 81.8, 69.2, 67.8, 67.3, 67.2, 64.0, 50.3, 47.0, 43.5, 41.4, 31.1. HRMS: $C_{28}H_{26}N_2O_6$ $[M + Na]^+$ calcd: 509.1689; obsd: 509.1687.

Epimer: ^1H NMR (500 MHz, $CDCl_3$): δ 7.78−7.74 (m, 2 H), 7.60−53 (m, 2 H), 7.41−7.29 (m, 9 H), 6.03−6.02 (m, 1 H), 5.20−5.17 (m, 3 H), 4.46−4.38 (m, 3 H), 4.34−4.30 (m, 1 H), 4.23−4.16 (m, 1 H), 4.09 (d, J = 17.5 Hz, 1 H), 3.91−3.89 (m,1 H), 2.96−2.90 (m, 1 H), 1.98 (d, J = 14.5 Hz, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.5, 168.8, 156.4, 143.6, 143.4, 141.3, 135.0, 129.7, 128.7, 128.5 (2 C), 128.4, 127.8, 127.7, 125.1, 125.0, 81.5, 67.3, 50.6, 47.0, 41.4, 35.5. HRMS: $C_{28}H_{26}N_2O_6$ $[M + Na]^+$ calcd: 509.1689; obsd: 509.1688.

Aspartimide ***7***. ^1H NMR (500 MHz, $CDCl_3$): δ 7.78 (d, J = 7.0 Hz, 2 H), 7.57 (bs, 2 H), 7.43−7.33 (m, 9 H), 5.57 (bs, 1 H), 5.18 (s, 2 H), 4.45 (bs, 3 H), 4.36 (s, 2 H), 1.15 (bs, 1 H), 3.20−3.15 (m, 1 H), 2.85−2.82 (m,1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.6, 173.1, 166.5, 155.8, 143.5, 141.3 (2 C), 134.7, 128.7 (2 C), 128.4, 127.8, 127.1, 125.0, 120.1, 67.8, 67.3, 50.4, 47.0, 39.7, 35.9. HRMS: $C_{28}H_{24}N_2O_6$ $[M + Na]^+$ calcd: 507.1532; obsd: 507.1529.

Tripeptide ***8***. Following the general procedure for solution phase peptide synthesis, Fmoc removal, and Trt removal, compound **8** was obtained in 78% yield from commercially available amino acids. ^1H NMR (500 MHz, $CDCl_3$): δ 7.74 (d, J = 7.5 Hz, 2 H), 7.68 (d, J = 8.5 Hz, 1 H), 7.59−7.55 (m, 3 H), 7.38 (t, J = 7.0 Hz, 2 H), 7.29−7.26 (m, 2 H), 6.10 (bs, 1 H), 4.88 (d, J = 5.5 Hz, 1 H), 5.51−4.48 (m, 1 H), 4.38 (d, J = 7.0 Hz, 2 H), 4.21−4.18 (m, 1 H), 3.97 (d, J = 5.0 Hz, 2 H), 4.74−3.63 (m, 5 H), 2.18−2.14 (m, 1 H), 2.06−2.05 (m, 1 H), 1.83−1.79 (m, 1 H), 0.92−0.88 (m, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.3, 172.0, 170.1, 156.8, 143.8, 141.2 (2 C), 127.7, 127.1, 125.1, 120.0, 67.3, 58.3, 57.6, 52.2, 50.5, 47.0, 44.3, 35.8, 30.8, 19.0, 17.8. HRMS: $C_{27}H_{33}N_3O_7$ $[2M+H]^+$ calcd: 1023.4715; obsd: 1023.4727.

Tripeptide ***9***. Following the general procedure for solution phase peptide synthesis, Fmoc removal, and Trt removal, compound **9** was obtained in 65% yield from commercially available amino acid building blocks. ^1H NMR (500 MHz, $CDCl_3$): δ 7.68 (d, J = 7.5 Hz, 2 H), 7.50 (d, J = 7.5 Hz, 2 H), 7.34−7.18 (m, 12 H), 6.85 (d, J = 8.5 Hz, 2 H), 6.77−6.73 (m, 3 H), 6.66 (d, J = 6.5 Hz, 1 H), 5.81 (d, J = 7.5 Hz, 1 H), 5.07 (d, J = 12.5 Hz, 1 H), 5.00 (d, J = 12.0 Hz, 1 H), 4.91 (s, 2 H), 4.78−4.74 (m, 1 H), 4.38−4.25 (m, 4 H), 4.13 (t, J = 7.0 Hz, 1 H), 3.60−3.53 (m, 2 H), 2.99−2.91 (m, 2 H), 1.74−1.87 (m, 1 H), 1.70−1.65 (m, 1 H), 1.58−1.49 (m, 2 H), 1.42−1.37 (m, 1 H), 0.79 (t, J = 6.0 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): 171.7, 171.5, 171.3, 157.9, 156.6, 143.7, 143.6, 141.3 (2 C), 136.9, 134.9, 130.4, 128.6 (3 C), 128.0, 127.8, 127.5, 127.1, 125.1, 125.0, 120.0 (2 C), 114.8, 69.9, 67.3, 67.2, 58.7, 53.4, 52.2, 52.0, 47.1, 40.9, 37.0, 35.8, 24.7, 22.8, 21.8. HRMS: $C_{48}H_{51}N_3O_8$ $[M + H]^+$ calcd: 798.3754; obsd: 798.3760.

Tripeptide ***12***. Following the general procedure for homoserine oxidation reaction, compound **12** was obtained in 78% yield from **8** (eluent: DCM/MeOH = 7/1). ^1H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, J = 7.5 Hz, 1 H), 7.78 (d, J = 7.5 Hz, 2 H), 7.54−7.51 (m, 3 H), 7.36 (t, J = 7.0 Hz, 2 H), 7.27−7.24 (m, 2 H), 6.13 (bs, 1 H), 4.95 (bs, 1 H), 4.43−4.42 (m, 1 H), 4.34−4.29 (m, 2 H), 4.18−4.16 (m, 1 H), 3.92 (bs, 2 H), 3.65 (s, 3 H), 2.92−2.87 (m, 1 H), 2.76−2.72 (m, 1 H), 2.12−2.11 (m, 1 H), 0.87−0.84 (m, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.4, 172.2, 170.8, 169.9, 156.9, 143.7 (2 C), 141.2 (2 C), 127.7, 127.1, 125.1, 119.9, 67.4, 57.8, 52.2, 49.6, 46.9, 44.3, 30.9, 18.9, 17.8. HRMS: $C_{27}H_{31}N_3O_8$ $[M + H]^+$ calcd: 526.2189; obsd: 526.2194.

Tripeptide ***13***. Following the general procedure for homoserine oxidation reaction, compound **13** was obtained in 80% yield from **9** (eluent: DCM/MeOH = 7/1). ^1H NMR (500 MHz, $CDCl_3$): δ 7.74 (d, J = 7.0 Hz, 2 H), 7.55 (d, J = 6.5 Hz, 2 H), 7.40−7.23 (m, 14 H), 6.91 (d, J = 7.0 Hz, 2 H), 6.80−6.79 (m, 3 H), 6.06 (bs, 1 H), 5.13 (d, J = 13.0 Hz, 1 H), 5.03 (d, J = 12.0 Hz, 1 H), 4.97 (s, 2 H), 4.84−4.82 (m, 1 H), 4.60 (bs, 1 H), 4.43−4.35 (m, 3 H), 4.18−4.17 (m, 1 H), 3.01 (d, J = 3.0 Hz, 2 H), 2.90−2.87 (m, 1 H), 2.77−2.73 (m, 1 H), 1.57−1.48 (m, 3 H), 0.81 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.9, 171.8, 171.4, 170.9, 157.9, 143.7, 141.2, 136.9, 134.8, 130.4, 128.6 (2 C), 128.0, 127.5, 127.1, 125.0, 120.0, 114.9, 69.9, 67.4, 53.5, 52.2, 47.0, 40.6, 36.9, 24.6, 22.7, 21.8. HRMS: $C_{48}H_{49}N_3O_9$ $[M - H]^-$ calcd: 810.3396; obsd: 810.3418.

Tetrapeptide ***14***. Following the general procedure for solid phase peptide synthesis and oxidation reaction, compound **14** was obtained in 67% yield (HPLC eluent: 30% ∼95% acetonitrile in water). ^1H NMR (500 MHz, CD_3OD): δ 7.80 (d, J = 7.5 Hz, 2 H), 7.66 (m, 1 H), 7.4 (t, J = 7.5 Hz, 2 H), 7.32−7.22 (m, 12 H), 4.83−4.79 (m, 1 H), 4.55−4.45 (m, 5 H), 4.41−4.38 (m, 2 H), 4.35−4.30 (m, 2 H), 4.23−4.20 (m, 1 H), 2.92 (dd, J = 6.5, 17.0 Hz, 1 H), 2.83 (dd, J = 6.0, 17.0 Hz, 1 H), 1.71−1.59 (m, 3 H), 0.90 (d, J = 6.0 Hz, 3 H), 0.88 (d, J = 6.0 Hz, 3 H). ^{13}C NMR (125 MHz, CD_3OD): δ 174.1, 172.8, 171.3 (2 C), 170.3, 141.2, 137.8, 128.0, 127.9, 127.4 (2 C), 127.3, 127.2, 126.8, 124.8, 119.5, 72.8, 69.1, 66.9, 55.3, 53.5, 53.4, 50.7, 49.9, 40.1, 34.8, 24.3, 20.2, 20.3. HRMS: $C_{45}H_{50}N_4O_{11}$ $[M - H]^-$ calcd: 821.3403; obsd: 821.3394.

Pentapeptide ***15***. Following the general procedure for solid phase peptide synthesis and homoserine oxidation reaction, compound **15** was obtained in 58% yield (HPLC eluent: 30%−95% acetonitrile in water). ^1H NMR (500 MHz, CD_3OD): δ 7.80 (d, J = 7.5 Hz, 2 H), 7.67−7.65 (m, 2 H), 7.40−7.20 (m, 14 H), 5.07 (s, 2 H), 4.58−4.51 (m, 2 H), 4.49−4.47 (m, 1 H), 4.44−4.41 (m, 1 H), 4.35−4.29 (m, 3 H), 4.21−4.14 (m, 2 H), 3.89−3.86 (m, 1 H), 3.70−3.65 (m, 3 H), 2.77−2.72 (m, 1 H), 2.61−2.54 (m, 1 H), 2.49−2.42 (m, 2 H), 2.14−1.80 (m, 8 H), 0.94−0.91 (m, 6 H). ^{13}C NMR (125 MHz, CD_3OD): δ 172.9, 172.8, 172.0, 171.9, 170.8, 143.8 (2 C), 141.1, 137.9, 136.1, 128.1, 128.0, 127.9 (2 C), 127.8 (2 C), 127.7, 127.5, 127.4 (2 C), 127.2, 126.8, 124.8, 119.5, 72.8, 68.7, 66.8, 66.0, 46.5, 30.0, 29.3, 24.3, 18.3, 17.0, 7.8. HRMS: $C_{51}H_{57}N_5O_{13}$ $[M - H]^-$ calcd: 946.3880; obsd: 946.3840.

Tripeptide ***18***. Following the general procedure for solution phase peptide synthesis, Fmoc removal, and Trt removal, the partially protected alcohol was obtained in 60% from commercially available amino acid building blocks. To a solution of the alcohol (25 mg, 0.05 mmol) in DCM/MeOH (2 mL, 1/1), Pd/C (10 mg) and NH_4OAc (2 mg) were added. The reaction was stirred under a H_2 atmosphere for 20 min. Then the reaction was filtered and concentrated, followed by flash column chromatography (eluent: DCM/MeOH = 4/1) to afford **18** in 80% yield. ^1H NMR (500 MHz, CD_3OD): δ 7.32−7.28 (m, 4 H), 7.25−7.22 (m, 1 H), 4.54−4.50 (m, 3 H), 4.42 (t, J = 4.0 Hz, 1 H), 3.94−3.83 (m, 4 H), 3.80 (dd, J = 3.0 Hz, 9.5 Hz, 1 H), 3.66−3.62 (m, 2 H), 2.08−2.02 (m, 3 H), 1.99 (s, 3 H), 1.89−1.84 (m, 1 H). ^{13}C NMR (125 MHz, CD_3OD): δ 172.7, 171.8, 170.4, 128.2, 127.9, 127.5, 127.1, 72.7, 70.4, 58.0, 51.4, 42.2, 34.1, 21.0. HRMS: $C_{18}H_{25}N_3O_7$ $[M - H]^-$ calcd: 394.1620; obsd: 394.1635.

Glycopeptide ***19***. Compound **17** (26 mg, 0.02 mmol) was dissolved in DCM/piperidine (4/1, 1 mL). After 15 min, the reaction was stopped by evaporating the solvents. The free amine was purified by Sephadex LH-20 (eluent: DCM/MeOH = 1/1). This amine was dissolved in anhydrous DMF (0.2 mL), followed by addition of a mixture of HATU (16 mg, 0.04 mmol), DIPEA (0.010 mL, 0.06 mmol) and compound **18** (16 mg, 0.04 mmol) in DMF (0.7 mL). The reaction was stirred at room temperature for 1 h. After purification by Sephadex LH-20 (eluent: DCM/MeOH = 1/1), compound **19** was obtained in 75% yield from **17**. ^1H NMR (500 MHz, $CDCl_3$): δ 8.05−7.98 (m, 6 H), 7.54−7.48 (m, 2 H), 7.45−7.29 (m, 18 H), 7.24−7.15 (m, 10 H), 6.64−6.62 (m, 1 H), 5.66 (t, J = 6.5 Hz, 1 H), 5.60−5.57 (m, 1 H), 5.49 (s, 1 H), 5.48 (m, 1 H), 5.19−5.16 (m, 1 H), 5.00−4.94 (m, 2 H), 4.82 (d, J = 8.0 Hz, 1 H), 4.69−4.68 (m, 2 H), 4.60−4.54 (m, 2 H), 4.36−4.35 (m, 2 H), 4.32−4.31 (d, J = 7.5 Hz, 1 H), 4.23−4.21 (d, J = 12.5 Hz, 1 H), 4.17 (dd, J = 2.0, 10.5 Hz, 1 H), 4.08 (d, J = 3.5 Hz, 1 H), 4.04−3.98 (m, 2 H), 3.96−3.93 (m, 1 H), 3.87−3.75 (m, 7 H), 3.69−3.66 (m, 1 H), 3.61−3.55 (m, 3 H), 3.39−3.32 (m, 4 H), 2.02−1.97 (m, 1 H), 1.91 (m, 3 H), 1.82−1.77 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.6, 171.2, 169.7, 169.6, 169.1, 166.0, 165.7, 165.3, 137.6, 137.5, 137.3, 135.1, 133.4 (2 C), 133.3, 130.0, 129.8, 129.6, 129.5 (2 C), 129.2, 129.0 (2 C), 128.6, 128.5 (3 C), 128.4, 128.3, 128.1, 127.8, 127.7, 126.6, 126.3, 104.3, 102.1, 101.2, 101.1, 99.9, 77.8, 77.2, 75.9, 75.6, 75.1, 73.2, 72.1, 71.3 (2 C), 70.9, 70.4, 69.1, 68.3, 67.2, 67.0, 66.7, 61.7, 59.0, 53.2, 53.0, 51.7, 43.3, 34.9, 22.8. HRMS: $C_{80}H_{84}N_4O_{26}$ $[M + Na]^+$ calcd: 1539.5272; obsd: 1539.5226.

Glycopeptide ***16***. Glycopeptide **19** (7.5 mg, 5 μmol) was oxidized following the general procedure for homoserine oxidation. The

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resulting carboxylic acid was purified by size exclusion chromatography using a Sephadex LH-20 column (eluent: DCM/MeOH = 1/1). HRMS: $C_{80}H_{82}N_4O_{27}$ $[M - H]^-$ calcd: 1529.5088; obsd: 1529.5016. This compound was dissolved in MeOH/DCM (1/1, 2 mL) followed by adding $Pd(OH)_2$ (15 mg). The mixture was stirred under a H_2 atmosphere for 6 h. Then the reaction was filtered. After concentration, the residue was dissolved in MeOH (0.4 mL), followed by adding hydrazine hydrate (0.1 mL) at room temperature. After 6 h, the reaction was quenched by acetone at 0 °C and purified by a Sephadex G-15 column to afford **16** (2.4 mg, 55% from **19**). ^{1}H NMR (500 MHz, D_2O): δ 4.53 (dd, J = 5.0 Hz, 8.0 Hz, 1 H), 4.45 (d, J = 8.0 Hz, 1 H), 4.37−4.35 (m, 2 H), 4.27−4.24 (m, 2 H), 4.05−4.02 (m, 2 H), 3.94 (dd, J = 5.5 Hz, 12.0 Hz, 1 H), 3.79−3.48 (m, 16 H), 3.46−3.41 (m, 2 H), 3.26−3.20 (m, 1 H), 3.18 (t, J = 8.5 Hz, 1 H), 2.58 (dd, J = 5.0 Hz, 16.0 Hz, 1 H), 2.49 (dd, J = 8.0 Hz, 16.0 Hz, 1 H), 1.90 (s, 3 H). ^{13}C NMR (125 MHz, D_2O): δ 175.4, 174.8, 173.5, 171.4, 170.8, 104.2, 103.0, 81.9, 76.2, 74.9, 74.8, 73.5, 72.6, 72.4, 70.9, 69.9, 69.7, 68.4, 68.3, 61.1, 60.9, 60.8, 55.3, 55.1, 51.4, 42.4, 21.6. HRMS: $C_{31}H_{50}N_4O_{24}$ $[M - H]^-$ calcd: 861.2737; obsd: 861.2723, $[M - 2H]^{2-}$ calcd: 430.1332; obsd: 430.1342.

Compound ***21***. To a solution of **17** (54 mg, 0.04 mmol) in pyridine (0.5 mL), Ac_2O (0.2 mL) was added at 0 °C. The reaction was stirred at room temperature for 2 h, and then it was extracted by EtOAc and washed with 10% HCl, $NaHCO_3$ (sat.), and brine. The organic phase was concentrated and purified by flash column chromatography. The resulting compound was dissolved in BH_3·THF (1.0 M in THF, 1 mL), followed by adding n-Bu_2BOTf (1.0 M in DCM, 0.2 mL) at 0 °C. The reaction was stirred at this temperature for 1.5 h. Then it was quenched with DIPEA and MeOH. The mixture was extracted by EtOAc and washed with $NaHCO_3$ (sat) and brine. The organic phase was concentrated and purified by flash column chromatography (eluent: toluene/acetone = 3/1) to afford **21** (49 mg, 85% from **17**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08−8.00 (m, 8 H), 7.77 (d, J = 7.5 Hz, 2 H), 7.63−7.37 (m, 15 H), 7.34−7.30 (m, 6 H), 7.25−7.22 (m, 4 H), 7.16−7.15 (m, 3 H), 5.65−5.56 (m, 3 H), 5.41−5.37 (m, 1 H), 5.16 (d, J = 12.0 Hz, 1 H), 5.09−5.08 (m, 1 H), 5.00−4.98 (d, J = 12.0 Hz, 1 H), 4.81−4.78 (d, J = 12.0 Hz, 1 H), 4.77−4.73 (m, 2 H), 4.67 (d, J = 3.0 Hz, 1 H), 4.62−4.60 (m, 2 H), 4.57 (d, J = 8.0 Hz, 2 H), 4.52−4.50 (m, 2 H), 4.49 (d, J = 12.0 Hz, 1 H), 4.40−4.31 (m, 2 H), 4.26−4.24 (m, 1 H), 4.20−4.17 (m, 1 H), 3.95−3.89 (m, 4 H), 3.76−3.70 (m, 3 H), 3.62−3.59 (m, 3 H), 3.54 (t, J = 6.0 Hz, 1 H), 3.18−3.09 (m, 1 H), 1.98 (s, 3 H), 1.79 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 169.6 (2 C), 166.5, 166.0, 165.4, 164.7, 156.0, 143.8, 143.7, 141.3, 138.2, 137.5, 133.3, 133.0, 130.2, 130.0 (2 C), 129.9, 129.7, 129.5, 129.3, 129.0, 128.5, 128.3, 128.1, 127.6, 127.1 (2 C), 125.2, 125.1, 112.0, 102.6, 102.0, 79.7, 76.1, 74.9, 74.7, 74.5, 73.8, 73.6, 73.5, 72.1, 70.6, 69.3, 69.2, 69.0, 68.6, 67.3 (2 C), 64.0, 62.1, 61.4, 54.2, 47.1, 20.8, 20.3. HRMS: $C_{81}H_{79}NO_{24}$ $[M + NH_4]^+$ calcd: 1467.5336; obsd: 1467.5325.

Compound ***22***. Compound **21** (14 mg, 0.01 mmol) was dissolved in DMF (0.7 mL), followed by addition of SO_3·Et_3N (10 mg). The mixture was stirred at 55 °C for 2 h, followed by purification by a Sephadex LH-20 column (eluent: DCM/MeOH = 1/1). Compound **22** (15 mg) was obtained in 95% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.34 (bs, 2 H), 8.00 (d, J = 7.5 Hz, 2 H), 7.96 (d, J = 7.0 Hz, 4 H), 7.76 (d, J = 7.5 Hz, 2 H), 7.59−7.56 (m, 1 H), 7.53−7.50 (m, 2 H), 7.48−7.44 (m, 3 H), 7.41−7.35 (m, 5 H), 7.33−7.28 (m, 5 H), 7.26−7.19 (m, 14 H), 5.54−5.48 (m, 2 H), 5.44 (t, J = 6.5 Hz, 1 H), 5.33−5.29 (m, 1 H), 5.12 (d, J = 12.5 Hz, 1 H), 5.06−5.01 (m, 2 H), 4.96 (d, J = 11.5 Hz, 1 H), 4.72−4.66 (m, 2 H), 4.63−4.55 (m, 4 H), 4.50−4.47 (m, 2 H), 4.33−4.30 (m, 1 H), 4.25−4.15 (m, 4 H), 4.14−4.11 (m, 1 H), 4.05−3.94 (m, 5 H), 3.91−3.83 (m, 3 H), 3.76 (d, J = 12.0 Hz, 1 H), 3.69 (d, J = 10.0 Hz, 1 H), 3.19−3.15 (m, 1 H), 1.87 (s, 3 H), 1.70 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.2, 169.5, 165.5, 165.4, 164.7, 155.9, 143.8, 143.7, 141.2, 138.7, 138.0, 135.1, 133.2, 130.0, 129.9, 129.8, 129.5, 129.1, 128.6, 128.5, 128.4, 128.3 (2 C), 128.1, 128.0, 127.9, 127.8, 127.7 (2 C), 127.6, 127.2, 127.1, 127.1 (2 C), 125.2, 120.0, 101.9, 101.3, 100.2, 79.6, 75.6, 75.0, 74.4, 74.0, 73.5, 73.1, 73.0, 71.7, 70.3, 69.4, 68.9, 67.3, 67.2, 66.1, 54.2, 53.4, 47.1, 20.7, 20.2. HRMS: $C_{81}H_{77}NO_{30}S_2^{2-}$ $[M]^{2-}$ calcd: 803.6992; obsd: 803.7006.

Tripeptide ***23***. Following the general procedure for solution phase peptide synthesis, Fmoc removal, and Trt removal, the protected tripeptide was obtained in 83% yield from commercial amino acids. This tripeptide (74 mg) was dissolved in MeOH/DCM (1/1, 3 mL), followed by addition of Pd/C (10 mg). This mixture was stirred under a H_2 atmosphere for 30 min. Then the reaction was filtered. After concentration, the residue was used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.41−4.38 (m, 1 H), 3.93−3.65 (m, 4 H), 1.94 (s, 3 H), 1.62−1.44 (m, 3 H), 0.86 (d, J = 6.5 Hz, 3 H), 0.83 (d, J = 6.0 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.0, 172.1, 171.6, 169.8, 51.5, 42.8, 40.9, 40.4, 24.6, 22.7, 22.3, 21.4. HRMS: $C_{12}H_{21}N_3O_5$ $[2M - H]^-$ calcd: 573.2884; obsd: 573.2903.

Glycopeptide ***24***. Compound **22** (16 mg, 0.01 mmol) was dissolved in DCM (0.8 mL), followed by addition of piperidine (0.2 mL). The reaction was stirred at room temperature for 15 min and the mixture was directly loaded onto a Sephadex LH-20 column. The resulting amine was dissolved in DMF (0.1 mL), followed by adding a mixture of HATU (7.8 mg, 0.02 mmol), DIPEA (0.009 mL, 0.05 mmol) and compound **23** (7 mg, 0.025 mmol) in DMF (0.7 mL). The reaction was stirred at room temperature for 1 h, then it was loaded onto a Sephadex LH-20 column (eluent: DCM/MeOH = 1/1) to afford coupling product in 91% yield. The resulting compound (20 mg) was dissolved in MeOH/DCM (1/1, 2 mL), followed by addition of $Pd(OH)_2$ (20 mg). This mixture was stirred under H_2 atmosphere for 16 h. Then the reaction was filtered. After concentration, the residue was purified by a Sephadex LH-20 (eluent: DCM/MeOH = 1/1) column to afford compound **24** in 99% yield. ^{1}H NMR (500 MHz, CD_3OD): δ 8.08 (d, J = 7.5 Hz, 2 H), 7.94 (t, J = 7.5 Hz, 4 H), 7.64−7.62 (m, 1 H), 7.57−7.49 (m, 4 H), 7.45−7.40 (m, 4 H), 5.49−5.44 (m, 1 H), 5.34 (t, J = 8.0 Hz, 1 H), 5.14−5.09 (m, 2 H), 4.82 (d, J = 7.0 Hz, 1 H), 4.73−4.65 (m, 3 H), 4.50−4.46 (m, 3 H), 4.37−4.34 (m, 1 H), 4.19−3.97 (m, 10 H), 3.63−3.52 (m, 4 H), 3.44−3.42 (m, 2 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.53 (s, 3 H), 0.96 (d, J = 5.0 Hz, 3 H), 0.92 (d, J = 5.0 Hz, 3 H). ^{13}C NMR (125 MHz, CD_3OD): δ 174.9, 174.0, 171.9, 171.7, 171.5, 170.9, 167.3, 166.8, 166.7, 134.6, 134.4, 134.3, 131.2, 130.9 (2 C), 130.7, 129.8, 129.6 (2 C), 129.5, 103.3, 101.9, 101.6, 81.2, 76.0, 75.0, 74.6, 73.8, 73.2, 72.5, 70.6, 70.1, 69.7, 67.7, 67.1, 66.4, 64.0, 53.4, 45.8, 43.8, 43.7, 43.2, 41.5, 25.8, 23.5, 22.6, 21.9, 20.6, 20.4, 18.8, 17.3, 13.3. HRMS: $C_{57}H_{68}N_4O_{32}S_2^{2-}$ $[M]^{2-}$ calcd: 692.1635; obsd: 692.1642.

Glycopeptide ***26***. The mixture of compound **24** (13.8 mg, 0.01 mmol) and **25** (5.5 mg, 0.015 mmol) was dissolved in DMF (0.8 mL), followed by adding HATU (7.8 mg, 0.02 mmol) and DIPEA (0.009 mL, 0.05 mmol). The reaction was stirred at room temperature for 1 h, then it was loaded onto a Sephadex LH-20 column (eluent: DCM/MeOH = 1/1) to afford compound **26** in 91% yield. ^{1}H NMR (500 MHz, CD_3OD): δ 8.06 (d, J = 7.0 Hz, 2 H), 7.95−7.89 (m, 4 H), 7.63−7.32 (m, 14 H), 5.52 (t, J = 9.5 Hz, 1 H), 5.36−5.32 (m, 1 H), 5.21−5.12 (m, 4 H), 4.85 (d, J = 8.0 Hz, 1 H), 4.75−4.70 (m, 2 H), 4.62 (s, 1 H), 4.55−4.51 (m, 1 H), 4.41 (t, J = 6.0 Hz, 1 H), 4.35−4.31 (m, 2 H), 4.27−4.10 (m, 6 H), 4.05−3.91 (m, 7 H), 3.88−3.75 (m, 5 H), 3.64−3.55 (m, 4 H), 2.18−2.14 (m, 1 H), 2.02−1.96 (m, 7 H), 1.88−1.84 (m, 1 H), 1.69−1.59 (m, 4 H), 0.96−0.89 (m, 12 H). ^{13}C NMR (125 MHz, CD_3OD): δ 133.1, 132.9, 129.4, 129.3, 128.4 (2 C), 128.2, 128.1, 101.7, 100.4, 100.3, 73.1, 72.4, 71.2, 69.2, 68.5, 65.9, 65.0, 57.9, 58.0, 54.3, 52.1, 42.2, 42.1, 22.1, 21.2, 20.5, 19.2, 18.9, 17.9, 15.9, 11.9. HRMS: $C_{75}H_{93}N_7O_{36}S_2^{2-}$ $[M]^{2-}$ calcd: 865.7557; obsd: 865.7549.

Glycopeptide ***20***. Glycopeptide **26** (6 mg, 3 μmol) was oxidized following the general procedure for homoserine oxidation. The resulting carboxylic acid was purified by size exclusion chromatography using a Sephadex LH-20 column (eluent: DCM/MeOH = 1/1). HRMS: $C_{75}H_{91}N_7O_{37}S_2$ $[M]^{2-}$ calcd: 872.7453; obsd: 872.7422. The carboxylic acid was dissolved in MeOH/DCM (1/1, 2 mL) followed by addition of $Pd(OH)_2$ (15 mg). This mixture was stirred under a H_2 atmosphere for 30 min. Then the reaction was filtered. After concentration, the residue was dissolved in MeOH (0.4 mL), followed

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by adding hydrazine hydrate (0.1 mL) at room temperature. After 6 h, the reaction was quenched by acetone at 0 °C and purified by Sephadex G-15 column to afford **20** (2.2 mg) in 50% overall yield over the three steps. ^{1}H NMR (500 MHz, D_2O): δ 4.52−4.45 (m, 3 H), 4.40−4.33 (m, 2 H), 4.29 (d, J = 8.0 Hz, 1 H), 4.25−4.22 (m, 1 H), 4.16−4.11 (m, 1 H), 4.08−3.93 (m, 7 H), 3.84−3.78 (m, 8 H), 3.74−3.67 (m, 2 H), 3.57−3.43 (m, 4 H), 3.28−3.17 (m, 2 H), 2.76−2.72 (m, 1 H), 2.63−2.57 (m, 1 H), 2.02−1.95 (m, 1 H), 1.90 (s, 3 H), 1.54−1.44 (m, 3 H), 0.78−0.72 (m, 12 H). ^{13}C NMR (125 MHz, D_2O): δ 174.8, 171.8, 171.7, 104.2, 102.9, 101.4, 82.0, 77.2, 73.9, 72.8, 72.5, 72.4, 70.9, 69.7, 68.2, 67.0, 63.0, 60.7, 52.4, 42.6, 42.5, 39.7, 30.8, 24.3, 22.2, 21.7, 20.6, 18.9, 17.2. HRMS: $C_{43}H_{69}N_7O_{32}S_2^{2-}$ $[M]^{2-}$ calcd: 629.6720; obsd: 629.6732.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02441.

Complete characterizations are provided, including ^{1}H and ^{13}C NMR spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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