

Supplementary Material

Peptidyl ω -Asp Selenoesters Enable Efficient Synthesis of *N*-linked Glycopeptides

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1. General Information

¹H-NMR spectra were recorded on a Varian Mercury Plus 400 MHz or 600 MHz instrument. ¹³C-NMR spectra were recorded on this same instrument at 100 MHz or 150 MHz. Chemical shifts (δ) are expressed in ppm downfield from TMS as internal standard. The letters s, brs, t, dd and m are used to indicate singlet, broad singlet, triplet, double of doublets, and multiplet, respectively. Electrospray ionization mass (ESI-MS) was performed on TSQ Quantum Access MAX (ThermoFisher Scientific). Analytical HPLC (Agilent, 1100 series HPLC system) analysis was carried out on a C18 reversed-phase analytical column (Agilent, 250 × 4.6 mm, particle size 5 μm) at 35°C, involved a mobile phase A (water + 0.1% TFA) and B (acetonitrile + 0.1% TFA) at a flow rate of 1.0 mL/min. Semi-preparative HPLC analysis was performed on C18 reversed-phase column (Agilent 250 × 9.4 mm, particle size 5 μm) at 35°C, involving a mobile phase A and B at a flow rate of 4.0 mL/min. All solvents and chemicals were obtained from standard commercial suppliers and without any further purification.

2-Chlorotriyl chloride resin, all amino acids and bezotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), *N,N*-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA) and triisopropylsilane (TIPS) were obtained from InnoChem Science & Technology Co., Ltd. *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), DCM and THF were purchased from Aldrich and dried before use. The glycosylamines (**1a-1c**), FmocGly-COOPh, FmocGly-COSPh, FmocGly-COS-*p*-PhNO₂, FmocGly-COSePh and FmocGly-COSe-*o*-CHO, FmocGly-COSe-*p*-CHO were synthesized and characterized by the reported procedure^[1,2].

General procedure for peptide synthesis

2-Chlorotriyl chloride resin (1.06 mmol/g) was swollen in dry DCM for 30 min, and washed with DCM (3 × 3 mL) and DMF (3 × 3 mL). Followed by a solution of Fmoc-AA-OH and DIPEA in DMF was added to the resin bubbling with N₂ and shaken at room temperature for 10 h. The resin was washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL) and subsequently submitted to iterative peptide assembly. After deprotection with 20% piperidine/DMF (3 mL, 3 × 5 min), washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL). Next, a solution of PyBOP and DIPEA in DMF were used for Fmoc/Boc-AA-OH coupling to resin, after shaking at room temperature for 2 h, the resin was washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL).

General procedure for the synthesis of peptidyl selenoester

Peptidyl selenoester was synthesized manually on 2-chlorotriyl chloride resin as the general procedure, followed by removal of allyl ester under the catalytic amounts of Pd(PPh₃)₄ (0.2 equiv) in the presence of PhSiH₃ (10.0 equiv) were dissolved in dry DCM bubbling with N₂ and shaken for 30 min. Next, the resin was washed with dry DCM and the process was repeated once. After 1 h, the resin was washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL). In addition, on-resin selenoesterification for the synthesis of peptidyl selenoester was conducted by adding diphenyl diselenide (DPDS, 2.0 equiv) in a dried round bottom flask, thoroughly flushed with the argon. Then, dry DMF and tributylphosphine (5.0 equiv) were added. The resulting suspension was bubbled for 4 h. The resin was then filtered and washed with DCM/DMF. A mixture of TFA and DCM (1:100 v/v) was added. After 1 h, the resin was washed with CH₂Cl₂ (2 × 2 mL). The combined filtrates were

concentrated and the crude product was triturated with cold diethyl ether to give a white suspension, which was centrifuged and the ether subsequently decanted to obtain crude peptidyl selenoester. The crude peptidyl selenoester was purified by semi-preparative HPLC and lyophilized.

General procedure for the synthesis of *N*-linked glycopeptide with monosaccharide or chitobiose

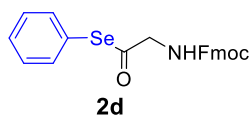
The monosaccharide (**1a**) or chitobiose (**1b**) (10 μ mol) was dissolved in DMSO (1 mL) and treated with DIPEA (10 μ mol). Then, the purified peptidyl selenoesters (**4b-9b**) (5 μ mol) were added, the resulting mixture was stirred at room temperature for 2 h. The reaction processes were monitored by HPLC. Upon completion of the coupling, crude products were purified by semi-preparative HPLC equipped with a C18 column (Agilent 250 \times 9.2 mm, particle size 5 μ m), involving mobile phases of 0.1% TFA (v/v) in water (solvent A)/0.1% TFA in acetonitrile (solvent B) at a flow rate of 4.0 mL/min, then lyophilized. The *N*-linked glycopeptides (**4c-7c**, **5d**, **8d** and **9d**) were obtained as white powders with 69 to 84% yield.

General procedure for the synthesis of complex *N*-linked glycopeptide with undecasaccharide

The undecasaccharide (**1c**, 1.5 μ mol) was dissolved in DMSO (0.5 mL) and 4 Å MS (2.0 mg), the peptidyl selenoester (1 μ mol) and DIPEA (2 μ mol) were added, the resulting mixture was stirred at room temperature for 6 h. The reaction processes were monitored by HPLC. Upon completion of the coupling, crude products were purified by semi-preparative HPLC equipped with a C18 column (Agilent 250 \times 9.2 mm, particle size 5 μ m), involving mobile phases of 0.1% TFA (v/v) in water (solvent A)/0.1% TFA in acetonitrile (solvent B) at a flow rate of 4.0 mL/min, then lyophilized. The complex *N*-linked Glycopeptides (**10e-12e**) were obtained as white powders with 59 to 65% yield.

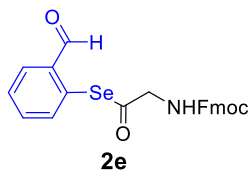
2. Characterization of Compounds

Seleno-phenyl ester (**2d**)



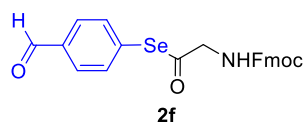
86% isolated yield. ^1H NMR (600 MHz, CDCl_3) δ (ppm) = 7.78 (d, J = 7.5 Hz, 2 H), 7.64 (d, J = 7.5 Hz, 2 H), 7.50 (d, J = 6.7 Hz, 2 H), 7.44–7.38 (m, 5 H), 7.33 (t, J = 7.1 Hz, 2 H), 5.52 (br. s., 1 H), 4.51 (d, J = 6.7 Hz, 2 H), 4.30–4.25 (m, 1 H), 4.14 (d, J = 5.9 Hz, 2 H). ^{13}C NMR (100 MHz, CDCl_3) δ = 200.0, 156.0, 143.4, 141.1, 135.8, 129.3, 129.0, 127.6, 126.9, 124.8, 119.9, 77.3, 76.7, 67.4, 53.6, 47.2. ESI-MS calcd for **2d** $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$ $[\text{M} + \text{H}]^+$ m/z = 438.06, found: 438.02.

Seleno-benzaldehyde ester (**2e**)



80% isolated yield. ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 10.08 (s, 1 H), 8.09–7.93 (m, 1 H), 7.74 (d, J = 7.3 Hz, 2 H), 7.64–7.48 (m, 5 H), 7.40–7.25 (m, 4 H), 5.90 (br. s., 1 H), 4.52 (d, J = 6.7 Hz, 2 H), 4.25 (t, J = 6.4 Hz, 1 H), 4.10 (d, J = 6.1 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl_3) δ = 199.4, 192.4, 156.1, 143.3, 141.0, 137.7, 136.6, 134.1, 129.7, 129.4, 129.2, 127.5, 126.9, 124.8, 119.8, 77.3, 76.7, 67.3, 53.5, 47.1. ESI-MS calcd for **2e** $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$ $[\text{M} + \text{H}]^+$ m/z = 466.05, found: 466.09.

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Seleno-benzaldehyde ester (**2f**)

83% isolated yield. ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 10.02 (s, 1 H), 7.87 (d, J = 8.5 Hz, 2 H), 7.77 (d, J = 7.3 Hz, 2 H), 7.68–7.59 (m, 4 H), 7.43–7.37 (m, 2 H), 7.35–7.30 (m, 2 H), 5.42 (br. s., 1 H), 4.56 (d, J = 6.7 Hz, 2 H), 4.32–4.23 (m, 1 H), 4.17 (d, J = 5.5 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl_3) δ = 191.3, 185.2, 156.1, 143.3, 141.1, 136.0, 130.6, 130.1, 129.8, 127.6, 126.9, 124.8, 119.9, 77.3, 76.7, 67.4, 53.6, 47.1. ESI-MS calcd for **2f** $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$ $[\text{M} + \text{H}]^+$ m/z = 466.05, found: 466.02.

N-linked glycopeptide (4c**)^[1,3]**

N-linked glycopeptide **4c** as a model of glycosylation reaction, ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ (ppm) = 8.53 (d, J = 8.1 Hz, 2H), 8.32 (d, J = 6.6 Hz, 2H), 8.19 (d, J = 9.1 Hz, 2H), 7.99 (t, J = 10.3 Hz, 3H), 7.78 (d, J = 10.0 Hz, 2H), 7.36–7.16 (m, 10H), 7.15–7.06 (m, 3H), 7.02–6.95 (m, 2H), 6.66 (s, 2H), 5.32 (dd, J = 5.5, 4.2 Hz, 3H), 4.81 (t, J = 9.3 Hz, 1H) (β -anomer), 4.55 (q, J = 6.5 Hz, 3H), 4.37 (q, J = 7.8 Hz, 2H), 4.22 (t, J = 7.7 Hz, 1H), 4.19–4.12 (m, 2H), 4.11–4.07 (m, 1H), 3.92 (q, J = 7.9 Hz, 3H), 3.64 (s, 1H), 3.57–3.49 (m, 3H), 3.08 (s, 3H), 2.98 (d, J = 10.3 Hz, 2H), 2.75–2.60 (m, 5H), 2.39–2.29 (m, 3H), 2.04–1.95 (m, 4H), 1.78 (s, 1H), 1.70 (q, J = 6.8 Hz, 3H), 1.51–1.42 (m, 5H), 1.36 (d, J = 14.9 Hz, 4H), 1.30–1.21 (m, 11H), 1.15 (dd, J = 21.8, 6.6 Hz, 4H), 1.04 (d, J = 6.2 Hz, 1H), 0.91–0.81 (m, 5H), 0.81–0.75 (m, 3H).

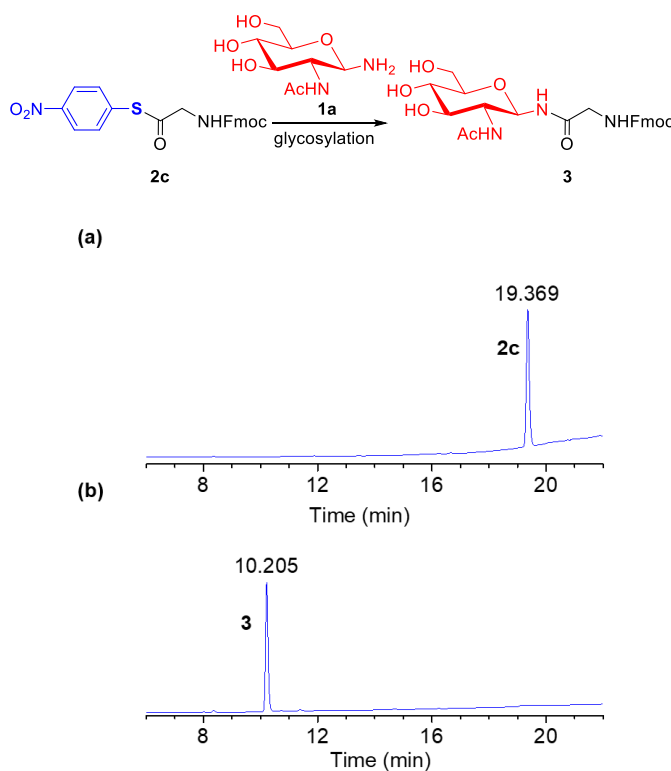
3. Analysis of the Process of Fmoc-Gly-ester-mediated Glycosylation^[1]

Figure S1. HPLC spectra of the starting material and the crude reaction mixture after 2 h of incubation; the product peak is labeled (gradient: 30% to 90% CH_3CN over 22 min, λ = 254 nm).

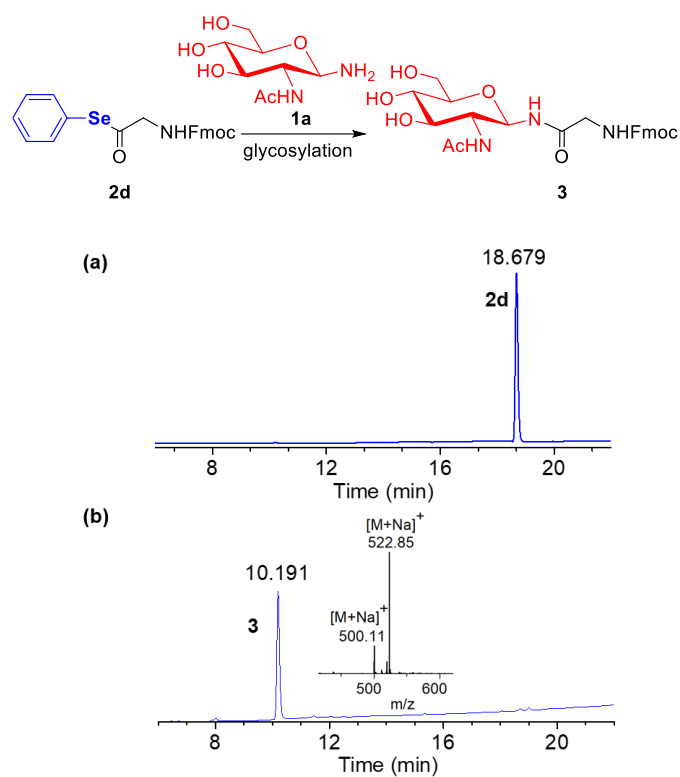


Figure S2. HPLC spectra of the starting material and the crude reaction mixture after 2 h of incubation; the product peak is labeled (gradient: 30% to 90% CH₃CN over 22 min, λ = 254 nm).

4. Analysis of the Process of Peptidyl Selenoester-mediated Glycosylation^[1]

4.1 Synthesis of glycopeptide 4c^[3]

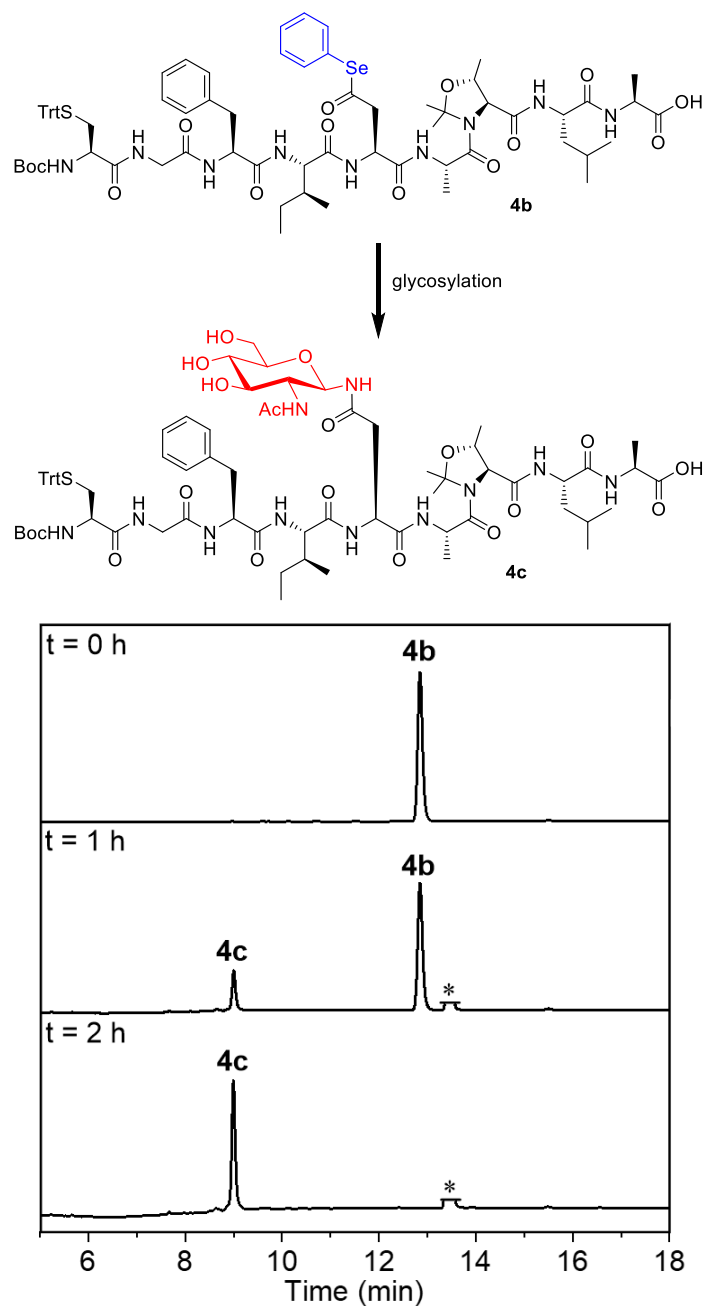


Figure S3. Analytical HPLC profiles of the ligation reaction (gradient: 80% to 100% CH₃CN over 18 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed 4b segment was not detected during the reaction).

4.2 Synthesis of glycopeptide 5c

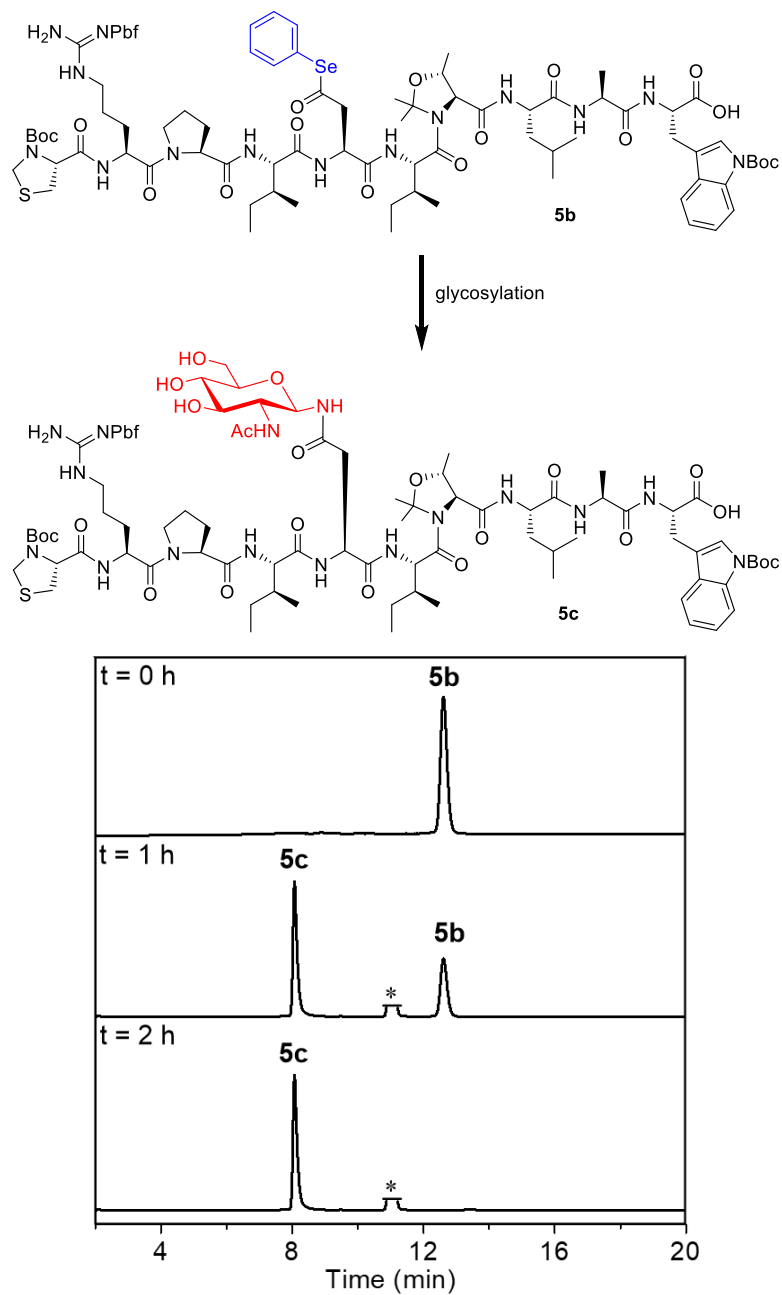


Figure S4. Analytical HPLC profiles of the ligation reaction (gradient: 90% CH₃CN over 20 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed **5b** segment was not detected during the reaction).

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4.3 Synthesis of glycopeptide 6c

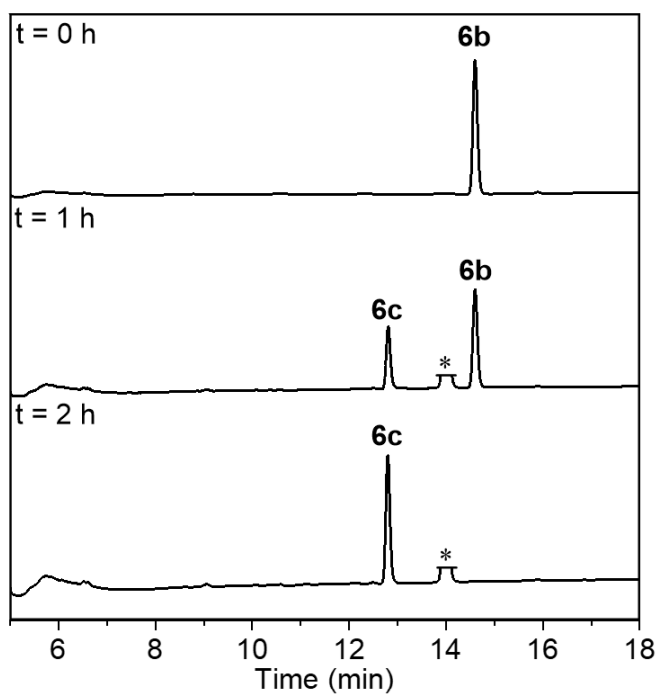
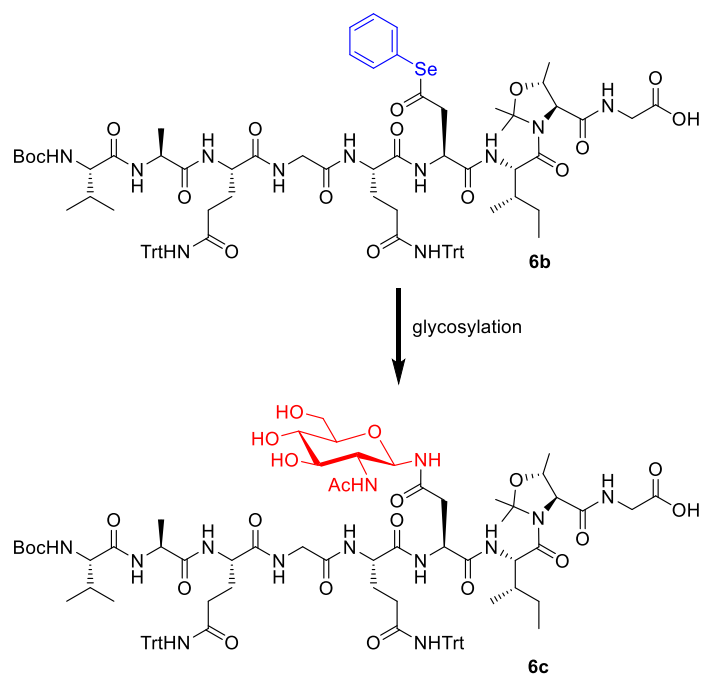


Figure S5. Analytical HPLC profiles of the ligation reaction (gradient: 75% to 100% CH₃CN over 18 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed **6b** segment was not detected during the reaction).

4.4 Synthesis of glycopeptide 7c

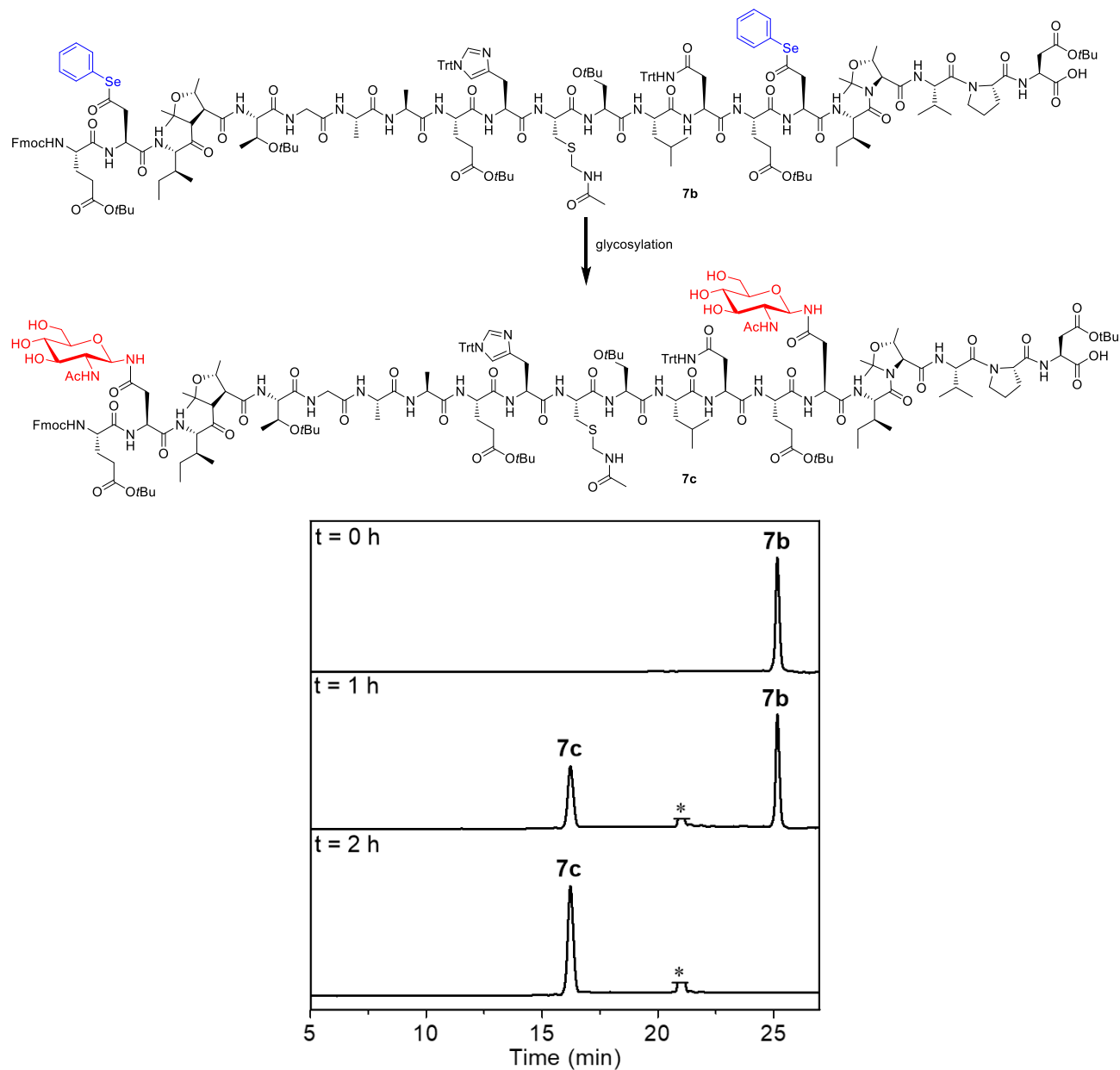


Figure S6. Analytical HPLC profiles of the ligation reaction (gradient: 50% to 100% CH₃CN over 27 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed **7b** segment was not detected during the reaction).

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4.5 Synthesis of glycopeptide 5d

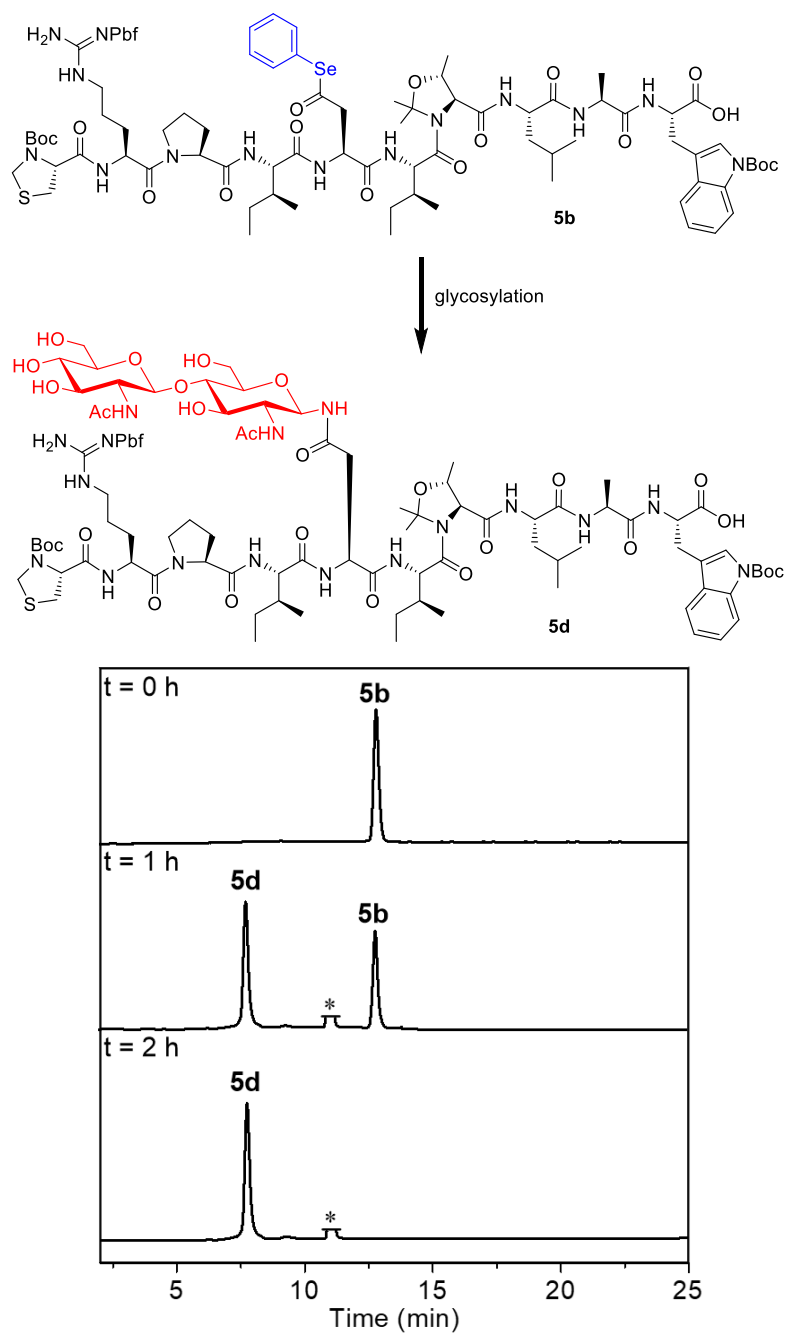


Figure S7. Analytical HPLC profiles of the ligation reaction (gradient: 90% CH₃CN over 25 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed 5b segment was not detected during the reaction).

4.6 Synthesis of glycopeptide 8d

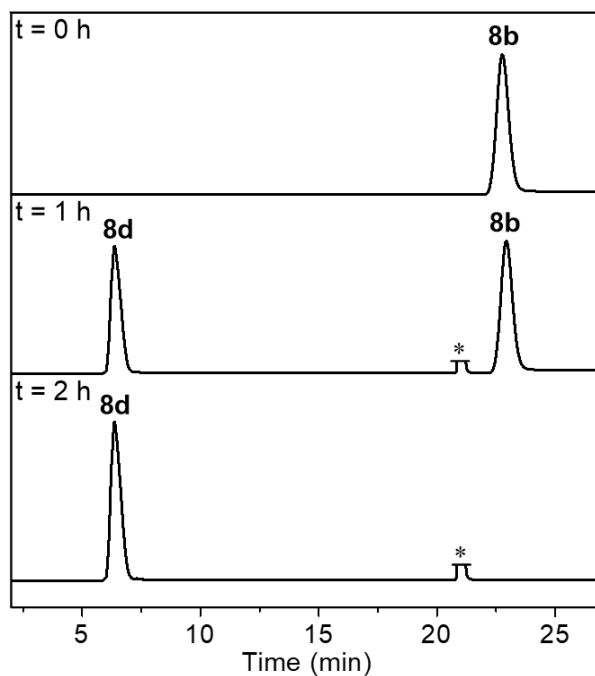
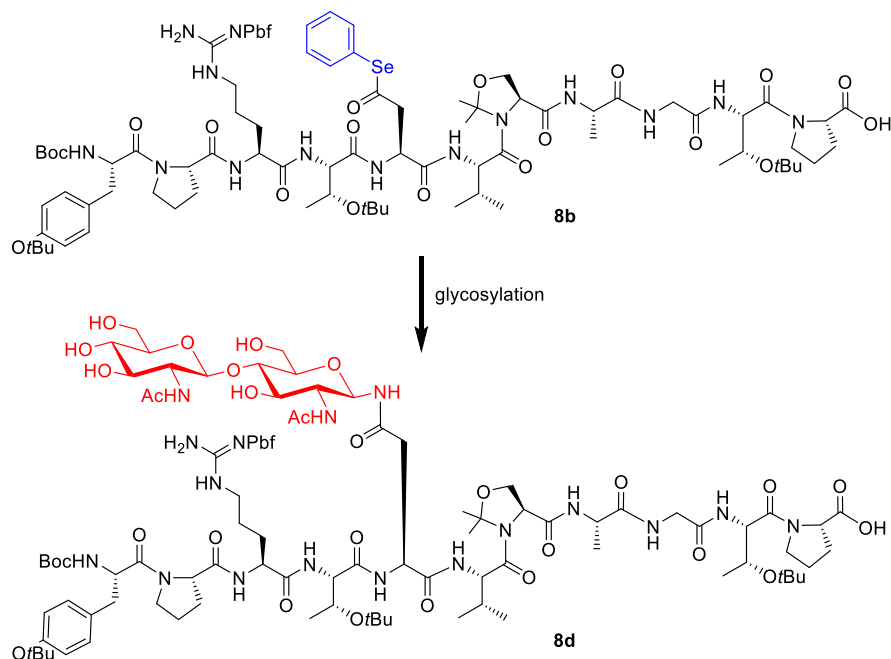


Figure S8. Analytical HPLC profiles of the ligation reaction (gradient: 50% to 100% CH₃CN over 27 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed **8b** segment was not detected during the reaction).

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4.7 Synthesis of glycopeptide 9d

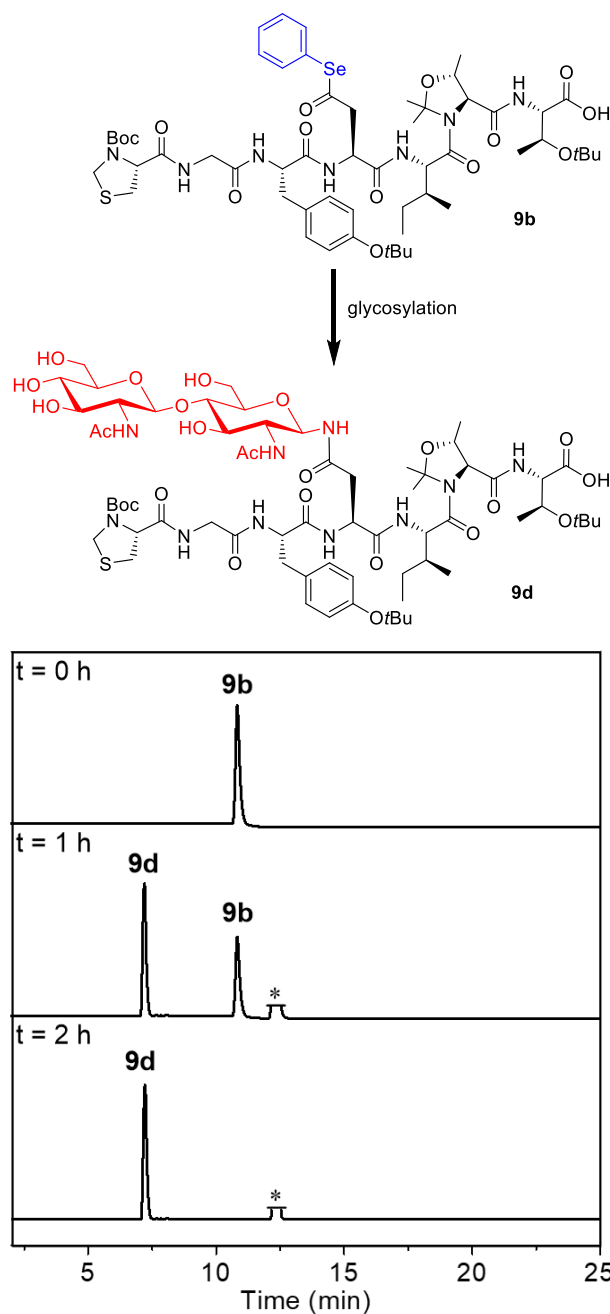
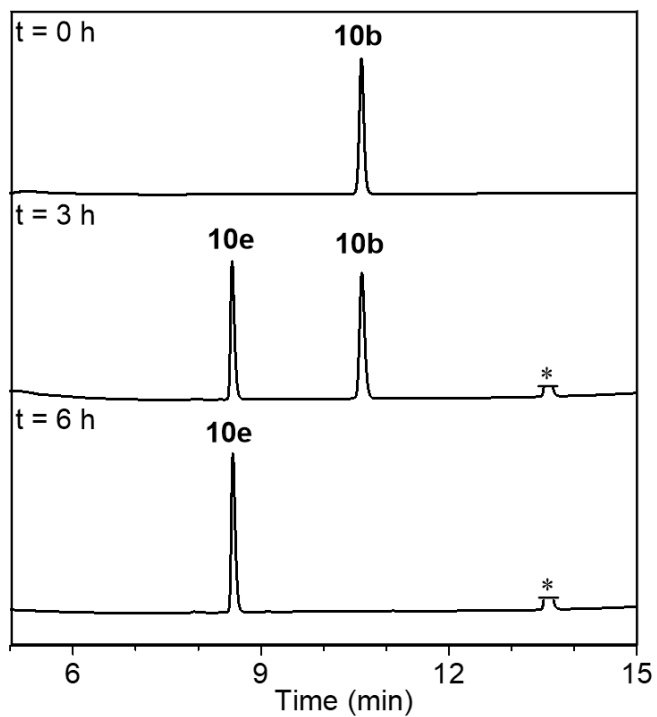
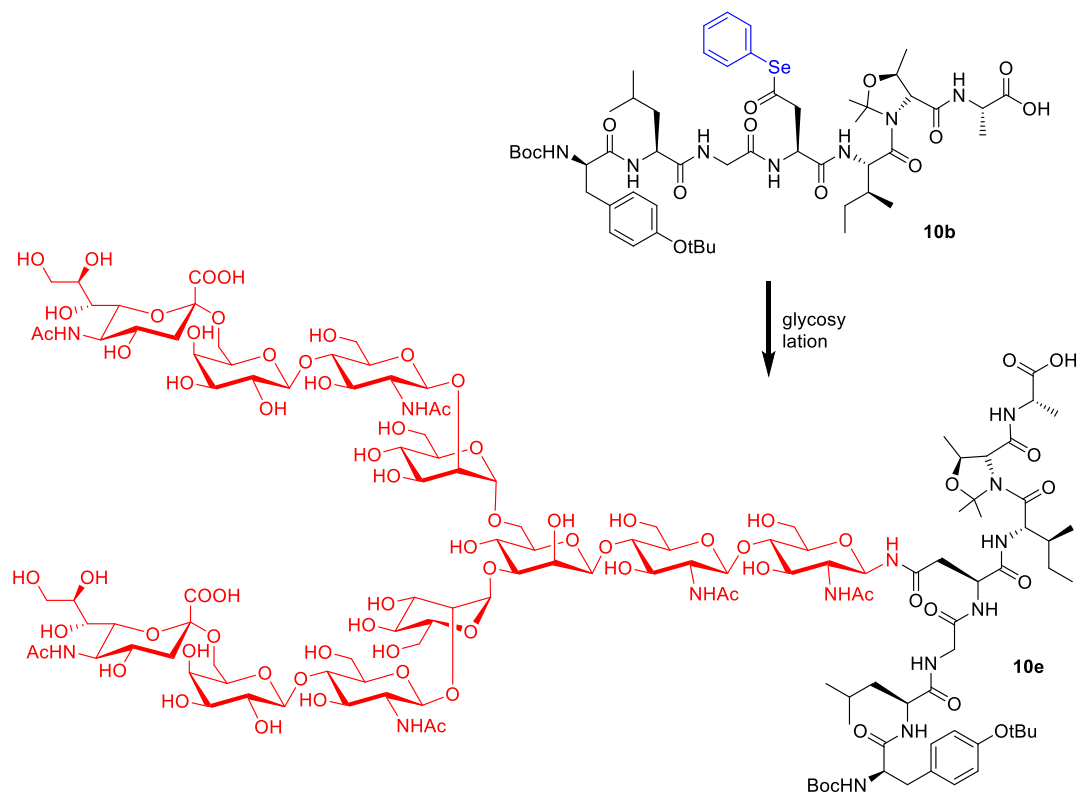


Figure S9. Analytical HPLC profiles of the ligation reaction (gradient: 85% to 100% CH₃CN over 25 min, $\lambda = 254$ nm): $t = 0$ h, $t = 1$ h, $t = 2$ h (* is the DPDS) (the hydrolyzed 9b segment was not detected during the reaction).

5. Analysis of the Process of Peptidyl Selenoester-mediated *N*-linked Glycosylation Containing the Complex Undecasaccharide^[4-6]

5.1. Synthesis of glycopeptide 10e



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Figure S10. Analytical HPLC profiles of the ligation reaction (gradient: 80% to 100% CH₃CN over 15 min, λ = 254 nm): t = 0 h, t = 3 h, t = 6 h (* is the DPDS) (the hydrolyzed **10b** segment was not detected during the reaction).

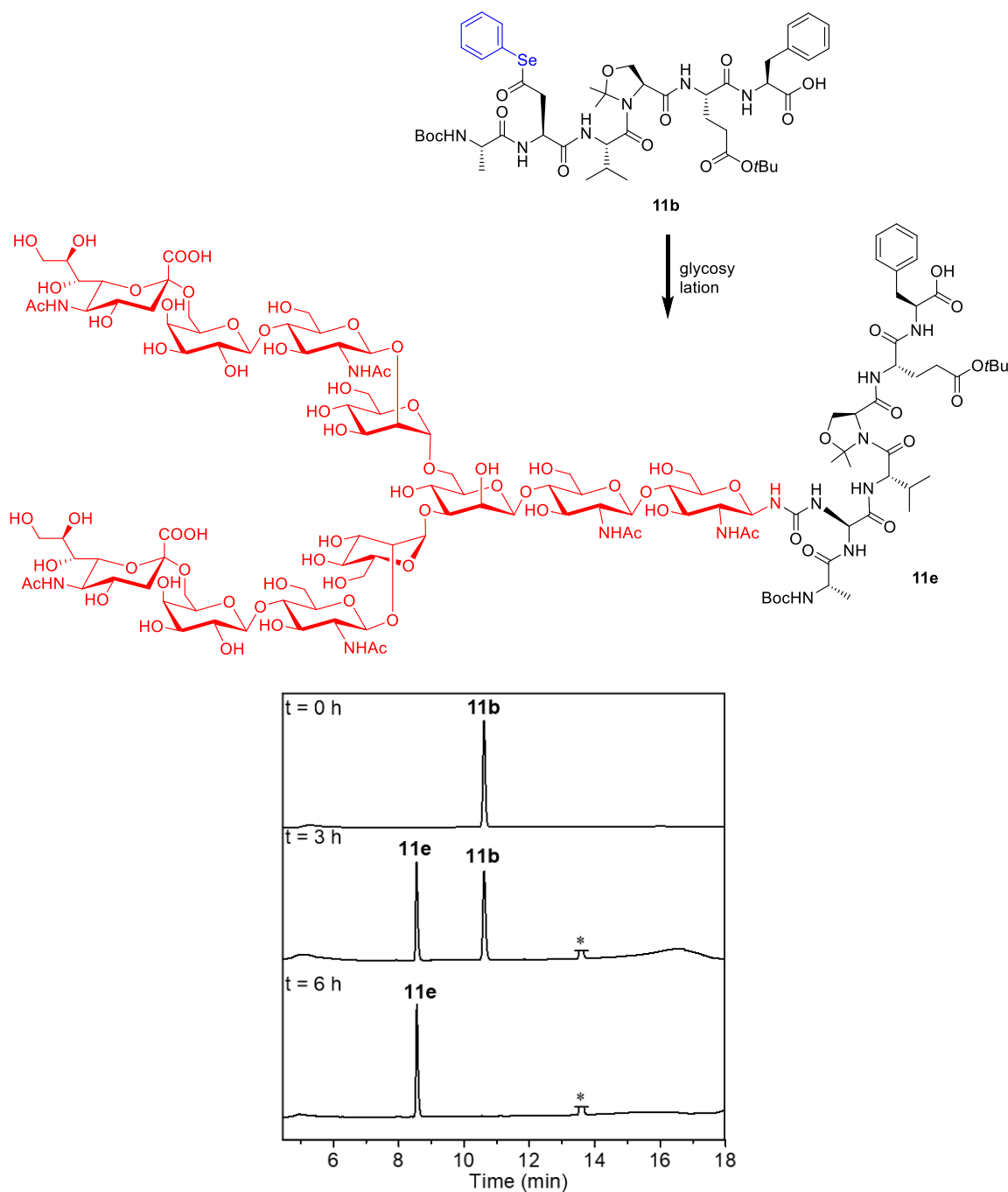
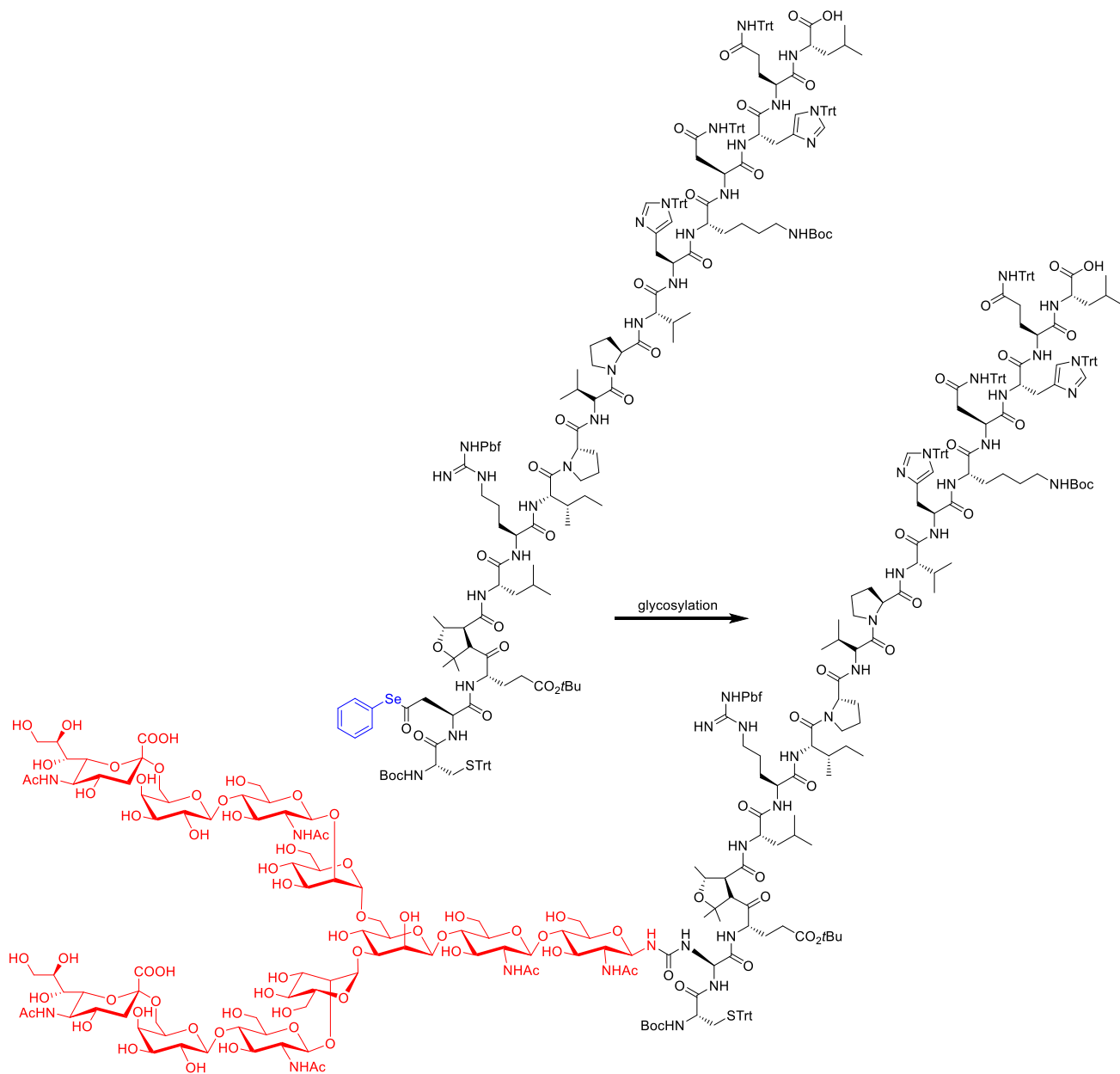
5.2. Synthesis of glycopeptide **11e**

Figure S11. Analytical HPLC profiles of the ligation reaction (gradient: 80% to 100% CH₃CN over 18 min, λ = 254 nm): t = 0 h, t = 2 h, t = 4 h (* is the DPDS) (the hydrolyzed **11b** segment was not detected during the reaction).

5.3. Synthesis of glycopeptide 12e



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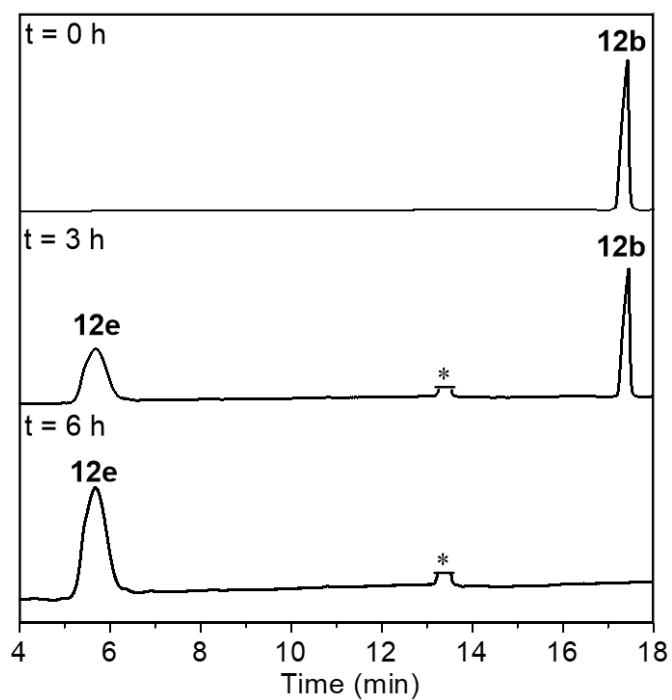


Figure S12. Analytical HPLC profiles of the ligation reaction (gradient: 80% to 100% CH₃CN over 18 min, 100% CH₃CN over 14-18 min, $\lambda = 254$ nm): $t = 0$ h, $t = 3$ h, $t = 6$ h (* is the DPDS) (the hydrolyzed **12b** segment was not detected during the reaction).

6. HPLC and ESI-MS spectra of peptidyl selenoesters

6.1. Peptidyl selenoester **4b**

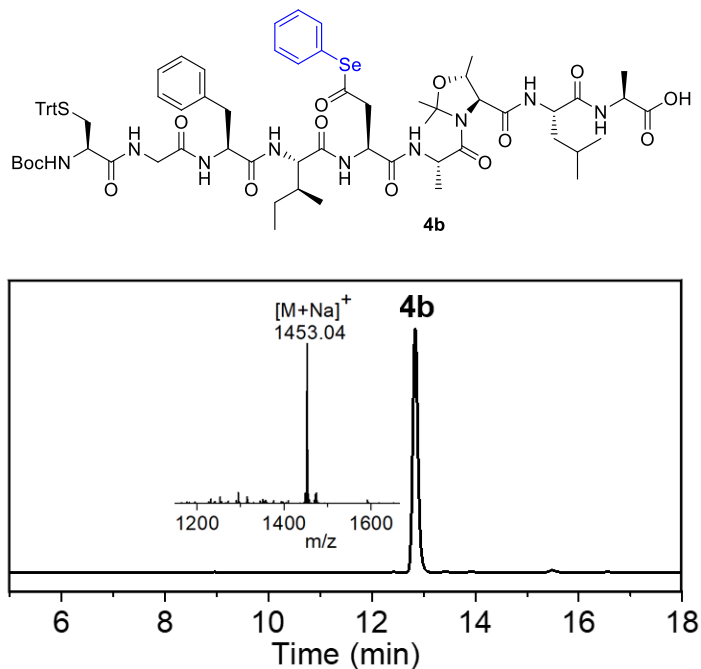
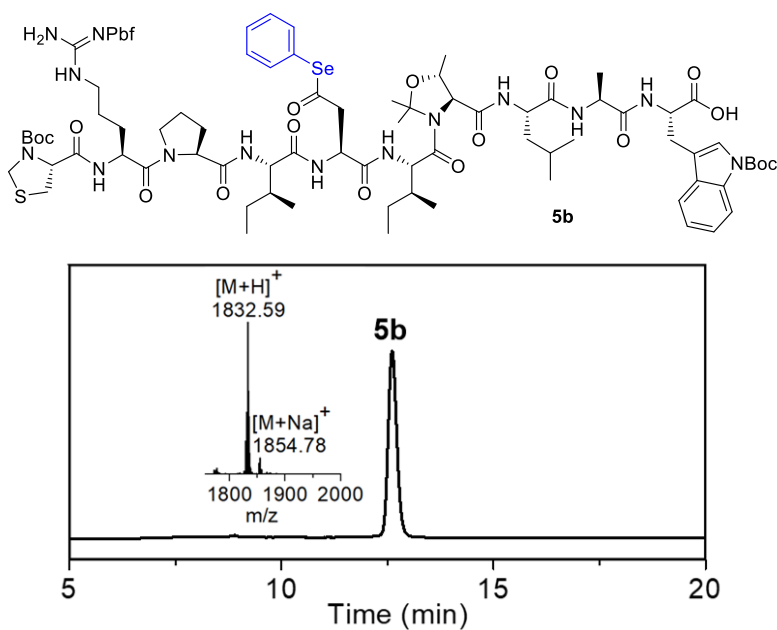


Figure S13. HPLC spectrum of purified selenoester **4b** (gradient: 80% to 100% CH₃CN over 18 min, λ = 254 nm). The isolated yield of **4b** by SPPS is about 80% (57 mg). ESI-MS calcd for **4b** C₇₃H₉₂N₉O₁₄SSe $[M+Na]^+$ m/z = 1453.57; observed: $[M+Na]^+$ m/z = 1453.04.

6.2. Peptidyl selenoester **5b**



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Figure S14. HPLC spectrum of purified selenoester **5b** (gradient: 90% CH₃CN over 20 min, λ = 254 nm). The isolated yield of **5b** by SPPS is about 83% (77 mg). ESI-MS calcd for **5b** C₈₇H₁₂₆N₁₄O₂₀S₂Se [M+H]⁺ m/z = 1832.12, [M+Na]⁺ m/z = 1854.12; observed: [M+H]⁺ m/z = 1832.59, [M+Na]⁺ m/z = 1854.78.

6.3. Peptidyl selenoester **6b**

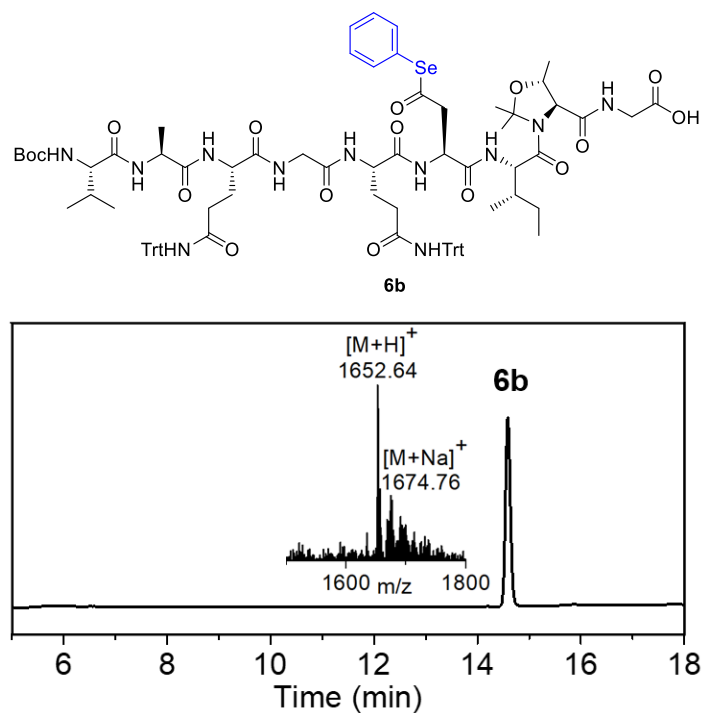


Figure S15. HPLC spectrum of purified selenoester **6b** (gradient: 75% to 100% CH₃CN over 18 min, λ = 254 nm). The isolated yield of **6b** by SPPS is about 75% (62 mg). ESI-MS calcd for **6b** C₈₈H₁₀₅N₁₁O₁₆Se [M+H]⁺ m/z = 1652.69, [M+Na]⁺ m/z = 1674.69; observed: [M+H]⁺ m/z = 1652.64, [M+Na]⁺ m/z = 1674.76.

6.4. Peptidyl selenoester **7b**

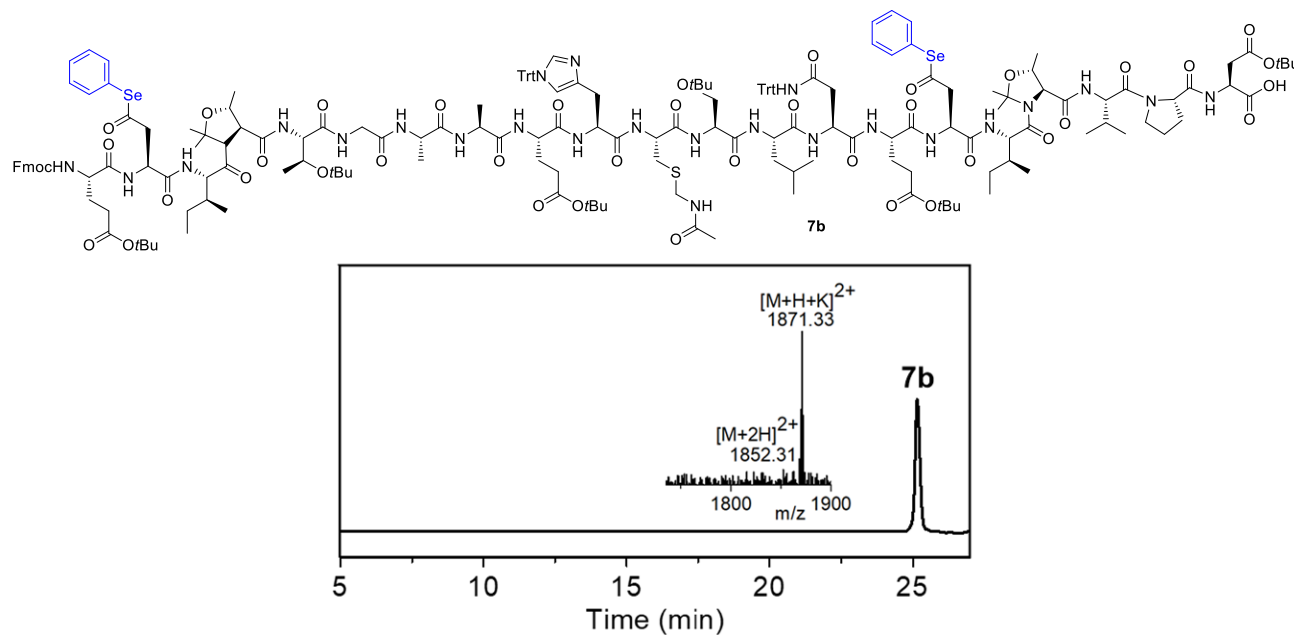
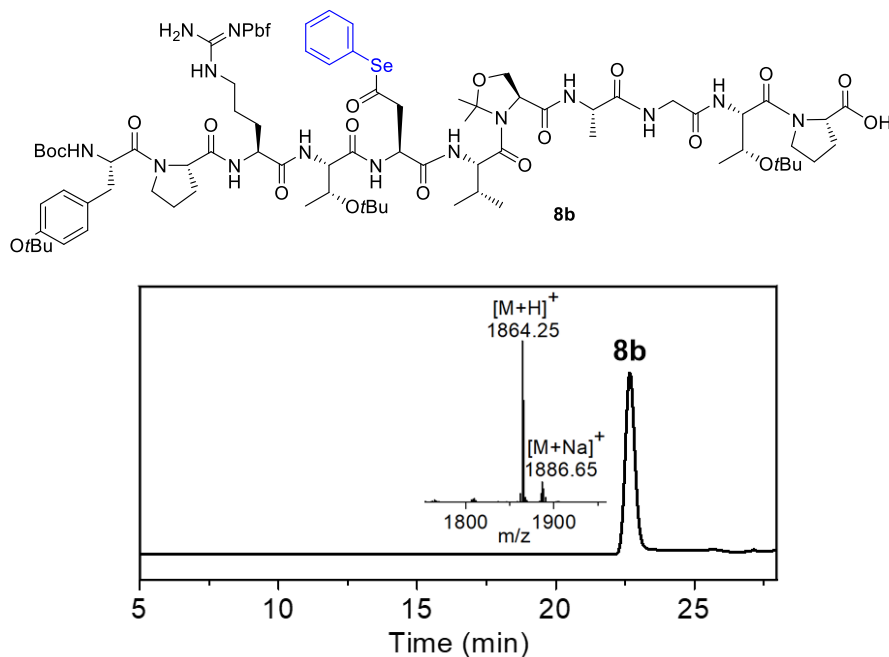


Figure S16. HPLC spectrum of purified selenoester **7b** (gradient: 50% to 100% CH₃CN over 27 min, λ = 254 nm). The isolated yield of **7b** by SPPS is about 67% (124 mg). ESI-MS calcd for **7b** C₁₉₀H₂₅₂N₂₄O₄₀SSe₂ $[M+2H]^{2+}$ m/z = 1852.11, $[M+H+K]^{2+}$ m/z = 1871.11; observed: $[M+2H]^{2+}$ m/z = 1852.31, $[M+H+K]^{2+}$ m/z = 1871.33.

6.5. Peptidyl selenoester **8b**



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Figure S17. HPLC spectrum of purified selenoester **8b** (gradient: 50% to 100% CH₃CN over 27 min, $\lambda = 254$ nm). The isolated yield of **8b** by SPPS is about 72% (67 mg). ESI-MS calcd for **8b** C₈₉H₁₃₄N₁₄O₂₂SSe [M+H]⁺ $m/z = 1864.14$, [M+Na]⁺ $m/z = 1886.14$; observed: [M+H]⁺ $m/z = 1864.25$, [M+Na]⁺ $m/z = 1886.65$.

6.6. Peptidyl selenoester **9b**

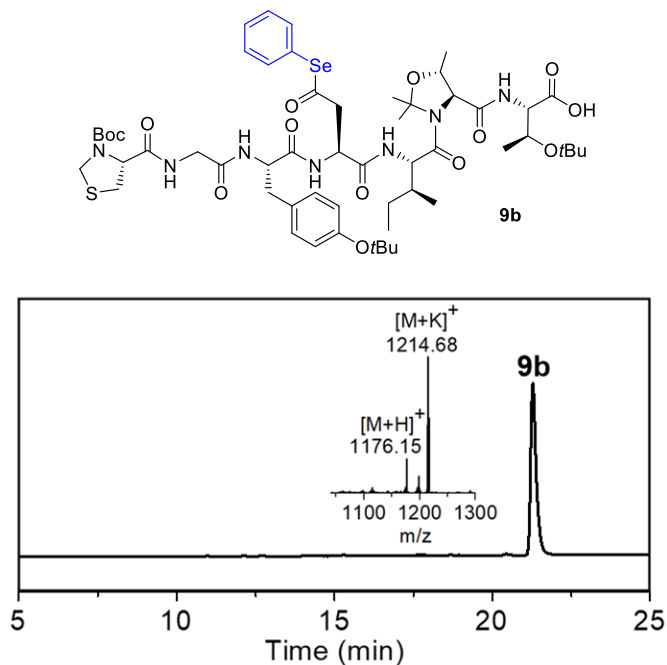


Figure S18. HPLC spectrum of purified selenoester **9b** (gradient: 35% to 100% CH₃CN over 25 min, $\lambda = 254$ nm). The isolated yield of **9b** by SPPS is about 81% (67 mg). ESI-MS calcd for **9b** C₅₅H₈₁N₇O₁₄SSe [M+H]⁺ $m/z = 1176.47$, [M+K]⁺ $m/z = 1214.47$; observed: [M+H]⁺ $m/z = 1176.15$, [M+K]⁺ $m/z = 1214.68$.

6.7. Peptidyl selenoester **10b**

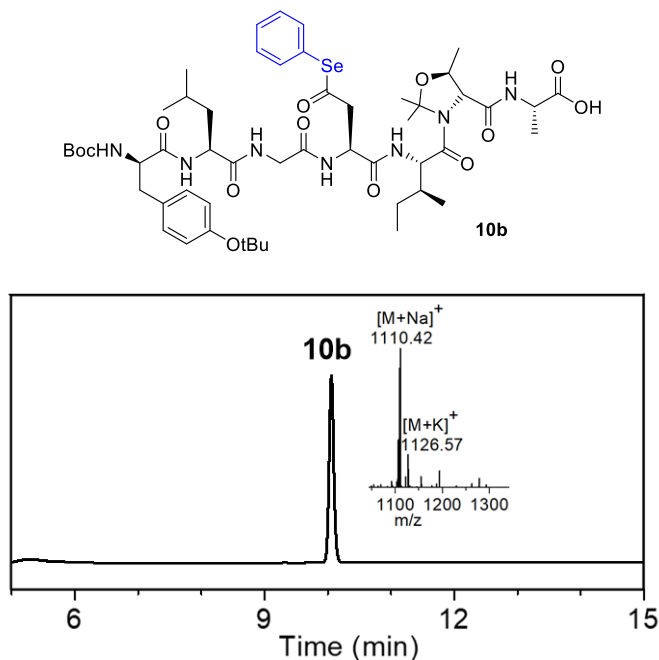
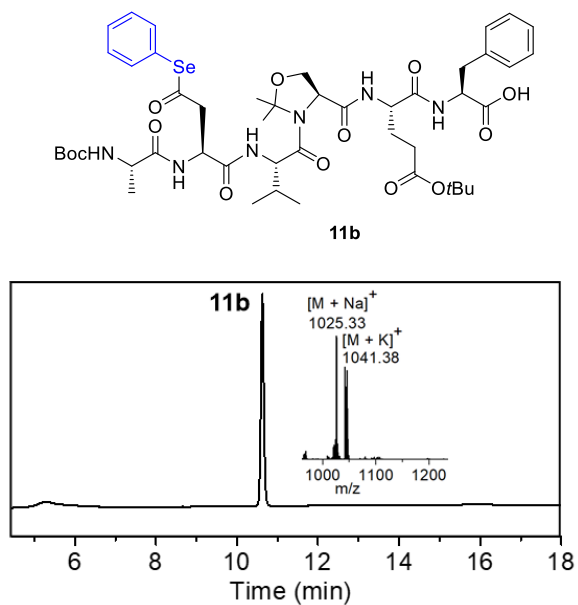


Figure S19. HPLC spectrum of purified selenoester **10b** (gradient: 80% to 100% CH₃CN over 15 min, λ = 254 nm). The isolated yield of **10b** by SPPS is about 77% (42 mg). ESI-MS calcd for **10b** C₅₂H₇₇N₇O₁₃Se $[M+Na]^+$ m/z = 1110.47, $[M+K]^+$ m/z = 1126.47; observed: $[M+Na]^+$ m/z = 1110.42, $[M+K]^+$ m/z = 1126.57.

6.8. Peptidyl selenoester **11b**



Supplementary Material

Figure S20. HPLC spectrum of purified selenoester **11b** (gradient: 80% to 100% CH₃CN over 18 min, $\lambda = 254$ nm). The isolated yield of **11b** by SPPS is about 79% (40 mg). ESI-MS calcd for **11b** C₄₇H₆₆N₆O₁₃Se [M+Na]⁺ $m/z = 1025.38$, [M+K]⁺ $m/z = 1041.38$; observed: [M+H]⁺ $m/z = 1025.33$, [M+Na]⁺ $m/z = 1041.38$.

6.9. Peptidyl selenoester **12b**

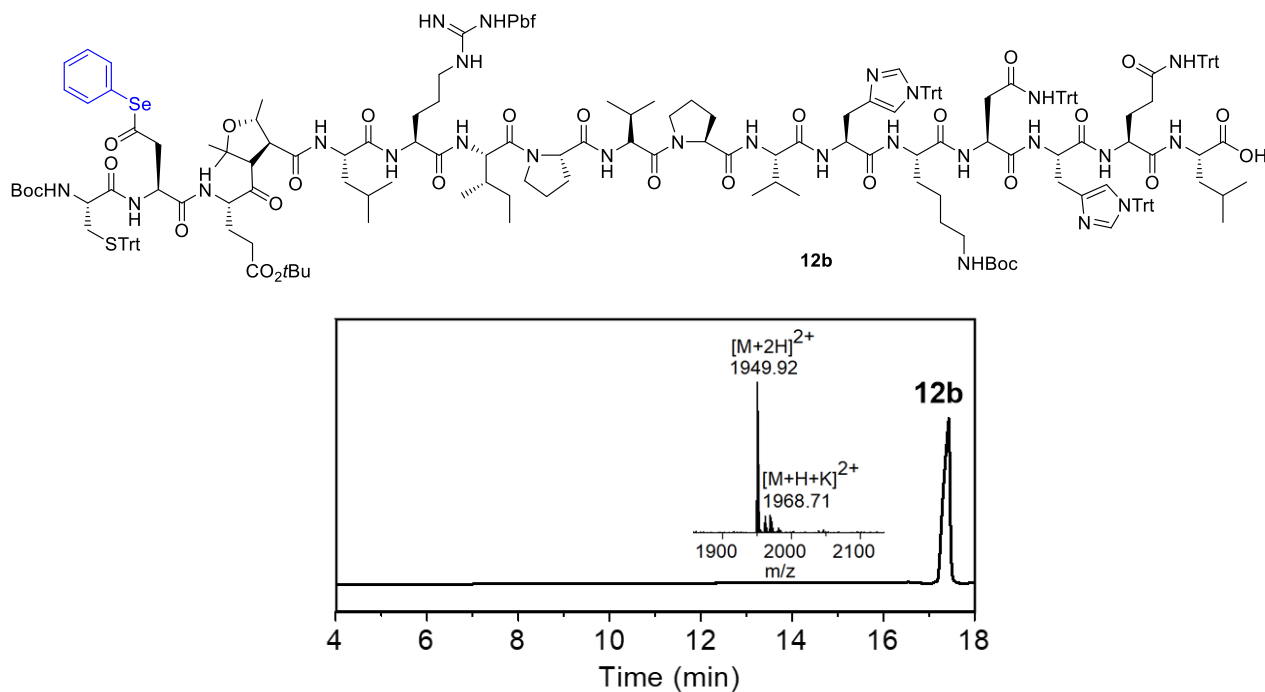


Figure S21. HPLC spectrum of purified selenoester **12b** (gradient: 80% to 100% CH₃CN over 18 min, 100% CH₃CN over 14-18 min, $\lambda = 254$ nm). The isolated yield of **12b** by SPPS is about 58% (113 mg). ESI-MS calcd for **12b** C₂₁₈H₂₆₁N₂₇O₃₁S₂Se [M+2H]²⁺ $m/z = 1949.92$, [M+H+K]²⁺ $m/z = 1968.91$; observed: [M+2H]²⁺ $m/z = 1949.92$, [M+H+K]²⁺ $m/z = 1968.71$.

7. HPLC and ESI-MS Spectra of *N*-linked Glycopeptides

7.1. Glycopeptide 4c

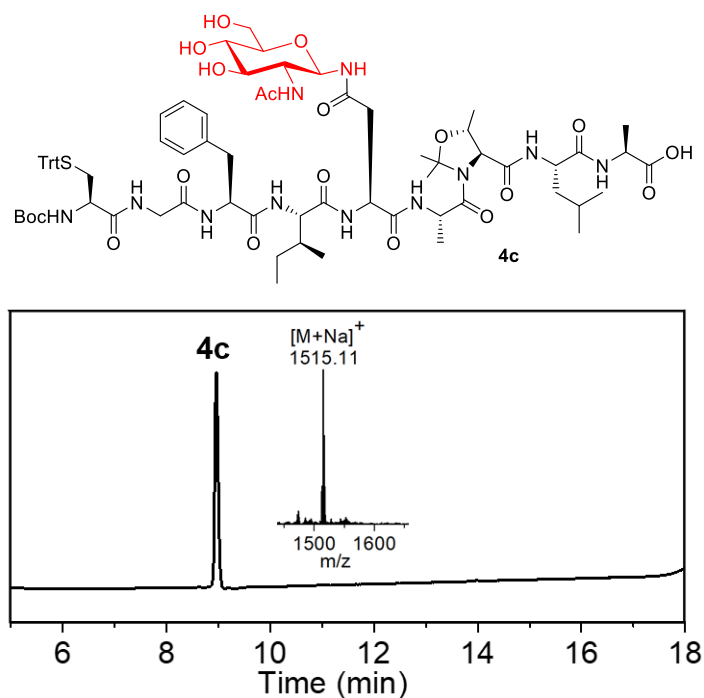
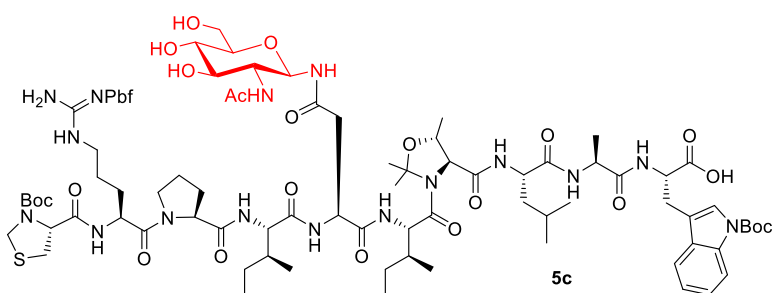


Figure S22. HPLC spectrum of purified glycopeptide **4c** (gradient: 80% to 100% CH₃CN over 18 min, $\lambda = 254$ nm). The isolated yield of **4c** is about 78% (6 mg, in solution) and 67% (50 mg, on resin), ESI-MS calcd for **4c** C₇₅H₁₀₂N₁₁O₁₉S $[M+Na]^+$ $m/z = 1515.71$; observed: $[M+Na]^+$ $m/z = 1515.11$.

7.2. Glycopeptide 5c



Supplementary Material

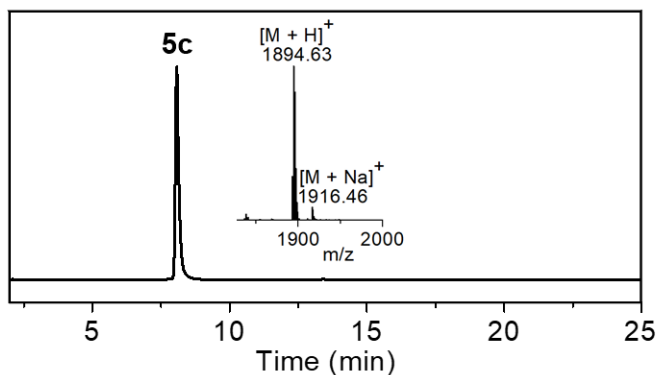


Figure S23. HPLC spectrum of purified glycopeptide **5c** (gradient: 90% CH₃CN over 25 min, λ = 254 nm). The isolated yield of **5c** is about 83% (8 mg, in solution) and 65% (61 mg, on resin), ESI-MS calcd for **5c** C₈₉H₁₃₆N₁₆O₂₅S₂ [M+H]⁺ m/z = 1894.93, [M+Na]⁺ m/z = 1916.93; observed: [M+H]⁺ m/z = 1894.63, [M+Na]⁺ m/z = 1916.46.

7.3. Glycopeptide 6c

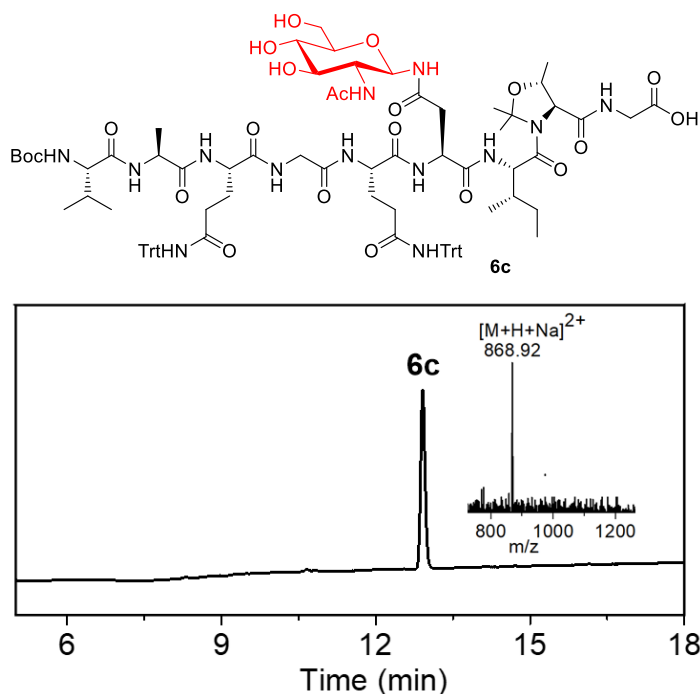


Figure S25. HPLC spectrum of purified glycopeptide **6c** (gradient: 75% to 100% CH₃CN over 18 min, λ = 254 nm). The isolated yield of **6c** is about 69% (6 mg), ESI-MS calcd for **6c** C₇₅H₁₀₂N₁₁O₁₉S [M+H+Na]²⁺ m/z = 868.91; observed: [M+H+Na]²⁺ m/z = 868.92.

7.4. Glycopeptide 7c

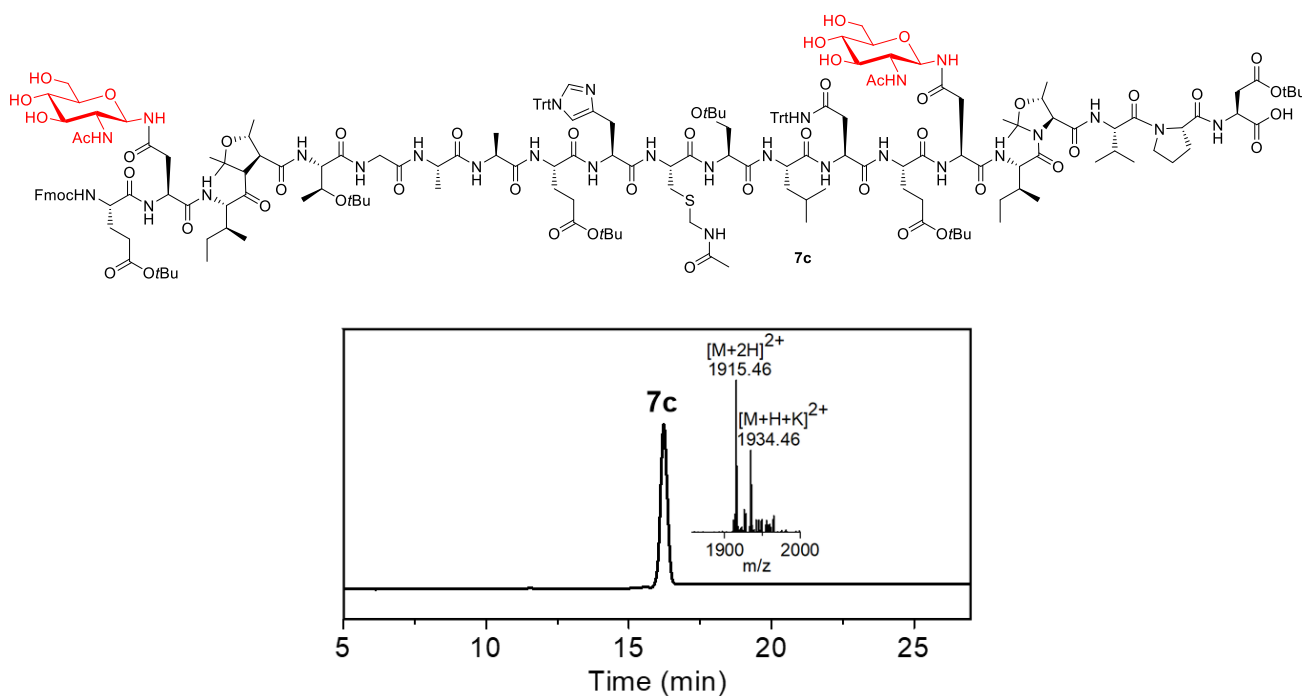
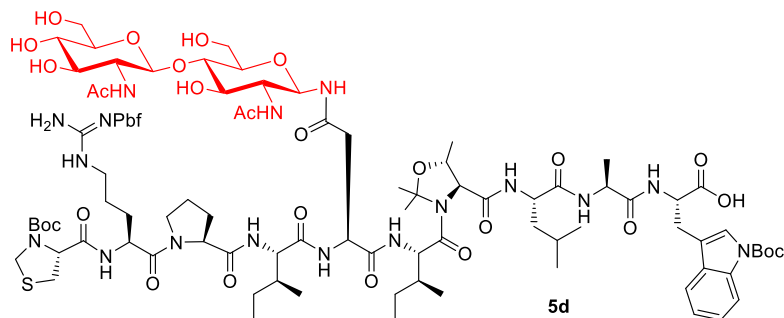


Figure S26. HPLC spectrum of purified glycopeptide **7c** (gradient: 50% to 100% CH₃CN over 27 min, λ = 254 nm). The isolated yield of **7c** is about 79% (15 mg, in solution). ESI-MS calcd for **11c** C₁₉₄H₂₇₂N₂₈O₅₀S $[M+2H]^{2+}$ m/z = 1915.26, $[M+H+K]^{2+}$ m/z = 1934.26; observed: $[M+2H]^{2+}$ m/z = 1915.46, $[M+H+K]^{2+}$ m/z = 1934.46.

7.5. Glycopeptide 5d



Supplementary Material

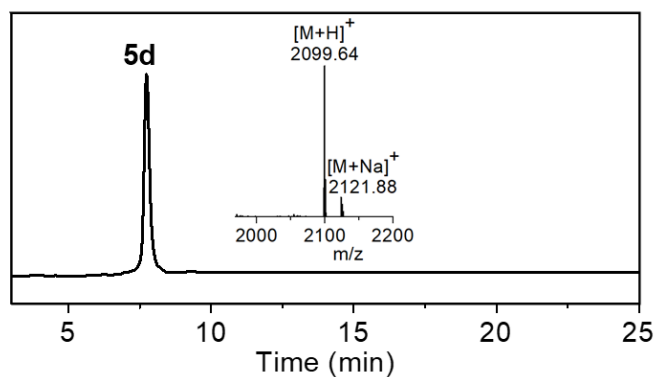


Figure S24. HPLC spectrum of purified glycopeptide **5d** (gradient: 90% CH₃CN over 25 min, λ = 254 nm). The isolated yield of **5d** is about 79% (8 mg, in solution). ESI-MS calcd for **5d** C₉₇H₁₅₁N₁₇O₃₀S₂ [M+H]⁺ m/z = 2099.46, [M+Na]⁺ m/z = 2121.46; observed: [M+H]⁺ m/z = 2099.64, [M+Na]⁺ m/z = 2121.88.

7.6. Glycopeptide 8d

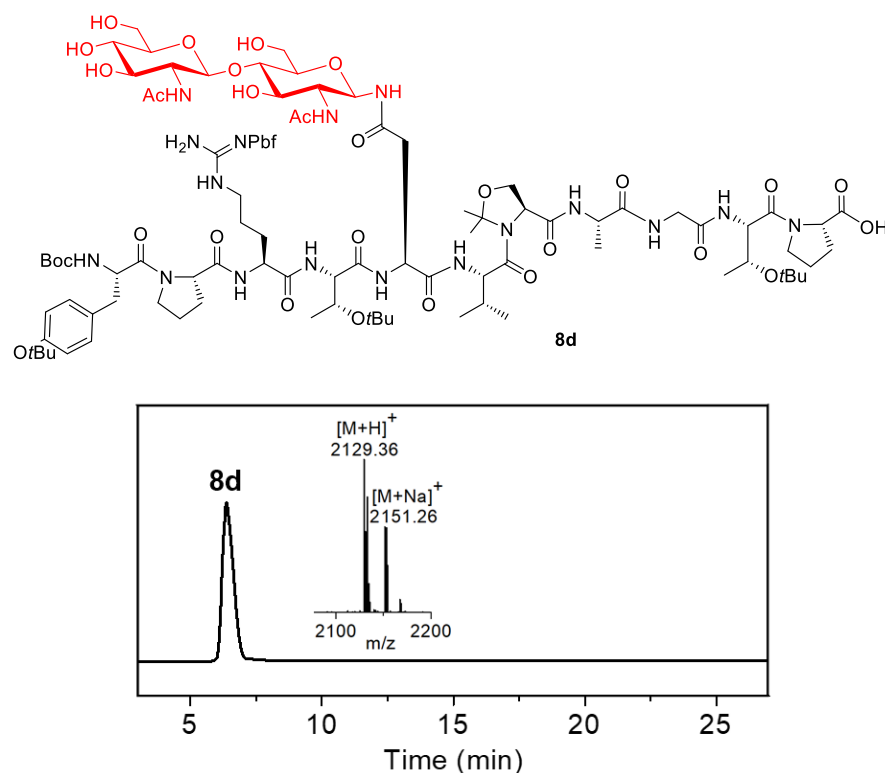


Figure S27. HPLC spectrum of purified glycopeptide **8d** (gradient: 50% to 100% CH₃CN over 27 min, λ = 254 nm). The isolated yield of **8d** is about 82% (8 mg). ESI-MS calcd for **8d** C₉₉H₁₅₇N₁₇O₃₂S [M+H]⁺ m/z = 2129.09, [M+Na]⁺ m/z = 2151.09; observed: [M+H]⁺ m/z = 2129.36, [M+Na]⁺ m/z = 2151.26.

7.7. Glycopeptide 9d

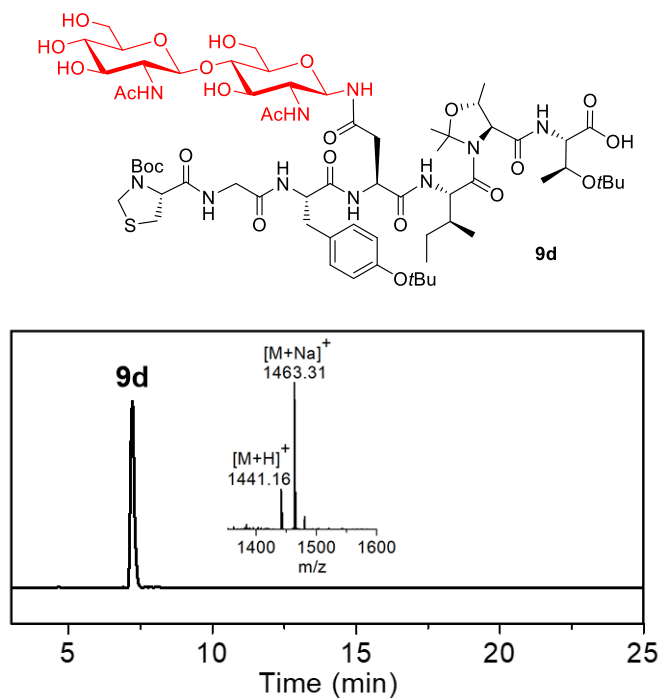
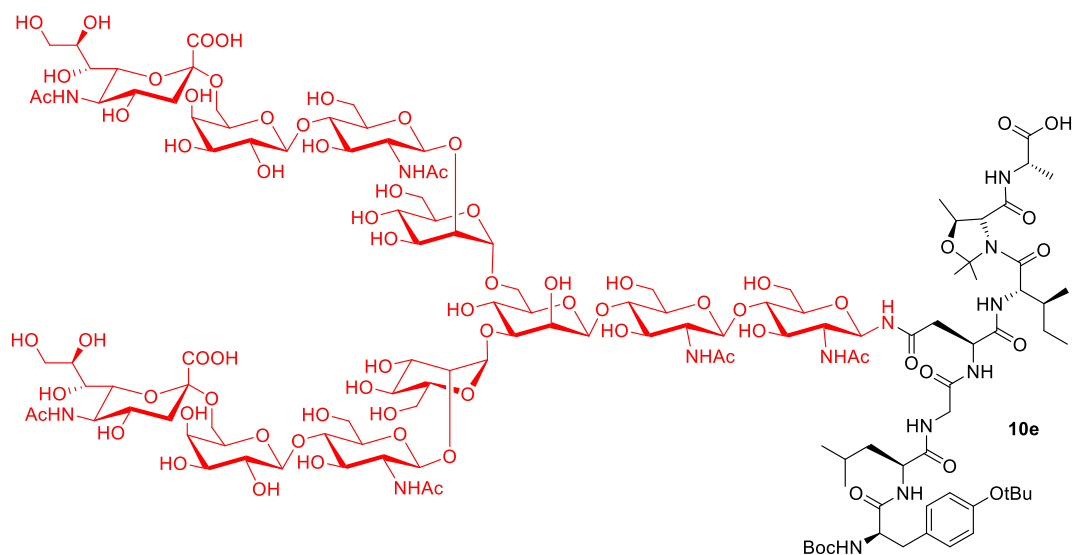


Figure S28. HPLC spectrum of purified glycopeptide **9d** (gradient: 85% to 100% CH₃CN over 25 min, λ = 254 nm). The isolated yield of **9d** is about 84% (6 mg). ESI-MS calcd for **9d** C₆₅H₁₀₄N₁₀O₂₄S $[M+H]^+$ m/z = 1441.69, $[M+Na]^+$ m/z = 1463.69; observed: $[M+H]^+$ m/z = 1441.16, $[M+Na]^+$ m/z = 1463.31.

7.8. Glycopeptide 10e



Supplementary Material

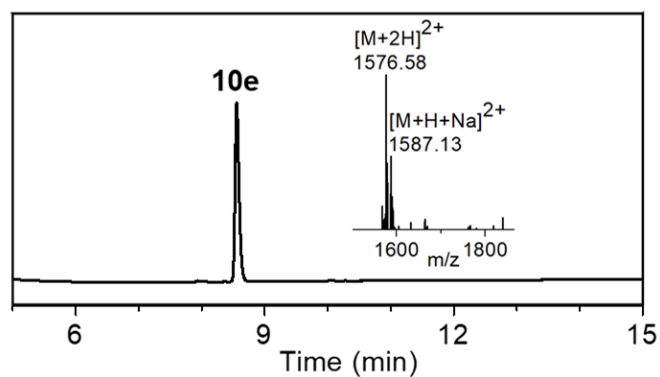


Figure S29. HPLC spectrum of purified glycopeptide **10e** (gradient: 80% to 100% CH₃CN over 15 min, λ = 254 nm). The isolated yield of **10e** is about 59% (4 mg). ESI-MS calcd for **10e** C₁₃₀H₂₁₀N₁₄O₇₄ [M+2H]²⁺ m/z = 1576.66, [M+H+Na]²⁺ m/z = 1587.66; observed: [M+2H]²⁺ m/z = 1576.58, [M+H+Na]²⁺ m/z = 1587.13.

7.9. Glycopeptide 11e

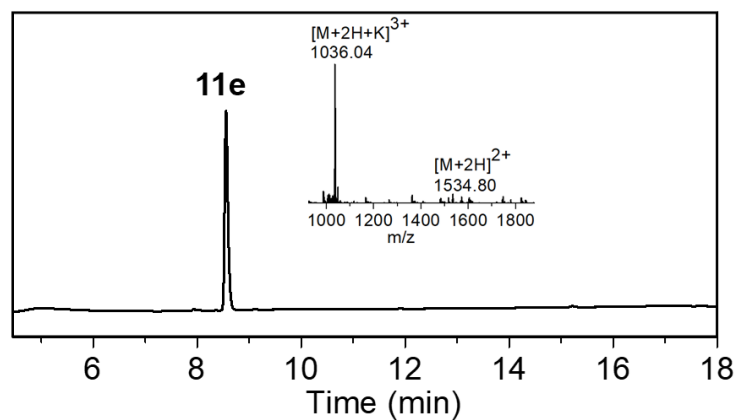
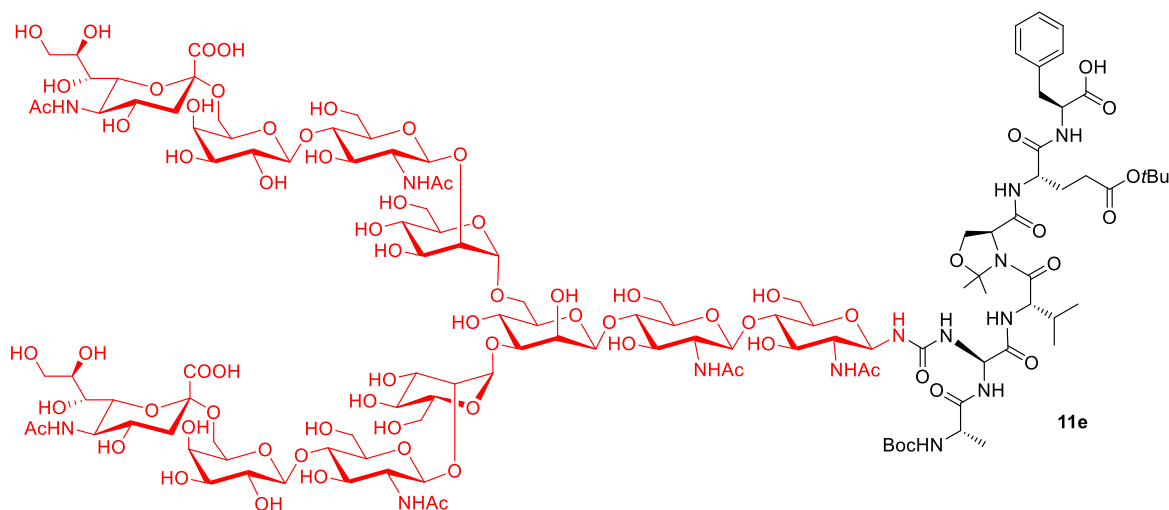


Figure S30. HPLC spectrum of purified glycopeptide **11e** (gradient: 80% to 100% CH₃CN over 18 min, λ = 254 nm). The isolated yield of **11e** is about 68% (4 mg). ESI-MS calcd for **11e** C₁₂₄H₁₉₈N₁₄O₇₄ [M+2H]²⁺ m/z = 1534.61, [M+2H+K]³⁺ m/z = 1036.07; observed: [M+2H]²⁺ m/z = 1534.80, [M+2H+K]³⁺ m/z = 1036.04.

7.10. Glycopeptide 12e

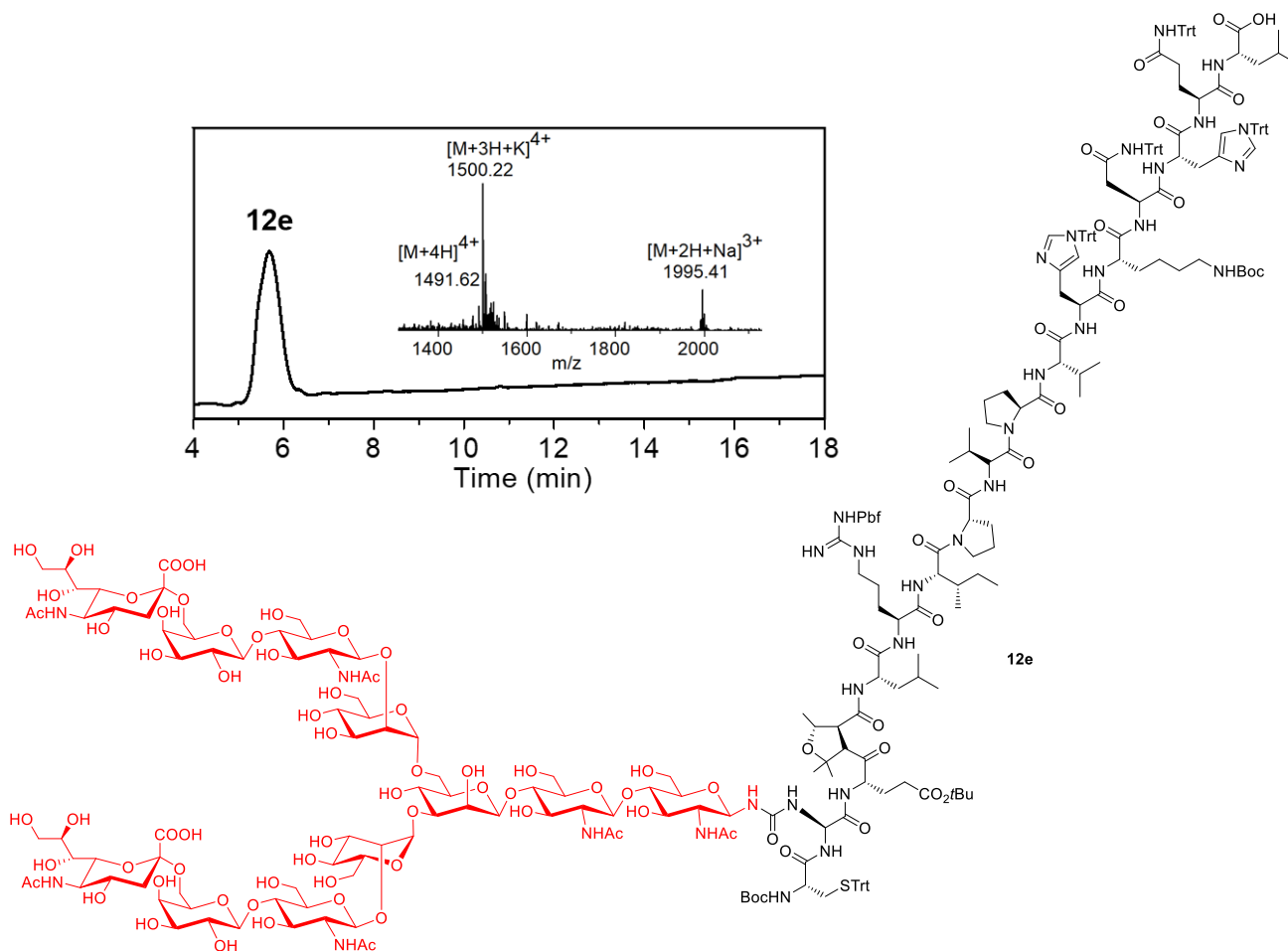
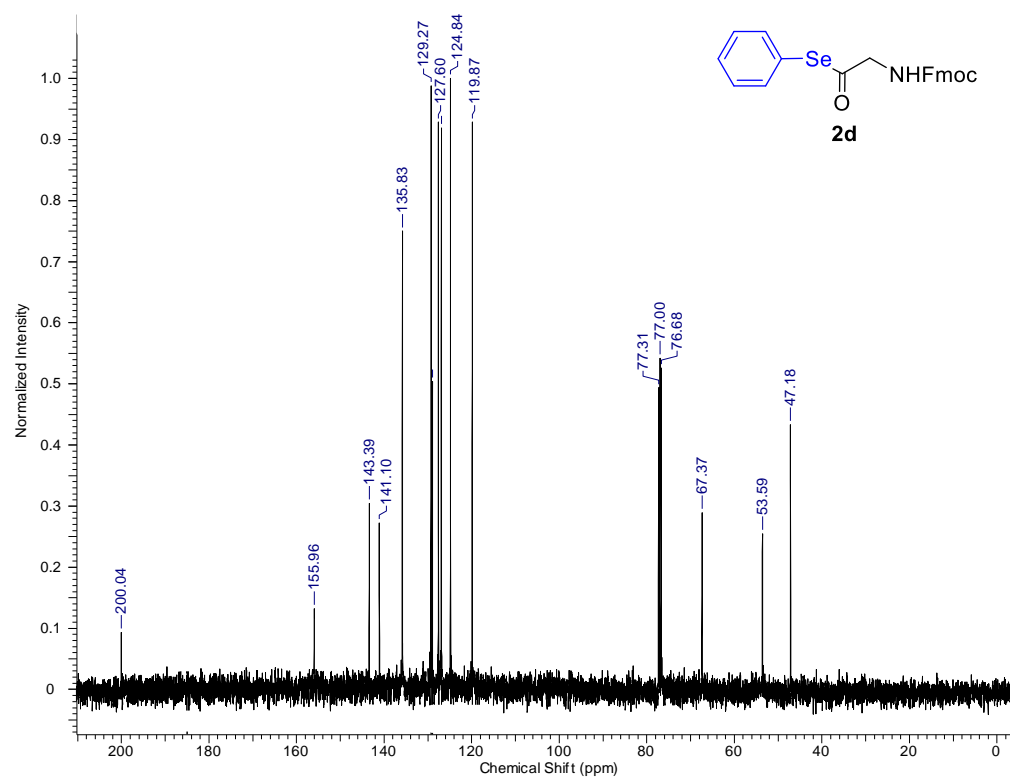
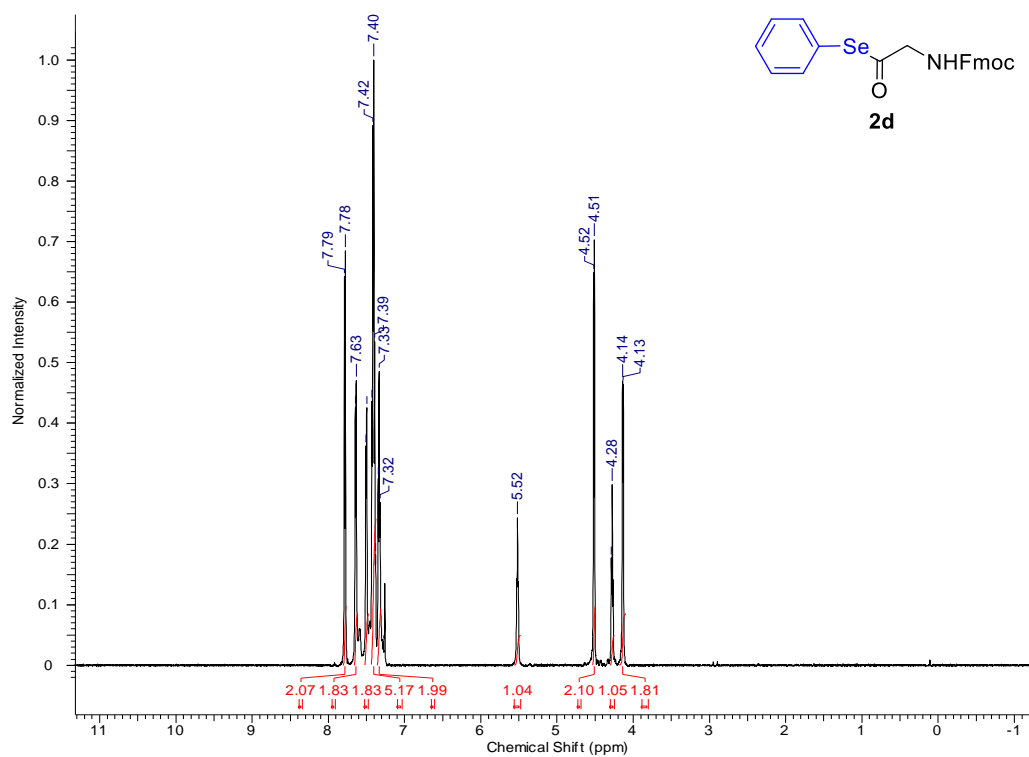
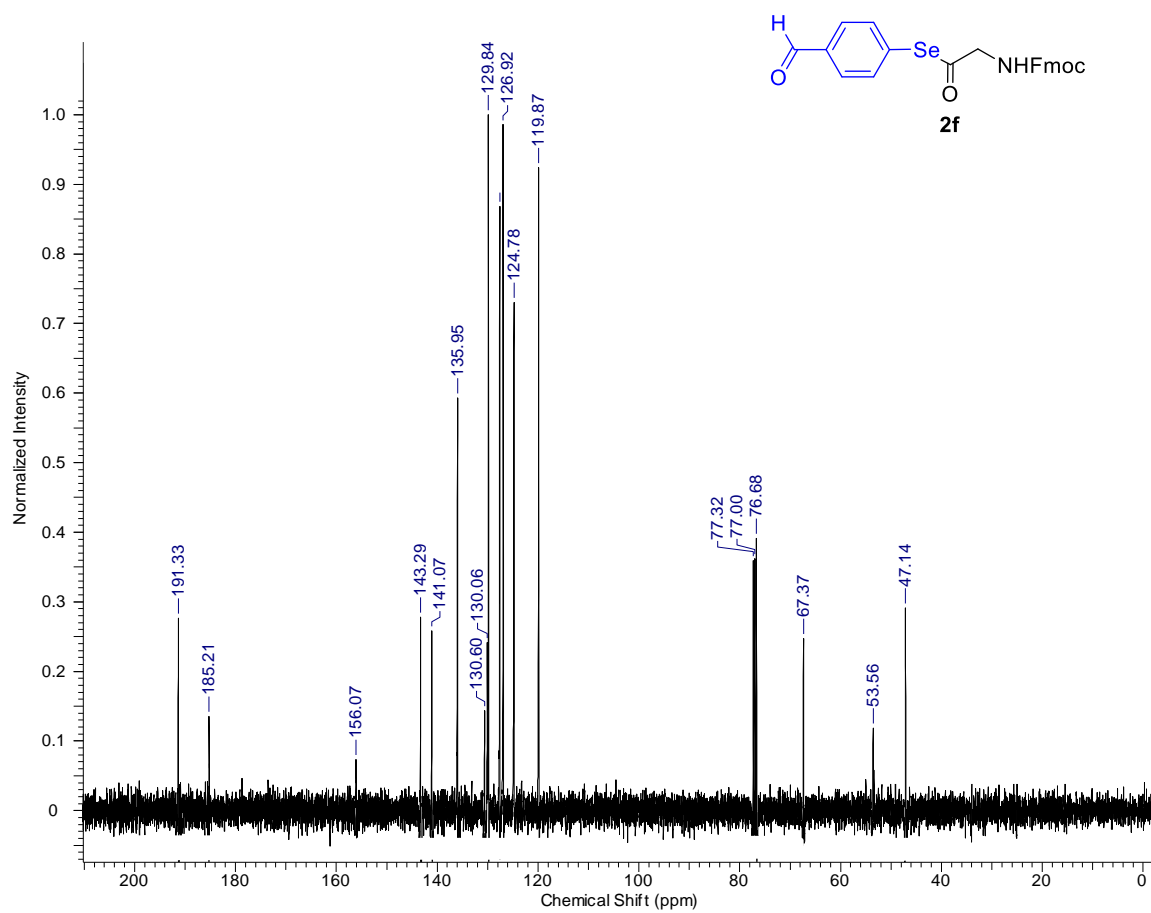
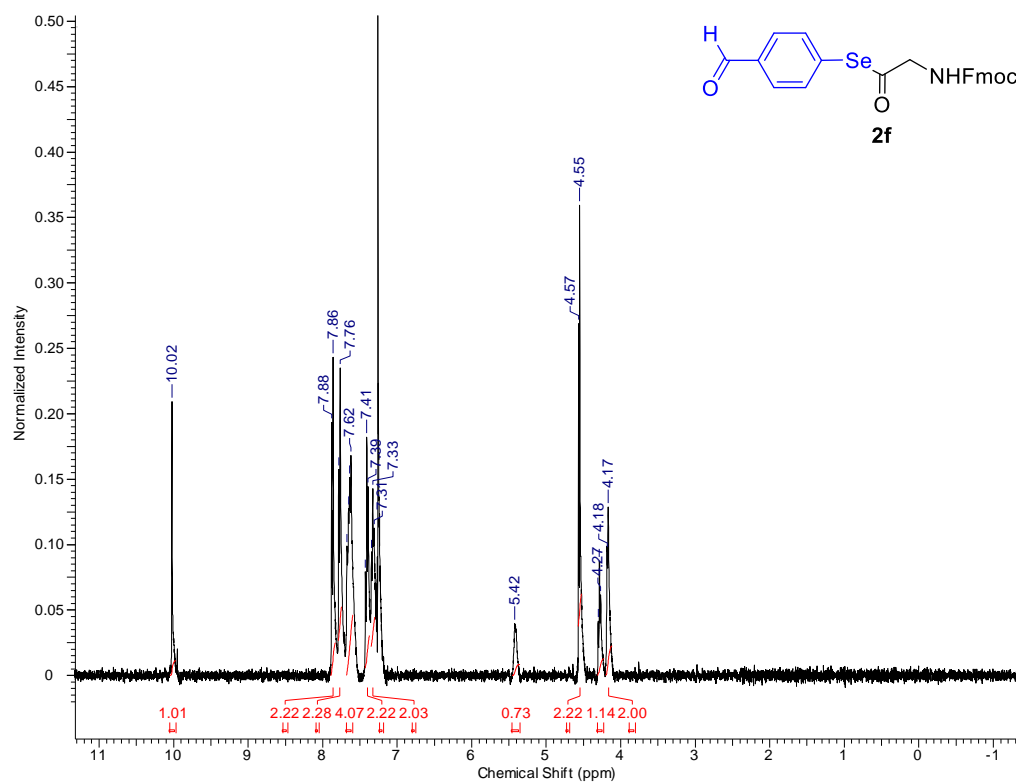


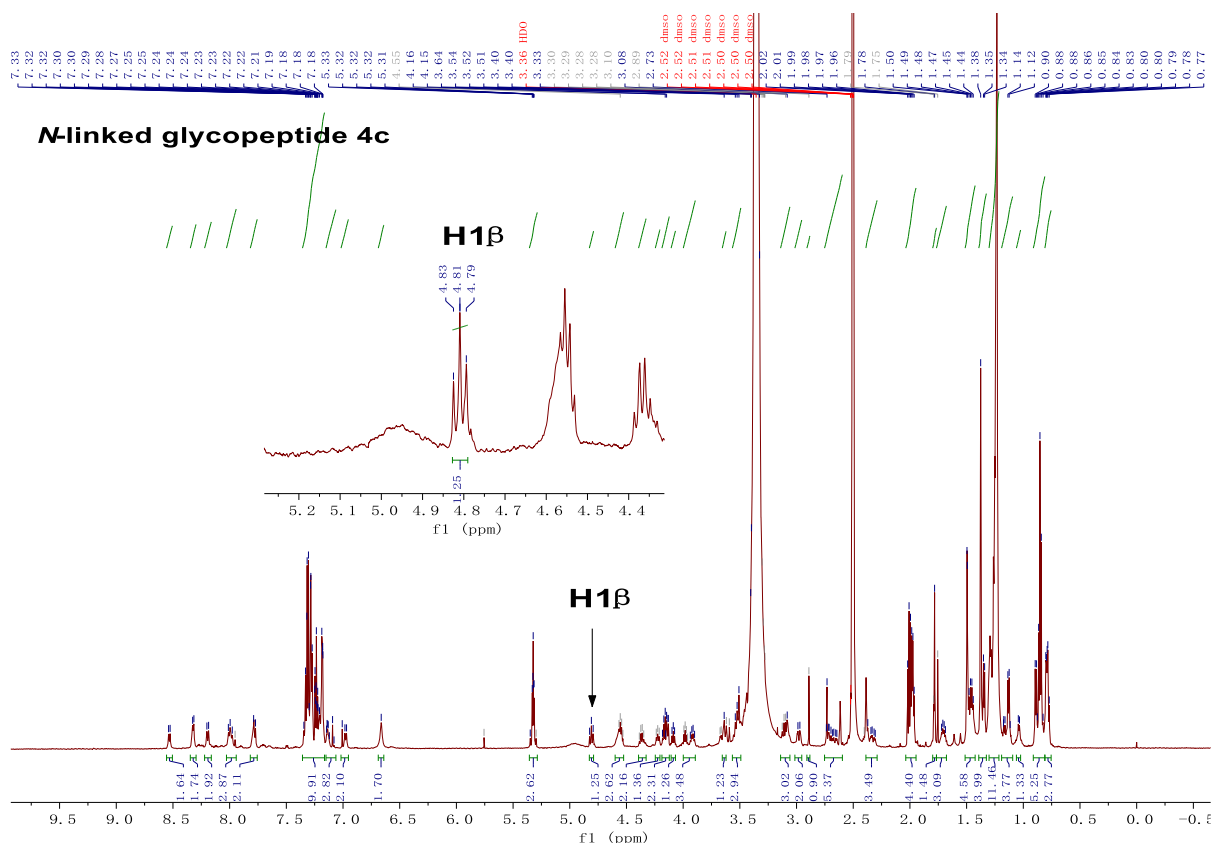
Figure S31. HPLC spectrum of purified glycopeptide **12e** (gradient: 80% to 100% CH₃CN over 14 min, 100% CH₃CN over 14-18 min, λ = 254 nm). The isolated yield of **12e** is about 62% (7 mg, in solution). ESI-MS calcd for **12e** C₂₉₇H₃₉₅N₃₃O₉₂S₂ [M+4H]⁴⁺ m/z = 1491.17, [M+3H+K]⁴⁺ m/z = 1500.66, [M+2H+Na]³⁺ m/z = 1995.22; observed: [M+4H]⁴⁺ m/z = 1491.62, [M+3H+K]⁴⁺ m/z = 1500.22, [M+2H+Na]³⁺ m/z = 1995.41.

8. ^1H NMR and ^{13}C NMR Spectra





Supplementary Material



9. Supplementary References

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