Supporting Information

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Second Generation Sugar-Assisted Ligation: An Effective New Method for the Synthesis of Cysteine-Containing Glycopeptides

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General procedures

Analytical HPLC was run on a Hitachi (D-7000 HPLC system) instrument using an analytical column (Grace Vydac “Protein & Peptide C18”, 150 x 4.6 mm, 10 µm particle size, flow rate 1.5 ml/min). Semi preparative HPLC was run on a Hitachi (D-7000 HPLC system) instrument using a semi preparative column (Grace Vydac “Protein & Peptide C18”, 250 x 10 mm, 10-15 µm particle size, flow rate 4 ml/min). Preparative HPLC was run on a Hitachi (D-7000 HPLC system) instrument using a preparative Column (Grace Vydac “Protein & Peptide C18”, 250 x 22 mm, 10-15 µm particle size, flow rate 8 ml/min). Detection of the signal was achieved with a photodiode array detector at a wavelength of $\lambda = 280$ nm. Eluents A (0.1 % TFA in water) and B (0.1 % TFA in acetonitrile) were used in a linear gradient at 50°C. Gradient A: 0 % B $\rightarrow$ 80 % B in 30 min.

Materials

Water was taken from a Milli-Q ultra pure water purification system (Millipore corp.). DMF was purchased in biotech grade. Commercial regents were purchased from Sigma-Aldrich or Acros Organics and were used without further purification. Tetrahydrofuran (THF) was distilled over sodium/benzophenone and dichloromethane ($\text{CH}_2\text{Cl}_2$) was distilled over calcium chloride. Other anhydrous-grade solvents were purchased from Sigma-Aldrich and used directly. Molecular sieves (AW 300) were freshly ground and flame-dried directly prior to glycosylation experiments. Analytical thin-layer chromatography (TLC) was performed using silica gel 60 $\text{F}_{254}$ glass plates. Compound spots were visualized by UV light (254 nm) and by staining with acidic ceric ammonium molybdate. Flash chromatography was performed on silica gel 60 Geduran (35-75 µm, EMD Science). Resins, protected amino acids and PyBOP were purchased from Novabiochem. Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc.
NMR Spectroscopy

$^1$H-NMR and $^{13}$C-NMR were recorded on a Bruker DRX-600 spectrometer equipped with a CryoProbe operating at 600 MHz and 150 MHz respectively. Coupling constants ($J$) are reported where possible in Hertz (Hz), and chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS, 0.00 ppm).

Mass spectrometry

MALDI-TOF mass spectra were measured on a Voyager-DE Pro biospectrometry workstation of PerSeptive Biosystems. A solution of 10 mg/ml $\alpha$-cyano-4-hydroxy cinnamic acid containing 0.1 % TFA was used for generating the probe-matrix mixture.

High resolution mass spectrometra were measured on an Agilent 6210 Time of Flight mass spectrometer.

Peptide synthesis

Solid-phase chemistry was carried out in syringes, equipped with teflon filters, purchased from Torviq.

Preloading of 3-(tritylthio) propanoic acid onto the MBHA-linker: Resin loadings were aimed at approximately 300 µmol/g by adding the resin in excess. First, the resin was washed (5x DCM, 3 min 5% DIPEA/DCM, 5x DCM, 5x DMF). For preactivation of the 3-(tritylthio) propanoic acid, PyBOP (1 eq.) was added to a 0.1 M solution of the 3-(tritylthio) propanoic acid in DMF containing 2 eq. NMM. After 5 min of preactivation, the mixture was added to the resin. After 2 h the resin was washed (5x DMF, 5x DCM, 3 min 5% DIPEA/DCM, 5x DCM, 5x DMF). For capping the resin was treated with acetic anhydride/pyridine (1:9) (2x 10 min), washed (5x DMF, 10x DCM) and finally dried in vacuo.
Solid-phase synthesis according to Boc-strategy: Boc Cleavage: After treatment with 5% m-Cresol/TFA (2x 4 min) the resin was washed with DCM (8x) and with DMF (5x). Coupling: After preactivation of 4 eq. protected amino acid (final concentration 0.1 M in DMF) for 5 min using 4 eq. PyBOP and 8 eq. NMM, the solution was added to the resin. After 30 min, the resin was washed with DMF (5x), DCM (5x) and DMF (5x). Capping: Acetic anhydride/pyridine (1:9) was added to the resin. After 5 min the resin was washed with DMF (5x) and DCM (5x). Terminal capping: Acetic anhydride/pyridine (1:9) was added to the resin. After 10 min the resin was washed with DMF (5x) and DCM (8x). Cleavage: A mixture of TFMSA/TFA/thioanisol (2:8:1) was added to the resin. After 2 h, the resin was washed with TFA (4x) Work-up: The combined solutions were concentrated in vacuo. The residue was dissolved in water, purified by preparative HPLC and analyzed by MALDI-TOF/MS (matrix: α-Cyano-4-hydroxycinnamic acid).

Preloading of the Rink amide resin: First, the resin was washed (5x DCM, 5x DMF), followed by removal of the Fmoc group by treating it with 10% piperidine/DMF (2x 5 min) and another washing step (5x DMF, 5x DCM, 5x DMF). For preactivation of the first protected amino acid, 4 eq. of PyBOP and 8 eq. of NMM were added to a solution of the building block (0.1 M) in DMF. After 5 min of preactivation, the mixture was added to the resin. After 2 h the resin was washed (5x DMF, 5x DCM, 5x DMF), capped with acetic anhydride/pyridine (1:9) (2x 5 min) and washed (5x DMF, 5x DCM, 5x DMF).

Glycopeptide solid-phase synthesis according to the Fmoc-strategy: Fmoc Cleavage: After treatment with 10% piperidine/DMF (2x 5 min) the resin was washed (5x DMF, 5x DCM, 5x DMF). Coupling: After preactivation of 4 eq. protected amino acid (final concentration 0.1 M in DMF) for 5 min using 4 eq. PyBOP and 8 eq. NMM, the solution was
added to the resin. After 30 min, the resin was washed with DMF (5x), DCM (5x) and DMF (5x). *Capping*: Acetic anhydride/pyridine (1:9) was added to the resin. After 5 min the resin was washed with DMF (5x), DCM (5x) and DMF (5x). *Coupling of the sugar containing monomer*: After preactivation of 1 eq. of building block (final concentration 0.1 M in DMF) for 5 min using 1 eq. PyBOP and 2 eq. NMM, the solution was added to the resin. After 6 h, the resin was washed with DMF (5x), DCM (5x) and DMF (5x). *Cleavage*: A mixture of TFA, thioanisole, triisopropylsilane and water (17:1:1:1) was added. After 2 h, the resin was washed with TFA (4x 4 mL) *Work-up*: The combined solutions were concentrated *in vacuo*. The residue was dissolved in water, purified by preparative HPLC and analyzed by MALDI-TOF/MS (matrix: α-Cyano-4-hydroxycinnamic acid).

**Glycopeptides**

Starting from 100 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 36 mg (43 µmol, 43%). ESI-TOF high-acc. (m/z): 843.3181 ([M+H]⁺, theor. 843.3189). HPLC: t<sub>R</sub>: 6.6 min (Gradient A); C<sub>34</sub>H<sub>50</sub>N<sub>8</sub>O<sub>15</sub>S (842.3116).
Starting from 100 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 35 mg (41 µmol, 41%). ESI-TOF high-acc. (m/z): 849.2737 ([M+H]^+, theor. 849.2753). HPLC: t_R: 5.6 min (Gradient A); C_{32}H_{48}N_{8}O_{15}S_{2} (848.2680).

Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 21.5 mg (24 µmol, 36%). ESI-TOF high-acc. (m/z): 907.2796 ([M+H]^+, theor. 907.2808). HPLC: t_R: 5.7 min (Gradient A); C_{34}H_{50}N_{8}O_{17}S_{2} (906.2735).

Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 25 mg (27 µmol, 41%). ESI-TOF high-acc. (m/z): 929.3123 ([M+H]^+, theor. 929.3128). HPLC: t_R: 5.9 min (Gradient A); C_{36}H_{52}N_{10}O_{15}S_{2} (928.3055).
Starting from 100 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 20 mg (21 µmol, 21%). ESI-TOF high-acc. (m/z): 942.3860 ([M+H]^+, theor. 942.3873). HPLC: t_R: 6.7 min (Gradient A); C_{39}H_{59}N_{9}O_{16}S (941.3800).

Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 11 mg (12 µmol, 18%). ESI-TOF high-acc. (m/z): 948.3421 ([M+H]^+, theor. 948.3437). HPLC: t_R: 6.6 min (Gradient A); C_{37}H_{57}N_{9}O_{16}S_{2} (947.3365).

Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 12 mg (12 µmol, 18%). ESI-TOF high-acc. (m/z): 1006.3484 ([M+H]^+, theor. 1006.3492). HPLC: t_R: 6.6 min (Gradient A); C_{39}H_{59}N_{9}O_{18}S_{2} (1005.3419).
Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 10 mg (10 µmol, 14%). ESI-TOF high-acc. \((m/z)\): 1028.3804 ([M+H]+, theor. 1028.3812). HPLC: \(t_R\): 6.5 min (Gradient A); C₄₁H₆₁N₁₁O₁₆S₂ (1027.3739).

Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 12 mg (11 µmol, 17%). ESI-TOF high-acc. \((m/z)\): 1061.4271 ([M+H]+, theor. 1061.4278). HPLC: \(t_R\): 9.0 min (Gradient A); C₄₃H₆₈N₁₀O₁₇S₂ (1060.4205).

Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 3 mg (3 µmol, 4%). ESI-TOF high-acc. \((m/z)\): 1118.4488 ([M+H]+, theor. 1118.4493). HPLC: \(t_R\): 8.8 min (Gradient A); C₄₅H₇₁N₁₁O₁₈S₂ (1117.4420).
Starting from 66.5 µmol Fmoc-Ser-Rink Amide-resin, the linear assembly was performed following the Fmoc-strategy. Yield: 7 mg (6 µmol, 9%). ESI-TOF high-acc. (m/z): 1175.4706 ([M+H]^+, theor. 1175.4707). HPLC: \( t_R \): 8.4 min (Gradient A); \( C_{47}H_{74}N_{12}O_{19}S_{2} \) (1174.4634).

**Peptide thioesters**

\[ \text{Ac-Leu Tyr Arg Ala Gly-S(CH}_2\text{)_2CONH}_2 \]

Starting from 100 µmol MBHA-resin preloaded with 3-(tritylthio) propanoic acid, the linear assembly was performed following the Boc-strategy. Yield: 50.6 mg (72 µmol, 72%). ESI-TOF high-acc. (m/z): 708.3499 ([M+H]^+, theor. 708.3497). HPLC: \( t_R \): 8.4 min (Gradient A); \( C_{31}H_{49}N_{9}O_{8}S \) (707.3425).

\[ \text{Ac-Leu Tyr Arg Ala Ala-S(CH}_2\text{)_2CONH}_2 \]
Starting from 100 µmol MBHA-resin preloaded with 3-(tritylthio) propanoic acid, the linear assembly was performed following the Boc-strategy. Yield: 39 mg (52 µmol, 52%). ESI-TOF high-acc. (m/z): 722.3644 ([M+H]^+, theor. 722.3654). HPLC: t_R: 8.8 min (Gradient A); C_{32}H_{51}N_{9}O_{8}S (721.3581).

\[
\text{Ac-Leu Tyr Arg Ala Tyr-S(CH}_2\text{)\text{2CONH}}_2
\]

Starting from 100 µmol MBHA-resin preloaded with 3-(tritylthio) propanoic acid, the linear assembly was performed following the Boc-strategy. Yield: 33.3 mg (41 µmol, 41%). ESI-TOF high-acc. (m/z): 814.3901 ([M+H]^+, theor. 814.3916). HPLC: t_R: 9.3 min (Gradient A); C_{38}H_{55}N_{9}O_{9}S (813.3843).

\[
\text{Ac-Leu Tyr Arg Ala His-S(CH}_2\text{)\text{2CONH}}_2
\]

Starting from 100 µmol MBHA-resin preloaded with 3-(tritylthio) propanoic acid, the linear assembly was performed following the Boc-strategy. Yield: 31 mg (39 µmol, 39%). ESI-TOF high-acc. (m/z): 788.3863 ([M+H]^+, theor. 788.3872). HPLC: t_R: 8.0 min (Gradient A); C_{35}H_{53}N_{11}O_{8}S (787.3799).

\[
\text{Ac-Leu Tyr Arg Ala Phe-S(CH}_2\text{)\text{2CONH}}_2
\]
Starting from 100 µmol MBHA-resin preloaded with 3-(tritylthio) propanoic acid, the linear assembly was performed following the Boc-strategy. Yield: 8.7 mg (11 µmol, 11%). ESI-TOF high-acc. (m/z): 798.3963 ([M+H]^+, theor. 798.3967). HPLC: t_R: 9.5 min (Gradient A); C_{38}H_{55}N_{9}O_{8}S (797.3894).

**Peptide Ligations**

Glycopeptides (1.5 equiv, approx. 3 µmol) were dissolved in 150µl of deoxygenated ligation buffer (4:1 v/v N-methyl-2-pyrrolidinone: 6M guanidine hydrochloride, 1 M HEPES, pH = 8.5). This solution was transferred to an eppendorf tube containing the thioester (approx. 2 µmol). Thiophenol (2% by volume, 3 µl) was added and the reaction mixed gently. The ligation mixture was incubated at 37 °C with gentle mixing every 12 h until the reaction was shown to be complete by LC-MS.
Ligation products:

Yield: 69%. MALDI-TOF (m/z): 1446.2 ([M+H]$^+$, theor. 1446.6). HPLC: $t_R$: 8.7 min (Gradient A); C$_{62}$H$_{92}$N$_{16}$O$_{22}$S (1445.6).

Yield: >95%. MALDI-TOF (m/z): 1371.8 ([M+H]$^+$, theor. 1372.5). HPLC: $t_R$: 7.8 min (Gradient A); C$_{60}$H$_{90}$N$_{16}$O$_{21}$ (1371.5).

Yield: 84%. MALDI-TOF (m/z): 1451.7 ([M+H]$^+$, theor. 1452.6). HPLC: $t_R$: 8.7 min (Gradient A); C$_{60}$H$_{90}$N$_{16}$O$_{22}$S$_2$ (1451.6).

Yield: >95%. MALDI-TOF (m/z): 1378.3 ([M+H]$^+$, theor. 1378.5). HPLC: $t_R$: 8.1 min (Gradient A); C$_{58}$H$_{88}$N$_{16}$O$_{21}$S (1377.5).
Yield: 28%. MALDI-TOF ($m/z$): 1533.1 ([M+H]$^+$, theor. 1532.7). HPLC: $t_R$: 8.9 min (Gradient A); C$_{64}$H$_{94}$N$_{18}$O$_{22}$S$_2$ (1531.7).

Yield: >95%. MALDI-TOF ($m/z$): 1458.0 ([M+H]$^+$, theor. 1458.6). HPLC: $t_R$: 8.3 min (Gradient A); C$_{62}$H$_{92}$N$_{18}$O$_{21}$S (1457.6).

Yield: 27%. MALDI-TOF ($m/z$): 1612.4 ([M+H]$^+$, theor. 1612.8). HPLC: $t_R$: 8.1 min (Gradient A); C$_{68}$H$_{99}$N$_{20}$O$_{22}$S$_2$ (1611.8).

Yield: >95%. MALDI-TOF ($m/z$): 1538.0 ([M+H]$^+$, theor. 1538.7). HPLC: $t_R$: 7.6 min (Gradient A); C$_{66}$H$_{96}$N$_{20}$O$_{21}$S (1537.7).
Yield: 38%. MALDI-TOF (m/z): 1509.3 ([M+H]^+, theor. 1510.6). HPLC: $t_R$: 9.3 min (Gradient A); C_{62}H_{92}N_{16}O_{24}S_{2} (1509.6).

Yield: >95%. MALDI-TOF (m/z): 1435.9 ([M+H]^+, theor. 1436.5). HPLC: $t_R$: 8.2 min (Gradient A); C_{60}H_{90}N_{16}O_{23}S (1435.5).

Yield: 22%. MALDI-TOF (m/z): 1590.5 ([M+H]^+, theor. 1590.7). HPLC: $t_R$: 8.3 min (Gradient A); C_{66}H_{97}N_{18}O_{24}S_{2} (1589.7).

Yield: >95%. MALDI-TOF (m/z): 1514.6 ([M+H]^+, theor. 1516.6). HPLC: $t_R$: 8.3 min (Gradient A); C_{64}H_{94}N_{18}O_{23}S (1515.6).
Yield: 40%. MALDI-TOF (m/z): 1616.4 ([M+H]$^+$, theor. 1616.7). HPLC: $t_R$: 9.9 min (Gradient A); C$_{60}$H$_{98}$N$_{16}$O$_{25}$S$_2$ (1615.7).

Yield: >95%. MALDI-TOF (m/z): 1541.8 ([M+H]$^+$, theor. 1542.6). HPLC: $t_R$: 8.8 min (Gradient A); C$_{67}$H$_{96}$N$_{16}$O$_{24}$S (1541.6).

Yield: 28%. MALDI-TOF (m/z): 1466.2 ([M+H]$^+$, theor. 1466.6). HPLC: $t_R$: 9.8 min (Gradient A); C$_{61}$H$_{92}$N$_{16}$O$_{22}$S$_2$ (1465.6).

Yield: >95%. MALDI-TOF (m/z): 1392.5 ([M+H]$^+$, theor. 1392.5). HPLC: $t_R$: 8.6 min (Gradient A); C$_{59}$H$_{90}$N$_{16}$O$_{21}$S (1391.5).
Yield: 32%. MALDI-TOF ($m/z$): 1637.5 ([M+H]$^+$, theor. 1638.8). HPLC: $t_R$: 9.6 min (Gradient A); C$_{71}$H$_{100}$N$_{18}$O$_{23}$S$_2$ (1637.8).

Yield: >95%. MALDI-TOF ($m/z$): 1564.6 ([M+H]$^+$, theor. 1564.7). HPLC: $t_R$: 8.4 min (Gradient A); C$_{69}$H$_{98}$N$_{18}$O$_{22}$S (1563.7).

Yield: 40%. MALDI-TOF ($m/z$): 1548.2 ([M+H]$^+$, theor. 1546.7). HPLC: $t_R$: 9.6 min (Gradient A); C$_{65}$H$_{96}$N$_{18}$O$_{22}$S$_2$ (1545.7).

Yield: >95%. MALDI-TOF ($m/z$): 1472.6 ([M+H]$^+$, theor. 1472.6). HPLC: $t_R$: 8.2 min (Gradient A); C$_{63}$H$_{94}$N$_{18}$O$_{21}$S (1471.6).
Yield: 59%. MALDI-TOF \((m/z)\): 1524.8 ([M+H]\(^+\), theor. 1524.7). HPLC: \(t_R\): 9.8 min (Gradient A); \(C_{63}H_{94}N_{16}O_{24}S_{2}\) (1523.6).

Yield: >95%. MALDI-TOF \((m/z)\): 1450.8 ([M+H]\(^+\), theor. 1450.5). HPLC: \(t_R\): 8.2 min (Gradient A); \(C_{61}H_{92}N_{16}O_{23}S\) (1449.5).

Yield: 62%. MALDI-TOF \((m/z)\): 1531.3 ([M+H]\(^+\), theor. 1532.7). HPLC: \(t_R\): 9.2 min (Gradient A); \(C_{64}H_{94}N_{18}O_{22}S_{2}\) (1531.7).

Yield: >95%. MALDI-TOF \((m/z)\): 1459.7 ([M+H]\(^+\), theor. 1458.6). HPLC: \(t_R\): 7.8 min (Gradient A); \(C_{62}H_{92}N_{18}O_{21}S\) (1457.6).
Yield: 65%. MALDI-TOF ($m/z$): 1559.6 ([M+H]$^+$, theor. 1559.7). HPLC: $t_R$: 9.8 min (Gradient A); C$_{68}$H$_{103}$N$_{17}$O$_{23}$S (1558.7).

Yield: >95%. MALDI-TOF ($m/z$): 1485.2 ([M+H]$^+$, theor. 1485.6). HPLC: $t_R$: 8.9 min (Gradient A); C$_{66}$H$_{101}$N$_{17}$O$_{22}$ (1484.6).

Yield: 58%. MALDI-TOF ($m/z$): 1625.7 ([M+H]$^+$, theor. 1625.8). HPLC: $t_R$: 9.3 min (Gradient A); C$_{71}$H$_{105}$N$_{19}$O$_{23}$S (1624.8).

Yield: >95%. MALDI-TOF ($m/z$): 1551.7 ([M+H]$^+$, theor. 1551.7). HPLC: $t_R$: 8.2 min (Gradient A); C$_{69}$H$_{104}$N$_{19}$O$_{22}$ (1550.7).
Yield: 48%. MALDI-TOF ($m/z$): 1565.4 ([M+H]$^+$, theor. 1565.7). HPLC: $t_R$: 10.0 min (Gradient A); C$_{66}$H$_{101}$N$_{17}$O$_{23}$S$_2$ (1564.7).

Yield: >95%. MALDI-TOF ($m/z$): 1491.7 ([M+H]$^+$, theor. 1491.6). HPLC: $t_R$: 9.0 min (Gradient A); C$_{64}$H$_{99}$N$_{17}$O$_{22}$S (1490.6).

Yield: 66%. MALDI-TOF ($m/z$): 1550.8 ([M+H]$^+$, theor. 1551.7). HPLC: $t_R$: 9.4 min (Gradient A); C$_{65}$H$_{99}$N$_{17}$O$_{23}$S$_2$ (1550.7).

Yield: >95%. MALDI-TOF ($m/z$): 1477.3 ([M+H]$^+$, theor. 1477.6). HPLC: $t_R$: 8.4 min (Gradient A); C$_{63}$H$_{97}$N$_{17}$O$_{22}$S (1476.6).
Yield: 68%. MALDI-TOF (m/z): 1545.5 ([M+H]^+, theor. 1545.7). HPLC: \( t_R: 9.9 \text{ min} \) (Gradient A); \( \text{C}_{67}\text{H}_{101}\text{N}_{17}\text{O}_{23}\text{S} \) (1544.7).

Yield: >95%. MALDI-TOF (m/z): 1471.7 ([M+H]^+, theor. 1471.6). HPLC: \( t_R: 8.5 \text{ min} \) (Gradient A); \( \text{C}_{65}\text{H}_{99}\text{N}_{17}\text{O}_{22} \) (1470.6).

Yield: 60%. MALDI-TOF (m/z): 1632.2 ([M+H]^+, theor. 1631.8). HPLC: \( t_R: 9.3 \text{ min} \) (Gradient A); \( \text{C}_{69}\text{H}_{103}\text{N}_{19}\text{O}_{23}\text{S}_2 \) (1630.8).

Yield: >95%. MALDI-TOF (m/z): 1557.9 ([M+H]^+, theor. 1557.7). HPLC: \( t_R: 8.3 \text{ min} \) (Gradient A); \( \text{C}_{67}\text{H}_{101}\text{N}_{19}\text{O}_{22}\text{S} \) (1556.7).
Yield: 45%. MALDI-TOF ($m/z$): 1657.5 ([M+H]$^+$, theor. 1657.8). HPLC: $t_R$: 10.6 min (Gradient A); C$_{72}$H$_{105}$N$_{17}$O$_{24}$S$_2$ (1656.8).

Yield: >95%. MALDI-TOF ($m/z$): 1583.1 ([M+H]$^+$, theor. 1583.7). HPLC: $t_R$: 9.6 min (Gradient A); C$_{70}$H$_{103}$N$_{17}$O$_{23}$S (1582.7).

Yield: 55%. MALDI-TOF ($m/z$): 1630.3 ([M+H]$^+$, theor. 1631.8). HPLC: $t_R$: 9.0 min (Gradient A); C$_{69}$H$_{103}$N$_{19}$O$_{23}$S$_2$ (1630.8).

Yield: >95%. MALDI-TOF ($m/z$): 1558.3 ([M+H]$^+$, theor. 1557.7). HPLC: $t_R$: 8.2 min (Gradient A); C$_{67}$H$_{101}$N$_{19}$O$_{22}$S (1556.7).
Yield: 23%. MALDI-TOF (m/z): 1647.3 ([M+H]^+, theor. 1645.8). HPLC: t_R: 10.2 min (Gradient A); C_{70}H_{105}N_{19}O_{23}S_{2} (1644.8).

Yield: >95%. MALDI-TOF (m/z): 1571.0 ([M+H]^+, theor. 1571.7). HPLC: t_R: 9.5 min (Gradient A); C_{68}H_{103}N_{19}O_{22}S (1570.7).

Yield: 40%. MALDI-TOF (m/z): 1712.4 ([M+H]^+, theor. 1711.9). HPLC: t_R: 8.5 min (Gradient A); C_{73}H_{107}N_{21}O_{23}S_{2} (1710.9).

Yield: >95%. MALDI-TOF (m/z): 1638.6 ([M+H]^+, theor. 1637.8). HPLC: t_R: 8.6 min (Gradient A); C_{71}H_{105}N_{21}O_{22}S (1636.8).
Yield: 39%. MALDI-TOF (m/z): 1737.5 ([M+H]^+, theor. 1737.9). HPLC: \( t_R \): 10.4 min (Gradient A); C\(_{76}\)H\(_{109}\)N\(_{19}\)O\(_{24}\)S\(_2\) (1736.9).

Yield: >95%. MALDI-TOF (m/z): 1663.4 ([M+H]^+, theor. 1663.8). HPLC: \( t_R \): 9.5 min (Gradient A); C\(_{74}\)H\(_{107}\)N\(_{19}\)O\(_{23}\)S (1662.8).

Yield: 81%. MALDI-TOF (m/z): 1608.5 ([M+H]^+, theor. 1609.8). HPLC: \( t_R \): 9.8 min (Gradient A); C\(_{67}\)H\(_{101}\)N\(_{17}\)O\(_{25}\)S\(_2\) (1608.7).

Yield: >95%. MALDI-TOF (m/z): 1535.2 ([M+H]^+, theor. 1535.7). HPLC: \( t_R \): 9.0 min (Gradient A); C\(_{65}\)H\(_{99}\)N\(_{17}\)O\(_{24}\)S (1534.6).
Yield: 50%. MALDI-TOF (m/z): 1623.3 ([M+H]$^+$, theor. 1623.8). HPLC: $t_R$: 10.2 min (Gradient A); C$_{68}$H$_{103}$N$_{17}$O$_{25}$S$_2$ (1622.8).

Yield: >95%. MALDI-TOF (m/z): 1548.8 ([M+H]$^+$, theor. 1549.7). HPLC: $t_R$: 9.3 min (Gradient A); C$_{66}$H$_{101}$N$_{17}$O$_{24}$S (1548.7).

Yield: 60%. MALDI-TOF (m/z): 1687.8 ([M+H]$^+$, theor. 1689.8). HPLC: $t_R$: 9.4 min (Gradient A); C$_{71}$H$_{105}$N$_{19}$O$_{25}$S$_2$ (1688.8).

Yield: >95%. MALDI-TOF (m/z): 1616.0 ([M+H]$^+$, theor. 1615.7). HPLC: $t_R$: 8.6 min (Gradient A); C$_{69}$H$_{103}$N$_{19}$O$_{24}$S (1614.7).
Yield: 37%. MALDI-TOF (m/z): 1714.9 ([M+H]^+, theor. 1715.9). HPLC: t_R: 10.6 min (Gradient A); C_{74}H_{107}N_{17}O_{26}S_{2} (1714.9).

Yield: >95%. MALDI-TOF (m/z): 1640.8 ([M+H]^+, theor. 1641.8). HPLC: t_R: 9.9 min (Gradient A); C_{72}H_{105}N_{17}O_{25}S (1640.8).

Yield: 62%. MALDI-TOF (m/z): 1664.0 ([M+H]^+, theor. 1664.9). HPLC: t_R: 11.3 min (Gradient A); C_{71}H_{110}N_{18}O_{24}S_{2} (1663.9).

Yield: >95%. MALDI-TOF (m/z): 1588.7 ([M+H]^+, theor. 1590.8). HPLC: t_R: 11.0 min (Gradient A); C_{69}H_{108}N_{18}O_{23}S (1589.8).
Ac-Leu Tyr Arg Ala His-Gly Val Leu Ser Cys Gly Tyr Ser-NH₂

Yield: 81%. MALDI-TOF (m/z): 1745.2 ([M+H]+, theor. 1745.0). HPLC: $t_R$: 10.8 min (Gradient A); C₇₅H₁₁₄N₂₀O₂₄S₂ (1744.0).

Ac-Leu Tyr Arg Ala His-Gly Val Leu Ser Cys Gly Tyr Ser-NH₂

Yield: >95%. MALDI-TOF (m/z): 1670.0 ([M+H]+, theor. 1670.9). HPLC: $t_R$: 9.5 min (Gradient A); C₇₃H₁₁₂N₂₀O₂₃S (1669.9).

Ac-Leu Tyr Arg Ala Gly-Gly Gly Val Leu Ser Cys Gly Tyr Ser-NH₂

Yield: 56%. MALDI-TOF (m/z): 1722.0 ([M+H]+, theor. 1721.9). HPLC: $t_R$: 11.4 min (Gradient A); C₇₃H₁₁₃N₁₉O₂₅S₂ (1720.9).

Ac-Leu Tyr Arg Ala Gly-Gly Gly Val Leu Ser Cys Gly Tyr Ser-S

Yield: >95%. MALDI-TOF (m/z): 1647.5 ([M+H]+, theor. 1647.8). HPLC: $t_R$: 10.0 min (Gradient A); C₇₁H₁₁₁N₁₉O₂₄S (1646.8).
Building Block Synthesis

The initial phase of the research involved the synthesis of monomer 1, which was essential for solid phase peptide synthesis (SPPS) of the desired auxiliary containing glycopeptides. The synthesis began from the known glycosyl amino acid 9, synthesized from (D)-glucosamine in five steps (Scheme 2).[1-3] Subsequent introduction of the 2-acetamide group was achieved by treatment with acetic anhydride and acetic acid in the presence of zinc dust and gave 10 in 91 % yield. Palladium-catalyzed allyl deprotection was necessary prior to removal of the acetate groups at the 3-, 4- and 6-positions with a methanolic sodium methoxide solution. Reallylation of the free acid followed by formation of a benzylidene acetal gave alcohol 11 in 51 % yield over four steps. Coupling of the free alcohol to S-trityl-2-mercaptoacetic acid gave 12 in 89 % over the two steps. With the desired thiol auxiliary in place, the final step involved palladium-catalyzed removal of the allyl protecting group to afford the desired glycosyl amino acid building block 1 in 91% yield.
Scheme 1. Synthesis of glycosyl amino acid building block 1 from (D)-glucosamine. a) Ac₂O, AcOH, Zn dust, 91 %; b) Pd(PPh₃)₄, NMA, THF; c) NaOMe, MeOH, pH 9; d) allyl bromide, DIEA, DMF; e) benzaldehyde dimethyl acetal, p-TsOH, MeCN, 51 % from 10; f) TrtSCH₂COOH, DIC, DMAP, CH₂Cl₂, 0°C, 91 %; g) Pd(PPh₃)₄, NMA, THF, 91 %. AcOH = acetic acid, NMA = N-methylaniline, DIEA = N,N-diisopropylethylamine, p-TsOH = p-toluene-4-sulfonic acid, Troc = 2,2,2-trichloroethyl carbamate, Trt = trityl, DIC = N,N'-Diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine. NB: Treatment of the allyl group with sodium methoxide in methanol caused conversion of the allyl ester to a methyl ester, which could not be subsequently removed in the presence of the Fmoc group. Therefore, steps b-d involved removal of the allyl protecting group prior to methanolysis of the acetate groups.
(3S,4R,5S,6R)-6-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(allyloxy)-3-oxopropoxy)-5-acetamido-2-(acetoxymethyl)tetrahydro-2H-pyran-3,4-diyl diacetate 10

Troc protected glycosyl amino acid 9 (1.07 g, 1.29 mmol) was dissolved in acetic anhydride (16 ml) and the reaction cooled to 0 °C. Pre-activated Zn dust [5 g, activated by washing with 1 M HCl (100 ml), water (2 x 100ml), diethyl ether (2 x 100 ml)] was added followed by acetic acid (4.8 ml) and the reaction stirred at rt for 90 min. The reaction was filtered through a plug of celite, washed with dichloromethane and the solvent removed in vacuo. Purification by column chromatography (eluent: 3:1 v/v ethyl acetate/hexane) gave the desired product 10 as a white solid (0.82 g, 91%).

R_f (3:1 v/v ethyl acetate/hexane) = 0.23; ¹H- and ¹³C-NMR spectroscopic data was consistent to that reported in the literature²³; HRMS (ESI-TOF) calcd for C₃₅H₄₀N₂O₁₃ [M+H]^+ 697.2603. Found: 697.2602.

(2R)-allyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-((6R,7S,8R,8aS)-7-acetamido-8-hydroxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-6-yloxy)propanoate 11
Glycosyl amino acid 10 (1.83 g, 2.63 mmol) was suspended in THF (37 ml). N-methylaniline (2.86 ml, 26.3 mmol) and Pd(PPh3)4 (303 mg, 0.26 mmol) were added and the reaction stirred at rt for 30 min. The solvent was removed in vacuo and the product purified by column chromatography (eluent: 9:1 v/v dichloromethane/methanol + 1% AcOH) to give the acid as a cream solid [1.7 g, 95%, \( R_f \) (9:1 v/v dichloromethane/methanol) = 0.07]. The resulting acid was dissolved in dry methanol (55 ml) and a solution of sodium methoxide in methanol (0.5 M solution) added until the pH reached 9.0. The reaction was stirred at rt for 2 h before neutralizing by the addition of Dowex 50H\(^+\) resin. The reaction was filtered and the solvent removed in vacuo to give the desired triol which was used in the next step without further purification. The triol was dissolved in DMF (16 ml) and cooled to 0 °C. DIEA (0.90 ml, 5.18 mmol) and allyl bromide (0.45 ml, 5.18 mmol) were added dropwise and the reaction stirred at rt for 6 h. The solvent was removed in vacuo and the product purified by column chromatography (eluent: 9:1 v/v dichloromethane/methanol) to give the allyl-protected compound as a white solid (0.77 g). The resulting triol (0.77 g, 1.35 mmol) was dissolved in MeCN (33 ml). Benzaldehyde dimethyl acetal (0.41 ml, 2.70 mmol) and \( p \)-toluenesulfonic acid (26 mg, 0.13 mmol) were added and the reaction stirred at rt for 3 h. The solvent was removed in vacuo and the product purified by column chromatography (eluent: 4:1 v/v ethyl acetate/hexane) to give the desired alcohol 11 as a white solid (0.87 g, 98% and 51% from 10).

\[ ^1H \text{ NMR (CDCl}_3, 600 MHz) \delta 7.73 (d, J = 7.8 \text{ Hz}, 2H), 7.61 (dd, J = 6.8, 6.9 \text{ Hz}, 2H), 7.45 (d, J = 5.4 \text{ Hz}, 2H), 7.41 (m, 2H), 7.36 (m, 2H), 7.33-7.26 (m, 3H), 5.85 (m, 1H), 5.50 (s, 1H), 5.30 (d, J = 16.8 \text{ Hz}, 1H), 5.20 (d, J = 10.8 \text{ Hz}, 1H), 4.62 (br. s, 1H), 4.55 (d, J = 7.8 \text{ Hz}, 1H), 4.45 (m, 2H), 4.30 (m, 2H), 4.25 (m, 1H), 4.18 (m, 2H), 3.85-3.77 (m, 2H), 3.69 (m, 1H), 3.58 (m, 1H), 3.46 (m, 1H), 3.39 (m, 1H), 1.90 (s, 3H); ^{13}C \text{ NMR (CDCl}_3, 150 MHz) \delta 172.6, 169.5, 156.5, 143.3, 140.9, 136.7, 131.1, 128.8, 127.8, 127.4, 126.7, 125.9, 124.6, 119.5, 118.0, 101.5, 101.0, 81.1, 70.6, 68.5, 68.1, 66.7, 66.0, 65.8, 56.5, 54.0, 46.7, 22.3; \text{ HRMS (ESI-TOF) calecd for } C_{36}H_{38}N_2O_{10} [M+H]^+ 659.2599. \text{ Found: 659.2597.} \]

\( (2R) \)-allyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-((6R,7S,8R,8aS)-7-acetamido-2-phenyl-8-(2-(tritylthio)acetox)hexahydropyrano[3,2-d][1,3]dioxin-6-ylxylo)propanoate 12
Alcohol 11 (142 mg, 0.22 mmol) was dissolved in dry dichloromethane (7.5 ml). S-Trityl-mercaptoacetic acid (110 mg, 0.32 mmol) was added and the reaction cooled to 0 °C. DIC (50 µl, 0.32 mmol) and DMAP (1.3 mg, 0.01 mmol) was added and the reaction stirred at 0 °C for 15 min. The solvent was removed in vacuo and the product purified by column chromatography (eluent: 2:1 hexane/ethyl acetate – 1:1 v/v hexane/ethyl acetate) to afford 12 as a white solid (190 mg, 91%).

R_f (1:1 v/v hexane/ethyl acetate) = 0.57; ^1H NMR (CDCl_3, 600 MHz) δ 7.87 (dd, J = Hz, 2H), 7.74 (dd, J = Hz 2H), 7.54-7.26 (m, 24H), 5.95 (m, 1H), 5.82 (d, J = 10.2, 1H), 5.68 (d, J = 10.8 Hz, 1H), 5.56 (s, 1H), 5.41 (dd, J = 1.2, 20.4 Hz, 1H), 5.34-5.29 (m, 2H), 4.74 (d, J = 6.6 Hz, 2H), 4.56 (m, 3H), 4.51 (d, J = 10.8 Hz, 1H), 4.39 (dd, J = 6.0, 12.6 Hz, 1H), 4.33-4.26 (m, 2H), 4.00 (dd, J = 10.2, 21.0 Hz, 1H), 3.80 (m, 2H), 3.69 (m, 1H), 3.55 (m, 1H), 3.08 (d, J = 16.2 Hz, 1H), 2.86 (d, J = 16.2 Hz, 1H), 1.89 (s, 3H); ^1H NMR (CDCl_3, 600 MHz) δ 170.5, 170.0, 169.4, 156.0, 143.9, 143.7, 141.4, 136.7, 131.5, 129.5, 128.2, 128.1, 127.8, 127.2, 127.0, 126.1, 125.2, 120.0, 119.4, 118.5, 102.0, 101.3, 78.3, 72.0, 69.1, 68.4, 67.3, 66.6, 66.3, 66.2, 54.4, 54.1, 47.3, 34.6, 23.0; HRMS (ESI-TOF) calcd for C_{57}H_{54}N_2O_{11}S [M+H]^+ 975.3521. Found: 935.3526.

(2R)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-((6R,7S,8R,8aS)-7-acetamido-2-phenyl-8-(2-(tritylthio)acetoxy)hexahydropyrano[3,2-d][1,3]dioxin-6-yloxy)propanoic acid 1
Allyl protected glycosyl amino acid 12 (135 mg, 0.14 mmol) was suspended in THF (6 ml). N-methylaniline (0.15 ml, 1.38 mmol) and Pd(PPh₃)₄ (16 mg, 0.01 mmol) were added and the reaction stirred at rt for 20 min. The solvent was removed in vacuo and the product purified by column chromatography (eluent: 95:5 v/v dichloromethane/methanol – 9:1 v/v dichloromethane/methanol) to give the desired acid 1 as a pale yellow solid (123 mg, 95%).

Rᵣ (95:5 v/v dichloromethane/methanol) = 0.26; ¹H-NMR (1:1 v/v CDCl₃/MeOD, 600 MHz): δ 7.73 (d, J = 6.6 Hz, 2H), 7.59 (d, J = 7.2 Hz 2H), 7.39 (d, J = 6.6 Hz 2H), 7.35 (m, 2H), 7.30-7.13 (m, 20 H), 5.45 (br. s, 1H), 5.17 (d, J = 10.2 Hz, 1H), 4.50 (d, J = 7.8 Hz, 1H), 4.42 (dd, J = 6.6, 10.2 Hz, 1H), 4.36 (dd, J = 6.6, 10.2 Hz, 1H), 4.26 (dd, J = 4.8, 10.2 Hz, 1H), 4.22 (br. s, 1H), 4.15 (m, 2H), 3.88 (m, 1H), 3.79 (d, J = 8.4 Hz, 1H), 3.68 (m, 1H), 3.59 (m, 1H), 3.44 (m, 1H), 2.90 (d, J = 13.8 Hz, 1H), 2.86 (d, J = 13.8 Hz, 1H), 1.56 (s, 3H); ¹³C-NMR (CDCl₃, 150 MHz) δ 172.0, 169.5, 169.0, 156.2, 143.4, 143.3, 140.8, 136.3, 128.9, 128.5, 127.5, 127.4, 127.2, 126.5, 126.4, 125.6, 124.4, 119.3, 101.3, 100.8, 78.2, 71.6, 69.3, 67.8, 66.7, 66.1, 65.6, 53.5, 46.6, 34.0, 29.0, 21.8; LC-MS Rᵣ = 10.5 min, MH⁺ = 935.4; HRMS (ESI-TOF) calcd for C₅₄H₅₀N₂O₁₁S [M+H]⁺ 935.3208. Found: 935.3211.

References and notes