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General information
The chemicals were obtained from Sigma Aldrich, Alfa Aesar, Acros Organics, ABCR, Fluoorochem, Iris Biochem, Carbosynth or VWR and were used without further purification. Reactions with air- and moisture-sensitive reactants were performed under argon atmosphere and in anhydrous solvents.

Solutions were concentrated on a rotary evaporator from Heidolph equipped with a PC3001 VARIOproo pump from Vacuubrand. Column chromatography was carried out on silica gel 60Å (particle size: 40-60 μm) from Acros Organics. Solvents in the p.a. quality from Lach-Ner and Penta were used for elution. Mixtures of solvents are each stated as volume fractions. For flash column chromatography a CombiFlash® RF from Teledyne ISCO was used. Thin-layer chromatography was performed on aluminum sheets from Merck (silica gel 60 F254, 20 × 20 cm). Chromatograms were visualized by UV light (λ = 254 nm/ 366 nm) or by staining with KMnO₄ solution, (NH₄)₂Ce(NO₃)₆/(NH₄)₆MoO₄·4H₂O solution, or PPh₃/ninhydrin method. For amino acid analysis or determination of resin loading, samples were hydrolyzed in 6 M HCl at 110~C overnight and analyzed on a Biochrom 30 amino acid analyzer (Biochrom Ltd., UK). ¹H- and ¹³C-NMR spectra were measured on a Bruker Avance III™ HD 500 MHz NMR system equipped with Prodigy cryo-probe or on a Bruker Avance III™ HD 500 MHz Cryo. CDCl₃, methanol-d₄ deuterium oxide and DMSO-d₆ from Sigma Aldrich or Eurisotop were used as solvents. Chemical shifts δ are quoted in ppm in relation to the chemical shift of the residual non-deuterated solvent peak (CDCl₃: δ(H) = 7.26, δ(C) = 77.2; methanol-d₄: δ(H) = 4.87, δ(C) = 49.0; DMSO-d₆: δ(H) = 2.50, δ(C) = 39.5; deuterium oxide: δ(H) = 4.79). J values are given in Hz. High-resolution mass spectra were recorded on an Agilent 5975C MSD Quadrupol, Q-Tof micro from Waters or LTQ Orbitrap XL from Thermo Fisher Scientific. HPCL-MS measurements were performed either on an LCMS-2020 system from Shimadzu equipped with Luna® C18(2) column (3µm, 100Å, 100 × 4.6 mm), or on HPLC-MS Infinity 1260 system equipped with 6120 Quadrupole LC/MS detector from Agilent Technologies and either preparative column Luna® 5 µm C18 (2), 100 Å, 250 x 21.2 mm (Phenomenex) or analytical column Poroshell 120, EC-C18 4 µm, 4.6 x 100 mm (Agilent Technologies). UV/VIS spectroscopy was performed on a Cary 60 UV/Vis spectrophotometer from Agilent Technologies. Data from experiments were processed using Microsoft Excell 2016 MSOS software. Fluorescence measurements were performed on Spark® microplate reader from Tecan, in 96-well half area black polystyrene microplates (Corning). Automated peptide synthesis was done on PS3™ Peptide Synthesizer, Protein Technologies, Inc.. For microwave irradiation, the standard kitchen microwave oven Daewoo KOR-9GPBC (Daewoo Electronics) was used.

Synthetic procedures
Compounds prepared according to literature:

**tert-Butyl-4-pentynoate**¹ [2] (4); HRMS [M+H]⁺ m/z calcd. for [C₉H₁₅O₂]⁺ 155.1072, found 155.1077

5-{(Triisopropylsilyl)-4-pentynoic acid}³ [7]; HRMS [M+H]⁺ m/z calcd. for [C₁₅H₂₅O₂Si]⁺ 253.16293, found 253.16335

5-{(Triethylsilyl)-4-pentynoic acid}³ [8]; HRMS [M+H]⁺ m/z calcd. for [C₁₅H₁₉O₂Si]⁺ 211.11598, found 211.11591
2'-Bromoethyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside\(^4\) (16); HRMS [M+Na]\(^+\) m/z calcd. for [C\(_{16}\)H\(_{23}\)O\(_{10}\)BrNa]\(^+\) 477.03668, found 477.03668

(E)-N-(2-azidoethyl)-4-((2-chloro-4-nitrophenyl)diazenyl)-N-ethylaniline\(^5\) (19) HRMS [M+H]\(^+\) m/z calcd. for [C\(_{18}\)H\(_{17}\)O\(_{2}\)N\(_{7}\)Cl]\(^+\) 374.11268, found 374.11253

**AA building blocks synthesis:**

![Chemical Diagram]

**Scheme S1: Synthesis of amino acid building blocks**

**General procedure 1 (for synthesis of 5 and 6):**

According to lit.\(^3\), with slight modifications. Alkyne was taken into dry THF and cooled to -78 °C in acetone/dry ice bath. The solution of n-BuLi (2.5M in hexane; 1.3 eq.) was added slowly and reaction mixture was stirred for 10 min. The cooling bath was replaced with ice bath (0 °C) and Silyl-Cl (1.2 eq.) was added dropwise. The reaction proceeded for 3 hrs at r.t., was quenched with sat. aq. NH\(_{4}\)Cl, THF was gently evaporated, resulting slurry diluted with water, extracted with EtOAc, and concentrated. The crude product was purified by column chromatography.
**tert-Butyl 5-(triisopropylsilyl)pent-4-ynoate (5)**

\[
\text{\textit{Si}}\text{\text{-C}_5\text{\text{-CO}_2\text{-CH}_3}\text{-CH\text{-CH}_3}\text{-CH\text{-CH}_3}}
\]

The general procedure 1 was followed, using 13 mmol of 4. The reaction was quenched with sat. aq. NH₄Cl (80 ml), THF was removed, resulting slurry diluted with water (100 ml), extracted with EtOAc (3 x 60 ml) and purified by silicagel column using PE-Et₂O (50 : 1). Yield: 2.8 g, 70 %. NMR data correspond to lit.⁴; HRMS [M+Na]⁺ m/z calcd. for [C₁₈H₃₄O₂NaSi]⁺ 333.22203, found 333.22212.

**tert-Butyl 5-(triethylsilyl)pent-4-ynoate (6)**

\[
\text{\textit{Si}}\text{\text{-C}_5\text{\text{-CO}_2\text{-CH}_3\text{-CH\text{-CH}_3\text{-CH\text{-CH}_3}}}}
\]

The general procedure 1 was followed, using 43 mmol of 4. The reaction was quenched with sat. aq. NH₄Cl (160 ml), THF was removed, resulting slurry diluted with water (200 ml), extracted with EtOAc (3 x 100 ml) and purified by silicagel column using PE-Et₂O (50 : 1). Yield: 3.3 g, 29 %. NMR data correspond to lit.³; HRMS [M+Na]⁺ m/z calcd. for [C₁₅H₂₈O₂NaSi]⁺ 291.17508, found 291.17531, 292.17898.

**General procedure 2 (for synthesis of 9, 10, and 11)**

According to lit.⁶, with slight modifications. To the solution of acid and N-hydroxy succinimide (1.05 eq.) in dry THF at 0 °C DCC (1.05 eq.) dissolved in dry THF was added slowly. The mixture was stirred for 40 min. at 0 °C, warmed gradually to r.t. and stirred for additional 2h. The reaction mixture was filtered, concentrated, dissolved in EtOAc, filtered, washed with saturated solution of NaHCO₃ and brine, and dried over Na₂SO₄. The residue was swiftly purified on column of silica.

**2,5-dioxopyrrolidin-1-yl pent-4-ynoate⁶ (pent-4-ynoic acid 1-oxysuccinimidyl ester (11))**

\[
\text{\textit{O}}\text{\text{\textit{O}}\text{-C}_5\text{\text{-CO}_2\text{-CH}_3\text{-CH\text{-CH}_3\text{-CH\text{-CH}_3}}}}
\]

The general procedure 2 was followed, using 4 mmol of 4-pentynoic acid in dry THF (14 ml) and DCC. For the workup, the reaction mixture was dissolved in EtOAc (100 ml), filtered, washed with NaHCO₃ sat. (30 ml) and brine (30 ml), dried over Na₂SO₄ and swiftly purified on column of silica in EtOAc - PE (1:1). Yield 670 mg, 86 %. Analytical data corresponded to lit.⁶
2,5-dioxopyrrolidin-1-yl 5-(triisopropylsilyl)pent-4-ynoate (TIPS-pent-4-ynoic acid 1-oxysuccinimidyl ester (9))

The general procedure 2 was followed, using 0.94 mmol of 7 in dry THF (3 ml). For the workup, the reaction mixture was dissolved in EtOAc (100 ml), filtered, washed with NaHCO₃ sat. (30 ml) and brine (30 ml), dried over Na₂SO₄ and swiftly purified on column of silica in EtOAc – PE (1 : 3). Yield 263 mg, 79 %.

δ u (400 MHz, Chloroform-d) 2.92 – 2.84 (2 H, m, OC(O)CH₂CH₃), 2.83 (4 H, s, NC(O)CH₂CH₂), 2.71 – 2.61 (2 H, m, OC(O)CH₂CH₂), 0.97 (9 H, t, J 7.9, CH₃), 0.57 (6 H, qd, J 7.9, 0.6, CH₂CH₃).

δ c (101 MHz, Chloroform-d) 169.01 (NC(O)CH₂), 167.15 (OC(O)CH₂CH₂), 105.02 (SiC≡C), 82.56 (SiC≡C), 30.99 (OC(O)CH₂CH₂), 25.71 (NC(O)CH₂CH₂), 18.70 (6C, CH₃), 15.60 (OC(O)CH₂CH₂), 11.29 (CH₂CH₃).

HRMS [M+Na]⁺ m/z calcd. for [C₁₉H₂₆NNaO₄Si]⁺ 374.17581, found 374.17597.

2,5-dioxopyrrolidin-1-yl 5-(triethylsilyl)pent-4-ynoate (TES-pent-4-ynoic acid 1-oxysuccinimidyl ester (10))

The general procedure 2 was followed, using 0.75 mmol of 8 in dry THF (4 ml). For the workup, the reaction mixture was dissolved in EtOAc (100 ml), filtered, washed with NaHCO₃ sat. (30 ml) and brine (30 ml), dried over Na₂SO₄ and swiftly purified by flash chromatography on silica in DCM - MeOH (200:1). Yield 148 mg, 64 %.

δ u (400 MHz, Chloroform-d) 2.94 – 2.87 (2 H, m, OC(O)CH₂CH₂), 2.84 (4 H, s, NC(O)CH₂CH₂), 2.72 – 2.64 (2 H, m, OC(O)CH₂CH₂), 0.99 (9 H, t, J 7.9, CH₃), 0.65 – 0.54 (6 H, m, CH₂CH₃).

δ c (101 MHz, Chloroform-d) 169.00 (NC(O)CH₂), 167.16 (OC(O)CH₂CH₂), 104.40 (SiC≡C), 83.86 (SiC≡C), 30.86 (OC(O)CH₂CH₂), 25.70 (NC(O)CH₂CH₂), 15.58 (OC(O)CH₂CH₂), 7.53 (CH₃), 4.47 (CH₂CH₃).

HRMS [M+Na]⁺ m/z calcd. for [C₁₉H₂₆NNaO₄Si]⁺ 332.12902, found 332.12886.

General procedure 3 (for synthesis of 1, 2 and 3)
A suspension of Fmoc-L-Lys-OH in the mixture of dioxane and sat. aq. NaHCO₃ (1 : 3) was cooled to 0 °C. A solution of active ester (1.2 eq.) in dioxane was added dropwise. In 5 min the ice bath was removed and reaction proceeded at r.t. for 20 hrs. Reaction mixture was diluted with EtOAc, washed
with sat. aq. citric acid, water, brine and dried over Na₂SO₄. Concentrated residue was purified by flash chromatography on C18 phase and lyophilized.

\[ N²-\text{(}(9H\text{-fluoren-9-yl)methoxy} \text{carbonyl})\text{-}N⁶-(pent-4-ynoyl)}\text{-L-lysine (Fmoc-L-Lys(pentynoyl)-OH, (1))} \]

\[
\text{FmocCHN} \quad \text{COOH} \quad \text{O} \quad \text{O} 
\]

The general procedure 3 was followed, using 0.54 mmol of Fmoc-L-Lys-OH in the mixture of dioxane and sat. aq. NaHCO₃ (1 : 3; 4 ml) and 11 in dioxane (2 ml). For the workup, the reaction mixture was diluted with EtOAc (30 ml), washed with sat. aq. citric acid (20 ml), water (20 ml), brine (20 ml) and dried over Na₂SO₄. Concentrated residue was purified by flash chromatography on C18 phase in H₂O-MeOH (5 → 95 %). Yield: 152 mg, 62 %.

Analytical data correspond to lit.7; HRMS [M+Na]⁺ m/z calcd. for [C₂₆H₂₈N₂NaO₄Si]⁺ 471.18904, found 471.18865.

\[ N²-\text{(}(9H\text{-fluoren-9-yl)methoxy} \text{carbonyl})\text{-}N⁶-\text{(5\text{-}(triisopropylsilyl)pent-4-ynoyl)}\text{-L-lysine (Fmoc-L-Lys(pentynoyl-TIPS)-OH, (2))} \]

\[
\text{N} \quad \text{N} 
\]

The general procedure 3 was followed, using 1.81 mmol of Fmoc-L-Lys-OH in the mixture of dioxane and sat. aq. NaHCO₃ (1 : 3, 12 ml) and 9 in dioxane (6 ml). For the workup, the reaction mixture was diluted with EtOAc (100 ml), washed with sat. aq. citric acid (25 ml), water (25 ml), brine (25 ml) and dried over Na₂SO₄. Concentrated residue was purified by flash chromatography on C18 phase in H₂O-ACN (60 → 90 % 20 min, 90 % 15 min). Yield: 1.1 g, 84 %.

\[ \delta \text{ H (400 MHz, Methanol-}d₄) \text{ 7.78 (2 H, d, J 7.5, 26, 29), 7.67 (2 H, t, J 6.7, 25, 30), 7.42 – 7.34 (2 H, m, 23, 32), 7.30 (2 H, td, J 7.5, 1.2, 24, 31), 4.87 (7 H, s), 4.44 – 4.33 (1 H, m), 4.34 (1 H, d, J 2.2, 20), 4.22 (1 H, t, J 7.0, 21), 4.13 (1 H, dd, J 9.4, 4.6, 3), 3.30 (3 H, p, J 1.6), 3.17 (2 H, t, J 6.9, 7', 7''), 2.55 (2 H, t, J 7.2, 13', 13''), 2.37 (2 H, t, J 7.2, 12', 12''), 1.91 – 1.79 (1 H, m, 4), 1.70 (1 H, dtd, J 14.1, 9.2, 5.5, 4), 1.50 (3 H, dtd, J 13.7, 14.3, 13.7, 6.9, 5', 5''), 1.46 (1 H, s, 6', 6''), 1.04 (18 H, d, J 6.0, 38, 39, 40, 41, 42, 43), 1.01 – 0.91 (2 H, m).} \]

\[ \delta \text{ C (101 MHz, Methanol-}d₄) \text{ 175.95, 173.91, 145.19, 142.59, 128.78, 128.17, 128.14, 126.28, 126.25, 120.91, 67.96, 55.19, 48.42, 40.24, 36.35, 32.31, 29.91, 24.34, 19.05, 17.10, 12.43.} \]

HRMS [M+Na]⁺ m/z calcd. for [C₃₅H₄₈N₂NaO₅Si]⁺ 627.32247, found 627.32270.
The general procedure 3 was followed, using 2.72 mmol of Fmoc-L-Lys-OH in the mixture of dioxane and sat. aq. NaHCO₃ (1 : 3, 20 ml) and 10 in dioxane (10 ml). For the workup, the reaction mixture was diluted with EtOAc (100 ml), washed with sat. aq. citric acid (25 ml), water (25 ml), brine (25 ml) and dried over Na₂SO₄. Concentrated residue was purified by flash chromatography on reverse phase in H₂O – ACN (60 → 90 % 20 min, 90 % 15 min). Yield: 1.1 g, 72 %.

δ H (400 MHz, Methanol-d₄) 7.78 (2 H, d, J 7.5, 26, 29), 7.67 (2 H, t, J 6.7, 25, 30), 7.42 – 7.34 (2 H, m, 23, 32), 7.30 (2 H, td, J 7.4, 1.2, 24, 31), 4.44 – 4.33 (1 H, m), 4.34 (1 H, d, J 2.4, 20), 4.22 (1 H, t, J 7.0, 21), 4.13 (1 H, dd, J 9.4, 4.6, 3), 3.17 (2 H, t, J 6.9, 7), 2.52 (2 H, t, J 7.2, 13), 2.36 (2 H, t, J 7.2, 12), 1.91 – 1.79 (1 H, m, 4''), 1.70 (1 H, dtd, J 14.1, 9.3, 5.7, 4'), 1.61 – 1.36 (3 H, m, 5'', 6''), 0.96 (9 H, t, J 7.9, 35'', 36'', 37'').

δ C (101 MHz, Methanol-d₄) 175.97 , 1, 173.96 , 11, 158.71 , 17, 145.35 , 22, 33, 145.19, 142.59 , 27, 28, 128.78 , 25, 30, 128.17 , 24, 31, 128.14, 126.28, 126.25 , 23, 32, 120.91 , 26, 29, 107.93 , 15, 82.86, 14, 67.95 , 20, 55.20 , 3, 48.42 , 21, 40.16 , 7, 36.28 , 12, 32.29 , 4, 29.94 , 6, 24.31 , 5, 17.14 , 13, 7.78 , 38, 39, 40, 5.38 , 35, 36, 37.

HRMS [M+Na]⁺ m/z calcd. for [C₁₂H₂₄N₂NaO₅Si]⁺ 585.27552, found 585.27595.
Saccharide building blocks synthesis:

**Scheme S2:** Synthesis of saccharide building blocks

\[ \text{A: BF}_3\cdot\text{Et}_2\text{O, dry DCM, r.t., 2.5h, 16\%; B: NaN}_3\cdot[\text{Bu}_4\text{N}]^+\text{Br}^-, \text{DMSO, r.t., 19.5h, 97\%; C: DowexR 1x8, MeOH, r.t., 16h, 93\%.} \]

\[ \text{A: BF}_3\cdot\text{Et}_2\text{O, dry DCM, 0°C-r.t., 23h, 50\% B: NaN}_3\cdot[\text{Bu}_4\text{N}]^+\text{Br}^-, \text{DMSO, r.t., 29h, 90\%; C: DowexR 1x8, MeOH, r.t., 16h, 87\%.} \]

\[ \text{A: BF}_3\cdot\text{Et}_2\text{O, dry DCM, 0°C-r.t., 23h, 67\% B: NaN}_3\cdot[\text{Bu}_4\text{N}]^+\text{Br}^-, \text{DMSO, r.t., 23.5h, 99\%; C: DowexR 1x8, MeOH, r.t., 16h, 84\%.} \]
(2R,3R,4S,5R,6R)-2-(acetoxy methyl)-6-(4-bromobutoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (4-Bromobutyltetra-0-acetyl-β-D-glucopyranoside (12))

Procedure according to lit.9, with slight modifications. To the solution of (2S,3R,4S,5R,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (3.9 g; 10 mmol) and 4-bromobutan-1-ol (2.15 ml; 15 mmol) in dry DCM (20 ml) boron trifluoride diethyl etherate (6.17 ml; 50 mmol) was added slowly under argon. After 74.5 hrs at room temperature the reaction mixture was poured into ice-cold H2O (40 ml) and extracted with DCM (3 x 20 ml). Organic layer was washed with H2O (25 ml), NaHCO3 sat. (30 ml), H2O (30 ml), and dried over Na2SO4. The product was purified on column of silica in DCM-MeOH (200 : 1) and by crystallization from iPrOH. Yield: 778 mg, 16 %.

\[\delta_{n} (400 \text{ MHz, Chloroform-}d)\]

δH: (400 MHz, Chloroform-d) 5.20 (1 H, t, J 9.5, 2-H), 5.12 – 5.03 (1 H, m, 4-H), 4.98 (1 H, dd, J 9.6, 7.9, 3-H), 4.49 (1 H, d, J 7.9, 1-H), 4.19 (2 H, dddd, 6-H2b), 3.94 – 3.87 (1 H, m, OCH2H2CH2), 3.69 (1 H, ddd, J 10.0, 4.8, 2.5, 5-H), 3.57 – 3.48 (1 H, m, OCH3H2CH2), 3.45 – 3.37 (2 H, m, CH2CH2Br), 2.08 (3 H, s, CH3), 2.05 (3 H, s, CH3), 2.02 (3 H, s, CH3), 2.00 (3 H, s, CH3), 1.96 – 1.86 (2 H, m, CH2CH2Br), 1.78 – 1.68 (2 H, m, OCH3H2CH2).

δC (101 MHz, Chloroform-d) 170.82 (C=O), 170.43 (C=O), 169.54 (C=O), 169.44 (C=O), 100.87 (CH-1), 72.94 (CH-2), 71.94 (CH-5), 71.40 (CH-3), 69.07 (OCH2CH2), 68.55 (CH-4), 62.08 (CH-6), 33.45 (CH2CH2Br), 29.39 (CH2CH2Br), 28.12 (OCH2CH2), 20.90 (CH3), 20.83 (CH3), 20.76 (CH3), 20.74 (CH3).

HRMS [M+Na]+ m/z calcd. for [C19H27O13BrNa]+ 505.06798, found 505.06811.

(2R,3S,4S,5R,6R)-2-(acetoxy methyl)-6-(3-bromopropoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (3-Bromopropy)-2,3,4,6-tetra-0-acetyl-β-D-galactopyranoside (14)

Procedure according to lit.4, with slight modifications. To the solution of (2S,3R,4S,5S,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (2.16 g; 5.52 mmol) in dry DCM (10 ml) containing activated molecular sieves (cca 600 mg) 3-bromopropan-1-ol (97%; 1 ml; 11.08 mmol) was added. Mixture was stirred for 1 h at room temperature, cooled to 0°C, and boron trifluoride diethyl etherate (3.48 ml; 28.67 mmol) was added in course of 10 min. The reaction mixture was stirred at room temperature for 23 hrs, diluted with DCM (200 ml), washed with H2O (50 ml), NaHCO3 sat. (40 ml), H2O (40 ml), dried over Na2SO4 and concentrated. Crude product was purified on column of silica in toluene – EtOAc (20 to 25 %). Yield: 1.3 g, 50 %. Analytical data correspond to lit.9.
(2R,3R,4S,5R,6R)-2-(acetoxyethyl)-6-(4-azidobutoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (13)

The general procedure 4 was followed, using 1.57 mmol of 12 in DMSO (3.7 ml). For the workup, the reaction mixture was diluted with DCM (150 ml), washed with H$_2$O (2 x 40 ml), brine (40 ml), dried over Na$_2$SO$_4$ and purified on column of silica in toluene → EtOAc, or by flash chromatography in toluene → EtOAc. 

δ$_N$ (400 MHz, Chloroform-d) 5.20 (1 H, t, J = 9.5, 2-H), 5.08 (1 H, dd, J = 10.0, 9.4, 4-H), 4.98 (1 H, dd, J = 9.6, 8.0, 3-H), 4.49 (1 H, d, J = 8.0, 1-H), 4.29 – 4.08 (2 H, m, 6-H$_{2,3}$), 3.94 – 3.85 (1 H, m, OCH$_2$CH$_2$), 3.68 (1 H, ddd, J = 9.9, 4.7, 2.5, 5-H), 3.51 (1 H, dt, J = 9.5, OCH$_2$CH$_3$), 3.28 (2 H, tt, J = 5.6, 2.5, CH$_3$CH$_2$N$_3$), 2.08 (3 H, s, CH$_3$), 2.04 (3 H, s, CH$_3$), 2.02 (3 H, s, CH$_3$), 2.00 (3 H, s, CH$_3$), 1.65 (4 H, tdd, J = 6.0, 3.6, 2.0, 0CH$_2$CH$_2$+CH$_2$CH$_2$N$_3$).

δ$_C$ (101 MHz, Chloroform-d) 170.81 (C=O), 170.43 (C=O), 169.54 (C=O), 169.42 (C=O), 100.87 (C-1), 72.94 (C-2), 71.94 (C-5), 71.41 (C-3), 69.36 (OCH$_2$CH$_3$), 68.55 (C-4), 62.07 (C-6), 51.19 (CH$_3$CH$_2$N$_3$), 26.73 (CH$_3$CH$_2$N$_3$), 25.59 (OCH$_2$CH$_3$), 20.88 (CH$_3$), 20.78 (CH$_3$), 20.76 (CH$_3$), 20.74 (CH$_3$).

HRMS [M+Na]$^+$ m/z calcd. for [C$_{16}$H$_{27}$N$_3$NaO$_{10}$]$^+$ 468.15887, found 468.15899.

(2R,3R,4S,5S,6S)-2-(acetoxyethyl)-6-(2-azidoethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (17)

The general procedure 4 was followed, using 3.61 mmol of 16 in DMSO (9 ml). For the workup, the reaction mixture was diluted with DCM (250 ml), washed with H$_2$O (2 x 60 ml), brine (60 ml), dried over Na$_2$SO$_4$ and purified by flash chromatography in PE → EtOAc (20 → 60 %). Yield: 1.5 g, 99 %

Analytical data corresponds to lit.$^4$; HRMS [M+Na]$^+$ m/z calcd. for [C$_{16}$H$_{27}$O$_{10}$N$_3$Na]$^+$ 440.12756, found 440.12717.
The general procedure 4 was followed, using 2.73 mmol of 14 in DMSO (8 ml). For the workup, the reaction mixture was diluted with DCM (200 ml), washed with H₂O (2 x 50 ml), brine (50 ml), dried over Na₂SO₄ and purified by flash chromatography in PE – EtOAc (20 → 60%). Yield: 1.1 g, 90 %

Analytical data corresponds to lit.¹¹

General procedure 5 (for synthesis of Glc-C⁴-N₃, Gal-C⁳-N₃ and Man-C²-N₃)
According to lit.¹², with slight modifications. Dowex® 1x8 (in OH⁻ cycle, washed, dried) was placed into syringe equipped with sintered filter. The solution of acetylated saccharide in MeOH was added under argon, syringe was sealed and rotated for 16 hrs. The solution was collected, the drained resin was washed with MeOH (12 x) and the washings together with collected solution were concentrated and co-distilled with toluene (2 x). Product was purified by flash chromatography.

The general procedure 5 was followed using 1.48 mmol of 13, 300 mg of Dowex® 1x8 and 10 ml of dry MeOH. The crude product was purified by flash chromatography in DCM - MeOH (5 → 20 %) and lyophilized. Yield: 382, 93 %.

δ_h (500 MHz, Methanol-d₄) 1.65 – 1.75 (4 H, m, OCH₂CH₂CH₂CH₂N₃); 3.17 (1 H, dd, J₂,3 9.2, J₂,1 7.8, 2-H); 3.23 – 3.38 (5 H, m, 3,4,5-H, OCH₂CH₂CH₂CH₂N₃); 3.58 (1 H, m, OCH₂H₆CH₂CH₂CH₂N₃); 3.66 (1 H, dd, J₆a,6a 11.9, J₆b,5 5.4, 6b-H); 3.87 (1 H, dd, J₆a,6a 11.9, J₆a,5 1.9, 6a-H); 3.94 (1 H, m, OCH₂H₆CH₂CH₂CH₂N₃); 4.25 (1 H, d, J₁,₂ 7.8, 1-H).

δ_c (126 MHz, Methanol-d₄) 26.71, 27.93 (OCH₂CH₂CH₂CH₂N₃); 52.29 (OCH₂CH₂CH₂CH₂N₃); 62.75 (CH₆-6); 70.06 (OCH₂CH₂CH₂CH₂N₃); 71.63 (4-C); 75.09 (2-C); 77.93 (5-C); 78.10 (3-C); 104.33 (1-C).

HRMS [M+Na]^+ m/z calcd. for [C₁₀H₁₉O₆N₃Na]^+ 300.11661, found 300.11660.
Preparation of model peptides Pep14 and Pep15:

(2S,3S,4S,5S,6R)-2-(2-azidopropoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (Man-C2-N3)

The general procedure 5 was followed, using 3.42 mmol of 17, 850 mg of Dowex® 1x8 and 15 ml of MeOH (HPLC grade). The crude product was purified by flash chromatography on C18 phase in H2O - ACN (10 %) and lyophilized. Yield: 714 mg, 84 %. Analytical data correspond to lit.4

δ_H (500 MHz, Methanol-d4) 3.38 – 3.45 (2 H, m, CH2N3); 3.57 (1 H, ddd, J5,4 9.8, J5,5 5.8, 2.2, 5-H); 3.60 – 3.65 (2 H, m, 4-H, CH2H2O); 3.71 (1 H, dd, J6b,6a 11.8, J6b,5 5.8, 6b-H); 3.73 (1 H, dd, J3,4 9.0, J3,2 3.4, 3-H); 3.84 (1 H, dd, J3,3 3.4, J1,1 1.8, 2-H); 3.85 (1 H, dd, J6a,6b 11.8, J6a,5 2.2, 6a-H); 3.92 (1 H, m, CH2H2O); 4.81 (1 H, d, J1,1 1.8, 1-H).

δ_C (126 MHz, Methanol-d4) 51.70 (CH3N3); 62.90 (6-C); 67.70 (CH2O); 68.48 (4-C); 72.03 (2-C); 72.43 (3-C); 74.89 (5-C); 101.77 (1-C).

HRMS [M+H]^+ m/z calcd. for [C9H14O6N3]^+ 248.08881, found 248.08905.

(2R,3R,4S,5R,6R)-2-(2-azidethoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (Gal-C3-N3)

The general procedure 5 was followed, using 2.39 mmol of 15, 550 mg of Dowex® 1x8 and 10 ml of MeOH (HPLC grade). The crude product was purified by flash chromatography on reverse phase in H2O - ACN (5→30 %) and lyophilized. Yield: 627 mg, 87 %. Analytical data in accordance with lit.13

δ_H (500 MHz, Methanol-d4) 1.87 (2 H, m, OCH2CH2CH2N3); 3.43 – 3.5 (5 H, m, 2,3,5-H, OCH2CH2CH2N3); 3.64 (1 H, dt, Jgem 10.0, Jvic 6.1, OCH2CH2CH2N3); 3.72 (1 H, dd, J6b,6a 11.3, J6b,5 5.6, H-6b); 3.75 (1 H, dd, J6a,6b 11.3, J6a,5 6.8, 6a-H); 3.83 (1 H, dd, J4,3 3.3, J4,5 1.1, 4-H); 3.97 (1 H, dt, Jgem 10.0, Jvic 6.0, OCH2H2OCH2CH2N3); 4.21 (1 H, d, J1,1 7.5, 1-H).

δ_C (126 MHz, Methanol-d4) 30.28 (OCH2CH2CH2N3); 49.38 (OCH2CH2CH2N3); 62.44 (6-C); 67.50 (OCH2CH2CH2N3); 70.25 (4-C); 72.51 (2-C); 74.94 (3-C); 76.64 (5-C); 105.07 (1-C).
Resin loading:
TentaGel-S-OH resin (269 mg, theor. 0.065 mmol) was soaked in dry DMF (2 ml) for 1.5 h and drained. In separate flask, Fmoc-Gly-OH (594.6 mg; 2 mmol) was taken into dry DMF (1.5 ml), dry DCM (6 ml) was added, the solution was cooled to 0 ºC, DIC (155 µl; 1 mmol) was added. The reaction mixture was stirred at 0 ºC for 45 min. DCM was removed, the residue was dissolved in dry DMF, and the resulting solution was added to the drained resin. DMAP (2.44 mg; 0.02 mmol) was added, the syringe was flushed with argon and rotated on rotary shaker for 2 hrs. Resin was drained, washed with DMF (5x), DCM (5x) and dried. Yield: 257 mg of dried loaded resin. Loading by Fmoc estimation: 0.188 mmol/g; AA analysis: 0.137 mmol/g.

Fmoc estimation method:
Done according lit.14 Dry loaded resin (approx. 5 mg, weighted precisely) was treated by DBU (2 % in DMF; 2 ml) for 40 min. The solution was collected, diluted to 10 ml with ACN, resulting solution was diluted for the second time (0.8 ml of solution to 10 ml by ACN), and its’ optical density measured on
UV spectrometer in optical cell (1 cm) at 304 nm. Blanc control sample was used to determine optical background. The resin load was calculated as

\[
\text{loading} \left[ \frac{\text{mmol}}{g} \right] = A_{304} \times \frac{16.4}{m_{\text{resin}} [\text{mg}]}
\]

**Automated SPPS**

Automated peptide synthesis was performed on PS3 peptide synthesizer, *Protein Technologies, Inc.* Synthesizer was loaded with glycine substituted resin (128 mg; 0.024 mmol for Pep14, 157 mg; 0.03 mmol for Pep15). Peptide was synthesized under standard automated Fmoc protocols (see below), using either 4 equivalents of each commercial amino acid and 4 equivalents of HBTU as coupling agent, or 2 equivalents of modified amino acids 2 or 3 and 2 equivalents of HBTU, in both cases activated by NMM (0.4 M in DMF). The remaining Fmoc-group was removed after the last coupling step using the automated protocol, the deprotection solution was piperidine : DMF (1 : 4).

The following Fmoc protected amino acids were utilized:

- **Step 1:** Fmoc-L-Lys(pentynoyl-TES)-OH (2) for Pep14, Fmoc-L-Lys(pentynoyl-TIPS)-OH (3) for Pep15
- **Step 2:** Fmoc-L-Tyr(tBu)-OH
- **Step 3:** Fmoc-L-Val-Oh
- **Step 4:** Fmoc-L-Lys(Boc)-OH
- **Step 5:** Deprotection – removal of Fmoc group

The resin was transferred from synthesizer reaction vessel to syringe (10 ml) equipped with sintered filter, washed with DCM (6x), drained, and dried. Yield: 143 mg (Pep14), resp. 166 mg (Pep15) of loaded resin. Small sample of resin (approx. 2 mg) was treated with NaOH following the general procedure bellow and the progress of the synthesis was verified by LC-MS analysis.

**Standard protocols for SP3 Peptide Synthesizer:**

Deprotection solution: piperidine (20 % in DMF)
Activation solution: NMM (0.4M in DMF)

| Coupling step: | FUNCTION | DURATION | REPEATED |
|---------------|----------|----------|----------|
| 1             | Wash with DMF | 30 s (10 min for the first coupling) | 3 (2 for the first coupling) |
| 2             | de-pr. of N-terminus by deprotection solution | 5 min. | 2 |
| 3             | Wash with DMF | 30 s | 6 |
| 4             | Amino acid + base dissolved in the activation solution | 30 s | 1 |
| 5             | amino acid coupling | 30 min | 1 |
| 6             | Wash with DMF | 30 s | 3 |

| Deprotection step: | FUNCTION | DURATION | REPEATED |
|-------------------|----------|----------|----------|
| 1                 | Wash with DMF | 30 s | 3 |
| 2                 | de-pr. of N-terminus by deprotection solution | 5 min. | 2 |
| 3                 | Wash with DMF | 30 s | 6 |
General procedure 6: Cleavage-off of TentaGel-S-OH resin with NaOH (for analytical samples)

To the loaded TentaGel-S-OH resin (approx. 1-2 mg), the solution of NaOH (0.1M in H₂O; 50 μl) was added, reaction mixture was shaken for 90 min., neutralized by HCl (0.2M in H₂O) and the liquids were collected. Drained resin was washed with warm ACN (2 x 20 μl), ACN washes were mixed with previously collected liquids and analyzed by LC-MS.

Figure S1: LC-MS analyses of compounds Pep14 (A) and Pep15 (B), at 270 nm. Molecular mass 830.7 corresponds to peptide with free alkyne. Note: silyl protective groups are not completely stable under cleavage conditions (0.1M NaOH for 1.5 hr)
Preparation of model peptide Pep16:

Resin loading:
TentaGel-S-OH resin (517 mg, theor. 0.124 mmol) was soaked in dry DMF (3 ml) for 1 hr and drained. In separate flask, Fmoc-Gly-OH (594.6 mg; 2 mmol) was taken into dry DMF (1 ml), dry DCM (8 ml) was added, the solution was cooled to 0 °C, DIC (155 µl; 1 mmol) was added. The reaction mixture was stirred for at 0 °C for 1 hr. DCM was removed, the residue was dissolved in dry DMF (4 ml), and 2/3 of the resulting solution was added to the drained resin. DMAP (1.6 mg; 0.013 mmol) was added, the syringe was flushed with argon and rotated on the rotary shaker for 1 hr. Resin was drained, the remaining 1/3 of anhydride solution together with DMAP (0.08 mg; 0.07 mmol) were transferred to the drained resin, the syringe was flushed with argon and rotated for 1 hr. The resin was drained, washed with DMF (5x), DCM (5x) and dried. Yield: 541.2 mg of dried loaded resin. Loading by Fmoc estimation: 0.139 mmol/g; AA analysis: 0.184 mmol/g.

SPPS
Solid phase peptide synthesis was performed “by hand” following the lit.15.

Fmoc-Glycine substituted TentaGel-S-OH resin (259 mg; 0.033 mmol) was swelled in DCM (15 min), and washed with DMF (1.5 ml; 30 s).

Fmoc removal:
Piperidine (20 % v/v in DMF; 1.5 ml) was added, resin was shaken for 2 min and drained.
Piperidine (20 % v/v in DMF; 1.5 ml) was added, resin was shaken for 12 min and drained.
Resin washed with DMF (4 x 2 ml)

Coupling step:
Fmoc-L-Lys(pentynoyl-TES)-OH (2) (37.2 mg; 0.066 mmol) and HBTU (25 mg; 0.066 mmol) were dissolved in DMF (1.5 ml), and added to the drained resin.
NMM (35 ml; 0.32 mmol) was added.
The resin was mixed with N₂ stream for 30 min.
The resin was drained; analytical sample was washed coupling conversion verified by ninhydrin test.
The resin was washed with DMF (6 x 2 ml) and DCM (4 x 2 ml).
Deprotection-coupling cycle was repeated using:

2. coupling: Fmoc-L-Lys(pentynoyl-TIPS)-OH (3) (39.9 mg; 0.066 mmol)
3. coupling: Fmoc-L-Tyr(tBu)-OH (60.66 mg; 0.066 mmol)
4. coupling: Fmoc-L-Val-OH (44.8 mg; 0.132 mmol)

Last Fmoc removal:

Piperidine (20 % v/v in DMF; 1.5 ml) was added, resin was shaken for 2 min and drained. Piperidine (20 % v/v in DMF; 1.5 ml) was added, resin was shaken for 12 min and drained. Resin washed with DMF (6 x 2 ml), DCM (4 x 2 ml), dried 2 h under vacuum.

Yield: 335 mg of substituted resin. Small sample of resin (approx. 2 mg) was treated with NaOH following the general procedure 6 and the progress of the synthesis was verified by LC-MS analysis.

Figure S2: LC-MS analysis of compound Pep16 at 270 nm.
Synthesis of model modified peptide *Pep1*

Peptide *Pep1* was synthesized on PS3 peptide synthesizer using L-glycine-loaded resin, standard Fmoc chemistry and 4 (resp. 2) equivalents of amino acid for coupling step. For detailed procedure see synthesis of *Pep14*.

The following Fmoc protected amino acids were utilized:
- Step 1: Fmoc-Lys(pentynoyl-TES)-OH (2)
- Step 2: Fmoc-L-Ala-OH *H₂O
- Step 3: Fmoc-Lys(pentynoyl-TIPS)-OH (3)
- Step 4: Fmoc-Asn(Trt)-OH
- Step 5: Fmoc-Lys(pentynoyl)-OH (1)
- Step 6: Fmoc-L-Tyr(tBu)-OH
- Step 7: Deprotection – removal of Fmoc group

The resin was transferred from synthesizer reaction vessel to syringe (10 ml) equipped with sintered filter, washed with DCM (4x), drained, and dried. Yield: 327 mg of loaded resin. Small sample of resin (approx. 2 mg) was treated with NaOH following the general procedure 6, using significantly shorter cleavage time (10 min), and analyzed by LC-MS (*Agilent*).
Figure S3: LC-MS analysis of compound Pep1 at 214 nm.

Expected mass: [M+2H]^2+ 808.57
Synthesis of glycosylated peptide **Pep10**:

![Diagram of the synthesis process](image-url)

**Scheme S5**: Synthesis of tripeptide-glycosylated peptide Pep10
Resin loading:

TentaGel-S-NH₂ resin (514 mg, theor. 0.134 mmol) was soaked in DMF (5 ml) for 1 h and drained. The solution of Fmoc-L-Met-OH (201.2 mg; 0.53 mmol) and HBTU (203.3 mg; 0.54 mmol) in NMM (0.4 M in DMF, 3 ml) was added, syringe was flushed with Ar and rotated for 75 min. Resin was drained, washed with DMF (6x), DCM (6x) and dried. Yield: 536 mg of dried loaded resin. Loading by Fmoc estimation: 0.197 mmol/g; AA analysis: 0.164 mmol/g. For Fmoc estimation method see synthesis of Pep14.

Automated SPPS

Automated peptide synthesis was performed on PS3 peptide synthesizer, Protein Technologies, Inc. Synthesizer was loaded with methionine substituted resin (250 mg; 0.049 mmol). Resin was capped with acetic anhydride under standard automated protocol, than peptide was synthesized under standard automated Fmoc protocols, using either 4 equivalents of each commercial amino acid and 4 equivalents of HBTU as coupling agent, or 2 equivalents of modified amino acid 2 or 3 and 2 equivalents of HBTU. The remaining Fmoc-group was removed after the last coupling step by piperidine : DMF (1 : 4).

The following Fmoc protected amino acids were utilized:
Step 1: Capping with acetic anhydride
Step 1: Fmoc-Gly-OH
Step 2: Fmoc-L-Lys(pentynoyl-TES)-OH (2)
Step 3: Fmoc-L-Arg(Pbf)-OH
Step 4: Fmoc-L-Lys(pentynoyl-TIPS)-OH (3)
Step 5: Fmoc-L-Asn(Trt)-OH
Step 6: Fmoc-L-Lys(pentynoyl)-OH (1)
Step 7: Fmoc-L-Trp(Boc)-OH
Step 8: Fmoc-Gly-OH
Step 9: deprotection – removal of Fmoc group

The resin was transferred from synthesizer reaction vessel to syringe (10 ml) equipped with sintered filter, washed with DCM (6x), drained, and dried. Yield: 324 mg of loaded resin. Small sample of resin (approx. 2 mg) was treated with CNBr following the general procedure and the progress of the synthesis was verified by LC-MS analysis.
Standard protocols for SP3 Peptide Synthesizer:
For deprotection and coupling protocol details see synthesis of Pep14.
Capping step:
Capping solution: acetic anhydride (2 ml)

| STEP | FUNCTION                              | DURATION | REPEATED |
|------|---------------------------------------|----------|----------|
| 1    | Wash with DMF                         | 30 s     | 6        |
| 2    | Mixing of capping solution in vial    | 30 s     | 1        |
| 3    | Capping                               | 20 min.  | 1        |
| 6    | Wash with DMF                         | 30 s     | 3        |

General procedure 7: Cleavage-off of TentaGel-S-NH₂ resin with CNBr (for analytical samples)
Small amount of the analyzed resin (approx. 2 mg) was soaked in water for 15 min and drained. H₂O (20 μl) and CNBr (0.5 M in 0.2M HCl; 20 μl) were added, the vial was flushed with argon, sealed and MW irradiated (900W 2 x 3 s with 27 s break). The resin was drained, washed with warm ACN (approx. 40-50°C; 2x), ACN fractions were collected and measured on LC-MS.

Modification of resin-bound protected peptide Pep4

Click I

Dry resin (71 mg; 0.014 mmol) was soaked in t-BuOH : H₂O (2 : 3) for 50 min. and drained. The solution of 3-azidopropyl β-d-galactopyranoside Gal-C3-N₃ (0.4 M in H₂O; 58.2 μl; 0.023 mmol) was added and syringe was thoroughly agitated. The coupling solution was prepared in separate vial: to the solution of CuSO₄ *5H₂O (0.1M in H₂O; 29.1 μl) the solution of BTTP (0.05M in t-BuOH; 116.4 μl) was added. Solution of sodium ascorbate (0.05M in H₂O; 116.4 μl) was added and the resulting colorless solution was transferred to the resin-azide mixture. DIPEA (4.46 ml; 0.026 mmol) was added, the vial was flushed with argon, sealed and gently rotated for 4h. The resin was drained, small sample treated with CNBr following the general procedure and the progress of the synthesis was verified by LC-MS analysis. The click procedure was repeated again, using the same amount of reagent and catalyst. The resin was washed with DMSO (10x), H₂O (3x), DMSO (3x), DCM (4x) and dried.
TES removal:

Dried resin was soaked in DMF:MeOH:H₂O (60:32:8) for 25 min. and drained. The solution of AgClO₄ (0.2M in DMF:MeOH:H₂O (60:32:8); 325 μl) was added under argon, the vial was sealed and rotated slowly for 2h with exclusion of light. Resin was drained, washed with DMF (5x), H₂O (2x), KCN (0.5 M in H₂O; 4x), H₂O (2x), DMSO (2x), DCM (3x) and dried.

Click II

Dry resin (48 mg; 0.0079 mmol) was soaked in t-BuOH:H₂O (2:3) for 50 min. and drained. The solution of 4-azidobutyl β-D-glucopyranoside Glc-C⁴-N₃ (0.4 M in H₂O; 39.4 μl; 0.016 mmol) was added and syringe was thoroughly agitated. The coupling solution was prepared in separate vial: to the solution of CuSO₄ *5H₂O (0.1M in H₂O; 18.7 μl) the solution of BTTP (0.05M in t-BuOH; 78.7 μl) was added. Solution of sodium ascorbate (0.05M in H₂O; 78.7 μl) was added and the resulting colorless solution was transferred to the resin-azide mixture. DIPEA (3.02 ml; 0.017 mmol) was added, the vial was flushed with argon, sealed and gently rotated for 4h. The resin was drained, small sample treated with CNBr following the general procedure and the progress of the synthesis was verified by LC-MS analysis. The resin was washed with DMSO (10x), H₂O (3x), DMSO (3x), DCM (4x) and dried.
**TIPS removal:**

To the dry resin (48 mg; 0.0079 mmol) TBAF (1 M in THF; 80 µl) was added. The vial was flushed with argon, sealed and shaken for 3h. The resin was drained, washed with DMSO (2x), H₂O (2x), DMF (2x) DCM (2x) and dried. Small sample was treated by CNBr following the general procedure and the progress of the synthesis was verified by LC-MS analysis.

**Click III**

Dry resin (34 mg; 0.0056 mmol) was soaked in t-BuOH : H₂O (2 : 3) for 45 min. and drained. The solution of 4-azidobutyl α-D-mannopyranoside **Man-C2-N₃** (0.4 M in H₂O; 27.9 µl; 0.011 mmol) was added and the mixture was thoroughly agitated. The coupling solution was prepared in separate vial: to the solution of CuSO₄ *5H₂O (0.1M in H₂O; 13.9 µl) the solution of BTTP (0.05M in t-BuOH; 55.8 µl) was added. Solution of sodium ascorbate (0.05M in H₂O; 55.8 µl) was added and the resulting colorless solution was transferred to the resin-azide mixture. DIPEA (2.14 ml; 0.012 mmol) was added, the vial was flushed with argon, sealed and gently rotated for 16h. The resin was drained, small sample treated with CNBr following the general procedure and the progress of the synthesis was verified by LC-MS analysis. The resin was washed with DMSO (10x), H₂O (3x), DMSO (3x), DCM (4x) and dried.
Deprotection of the modified peptide:

To the dry resin, the mixture of TFA : H₂O : TIS (95 : 2.5 : 2.5; 0.4 ml) was added. The syringe was gently rotated for 1 hr, TFA cocktail was replaced by a fresh batch (0.4 ml), reaction mixture was rotated for additional 1 hr, drained, washed with DMSO (5x), DCM (5x), DMSO (3x), H₂O (3x). NaOH (1.1M on H₂O; 0.3 ml) was added and reaction mixture was rotated for 1 hr. The resin was drained, washed with H₂O (5x), DMSO (3x), DCM (5x) and dried. Small sample was treated with CNBr following the general procedure, and resulting solution analyzed by LC-MS analysis.
Figure S4: Synthesis of Pep10. LC-MS analyses of peptide modifications. A: Click I (galactose), after first CuAAC round - detected mass 1081.8 corresponds to remaining non-modified peptide; B: Click I (galactose), after repeated CuAAC reaction; C: TES removal; D: Click II (glucose); E: TIPS removal; F: Click III (mannose); G: peptide deprotection in TFA cleavage cocktail - detected mass 1092.0 corresponds to TFA acetate; H: deprotected peptide after hydrolysis of TFA acetate by NaOH. UV absorption measured at 266 nm.
Synthesis of Pep11:

TentaGel-S-NH₂ resin (500 mg, theor. 0.13 mmol) was soaked in DMF (5 ml) for 40 min and drained. The solution of Fmoc-Gly-OH (154.6 mg; 0.52 mmol) and HBTU (197.2 mg; 0.52 mmol) in NMM (0.4 M in DMF, 3 ml) was added, syringe was flushed with Ar and rotated for 75 min. Resin was drained, washed with DMF (6x), DCM (6x) and dried. Yield: 571 mg of dried loaded resin. Loading by Fmoc estimation (for details see synthesis of Pep14): 0.184 mmol/g; AA analysis: 0.176 mmol/g.

Scheme S6: Synthesis of double modified fluorogenic peptide AK-280
Automated SPPS

Automated peptide synthesis was performed on PS3 peptide synthesizer, Protein Technologies, Inc. Synthesizer was loaded with glycine-substituted resin (275 mg; 0.046 mmol). Resin was capped with acetic anhydride under standard automated protocol, than peptide was synthesized under standard automated Fmoc protocols, using either 4 equivalents of each commercial amino acid and 4 equivalents of HBTU as coupling agent, or 2 equivalents of modified amino acid 1 or 2 and 2 equivalents of HBTU. The remaining Fmoc-group was removed after the last coupling step, the deprotection solution was piperidine : DMF (1 : 4). For the standard protocol details see synthesis of Pep14 (coupling and deprotection and capping step)

The following Fmoc protected amino acids were utilized:
Step 1: Fmoc-L-Met-OH
Step 2: Fmoc-Gly-OH
Step 3: Fmoc-L-Lys(pentynoyl)-OH (1)
Step 4: Fmoc-L-Phe-OH,
Step 5: Fmoc-L-Ala-OH * H2O
Step 6: Fmoc-L-Lys(Boc)-OH
Step 7: Fmoc-L-Ala-OH * H2O
Step 8: Fmoc-L-Lys(pentynoyl-TES)-OH (2)
Step 9: Fmoc-L-Tyr(tBu)-OH

The resin was transferred from synthesizer reaction vessel to syringe (10 ml) equipped with sintered filter, washed with DCM (6x), drained, and dried. Yield: 362 mg of loaded resin. Small sample of resin (approx. 2 mg) was treated with CNBr following the general procedure and the progress of the synthesis was verified by LC-MS analysis.

Modification of resin-bound protected peptide

Click I – 3-azido-7-hydroxycoumarin

Dry resin was soaked in t-BuOH : H2O (2 : 3) for 30 min. and drained. The solution of 3-azido-7-hydroxycoumarin (0.4 M in DMSO; 253 µl) was added under argon and syringe was shortly agitated. The coupling solution was prepared in separate vial: to the solution of CuSO4 *5H2O (0.1M in H2O; 127 µl) the solution of BTTP (0.05M in t-BuOH, 506 µl) was added. Solution of sodium ascorbate (0.05M in H2O; 506 µl) was added and the resulting colorless solution was transferred to the resin-azide mixture under argon. The syringe was sealed and gently shaken each approx. 30 min (4x), than stood overnight. The resin was drained, small sample treated with CNBr following the general procedure, and the progress of the synthesis was verified by LC-MS analysis. The click procedure was repeated again with the same amount of reactants and reagents. The resin was washed with DMSO (10x), H2O (3x), DMSO (3x), DCM (4x) and dried. Yield: 361 mg of loaded resin.
TES removal:

Dried resin was soaked in DMF : MeOH : H₂O (60 : 32 : 8) for 40 min. and drained. The solution of AgClO₄ (0.2 M in DMF : MeOH : H₂O (60 : 32 : 8); 1956 μl) was added under argon, syringe with resin was slowly rotated for 2 hr with exclusion of light. Resin was drained, washed with DMF (5x), H₂O (2x), KCN (0.5 M in H₂O; 4x), H₂O (2x), DMSO (2x), DCM (3x), blow dried with nitrogen, for 60 min. Yield: 337 mg of loaded resin. The progress of the synthesis was verified by LC-MS analysis.

Click II - N-ethyl-N-(2-azidoethyl)-4-(2-chloro-4-nitrophenylazo)phenylamine

Dry resin was soaked in t-BuOH : H₂O (2 : 3) for 20 min. and drained. The solution of N-ethyl-N-(2-azidoethyl)-4-(2-chloro-4-nitrophenylazo)phenylamine⁵ (0.4 M in DMSO; 253 μl) was added under argon and syringe was shortly agitated. The coupling solution was prepared in separate vial: To the solution of CuSO₄·5H₂O (0.1 M in H₂O; 127 μl) the solution of BTTP (0.05 M in t-BuOH, 506 μl) was added. Solution of sodium ascorbate (0.05 M in H₂O; 506 μl) was added and the resulting colorless solution was transferred to the resin-azide mixture under argon. The syringe was sealed and gently rotated overnight. The resin was drained, small sample treated with CNBr following the general procedure, and the progress of the synthesis was verified by LC-MS analysis. The resin was washed with DMSO (25x), DCM (10x) and dried. Yield: 420 mg of loaded resin.
Deprotection of the modified peptide:

To the dry resin, the mixture of TFA : H₂O : TIS (95 : 2.5 : 2.5; 3 ml) was added. The syringe was gently rotated for 1 hr, TFA cocktail was replaced by a fresh batch (3 ml), reaction mixture was rotated for additional 1 hr, drained, washed with DMSO (5x), DCM (5x), DMSO (3x), H₂O (3x). Small sample was treated with CNBr following the general procedure, and resulting solution was analyzed by LC-MS analysis.

Cleavage-off from the resin:

To the resin, soaked in H₂O from previous step, H₂O (2 ml) and CNBr (0.5 M in 0.2M HCl; 2 ml) was added under argon. Syringe was gently rotated overnight. The resin was drained and washed with H₂O. The resin was extracted by warm ACN (approx. 50°C; 3 ml, 3x), DMSO (cca 2 ml), organic fractions were collected and concentrated. HPLC purification (5 →83% ACN in H₂O (+ 0.1% TFA)) afforded 26 mg (34.1% over whole synthesis, calculated with resin load 0.176 mmol/g) of the modified peptide **Pep11**. HRMS [M+Na]⁺ m/z calcd. for [C₈₃H₁₀₃N₂₂NaO₁₈]⁺ 1753.74014, found 1753.74082.
Synthesis of 18 – the positive standard for fluorescence assay

The solution of CuSO₄·5H₂O (0.1 M in H₂O, 383 µl; 0.038 mmol) was mixed with BTTP (0.05 M in t-BuOH, 1.53 ml, 0.077 mmol), than with sodium ascorbate solution (0.05 M in H₂O, 1.53 ml, 0.077 mmol). 3-azido-7-hydroxy-2H-chromen-2-one (3-azido-7-hydroxy-coumarin, Santiago) (0.4 M in DMSO, 420.8 µl; 0.168 mmol) was added. To the well stirred solution 1 (0.4 M in DMSO; 382.5 µl; 0.153 mmol) was dropped, reaction mixture was stirred for 2 h and stored for 14 h at -12 °C for 14 h. Liquid was discarded, remaining syrupy residue was extracted with DCM (2x), EDTA (1x), H₂O (1x), EtOH (1x) and concentrated. Crude residue was taken into DMF (3 ml), precipitated with Et₂O (30 ml), centrifuged and dried. Yield: 104 mg, (dark green foam) [M+Na]+ m/z calcd. for [C₃₅H₃₃O₈N₅Na]+ 674.2213, found 674.2188.

¹H NMR spectra of 18 showed very broad signals that made signal assignment impossible. In order to improve spectrum resolution, we measured ¹H and ¹³C spectra at elevated temperatures (50, 80, 100 °C), however we observed decomposition of the sample. Therefore, identity and purity of Fmoc-protected Pep12 was proven by HPLC/MS (see below).
HPLC chromatogram of compound 18 used as positive control during trypsin digestion experiment with Pep11. The observed and expected mass is indicated.

Methods optimizations:

Optimization of TES removal in the presence of TIPS:
Based on lit. 16

In order to find optimal conditions for selective removal of TES protective group in presence of TIPS, initially tested in solution, various substrate/silver salts ratios were used to reach maximal efficiency. AgNO₃, AgClO₄ and AgF were selected as promising cleavage agents. The other silver salts were either of limited efficiency (AgNO₂) or not effective at all (AgOCN, Ag₂SO₄) at given concentration. In our hands, bases (1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), Cs₂CO₃) in combination with MeOH did not give useful results.

Sample preparation: In the 200 µl HPLC sample vial, 2 (0.8 µmol; 1 eq.) and 3 (if used - 0.8 µmol; 1 eq.) were dissolved in DMF (60 µl). Silver salt (0.1-10 eq, in MeOH : H₂O (4 : 1); 40 µl) was added and the reaction mixture was monitored on LC-MS system (Shimadzu) in indicated time points. Equivalents of salt marked in graph headings are related to amino acid 2.
Optimization of TES removal from resin bound peptides:
As AgClO₄ showed up to be the most convenient cleaving agent for removal of TES in the presence of TIPS, we used it to establish the procedure for semiorthogonal deprotection of resin-bound glycopeptides. We prepared the model resin-bound alkyne-modified peptides Pep14 (TES), Pep15 (TIPS), and Pep16 (TES + TIPS) and exposed them to AgClO₄ solutions of varying concentrations. Deprotected peptides were cleaved-off from the resin and analyzed by LC-MS (Shimadzu).
**Typical procedure:**

Sample of resin-bound peptide (2 mg; 0.37 μmol) was soaked in DMF : MeOH : H₂O (60 : 32 : 8) for 20 min., drained, the solution of AgClO₄ (of given concentration in DMF : MeOH : H₂O (60 : 32 : 8); 10 μl) was added, reaction vial was flushed with argon and gently shaken each 15 min for overall time of 2h with exclusion of light. The resin was drained, washed with DMF : MeOH : H₂O (60 : 32 : 8, 5x), KCN (0.1M in H₂O; 4x), H₂O (3x), and drained. For cleavage of the peptide from the TentaGel-S-OH resin, the solution of NaOH (0.1M in H₂O; 30 μl) was added, reaction mixture was shaken for 10 min.,
neutralized by HCl (0.2M in H₂O) and the liquids were collected. Drained resin was washed with warm ACN (2x), ACN washes were mixed with previously collected liquids and analyzed by LC-MS.

Figure S7: Deprotection of resin bound TES-containing peptide Pep14 using different concentrations of AgClO₄ for 2h in DMF:MeOH:H₂O (60:32:8). The figure shows HPLC chromatograms of the crude peptide after cleavage from the resin using 0.1M NaOH. Crude peptide without treatment with AgClO₄ (black line), using 0.08M AgClO₄ (purple line), using 0.16M AgClO₄ (blue line) and using 0.24M AgClO₄ (orange line). Complete deprotection was observed using 0.16M solution of AgClO₄. Note. The TES group is not completely stable to the cleavage conditions (0.1M NaOH for 90 min) and therefore the crude peptide (black line) also contains the deprotected peptide.
Figure S8: Deprotection of resin bound TES-containing peptide **Pep15** using different concentrations of AgClO₄ for 2h in DMF : MeOH : H₂O (60 : 32 : 8). The figure shows HPLC chromatograms of the crude peptide after cleavage from the resin using 0.1M NaOH. Crude peptide without treatment with AgClO₄ (black line), using 0.08M AgClO₄ (purple line), using 0.16M AgClO₄ (blue line) and using 0.24M AgClO₄ (orange line). The TIPS group is stable towards AgClO₄. Note. The TIPS group is not completely stable to the cleavage conditions (0.1M NaOH for 90 min) and therefore the crude peptide (black line) also contains traces of the deprotected peptide.

Figure S9: Conversion of TES removal from resin-bound peptide **Pep16**, traced by LC-MS after cleavage-off. Concentrations of AgClO₄ solutions: 0.08M, 0.16M, and 0.24M in DMF:MeOH:H₂O (60:32:8), 5ml/g or dry substituted resin, 2h. TES group was once more fully removed by 0.16M solution of AgClO₄.
Optimization of CuAAC reaction for resin-bound peptides:

In order to find the optimal conditions to modify peptides by CuAAC on solid phase, model resin-bound heptapeptide **Pep1** was “clicked” with saccharide analog **Gal-C3-N₃**. In our hands, the first modification of peptide backbone was the most challenging one. LC-MS was used to analyze reaction mixture after product was cleaved-off from the resin, conversion was determined from peak areas of product **Pep17** and remaining unmodified **Pep1**.

**Scheme 9** Model reaction used for optimisation of final CuAAC click reaction on solid phase

**Typical procedure:**

Resin-bound **Pep1** (5 mg; 0.77 µmol) was soaked in solvent mixture for 30 min and drained. The solution of 3-azidopropyl β-D-galactopyranoside **Gal-C3-N₃** (0.4 M in H₂O; 3.83 µl; 1.53 µmol) was added and vial was thoroughly agitated. The click activation solution was prepared in separate vial: to the solution of CuSO₄ *5H₂O (0.1M in H₂O; 1.91 µl; 0.19 µmol) the solution of BTTP (0.05M in t-BuOH; 7.65 µl; 0.38 µmol) was added, solution of sodium ascorbate (0.05M in H₂O; 7.65 µl; 0.38 µmol) was added and the resulting colorless solution was transferred to the resin-azide mixture. Detergent or base was added (if relevant), the vial was flushed with argon, sealed and gently rotated for 24h. The resin was drained, washed with used solvent mixture (2x), DMSO (5x), H₂O (3x) and drained. For cleavage of the peptide from the TentaGel-S-OH resin, the solution of NaOH (0.1M in H₂O; 50 µl) was added, reaction mixture was shaken for 10 min., neutralized by HCl (0.2M in H₂O) and the liquids were collected. Drained resin was washed with warm ACN (2x), ACN washes were mixed with previously collected liquids and analyzed by LC-MS.
Figure S10: Click modification of resin bound Pep1 with Gal-C3-N3 by CuAAC following general procedure described above (2 equiv. of Gal-C3-N3, 25mol% of CuSO4, 50mol% of BTTIP ligand and 50mol% of sodium ascorbate). The conversion of the peptide was followed by HPLC–MS after cleavage of the peptide from the resin by 0.1M NaOH. A) performed in DMSO : H2O (1 : 2); B) performed in DMSO : H2O (1 : 2) + 0.5% Tween 20; C) performed in DMSO : H2O (1 : 2) + 0.5% Triton X; D) performed in t-BuOH : H2O (2 : 3); E) performed in t-BuOH : H2O (2 : 3) + 1% Tween 20; F) performed in t-BuOH : H2O (2 : 3) + 5% Tween 20; G) performed in t-BuOH : H2O (2 : 3) + 10% Tween 20; H) performed in t-BuOH : H2O (2 : 3) and heated up at 40°C; I) performed in t-BuOH : H2O (2 : 3) + DIPEA (2.2 equiv.); J) performed in t-BuOH : H2O (2 : 3) + NMM (2.2 equiv.).

Fluorescence assay – Cleavage of fluorogenic substrate Pep11 by trypsin
Peptide Pep11 was subjected to the brief “proof-of-concept” assay with trypsin. Enzyme trypsin is able to cleave peptide chains after polar amino acid residues (arginine and lysine). The peptide Pep11, contains the fluorophore and the quencher molecule in close proximity so that the fluorescence of the coumarin is quenched. Cleavage of the peptide Pep11 by trypsin after the remaining lysine residue yields two short peptide sequences where the two molecules (the dye and the quencher) are apart. As consequence the fluorescence of the free coumarin can be observed and detected.
Conditions:
The fluorescence assay of modified peptide Pep11 cleaved by Trypsin (Trypsin Gold, Promega) was performed at 37°C in 96-well non-treated flat bottom half area black polystyrene plates (Corning). The conditions were as follows: 50 mM HEPES, pH 7.4, 150 mM NaCl, 5 % v/v DMSO, 0.02 % (w/v; corresponds to enzyme : substrate ratio 1:100) or 0.01 % (w/v; corresponds to enzyme : substrate ratio 1:200) trypsin, 10 μM fluorogenic peptide substrate in a final volume of 50 μl. Fluorescence was read continuously in a plate reader (Tecan Spark®). Excitation and emission wavelegths were 404 and 477 nm. Controls: same conditions. Negative controls: no enzyme. Positive controls: fluorogenic substrate was surrogated with the mixture of 18 and 19 (1:1).

Scheme S10: Trypsine cleavage of fluorogenic peptide AK-280

Conditions:
The fluorescence assay of modified peptide Pep11 cleaved by Trypsin (Trypsin Gold, Promega) was performed at 37°C in 96-well non-treated flat bottom half area black polystyrene plates (Corning). The conditions were as follows: 50 mM HEPES, pH 7.4, 150 mM NaCl, 5 % v/v DMSO, 0.02 % (w/v; corresponds to enzyme : substrate ratio 1:100) or 0.01 % (w/v; corresponds to enzyme : substrate ratio 1:200) trypsin, 10 μM fluorogenic peptide substrate in a final volume of 50 μl. Fluorescence was read continuously in a plate reader (Tecan Spark®). Excitation and emission wavelegths were 404 and 477 nm. Controls: same conditions. Negative controls: no enzyme. Positive controls: fluorogenic substrate was surrogated with the mixture of 18 and 19 (1:1).
Figure S11: Fluorescence readings of Pep11–trypsin assay. Concentration of fluorogenic substrate 10 μM, measured at 477 nm.
Copies of NMR spectra

Compound 4
Compound 8
Compound 16
Compound 5
Compound 6
Compound 11
Compound 9
Compound 10
Compound 1
Compound 2
Compound 3
Compound 12
Compound 15
Compound Glc-C4-N3
Compound Man-C2-N$_3$
Compound Gal-C3-N₃

[Chemical Structure Image]

Parameter Value
Solvent: DMSO
Temperature: 298.15K
Nucleus: 1H,

[1H NMR Spectrum Image]

Parameter Value
Solvent: DMSO
Temperature: 298.15K
Nucleus: 13C,

[13C NMR Spectrum Image]
Abbreviations:

ACN  Acetonitrile
Boc  tert-Butyloxycarbonyl
BTTP 3-[4-{[Bis[1-tert-butyl-1H,1,2,3-triazol-4-yl]methyl]amino}methyl]-1H,1,2,3-triazol-1-yl]propanol
Bu  Butyl
CuAAC  The Copper(I)-Catalyzed Azide Alkyne Cycloaddition
DCC  N,N′-Dicyclohexylcarbodiimide
DCM  Dichloromethane
DIC  N,N′-Diisopropylcarbodiimide
DIPEA  N,N-Diisopropylethylamine
DMAP  4-(Dimethylamino)pyridine
DMF  Dimethylformamide
DMSO  Dimethyl sulfoxide
EDTA  Ethylenediaminetetraacetic acid
Eq.  Equivalent
Fmoc  9-Fluorenylmethoxycarbonyl
HBTU  N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
HEPES  4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HPLC  High Pressure Liquid Chromatography
HRMS  High Resolution Mass Spectroscopy
iPr  Isopropyl
iPrOH  2-Propanol
LC-MS  Liquid Chromatography – Mass Spectroscopy
Me  Methyl
NHS  N-Hydroxysuccinimide
NMM  4-Methylmorpholine
NMR  Nuclear Magnetic Resonance
Pbf  2,2,4,6,7-Pentamethylidihydrobenzofuran-5-sulfonyl
PE  Petroleum
SPPS  Solid Phase Peptide Synthesis
TBAF  Tetra-butylammonium fluoride
t-Bu  Tert-Butyl
t-BuOH  2-Methyl-2-propanol
TES  Triethylsilyl
TFA  Trifluoroacetic acid
THF  Tetrahydrofuran
TIPS  Triisopropylsilyl
TIS  Triisopropylsilane
Trt  Trityl
References:
1. G. M. Fischer, C. Jungst, M. Isomaki-Krondahl, D. Gauss, H. M. Moller, E. Daltrozzo and A. Zumbusch, *Chem Commun*, 2010, **46**, 5289-5291.
2. B. Neises and W. Steglich, *Angewandte Chemie International Edition in English*, 1978, **17**, 522-524.
3. P. R. Werkhoven, M. Elwakiel, T. J. Meuleman, H. C. Q. van Ufford, J. A. W. Kruijtzer and R. M. J. Liskamp, *Org Biomol Chem*, 2016, **14**, 701-710.
4. S. Combemale, J. N. Assam-Evoung, S. Houaidji, R. Bibi and V. Barragan-Montero, *Molecules*, 2014, **19**, 1120-1149.
5. M. Malkoch, K. Schleicher, E. Drockenmuller, C. J. Hawker, T. P. Russell, P. Wu and V. V. Fokin, *Macromolecules*, 2005, **38**, 3663-3678.
6. A. Horatscheck, S. Wagner, J. Ortwein, B. G. Kim, M. Lisurek, S. Beligny, A. Schutz and J. Rademann, *Angew. Chem. Int. Ed. Engl.*, 2012, **51**, 9441-9447.
7. K. Shinoda, Y. Sohma and M. Kanai, *Bioorg Med Chem Lett*, 2015, **25**, 2976-2979.
8. J. Dahmen, T. Frejd, G. Gronberg, T. Lave, G. Magnusson and G. Noori, *Carbohyd Res*, 1983, **116**, 303-307.
9. V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin and D. M. Haddleton, *J Am Chem Soc*, 2006, **128**, 4823-4830.
10. S. Park and L. Shin, *Org Lett*, 2007, **9**, 1675-1678.
11. C. C. Lee, G. Grandinetti, P. M. McLendon and T. M. Reineke, *Macromol Biosci*, 2010, **10**, 585-598.
12. B. Ren, M. Y. Wang, J. Y. Liu, J. T. Ge, X. L. Zhang and H. Dong, *Green Chem*, 2015, **17**, 1390-1394.
13. E. Calatrava-Perez, S. A. Bright, S. Achermann, C. Moylan, M. O. Senge, E. B. Veale, D. C. Williams, T. Gunnlaugsson and E. M. Scanlan, *Chem Commun (Camb)*, 2016, **52**, 13086-13089.
14. M. Gude, J. Ryf and P. D. White, *Lett Pept Sci*, 2002, **9**, 203-206.
15. I. Coin, M. Beyermann and M. Bienert, *Nat Protoc*, 2007, **2**, 3247-3256.
16. I. E. Valverde, A. F. Delmas and V. Aucagne, *Tetrahedron*, 2009, **65**, 7597-7602.