Electronic Supplementary Material (ESI) for ChemComm.

Glycan Shields for Penetrating Peptides

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1. Materials, instrumentations and methods

Fmoc-Rink Amide AM resin, *N*-HBTU and Fmoc-*L*-Lys(Mtt)-OH were obtained from Iris Biotech GmbH. (Boc-aminooxy)acetic acid were purchased from TCI Chemicals. *N*-HATU was provided by Glentham life sciences. Fmoc-*L*-Leu-OH, Fmoc-*L*-Arg(Pbf)-OH, Fmoc-6Ahx-OH, Dulbecco′s Modified Eagle′s Medium, Fetal bovine serum, Thiazolyl Blue Tetrazolium Bromide (MTT), Lithium hydroxide and Heparin sodium salt were purchased from Sigma-Aldrich®. 5-Carboxytetramethlyrhodamine was purchased from Carbosynth. Hoechst 33342 Trihydrochloride Trihydrate was purchased in ThermoFisher. Methyl 3,5-diaminobenzoate was obtained from Alfa Aesar. Gibco™ Penicillin-Streptomycin-Glutamine was acquired from Fisher scientific. 96-well Glass Bottom Black Plate (P96-1.5H-N) was obtained from Cellvis. Deuterated solvents (D_2O, CD_3OD, $CDCl_3$) were from EMD Millipore Corporation. *N,N*-Dimethylformamide, for peptide synthesis, was purchased from Scharlau. All the other solvents were HPLC grade, purchased from Sigma-Aldrich® or Fisher Scientific® and used without further purification.

A JASCO HPLC with an Agilent Eclipse XDB-C18 column, 9.4x250 mm, was used for semi-preparative purification using gradient 95:5→5:95 (0→35 min) H_2O (0.1% TFA)/CH_3CN (0.1% TFA).

High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) analyses were carried out on Agilent Technologies 1260 Infinity II associated with a 6120 Quadrupole LC-MS using an Agilent SB-C18 column with gradient between 95:5→5:95 (0→35 min) H_2O (0.1% TFA)/CH_3CN (0.1% TFA). Accurate mass determinations (HRMS) using ESI-MS were performed on a Sciex QSTAR Pulsar mass spectrometer and are reported as mass-per-charge ratio (*m/z*).

Nuclear Magnetic Resonance (NMR) spectra were recorded on either a Varian Mercury 300 MHz or a Varian Inova 500 MHz spectrometer. Chemical shifts are reported in ppm (δ units) referenced to the following solvent signals: ^{1}H-RMN: D_2O 4.79, CD_3OD 3.31, $CDCl_3$ 7.26. ^{13}C-RMN: CD_3OD 49.0, $CDCl_3$ 77.2. Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t) doublet of doublet (dd) with coupling constants (*J*) given in Hz, or multiplet (m).

Calculation of the labelled peptides concentrations was performed by measuring the absorbance on a Biochrom Libra S60 UV-VIS Spectrophotometer.

Circular Dichroism (CD) measurements were performed with a Jasco J-1100 CD Spectrometer equipped with a Jasco MCB-100 Mini Circulation Bath.

Cells were maintained in an INCO 108 incubator (Memmert). For the acquisition of cell microscopy images a Dragonfly confocal spinning-disk mounted on a Nikon Eclipse Ti-E equipped with an Andor Sona sCMOS digital camera was used. Images were analyzed with Fiji.[S1]

For cell viability assays, an Infinite F200pro microplate reader (Tecan) was used.

A Guava EasyCyte™ cytometer (EMD Millipore) was used for all flow cytometry experiments and data analysed with InCyte software included in GuavaSoft 3.2 (Millipore) or with R[S2] 4.0.3 (packages CytoExploreR[S3] 1.0.8 and ggcyto[S4] 1.18.0).

Mouse model assays were performed with an IVIS Spectrum in vivo imaging system (Caliper Life Sciences, Alameda, USA) and quantified with Image software (Caliper Life Sciences).

2. Abbreviations

Peptide abbreviations: Tm**P**$(X)_Z$ where Tm = TAMRA, X = Man (*α*-D-Mannose), Glu (*β*-D-Glucose) or Gal (*β*-D-Galactose) and Z= 4 or 6. Aa: Amino acids; Boc: *tert*-Butoxycarbonyl; DCM: Dichloromethane; DIEA: *N,N*-Diisopropylethylamine; DMEM: Dulbecco's Modified Eagle Medium; DMF: *N,N*-Dimethylformamide; FBS: Fetal Bovine Serum; Fmoc-6Ahx-OH: *N*-Fmoc-6-aminohexanoic acid; Fmoc: 9-fluorenylmethoxycarbonyl; HEPES: 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethane-1-sulfonic acid; HFIP: 1,1,1,3,3,3-Hexafluoro-2-propanol; HKR: HEPES-Krebs-Ringer buffer (5 mM HEPES, 137 mM NaCl, 2.68 mM KCl, 2.05 mM $MgCl_2$, 1.8 mM $CaCl_2$, pH 7.4); Hz: Hertz; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Mtt: 4-Methyltrityl; Alkoxyamine: (Boc-aminooxy)acetic acid; *N*-HATU: N-[(Dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminiun hexafluorophosphate N-oxide; *N*-HBTU: N-[(1HBenzotriazol-1-yl)4-(dimethylamino)methylene]-N-methyl methanaminiumhexafluoro phosphate N-oxide; Pbf: 2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl; SPPS: Solid phase peptide synthesis; 5-TAMRA: 5-Carboxytetramethyl rhodamine; ^{t}Bu: *tert*-Butyl; TFA: Trifluoroacetic acid; TFE: Trifluoroethanol; TIS: Triisopropylsilane; TNBS: 2,4,6-Trinitrobenzenesulfonic acid; Trt: Trityl.

3. Di-alkoxyamine connector synthesis and characterization

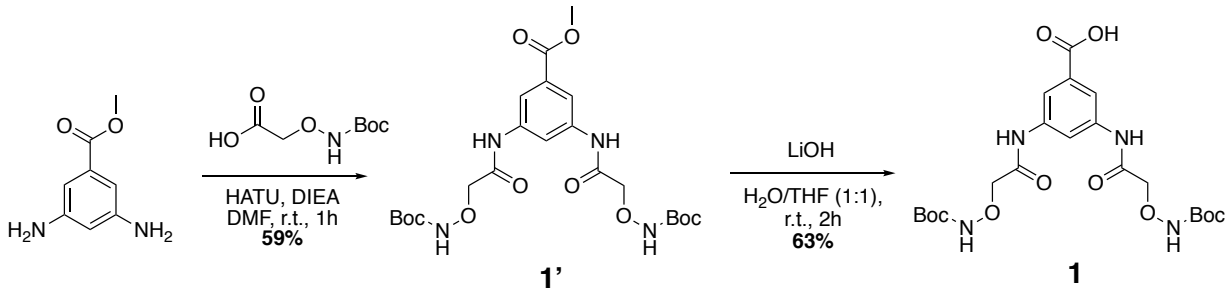

Figure S1. Synthetic scheme and reaction conditions for the synthesis of di-alkoxyamine (compound **1**).

3.1 Synthesis of methyl 3,5-di-((Boc-aminooxy)acetamido)benzoate (1')

Following the synthetic scheme of **Fig. S1**, the commercial starting reagent methyl 3,5-diaminobenzoate (100 mg, 0.6 mmol, 1 equiv) in DMF was added to a preactivated mixture of (Boc-aminooxy)acetic acid (287 mg, 1.5 mmol, 2.5 equiv), *N*-HATU (570 mg, 1.5 mmol, 2.5 equiv) and DIEA (257 µL, 1.5 mmol, 2.5 equiv) in DFM (10 mL) and stirred for 1 h. The DMF excess was then

removed by evaporation at the rotary evaporator and the oil obtained was dried under high vacuum. The crude was purified by silica gel column chromatography (40:60 AcOEt/Hexane) providing intermediate **1'**. Yield: 181 mg (59%). R_f = 0.68 (80:20 AcOEt:Hex). **^{1}H-NMR** (300 MHz, $CDCl_3$, δ): 10.29 (s, 2H), 8.37 (t, *J* = 2.0 Hz, 1H), 8.12 (m, 2H), 8.07 (d, *J* = 1.9 Hz, 2H), 4.46 (s, 4H), 3.89 (s, 3H), 1.50 (s, 18H). **^{13}C-NMR** (75 MHz, $CDCl_3$, δ): 167.5 (HN*C*O), 166.5 (*C*OO), 158.3 (HN*C*OO), 138.4 ($C_{q\text{-}3,5}$), 131.30 ($C_{q\text{-}1}$), 117.1 ($Csp^2{}_4$), 116.1 ($Csp^2{}_{2,5}$), 83.6($C(CH_3)_3$), 77.6 (CH_2) under $CDCl_3$, 52.2 (COO*C*H_3), 28.1 (C(*C*H_3)$_3$). **135-DEPT** (75 MHz, $CDCl_3$, δ): 117.1 ($Csp^2{}_4$), 116.1 ($Csp^2{}_{2,5}$), 76.5 (CH_2), 52.2 (COO*C*H_3), 28.1 (C(*C*H_3)$_3$). **HRMS** (ESI): Calculated for $C_{22}H_{32}N_4O_{10}Na$ ($[M+Na]^+$): 535.2011; found: 535.2010. **Fig. S2-S4.**

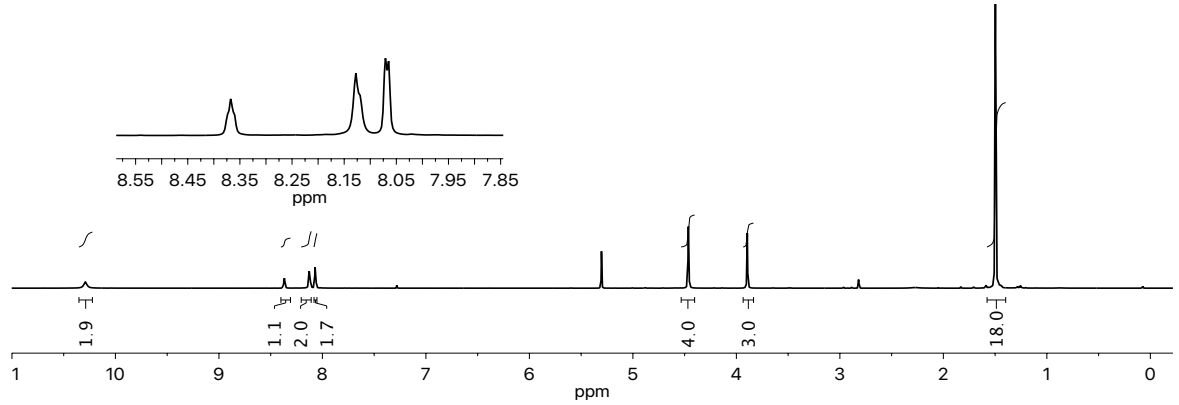

Figure S2. Intermediate **1'**: ^{1}H-NMR spectra in $CDCl_3$, solvent signals: residual peak of $CDCl_3$ at 7.26 ppm and DCM at 5.30 ppm.

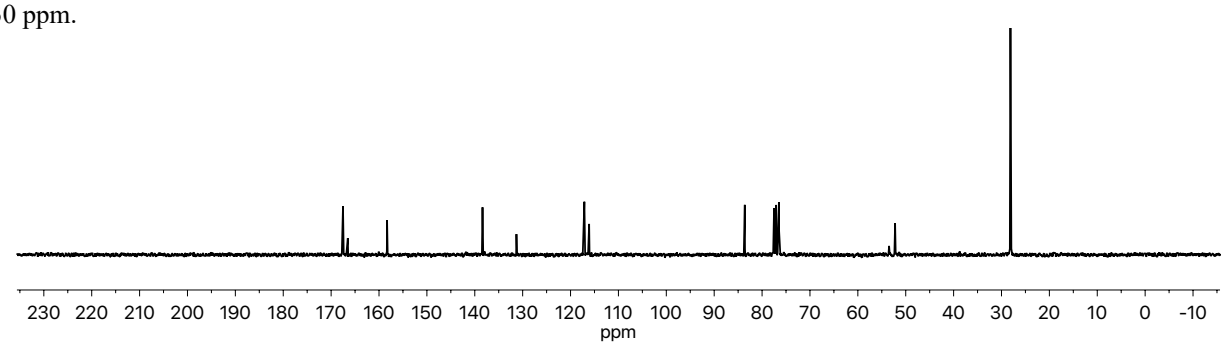

Figure S3. Intermediate **1'**: ^{13}C-NMR spectra in $CDCl_3$ solvent signals: residual peak of $CDCl_3$ at 77.1 ppm.

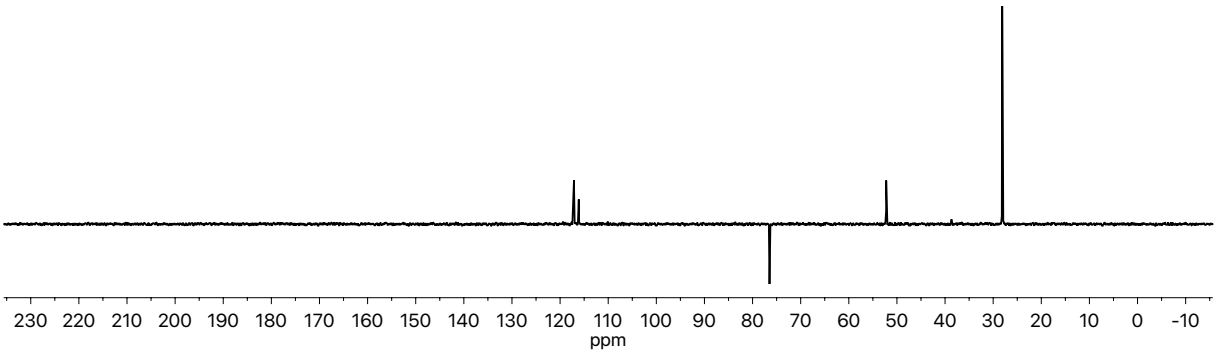

Figure S4. Intermediate **1'**: 135-DEPT spectra in $CDCl_3$, -CH_3 and -CH (↑), -CH_2 (↓).

3.2 Synthesis of 3,5-di-((Boc-aminooxy)acetamido)benzoic acid (1)

Following the synthetic scheme of **Fig. S1**, compound **1** was obtained after deprotection of the methyl ester of 180 mg of intermediate **1'** by treatment with LiOH (20 mg) in H_2O/THF (1:1, 10

mL). The reaction was stirred for 2 h at room temperature and the THF was evaporated by rotary evaporation. The remaining aqueous solution was acidified with HCl (5%) to pH=3-4 observing the appearance of a white precipitate corresponding to compound **1**. The final product was extracted to the organic phase with DCM (3x15 mL), dried with anhydrous $MgSO_4$, filtered, and concentrated at the rotary evaporator. Yield: 110 mg (63%). R_f = 0.25 (80:20 AcOEt:Hex). **^{1}H-NMR** (300 MHz, CD_3OD, δ): 10.60 (s, 1H), 8.40 (t, *J* = 1.8 Hz, 1H), 8.12 (d, *J* = 1.9 Hz, 2H), 4.43 (s, 4H), 1.52 (s, 18H). **^{13}C-NMR** (75 MHz, CD_3OD, δ): δ 172.5 (HN*C*O), 171.5 (*C*OOH), 162.8 (HN*C*OO), 142.4 ($C_{q\text{-}3,5}$), 135.8 ($C_{q\text{-}1}$), 120.7 ($Csp^2{}_4$), 119.3 ($Csp^2{}_{2,5}$), 86.0 (*C*(CH_3)$_3$), 79.5 (*C*H_2), 31.1 (C(*C*H_3)$_3$). **135-DEPT** (75 MHz, CD_3OD, δ) 117.8 ($Csp^2{}_4$), 115.9 ($Csp^2{}_{2,5}$), 76.4 (*C*H_2), 28.3 (C(*C*H_3)$_3$). **HRMS** (ESI): Calculated for $C_{21}H_{30}N_4O_{10}Na$ ([M+Na]$^+$): 521.1854; found: 521.1854. **Fig. S5-S7.**

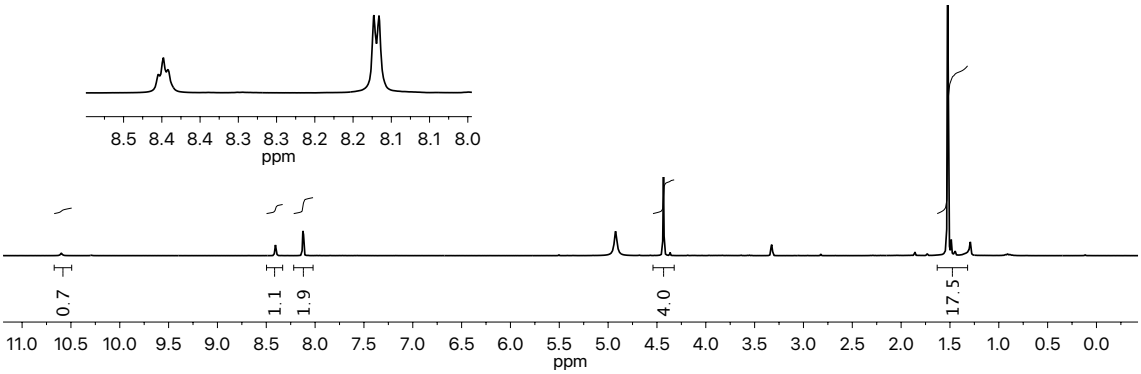

Figure S5. Compound **1**: ^{1}H-NMR spectra in CD_3OD, solvent signals: residual peak of CD_3OD at 3.31 ppm and H_2O signal at 4.87 ppm.

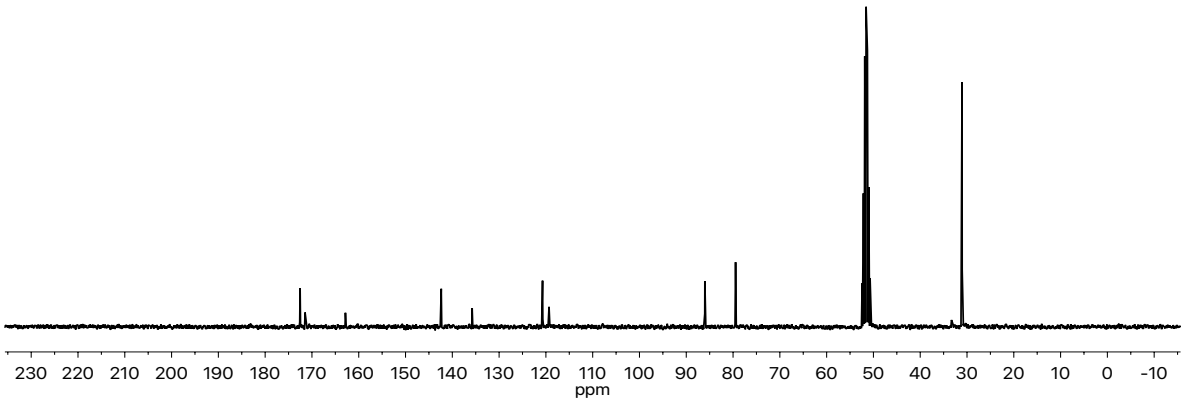

Figure S6. Compound **1**: ^{13}C-NMR spectra in CD_3OD, solvent signals: residual peak of CD_3OD at 52.0 ppm.

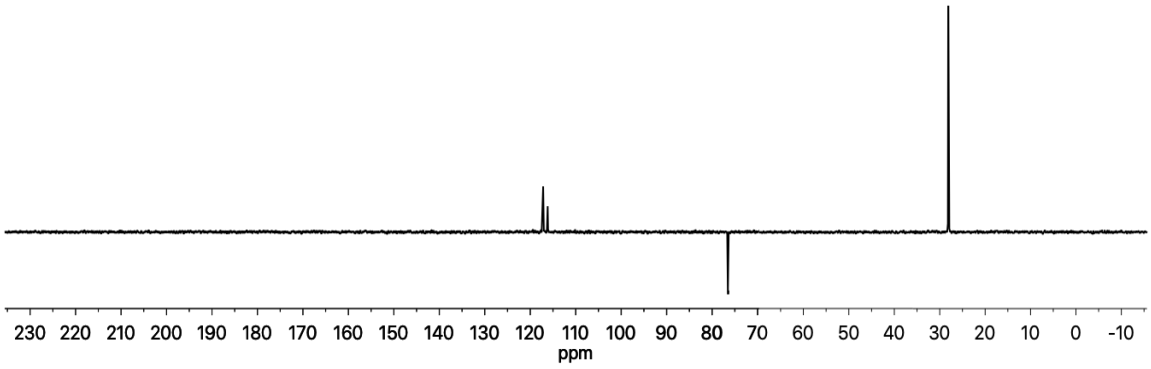

Figure S7. Compound **1**: 135-DEPT spectra in CD_3OD, -CH_3 and -CH (↑), -CH_2 (↓).

4. General protocols

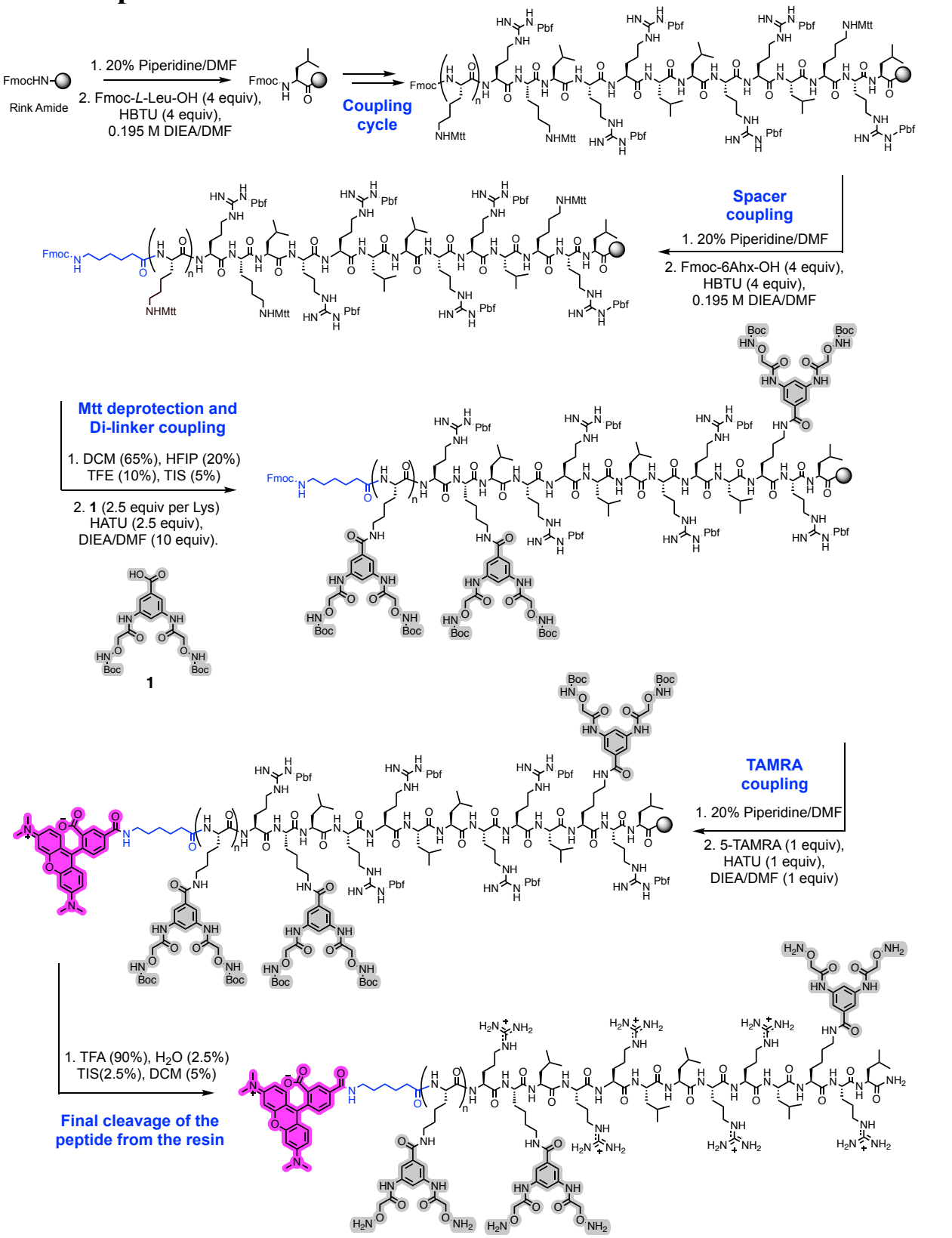

Figure S8. General synthetic scheme for solid phase synthesis of peptides: with two lysines (n = 0), peptide sequences: RKLRRLLRRLKRL, these peptides will be the precursors of the glycopeptides with formula Tm**P**$(X)_4$, or with three lysines (n = 1), peptide sequences: KRKLRRLLRRLKRL, these peptides will be the precursors of the glycopeptides with formula Tm**P**$(X)_6$.

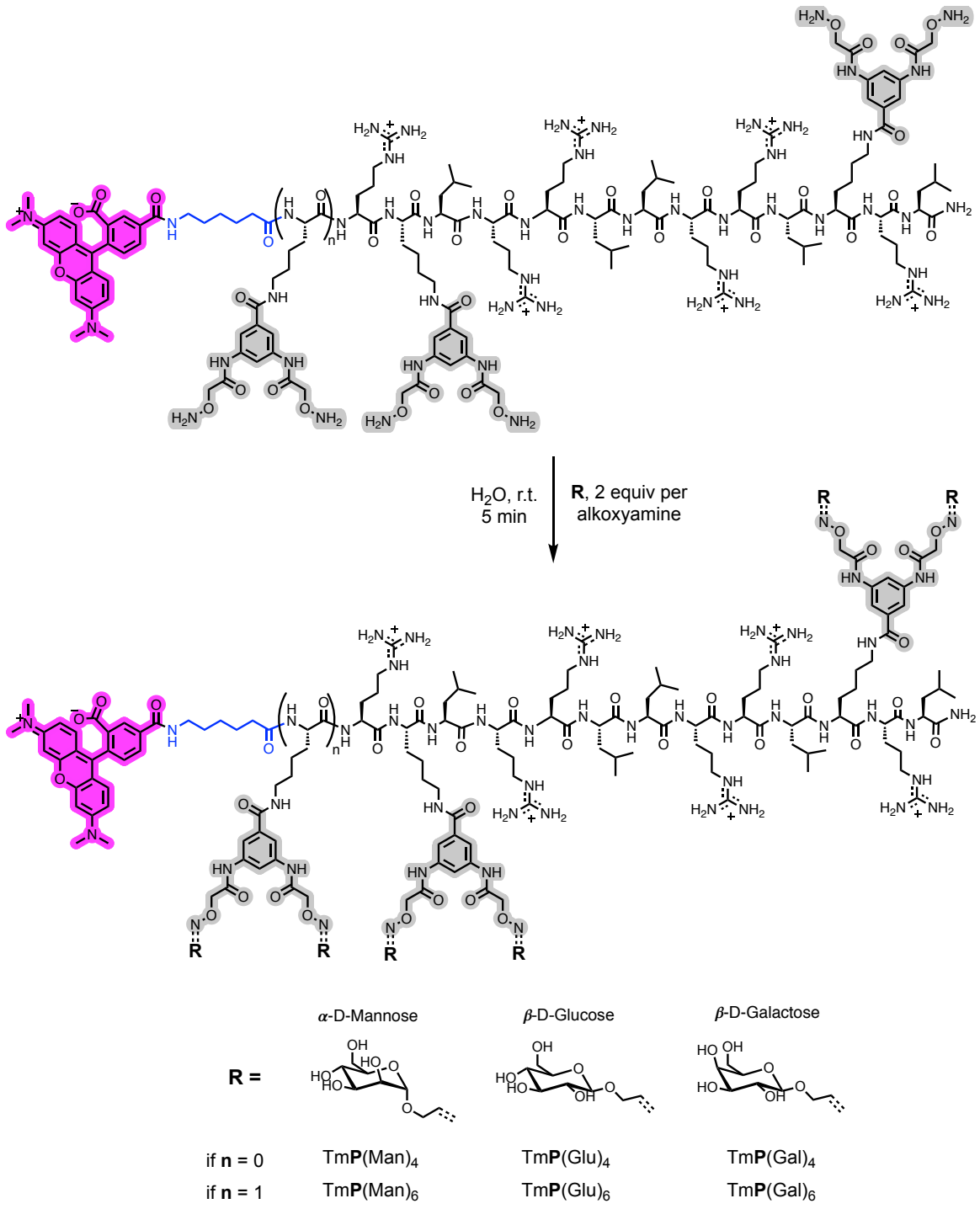

Figure S9. General synthetic scheme for the coupling of the carbohydrate ligands *α*-D-Mannose (Man), *β*-D-Glucose (Glu) and *β*-D-Galactose (Gal) to obtain the glycopeptides: if n = 0, Tm**P**(Man)$_4$, Tm**P**(Glu)$_4$, and Tm**P**(Gal)$_4$; if n= 1, Tm**P**(Man)$_6$, Tm**P**(Glu)$_6$, and Tm**P**(Gal)$_6$.

Amino acid coupling cycle: All peptides were synthesized via manual solid phase peptide synthesis[S5,S6] by the Fmoc method, using Fmoc-Rink Amide resin (0.19 mmol/g loading). 0.05 mmol of the resin were swollen in DMF (2 mL) for 20 min in a peptide synthesis vessel prior to synthesis. The coupling cycle consisted of removal of the Fmoc protecting group with a piperidine solution in DMF (20%, 2 mL) for 20 min; then, the mixture was filtered and the resin was washed with DMF (3x2 mL, 1 min). Amino acid coupling was performed by treatment with a solution of α-amino acids

(4 equiv), *N*-HBTU (3.95 equiv) in DMF (2 mL), which was mixed with DIEA (0.195 M in DMF, 1.2 equiv) 1 min before addition; the resulting mixture was stirred by Ar bubbling for 20 min. Finally, the resin was washed with DMF (3x2 mL, 1 min). The efficiency of each amino acid coupling and deprotection was monitored using the TNBS test.

Peptide termination protocol: Upon completion of the linear peptide sequence, "spacer coupling" was used as the termination protocol. After the final removal of the Fmoc group with piperidine/DMF (20%, 2 mL), the linear peptide was treated with a solution of *N*-Fmoc-6-aminohexanoic acid (4 equiv), *N*-HBTU (3.95 equiv), and DIEA (0.195 M in DMF, 1.2 equiv) in DMF.

General protocol for the incorporation of the di-alkoxyamine connector: The resin was washed with DCM (3x2 mL, 5 min), and the Mtt protecting group was selectively removed by mechanical agitation of the resin with a mixture of DCM/HFIP/TFE/TIS (6.5:2:1:0.5, 3x2 mL, 2 h). Finally, the mixture was filtered, and the resin was washed with DCM (3x2 mL, 2 min) and DMF (3x2 mL, 20 min). Then, a pre-activated solution of the linker di-alkoxyamine (Compound **1**, 2.5 equiv per free amine) and *N*-HATU (2.5 equiv per free amine) and DIEA (10 equiv) in DMF (2 mL) was added to the resin. The resin was stirred by Ar bubbling for 40 min and finally washed with DMF (3x2 mL, 2 min) and DCM (3x2 mL, 2 min).

General protocol for fluorescent labelling of the peptides: the Fmoc protecting group of the previously attached “spacer” was removed using a solution of piperidine in DMF (20%, 2 mL) for 20 min and the resin was washed with DMF (3x2 mL). Coupling was carried out by adding a solution of 5-TAMRA (1 equiv), *N*-HATU (1 equiv) and DIEA (0.195 M, 1 equiv) in DMF (2 mL) and the mixture was stirred with Ar bubbles for 2 h. Finally, the resin was washed with DMF (3x3 mL) and DCM (3x3 mL).

General protocol for peptide cleavage: the peptides were deprotected and separated from the resin by the standard procedure in TFA at room temperature using TFA/DCM/H_2O/TIS (90:5:2.5:2.5, 3 mL) for 2 h. Subsequently, the mixture was filtered, washed with TFA (1 mL) and the peptide was precipitated with Et_2O (25 mL). The precipitate was centrifuged and dissolved in H_2O (3-5 mL). The peptide structure was then purified by reverse phase HPLC [H_2O (0.1% TFA)/CH_3CN (0.1% TFA), 95:5→5:95 (0→35 min)]; the collected fractions were concentrated and treated with different carbohydrate ligands.

General protocol for the coupling of carbohydrate ligands: A solution of the peptide in H_2O (5 mM) was reacted with a solution of the corresponding aldehyde ligands[S6,S7] (2 equiv per alkoxyamine) in H_2O (120 mM) for 5 min. The peptides were then purified by reversed phase HPLC to remove excess ligand. Purification was carried out on a reverse phase HPLC [H_2O (0.1% TFA)/CH_3CN (0.1% TFA), 95:5→5:95 (0→35 min)]; the collected fractions were concentrated, freeze-dried and stored at -20°C. Purity and identity were confirmed by HPLC, ^{1}H-NMR, MS and HRMS.

5. Peptide synthesis

5.1 TmP(Glu)$_4$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide with four *β*-D-glucoses, Tm**P**(Glu)$_4$ was obtained after RP-HPLC purification [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 8% (14 mg) and 99% purity. It was characterized on a HPCL-MS [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 7.44 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.28-8.19 (m, 1H), 7.99-7.93 (m,1H), 7.84-7.75 (m,1H), 7.76-7.66 (m, 4H), 7.57-7.49 (m, 2H), 7.46-7.41 (m, 1H), 7.43-7.35 (m, 1H), 7.17-7.09 (m, 1H), 7.13-7.06 (m, 1H), 7.08-7.00 (m, 2H), 6.79-6.67 (m, 1H), 6.59-6.52 (m, 4H), 4.39-4.01 (m, 13H), 3.86–3.76 (m, 8H), 3.65 (s, 8H), 3.55-3.19 (m, 24H), 3.13 (s, 12H), 3.10-2.99 (m, 18H), 2.32 (t, *J* = 7.4 Hz 2H), 1.81-1.14 (m, 57H), 0.88-0.72 (m, 30H). **MS** (ESI, H_2O): 1954.1 (7, $[M+2H+2TFA]^{+2}$), 1303.6 (55, $[M+3H+2TFA]^{+3}$), 1265.1 (100, $[M+3H+TFA]^{+3}$), 1226.7 (21, $[M+3H]^{+3}$), 949.2 (95, $[M+4H+TFA]^{+4}$), 920.7 (20, $[M+4H]^{+4}$), 736.7 (25, $[M+5H]^{+5}$). **HRMS** (ESI): Calculated for $C_{163}H_{260}N_{45}O_{52}$ ($[M+3H]^{+3}$): 1226.6356; found: 1226.6355. **Fig. S15.**

5.2 TmP(Gal)$_4$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide with four *β*-D-galactoses, Tm**P**(Gal)$_4$ was obtained after RP-HPLC purification [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 9% (16 mg) and 98% purity. It was characterized on a HPLC-MS [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 7.50 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.28-8.21 (m, 1H), 7.99-7.91 (m, 1H), 7.83-7.76 (m, 1H), 7.74-7.66 (m, 4H), 7.54-7.45 (m, 1H), 7.49-7.38 (m, 1H), 7.43-7.37 (m, 1H), 7.13-7.10 (m, 1H), 7.11-7.08 (m, 1H), 7.08-6.95 (m, 2H), 6.78-6.61 (m, 1H), 6.60-6.49 (m, 4H), 4.46-3.94 (m, 13H), 3.89-3.71 (m, 8H), 3.64 (s, 8H), 3.54-3.19 (m, 24H), 3.12 (s, 12H), 3.11-2.89 (m, 18H), 2.37-2.29 (m, 2H), 1.92-1.13 (m, 57H), 0.91-0.70 (m, 30H). **MS** (ESI, H_2O): 1303.3 (50, $[M+3H+2TFA]^{+3}$), 1265.3 (78, $[M+3H+TFA]^{+3}$), 1226.9 (32, $[M+3H]^{+3}$), 949.2 (100, $[M+4H+TFA]^{+4}$), 920.2 (33, $[M+4H]^{+4}$), 736.5 (40, $[M+5H]^{+5}$). **HRMS** (ESI): Calculated for $C_{163}H_{260}N_{45}O_{52}$: ($[M+3H]^{+3}$): 1226.6356; found: 1226.6353. **Fig. S16.**

5.3 TmP(Man)$_4$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide with four *α*-D-mannoses, Tm**P**(Man)$_4$ was obtained after RP-HPLC purification [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 6% (11 mg) and 99% purity. It was characterized on a HPLC-MS [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 7.46 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.25-8.17 (m, 1H), 7.94 (dd, *J* = 7.4, 1.3 Hz, 1H), 7.77 (s, 1H), 7.72-7.64 (m, 4H), 7.54-7.43 (m, 1H), 7.45-7.36 (m, 1H), 7.38-7.34 (m, 1H), 7.13-7.06 (m, 1H), 7.11-7.04 (m, 1H), 7.06-6.98 (m, 2H), 6.77-6.67 (m, 1H), 6.58-6.49 (m, 4H), 4.46-3.97 (m, 13H), 3.89-3.68 (m, 8H), 3.62 (s, 8H), 3.51-3.16 (m, 24H), 3.11 (s, 6H), 3.09 (s, 6H), 3.07-2.97 (m, 18H), 2.31 (t, *J* = 7.7 Hz, 2H), 1.81-1.18 (m, 57H), 0.86-0.68 (m, 30H). **MS** (ESI, H_2O): 1303.2 (78

$[M+3H+2TFA]^{+3}$), 1265.1 (100, $[M+3H+TFA]^{+3}$), 1227.3 (29, $[M+3H]^{+3}$), 949.0 (86, $[M+4H+TFA]^{+4}$), 920.6 (31, $[M+4H]^{+4}$), 736.8 (20, $[M+5H]^{+5}$). **HRMS** (ESI): Calculated for $C_{163}H_{260}N_{45}O_{52}$ ($[M+3H]^{+3}$): 1226.6356; found: 1226.6353 ($[M+3H]^{+3}$). **Fig. S17.**

5.4 TmP(Glu)$_6$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide with six *β*-D-glucoses, Tm**P**$(Glu)_6$ was obtained after RP-HPLC purification [H_2O (0.1% TFA)/ CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 7% (16 mg) and 99% purity. It was characterized on a HPLC-MS [H_2O (0.1% TFA)/ CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 7.40 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.33-8.26 (m, 1H), 8.08-7.94 (m, 1H), 7.80-7.74 (m, 1H), 7.74-7.69 (m, 6H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.34-7.26 (m, 1H), 7.12-7.08 (m, 2H), 7.09-7.06 (m, 1H), 7.04-6.93 (m, 3H), 6.72-6.57 (m, 1H), 6.49-6.39 (m, 6H), 4.34-4.06 (m, 14H), 3.95-3.86 (m, 12H), 3.81-3.53 (m, 36H), 3.11 (s, 12H), 3.09-3.04 (m, 20H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.88-1.09 (m, 63H), 0.90-0.71 (m, 30H). **MS** (ESI, H_2O): 1575.7 (32, $[M+3H+2TFA]^{+3}$), 1537.6 (47, $[M+3H+TFA]^{+3}$), 1499.2 (17, $[M+3H]^{+3}$), 1182.0 (21, $[M+4H+2TFA]^{+4}$), 1153.3 (100, $[M+4H+TFA]^{+4}$), 1124.8 (58, $[M+4H]^{+4}$), 922.8 (25, $[M+5H+TFA]^{+5}$), 900.0 (20, $[M+5H]^{+5}$). **HRMS** (ESI): Calculated for $C_{196}H_{309}N_{51}O_{70}$ ($[M+4H]^{+4}$): 1124.3041; found: 1124.3040. **Fig. S18.**

5.5 TmP(Gal)$_6$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide with six *β*-D-galactoses, Tm**P**$(Gal)_6$ was obtained after RP-HPLC purification [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 6% (13 mg) and 99% purity. It was characterized on a HPLC-MS [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 7.59 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.33-8.26 (m, 1H), 8.00-7.95 (m, 1H), 7.80-7.74 (m, 1H), 7.74-7.67 (m, 6H), 7.58-7.49 (m, 1H), 7.44-7.37 (m, 2H), 7.35-7.30 (m, 1H), 7.12-7.05 (m, 3H), 7.01-6.90 (m, 1H), 6.65-6.55 (m, 1H), 6.49-6.39 (m, 6H), 4.32-4.00 (m, 14H), 3.96-3.83 (m, 12H), 3.84-3.51 (m, 36H), 3.12 (s, 12H), 3.09-3.00 (m, 20H), 2.37-2.28 (m, 2H), 1.88-1.16 (m, 63H), 0.90-0.67 (m, 30H). **MS** (ESI, H_2O): 1575.8 (32, $[M+3H+2TFA]^{+3}$), 1537.3 (43, $[M+3H+TFA]^{+3}$), 1499.8 (14, $[M+3H]^{+3}$), 1182.0 (22, $[M+4H+2TFA]^{+4}$), 1153.2 (100, $[M+4H+TFA]^{+4}$), 1124.6 (70 $[M+4H]^{+4}$), 922.3 (43, $[M+5H+TFA]^{+5}$), 900.6 (14, $[M+5H]^{+5}$). **HRMS** (ESI): Calculated for $C_{196}H_{309}N_{51}O_{70}$ ($[M+4H]^{+4}$): 1124.3041; found: 1124.3039. **Fig. S19.**

5.6 TmP(Man)$_6$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide with six *α*-D-mannoses, Tm**P**$(Man)_6$ was obtained after RP-HPLC purification [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 5% (11 mg) and 99% purity. It was characterized on a HPLC-MS [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 7.36 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.31-8.19 (m, 1H), 8.06-7.93 (m, 1H), 7.79-7.73 (m, 1H), 7.72-7.66 (m, 6H), 7.53-7.49 (m, 1H), 7.37-7.33 (m, 1H), 7.32-7.26 (m, 1H), 7.10-7.05 (m, 3H), 7.01-6.89 (m, 1H), 6.64-6.53 (m, 1H), 6.48-6.37 (m, 6H), 4.36-3.97 (m, 14H), 3.96-3.82 (m, 12H),

3.81-3.49 (m, 36H), 3.10 (s, 12H), 3.08-3.02 (m, 20H), 2.31 (t, J = 7.7 Hz, 2H), 1.87-1.09 (m, 63H), 0.93-0.64 (m, 30H). **MS** (ESI, H_2O): 1575.7 (26, $[M+3H+2TFA]^{+3}$), 1537.5 (52, $[M+3H+TFA]^{+3}$), 1499.9 (24, $[M+3H]^{+3}$), 1181.8 (23, $[M+4H+2TFA]^{+4}$), 1153.3 (100, $[M+4H+TFA]^{+4}$), 1125.1 (41, $[M+4H]^{+4}$), 922.9 (25, $[M+5H+TFA]^{+5}$). **HRMS** (ESI): Calculated for $C_{196}H_{309}N_{51}O_{70}$ ($[M+4H]^{+4}$): 1124.3041; found: 1124.3040. **Fig. S20.**

5.7 TmR$_8$

Following the general protocol of the SPPS for synthesizing a 5-TAMRA labelled peptide, Tm**R$_8$** was obtained after RP-HPLC purification [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→35 min)] with an overall yield of 17% (15 mg) and 99% purity. It was characterized on a HPLC-MS [H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min)]. t_R 5.96 min. **^{1}H-NMR** (500 MHz, D_2O, δ): 8.17 (d, J = 1.9 Hz, 1H), 7.97 (dd, J = 8.5, 1.9 Hz, 1H), 7.45 (d, J = 8.5 Hz, 1H), 7.27 (d, J = 8.3 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 6.94 (dd, J = 8.8, 2.4 Hz, 1H), 6.89 (d, J = 2.5 Hz, 1H), 6,88 (d, J = 2.4 Hz, 1H), 6.83 (dd, J = 8.3, 2.4 Hz, 1H), 4.28-3.97 (m, 4H), 3.52-3.36 (m, 2H), 3.21 (s, 12H), 3.16-2.97 (m, 20H), 2.30 (t, J = 7.3 Hz, 2H), 1.81-1.43 (m, 38H). **MS** (ESI): 1124.7 (10, $[M+2H+4TFA]^{+2}$), 1067.9 (18, $[M+2H+3TFA]^{+2}$), 1011.0 (15, $[M+2H+2TFA]^{+2}$), 712.3 (37, $[M+3H+3TFA]^{+3}$), 674.2 (100, $[M+3H+2TFA]^{+3}$), 636.3 (95, $[M+3H+TFA]^{+3}$), 598.2 (38, $[M+3H]^{+3}$), 534.5 (25, $[M+4H+3TFA]^{+4}$), 506.0 (37, $[M+4H+2TFA]^{+4}$), 477.5 (48, $[M+4H+TFA]^{+4}$), 449.0 (62, $[M+4H]^{+4}$), 382.3 (12, $[M+5H+TFA]^{+5}$), 359.5 (30, $[M+5H]^{+5}$). **HRMS** (ESI): Calculated for $C_{79}H_{133}N_{36}O_{13}$ ($[M+3H]^{+3}$): 598.0279; found: 598.0277. **Fig. S21.**

6. General procedure for circular dichroism

Circular dichroism measurements were carried out using the following settings: acquisition range: 300-190 nm; bandwidth: 1.0 nm; accumulation: 3 scans; data pitch: 1 nm; CD scale: 200 mdeg/1.0 dOD; D.I.T. (Data Integration Time): 1s; scanning mode: continuous; scanning speed: 200 nm/min. Measurements were done from 10ºC to 60ºC (data interval: 10ºC; temp. gradient: 5ºC/min) in a quartz cell of 0.2 cm path length at a final volume of 0.5 mL (HKR buffer or TFE) with a final peptide concentration of 50 μM.

Results are expressed as the mean residue molar ellipticity $[\theta]_{MRt}$ with units of degrees·cm^2·dmol^{-1} and calculated using the **eq. 1**, where θ is the ellipticity (deg), C is the peptide concentration (M) and l is the cell path length (cm).[S8,S9]

$$[\theta]_{MRt} = \frac{100 \cdot \theta}{C \cdot l \cdot N^o of\ residues} \qquad [1]$$

To calculate the percent of helicity (%[θ]), we use **eq. 2**, in which the molar ellipticity at 222 nm is an absolute value.[S10]

$$\%[\theta] = \left(\frac{|[\theta]_{MRt(222)}| - 2340}{30300}\right) \cdot 100 \qquad [2]$$

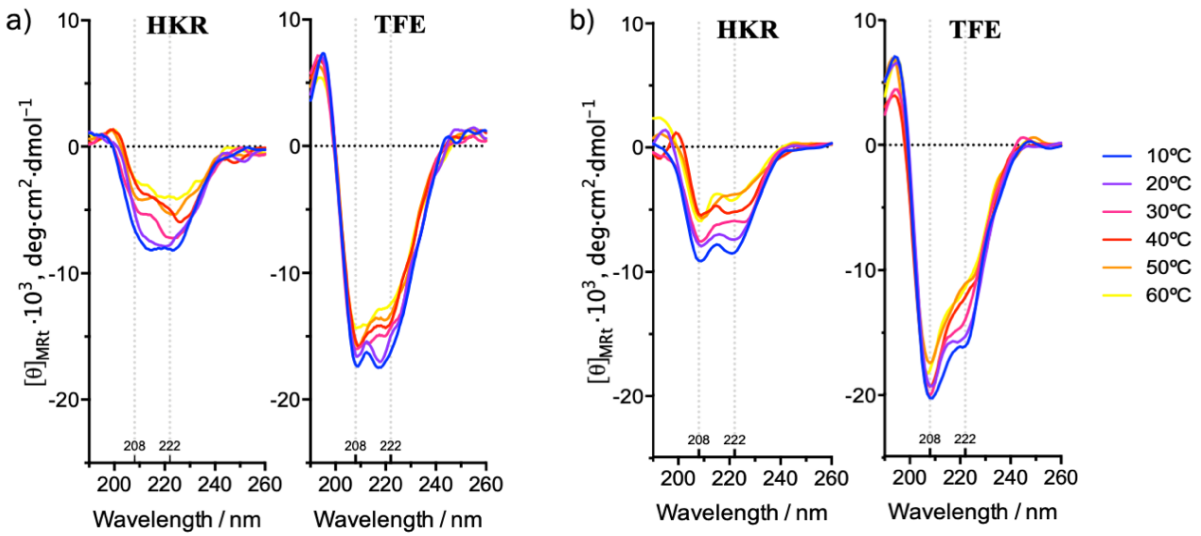

Figure S10. CD spectra of a) Tm**P**(Glu)$_4$ and b) Tm**P**(Glu)$_6$ under different temperatures in HKR buffer (pH 7.4) and TFE. CD results in percent helicity at 20°C in HKR buffer for Tm**P**(Glu)$_4$: 17.9% and Tm**P**(Glu)$_6$: 20.7%, and in TFE for Tm**P**(Glu)$_4$: 41.7% and Tm**P**(Glu)$_6$: 46.9%.

7. Cells lines and culture

HeLa cells were incubated at 37ºC, 5% CO_2, 95% humidity in the incubator with Dulbecco's Modified Eagle's Medium (DMEM, 4500 mg/L glucose, L-glutamine, sodium pyruvate and sodium bicarbonate), supplemented with 10% fetal bovine serum and 1% of Penicillin-Streptomycin-Glutamine Mix. For microscopy studies, cells were grown on four chamber glass bottom dishes and washed with HKR buffer and nuclei were stained by incubation with 1μM Hoechst 33342 in HKR for 30 min. The peptide was diluted in HKR and concentration determined by UV-Vis absorbance (λ = 555, ε = 91,000 $M^{-1}cm^{-1}$). Cells were incubated with the peptide for 30 min and then washed to remove excess peptide using a heparin-containing solution (1x500 μL, 0.1 mg/ml heparin in HKR buffer) and HKR buffer (2x500 μL) before being observed at the microscope.

8. Cell viability: MTT assay

Cell viability was established by a standard MTT assay. One day before the assay, a suspension of HeLa cells was plated in 96-well tissue culture plates (Costar 96 Flat Bottom Transparent Polystyrol) by adding 100 μL (150,000 cells/mL) per well. The next day, the medium was aspirated and cells were incubated with different concentrations of peptide diluted in HKR (50 μL/well). After 1 hour of incubation at 37ºC, the medium was aspirated and cells were washed with heparin solution (1x100 μL, 0.1 mg/ml heparin in HKR) and HKR buffer (2x100 μL/well). Then, fresh medium (DMEM) containing 10% FBS (100 μL) was added to the cells during 3h. Controls (100% and 0% viability) were performed with only cell culture medium (100 μL final medium) or medium supplemented with 1% Triton X-100, respectively. The viability was measured by quantifying the cellular ability to reduce the water-soluble tetrazolium dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) to its insoluble formazan salt as follows. MTT (5 mg/mL in PBS, 10 μL/well) was added to the wells containing 100 μL medium, and the cells were further incubated for 4 h. The

supernatant was carefully removed and the water-insoluble formazan salt was dissolved in DMSO (100 µL/well). The absorbance was measured at 570 nm using a microplate reader.

MTT assays were performed in duplicate with all glycopeptides and with control peptides Tm**R**$_8$ and Tm**P**(Acetone)$_2$ performing three replicates for each concentration in each of the experiments. Data points were expressed as normalized values for untreated control cells (100% and 0%) and were presented as the average percentage of three replicates ± standard deviation.

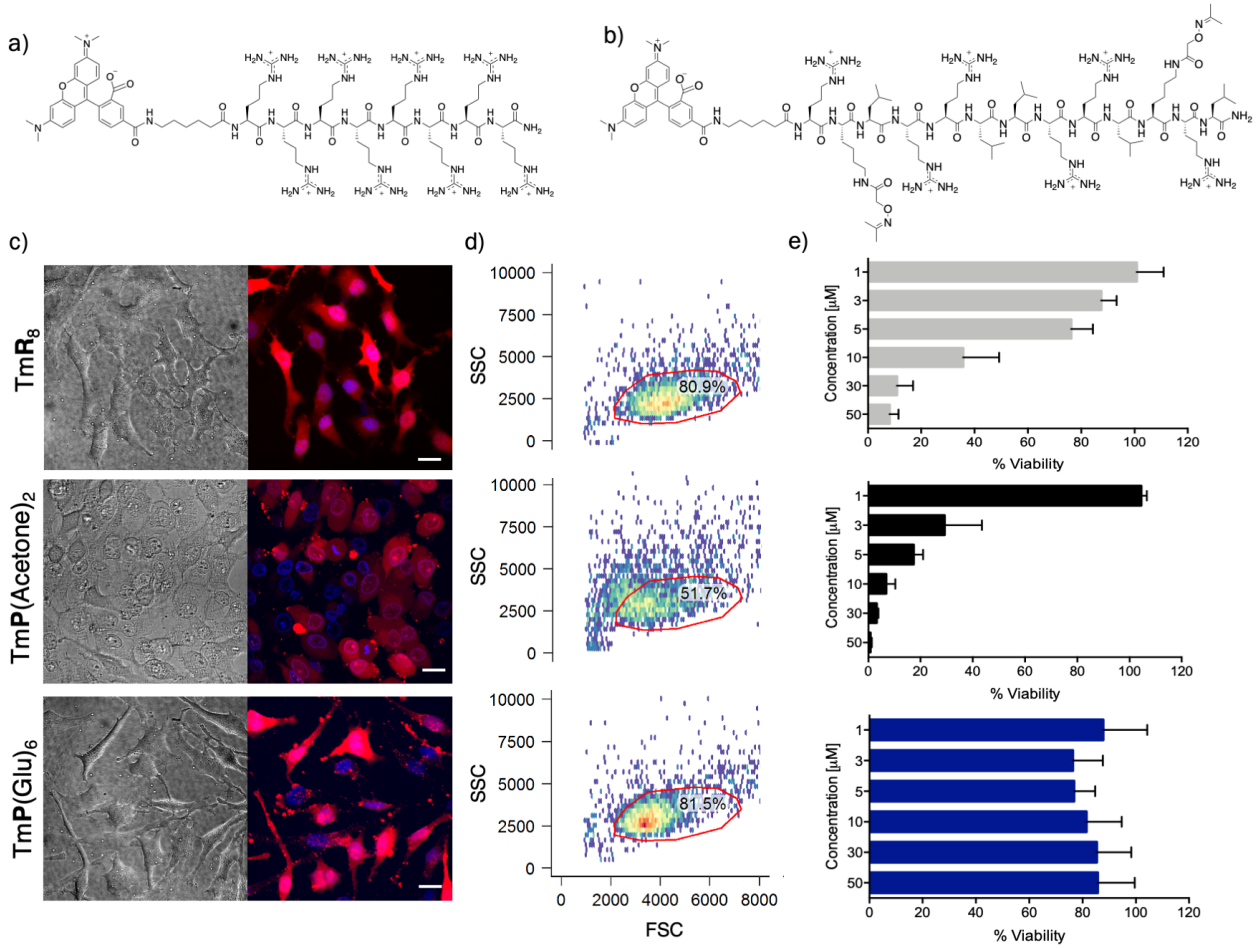

Figure S11. Structure of control peptides a) Tm**R**$_8$ and b) Tm**P**(Acetone)$_2$[S6]. c) Brightfield (left) and confocal (right) microscopy images of the control peptides and glycopeptide Tm**P**(Glu)$_6$ incubated at 5 µM for 30 min at 37ºC in HeLa cells. Chromatin condensation, suggestive of early stage apoptosis, can be observed in most of the cells treated with the Tm**P**(Acetone)$_2$ peptide. TAMRA fluorescence is shown in red, nuclei were counterstained with Hoechst in blue; scale bars: 25 µm. d) Forward versus side scatter plot of HeLa cells incubated with 5 µM of each of the peptides and analyzed by flow cytometry. Cells incubated with Tm**P**(Acetone)$_2$ show a change of morphology characterized by lower FSC and higher SSC, also associated with early stage apoptosis.[S11] e) Cellular viability determined by MTT assay at different concentrations (1, 3, 5, 10, 30 and 50 µM) in HeLa cells. Panels (c) and (e) of Tm**R**$_8$ and Tm**P**(Glu)$_6$ also form part of Fig. 2.

9. General protocol for flow cytometry

One day before the assay, HeLa cells were plated in 96-well tissue culture plates (Costar 96 Flat Bottom Transparent Polystyrol) by adding 100 µL (150,000 cells/mL) per well. The next day, the medium was aspirated, and cells were incubated with different concentrations of peptide (50

µL/well) in HKR buffer. After 30 min of incubation at 37ºC, the buffer was aspirated; cells were washed with heparin solution (1x100 µL, 0.1 mg/mL) and HKR buffer (2x100 µL). Finally, 100 µL of trypsin-EDTA was added in each well and cells were incubated for 15 min at 37ºC. After this, 100 µL of a solution of 2% FBS and 5 mM EDTA in PBS were added to the cells to neutralize the trypsin. Then, 2,500 cells from each well were analyzed in the cytometer. The median fluorescence intensity of the Yellow-G channel (λ_{ex} = 532 nm, λ_{em} = 575/25 nm) was determined for the population of cells with normal FSC/SSC parameters. Data are presented as the average of the median fluorescence intensity of three replicates ± standard deviation.

10. *In vivo* experiments in mouse models

Animal experiments were performed at the CEBEGA (USC) in accordance with the European Communities Council Directive 2010/63/EU and the Spanish Royal Decree 53/2013, on the protection of animals used for experimental and other scientific purposes and were approved by USC Ethical Committee and the competent authority (Xunta de Galicia). The biodistribution of Tm**P**$(X)_6$ compounds, where X = Glu, Gal, Man, was determined in 7-week-old female ICR (Swiss) mice (CEBEGA, USC, Santiago de Compostela, Spain) using an IVIS Spectrum "*in vivo"* imaging system (Caliper Life Sciences, Alameda, USA). Animals were anaesthetized with isofluorane (2.5%) using an inhalation anesthesia machine. 50 µg of each glycopeptide in PBS were administered to a total of three mice by intravenous injection in a total volume of 50 µL and one control mouse was injected with the same volume of PBS. *In vivo* images (λ_{ex} = 535 nm; λ_{em} = 580 nm) were acquired at 1 h post-injection and then at endpoint (3h) the animals were sacrificed for necropsy and *ex vivo* study of the following organs: lungs, heart, liver, kidneys and spleen. Glycopeptide fluorescence was quantified through an intensity map obtained using Image software (Caliper Life Sciences). The software uses a colour-based scale to represent the intensity of each pixel from red (representing low intensity) to yellow (representing high intensity).

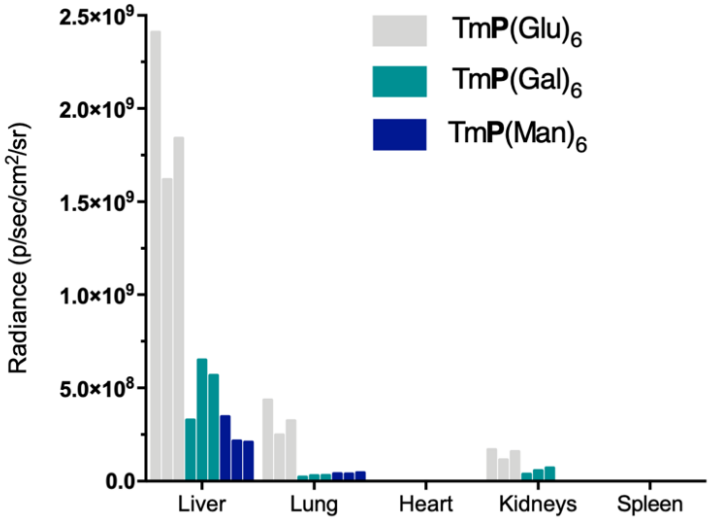

Figure S12. Quantification of the amount of each glycopeptide in the different organs studied. *Ex vivo* fluorescence quantification of three mice 3 h after i.v. administration of 50 µg in PBS of Tm**P**$(X)_6$ glycopeptides (X = Glu, Gal or Man).

	PBS	Tm**P**$(Glu)_6$			PBS	Tm**P**$(Gal)_6$			PBS	Tm**P**$(Man)_6$		
White blood cells (10^9/L) [0.8-10.6]	3.47 ±0.02	5.89 ±0.20	5.08 ±0.19	4.42 ±0.22	3.66 ±0.62	3.64 ±0.04	8.22 ±0.23	12.15 ±0.15	4.98 ±0.26	6.21 ±0.16	5.22 ±0.13	5.85 ±0.87
Neutrophils (10^9/L) [0.23-3.6]	0.34 ±0.04	1.69 ±0.16	1.02 ±0.07	1.28 ±0.07	0.69 ±0.06	1.59 ±0.01	3.05 ±0.24	3.62 ±0	0.82 ±0.13	1.85 ±0	1.00 ±0.05	1.23 ±0.20
Lymphocytes (10^9/L) [0.6-8.9]	2.92 ±0.06	2.95 ±0.1	3.01 ±0.29	2.35 ±0.21	2.64 ±0.44	1.54 ±0.10	4.02 ±0.05	5.88 ±0.13	3.95 ±0.14	3.92 ±0.07	3.80 ±0.04	4.13 ±0.57
Monocytes (10^9/L) [0.04-1.4]	0.18 ±0.07	1.20 ±0.06	0.93 ±0.16	0.74 ±0.08	0.28 ±0.08	0.47 ±0.06	1.09 ±0.04	2.62 ±0.01	0.15 ±0	0.39 ±0.05	0.39 ±0.02	0.41 ±0.08
Eosinophils (10^9/L) [0-0.51]	0.03 ±0	0.04 ±0	0.11 ±0.01	0.04 ±0	0.06 ±0.04	0.05 ±0.01	0.07 ±0.01	0.03 ±0	0.07 ±0.02	0.06 ±0.03	0.04 ±0.01	0.08 ±0
Basophils (10^9/L) [0-0.12]	0.00 ±0	0.01 ±0	0.005 ±0.01	0.005 ±0.01	0.00 ±0	0.00 ±0	0.00 ±0	0.005 ±0.01	0.00 ±0	0.00 ±0	0.01 ±0	0.01 ±0
Red Blood Cells (10^{12}/L) [6.5-11.5]	10.1 ±0.16	9.48 ±0.08	10.56 ±0.23	9.42 ±0.20	8.98 ±1.21	7.98 ±0.16	8.94 ±0.08	9.94 ±0.23	10.01 ±0.16	10.18 ±0.21	11.00 ±0.11	11.51 ±0.63
Hemoglobina (g/dL) [11.0-16.5]	14.8 ±0	15.15 ±0.07	16.75 ±0.21	14.70 ±0.14	14.0 ±1.98	12.05 ±0.07	13.60 ±0	14.0 ±0	15.4 ±0.71	14.6 ±0.71	16.1 ±0	15.3 ±7.07
Platelet (10^9/L) [400-1600]	483.0 ±33.94	1067.5 ±13.44	1071.5 ±0.71	1005.5 ±4.95	887.0 ±144.25	915.5 ±28.99	1301.0 ±4.24	1607.5 ±20.51	489.0 ±9.89	1551.5 ±113.84	1425.0 ±15.56	1556.5 ±36.06

Figure S13. Hematological analysis of mice blood 3 h after i.v. administration of 50 µg of Tm**P**$(X)_6$ glycopeptides (X = Glu, Gal or Man) to a total of three mice and one control mouse with PBS. Reference intervals are indicated in grey in the first column. For each mouse, each value was measured in duplicate and is plotted as the mean ± standard deviation. Slight deviations from reference values have been highlighted in yellow and more significant changes are shown in red. Tm**P**$(Gal)_6$ causes high values of white blood cells compared to the control (monocytosis).

	PBS	Tm**P**$(Glu)_6$			PBS	Tm**P**$(Gal)_6$			PBS	Tm**P**$(Man)_6$		
Albumin (g/dL)	5.2 ±0.1	5.0 ±0.1	5.1 ±0.3	5.1 ±0.3	4.2 ±0.1	4.0 ±0.1	4.4 ±0.1	4.4 ±0.1	4.5 ±0.5	5.1 ±0.6	4.6 ±0.1	5.1 ±0.4
Alkaline phosphatase (U/L)	110 ±4	77 ±5	126 ±3	110 ±6	109 ±8	110 ±11	190 ±5	101 ±6	112 ±4	140 ±6	152 ±2	163 ±7
Alanine aminotransferase (U/L)	56 ±6	52 ±9	85 ±13	64 ±3	48 ±6	42 ±9	69 ±13	47 ±3	65 ±3	75 ±2	59 ±2	62 ±6
Amylase (U/L)	924 ±16	1060 ±25	1043 ±33	1097 ±41	1071 ±12	1190 ±13	1045 ±21	1611 ±9	1113 ±8	1170 ±15	1248 ±27	1189 ±23
Total Bilirubin (mg/dL)	0.3 ±0.1	0.3 ±0	0.3 ±0.1	0.3 ±0	0.3 ±0.1	0.3 ±0	0.4 ±0	0.3 ±0	0.3 ±0	0.3 ±0	0.3 ±0	0.3 ±0
Blood Urea Nitrogen (mg/dL)	21 ±2	19 ±4	21 ±1	22 ±3	25 ±0	23 ±1	19 ±6	23 ±3	18 ±3	18 ±1	19 ±6	18 ±2
Total protein (g/dL)	6.1 ±0.6	6.3 ±0.7	6.5 ±0.3	6.4 ±0.2	5.3 ±0.5	5.1 ±0	5.4 ±0	5.6 ±0	5.6 ±0.7	6.6 ±0.3	5.9 ±0.6	6.4 ±0.2
Globulin (g/dL)	0.9 ±0.2	1.3 ±0.3	1.4 ±0.1	1.2 ±0	1.1 ±0.1	1.1 ±0.1	1.0 ±0.1	1.2 ±0	1.1 ±0.1	1.4 ±0	1.3 ±0	1.3 ±0
Calcium (mg/dL)	12.8 ±1.7	13.5 ±0	14.6 ±0	13.8 ±0	11.5 ±0.2	11.0 ±0.5	11.3 ±0.1	11.8 ±0.3	12.8 ±0.5	13.5 ±0.9	12.6 ±0.4	13.1 ±1.1
Na^+ (mmol/dL)	149 ±2	154 ±3	153 ±0	156 ±1	153 ±2	156 ±5	154 ±4	160 ±5	153 ±1	156 ±1	155 ±0	158 ±2
K^+ (mmol/dL)	8.1 ±0.1	8.0 ±0.4	8.2 ±0	8.5 ±0.2	8.5 ±0.2	8.5 ±0	8.5 ±0	8.5 ±0	8.5 ±0.5	8.5 ±0.3	8.6 ±0	8.1 ±0.4

Figure S14. Biochemical analysis of blood from mice 3 h after i.v. administration of 50 µg of Tm**P**$(X)_6$ glycopeptides (X = Glu, Gal or Man) to a total of three mice and one control mouse with PBS. For each mouse, each value was measured in duplicate and is plotted as the mean ± standard deviation. Tm**P**$(Gal)_6$ shows slight increase in alkaline phosphatase and alanine aminotransferase values (in yellow, hepatic injury) and hight increase in amylase values (in red, pancreas disorder).

11. Peptide characterization

11.1 With TmP(X)$_4$ structure

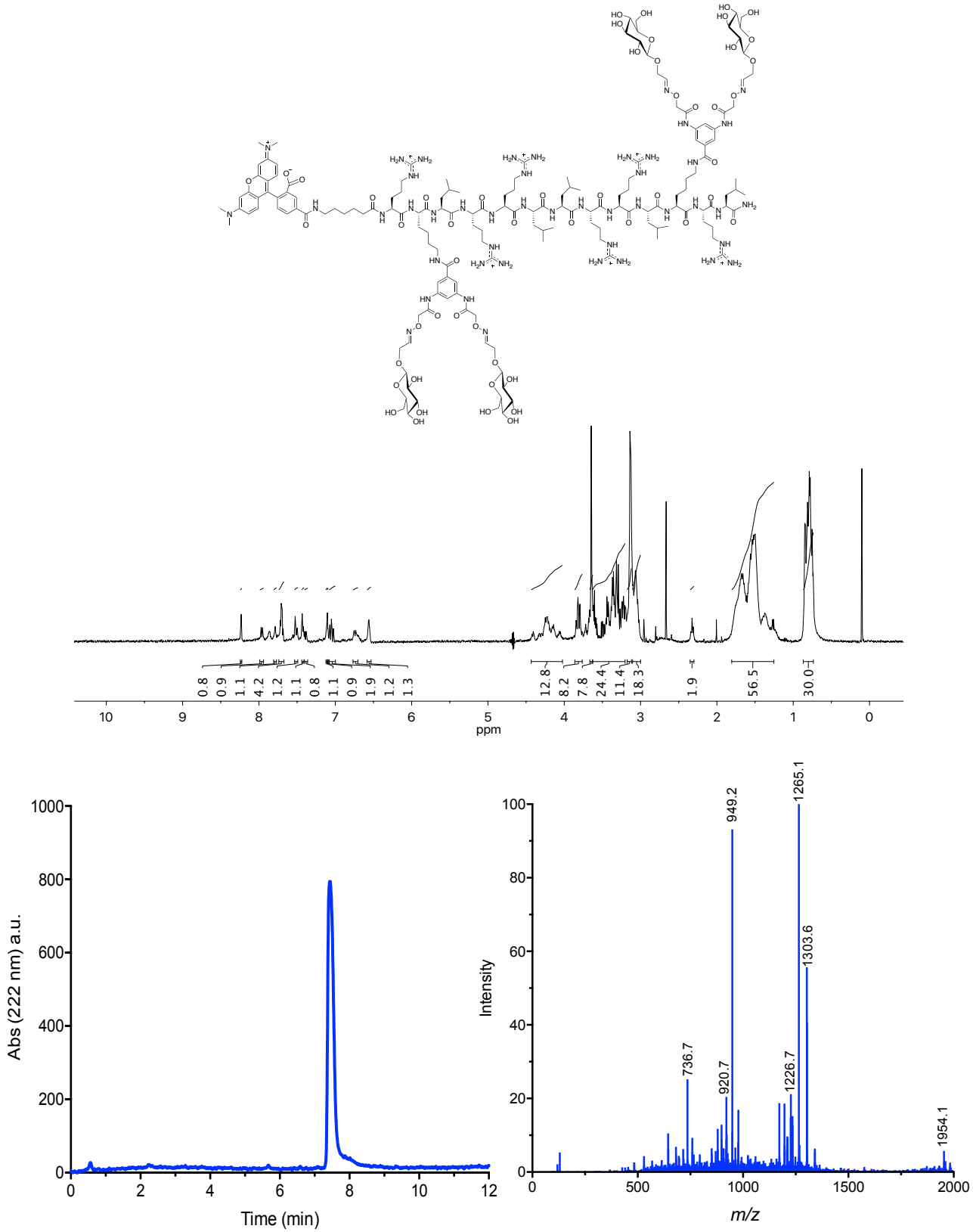

Figure S15. Peptide Tm**P**(Glu)$_4$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 7.44 min.

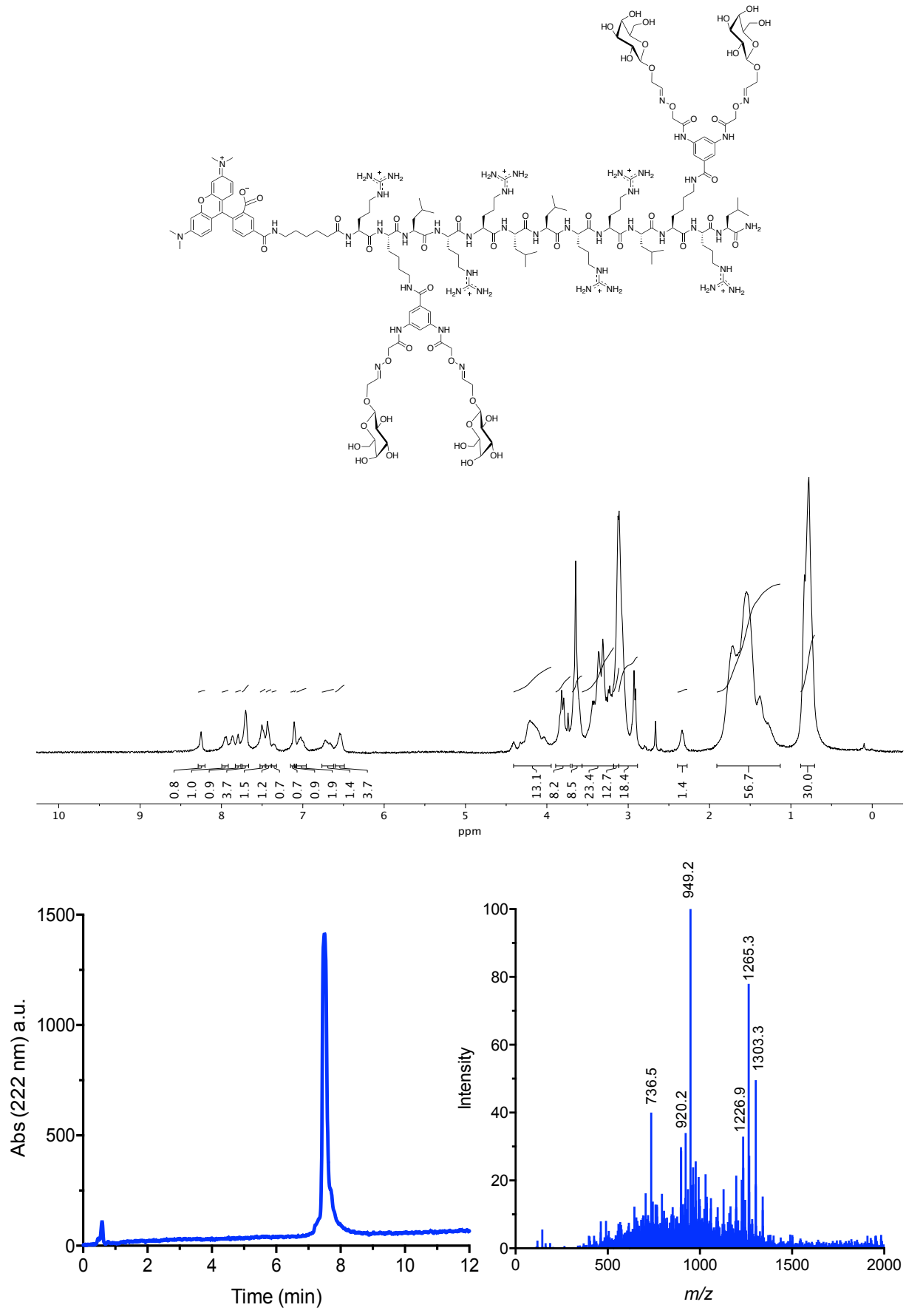

Figure S16. Peptide Tm**P**(Gal)$_4$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 7.50 min.

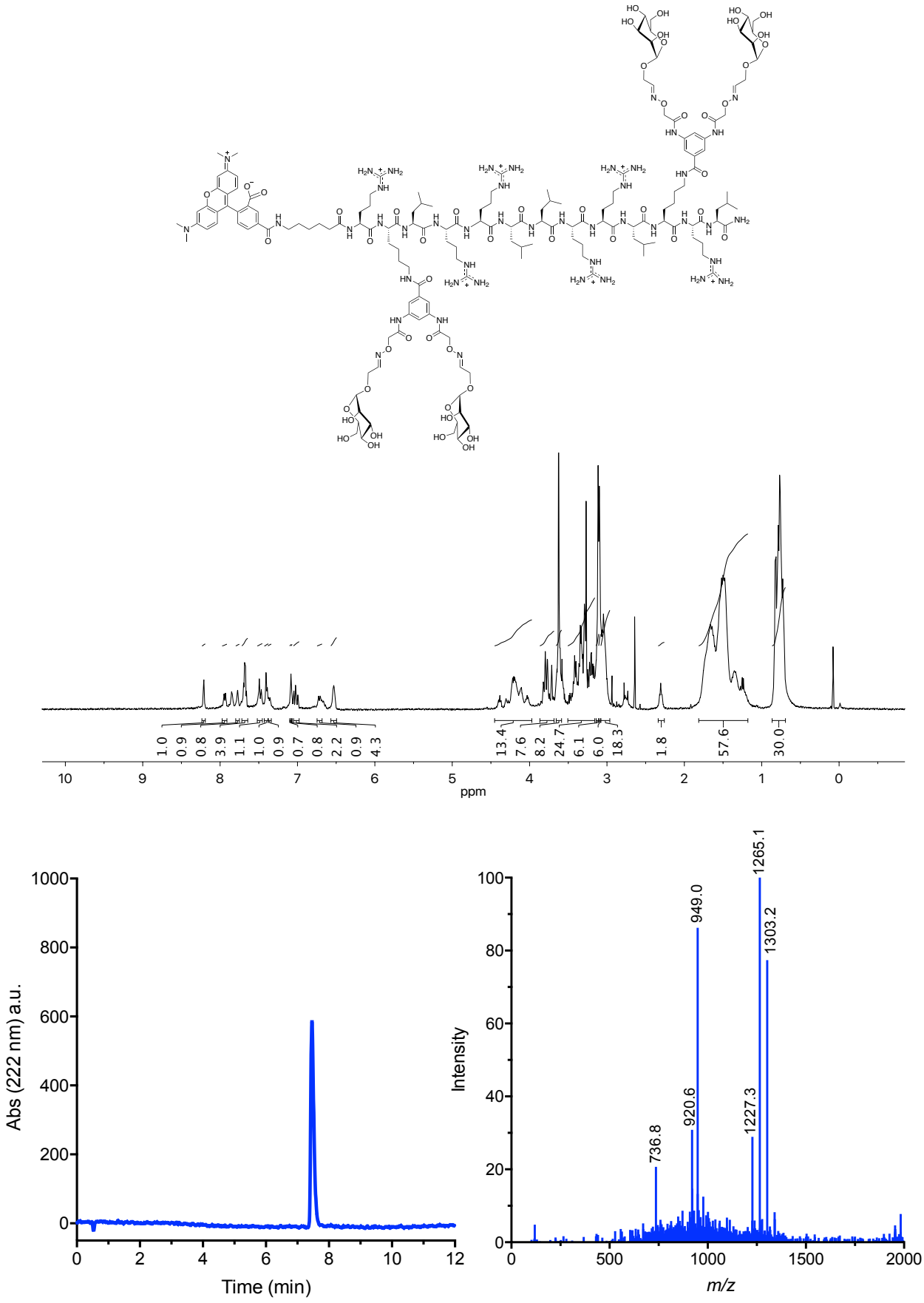

Figure S17. Peptide Tm**P**(Man)$_4$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 7.46 min.

11.2 With TmP(X)$_6$ structure

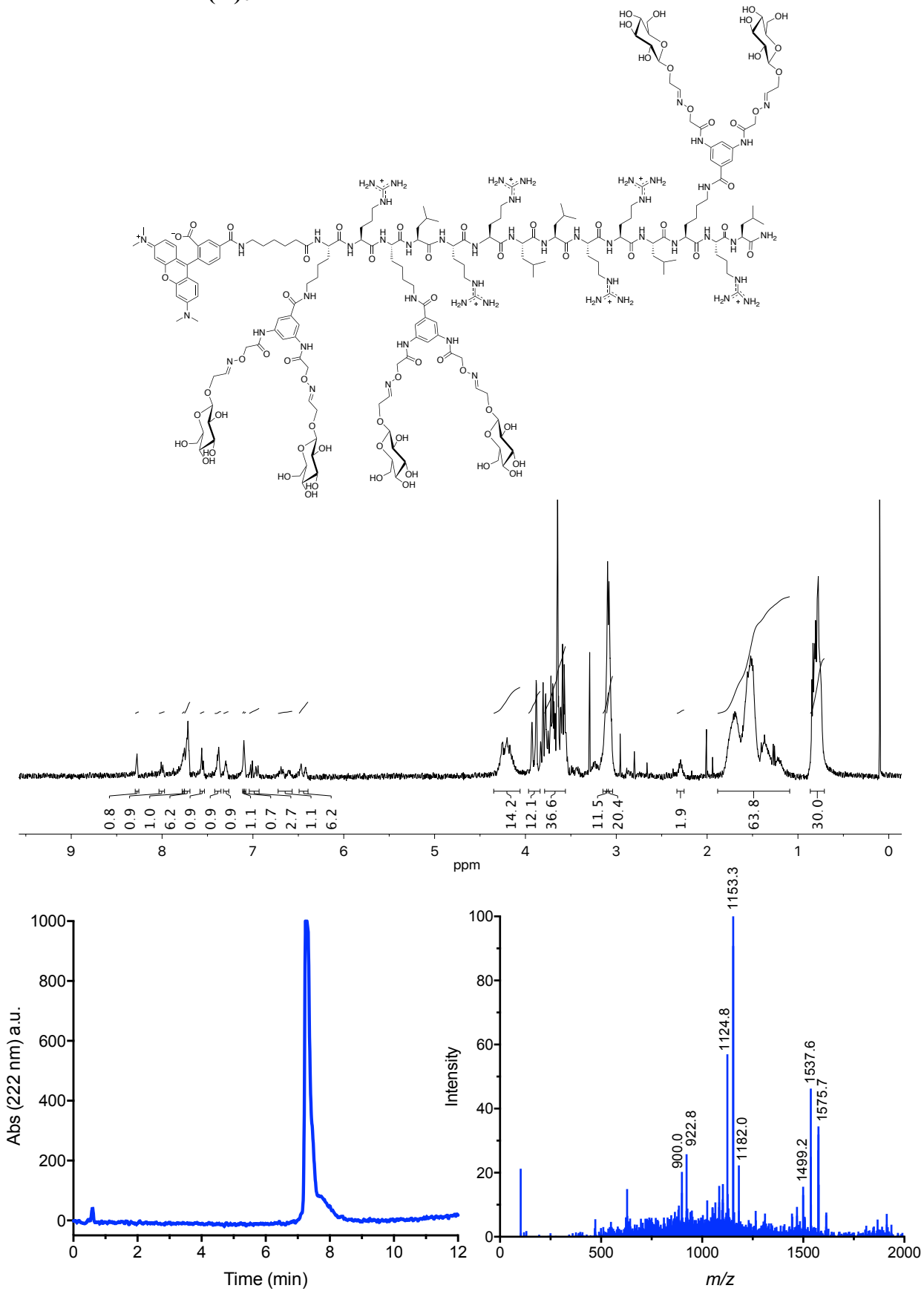

Figure S18. Peptide Tm**P**(Glu)$_6$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 7.40 min.

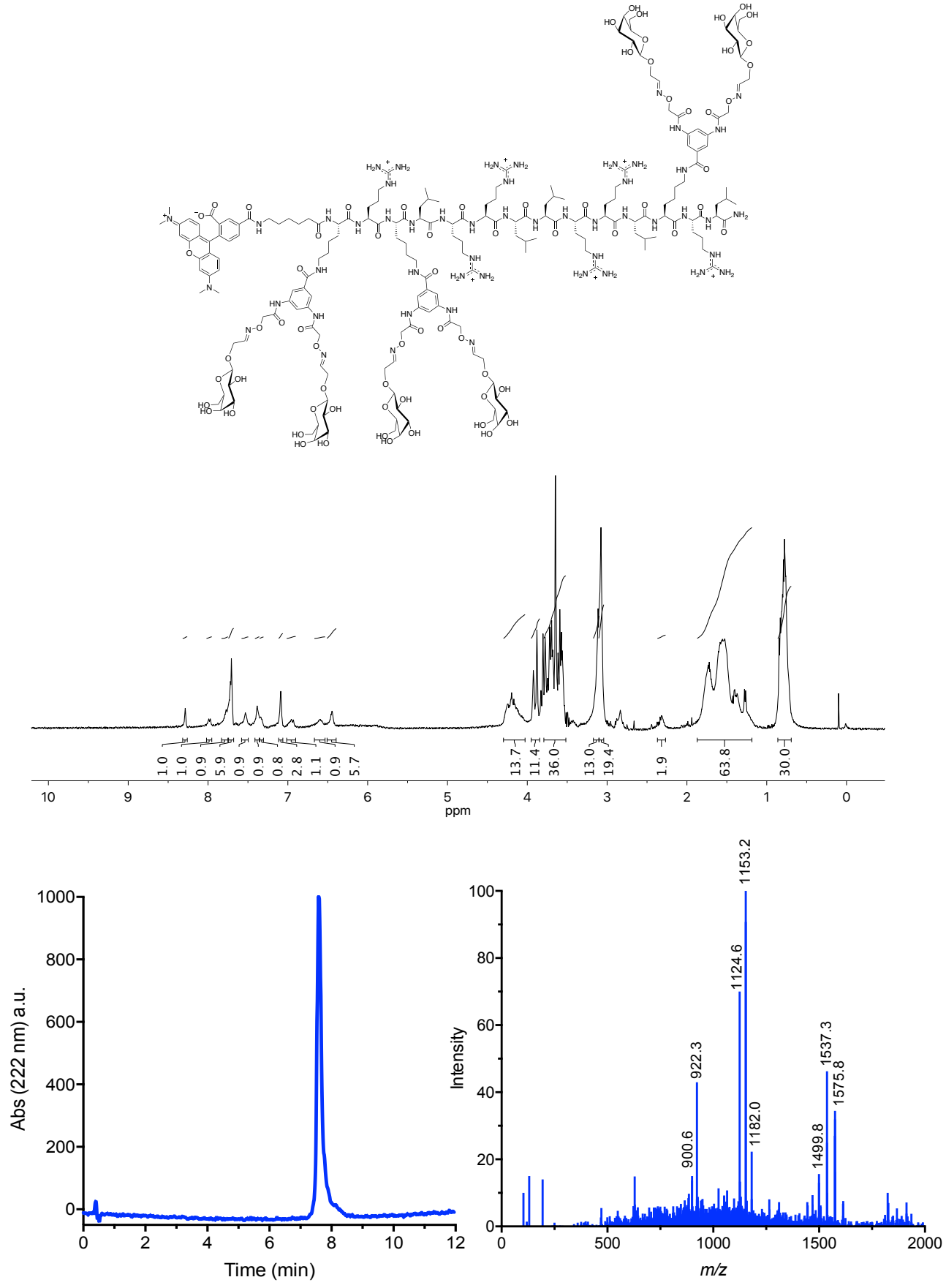

Figure S19. Peptide Tm**P**$(Gal)_6$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 7.59 min.

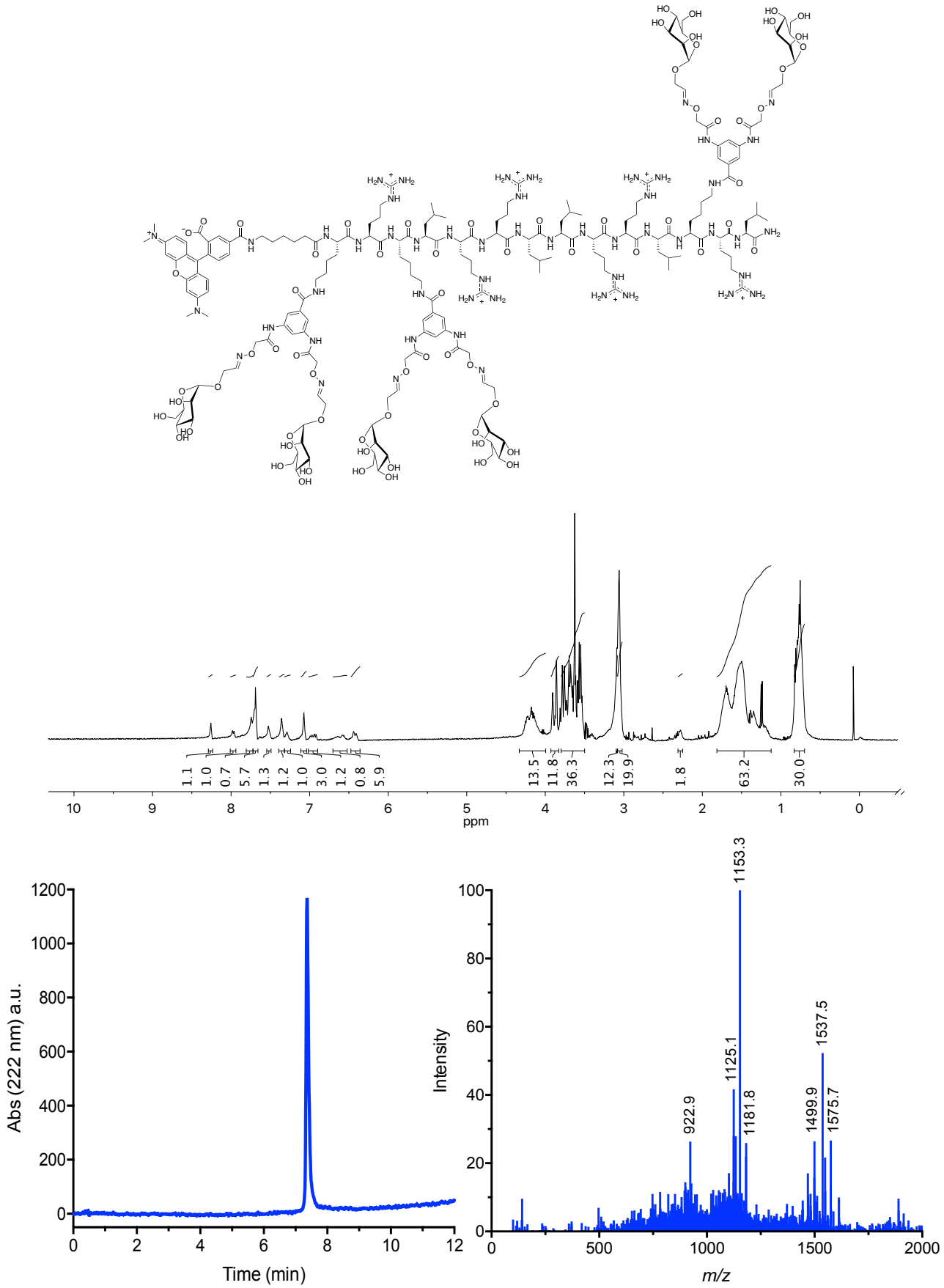

Figure S20. Peptide Tm**P**(Man)$_6$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 7.36 min.

11.3 Control peptide TmR$_8$

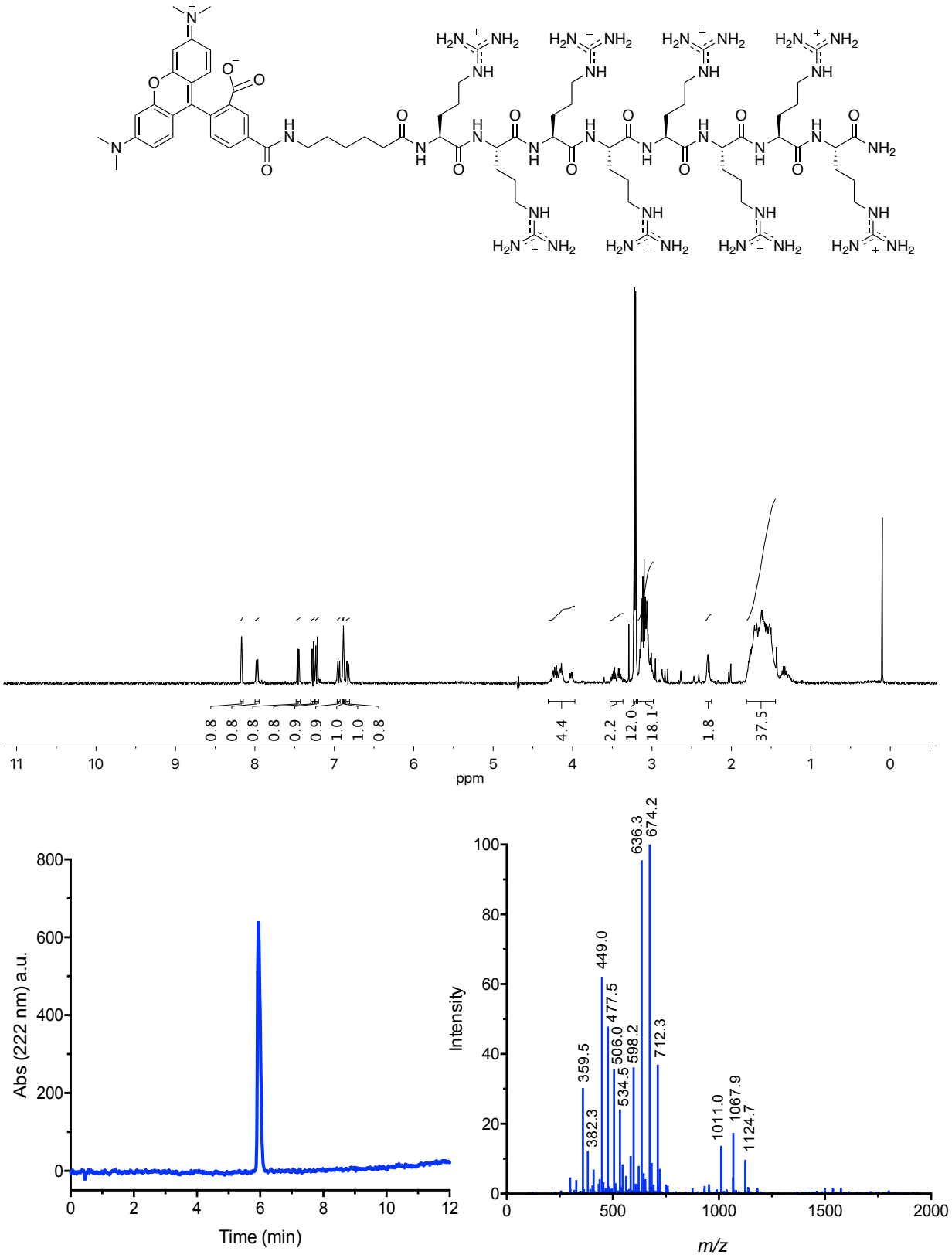

Figure S21 Peptide Tm**R**$_8$. ^{1}H-RMN spectra in D_2O and HPLC-MS (ESI) in H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (0→12 min), t_R 5.96 min.

12. Supporting references

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