Supplementary Material (ESI) for Chemical Communications

Supporting Information

for

"Rational design of a Tn antigen mimic"

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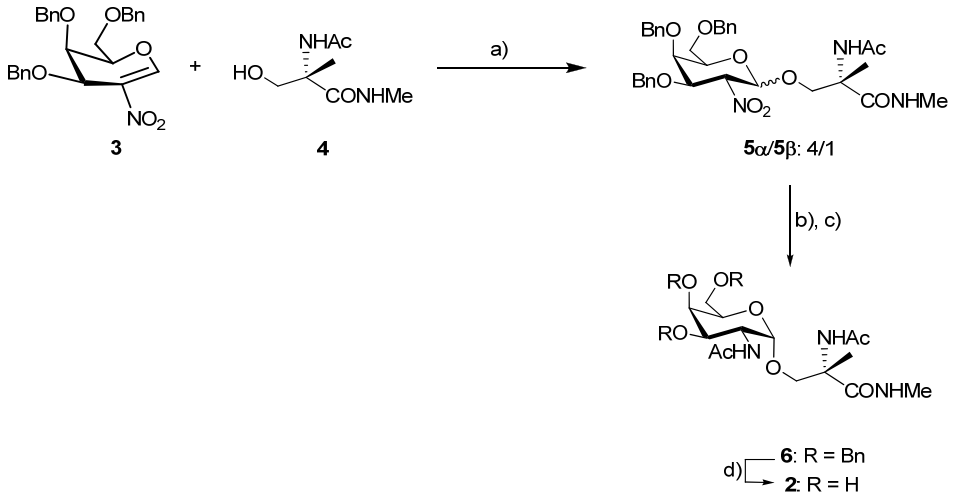
Experimental data

General Procedures. All manipulations with air-sensitive reagents were carried out under a dry argon atmosphere using standard Schlenk techniques. Solvents were purified according to standard procedures. The chemical reagents were purchased from Aldrich Chemical Co. Analytical TLC was performed using Polychrom SI F_{254} plates. Column chromatography was performed using Kieselgel 60 (230–400 mesh). Organic solutions were dried over anhydrous Na_2SO_4 and, when necessary, concentrated under reduced pressure using a rotary evaporator. NMR spectra were recorded at 300 or 400 MHz (^1H) and at 100 MHz (^{13}C) and signals are reported in ppm downfield. Optical rotations were measured on a Perkin-Elmer 341 polarimeter in a 1 dm cell of 1 mL capacity. Microanalyses were carried out on a CE Instruments EA-1110 analyser and are in good agreement with the calculated values. IR experiments were carried out in a Nicolet Nexus FT-IR spectrophotometer using an ATM module.

Synthesis

The synthesis of compound **1** was achieved following the methodology described in ref. S1. Compound **4** was obtained following the procedure described in ref. S2.

Synthesis of compound **2**



Scheme S1. Synthesis of derivative **2**. (a) *t*BuOK, THF, 25 ºC, 12 h, 44%; (b) i) H_2/Ni Raney (T4), EtOH, 25 ºC, 4 h. ii) Ac_2O/Py (1:2), 25 ºC, 3 h, 38% overall yield; (c) column chromatography, 71%; (d) H_2/Pd-C, MeOH/ethyl acetate (4:1), 25 ºC, 12 h, 90%.

Synthesis of compounds 5α/5β. 3,4,6-Tri-*O*-benzyl-2-nitrogalactal (905 mg, 1.96 mmol) and compound **4**[S2] (410 mg, 2.35mmol) were dissolved in THF (30 mL) under an argon atmosphere in presence of molecular sieves. The reaction mixture was stirred at 25 ºC for 30 min and a 1M potassium *tert*-butoxide solution in THF (0.3 mL, 0.3 mmol) was then added. After the reaction was stirred for an additional 12 h, the molecular sieves were filtered off and all solvents were removed by evaporation. The residue was purified by a silica gel column chromatography (hexane/ethyl acetate, 4:1) to give a mixture 4:1 of **5α/5β** (553 mg, 44%) as a colorless oil. Elemental analysis: calcd (%) for $C_{34}H_{41}N_3O_9$: C, 64.24; H, 6.50; N, 6.61; found: C, 64.20; H, 6.55; N, 6.71.

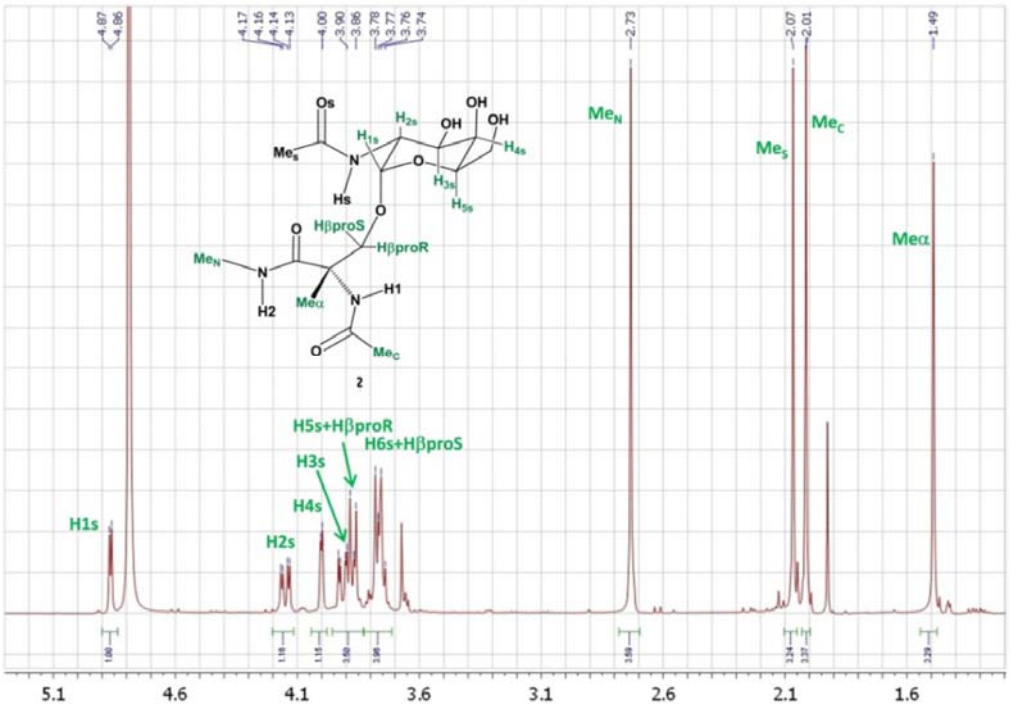
Synthesis of compound 6. Platinized Raney-nickel (T4) catalyst was freshly prepared as described in the literature.[S3] The catalyst obtained by using 2.0 g of Raney nickel/aluminum alloy was suspended in ethanol (20 mL) and pre-hydrogenated for 10 min before the addition of the mixture of **5α/5β** (225 mg, 0.35mmol) in ethanol/ethyl acetate (4:1, 5 mL). The reaction mixture was shaken

under H_2 (1 atm) for 4 h at 25 ºC. The catalyst was filtered off and the solvent evaporated. The residue was dissolved in pyridine/acetic anhydride (2:1, 6 mL) and stirred at 25 ºC for 3 h. Removal of the volatiles and silica gel column chromatographic purification (ethyl acetate/hexane, 7:3) gave **6** (62 mg, 27%) as a colorless oil. $[\alpha]^{25}_D$ (c = 1. 15, CH_3OH): +63.1. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.51 (s, 3H, CH_3), 1.94 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.75 (d, 3H, *J* = 4.7 Hz, NHC$\underline{H}_3$), 3.56 (d, 2H, *J* = 6.5 Hz, CH_2), 3.67 (dd, 1H, J_1 = 11.0 Hz, J_2 = 2.4 Hz, H_{3s}), 3.81-3.87 (m, 3H, H_β, $H_{\beta'}$, H_{5s}), 3.97-4.00 (m, 1H, H_{4s}), 4.41 (d, 1H, *J* = 11.6 Hz, C$\underline{H}_2$Ph), 4.45 (d, 1H, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.47-4.53 (m, 2H, C$\underline{H}_2$Ph, H_{2s}), 4.58 (d, 1H, *J* = 11.6 Hz, C$\underline{H}_2$Ph), 4.71 (d, 1H, *J*= 12.0 Hz, C$\underline{H}_2$Ph), 4.93 (d, 1H, *J*= 11.6 Hz, C$\underline{H}_2$Ph), 4.96 (d, 1H, *J* = 3.6 Hz, H_{1s}), 5.70 (d, 1H, *J* = 7.8 Hz, $NHAc_s$), 6.54 (s, 1H, NHAc), 6.80 (d, 1H, *J* = 4.7 Hz, NHMe), 7.27-7.38 (m, 15H, 3 Ph). ^{13}C NMR ($CDCl_3$) δ (ppm): 19.9 (CH_3), 23.5, 24.2 (2 CH_3CO), 26.5 ($NHCH_3$), 49.8 (C_{2s}), 60.4 (C_α), 68.9 (CH_2), 70.3 (C_{5s}), 71.4 (CH_2Ph), 72.1 (C_β), 72.3 (C_{4s}), 73.6 (CH_2Ph), 74.5 (CH_2Ph), 76.6 (C_{3s}), 99.2 (C_{1s}), 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.2, 128.4, 128.5, 137.6, 137.9, 138.3 (3 Ph), 170.4, 170.5, 173.1 (3 CO). Elemental analysis: calcd (%) for $C_{36}H_{45}N_3O_8$: C, 66.75; H, 7.00; N, 6.49; found: C, 66.70; H, 6.94; N, 6.50.

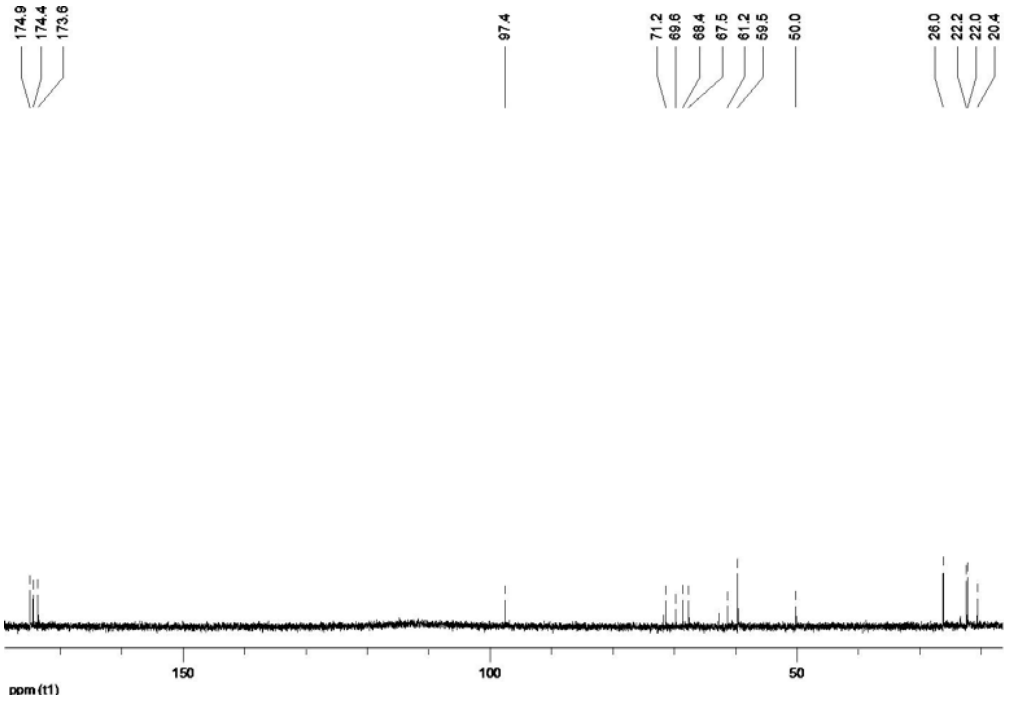
Synthesis of compound 2. A solution of compound **6** (33 mg, 0.05mmol) in MeOH/ethyl acetate (4:1, 5 mL) was treated with 10% Pd/C (15 mg) as a catalyst. The reaction mixture was shaken under H_2 (1 atm) for 12 h at 25 ºC. Removal of the catalyst and the solvent gave 17 mg of compound **2**, as a colorless oil in 90% yield. $[\alpha]^{25}_D$ (c = 0.56, CH_3OH): +21.8. IR (H_2O): 1630 cm^{-1}. ^{1}H NMR (D_2O) δ (ppm) : 1.49 (s, 3H, Meα), 2.01 (s, 3H, Me_C), 2.07 (s, 3H, Me_S), 2.73 (s, 3H, Me_N), 3.73-3.79 (m, 3H, CH_2, $H_{\beta proS}$), 3.85-3.89 (m, 2H, H_{5s}, $H_{\beta proR}$), 3.91 (dd, 1H, J_1 = 11.0 Hz, J_2 = 3.0 Hz, H_{3s}), 4.00 (‘d’, 1H, *J* = 2.9 Hz, H_{4s}), 4.15 (dd, 1H, J_1 = 11.0 Hz, J_2 = 3.6 Hz, H_{2s}), 4.87 (d, 1H, *J* = 3.6 Hz, H_{1s}). ^{1}H NMR (H_2O/D_2O) δ (ppm) : 1.47 (s, 3H, Meα), 1.99 (s, 3H, Me_C), 2.05 (s, 3H, Me_S), 2.71 (d, 3H, *J* = 4.6 Hz, Me_N), 3.70-3.78 (m, 3H, CH_2, $H_{\beta proS}$), 3.82-3.92 (m, 3H, H_{5s}, $H_{\beta proR}$, H_{3s}), 3.96-4.01 (m, 1H, H_{4s}), 4.08-4.17 (m, 1H, H_{2s}), 7.87-7.96 (m, 1H, NH2), 8.08 (d, 1H, *J* = 8.8 Hz, NH_S), 8.19 (s, 1H, NH1). ^{13}C NMR (D_2O) δ (ppm): 20.4 (Meα), 22.0 (Me_S), 22.2 (Me_C), 26.0 (Me_N), 50.0 (C_{2s}), 59.5 ($C_{\alpha 1}$), 61.2 (CH_2), 67.5 (C_{3s}), 68.4 (C_{4s}), 69.6 (C_β), 71.2 (C_{5s}), 97.4 (C_{1s}), 173.6, 174.4, 174.9 (3 CO). Elemental analysis: calcd (%) for $C_{15}H_{27}N_3O_8$: C, 47.74; H, 7.21; N, 11.13; found: C, 47.70; H, 7.25; N, 11.20.

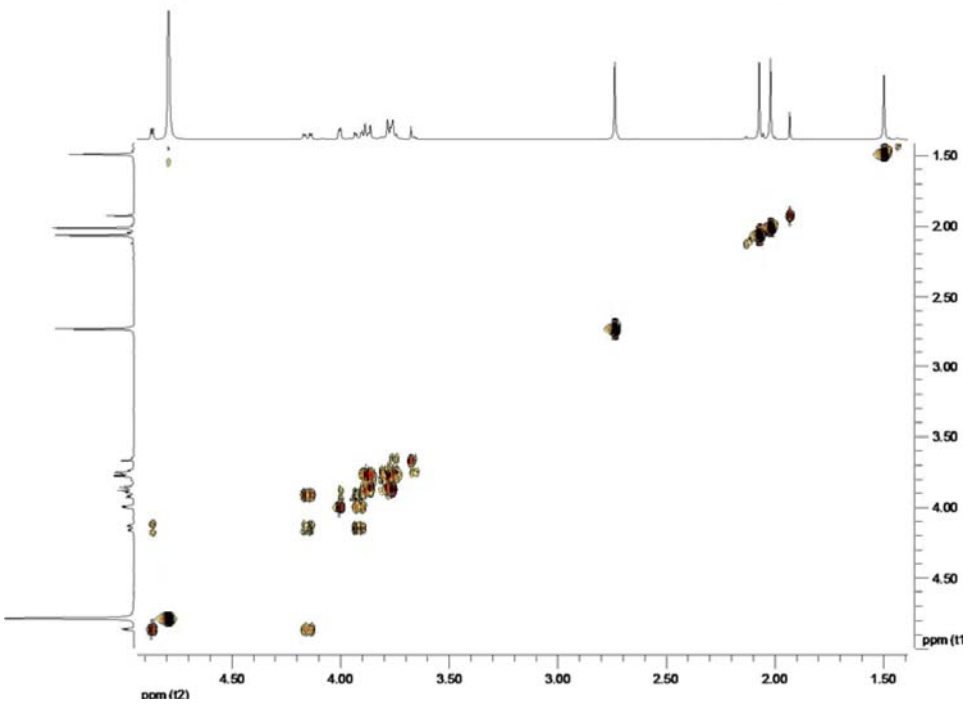
Compound 1. IR (H_2O): 1636 cm^{-1}.

^{1}H NMR (400 MHz, D_2O)



^{13}C NMR (100 MHz, D_2O)

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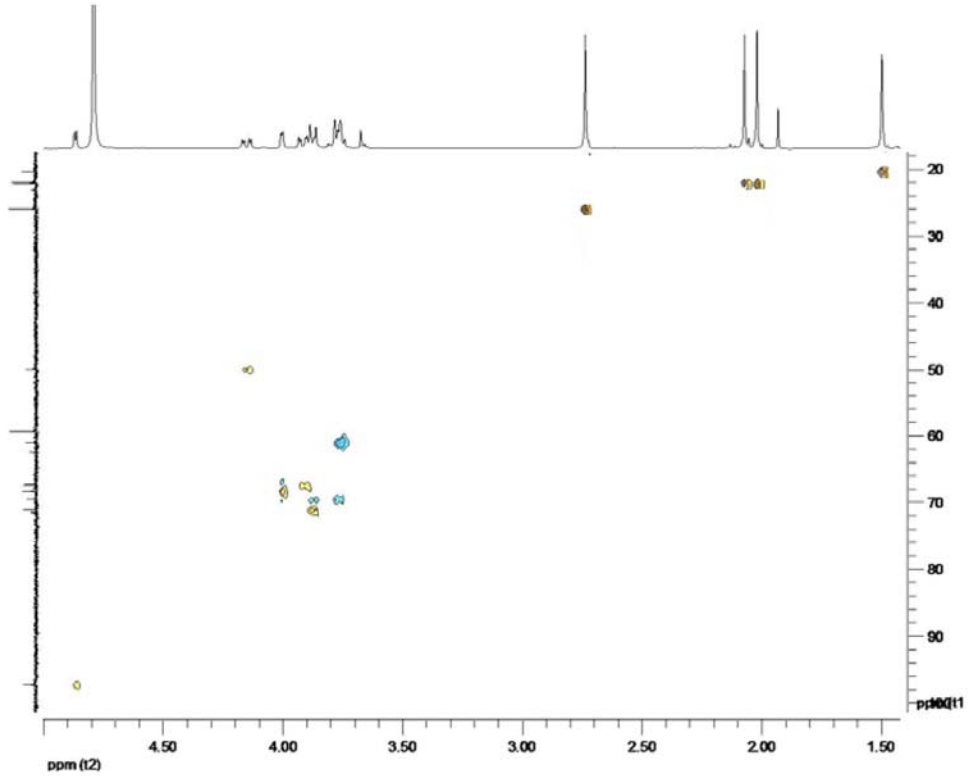
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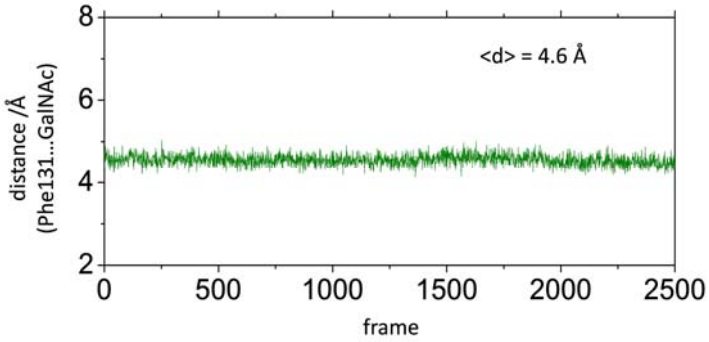

Figure S1. Distance time series between the geometrical center of the phenyl ring of Phe131 and the six-membered ring of GalNAc for derivative **2** bound to EcorL. The average distance (<d>) over the trajectory is also shown.

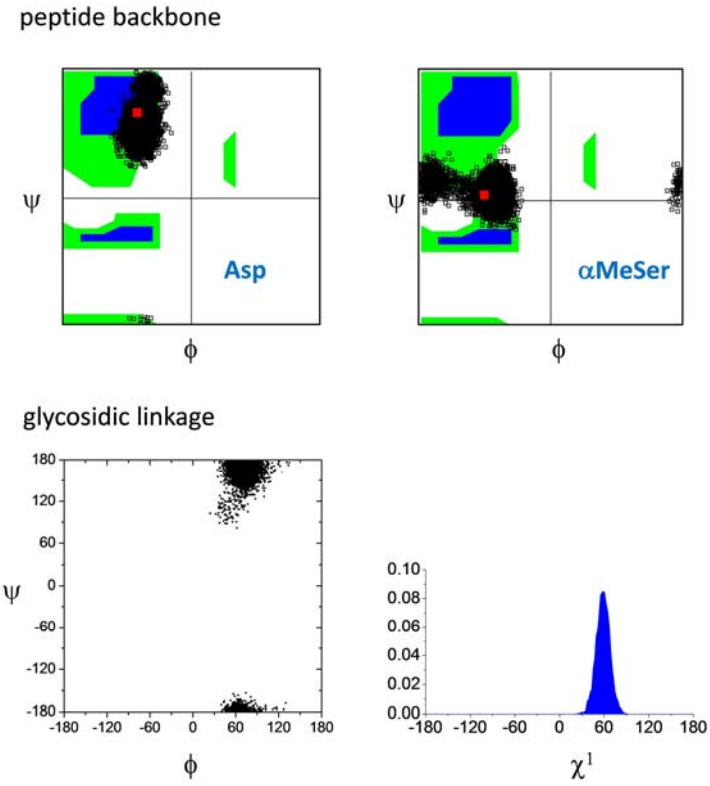

Figure S2. ϕ/ψ distributions for Asp and αMeSer residues (peptide backbone), conformation of the glycosidic linkage (ϕ=O5-C1-O1-Cβ, ψ=C1-O1-Cβ-Cα), and lateral chain (χ^1=O1-Cβ-Cα-N) obtained from the unrestrained 25 ns MD simulations carried out on the complex **SM3:Pro-Asp-MeSer*-Arg** (* =αGalNAc). The red squares show the conformation of Asp and Thr residues in the crystal structure (code name: 1sm3)

STD experiments

STD experiments were recorded at 298K on a Bruker Avance DRX 500MHz spectrometer. **EcorL** lectin was purchased from Sigma-Aldrich and was dissolved in D_2O buffer (20mM sodium phosphate buffer, 100mM NaCl, pH=7.4) to a final concentration of 40 μM for the binding studies.

The binding of each glycopeptide was evaluated by STD experiments performed with 35:1 molar ratios of the glycopeptide/**EcorL**. The competition STD experiments between glycopeptides **1** and **2** were performed with 25:25:1 molar ratios of **1**/**2**/**EcorL** mixture. An off-resonance frequency of δ=40 ppm and on-resonance frequency of δ=7.0 ppm (protein aromatic signals region) were applied. A total number of 5120 scans were acquired and the spectra were multiplied by an exponential line broadening function of 1Hz prior to Fourier transformation. All experiments were recorded with a 15 ms spin lock pulse, which minimizes the protein background resonances. Spectra processing were performed on PC station using Topspin 2.0 software (Bruker). The next step was to confirm that the glycopeptides and *N*-acetylgalactosamine (GalNAc) compete for the same binding site. To this end, a mixture of α/β anomers of GalNAc was added to a mixture of **1**/**2**/**EcorL** to obtain a **25**:**25**:**50**:**1** molar ratios of **1**/**2**/GalNAc/**EcorL** (Figure S3). A total number of 1024 scans were acquired in this case.

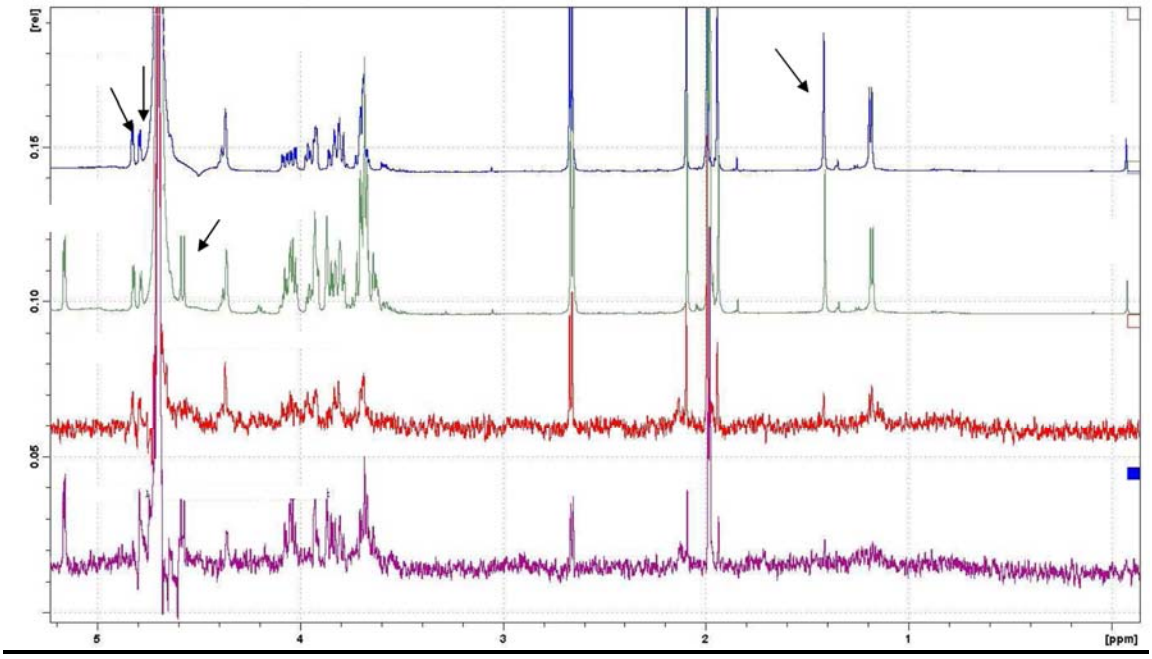

Figure S3. Competition STD experiments.

ELISA experiments

The E90413Mu 96 Tests Enzyme-linked Immunosorbent Assay Kit for Mouse Mucin 1 (MUC1) was purchased from SCNK Life Science Inc. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to MUC1. The standard protocol provide by SCNK Life Science Inc. was used in all the experiments. Thus, standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for MUC1 and derivatives. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. A TMB substrate solution was then added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm.

Different concentrations of the glycopeptides **1** and **2** in water were used, varying from 0.85 to 22 mM (serial two-fold dilutions). The microtiter plate was coated with 100 μL/well. In general, negative results were observed for both derivatives, however a slight optical density (0.03) was observed only at high concentration (22 mM) of the glycopeptide.

References

[S1] F. Corzana, J. H. Busto, G. Jiménez-Osés, M. García de Luis, J. L. Asensio, J. Jiménez-Barbero, J. M. Peregrina and A. Avenoza*, J. Am. Chem. Soc.,* 2007, **129**, 9458.

[S2] A. Fernández-Tejada, F. Corzana, J. H. Busto, G. Jiménez-Osés, J. M. Peregrina and A. Avenoza, *Chem. Eur. J.*, 2008, **14**, 7042.

[S3] S. Nishimura, *Bull. Chem. Soc. Jpn.*, 1959, **32**, 61.