Supporting Information

Conformationally Constrained Functional Peptide Monolayers for the Controlled Display of Bioactive Carbohydrate Ligands

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1. Methods. Melting points were determined using a Leitz model Laborlux 12 apparatus. Column chromatography was performed on MN Kieselgel 60M (0.040-0.063 mm, Macherey-Nagel). Solid-state IR absorption spectra (KBr disk) were recorded with a Perkin-Elmer model 1720X FT-IR spectrophotometer, nitrogen-flushed, equipped with a sample-shuttle device, at 2 cm⁻¹ nominal resolution, averaging 20 scans. ¹H-NMR were obtained with Bruker model AC 200 and Bruker model AM 400 spectrometers. Deuteriochloroform (99.8%, *d*; Euriso-Top), deuterated acetonitrile (99.95%, *d*₃; Acros Organics), methanol (99.8%, *d*₄; Acros Organics), and deuterated dimethyl sulfoxide (99.8%, *d*₆; Euriso-Top), with tetramethylsilane as the internal standard, were used as solvents. HRMS data was obtained with an Agilent 1260 LC/MS and a 6520B Q-TOF mass spectrometer with electrospray ionization.

2. Chemicals. All chemicals were commercially available and used as received. Electrochemistry experiments: ethanol (HPLC grade, ≥99.8%), hydrogen peroxide 30%, and sodium hydroxide monohydrate (≥99.9995%) were purchased from Fluka; ethanol 200 Proof was from Decan Labs, Inc.; potassium chloride (99.0%) and hexammineruthenium(III) chloride (98%) were from Aldrich; sulfuric acid 98% was from BDH (Aristar Grade); the chemical reagents for making buffers were purchased from Sigma-Aldrich and Acros Organics; bovine serum albumin (BSA) was from Sigma-Aldrich; Concanavalin A was purchased from MP Biomedicals (Solon, OH); Ricin (RCA120) was purchased from EY Laboratories (San Mateo, CA); low conductivity water was milliQ Water pro analysis (Merck) or AnalaR Normapur (NDH). Peptide synthesis: potassium bisulfate, anhydrous sodium sulfate, diethyl ether, ethyl acetate, petroleum ether, methanol, methylene chloride (Carlo Erba); *N,N*-diisopropylethylamine (DIEA), trifluoroacetic acid, triisopropylsilane (Acros Organics); acetonitrile, *N,N*-dimethylformamide (DMF), benzyl chloroformate (Sigma-Aldrich); 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (GL Biochem, Shanghai); hexafluoroisopropanol (Oakwood); 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (Genscript). Ph₃C-S-(CH₂)₂-COOH and peptides **1** and **3** were prepared as already described.^{S1-S3} Mono-4-methoxytrityl-

protected 3-mercaptopropionic acid and tetra-*O*-acetyl-3-aminopropyl D-mannoside were prepared as described.^{S4,S5} The syntheses of thiolated peptides **2** and **4** were carried out as shown in sections 3 and 4. The synthesis of the galactose-equivalent of glycopeptide **4**, used here for the control experiments with Ricin, will be described elsewhere. The free acid corresponding to **1** was obtained by deprotection of **9-1**, carried out as described below for the deprotection of **10** to give **4**. Z indicates the benzyloxycarbonyl group.

3. Synthesis of **2**

Z-(Aib)₂-OH. To a solution of Z-(Aib)₂-OtBu (430 mg, 1.14 mmol) in 10 ml of anhydrous CH₂Cl₂, 10 ml of trifluoroacetic acid were added under stirring. After 1.5 h the solvent was evaporated and the remaining traces of trifluoroacetic acid were removed by repetitive evaporations with diethyl ether. The product was recrystallized from diethyl ether to give a colorless solid (yield = 90%) having mp = 135-137 °C. IR (KBr) 3480, 3336, 2944, 1724, 1706, 1612, 1536 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ 1.34 (s, 12H), 7.29 (s, 1H), 7.35 (s, 5H), 7.47 (s, 1H).

Z-(Aib)₅-NHtBu. To a solution of Z-Aib₂-OH (322 mg, 1.00 mmol) in 5 ml of anhydrous acetonitrile, cooled to 0° C, EDC·HCl (192 mg, 1.00 mmol) was added. The reaction mixture was stirred for 30 min at room temperature. The solvent was evaporated and the oily residue was dissolved in ethyl acetate. The solution was washed with 10% KHSO₄, dried over anhydrous Na₂SO₄ and the solvent was evaporated. The oily residue was dissolved in 5 ml of anhydrous acetonitrile and H-Aib₃-NHtBu (obtained, and used as such, by catalytic hydrogenation of 400 mg, 0.86 mmol of Z-Aib₃-NHtBu^{S2} in MeOH) in 20 ml of anhydrous acetonitrile was added. After stirring for 72 h at 80° C under reflux, the solvent was evaporated to dryness. The residue was then purified by column chromatography using as the eluant dichloromethane/ethanol mixture (v/v 9/1) to give Z-(Aib)₅-NHtBu as a colorless solid (yield 40%) having mp = 170-172 °C. IR (KBr) 3409, 3378, 3279, 1708, 1673, 1530 cm⁻¹.

¹. ¹H NMR (CDCl₃) δ 1.31 (s, 6H), 1.39 (s, 9H), 1.44 (s, 6H), 1.47 (s, 12H), 1.49 (s, 6H), 5.11 (s, 2H), 6.45 (s, 1H), 6.88 (s, 2H), 7.36 (m, 5H), 7.44 (s, 3H).

Ph₃C-S-(CH₂)₂-CO-(Aib)₅-NHtBu. To an ice-cold solution of Ph₃C-S-(CH₂)₂-COOH (33 mg, 0.09 mmol) in 10 ml of anhydrous CH₂Cl₂, HOAt (13 mg, 0.09 mmol) and EDC·HCl (18 mg, 0.09 mmol) were added, followed by addition of a solution of H-(Aib)₅-NHtBu (obtained, and used as such, by catalytic hydrogenation of 50 mg, 0.08 mmol, of Z-(Aib)₅-NHtBu in MeOH) in anhydrous CH₂Cl₂ containing DIEA (16 μl, 0.09 mmol). After stirring for 3 days at room temperature, the reaction mixture was evaporated to dryness. The crude product was purified by column chromatography using as the eluant petroleum ether/ethyl acetate mixtures (v/v 1/1, 1/2, 1/3, 1/4, 1/5) and recrystallized from ethyl acetate/petroleum ether to give a colorless solid (yield = 76%) having mp = 243-245°C. IR (KBr) 3426, 3333, 1687, 1667, 1651, 1536, 741, 699 cm⁻¹. ¹H NMR (CD₃CN) δ 1.28 (s, 6H), 1.31 (s, 9H), 1.37 (s, 6H), 1.39 (s, 18H), 2.20 (t, 2H), 2.45 (t, 2H), 6.80, 6.87, and 6.92 (3s, 3H), 7.22-7.42 (m, 15H), 7.59 (s, 2H).

HS-(CH₂)₂-CO-(Aib)₅-NHtBu (**2**). Ph₃C-S-(CH₂)₂-CO-(Aib)₅-NHtBu (45 mg, 0.05 mmol) was dissolved in 10 ml of a 7:2:1 mixture of CH₂Cl₂/trifluoroacetic acid/triisopropylsilane. After stirring for 20 min at room temperature, the solvent was evaporated and the remaining traces of trifluoroacetic acid were removed by repetitive evaporations with diethyl ether. The product was purified by column chromatography using the eluant CH₂Cl₂ and then CH₂Cl₂/MeOH 9/1. After crystallization from diethyl ether, HS-(CH₂)₂-CO-(Aib)₅-NHtBu was obtained as a colorless solid (yield 85%) having mp = 141-143 °C. IR (KBr) 3425, 3353, 3275, 2555, 1688, 1645, 1539 cm⁻¹. ¹H NMR (CDCl₃) δ 1.41 (s, 9H), 1.45 (s, 6H), 1.52-1.58 (m, 24H), 1.79 (t, 1H), 2.65 (t, 2H), 2.86 (q, 2H), 6.80, 6.91, 7.50, 7.59, 7.77, 7.80 (6s, 6H).

4. Synthesis of **4**

The synthesis of thiolated, glycan-functionalized tetra-peptide **4** is outlined in Scheme 2. Coupling of methyl α -aminoisobutyrate (**5**) with *N*-Cbz protected Aib (**6**) under the agency of HATU [(2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)] afforded dipeptide **7** in 87% yield. Hydrogenolysis of the benzyloxycarbonyl protecting group (Cbz or Z) in **7** and coupling with monomer **6** furnished tripeptide **8** in 85% yield over two steps. A final reiteration of this two-step sequence delivered a tetrapeptide intermediate in 78% yield. Hydrogenolysis and HATU-mediated coupling with mono-4-methoxytrityl (MmT)-protected 3-mercaptopropionic acid (**11**)^{S4} afforded thiolated tetrapeptide **9** in 81% yield. Saponification of the methyl ester in **9** and HATU-mediated coupling with known tetra-*O*-acetyl-3-aminopropyl D-mannoside (**12**)^{S5} successfully installed the glycan moiety. Removal of all acetates with NaOMe/MeOH furnished glycopeptide **10** in 68% over three steps. Treatment of **10** with a mixture of hexafluoroisopropanol (HFIP), trifluoroacetic acid (TFA), dichloromethane, and triisopropylsilane (TIPS) in a 10:7:2:1 ratio smoothly removed the MmT thiol protecting group, delivering target molecule **4** in 65% yield. The details are as follows.

^a Reagents and conditions: (a) HATU, **6**, DMF, rt; (b) H_2 , Pd/C, MeOH; (c) HATU, **11**, DMF, rt; (d) NaOH, H_2O , MeOH; (e) HATU, **12**, DMF, rt; (f) NaOMe, MeOH, rt; (g) HFIP/ CH_2Cl_2 /TFA/TIPS (10:7:2:1), rt, 40 min.

Aib (5.25g, 50.9 mmol) was suspended in dry methanol (50 ml) and cooled using an ice bath. While stirring, thionyl chloride (3.9 ml, 53.4 mmol) was added dropwise over 20 minutes and the reaction was allowed to stir at room temperature overnight. The solution was concentrated, redissolved in dichloromethane, and allowed to recrystallize into a white solid to provide **5** in 96% yield. IR (neat) 3458, 2985, 2939, 1647, 1473, 1422, 1231 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 1.60 (s, 6H), 3.85 (s, 3H), 4.89 (s, 3H) ^{13}C NMR (125 MHz, CD_3OD) δ 22.8 (2 C), 52.7, 56.7, 171.8 HRMS (ESI) calc'd for $\text{C}_5\text{H}_{11}\text{NO}_2+\text{H}^+$ = 118.0868, found 118.0864.

Aib (8.0 g, 7.8 mmol) and sodium carbonate (4.9 g, 46.5 mmol) were dissolved in water (260 ml). The reaction vessel was cooled to 0 $^\circ\text{C}$ and benzyl chloroformate (14.5 g, 8.53 mmol) in tetrahydrofuran (100 ml) was added dropwise over a period of 15 min. The solution was stirred at 0 $^\circ\text{C}$ for 1.5 h then allowed to warm to r.t. overnight. Washed with diethyl ether (200 ml) acidified with conc. HCl (pH 1-2), and extracted with ethyl acetate (300 ml). The organic layers were combined, dried (Na_2SO_4), and concentrated to yield the crude product as a white solid. Purified by column chromatography (0-100% EtOAc/Hexanes) to yield compound **6** in 91% yield. IR (neat) 3325, 3032, 2988, 2950, 1731, 1653, 1461, 1422, 1258 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 1.58 (s, 6H), 5.10 (s, 2H), 5.46 (bs, 1H), 7.29-7.36 (m, 5H), 10.11 (bs, 1H) ^{13}C NMR (125 MHz, CDCl_3) δ 25.1 (2 C), 56.4, 66.9, 128.0 (2 C), 128.1, 128.5 (2 C), 136.2, 155.3, 179.5 HRMS (ESI) calc'd for $\text{C}_{12}\text{H}_{15}\text{NO}_4+\text{Na}^+$ = 260.0899, found 260.0895.

To a stirred solution of **6** (12.3 g, 52.1 mmol) in dry DMF (300 ml) was added HATU (20.0 g, 52.1 mmol), DIEA (20.2 g, 156.3 mmol) and allowed to stir for 20 min to activate the acid. Then, compound **5** (8.0 g, 52.1 mmol) was added and allowed to stir overnight. The reaction was diluted with ethyl acetate (400 ml) and washed with sat. NH_4Cl (2 x 300 ml), sat. NaHCO_3 (2 x 300 ml),

water (300 ml), brine (300 ml), dried (Na_2SO_4) and concentrated. Purified by column chromatography (30-35% EtOAc/Hexanes) to afford compound **7** as a white solid in 87% yield. IR (neat) 3321, 3032, 2986, 2948, 1724, 1660, 1513, 1454, 1385, 1251 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 1.51 (s, 6H), 1.52 (s, 6H) 3.72 (s, 3H) 5.10 (s, 2H), 5.30 (bs, 1H), 6.91 (bs, 1H) 7.29-7.37 (m, 5H) ^{13}C NMR (125 MHz, CDCl_3) δ 24.5 (2 C), 25.1 (2 C), 52.5, 56.5, 57.0, 66.7, 128.0 (2 C), 128.1, 128.5 (2 C), 136.4, 155.1, 173.4, 175.0 HRMS (ESI) calc'd for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_5 + \text{H}^+ = 337.1763$, found 337.1756.

Compound **7** (14.5 g, 43.1 mmol) was dissolved in dry methanol (215 ml) and 10% Pd/C (150 mg) was added slowly to the reaction vessel. Then a H_2 balloon was carefully fitted to the reaction vessel and allowed to stir overnight at room temperature. The H_2 balloon was removed and the solution filtered through a celite bed and concentrated to dryness to afford free amine as a gummy solid in 92% yield and taken directly into the next reaction without purification. The free amine was dissolved in dry DMF (50 ml) and added dropwise to the acid **6** (8.0 g, 39.5 mmol) in DMF (250 mL) HATU (15.0 g, 39.5 mmol) and DIEA (15.3 g, 118.5 mmol) after pre-activation for 20 min. The reaction was allowed to stir overnight at r.t.. The reaction was then diluted with ethyl acetate (400 ml) and washed with sat. NH_4Cl (2 x 300 ml), sat. NaHCO_3 (2 x 300 ml), water (300 ml), brine (300 ml), dried (Na_2SO_4) and concentrated. Purified by column chromatography (50-70% EtOAc/Hexanes) to afford compound **8** as a white solid in 92% yield. IR (neat) 3335, 2987, 2946, 1733, 1705, 1657, 1527, 1455, 1385, 1263 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 1.42 (s, 6H), 1.48 (s, 6H) 1.51 (s, 6H) 3.71 (s, 3H) 5.10 (s, 2H), 5.15 (bs, 1H), 6.33 (bs, 1H), 7.24 (bs, 1H), 7.33-7.39 (m, 5H) ^{13}C NMR (125 MHz, CDCl_3) δ 24.7 (2 C), 25.2 (2 C), 25.4 (2 C) 52.2, 56.1, 56.8, 57.3, 67.1, 128.2 (2 C), 128.4, 128.7 (2 C), 136.0, 155.4, 172.6, 173.3, 175.1 HRMS (ESI) calc'd for $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_6 + \text{H}^+ = 422.2291$, found 422.2484.

Compound **8** (13.0 g, 30.8 mmol) was dissolved in dry methanol (200 ml) and 10% Pd/C (150 mg) was added slowly to the reaction vessel. Then a H₂ balloon was carefully fitted to the reaction vessel and allowed to stir overnight at room temperature. The H₂ balloon was removed and the solution filtered through a celite bed and concentrated to dryness to afford free amine as a gummy solid in 91% yield and taken directly into the next reaction without purification. The free amine was dissolved in dry DMF (50 ml) and added dropwise to the acid **6** (6.6 g, 27.9 mmol) in DMF (150 ml) HATU (10.6 g, 27.9 mmol) and DIEA (10.8 g, 83.58 mmol) after pre-activation for 20 min. The reaction was allowed to stir overnight at r.t.. The reaction was then diluted with ethyl acetate (300 ml) and washed with sat. NH₄Cl (2 x 200 ml), sat. NaHCO₃ (2 x 200 ml), water (200 ml), brine (200 ml), dried (Na₂SO₄) and concentrated. Purified by column chromatography (80-100% EtOAc/Hexanes) to afford compound **8-1** as a white solid in 85% yield. IR (neat) 3324, 2985, 2942, 1730, 1703, 1652, 1519, 1454, 1384, 1262 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 1.33 (s, 6H), 1.46 (s, 6H), 1.47 (s, 6H) 1.51 (s, 6H) 3.68 (s, 3H) 5.11 (s, 2H), 5.30 (bs, 1H), 6.33 (bs, 1H), 7.10 (bs, 1H), 7.30 (bs, 1H) 7.33-7.37 (m, 5H) ¹³C NMR (125 MHz, CDCl₃) δ 24.9 (2 C), 25.2 (4 C), 25.3 (2 C) 51.9, 55.8, 56.8, 56.9, 57.3, 67.4, 128.2 (2 C), 128.6, 128.7 (2 C), 136.1, 155.7, 172.4, 173.6, 174.2, 175.4 HRMS (ESI) calc'd for C₂₅H₃₈N₄O₇+H⁺ = 507.2819, found 507.2806.

Compound **8.1** (10.8 g, 21.3 mmol) was dissolved in dry methanol (175 ml) and 10% Pd/C (150 mg) was added slowly to the reaction vessel. Then a H₂ balloon was carefully fitted to the reaction vessel and allowed to stir overnight at room temperature. The H₂ balloon was removed and the solution filtered through a celite bed and concentrated to dryness to afford free amine as a gummy solid in 98% yield and taken directly into the next reaction without purification. The free amine was

dissolved in dry DMF (50 ml) and added dropwise to mono-4-methoxytrityl-protected 3-mercaptopropionic acid **11** (7.9 g, 20.8 mmol) in DMF (100 ml) HATU (7.9 g, 20.8 mmol) and DIEA (8.1 g, 62.5 mmol) after pre-activation for 20 min. The reaction was allowed to stir overnight at room temperature. The reaction was then diluted with ethyl acetate (200 ml) and washed with sat. NH_4Cl (2 x 150 ml), sat. NaHCO_3 (2 x 150 ml), water (150 ml), brine (150 ml), dried (Na_2SO_4) and concentrated. Purified by column chromatography (80-100% EtOAc/Hexanes) to afford compound **9** as a white solid in 83% yield. IR (neat) 3309, 2985, 2935, 1729, 1645, 1507, 1441, 1383, 1249 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 1.35 (s, 6H), 1.46 (s, 6H), 1.52 (s, 12H), 1.99 (t, $J = 6.5$ Hz, 2H), 2.64 (t, $J = 6.5$ Hz, 2H), 3.69 (s, 3H), 3.81 (s, 3H), 6.12 (s, 1H), 6.50 (s, 1H), 6.85 (d, $J = 7.0$ Hz, 2H), 7.24-7.27 (m, 2H), 7.29-7.35 (m, 7H) 7.38 (s, 1H), 7.43-7.45 (m, 4H) ^{13}C NMR (125 MHz, CDCl_3) δ 24.9 (2 C), 25.1 (2 C), 25.3 (2 C), 25.4 (2 C), 28.0, 35.7, 51.9, 55.3, 55.8, 56.8, 57.0, 57.2, 66.7, 113.4, 126.9 (2 C), 128.0 (4 C), 129.4 (4 C), 130.7 (2 C), 136.4, 144.8, 158.4, 171.7, 172.8, 173.4, 174.5, 175.4 HRMS (ESI) calc'd for $\text{C}_{40}\text{H}_{52}\text{N}_4\text{O}_7\text{S}+\text{H}^+ = 733.3635$, found 733.3630.

Compound **9** (14.0 g, 19.11 mmol) was dissolved in methanol (100 ml), 6 N NaOH (100 ml) and stirred overnight at room temperature. The following day the reaction solution was heated to 60 °C for 24 h. Then, it was heated to 100 °C for 2 h, cooled to 60 °C for 12 h, cooled to 0 °C, and the pH adjusted (6-7). The solution was extracted with chloroform (3 x 200 ml). The organic layer was washed with brine (200 ml), dried (Na_2SO_4), and purified by column chromatography (0-10% MeOH/ CH_2Cl_2) to afford **9-1** in 87% yield as a white solid. IR (neat) 3292, 2985, 2935, 1739, 1652, 1531, 1508, 1442, 1385, 1250 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 1.33 (s, 6H), 1.41 (s, 6H), 1.45 (s, 6H), 1.56 (s, 6H), 2.17 (t, $J = 6.5$ Hz, 2H), 2.54 (t, $J = 6.5$ Hz, 2H), 3.78 (s, 3H), 6.80 (d, $J = 9.0$ Hz, 2H), 6.92 (bs, 1H), 7.19-7.21 (m, 2H), 7.25-7.30 (m, 7H) 7.38-7.39 (m, 4H), 7.43-7.45 (m, 4H), 7.63 (bs, 1H), 7.78 (bs, 1H) ^{13}C NMR (125

MHz, CDCl₃) δ 24.8 (2 C), 24.9 (2 C), 25.0 (2 C), 25.2 (2 C), 27.8, 35.2, 55.2, 56.8, 56.9, 57.0, 57.4, 66.6, 113.3, 126.8 (2 C), 127.9 (4 C), 129.5 (4 C), 130.8 (2 C), 136.7, 145.0, 158.3, 172.3 (2 C), 174.4, 175.6, 175.7 HRMS (ESI) calc'd for C₃₉H₅₀N₄O₇S+Na⁺ = 741.3298, found 741.3296.

To a solution of compound **9-1** (0.05 g, 0.07 mmol) in dry DMF (0.4 ml), HATU (0.027 g, 0.07 mmol) and DIPEA (0.02 g, 0.14 mmol) were added. The reaction was allowed to stir for 30 min to

activate the acid. Then tetra-*O*-acetyl-3-aminopropyl D-mannoside (0.03 g, 0.08 mmol) was added and allowed to stir overnight. The reaction was diluted with ethyl acetate (400 ml) and washed with sat. NH₄Cl (2 x 300 ml), sat. NaHCO₃ (2 x 300 ml), water (300 ml), brine (300 ml), dried (Na₂SO₄) and concentrated. The material was then redissolved in dry methanol (2 ml) and sodium methoxide (1 mg, 0.02 mmol) and allowed to stir overnight. This material was then purified directly by HPLC. After lyophilization, compound **10** was afforded as a white solid in 78% yield over 2 steps. $[\alpha]_D^{23}$ -4° (c 1.0, CH₃OH) IR (neat) 3311, 2983, 2933, 1646, 1508, 1423, 1381, 1250 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 1.32 (s, 6H), 1.38 (s, 6H), 1.41 (s, 6H), 1.49 (s, 6H), 1.84 (p, *J* = 6.5 Hz, 2H), 2.34 (t, *J* = 7.0 Hz, 2H), 2.47 (t, *J* = 7.0 Hz, 2H), 3.49-3.55 (m, 2H), 3.62 (t, *J* = 9.5 Hz, 1H), 3.69-3.73 (m, 2H), 3.79-3.84 (m, 3H), 4.76 (d, *J* = 1.5 Hz, 1H), 6.82 (d, *J* = 10 Hz, 2H), 7.19-7.22 (m, 2H), 7.24-7.28 (m, 6H), 7.35-7.37 (m, 4H), 7.64-7.66 (m, 3H), 7.86 (bs, 1H), 8.25 (bs, 1H) ¹³C NMR (125 MHz, CDCl₃) δ 23.6 (2 C), 24.0 (2 C), 24.2 (2 C), 24.7 (2 C), 27.5, 28.9, 34.4, 36.2, 36.4, 56.2 (2 C), 56.3, 56.4, 56.8, 56.9, 61.6, 66.3, 67.4, 70.9, 71.4, 73.1, 100.4, 112.9, 126.4 (2 C), 127.5 (4 C), 129.3 (4 C), 130.6 (2 C), 136.7, 145.1, 158.5, 172.4, 175.1, 175.2, 175.9, 176.2 HRMS (ESI) calc'd for C₄₈H₆₇N₅O₁₂S+H⁺ = 938.4585, found 938.4568.

Compound **10** was dissolved in a solution of hexafluoroisopropanol, dichloromethane, trifluoroacetic acid, triisopropylsilane 10:7:2:1. The reaction was allowed to stir for 45 min. The solvent was then concentrated and remaining traces of trifluoroacetic acid

were removed by repetitive co-evaporations with diethyl ether. This material was then purified on an HPLC. After lyophilization, compound **4** was afforded as a white solid in 65% yield. $[\alpha]_D^{23} -6^\circ$ (c 1.0, CH₃OH) IR (neat) 3400, 2983, 2935, 1647, 1473, 1419, 1379, 1229 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 1.41 (s, 6H), 1.42 (s, 6H), 1.45 (s, 6H), 1.50 (s, 6H), 1.85 (p, $J = 7.0$ Hz, 2H), 2.56 (t, $J = 6.5$ Hz, 2H), 2.79 (t, $J = 6.5$ Hz, 2H), 3.50-3.55 (m, 2H), 3.61 (t, $J = 9.5$ Hz, 1H), 3.69-3.72 (m, 2H), 3.76-3.83 (m, 3H), 4.76 (d, $J = 1.0$ Hz, 1H), 7.71 (bs, 1H), 7.79 (bs, 1H), 7.96 (bs, 1H), 8.35 (bs, 1H) ¹³C NMR (125 MHz, CDCl₃) δ 23.7, 23.8, 23.9, 24.0, 24.1, 24.2, 24.6, 24.8, 28.9, 36.2, 38.9, 56.3, 56.5, 56.7, 56.8, 56.9, 61.6, 64.7, 67.4, 70.9, 71.3, 73.1, 100.3, 172.4, 175.2, 175.4, 176.0, 176.3 HRMS (ESI) calc'd for C₂₈H₅₁N₅O₁₁S+H⁺ = 666.3384, found 666.3506.

5. Gold Preparation and SAM Formation. For the IRRAS, XPS, and electrochemistry experiments we used 11 mm x 11 mm ArrandeeTM gold plates, which consist of a borosilicate glass substrate (thickness 0.7 ± 0.1 mm), a thin chromium interlayer (2.5 ± 1.5 nm), and a gold layer (250 ± 50 nm). The plates were first immersed in ethanol for 5 min, dried in vacuum for 30 min, immersed for 5 min in a freshly prepared but already cold piranha solution (3:1 v/v solution of concentrated sulfuric acid and hydrogen peroxide. *Extreme caution must be exercised when using piranha solution, as it is a very strong oxidant and reacts violently with organic matter*), and then carefully washed with a stream of bi-distilled water. The plates were then dipped into ethanol for 5 min and dried in a desiccator under vacuum for 30 min. Annealing was carried out using a butane-air flame in a dark room. Annealing eventually caused appearance of dark red glowing on the gold surface. After waiting a few seconds to allow the surface to cool down, the same sequence step was repeated for an overall 3 min. The plates were left to

cool down for 30 min, under a protecting glass cap. The annealing/cooling procedure was repeated three times. After annealing and washing (first in water and then in ethanol), the freshly prepared gold plates were immersed for 48 h in a 0.5 mM solution of the peptide of choice (for **1-3**, in ethanol; for **4**, in 1:1 water/ethanol). To remove any trace of the incubation solution and loose molecules adsorbed on the monolayer, the gold plates were washed with a stream of ethanol and then immersed in ethanol for 5 min; these washing steps were repeated three times. The plates were finally dried under vacuum for 30 min. The whole procedure was applied just before carrying out the electrochemistry, IRRAS, PM-IRRAS, or XPS experiment. For the electrochemical experiments the plates were clamped to a small gold alligator and most of the electrode surface was wrapped with very Teflon tape to yield an exposed electrode area of typically $\sim 0.15 \text{ cm}^2$.

The gold chips for SPR were prepared via electron beam evaporation at the University of Washington Microfabrication Facility (Seattle, WA). Titanium (2 nm) and gold (45 nm) films were deposited onto cleaned SF-10 glass (18 mm $\times$ 18 mm; Schott Glass Technology). The SPR gold chips were attached to a nine-element silicone mask (culture-well silicone sheets were purchased from Grace Bio-Laboratories). Each of the nine-element wells was spotted with 0.5 mM peptide solutions (total concentration of the two peptides) in 1:1 water/ethanol. After 48 h incubation, the gold chips were washed with ethanol and dried under a stream of nitrogen.

6. Electrochemistry. The measurements were conducted in an all-glass heart-shaped electrochemical cell thermostated at 25 °C. Prior to each measurement, the electrolytic solution was purged with argon for 15 min, and then a blanket of argon was maintained over the solution during the entire experiment. For the reductive desorption experiments, a CHI 660 C electrochemical workstation was used. The electrolytic solution was aqueous 0.1 M NaOH and thus we used a mercury oxide reference electrode (MOE), known to be particularly suitable for measurements in alkaline solutions; the specific electrode we used had a potential of -132 mV vs the saturated calomel reference electrode (SCE), in line with the official value of -140 mV.^{S6} A Pt ring was used as the counter-electrode. After immersion of the electrode into the solution, the SAM was stabilized by

multiscan CV (20 cycles at 0.1 V s⁻¹) from -0.1 to -0.45 V MOE. The reductive desorption CV curve was recorded at a scan rate of 0.05 V s⁻¹, from -0.1 to -1.15 V. The peak potential values are the result of at least three separate experiments. To ensure complete removal of the thiolated peptide from the surface (because of their limited solubility), we applied to the electrode 50 more cycles at 0.5 V s⁻¹ in the same potential range. Determination of the electrochemical area of each electrode was then carried out by running CV experiments at 0.1 – 1 V s⁻¹ in 0.5 M KCl containing 3 mM hexammineruthenium(III) chloride, whose diffusion coefficient is $6.84 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The geometrical areas were calculated by integration from pictures of the exposed electrode surface in comparison with area standards. The roughness factor was calculated from the ratio between the electrochemical and the geometrical area, and found to be 1.16 ± 0.08 . Current and charge values were normalized relative to the electrochemical area.

7. Infrared Reflection Absorption Spectroscopy. To correct each spectrum for the background, we built a baseline using the spline interpolation technique.^{S7} To determine the tilt of the peptides inside the SAMs, we employed a method using a relationship between the experimental amide 1 / amide 2 absorbance ratio (A_1/A_2) and a function of the tilt angles of the helical axis (θ) and the transition moments related to the amide 1 (β_1) and amide 2 (β_2) modes with respect to the surface normal.^{S8} The orientation of the peptides bound to the gold surface was thus determined according to eq S1,^{S9} assuming an isotropic peptide monolayer:

$$(S1) \quad \frac{A_1}{A_2} = K \frac{2 \left[\frac{1}{2} (3 \cos^2 \theta - 1) \right] \left[\frac{1}{2} (3 \cos^2 \beta_1 - 1) \right] + 1}{2 \left[\frac{1}{2} (3 \cos^2 \theta - 1) \right] \left[\frac{1}{2} (3 \cos^2 \beta_2 - 1) \right] + 1}$$

where K is the scaling factor related to the intrinsic “oscillator strength” of the amide 1 and amide 2 vibrational modes. K was calculated from the amide 1 / amide 2 absorbance ratio in a KBr pellet: for our peptides, $K = 1.75 \pm 0.2$. For the transition moments, we bracketed the β_1 and β_2 angles between 39 and 45° and 75 and 90°, which correspond to

available and/or adopted values for the 3_{10} -helix axis.^{S10-S13} Use of the two opposite combinations (39 and 90°; 45 and 75°) makes the tilt angles to change by no more than 2.5 and 8.4° for the most and the least inclined peptides, respectively. The error on K introduces a maximum $\pm 3^\circ$ uncertainty. To the tilt angles we associate an error of $\pm 5^\circ$, also considering the contributions of possible imperfections of surface and monolayer.

8. Surface Plasmon Resonance. Figure S1 shows the outcome of SPR binding experiments carried out by exposing SAMs containing different amounts of **4** or its galactose-equivalent (with peptide **1** as the diluent) to a PBS buffer solution of 500 nM Ricin, previously dialyzed overnight against PBS.

Figure S1. Time dependence of the SPR response of 500 nM Ricin, in PBS buffer, to the SAMs containing thiolated mannose-peptide **4** (50 and 100%) or its galactose-equivalent (50 and 100%), and peptide **1** (50 and 100%) as indicated in the legend. Percentages refer to the amount of glycopeptide in the incubating solutions. The dissociation curves (~750 s) correspond to washing the surfaces with PBS buffer.

Figure S2 shows the time dependence of the SPR response of 100 nM ConA, in HEPES buffer, to the SAMs prepared from thiolated mannose-peptide **4** and diluent peptide HS(CH₂)₂CO-(Aib)₄OH in place of **1**. It is interesting to note that, compared to Figure 3, which includes **1** as the diluent, the presence of carboxylate groups (at the working pH value, the free acids are deprotonated) appears to facilitate adsorption of ConA at low mannose surface concentrations.

Figure S2. Time dependence of the SPR response of 100 nM ConA, in HEPES buffer, to the SAMs containing thiolated mannose-peptides **4** and diluent peptide HS(CH₂)₂CO-(Aib)₄OH. The curves correspond to the following percentages of **4** in the incubating solutions (bottom to top): 0, 5, 10, 25, 50, 75, 90, and 100%. The decreasing curves correspond to washing the surfaces with HEPES buffer.

The analysis of the SPR association and dissociation curves was carried out with SigmaPlot 12. We based the analysis on exponential kinetics.^{S14} The association rate constant k_a and the dissociation rate constant k_d define the association equilibrium constant K_a , i.e., $K_a = k_a/k_d$. K_a refers to equilibrium S2:

where $[C]$ is the concentration of ConA (because of the continuous flow of solution over the chip, this surface concentration is assumed to be constant during the association step and the same as the ConA bulk concentration), $[S]$ is the concentration of free mannose binding sites, and $[CS]$ is the concentration of bound ConA on the surface. For each experimental graph (cf Figure 3), the curves were fitted using an exponential increase association model (eq S3) and an exponential decrease for the dissociation of the adsorbed species (eq S4). $[S]_0$ is the total concentration of available surface sites, i.e., $[CS] = [S]_0 - [S]$. Eq S3 is based on the assumption that $k_a \gg k_d$ (in our case, $K_a \sim 10^6$ - 10^7 M^{-1}).

$$(S3) \quad [CS] = [S]_0 [1 - \exp(-kt)]$$

$$(S4) \quad [CS] = [CS]_0 \exp(-k_d t)$$

$$(S5) \quad k = k_d + k_a [C]$$

A series of such simulations is shown in Figure S3. k (eq S5) is the observed rate constant of eq S3. We determined k_a as the slope of the k versus $[C]$ plots obtained from each SAM studied (from 10 to 100% nominal concentration of **4** in the SAM). Figure S4 shows typical plots. For the calculation of K_a , plotted in Figure S5, we used the k_d values obtained from eq S4: this approach provides more precise k_d values than those obtained from eq S5, as intercepts are affected by relatively large errors. The K_a values here determined are in agreement with values reported for other SAM systems, ranging from 10^6 to 10^7 M^{-1} .^{S15} The increase of K_a with an increase of the concentration of peptide **4** in the monolayer is associated with both a slight decrease of k_d (Figure S6) and a slight increase of k_a (Figure S7).

Figure S3. Time dependence of the SPR response of 100 nM ConA, in HEPES buffer, to the SAMs containing thiolated mannose-peptides **4** and peptides **1** (red curves). The curves correspond to the following percentages of **4** in the incubating solutions (bottom to top): 0, 10, 25, 32.5, 50, 62.5, 75, 90 and 100%. The decreasing curves correspond to washing the surfaces with HEPES buffer. The corresponding exponential simulations are shown as black lines.

Figure S4. Examples of determination of k_a and k_d values from experiments carried out at (bottom to top) 10, 50, and 100% mannose-peptide **4** in the SAM. Percentages refer to the amount of **4** in the incubating solutions.

Figure S5. Dependence of the value of K_a on the amount of mannose-peptide **4** in the SAM. Percentages refer to the amount of **4** in the incubating solutions.

Figure S6. Dependence of the value of k_d on the amount of mannose-peptide **4** in the SAM. Percentages refer to the amount of **4** in the incubating solutions.

Figure S7. Dependence of the value of k_a on the amount of mannose-peptide **4** in the SAM. Percentages refer to the amount of **4** in the incubating solutions.

9. X-Ray photoelectron spectroscopy (XPS). The surface chemical composition of the SAMs was investigated by X-ray Photoelectron Spectroscopy. The samples were transferred into the fast-entry lock system of the XPS vacuum chamber without ambient exposure, to avoid surface modification or atmospheric contamination. Analysis was performed on a Perkin-Elmer Φ 5600-ci spectrometer using non-monochromatized Al-K α radiation (1486.6 eV) working at 280 W. The sample analysis area was 800 μm in diameter and the working pressure lower than 10^{-9} mbar. The spectrometer was calibrated by assuming the binding energy (BE) of the Au 4f $_{7/2}$ line to be 83.9 eV with respect to the Fermi level. The standard deviation for the BEs values was ± 0.2 eV. Survey scans were obtained in the 0–1350 eV range (187.85 eV pass energy, 0.5 eV/step, 0.025 s/step). Detailed scans were recorded for the C1s, N1s, O1s, S2p and Au4f regions (58.70 eV pass energy, 0.125 eV/step, 0.05 s/step). No further element was detected. The analysis involved Shirley-type background subtraction^{S16} and peak area determination by integration. The atomic compositions were evaluated from peak areas using sensitivity factors supplied by Perkin-Elmer, taking into account the geometric configuration of the

apparatus.^{S17} The experimental uncertainty on the atomic composition values was within $\pm 5\%$.

The XPS analysis was carried out on SAM samples prepared from 0.5 mM peptide solutions (total concentration of the two peptides) in 1:1 water/ethanol. The solutions contained peptide **4** in 0, 50, 75, and 100% concentration. Peptides **1** and **4** have the same number of nitrogen (5) and sulfur (1) atoms. Despite the weakness of the sulfur signal, which makes the N/S ratio ca. 10% larger than the theoretical value of 5, the XPS results show that the N/S ratio does not change, within 7%, which points to the integrity of the peptides in the SAMs. To assess the concentration of peptide **4** in the various SAMs, we relied on the ratio between oxygen and nitrogen, where the latter served as an internal normalization factor. XPS analysis shows that both oxygen and, to a lesser extent, carbon contents increase as the amount of glycopeptide **4** in the incubating solutions also increases. Since the relative increase of oxygen is larger than that of carbon, we focused on the former parameter. Figure S8 shows the trend of the relative increase of the O/N

Figure S8. Dependence of the SAMs' O/N ratio on the corresponding ratios calculated using the composition of the original solutions, in which the amount of **4** was (left to right) 0, 50, 75, and 100%. The values are reported as relative increases from the value obtained or calculated for the 0% case (only peptide **1**). The dashed lines are meant to evidence the experimental trend (see text).

ratio with respect to the 0% SAM, plotted versus the corresponding calculated increase, assuming that the SAM composition is the same as in the original solutions. The plot shows that the relative amount of oxygen increases, as expected. Two trend lines are displayed. Linear regression shows that the SAM composition is within ca. 20% as in solution, which, given the various errors associated to these determinations, can be taken as a reasonable overall error. The curved trend would point to a more pronounced coverage of the gold surface by the smaller diluent peptide **1** with respect to glycopeptide **4** in the mixed SAMs. Both trends, however, are consistent with the expected increase in O/N ratio within XPS experimental variance.

10. References

- (S1) Peggion, C.; Formaggio, F.; Toniolo, C.; Becucci, L.; Moncelli, M. R.; Guidelli, R. *Langmuir* **2001**, *17*, 6585-6592.
- (S2) Fabris, L.; Antonello, S.; Armelao, L.; Donkers, R. L.; Polo, F.; Toniolo, C.; Maran, F. *J. Am. Chem. Soc.* **2006**, *128*, 326-336.
- (S3) Becucci, L.; Guryanov, I.; Maran, F.; Guidelli, R. *J. Am. Chem. Soc.* **2010**, *132*, 6194-6204.
- (S4) Mourtas, S. ; Dimitrios, K. ; Vagiani, K. ; Katakalous, C. ; Barlos, K. *Tetrahedron Lett.* **2001**, *42*, 6965-6968.
- (S5) Appeldoorn, C. M. ; Joosten, J. A. F. ; el Maate, F. A. ; Dobrindt, U. ; Hacker, J. ; Liskamp, R. M. J. ; Khan, A. S. ; Pieters, R. J. *Tetrahedron: Asymmetry* **2005**, *16*, 361-372.
- (S6) Rondinini, S.; Longhi, P.; Mussini, P. R.; Mussini, T. *Pure & Appl. Chem.* **1994**, *66*, 641-647.
- (S7) Zamylny, V.; Zawisza, I.; Lipkowski, J. *Langmuir* **2003**, *19*, 132-145.
- (S8) (a) Enriquez, E. P.; Samulski, E. T. *Mat. Res. Soc. Symp. Proc.* **1992**, *255*, 423-434. (b) Wen, X.; Linton, R.W.; Formaggio, F.; Toniolo, C.; Samulski, E. T. *J. Phys. Chem. A* **2004**, *108*, 9673-9681.
- (S9) Miura, Y.; Kimura, S.; Imanishi, Y.; Umemura, J. *Langmuir* **1998**, *14*, 6935-6940.

- (S10) Malcolm, B. R.; Walkinshaw, M. D. *Biopolymers* **1986**, *25*, 607–625.
- (S11) Goormaghtigh, E.; Raussens, V.; Ruyschaert, J.-M. *Biochimica et Biophysica Acta*. **1999**, *1422*, 105-185.
- (S12) Nguyen, K. T.; Le Clair, S.; Ye, S. J.; Chen, Z. *J. Phys. Chem. B* **2009**, *113*, 12169–12180.
- (S13) Arikuma, Y.; Nakayama, H.; Morita, T.; Kimura, S. *Angew. Chem. Int. Ed.* **2010**, *49*, 1800-1804.
- (S14) *Surface Plasmon Resonance Based Sensors*, Homola, J. Ed.; Springer: 2006.
- (S15) (a) Smith, E. A.; Thomas, W. D.; Kiessling, L. L.; Corn, R. M. Surface Plasmon Resonance Imaging Studies of Protein-Carbohydrate Interactions. *J. Am. Chem. Soc.* **2003**, *125*, 6140-6148. (b) Zhang, Y.; Luo, S.; Tang, Y.; Yu, L.; Hou, K.-Y.; Cheng, J.-P.; Zeng, X.; Wang, P. G. *Anal. Chem.* **2006**, *78*, 2001-2008. (c) Liang, P. H.; Wang, S.-K.; Wong, C.-H. *J. Am. Chem. Soc.* **2007**, *129*, 11177-11184. (d) Jaipuri, F. A.; Collet, B. Y. M.; Pohl, N. L. *Angew. Chem.* **2008**, *120*, 1731-1734. (e) Mori, T.; Toyoda, M.; Ohtsuka, T.; Okahata, Y. *Anal. Biochem.* **2009**, *395*, 211-216. (f) Schlick, K. H.; Cloninger, M. J. *Tetrahedron* **2010**, *66*, 5305-5310. (g) Cheng, F.; Shang, J.; Ratner, D. A. *Bioconjugate Chem.* **2011**, *22*, 50-57. (h) Pandey, B.; Tan, Y. H.; Fujikawa, K.; Demchenko, A. V.; Stine, K. J. *J. Carbo. Chem.* **2012**, *31*, 466-503. (i) Sato, Y.; Yoshioka, K.; Murakami, T.; Yoshimoto, S.; Niwa, O. *Langmuir* **2012**, *28*, 1846–1851.
- (S16) Shirley, D. A. *Phys. Rev. B* **1972**, *5*, 4709-4714.
- (S17) Moulder, J. F.; Stickle, W. F.; Sobol, P. W.; Bomben, K. D. *Handbook of X-ray Photoelectron Spectroscopy*; Perkin-Elmer: Eden Prairie, MN, 1992.