Electronic Supplementary Material (ESI) for Chemical Communications

Selection of a Synthetic Glycan Oligomer from a Library of DNA-Templated Fragments Against DC-SIGN and Inhibition of HIVgp120 Binding to Dendritic Cell

Supporting information

Contents

General Techniques.

All reactions performed in solution were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through commercially available alumina columns (Innovative technology, Inc., MA). Reactions in solution were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and 10% ethanolic phosphomolybdic acid or vanillin solution and heat as developing agents and by LC-MS. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. Resins were purchased from Novabiochem. NMR spectra were recorded on Bruker Advance-400 instrument at 400 (^{1}H), 100 (^{13}C) MHz. Chemical shifts are given in parts per million (δ) and calibrated using residual un-deuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. LC-MS were recorded using an Agilent 1100 HPLC and Surveyor MSQ spectrometer (Thermo Scientific). Unless otherwise stated, a Supelco C18 (5 cm x 4.6mm, 5 μm particles) column was used with a linear elution gradient from 95% H_2O (0.01% TFA) to 100% MeCN in 8 min at a flow rate of 0.7 mL/min. PNAs were synthesized on an Intavis MultiPep instrument in a fully automated fashion using NovaPEG Rink Amide resin loaded at 0.2 mmol/g in 500 μL fritted tubes. The reactions were monitored by cleaving an aliquot of resin and analyzing the cleavage product by LC-MS and MALDI. The MALDI spectra were measured using a Brucker Daltonics AutoflexII TOF spectrometer. Dialyses were performed using a Spectrum Laboratories Spectra/Por® Regenerated Cellulose Dialysis Membrane with a MW cutoff of 6-8.000. The BIACORE 3000 system, sensor chip CM5, surfactant P20, amine coupling kit containing N-hydroxysuccinimide (NHS) and N-Ethyl-N'-dimethylaminopropyl carbodiimide (EDC), were from BIACORE (Upsala, Sweden).

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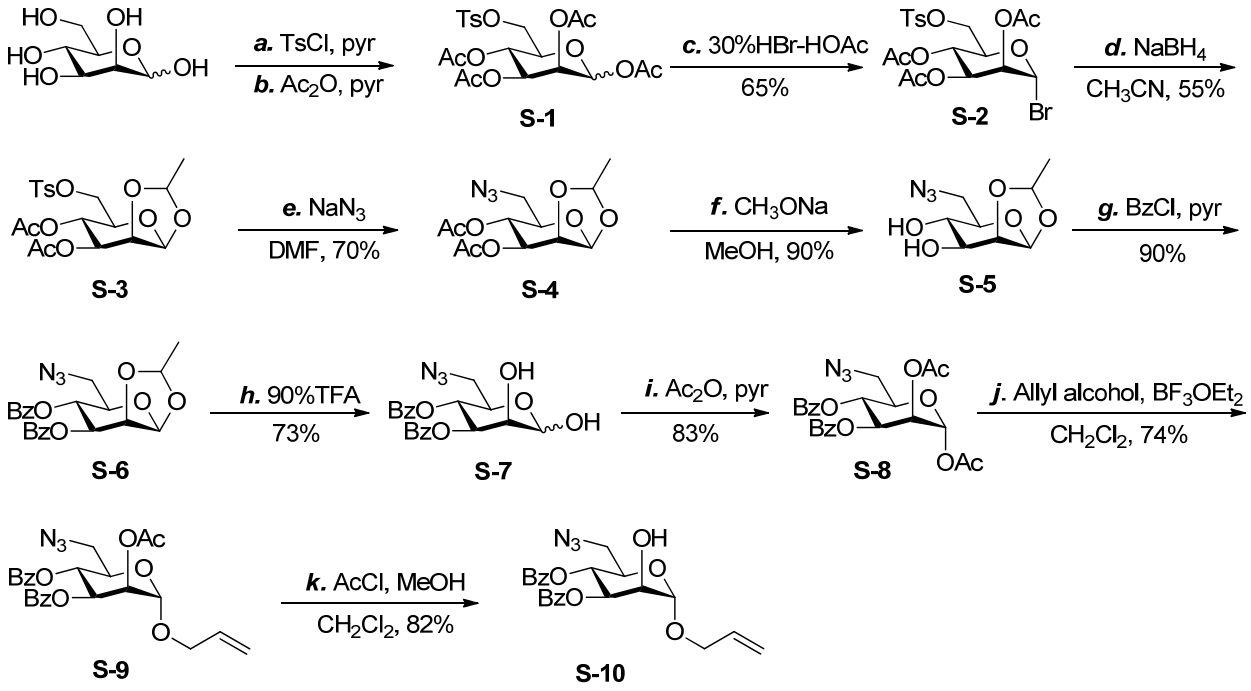
Synthesis of monosaccharide S-10



3,4-di-*O*-acetyl-6-*O*-tosyl-1,2-ethylidene-*β*-D-mannopyranoside (S-3)[1]: This compound was prepared following known procedures in an overall 55% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (d, J = 4.8 Hz, 3H), 1.92 (s, 3H), 2.01 (s, 3H), 2.36 (s, 3H), 3.68-3.71 (m, 1H), 4.00-4.09 (m, 2H), 4.16 (t, J = 2.4 Hz, 1H), 5.14-5.18 (m, 3H), 5.21 (d, J = 2.4 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 21.3, 21.5, 66.2, 68.2, 70.1, 71.2, 77.1, 96.5, 104.4, 128.0, 129.8, 132.4, 145.0, 169.5, 170.1; HRMS (MALDI-TOF): *m/z*: calcd for $C_{19}H_{24}O_{10}SNa$ $(M+Na)^+$: 467.0988; found 467.1010.

3,4-di-*O*-acetyl-6-azido-1,2-ethylidene-*β*-D-mannopyranoside (S-4)[2]: This compound was prepared following a known procedure in a 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, J = 4.8 Hz, 3H), 1.96 (s, 3H), 2.02 (s, 3H), 3.23 (dd, J = 13.0, 6.0 Hz, 1H), 3.33 (dd, J = 13.2, 3.2 Hz, 1H), 3.57-3.61 (m, 1H), 4.17 (dd, J = 3.6, 2.4 Hz, 1H), 5.13-5.26 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 21.4,

[1] a) Jeong, L. S.; Schinazi, R. F.; Beach, J. W.; Kim, H. O.; Shanmuganathan, K.; Nampalli, S.; Chun, M. W.; Chung, W. K.; Choi, B. G.; Chu, C. K.; *J. Med. Chem.* **1993**, *36*, 2627-2638. b) Mori, T.; Hatano, K.; Matsuoka, K.; Esumi, Y.; Tooned, E. J.; Terunuma, D. *Tetrahedron,* **2005**, *61*, 2751-2760.
[2] Kong, D. C. M.; Itzstein, M. Von; *Carbohydr. Res.* **1998**, *305*, 323-329.

51.0, 66.8, 70.3, 72.6, 77.2, 96.4, 104.6, 169.4, 170.1; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{12}H_{17}O_7N_3Na$ $(M+Na)^+$: 338.0964; found 338.0946.

6-azido-1,2-ethylidene-*β*-D-mannopyranoside (S-5): Compound **S-4** (31.6 mmol) was dissolved in MeOH (80 mL) and NaOMe (4.74 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was neutralized by adding Amberlite IRC-50. After filtration, the filtrates were concentrated. The residue was purified by flash silica gel column chromatography to afford compound **S-5** in a 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (d, J = 4.8 Hz, 3H), 3.38-3.45 (m, 2H), 3.56-3.61 (m, 1H), 3.73 (t, J = 8.8 Hz, 1H), 3.80 (dd, J = 7.2, 4.0 Hz, 1H), 4.14 (dd, J = 3.8, 2.4 Hz, 1H), 5.28-5.31 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 51.0, 67.6, 71.4, 74.5, 80.4, 96.6, 104.0; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_8H_{13}O_5N_3Na$ $(M+Na)^+$: 254.0753; found 254.0757.

3,4-di-*O*-benzoyl-6-azido-1,2-ethylidene-*β*-D-mannopyranoside (S-6)[3]: This compound was prepared following a known procedure in a 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (d, J = 4.8 Hz, 3H), 3.48 (dd, J = 13.2, 6.0 Hz, 1H), 3.55 (dd, J = 13.2, 2.8 Hz, 1H), 3.90-3.94 (m, 1H), 4.53 (dd, J = 3.6, 2.4 Hz, 1H), 5.35 (dd, J = 9.8, 4.8 Hz, 1H), 5.49 (d, J = 2.8 Hz, 1H), 5.64 (dd, J = 10.0, 4.0 Hz, 1H), 5.86 (t, J = 9.6 Hz, 1H), 7.39-7.43 (m, 4H), 7.52-7.57 (m, 2H), 7.96-8.04 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 51.3, 67.3, 71.2, 73.2, 77.4, 96.7, 104.9, 128.5, 128.5, 128.9, 128.9, 129.8, 130.0, 133.5, 133.6, 165.4, 166.0; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{22}H_{21}O_7N_3Na$ $(M+Na)^+$: 462.1277; found 462.1257.

3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S-7)[3]: This compound was prepared following a known procedure in a 73% yield ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (dd, J = 13.2, 2.8 Hz, 1H), 3.48 (dd, J = 13.2, 6.0 Hz, 1H), 4.26 (dd, J = 3.0, 2.0 Hz, 1H), 4.41-4.46 (m, 1H), 5.28 (d, J = 2.0 Hz, 1H), 5.68 (dd, J = 10.0, 3.2 Hz, 1H), 5.80 (t, J = 10.0 Hz, 1H), 7.33-7.53 (m, 6H), 7.91-7.98 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.4, 68.1, 69.5, 72.6, 94.6, 128.0, 128.2, 129.1, 129.2, 129.3, 129.5, 133.0, 133.2, 165.8, 165.9; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{20}H_{19}O_7N_3Na$ $(M+Na)^+$: 436.1121; found 436.1086.

1,2-di-*O*-acetyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S-8)[3]: This compound was prepared following a known procedure in an 83% yield ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.23 (s, 3H), 3.44 (dd, J = 13.6, 5.6 Hz, 1H), 3.53 (dd, J = 13.6, 2.8 Hz, 1H), 4.26-4.31 (m, 1H), 5.51 (dd, J = 3.2, 2.0 Hz, 1H), 5.79 (dd, J = 10.0, 3.2 Hz, 1H), 5.87 (t, J = 10.0 Hz, 1H), 6.26 (d, J = 2.0 Hz, 1H), 7.34-7.54 (m, 6H), 7.89-7.99 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.8, 50.8, 66.9, 68.6, 69.3, 72.1, 90.4, 128.5, 128.5, 128.7, 128.9, 129.6, 129.8, 133.5, 133.7, 165.3, 165.5, 168.0, 169.6; HRMS (MALDI-

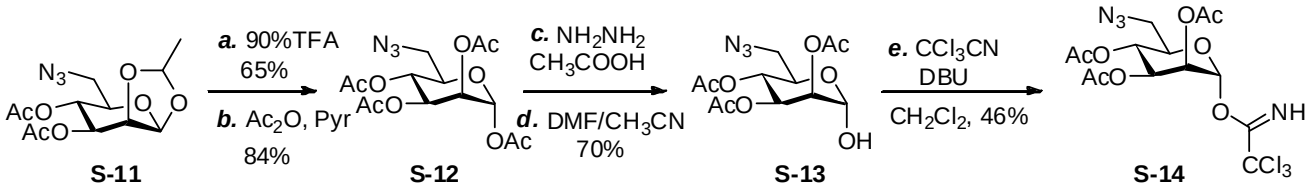
[3] Heng, L.; Ning, J.; Kong, F. *J. Carbohy. Chem.* **2001**, *20*, 285-296.

TOF): *m*/*z*: calcd for $C_{24}H_{23}O_9N_3Na$ $(M+Na)^+$: 520.1332; found 520.1282

Allyl 2-*O*-acetyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S-9): Compound **S-8** (9.02 mmol) was dissolved in CH_2Cl_2 (50 mL), cooled to 0°C and allyl alcohol (18.05 mmol) was added. BF_3Et_2O (36.1 mmol) was added dropwise over the course of 5 min, and the reaction mixture was stirred at room temperature for 24 h. The mixture was neutralized with saturated aqueous sodium bicarbonate solution and diluted with CH_2Cl_2. After washing with water and brine, the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-9** in a 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.42 (dd, J = 13.4, 2.4 Hz, 1H), 3.52 (dd, J = 13.4, 7.2 Hz, 1H), 4.15 (dd, J = 12.6, 6.0 Hz, 1H), 4.24-4.29 (m, 1H), 4.33 (dd, J = 12.8, 5.2 Hz, 1H), 5.04 (d, J = 2.8 Hz, 1H), 5.29 (dd, J = 10.4, 1.2 Hz, 1H), 5.41 (dd, J = 17.2, 1.6 Hz, 1H), 5.52 (dd, J = 3.2, 2.0 Hz, 1H), 5.77 (t, J = 10.0 Hz, 1H), 5.84 (dd, J = 10.0, 3.2 Hz, 1H), 5.92-6.02 (m, 1H), 7.31-7.52 (m, 6H), 7.90-7.98 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 51.2, 67.7, 68.8, 69.7, 69.9, 70.4, 96.5, 118.5, 128.4, 128.5, 128.8, 129.2, 129.6 129.8, 132.9, 133.3, 133.6, 165.3, 165.5, 169.8; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{25}H_{25}O_8N_3Na$ $(M+Na)^+$: 518.1539; found 518.1581.

Allyl 3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S-10)[4]: This compound was prepared following a known procedure in an 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24 (br, 1H), 3.34 (dd, J = 12.8, 2.4 Hz, 1H), 3.57 (dd, J = 13.4, 7.6 Hz, 1H), 4.16 (dd, J = 13.0, 6.4 Hz, 1H), 4.21-4.26 (m, 1H), 4.33-4.38 (m, 2H), 5.06 (d, J = 1.6 Hz, 1H), 5.30 (dd, J = 10.4, 1.6 Hz, 1H), 5.43 (dd, J = 17.2, 1.6 Hz, 1H), 5.70 (dd, J = 9.8, 2.8 Hz, 1H), 5.82 (t, J = 10.0 Hz, 1H), 5.95-6.05 (m, 1H), 7.29-7.53 (m, 6H), 7.94-7.97 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.4, 67.9, 68.6, 69.3, 70.4, 72.5, 98.7, 118.3, 128.4, 128.5, 128.9, 129.2, 129.8, 133.2, 133.3, 133.5, 165.7, 165.8; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{23}H_{23}O_7N_3Na$ $(M+Na)^+$: 476.1434; found 476.1455.

Synthesis of monosaccharide S-14



1,2,3,4-tetra-*O*-acetyl-6-azido-*α*-D-mannopyranoside (S-12)[3]: This compound was prepared following

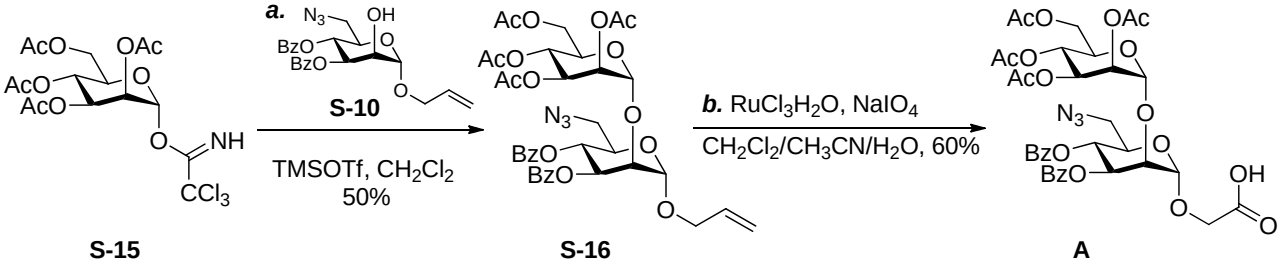
[4] Kwon, Y.-U.; Soucy, R. L.; Snyder, D. A.; Seeberger, P. H. *Chem. Eur. J.* **2005**, 11, 2493-2504.

a known procedure in an overall 84% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H), 1.97 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 3.23 (dd, J = 13.6, 5.6 Hz, 1H), 3.32 (dd, J = 13.6, 3.2 Hz, 1H), 3.92-3.93 (m, 1H), 5.16 (t, J = 2.0 Hz, 1H), 5.24-5.25 (m, 2H), 6.00 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.6, 20.6, 50.5, 66.3, 68.2, 68.5, 71.7, 90.2, 168.0, 169.4, 169.6, 169.8; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{14}H_{19}O_9N_3Na$ $(M+Na)^+$: 396.1019; found 396.1018.

2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranoside (S-13)[5]: This compound was prepared following a known procedure in a 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (s, 3H), 1.99 (s, 3H), 2.10 (s, 3H), 3.24-3.33 (m, 2H), 4.11-4.16 (m, 1H), 4.82 (d, J = 3.6 Hz, 1H), 5.15-5.20 (m, 3H), 5.33 (dd, J = 10.0, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.8, 50.9, 67.2, 68.8, 69.4, 70.2, 91.7, 170.2, 170.4, 170.5; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{12}H_{17}O_8N_3Na$ $(M+Na)^+$: 354.0913; found 354.0927.

2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl trichloroacetimidate (S-14): Compound **S-13** (0.58 mmol) was dissolved in CH_2Cl_2 (8 mL), then trichloroacetonitrile (1.74 mmol) and DBU (0.174) were added. The reaction mixture was stirred at room temperature overnight. The solution was concentrated *in vacuo* and the residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-14** in a 46% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.01 (s, 3H), 2.14 (s, 3H), 3.27-3.36 (m, 2H), 4.09-4.12 (m, 1H), 5.29-5.36 (m, 2H), 5.40 (t, J = 2.0 Hz, 1H), 6.24 (d, J = 1.2 Hz, 1H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 50.6, 66.2, 67.7, 68.5, 72.4, 90.4, 94.1, 159.4, 169.5, 169.6, 169.7.

Preparation of Glycan A-Man-α1,2-Man(6-N$_3$)



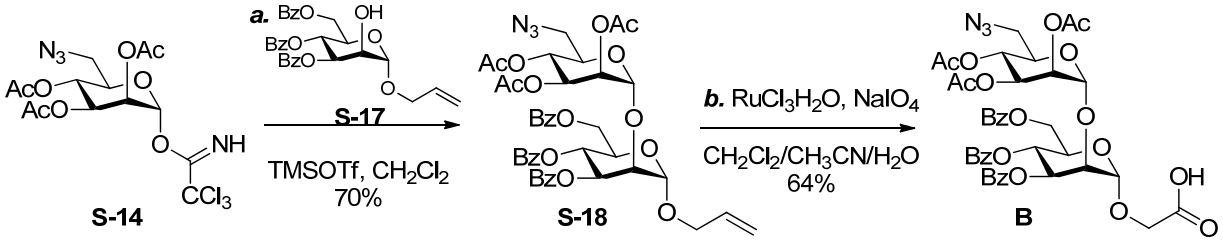
(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S-16): Glycosyl donor **S-10** (0.69 mmol) and glycosyl trichloroacetimidate **S-15**(1.03 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled to -30°C and treated with TMSOTf

[5] Utille, J.-P.; Priem, B. *Carbohydr. Res.* **2000**, *329*, 431-439.

(31 μL, 0.17 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by addition of Et_3N (0.1 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-16** in a 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 3.37 (dd, J = 13.4, 2.0 Hz, 1H), 3.52 (dd, J = 13.6, 3.2 Hz, 1H), 4.08-4.35 (m, 7H), 4.94 (d, J = 1.6 Hz, 1H), 5.11 (d, J = 1.6 Hz, 1H), 5.24-5.42 (m, 4H), 5.47 (dd, J = 10.0, 3.2 Hz, 1H), 5.68 (t, J = 10.0 Hz, 1H), 5.78 (dd, J = 10.2, 3.2 Hz, 1H), 5.91-6.01 (m, 1H), 7.32-7.49 (m, 6H), 7.90-7.93 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 51.3, 61.0, 62.5, 66.2, 67.2, 67.5, 67.8, 68.4, 68.7, 68.8, 69.2, 69.3, 70.5, 72.6, 75.8, 76.7, 97.4, 99.4, 118.3, 128.4, 128.5, 128.8, 128.8, 129.8, 129.8, 133.0, 133.4, 165.3, 165.4, 169.4, 169.5, 169.8, 170.5; HRMS (MALDI-TOF): m/z: calcd for $C_{37}H_{41}O_{16}N_3Na$ $(M+Na)^+$: 806.2385; found 806.2312.

(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (A)[6]: This compound was prepared following a known procedure in a 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.07 (s, 3H), 3.42 (dd, J = 13.6, 2.4 Hz, 1H), 3.51 (dd, J = 9.8, 6.4 Hz, 1H), 4.13-4.29 (m, 4H), 4.38-4.44 (m, 3H), 5.01 (d, J = 1.2 Hz, 1H), 5.25 (s, 1H), 5.30 (t, J = 10.0 Hz, 1H), 5.44-5.50 (m, 2H), 5.72 (t, J = 10.0 Hz, 1H), 5.82 (dd, J = 10.0, 3.2 Hz, 1H), 7.32-7.49 (m, 6H), 7.91-7.94 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 51.2, 62.5, 64.1, 66.2, 67.6, 68.9, 69.2, 70.2, 71.0, 75.9, 98.5, 99.2, 128.4, 128.5, 128.7, 129.8, 129.9, 133.5, 165.3, 165.5, 169.7, 169.8, 170.0, 171.1, 172.5; HRMS (MALDI-TOF): m/z: calcd for $C_{36}H_{39}O_{18}N_3Na$ $(M+Na)^+$: 824.2127; found 824.2185.

Preparation of Glycan B- Man(6-N$_3$)-α1,2-Man



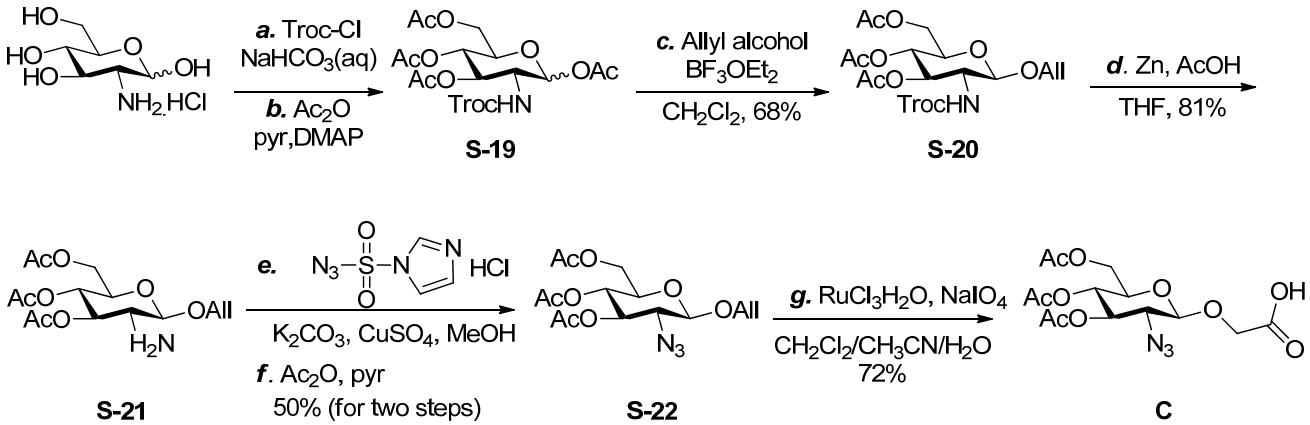
2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (S-18): Glycosyl donor **S-17** (0.69 mmol) and glycosyl trichloroacetimidate **S-14** (1.03 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled to -30°C and treated with TMSOTf

[6] Buskas, T.; Söderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958-963.

(31 μL, 0.17 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by addition of Et_3N (0.1 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-18** in a 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 3.28 (dd, J = 13.2, 2.4 Hz, 1H), 3.36 (dd, J = 13.4, 6.8 Hz, 1H), 4.12-4.17 (m, 2H), 4.29-4.34 (m, 2H), 4.39-4.43 (m, 1H), 4.51 (dd, J = 12.0, 5.2 Hz, 1H), 4.62 (dd, J = 12.2, 2.4 Hz, 1H), 4.98 (d, J = 1.2 Hz, 1H), 5.20-5.27 (m, 3H), 5.35 (dd, J = 17.2, 1.2 Hz, 1H), 5.46-5.48 (m, 2H), 5.87 (dd, J = 10.0, 3.2 Hz, 1H), 5.92-6.02 (m, 2H), 7.29-7.53 (m, 9H), 7.92-8.08 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 51.1, 63.6, 67.1, 67.4, 68.7, 68.7, 68.8, 69.2, 70.7, 70.9, 77.0, 77.3, 97.4, 99.5, 118.3, 128.3, 128.4, 128.5, 128.9, 129.0, 129.7, 129.8, 129.9, 133.0, 133.1, 133.3, 133.4, 165.2, 165.5, 166.2, 169.4, 169.4, 169.8; HRMS (MALDI-TOF): m/z: calcd for $C_{42}H_{43}O_{16}N_3Na$ $(M+Na)^+$: 868.2541; found 868.2459.

(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (B)[6]: This compound was prepared following a known procedure in a 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.04 (s, 6H), 3.31 (s, 2H), 4.12-4.14 (m, 1H), 4.39-4.62 (m, 6H), 5.03 (s, 1H), 5.23 (t, J = 9.6 Hz, 1H), 5.30 (s, 1H), 5.44-5.48 (m, 2H), 5.90 (d, J = 10.0 Hz, 1H), 5.99 (t, J = 10.0 Hz, 1H), 7.28-7.51 (m, 9H), 7.90-8.04 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 51.0, 63.5, 65.0, 67.0, 67.3, 68.7, 69.0, 69.2, 70.7, 76.6, 98.8, 99.2, 128.3, 128.4, 128.5, 128.8, 129.0, 129.6, 129.8, 129.9, 133.0, 133.3, 133.4, 165.2, 165.5, 166.5, 169.5, 170.0, 170.0, 174.9; HRMS (MOLDI-TOF): m/z: calcd for $C_{41}H_{41}O_{18}N_3Na$ $(M+Na)^+$: 886.2283; found 886.2224.

Preparation of Glycan C-Gln (2-N_3)



1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (S-19):
This compound was prepared following a known procedure.[7] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.15 (s, 3H), 3.98-4.04 (m, 2H), 4.13-4.24 (m, 2H), 4.58 (d, J = 12.0 Hz, 1H), 4.78 (d, J = 12.0 Hz, 1H), 5.14 (t, J = 10.0 Hz, 1H), 5.25 (t, J = 10.0 Hz, 1H), 5.53 (d, J = 9.2 Hz, 1H), 6.18 (d, J = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.8, 53.1, 61.5, 67.6, 69.6, 70.3, 74.5, 90.4, 95.3, 154.1, 168.7, 169.2, 170.6, 171.1; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{17}H_{22}O_{11}NCl_3Na$ $(M+Na)^+$: 544.0156; found 544.0104.

Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D- glucopyranose (S-20): Compound **S-19** (9.02 mmol) was dissolved in CH_2Cl_2 (50 mL), cooled to 0°C and allyl alcohol (18.05 mmol) was added. BF_3Et_2O (36.1 mmol) was added dropwise over the course of 5 min, and the reaction mixture was stirred at room temperature for 24 h. The mixture was neutralized with saturated aqueous sodium bicarbonate solution and diluted with CH_2Cl_2. After washing with water and brine, the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-20** in a 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.05 (s, 3H), 2.12 (s, 3H), 4.00-4.14 (m, 4H), 4.22 (dd, J = 12.6, 5.2 Hz, 1H), 4.28 (dd, J = 12.2, 4.8 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.96 (d, J = 4.0 Hz, 1H), 5.13 (t, J = 10.0 Hz, 1H), 5.26-5.36 (m, 4H), 5.87-5.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 20.7, 53.9, 61.9, 67.9, 68.2, 68.9, 71.0, 74.6, 95.4, 96.3, 118.8, 132.9, 154.2, 169.4, 170.6, 170.9; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{18}H_{24}O_{10}NCl_3Na$ $(M+Na)^+$: 542.0363; found 542.0335.

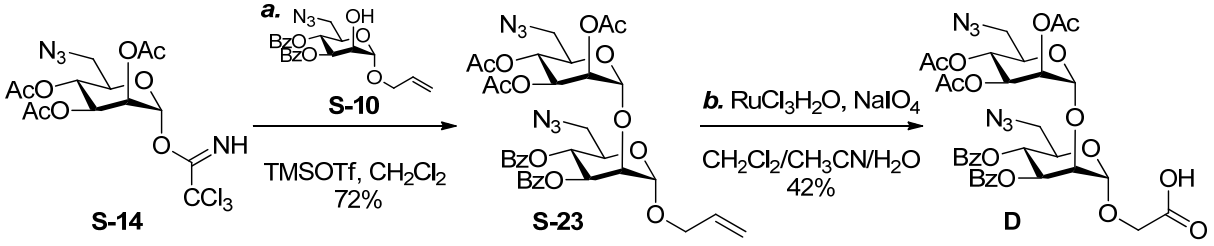
Allyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-*β*-D-glucopyranose (S-21): Compound **S-20** (5.40 mmol) was dissolved in THF (10 mL) and acetic acid (10 mL). Zinc (2.0 g) was added to the solution. After 6 hours, the reaction was complete and the mixture was filtered through celite. The solution was concentrated and the crude product purified by flash chromatography ($MeOH/CHCl_3$) to give the compound **S-21** in an 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H), 1.91 (s, 3H), 1.92 (s, 3H), 2.78 (dd, J = 10.2, 4.0 Hz, 1H), 3.84-3.92 (m, 3H), 4.03-4.14 (m, 2H), 4.75 (d, J = 3.6 Hz, 1H), 4.79 (t, J = 10.0 Hz, 1H), 4.98 (t, J = 10.0 Hz, 1H), 5.07 (dd, J = 10.4, 1.2 Hz, 1H), 5.17 (dd, J = 17.2, 1.6 Hz, 1H), 5.73-5.83 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.7, 54.4, 62.1, 67.7, 68.6, 68.7, 74.6, 98.7, 117.7, 133.4, 169.5, 170.3, 170.6; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{15}H_{23}O_8NNa$ $(M+Na)^+$: 368.1321; found 368.1306.

[7] Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296,* 135-147.

Allyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranose (S-22)[8]: Imidazole-1-sulfonyl azide hydrochloride (1.2 mmol) was added to **S-21** (1.0 mmol), K_2CO_3 (1.5 mmol) and $CuSO_4$(10 µmol) in MeOH (20 mL) and the mixture stirred at room temperature for 3 h. The mixture was concentrated and co-evaporated with toluene. Acetic anhydride (9.0 mmol) was added to the residue in pyridine (5 mL) and the mixture stirred (2 h). The mixture was concentrated, diluted with H_2O and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and concentrated. Flash chromatography gave compound **S-22** in a 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.02 (s, 6H), 3.29 (dd, J = 10.6, 3.2 Hz, 1H), 3.98-4.06 (m, 3H), 4.16-4.24 (m, 2H), 4.98 (dd, J = 11.4, 8.0 Hz, 1H), 5.21 (dd, J = 10.6, 1.2 Hz, 1H), 5.30 (dd, J = 17.2, 1.6 Hz, 1H), 5.44 (t, J = 9.6 Hz, 1H), 5.83-5.93 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 60.7, 61.7, 67.6, 68.5, 68.8, 70.3, 96.7, 118.3, 132.7, 169.5, 169.7, 170.3; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{15}H_{21}O_8N_3Na$ $(M+Na)^+$: 394.1226; found 394.1266.

Carboxymethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranose (C)[6]**:** This compound was prepared following a known procedure in a 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 3.36 (dd, J = 10.8, 3.2 Hz, 1H), 4.09-4.13 (m, 1H), 4.16-4.31 (m, 3H), 4.97 (t, J = 9.6 Hz, 1H), 5.06 (d, J = 3.6 Hz, 1H), 5.41 (dd, J = 10.4, 9.2 Hz, 1H), 9.36 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 60.6, 61.8, 64.2, 68.1, 68.3, 70.2, 97.8, 169.9, 170.2, 171.0, 172.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{14}H_{19}O_{10}N_3Na$ $(M+Na)^+$: 412.0968; found 412.0984.

Preparation of Glycan D-Man (6-N$_3$)-α1,2-Man(6-N$_3$)



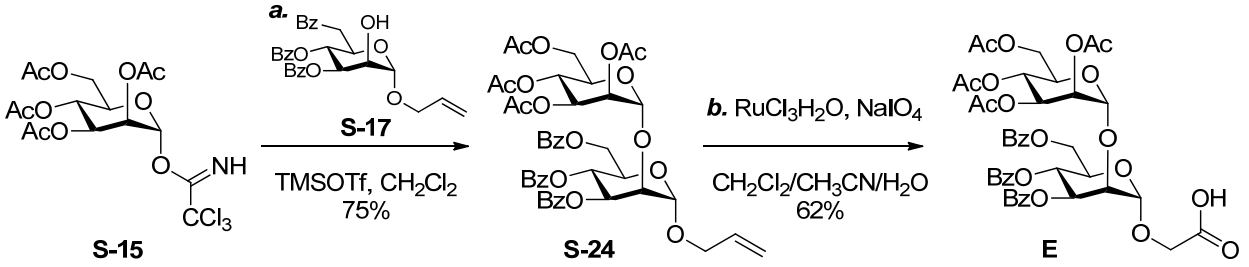
(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S-23): Glycosyl donor **S-10** (0.69 mmol) and glycosyl trichloroacetimidate **S-14** (1.03 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled to -30°C and treated with TMSOTf (31 µL, 0.17 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by addition of Et_3N (0.1 mL) and then concentrated under reduced pressure. The

[8] Goddard-Borger, E. D.; Stick, R. V.; *Org. Lett.* **2007**, *9*, 3797-3800.

residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-23** in a 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 3.30-3.41 (m, 3H), 3.51 (dd, J = 13.4, 6.8 Hz, 1H), 4.13-4.22 (m, 3H), 4.26 (t, J = 2.0 Hz, 1H), 4.33 (dd, J = 12.8, 5.2 Hz, 1H), 4.96 (d, J = 1.6 Hz, 1H), 5.19 (d, J = 1.6 Hz, 1H), 5.23 (t, J = 10.0 Hz, 1H), 5.29 (d, J = 10.4 Hz, 1H), 5.37-5.44 (m, 2H), 5.49 (dd, J = 9.8, 3.2 Hz, 1H), 5.71 (t, J = 10.0 Hz, 1H), 5.82 (dd, J = 10.0, 3.2 Hz, 1H), 5.92-6.02 (m, 1H), 7.31-7.49 (m, 6H), 7.91-7.95 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.7, 51.2, 51.3, 67.1, 67.9, 68.6, 68.7, 69.2, 70.5, 70.7, 76.9, 77.3, 97.2, 99.3, 118.4, 128.4, 128.5, 128.9, 129.8, 129.8, 133.0, 133.4, 165.3, 165.4, 169.4, 169.9; HRMS (MALDI-TOF): m/z: calcd for $C_{35}H_{38}O_{14}N_6Na$ $(M+Na)^+$: 789.2344; found 789.2356.

(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S5-29)[6]: This compound was prepared following a known procedure in a 42% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 3.37-3.46 (m, 4H), 4.22-4.43 (m, 5H), 5.02 (s, 1H), 5.26 (t, J = 10.0 Hz, 1H), 5.33 (s, 1H), 5.46 (s, 1H), 5.51 (dd, J = 9.8, 3.2 Hz, 1H), 5.76 (t, J = 10.0 Hz, 1H), 5.86 (dd, J = 10.0, 2.4 Hz, 1H), 7.33-7.49 (m, 6H), 7.93-7.96 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 20.7, 51.0, 51.1, 64.9, 67.1, 67.7, 68.7, 69.2, 70.3, 70.8, 76.7, 98.3, 99.2, 128.4, 128.5, 128.8, 128.9, 129.8, 129.9, 133.4, 165.3, 165.5, 169.6, 169.7, 170.0, 174.7; HRMS (MALDI-TOF): m/z: calcd for $C_{34}H_{36}O_{16}N_6Na$ $(M+Na)^+$: 807.2086; found 807.2097.

Preparation of Glycan E-Man -α1,2-Man

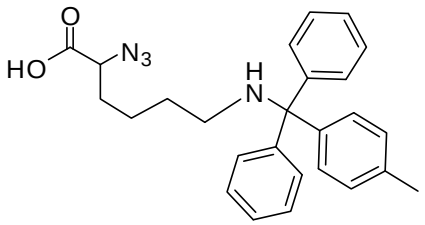


(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (S-24): Glycosyl donor **S-17** (0.69 mmol) and glycosyl trichloroacetimidate **S-15** (1.03 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled to -30°C and treated with TMSOTf (31 μL, 0.17 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by addition of Et_3N (0.1 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S-**

24 in a 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.01 (s, 6H), 2.05 (s, 3H), 4.06-4.51 (m, 7H), 4.49 (dd, J = 12.0, 5.6 Hz, 1H), 4.61 (dd, J = 12.2, 2.4 Hz, 1H), 4.97 (s, 1H), 5.13 (s, 1H), 5.23-5.36 (m, 3H), 5.45-5.48 (m, 2H), 5.84 (dd, J = 10.2, 3.2 Hz, 1H), 5.91-6.00 (m, 2H), 7.28-7.51 (m, 9H), 7.91-8.06 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 62.5, 63.5, 66.2, 67.3, 68.7, 68.8, 68.9, 69.2, 69.3, 70.9, 76.8, 97.6, 99.4, 118.2, 128.3, 128.4, 128.5, 128.8, 129.0, 129.7, 129.8, 129.9, 133.0, 133.1, 133.3, 133.4, 165.2, 165.5, 166.2, 169.3, 169.4, 169.7, 170.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{44}H_{46}O_{18}Na$ $(M+Na)^+$: 885.2582; found 885.2543.

(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (E)[6]: This compound was prepared following a known procedure in a 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 4.14-4.23 (m, 3H), 4.35-4.48 (m, 5H), 4.60 (d, J = 9.2 Hz, 1H), 5.02, (s, 1H), 5.27-5.47 (m, 4H), 5.86 (d, J = 10.0 Hz, 1H), 6.00 (t, J = 10.0 Hz, 1H), 7.28-7.52 (m, 9H), 7.90-8.05 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 62.5, 63.4, 64.9, 66.2, 67.2, 68.9, 69.1, 69.2, 70.7, 76.3, 98.5, 99.4, 128.3, 128.5, 128.5, 128.8, 129.0, 129.6, 129.7, 129.8, 129.9, 133.0, 133.2, 133.4, 165.1, 165.5, 166.4, 169.4, 169.5, 169.8, 171.5, 14.7; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{43}H_{44}O_{20}Na$ $(M+Na)^+$: 903.2324; found 903.2303

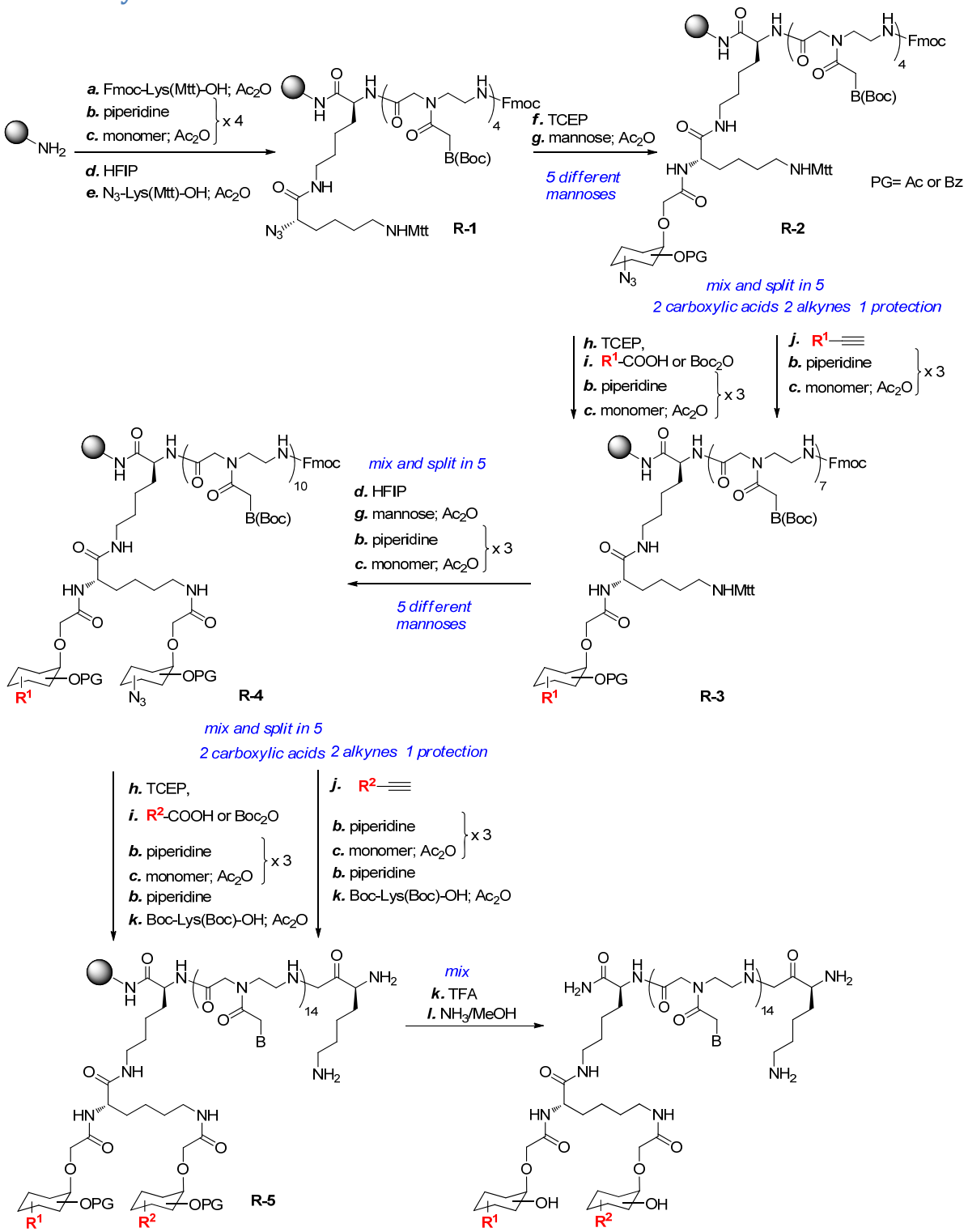
N_3-Lys(Mtt)-OH synthesis



To a solution of 625 mg of Fmoc-Lys(Mtt)-OH (1 mmol, 1 equiv) in 20 mL MeOH were added 415 mg of K_2CO_3 (3 mmol, 3 equiv) and the solution was stirred until complete Fmoc deprotection. Then, 208 mg of K_2CO_3 (1.5 mmol, 1.5 equiv) were added, followed by 32 mg of $CuSO_4$ (0.02 mmol, 20% equiv) and 252 mg of imidazole-1-sulfonyl azide hydrochloride (1.2 mmol, 1.2 equiv – prepared according to a described procedure - Goddard-Borger, E. D.; Stick, R. V.; Org. Lett. 2007, 9, 3797-3800), and the reaction mixture was stirred overnight. The MeOH was evaporated and the residue was suspended in 100 ml of H_2O. 60 mL of citric acid 5% aq. were added (to adjust the pH to 3-4), and the crude was extracted with 3x100 mL of AcOEt. The combined organic phases were washed with 2x100 mL of H_2O, dried with Na_2SO_4 and concentrated under reduced pressure. Silica gel chromatography (DCM-MeOH 100-0 to 90-10) afforded 340 mg of N_3-Lys(Mtt)-OH (80%). ^{1}H NMR (400 MHz, CDCl3) δ 1.27 (m, 2H), 1.52 (m, 2H), 1.62 (m, 2H), 2.29 (s, 3H), 2.34 (m, 2H), 3.60 (m, 1H) 7.10-7.44 (m, 14H); LC-MS: m/z: calcd for C26H29O2N4 (M+H)+: 429.53; found: 429.30.

2.-PNA-encoded mannose library synthesis

Detailed Synthetic Scheme



library of 441 PNA tagged modified mannoses

General procedures.#

All procedures were individually validated by monitoring the cleavage product by LC/MS and/ or MALDI. Washing were typically performed with 10x the resin volume. The PNA monomers were protected were prepared according to previously reported protocols.[9]

Procedure 1. General procedure for resin loading.

NovaPEG Rink amide resin (0.2 mmol/g, NovaBiochem) was swollen in CH_2Cl_2 for 20 min. Fmoc-Lys(Mtt)-OH (1.0 equiv) was dissolved in anhydrous NMP and HOBt (5.0 equiv) followed by diisopropylcarbodiimide (DIC, 15.0 equiv) were added. The mixture was stirred for 15 min prior the addition to the resin and then shaken for 8 h with the resin. Next, the resin was washed extensively with DMF and CH_2Cl_2, and dried.

Procedure 2. General procedure for capping the resin.

To 50 mg of resin was added 1.0 mL of capping mixture (9.2 mL of acetic acid and 13 mL of 2,6-lutidine in 188 mL of DMF) and the mixture was shaken for 15 min. Subsequently, the resin was washed with DMF and CH_2Cl_2.

Procedure 3. General procedure for capping in Intravis AG Multipep RS Synthesizer.

To 10 mg of the resin were added 100 µL of capping mixture (9.2 mL of acetic acid and 13 mL of 2,6-lutidine in 188 mL of DMF). After 5 min, the resin was washed with 2 x 250 µL of DMF.

Procedure 4. General procedure for Fmoc deprotection.

To 50 mg of resin was added 1.0 mL of 20% piperidine solution in DMF, and the resin was shaken for 5 min. Subsequently, the resin was washed with 3 x 2 mL of DMF and 3 x 2 mL of CH_2Cl_2 (repeated twice).

Procedure 5. General procedure for Fmoc deprotection in Intravis AG Multi pep RS Synthesizer

To 10 mg of resin were added 100 µL of 20% piperidine solution in DMF. After 2 min, the resin was washed with 250 µL of DMF and the sequence was repeated a second time. Finally, the resin was washed with 5 x 250 µL of DMF and 3 x 250 µL of CH_2Cl_2.

Procedure 6. General procedure for PNA synthesis or aminoacid coupling on NovaPEG resin in Intavis AG Multipep RS Synthesizer

Up to 7 monomers: To a solution of 0.008 mmol (4.0 equiv) of Fmoc-protected PNA monomer (or Fmoc and side protected aminoacid) in 40 µL of NMP were added 14 µL (0.007 mmol, 3.5 equiv) of HATU 0.5 M in NMP, followed by 6.7 µL of solution of 1.2 M DIPEA (0.008 mmol, 4.0 equiv) and 1.8 M 2,6 lutidine (0.012 mmol, 6.0 equiv) in NMP. The mixture was agitated for 5 min at room temperature, and

[9] S. Pothukanuri, Z. Pianowski, N. Winssinger, *Eur. J. Org. Chem.*, **2008**, *18*, 3141-48; Z. Pianowski, K. Gorska, L. Oswald, C. A. Merten, N. Winssinger, *J. Am. Chem. Soc.,* **2009**, 6492-6497.

then added to 10 mg (0.002 mmol, 1.0 equiv) of resin. After 20 min the coupling procedure was repeated, without washing and then washed with 6 x 250 µL of DMF and 6 x 250 µL of DCM. Finally, the resin was capped and Fmoc was deprotected according to the general procedures described above.

From 8 monomers above: To a solution of (0.01 mmol, 5.0 equiv) of Fmoc-protected PNA monomer (or Fmoc and side protected aminoacid) in 50 µL of NMP were added 17.6 µL (0.0088 mmol, 4.4 equiv) of HATU 0.5 M in NMP, followed by 8.3 µL of base solution DIPEA 1.2 M (0.01 mmol, 5.0 equiv) and 2,6 lutidine 1.8 M (0.015 mmol, 7.5 equiv) in NMP. The mixture was agitated for 5 min at room temperature, and then added to 10 mg (0.002 mmol, 1.0 equiv) of resin. After 20 min the coupling procedure was repeated, and after 25 min more the resin was washed with 6 x 250 µL of DMF and 6 x 250 µL of DCM. Finally, the resin was capped and Fmoc was deprotected according to the general procedures described above.

Procedure 7. General procedure for Mtt deprotection

10 mg of resin were successively treated with 5 x 200 µL hexafluoroisopropanol (50% solution in DCE) 5 x 5 min, and then washed with 3 x 250 µL of DMF and 3 x 250 µL of DCE. The above procedure was repeated 2 more times. Finally the resin was washed with 6 x 250 µL of DMF and 6 x 250 µL of DCM**.**

Procedure 8. General procedure for azide reduction

10 mg of resin (0.002 mmol, 1 equiv) were treated with 180 µL of a solution of TCEP (1M in THF) and 20 µL of H_2O for 1 h. The resin was successively washed with 2 x 200 µL of THF, 2 x 200 µL of H_2O, 3 x 200 µL of DMF and 3 x 200 µL of CH_2Cl_2.

Procedure 9. General procedure for carboxylic acid coupling.

The corresponding carboxylic acid (0.01 mmol, 5.0 equiv) was dissolved in 200 µL of NMP, HOBt (1.5 mg, 0.01 mmol, 5.0 equiv) followed by diisopropylcarbodiimide (4.7 µL, 0.03 mmol, 15.0 equiv) were added and agitated for 10 min at room temperature and then added to 10 mg of resin (0.002 mmol). The reaction was shaken for 8 h at r.t. and was washed after with 6 x 200 mL of DMF and 6 x 200 mL of CH_2Cl_2.

Procedure 10. General procedure for Boc protection

10 mg (0.002 mmol, 1 equiv) of resin were treated with a solution of Boc_2O (11.6 µl, 50 equiv) and 2,6-lutidine (11.6 µL, 100 equiv) in 200 µL of NMP during 8 h at 40 ^oC. After, the resin was washed with DMF (6 x 200 µL) and CH_2Cl_2 (6 x 200 µL).

Procedure 11. General procedure for Click cycloaddition reaction

To 10 mg (0.002 mmol, 1 equiv) of resin were added successively 150 µL (0.015 mmol, 7.5 equiv) of alkyne 0.2 M in NMP, 15 µL (0.015 mmol, 7.5 equiv) of sodium ascorbate 198 mg/mL in H_2O, 3.8 µL (0.0005 mmol, 0.25 equiv) of copper sulfate 21.4 mg/mL in H_2O and 38 µL (0.001 mmol, 0.50 equiv) of TBTA 15 mg/mL in NMP, and the reaction was shaken overnight. Finally, the resin was washed with

8x250 µL of sodium diethyldithiocarbamate 0.02 M in DMF, 12x250 µL of DMF and 12x250 µL of DCM.

Procedure 12. General procedure for the cleavage from the resin

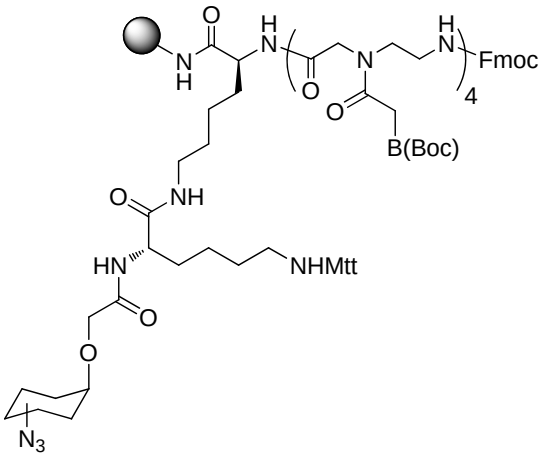
The resin (10 mg) was treated with TFA (95%, 200 µL) for 3 h. The TFA solution was precipitated in 2 ml of Et_2O and centrifuged to recover the product as a pellet. The precipitate was dissolved in 3 mL of water and filtered finally; the water was removed by freeze-dried.

Procedure 13. General procedure for the glycan deprotection

The precipitate was dissolved in H_2O (500 µL for crude cleaved from 10 mg of resin) and 800 µL of 2M ammonia in MeOH were added. The solution was stirred for 4 days at room temperature, then diluted with 1 mL of water and freeze-dried.

Library synthesis and characterization.#

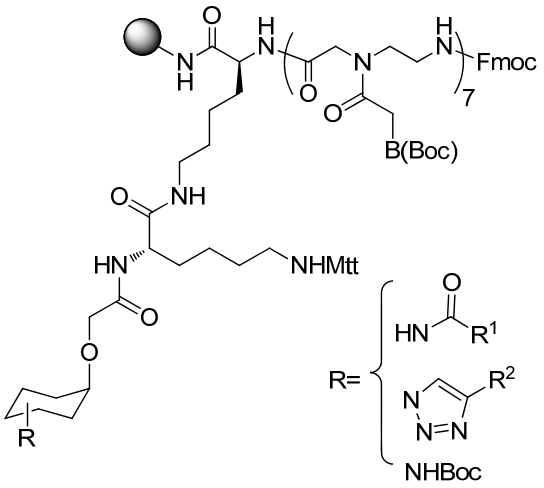
First point of diversity (R-2): NovaPEG Rink amine resin (50 mg) was loaded with FmocLys(Mtt)OH (***procedure 1***) followed by capping of the un-reacted amino groups (***procedure 2***). The resin was split into 5 different columns (10 mg each) and after Fmoc deprotection (***procedure 5***) the corresponding 4-mers were loaded using the Multipep Synthesizer (***procedure 6***). The Mtt protecting group on the side chain of the Lysine was deprotected using ***procedure 7*** and N_3-Lys(Mtt)OH was loaded following ***procedure 9***. The azide group was reduced using the protocol described in ***procedure 8*** and 5 different glycans (A-E) functionalized as carboxylic acids were loaded (***procedure 9***). MALDI of an analytical cleavage from each resin confirmed the completion of each sequence.



Entry	Glycan	CODON	*m/z* calculated	*m/z* found
1	A	GCCG	2363.14	2364.91

2	B	GGAA	2473.18	2474.92
3	C	CGGC	1951.23	1951.73
4	D	AAGG	2394.62	2395.77
5	E	GAAC	2450.73	2451.85

Second point of diversity: Boc protection, 2 alkynes and 2 carboxylic acids (R-3). The 5 resins prepared in the previous step were suspended in CH_2Cl_2 and mixed together in a SPE tube. The resin was shaken for 20 min and distributed in 5 different columns. Then the azide group was reduced following ***procedure 8*** (columns 1, 2 and 5) and the resulting amine was protected as Boc (***procedure 10***, column 5) or the corresponding carboxylic acid was loaded (columns 1 and 2, ***procedure 9***). After click cycloaddition reaction with 2 different alkynes (columns 3 and 4, ***procedure 11***) the second PNA codon (3mer) was introduced in all the columns following ***procedure 6***. The success of the reactions in each pool was assessed by MALDI showing complete conversions.

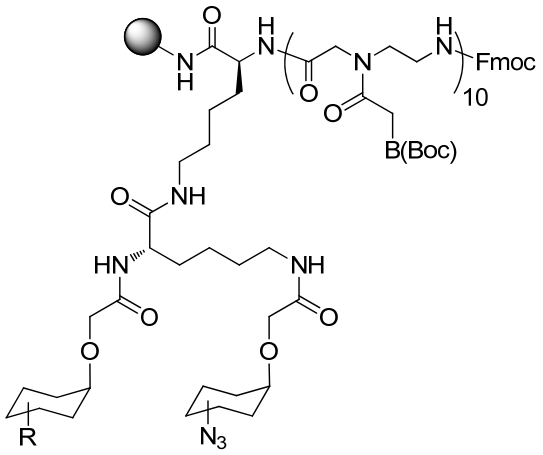


Entry	Reagent	CODON	*m/z* calculated range	*m/z* found
1	Acetic acid	G*GC	2899.57-3421.52	2900.22-3422.76
2	Benzoic acid	C*GA	2945.64-3467.59	2945.55-3468.42
3	Propargyl acetate	G*CA	2965.67-3507.16	2966.66-3508.56
4	Phenylacetylene	C*CG	2945.69-3491.21	2946.90-3399.45
5	Boc_2O	T*GG	2872.58-3398.07	2872.61-3399.45

*The star denotes a GPNA residue

Third point of diversity (R-4): The 5 resins prepared in the previous step were suspended in CH_2Cl_2 and mixed together in a SPE tube. The resin was shaken for 20 min and distributed in 5 different columns.

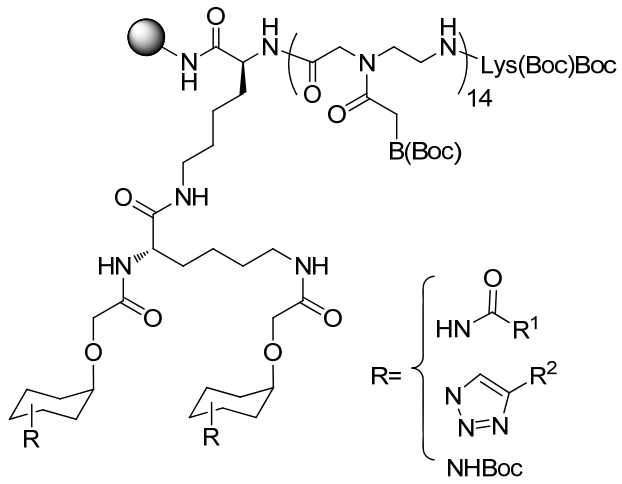
Then the Mtt protecting group was removed following ***procedure 7*** and the corresponding 5 mannoses were introduced using the conditions described in ***procedure 9***. After deprotection of Fmoc group (***procedure 5***), the third PNA codon (3mer) was introduced (***procedure 6***). The success of the reactions in each pool was assessed by MALDI showing complete conversions.



Entry	Glycan	CODON	*m/z* calculated range	*m/z* found
1	A	GTG*	4604.09-5238.67	4550-5300
2	B	GCA*	4608.11-5241.69	4550-5300
3	C	ACG*	4161.78-4795.76	4100-4900
4	D	CGA*	4556.55-5191.13	4500-5200
5	E	AGC*	4652.66-5287.24	4600-5300

*The star denotes a GPNA residue

Fourth point of diversity: Boc, 2 alkynes and 2 carboxylic acids (R-5). The 5 resins prepared in the previous step were suspended in CH_2Cl_2 and mixed together in a SPE tube. The resin was shaken for 20 min and distributed in 5 different columns. Then the azide group was reduced following ***procedure 9*** (columns 1, 2 and 5) and the amine was protected as Boc (***procedure 10***, column 5) or the corresponding carboxylic acid was loaded (columns 1 and 2, ***procedure 9***). Columns 3 and 4 underwent a click cycloaddition reaction with 2 different alkynes (***procedure 11***). Finally, the last PNA codon (4mer) followed by a Boc-Lys(Boc)-OH were introduced in all the columns following ***procedure 6***. The success of the reactions in each pool was assessed by MALDI.



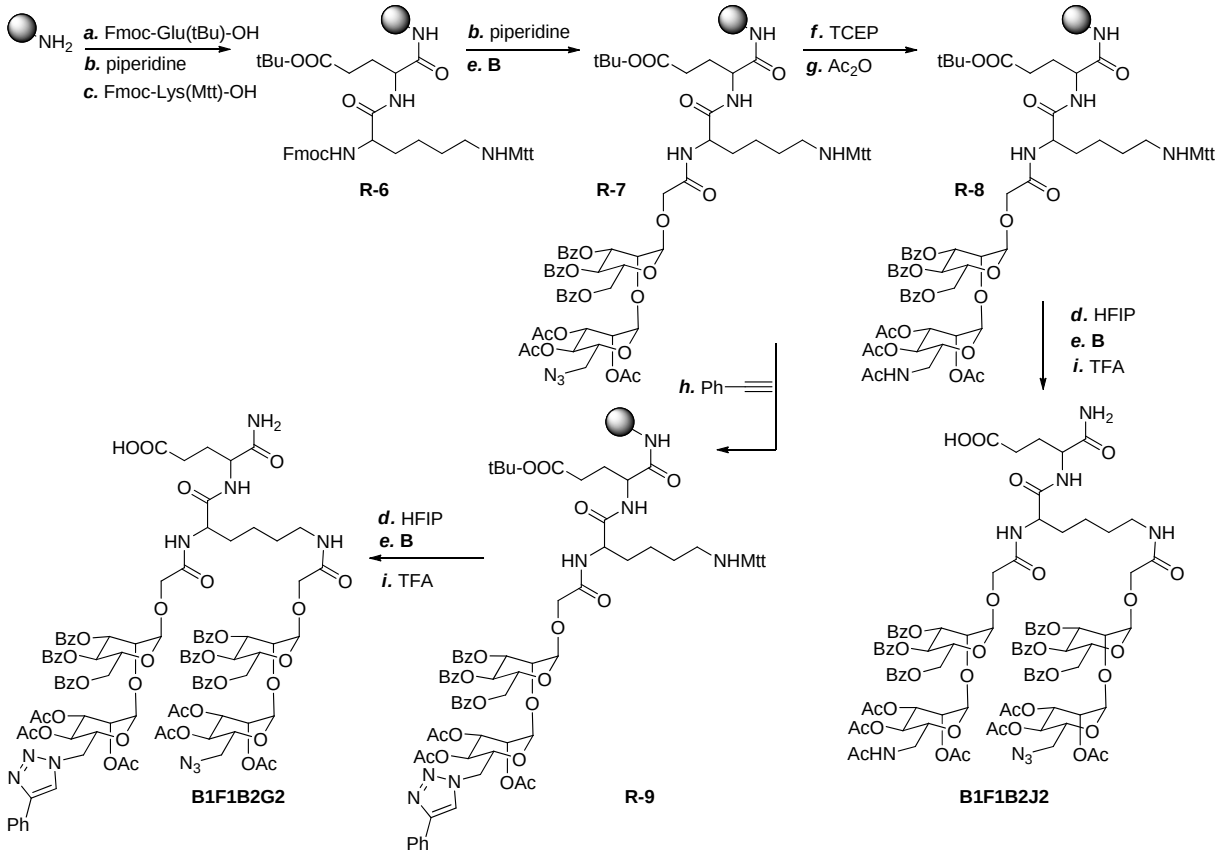
Entry	Reagent	CODON	*m/z* calculated range	*m/z* found
1	Acetic acid	CGAA	5191.48-6333.54	5100-6400
2	Benzoic acid	GAGA	5253.55-6457.68	5200-6500
3	Propargyl acetate	CAGG	5273.58-6497.74	5200-6500
4	Phenylacetylene	GACG	5301.62-6529.81	5200-6500
5	Boc_2O	AGGC	5133.50-6240.02	5100-6400

Cleavage and deprotection.

The cleavage of the 5 resins prepared in the previous step following ***procedure 12*** and the deprotection of the hydroxyl groups (***procedure 13***) afforded the final mixture of compounds that were analyzed by MALDI. A mix of the 5 pools generated the library of 441 members.

Entry	*m/z* calculated range	*m/z* found
1	4939.24-5558.30	4900.5700
2	5001.31-5682.44	4950.5800
3	4979.29-5638.42	4950-5800
4	5049.38-5754.57	5000-5900
5	4881.26-5458.33	4850-5700
mix	4881.26-5754.57	4850-5900

3.-Dendrimer synthesis.#



Preparation of resin R-6: To a solution of 212.8 mg (0.5 mmol, 5.0 equiv) of Fmoc-Glu(tBu)-OH in 700 µL of NMP were added 875 µL (0.44 mmol, 4.0 equiv) of HATU 0.5 M in NMP, followed by 420 µL of base solution (DIPEA 1.2 M (0.5 mmol, 5.0 equiv) and 2,6 lutidine 1.8 M (0.75 mmol, 7.5 equiv) in NMP). The resulting solution was agitated for 10 min, and then added to 200 mg (0.1 mmol, 1 equiv) of NovaPEG rink amide resin. The mixture was shaken for 3 h and subsequently washed with 6 x 1500 µL of DMF and 6 x 1500 µL of CH_2Cl_2. Fmoc was removed by shaking the resin for 10 minutes with 2 mL of a 20% piperidine/DMF solution. Fmoc-Lys(Mtt)-OH was activated and coupled to the resin in a similar manner to Fmoc-Glu(tBu)-OH. LC-MS: $(M+H)^+$ calculated: 497.57, obtained: 497.34

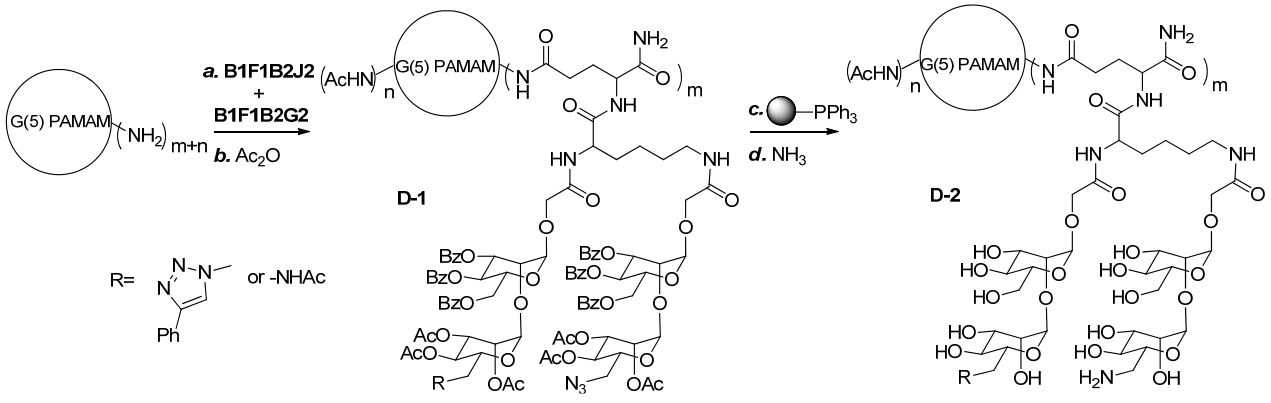
Preparation of resin R-7: The Fmoc was removed by shaking the resin for 10 minutes with 2 mL of a 20% piperidine/DMF solution. Subsequently, to a solution of 16.8 mg (0.0195 mmol, 1.3 equiv) of glycan **B** in 200 µL of NMP were added 9.0 mg (0.059 mmol, 3.9 mg) of HOBt, followed by 28 µL (0.176 mmol, 11.7 eq.) of DIC. The resulting solution was agitated for 10 min, and then added to 30 mg (0.015 mmol, 1 eq.) of resin **R-6**. The mixture was shaken for 16 h at room temperature and subsequently washed with 6

x 250 µL of DMF and 6 x 250 µL of CH_2Cl_2. LC-MS: $(M+H)^+$ calculated: 1121.09, obtained: 1120.70

Preparation of resin R-8: To 15 mg (0.0075 mmol, 1 equiv) of resin **R-7** 300 µl of a 1.0 M solution of Tris(2-carboxyethyl)phosphine in 10%H_2O/DMF was added, the mixture was shaken for 1.5 h and subsequently the resin washed with 8 x 250 µL of H_2O, 8 x 250µL of THF, followed by 8 x 250 µL of DMF and 8 x 250µL of CH_2Cl_2. Subsequently the resin was treated with 500 µL of a solution containing 13.8 µL (0.25 mmol, 34 equiv) of acetic anhydride and 19.5 µL of 2,6 lutidine (0.17 mmol, 23 equiv) for 2.5 h. Finally the resin was washed with 6 x DMF and 6 x CH_2Cl_2. LC-MS: $(M+H)^+$ calculated: 1137.12, obtained: 1136.74

Preparation of resin R-9: To 15 mg (0.0075 mmol, 1 equiv) of resin **R-7** were added successively 6.2 µL (0.056 mmol, 7.5 equiv) of phenylacetylene in 228 µL NMP, 23.4 µL of sodium ascorbate 477 mg/mL in H_2O (0.056 mmol, 7.5 equiv), 6 µL of copper sulfate 51.6 mg/mL in H_2O (0.0019 mmol, 0.25 equiv) and 60 µL of TBTA 36 mg/mL in NMP (0.0038 mmol, 0.50 equiv), and the reaction was shaken overnight. Finally, the resin was washed with 8 x 250 µL of sodium diethyldithiocarbamate 0.02 M in DMF, 12 x 250 µL of DMF and 12 x 250 µL of DCM. LC-MS: $(M+H)^+$ calculated: 1223.22, obtained: 1222.79

Preparation of B1F1B2J2 and B1F1B2G2 acids: Mtt was deprotected by repeatedly shaking 15 mg of resins **R-7** and **R-9** with 500 µL of a 50% HFIP/DCE solution, until the yellow color disappeared (5 x 5 minutes). The second glycan **B** was coupled according to the procedure described above. Subsequently, 300 µL of TFA were added and after 3 h, the TFA solution was added to 4 mL of water. The resulting suspension was lyophylized to afford 3.0 mg of **B1F1B2J2** and 5.0 mg of **B1F1B2G2**. LC-MS: $(M+H)^+$ **B1F1B2J2**: calculated: 1982.88, obtained: 1983.26, **B1F1B2G2** $(M+2H)^{2+}$: calculated: 1034.99, obtained: 1034.94.



Coupling of glycan acids B1F1B2J2 and **B1F1B2G2 to PAMAM dendrimer G5:** To a solution of 3.0 mg (1.5 µmol) of **B1F1B2J2** and 3.2 mg (1.5 µmol) of **B1F1B2G2** in 100 µL of DMSO, were added 1.6 mg (4.3 µmol, 1.2 equiv) of HATU in 100 µL of DMSO, followed by 21 µL of DIPEA (to adjust the pH to 8-9). The resulting solution was agitated for 15 min, and then added to a suspension of 0.91 mg (1equiv) of PAMAM dendrimer ethylenediamine core - generation 5 (obtained from the drying of 22.8 µL of a 5% methanolic solution) in 100µL of DMSO. The reaction mixture was stirred for 3 days, then dialysed against DMSO (16 hours), and finally freeze-dried. MALDI: average MW obtained ~38000 suggesting an amine conversion of ~6%. The remaining free amines were acetylated with 200 µL of a methanolic solution containing 9.9 µL of acetic anhydride (105 µmol, 35 equiv) and 17.1 µL of pyridine (210 µmol, 70 equiv). The reaction was stirred for 3 days, then dialysed twice against H_2O (16 + 10 hours), and finally freeze-dried, to afford 1.21 mg of dendrimer **7** MALDI: average MW calculated: ~42000, obtained: ~42000

Azide reduction and deprotection: To a suspension of dendrimer **D-1** (0.21 µmol of azide, 1equiv) in 300 µL THF-H_2O (3-1) were added 8 mg of polymer-bound triphenylphosphine (24 µmol, 110 equiv) and the reaction was shaken for 3 days. THF was subsequently removed under reduced pressure, the residue was suspended in 1 ml of MeCN-H_2O (1-1), filtered and finally freeze-dried, to afford 0.75 mg of the free-amine product. Then the dendrimer was suspended in 300 µL of a 2M solution of NH_3 in MeOH, and the reaction was stirred for 7 days. The mixture was concentrated under reduced pressure, subsequently the residue was suspended in 1 ml of MeCN-H_2O (1-1) and finally freeze-dried, to afford 0.49 mg of dendrimer **D-2**. Average MW calculated: ~36800, obtained: ~37000

4.-Procedure for the selection of the fittest combination in a PNA-DNA hybrid mannose library against recombinant DC-SIGN lectin.#

Protocol for the Selection.

A library of DNA containing all permutations of codons complimentary to the PNA encoded glycan library, flanked by 20-mer primer (68mer DNA) was obtained by split and pool synthesis from commercial supplier (Microsynth AG, Switzerland). The self assembled library was prepared by mixing the modified PNA encoded glycan library with the DNA library as well as sequences complimentary to the primers on the DNA library (P5' block 5'-ACGAGAGGCTCACAACAGGC-3' and P3' block, 5'-GGATAGACAATAACGACGAC-3'). All the components were mixed at equal molar ratio to obtain a final concentration 10 µM in HBS–$CaCl_2$ –T buffer (10 mM, Hepes, pH 7.4, 150 mM NaCl, 1mM EDTA, 5mM $CaCl_2$, Tween 20, 0.05%) in a final volume of 100 µL. The mixture was heat denatured (10 min at 95 °C) and cooled down to room temperature for one hour to allow for sequence specific annealing.

Based on the library of DNA templates, the codon for the last element of diversity in the first PNA was fixed as J (a subset of 105 combinations within the library) whereas the codon of the last element of diversity for the second PNA was F-I (a subset of 357 combinations within the library).

Recombinant soluble DC-SIGN was isolated as the full extracellular domain portion of the lectin, expressed in *E. coli* and purified by affinity chromatography on Mannose-Sepharose[10] followed by purification via ion-exchange chromatography. The isolated DC-SIGN is a homotetramer with a molecular weight around 156 kD. On SDS-PAGE in reducing conditions, it appears as a single band around 39 kD.

DC-SIGN was dissolved in HBS-$CaCl_2$ at 1.5 mg/mL and immobilized on carboxylic acid activated magnetic beads (Dynal Invitrogen) following the manufacturer recommendations. 50 µL of beads were washed twice with cold, 25 mM MES , pH 5 to eliminate the conservation buffer containing BSA. The bead slurry was then magnetically separated and resuspended on 25 µL of a 50 µg/mL EDC (Sigma) solution in 25 mM MES cold buffer and 25 µL of a 50 µg/mL NHS (Sigma) solution in 25 mM MES cold buffer. The beads were EDC/ NHS activated for 30 min with constant agitation at RT. 100µL of beads were divided in two aliquots 50 µL each, magnetically separated and 25mM MES washed. Then we added to one of the tubes 50 µg of DC-SIGN to be immobilized into and to the other tube we added immobilization buffer without protein. After 30 min incubation at RT with gentle agitation, both tubes were placed on magnetic stand, and the magnetic beads were washed once with cold 25 mM MES and then incubated for one hour on Tris-HCl pH 7 for 30 min in order to quench the non reacted carboxylic groups. Then both resins (DC-SIGN and Control beads were kept at 4 ° C during the selection procedure.

[10] Mitchell, D. A.; Fadden, A. J.; Drickamer, K. *J. Biol. Chem.* **2001**, 276, 28939-28945.

A quantity of 10 µg of DC-SIGN was immobilized for each round of selection.

The DNA/PNA hybrids solution (50 µL) was incubated with the target immobilized protein slurry (10 µL) for 30 min at RT with gentle agitation. After incubation the tubes were placed on a magnetic stand for 2 minutes and the supernatant containing the unbound molecules was pipetted off and discarded. The retained candidates were washed 3 times with 100 µL of HBS-$CaCl_2$-Tween 20, 0.05% (HBS-T), to eliminate PNA/DNA hybrids non-specifically interacting with the target. After 3 washes, the DC-SIGN functionalized magnetic beads were re-suspended on 50 µL of distilled water and heated at 94 °C for 5 min. Then the tubes were placed on a magnetic stand and the supernatant containing the selected candidates was recovered in a new tube, diluted 100 times and 1 µL of this dilution was used as template for PCR amplification. The same procedure was carried out in parallel with non functionalized magnetic beads as control.

PCR amplification and ssDNA preparation.

DNA/PNA hybrids recovered after selection by heat elution were used as templates on PCR amplification under the following conditions. PCR reactions containing 2µM primer P3' Cy3-5'-GGATAGACAATAACGACGAC-3', 2µM primer P5' Biot-5'- GCCTGTTGTGAGCCTCTCGT-3', 0.2 units of AmpliTaq Gold (Applied Biosystems), 1.5 mM $MgCl_2$, Amplitaq Gold Buffer II, were amplified as follows: one first incubation at 95°C for 10 min, 95 °C during 1 min, 52 °C for 30 s, 68 °C for 30 s (25 cycles) and a final elongation 10 min at 72 °C.

The PCR reactions were analyzed on 3.5% agarose electrophoresis to confirm the amplification of a specific 68 nucleotides product, stained with ethidium bromide and visualized by UV transilumination.

A quantity of 100 µl of PCR reaction from each round of selection was purified using Quiaquick PCR purification kit (Qiagen). The purified PCR product was immobilized on Dynal Streptavidine magnetic beads, and single strand DNA was prepared following a known protocol.[11]

The Cy3 labeled DNA strand (ss-FDNA) was conserved at -20°C until hybridization on microarray slides while the immobilized biotinylated strand (template strand) was rehybridized with the modified mannose PNA encoded library (5 µM) during 30 min at 50 °C. The non hybridized PNA molecules were washed out of the magnetic beads by five successive washes with 100 µl HBS-T at room temperature. The new recruited PNA/DNA hybrids were eluted from the magnetic beads by heating the beads at 95 °C (50 µl) for ten minutes on HBS- $CaCl_2$-T buffer, 5 mM biotin. The eluted DNA/PNA hybrids were engaged in further rounds of selection by using the recover solution to incubate with the immobilized target.

The Cy3 labeled DNA strands corresponding to several rounds of selection were hybridized to custom

[11] Beaulieu, M.; Larson, G. P.; Geller, L.; Flanagan, S. D.; Krontiris ,T. G.; *Nucleic Acids Research*, **2001**, *29*, 1114-1124.

array containing the complementary sequences as previously described.[12]

Cloning and sequencing of PCR products.

PCR products corresponding to the 4th round of selection were inserted into plasmid TOPO TA cloning systems (Invitrogen ™; 200 ng of PCR product were mixed with TOPO TA cloning vector as indicated by the manufacturer at room temperature for 15 minutes). The ligation mix was transformed into *E. coli* chemical competent cells (Invitrogen). Blue/white colony selection was performed by plating the transformed bacteria in LB plates containing 10 mg/ml Xgal, 1 mM IPTG, 50 µg/ml ampicillin. We obtained approximately 50-70 colonies by transformation. White colonies were systematically replicated into fresh plates and PCR amplified wit universal oligonucleotides M13 forward and reverse. The purified PCR product was sequenced by GATC Biotech AG sequencing services.

The following sequences were obtained:

CGGC ACC TGC TCCG CCTT CCG TGC GTCC

CCTT ACC CGT TCCG CCTT ACC CGT CTCT

CGGC CCG TCG TCCG CCTT ACC CAC CTCT

GCCG GGC GCT TCCG GCCG ACC TGC GCTT

CCTT GCT CAC TCCG CCTT ACC GCT GTCC

CCTT GGC TCG TCCG TTCC CGT GCT GTCC

CCTT GGC TCG TCCG CCTT CCG CGT CTGC

CCTT ACC GCT TCCG CCTT ACC GCT CTGC

CCTT GGC GCT TCCG TTCC GCT TGC CTCT

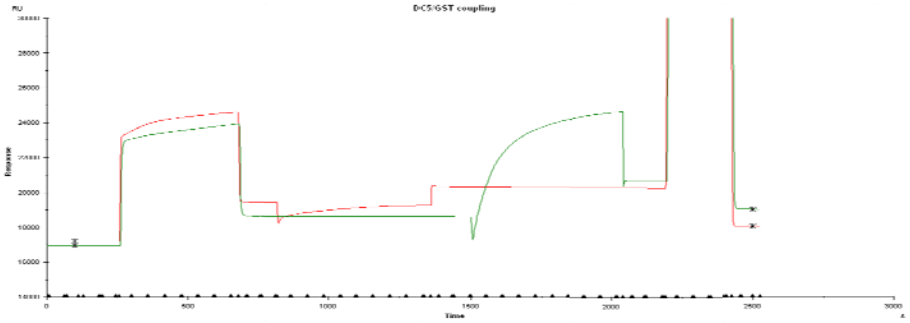
GCCG CCG CGT TCCG TTCC GCT CGT GTCC

[12] Urbina, H. D.; Debaene, F.; Jost, B.; Bole-Feysot, C.; Kuzmic, P.; Harris, J. L.; Winssinger, N. *ChemBioChem*, **2006**, 1790-1797.

5.- Surface plasmon resonance (SPR) æ ˇ°¹ºß æ ˇ¹–¹.#

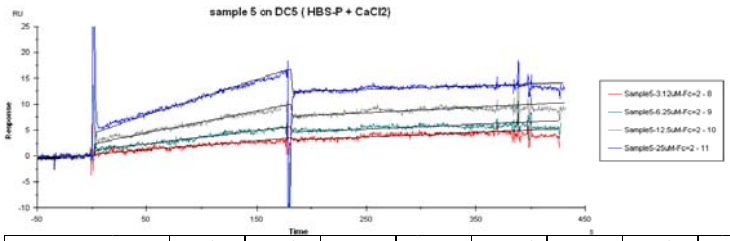
All biosensor assays were performed with Hepes-buffered saline (HBS-P) as running buffer (10 mM Hepes, 150mM sodium acetate, 3mM magnesium acetate, 0.005% surfactant P20 with or without 5mM $CaCl_2$, pH 7.4). The different compounds were dissolved in the running buffer. Immobilizations were performed by injecting, onto the activated surface by EDC/NHS of a sensor chip CM5, 45 µl of DC-SIGN (same source as before) or GST (100 µg/ml in formate buffer, pH 4.3), which gave a signal of approximately 2100 RU and 900 RU respectively, followed by 20 µL of ethanolamine hydrochloride, pH 8.5, to saturate the free activated sites of the matrix. All the binding experiments were carried out at 25°C with a constant flow rate of 20 µl/mn. The sensor chip surface was regenerated after each experiment by injection of 10 µl of 10mM HCl. The kinetic parameters were calculated using the BIAeval 4.1 software on a personal computer. Global analysis was performed using the simple Langmuir binding model. The specific binding profiles were obtained after subtracting the response signal from the channel control (GST). The fitting to each model was judged by the reduced chi square and randomness of residue distribution.

Protein loading



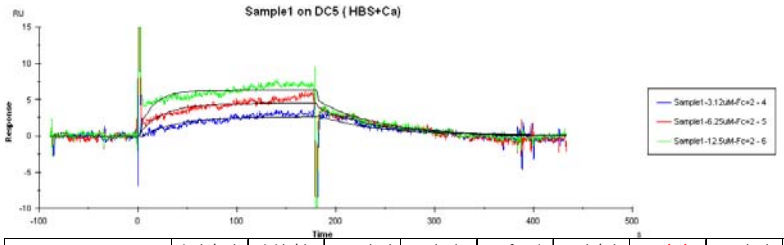
DC-SIGN: 2100 RU
GST: 900 RU

Entry 1 (from Fig 3 in the manuscript)



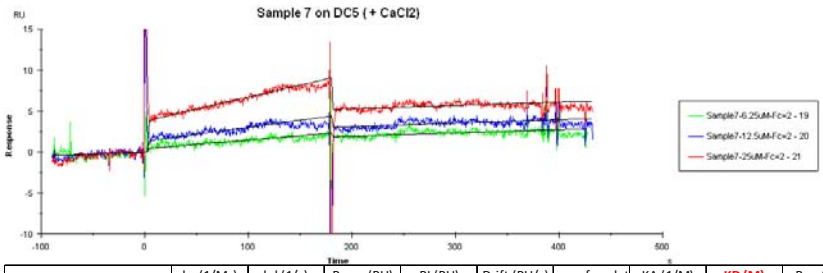
	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Drift (RU/s)	onc of analyt	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	33,5	4,24E-06	78,7				7,90E+06	1,27E-07			0,138
Sample5-3.12uM-Fc=2 - 8				0,401	8,93E-03	3,12u			75,7	1,09E-04	
Sample5-6.25uM-Fc=2 - 9				1,13	9,31E-03	6,25u			77,2	2,13E-04	
Sample5-12.5uM-Fc=2 - 10				2,39	0,0108	12,5u			78	4,23E-04	
Sample5-25uM-Fc=2 - 11				4,5	7,47E-03	25u			78,4	8,41E-04	

Entry 3 (from Fig 3 in the manuscript)



	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	onc of analyt	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	3,94E+03	0,0161	6,47			2,45E+05	4,09E-06			0,202
Sample1-3.12uM-Fc=2 - 4				-0,15	3,12u			2,8	0,0284	
Sample1-6.25uM-Fc=2 - 5				0,599	6,25u			3,91	0,0407	
Sample1-12.5uM-Fc=2 - 6				1,46	12,5u			4,87	0,0653	

Entry 5 (from Fig 3 in the manuscript)



	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Drift (RU/s)	onc of analyt	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	18,2	3,80E-05	58,2				4,77E+05	2,09E-06			0,13
Sample7-6.25uM-Fc=2 - 19				0,475	4,06E-03	6,25u			43,6	1,52E-04	
Sample7-12.5uM-Fc=2 - 20				1,38	4,27E-03	12,5u			49,8	2,65E-04	
Sample7-25uM-Fc=2 - 21				3,85	4,13E-03	25u			53,7	4,92E-04	

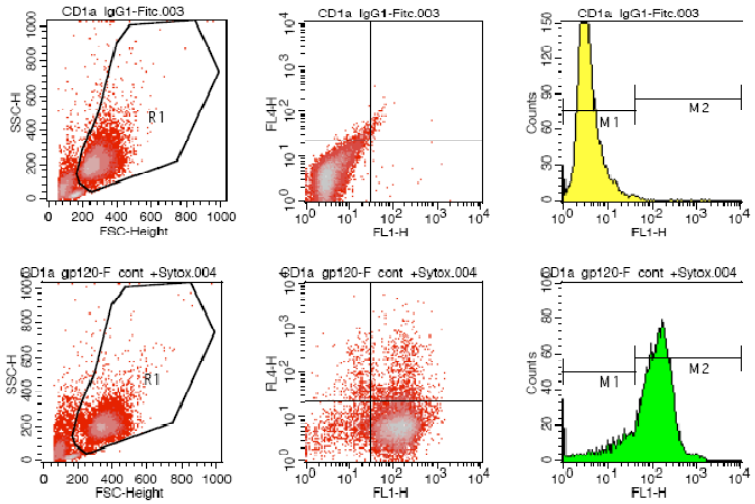
6. Inhibition of gp120 binding to dendritic cells.

Generation of human monocyte-derived dendritic cells (MDDCs)

Elutriated human monocytes were obtained from the French Blood Bank (Etablissement Français du Sang, Strasbourg, France). To obtain MDDCs, 3 x10^6 monocytes were cultured at 37°C and 5% CO_2 in 5 ml of complete RPMI 1640 medium (Lonza, containing 10% fetal bovine serum (FBS) and a mixture of penicillin and streptomycin) in the presence of 50 ng/ml recombinant human granulocyte macrophage colony stimulating factor (ImmunoTools) and 10 ng/ml recombinant human interleukin-4 (ImmunoTools), with readdition of cytokines at day 3. Non-adherent cells were harvested on day 5, and specific cell marker expression (CD1a, DC-SIGN) were characterized by flow cytometry on a FACS Calibur flow cytometer (Beckton-Dickinson) and analysed with the CellQuest Pro software (BD Biosciences).

Competition assay between DC-SIGN ligands and gp120 for binding to MDDCs.

Human MDDCs (1 x10^5 in 100 µl) were aliquoted in a 96-well microtiter plate in RPMI 1640 medium without FBS. To assess the binding of HIV-1 glycoprotein 120, FITC-conjugated recombinant gp120 HIV-1 IIIB (ImmunoDiagnostics, Inc, Woburn, MA, USA) was added to the cells (final concentration of 5 µg/ml) and incubated for 45 min at 37°C. For competition assays, cells were pretreated with either mannan (100 µg/ml final) or dendrimers (at the indicated concentrations) for 30 min at 37°C, followed by incubation with gp120-FITC (gp120-fl)for 45 min at 37°C. After washing with HBSS buffer (Lonza), expression of fluorescence intensity was analyzed on a FACS Calibur flow cytometer with the CellQuest Pro software.



Histogram Statistics

File: CD1a IgG1-Fitc.003 — Log Data Units: Linear Values
Sample ID: CD1a IgG1-Fitc — Acquisition Date: 26-Apr-11
Gate: G1 — Gated Events: 17303
Total Events: 27841 — X Parameter: FL1-H (Log)

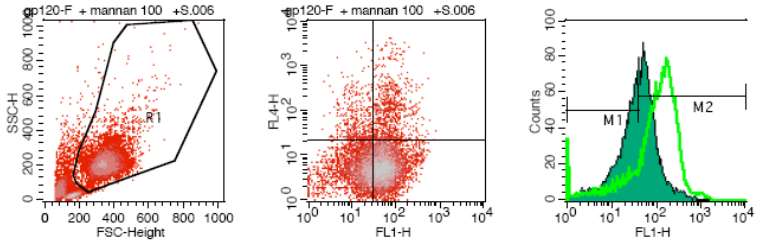
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean
All	1, 9910	17303	100.00	62.15	4.77	3.58
M1	1, 41	17260	99.75	61.99	4.21	3.55
M2	41, 9910	46	0.27	0.17	219.37	90.24

Histogram Statistics

File: CD1a gp120-F cont +Sytox.004 — Log Data Units: Linear Va
Sample ID: CD1a gp120-F cont +Sytox — Acquisition Date: 26-Apr-
Gate: G1 — Gated Events: 12221
Total Events: 19586 — X Parameter: FL1-H (Log

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean
All	1, 9910	12221	100.00	62.40	140.07	97.42
M1	1, 41	1697	13.89	8.66	19.24	13.74
M2	41, 9910	10543	86.27	53.83	159.34	133.32

DC cells (Top), DC cells +gp120-fl (bottom)

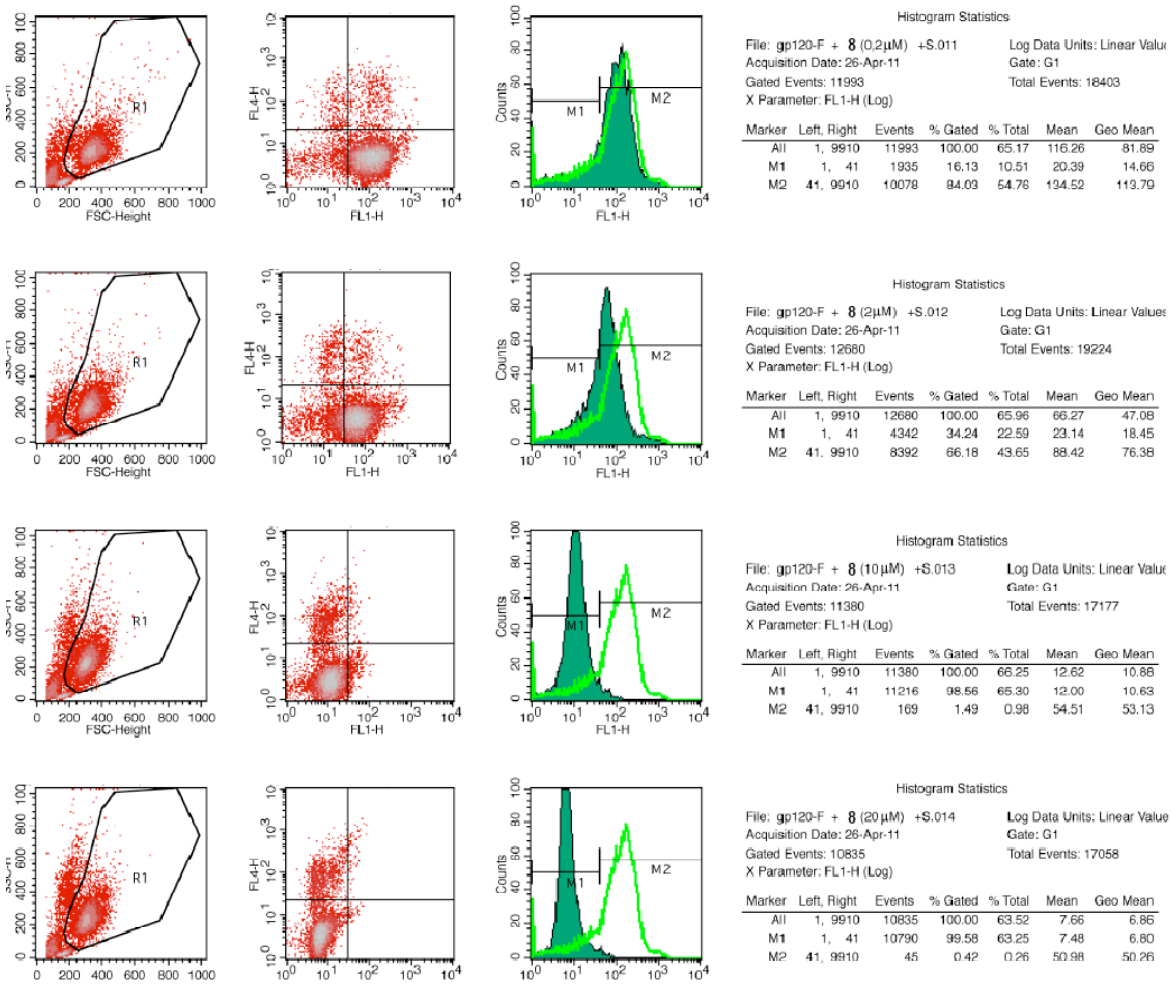


Histogram Statistics

File: gp120-F + mannan 100 +S.006 — Log Data Units: Linear Val
Acquisition Date: 26-Apr-11 — Gate: G1
Gated Events: 12214 — Total Events: 19105
X Parameter: FL1-H (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean
All	1, 9910	12214	100.00	63.93	50.72	36.25
M1	1, 41	6232	51.02	32.62	23.08	19.18
M2	41, 9910	6039	49.44	31.61	79.14	70.00

DC cells (Top), DC cells +gp120-fl (bottom) + mannan (100 µg/ml, i.e. 500 µM in mannos)



DC cells (Top), DC cells + gp120-fl (bottom) + dendron **8** at 0.2 µM, 2 µM, 10 µM, 20 µM (from top to bottom)