Potent divalent inhibitors with rigid glucose click spacers for *Pseudomonas aeruginosa* lectin LecA

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General procedure for the “click reaction”, step a. Preparation of compounds 11, 13, 15, 17

Physical data of 11

Physical data of 13

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$^1$H NMR of 2

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$^1$H NMR of 3

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$^1$H NMR of 4

$^{13}$C NMR of 4

$^1$H NMR of 6a

$^{13}$C NMR of 6a

$^1$H NMR of 6

$^{13}$C NMR of 6

$^1$H NMR of 7a
\begin{align*}
&^{13}\text{C NMR of } 7a & S-30 \\
&^{1}\text{H NMR of } 7 & S-31 \\
&^{13}\text{C NMR of } 7 & S-31 \\
&^{1}\text{H NMR of } 9a & S-32 \\
&^{13}\text{C NMR of } 9a & S-32 \\
&^{1}\text{H NMR of } 9 & S-33 \\
&^{13}\text{C NMR of } 9a & S-33 \\
&^{1}\text{H NMR of } 8a & S-34 \\
&^{1}\text{H NMR of } 10a & S-34 \\
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&^{13}\text{C NMR of } 8 & S-35 \\
&^{1}\text{H NMR of } 10 & S-36 \\
&^{13}\text{C NMR of } 10 & S-36 \\
&^{1}\text{H NMR of } 11 & S-37 \\
&^{13}\text{C NMR of } 11 & S-37 \\
&^{1}\text{H NMR of } 13 & S-38 \\
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&^{1}\text{H NMR of } 18 & S-44 \\
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&^{1}\text{H NMR of } 19c & S-46 \\
&^{13}\text{C NMR of } 19c & S-46 \\
\end{align*}
$^1$H NMR of 19
HSQC/HMBC NMR of 19
$^1$H NMR of 20
$^{13}$C NMR of 20
$^1$H NMR of 21
$^{13}$C NMR of 21
$^1$H NMR of 24
$^{13}$C NMR of 24
$^1$H NMR of 22
HSQC NMR of 22
IC$_{50}$ measurement of 12
IC$_{50}$ measurement of 14
IC$_{50}$ measurement of 16
IC$_{50}$ measurement of 18
IC$_{50}$ measurement of 19
IC$_{50}$ measurement of 20
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Experimental Section

General: Unless stated otherwise, chemicals were obtained from commercial sources and were used without further purification. Solvents were purchased from Biosolve (Valkenswaard, The Netherlands). All moisture-sensitive reactions were performed under nitrogen atmosphere. Anhydrous THF was dried over Na/benzophenone and freshly distilled prior to use. All the other solvents were dried over molecular sieves 4 Å or 3 Å. TLC was performed on Merck precoated Silica 60 plates. Spots were visualized by UV light, 10% H2SO4 in MeOH and triphenylphosphine in THF followed by Ninhydrine. Microwave reactions were carried out in a Biotage microwave Initiator (Uppsala, Sweden). The microwave power was limited by temperature control once the desired temperature was reached. Sealed vessels of 2-5 mL and 10-20 mL were used. Analytical HPLC runs were performed on a Shimadzu automated HPLC system with a reversed-phase column (Alltech, C8, 90 M, 5 mm, 250 L, 4.6 mm, Deerfield, IL, USA) that was equipped with an evaporative light-scattering detector (PLELS 1000, Polymer Laboratories, Amherst, MA, USA) and a UV/Vis detector operating at 220 nm and 250 nm. Preparative HPLC runs were performed on an Applied Biosystems workstation. Elution was effected by using a linear gradient of 5% MeCN/0.1% TFA in H2O to 5% H2O/0.1% TFA in MeCN or by a gradient of 0.1% in H2O to 30% MeCN/0.1% TFA in H2O. 1H NMR (300 MHz) and 13C (75.5 MHz) were performed on a Varian G-300 spectrometer. HSQC, HMBC and TOCSY NMR (500 MHz) were performed with a VARIAN INOVA-500. Electrospray Mass experiments were performed in a Shimadzu LCMS QP-8000. High resolution mass spectrometry (HRMS) analysis was performed using an Applied Biosystems 4700 MALDI TOF/TOF instrument.

LecA inhibition assay: The lectin LecA was obtained from Sigma-Aldrich and it was FITC labeled according to the procedure of Sigma-Aldrich. Microarray experiments were performed by using PamChip arrays run on a PamStation12 instrument (Pam-Gene B.V., ’s Hertogenbosch, The Netherlands). Data were obtained by real-time imaging of the fluorescence signal by a CCD camera. Images were analyzed by using BioNavigator software (Pam-Gene). Each array slide contains spots in duplicate. The fluorescence intensities were expressed in arbitrary units and the relative intensities
were the average of the two duplicate spots. Aliquots of a solution of FITC-labeled LecA (20 mg mL⁻¹) in HEPES/PBS buffer (10 mM HEPES, 100 mM NaCl, 0.1% BSA. pH 7.4), containing different concentrations of the inhibitors were incubated for 1 h at r.t. and subsequently added to the glycodendrimer chip. The binding process was monitored for 2 h and the end values of the fluorescence detection were taken for the determination of the IC₅₀ by using Prism 5 (Graphpad Software, Inc.).

Scheme 1. Preparation of the building block 4. a) i. BCl₃·SMe₂, DCM, microwave, 80°C, 20'; ii. Ac₂O, Py, 70% 2 steps; b) MeONa, MeOH, quant.; c) BzCl, Py, -40 °C, 30%

3,7-Anhydro-4,5,6,8-tetra-O-acetyl-1,1,2,2-tetrahydro-1,2-D-glycero-L-mannooctitol, 2. A BCl₃·SMe₂ solution (2M, 6.44 mL, 12.88 mmol) in CH₂Cl₂ was added to a solution of 1 (1 g, 1.61 mmol) in CH₂Cl₂ (12 mL). The mixture was heated under microwave irradiation at 80°C for 20 min. The resulting black solution was neutralized with a saturated NaHCO₃ solution (80 mL). The liquid was evaporated, methanol was added and the suspension was filtered. The solvent was evaporated under vacuum and pyridine (15 mL) was added to the residue. The solution was treated with acetic anhydride (2.46 mL, 26 mmol) and the mixture was stirred overnight at r.t.. The mixture was concentrated under vacuum and the residue was dissolved in CH₂Cl₂, washed with 1M KHSO₄ solution (15 mL), H₂O (15 mL) and brine (15 mL). The organic layer was dried over sodium sulfate. After evaporation of the solvent the product was purified by column chromatography (ethyl acetate/hexane, 4:6) to give 2 as a white solid (0.430 g, 1.13 mmol, 70%). ¹H NMR (300MHz, CDCl₃): δ, 5.39 (d, 1H, J₆,₅=3.34 Hz, H-6), 5.38 (t, 1H, J₄,₃=J₄,₅=9.95 Hz, H-4), 4.97 (dd, 1H, J₅,₄=9.95 Hz, J₅,₆=3.34 Hz, H-5), 4.16 (dd, 1H, J₃,₁=2.19, J₃,₄=9.95 Hz, H-3), 4.09 (d, 2H, J₃,₈a,J₃,₈b=6.40 Hz, H-8ab), 3.88 (t, 1H, J₇,₈a=J₇,₈b=6.40 Hz, H-7), 2.50 (d, J₈a,J₈b=2.19 Hz, H-1), 2.13, 2.05, 2.02 and 1.96 (4s, 4H, COCH₃). ¹³C NMR (75.5 MHz, CDCl₃): δ ppm 170.59 (C=O), 170.40 (C=O), 170.26 (C=O), 169.53 (C=O), 78.10 (C-1), 75.45 (C-2), 74.79 (C-7), 71.62 (C-5), 69.07 (C-3), 68.44 (C-4), 67.48
(C-6), 61.78 (C-8), 20.09 (COCH$_{3}$). MS (ESI) m/z calc for C$_{16}$H$_{21}$O$_{5}$ (M+H)$^{+}$ 357.12, found 357.32.

3.7-Anhydro-1,1,2,2-tetrahydro-1,2-D-glycero-L-mannoctitol, 3. A solution of 2 (3 g, 8.42 mmol) in methanol (25 mL) was treated with a solution of NaOMe in methanol (30%, 1 mL) and the mixture was stirred for 4 h at r.t. After neutralization with DowexH$^{+}$, the mixture was filtered and the methanol evaporated in vacuum to give compound 3 as a white foam (1.57 g, 8.35 mmol, quant.) $^{1}$H NMR (300MHz, CD$_{3}$OD): δ, 3.86 (d, 1H, J$_{6,5}$=3.2 Hz, H-6), 3.86 (dd, 1H, J$_{3,1}$=2.19, J$_{3,4}$=9.55 Hz, H-3), 3.73 (d, 1H, J$_{8a,7}$=6.05 Hz, J$_{8a,8b}$=11.45 Hz, H-8a), 3.66 (d, 1H, J$_{8b,7}$=6.05 Hz, J$_{8b,8a}$=11.45 Hz, H-8b), 3.65 (t, 1H, J$_{4,3}$=J$_{4,5}$=9.55 Hz, H-4), 3.48 (t, 1H, J$_{7,8a}$=J$_{7,8b}$=6.05 Hz, H-7), 3.40 (dd, 1H, J$_{5,4}$=9.55 Hz, J$_{5,6}$=3.30 Hz, H-5), 2.85 (d, J$_{1,3}$=2.19 Hz, H-1). $^{13}$C NMR (75.5 MHz, CD$_{3}$OD): δ ppm 82.04 (C-1), 80.67 (C-7), 75.70 (C-5), 75.06 (C-2), 72.43 (C-6), 72.25 (C-4), 70.54 (C-3), 62.69 (C-8). MS (ESI) m/z calc for C$_{8}$H$_{13}$O$_{5}$ (M+H)$^{+}$ 189.08, found 189.40.

3.7-Anhydro-4,5,8-tri-O-acetyl-1,1,2,2-tetrahydro-1,2-D-glycero-L-mannoctitol, 4. Benzoyl chloride (1.97 mL, 17 mmol) in pyridine (10 mL) was added dropwise to a solution of 3 (1 g, 5.3 mmol) in pyridine (25 mL) previously cooled at -40 C. The mixture was kept at -40 C for 30 min and the reaction was quenched with water (50 mL). The product was extracted three times with CH$_{2}$Cl$_{2}$ (30 mL). After evaporation of the liquid, the residue was dissolved in CH$_{2}$Cl$_{2}$ and washed with 1M KHSO$_{4}$, H$_{2}$O and brine. The organic layer was dried over sodium sulfate. TLC analysis showed the presence of four different benzoylated compounds, monitored by TLC. The desired compound 4 (toluene/ethylacetate, 4/1; R$_{f}$=0.52) was obtained after column chromatography (toluene/ethylacetate, 9:1, 0.796 g, 1.59 mmol, 30%). $^{1}$H NMR (300 MHz, CDCl$_{3}$): δ 8.05-7.26 (m, 15H, Ar), 6.04 (t, 1H, J$_{4,3}$=J$_{4,5}$=10.18 Hz, H-4), 5.37 (dd, 1H, J$_{5,4}$=10.18 Hz, J$_{5,6}$=2.95 Hz, H-5), 4.70 (dd, 1H, J$_{8a,7}$=5.98 Hz, J$_{8a,8b}$=11.60 Hz H-8a), 4.59 (dd, 1H, J$_{8b,7}$=5.98 Hz, J$_{8b,8a}$=11.60 Hz, H-8b), 4.48 (dd, 1H, J$_{3,1}$=2.05, J$_{3,4}$=10.18 Hz, H-3), 4.45 (d, 1H, J$_{6,5}$=2.95 Hz, H-6), 4.10 (t, 1H, J$_{7,8a}$=J$_{7,8b}$=5.98 Hz, H-7), 3.22 (s, 1H, OH), 2.47 (d, 1H, J$_{1,3}$=2.05 Hz H-1). $^{13}$C NMR (75.5 MHz, CDCl$_{3}$): δ ppm 166.62 (C=O), 165.94 (C=O), 165.39 (C=O), 133.47 (C, Ar), 133.32 (C, Ar), 133.30 (C, Ar), 129-129.79 (CH, Ar), 129.50
Recovery of compound 2. The crude benzoylated compounds (1.9 g) were dissolved in methanol (20 mL) and treated with 500 µL of 30% MeONa solution in methanol. After 4 h the reaction mixture was neutralized with Dowex and the methanol was removed under vacuum. The residue obtained was dissolved in pyridine 10 mL and 3 mL of acetic anhydride was added. The mixture was stirred at r.t. and it was concentrated under vacuum. The residue was dissolved in CH₂Cl₂ and washed with 1M KHSO₄, H₂O and brine. The organic layer was dried over sodium sulfate. After evaporation of the solvent the product was purified by column chromatography (ethyl acetate/hexane, 4:6) to give 2 (1.23 g, 3.45 mmol, 65%).

Scheme 2. Synthesis and elongation of the spacer. a) CuSO₄, Na-ascorbate, DMF, 10% H₂O, 30°, 80°C, microwave, 85-91%; b) i. Tf₂O, Py, DCM, 0°C, 1h; ii. NaN₃, DMF, r.t., 4h, 80-85% 2 steps.
6a. Compound 5\(^2\) (0.225 g, 1.2 mmol), CuSO\(_4\)-5H\(_2\)O (0.038 g, 0.15 mmol) and sodium ascorbate (0.060 g, 0.3 mmol) were added to a solution of 4 (0.5 g, 1 mmol) in DMF (15 mL) containing 10% water. The mixture was heated under microwave irradiation at 80°C for 30 min. After evaporation of the solvent the residue was dissolved in CH\(_2\)Cl\(_2\). The organic solution was washed three times with water and brine and it was dried over sodium sulfate. The solvent was removed and the white solid compound 5 was purified by column chromatography (EtOAc/Hexane, 2:3) (0.625 g, 0.91 mmol, 91%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 8.02-7.29 (m, 15H, Ar), 7.80 (s, 1H, H-3), 6.07 (t, 1H, J\(_{6,5}\)=J\(_{7,6}\)=9.95 Hz, H-6), 5.56 (dd, 1H, J\(_{7,6}\)=9.95, J\(_{5,4}\)=3.15 Hz, H-7), 5.04 (d, 1H, J\(_{6,5}\)=9.95 Hz, H-5), 4.94 (t, 1H, J\(_{NH,1ab}\)=4.55 Hz, NH), 4.71 (dd, 1H, J\(_{10a,9}\)=5.94 Hz, J\(_{10a,10b}\)=11.38 Hz, H-10a), 4.56 (dd, 1H, J\(_{10b,9}\)=5.94 Hz, J\(_{10b,10a}\)=11.38 Hz, H-10b), 4.53 (d, 1H, J\(_{8,7}\)=3.15 Hz, H-8), 4.34 (m, 2H, H-2ab), 4.27 (t, 1H, J\(_{9,10a}\)=J\(_{9,10b}\)=5.94 Hz, H-9), 3.49 (m, 2H, H-2ab), 3.38 (s, 1H, OH), 1.42 (s, 9H, C(CH\(_3\))\(_3\)). \(^1^3\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta\) 166.84 (C=O), 166.02 (C=O), 165.92 (C=O), 156.16 (NH\(_3\)=O), 145.04 (C-4), 133.80-133.36 (C, Ar), 130.23-128.41 (CH, Ar), 123.52 (C-3), 77.60 (C-9), 75.29 (C-7), 74.15 (C-5), 70.09 (C-6), 68.00 (C-8), 63.51 (C-10), 50.56 (C-2), 40.83 (C-1), 80.17 (C(CH\(_3\))\(_3\)), 28.53 (C(CH\(_3\))\(_3\)). MS (ESI) \(m/z\) calcd for C\(_{36}\)H\(_{39}\)N\(_4\)O\(_{10}\) 687.27 (M+H\(^+\)), found 687.00.

**General procedure for the introduction of the azide, step b. Preparation of compounds 6, 7 and 9:** Triflic anhydride (10 equiv) was added dropwise to a solution of the carbohydrate (1 equiv) in CH\(_2\)Cl\(_2\) (20 mL) containing pyridine 10 %, previously cooled at 0°C. The solution was stirred at 0°C for 1.5 h and the reaction was quenched adding cold 1 M KHSO\(_4\) (20 mL). The organic layer was washed two times with cold water and cold brine and dried over sodium sulfate. The solvent was evaporated giving the triflate-carbohydrate compound as a yellow solid which was used without any further purification. The solid was dissolved in DMF (20 mL) and sodium azide (5 equiv) was added. The reaction mixture was stirred at r.t. for 5 h. The solvent was evaporated under vacuum and the residue was dissolved in CH\(_2\)Cl\(_2\) and washed three times with water and brine. The organic layer was dried over sodium sulfate. The solvent was evaporated and the residue purified by column chromatography to give a yellowish solid.
General procedure for the “click reaction”, step a. Preparation of compounds 7a and 9a: The reactions between compound 4 and the azide–carbohydrate compounds 6 and 7, respectively, were performed following the experimental procedure reported for the synthesis of the compound 6a.

6. (0.527 g, 0.74 mmol, 85%) \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 8.09-7.28 (m, 15H, Ar), 7.66 (s, 1H, H-3), 5.84 (t, 1H, \(J_{7,8}=9.84\) Hz, H-7), 5.65 (t, 1H, \(J_{6,5}=J_{6,7}=9.84\) Hz, H-6), 5.02 (d, 1H, \(J_{5,6}=9.84\) Hz, H-5), 4.85 (t, 1H, \(J_{NH,1ab}=4.55\) Hz, NH), 4.74 (dd, 1H, \(J_{10a,6}=2.16\) Hz, \(J_{10a,10b}=12.53\) Hz, H-10a), 4.62 (dd, 1H, \(J_{10b,9}=4.53\) Hz, \(J_{10b,10a}=12.53\) Hz, H-10b), 4.39 (m, 2H, H-2ab), 4.03 (t, 1H, \(J_{8,9}=9.84\) Hz, H-8); 3.98 (m, 1H, H-9), 3.52 (m, 2H, H-1ab), 1.40 (s, 9H, C(CH\(_3\))\(_3\)). \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta\) 166.35 (C=O), 165.82 (C=O), 165.68 (C=O), 156.03 (NH\(=\)O), 144.28 (C-4), 133.97-133.24 (C, Ar), 130.52-128.05 (CH, Ar), 123.34 (C-3), 80.17 (C(CH\(_3\))\(_3\)), 77.33 (C-9), 73.95 (C-5), 75.11 (C-7), 72.36 (C-6), 63.88 (C-10), 61.35 (C-8), 50.52 (C-2), 40.81 (C-1), 28.53 (C(CH\(_3\))\(_3\)). MS (ESI) \(m/z\) caled for C\(_{36}H\(_{38}\)N\(_7\)O\(_9\) (M+H)\(^+\) 712,27, found 712.25.

7a. (0.775 g, 0.64 mmol, 86%) \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 8.1 (1H, H-3’), 7.99-7.11 (m, 30H, Ar), 7.71 (1H, H-3), 6.31 (1H, H-7), 5.93 (1H, H-6’), 5.81 (1H, H-6), 5.52 (1H, H-7’), 5.30 (1H, H-8), 5.28 (1H, H-5), 4.95 (1H, H-5’), 4.89 (1H, NH), 4.71 (1H, H-9), 4.65 (1H, H-10a’), 4.52 (1H, H-10b’), 4.45 (1H, H-10a), 4.40 (1H, H-8’), 4.38 (2H, H-2ab), 4.20 (1H, H-9’), 3.93 (1H, H-10b), 3.50 (2H, H-1ab), 3.50 (1H, OH), 1.38 (9H, C(CH\(_3\))\(_3\)). \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta\) 166.75 (C=O), 166.02 (C=O), 165.88 (C=O), 165.73 (C=O), 165.63 (C=O), 165.17 (C=O), 165.08 (NH\(=\)O), 156.08 (NH\(=\)O), 145.88 (C-4’), 144.29 (C-4), 134.36-132.83 (Ar), 130.01-127.89 (Ar), 123.90 (C-3), 123.02 (C-3’), 80.13 (C(CH\(_3\))\(_3\)), 77.33 (C-9), 76.87 (C-9’), 75.27 (C-7’), 74.32 (C-5’), 74.20 (C-5), 73.97 (C-7), 72.97 (C-6), 70.32 (C-6’), 68.03 (C-8’), 63.49 (C-10’), 62.68 (C-10), 61.07 (C-8), 50.53 (C-2), 40.77 (C-1), 28.52 (C(CH\(_3\))\(_3\)). MS (ESI) \(m/z\) caled for C\(_{65}H\(_{62}\)N\(_7\)O\(_{17}\) (M+H)\(^+\) 1212,42, found 1212.25.
7. (0.593 g, 0.48 mmol, 83%) $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.09-7.14 (m, 15H, Ar), 7.85 (1H, H-3'), 7.69 (1H, H-3), 6.25 (1H, H-7), 5.53 (1H, H-6'), 5.81 (1H, H-6), 5.80 (1H, H-7'), 5.23 (1H, H-8), 5.25 (1H, H-5), 4.95 (1H, H-5'), 4.86 (1H, NH), 4.64 (1H, H-9), 4.66 (1H, H-10a'), 4.61 (1H, H-10b'), 4.40 (1H, H-10a), 3.97 (1H, H-8'), 4.39 (2H, H-2ab), 3.90 (1H, H-9'), 3.98 (1H, H-10b), 3.52 (2H, H-lab), 1.39 (9H, C(CH$_3$)$_3$). $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ 166.36 (C=O), 165.88 (C=O), 165.80 (C=O), 165.55 (C=O), 165.49 (C=O), 165.07 (C=O), 156.03 (NH$_3$=O), 145.08 (C-4'), 144.06 (C-4). 133.80-133.24 (C, Ar), 130.24-128.29 (CH, Ar), 123.40 (C-3), 122.27 (C-3'), 80.18 (C(CH$_3$)$_3$), 77.32 (C-9), 77.25 (C-9'), 75.11 (C-7'), 74.25 (C-5), 73.89 (C-5'), 73.82 (C-7), 72.78 (C-6), 72.59 (C-6'), 64.02 (C-10'), 62.73 (C-10), 61.44 (C-8'), 61.13 (C-8), 50.53 (C-2), 40.77 (C-1), 28.52 (C(CH$_3$)$_3$). MS (ESI) m/z calcd for C$_{58}$H$_{56}$N$_{10}$O$_{15}$ (M+H)$^+$ 1237.43, found 1238.00.

9a. (0.618 g, 0.36 mmol, 80%) $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.03-7.10 (m, 45H, Ar), 7.93 (2H, H-3', H-3''), 7.65 (1H, H-3), 6.25 (2H, H-7, H-7'), 5.93 (1H, H-6''), 5.76 (1H, H-6), 5.68 (1H, H-6'), 5.48 (1H, H-7''), 5.23 (1H, H-5), 5.22 (1H, H-8'), 5.19 (1H, H-8'), 5.17 (1H, H-5'), 4.91 (1H, H-5''), 4.84 (1H, NH), 4.70 (1H, H-9), 4.68 (1H, H-10a''), 4.65 (1H, H-9'), 4.47 (1H, H-10b''), 4.40 (2H, H-10a, H-8''), 4.38 (3H, H-2ab, H-10a'), 4.15 (1H, H-9''), 4.00 (1H, H-10b'), 3.95 (1H, H-10b), 3.48 (2H, H-lab), 1.38 (s, 9H, C(CH$_3$)$_3$). $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ 166.74-165.00 (C=O), 156.06 (NH$_3$=O), 145.80 (C-4''), 145.06 (C-4'), 144.16 (C-4), 134.00-132.82 (Ar), 130.30-128.00 (Ar), 123.41 (C-3), 122.51 (C-3', C-3''), 80.16 (C(CH$_3$)$_3$), 77.45 (C-9, C-9''), 77.00 (C-9'''), 75.28 (C-7''), 74.14 (C-5''), 73.97 (C-5, C-5''), 73.67 (C-7, C-7'), 72.91 (C-6''), 72.72 (C-6), 70.19 (C-6'''), 67.88 (C-8''), 63.41 (C-10'''), 62.87 (C-10'), 62.64 (C-10), 61.08 (C-8, C-8'), 50.49 (C-2), 40.79 (C-1), 28.53 (C(CH$_3$)$_3$). MS (ESI) m/z calcd for C$_{94}$H$_{88}$N$_{10}$O$_{24}$ (M+2H)$^{2+}$ 869.29, found 869.90.

9. (0.486 g, 0.28 mmol, 80%) $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.10-7.11 (m, 45H, Ar), 7.88 (s, 1H, H-3'), 7.81 (s, 1H, H-3''), 7.69 (s, 1H, H-3), 6.24 (1H, H-7), 6.21 (1H, H-7'), 5.79 (1H, H-6'''), 5.78 (1H, H-7'''), 5.63 (1H, H-6), 5.50 (1H, H-6'), 5.23 (1H, H-5), 5.21 (1H, H-8), 5.14 (1H, H-8'), 5.09 (1H, H-5'), 4.91 (1H, H-5''), 4.87 (1H, NH),
4.65 (1H, H-10a’’), 4.63 (1H, H-9), 4.61 (1H, H-10b’’), 4.53 (1H, H-9’’), 4.42 (3H, H-10a, H-2ab), 4.32 (1H, H-10a’), 3.95 (3H, H-10b’, H-10b, H-8’’), 3.86 (1H, H-9’’), 3.50 (2H, H-1ab), 1.39 (s, 9H, C(CH$_3$)$_3$). $^{13}$C NMR (75.5 MHz, CDCl$_3$): δ 166.35-165.02 (C=O), 156.02 (NH$_2$C=O), 145.04 (C-4’’), 144.06 (C-4), 133.99-133.05 (C, Ar), 130.43-128.00 (CH, Ar), 123.41 (C-3), 122.39 (C-3’’), 122.26 (C-3’), 80.19 (C(CH$_3$)$_3$), 77.50 (C-9, C-9’), 77.30 (C-9’’), 74.95 (C-7’’), 74.22 (C-5), 74.05 (C-5’), 73.80 (C-5’’), 73.70 (C-7), 73.55 (C-7’), 72.66 (C-6, C-6’’), 72.36 (C-6’), 63.88 (C-10’’), 62.71 (C-10’), 62.61 (C-10), 61.34 (C-8’’), 61.19 (C-8), 61.02 (C-8’), 50.54 (C-2), 40.81 (C-1), 28.53 (C(CH$_3$)$_3$). MS (ESI) m/z calcd for C$_{87}$H$_{78}$N$_{13}$O$_{21}$ (M+2H)$^{2+}$ 881.80, found 882.50.

Scheme 3. a) TFA, DCM, quant. b) imidazole-1-sulfonyl azide, K$_2$CO$_3$, CuSO$_4$, MeOH, 82-85%.

**General procedure for the removal of the Boc, step a. Preparation of compounds 8a and 10a.** Trifluoroacetic acid (5 mL, 50%) was added to a solution of 7 and 9 (0.16 mmol), respectively, in CH$_2$Cl$_2$ (10 mL). After 2 h the solvent was evaporated under vacuum to give the compounds 8a and 10a, respectively, as yellowish solids that were used without further purification.

8a. MS (ESI) m/z calcd for C$_{60}$H$_{52}$N$_{10}$O$_{14}$ (M+H)$^+$ 1137.57, found 1137.37.

10a. MS (ESI) m/z calcd for C$_{89}$H$_{75}$N$_{13}$O$_{21}$ (M+2H)$^{2+}$ 831.77, found 832.30.
General procedure for the diazotransfer, step b. Preparation of compounds 8 and 10. Imidazole-1-sulfonyl azide hydrochloride (1.2 equiv) was added to the ammonium salt 8a and 10a (1 equiv), respectively, K₂CO₃ (2.5 equiv) and CuSO₄·5H₂O (0.01 equiv) in MeOH (5 mL) and the mixture was stirred at r.t. for 4 h. The solvent was removed under vacuum and the residue was dissolved in CH₂Cl₂ and washed three times with water and brine. After drying over sodium sulfate the solvent was removed and the compound purified by column chromatography.

8. (0.160 g, 0.14 mmol, 85% from 7) ¹H NMR (300 MHz, CDCl₃): δ 8.08-7.12 (m, 30H, Ar), 7.92 (1H, H-3'), 7.78 (1H, H-3’), 6.28 (1H, H-7), 5.55 (1H, H-6'), 5.91 (1H, H-6), 5.81 (1H, H-7'), 5.26 (1H, H-8), 5.32 (1H, H-5), 4.96 (1H, H-5'), 4.67 (1H, H-9), 4.63 (1H, H-10a'), 4.59 (1H, H-10b'), 4.39 (1H, H-10a), 3.97 (1H, H-8'), 4.33 (2H, H-2ab), 3.90 (1H, H-9'), 3.98 (1H, H-10b), 3.60 (2H, H-1ab). ¹³C NMR (75.5 MHz, CDCl₃): δ 166.37 (C=O), 165.89 (C=O), 165.86 (C=O), 165.54 (C=O), 165.46 (C=O), 165.14 (C=O), 145.03 (C-4'), 144.14 (C-4), 134.26-133.92 (Ar), 130.46-128.91 (Ar), 123.72 (C-3), 122.51 (C-3'), 77.22 (C-9'), 77.22 (C-9), 75.16 (C-7'), 74.00 (C-5), 73.87 (C-5'), 74.10 (C-7), 72.63 (C-6'), 72.38 (C-6), 64.07 (C-10'), 62.76 (C-10), 61.43 (C-8'), 61.14 (C-8), 50.66 (C-1), 49.59 (C-2). MS (ESI) m/z calcd for C₆₀H₅₁N₁₂O₁₄ (M+H)⁺ 1163.36, found 1163.60

10. (0.238 g, 0.14 mmol, 83% from 9) ¹H NMR (300 MHz, CDCl₃): δ 8.10-7.13 (m, 45H, Ar), 7.87 (s, 1H, H-3’”), 7.80 (s, 1H, H-3’), 7.77 (s, 1H, H-3), 6.22 (1H, H-7), 6.18 (1H, H-7’), 5.86 (1H, H-6’”), 5.77 (1H, H-7’”), 5.63 (1H, H-6), 5.49 (1H, H-6’), 5.25 (1H, H-5), 5.19 (1H, H-8), 5.11 (2H, H-5’, H-8’), 4.90 (1H, H-5’”), 4.63 (1H, H-10a’”), 4.61 (1H, H-9), 4.58 (1H, H-10b’”), 4.52 (1H, H-9’), 4.38 (3H, H-10a, H-2ab), 4.31 (1H, H-10a’), 3.95 (3H, H-10b’, H-10b, H-8’”), 3.85 (1H, H-9’’), 3.68 (2H, H-1ab). ¹³C NMR (75.5 MHz, CDCl₃): δ 166.14-164.83 (C=O), 144.78 (C-4’’), 144.53 (C-4’), 143.88 (C-4), 133.49-133.12 (C, Ar), 129.80-128.23 (CH, Ar), 123.47 (C-3), 122.40 (C-3’’), 122.15 (C-3’), 76.82 (C-9, C-9’, C-9’’), 74.73 (C-7’’), 73.66 (C-5), 73.62 (C-5’), 73.51 (C-5’’), 73.46 (C-7), 73.39 (C-7’), 72.47 (C-6), 72.18 (C-6’), 71.94 (C-6’’), 63.68 (C-10’’), 62.49 (C-10’), 62.39 (C-10), 61.10 (C-8’’), 60.86 (C-8,
C-8'), 50.41 (C-1), 49.35 (C-2). MS (ESI) m/z calcd for C_{89}H_{74}N_{15}O_{21} (M+2H)^{2+} 844.77, found 845.40

Scheme 4. Synthesis of the ligands. a) CuSO_4, Na-ascorbate, DMF, 10% H_2O, 30', 80°C, microwave, 80-85%. b) MeONa, MeOH, 60-87%.
General procedure for the “click reaction”, step a. Preparation of compounds 11, 13, 15, 17: Compound 21 or compound 2 (1.2 equiv), CuSO₄·5H₂O (0.15 equiv) and sodium ascorbate (0.3 equiv) were added to a solution of 8 or 10 (1 equiv) in DMF (3 mL) with 10% water. The mixture was heated under microwave irradiation at 80°C for 30 min. After evaporation of the solvent the residue was dissolved in CH₂Cl₂ and washed three times with water and brine. After drying over sodium sulfate the solvent was removed and the compound purified by column chromatography to give a white solid.

11. (0.04 g, 0.02 mmol, 83%) ¹H NMR (300 MHz, CDCl₃): 8.10-7.10 (30H, Ar), 7.93, 7.73, 7.48, 7.44 (4H, 4×H-3), 6.26, 6.24 (2H, 2×H-7), 5.82, 5.66 (2H, 2×H-6), 5.52-5.41 (3H, 2×H-14, 1×H-12), 5.32-5.06 (7H, 2×H-5, 2×H-13, 2×H-8, 1×H-12), 4.88-4.56 (8H, H-1ab, H-2ab, 2×H-9, 2×H-11), 4.38-4.26 (2H, 2×H-10a), 4.15-3.95 (6H, 2×H-10b, 2×H-16ab, 2×H-15), 2.11, 2.00, 2.02, 1.99, 1.98, 1.93, 1.87, 1.61 (6×CH₂C=O). ¹³C NMR (75.5 MHz, CDCl₃): 170.80-170.17, 169.99, 169.73 (C=O, acetyl), 166.04, 165.88, 165.35, 165.08 (C=O, benzoyl), 145.20, 145.13, 144.78, 144.57 (4×C-4), 133.92-133.15 (C, Ar), 130.27-129.45, 128.97-128.18 (CH, Ar), 123.76, 123.74, 122.70, 122.49 (4×C-3), 77.12 (2×C-9), 75.08 (2×C-15), 74.05-73.60 (2×C-5, 2×C-7, 2×C-11), 72.79 (1×C-6), 72.37-71.90 (1×C-6, 2×C-13), 69.00 (2×C-12), 67.80 (2×C-14), 63.04, 62.76 (2×C-10), 61.64-61.10 (2×C-8, 2×C-16), 49.89 (C-2, C-1), 20.93 (CH₃C=O). MS (ESI) m/z calcd for C₈₉H₇₄N₁₅O₂₁ (M+2H)⁺ 938.30, found 939.15.

13. (0.04 g, 0.02 mmol, 85%) ¹H NMR (300 MHz, CDCl₃): 8.00-7.05 (30H, Ar), 7.91, 7.52, 7.36, 6.89 (4H, 4×H-3), 6.22, 6.19 (2H, 2×H-7), 5.74, 5.67 (2H, 2×H-6), 5.40-5.35 (2H, 2×H-17), 5.23-5.10 (5H, 2×H-5, 2×H-15, 1×H-8), 5.08-4.92 (3H, 2×H-16, 1×H-8), 4.83-4.60 (6H, 2×H-9, H-1ab, H-2ab), 4.45 (1H, 1×H-14), 4.41-4.10 (3H, 2×H-10a, 1×H-14), 4.23-4.03 (5H, 2×H-19ab, 1×H-10b), 4.00-3.69 (5H, 2×H-13a, 2×H-18, 1×H-10b) 3.50-3.31 (2H, 2×H-13b), 2.59-2.51 (4H, 2×H-11ab), 2.12, 2.09, 2.02, 2.01, 2.00, 1.97, 1.96, 1.95 (6×CH₂C=O), 2.00-1.58 (4H, 2×H-12ab). ¹³C NMR (75.5 MHz, CDCl₃): 170.70, 170.51, 170.39, 169.75 (C=O, acetyl), 166.08, 165.86, 165.35, 165.06 (C=O, benzoyl), 147.75 (2×C-4), 144.80, 144.45 (2×C-4), 133.85-133.29 (C, Ar), 130.21-129.45 (CH, Ar) 128.94-128.28 (CH, Ar), 122.67, 122.64, 122.34, 122.26 (4×C-3), 101.44 (2×C-14), 77.06, 76.66 (2×C-9), 73.82 (2×C-5, 2×C-
7), 72.50 (2×C-6), 71.9 (2×C-16), 70.80 (2×C-18), 69.20 (2×C-15, 2×C-13), 67.33 (2×C-17), 63.38, 62.64 (2×C-10), 61.50-61.00 (2×C-8, 2×C-19), 49.93 (C-2), 49.45 (C-1), 29.18 (2×C-12), 21.90, 21.78 (2×C-11), 20.89 (CH3C=O). MS (ESI) m/z calcd for C80H74N15O21 (M+2H)2+ 996.34, found 996.95.

15. (0.07 g, 0.03 mmol, 80%) 1H NMR (300 MHz, CDCl3): 8.10-7.10 (45H, Ar), 7.92, 7.85, 7.74, 7.48, 7.44 (5H, 4×H-3), 6.326.15 (3H, 3×H-7), 5.85, 5.67, 5.64 (3H, 3×H-6), 5.52-5.42 (3H, 2×H-14, 1×H-12), 5.32-5.06 (7H, 3×H-5, 2×H-13, 3×H-8, 1×H-12), 4.85-4.50 (9H, H-1ab, H-2ab, 3×H-9, 2×H-11), 4.38-4.25 (3H, 3×H-10a), 4.15-3.90 (7H, 3×H-10b, 2×H-16ab, 2×H-15), 2.11, 2.01, 1.99, 1.97, 1.93, 1.87, 1.85, 1.61 (6×CH3C=O). 13C NMR (75.5 MHz, CDCl3): 170.79-170.21, 169.98, 169.71 (C=O, acetyl), 166.03, 165.87, 165.33, 165.06 (C=O, benzoyl), 145.19, 145.12, 144.81, 144.74, 144.58 (5×C-4), 133.97-133.25 (C, Ar), 130.29-129.57, 129.46-128.19 (CH, Ar), 123.77, 123.76, 122.72, 122.51, 122.50 (5×C-3), 77.06 (3×C-9), 75.05 (2×C-15), 74.05-73.60 (3×C-5, 3×C-7, 2×C-11), 72.80-71.90 (3×C-6, 2×C-13), 69.00 (2×C-12), 67.82 (2×C-14), 62.80 (3×C-10), 61.85-61.00 (3×C-8, 2×C-16), 49.86 (C-2, C-1), 20.93 (CH3C=O). MS (ESI) m/z calcd for C80H74N15O21 (M+2H)2+ 1201.37, found 1202.05.

17. (0.07 g, 0.03 mmol, 82%) 1H NMR (300 MHz, CDCl3): 8.00-7.10 (45H, Ar), 7.86 (2H, 2×H-3), 7.50, 7.34, 6.89 (3H, 3×H-3), 6.26-6.15 (3H, 3×H-7), 5.78-, 5.58 (3H, 3×H-6), 5.40-5.35 (2H, 2×H-17), 5.23-4.95 (10H, 3×H-5, 2×H-15, 3×H-8, 2×H-16), 4.83-4.67 (5H, 1×H-9, H-1ab, H-2ab), 4.61, 4.54 (2H, 2×H-9), 4.45 (1H, 1×H-14), 4.40-4.25 (3H, 3×H-10a, 1×H-14), 4.23-4.05 (5H, 2×H-19ab, 1×H-10b), 4.00-3.69 (6H, 2×H-13a, 2×H-18, 2×H-10b), 3.50-3.33 (2H, 2×H-13b), 2.57-2.51 (4H, 2×H-11ab), 2.12, 2.09, 2.02, 2.01, 2.00, 1.98, 1.97, 1.96 (6×CH3C=O), 1.67-1.57 (4H, 2×H-12ab). 13C NMR (75.5 MHz, CDCl3): 170.69, 170.51, 170.38, 169.74 (C=O, acetyl), 166.07, 165.83, 165.34, 165.03 (C=O, benzoyl), 147.75 (2×C-4), 144.82, 144.74, 144.45 (3×C-4), 133.92-133.15 (C, Ar), 130.27-129.45 (CH, Ar) 128.97-128.18 (CH, Ar), 123.66 (1×C-3), 122.59 (2×C-3), 122.35, 121.28 (2×C-3), 101.65 (2×C-14), 77.09 (2×C-9), 76.67 (1×C-9), 74.00-73.70 (3×C-5, 3×C-7), 72.60 (3×C-6), 71.21 (2×C-16), 70.83 (2×C-18), 69.24 (2×C-15, 2×C-13), 67.36 (2×C-17), 63.30, 62.70 (3×C-10), 61.55-61.00 (3×C-8, 2×C-19), 49.96 (C-2), 49.46 (C-1), 29.92, 29.15
(2xC-12), 21.93, 21.78 (2xC-11), 20.93 (CH3C=O). MS (ESI) m/z calcd for C89H74N15O21 (M+2H)2+ 1258.92, found 1259.70.

**General procedure for the removal of acetyl and benzoyl protecting groups, step b. Preparation of compounds 12, 14, 16, 18.** A solution of the protected carbohydrate compounds 11, 13, 15, 17 in methanol (5 mL) was treated, respectively, with a solution of NaOMe in methanol (30%, 200 μL) and the mixture was stirred for 4 h at r.t. After neutralization with Dowex, the mixture was filtered and the methanol evaporated in vacuum to give the desired compound, which was purified by preparative HPLC.

**12.** (0.017 g, 0.017 mmol, 87%) 1H NMR (300 MHz, D2O): 8.34, 8.29, 8.00, 7.96 (4H, 4×H-3), 5.01 (4H, H-1ab, H-2ab), 4.95-4.75 (4H, 2×H-5, 2×H-8), 4.54, 4.45 (2H, 2×H-11), 4.34-4.27 (4H, 2×H-9, 2×H-7), 4.05-3.70 (14H, 2×H-14, 2×H-12, 2×H-6, 2×H-15, 2×H-13, 2×H-16ab), 3.56 (2H, 2×H-10a), 3.34-3.27 (2H, 2×H-10b). 13C NMR (125 MHz, D2O): 143.40, 143.50, 142.30, 142.20 (4×C-4), 124.22, 124.15, 123.85, 123.83 (4×C-3), 77.98 (2×C-15), 76.98 (2×C-9), 73.41 (3×C-7), 72.80-72.38 (2×C-5, 2×C-11, 2×C-13), 71.80 (2×C-6), 68.98 (2×C-12), 67.85 (2×C-14), 60.99 (2×C-8), 59.98 (2×C-16), 58.75 (2×C-10), 48.90 (C-2, C-1). HRMS (MALDI TOF/TOF) m/z calcd for C34H51N12O18 (M+H)+ 915.3444 found 915.3411.

**14.** (0.012 g, 0.012 mmol, 80%) 1H NMR (300 MHz, D2O): 8.33, 7.99, 7.93, 7.59 (4H, 4×H-3), 4.99-4.92 (4H, H-1ab, H-2ab), 4.85-4.70 (4H, 2×H-5, 2×H-8), 4.39-4.25 (6H, 2×H-9, 2×H-14, 2×H-7), 3.96-3.85 (6H, 2×H-6, 2×H-13a, 2×H-17), 3.79-3.62 (10H, 2×H-13b, 2×H-15, 2×H-16, 2×H-19ab), 3.56-3.49 (4H, 2×H-10a, 2×H-18), 3.31-3.24 (2H, 2×H-10b), 2.95, 2.74 (4H, 2×H-11ab), 1.99, 1.90 (4H, 2×H-12ab). 13C NMR (125 MHz, D2O): 146.30, 143.30, 143.14 (4×C-4), 123.94, 123.75, 122.12, 122.05 (4×C-3), 101.34 (2×C-14), 76.90 (2×C-9), 73.90-73.00 (2×C-16, 2×C-7), 72.30-71.10 (2×C-15, 2×C-6, 2×C-5), 69.25 (2×C-18), 67.70-67.00 (2×C-17, 2×C-13), 60.90-60.50 (2×C-8), 59.49 (2×C-19), 58.53 (2×C-10), 48.75, 48.52 (C-2, C-1), 27.03, 26.92 (2×C-12), 19.54, 19.35 (2×C-11). HRMS (MALDI TOF/TOF) m/z calcd for C40H63N12NaO20 (M+Na)+ 1053.4096 found 1053.4061.
16. (0.017 g, 0.015 mmol, 75%) $^1$H NMR (300 MHz, D$_2$O): 8.39, 8.38, 8.32, 8.02, 7.99 (5H, 5×H-3), 5.05 (4H, H-1ab, H-2ab), 4.92-4.78 (6H, 3×H-5, 3×H-8), 4.59, 4.49 (2H, 2×H-11), 4.40-4.32 (6H, 3×H-9, 3×H-7), 4.10-3.73 (15H, 2×H-14, 2×H-12, 3×H-6, 2×H-15, 2×H-13, 2×H-16ab), 3.63-3.58 (3H, 3×H-10a), 3.38-3.32 (3H, 3×H-10b). $^{13}$C NMR (125 MHz, D$_2$O): 144.57-143.10 (5×C-4), 124.01-123.40 (5×C-3), 77.83 (2×C-15), 76.81 (3×C-9), 73.24 (3×C-7), 72.53-72.18 (3×C-5, 2×C-11, 2×C-13), 71.70-71.60 (3×C-6), 68.90-68.70 (2×C-12), 67.70 (2×C-14), 60.82 (3×C-8), 59.75 (2×C-16), 58.55 (3×C-10), 48.70 (C-2, C-1). HRMS (MALDI TOF/TOF) m/z calcd for C$_{42}$H$_{62}$N$_{15}$O$_{22}$ (M+H)$^+$ 1128.4193 found 1128.4012.

18. (0.016 g, 0.013 mmol, 70%) $^1$H NMR (300 MHz, D$_2$O): 8.34 (2H, 2×H-3), 8.02, 7.96, 7.67 (3H, 3×H-3), 4.99-4.94 (4H, H-1ab, H-2ab), 4.87-4.72 (6H, 3×H-5, 3×H-8), 4.37-4.25 (8H, 3×H-9, 2×H-14, 3×H-7), 3.99-3.85 (7H, 3×H-6, 2×H-13a, 2×H-17), 3.78-3.60 (10H, 2×H-13b, 2×H-15, 2×H-16, 2×H-19ab), 3.57-3.47 (5H, 3×H-10a, 2×H-18), 3.32-3.24 (3H, 3×H-10b), 2.95, 2.76 (4H, 2×H-11ab), 1.99, 1.91 (4H, 2×H-12ab). $^{13}$C NMR (125 MHz, D$_2$O): 145.12, 145.09, 143.52, 143.25 (5×C-4), 123.95, 123.82, 122.54, 122.39 (5×C-3), 101.32 (2×C-14), 76.75 (3×C-9), 73.70-73.10 (2×C-16, 3×C-7), 72.50-71.20 (2×C-15, 3×C-6, 3×C-5), 69.27 (2×C-18), 67.60-67.10 (2×C-17, 2×C-13), 60.72 (3×C-8), 59.49 (2×C-19), 58.55 (3×C-10), 48.53 (C-2, C-1), 26.89 (2×C-12), 19.42, 19.15 (2×C-11). HRMS (MALDI TOF/TOF) m/z calcd for C$_{48}$H$_{74}$N$_{15}$O$_{24}$ (M+H)$^+$ 1244.5031 found 1244.4996.
**Scheme 5.** Synthesis of the PEG based ligand. i) DMAP, propargyl amine, Et₃N, EDCI, DCM, 93%; ii) CuSO₄, Na-ascorbate, DMF, 10% H₂O, 30', 80°C, microwave, 65%. b) MeONa, MeOH, 95%.

**19b.** Propargyl amine (0.05 mL, 0.70 mmol) was added to a solution of 19a (0.20 g, 0.30 mmol), 4-dimethyl aminopyridine (DMAP, 0.01 g, 0.08 mmol) and triethylamine (0.01 mL, 0.03 mmol) in CH₂Cl₂ (15 mL). The mixture was cooled to 0°C and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.11 g, 0.60 mmol) was added. The mixture was stirred for 1 h at 0°C and overnight at r.t.. The reaction was quenched with a 1M KHSO₄ solution (15 mL) and the organic layer was washed with NaHCO₃ saturated solution, water and brine (20 mL). The organic solution was dried over sodium sulfate and after evaporation of the solvent, 19b was obtained as an oily product (0.21 g, 0.28 mmol, 93%). ¹H NMR (300 MHz, CDCl₃): 7.00 (2H, NH), 3.95 (m, 4H, H-3), 3.65 (t, 4H, J₆,₅ = 5.70 Hz, H-6), 3.60-3.50 (48H, (OCH₂CH₂)₁₂O), 2.41 (t, 4H, H-5, J₆,₅ = 5.70 Hz), 2.16 (t, 2H, H-1, J₁,₃ = 2.50 Hz). ¹³C NMR (75.5 MHz, CDCl₃): 171.28, 80.08, 71.01, 70.53-70.25, 70.21, 70.16, 66.92, 36.56, 28.76 MS (ESI) m/z calcd for C₃₆H₆₅N₂O₁₅ (M+H)⁺ 765.44, found 765.75.

**19c.** The general procedure for the “click reaction” was applied. Starting from compound 19b (0.19 g, 0.25 mmol) and compound 25⁻¹¹ (0.24 g, 0.55 mmol), after column chromatography, 19c was obtained (0.19 g, 0.16 mmol, 65%). ¹H NMR (300 MHz, CDCl₃): 7.53 (s, 2H, H-6), 7.29 (2H, NH), 5.31 (d, 2H, J₁₃,₁₂ = 3.15 Hz, H-13), 5.15 (dd, 2H, J₁₁,₁₀ = 8.12 Hz, J₁₁,₁₂ = 10.37 Hz, H-11), 4.95 (dd, 2H, J₁₂,₁₁ = 10.37, Hz
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NMR (75.5 MHz, CDCl₃): δ ppm 81.43, 70.19, 170.10, 169.56, 169.31, 61.18, 46.61, 36.71, 34.91, 30.13, 20.81, 20.65, 20.61, 20.5. MS (ESI) m/z calcld for C₇₀H₁₁₆N₀₃S (M+H)⁺ 814.33, found 814.70.

19. The general procedure for the removal of acetyl protecting groups was applied. Starting from 19c (0.07 g, 0.04 mmol), compound 19 was obtained (0.05 g, 0.04 mmol, 95%). ¹H NMR (300 MHz, D₂O): 7.95 (s, 2H), 4.56 (t, 4H, J=6.85 Hz), 4.49 (s, 4H), 4.37 (d, 2H, J=7.74 Hz), 3.96-3.87 (m, 4H), 3.84-3.50 (m, 6H), 2.58 (t, 4H, J=5.93 Hz), 2.23 (p, 4H, J=6.39 Hz). ¹³C NMR (125 MHz, D₂O): 171.30 (NHC=O), 143.68 (C, Tr), 122.40 (CH, Tr), 101.14, 73.47, 71.06, 69.07, 68.75-67.20, 66.98, 65.04, 64.65, 59.33, 45.46, 34.28, 32.84, 27.92. HRMS (MALDI TOF/TOF) m/z calcld for C₅₄H₉₀N₀₂S (M+H)⁺ 1291.6619 found 1291.6371.

21. BF₃·Et₂O (2.6 mL, 20.48 mmol) was added dropwise to a solution of β-D-galactose pentaacetate (2 g, 5.12 mmol) and 3-butyne-1-ol (1.55 mL, 20.48 mmol) in CH₂Cl₂ (50 mL) previously cooled at 0°C. The mixture was stirred overnight at room temperature. The solution was neutralized with a saturated NaHCO₃ solution and the organic phase was washed with water (30 mL) and once with brine (30 mL). After drying over sodium sulfate, the solvent was removed and the compound purified by column chromatography to give 20 as a white solid (1.48 g, 3.58 mmol, 70%). ¹H NMR (300 MHz, CDCl₃): δ 5.30 (d, 1H, J₉₂=2.57 Hz, H-9), 5.10 (t, 1H, J₇₆=J₇₈=9.10 Hz, H-7), 4.94 (dd, 1H, J₈₇=9.10 Hz, J₈₂=2.57 Hz, H-8), 4.41 (d, 1H, J₆₇=9.10 Hz, H-6), 4.07 (m, 2H, H-11ab), 3.88 (m, 2H, H-10, H-5a), 3.56 (m, 1H, H-5b), 2.18 (m, 2H, H-3), 2.18 (1H, H-1), 2.06, 1.99, 1.96, 1.90 (C=OCH₃), 1.73 (m, 2H, H-4). ¹³C NMR (75.5 MHz, CDCl₃): δ ppm 170.19, 170.10, 169.95, 169.31 (4×C=O), 101.37,
83.29, 70.75, 70.49, 68.83, 68.77, 68.18, 66.96, 61.18, 28.11, 20.61 (C=OCH$_3$), 20.53 (2×C=OCH$_3$), 20.45 (C=OCH$_3$), 14.63. MS (ESI) m/z calcd for C$_{19}$H$_{28}$KO$_9$ (M+K)$^+$ 437.12, found 437.15.

**20.** The general procedure for the removal of acetyl protecting groups was applied. Starting from 21 (0.19 g, 0.48 mmol), compound 20$^{22}$ was obtained (0.12 g, 0.47 mmol, 98%). $^1$H NMR (300 MHz, CD$_3$OD): δ ppm 4.21 (d, 1H, $J_{6,7} = 6.83$ Hz, H-6), 3.96 (dt, 1H, $J_{5a,4} = 6.50$ Hz, $J_{5a,5b} = 9.90$ Hz, H-5a), 3.84 (s, 1H, H-9), 3.73 (d, 2H, $J_{11ab,10} = 6.36$ Hz, H-11ab), 3.65 (dt, 1H, $J_{5b,4} = 6.50$ Hz, $J_{5b,5a} = 9.90$ Hz, H-5b), 3.55-3.43 (m, 3H, H-7, H-8, H-10), 2.30 (dt, 2H, $J_{3,4} = 7.00$ Hz, J$_{3,1}$ = 2.60 Hz, H-3), 2.20 (t, 1H, $J_{1,3} = 2.60$ Hz, H-1), 1.80 (p, 2H, $J_{4,3} = J_{4,5} = 6.50$ Hz, H-4). $^{13}$C NMR (75.5 MHz, CD$_3$OD): δ ppm 104.94, 84.70, 76.45, 74.86, 72.47, 70.17, 69.63, 69.24, 62.34, 29.99, 15.75. HRMS (MALDI TOF/TOF) m/z calcd for C$_{11}$H$_{18}$NaO$_6$ (M+Na)$^+$ 269.0996 found 269.0928.

[Scheme 6](#).

**24.** The general procedure for the “click reaction” was applied. Starting from compound 2 (0.05 g, 0.15 mmol) and compound 23$^{23}$ (0.02 g, 0.22 mmol), after column chromatography, 24 was obtained (0.04 g, 0.09 mmol, 60%). $^1$H NMR (300 MHz, CDCl$_3$): 7.77 (s, 1H, H-3), 5.49 (d, 1H, $J_{8,7} = 3.15$ Hz, H-8), 5.35 (t, 1H, $J_{6,5} = J_{6,7} = 10.10$ Hz, H-6), 5.16 (dd, 1H, $J_{7,6} = 10.10$ Hz $J_{7,8} = 3.15$ Hz, H-7), 4.74 (d, 1H, $J_{5,6} = 10.10$ Hz, H-5), 4.48 (m, 2H, H-1ab), 4.17-4.08 (m, 3H, H-9, H-10ab), 3.98 (m, 2H, H-1ab), 2.70 (s, 1H, OH), 2.19, 2.04, 2.00, 1.91 (12H, C=OCH$_3$). $^{13}$C NMR (75.5 MHz, CD$_3$Cl): 170.77 (C=O), 170.51 (C=O), 170.45 (C=O), 144.69 (C-4), 124.04 (C-3), 75.11 (C-9), 74.09 (C-5), 72.00 (C-7) 69.47 (C-6), 67.93 (C-8), 61.96 (C-10), 61.48 (C-1), 53.14 (C-2), 20.99(C=OCH$_3$). MS (ESI) m/z calcd for C$_{18}$H$_{23}$N$_3$O$_{10}$ (M+H)$^+$ 444.16, found 444.70.
The general procedure for the removal of acetyl protecting groups was applied. Starting from 24 (0.03 g, 0.07 mmol), compound 22 was obtained (0.02 g, 0.07 mmol, quant). $^1$H NMR (300 MHz, CD$_3$OD): δ ppm 8.05 (s, 1H, H-3), 4.49 (t, 2H, $J_{2ab,1ab}$=5.55 Hz, H-2ab), 4.34 (d, 1H, $J_{5,6}$=9.55 Hz, H-5), 3.97-3.83 (m, 4H, H-8, H-9, H-10ab), 3.80-3.63 (m, 3H, H-1ab, H-6), 3.60 (dd, 1H, $J_{7,8}$=3.15 Hz, $J_{7,6}$=9.55 Hz, H-7). $^{13}$C NMR (75.5 MHz, CD$_3$OD): δ ppm 147.00 (C-4), 125.43 (C-3), 80.93 (C-9), 76.24 (C-5), 76.20 (C-7) 72.23 (C-6), 70.91 (C-8), 62.83 (C-10), 61.58 (C-1), 54.02 (C-2). HRMS (MALDI TOF/TOF) m/z calcd for C$_{10}$H$_{18}$N$_3$O$_6$ (M+H)$^+$ 276.1196 found 269.1135.

**Calculation of the effective length of the spacer of compound 19**

The spacer of compound 19 contains one of the longest commercially available homogeneous PEG molecule. The spacer is of similar length as an all PEG spacer with 20 PEG units (CH$_2$CH$_2$O), since they both contain 61 atoms. Using the Flory equation$^{24}$ for PEG, $R_f = aN^3$, where $R_f$ is the length, $N$ is the number of PEG units, and $a$ is the length of one monomer (taken to be 3.5 Å), an effective length of 21.1 Å is calculated. Considering that the spacer of 19 contain two amide bonds and two triazole units, it is likely that its effective length is longer than that. Furthermore flexible PEG and PEG-hybrid spacers are known to give a broad distance distribution, both theoretically$^{25}$ and experimentally.$^9$ Therefore the spacer of 19 is expected to adequately cover the distance between the two binding sites of LecA of 26 Å, measured between the anomeric oxygens of bound galactosides of X-ray structure with pdb code 1OKO.$^{12b}$

**Estimating the potential for bivalent binding of 12 and 14**

The complex between the rigid and flexible molecule and lectin 1 from *Pseudomonas aeruginosa* was modeled using the X-structure of this lectin complexed to galactose as a template (PDB ID: 1OKO). First, one of the terminal sugar moieties of either the rigid or flexible molecule was superimposed onto the galactose moiety of molecule A in the X-structure with respect to the atoms comprising the sugar-ring. Subsequently, the galactose moieties of molecules A and B were deleted from the structure and the other terminal sugar of either the rigid or the flexible molecule was pulled to the binding site of galactose B using restrained molecular dynamics. The superimposed
sugar and the protein units A and B were kept in a fixed position during the simulation. Restraints were used based on a limited number of hydrogen bonds present between residues of protein unit B and the galactose of B. In the top structure (A) our worst ligand, compound 12 was modeled (see yellow structure). Clearly the left galactose of 12 cannot reach the position of galactose B of the X-ray structure (shown in blue). In contrast, as shown in the bottom structure (B), both galactosides of our best ligand 14 can bind in both binding sites simultaneously. Modeling was accomplished using the Yasara Structure software (version 11.9.18).

A

B

References


2 in CDCl$_3$
3 in CD$_3$OD
4 in CDCl$_3$
6a in CDCl₃
6 in CDCl$_3$
7a in CDCl₃
7 in CDCl$_3$
9a in CDCl₃
9 in CDCl$_3$
8a in CDCl$_3$

![NMR spectrum of 8a in CDCl$_3$]

10a in CDCl$_3$

![NMR spectrum of 10a in CDCl$_3$]
8 in CDCl$_3$
10 in CDCl₃
11 in CDCl₃
13 in CDCl₃

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15 in CDCl$_3$
17 in CDCl₃

H Standard 1H spectrum

13C

13C OBSERVE
12 in D$_2$O
14 in D$_2$O
16 in D$_2$O
18 in D$_2$O
19b in CDCl$_3$
19c in CDCl$_3$
19 in D$_2$O
20 in CD$_3$OD
24 in CDCl₃
24 in CDCl₃
22 in CD$_3$OD
22 in CD$_3$OD
12 IC₅₀ = 314 ± 62 µM

LOGIC₅₀ = -3,503
IC₅₀ (M) = 2,23 × 10⁻⁷
LOGIC₅₀ (error) = 0,07849
R² = 0,9915

14 IC₅₀ = 223 ± 23 nM

LOGIC₅₀ = -6,655
IC₅₀ (M) = 2,213 × 10⁻⁷
LOGIC₅₀ (error) = 0,04342
R² = 0,9971
16  \( \text{IC}_{50}=1.76 \pm 0.34 \, \mu\text{M} \)

\[ \begin{align*}
\text{LOGIC50} & \quad -5.753 \\
\text{IC50 (M)} & \quad 1.766 \times 10^{-6} \\
\text{LOGIC50 (error)} & \quad 0.07599 \\
R^2 & \quad 0.9957
\end{align*} \]

18  \( \text{IC}_{50}=383 \pm 61 \, \text{nM} \)

\[ \begin{align*}
\text{LOGIC50} & \quad -6.417 \\
\text{IC50 (M)} & \quad 3.830 \times 10^{-7} \\
\text{LOGIC50 (error)} & \quad 0.06460 \\
R^2 & \quad 0.9929
\end{align*} \]
19 \( IC_{50} = 2.06 \pm 0.59 \, \mu M \)

![Graph showing IC50 for compound 19.]

LOGIC50 = -5.687
IC50 (M) = 2.056e-006
LOGIC50 (error) = 0.1108
R² = 0.9902

20 \( IC_{50} = 133 \pm 60 \, \mu M \)

![Graph showing IC50 for compound 20.]

LOGIC50 = -3.877
IC50 (M) = 0.0001327
LOGIC50 (error) = 0.1646
R² = 0.9644
IC$_{50}$=92 ± 37 µM

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14

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