Supporting Information

Catechol-functionalized sequence-defined glycomacromolecules as covalent inhibitors of bacterial adhesion

Lukas Fischer,[a] Ricarda Steffens [a], Tanja J. Paul,[a] Laura Hartmann [a*]

[a] *Institut für Organische und Makromolekulare Chemie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany*

Materials

2-Bromoethanol (Carbolution), 2-chloroethanol (Sigma Aldrich), 3-(3,4-dihydroxyphenyl)propionic acid (abcr), acetic anhydride (VWR Chemicals), aceton (Carl Roth), acetonitrile (Sigma Aldrich), succinic anhydride (99%, Acros), boron trifluoride diethyl etherate (98%, Alfa Aesar), calcium chloride (Panreac AppliChem), chloroform-d (Deutero), citric acid (Fisher Chemical), Concanavalin A type IV (Sigma Aldrich), deuterium oxide (Deutero), 1,8-diazabiscyclo[5.4.0]undec-7-ene (Fluorochem), dichloromethane (VWR Prolabo), diethylenetriamine (Carl Roth), diethyl ether (VWR Prolabo), dimethylsulfoxide-d_6 (Deutero), 1,4-Dioxane (Fisher Chemical), D(+)-mannose (99%, Acros), acetic acid (VWR Chemicals), ethanol (Carl Roth), ethyl acetate (VWR-Prolabo), ethylenediamine (Sigma Aldrich), galactose pentaacetate (Fluorochem), 2-(4-(2-hydroxyethyl)-1-piperazinyl)-ethanesulfonic acid (Fisher Scientific), potassium carbonate (Fisher Scientific), copper sulfate (Acros), magnesium sulfate (Fisher Chemical), manganese chloride (Sigma Aldrich), methanol (VWR Prolabo), methanol-d_4 (Deutero), *N,N*-dimethylformamide (VWR Prolabo), sodium ascorbate (Panreac AppliChem), sodium azide (Panreac AppliChem), sodium chloride (98%, Sigma Aldrich), sodium diethyldithiocarbamate (Alfa Aesar), sodium dodecyl sulfate (Carl Roth), sodium bicarbonate (VWR-Chemicals), sodium methoxide (Sigma Aldrich), hexane (VWR Prolabo), oxalyl chloride (Alfa Aesar), 4-pentynoic acid (Sigma Aldrich), phosphorus trichloride (Sigma Aldrich), piperidine (Acros), *p*-toluenesulfonic acid (Sigma Aldrich), (benzotriazol-1-yloxy)-tripyrrolidin-phosphonium hexafluorophosphate (PyBOP) (Carbolution), hydrochloric acid (37%, VWR Chemicals), sulphuric acid (Sigma Aldrich), Tentagel® S RAM (Rapp Polymere), tetrahydrofuran (Sigma Aldrich), trichloroacetonitrile (Fluorochem), triethylamine (Acros Organics), triethylsilane (TCI Chemicals), trifluororacetic acid (Acros), trifluoroacetate (Acros Organics) triisopropylsilane (Sigma Aldrich), trimethylsilyl azide (Sigma Aldrich), trityl chloride (Acros Organics), vanillin (Caelo), tin(IV) chloride (Fisher Scientific).

Instrumentation

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR and ^{13}C NMR were recorded on a Bruker Avance III 300, a Bruker Avance DRX-500 or a Bruker Avance III 600. Chemical shifts were reported as delta (δ) in parts per million (ppm) and coupling constants as *J* in Hertz (Hz). Multiplicities are stated as following: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

High Resolution-Mass Spectrometry (HR-MS)

HR-MS measurements were conducted on a Bruker UHR-QTOF maxis 4G with a direct inlet via syringe pump, an ESI source and a quadrupole Time of Flight (QTOF) analyzer. Samples were dissolved in water with a concentration of 1 mg/ml.

Matrix-assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF)-Mass Spectrometry

MALDI-TOF measurements were conducted on a Ultraflex I from Bruker Daltonics. The samples were measured in linear mode with cyano-4-hydroxycinnamic acid (HCCA) as matrix in a ratio of 1:2. As a solvent acetonitrile with 0.1% TFA was used.

Reversed Phase-High Pressure Liquid Chromatography (RP-HPLC)

RP-HPLC was performed with an Agilent 1260 Infinity instrument coupled to a variable wavelength detector (VWD) set to 214 nm. As a column a Poroshell 120 EC-C18 1.8 µM (3.0x50 mm, 2.5 µM) reversed phase column was used. The mobile phase A consisted of 95/5 H_2O/MeCN with 0.1% formic acid and mobile phase B consisted of 95/5 MeCN/H_2O with 0.1% formic acid. The flowrate for all measurements was 0.4 ml/min.

UV/Vis-Spectroscopy

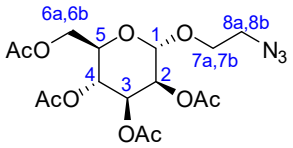
The UV/Vis measurements were done on a „Specord 210 Plus“ from Analytik Jena AG. For the measurement a quartz cuvette from Hellma Analytics with a thickness of 1 cm and a volume of 1 ml was used.

Freeze Dryer

Lyophilization of the final structures was conducted on an Alpha 1-4 LD plus instrument from Martin Christ Freeze Dryers GmbH. The lyophilisation was done at a pressure of 0.1 mbar.

Building Block Synthesis and Analytics

1-(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-α-D-mannose



The synthesis was done following literature.[1]

Yield: 19.48 g (46.60 mmol, 77%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 5.35 (dd, J_{HH} = 10.0 Hz, 3.2 Hz, 1H, H3), 5.32-5.24 (m, 2H, H2, H4), 4.86 (d, J_{HH} = 1.7 Hz, 1H, H1), 4.28 (dd, J_{HH} = 12.3, 5.3 Hz, 1H, H5), 4.11 (dd, J_{HH} = 12.2 Hz, 2.5 Hz, 1H, H6a), 4.03 (ddd, J_{HH} = 9.5 Hz, 5.3 Hz, 2.4 Hz, 1H, H6b), 3.86 (ddd, J_{HH} = 10.6 Hz, 6.6 Hz, 4.0 Hz, 1H, H7a), 3.66 (ddd, J_{HH} = 10.6 Hz, 5.8 Hz, 3.7 Hz, 1H, H7b), 3.54-3.40 (m, 2H, H8a, H8b), 2.15 (s, 3H, C(O)CH_3), 2.09 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3).

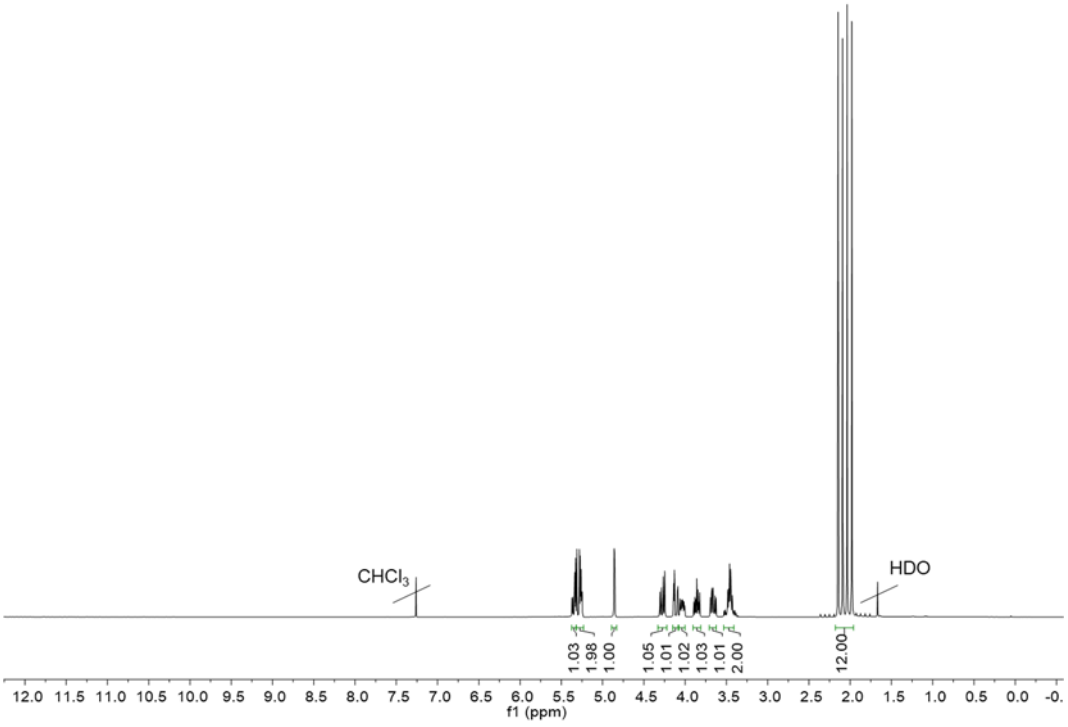
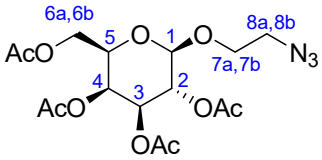


Figure S1: ^{1}H-NMR (300 MHz, $CDCl_3$) of *1-(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-α-D-mannose.*

1-(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-galactose



The synthesis was done following literature.[2]

Yield: 2.20 g (5.28 mmol, 72%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 5.36 (dd, J_{HH} = 3.4 Hz, 1.2 Hz, 1H, H3), 5.20 (dd, J_{HH} =10.5 Hz, 7.9 Hz, 1H, H2), 4.99 (dd, J_{HH} = 10.5 Hz, 3.4 Hz, 1H, H4), 4.53 (d, J_{HH} = 7.9 Hz, 1H, H1), 4.17-3,97 (m, 3H, H5, H6a, H6b), 3.90 (td, J_{HH} = 6.6 Hz, 1.2 Hz, 1H, H7a), 3.66 (ddd, J_{HH} = 10.6 Hz, 8.4 Hz, 3.4 Hz, 1H, H7b), 3.47 (ddd, J_{HH} = 13.3 Hz, 8.4 Hz, 3.5 Hz, 1H, H8a), 3.27 (ddd, J_{HH} = 13.4 Hz, 4.8 Hz, 3.4 Hz, 1H, H8b), 2.12 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.95 (s, 3H, C(O)CH_3).

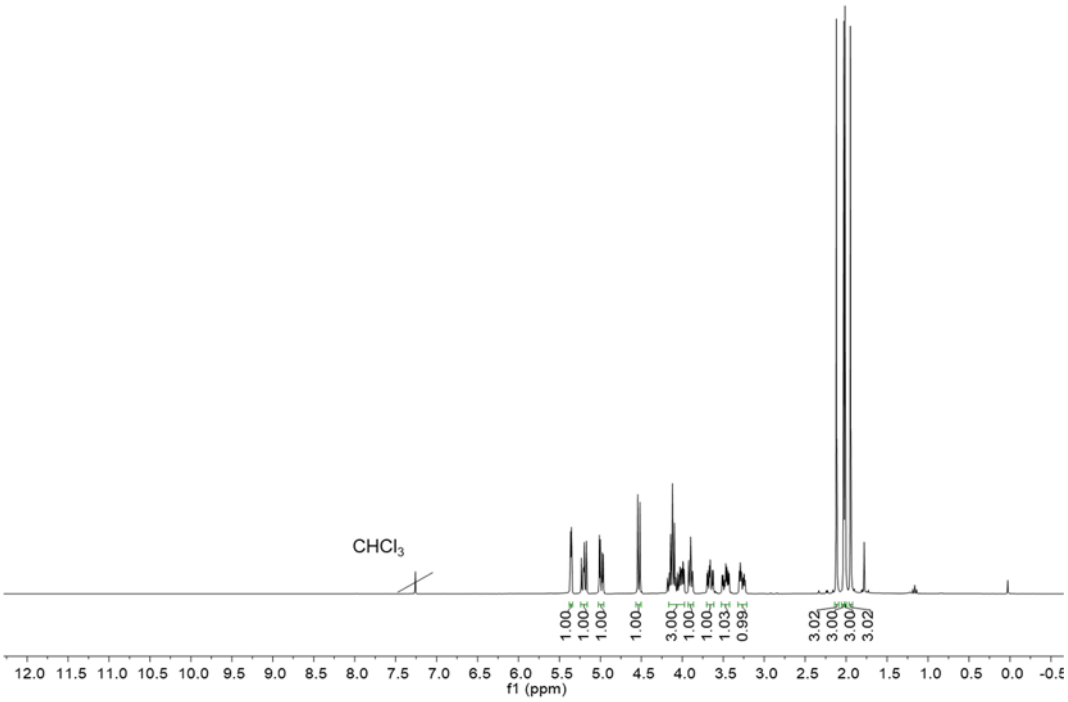
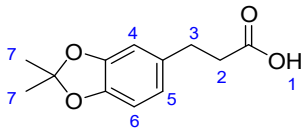


Figure S2: ^{1}H-NMR (300 MHz, $CDCl_3$) of *1-(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-galactose.*

3-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)propionic acid



The synthesis was done following literature.[3]

Yield: 10.68 g (51.45 mmol, 57%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm) 6.73-6.70 (m, 3H, H4, H5, H6), 2.74 (t, J_{HH} = 7.5 Hz, 2H, H3), 2.50 (t, J_{HH} = 7.5 Hz, 2H, H2), 1.60 (s, 6H, H7).

RP-HPLC (Eluent B from 0% to 100% in 17 min): t_r = 9.95 min, relative purity 99%.

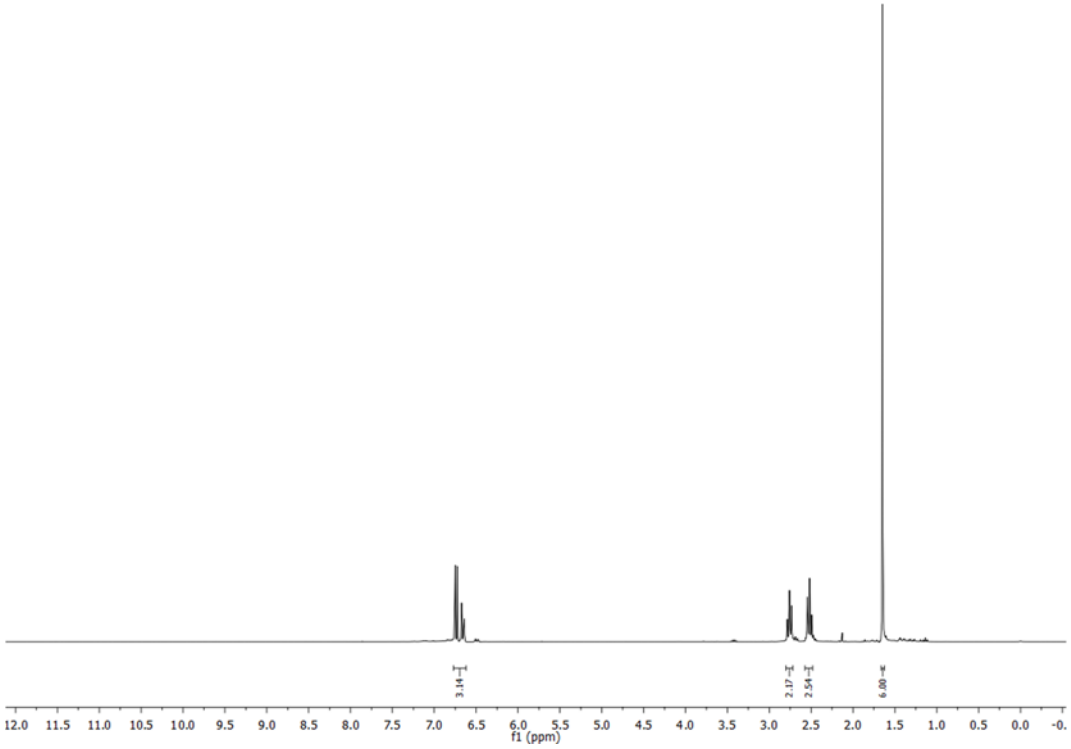
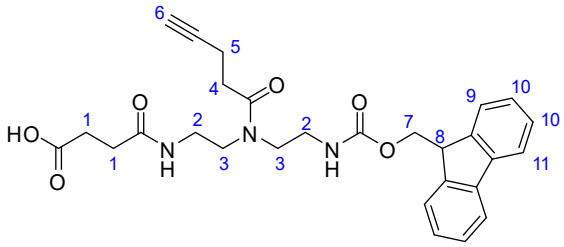


Figure S3: ^{1}H-NMR (300 MHz, DMSO-d_6) of *3-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)propionic acid*.

1-(9H-fluoren-9-yl)-3,11-dioxo-7-(pent-4-ynoyl)-2-oxa-4,7,10-triazatetradecan-14-oic acid (TDS)



The synthesis was done following literature.[4]

Yield: 12.46 g (24.7 mmol, 38%).

^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 8.03 (t, J_{HH} = 6.2 Hz, 1H, NH), 7.88 (d, J_{HH} = 7.5 Hz, 2H, H11), 7.67 (dd, J_{HH} = 7.5 Hz, 3.0 Hz, 2H, H9), 7.47-7.38 (m, 4H, H10, NH), 7.36-7.30 (m, 4H, H10), 4.30 (dd, J_{HH} = 17.0 Hz, 6.9 Hz, 2H, H7), 4.20 (t, J_{HH} = 6.9 Hz, 1H, H8), 3.33-3.23 (m, 4H, H2), 3.18-3.03 (m, 4H, H3), 2.73 (t, J_{HH} = 2.8 Hz, 1H, H6), 2.41-2.24 (m, 6H, H4, H1).

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 10.28 min, relative purity 99%.

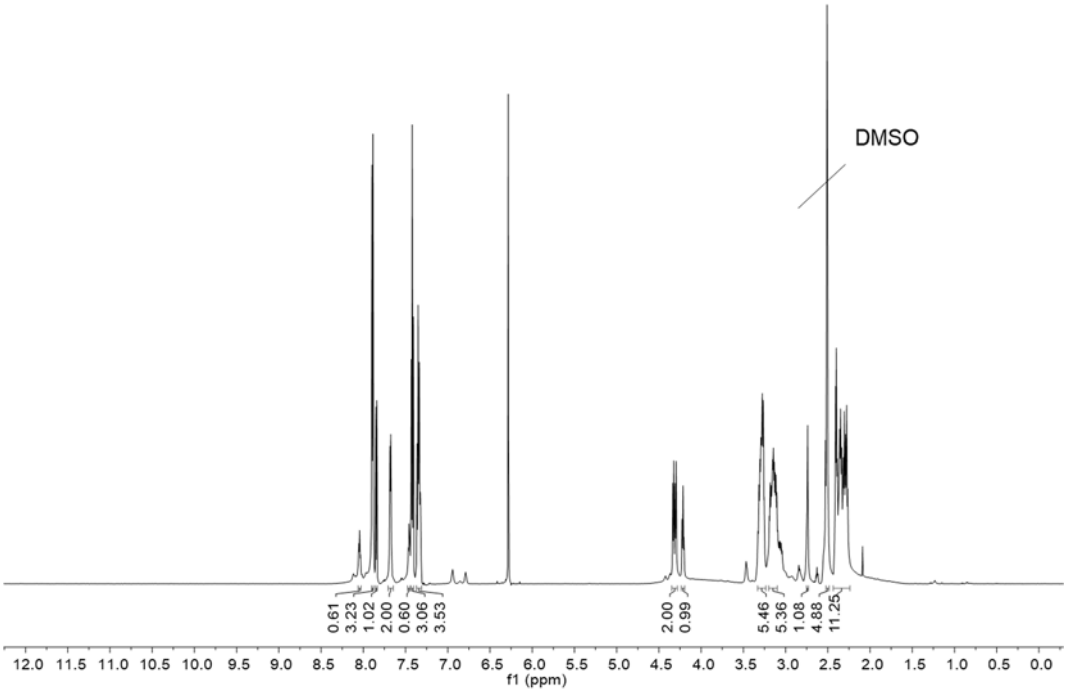
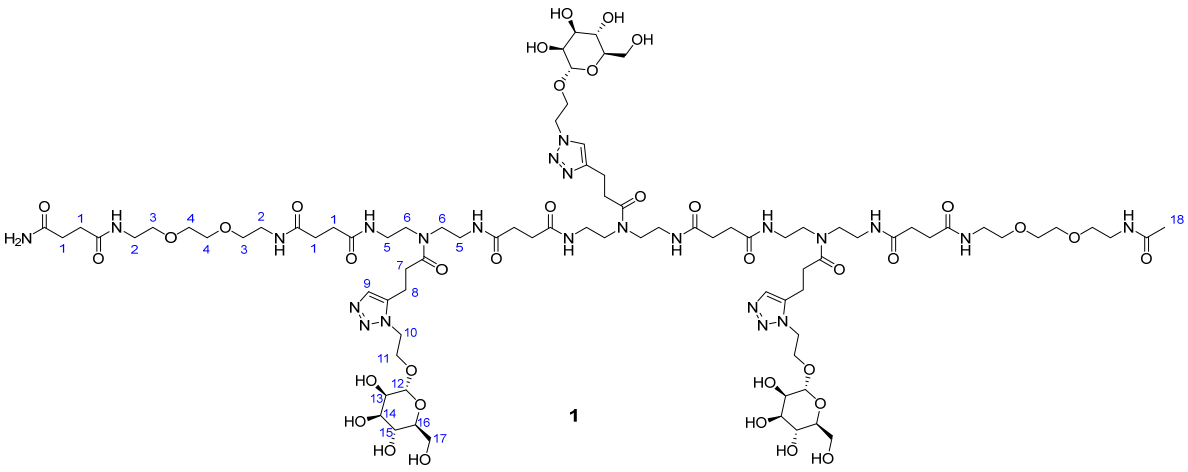


Figure S4: ^{1}H-NMR (300 MHz, DMSO-d_6) of *1-(9H-fluoren-9-yl)-3,11-dioxo-7-(pent-4-ynoyl)-2-oxa-4,7,10-triazatetradecan-14-oic acid (TDS).*

Solid Phase Synthesis and Analytics

All glycomacromolecules were synthesized on solid support according to literature using the building blocks EDS, TDS and Fmoc-Lys(Boc).[5] For functionalization the lysine was deprotected on solid support using 4 M HCl in dioxane for 30 min. The catechol moiety was introduced at the terminal amine or deprotected lysine or both. For this the resin was treated with 5 eq. 3-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)propionic acid, 5eq. PyBOP and 10 eq. DIPEA in DMF for 1 h. For glycomacromolecules without catechol the terminal amine was capped with acetic anhydride for 5 min. After assembly of the scaffold and either catechol coupling or end capping, sugars were introduced via an established CuAAC protocol.[5] Afterwards the sugars were deprotected on solid support using 0.1 M sodium methoxide in methanol. For final cleavage the resin was treated with 95% TFA, 2.5% TIPS and 2.5% DCM for 1h. The glycomacromolecules were precipitated in diethyl ether and freeze dried. For all structures Tentagel® S RAM as a resin was used.

3Man (**1**)



1

Yield: 233 mg (75%).

^{1}H-NMR (600 MHz, D_2O) δ (ppm) 7.97-7.95 (m, 3H, H9), 4.67-4.64 (m, 6H, H10), 4.11-4.08 (m, 3H, H11), 3.94 -3.88 (m, 3H, H11), 3.85-3.82 (m, 3H, H13), 3.74-3.72 (m, 3H, H14), 3.67-3.56 (m, 25H, H3, H4, H15, H17), 3.47-3.33 (m, 32H, H2, H5, H6), 3.08-3.04 (m, 3H, H16), 3.02-2.98 (m, 6H, H7), 2.81-2.77 (m, 6H, H8), 2.56-2.40 (m, 20H, H1), 1.97 (s, 3H, H18).

^{13}C-NMR (150 MHz, D_2O) δ (ppm) 178.59, 175.91, 175.81, 175.76, 175.73, 175.65, 175.62, 175.57, 175.54, 175.41, 175.15, 163.98, 163.75, 163.51, 147.07, 147.07, 125.60, 125.58, 125.55, 118.24, 116.31, 100.50, 73.83, 71.43, 70.87, 70.56, 70.41, 70.39, 69.82, 69.80, 69.75, 67.36, 66.24, 61.68, 51.52, 51.50, 48.08, 48.06, 46.08, 46.06, 46.04, 39.94, 39.88, 38.28, 37.85, 32.71, 31.98, 31.89, 31.85, 31.80, 31.76, 31.72, 31.58, 31.32, 22.77, 21.22.

HR-ESI-MS: calculated mass for $C_{85}H_{143}N_{23}O_{36}$ $[M+3H]^{3+}$: 688.3428, found 688.3427.

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 8.41 min, relative purity 94%.

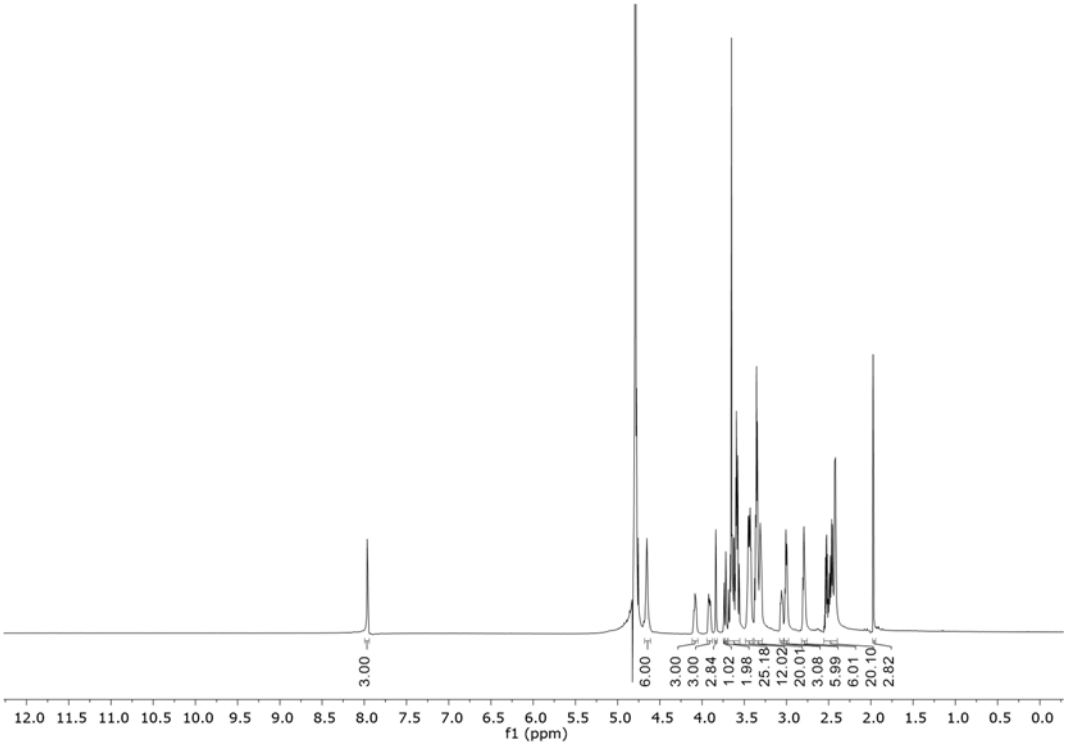


Figure S5: ^{1}H-NMR (600 MHz in D_2O) of 3Man (**1**).

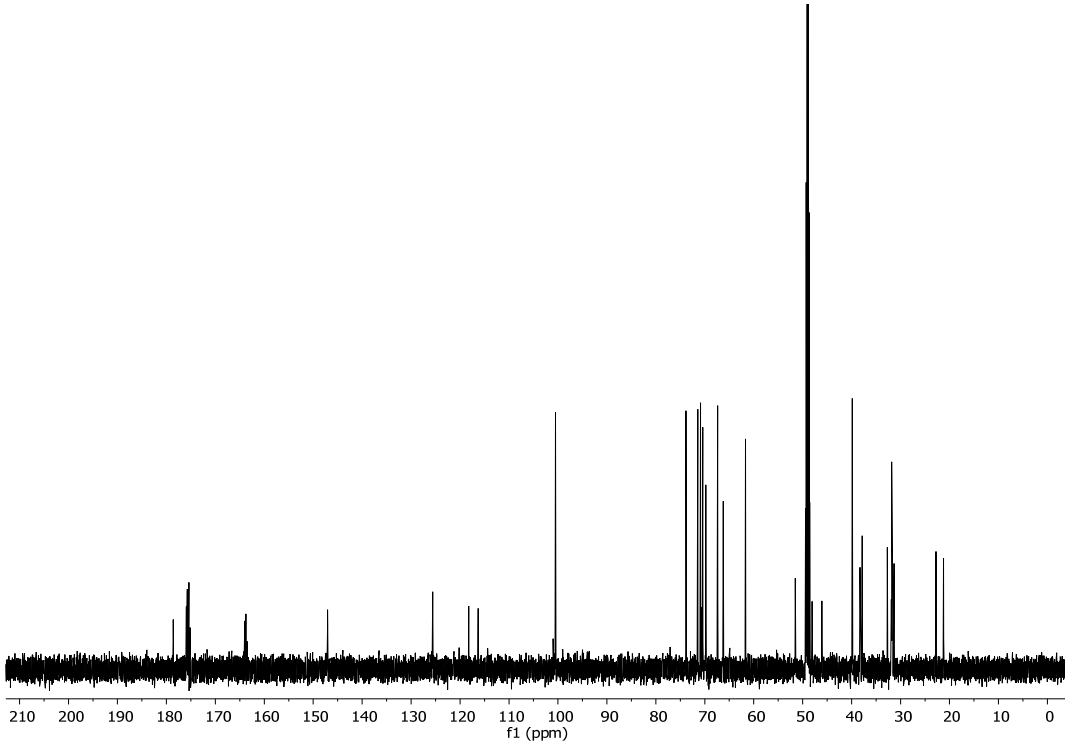


Figure S6: ^{13}C-NMR (150 MHz in D_2O) of 3Man (**1**).

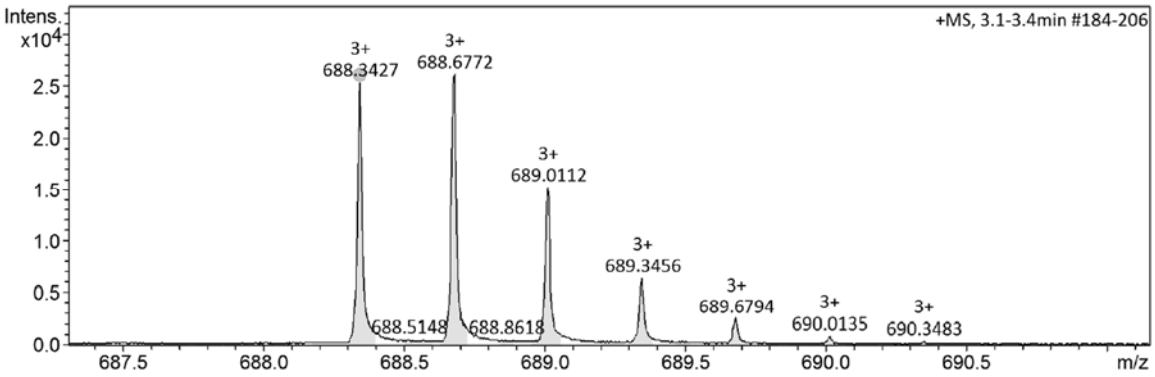


Figure S7: HR-ESI (ESI$^+$ Q-TOF) of 3Man (**1**).

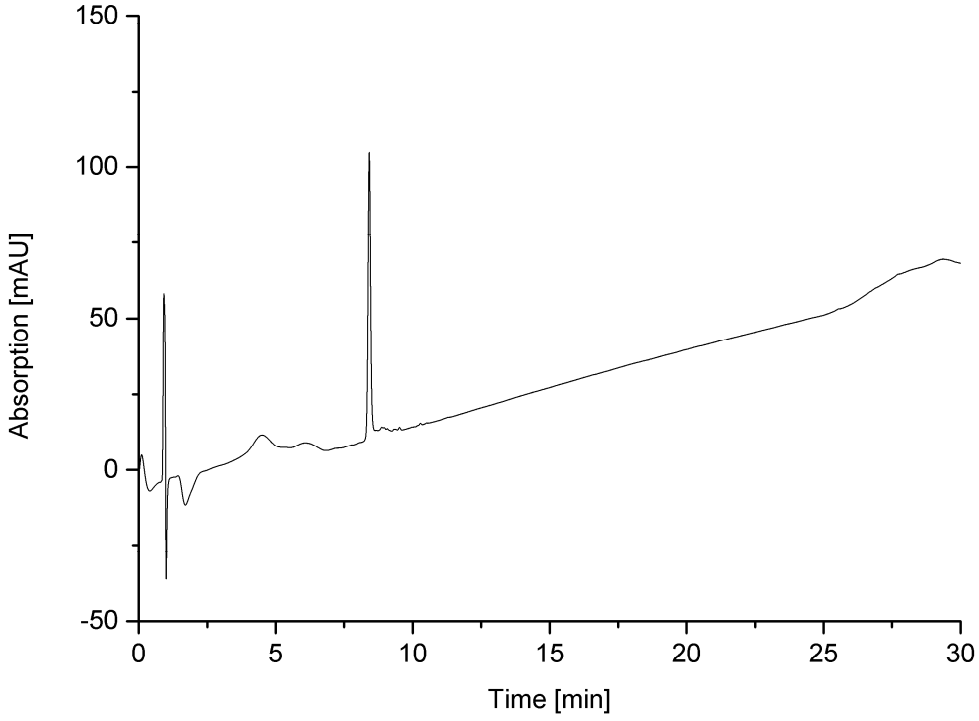
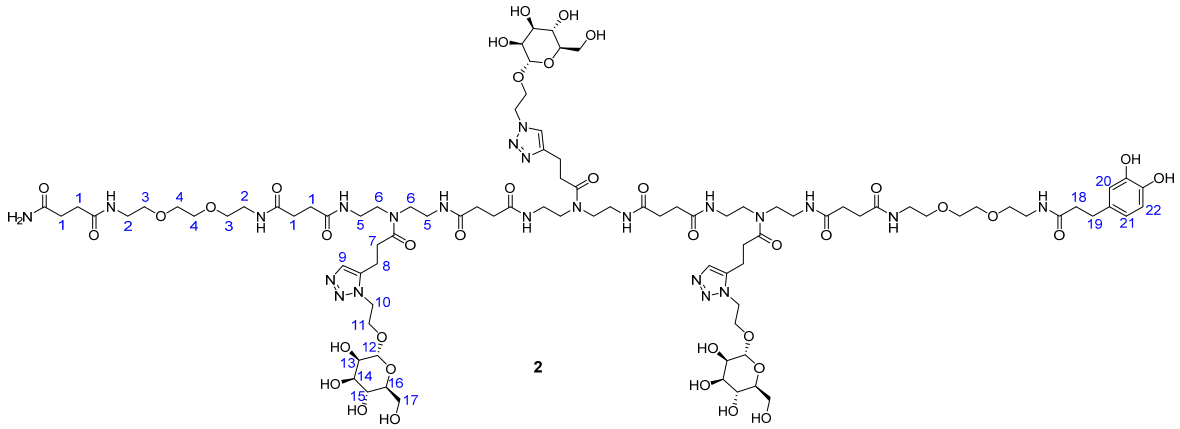


Figure S8: RP-HPLC chromatogram of 3Man (**1**) (gradient from 0% to 50% eluent B over 30 min at 25°C).

3Man-1Cat (**2**)



Yield: 315 mg (96%).

^{1}H-NMR (600 MHz, D_2O) δ (ppm) 8.01-7.95 (m, 3H, H9), 6.85 (d, J_{HH} = 8.0 Hz, 1H, H20), 6.78 (d, J_{HH} = 1.9 Hz, 1H, H22), 6.69 (dd, J_{HH} = 8.2 Hz, 2.1 Hz, 1H, H21), 4.71-4.67 (m, 6H, H10), 4.17-4.10 (m, 3H, H11), 4.00-3.93 (m, 3H, H11), 3.91-3.88 (m, 3H, H13), 3.82-3.78 (m, 3H, H14), 3.75-3.60 (m, 25H, H3,

H4, H15, H17), 3.56-3.34 (m, 32H, H2, H5, H6), 3.32 (t, J_{HH} = 5.3 Hz, 2H, H18), 3.15-3.10 (m, 3H, H16), 3.08-3.02 (m, 6H, H7), 2.88-2.80 (m, 8H, H8, H19), 2.61-2.46 (m, 20H, H1).

^{13}C-NMR (150 MHz, D_2O) δ (ppm) 176.61, 175.73, 175.71, 175.64, 175.62, 175.57, 175.55, 175.47, 175.42, 147.17, 144.78, 143.19, 125.37, 125.32, 121.52, 118.42, 117.06, 117.03, 116.10, 100.48, 73.79, 71.43, 70.85, 70.40, 70.36, 69.90, 69.76, 69.71, 67.37, 66.24, 61.66, 51.35, 48.09, 48.06, 46.10, 46.06, 39.85, 38.50, 38.30, 37.86, 32.70, 31.98, 31.88, 31.86, 31.81, 31.74, 31.61, 31.56, 31.32, 21.27.

HR-ESI-MS: calculated mass for $C_{92}H_{149}N_{23}O_{38}$ $[M+3H]^{3+}$: 729.0217, found 729.0213.

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 10.17 min, relative purity 98%.

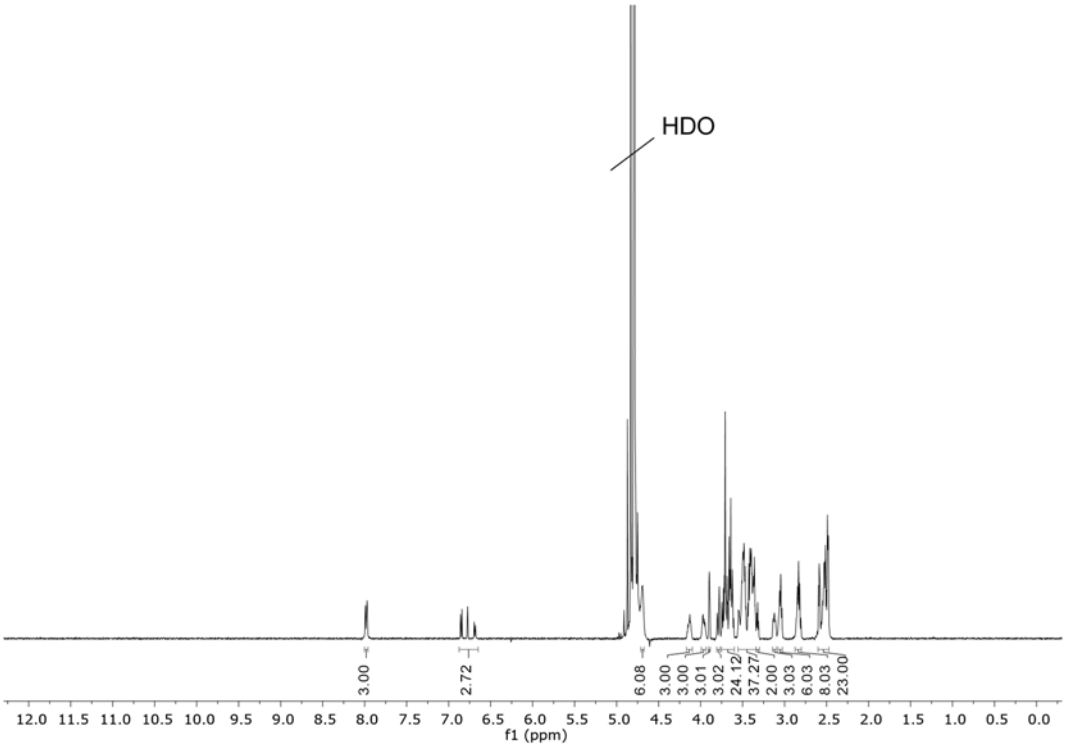


Figure S9: ^{1}H-NMR (600 MHz in D_2O) of 3Man-1Cat (**2**).

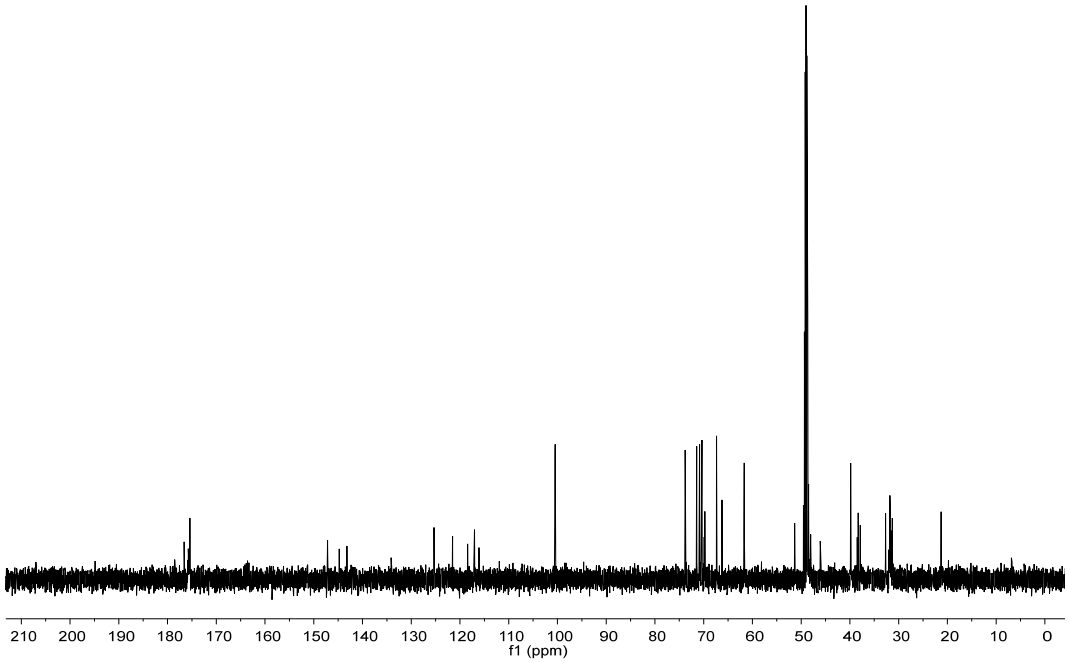


Figure S10: ^{13}C-NMR (150 MHz in D_2O) of 3Man-1Cat (**2**).

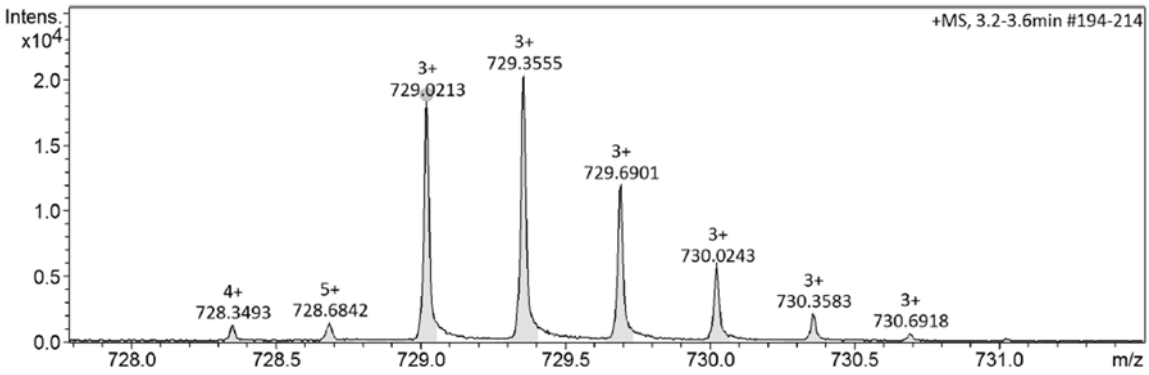


Figure S11: HR-ESI (ESI$^+$ Q-TOF) of 3Man-1Cat (**2**).

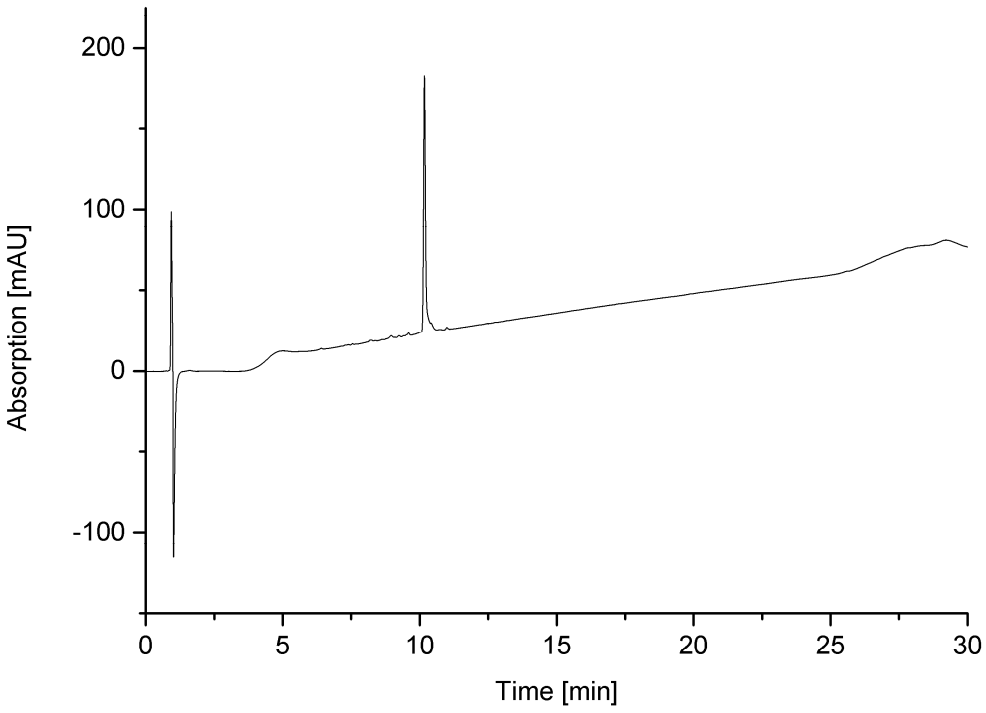
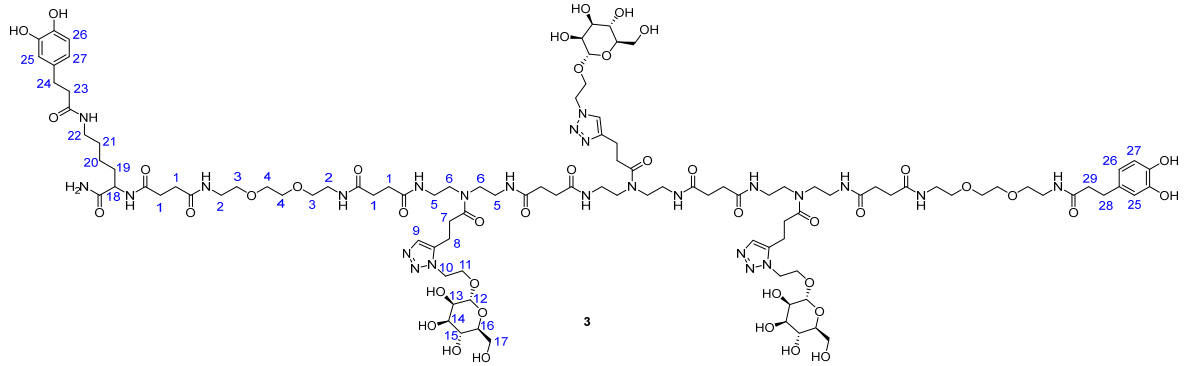


Figure S12: RP-HPLC chromatogram of 3Man-1Cat (**2**) (gradient from 0% to 50% eluent B over 30 min at 25°C).

3Man-2Cat (**3**)



Yield: 214 mg (58%).

^{1}H-NMR (600 MHz, D_2O) δ (ppm) 7.94-7.89 (m, 3H, H9), 6.81 (dd, J_{HH} = 8.1 Hz, 4.5 Hz, 2H, H25), 6.74 (s, 2H, H26), 6.68-6.63 (m, 2H, H27), 4.66-4.63 (m, 6H, H10), 4.16 (dd, J_{HH} = 9.3 Hz, 5.0 Hz, 1H, H18), 4.12-4.06 (m, 3H, H11), 3.95-3.89 (m, 3H, H11), 3.87-3.84 (m, 3H, H13), 3.78-3.73 (m, 3H, H14), 3.72-3.56 (m, 25H, H3, H4, H15, H17), 3.54-3.28 (m, 44H, H2, H5, H6, H29), 3.12-3.05 (m, 5H, H16, H23),

3.03-2.98 (m, 6H, H7), 2.83-2.76 (m, 10H, H8, H28), 2.61-2.55 (m, 4H, H22, H24), 2.52-2.42 (m, 20H, H1), 1.76-1.68 (m, 1H, H19), 1.65-1.57 (m, 1H, H19), 1.35-1.28 (m, 2H, H21), 1.21-1.08 (m, 2H, H20).

^{13}C-NMR (150 MHz, D_2O) δ (ppm) 175.72, 175.58, 175.43, 175.42, 147.38, 144.86, 143.27, 125.17, 125.14, 125.13, 121.56, 121.48, 118.48, 117.18, 117.11, 117.10, 117.07, 117.05, 117.00, 116.16, 100.54, 73.84, 71.50, 70.93, 70.57, 70.41, 69.94, 69.80, 67.42, 66.30, 61.72, 51.21, 48.06, 46.13, 39.90, 39.86, 39.67, 38.65, 38.34, 37.90, 32.79, 31.91, 31.89, 31.80, 31.76, 31.73, 31.66, 31.64, 31.59, 31.55, 31.55, 23.29, 21.41.

HR-ESI-MS: calculated mass for $C_{107}H_{169}N_{25}O_{42}$ $[M+3H]^{3+}$: 826.7370, found 826.7370.

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 11.70 min, relative purity 90%.

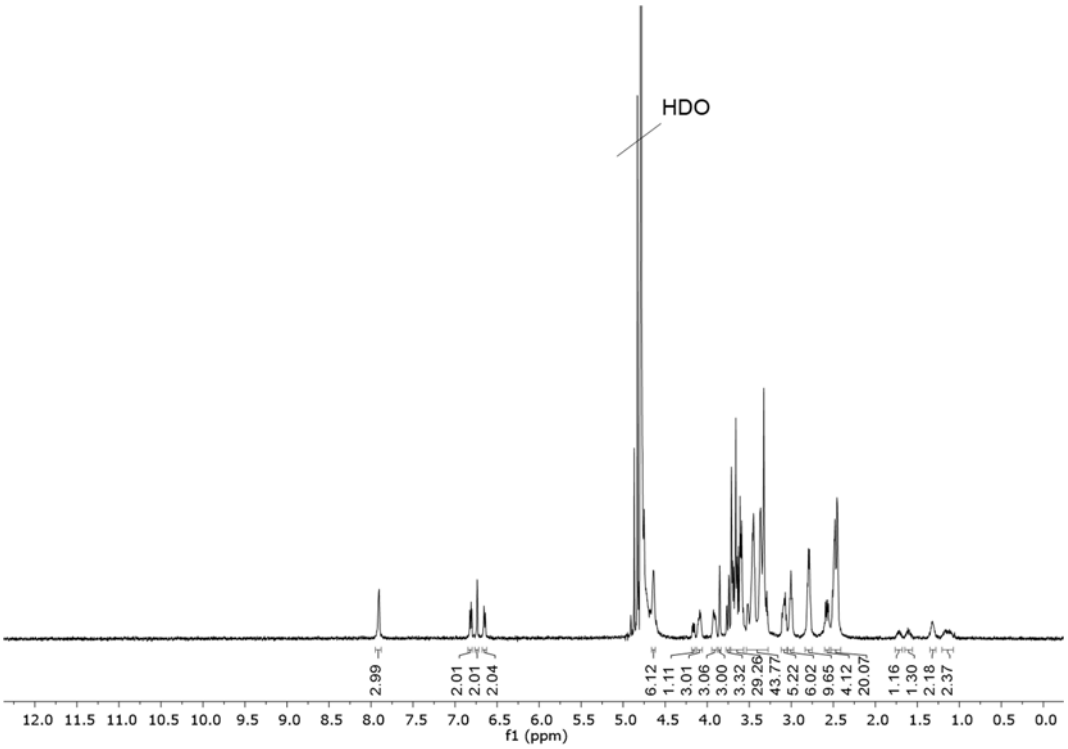


Figure S13: ^{1}H-NMR (600 MHz in D_2O) of 3Man-2Cat (**3**).

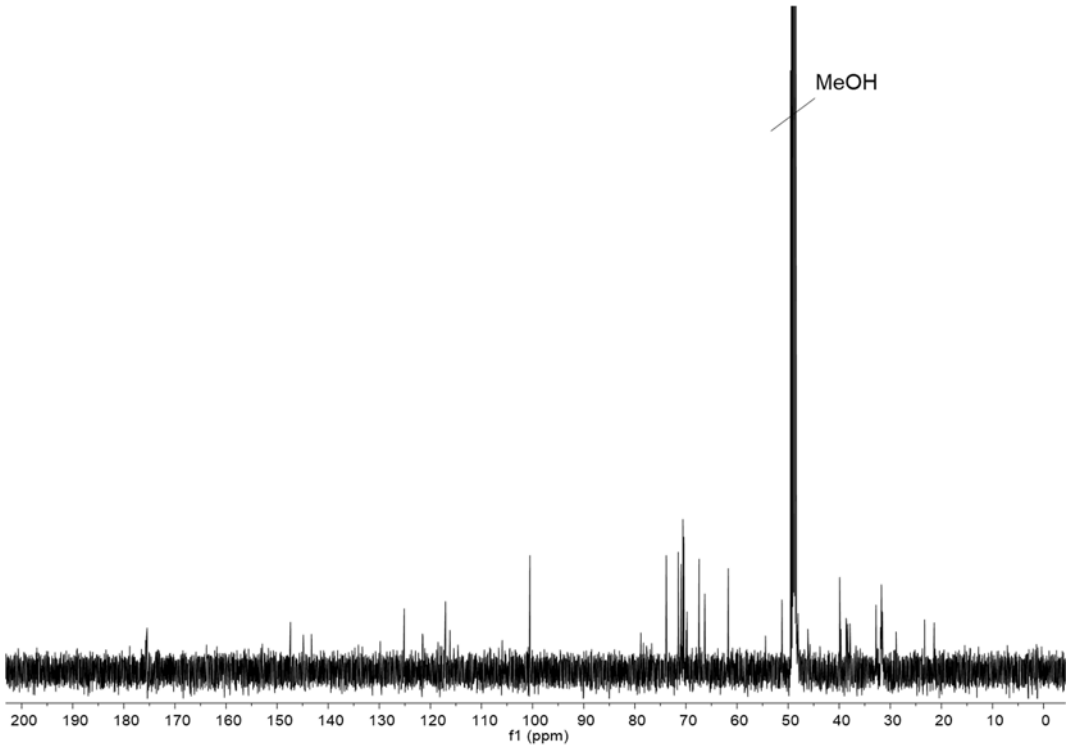


Figure S14: ^{13}C-NMR (150 MHz in D_2O) of 3Man-2Cat (**3**).

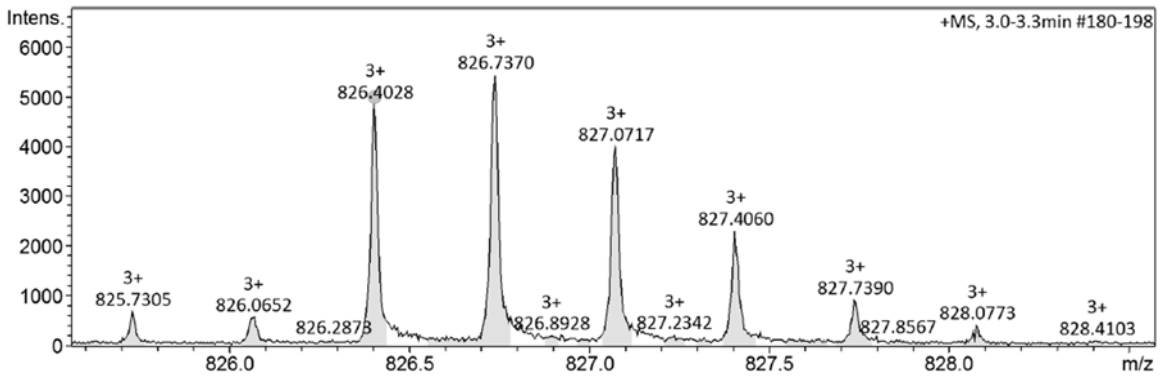


Figure S15: HR-ESI (ESI$^+$ Q-TOF) of 3Man-2Cat (**3**).

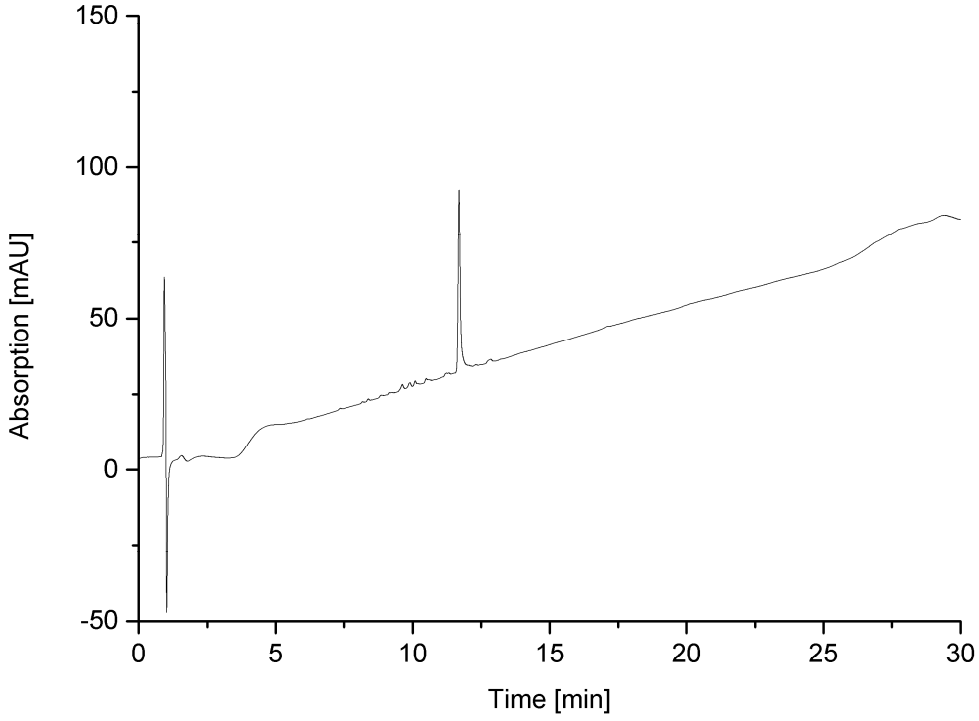
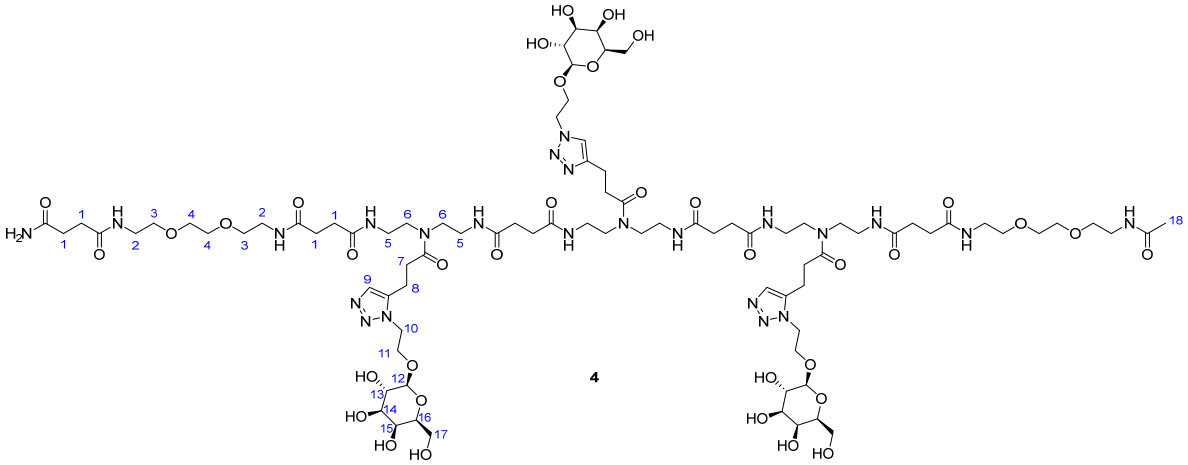


Figure S16: RP-HPLC chromatogram of 3Man-2Cat (**3**) (gradient from 0% to 50% eluent B over 30 min at 25°C).

3Gal (**4**)



Yield: 131 mg (85%).

^{1}H-NMR (600 MHz, D_2O): δ (ppm) 8.13-8.09 (m, 3H, H9), 4.76-4.74 (m, 6H, H10), 4.41 (d, J_{HH} = 7.9 Hz, 3H, H12), 4.37-4.31 (m, 3H, H11), 4.18-4.12 (m, 3H, H11), 3.94 (d, J_{HH} = 3.5 Hz, 3H, H13), 3.79-3.61 (m,

28H, H3, H4, H14, H15, H17), 3.54-3.33 (m, 32H, H2, H5, H6, H16), 3.11-3.06 (m, 6H, H7), 2.89-2,82 (m, 6H, H8), 2.59-2.44 (m, 20H, H1), 2.02 (s, 3H, H18).

^{13}C-NMR (150 MHz, D_2O): δ (ppm) 178.21, 178.14, 175.48, 175.37, 175.30, 175.28, 175.20, 175.17, 175.12, 175.09, 174.82, 174.70, 163.43, 163.15, 145.92, 145.90, 125.82, 125.79, 125.75, 117.97, 115.65, 103.52, 75.66, 73.18, 71.11, 70.08, 69.92, 69.33, 69.27, 69.08, 68.18, 66.43, 61.45, 51.77, 51.75, 51.73, 51.70, 47.62, 47.60, 45.66, 45.64, 45.61, 39.48, 39.42, 37.85, 37.41, 32.06, 31.55, 31.43, 31.38, 31.32, 31.29, 31.29, 31.14, 30.89, 22.33, 20.56, 20.52.

HR-ESI-MS: calculated mass for $C_{85}H_{143}N_{23}O_{36}$ $[M+3H]^{3+}$: 688.3428, found 688.3432.

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 8.47 min, relative purity 94%.

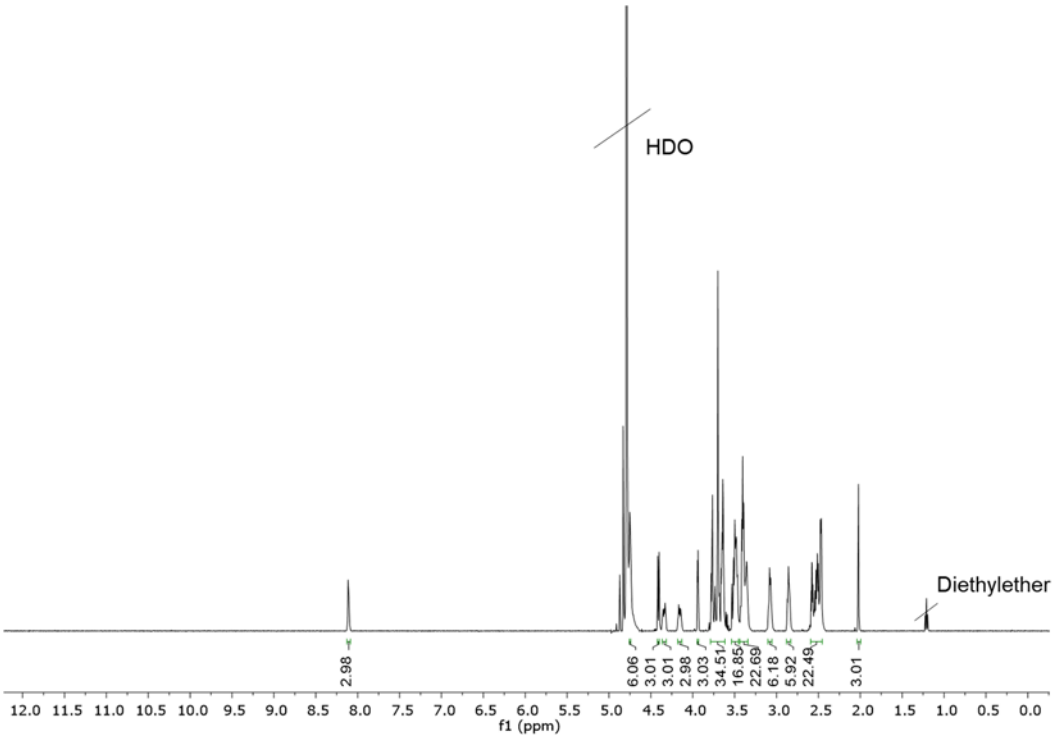


Figure S17: ^{1}H-NMR (600 MHz in D_2O) of 3Gal (**4**).

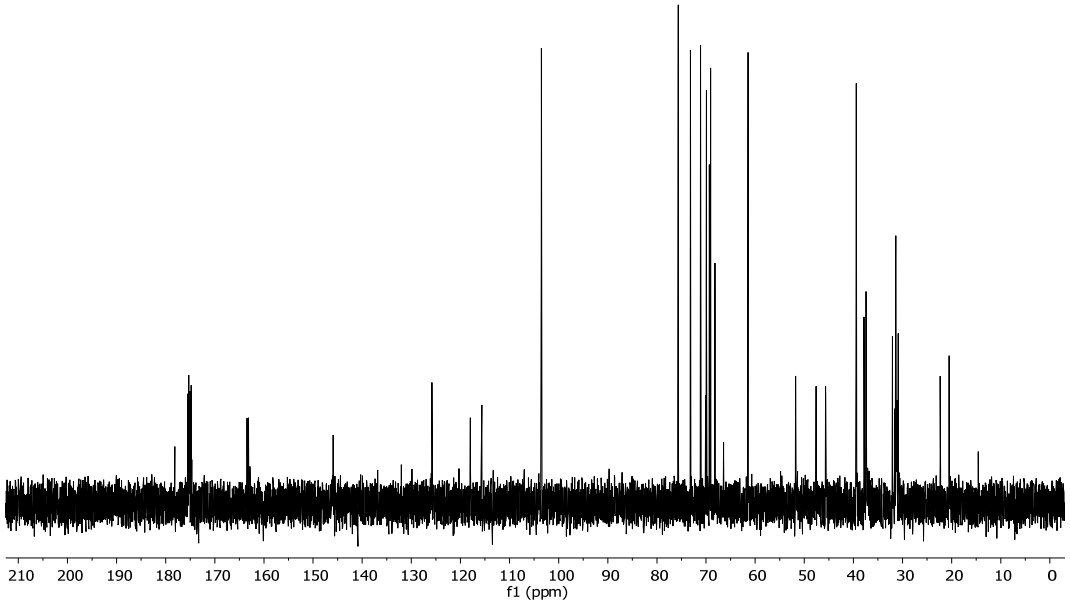


Figure S18: ^{13}C-NMR (150 MHz in D_2O) of 3Gal (**4**).

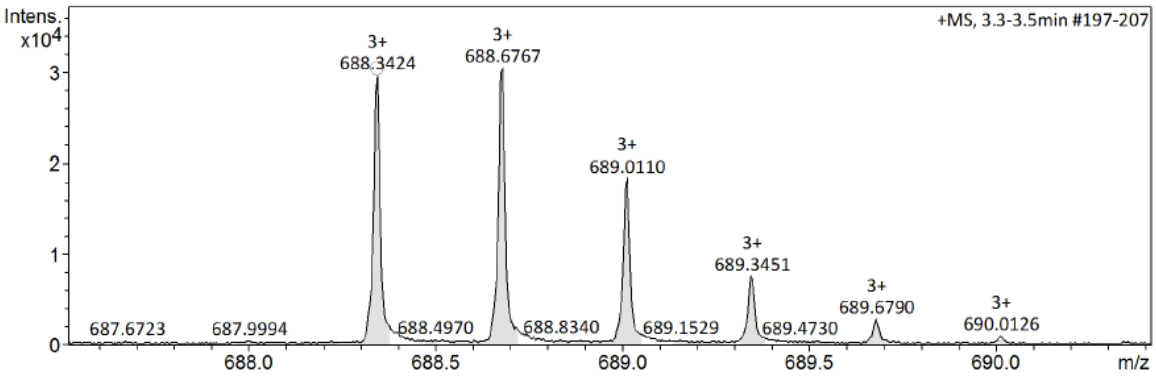


Figure S19: HR-ESI (ESI$^+$ Q-TOF) of 3Gal (**4**).

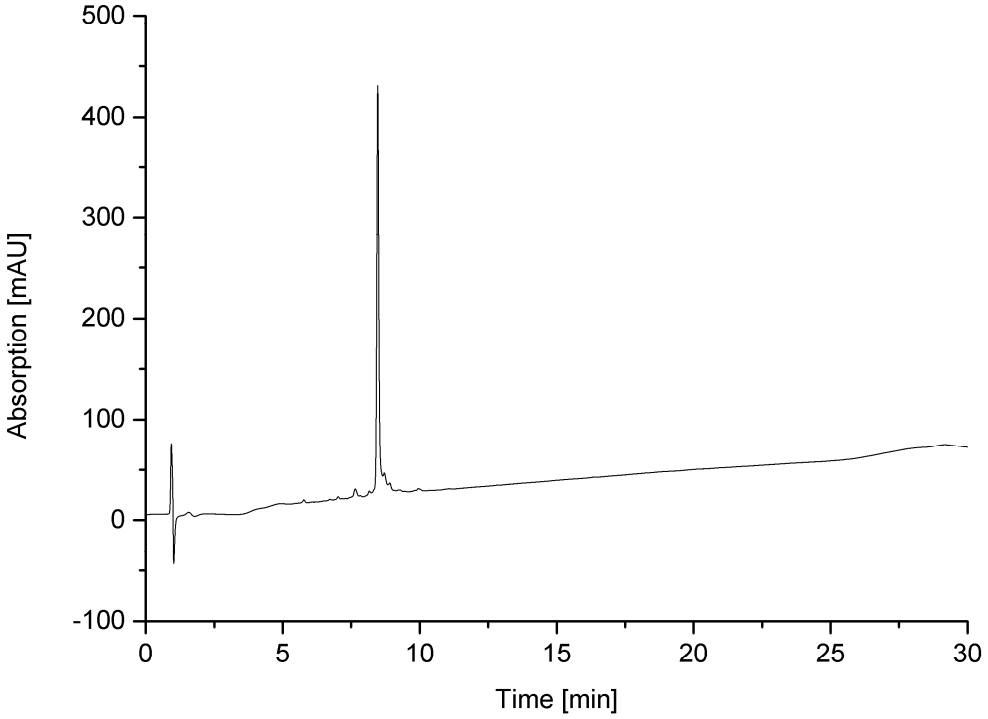
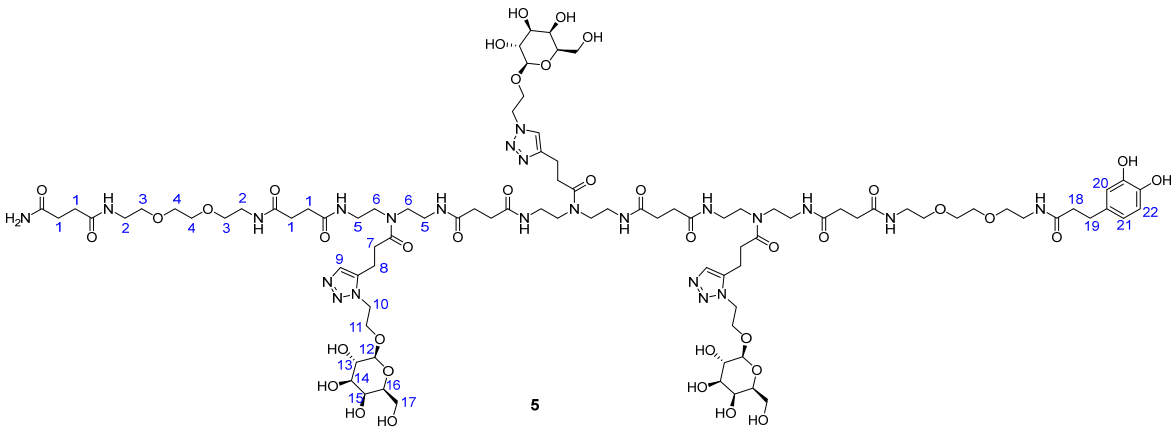


Figure S20: RP-HPLC chromatogram of 3Gal (**4**) (gradient from 0% to 50% eluent B over 30 min at 25°C).

3Gal-1Cat (**5**)



Yield: 138 mg (85%).

^{1}H-NMR (600 MHz, D_2O) δ (ppm) 8.03-7.96 (m, 3H, H9), 6.77 (d, J_{HH} = 8.1 Hz, 1H, H20), 6.70 (d, J_{HH} = 2.1 Hz, 1H, H22), 6.61 (dd, J_{HH} = 8.1 Hz, 2.1 Hz, 1H, H21), 4.67-4.63 (m, 6H, H10), 4.36-4.31 (m, 3H, H12), 4.29-4.23 (m, 3H, H11), 4.10-4.03 (m, 3H, H11), 3.87 (d, J_{HH} = 3.4 Hz, 3H, H13), 3.74-3.53 (m, 28H,

H3, H4, H14, H15, H17), 3.49-3.23 (m, 44H, H2, H5, H6, H18), 3.02-2.95 (m, 6H, H7), 2.81-2.71 (m, 8H, H8, H19), 2.53-2.37 (m, 20H, H1).

^{13}C-NMR (150MHz, D_2O) δ (ppm) 175.67, 175.65, 175.54, 175.48, 175.44, 146.81, 146.78, 144.87, 125.78, 125.76, 125.73, 121.51, 104.01, 76.09, 73.66, 71.59, 70.41, 70.39, 69.94, 69.92, 69.79, 69.78, 69.73, 69.56, 69.54, 68.73, 61.89, 51.83, 51.82, 39.89, 39.86, 38.52, 38.31, 37.91, 37.88, 32.65, 31.91, 31.90, 31.88, 31.82, 31.78, 31.75, 31.59, 31.57, 31.33, 21.27, 21.23, 21.22.

HR-ESI-MS: calculated mass for $C_{92}H_{149}N_{23}O_{38}$ $[M+3H]^{3+}$: 729.0217, found 729.0205.

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 9.78 min, relative purity 99%.

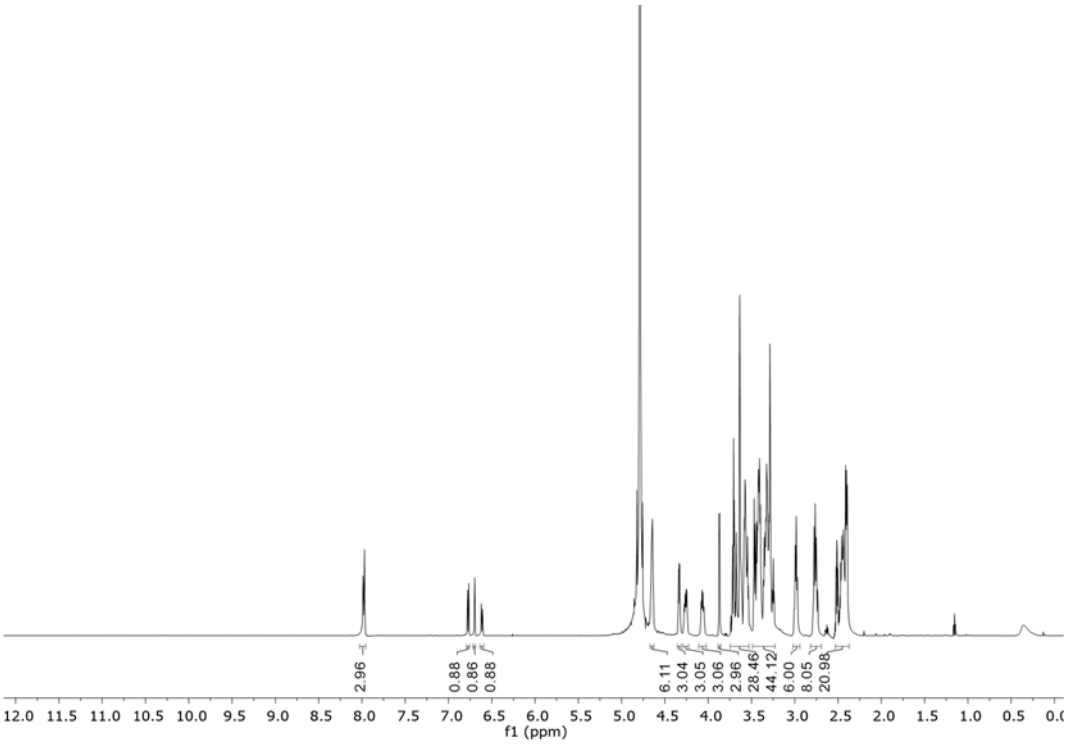


Figure S21: ^{1}H-NMR (600 MHz in D_2O) of 3Gal-1Cat (**5**).

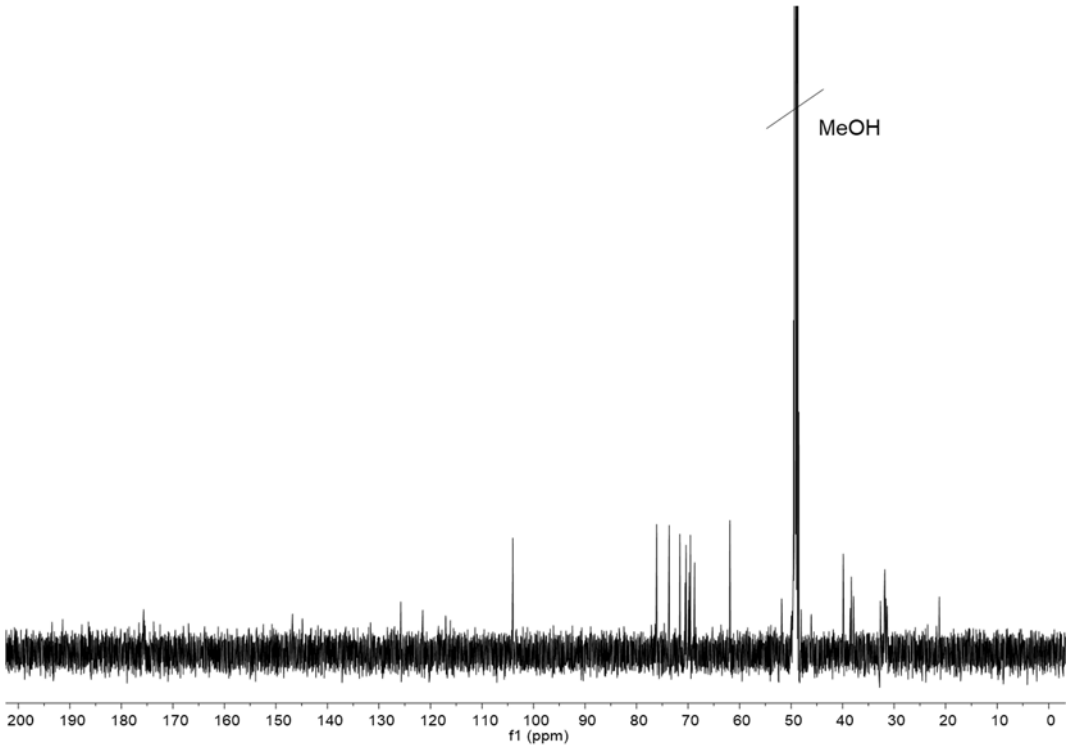


Figure S22: ^{13}C-NMR (150 MHz in D_2O) of 3Gal-1Cat (**5**).

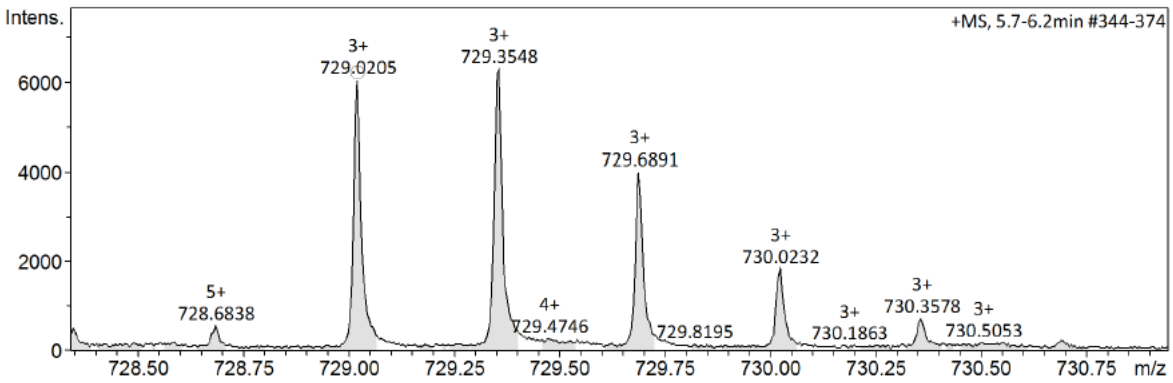


Figure S23: HR-ESI (ESI$^+$ Q-TOF) of 3Gal-1Cat (**5**).

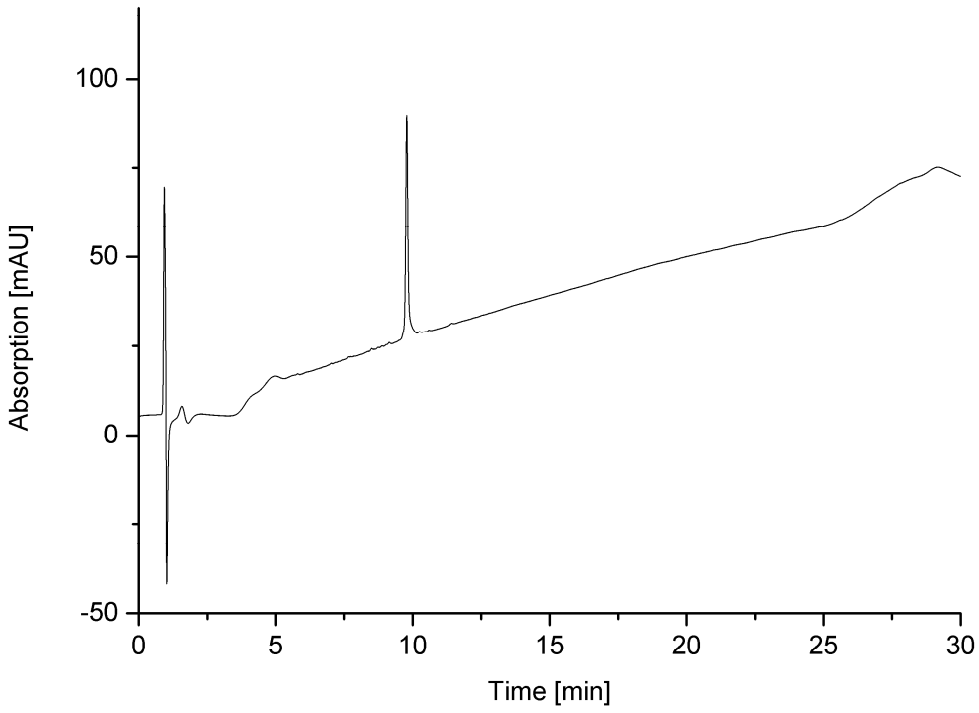
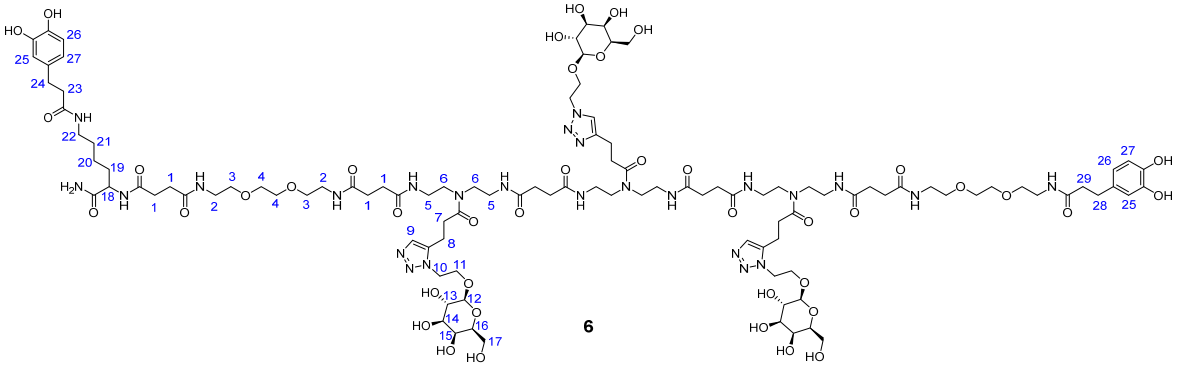


Figure S24: RP-HPLC chromatogram of 3Gal-1Cat (**5**) (gradient from 0% to 50% eluent B over 30 min at 25°C).

3Gal-2Cat (**6**)



Yield: 91 mg (49%),

^{1}H-NMR (600 MHz, D_2O): δ (ppm) 7.97-7.92 (m, 3H, H9), 6.81 (dd, J_{HH} = 8.1 Hz, 5.9 Hz, 2H, H25), 6.73 (d, J_{HH} = 1.9 Hz, 2H, H27), 6.64 (dt, J_{HH} = 8.1 Hz, 2.2 Hz, 2H, H26), 4.67-4.63 (m, 6H, H10), 4.38-4.34 (m, 3H, H12), 4.30-4.25 (m, 3H, H11), 4.14 (dd, J_{HH} = 9.4 Hz, 5.0 Hz, 1H, H18), 4.11-4.06 (m, 3H, H11), 3.90 (d, J_{HH} = 3.4 Hz, 3H, H13), 3.77-3.56 (m, 28H, H3, H4, H14, H15, H17), 3.51-3.25 (m, 34H, H2, H5, H6,

H29), 3.08-2.95 (m, 9H, H23, H7), 2.80-2.74 (m, 10H, H8, H28, H24), 2.61-2.53 (m, 6H, H16, H22), 2.51-2.39 (m, 20H, H1), 1.73-1.67 (m, 1H, H19), 1.62-1.56 (m, 1H, H19), 1.33-1.03 (m, 4H, H20, H21).

^{13}C-NMR (150 MHz, D_2O) δ (ppm) 175.40, 175.23, 175.21, 175.18, 175.15, 175.04, 175.02, 175.01, 146.44, 146.41, 146.38, 144.32, 125.23, 125.21, 125.16, 125.13, 116.70, 116.68, 116.65, 116.63, 116.60, 116.56, 115.66, 103.53, 75.63, 73.17, 71.11, 70.08, 69.92, 69.31, 69.06, 68.32, 61.43, 51.28, 47.63, 47.60, 45.62, 39.40, 38.16, 38.06, 37.85, 37.42, 32.22, 32.21, 32.20, 31.44, 31.43, 31.36, 31.33, 31.28, 31.24, 31.19, 31.14, 31.13, 31.02, 28.34, 22.76, 20.82.

HR-ESI-MS: calculated mass for $C_{107}H_{169}N_{25}O_{42}$ $[M+3H]^{3+}$: 826.7370, found 826.7371.

RP-HPLC (Eluent B from 0% to 100% in 30 min): t_r = 11.38 min, relative purity 96%.

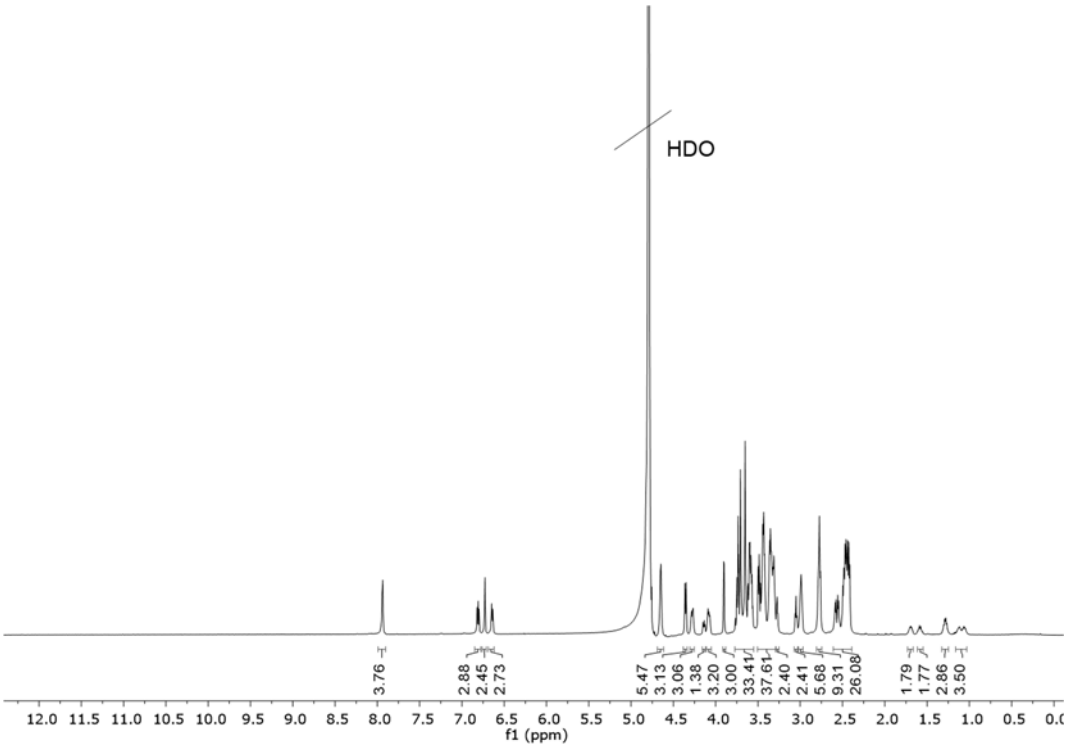


Figure S25: ^{1}H-NMR (600 MHz in D_2O) of 3Gal-2Cat (**6**).

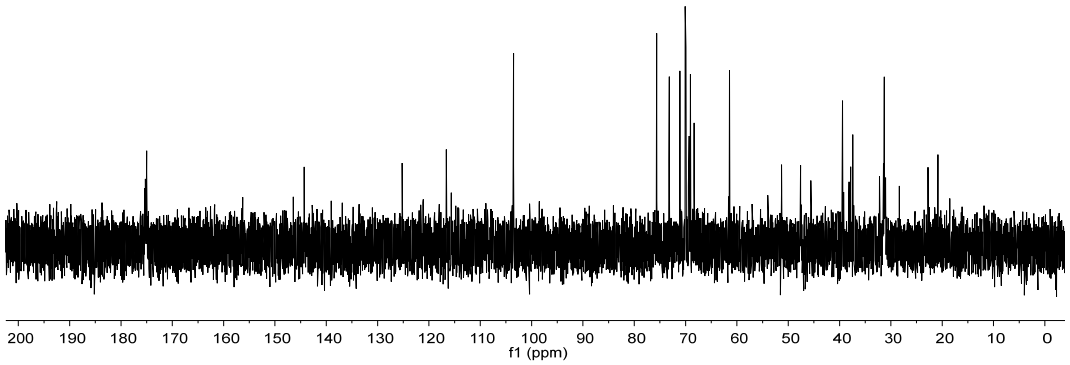


Figure S26: ^{13}C-NMR (150 MHz in D_2O) of 3Gal-2Cat (**6**).

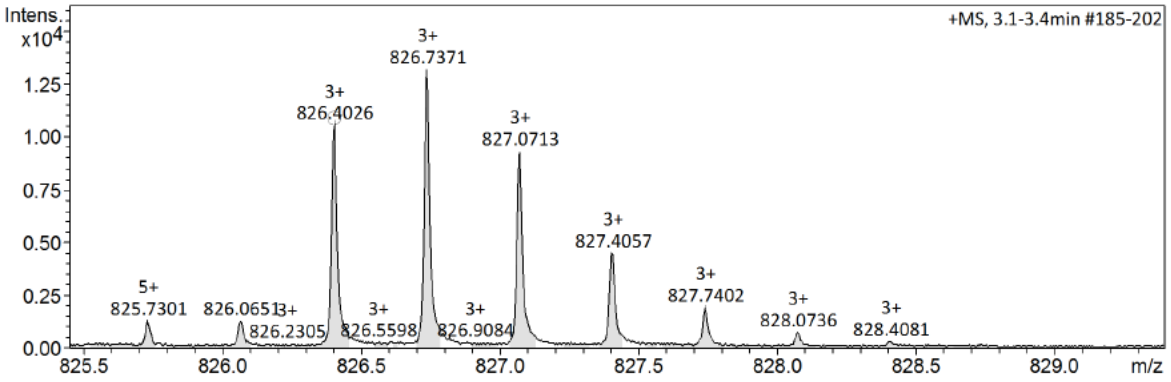


Figure S27: HR-ESI (ESI$^+$ Q-TOF) of 3Gal-2Cat (**6**).

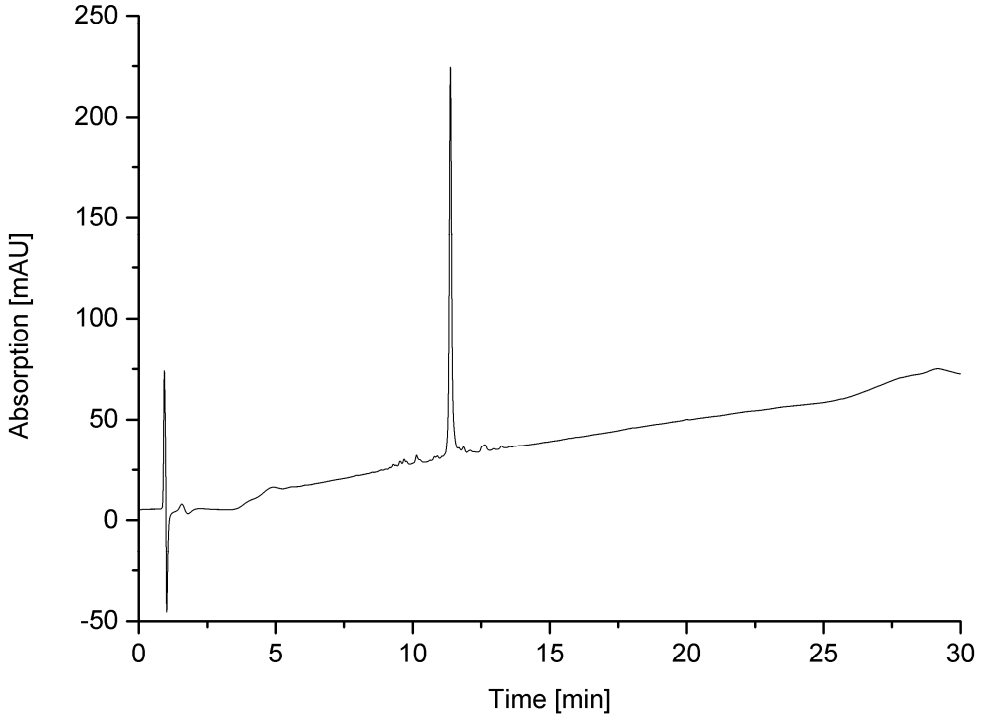


Figure S28: RP-HPLC chromatogram of 3Gal-2Cat (**6**) (gradient from 0% to 50% eluent B over 30 min at 25°C).

Concentration Dependent Turbidity Assay

A solution of 5 µM ConA in LBB buffer (10 mM HEPES, 50 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4) was prepared. The transmission of 1 ml of this solution was measured as 100% transmission baseline. Afterwards glycomacromolecules were stepwise titrated to the ConA solution and after 20 min incubation the transmission was measured. Every glycomacromolecule was measured three times.

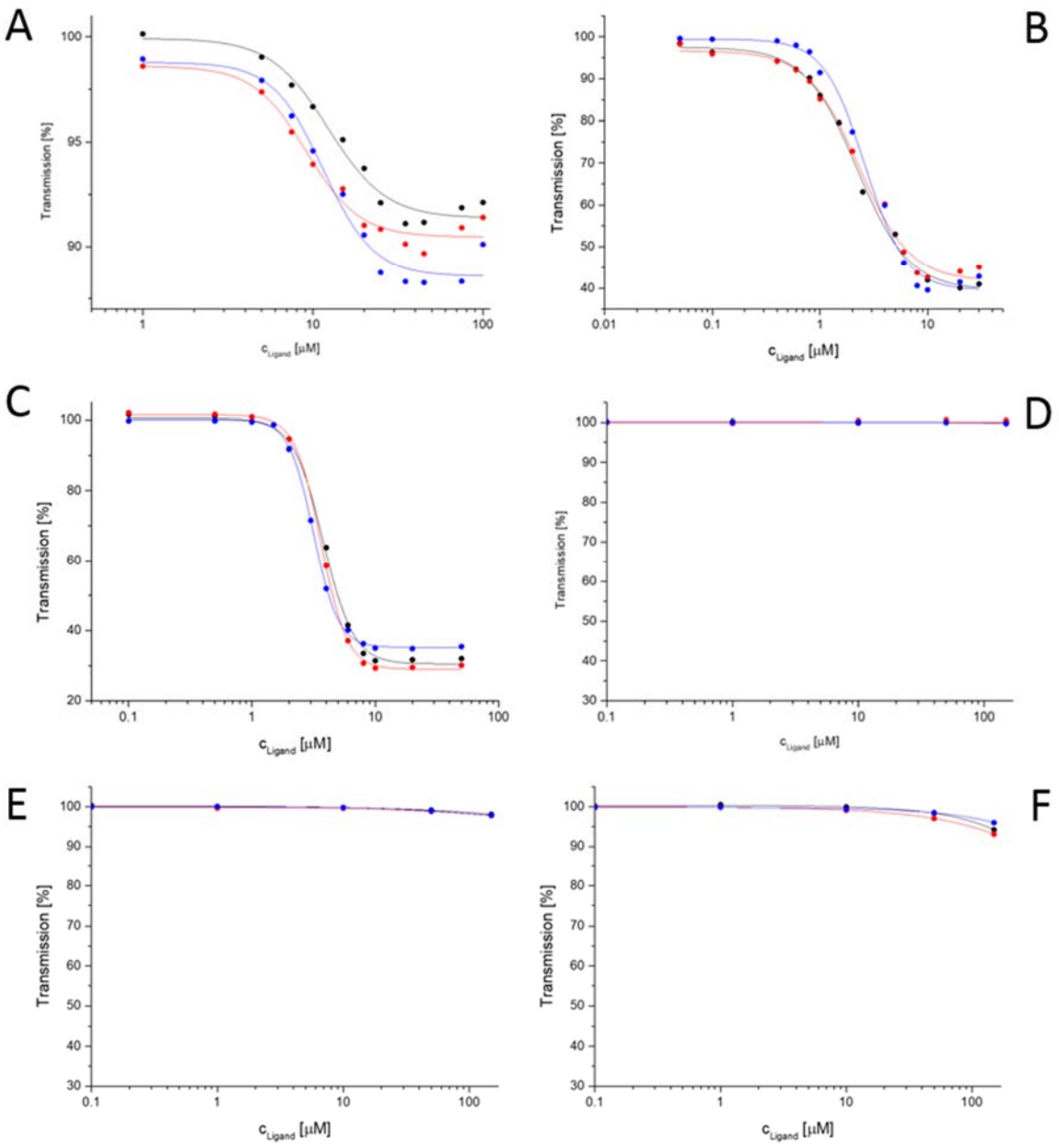


Figure S29: Transmission values obtained in the concentration dependent turbidity assay for different concentrations of glycomacromolecules. A) 3Man (**1**), B) 3Man-1Cat (**2**), C) 3Man-2Cat (**3**), D) 3Gal (**4**), E) 3Gal-1Cat (**5**), F) 3Gal-2Cat (**6**)

Table S1: Results from the concentration dependent turbidity assay.

Ligand	$1/2T_{max}$ [%]	$c_{1/2Tmax}$ [µM]	$1/c_{1/2Tmax}$ [1/µM]
3Man (**1**)	94.62±0.81	11.01±1.34	0.091±0.01
3Man-1Cat (**2**)	69.08±0.40	2.37±0.17	0.42±0.03
3Man-2Cat (**3**)	66.10±1.09	3.53±0.27	0.28±0.02

Quantitative Precipitation Assay

A solution of 15 µM ConA in LBB buffer (10 mM HEPES, 50 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4) was prepared and the concentration was measured at 280 nm. Afterwards aliquots of this solution were mixed with different concentrations of glycomacromolecules, incubated for 24 h and centrifuged for 5 min at 4400 rpm. The precipitate was resuspended in LBB buffer with 50 mM α-methyl D-mannoside and the ConA concentration was determined at 280 nm. To calculate the amount of ConA precipitated per glycomacromolecule the linear slope between 1 and 5 µM ligand was used.

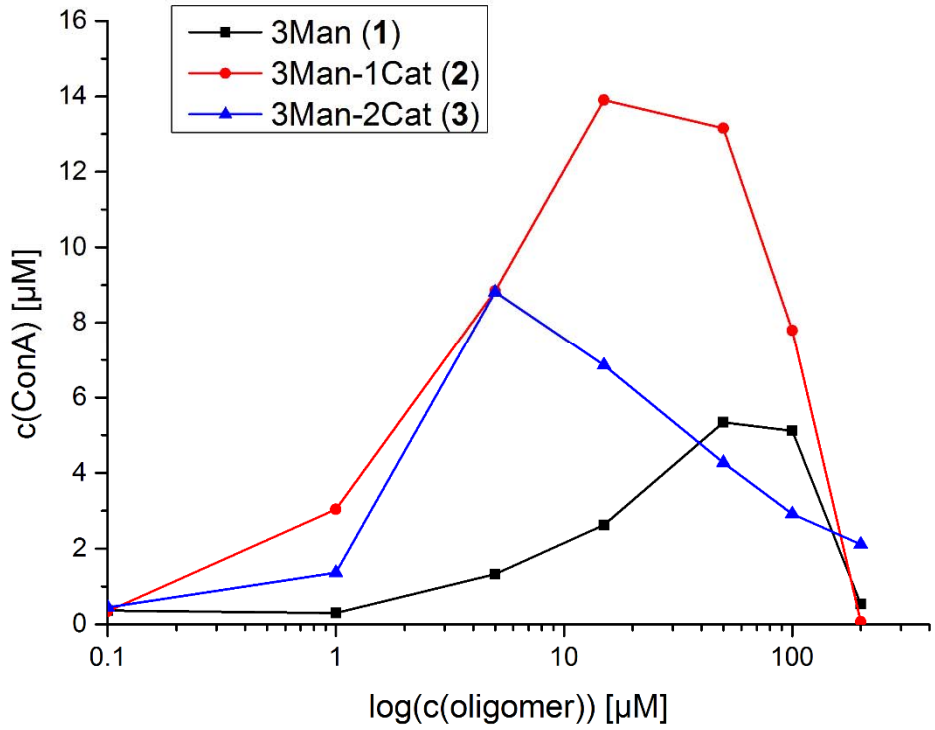


Figure S30: Amount of ConA precipitated per glycomacromolecule in the quantitative precipitation assay.

Table S2: Results from the quantitative precipitation assay.

Ligand	ConA/Ligand
3Man (**1**)	0.10±0.01
3Man-1Cat (**2**)	0.72±0.02
3Man-2Cat (**3**)	1.86±0.02

Covalent Binding Assay (MALDI-TOF)

For the determination of a covalent bond between ConA and ligand, equimolar amounts of ConA (8 µM) and ligand (8 µM) were incubated in LBB buffer (10 mM HEPES, 50 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4) for 24 h. Afterwards the samples were filtrated and measured via MALDI-TOF in linear mode.

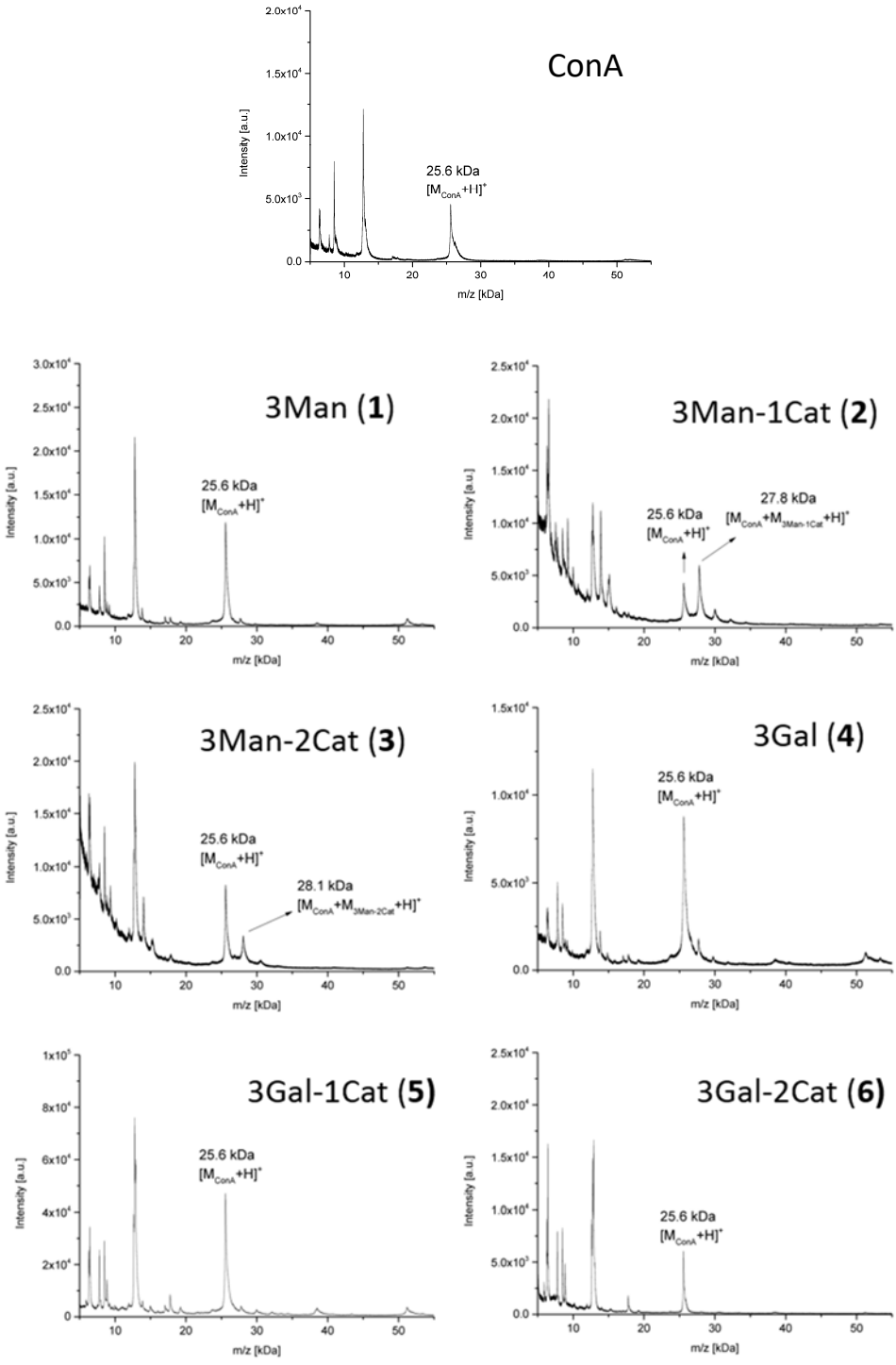


Figure S31: Results from the covalent binding assay via MALDI-TOF for structures **1**-**6**.

Covalent Binding Assay (SDS-PAGE)

Equimolar amounts of ConA (8 µM) and ligand (8 µM) were incubated in LBB buffer (10 mM HEPES, 50 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4) for 24 h. Afterwards the samples were treated with sample buffer (40% glycerol, 4 mg/ml SDS, 0.02% bromophenol blue) and injected into a 15% polyacrylamide gel. The seperation was done over 2 h by 120 V (0.2 A, 300 W) and samples were stained with Coomassie®. As standard Page-Ruler® Prestained Protein Ladder (P/N) 26616 was used.

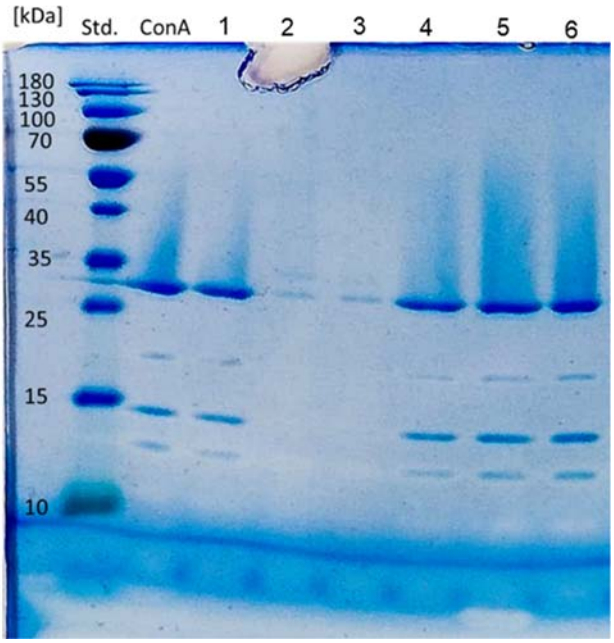


Figure S32: SDS-PAGE of ConA and oligomers **1**-**6**.

Bacterial Adhesion-Inhibition Assay

The *E.coli* strain PKL1162 was cultured from a stock in LB media (ampicillin 100 mg/ml and chloramphenicol 50 mg/ml) at 37°C overnight. The bacterial cells were centrifuged and washed twice and suspended in PBS buffer to a cell concentration of OD_{600} = 0.4. The adhesion-inhibition assay was conducted as described prior in this working group.[6] Black 96-well microtiter plates (Nunc, MaxiScorp) were treated with mannan (1.2 mg/ml in carbonate buffer pH 9.6) for 12 h at 37°C until full evaporation of water. The plates were washed three times with PBST buffer (PBS buffer + 0.05% v/v Tween®20) and blocked with PVA (1% in PBS) for 2 h. Afterwards the plates were washed with PBST twice and PBS once. For the measurement a serial dilution of glycomacromolecules on the mannan-coated microtiter plates was performed (50 µl). The bacterial suspension was added (50 µl) and the plates were incubated for either 1 h or 24 h at 37°C. After incubation the microtiter plates were washed three times with PBS and refilled with PBS (100 µl) to measure the fluorescence intensity (excitation 485 nm, emission 535 nm).

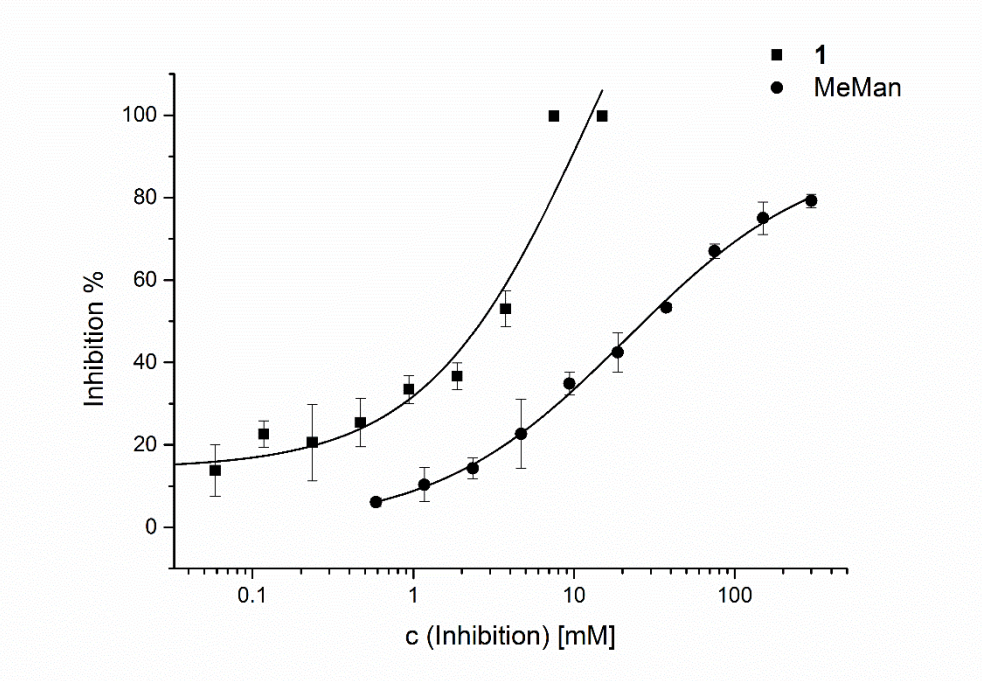


Figure S33: Inhibition curves of structure **1** and MeMan obtained in the bacterial adhesion-inhibtion assay after 1 h incubation.

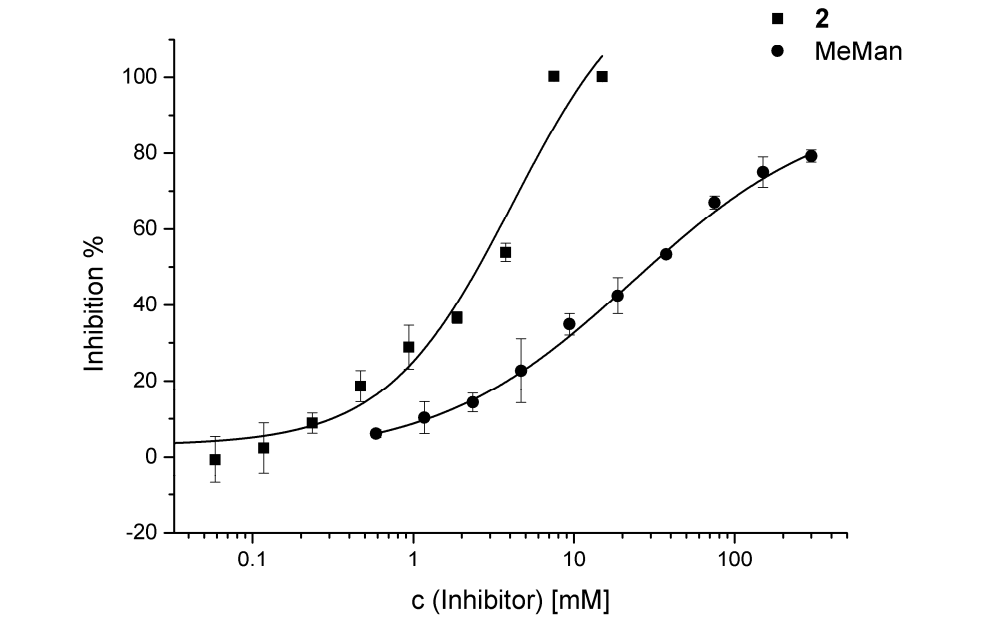


Figure S34: Inhibition curves of structure **2** and MeMan obtained in the bacterial adhesion-inhibition assay after 1 h incubation.

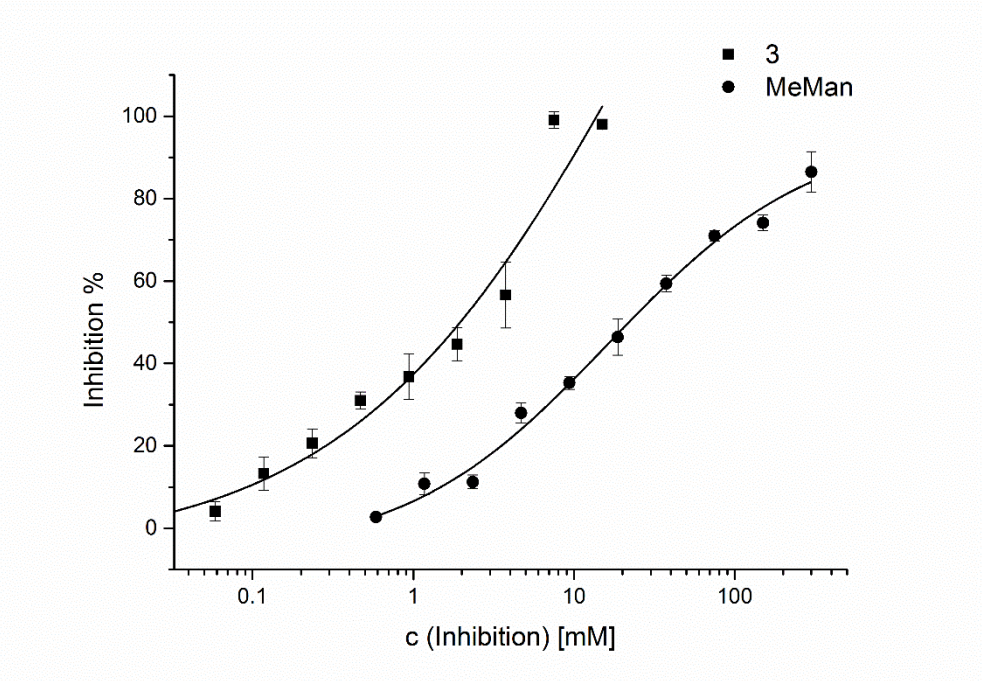


Figure S35: Inhibition curves of structure **3** and MeMan obtained in the bacterial adhesion-inhibition assay after 1 h incubation.

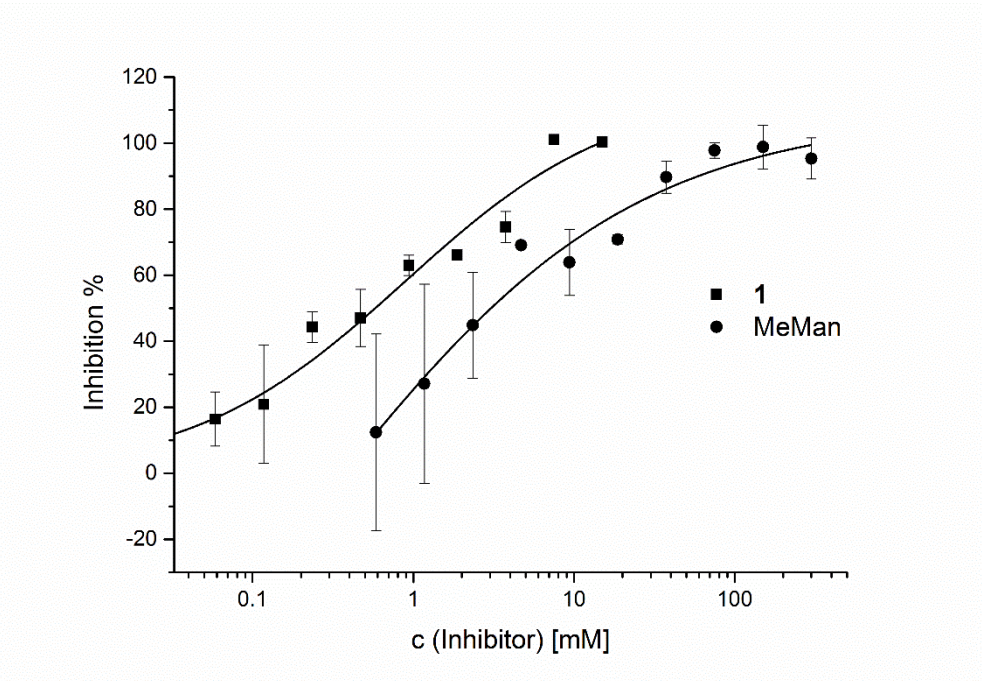


Figure S36: Inhibition curves of structure **1** and MeMan obtained in the bacterial adhesion-inhibition assay after 24 h incubation.

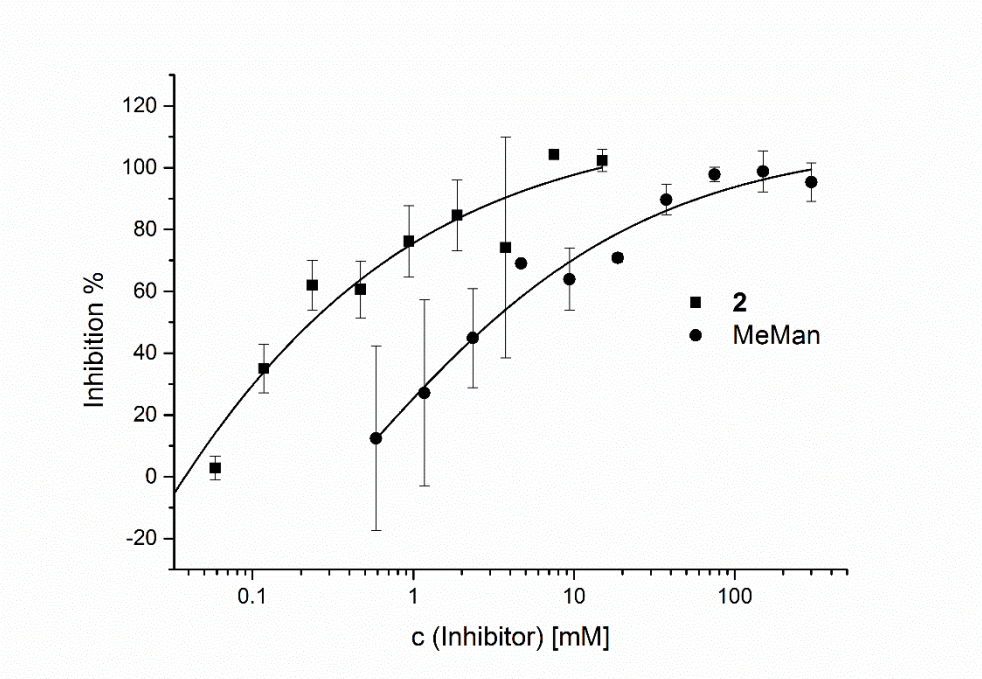


Figure S37: Inhibition curves of structure **2** and MeMan obtained in the bacterial adhesion-inhibition assay after 24 h incubation.

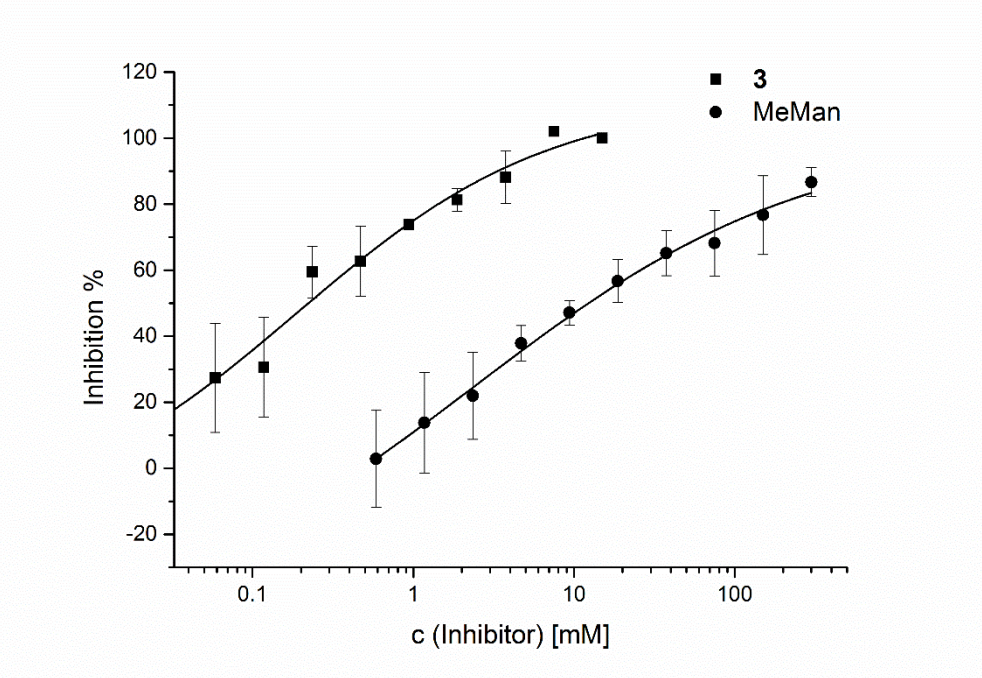


Figure S38: Inhibition curves of structure **3** and MeMan obtained in the bacterial adhesion-inhibition assay after 24 h incubation.

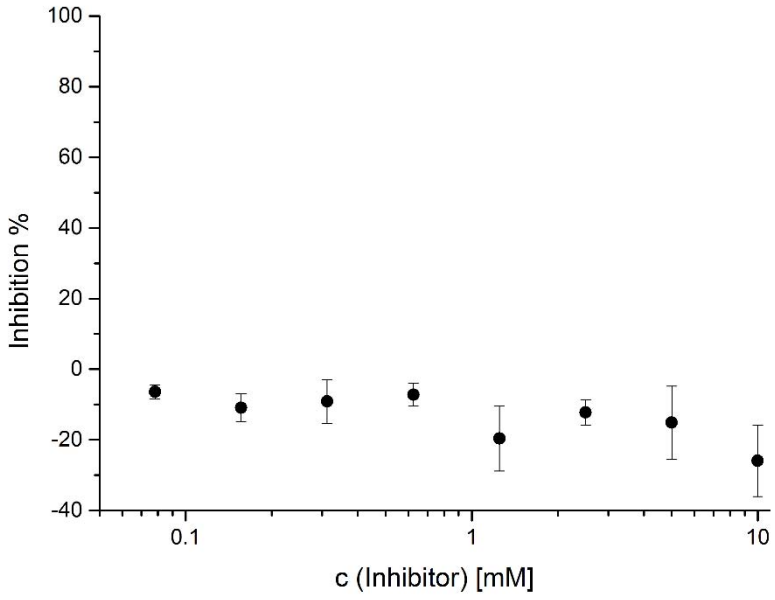


Figure S39: Inhibition curve of structure **4** obtained in the bacterial adhesion-inhibition assay after 1 h incubation.

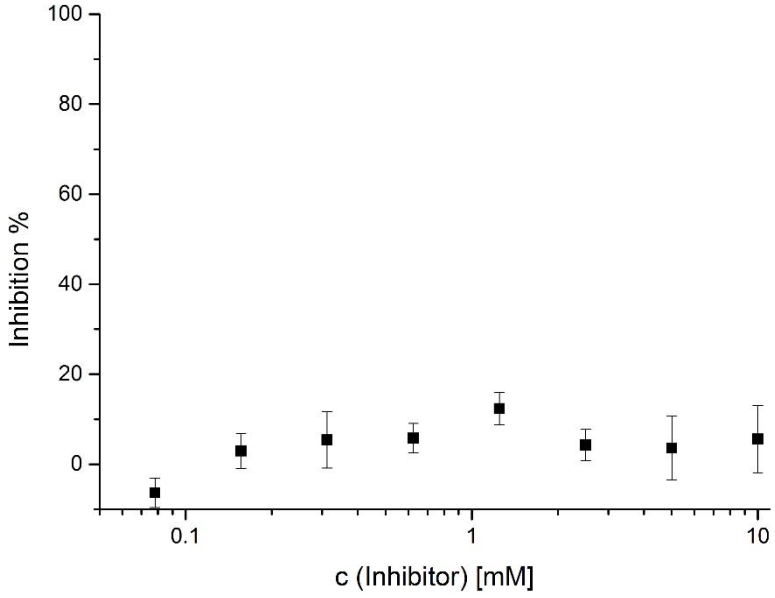


Figure S40: Inhibition curve of structure **5** obtained in the bacterial adhesion-inhibition assay after 1 h incubation.

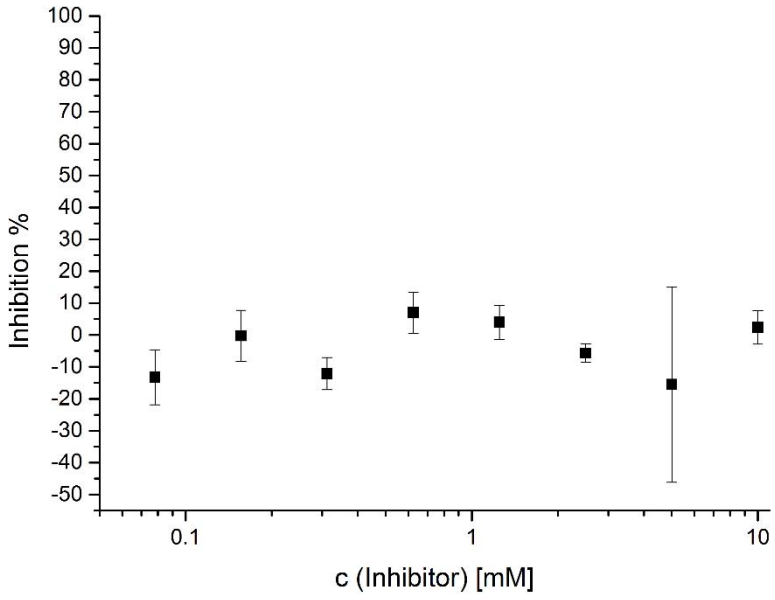


Figure S41: Inhibition curve of structure **6** obtained in the bacterial adhesion-inhibition assay after 1 h incubation.

(1) Hayes, W.; Osborn, H. M.; Osborne, S. D.; Rastall, R. A.; Romagnoli, B. *Tetrahedron* **2003**, *59*, 7983.

(2) Wu, L.; Sampson, N. S. *ACS Chemical Biology* **2014**, *9*, 468.

(3) Wei, Q.; Achazi, K.; Liebe, H.; Schulz, A.; Noeske, P. L. M.; Grunwald, I.; Haag, R. *Angewandte Chemie International Edition* **2014**, *53*, 11650.

(4) Ponader, D.; Wojcik, F.; Beceren-Braun, F.; Dernedde, J.; Hartmann, L. *Biomacromolecules* **2012**, *13*, 1845.

(5) Gerke, C.; Ebbesen, M. F.; Jansen, D.; Boden, S.; Freichel, T.; Hartmann, L. *Biomacromolecules* **2017**, *18*, 787.

(6) Boden, S.; Reise, F.; Kania, J.; Lindhorst, T. K.; Hartmann, L. *Macromolecular Bioscience* **2019**, *19*, e1800425.