Supplementary Information

Cyclooligomerisation of azido-alkyne-functionalised sugars: synthesis of 1,6-linked cyclic *pseudo*-galactooligosaccharides and assessement of their sialylation by *Trypanosoma cruzi trans*-sialidase†

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Experimental

General procedures

All chemicals were purchased as reagent grade and used without further purification. Solvents were dried according to standard methods. MuNANA (2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid sodium salt), used as a donor substrate for sialylation reactions, was acquired from Toronto Research Chemicals Inc. The *trans*-sialidase used in this study was a His-tagged 70 kDa recombinant material truncated to remove *C*-terminal repeats but retaining the catalytic *N*-terminal domain of the enzyme.[1] Reactions were monitored by thin layer chromatography (TLC) on 0.25 nm precoated silica gel plates (Whatman, AL SIL G/UV, aluminium backing) with the indicated eluents. Compounds were visualized under UV light (254 nm) and/or dipping in ethanol–sulfuric acid (95:5, v/v) or orcinol (2%) in aqueous sulfuric acid (10%), followed by heating the plate for a few minutes. Column chromatography was performed on silica gel 60 (Fluorochem, 35–70 mesh) or on a Biotage Horizon High-Performance FLASH Chromatography system using 12 mm or 25mm flash cartridges with the eluents indicated. The microwave-assisted reactions were carried out in a Biotage Initiator System, using sealed tubes. HPLC purifications were performed in the Dionex DX-500 HPLC system using a Phenomenex C18 semi-preparative reverse phase column (250 x 10.0 mm). Nuclear magnetic resonance spectra were recorded on a JEOL Lambda 400MHz spectrometer. ^{1}H-NMR spectra recorded at 400MHz were referenced to δH 7.27 for $CDCl_3$, δH 3.35 for CD_3OD, δH 4.63 ppm for D_2O, and ^{13}C NMR spectra recorded at 100 MHz were referenced to δC 77.0 for $CDCl_3$ and δC 49.15 for CD_3OD. Assignments were made with the aid of HSQC and COSY experiments. Optical rotations were measured at ambient temperature on a Perkin-Elmer model 341 polarimeter using a sodium lamp. Accurate mass electrospray ionization mass spectra (ESI-MS) and high resolution ESI-MS (HRESI MS) were obtained from the John Innes Centre metabolite analysis service on a Thermo Finningan DecaXP^{plus} using positive or negative ionization mode, and Thermofisher LTQ Orbitrap XL mass spectrometers, respectively.

Molecular modelling procedure

A conformational search was performed for cyclic pseudotrisaccharides **15** and **19** using the MONTE CARLO method, using the MMFF molecular mechanics model implemented in the Spartan'06 1.1.2 software[2] using non-solvated conditions (gas phase). For initial modelling of glycomacrocycle **15** the crystallographic data for 4-(β-D-glucopyranosyl)-1-methoxycarbonylmethyl-triazole[3] was used as a basis to build the input structure. On the other hand, no crystallographic structure has been reported for the

galactose methylene triazole moiety of glycomacrocycle **19**, and thus, a simple input 3D structure was built from the 2D structure, and then energy minimized. Once relaxed the macrocyclic structures of the two glycopolymers were subjected to a conformational search and the lowest energy conformers for each compound was selected from among 10,000 conformations analyzed by the MONTE CARLO method. Sets of six superimposed lowest energy conformations of **15** and **19** are presented in Fig S20.

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose 3[4]

Compound **3** was prepared according to ref.[5] A suspension of finely powdered galactose (10 g, 55.5 mmol) and iodine (3 g, 11.8 mmol) in acetone (500 mL) was stirred at room temperature until TLC [toluene:EtOAc (2:1)] indicated that the reaction was completed (approx. 20 h). A 10% aq $Na_2S_2O_3$ solution was added with stirring until the mixture was decolorized, acetone was removed under reduced pressure and the residue was extracted with CH_2Cl_2. The organic extract was washed with water, dried ($MgSO_4$) and concentrated to give the known title compound **3** as a syrup (12.8 g, 89%); δ_H ($CDCl_3$, 400 MHz), δ (ppm): 5.57 (1H, d, $J_{1,2}$ 5.1 Hz, H-1), 4.62 (1H, dd, $J_{2,3}$ 2.4 Hz; $J_{3,4}$ 8.0 Hz, H-3), 4.34 (1H, dd, $J_{1,2}$ 5.1 Hz, $J_{2,3}$ 2.4 Hz, H-2), 4.28 (1H, d, $J_{3,4}$ 8.0 Hz, H-4), 3.90-3.72 (2H, m, H-6, H-6'), 2.45 (1H, dd, $J_{5,6}$ 10.2 Hz; $J_{5,6'}$ 3,0 Hz, H-5), 2.30 (1H, OH), 1.54, 1.46, 1.34 (12H, 3s, 4×CH_3). NMR data were in accord with the literature.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2-propynyl)-α-D-galactopyranose 4[6-8]

To a solution of alcohol **3** (12.8 g, 49.2 mmol) in anhydrous DMF (50 mL) was added NaH (2.36 g, 59 mmol, 60% oil suspension in oil), the mixture was stirred for 15 min at 0 ºC, then an 80% solution of propargyl bromide in toluene (6 mL, 54 mmol) was added dropwise (approx. 20 min.) the mixture was brought to room temperature and stirred for 1 h. The reaction mixture was quenched with methanol (25 mL), diluted with water and extracted with diethyl ether. The organic extract was dried ($MgSO_4$) and concentrated to give known 6-*O*-propargyl ether **4** (13.1 g, 89%); δ_H ($CDCl_3$, 400 MHz): 5.54 (1H, d, $J_{1,2}$ 5.1 Hz, H-1), 4.60 (1H, dd, $J_{2,3}$ 2.6 Hz, $J_{3,4}$ 8.0 Hz; H-3), 4.31(1H, dd, $J_{1,2}$ 5.2 Hz, $J_{2,3}$ 2.6 Hz, H-2), 4.27-4.16 (3H, m, H-4, C*H*$_2$C≡CH), 3.99 (1H, dt, $J_{4,5}$ 2.4 Hz, $J_{5,6}$ 7.0 Hz, $J_{5,6'}$ 5.2 Hz, H-5), 3.77 (1H, dd, $J_{5,6'}$ 5.2 Hz, $J_{6,6'}$ 10.2 Hz, H-6'), 3.67 (1H, dd, $J_{5,6}$ 7.0 Hz; $J_{6,6'}$ 10.2 Hz, H-6), 2.45 (1H, s, CH_2C≡C*H*), 1.54, 1.45, 1.34, 1.32 (12H, 4s, 4xCH_3). δ_C ($CDCl_3$, 100 MHz): 109.2, 108.5 (*C*CH_3)$_2$), 96.3 (C-1), 79.6 (CH_2*C*≡CH), 74.5 (CH_2C≡*C*H), 71.1, 70.5, 70.4, 68.6 (C-2, C-3, C-4, C-5), 66.7 (C-6), 58.4 (*C*H_2C≡CH), 25.9, 25.8, 24.8, 24.4 (*C*H_3); IR (KBr) ν_{max} 3273, 2925, 1382, 1211, 1070 cm^{-1}; ESI-MS: *m/z* 299.0 $[M+H]^+$, 316.1 $[M+ NH_4]^+$.

6-*O*-(2-Propynyl)-D-galactopyranose 5

A solution of di-*O*-isopropylidene derivative **4** (1.0 g, 3.35 mmol) in 80% aqueous TFA (10 mL) was stirred at 22 ºC until reaction was complete (~2 h, TLC in CH_2Cl_2:MeOH 7:3) and the mixture was concentrated under reduced pressure several times with addition of *i*-PrOH to give a yellow syrup. Purification by column chromatography (CH_2Cl_2–MeOH 9:1) gave product **5** as a mixture of anomers (680 mg, 93%); δ_H (CD_3OD, 400 MHz): 5.14 (1H, d, $J_{1,2}$ 3.6 Hz, H-1, α-anomer), 4.44 (1H, d, $J_{1,2}$ 7.4 Hz, H-1, β-anomer), 4.21-4.17 (3H, m, C*H*$_2$C≡CH, H-5), 3.85-3.44 (4H, m, H-2, H-3, H-4, H-6), 3.46 (1H, m, H-6'), 2.84 (1H, m, CH_2C≡C*H*). δ_C (CD_3OD, 100 MHz): 98.1 (C-1, β-anomer), 94.1 (C-1, α-anomer), 76.0 (CH_2*C*≡CH), 74.9 (CH_2C≡*C*H), 71.2, 70.5, 70.4, 70.3 (C-5, C-3, C-2, C-4), 69.9 (C-6), 59.3 (*C*H_2C≡CH); ESI-MS: *m/z* 236.0 $[M+NH_3]^+$, 241.0 $[M+Na]^+$.

1,2,3,4-Tetra-*O*-acetyl-6-*O*-(2-propynyl)-D-galactopyranose 6

A solution of **5** (530 mg, 2.43 mmol), anhydrous pyridine (5.3 mL) and acetic anhydride (2.7 mL) was stirred at room temperature for 6 h, then concentrated with toluene and the residues was dissolved in CH_2Cl_2. The resulting solution was washed successively with 10% HCl and satd $NaHCO_3$ solution, dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **6** as a yellow syrup (980 mg, 100%) which was a 2:5 mixture of α- and β-anomers. Additional purification by column chromatography (hexane–EtOAc) afforded **6-β** (730 mg, 74%): $[\alpha]_D^{25}$ + 32.1 (*c* 1.0, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 5.70 (1H, d, $J_{1,2}$ 8.3 Hz, H-1), 5.48 (1H, dd, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1.0 Hz, H-4), 5.35-5.30 (1H, m, H-2), 5.08 (1H, dd, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.5 Hz, H-3), 4.18-4.08 (2H, m, C*H*$_2$C≡CH), 4.01 (1H, t, $J_{5,6}$ 6.8 Hz, H-5), 3.63 (1H, dd, $J_{5,6'}$ 4.4 Hz, $J_{6,6'}$ 6.0 Hz, H-6'), 3.57 (1H, d, $J_{6,6'}$ 6.0 Hz, H-6), 2.43 (1H, s, CH_2C≡C*H*), 2.17, 2.16, 2.01, 1.99 (12H, 4s, 4xCH_3); δ_C ($CDCl_3$, 100 MHz): 170.0 (*C*OCH_3), 169.9 (*C*OCH_3), 169.4 (*C*OCH_3), 168.9 (*C*OCH_3), 92.2 (C-1), 78.8 (CH_2*C*≡CH), 75.0 (CH_2C≡*C*H), 72.7 (C-5), 70.9 (C-3), 68.0, 67.3, 66.5 (C-6, C-4; C-2), 58.5 (*C*H_2C≡CH), 20.8, 20.7, 20.6, 20.5 (CO*C*H_3); IR (KBr) ν_{max} 3276, 2937, 2360, 1747, 1373, 1220 cm^{-1}; HRESI MS: *m/z* found 404.1553 $[M+NH_4]^+$; calcd for $C_{17}H_{22}O_{10}NH_4^+$: 404.1551.

2,3,4-Tri-*O*-acetyl-6-*O*-(2-propynyl)-α-D-galactopyranosyl bromide 7

To a solution of acetate **6** (980 mg, 2.54 mmol) in anhydrous CH_2Cl_2 (20 mL) cooled to 0 ºC was slowly added 33% HBr in AcOH (2.3 mL), the mixture was stirred for 10 h at room temperature, diluted with toluene and concentrated under reduced pressure to give crude bromide **7** as an amber syrup (920 mg, 89%): δ_H ($CDCl_3$, 400 MHz): 6.71 (1H, d, $J_{1,2}$,3.9 Hz, H-1), 5.55 (1H, m, H-4), 5.40 (1H, dd, $J_{3,4}$ 3.2 Hz; $J_{2,3}$ 11.0 Hz, H-3), 5.05 (1H, dd, $J_{1,2}$ 3.9 Hz $J_{2,3}$ 11.0 Hz, H-2), 4.45 (1H, t, $J_{5,6}$ 6.1 Hz, H-5), 4.20-4.13

(2H, m, C*H*$_2$C≡CH), 3.64-3.56 (2H, m, H-6, H-6'), 2.47 (1H, s, CH$_2$C≡C*H*), 2.15, 2.13, 2.00 (9H, 3s, 3×CH$_3$). This compound was unstable and was used directly in the next step.

2,3,4-Tri-*O*-acetyl-6-*O*-(2-propynyl)-β-D-galactopyranosyl azide 8

A mixture of glycosyl bromide **7** (8.85 g mg, 21.74 mmol), Bu$_4$NHSO$_4$ (7.38 g, 21.74 mmol), NaN$_3$ (4.24 g, 65.2 mmol) and satd aq NaHCO$_3$ solution (90 mL) in CH$_2$Cl$_2$ (90 mL) was vigorously stirred at room temperature for 2 h, after which time TLC indicated complete transformation of the halide [Hex-EtOAc (1:1)]. The mixture was diluted with EtOAc, the organic phase was separated, washed successively with sat. aq NaHCO$_3$, H$_2$O (×2), brine then dried (MgSO$_4$), filtered and concentrated. The residue was purified by flash chromatography (Hexane–EtOAc 3:4) affording compound **8** (4.18 g, mmol, 52%); $[\alpha]_D^{25}$ –22 (*c* 1.0, CHCl$_3$); δ_H (CDCl$_3$, 400 MHz): 5.46 (1H, br d, $J_{3,4}$ 3.4 Hz, H-4), 5.16 (1H, dd, $J_{1,2}$ 8.8 Hz, $J_{2,3}$ 10.2 Hz, H-2), 5.04 (1H, dd, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.4 Hz, H-3), 4.61 (1H, d, $J_{1,2}$ 8.8 Hz, H-1), 4.17 (2H, m, C*H*$_2$C≡CH), 3.97 (1H, dd, $J_{5,6}$=$J_{5,6'}$ 6.1 Hz, H-5), 3.66–3.62 (2H, m, H-6, H-6'), 2.47 (1H, t, *J* 2.4 Hz, CH$_2$C≡C*H*), 2.17, 2.09, 2.00 (9H, 3s, 3xCH$_3$); δ_C (CDCl$_3$, 100 MHz): 170.0 (*C*OCH$_3$), 169.4 (*C*OCH$_3$), 88.2 (C-1), 78.9 (CH$_2$*C*≡CH), 75.1 (CH$_2$C≡*C*H), 74.1 (C-5), 70.7 (C-3), 68.1 (C-2), 67.3(C-6), 67.2 (C-4), 58.6 (*C*H$_2$C≡CH), 20.6, 20.6, 20.5 (CO*C*H$_3$); IR (KBr) ν_{max} 3278, 2937, 2119, 1749, 1371, 1222 cm^{-1}. HRESI MS: *m/z* found 392.1061 [M+Na]$^+$; calcd for $C_{15}H_{19}N_3O_8Na$: 392.10644.

6-*O*-(2-Propynyl)-β-D-galactopyranosyl azide 1

To a solution of compound **13** (279 mg, 0.76 mmol) in anhydrous MeOH (1.5 mL) was added 1M NaOMe until pH 9-10. The mixture was stirred for 1 h at room temperature, neutralized with ion exchange resin (Amberlitet IR 120, H$^+$) until neutral pH, filtered and concentrated under reduced pressure to give glycosyl azide **1** as a white solid (165.5 mg, 90%); $[\alpha]_D^{25}$ –15.5 (*c* 0.6, MeOH); δ_H (D$_2$O, 400 MHz): 4.49 (1H, d, $J_{1,2}$ 8.8 Hz, H-1), 4.11 (2H, t, *J* 2.4 Hz, C*H*$_2$C≡CH), 3.80-3.76 (2H, m, H-4, H-5), 3.65-3.64 (2H, m, H-6; H-6'), 3.52 (1H, dd, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.4 Hz, H-3), 3.36 (1H, dd, $J_{1,2}$ 8.8 Hz, $J_{2,3}$ 9.8 Hz, H-2), 2.76 (1H, t, *J* 2.4 Hz, CH$_2$C≡C*H*); δ_C (D$_2$O, 100 MHz): 91.3 (C-1), 80.0 (CH$_2$*C*≡CH), 77.0 (CH$_2$C≡*C*H), 76.1 (C-5), 73.3 (C-3), 71.0 (C-2), 70.0 (C-6), 69.5 (C-4), 59.0 (*C*H$_2$C≡CH); HRESI MS: *m/z* found 266.0749 [M+Na]$^+$; calcd for $C_9H_{13}N_3O_5Na^+$ [M+Na]$^+$: 266.0747.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-galactopyranose 9[9,10]

To a cold (0 ºC) solution of alcohol **3** (10.7 g, 41.3 mmol) in a mixture of dry acetone (11.5 mL) and anhydrous pyridine (13 mL) was added *p*-TsCl (9.36 g, 49.1 mmol) in portions over 0.5 h. The reaction mixture was stirred for 38 h at room temperature then cooled to 0 ºC and water (4 mL) was added. Stirring the mixture resulted in crystallisation of the product, which was separated by filtration, washed

with water and dried under vaccum. Recrystallization from isopropanol gave compound **9** as a white needles (10.27 g, 62%); m.p: 89-91 ºC; Lit.[9] m.p. 91-92 ºC; $[\alpha]_D^{25}$ –67.0 (*c* 1.0, $CHCl_3$); Lit.[10] $[\alpha]_D^{20}$ –66 ($CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 7.81 (2H, d, *J* 8.3 Hz, Ar), 7.33 (2H, d, *J* 8.0 Hz, Ar), 5.46 (1H, d, $J_{1,2}$ 5.0 Hz, H-1), 4.59 (1H, dd, $J_{3,4}$ 7.8; Hz, $J_{2,3}$ 2.4 Hz, H-3), 4.30 (1H, dd, $J_{1,2}$ 5.0 Hz, $J_{2,3}$ 2.4 Hz, H-2), 4.18-4.22 (2H, m, H-4, H-6), 4.11-4.03 (2H, m, H-6', H-5), 2.44 (3H, s, CH_3Ar), 1.50, 1.35, 1.32, 1.28 (12H, 4s, 4×CH_3); IR (KBr) ν_{max} 2987, 2923, 1369, 1176, 1070 cm^{-1}; ESI-MS: *m/z* 432.1 $[M+NH_3]^+$, 437.1 $[M+Na]^+$.

6-Azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose 10[11]

A suspension of compound **9** (8.8 g, 21 mmol) and NaN_3 (20.47 g, 315 mmol) in DMF (200 mL) was heated (120-130 ºC) for 6 h until complete conversion of starting material, as judged by TLC (hexane-EtOAc 6:1).The solution was allowed to cool, diluted with water (300 mL) and extracted with CH_2Cl_2. The organic phase was washed with water, satd aq $NaHCO_3$ solution and water, dried ($MgSO_4$) and concentrated under reduced pressure to give known compound **10** as syrup (6.1 g, 100%); $[\alpha]_D^{25}$ –95.4 (*c* 1.0, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 5.55 (1H, d, $J_{1,2}$ 5.1 Hz, H-1), 4.63 (1H, dd, $J_{2,3}$ 2.4 Hz, $J_{3,4}$ 7.8 Hz, H-3), 4.34 (1H, dd, $J_{1,2}$ 4.9 Hz, $J_{2,3}$ 2.4 Hz, H-2), 4.19 (1H, dd, $J_{3,4}$ 7.8 Hz, $J_{4,5}$ 1.9 Hz, H-4), 3.92 (1H, ddd, $J_{4,5}$ 1.9 Hz, $J_{5,6}$ 7.8 Hz, $J_{5,6'}$ 5.4 Hz, H-5), 3.51 (1H, dd, $J_{5,6}$ 7.8 Hz, $J_{6,6'}$12.7 Hz, H-6), 3.36 (1H, dd, $J_{5,6'}$ 5.4 Hz, $J_{6,6'}$ 12.7 Hz; H-6'), 1.55, 1.46, 1.34, 1.35 (12H, 4s, 4xCH_3); δ_C ($CDCl_3$, 100 MHz): 109.6, 108.8 ($C(CH_3)_2$), 96.3 (C-1), 71.1 (C-4), 70.8 (C-2), 70.3 (C-3), 66.9 (C-5), 50.6 (C-6), 26.0, 25.9, 24.8, 24.4 (CH_3) IR (KBr) ν_{max}: 3000, 2102, 1382, 1211, 1070 cm^{-1}.

6-Azido-6-deoxy-α-D-galactopyranose 11[11]

A solution of compound **10** (6.15 g, 21.6 mmol) in 80% aqueous TFA (61.5 mL) was stirred until TLC (DCM-methanol 7:3) showed (~2 h) that the reaction was completed and the resulting solution was evaporated several times with *i*-PrOH to give compound **11** as white solid material (3.97 g, 90 %); $[\alpha]_D^{25}$ +23.0 (*c* 1.0, MeOH); δ_H (CD_3OD, 400 MHz): 5.15 (1H, d, $J_{1,2}$ 3.4 Hz, H-1), 4.12 (1H, dd, $J_{5,6}$ 8.0 Hz, $J_{5,6'}$ 4.9 Hz, H-5), 3.79-3.76 (2H, m, H-3, H-4), 3.74 (1H, dd, $J_{1,2}$ 3.4 Hz; $J_{2,3}$ 10.3 Hz, H-2), 3.50 (1H, dd, $J_{5,6}$ 8.0 Hz, $J_{6,6'}$ 12.7 Hz, H-6), 3.31 (1H, dd, $J_{5,6'}$ 4.9 Hz, $J_{6,6'}$ 12.7 Hz, H-6'); δ_C (CD_3OD, 100 MHz): 94.1 (C-1), 71.3 (C-2), 70.9, 70.4 (C-3, C-4), 70.1 (C-5), 52.6 (C-6); ESI-MS: *m/z* 249.9 $[M+HCO_2]^-$.

1,2,3,4-Tetra-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranose 12[12,13]

A solution of the unprotected sugar **11** (3.36 g, 16.38 mmol), anhydrous pyridine (34 mL) and acetic anhydride (17 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated

several times with toluene and the residue was dissolved in CH_2Cl_2. The solution was washed with 1M HCl, and satd aq $NaHCO_3$ solution, dried over Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (EtOAc–hexane 1:1) to give **12** as a pale oil (5.74 g, 94%), $[\alpha]_D^{25}$ +87.8 (*c* 1.0, $CHCl_3$); Lit.[13] $[\alpha]_D$ +97 ($CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 6.41 (1H, s, H-1), 5.48 (1H, br s, H-4), 5.34 (2H, m, H-2, H-3), 4.24 (1H, br dd, $J_{5,6}$ 7.6 Hz, $J_{5,6'}$ 5.4 Hz, H-5), 3.45 (1H, dd, $J_{5,6}$ 7.6 Hz, $J_{6,6'}$ 12.9 Hz, H-6), 3.22 (1H, dd, $J_{5,6'}$ 5.4 Hz, $J_{6,6'}$ 12.9 Hz, H-6′), 2.05, 1.93, 1.91 (12H, 3s, 4×CH_3); δ_C ($CDCl_3$, 100 MHz): 170.0, 169.8, 168.8 (*C*OCH_3), 89.5 (C-1), 70.0 (C-2), 68.0 (C-3), 67.3 (C-4), 66.3 (C-5), 50.2 (C-6), 20.8, 20.5, 20.4 (CO*C*H_3); IR (KBr) ν_{max} 2979, 2106, 1751, 1373, 1224, 1074 cm^{-1}; HRESI MS: *m/z* found 396.1012 $[M+Na]^+$; calcd for $C_{14}H_{19}N_3O_9\ Na^+$: 396.1014.

2-Propynyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranoside 13[14]

To a cold (0 ºC) solution of compound **12** (956 mg, 2.56 mmol) in CH_2Cl_2 (20 mL) was added propargyl alcohol (0.2 mL, 3.07 mmol) and $BF_3{\cdot}Et_2O$ (0.5 mL, 3.84 mmol). The reaction mixture was stirred for 6 h at 52–54 ºC, then more propargyl alcohol (0.2 mL, 3.07 mmol) was added and the mixture was stirred for 20 h at 52–54 ºC before being quenched with K_2CO_3 (480 mg). After stirring for 30 min the solid precipitate was filtered off, washed with CH_2Cl_2, the filtrates were combined, washed with water (×2), the organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (toluene/EtOAc) to yield glycoside **13** (360 mg, 38%); $[\alpha]_D^{25}$ –21.5 (c 1.0, $CHCl_3$); Lit.[14] $[\alpha]_D^{20}$ –32.5 ($CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 5.36 (1H, br d, $J_{3,4}$ 3.4 Hz, H-4), 5.24 (1H, dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.2 Hz, H-2), 5.07 (1H, dd, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.4 Hz, H-3), 4.80 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 4.42 (2H, d, *J* 2.2 Hz, C*H*$_2$C≡CH), 3.89 (1H, dd, $J_{5,6}$ 8.3 Hz, $J_{5,6'}$ 4.1 Hz, H-5), 3.55 (1H, dd, $J_{5,6}$ 8.3 Hz, $J_{6,6'}$ 12.9 Hz, H-6), 3.14 (1H, dd, $J_{5,6}$ 4.1 Hz, $J_{6,6'}$ 12.9 Hz, H-6'), 2.49 (1H, t, *J* 2.2 Hz, $CH_2C{\equiv}CH$), 2.18 (3H, s, CH_3), 2.15 (3H, s, CH_3), 1.99 (3H, s, CH_3); δ_C ($CDCl_3$, 100 MHz): 170.2, 169.5, 169.4 (*C*OCH_3), 98.5 (C-1), 78.0 ($CH_2C{\equiv}CH$), 75.5, 73.1, 71.5, 68.4, 67.9 (C-2, C-3, C-4, C-5, $CH_2C{\equiv}CH$), 55.9 ($CH_2C{\equiv}CH$), 51.0 (C-6), 20.7, 20.6, 20.5 (CO*C*H_3); IR (KBr) ν_{max} 3278, 2104, 1749, 1222, 1072 cm^{-1}; HRESI MS: *m/z* found 392.10616 $[M+Na]^+$; calcd for $C_{15}H_{19}N_3O_8\ Na^+$: 392.10644.

2-Propynyl 6-azido-6-deoxy-β-D-galactopyranoside 2

To a solution of compound **13** (265 mg, 0.71 mmol) in anhydrous MeOH (1.5 mL) was added 1M NaOMe until pH 9-10 was achieved. The mixture was stirred for 1 h at room temperature, neutralized with ion exchange resin (Amberlite IR-120, H^+), filtered and concentrated under reduced pressure. The product **2** was obtained as a white solid (150 mg, 86%); $[\alpha]_D^{25}$ –37.5 (*c* 0.24, MeOH); δ_H (D_2O, 400 MHz): 4.44 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 4.37 (2H, dd, *J* 2.0 Hz, *J* 6.8 Hz, *C*$H_2C{\equiv}CH$), 3.71 (1H, d, $J_{3,4}$ 2.6

Hz, H-4), 3.69 (1H, dd, $J_{5,6}$ 9.2 Hz, $J_{5,6'}$ 3.6 Hz; H-5), 3.64 (1H, dd, $J_{5,6}$ 9.2 Hz; $J_{6,6'}$ 12.2 Hz, H-6), 3.59-3.47 (2H, m, H-2, H-3), 3.17 (1H, dd, $J_{5,6'}$ 3.6 Hz; $J_{6,6'}$ 12.2 Hz, H-6'), 2.83 (1H, t, *J* 2.2 Hz, $CH_2C{\equiv}CH$); δ_C (D_2O, 100 MHz): 101.8 (C-1), 78.0 ($CH_2C{\equiv}CH$), 77.2 ($CH_2C{\equiv}CH$), 75.0 (C-5), 73.4 (C-3), 71.2 (C-2), 69.8 (C-4), 57.4 ($CH_2C{\equiv}CH$), 51.6 (C-6); HRESI MS: *m/z* found 266.0747 $[M+Na]^+$; calcd for $C_9H_{13}N_3O_5\ Na^+$: 266.0747.

Table S1 Reaction conditions and product composition for CuAAC and thermal 1,3-dipolar cycloaddition applied for macrocyclization of monomers **1** and **2**

Entry	Monomer (concentration)[a]	Catalytic system	MW Temperature/Time	Obtained glycomacrocycles[b]
1	**1** (0.17 M)	$CuSO_4$-Cu turnings	100 ºC/15 min	**14**, **15** and **16**
2	**1** (1.00 M)	$CuSO_4$-Cu turnings	100 ºC/15 min	**14**, **15**, **16** and **17**
3	**1** (1.00 M)	$CuSO_4$-sodium ascorbate	100 ºC/30 min	**14**, **15**, **16** and **17**
4	**1** (1.00 M)	Cu wire	120/90 min then 150 ºC/120 min	**14**, **15** and **16**
5	**1** (1.00 M)	no catalyst	120/60 min then 150 ºC/120 min	**14**, **15** and **16**
6	**2** (0.18 M)	$CuSO_4$-Cu turnings	100 ºC/15 min	**18** and **19**
7	**2** (1.00 M)	$CuSO_4$-Cu turnings	100 ºC/15 min	**18** and **19**
8	**2** (1.00 M)	$CuSO_4$-sodium ascorbate	100 ºC/ 30 min	**18** and **19**
9	**2** (1.00 M)	Cu wire	120/90 min then 150 ºC/120 min	**18** and **19**
10	**2** (1.00 M)	No catalyst	120/60 min then 150 ºC/120 min	**18** and **19**

[a]All reactions were conducted in DMF as a solvent. [b]Products formation was monitored by TLC (CH_3CN–EtOAc–iProOH–H_2O 85:20:50:50) and confirmed by HPLC (RP C18 column: 10×250 mm, Jupiter, Phenomenex; gradient elution: 0.1% aq TFA (A) – CH_3CN (B), 0% to 35% B in 40 min).

Table S2 Retention times and ESI MS data for sialylation products of cyclic dimers **14** and **18** and cyclic trimers **15** and **19**

Starting material	Retention time[a] (min)	Product	Retention time[a] (min)	*m/z* Found[b]	*m/z* Calculated (Mol. Formula)
14	12.33	**20**	12.58	533.5 $[M–2H]^{2–}$ 1067.4 $[M–H]^{–}$ 1089.4 $[M–2H+Na]^{–}$	1066.4 ($C_{40}H_{58}N_8O_{26}^{2–}$) 1067.4 ($C_{40}H_{58}N_9O_{26}^{–}$) 1089.3 ($C_{40}H_{58}N_8NaO_{26}^{–}$)
15	13.68	**21**	12.62	800.5 $[M–2H]^{2–}$	1600.5 ($C_{60}H_{88}N_{12}O_{39}^{2–}$)
18	12.28	**22**	12.34	533.4 $[M–2H]^{2–}$ 1067.3 $[M–H]^{–}$	1066.4 ($C_{40}H_{58}N_8O_{26}^{2–}$) 1067.4 ($C_{40}H_{58}N_9O_{26}^{–}$)
19	13.70	**23**	12.61	800.5 $[M–2H]^{2–}$	1600.5 ($C_{60}H_{88}N_{12}O_{39}^{2–}$)

[a] Analytical HPLC was performed on Phenomenex Jupiter HPLC column (C18, 250×3 mm) with 0.1% TFA in H_2O–MeCN (gradient elution from 0 to 27% MeCN in 40 min) using Dionex HPLC system equipped with variable wavelength UV detector (230 nm). [b] All data are obtained in ESI MS negative mode.

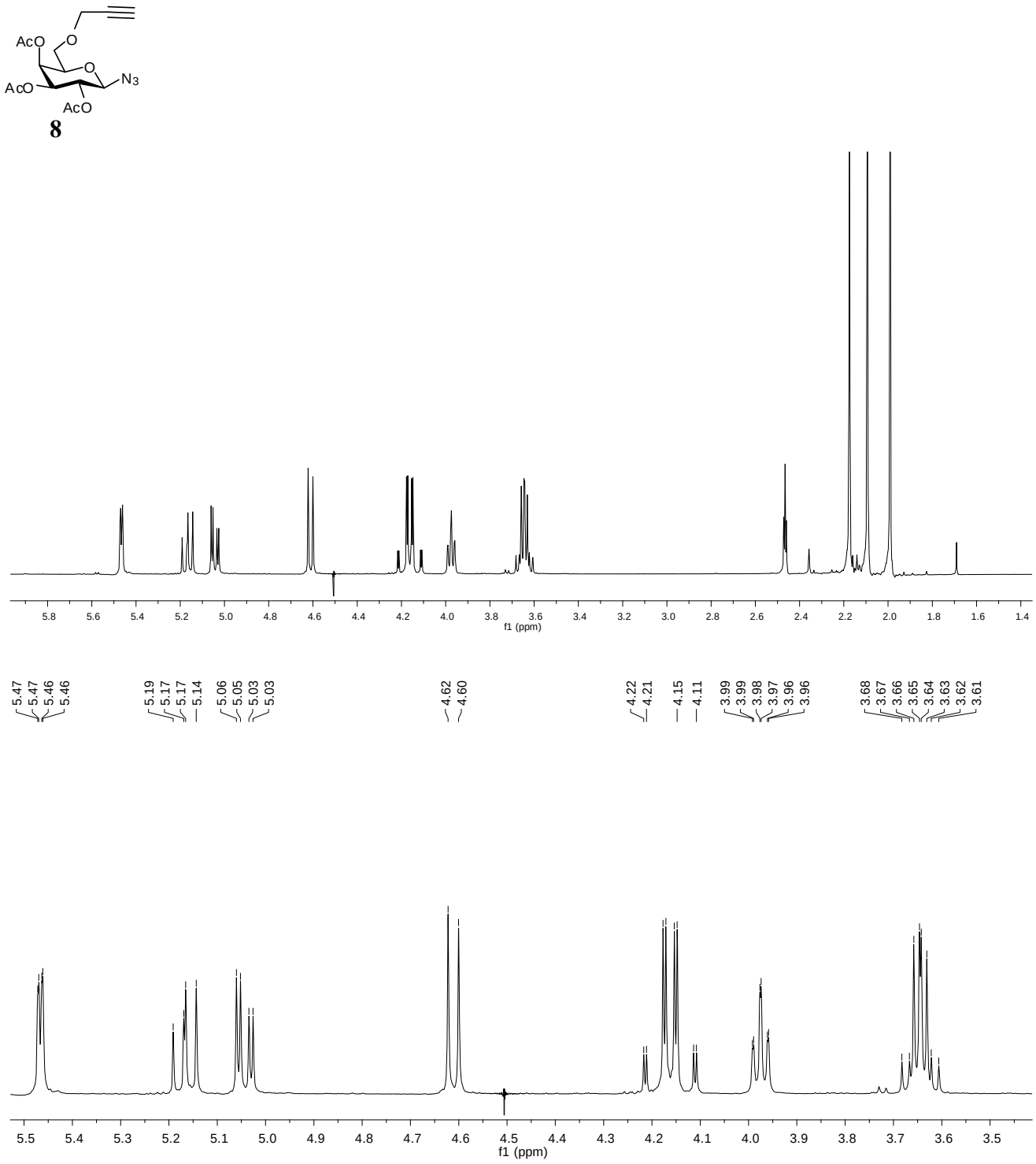
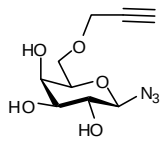


Fig. S1 ^{1}H NMR spectrum of compound **8**.



1

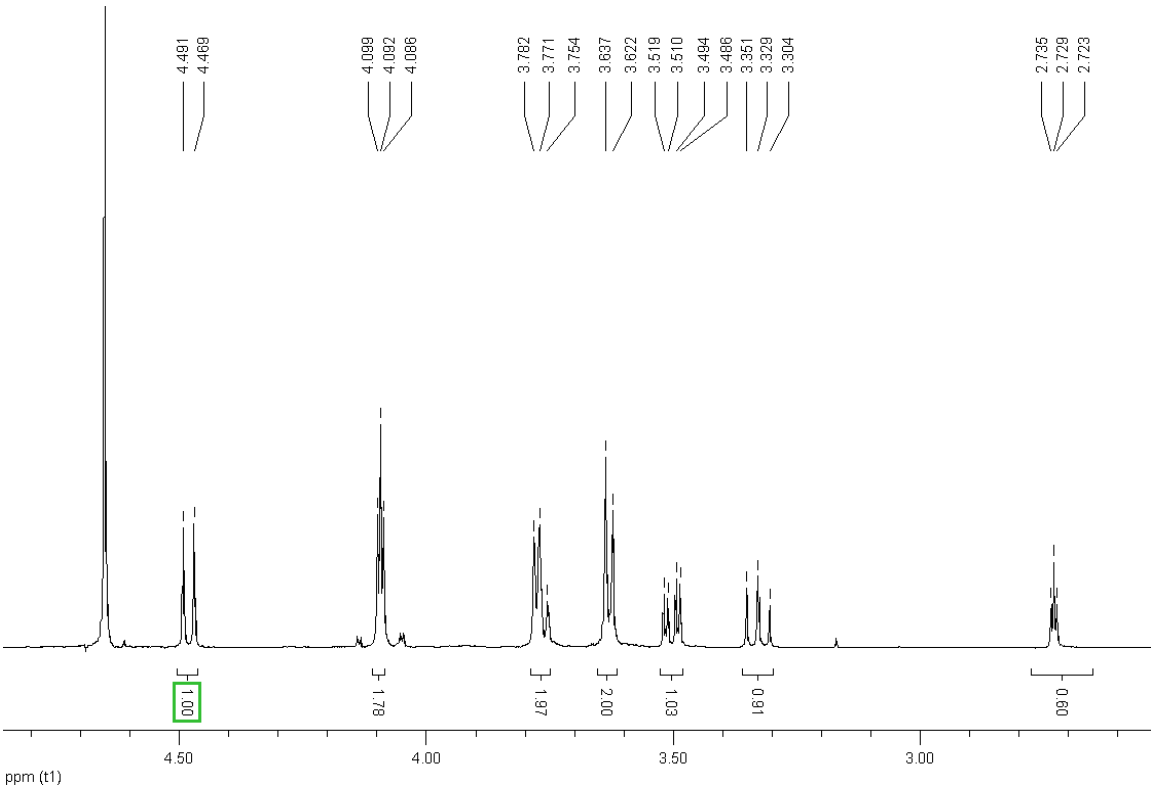
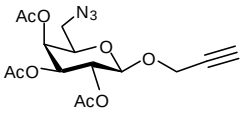
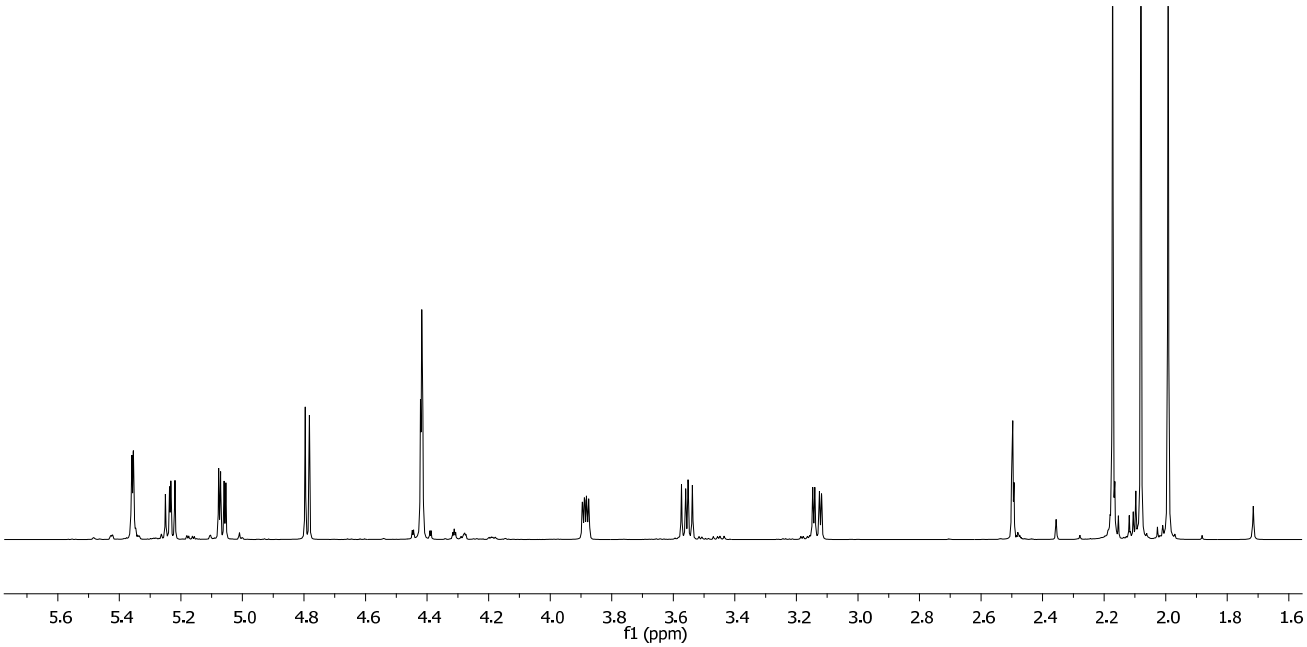


Fig. S2 ^{1}H NMR spectrum of compound **1**.



13



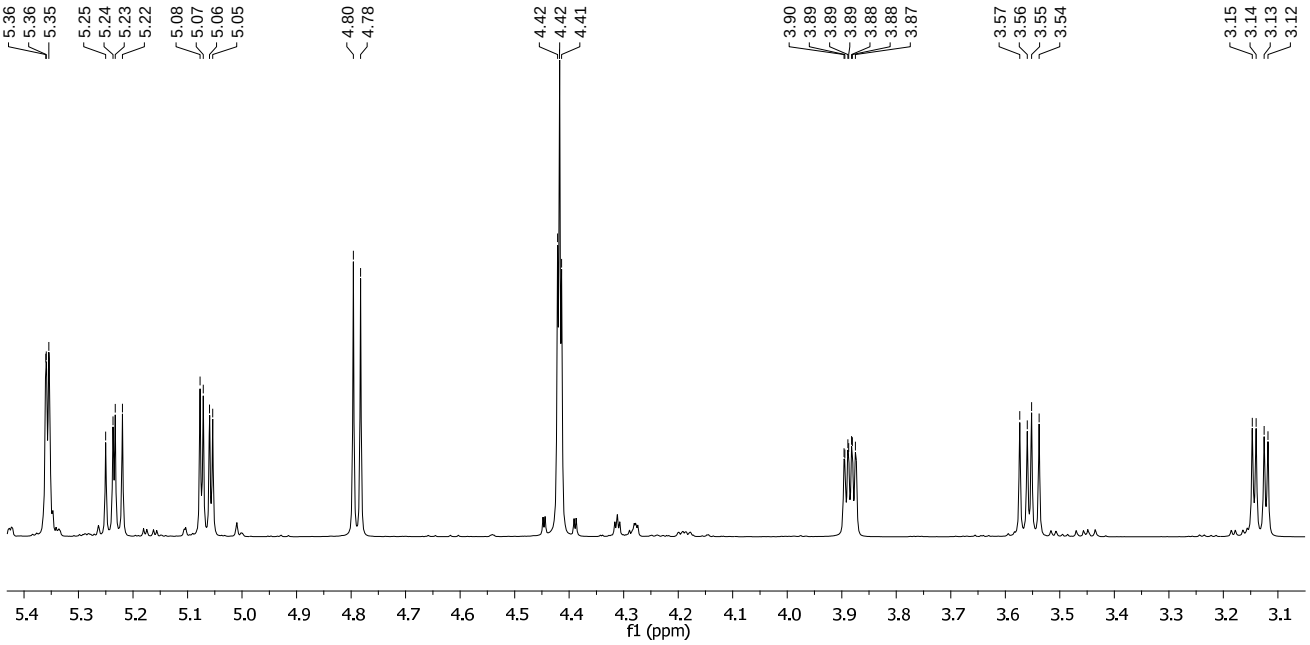
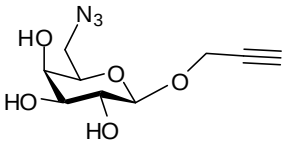


Fig. S3 ^{1}H NMR spectrum of compound **13**.



2

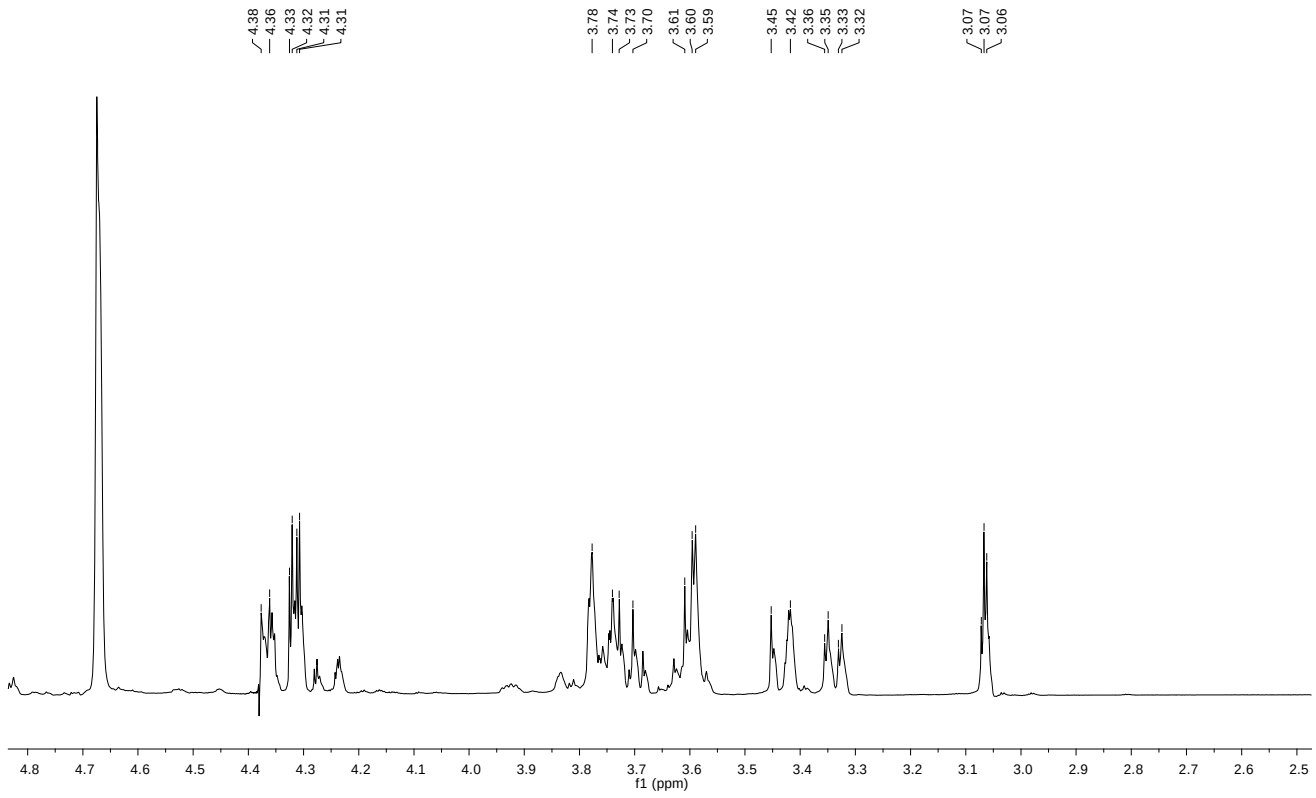


Fig. S4 ^{1}H NMR spectrum of compound **2**.

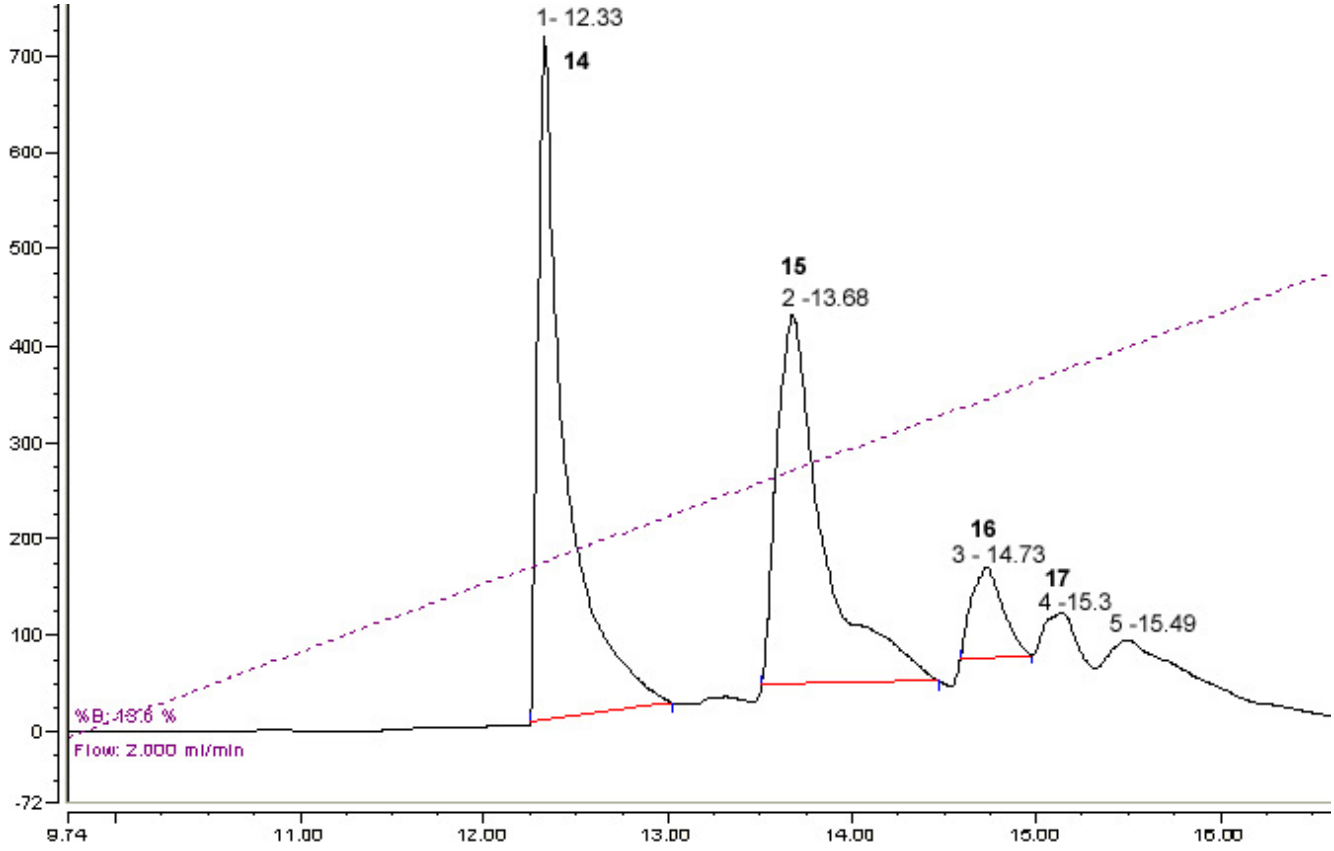


Fig. S5 HPLC trace for the separation of the cyclooligomerization products of building block **1**.

The elution profile of the products of CuAAC reaction of monosaccharide monomer **1** (c=0.17 M) obtained from Phenomenex Jupiter HPLC column (C18, 250×10 mm) with 0.1% TFA in H_2O–MeCN (gradient elution from 0 to 27% MeCN in 40 min) using Dionex HPLC system equipped with variable wavelength UV detector (230 nm). Peaks corresponding to macrocyclic compounds **14** (peak 1, 12.33 min), **15** (peak 2, 13.68 min) and **16** (peak 3, 14.73 min) were collected and analyzed by ESI MS and ^{1}H NMR. Peak 4 at 15.13 min and peak 5 at 15.49 min were analyzed by ESI-MS only since combined fractions accounted for less than 1 mg in each case.

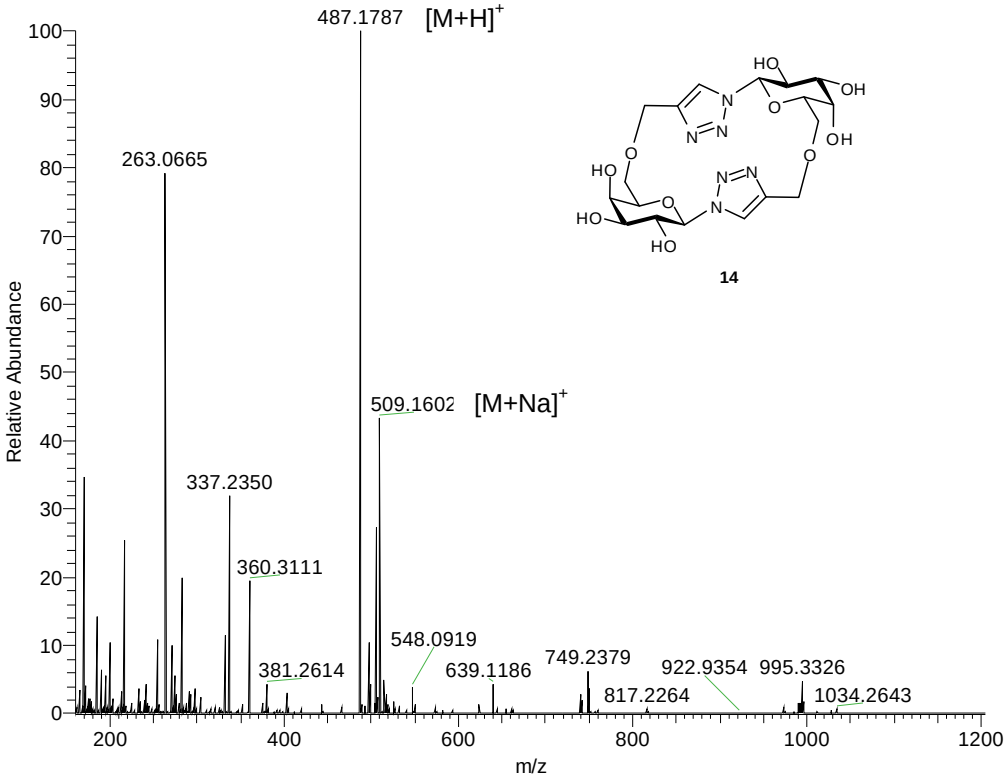


Fig. S6 ESI MS Analysis of cyclic dimer **14**.

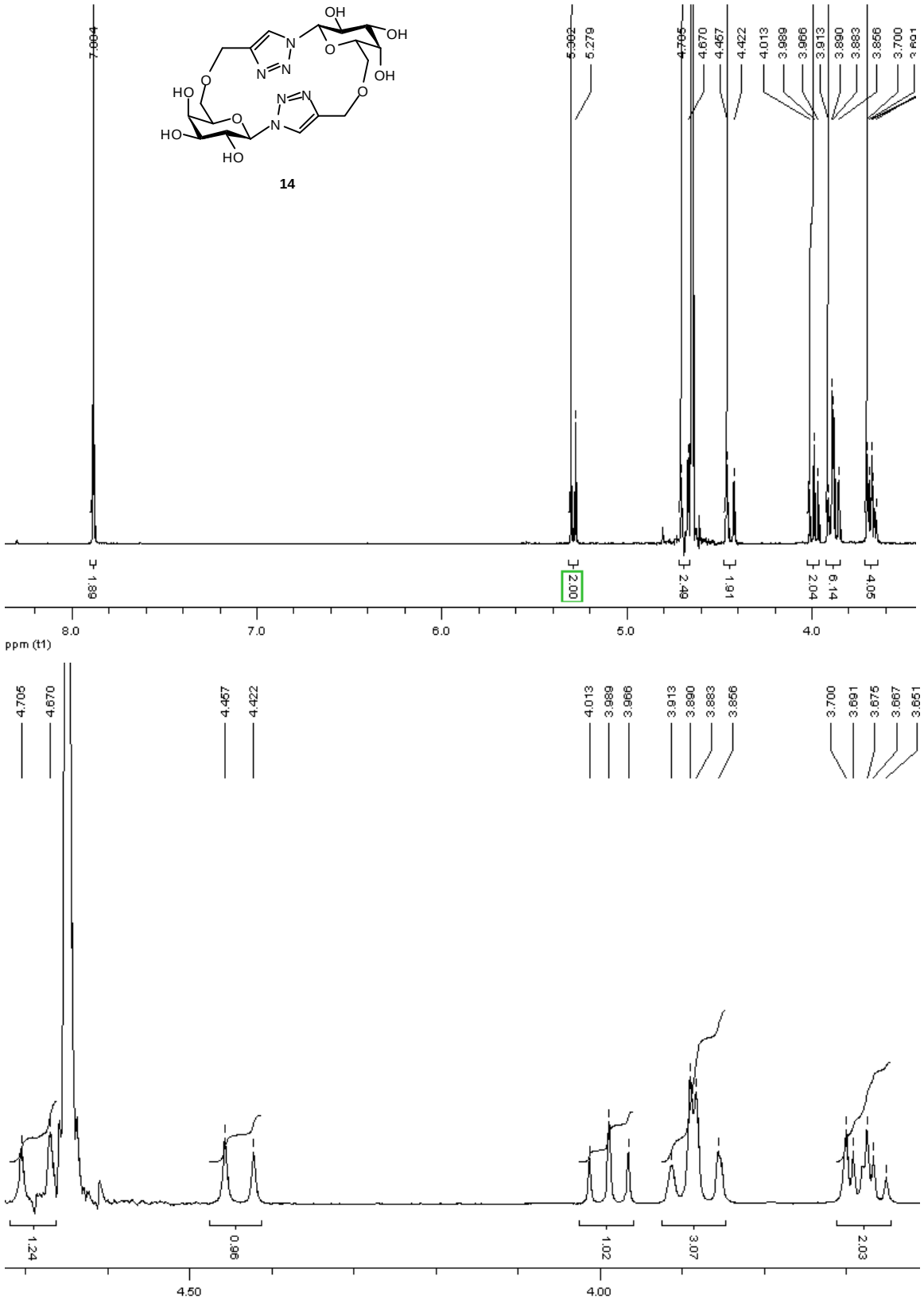


Fig. S7 ^{1}H NMR Spectrum of cyclic dimer **14**.

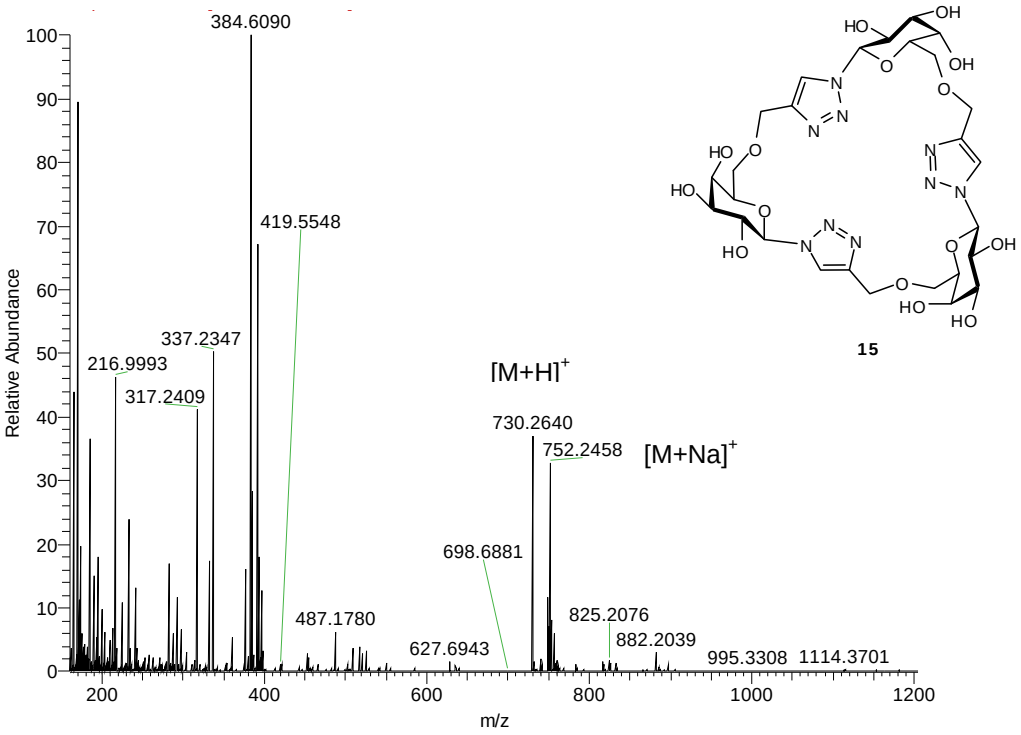
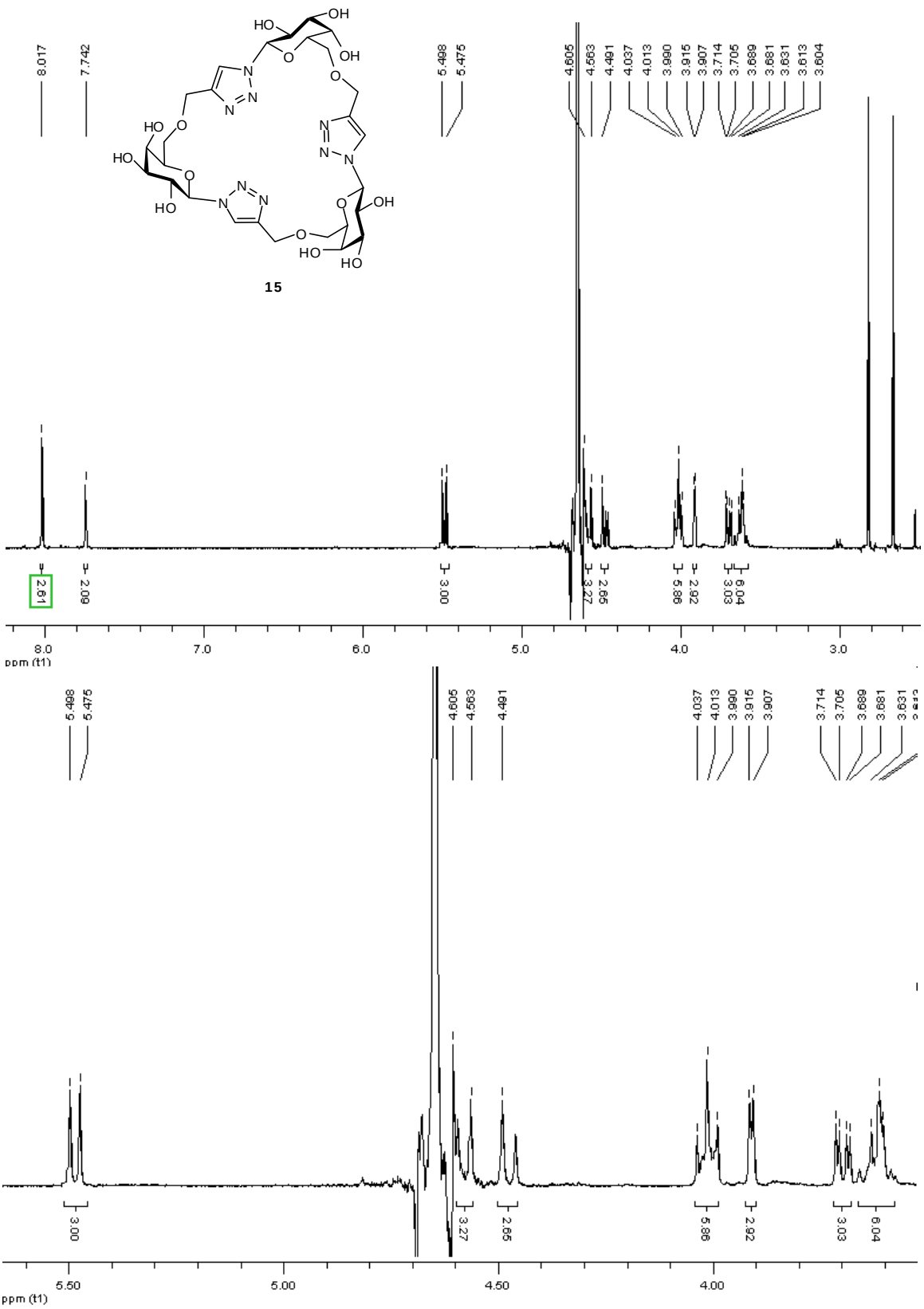


Fig. S8 ESI MS Analysis of cyclic trimer **15**.



15

Fig. S9 ^{1}H NMR Spectrum of cyclic trimer **15**.

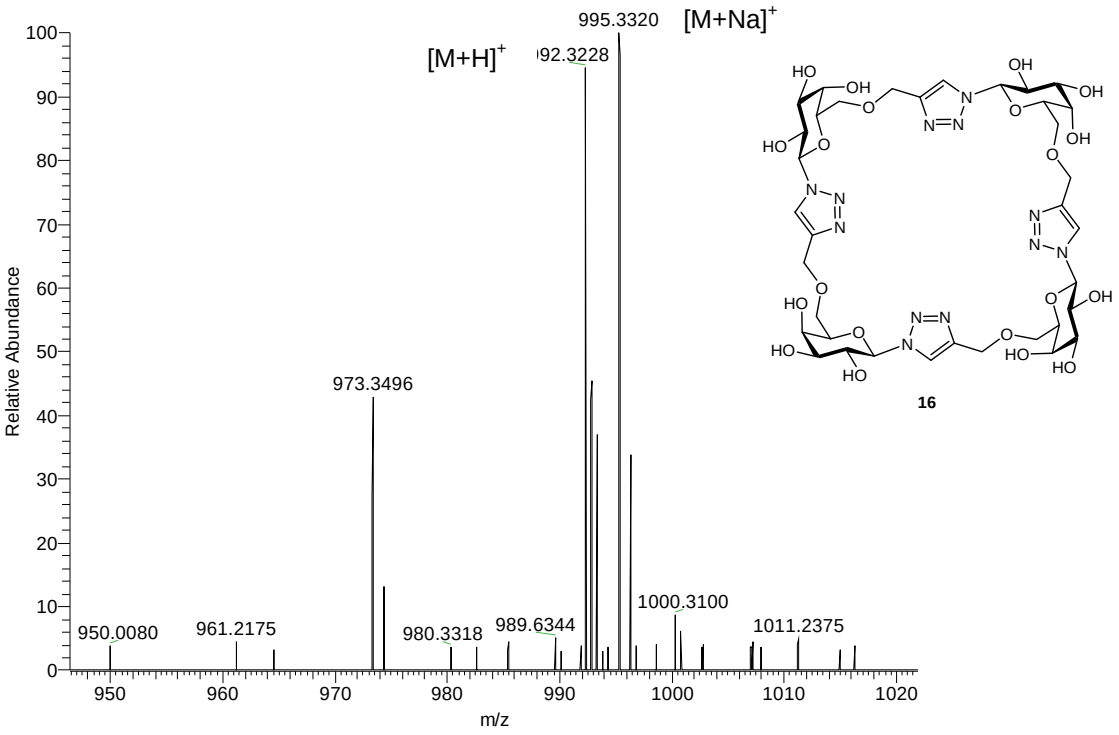
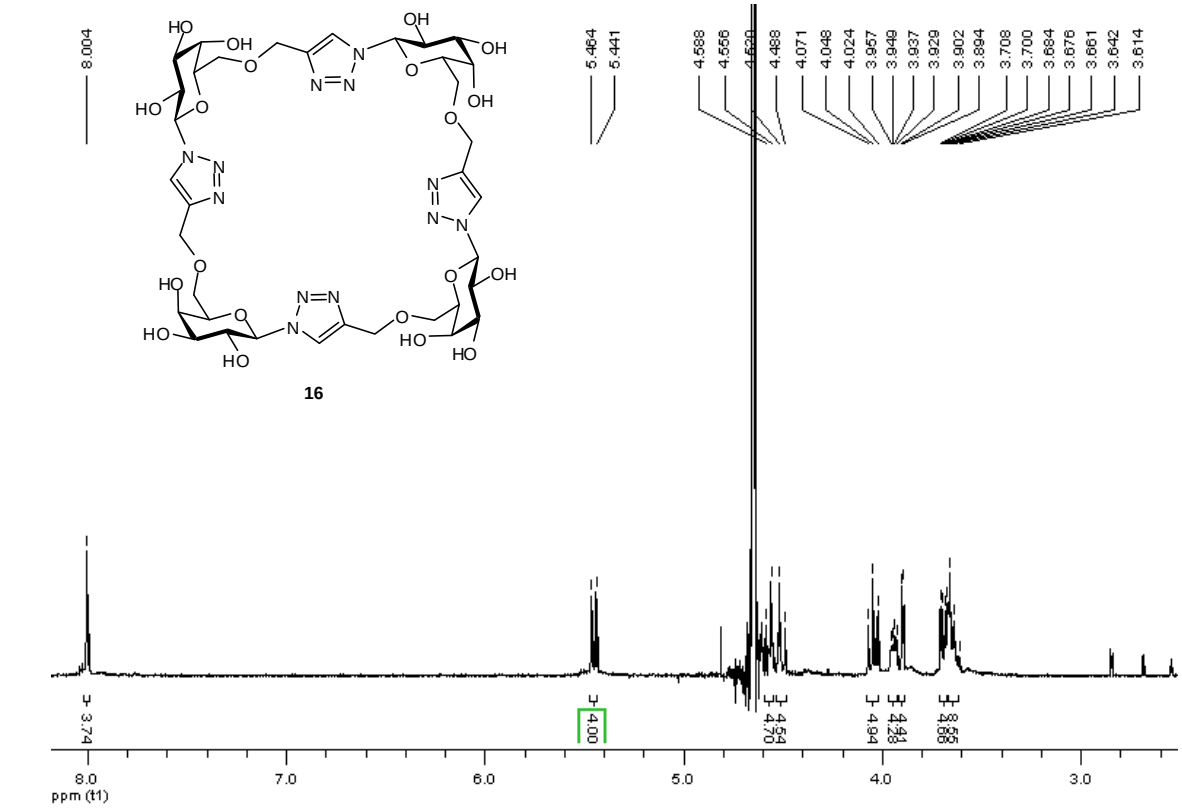


Fig. S10 ESI MS Analysis of cyclic tetramer **16**.



16

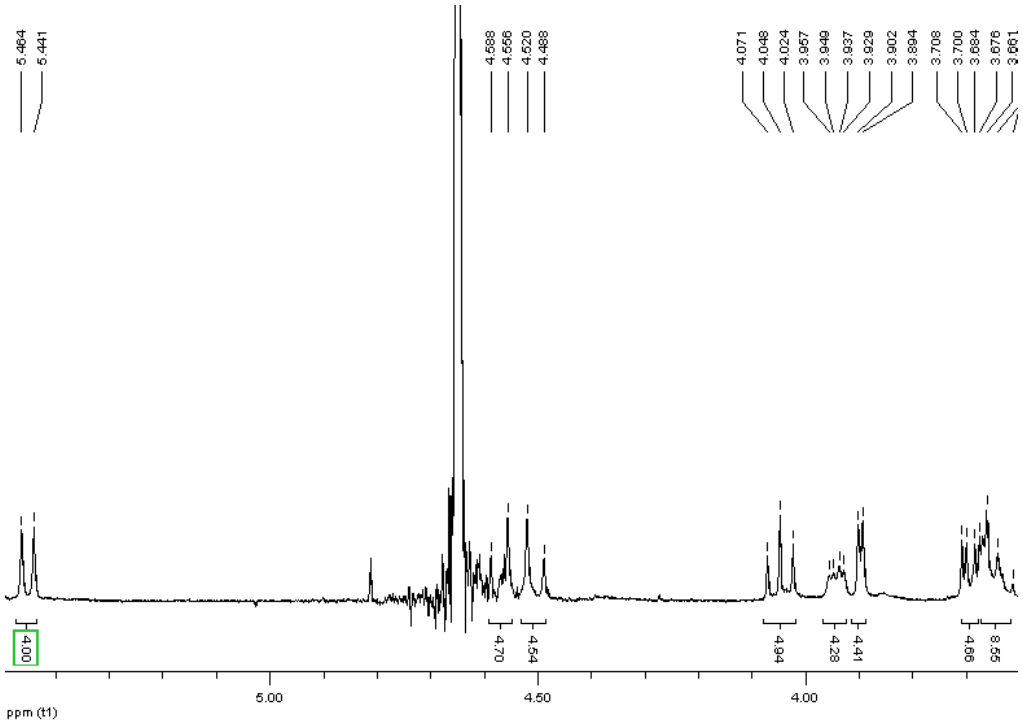


Fig. S11 ^{1}H NMR Spectrum of cyclic tetramer **16**.

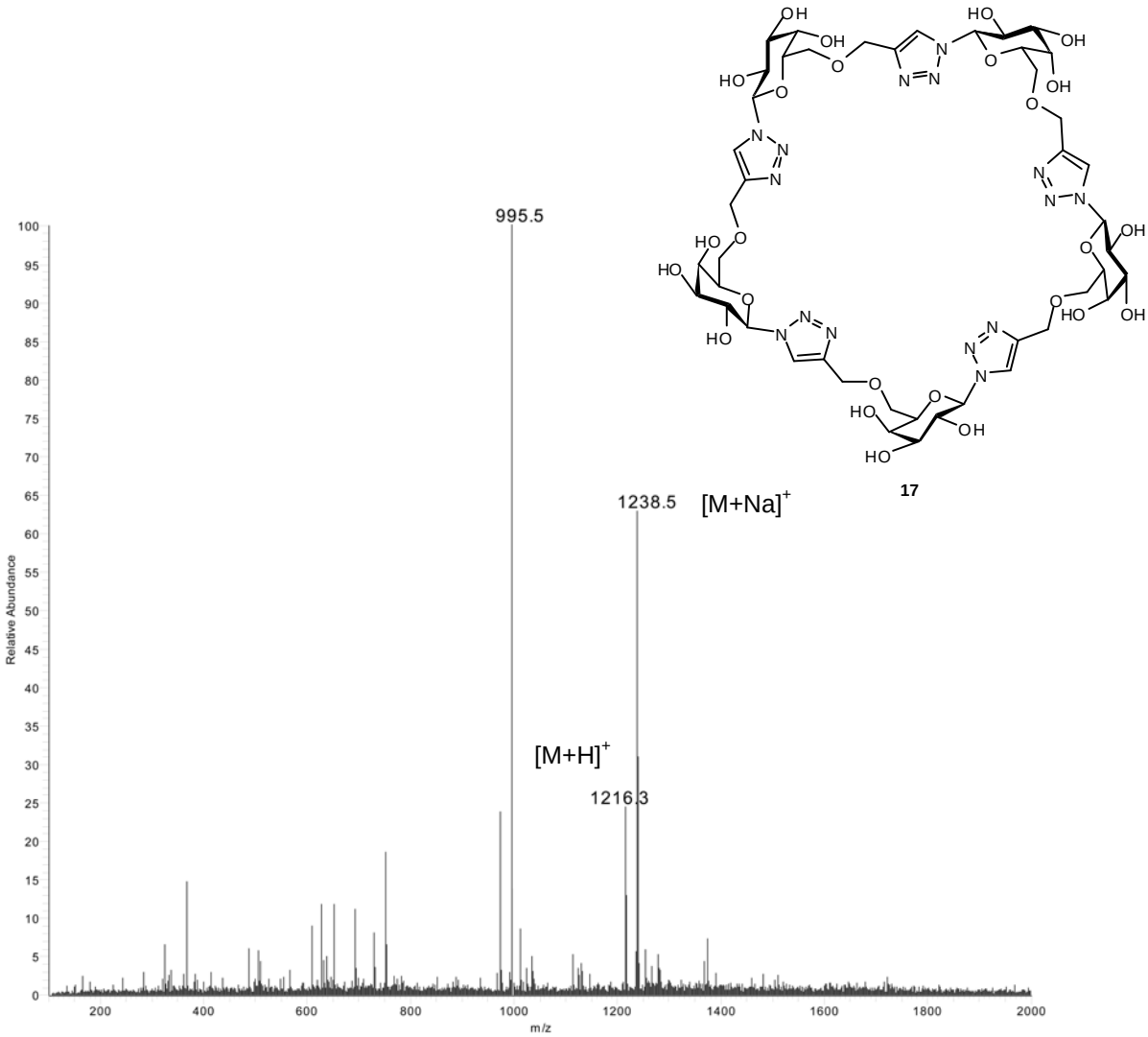


Fig. S12 ESI MS Analysis of the cyclic pentamer **17**.

Compound **17** was isolated as fractions corresponding to peak 4 (retention time 15.13 min, see HPLC trace in Fig. S5) from the products of cyclooligomerization of building block **1.** Peaks *m/z* 1216.3 $[M+Na]^+$ and 1238.5 $[M+K]^+$ can be tentatively assigned to cyclic pentamer **17**. Peak *m/z* 995.3 corresponds to compound **16** is present in the HPLC fractions due to incomplete separation.

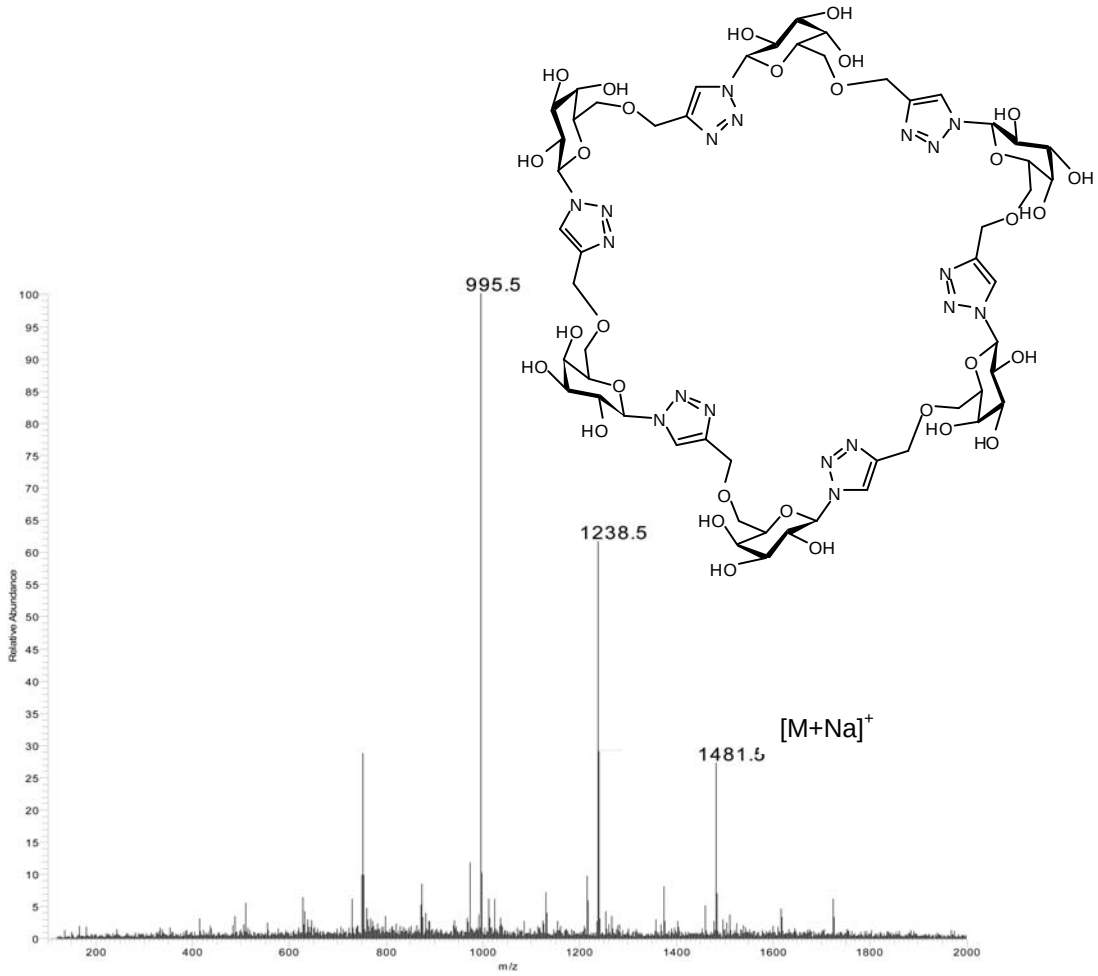


Fig. S13 ESI MS Analysis of the cyclic hexamer from building block **1**.

The cyclic hexamer was found in the mixture of cyclooligomerization products of building block **1** and isolated as peak 5 (retention time 15.49 min, see HPLC trace in Fig S5). The peak at *m/z* 1481.5 can be tentatively assigned to hexamer formed as a result of cyclooligopolimerisation of building block **1**. Peaks 995.5 $[M+Na]^+$ and 1216.3 $[M+Na]^+$can be assigned to a tetramer and pentamer, respectively, which are present in the HPLC fractions due to incomplete separation.

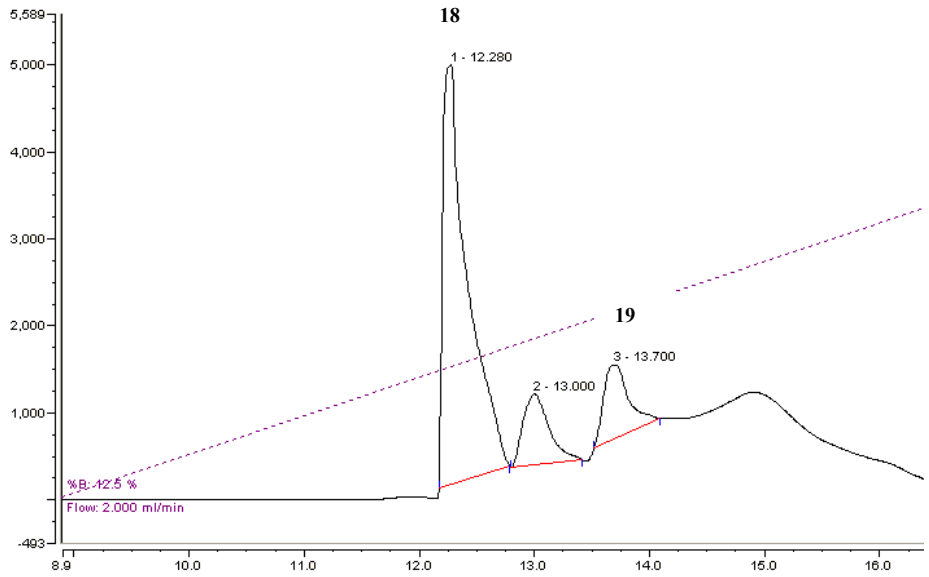


Fig. S14 HPLC trace for the separation of the cyclooligomerization products of building block **2**.

The elution profile of the products of CuAAC reaction of monosaccharide monomer **2** obtained from Phenomenex Jupiter HPLC column (C18, 250×10 mm) with 0.1% TFA in H_2O–MeCN (gradient elution from 0 to 27% MeCN in 40 min) using Dionex HPLC system equipped with variable wavelength UV detector (230 nm). Peaks 1 (12.28 min) and 3 (13.70 min) correspond to macrocyclic compounds **18** and **19** whose structures were confirmed by ESI MS and ^{1}H MNR. Fractions corresponding to peak 2 (13.00 min) accounted for less than 1% and were analyzed by ESI MS (see page S30).

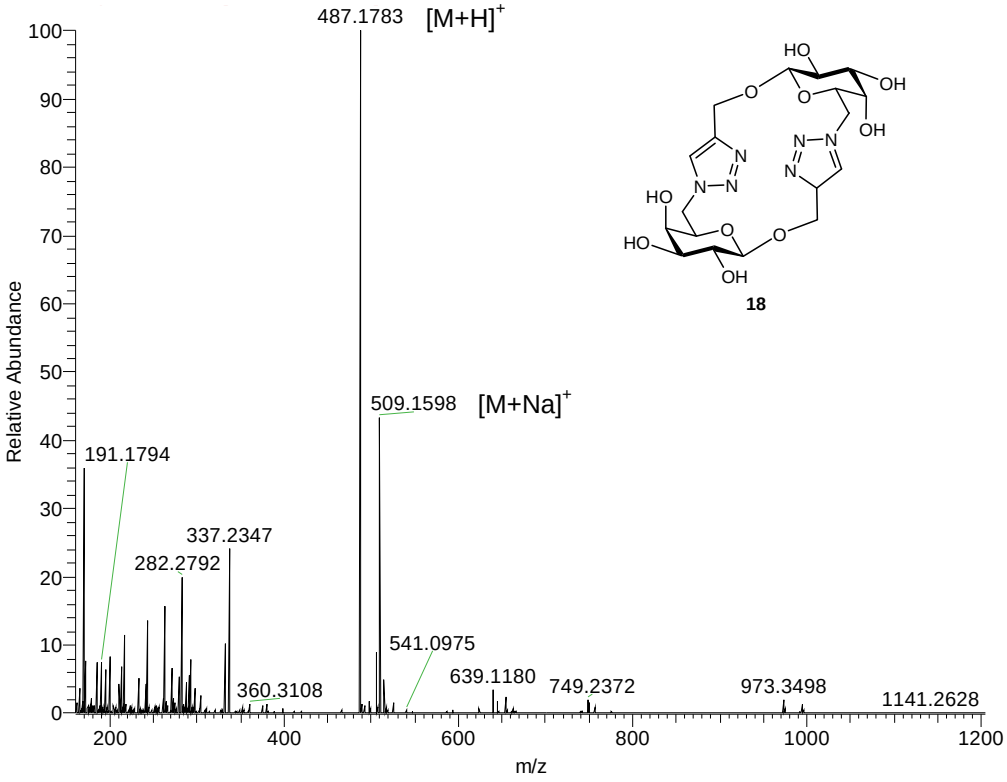


Fig. S15 ESI MS Analysis of **18**.

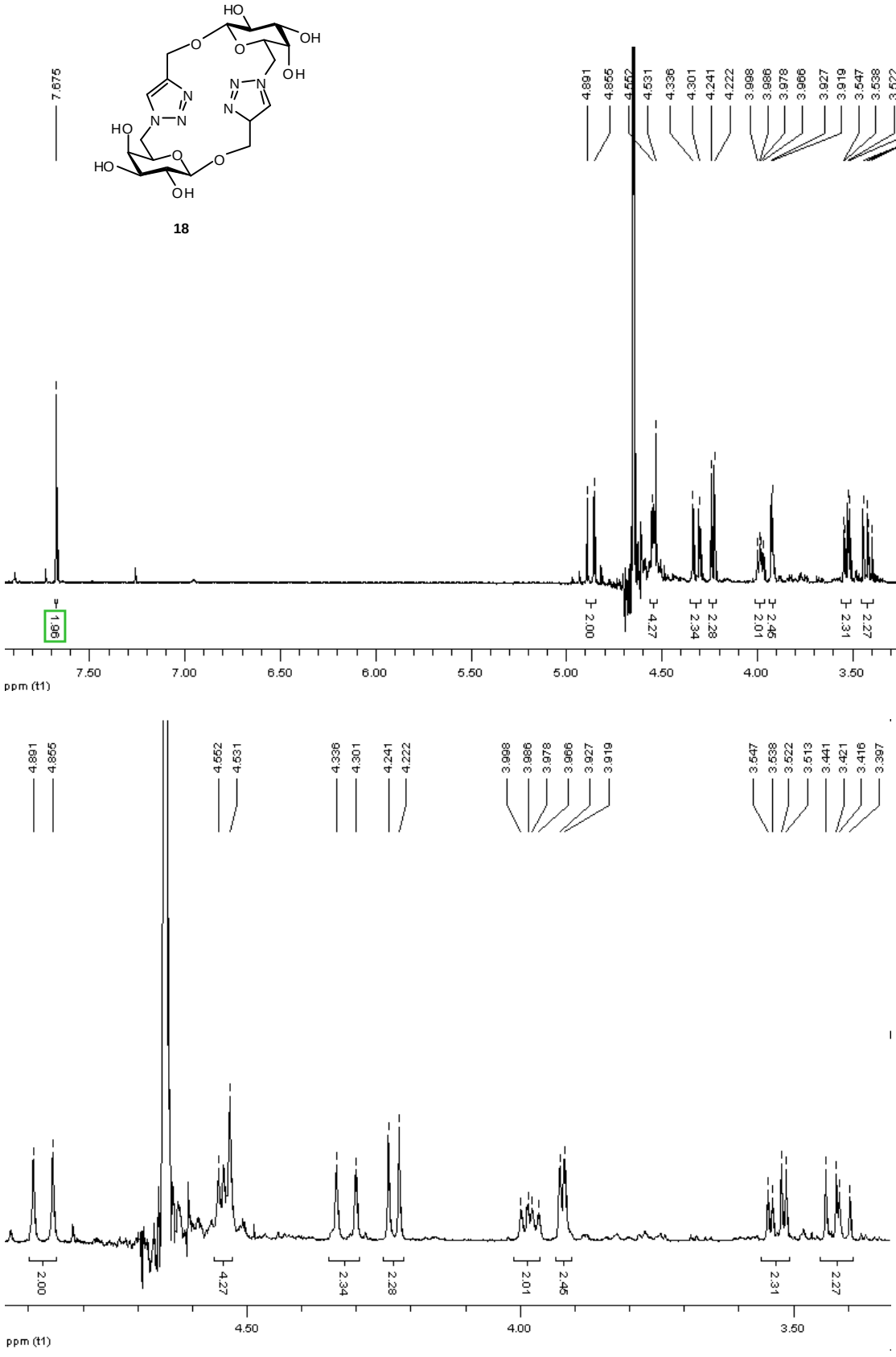


Fig. S16 ^{1}H NMR Spectrum of cyclic dimer **18**.

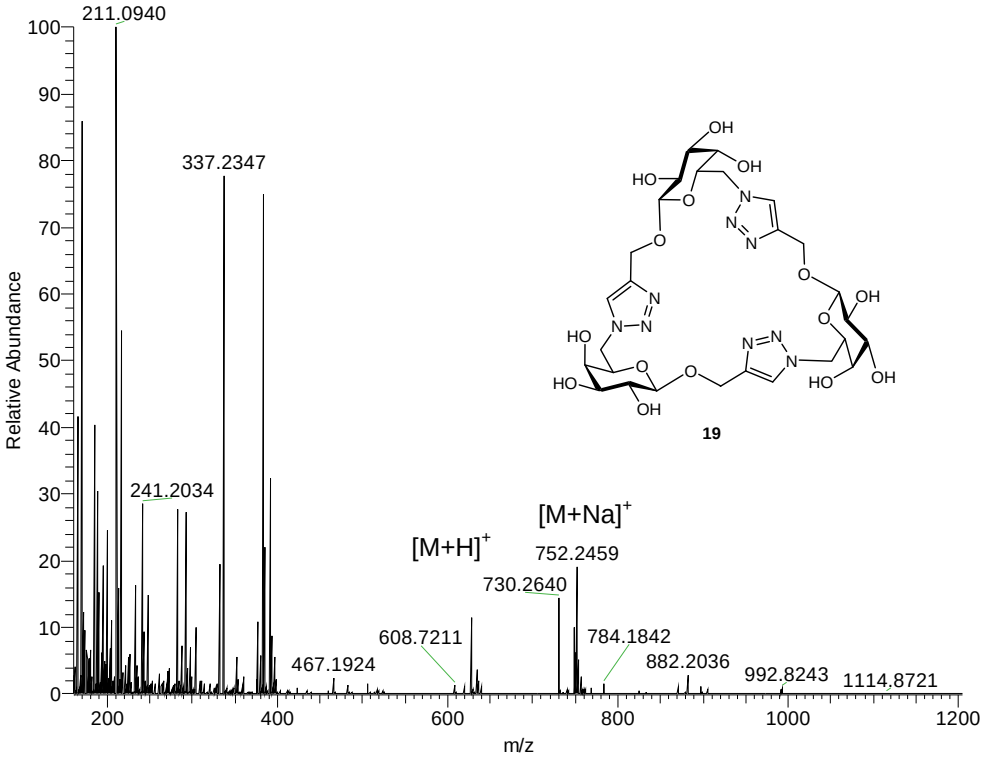


Fig. S17 ESI MS Analysis of cyclic trimer **19**.

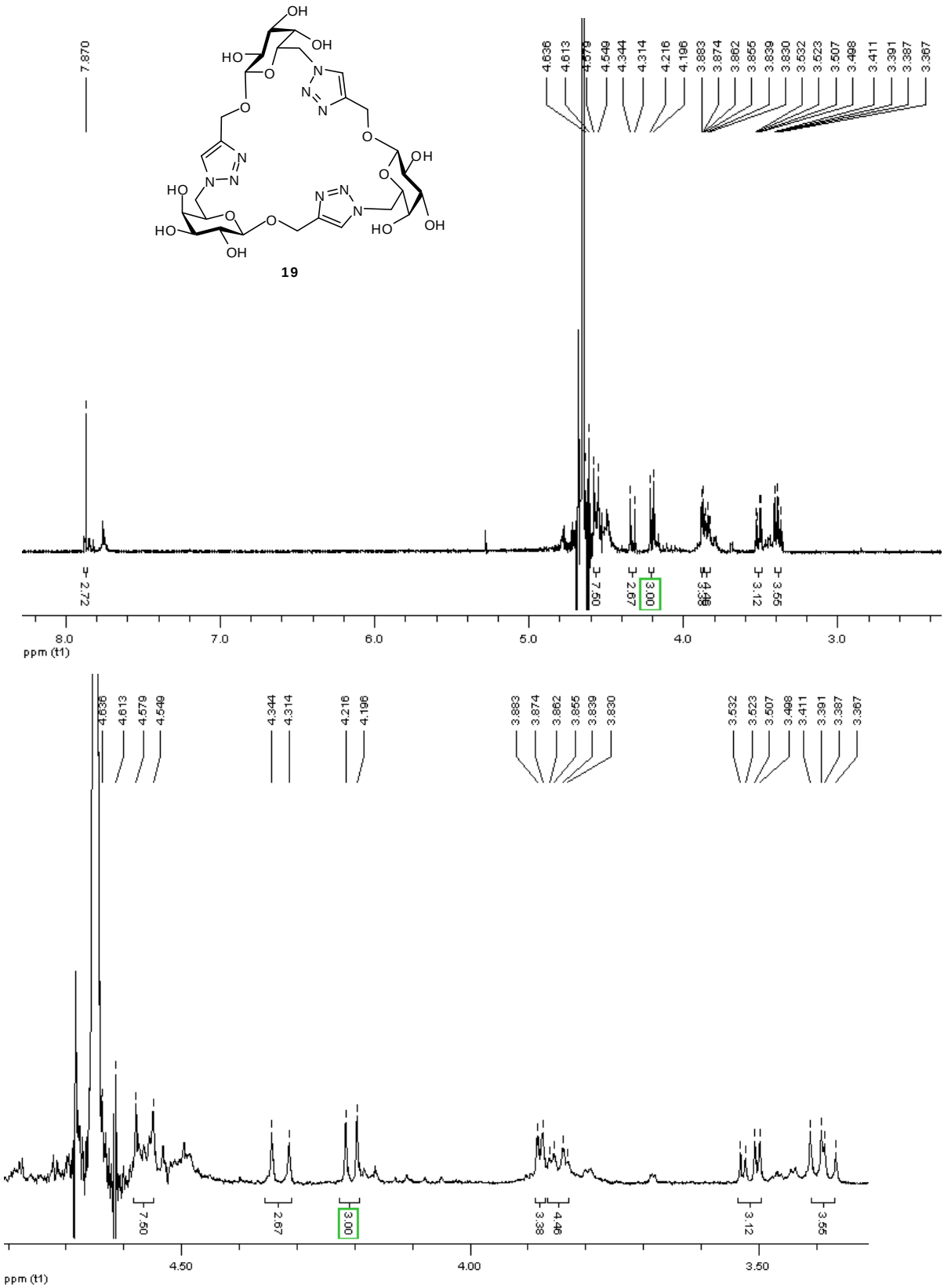


Fig. S18 ^{1}H NMR Spectrum of cyclic trimer **19**.

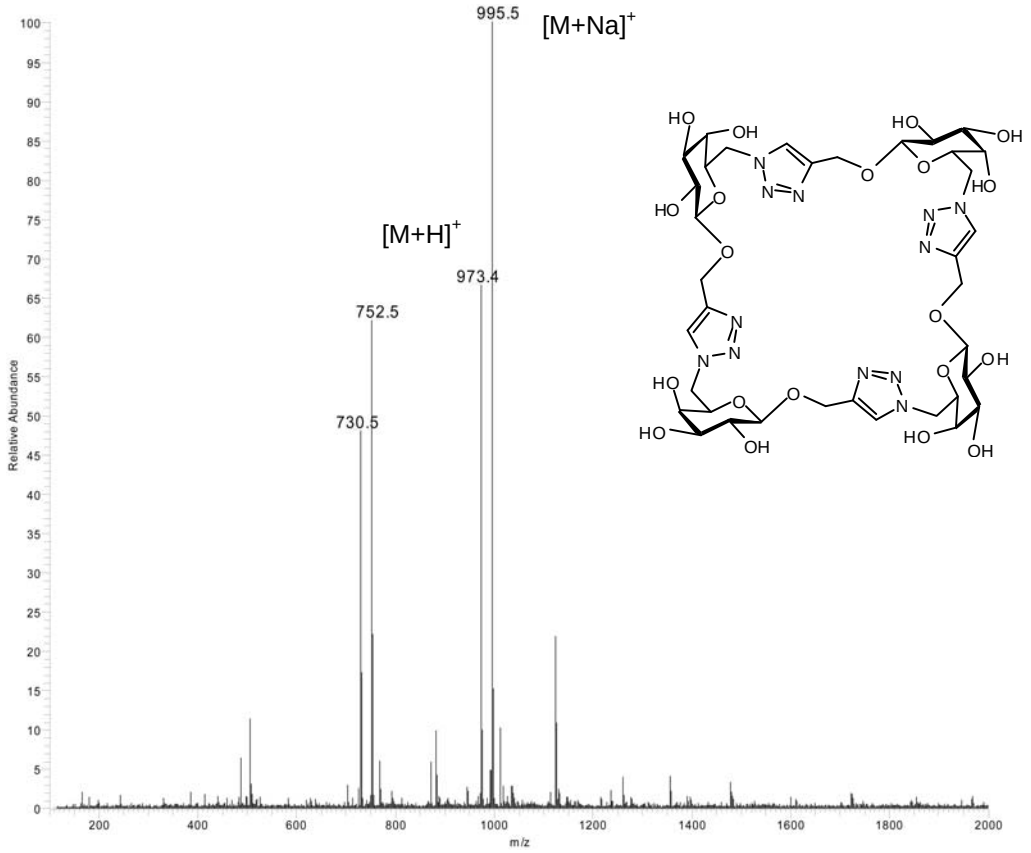


Fig. S19 ESI MS Analysis of the tentative cyclic tetramer obtained by cyclooligomerization of building block **2**

A product isolated by HPLC as peak 2 (retention time 13.00 min, see HPLC trace in Fig S15) was analyzed by ESI MS to show peak *m/z* 995.5 which can be tentatively assigned to Na^+ adduct of a tetramer formed as a result of cyclooligomerisation of building block **2**. Peak 752.5 $[M+Na]^+$ corresponds to trimer **19** which may present as a result of the incomplete HPLC separation.

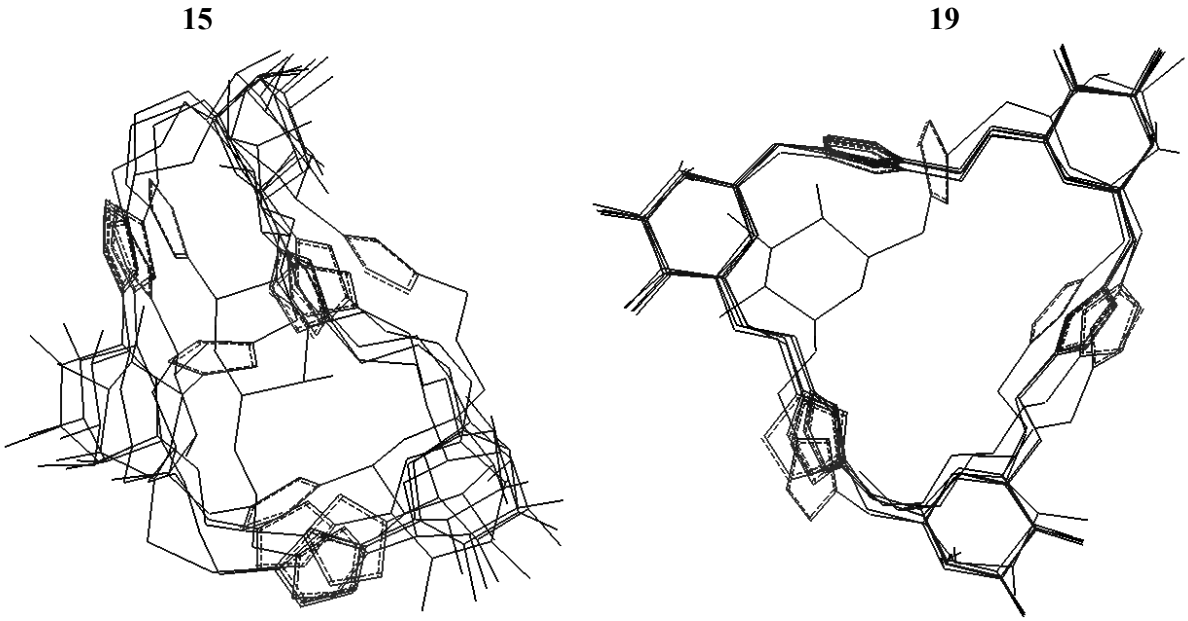


Fig S20 Molecular modeling of cyclic trimers **15** and **19**.

Superimposed structures of six lowest energy conformations for cyclic trimers **15** and **19** obtained by conformational searches carried out in MMFF molecular mechanics model implemented in the Spartan ’06 software.

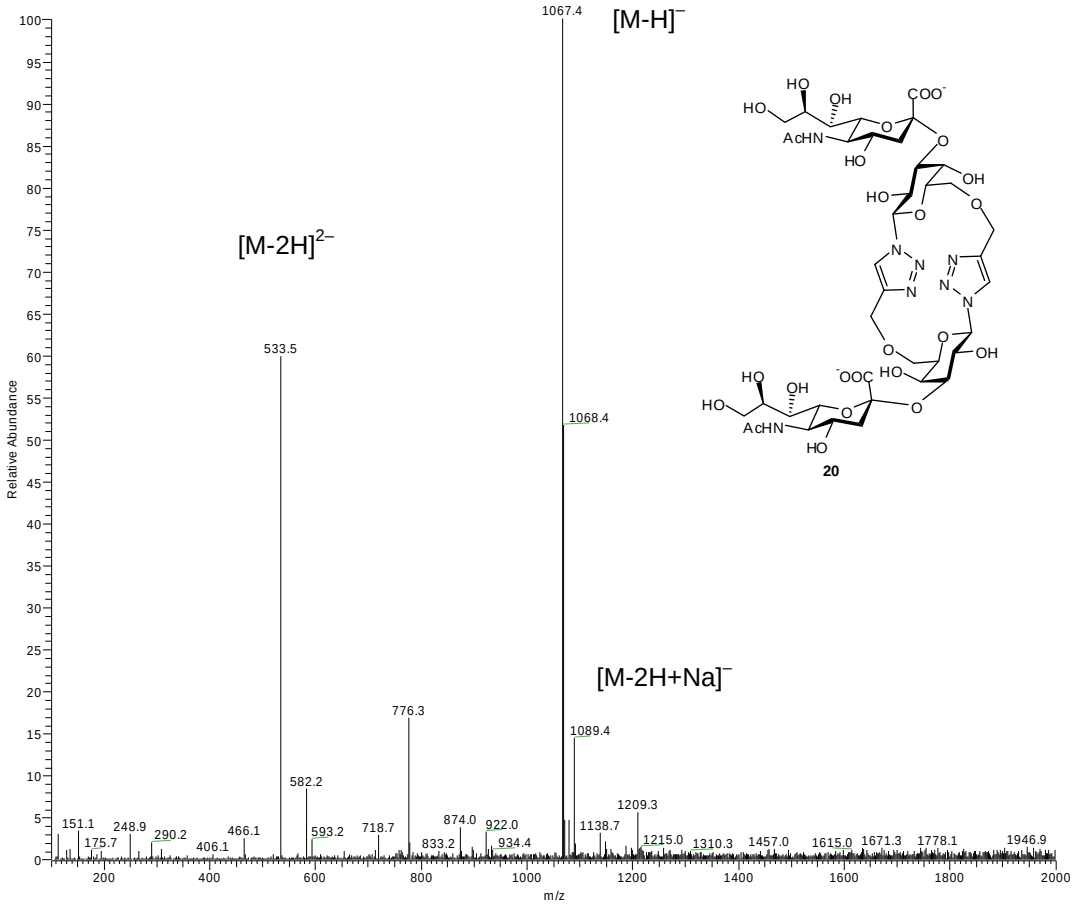


Fig S21 ESI MS Analysis of enzymatically sialylated cyclic pseudooligosachharides **20**.

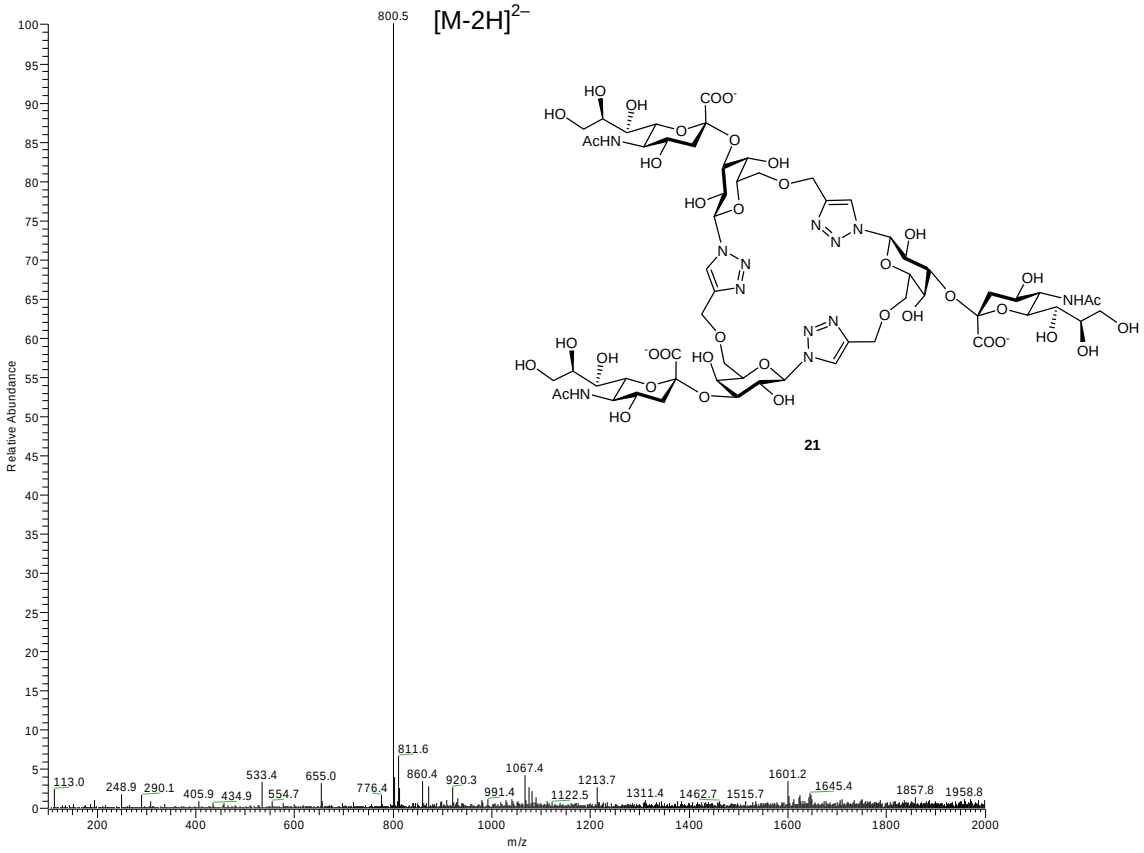


Fig S22 ESI MS Analysis of enzymatically sialylated cyclic pseudooligosachharides **21**.

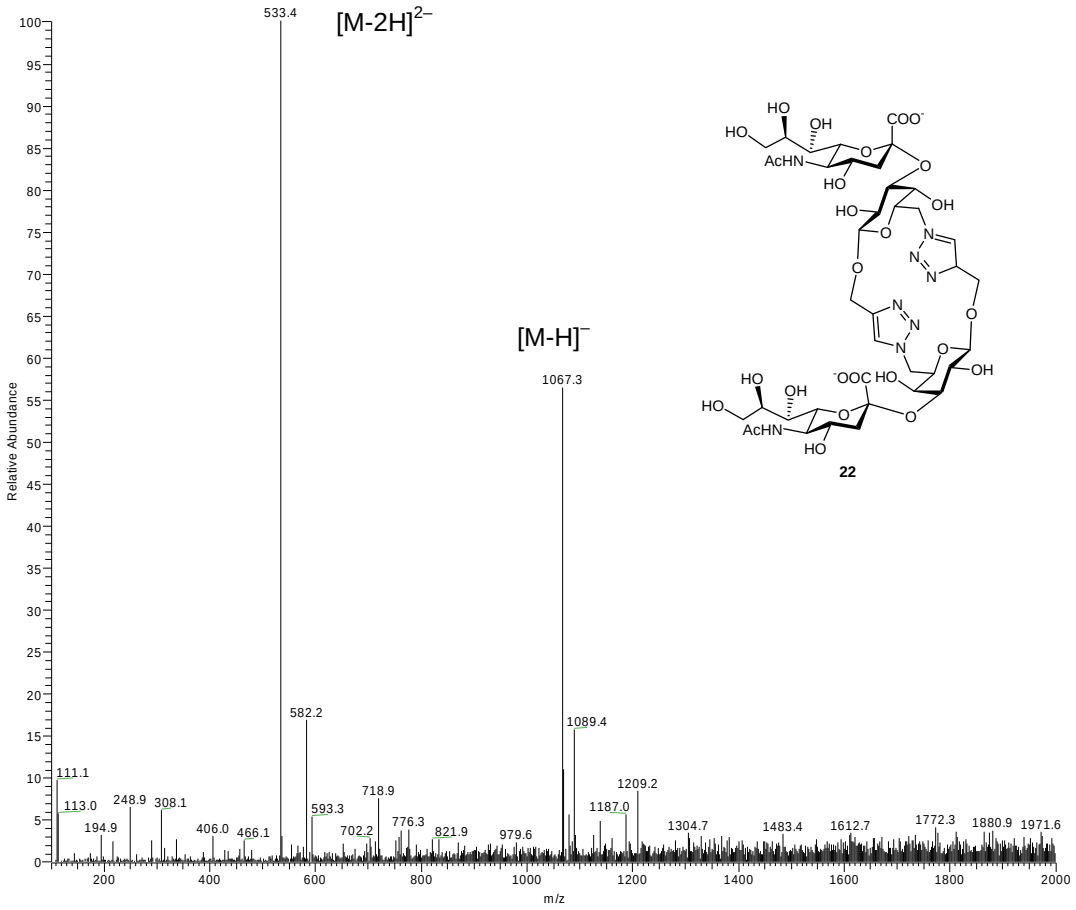


Fig. S23 ESI MS Analysis of enzymatically sialylated cyclic pseudooligosachharides **22**.

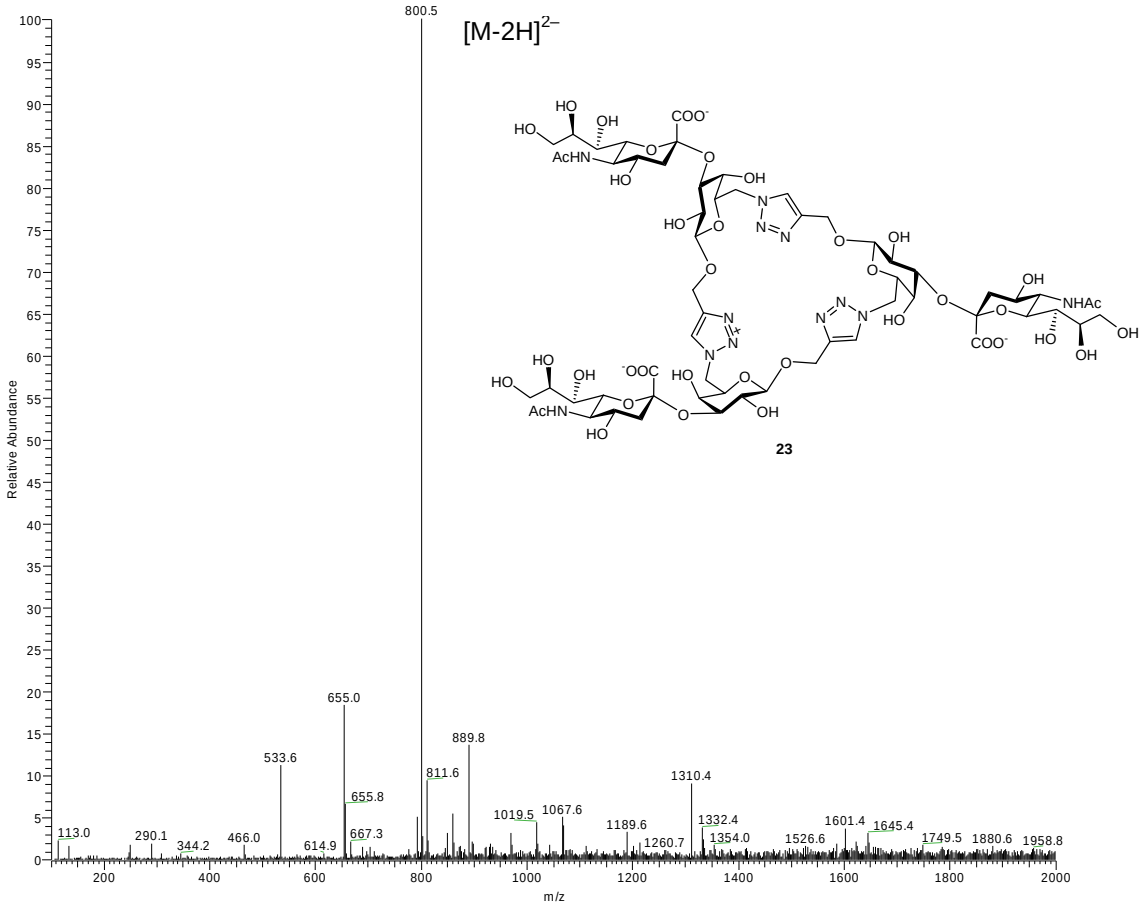


Fig. S24 ESI MS Analysis of enzymatically sialylated cyclic pseudooligosachharides **23**.

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