Topological effects and binding modes operating with multivalent iminosugar-based glycoclusters and mannosidases

Yoan Brissonnet,† Carmen Ortiz Mellet,‡ Sandrine Morandat,├ M. Isabel Garcia Moreno,‡ David Deniaud,† Susan E. Matthews, ∀ Sébastien Vidal,I Sergej Šesták,II Karim El Kirat‖ and Sébastien G. Gouin.*†

† LUNAM Université, CEISAM, Chimie Et Interdisciplinarité, Synthèse, Analyse, Modélisation, UMR CNRS 6230, UFR des Sciences et des Techniques, 2, rue de la Houssinière, BP 92208, 44322 NANTES Cedex 3, France.

‡ Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, C/ Profesor García González nº 1, 41012 Sevilla, Spain,

├ Dr. S. Morandat, Laboratoire de Génie Enzymatique et Cellulaire, FRE-CNRS 3580, Université de Technologie de Compiègne (UTC), BP 20529, 60205 Compiègne Cedex, France,

∀ Dr S.E. Matthews School of Pharmacy - University of East Anglia - Norwich, NR4 7TJ, United Kingdom

I Dr. S. Vidal Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Laboratoire de Chimie Organique 2 Glycochimie, UMR 5246, CNRS, Université Claude Bernard Lyon 1, F-69622 Villeurbanne, France,

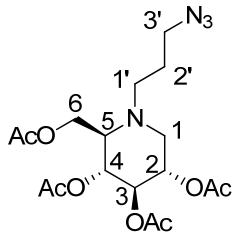
II Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravska cesta 9, 845 38 Bratislava, Slovakia

‖ Dr. K. El Kirat, Laboratoire de Biomécanique et Bioingénierie, UMR-CNRS 7338, Université de Technologie de Compiègne (UTC), BP 20529, 60205 Compiègne Cedex, France.

Contents

Materials NMR spectra were recorded at room temperature with a Bruker Avance 300 Ultra Shield or eBruker Avance III 400 spectrometer and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak peak ($CHCl_3$: ^1H: δ=7.26, ^{13}C: δ=77.2; DMSO-d6: ^1H: δ=2.54, ^{13}C: δ=40.4). Peak multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). High resolution mass spectra HRMS where obtained by Electrospray Ionisation (ESI) on a Micromass-Waters Q-TOF Ultima Global or with a Bruker Autoflex III SmartBeam spectrometer (MALDI). Low-resolution mass spectra (MS) were recorded with a Thermo electron DSQ spectrometer. All reagents were purchased from Acros Organics or Aldrich and were used without further purification. Column chromatography was conducted on silica gel Kieselgel SI60 (40-63 μm) from Merck. Reactions requiring anhydrous conditions were performed under argon. Dichloromethane was distilled from calcium hydride under nitrogen prior to use. Microwave experiments were conducted in sealed vials in commercial microwave reactors especially designed for synthetic chemistry. (MultiSYNTH, Milestone). The instrument features a special shaking system that ensures high homogeneity of the reaction mixtures. Optical rotations were measured on a 343 PERKIN ELMER at 20°C in a 1cm cell in the stated solvent; $[\alpha]_D$ values are given in 10^{-1} deg.cm^2 g^{-1} (concentration c given as g/100 mL).

N-(3-Azidopropyl)-2,3,4,6-tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucitol (14)

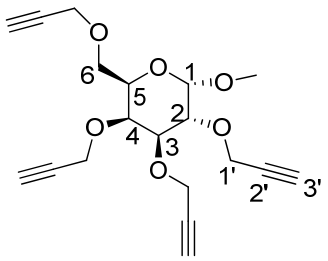


Conventional catalytic hydrogenolysis of **11** (760 mg, 1.25 mmol) was carried out with $Pd(OH)_2$ (875 mg, 1.25 mmol) in MeOH-1M aq. HCl solution (8 mL, 3:1) at 1 atm for 15 h. Then, the catalyst was filtered over celite and the solvent removed under reduced pressure to give **12**(quant) as a hygroscopic brown gum.
(For analytical data see: J. D. Diot, I. G. Moreno, G. Twigg, C. O. Mellet, K. Haupt, T. D. Butters, J. Kovensky, S. G. Gouin, *J. Org. Chem;* **2011,** *76*, 7757-7768.)

Imidazole-1-sulfonyl azide hydrochloride **13** (326 mg, 1.25 mmol) was added to the crude mixture (275 mg, 1.25 mmol) with K_2CO_3 (207 mg, 1.5 mmol) and $CuSO_4.5H_2O$ (3.2 mg, 12.5 µmol) in MeOH (15 mL) and the mixture was stirred overnight at room temperature.
The solvent was evaporated under reduced pressure and crude **14** was dissolved in pyridine-acetic anhydride (1:1, 14 mL) was stirred for 24 hours. Water (9mL) was added slowly at 0°C to quench the reaction. The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were then washed with an aqueous solution of HCl (1M, 2 x 10 mL) and a saturated aqueous $NaHCO_3$ solution (1 x 15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by flash chromatography (AcOEt - petroleum spirit 4 - 6) to give **14** as yellow oil (180 mg, 35% for three steps).

$[\alpha]_D$ = +3.8 (c= 1, Chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.04-4.92 (m, 2H, H-3 and H-4), 4.92-4.80 (m, 1H, H-2), 4.10 (d, *J* = 2.7 Hz, 2H, H-6), 3.28 (t, *J* = 6.6 Hz, 2H, H-3'), 3.12 (dd, *J* = 11.4 and 5 Hz, 1H, H-1$_a$), 2.89-2.75 (m, 1H, H-1'), 2.63-2.43 (m, 2H, H-1'$_b$,5), 2.22 (dd, *J* = 1.2, *J* = 11.4, 1H, H-1$_b$), 2.00, 1.95, 1.93 (3s, 12H, C=OC*H3*), 1.71-1.53 (m, 2H, H-2').; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 170.2, 170.0, 169.6 (C=O), 74.4 (C-4), 69.2 (C-2), 69.1 (C-3), 61.9 (C-5), 59.5 (C-6), 52.7 (C-1), 49.0 (C-3'), 48.4 (C-1'), 25.2 (C-2'), 20.7 (CH_3).; HRMS (MALDI): Found 415.1816 $C_9H_{19}O_4N_4Na$ requires 415.1823.

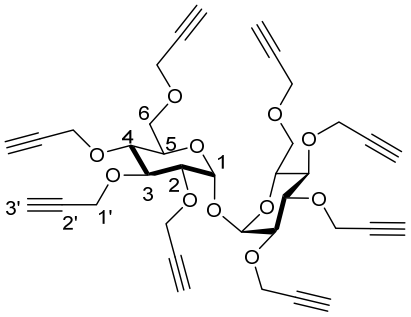
Methyl 2,3,4,6-tetra-*O*-propargyl-α-D-galactopyranoside (18)



NaH (412 mg, 10.3 mmol, dispersed in oil, 60%) was added in a small portion at 0°C to a solution of methyl α-D-galactopyranoside (200 mg, 1.03 mmol) in anhydrous DMF (10 mL). The suspension was stirred for one hour at room temp. Propargyl bromide (690 μL, 6.18 mmol, dispersed in toluene 80%) was added dropwise, and the reaction mixture was maintained for 3 hours. The mixture was cooled, MeOH was added 10 ml, and the solvent removed under reduced pressure after 30 min. Water 10 mL was added and the compound was extracted with diethyl ether (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered, and the solvent were evaporated under reduced pressure. The crude material was purified by column chromatography (8-2 EtOAc - cyclohexane) to give **18** as a yellow oil (306 mg, 86%).

$[\alpha]_D$ = +31 (c= 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.94 (1 H, d, *J* = 3.6 Hz, H-1), 4.49 (2 H, d, *J* = 2.3 Hz, C*H*$_2$CCH), 4.41(4 H, m, C*H*$_2$CCH), 4.24-4.18 (2 H, dd, *J* = 2.4 Hz, *J* = 7.9 Hz, C*H*$_2$CCH), 4.12-4.07 (1 H, m, H-4), 4.03-3.88 (3 H, m, H-2,3,5), 3.78 (1 H, dd, *J* = 5.7 Hz, *J* = 9.6 Hz, H-6$_A$), 3.66 (1 H, dd, *J* = 6.6 Hz, H-6$_B$), 3.42 (3 H, s, OMe), 2.44 (4 H, m, CCH).; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 98.8 (C-1), 80.4, 80.2, 79.7 (C-2'), 78.6 (C-3), 76.2 (C-2), 74.7 (C-3'), 74.6 (C-4), 69.3 (C-6), 69.1 (C-5), 59.9, 59.1, 58.9, 58.8 (C-1'), 55.5 (OMe); HRMS (MALDI) $[M+Na]^+$: Found 369.1305 $C_{19}H_{22}O_6Na$ requires 369.1309.

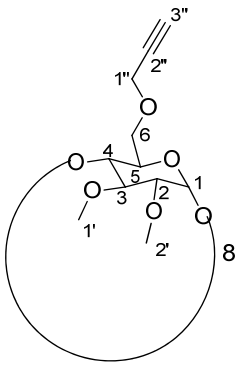
Propargyl-α-D-glucopyranosyl-α-D-glucopyranoside (20)



NaH (280 mg, 7.01 mmol, dispersed in oil, 60%) was added in a small portion at 0°C to a solution of α-D-glucopyranosyl-α-D-glucopyranoside (150 mg, 0.44 mmol) in anhydrous DMF (10 mL). The suspension was stirred for one hour at room temp. Propargyl bromide (490 μL, 4.38 mmol, dispersed in toluene 80%) was added dropwise, and the reaction mixture was stirred for 6 hours. mixture was cooled, MeOH was added 10 mL, and the solvent removed under reduced pressure after 30 min. Water 10 mL was added and the compound was extracted with diethyl ether (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered, and the solvent were evaporated under reduced pressure. The crude material was purified by column chromatography (EtOAc - cyclohexane, 7-3) to give **20** as a yellow oil (130 mg, 46%).

$[\alpha]_D$ = +125 (c= 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.18 (2 H, d, *J* = 3.6 Hz, H-1), 4.56-4.35 (8 H, br, H-1'), 4.35-4.10 (8 H, br, H-1'), 4.13-4.01 (2 H, m, H-5), 3.83 (2 H, dd, *J* = 3.5 Hz, 10.5 Hz, H-6_A), 3.75 (2 H, t, *J* = 9.3 Hz, H-3-3'), 3.70 (2 H, dd, *J* = 2.1 Hz, *J* = 10.5 Hz, H-6_B), 3.56 (2 H, dd, *J* = 9.5, 3.6 Hz, H-2), 3.48 (2 H, m, H-4), 2.49-2.38 (8 H, m, H-1'); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 94.0 (C-1), 81.3 (C-3), 80.2-80.1-79.7-79.5 (C-3'), 78.9 (C-2), 76.6 (C-4), 75.0 (C-2'), 74.3 (C-3'), 69.9 (C-5), 67.8 (C-6), 60.5, 60.3, 58.6, 58.4 (C-1'); HRMS (MALDI) $[M+Na]^+$: Found 669.2317 $C_{36}H_{38}O_{11}Na$ requires 669.2306.

Octakis(6-O-propargyl-2,3-di-O-methyl)cyclomaltooctaose (21)



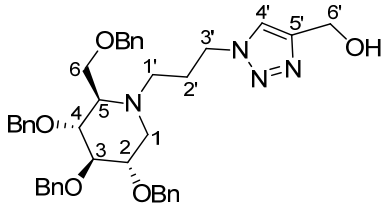
NaH (105 mg, 2.62 mmol, dispersed in oil, 60%) was added in small portions at 0°C to a solution of octakis(2,3-di-*O*-methyl)cyclomaltooctaose (200 mg, 0.131 mmol - K. Takeo, H. Mitoh, K. Uemura, *Carbohydrate Res.* **1989**, *187*, 203-221) in anhydrous DMF (15 mL). The suspension was magnetically stirred for one hour at room temp. Propargyl bromide (180 µL, 1.57 mmol,) dispersed in toluene 80%, was then added dropwise, and the reaction mixture was stirred for 6 hours. The mixture was cooled, MeOH was added (5 mL), and the solvent removed under reduced pressure after 30 min. Water (5 mL) was added and the compound was extracted with diethyl ether (2 x 15 mL). The organic phase was dried ($MgSO_4$), filtered, and the solvent were evaporated under reduced pressure. The crude material was purified by column chromatography (EtOAc - cyclohexane 96 - 4) to give **21** as a brown gum (192 mg, 80%).

$[\alpha]_D$ = +101 (c= 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.23 (8 H, d, *J* = 3.6 Hz, H-1), 4.22 (16 H, dq, *J* = 2.61 Hz, *J* = 15.6 Hz, H-1'), 3.96-3.78 (24 H, m, H-5,-6), 3.75-3.66 (8 H, m, H-5), 3.72-3.58 (32 H, m, H-1',-4), 3.58-3.46 (32 H, m, H-,2', -3), 3.20 (8 H, dd, *J* = 3.4 Hz, *J* = 9.66 Hz, H-2,), 2.49 (8 H, t, *J* = 2.3 Hz, H-3''); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 98.3 (C-1), 82.2 (C-3), 82.0 (C-2), 79.8 (C-2''), 79.0 (C-4), 75.1 (C-3''), 70.9 (C-5), 68.7 (C-6), 61.6 (C-1'), 58.9 (C-2'), 58.6 (C-1''); HRMS (MALDI) $[M+Na]^+$: Found 1847.7832 $C_{88}H_{128}O_{40}Na$ requires 1847.7874.

General procedure for CuAAC

To a solution of the **18** (30 mg, 86.6 μmol) and the azido-derivative (230 mg, 0.38 mmol) in dioxane-H_2O (5 mL, 4–1) copper sulfate (65 mg, 0.26 mmol) and sodium ascorbate (103 mg, 0.52 mmol) were added and the mixture was irradiated at 80 °C for 45 min in a sealed vessel. The mixture was poured into a NH_4Cl satd. solution (20 mL) and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (AcOEt – MeOH, 99 - 1), colorless oil, 66% yield.

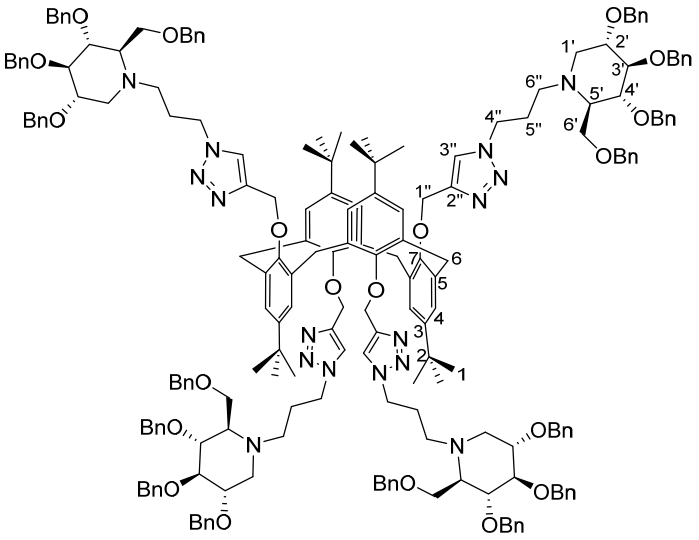
Compound (22)



22 was purified by flash chromatography on silica gel (AcOEt – cyclohexane, 99 - 1), colorless oil, 92% yield.

$[\alpha]_D$ = +1 (c= 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33 (1 H, s, H-4'),7.31-7.05 (20 H, m, 20 × Har), 4.95-4.71(3 H, m, C*H*HPh), 4.71-4.55 (4 H, m, 2 x CH_2Ph, 2 x H-6'), 4.45-4.28 (3 H, m, CH*H*Ph), 4.25-4.05 (2 H, m, H-3'), 3.61 (1 H, dd, *J* = 3.7 Hz, *J* = 10.8 Hz, H-6_A), 3.55-3.37 (4 H, m, H-2, -3, -4, -6_B), 2.93 (1 H, dd, *J* = 4.14 Hz, *J* = 11.5 Hz, H-1_A), 2.83-2.65 (1 H, m, H-1'$_A$), 2.52-2.35 (1 H, m, H-1'$_B$), 2.35-2.25 (1 H, m, H-5), 2.08 (t, *J* = 10.5 Hz, 1 H, H-1_B), 2.01-1.85 (m, 2H, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.7 (C-5'), 139.0, 138.5, 137.9 (Car), 128.6-127.5 (CHar), 122.1 (C-4'), 87.1 (C-3), 78.6 (C-2), 78.4 (C-4), 75.4, 75.3, 73.3, 72.9 (CH_2Ph), 66.6 (C-6), 64.7 (C-5), 56.5 (C-6'), 54.4 (C-1), 49.3 (C-1'), 48.5 (C-3'), 26.2 (C-2'); HRMS (MALDI) $[M+Na]^+$: Found 663.3533 $C_{40}H_{46}N_4O_5$ Na requires 663.3541.

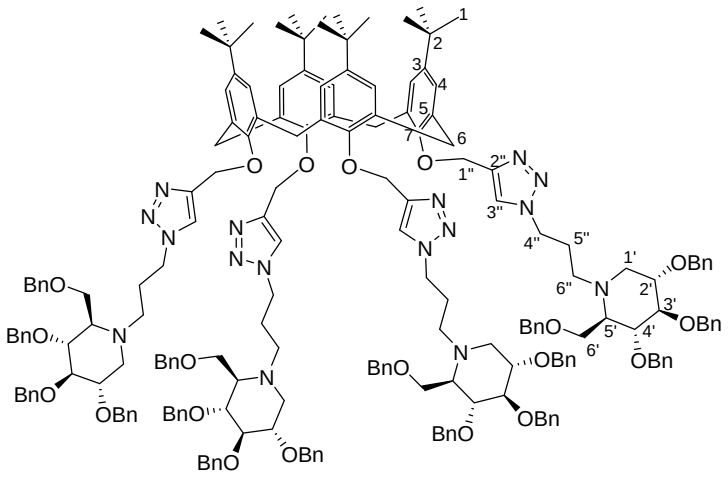
Compound (23)



23 was purified by flash chromatography on silica gel (AcOEt – cyclohexane, 95 - 5), colorless oil, 75% yield.

$[\alpha]_D$ = +4 (c= 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33-7.05 (80 H, m, Har), 6.92-6.84 (12 H, m, 8 x H-4, 4 x H-3''), 5.02-4.77(12 H, m, CH_2Ph), 4.72-4.60 (8 H, m, CH_2Ph), 4.54-4.37 (20 H, m, 12 x CH_2Ph, 4 x H-1''), 4.33-4.11 (8 H, m, H-4''), 3.74-3.41 (28 H, m, 4 x H-2',3',4',6', 8 x H-6), 3.08 (4 H, dd, *J* = 3.7 Hz, *J* = 10.8 Hz, H-1'$_A$), 2.93-2.79 (4 H, m, H-6''$_A$), 2.70-2.58 (4 H, m, H-6''$_B$), 2.40-2.30 (4 H, m, H-5'), 2.22 (4 H, t, *J* = 10.5 Hz, H-1'$_B$), 2.09-1.90 (8 H, m, H-5''),1.09 (36 H, s, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.5 (C_{IV}), 144.8, 144.7 (C-2''), 139.0, 138.6, 137.9 (Car), 133.6 (C_{IV}), 128.8-127.3 (CHar), 126.8 (C-4), 123.0 (C-3''), 87.2 (C-3'), 78.7 (C-2'), 78.5 (C-4'), 75.4, 75.3, 73.3, 72.8 (CH_2Ph), 66.4 (C-6'), 64.5 (C-1''), 64.4 (C-5'), 54.7 (C-1'), 49.5 (C-6''), 48.3 (C-4''), 38.6 (C-6), 33.9 (C-2), 31.6 (C-1), 25.9 (C-5''); HRMS (ES) $[M+2H]^{2+}$: Found 1076.2614 $C_{204}H_{234}N_{16}O_{20}$ requires 1076.2616.

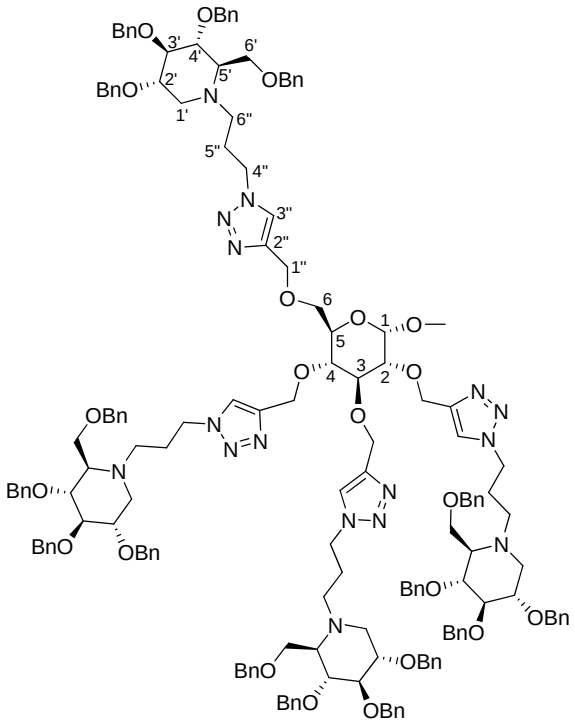
Compound (24)



24 was purified by flash chromatography on silica gel (AcOEt – cyclohexane, 98 - 2), colorless oil, 64% yield.

$[\alpha]_D$ = +5 (c= 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ = 7.67 (4 H, m, 4 x H-3’’), 7.31-7.05 (80 H, m, Har), 6.72 (8 H, s, H-4), 5.05 (8 H, m, H-1’’), 4.97-4.67(12 H, m, CH_2Ph), 4.69-4.59 (8 H, m, CH_2Ph), 4.45-4.31 (12 H, m, CH_2Ph,), 4.29-4.09 (12 H, m, 4 x H-6_B, 8 x H-4’’), 3.68-3.41 (20 H, m, H-2’,3’,4’,6’,), 3.04 (4 H, dd, *J* = 4.8 Hz, *J* = 11.1 Hz, H-$1'_A$), 2.87 (4 H, d, *J* = 12.7 Hz, 4 x H-6_B), 2.82-2.69 (4 H, m, H-$6''_A$), 2.61-2.46 (4 H, m, H-$6''_B$) 2.36-2.27 (4 H, m, H-5’), 2.16 (4 H, t, *J* = 10.8 Hz, H-$1'_B$), 2.04-1.87 (8 H, m, H-5’’),1.06 (36 H, s, H-1).; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.2 (C_{IV}), 145.1, 144.6 (C-2’’), 139.1, 138.7, 137.9 (Car), 134.2 (C_{IV}), 128.4-127.3 (CHar), 125.1(C-4), 124.5 (C-3’’), 87.3 (C-3’), 78.7 (C-2’), 78.5 (C-4’), 75.4, 75.2, 73.3, 72.8 (CH_2Ph), 66.5 (C-6’,C-1’’), 64.4 (C-5’), 54.6 (C-1’), 49.4 (C-6’’), 48.4 (C-4’’), 34.0 (C-2), 31.6 (C-6), 31.4 (C-1), 26.1 (C-5’’); HRMS (ES) $[M+2H]^{2+}$: Found 1613.8887 $C_{204}H_{234}N_{16}O_{20}$ requires 1613.8854.

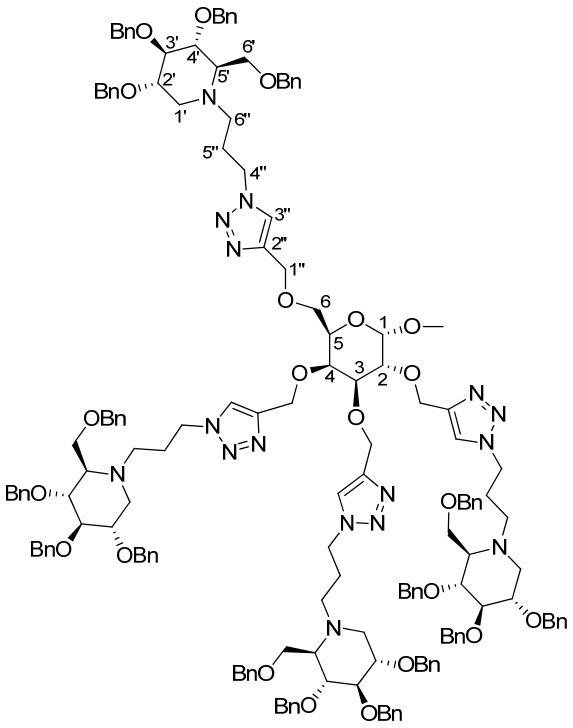
Compound (25)



25 was purified by flash chromatography on silica gel (AcOEt – MeOH, 99 - 1), colorless oil, 69% yield.

$[\alpha]_D$ = +28 (c= 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ = 7.96, 7.78, 7.76, 7.54 (4 H, s, 4 x H-3''), 7.45-7.12 (80 H, m, Har), 5.1-4.75 (18 H, m, 12 x C*H*HPh, 5 x H-1'', H-1), 4.78-4.63 (11 H, m, 4 × CH_2Ph, 3 x H-1''), 4.50-4.32 (12 H, m, CH*H*Ph), 4.34-4.13 (8 H, m, H-4''), 3.92 (1 H, t, *J* = 9.2 Hz, H-3), 3.84 (1 H, dd, *J* = 3.7 Hz, *J* = 10.5 Hz, H-6_A), 3.78-3.45 (24 H, m, H-2',3',4',6', H-2,4,5,6_B), 3.38 (3 H, s, OCH_3), 2.93-2.78 (4 H, m, H-1'$_A$), 2.69-2.59 (4 H, m, H-6''$_A$), 2.46-2.29 (4 H, m, H-6''$_B$), 2.20-2.09 (4 H, m, H-5'), 2.05-1.92 (4 H, m, H-1'$_B$), 1.89-1.71 (8 H, m, H-5''); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 145.0, 144.8, 144.7, 144.6 (C-2''), 139.0, 138.5, 137.8 (Car), 128.7-127.5 (CHar), 123.8, 123.5, 123.0 (C-3''), 97.8 (C-1), 87.2 (C-3'), 81.6 (C-3), 79.8 (C-2), 78.6 (C-2'), 78.4 (C-4'), 77.5 (C-4), 75.3, 75.2, 73.3, 72.8 (CH_2Ph), 70.0 (C-5), 68.8 (C-6), 66.4 (C-1''), 64.8 (C-6'), 64.4 (C-5'), 55.2 (OCH_3), 54.4 (C-1'), 49.2 (C-6''), 48.4 (C-4''), 25.9 (C-5''); HRMS (MALDI) $[M+Na]^+$: Found 2794.4184 $C_{167}H_{190}N_{16}O_{22}Na$ requires 2794.4133.

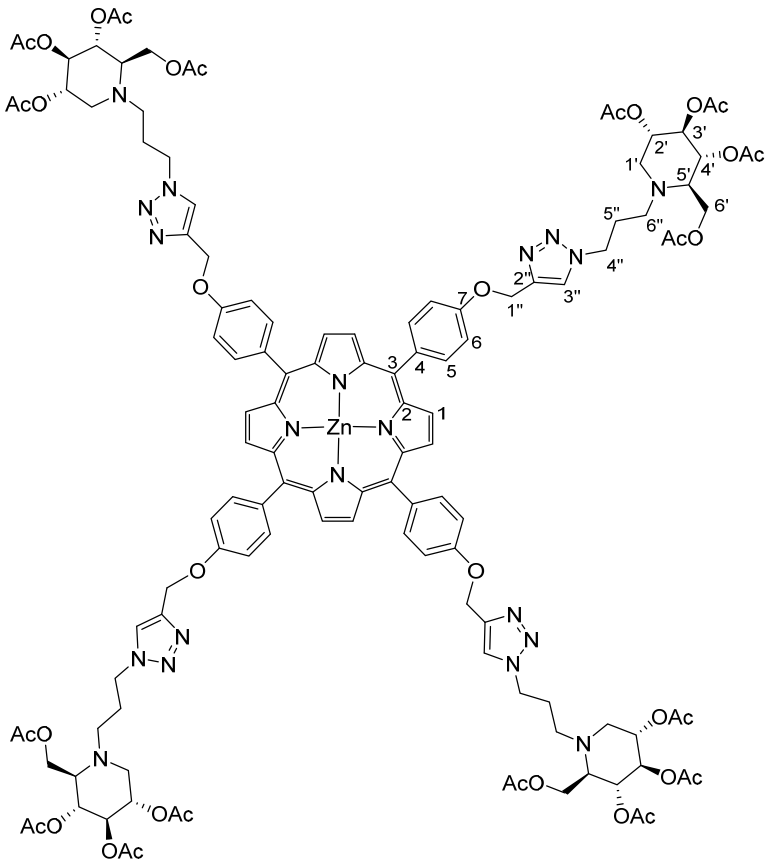
Compound (26)



26 was purified by flash chromatography on silica gel (AcOEt – MeOH, 99 - 1), colorless oil, 66% yield.

$[\alpha]_D$ = +14 (c= 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ = 7.75, 7.61, 7.58, 7.48 (4 H, s, H-3''), 7.40-7.08 (80 H, m, Har), 5.02-4.72 (18 H, m, 12 x C*H*HPh, 5 x H-1'', H-1), 4.72-4.50 (11 H, m, 4 x CH_2Ph, 3 x H-1''), 4.49-4.32 (12 H, m, CH_2Ph), 4.32-4.08 (8 H, m, H-4''), 4.04-4.96 (2 H, m, H-2, -3), 4.93-4.83 (2 H, m, H-4, -5), 3.70-3.40 (22 H, m, 4 x H-2',3',4',6', H-6), 3.34 (3 H, s, OCH_3), 3.12-2.98 (4 H, m, H-1'$_A$), 2.83-2.71 (4 H, m, H-6''$_A$), 2.64-2.46 (4 H, m, H-6''$_B$), 2.39-2.28 (4 H, m, H-5'), 2.23-2.10 (4 H, m, H-1'$_B$), 2.07-1.90 (8 H, m, H-5''); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 145.0, 144,9, 144.5 (C-2''), 139.0, 138.5, 137.8 (Car), 128.7-127.5 (CHar), 123.2-122.9 (C-3''), 98.5 (C-1), 87.1 (C-3'), 79.3 (C-3), 78.9 (C-2), 78.6 (C-2'), 78.4 (C-4'), 77.4 (C-4), 75.3, 75.0, 73.3, 72.8 (CH_2Ph), 69.2 (C-5), 69.0 (C-6), 66.2 (C-1''), 65.0 (C-6'), 64.5 (C-5'), 55.3 (OCH_3), 54.4 (C-1'), 49.2 (C-6''), 48.3 (C-4''), 25.9 (C-5''); HRMS (MALDI) $[M+Na]^+$: Found 2794.4111 $C_{167}H_{190}N_{16}O_{22}Na$ requires 2794.4133.

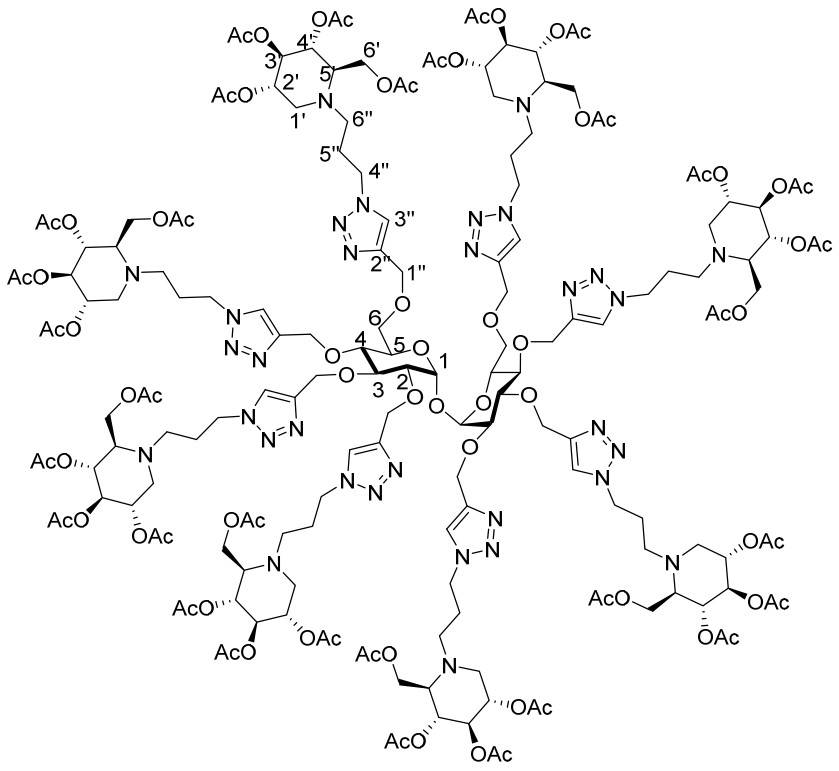
Compound (27)



27 was purified by flash chromatography on silica gel (DCM – MeOH, 8 - 2), purple solid, 66% yield.

$[\alpha]_D$ = too dark for analysis; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.93 (8 H, s, H-1), 8.11 (8 H, d, *J* = 8.3 Hz, H-5),7.43 (4 H, s, H-3’’), 7.22 (8 H, d, *J* = 8.3 Hz, H6), 5.09-4.97 (8 H, m, H-3’, -4’), 4.97-4.83 (4 H, m, H-2’), 4.67 (8 H, s, H-1’’), 4.22-3.99 (16 H, m, H-6’, -4’’), 3.11 (4 H, dd, *J* = 4.9 Hz, *J* = 11.8 Hz, H-1’$_A$), 2.77-2.65 (4 H, m, H-6’’$_B$), 2.61-2.51 (4 H, m, H-5’), 2.42-2.28 (4 H, m, H-6’’$_A$), 2.25-2.15 (4 H, m, H-1’$_B$), 2.06-1.96 (48 H, m, CH_3), 1.94-1.84 (8 H, m, H-5’’); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7-169.8 (C=O), 157.9, 150.5, 143.9, 136.5 (C_{IV}), 135.9 (C-5), 131.8 (C1), 122.5 (C-2’’), 120.4 (C_{IV}), 112.9 (C-6), 74.4 (C-4’), 69.4 (C-3’), 69.2 (C-2’), 62.4 (C-5’), 61.9 (C-1’’), 59.6 (C-6’), 52.7 (C-1’), 48.4 (C-6’’), 47.9 (C-4’’), 27.1 (C-5’’), 20.9 (CH_3); HRMS (ES) $[M+2H]^{2+}$: Found 1275.4588 $C_{124}H_{142}N_{20}O_{36}Zn$ requires 1275.4577.

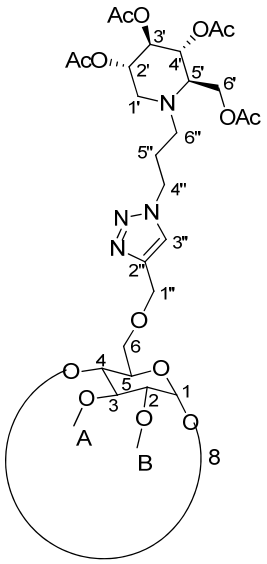
Compound (28)



28 was purified by flash chromatography on silica gel (DCM – MeOH, 98 - 2), colorless oil, 58% yield.

$[\alpha]_D$ = +24 (c= 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.07, 8.04, 7.98, 7.74 (8 H, s, H-3’’), 5.18 (2 H, d, *J* = 3.46 Hz, H-1), 5.08-4.98 (16 H, m, H-3’, -4’), 4.98-4.74 (16 H,m, 8 x H-2’, 8 x H-1’’), 4.73-4.57 (8 H, m, H-1’’), 4.50-4.28 (16 H, m, H-4’’), 4.19-4.01 (18 H, m, 16 x H-6’, 2 x H-5), 3.80 (2 H, t, *J* = 9.3 Hz, H-3), 3.75-3.65 (4 H, m, H-6), 3.56-3.41 (4 H, m, H-2, -4), 3.26-3.13 (8 H, m, H-1’$_A$), 2.97-2.79 (8 H, m, H-6’’), 2.72-2.47 (16 H, m, H-5’, -6’’), 2.41-2.22 (8 H, m, H-1’$_B$), 2.18-1.89 (112 H, m, H-5’’, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3-169.7 (C=O), 145.2-144.3 (C-2’’), 124.3-123 (C-3’’), 93.6 (C-1), 81.1 (C-3), 79.4 (C-2), 77.3 (C-4), 74.5 (C-4’), 70.7 (C-5), 69.4 (C-6,2’,3’), 66.4, 66.0, 64.8, 64.4 (C-1’’), 62.1 (C-5’), 59.8 (C-6’), 52.8 (C-1’), 48.6 (C-6’’), 48.0 (C-4’’), 26.9 (C-5’’), 20.9 (CH_3); HRMS (MALDI) $[M+4H]^{4+}$: Found 990.9178 $C_{172}H_{250}N_{32}O_{75}$ requires 990.9216.

Compound (29)



29 was purified by flash chromatography on silica gel (DCM – MeOH, 99 - 1), colorless oil, 57% yield.

$[\alpha]_D$ = +57 (c= 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (8 H, s, H-3’’), 5.19 (8 H, d, *J* = 3.43 Hz, H-1), 5.08-4.99 (16 H, m, H-3’, -4’), 4.98-4.87 (8 H, m, H-2’), 4.64-4.50 (16 H, m, H-1’’), 4.45-4.30 (16 H, m, H-4’’), 4.14 (16 H, d, *J* = 2.2 Hz, H-6’), 4.01-3.90 (8 H, m, H-6_A), 3.82-3.44 (80 H, m, H-3, -4, -5, -6_B, A, B), 3.25-3.08 (16 H, m, H-2, -1’$_A$), 2.94-2.83 (8 H, m, H-6’’$_A$), 2.71-2.53 (16 H, m, H-5’, -6’’$_B$), 2.36-2.26 (8 H, m, H-1’$_B$), 2.08-1.97 (112 H, m, H-5’’, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6-169.7 (C=O), 145.0 (C-2’’), 123.0 (C-3’’), 98.2 (C-1), 82.1 (C-2,-3), 78.6 (C-4), 74.4 (C-4’), 71.2 (C-5), 69.2 (C-6, -2’, -3’), 64.9 (C-1’’), 61.9 (C-5’), 61.4 (C-A), 59.6 (C-6’), 58.8 (C-B), 52.7 (C-1’), 48.6 (C-6’’), 47.9 (C-4’’), 26.8 (C-5’’), 20.7 (CH_3).; HRMS (ES) $[M+5H]^{5+}$: Found 1028.6470 $C_{224}H_{341}N_{32}O_{104}$ requires 1028.6488.

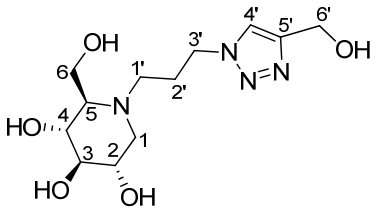
General procedure for benzyl hydrogenolysis

Catalytic hydrogenation of compound **26** (80 mg, 28.8 μmol) was carried out with $Pd(OH)_2$ (73 mg) in MeOH-1M aq. HCl solution (10 mL, 8:2) at 1 atm for 48 h. The catalyst was filtered over celite and the solvent removed under reduced pressure to give compound **5** (quant) as a sticky solid.

General procedure for acetates deprotection

Compound **29** (30 mg, 0.0058 mmol) was dissolved in MeOH-H_2O (1-1, 5 mL). Amberlite resin IRA 400 OH- 1.25 meq/mL (780 mg) was added to the solution, and the mixture was stirred overnight at room temperature. The resin was filtered off and washed with methanol and water. The solvent was evaporated under reduced pressure to give compound **8** (20 mg, 91%) as pale yellow solid.

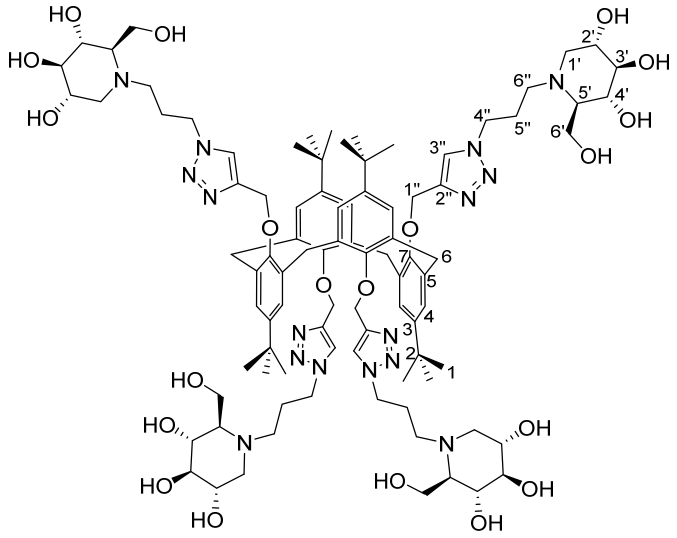
Compound (1)



1 was purified by flash chromatography on silica gel (AcOEt – cyclohexane, 99 - 1), white solid, 92% yield.

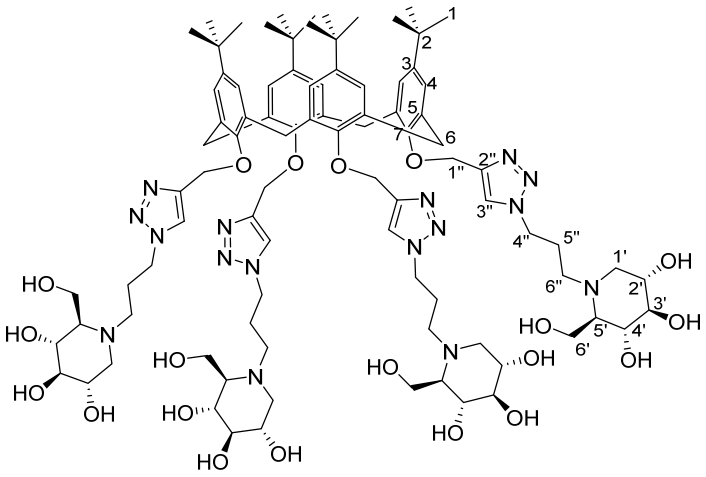
$[\alpha]_D$ = -8 (c= 1, H_2O); ^1H NMR (400 MHz, D_2O) δ = 8.05 (1 H, s, H-4'), 4.79 [under solvent peak] (2 H, H-6'), 4.57-4.47 (2 H, m, H-3'), 3.82 (2 H, d, *J* =2.57 Hz, H-6), 3.62-3.54 (1 H, m, H-2), 3.42 (1 H, t, *J* = 9.4 Hz, H-4), 3.31 (1 H, t, *J* = 9.2 Hz, H-3), 3.02 (1 H, dd, *J* = 4.96 Hz, *J* = 11.3 Hz, $H\text{-}1_A$), 2.87-2.77 (1 H, m, $H\text{-}1'_A$), 2.75-2.55 (1 H, m, $H\text{-}1'_B$), 2.39-2.27 (2 H, m, $H\text{-}1_B$, -5), 2.24-2.14 (2 H, m, H-2'); ^{13}C NMR (100 MHz, D_2O): δ = 147.0 (C-5'), 124.1 (C-4'), 78.3 (C-3), 70.0 (C-4), 68.9 (C-2), 64.8 (C-5), 57.6 (C-6), 55.3 (C-1), 54.7 (C-6'), 49.3 (C-3'), 48.6 (C-1'), 23.2 (C-2'); HRMS (MALDI) $[M+H]^+$: Found 303.1655 $C_{12}H_{22}N_4O_5$ requires 303.1663.

Compound (2)



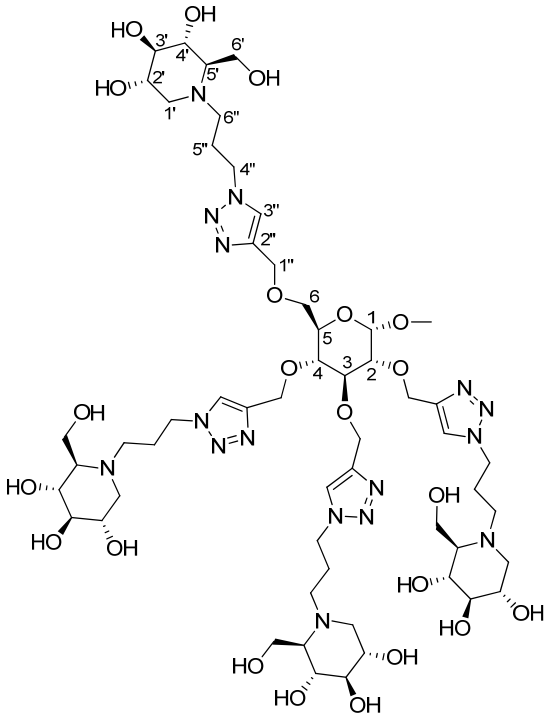
White solid, $[\alpha]_D$ = -4 (c= 0.3, H_2O); ^1H NMR (400 MHz, D_2O) δ = 7.99 (4 H, s, H-3’’), 7.03 (8 H, m, H-4), 4.75-4.72 (8 H, m, H-1’’), 4.70-4.59 (8 H, m, H-4’’), 4.08 (4 H, d, *J* = 13 Hz, H-6’$_A$), 4.02-3.94 (4 H, m, H-6’$_B$), 3.93-3.85 (4 H, m, H-2’), 3.77-3.70 (4 H, m, H-4’), 3.70-3.52 (20 H, m, H-6, -1’$_A$, -3’, -6’’$_A$), 3.49-3.37 (4 H, m, H-6’’$_B$), 3.29 (4 H, d, H-5’), 3.19 (4 H, t, *J* = 11.8 Hz, H-1’$_B$), 2.62-2.45 (8 H, m, H-5’’), 1.06 (36 H, s, H-1); ^{13}C NMR (100 MHz, D_2O): δ = 153.6 (C_{IV}), 145.0,144.4 (C-2’’), 133.4 (C_{IV}), 127.1 (C-4), 125.9 (C-3’’), 75.8 (C-3’), 67.1 (C-4’), 65.9 (C-2’), 65.7 (C-5’), 63.8 (C-1’’), 53.9 (C-6’), 53.3 (C-1’), 50.1 (C-6’’), 47.3 (C-4’’), 36.8 (C-6), 33.4 (C-2), 30.8 (C-1), 23.6 (C-5’’); HRMS (ES) $[M+2Na]^{2+}$: Found 915.4951 $C_{92}H_{136}N_{16}Na_2O_{20}$ requires 915.4968.

Compound (3)



White solid, $[\alpha]_D$ = -8 (c= 0.1, MeOH); ^{1}H NMR (400 MHz, MeOD) δ = 8.08 (4 H, s, H-3’’), 6.79 (8 H, s, H-4), 5.05 (8 H, s, H-1’’), 4.51-4.43 (8 H, m, H-4’’), 4.22 (4 H, d, H-6$_A$), 3.86-3.75 (8 H, m, H-6’), 3.50-3.42 (4 H, m, H-2’), 3.36 (4 H, t, *J* = 9.2 Hz, H-4’), 3.15 (4 H, t, *J* = 9 Hz, H-3’), 3.03-2.94 (8 H, m, H-6$_B$, -1’$_A$), 2.91-2.78 (4 H, m, H-6’’$_A$), 2.53-2.43 (4 H, m, H-6’’$_B$), 2.15-2.00 (16 H, m, H-1’$_B$, -5’, -5’’), 0.97 (36 H, s, H-1); ^{13}C NMR (100 MHz, MeOD): δ = 153.4, 146.5, 145.7, 135.5 (C$_{IV}$), 126.6 (C-3’’), 126.3 (C-4), 80.5 (C-3’), 72.1 (C-4’), 70.8 (C-2’), 67.9 (C-5’), 67.4 (C-1’’) 59.6 (C-6’), 57.8 (C-1’), 50.3 (C-6’’), 49.5 (C-4’’), 34.8 (C-2), 32.9 (C-6), 32.0 (C-1), 27.3 (C-5’’); HRMS (ES) $[M+2Na]^{2+}$: Found 915.4951 cf comp prec $C_{92}H_{136}N_{16}Na_2O_{20}$ requires 915.4949.

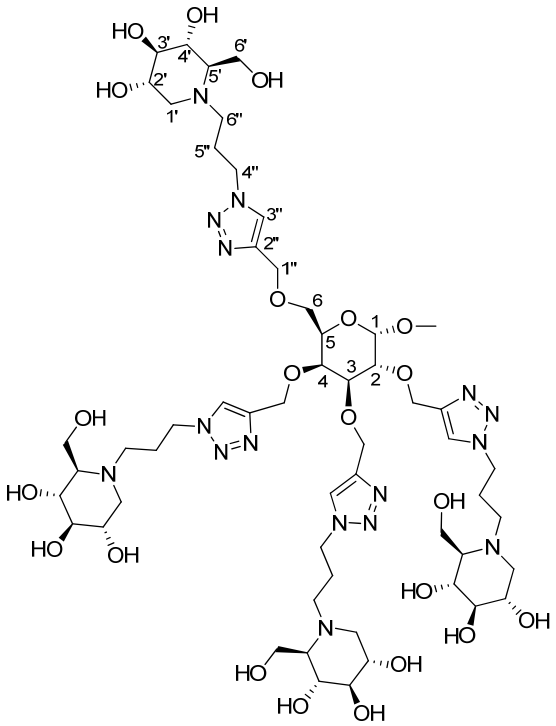
Compound (4)



White solid, $[\alpha]_D$ = -15 (c= 1, H_2O); ^{1}H NMR (400 MHz, D_2O) δ = 8.17 (3 H, s, H-3’’), 8.12 (1 H, s, H-3’’),4.91-4.55 (17 H,m, H-1’’, -4’’, -1), 4.05 (4 H, d, *J* = 12.9 Hz, H-6’$_A$), 3.93-3.69 (12 H, m, H-2, -5, -6, -H-4’, -6’$_B$), 3.69-3.42 (18 H, m, H-3, -4, -1’, -2’, -3’, -6’’$_A$), 3.36 (3 H, s, OCH_3), 3.35-3.18 (8 H, m, H-5’, -6’’$_B$), 3.12 (4 H, t, *J* = 11.6 Hz, H-1’), 2.56-2.35 (8 H, m, H-5’’); ^{13}C NMR (100 MHz, D_2O): δ = 143.6-143.4 (C-2’’), 125.8-125.4 (C-3’’), 97.0 (C-1), 80.4 (C-2), 78.9 (C-3), 76.7 (C-4), 75.7 (C-3’), 69.1 (C-5), 68.0 (C-6), 67.0 (C-2’), 66.0 (C-4’), 65.5 (C-5’), 64.9, 64.7, 62.9, 62.8 (C-1’’), 55.1 (OCH_3), 53.8 (C-6’), 53.3 (C-1’), 50.1

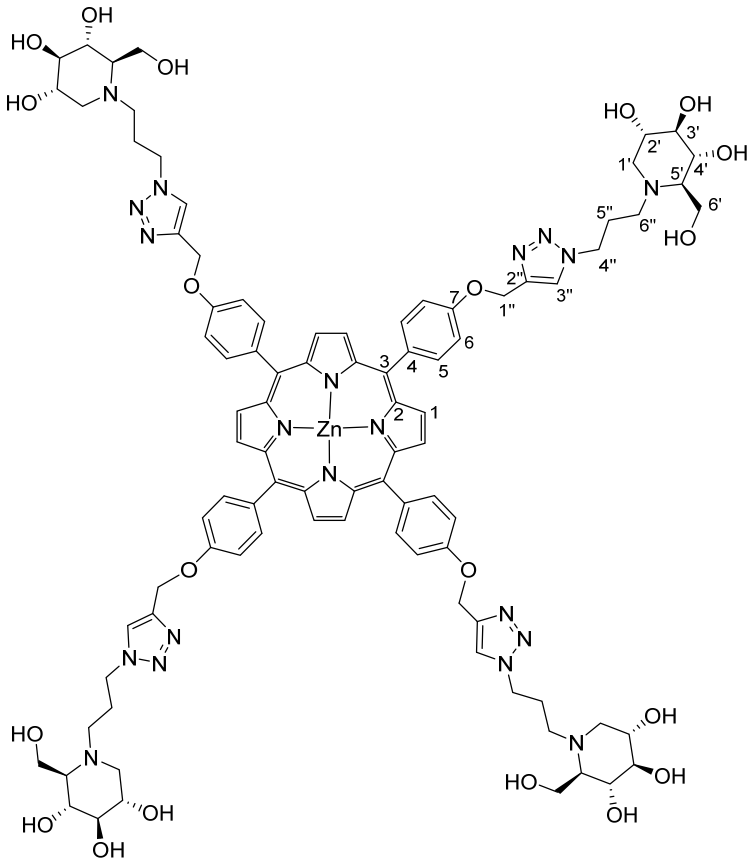
(C-6’’), 47.6 (C-4’’), 23.3 (C-5’’); HRMS (MALDI) $[M+Na]^+$: Found 1353.6590 $C_{92}H_{136}N_{16}NaO_{20}$ requires 1353.6621.

Compound (5)



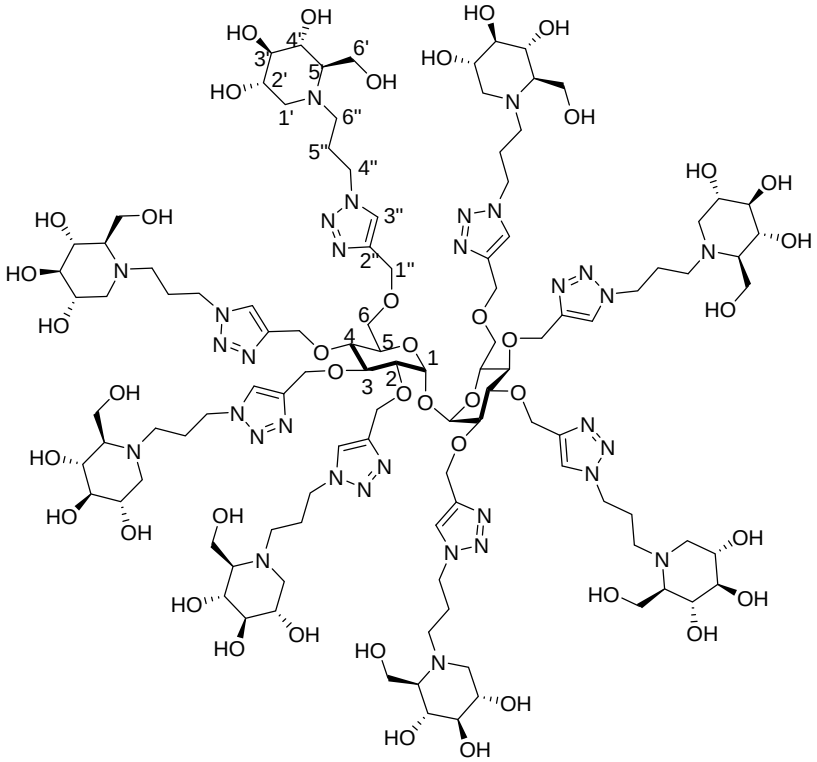
White solid, $[\alpha]_D$ = +32 (c= 1, H_2O); ^1H NMR (400 MHz, D_2O) δ = 8.15, 8.12 (4 H, 2 s, H-3’’), 4.93-4.66 (9 H, m, H-1’’, H-1), 4.64-4.61 (8 H, m, H-4’’), 4.23-4.18 (1 H, m, H-3), 4.10-4.04 (1 H, m, H-5), 4.00-3.82 (10 H, m, H-2, -4, -6’), 3.76-3.61 (5 H, m, H-6_A, -2’), 3.59-3.49 (5 H, m, H-6_B, -4’), 3.47-3.35 (7-H, m, H-3’, OMe) , 3.32-3.22 (4 H, m, H-1’$_A$), 3.17-3.05(4 H, m, H-6’’$_A$), 3.03-2.87 (4 H, m, H-6’’$_B$), 2.75-2.06 (8 H, m, H-1’$_B$, -5’), 2.41-2.24 (8 H, m, H-5’’); ^{13}C NMR (100 MHz, D_2O): δ = 144.1 (C-2’’), 125.3 (C-3’’), 97.7 (C-1), 77.5 (C-3), 77.3 (C-3’), 75.7 (C-2), 74.8 (C-4), 68.9 (C-6, 2’), 68.5 (C-5), 67.7 (C-4’), 65.1 (C-5’), 65.0, 63.5, 63.4, 63.0 (C-1’’), 56.1 (OCH_3), 55.1 (C-6’), 54.5 (C-1’), 49.2 (C-6’’), 48.2 (C-4’’), 23.8 (C-5’’); HRMS (MALDI) $[M+Na]^+$: Found 1353.6659 $C_{92}H_{136}N_{16}NaO_{20}$ requires 1353.6621.

Compound (6)



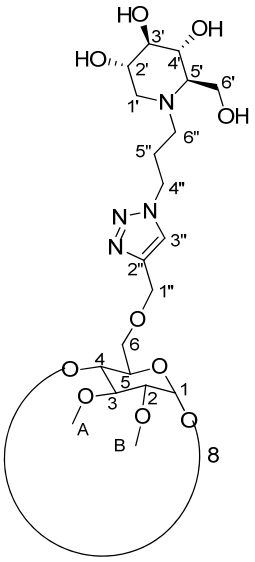
Purple solid, $[\alpha]_D$ = Too dark for analysis; ^{1}H NMR (400 MHz, DMSO+εD_2O) δ = 8.73 (8 H, s, H-1), 8.26 (4 H, s, H-3’’), 7.95 (8 H, s, H-5), 7.30 (8 H, s, H-6), 5.26 (8 H, s, H-1’’), 4.55-4.30 (8 H, m, H-4’’), 3.80-3.58 (8 H, m, H-6’), 3.38-3.31 (4 H, m, H-2’), 3.19 (4 H, t, *J* = 9.4 Hz, H-4’), 3.04 (4 H, t, *J* = 9 Hz, H-3’), 3.00-2.84 (8 H, m, H-1’$_A$, H-6’’$_A$), 2.71-2.58 (4 H, m, H-6’’$_B$), 2.30-2.18 (8 H, m, H-1’$_B$, -5’), 2.17-2.03 (8 H, m, H-5’’); ^{13}C NMR (100 MHz, DMSO+εD_2O): δ = 158.2, 150.4, 143.7 (C_{IV}), 136.1 (C-5), 132.3 (C1), 125.7 (C-3’’), 120.8 (C_{IV}), 113.6 (C-6), 78.3 (C-3’), 69.8 (C-4’), 68.5 (C-2’), 66.7 (C-5’), 61.8 (C-1’’), 57.4 (C-6’), 55.9 (C-1’), 49.8 (C-6’’), 48.5 (C-4’’), 25.4 (C-5’’); HRMS (ES) $[M+2H]^{2+}$: Found 939.3743 $C_{92}H_{110}N_{20}O_{20}Zn$ requires 939.3767.

Compound (7)



White solid, $[\alpha]_D$ = + 15 (c= 0.5, H_2O); ^1H NMR (400 MHz, D_2O) δ = 8.10-8.04 (8 H, s, H-3''), 5.26 (2 H, d, *J* = 3.3 Hz, H-1), 4.99-4.58 (16 H, m, H-1''), 4.57-4.35 (16 H, m, H-4''), 4.07-3.89 (4 H, m, H-3, -5), 3.88-3.74 (16 H, m, H-6'), 3.73-3.52 (16 H, m, H-2, -4, -6, -2'), 3.46-3.36 (8 H, m, H-4'), 3.34-3.25 (8 H, m, H-3'), 3.07-2.95 (8 H, m, H-1'$_A$), 2.90-2.57 (16 H, m, H-6''), 2.38-2.21 (16 H, m, H-1'$_B$, -5'), 2.21-2.04 (16 H, m, H-5''); ^{13}C NMR (100 MHz, D_2O): δ = 144.3-143.7 (C-2''), 125.2-124.8 (C-3''), 92.4 (C-1), 80.4 (C-3), 78.5 (C-2), 78.3 (C-3'), 76.7 (C-4), 70.2 (C-5),70.1 (C-4'), 68.9 (C-2'), 67.8 (C-6), 65.5 (C-1''), 64.9 (C-5'), 63.6, 63.3 (C-1''), 57.6 (C-6'), 55.4 (C-1'), 48.6 (C-4'',6''), 24.0 (C-5''); HRMS (ES) $[M+2Na]^{2+}$: Found 1330.6411 $C_{108}H_{182}N_{32}Na_2O_{43}$ requires 1330.6445.

Compound (8)



White solid, $[\alpha]_D$ = + 37 (c= 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ = 8.11 (8 H, s, H-3’’), 5.29 (8 H, s, H-1), 4.75-4.54 (16 H, m, H-1’’), 4.54-4.38 (16 H, m, H-4’’), 4.09-3.51 (112 H, m, H-3, -4, -5, -6, -2’, -6’, -7’$_A$, -7’$_B$), 3.48-3.37 (8 H, m, H-4’), 3.37-3.25 (16 H, m, H-2, -3’), 3.02 (8 H, dd, *J* = 4.75 Hz, *J* = 11 Hz, H-1’$_A$), 2.94-2.78 (8 H, m, H-6’’$_A$), 2.77-2.64 (8 H, m, H-6’’$_B$), 2.42-2.26 (m, 16 H, H-1’$_B$,5’), 2.22-2.07 (16 H, m, H-5’’); ^{13}C NMR (100 MHz, D_2O): δ = 144.0 (C-2’’), 125.0 (C-3’’), 96.6 (C-1), 81.4-79.8 (C-3,2), 78.4 (C-3’), 75.8 (C-4), 70.0 (C-5,4’), 68.8 (C-6,2’), 65.0 (C-5’), 63.6 (C-1’’), 59.8-58.8 (C-A), 58.4 (C-B), 57.7 (C-6’), 55.5 (C-1’), 48.8 (C-6’’), 48.6 (C-4’’), 24.2 (C-5’’).

General procedure for the inhibition assays

Inhibition constant (K_i) values were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-gluco/β-galactosidase from bovine liver) or *p*-nitrophenyl α- or β-D-glycopyranoside (for other glycosidases) or α,α'-trehalose (for trehalase). Each assay was performed in phosphate buffer or phosphate-citrate buffer (for α- or β-mannosidase and amyloglucosidase) at the optimal pH for the enzymes. The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. The mixture was incubated for 10-30 min at 37 ºC or 55 ºC (for amyloglucosidase) and the reaction was quenched by addition of 1 M Na_2CO_3. Reaction times were appropriate to obtain 10-20% conversion of the substrate in order to achieve linear rates. The absorbance of the resulting mixture was determined at 405 nm . Approximate values of K_i were determined using a fixed concentration of substrate (around the K_M value for the different glycosidases) and various concentrations of inhibitor. Full K_i determinations and enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis.

Representative Lineweaver-Burk Plot

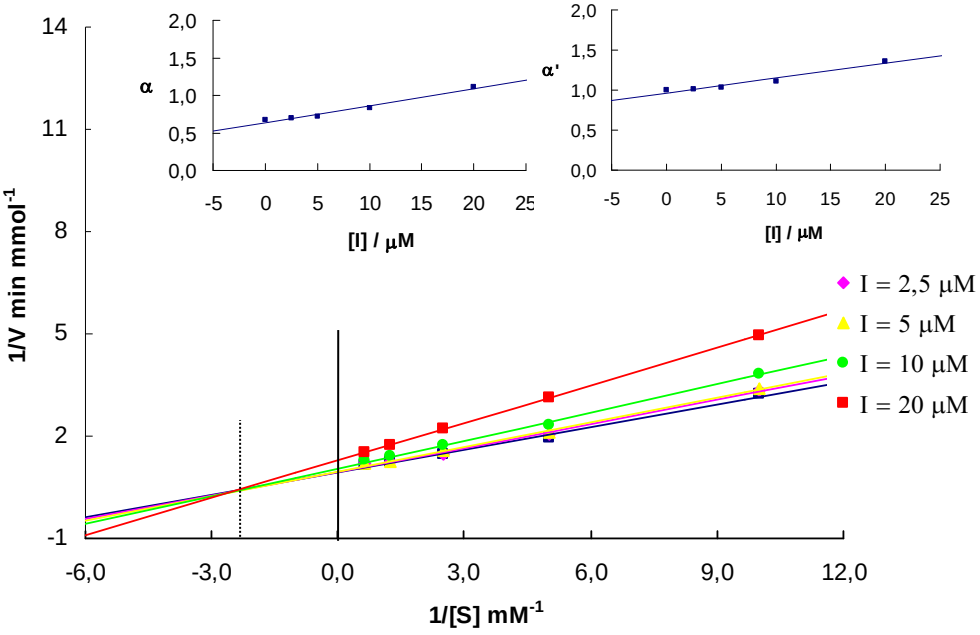


Figure S1. Lineweaver-Burk Plot for K_i determination (45 µM) and K_i' determination (54 µM) of **2** against *baker yeast α-glucosidase* (pH 6.8).

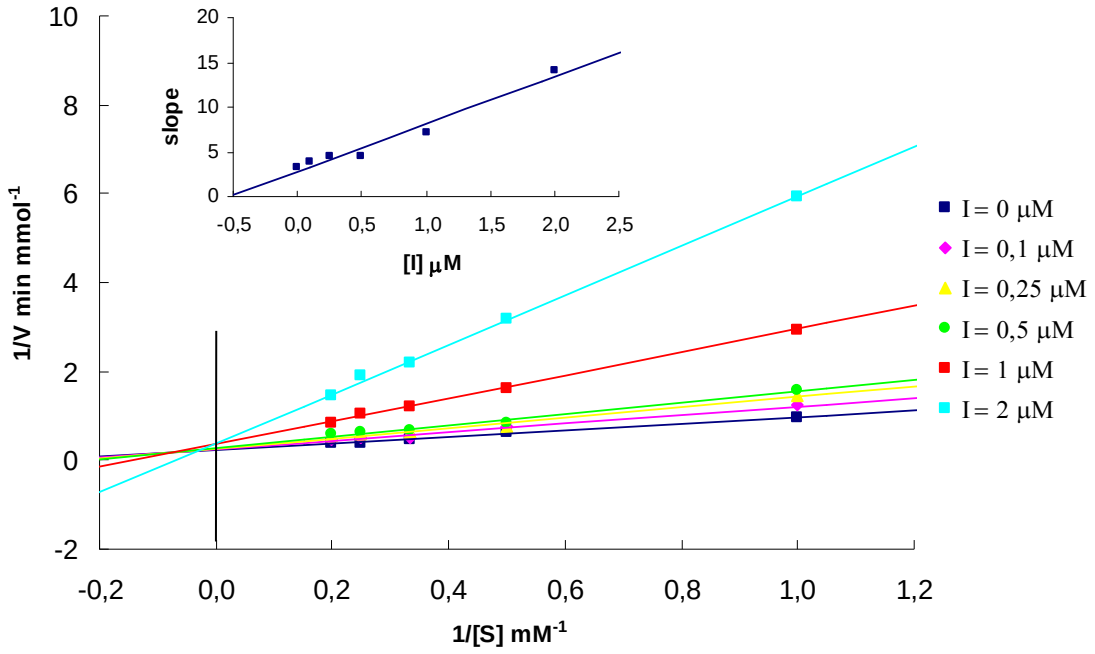


Figure S2. Lineweaver-Burk Plot for K_i determination (0.5 µM) of **6** against *Jack bean α-mannosidase* (pH 5.5).

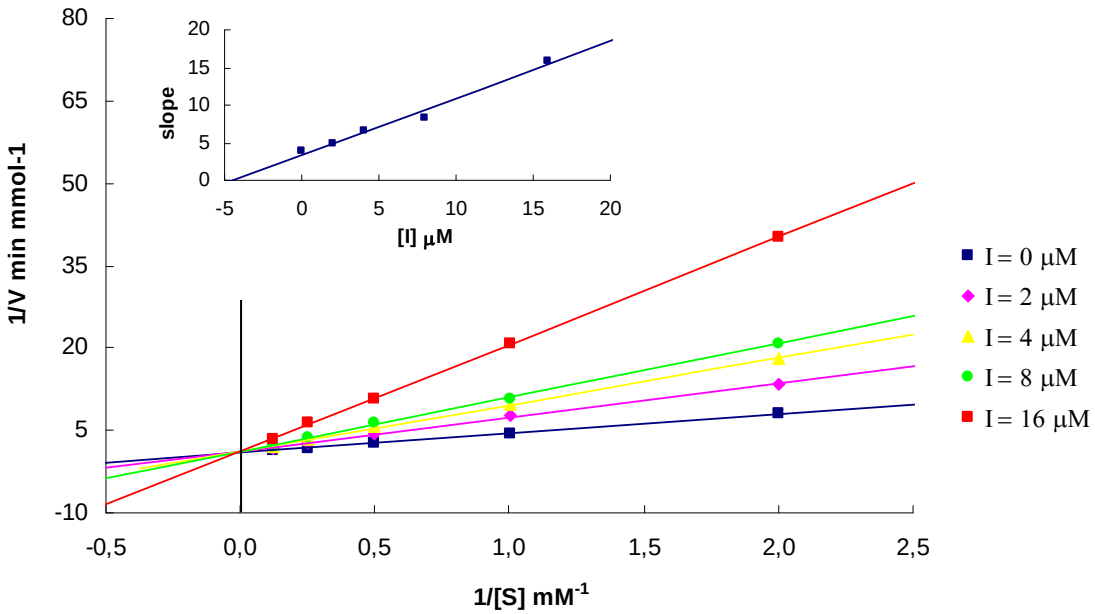


Figure S3. Lineweaver-Burk Plot for K_i determination (4.6 µM) of **7** against *Aspergillus niger amyloglucosidase* (pH 5.5).

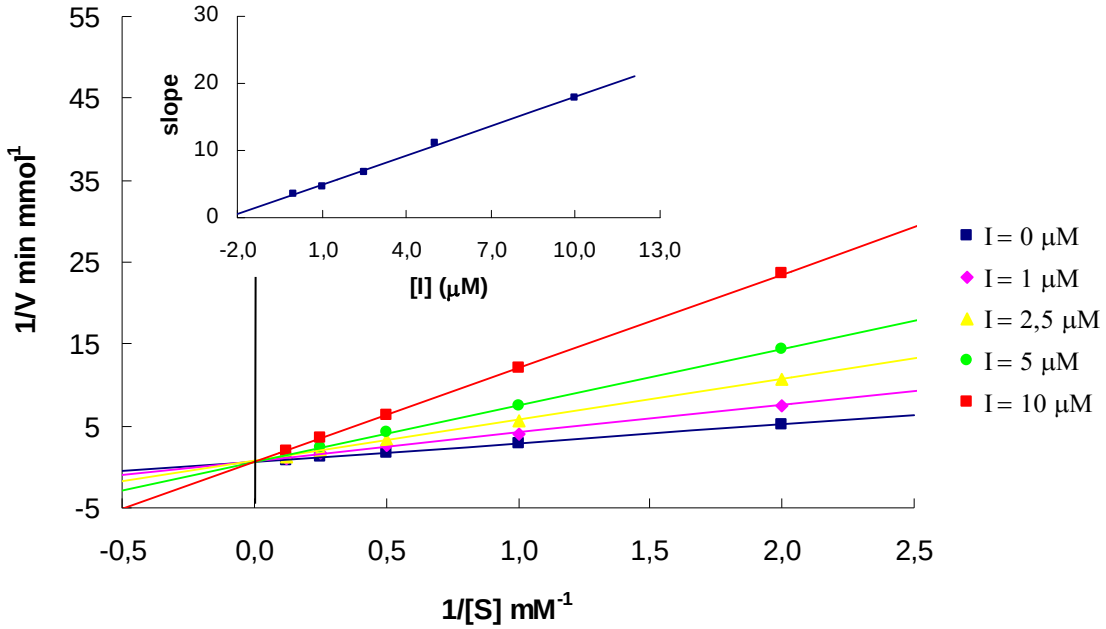


Figure S4. Lineweaver-Burk Plot for K_i determination (2.3 µM) of **5** against *Aspergillus niger amyloglucosidase* (pH 5.5).

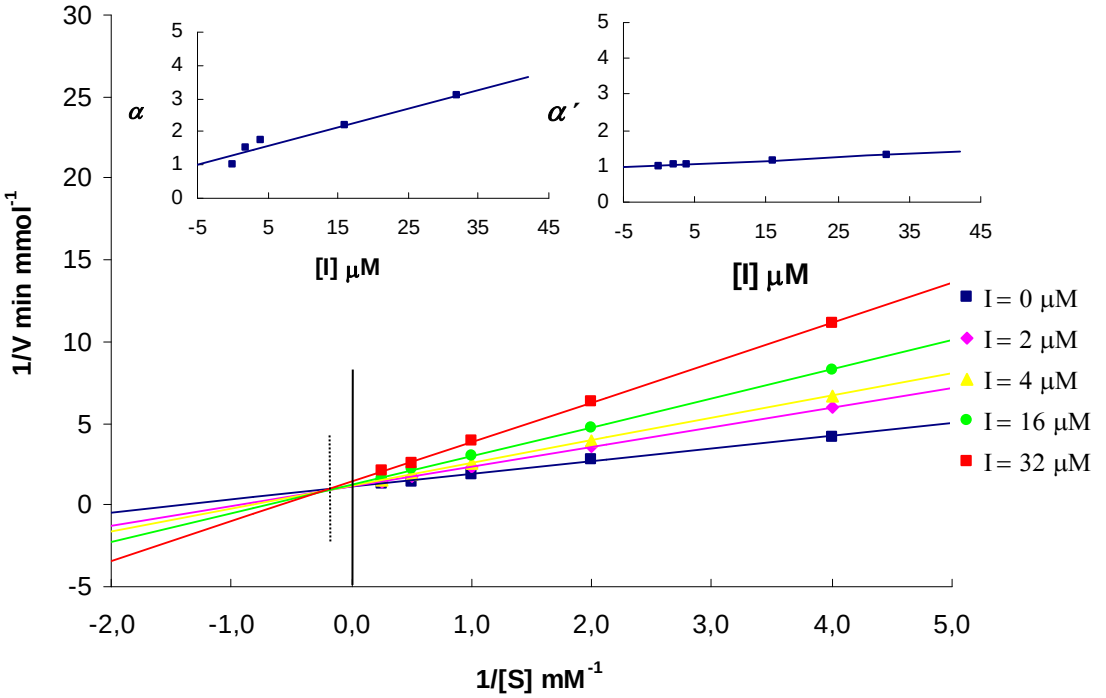


Figure S5. Lineweaver-Burk Plot for K_i determination (18 µM) and K_i' determination (108 µM) of **6** against *green coffee α-galactosidase* (pH 6.8).

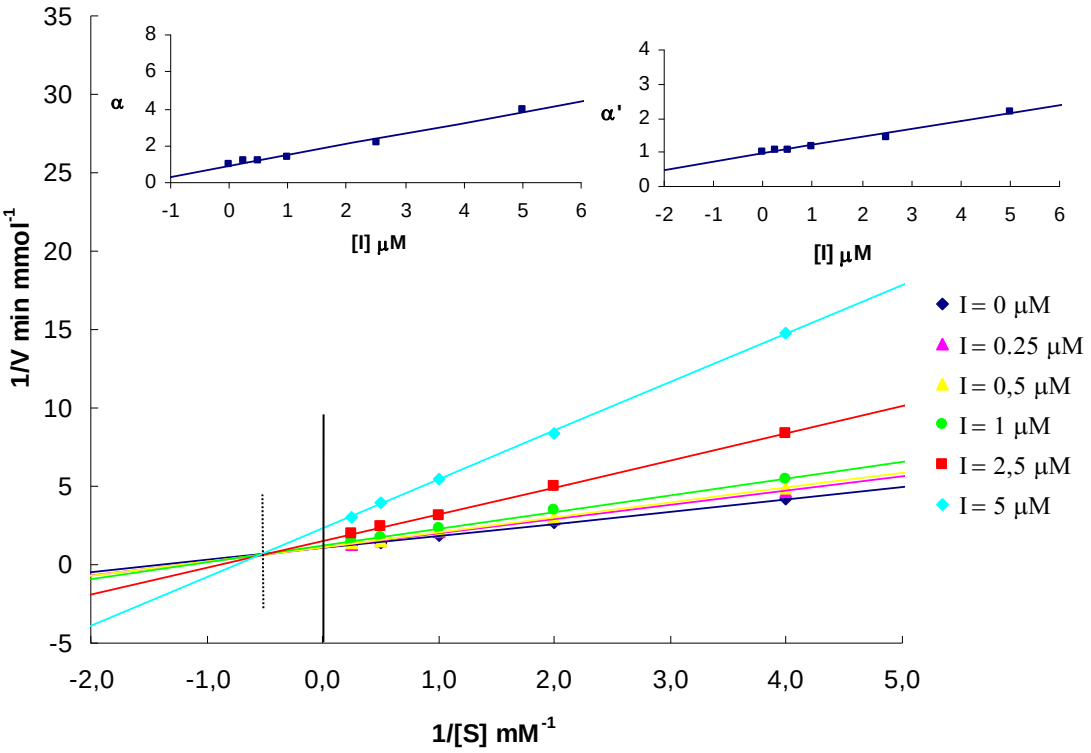


Figure S6. Lineweaver-Burk Plot for K_i determination (1.7 µM) and K_i' determination (4.3 µM) of **7** against *green coffee α-galactosidase* (pH 6.8).

GM and LM inhibition

GM and LM inhibition were measured as previously described (I. Nemčovičová, S. Šesták, D. Rendić, M. Plšková, J. Mucha, I. B. H.Wilson, *Glycoconj. J.* **2013**, *in press*). Briefly, mannosidase activities were measured with p-nitrophenyl-α-D-mannopyranoside (pNP-Man; Sigma) as substrate at a concentration of 2 mM (diluted from a 100 mM stock solution in dimethylsulfoxide) in 100 mM of acetate buffer at pH 6.0 or at pH 5.2 for GM and LM, respectively. 0.5-2 µl of enzyme and 10 µl of the inhibitor in 50% DMSO (or the same volume of 50% DMSO in a control reaction) were incubated for 3 h at 37 °C (total volume of 50 µl). In case of GM, the reaction was supplemented with 0.2 mM $CoCl_2$ (final concentration). The reactions were terminated with ten volumes (0.5 ml) of a 100 mM sodium carbonate solution. The formation of p-nitrophenol was measured at 410 nm with a spectrophotometer.

Sample preparation for AFM imaging

JBαMan was suspended in 20 mM citrate buffer pH 5.5. The multivalent iminosugars were suspended either in citrate or citrate/DMSO (or methanol) mixtures. Mixtures of JBαMan/DNJ 1:1 (mol/mol) in 20 mM citrate buffer pH 5.5 (final concentration of JBαMan and DNJ 4.54 nM) and incubated for 1h at room temperature. Freshly cleaved mica squares (16 mm^2) were glued onto steel sample discs (Agar Scientific, England) using Epotek 377 (Polytec, France). A 150 µL portion of the JBαMan/DNJ suspensions were then deposited onto the mica samples, and were allowed to adsorb on the solid surface for 20 h at room temperature. Subsequently, samples were rinsed 3 times with ultrapure water and they were dried in a dessicator under vacuum for 1 h. The samples were then left overnight under ambient conditions before AFM imaging.

AFM imaging

The samples were investigated using a commercial AFM (NanoScope III MultiMode AFM, Veeco Metrology LLC, Santa Barbara, CA) equipped with a 125 µm × 125 µm × 5 µm scanner (J-scanner). Topographic images were recorded in air in Tapping™ mode using RTESP cantilevers (Veeco Metrology Group, Santa Barbara, CA) with a nominal spring constant of 40 N/m (manufacturer specified), with a minimal applied force (<200 pN), at a scan rate of 2 Hz and a drive amplitude of ~300 kHz. The curvature radius of silicon nitride tips was ~10 nm. Images were obtained at room temperature (21–22°C) in air. All images (512 × 512 pixels) shown in this paper are flattened raw data.

RESULTS

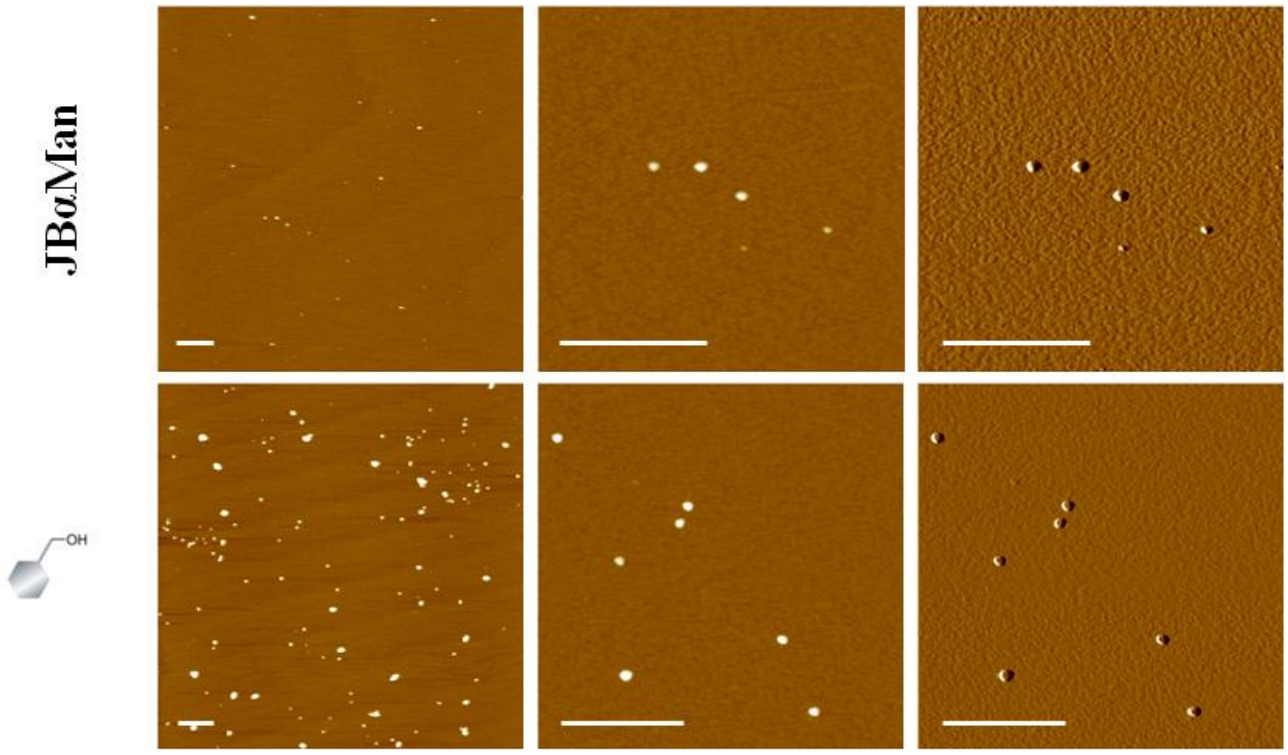


Figure S7: AFM height (left and middle) and deflection (right column) images of JBαMan alone or interacting with monovalent ligand (z=10 nm in height images ; scale bar is 500 nm).

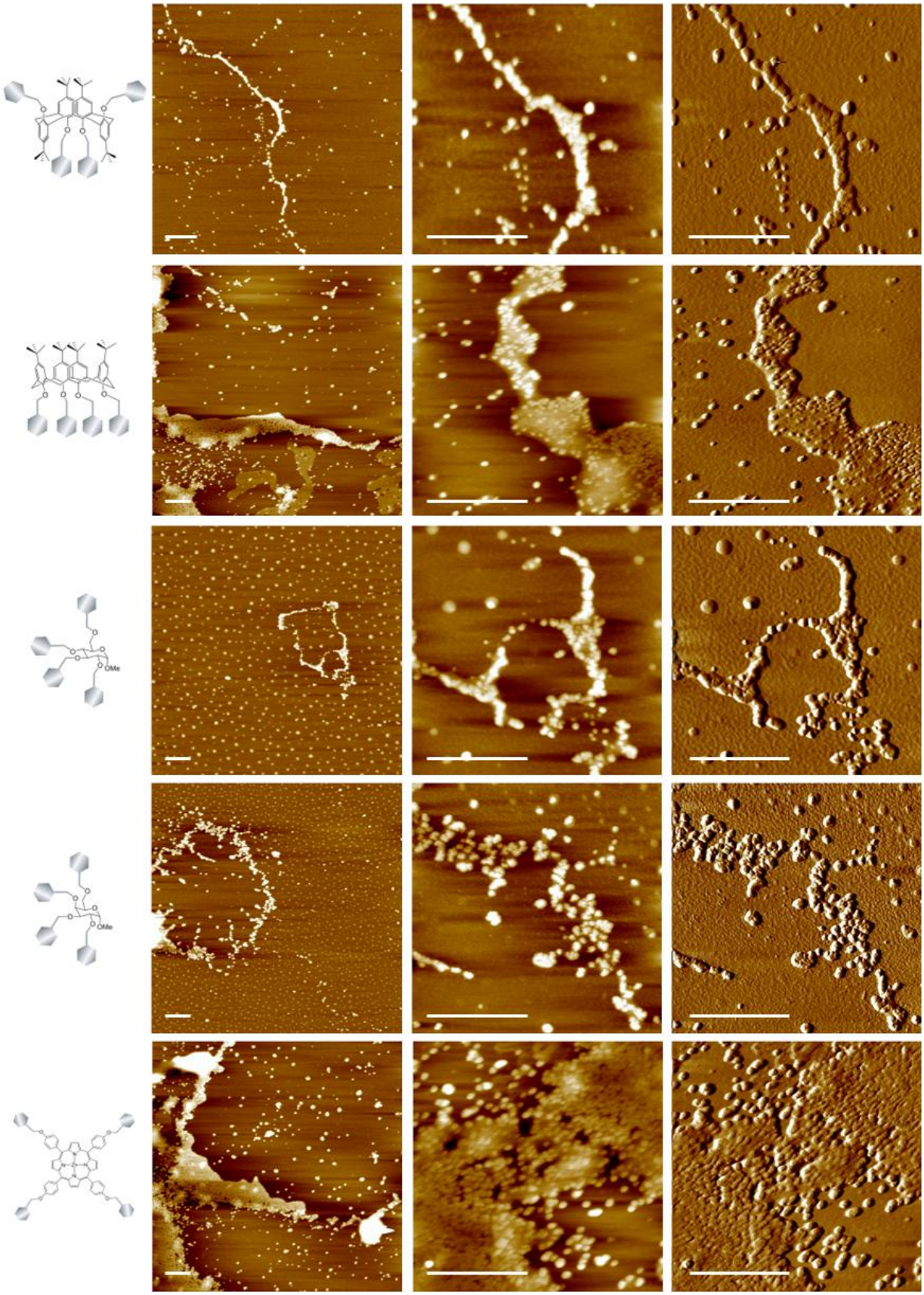


Figure S8: AFM height (left and middle) and deflection (right column) images of JBαMan interacting with tetravalent ligands (z=10 nm in height images ; scale bar is 500 nm).

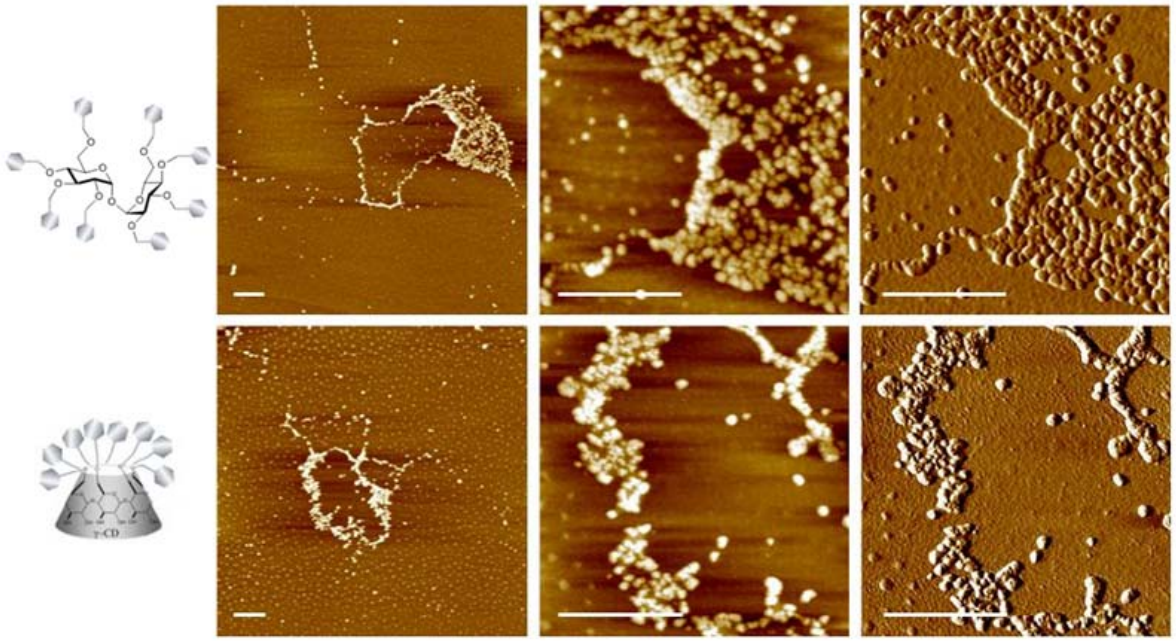


Figure S9: AFM height (left and middle) and deflection (right column) images of JBαMan interacting with mannose octavalent ligands (z=10 nm in height images ; scale bar is 500 nm).

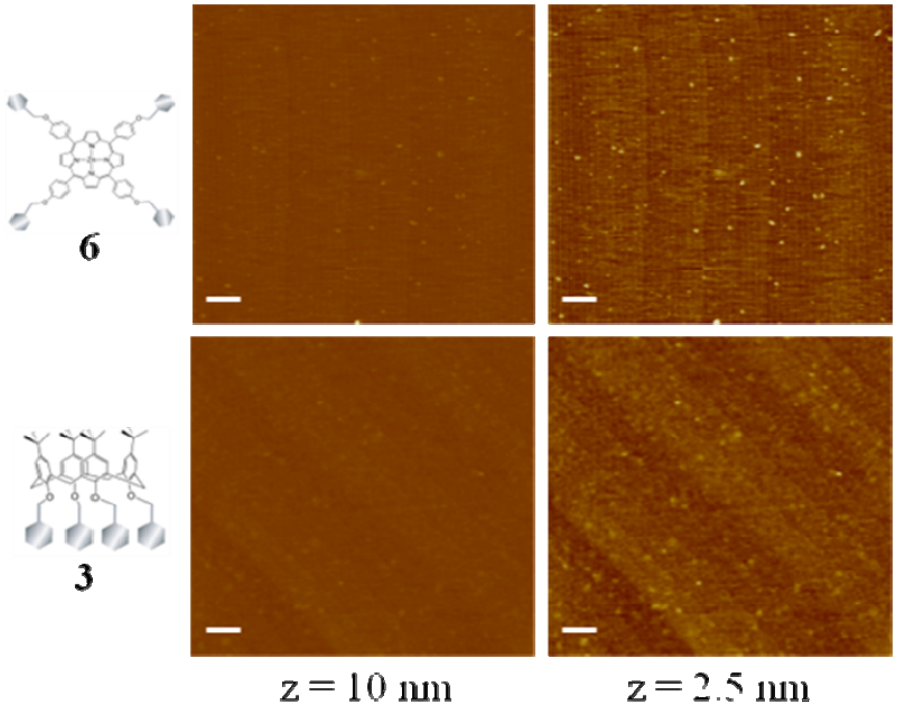


Figure S10: AFM height images tetravalent ligands **3** and **6** adsorbed on mica without JBαMan (scale bar is 500 nm). Note that left and right column are the same images. Indeed, images of the left column are presented with the same z-scale as the ones with proteins (see previous figures) and the z-scale was adjusted at 2.5 nm to reveal the presence of very thin structures (right column).

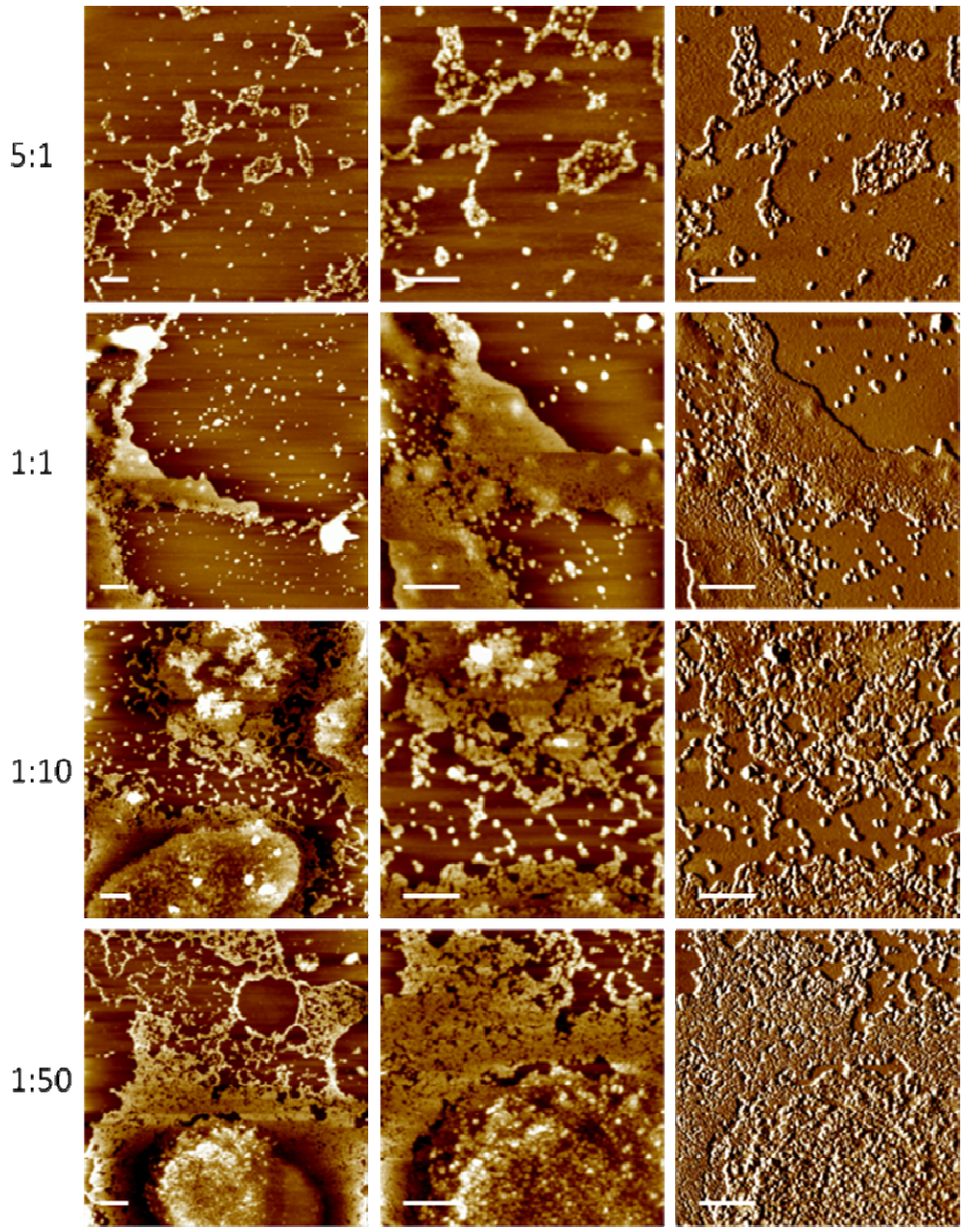


Figure S11: AFM height (left and middle) and deflection (right column) images of JBαMan interacting with tetravalent ligands at different JBαMan/ligand molar ratios (z=10 nm in height images ; scale bar is 500 nm). By increasing the amount of ligand, the surface covered with aggregates increases in AFM images.

DLS measurements

Dynamic light scattering (DLS) was used to determine the hydrodynamic diameter of protein/ligands complexes in solution (20 mM citrate buffer pH 5.5). Samples were prepared by incubating the protein with the different ligands (1:10, molar ratio) at 4.54 µM during one hour at ambient temperature under gentle agitation. The measurements were performed with the Zetasizer (Nano ZS, Malvern Instruments Ltd., UK). The mean hydrodynamic diameter was determined from the autocorrelation function of the intensity of light scattered from the particles. The software used was DTS Nano version 5.03, supplied by the manufacturer (Malvern Instruments Ltd.).

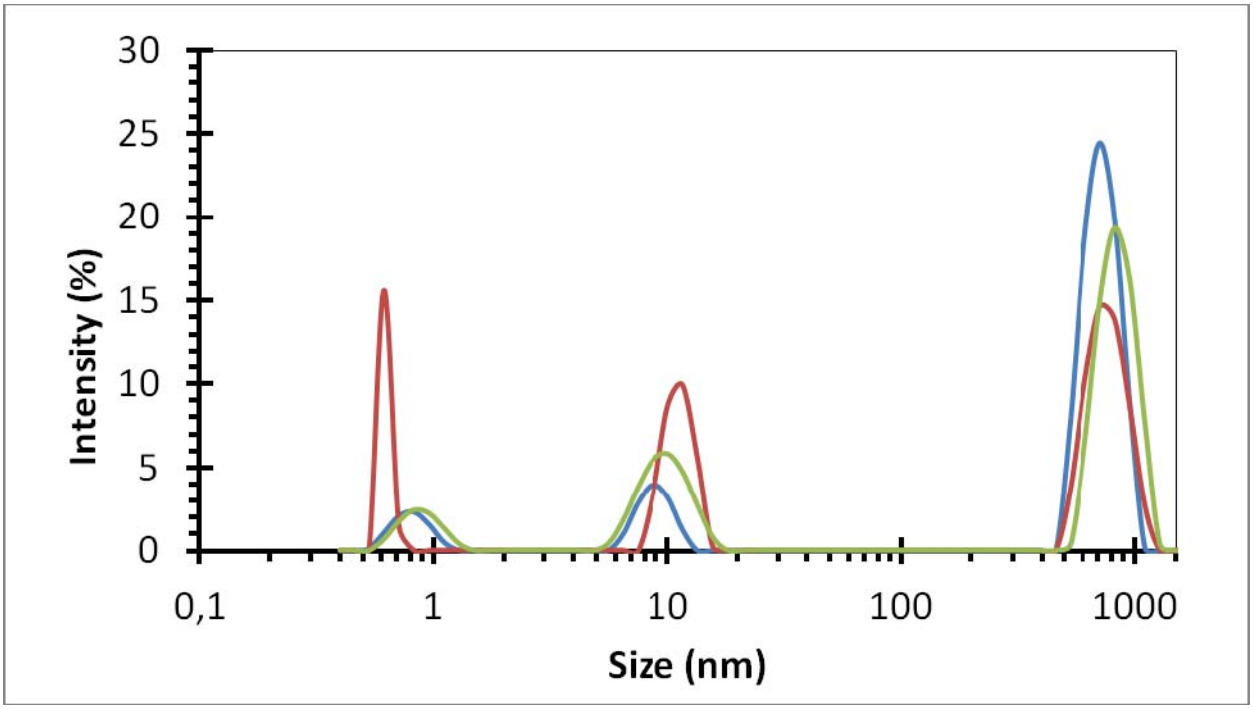
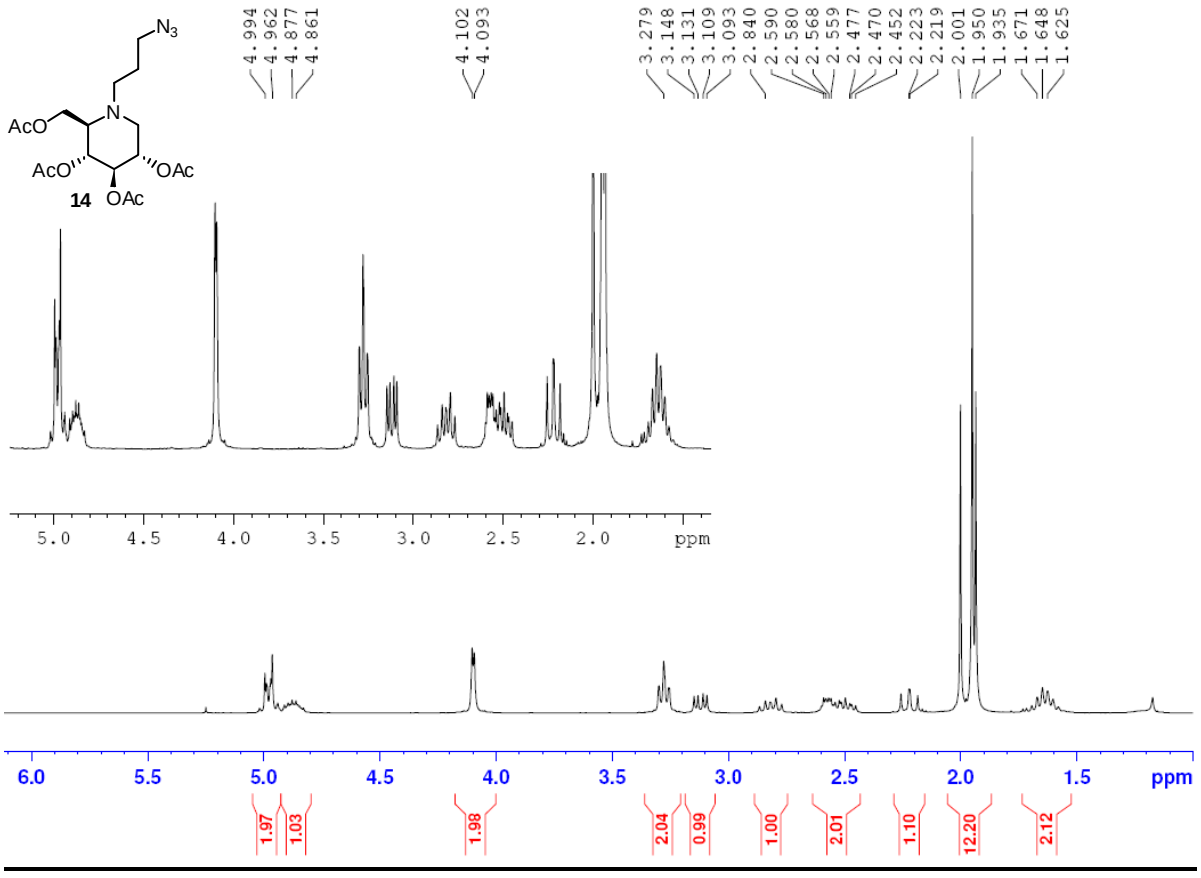
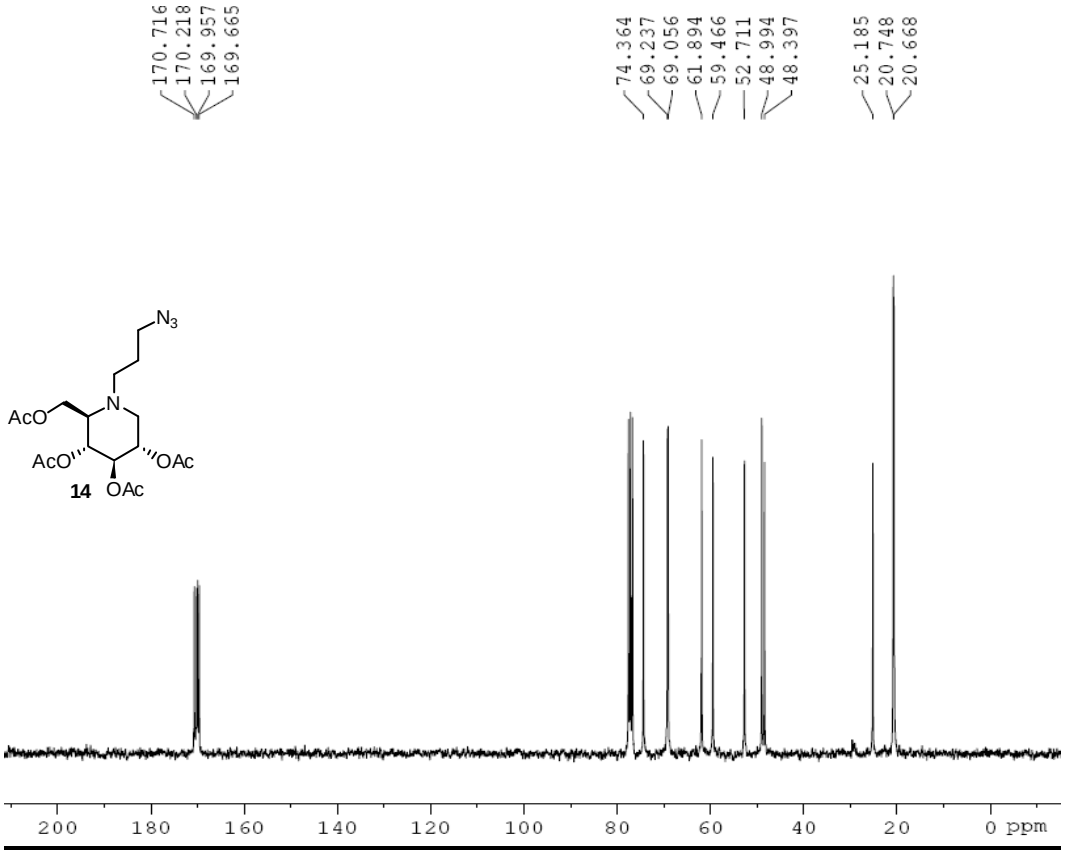
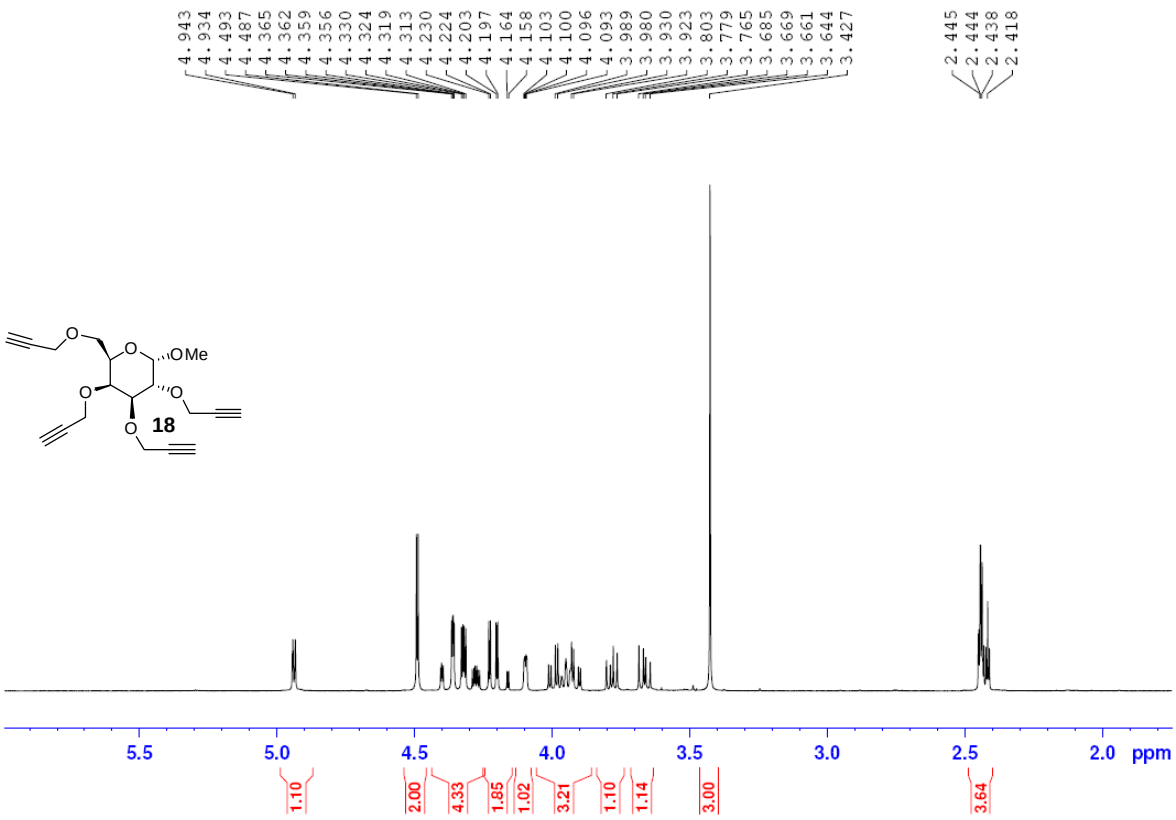
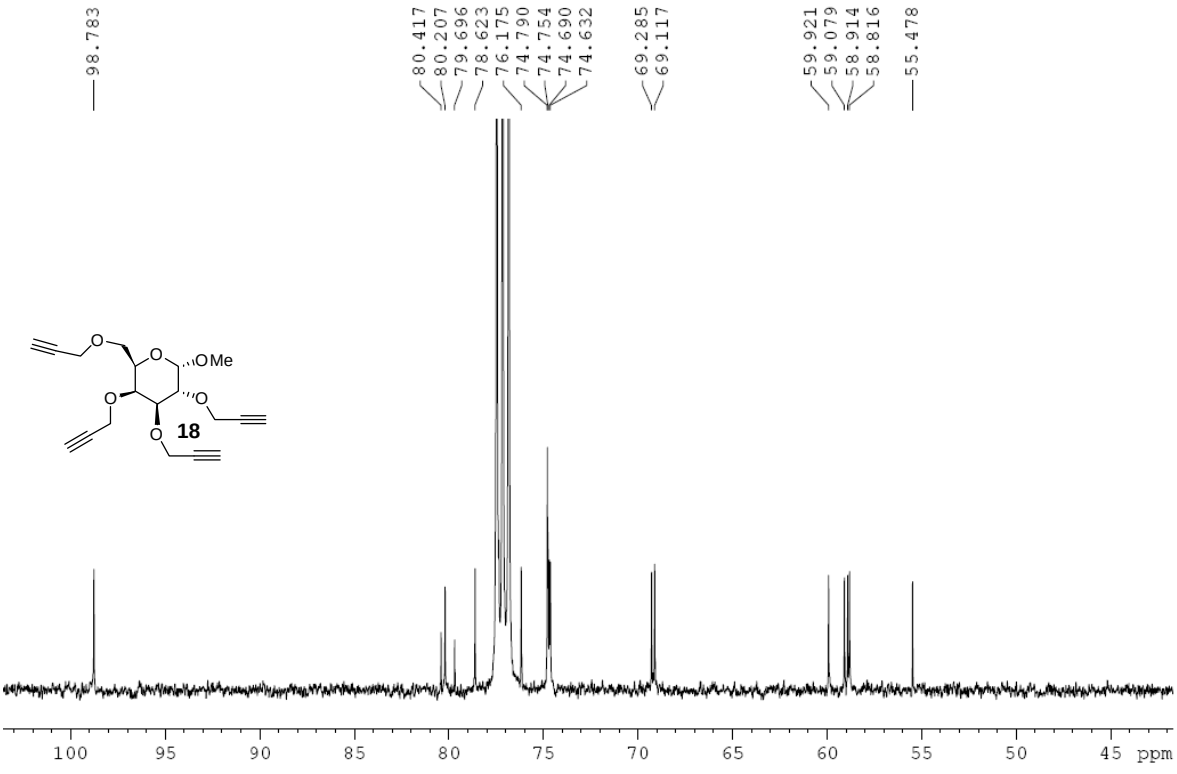


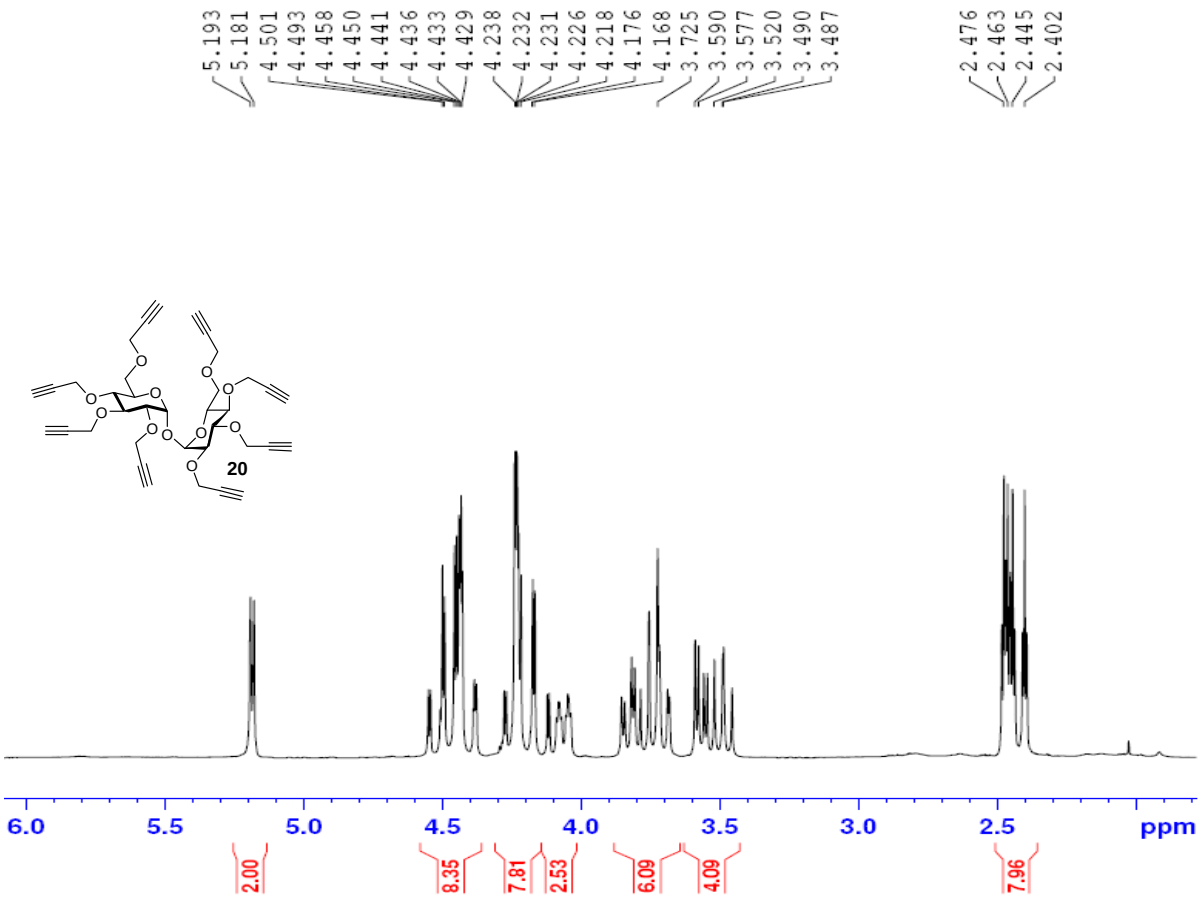
Figure S12: Typical size distribution graph obtained by DLS on mixtures of JBαMan/**6** in 1:10 molar ratio. Theses results show the coexistence of large complexes (731.9 ± 161.9) with the free protein (10.4 ± 2.2) and the free ligand **6** (less than 1 nm).

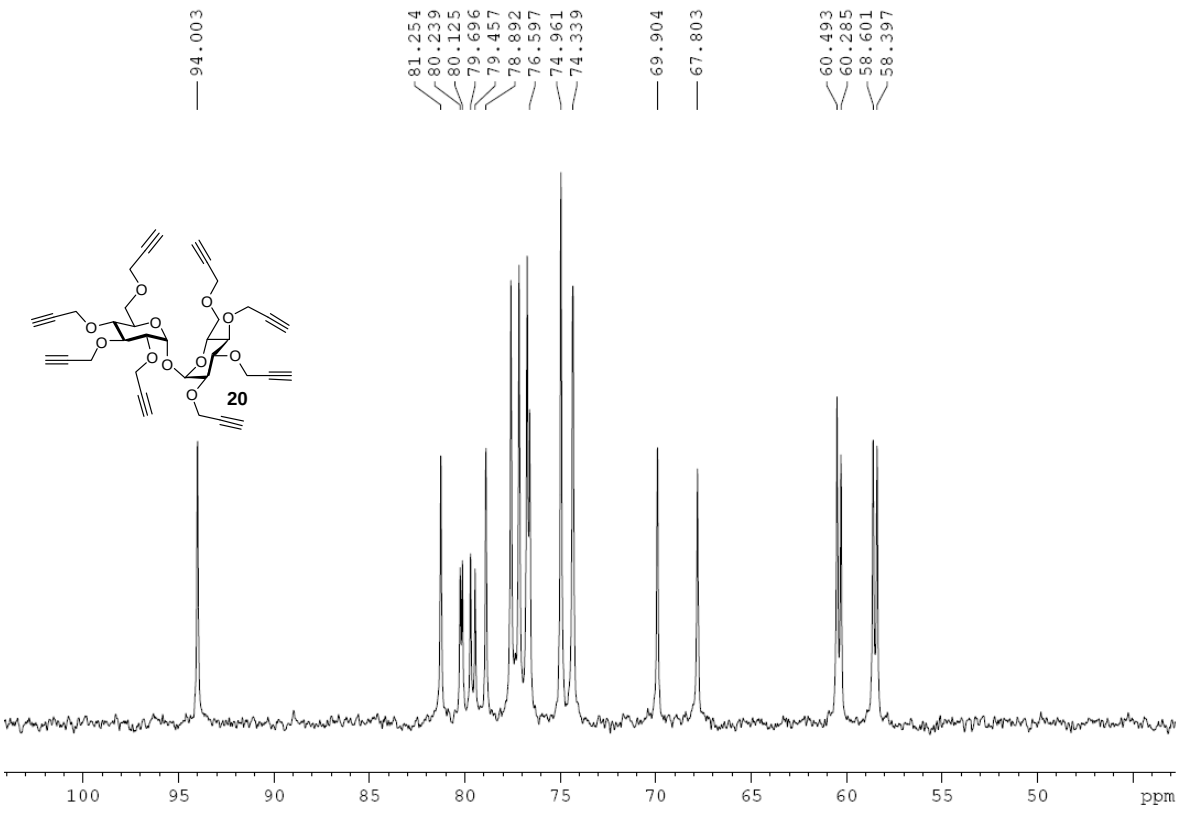

4.994
4.962
4.877
4.861
4.102
4.093
3.279
3.148
3.131
3.109
3.093
2.840
2.590
2.580
2.568
2.559
2.477
2.470
2.452
2.223
2.219
2.001
1.950
1.935
1.671
1.648
1.625
N3
AcO
AcO
OAc
OAc
14
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.97
1.03
1.98
2.04
0.99
1.00
2.01
1.10
12.20
2.12

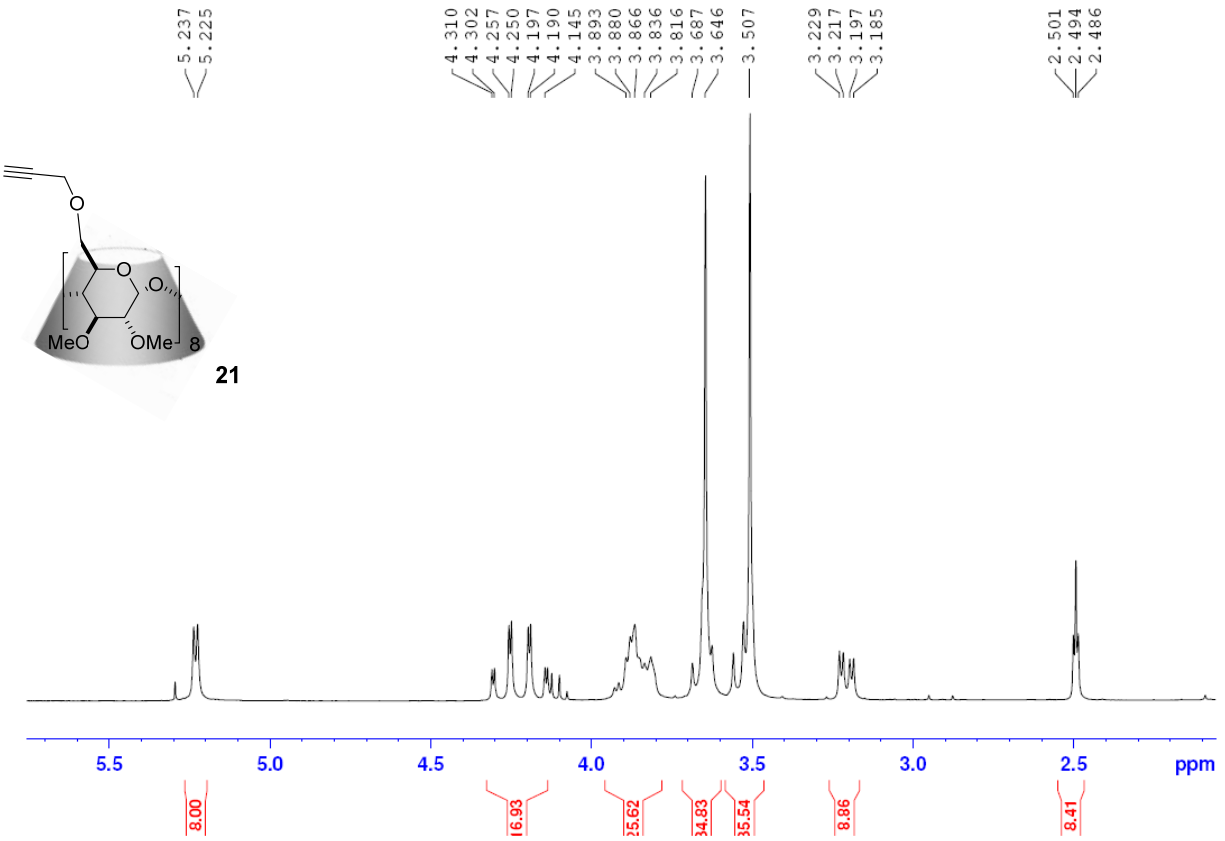

170.716
170.218
169.957
169.665
74.364
69.237
69.056
61.894
59.466
52.711
48.994
48.397
25.185
20.748
20.668
N3
AcO
AcO
OAc
OAc
14
200
180
160
140
120
100
80
60
40
20
0 ppm

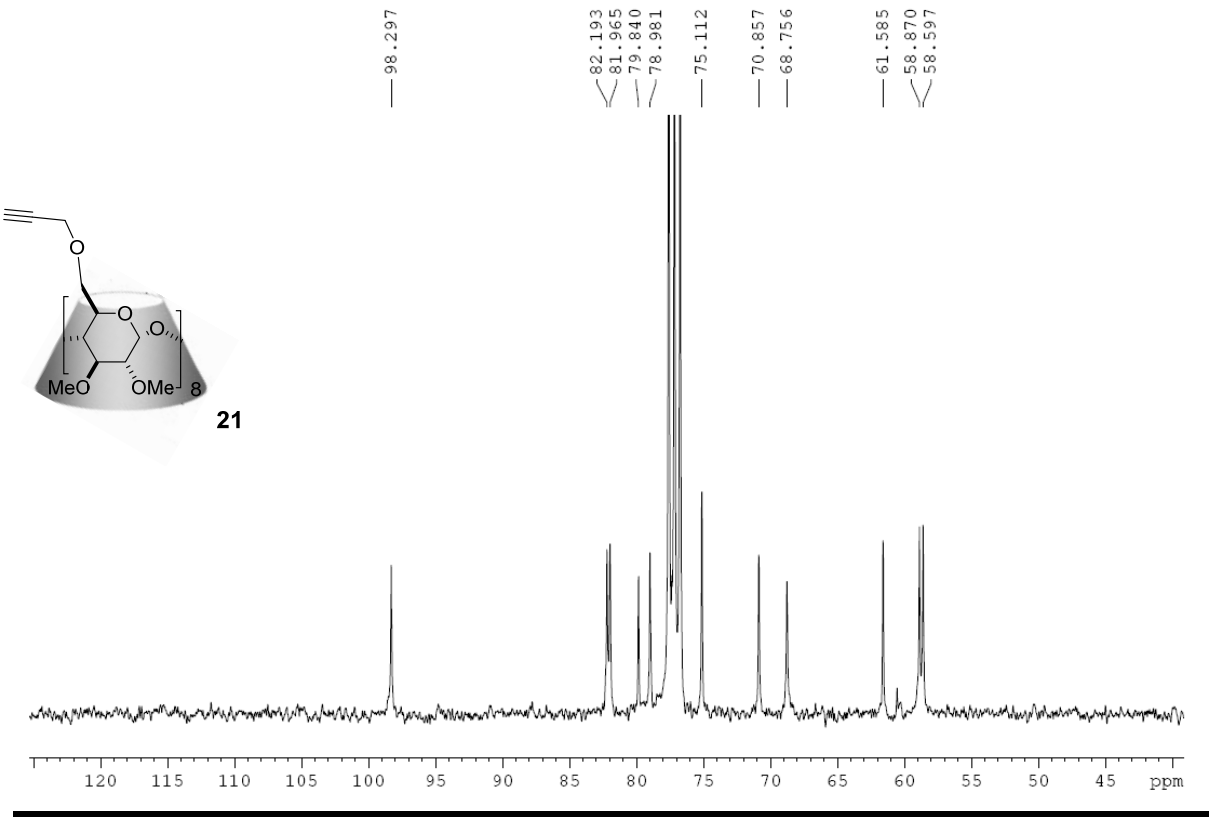

4.943
4.934
4.493
4.487
4.365
4.362
4.359
4.356
4.330
4.324
4.319
4.313
4.230
4.224
4.203
4.197
4.164
4.158
4.103
4.100
4.096
4.093
3.989
3.980
3.930
3.923
3.803
3.779
3.765
3.685
3.669
3.661
3.644
3.427
2.445
2.444
2.438
2.418
OMe
18
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0 ppm
1.10
2.00
4.33
1.85
1.02
3.21
1.10
1.14
3.00
3.64

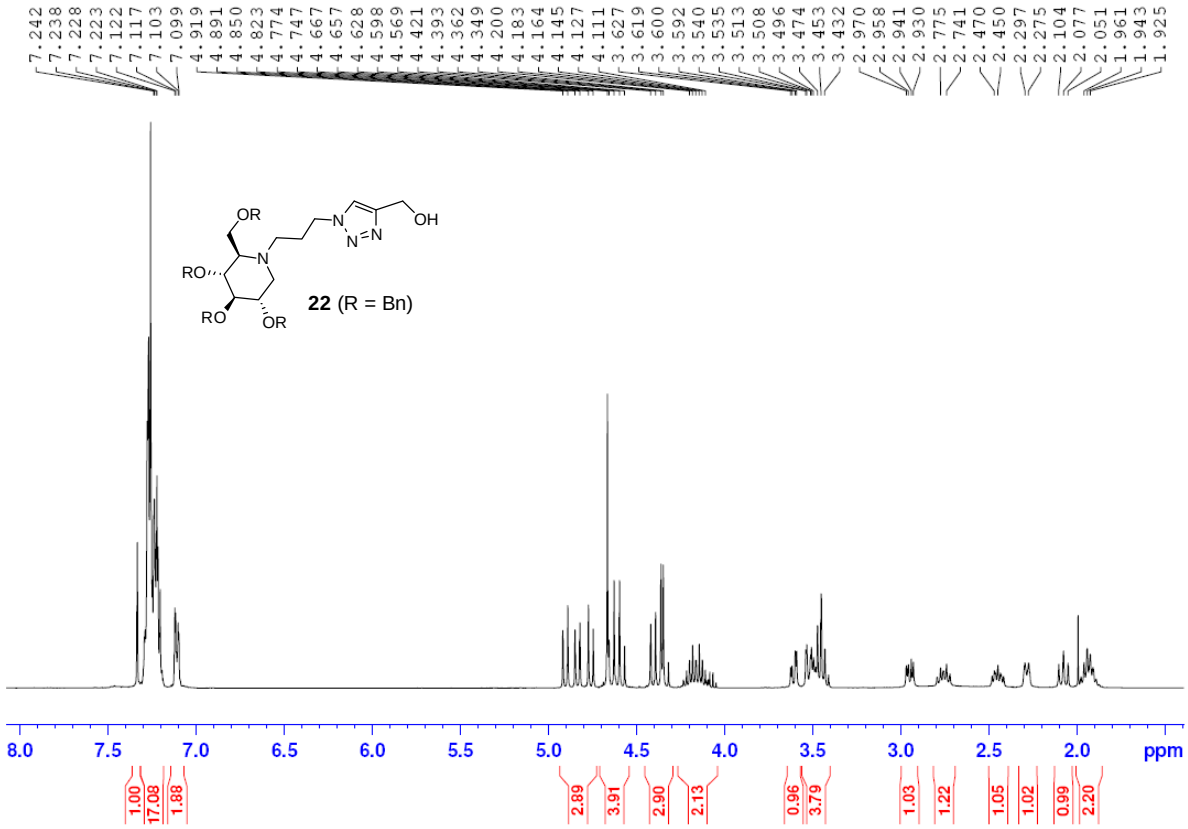

98.783
80.417
80.207
79.696
78.623
76.175
74.790
74.754
74.690
74.632
69.285
69.117
59.921
59.079
58.914
58.816
55.478
OMe
18
100
95
90
85
80
75
70
65
60
55
50
45 ppm

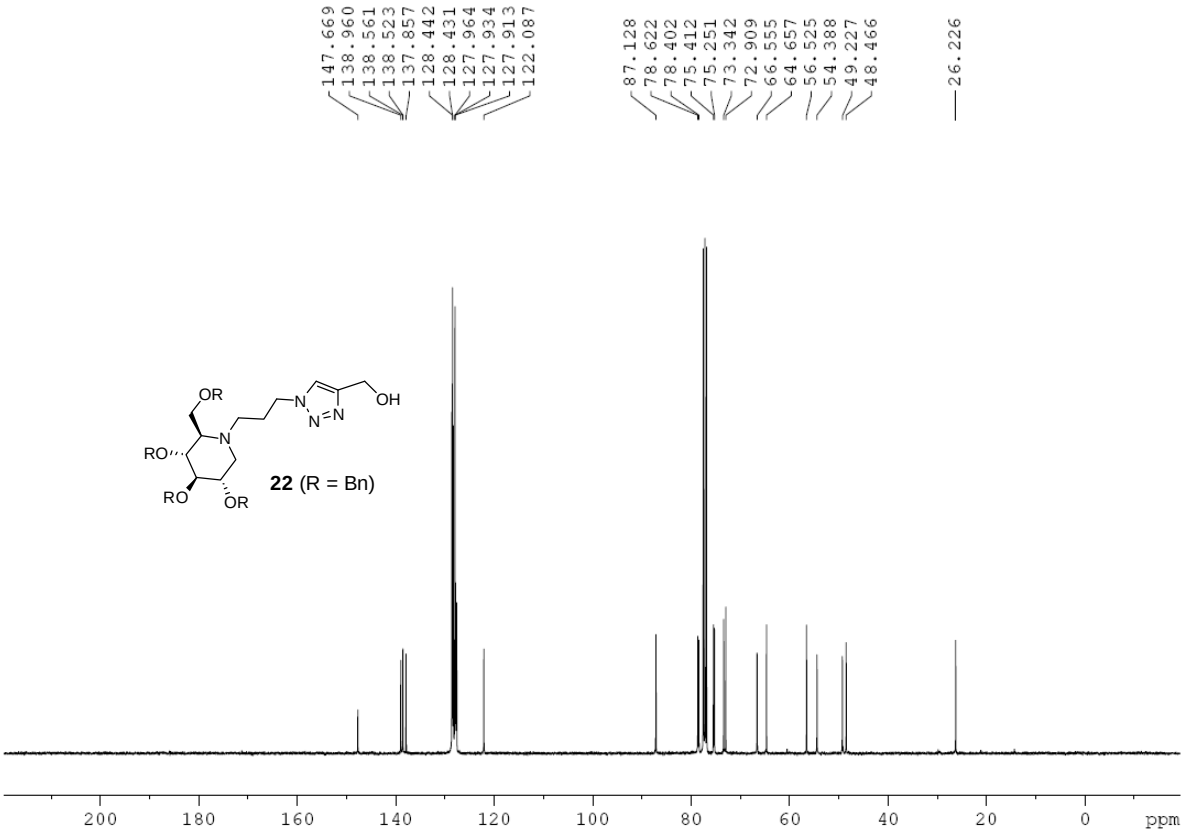

5.193
5.181
4.501
4.493
4.458
4.450
4.441
4.436
4.433
4.429
4.238
4.232
4.231
4.226
4.218
4.176
4.168
3.725
3.590
3.577
3.520
3.490
3.487
2.476
2.463
2.445
2.402
20
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
2.00
8.35
7.81
2.53
6.09
4.09
7.96

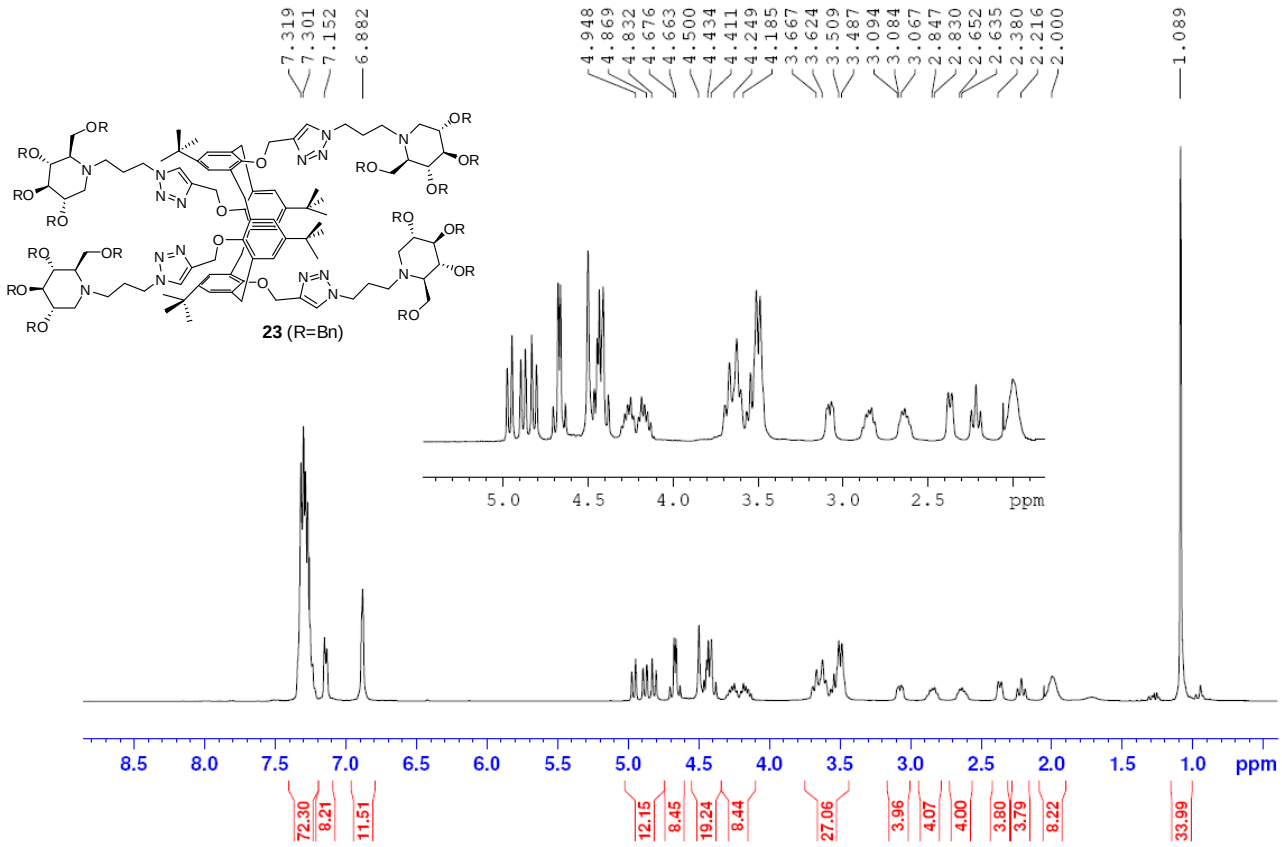

94.003
81.254
80.239
80.125
79.696
79.457
78.892
76.597
74.961
74.339
69.904
67.803
60.493
60.285
58.601
58.397
20
100
95
90
85
80
75
70
65
60
55
50
ppm

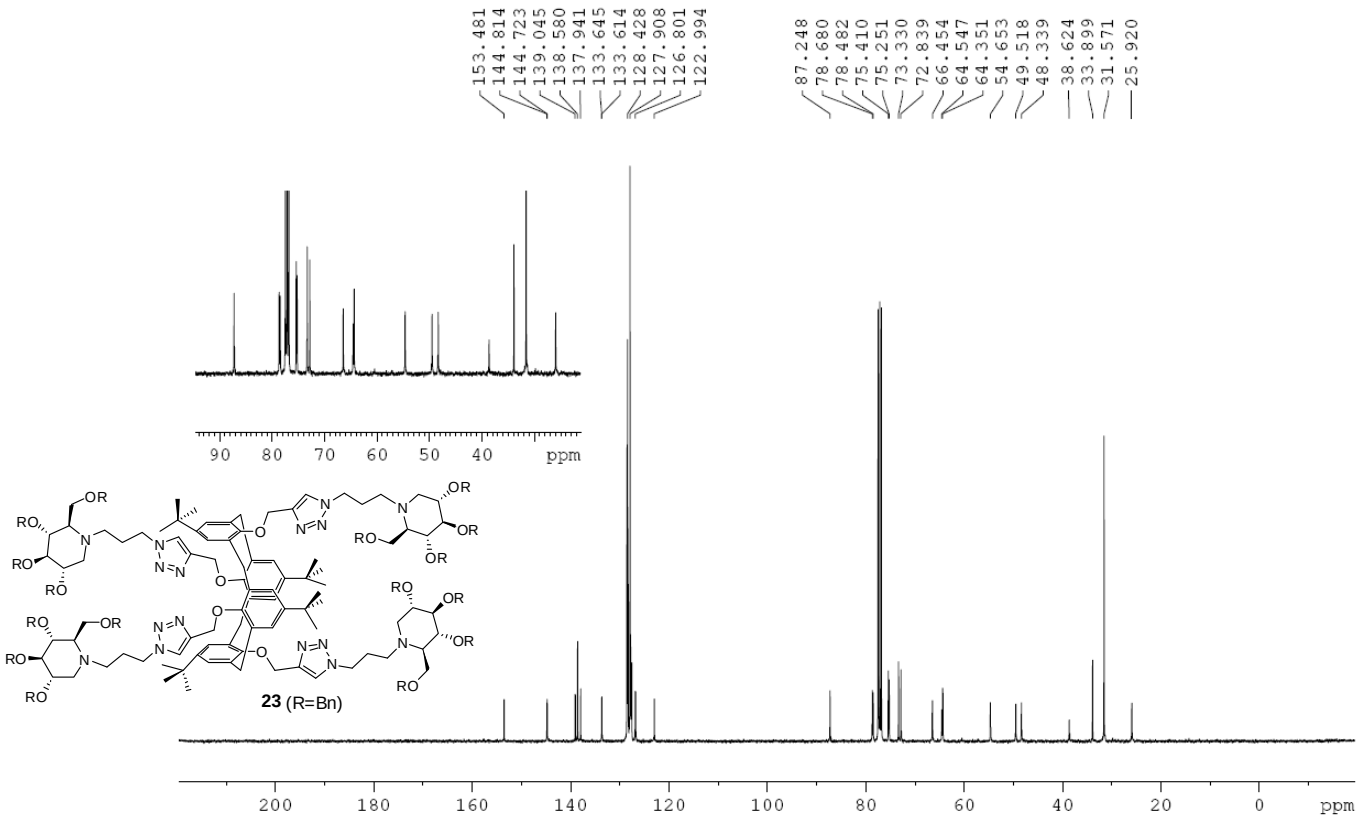

5.237
5.225
4.310
4.302
4.257
4.250
4.197
4.190
4.145
3.893
3.880
3.866
3.836
3.816
3.687
3.646
3.507
3.229
3.217
3.197
3.185
2.501
2.494
2.486
MeO
OMe
8
21
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
8.00
16.93
25.62
34.83
35.54
8.86
8.41

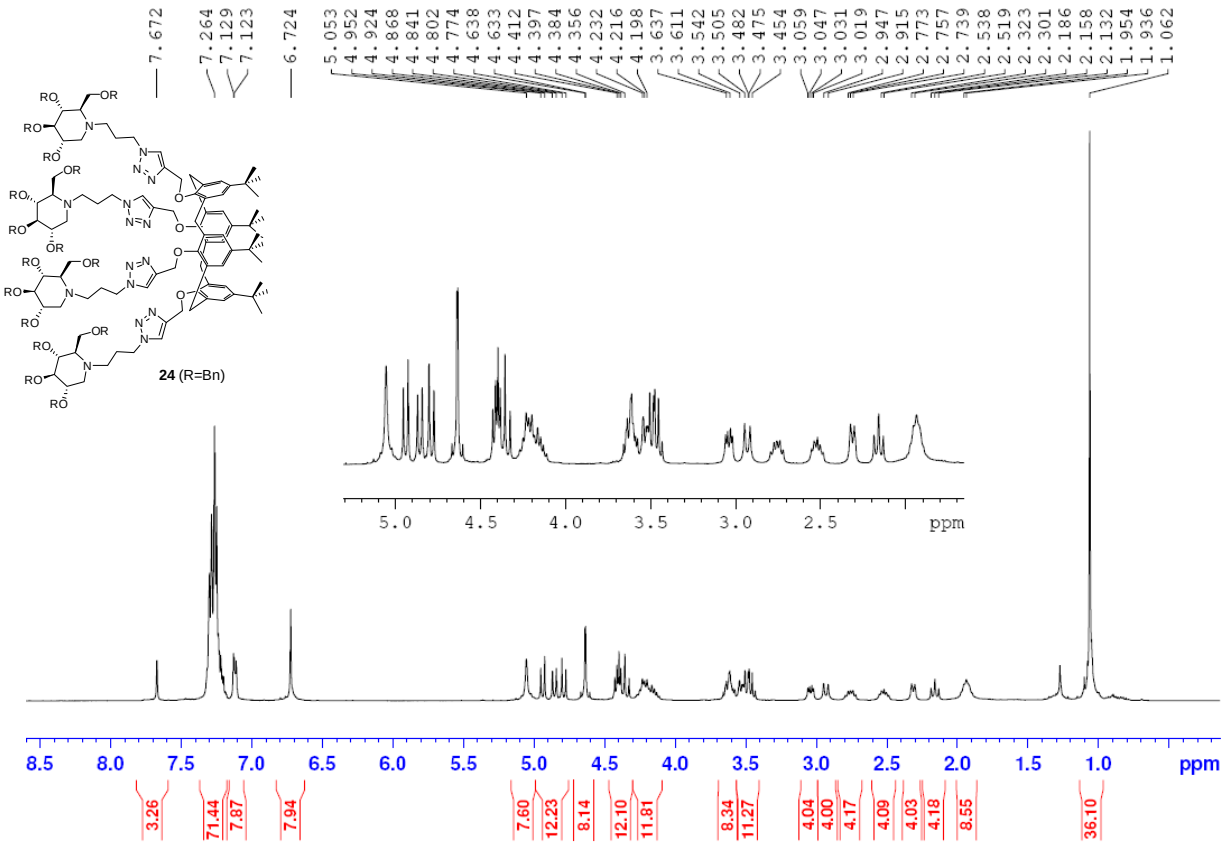

98.297
82.193
81.965
79.840
78.981
75.112
70.857
68.756
61.585
58.870
58.597
MeO
OMe
8
21
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
ppm

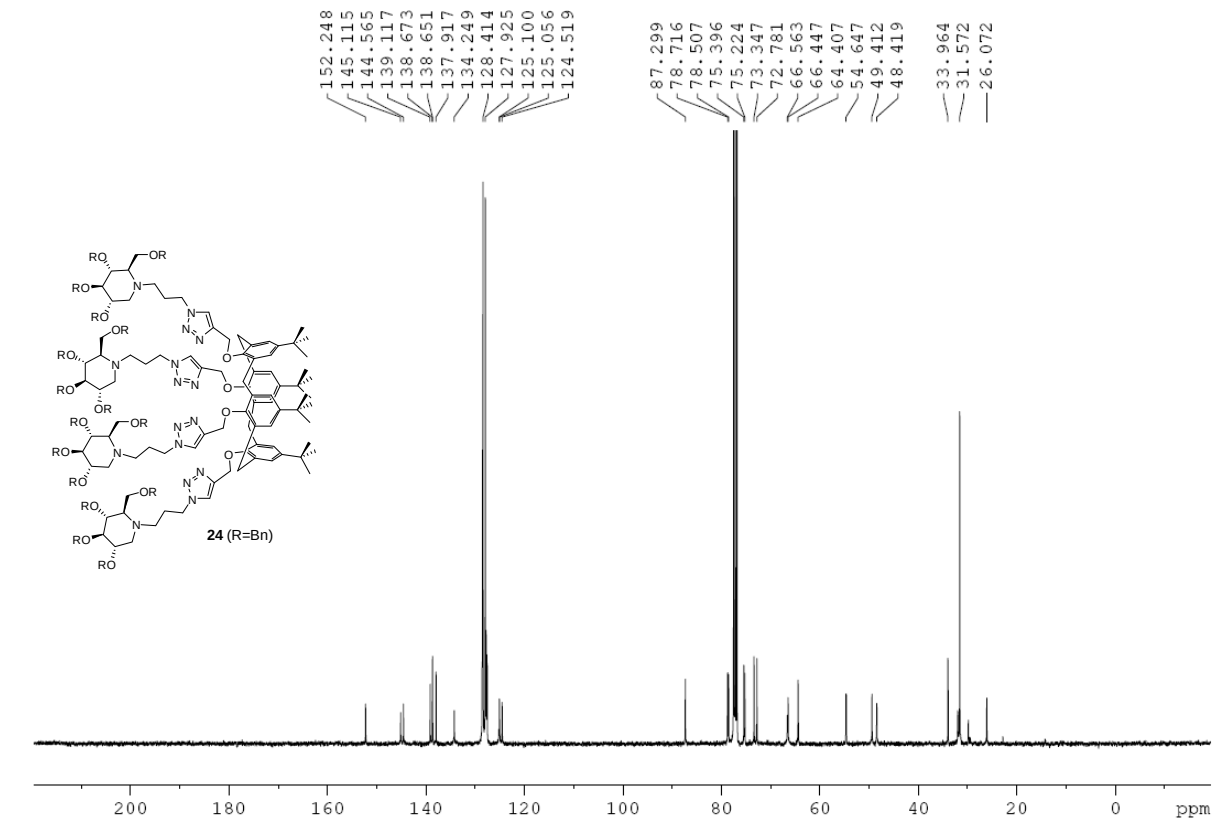

7.242
7.238
7.228
7.223
7.122
7.117
7.103
7.099
4.919
4.891
4.850
4.823
4.774
4.747
4.667
4.657
4.628
4.598
4.569
4.421
4.393
4.362
4.349
4.200
4.183
4.164
4.145
4.127
4.111
3.627
3.619
3.600
3.592
3.540
3.535
3.513
3.508
3.496
3.474
3.453
3.432
2.970
2.958
2.941
2.930
2.775
2.741
2.470
2.450
2.297
2.275
2.104
2.077
2.051
1.961
1.943
1.925
OR
OH
RO
RO
OR
22 (R = Bn)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
17.08
1.88
2.89
3.91
2.90
2.13
0.96
3.79
1.03
1.22
1.05
1.02
0.99
2.20

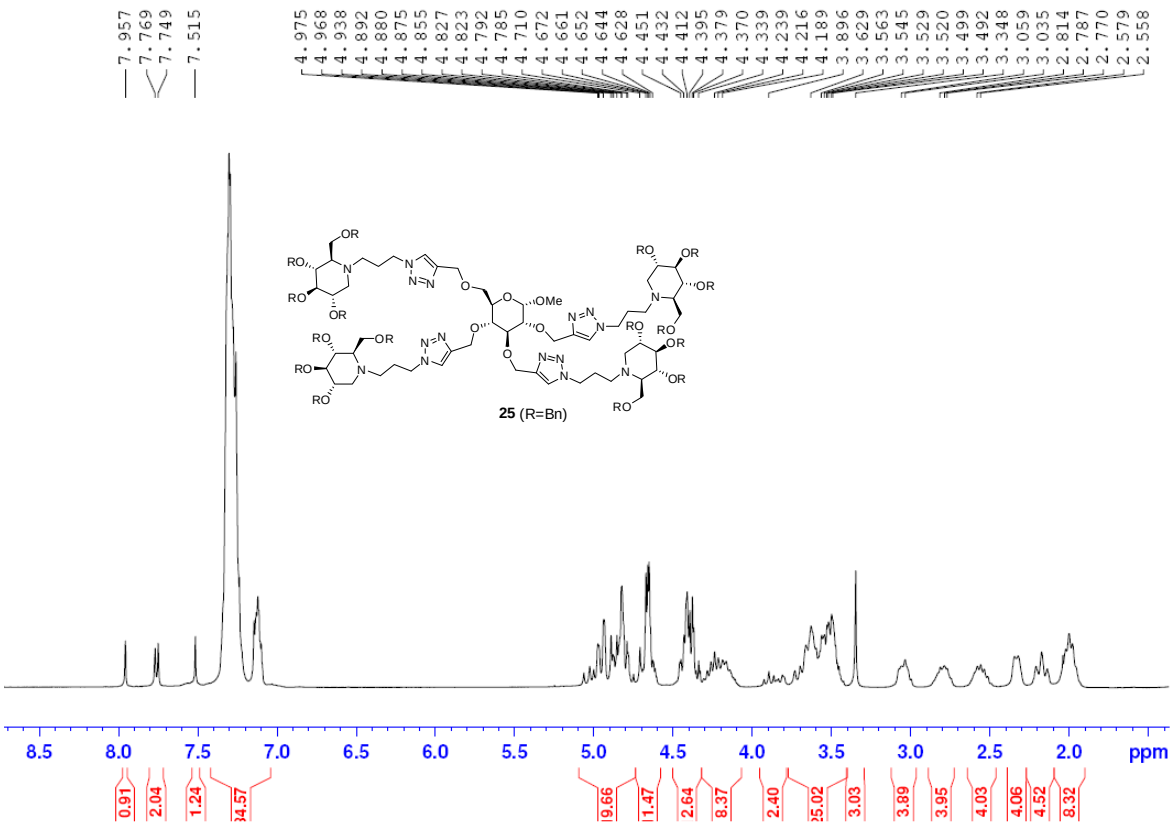

147.669
138.960
138.561
138.523
137.857
128.442
128.431
127.964
127.934
127.913
122.087
87.128
78.622
78.402
75.412
75.251
73.342
72.909
66.555
64.657
56.525
54.388
49.227
48.466
26.226
OR
OH
RO
RO
OR
22 (R = Bn)
200
180
160
140
120
100
80
60
40
20
0
ppm

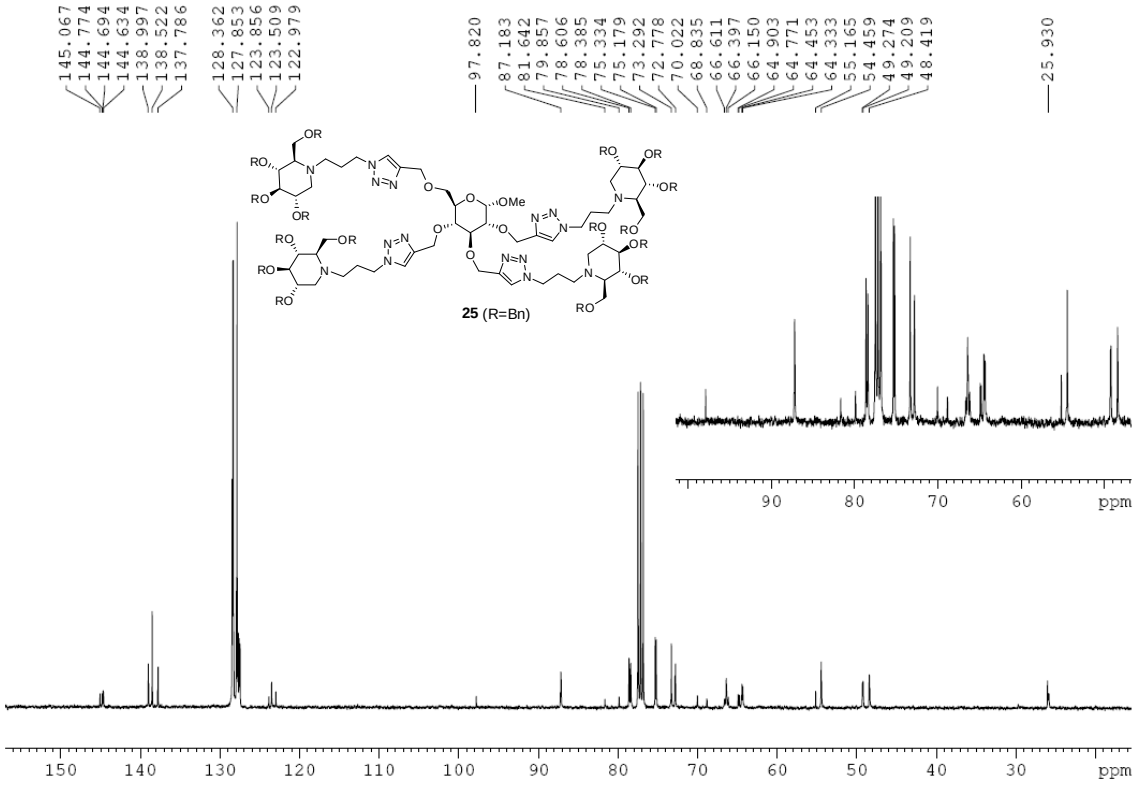

7.319
7.301
7.152
6.882
4.948
4.869
4.832
4.676
4.663
4.500
4.434
4.411
4.249
4.185
3.667
3.624
3.509
3.487
3.094
3.084
3.067
2.847
2.830
2.652
2.635
2.380
2.216
2.000
1.089
23 (R=Bn)
5.0
4.5
4.0
3.5
3.0
2.5
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
72.30
8.21
11.51
12.15
8.45
19.24
8.44
27.06
3.96
4.07
4.00
3.80
3.79
8.22
33.99

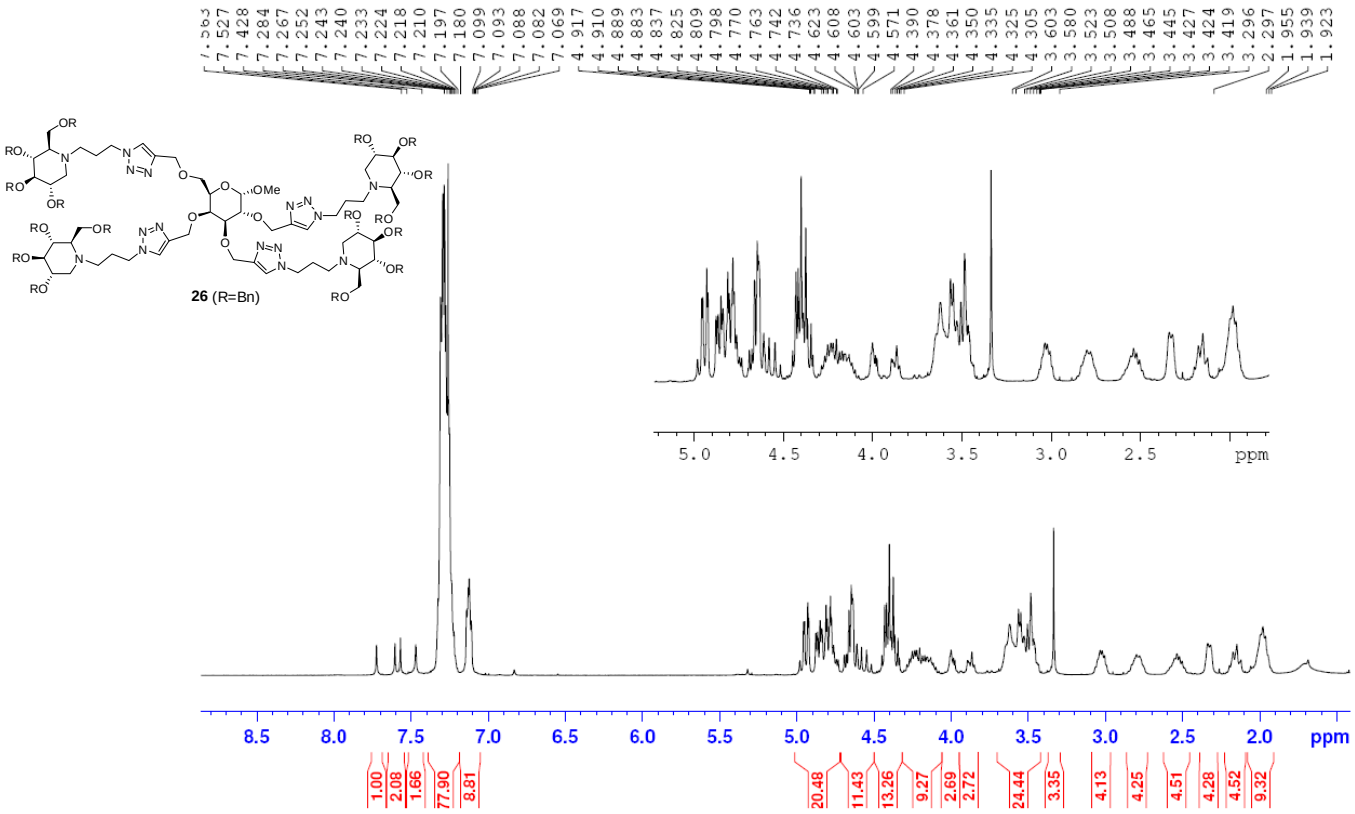

153.481
144.814
144.723
139.045
138.580
137.941
133.645
133.614
128.428
127.908
126.801
122.994
87.248
78.680
78.482
75.410
75.251
73.330
72.839
66.454
64.547
64.351
54.653
49.518
48.339
38.624
33.899
31.571
25.920
23 (R=Bn)
90
80
70
60
50
40
ppm
200
180
160
140
120
100
80
60
40
20
0
ppm

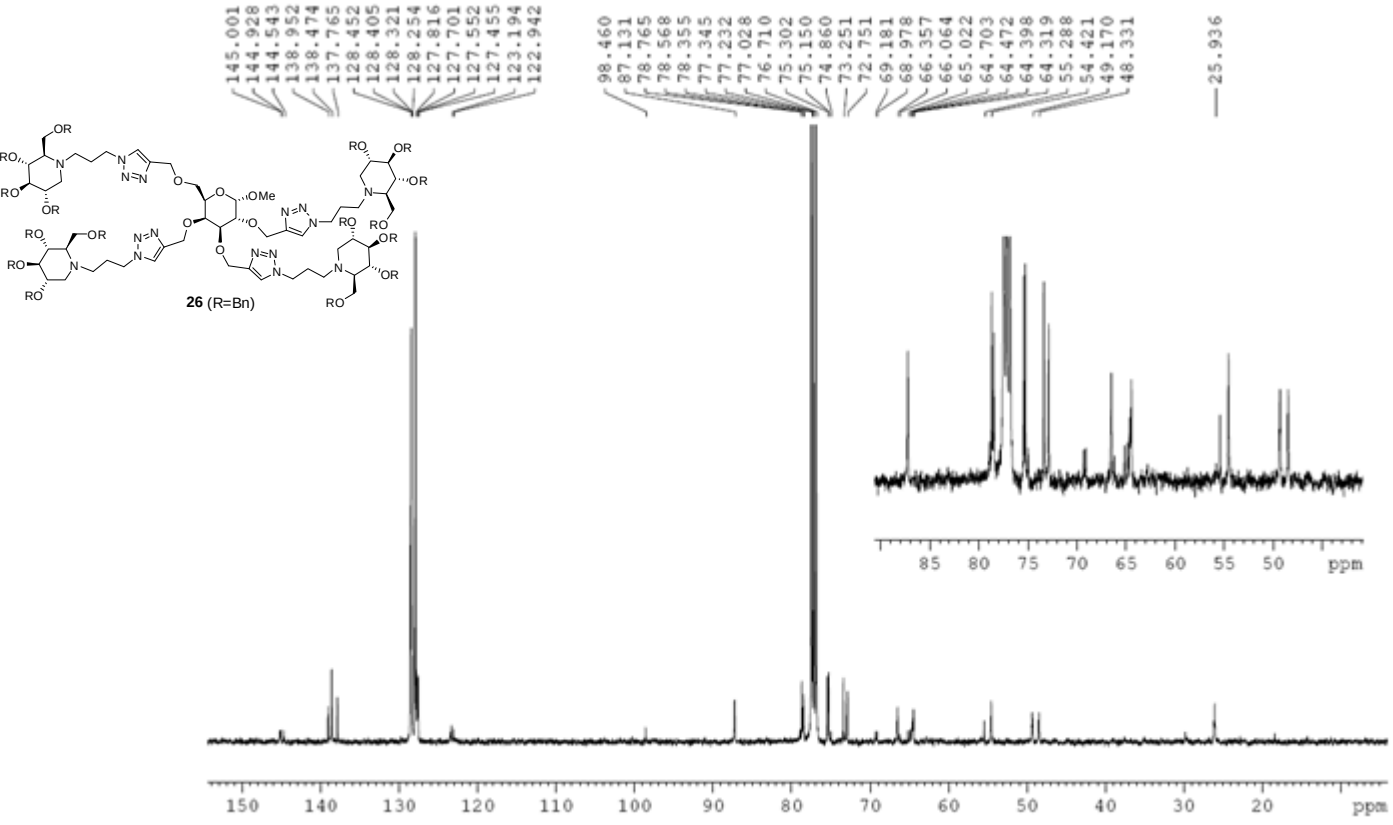

7.672
7.264
7.129
7.123
6.724
5.053
4.952
4.924
4.868
4.841
4.802
4.774
4.638
4.633
4.412
4.397
4.384
4.356
4.232
4.216
4.198
3.637
3.611
3.542
3.505
3.482
3.475
3.454
3.059
3.047
3.031
3.019
2.947
2.915
2.773
2.757
2.739
2.538
2.519
2.323
2.301
2.186
2.158
2.132
1.954
1.936
1.062
24 (R=Bn)
5.0
4.5
4.0
3.5
3.0
2.5
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
3.26
71.44
7.87
7.94
7.60
12.23
8.14
12.10
11.81
8.34
11.27
4.04
4.00
4.17
4.09
4.03
4.18
8.55
36.10

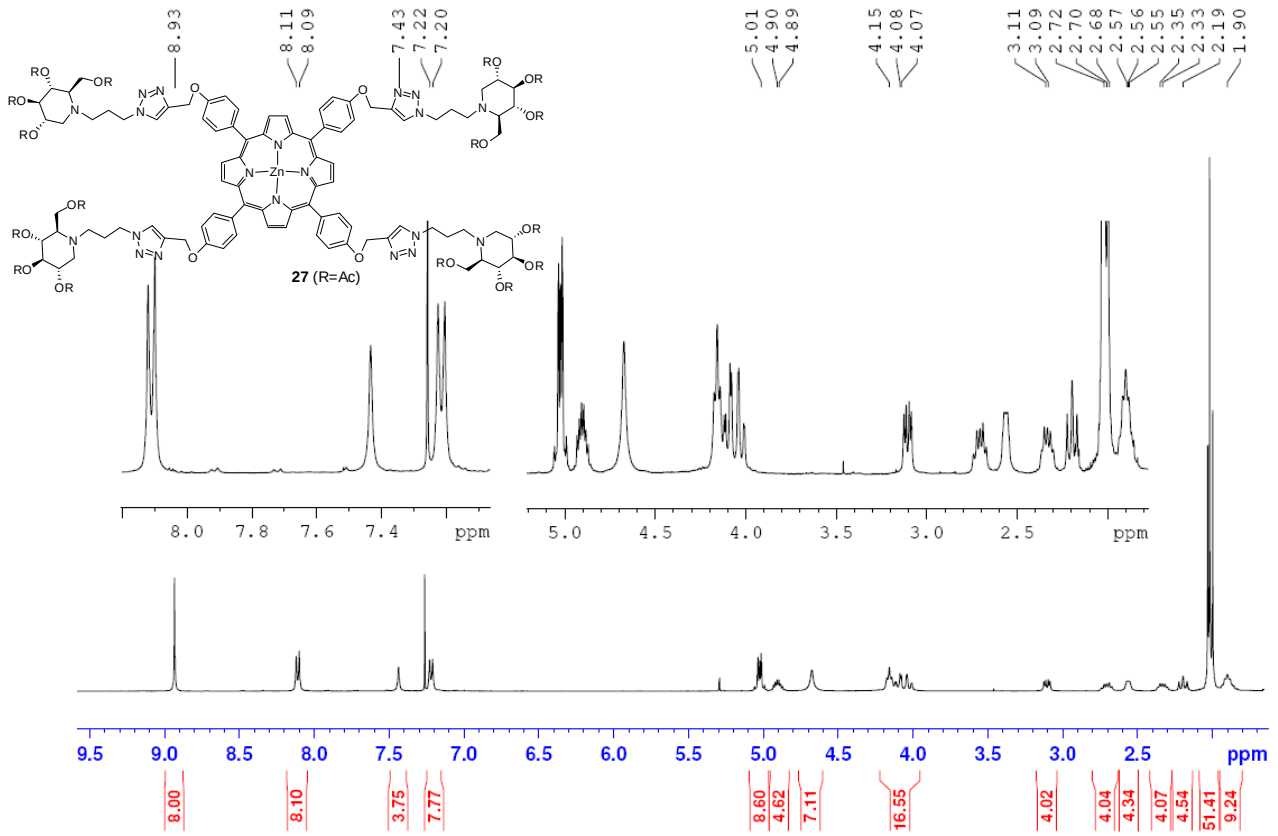

152.248
145.115
144.565
139.117
138.673
138.651
137.917
134.249
128.414
127.925
125.100
125.056
124.519
87.299
78.716
78.507
75.396
75.224
73.347
72.781
66.563
66.447
64.407
54.647
49.412
48.419
33.964
31.572
26.072
24 (R=Bn)
200
180
160
140
120
100
80
60
40
20
0
ppm

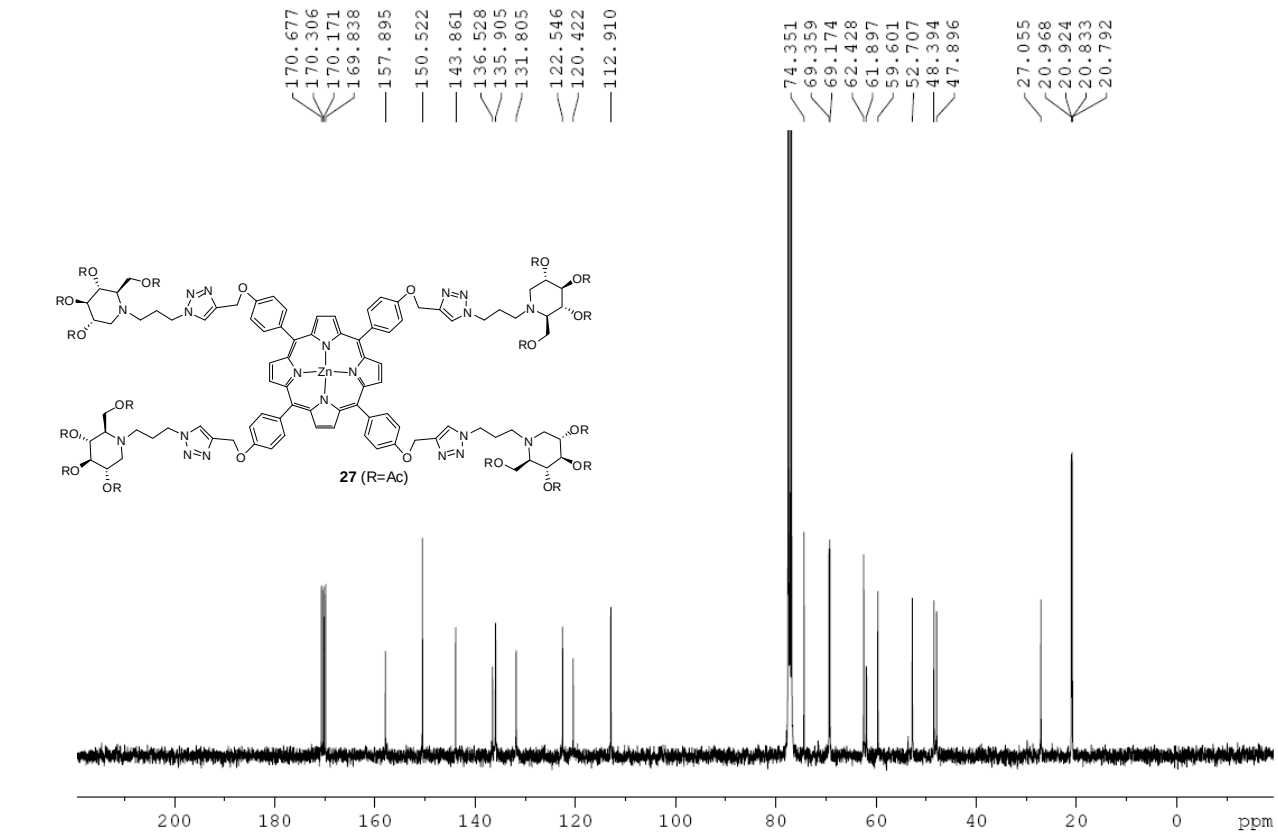

7.957
7.769
7.749
7.515
4.975
4.968
4.938
4.892
4.880
4.875
4.855
4.827
4.823
4.792
4.785
4.710
4.672
4.661
4.652
4.644
4.628
4.451
4.432
4.412
4.395
4.379
4.370
4.339
4.239
4.216
4.189
3.896
3.629
3.563
3.545
3.529
3.520
3.499
3.492
3.348
3.059
3.035
2.814
2.787
2.770
2.579
2.558
25 (R=Bn)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
0.91
2.04
1.24
4.57
9.66
1.47
2.64
8.37
2.40
5.02
3.03
3.89
3.95
4.03
4.06
4.52
8.32

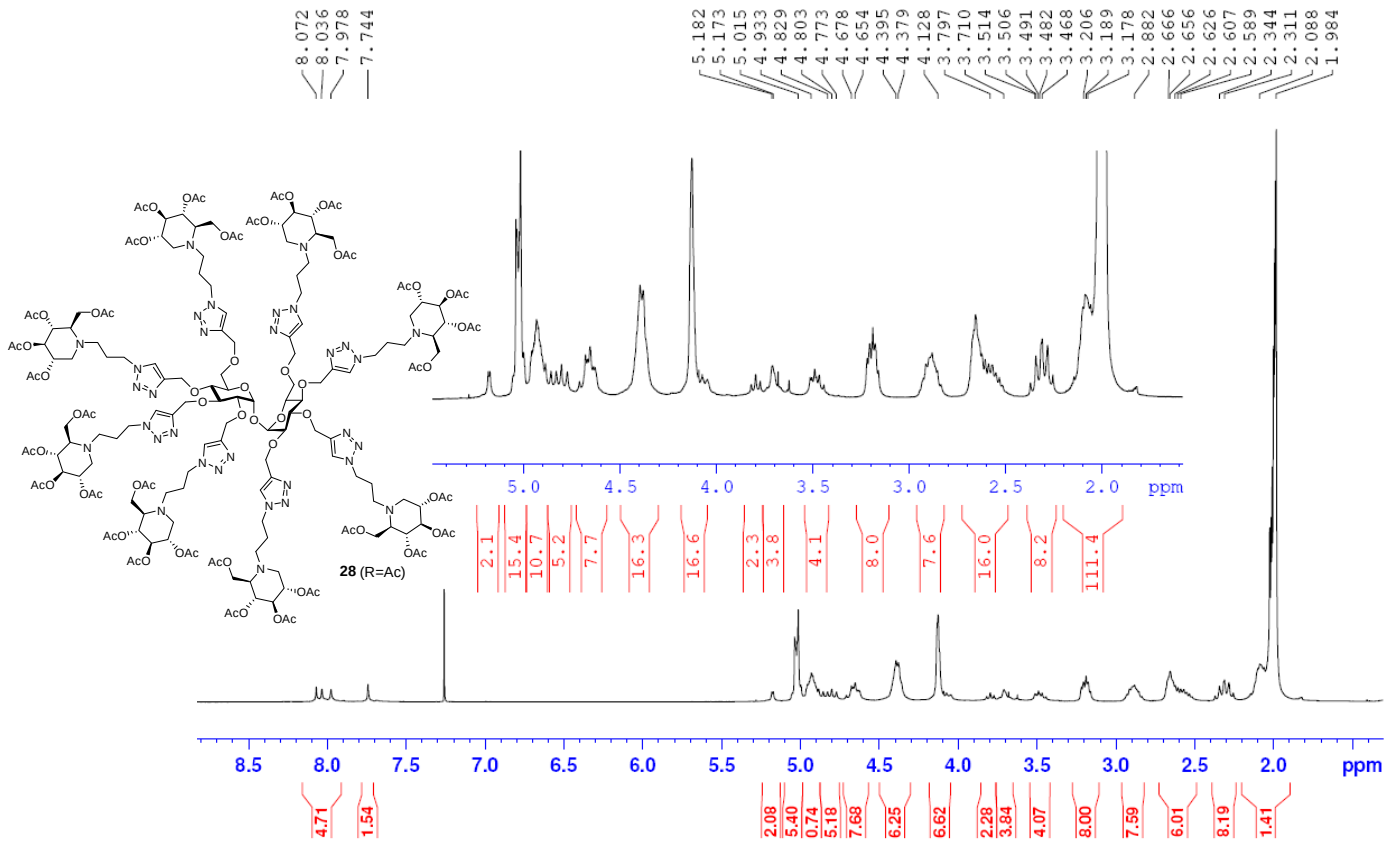

145.067
144.774
144.694
144.634
138.997
138.522
137.786
128.362
127.853
123.856
123.509
122.979
97.820
87.183
81.642
79.857
78.606
78.385
75.334
75.179
73.292
72.778
70.022
68.835
66.611
66.397
66.150
64.903
64.771
64.453
64.333
55.165
54.459
49.274
49.209
48.419
25.930
25 (R=Bn)
90
80
70
60
ppm
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

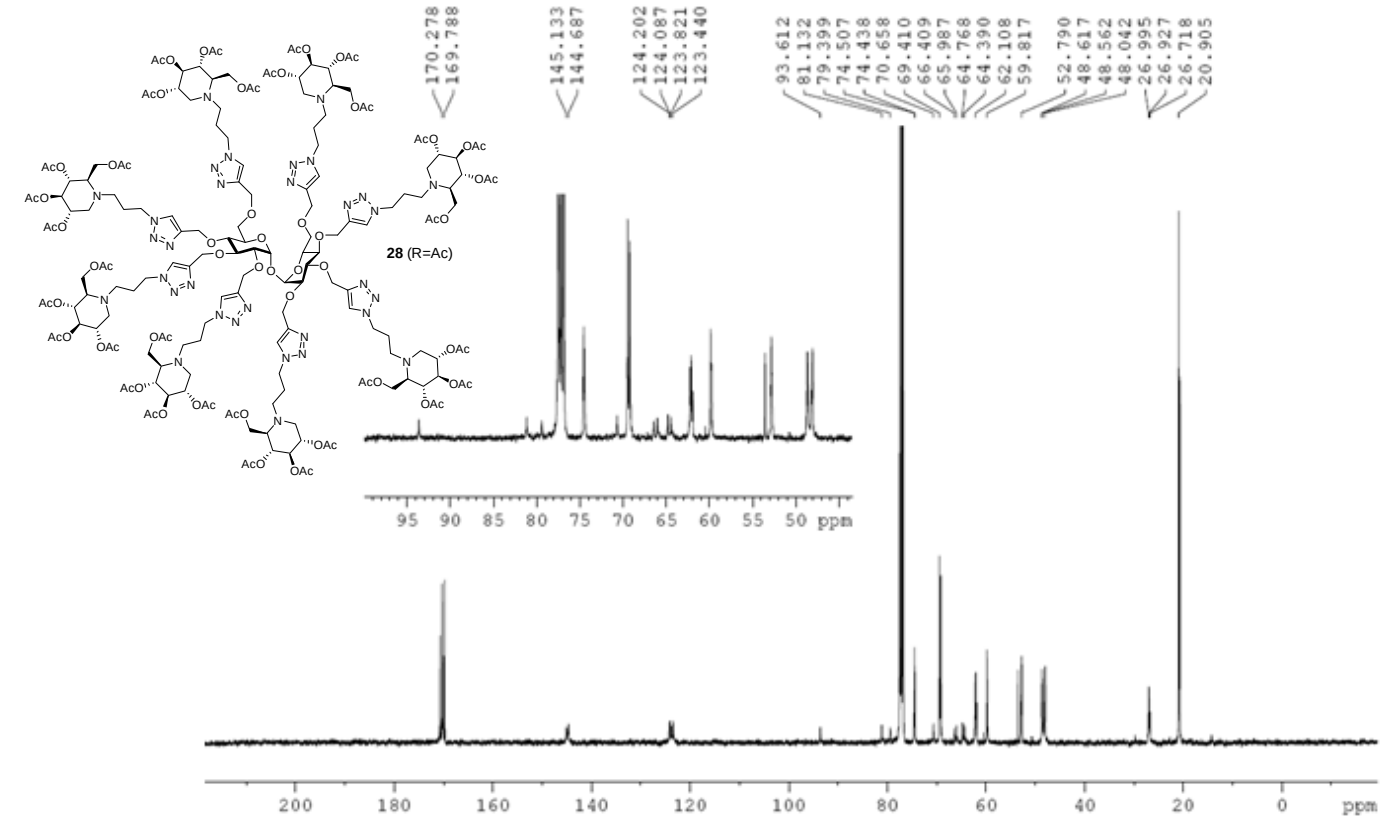

26 (R=Bn)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
1.00 2.08 1.66 77.90 8.81 20.48 11.43 13.26 9.27 2.69 2.72 24.44 3.35 4.13 4.25 4.51 4.28 4.52 9.32

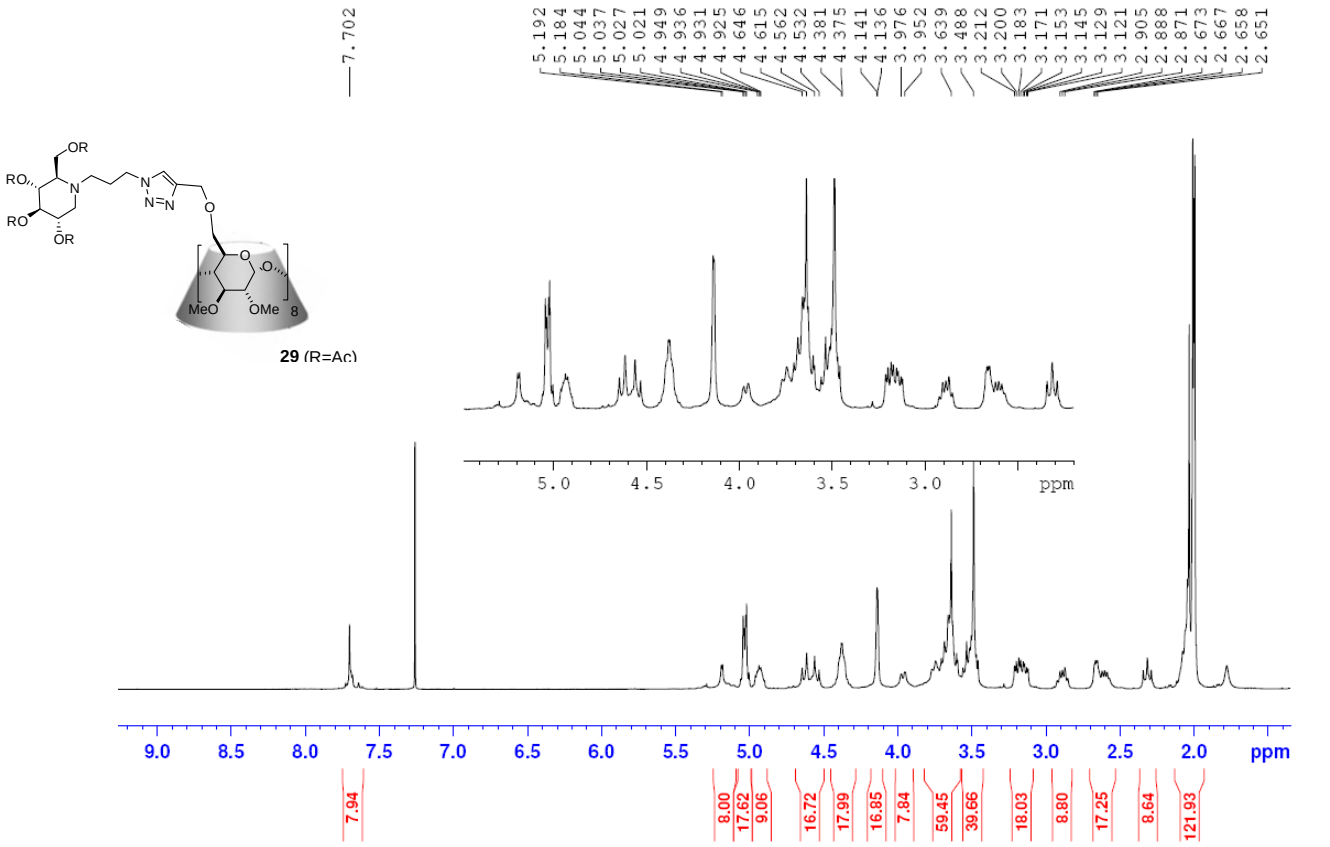

26 (R=Bn)
150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

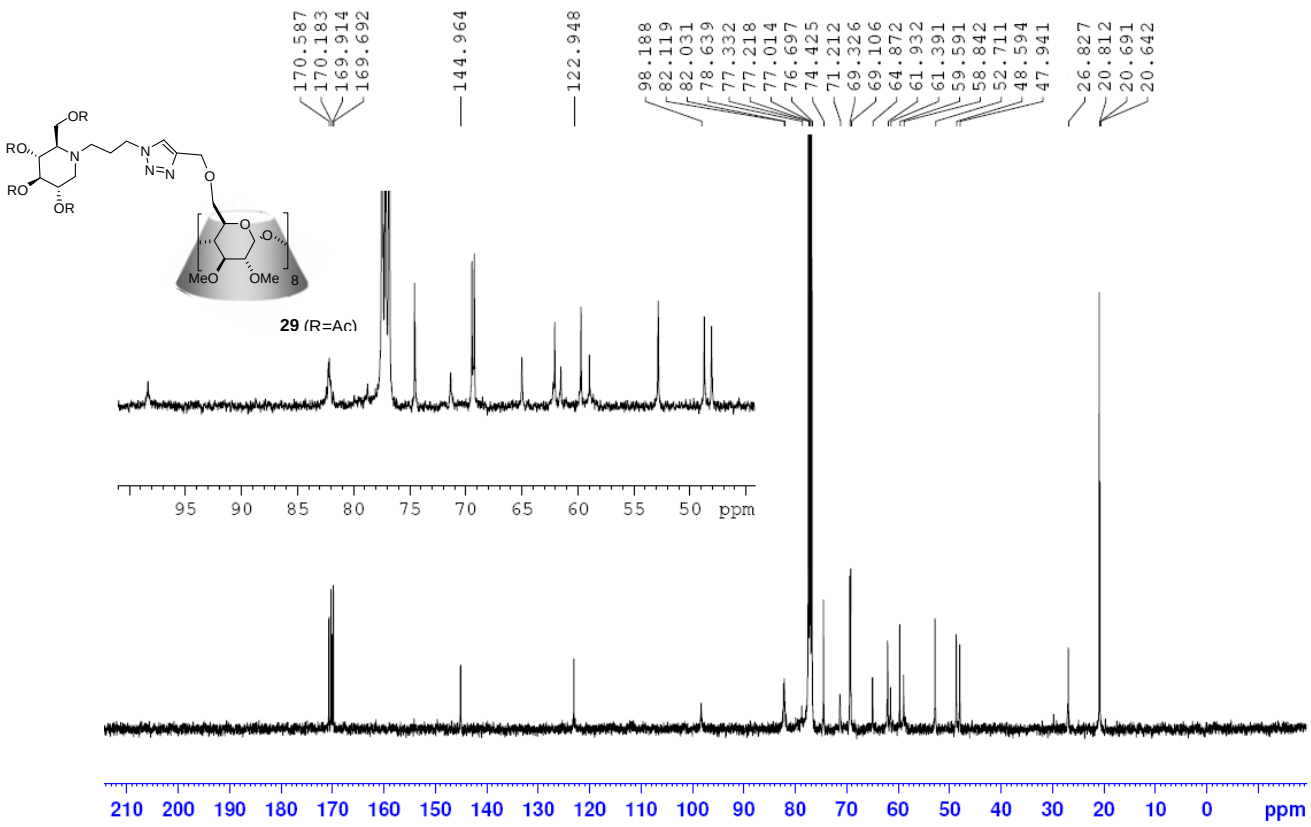

8.93
8.11
8.09
7.43
7.22
7.20
5.01
4.90
4.89
4.15
4.08
4.07
3.11
3.09
2.72
2.70
2.68
2.57
2.56
2.55
2.35
2.33
2.19
1.90
27 (R=Ac)
8.0 7.8 7.6 7.4 ppm
5.0 4.5 4.0 3.5 3.0 2.5 ppm
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 ppm
8.00
8.10
3.75
7.77
8.60
4.62
7.11
16.55
4.02
4.04
4.34
4.07
4.54
51.41
9.24

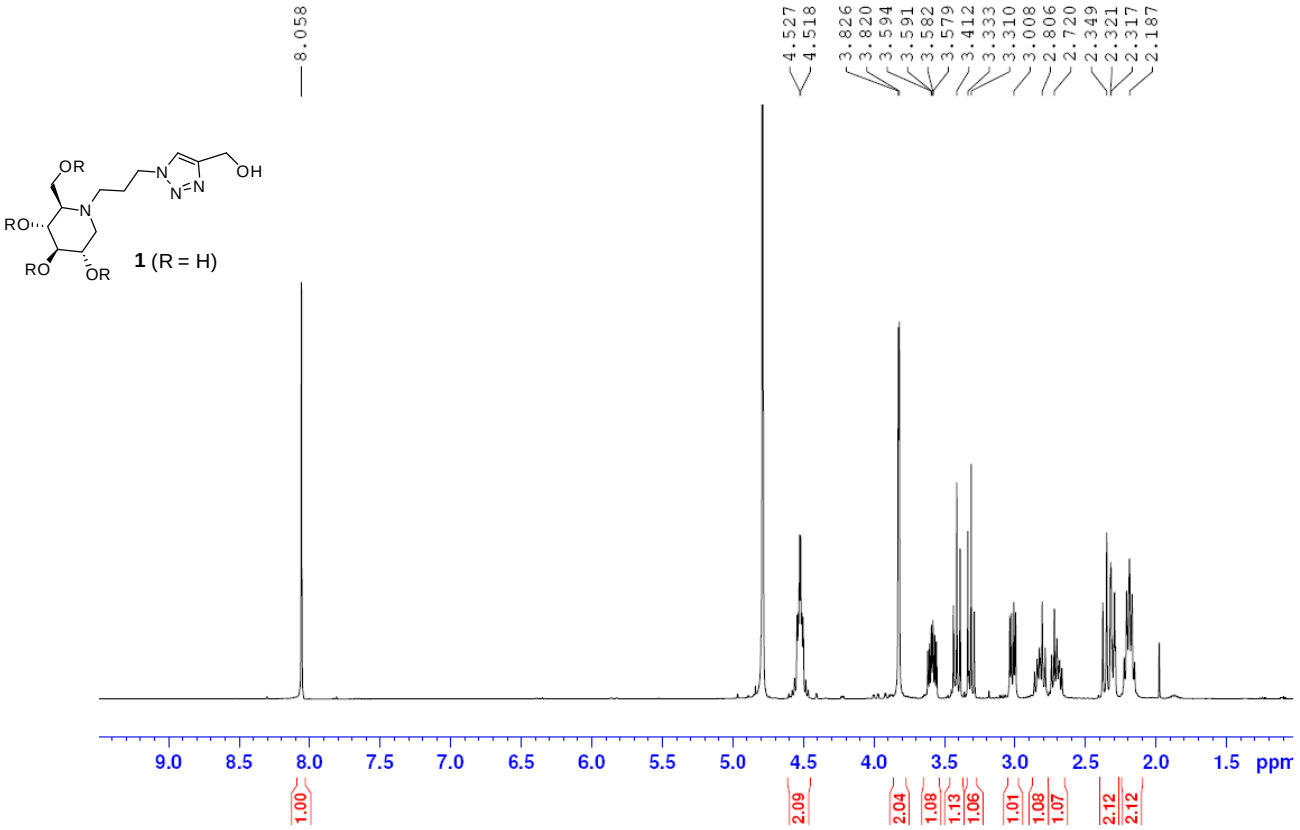

170.677
170.306
170.171
169.838
157.895
150.522
143.861
136.528
135.905
131.805
122.546
120.422
112.910
74.351
69.359
69.174
62.428
61.897
59.601
52.707
48.394
47.896
27.055
20.968
20.924
20.833
20.792
27 (R=Ac)
200 180 160 140 120 100 80 60 40 20 0 ppm

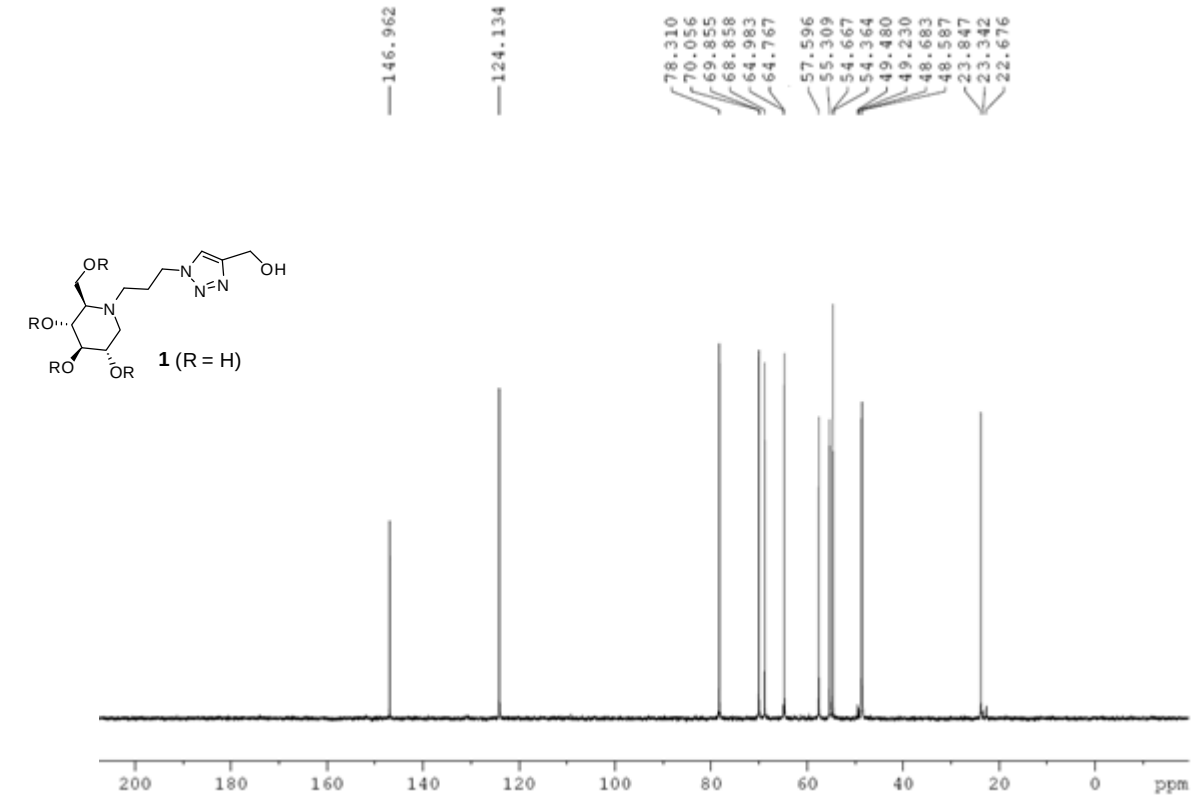

8.072
8.036
7.978
7.744
5.182
5.173
5.015
4.933
4.829
4.803
4.773
4.678
4.654
4.395
4.379
4.128
3.797
3.710
3.514
3.506
3.491
3.482
3.468
3.206
3.189
3.178
2.882
2.666
2.656
2.626
2.607
2.589
2.344
2.311
2.088
1.984
28 (R=Ac)
ppm

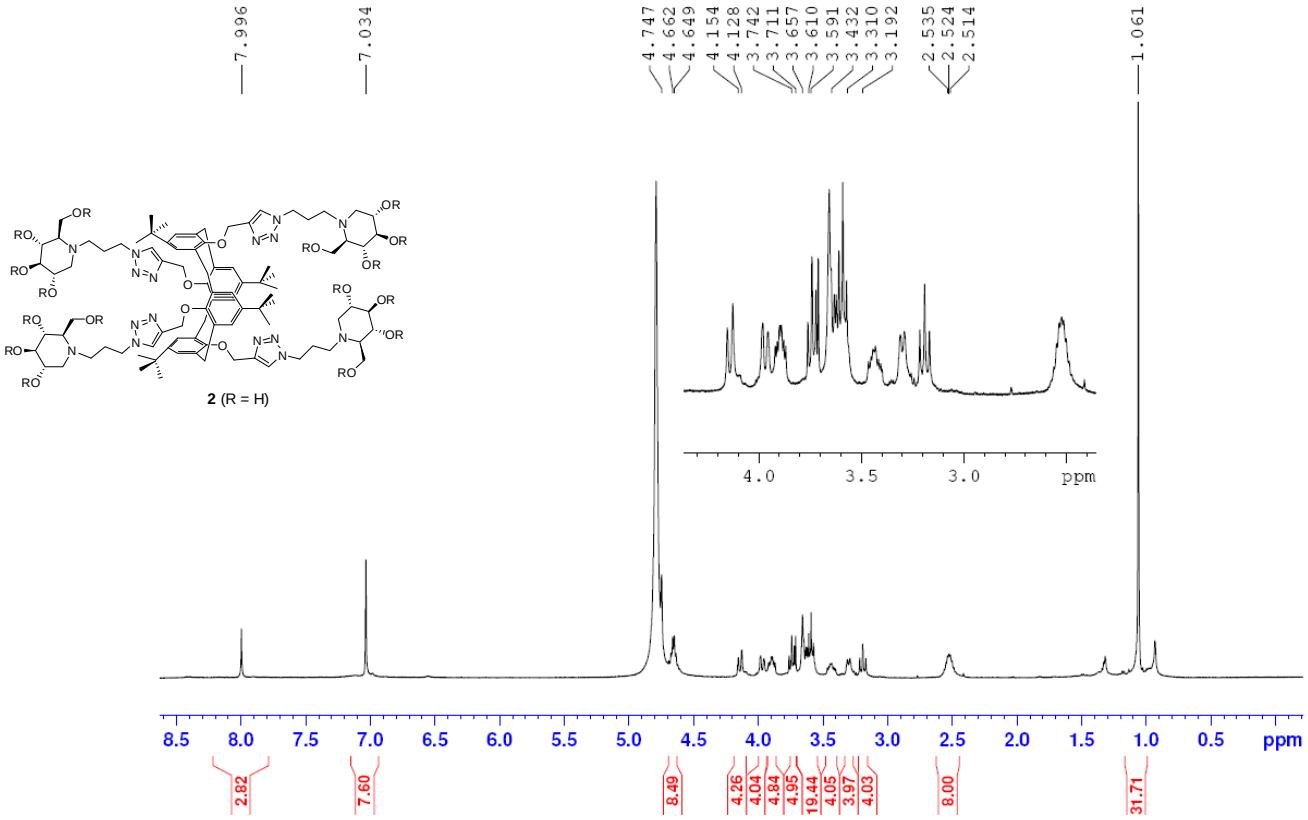

170.278
169.788
145.133
144.687
124.202
124.087
123.821
123.440
93.612
81.132
79.399
74.507
74.438
70.658
69.410
66.409
65.987
64.768
64.390
62.108
59.817
52.790
48.617
48.562
48.042
26.995
26.927
26.718
20.905
28 (R=Ac)
ppm


7.702
5.192
5.184
5.044
5.037
5.027
5.021
4.949
4.936
4.931
4.925
4.646
4.615
4.562
4.532
4.381
4.375
4.141
4.136
3.976
3.952
3.639
3.488
3.212
3.200
3.183
3.171
3.153
3.145
3.129
3.121
2.905
2.888
2.871
2.673
2.667
2.658
2.651
OR
RO
RO
OR
N
N=N
O
MeO
OMe
8
29 (R=Ac)
5.0
4.5
4.0
3.5
3.0
ppm
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
7.94
8.00
17.62
9.06
16.72
17.99
16.85
7.84
59.45
39.66
18.03
8.80
17.25
8.64
121.93

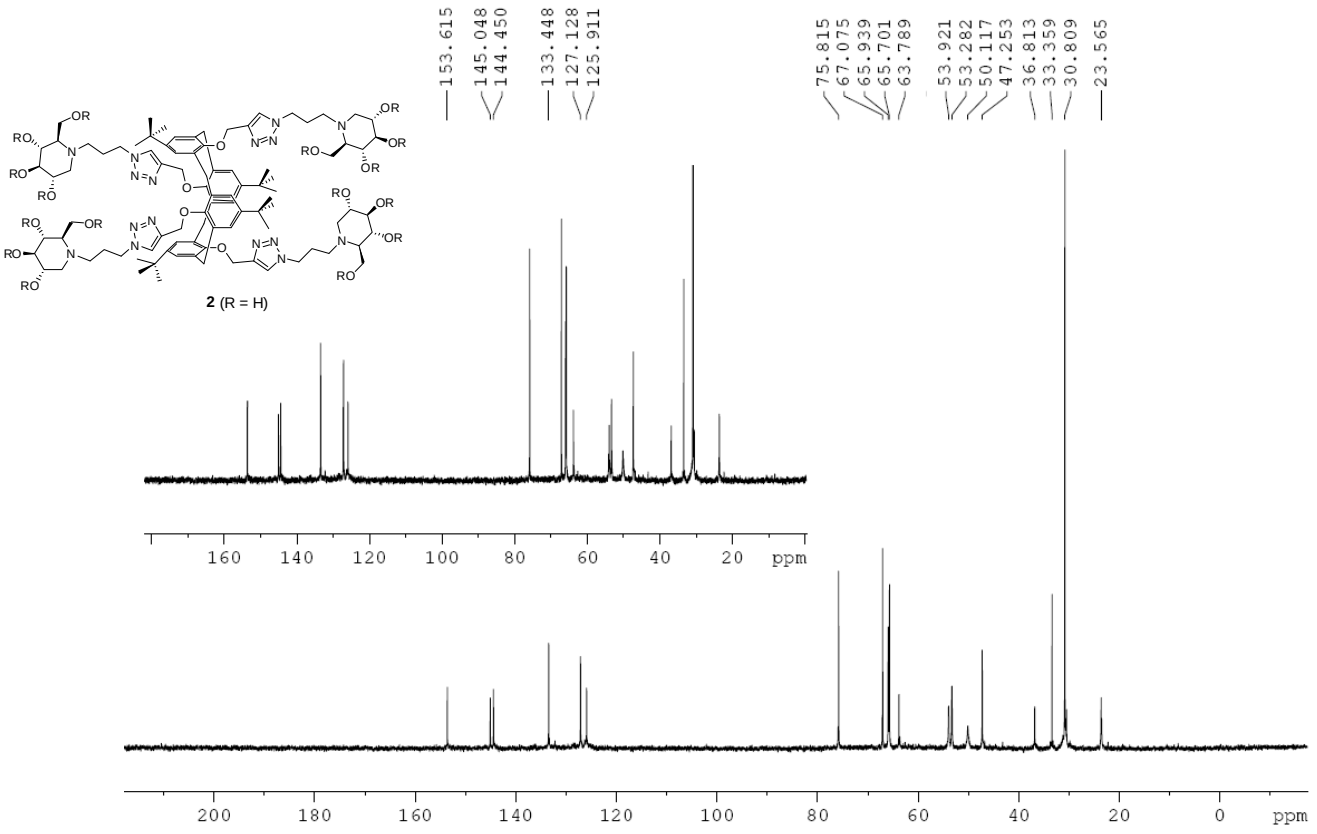

170.587
170.183
169.914
169.692
144.964
122.948
98.188
82.119
82.031
78.639
77.332
77.218
77.014
76.697
74.425
71.212
69.326
69.106
64.872
61.932
61.391
59.591
58.842
52.711
48.594
47.941
26.827
20.812
20.691
20.642
OR
RO
RO
OR
N
N=N
O
MeO
OMe
8
29 (R=Ac)
95
90
85
80
75
70
65
60
55
50
ppm
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


8.058
4.527
4.518
3.826
3.820
3.594
3.591
3.582
3.579
3.412
3.333
3.310
3.008
2.806
2.720
2.349
2.321
2.317
2.187
OR
OH
RO
RO
OR
1 (R = H)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
2.09
2.04
1.08
1.13
1.06
1.01
1.08
1.07
2.12
2.12

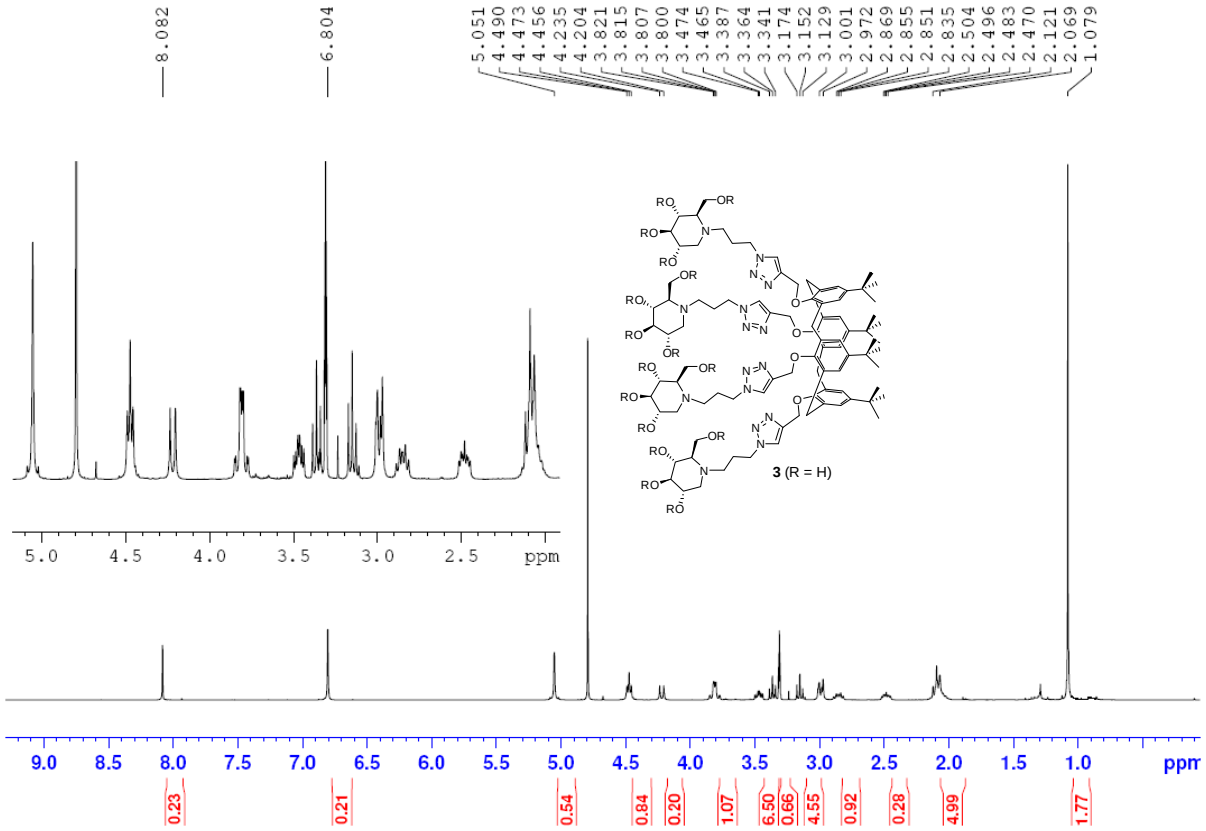

146.962
124.134
78.310
70.056
69.855
68.858
64.983
64.767
57.596
55.309
54.667
54.364
49.480
49.230
48.683
48.587
23.847
23.342
22.676
OR
OH
RO
RO
OR
1 (R = H)
200
180
160
140
120
100
80
60
40
20
0
ppm

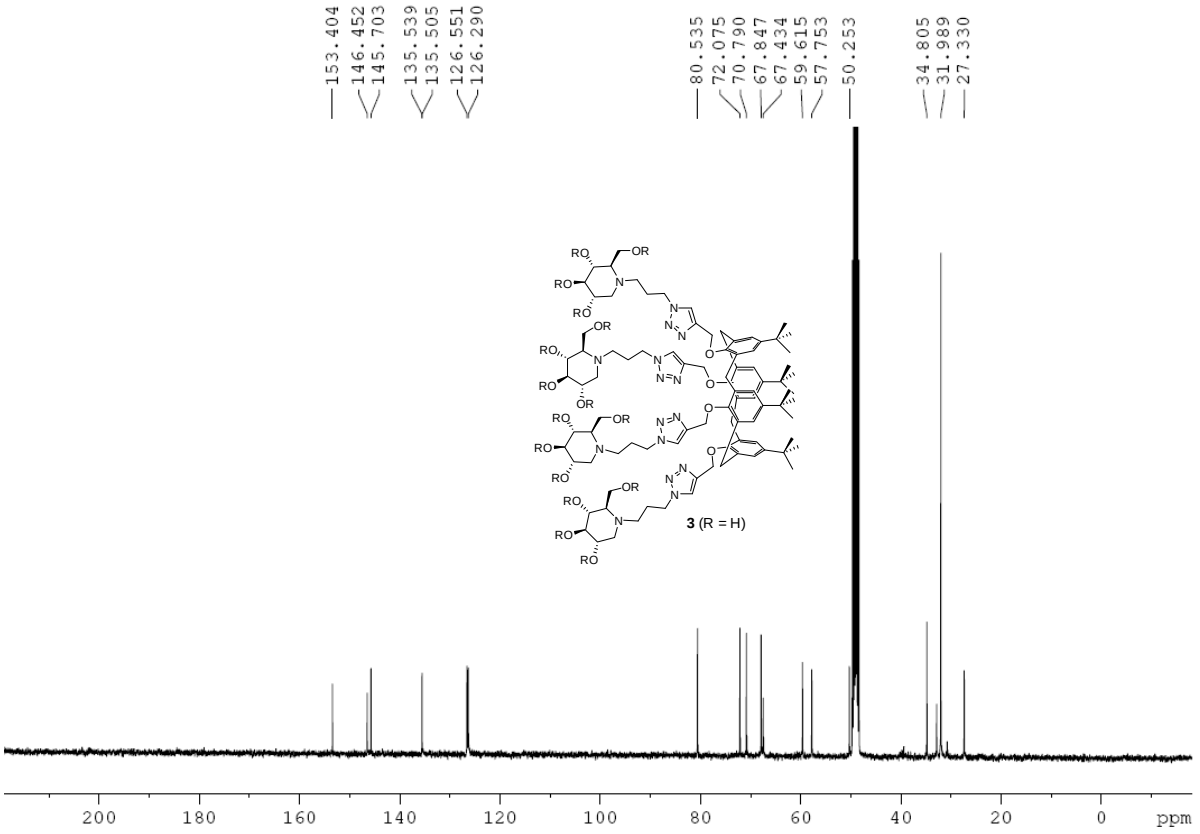

7.996
7.034
4.747
4.662
4.649
4.154
4.128
3.742
3.711
3.657
3.610
3.591
3.432
3.310
3.192
2.535
2.524
2.514
1.061
4.0
3.5
3.0
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.82
7.60
8.49
4.26
4.04
4.84
4.95
19.44
4.05
3.97
4.03
8.00
31.71

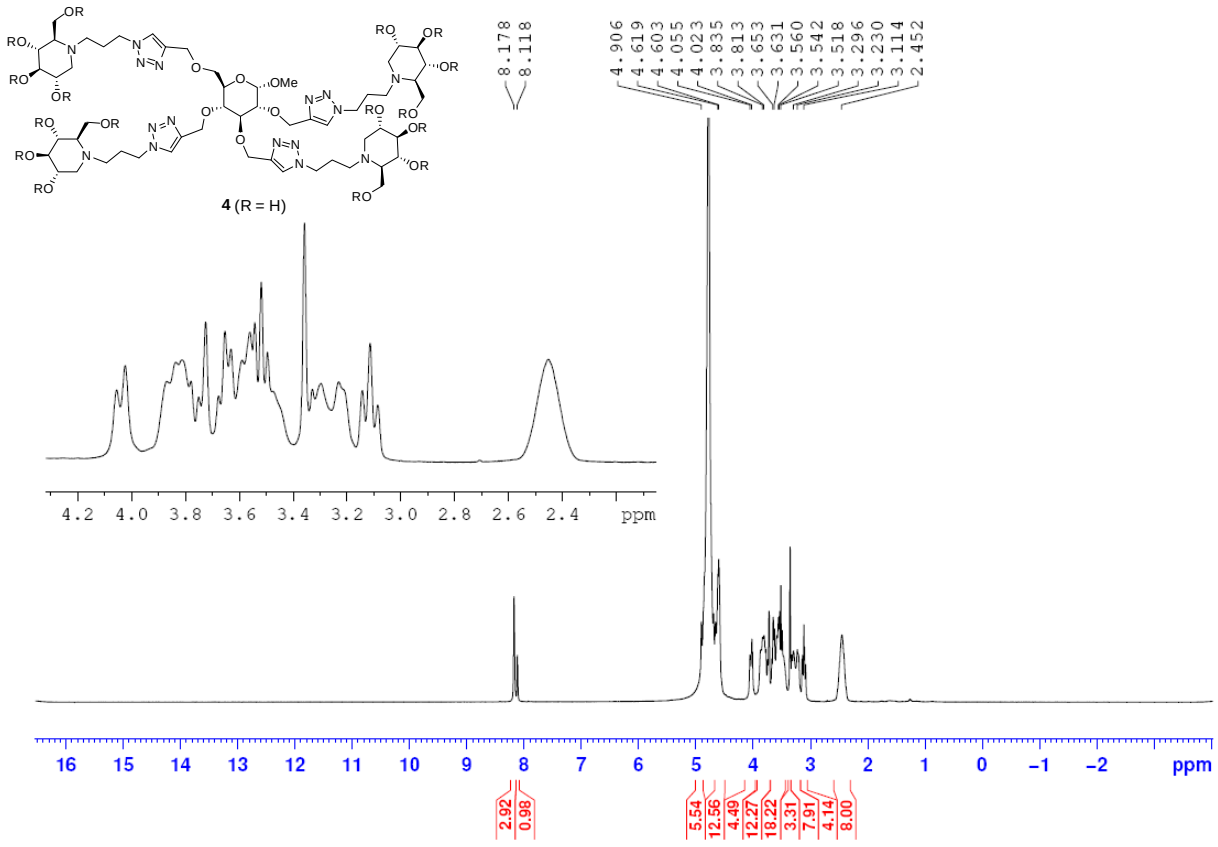
2 (R = H)


153.615
145.048
144.450
133.448
127.128
125.911
75.815
67.075
65.939
65.701
63.789
53.921
53.282
50.117
47.253
36.813
33.359
30.809
23.565
160
140
120
100
80
60
40
20
ppm
200
180
160
140
120
100
80
60
40
20
0
ppm

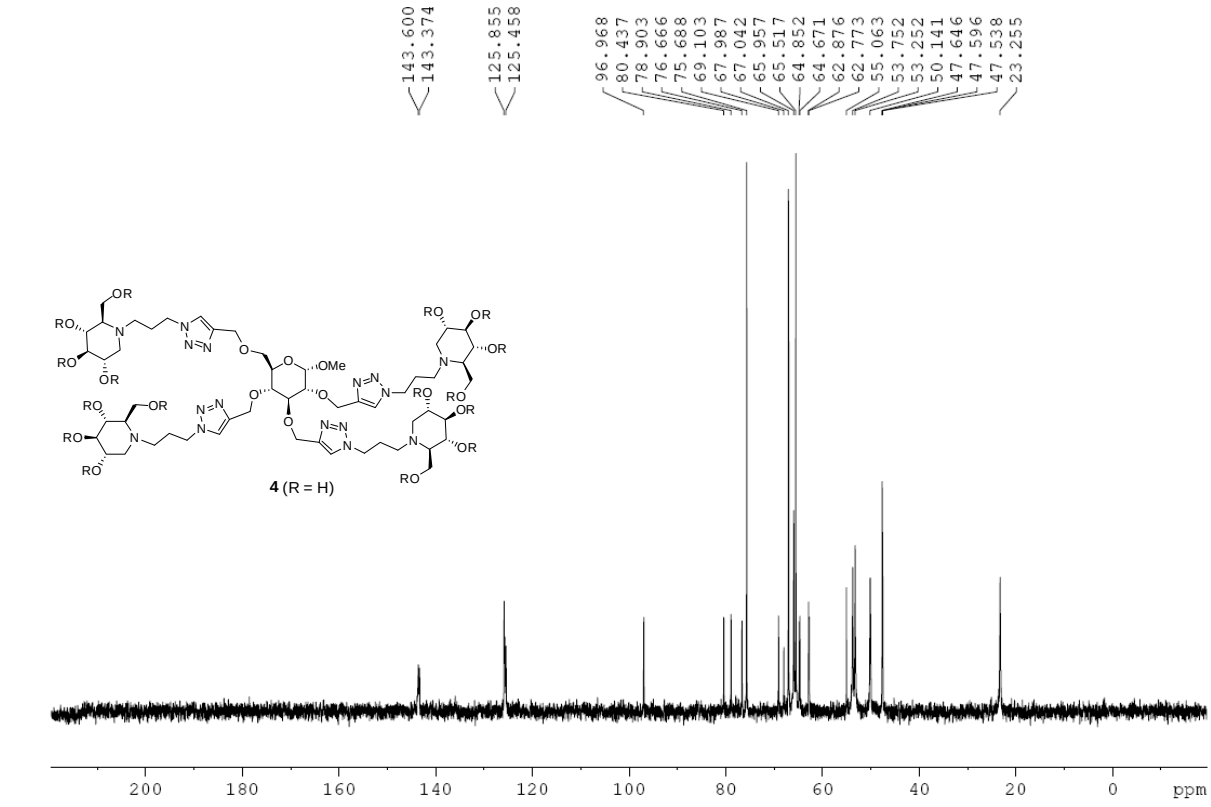
2 (R = H)


8.082
6.804
5.051
4.490
4.473
4.456
4.235
4.204
3.821
3.815
3.807
3.800
3.474
3.465
3.387
3.364
3.341
3.174
3.152
3.129
3.001
2.972
2.869
2.855
2.851
2.835
2.504
2.496
2.483
2.470
2.121
2.069
1.079
3 (R = H)
5.0
4.5
4.0
3.5
3.0
2.5
ppm
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.23
0.21
0.54
0.84
0.20
1.07
6.50
0.66
4.55
0.92
0.28
4.99
1.77

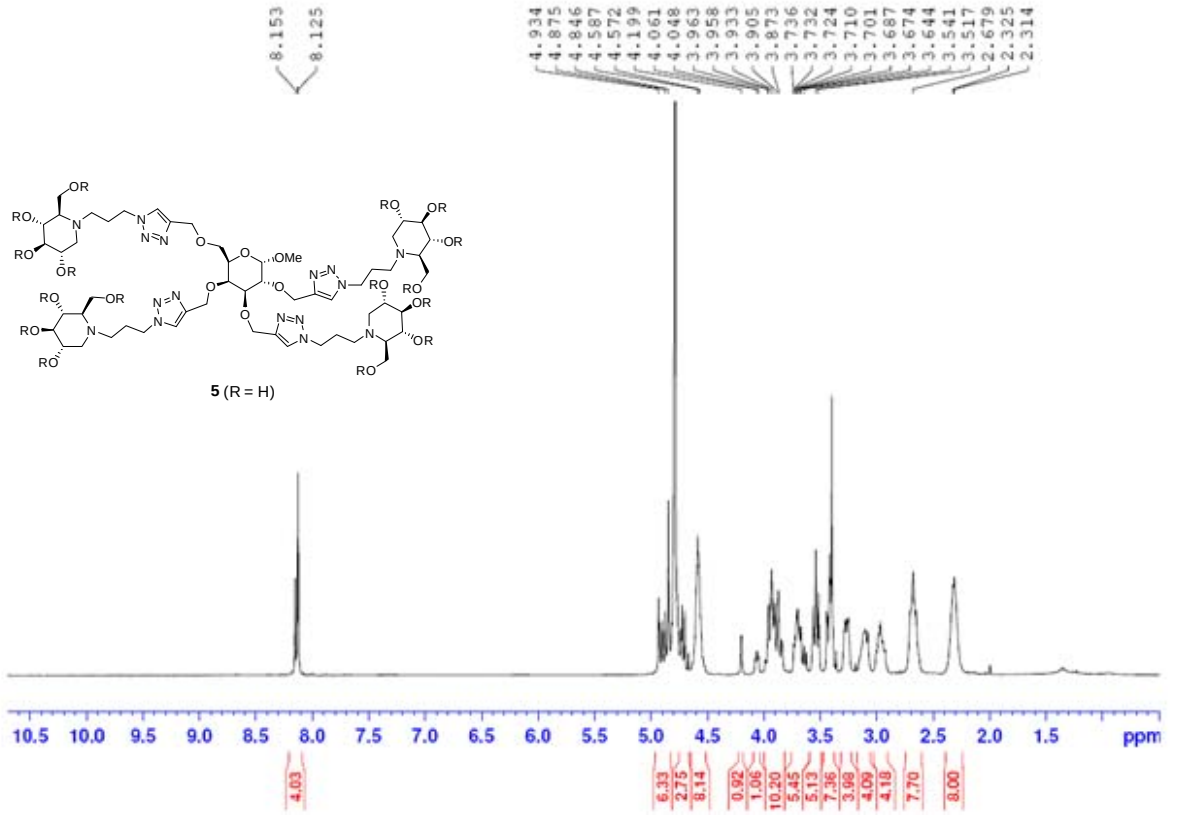

153.404
146.452
145.703
135.539
135.505
126.551
126.290
80.535
72.075
70.790
67.847
67.434
59.615
57.753
50.253
34.805
31.989
27.330
3 (R = H)
200
180
160
140
120
100
80
60
40
20
0
ppm


8.178
8.118
4.906
4.619
4.603
4.055
4.023
3.835
3.813
3.653
3.631
3.560
3.542
3.518
3.296
3.230
3.114
2.452
4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 ppm
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 ppm
2.92
0.98
5.54
12.56
4.49
12.27
18.22
3.31
7.91
4.14
8.00

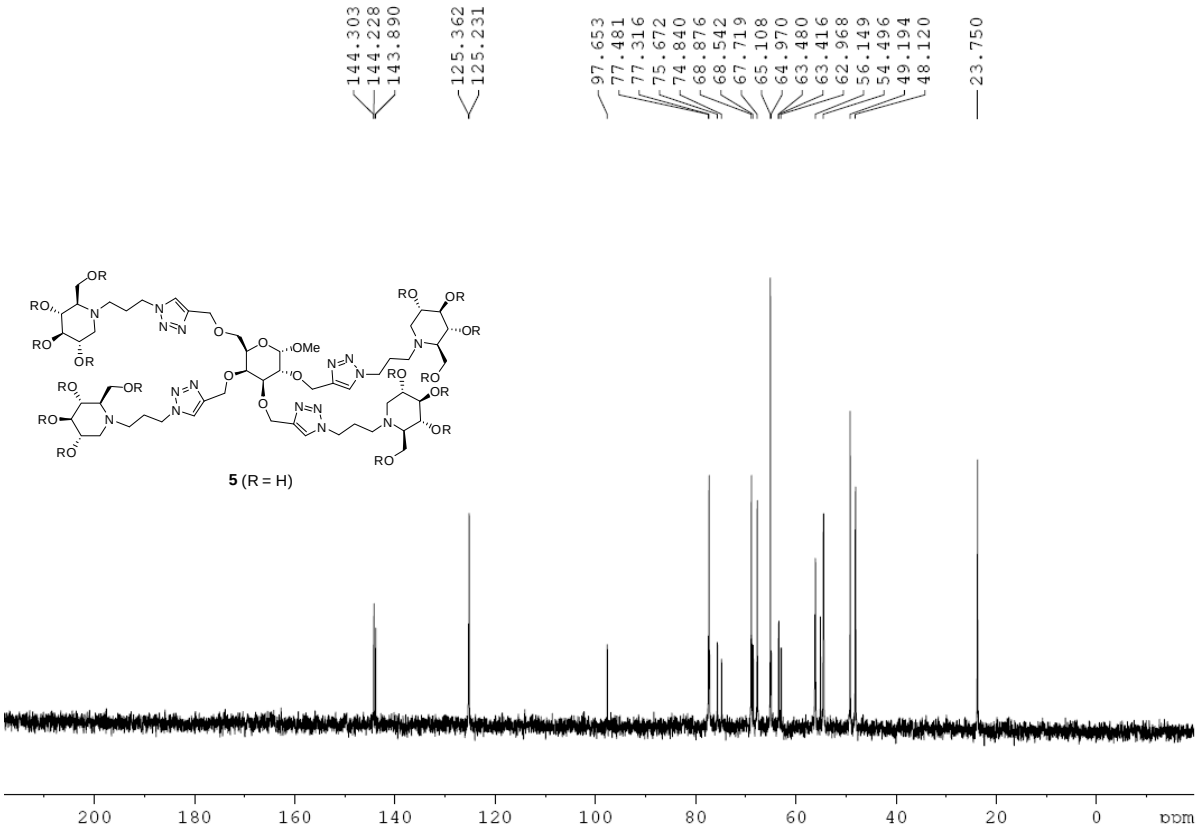
4 (R = H)


143.600
143.374
125.855
125.458
96.968
80.437
78.903
76.666
75.688
69.103
67.987
67.042
65.957
65.517
64.852
64.671
62.876
62.773
55.063
53.752
53.252
50.141
47.646
47.596
47.538
23.255
200 180 160 140 120 100 80 60 40 20 0 ppm

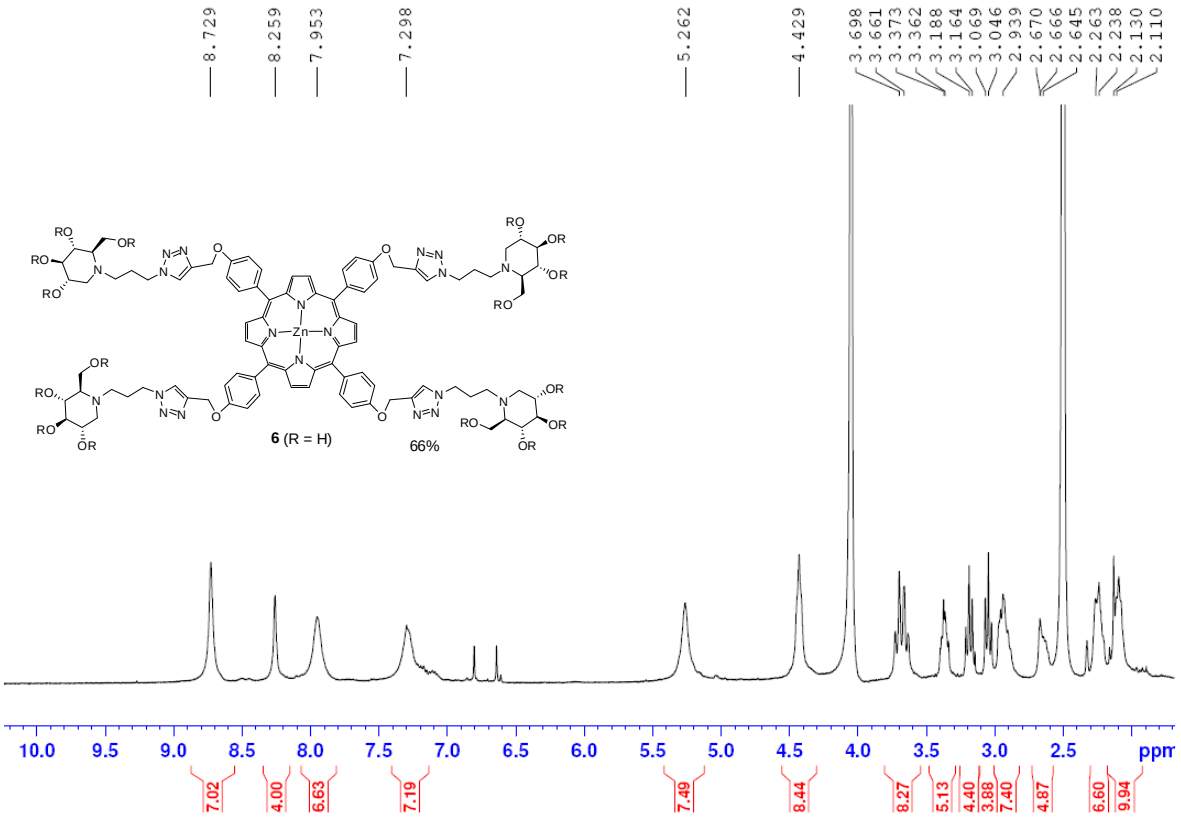
4 (R = H)

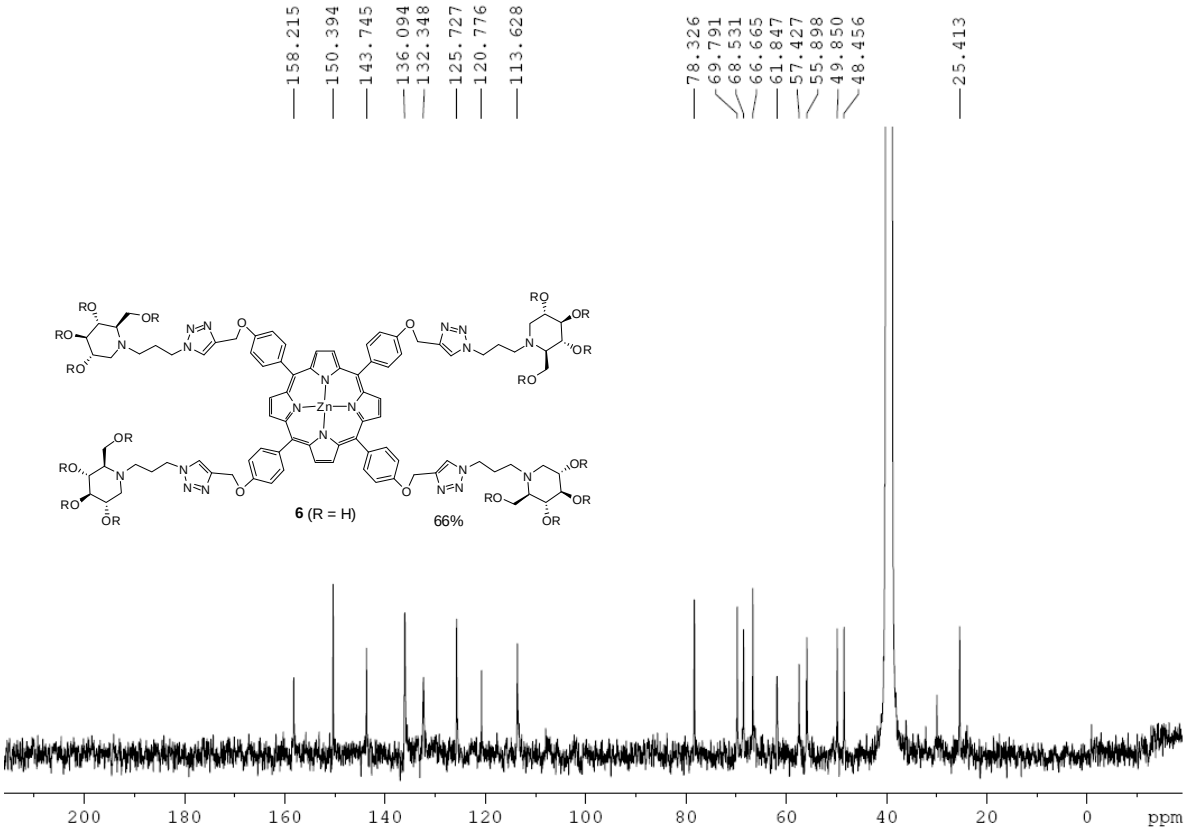

8.153
8.125
4.934
4.875
4.846
4.587
4.572
4.199
4.061
4.048
3.963
3.958
3.933
3.905
3.873
3.736
3.732
3.724
3.710
3.701
3.687
3.674
3.644
3.541
3.517
2.679
2.325
2.314
OMe
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
4.03
6.33
2.75
8.14
0.92
1.06
10.20
5.45
5.13
7.36
3.98
4.09
4.18
7.70
8.00

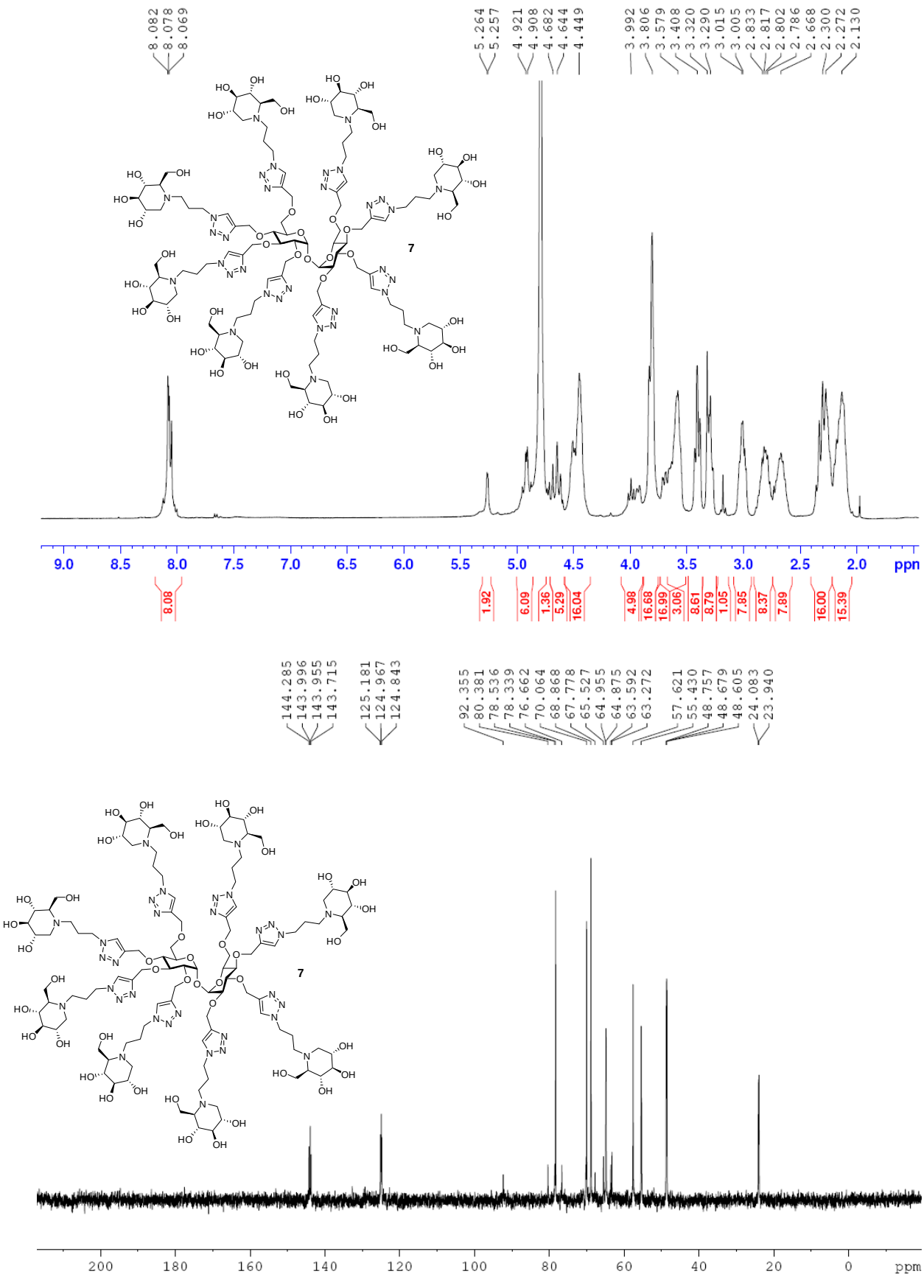
5 (R = H)

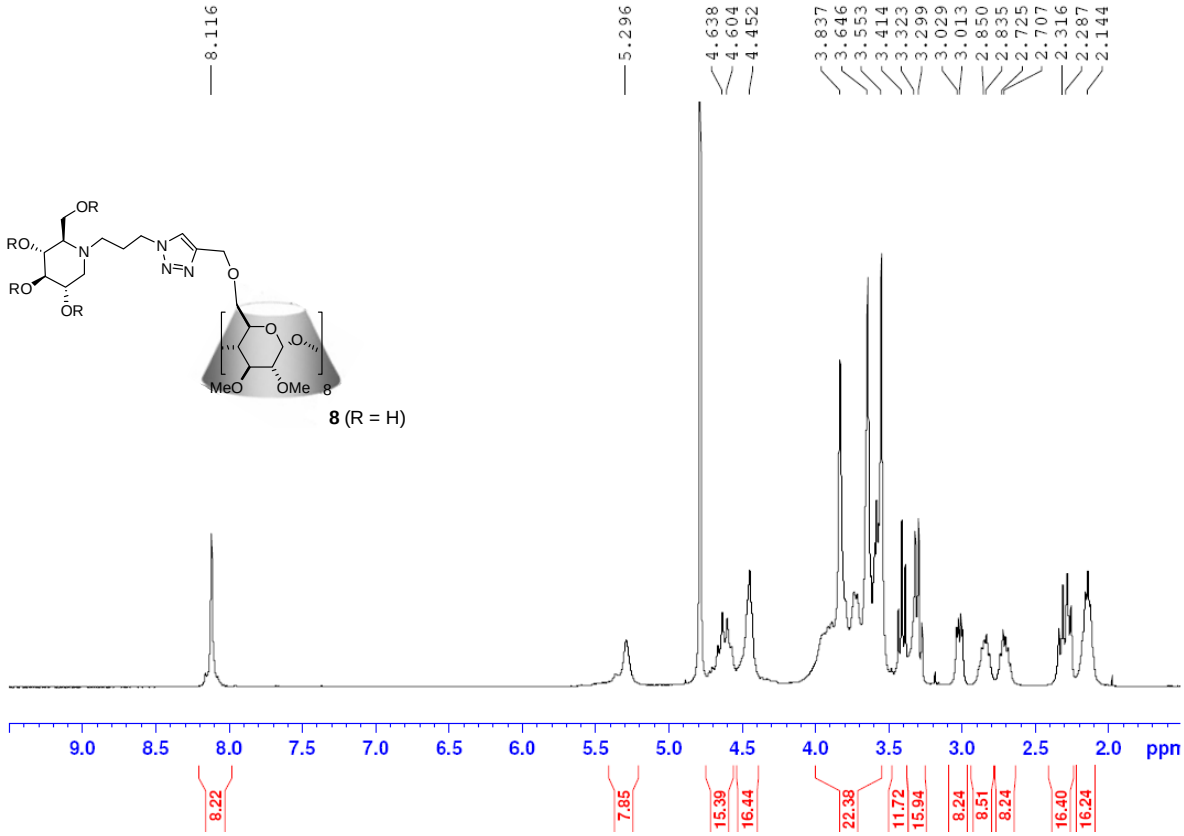

144.303
144.228
143.890
125.362
125.231
97.653
77.481
77.316
75.672
74.840
68.876
68.542
67.719
65.108
64.970
63.480
63.416
62.968
56.149
54.496
49.194
48.120
23.750
OMe
200 180 160 140 120 100 80 60 40 20 0 ppm

5 (R = H)

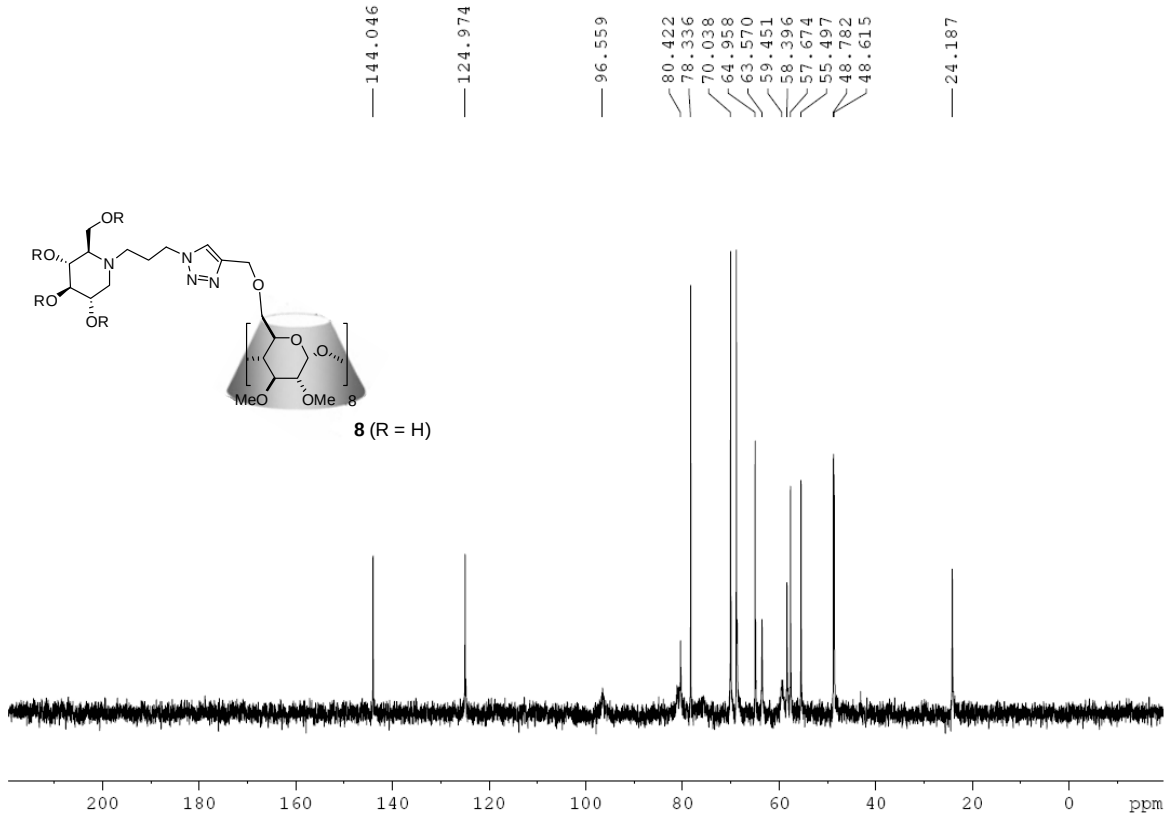

8.729
8.259
7.953
7.298
5.262
4.429
3.698
3.661
3.373
3.362
3.188
3.164
3.069
3.046
2.939
2.670
2.666
2.645
2.263
2.238
2.130
2.110
6 (R = H)
66%
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
7.02
4.00
6.63
7.19
7.49
8.44
8.27
5.13
4.40
3.88
7.40
4.87
6.60
9.94


158.215
150.394
143.745
136.094
132.348
125.727
120.776
113.628
78.326
69.791
68.531
66.665
61.847
57.427
55.898
49.850
48.456
25.413
6 (R = H)
66%
200
180
160
140
120
100
80
60
40
20
0
ppm


8.082
8.078
8.069
5.264
5.257
4.921
4.908
4.682
4.644
4.449
3.992
3.806
3.579
3.408
3.320
3.290
3.015
3.005
2.833
2.817
2.802
2.786
2.668
2.300
2.272
2.130
7
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppn
8.08
1.92
6.09
1.36
5.29
16.04
4.98
16.68
16.99
3.06
8.61
8.79
1.05
7.85
8.37
7.89
16.00
15.39
144.285
143.996
143.955
143.715
125.181
124.967
124.843
92.355
80.381
78.536
78.339
76.662
70.064
68.868
67.778
65.527
64.955
64.875
63.592
63.272
57.621
55.430
48.757
48.679
48.605
24.083
23.940
7
200
180
160
140
120
100
80
60
40
20
0
ppm


8.116
5.296
4.638
4.604
4.452
3.837
3.646
3.553
3.414
3.323
3.299
3.029
3.013
2.850
2.835
2.725
2.707
2.316
2.287
2.144
OR
RO
RO
OR
N
N
N=N
O
O
O
MeO
OMe
8
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
8.22
7.85
15.39
16.44
22.38
11.72
15.94
8.24
8.51
8.24
16.40
16.24

8 (R = H)


144.046
124.974
96.559
80.422
78.336
70.038
64.958
63.570
59.451
58.396
57.674
55.497
48.782
48.615
24.187
OR
RO
RO
OR
N
N
N=N
O
O
O
MeO
OMe
8
200
180
160
140
120
100
80
60
40
20
0
ppm

8 (R = H)