Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Cyclo-oligo-(1→6)-β-D-Glucosamine Based Artificial Channels for Tunable Transmembrane Ion Transport

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I. General Methods:

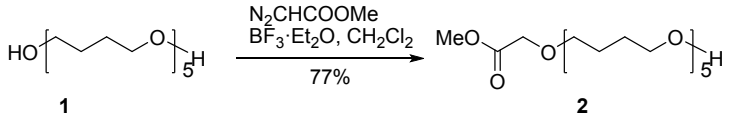
All reagents for synthesis were commercial and used without further purification. Egg yolk phosphatidylcholine (EYPC) was obtained from Avanti Polar Lipids as a solution in $CHCl_3$ (25mg/ml), HEPES buffer, HPTS, Triton X-100, NaOH and inorganic salts were of molecular biology grade from Sigma. Solvents were distilled over CaH_2 (CH_2Cl_2), Mg/I_2 (MeOH) or purchased as dry. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F_{254} (Merck). Silica gel column chromatography was performed with Silica Gel 60 (40-63 μm, E. Merck). Reversed phase column chromatography was performed on a Supelco C-18 column (10×250 mm) or on a VYDAC 218TP1010 column (10×250 mm) with a flow rate of 2 mL/min and accompanied with UV-detection (λ = 205 nm, 254 nm). Gel-permeation chromatography was performed on a column of LH-20 gel (25×300 mm, V_0 = 25 mL) in $CHCl_3$/MeOH (1:1, v/v) or on a column of TSK HW-40 (S) gel (25×400 mm, V_0 = 60 mL) in 0.1 M AcOH. Large unilamellar vesicles (LUV) were prepared by using mini extruder, equipped with a polycarbonate membrane of 100 nm pore size, obtained from Avanti Polar Lipids.

II. Physical Measurements:

NMR spectra were recorded at 293-305 K using a 400 MHz or a 600 MHz Spectrometer. Shifts are referenced relative to deuterated solvent residual peaks. Chemical shifts are reported relative to chloroform (δ 7.26) or methanol (δ 3.31) for ^{1}H NMR and chloroform (δ 77.2) or methanol (δ 49.0) for ^{13}C NMR. The following abbreviations are used to explain the observed multiplicities: s (singlet), d (doublet), t (triplet), m (multiplet) and br. t (broad triplet). High resolution (HR-ESI-QTOF) mass spectra were recorded using a Bruker MicrOTOF-Q II XL spectrometer. TLC plates were inspected by UV light (λ = 254 nm) and developed by treatment with a mixture of 10% H_2SO_4 in EtOH/H_2O (95:5, v/v) followed by heating or with a mixture of *p*-anisaldehyde/H_2SO_4/AcOH (8/10/3, v/v/v) in EtOH followed by heating. Fluorescence spectra were recorded from Fluoromax-4 from JobinYvon Edison equipped with an injector port and a magnetic stirrer. The buffer used for fluorescence experiment comprised 10 mM HEPES and 100 mM NaCl and the pH of the buffer was adjusted to 7.0 by NaOH using Helmer pH meter. All data from fluorescence studies were processed either by either of KaleidaGraph, and Origin 8.0.

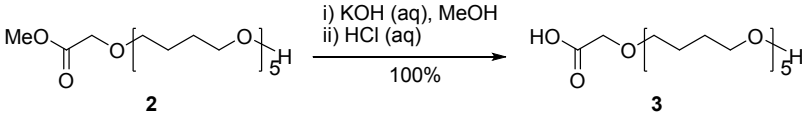
III. Synthesis:

Scheme S1. Synthesis of Ester **2**.



A solution of methyl diazoacetate (47 mg, 0.47 mmol) in anhydrous CH_2Cl_2 (15 mL) was added dropwise to a solution of alcohol **1**[S1] (358 mg, 0.95 mmol) and $BF_3{\cdot}Et_2O$ (10 μL, 0.17 mmol) in anhydrous CH_2Cl_2 (30 mL) during 3 h and with intensive stirring at room temperature. Then the reaction mixture was washed with brine and water. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (toluene/EtOAc, 1:1) to give **2** (164 mg, 77%) as a colorless oil. R_f = 0.49 (EtOAc).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (s, 2H, $CH_2OC\mathit{H}_2COOMe$), 3.74 (s, 3H, $COOCH_3$), 3.62 (t, J = 5.9 Hz, 2H, $C\mathit{H}_2OH$), 3.53 (t, J = 6.4 Hz, 2H, $C\mathit{H}_2OCH_2COOMe$), 3.46-3.38 (m, 16H, 8 OCH_2), 1.72-1.57 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.05 (CO), 71.78 ($\mathit{C}H_2OCH_2COOMe$), 70.92 (OCH_2), 70.71 (OCH_2), 70.61 (OCH_2), 70.50 (OCH_2), 68.31 ($CH_2O\mathit{C}H_2COOMe$), 62.81 (CH_2OH), 51.81 ($COO\mathit{C}H_3$), 30.44 (CH_2), 26.99 (CH_2), 26.60 (CH_2), 26.56 (CH_2), 26.53 (CH_2), 26.42 (CH_2) ppm. HRMS (ESI) calcd. for $C_{23}H_{46}O_8$ $[M + Na]^+$ 473.3085; found 473.3092.

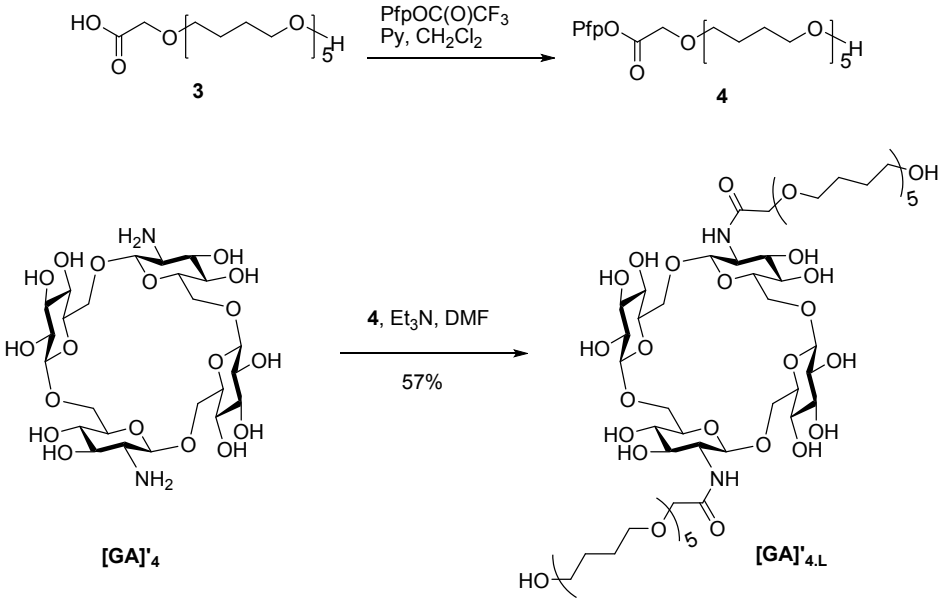
Scheme S2. Synthesis of Acid **3**.



To a solution of ester **2** (164 mg, 0.36 mmol) in a mixture of CH_2Cl_2 (1 mL) and MeOH (5 mL) was added 1.5 M aqueous KOH (0.5 mL). The reaction mixture was stirred for 25 min at room temperature, quenched with 1 M aqueous HCl up to pH < 7, diluted with CH_2Cl_2 and washed with water. The organic layer was dried with Na_2SO_4, filtered, and concentrated to give **3**. Yield 159 mg (100%), colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.06 (s, 2H, $CH_2OC\mathit{H}_2COOH$), 3.62 (t, J = 5.9 Hz, 2H, $C\mathit{H}_2OH$), 3.57 (t, J = 6.3 Hz, 2H, $C\mathit{H}_2OCH_2COOH$), 3.47-3.39 (m, 16H, 8 $OC\mathit{H}_2$), 1.73-1.57 (m, 20H, 10 $C\mathit{H}_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.41 ($\mathit{C}O$), 71.78 ($\mathit{C}H_2OCH_2COOH$), 70.94 (OCH_2), 70.92 (OCH_2), 70.73 (OCH_2), 70.71 (OCH_2), 70.68 (OCH_2), 70.59 (OCH_2), 70.47 (OCH_2), 68.02 ($CH_2O\mathit{C}H_2COOH$), 62.74 (CH_2OH), 30.32 (CH_2), 27.02 (CH_2), 26.53 (CH_2), 26.48 (CH_2),

26.44 (CH_2), 26.26 (CH_2) ppm. HRMS (ESI) calcd. for $C_{22}H_{44}O_8$ $[M + Na]^+$ 459.2928; found 459.2924.

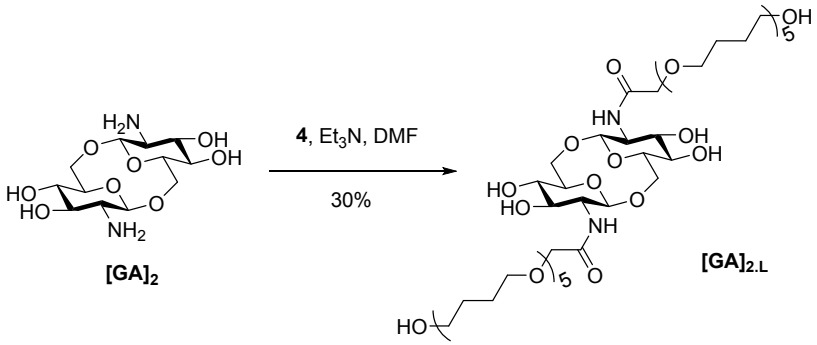
Scheme S3. Synthesis of Conjugate **[GA]′$_{4.L}$**.



Pyridine (0.31 mL, 3.4 mmol) and pentafluorophenyl trifluoroacetate (141 µL, 0.68 mmol) was added to a solution of **3** (73 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (1 mL) at room temperature. Upon completion (60 min), the reaction mixture was co-evaporated with toluene (3×5 mL), dried and purified by column chromatography on silica gel (toluene/EtOAc, 3:1) to give **4** (47 mg, 64%) as a colorless oil. Activated ester **4** (R_f = 0.47, toluene/EtOAc, 3:1) was immediately used in the next step. A solution of ester **4** (47 mg, 0.064 mmol) and Et_3N (20 µL) in DMF (1 mL) was added to a solution of **[GA]′$_4$**[S2] (13 mg, 0.017 mmol) in DMF (1 mL). The reaction mixture was stirred at room temperature for 24 h and DMF was co-evaporated with toluene (3 × 5 mL). The residue was dissolved in MeOH (2 mL) and 1 M MeONa in MeOH (0.6 mL) was added. After 60 min, the reaction mixture was made neutral by addition of 1 M aqueous AcOH (0.6 mL) and concentrated. Purification of **[GA]′$_{4.L}$** was carried out by RP chromatography on a Supelco C-18 column using 40% aqueous MeCN as eluent. Yield 14.4 mg (57%), colorless oil. R_f = 0.41 (MeCN/H_2O, 3:1). $[\alpha]_D$ = -4 (*c* = 1, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 4.8 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1^{GlcN}), 4.34 (d, $J_{1,2}$ = 7.5 Hz, 1H, H-1^{Glc}), 4.03 (dd, $J_{6a,5}$ = 1.8 Hz, $J_{6a,6b}$ = 11.8 Hz, 1H, H-6a^{GlcN}), 4.00-3.94 (m, 4H, H-6^{Glc}, H-6^{GlcN}, OCH_2CO), 3.91 (dd, $J_{6b,5}$ = 5.2 Hz, $J_{6b,6a}$ = 11.9 Hz, 1H, H-6b^{Glc}), 3.72 (dd, $J_{3,2}$ = 8.7 Hz, $J_{3,4}$ = 10.2 Hz, 1H, H-3^{GlcN}), 3.63 (dd, $J_{2,1}$ = 8.2 Hz, $J_{2,3}$ = 10.3 Hz, 1H, H-2^{GlcN}),

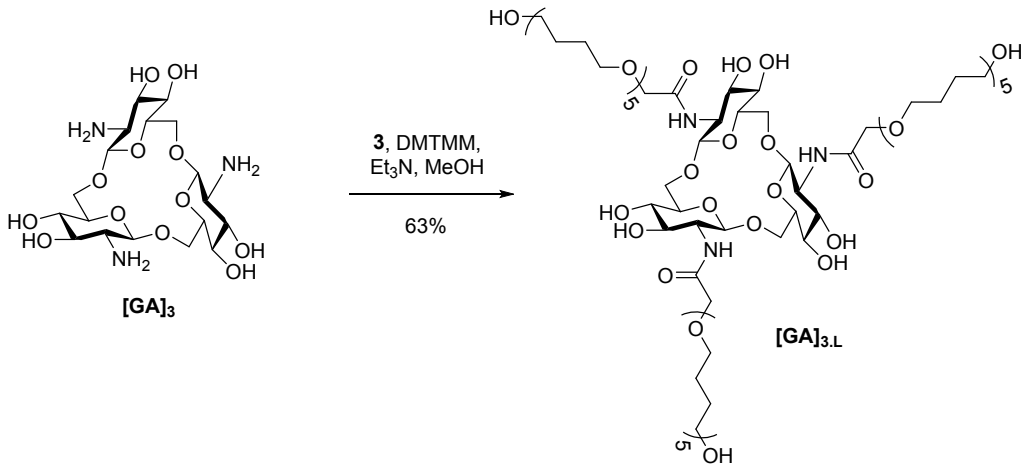
3.61-3.57 (m, 3H, H-5^{GlcN}, OCH_2), 3.56 (t, J = 6.2 Hz, 2H, CH_2OH), 3.48-3.43 (m, 16H, 8 OCH_2), 3.42-3.39 (m, 1H, H-5^{Glc}), 3.35-3.28 (m, 3H, H-3^{Glc}, 2 H-4), 3.25 (dd, $J_{2,1}$ = 7.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, H-2^{Glc}), 1.74-1.55 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (150 MHz, CD_3OD) δ 173.52 (CO), 105.74 (C-1^{Glc}), 102.70 (C-1^{GlcN}), 78.29 (C-5^{Glc}), 78.14 (C-3^{Glc}), 77.81 (C-5^{GlcN}), 75.14 (C-3^{GlcN}), 74.87 (C-2^{Glc}), 72.88 (OCH_2), 72.40 (C-4^{GlcN}), 71.90, 71.87, 71.82, 71.75, 71.24 (O*C*H_2CO), 71.20 (C-4^{Glc}), 70.0 (C-6^{Glc}), 62.97 (CH_2OH), 57.74 (C-2^{GlcN}), 30.60 (CH_2), 27.69 (CH_2), 27.51 (CH_2), 27.43 (CH_2) ppm. HRMS (ESI) calcd. for $C_{68}H_{126}N_2O_{32}$ $[M + NH_4]^+$ 1500.8632; found 1500.8640.

Scheme S4. Synthesis of Conjugate **[GA]$_{2.L}$**.



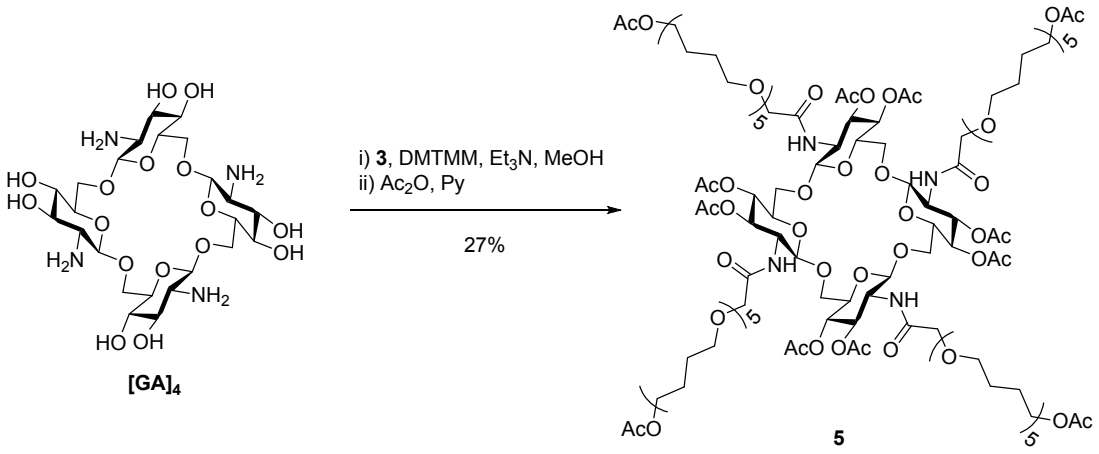
Obtained from cyclodisaccharide **[GA]$_2$**[S3] (14.5 mg, 0.033 mmol) and activated ester **4** (84 mg, 0.11 mmol) as described for **[GA]′$_{4.L}$**. Purification of **[GA]$_{2.L}$** was achieved by RP chromatography on a Supelco C-18 column using gradient elution (50 → 80%) with aqueous MeCN. Yield 11.5 mg (30%), colorless oil. R_f = 0.44 ($CHCl_3$/MeOH, 6:1). $[\alpha]_D$ = -2 (c = 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 4.63 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.15 (dd, $J_{4,3}$ = 7.3 Hz, $J_{4,5}$ = 8.5 Hz, 1H, H-4), 3.99 (dd, $J_{6a,5}$ = 2.3 Hz, $J_{6a,6b}$ = 11.5 Hz, 1H, H-6a), 3.96 (s, 2H, OCH_2CO), 3.92 (dd, $J_{2,1}$ = 1.5 Hz, $J_{2,3}$ = 6.5 Hz, 1H, H-2), 3.77-3.69 (m, 2H, H-5, H-6b), 3.62 (dd, $J_{3,2}$ = 6.5 Hz, $J_{3,4}$ = 8.6 Hz, 1H, H-3), 3.59-3.54 (m, 4H, OCH_2, CH_2OH), 3.50-3.42 (m, 16H, 8 OCH_2), 1.74-1.57 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 173.71 (CO), 102.92 (C-1), 79.99 (C-5), 73.12 (*C*H_2OCH_2CO), 72.88 (C-3), 72.10 (OCH_2), 72.02 (OCH_2), 71.41 (CH_2OCH_2CO), 70.15 (C-6), 68.91 (C-4), 63.21 (CH_2OH), 58.11 (C-2), 30.65 (CH_2), 27.72 (CH_2), 27.55 (CH_2), 27.53 (CH_2), 27.46 (CH_2) ppm. HRMS (ESI) calcd. for $C_{56}H_{106}N_2O_{22}$ $[M + Na]^+$ 1181.7129; found 1181.7118.

Scheme S5. Synthesis of Conjugate **[GA]$_{3.L}$**.



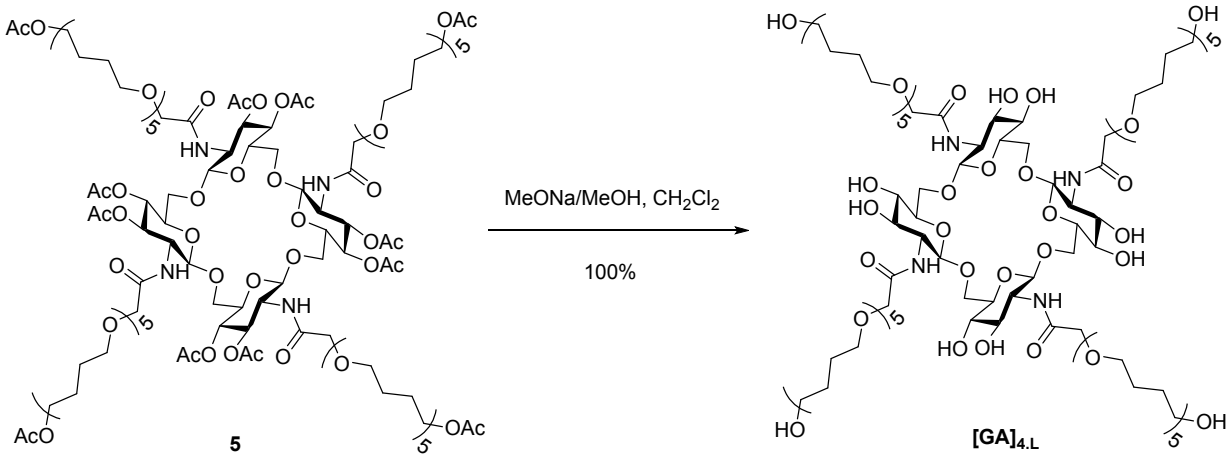
A mixture of cyclotrisaccharide **[GA]$_3$**[S3] (10 mg, 0.017 mmol), acid **3** (33 mg, 0.077 mmol), DMTMM (22 mg, 0.078 mmol) and Et_3N (15 μL) in methanol (2 mL) was stirred at room temperature for 24 h, then concentrated and subjected to gel-permeation chromatography on a column of LH-20 gel. Carbohydrate-containing eluate was evaporated and purified firstly by gel-permeation chromatography on a column of TSK HW-40 (S) gel (25×400 mm) in 0.1 M AcOH, then by chromatography on silica gel ($CHCl_3$/MeOH, 4:1) to afford conjugate **[GA]$_{3.L}$** (13.5 mg, 63%) as colorless oil. R_f = 0.21 ($CHCl_3$/MeOH, 4:1). $[\alpha]_D$ = -13.5 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 4.60 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.16 (dd, $J_{6a,5}$ = 2.5 Hz, $J_{6a,6b}$ = 11.4 Hz, 1H, H-6a), 4.01(s, 2H, OCH_2CO), 3.87 (dd, $J_{6b,5}$ = 1.9 Hz, $J_{6b,6a}$ = 11.3 Hz, 1H, H-6b), 3.81 (t, $J_{2,1} = J_{2,3}$ = 8.7 Hz, 1H, H-2), 3.69 (t, $J_{4,3} = J_{4,5}$ = 9.2 Hz, 1H, H-4), 3.61-3.54 (m, 5H, H-3, OCH_2, C*H*$_2$OH), 3.50-3.41 (m, 16H, 8 OCH_2), 3.40-3.34(m, 1H, H-5), 1.76-1.55 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 174.03 (CO), 102.53 (C-1), 76.11 (C-5), 75.61 (C-3), 72.69 (OCH_2), 71.73 (OCH_2), 71.66 (OCH_2), 71.61 (OCH_2), 71.12 (O*C*H$_2$CO), 70.72 (C-4), 69.32 (C-6), 62.82 (CH_2OH), 56.50 (C-2), 30.46 (CH_2), 27.55 (CH_2), 27.37 (CH_2), 27.35 (CH_2), 27.29 (CH_2) ppm. HRMS (ESI) calcd. for $C_{84}H_{159}N_3O_{33}$ [M + 2Na]$^{2+}$ 892.0320; found 892.0323.

Scheme S6. Synthesis of Conjugate **5**.



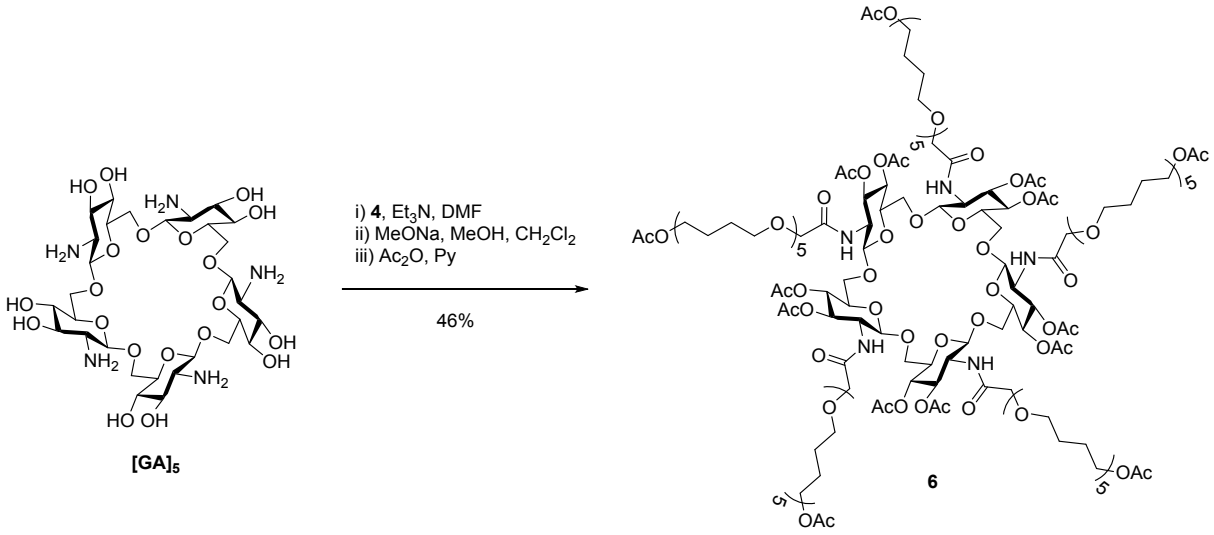
A mixture of cyclotetrasaccharide **[GA]$_4$**[3] (12.2 mg, 0.017 mmol), acid **3** (48 mg, 0.11 mmol), DMTMM (32 mg, 0.11 mmol) and Et_3N (10 μL) in methanol (2 mL) was stirred at room temperature for 24 h, then concentrated and subjected to gel-permeation chromatography on a column of LH-20 gel. Carbohydrate-containing eluate was evaporated, the residue was dissolved in pyridine (2 mL) and Ac_2O (1 mL) was added. After 3 h, the reaction mixture was co-evaporated with toluene (3 × 5 mL) and dried *in vacuo*. The residue was purified by RP chromatography on a column VYDAC 218TP1010 (10 × 250 mm) using 95% aqueous MeCN as eluent. Yield 13 mg (27%), colorless oil. R_f = 0.53 (toluene/acetone, 2:3). $[\alpha]_D$ = 14.5 (*c* = 1, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (d, $J_{NH,2}$ = 7.9 Hz, 1H, NH), 5.47 (t, $J_{3,2} = J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.01 (br. t, $J_{4,3} = J_{4,5}$ = 8.6 Hz, 1H, H-4), 4.97 (d, $J_{1,2}$ = 7.4 Hz, 1H, H-1), 4.08 (t, *J* = 6.4 Hz, 2H, AcOCH_2), 3.91-3.75 (m, 6H, H-2, H-5, 2 H-6, OCH_2CO), 3.50-3.36 (m, 18H, 9 OCH_2), 2.03 (s, 6H, 2 OAc), 1.99 (s, 3H, OAc), 1.72-1.56 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.21 (CO), 170.50 (CO), 170.26 (CO), 169.46 (CO), 100.40 (C-1), 73.49 (C-5), 71.86 (C-3), 71.72 (OCH_2), 70.81, 70.71, 70.42, 70.27, 70.17, 69.01 (C-4), 67.87 (O*C*H_2CO), 64.43 (*C*H_2OAc), 54.31 (C-2), 26.62 (CH_2), 26.40 (CH_2), 26.36 (CH_2), 25.62 (CH_2), 21.05 (OAc), 20.77 (OAc) ppm. HRMS (ESI) calcd. for $C_{136}H_{236}N_4O_{56}$ $[M + NH_4]^+$ 1428.8209; found 1428.8190.

Scheme S7. Synthesis of Conjugate **[GA]$_{4.L}$**.



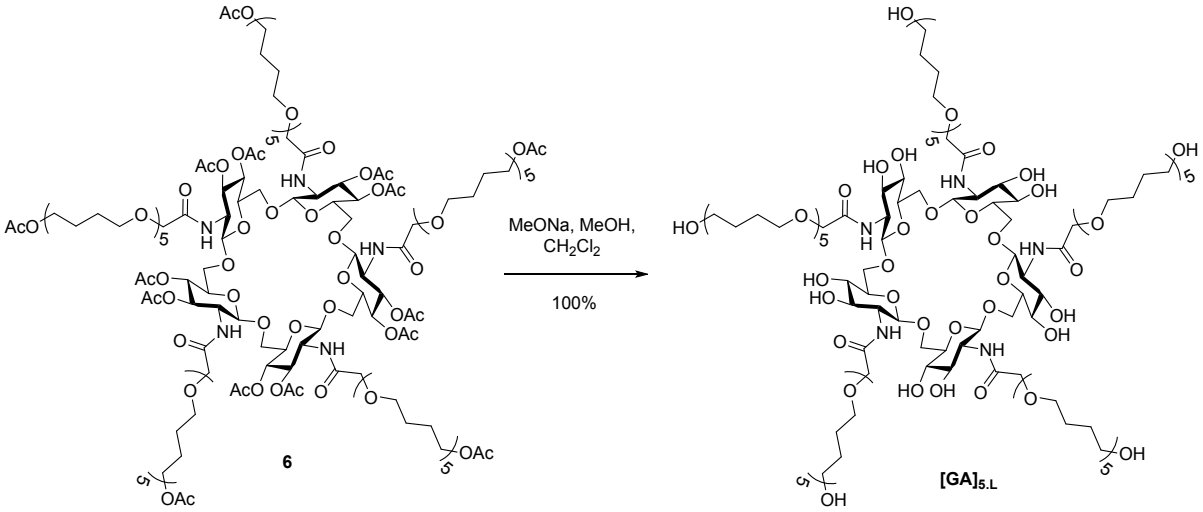
Acetylated conjugate **5** (23 mg, 0.0082 mmol) was dissolved in a mixture of MeOH (1.5 mL) and CH_2Cl_2 (1 mL) and 1 M MeONa in MeOH (20 μL) was added. After 24 h, the reaction mixture was made neutral by addition of 1 M aqueous AcOH (20 μL) and concentrated. The residue was purified by gel-permeation chromatography on a column of LH-20 gel to afford **[GA]$_{4.L}$** (19 mg, 100%) as a colorless oil. R_f = 0.55 ($CHCl_3$/MeOH, 3:1). $[\alpha]_D$ = -3 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 4.67 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.04-3.92 (m, 4H, 2 H-6, OCH_2CO), 3.72-3.61 (m, 2H, H-2, H-3), 3.61-3.54 (m, 4H, OCH_2, C*H*$_2$OH), 3.50-3.40 (m, 18H, H-4, H-5, 8 OCH_2), 1.75-1.55 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 173.21 (CO), 102.92 (C-1), 76.97 (C-5), 74.87 (C-3), 72.59 (OCH_2), 71.59 (OCH_2), 71.51 (OCH_2), 71.46 (OCH_2), 71.33 (C-4), 70.93 (O*C*H$_2$CO), 70.29 (C-6), 62.69 (CH_2OH), 57.04 (C-2), 30.26 (CH_2), 27.34 (CH_2), 27.15 (CH_2), 27.11 (CH_2) ppm. HRMS (ESI) calcd. for $C_{112}H_{212}N_4O_{44}$ $[M + Na]^+$ 2340.4367; found 2340.4363.

Scheme S8. Synthesis of Conjugate **6**.



A solution of ester **4** (49 mg, 0.081 mmol) and Et_3N (20 μL) in DMF (1 mL) was added to a solution of **[GA]$_5$**[S3] (9 mg, 0.0081 mmol) in DMF (1 mL). The reaction mixture was stirred at room temperature for 24 h and DMF was co-evaporated with toluene (3 × 5 mL). The residue was dissolved in MeOH (2 mL) and 1 M MeONa in MeOH (0.1 mL) was added. After 60 min, the reaction mixture was made neutral by addition of 1 M aqueous AcOH (0.1 mL) and concentrated. The residue was subjected to gel-permeation chromatography on a column of LH-20 gel. Carbohydrate-containing eluate was evaporated, dissolved in pyridine (2 mL) and Ac_2O (1 mL) was added. After 3 h, the reaction mixture was co-evaporated with toluene (3 × 5 mL), dried *in vacuo* and purified by RP chromatography on a column VYDAC 218TP1010 (10 × 250 mm) using 95% aqueous MeCN as eluent. Yield 13 mg (46%), colorless oil. R_f = 0.5 (toluene/acetone, 4:5). $[\alpha]_D$ = 6 (*c* = 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.68 (d, $J_{NH,2}$ = 7.9 Hz, 1H, NH), 5.39 (t, $J_{3,2} = J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.0 (br. t, $J_{4,3} = J_{4,5}$ = 9.3 Hz, 1H, H-4), 4.95 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.09 (t, *J* = 6.5 Hz, 2H, AcOC*H*$_2$), 3.93-3.84 (m, 3H, H-6a, OCH_2CO), 3.79-3.71 (m, 3H, H-2, H-5, H-6b), 3.55-3.39 (m, 18H, 9 OCH_2), 2.05 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.73-1.58 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.36 (CO), 170.28 (CO), 169.42 (CO), 99.85 (C-1), 73.42 (C-5), 71.69 (C-3), 71.58 (OCH_2), 70.72 (OCH_2), 70.70 (OCH_2), 70.64 (OCH_2), 70.62 (OCH_2), 70.60 (OCH_2), 70.31 (OCH_2), 70.17 (OCH_2), 70.05 (O*C*H$_2$CO), 69.09 (C-4), 67.39 (C-6), 64.33 (*C*H$_2$OAc), 54.18 (C-2), 26.51 (CH_2), 26.29 (CH_2), 26.25 (CH_2), 26.22 (CH_2), 25.51 (CH_2), 20.95 (OAc), 20.65 (OAc) ppm. HRMS (ESI) calcd. for $C_{170}H_{295}N_5O_{70}$ $[M + Na]^+$ 1786.4731; found 1786.4751.

Scheme S9. Synthesis of Conjugate **[GA]$_{5.L}$**.



Obtained from acetylated conjugate **6** (13 mg, 0.0037 mmol) as described for **5**. Yield 12 mg (100%), colorless oil. R_f = 0.59 ($CHCl_3$/MeOH, 4:1). $[\alpha]_D$ = -7 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 4.68 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.08-3.95 (m, 3H, H-6a, OCH_2CO), 3.90 (dd, $J_{6b,5}$ = 5.1 Hz, $J_{6b,6a}$ = 11.6 Hz, 1H, H-6b), 3.70 (br. t, $J_{2,1} = J_{2,3}$ = 8.4 Hz, 1H, H-2), 3.65-3.54 (m, 5H, H-3, OCH_2, C*H*$_2$OH), 3.52-3.37 (m, 18H, H-4, H-5, 8 OCH_2), 1.75-1.56 (m, 20H, 10 CH_2) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 173.37 (CO), 103.19 (C-1), 77.31 (C-5), 75.17 (C-3), 72.75 (OCH_2), 71.92 (C-4), 71.73 (OCH_2), 71.67 (OCH_2), 71.16 (O*C*H$_2$CO), 70.41 (C-6), 62.82 (CH_2OH), 57.36 (C-2), 30.46 (CH_2), 27.55 (CH_2), 27.33 (CH_2), 27.30 (CH_2) ppm. HRMS (ESI) calcd. for $C_{140}H_{265}N_5O_{55}$ $[M + 2Na]^{2+}$ 1471.3939; found 1471.3902.

IV. Ion Channel Studies

Ion transporting activity studies:

Buffer and stock solution preparation: HEPES buffer was prepared by dissolving solid HEPES (10 mM) and NaCl (100 mM) in autoclaved water, followed by adjustment of pH (pH = 7.0) by adding NaOH solution. Stock solution of all channel forming molecules were prepared in HPLC grade DMSO.

Preparation of EYPC-LUVs⊃HPTS: A thin transparent film of egg yolk phosphatidylcholine (EYPC) was prepared by drying of 1 mL of EYPC (25 mg/mL in $CHCl_3$) with purging of nitrogen and continuous rotation in a clean and dry small round bottomed flask. The transparent film was kept in high vacuum for 3 h to remove all trace of $CHCl_3$. The resulting films were hydrated with 1 mL buffer (1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0) for 1 h with 4-5 times vortexing occasionally and subjected to freeze-thaw cycle (≥ 15 times). Extrusions were done 19 times (must be an odd number) by a Mini-extruder with a polycarbonate membrane, pore size 100 nm (Avanti). Extravesicular dyes were removed by gel filtration (Sephadex G-50) with buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) and diluted to 6 mL to get EYPC-LUVs⊃HPTS: ~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0, outside: 10 mM HEPES, 100 mM NaCl, pH = 7.0.[S4]

Ion transport activity: In a clean and dry fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was added followed by addition of 25 μL of EYPC-LUVs⊃HPTS in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$). The time course of HPTS fluorescence emission intensity, F_t was observed at λ_{em} = 510 nm (λ_{ex} = 450 nm). 20 μL of 0.5 M NaOH was added to the cuvette at t = 20 second to make the pH gradient between the intra and extra vesicular system. Channel forming molecules were added at t = 100 s in different concentration and finally at t = 300 s 25 μL of 10% Triton X-100 was added to lyze those vesicles resulting destruction of pH gradient (Figure **3a, b**).

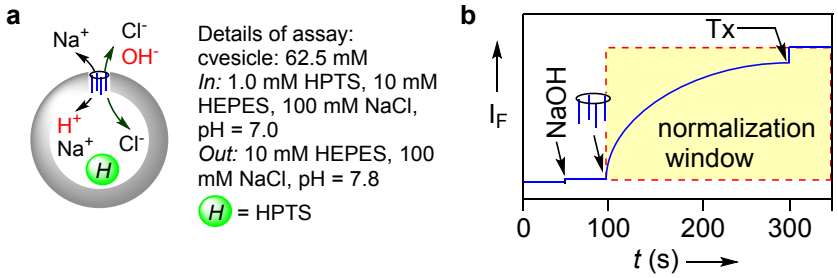


Fig. S1 Representation of ion transport activity assay using EYPC vesicles (**a**). Representation of ion transport kinetics experiment using fluorescence (**b**).

Fluorescence time courses (F_t) were normalized to fractional emission intensity I_F using Equation S1.

$$I_F = [(F_t - F_0) / (F_\infty - F_0)] \times 100 \qquad \text{(S1)}$$

Where F_0 = Fluorescence intensity just before the channel addition (at 0 s). F_∞ = Fluorescence intensity at saturation after complete leakage (at 320 s). F_t = Fluorescence intensity at time t.

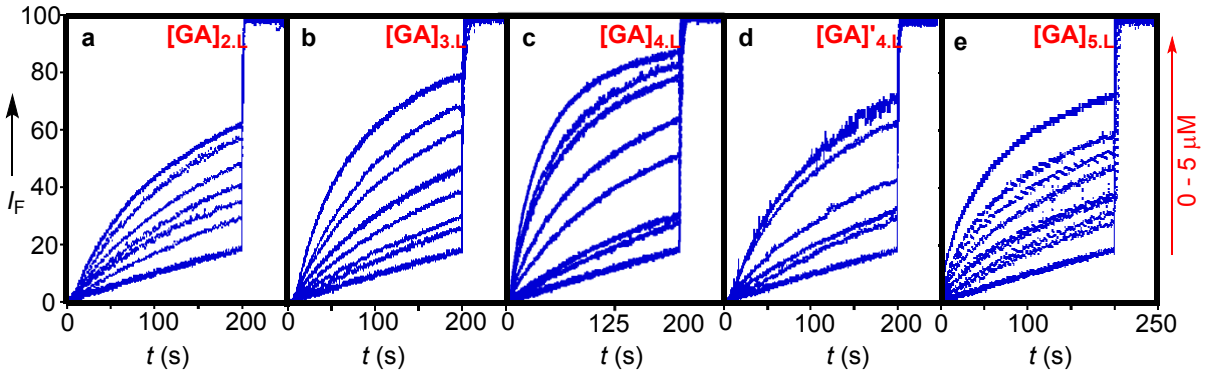


Fig. S2 Ion transport assay of neoglycolipid derivatives (**a** for **[GA]$_{2.L}$**, **b** for **[GA]$_{3.L}$**, **c** for **[GA]'$_{4.L}$**, and **d** for **[GA]$_{5.L}$**) using EYPC LUVs⊃HPTS, where the time of compound addition were taken as 0 s and Triton X-100 was added at 200 s.

The concentration profile data were analysed by Hill Equation (Equation **S2**) to get the Effective concentration (EC_{50}) and Hill Coefficient (n), (Figure **4a**).

$$Y = Y_\infty + (Y_0 - Y_\infty) / [1 + (c / EC_{50})^n] \qquad \text{(S2)}$$

Where, Y_0 = Fluorescence intensity just before the channel addition (at 0 s). Y_∞ = Fluorescence intensity with excess channel concentration, c = Concentration of channel forming molecule.

The unimolecularity of channels was proved by the following linear correlation plot. The rate obtained from first order equation fitting to the individual concentration curve of **[GA]$_{4.L}$** (Fig. 2C) was plotted against concentration. Linear correlation of rate verses concentration curve signifies unimolecularity of channel.

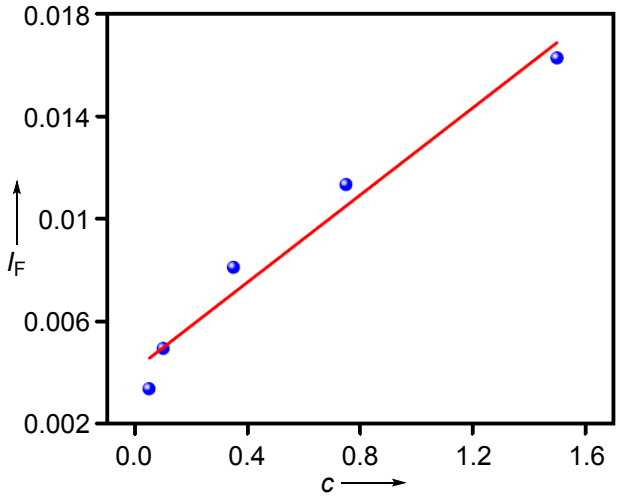


Fig. S3 Linear correlation between the rate of transport and the concentration of the channel.

Ion Selectivity studies by competitive inhibition assay:

Buffer and stock solution preparation: HEPES buffer was prepared by dissolving solid HEPES (10 mM) and appropriate salt (100 mM) in autoclaved water, followed by adjustment of pH (pH = 7.0) by adding NaOH solution. Stock solution of all channel forming molecules were prepared in HPLC grade DMSO.

Preparation of EYPC-LUVs⊃HPTS: A thin transparent film of egg yolk phosphatidylcholine (EYPC) was prepared by drying of 1 mL of EYPC (25 mg/mL in $CHCl_3$) with purging of nitrogen and continuous rotation in a clean and dry small round bottomed flask. The transparent film was kept in high vacuum for 3 h to remove all trace of $CHCl_3$. The resulting films were hydrated with 1 ml buffer (1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0) for 1 h with 4-5 times vortexing occasionally and subjected to freeze-thaw cycle (≥ 15 times). Extrusions were done 19 times (must be an odd number) by a

Mini-extruder with a polycarbonate membrane, pore size 100 nm (Avanti). Extravesicular dyes were removed by gel filtration (Sephadex G-50) with buffer (10 mM HEPES, pH = 7.0) and diluted to 6 mL to get EYPC-LUVs⊃HPTS: ~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0, outside: 10 mM HEPES, pH = 7.0.

Ion Selectivity Assay: In a clean and dry fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM MX, pH = 7.0) was added followed by addition of 25 μL of EYPC-LUVs⊃HPTS in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at t = 0). The time course of HPTS fluorescence emission intensity, F_t was observed at λ_{em}= 510 nm (λ_{ex}= 450 nm). 20 μL of 0.5 M NaOH was added to the cuvette at t = 20 s to make the pH gradient between the intra and extra vesicular system. Channel forming moleFcules were added at t = 100 s and at t = 300 s, of 10% Triton X-100 (25 μL) was added to lyse vesicles for complete destruction of pH gradient.

All fluorescence time dependent spectra were normalized between 0 (at 96 s) and 100 s (at 320 second) by using Equation S1.

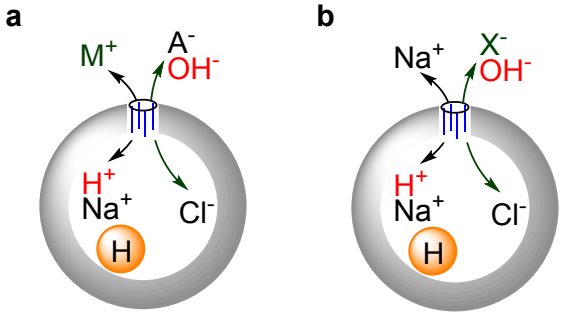


Fig. S4 Schematic representations of the fluorescence based ion selectivity assay. Vesicle leakage assay for cation selectivity (**a**) and anion selectivity (**b**).

Cation selectivity was checked by varying the extravesicular chloride salts (MCl) of different alkali matal cations (Fig. **S4a**). The difference in rate observed for different cation (Li^+, Na^+, K^+, Rb^+, Cs^+) is characteristics of cation selectivity of Ion channel. Under the applied conditions no cation selectivity sequence was observed for either of glucosamine derivatives $\mathbf{[GA]_{2.L}}$, $\mathbf{[GA]_{3.L}}$, $\mathbf{[GA]_{4.L}}$, $\mathbf{[GA]'_{4.L}}$ and $\mathbf{[GA]_{5.L}}$.

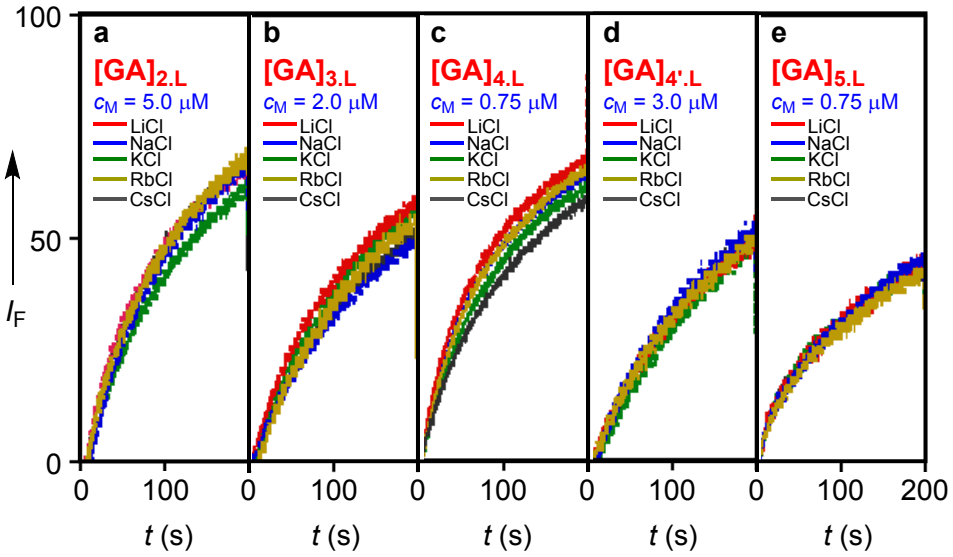


Fig. S5 Studies on cation selectivities of ion channels **[GA]$_{2.L}$** (**a**), **[GA]$_{3.L}$** (**b**), **[GA]$_{4.L}$** (**c**), **[GA]'$_{4.L}$** (**d**) and **[GA]$_{5.L}$** (**e**) evaluated by competitive inhibition assay.

Anion selectivity was evaluated by varying the extravesicular sodium salts of different halides (NaX) (Fig. **S2b**). In these assays, NaCl, NaBr and NaI were used. Difference in transport activity was observed due to the competitive transport of X^-/OH^-. Selectivity for anion was observed for all glucosamine derivatives (Figure 5).

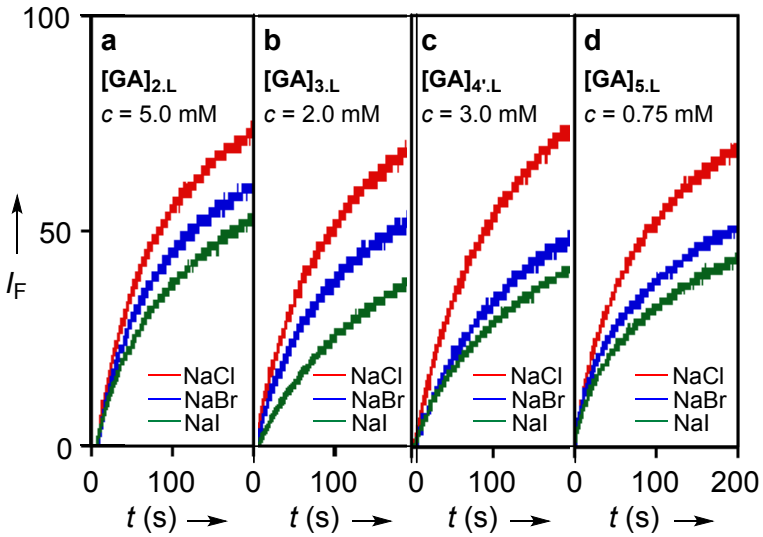


Fig. S6 Anion selectivities of **[GA]$_{2.L}$**, **[GA]$_{3.L}$**, **[GA]'$_{4.L}$** and **[GA]$_{5.L}$** evaluated by competitive inhibition assay.

V. Molecular Modelling Data

MD calculations were carried out using MM3 force field as implemented in TINKER v. 5.0 package.[S6] Solvent Accessible Surface Area (SASA)[S7] model was used to account for solvation. Global minima found as described in the previous communication[S3] were used as starting points. Simulation lengths were 10 ns in each case with the standard step size of 1 fs. Constant temperature mode at 300 K with Berendsen thermostat was employed. Structural snapshots were taken each 0.002 ns, resulting in 5000 structures for each molecule.

Table S1. Characteristic dimensions of internal cavities in compounds **[GA]$_{2.L}$**, **[GA]$_{3.L}$**, **[GA]$_{4.L}$** and **[GA]$_{5.L}$** measured for energy optimized conformations.

[GA]$_{n.L}$	O5-O5', Å	O5''-O5''', Å	O6-O6', Å	O6''-O6''', Å
[GA]$_{2.L}$	4.70	-	2.24	-
[GA]$_{3.L}$	3.61	-	5.00	-
[GA]$_{4.L}$	5.96	5.96	6.80	6.80
[GA]$_{5.L}$	6.95[a]	5.35[a]	6.89[a]	7.61[a]

[a] Data may not characterize ring shape well because of the flexibility.

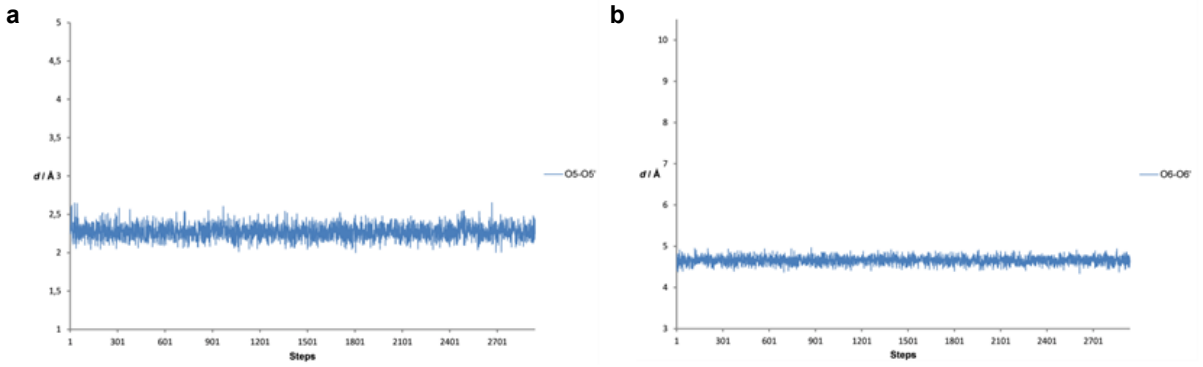


Fig. S7 MD graphs for interatomic distances in structure **[GA]$_{2.L}$**.

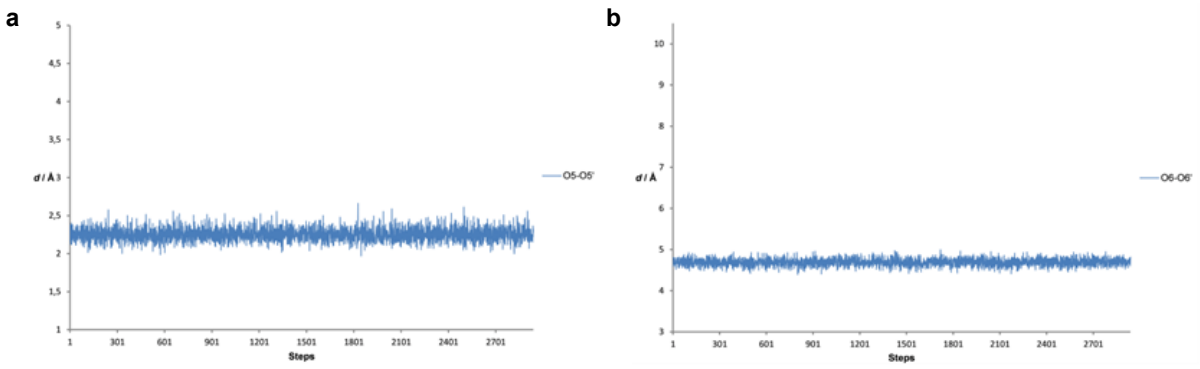


Fig. S8 MD graphs for interatomic distances for the scaffold disaccharide $\mathbf{[GA]_{2.Ac}}$.

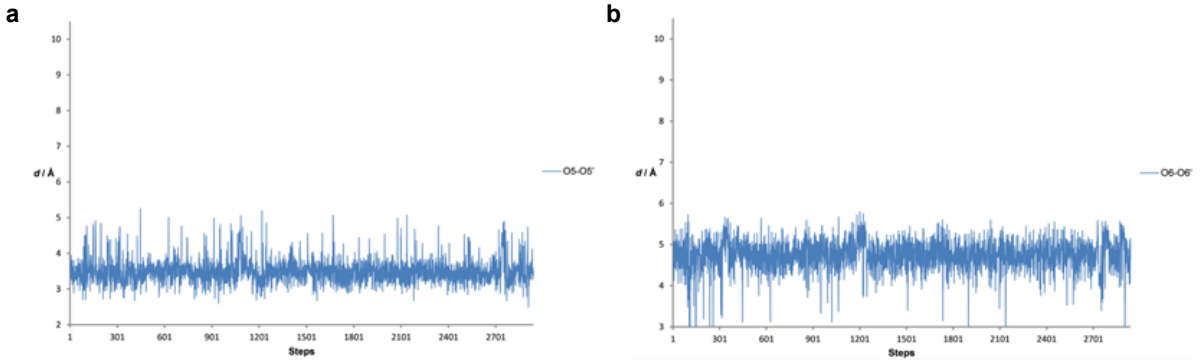


Fig. S9 MD graphs for interatomic distances in structure $\mathbf{[GA]_{3.L}}$.

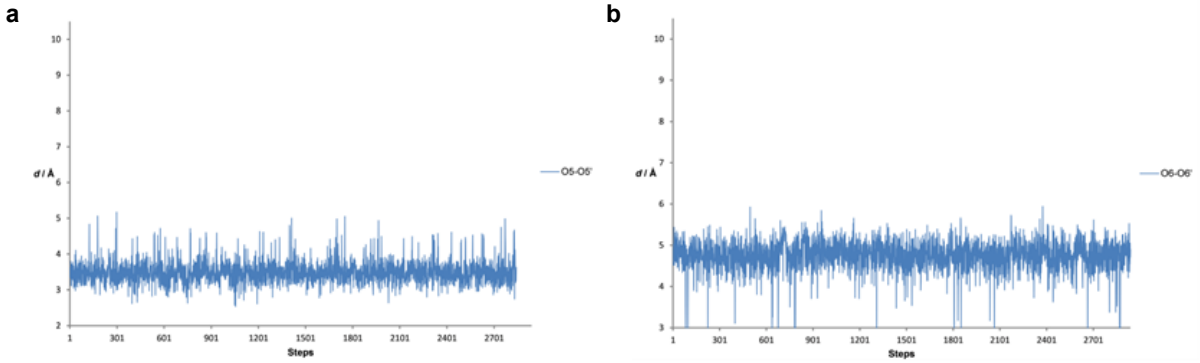


Fig. S10 MD graphs for interatomic distances for the scaffold trisaccharide $\mathbf{[GA]_{3.Ac}}$.

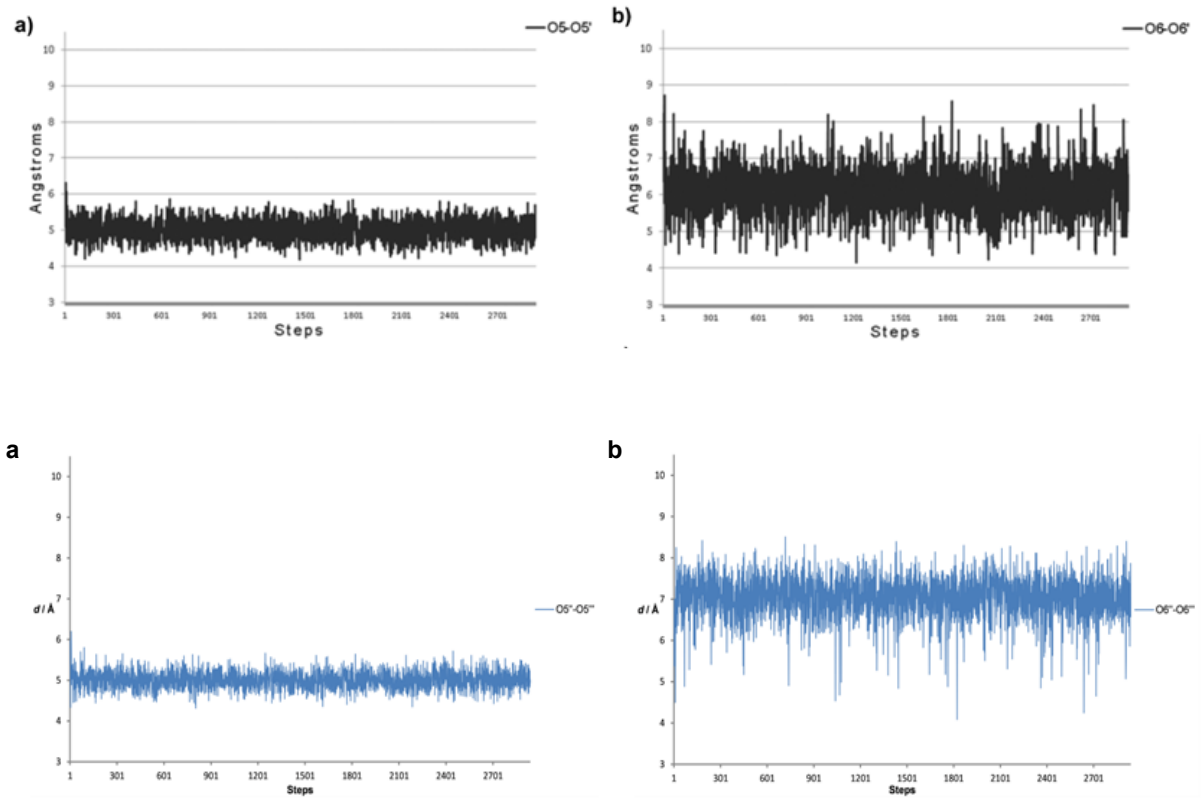


Fig. S11 MD graphs for interatomic distances in structure $\mathbf{[GA]_{4.L}}$.

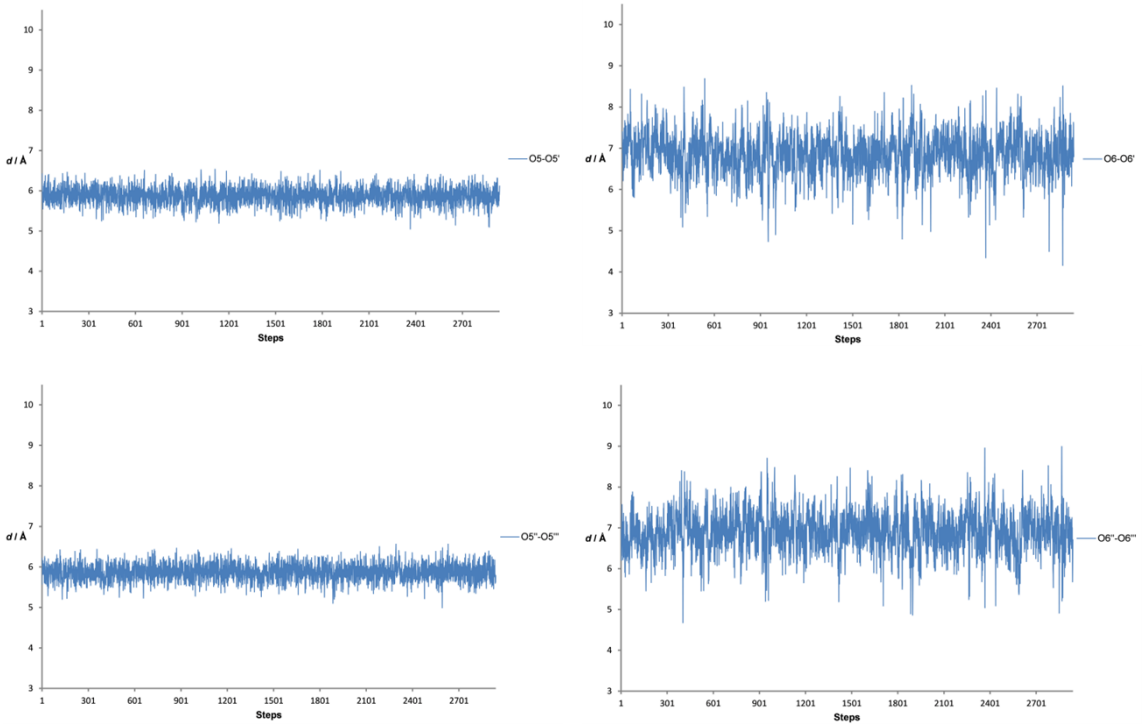


Fig. S12 MD graphs for interatomic distances for the scaffold tetrasaccharide $\mathbf{[GA]_{4.Ac}}$.

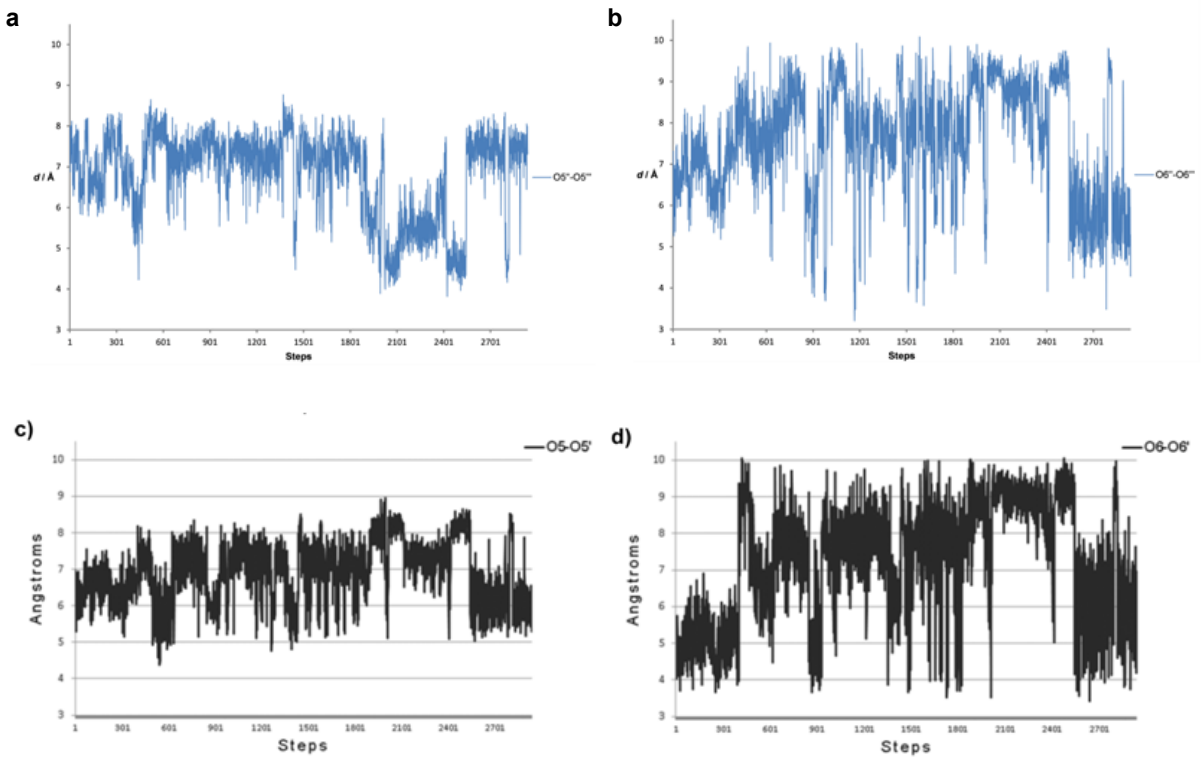


Fig. S13 MD graphs for interatomic distances in structure $[GA]_{5.L}$

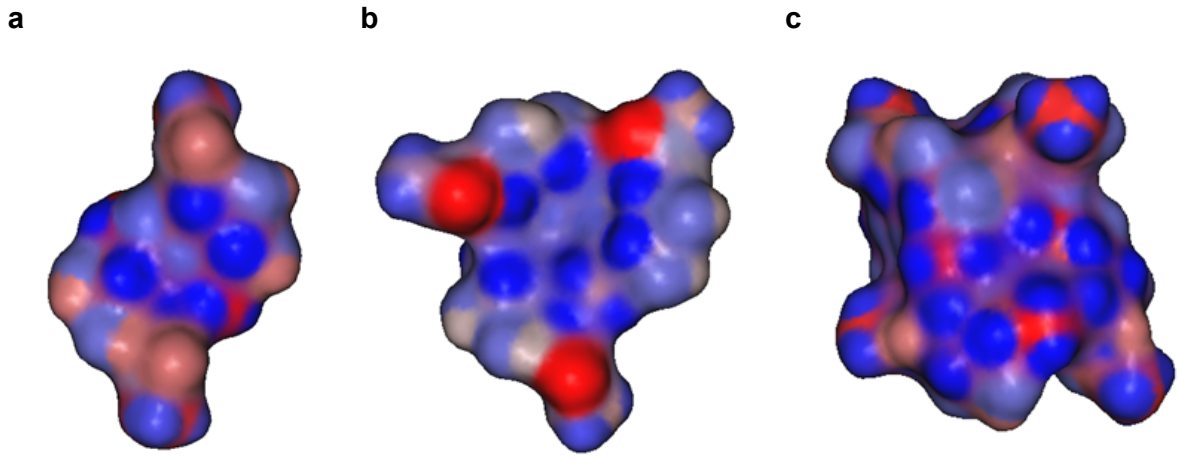


Fig. S14 Distributions of hydrophobic (blue) and hydrophilic (red) indentation for the cyclic di- (**a**), tri- (**b**) and pentaglucosamine (**c**) matrices $[GA]_{2.Ac}$, $[GA]_{3.Ac}$, and $[GA]_{5.Ac}$, respectively.

VI. NMR Data

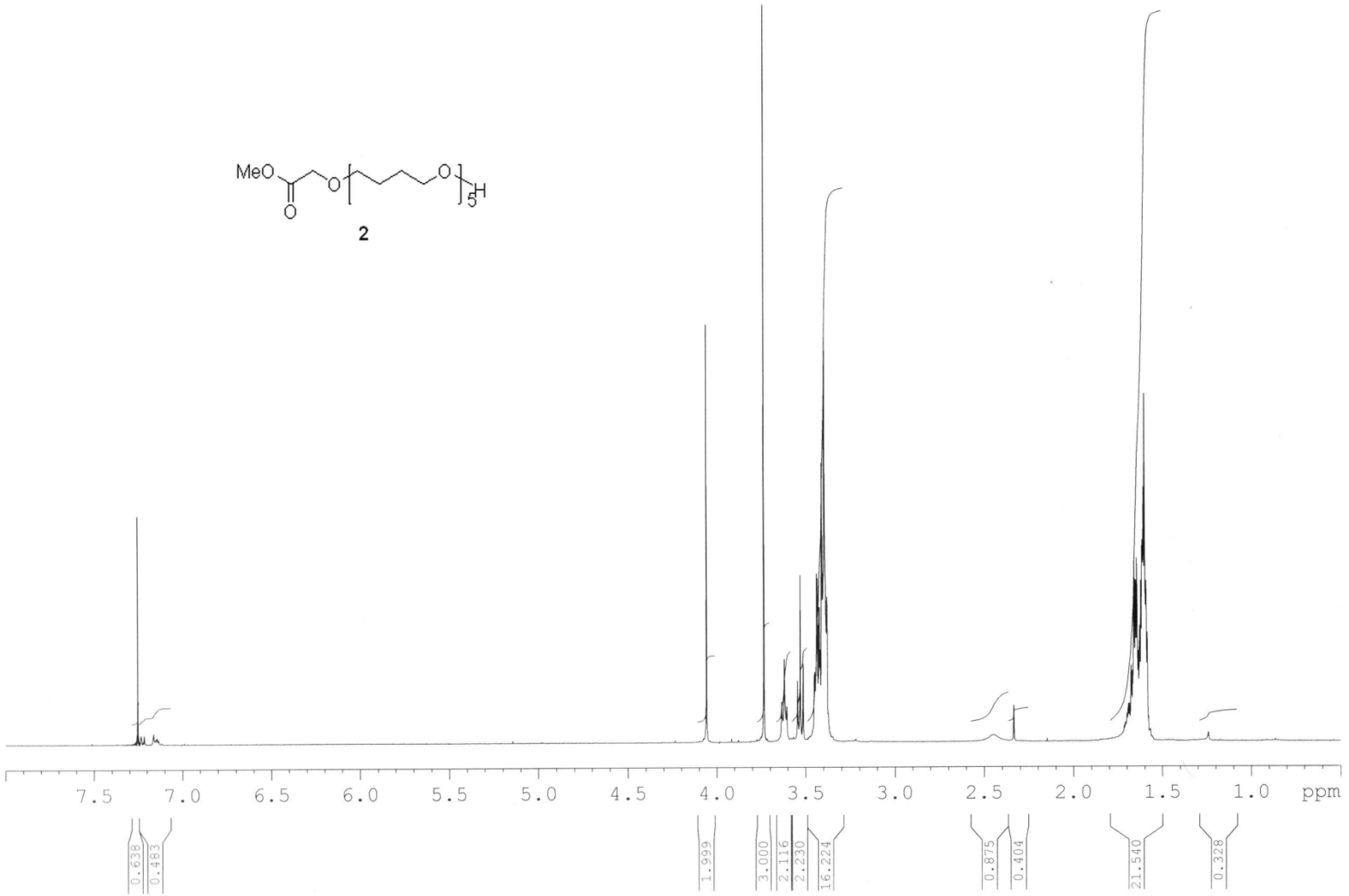


Fig. S15. ^{1}H NMR spectrum of ester **2.**

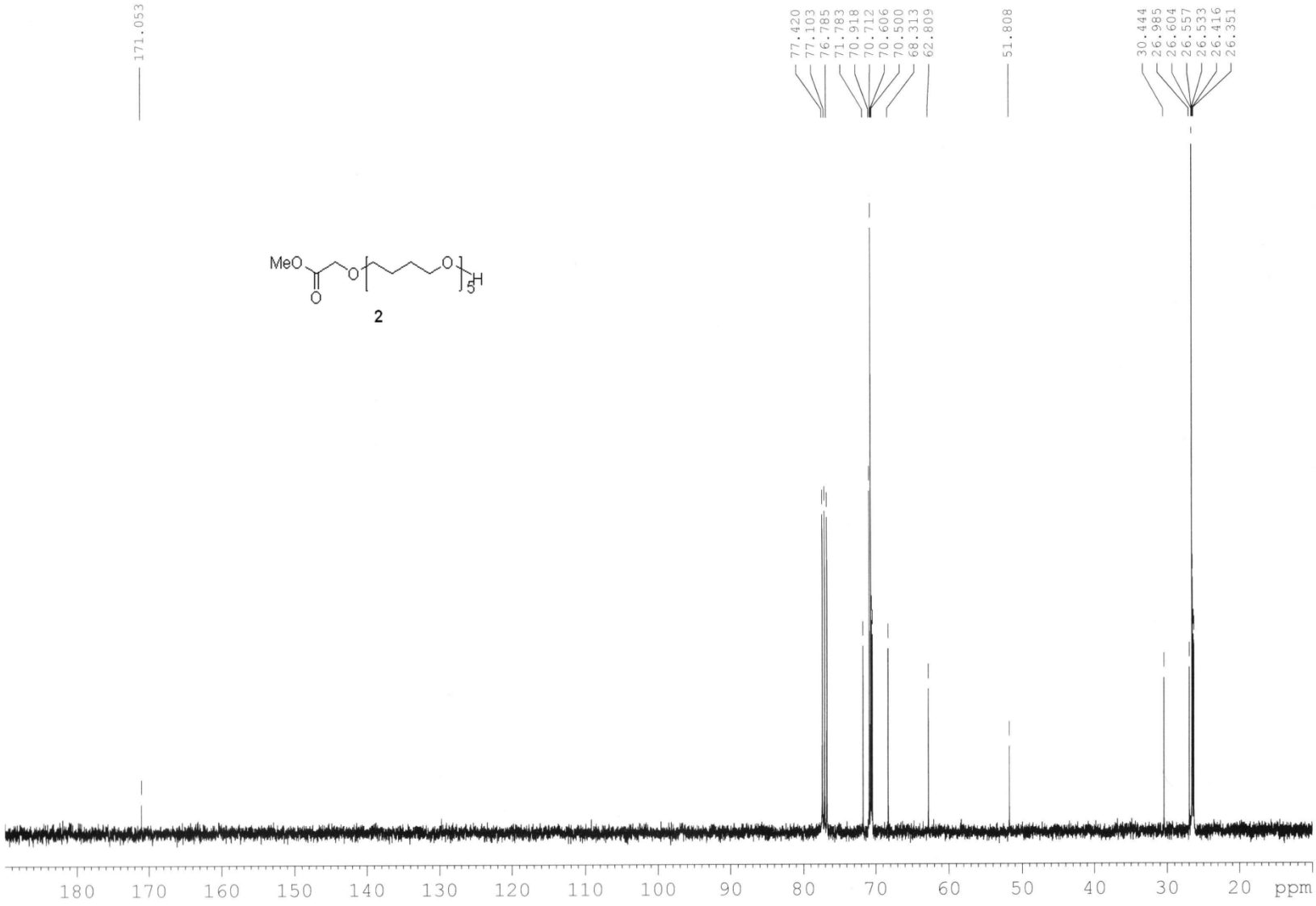


Figure S16. ^{13}C NMR spectrum of ester **2.**

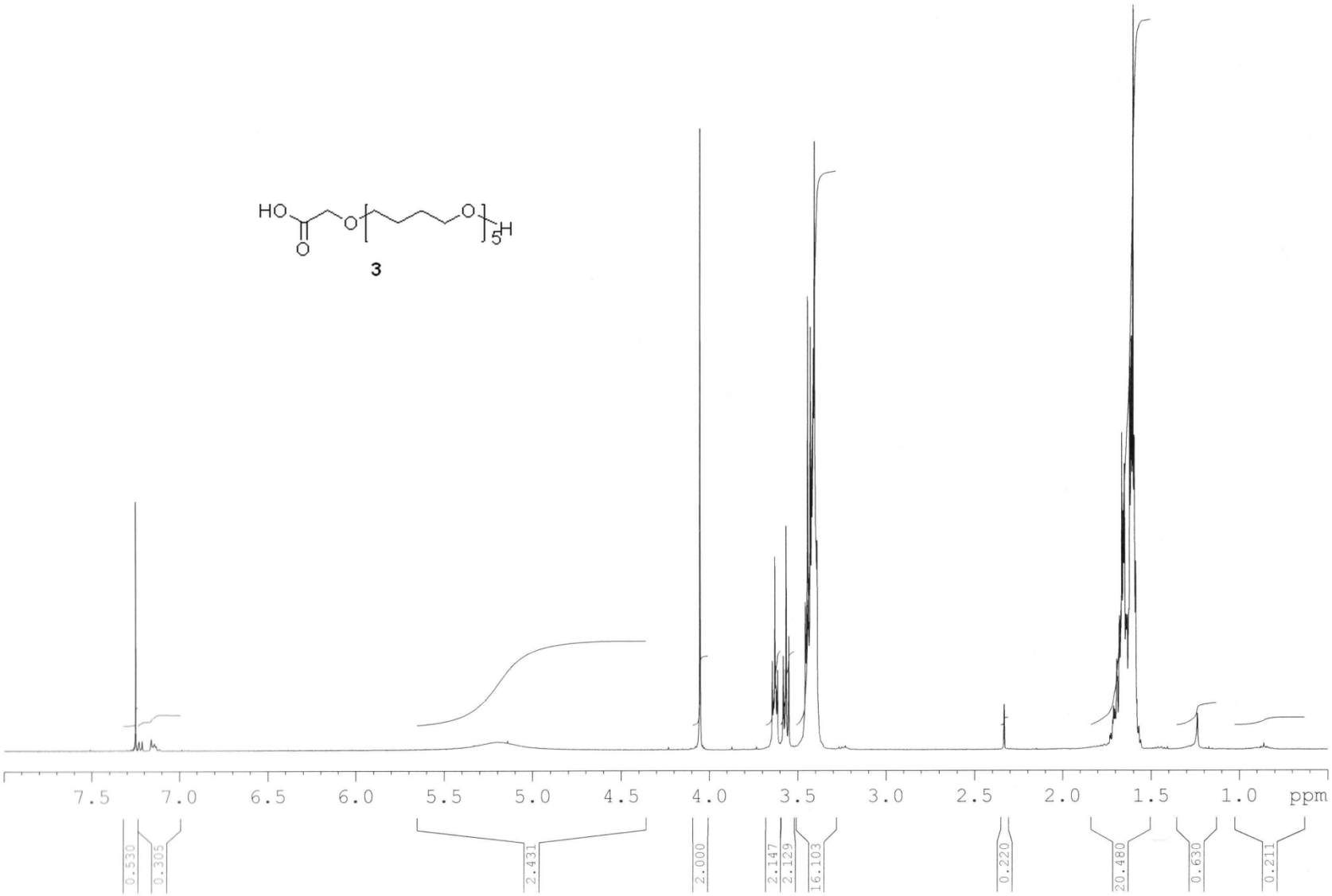


Fig. S17 ^{1}H NMR spectrum of acid **3.**

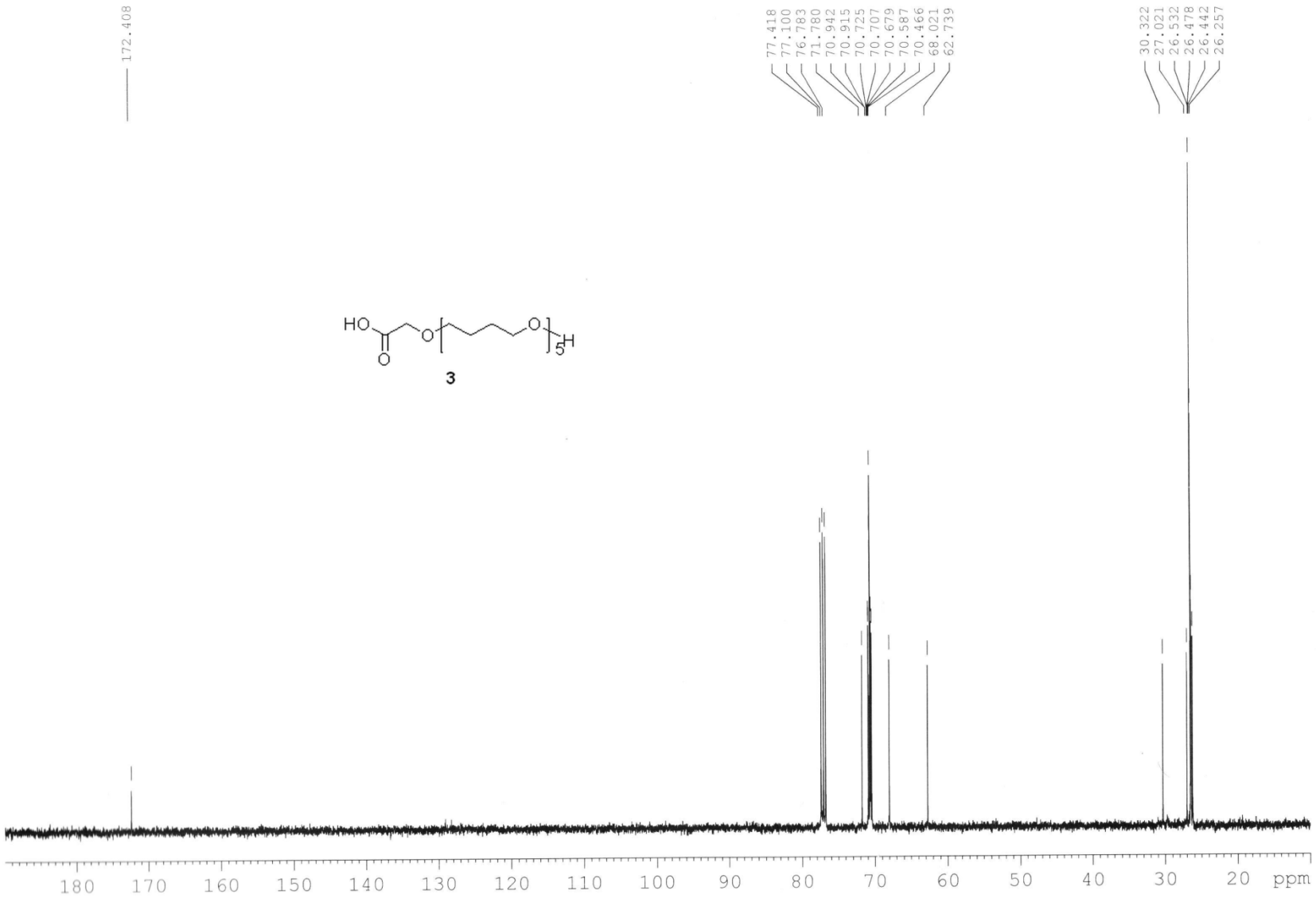


Fig. S18 ^{13}C NMR spectrum of acid **3.**

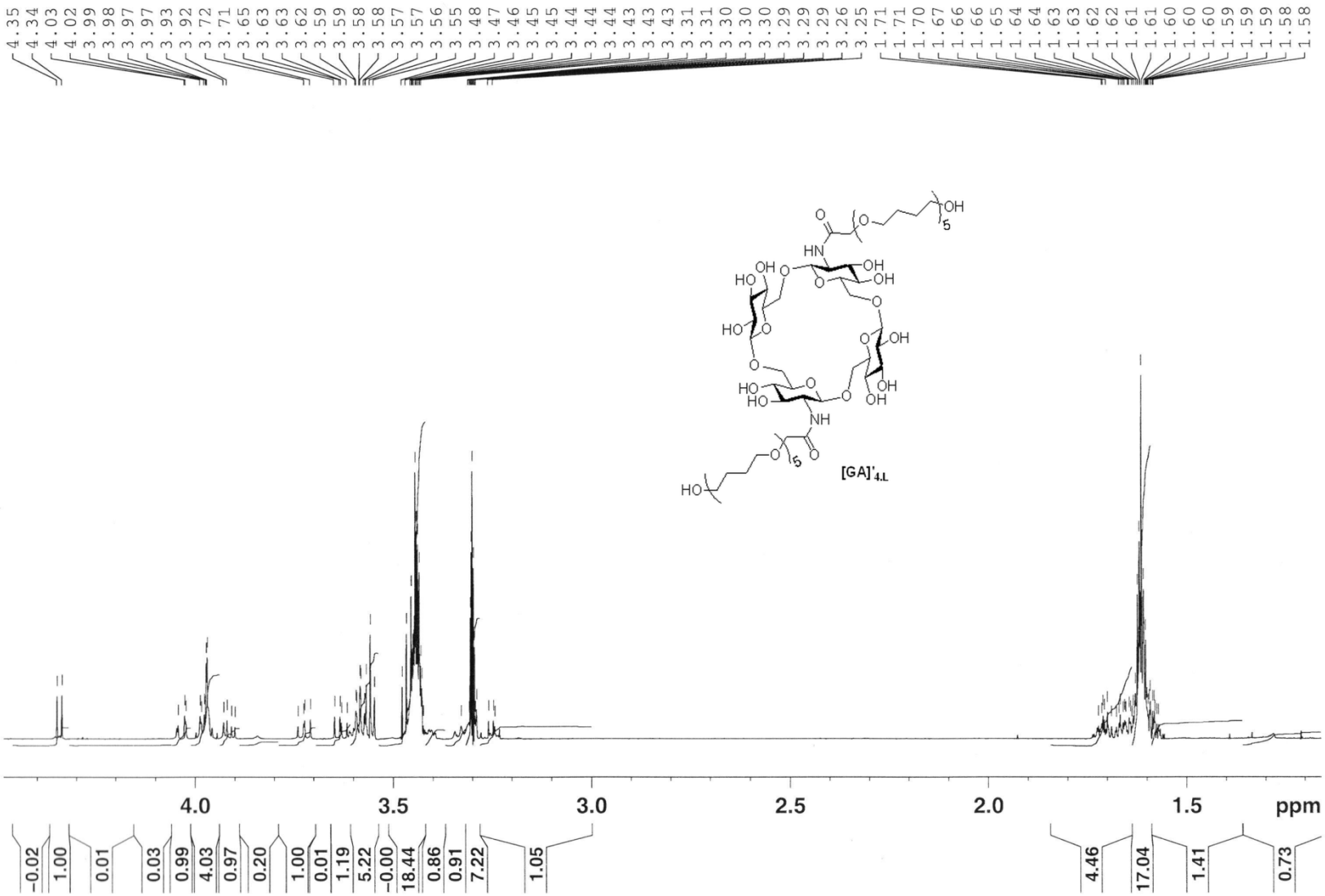


Fig. S19 ^{1}H NMR spectrum of conjugate **[GA]'$_{4.L}$.**

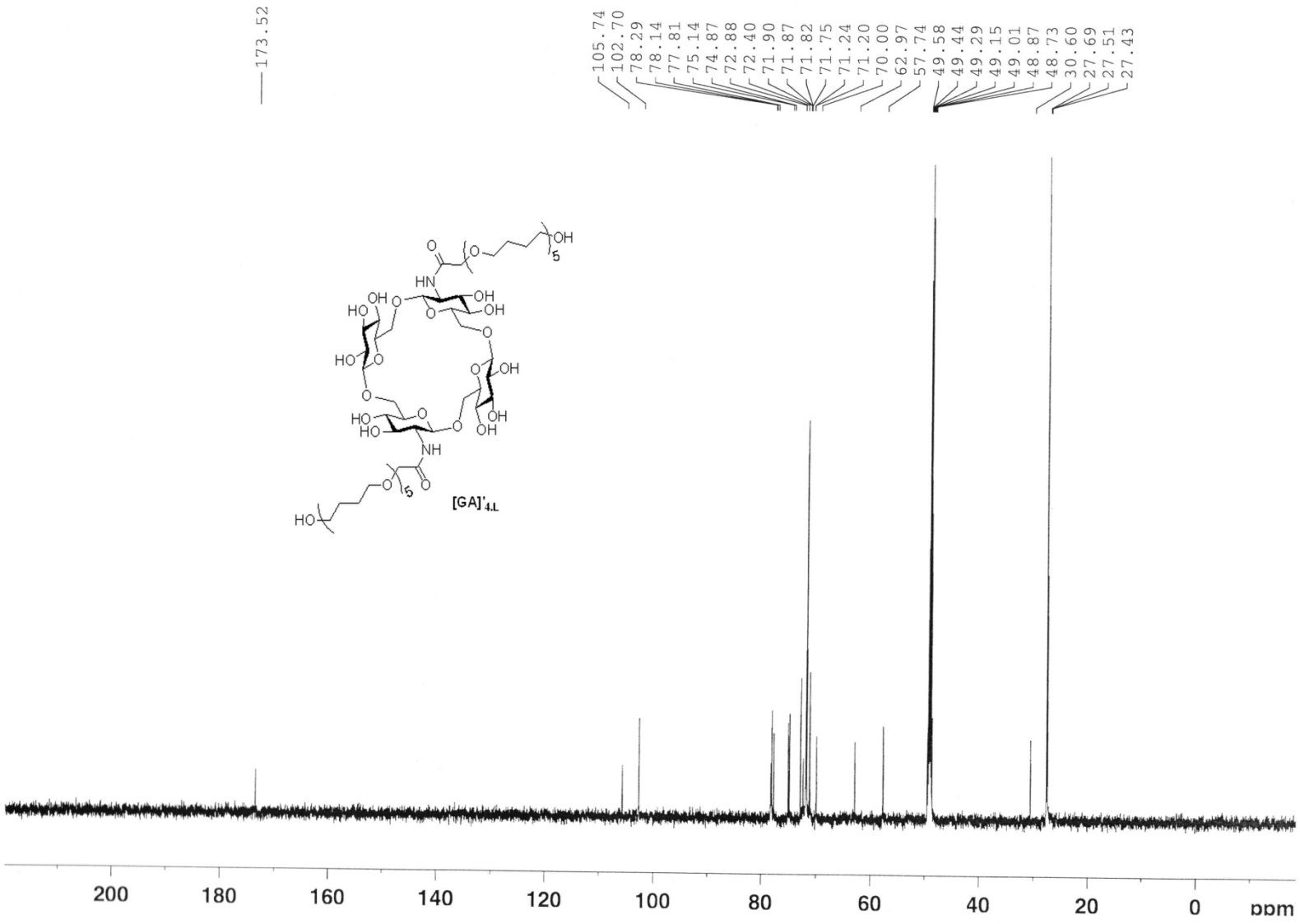


Fig. S20 ^{13}C NMR spectrum of conjugate **[GA]'$_{4.L}$.**

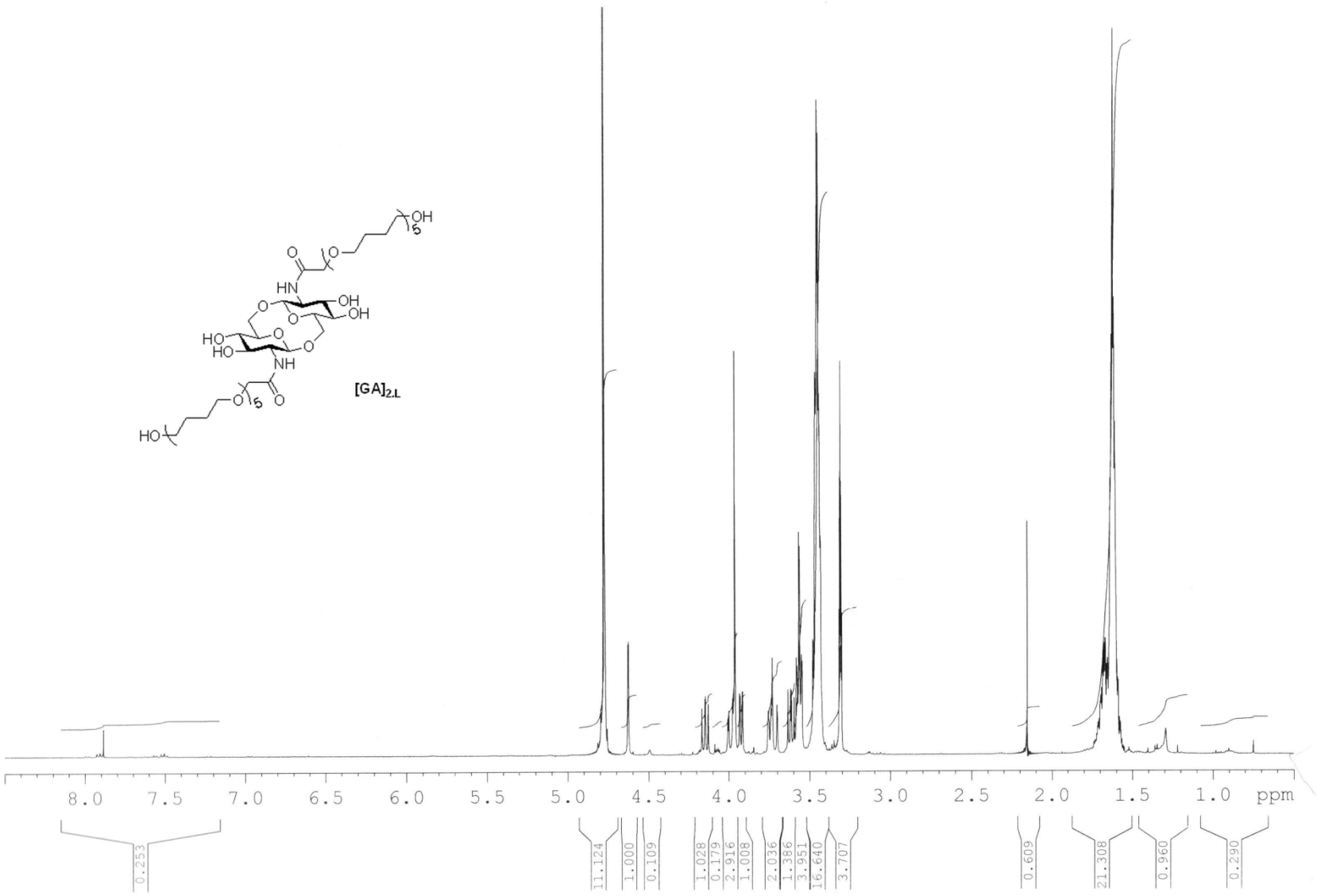


Fig. S21 ^{1}H NMR spectrum of conjugate **[GA]$_{2.L}$.**

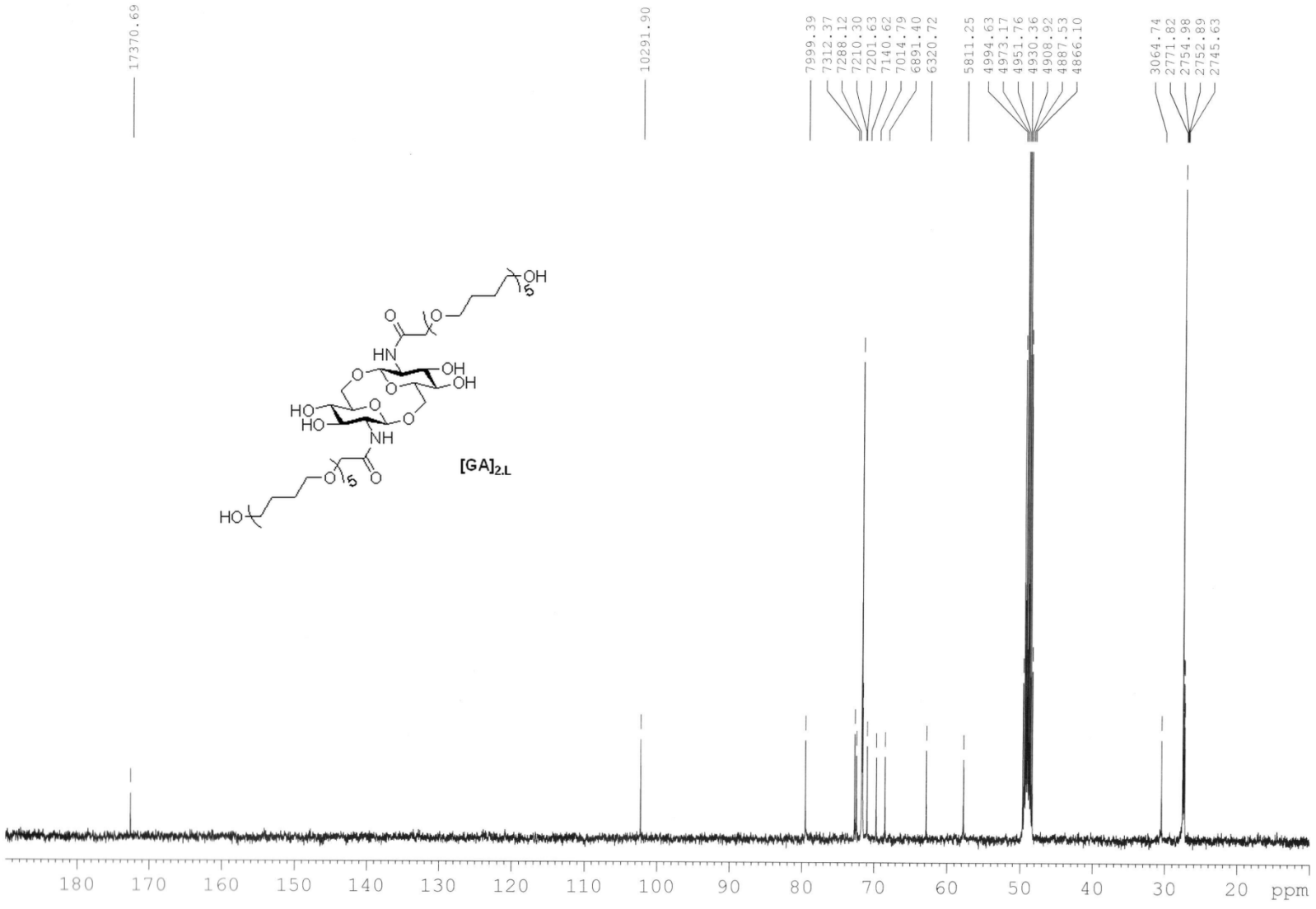


Fig. S22 ^{13}C NMR spectrum of conjugate **$[GA]_{2.L}$.**

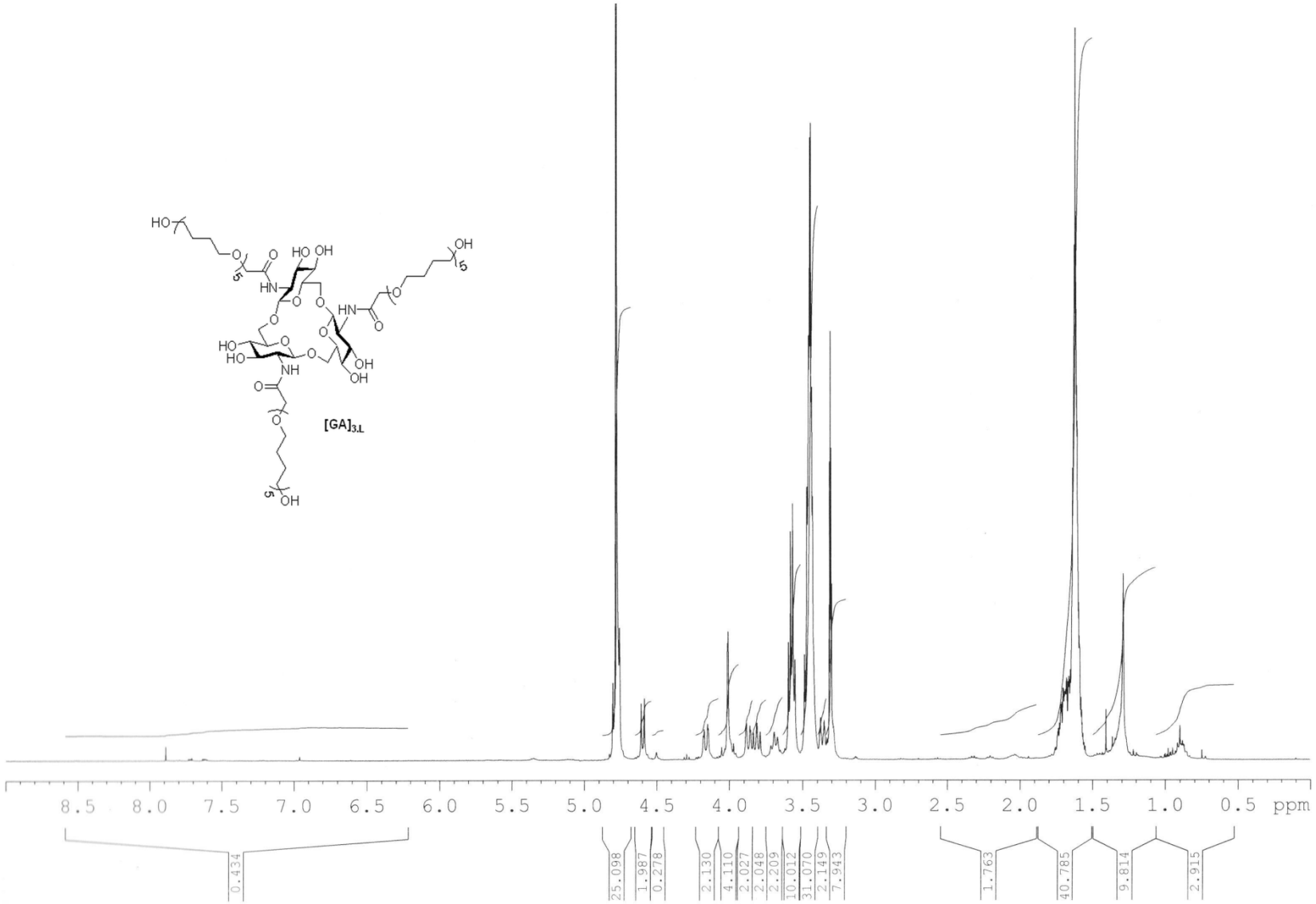


Fig. S23 ^{1}H NMR spectrum of conjugate **$[GA]_{3.L}$.**

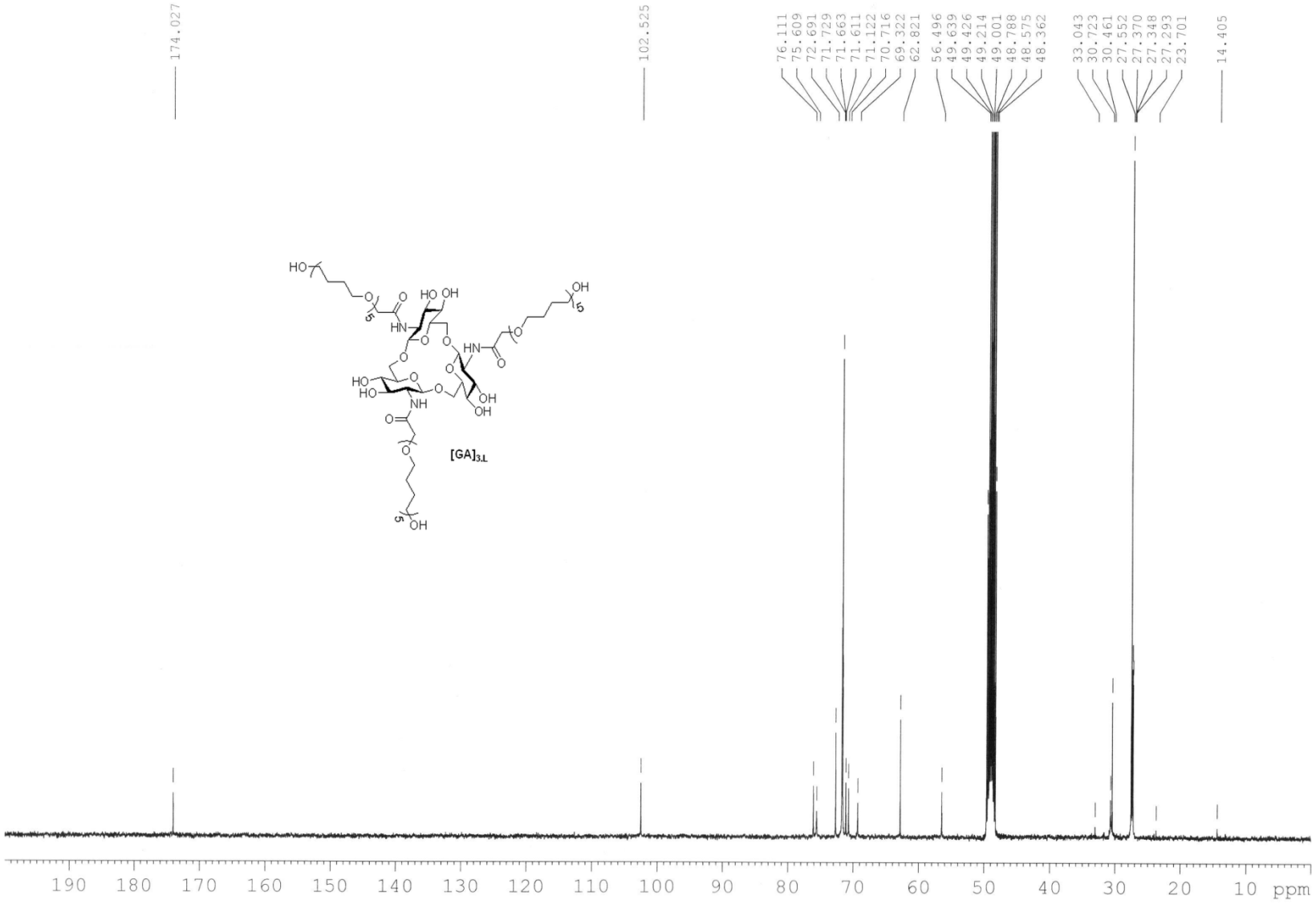


Fig. S24 ^{13}C NMR spectrum of conjugate **$[GA]_{3.L}$.**

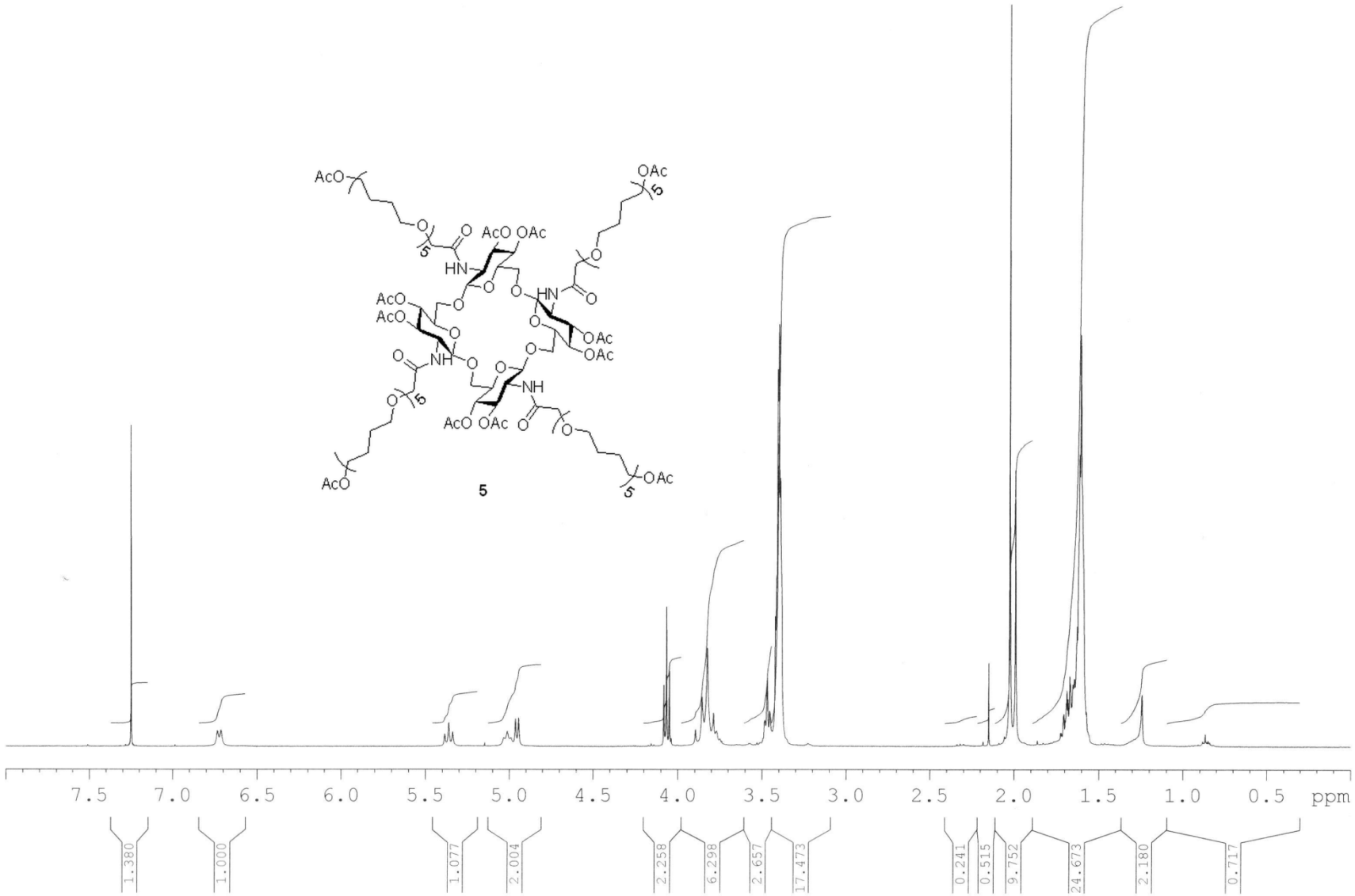


Fig. S25 ^{1}H NMR spectrum of conjugate **5.**

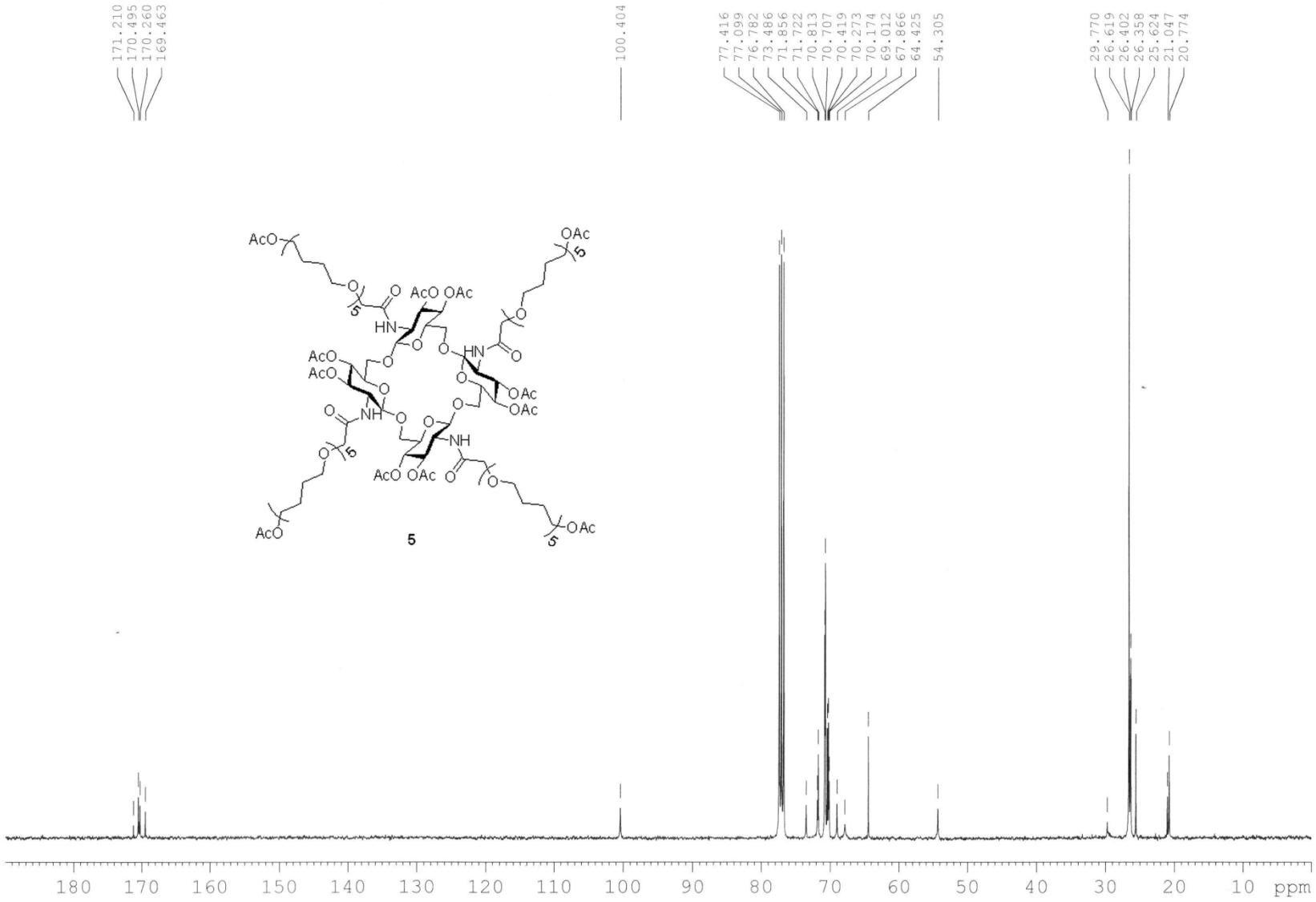


Fig. S26 ^{13}C NMR spectrum of conjugate **5.**

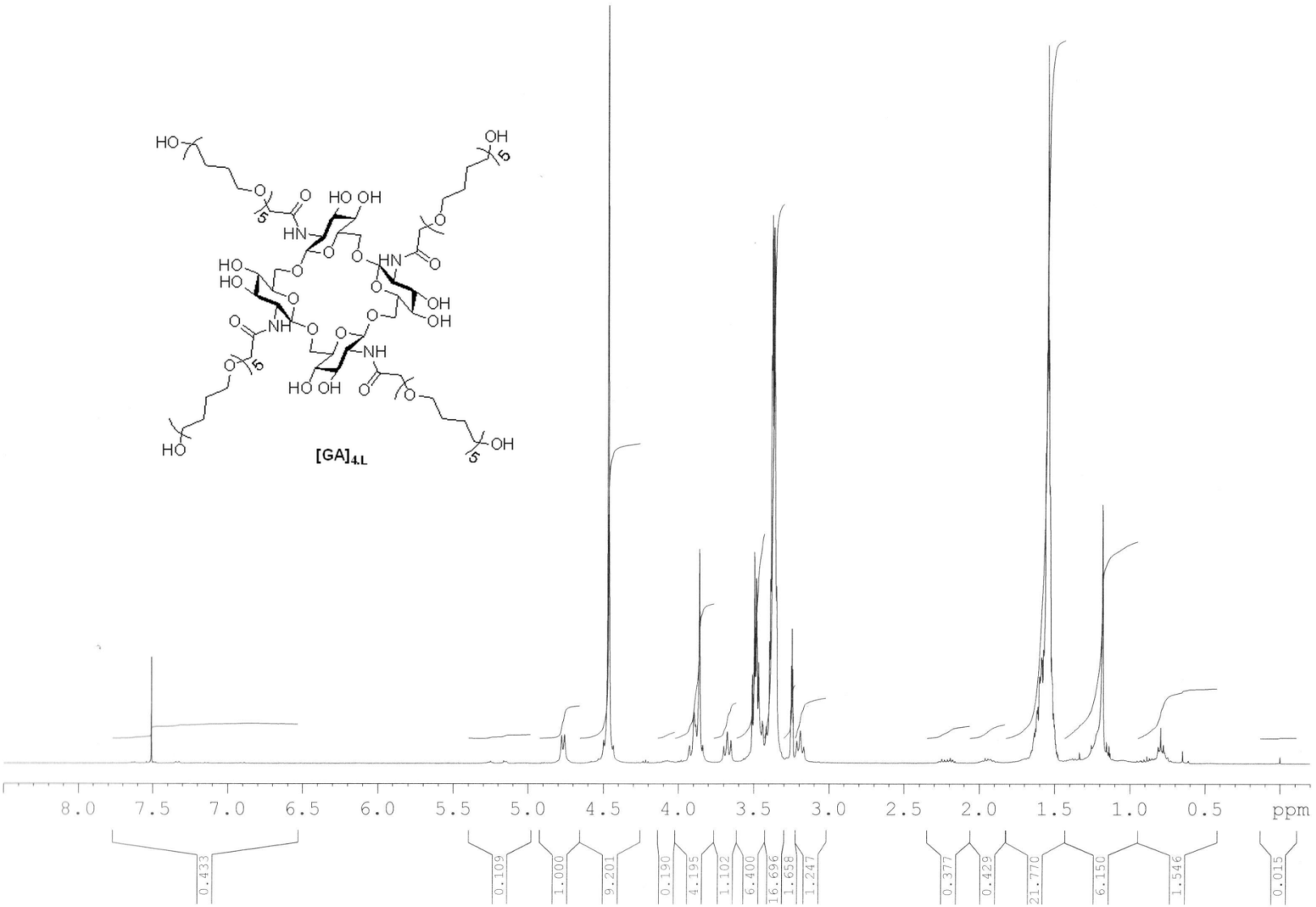


Fig. S27 ^{1}H NMR spectrum of conjugate **[GA]$_{4.L}$.**

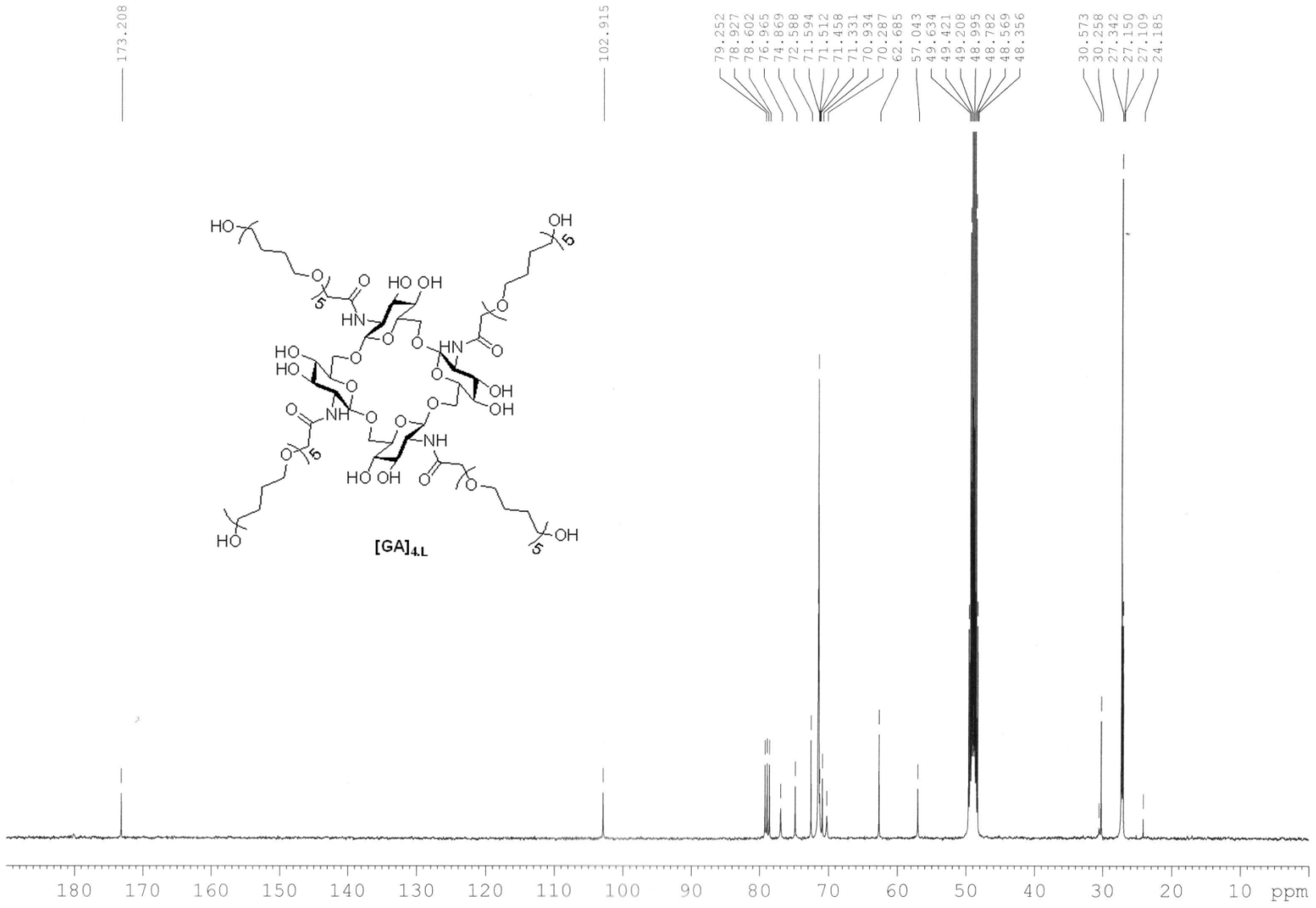


Fig. S28 ^{13}C NMR spectrum of conjugate **[GA]$_{4.L}$.**

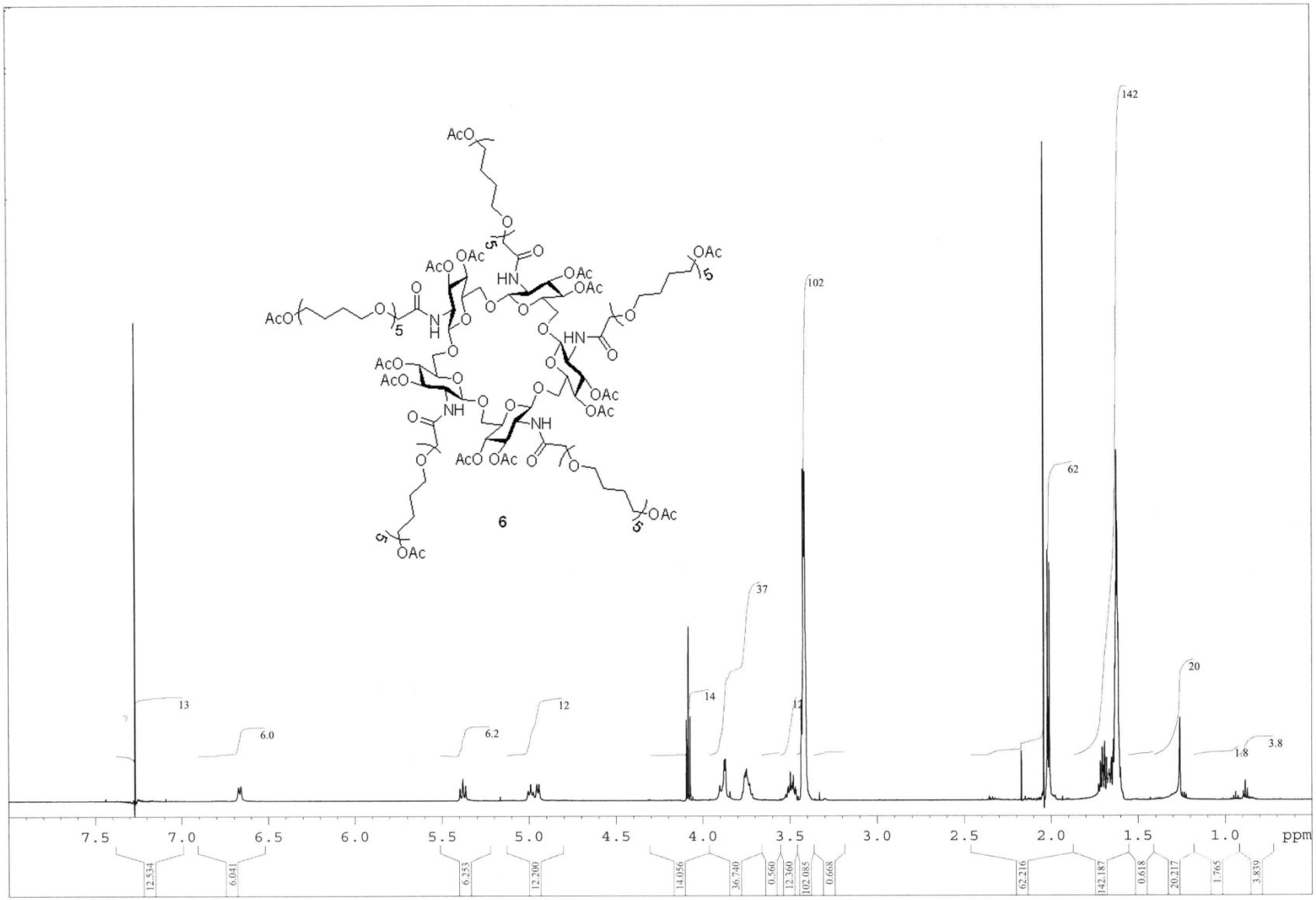


Fig.S29 ^{1}H NMR spectrum of conjugate **6.**

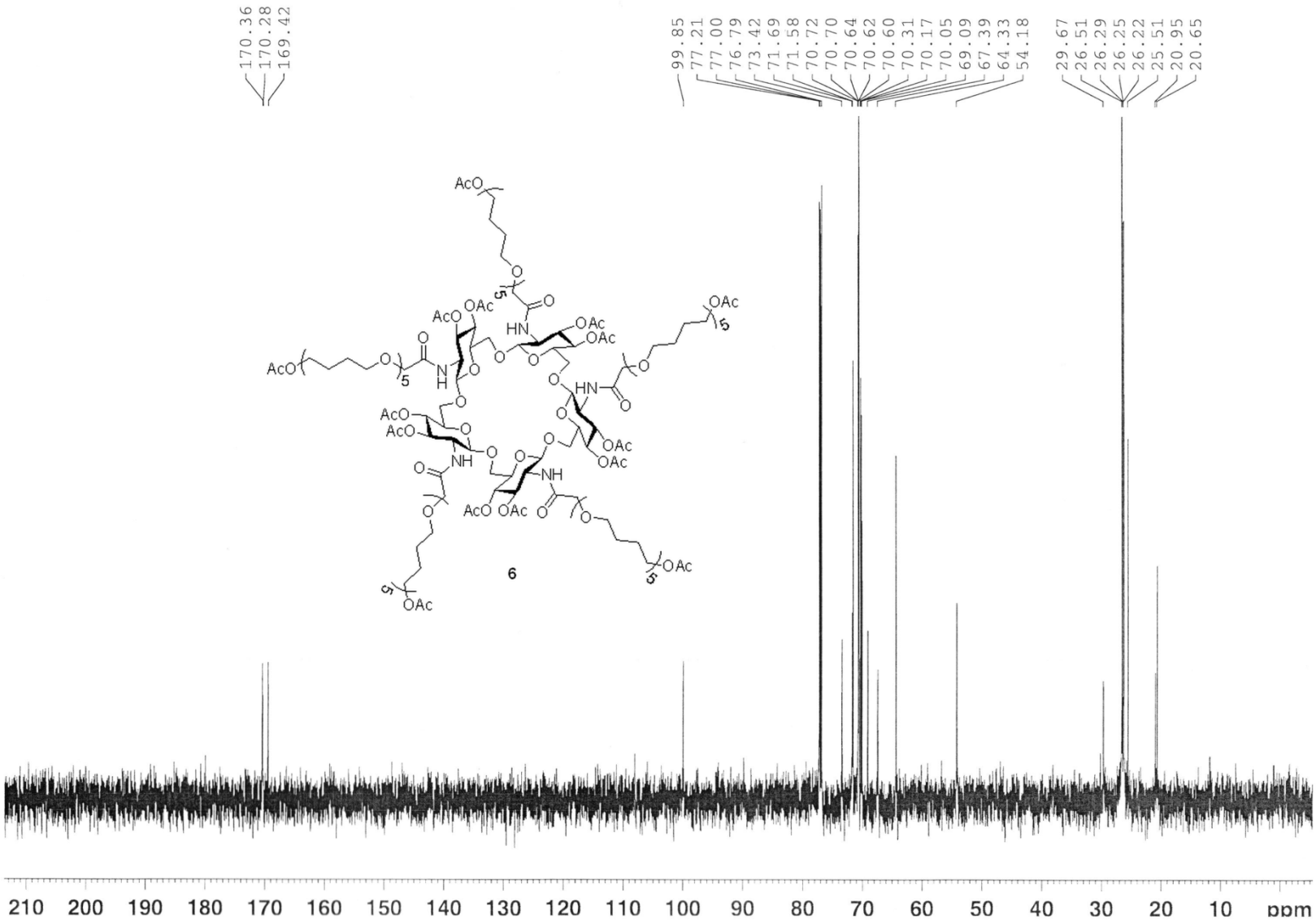


Fig. S30 ^{13}C NMR spectrum of conjugate **6.**

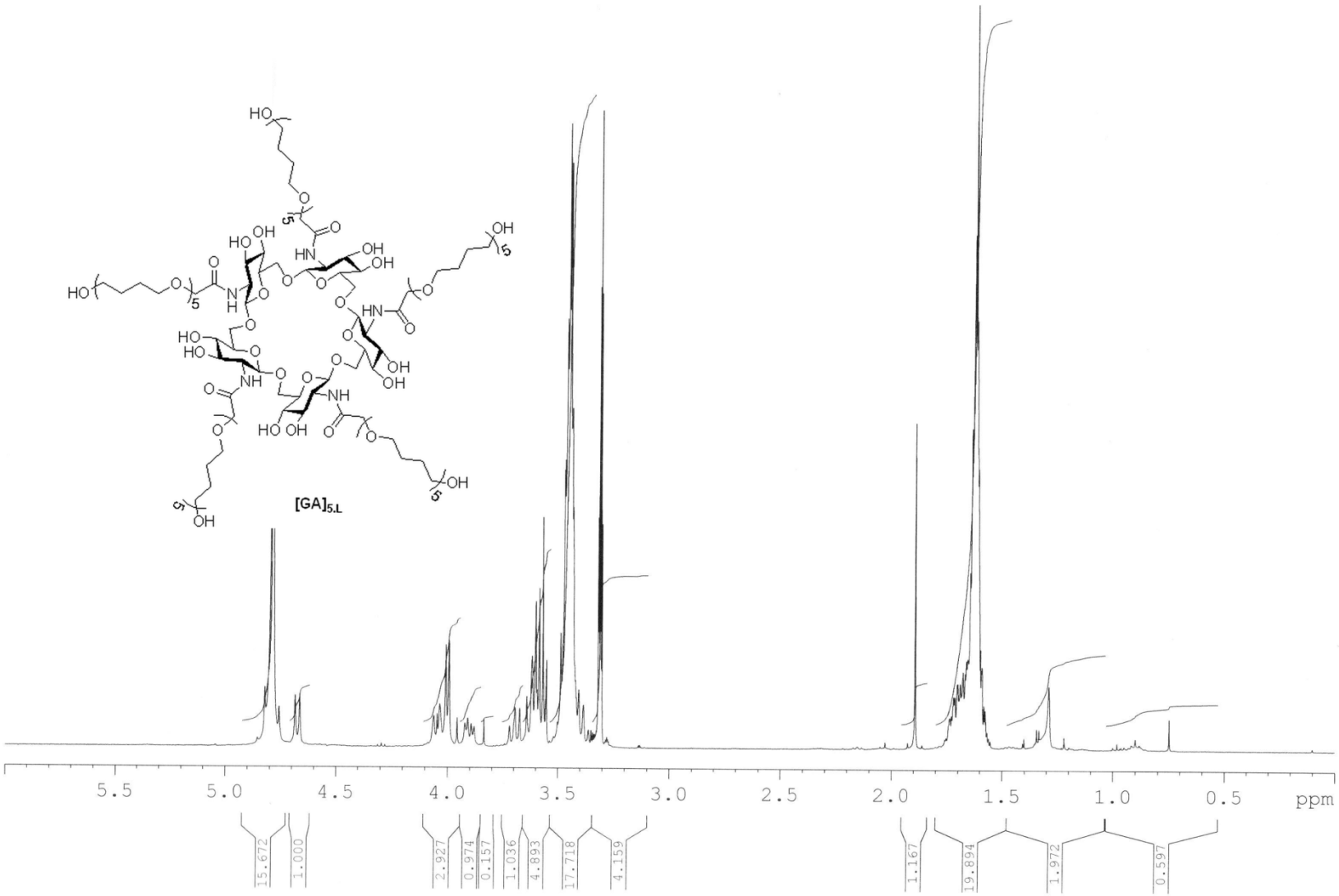


Fig. S31 ^{1}H NMR spectrum of conjugate **$[GA]_{5\text{-}L}$**.

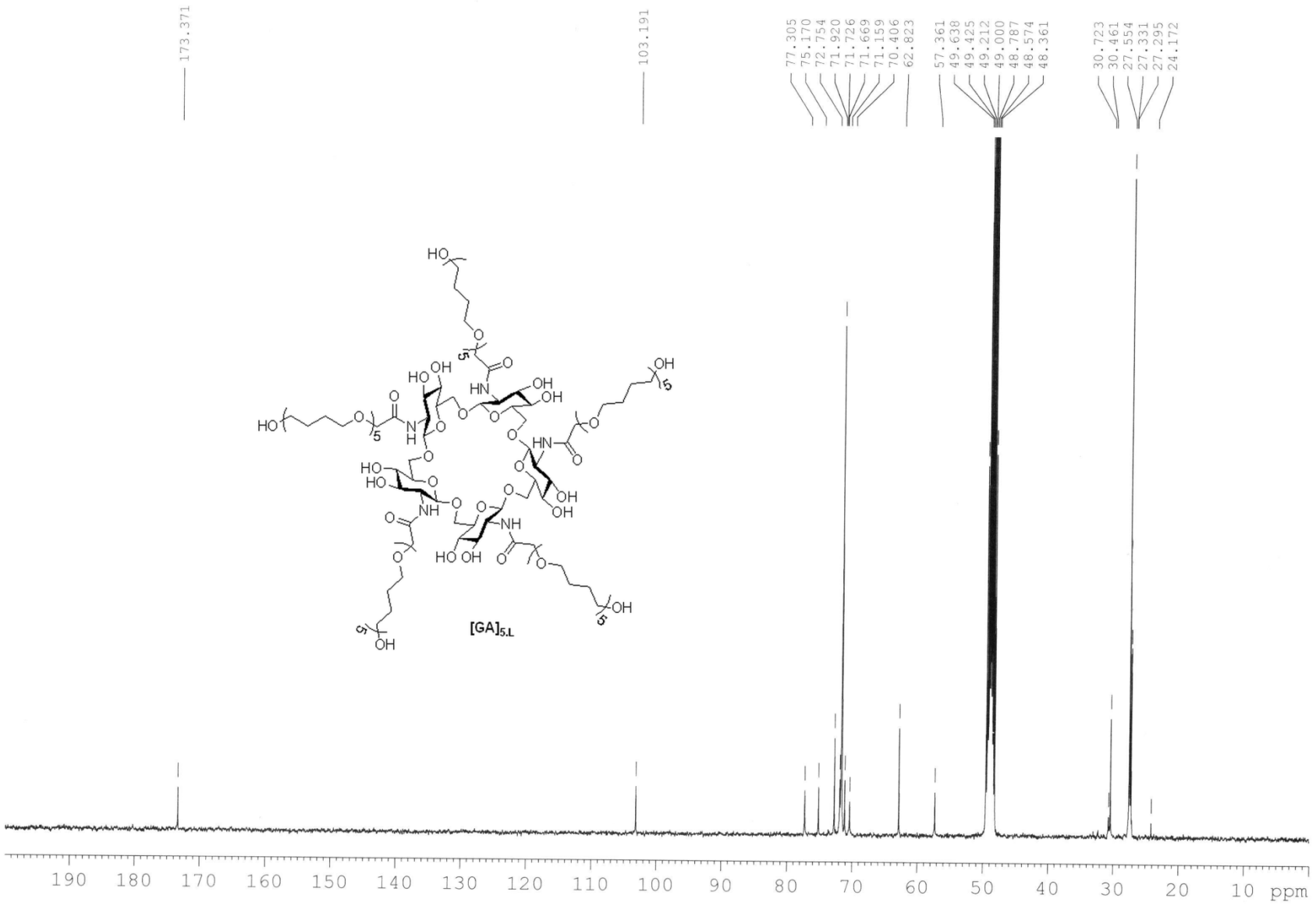


Fig. S32 ^{13}C NMR spectrum of conjugate **[GA]$_{5.L}$.**

VII. References:

(S1) Knuf, E. C.; Jiang, J.-K.; Gin, M. S. *J. Org. Chem.* **2003**, *68*, 9166-9169.

(S2) Titov, D. V.; Gening, M. L.; Gerbst, A. G.; Chizhov, A. O.; Tsvetkov, Y. E.; Nifantiev, N. E. *Carbohydr. Res.,* in press. *DOI: 10.1016/j.carres.2012.12.005*.

(S3) Gening, M. L.; Titov, D. V.; Grachev, A. A.; Gerbst, A. G.; Yudina, O. N.; Shashkov, A. S.; Chizhov, A. O.; Tsvetkov, Y. E.; Nifantiev, N. E. *Eur. J. Org. Chem.* **2010**, 2465-2475.

(S4) (a) Talukdar, P.; Bollot, G.; Mareda, J.; Sakai N.; Matile, S. *J. Am. Chem. Soc.* **2005**, *127*, 6528-6529; (b) Sakai N.; Matile, S. *J. Am. Chem. Soc.* **2003**, *125*, 14348-14356; (c) Sidorov, V.; Kotch, F. W.; Abdrakhmanova, G.; Mizani, R.; Fettinger J. C.; Davis, J. T. *J. Am. Chem. Soc.* **2002**, *124*, 2267-2278.

(S5) Madhavan, N.; Robert, E. C.; Gin, M. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 7584-7587.

(S6) Software developed in Professor Jay Ponder's Lab. http://dasher.wustl.edu/tinker.

(S7) Ooi, T.; Oobatake, M.; Némethy, G.; Scheraga, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3086–3090.