Immobilization of calix[4]arene-based glycoclusters on TiO_2 nanoparticles via click Cu(I)-catalyzed azide-alkyne coupling

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Supporting Information

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General Experimental Section. All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Anhydrous solvents were dried over standard drying agents[17] and freshly distilled prior to use. Reactions were monitored by TLC on silica gel 60 F_{254} with detection by charring with sulfuric acid. Flash column chromatography[18] was performed on silica gel 60 (40-63 μm). Optical rotations were measured at 20 ± 2 °C in the stated solvent; $[\alpha]_D$ values are given in deg·mL·g^{-1}·dm^{-1}. ^{1}H NMR (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded from $CDCl_3$ solutions at room temperature unless otherwise specified. Peak assignments were aided by ^{1}H-^{1}H COSY and gradient-HMQC experiments. In the ^{1}H NMR spectra reported below, the *n* and *m* values quoted in geminal or vicinal proton-proton coupling constants $J_{n,m}$ refer to the number of the corresponding sugar protons.

High Resolution MS Analysis. For accurate mass measurements the compounds were analyzed in positive ion mode by electrospray hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF) fitted with a Z-spray electrospray ion source (Waters, Manchester, UK). The capillary source voltage and the cone voltage were set at 3500 V and 35 V, respectively; the source temperature was kept at 80 °C; nitrogen was used as a drying gas at a flow rate of ca. 50 L/h. The time-of-flight analyzer was externally calibrated with NaI from *m/z* 300 to 2000 to yield an accuracy near to 5 ppm. When necessary an internal lock mass was used to further increase the mass accuracy. Accurate mass data were collected by directly infusing samples (10 pmol/μL in 1:1 CH_3CN-H_2O containing 10 mM ammonium formate) into the system at a flow rate of 5 μL/min. The acquisition and data processing were performed with the MassLynx 4.1 software (Waters, Manchester, UK).

Thermogravimetric analysis (TGA). The thermogravimetric analysis was carried out with a Mettler Toledo TGA/SDTA mod. 851^e thermobalance, calibrated by using high purity indium and zinc standards, under nitrogen flow (from 308 to 873 K) and air flow (from 873 to 1173 K) maintained constant at 70 ml min^{-1}. The alumina pans (70 μL) were filled by powder samples (about 25 mg) and heated from 308 to 1173 K at a variable heating rate of 1 K min^{-1} or 20 K min^{-1} depending on the weight losses (higher than 2 μg s^{-1} or lower than 1 μg s^{-1}, respectively).
The TGA analyses of all samples showed two weight loss steps (Figure 3). The first weight loss was attributed to the solvent residue evaporation and sample pyrolysis (4-5% for each sample), while the second one was due to the oxidation of carbon residues remained under atmosphere nitrogen (about 2% for each sample) after pyrolysis treatment below 873 K. All samples showed an ash content of 92-94% due to TiO_2 after a thermal treatment in air up to 1173 K.

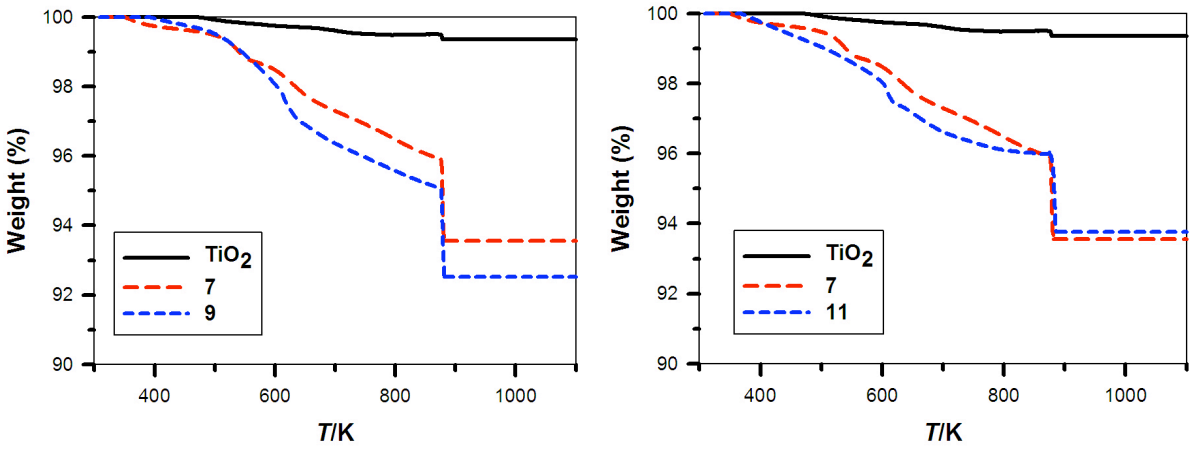


Figure 3 TGA curves under nitrogen and air (T >873 K) for TiO_2 powder, nanoparticles **7** and glyconanoparticles **9** (left) and TiO_2 powder, nanoparticles **7** and glyconanoparticles **11** (right).

The experimental TGA data showed that the % by weight of the organic compounds (oc) on the TiO_2 surfaces ranging from 6.2 to 7.5 wt%. Accordingly to these results, the weight fraction of the organic compounds (wt_{oc}) can be written as:

$$wt_{oc} = g_{oc} / g_{TiO2} = \frac{W_{oc}}{W_{ot}} \cong \frac{W_{oc}}{W_{TiO2}} \quad (1)$$

where W_{oc} and W_{ot} were the weight of organic compounds on the TiO_2 surfaces and the total weight of the sample, respectively. It is quite reasonable assuming that the $W_{ot} = W_{oc}+W_{TiO2} \cong W_{TiO2}$ under our experimental conditions. Since the molecular weights (M_i) of the organic compounds (i = **7**, **9**, and **11**) are known, the molar concentration of the organic compounds on TiO_2 nanoparticles, reported as mmol/g_{TiO2}, can be calculated by eq(2):

$$[m_{oc}]_i = \frac{10^3 \cdot wt_{oc}}{M_i} \quad (2)$$

Finally, the number of the organic compounds (n_{oc}) per unit of TiO_2 surfaces (in nm^2) can be easily calculated by eq(3):

$$[n_{oc}]_i = \frac{10^{-3} \cdot N}{A \cdot 10^{+18} nm^2 / g} \cdot [m_{oc}]_i \quad (3)$$

where N is the Avogadro number $6.023 \cdot 10^{23}$ mol^{-1} and A is the surface area of the commercially available TiO_2 nanoparticles (50 ± 15 m^2/g) . The results were summarized in Table 2.

Table 2. Weight fractions and molar concentrations per gram of TiO_2 nanoparticles and number of organic compounds **7**, **9**, and **11** per TiO_2 surface units.

Products	[1] M_i (g/mol)	[2] w_{toc} g_{oc}/g_{TiO2}	[3] m_{toc} (mmol/g_{TiO2})	[4] n_{toc} (number$_{oc}$/nm^2)
7	989.00	0.065	0.06_6	0.8_2
9	1861.81	0.075	0.04_0	0.5_0
11	2378.27	0.062	0.02_6	0.3_2

[1]Molecular weights of the prepared compounds. [2] TGA experimental signal rewritten as reported in eq(1). [3] Calculated using eq(2). [4] Calculated using eq(3).

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) analysis. The spectroscopic characterization of adsorbed and chemically modified TiO_2 powder with calixsugars was carried out by DRIFTS using a FTIR Bruker spectrometer mod. IFS 88 equipped with a MCT detector and diffuse reflectance device purchased by Graseby-Specac. The samples were prepared by mixing homogeneously at least 25% by wt. of organic compound with anhydrous KBr. The DRIFT spectra were obtained between 650 and 4000 cm^{-1} by collecting at least 1000 interpherograms with a resolution of 16 cm^{-1} under dry air flow at room temperature.

The diffuse reflectance spectroscopy of weakly absorbing samples is employed to find a correlation between reflectance intensities and sample concentrations. Under these conditions the absorbing sample concentrations can be linearly correlated to reflectance intensities, by Kubelka-Munk units (KM), using the following well-known eq(4), where *KM* is the diffuse reflectance at a given wavenumber, *s* is the scattering coefficient and *k* is the absorption coefficient at a specific wavenumber related to the sample concentration.

$$F(R) = \frac{(1-R)^2}{2 \times R} = \frac{k}{s} \qquad (4)$$

In order to develop a suitable calibration method, some mixtures of TiO_2 powder and azidocalixarenes **6** (25% by wt.) were homogeneously dispersed in anhydrous KBr (75% by wt.). A calibration straight line was determined in a concentration range between 0 and 10% by weight of the above-mentioned organic compounds (Figure 4).

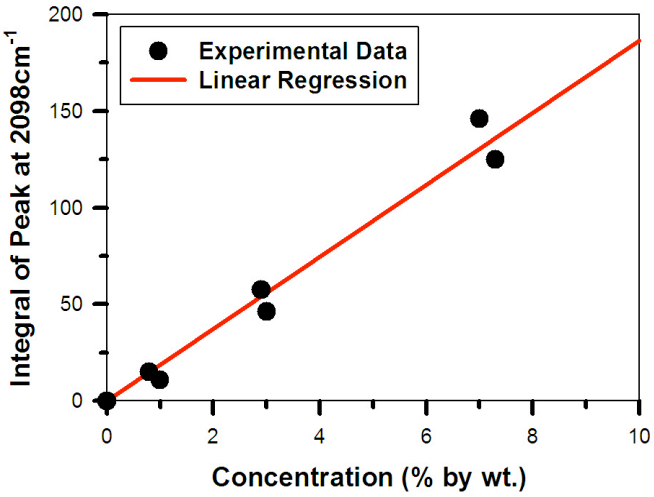


Figure 4 Calibration plot obtained by DRIFT spectra.

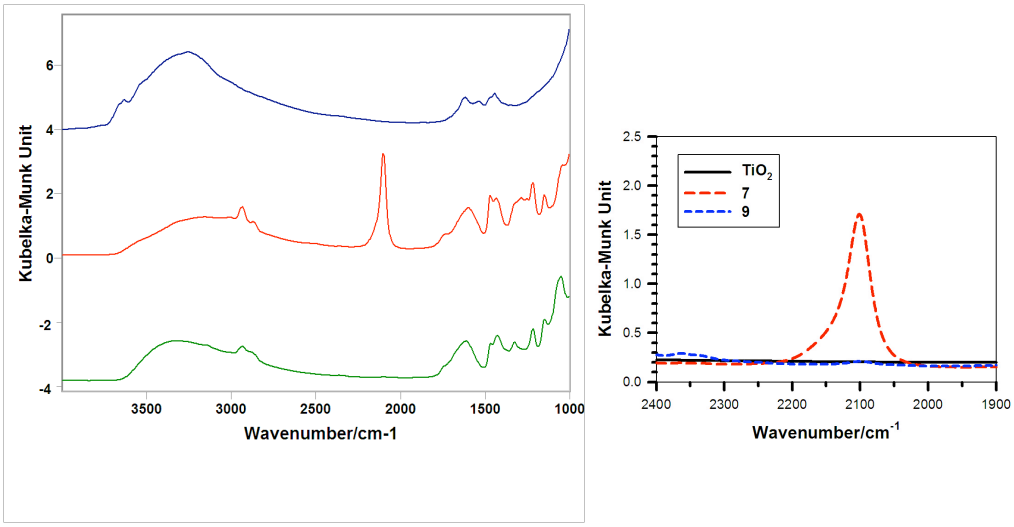


Figure 5 DRIFT spectra of TiO_2, nanoparticles **7** and glyconanoparticles **9**.

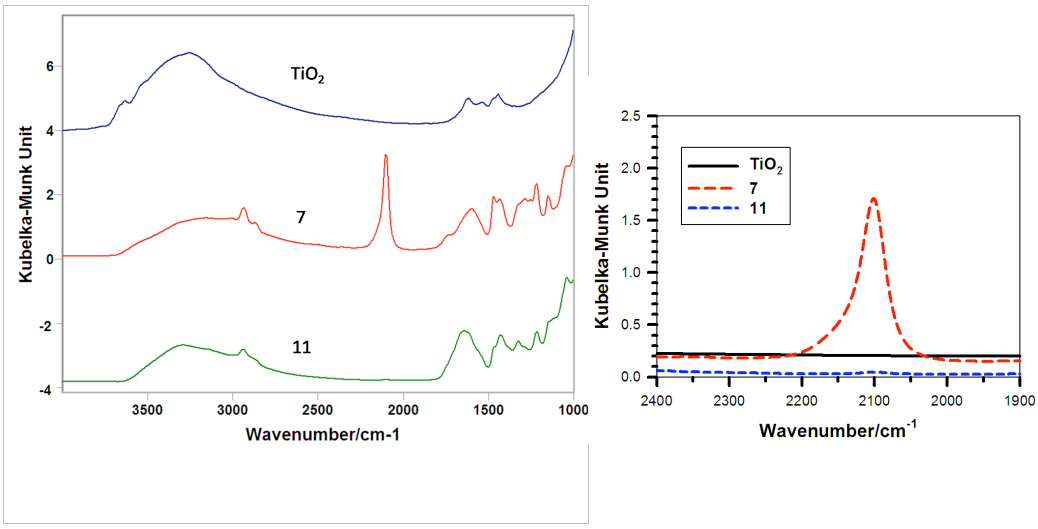


Figure 6 DRIFT spectra of TiO_2, nanoparticles **7** and glyconanoparticles **11**.

Morphological characterization of TiO_2 nanoparticles. Morphological studies of the TiO_2 nanoparticles were performed by Scanning Electron Microscopy using a Cambridge Stereocam S-360 instrument (Figure 7). A detailed analysis of the SEM micrographs indicated that TiO_2 nanoparticles were aggregated in a complex nanostructured system with dimensions as large as 200 nm and a surface area of 50 ± 15 m^2/g.

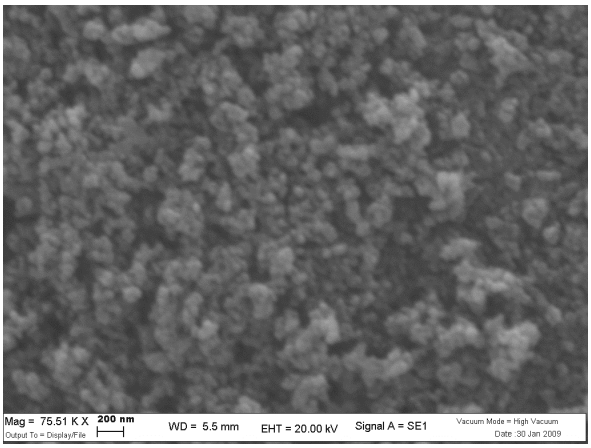
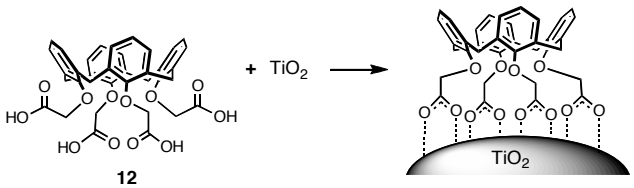


Figure 7 SEM micrograph of a commercially available TiO_2 sample used as received.

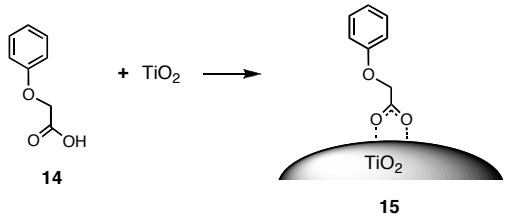
Adsorption/desorption on TiO_2 surface using model compounds.



12 **13**

Synthesis of nanoparticles **13**. To a solution of known[9] tetrakis(carboxylmethoxy)-calix[4]arene (0.20 g, 0.30 mmol) in in 4:1 CH_3OH-toluene (20 mL) was added commercially available TiO_2 nanoparticles (0.50 g). The suspension was stirred in the dark at room temperature for 16 h, then centrifuged (4,000 rpm, 5 min) and decanted. The solid was suspended in 4:1 CH_3OH-toluene (10 mL), centrifuged (4,000 rpm, 5 min) and decanted. The solid was dried under vacuum at room temperature to give the nanoparticles **13** (0.49 g) as a white powder, which was characterized by TGA (weight fraction: 3.93% w/w; molar concentration per gram of TiO_2 nanoparticles: 0.058 mmol/g; number of molecules per TiO_2 surface unit: 0.70 ligand/nm^2).

Desorption of **12** *from nanoparticles* **13**. A suspension of **13** (0.20 g) in 1:1 CH_3OH-H_2O (2 mL) was stirred in the dark at room temperature for 24 h, then centrifuged (4,000 rpm, 5 min) and decanted. The solid was dried under vacuum at room temperature and characterized by TGA (weight fraction: 3.93% w/w; molar concentration per gram of TiO_2 nanoparticles: 0.058 mmol/g; number of molecules per TiO_2 surface unit: 0.70 ligand/nm^2).



14 15

Synthesis of nanoparticles **15**. To a solution of commercial phenoxyacetic acid **14** (0.45 g, 0.30 mmol) in 4:1 CH_3OH-toluene (20 mL) was added commercially available TiO_2 nanoparticles (0.50 g). The suspension was stirred in the dark at room temperature for 16 h, then centrifuged (4,000 rpm, 5 min) and decanted. The solid was suspended in 4:1 CH_3OH-toluene (10 mL), centrifuged (4,000 rpm, 5 min) and decanted. The solid was dried under vacuum at room temperature to give the nanoparticles **15** (0.43 g) as a white powder, which was characterized by TGA (weight fraction: 1.76% w/w; molar concentration per gram of TiO_2 nanoparticles: 0.12 mmol/g; number of molecules per TiO_2 surface unit: 1.45 ligand/nm^2).

Desorption of **14** *from nanoparticles* **15**. A suspension of **15** (0.20 g) in 1:1 CH_3OH-H_2O (2 mL) was stirred in the dark, at room temperature for 24 h, then centrifuged (4,000 rpm, 5 min) and decanted. The solid was dried under vacuum at room temperature and characterized by TGA (weight fraction: 1.89% w/w; molar concentration per gram of TiO_2 nanoparticles: 0.12 mmol/g; number of molecules per TiO_2 surface unit: 1.45 ligand/nm^2).

5,11,17,23-Tetraallyl-25,26,27,28-tetramethoxyethoxymethoxy-calix[4]arene (2). To a cooled (0 °C), stirred solution of known[9] tetraallyl calixarene **1** (1.00 g, 1.70 mmol) in anhydrous DMF (20 mL) was added NaH (0.27 g, 6.80 mmol, of a 60% dispersion in oil) and, after 10 min, methoxyethoxymethyl chloride (0.77 mL, 6.80 mmol). The mixture was stirred at room temperature for 1 h and then cooled to 0 °C. NaH (0.27 g, 6.80 mmol, of a 60% dispersion in oil) and methoxyethoxymethyl chloride (0.77 mL, 6.80 mmol) were added, the suspension was stirred at room temperature for an additional 1 h, then diluted with CH_3OH (1 mL), and after 30 min, with 1M phosphate buffer at pH 7 (80 mL) and extracted with Et_2O (2 x 100 mL). The combined organic phases were dried (Na_2SO4) and concentrated. The residue was eluted from a column of silica gel with 3:1 AcOEt-cyclohexane containing 0.3% of Et_3N to give **2** (1.53 g, 96%) as a syrup. ^{1}H NMR: δ 6.50 (s, 8 H, Ar), 5.81 (ddt, 4 H, J = 6.5 Hz, J_{cis} =10.0 Hz, J_{trans} = 16.8 Hz, 4 CH_2CH=CH_2), 5.17 (s, 8H, 4 OCH_2OCH$_2$CH$_2$OCH$_3$), 4.98 (ddt, 4 H, J = 1.2 Hz, J_{gem} = 2.0 Hz, H_{cis} of 4 CH_2CH=CH_2), 4.90 (ddt, 4H, J = 1.5 Hz, H_{trans} of 4 CH_2CH=CH_2), 4.40 and 3.14 (2 d, 8H, J = 13.2 Hz, 4 ArCH_2Ar), 3.98-3.94 and 3.60-3.55 (2 m, 16H, 4 OCH$_2$OCH_2CH_2OCH$_3$), 3.40 (s, 12H, 4 OCH$_2$OCH$_2$CH$_2$OCH_3), 3.08 (ddd, 8H, 4 CH_2CH=CH$_2$). ^{13}C NMR: δ 152.5 (C), 138.1 (CH), 134.5

(C), 133.9 (C), 128.5 (CH), 114.9 (CH_2), 99.2 (CH_2), 72.1 (CH_2), 69.6 (CH_2), 59.0 (CH_3), 39.3 (CH_2), 31.5 (CH_2). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{56}H_{76}NO_{12}$ $(M+NH_4)^+$ 954.5368, found 954.5333.

5,11,17,23-Tetrakis(3-hydroxypropyl)-25,26,27,28-tetramethoxyethoxymethoxy-calix[4]arene (3). To a cooled (0 °C), stirred solution of **2** (1.53 g, 1.63 mmol) in anhydrous THF (10 mL) was added dropwise 9-boracyclo[3.3.1]nonane (52 mL, 26.0 mmol, of a 0.5 M solution in THF). The solution was allowed to reach room temperature in 1 h and then cooled to 0 °C and slowly diluted with 10 M NaOH (2.0 mL) and 30% H_2O_2 (6.0 mL). The mixture was stirred at room temperature for 30 min and then warmed to 60 °C. Stirring was continued for additional 2 h, then the mixture was cooled to room temperature, diluted with 1 M phosphate buffer at pH 7 (50 mL), concentrated to remove the organic solvents, and extracted with AcOEt (2 x 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a short column of silica gel (5 cm), with AcOEt, 1:1 AcOEt-acetone, acetone, and 9:1 acetone-methanol (all containing 0.3% of Et_3N), to give **4** (1.25 g, 76%) as a syrup. ^{1}H NMR: δ 6.56 (s, 8H, Ar), 5.19 (s, 8H, 4 O*C*H_2OCH$_2$CH$_2$OCH$_3$), 4.42 and 3.14 (2 d, 8H, *J* = 13.0 Hz, 4 ArC*H*$_2$Ar), 3.98-3.94 and 3.61-3.56 (2 m, 16H, 4 OCH$_2$O*CH*$_2$*C*H$_2$OCH$_3$), 3.55 (t, 8H, *J* = 6.5 Hz, 4 CH$_2$CH$_2$*CH*$_2$OH), 3.40 (s, 12H, 4 OCH$_2$OCH$_2$CH$_2$O*CH*$_3$), 2.80 (bs, 4H, 4 OH), 2.40 (t, 8H, *J* = 7.0 Hz, 4 *CH*$_2$CH$_2$CH$_2$OH), 1.70 (tt, 8H, *J* = 6.5, 7.0 Hz, 4 CH$_2$*CH*$_2$CH$_2$OH). ^{13}C NMR: δ 152.1 (C), 135.9 (C), 134.4 (C), 128.1 (CH), 99.3 (CH_2), 72.1 (CH_2), 69.6 (CH_2), 62.1 (CH_2), 59.0 (CH_3), 33.9 (CH_2), 31.4 (CH_2), 31.3 (CH_2). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{56}H_{84}NO_{16}$ $(M+NH_4)^+$ 1026.5790, found 1026.5797.

5,11,17,23-Tetrakis(3-azidopropyl)-25,26,27,28-tetrahydroxy-calix[4]arene (4). A mixture of **3** (1.25 g, 1.24 mmol), sodium azide (0.65 g, 9.98 mmol), diphenyl phosphoryl azide (1.60 mL, 7.44 mmol), 1,8-diazabicyclo[5.4.0.]undec-7-ene (0.74 mL, 4.96 mmol), and anhydrous DMF (10 mL) was stirred at 120 °C for 14 h, then cooled to room temperature, diluted with Et_2O (150 mL), washed with H_2O (30 mL), dried (Na_2SO_4), and concentrated to give crude 5,11,17,23-tetrakis(3-azidopropyl)-25,26,27,28-tetrakis(methoxyethoxymethoxy)-calix[4]arene. An analytical sample was obtained by column chromatography on silica gel (2:1 AcOEt-cyclohexane, containing 0.3% of Et3N). ^{1}H NMR: δ 6.49 (s, 8H, Ar), 5.18 (s, 8H, 4 O*C*H_2OCH$_2$CH$_2$OCH$_3$), 4.41 and 3.14 (2 d, 8H, *J* = 13.4 Hz, 4 ArC*H*$_2$Ar), 3.98-3.94 and 3.60-3.56 (2 m, 16H, 4 OCH$_2$O*CH*$_2$*C*H$_2$OCH$_3$), 3.41 (s, 12H, 4 OCH$_2$OCH$_2$CH$_2$O*CH*$_3$), 3.18 (t, 8H, *J* = 6.7 Hz, 4 CH$_2$CH$_2$*CH*$_2$N$_3$), 2.40 (t, 8H, *J* = 7.3 Hz, 4 *CH*$_2$CH$_2$CH$_2$N$_3$), 1.72 (tt, 8H, *J* = 6.7, 7.3 Hz, 4 CH$_2$*CH*$_2$CH$_2$N$_3$). ^{13}C NMR: δ 152.6 (C), 134.9 (C), 134.6 (C), 128.3 (CH), 99.3 (CH_2), 72.1 (CH_2), 69.6 (CH_2), 59.1 (CH_3), 50.5 (CH_2), 32.0 (CH_2),

31.5 (CH_2), 30.5 (CH_2). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{56}H_{80}N_{13}O_{12}$ $(M+NH_4)^+$ 1126.6049, found 1126.5996.

To a warmed (100 °C) solution of crude 5,11,17,23-tetrakis(3-azidopropyl)-25,26,27,28-tetrakis(methoxyethoxymethoxy)-calix[4]arene in CH_3CO_2H (16 mL) was slowly added H_2O (4 mL). The mixture was stirred at 100 °C for 1 h, then cooled to room temperature and concentrated. The residue was triturated with Et_2O (2 x 5 mL) to give **4** (0.63 g, 60% overall yield) as a white solid. Mp 180-181 °C (Et_2O). ^{1}H NMR: δ 10.21 (s, 4H, 4 OH), 6.89 (s, 8H, Ar), 4.25 and 3.48 (2 bd, 8H, *J* = 13.0 Hz, 4 ArC*H*$_2$Ar), 3.26 (t, 8H, *J* = 6.7 Hz, 4 $CH_2CH_2CH_2N_3$), 2.53 (t, 8H, *J* = 7.5 Hz, 4 $CH_2CH_2CH_2N_3$), 2.03 (tt, 8H, *J* = 6.7, 7.5 Hz, 4 $CH_2CH_2CH_2N_3$). ^{13}C NMR: δ 147.1 (C), 134.4 (C), 128.9 (CH), 128.2 (C), 50.5 (CH_2), 31.8 (CH_2), 30.4 (CH_2). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{40}H_{48}N_{13}O_4$ $(M+NH_4)^+$ 774.3952, found 774.3978.

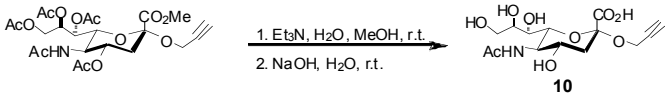
5,11,17,23-Tetrakis(3-azidopropyl)-25,26,27,28-tetrakis(ethoxycarbonylmethoxy)-calix[4]arene (5). A mixture of **4** (600 mg, 0.79 mmol), anhydrous K_2CO_3 (650 mg, 4.70 mmol), ethyl bromoacetate (0.70 mL, 6.32 mmol), and anhydrous acetonitrile (15 mL) was stirred under reflux overnight in a nitrogen atmosphere, then cooled to room temperature, diluted with 1 M phosphate buffer at pH 7 (50 mL), concentrated to remove the organic solvents, and extracted with AcOEt (2 x 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 4:1 cyclohexane-AcOEt to give **5** (695 mg, 80%) as a syrup. ^{1}H NMR: δ 6.50 (s, 8H, Ar), 4.85 and 3.16 (2 d, 8H, *J* = 13.2 Hz, 4 ArC*H*$_2$Ar), 4.75 (s, 8H, 4 *CH*$_2$CO$_2$Et), 4.22 (q, 8H, *J* = 7.0 Hz, 4 $CH_2CO_2CH_2CH_3$), 3.18 (t, 8H, *J* = 6.5 Hz, 4 $CH_2CH_2CH_2N_3$), 2.40 (t, 8H, *J* = 7.5 Hz, 4 $CH_2CH_2CH_2N_3$), 1.72 (tt, 8H, *J* = 6.5, 7.5 Hz, 4 $CH_2CH_2CH_2N_3$), 1.30 (t, 12H, *J* = 7.0 Hz, 4 $CH_2CO_2CH_2CH_3$). ^{13}C NMR: δ 170.3 (C), 154.1 (C), 134.9 (C), 134.3 (C), 128.4 (CH), 71.3 (CH_2), 60.4 (CH_2), 50.5 (CH_2), 32.0 (CH_2), 31.5 (CH_2), 30.5 (CH_2), 14.2 (CH_3). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{56}H_{72}N_{13}O_{12}$ $(M+NH_4)^+$ 1118.5423, found 1118.5359.

5,11,17,23-Tetrakis(3-azidopropyl)-25,26,27,28-tetrakis(carboxylmethoxy)-calix[4]arene (6). A solution of **5** (390 mg, 0.35 mmol) in anhydrous THF (10 mL) and 2 M aqueous NaOH (1.5 mL) was kept at room temperature for 18 h in a nitrogen atmosphere, then acidified with 1 M HCl (15 mL), concentrated to remove the organic solvents, and extracted with AcOEt (2 x 80 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was triturated with MeOH, to give **6** (300 mg, 86%) as a white solid. Mp 250-253 °C (dec.). ^{1}H NMR (DMSO-*d*6, 160 °C): δ 6.69 (s, 8H, Ar), 4.72 and 3.22 (2 d, 8H, *J* = 13.0 Hz, 4 ArC*H*$_2$Ar), 4.60 (s, 8H, 4 *CH*$_2$CO$_2$H), 3.20 (t, 8H, *J* = 6.5 Hz, 4 $CH_2CH_2CH_2N_3$), 2.42 (t, 8H, *J* = 7.5 Hz, 4 $CH_2CH_2CH_2N_3$), 1.72 (tt, 8H, *J*

= 6.5, 7.5 Hz, 4 $CH_2CH_2CH_2N_3$). ^{13}C NMR (DMSO-*d*6): δ 171.0 (C), 153.2 (C), 135.0 (C), 134.3 (C), 128.7 (CH), 71.7 (CH_2), 50.2 (CH_2), 31.5 (CH_2), 30.8 (CH_2), 29.8 (CH_2). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{48}H_{56}N_{13}O_{12}$ $(M+NH_4)^+$ 1006.4171, found 1006.4146.

Synthesis of nanoparticles 7. To a solution of **6** (0.30 g, 0.30 mmol) in acetone (10 mL) was added commercially available TiO_2 nanoparticles (1.00 g). The suspension was stirred in the dark at room temperature for 16 h, then centrifuged (4,000 rpm, 5 min) and decanted. The solid was suspended in acetone (10 mL), centrifuged (4,000 rpm, 5 min) and decanted. The solid was dried under vacuum at room temperature to give **7** (1.02 g) as a white powder, which was characterized by DRIFTS and TGA. The combined surnatant phases were concentrated to give pure tetraazide calixarene **6** (0.23 g).

Synthesis of glyconanoparticles 9. To a solution of propargyl galactoside **8** (27.5 mg, 0.12 mmol) in 1:1 CH_3OH-H_2O (2 mL) were added $CuSO_4$·$5H_2O$ (0.012 mmol, 12 μL of a 1 M solution in H2O), sodium ascorbate (0.063 mmol, 63 μL of a freshly prepared 1 M solution in H_2O), and then the nanoparticles **7** (300 mg). The reaction mixture was stirred in a nitrogen atmosphere, in the dark, at room temperature for 24 h, then centrifuged (4,000 rpm, 5 min) and decanted. The solid was washed with 1:1 CH_3OH-H_2O (4 mL), an aqueous solution of EDTA disodium salt (50 mM, 2 mL), and finally H_2O (2 mL). The solid was dried under vacuum at room temperature for 8 h to give **9** (280 mg) as a white powder, which was characterized by DRIFTS and TGA.



10

Propargyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (10). A solution of known[19] methyl (propargyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (0.20 g, 0.38 mmol) in 1:1:5 triethylamine, water and methanol (3 mL) was kept at room temperature for 24 h, then concentrated. A solution of the methyl ester derivative in 0.2 M aqueous NaOH (2 mL) was kept at room temperature for 18 h in a nitrogen atmosphere, then neutralized with Amberlist IR-120 resin (H^+ form, activated and washed with H_2O and MeOH immediately before the use), and filtered through a sintered glass filter. The resin was washed with H_2O, and the solution was concentrated. The residue was eluted from a C18 silica gel cartridge with H_2O, and dried under high vacuum to give **10** (115 mg, 90%) as an amorphous solid; $[\alpha]_D$ = -4.5 (*c* 0.9, MeOH). ^{1}H NMR (400 MHz, CD_3OD) selected data: δ 4.42 and 4.22 (2 dd, 2H, *J*

= 2.5, 15.0 Hz, OC*H*$_2$C≡CH), 2.79 (dd, 1H, *J* = 2.5, 2.5 Hz, OCH$_2$C≡C*H*), 2.74 (dd, 1H, $J_{3eq,4}$ = 4.8 Hz, $J_{3ax,3eq}$ = 12.8 Hz, H-3eq), 1.98 (s, 3H, Ac), 1.68 (dd, 1H, $J_{3ax,4}$ = 11.2 Hz, H-3ax). ^{13}C NMR (75 MHz, CD_3OD): δ 175.3 (C), 171.9 (C), 80.3 (CH), 75.4 (C), 74.9 (CH), 72.7 (CH), 70.1 (CH), 68.8 (CH), 64.5 (CH_2), 53.8 (CH), 53.0 (CH_2), 41.7 (CH_2), 22.6 (CH_3). HRMS (ESI/Q-TOF) *m/z* calcd for $C_{14}H_{22}NO_9$ $(M+H)^+$ 348.1294, found 348.1274.

Synthesis of glyconanoparticles 11. To a solution of propargyl sialoside **10** (78.5 mg, 0.22 mmol) in 1:1 CH_3OH-H_2O (4 mL) were added $CuSO_4$·5H_2O (0.023 mmol, 23 μL of a 1 M solution in H2O), sodium ascorbate (0.113 mmol, 113 μL of a freshly prepared 1 M solution in H_2O), and then the nanoparticles **7** (500 mg). The reaction mixture was stirred in a nitrogen atmosphere, in the dark, at room temperature for 5 days, then centrifuged (15,000 rpm, 4 min) and decanted. The solid was washed with 1:1 CH_3OH-H_2O (4 mL), an aqueous solution of EDTA disodium salt (50 mM, 2 mL), and finally H_2O (4 mL). The solid was dried under vacuum at room temperature for 8 h to give **11** (400 mg) as a white powder, which was characterized by DRIFTS and TGA.

References

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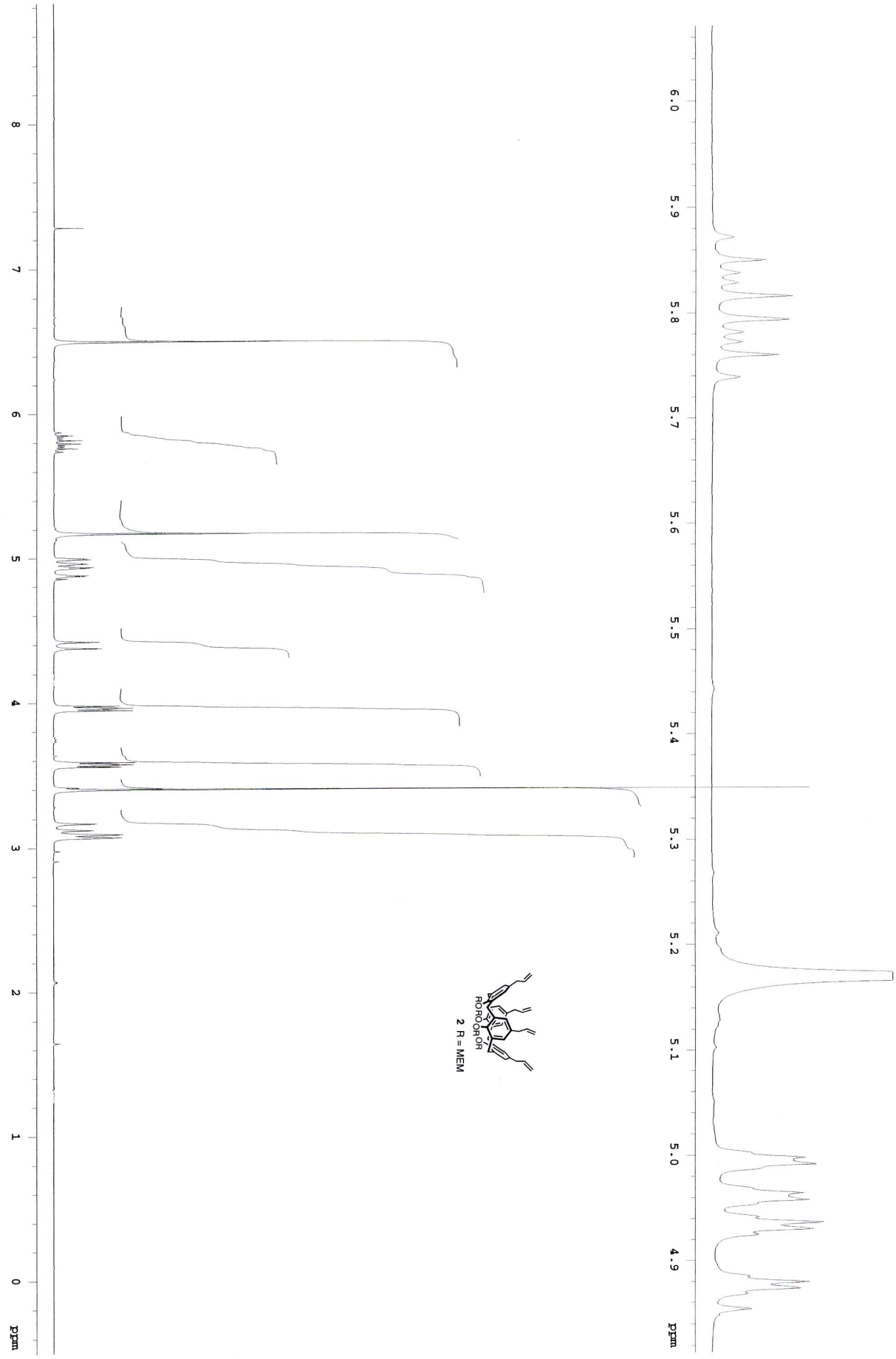
19. Z. Gan and R. Roy, *Can. J. Chem.* 2002, **80**, 908-916.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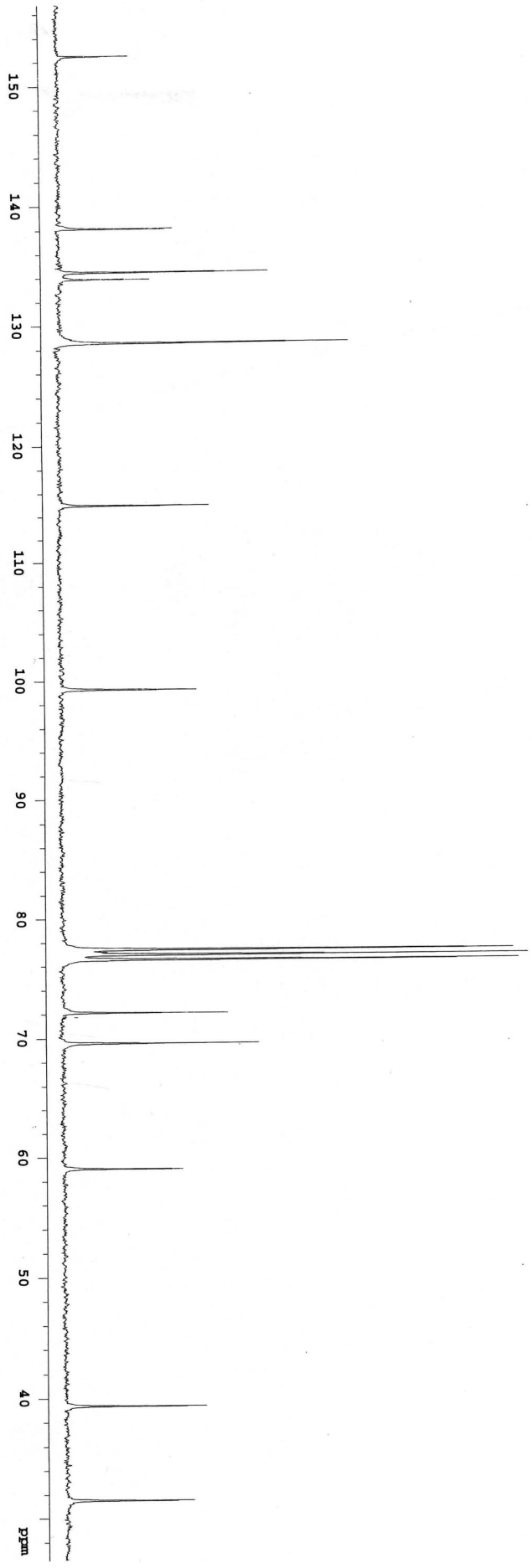

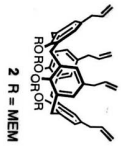

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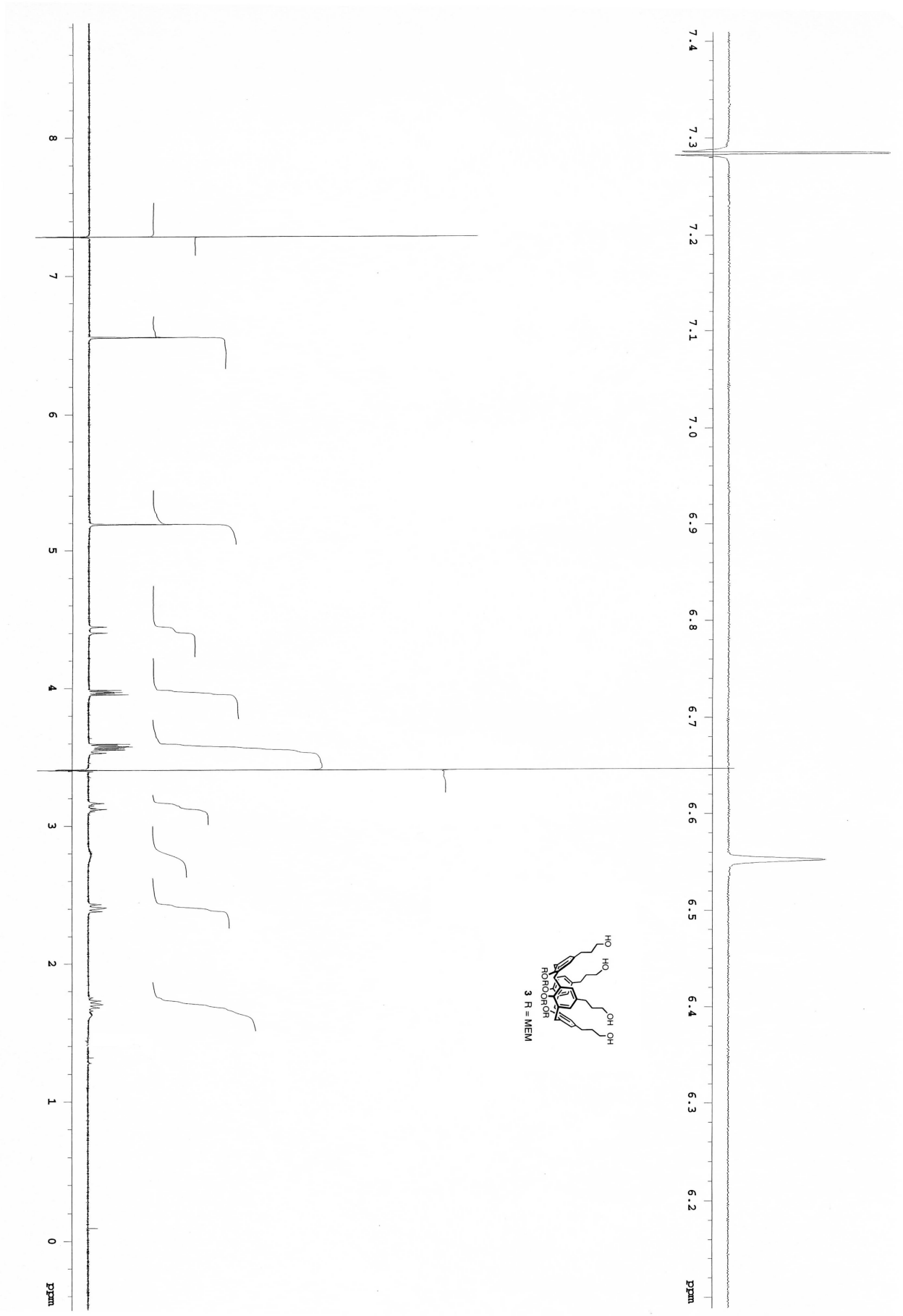
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RO
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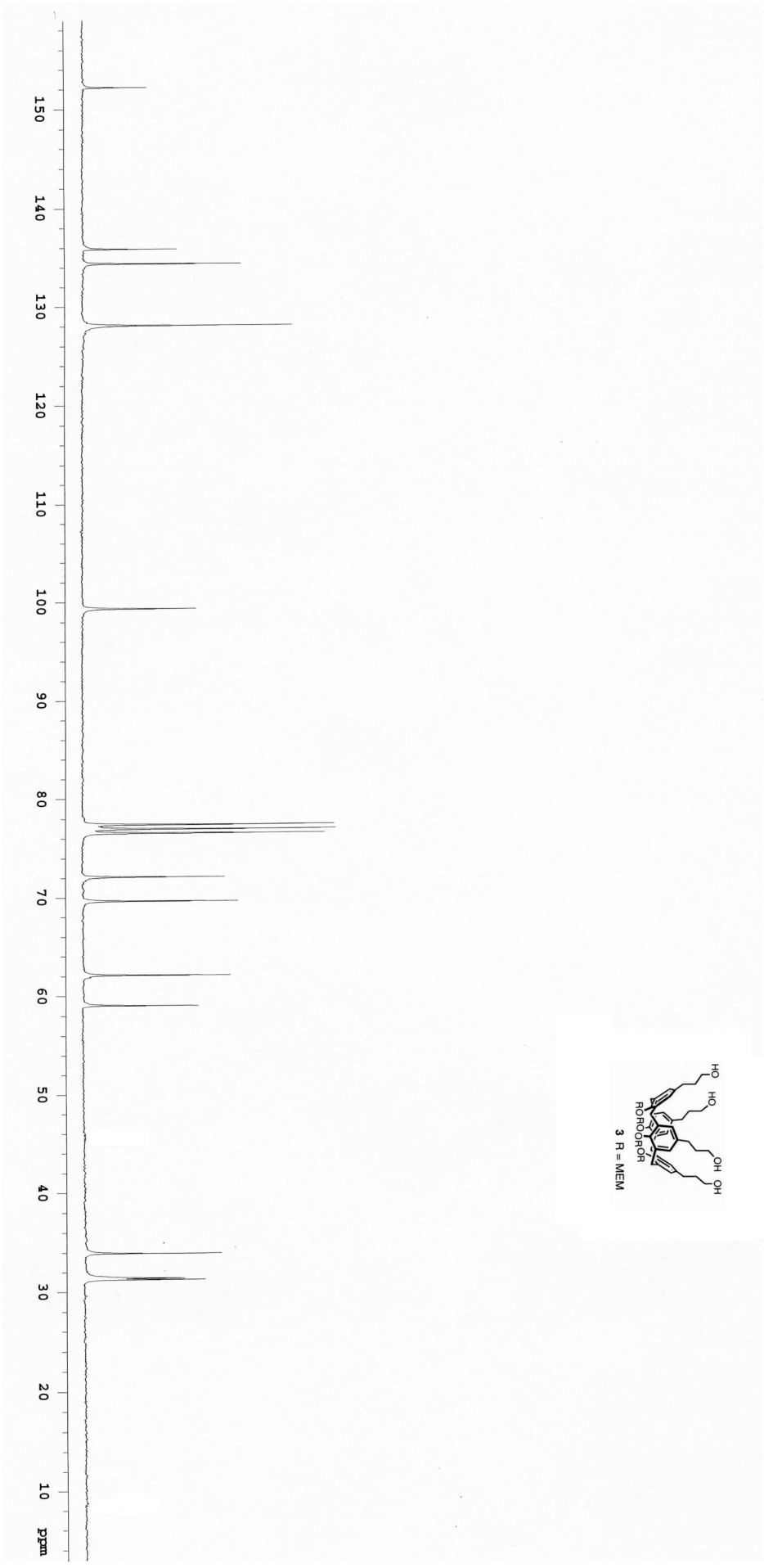
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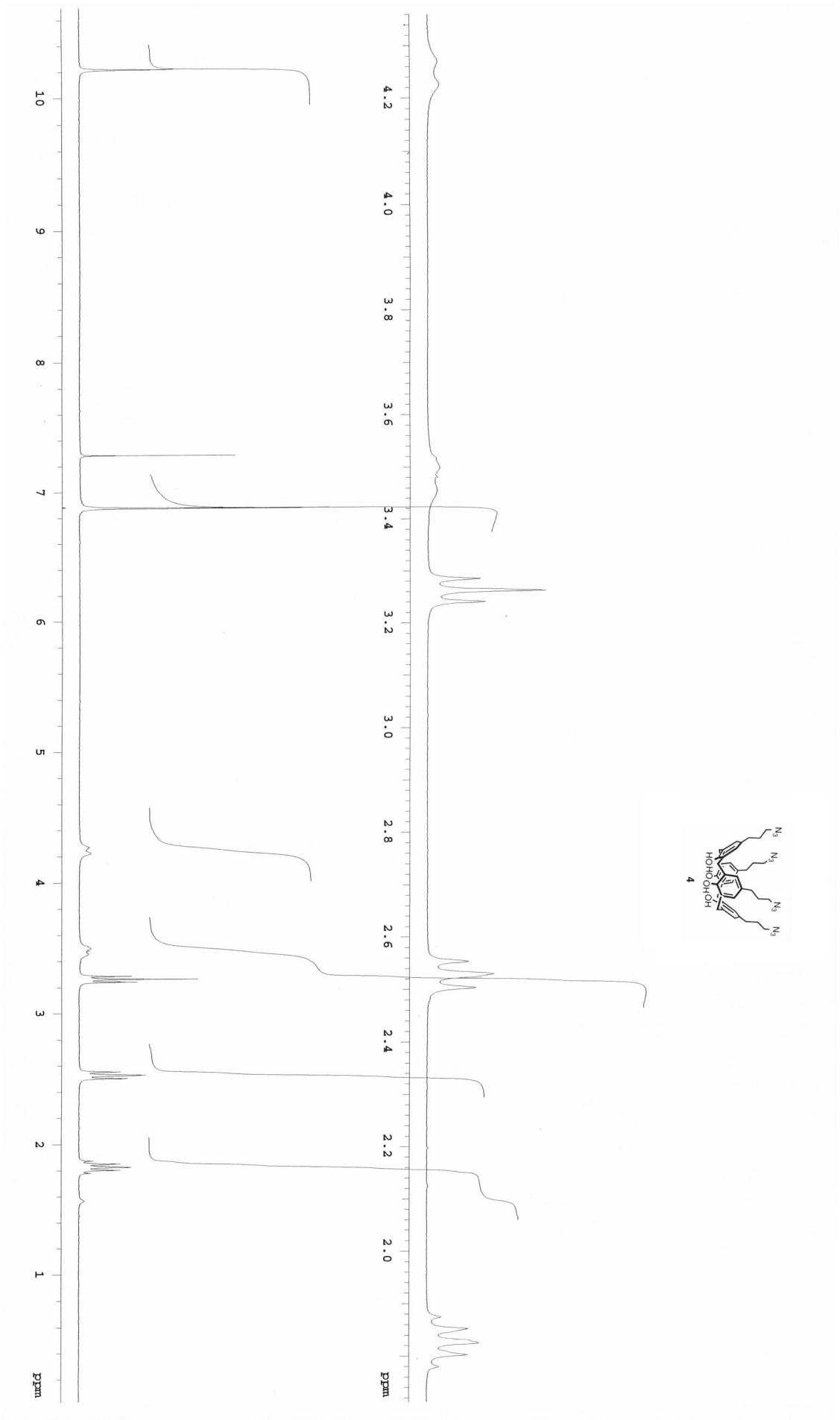
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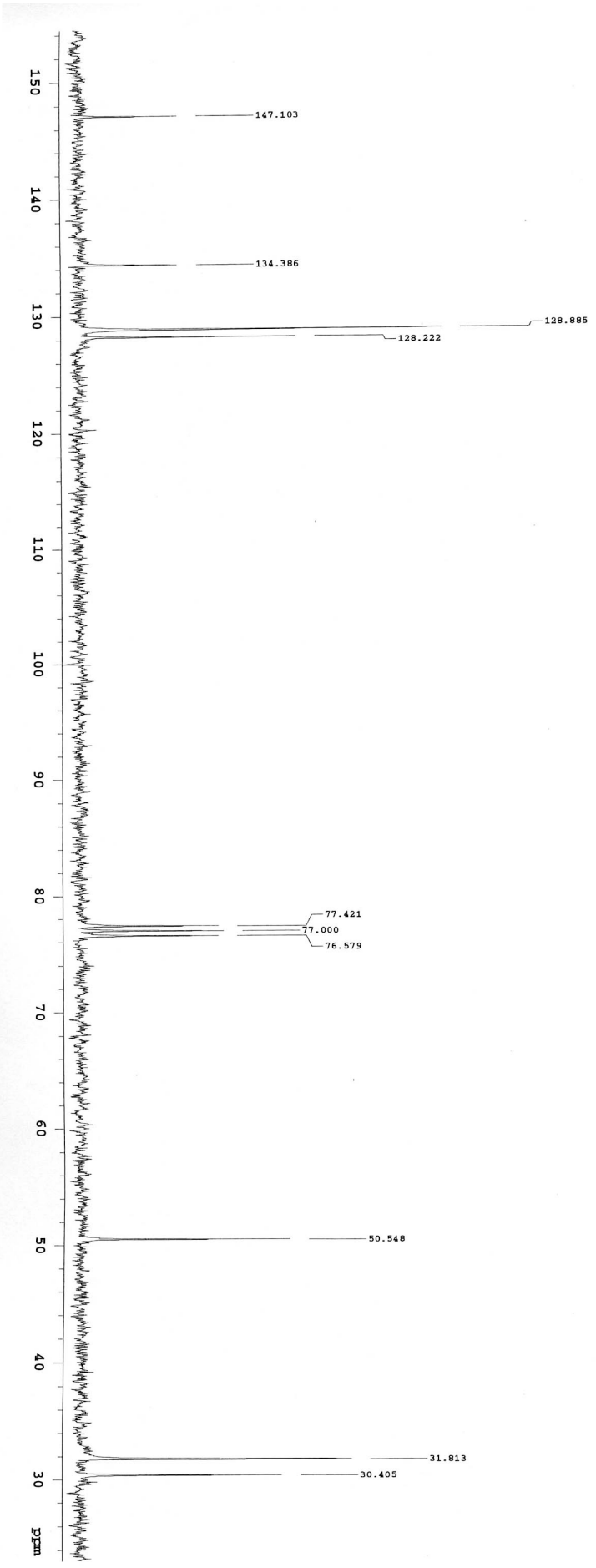
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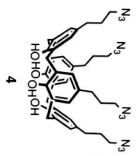
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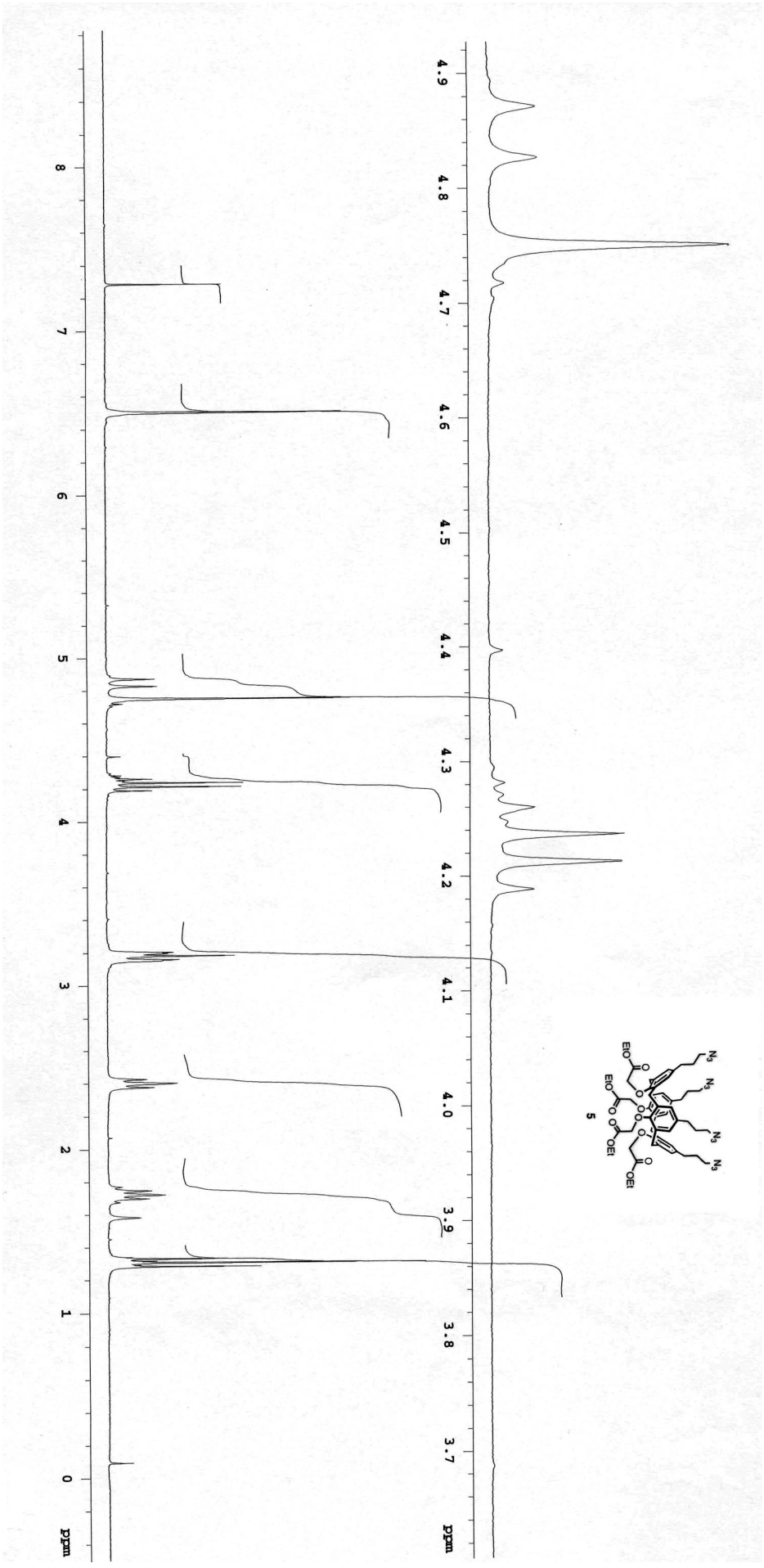
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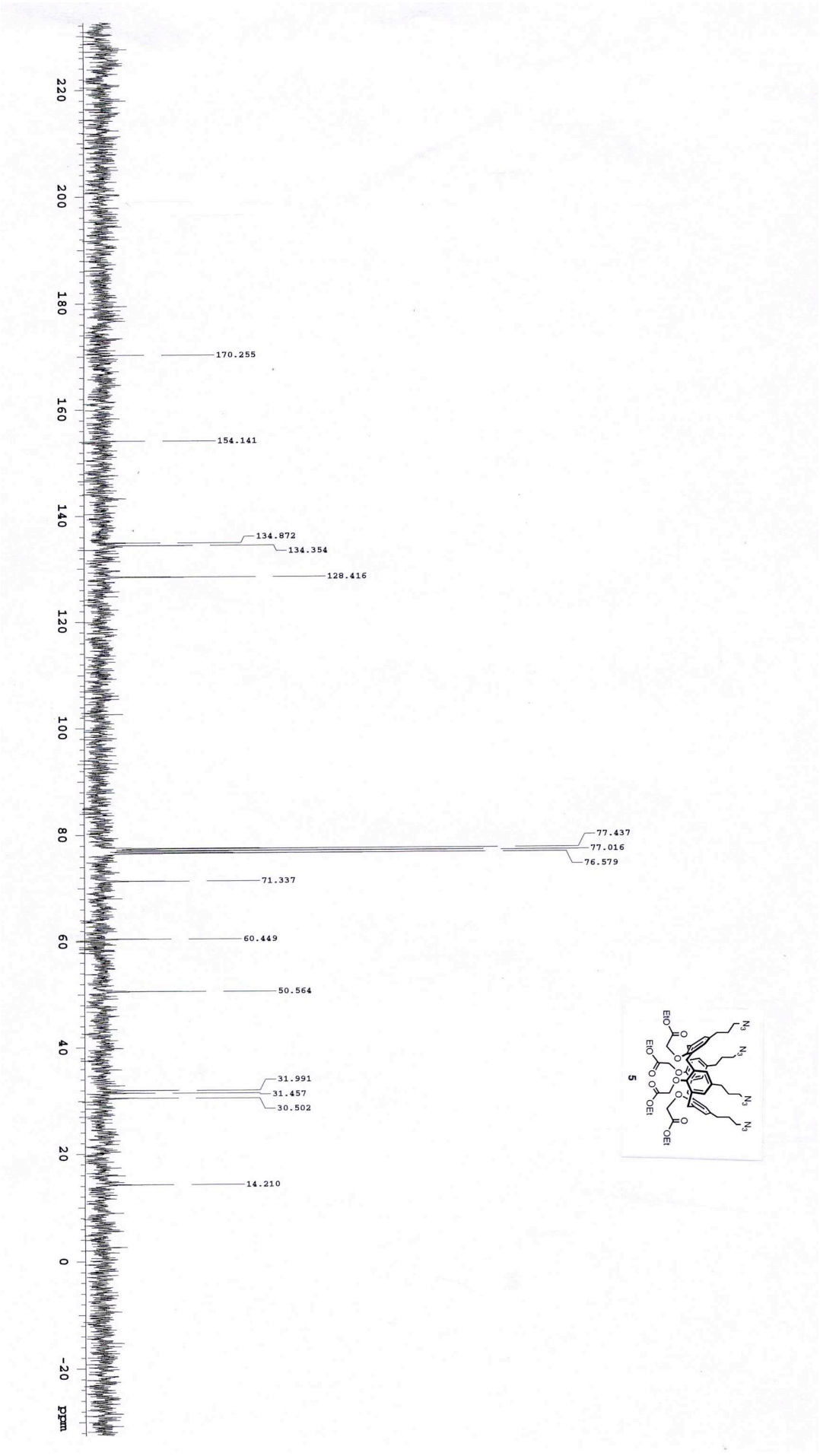
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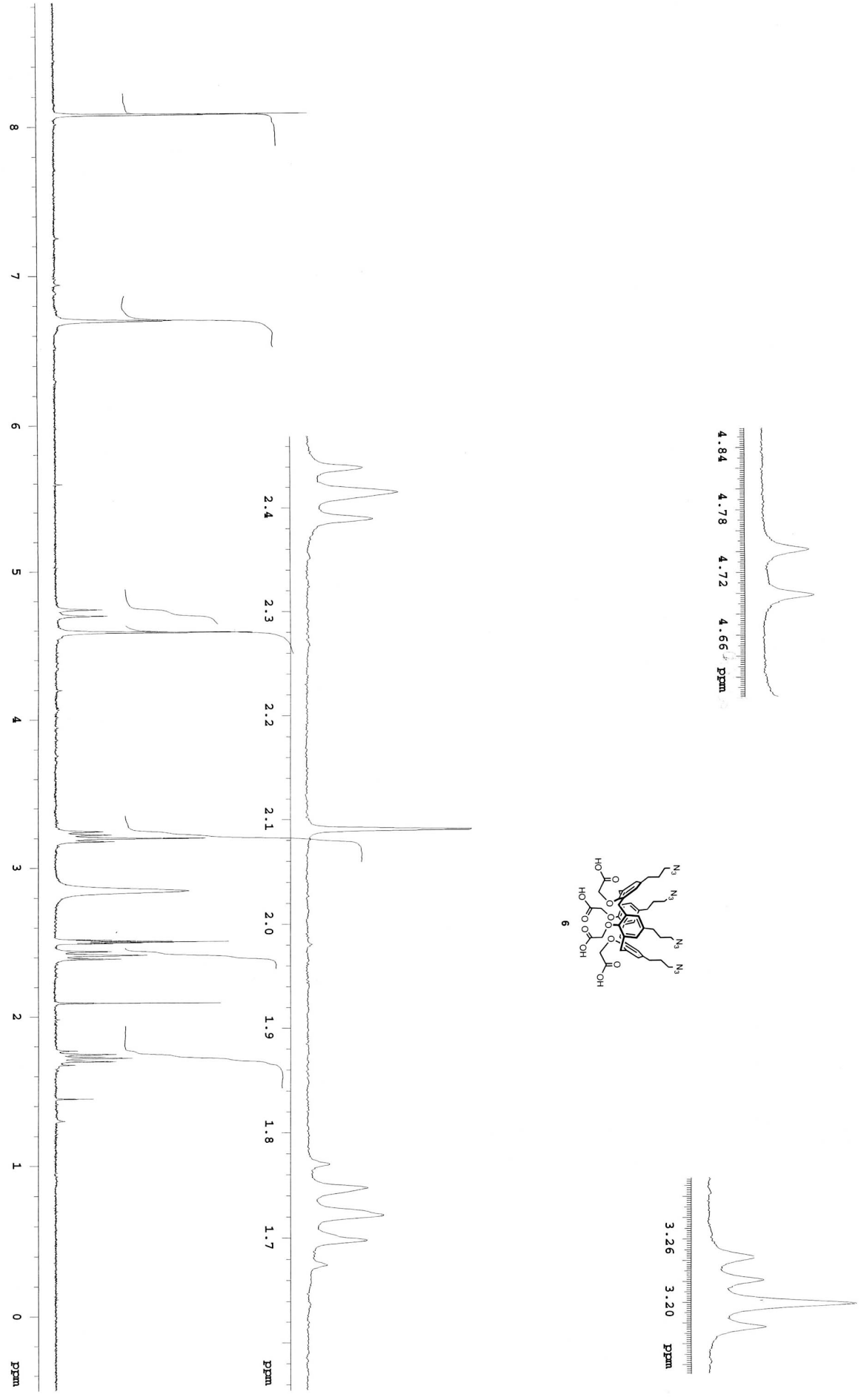
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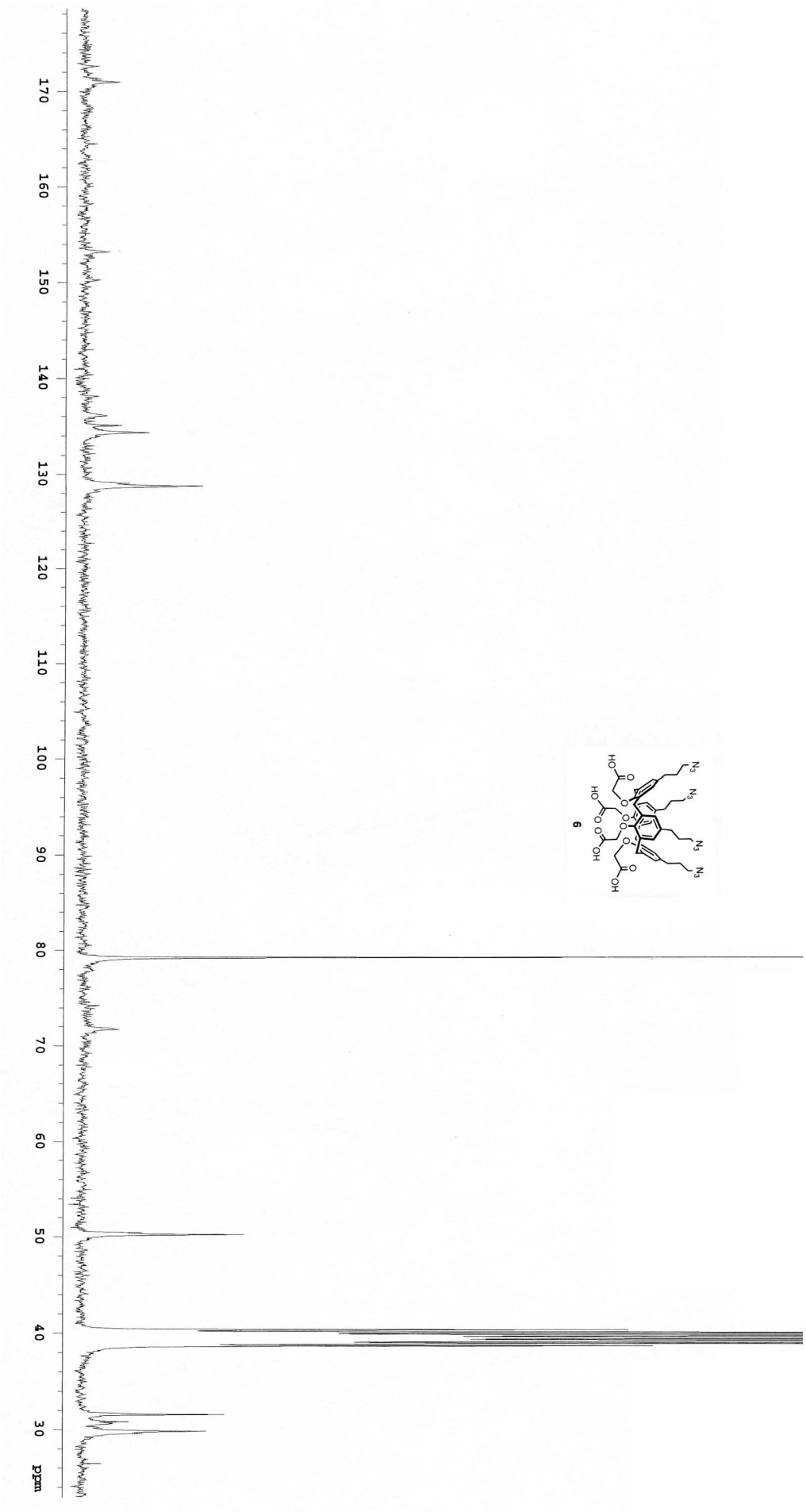
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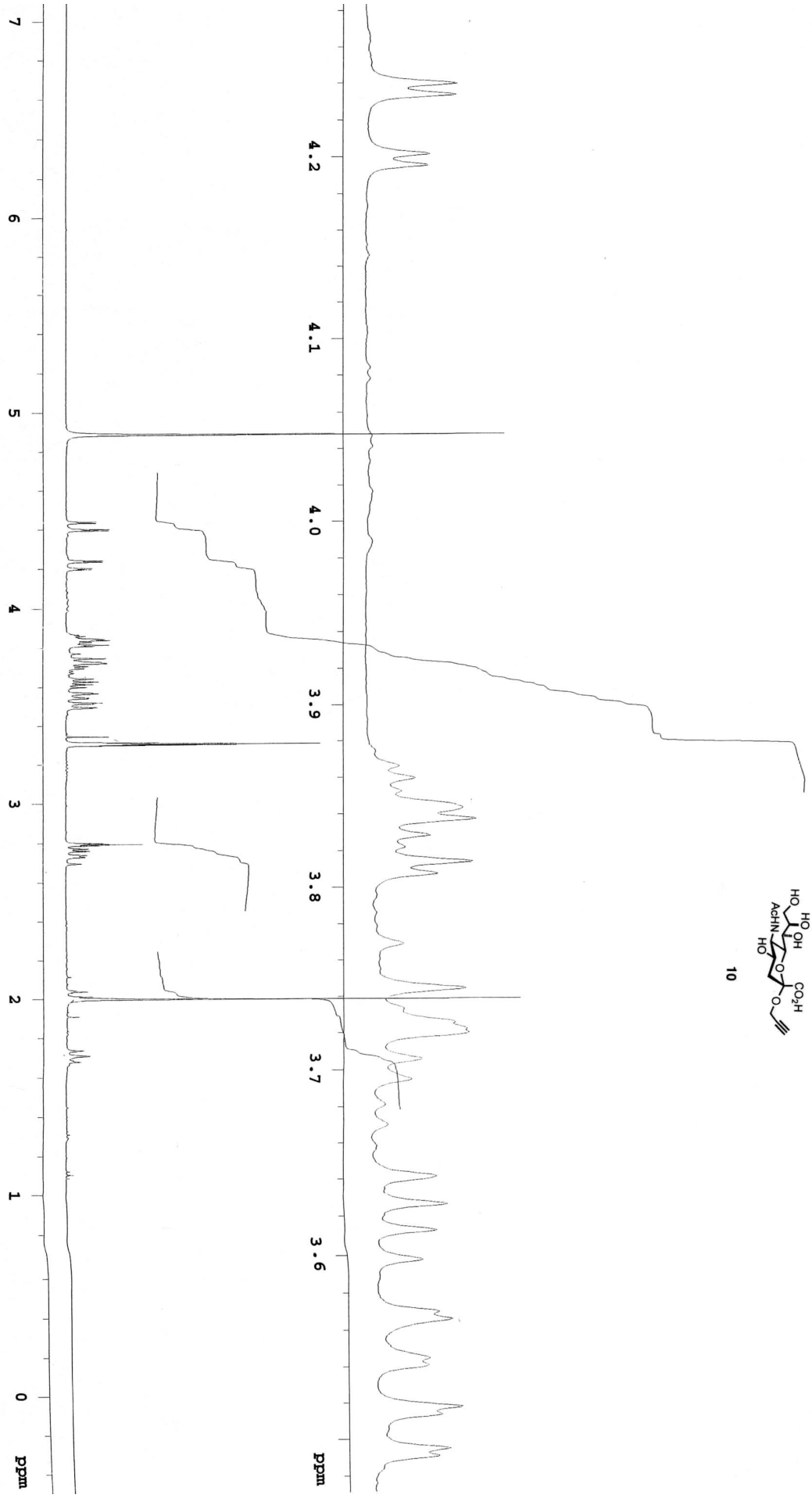
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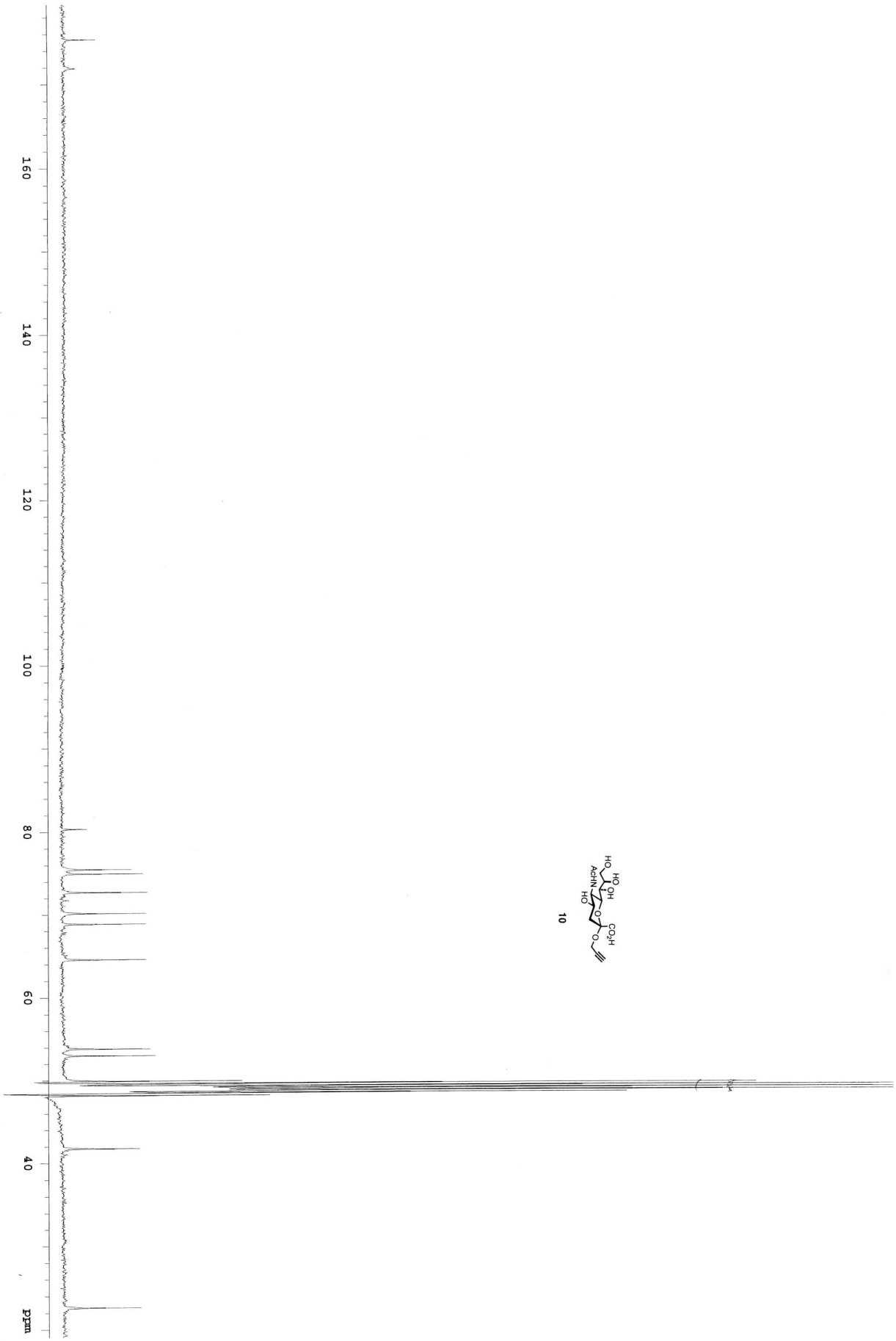
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