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Supporting Information

for

Glycosphingolipid synthesis employing a combination of recombinant glycosyltransferases and an endoglycoceramidase glycosynthase.

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Table of Contents

General	3
Enzymes	4
Glycosyltransferase catalyzed synthesis of **4**,**5** and **6**	5-6
Synthesis of **7**-**8** with EGCase glycosynthase	6-7
Synthesis of **9**-**11**	7-9
Acylation of *lyso*-G_{M3}**10** – preparation of **12** and **13**	9-11
References	11-12
^{1}H and ^{13}C NMR spectra for compounds **7**-**12**	13

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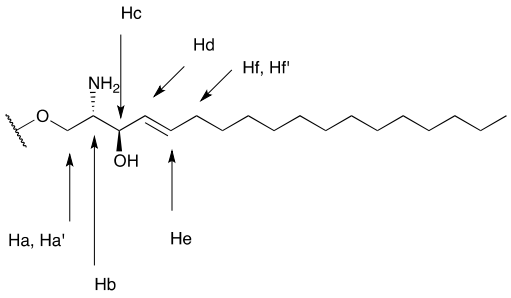
General

Solvents and chemicals were of analytical grade and were purchased from the Sigma-Aldrich company, with the exception of D-*erythro*-sphingosine (Avanti Polar Lipids). *N,N*-Dimethylformamide and triethylamine were dried and stored over 4Å molecular sieves. 1,2-Dimethoxyethane (≥99%) and acetonitrile (HPLC grade) were used without further purification.

Analytical thin-layer chromatography (t.l.c.) was performed on MerckSilica Gel 60F_{254} (0.2 mm thickness on aluminum. T.l.c. plates were visualized using UV light (254 or 365 nm) and by immersion in either 10% ammonium molybdate in 2 M H_2SO_4, 5% H_2SO_4, or 0.2% (w/v) ethanolic ninhydrin prior to heating. Reversed-phase silica gel chromatography was performed using Waters Sep-Pak® tC18 cartridges of varying size (water/acetonitrile gradient elution – see experimental for specific information). Cartridges were preconditioned by washing with 10 bed volumes of acetonitrile followed by 20 volumes of deionized water. Anion exchange chromatography was performed using Waters Sep-Pak® QMA light cartridges with gradient elution (50-500 mMammonium formate) according to the manufacturers instructions. Alternative chromatographic techniques are described in detail in the experimental section.

^1H and ^{13}C NMR spectra were recorded on aBrukerAvance 600inv Fourier Transform spectrometer fitted with a TCI-Z cryoprobe. All spectra were recorded using an internal lock (deuterium) and are referenced internally to a residual solvent peak. ^1H and ^{13}Cchemical shifts are quoted in parts per million (ppm) downfield of tetramethylsilane, and chemical shifts (δ_x) are rounded to the nearest 0.1 ppm unless increased precision was required to distinguish resonances. Coupling constants (J) are given in Hertz (Hz) and are quoted to the nearest 0.1 Hz. Proton and carbon spectral assignments were made based on COSY, TOCSY, APT,^1H-^{13}C-HSQC, and ^1H-^{13}C-HMBC experiments as required. Reported ^{13}C resonances marked with an asterisk* were extracted from HMBC spectra. All NMR spectra were acquired using deuterated methanol as solvent. Sphingosine ^1H NMR resonances were annotated Hx as described in the figure below.



Mass spectra were acquired from sample dissolved in aqueous methanol on a Waters/Micromassinstrument (electrospray ionization) and recorded using Time-of-Flight method.

Enzymes

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Endoglycoceramidase II glycosynthases E351S and E351S/D314Y,[1,2] CMP-sialic acid synthetase NSY-05,[3] α2,3-sialyltransferase Cst-I,[4] β1,3-*N*-acetylglucosaminyl transferase HP-39,[5] β1,4-galactosyltransferaseHP-21,[6] and α2,3-sialyltransferase Cst-I (construct Cst-06)[7] were expressed in *E. coli* as previously described. Bovine α1,3-galactosyltransferase (construct BOV-10) is a truncated version (aa 80-368) of the previously described enzyme,[8] cloned in pCWori+. Details will be reported elsewhere.

EGCase glycosynthases E351S and E351S/D314Y could be used interchangeably for the purposes of synthesis in this work. Enzyme concentrations used for EGCase glycosynthase reactions (typically 1.0 mg/mL) could be reduced at least 10-fold without altering reaction yields.

CMP-sialic acid synthetase NSY-05 crude cell lysate was used without further purification. NSY-05 used in this work had been stored at 4 °C for in excess of 2 years. Although reactions described below employ purified Cst-I for synthesis of **10** and **11**, crude cell lysate could also be employed without noticeable changes in reaction outcome. Both crude and purified Cst-I used in the synthesis of **10** and **11** had been stored at 4 °C for in excess of two years.

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Synthesis ofLNnT-F (4),Galili-F (5) and LM1-F (6)

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylfluoride (**4**)

The reaction mixture included 31 mg of α-lactosyl fluoride (10 mM final concentration), 20 mM UDP-GlcNAc, 50 mM HEPES pH 7.5, 10 mM $MgCl_2$ and 3.5 units of β1,3-GlcNAcT HP-39. The reaction was incubated overnight at 37°C and looked complete as judged by TLC with detection by charring in 5% H_2SO_4. . The enzyme (HP-39) was removed by running the reaction mix through a membrane with a 100,000 nominal molecular weight limit using an Amicon Ultra-15 (PL-100) centrifugal filter device. The remaining donor was removed by loading the filtrate onto an anion-exchange column (AG1-X2 resin, acetate form, BioRad). The column was developed with H_2O and the fractions containing the product were pooled and used directly for the addition of the β-D-galactopyranosyl residue by HP-21. The reaction mixture contained 2.5 mMGlcNAcβ-1,3-Galβ-1,4-Glc-F, 5 mM UDP-Gal, 50 mMHepes pH 7.5, 10 mM $MnCl_2$ and 28 units of HP-21. The reaction was incubated at 37°C. TLC analysis showed that the reaction was complete within 45 min. The reaction mixture was stored at -20°Cprior to further use.

α-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylfluoride (**5**)

α1,3-Galactosyltransferase BOV-10 (2.5 mL of an extract clarified by centrifugation at 149,000 X *g*) and UDP-Gal (5 mM final concentration) were added to two thirds of the LNnT-F (**4**) reaction mixture (22 mL in 50 mMHepes pH 7.5, 10 mM $MgCl_2$).The reaction was complete after 2 h of incubation at 37°C. The reaction mixture was lyophilized and re-suspended in 4 mL H_2O before application to a Superdex Peptide column (GE Healthcare;1x30 cm). The column was developed with 50 mM NH_4HCO_3(9 runs total) and the fractions containing Galili-F**5** were pooled and lyophilized (65.2 mg).

(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylfluoride (**6**)

α2,3-SialyltransferaseCST-06 (1.8 unit) and CMP-Neu5Ac (5 mM final concentration) were added to one third of the LNnT-F (**4**) reaction mix (11 mL in 50 mMHepes pH 7.5, 10 mM $MgCl_2$). The reaction was judged complete after 2 h of incubation at 37°C. The mixture was lyophilized and re-suspended in 2.5 mL H_2Obefore application to a Superdex Peptide column (GE Healthcare;1x30 cm). The column was developed with 50 mM NH_4HCO_3(5 runs total) and the fractions containing LM1-F were pooled and lyophilized to afford **6** (32.7 mg).

General comment regarding glycosyl fluoride purity: Extensive purification of the oligosaccharide fluorides was avoided owing to concerns regarding decomposition.

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Recovered masses reflect yields in excess of 100% owing to the presence of salts. A small amount of hemiacetal (<5% as judged by TLC) was also evident.

Synthesis of *lyso*-glycosphingolipids 7 and 8

*O-(α-D-Galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol (**7**)*

D-*Erythro*-sphingosine hydrochloride (7.7 mg; 23 μmol) was dissolved in 35 mM sodium acetate (1.60 mL; pH 5.3) with sonication. This solution was then added to a glass vial containing α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-glucopyranosyl-fluoride (**5**;24.0 mg; 27μmol), before adding EGCase glycosynthase (~1.5 mg of E351S/D314Y in 0.45 mL 25 mMNaOAc, pH 5.3) and 1,2-dimethoxyethane (0.20 mL). The reaction mixture, in which some precipitate was evident, was allowed to stand at 37 °C. All of the components were dissolved within three hours. After ~ 48 hours no glycosyl fluoride was evident by t.l.c. (aliquots were diluted in $CHCl_3$/MeOH before spotting on plates eluted with in 5/4/1 $CHCl_3$/MeOH/0.2% $CaCl_2$). The reaction mixture was freeze dried and the solid extracted with $CHCl_3$/MeOH (~ 10 mL). The organic extract was concentrated and then dissolved in water before purification on a Waters tC18 SepPak cartridge (2g) using a gradient of acetonitrile/water. The target glycolipid **7** (16.1 mg; 13 μmol; 61%) was isolated after lyophilization. ^{1}H NMR (600 MHz; CD_3OD) δ_H: 5.90-5.85 (m, 1H, He), 5.51-5.46 (m, 1H, Hd), 5.05 (s br, 1H, H-1′′′′), 4.67 (d, 1H, H-1′′, J 8.4), 4.44 (d, 1H, H-1′, J 7.3), 4.37 (d, 1H, H-1, J 8.1), 4.36 (d, 1H, H-1′′′, J 7.7), 4.32 (dd, 1H, Hc, J ~ 4.5, 6.5), 4.21 (dd, 1H, J~6), (m, 2H, H-3′, H-3′′′), 3.99 (dd, 1H, Ha, J 8.4, 11.0), 3.96-3.89 (m, 4H, Ha′, H4′′′′, C*H*$_2$), 3.89-3.82 (m, 4H), 3.81-3.74 (m, 3H, C-2′′), 3.74-3.67 (m, 5H), 3.67-3.63 (m, 3H), 3.63-3.53 (m, 6H), 3.48-3.45 (m, 1H, H-5), 3.45-3.42 (m, 1H, H-5′′), 3.42-3.38 (m, 1H, Hb), 3.30 (m, 1H, H-2 (obscured)), 2.13-2.08 (m, 2H, Hf, Hf′), 2.00 (s, 3H, NHAc), 1.45-1.39 (m, 2H, C*H*$_2$), 1.35-1.25 (m, 20H, 10 x CH_2), 0.90 (t, 3H, CH_3-r). ^{13}C NMR (151 MHz; CD_3OD) δ_C: 174.7(C=O), 137.1 ($C5_S$), 128.3($C4_S$), 105.1 (C1′′′), 105.0 (C1′), 104.3(C1′′), 103.8(C1), 97.8(C1′′′′), 83.5, 80.8, 80.2, 80.0, 76.8, 76.77, 76.72, 76.6, 76.4, 74.6 (C2), 74.0, 72.4, 71.7, 71.5, 71.22, 71.18, 71.0, 70.2, 70.0, 67.2, 66.8, 62.8, 62.62, 62.60, 61.8, 61.7, 57.0(C2′′), 56.7($C2_S$), 33.5, 33.2, 30.91, 30.90 (mult. C), 30.87, 30.86, 30.7, 30.6, 30.5, 30.3, 23.2, 14.6. ESI HRMS (m/z) calculated for$C_{50}H_{91}N_2O_{27}$: 1151.5809. Found: 1151.5825 [M+H].

*O-[(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid]-(2→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol (**8**)*

D-*Erythro*-sphingosine hydrochloride (7.0 mg; 21μmol) was dissolved in 35 mM sodium acetate (1.50 mL; pH 5.3) with sonication. This solution was then added to a glass vial containing (5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-

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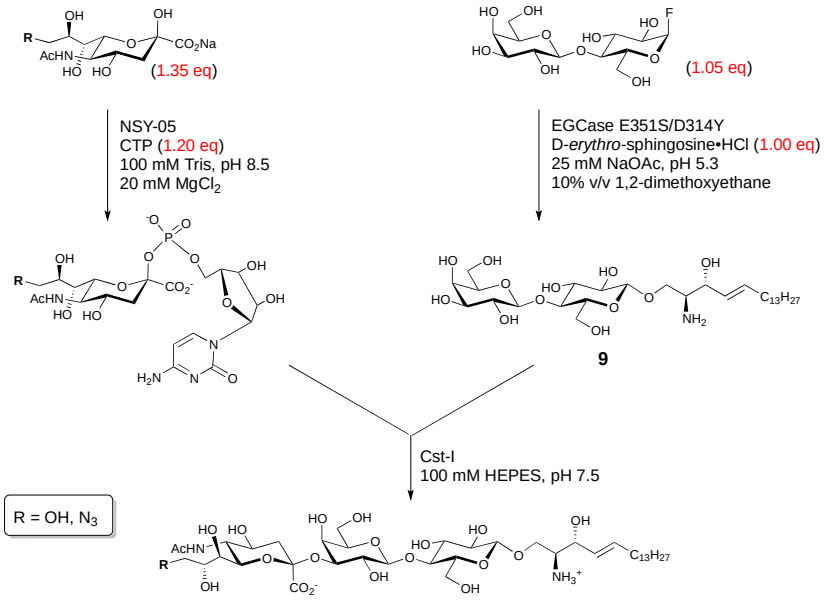
galactopyranosyl-(1→4)-α-D-glucopyranosyl-fluoride (**6**;25.0 mg; 25μmol), before adding EGCase glycosynthase (~1.3 mg of E351S/D314Y in 0.40 mL 25 mMNaOAc, pH 5.3) and 1,2-dimethoxyethane (0.20 mL). The reaction mixture, in which some precipitate was evident, was allowed to stand at 37 °C. All of the components were dissolved within four hours. After ~ 45 hours no glycosyl fluoride was evident by t.l.c. (aliquots were diluted in $CHCl_3$/MeOH before spotting on plates eluted with in 5/4/1.2 $CHCl_3$/MeOH/0.2% $CaCl_2$). The reaction mixture was freeze dried and the solid extracted with $CHCl_3$/MeOH (~ 10 mL). The organic extract was concentrated and then dissolved in water before purification on a Waters tC18 SepPak cartridge (2g) using a gradient of acetonitrile/water containing 0.05%TFA. The target trifluoracetic acid salt of**8** (23.1 mg; 23 μmol; 80%) was isolated after lyophilization.^{1}H NMR (600 MHz; CD_3OD) δ_H: 5.90-5.84 (m, 1H, He), 5.51-5.46 (m, 1H, Hd), 4.65 (d, 1H, H-1′′, J 8.4), 4.45 (d, 1H, H1′, J 8.1), 4.36 (d, 1H, H-1, J 8.1), 4.35 (d, 1H, H-1′′′, J 7.7), 4.31 (dd, 1H, Hc, J ~ 4.5, 6), 4.07-4.03 (m, 2H, H-3′, H-3′′′), 3.99-3.94 (m, 2H, Ha, C*H*$_2$), 3.94-3.88 (m, 4H, Ha′, C*H*$_2$), 3.86-3.74 (m, 8H), 3.74-3.61 (m, 8H), 3.61-3.53 (m, 7H), 3.50 (dd, 1H, J 1.0, 8.8), 3.48-3.44 (m, 1H, H-5), 3.43-3.38 (m, 2H, H-5′′, Hb), 3.30 (m, 1H, H-2 (obscured)), 2.77 (dd, 1H, $H3_{eq}$′′′′, J4.4, 13.2), 2.13-2.08 (m, 2H, Hf, Hf′), 2.00 (s, 3H, NHAc), 1.99 (s, 3H, NHAc), 1.90 (dd, 1H, $H\text{-}3_{ax}$′′′′, J 11.4),1.45-1.40 (m, 2H, C*H*$_2$), 1.35-1.25 (m, 20H, 10 x CH_2), 0.90 (t, 3H, CH_3-r).^{13}C NMR (151 MHz; CD_3OD) δ_C: 175.4, 174.5, 172.2, 137.0, 128.3, 105.1 (2C, C1′, C1′′′), 104.4 (C1′′), 103.9 (C1), 99.9 (C2′′′′), 83.6, 80.7, 80.2, 78.0, 77.1, 76.83, 76.78, 76.6, 76.5, 75.6, 74.6, 74.0, 72.7, 71.7, 71.0, 70.9, 70.2, 70.1, 69.7, 68.8, 67.2, 65.0, 62.64, 62.59, 61.78, 61.76, 57.0, 56.8, 53.8, 41.3 (C3′′′′), 33..5, 33.2, 30.95 (mult. C), 30.91, 30.8, 30.63, 30.56, 30.3, 23.9, 23.2 (NHAc), 22.8 (NHAc), 14.6. ESI HRMS (m/z) calculated for$C_{55}H_{98}N_3O_{30}$: 1280.6235. Found: 1280.6256 [M+H].

Synthesis of*lyso*-G_{M3} and 9-azido-*lyso*-G_{M3}(10 and 11):

Sialyllactoside**10** and its 9-azido counterpart **11** were prepared in parallel using EGCase E351S/D314Y to generate the common lactosyl sphingosine intermediate **9**. CMP-sialic acid and CMP-(9-azido-9-deoxy-sialic acid) were prepared via reaction of cytidine triphosphate and the appropriate sialic acid, catalyzed by *Neisseria meningitidis* CMP-sialic acid synthetase NSY-05. The *crude* sialic acid donors were coupled with *crude***9** using the α(2→3)-sialyltransferase Cst-I from *Campylobacter jejuni*.



*O-(β-D-Galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol (**9**)*

β-D-Galactopyranosyl-(1→4)-α-D-glucopyranosyl fluoride (0.070 g; 0.20 mmol) and D-*erythro*-sphingosine hydrochloride (0.065 g; 0.19 mmol) were combined in water (15.25 mL) containing NaOAc (0.45 mL of a 1M solution, pH 5.3). After sonication to dissolve the solids, 1,2-dimethoxyethane (1.8 mL) was added, followed by EGCase E351S/D314Y (2 mg in 25 mMNaOAc, pH 5.3). The mixture was allowed to stand at 37 °C for 24 hours before addition of a second aliquot of EGCase (1.6 mg in 0.40 mL). After a total of ~ 72 hours the reaction mixture was freeze dried, yielding 175 mg of crude solid. This material was used without purification for the preparation of **10** and **11**.

CMP-sialic acid

Cytidine triphosphate disodium salt (0.067 g; 127μmol) and *N*-acetyl neuraminic acid sodium salt (0.051 g; 154μmol) were dissolved in water (3.2 mL), 500 mMTris (1.28 mL, pH 8.5), and 100 mM $MgCl_2$ (1.28 mL), before adding NSY-05 (0.60 mL of crude cell lysate comprising 0.5% of a 2 year old crude cell lysate from a 2L culture). The pH of the mixture was adjusted to ~ 7.5 (pH paper) by addition of NaOH solution, and the mixture was tumbled overnight at room temperature. After brief centrifugation (2 min. at ~ 10^4 rpm) to remove solids the mixture was freeze dried.

CMP-(9-azido-9-deoxy-sialic acid)

Cytidine triphosphate disodium salt (0.042 g; 80 μmol) and 9-azido-*N*-acetyl neuraminic acid (0.030 g; 90 μmol) were dissolved in water (2.0 mL), 500 mMtris (0.80 mL, pH 8.5), and 100 mM $MgCl_2$ (0.80 mL), before adding NSY-05 (0.40 mL of crude cell lysate comprising 0.5% of a 2 year old crude cell lysate from a 2L culture). The pH of the mixture

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was estimated to be between 7.0 and 7.5 (pH paper), and the mixture was tumbled overnight at room temperature. After brief centrifugation (2 min. at ~ 10^4 rpm) to remove solids the mixture was freeze dried.

*O-[(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid]-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol (**10**)*

Crude lactosyl sphingosine (**9**, 0.115 g of 0.175 g crude material obtained in the reaction described above; theoretical maximum of 127μmol) and CMP-sialic acid (crude product after lyophilization; theoretical maximum of 127 μmol)were combined and dissolved in HEPES (6.00 mL; 83 mM, pH 7.5). The pH of the solution was adjusted to 7.5 by addition of ~200 μL of 1 M NaOH solution before adjusting the total volume to 9.80 mL with water. Sialyltransferase Cst-I (~ 10 mg in 200 μL) was added, and reaction progress was monitored by t.l.c. using 5/3.5/0.8 $CHCl_3$/MeOH/0.2% $CaCl_2$). After six hours reaction progress had ceased according to t.l.c.. The reaction mixture wasfreeze dried for storage prior to purification. Purification was conducted on a Waters tC18 SepPak (2g) and then repeated on a larger (5g) cartridge to afford **10** as the trifluoroacetic acid salt (66 mg; 65 μmol;51%).^{1}H NMR (600 MHz; CD_3OD) δ_H: 5.90-5.84 (m, 1H, He), 5.51-5.46 (m, 1H, Hd), 4.42 (d, 1H, H-1′, $J_{1,2}$7.7), 4.36 (d, 1H, H-1, $J_{1,2}$ 7.7), 4.31 (dd, 1H, Hc, J ~ 7), 4.05 (dd, 1H, H-3′, $J_{3,4}$ 3.3, $J_{3,2}$ 9.9), 3.98-3.94 (m, 2H, CH_2, Ha), 3.93-3.87 (m, 3H, Ha′, H-4′, CH_2), 3.86-3.81 (m, 2H, H9a), 3.79-3.74 (m, 3H, CH_2, H-5′′), 3.68-3.53 (m, 7H, CH_2, H-9b, H-2, H-3, H-4, H-5′), 3.51-3.48 (dd, 1H, J 1.5, J 8.8), 3.47-3.44 (m, 1H, H-5), 3.41-3.38 (m, 1H, Hb), 3.29 (dd, 1H, H-2, $J_{2,3}$ 9.2), 2.79 (dd, 1H, $H3_{eq}$′′, $J_{3eq,4}$ 4.0, $J_{3eq,3ax}$ 12.6), 2.13-2.08 (m, 2H, Hf, Hf′), 2.01 (s, 3H, NHAc), 1.86 (dd, 1H, H-3_{ax}′′, $J_{3ax,4}$ 11.4), 1.46-1.40 (m, 2H, Hg, Hg′), 1.37-1.25 (m, 20H), 0.90 (t, 3H, J ~ 7). ^{13}C NMR (151 MHz; CD_3OD) δ_C: 175.4, 172.7, 136.8, 128.2, 105.1 (C1′), 103.8 (C1), 100.1 (C2′′), 80.5, 77.8, 77.2, 76.9, 76.3, 75.3, 74.5, 72.7, 70.9, 70.7, 70.1, 69.4, 68.8, 67.1($C1_S$), 64.8 (C9′′), 62.5, 61.6, 56.7, 53.7 (C5′′), 41.4 (C3′′), 33.4, 33.1, 30.81 (mult. C), 30.77, 30.66, 30.5, 30.4, 30.2, 23.8, 22.6 (NHAc), 14.5 ($C1_S$). ESI HRMS (m/z) calculated for$C_{41}H_{75}N_2O_{20}$: 915.4913. Found: 915.4901 [M+H].

*O-[(5-Acetamido-9-azido-3,5,9-trideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid]-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol (**11**)*

Crude lactosyl sphingosine (**9**, 0.060 g of 0.175 g crude material obtained in the reaction described above; theoretical maximum of 66 μmol) and CMP-(9-azido-9-deoxy-sialic acid) [crude product after lyophilization; theoretical maximum of 80 μmol] were combined and dissolved in water (4.00 mL). To this solution was added $MnCl_2$ (0.80 mL of a 100 mM solution) and HEPES (0.80 mL of a 1.0 M solution). The pH of the solution was adjusted from ~6.5 to ~7.5 by addition of 1 M NaOH solution (~0.2 mL) before addition of sialyltransferase Cst-I (~12 mg in 0.25 mL). After diluting the mixture to a volume of 8.0 mL with water, alkaline phosphatase (3 μL) was added. Reaction progress was monitored by t.l.c. using 5.5/3/0.5 $CHCl_3$/MeOH/0.2% $CaCl_2$). After twenty hours the reaction mixture was applied to a Waters tC18 SepPak (2g) and eluted as described in the general methods.

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Purification was repeated on a larger (5g) cartridge to afford**11** as the trifluoroacetic acid salt(0.038 g; 37 μmol; 55%).^{1}H NMR (600 MHz; CD_3OD) δ_H: 5.91-5.84 (m, 1H, He), 5.51-5.46 (m, 1H, Hd), 4.41 (d, 1H, H-1′, $J_{1,2}$8.1), 4.36 (d, 1H, H-1, $J_{1,2}$ 7.7), 4.31 (dd, 1H, Hc, J 4.0, 6.1), 4.03 (dd, 1H, H-3′, $J_{3,4}$ 2.8, $J_{3,2}$ 9.5), 4.00-3.94 (m, 3H, CH_2, Ha), 3.92 (dd, 1H, Ha′, J 3.7, 11.4), 3.89 (d br, 1H, H-4′), 3.85 (dd, 1H, CH_2), 3.79-3.75 (m, 3H, H-5′′, CH_2), 3.70-3.64 (m, 2H, H-4′′, CH_2), 3.60 (dd, 1H, H-2′), 3.58-3.52 (m, 4H, H-3, H-9a′′, H4), 3.48-3.45 (m, 2H), 3.41-3.38 (m, 1H, Hb), 3.37 (dd, 1H, H-9b′′, $J_{9,8}$ 6.6, $J_{9,9}$ 12.8), 3.30 (dd, obscured, 1H, H-2), 2.78 (dd, 1H, H3$_{eq}$′′, $J_{3eq,4}$ 3.7, $J_{3eq,3ax}$ 13.6), 2.14-2.08 (m, 2H, Hf, Hf′), 2.02 (s, 3H, NHAc), 1.87 (dd, 1H, H-3$_{ax}$′′, $J_{3ax,4}$ ~ 11.8), 1.46-1.40 (m, 2H, Hg, Hg′), 1.36-1.26 (m, 20H), 0.90 (t, 3H, J ~ 7). ^{13}C NMR (151 MHz; CD_3OD) δ_C: 175.5, 172.7*, 137.0, 128.4, 105.2 (C1′), 103.9 (C1), 100.3* (C2′′), 80.9, 78.0, 77.1, 76.8, 76.5, 75.2, 74.7, 71.9, 71.0, 70.94, 70.91, 69.5, 68.9, 67.3(C1$_S$), 62.6, 61.9, 56.9, 55.4 (C9′′), 53.9 (C5′′), 41.5 (C3′′), 33.5, 33.2, 30.96, 30.95 (mult. C), 30.92, 30.91, 30.8, 30.65, 30.55, 30.33, 23.9, 22.8 (NHAc), 14.6 (C1$_S$).ESI HRMS (m/z) calculated for$C_{41}H_{74}N_5O_{19}$: 940.4978. Found: 940.4996 [M+H].

*O-[(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid]-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2S, 3R, 4E)-2-(1-adamantaneacetamido)-octadec-4-ene-1,3-diol(**12**)*

N-Succinimidyl adamantan-1-yl acetic acid (0.005 g; 18 μmol) and the ammonium salt **11** (0.0075g; 7 μmol) were combined and dissolved in *N*,*N*-dimethylformamide (0.50 mL). To this solution was added triethylamine (1.0 μL) and a small amount of *N*,*N*-dimethylaminopyridine. The mixture was stirred overnight. After 18 hours additional activated ester (0.008 g; 27 μmol) was added. After a total of 42 hours the reaction mixture was diluted with 7.5 mL of deionized water. Precipitate was removed by centrifugation before the supernatant was applied to a preconditioned Waters tC18 SepPak (2g). Elution with a gradient of H_2O and acetonitrile yielded material that was further purified on a Waters QMA Light anion exchange cartridge (130 mg sorbent weight; gradient elution with ammonium formate, 100 mM-500 mM) to give, after repeated lyophilization, **12** (0.005 g; 5μmol; 67%).^{1}H NMR (600 MHz; CD_3OD) δ_H: 5.74-5.68 (m, 1H, He), 5.50-5.45 (m, 1H, Hd), 4.42 (d, 1H, H1′, $J_{1,2}$ 7.6), 4.30 (d, 1H, H-1, $J_{1,2}$ 7.8), 4.17-4.13 (m, 1H, Ha), 4.08 (dd, 1H, Hc), 4.07-4.01 (m, 1H), 4.02-3.97 (m, 1H, Hb), 3.94-3.82 (m, 4H), 3.79-3.73 (m, 2H, H-5′′), 3.69-3.62 (m, 2H), 3.61-3.51 (m, 5H), 3.49 (d, 8.5 Hz), 3.42-3.39 (m, 1H). 3.28 (dd, 1H, H-2, J 8.1, 8.6), 2.79 (s br, 1H, H3$_{eq}$′′), 2.06-2.01 (m, 2H, Hf, Hf′), 2.00 (s, 3H, NHAc), 1.97-1.93 (m, 5H), 1.92-1.88 (m, 1H, H-3$_{ax}$′′), 1.77-1.72 (m, 3H), 1.70-1.66 (m, 3H), 1.66-1.61 (m, 6H), 1.42-1.36 (m, 2H, CH_2), 1.34-1.26 (m, 20H), 0.90 (t, 3H, J ~ 7); ^{13}C NMR (151 MHz; CD_3OD) δ_C: 176.3*, 175.3, 173.7, 135.1, 131.4, 105.1 (C1′), 104.6 (C1), 80.8, 77.8, 76.9, 76.4, 76.2, 75.2, 74.8, 72.8, 72.7, 70.7, 70.1, 69.4, 68.9, 64.7, 62.5, 61.8, 54.9, 53.7, 52.0, 43.8, 41.4, 37.9, 34.0, 33.4, 33.1, 30.8 (mult. C), 30.76, 30.71, 30.5, 30.4, 30.3, 30.2, 23.7, 22.6, 14.5. ESI HRMS (m/z) calculated for$C_{51}H_{78}N_2NaO_{24}$: 1125.4842. Found: 1125.4810 [M+Na].

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*O-[(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onic acid]-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2S, 3R, 4E)-2-(1-adamantaneacetamido)-octadec-4-ene-1,3-diol (**13**)*

N-Succinimidyl7-hydroxycoumarin-3-carboxylate (0.008 g; 26 μmol) and the ammonium salt **10** (0.011g; 11 μmol) were combined and dissolved in *N*,*N*-dimethylformamide (0.75 mL). To this solution was added triethylamine (0.8 μL) and a small amount of *N*,*N*-dimethylaminopyridine. The mixture was protected from the light and stirred overnight. After 24 hours additional *N*-succinimidyl7-hydroxycoumarin-3-carboxylate (0.006 g; 20 μmol) and triethylamine (1.0 μL) were added. After a total of 40 hours the reaction mixture was diluted with 7 mL of deionized water and applied to a preconditioned Waters tC18 SepPak (2g). Elution with a gradient of H_2O and acetonitrile yielded material that was further purified on a Waters QMA Light anion exchange cartridge (130 mg sorbent weight; gradient elution with ammonium formate, 100 mM-500 mM) to give, after repeated lyophilization, **13** (0.009 g; 8 μmol; 74%).^{1}H NMR (600 MHz; CD_3OD) δ_H: 8.76 (s, 1H), 7.67 (d, 1H, J 8.6), 6.88 (dd, 1H, J 2.2, 8.6), 6.77 (d, 1H, J 2.2), 5.77-5.70 (m, 1H, He), 5.54-5.48 (m, 1H, Hd), 4.43 (d, 1H, H-1′, $J_{1,2}$7.8), 4.35 (d, 1H, H-1, $J_{1,2}$ 7.8), 4.28 (dd, 1H, Hc, J ~7.1), 4.26-4.23 (m, 1H, Hb), 4.14 (dd, 1H, J 5.5, 10.4), 4.05 (dd, 1H, H-3′, $J_{3,4}$ 3.0, $J_{3,2}$ 9.8), 3.94 (dd, 1H, CH_2, J 2.5, 12.2), 3.91-3.83 (m, 4H), 3.80-3.74 (m, 2H), 3.72-3.69 (m, 2H, H-5′), 3.65 (dd, 1H, J 4.0, 11.6), 3.63-3.55 (m, 5H), 3.54 (dd, 1H, J 8.6, 8.8), 3.48 (d br, 1H, J 8.9), 3.44-3.41 (m, 1H), 3.28 (dd, 1H, H-2, J 8.2, 8.6), 2.86 (d br, 1H, $H3_{eq}$′′, J ~ 11.5), 2.09-2.03 (m, 2H, Hg, Hg′), 2.01 (s, 3H, NHAc), 1.73 (ddb r, J~ 11.5), 1.33-1.10 (m, 22H), 0.90 (t, 3H, CH_3). ^{13}C NMR (151 MHz; CD_3OD) δ_C: 175.5, 166.1, 164.4, 163.2, 158.3, 149.9, 135.4, 133.0, 130.7, 115.9, 114.4, 112.7, 105.1, 104.6, 103.1, 80.9, 77.7, 77.0, 76.5, 76.2, 74.9, 74.6, 72.96, 72.94, 70.9, 70.1, 69.8, 69.4, 69.8, 69.4, 69.0, 64.6, 62.7, 61.9, 55.6, 53.9, 42.1, 34.8, 33.4, 33.1, 30.82, 30.76 (mult. C) 30.73, 30.5, 30.4, 30.2, 23.8, 22.6, 14.5. ESI HRMS (m/z) calculated for $C_{53}H_{89}N_2O_{21}$: 1089.5963. Found: 1089.5958 [M-H].

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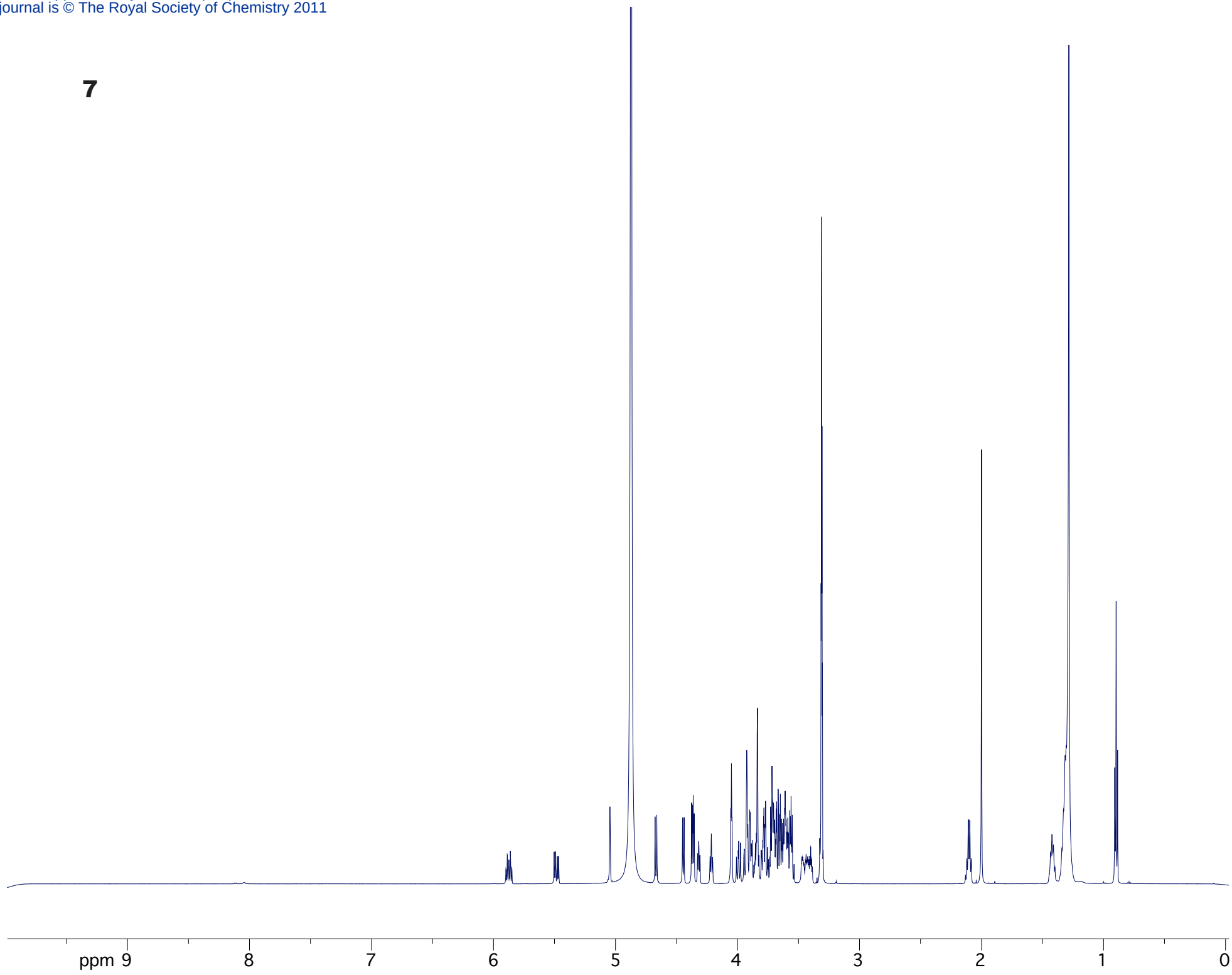

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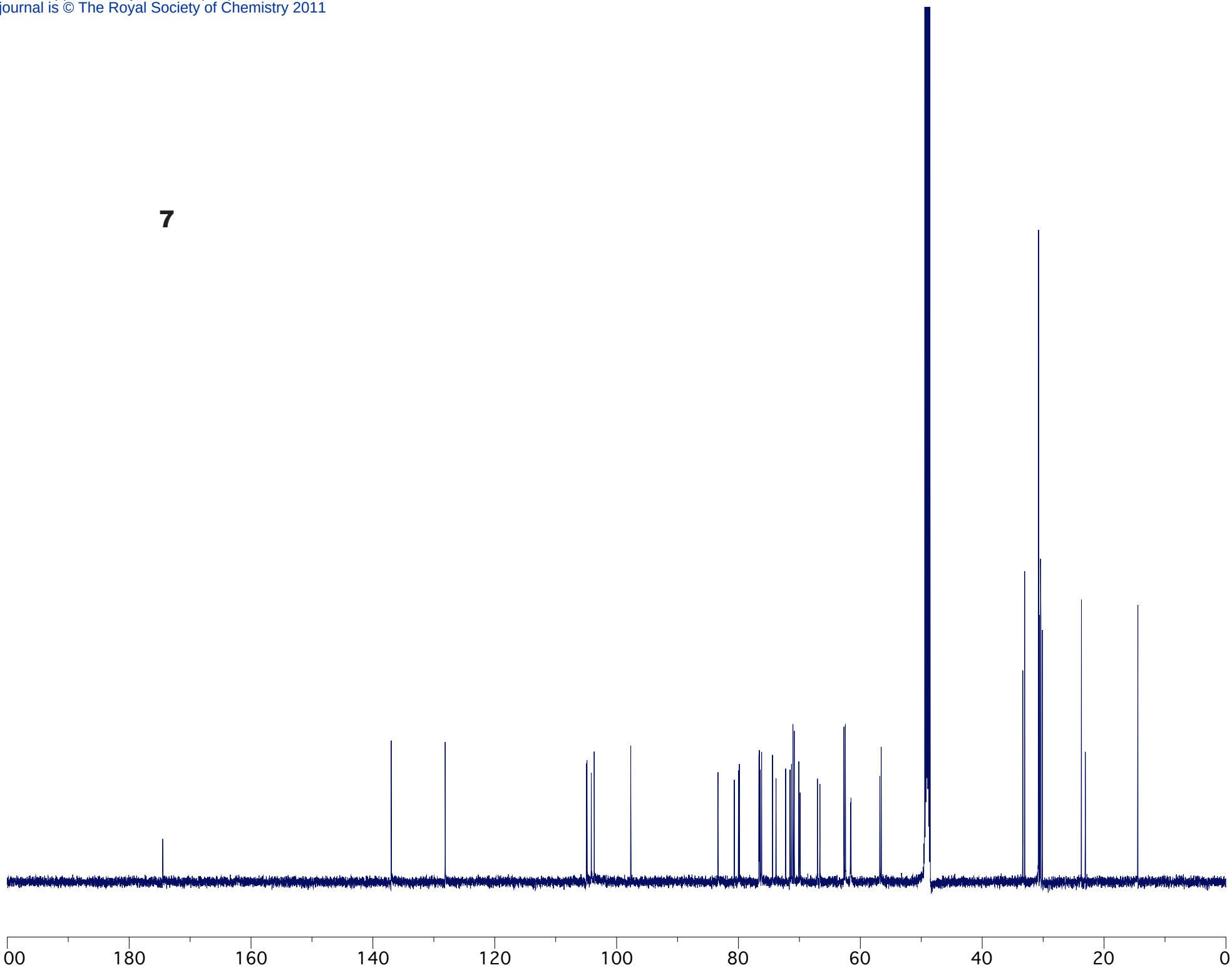

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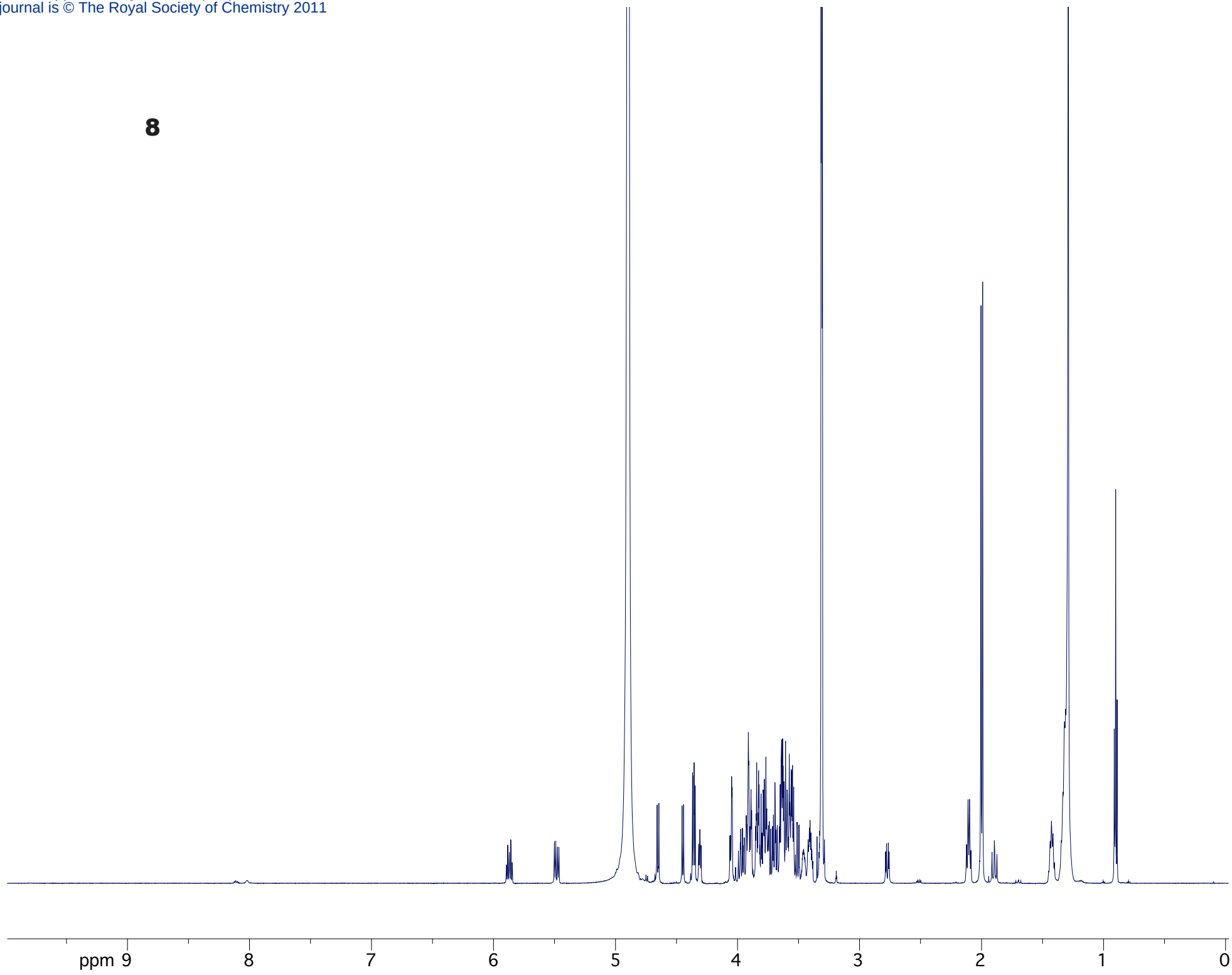

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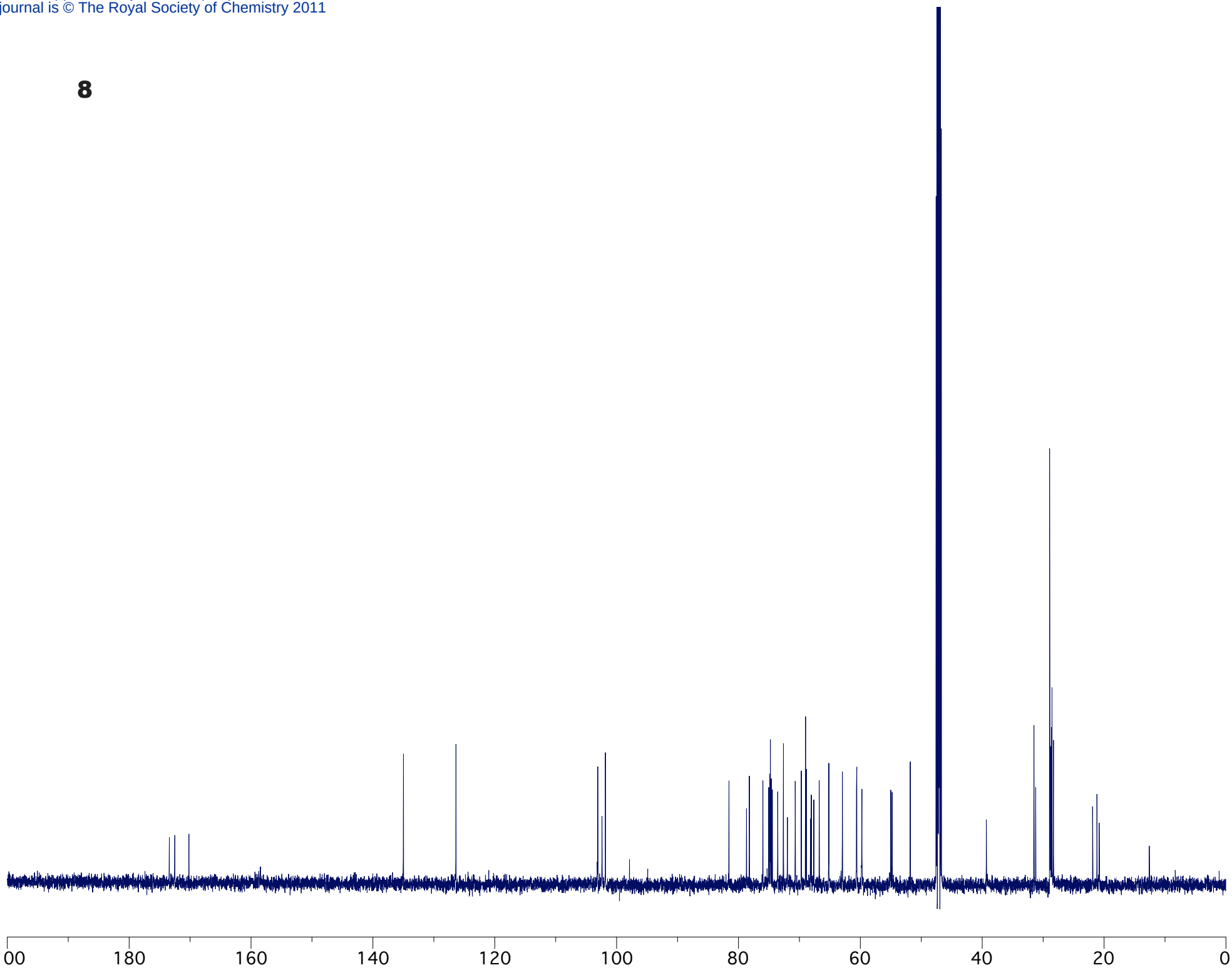

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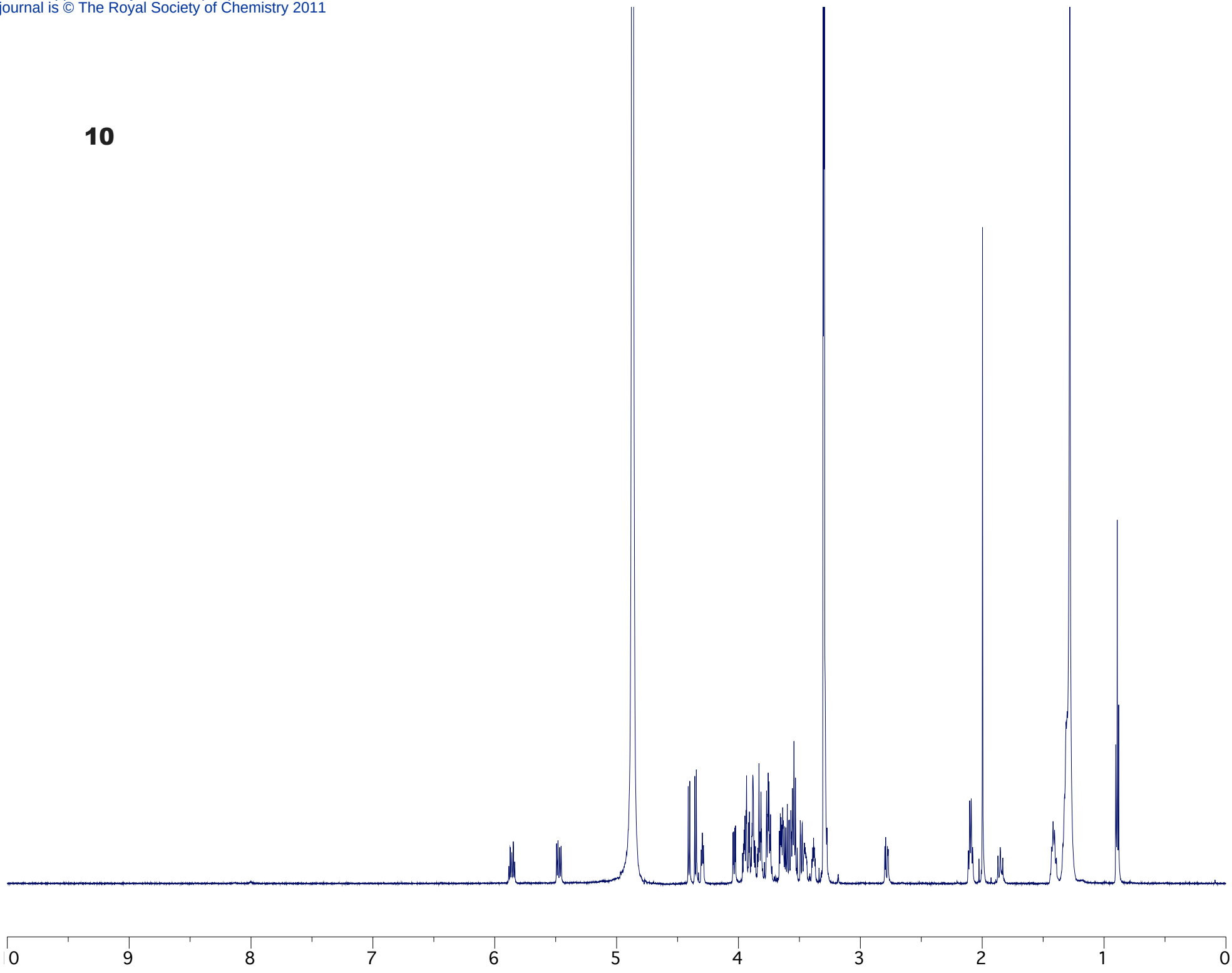

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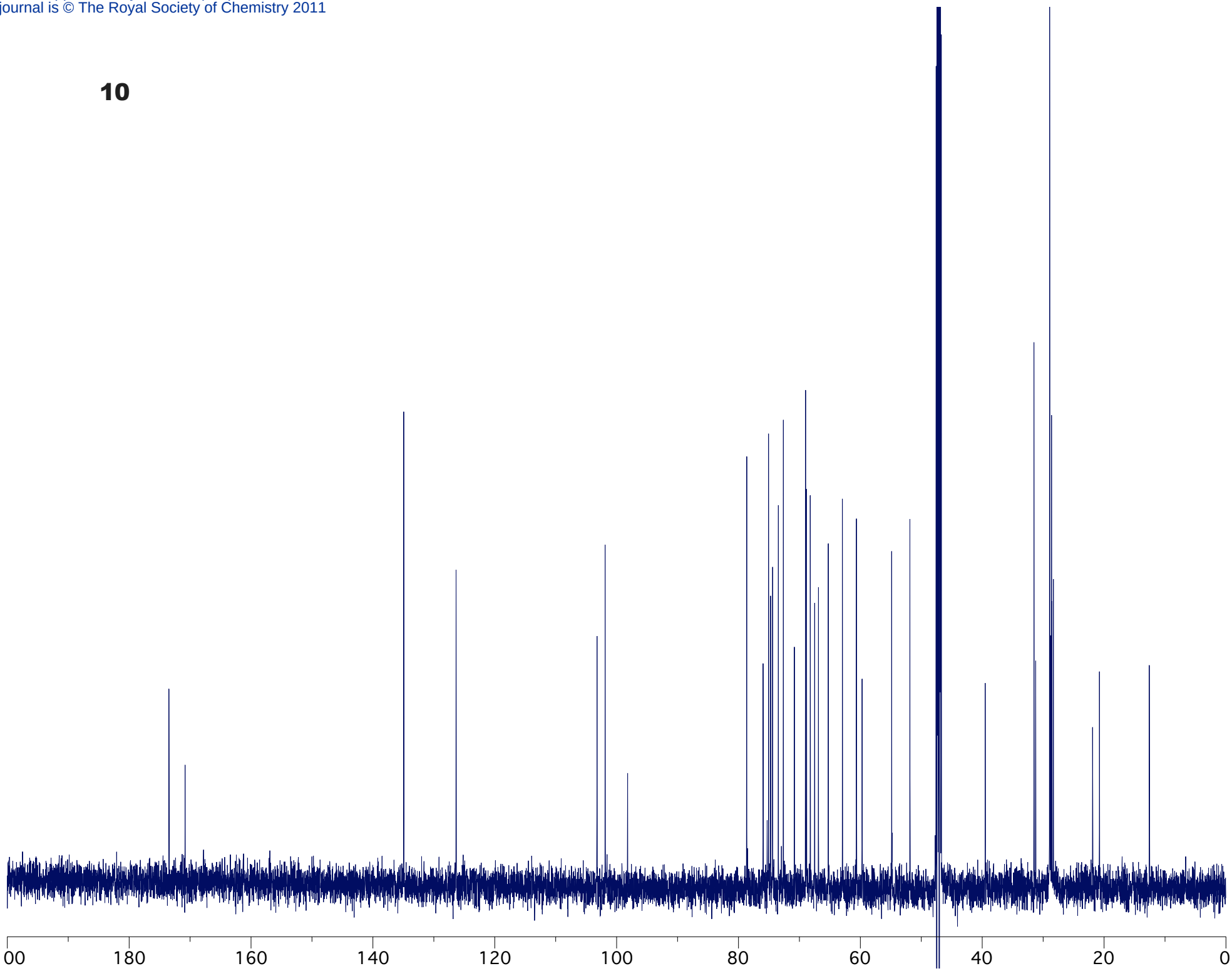

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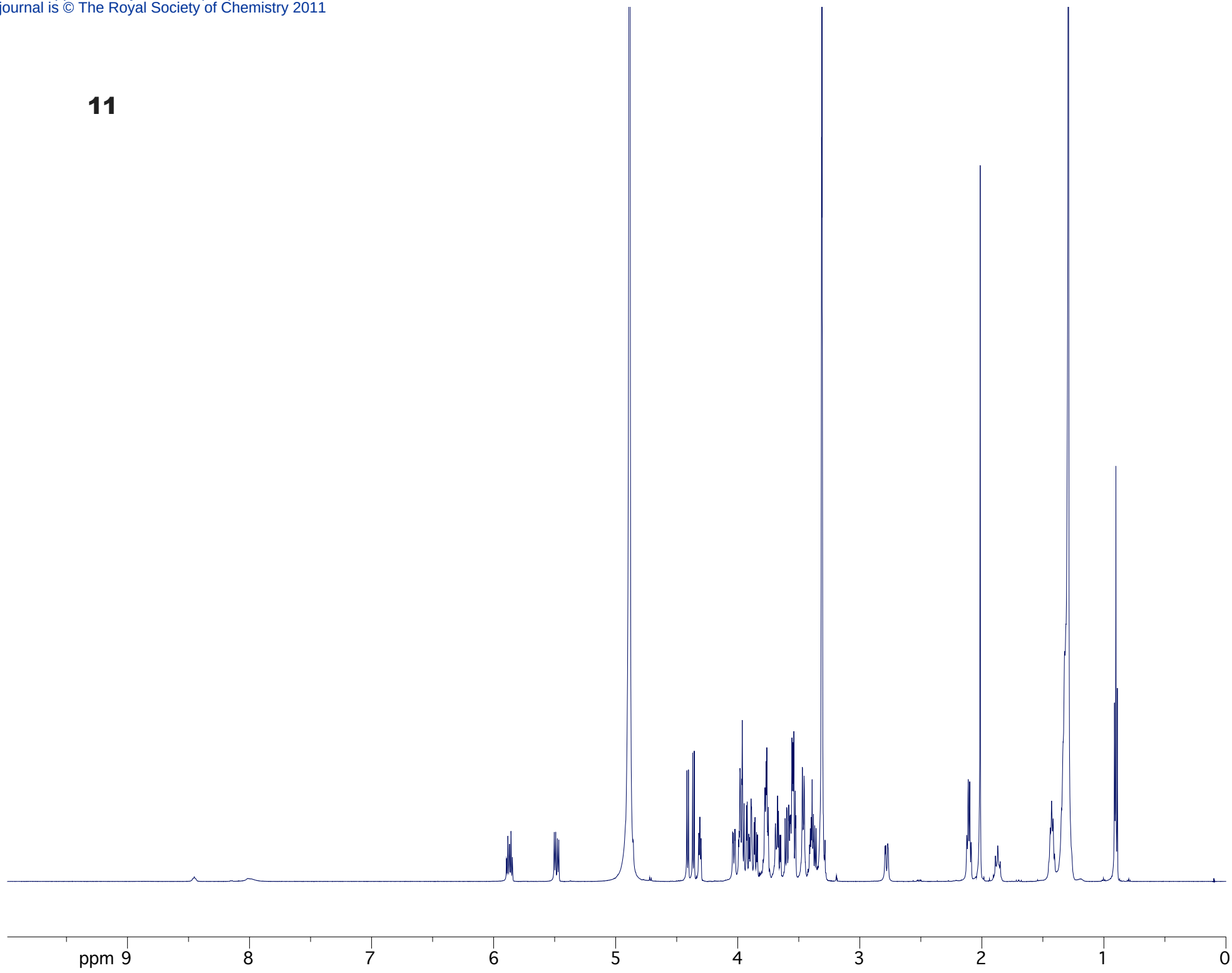

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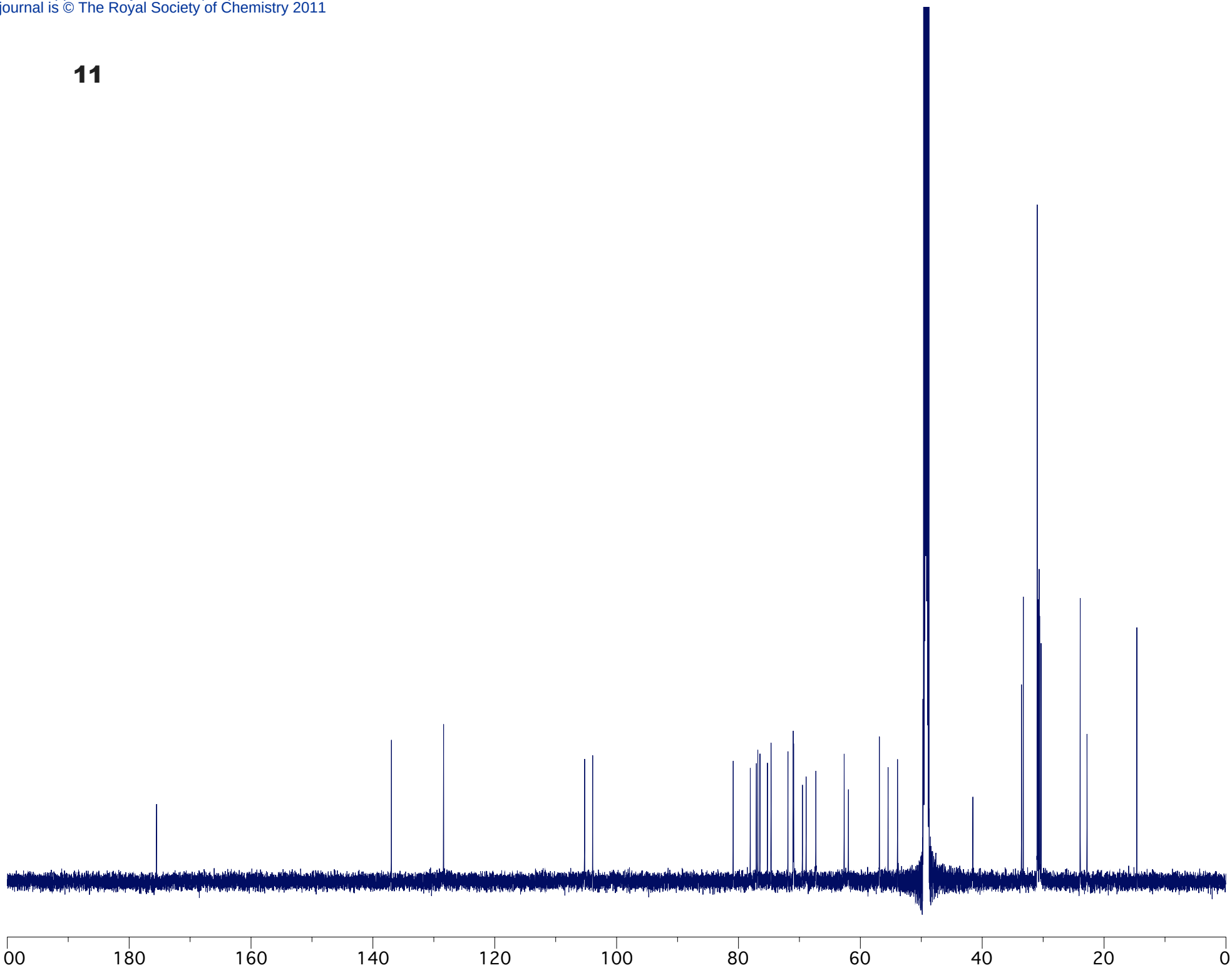

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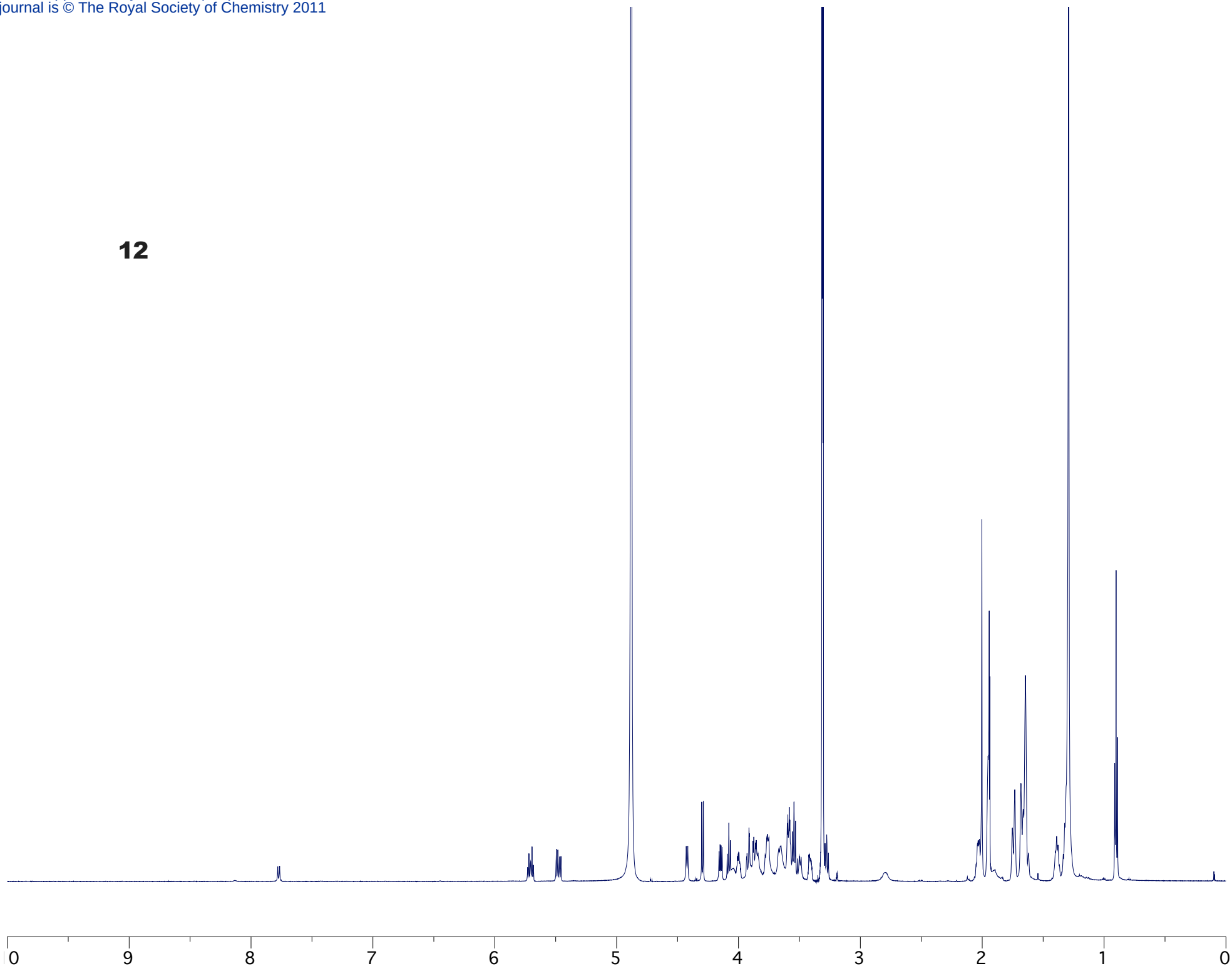

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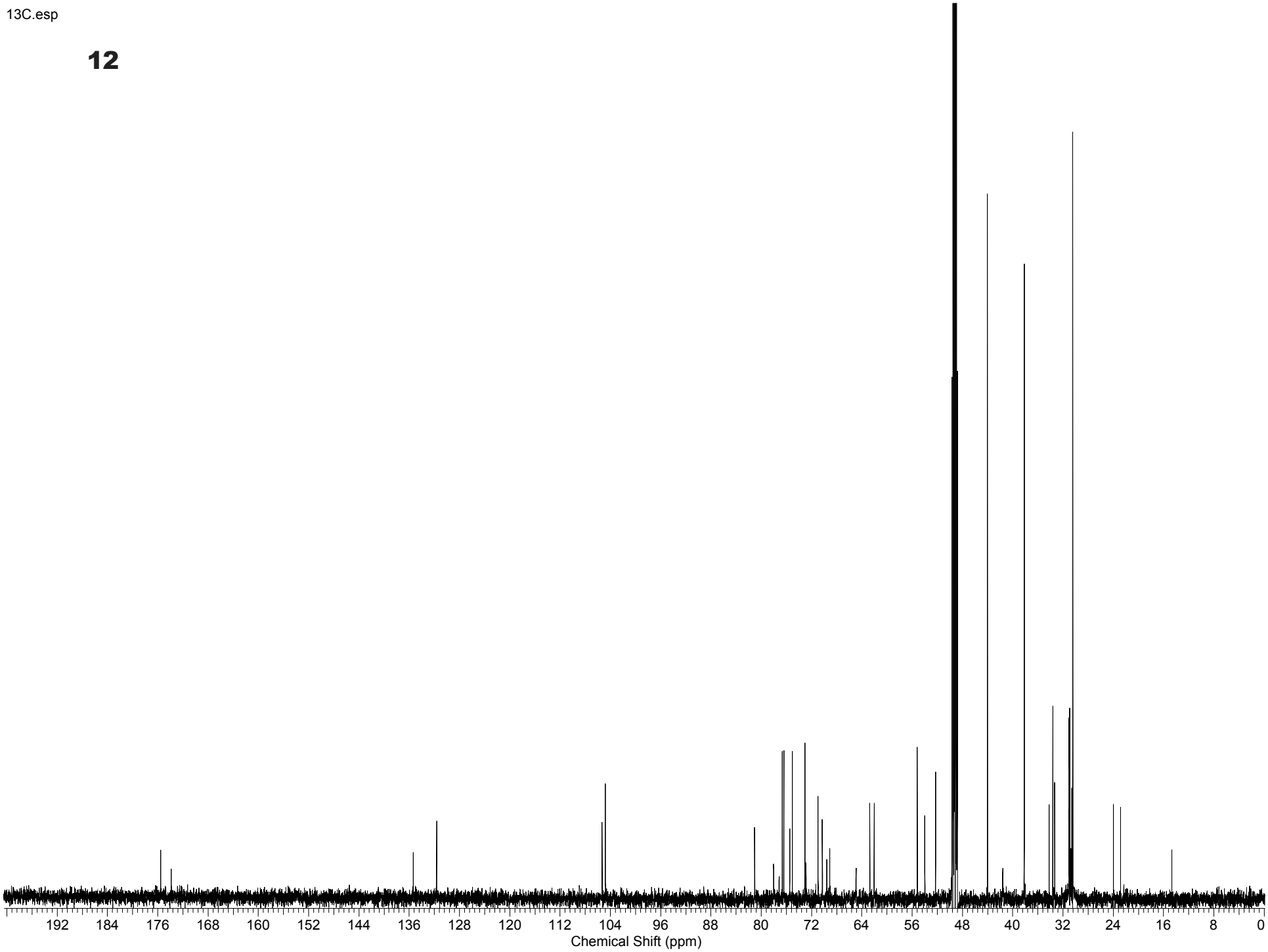

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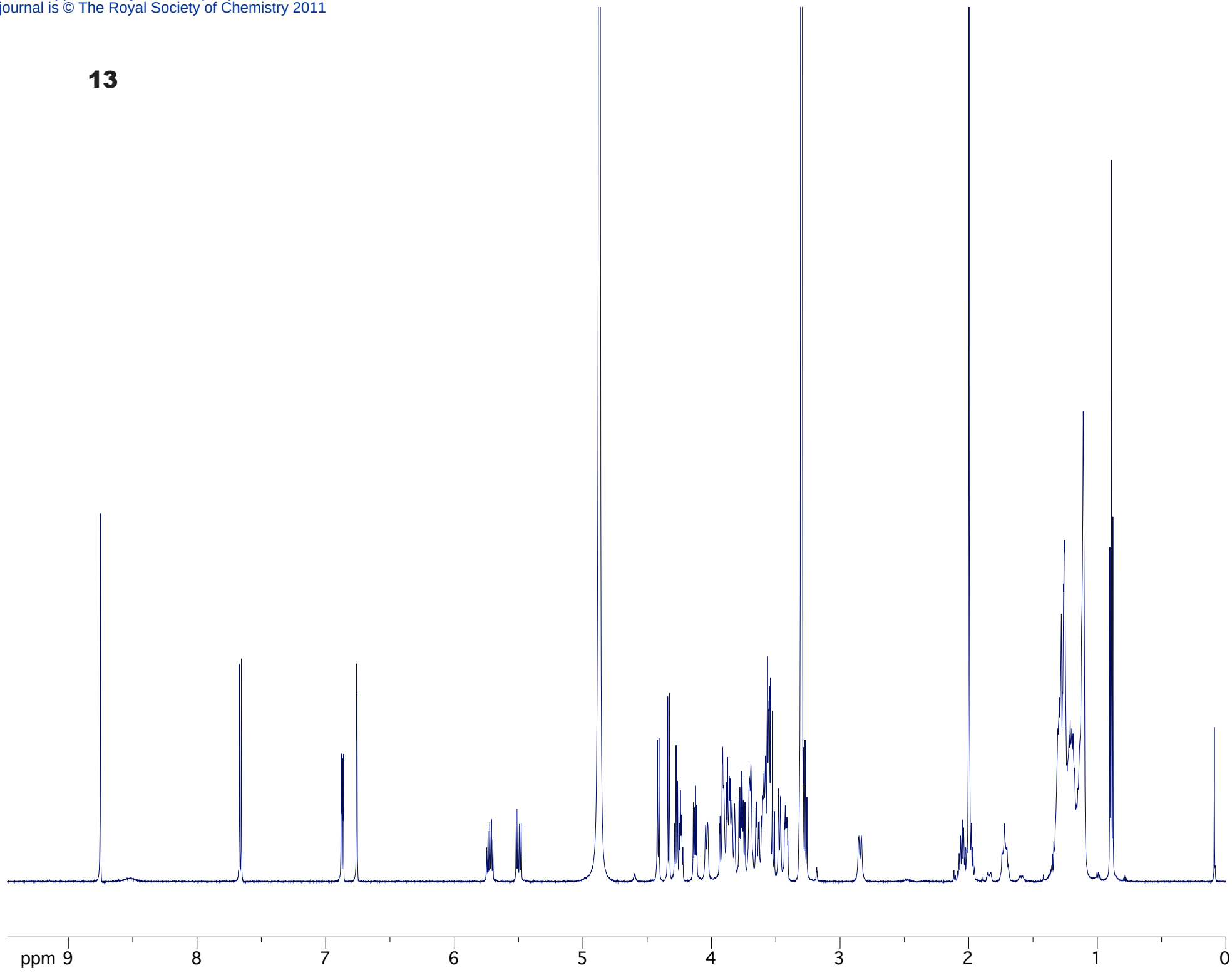

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