Supporting Information

A Chemoenzymatic Route to *N*-acetyl-Glucosamine-1-Phosphate Analogues: Substrate Specificity Investigations of *N*-Acetylhexosamine 1-Kinase

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Contents:

A. Materials and Chemical Reagents. Restriction enzymes were purchased from New England Biolabs (Beverly, MA). Quick-stick ligase was purchased from Bioline (London, United Kingdom). QIAprep miniprep kit was purchased from Qiagen (Santa Clarita, CA). Illustra GFX PCR DNA and gel band purification kit, HisTrap HP column (5 mL) and HiTrap Desalting column (5mL) were purchased from GE healthcare (Piscataway, NJ). Isopropyl-1-thio-β-D-galactopyranoside (IPTG) was purchased from Acros Organics (Geel, Belgium). Adenosine 5′-triphosphate disodium salt hydrate (ATP), D-Glucosamine hydrochloride, *N*-acetylmuramic acid, imidazole and Dowex 50WX8-400 ion exchange resin (hydrogen form) were purchased from Sigma-Aldrich. *N*-propionyl-D-glucosamine was purchased from TCI America. Amicon Ultra 10,000 MWCO was purchased from Millipore (Billerica, MA). Unless indicated, all commercial reagents were used as received without further purification. All reaction solvents were purified before use.

B. Bacterial Strains and Plasmids. *Escherichia coli* DH5α [*lacZDM15 hsdR recA*] was purchased from Gibco-BRL (Gaithersburg, MD). BL21 (DE3) [F^- *ompT* $hsdS_B(r^-_B m^-_B)$ *gal dcm* (DE3)] and plasmid pET22b were purchased from Novagen (Carlsbad, CA). Plasmid pCR2.1-BL1642 was kindly provided by Dr. Kitaoka in National Food Research Institute of Japan.

C. Construction of Recombinant Plasmid. The *nahK* gene from *Bifidobacterium longum* JCM1217 was cut out from plasmid pCR2.1-BL1642 with *Nde*I and *Xho*I, and ligated into pET22b at *Nde*I/*Xho*I sites. The recombinant plasmid was transformed into *E. coli* DH5α cells for DNA sequencing. After sequence confirmation, the plasmid was transformed into *E. coli* BL21 (DE3) for protein expression.

D. Overexpression and Purification of NahK. *E. coli* BL21 (DE3) transformant containing the pET22b-*nahK* plasmid was grown in LB broth containing 100 μg/ml ampicillin at 37°C. When OD_{600} reached 0.7, IPTG was added to a final concentration of 0.1 mM to induce protein to express at 16°C for 20 h. The cells were harvested by centrifugation at 4,000 × *g* for 30 min. The cell pellet was resuspended in 20 mM sodium phosphate (pH 7.4) and sonicated on ice. The lysate was clarified by centrifugation at 20,000 × *g* for 40 min at 4°C and the supernatant was applied to HisTrap™ HP column. The column was then washed with washing buffer (20 mM sodium phosphate, pH 7.4, 100 mM NaCl, 10 mM imidazole) and the enzyme was eluted with elution buffer (20 mM sodium phosphate, pH 7.4, 100 mM NaCl, 500 mM imidazole). The eluant was desalted by HiTrap™ Desalting HP column with 50 mM Tris-HCl buffer, pH 7.5. The fractions containing the purified enzyme were combined and concentrated with Amicon Ultra and the enzyme was stored in 20% glycerol (50 mM Tris-HCl, pH 7.5) at -20°C.

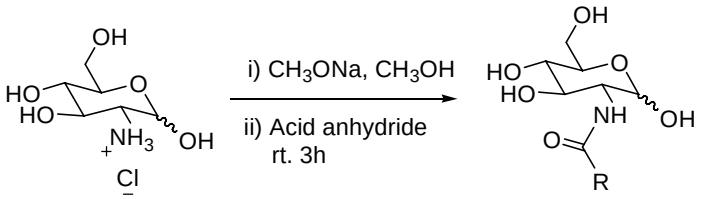
E. Activity Measurement. The activity of recombinant NahK was assayed in a cocktail containing 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 5 mM GlcNAc, 5 mM ATP, and various amount of NahK incubated at 37°C for 30 min. The reaction was terminated by boiling the mixture for 2 min followed by centrifugation. The supernatant was analyzed

by normal phase silica gel thin-layer chromatography [n-butanol/acetic acid/water = 2:1:1 (v/v/v)].

F. Phosphorylation of GlcNAc and its analogues. The reaction mixture (10 mL) contained 40 mM GlcNAc or its analogues, 50 mM ATP, 10 mM $MgCl_2$, and 1.5 mg/mL NahK in 100 mM Tris-HCl buffer (pH 9.0). After incubation at 37°C for 19 h, the mixture was briefly boiled for 2 min and then centrifuged to remove protein. The supernatant was concentrated under reduced pressure and the residue was purified by normal phase silica gel column chromatography using gradient CH_2Cl_2/5 mM NH_4HCO_3 in methanol as eluent (CH_2Cl_2/5 mM NH_4HCO_3 in methanol 1:1 to 0:1). The fractions containing the products were collected and concentrated under reduced pressure.

G. Physical Properties and Spectroscopic Measurements. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 (^{13}C NMR) spectra were recorded on a Bruker DPX 400 spectrometer at 400 MHz and 100 MHz respectively, or on a Bruker DRX 500 spectrometer at 500 MHz and 125 respectively. Chemical shifts and coupling constants were reported in ppm and Hz respectively. The high-resolution mass spectra were recorded on a Bruker MicrOTOF spectrometer.
Analytical TLC was carried out on silica gel 60 F254 aluminum-backed plates (E. Merck). The 200–400 mesh size of the same absorbent was utilized for all chromatographic purifications. Unless noted, all compounds isolated by chromatography were sufficiently pure by ^{1}H NMR analysis for use in subsequent reactions.

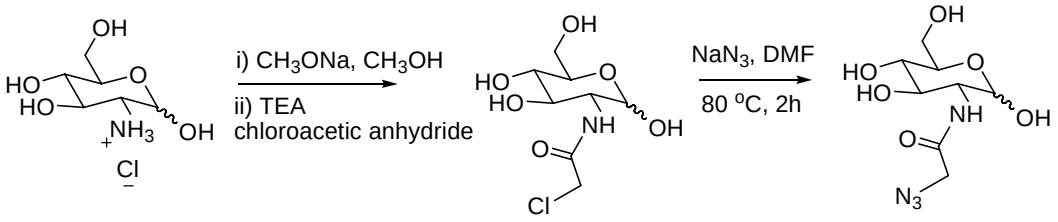
H. Synthetic Experimental



4: R = Pr
5: R= Ph

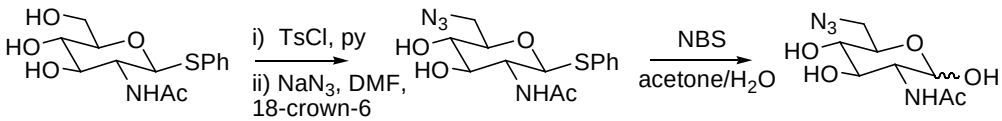
2-Butyramido-2-deoxy-D-glucopyranose (4): D-Glucosamine hydrochloride (1 g, 4.637 mmol) was dissolved in 5 mL anhydrous methanol in which an equivalent amount of sodium had been added. The mixture was stirred for 10 min before butyric anhydride (0.99 mL, 6.075 mmol) was added and the resulting reaction mixture was allowed to stir at room temperature for approximately 3 h, the precipitate was filtered off and washed with dry methanol. The combined filtrates and washings were evaporated under reduced pressure and purified by silica gel column chromatography (EtOAc:2-propanol:water 9:3:1) to give **4** as a white solid (700 mg, 61%). NMR data see ref 1.

2-Benzamido-2-deoxy-D-glucopyranose (5): Compound **5** was prepared as described for compound **4**, white solid (53% yield). NMR data see ref 2.



12 **2**

2-azidoacetamido-2-deoxy-D-glucopyranose (2): Glucosamine hydrochloride (10 g, 45 mmol) was dissolved in MeOH (200 mL) and 1MNaOMe in MeOH was added (45 mL, 45 mmol). The reaction mixture was stirred at rt for 1 h at which point TEA (0.14 mL, 0.93 mmol) and chloroacetic anhydride (25 g, 146 mmol) were added. The reaction mixture was stirred for another 6 h. Removal of solvent provided chloro-intermediate **12** which was partially purified by silica gel chromatography eluting with a gradient of CH_2Cl_2:MeOH (20:1 to 7:1). The resulting yellow oil was dissolved in DMF (100 mL), NaN_3 (30 g, 465 mmol) was added and the reaction mixture was heated to 80 °C for 2 h. Upon cooling to rt, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 7:1 to 5:1) to give **2** as a white solid. NMR data see ref 3.

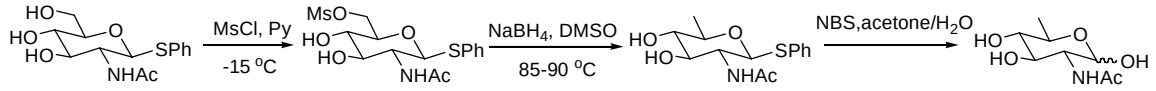


13 **16** **7**

Phenyl 2-Acetamido-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranoside (16): Thio glycoside **13** (626 mg, 2 mmol) was dissolved in anhydrous pyridine (10 mL) and a solution of p-toluenesulfonyl chloride (380 mg, 2 mmol) in CH_2Cl_2 (4.0 mL) was added at 0 °C and the reaction mixture was allowed to stir at room temperature. After 2h, another amount of p-toluenesulfonyl chloride (380 mg, 2 mmol) was added. The reaction was monitored by TLC and was diluted by EtOAc (100 mL) at completion and then washed with 1N HCl (50 mL), sa. $NaHCO_3$ (50 mL). and brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated under *vacuo*. The residue was redissolved in anhydrous DMF (10 mL) and treated with NaN_3 (520 mg, 8 mmol) and 18-crown-6 (60 mg). The resulting mixture was stirred at 40-50 °C for 48 h before the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 25:1 to 20:1) to give **16** as a white solid (520 mg, 77% over two steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.52-7.54 (m, 2H), 7.28-7.34 (m, 3H), 4.80 (d, *J* = 10.4 Hz, 1H), 3.76 (t, *J* = 10.0 Hz, 1H), 3.58 (m, 1H), 3.40-3.50 (m, 3H), 3.31 (m, 1H, in solvent peak), 2.03 (s, 3H), ^{13}C NMR (100 MHz, CD_3OD) δ 173.5, 134.9, 133.2, 129.9, 128.6, 88.2, 80.5, 77.2, 72.6, 56.2, 53.0, 23.0; HRMS (ESI) calcd for $C_{14}H_{18}N_4O_4SNa$ $(M+Na)^+$ 361.0941, found 361.0929 *m*/*z*.

2-Acetamido-6-azido-2,6-dideoxy-D-glucopyranose (7): Compound **16** (208 mg, 0.612 mmol) was dissolved in acetone/H_2O (v/v 9:1 10 mL) and treated with *N*-bromosuccinimide (NBS) (240 mg, 1.347 mmol) and the reaction was stirred at room temperature for 1 h. Upon completion, $NaHCO_3$ was added to neutralize the reaction and

the solvent was removed and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 25:1 to 20:1) to give **7** as a white solid (α and β mixture, 130 mg, 86%). NMR data see ref 4.

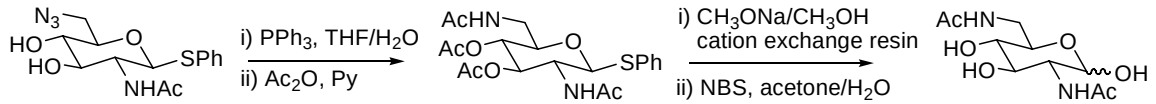


13 **14** **15** **6**

Phenyl 2-Acetamido-2-deoxy-6-O-methylsulfonyl-1-thio-β-D-glucopyranoside (14): Compound **13** (476 mg, 1.52 mmol) was dissolved in anhydrous pyridine (8 mL) and the solution was cooled to -15 °C. Mesyl chloride (125 μL, 1.60 mmol) was added dropwise. After 2h, the reaction was quench by adding methanol and the solvent was removed and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 20:1 to 15:1) to give **14** as a white solid (438 mg, 74%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.48-7.50 (m, 2H), 7.26-7.32 (m, 3H), 4.85 (d, 1H, in H_2O peak), 4.54 (dd, *J* = 11.3, 1.8 Hz, 1H), 4.37 (dd, *J* = 11.3, 6.0 Hz, 1H), 3.77 (t, *J* = 10.0, 1H), 3.54-3.58 (m ,1H), 3.50 (t, *J* = 8.9 Hz, 1H), 3.31-3.38 (m, 1H), 3.03 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.7, 135.4, 132.7, 130.2, 128.6, 88.2, 79.2, 77.3, 71.6, 71.0, 56.3, 37.7, 23.1; HRMS (ESI) calcd for $C_{15}H_{21}NO_7S_2Na$ $(M+Na)^+$ 414.0652, found 414.0636 *m*/*z*.

Phenyl 2-Acetamido-2,6-dideoxy-1-thio-β-D-glucopyranoside (15): Compound **14** (100 mg, 0.255 mmol) was dissolved in dry DMSO (5 mL) and $NaBH_4$ (94 mg, 25.5 mmol) was added. The reaction was heated at 85-95 °C for 18 h. The reaction was quenched by adding 5% acetic acid solution at 0 °C and the solution was diluted with more water and extracted several times with ethyl acetate (200 mL total). The combined extracts were evaporated under *vacuo* and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 20:1 to 15:1) to give **15** as a white solid (66 mg, 87%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.43-7.47 (m, 2H), 7.25-7.32 (m, 3H), 4.74 (d, *J* = 10.3 Hz, 1H), 3.75 (t, *J* = 10.0 Hz, 1H), 3.42 (dd, *J* = 9.7, 8.9 Hz, 1H), 3.32 (m, 1H, in solvent peak), 3.06 (t, *J* = 9.1 Hz, 1H), 1.99 (s, 3H), 1.30 (d, *J* = 6.2, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.7, 135.7, 132.6, 130.0, 128.5, 88.2, 77.6, 77.3 (two carbons), 56.7, 23.1, 18.5; HRMS (ESI) calcd for $C_{14}H_{19}NO_4SNa$ $(M+Na)^+$ 320.0927, found 320.0924 *m*/*z*.

2-Acetamido-2,6-dideoxy-D-glucopyranose (6): The thio glycoside was deprotected as described for compound **7**, white solid (α and β mixture, 90%). NMR data see ref 5.

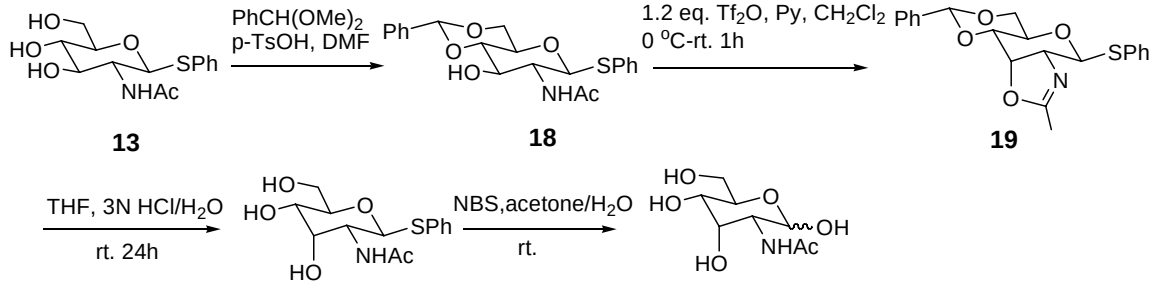


16 **17** **8**

Phenyl 2,6-di-Acetamido-2,6-dideoxy-3,4-di-O-acetyl-1-thio-β-D-glucopyranoside (17): To a solution of compound **16** (100 mg, 0.296 mmol) in THF (5 mL), was added Ph_3P (85 mg, 0.325mmol) and water (80 μL). The mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure. The residue was dissolved in 5 mL anhydrous pyridine and acetic anhydride 1.0 mL was added. The mixture was stirred at room temperature for overnight. Upon completion, the solution was diluted by 50 mL

EtOAc and washed H_2O, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 50:1 to 40:1) to give **17** as a white foam (94 mg, 72 % over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.45 (m, 2H), 7.24-7.27 (m, 3H), 6.00-6.06 (m, 2H), 5.17 (app t, J = 10.0 Hz, 1H), 4.87-4.93 (m, 2H), 4.13-4.20 (m, 1H), 3.60-3.71 (m, 2H), 3.11-3.17 (m, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.4, 170.3, 170.0, 133.0, 132.2, 129.2, 128.1, 86.8, 76.7, 73.8, 69.7, 53.5, 40.2, 23.5, 23.3, 20.9 (two carbons); HRMS (ESI) calcd for $C_{20}H_{26}N_2O_7SNa$ $(M+Na)^+$ 461.1353, found 461.1332 *m*/*z*.

2,6-di-Acetamido-2,6-dideoxy-D-glucopyranose (8): Compound **17** (85 mg, 0.194 mmol) was dissolved in anhydrous methanol (5 mL) and the solution of MeONa in methanol (0.5 M, 0.3 mL) was added. The reaction solution was stirred at room temperature for 2h. Cation exchange resin (H form) was added to adjust the pH to ~7 and the resin was removed by filtration and washed several times with methanol. The combined filtrates were concentrated under reduced pressure and the resulting residue was dissolved in acetone/H_2O (v/v 9:1 5 mL) and treated with N-bromosuccinimide (76 mg, 0.426 mmol) and the reaction was stirred at room temperature for 2 h. Upon completion, the solvent was removed and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 10:1 to 5:1) to give **8** as a white solid (α and β mixture, 32 mg, 63% over two steps). ^{1}H NMR (400 MHz, D_2O, α and β mixture) δ 5.22 (d, J = 3.5 Hz, 1H, α anomeric H), 4.73 (d, J = 8.5 Hz, 0.7H, β anomeric H), 3.90-3.96 (m, 2H), 3.37-3.81 (m, 8H), 2.06-2.09 (m, 10H); HRMS (ESI) calcd for $C_{10}H_{18}N_2O_6Na$ $(M+Na)^+$ 285.1057, found 285.1052 *m*/*z*. Also see ref 6.



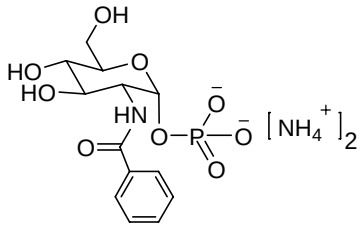
13 **18** **19** **20** **11**

Phenyl 4,6-O-Benzylidene-2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (18): To a solution of compound **13** (330 mg, 1.053 mmol) in dry DMF (5 mL) was added benzaldehyde dimethyl acetyl (0.32 mL, 2.11 mmol) and *p*-toluenesulfonic acid (20 mg, 1.053 mmol), and the solution was stirred for 13 hrs at rt. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 40:1) to give **18** as a white solid (360 mg, 85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.98 (d, J = 9.0 Hz, 1H), 7.31-7.45 (m, 9H), 7.23-7.26 (m, 1H), 5.61 (s, 1H), 5.42 (d, J = 5.5 Hz, 1H), 4.97 (d, J = 10.2 Hz, 1H), 4.19-4.22 (m, 1H), 3.62-3.74 (m, 3H), 3.46-3.53 (m, 2H), 1.84 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.3, 137.7, 134.5, 129.5, 129.1, 128.9, 128.1, 126.7, 126.4, 100.7, 86.4, 80.9, 71.7, 70.0, 67.7, 54.9, 23.1; HRMS (ESI) calcd for $C_{21}H_{23}NO_5SNa$ $(M+Na)^+$ 424.1189, found 424.1176 *m*/*z*.

Phenyl 4,6-O-Benzylidene-1-thio-β-D-allopyranosido[2,3:4′,5′]-2′-methyl-2′-oxazoline (19): Compound **18** (300 mg, 0.747 mmol) was dissolved in 5 mL anhydrous pyridine and a solution of trifluoromethanesulfonic anhydride (0.15 mL, 0.896 mmol) in CH_2Cl_2 (2 mL) was added at 0 °C. The reaction mixture was stirred at rt. for 1h then was diluted with CH_2Cl_2 (50 mL) and washed with water (2*50 mL). The organic layer was collected and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:EtOAc 4:1 to 3:1) to give **19** as a white solid (195 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50-7.53 (m, 4H), 7.29-7.39 (m, 6H), 5.63 (s, 1H), 4.79-4.82 (m, 2H), 4.39 (dd, *J* = 9.9, 4.6 Hz, 1H), 4.33 (dd, *J* = 9.6, 3.7 Hz, 1H), 4.26-4.29 (m, 1H), 3.83-3.88 (m, 1H), 3.80 (t, *J* = 10.0 Hz, 1H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.3, 137.0, 133.5, 132.1, 129.4, 129.2, 128.5, 128.0, 126.4, 102.8, 86.8, 76.4, 75.2, 69.6, 68.9, 65.3, 14.4; HRMS (ESI) calcd for $C_{21}H_{21}NO_4SNa$ $(M+Na)^+$ 406.1083, found 406.1071 *m*/*z*.

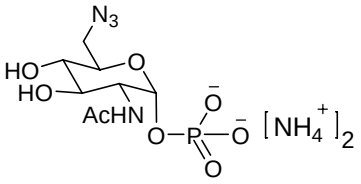
Phenyl 2-Acetamido-2-deoxy-1-thio-β-D-allopyranoside (20): Compound **19** (187 mg, 0.488 mmol) was dissolved in THF (10 mL) and treated with 3N HCl/H_2O. The reaction was stirred at rt. for 24 h and neutralized with $NaHCO_3$. The solvent was removed and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH 10:1 to 5:1) to give **20** as a white solid (140 mg, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43-7.46 (m, 2H), 7.23-7.35 (m, 3H), 5.10 (d, *J* = 4.1 Hz, 1H), 4.93 (d, *J* = 7.0 Hz, 1H), 4.71 (d, *J* = 10.1 Hz, 1H), 4.30 (dd, *J* = 11.9, 1.9 Hz, 1H), 4.04 (dd, *J* = 11.9, 7.0 Hz, 1H), 3.82-3.84 (m, 1H), 3.75-3.79 (m, 1H), 3.29-3.34 (m, 1H), 2.52 (m, 1H, in solvent), 2.02 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 134.2, 130.2, 128.9, 126.7, 86.7, 73.6, 70.8, 67.8, 64.0, 53.8, 20.7; HRMS (ESI) calcd for $C_{14}H_{20}NO_5S$ $(M+H)^+$ 314.1057, found 314.1055 *m*/*z*.

2-Acetamido-2-deoxy-D-allopyranoside (11): Compound **20** was deprotected as described for compound **7**, white solid (pyranose α, β and furanose mixture, 90%). HRMS (ESI) calcd for $C_8H_{16}NO_6$ $(M+H)^+$ 222.0972, found 222.0979 *m*/*z*. NMR data see ref 7.



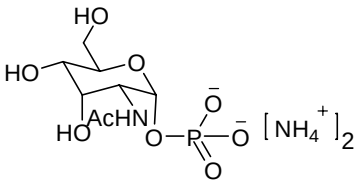
21

2-benzamido-2-deoxy-α-D-glucosyl diammonium Phosphate (21): ^{1}H NMR (400 MHz, D_2O) δ 7.88-7.90 (m, 2H), 7.56-7.69 (m, 3H), 5.58 (dd, *J* = 7.4, 3.3 Hz, 1H), 4.23-4.26 (m, 1H), 3.94-4.06 (m, 3H), 3.86 (dd, *J* = 12.3, 4.7 Hz, 1H), 3.63 (app t, *J* = 9.6 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 171.6, 133.6, 132.2, 128.7, 127.5, 93.2 (d), 72.5, 71.1, 70.0, 60.6, 54.6 (d); ^{31}P NMR (162 MHz, D_2O) δ 0.71; HRMS (ESI) calcd for $C_{13}H_{17}NO_9P$ $(M-H)^-$ 362.0646, found 362.0643 *m*/*z*.



22

2-Acetamido-6-azido-2,6-dideoxy-α-D-glucosyl diammonium Phosphate (22): ^{1}H NMR (400 MHz, D_2O) δ 5.42 (dd, *J* = 7.2, 3.3 Hz, 1H), 4.05-4.09 (m, 1H), 3.97-4.01 (m, 1H), 3.76-3.85 (m, 2H), 3.67 (dd, *J* = 13.6, 4.2 Hz, 1H), 3.59 (app t, *J* = 9.3 Hz, 1H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.7, 93.1 (d), 71.0, 70.9, 70.4, 54.0 (d), 50.8, 22.0; ^{31}P NMR (162 MHz, D_2O) δ 0.60; HRMS (ESI) calcd for $C_8H_{14}N_4O_8P$ $(M-H)^-$ 325.0555, found 325.0559 *m*/*z*.



23

2-Acetamido-2-deoxy-α-D-allosyl diammonium Phosphate (23): ^{1}H NMR (400 MHz, D_2O) δ 5.39 (dd, *J* = 7.7, 3.6 Hz, 1H), 4.02-4.13 (m, 3H), 3.94 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.79 (dd, *J* = 12.3, 5.3 Hz, 1H), 3.70 (dd, *J* = 10.5, 3.4 Hz, 1H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.1, 92.3 (d), 69.9, 67.3, 66.1, 60.9, 50.3 (d), 22.0; ^{31}P NMR (162 MHz, D_2O) δ 2.17; HRMS (ESI) calcd for $C_8H_{15}NO_9P$ $(M-H)^-$ 300.0490, found 300.0481 *m*/*z*.

References:

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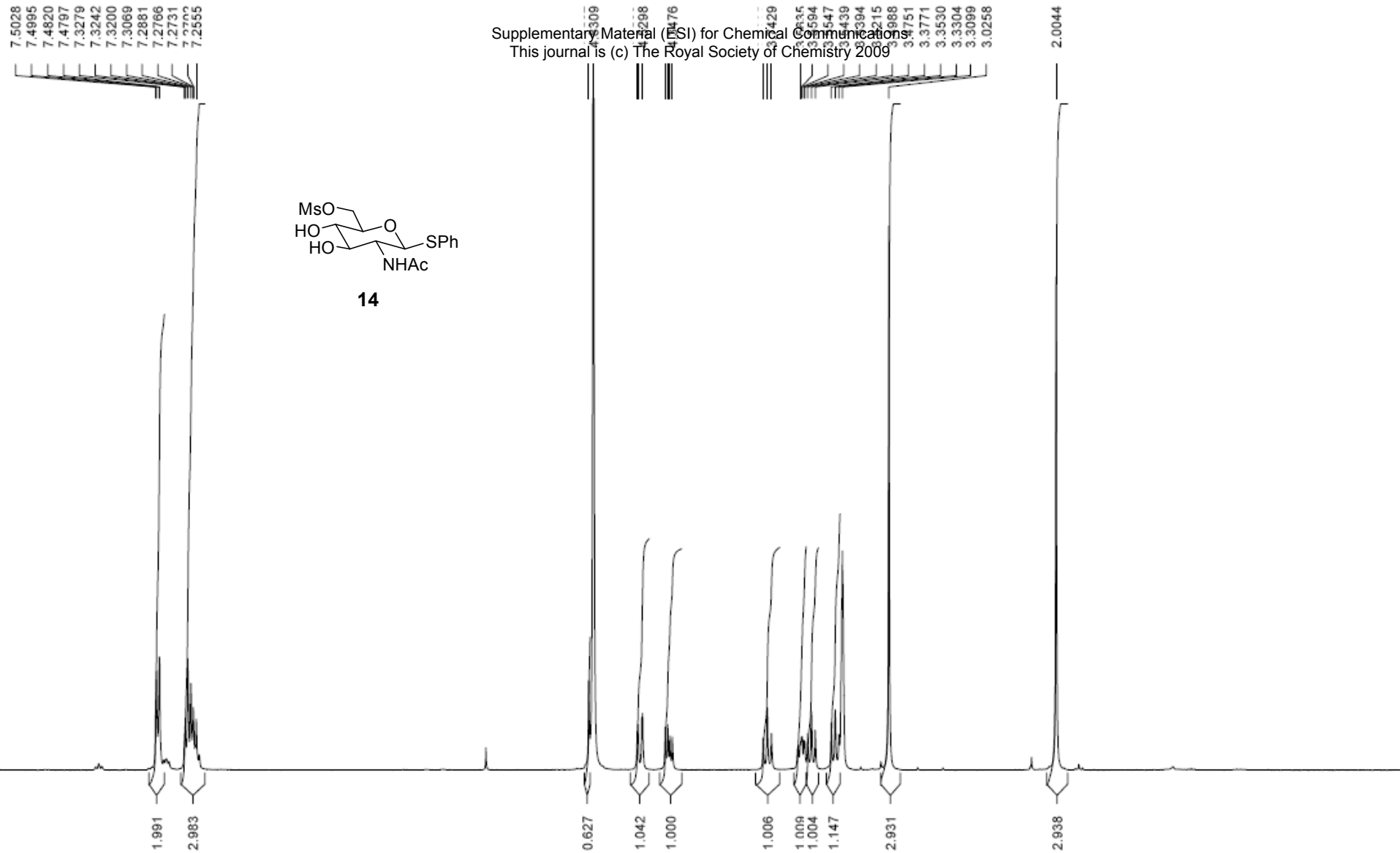
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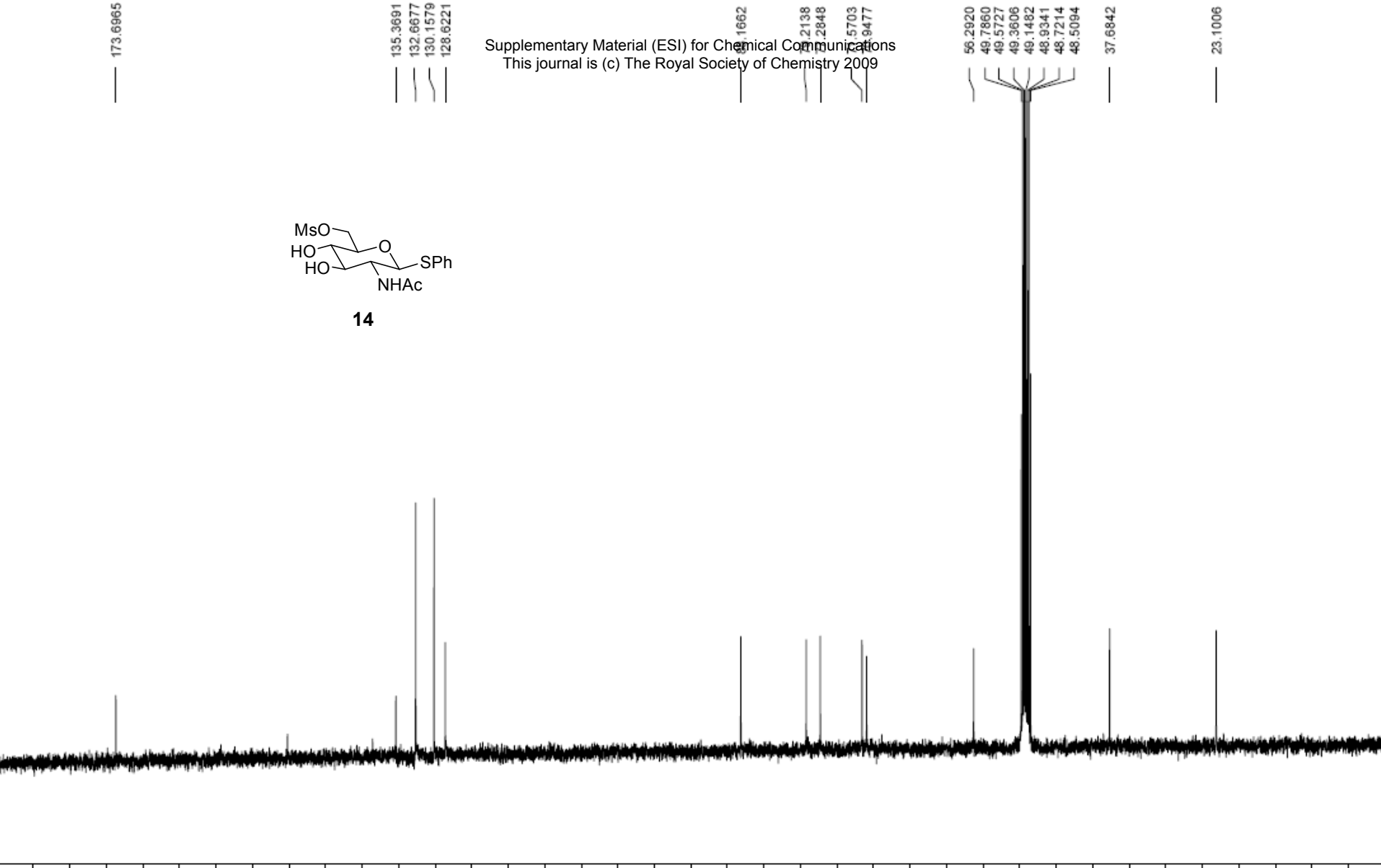
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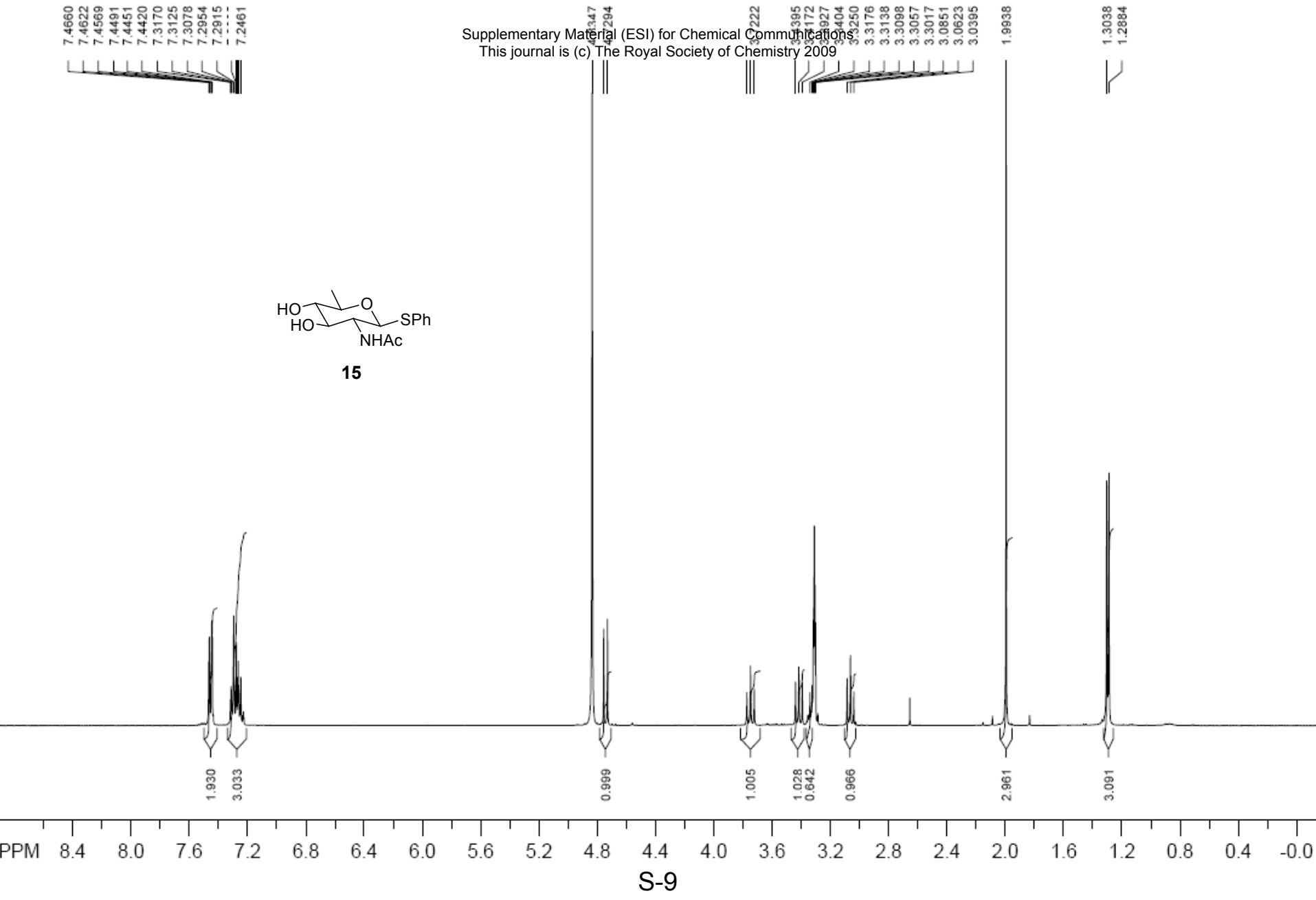
14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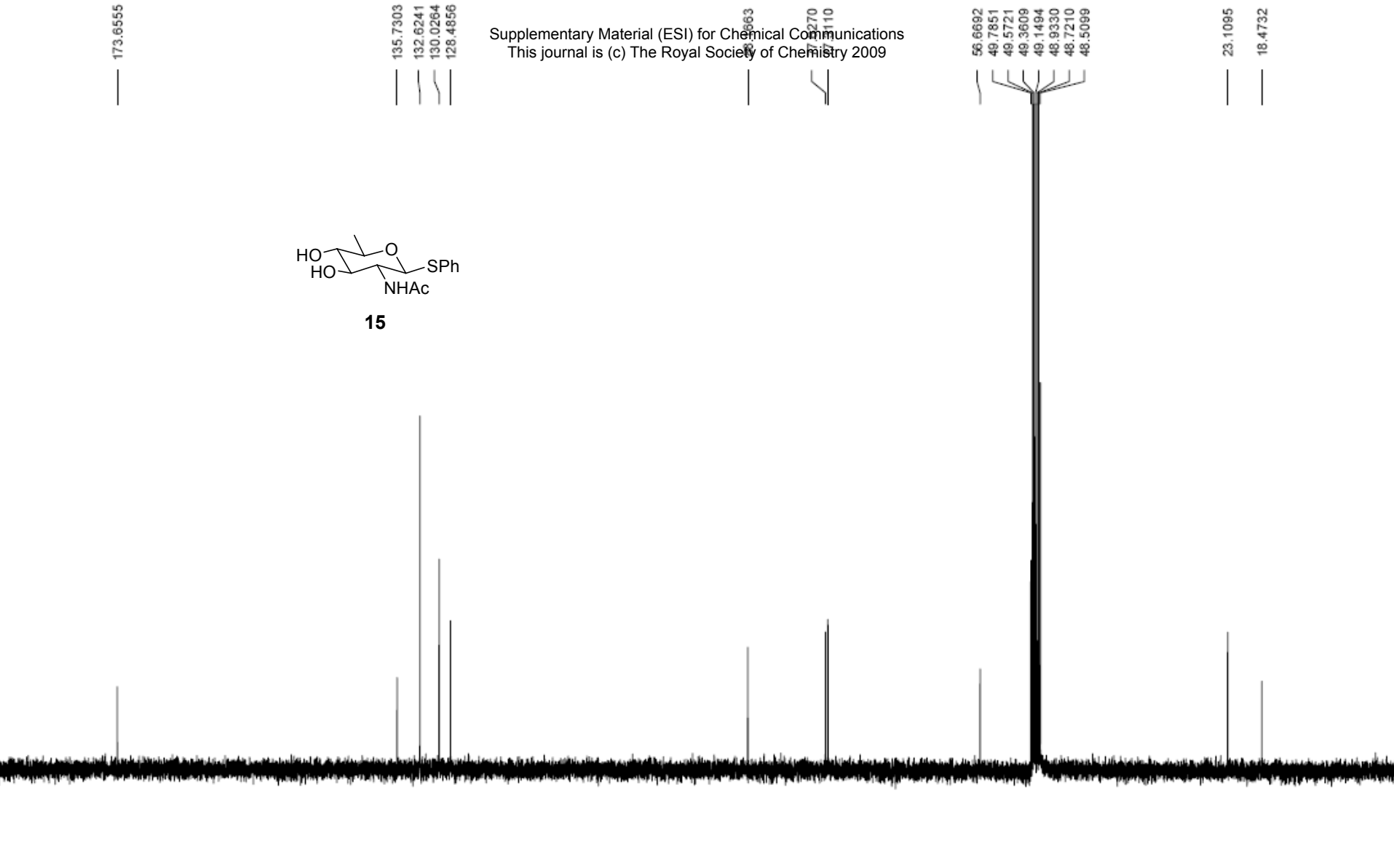
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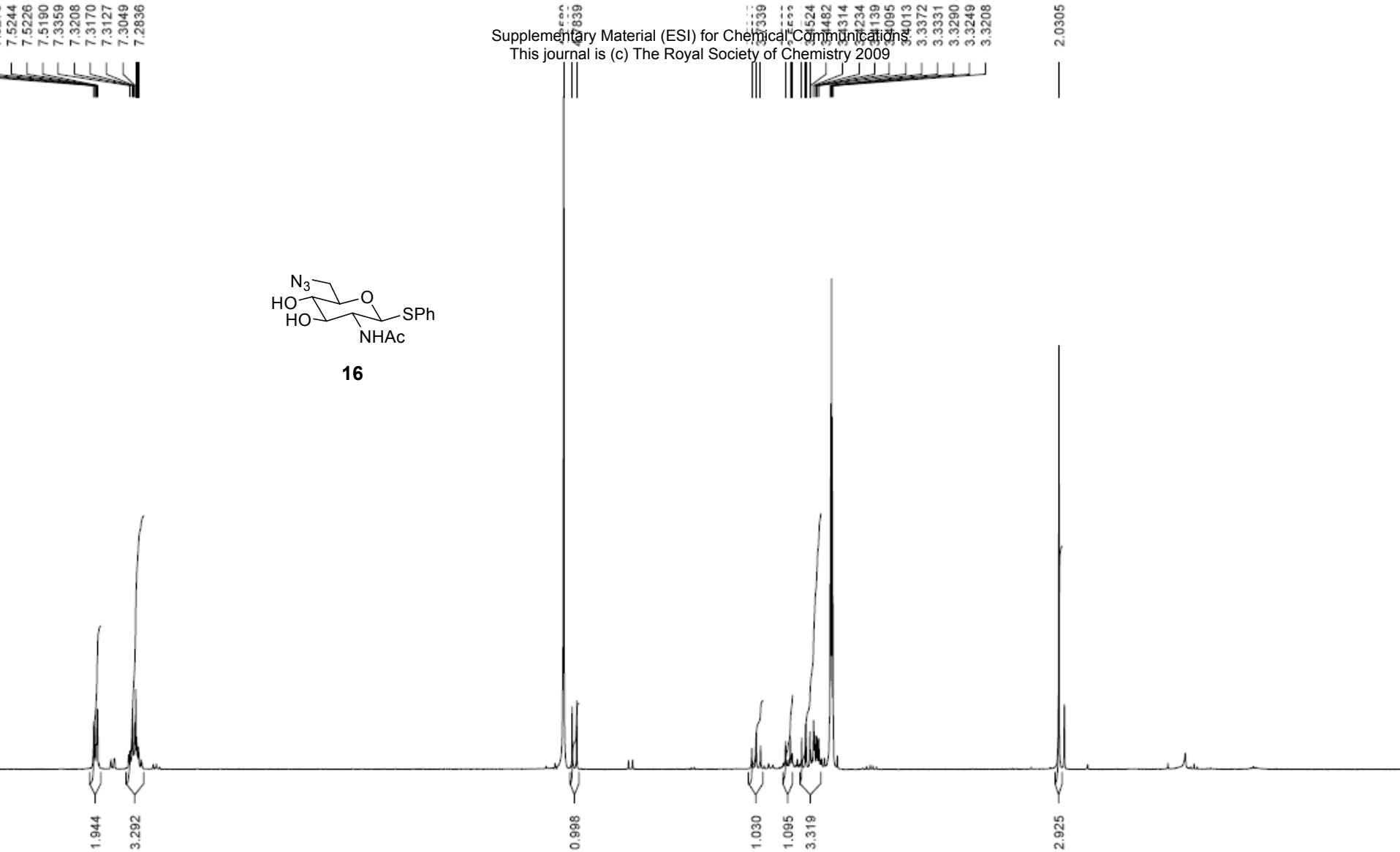
15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0.4

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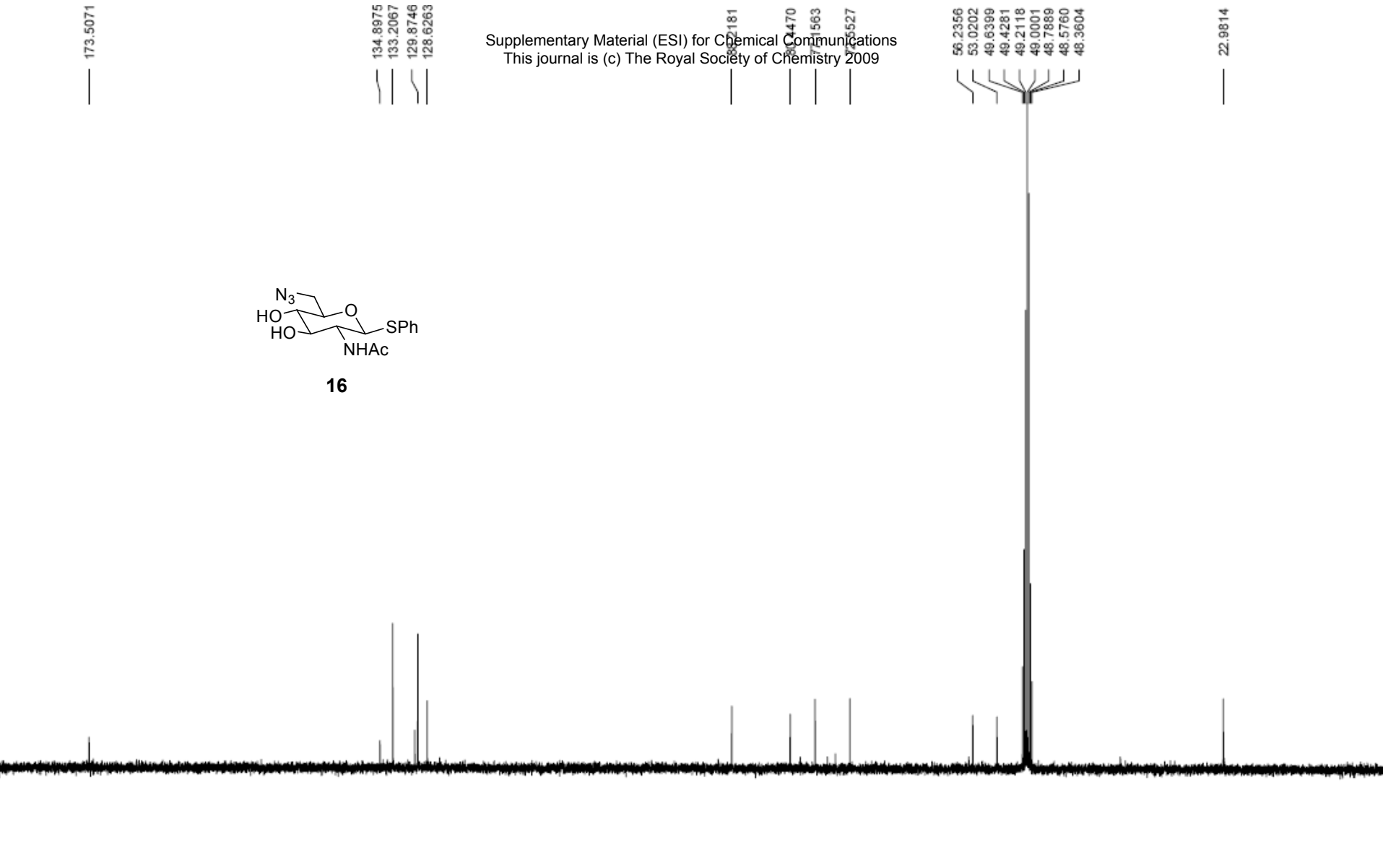
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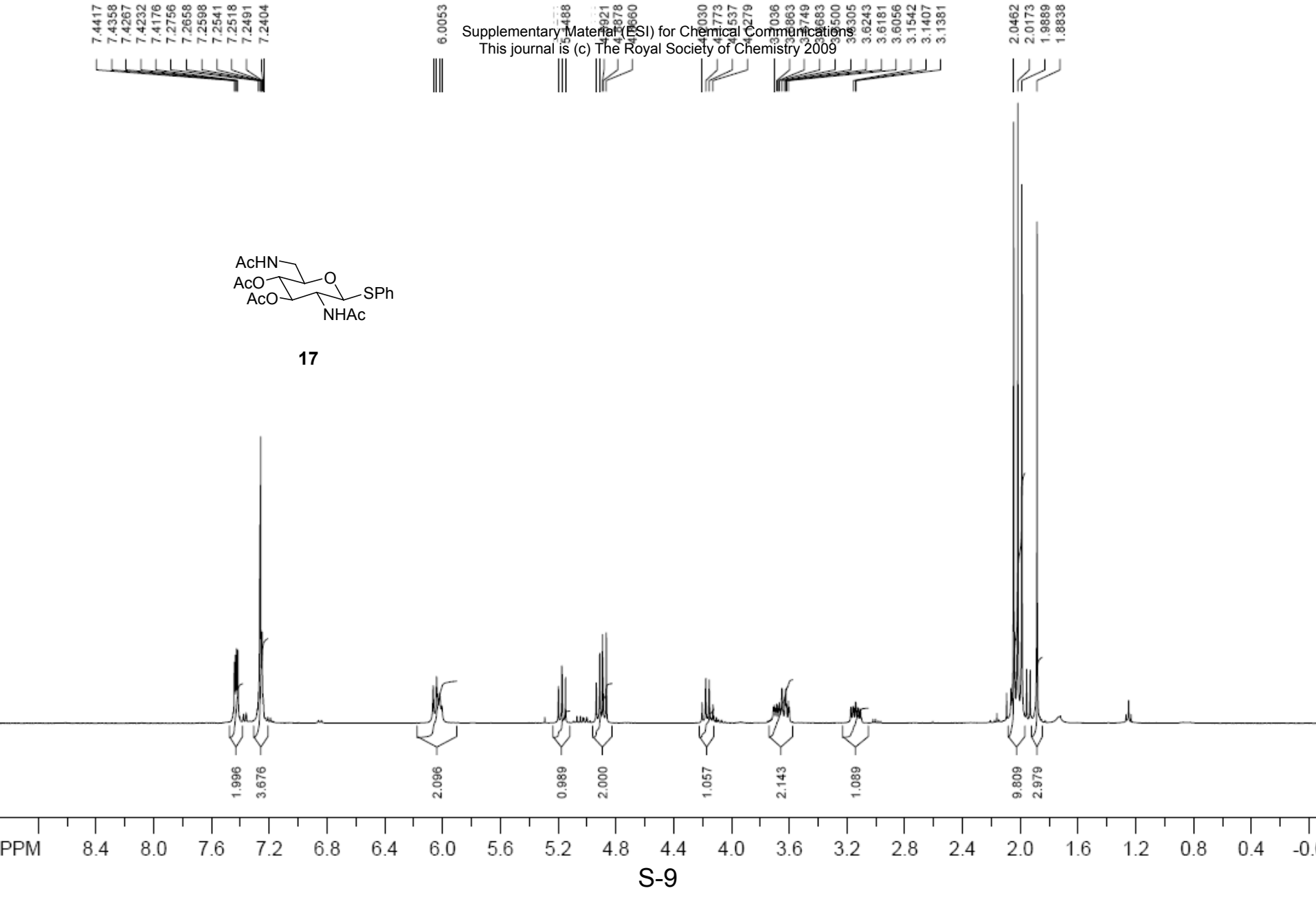
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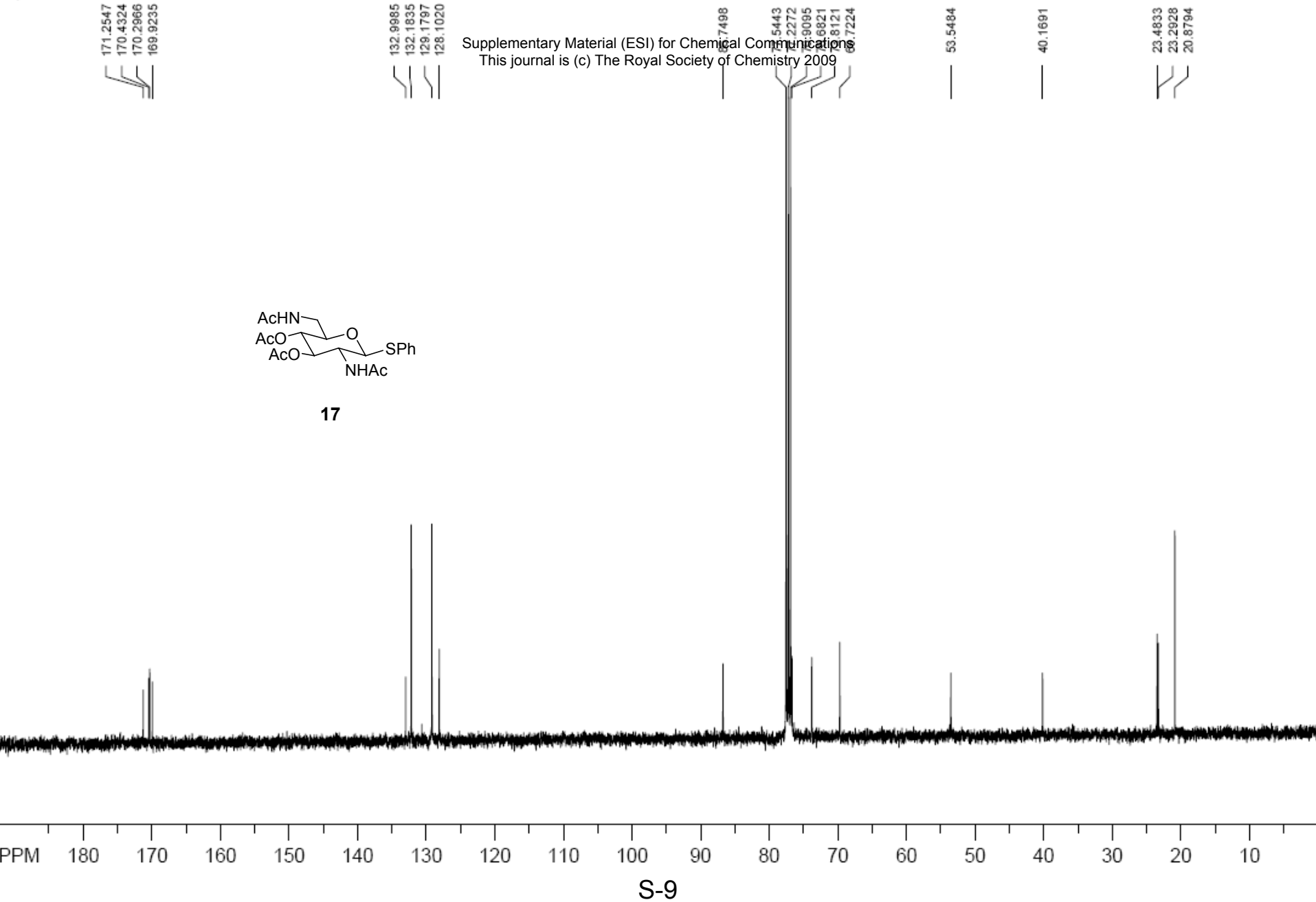
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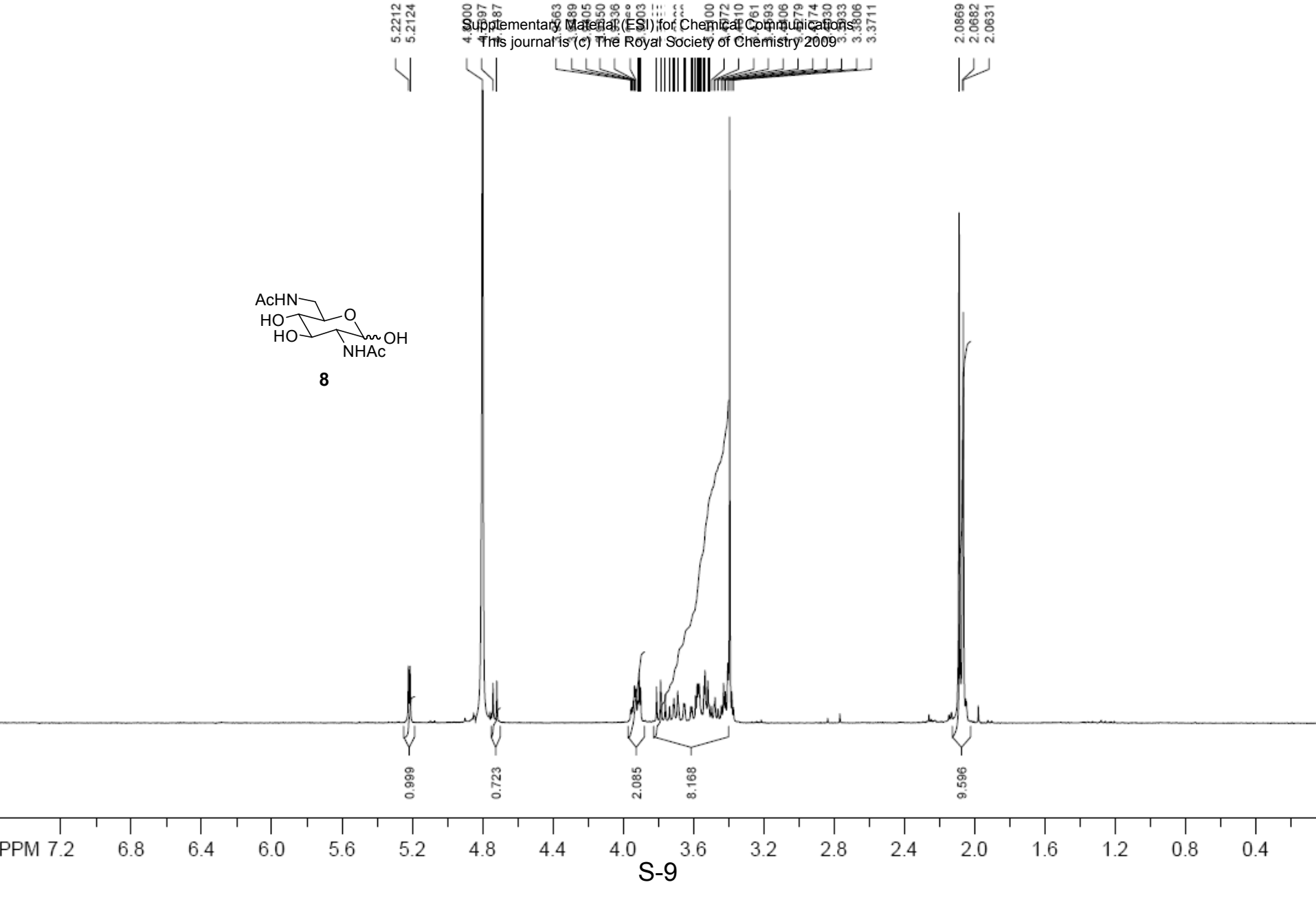

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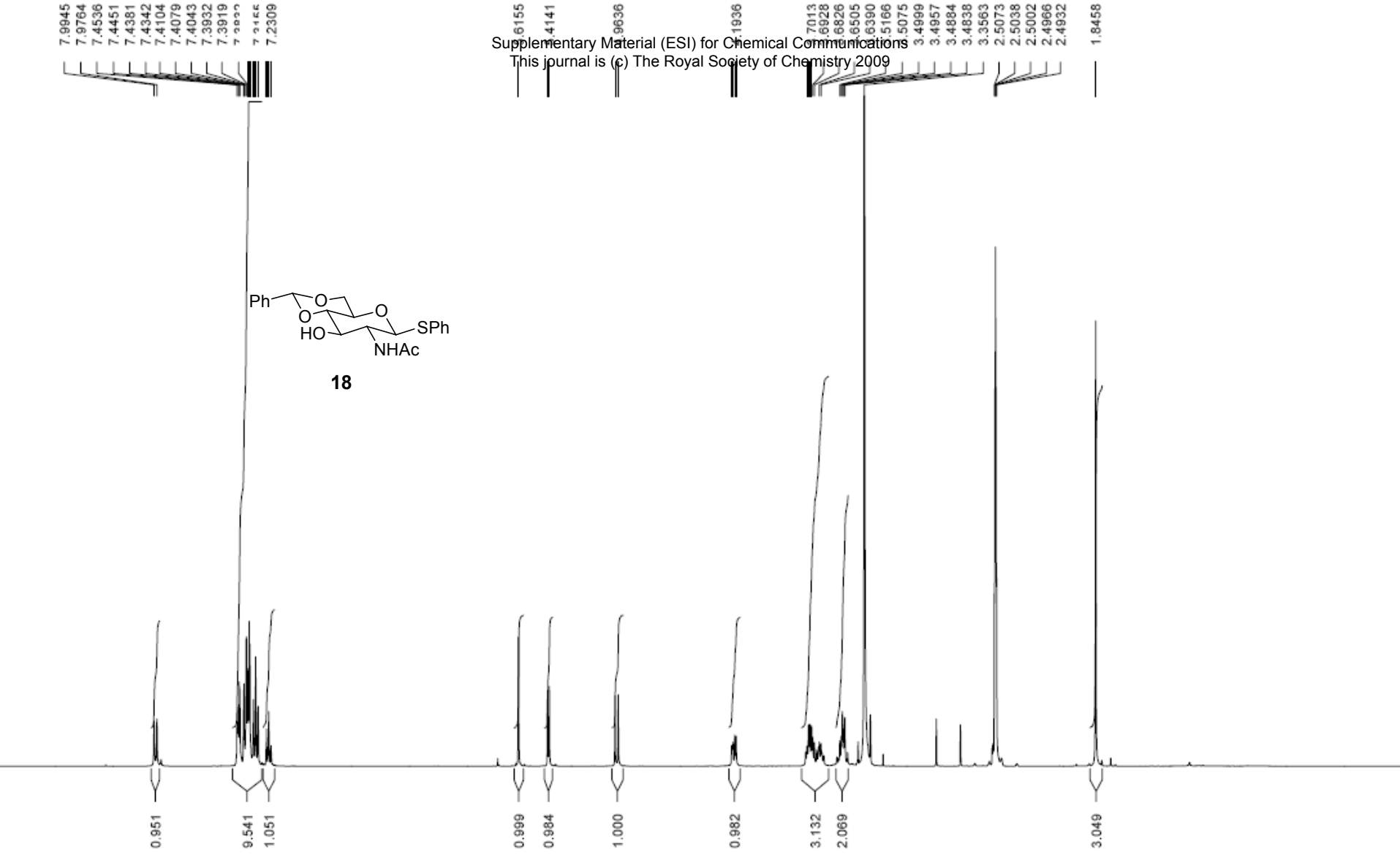
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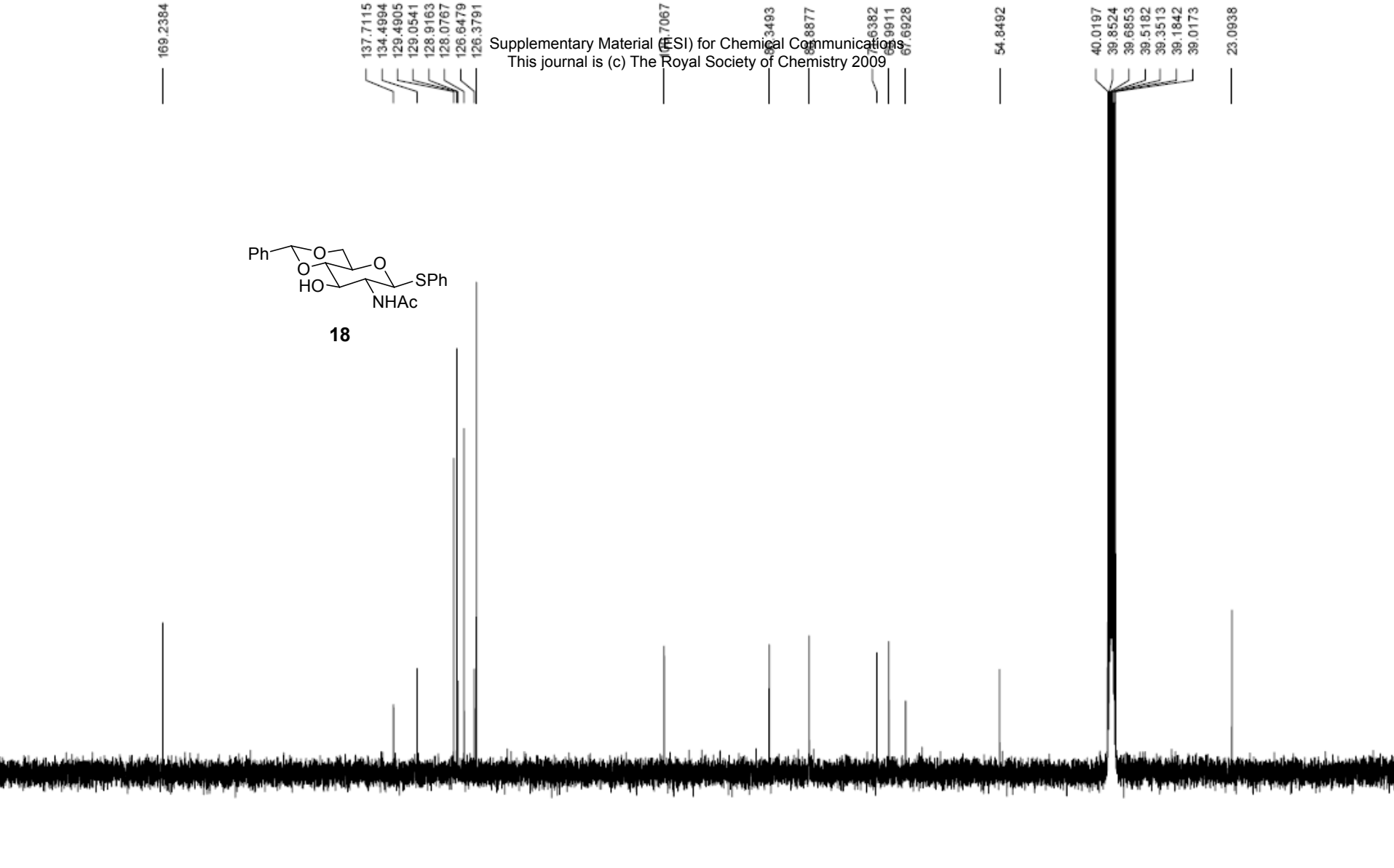
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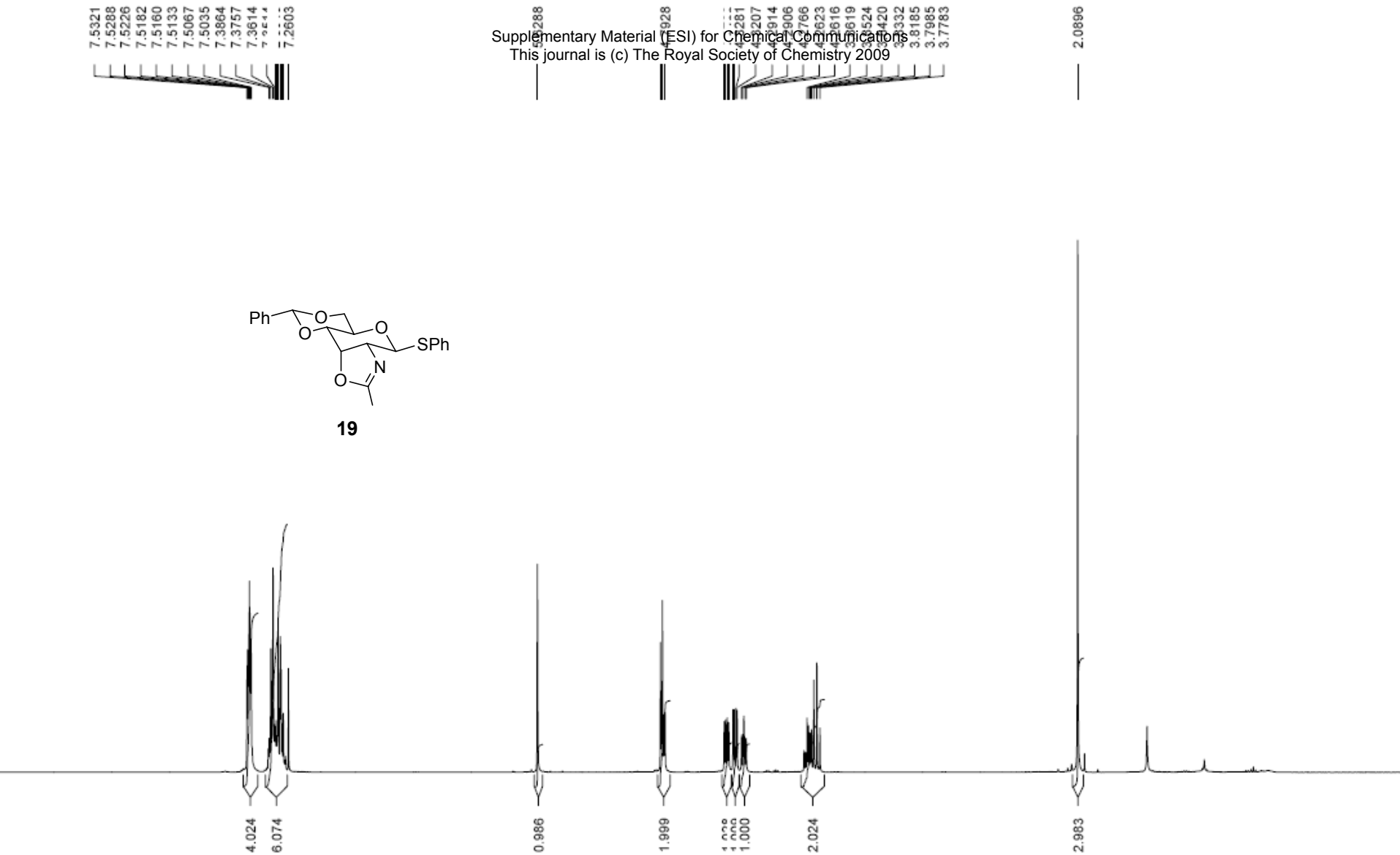
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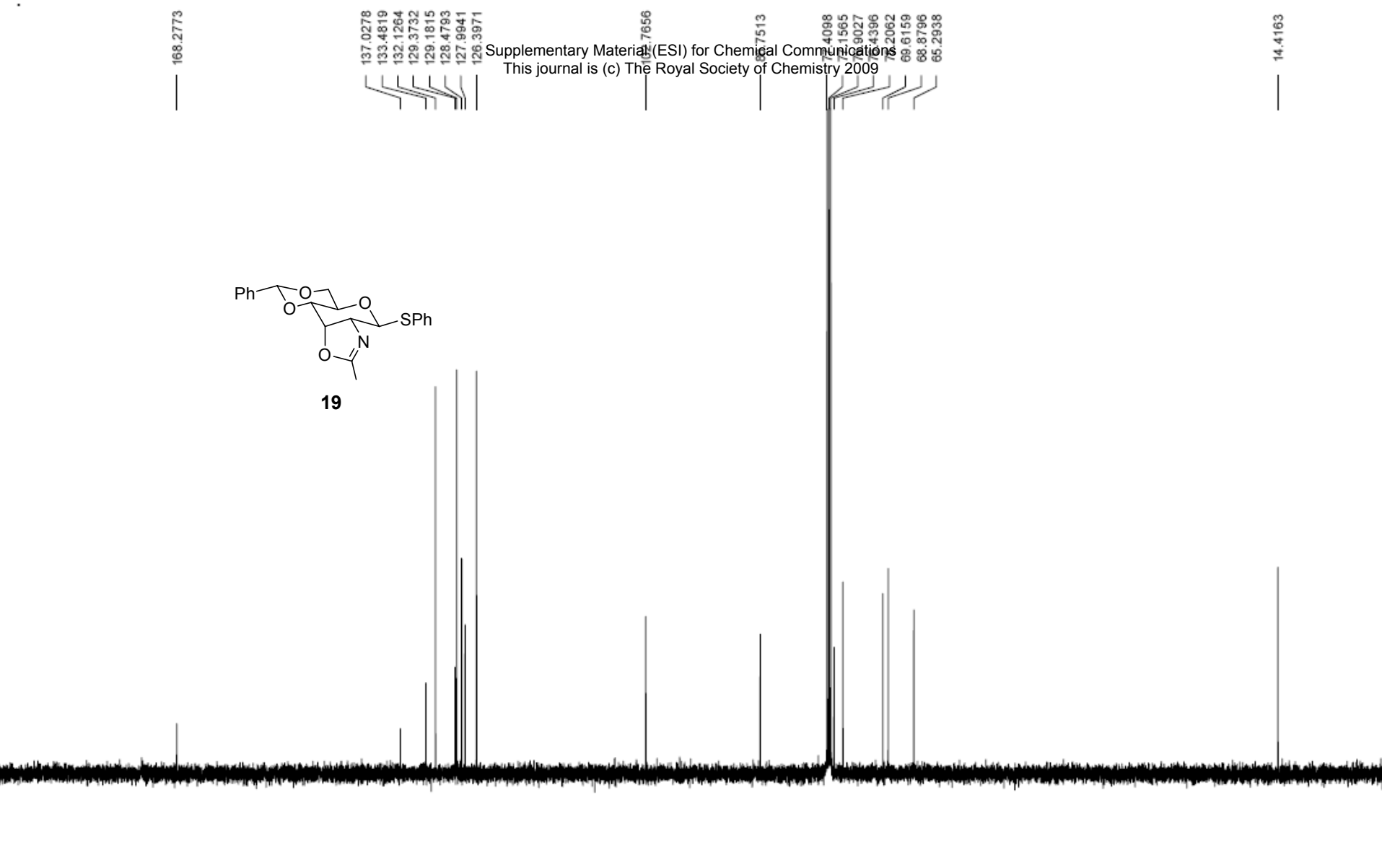
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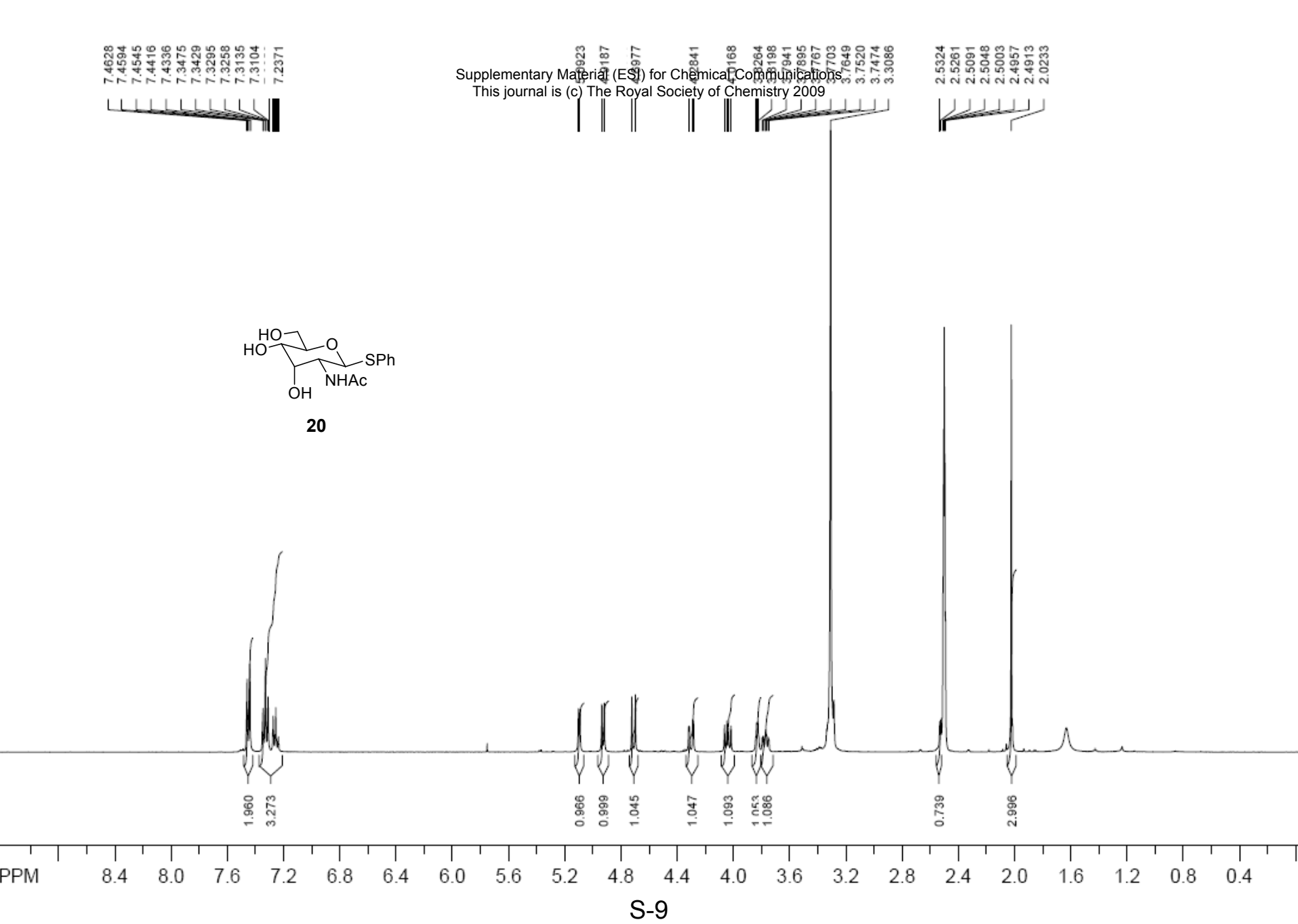
19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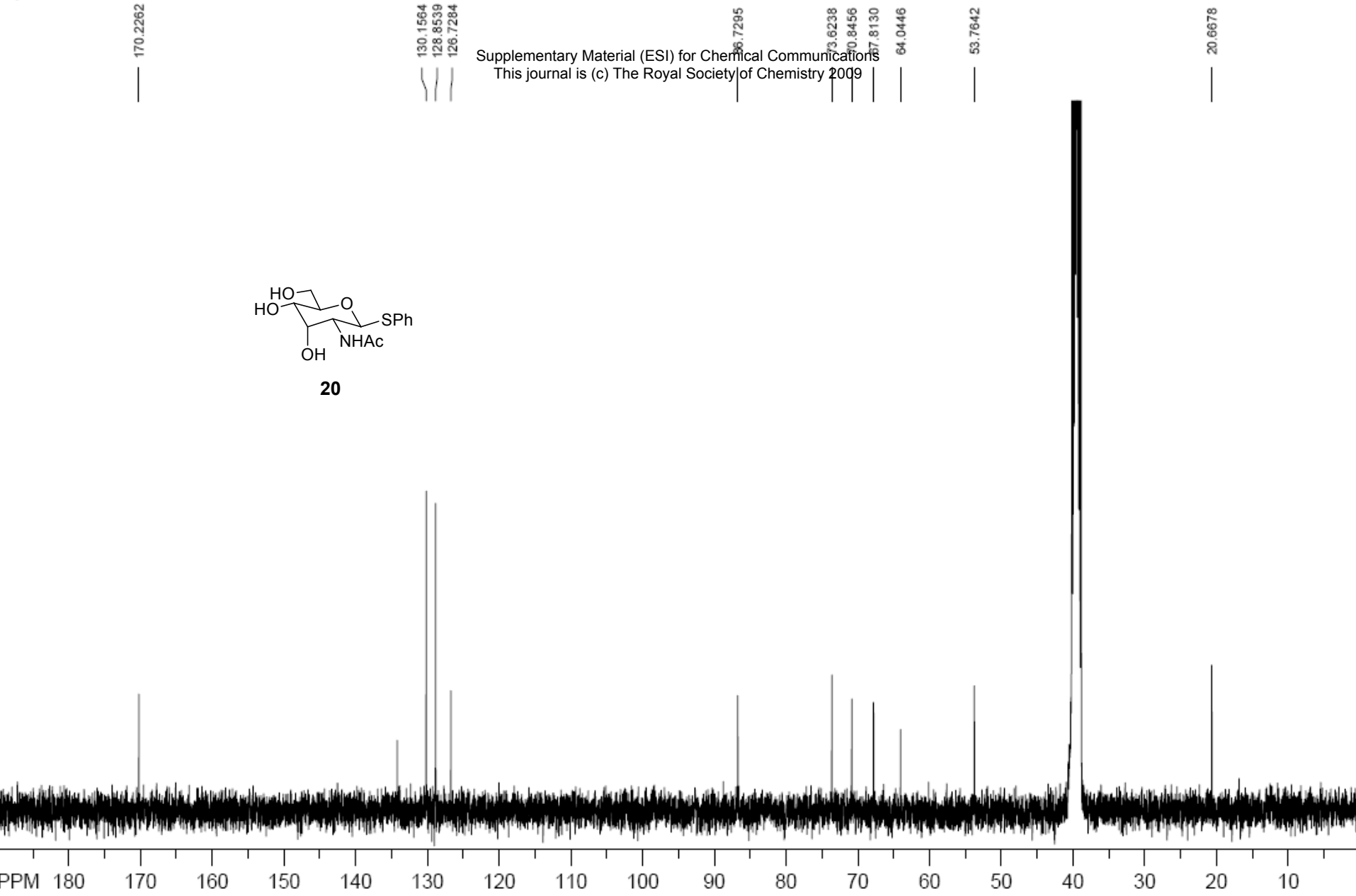
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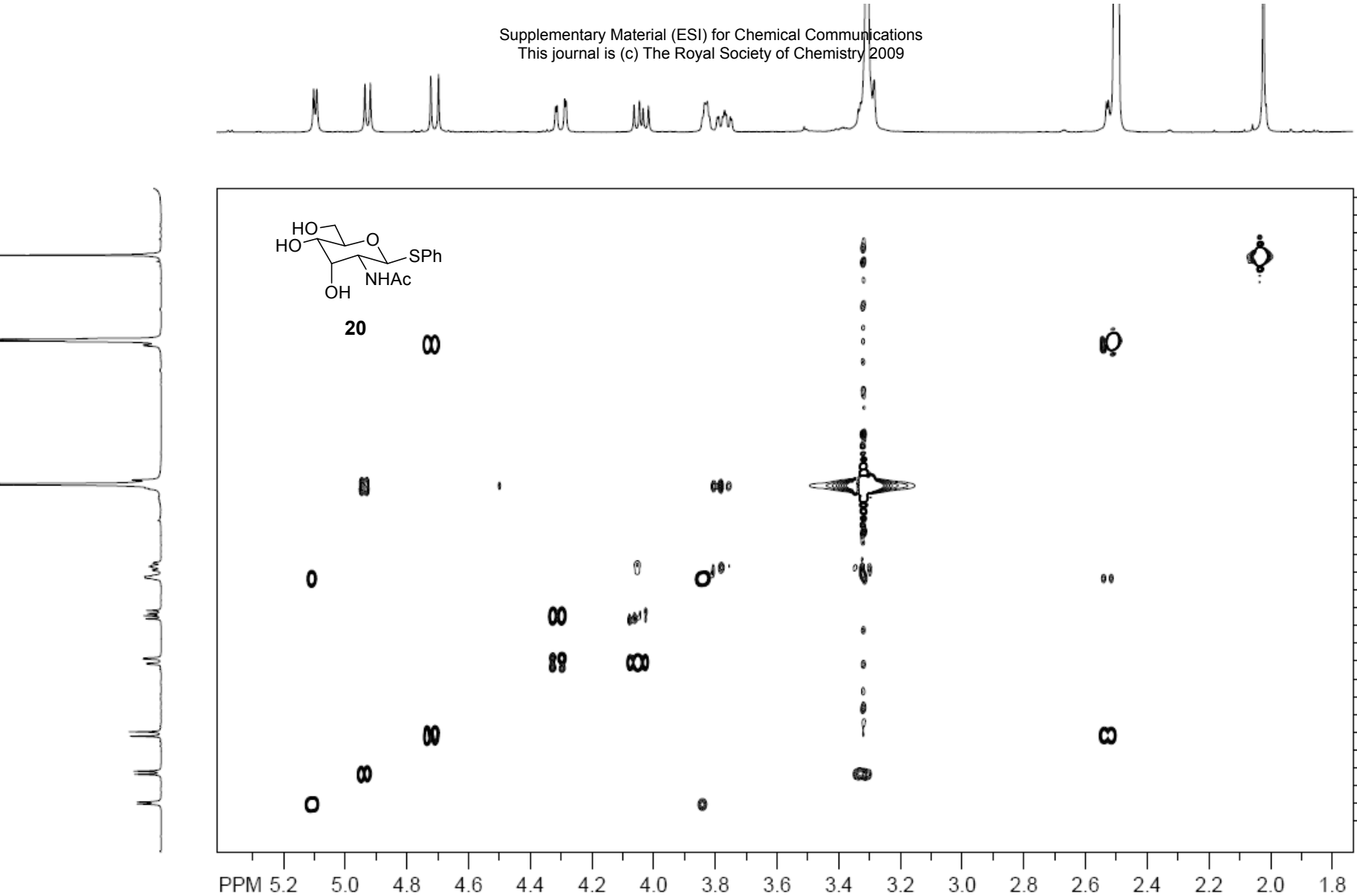
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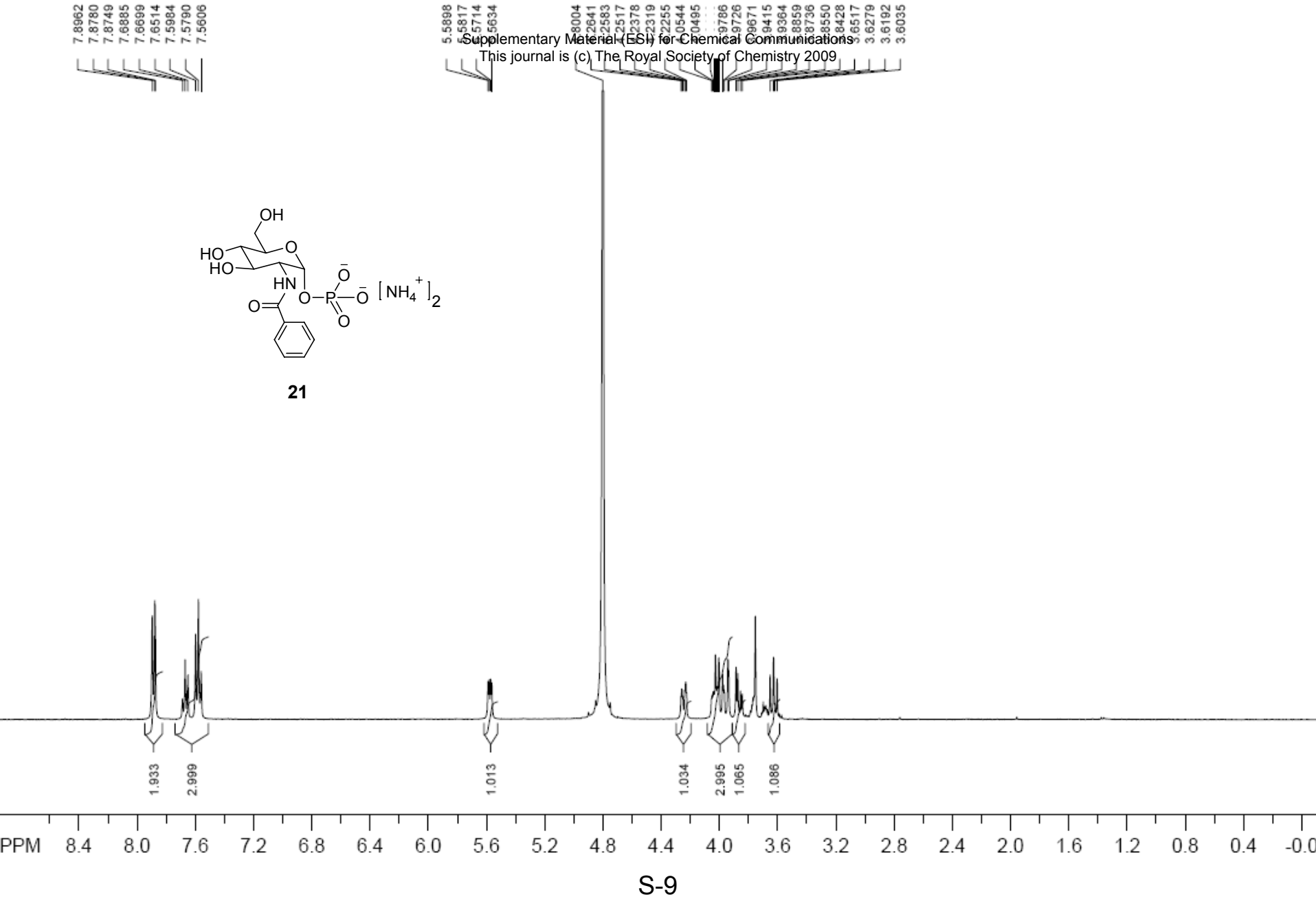
PPM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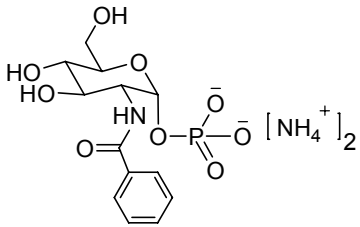

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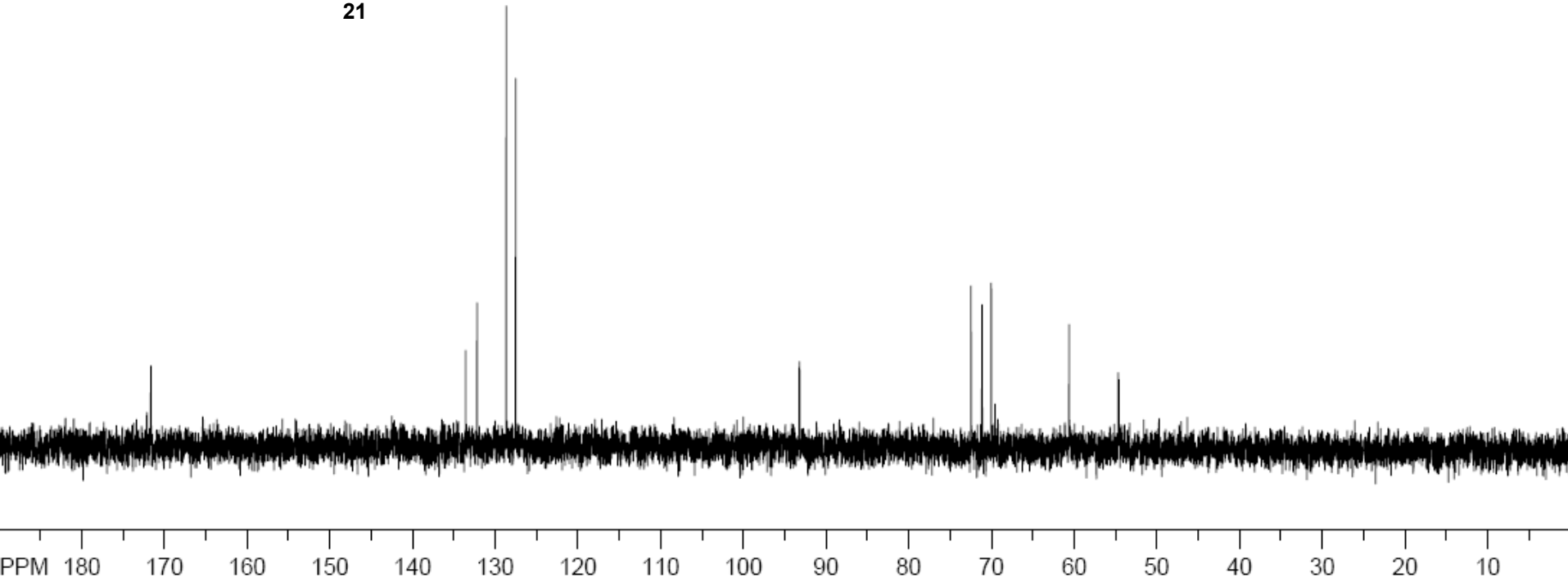

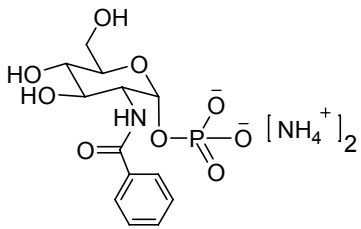

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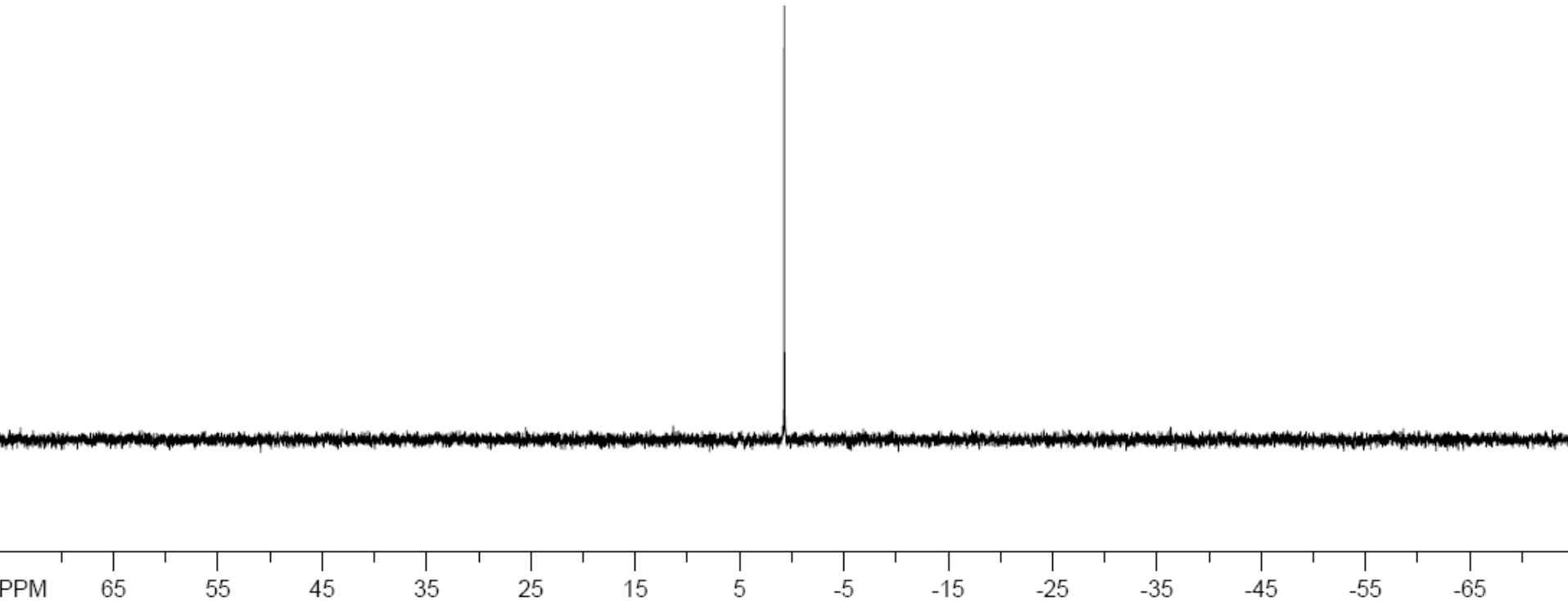

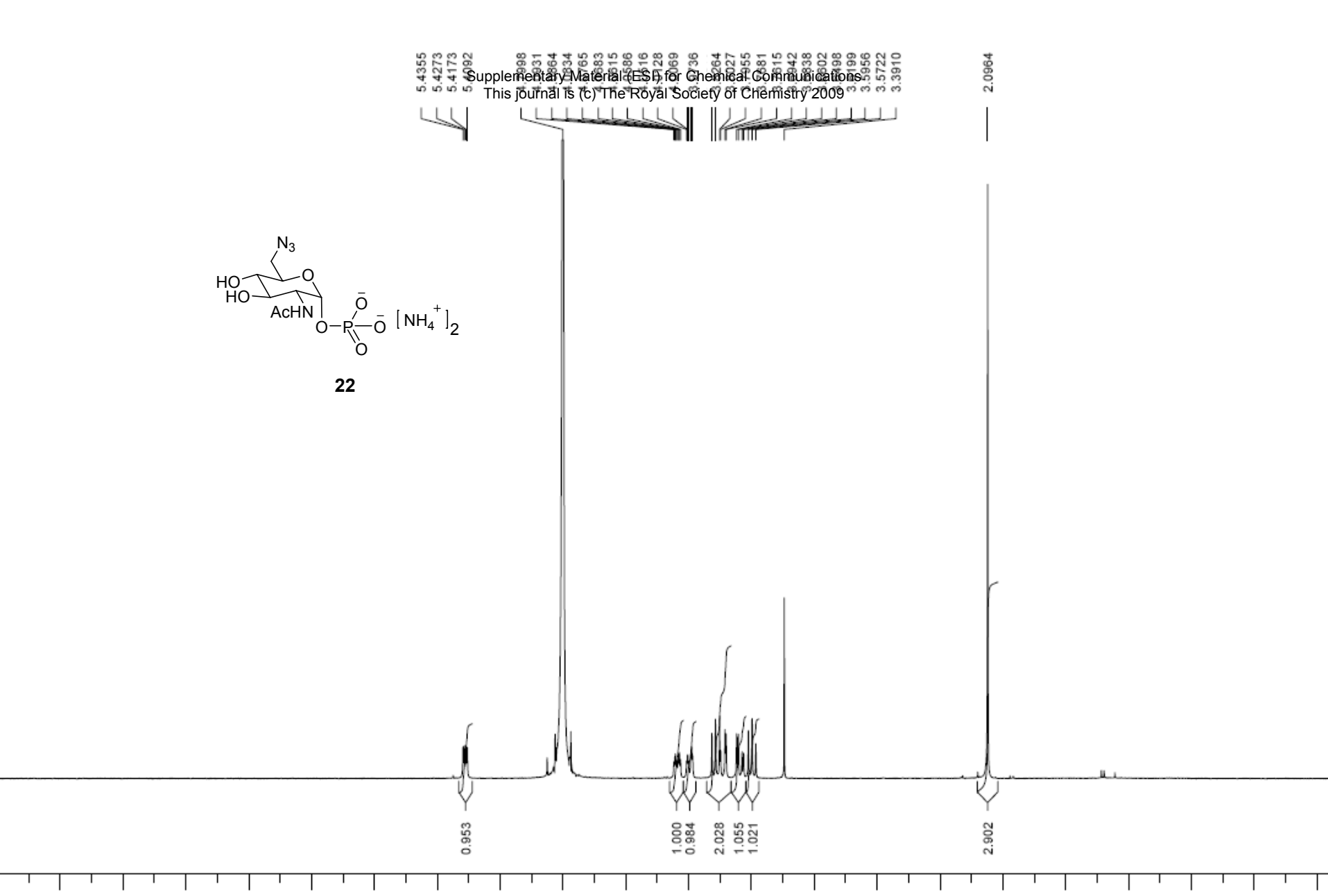
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2.902
PPM 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4

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AcHN
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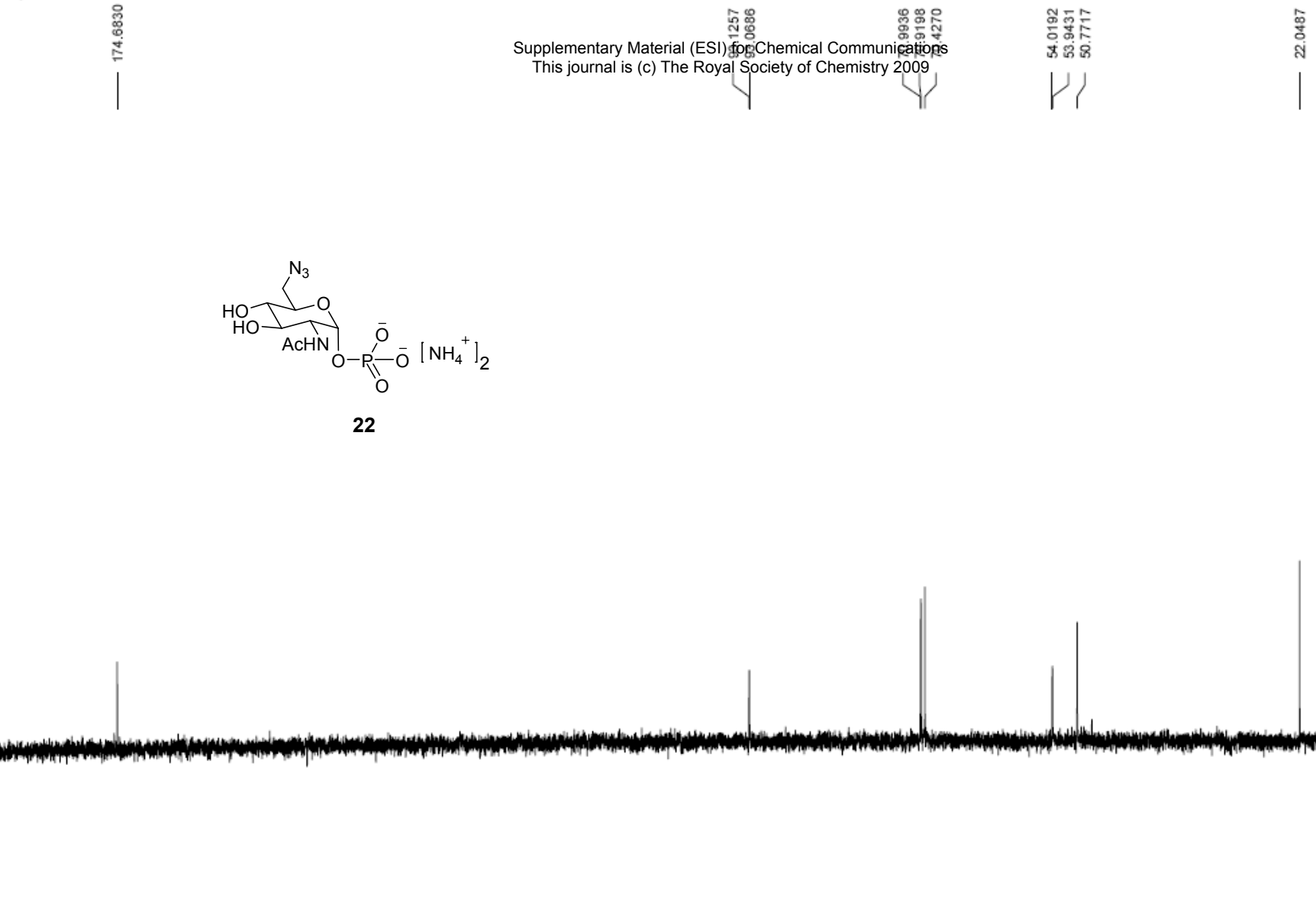
O
P
O
O
O
[NH4+]2

22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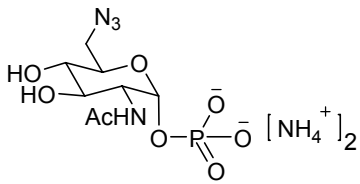
22



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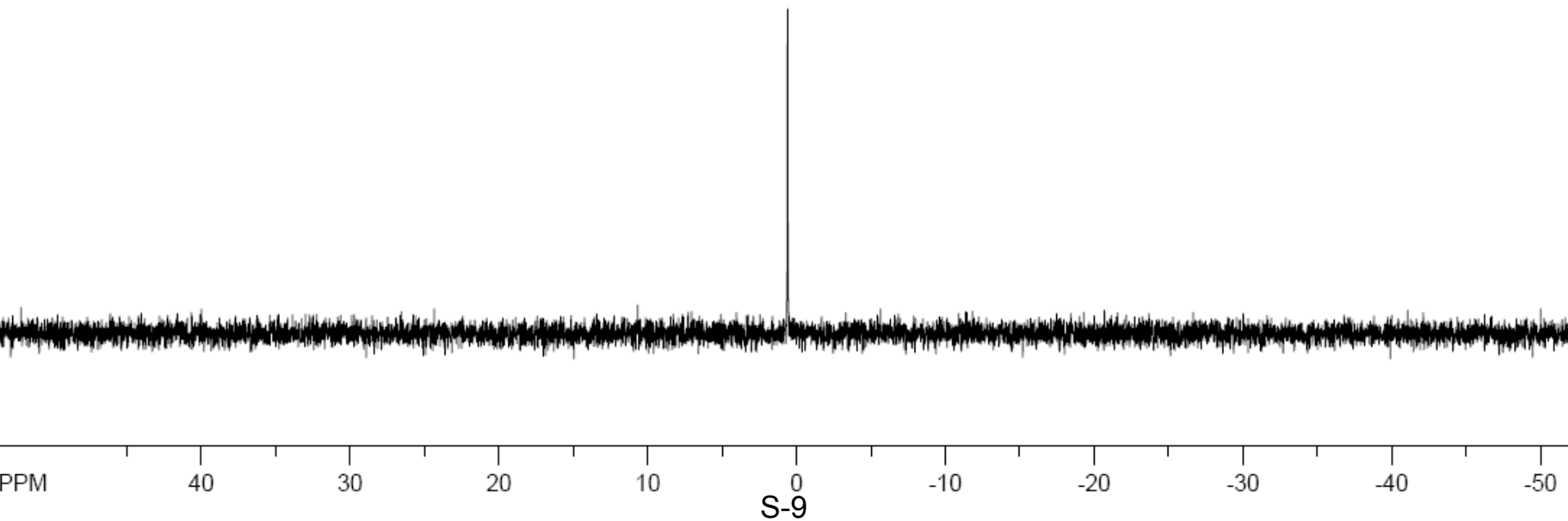
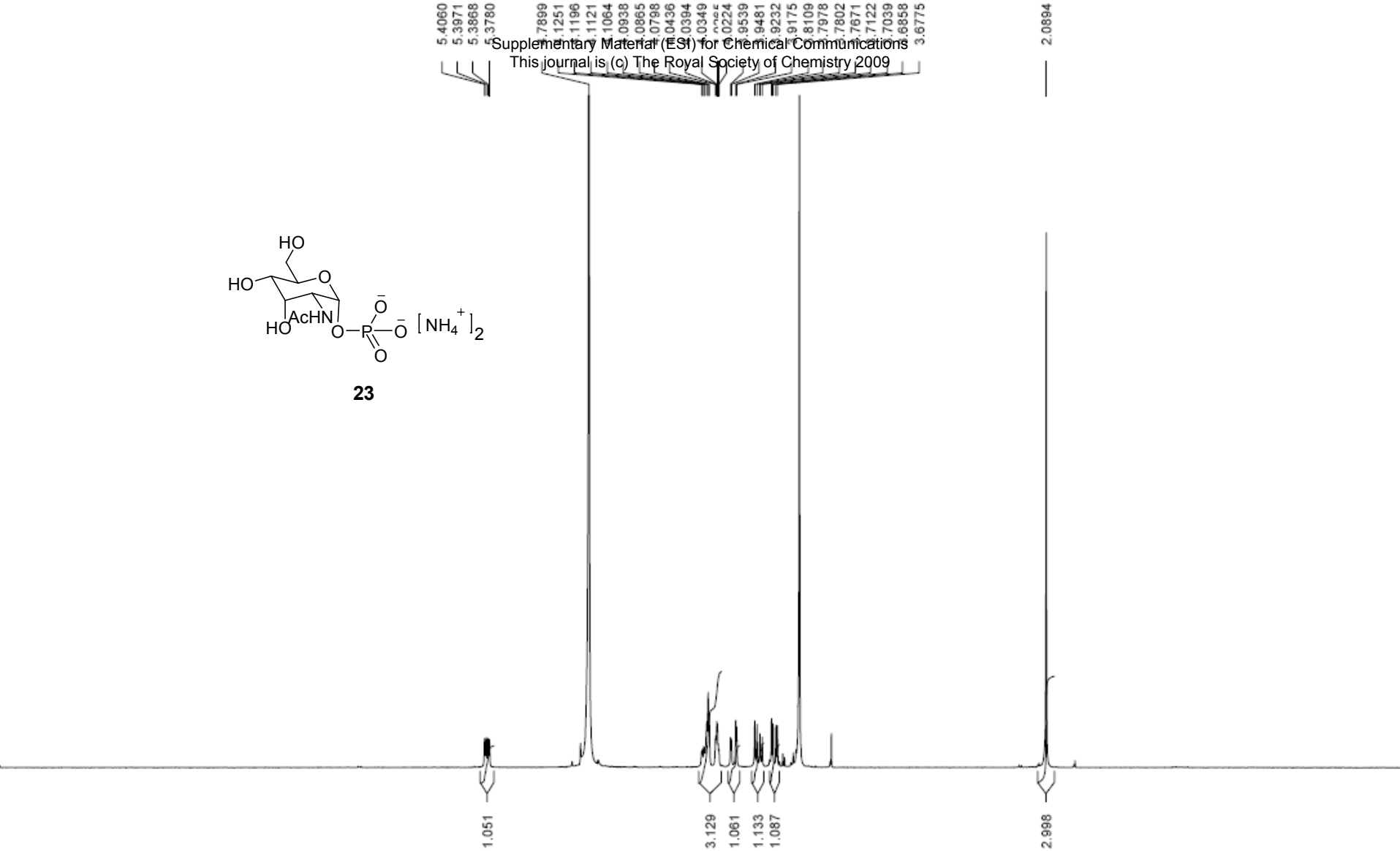
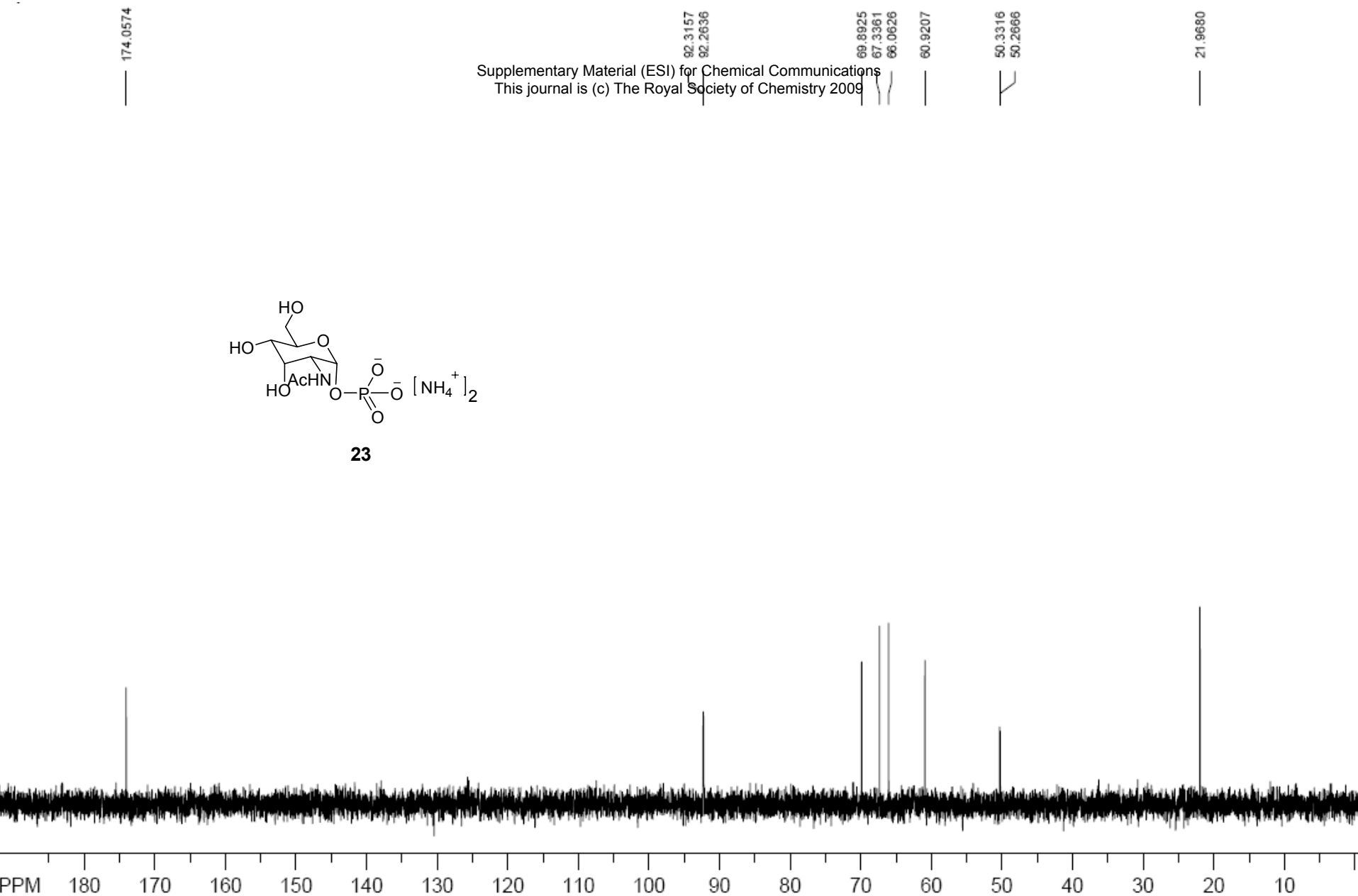


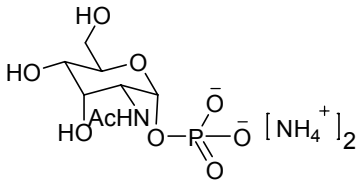

5.4060
5.3971
5.3868
5.3780
2.0894
3.6775
1.051
3.129
1.061
1.133
1.087
2.998
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4

HO
HO
O
AcHN
HO
O
P
O
O
O
[NH4+]2

23


174.0574
92.3157
92.2636
69.8925
67.3361
66.0626
60.9207
50.3316
50.2666
21.9680
HO
HO
O
HO
AcHN
O
P
O
O
O
[NH4+]2
PPM
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

23



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