Electronic Supplementary Information (ESI) for

Synthesis of Sulfone-based Nucleotide Isosteres: Identification of CMP-Synthetase Inhibitors

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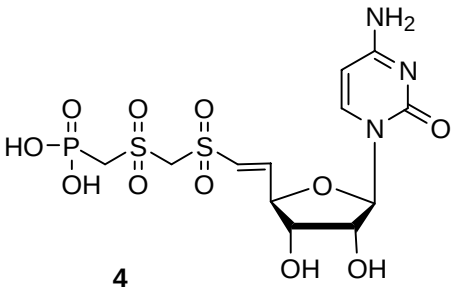
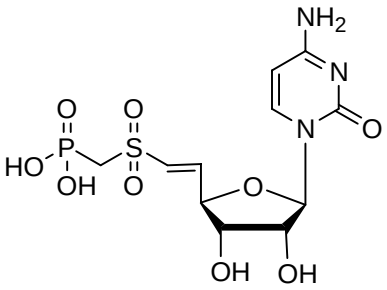
Materials and General Methods.

All chemicals were used as supplied without further purification. Solvents (MeOH 99.8%, CH_2Cl_2 99.8%, DMF 99.8%, THF 99.9%) were purchased in anhyd DriSolv™, used without further purification, and stored under argon. Glass-backed TLC plates (Silica Gel 60 with a 254 nm fluorescent indicator) were cut into 2 cm x 5 cm portions, used without further manipulation, and stored over desiccant. Developed TLC plates were visualized under a short-wave UV lamp, treated with a cerium-molybdate solution and charred. Column chromatography was conducted using flash silica gel (32-63 um). NMR experiments (1D and 2D) were conducted on Varian NMRS 600 and Bruker Advance-III 600 MHz spectrometers at 295K. Optical rotation was recorded on 370 Polarimeter and Autopol IV Automatic Polarimeter at 589 nm wavelength. High resolution mass spectra were recorded in ESI-Orbitrap instrument. Final compounds were purified on reverse-phase HPLC (Vydac, C18, 10 mm X 250 mm). All reactions were carried out in dry glassware that was flamed dried and put under argon. All enzymatic reactions were analyzed using a P/ACE^{TM} MDQ Capillary Electrophoresis System equipped with a UV detector of 200 and 254 nm wavelengths.



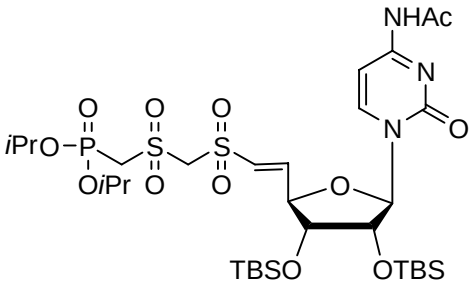
4

(*E*)-2-((*2R,3S,4S,5R*)-5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]-vinylsulfonylmethylsulfonylmethyl)-phosphonic acid (4). To a stirred solution of compound **7** (21 mg, 0.03 mmol) in dry CH_2Cl_2 (0.5 mL) was added TMSBr (71 uL, 0.6 mmol) at 0 ºC dropwise via a syringe. The mixture was warmed to room temperature and stirred for 36 h. The volatiles were then removed by rotary evaporation. The residue was dissolved in MeOH (1 mL), stirred for 40 min, and then concentrated in vacuo. The residue was co-evaporated with MeOH (1 mL) three more times. The mixture was then subjected to RP HPLC (water:methanol 95%:5%) to give compound **4** (10 mg) in 84% yield. $[\alpha]_D^{24}$= 3.6° (*c*. 0.07, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.65 (d, *J* = 7.8 Hz, 1H, H-6), 7.17 (dd, *J* = 15, 3.6 Hz, 1H, H-5'), 7.00 (dd, *J* = 15, 1.8 Hz, 1H, H-6'), 6.20 (d, *J* = 7.8 Hz, 1H, H-5), 5.99 (d, *J* = 3 Hz, 1H, H-1'), 4.68 (app m, 1H, H-4'), 4.25 (dd, *J* = 5.4, 1.2 Hz, 1H, H-2'), 4.11 (dd, *J* = 6.6, 4.8 Hz, 1H, H-3'), 4.05 (d, *J* = 15.0 Hz, 2H, PCH_2S), hydrogens between S and S (SCH_2S) were not observed due to exchanged with deuterium. ^{13}C NMR (150 MHz, CD_3OD): δ 167.7, 158.4, 147.6, 142.7, 130.1, 96.8, 92.8, 82.2, 75.3, 74.0, 67.0. Carbons between S and S were not observed due to exchanged with deuterium. HRMS (ESI+) *m/z* calcd for $C_{12}H_{18}N_3O_{11}PS_2Na$ $(M + Na)^+$ is 498.0018, found $(M + Na)^+$ 498.0029.



5

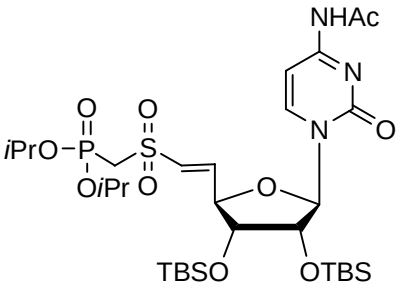
(*E*)-2-((*2R,3S,4S,5R*)-5-(4-Amino-2-oxo-*2H*-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)vinylsulfonyl)methylphosphonic acid (5). To a stirred solution of compound **8** (63 mg, 0.08 mmol) in dry CH_2Cl_2 (0.5 mL) was added TMSBr (220 uL, 1.7 mmol) at 0 ºC dropwise via a syringe. The mixture was warmed to room temperature and stirred for 36 h. The volatiles were then removed by rotary evaporation. The residue was dissolved in MeOH, stirred for 40 min, and then concentrated in vacuo. The residue was coevaporated with MeOH (1 mL) three more times. The mixture was then subjected to RP HPLC (water:methanol 95%:5%) to give compound **5** (27 mg, 82%). $[\alpha]_D^{31}$= + 19° (*c*. 0.6, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 8.01 (d, *J* = 7.2 Hz, 1H, H-6), 7.04 (app s, 2H, H-5' and H-6'), 6.26 (d, *J* = 7.8 Hz, 1H, H-5), 5.92 (d, *J* = 3.6 Hz, 1H, H-1'), 4.71 (d, *J* = 6.6 Hz, 1H, H-4'), 4.30 (dd, *J* = 8.4 Hz, 3.6 Hz, 1H, H-2'), 4.16 (dd, *J* = 11.4, 6.6 Hz, 1H, H-3'), 3.62 (dd, *J* = 15.6, 6.6 Hz, 2H, PCH_2S). ^{13}C NMR (150 MHz, CD_3OD): δ 161.1, 149.1, 146.3, 144.0, 131.2, 106.1, 96.0, 92.6, 82.8, 74.6, 73.8. HRMS (ESI+) *m/z* calcd for $C_{11}H_{17}N_3O_9PS$ $(M + H)^+$ is 398.0423, found $(M + H)^+$ 398.0420.



7

Diisopropyl-(*E*)-2-((*2R,3R,4S,5R*)-5-(4-acetamido-2-oxo-2*H*-pyrimidin-1-yl)-3, 4-bis-(*tert*-butyl-dimethyl-silyloxy)-tetrahydrofuran-2-yl)vinylsulfonylmethyl sulfonyl)methylphosphonate (7). To a stirred solution of disulfone reagent **2**[1] (50 mg, 0.1 mmol) in dry THF (0.5 mL) was added LiBr (26 mg, 0.3 mmol) until dissolved, followed by diisopropylethylamine (50 uL, 0.3 mmol) under argon at room temperature. The solution was stirred for 15 min and a solution of the crude 5'-cytidine aldehyde, **6**[2] (34 mg, 0.07 mmol), in THF (0.5 mL) was added dropwise via a syringe. The solution was stirred for 3 h at room temperature under argon and concentrated in vacuo. After flash-silica chromatography (75% EtOAc: 25% hexane), compound **7** (37 mg), an amorphous solid, was obtained in 67% yield. $[\alpha]_D^{24}$ = + 73.5° (*c*. 0.26, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.97 (s, 1H, NH), 7.59 (app m. 2H, H-6 and H-6'), 7.06 (dd, *J* = 15, 2.4 Hz, 1H, H-5'), 5.81 (app s, 1H, H-1'), 5.56 (d, *J* = 15.6 Hz, 1H, SCHS), 5.33 (d, *J* = 15 Hz, 1H, SCHS), 4.90-4.84 (m, 2H, CH of isopropyls), 4.80 (d, *J* = 4.8 Hz, 1H, H-4'), 4.23-4.18 (m, 2H, H-2' and PCHS), 3.87 (app t, *J* = 16.2 Hz, 1H, PCHS), 3.80 (dd, *J* = 8.4, 4.2 Hz, 1H, H-3'), 2.21 (s, 3H), 1.39 (d, *J* = 6 Hz, 12 H, isopropyls), 0.89 (d, *J* = 4.2 Hz, 18H, CH_3 of *tert*-butyl groups), 0.22 (s, 3H, CH_3 attached to silyls), 0.12 (s, 3H, CH_3 attached to silyls), 0.05 (s, 3H, CH_3 attached to silyls), 0.03 (s, 3H, CH_3 attached to silyls). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.6, 162.6, 155.0,

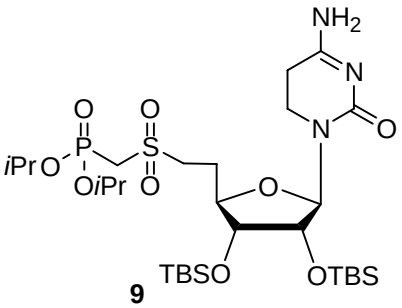
144.7, 144.1, 130.1, 96.9, 93.4, 80.3, 75.0, 73.9, 73.6, 73.5, 70.0, 51.4, 25.94 (3), 25.91 (3), 25.0, 24.31, 24.29, 23.92, 23.89, 18.21, 18.16, -4.2 (2), -4.9 (2). HRMS (ESI+) *m/z* calcd for $C_{32}H_{61}N_3O_{12}PS_2Si_2$ $(M + H)^+$ is 830.2973, found $(M + H)^+$ 830.2967.



8

Diisopropyl-(*E*)-2-((*2R,3R,4S,5R*)-5-(4-acetamido-2-oxo-2*H*-pyrimidin-1-yl)-3,4-bis-(*tert*-butyl-dimethyl-silyloxy)-tetrahydrofuran-2-yl)vinylsulfonyl) methylphosphonate (8). To a stirred solution of monosulfone reagent **3**[3] (495 mg, 1.2 mmol) in dry THF (1.7 mL) was added LiBr (305 mg, 3.5 mmol) until dissolved, followed by diisopropylethylamine (580 uL, 3.5 mmol) under argon at room temperature. The solution was stirred for 15 min and a solution of the crude 5'-cytidine aldehyde, **6**[2] (405 mg, 0.8 mmol), in THF (1.7 mL) was added dropwise via a syringe. The solution was stirred 3 h and concentrated in vacuo. After flash-silica chromatography (75% EtOAc: 25% hexane), an amorphous solid product, **8** (416 mg, 70% yield) was obtained. $[\alpha]_D^{24}$ = + 84.3° (*c*. 0.34, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.45 (s, 1H, NH), 7.98 (d, *J* = 7.8 Hz, 1H, H-6), 7.45 (d, *J* = 7.2 Hz, 1H, H-5), 7.13 (dd, *J* = 15, 1.8 Hz, 1H, H-6'), 7.04 (dd, *J* = 15.6, 4.2 Hz, 1H, H-5'), 5.80 (s, 1H, H-1'), 4.86-4.81 (m, 2H, CH of isopropyls),

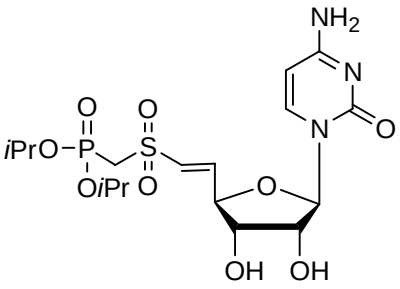
4.27 (app. d, *J* = 2.4 Hz, 1H, H-4'), 3.79 (dd, *J* = 8.4, 4.2 Hz, 1H, H-2'), 3.64 (app t, *J* = 15.9 Hz, 1H, PCHS), 3.55 (app t, *J* = 15.9 Hz, 1H, PCHS), 3.49 (d, *J* = 4.8 Hz, 1H, H-3'), 2.23 (s, 3H, CH_3 of acetate), 1.38 (app t, *J* = 6.6 Hz, 12H, isopropyls), 0.91 (d, *J* = 15.6 Hz, 18 H, *tert*-butyl groups) 0.24 (s, 3H, CH_3 from silyls), 0.13 (s, 3H, CH_3 from silyls), 0.06 (s, 3H, CH_3 from silyls), 0.05 (s, 3H, CH_3 from silyls). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.3, 162.7, 155.1, 144.6, 143.7, 130.7, 96.8, 93.0, 80.4, 75.4, 74.4, 73.1, 73.0, 53.9, 26.0 (6), 25.2, 24.33, 24.29, 24.02, 23.97, 18.21, 18.18, -4.1, -4.2, -4.7, -4.9. HRMS (ESI+) *m/z* calcd for $C_{31}H_{59}N_3O_{10}PSSi_2$ $(M + H)^+$ is 752.3197, found $(M + H)^+$ 752.3190.



9

Diisopropyl (2-((*2R,3R,4S,5R*)-5-(4-amino-2-oxo-5,6-dihydro-2*H*-pyrimidin-1-yl)-3,4-bis-(*tert*-butyl-dimethyl-silyloxy)-tetrahydrofuran-2-yl)ethylsulfonyl) methylphosphonate (9). To a solution of compound **8** (33 mg, 0.04 mmol) in THF:MeOH (1:1, 0.8 mL) was added 10% Pd/C (15 mg). Hydrogen gas was introduced via a balloon to the reaction vessel. The reaction was stirred for 1 h at 1 atm under a hydrogen atmosphere. Afterward, the reaction mixture was filtered through celite and washed with EtOAc (3 X 2 mL). The contents were then concentrated in vacuo to give an oil product **9** (26 mg) in 84 % yield. $[\alpha]_D^{23}$ = 3.0° (*c*. 0.13, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.56 (s, 1H, NH), 5.56 (d,

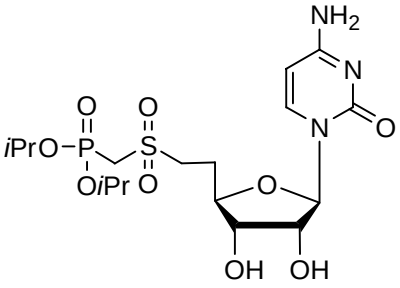
J = 4.8 Hz, 1H, H-1'), 4.84-4.78 (m, 2H, CH of isopropyls), 4.27 (app t, *J* = 4.8 Hz, 1H, H-2'), 3.90 (dt, *J* = 10.8, 3.6 Hz, 1H, H-4'), 3.82 (app t, *J* = 4.2 Hz, 1H, H-3'), 3.65-3.46 (m, 4H, PCH_2S and H-6'), 3.41-3.37 (m, 2H, H-6), 2.69-2.65 (m, 2H, H-5), 2.19-2.15 (m, 1H, H-5'), 2.01-1.96 (m, 1H, H-5'), 1.37 (app t, *J* = 4.8 Hz, 12H, isopropyls), 0.9 (d, *J* = 11.4 Hz, 18H, *tert*-butyls), 0.09 (s, 6H, methyls of silyls), 0.07 (s, 6H, methyls of silyls). ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.5, 152.4, 91.5, 81.0, 75.8, 73.14, 73.09, 73.05, 73.00, 72.91, 72.87, 72.7, 39.3, 31.3, 26.0, 25.8, 24.3, 24.24, 24.23, 23.93, 23.91, 23.90, 23.89, 18.18, 18.12, -4.2, -4.3, -4.5, -4.5. HRMS (ESI+) *m/z* calcd for $C_{29}H_{62}N_3O_9PS_2Si_2$ $(M + 2H)^+$ is 715.3483, found $(M + 2H)^+$ 715.3242.



10

Diisopropyl-(*E*)-2-((*2R,3S,4S,5R*)-5-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)vinylsulfonyl)methylphosphonate (10). To a stirred solution of compound **8** (41 mg, 0.05 mmol) in dry THF (300 uL) was added TBAF (1 M in THF, 125 uL) and glacial acetic acid (10 uL, 0.16 mmol). The solution was stirred for 1 h at room temperature. The solution was then concentrated in vacuo. After flash-silica chromatography (5% MeOH: 95% CH_2Cl_2), an amorphous solid (34 mg) was obtained. To a stirred solution of the

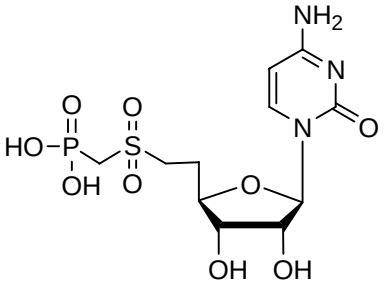
amorphous solid (34 mg, 0.06 mmol) in CH_2Cl_2, TFA (25 uL, 0.32 mmol) and triethylsilane (10 uL, 0.06 mmol) were added at 0 °C. The reaction was then warmed to room temperature and stirred for overnight. The reaction was then concentrated in vacuo and after flash-silica chromatography (5% MeOH: 95% CH_2Cl_2 to 10% MeOH: 90% CH_2Cl_2), an amorphous solid, compound **10** (23 mg), was obtained in 72% yield. $[\alpha]_D^{24}$= + 70.0° (*c*. 0.18, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.64 (d, *J* = 7.8 Hz, 1H, H-6), 7.10 (dd, *J* = 15.6, 4.2 Hz, 1H, H-6'), 7.00 (dd, *J* = 15, 1.2 Hz, 1H, H-5'), 5.94 (d, *J* = 7.8 Hz, 1H, H-5), 5.88 (d, *J* = 3Hz, 1H, H-1'), 4.81-4.77 (m, 2H, CH of isopropyls), 4.63-4.61 (app m, 1H, H-4'), 4.22 (dd, *J* = 4.8, 3Hz, 1H, H-2'), 4.11 (dd, *J* = 6.6, 5.4 Hz, 1H, H-3'), 4.04 (d, *J* = 17.4 Hz, 2H, PCH_2S), 1.36 (d, *J* = 7.8 Hz, 12H, isopropyls). NH_2 and 2 hydroxyls were exchanged with deuterium. ^{13}C NMR (150 MHz, CD_3OD): δ 167.7, 158.1, 145.5, 143.2, 131.5, 106.2, 96.5, 94.0, 82.1, 75.1, 74.5, 74.33, 74.29, 24.4, 24.3, 24.09, 24.05. HRMS (ESI+) *m/z* calcd for $C_{17}H_{29}N_3O_9PS$ $(M + H)^+$ is 482.1362, found $(M + H)^+$ 482.1347.



11

Diisopropyl-(*2*-((*2R,3S,4S,5R*)-5-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)ethylsulfonyl)methylphosphonate (11). To a stirred solution of compound **10** (18 mg, 0.04 mmol) in dry THF and MeOH (1:1,

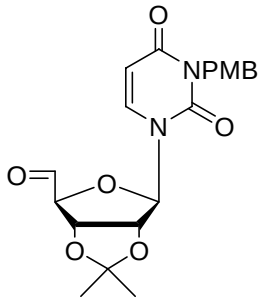
1 mL) was added 10% Pd/C (10 mg). Hydrogen gas in a balloon was introduced to the reaction vessel. The solution was stirred for 2 h at room temperature at 1 atm under a hydrogen atmosphere. The solution was then filtered through celite and washed three times with MeOH (3 X 3 mL). Flash-silica chromatography (5% MeOH: 95% CH_2Cl_2 to 10% MeOH: 90% CH_2Cl_2) to obtain an amorphous solid, **11** (12 mg), in 67 % yield. $[\alpha]_D^{24}$ = + 39.3° (*c*. 0.21, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.61 (d, *J* = 7.2 Hz, 1H, H-6), 5.90 (d, *J* = 7.8 Hz, 1H, H-5), 5.74 (d, *J* = 3Hz, 1H, H-1'), 4.81-4.76 (m, 2H, CH of isopropyls), 4.16 (dd, *J* = 5.4, 3 Hz, 1H, H-2'), 3.99-3.91 (app m, 1H, H-4'), 3.92 (dd, *J* = 6.6, 6 Hz, 1H, H-3'), 3.58-3.53 (m, 1H, H-6'), 3.47-3.42 (m, 1H, H-6'), 2.32-2.27 (m, 1H, H-5'), 2.18-2.13 (m, 1H, H-5'), 1.34 (d, *J* = 6.6 Hz, 12H, isopropyls). NH_2, PCH_2S and 2 hydroxyls were exchanged with deuterium. ^{13}C NMR (150 MHz, CD_3OD): δ 167.7, 158.3, 143.2, 96.3, 93.8, 82.4, 75.5, 74.6, 74.4, 74.36, 74.33, 52.6, 26.4, 24.37, 24.36, 24.06, 24.02. HRMS (ESI+) *m/z* calcd for $C_{17}H_{31}N_3O_9PS$ $(M + H)^+$ is 484.1519, found $(M + H)^+$ 484.1506.



12

(2-((*2R,3S,4S,5R*)-5-(4-amino-2-oxo-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetra hydrofuran-2-yl)-ethylsulfonyl)methylphosphonic acid (12). Compound **11** (10 mg, 0.02 mmol) was dissolved in CH_2Cl_2 (0.2 mL) and TMSBr (390 uL, 2.6

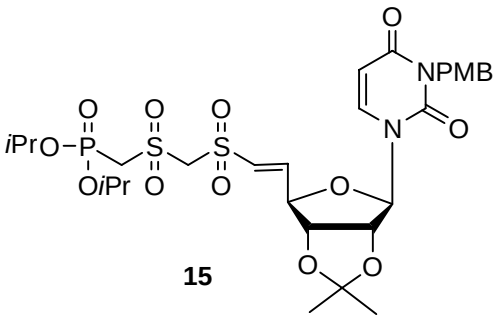
mmol) was added at 0 ºC via a syringe. The reaction was warmed to room temperature and stirred for 36 h after which the reaction was concentrated in vacuo. MeOH (1 mL) was added, stirring was continued for 40 min, and then the reaction was concentrated in vacuo. The residue was co-evaporated with MeOH (3 X 1 mL) three more times. The mixture was then subjected to RP HPLC (water:methanol) to give compound **12** (7 mg, 84%). $[\alpha]_D^{31}$= + 2.3° (*c*. 0.3, MeOH). ^{1}H (600 MHz, CD_3OD): δ 7.71 (d, *J* = 7.8 Hz, 1H, H-6), 6.06 (d, *J* = 7.8 Hz, 1H, H-5'), 5.64 (d, *J* = 4.2 Hz, 1H, H-1'), 4.39 (dd, *J* = 5.4, 4.2 Hz, 1H, H-2'), 4.17-4.14 (m, 1H, H-4'), 4.07 (app t, *J* = 6 Hz, 1H, H-3'), 3.69 (d, *J* = 15.5 Hz, 2H, PCH_2S), 3.56-3.50 (m, 2 H, H-6'), 2.35-2.30 (m, 1H, H-5'), 2.24-2.19 (m, 1H, H-5'). NH_2 and 2 hydroxyls were exchanged with deuterium. ^{13}C NMR (150 MHz, D_2O): δ 159.5, 148.6, 144.6, 95.2, 91.5, 81.8, 73.6, 72.7, 51.4 (d, *J*= 120.8 Hz), 50.5, 25.1. HRMS (ESI+) *m/z* calcd for $C_{11}H_{19}N_3O_9PS$ $(M + H)^+$ is 400.0580, found $(M + H)^+$ 400.0602.



14

(*3aR,4S,6R,6aS*)-6-(3-(4-methoxybenzyl)-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)-tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]dioxole-4-carbaldehyde (14). To a stirred solution of Dess-Martin periodinane (1.76 g, 4.17 mmol) in dry

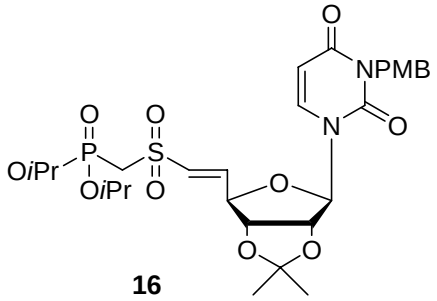
dichloromethane (7 mL), a solution of compound **13**[4] (844 mg, 2.08 mmol) in dry dichloromethane (5 mL) was added dropwise over 5 min. at room temperature under argon. TLC monitoring showed no more starting material after 3 h. The solvent was removed under vacuum. The residue was taken up in ethyl acetate (50 mL), washed with 5% Na_2SO_3 solution (30 mL), saturated aqueous $NaHCO_3$ followed by water (30 mL), and brine (15 mL) and dried over Na_2SO_4 to obtain crude yellow oil. The product was placed on a flash silica gel column and eluted, respectively, with 1:1, 3:2, and 4:l ethyl acetate-hexanes, to afford 80% of the aldehyde **14**. $[\alpha]_D^{24}$= - 14.3^o (*c*. 0.07, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.24 (s, 1H, H-5' aldehyde peak), 7.37 (d, *J* = 8.6 Hz, 2H, phenyl), 7.16 (d, *J* = 8.0 Hz, 1H, H-6), 6.83 (d, *J* = 8.6 Hz, 2H, phenyl), 5.78 (d, *J* = 8.0 Hz, 1H, H-5), 5.44 (s, 1H, H-1'), 5.26 (d, *J* = 6.0 Hz, 1H, H-4'), 5.08 (d, *J* = 12.0 Hz, 1H, H-3'), 5.03 (d, *J* = 12.0 Hz, 1H, NCH), 4.88 (d, *J* = 12.0 Hz, 1H, NCH), 4.53 (s, 1H, H-2'), 3.78 (s, 3H, OCH_3), 1.53 (s, 3H, isopropylidene), 1.37 (s, 3H, isopropylidene). ^{13}C NMR (150 MHz, $CDCl_3$): δ 199.6, 162.4, 159.5, 151.3, 141.8, 136.3, 131.6, 131.2, 129.5, 128.5, 113.9, 102.8, 101.3, 94.4, 85.2, 84.4, 55.5, 43.7, 26.6, 25.0. HRMS (MALDI) *m/z* calcd for $C_{20}H_{22}N_2O_7$ $[M + H]^+$ is 403.1505, found $[M + H]^+$ 403.1449.



15

Diisopropyl-(*E*)-2-(*3aR,4R,6R,6aS*)-6-(3-(4-methoxybenzyl)-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2,2-dimethyl-tetrahydro-furo[3,4-*d*][1,3]dioxol-4-yl) vinylsulfonyl)methylsulfonyl)methylphosphonate (15). To a stirred solution of disulfone reagent **2**[1] (357 mg, 0.71 mmol), *N,N*-diisopropylethylamine (148.5 μL, 0.85 mmol), and lithium bromide (0.85 mmol) in dry THF (15 mL) was added dropwise under argon compound **14** (114.6 mg, 0.28 mmol) in dry THF and stirred for 2.5 h at r. t. The reaction mixture was acidified with diluted (1:1) acetic acid, extracted with ethyl acetate, washed with water, dried over sodium sulfate, and chromatographed on silica-gel (hexane-ethyl acetate 2:8) to afford 64% of the main product **15**. $[\alpha]_D^{24}$= + 24.5^o (*c*. 0.51, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40 (d, *J* = 9.0 Hz, 2H, phenyl), 7.13 (dd, *J* = 15.6, 4.2 Hz, 1H, H-6'), 7.10 (d, *J* = 7.8 Hz, 1H, H-6), 6.84 (d, *J* = 9.0 Hz, 2 H, phenyl), 6.79 (dd, *J* = 15.6, 1.8 Hz, 1H, H-5'), 5.78 (d, *J* = 7.8 Hz, 1H, H-5), 5.55 (s, 1H, H-1'), 5.11-4.77 (m, 9H, NCH_2, SCH_2S, H-2', H-3', H-4', CH of isopropyls), 4.04-3.85 (m, 2H, PCH_2S), 3.77 (s, 3H, OCH_3), 1.55 (s, 3H, isopropylidene), 1.34-1.37 (m, 15H, isopropyls and isopropylidenes). ^{13}C NMR (150 MHz, $CDCl_3$): δ 162.5, 159.4, 150.9, 147.7, 141.0, 130.9, 128.8, 128.7, 114.9, 114.1, 103.0, 97.6, 86.9, 84.8, 84.3, 77.6, 73.62, 73.58, 73.53, 70.5, 55.5, 51.9, 43.9, 27.3, 25.5, 24.39, 24.37,

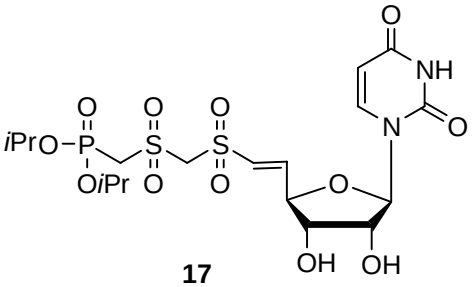
23.97, 23.92. HRMS (MALDI) *m/z* calcd for $C_{29}H_{41}N_2O_{13}PS_2$ $[M + Na]^+$ is 743.1685, found $[M + Na]^+$ 743.1905.



16

Diisopropyl ((*E*)-2-((*3aS,4R,6R,6aS*)-6-(3-(4-methoxybenzyl)-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2,2-dimethyl-tetrahydro-furo[3,4-*d*][1,3]dioxol-4-yl)-vinylsulfonyl)methylphosphonate (16). To a stirred solution of monosulfone reagent **3**[3] (280.4 mg, 0.56 mmol), *N,N*-diisopropylethylamine (98 μL, 0.57 mmol), and lithium bromide (0.57 mmol) in dry THF (5 mL) was added dropwise under argon compound **14** (73 mg, 0.19 mmol) in dry THF and stirred for 6 h at r. t. The reaction mixture was acidified with diluted 2 mL (1:1) acetic acid and water, extracted with ethyl acetate, washed with water, dried over sodium sulfate, and chromatographed on silica-gel (hexane-ethyl acetate 2:8) to afford 67% of the main product **16**. $[\alpha]_D^{24}$= + 21.7° (*c.* 0.06, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (d, *J* = 9.0 Hz, 2H, phenyl), 7.16 (d, *J* = 8.4 Hz, 1H, H-6), 7.06 (dd, *J* = 15.6, 5.4 Hz, 1H, H-6'), 6.88 (dd, *J* = 15.6, 1.8 Hz, 1H, H-5'), 6.84 (d, *J* = 9.0 Hz, 2H, phenyl), 5.77 (d, *J* = 7.8 Hz, 1H, H-5), 5.65 (d, *J* = 1.8 Hz, 1H, H-1'), 5.05-4.73 (m, 7H, NCH_2, H-2', H-3', CH of isopropyls, H-4'), 3.76 (s, 3H, OCH_3), 3.52 (dd, *J* = 16.2, 4.2 Hz, 2H, PCH_2S), 1.55 (s, 3H, isopropylidene), 1.32-1.36 (m, 15H, isopropyls and isopropylidenes). ^{13}C NMR (150 MHz, $CDCl_3$):

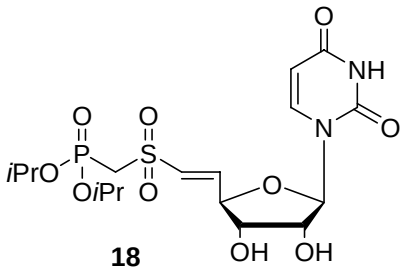
δ 162.5, 159.4, 150.8, 144.5, 140.7, 131.1, 130.6, 128.8, 115.1, 114.1, 102.9, 96.4, 86.3, 84.8, 84.2, 73.16, 73.14, 73.09, 55.5, 54.2, 53.3, 43.9, 27.3, 25.6, 24.33, 24.30, 23.99, 23.98. HRMS (MALDI) *m/z* calcd for $C_{28}H_{39}N_2O_{11}PS$ [M + Na]$^+$ is 665.1910, found [M + Na]$^+$ 665.2191.



17

Disopropyl-((*E*)-2-((*2R,3S,4S,5R*)-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydro-furan-2-yl)vinylsulfonyl)methylsulfonyl)methyl phosphonate (17). To a solution of **15** (474 mg, 0.64 mmol) in 46.9 mL of acetonitrile was added ceric ammonium nitrate (1.74 g, 3.17 mmol) in 4.69 mL of water, and the mixture was heated to reflux for 30 min., evaporated the solvent and then diluted with water, and extracted with ethyl acetate (3 X 5 mL). Flash chromatography over silica gel (ethyl acetate:MeOH:water = 6:0.5:0.5) afforded **17** in 65% yield. The residue was purified on reverse phase HPLC using methanol and water. $[\alpha]_D^{24}$= + 47.1^o (*c*. 0.03, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.59 (d, *J* = 8.4 Hz, 1H, 6-H), 7.17 (dd, *J* = 15.6, 5.4 Hz, 1H, H-6'), 6.95 (dd, *J* = 15.6, 1.8 Hz, 1H, H-5'), 5.90 (d, *J* = 1.7 Hz, 1H, H-1'), 5.73 (d, *J* = 8.4 Hz, 1H, H-5), 4.80 (m, 2H, CH of isopropyl), 4.65 (m, 1H, H-2'), 4.23 (m, 1H, H-4'), 4.15 (m, 1H, H-3'), 1.36 (d, *J* = 5.4 Hz, 12H, isopropyls). Hydrogens between P and S (PCH_2S) and S and S (SCH_2S) were exchanged with deuterium

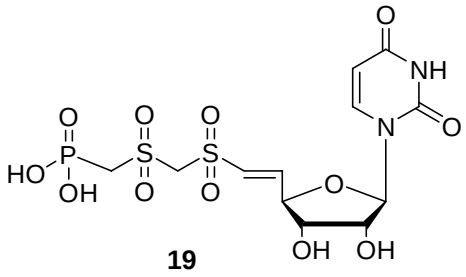
and not observed. ^{13}C NMR (150 MHz, CD_3OD): δ 164.8, 151.0, 147.1, 141.6, 128.4, 102.2, 91.2, 81.5, 73.66, 73.64, 73.18, 73.07, 70.5, 50.2 (d), 23.18, 23.15, 22.84, 22.81. HRMS (MALDI) *m/z* calcd for $C_{18}H_{29}N_2O_{12}PS_2$ $[M + H]^+$ is 561.0978, found $[M + H]^+$ 561.0966.



18

Diisopropyl ((*E*)-2-((*2R,3R,4S,5R*)-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]-vinylsulfonyl)methylphosphonate (18). To a solution of **16** (145 mg, 0.23 mmol) in 13 mL of acetonitrile was added ceric ammonium nitrate (652 mg, 1.19 mmol) in 1.3 mL of water, and the mixture was heated to reflux for 30 min. The solvent was then evaporated; the residue was diluted with water, and extracted with ethyl acetate (3X5 mL). Flash chromatography over silica gel (ethyl acetate:MeOH:water = 6:0.5:0.5) afforded **18** in 72% yield. The residue was purified on reverse phase HPLC using methanol and water. $[\alpha]_D^{24}$= + 20.0^o (*c*. 0.03, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.64 (d, *J* = 8.4 Hz, 1H, H-6), 7.05 (dd, *J* = 15.6, 5.4 Hz, 1H, H-6'), 6.95 (dd, *J* = 15.6, 1.8 Hz, 1H, H-5'), 5.86 (d, *J* = 1.7 Hz, 1H, H-1'), 5.73 (d, *J* = 8.4 Hz, 1H, H-5), 4.79 (m, 2H, CH of isopropyl), 4.58 (m, 1H, H-4'), 4.28 (m, 1H, H-2'), 4.14 (m, 1H, H-3'), 4.01 (d, *J* = 16.8 Hz, 2H, SCH_2P), 1.34-1.36 (d, *J* = 6.0 Hz, 12H, isopropyls). ^{13}C NMR (150 MHz, CD_3OD): δ 164.8, 150.9, 144.1, 142.0,

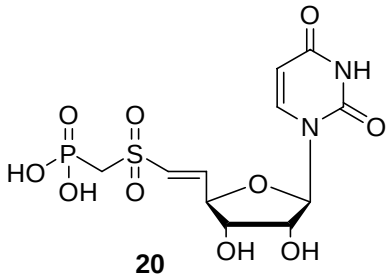
130.3, 102.0, 91.7, 81.3, 73.24, 73.15, 73.12, 73.07, 23.17, 23.15, 22.89, 22.88. A carbon between P and S is not observed due to exchanged with deuterium. HRMS (MALDI) *m/z* calcd for $C_{17}H_{27}N_2O_{10}PS$ $[M + H]^+$ is 483.1202, found $[M + H]^+$: 483.1213.



19

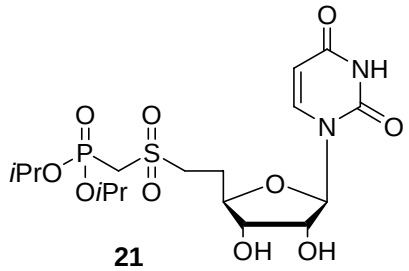
(*E*)-2-((*2R,3S,4S,5R*)-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)vinylsulfonyl)methylsulfonyl)methyl phosphonic acid (19). To a stirred solution of **17** (33 mg) in dichloromethane was added TMSBr (0.11 mL) drop wise at 0 °C under argon. The reaction mixture was stirred overnight at room temperature, and then it was evaporated. Working up with methanol gave compound **19** in quantitative yields. The residue was purified on reverse phase HPLC using methanol and water to give 76% of the desired product **19**. $[\alpha]_D^{24}$= + 47.0° (*c.* 0.03, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.60 (d, *J* = 8.4 Hz, 1H, H-6), 7.16 (dd, *J* = 15.6, 5.4 Hz, 1H, H-6'), 6.95 (dd, *J* = 15.6, 1.8 Hz, 1H, H-5'), 5.94 (d, *J* = 1.7 Hz, 1H, H-1'), 5.75 (d, *J* = 8.4 Hz, 1H, H-5), 5.37 (s, 2H, SCH_2S), 4.65 (m, 1H, H-4'), 4.23 (t, 1H, H-2'), 4.15 (t, 1H, H-3'), 4.09 (d, *J*= 16.2 Hz, 2H, PCH_2S). ^{13}C NMR (150 MHz, CH_3OD): δ 164.9, 151.0, 146.9, 141.6, 128.7, 102.2, 91.2, 81.5, 73.2, 73.0. Carbons between P and S and also carbons between S and S are not observed due to exchanged with

deuterium. Low intensity doublet is seen around 50.3 for carbons between P and S. HRMS *m/z* calcd for $C_{12}H_{17}N_2O_{12}PS_2$ $[M + H]^+$ is 477.0039, found $[M + H]^+$ 477.0031.



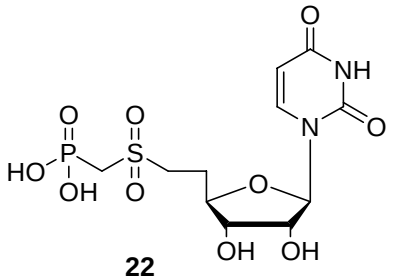
20

(*E*)-2-((*2R,3R,4S,5R*)-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-3,4-dihydro xy-tetrahydrofuran-2-yl)vinylsulfonyl)methylphosphonic acid (20). To a stirred solution of **18** (60 mg) in dichloromethane was added TMSBr (0.23 mL) drop wise at 0 °C under argon. The reaction mixture was stirred overnight at room temperature, and then it was evaporated. Working up with methanol gave compound **20** in quantitative yields. The residue was purified on reverse phase HPLC using methanol and water to give 80% of the desired product. $[\alpha]_D^{24}$= + 33.3° (*c*. 0.02, MeOH). ^{1}H NMR (600 MHz, CH_3OD): δ 7.55 (d, *J* = 8.4 Hz, 1H, H-6), 6.98 (dd, *J* = 15.6, 5.4 Hz, 1H, H-6'), 6.88 (dd, *J* = 15.6, 1.8 Hz, 1H, H-5'), 5.81 (d, *J* = 1.7 Hz, 1H, H-1'), 5.66 (d, *J* = 8.4 Hz, 1H, H-5), 4.51 (m, 1H, H-4'), 4.17 (m, 1H, H-2'), 4.05 (m, 1H, H-3'), 3.72 (dd, *J* = 16.2, 3.8 Hz, 2H, SCH_2P). ^{13}C NMR (150 MHz, CD_3OD): δ 164.9, 151.0, 144.1, 141.9, 130.2, 102.1, 91.4, 91.2, 81.4, 73.2, 48.7. HRMS *m/z* calcd for $C_{11}H_{15}N_2O_{10}PS$ $[M + Na]^+$ is 421.0083, found $[M + Na]^+$: 421.0093.



21

Diisopropyl (2-((*2R,3R,4S,5R*)-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)ethylsulfonyl)methylphosphonate (21). Compound **18** in abs. ethanol was hydrogenated (pressure 1 atm, 10% Pd/C) at room temperature for 3 h. Then, the catalyst was removed by filteration through celite, solvent evaporated in vacuum. The residue was chromatographed on silica-gel (ethyl acetate:MeOH:H_2O = 6:0.5:0.5) to afford 43% of the main product **21.** It was purified on reverse phase HPLC using methanol and water. ^{1}H NMR (600 MHz, CH_3OD): δ 7.60 (d, *J* = 12.0 Hz, 1H, H-6), 5.75 (d, *J* = 6.0 Hz, 1H, H-1'), 5.70 (d, *J* = 12.0 Hz, 1H, H-5), 4.78 (m, 2H, CH of isopropyl), 4.24 (m, 1H, H-2'), 3.96 (m, 2H, H-4', H-3'), 3.40-3.54 (m, 2H, H-6'), 2.13-2.29 (m, 2H, H-5'), 1.35 (d, *J*= 6.0 Hz, 12H, isopropyls). Protons between P and S (PCH2S) were exchanged with deuterium and not observed. ^{13}C NMR (150 MHz, CH_3OD): δ 164.9, 151.0, 142.0, 105.0, 101.8, 91.4, 81.7, 73.2 (4-carbons), 48.0, 25.3, 23.2 (2-carbons), 22.9 (2-carbons). HRMS (MALDI) *m/z* calcd for $C_{17}H_{29}N_2O_{10}PS$ $[M + H]^+$ is 485.1359, found $[M + H]^+$ 485.1355.



22

(2-((*2R,3R,4S,5R*)-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)ethylsulfonyl)methylphosphonic acid (22). Compound **20** in abs. ethanol was hydrogenated (pressure 1 atm, 10% Pd/C) at room temperature for 3 h. Then, the catalyst was removed by filtration through celite. The contents were then concentrated in vacuo to give an oil product **22** in 65% yield. The residue was purified on reverse phase HPLC using methanol and water. $[\alpha]_D^{24}$= + 12.5° (*c*. 0.04, MeOH). ^{1}H NMR (600 MHz, CH_3OD): δ 7.51 (d, *J* = 8.4 Hz, 1H, H-6), 5.67 (d, *J* = 1.7 Hz, 1H, H-1'), 5.62 (d, *J* = 8.4 Hz, 1H, H-5), 4.14 (m, 1H, H-2'), 3.88 (m, 1H, H-4'), 4.05 (m, 1H, H-3'), 3.31-3.48 (m, 2H, H-6'), 2.03-2.23 (m, 2H, H-5'). Protons between P and S (PCH2S) were exchanged with deuterium and not observed. ^{13}C NMR (150 MHz, CH_3OD): δ 164.1, 151.0, 141.9, 101.8, 91.3, 81.8, 73.55, 73.29, 50.8, 48.37, 25.4. HRMS (MALDI) *m/z* calcd for $C_{11}H_{17}N_2O_{10}PS$ $[M + H]^+$ is 401.0419, found $[M + H]^+$ 401.0411.

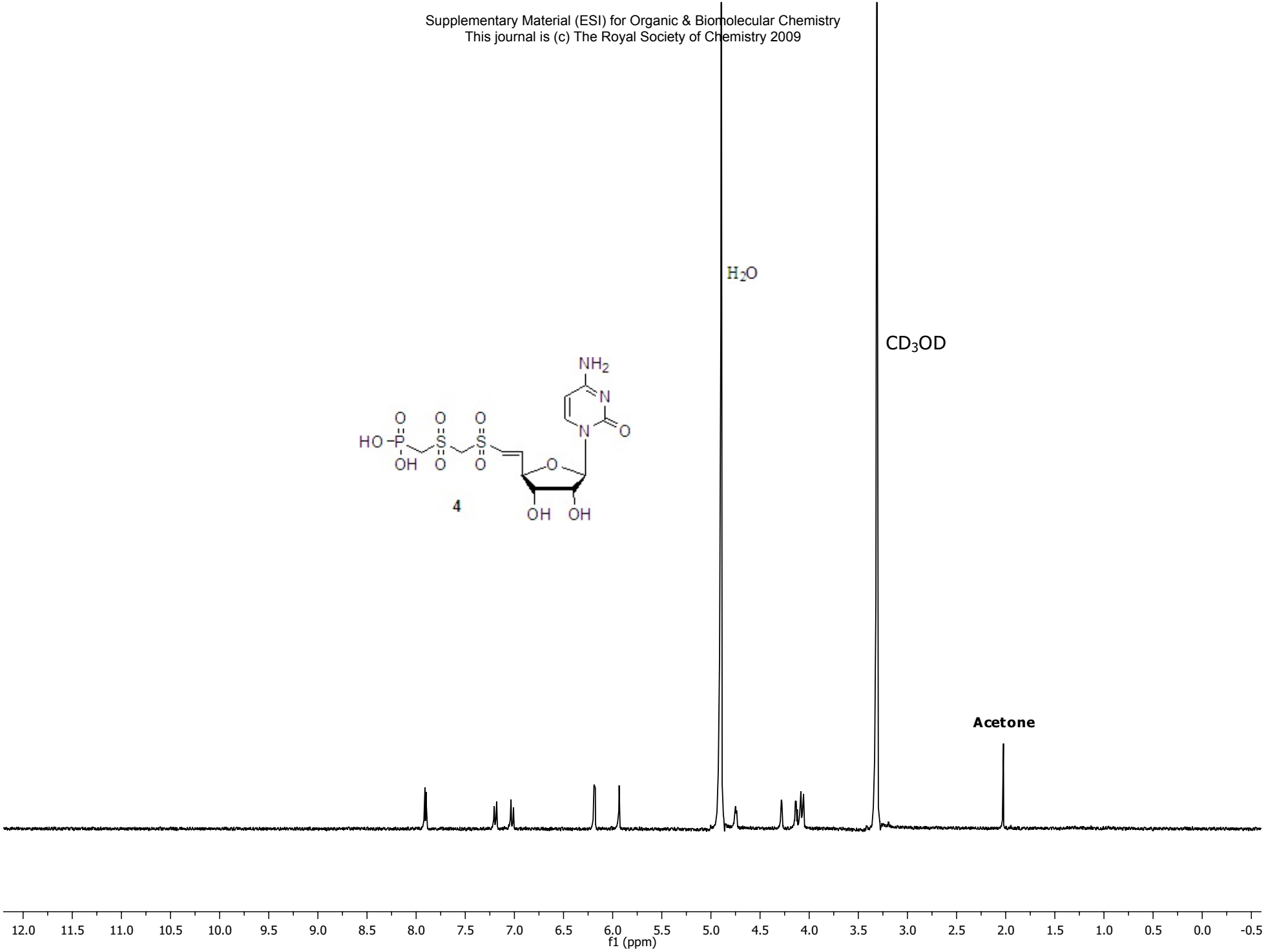
NmCSS Substrate Assay:

The reaction mixtures (20 uL) in Tris-HCl buffer (200 mM, pH 8.5) containing purified *Neisseria meningitidis* CSS recombinant enzyme (0.09 ug),[5] CTP (1 mM), CDP (1 mM), UDP (1 mM), or UTP (1 mM), and *N*-acetylneuraminic acid

(Neu5Ac, 1 mM) and $MgCl_2$ (20 mM) were incubated at 37 °C for 30 min and the reactions were stopped by adding 10% SDS on ice to give a final concentration of 1% SDS. The samples were analyzed using a P/ACE^{TM} MDQ Capillary Electrophoresis System equipped with a UV detector at 200 nm wavelength. The percentage conversion for the formation of CMP-Neu5Ac was calculated based on UV response standard curve of CTP and CMP-Neu5Ac obtained using mixtures of different ratios of CTP and CMP-Neu5Ac (data not shown). The reaction was also carried out at higher enzyme concentrations (7.5 mg, data not shown).

NmCSS Inhibition Assay:

The reaction mixtures (20 uL) in Tris-HCl buffer (200 mM, pH 8.5) containing purified *Neisseria meningitidis* CSS recombinant enzyme (1.25 ng),[5] CTP (1 mM), *N*-acetylneuraminic acid (Neu5Ac, 1 mM), $MgCl_2$ (20 mM), with or without an inhibitor (1 mM) were incubated at 37 °C for 15 min and the reactions were stopped by adding 10% SDS on ice to give a final concentration of 1% SDS. The samples were analyzed using a P/ACE^{TM} MDQ Capillary Electrophoresis System equipped with a UV detector at 254 nm wavelength. The percentage conversion for the formation of CMP-Neu5Ac was calculated based on UV response standard curve of CTP and CMP-Neu5Ac obtained using mixtures of different ratios of CTP and CMP-Neu5Ac (data not shown).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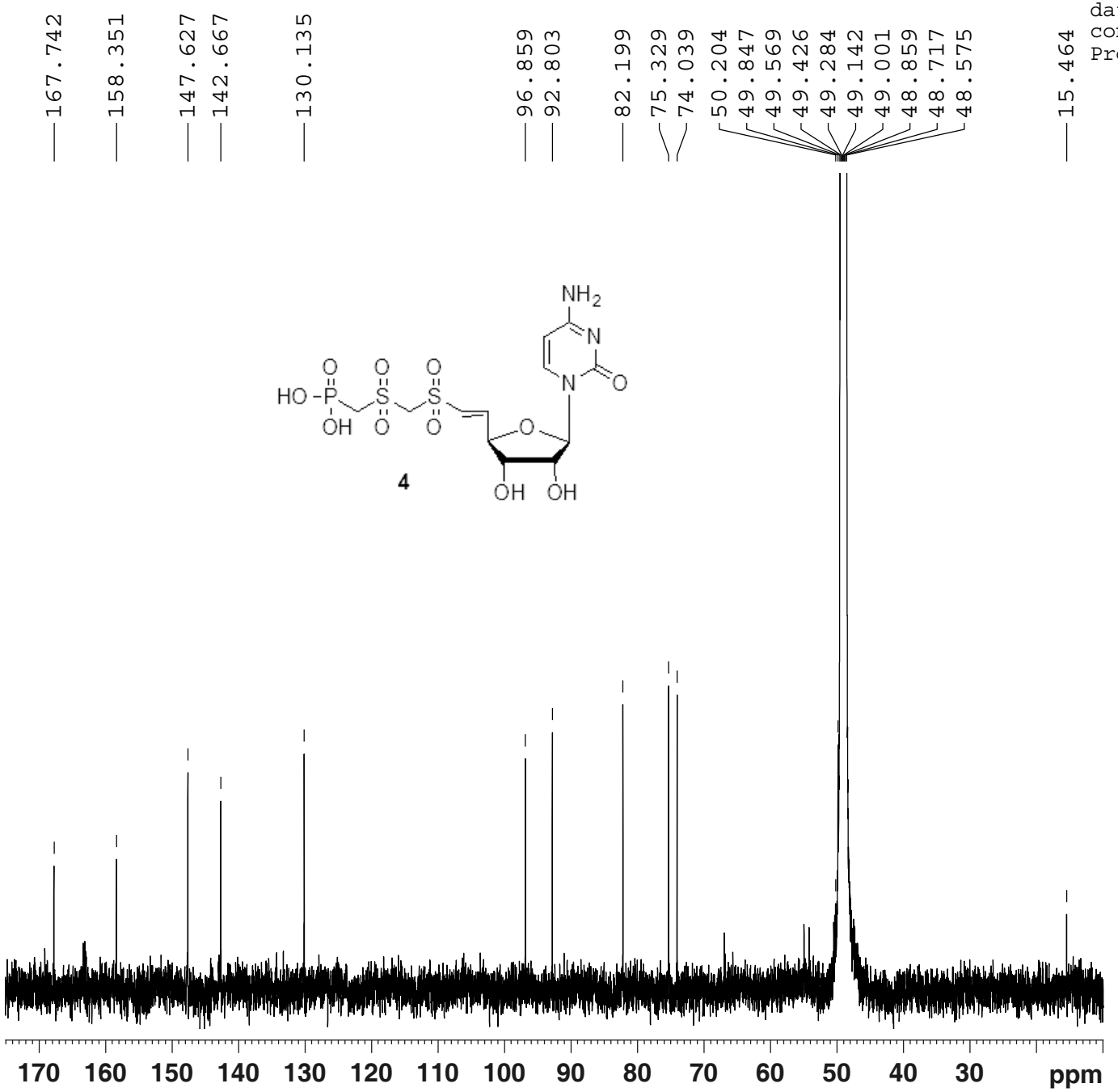


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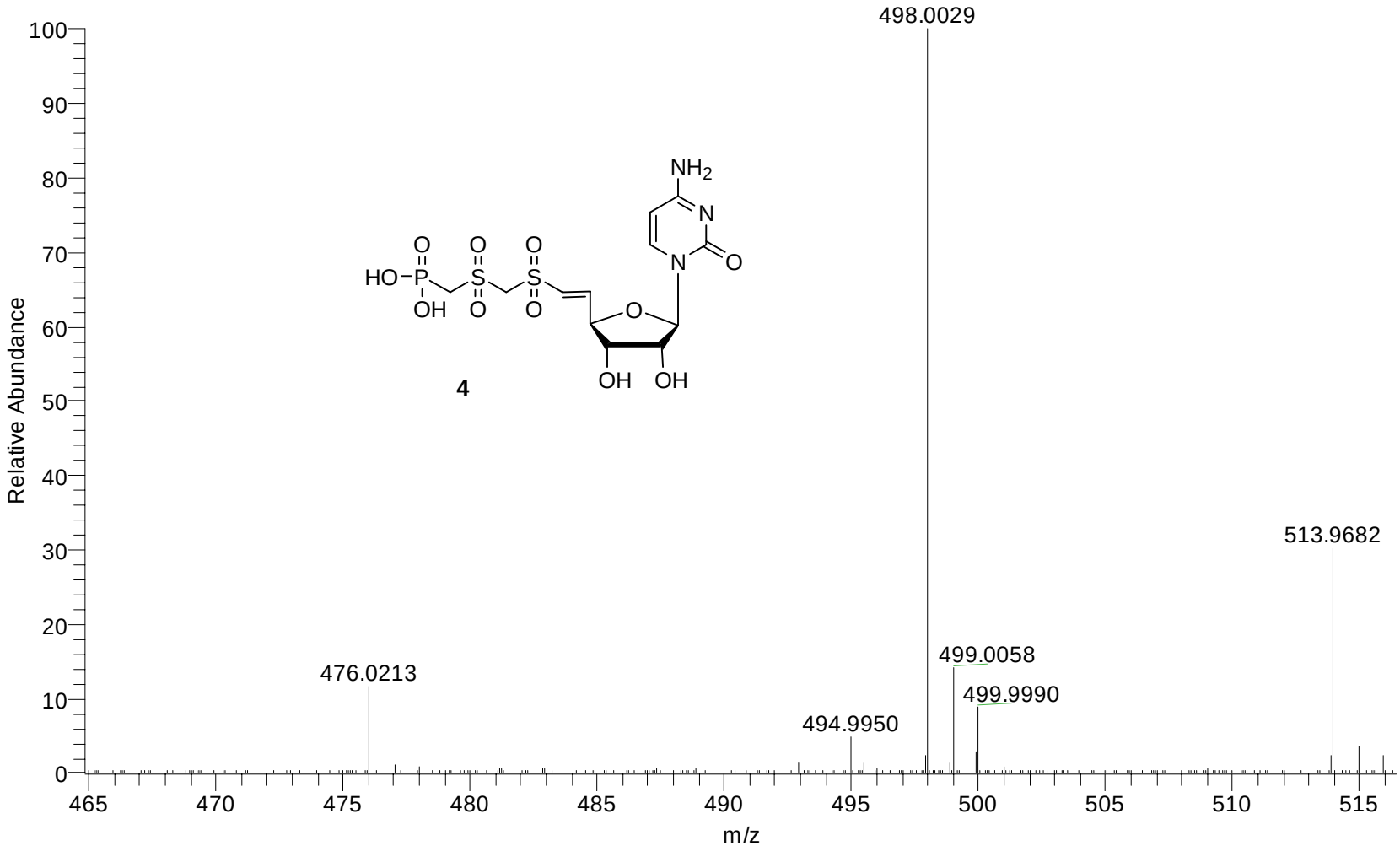
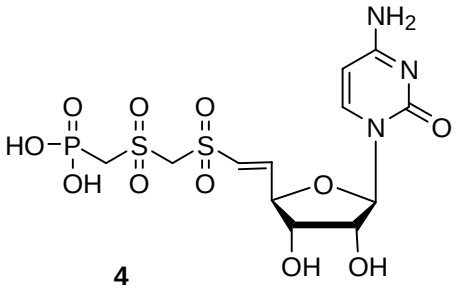
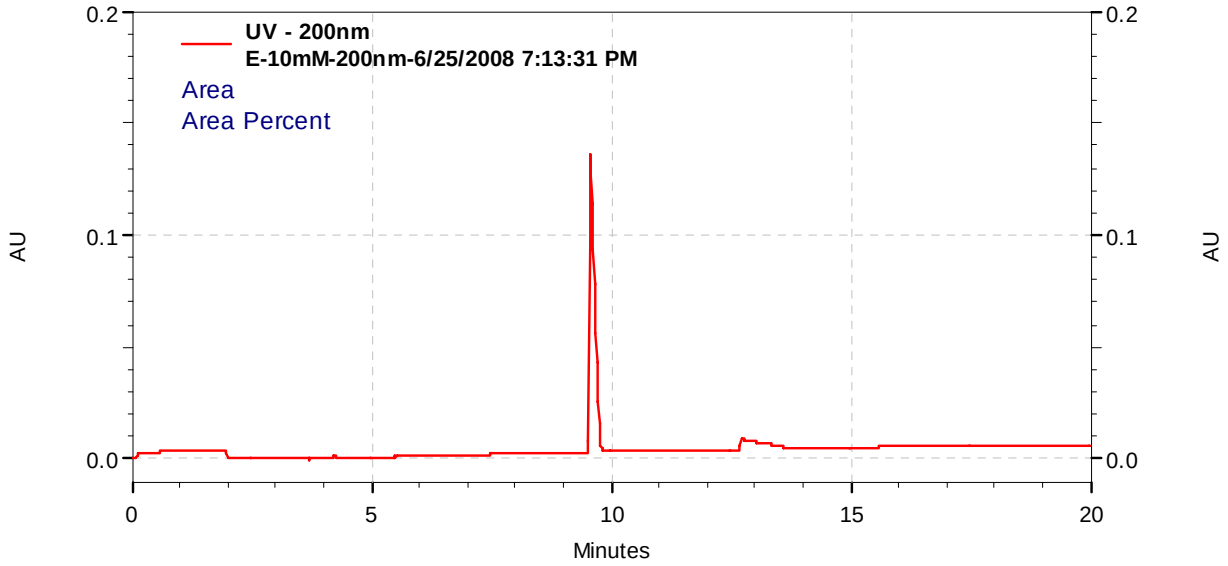
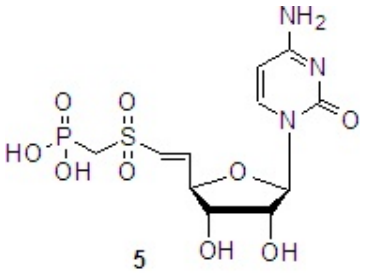


4


NH2
N
N
O
HO
P
OH
O
S
S
O
O
O
O
O
OH
OH

4


UV - 200nm
E-10mM-200nm-6/25/2008 7:13:31 PM
Area
Area Percent
AU
0.2
0.1
0.0
0
5
10
15
20
Minutes


NH2
N
N
O
HO
P
OH
O
S
O
O
O
OH
OH

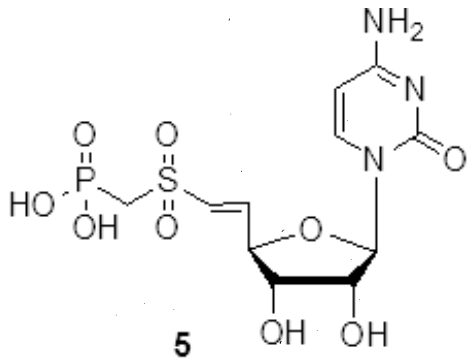
5

H2O
CD3OD
1.00
1.72
0.89
0.91
0.96
0.79
0.79
1.40
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

Std proton

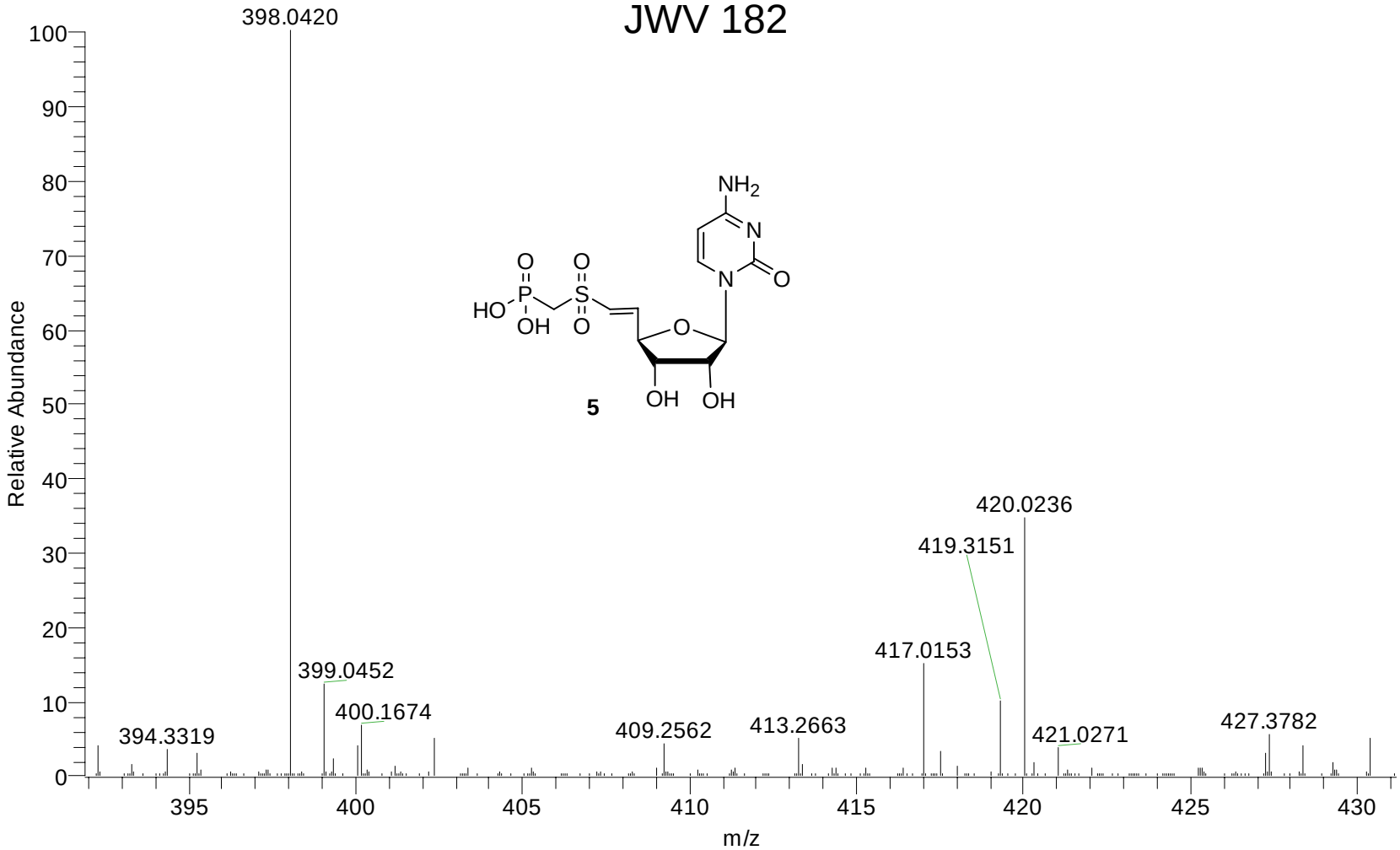
Automation directory:

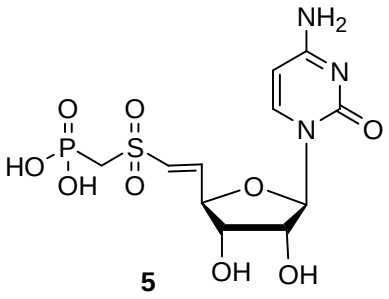
Pulse Sequence: s2pul



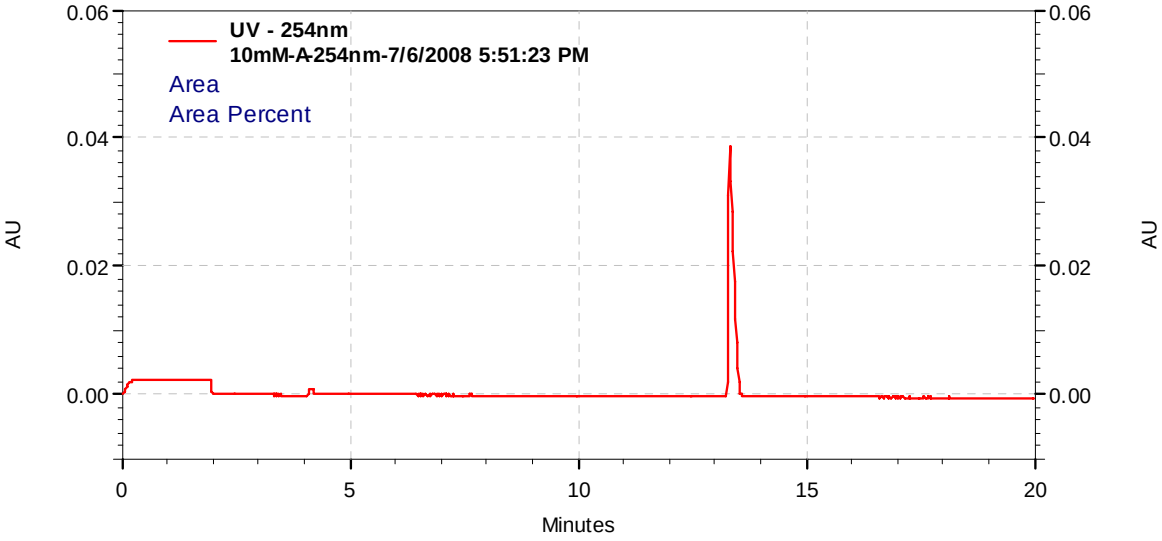
5

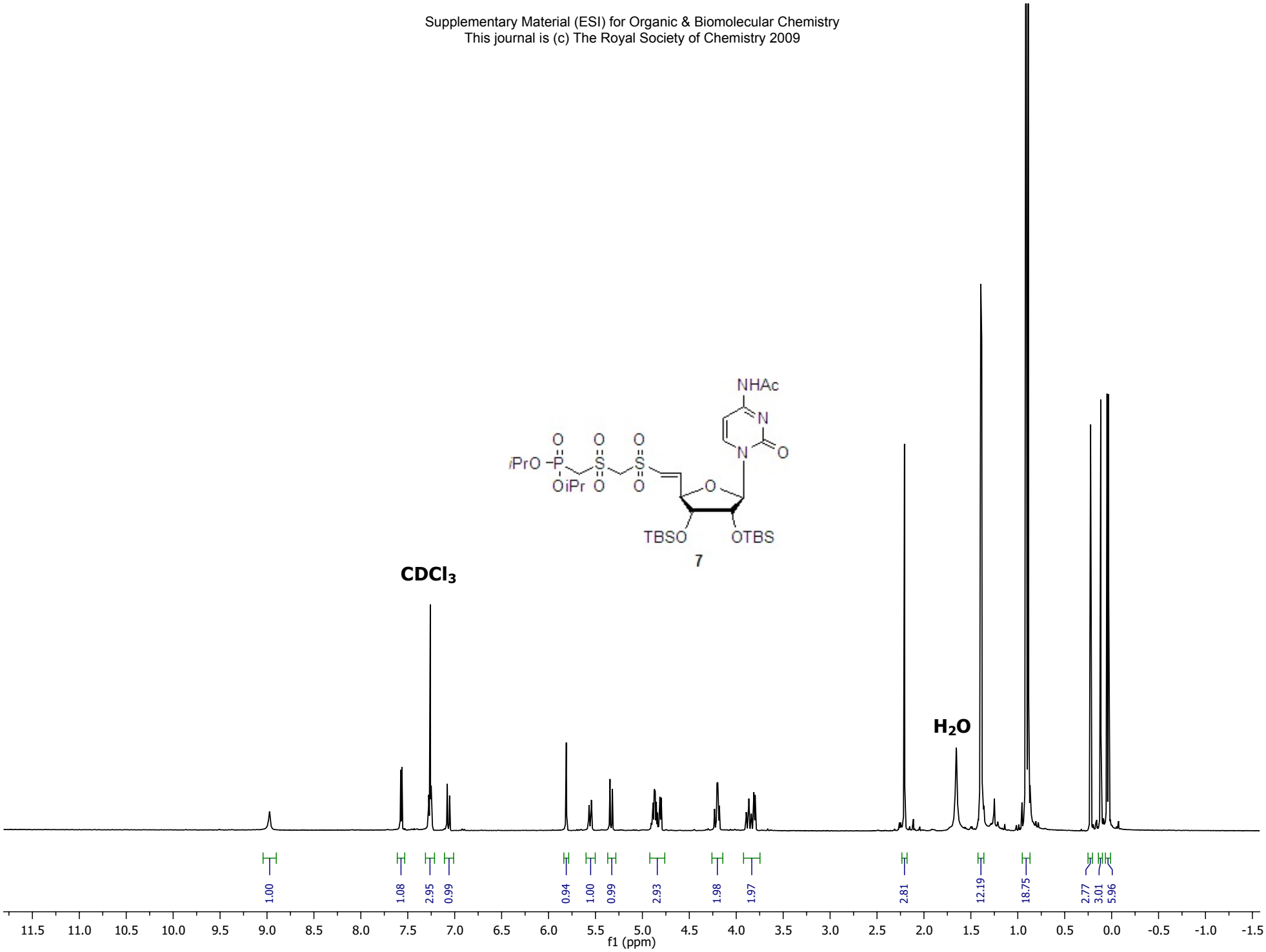
1020-3 #12-19 RT: 0.17-0.27 AV: 8 NL: 2.02E7
T: FTMS + c NSI Full ms [200.00-1000.00]

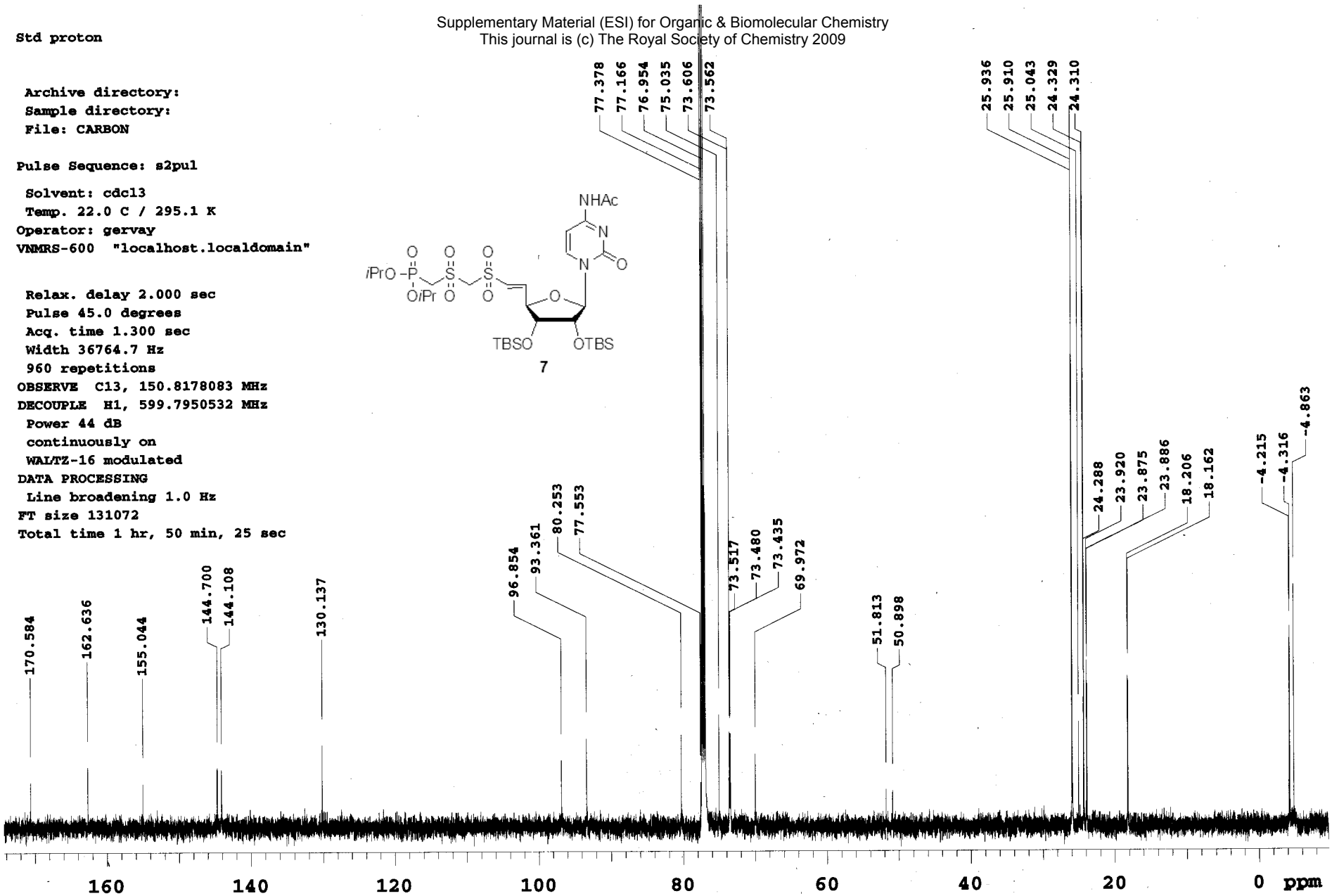



NH2
N
N
O
HO
P
OH
O
O
S
O
O
OH
OH

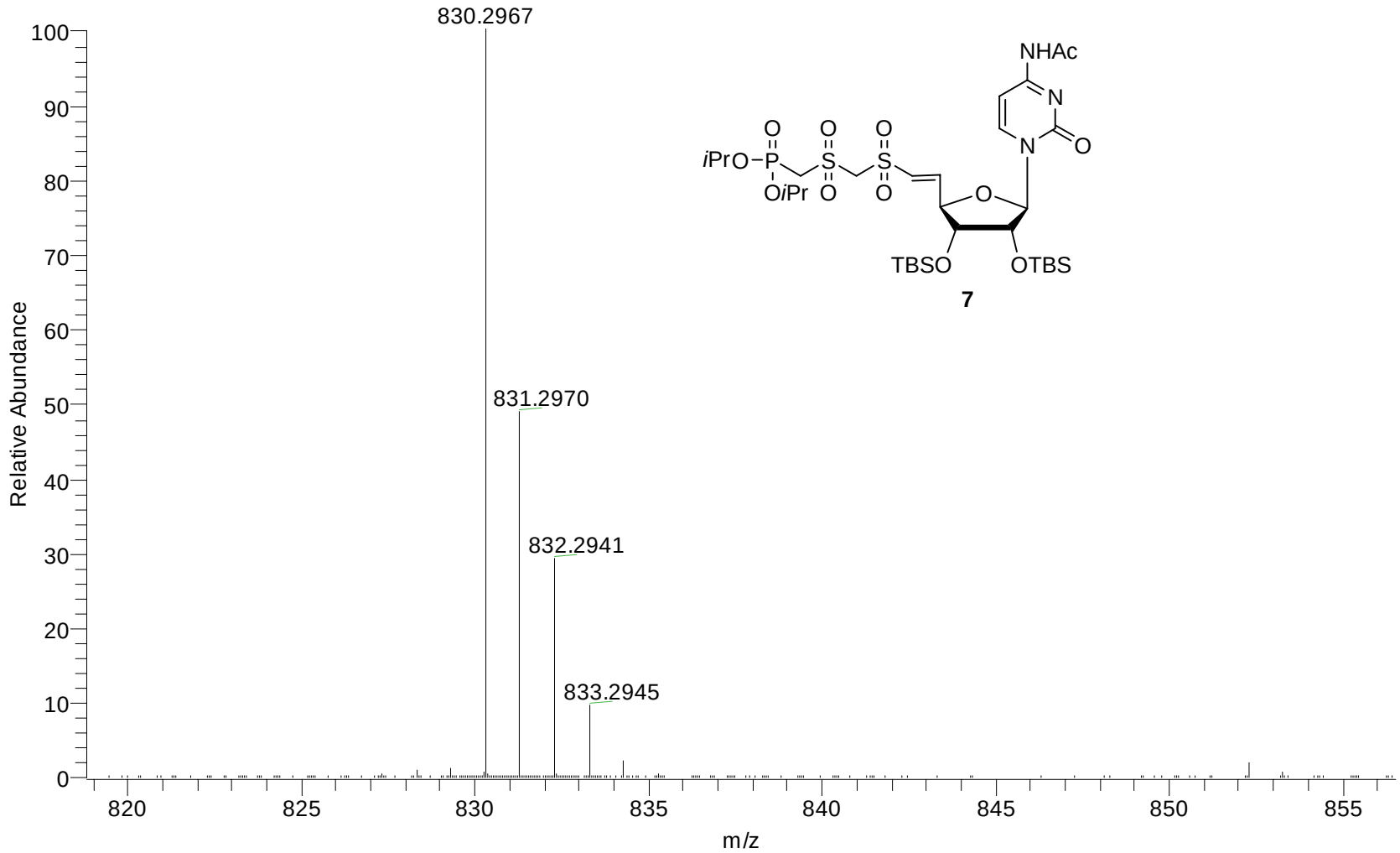
5


UV - 254nm
10mM-A-254nm-7/6/2008 5:51:23 PM
Area
Area Percent
AU
0.06
0.04
0.02
0.00
0
5
10
15
20
Minutes

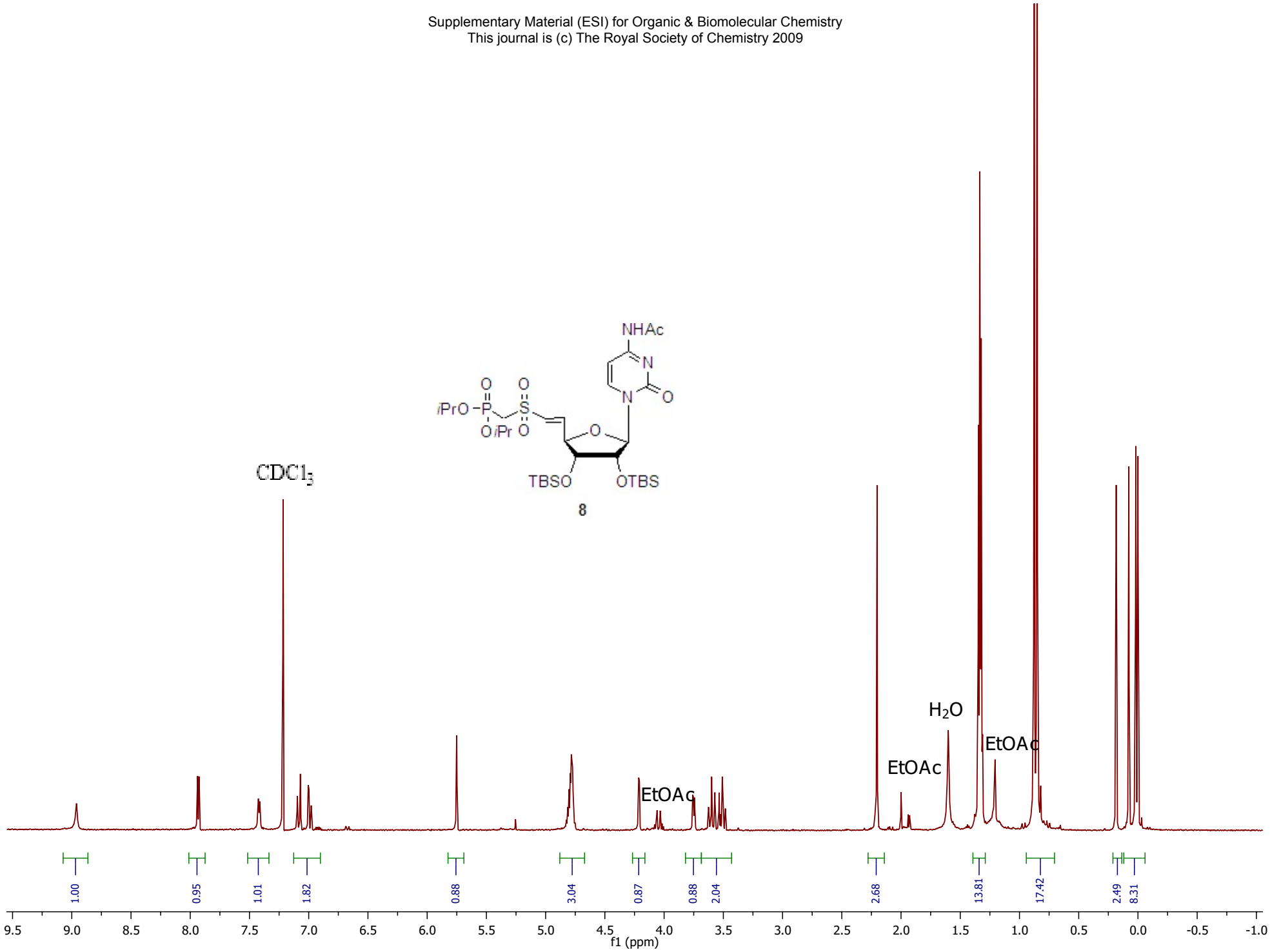

NHAc
N
N
O
iPrO
P
O
OiPr
O
S
O
O
S
O
O
O
TBSO
OTBS
7
CDCl3
H2O
1.00
1.08
2.95
0.99
0.94
1.00
0.99
2.93
1.98
1.97
2.81
12.19
18.75
2.77
3.01
5.96
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
f1 (ppm)

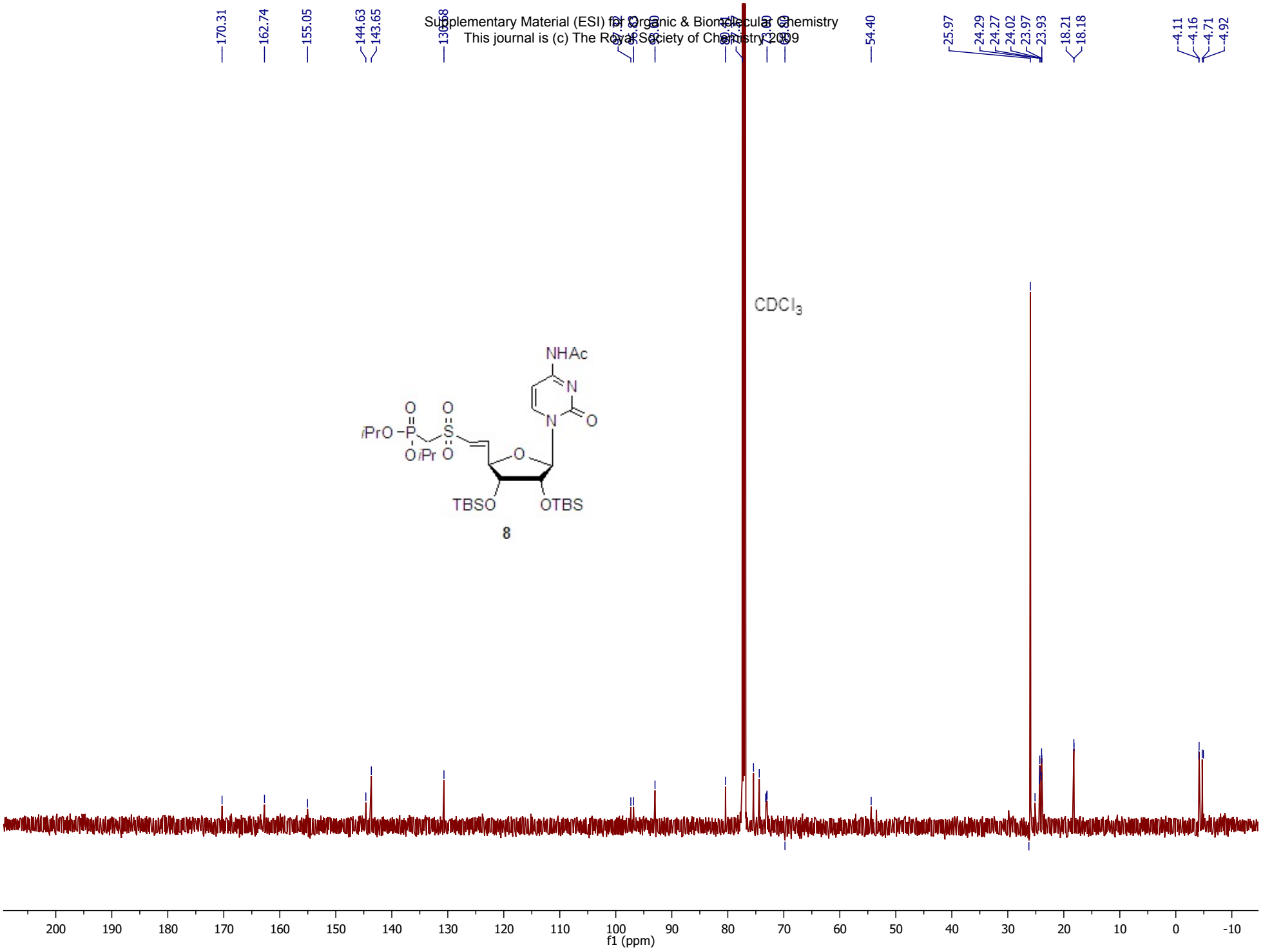

Std proton
Archive directory:
Sample directory:
File: CARBON
Pulse Sequence: s2pul
Solvent: cdcl3
Temp. 22.0 C / 295.1 K
Operator: gervay
VNMRS-600 "localhost.localdomain"
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 36764.7 Hz
960 repetitions
OBSERVE C13, 150.8178083 MHz
DECOUPLE H1, 599.7950532 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 1 hr, 50 min, 25 sec
NHAc
iPrO
OiPr
TBSO
OTBS
7
77.378
77.166
76.954
75.035
73.606
73.562
25.936
25.910
25.043
24.329
24.310
170.584
162.636
155.044
144.700
144.108
130.137
96.854
93.361
80.253
77.553
73.517
73.480
73.435
69.972
51.813
50.898
24.288
23.920
23.875
23.886
18.206
18.162
-4.215
-4.316
-4.863
160
140
120
100
80
60
40
20
0
ppm

1062 #15-19 RT: 0.23-0.29 AV: 5 NL: 3.86E7
T: FTMS + c ESI Full ms [250.00-1000.00]

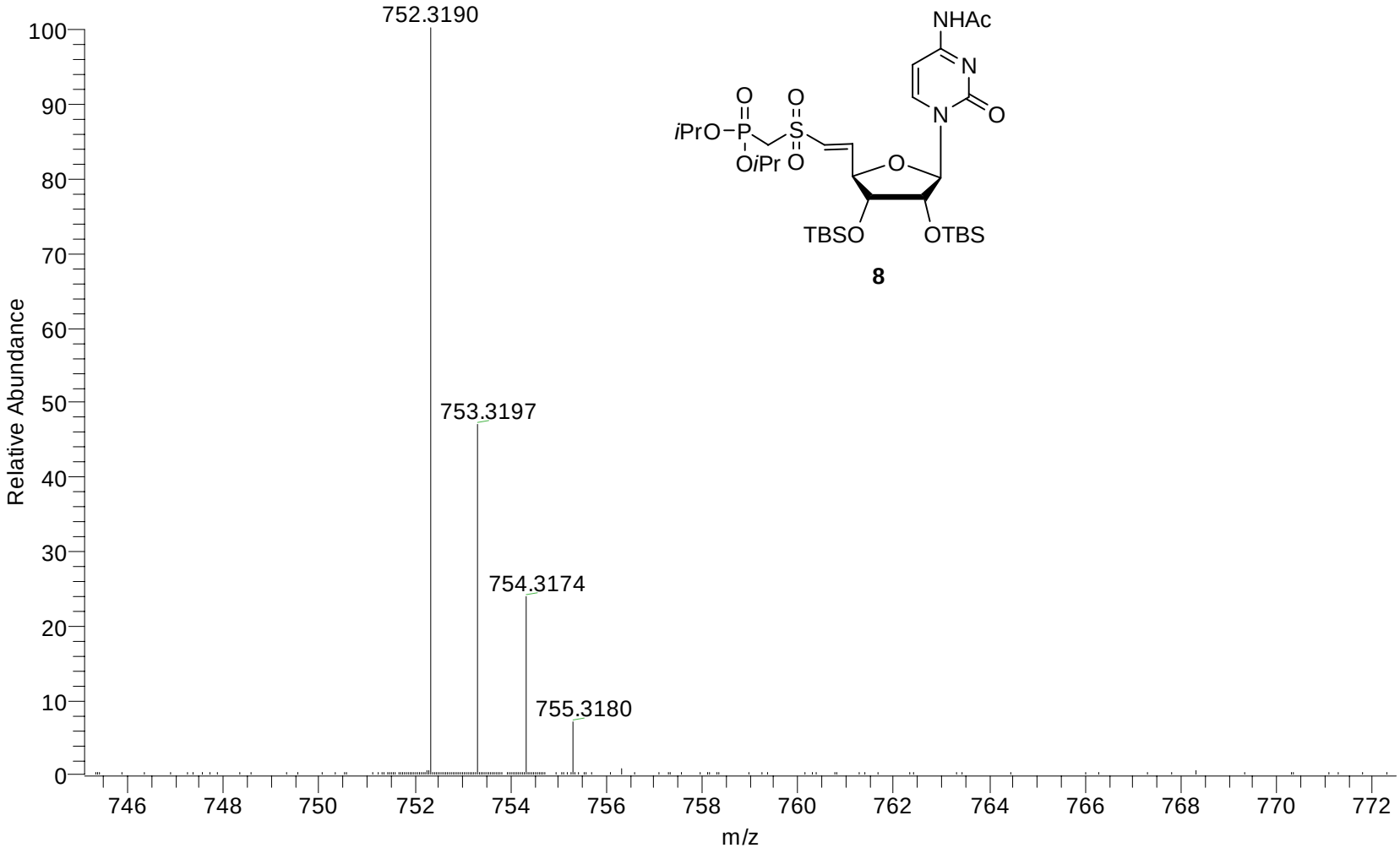


7

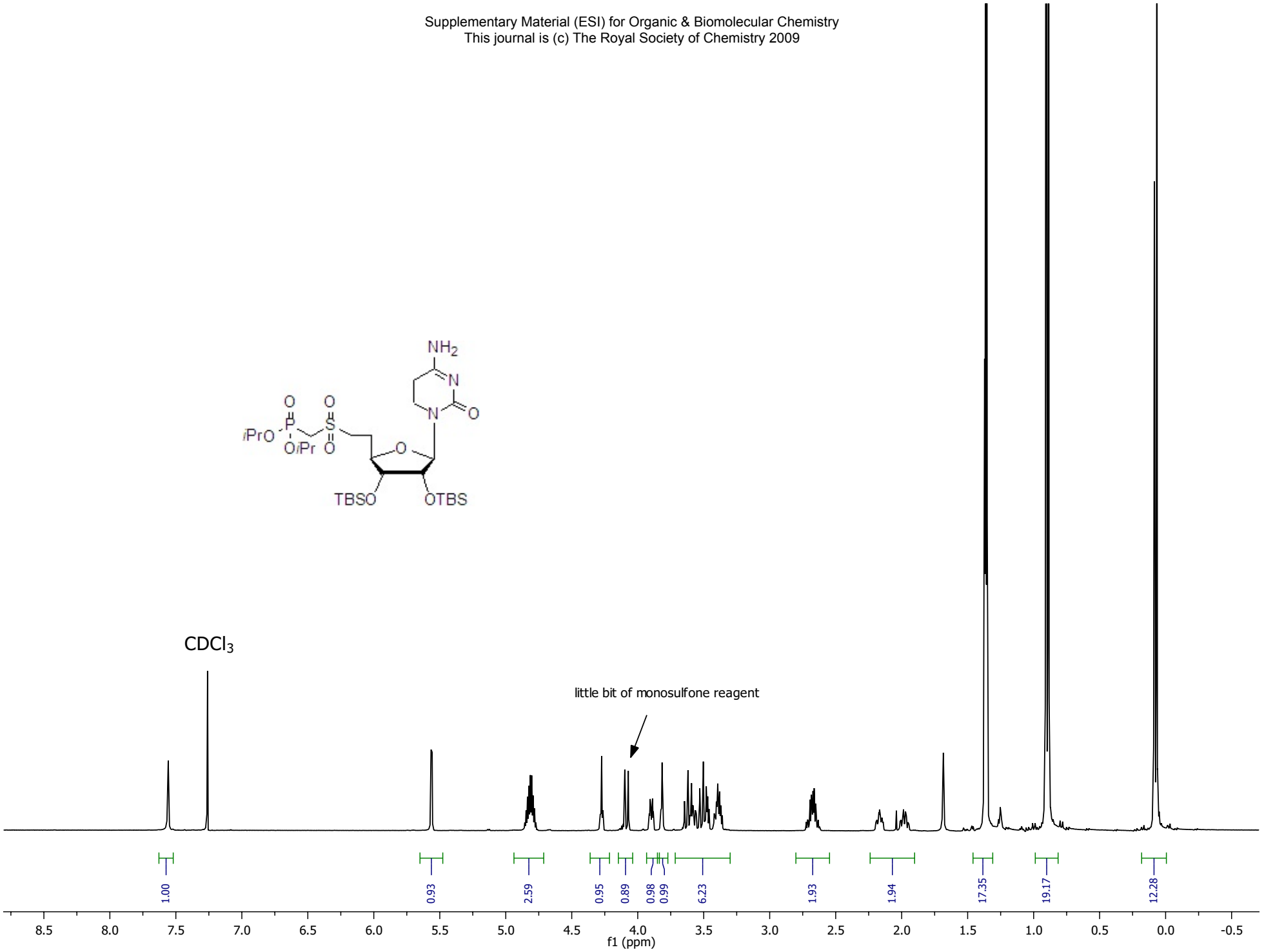

NHAc
N
N
O
iPrO
P
O
O
S
O
O iPr O
O
TBSO
OTBS
8
CDCl3
EtOAc
EtOAc
H2O
EtOAc
1.00
0.95
1.01
1.82
0.88
3.04
0.87
0.88
2.04
2.68
13.81
17.42
2.49
8.31
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

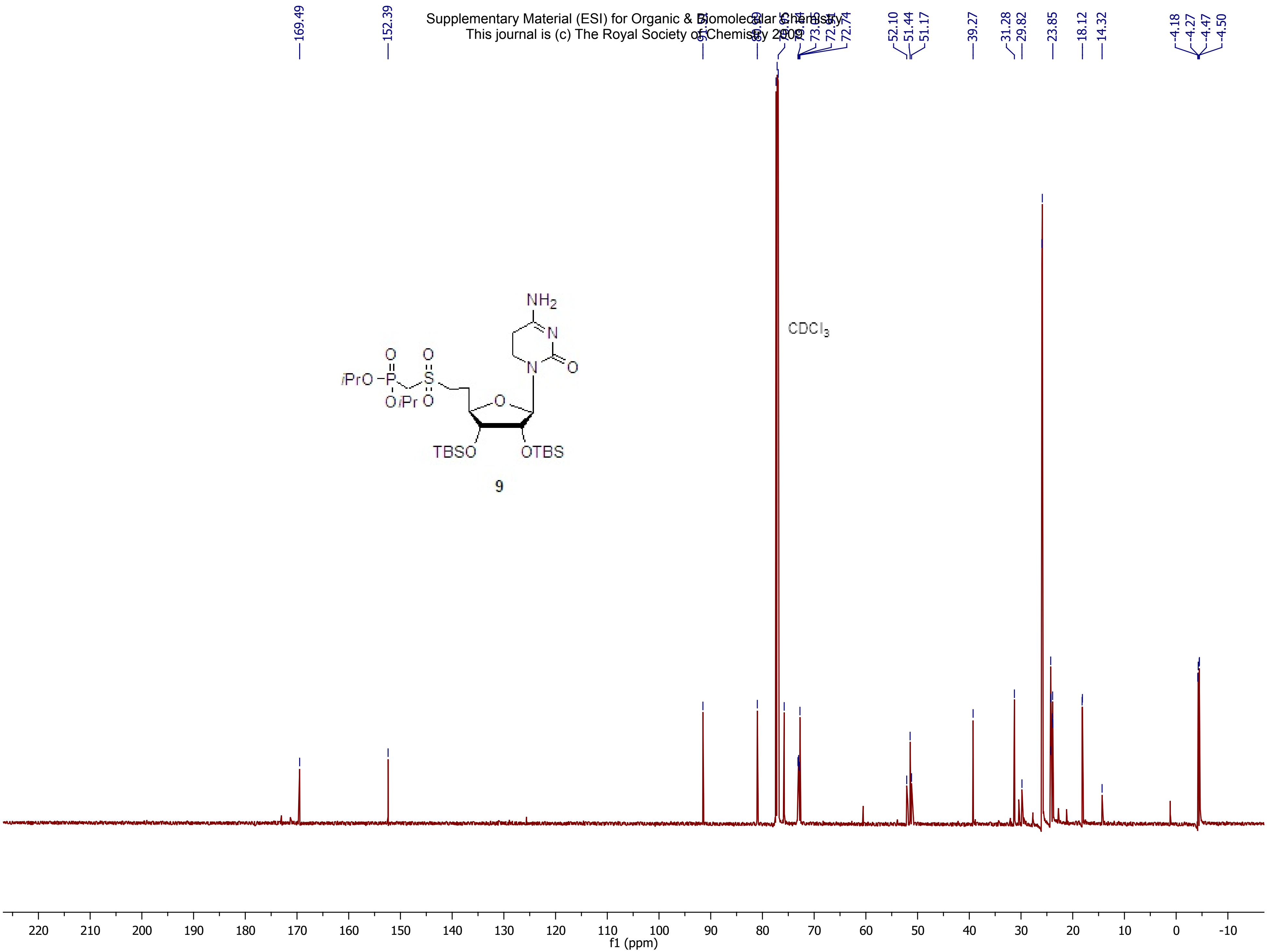

170.31
162.74
155.05
144.63
143.65
130.68
54.40
25.97
24.29
24.27
24.02
23.97
23.93
18.21
18.18
-4.11
-4.16
-4.71
-4.92
CDCl3
NHAc
N
N
O
iPrO
P
O
O
S
O
O
iPr
O
TBSO
OTBS
8
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

1061 #7-10 RT: 0.10-0.15 AV: 4 NL: 1.24E8
T: FTMS + c ESI Full ms [250.00-1000.00]

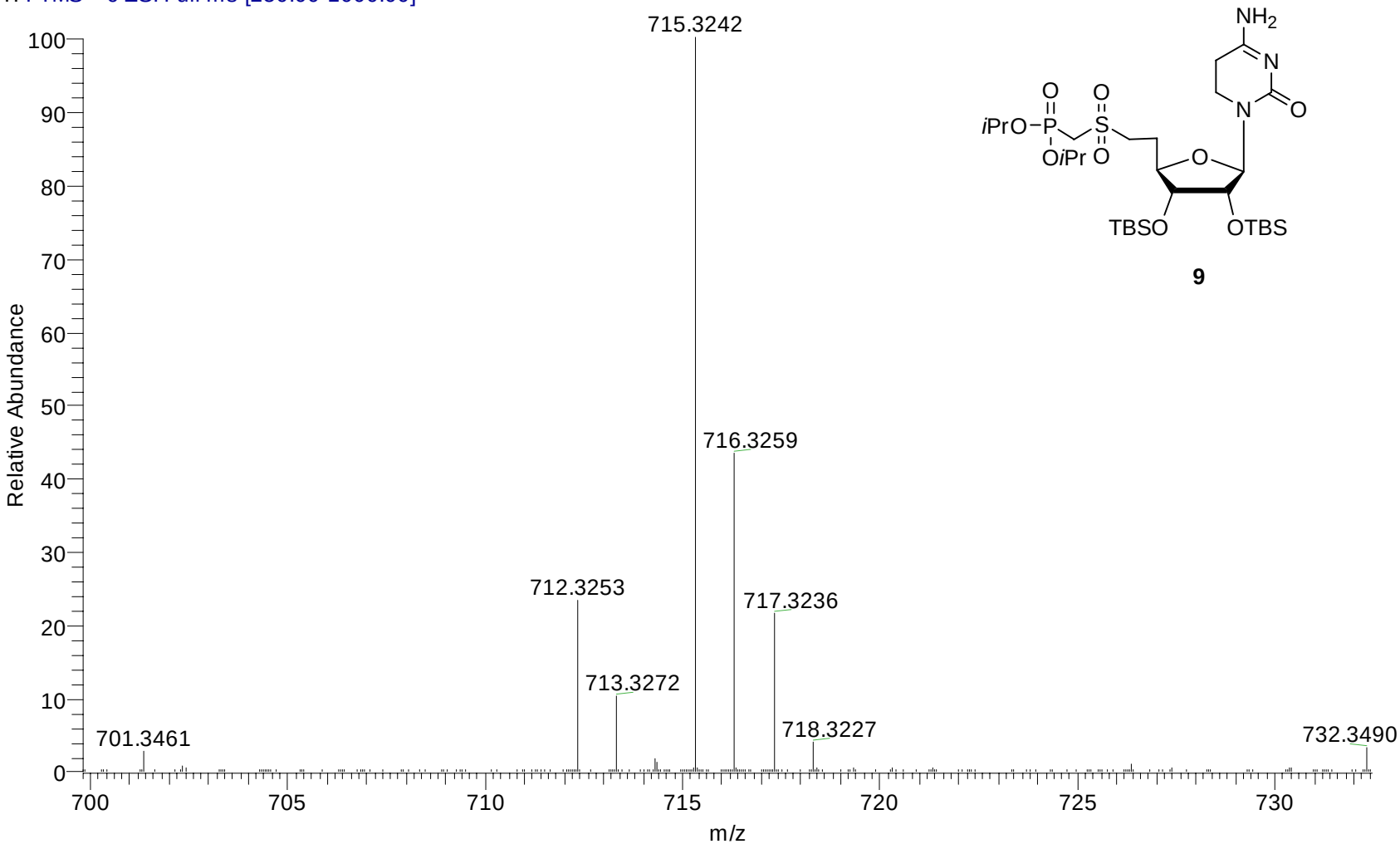


8

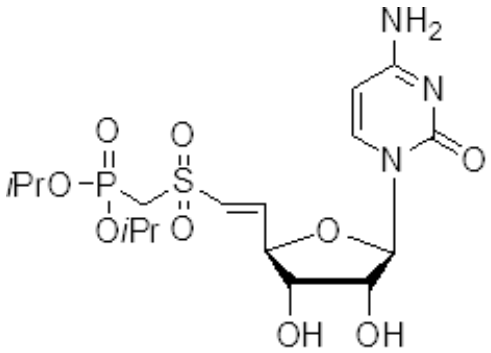

NH2
N
N
O
O
O
O
iPrO
P
S
OiPr
O
O
TBSO
OTBS
CDCl3
little bit of monosulfone reagent
1.00
0.93
2.59
0.95
0.89
0.98
0.99
6.23
1.93
1.94
17.35
19.17
12.28
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



1063 #73-94 RT: 1.09-1.41 AV: 22 NL: 2.34E7
T: FTMS + c ESI Full ms [250.00-1000.00]

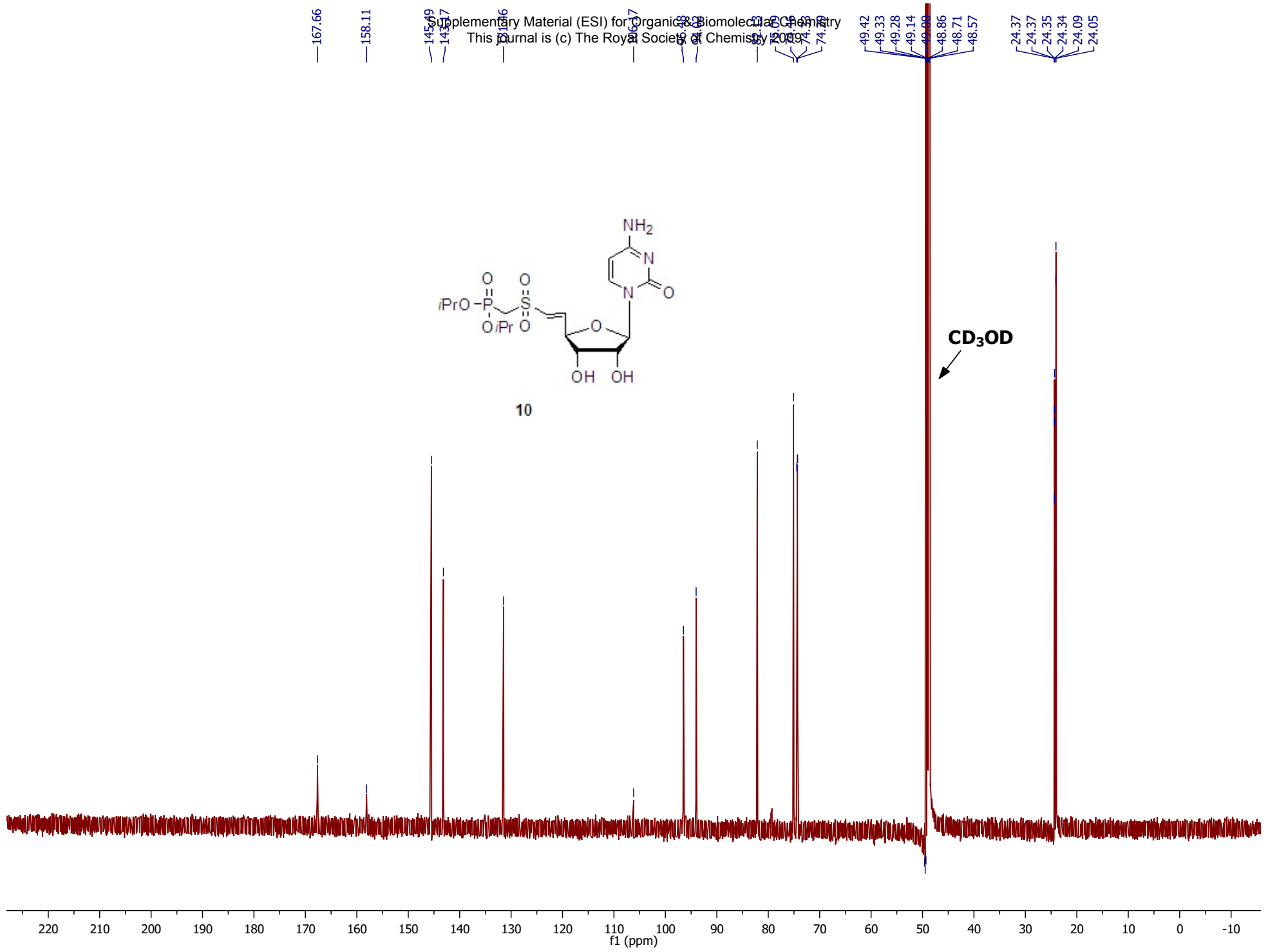


9


NH2
N
N
O
O
iPrO
P
S
O
OiPr
O
O
OH
OH

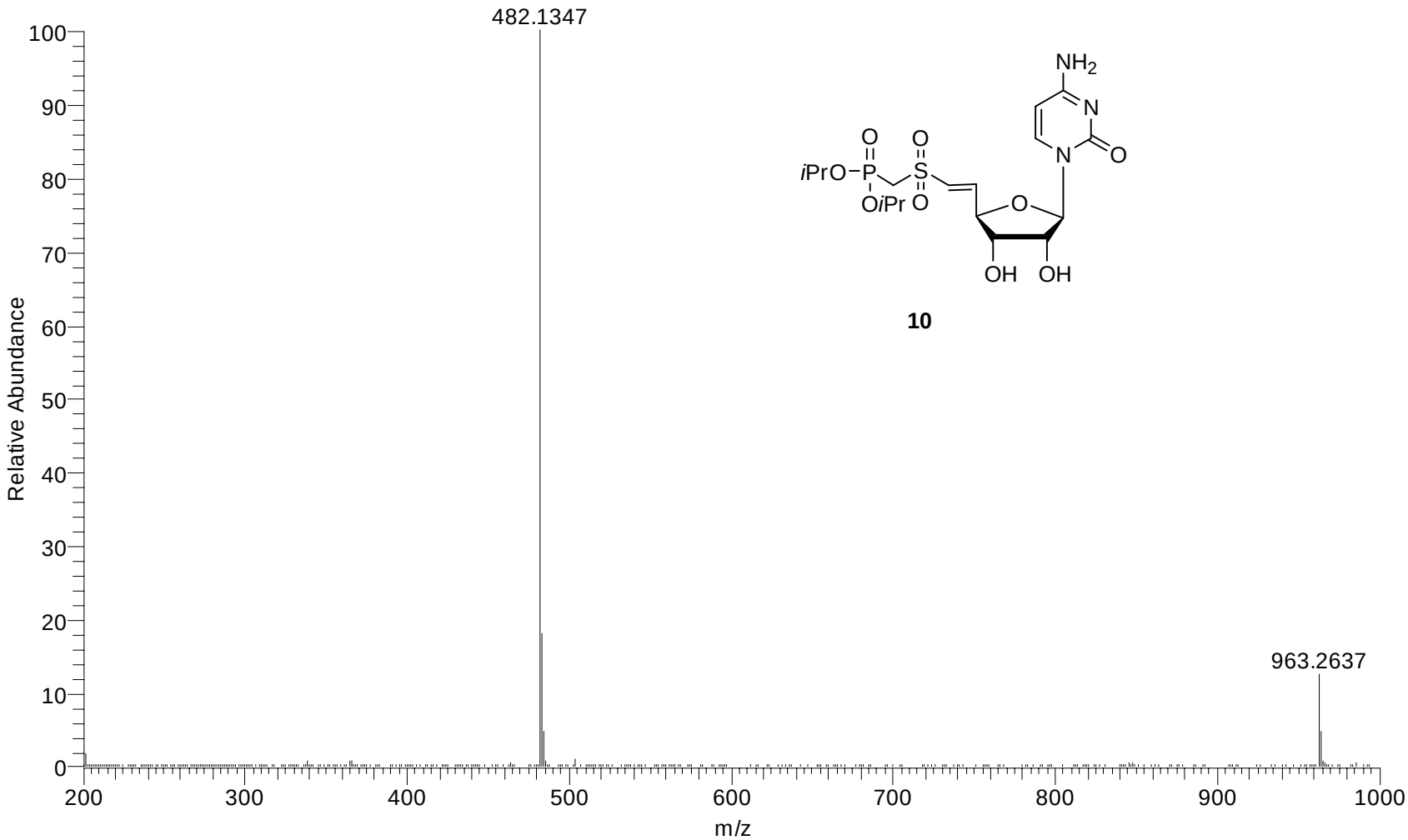
10

D2O
CD3OD
1.00
1.87
0.95
0.96
1.97
0.98
0.99
1.02
11.59
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

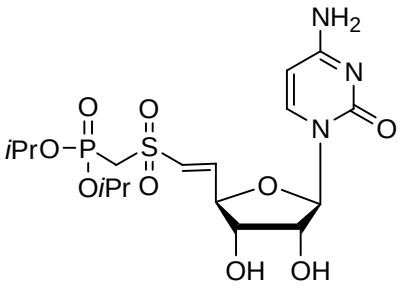

167.66
158.11
145.49
143.17
116.46
96.07
49.42
49.33
49.28
49.14
49.00
48.86
48.71
48.57
24.37
24.37
24.35
24.34
24.09
24.05
CD3OD
NH2
N
N
O
iPrO
P
O
O
S
O
O
iPr
O
OH
OH
10
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

JWV 138

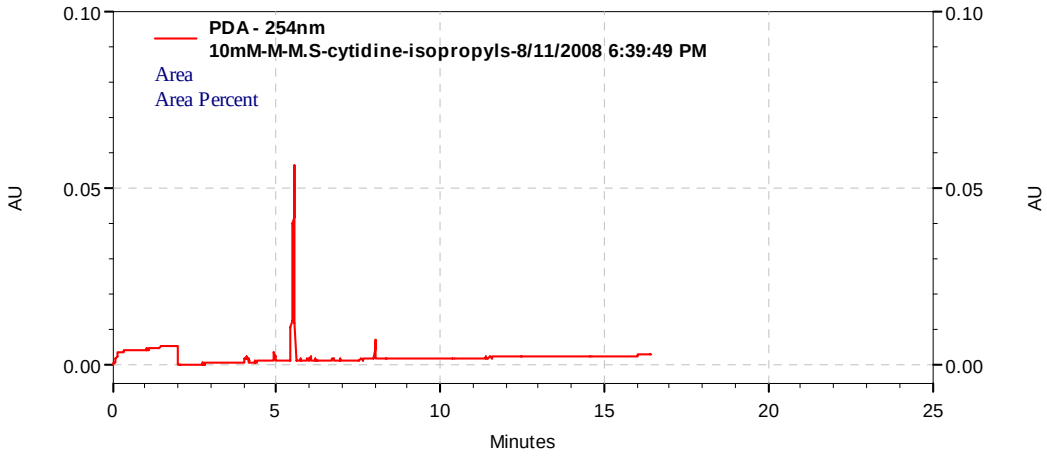
1027 #5-9 RT: 0.08-0.15 AV: 5 NL: 1.07E6
T: FTMS + c ESI Full ms [200.00-1000.00]

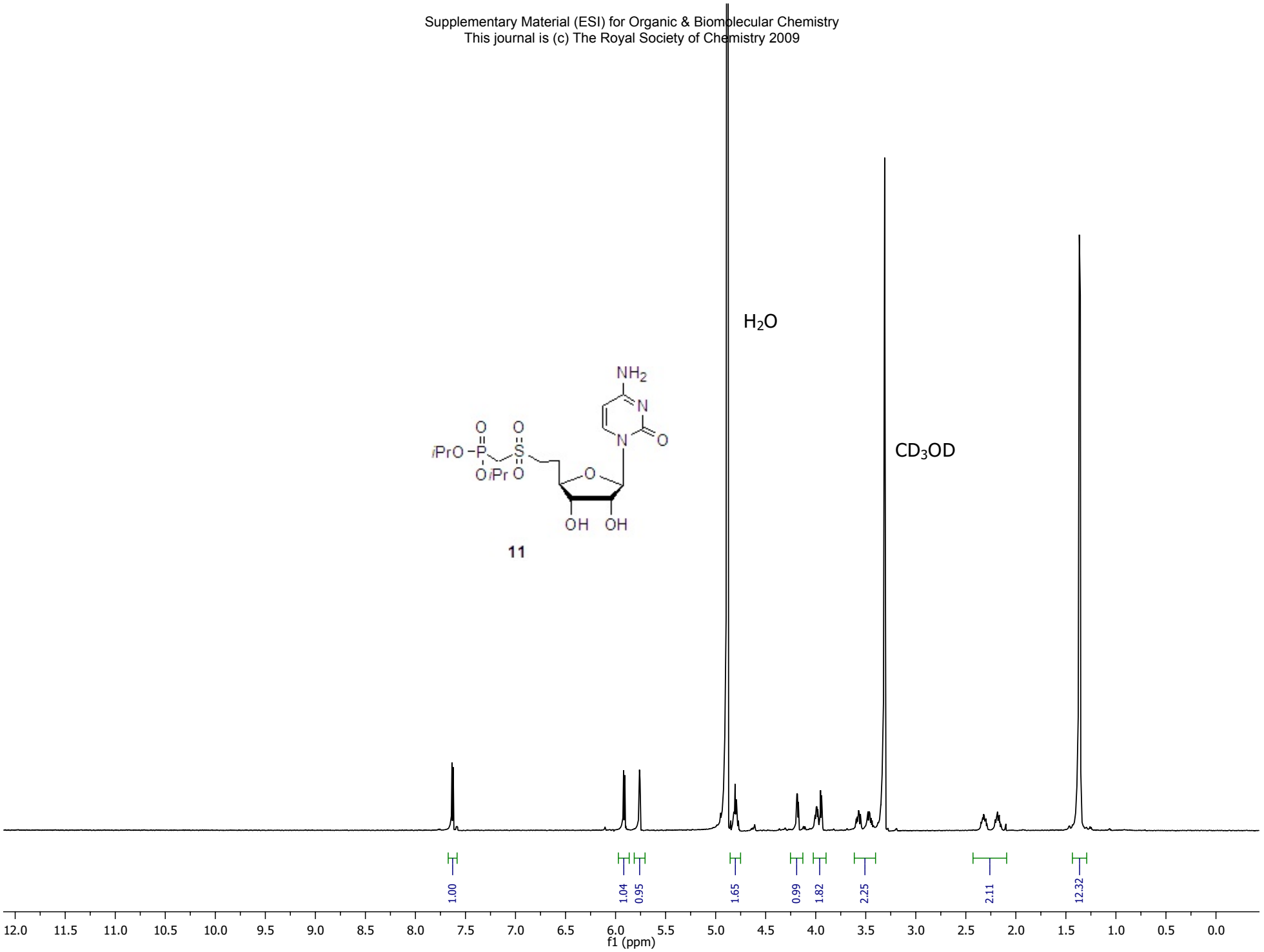


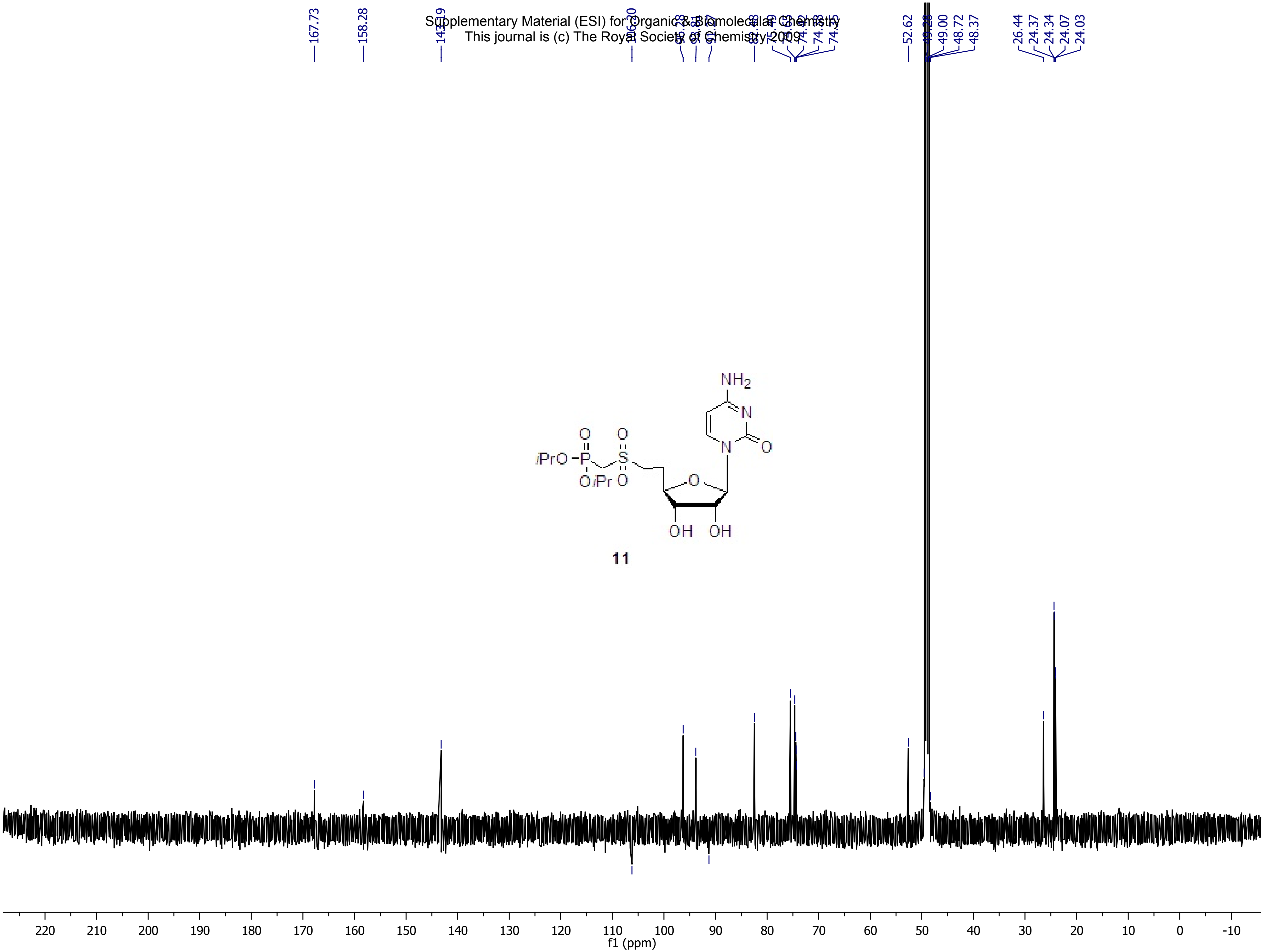
10


NH2
N
N
O
O
O
iPrO–P
S
OiPr
O
O
OH
OH

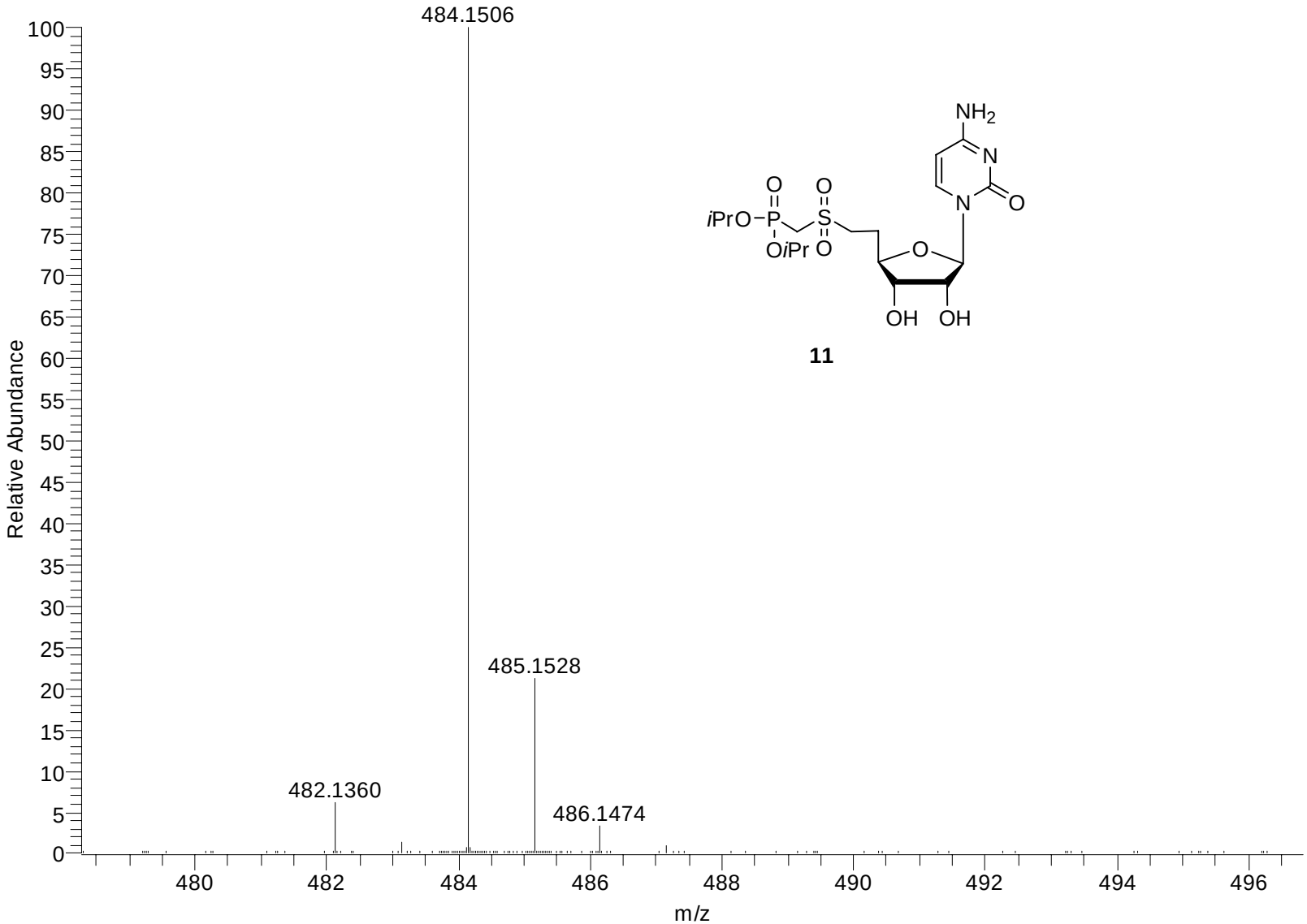
10


PDA - 254nm
10mM-M-M.S-cytidine-isopropyls-8/11/2008 6:39:49 PM
Area
Area Percent
AU
0.10
0.05
0.00
0
5
10
15
20
25
Minutes

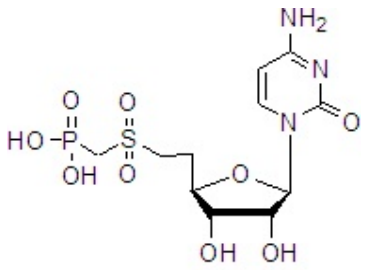

H2O
CD3OD
NH2
N
N
O
iPrO
P
O
O
S
O
O
iPr
O
O
OH
OH
11
1.00
1.04
0.95
1.65
0.99
1.82
2.25
2.11
12.32
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


167.73
158.28
52.62
49.00
48.72
48.37
26.44
24.37
24.34
24.07
24.03
NH2
N
N
O
iPrO
P
S
O
OiPr
O
OH
OH
11
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

1080 #1-4 RT: 0.01-0.06 AV: 4 NL: 1.28E8
T: FTMS + c ESI Full ms [200.00-1000.00]

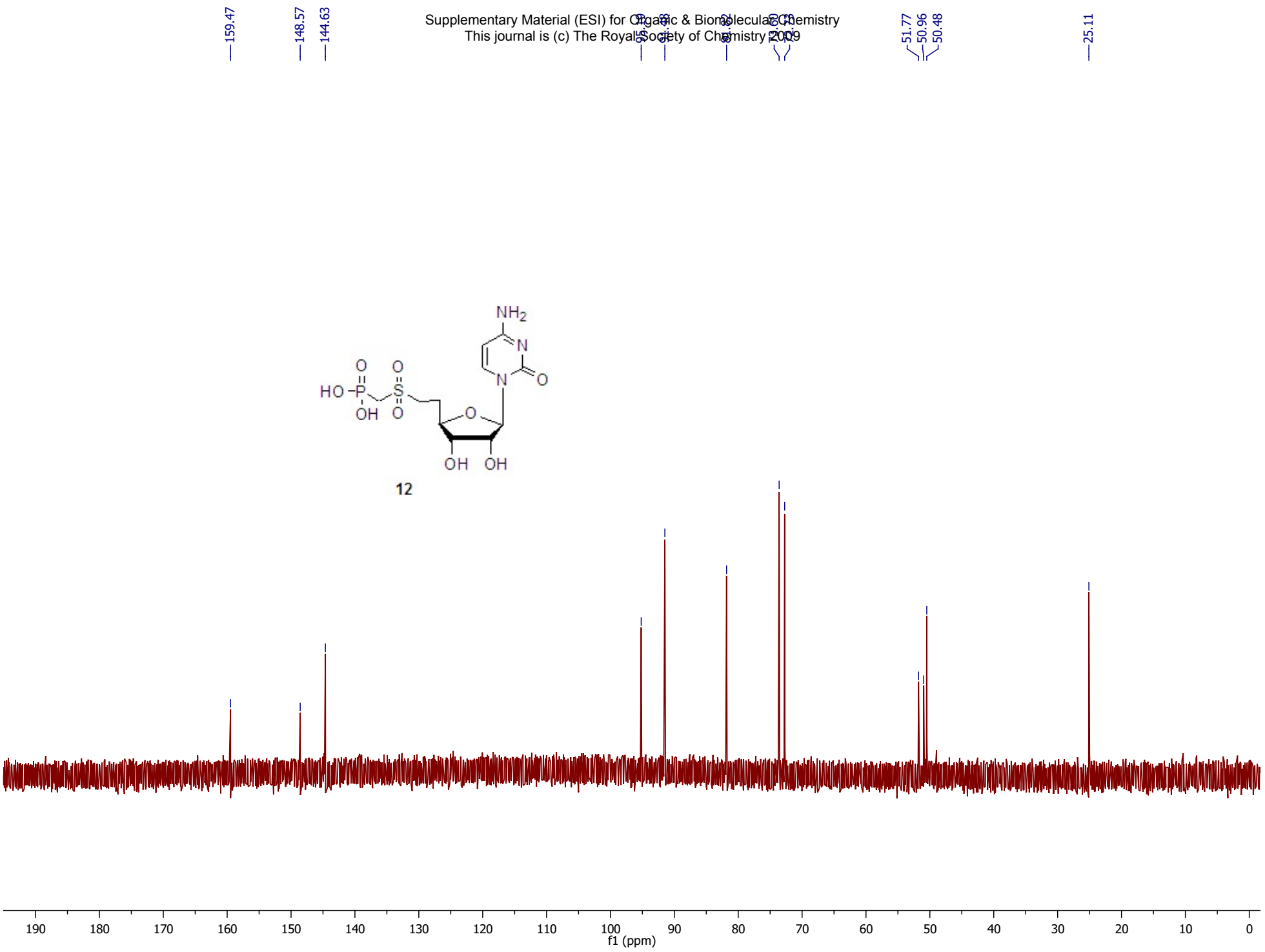


11


NH2
N
N
O
HO
P
OH
O
S
O
O
O
OH
OH

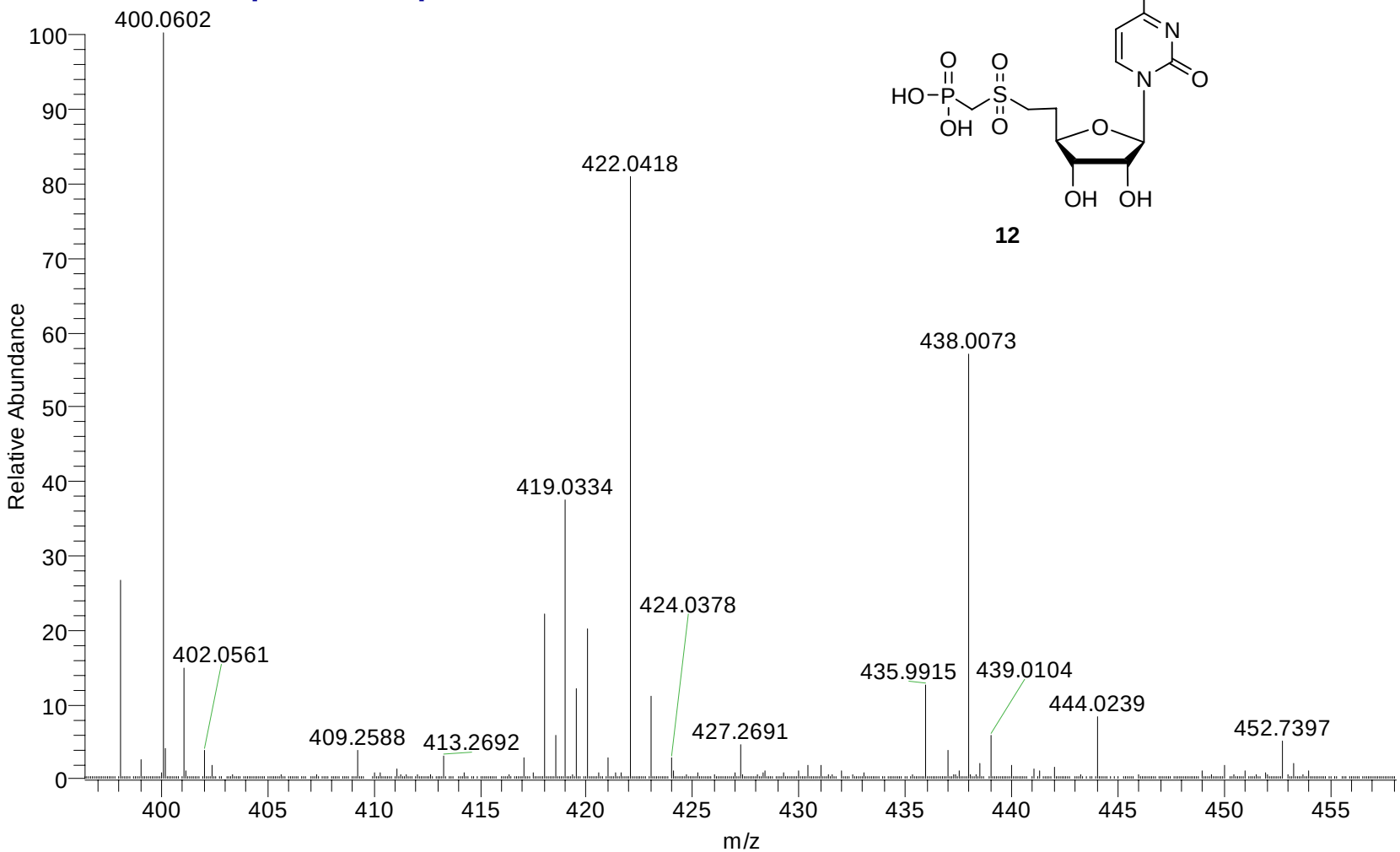
12

H2O
CD3OD
EtOAc
EtOAc
1.00
0.92
0.82
0.84
1.77
1.62
1.80
1.58
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

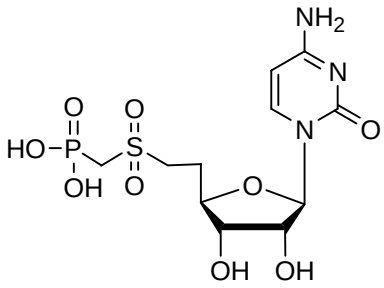

159.47
148.57
144.63
51.77
50.96
50.48
25.11
NH2
N
N
O
HO
P
OH
O
S
O
O
O
OH
OH
12
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

JWV 196

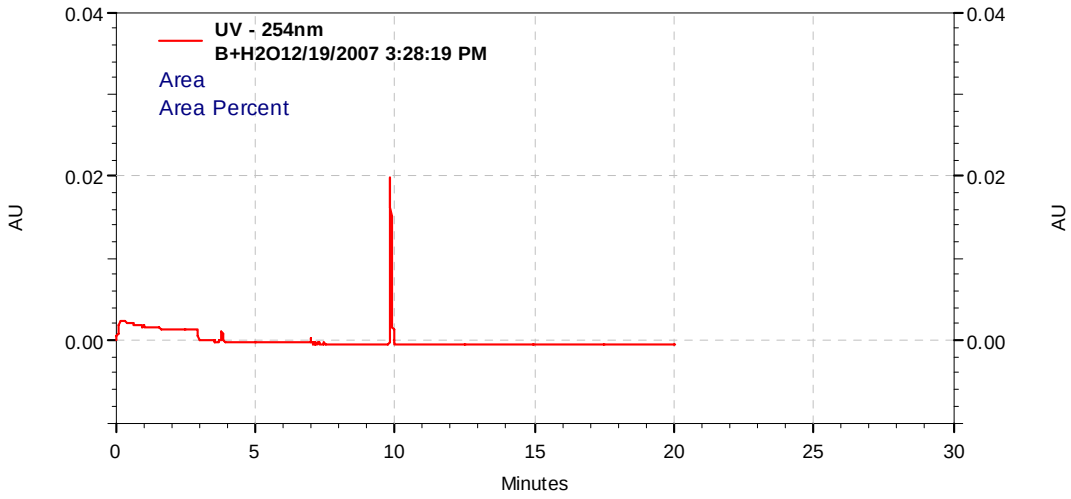
1021-2 #217-267 RT: 3.65-4.49 AV: 51 NL: 1.44E7
T: FTMS + c NSI Full ms [200.00-1000.00]

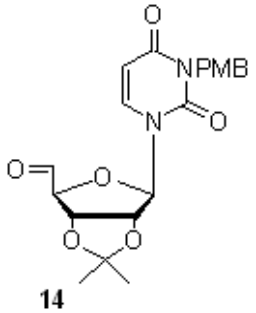


12

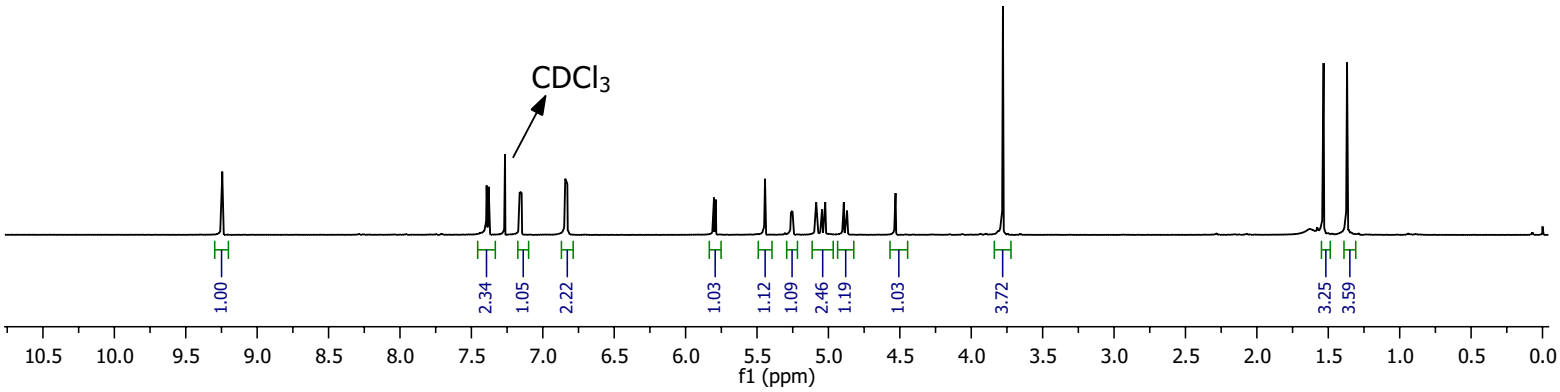

NH2
N
O
N
HO
P
O
OH
S
O
O
O
OH
OH

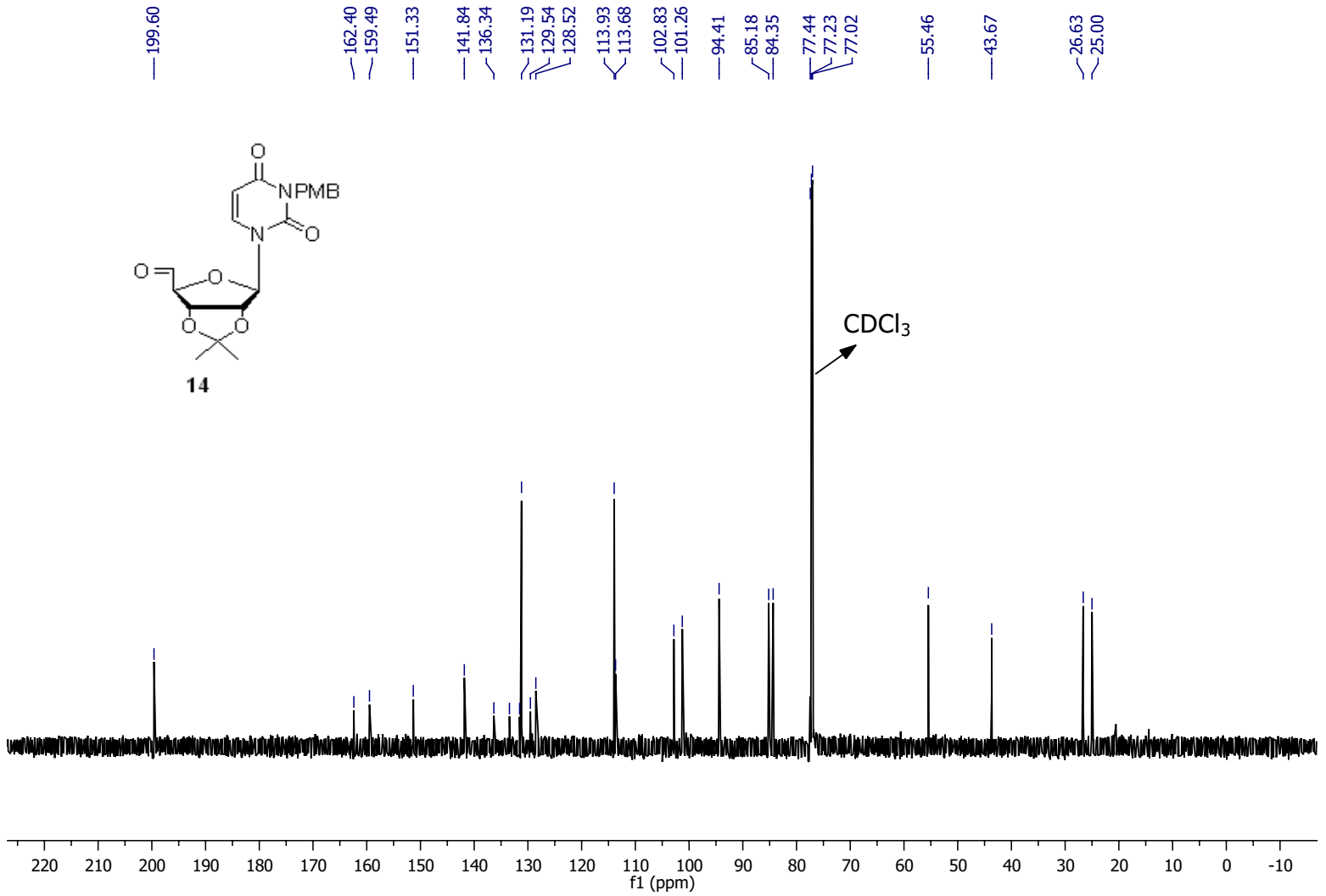
12

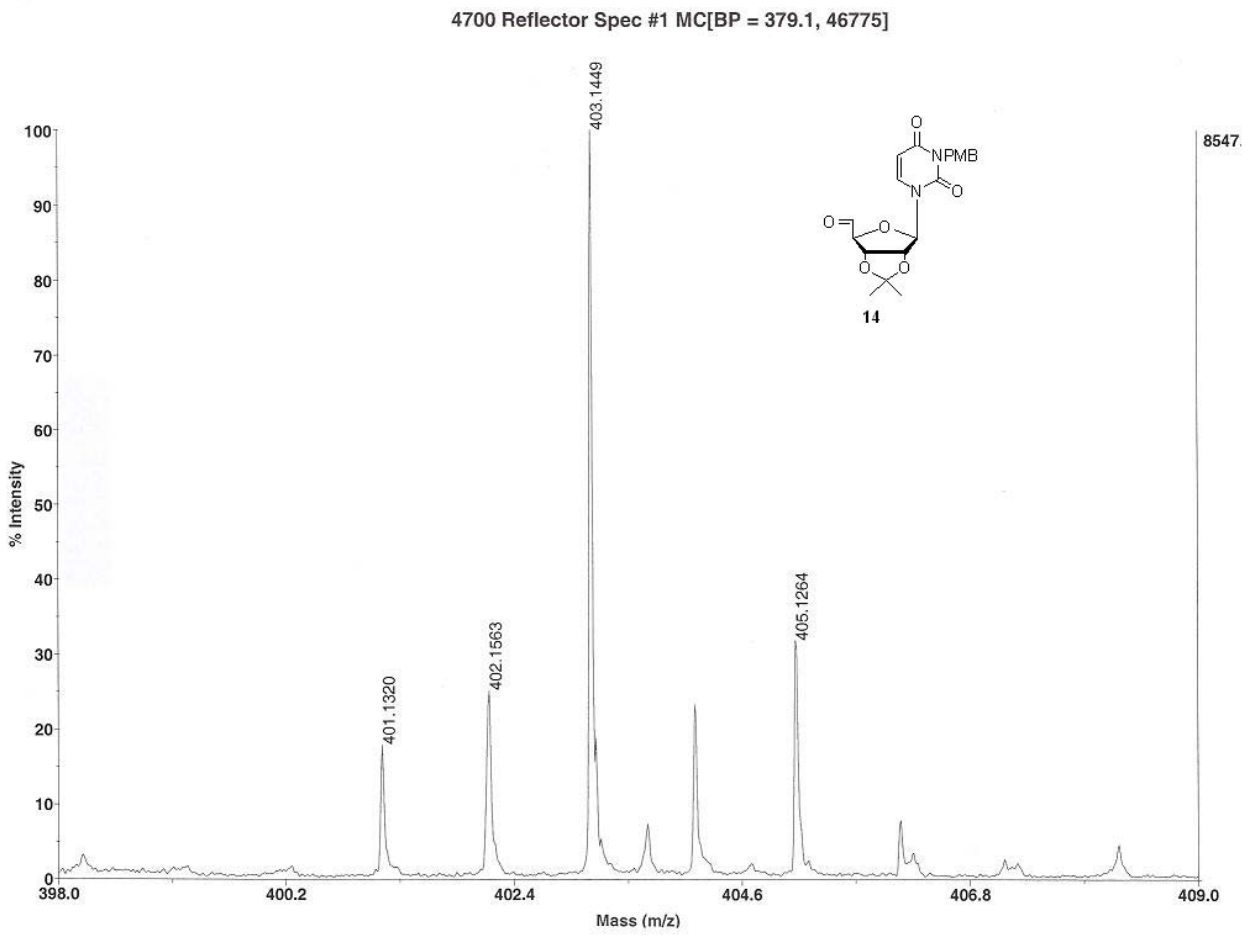

UV - 254nm
B+H2O12/19/2007 3:28:19 PM
Area
Area Percent
0.04
0.02
0.00
AU
0
5
10
15
20
25
30
Minutes

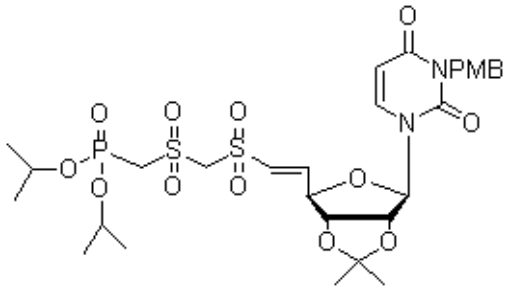

O
NPMB
N
O
O
O
O
O

14

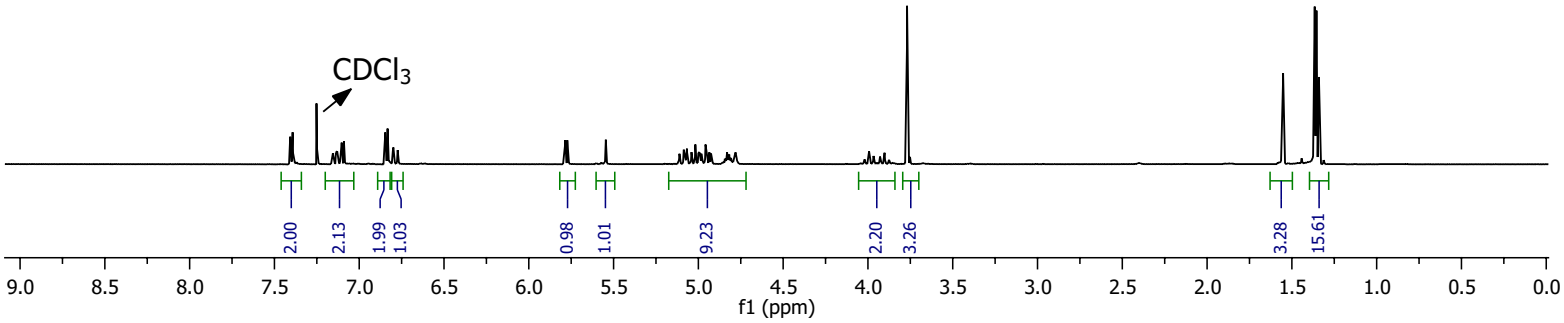

CDCl3
1.00
2.34
1.05
2.22
1.03
1.12
1.09
2.46
1.19
1.03
3.72
3.25
3.59
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

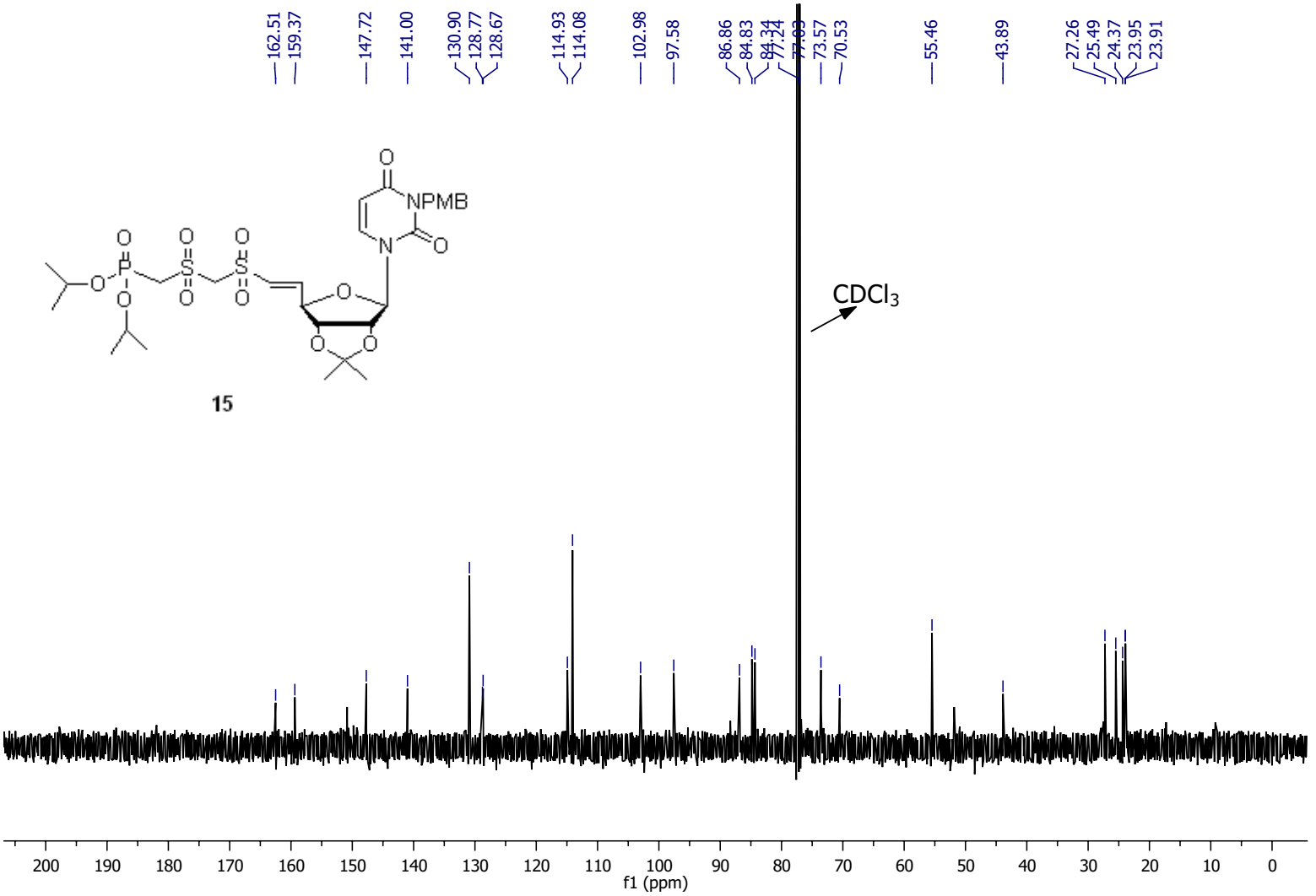

199.60
162.40
159.49
151.33
141.84
136.34
131.19
129.54
128.52
113.93
113.68
102.83
101.26
94.41
85.18
84.35
77.44
77.23
77.02
55.46
43.67
26.63
25.00
NPMB
O
N
O
O
O
O
O
14
CDCl3
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

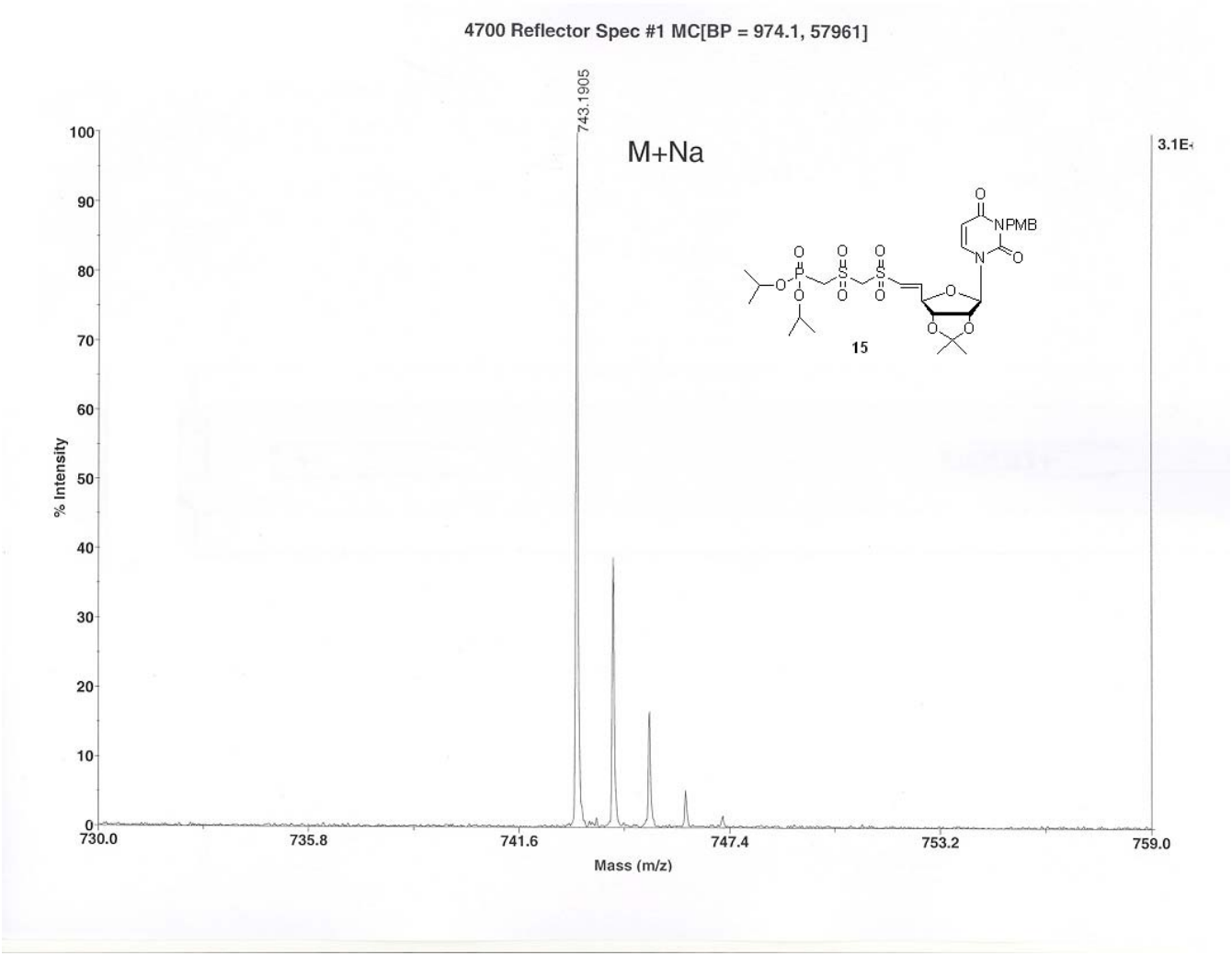

4700 Reflector Spec #1 MC[BP = 379.1, 46775]
403.1449
8547.
NPMB
O
O
O
O
O
O
14
100
90
80
70
60
50
40
30
20
10
0
% Intensity
401.1320
402.1563
405.1264
398.0
400.2
402.4
404.6
406.8
409.0
Mass (m/z)

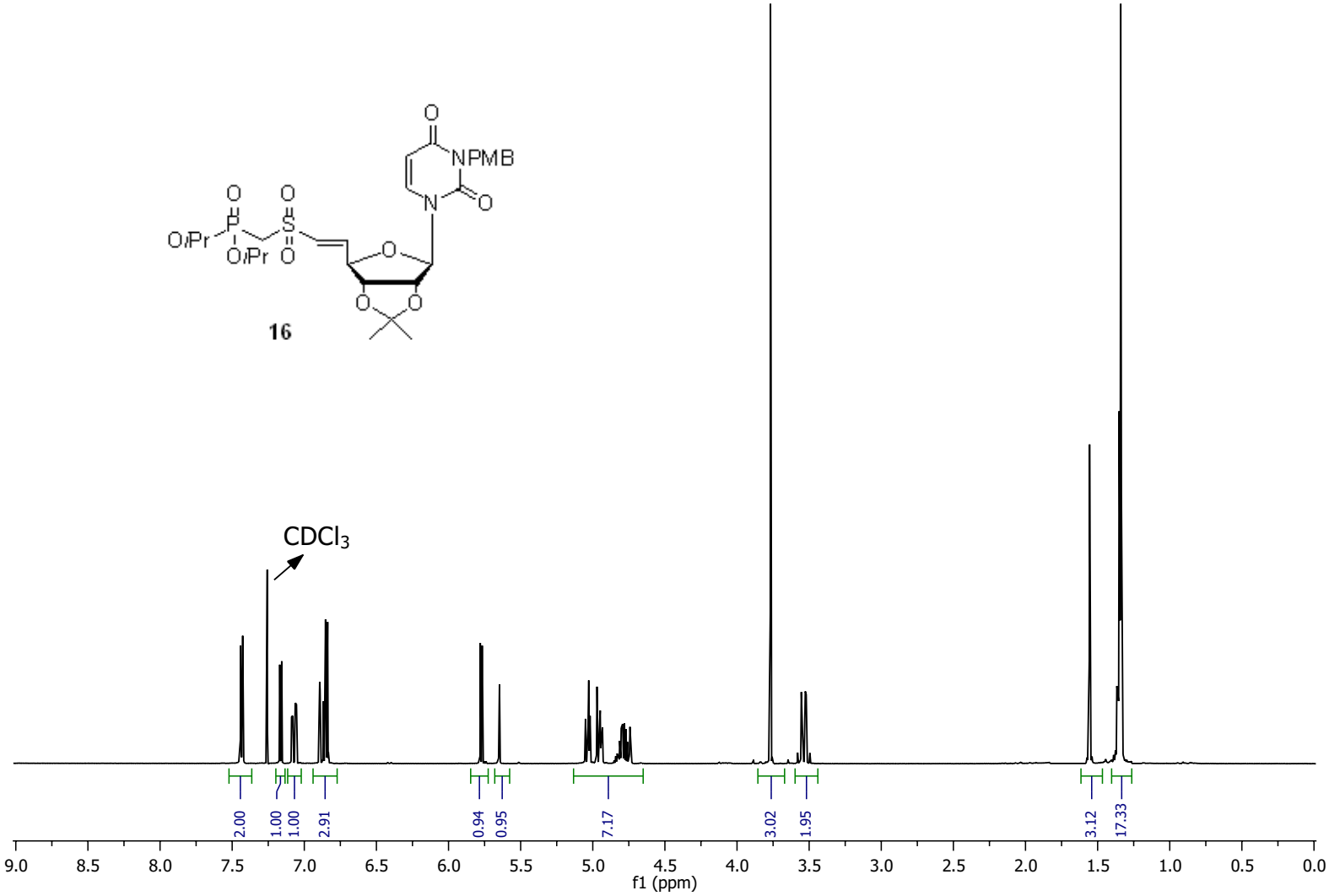

O
NPMB
N
O
O
P
O
O
S
O
O
S
O
O
O
O
O
O

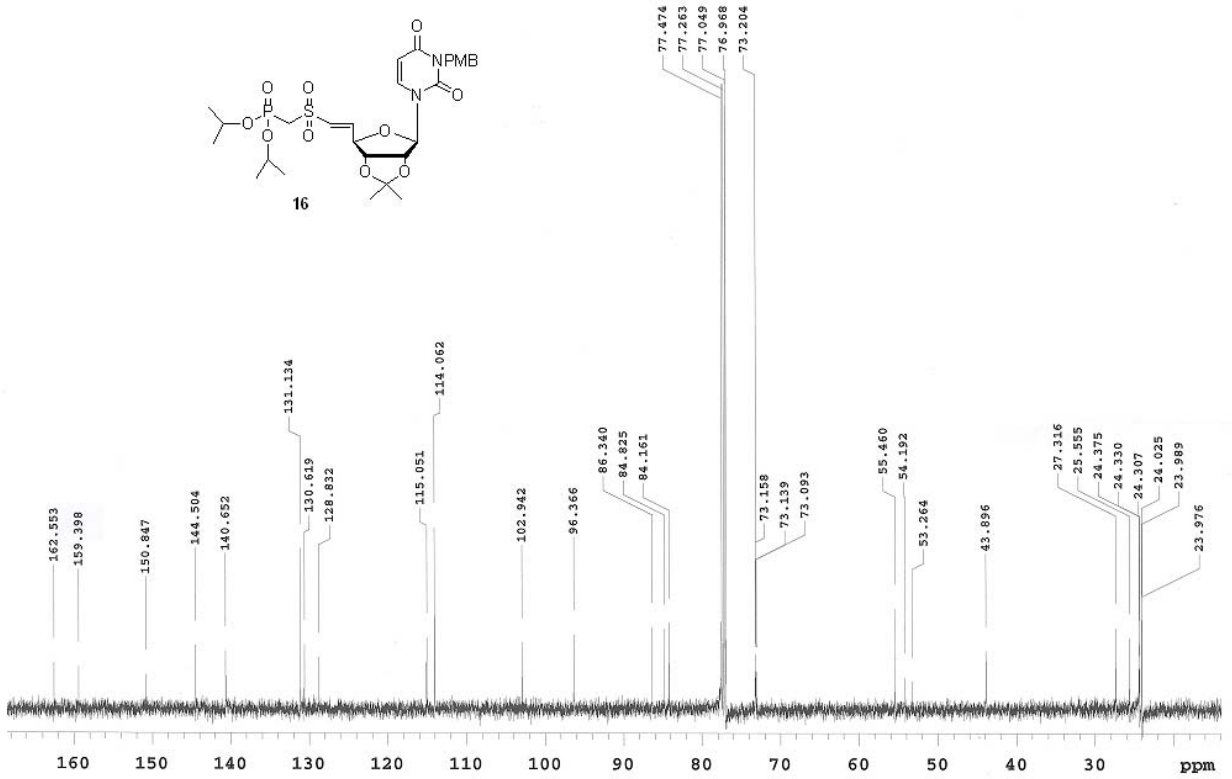
15

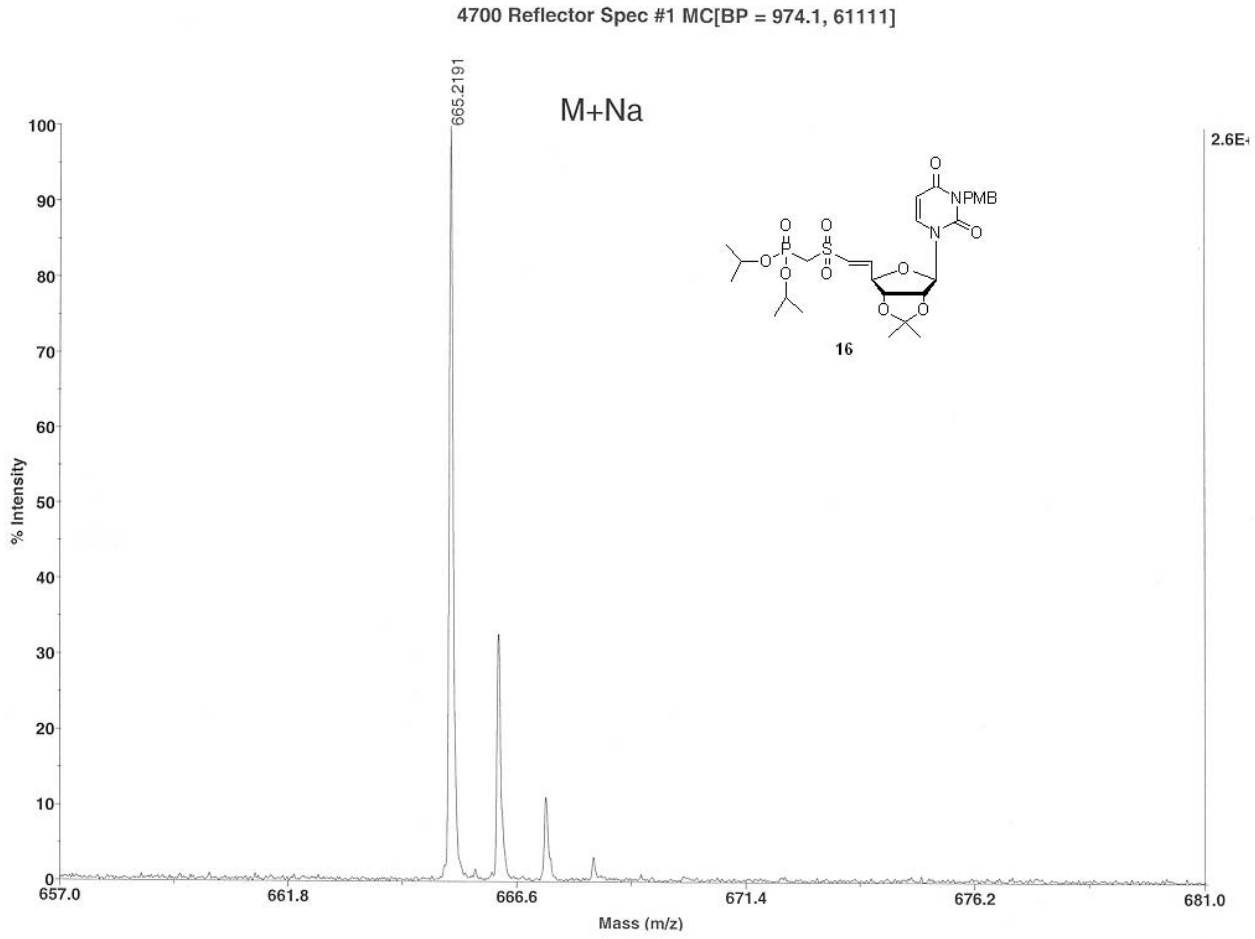

CDCl3
2.00
2.13
1.99
1.03
0.98
1.01
9.23
2.20
3.26
3.28
15.61
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

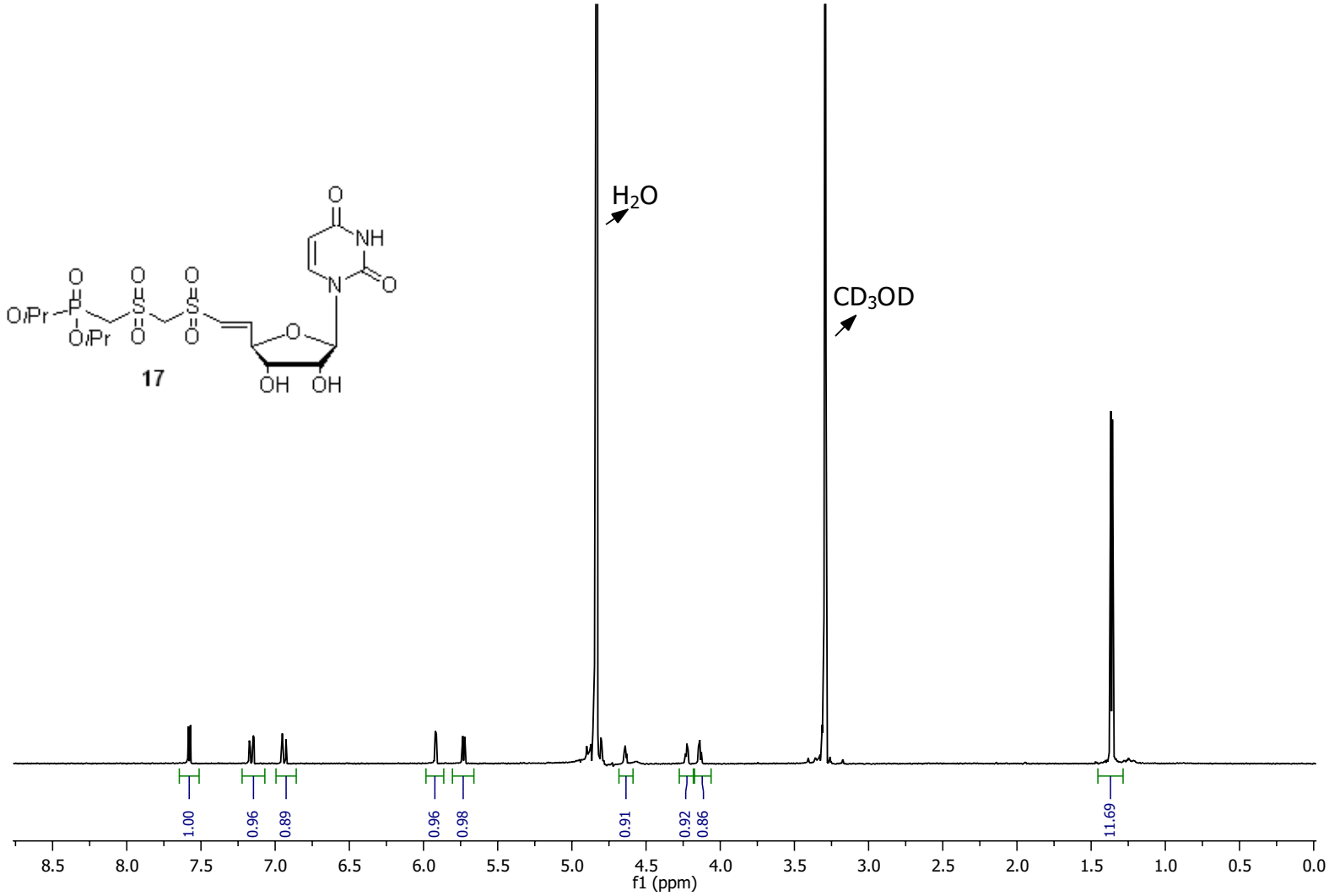

162.51
159.37
147.72
141.00
130.90
128.77
128.67
114.93
114.08
102.98
97.58
86.86
84.83
84.34
77.24
77.03
73.57
70.53
55.46
43.89
27.26
25.49
24.37
23.95
23.91
NPMB
CDCl3
15
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

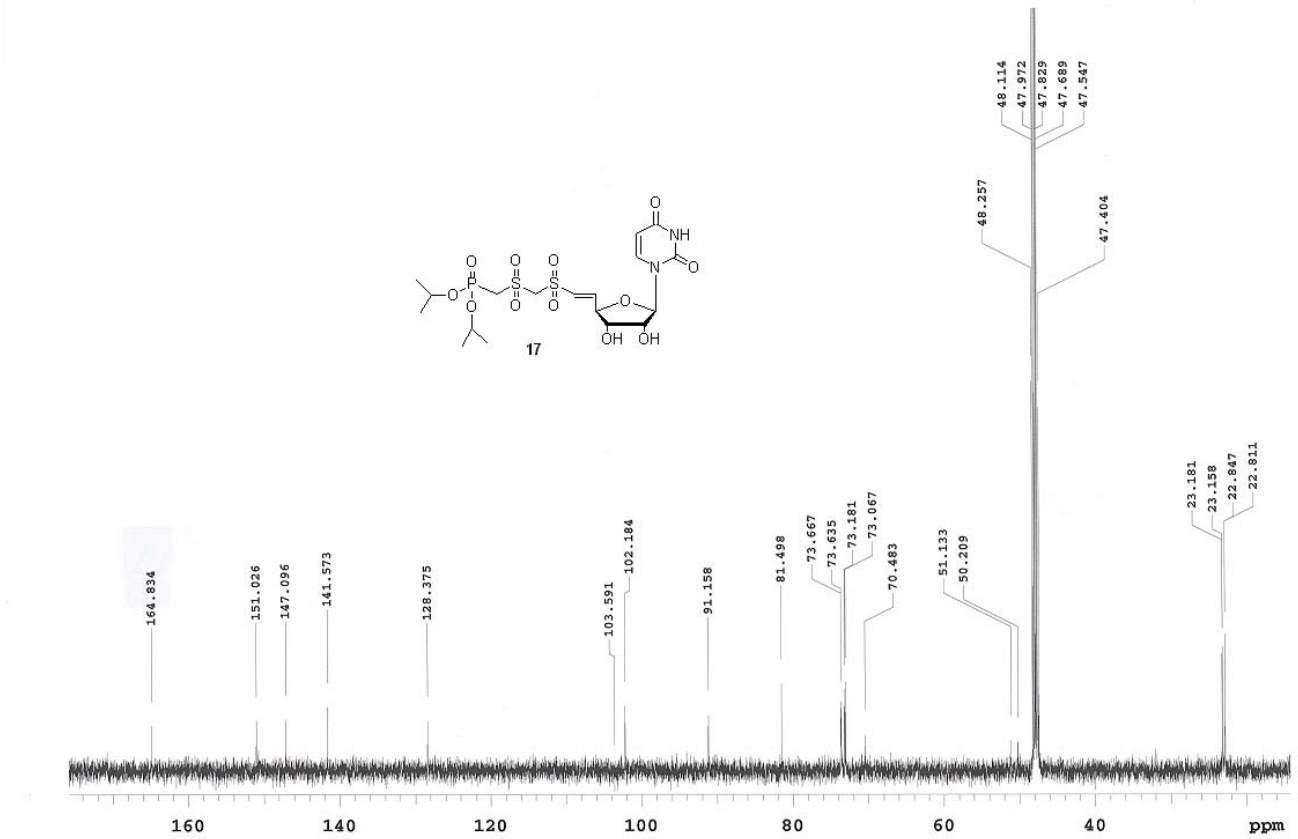

4700 Reflector Spec #1 MC[BP = 974.1, 57961]
743.1905
M+Na
3.1E4
NPMB
15
% Intensity
100
90
80
70
60
50
40
30
20
10
0
730.0
735.8
741.6
747.4
753.2
759.0
Mass (m/z)

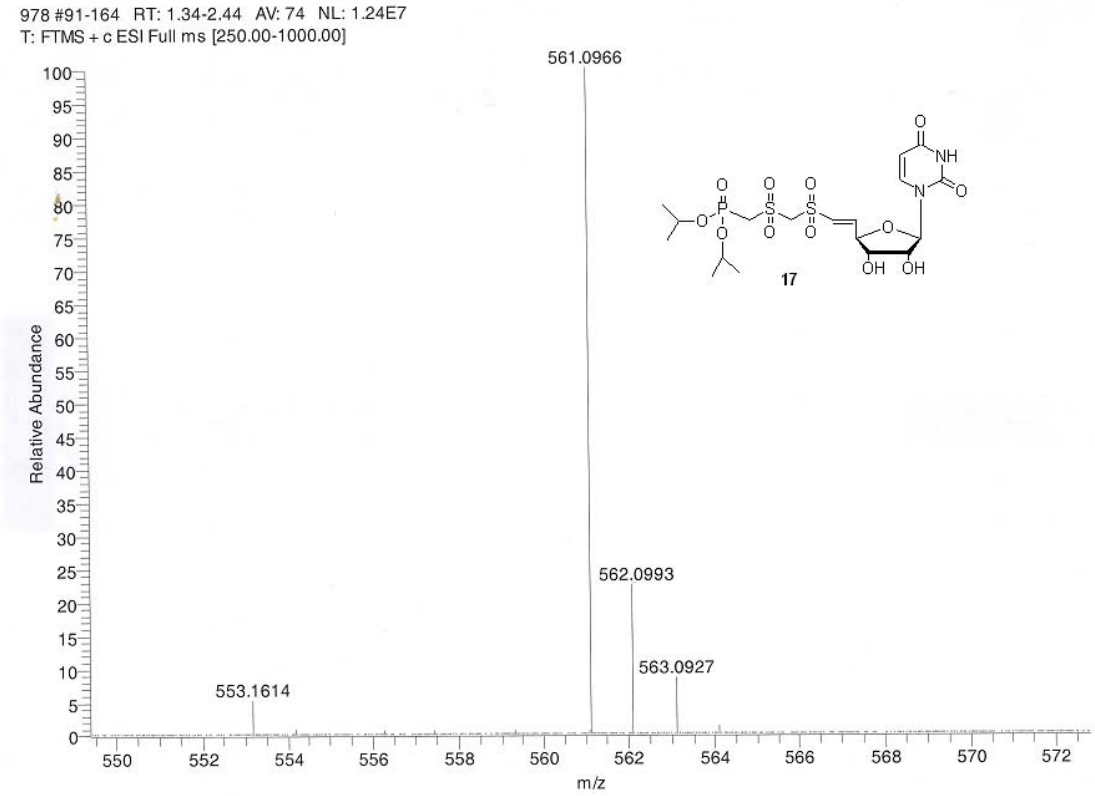

O
NPMB
N
O
O
O
OiPr
P
OiPr
O
S
O
O
O
O
O
16
CDCl3
2.00
1.00
1.00
2.91
0.94
0.95
7.17
3.02
1.95
3.12
17.33
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

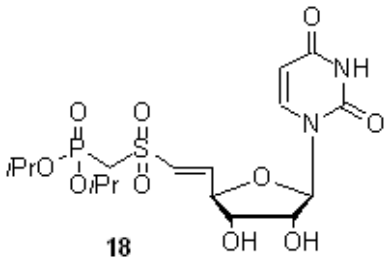

NPMB
16
77.474
77.263
77.049
76.968
73.204
162.553
159.398
150.847
144.504
140.652
131.134
130.619
128.832
115.051
114.062
102.942
96.366
86.340
84.825
84.161
73.158
73.139
73.093
55.460
54.192
53.264
43.896
27.316
25.555
24.375
24.330
24.307
24.025
23.989
23.976
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm


4700 Reflector Spec #1 MC[BP = 974.1, 61111]
665.2191
M+Na
NPMB
16
% Intensity
Mass (m/z)
657.0
661.8
666.6
671.4
676.2
681.0

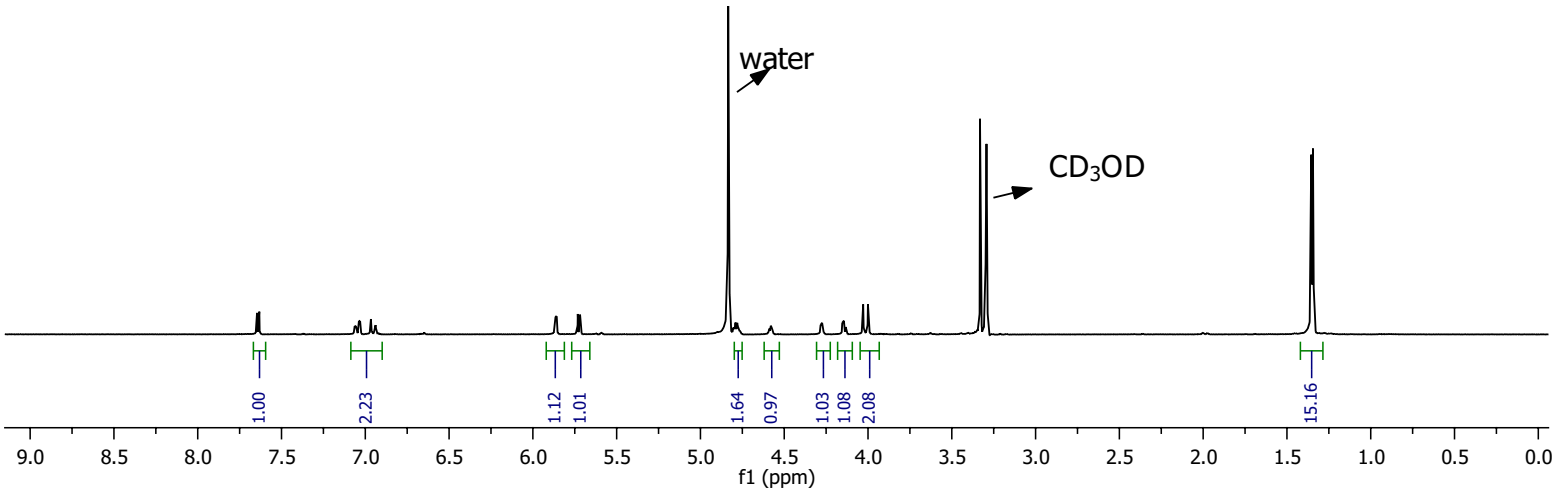

17
H2O
CD3OD
1.00
0.96
0.89
0.96
0.98
0.91
0.92
0.86
11.69
f1 (ppm)

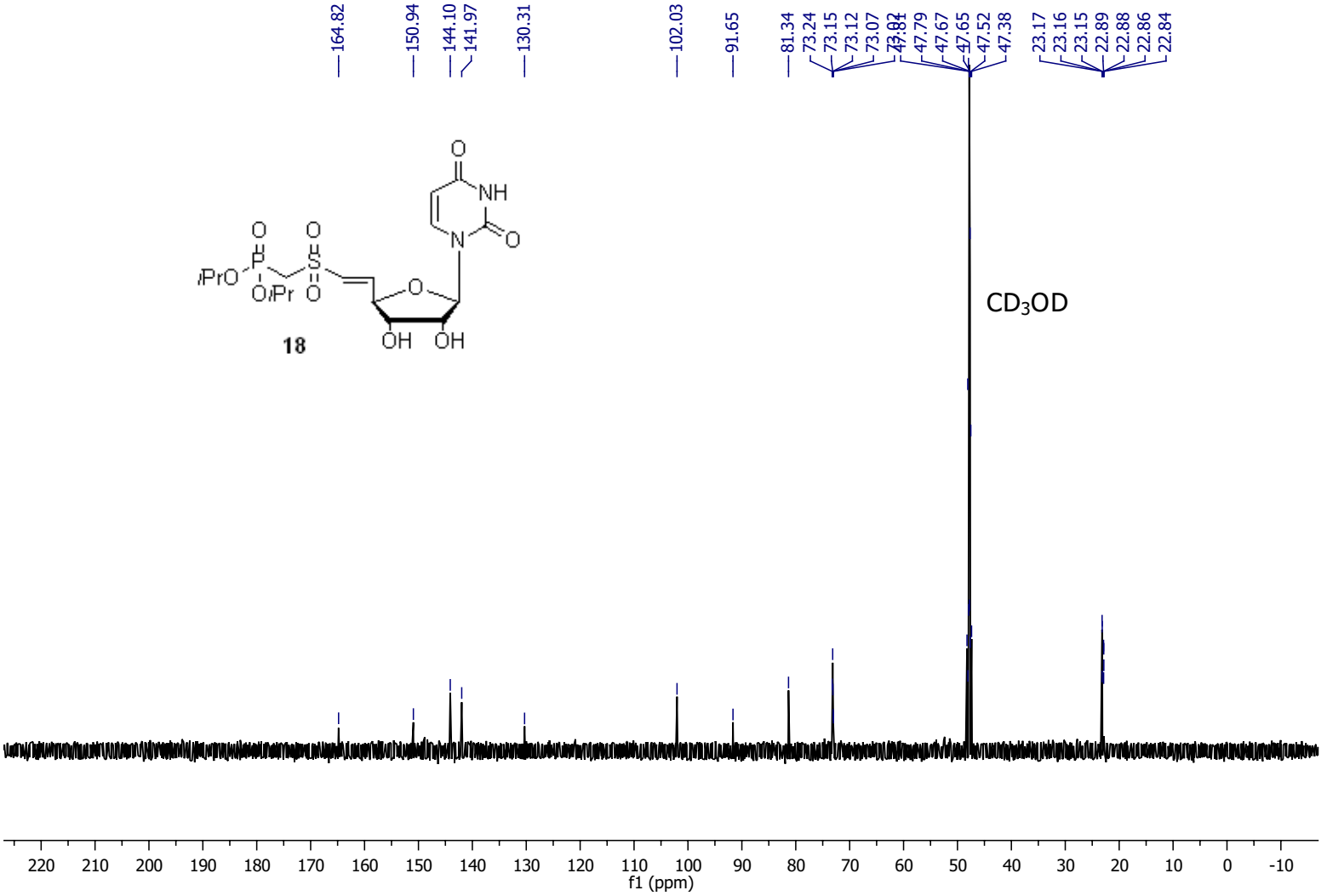

17
164.834
151.026
147.096
141.573
128.375
103.591
102.184
91.158
81.498
73.667
73.635
73.181
73.067
70.483
51.133
50.209
48.257
48.114
47.972
47.829
47.689
47.547
47.404
23.181
23.158
22.847
22.811
160
140
120
100
80
60
40
ppm

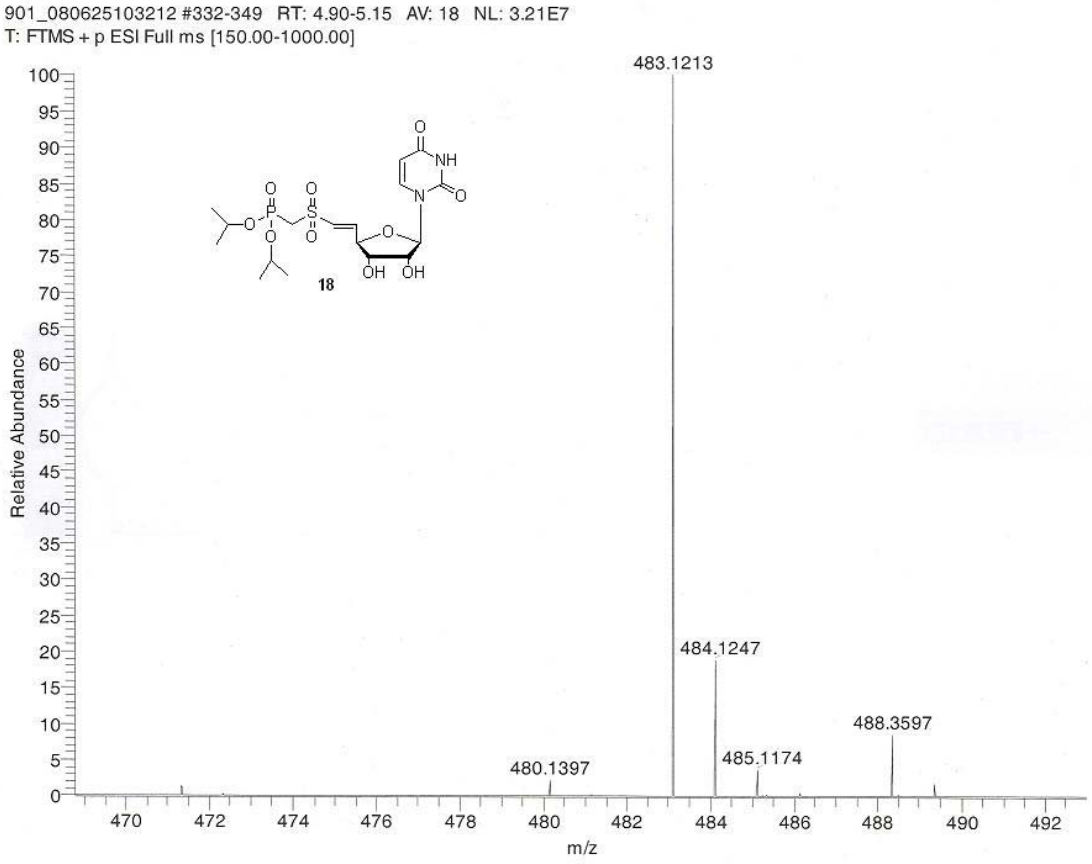

978 #91-164 RT: 1.34-2.44 AV: 74 NL: 1.24E7
T: FTMS + c ESI Full ms [250.00-1000.00]
561.0966
562.0993
563.0927
553.1614
Relative Abundance
m/z
17

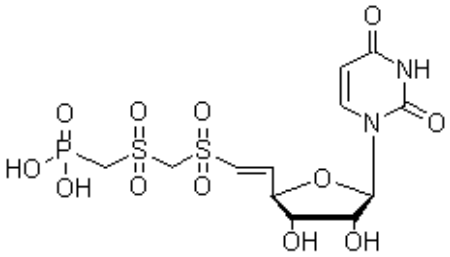

O
NH
N
O
O
O
P
O
iPrO
OiPr
S
O
O
OH
OH

18

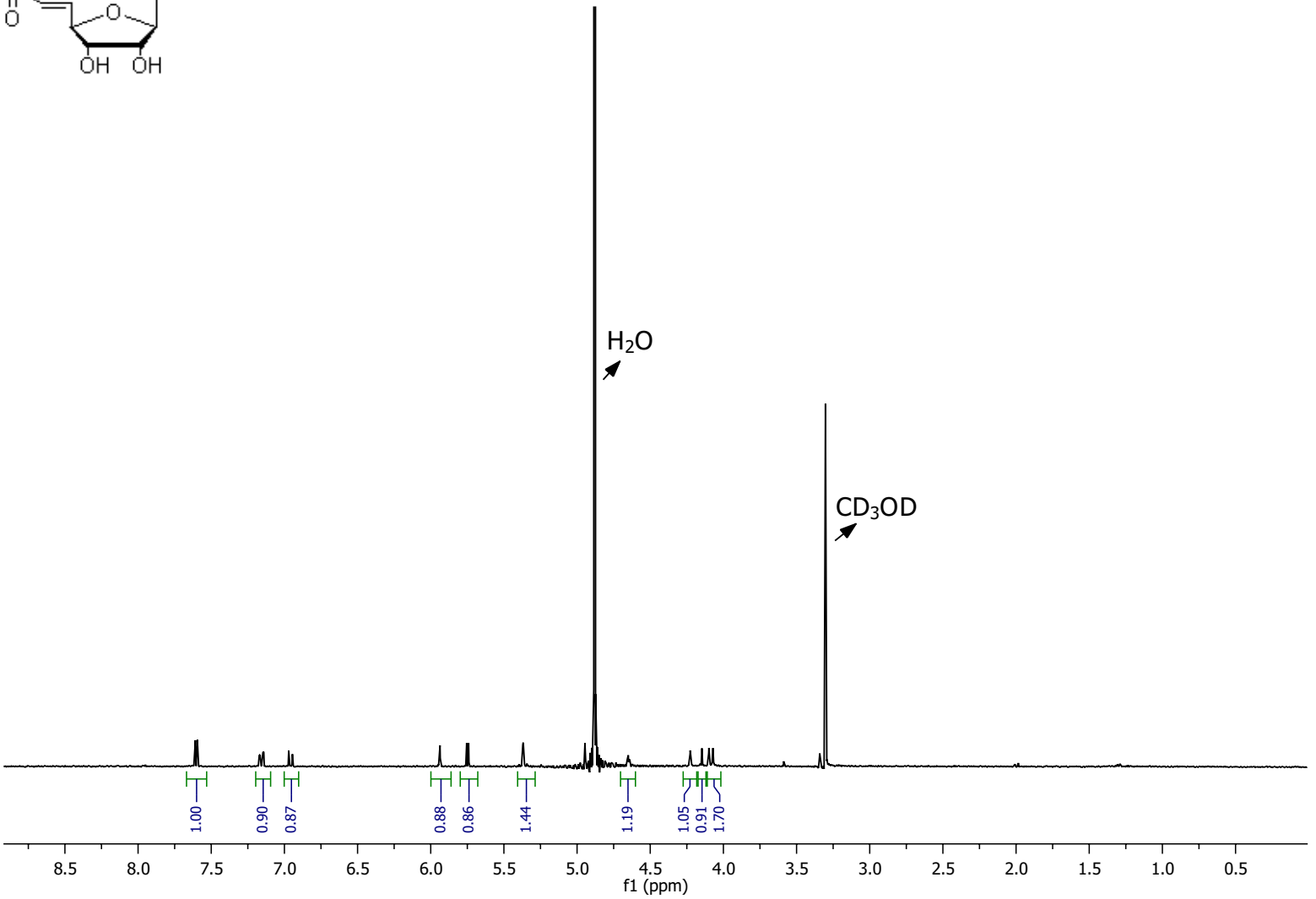

water
CD3OD
1.00
2.23
1.12
1.01
1.64
0.97
1.03
1.08
2.08
15.16
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

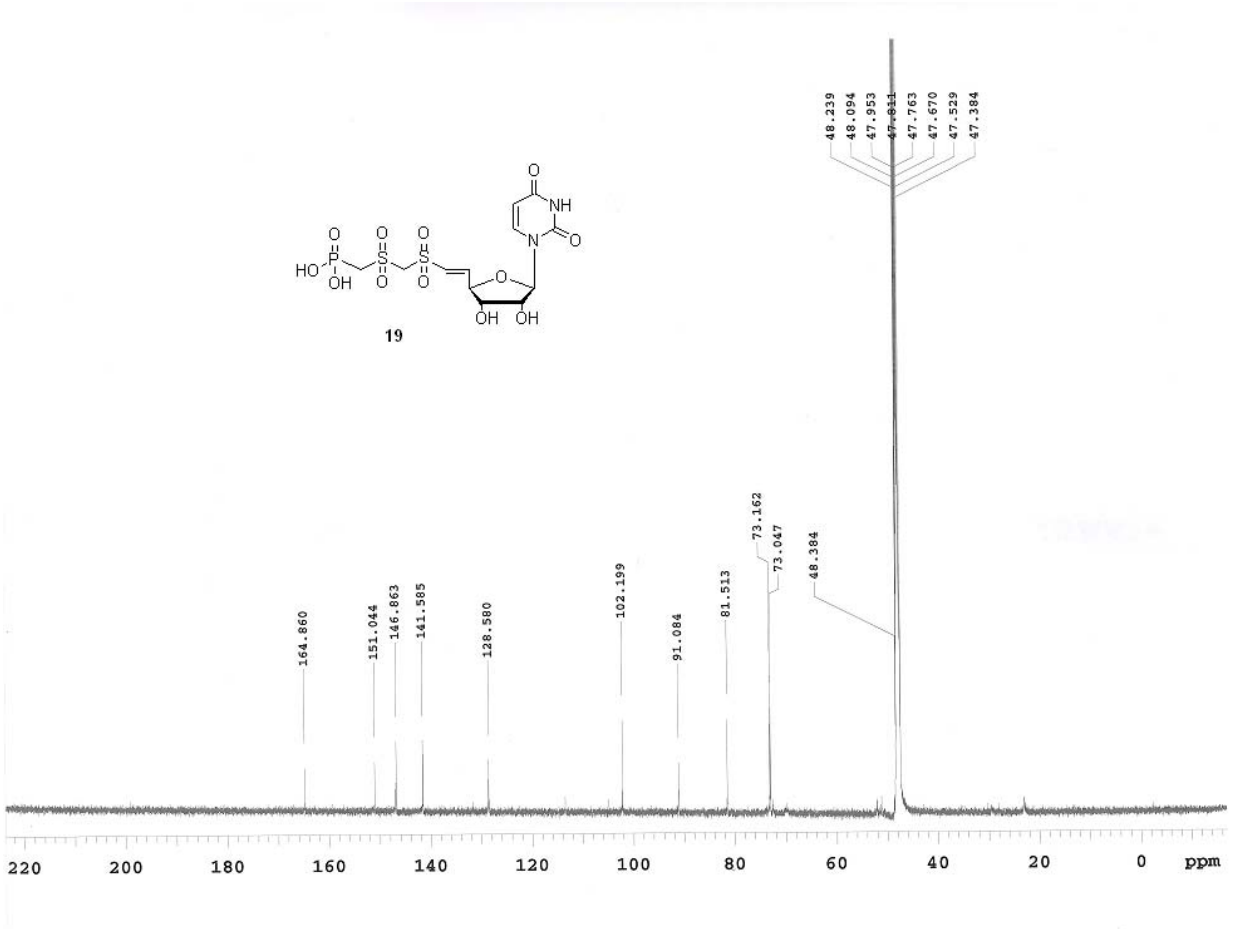

164.82
150.94
144.10
141.97
130.31
102.03
91.65
81.34
73.24
73.15
73.12
73.07
47.79
47.67
47.65
47.52
47.38
23.17
23.16
23.15
22.89
22.88
22.86
22.84
CD3OD
18
f1 (ppm)

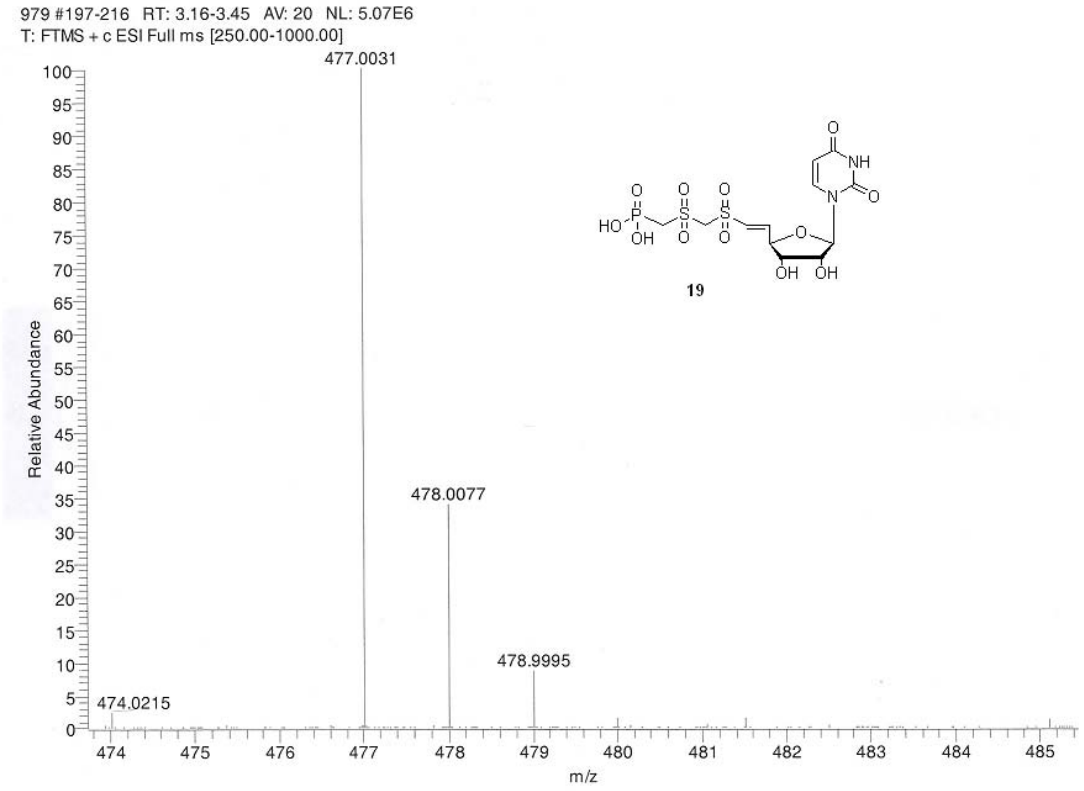

901_080625103212 #332-349 RT: 4.90-5.15 AV: 18 NL: 3.21E7
T: FTMS + p ESI Full ms [150.00-1000.00]
483.1213
484.1247
488.3597
485.1174
480.1397
Relative Abundance
m/z
18

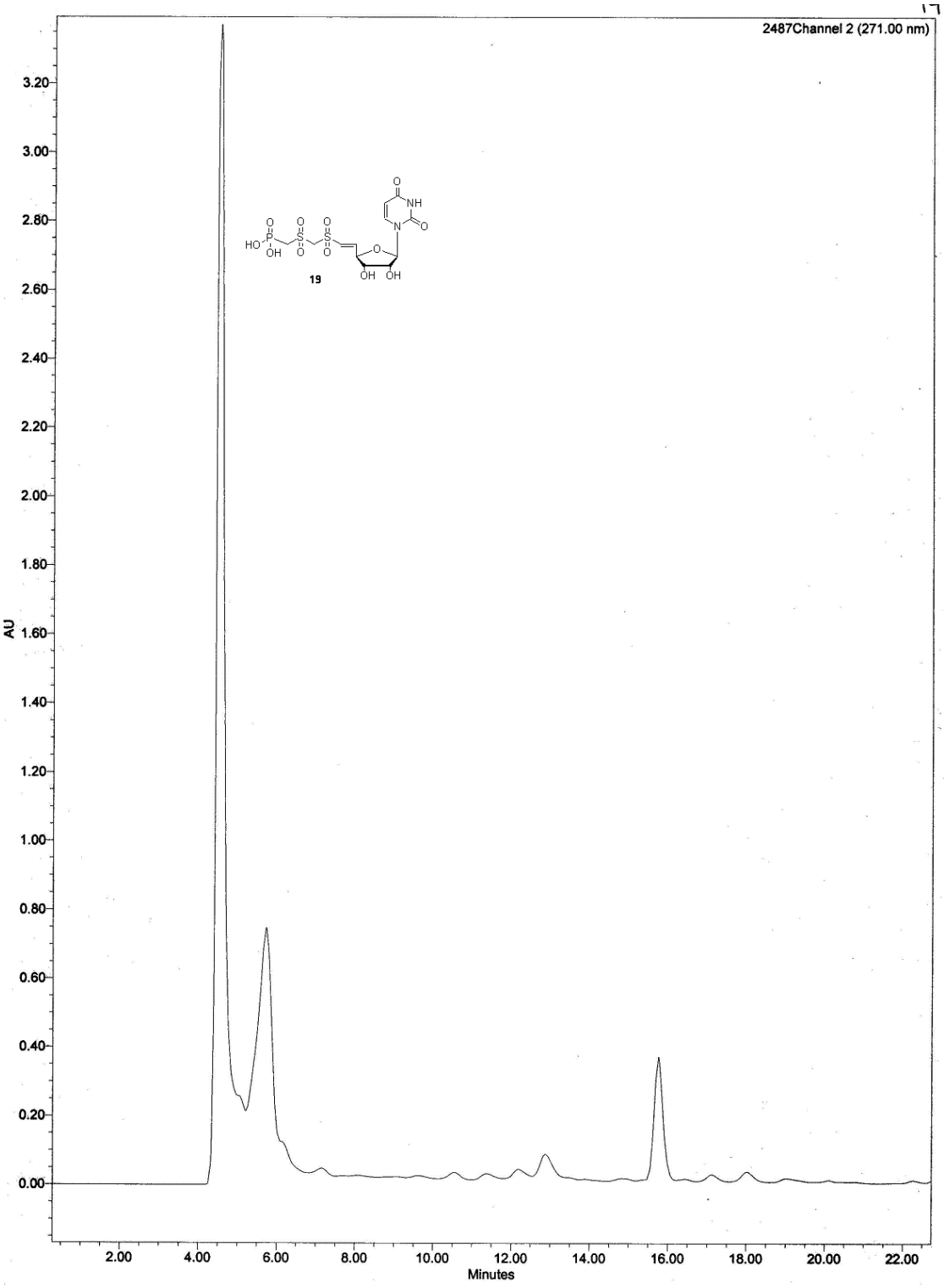

O
NH
N
O
HO
P
OH
O
S
O
O
S
O
O
O
OH
OH

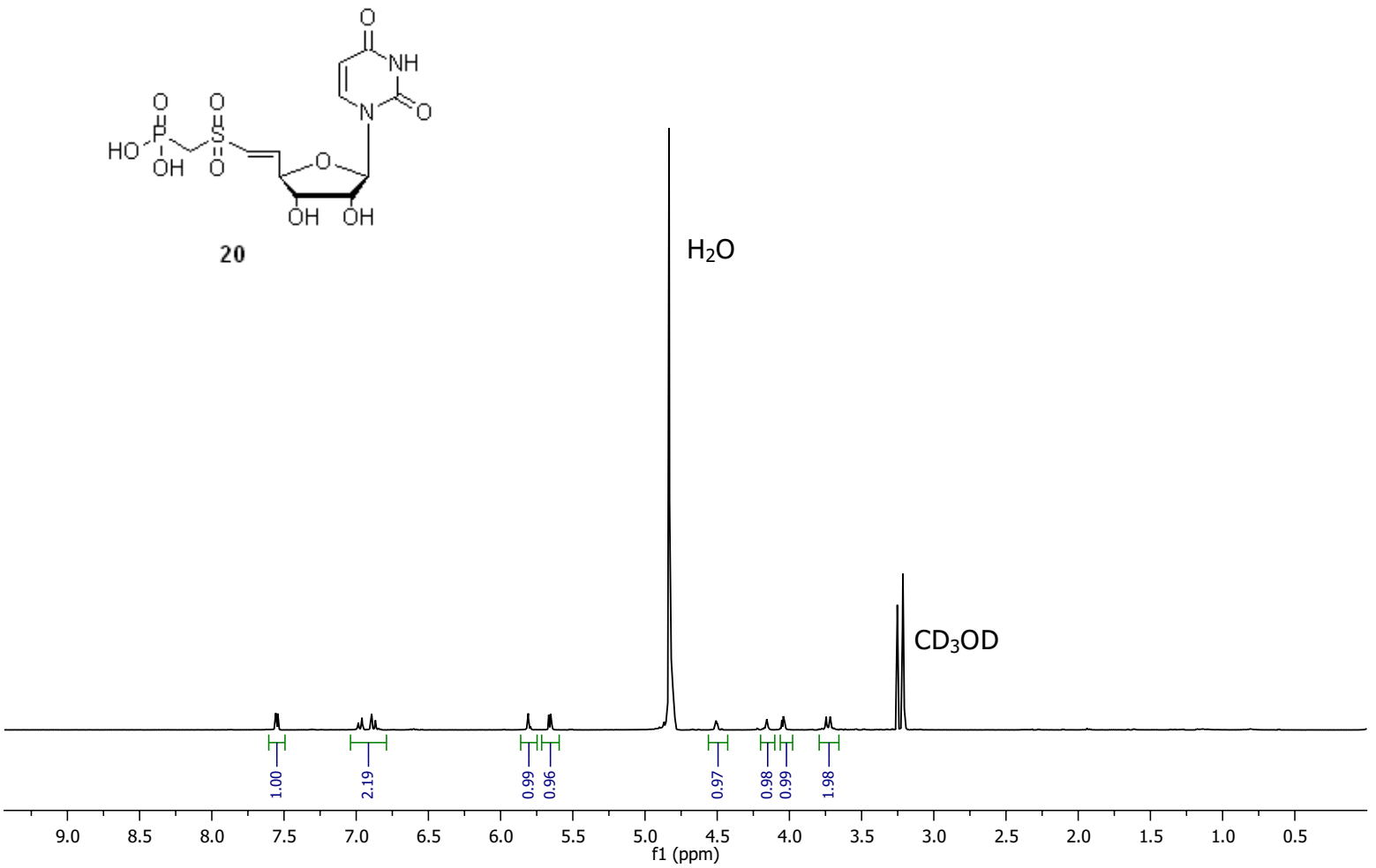
19


H2O
CD3OD
1.00
0.90
0.87
0.88
0.86
1.44
1.19
1.05
0.91
1.70
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

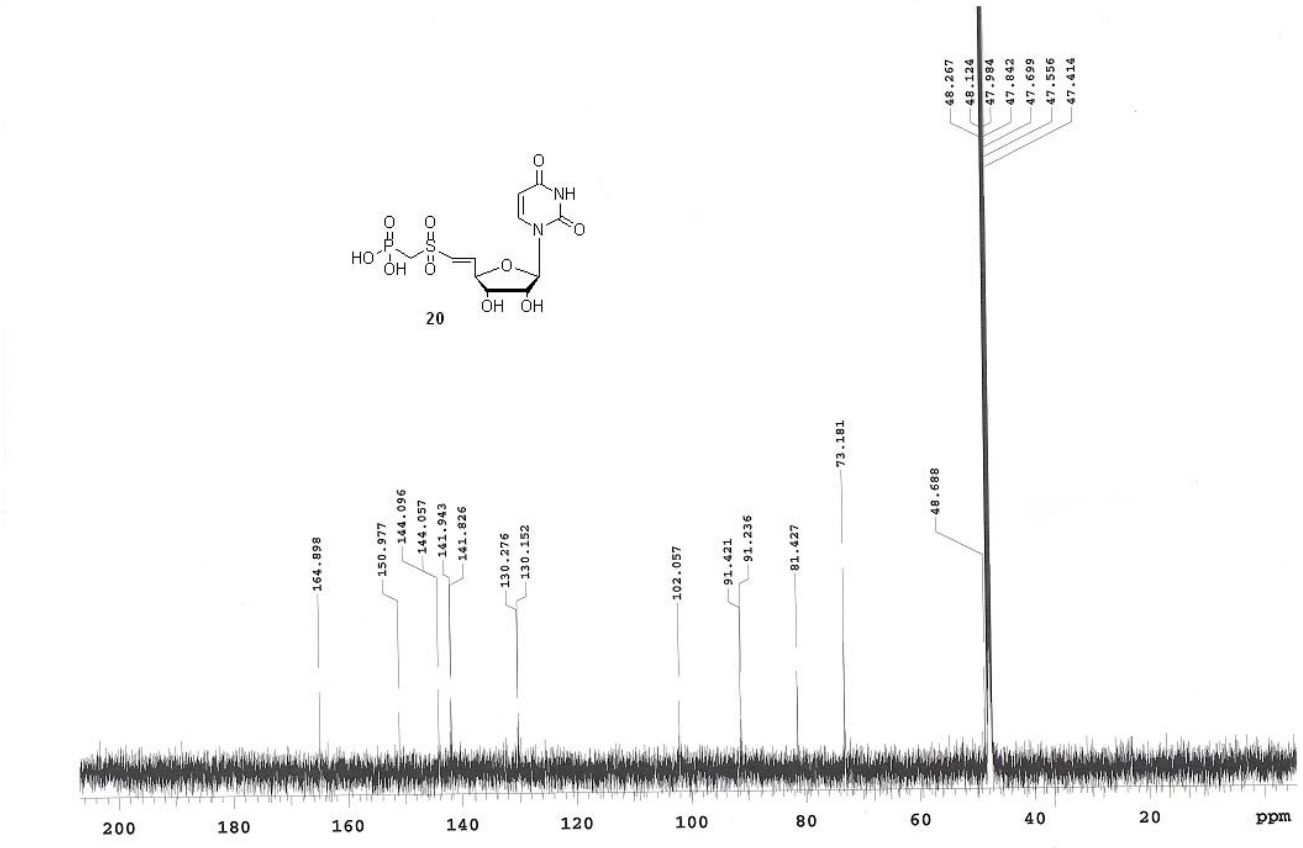

19
48.239
48.094
47.953
47.631
47.763
47.670
47.529
47.384
164.860
151.044
146.863
141.585
128.580
102.199
91.084
81.513
73.162
73.047
40.384
220
200
180
160
140
120
100
80
60
40
20
0
ppm


979 #197-216 RT: 3.16-3.45 AV: 20 NL: 5.07E6
T: FTMS + c ESI Full ms [250.00-1000.00]
477.0031
478.0077
478.9995
474.0215
Relative Abundance
m/z
19

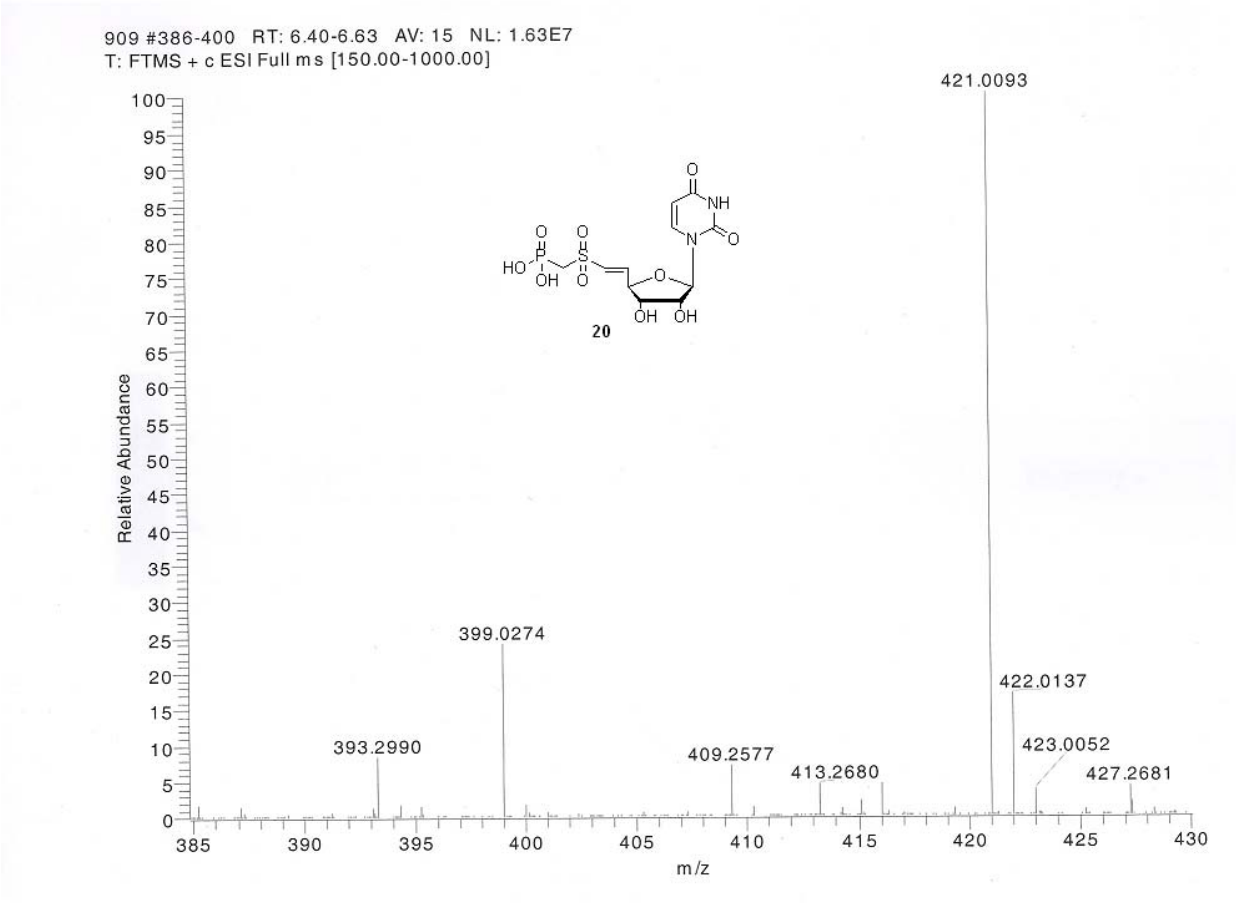

2487Channel 2 (271.00 nm)
19
AU
Minutes
3.20
3.00
2.80
2.60
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00

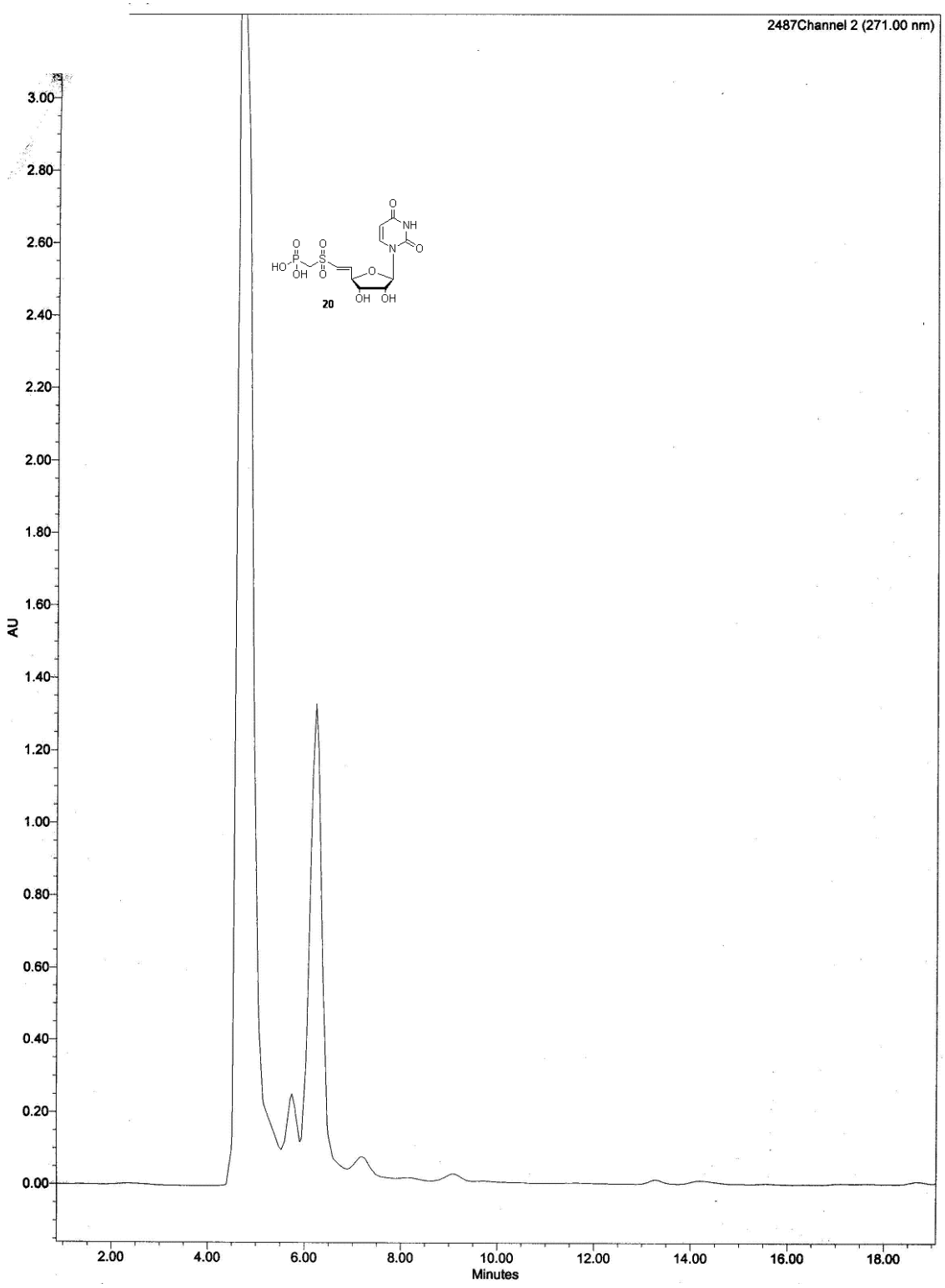

O
NH
N
O
HO
P
OH
O
S
O
O
OH
OH
H2O
CD3OD
1.00
2.19
0.99
0.96
0.97
0.98
0.99
1.98
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

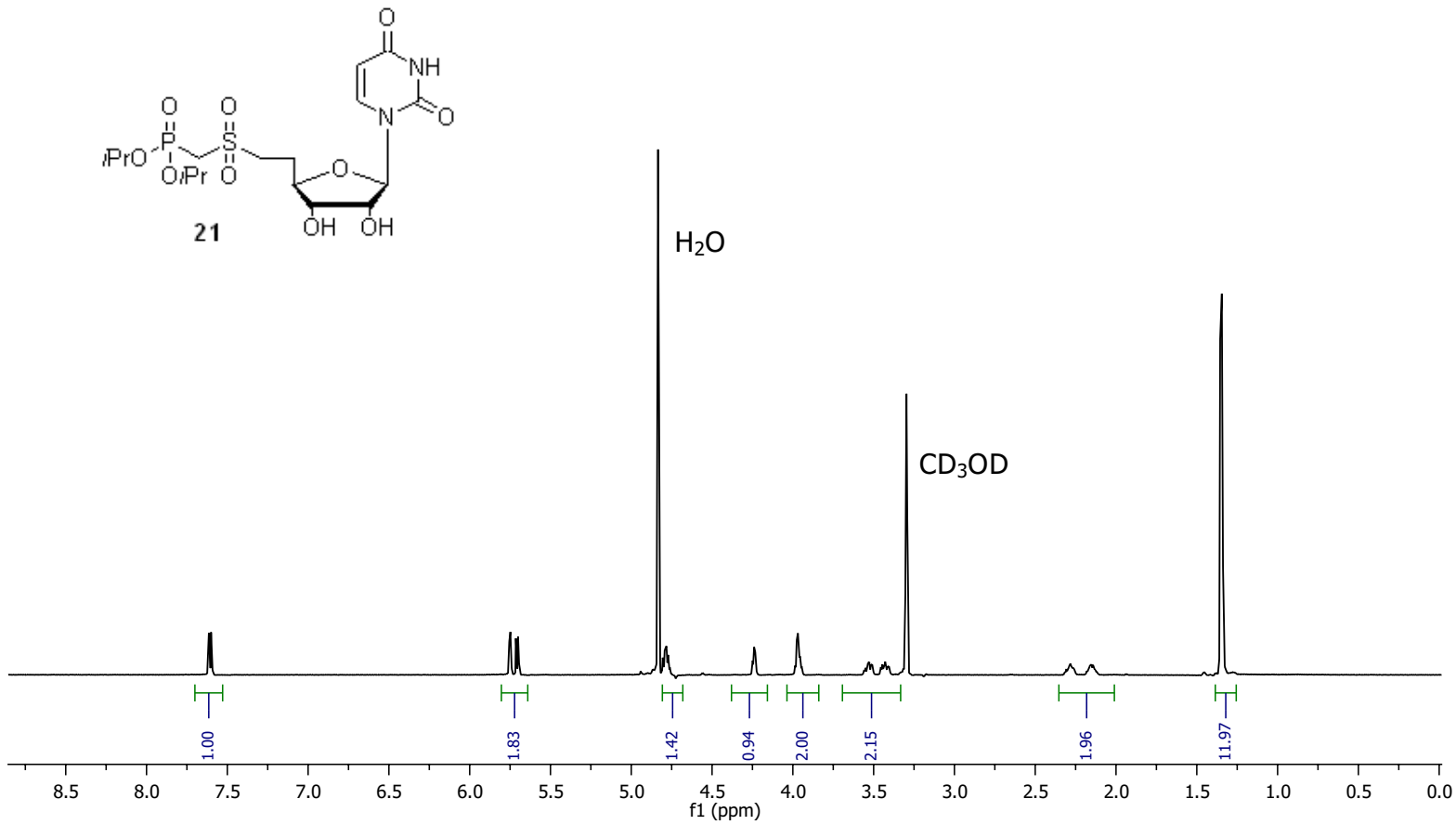
20

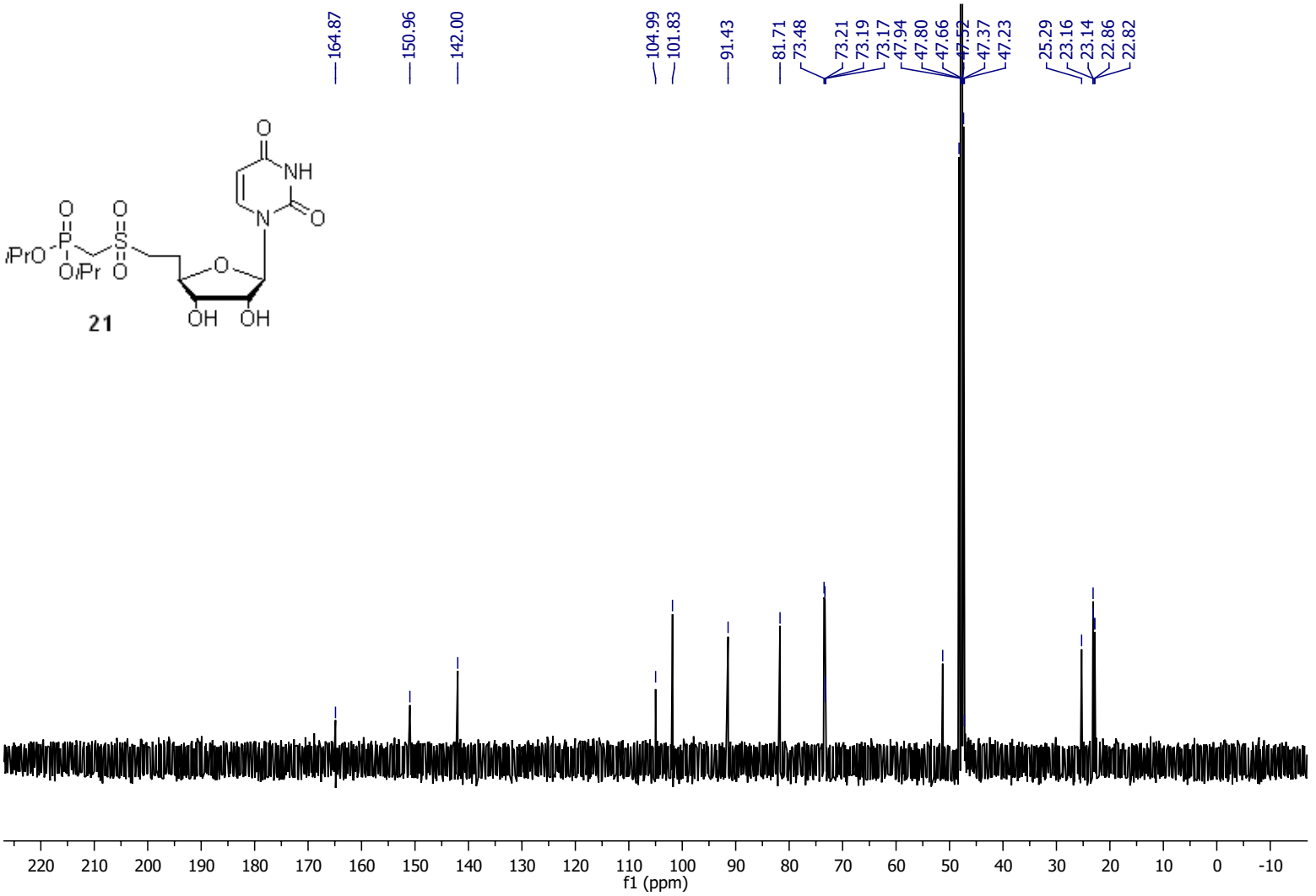

48.267
48.124
47.984
47.842
47.699
47.556
47.414
73.181
48.688
164.898
150.977
144.096
144.057
141.943
141.826
130.276
130.152
102.057
91.421
91.236
81.427
200
180
160
140
120
100
80
60
40
20
ppm

20

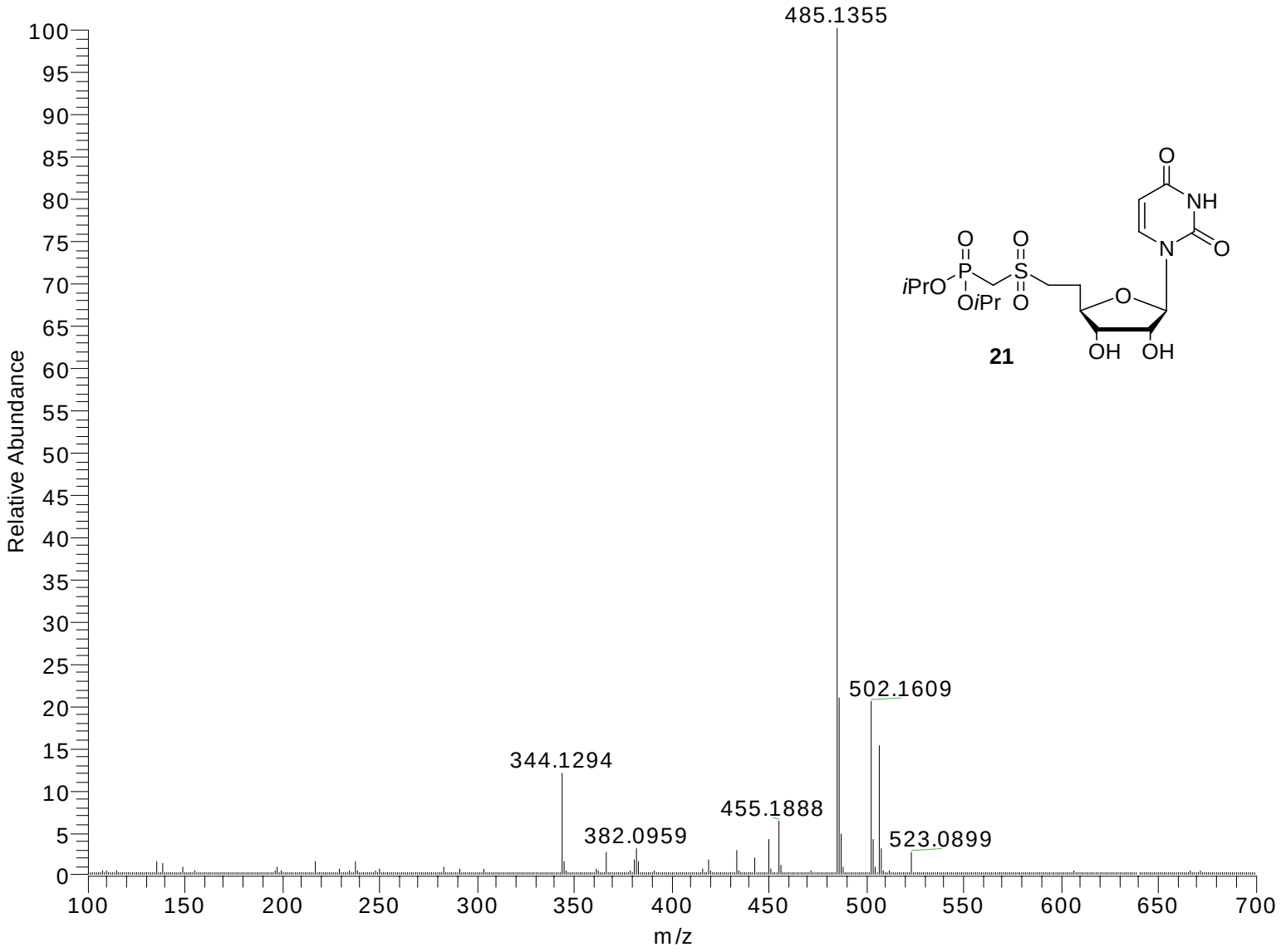

909 #386-400 RT: 6.40-6.63 AV: 15 NL: 1.63E7
T: FTMS + c ESI Full ms [150.00-1000.00]
421.0093
399.0274
422.0137
393.2990
409.2577
423.0052
413.2680
427.2681
Relative Abundance
m/z
20


2487Channel 2 (271.00 nm)
AU
Minutes
3.00
2.80
2.60
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20

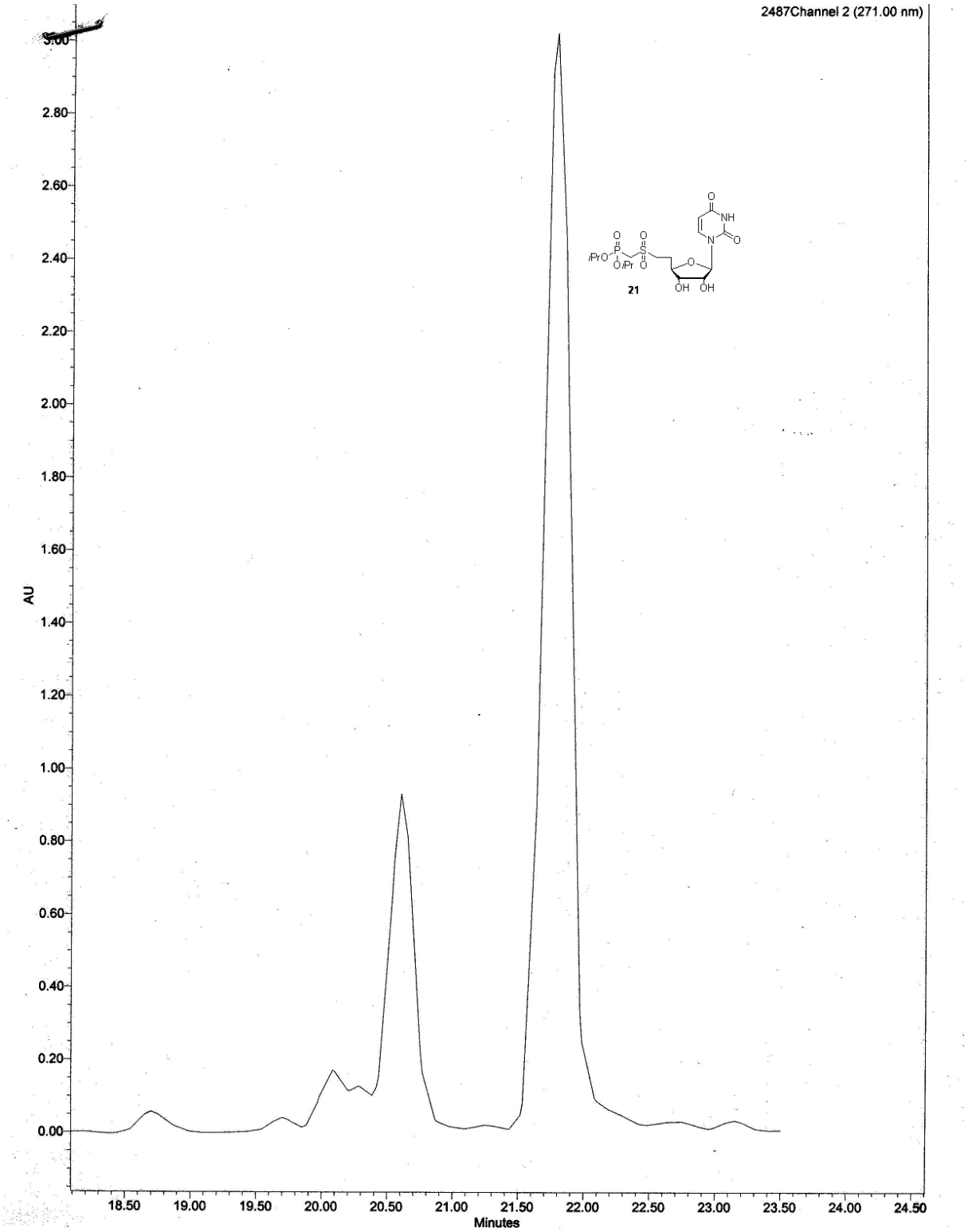

21
H2O
CD3OD
1.00
1.83
1.42
0.94
2.00
2.15
1.96
11.97
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

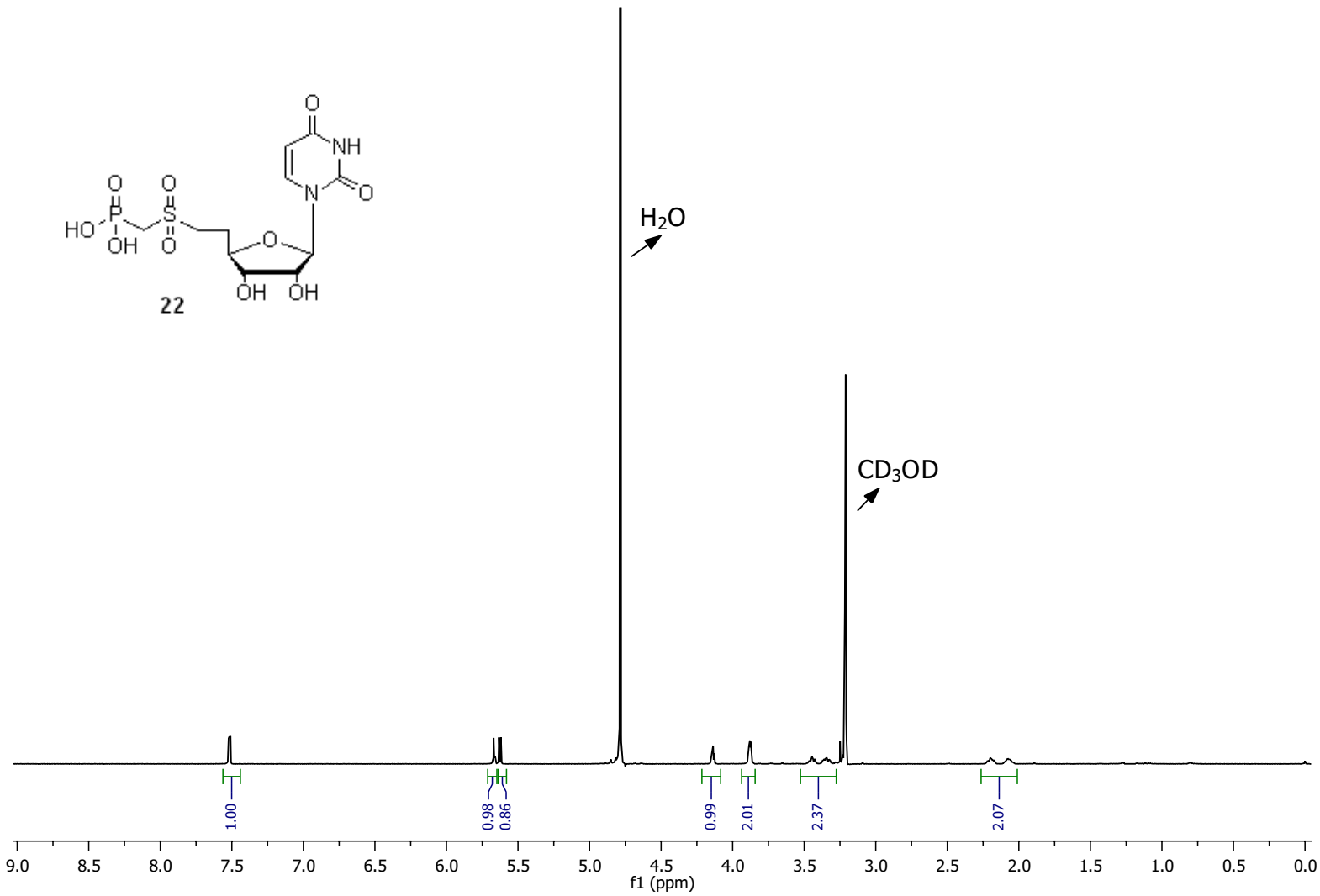

164.87
150.96
142.00
104.99
101.83
91.43
81.71
73.48
73.21
73.19
73.17
47.94
47.80
47.66
47.52
47.37
47.23
25.29
23.16
23.14
22.86
22.82
O
NH
N
O
iPrO
O
P
O
O
iPr
S
O
O
O
OH
OH
21
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

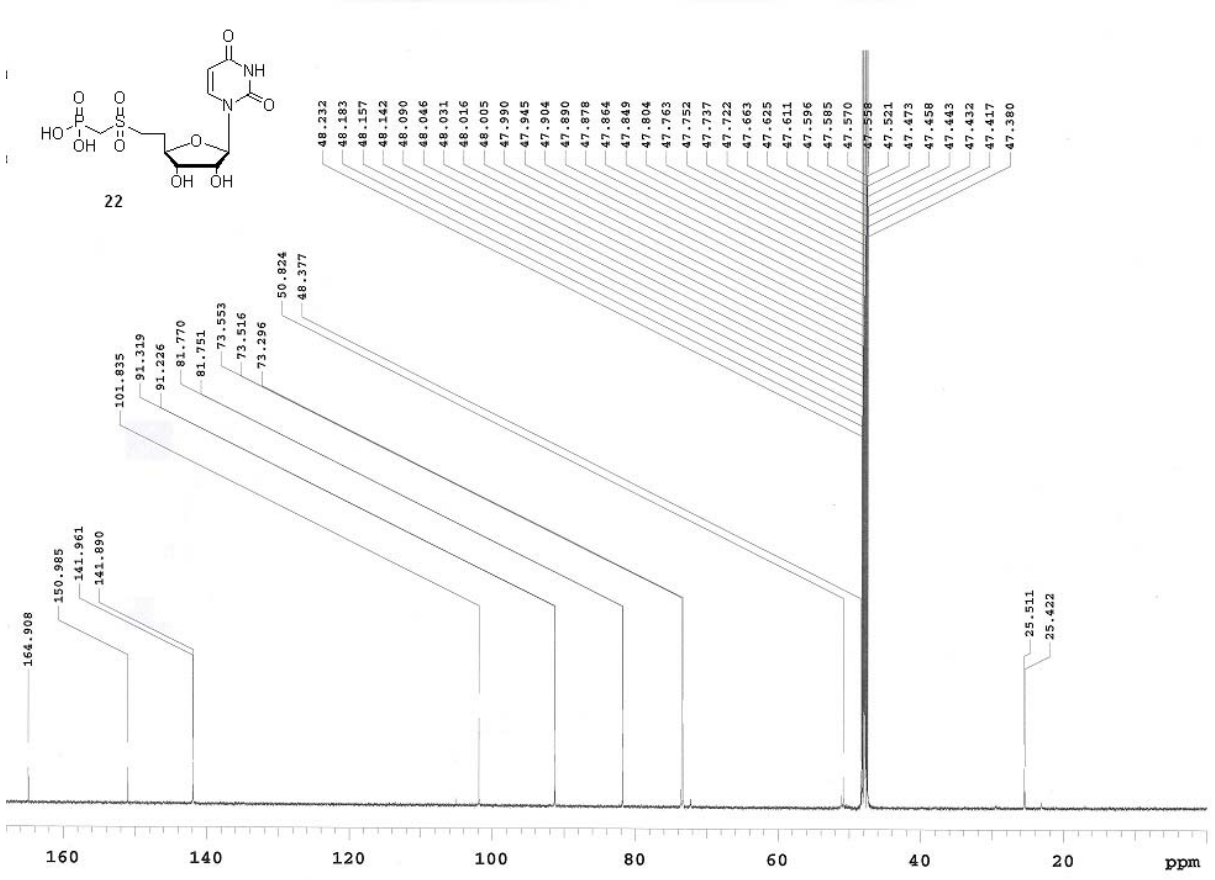
913 #315-332 RT: 4.68-4.95 AV: 18 NL: 1.32E8
T: FTMS + c ESI Full ms [100.00-700.00]

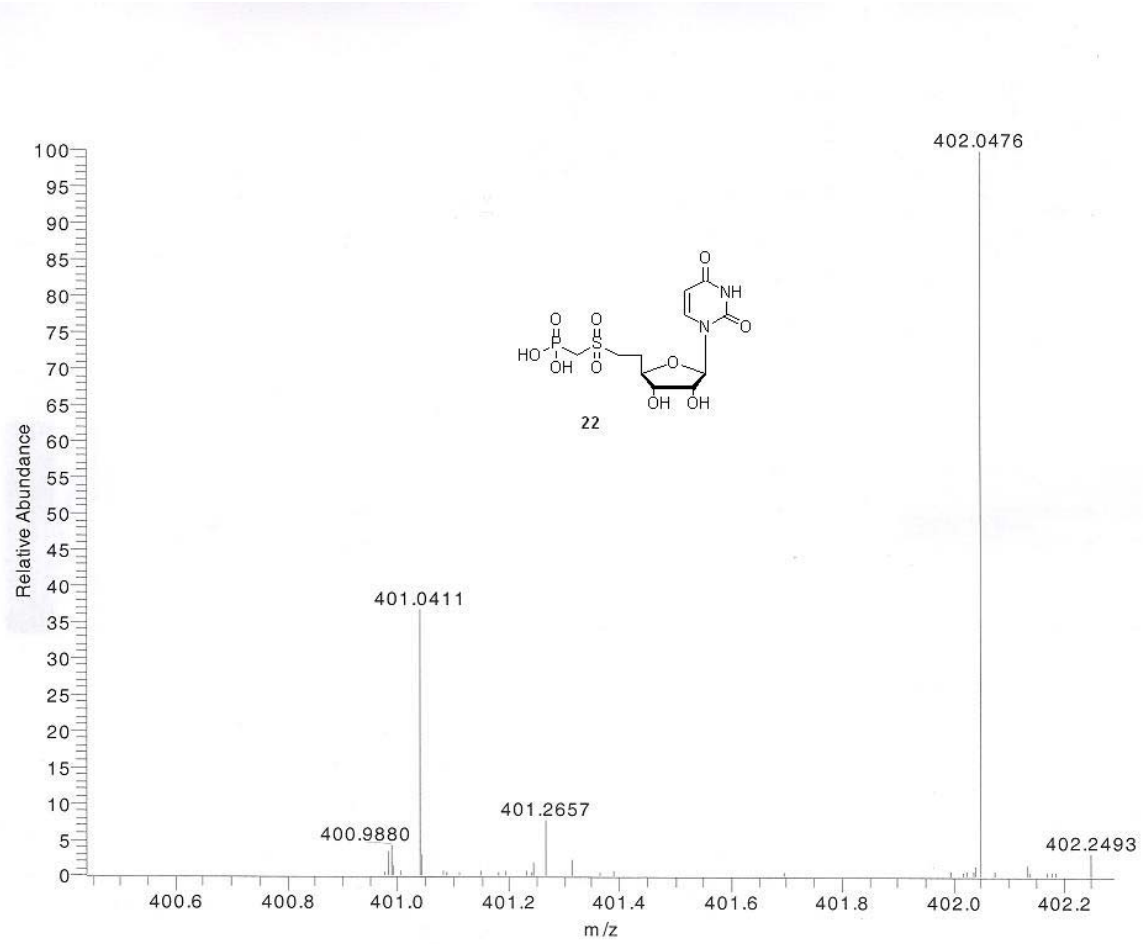


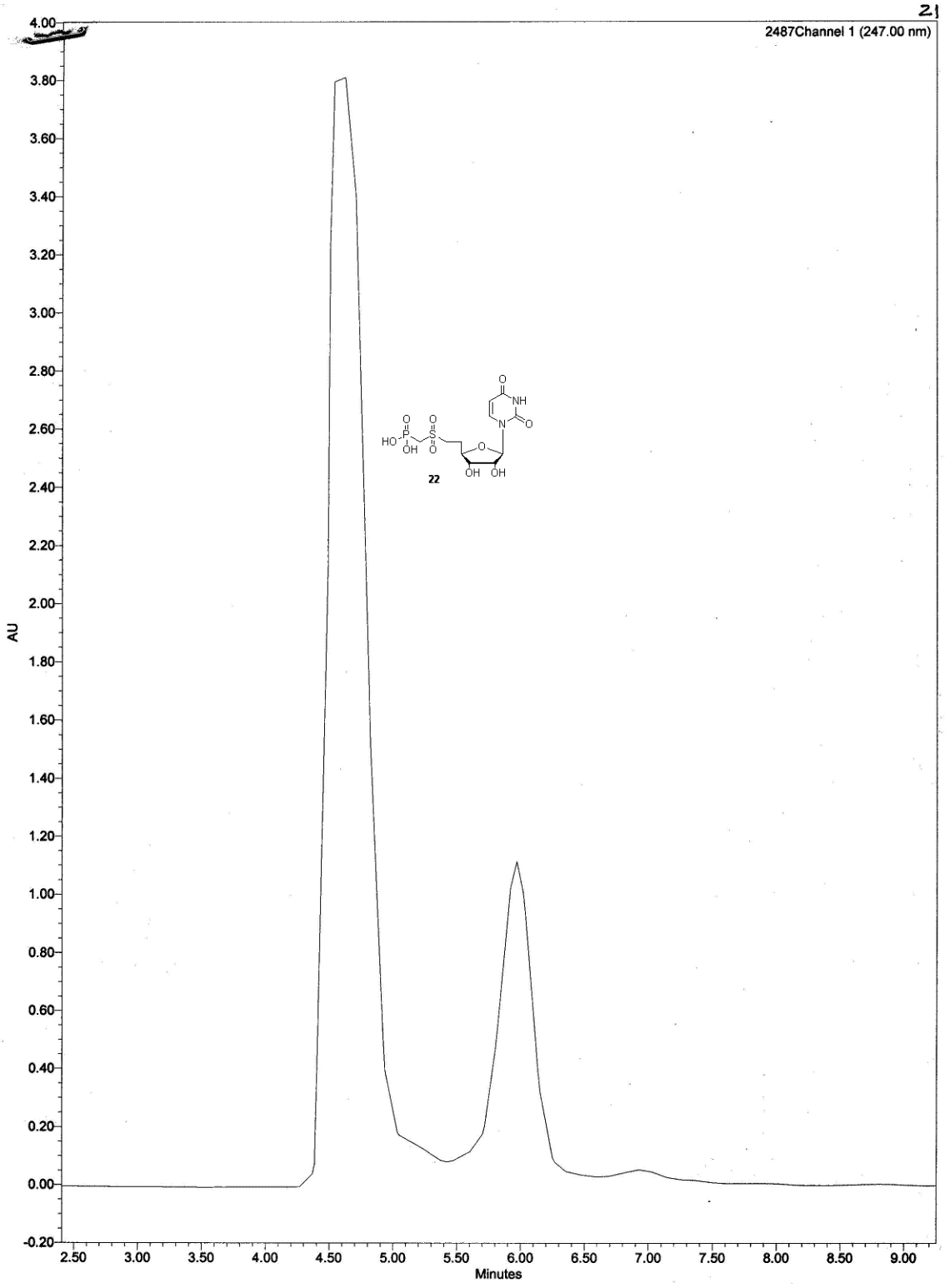
21


2487Channel 2 (271.00 nm)
AU
Minutes
21

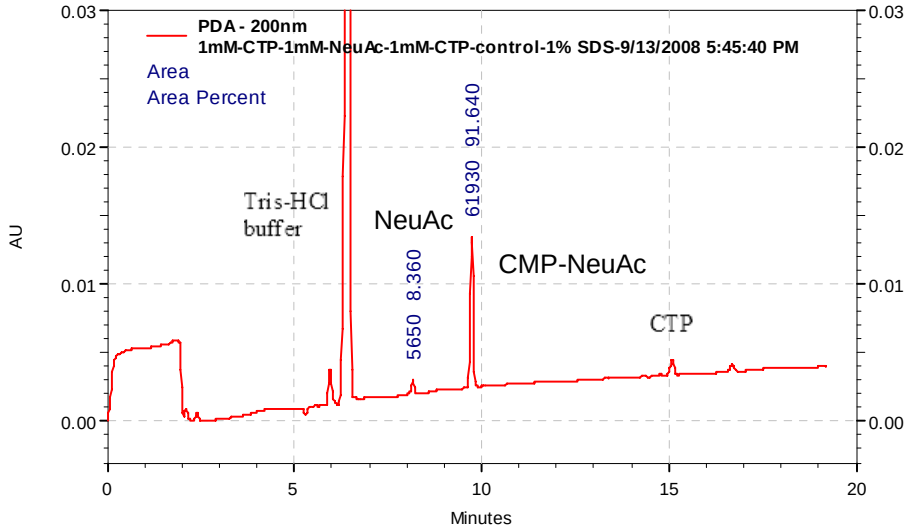

22
H2O
CD3OD
1.00
0.98
0.86
0.99
2.01
2.37
2.07
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

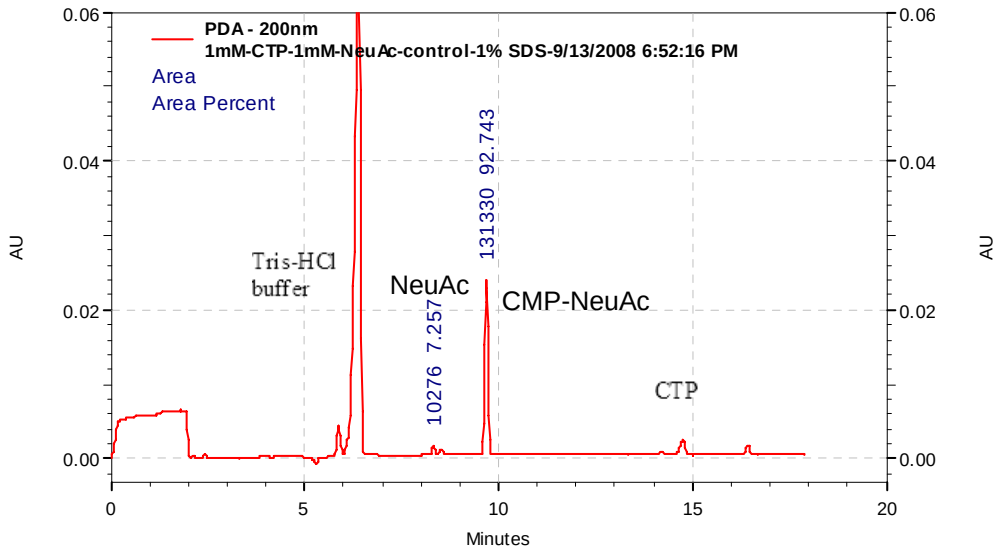

22
48.232
48.183
48.157
48.142
48.090
48.046
48.031
48.016
48.005
47.990
47.945
47.904
47.890
47.878
47.864
47.849
47.804
47.763
47.752
47.737
47.722
47.663
47.625
47.611
47.596
47.585
47.570
47.558
47.521
47.473
47.458
47.443
47.432
47.417
47.380
50.824
48.377
101.835
91.319
91.226
81.770
81.751
73.553
73.516
73.296
164.908
150.985
141.961
141.890
25.511
25.422
160
140
120
100
80
60
40
20
ppm


402.0476
401.0411
401.2657
400.9880
402.2493
Relative Abundance
m/z
22

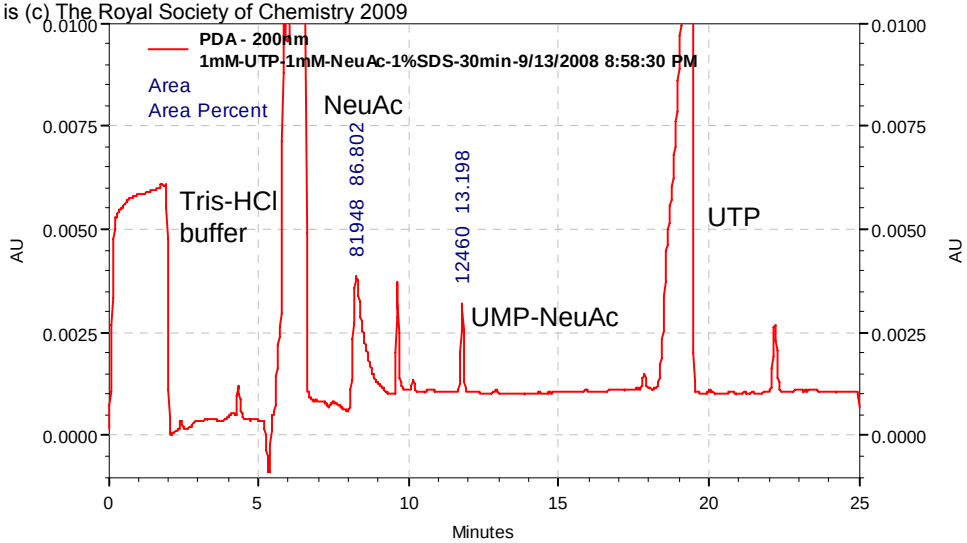

2487Channel 1 (247.00 nm)
AU
Minutes
22

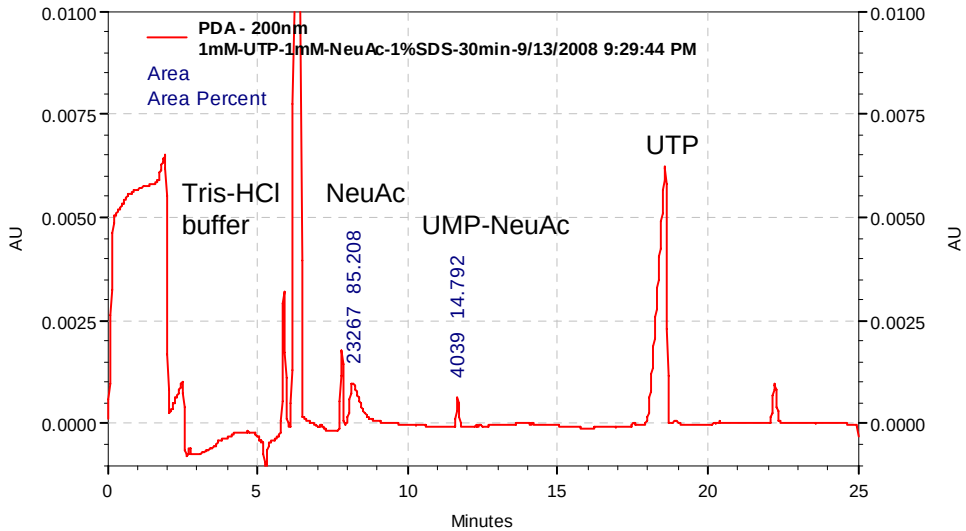
CTP at 1 mM


PDA - 200nm
1mM-CTP-1mM-NeuAc-1mM-CTP-control-1% SDS-9/13/2008 5:45:40 PM
Area
Area Percent
Tris-HCl buffer
NeuAc
CMP-NeuAc
CTP
5650 8.360
61930 91.640
AU
Minutes

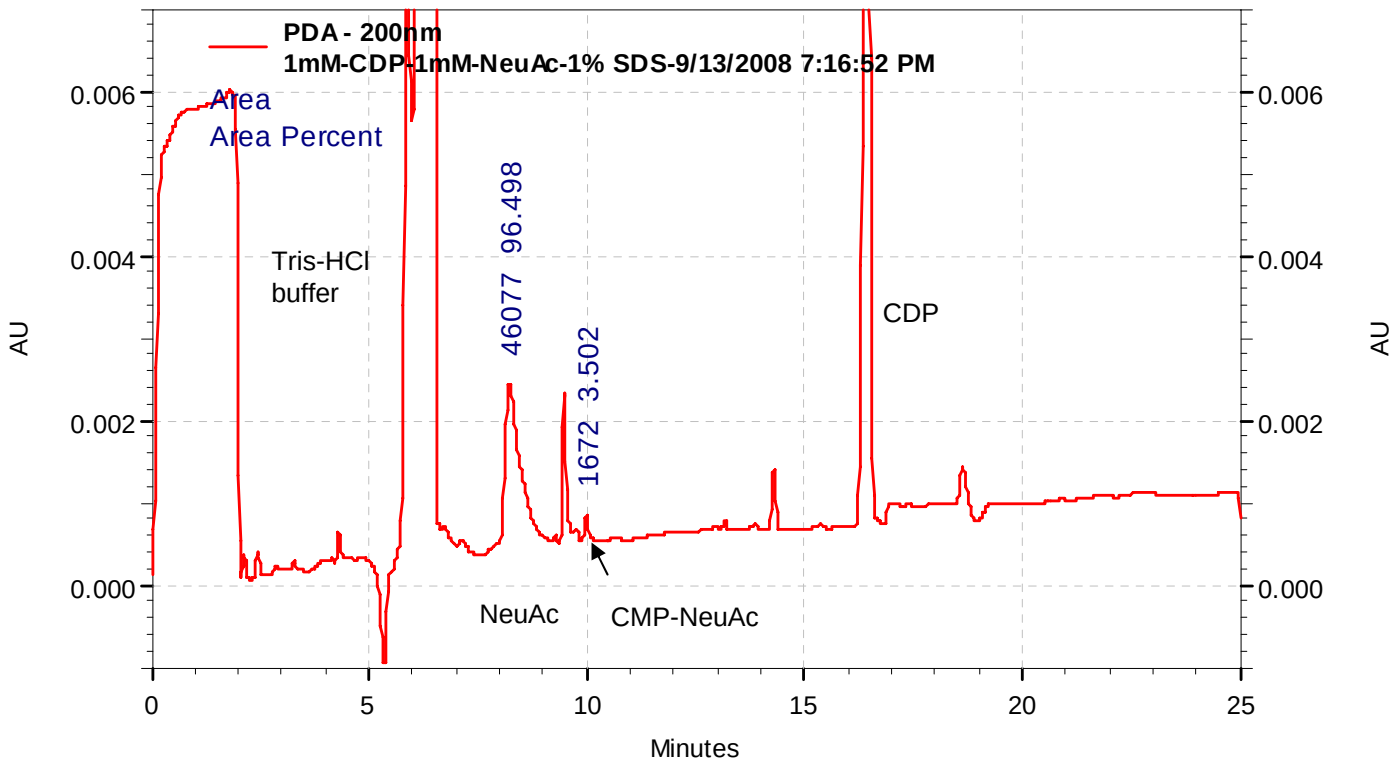

PDA - 200nm
1mM-CTP-1mM-NeuAc-control-1% SDS-9/13/2008 6:52:16 PM
Area
Area Percent
Tris-HCl buffer
NeuAc
CMP-NeuAc
CTP
10276 7.257
131330 92.743
AU
Minutes

UTP at 1 mM

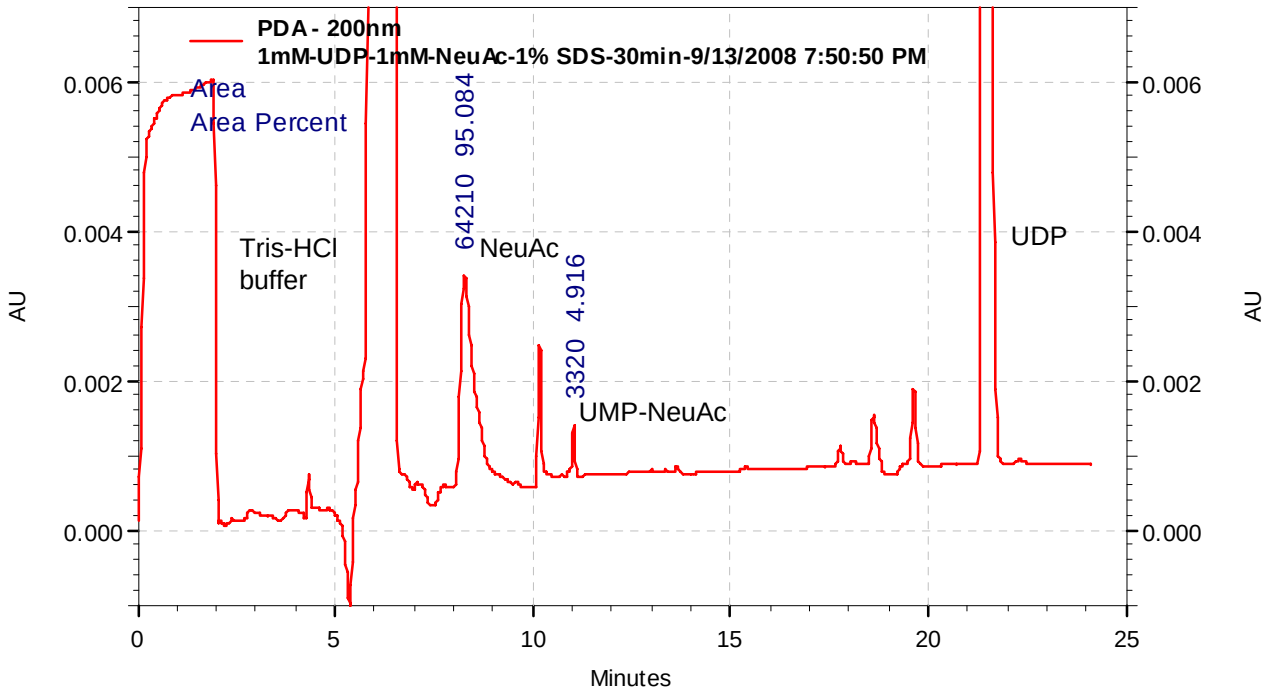

PDA - 200nm
1mM-UTP-1mM-NeuAc-1%SDS-30min-9/13/2008 8:58:30 PM
Area
Area Percent
Tris-HCl buffer
NeuAc
81948 86.802
12460 13.198
UMP-NeuAc
UTP
AU
Minutes
0.0100
0.0075
0.0050
0.0025
0.0000
0
5
10
15
20
25


PDA - 200nm
1mM-UTP-1mM-NeuAc-1%SDS-30min-9/13/2008 9:29:44 PM
Area
Area Percent
Tris-HCl buffer
NeuAc
UMP-NeuAc
UTP
23267 85.208
4039 14.792
AU
Minutes
0.0100
0.0075
0.0050
0.0025
0.0000
0
5
10
15
20
25

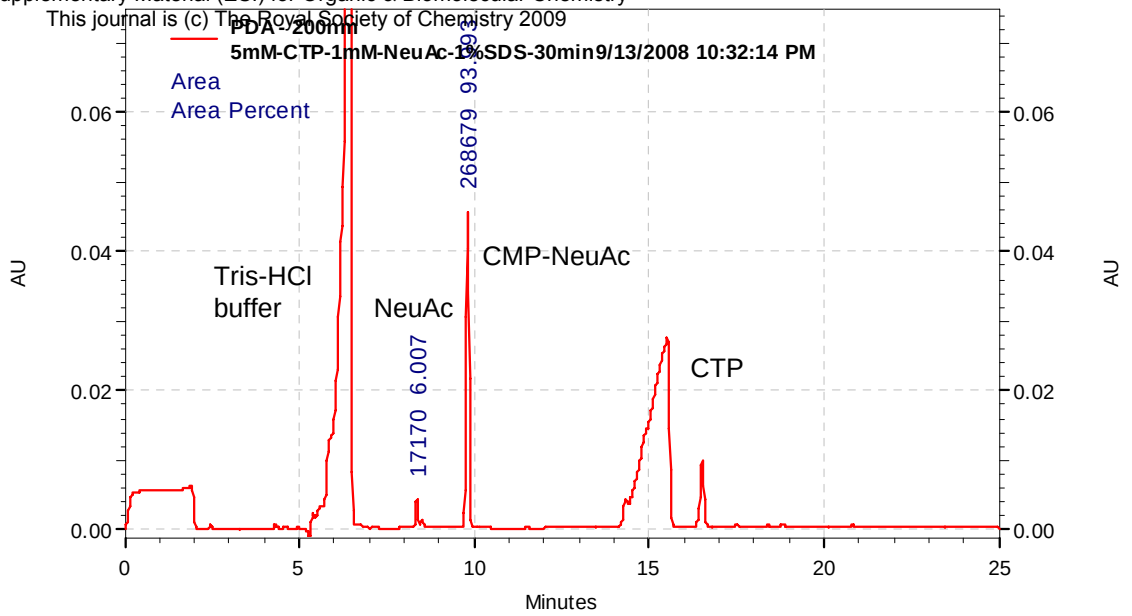
CDP at 1 mM

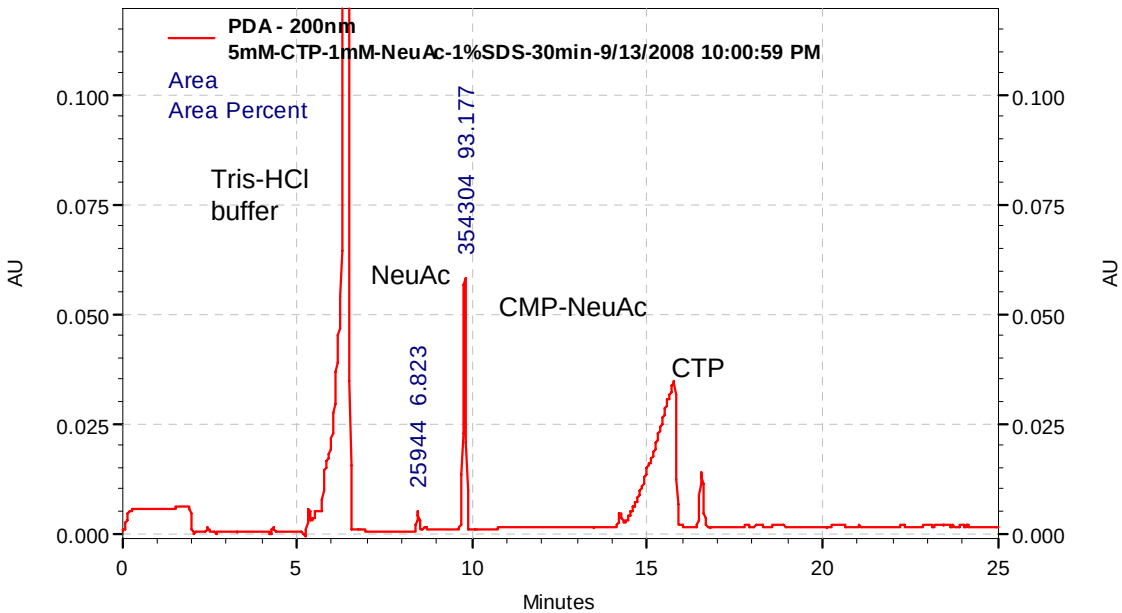

PDA - 200nm
1mM-CDP-1mM-NeuAc-1% SDS-9/13/2008 7:16:52 PM
Area
Area Percent
Tris-HCl
buffer
96.498
46077
3.502
1672
NeuAc
CMP-NeuAc
CDP
AU
Minutes
0.006
0.004
0.002
0.000
0
5
10
15
20
25

UDP at 1 mM

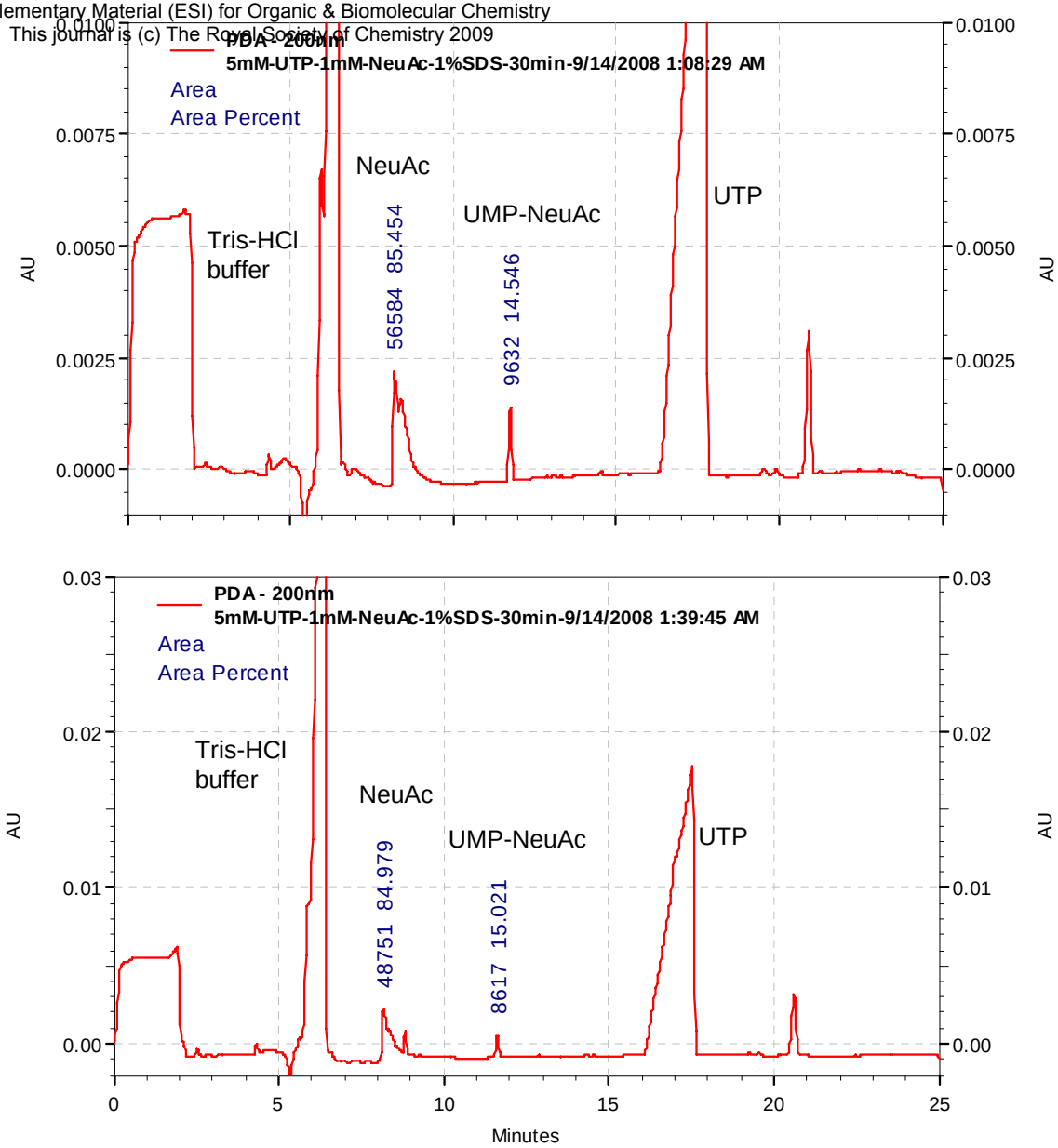

PDA - 200nm
1mM-UDP-1mM-NeuAc-1% SDS-30min-9/13/2008 7:50:50 PM
Area
Area Percent
64210 95.084
3320 4.916
Tris-HCl
buffer
NeuAc
UMP-NeuAc
UDP
AU
Minutes
0.000
0.002
0.004
0.006
0
5
10
15
20
25

CTP at 5 mM

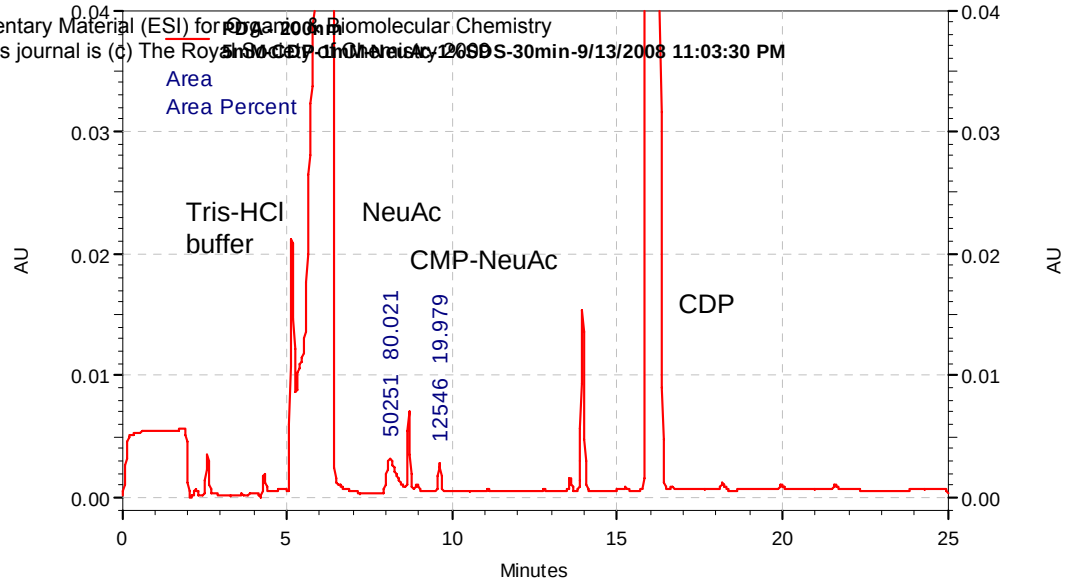

PDA - 200nm
5mM-CTP-1mM-NeuAc-1%SDS-30min9/13/2008 10:32:14 PM
Area
Area Percent
268679 93.993
17170 6.007
Tris-HCl buffer
NeuAc
CMP-NeuAc
CTP
AU
Minutes
0.00
0.02
0.04
0.06
0
5
10
15
20
25

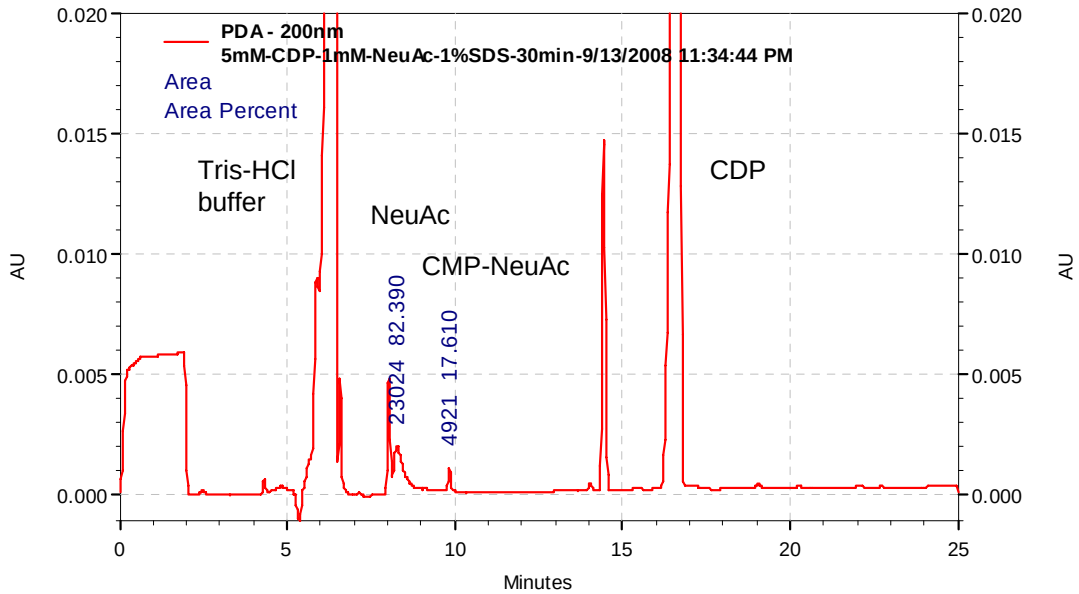

PDA - 200nm
5mM-CTP-1mM-NeuAc-1%SDS-30min-9/13/2008 10:00:59 PM
Area
Area Percent
354304 93.177
25944 6.823
Tris-HCl buffer
NeuAc
CMP-NeuAc
CTP
AU
Minutes
0.000
0.025
0.050
0.075
0.100
0
5
10
15
20
25

UTP at 5 mM

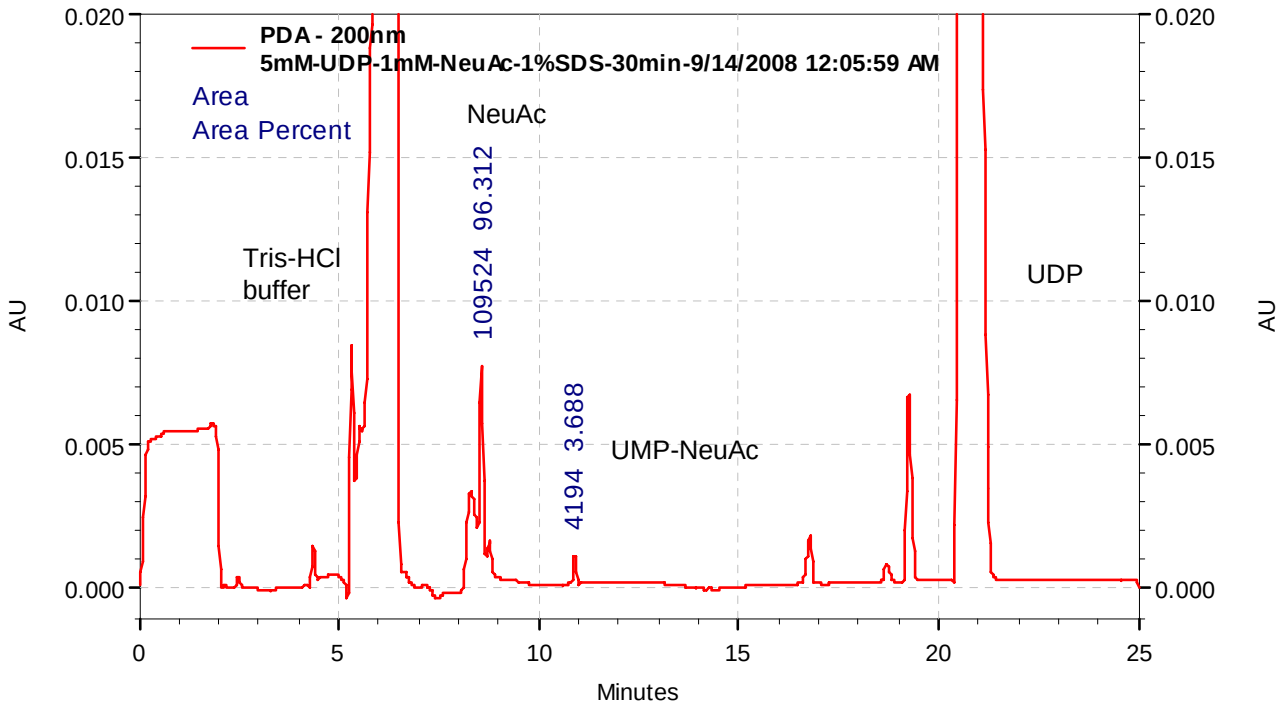


CDP at 5 mM

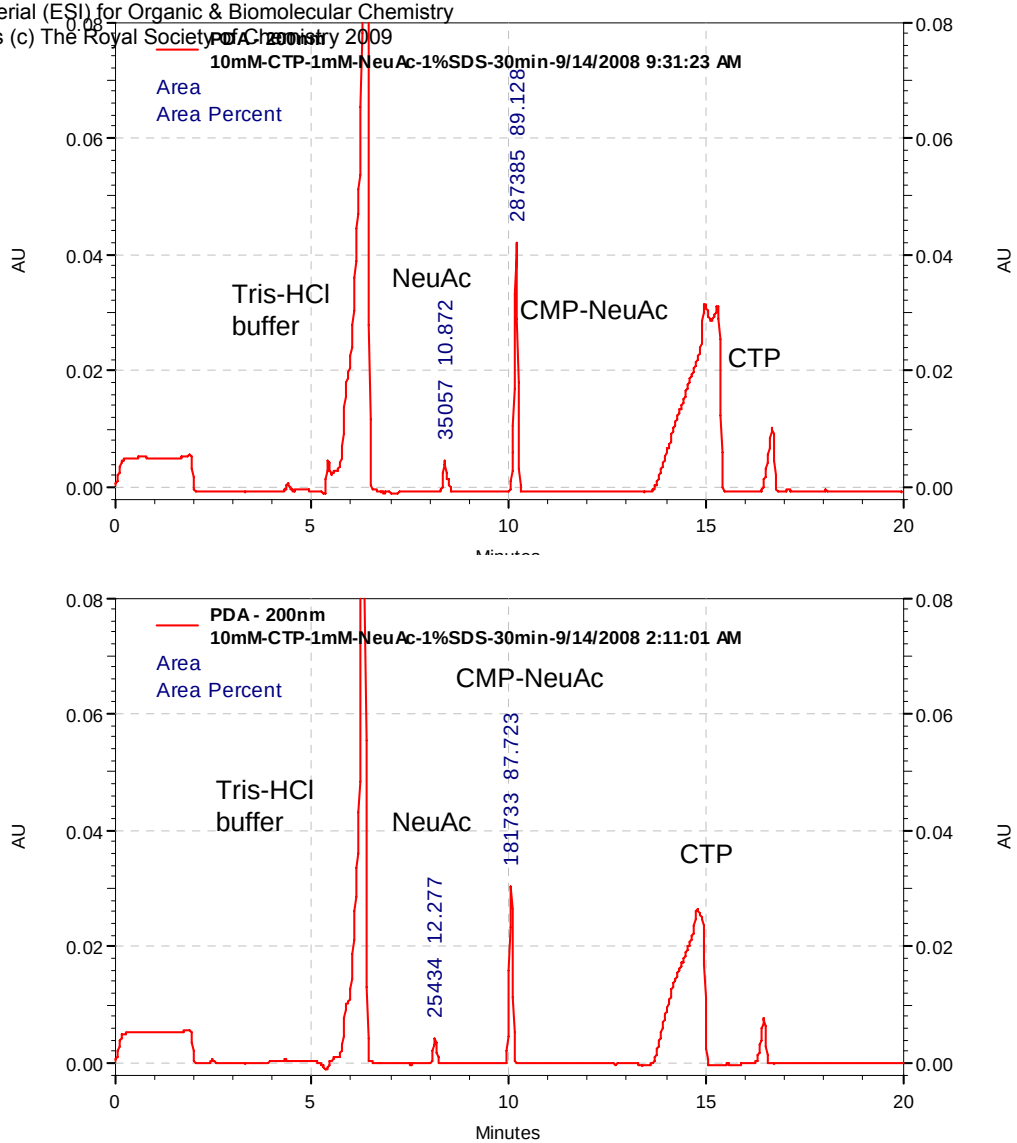

PDA - 200nm
5mM-CDP-1mM-NeuAc-1%SDS-30min-9/13/2008 11:03:30 PM
Area
Area Percent
Tris-HCl buffer
NeuAc
CMP-NeuAc
CDP
80.021
19.979
50251
12546
AU
Minutes


PDA - 200nm
5mM-CDP-1mM-NeuAc-1%SDS-30min-9/13/2008 11:34:44 PM
Area
Area Percent
Tris-HCl buffer
NeuAc
CMP-NeuAc
CDP
82.390
17.610
23024
4921
AU
Minutes

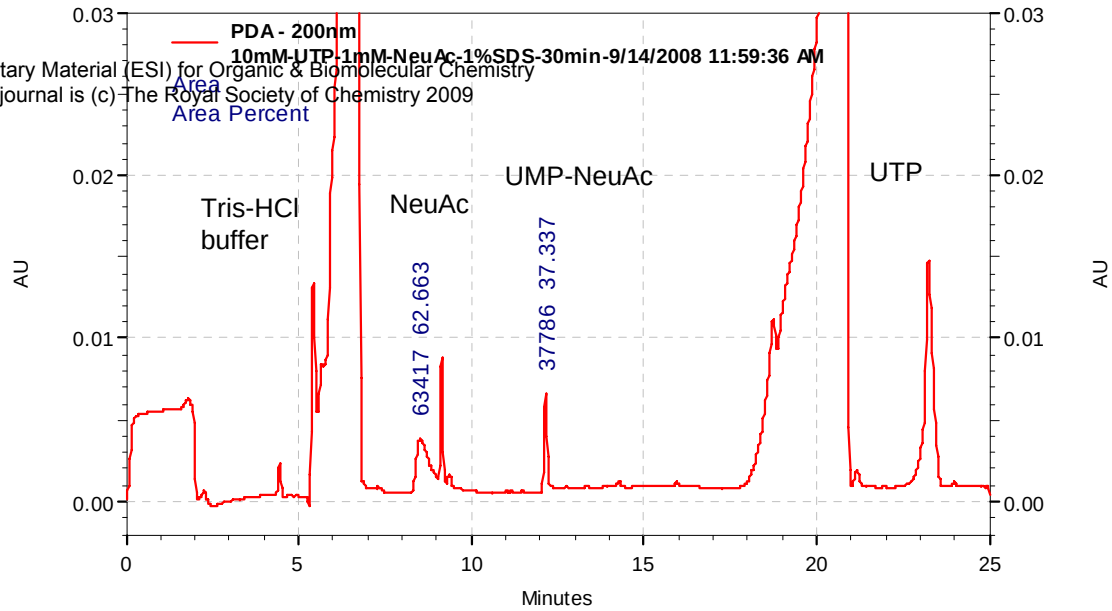
UDP at 5 mM

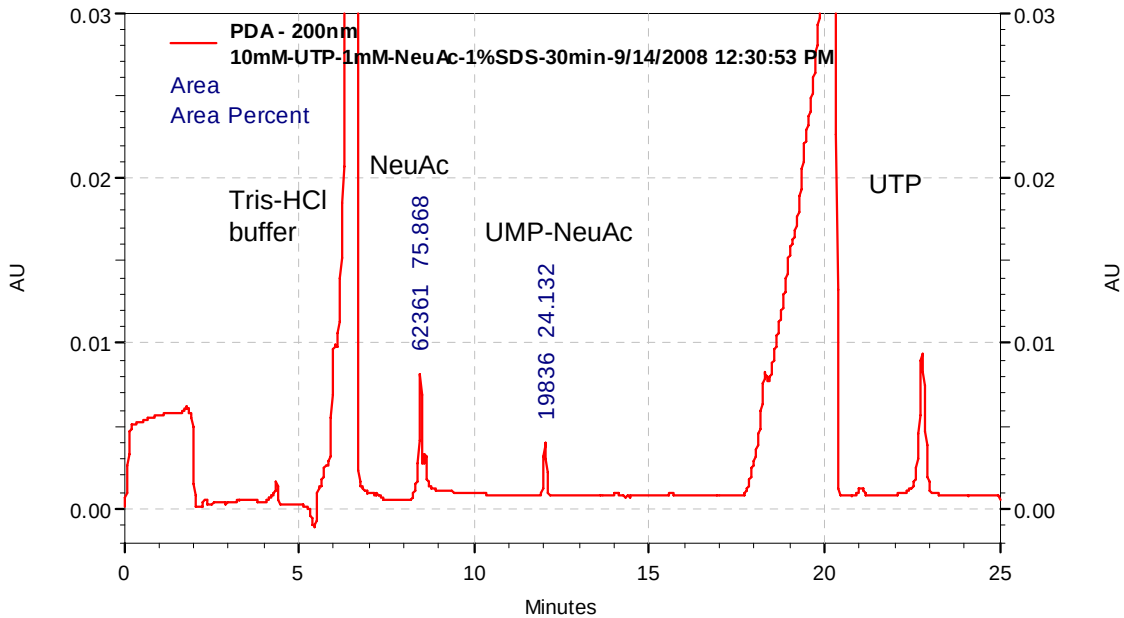

PDA - 200nm
5mM-UDP-1mM-NeuAc-1%SDS-30min-9/14/2008 12:05:59 AM
Area
Area Percent
NeuAc
109524 96.312
4194 3.688
Tris-HCl
buffer
UMP-NeuAc
UDP
AU
Minutes
0.020
0.015
0.010
0.005
0.000
0
5
10
15
20
25

CTP at 10 mM

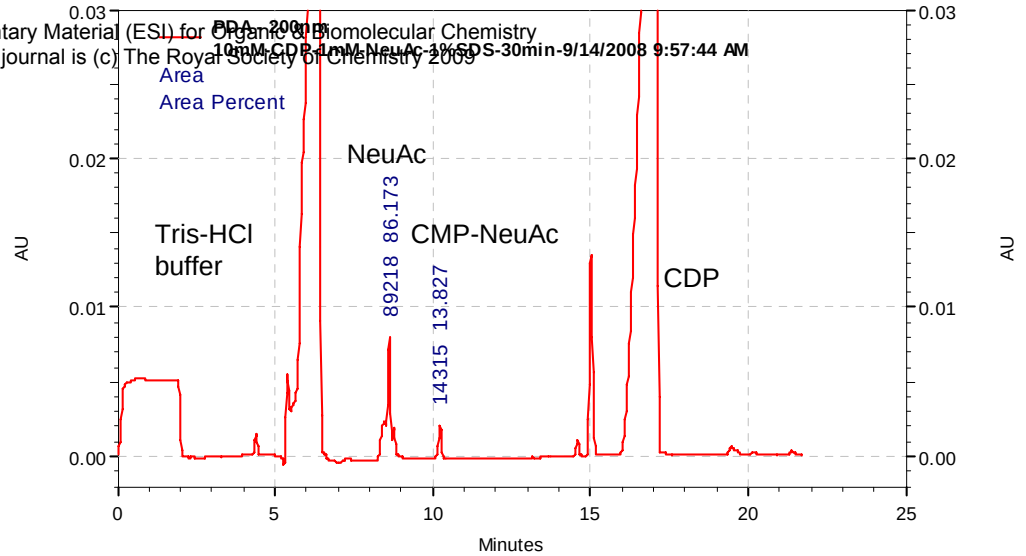

PDA - 200nm
10mM-CTP-1mM-NeuAc-1%SDS-30min-9/14/2008 9:31:23 AM
Area
Area Percent
89.128
287385
10.872
35057
Tris-HCl
buffer
NeuAc
CMP-NeuAc
CTP
AU
0.08
0.06
0.04
0.02
0.00
0
5
10
15
20
PDA - 200nm
10mM-CTP-1mM-NeuAc-1%SDS-30min-9/14/2008 2:11:01 AM
Area
Area Percent
87.723
181733
12.277
25434
Tris-HCl
buffer
NeuAc
CMP-NeuAc
CTP
AU
Minutes

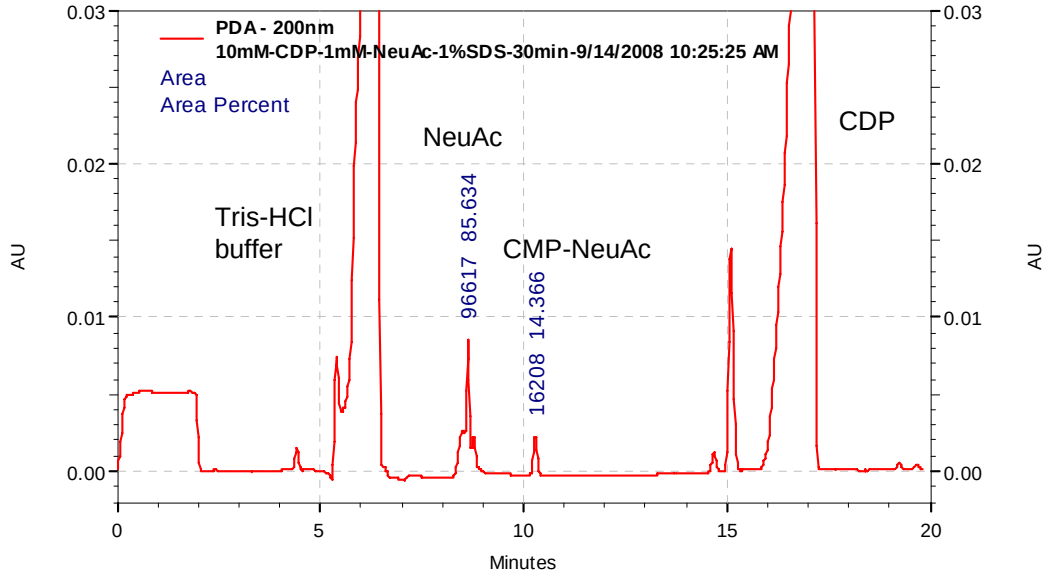
UTP at 10 mM



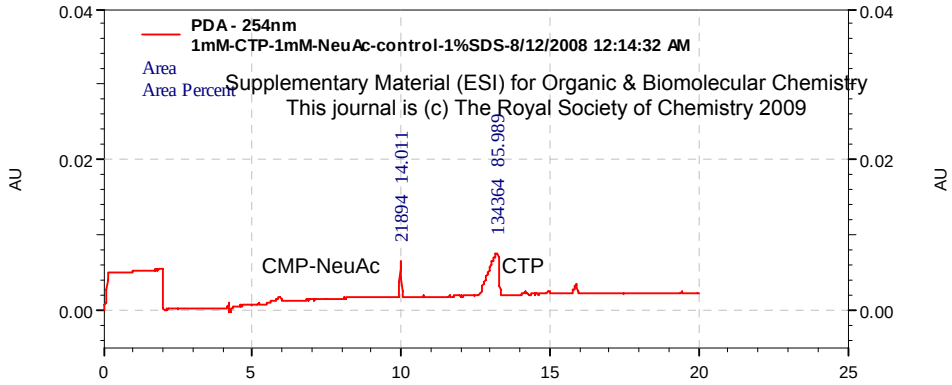


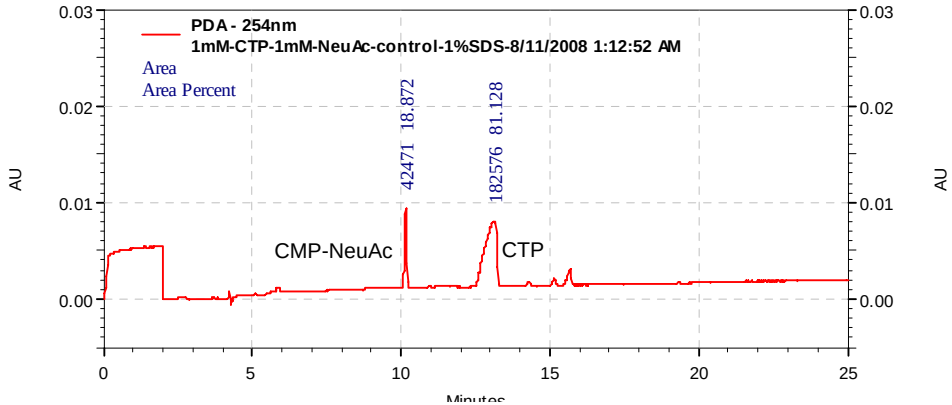
CDP at 10 mM

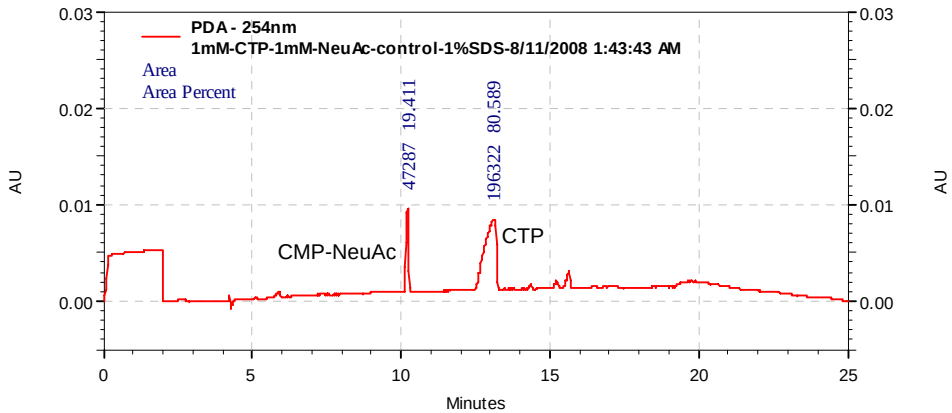




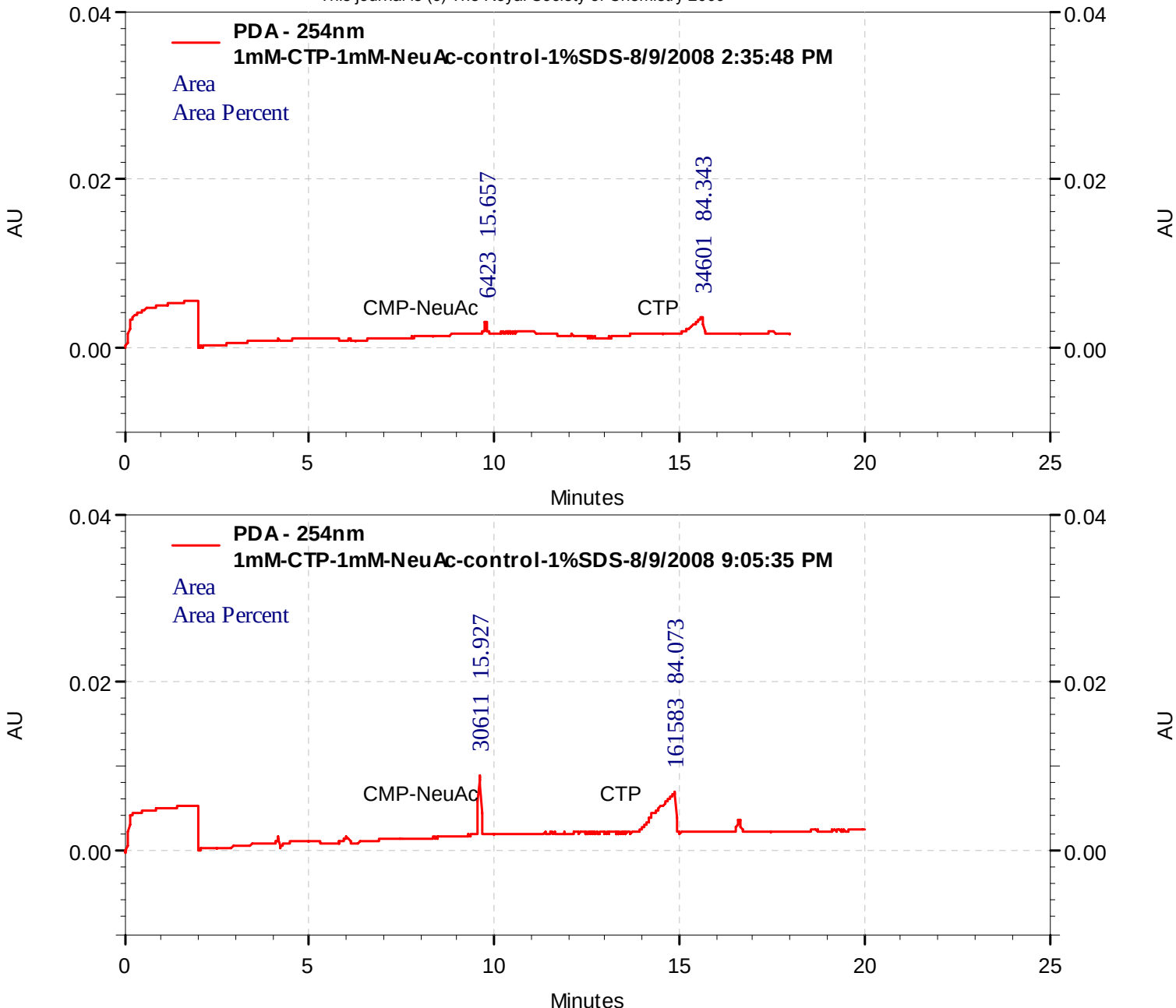
Controls
With no
Inhibitors

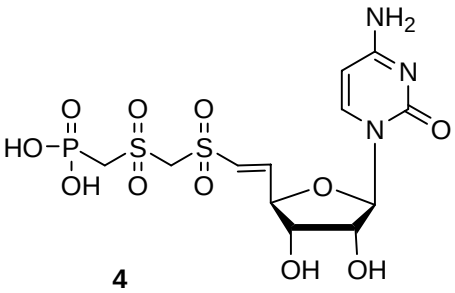




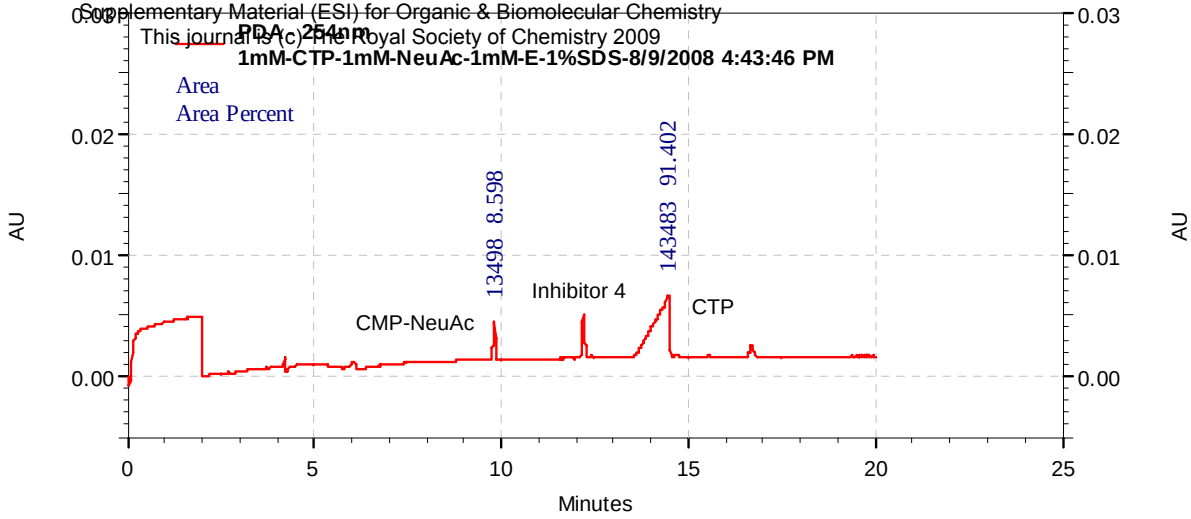


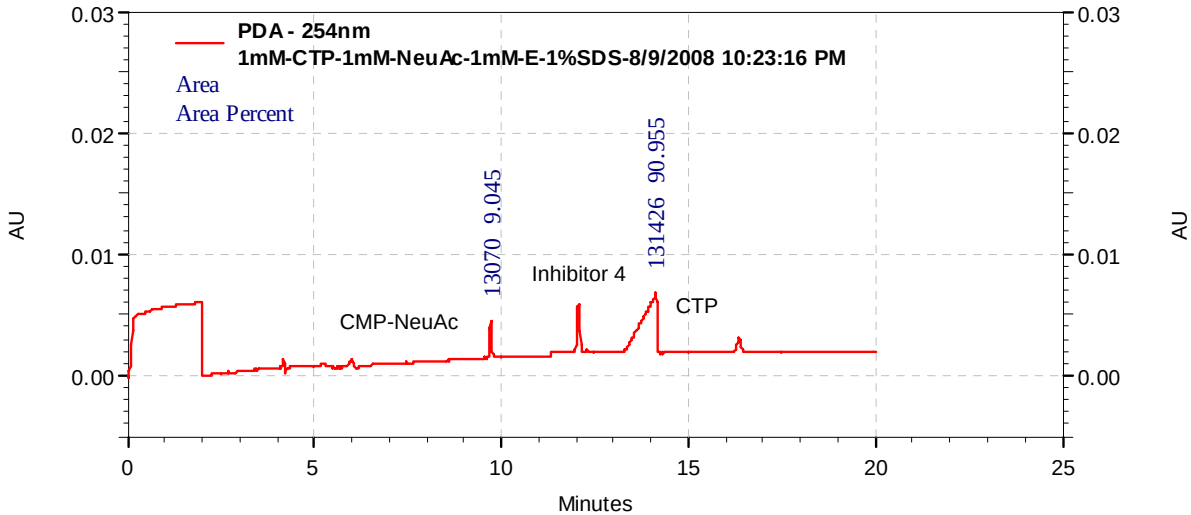
Controls
With no
Inhibitors

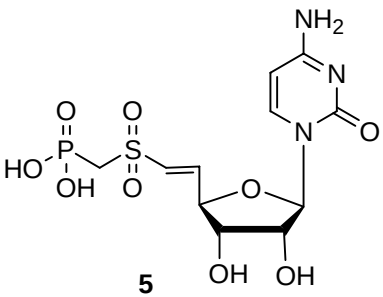




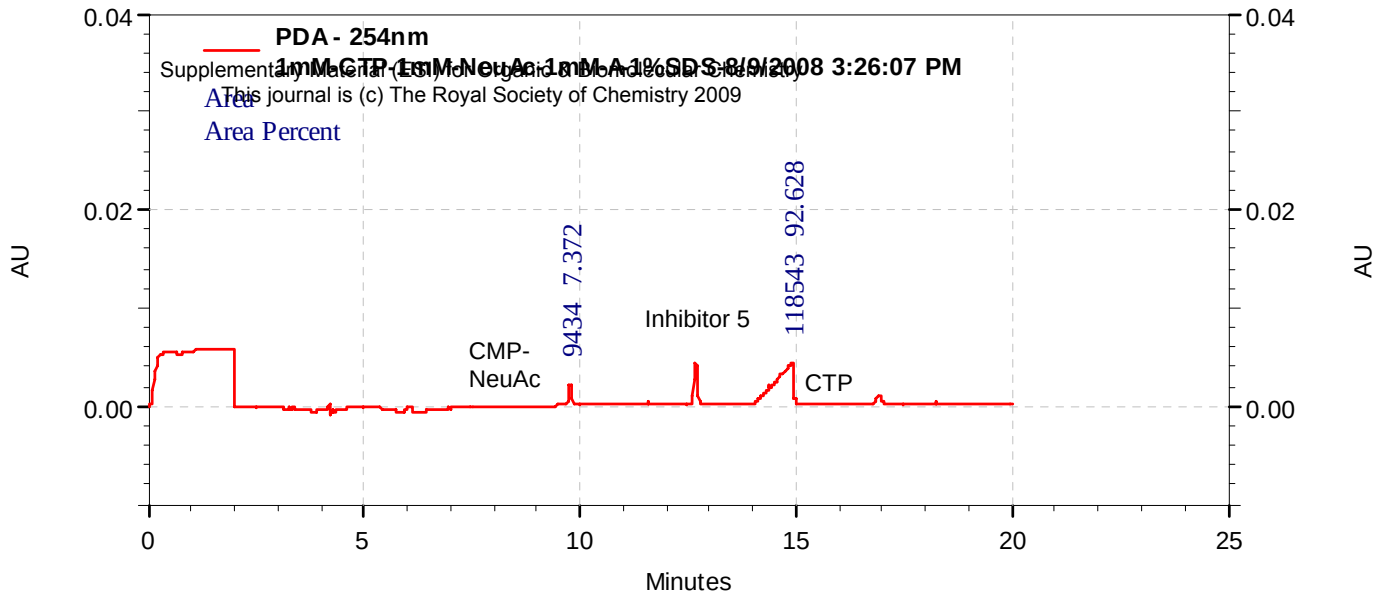
4

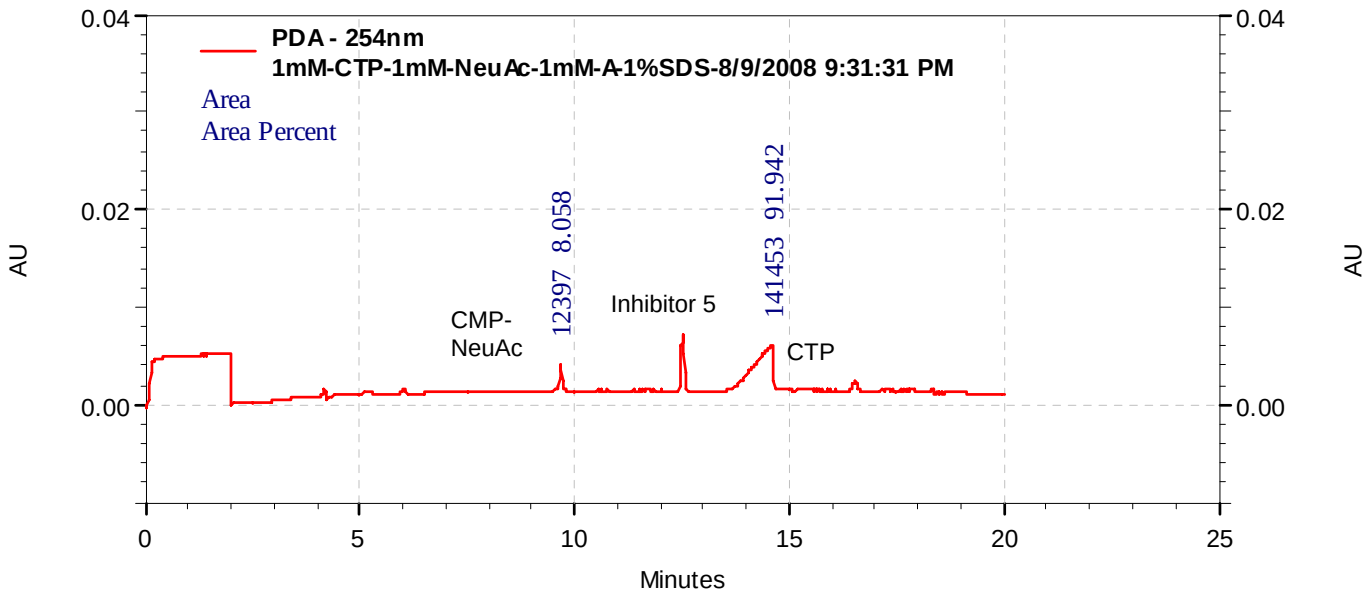


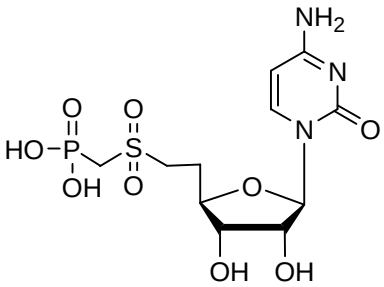




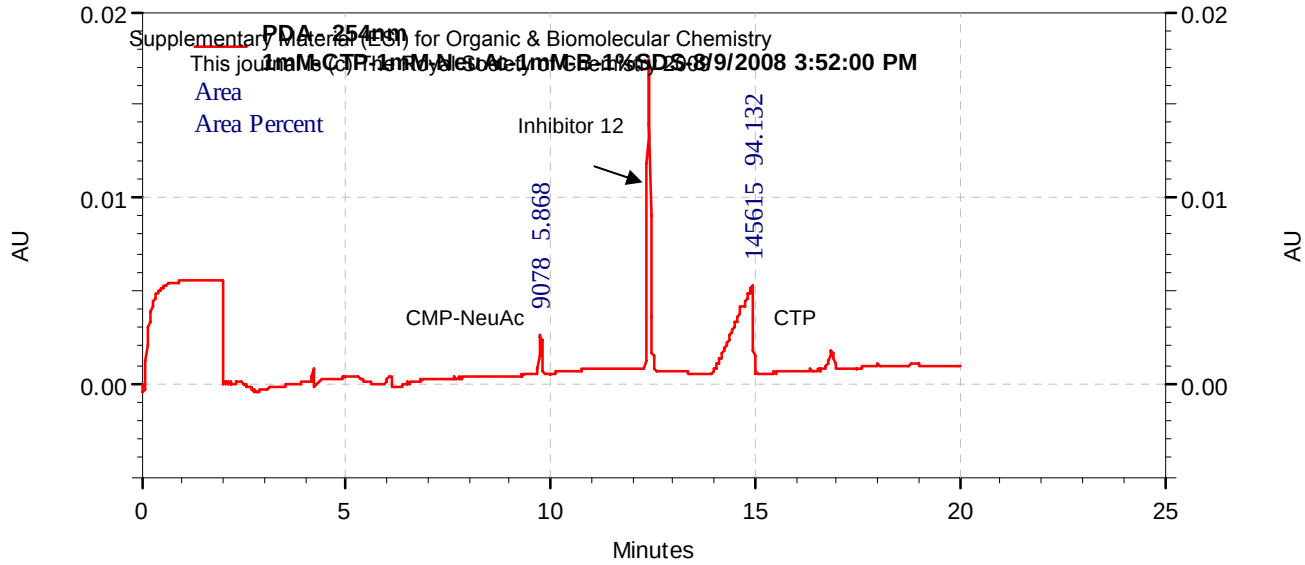
5

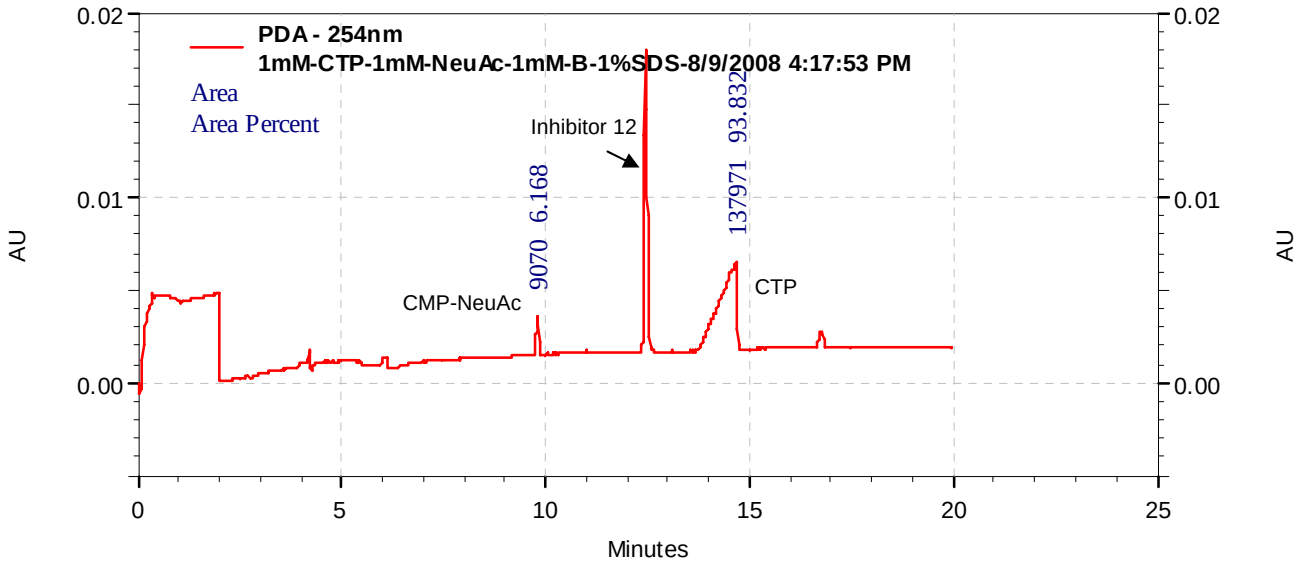


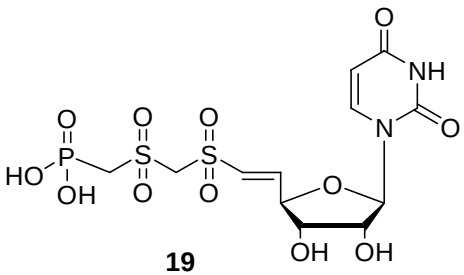



NH2
N
O
N
O
HO
P
O
OH
S
O
O
O
OH
OH

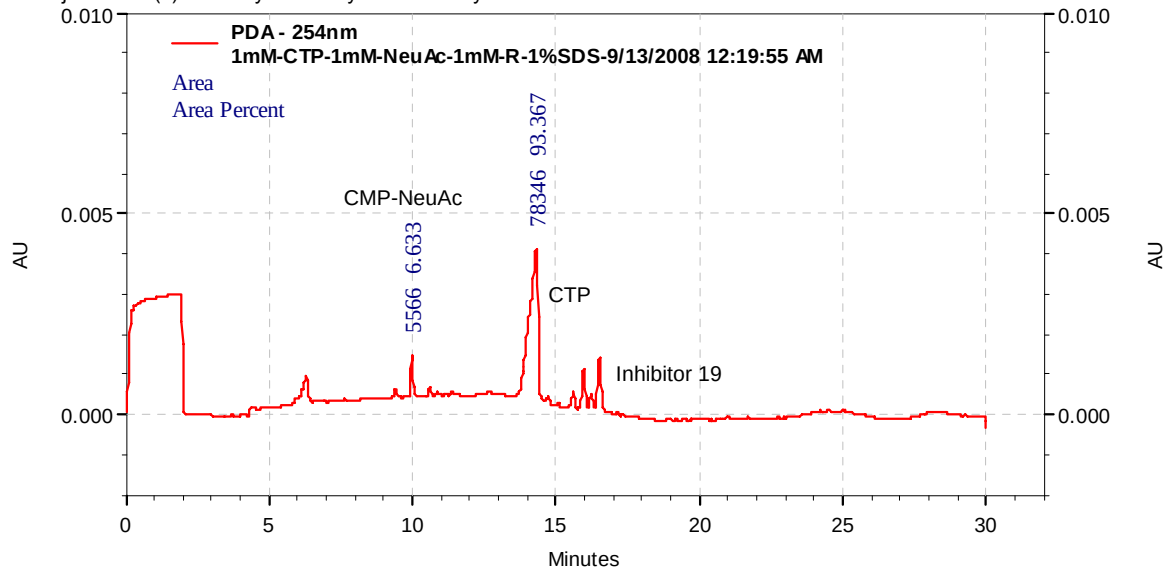
12

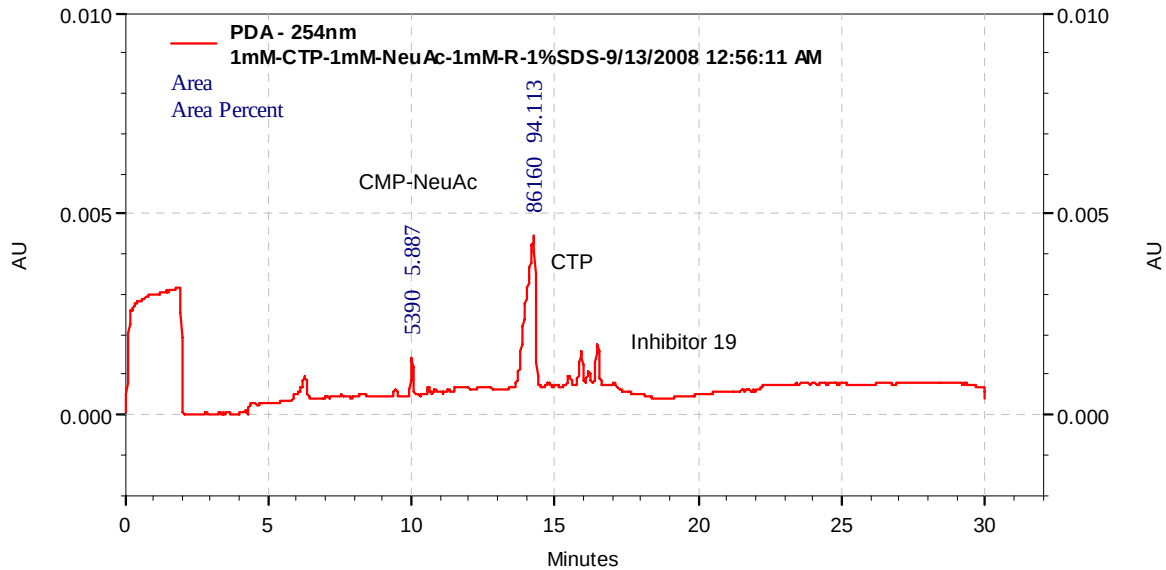

PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-B-1%SDS-8/9/2008 3:52:00 PM
Area
Area Percent
Inhibitor 12
5.868
9078
94.132
145615
CMP-NeuAc
CTP
AU
Minutes
0.02
0.01
0.00
0
5
10
15
20
25

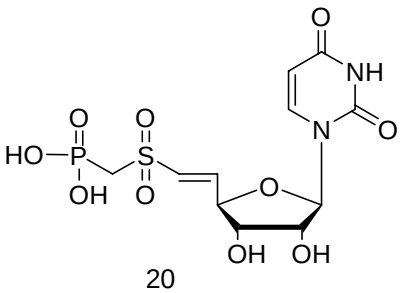

PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-B-1%SDS-8/9/2008 4:17:53 PM
Area
Area Percent
Inhibitor 12
6.168
9070
93.832
137971
CMP-NeuAc
CTP
AU
Minutes
0.02
0.01
0.00
0
5
10
15
20
25


HO
P
OH
O
S
S
O
NH
N
O
OH
OH

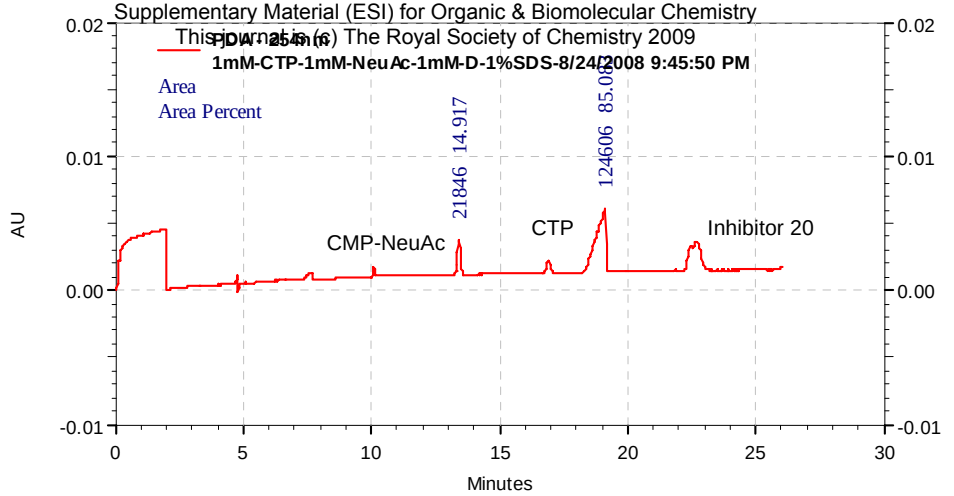
19

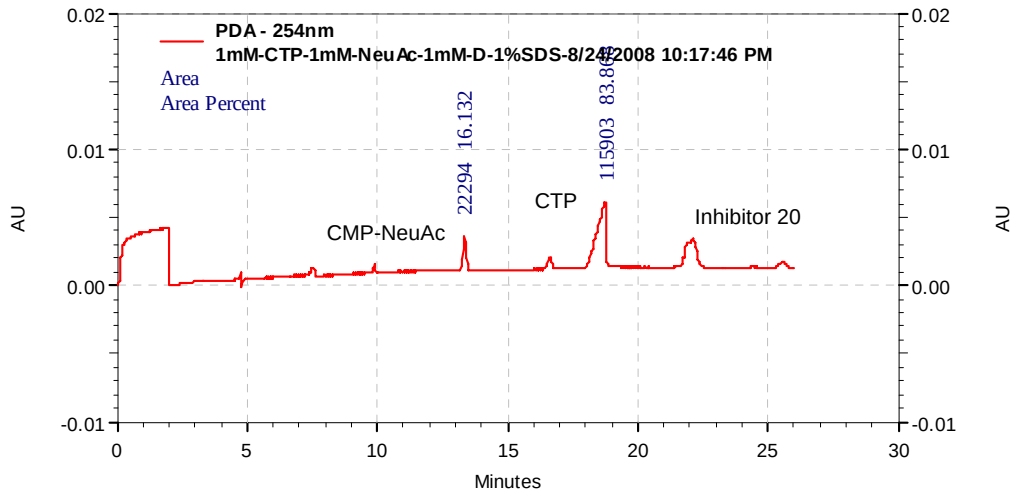

PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-R-1%SDS-9/13/2008 12:19:55 AM
Area
Area Percent
CMP-NeuAc
5566 6.633
78346 93.367
CTP
Inhibitor 19
AU
Minutes
0.010
0.005
0.000
0
5
10
15
20
25
30

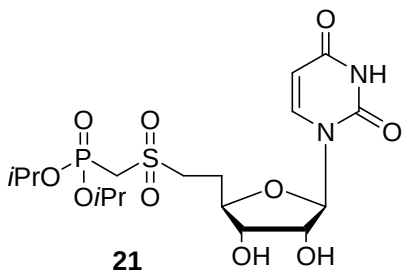

PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-R-1%SDS-9/13/2008 12:56:11 AM
Area
Area Percent
CMP-NeuAc
5390 5.887
86160 94.113
CTP
Inhibitor 19
AU
Minutes
0.010
0.005
0.000
0
5
10
15
20
25
30


O
NH
N
O
HO
P
S
O
OH
O
O
OH
OH

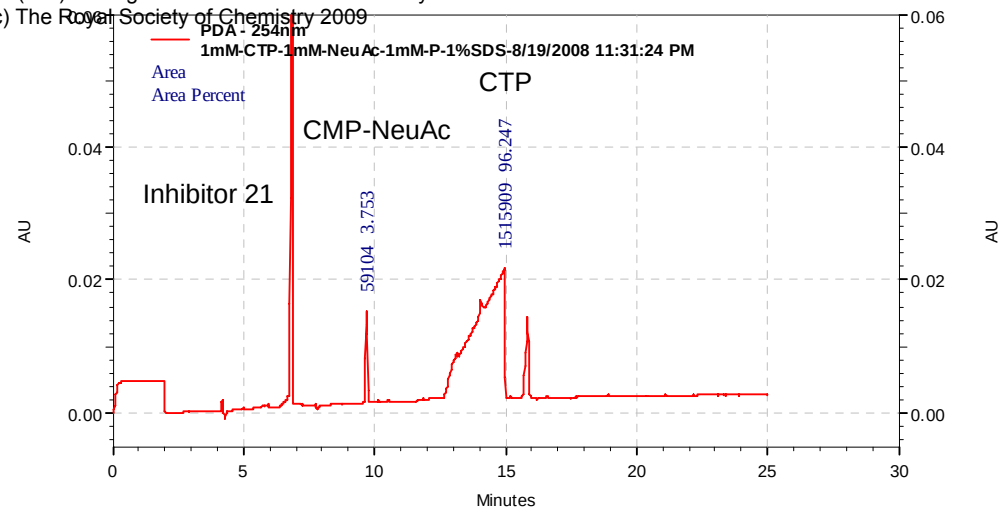
20

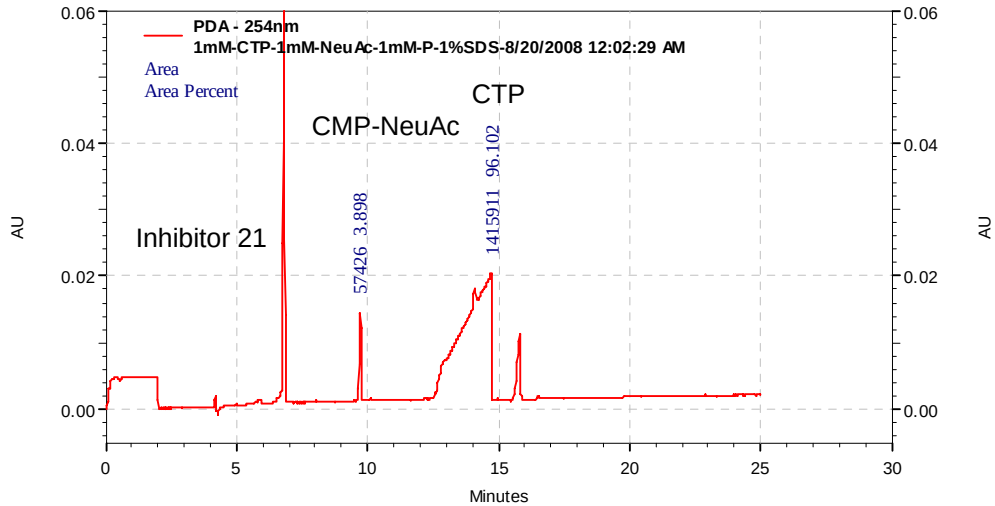

PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-D-1%SDS-8/24/2008 9:45:50 PM
Area
Area Percent
14.917
21846
85.083
124606
CMP-NeuAc
CTP
Inhibitor 20
AU
Minutes
0.02
0.01
0.00
-0.01
0
5
10
15
20
25
30

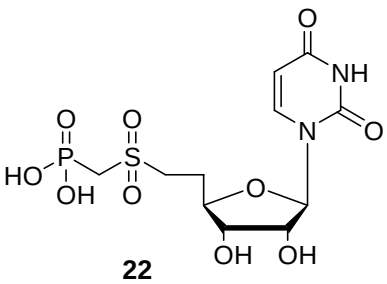

PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-D-1%SDS-8/24/2008 10:17:46 PM
Area
Area Percent
16.132
22294
83.868
115903
CMP-NeuAc
CTP
Inhibitor 20
AU
Minutes
0.02
0.01
0.00
-0.01
0
5
10
15
20
25
30



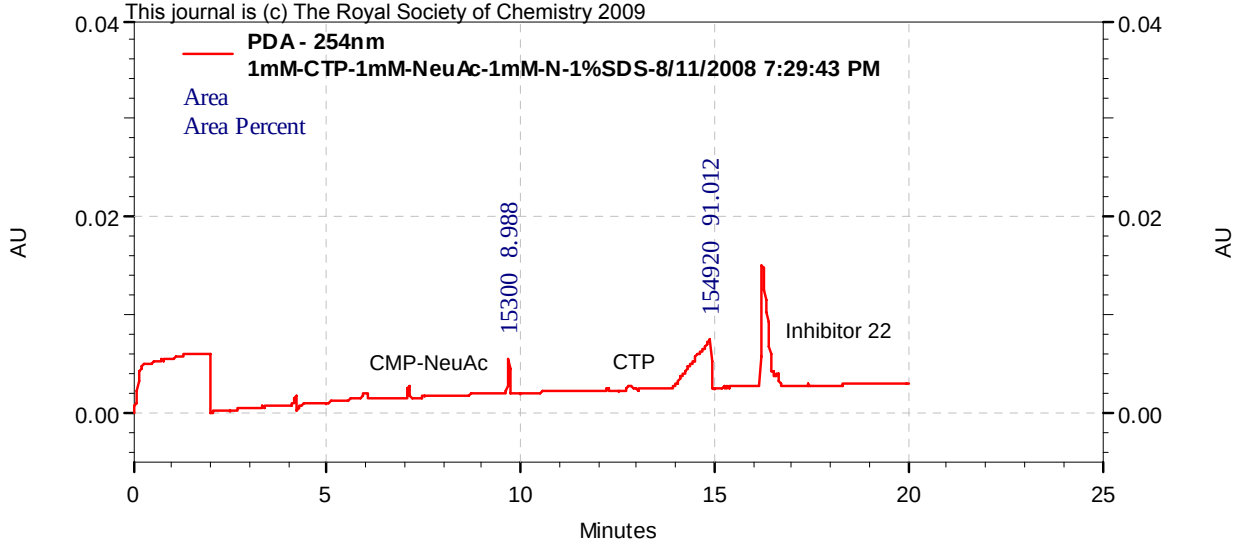
21

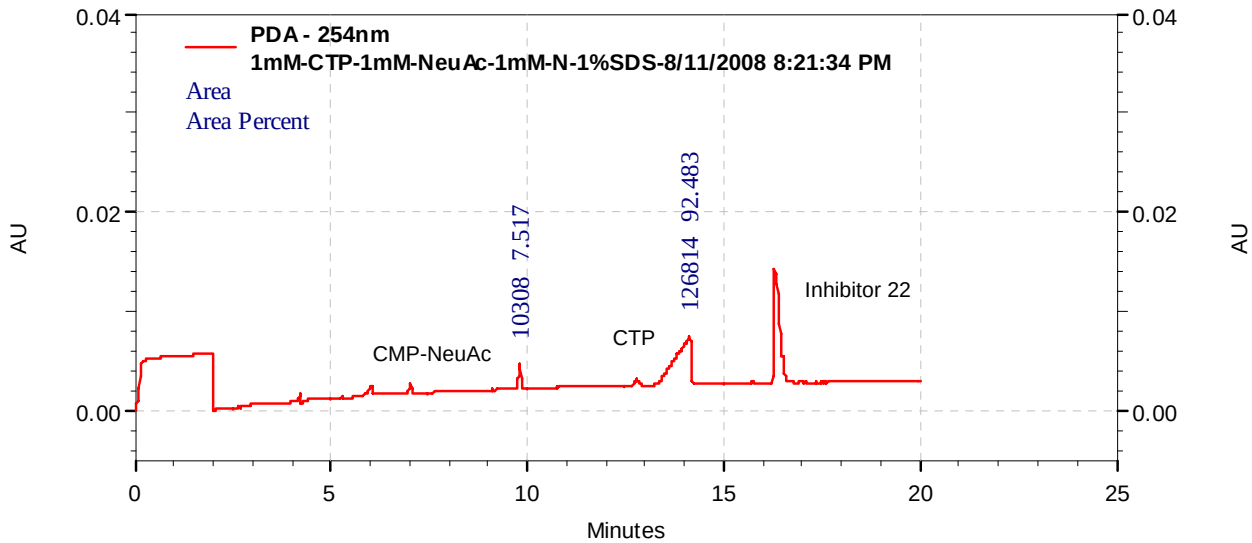





O
NH
O
N
HO
P
OH
O
S
O
O
OH
OH

22


PDA - 254nm
1mM-CTP-1mM-NeuAc-1mM-N-1%SDS-8/11/2008 7:29:43 PM
Area
Area Percent
15300 8.988
154920 91.012
CMP-NeuAc
CTP
Inhibitor 22
AU
Minutes
0.04
0.02
0.00
0
5
10
15
20
25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1mM-CTP-1mM-NeuAc-1mM-N-1%SDS-8/11/2008 8:21:34 PM
Area
Area Percent
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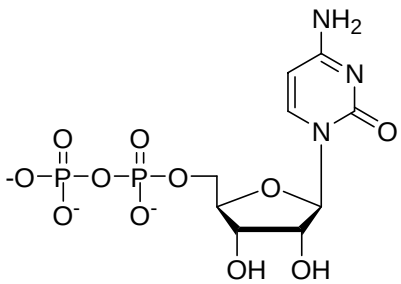
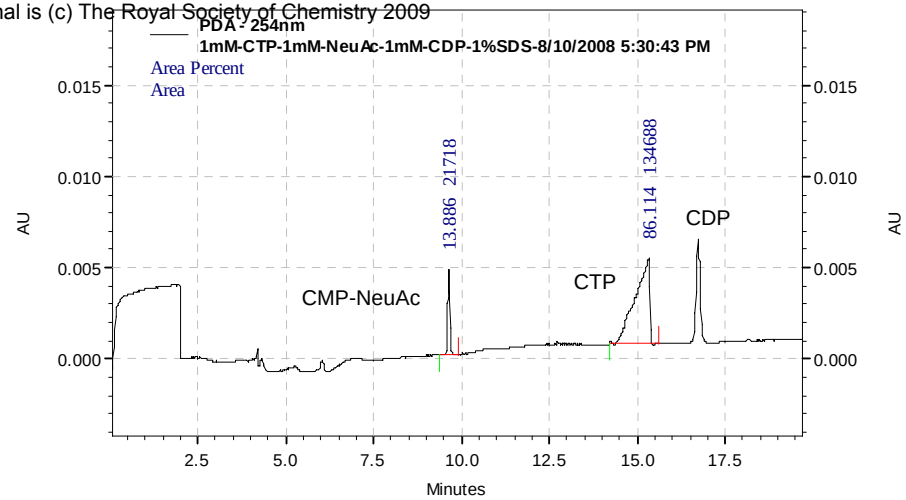
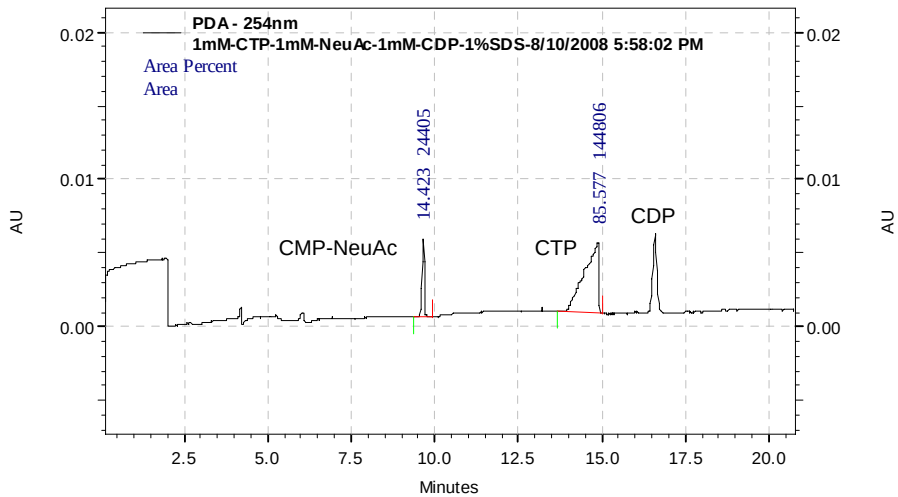
CMP-NeuAc
CTP
Inhibitor 22
AU
Minutes
0.04
0.02
0.00
0
5
10
15
20
25



CDP





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