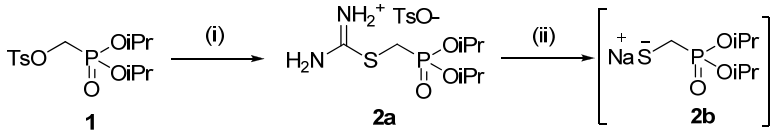
- Supplementary Data -

Synthesis of Novel Deoxynucleoside *S*-methylphosphonic Acids using *S*-(Diisopropylphosphonomethyl)iso-thiouronium tosylate, a New Equivalent of Mercaptomethylphosphonate

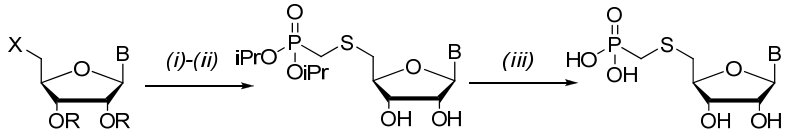
Ivana Kóšiová, Miloš Buděšínský, Natalya Panova and Ivan Rosenberg

Institute of Organic Chemistry and Biochemistry, Academy of Sciences v. v. i., Flemingovo 2, 166 10 Prague 6, Czech Republic



(i) thiourea, ethanol, reflux; (ii) sodium *iso*-propoxide

Scheme 1



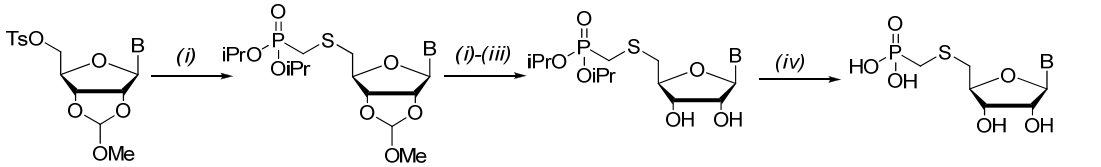
3a B= A, X= Br, R= H
3b B= G, X= I, R= H
3c B= U, X= I, R= $CH(CH_3)_2$

4a 62%
4b 48%
4c 64%

5a 64%
5b 48%
5c 84%

(i) **2a**, sodium *iso*-propoxide, *iso*-propanol; for **4c**: (ii) Dowex H^+, ethanol;
(iii) bromotrimethylsilane, 2,6-lutidine, acetonitrile

Scheme 2



6 B= A^{Bz}
7 B= C^{Bz}

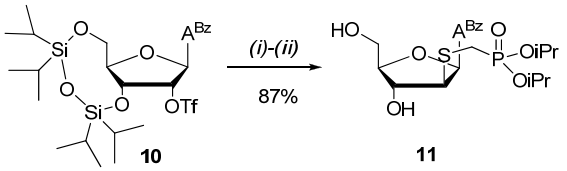
8 B= A^{Bz}
9 B= C^{Bz}

4a B= A, 76%
4d B= C, 72%

5a B= A, 77%
5d B= C, 80%

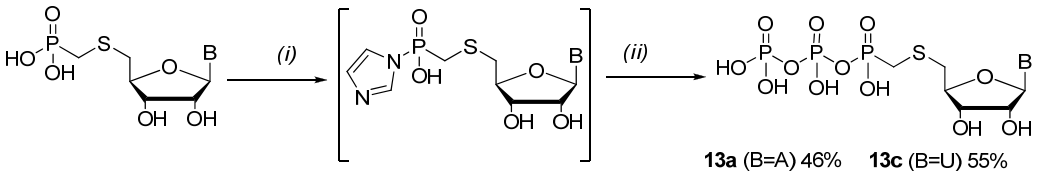
(i) **2a**, sodium *iso*-propoxide, DMF; (ii) aq. 50% acetic acid; (iii) NH_3(aq), ethanol;
(iv) bromotrimethylsilane, 2,6-lutidine, acetonitrile

Scheme 3



(i) **2a**, sodium i*so*-propoxide, DMF; (ii) 0.5 M TBAF, THF

Scheme 4



(i) imidazole, PPh_3, dipyridyl disulfide, tri-*n*-octylamine, DMF, (ii) bis(tributylamonium)pyrophosphate (0.5 M in DMSO)

Scheme 5

Experimental

General

The course of the reactions was followed by TLC on Merck Silica gel 60 F_{254} aluminum sheets and the products were visualized both by UV monitoring (254 nm) and by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine (PNBP) which, after short heating and exposing to ammonia vapors, showed the phosphonate esters as blue spots. Preparative column chromatography (PLC) was performed on silica gel (40-60 μm, Fluka). Elution was performed at the rate of 40 ml/min. PLC and TLC were carried out with the following solvent systems (v/v): chloroform-ethanol 9:1 (C-1), ethyl acetate-acetone-ethanol-water 4:1:1:1 (H-1), and ethyl acetate-acetone-ethanol-water 12:2:2:1 (H-3).

HPLC analyses were performed on Alliance (Waters) instrument using Luna C18 column (5 μm, 4.6 x 150 mm, Phenomenex) under gradient elution with acetonitrile in 0.1 M-triethylammonium acetate buffer (TEAA).

The preparative reversed-phase HPLC was performed on an Axia column (20x200 mm, Luna C18(2), 5 μm, Phenomenex), using a linear gradient of methanol in 0.1M triethylammonium hydrogencarbonate buffer.

The ion exchange chromatography was performed on a POROS 50 HQ (Appl. Biosys.) anion exchanger column in ammonium hydrogencarbonate,

MS HR ESI spectra (m/z) were recorded on LTQ Orbitrap XL (Thermo Fischer Scientific) instrument. IR spectra were recorded on FTIR spectrometer (Bruker Equinox 55, Germany). ^{1}H and ^{13}C NMR spectra were measured on Bruker AVANCE-600 spectrometer (^{1}H at 600.13 MHz, ^{13}C at 150.9 MHz) in DMSO or D_2O at 300 K. Chemical shifts and coupling constants were obtained from a first-order analysis of spectra. Signals in the ^{1}H NMR spectra were assigned to protons on the basis of chemical shifts, observed multiplicities and homonuclear 2D-COSY experiments.

Diisopropyl phosphonomethylisothiouronium tosylate (2a)

A mixture of diisopropyl tosyloxymethylphosphonate[1] (**1**, 20.0 g, 0.057 mol) and thiourea (6.5 g, 0.087 mol) in ethanol (200 ml) was heated under reflux for 18 h (TLC in C-1) and then concentrated at reduced pressure. The title product was precipitated from ethanol by diethyl ether.

Yield: 21.0 g (0.049 mol, 86 %); HRMS $(M+H)^+$ for $C_8H_{20}N_2O_3PS$: calcd *m/z* 255.0927, obs. 255.0927; IR (KBr, cm^{-1}): 3121, 2982, 1666, 1400, 1216, 1192, 1128, 1038, 1013, 996, 815, 690, 570.

^{1}H NMR (D_2O): 1.36 (d, J = 6.2 Hz, 12H, 4x CH_3); 2.39 (bs, 3H, CH_3 (Tos)); 3.58 (d, 2H, J(H,P) = 12.6 Hz, S-CH_2-P); 4.80 (m, 2H, 2x O-CH<); 7.37 (m, 2H, Ar-H (Tos)); 7.68 (m, 2H, Ar-H (Tos)); ^{13}C NMR (D_2O): 23.21 (CH_3 (Tos)); 25.74 (d, J(C,P) = 4.7 Hz, 2xCH_3); 25.83 (d, J(C,P) = 4.1 Hz, 2xCH_3); 27.11 (d, J(C,P) = 151.6 Hz, S-CH_2-P); 128.07 (2xAr-CH); 132.18 (2xAr-CH); 142.06 (Ar-C); 145.21 (Ar-C).

Diisopropyl 5'-deoxyadenosine-5'-*S*-methylphosphonate (4a)

Method A

5'-Bromo-5'-deoxyadenosine (**3a**). *N*-Methylimidazole (6 ml, 74.8 mmol) was added dropwise to the stirred suspension of adenosine (10.0 g, 34.4 mmol), triphenyl phosphine (19.6 g, 74.8 mmol), and carbon tetrabromide (24.8 g, 74.8 mmol) in pyridine (350 ml). The resulting mixture was stirred at room temperature for 24 h (TLC in H-3), concentrated at reduced pressure, and the product was purified by silica gel chromatography (elution with a linear gradient of 0-100% H-3 in ethyl acetate. Crystalisation from methanol afforded 5.5 g (16.7 mmol, 48 %) of the TLC- and HPLC-pure **3a**. This product was used without characterization for the next reaction.

(Diisopropylphosphonomethyl)isothiouronium tosylate **2a** (5.8 g, 13.5 mmol) was added to the solution (deoxygenated by argon bubling) of sodium *iso*-propoxide (2.2 g, 27 mmol) in *iso*-propanol (120 ml) and the resulting mixture was stirred at room temperature for 30 min. 5'-Bromo-5'-deoxyadenosine (**3a**, 1.8 g, 5.4 mmol) was added subsequently to the mixture and the resulting suspension was stirred at room temperature overnight (TLC in H-3). Reaction mixture was concentrated at reduced pressure, the residue was adsorbed on a silica gel (50 g) from methanol solution, and the product was purified first by silica gel chromatography (elution with a gradient of 0-100% H-3 in ethyl acetate) and subsequently by RP-HPLC (elution with a gradient of 0-75% methanol in 0.1 M TEAB).

Yield: 1.5 g (3.35 mmol, 62 %); HRMS $(M-H)^-$ for $C_{17}H_{27}N_5O_6PS$: calcd *m/z* 460.1420, obs. 460.1427; IR (KBr, cm^{-1}): 3333, 2980, 2932, 2361, 1644, 1600, 1577, 1475, 1421, 1386, 1375, 1239, 1103, 990, 889, 799, 536.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

Method B

(Diisopropylphosphonomethyl)isothiouronium tosylate **2a** (7.2 g, 16.9 mmol) was added to the solution (deoxygenated by argon bubling) of sodium *iso*-propoxide (2.8 g, 33.8 mmol) in DMF (40 ml), and the resulting mixture was stirred at room temperature for 30 min. 6-*N*-Benzoyl-5'-deoxy-2',3'-*O*-methoxymethylidene-5'-*O*-tosyl-adenosine (**6**[2], 6.4 g, 11.3 mmol) in DMF (10 ml) was added subsequently, and the resulting mixture was stirred at room temperature for 1 h (TLC in C-1). The reaction mixture was concentrated at reduced pressure, the residue was diluted with ethyl acetate (100 ml), and the solution was washed with water (3x 50 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated, and the crude product **8** was purified by chromatography on a silica gel column (elution with a gradient of 0-10% ethanol in chloroform).

The protected phosphonate **8** was dissolved in 50% aqueous acetic acid (50 ml), the solution was stirred at room temperature for 2 h (TLC in C-1), then concentrated at reduced pressure, and the residue was co-evaporated with 2M TEAB (10 ml) and finally with methanol. The residue was diluted with concentrated aqueous ammonia (40 ml) and stirred at room temperature overnight (TLC in C-1). The solution was then concentrated at reduced pressure, and the product was purified by chromatography on silica gel.

Yield: 3.7 g (8.1 mmol, 72%).

Diisopropyl 5'-deoxyguanosine-5'-*S*-methylphosphonate (4b)

Compound **4b** was prepared from **3b**.[3] (2.0 g, 5.1 mmol) by the same procedure as compound **4a** (Method A).

Yield: 1.17 g (2.45 mmol, 48%); HRMS $(M-H)^-$ for $C_{17}H_{27}N_5O_7PS$: calcd *m/z* 476.1369, obs. 476.1375; IR (KBr, cm^{-1}): 3412, 3133, 2980, 2930, 2360, 1691, 1633, 1606, 1534, 1485, 1375, 1233, 1178, 1103, 993, 889, 811, 537.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

Diisopropyl 5'-deoxyuridine-5'-S-methylphosphonate (4c)

Compound **4c** was prepared from **3c**.[4] (1.8 g, 4.6 mmol) by the same procedure as compound **4a** (Method A, but the intermediate was purified only by chromatography on silica gel), followed by deprotection with Dowex 50 H^+. To a solution of protected phosphonate in ethanol, Dowex 50 in H+ was added. Reaction mixture was stirred at 45 °C for 1h (TLC in C-1), then filtered, and the resin was washed with warm ethanol. The residue was evaporated at reduced pressure and the product was purified by HPLC (elution with a 0-100% gradient of methanol in 0.1M TEAB).

Yield: 1.3 g (2.9 mmol, 64 %); HRMS $(M-H)^-$ for $C_{16}H_{26}N_2O_8PS$: calcd *m/z* 437.1148, obs. 437.1154; IR (KBr, cm^{-1}): 3430, 3064, 2981, 2934, 2360, 1697, 1635, 1465, 1459, 1387, 1377, 1233, 1103, 989, 889, 810, 538.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

Diisopropyl 5'-deoxycytidine-5'-*S*-methylphosphonate (4d)

Compound **4d** was prepared from **7**[5] (2.0 g, 3.7 mmol) by the same procedure as compound **4a** (Method B).

Yield 1.2 g (2.8 mmol, 76 %); HRMS $(M-H)^-$ for $C_{16}H_{28}N_3O_7PS$: calcd *m/z* 436.1308, obs. 436.1315; IR (KBr, cm^{-1}): 3428, 2980, 2360, 2342, 1647, 1525, 1490, 1376, 1236, 1102, 993, 890, 788, 539.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

5'-Deoxyadenosine-5'-*S*-methylphosphonic acid (5a)

Diisopropylester **4a** (0.23 g, 0.5 mmol) was treated with bromotrimethylsilane (0.264 ml, 2.0 mmol) and 2,6-lutidine (0.74 ml, 4.0 mmol) in dry acetonitrile (5 ml) at room temperature overnight. The course of the reaction was checked by HPLC after dilution of the sample with TEAB-ethanol. Reaction mixture was concentrated under reduced pressure, the residue treated with 0.1M-TEAB for 1 h, and the crude product **5a** was purified by preparative HPLC on Axia C18 column by elution with a linear gradient of methanol (0-50%) in 0.1M TEAB.

Yield: 0.12 g (0.32 mmol, 64 %); HRMS $(M-H)^-$ for $C_{11}H_{15}N_5O_6PS$: calcd *m/z* 376.0481, obs. 376.0483; IR (KBr, cm^{-1}): 3425, 2921, 2361, 2343, 1646, 1607, 1576, 1476, 1421, 13334, 1089, 1058, 972, 796, 648, 551.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

5'-Deoxyguanosine-5'-*S*-methylphosphonic acid (5b)

Compound **5b** was prepared from **4b** (0.24 g, 0.5 mmol) by the same procedure as compound **5a**.

Yield: 0.94 g (0.24 mmol, 48 %); HRMS $(M-H)^-$ for $C_{11}H_{15}N_5O_7PS$: calcd *m/z* 392.0430, obs. 392.0438; IR (KBr, cm^{-1}): 3425, 3148, 2908, 1695, 1632, 1534, 1482, 1357, 1179, 1056, 973, 833, 780, 553.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

5'-Deoxyuridine-5'-*S*-methylphosphonic acid (5c)

Compound **5c** was prepared from **4c** (219 mg, 0.5 mmol) by the same procedure as compound **5a**.

Yield: 0.15 g (0.42 mmol, 84 %); HRMS $(M-H)^-$ for $C_{10}H_{14}N_2O_8PS$: calcd *m/z* 353.0209, obs. 353.0217; IR (KBr, cm^{-1}): 3426, 2960, 2923, 2581, 1694, 1465, 1446, 1396, 1387, 1097, 1057, 972, 814, 767, 549.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

5'-Deoxycytidine-5'-*S*-methylphosphonic acid (5d)

Compound **5d** was prepared from **4d** (0.22 g, 0.5 mmol) by the same procedure as compound **5a**.

Yield: 0.16 g (0.45 mmol, 80 %); HRMS $(M-H)^-$ for $C_{10}H_{15}N_3O_7PS$: calcd *m/z* 352.0368, obs. 352.0373; IR (KBr, cm^{-1}): 3427, 2905, 2577, 1644, 1526, 1494, 1404, 1373, 1289, 1097, 1053, 970, 758, 599, 549.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

Diisopropyl 6-*N*-benzoyl-2′-deoxyarabinoadenosin-2′-*S*-methylphosphonate (11)

Compound **11** was prepared from **10**[6] (6.0 g, 8.1 mmol) by the same procedure as compound **8** (Method B). The obtained fully protected product was partitioned between chloroform and water. The organic layer was dried with sodium sulfate and concentrated *in vacuo*. The residue was treated with 0.5M TBAF in THF (75 ml) at r. t. for 30 minutes (TLC in C-1). The mixture was quenched by addition of water (10 ml), and the product **12** was purified by chromatography on a silica gel column by elution with a linear gradient of ethanol (0-10%) in chloroform.

Yield: 4.0 g (7.0 mmol, 87 %); HRMS $(M+H)^+$ for $C_{24}H_{33}N_5O_7PS$: calcd *m/z* 566.1838, obs. 566.1833; IR (KBr, cm^{-1}): 3403, 3306, 3007, 2986, 2935, 1708, 1612, 1586, 1456, 1377, 1353, 1332, 1297, 1240, 1102, 1080, 1000, 799, 707, 666, 539.

^1H and ^{13}C NMR data – see Table S1 and Table S2.

5'-Deoxyadenosine-5'-*S*-methylphosphonyldiphosphate (13a)

2,2'-Dipyridyldisulphide (0.044 g, 0.199 mmol) was added to a solution of **5a** (0.015 g, 0.040 mmol), imidazole (0.032 mg, 0.477 mmol), triphenyl phosphine (0.052 g, 0.199 mmol) and tri-*n*-octylamine (0.087 ml, 0.199 mmol) in DMF. The reaction mixture was stirred at room temperature for 2 h (TLC in IPAW), and then poured into the solution of $NaIO_4$ (0.042 g) in acetone-diethyl ether-triethylamine mixture (4 ml, 12:7:1). The resulting precipitate was left aside in an ice bath for 1 h and then centrifuged. The sediment was washed once with cold precipitating solution and then twice with the cold mixture of acetone – diethyl ether – triethylamine (12:7:1), and finally it was dried over P_2O_5 *in vacuo*. The obtained phosphonoimidazolide **12** was dissolved in 0.5 M -bis(tributylamonium) pyrophosphate in DMSO (0.24 ml, 0.12 mmol) and the reaction mixture was left aside at room temperature overnight (analysis performed by HPLC). The reaction mixture was diluted with 0.05 M ammonium hydrogencarbonate and the product was purified on POROS 50 HQ by elution with a linear gradient of ammonium hydrogencarbonate (0.05-0.5 M). The corresponding fractions were pooled and evaporated at reduced pressure. The residue was co-distilled twice with methanol and subsequently treated with Dowex 50 (Na^+) form. Sodium salt of **13a** was obtained as a white lyophylizate. Yield: 0.011 g (0.018 mmol, 46%); HRMS $(M+H)^+$ for $C_{11}H_{15}N_5Na_4O_{12}P_3S$: calcd *m/z* 625.9244, obs. 625.9234.

^1H NMR (600 MHz; D_2O):

8.42 s (1H, H-2);
8.25 s (1H, H-8);
5.88 d (1H; *J*(1',2') = 6.0; H-1');
4.76 dd (1H; *J*(2',1') = 6.0, *J*(2',3') = 5.5; H-2');
4.26 ddd (1H; *J*(4',3') = 3.4, *J*(4',5') = 5.4, *J*(4',5'') = 7.1; H-4');
4.19 dd (1H; *J*(3',2') = 5.5, *J*(3',4') = 3.4; H-3');
3.15 bdd (1H; *J*(5',5'') = 13.7, *J*(5',4') = 5.4; H-5');
3.05 bdd (1H; *J*(5'',5') = 13.7, *J*(5'',4') = 7.1; H-5'');
2.91 m (2H, P-CH_2-S).
^{31}P NMR (202.3 MHz; D_2O):
11.64, -8.09 and -22.08.

5'-Deoxyguanosine-5'-*S*-methylphosphonyldiphosphate (13b)

Compound **13b** was prepared from **5b** (0.02 g, 0.034 mmol) by the same procedure as compound **8a**. Yield: 0.015g (0.024 mmol, 69 %); HRMS $(M+H)^+$ for $C_{11}H_{15}N_5Na_4O_{13}P_3S$: calcd *m/z* 641.9193, obs. 641.9183.

^{1}H NMR (600 MHz; D_2O):
7.97 s (1H, H-2);
5.71 d (1H; *J*(1',2') = 6.2; H-1');
4.65 dd (1H; *J*(2',1') = 6.2, *J*(2',3') = 5.5; H-2');
4.22 ddd (1H; *J*(4',3') = 3.4, *J*(4',5') = 5.6, *J*(4',5'') = 6.9; H-4');
4.16 dd (1H; *J*(3',2') = 5.5, *J*(3',4') = 3.4; H-3');
3.12 bdd (1H; *J*(5',5'') = 13.7, *J*(5',4') = 5.6; H-5');
3.02 bdd (1H; *J*(5'',5') = 13.7, *J*(5'',4') = 6.9; H-5'');
2.92 m (2H, P-CH_2-S).
^{31}P NMR (202.3 MHz; D_2O):
11.76, -7.26 and -21.63.

5'-Deoxyuridine-5'-*S*-methylphosphonyldiphosphate (13c)

Compound **13c** was prepared from **5c** (0.015 g, 0.042 mmol) by the same procedure as compound **8a**.Yield: 0.014 g (0.023 mmol, 55%); HRMS $(M+H)^+$ for $C_{10}H_{14}N_2Na_4O_{14}P_3S$: calcd *m/z* 602.8958, obs. 602.8964.

^{1}H NMR (600 MHz; D_2O):
7.63 d (1H, *J*(6,5) = 7.6; H-6);
5.85 d (1H; *J*(1',2') = 5.1; H-1');
5.82 d (1H, *J*(5,6) = 7.6; H-5);
4.21 dd (1H; *J*(2',1') = 5.1, *J*(2',3') = 5.7; H-2');
4.07 ddd (1H; *J*(4',3') = 5.1, *J*(4',5') = 4.8, *J*(4',5'') = 7.3; H-4');

4.02 dd (1H; *J*(3',2') = 5.7, *J*(3',4') = 5.1; H-3');
3.14 ddd (1H; *J*(5',5'') = 13.8, *J*(5',4') = 4.8, *J*(5',P) = 0.8; H-5');
3.00 bdd (1H; *J*(5'',5') = 13.8, *J*(5'',4') = 7.3, *J*(5'',P) = 0.8; H-5'');
2.93 d (2H, *J*(CH_2,P) = 14.0; P-CH_2-S).
^{31}P NMR (202.3 MHz; D_2O):
11.72, -8.58 and -22.06.

5'-Deoxycytidine-5'-*S*-methylphosphonyldiphosphate (13d)

Compound **13d** was prepared from **5d** (0.02 g, 0.056 mmol) by the same procedure as compound **8a**. Yield: 0.024g (0.04mmol, 72%); HRMS $(M+H)^+$ for $C_{10}H_{15}N_3Na_4O_{13}P_3S$: calcd *m/z* 601.9131, obs. 601.9124.

^{1}H NMR (600 MHz; D_2O):
7.76 d (1H, *J*(6,5) = 7.6; H-6);
6.08 d (1H, *J*(5,6) = 7.6; H-5);
5.84 d (1H; *J*(1',2') = 4.7; H-1');
4.21 dd (1H; *J*(2',1') = 4.7, *J*(2',3') = 5.6; H-2');
4.12 ddd (1H; *J*(4',3') = 5.1, *J*(4',5') = 4.6, *J*(4',5'') = 7.3; H-4');
4.04 dd (1H; *J*(3',2') = 5.6, *J*(3',4') = 5.1; H-3');
3.15 bdd (1H; *J*(5',5'') = 13.8, *J*(5',4') = 4.6; H-5');
3.03 bdd (1H; *J*(5'',5') = 13.8, *J*(5'',4') = 7.3; H-5'');
2.94 d (2H, *J*(CH_2,P) = 14.0; P-CH_2-S).
^{31}P NMR (202.3 MHz; D_2O):
11.82, -9.00 and -22.12.

Table 1. Proton NMR data of compounds **4a-4d, 5a-5d** and **11**

Compound	Solvent	H-1'	H-2'	H-3'	H-4'	H-5'a	H-5'b	Base	S-CH_2-P(OR)$_2$
4a [a]	DMSO	5.88 d (6.0)	4.72 m (6.0;5.6;5.2)	4.15 td (5.2;5.2;4.0)	4.06 ddd (7.1;5.5;4.0)	3.05 ddd (13.9;5.5;1.1)	3.02 ddd (13.9;7.1;1.0)	8.34 s (H-8) 8.15 s (H-2)	4.53 m (2H); 2.85 dd (*J*=15.1,13.0) and 2.83 dd (*J*=15.1,13.3) (P-CH_2-S); 1.19 d, 1.20 d, 1.206 d, 1.208 d, *J*=6.2 (4x CH_3)
4b [b]	DMSO	5.68 d (6.0)	4.56 m (6.1;6.0;4.8)	4.05 td (4.8;4.8;3.6)	4.00 ddd (7.0;5.7;3.6)	3.06 ddd (13.8;5.7;1.1)	2.98 ddd (13.8;7.0;0.8)	7.89 s (H-8) 10.67 bs (NH)	4.56 m (2H); 2.88 dd (*J*=15.2,13.0) and 2.85 dd (*J*=15.2,13.2) (P-CH_2-S); 1.215 d, 1.222 d and 1.226 d, *J*=6.2 (4x CH_3)
4c [c]	DMSO	5.75 d (5.5)	4.09 t (5.5;5.4)	3.87 dd (5.4;4.4)	3.95 ddd (6.8;5.4;4.4)	3.00 ddd (13.9;5.4;1.0)	2.97 ddd (13.9;6.8;1.0)	7.65 d, *J*=8.1 (H-6); 5.64 d, *J*=8.1 (H-5)	4.58 m (2H); 2.91 dd (*J*=15.1,12.9) and 2.86 dd (*J*=15.1,13.4) (P-CH_2-S); 1.237 d, 1.242 d and 1.248 d, *J*=6.0 (4x CH_3)
4d [d]	DMSO	5.78 (4.4)	3.97 td (5.5;5.5;4.4)	3.82 q (5.9;5.5;5.4)	3.93 q (5.4 (3x))	2.99 m	2.99 m	7.58 d, *J*=7.4 (H-6); 5.73 d, *J*=7.4 (H-5)	4.58 m (2H); 2.91 dd (*J*=15.1,12.9) and 2.86 dd (*J*=15.1,13.4) (P-CH_2-S); 1.237 d, 1.242 d and 1.248 d, *J*=6.0 (4x CH_3)
5a	D_2O	6.07 d (5.7)	4.82 t (5.7; 5.5)	4.45 dd (5.5;4.2)	4.36ddd (6.3;5.9;4.2)	3.07 ddd (14.0;5.9;1.0)	2.99 bdd (14.0;6.3;<1)	8.21 s (H-2); 8.40 s (H-8)	2.61 d, *J*=13.7 (P-CH_2-S)
5b	D_2O	5.89 d (5.9)	4.82 dd (5.9;5.4)	4.43 dd (5.4;4.0)	4.31 ddd (6.6;6.0;4.0)	3.04 ddd (13.9;6.0;0.8)	2.98 bdd (13.9;6.6;<1)	8.01 s (H-8)	2.60 d, *J*=13.6 (P-CH_2-S)
5c	D_2O	5.89 d (4.9)	4.27 t (5.2;4.9)	4.12 m	4.12 m	3.02 m	2.92 m	7.62 d, *J*=7.6 (H-6); 5.82 d, *J*=7.6 (H-5)	2.62 m (P-CH_2-S)
5d	D_2O	5.91 d (4.3)	4.33 dd (5.3;4.3)	4.21 dd (5.9;5.3)	4.25 ddd (6.4;5.9;4.9)	3.08 ddd (14.1;4.9;0.7)	2.98 ddd (14.1;6.4;0.7)	7.78 d, *J*=7.6 (H-6); 6.07 d, *J*=7.6 (H-5)	2.64 m (P-CH_2-S)
11 [e]	DMSO	6.65 d (7.2)	4.12 dd (10.0;7.2)	4.40 ddd (10.0;8.2;5.8)	3.82 ddd (8.2;4.2;2.3)	3.77 ddd (12.3;5.2;2.3)	3.69 ddd (12.3;4.2;5.2)	8.74 s (H-2); 8.67 s (H-8)	4.50 m and 4.43 m (2x O-CH<); 2.91 dd (*J*=14.8,14.0) and 2.86 dd (*J*=14.8,13.7) (P-CH_2-S); 1.086 d, 1.163 d, 1.177 d and 1.179 d, *J*=6.2 (4x CH_3)

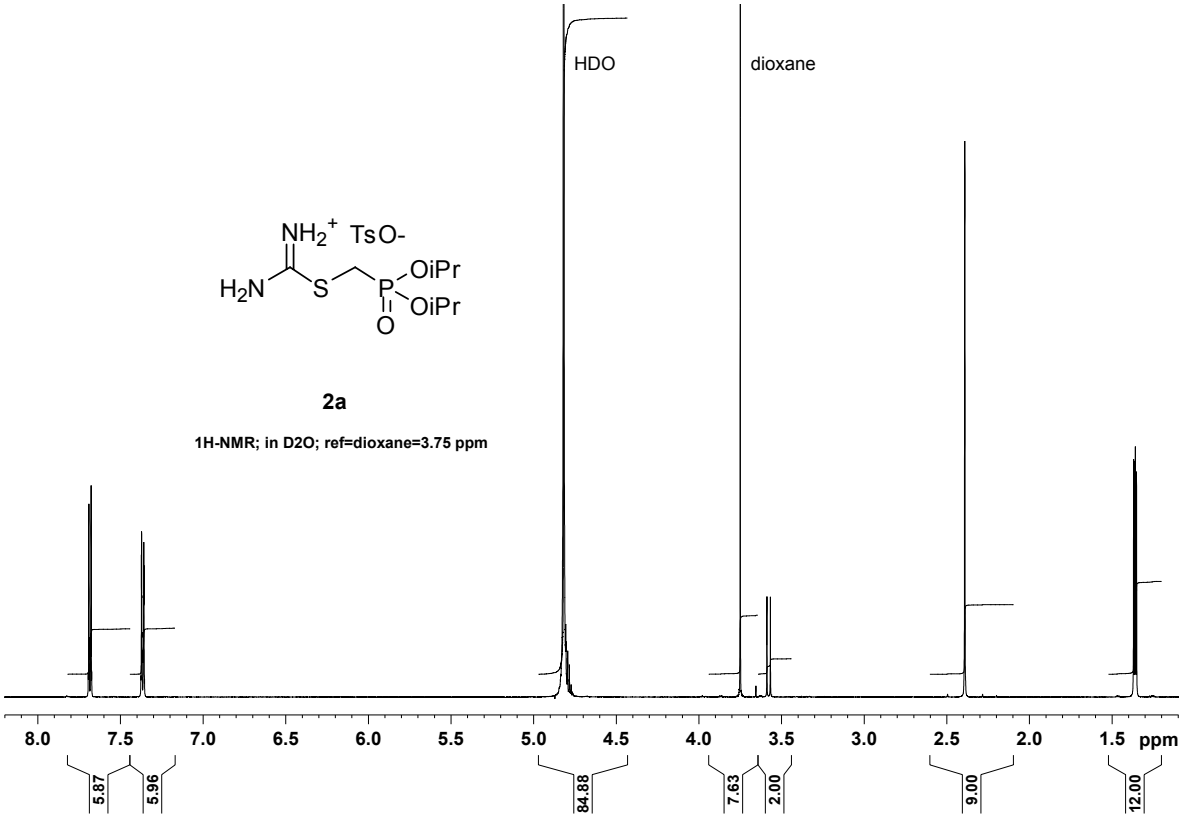
Additional signals: [a] 7.31 b (NH_2); 5.52 d, *J*=6.0 Hz (2-OH); 5.33 d, *J*=5.2 Hz (3-OH); [b] 6.51 bs (NH_2); 5.50 d, *J*=6.1 Hz (2-OH); 5.28 d, *J*=4.8 Hz (3-OH);

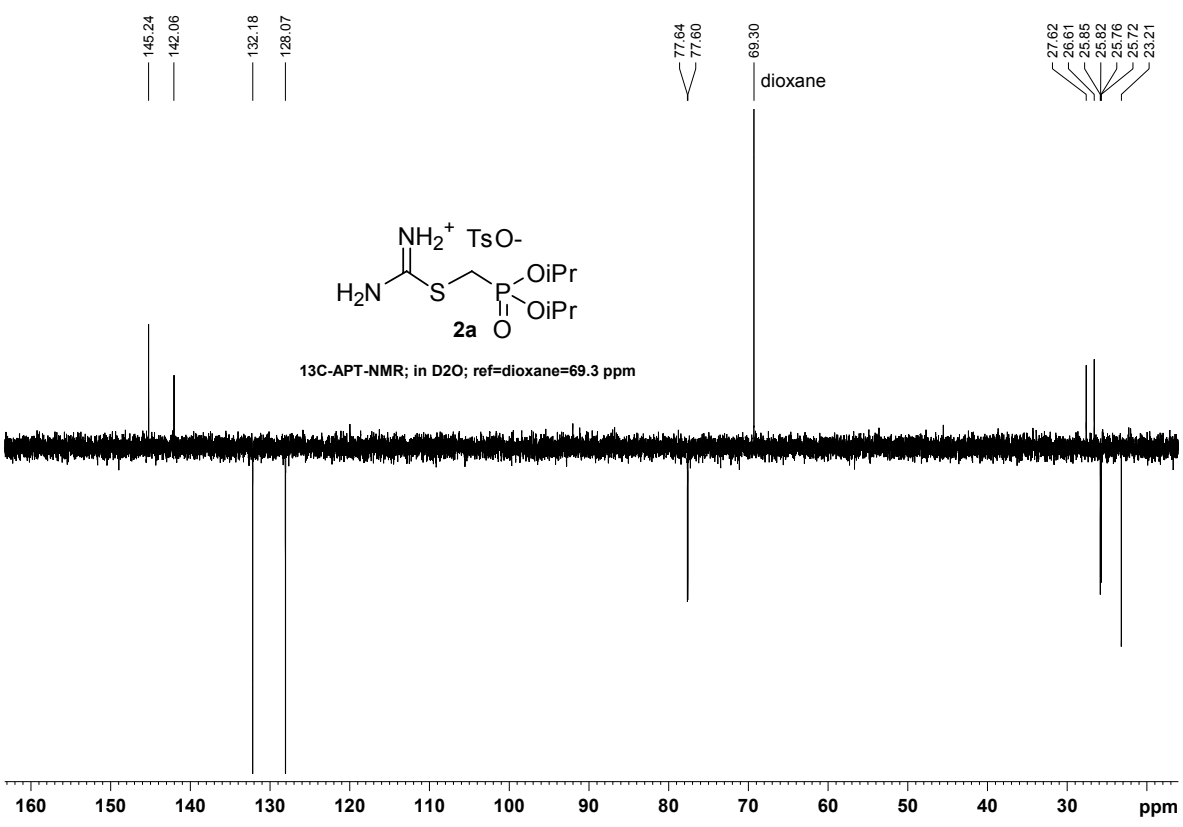
[c] 11.36 bs (NH); 5.46 bs (2-OH); 5.27 bs (3-OH); [d] 7.23 b and 7.15 b (NH_2); 5.26 d, *J*=5.5 Hz (2-OH); 5.16 d, *J*=5.9 Hz (3-OH); [e] 11.22 bs (NH); 8.04 m, 2H, 7.65 m, 1H and 7.55 m, 2H (C_6H_5); 5.97 d, *J*=6.0 Hz (3-OH); 5.20 t, *J*=5.2 Hz (3-OH).

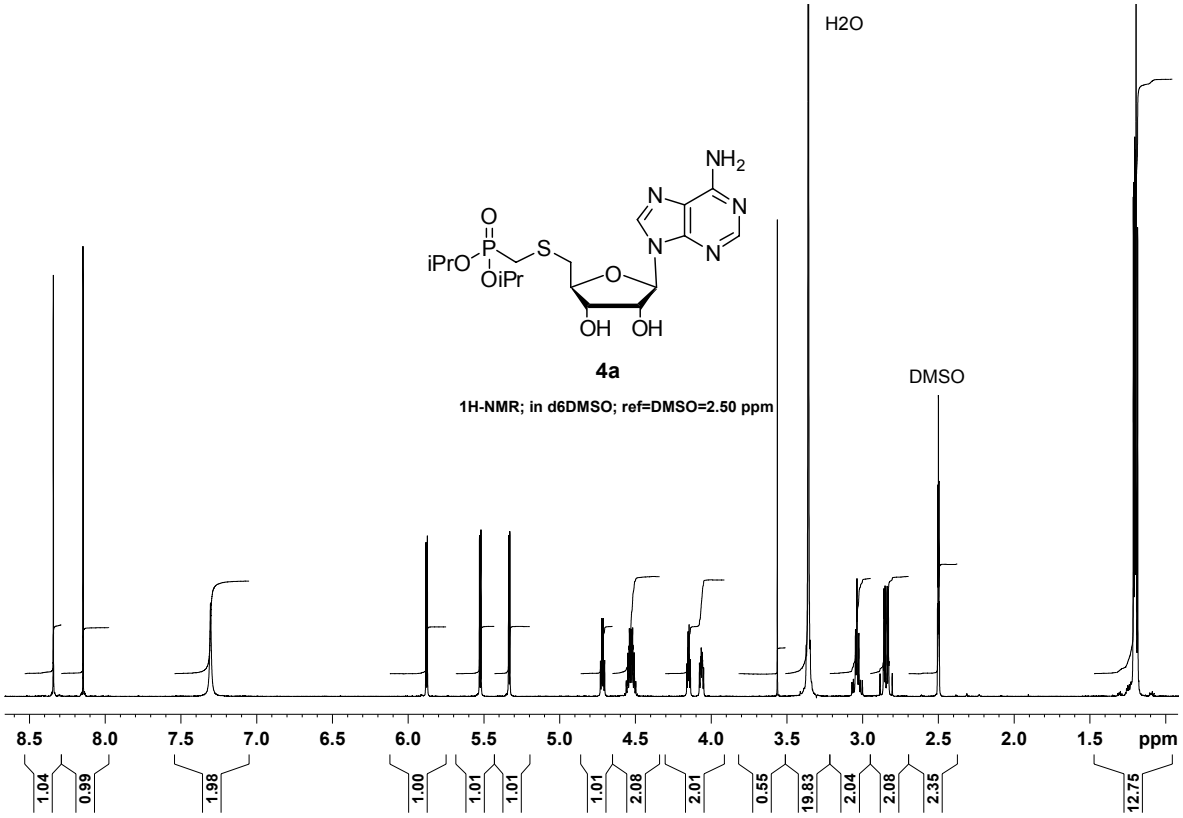
Table 2. Carbon-13 NMR data of compounds **4a-4d, 5a-5d** and **11**

Compound	Solvent	C-1'	C-2'	C-3'	C-4'	C-5'	Base	$P(OR)_2$
4a	DMSO	87.71	72.83	72.82	84.10	35.24 (3.7)	152.90 (C-2); 149.62 (C-4); 119.38 (C-5); 156.30 (C-6); 140.03 (C-8)	70.44 d, *J*=3.4 and 70.39 d, *J*=3.4 (2x O-CH<); 25.54 d, *J*=146.9 (P-CH_2-S); 24.02 d, *J*=3.4 and 23.86 d, *J*=4.8 (4x CH_3)
4b	DMSO	86.86	72.80	72.76	83.97	35.22 (3.7)	153.89 (C-2); 151.58 (C-4); 117.06 (C-5); 156.98 (C-6); 136.09 (C-8)	70.53 d, *J*=6.6 and 70.49 d, *J*=6.5 (2x O-CH<); 25.57 d, *J*=146.8 (P-CH_2-S); 24.04 d, *J*=3.6 and 23.89 d, *J*=3.7 (4x CH_3)
4c	DMSO	88.58	72.54	72.40	83.34	35.00 (3.7)	150.93 (C-2); 163.27 (C-4); 102.34 (C-5); 141.25 (C-6)	70.54 d, *J*=6.8 and 70.49 d, *J*=6.7 (2x O-CH<); 25.57 d, *J*=147.0 (P-CH_2-S); 24.06 d, *J*=3.6 and 23.92 d, *J*=3.6 (4x CH_3)
4d	DMSO	89.85	73.35	72.56	82.89	35.02 (3.5)	155.46 (C-2); 165.76 (C-4); 94.55 (C-5); 141.66 (C-6)	70.51 d, *J*=6.8 and 70.46 d, *J*=6.8 (2x O-CH<); 25.64 d, *J*=146.9 (P-CH_2-S); 24.06 d, *J*=3.5 and 23.93 d, *J*=4.8 (4x CH_3)
5a	D_2O	89.81	76.20	74.89	86.28	36.58 (7.4)	155.59 (C-2); 151.67 (C-4); 121.49 (C-5); 158.32 (C-6); 142.78 (C-8)	32.82 d, *J*=128.9 (P-CH_2-S)
5b	D_2O	89.72	75.76	75.03	86.24	38.56 (7.4)	157.63 (C-2); 154.46 (C-4); 119.37 (C-5); 163.40 (C-6); 140.26 (C-8)	32.64 d, *J*=128.8 (P-CH_2-S)
5c	D_2O	92.63	76.88	75.52	85.28	38.94 (7.7)	162.51 (C-2 + C-4); 105.68 (C-5); 143.34 (C-6)	32.93 d, *J*=128.5 (P-CH_2-S)
5d	D_2O	92.79	76.54	74.54	85.05	38.31 (7.2)	160.39 (C-2); 168.94 (C-4); 99.22 (C-5); 144.38 (C-6)	32.73 d, *J*=130.0 (P-CH_2-S)
11 [a]	DMSO	84.03	55.11	72.74	85.13	59.92	151.82 (C-2); 152.28 (C-4); 125.24 (C-5); 150.58 (C-6); 143.35 (C-8)	70.83 d, *J*=6.6 and 70.79 d, *J*=6.7 (2x O-CH<); 25.45 d, *J*=146.0 (P-CH_2-S); 23.97 d, *J*=3.5, 23.95 d, *J*=3.5, 23.80 d, *J*=4.7 and 23.69 d, *J*=4.9 (4x CH_3)

Additional signals: [a] 165.77 (C=O); 133.59; 128.72(2), 128.72(2) and 132.71 (C_6H_5).

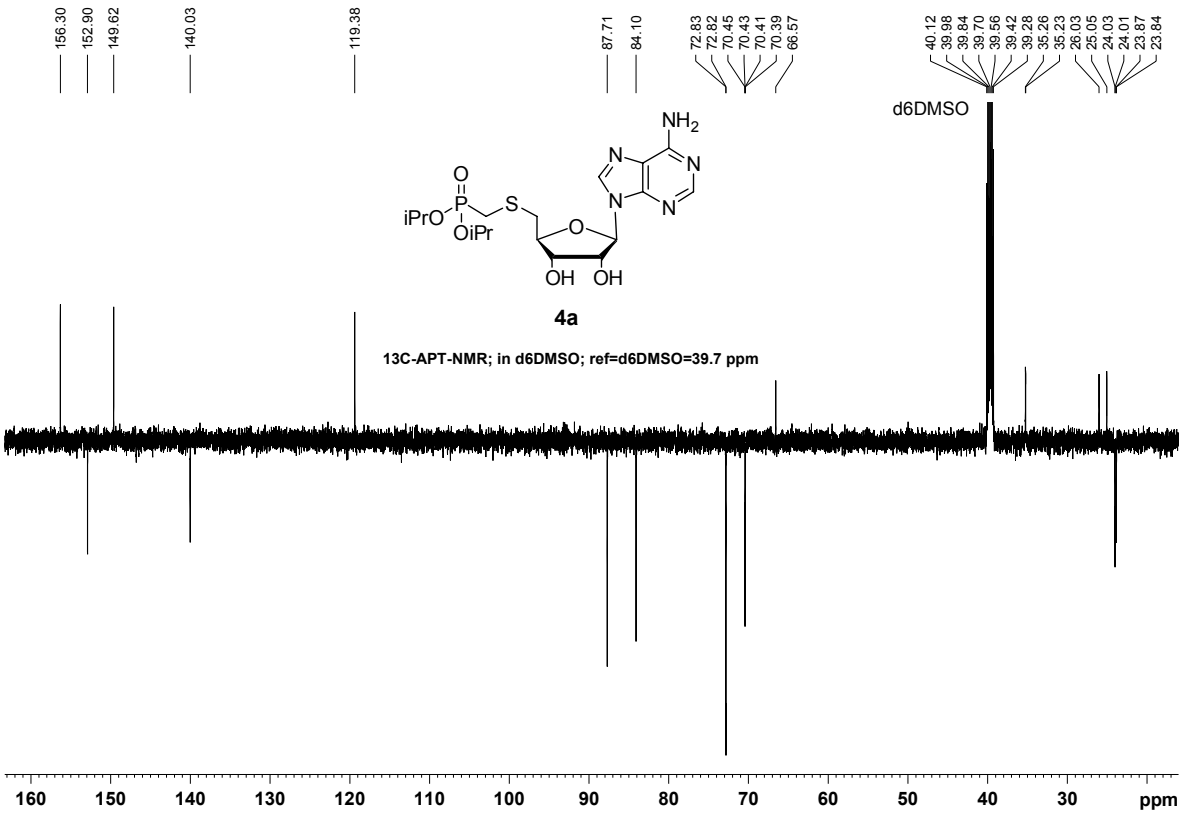

HDO
dioxane
NH2+ TsO-
H2N
S
P
OiPr
OiPr
O
2a
1H-NMR; in D2O; ref=dioxane=3.75 ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
5.87
5.96
84.88
7.63
2.00
9.00
12.00


145.24
142.06
132.18
128.07
77.64
77.60
69.30
dioxane
27.62
26.61
25.85
25.82
25.76
25.72
23.21
NH2+ TsO-
H2N
S
P
OiPr
OiPr
2a O
13C-APT-NMR; in D2O; ref=dioxane=69.3 ppm
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm



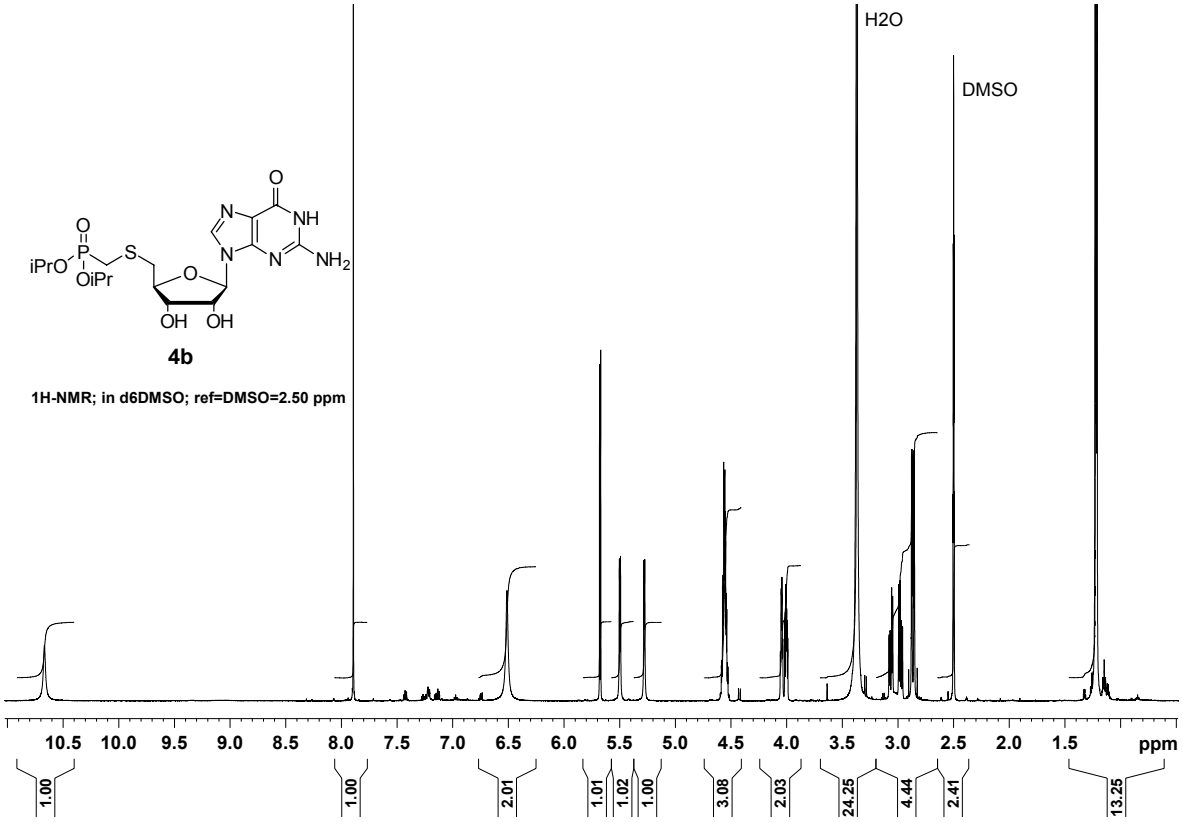
4a

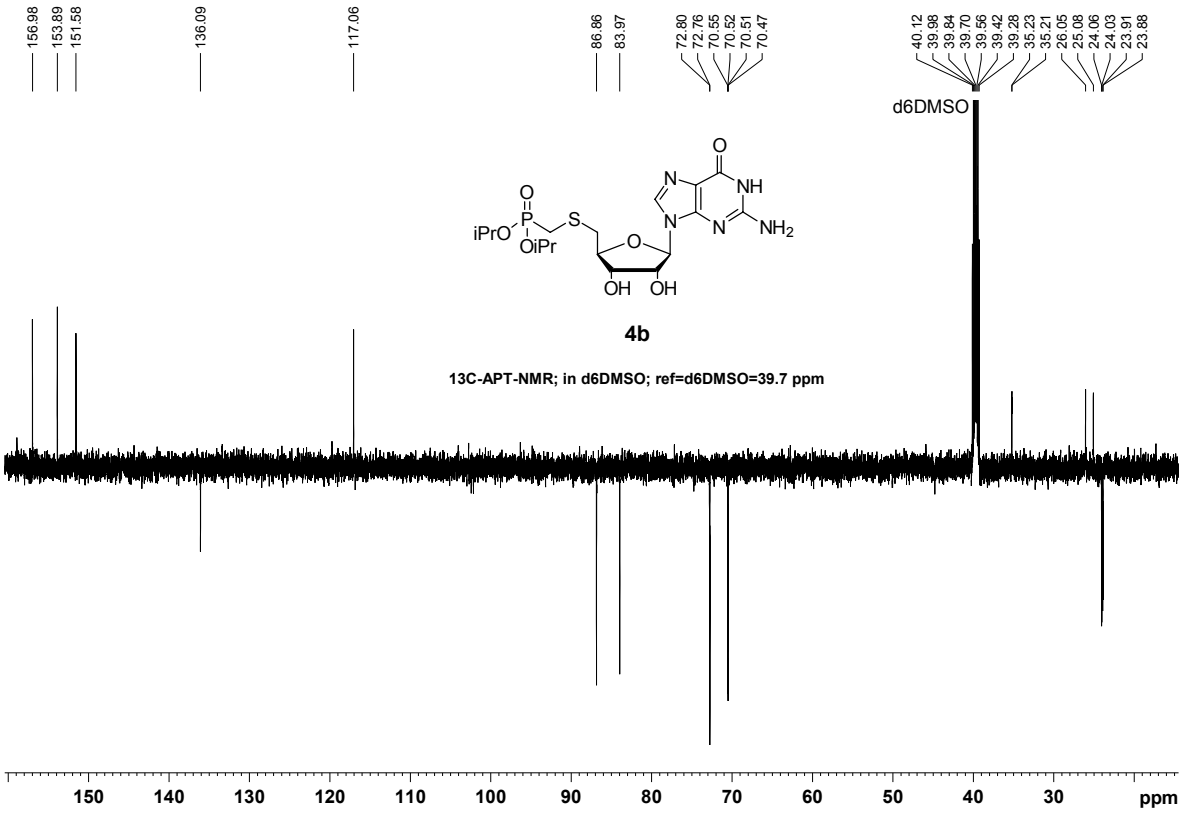
1H-NMR; in d6DMSO; ref=DMSO=2.50 ppm

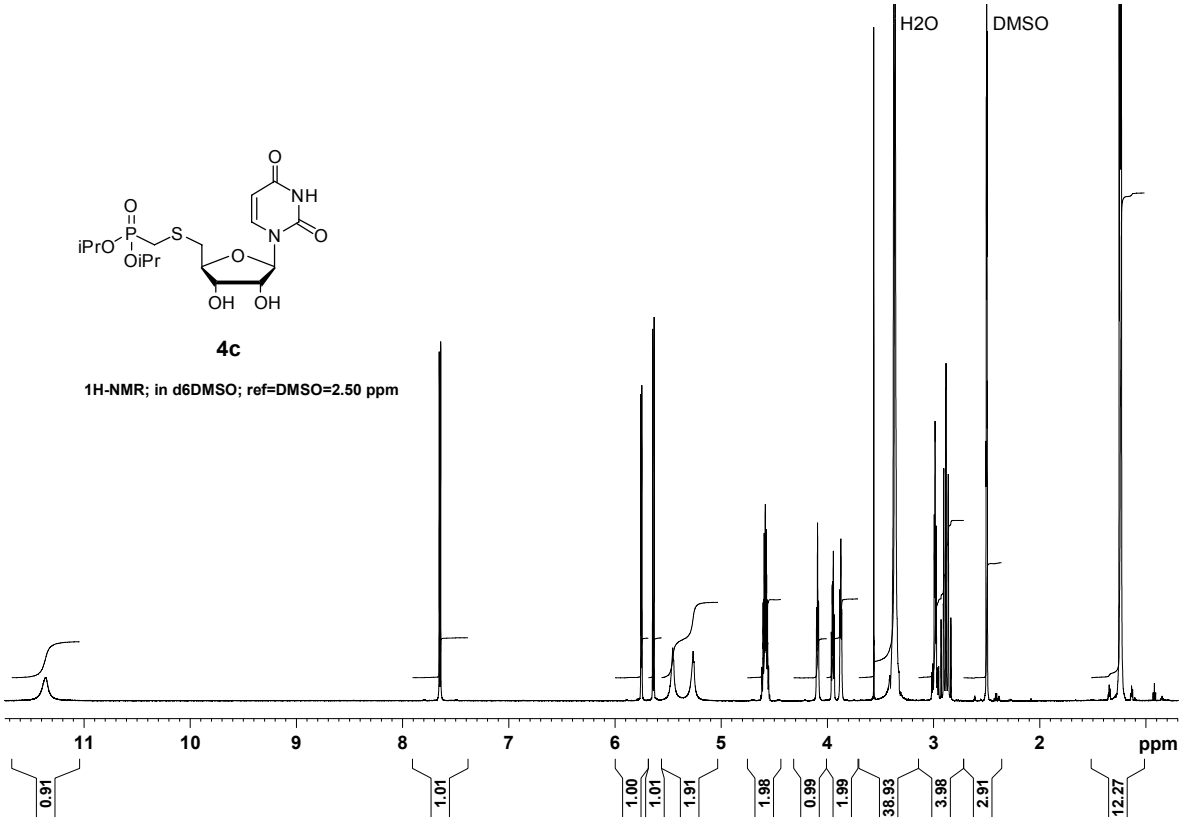


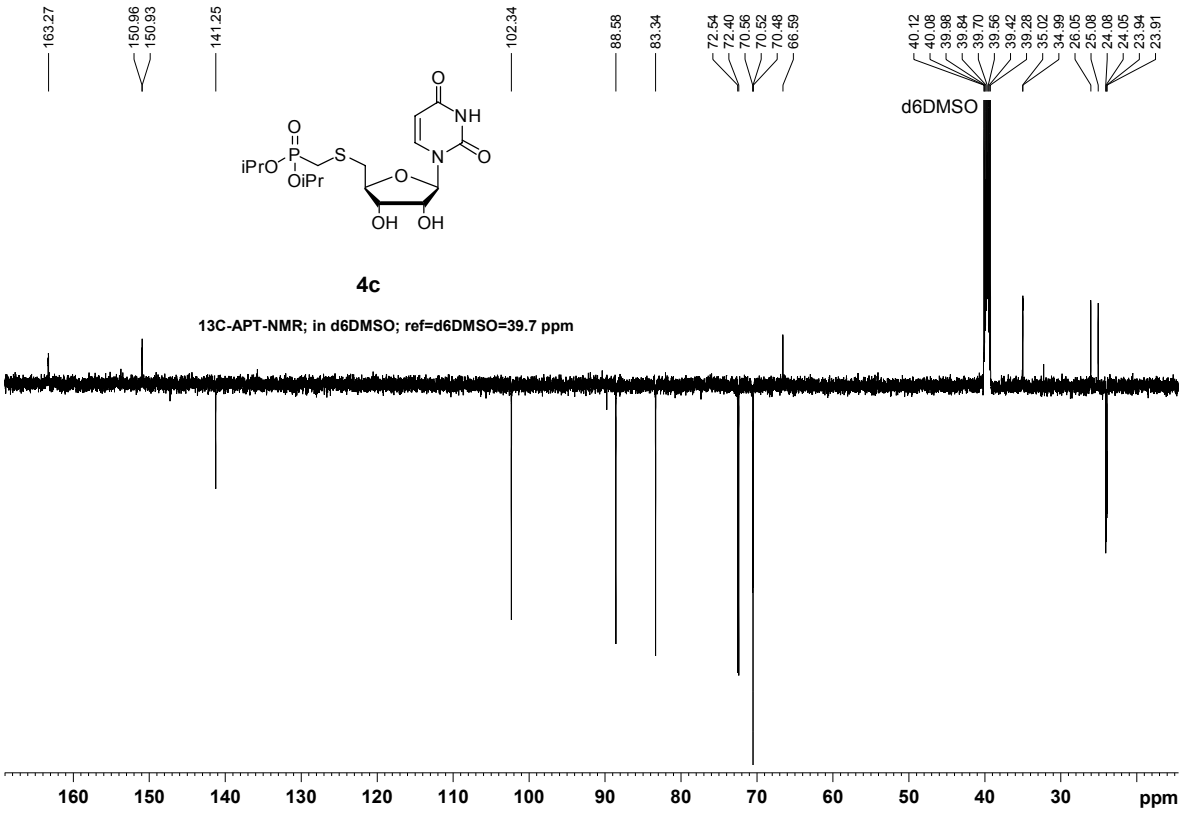
4a

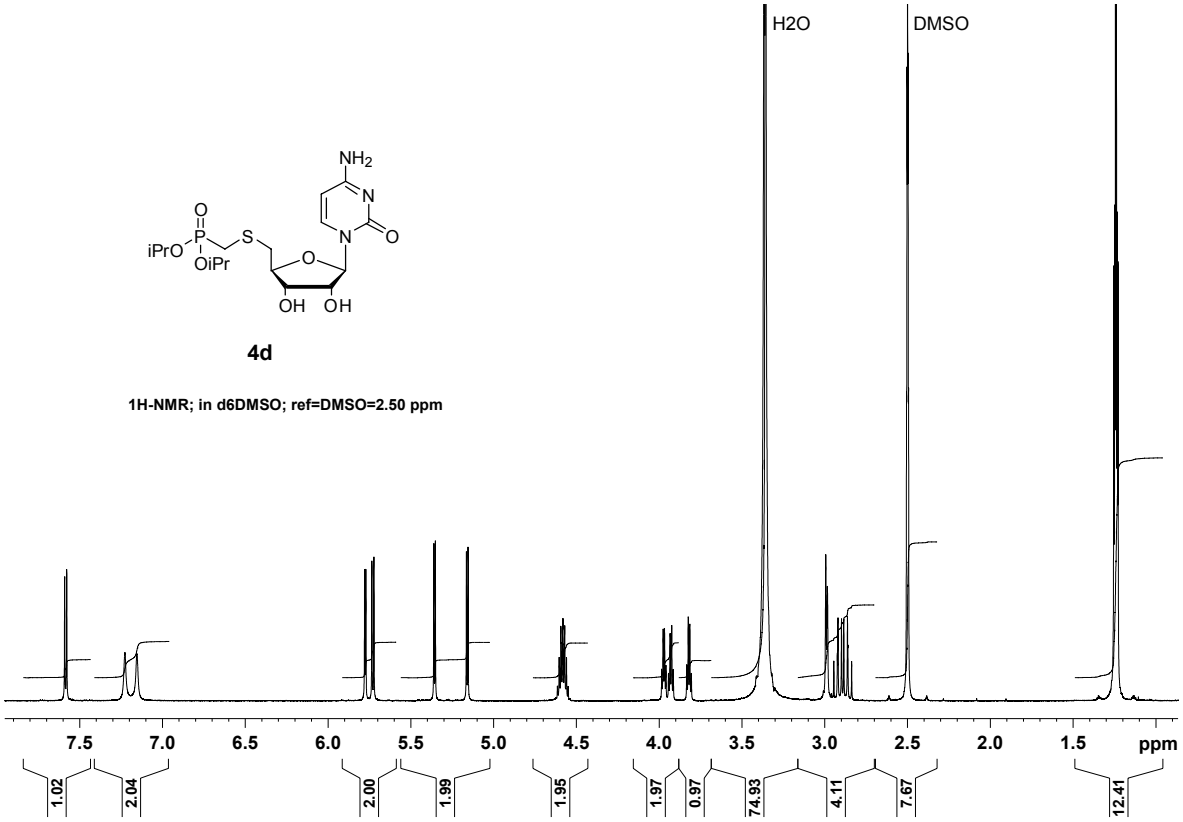
13C-APT-NMR; in d6DMSO; ref=d6DMSO=39.7 ppm

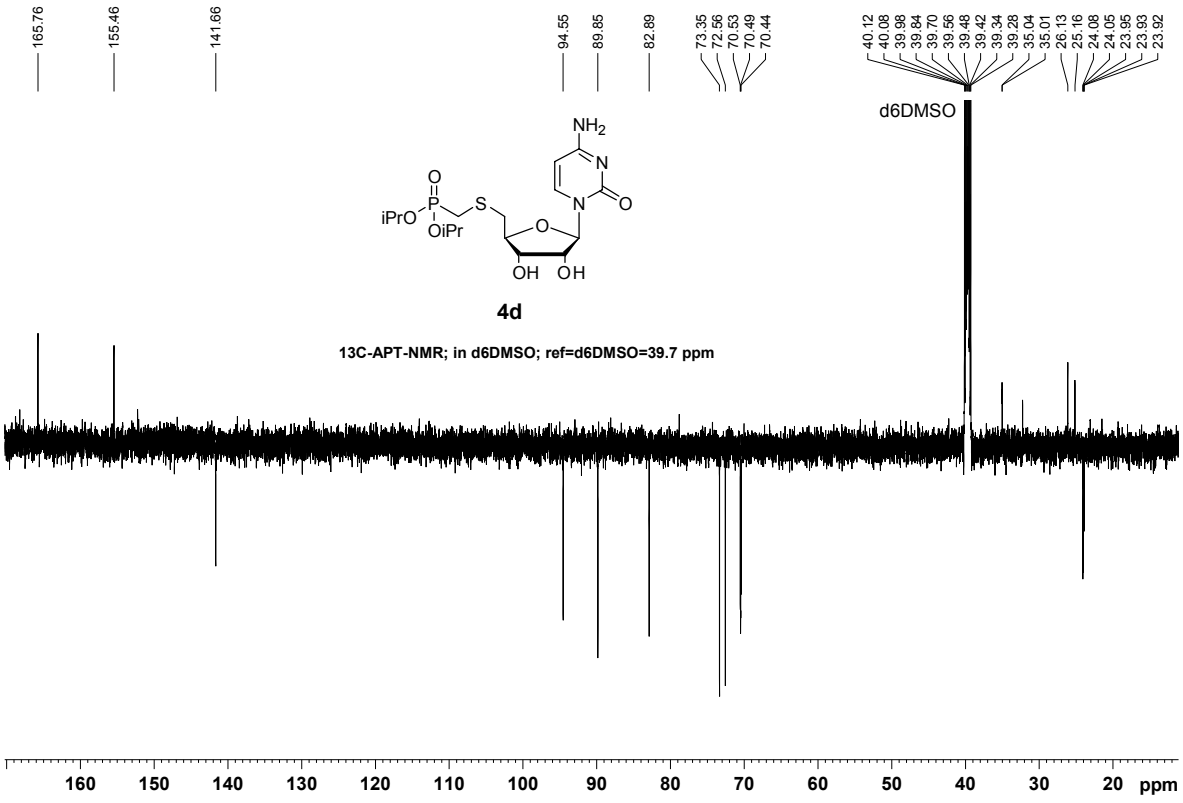

H2O
DMSO
4b
1H-NMR; in d6DMSO; ref=DMSO=2.50 ppm
ppm

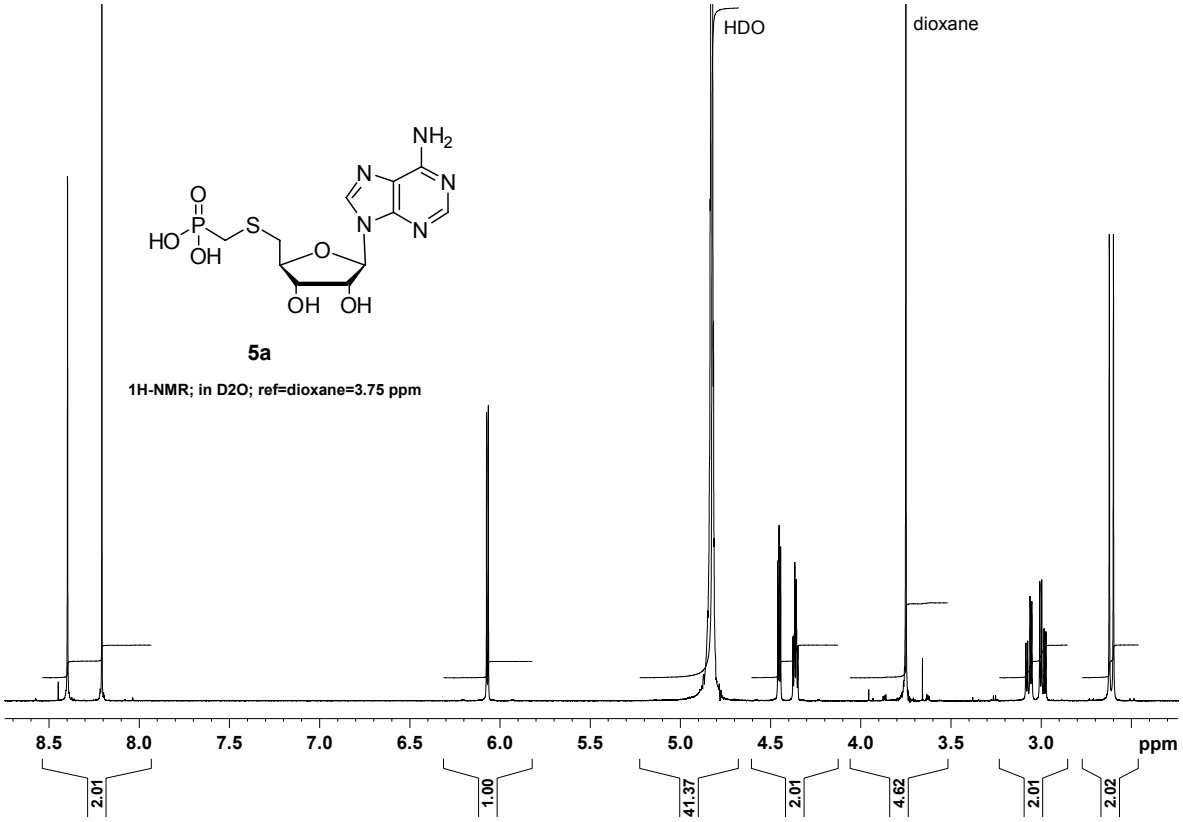

156.98
153.89
151.58
136.09
117.06
86.86
83.97
72.80
72.76
70.55
70.52
70.51
70.47
40.12
39.98
39.84
39.70
39.56
39.42
39.28
35.23
35.21
26.05
25.08
24.06
24.03
23.91
23.88
d6DMSO
4b
13C-APT-NMR; in d6DMSO; ref=d6DMSO=39.7 ppm
ppm





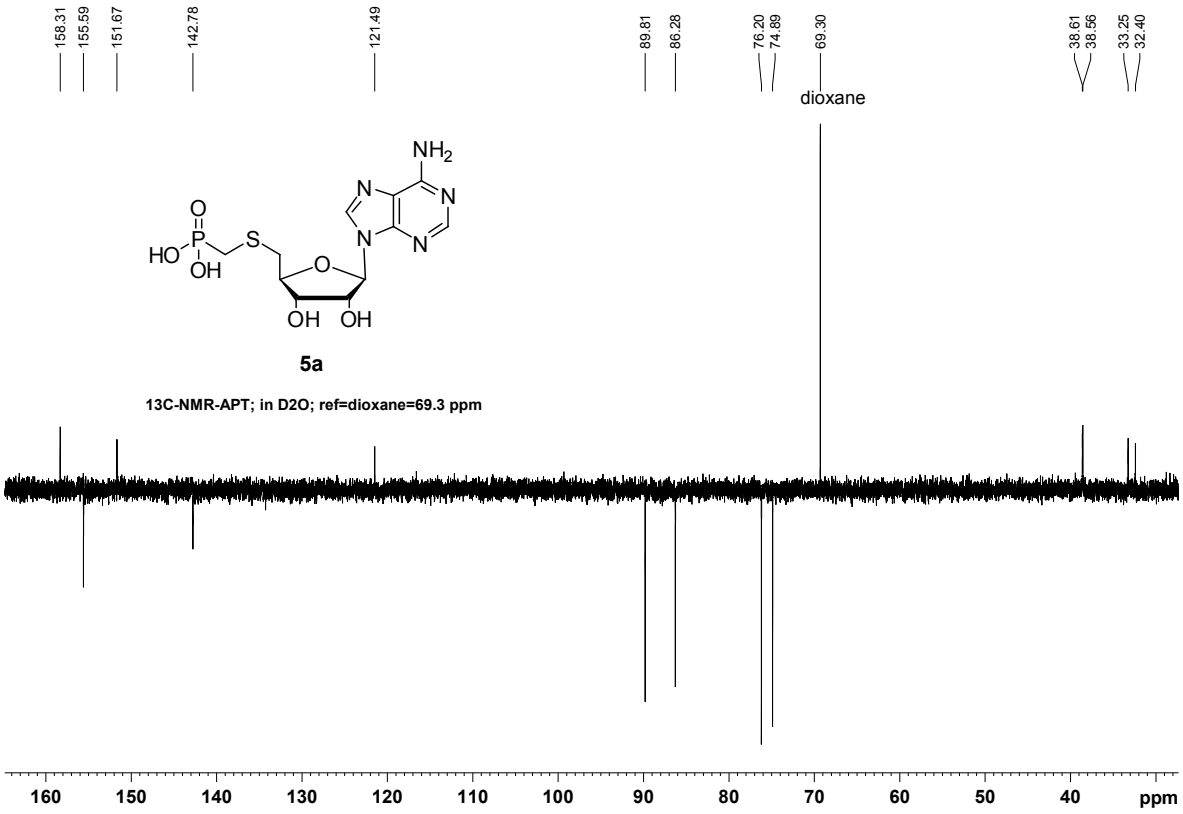






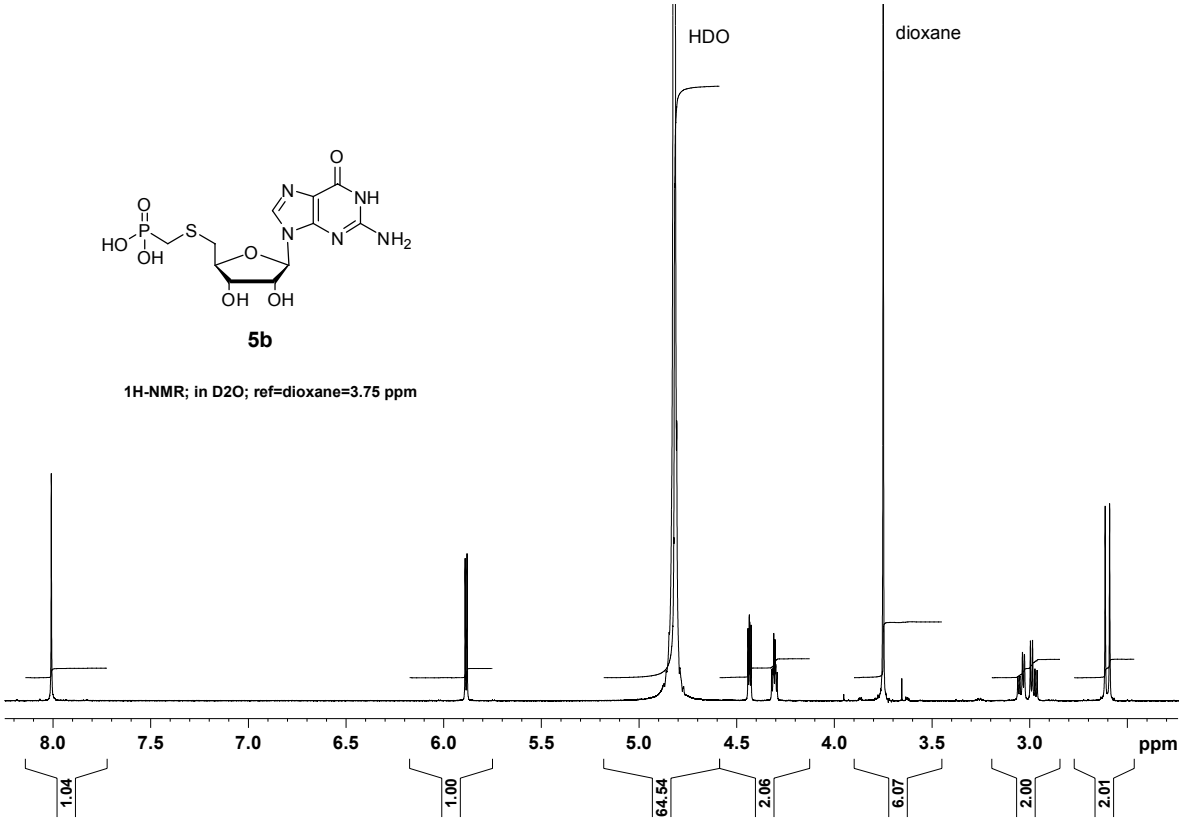
5a

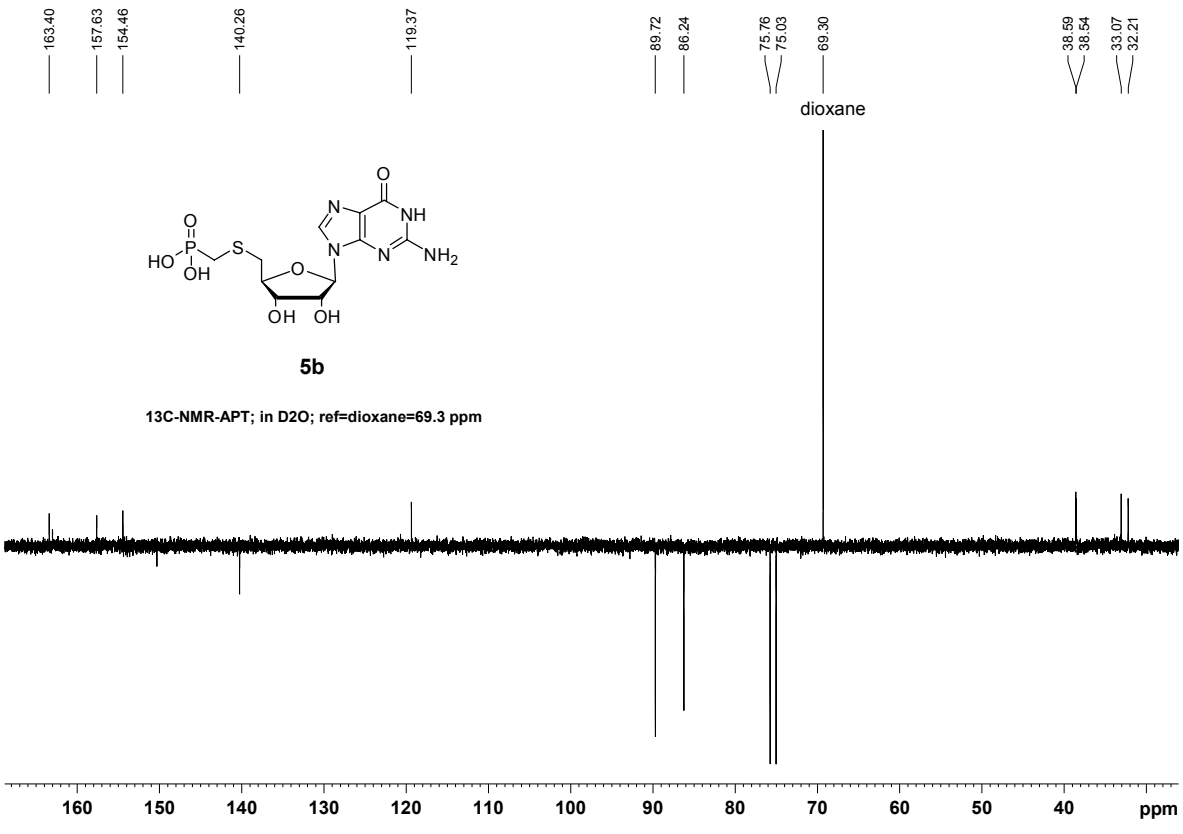
1H-NMR; in D2O; ref=dioxane=3.75 ppm

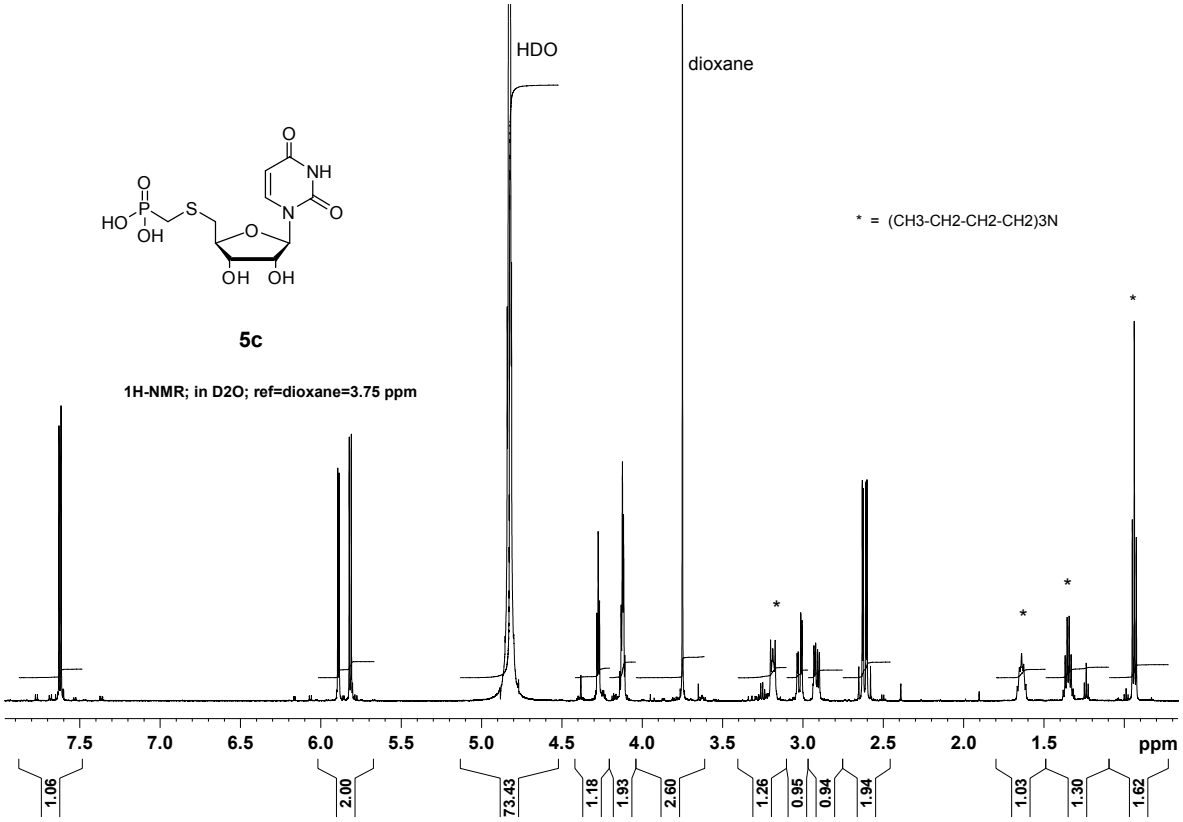


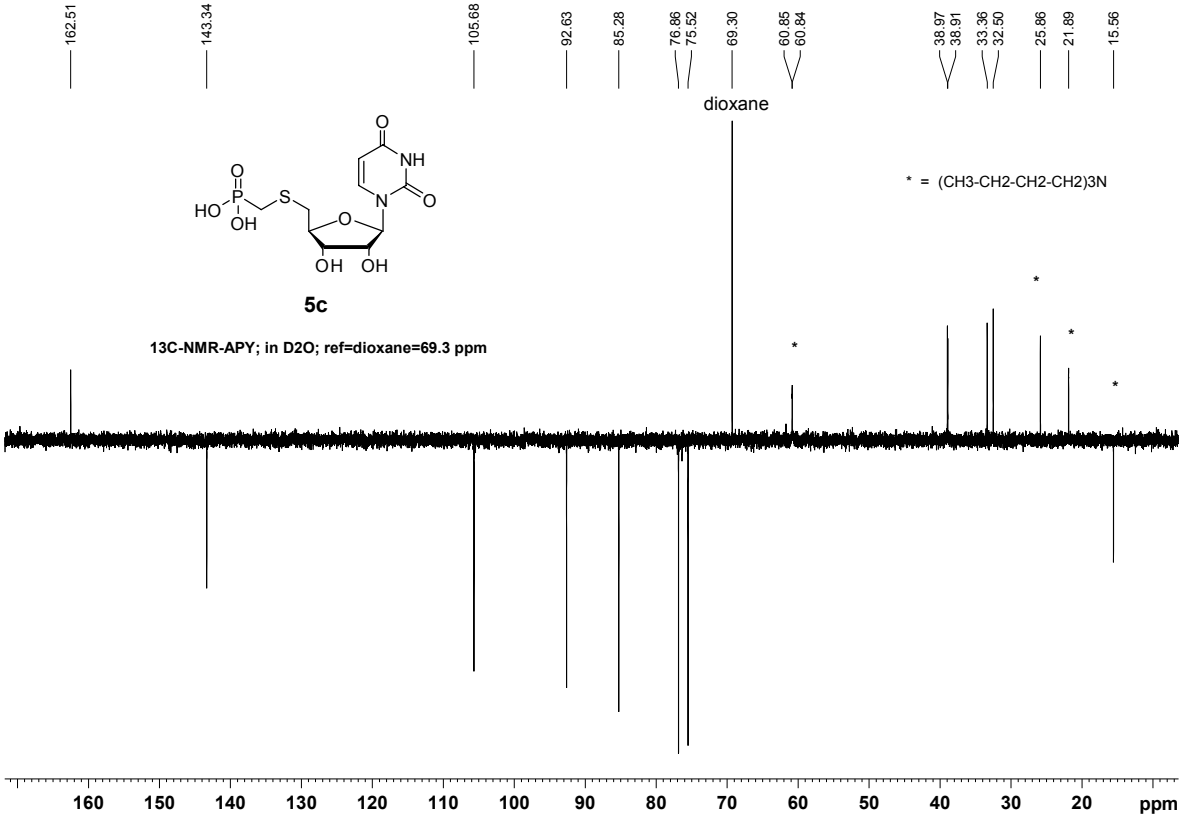
5a

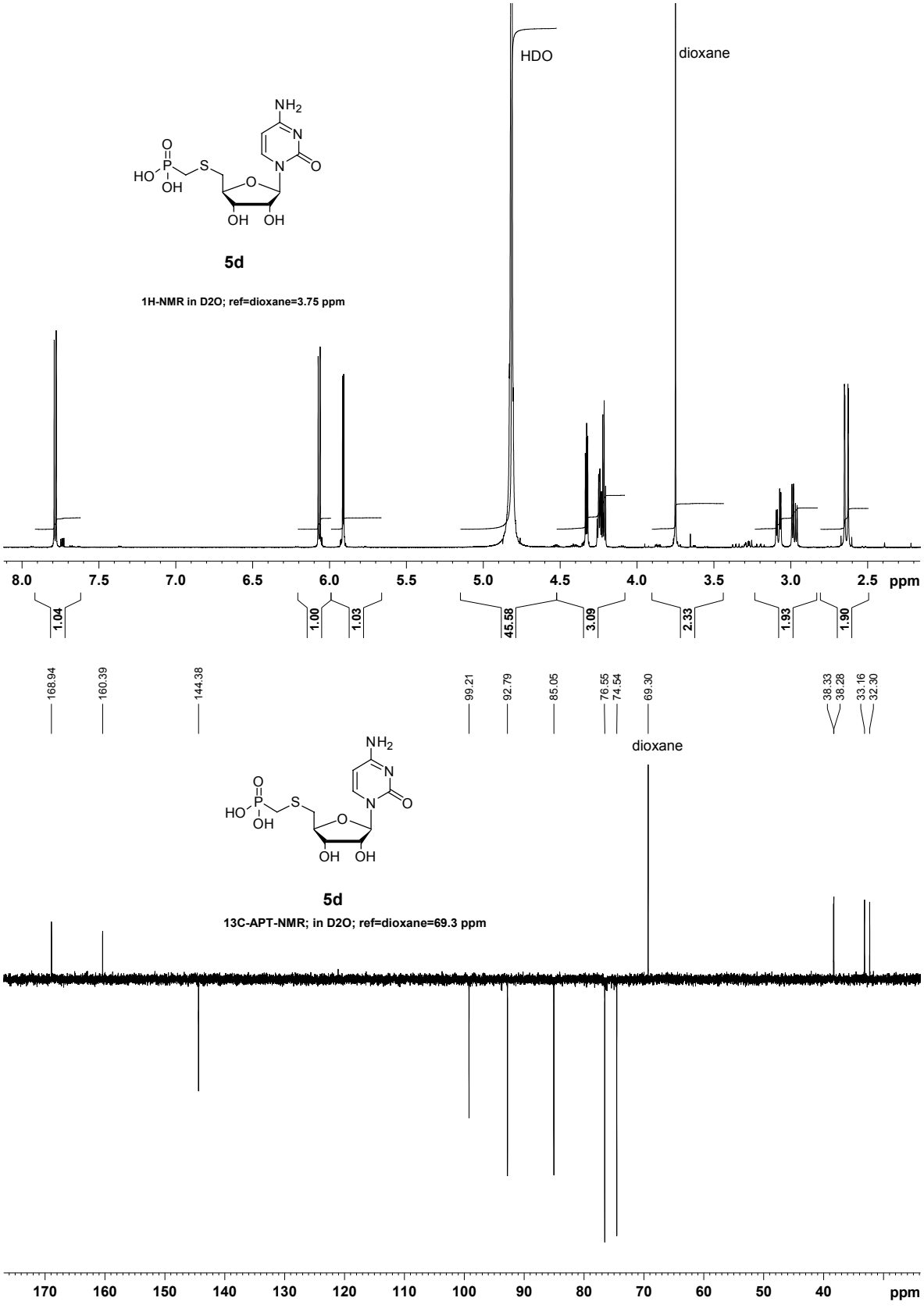
13C-NMR-APT; in D2O; ref=dioxane=69.3 ppm

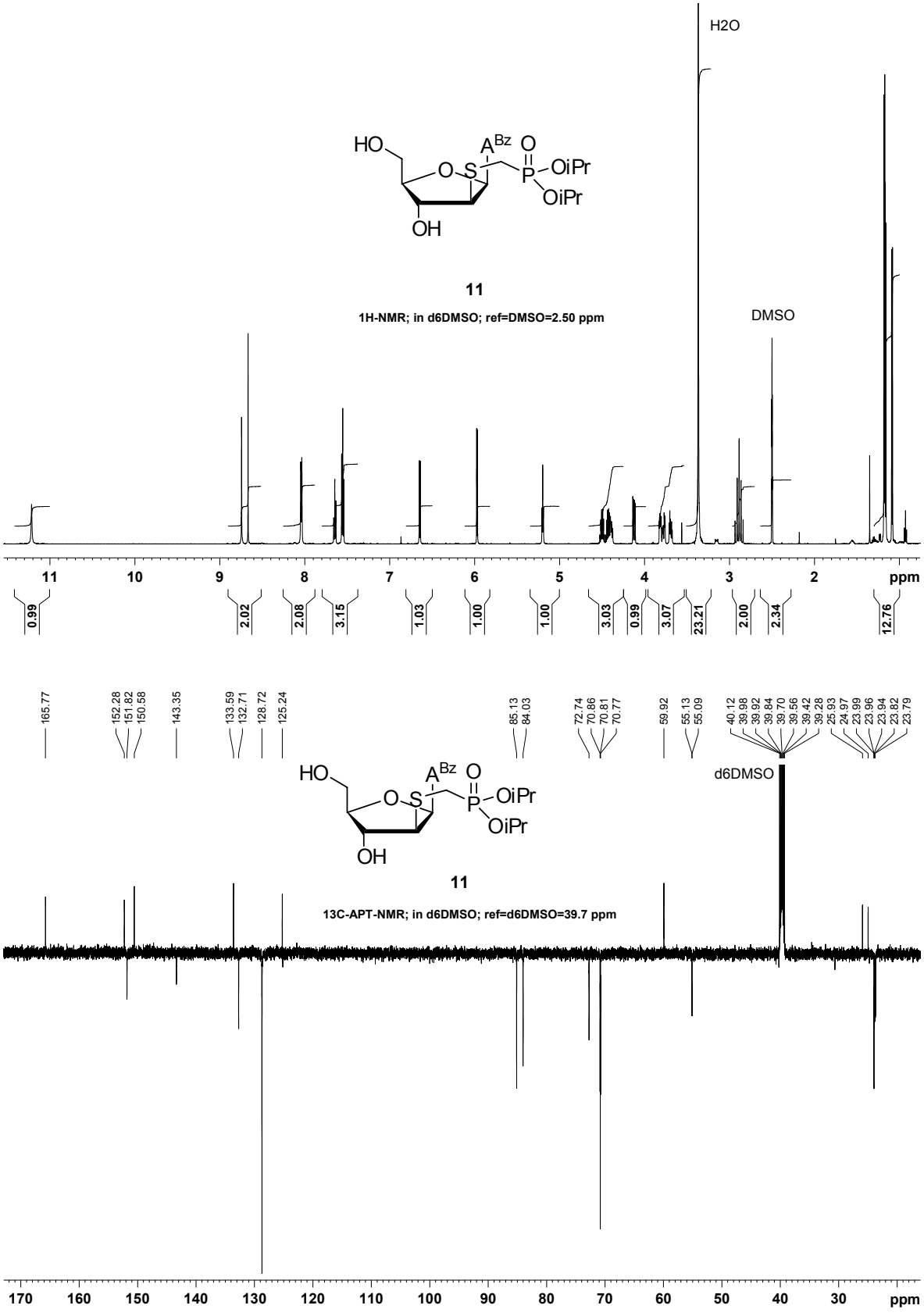


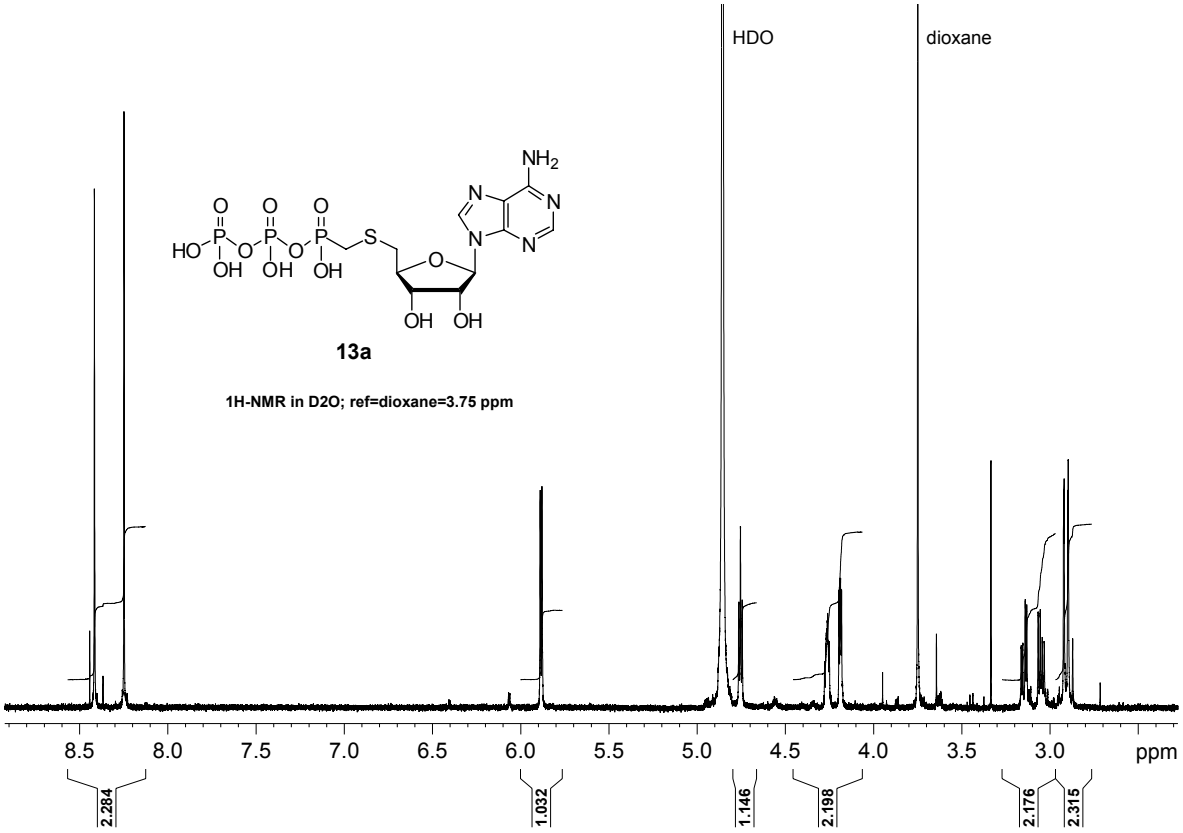


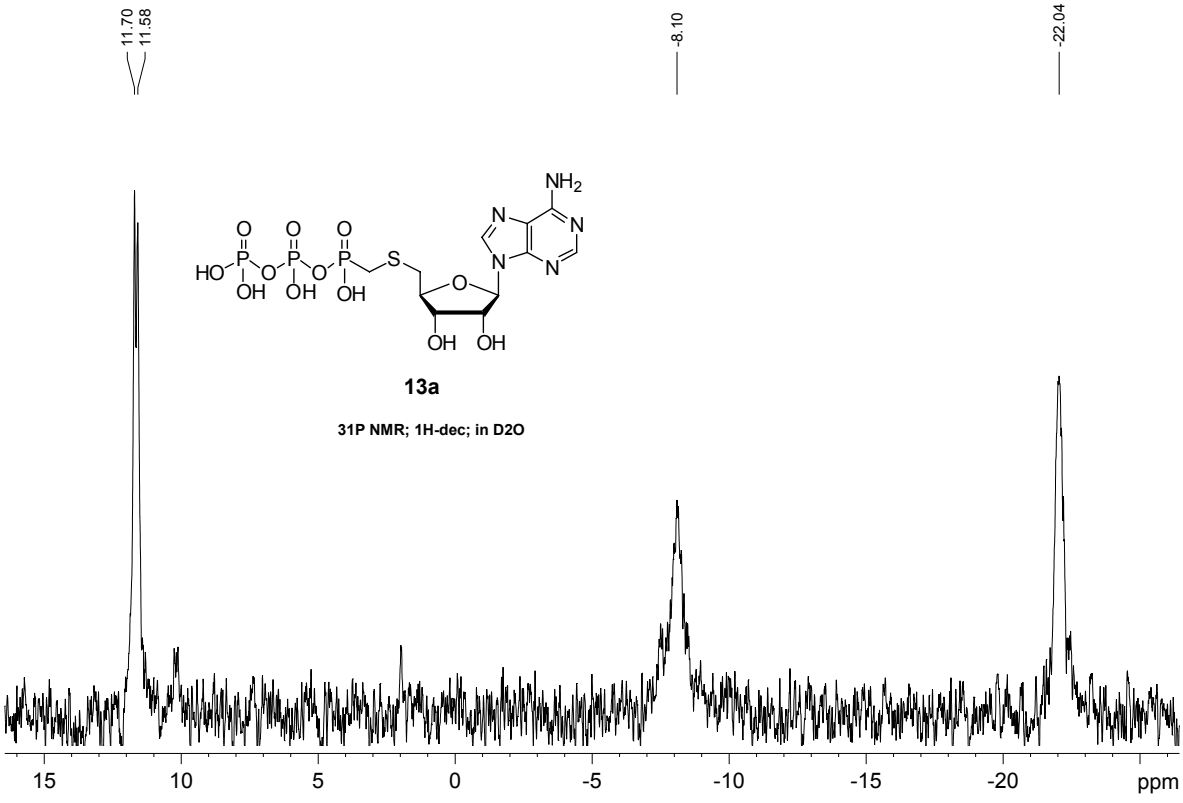

HDO
dioxane
* = (CH3-CH2-CH2-CH2)3N
5c
1H-NMR; in D2O; ref=dioxane=3.75 ppm
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
1.06 2.00 73.43 1.18 1.93 2.60 1.26 0.95 0.94 1.94 1.03 1.30 1.62

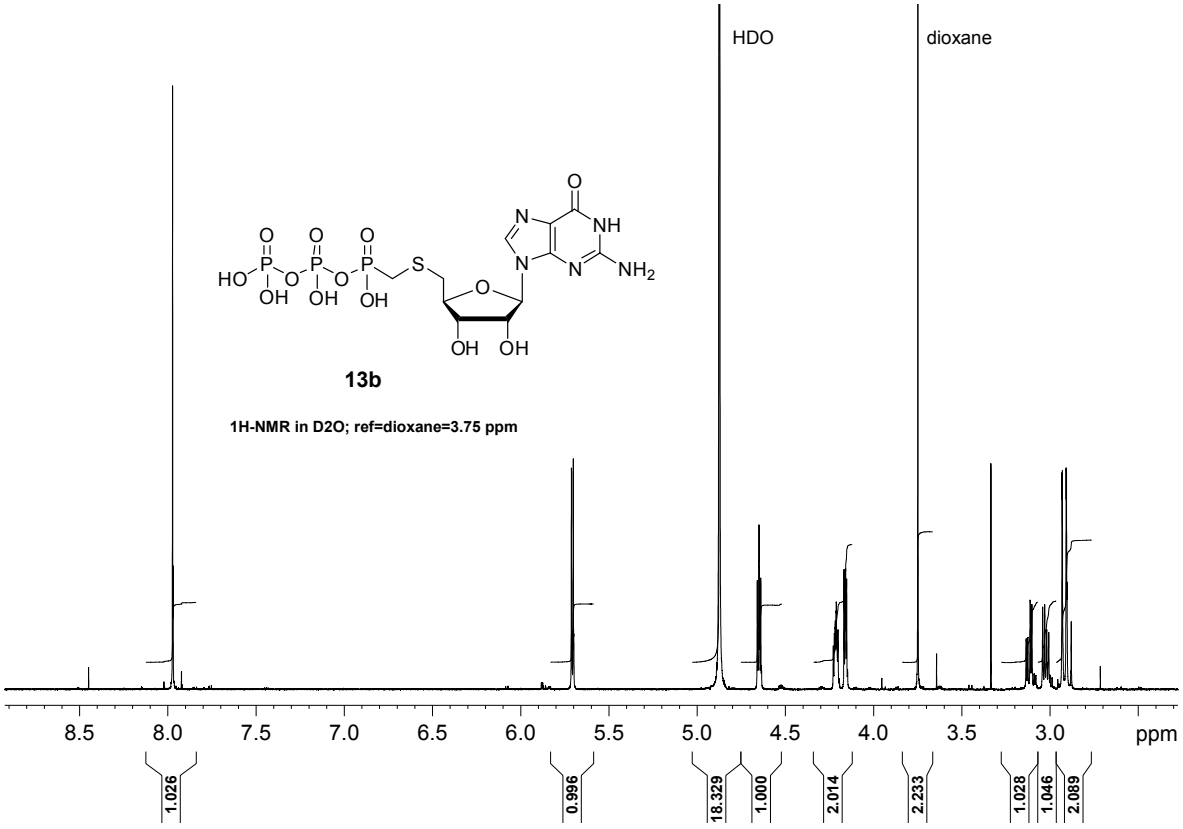

162.51 143.34 105.68 92.63 85.28 76.86 75.52 69.30 60.85 60.84 38.97 38.91 33.36 32.50 25.86 21.89 15.56
dioxane
* = (CH3-CH2-CH2-CH2)3N
5c
13C-NMR-APY; in D2O; ref=dioxane=69.3 ppm
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

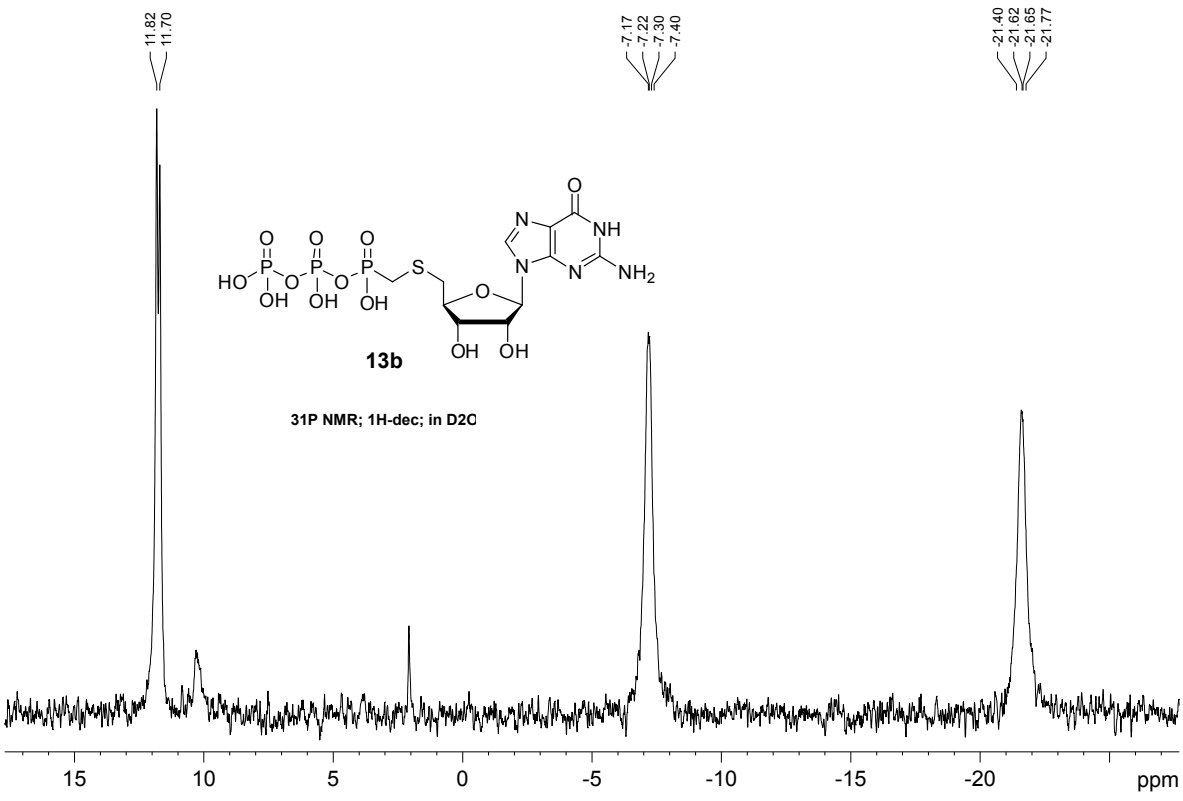

HDO
dioxane
NH2
N
O
HO
P
OH
S
O
N
O
OH OH
5d
1H-NMR in D2O; ref=dioxane=3.75 ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 ppm
1.04 1.00 1.03 45.58 3.09 2.33 1.93 1.90
168.94 160.39 144.38 99.21 92.79 85.05 76.55 74.54 69.30 38.33 38.28 33.16 32.30
dioxane
5d
13C-APT-NMR; in D2O; ref=dioxane=69.3 ppm
170 160 150 140 130 120 110 100 90 80 70 60 50 40 ppm

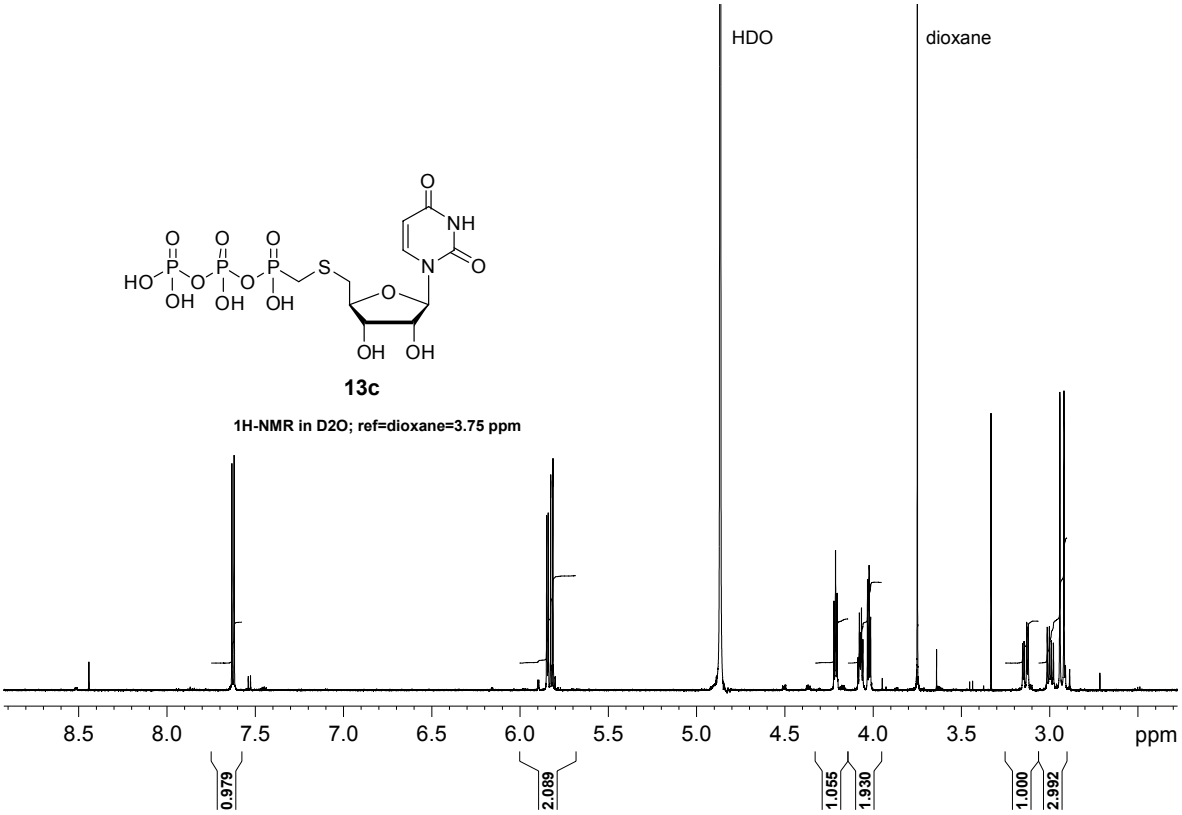

H2O
HO
A^Bz
O
O
S
P
OiPr
OiPr
OH
11
1H-NMR; in d6DMSO; ref=DMSO=2.50 ppm
DMSO
11
10
9
8
7
6
5
4
3
2
ppm
0.99
2.02
2.08
3.15
1.03
1.00
1.00
3.03
0.99
3.07
23.21
2.00
2.34
12.76
165.77
152.28
151.82
150.58
143.35
133.59
132.71
128.72
125.24
85.13
84.03
72.74
70.86
70.81
70.77
59.92
55.13
55.09
40.12
39.98
39.92
39.84
39.70
39.56
39.42
39.28
25.93
24.97
23.99
23.96
23.94
23.82
23.79
d6DMSO
HO
A^Bz
O
O
S
P
OiPr
OiPr
OH
11
13C-APT-NMR; in d6DMSO; ref=d6DMSO=39.7 ppm
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

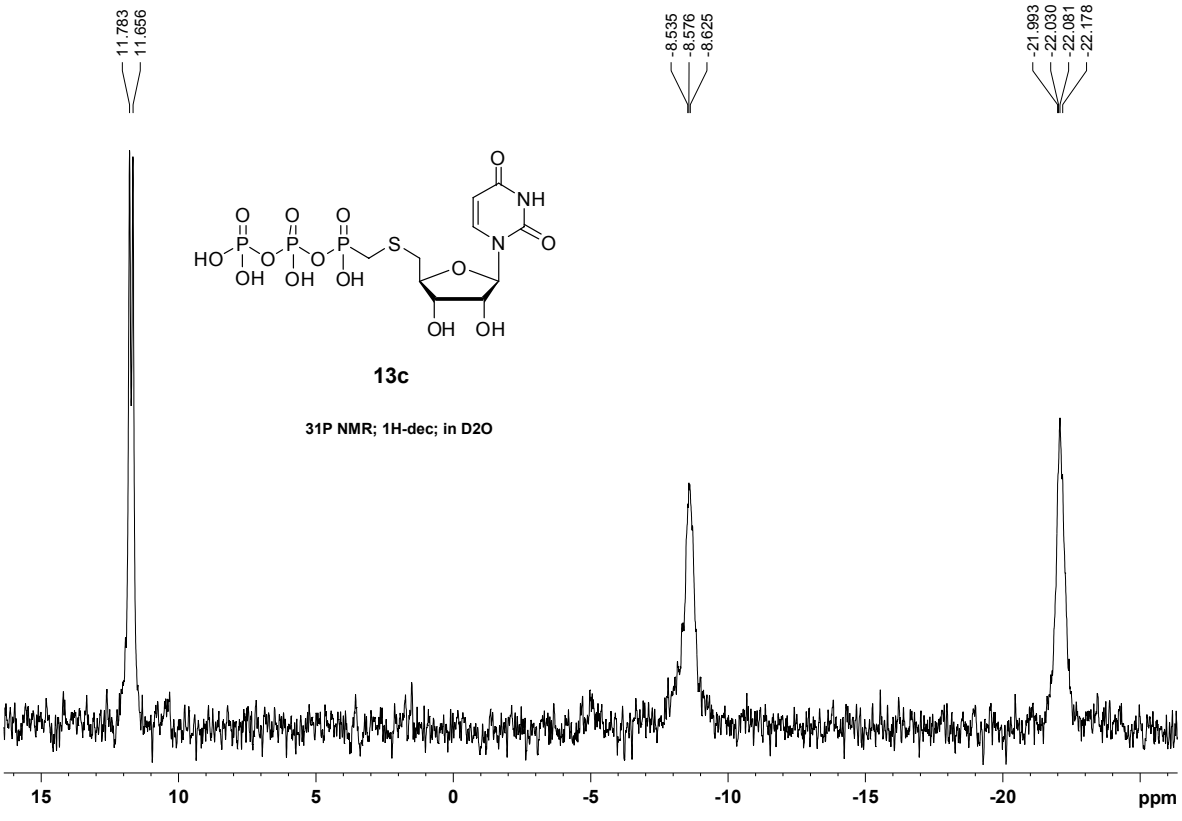

HDO
dioxane
NH2
HO
OH OH OH
OH OH
13a
1H-NMR in D2O; ref=dioxane=3.75 ppm
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 ppm
2.284
1.032
1.146
2.198
2.176
2.315

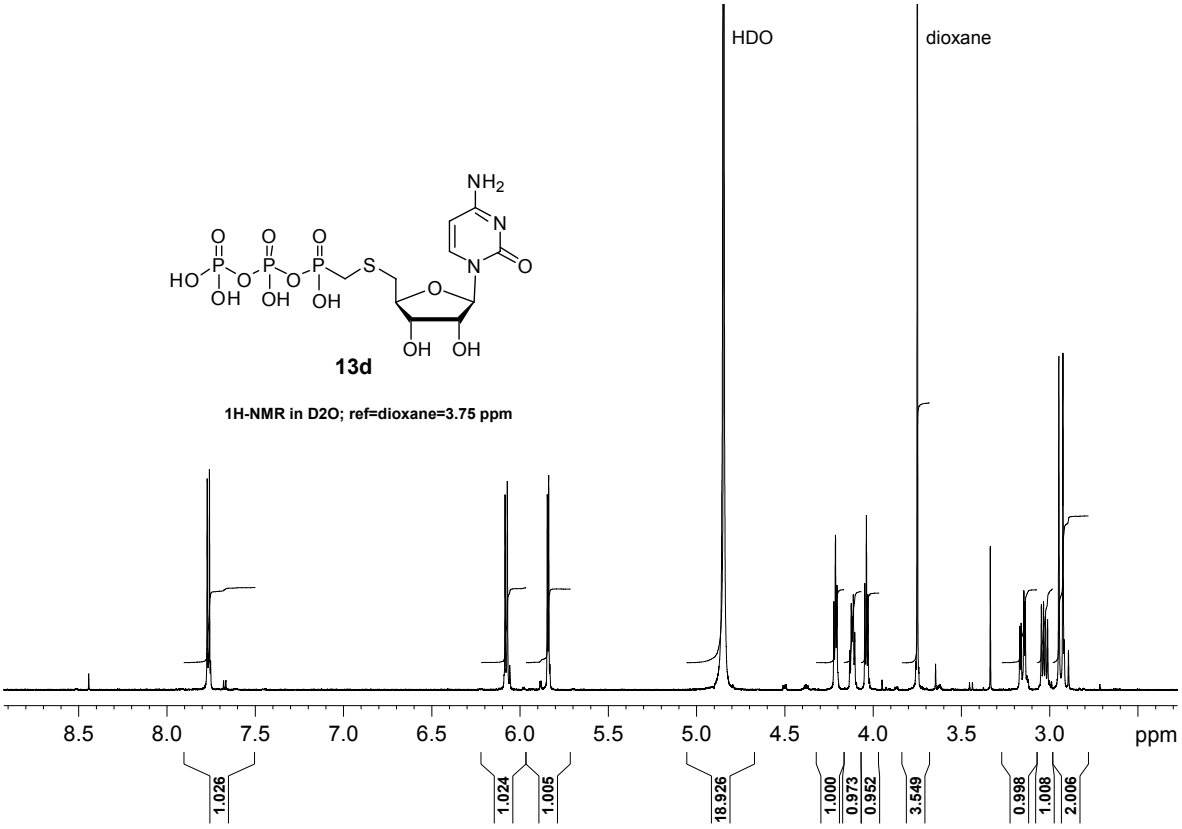

11.70
11.58
-8.10
-22.04
NH2
HO
OH OH OH
OH OH
13a
31P NMR; 1H-dec; in D2O
15 10 5 0 -5 -10 -15 -20 ppm

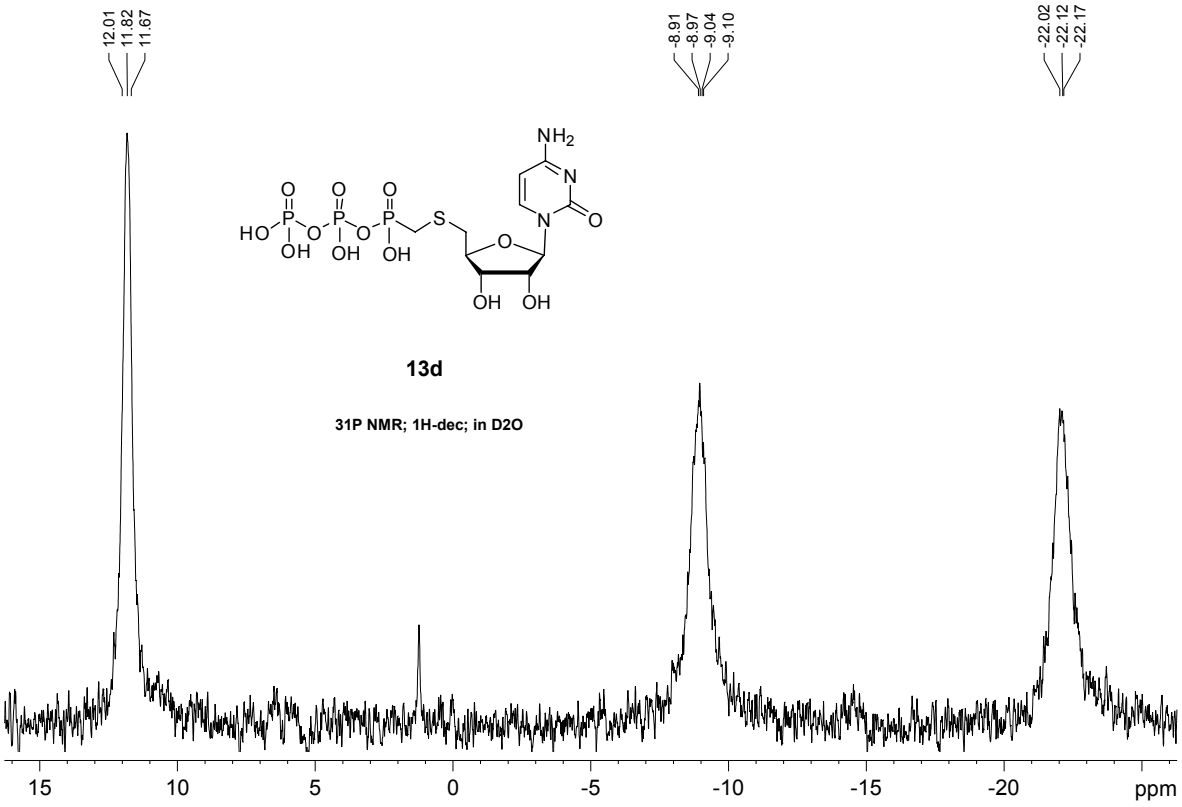

HDO
dioxane
13b
1H-NMR in D2O; ref=dioxane=3.75 ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm
1.026
0.996
18.329
1.000
2.014
2.233
1.028
1.046
2.089


11.82
11.70
-7.17
-7.22
-7.30
-7.40
-21.40
-21.62
-21.65
-21.77
13b
31P NMR; 1H-dec; in D2O
15
10
5
0
-5
-10
-15
-20
ppm


HDO
dioxane
13c
1H-NMR in D2O; ref=dioxane=3.75 ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm
0.979
2.089
1.055
1.930
1.000
2.992


11.783
11.656
-8.535
-8.576
-8.625
-21.993
-22.030
-22.081
-22.178
13c
31P NMR; 1H-dec; in D2O
15
10
5
0
-5
-10
-15
-20
ppm





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