Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Addressing Regio- and Stereo-Specificity Challenges in the Synthesis of Nucleoside 2',3'-Cyclic Monophosphate Analogs – a Rapid and Facile Synthesis of Nucleosides-2',3'-*O,O*-phosphoro-thioate or -selenoate, and Elucidation of the Origin of the Rare Specificity

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All authors have approved the final article

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Experimental

General

All commercial reagents were used without further purification, unless otherwise noted. All air- and moisture sensitive reactions were conducted in flame-dried, nitrogen-flushed, two-neck flasks sealed with rubber septa, and the reagents were introduced with a syringe. Progress of the reactions was monitored by TLC using precoated Merck silica gel plates (60F-253). Reactants and products were visualized using UV light. Compounds were characterized by NMR using a Bruker DMX-600 spectrometer. ^{1}H NMR spectra were recorded at 600 MHz. Nucleotides were also characterized by ^{31}P NMR in D_2O at 161.96 MHz. High-resolution mass spectra were recorded on an AutoSpec-ESI mass spectrometer. Nucleotides were analyzed using electron spray ionization (ESI) on a Q-TOF micro-instrument (Waters). Primary purification of the nucleotides was achieved on a combi flash RF+ Teledyne ISCO system using a column of Sephadex DEAE-A25, swollen in 1 M $NaHCO_3$ at 4 °C for 24 h. The resin was washed with deionized water before use. LC separation was monitored by UV detection at 260 nm and 220 nm. Final purification of the nucleotides was achieved on an HPLC (Merck-Hitachi) system using a semipreparative reversed-phase column [Gemini 5u C-18 110A, 250 mm × 10 mm, 5 µm (Phenomenex, Torrance, CA)]. The details of the solvent system gradients used for the separation of each product are provided below. Specific rotation was measured by a Jasco P-2000 polarimeter using an aperture of 8 mm with a cuvette of 0.5 dm, in H_2O solution at 22 ^{0}C, with Na as a light source at 589 nm.

2-Cyanoethoxy-dithiophospholane, 8, and 2-cyanoethoxy-seleno-thiophospholane, 9

Hydroxypropionitrile was added dropwise into 2-chloro-dithiaphospholane, **6**, in pyridine. The reaction mixture was stirred at room temperature for 30 min. Then excess of S_8 or Se_0 was added and the reaction mixture was stirred for 2 h. The solvent was removed under vaccuo. Toluene (15 mL) was added to the residue. The solution was stirred and then the solvent was evaporated. Finally, acetonitrile (20 mL) was added to the residue and the excess of sulfur/ selenium was filtered off. The crude product was purified by silica gel column chromatography using chloroform as an eluent.

2-Cyanoethoxy-thio-dithiophospholane, 8

Starting from 3-hydroxypropionitrile (5.2 g, 5 mL, 0.07 mol) and 2-chloro-di-thiophospholane (10.9 g, 6.86 mL, 0.07 mol), product **8** was obtained as a colorless oil (10.5 g, 67% yield, 0.04 mol). ^{31}P NMR ($CDCl_3$): 123 (s) ppm. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.3 (m, 2H), 3.6 (s, 4H), 2.8 (m, 2H). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ: 116, 61, 41, 19 ppm. HRMS ESI (negative) m/z: calculated: 223.94329, found: 223.94358.

2-Cyanoethoxy-seleno-dithiophospholane, 9

Starting from 3-hydroxypropionitrile (5.2 g, 5 mL, 0.07 mol) and 2-chloro-di-thiophospholane (10.9 g, 6.86 mL, 0.07 mol), product **9** was obtained in 72% yield (13.8 g, 0.05 mol) as a colorless oil: ^{31}P NMR ($CDCl_3$): 106 (s) ppm. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.2 (m, 2H), 3.7 (s, 4H), 2.8 (m, 2H). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ: 116, 62, 43, 19 ppm. HRMS ESI (negative) m/z: calculated: 271.8876, found: 271.8875.

2-Cyanoethoxy-thio-1,3,2-dithiaphosphinane, 10

Starting from 3-hydroxypropionitrile (5.2 g, 5 mL, 0.07 mol) and 2-chloro-1,3,2-dithiaphosphorinane, (12.04 g, 0.07 mol), and following the above general procedure, product **10** was obtained in 90 % yield (14.9 g, 0.06 mol) as a white solid. ^{31}P NMR ($CDCl_3$): 85 (s) ppm. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.2 (m, 2H), 3.3 (m, 4H), 3.1(t, 2H), 2.4 (d, 1H), 1.9 (d, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ: 117, 57, 35, 25, 19 ppm. HRMS ESI (negative) m/z: calculated: 238.9876, found: 238.9869.

Nucleosides-2',3'-O,O-phosphorothioate (S*p*, exo), 1A and 12-15. General procedure

2-Cyanoethoxy-dithiophospholane, **8** (2.05 mmol), dry triethylamine (0.2 mL), and nucleoside (2.05 mmol) were suspended in dry acetonitrile (15 mL). Then, DBU (0.5 mL, 3 mmol) was added, the reaction was stirred for 1 min. Finally, the reaction mixture was cooled in an ice bath and 0.5 M TEAB (10 mL) was added dropwise and the reaction mixture was stirred for 20 min. The reaction solution was evaporated. The aqueous phase was freeze-dried and separated by ion-exchange liquid chromatography on a Sephadex® DEAE A-25 column with a linear gradient of 0-0.1 M NH_4HCO_3 buffer (pH 7.5). The product was further purified on RP18 (eluent: 0.5 M TEAA buffer (pH 7) and 10% CH_3CN).

Uridine-2',3'-O,O-phosphorothioate (S*p*, exo), 1A

2-Cyanoethoxy-dithiophospholane, **8** (460 mg, 2.05 mmol), dry triethylamine (0.2 mL), and uridine (500 mg, 2.05 mmol) were suspended in dry acetonitrile (15 mL). DBU (0.5 mL, 3 mmol) was then added. The product was obtained as the triethylammonium salt at 68 % overall yield (430 mg, 1.32 mmol). ^{1}H NMR (D_2O, 400 MHz) δ: 7.6 (d, 1H, J 10.8 Hz), 5.86 (s, 1H), 5.78 (d, 1H, J 10.8 Hz), 5.18 (t, 1H, J 6.9 Hz), 4.9 (q, 1H, J 4.6 Hz), 4.28 (s, 1H), 3.7 (m, 2H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 77.26 (s) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 168, 152, 146, 104, 94, 87, 84, 80, 63 ppm. HRMS ESI (negative) m/z: calculated: 323.9813, found: 323.9824. $[\alpha]_{589}^{22}$ (L 0.05 dm) -16.68 cm^2g^{-1}

Uridine-2',3'-O,O-phosphoroselenoate (*Sp*, exo), 11

2-Cyanoethoxy-seleno-thiophospholane, **9** (480 mg, 2.05 mmol), dry triethylamine (0.2 mL), and uridine (500 mg, 2.05 mmol) were suspended in dry acetonitrle (15 mL). DBU (0.5 ml, 3 mmol) was then added to the reaction solution. The reaction mixture was stirred for 1 min. Finally, the reaction mixture was cooled in an ice bath and 0.5 M TEAB (10 mL) was added dropwise and the reaction mixture was stirred for 20 min. The reaction solution was evaporated. The residue was freeze dried and separated by liquid chromatography on a Sephadex® DEAE A-25 column with a linear gradient of 0- 0.1 M NH_4HCO_3 buffer (pH 7.5), followed by separation on RP18 (eluent: 0.5 M TEAA buffer (pH 7) and 10% acetonitryl). The product was obtained as the triethylammonium salt at 61 % overall yield (459 mg, 1.24 mmol). ^{1}H NMR (D_2O, 400 MHz) δ: 7.6 (d, 1H, J 10.8 Hz), 5.8 (s, 1H), 5.78 (d, 1H, J 10.8Hz), 5.2 (t, 1H, J 6.3 Hz), 4.9 (q, 1H, J 4.1 Hz), 4.3 (s, 1H), 3.8 (m, 2H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 66.9 (s) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 167, 152, 145, 103, 94, 86, 83, 79, 62 ppm. HRMS ESI (negative) m/z: calculated: 368.9352, found: 368.9391, $[\alpha]_{589}^{22}$ (L 0.05 dm) -51.41 cm^2g^{-1}

Adenosine-2',3'-O,O-phosphorothioate (S*p*, exo), 12

2-Cyanoethoxy-dithiophospholane, **8** (460 mg, 2.05 mmol), dry triethylamine (0.2 mL), and adenosine (0.54 g, 2.05 mmol) were suspended in dry acetonitrile (15 mL). DBU (0.5 mL, 3 mmol) was then added. The product was obtained as the triethylammonium salt at 62 % overall yield (438 mg, 1.26 mmol). ^{1}H NMR (D_2O, 400 MHz) δ: 8.34 (s, 1H,), 8.26 (s, 1H), 6.34 (s, 1H), 5.54 (t, 1H), 5.24 (t, 1H), 4.5 (q, 2H), 3.9 (q, 2H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 77.34 (s) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 157, 154, 149,142, 120, 90, 86, 82, 80, 62 ppm. HRMS ESI (negative) m/z: calculated: 345.0296, found: 345.0290. $[\alpha]_{589}^{22}$ (L 0.05 dm) -38.8 cm^2g^{-1}

Thymidine-2',3'-O,O-phosphorothioate (S*p*, exo), 13

2-Cyanoethoxy-dithiophospholane, **8** (460 mg, 2.05 mmol), dry triethylamine (0.2 mL), and thymidine (520mg, 2.05 mmol) were suspended in dry acetonitrile (15 mL). DBU (0.5 mL, 3 mmol) was then added. The product was obtained as the triethylammonium salt at 68 % overall yield (468 mg, 1.39 mmol). ^{1}H NMR (D_2O, 400 MHz) δ: 7.32 (s, 1H), 5.82 (s, 1H), 5.1 (t, 1H), 4.8 (q, 1H), 4.18 (s, 1H), 3.7 (m, 2H), 1.7 (s, 3H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 77.23 (s) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 182, 175, 158, 140, 113, 93, 86, 83, 82, 79, 62, 13 ppm. HRMS ESI (negative) m/z: calculated: 336.0181, found: 336.0179. $[\alpha]_{589}^{22}$ (L 0.05 dm) -9.132 cm^2g^{-1}

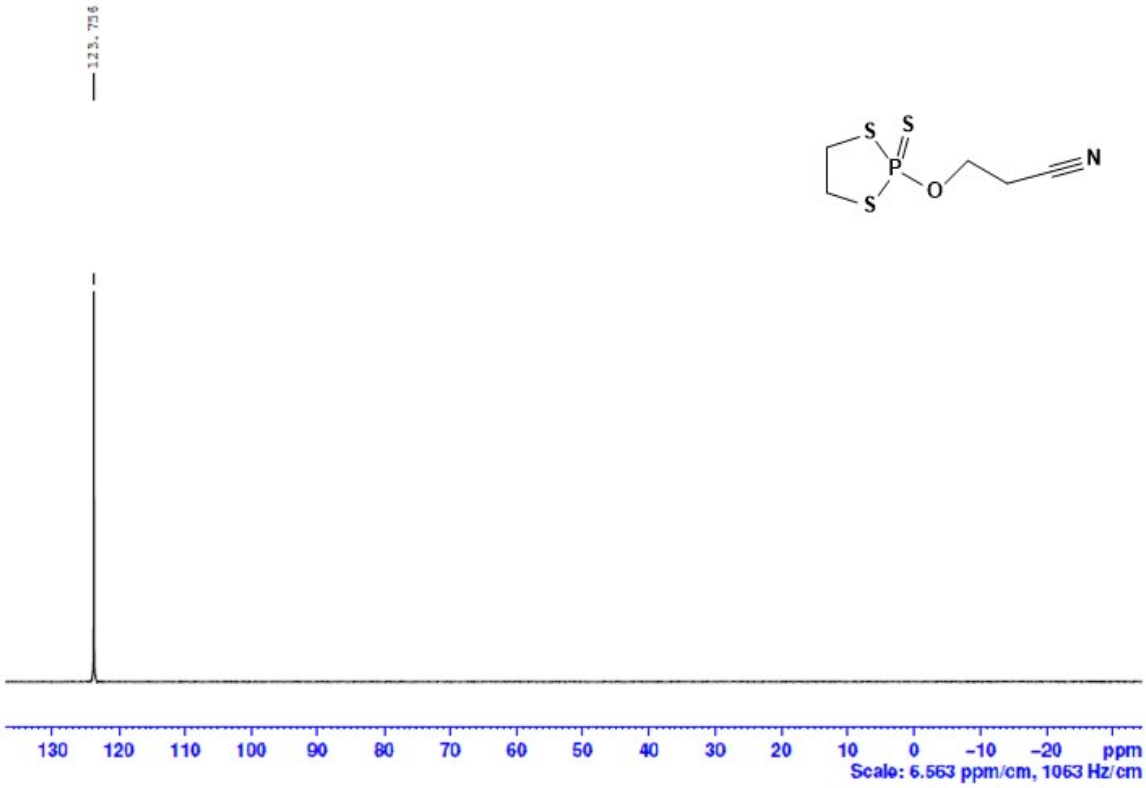
Cytosine-2',3'-O,O-phosphorothioate (S*p*, exo), 14

2-Cyanoethoxy-dithiophospholane, **8** (460 mg, 2.05 mmol), dry triethylamine (0.2 mL), and cytidine (490 mg, 2.05 mmol) were suspended in dry acetonitrile (15 mL). DBU (0.5 mL, 3 mmol) was then added. The product was obtained as the triethylammonium salt at 50 % overall yield (329 mg, 1.02 mmol). ^{1}H NMR (D_2O, 400 MHz) δ: 7.61 (s, 1H), 5.95 (s, 1H), 5.84 (s, 1H), 5.17 (t, 1H), 4.97 (q, 1H), 4.38 (s, 1H), 3.7 (m, 2H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 77.9 (s) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 168, 158, 145, 97, 95, 86, 83, 80, 62, 42 ppm. HRMS ESI (negative) m/z: calculated: 321.0184, found: 321.0193. $[\alpha]_{589}^{22}$ (L 0.05 dm) -45.83 cm^2g^{-1}

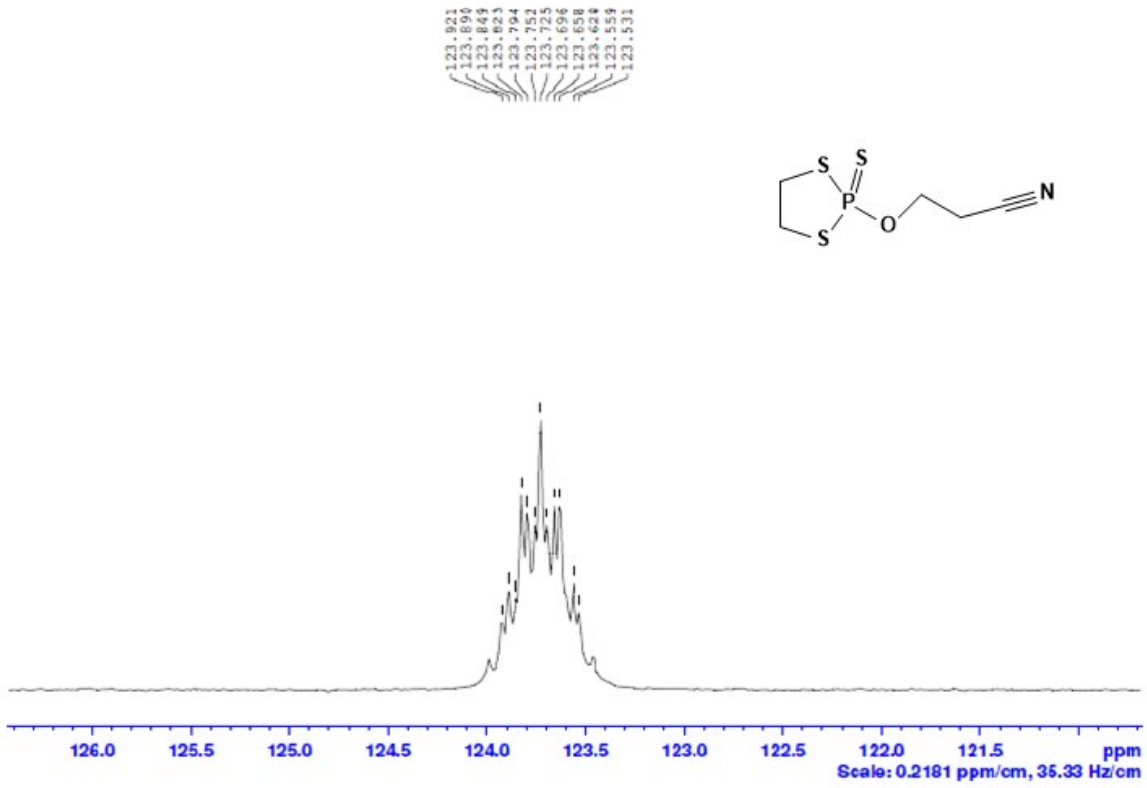
Guanosine-2',3'-O,O-phosphorothioate (S*p*, exo), 15

2-Cyanoethoxy-dithiophospholane, **8** (460 mg, 2.05 mmol), dry triethylamine (0.2 mL), and guanosine (580 mg, 2.05 mmol) were suspended in dry acetonitrile (15 mL). DBU (0.5 mL, 3 mmol) was then added. The product was obtained as the triethylammonium salt at 20 % overall yield (148 mg, 4.09 mmol). ^{1}H NMR (D_2O, 400 MHz) δ: 8.48 (s, 1H), 6.07 (s, 1H), 5.54 (s, 1H), 5.22 (t, 1H), 4.55 (q, 1H), 4.96 (s, 1H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 77.9 (s) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 168, 158, 145, 97, 95, 86, 83, 80, 62, 42 ppm. HRMS ESI (negative) m/z: calculated: 361.0245, found: 361.0240. $[\alpha]_{589}^{22}$ (L 0.05 dm) -29.8 cm^2g^{-1}

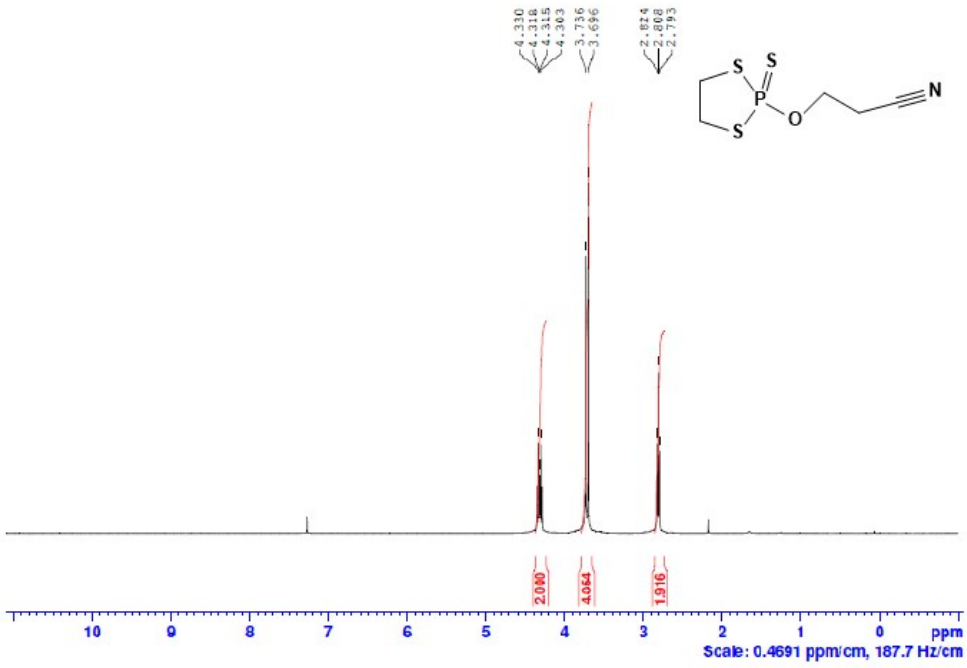
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of 2-cyanoethoxy-dithiophospholane, 8



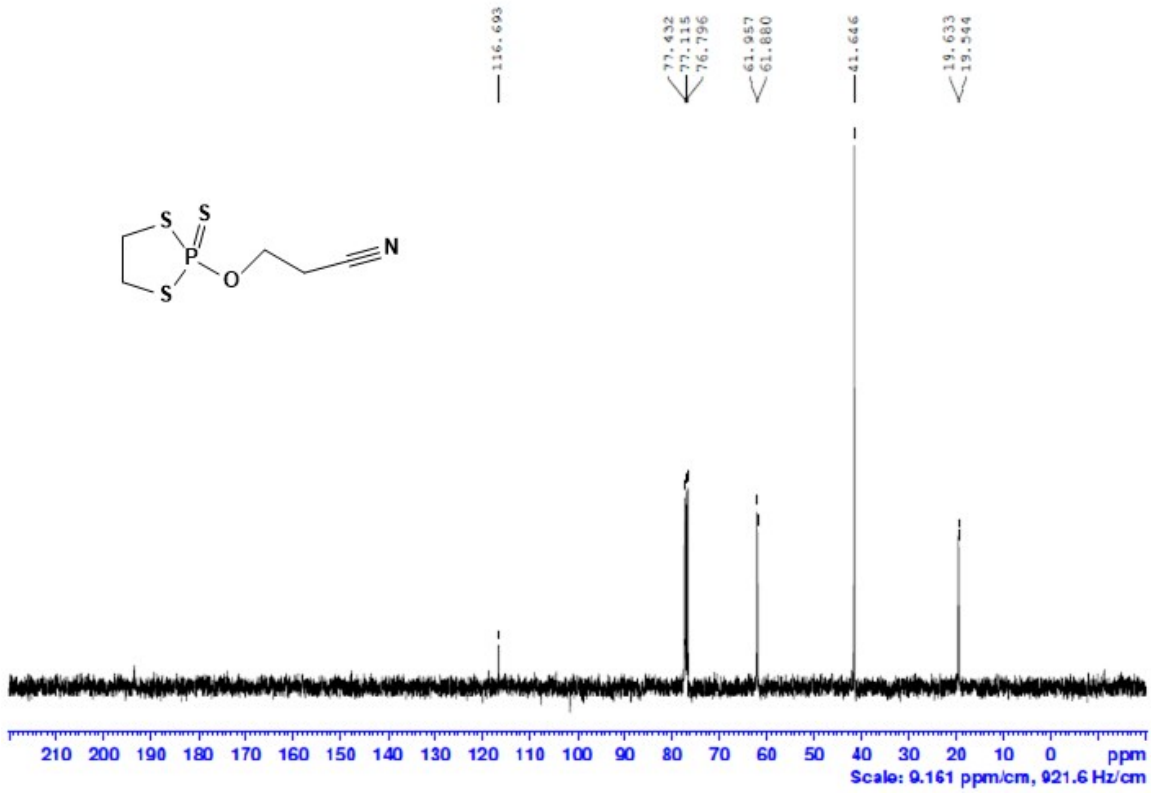
^{31}P NMR ($CDCl_3$, 161.96 MHz)



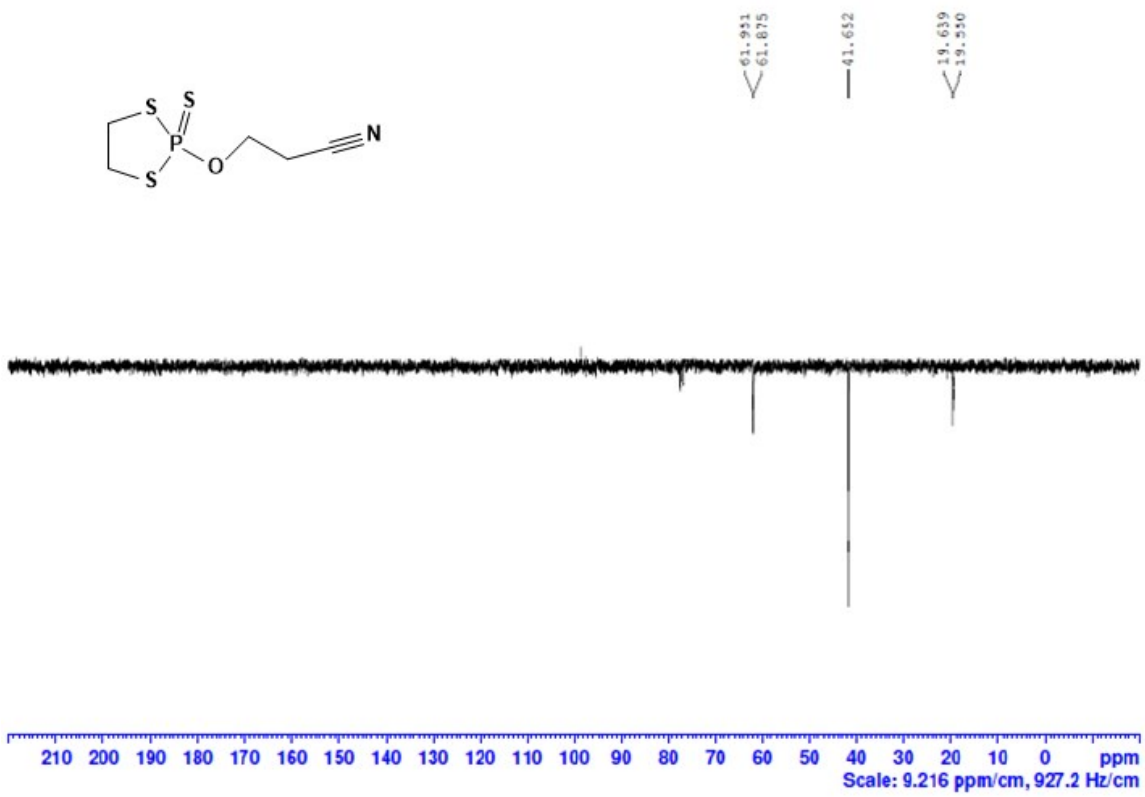
^{31}P NMR ($CDCl_3$, 161.96 MHz)



^{1}H NMR ($CDCl_3$, 400 MHz)

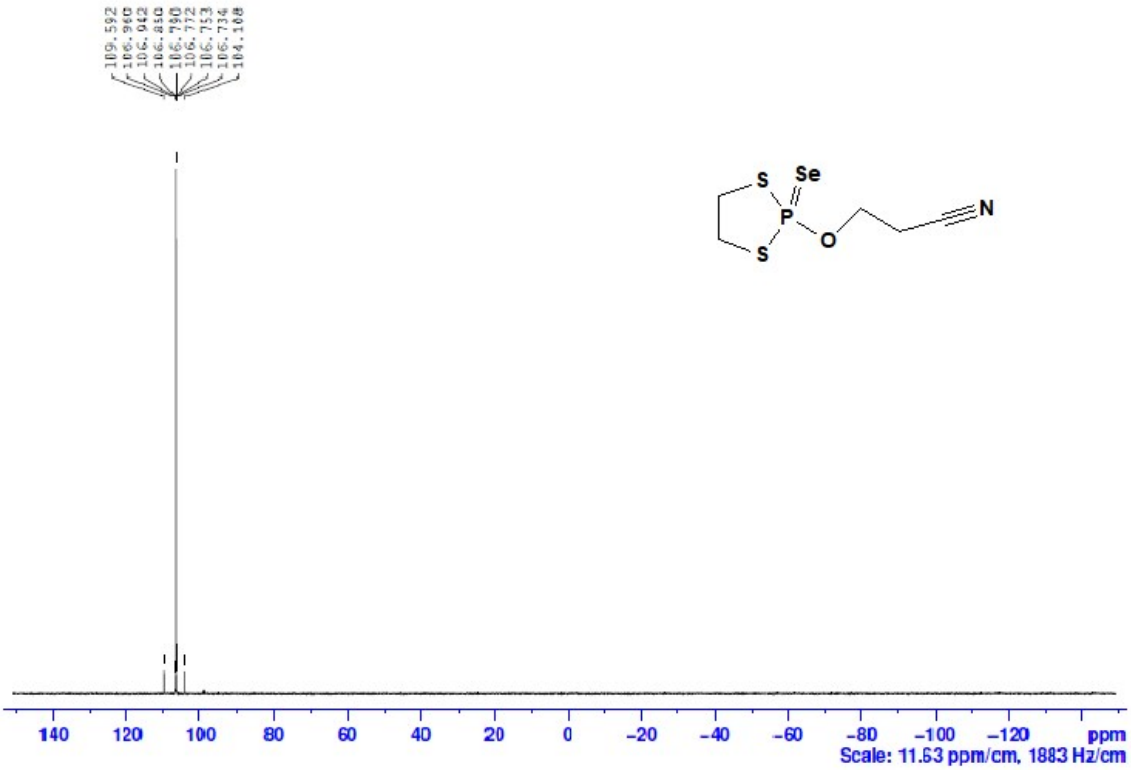


^{13}C NMR ($CDCl_3$, 100.61 MHz).

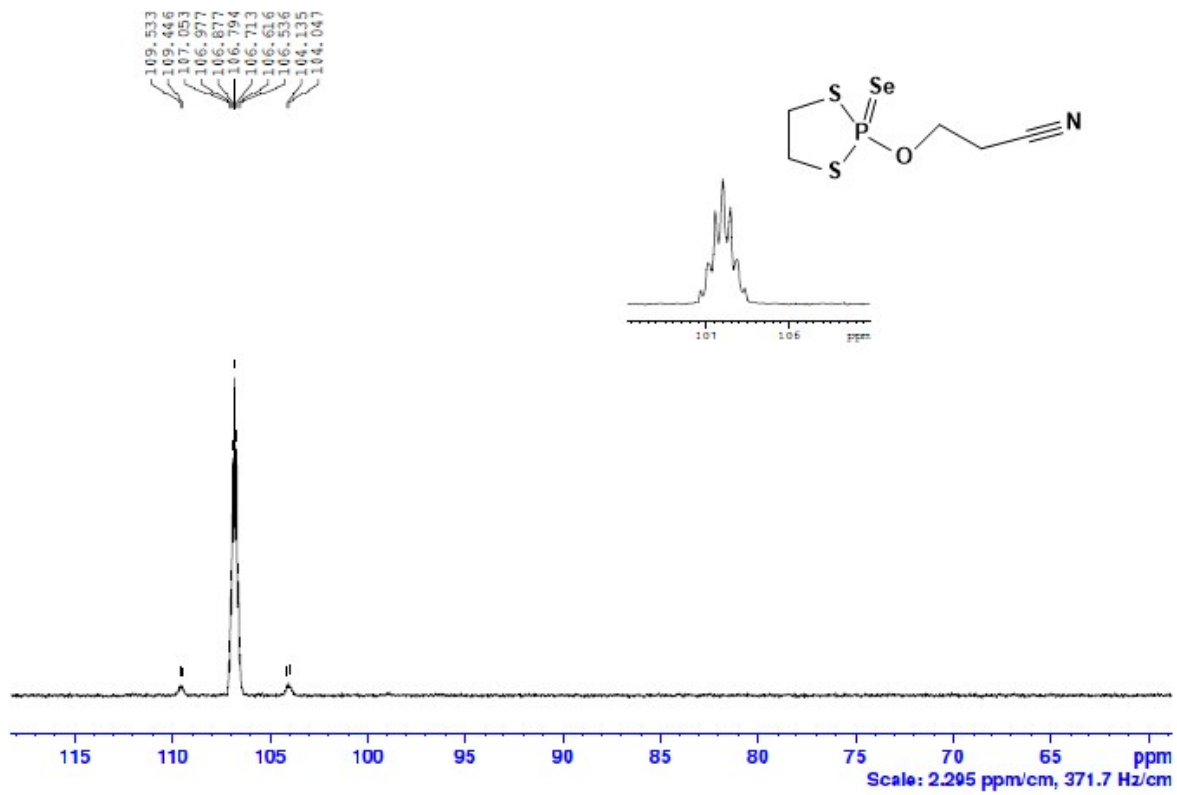


^{13}C NMR ($CDCl_3$, 100.61 MHz).

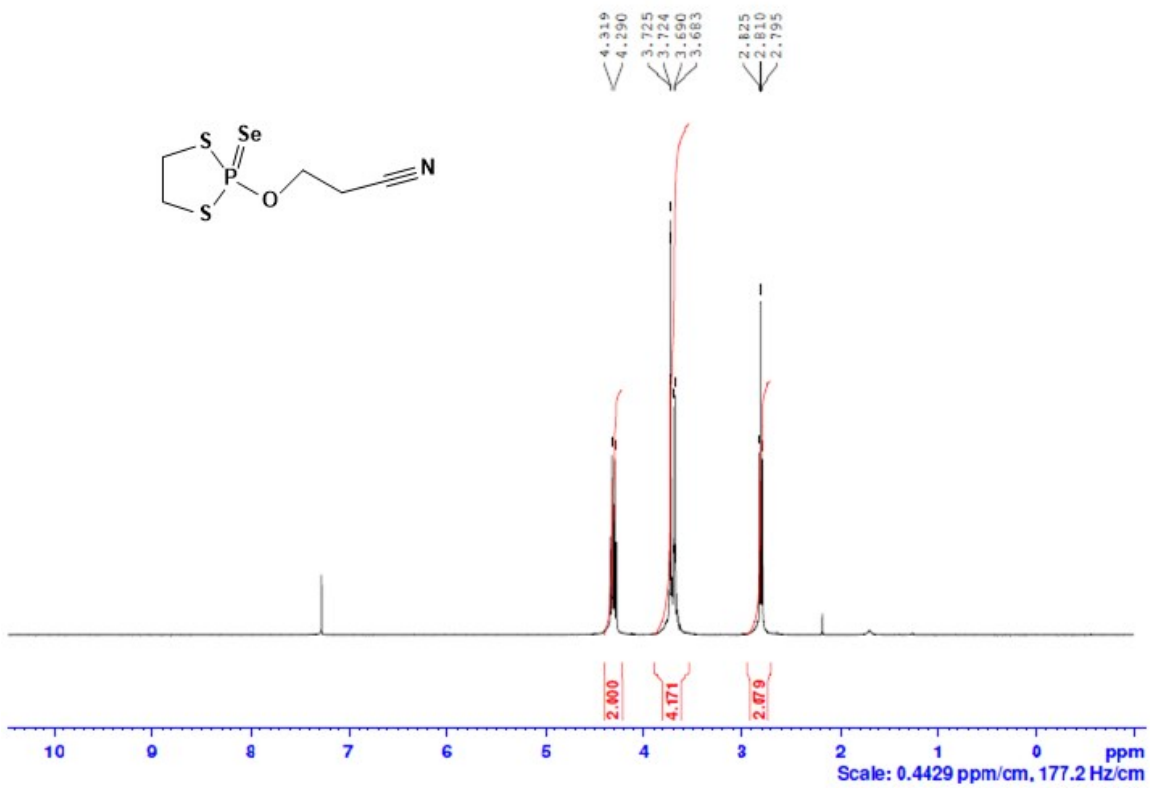
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of 2-Cyanoethoxy-seleno-thiophospholane, 9



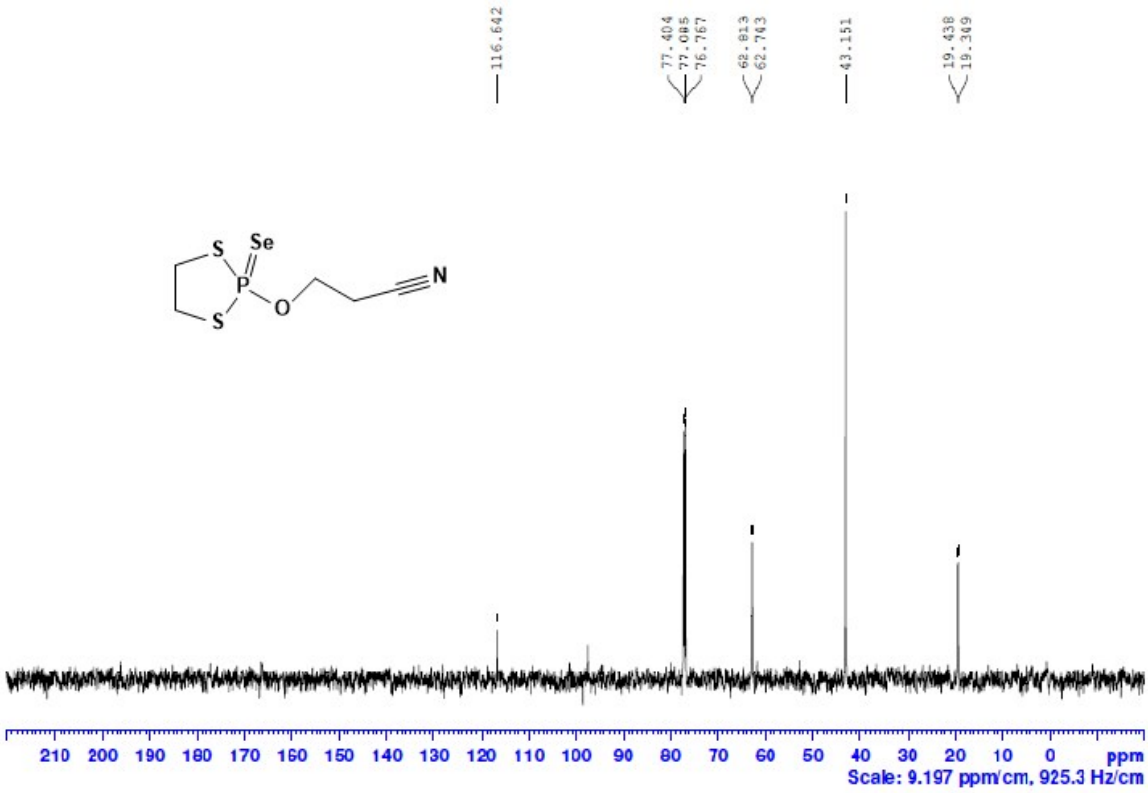
^{31}P NMR ($CDCl_3$, 161.96 MHz)



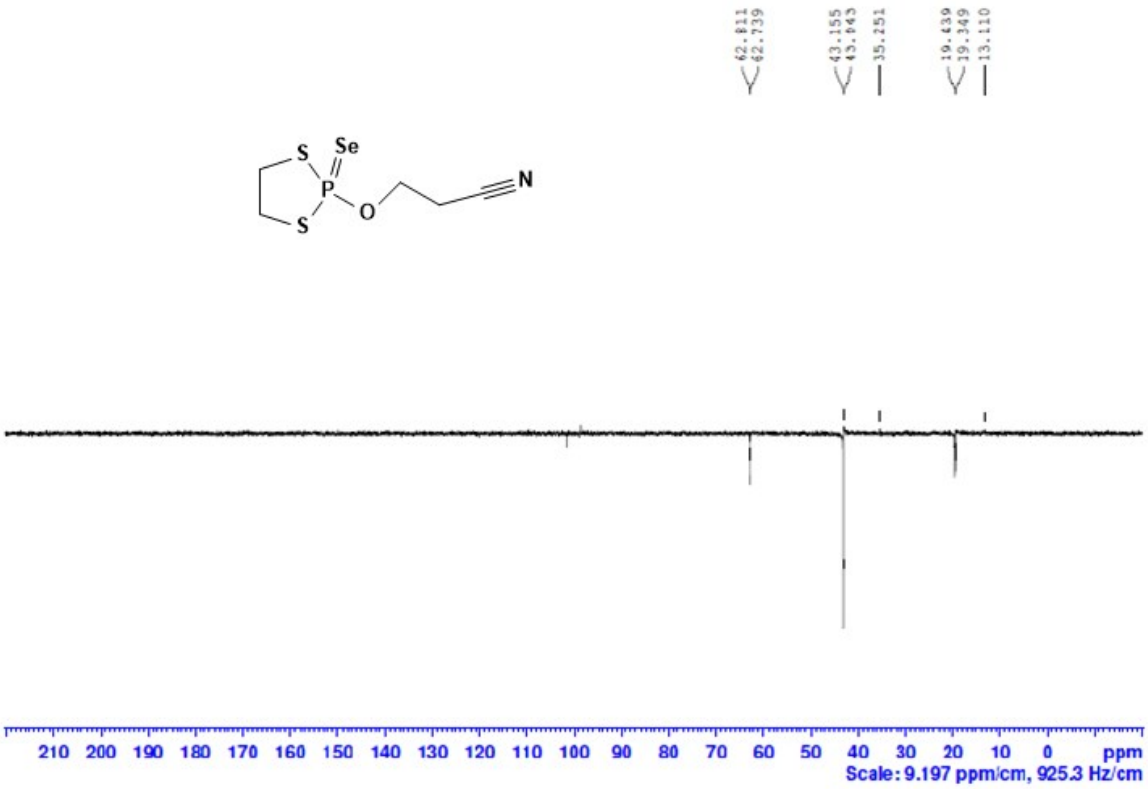
^{31}P NMR ($CDCl_3$, 161.96 MHz)



^{1}H NMR ($CDCl_3$, 400 MHz)

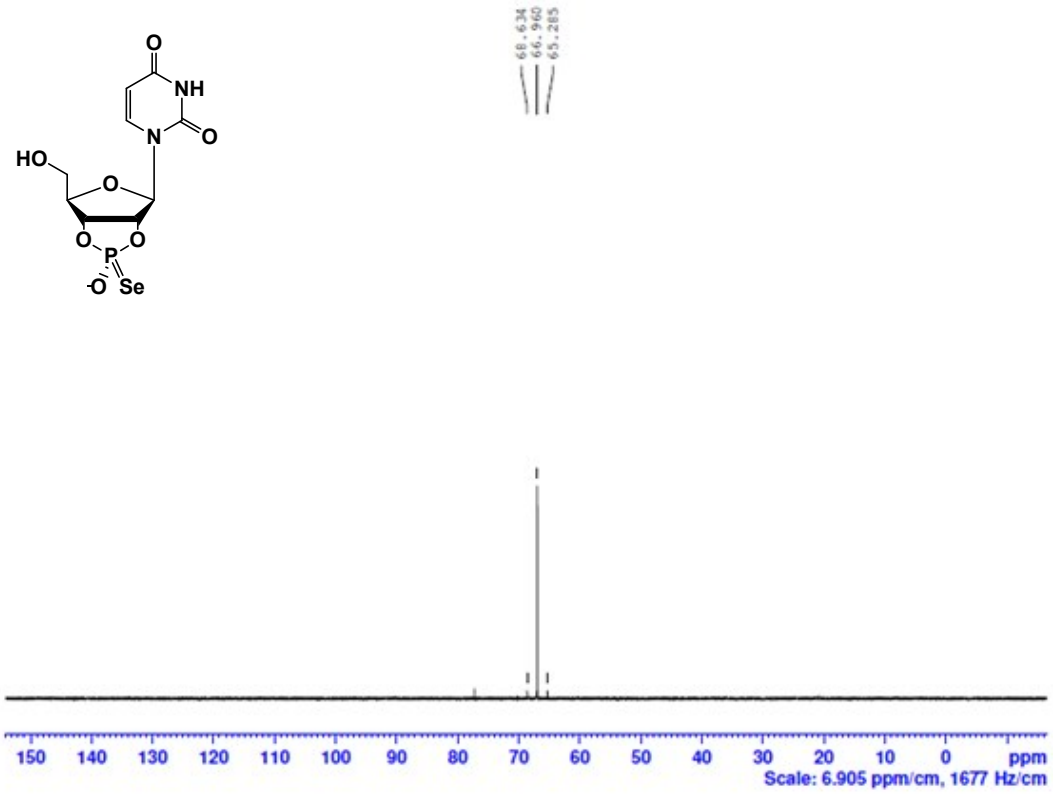


^{13}C NMR ($CDCl_3$, 100.61 MHz).

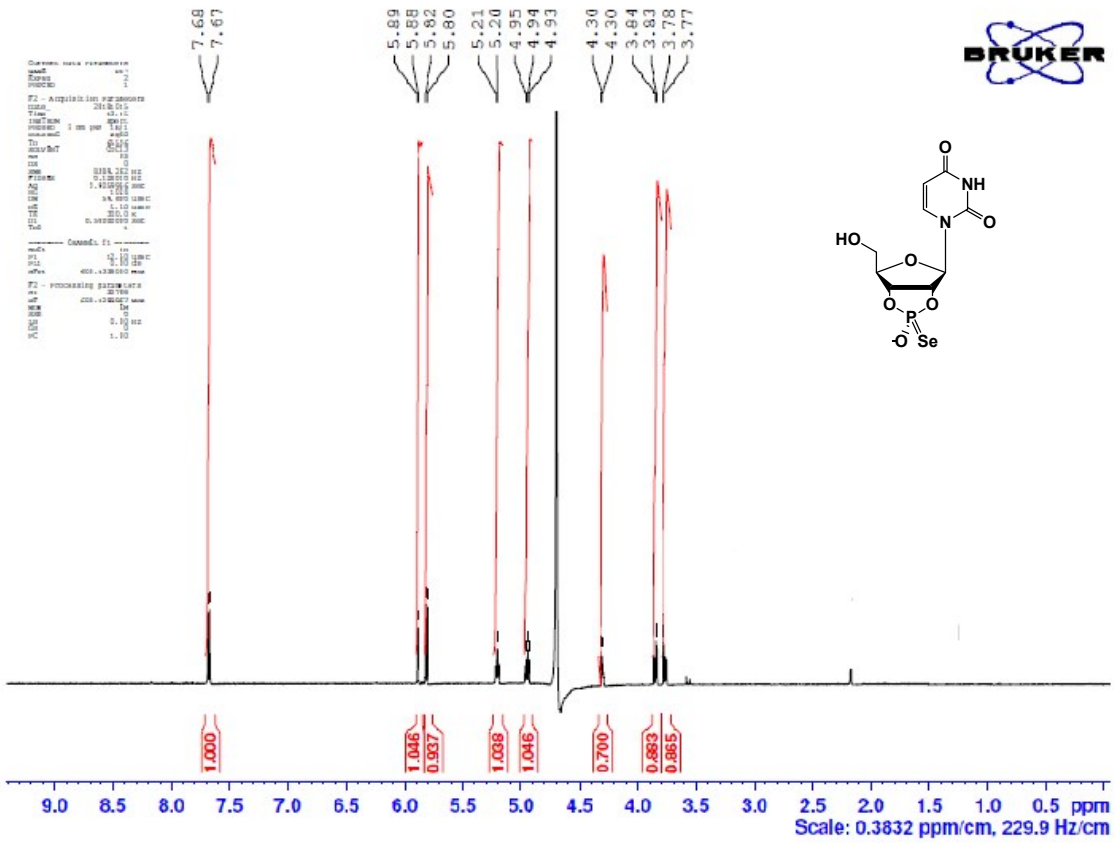


^{13}C NMR ($CDCl_3$, 100.61 MHz).

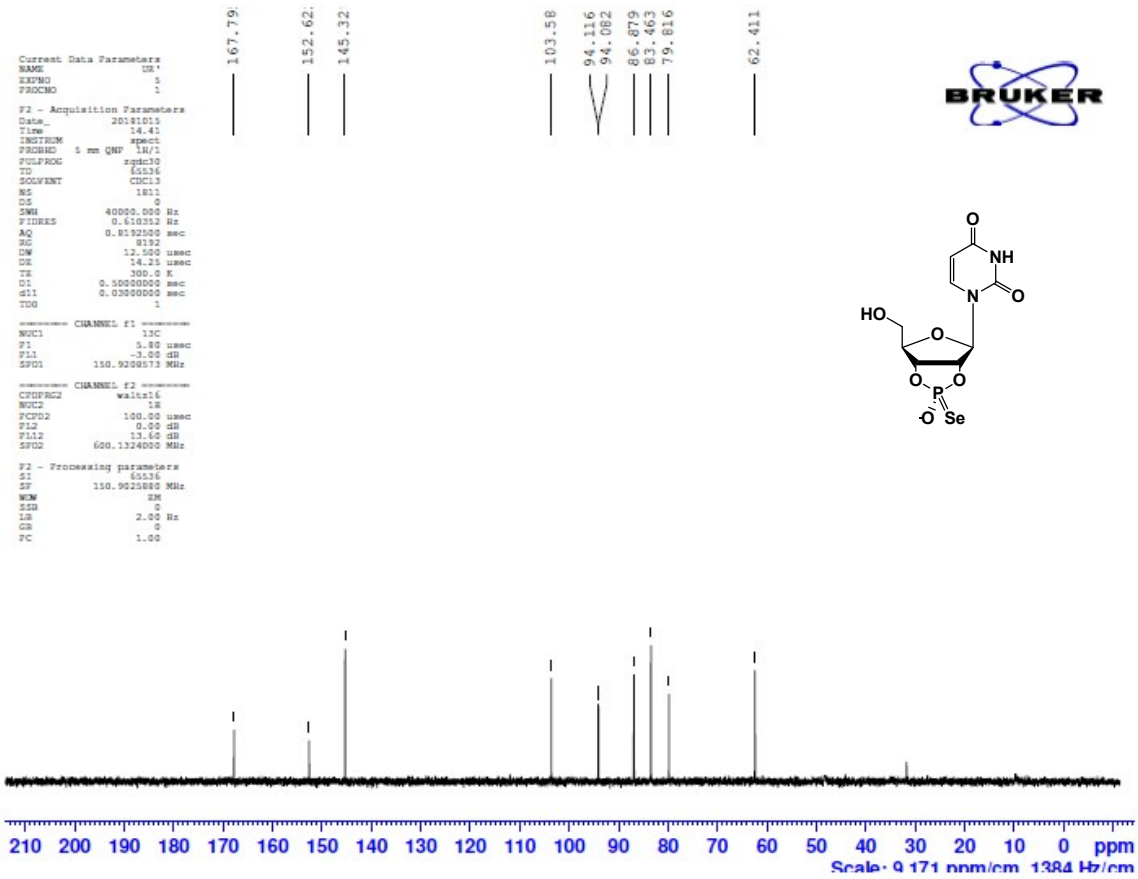
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of Uridine-2',3'-O,O-phosphoroselenoate (*Sp*, exo) **11**.



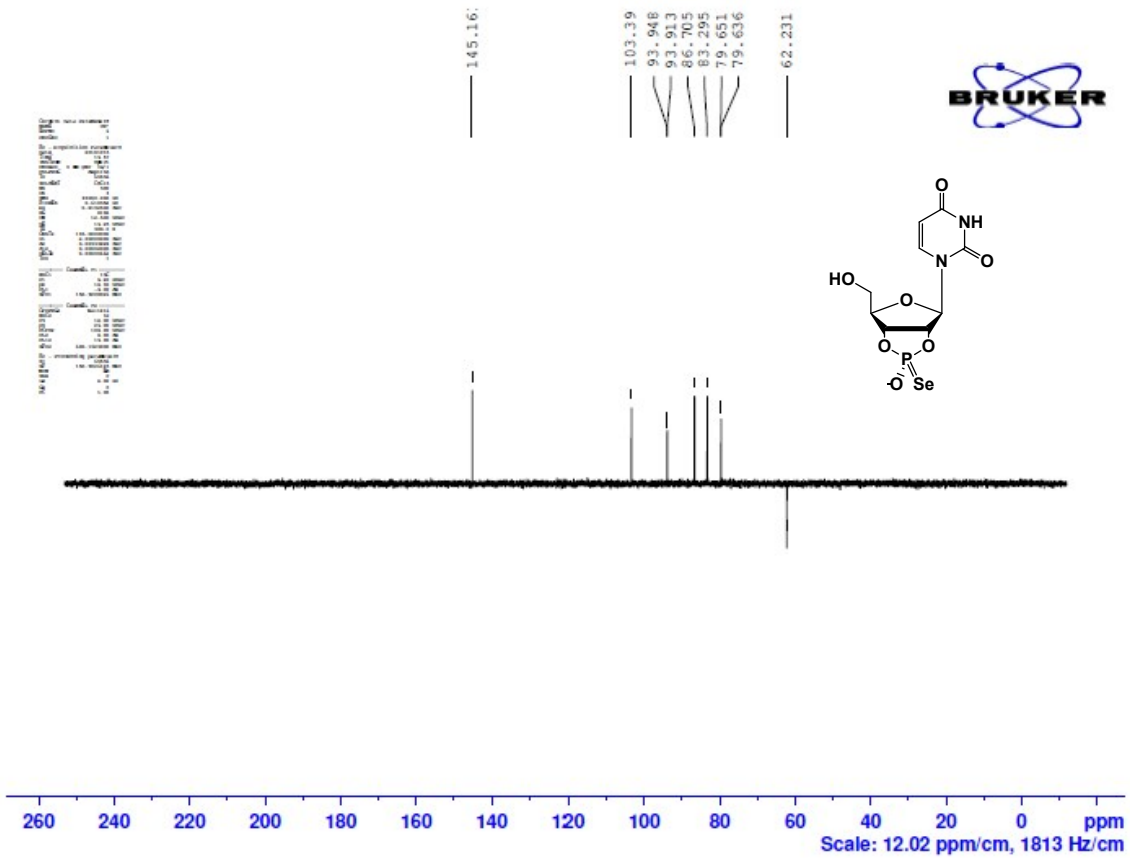
^{31}P NMR (D_2O, 161.96 MHz).



^{1}H NMR (D_2O, 400 MHz)

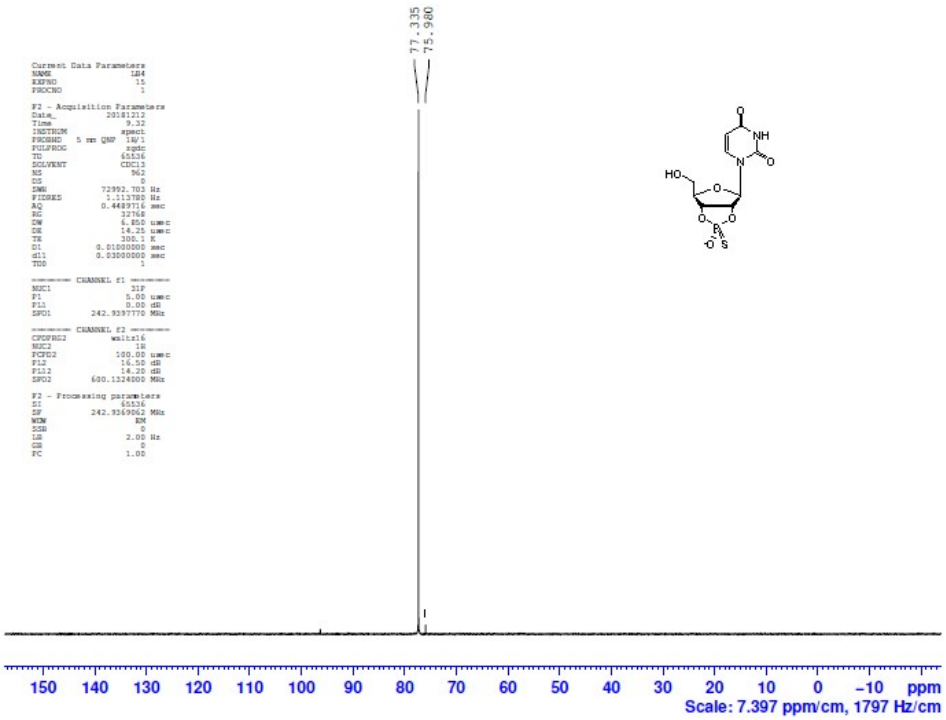


^{13}C NMR (D_2O, 100.61 MHz).

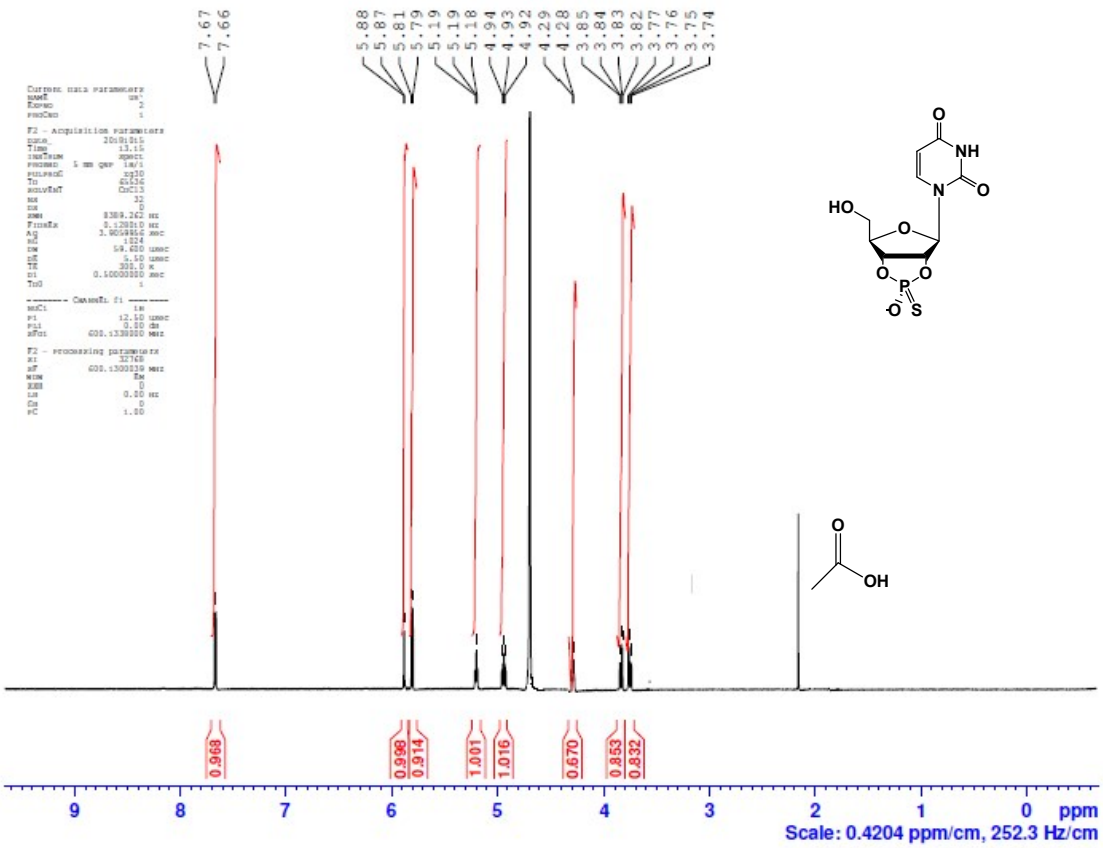


^{13}C NMR (D_2O, 100.61 MHz).

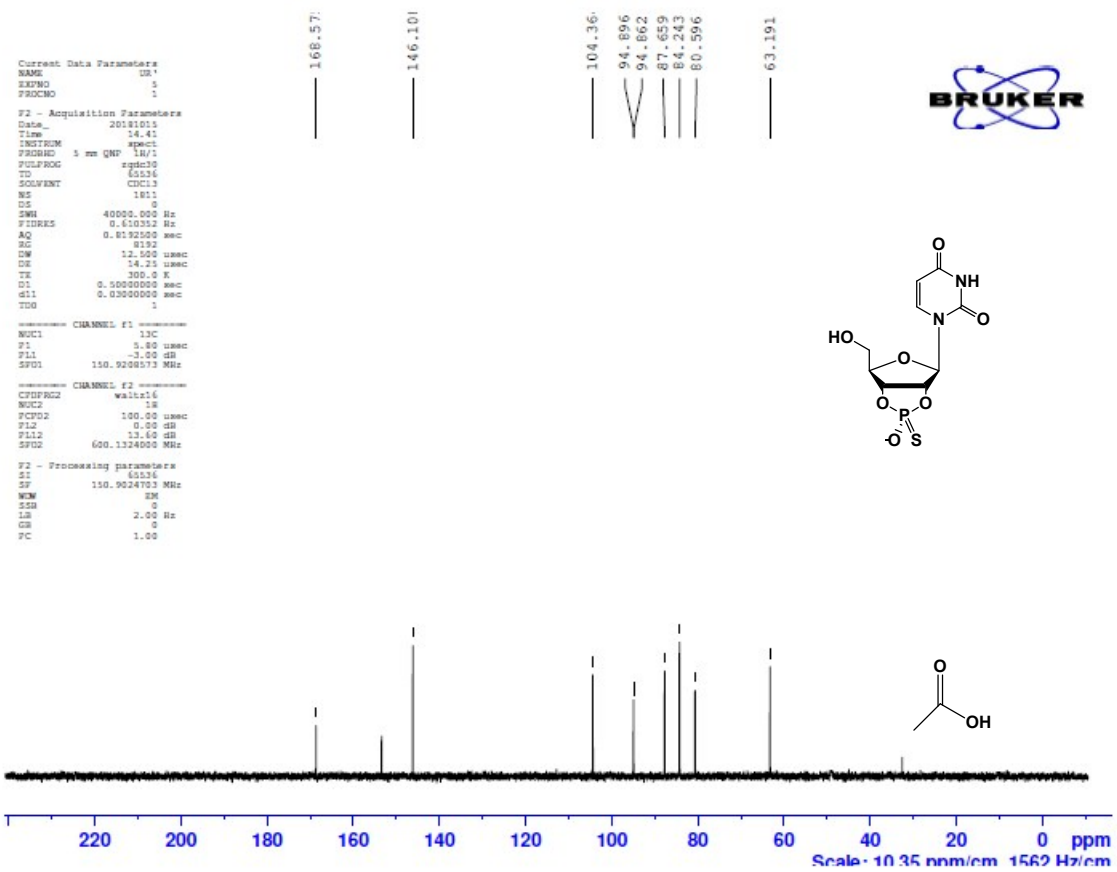
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of Uridine-2',3'-O,O-phosphorothioate (S*p*, exo), **10**



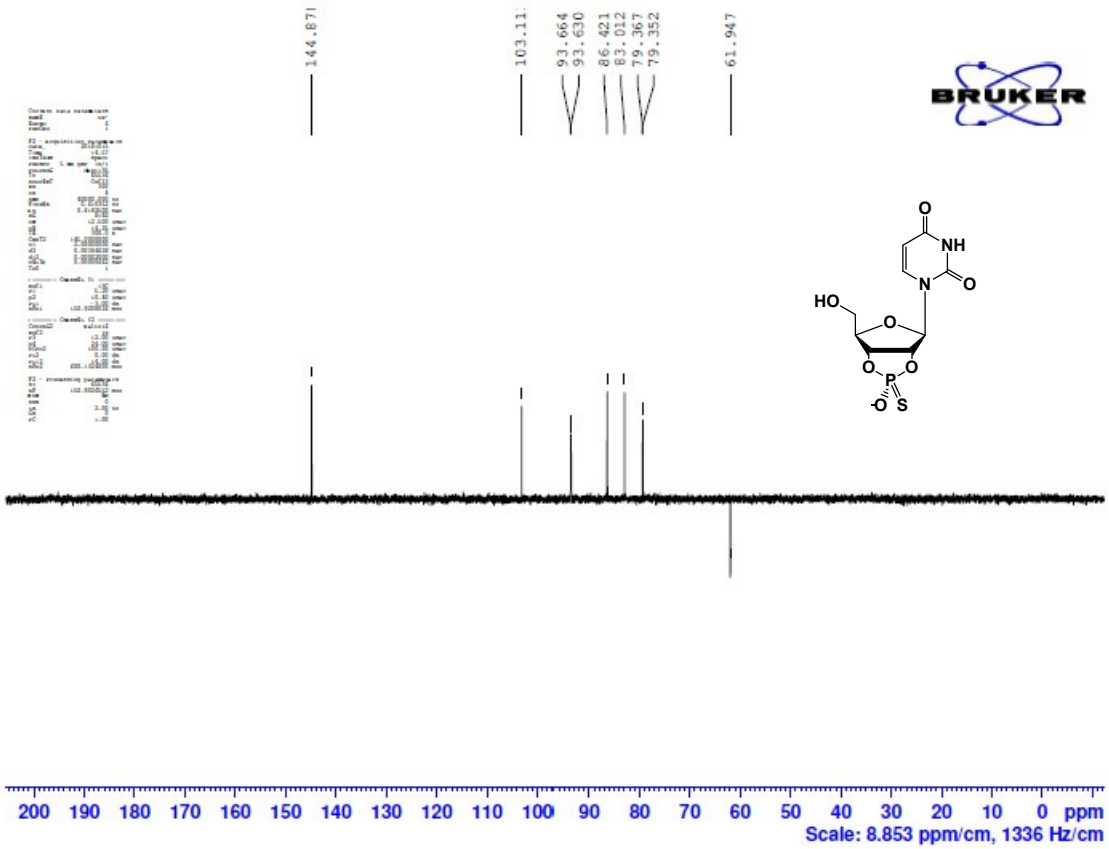
^{31}P NMR (D_2O, 161.96 MHz)



^{1}H NMR (D_2O, 400 MHz).

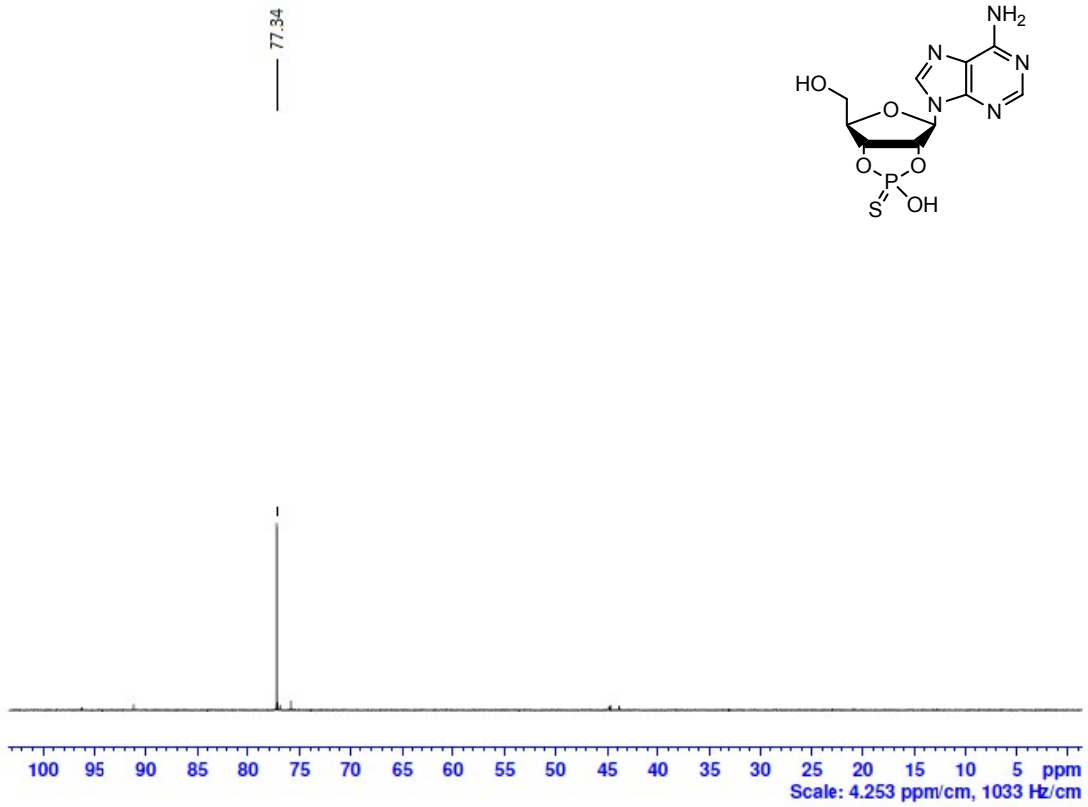


^{13}C NMR (D_2O, 100.61 MHz).

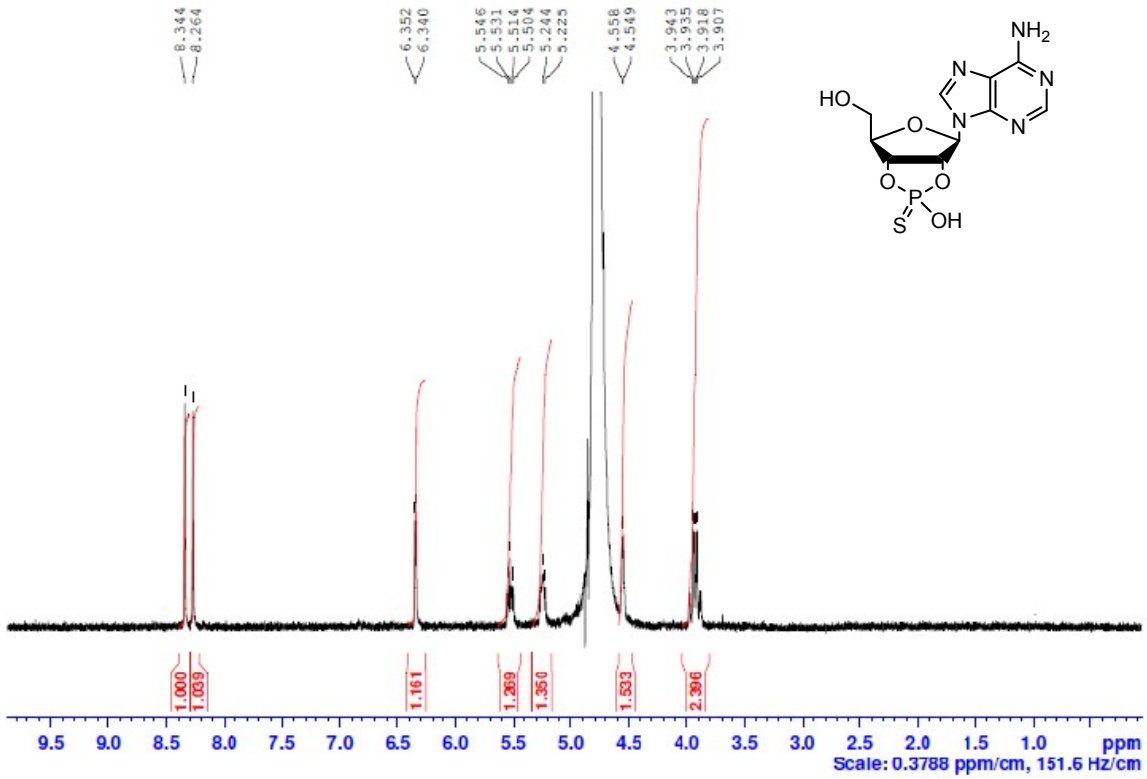


^{13}C NMR (D_2O, 100.61 MHz).

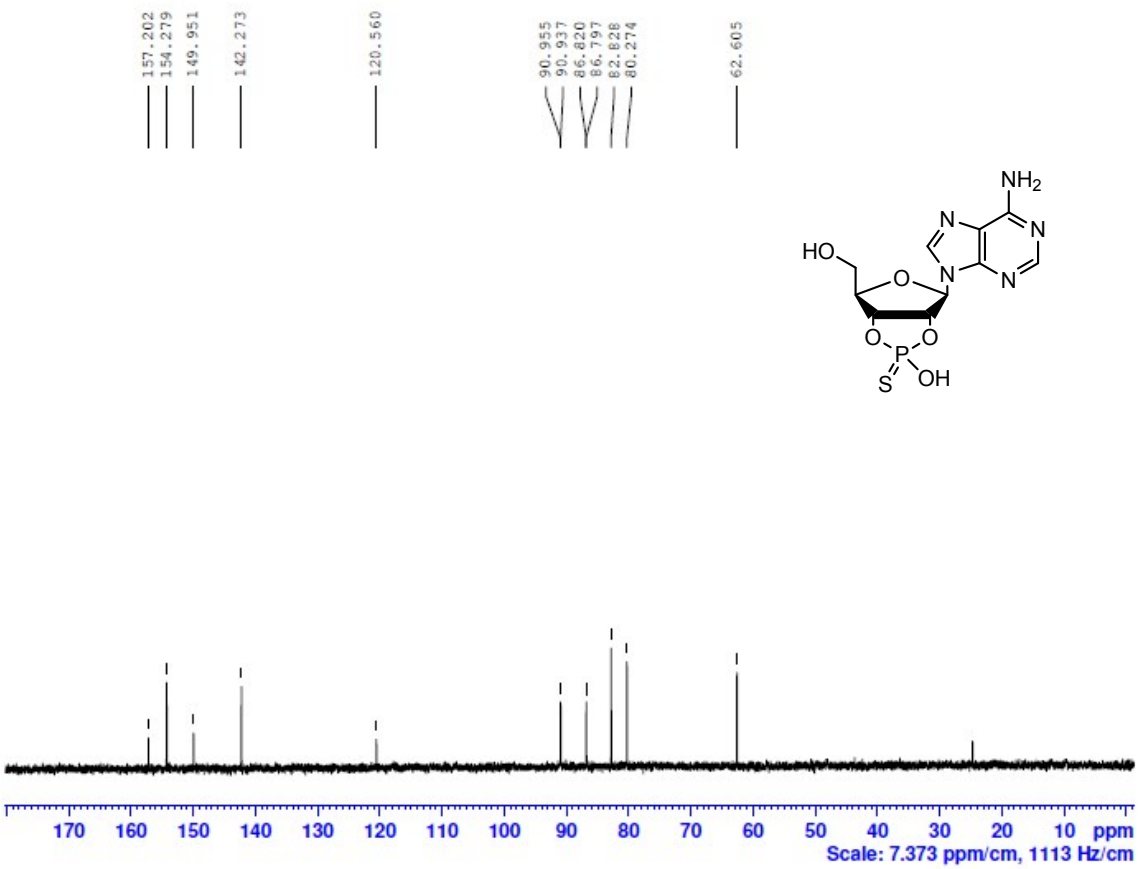
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of adenine-2',3'-O,O-phosphorothioate (S*p*, exo), 12.



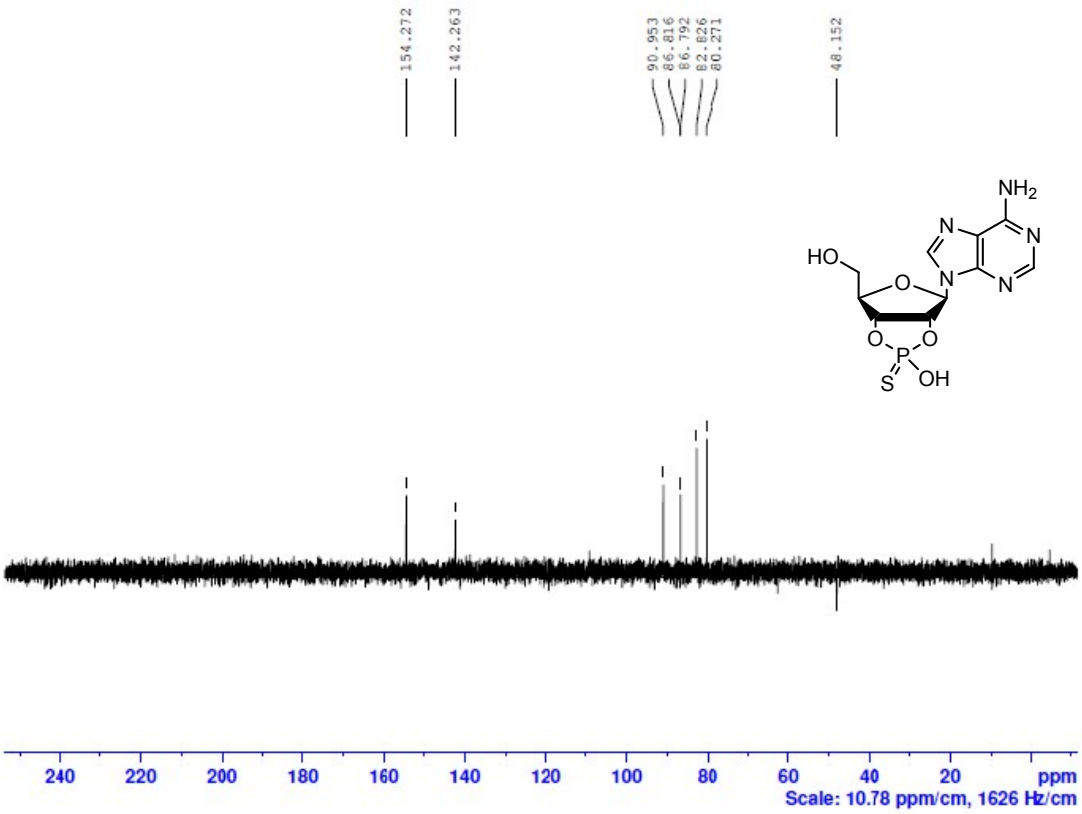
^{31}P NMR (D_2O, 161.96 MHz)



^{1}H NMR (D_2O, 400 MHz)

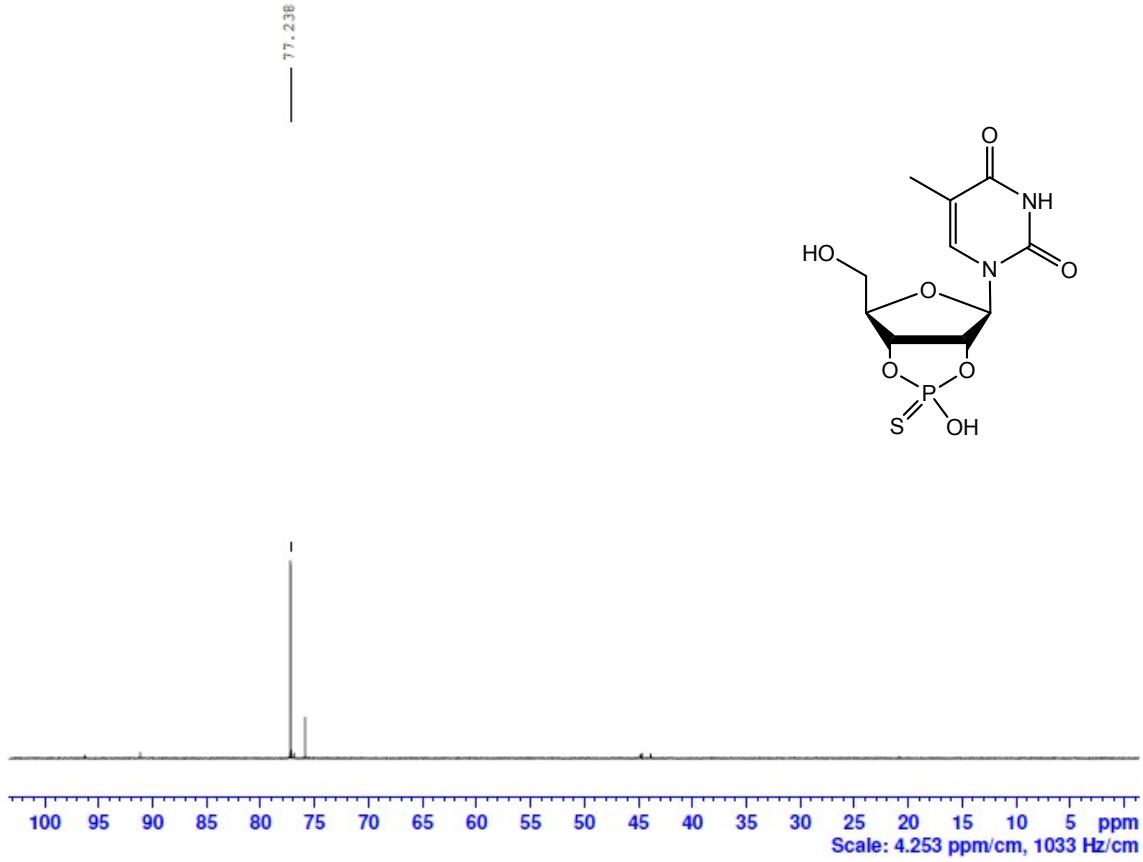


^{13}C NMR (D_2O, 100.61 MHz).

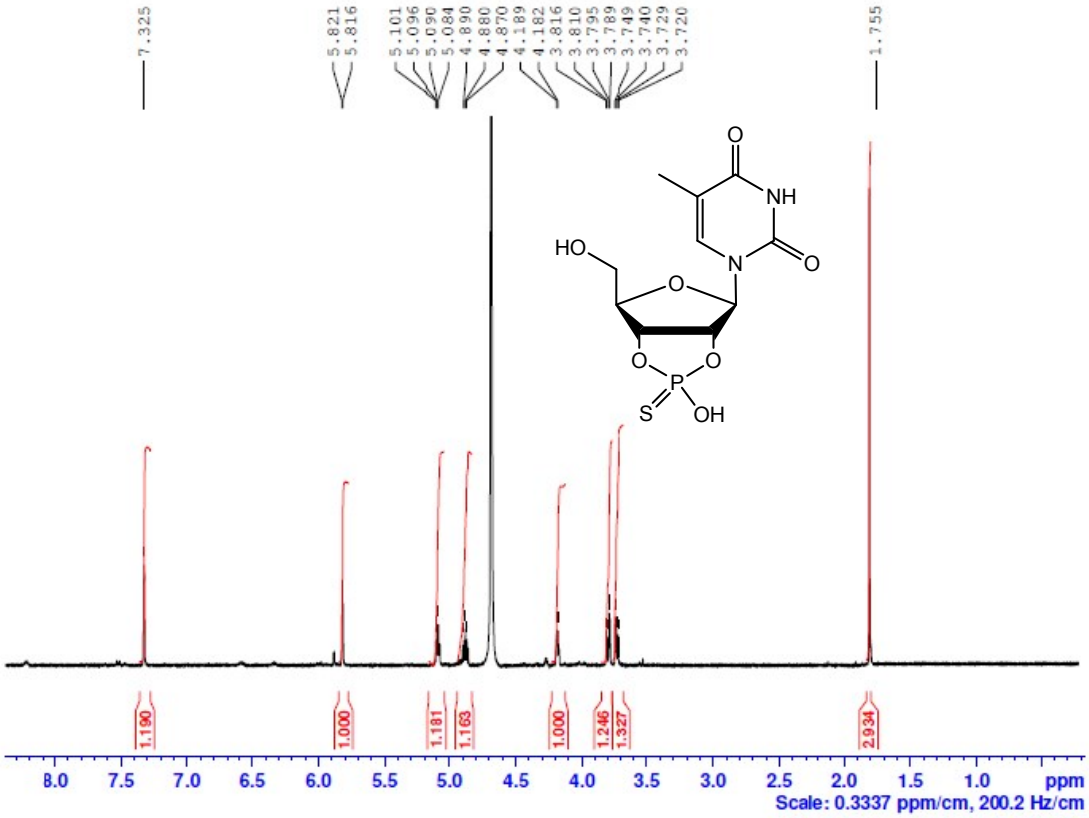


^{13}C NMR (D_2O, 100.61 MHz).

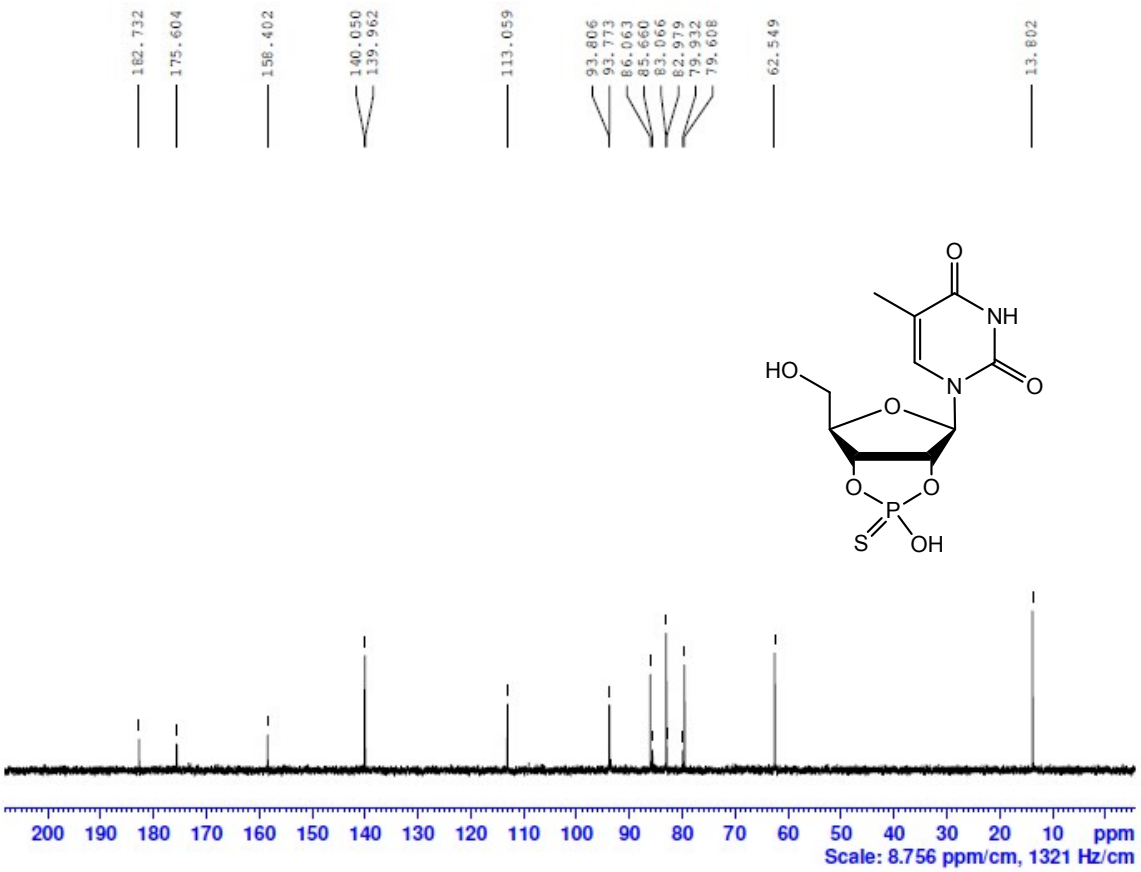
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of thymine-2',3'-O,O-phosphorothioate (S*p*, exo), **13**.



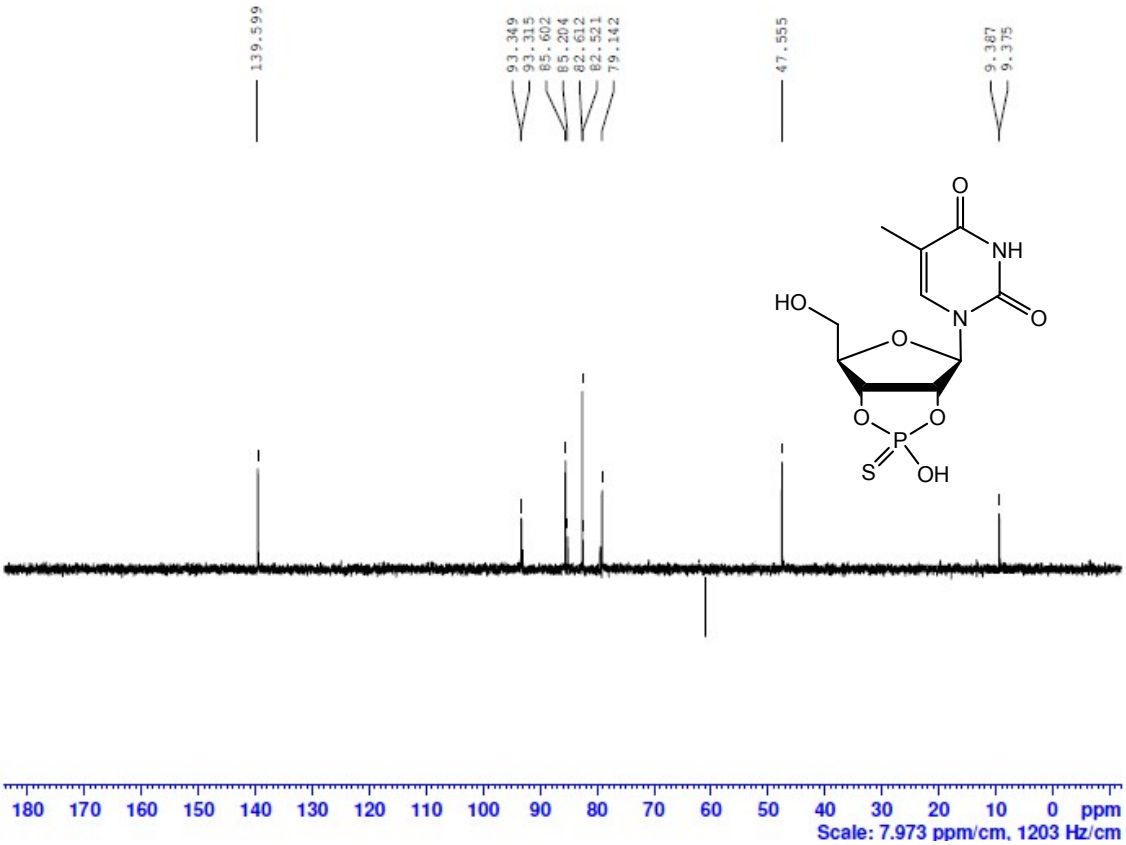
^{31}P NMR (D_2O, 161.96 MHz)



^{1}H NMR (D_2O, 400 MHz)

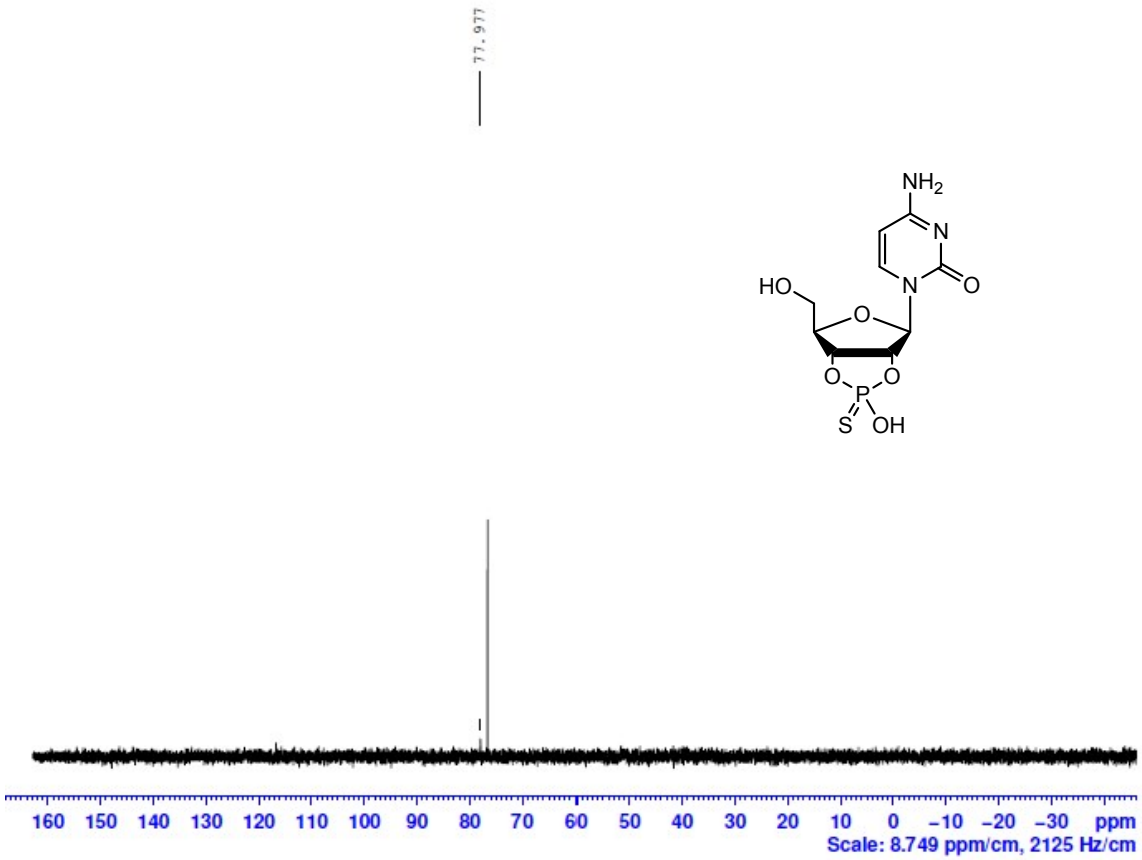


^{13}C NMR (D_2O, 100.61 MHz).

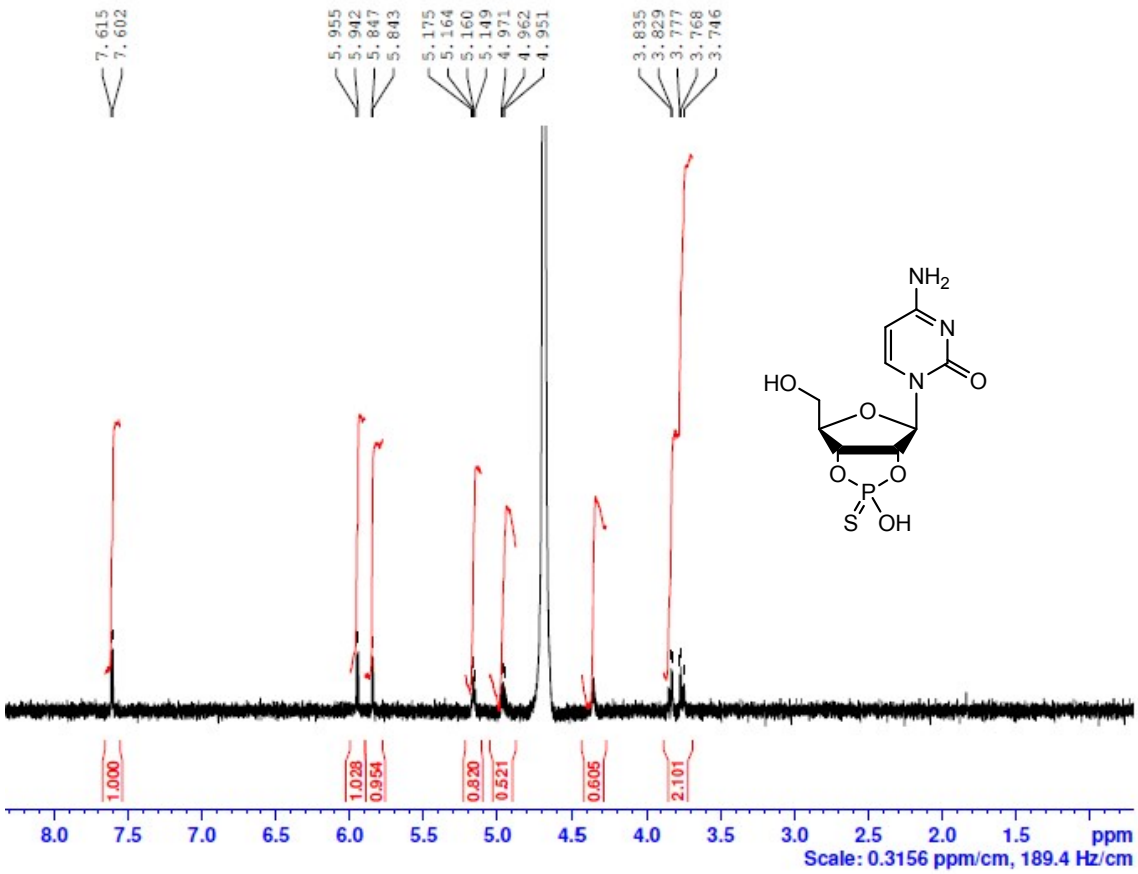


^{13}C NMR (D_2O, 100.61 MHz).

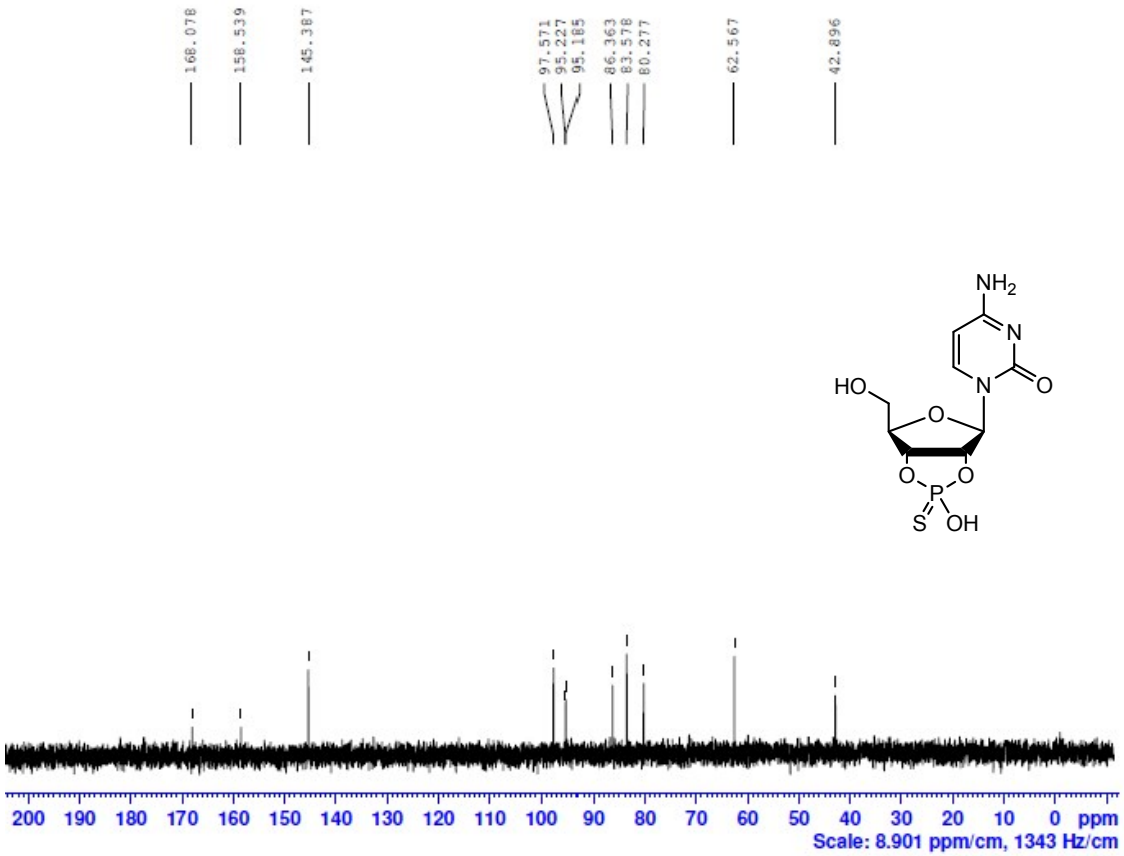
$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of cytosine-2',3'-O,O-phosphorothioate (S*p*, exo), **14**.



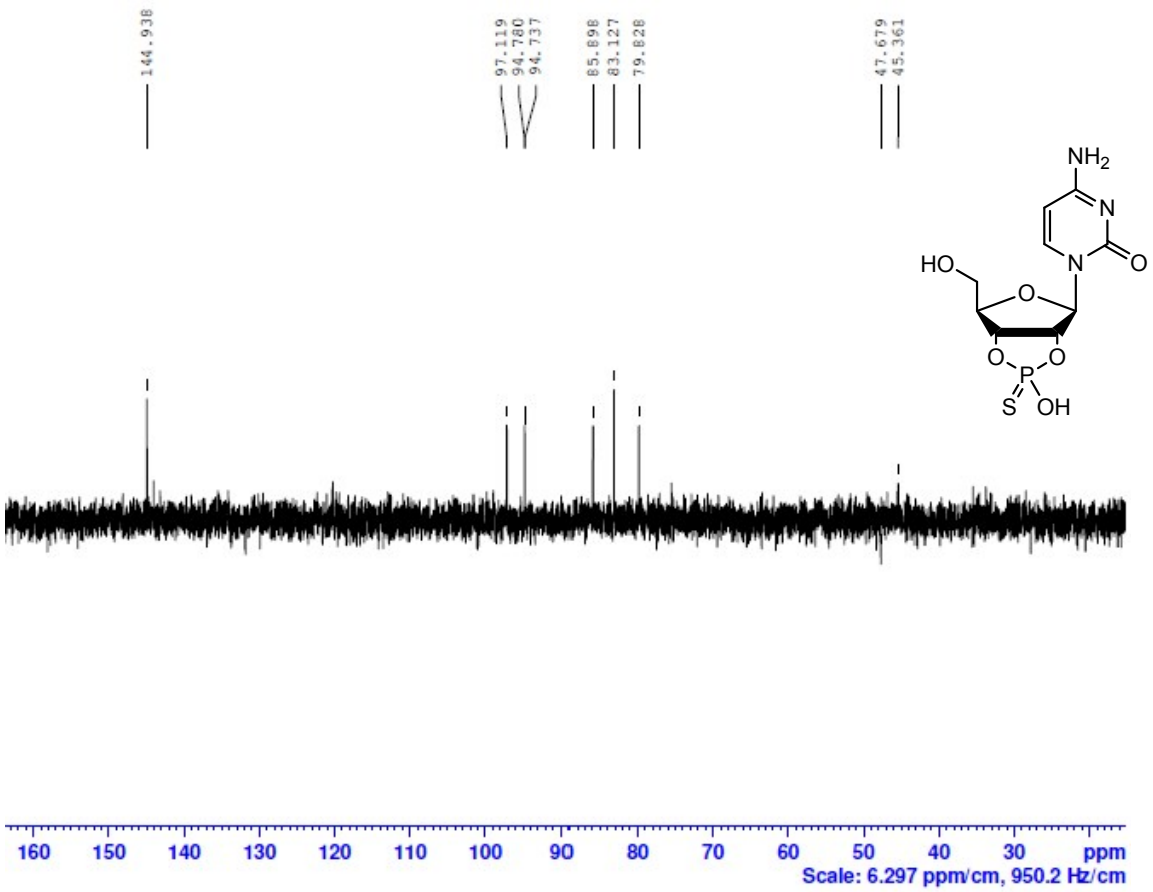
^{31}P NMR (D_2O, 161.96 MHz)



^{1}H NMR (D_2O, 400 MHz

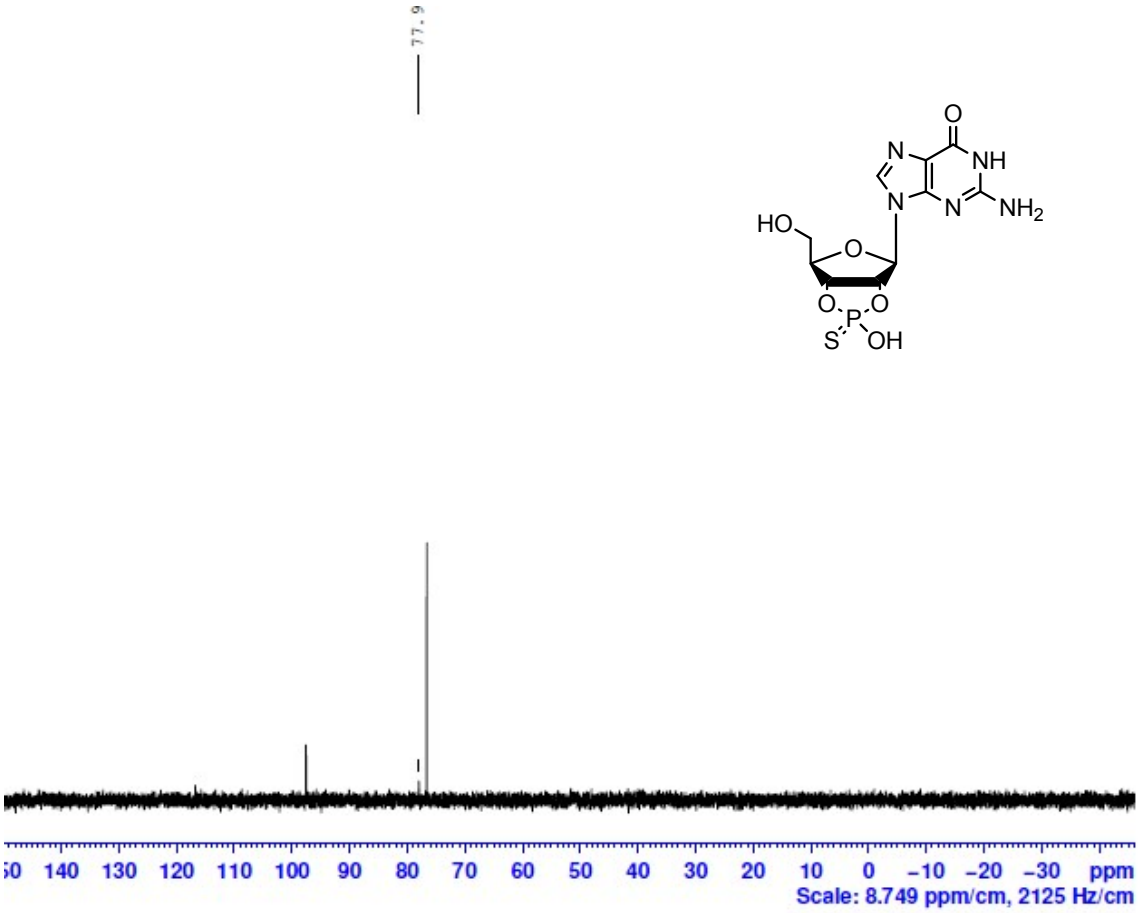


^{13}C NMR (D_2O, 100.61 MHz).

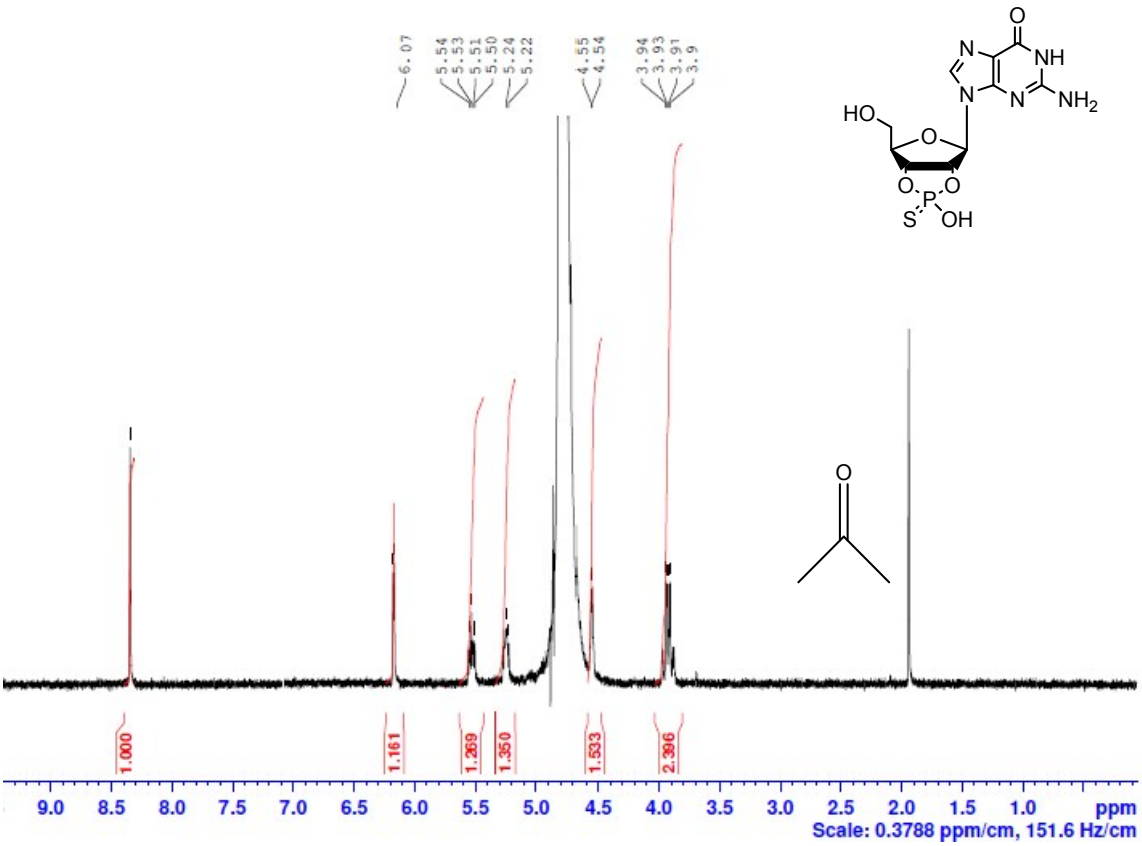


^{13}C NMR (D_2O, 100.61 MHz).

$^{31}P/^{1}H/^{13}C$ and DEPT NMR spectra of guanine- 2',3'-O,O-phosphorothioate (S*p*, exo), **15**.

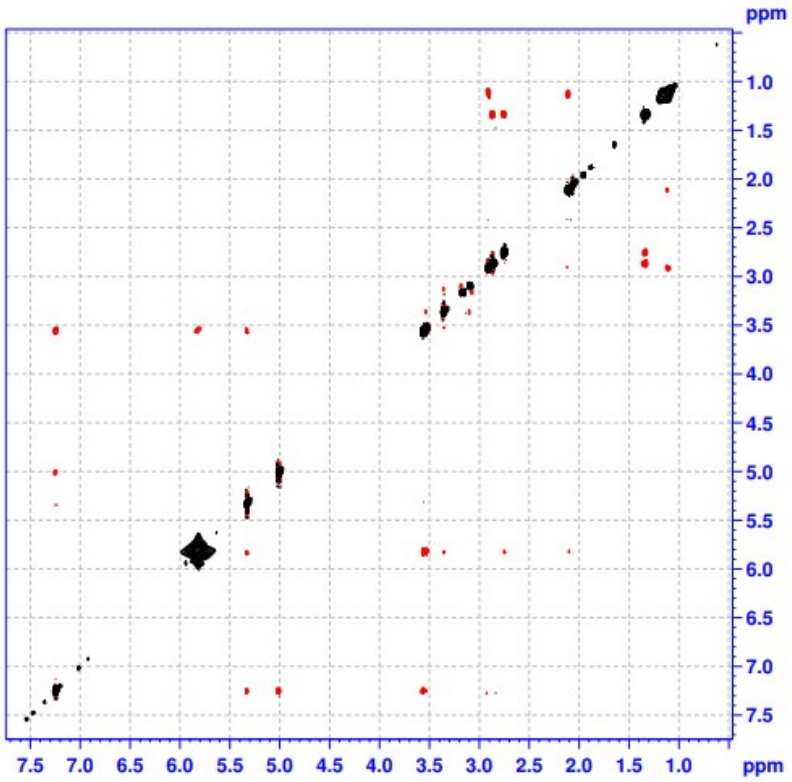


^{31}P NMR (D_2O, 161.96 MHz)



^{1}H NMR (D_2O, 400 MHz)

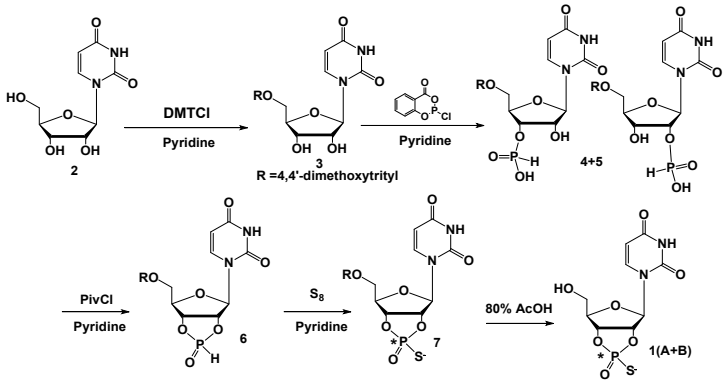
Nosey spectrum of solutions containing 1M of uridine and 1M of DBU



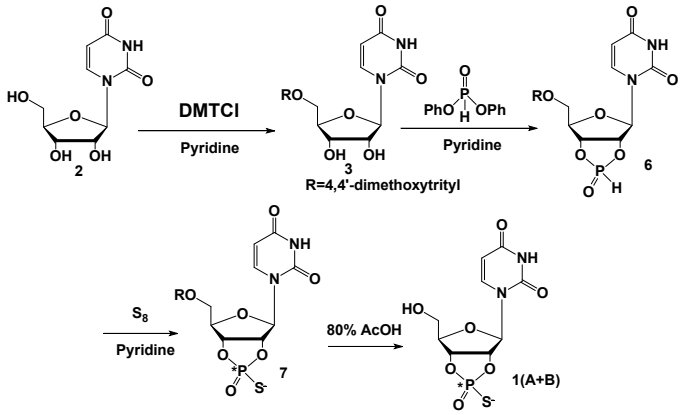
BRUKER

NAME	DBU uridine
EXPNO	6
PROCNO	1
Date_	20191202
Time	10.47
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	noesyph
TD	2048
SOLVENT	Acetone
NS	8
DS	8
SWH	2913.753 Hz
FIDRES	1.422731 Hz
AQ	0.3514868 sec
RG	28.5
DW	171.600 usec
DE	6.50 usec
TE	300.0 K
D0	0.00015874 sec
D1	2.50000000 sec
D8	1.50000000 sec
IN0	0.00034320 sec
========	CHANNEL f1 ========
NUC1	1H
P1	10.10 usec
PL1	0.00 dB
SFO1	400.1016404 MHz
ND0	1
TD	128
SFO1	400.1016 MHz
FIDRES	22.763596 Hz
SW	7.282 ppm
FnMODE	TPPI
SI	1024
SF	400.0999988 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00
SI	1024
MC2	TPPI
SF	400.0999978 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0

Fig1. All the NOE interactions observed are intramolecular, no NOE interactions are observed between uridine and DBU protons. ^{1}H NMR (D_2O, 400 MHz), ^{13}C NMR (D_2O, 100.61 MHz).



Scheme S1. Ramsay-Shaw's synthesis of epimeric uridine-2',3'-cyclic thio-phosphate, **1(A+B)**.



Scheme S2. Kraszewski's synthesis of epimeric uridine-2',3'-cyclic thio-phosphate. **1(A+B)**. All reported syntheses of nucleoside 2',3'-cyclic (thio or dithio)monophosphate have involved a 5'-OH protection/deprotection strategy.