

A Novel Membrane Permeant cADPR Antagonist

Modified in the Pyrophosphate Bridge

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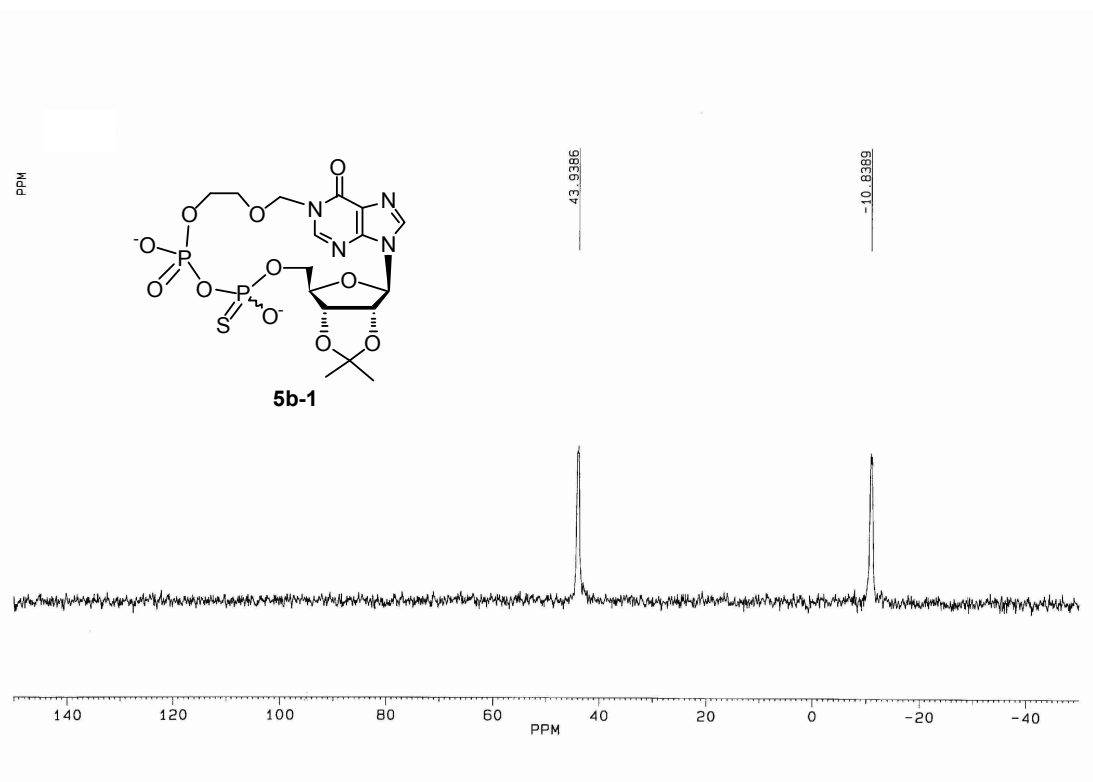
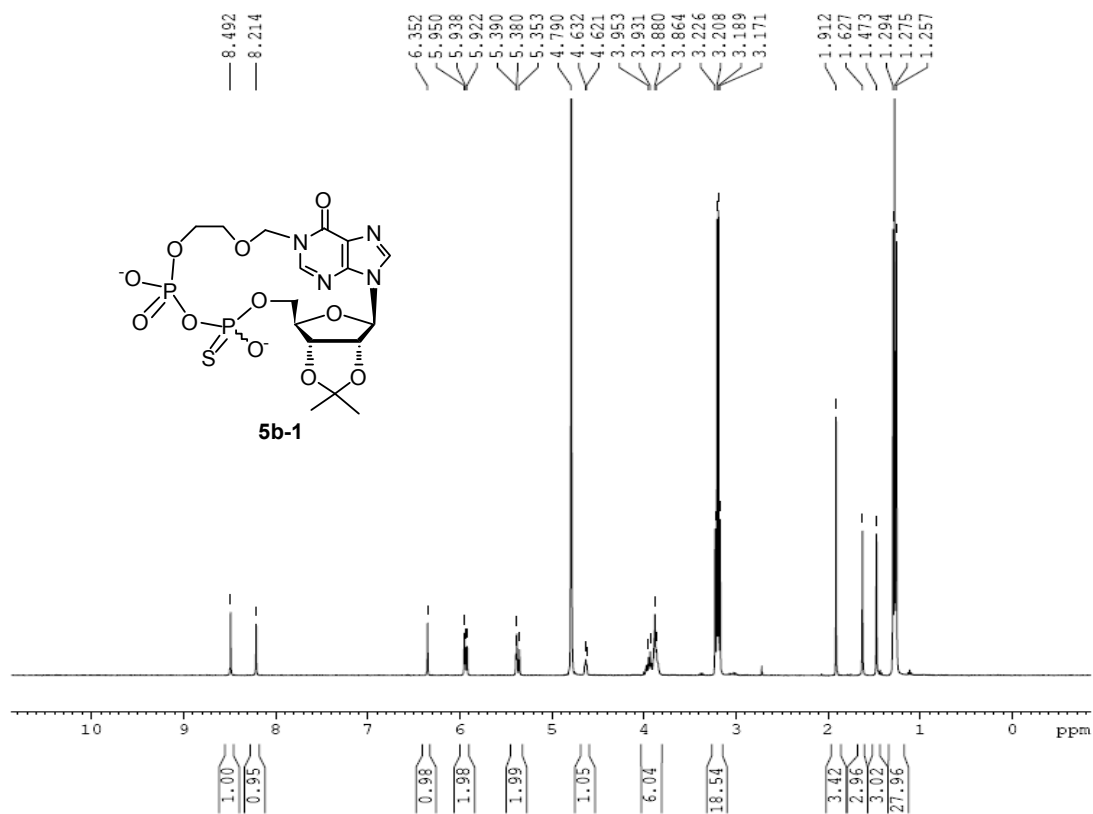
[¶]Equal contribution

*Corresponding author

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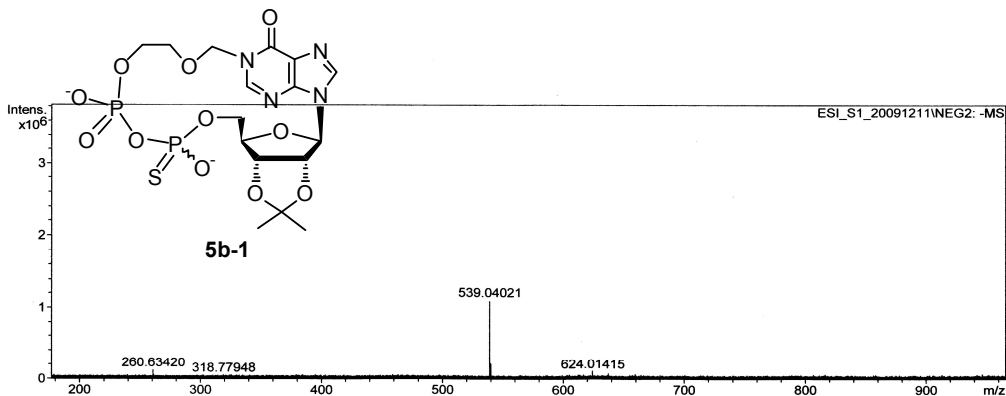
1. ^1H NMR, ^{31}P NMR and HRMS of 5b-1



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 475.32548;509.25407;509.25407;566.42760;
 588.40954;679.51166;701.49361;826.47121;
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-2880;
 mg/mL; 150uL/h

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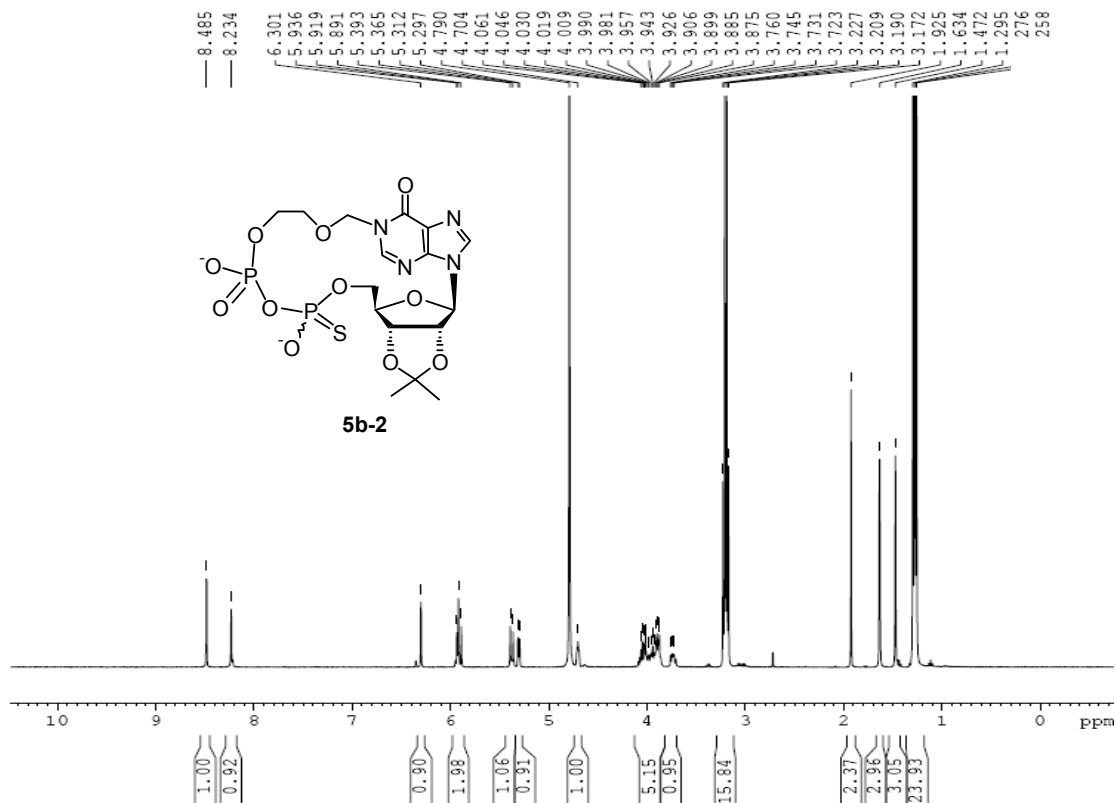


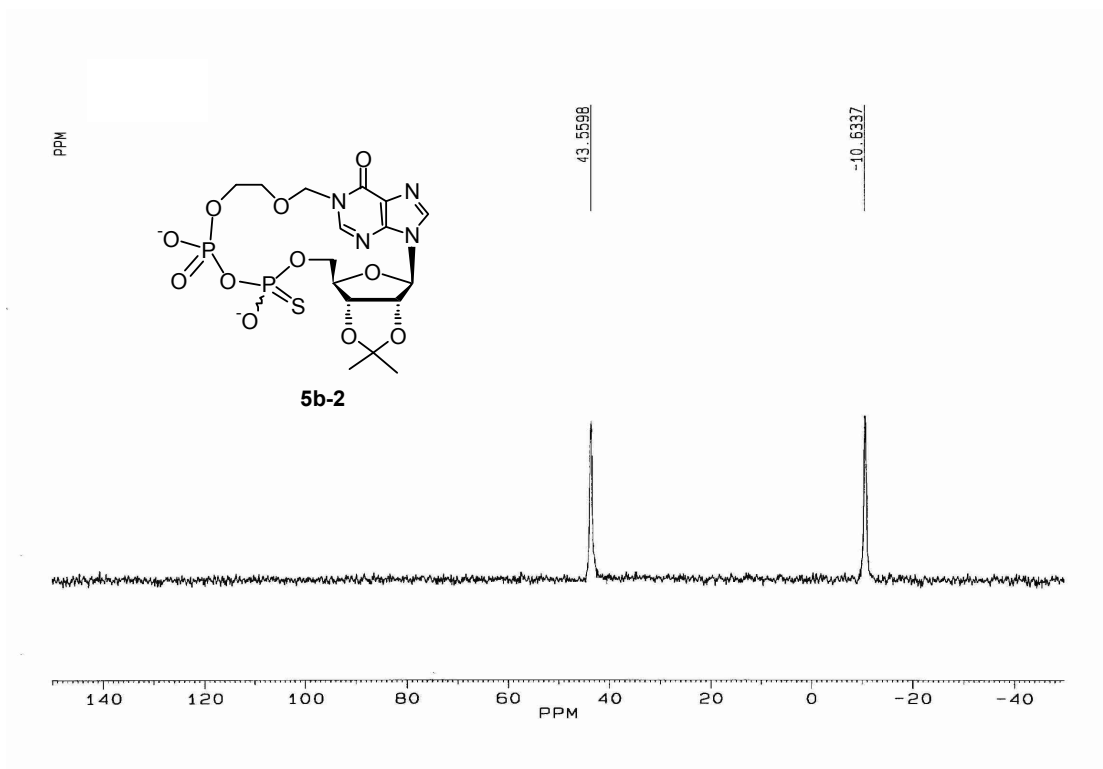
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printed: 11/12/2009 10:41:00

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2. ¹H NMR, ³¹P NMR and HRMS of 5b-2

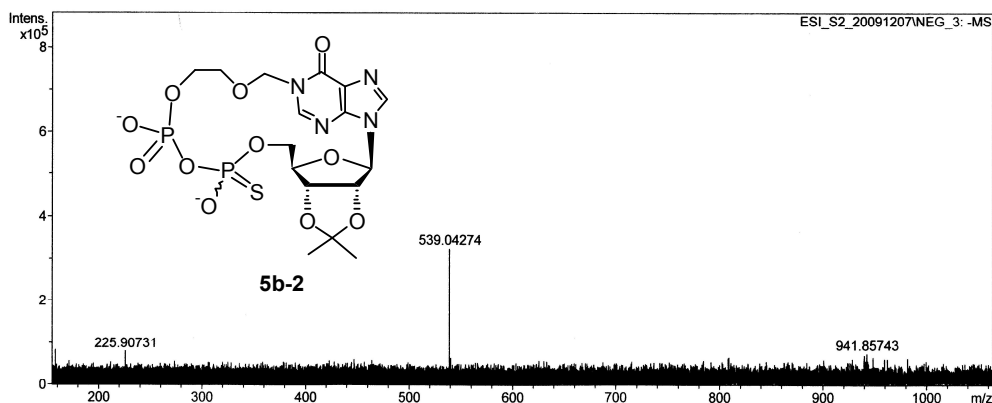




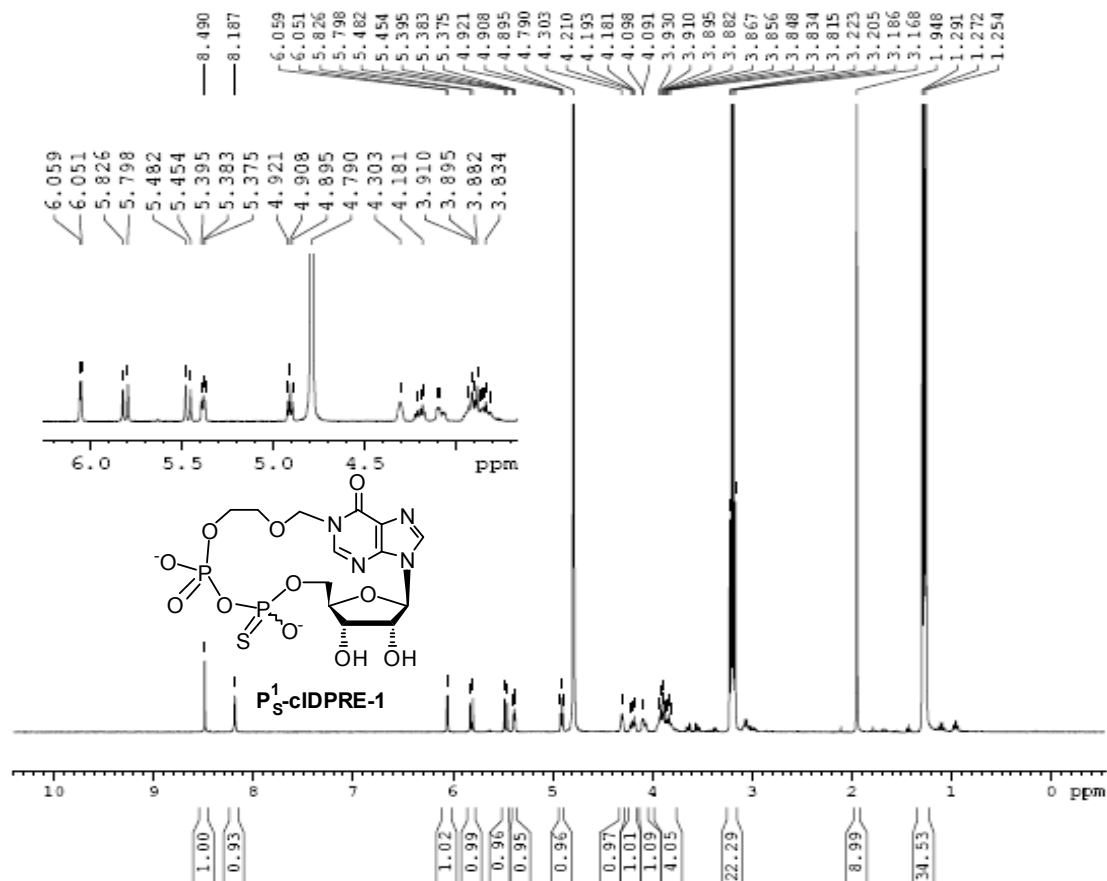
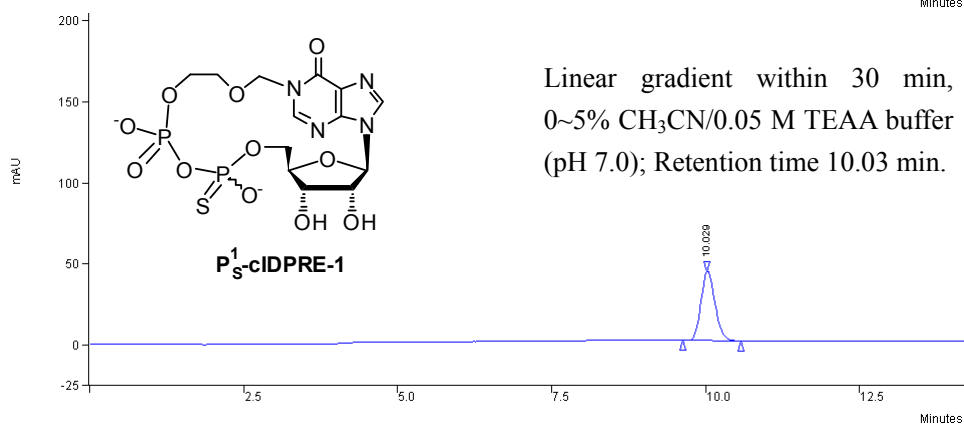
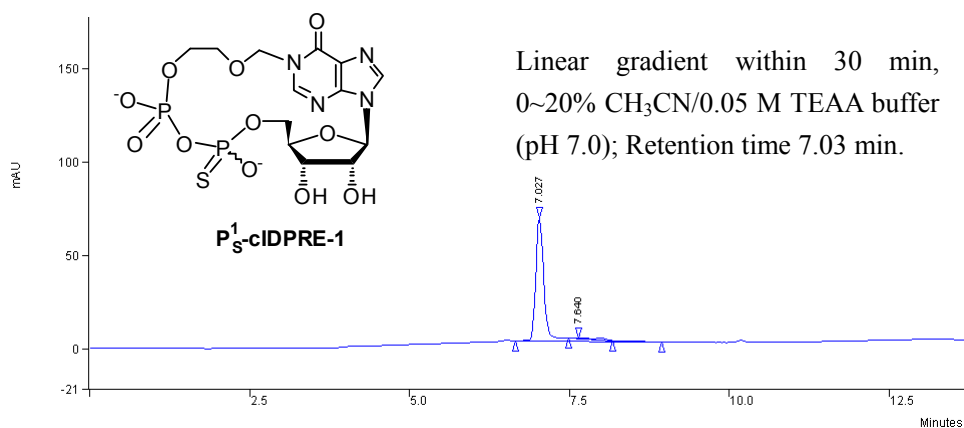
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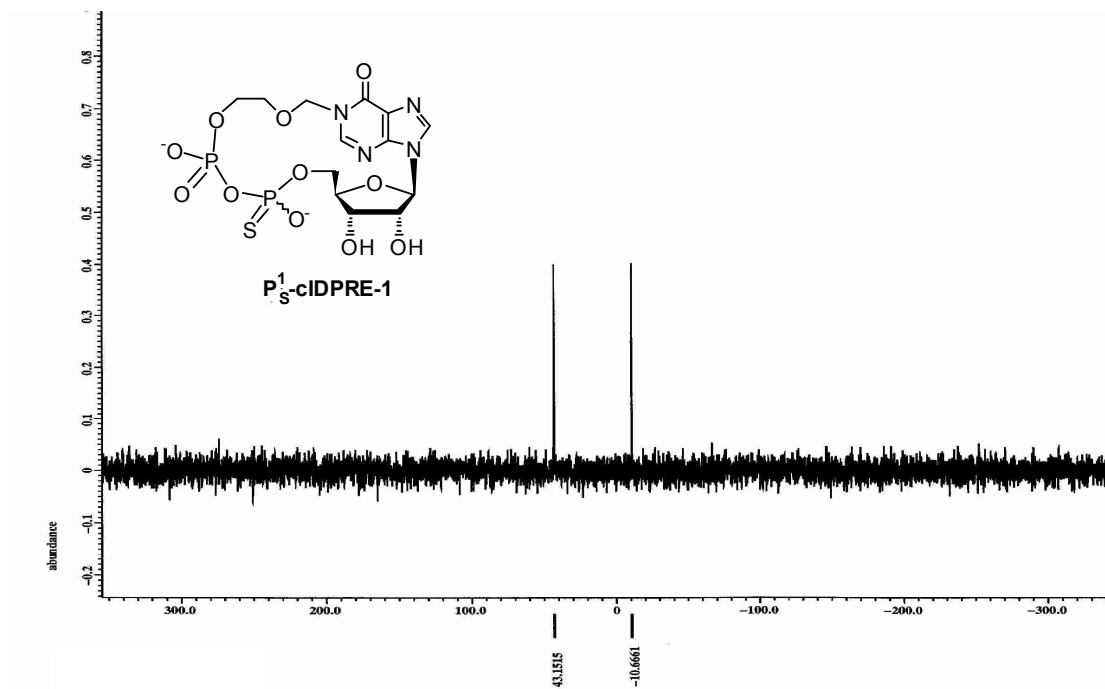
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 475.32548;509.25407;509.25407;566.42760;
 588.40954;679.51166;701.49361;826.47121;
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-2880;
 mg/mL; 150uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
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3. HPLC, ^1H NMR, ^{31}P NMR and HRMS of $\text{P}_S^1\text{-cIDPRE-1}$

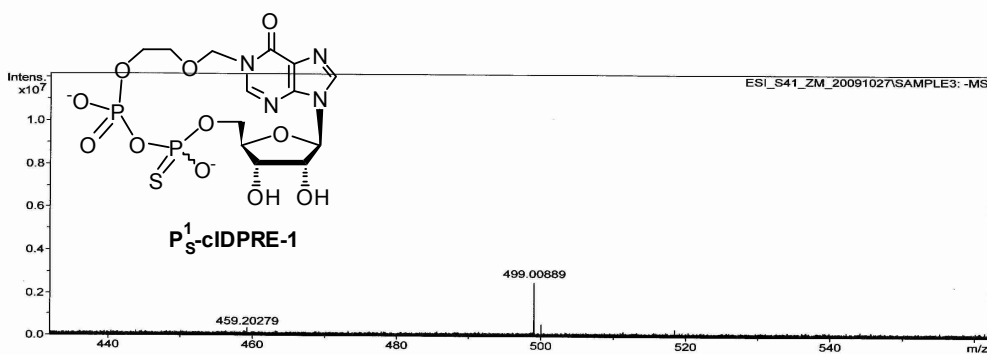




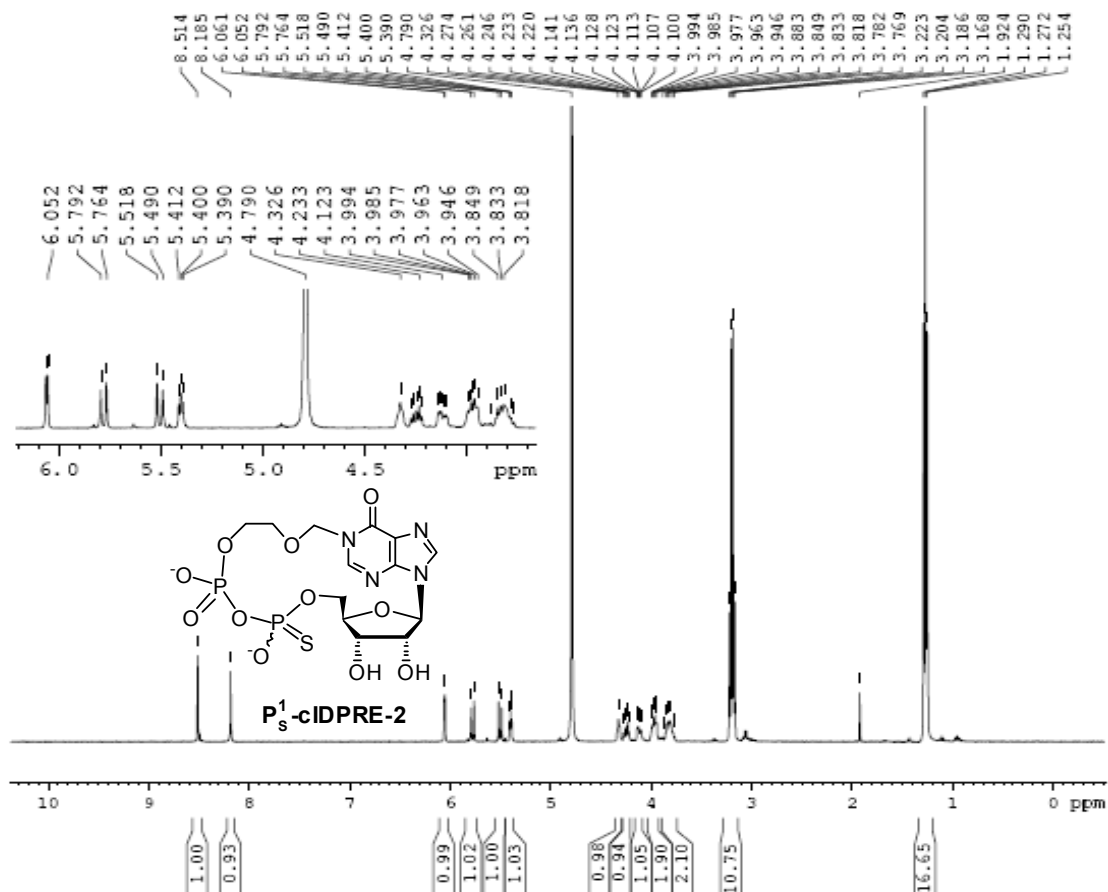
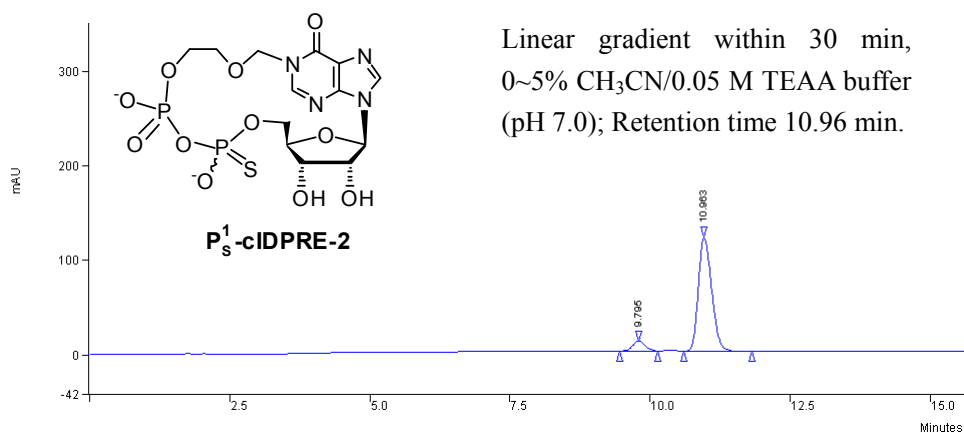
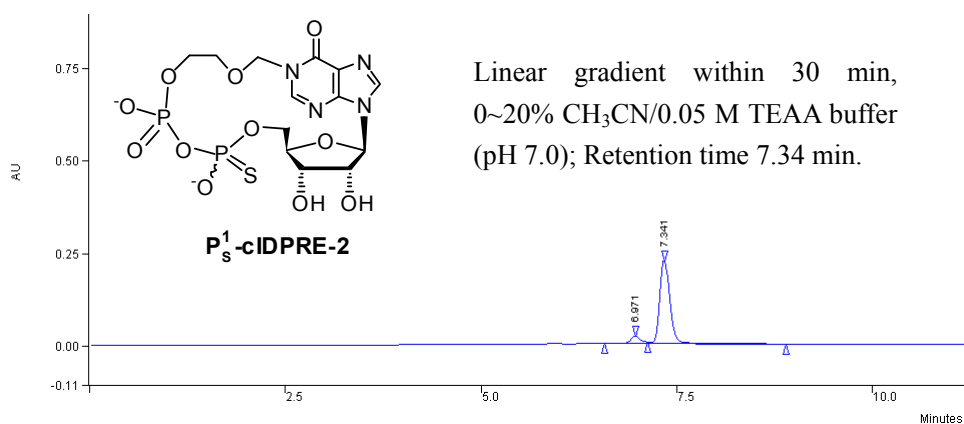
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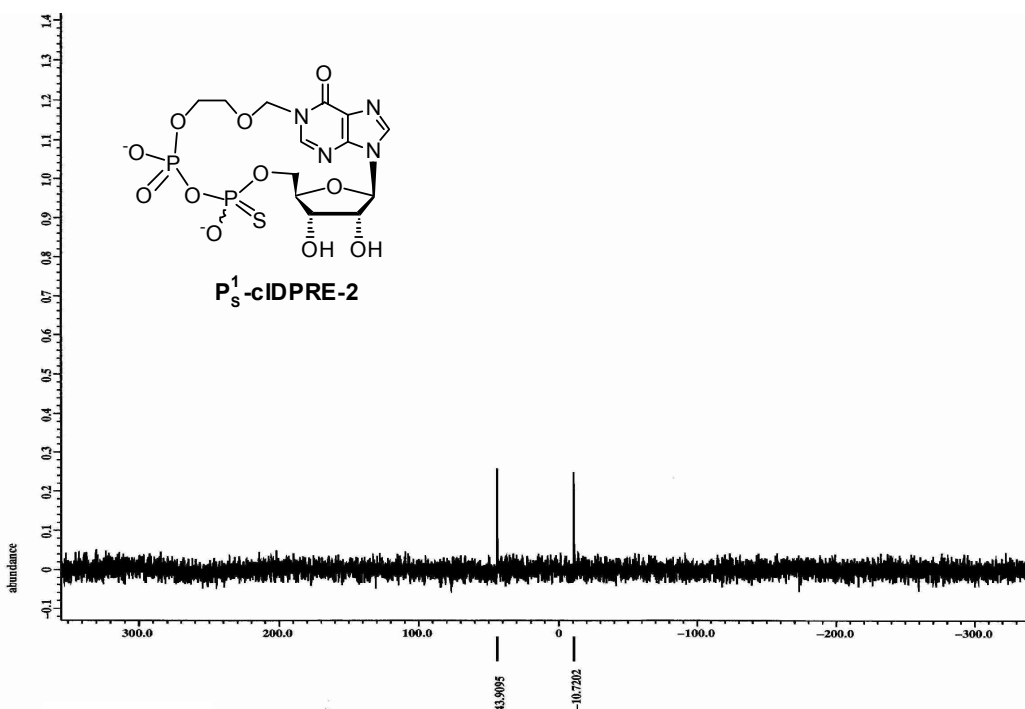
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 589.40954;679.51166;701.49361;826.47121;
 in 1:1 Tol:MeOH; CapExit 150V; m/z 150-1500; 1024K; D1 2s
 0.05 mg/mL; 150uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	499.00953	499.00889	0.64	1.28	M-H; ⁺ e



4. HPLC, ^1H NMR, ^{31}P NMR and HRMS of $\text{P}_s^1\text{-cIDPRE-2}$

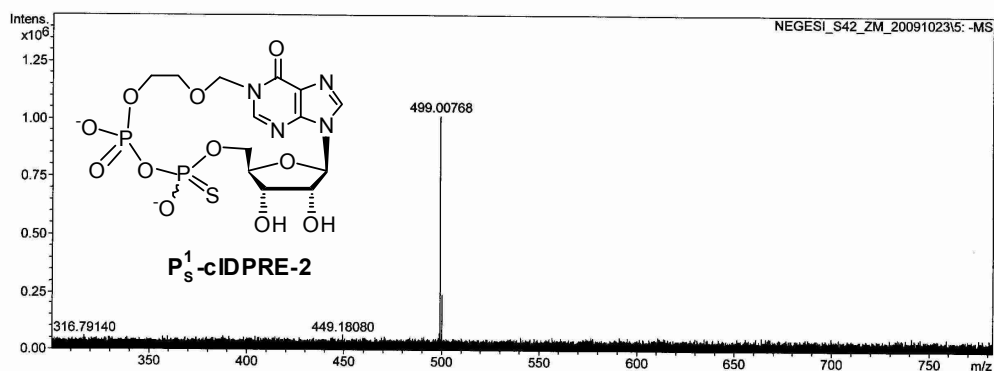




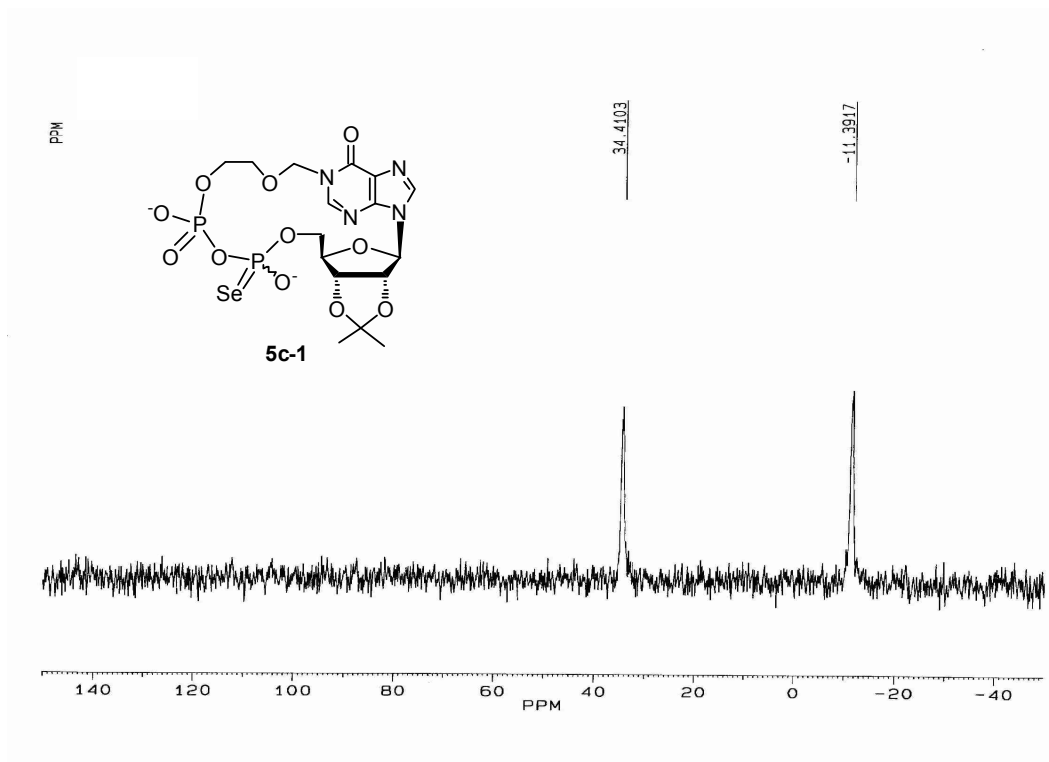
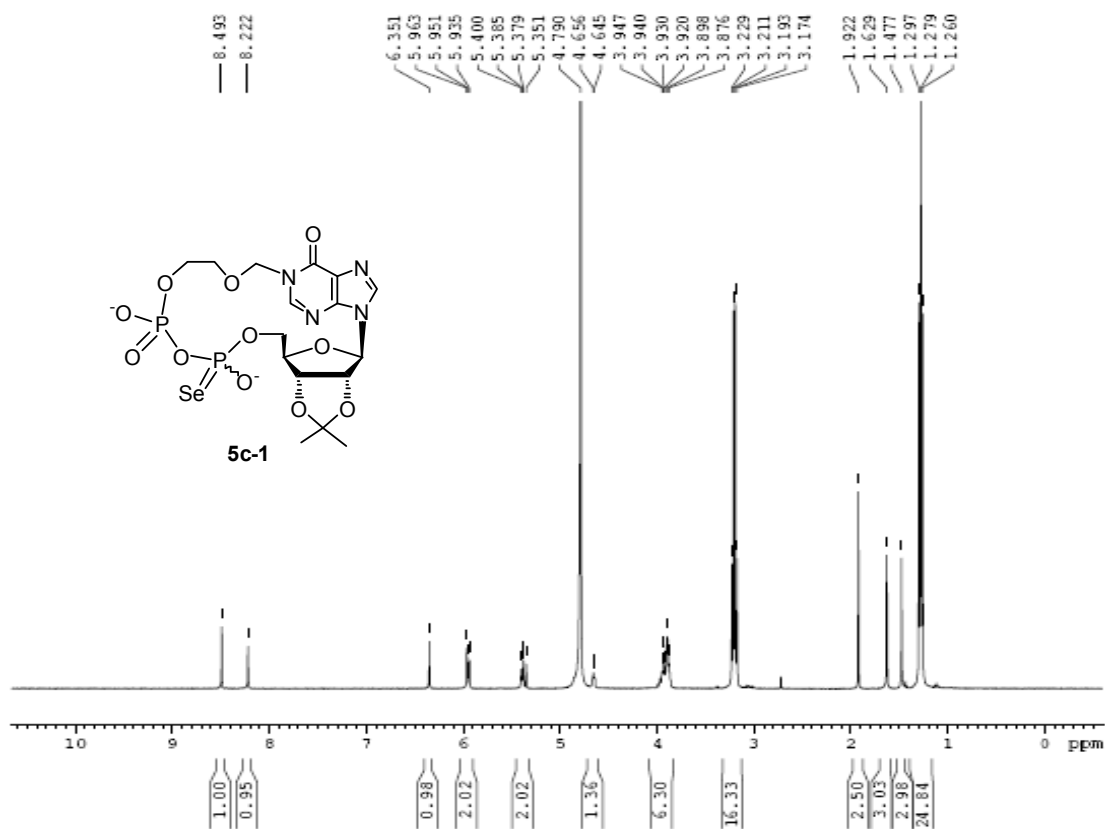
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 Comment NEGESI C13H18N4O11P2S1 MW 500.0168
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 475.32548;509.25407;509.25407;566.42760;
 588.40954;679.51166;701.49361;826.47121;
 in 1:1 Tol:MeOH; CapExit 150V; m/z 150-1500; 1024K; D1 2s
 0.05 mg/mL; 150uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
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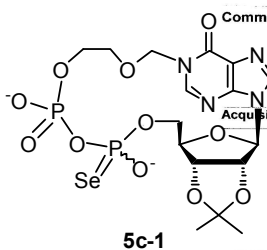


5. ^1H NMR, ^{31}P NMR and HRMS of 5c-1



BJMU - BRUKER APEX IV FT-MS (7.0T) Spectrum Report

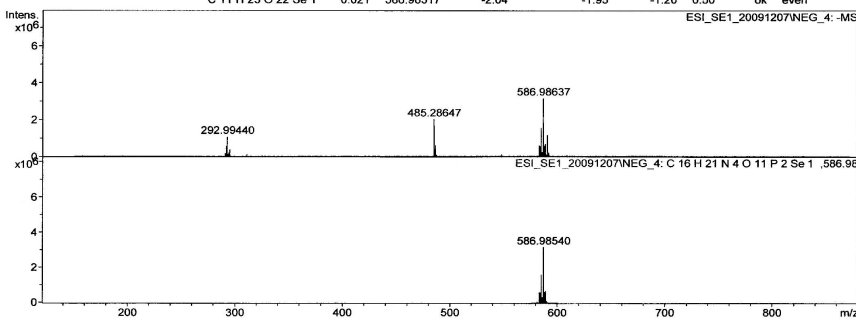
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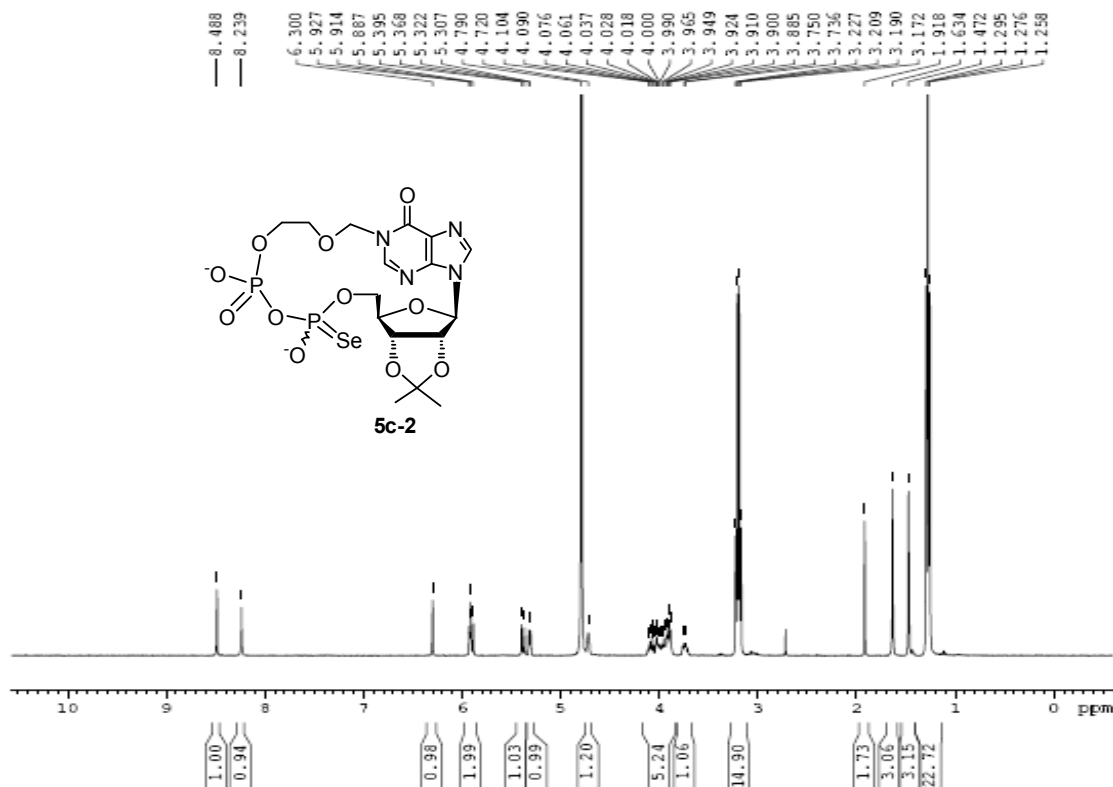
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 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-2880;
 mg/mL; 150uL/h

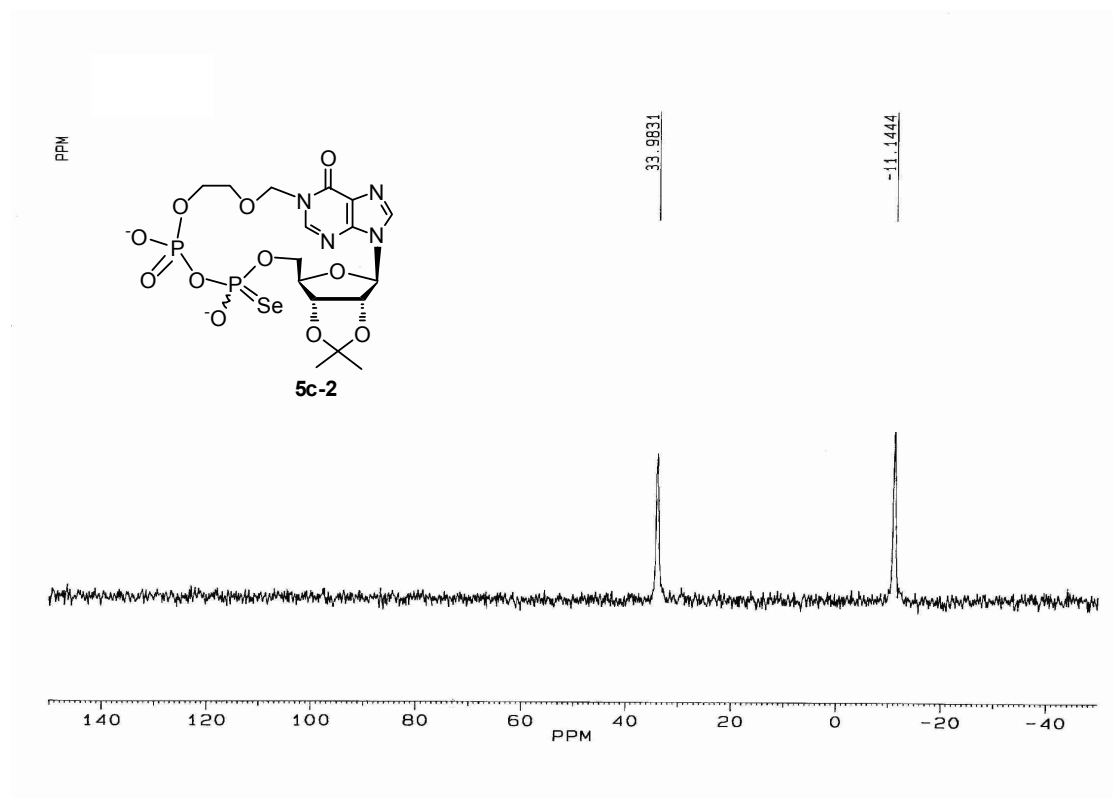
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C 15 H 25 O 15 P 2 Se 1	0.010	586.98394	-4.14	-4.06	-2.43	4.50	ok	even
C 14 H 21 N 1 O 19 Se 1	0.011	586.98785	2.52	2.63	1.48	5.00	ok	odd
C 11 H 23 O 22 Se 1	0.021	586.98517	-2.04	-1.95	-1.20	0.50	ok	even



6. ¹H NMR, ³¹P NMR and HRMS of 5c-2

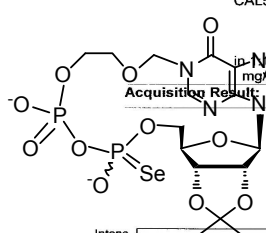




BJMU - BRUKER APEX IV FT-MS (7.0T) Spectrum Report

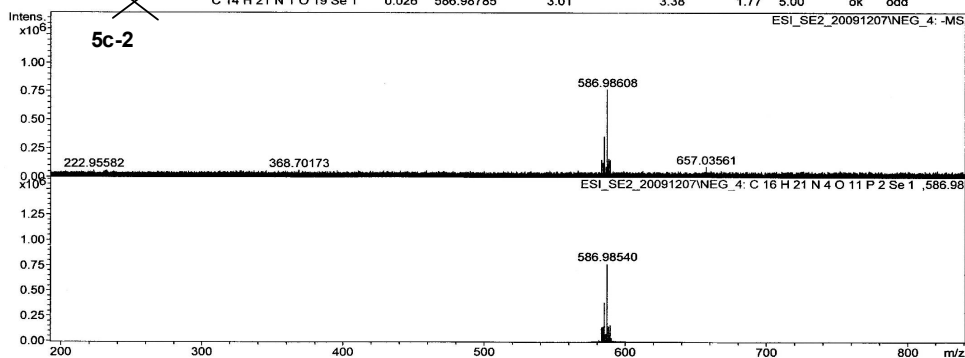
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 mg/mL; 150uL/h

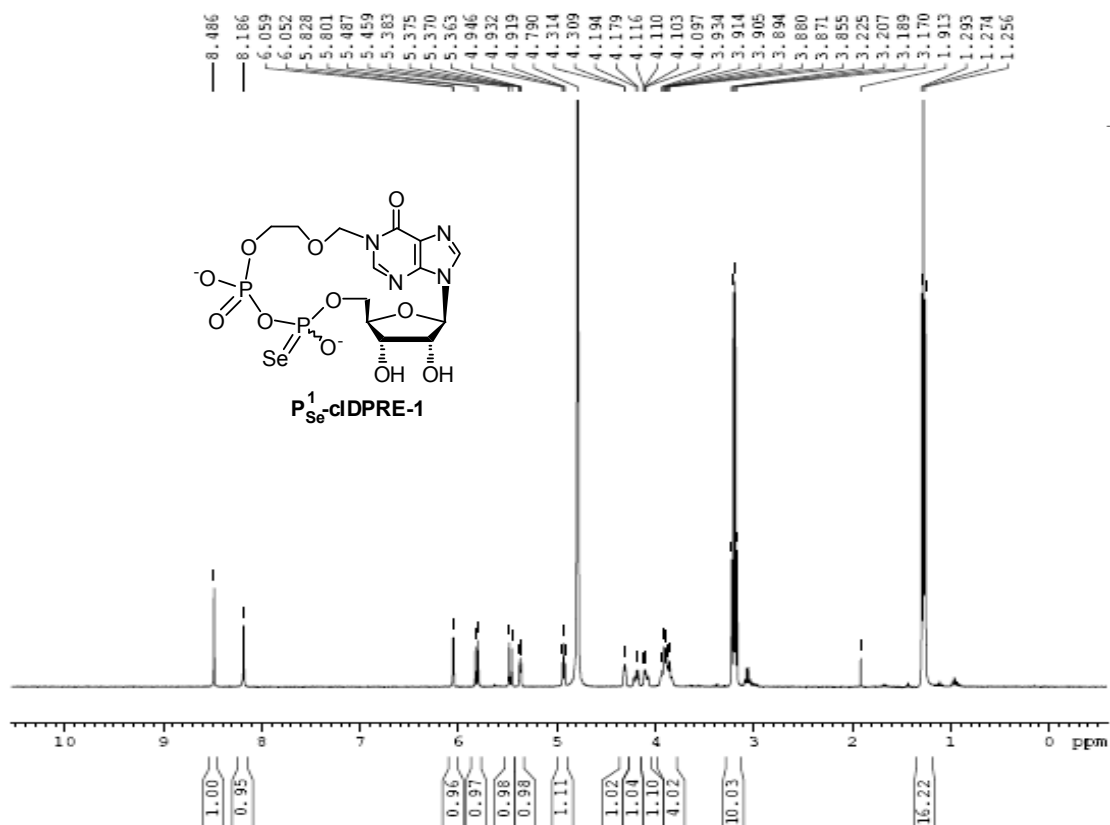
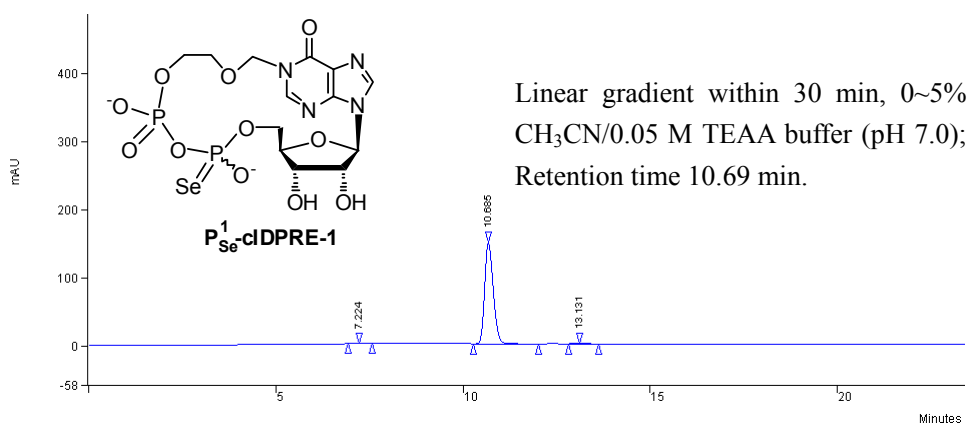
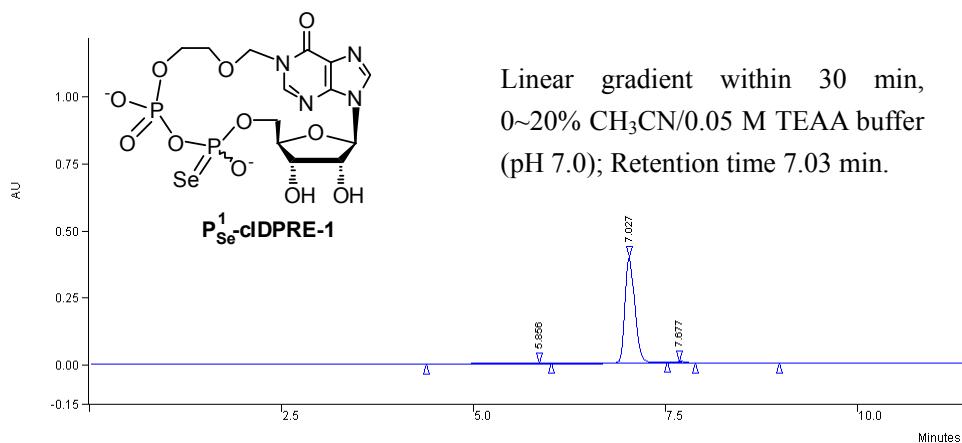


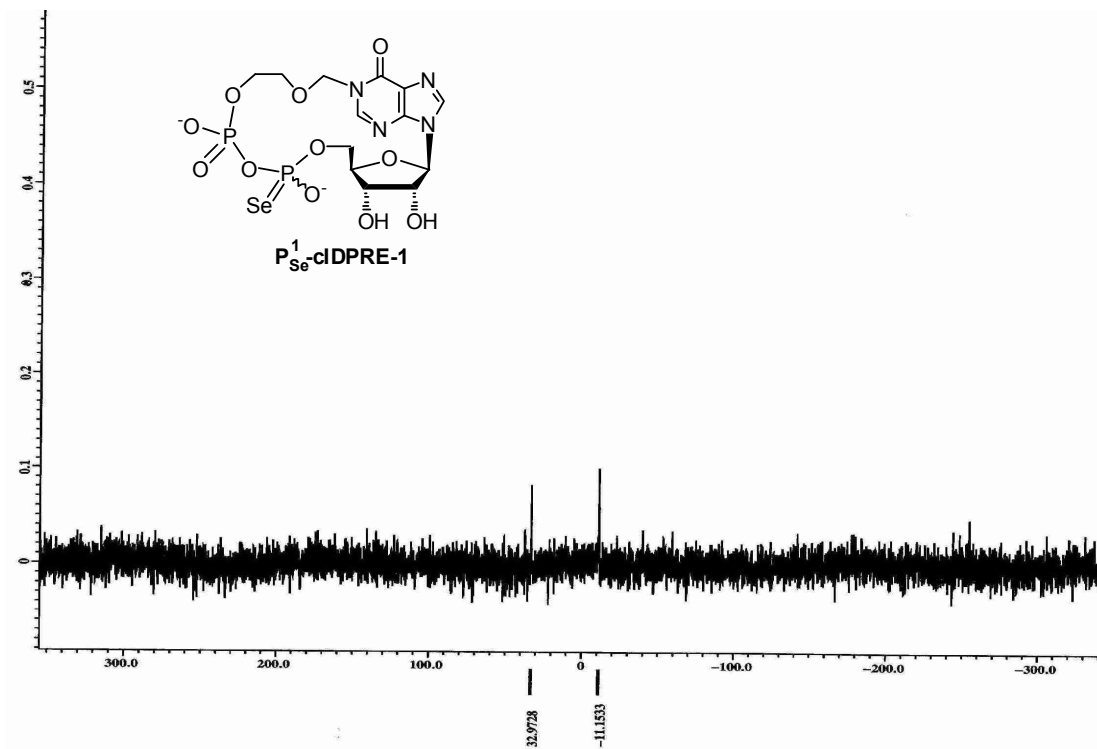
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C 15 H 25 O 15 P 2 Se 1	0.024	586.98394	-3.65	-3.32	-2.14	4.50	ok	even
C 14 H 21 N 1 O 19 Se 1	0.026	586.98785	3.01	3.38	1.77	5.00	ok	odd



7. HPLC, ¹H NMR, ³¹P NMR and HRMS of P¹_{Se}-cIDPRE-1

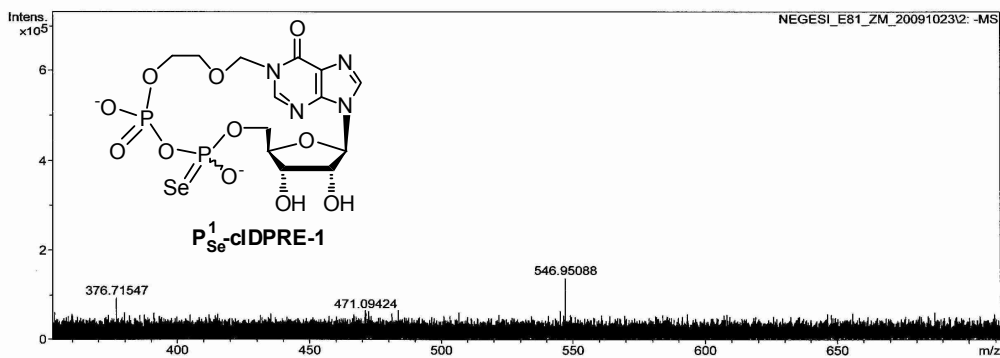




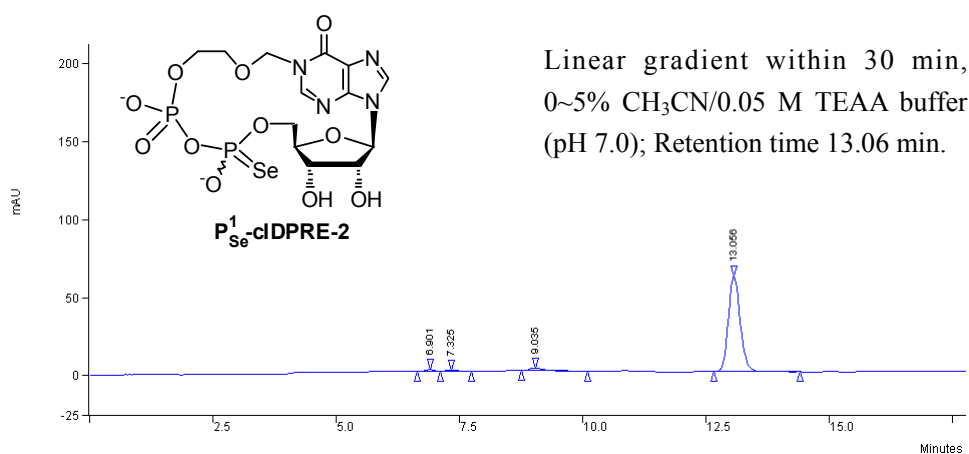
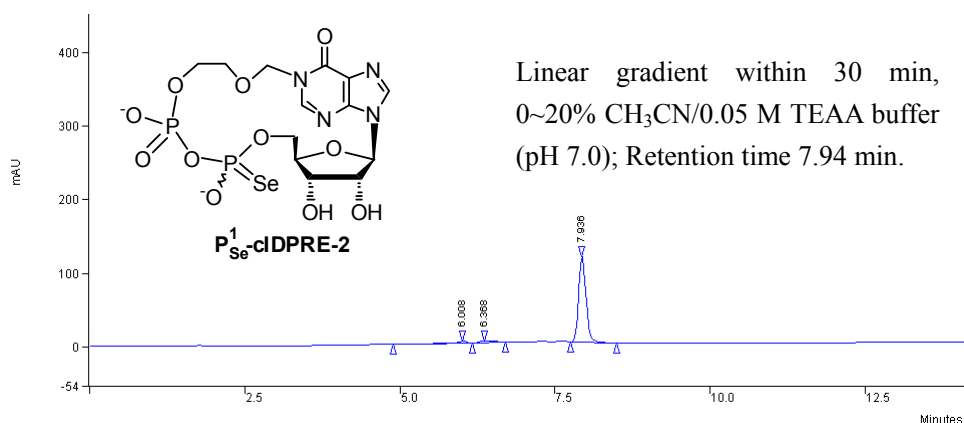
Bruker APEX IV FT_MS (7.0 T) Report (all)

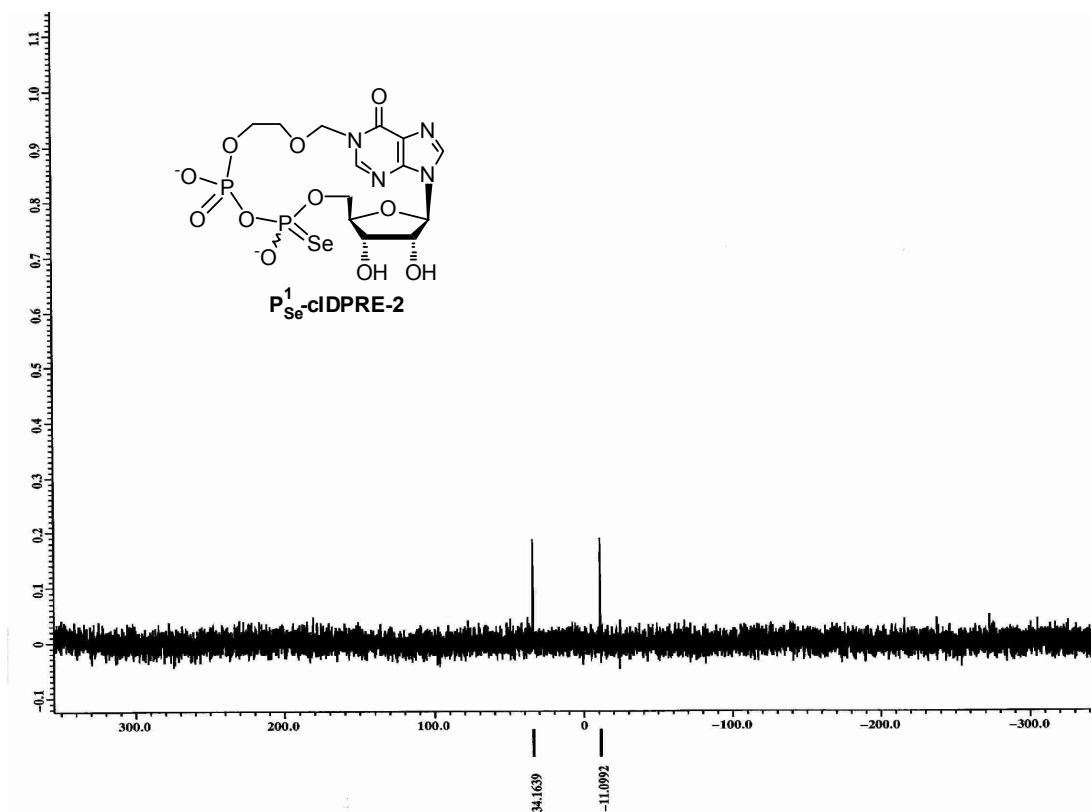
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475.32548;509.25407;509.25407;566.42760;
588.40954;679.51166;701.49361;826.47121;
in 1:1 Tol:MeOH; CapExit 150V; m/z 150-1500; 1024K; D1 2s
0.05 mg/mL; 150uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
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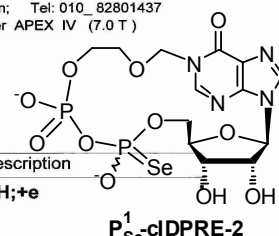
8. HPLC, ^1H NMR, ^{31}P NMR and HRMS of $\text{P}_{\text{Se}}^1\text{-cIDPRE-2}$



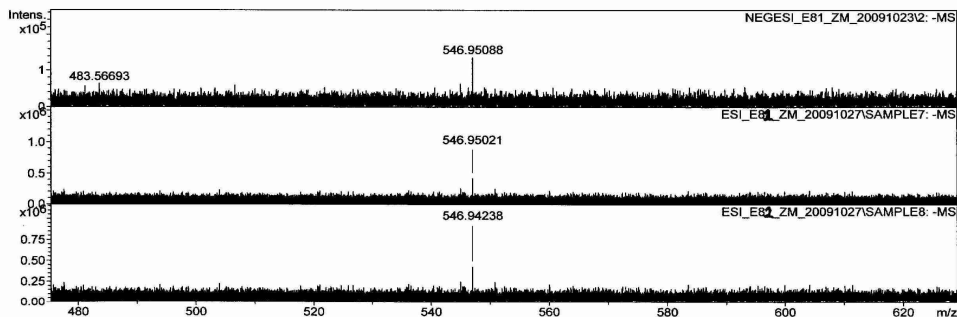


Bruker APEX IV FT_MS (7.0 T) Report (all)

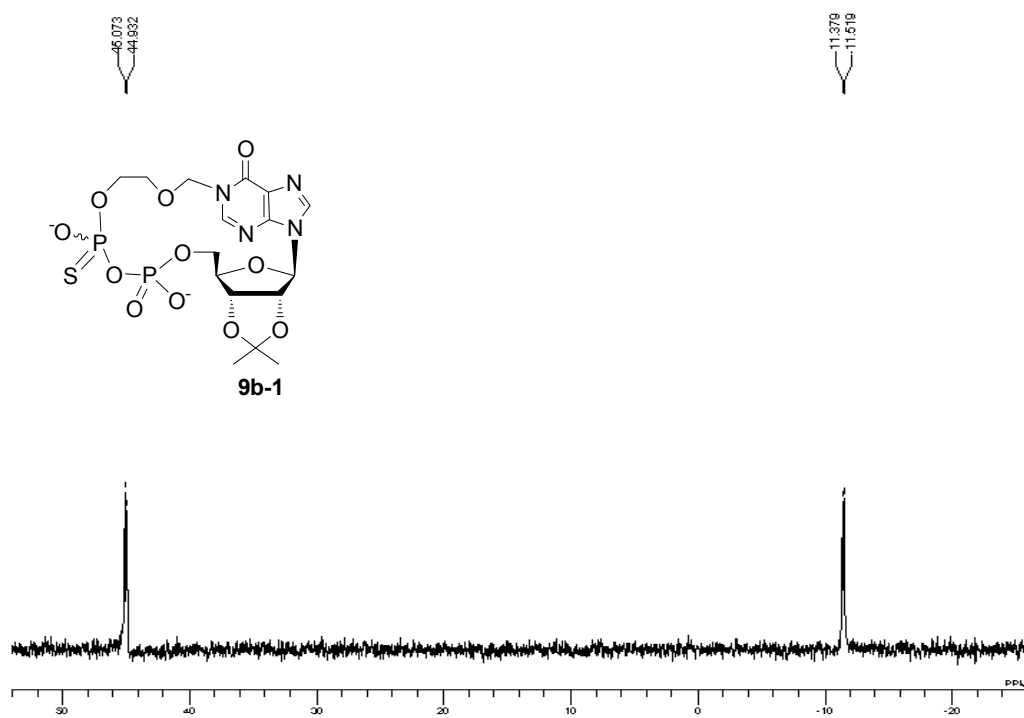
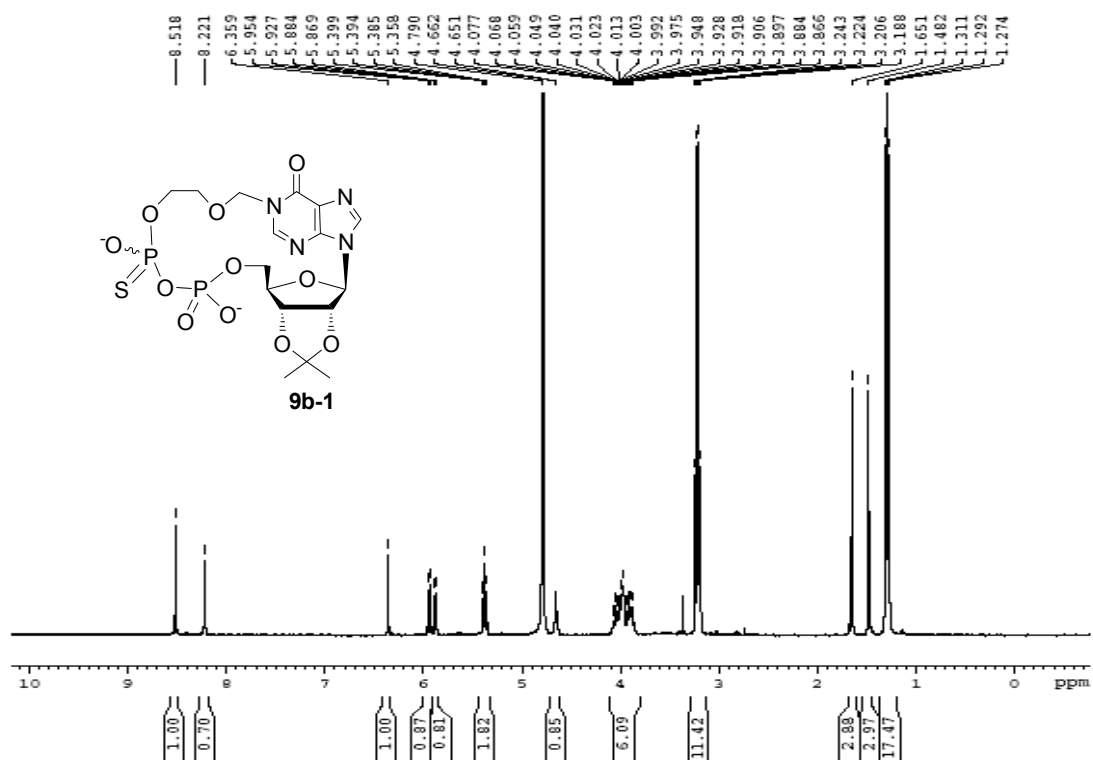
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 475.32548;509.25407;509.25407;566.42760;
 588.40954;679.51165;701.49361;826.47121;
 in 1:1 Tol:MeOH; CapExit 150V; m/z 150-1500; 1024K; D1 2s
 0.05 mg/mL; 150uL/h



Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	546.95343	546.95088	2.55	4.66	M-H; ⁺ e



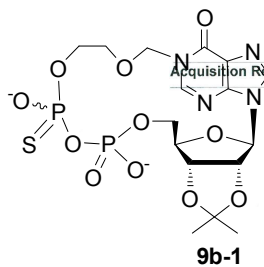
9. ^1H NMR, ^{31}P NMR and HRMS of 9b-1



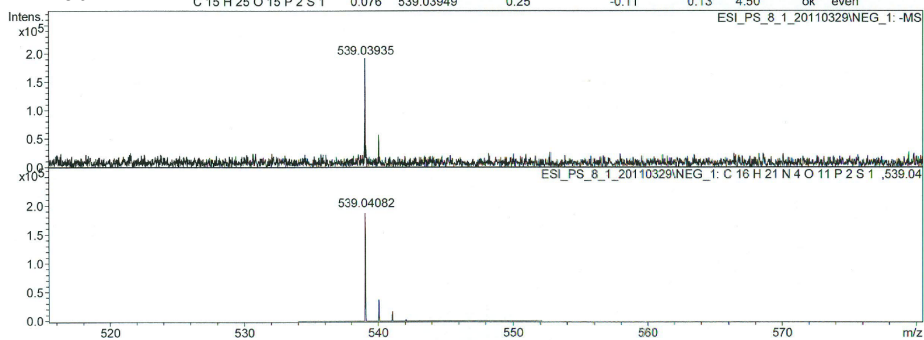
BJMU - BRUKER APEX IV FT-MS (7.0T) Spectrum Report

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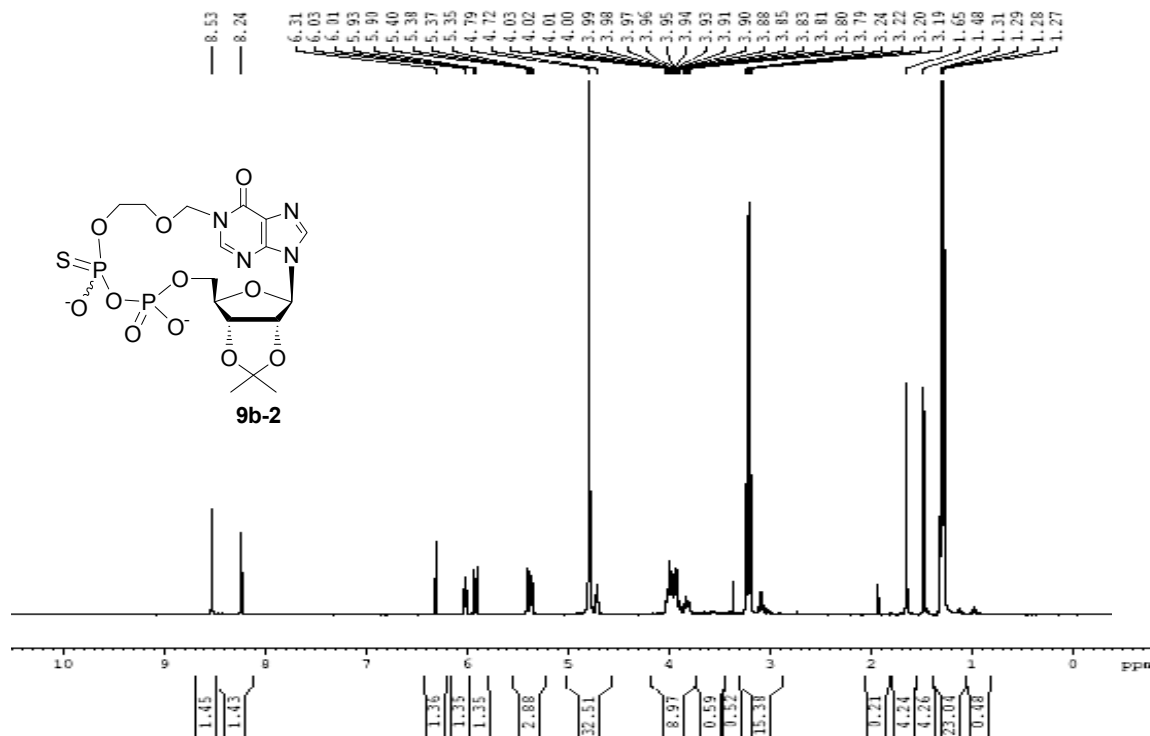
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 335.99207; 414.00562; 509.25407; 826.47121351; 96445□
 in 1:1 H2O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
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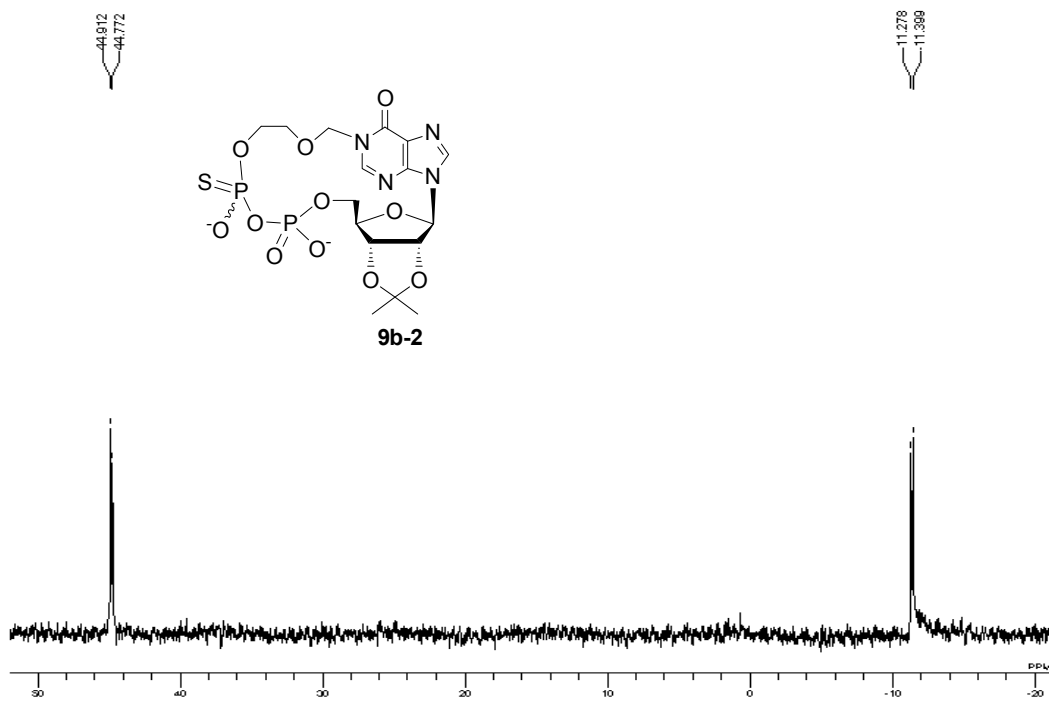


Exact Mass	Measured Mass	Error (mDa)	Error (ppm)	Description
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C 28 H 17 N 2 O 4 P 2 S 1	0.054	539.03897	-0.70	-1.12
C 29 H 13 N 6 P 2 S 1	0.057	539.04031	1.78	1.21
C 15 H 15 N 11 O 6 P 2 S 1	0.058	539.04082	2.72	1.94
C 31 H 15 N 3 O 1 P 2 S 1	0.053	539.04165	4.27	3.73
C 16 H 21 N 4 O 11 P 2 S 1	0.066	539.04082	2.73	2.28
C 15 H 25 O 15 P 2 S 1	0.076	539.03949	0.25	-0.11



10. ¹H NMR, ³¹P NMR and HRMS of 9b-2





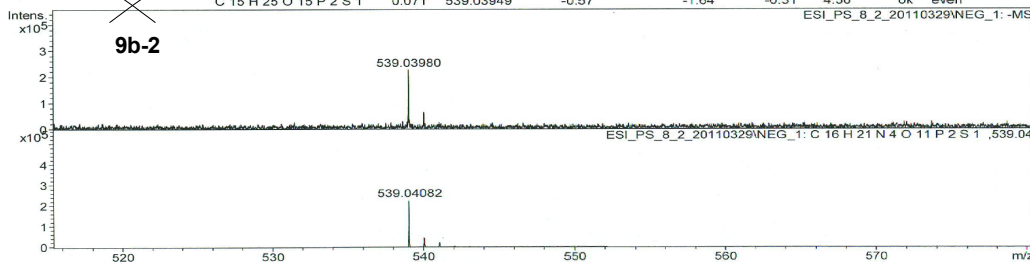
BJMU - BRUKER APEX IV FT-MS (7.0T) Spectrum Report

Analysis Info Acquisition Date 29/03/2011 10:17:24
Analysis Name C:\data_sample\ESI_20110320_30\ESI_PS_8_2_20110329\NE G_1 Operator bpfxsh@bjmu.edu.cn
 Instrument FT_MS_Bruker APEX IV (7.0 T)

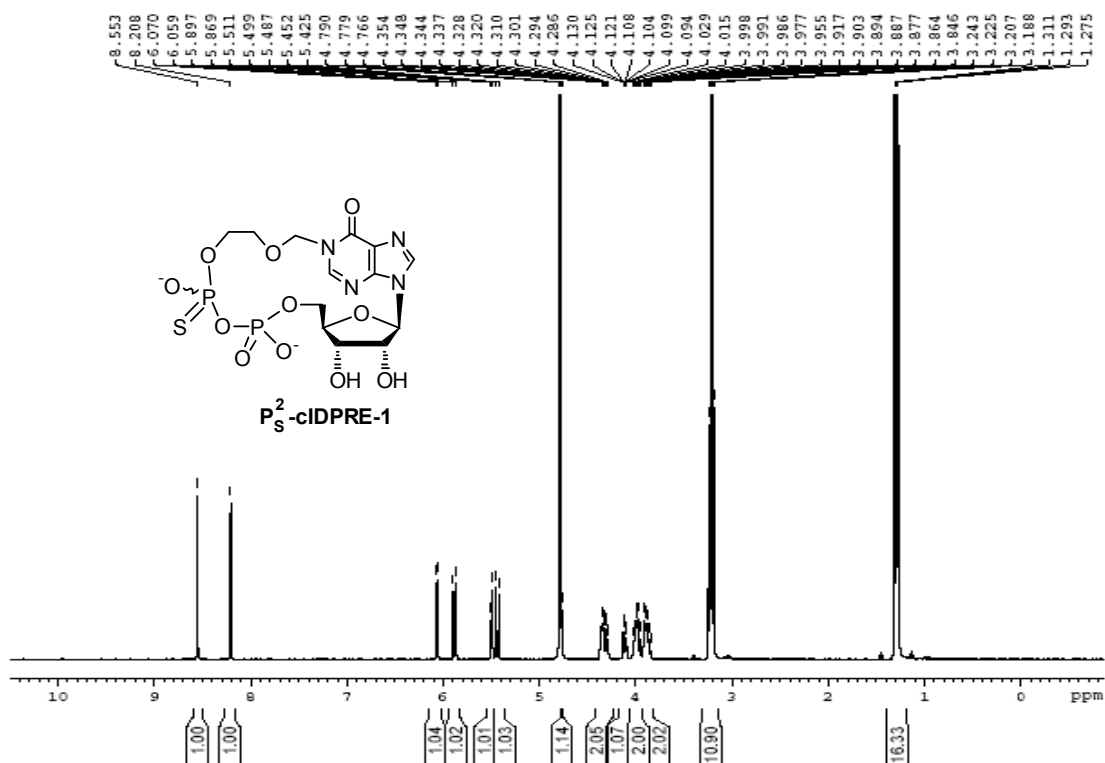
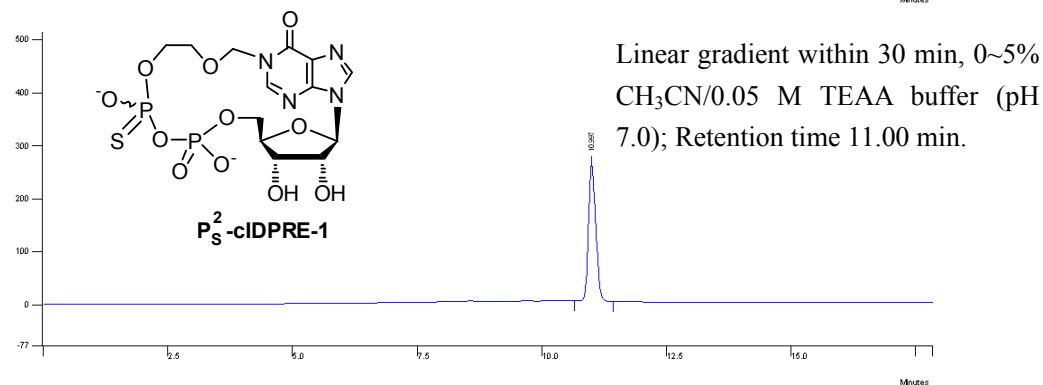
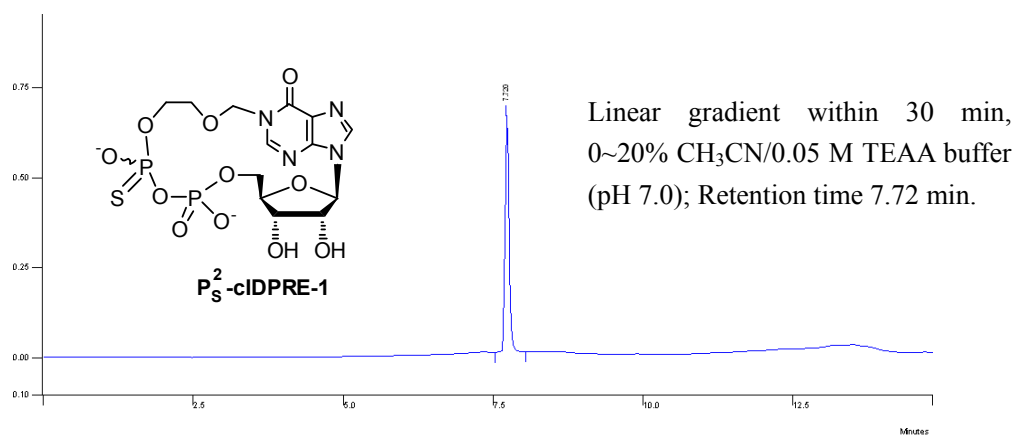
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 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451;
 335.99207; 414.00562; 509.25407; 826.47121351; 96445
 in 1:1 H2O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
 D1= 2s ; ns=8 100uL/h

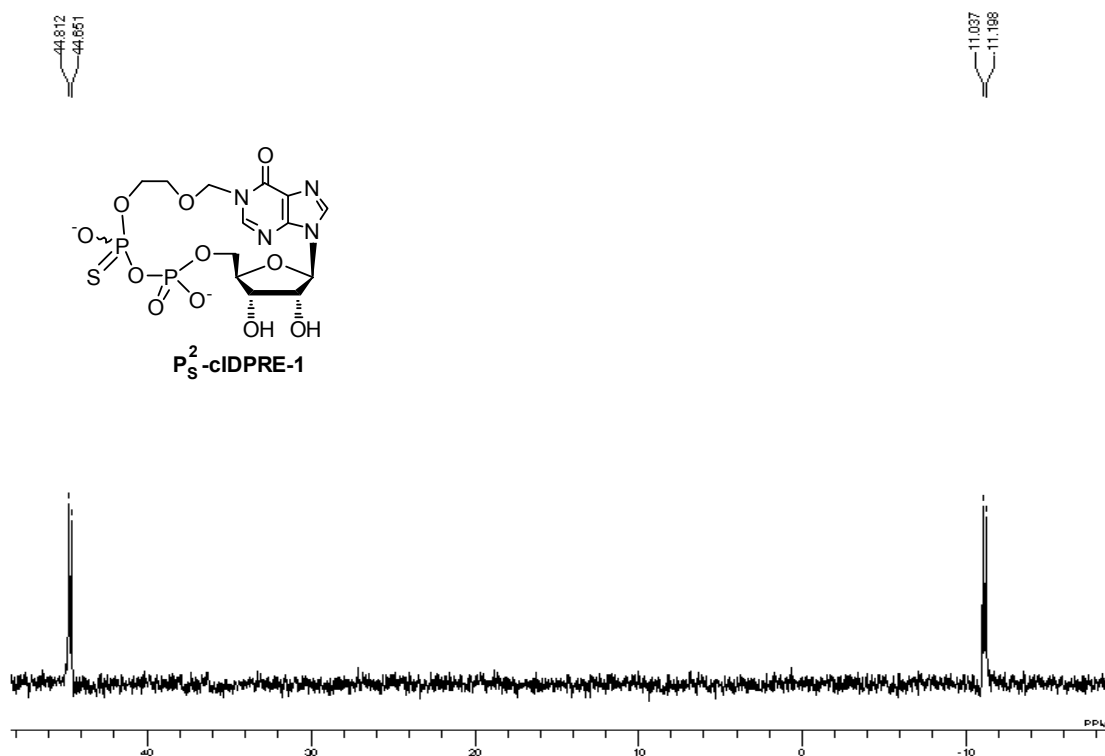
Acquisition Result: Exact Mass Measured Mass Error (mDa) Error (ppm) Description

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e ⁻
C 16 H 11 N 15 O 2 P 2 S 1	0.046	539.04216	4.38	2.82	2.36	20.00	ok	odd
C 26 H 15 N 5 O 3 P 2 S 1	0.051	539.03763	-4.02	-5.17	-2.17	23.00	ok	odd
C 17 H 17 N 8 O 7 P 2 S 1	0.052	539.04216	4.39	3.12	2.37	14.50	ok	even
C 15 H 15 N 11 O 6 P 2 S 1	0.054	539.04082	1.90	0.42	1.02	15.00	ok	odd
C 28 H 17 N 2 O 4 P 2 S 1	0.056	539.03897	-1.53	-2.60	-0.82	22.50	ok	even
C 18 H 23 N 1 O 12 P 2 S 1	0.069	539.04217	4.40	3.46	2.37	9.00	ok	odd
C 29 H 13 N 6 P 2 S 1	0.060	539.04031	0.95	-0.28	0.51	27.50	ok	even
C 16 H 21 N 4 O 11 P 2 S 1	0.061	539.04082	1.91	0.75	1.03	9.50	ok	even
C 31 H 15 N 3 O 1 P 2 S 1	0.066	539.04165	3.45	2.23	1.86	27.00	ok	odd
C 15 H 25 O 15 P 2 S 1	0.071	539.03949	-0.57	-1.64	-0.31	4.50	ok	even



11. HPLC, ¹H NMR, ³¹P NMR and HRMS of P_S²-cIDPRE-1

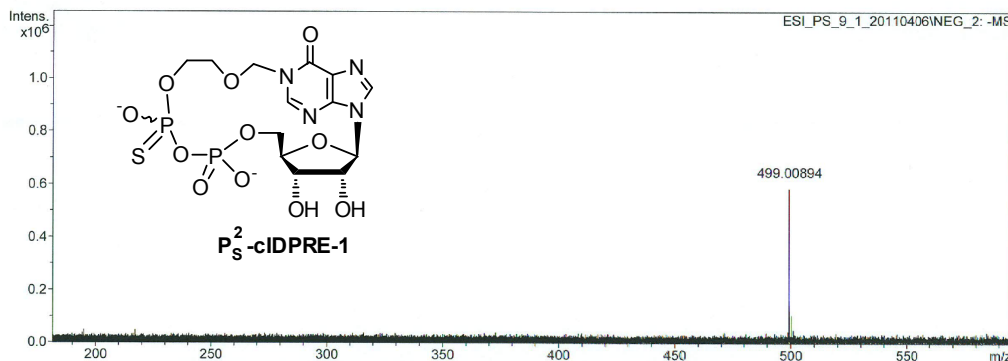




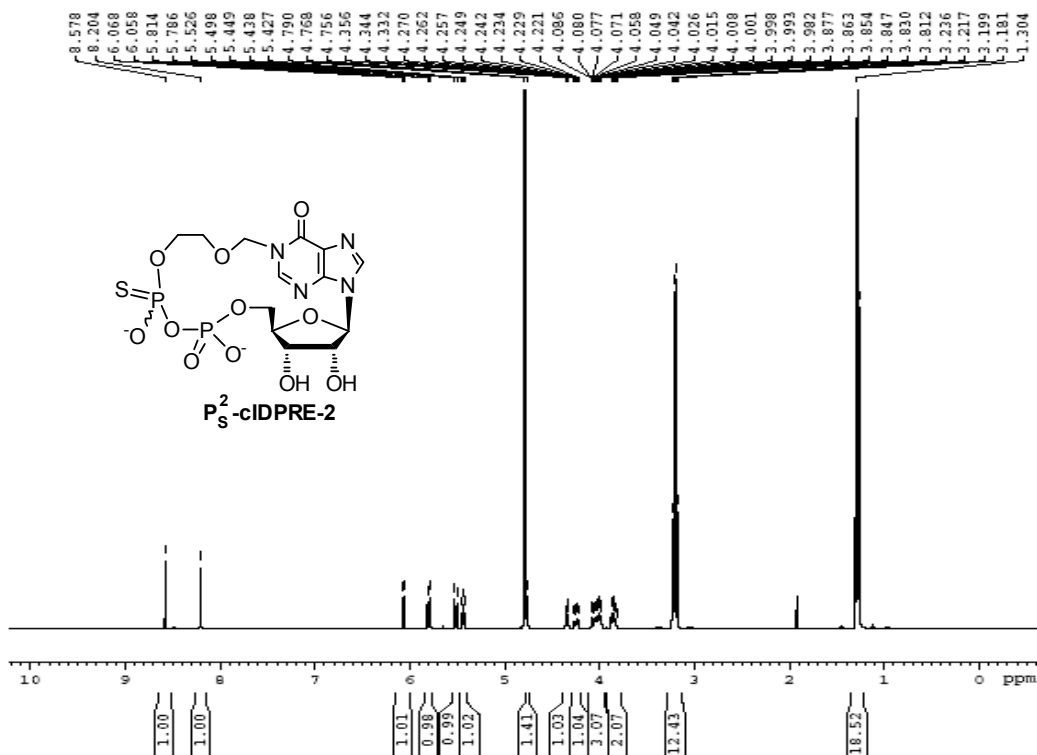
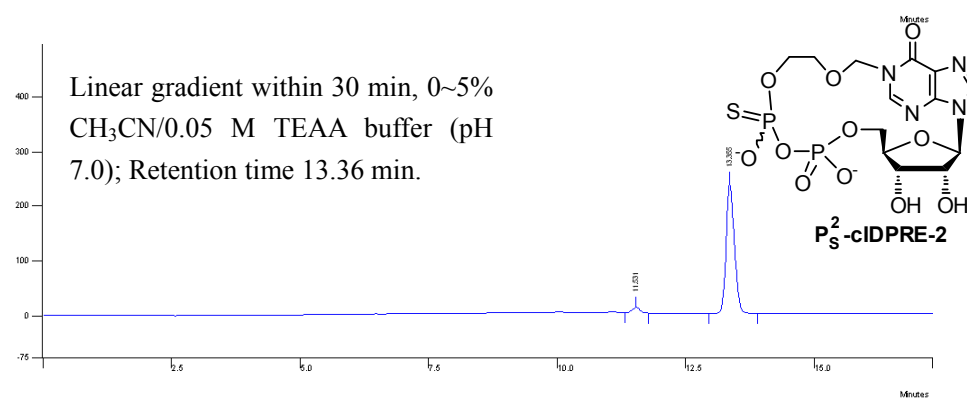
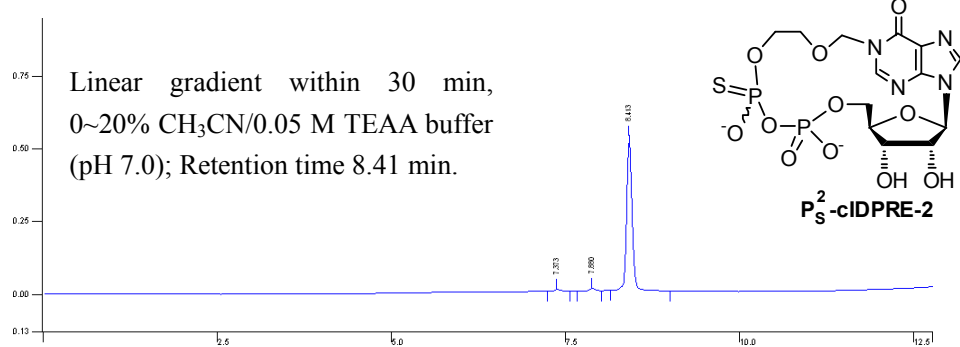
STATE KEY LABORATORY OF NATURAL AND BIOMIMETIC DRUGS (PEKING UNIVERSITY)

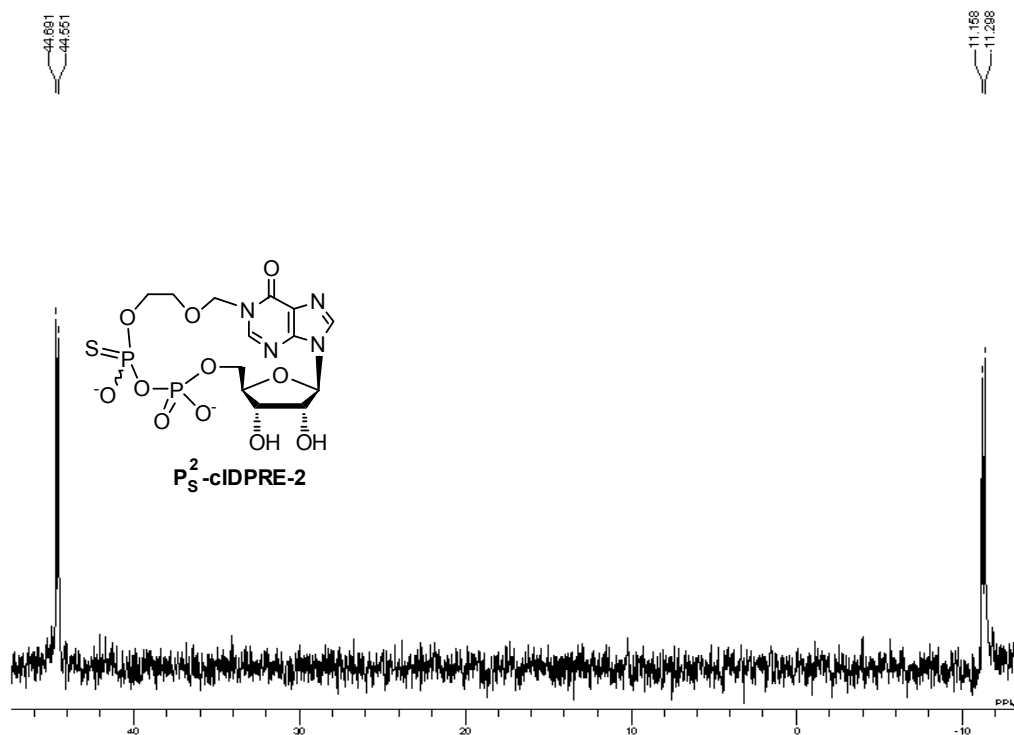
Analysis Name C:\data_sample\ESI_20110401_10\ESI_PS_9_1_20110406\NEG_ Acquisition Date 06/04/2011 14:32:36
 Method 2 Operator bpfxsh@bjmu.edu.cn; Tel: 010_82801437
 Sample Name Instrument FT_MS_Bruker APEX IV (7.0 T)
 Comment ESI C13H18N4O11P2S1 MW 500.0168
 CALSAME: NEG 216.98461; 615.12089; 833.11525
 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451;
 335.99207; 414.00562; 509.25407; 826.47121351; 96445
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
 D1= 2s; ns=8 100uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	499.00953	499.00894	0.59	1.18	M-H; ⁺ e



12. HPLC, ¹H NMR, ³¹P NMR and HRMS of P_S²-cIDPRE-2

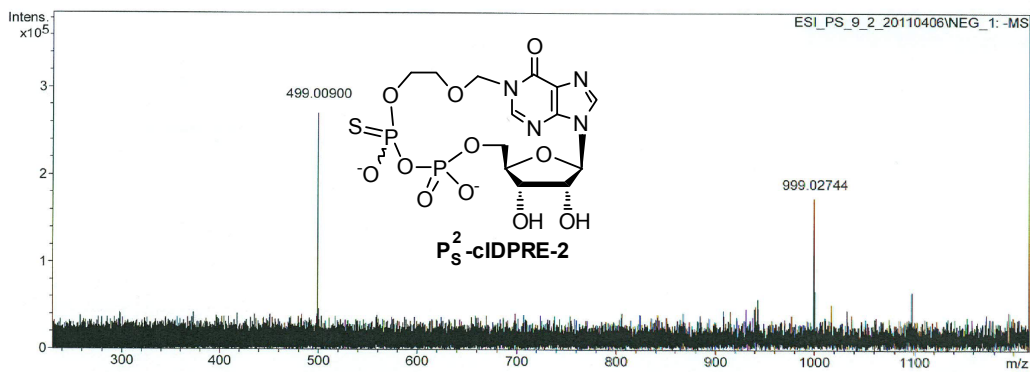




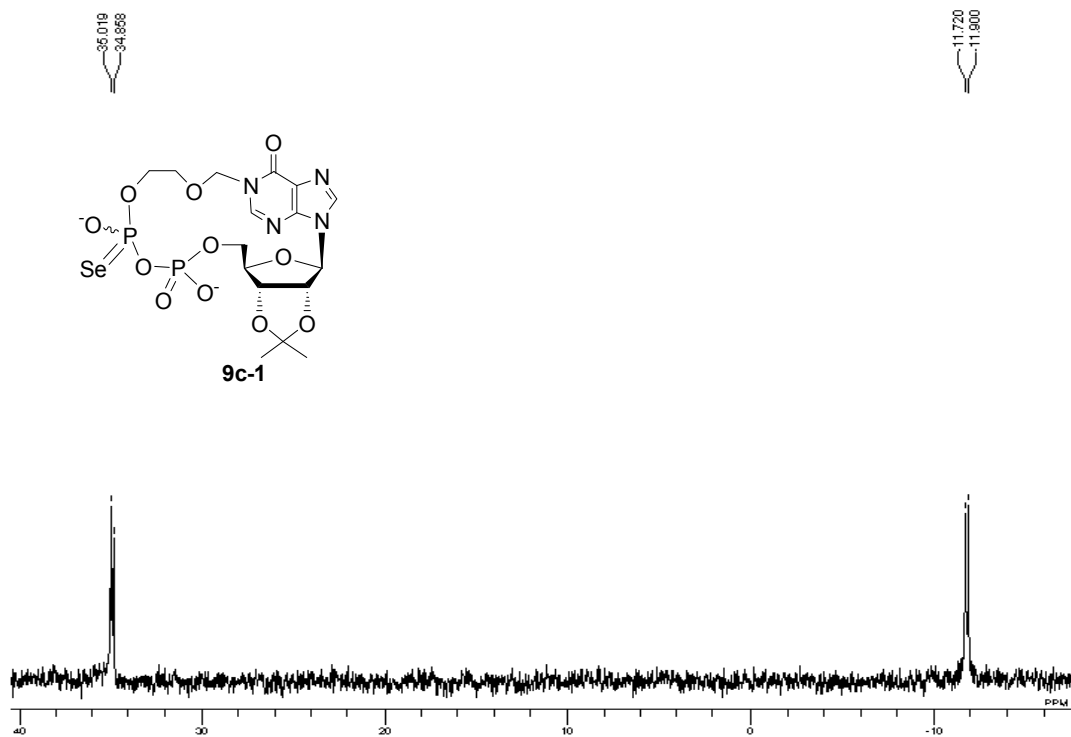
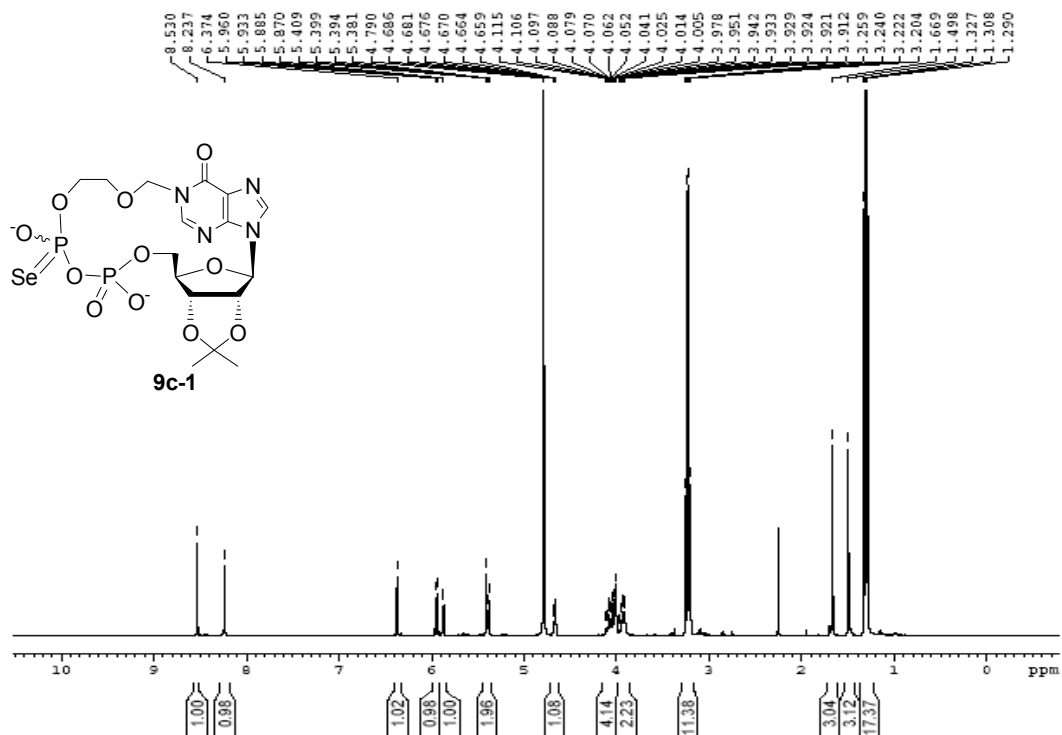
Bruker APEX IV FT_MS (7.0 T) Report (all)

<p>Analysis Info</p> <p>Analysis Nam C:\data_sample\ESI_20110401_10\ESI_PS_9_2_20110406\NEG_1</p> <p>Method Operator bpfxsh@bjmu.edu.cn; Tel: 010_82801437</p> <p>Sample Name Instrument FT_MS_Bruker APEX IV (7.0 T)</p> <p>Comment ESI C₁₃H₁₈N₄O₁₁P₂S₁ MW 500.0168 CALSAME; NEG 216.98461; 615.12089; 833.11525 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451; 335.99207; 414.00562; 509.25407; 826.47121351; 96445 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-3000; 1024K; D1= 2s ; ns=8 100uL/h</p>	<p>Acquisition Date 06/04/2011 14:38:47</p>
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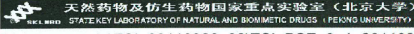
Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
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	999.02633	999.02744	-1.11	-1.11	2M-H; ⁺ e



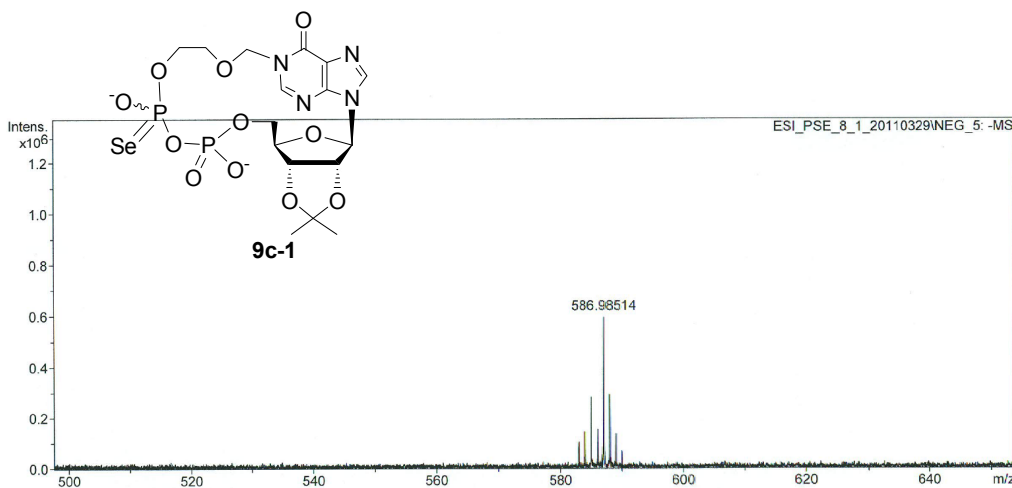
13. ^1H NMR, ^{31}P NMR and HRMS of 9c-1



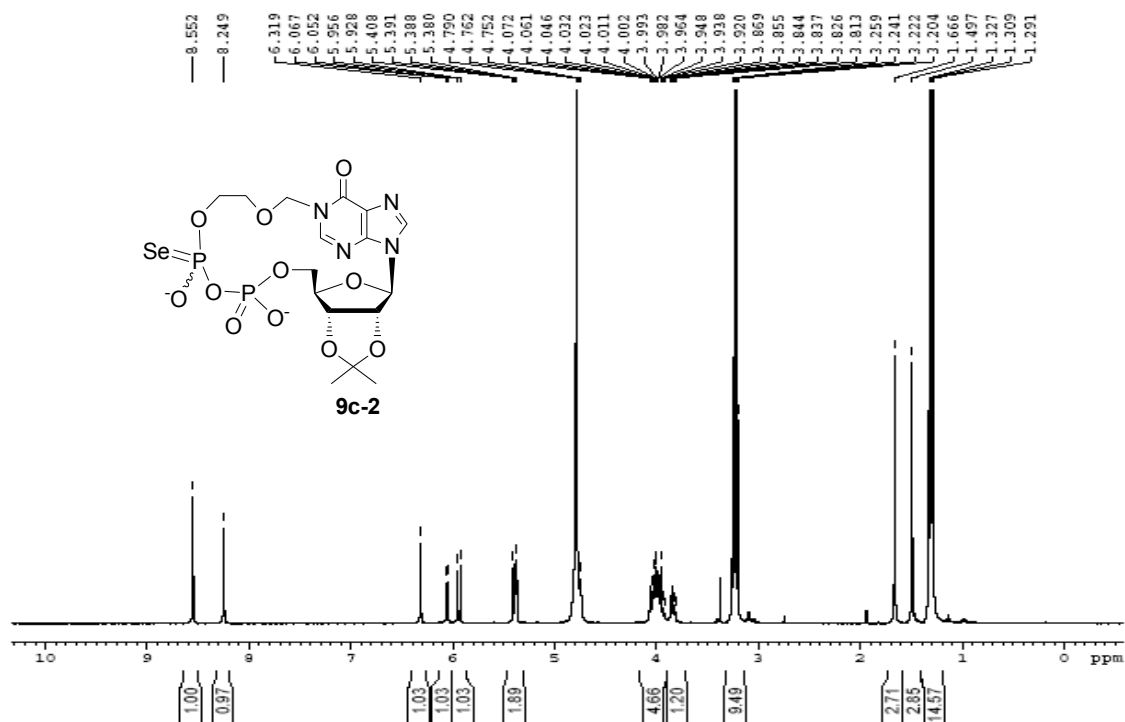
Bruker APEX IV FT_MS (7.0 T) Report (all)

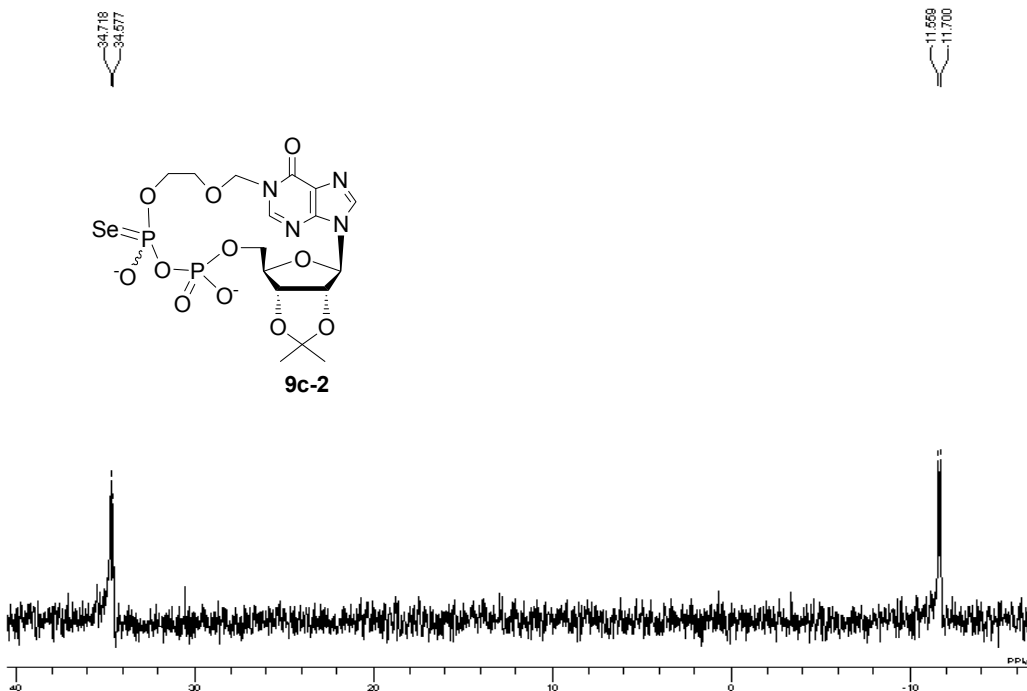
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 Method Operator bpfxsh@bjmu.edu.cn; Tel: 010_82801437
 Sample Name Instrument FT_MS_Bruker APEX IV (7.0 T)
 Comment ESI C₁₆H₂₂N₄O₁₁P₂Se MW 587.99201
 CALSAME; NEG 216.98461; 615.12089; 833.11525
 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451;
 335.99207; 414.00562; 509.25407; 826.47121351; 96445
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
 D1= 2s; ns=8 100uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	586.98473	586.98514	-0.41	-0.70	M-H;+e



14. ¹H NMR, ³¹P NMR and HRMS of 9c-2





Bruker APEX IV FT_MS (7.0 T) Report (all)

Analysis Info 天然药物及仿生药物国家重点实验室 (北京大学)
 STATE KEY LABORATORY OF NATURAL AND BIOMIMETIC DRUGS, PEKING UNIVERSITY

Acquisition Date: 29/03/2011 10:07:31

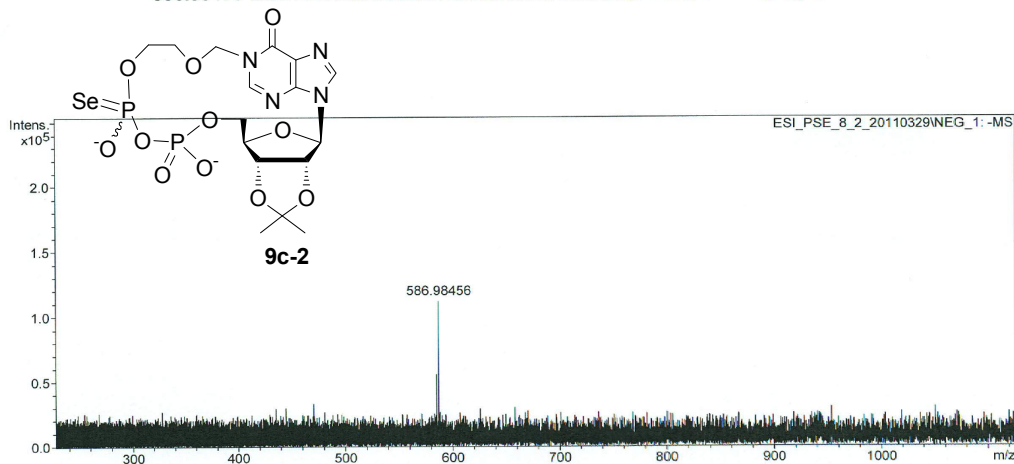
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Method: Operator: bpfxsh@bjmu.edu.cn; Tel: 010_82801437

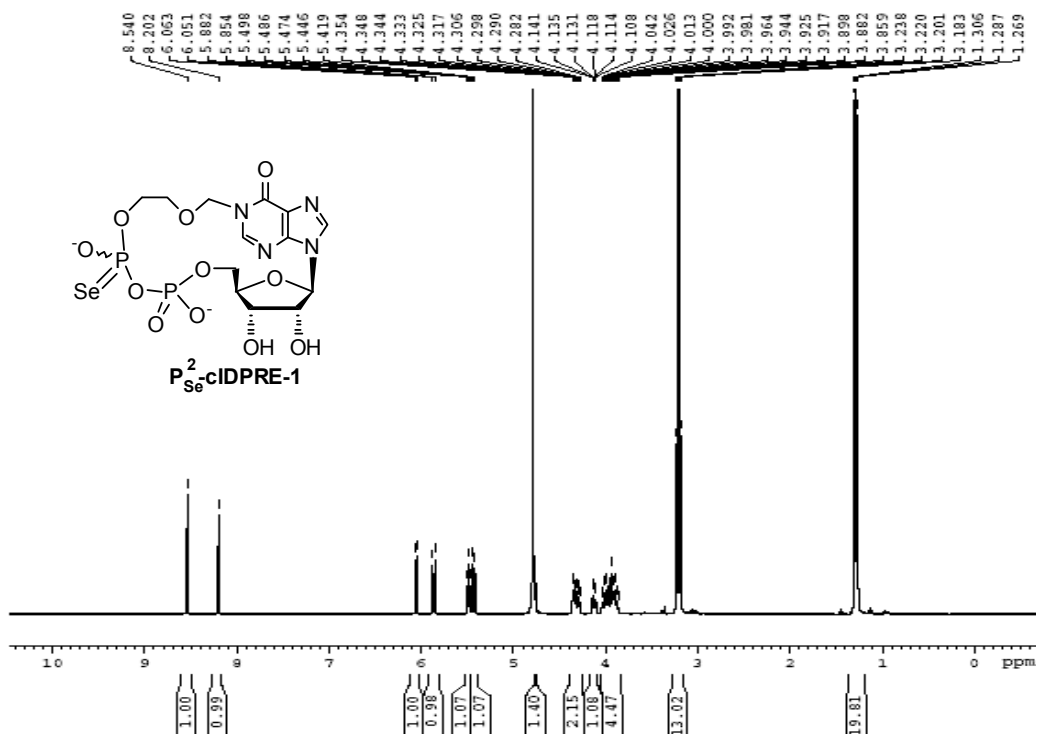
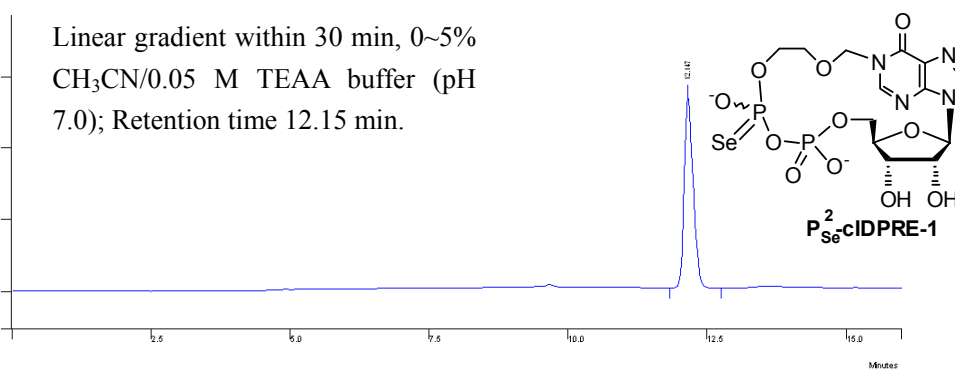
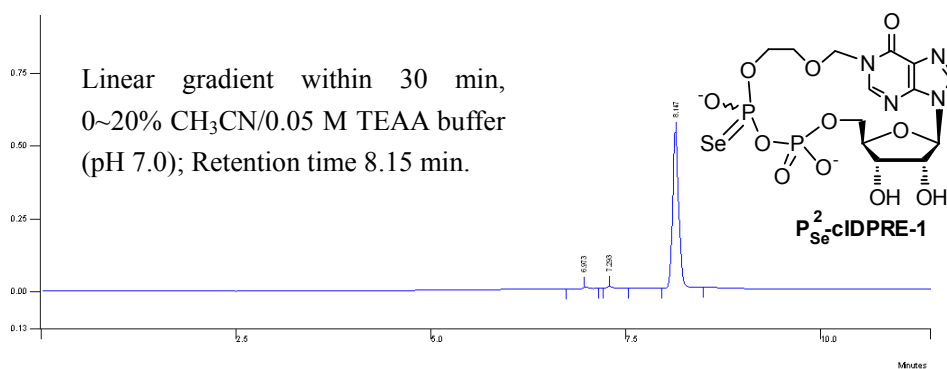
Sample Name: Instrument: FT_MS_Bruker APEX IV (7.0 T)

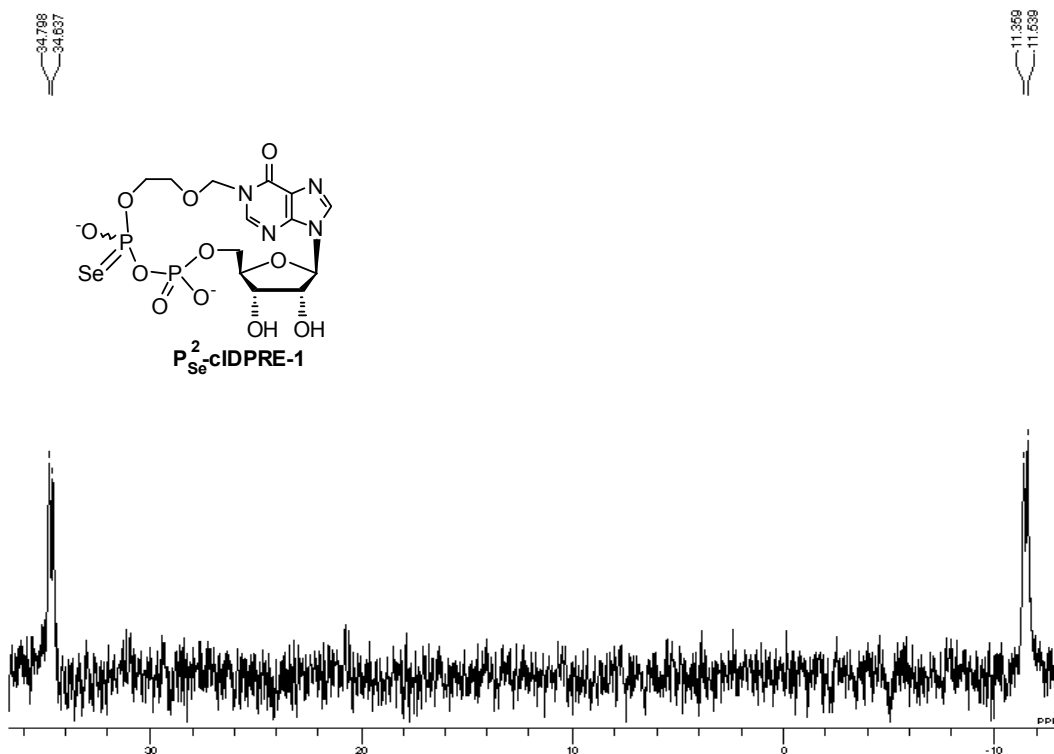
Comment: ESI C16H22N4O11P2SE MW 587.99201
 CALSAME: NEG 216.98461; 615.12089; 833.11525
 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451;
 335.99207; 414.00562; 509.25407; 826.47121351; 96445
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
 D1= 2s; ns=8 100uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	586.98473	586.98456	0.17	0.29	M-H; ⁺ e



15. HPLC, ¹H NMR, ³¹P NMR and HRMS of P_{Se}²-cIDPRE-1





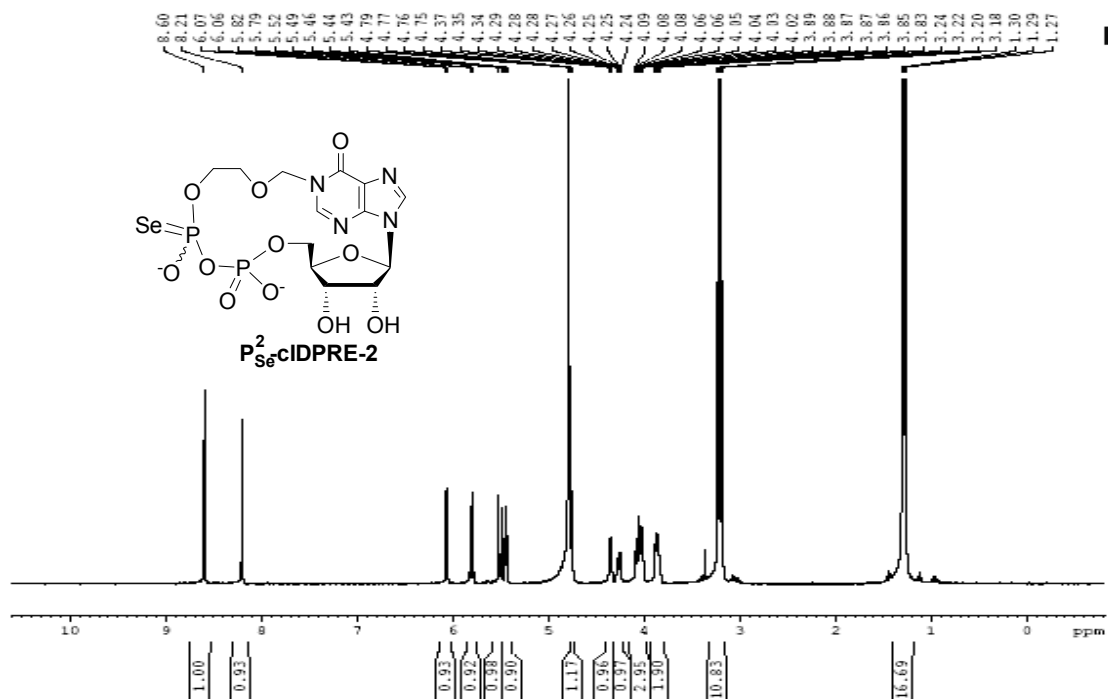
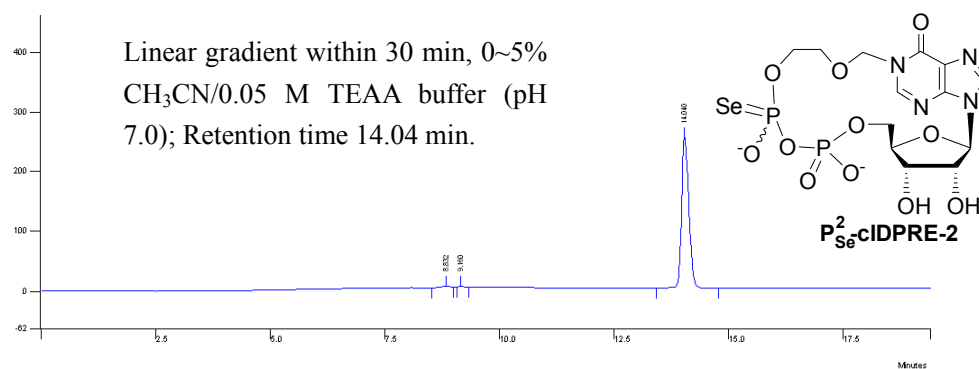
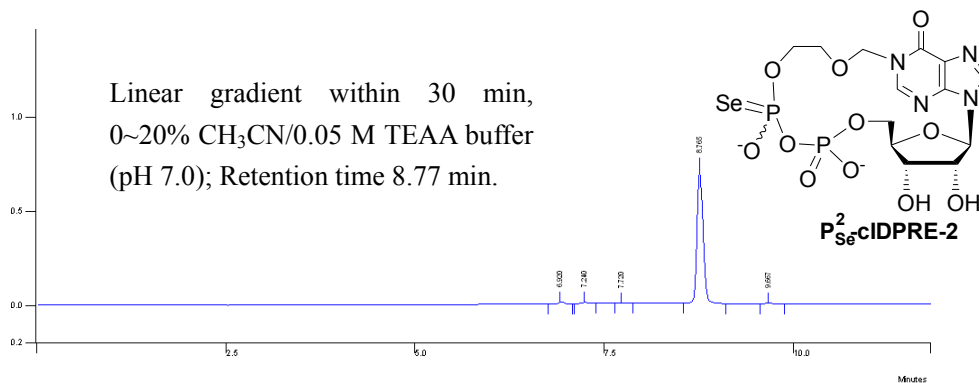
STATE KEY LABORATORY OF NATURAL AND BIOMIMETIC DRUGS (PEKING UNIVERSITY)

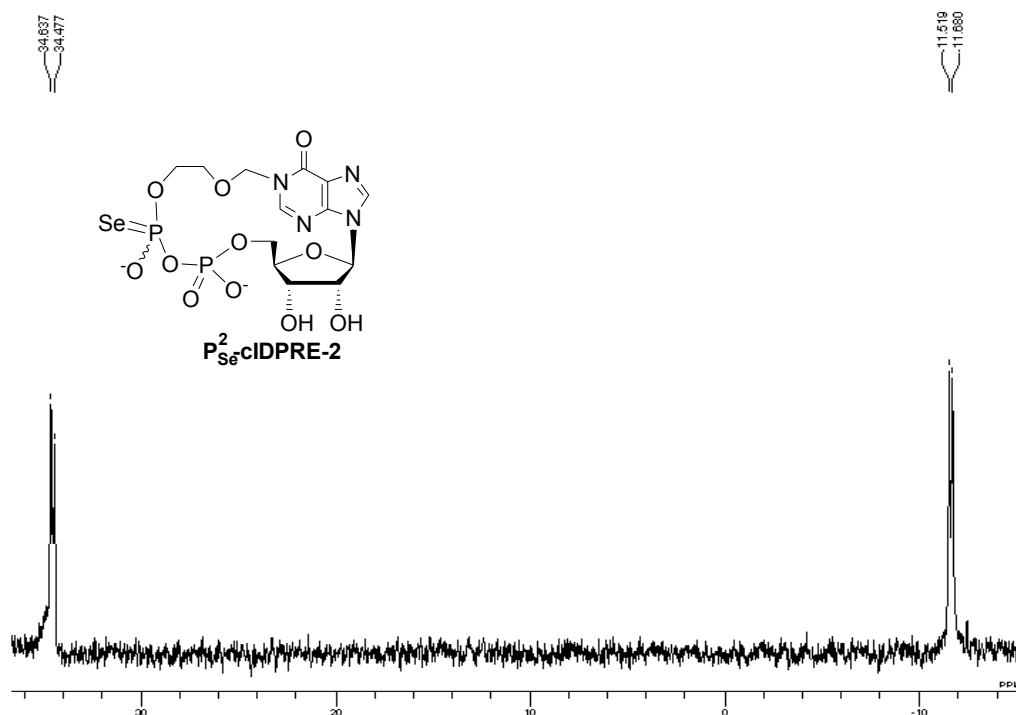
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 Method _2 Operator bpxsh@bjmu.edu.cn; Tel: 010_82801437
 Sample Name Instrument FT_MS_Bruker APEX IV (7.0 T)
 Comment ESI C₁₃H₁₈N₄O₁₁P₂Se₁ MW 547.96071
 CALSAME; NEG 216.98461; 615.12089; 833.11525
 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451;
 335.99207; 414.00562; 509.25407; 826.47121351.96445
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
 D1= 2s ; ns=8 100uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	546.95343	546.95402	-0.59	-1.08	M-H; ⁺ e



16. HPLC, ¹H NMR, ³¹P NMR and HRMS of P_{Se}²-cIDPRE-2

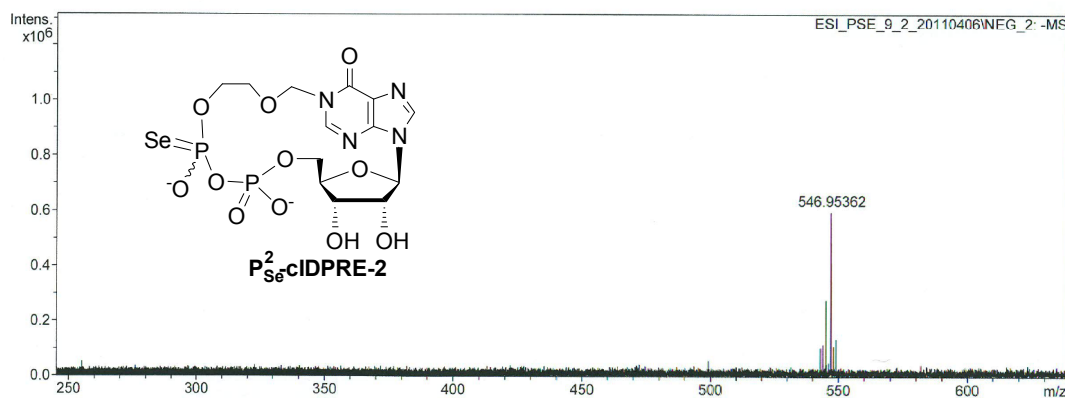




STATE KEY LABORATORY OF NATURAL AND BIOMIMETIC DRUGS (PEKING UNIVERSITY)

Analysis Name C:\data_sample\ESI_20110401_10\ESI_PSE_9_2_20110406\NEG Acquisition Date 06/04/2011 14:54:35
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 Sample Name Instrument FT_MS_Bruker APEX IV (7.0 T)
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 CALSAME; NEG 216.98461; 615.12089; 833.11525
 POS 399.14451; 617.13639; 835.12827; 1053.12015; 1271.11203; 1489.10391; 1707.09579320.01451;
 335.99207; 414.00562; 509.25407; 826.47121351; 96445
 in 1:1 H₂O:MeOH; CapExit 150V; m/z 150-3000; 1024K;
 D1= 2s ; ns=8 100uL/h

Acquisition Result:	Exact Mass	Measured Mass	Error (mm/e)	Error (ppm)	Description
	546.95343	546.95362	-0.19	-0.35	M-H; ⁺ e



17. Bioassay results of P_S¹-cIDPRE-1 and P_S¹-cIDPRE-2

The bioassay was carried out with similar procedures of reference **6e** and **6f** (Reference **6e**. M. Dong, T. Kirchberger, X. C. Huang, Z. J. Yang, L. R. Zhang, A. H. Guse and L. H. Zhang, *Org. Biomol. Chem.*, 2010, **8**, 4705-4715; **6f**. L. J. Li, C. C. Siebrands, Z. J. Yang, L. R. Zhang, A. H. Guse and L. H. Zhang, *Org. Biomol. Chem.*, 2010, **8**, 1843-1848).

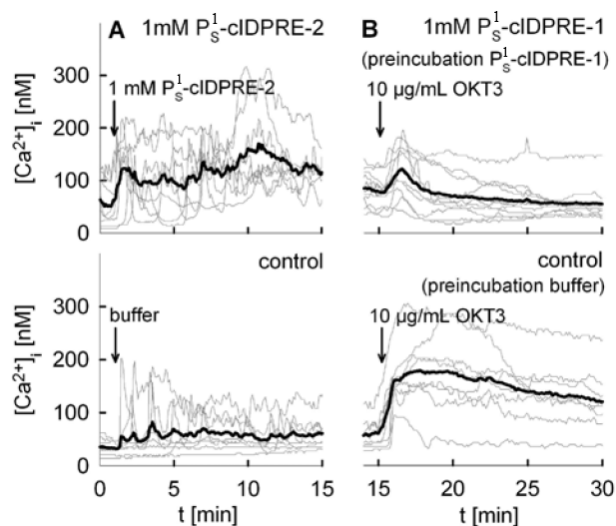


Fig. S-1 Effects of P_S¹-cIDPRE-1 and P_S¹-cIDPRE-2 on the free cytosolic Ca²⁺ concentration. Jurkat T-cells loaded with the fluorescent Ca²⁺ dye Fura2-AM were analyzed over 14 minutes after addition of compounds or vehicle. (A) Stimulation with 1 mM compound P_S¹-cIDPRE-2 (n = 8) evoked Ca²⁺ release in contrast to buffer control (n = 8); (B) Application of OKT3 (10 μg/mL) in the presence of 1 mM cIDPRE-analogue P_S¹-cIDPRE-1 (n = 11) (14 minutes preincubation) resulted in significantly lower Ca²⁺ signaling as compared to OKT3 alone (n = 8). Gray line: time courses of single cells; black line: mean of 8-11 cells. The arrow indicates time of compound or vehicle application.

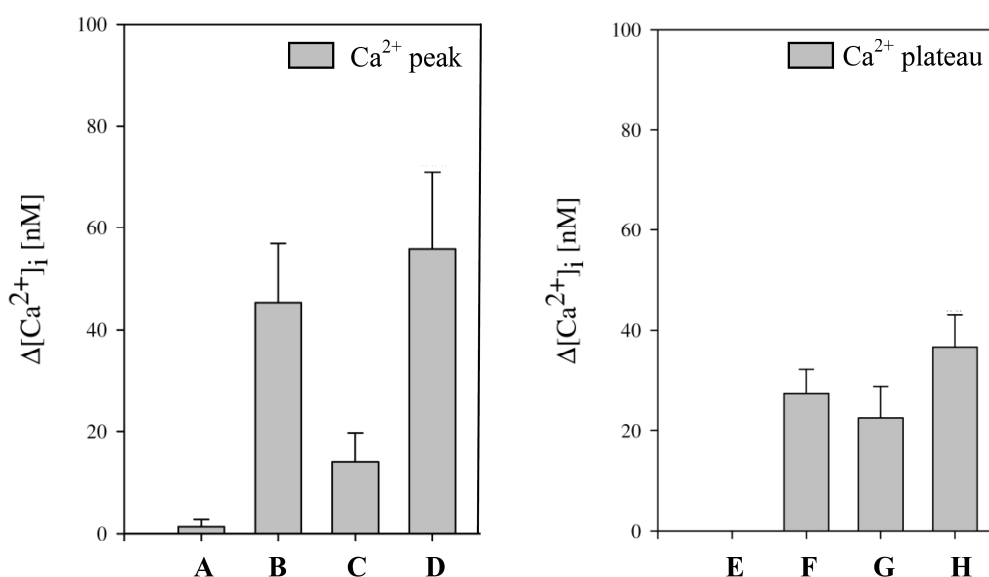


Fig. S-2 Comparison of effects of cIDPRE, P_s¹-cIDPRE-1 and P_s¹-cIDPRE-2 on Ca²⁺ release. Jurkat T-cells loaded with the fluorescent Ca²⁺ dye Fura2-AM were analyzed over 14 minutes after addition of compounds or vehicle. Ca²⁺ peak: difference between the Ca²⁺ concentration 0.4 minutes after compound application and the basal Ca²⁺ concentration (after one minute); Ca²⁺ plateau: difference between the Ca²⁺ concentration ten minutes after compound application and the basal Ca²⁺ concentration (after one minute). (A) Ca²⁺ peak stimulated by buffer control (n = 57); (B) Ca²⁺ peak stimulated by 1 mM cIDPRE (n = 58); (C) Ca²⁺ peak stimulated by 1 mM P_s¹-cIDPRE-1 (n = 39); (D) Ca²⁺ peak stimulated by 1 mM P_s¹-cIDPRE-2 (n = 33); (E) Ca²⁺ plateau stimulated by buffer control (n = 57); (F) Ca²⁺ plateau stimulated by 1 mM cIDPRE (n = 58); (G) Ca²⁺ plateau stimulated by 1 mM P_s¹-cIDPRE-1 (n = 39); (H) Ca²⁺ plateau stimulated by 1 mM P_s¹-cIDPRE-2 (n = 33).

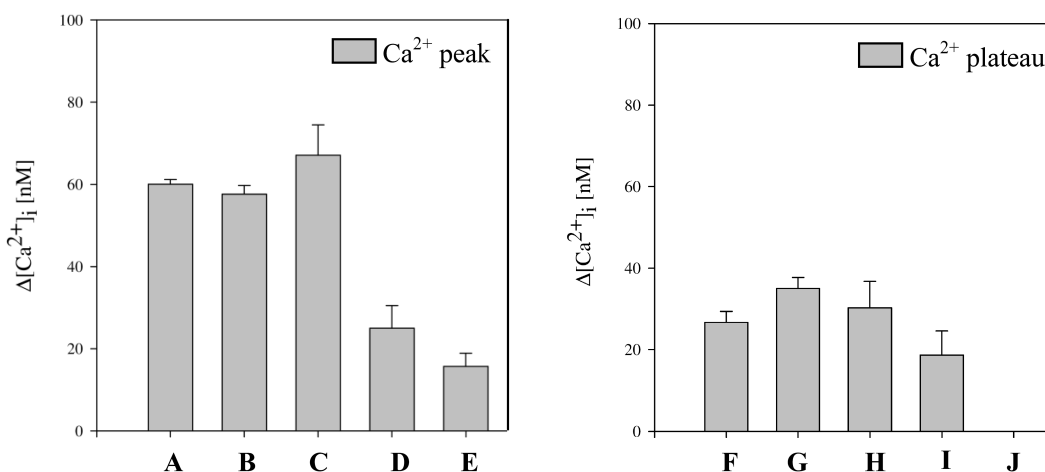


Fig. S-3 Antagonistic effects of P_s¹-cIDPRE-1 on Ca²⁺ release. Jurkat T-cells loaded with the fluorescent Ca²⁺ dye Fura2-AM were analyzed over 14 minutes after addition of compounds or vehicle. Ca²⁺ Peak: difference between the Ca²⁺ concentration 2.5 minutes after OKT3 application and the basal Ca²⁺ concentration (after 15 minutes); Ca²⁺ Plateau: difference between the Ca²⁺ concentration ten minutes after OKT3 application and the basal Ca²⁺ concentration (after 15 minutes). (A) Ca²⁺ peak stimulated by 1 mM cIDPRE (n = 58); (B) Ca²⁺ peak stimulated by OKT3 (10 μg/mL) in the presence of buffer (14 minutes

preincubation) (n = 41); (C) (D) (E) Ca²⁺ peak stimulated by OKT3 (10 µg/mL) in the presence of 100 µM (n = 35), 500 µM (n = 31) or 1 mM (n = 39) P_S¹-cIDPRE-1 (14 minutes preincubation); (F) Ca²⁺ plateau stimulated by 1 mM cIDPRE (n = 58); (G) Ca²⁺ plateau stimulated by OKT3 (10 µg/mL) in the presence of buffer (14 minutes preincubation) (n = 41); (H) (I) (J) Ca²⁺ plateau stimulated by OKT3 (10 µg/mL) in the presence of 100 µM (n = 35), 500 µM (n = 31) or 1 mM (n = 39) P_S¹-cIDPRE-1 (14 minutes preincubation).

18. Procedures for preparation of P_S¹-cIDPRE-1, P_S¹-cIDPRE-2, P_{Se}¹-cIDPRE-1, P_{Se}¹-cIDPRE-2, P_S²-cIDPRE-1, P_S²-cIDPRE-2, P_{Se}²-cIDPRE-1 and P_{Se}²-cIDPRE-2

All solvents were dried and distilled prior to use. Unless otherwise noted, materials were obtained from commercial suppliers and were used as provided. Evaporations were carried out under reduced pressure with a bath temperature of 35 °C. ¹H NMR data were recorded with Bruker Avance III 400 spectrometer; Chemical shifts were reported in parts per million downfield from TMS. ³¹P NMR spectra were recorded at room temperature using Bruker Avance 300 (121.5 MHz) or JNM-ECA 600 (243.0 MHz) spectrometer; Orthophosphoric acid (85%) was used as an external standard. HR-ESI-MS (electrospray ionization) were attained with Bruker APEX IV. Optical rotation was determined with Perkin-Elmer 243B polarimeter. The analysis of compounds was performed on Welch XB analytical C₁₈ reversed-phase column (5 µm, 4.6 x 150 mm) with Varian HPLC by the buffer system: MeCN/TEAA (pH = 7.0). The purification of compounds was performed on Agela Venusil XBP preparative C₁₈ reversed-phase column (10 µm, 22 ×250 mm) with Gilson HPLC by the buffer system: MeCN/TEAA (pH = 7.0).

Methods for preparative HPLC:

Method A: performed with C₁₈ reversed-phase column, eluting with a linear gradient of 0-40% CH₃CN in TEAA buffer solution (0.05 M, pH 7.0), 5 mL/min of flow rate, 260 nm of detection wavelength.

Method B: performed with C₁₈ reversed-phase column, eluting with a linear gradient of 0-15% CH₃CN in TEAA buffer solution (0.05 M, pH 7.0), 5 mL/min of flow rate, 260 nm of detection wavelength.

***N*¹-[(5''-*O*-phosphorylethoxy)methyl]-5'-*O*-phosphorothioate-2',3'-*O*-isopropylideneinosine-5',5''-cyclicpyrophosphate **5b-1** and **5b-2**.** 2-Cyanoethoxy-*N,N,N',N'*-tetra-isopropylphosphoramidite (41 μ L, 0.13 mmol) was added to a solution of **2** (50 mg, 89 μ mol) and 1*H*-tetrazole (19 mg, 0.27 mmol) in CH₃CN (30 mL) under argon. After the reaction was continued for 0.5 h, CS₂ solution (30 mL) of S₈ (32 mg, 1 mmol) was added and the reaction was continued for 8 h. After the reaction mixture was evaporated, the residue resolved in H₂O and extracted with CHCl₃. The aqueous layer was concentrated. The purification was performed with method A for preparative HPLC to give compound **4b** (a pair of diastereoisomers, unseparated, 37.6 mg, 61%). Compound **4b** was resolved in 1M TEAB bicarbonate buffer (pH = 7.5, 5 mL) and the reaction was stirred for 3h at room temperature. After evaporation, the separation of the mixture was performed with method A for preparative HPLC. The fractions of **5b-1** (13.4 mg, 32%) and **5b-2** (23.0 mg, 58%) were collected separately. Data for **5b-1**: δ_{H} (400MHz; D₂O) 1.47, 1.63 (each s, each 3H, (CH₃)₂C), 3.86-3.95 (m, 6H, H₅'', OCH₂CH₂OP), 4.62-4.63 (m, 1H, H₄''), 5.35-5.39 (m, 2H, H₁'',a, H₃''), 5.92-5.95 (m, 2H, H₁'',b, H₂''), 6.35 (s, 1H, H₁''), 8.21 (s, 1H, H₈), 8.49 (s, 1H, H₂); δ_{P} (121.5 MHz; D₂O; decoupled with ¹H) -10.84 (s), 43.94 (s); m/z (ESI-TOF⁻) 539.0402 (M⁻ requires 539.0408). Data for **5b-2**: δ_{H} (400MHz; D₂O) 1.47, 1.63 (each s, each 3H, (CH₃)₂C), 3.75-3.76 (m, 1H, H_{5a}''), 3.88-4.06 (m, 5H, H_{5b}'', OCH₂CH₂OP), 4.70 (m, 1H, H₄''), 5.30-5.31 (m, 1H, H₃''), 5.38 (d, 1H, J_{H₁'',a, H₁'',b} = 11.2 Hz, H₁'',a), 5.90 (d, 1H, J_{H₁'',b, H₁'',a} = 11.2 Hz, H₁'',b), 5.94 (m, 1H, H₂''), 6.30 (s, 1H, H₁''), 8.23 (s, 1H, H₈), 8.49 (s, 1H, H₂); δ_{P} (121.5 MHz; D₂O; decoupled with ¹H) -10.63 (s), 43.56 (s); m/z (ESI-TOF⁻) 539.0427 (M⁻ requires 539.0408).

***N*¹-[(5''-*O*-phosphorylethoxy)methyl]-5'-*O*-phosphorothioate-inosine-5',5''-cyclic pyrophosphate **P_S¹-cIDPRE-1** and **P_S¹-cIDPRE-2**.** The solution of **5b-1** (13.4 mg, 18 μ mol) in 60% HCOOH (5 mL) was stirred for 12 h and then evaporated under reduced pressure. The purification of the residue was performed with method A for preparative HPLC, and the main fraction was collected to give the target molecule **P_S¹-cIDPRE-1** (10.8 mg, 85%). The same treatment was imposed on **5b-2** to give **P_S¹-cIDPRE-2** (18.5 mg, 85%), which was purified with the same procedure. Data for **P_S¹-cIDPRE-1**: δ_{H} (400MHz; D₂O) 3.88-3.91 (m, 4H, OCH₂CH₂OP), 4.09-4.10 (m, 1H, H_{5a}''), 4.18-4.21 (m, 1H, H_{5b}''), 4.30 (m, 1H, H₄''), 4.90-4.92 (m, 1H, H₃''), 5.38-5.40 (m, 1H, H₂''), 5.46 (d, 1H, J_{H₁'',a, H₁'',b} = 11.2 Hz, H₁'',a), 5.81 (d, 1H, J_{H₁'',b, H₁'',a} = 11.2 Hz, H₁'',b), 6.05 (d, 1H, J_{H₁'', H₂'} = 3.2 Hz, H₁''), 8.19 (s, 1H, H₈), 8.49 (s, 1H,

H₂); δ_P (243.0 MHz; D₂O; decoupled with ¹H) -10.67 (s), 43.15 (s); m/z (ESI-TOF⁻) 499.0089 (M⁻ requires 499.0095). Data for **P¹_S-cIDPRE-2**: δ_H (400MHz; D₂O) 3.82-3.85 (m, 2H, OCH₂), 3.95-3.99 (m, 2H, CH₂OP), 4.10-4.14 (m, 1H, H_{5a}'), 4.22-4.27 (m, 1H, H_{5b}'), 4.33 (m, 1H, H₄'), 4.79 (buried in H₂O residue peak, 1H, H₃'), 5.39-5.41 (m, 1H, H₂'), 5.51 (d, 1H, $J_{H1''a, H1''b} = 11.2$ Hz, H₁''a), 5.78 (d, 1H, $J_{H1''b, H1''a} = 11.2$ Hz, H₁''b), 6.06 (d, 1H, $J_{H1', H2'} = 3.6$ Hz, H₁'), 8.19 (s, 1H, H₈), 8.51 (s, 1H, H₂); δ_P (243.0 MHz; D₂O; decoupled with ¹H) -10.72 (s), 43.91 (s); m/z (ESI-TOF⁻) 499.0077 (M⁻ requires 499.0095).

N¹-[(5''-O-phosphorylethoxy)methyl]-5'-O-phosphoroselenoate-2',3'-O-isopropylidene-inosine-5',5''-cyclicpyrophosphate 5c-1 and 5c-2. 2-Cyanoethoxy-*N,N,N',N'*-tetra-isopropylphosphoramidite (41 μ L, 0.13 mmol) was added to a solution of **2** (50 mg, 89 μ mol) and 1*H*-tetrazole (19 mg, 0.27 mmol) in CH₃CN (30 mL) under argon. After the reaction was continued for 0.5 h, the CHCl₃ solution (30 mL) of Se (79 mg, 1 mmol) was added and the reaction was continued for 8 h. After the reaction mixture was evaporated, the residue resolved in H₂O and extracted with CHCl₃ and the aqueous layer was concentrated. The purification was performed with method A for preparative HPLC to give compound **4c** (a pair of diastereoisomers, unseparated, 23.0 mg, 35%). Compound **4c** was resolved in 1M TEAB bicarbonate buffer (pH = 7.5, 5 mL) and the reaction was stirred for 3h at room temperature. After evaporation, the separation of the mixture was performed with method A for preparative HPLC. The fractions of **5c-1** (7.9 mg, 32%) and **5c-2** (13.4 mg, 54%) were collected separately. Data for **5c-1**: δ_H (400MHz; D₂O) 1.48, 1.63 (each s, each 3H, (CH₃)₂C), 3.88-3.95 (m, 6H, H₅', OCH₂CH₂OP), 4.65-4.66 (m, 1H, H₄'), 5.37 (d, 1H, $J_{H1''a, H1''b} = 11.2$ Hz, H₁''a), 5.39-5.40 (m, 1H, H₃'), 5.94-5.96 (m, 2H, H₁''b, H₂'), 6.35 (s, 1H, H₁'), 8.22 (s, 1H, H₈), 8.49 (s, 1H, H₂); δ_P (121.5 MHz; D₂O; decoupled with ¹H) -11.39 (s), 34.41 (s); m/z (ESI-TOF⁻) 586.9864 (M⁻ requires 586.9847). Data for **5c-2**: δ_H (400MHz; D₂O) 1.47, 1.63 (each s, each 3H, (CH₃)₂C), 3.74-3.75 (m, 1H, H_{5a}'), 3.89-4.10 (m, 5H, H_{5b}', OCH₂CH₂OP), 4.72 (m, 1H, H₄'), 5.31-5.32 (m, 1H, H₃'), 5.38 (d, 1H, $J_{H1''a, H1''b} = 10.8$ Hz, H₁''a), 5.89-5.93 (m, 2H, H₁''b, H₂'), 6.30 (s, 1H, H₁'), 8.24 (s, 1H, H₈), 8.49 (s, 1H, H₂); δ_P (121.5 MHz; D₂O; decoupled with ¹H) -11.14 (s), 33.98 (s); m/z (ESI-TOF⁻) 586.9861 (M⁻ requires 586.9847).

N¹-[(5''-O-phosphorylethoxy)methyl]-5'-O-phosphoroselenoate-inosine-5',5''-cyclic-pyrophosphate P¹_{Sc}-cIDPRE-1 and P¹_{Sc}-cIDPRE-2. The solution of **5c-1** (7.9 mg, 10 μ mol) in 60% HCOOH (5 mL) was stirred for 12 h and then evaporated under reduced pressure. The

purification of the residue was performed with method A for preparative HPLC, and the main fraction was collected to give the target molecule **P¹_{sc}-cIDPRE-1** (6.5 mg, 87%). The same treatment was imposed on **5c-2** (13.4 mg, 17 μmol) to give **P¹_{sc}-cIDPRE-2** (10.8 mg, 85%), which was purified with the same procedure. Data for **P¹_{sc}-cIDPRE-1**: δ_H(400MHz; D₂O) 3.86-3.93 (m, 4H, OCH₂CH₂OP), 4.10-4.12 (m, 1H, H_{5a}'), 4.18-4.19 (m, 1H, H_{5b}'), 4.31 (m, 1H, H₄'), 4.92-4.95 (m, 1H, H₃'), 5.36-5.38 (m, 1H, H₂'), 5.47 (d, 1H, J_{H₁'_a,H₁'_b} = 11.2 Hz, H₁'_a), 5.82 (d, 1H, J_{H₁'_b,H₁'_a} = 11.2 Hz, H₁'_b), 6.06 (d, 1H, J_{H₁',H₂'} = 2.8 Hz, H₁'), 8.19 (s, 1H, H₈), 8.49 (s, 1H, H₂); δ_P(121.5 MHz; D₂O; decoupled with ¹H) -11.15 (s), 32.97 (s); m/z (ESI-TOF⁻) 546.9509 (M⁻ requires 546.9534). Data for **P¹_{sc}-cIDPRE-2**: δ_H(400MHz; D₂O) 3.81-3.85 (m, 2H, OCH₂), 3.97-4.01 (m, 2H, CH₂OP), 4.11-4.15 (m, 1H, H_{5a}'), 4.24-4.29 (m, 1H, H_{5b}'), 4.33 (m, 1H, H₄'), 4.83 (m, 1H, H₃'), 5.37-5.39 (m, 1H, H₂'), 5.50 (d, 1H, J_{H₁'_a,H₁'_b} = 10.8 Hz, H₁'_a), 5.79 (d, 1H, J_{H₁'_b,H₁'_a} = 10.8 Hz, H₁'_b), 6.06 (d, 1H, J_{H₁',H₂'} = 3.6 Hz, H₁'), 8.19 (s, 1H, H₈), 8.52 (s, 1H, H₂); δ_P(121.5 MHz; D₂O; decoupled with ¹H) -11.10 (s), 34.16 (s); m/z (ESI-TOF⁻) 546.9502 (M⁻ requires 546.9534).

N¹-[(5''-O-(phosphorothioate)ethoxy)methyl]-5'-O-phosphoryl-2',3'-O-isopropylideneinosine-5',5''-cyclicpyrophosphate 9b-1 and 9b-2. 2-Cyanoethoxy-*N,N,N',N'*-tetraisopropyl phosphoramidite (0.1 mL, 0.33 mmol) was added to a solution of **6** (0.12 g, 0.22 mmol) and 1*H*-tetrazole (46 mg, 0.66 mmol) in CH₃CN (80 mL) under argon. After the reaction was continued for 0.5 h, CS₂ solution (30 mL) of S₈ (69 mg, 2.16 mmol) was added and the reaction was continued for 8 h. After the reaction mixture was evaporated, the residue resolved in H₂O and extracted with CHCl₃. The aqueous layer was concentrated. The purification was performed with method A for preparative HPLC to give compound **8b** (a pair of diastereoisomers, unseparated, 84 mg, 57%). Compound **8b** was resolved in 1M TEAB bicarbonate buffer (pH = 7.5, 10 mL) and the reaction was stirred for 6h at 35°C. After evaporation, the separation of the mixture was performed with method B for preparative HPLC. The fractions of **9b-1** (37 mg, 41%) and **9b-2** (48 mg, 54%) were collected separately. Data for **9b-1**: δ_H(400 MHz; D₂O) 1.48, 1.65 (each s, each 3H, (CH₃)₂C), 3.87-4.08 (m, 6H, H₅', OCH₂CH₂OP), 4.65-4.66 (m, 1H, H₄'), 5.36-5.40 (m, 2H, H₃', H₁'_a), 5.87-5.88 (m, 1H, H₂'), 5.94 (d, 1H, J_{H₁'_b,H₁'_a} = 10.8 Hz, H₁'_b), 6.36 (s, 1H, H₁'), 8.22 (s, 1H, H₈), 8.52 (s, 1H, H₂); δ_P(121.5 MHz; D₂O; decoupled with ¹H) -11.45 (d, J_{p,p} = 17.0 Hz), 45.00 (d, J_{p,p} = 17.0 Hz); m/z (ESI-TOF⁻) 539.0394 (M⁻ requires 539.0408). Data for **9b-2**: δ_H(400 MHz; D₂O)

1.48, 1.65 (each s, each 3H, (CH₃)₂C), 3.79-4.03 (m, 6H, H₅, OCH₂CH₂OP), 4.72 (m, 1H, H₄'), 5.35-5.37 (m, 1H, H₃'), 5.39 (d, 1H, J_{H1''a, H1''b} = 10.8 Hz, H₁''a), 5.92 (d, 1H, J_{H1''b, H1''a} = 10.8 Hz, H₁''b), 6.01-6.03 (m, 1H, H₂'), 6.31 (s, 1H, H₁'), 8.24 (s, 1H, H₈), 8.53 (s, 1H, H₂); δ_p(121.5 MHz; D₂O; decoupled with ¹H) -11.34 (d, J_{p,p} = 17.0 Hz), 44.84 (d, J_{p,p} = 17.0 Hz); m/z (ESI-TOF⁻) 539.0398 (M⁻ requires 539.0408).

N¹-[(5''-O-(phosphorothioate)ethoxy)methyl]-5'-O-phosphoryl-inosine-5',5''-cyclic-pyrophosphate P_S²-cIDPRE-1 and P_S²-cIDPRE-2 The solution of **9b-1** (25 mg, 34 μmol) in 60% HCOOH (10 mL) was stirred for 20 h and then evaporated under reduced pressure. The purification of the residue was performed with method B for preparative HPLC, and the main fraction was collected to give the target molecule **P_S²-cIDPRE-1** (20 mg, 85%); The same treatment was imposed on **9b-2** (25 mg, 34 μmol) to give **P_S²-cIDPRE-2** (22 mg, 95%), which was purified with the same procedure. Data for **P_S²-cIDPRE-1**: δ_H(400 MHz; D₂O) 3.85-3.92 (m, 2H, OCH₂), 3.96-4.03 (m, 2H, CH₂OP), 4.09-4.13 (m, 1H, H_{5a}'), 4.29-4.35 (m, 2H, H₄', H_{5b}'), 4.77-4.78 (m, 1H, H₃'), 5.44 (d, 1H, J_{H1''a, H1''b} = 11.2 Hz, H₁''a), 5.49-5.51 (m, 1H, H₂'), 5.88 (d, 1H, J_{H1''b, H1''a} = 11.2 Hz, H₁''b), 6.06 (d, 1H, J_{H1', H2'} = 4.4 Hz, H₁'), 8.21 (s, 1H, H₈), 8.55 (s, 1H, H₂); δ_p(121.5 MHz; D₂O; decoupled with ¹H) -11.12 (d, J_{p,p} = 19.6 Hz), 44.73 (d, J_{p,p} = 19.6 Hz); m/z (ESI-TOF⁻) 499.0089 (M⁻ requires 499.0095). Data for **P_S²-cIDPRE-2**: δ_H(400 MHz; D₂O) 3.81-3.88 (m, 2H, OCH₂), 3.98-4.09 (m, 3H, H_{5a}', CH₂OP), 4.22-4.27 (m, 1H, H_{5b}'), 4.33-4.36 (m, 1H, H₄'), 4.76-4.77 (m, 1H, H₃'), 5.43-5.45 (m, 1H, H₂'), 5.51 (d, 1H, J_{H1''a, H1''b} = 11.2 Hz, H₁''a), 5.80 (d, 1H, J_{H1''b, H1''a} = 11.2 Hz, H₁''b), 6.06 (d, 1H, J_{H1', H2'} = 4 Hz, H₁'), 8.20 (s, 1H, H₈), 8.58 (s, 1H, H₂); δ_p(121.5 MHz; D₂O; decoupled with ¹H) -11.23 (d, J_{p,p} = 17.0 Hz), 44.62 (d, J_{p,p} = 17.0 Hz); m/z (ESI-TOF⁻) 499.0090 (M⁻ requires 499.0095).

N¹-[(5''-O-(phosphoroselenoate)ethoxy)methyl]-5'-O-phosphoryl-2',3'-O-isopropylidene-inosine-5',5''-cyclicpyrophosphate 9c-1 and 9c-2. 2-Cyanoethoxy-*N,N,N',N'*-tetraisopropylphosphoramidite (0.1 mL, 0.33 mmol) was added to a solution of **6** (0.12 g, 0.22 mmol) and 1*H*-tetrazole (46 mg, 0.66 mmol) in CH₃CN (80 mL) under argon. After the reaction was continued for 0.5 h, the CHCl₃ solution (30 mL) of Se (0.17 g, 2.16 mmol) was added and the reaction was continued for 8 h. After the reaction mixture was evaporated, the residue resolved in H₂O and extracted with CHCl₃ and the aqueous layer was concentrated. The purification was performed with method A for preparative HPLC to give compound **8c** (a pair of diastereoisomers, unseparated, 47 mg, 30%). Compound **8c** was resolved in 1M TEAB

bicarbonate buffer (pH = 7.5, 10 mL) and the reaction was stirred for 6h at 35°C. After evaporation, the separation of the mixture was performed with method B for preparative HPLC. The fractions of **9c-1** (18 mg, 36%) and **9c-2** (30 mg, 59%) were collected separately. Data for **9c-1**: δ_{H} (400 MHz; D₂O) 1.50, 1.67 (each s, each 3H, (CH₃)₂C), 3.91-3.95 (m, 2H, OCH₂), 3.99-4.12 (m, 4H, H₅' , CH₂OP), 4.66-4.69 (m, 1H, H₄'), 5.38-5.41 (m, 2H, H₃' , H₁'a), 5.87-5.89 (m, 1H, H₂'), 5.95 (d, 1H, $J_{\text{H}_1''\text{b}, \text{H}_1''\text{a}} = 10.8$ Hz, H₁'b), 6.37 (s, 1H, H₁'), 8.24 (s, 1H, H₈), 8.53 (s, 1H, H₂); δ_{P} (121.5 MHz; D₂O; decoupled with ¹H) -11.81 (d, $J_{\text{p,p}} = 19.6$ Hz), 34.94 (d, $J_{\text{p,p}} = 19.6$ Hz); m/z (ESI-TOF⁻) 586.9851 (M⁻ requires 586.9847). Data for **9c-2**: δ_{H} (400 MHz; D₂O) 1.49, 1.67 (each s, each 3H, (CH₃)₂C), 3.81-4.07 (m, 6H, H₅' , OCH₂CH₂OP), 4.75-4.76 (m, 1H, H₄'), 5.38-5.41 (m, 2H, H₃, H₁'a), 5.94 (d, 1H, $J_{\text{H}_1''\text{b}, \text{H}_1''\text{a}} = 11.2$ Hz, H₁'b), 6.05-6.07 (m, 1H, H₂'), 6.32 (s, 1H, H₁'), 8.25 (s, 1H, H₈), 8.55 (s, 1H, H₂); δ_{P} (121.5 MHz; D₂O; decoupled with ¹H) -11.63 (d, $J_{\text{p,p}} = 17.1$ Hz), 34.65 (d, $J_{\text{p,p}} = 17.1$ Hz); m/z (ESI-TOF⁻) 586.9846 (M⁻ requires 586.9847).

N¹-[(5''-O-(phosphoroselenoate)ethoxy)methyl]-5'-O-phosphoryl-inosine-5',5''-cyclic-pyrophosphate P_{Se}²-cIDPRE-1 and P_{Se}²-cIDPRE-2. The solution of **9c-1** (18 mg, 23 μmol) in 60% HCOOH (10 mL) was stirred for 20 h and then evaporated under reduced pressure. The purification of the residue was performed with method B for preparative HPLC, and the main fraction was collected to give the target molecule **P_{Se}²-cIDPRE-1** (15 mg, 90%). The same treatment was imposed on **9c-2** (25.0 mg, 32 μmol) to give **P_{Se}²-cIDPRE-2** (22 mg, 95%), which was purified with the same procedure. Data for **P_{Se}²-cIDPRE-1**: δ_{H} (400 MHz; D₂O) 3.86-4.04 (m, 4H, OCH₂CH₂OP), 4.11-4.14 (m, 1H, H_{5a}'), 4.28-4.35 (m, 2H, H_{5b}' , H₄'), 4.76-4.78 (m, 1H, H₃'), 5.43 (d, 1H, $J_{\text{H}_1''\text{a}, \text{H}_1''\text{b}} = 11.2$ Hz, H₁'a), 5.47-5.50 (m, 1H, H₂'), 5.87 (d, 1H, $J_{\text{H}_1''\text{b}, \text{H}_1''\text{a}} = 11.2$ Hz, H₁'b), 6.06 (d, 1H, $J_{\text{H}_1', \text{H}_2'} = 6$ Hz, H₁'), 8.20 (s, 1H, H₈), 8.54 (s, 1H, H₂); δ_{P} (121.5 MHz; D₂O; decoupled with ¹H) -11.45 (d, $J_{\text{p,p}} = 19.6$ Hz), 34.72 (d, $J_{\text{p,p}} = 19.6$ Hz); m/z (ESI-TOF⁻) 546.9540 (M⁻ requires 546.9534). Data for **P_{Se}²-cIDPRE-2**: δ_{H} (400 MHz; D₂O) 3.83-3.89 (m, 2H, OCH₂), 4.02-4.09 (m, 3H, H_{5a}' , CH₂OP), 4.24-4.29 (m, 1H, H_{5b}'), 4.34-4.37 (m, 1H, H₄'), 4.75-4.77 (m, 1H, H₃'), 5.43-5.46 (m, 1H, H₂'), 5.51 (d, 1H, $J_{\text{H}_1''\text{a}, \text{H}_1''\text{b}} = 11.2$ Hz, H₁'a), 5.81 (d, 1H, $J_{\text{H}_1''\text{b}, \text{H}_1''\text{a}} = 11.2$ Hz, H₁'b), 6.06 (d, 1H, $J_{\text{H}_1', \text{H}_2'} = 4$ Hz, H₁'), 8.21 (s, 1H, H₈), 8.60 (s, 1H, H₂); δ_{P} (121.5 MHz; D₂O; decoupled with ¹H) -11.60 (d, $J_{\text{p,p}} = 19.6$ Hz), 34.56 (d, $J_{\text{p,p}} = 19.6$ Hz); m/z (ESI-TOF⁻) 546.9536 (M⁻ requires 546.9534).