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Electronic Supplementary Information

Outstanding effects on antithrombin activity of modified TBA diastereomers containing an optically pure acyclic nucleotide analogue

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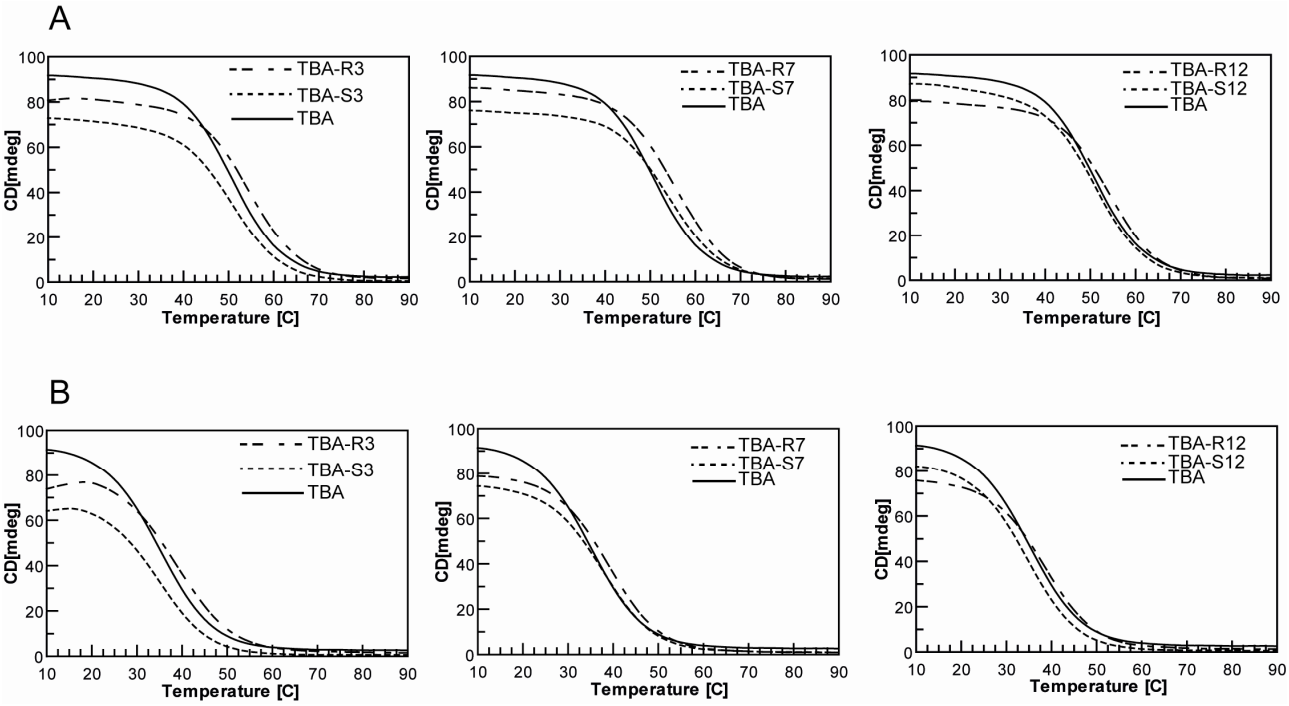


Figure S1. CD melting curves from 10 to 90 °C of TBA and modified ONs in K^+ phosphate buffer (A) and PBS (B). The temperature scan rate was 1.0 °C /min.

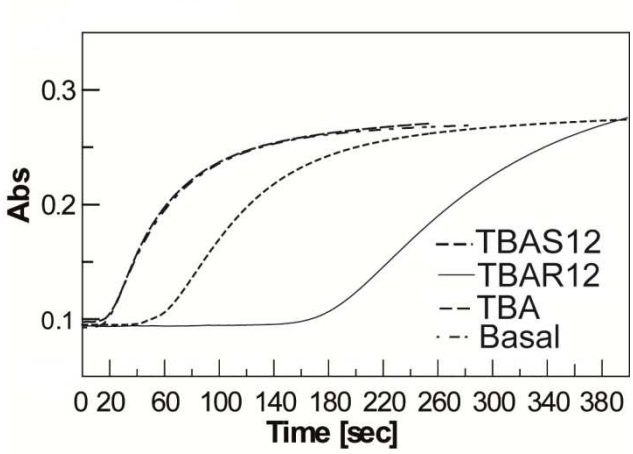


Figure S2. One example of spectroscopic measurement of the scattering produced by the fibrinogen clotting. The curves were obtained measuring, as a function of the time, the UV scattering caused by fibrin polymerization. 1.0 mL of fibrinogen (2 mg/1 mL) in PBS solution was incubated for 1 min with 20 nM of each ON and clot formation was triggered by addition of 1.0 NIH of human thrombin. The basal curves was registered starting the polymerization reaction in absence of any inhibitor. Wavelength was fixed at 380 nm.

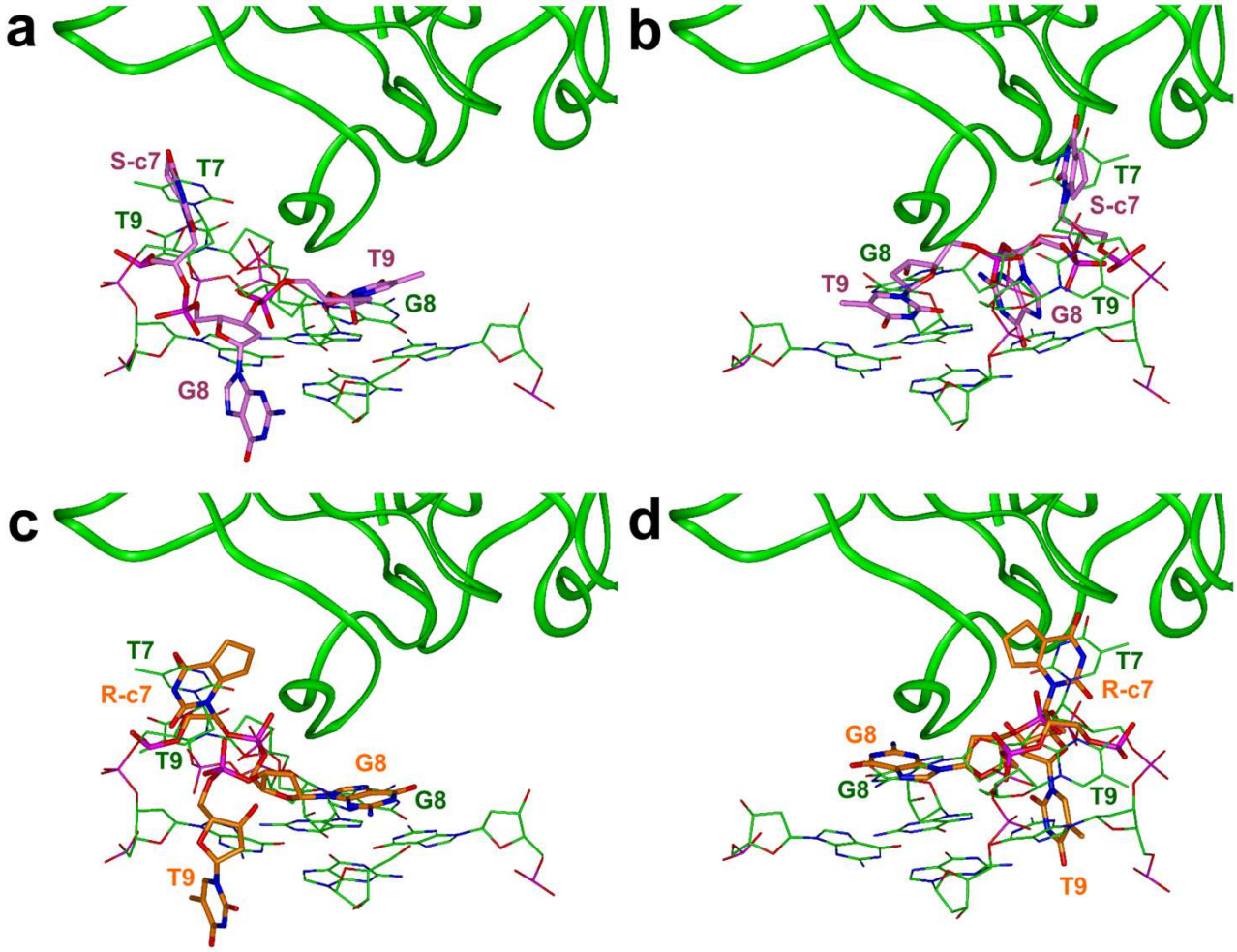


Figure S3. (a; binding mode I) and (b; binding mode II): TBA-S_7 (carbons = pink) superimposed on the bioactive conformation of TBA in complex with thrombin (carbons = green; PDB ID: 1HAP). (c; binding mode I) and (d; binding mode II): TBA-R_7 (carbons = orange) superimposed on the bioactive conformation of TBA (carbons = green; PDB ID: 1HAP). Heteroatoms are coloured as follows: O = red; N = blue; P = magenta. Thrombin ABE I is shown as green ribbon.

Table S1. Calculated occurrence rates (%) of conformers presenting TGT and TT nucleobases "stacked" on the guanine planes.

	TBA[a]	TBA-S_3	TBA-R_3	TBA-S_7	TBA-R_7	TBA-S_{12}	TBA-R_{12}
T3[b]	62.5	38.0	32.5	22.5	47.0	46.5	43.0
T4	46.0	43.5	68.0	55.0	48.0	52.5	41.0
T7[c]	14.0	20.0	6.50	18.0	23.5	20.5	12.5
G8	78.5	67.5	74.5	46.0	62.0	59.0	61.0
T9	26.0	33.0	40.5	63.5	38.0	36.5	42.5
T12[d]	33.5	37.5	29.5	25.0	18.5	18.5	28.5
T13	38.0	55.5	57.0	79.5	69.5	65.5	59.5

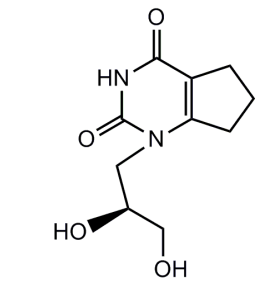
[a] Data from reference 17. [b] T3 residue in TBA-S_3 and in TBA-R_3 is replaced by the acyclic nucleoside **c** (**S-c** and **R-c**, respectively). [c] T7 residue in TBA-S_7 and in TBA-R_7 is replaced by the acyclic nucleoside **c** (**S-c** and **R-c**, respectively). [d] T12 residue in TBA-S_{12} and TBA-R_{12} is replaced by the acyclic nucleoside **c** (**S-c** and **R-c**, respectively)**.**

Table S2. Calculated occurrence rates (%) of *syn*, *anti*, and s/a conformers of TGT and TT glycosidic bonds.

	TBA[a]			$TBA\text{-}S_3$			$TBA\text{-}R_3$			$TBA\text{-}S_7$			$TBA\text{-}R_7$			$TBA\text{-}S_{12}$			$TBA\text{-}R_{12}$		
	syn	*anti*	s/a	*syn*	*anti*	s/a	*syn*	*anti*	s/a	*syn*	*anti*	s/a	*syn*	*anti*	s/a	*syn*	*anti*	s/a	*syn*	*anti*	s/a
T3[b]	28.5	69.5	2.00	-	-	-	-	-	-	37.5	59.0	3.50	40.5	57.0	2.50	47.0	50.0	3.00	32.0	65.0	3.00
T4	38.5	56.0	5.50	45.0	52.0	3.00	35.5	59.5	5.00	35.5	61.5	3.00	37.0	59.0	4.00	40.5	54.0	5.50	38.5	60.5	1.00
T7[c]	39.0	59.5	1.50	36.0	59.5	4.50	47.0	51.0	2.00	-	-	-	-	-	-	36.0	61.5	2.50	38.5	61.0	0.50
G8	39.5	38.0	22.5	29.5	42.0	28.5	34.5	43.5	22.0	30.5	50.5	19.0	31.0	45.0	24.0	39.5	35.5	25.0	41.0	37.5	21.5
T9	41.5	50.0	8.50	43.0	50.5	6.50	36.5	55.0	8.50	34.5	58.0	7.50	37.0	53.5	9.50	33.5	60.0	6.50	38.5	53.0	8.50
T12[d]	32.0	66.0	2.00	40.5	56.5	3.00	47.0	51.5	1.50	28.5	71.0	0.50	35.5	64.0	0.50	-	-	-	-	-	-
T13	37.0	59.0	4.00	37.0	57.0	6.00	45.5	49.0	5.50	45.0	52.0	3.00	50.0	45.0	5.00	53.5	41.5	5.00	54.5	43.0	2.50

[a] Data from reference 17. [b] T3 residue in $TBA\text{-}S_3$ and in $TBA\text{-}R_3$ is replaced by the acyclic nucleoside **c** (**S-c** and **R-c**, respectively). [c] T7 residue in $TBA\text{-}S_7$ and in $TBA\text{-}R_7$ is replaced by the acyclic nucleoside **c** (**S-c** and **R-c**, respectively). [d] T12 residue in $TBA\text{-}S_{12}$**c** and $TBA\text{-}R_{12}$ is replaced by the acyclic nucleoside **c** (**S-c** and **R-c**, respectively).

Figure S4 ^{1}H NMR **S-c** (Py-D5, 400 MHz)



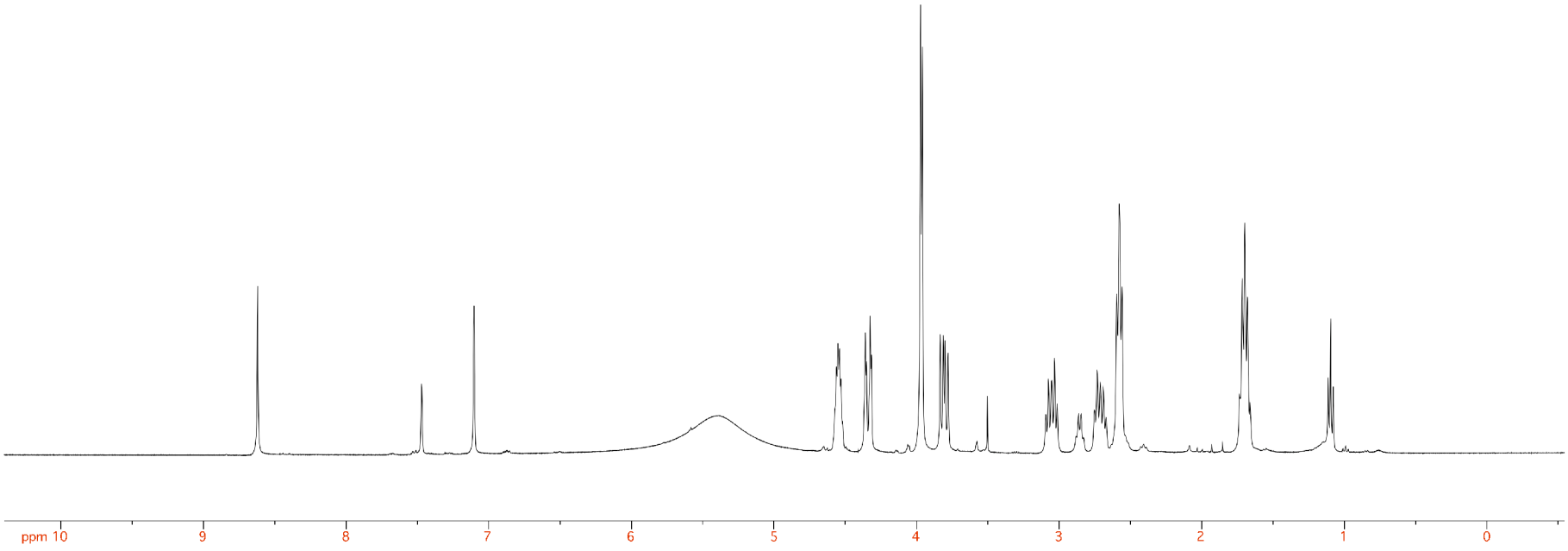
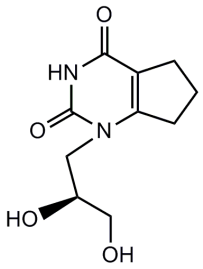


Figure S4 ^{1}H NMR **S-c** (Py-D5, 400 MHz)



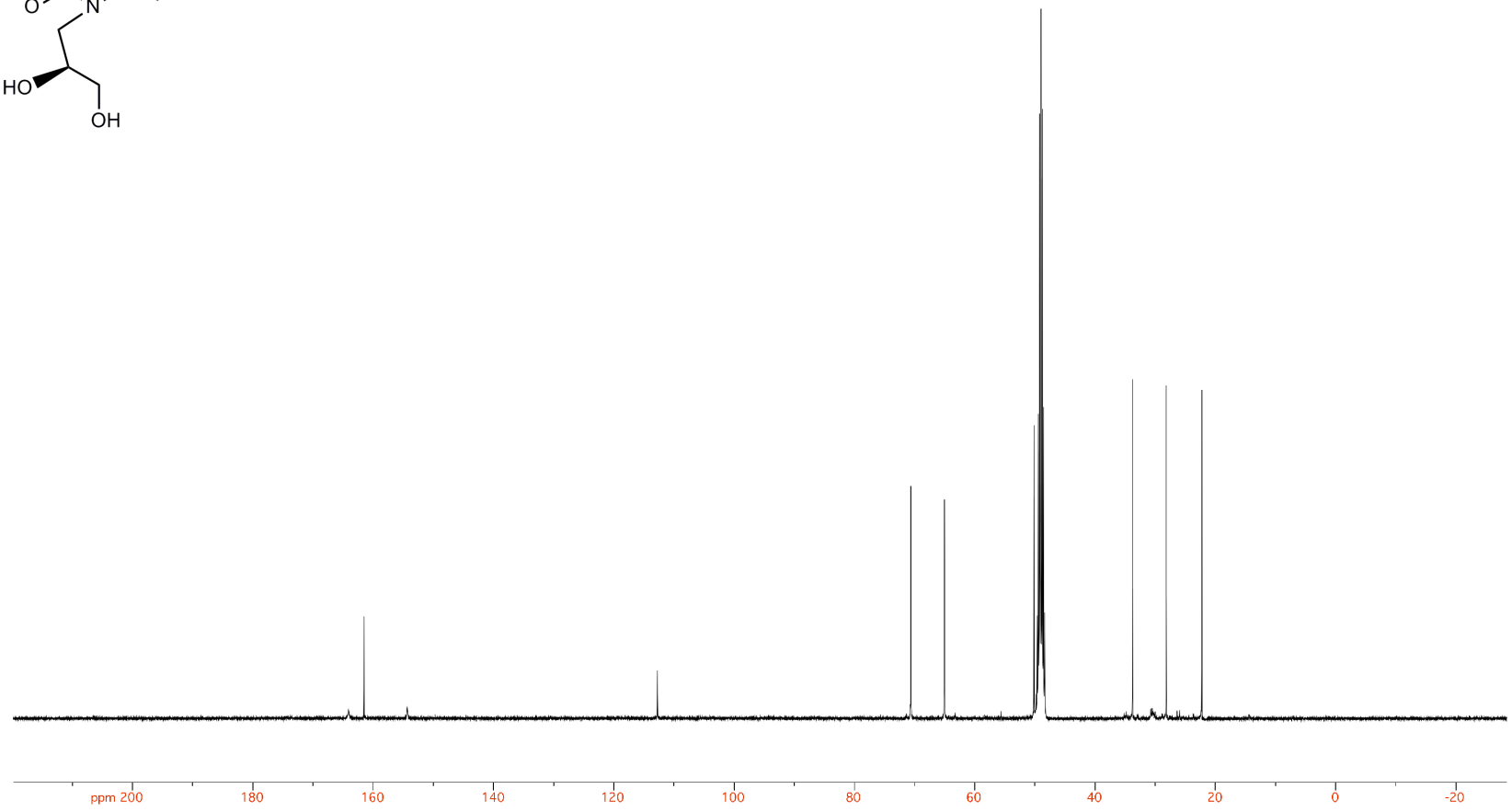
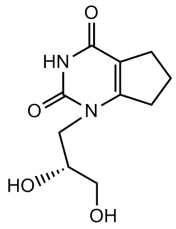


Figure S 6: ^{1}H NMR **R-c** (Py-D5, 400 MHz)



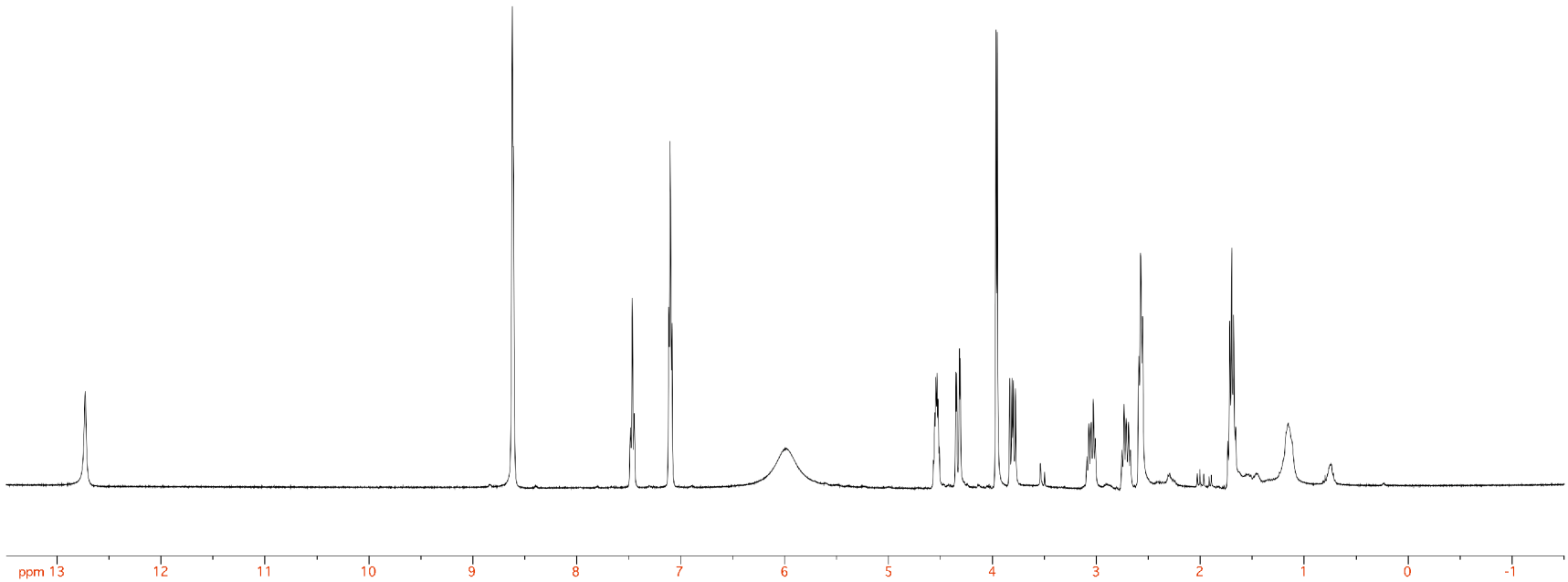
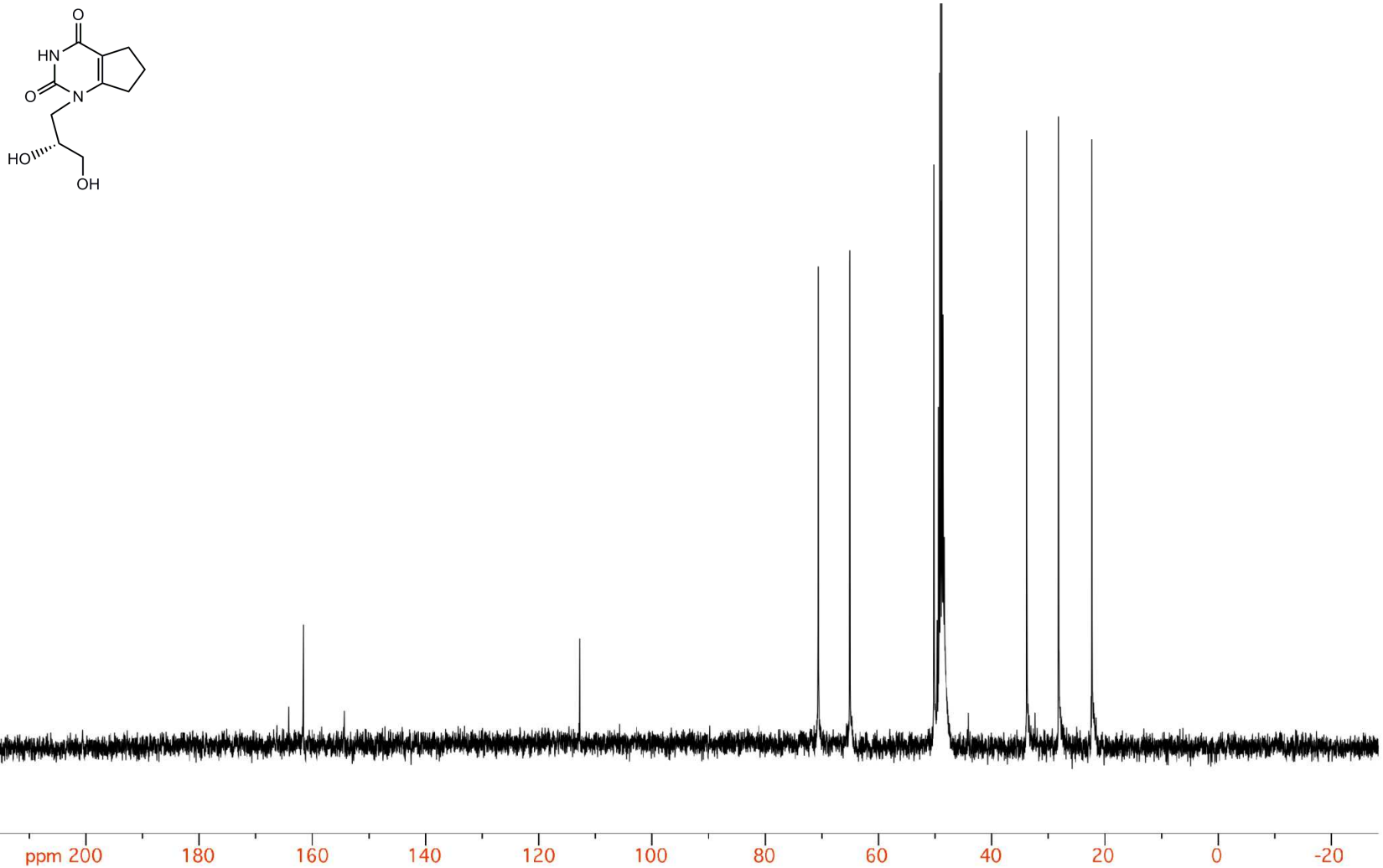


Figure S7 ^{13}C NMR **R-c** (CD_3OD, 100 MHz)



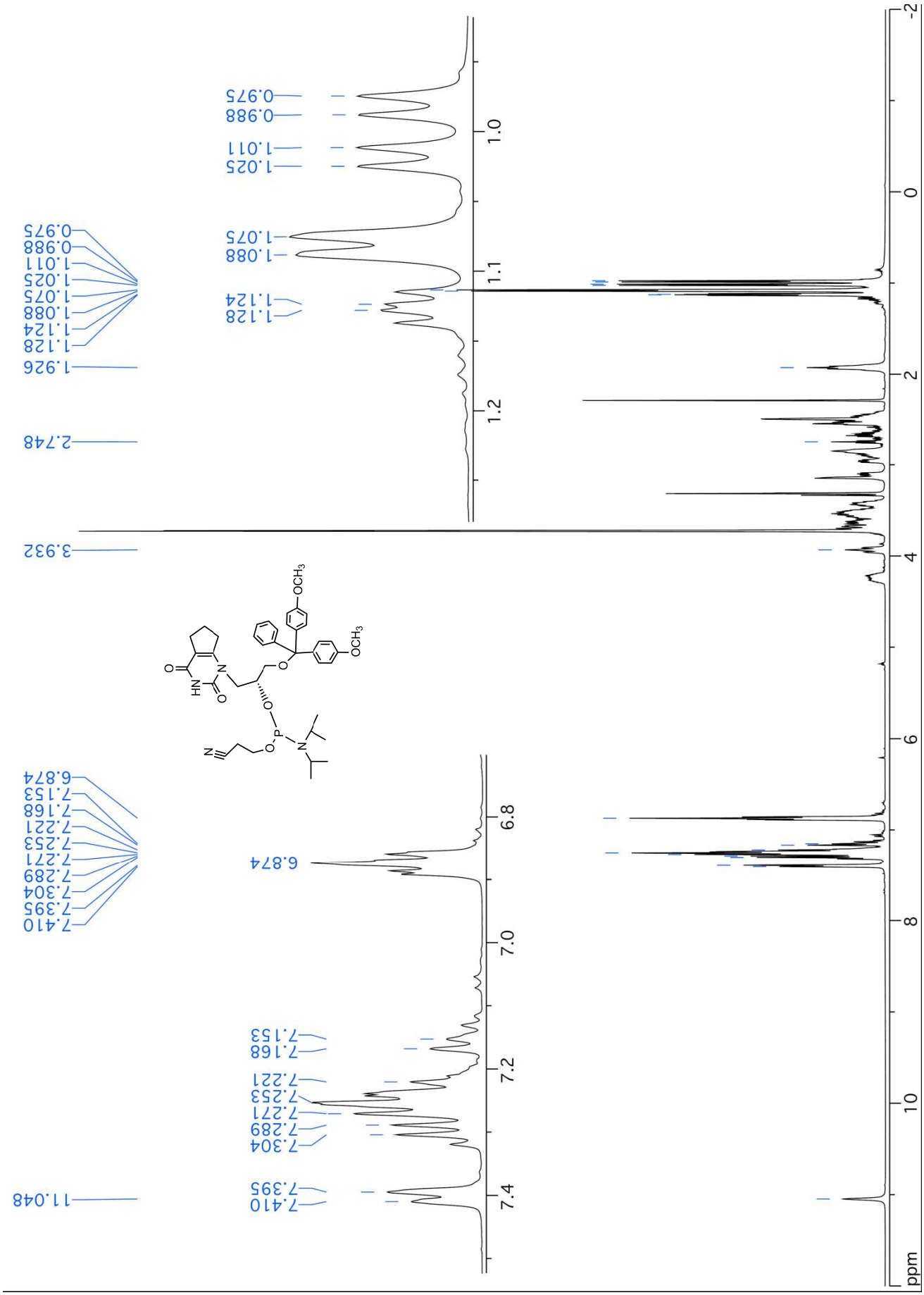


Figure S8: ^{1}H NMR **5a** (DMSO-D6, 500Mz)

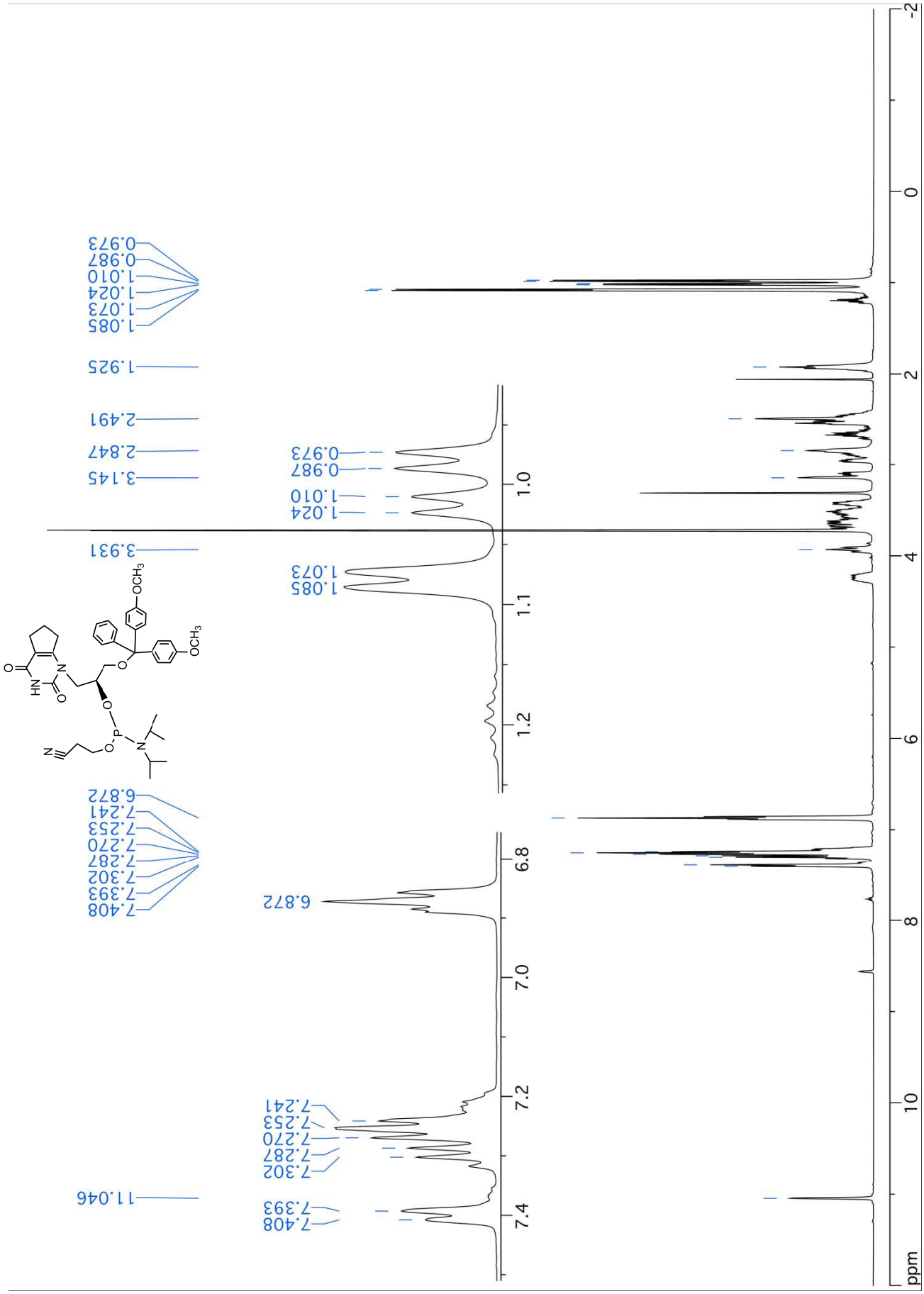


Figure S9: ^{1}H NMR **5b** (DMSO-D6, 500Mz)

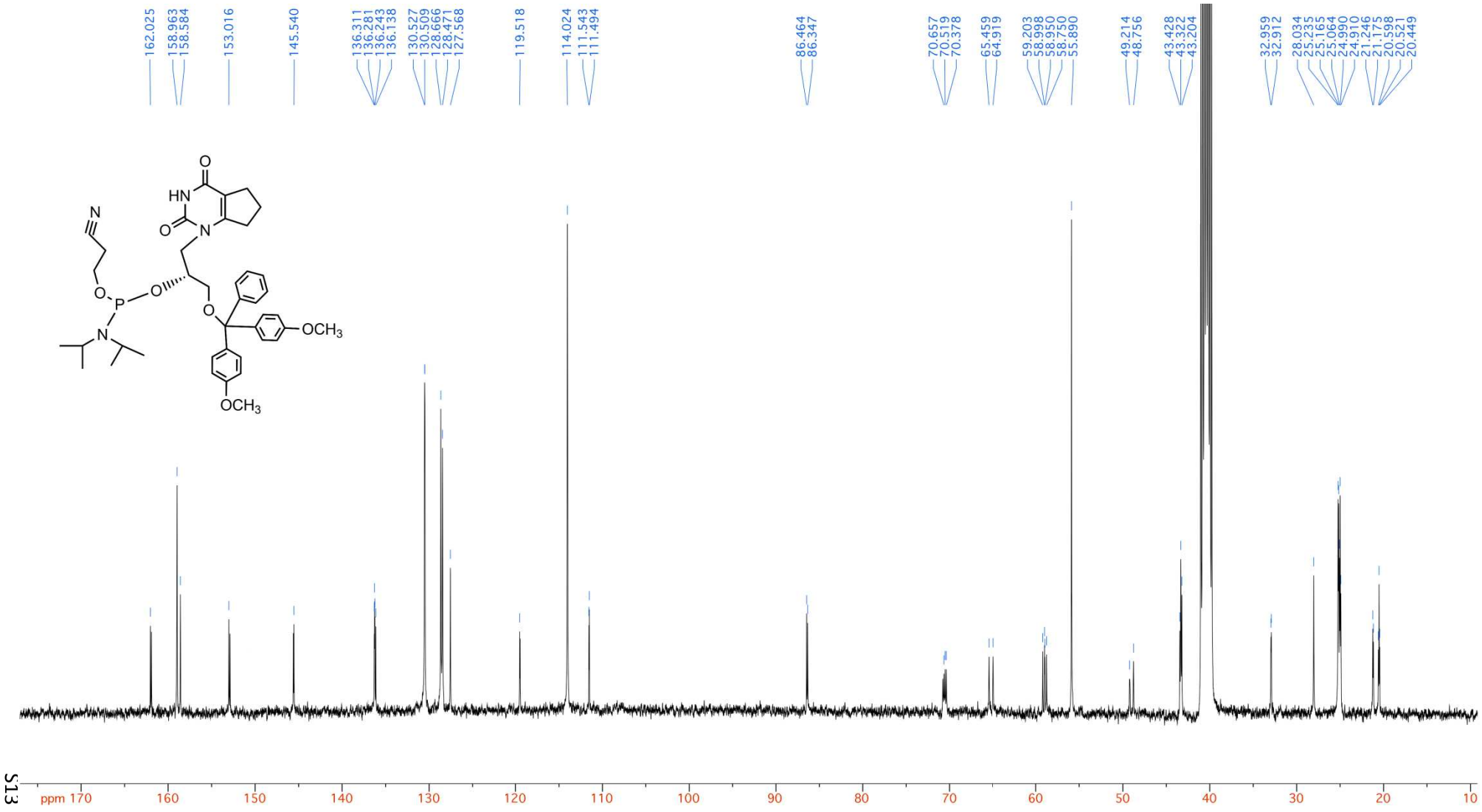


Figure S10: ^{13}C NMR **5a** (DMSO-D6,100MHz)

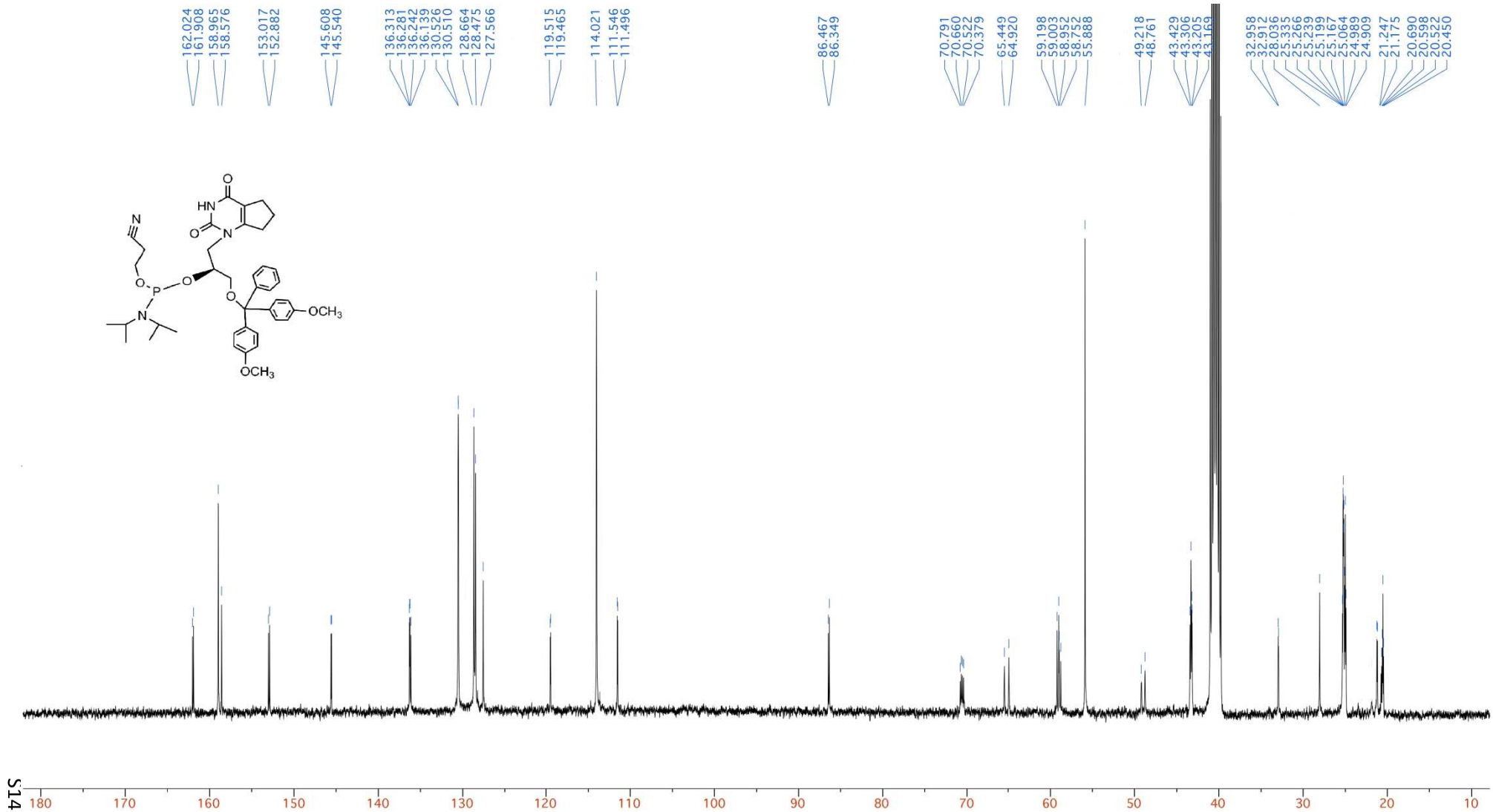


Figure S11: ^{13}C NMR **5b** (DMSO-D6,100MHz)

Figure S12: ^{31}P NMR **5a** (DMSO-D6,202 MHz)

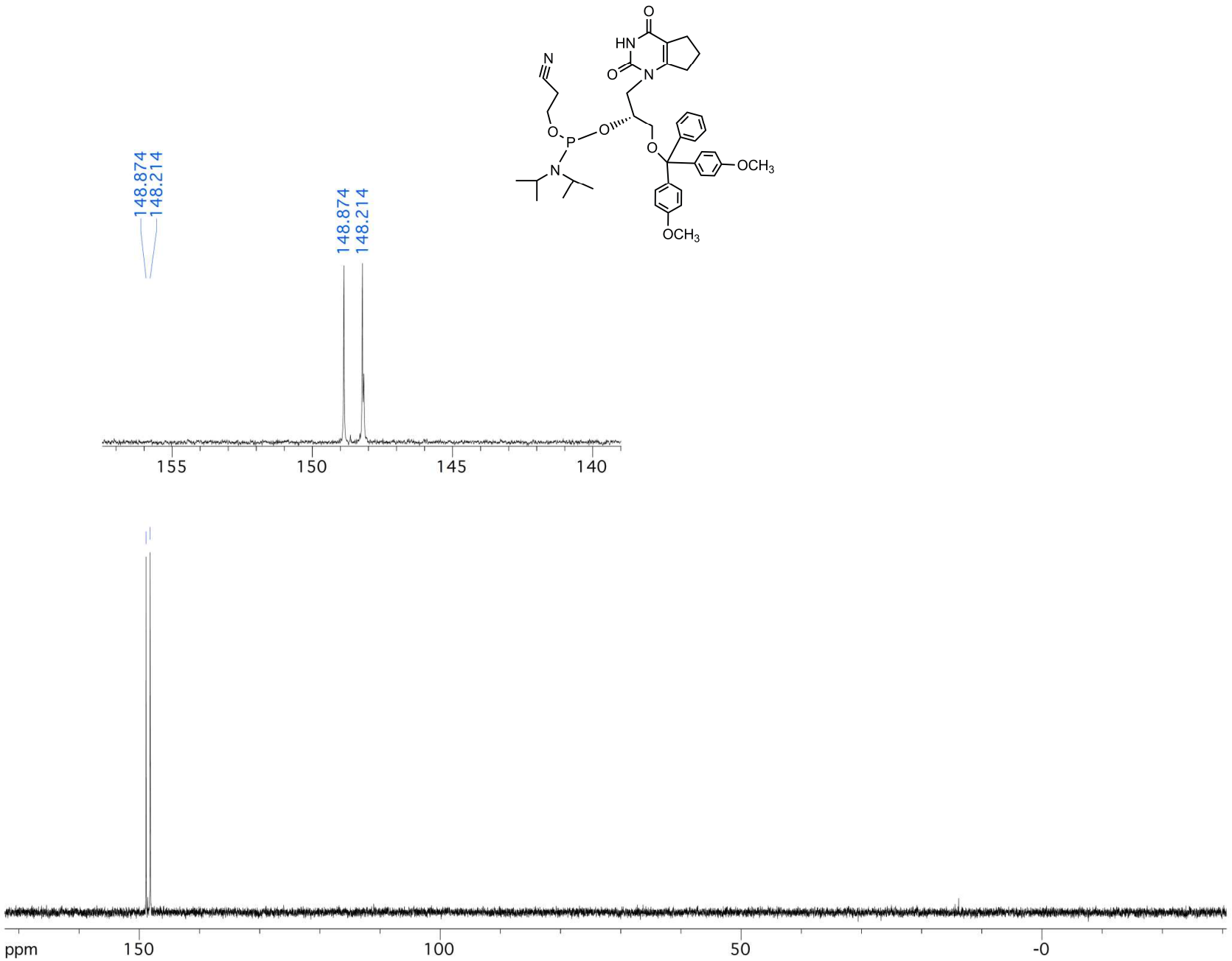
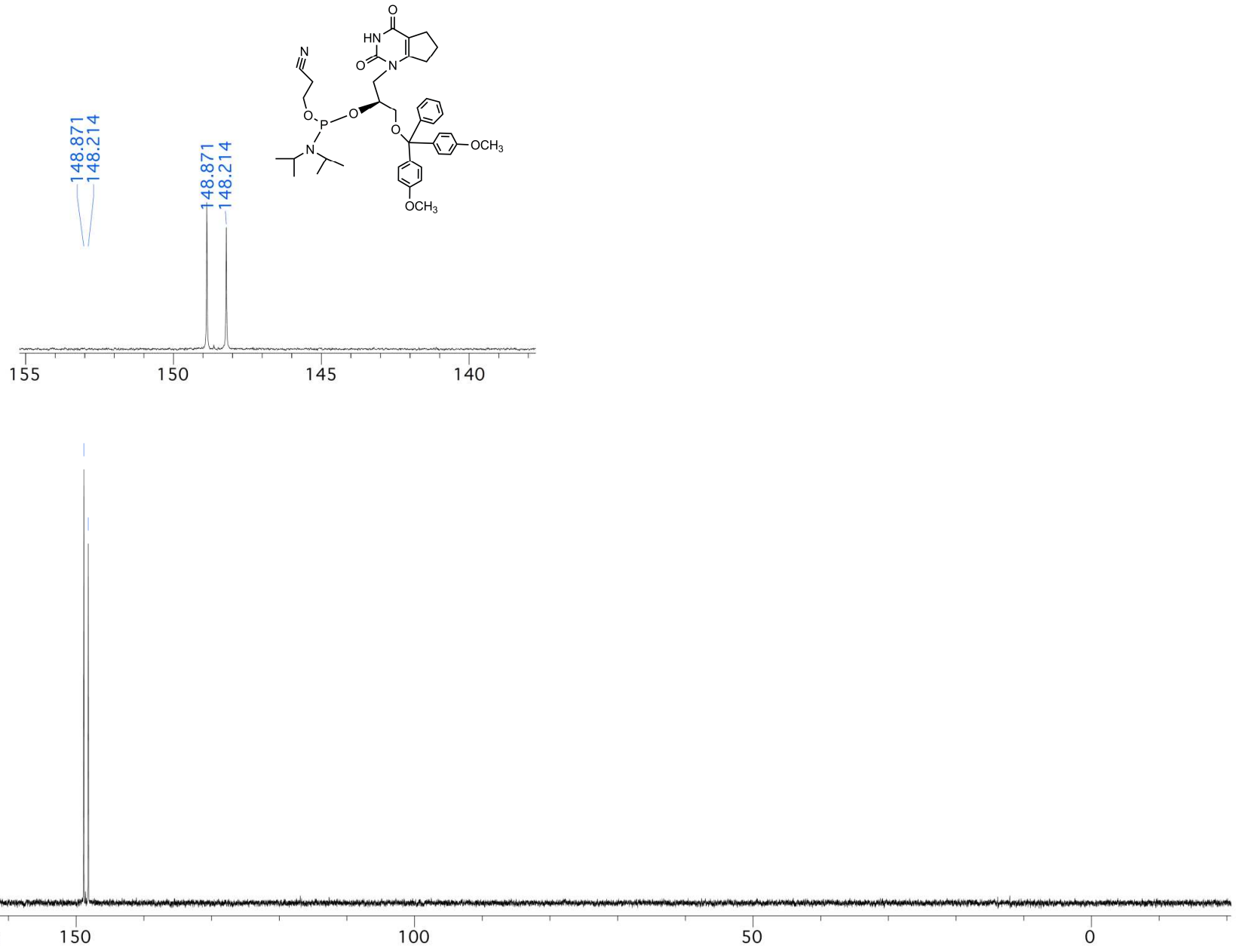


Figure S13: ^{31}P NMR **5b** (DMSO-D6,202 MHz)



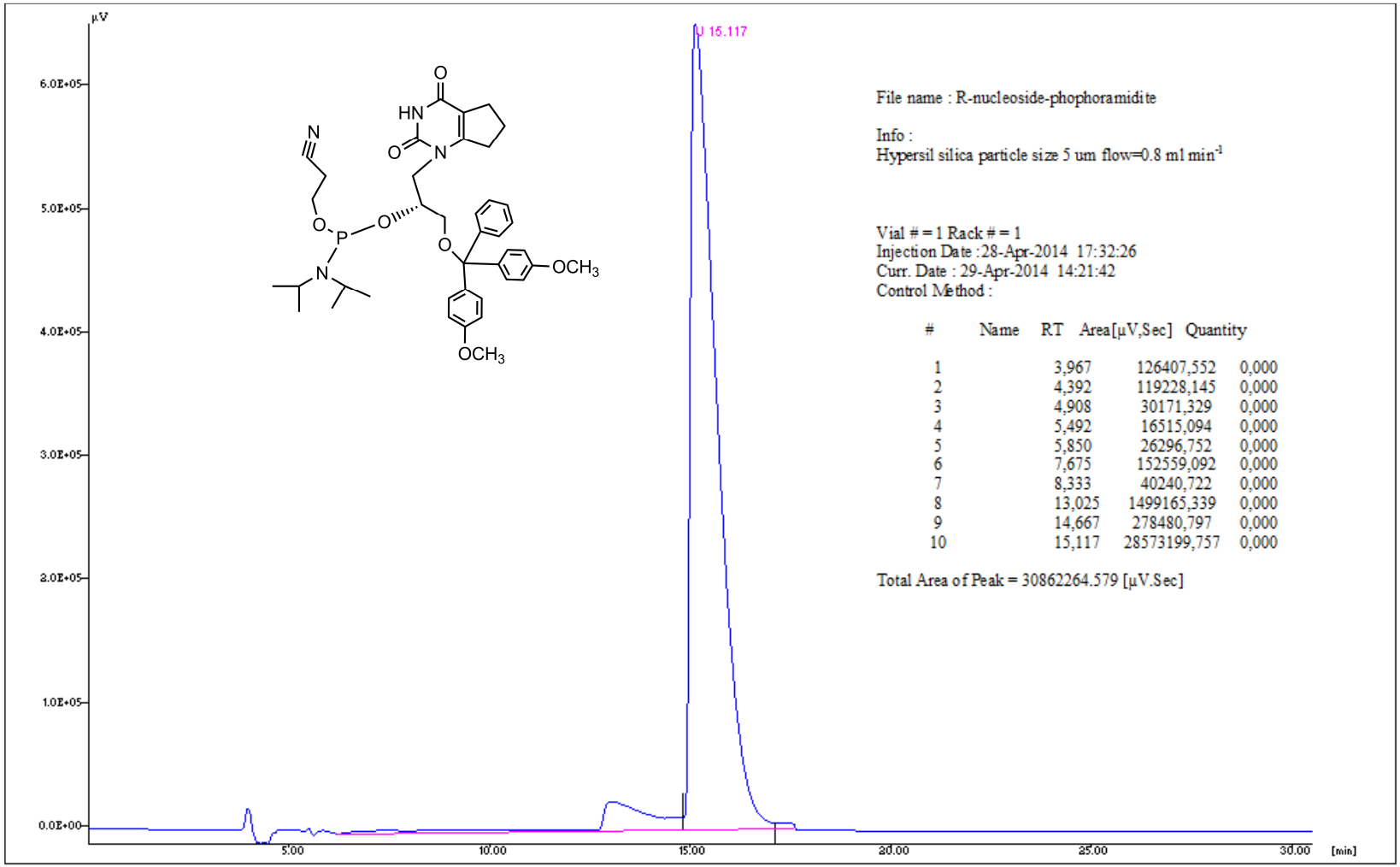


Figure S14 HPLC chromatogram of the mixture of conversion of 4a in **5a.** Hipersil silica column particle size 5 um; eluent:(50:50; V/V) n-Hexane-Ethyl Acetate.

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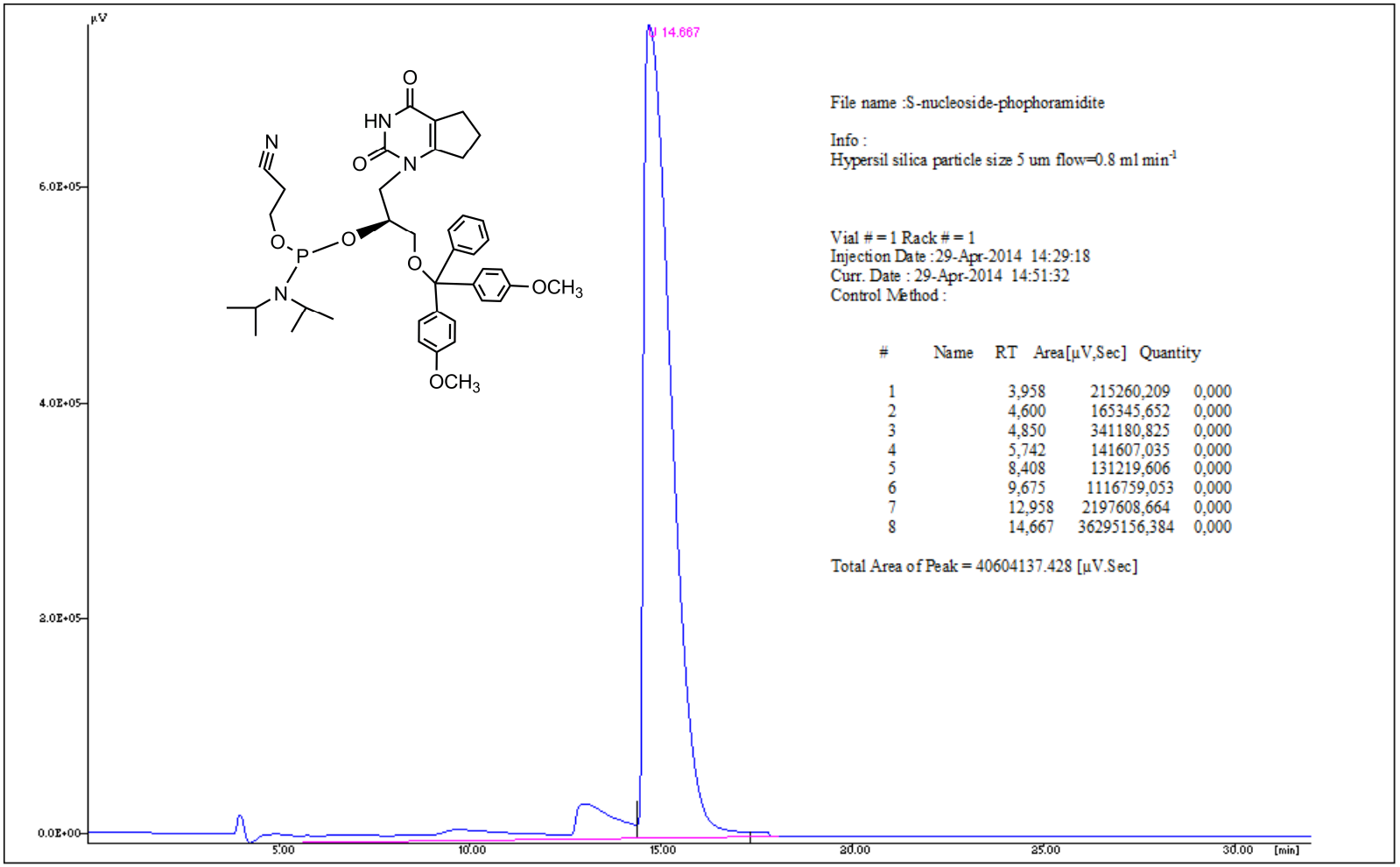

Figure S15: HPLC chromatogram of the mixture of conversion of 4b in **5b**. Hipersil silica column particle size 5 um; eluent:(50:50; V/V) n-Hexane-Ethyl Acetate.

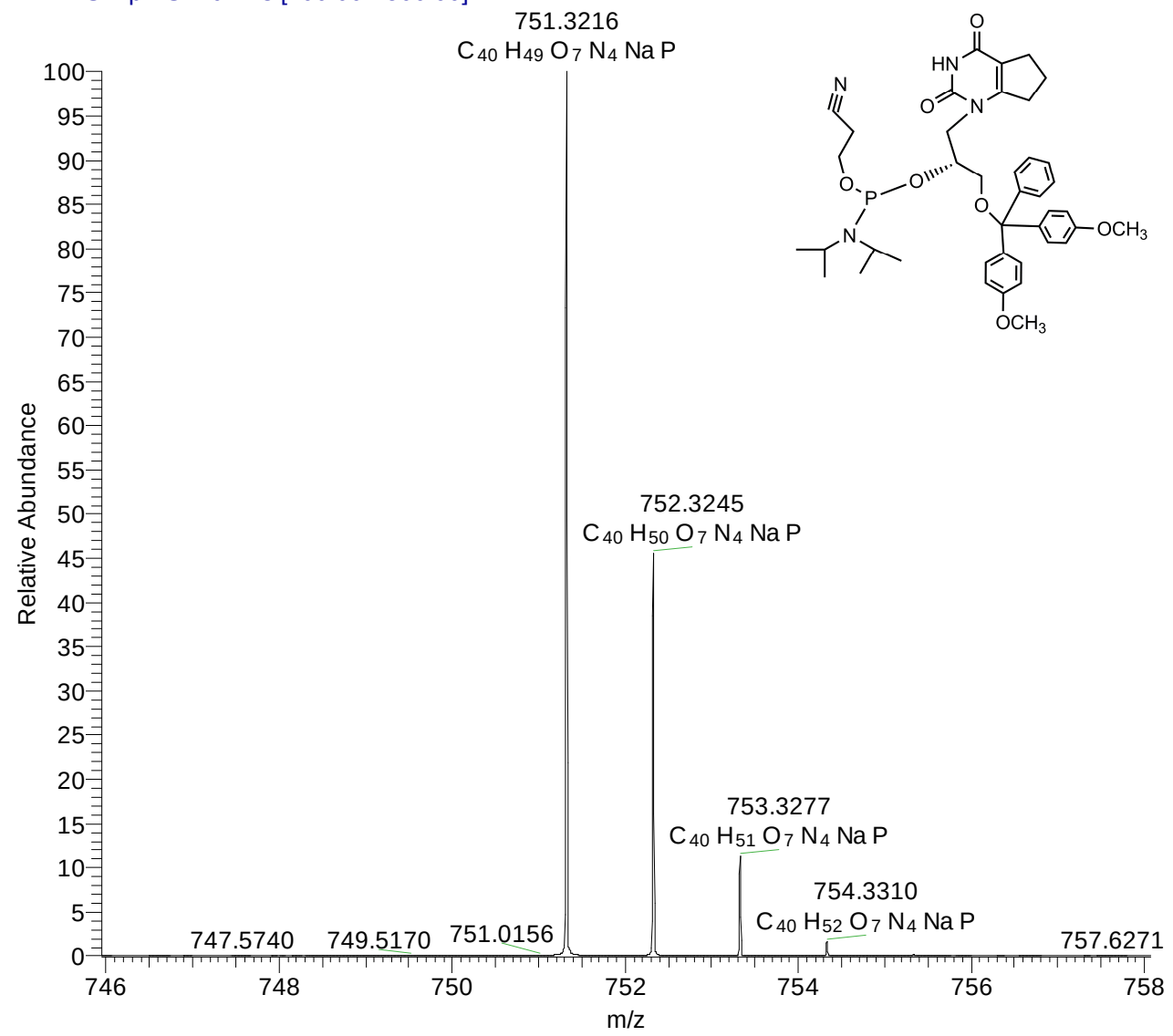

Figure S16: High resolution ESI-MS mass spectrum of **5a** was performed on a Thermo LTQ Orbitrap XL mass spectrometer (positive mode). The spectra was recorded by infusion into the ESI source using MeOH as the solvent.

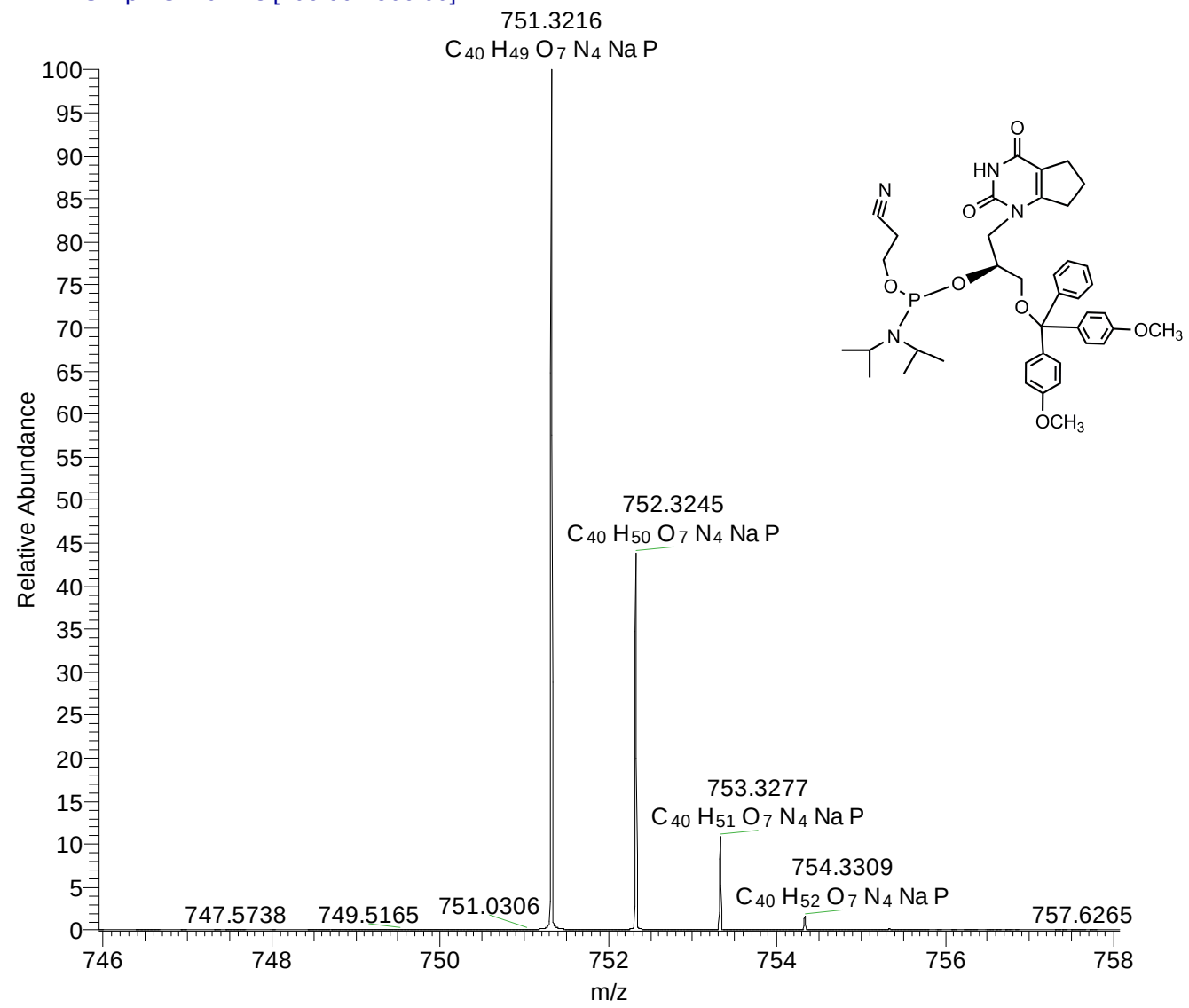

Figure S17: High resolution ESI-MS mass spectrum of **5b** was performed on a Thermo LTQ Orbitrap XL mass spectrometer (positive mode). The spectra was recorded by infusion into the ESI source using MeOH as the solvent.