Electronic Supplementary materials

Cationic lipo-thiophosphoramidates for gene delivery: synthesis, physico-chemical characterization and gene transfection activity - comparison with lipo-phosphoramidates.

Aurore Fraix, Tristan Montier, Nathalie Carmoy, Damien Loizeau, Laure Burel-Deschamps, Tony Le Gall, Philippe Giamarchi, Hélène Couthon-Gourvès, Jean-Pierre Haelters, Pierre Lehn, Paul-Alain Jaffrès

Summary

S1 Determination of CLC.

Material

Nile Red ((9-diethylamino-5H-benzo[alpha]phenoxazine-5-one) fluorescence measurements were done on a Cary Eclipse Varian spectrofluorimeter. Abs/ Em = 552/ 636 nm.

Protocol

Aliquots of stock solutions of lipids in chloroform were dried under vacuum in presence of Nile Red. A pH 7 Hepes buffer (5.10^{-3} mol.L^{-1}) was then added and samples were kept at 4°C for 4 days. They were then sonicated for 30 minutes at 30°C. The final Nile Red concentration was 5.10^{-7} mol.L^{-1} and lipids final concentrations were between 5.10^{-7} and 3.10^{-5} mol.L^{-1}.

Result

The concentration from which phospholipids aggregates are formed is determined by measuring the fluorescence of the Nile Red probe (9-diethylamino-5H-benzo[alpha]phenoxazine-5-one; Molecular Probe, France). Nile red is an hydrophobic fluorescent probe with a low solubility and low fluorescence in water[1]. It is highly solvatochromic exhibiting an increase in fluorescence quantum yield with decreasing solvent polarity accompanied by a blue shift in the peak emission.[2] Then, Nile Red fluorescence intensity suddenly rises upon the formation of phospholipids aggregates, where Nile Red locates because of its hydrophobic character. As an illustration, figure S1 displays the fluorescence intensity of Nile Red *versus* the concentration of phospholipids **7b** in water. It can be seen that the fluorescence intensity rises with increasing phospholipids concentration while the maximum emission wavelength exhibits a blue shift. The CLC value can be seen as the onset of the fluorescence intensity increase. It corresponds to the critical concentration that is required for the transition of monomer phospholipids to a supra-molecular assembly. The repetition of such kind of experiment for the other lipids allows determining the CLC of these lipids (Table S1).

[1] M. C. A. Stuart, J. C. Van de Pas., J. B. F. N. Engberts. *J. Phys. Org. Chem.* **2005,** *18*, 929-934.
[2] G. Cevc. Phospholipids Handbook**. 1993**, Marcel Dekker, Inc. (New York) .

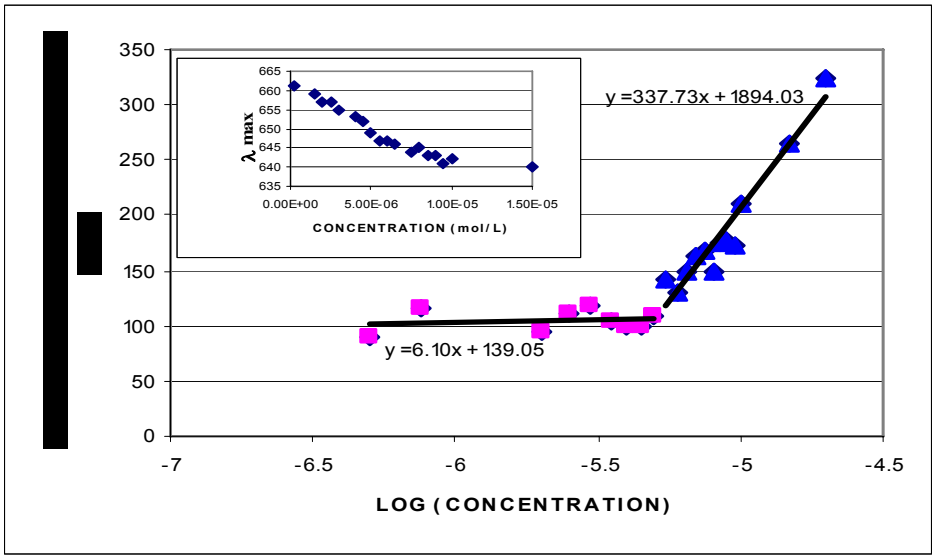


Figure S1 : fluorescence intensity of Nile Red *versus* the concentration of the lipothiophosphoramidate **7b** in Hepes buffer at 20°C.

Cationic lipid	CLC ($mol.L^{-1}$)
3a	$6.4.10^{-6}$
3b	$6.9.10^{-6}$
7a	$3.3.10^{-6}$
7b	$4.4.10^{-6}$

Table S1 : CLC values of the lipids **3a-b** and **7a-b**

S2 Fluorescence anisotropy measurements.

Material

Laurdan (6-Dodecanoyl-2- dimethylaminonaphthalene, Abs/ Em = 364/ 497 nm) fluorescence measurements were done on a Cary Eclipse Varian spectrofluorimeter using manual polarizers to record I_{vv}, I_{vh}, I_{hv} and I_{hh} and fluorescence anisotropy was calculated as follows:

$$r = [(I_{vv} - GI_{vh})/(I_{vv} + 2GI_{vh})] \quad \text{with } G = I_{hv}/I_{hh}$$

Protocol

Liposomes were prepared has described before at a final lipid concentration of 2.10^{-4} mol.L^{1}. Laurdan concentration was 2.10^{-6} mol.L^{-1}.
The results are summarized in figure 5 and discussed in the main text.

The results are summarized in figure 5 and discussed in the main text.

S3 Size of liposomes and lipoplexes.

Protocol

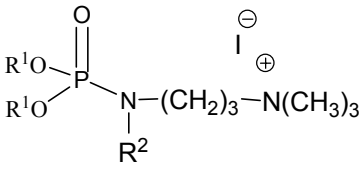
Liposome:

200 µL of liposomal solution and 3.5 mL of sterile water are introduced into a cuvette receptacles. The whole content is taken by a syringe, reintroduced in a cuvette receptacle after filtration over a 1.2 µm filter and used for size measurements.

Lipoplexe:

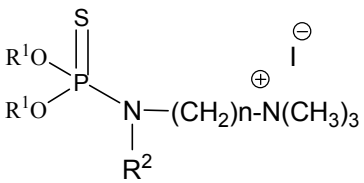
A pDNA solution **A** is obtained by mixing 6.06 µL (corresponding to 40 µg of DNA) of a mother pDNA solution (6.6 mg/mL) with 993.94 µL of sterile water. To prepare a lipoplexe solution possessing a Charge Ratio of 2 (CR = 2), 2.160 µL of a liposomal solution (1.5 µmol/mL) is diluted with distilled water to obtained the solution **B** with a final volume of 1 mL (in that case 840 µL of water were added). Then the pDNA solution **A** is added dropwise to the liposomal solution **B** and the mixture is vortexed. After, at least, 45 min of incubation, the whole solution is introduced in a cuvette receptacles and 1 mL of distilled water is added. The content is taken in a syringe, filtrated over a 1.2 µm filter and used for size measurements.

For the other CR, the quantity of cationic lipid used is adapted.



3a (KLN10) $R^2 = H$

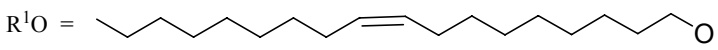
3b (KLN6) $R^2 = CH_3$



7a (BSV44) $R^2 = H$ n = 3

7b (BSV25) $R^2 = CH_3$ n = 3

7c (BSV17) $R^2 = H$ n = 2



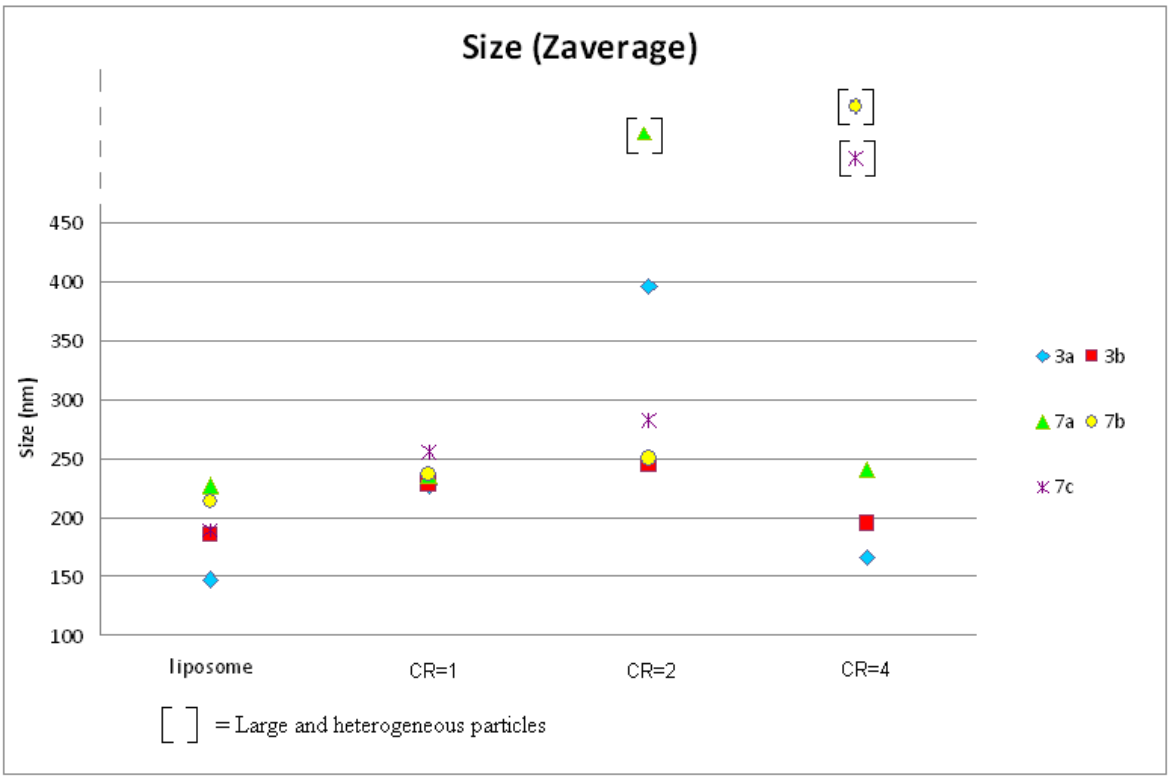


Figure S3-1: graphical representation of the size of liposomes and lipoplexes **3a-b** and **7a-c**.

				lipoplexe								
	liposome			CR=1			CR=2			CR=4		
	Z average	poly index	intensity	Z average	poly index	intensity	Z average	poly index	intensity	Z average	poly index	intensity
3b	191	0,388	237	231	0,158	260	248	0,041	242	198	0,106	220
	181	0,422	261	225	0,107	252	240	0,128	253	192	0,107	215
	185	0,35	254									
average	185,7	0,387	250,7	228,0	0,133	256,0	244,0	0,085	247,5	195,0	0,107	217,5
3a	151	0,604	209	230	0,178	274	376	0,478	358	168	0,114	188
	145	0,407	216	224	0,168	261	391	0,369	451	164	0,131	190
	145	0,393	210				420	0,438	489	165	0,1	176
average	147,0	0,468	211,7	227,0	0,173	267,5	395,7	0,428	432,7	165,7	0,115	184,7
7a	230	0,244	297	241	0,169	283				268	0,59	169
	225	0,209	269	230	0,169	252	Check poly index			226	0,268	234
	225	0,242	283		0,107					227	0,249	235
average	226,7	0,232	283,0	235,5	0,148	267,5				240,3	0,369	212,7
7b	200	0,206	251	243	0,175	227	251	0,127	284			
	218	0,234	268	234	0,142	266	250	0,118	282	Check poly index		
	221	0,22	270	232	0,158	266	247	0,138	281			
average	213,0	0,220	263,0	236,3	0,158	253,0	249,3	0,128	282,3			
7c	195	0,204	223	264	0,203	290	288	0,117	296			
	187	0,199	224	253	0,185	303	276	0,088	301	Check poly index		
	186	0,161	221	251	0,174	295						
average	189,3	0,188	222,7	256,0	0,187	296,0	282,0	0,103	298,5			

Table S3-1: full data of the size of liposomes and lipoplexes (CR = 1, 2 and 4).

S4 Zeta potentials of liposomes and lipoplexes.

Protocol:
The zeta potentials have been recorded after size measurements. 1.5 mL of the solution used for size measurements are placed in the zeta potential measurement cell.

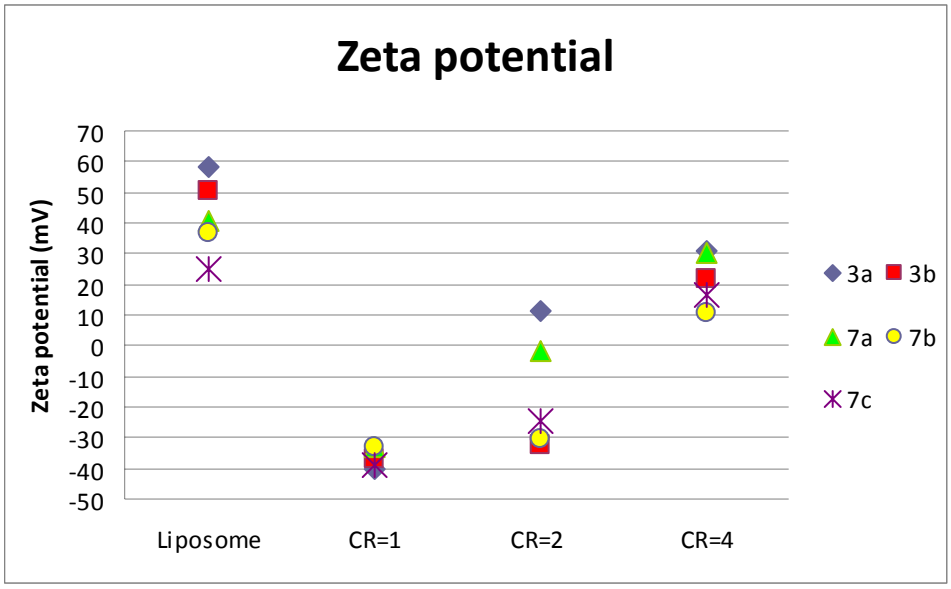


Figure S4-1: graphical representation of the zeta potential of liposomes obtained from **3a-b** and **7a-c** and lipoplexes at CR=1, 2 and 4.

	Zeta potential (mV)			
	Liposome	CR=1	CR=2	CR=4
3b	50 51 51	-35 -39	-31 -34	22 22
average	50,7	-37,0	-32,5	22,0
3a	57 60 57	-40 -41	10 13	30 32
average	58,0	-40,5	11,5	31,0
7a	40 41	-32 -34	9 -15 0	33 27 30
average	40,5	-33,0	-2,0	30,0
7b	35 39	-33 -33	-30 -31	14 -7 24
average	37,0	-33,0	-30,5	10,3
7c	28 22	-36 -39 -42	-26 -23	25 11 13
average	25,0	-39,0	-24,5	16,3

Table SM4-1: zeta potentials of liposomes and lipoplexes - full data (CR = 1, 2 and 4).

S5 Stability of lipoplexes at CR = 1

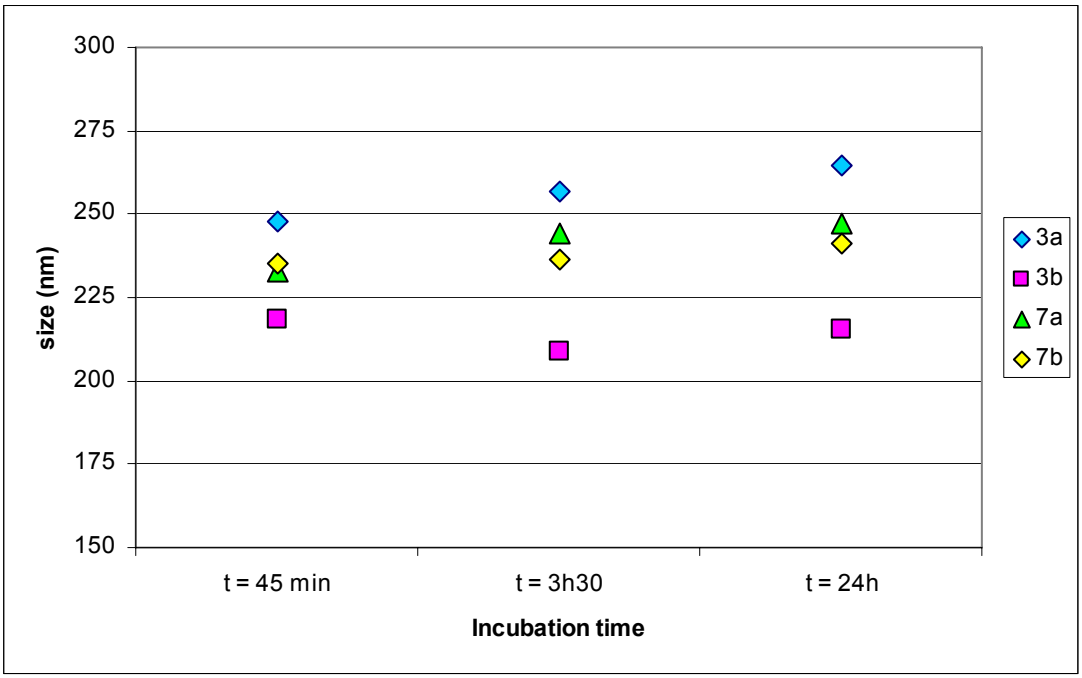


Figure S5-1: Determination of the size of lipoplexes (at CR = 1) formed with the cationic lipids **3a-b** or **7a-b** at 45 minutes, 3h30 and 24 h.

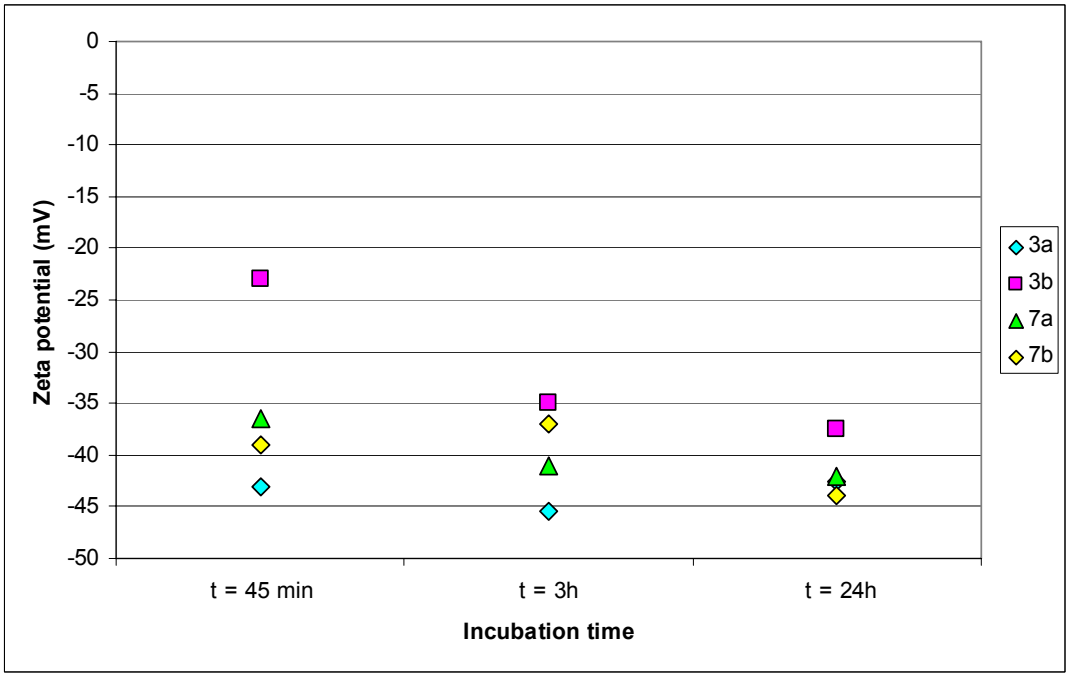


Figure S5-2: Determination of the zeta potential of lipoplexes (at CR = 1) formed with the cationic lipids **3a-b** or **7a-b** at 45 minutes, 3h30 and 24 h.

S6 Formulation as liposomes

The cationic lipids **3a-b** and **7a-c** were formulated as liposome by using the hydratation of lipid film. The hydratation times are reported in the table S6-1.

Cationic lipid	Hydratation time at 4°C
3a	4 days
3b	4 days
7a	4 days
7b	5 days
7c	3 days

S7 NMR Data

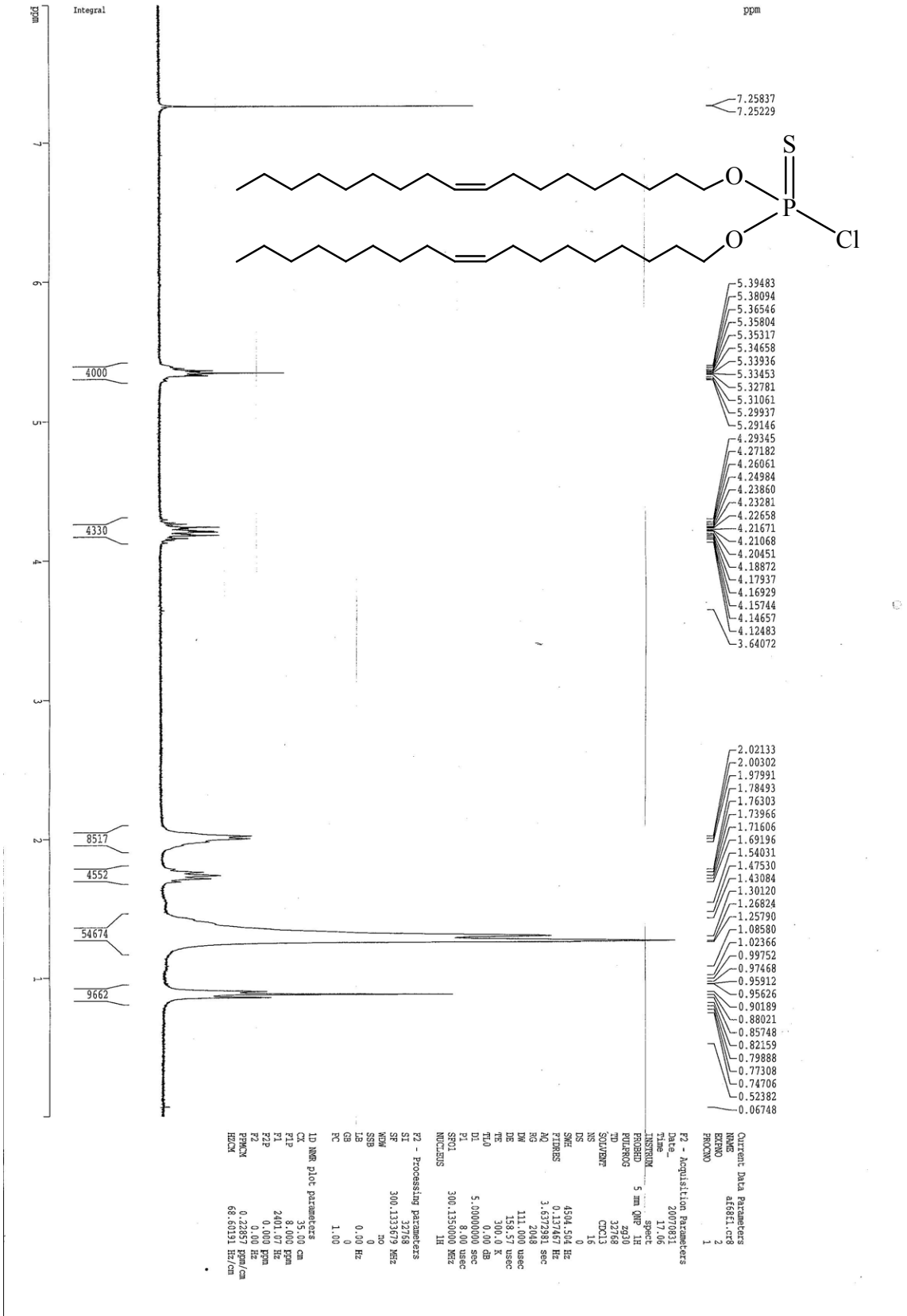


Figure S7-1 : ^{1}H NMR spectrum of compound **5**.

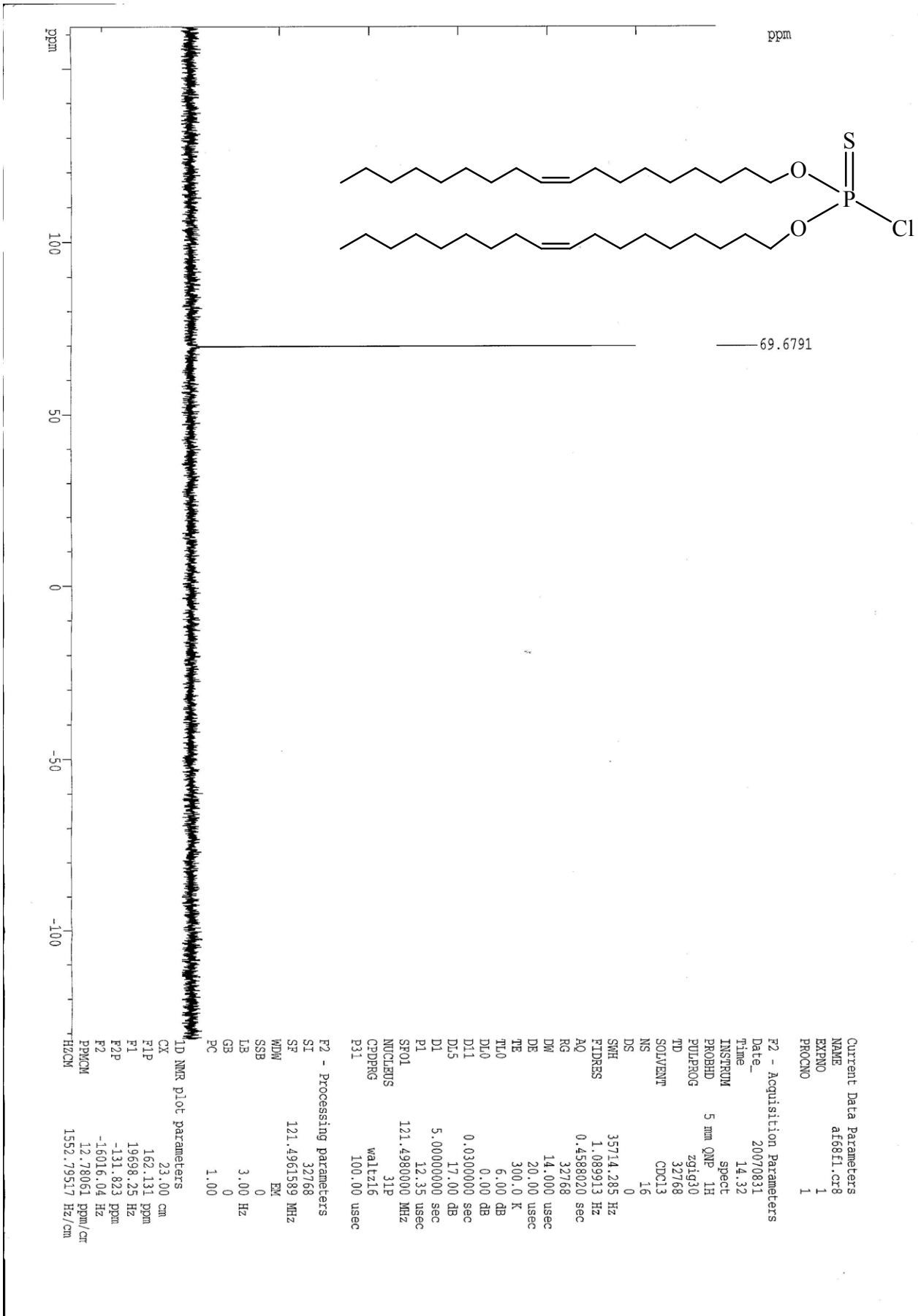


Figure S7-2 : ^{31}P NMR spectrum of compound **5**.

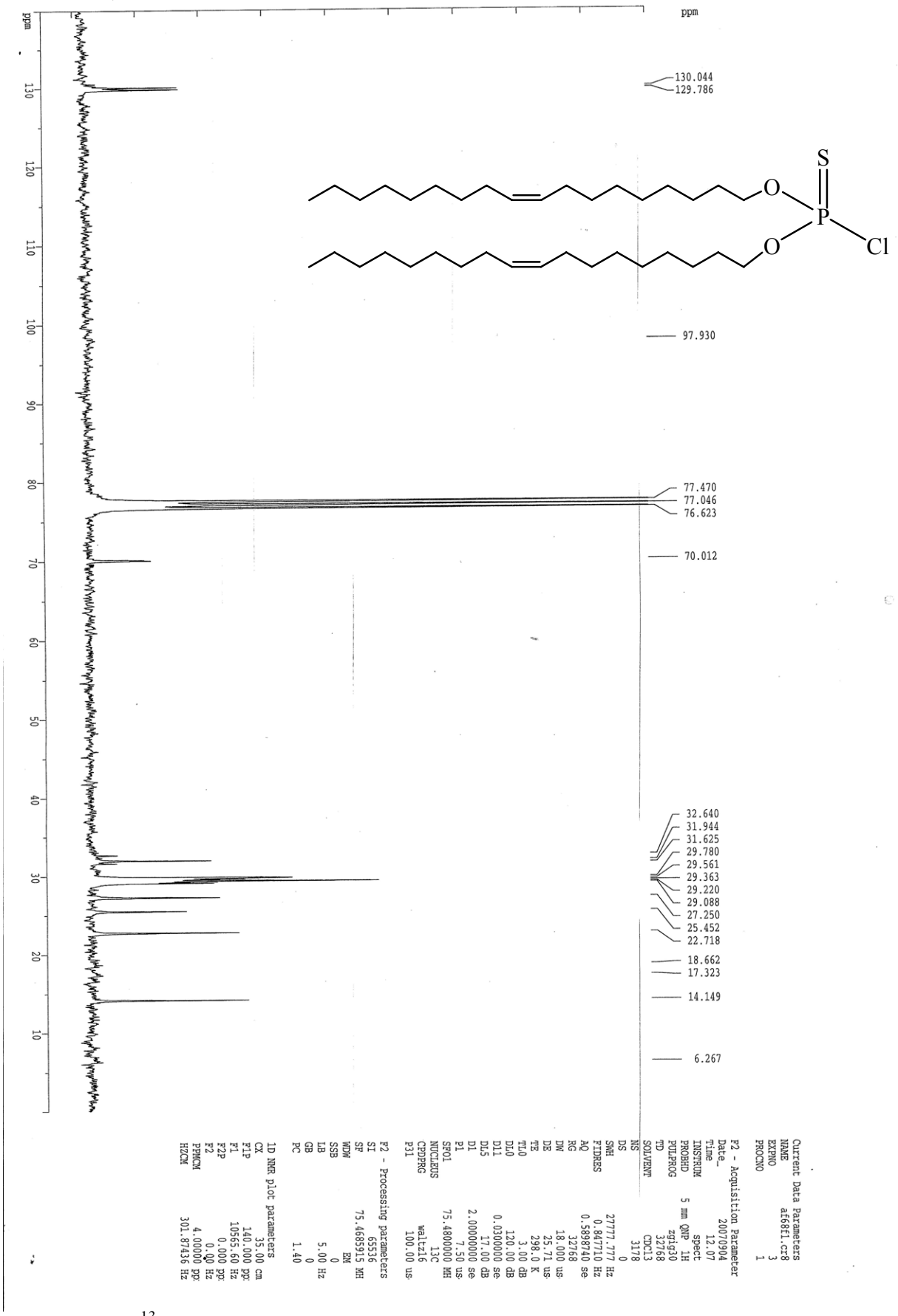


Figure S7-3 : ^{13}C NMR spectrum of compound **5**.

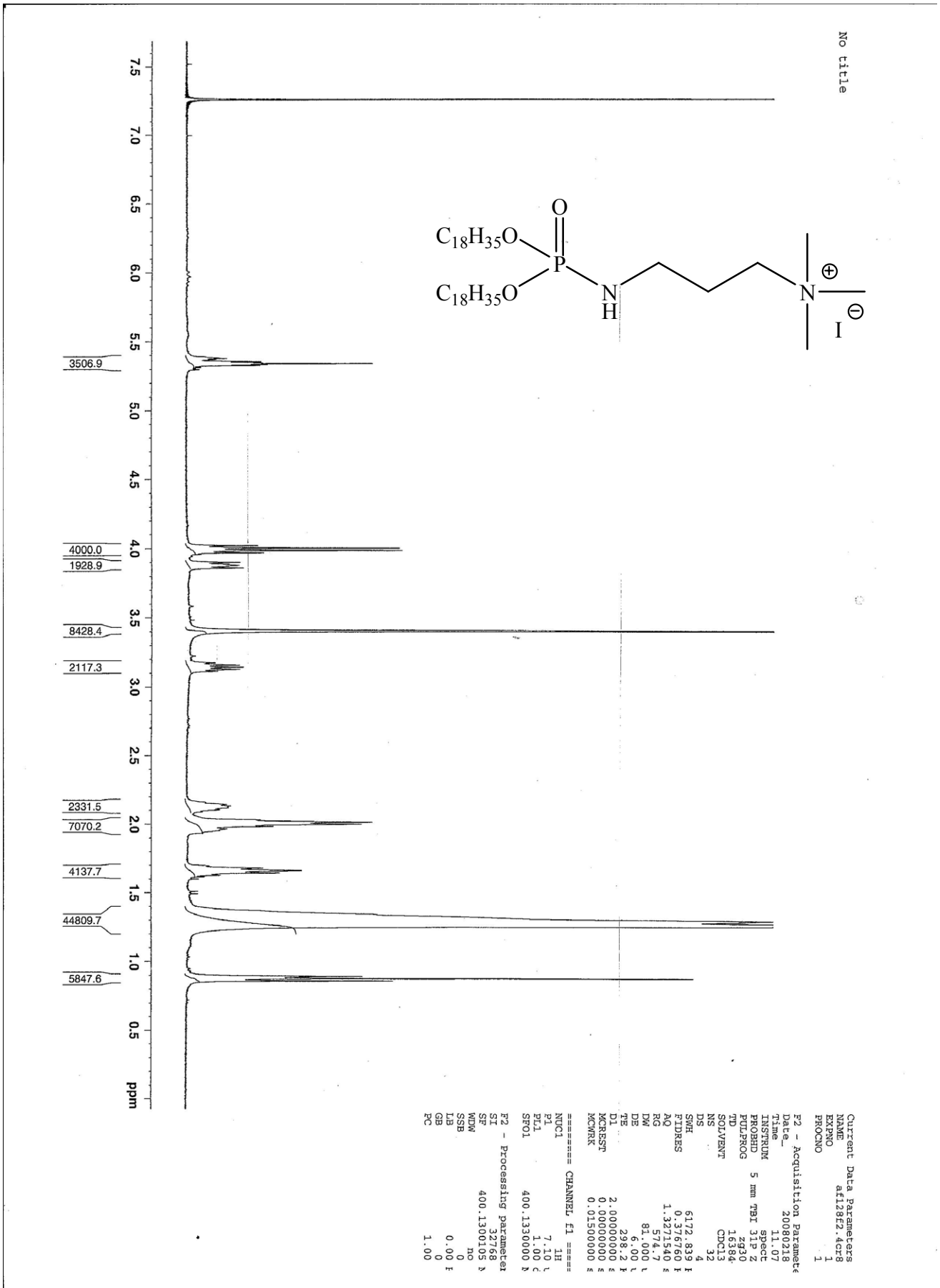


Figure S7-4 : ^{1}H NMR spectrum of compound **3a**.

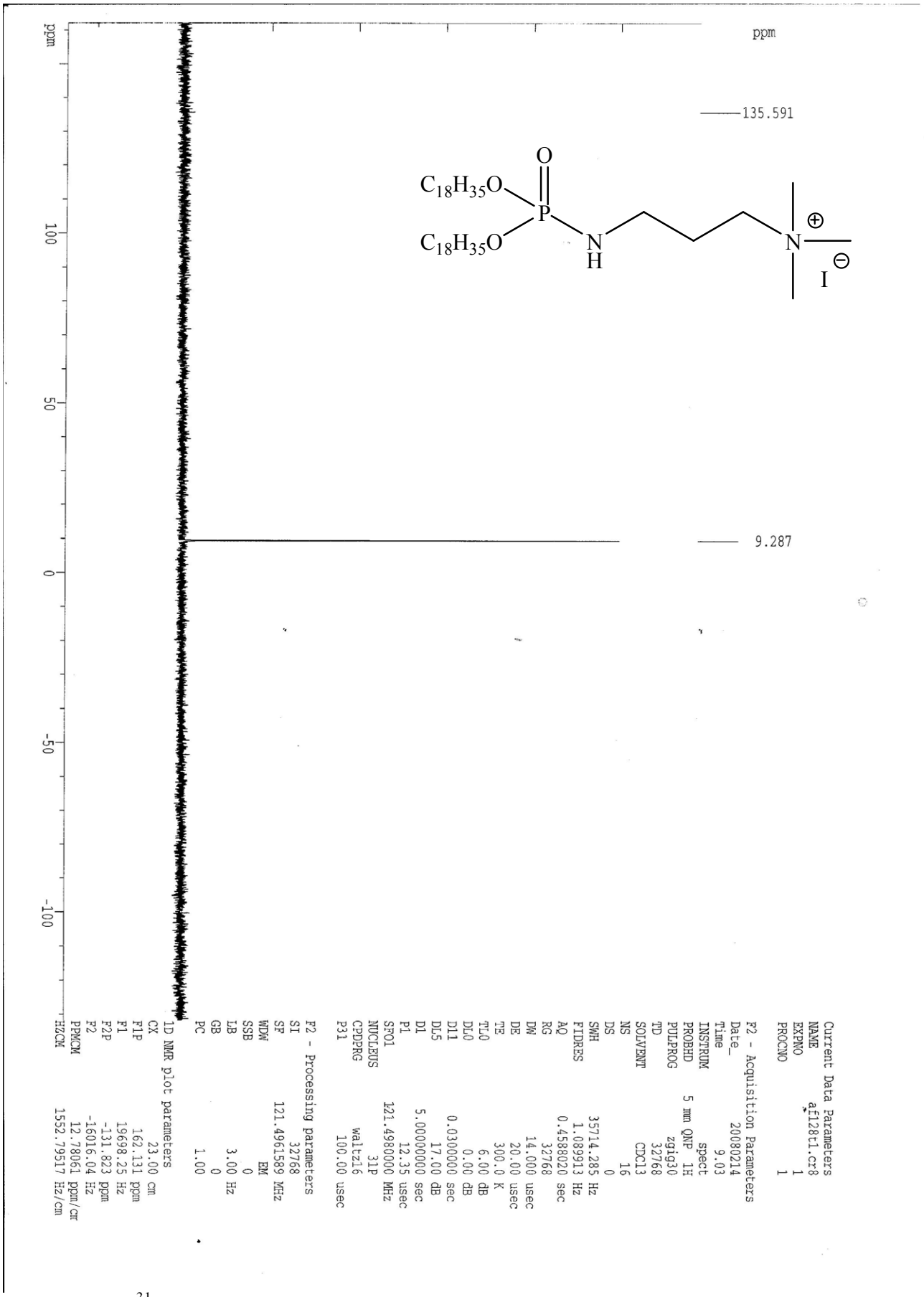


Figure S7-5 : ^{31}P NMR spectrum of compound **3a**.

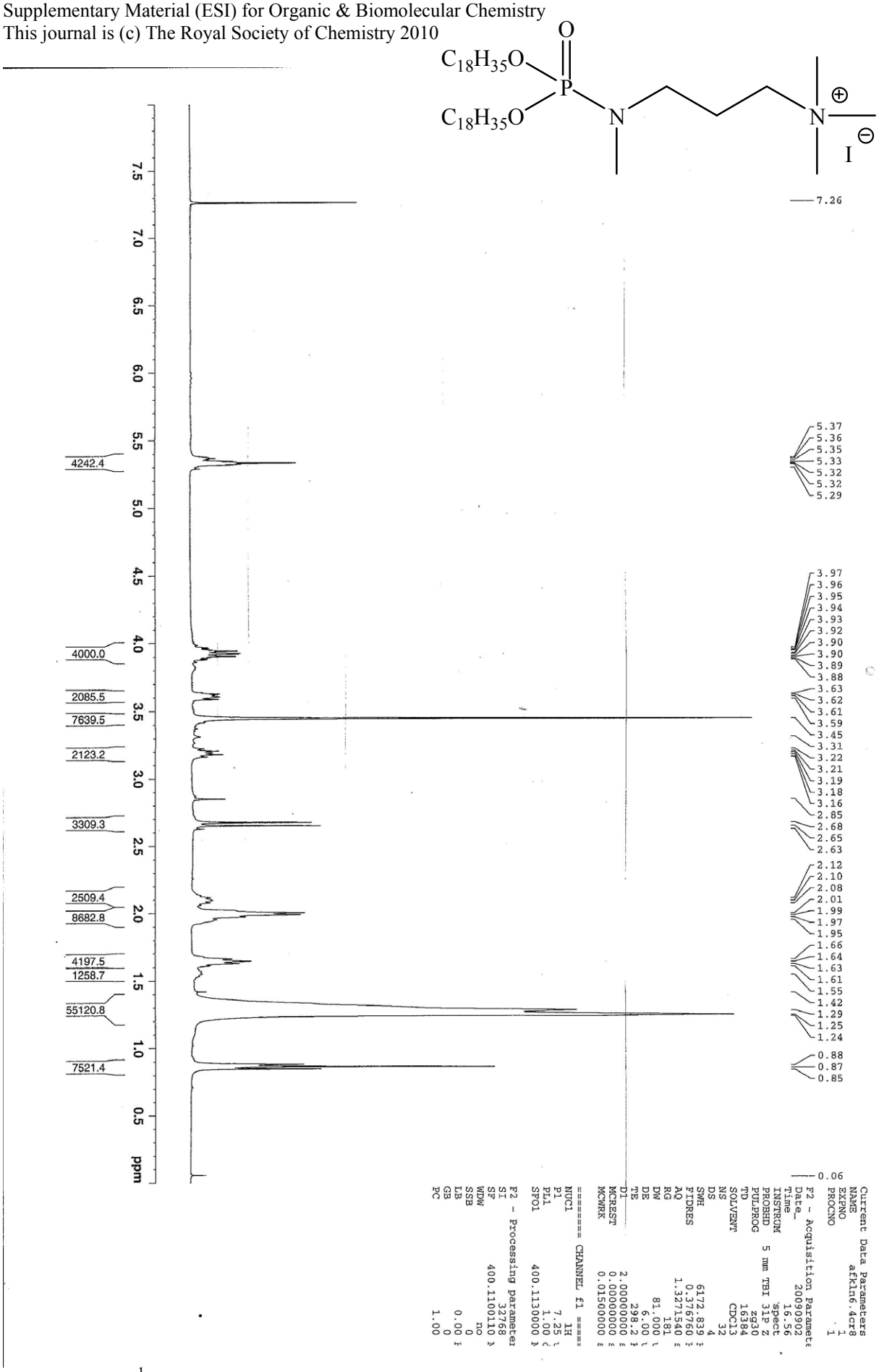


Figure S7-6 : ^{1}H NMR spectrum of compound **3b**.

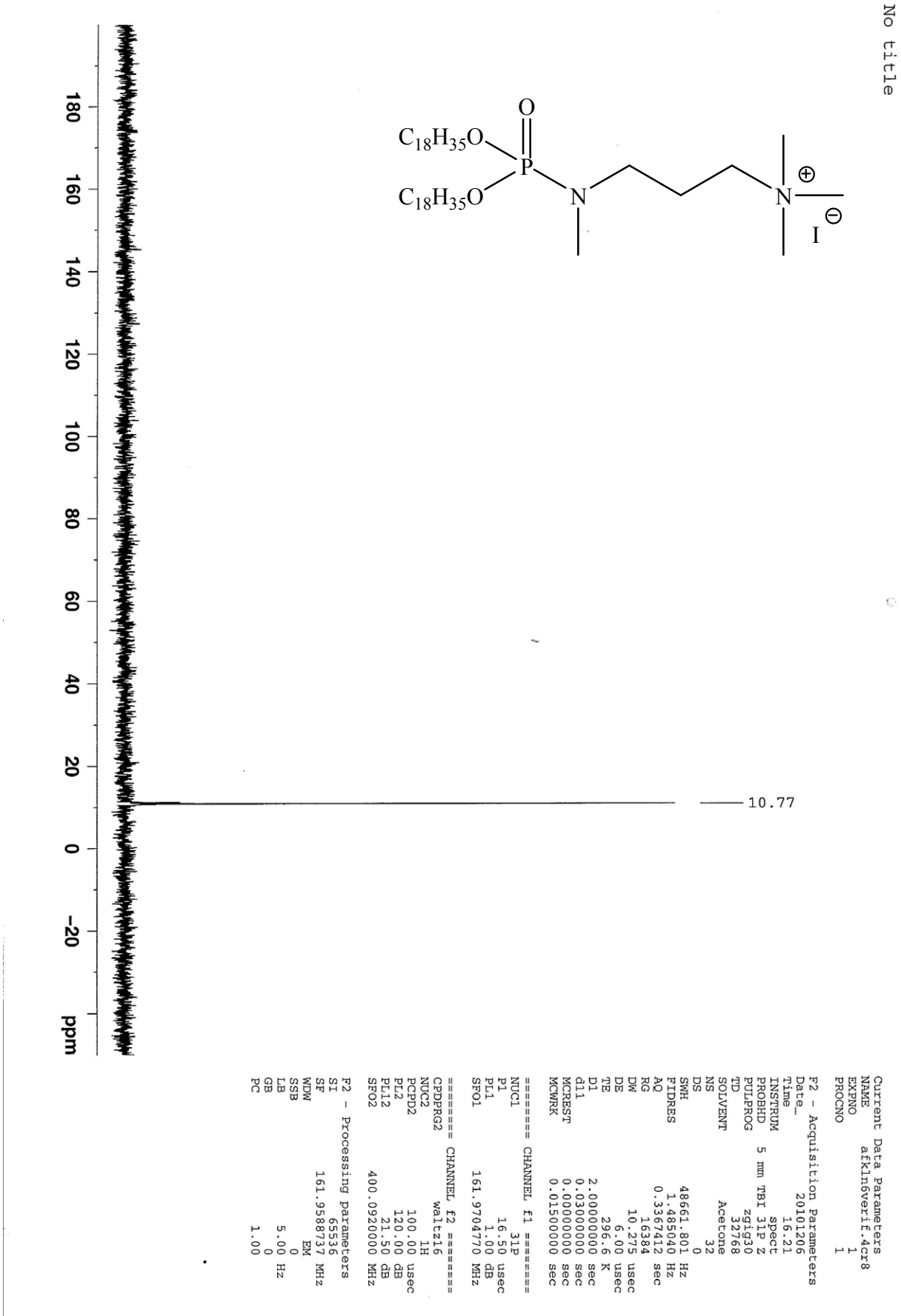


Figure S7-7 : ^{31}P NMR spectrum of compound **3b**.

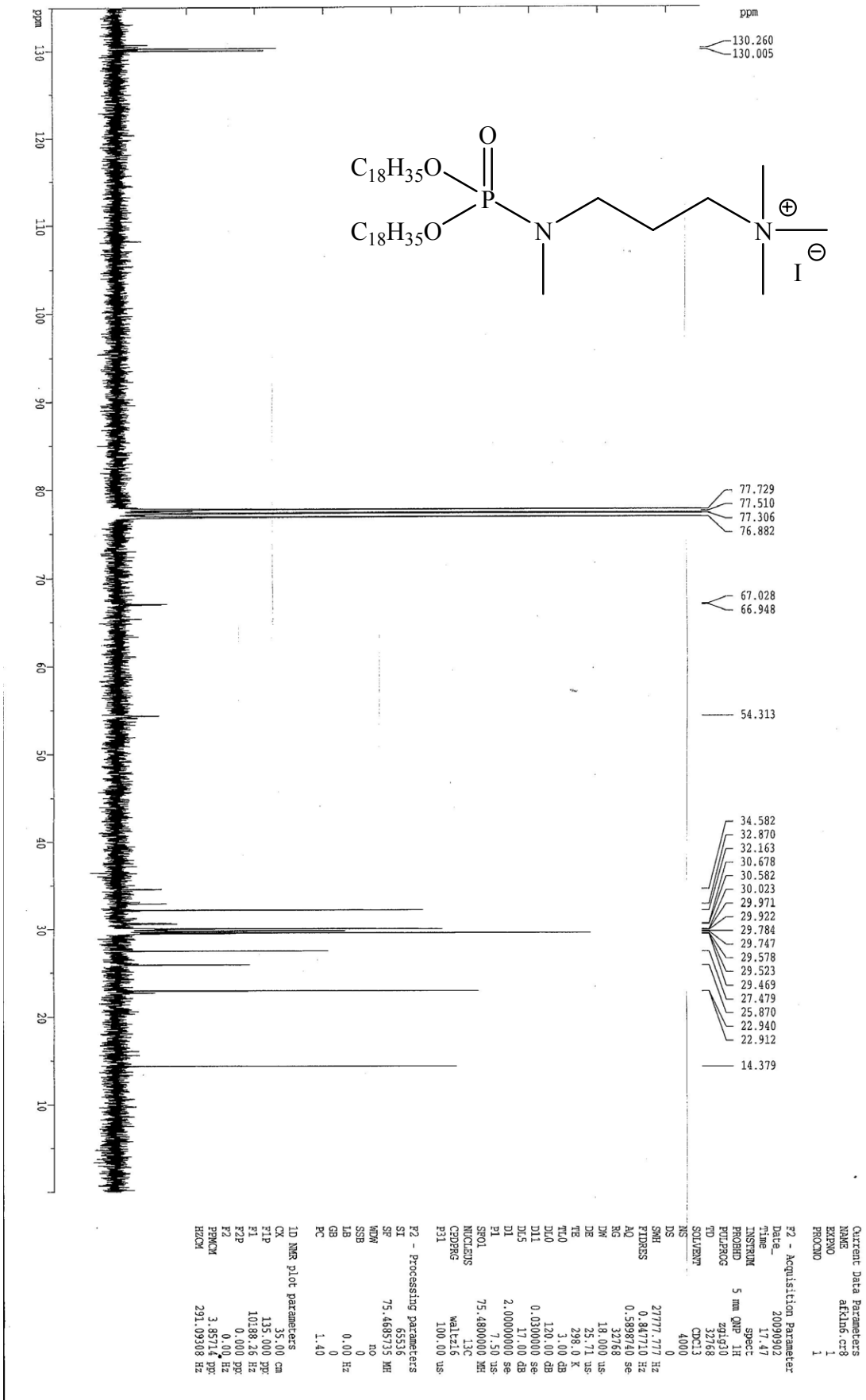


Figure S7- 8: ^{13}C NMR spectrum of compound **3b**.

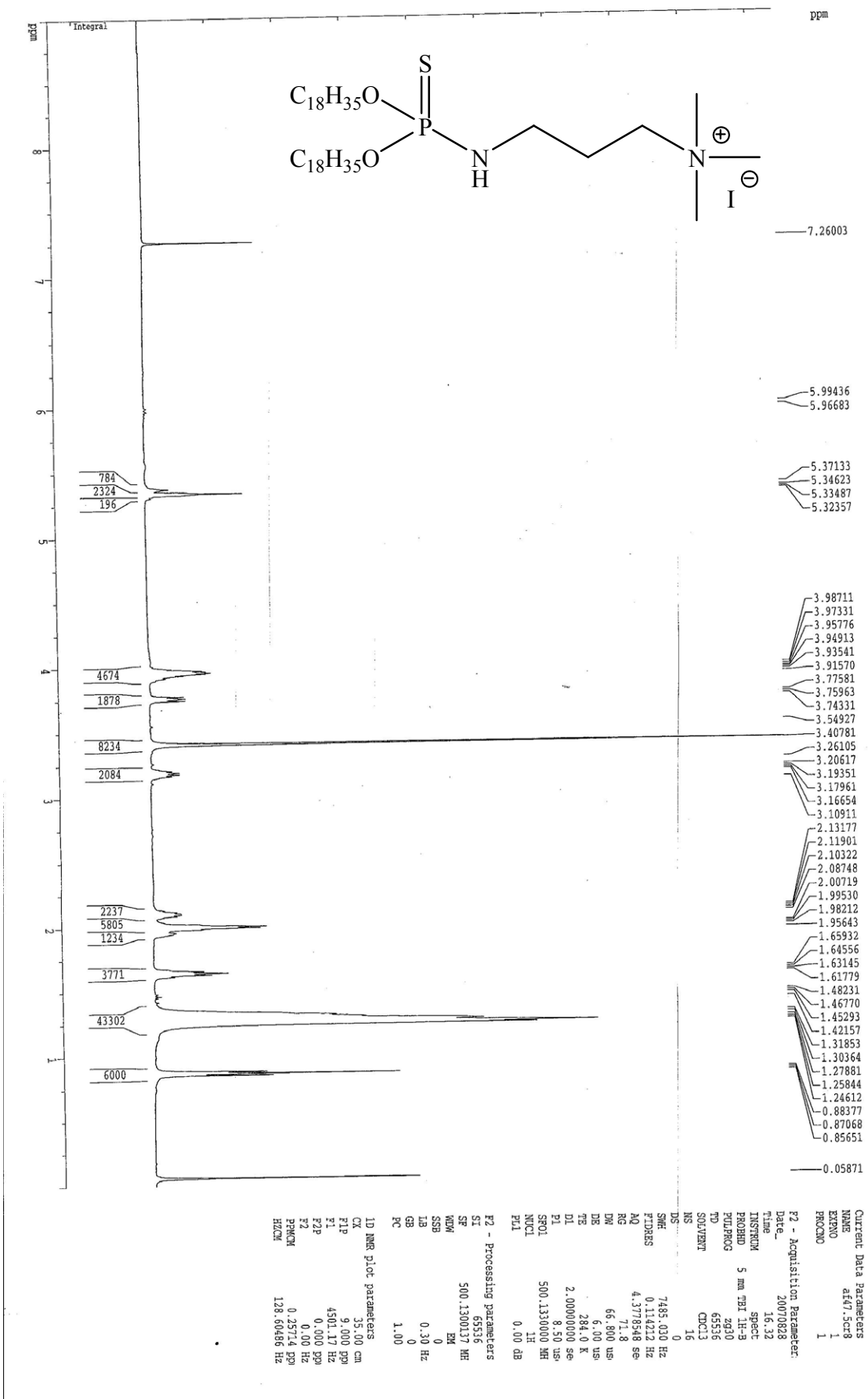


Figure S7- 9: ^{1}H NMR spectrum of compound **7a**.

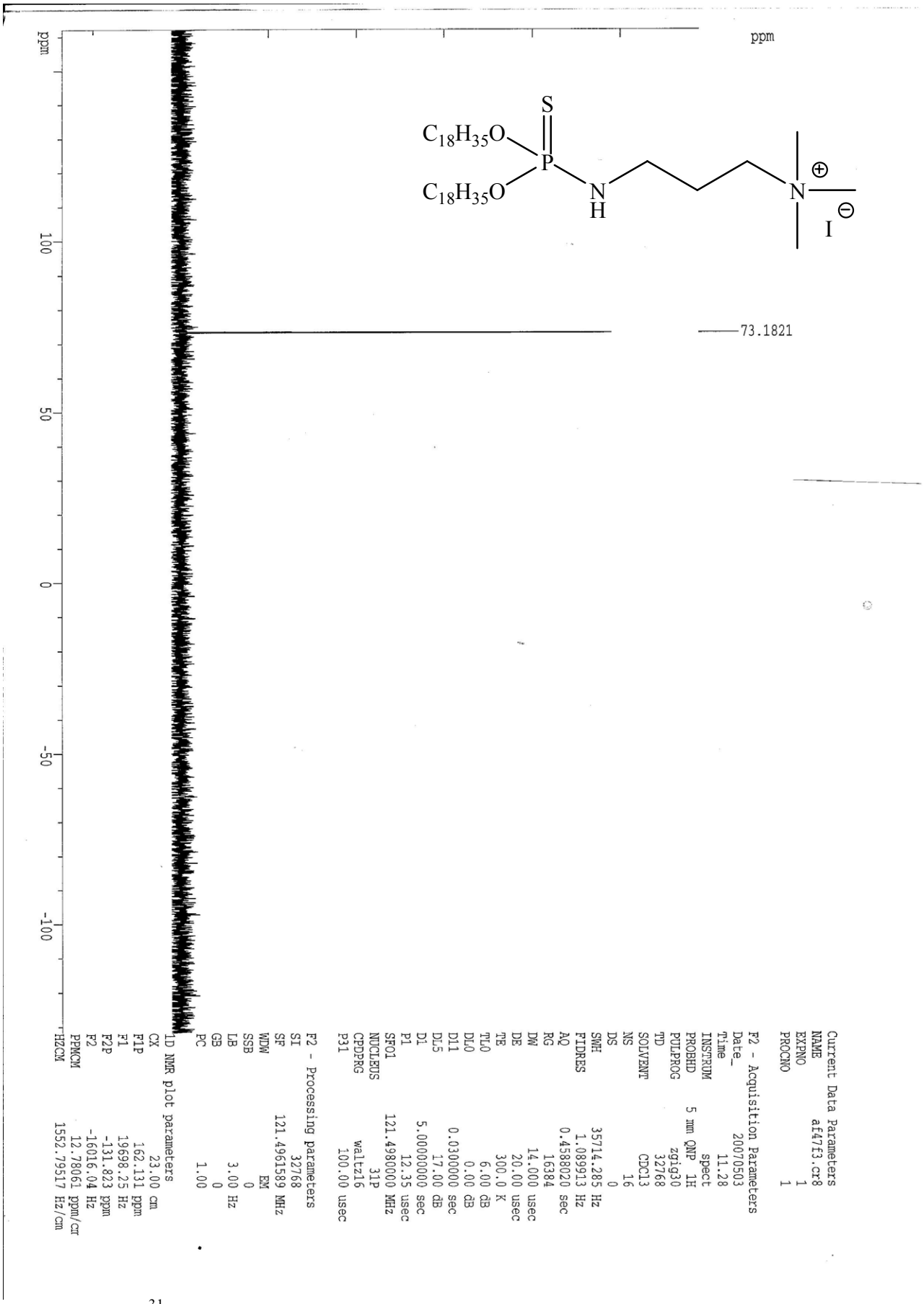


Figure S7- 10: ^{31}P NMR spectrum of compound **7a**.

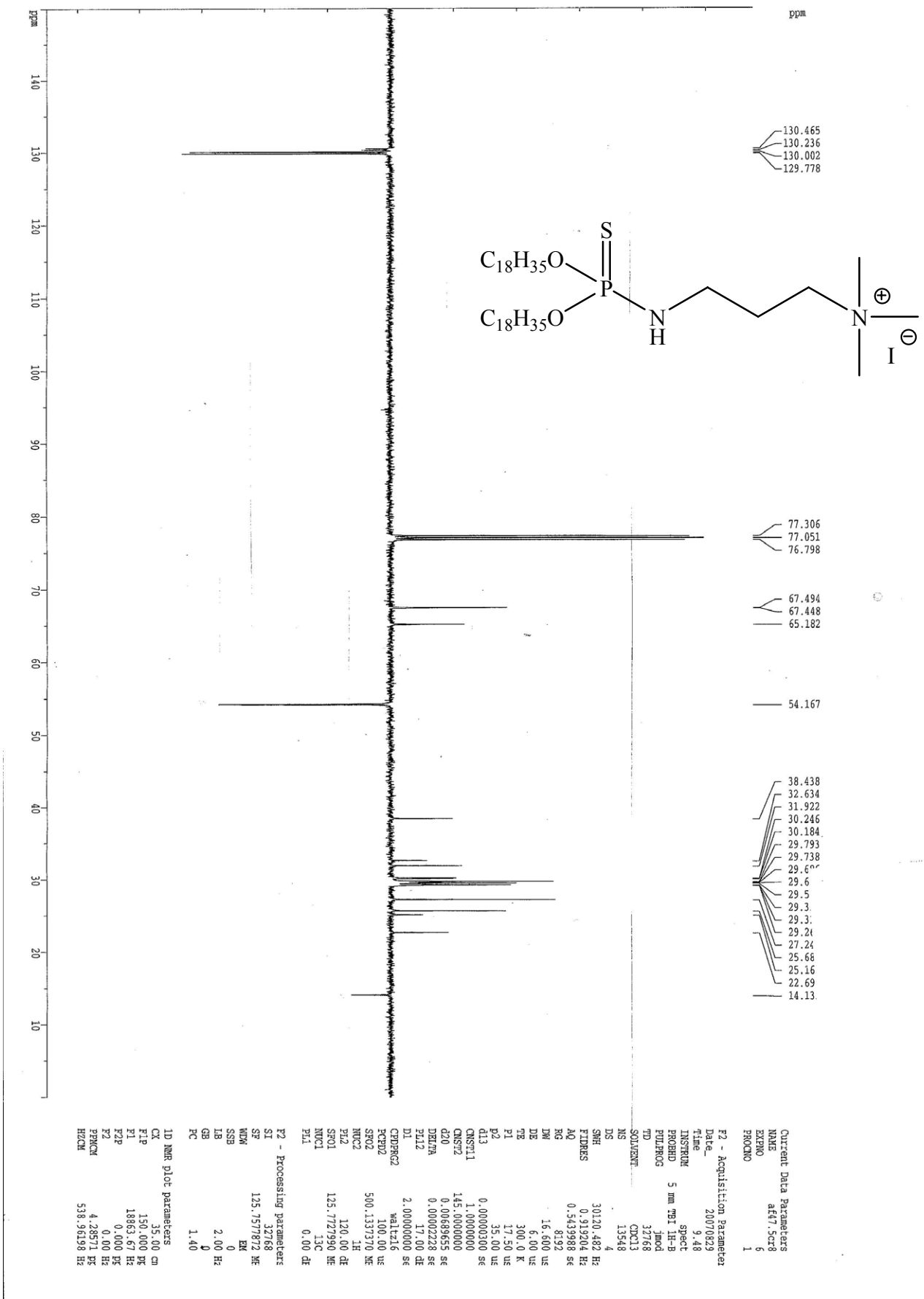


Figure S7- 11: ^{13}C NMR spectrum of compound **7a**.

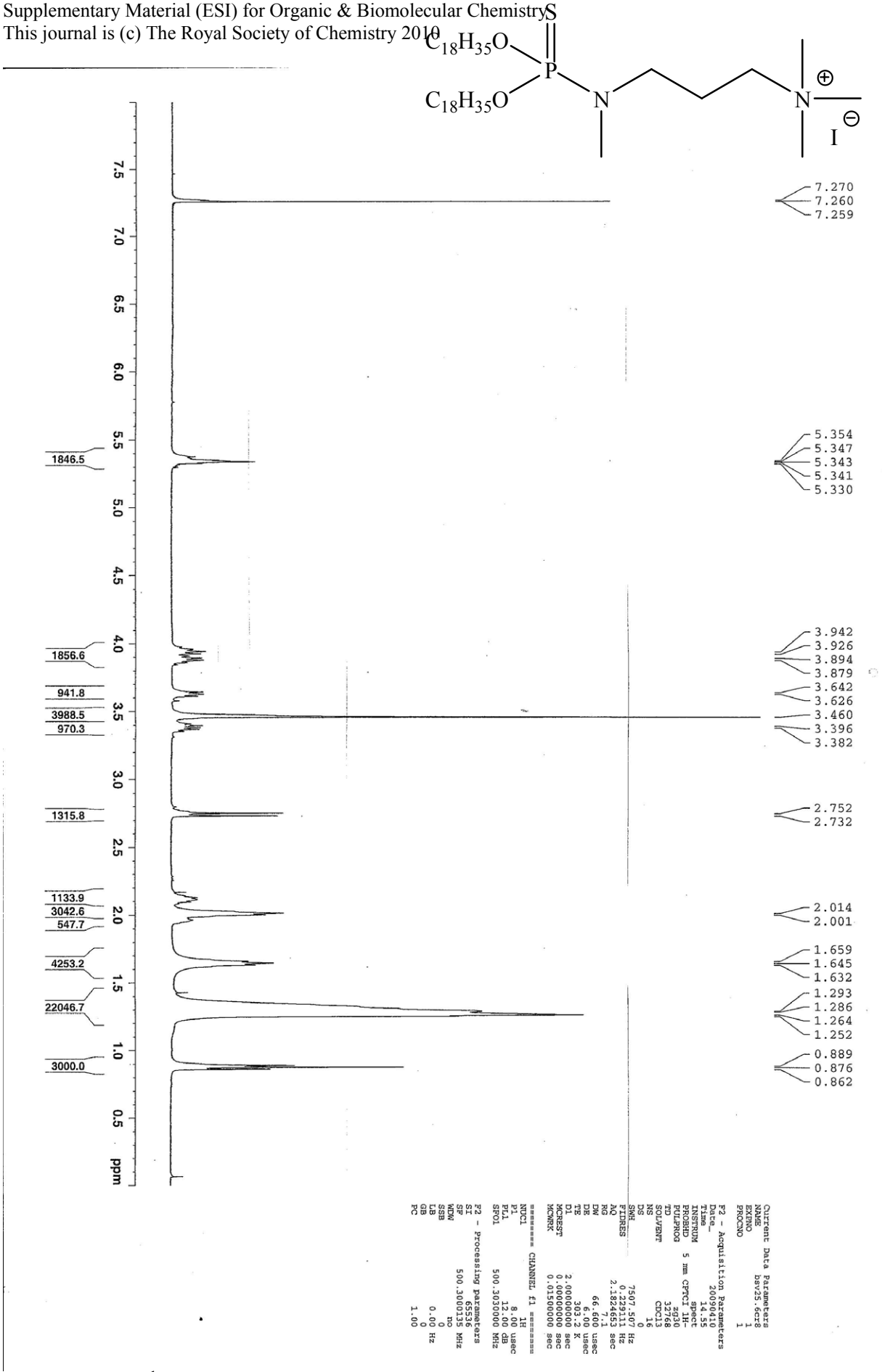


Figure S7- 12: ^{1}H NMR spectrum of compound **7b**.

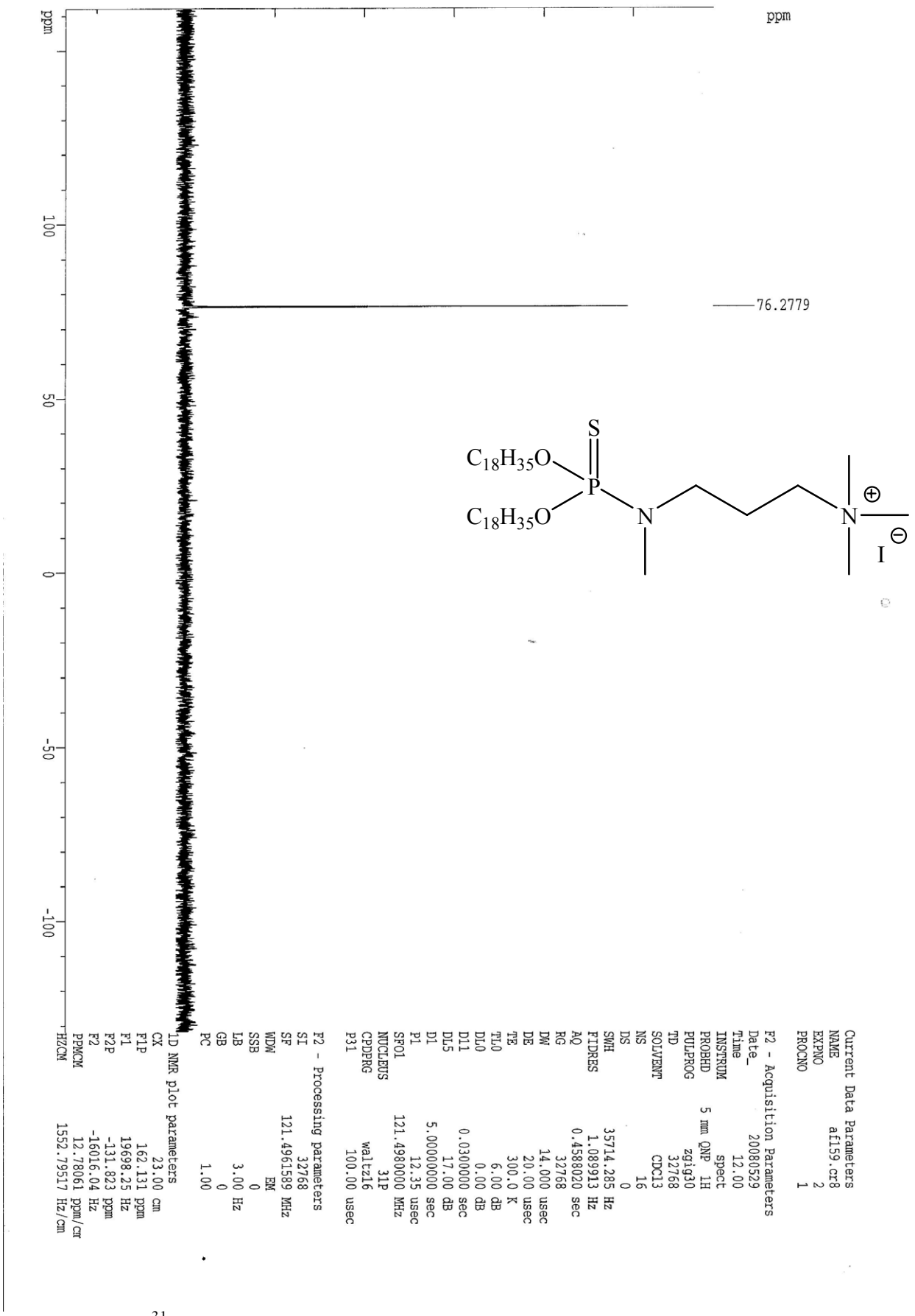


Figure S7- 13: ^{31}P NMR spectrum of compound **7b**.

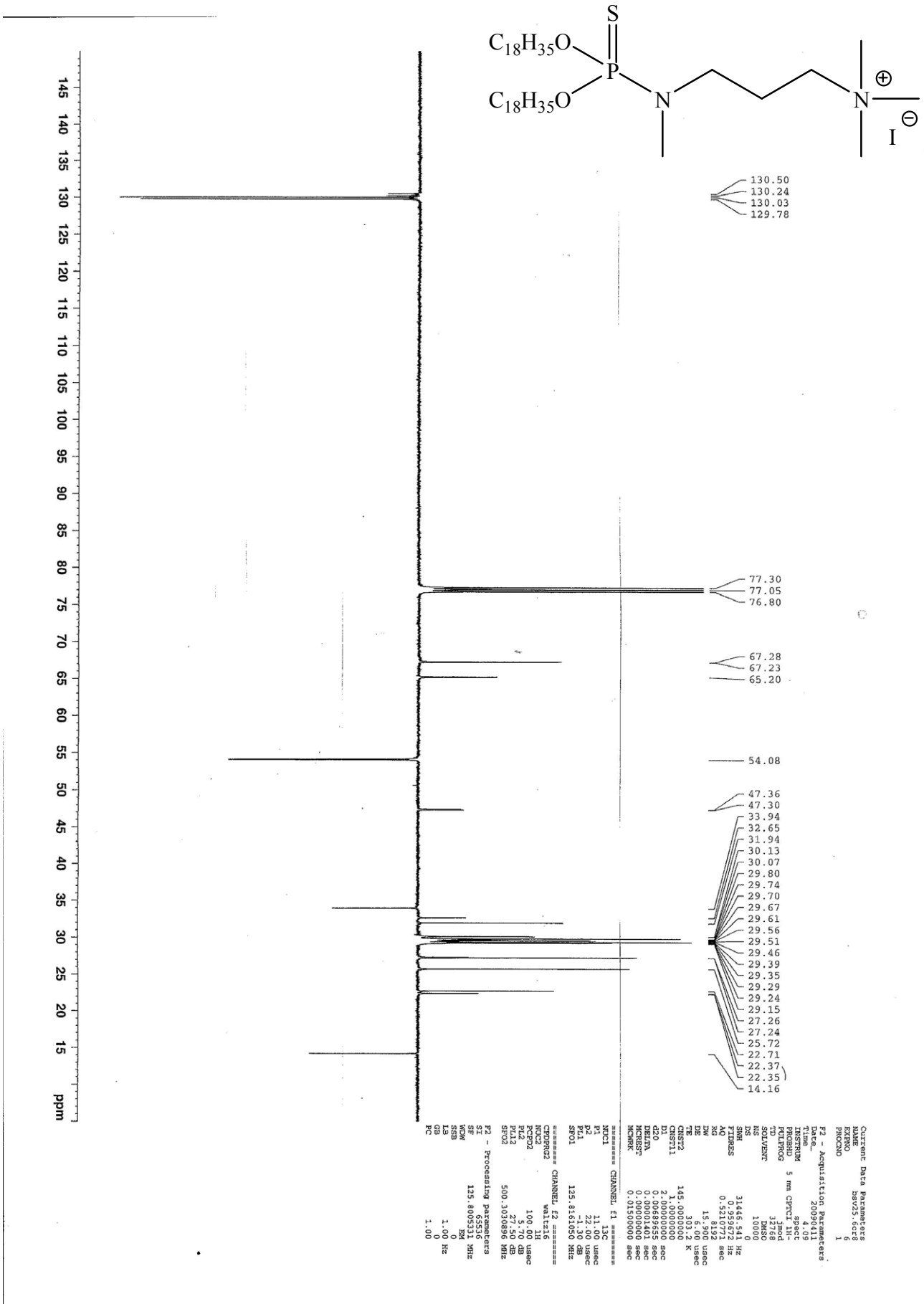


Figure S7- 14: ^{13}C NMR spectrum of compound **7b**.

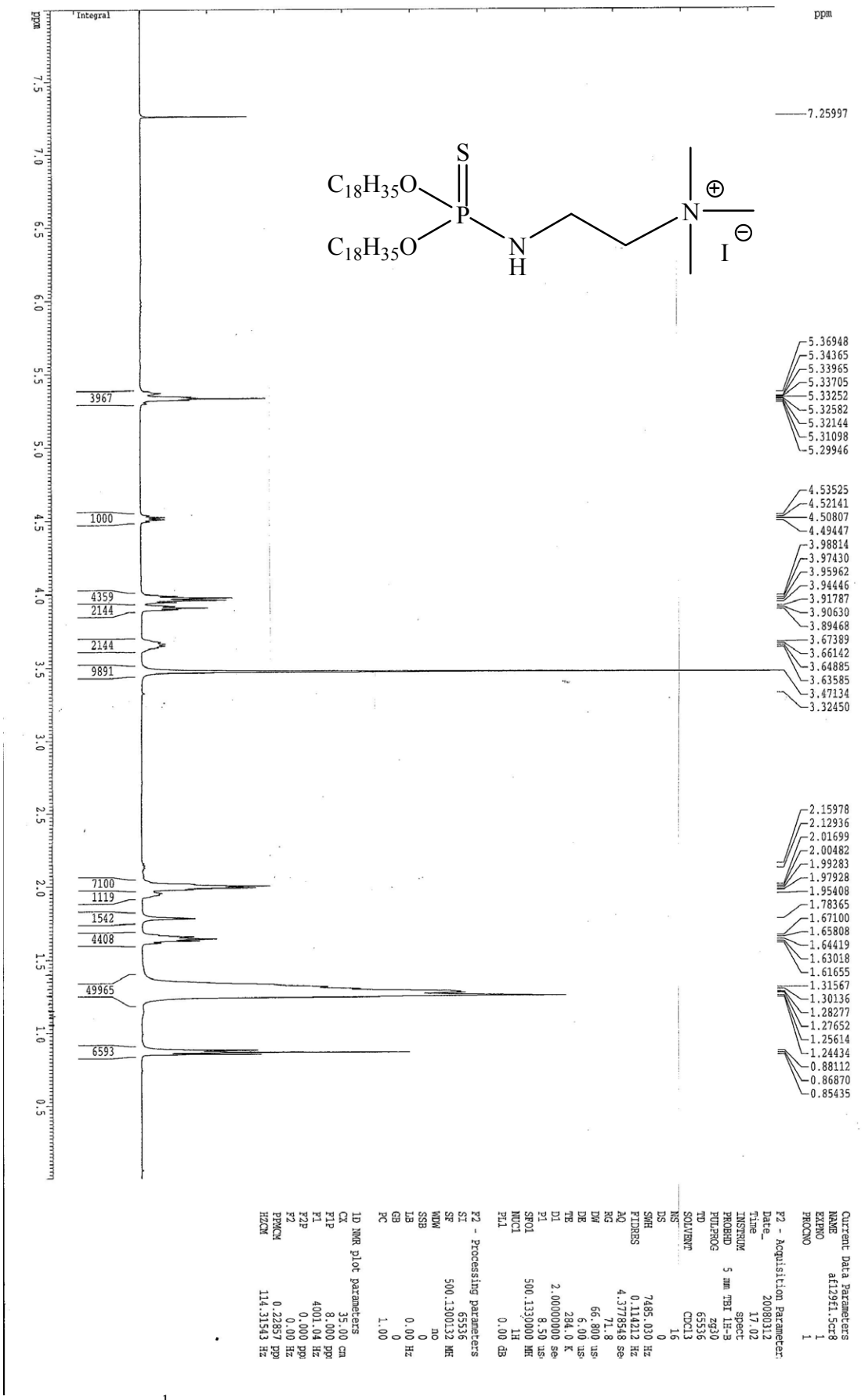


Figure S7- 15: ^{1}H NMR spectrum of compound **7c**.

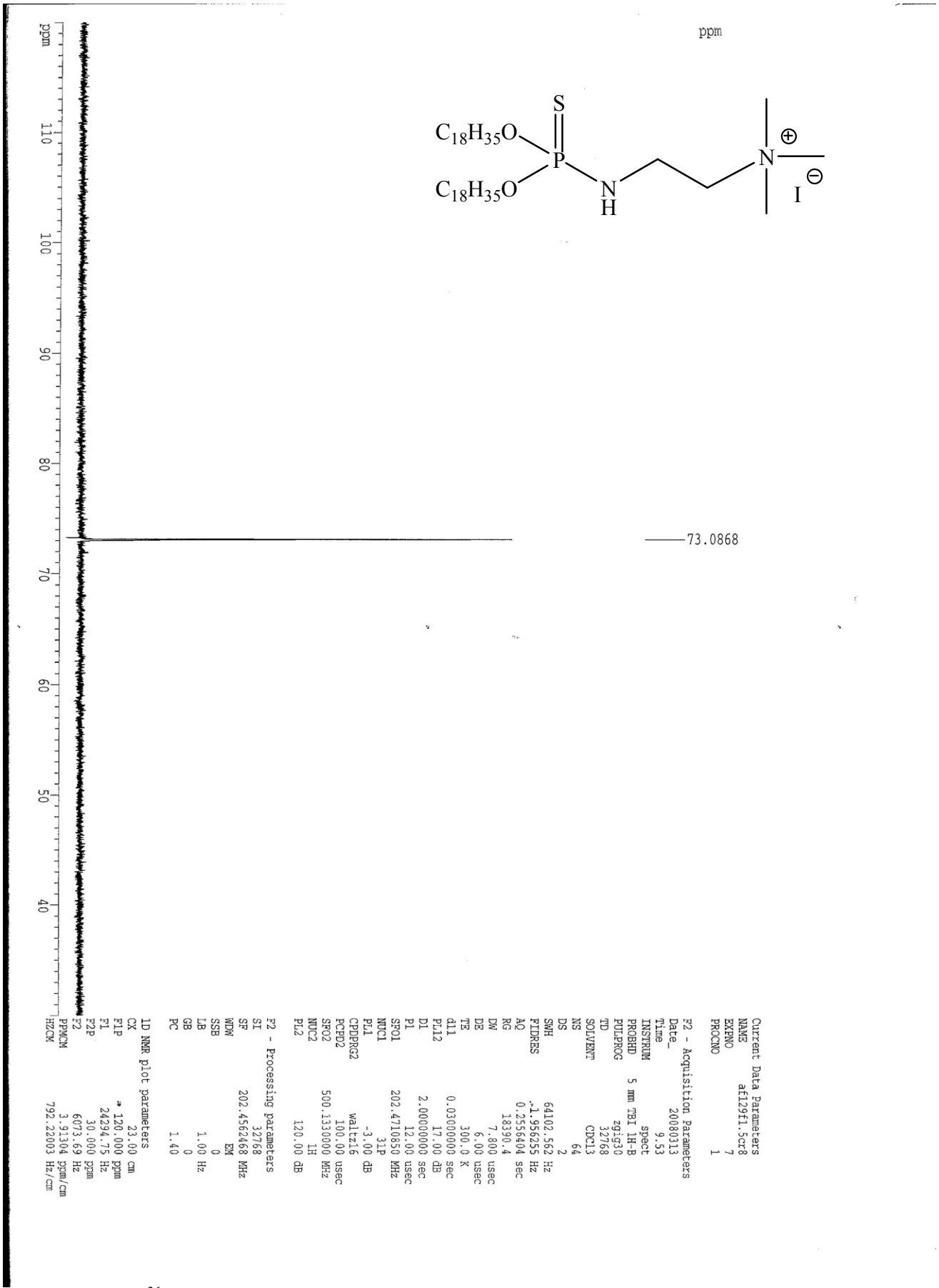


Figure S7- 16: ^{31}P NMR spectrum of compound **7c**.

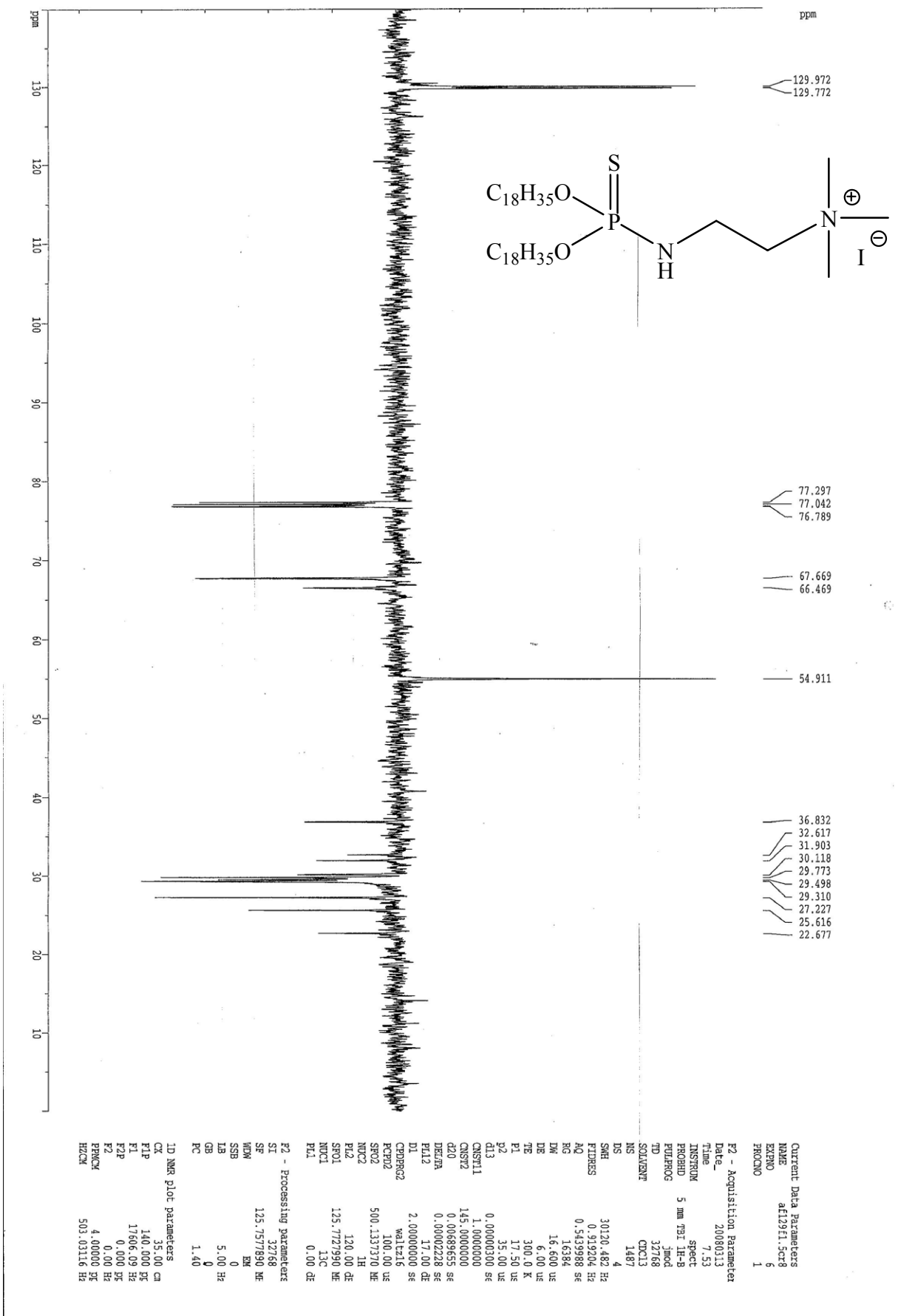


Figure S7- 17: ^{13}C NMR spectrum of compound **7c**.