

Electronic Supplementary Materials

Lipothiophosphoramidates for gene delivery: critical role of the cationic polar headgroup.

Aurore Fraix,^a Tristan Montier,^{b,c} Tony Le Gall,^b Charlotte Sevrain,^a Nathalie Carmoy,^{b,c} Mattias Lindberg,^b Pierre Lehn,^{b,c} Paul-Alain Jaffrès^{a,b*}

a) Université de Brest, Université Européenne de Bretagne, CEMCA, CNRS UMR 6521, IFR 148 ScInBioS, , 6 Avenue Le Gorgeu, 29238 Brest, France. e-mail : pjaffres@univ-brest.fr

b) IBiSA SynNanoVect platform, IFR 148 ScInBioS, Université de Bretagne Occidentale, Faculté de médecine Morvan, avenue Camille Desmoulins, 46 rue Félix Le Dantec, CS 51819, 29218 Brest Cedex 2, France.

c) INSERM U613, IFR 148 ScInBioS, Université de Bretagne Occidentale, Faculté de médecine Morvan, avenue Camille Desmoulins, 46 rue Félix Le Dantec, CS 51819, 29218 Brest Cedex 2, France.

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ESI-1 Formulation as liposomes – hydration time

compound	2 (BSV-17)	6a (BSV-14)	6b (BSV-28)	7 (BSV-21)	7 + DOPE (BSV-21 + DOPE)
formulation method	film hydration (3 days)	film hydration (13 days)	film hydration (13 days)	ethanolic injection	film hydration (3 days)

Table S1-1: Formulation method used for compounds 2, 6a-b, 7 and 7+DOPE.

ESI-2 Formulation as liposomes

Lipoplexes preparation

For each CR, the appropriate volume of liposomal solution ($1.5 \mu\text{mol.mL}^{-1}$) (e.g. CR=1, 80 μL) was introduced in a test tube and completed with water to obtain 1 mL of final volume (e.g. 920 μL). Forty μg of DNA (plasmid: pCMV-luc, 6.6 mg.mL^{-1} , 6.06 μL) was introduced in 993.94 μL of water. DNA solution was added dropwise to the liposomal solution. After mild homogenization, the mixture was incubated for 1h at R.T..

Size and Zeta potential measurements.

Size and Zeta potential measurements were performed on a ZetaSizer 3000 HSa (Malvern Instruments).

For size measurements, 100 μL of liposomal solution were diluted in 3.5 mL of water or the lipoplex solution was diluted in 1 mL of water. The dilution was filtered and introduced in the appropriate cuvette. The size of liposomes and lipoplexes was measured by quasi-elastic laser light scattering (QELS) in water with a sample refractive index of 1.59, a viscosity of 0.89 and a temperature of 25°C . The system was calibrated with the $200 \pm 5 \text{ nm}$ polystyrene polymer (Duke Scientific Corps Palo Alto, CA). The diameter of liposomes and lipoplexes was calculated in the contin mode.

Zeta potential measurement cell was filled with the solution used for size measurement.

ESI-3 Transfection efficiency and toxicity of 7 and 7/DOPE (1/1 molar ratio)

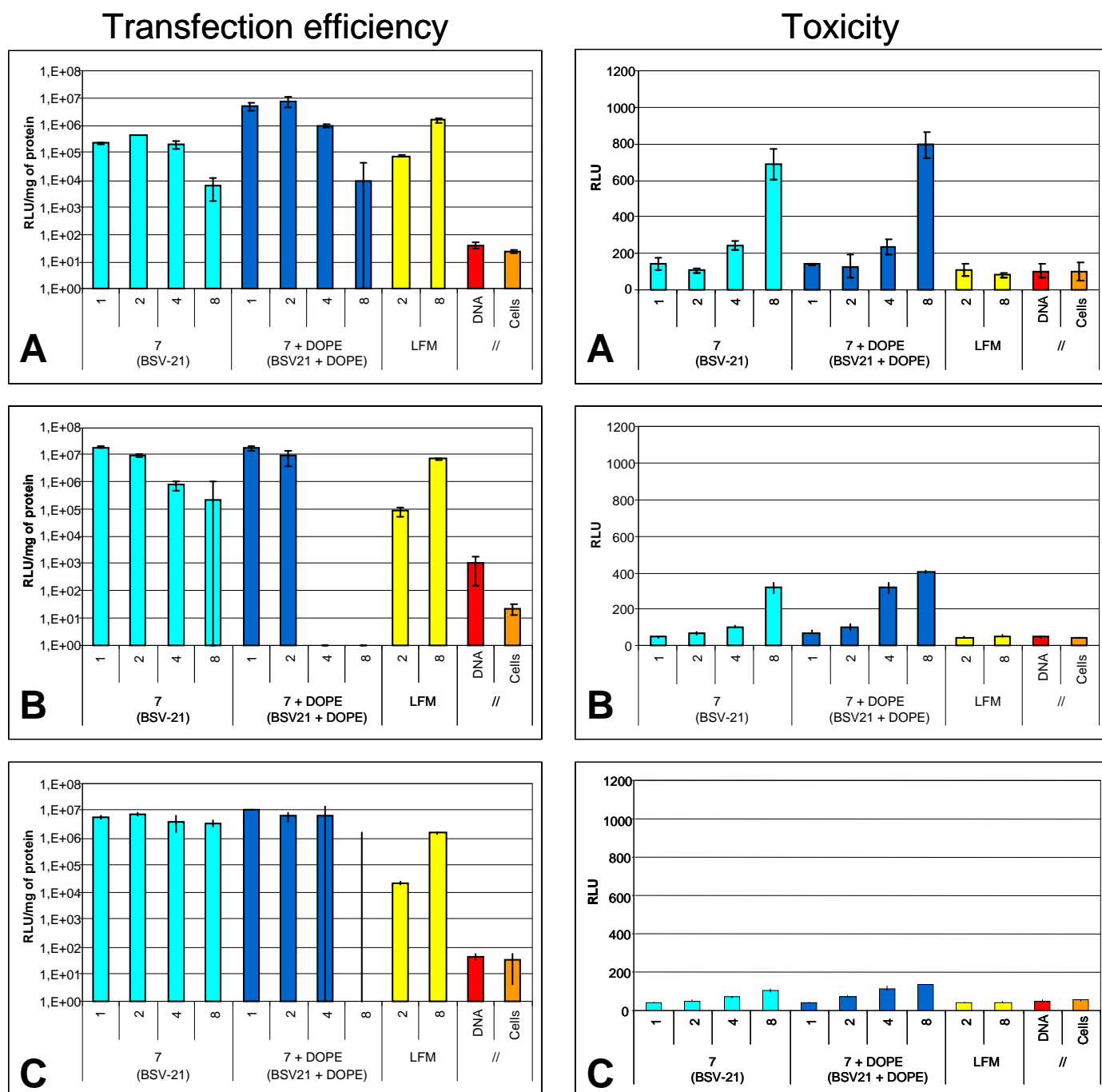


Figure S3-1: *In vitro* transfection efficiency and early toxicity of 7 (BSV21), 7 + DOPE (BSV21 + DOPE) and Lipofectamine as reference. **A-**HeLa cell line. **B-** A549 cell line; **C-** 16HBE14o(-).

ESI-4 NMR Data

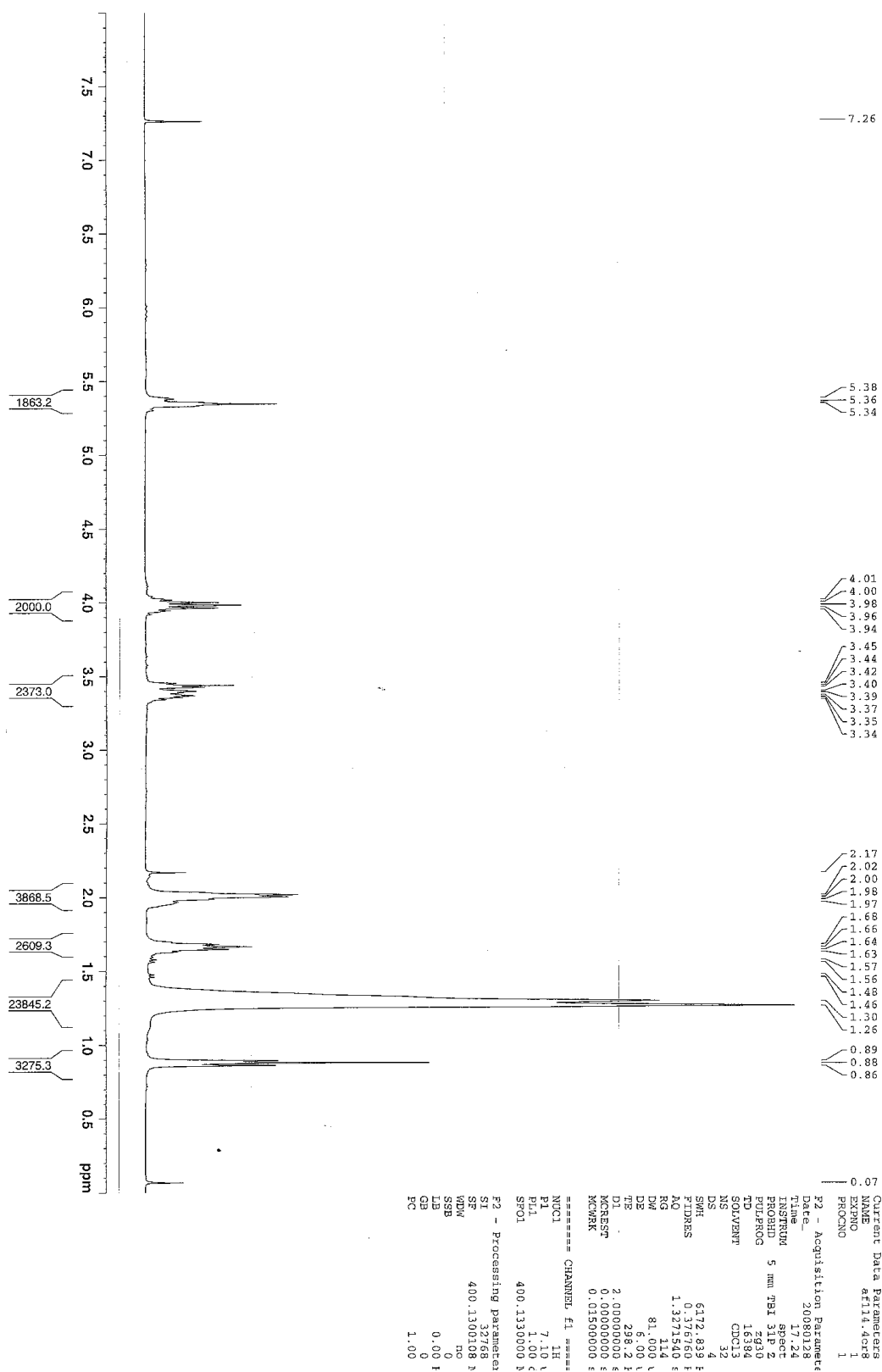


Figure S4-1: ¹H NMR (CDCl₃) spectrum of compound 4a.

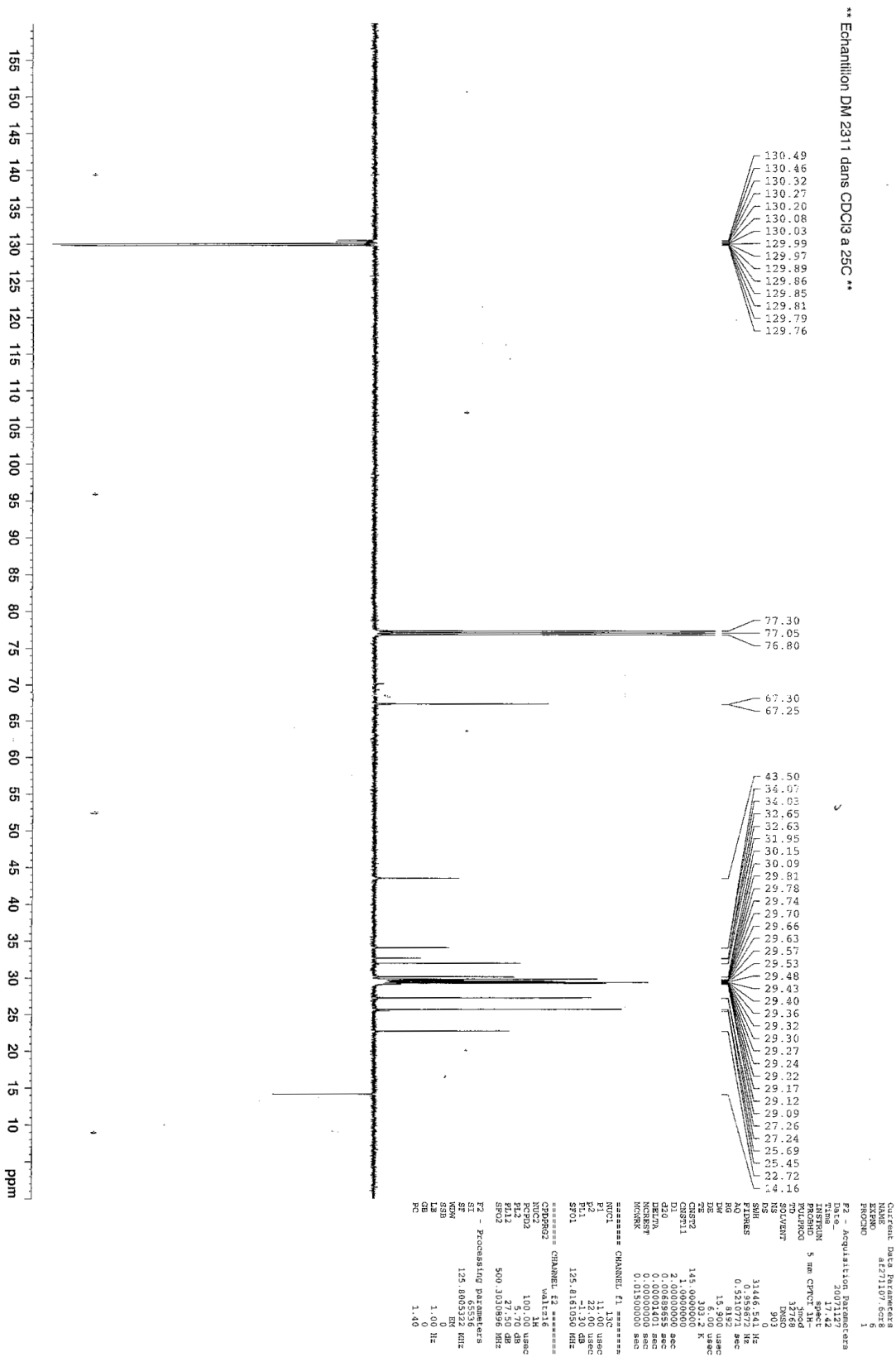


Figure S4-2: ¹³C NMR (CDCl₃) spectrum of compound **4a**.

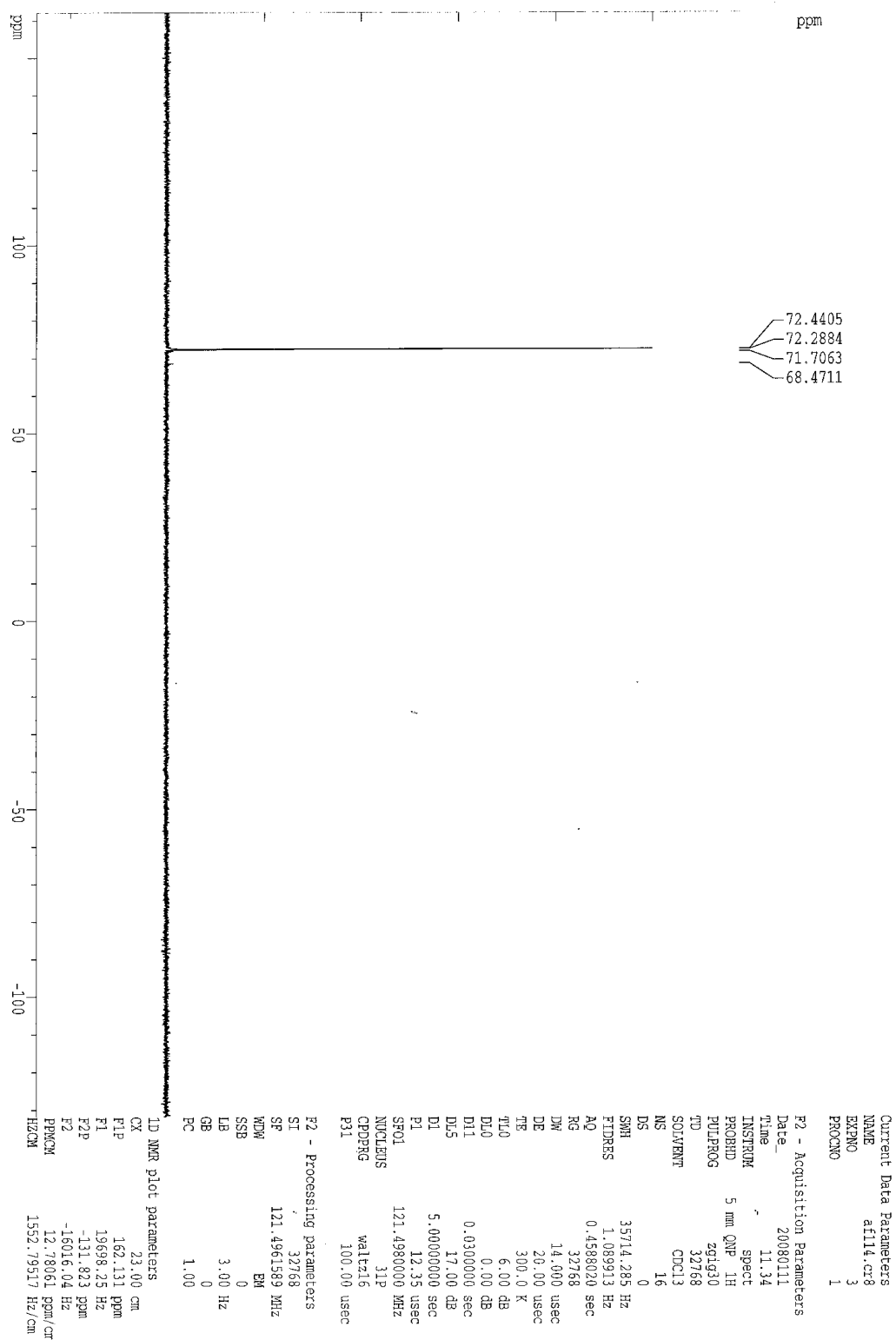


Figure S4-3: ^{31}P NMR (CDCl_3) spectrum of compound **4a**.

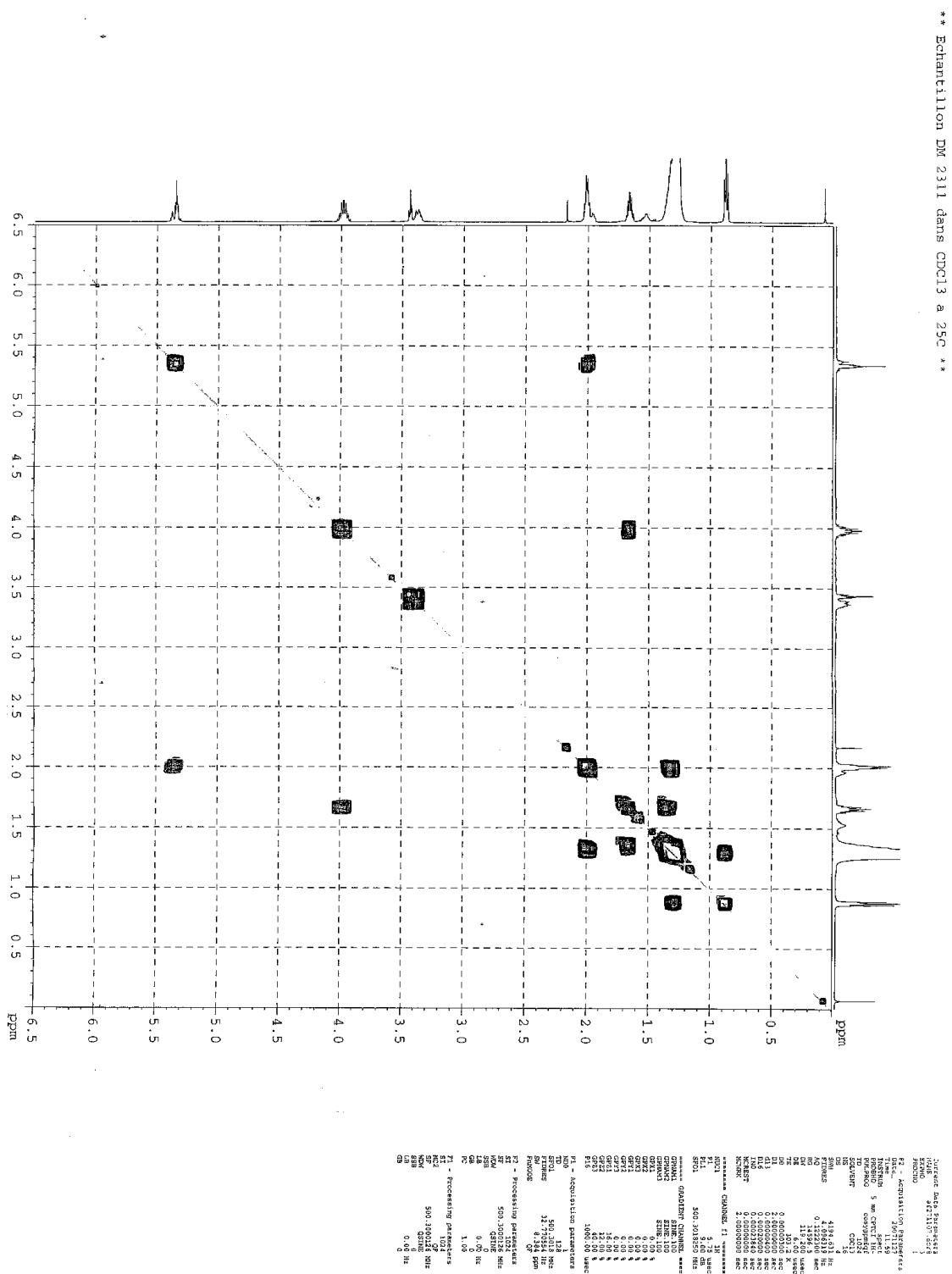
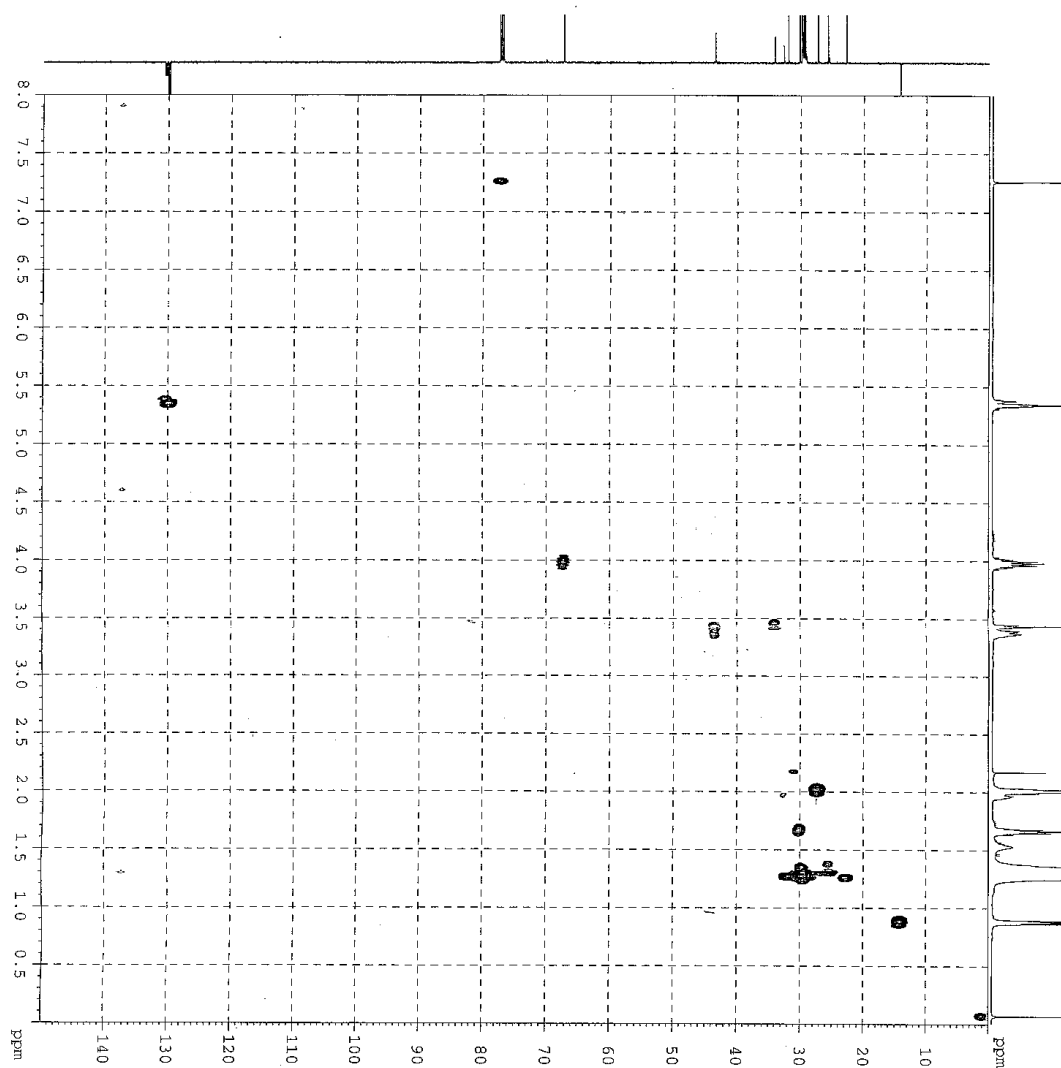


Figure S4-4: 2D Cosy NMR (CDCl₃) spectrum of compound 4a.

** Echantillon DM 2311 dans CDCl3 a 25C **



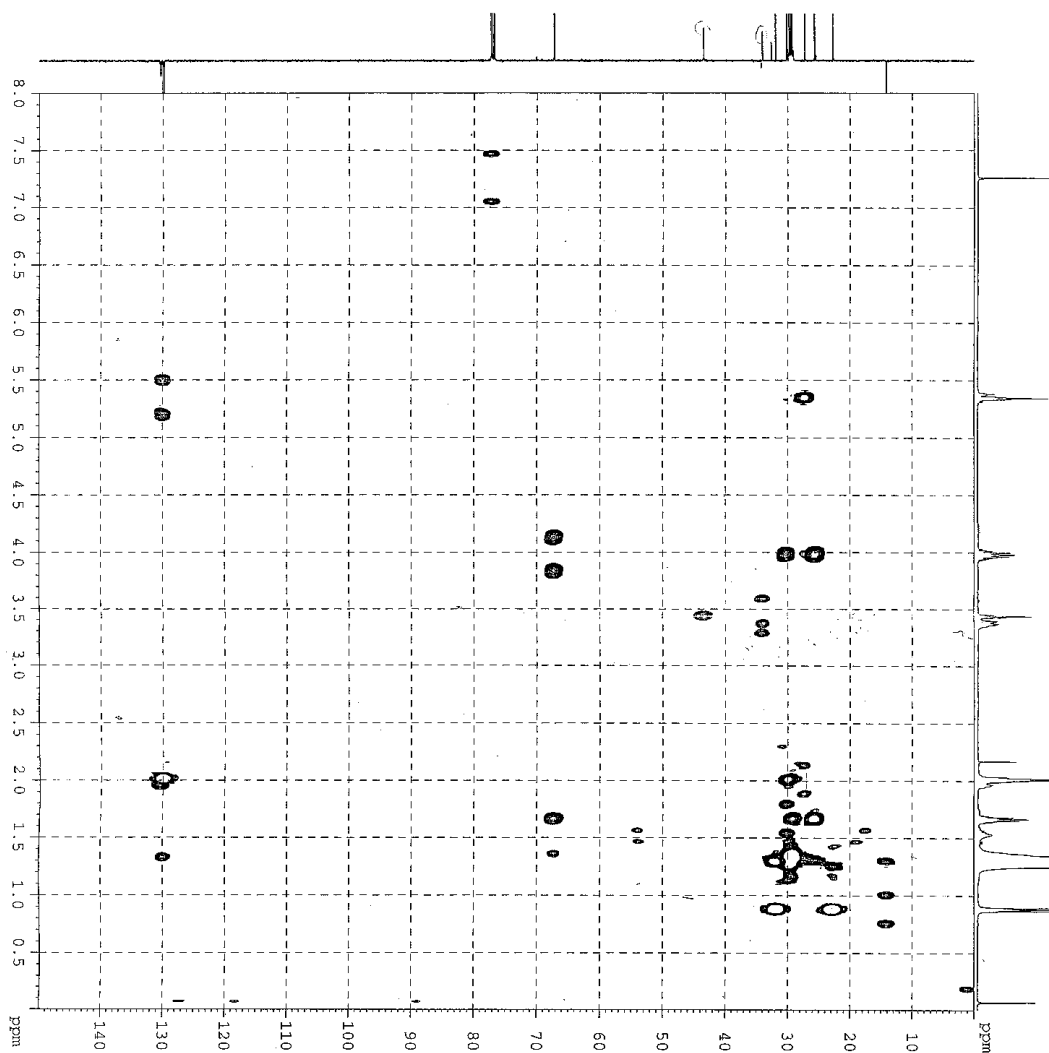
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PULPROG: zgpg30
TD: 65536
SOLVENT: CDCl3
AQ: 1.10000000
RG: 512
AQ2: 0.04000000
RG2: 4096
FIDRES: 0.32000000
AQ3: 0.00000000
RG3: 32768
AQ4: 0.00000000
RG4: 32768
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SF: 125.7601400
WDW: EM
SSB: 0
GB: 0
PC: 1.00
SC: 1.00
OC: 0
MC: 0
MD: 0
HM: 0
HSM: 0
AQ5: 0.00000000
AQ6: 0.00000000
F2 - Acquisition parameters
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EXPNO: 2
PROCNO: 1
PROCNAME: 4a
F1 - Acquisition Parameters
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PROBHD: 5 mm QNP1H/1
PULPROG: zgpg30
TD: 65536
SOLVENT: CDCl3
AQ: 1.10000000
RG: 512
AQ2: 0.04000000
RG2: 4096
FIDRES: 0.32000000
AQ3: 0.00000000
RG3: 32768
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HM: 0
HSM: 0
AQ5: 0.00000000
AQ6: 0.00000000
=====

```

Figure S-4-5: 2D HMQC NMR (CDCl₃) spectrum of compound 4a.

** Echantillon DW 2311 dans CDCl3 a 25C **



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EXPNO: 4281107
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Process:
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F2: Processing parameters
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Time: 21:32
Name: f2
Expno: 4281107
Procno: 1
Filename: f2
Directory:
Process:
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F3: Acquisition Parameters
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Time: 11:53
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Expno: 4281107
Procno: 1
Filename: f2
Directory:
Process:
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F4: Processing parameters
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Expno: 4281107
Procno: 1
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Process:
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Expno: 4281107
Procno: 1
Filename: f2
Directory:
Process:
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Name: DW_23110849
Expno: 4281107
Procno: 1
Filename: f2
Directory:
Process:
-----
Name: DW_23110849
Expno: 4281107
Procno: 1
Filename: f2
Directory:
Process:
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Figure S-4-6: 2D HMBC NMR (CDCl₃) spectrum of compound 4a.

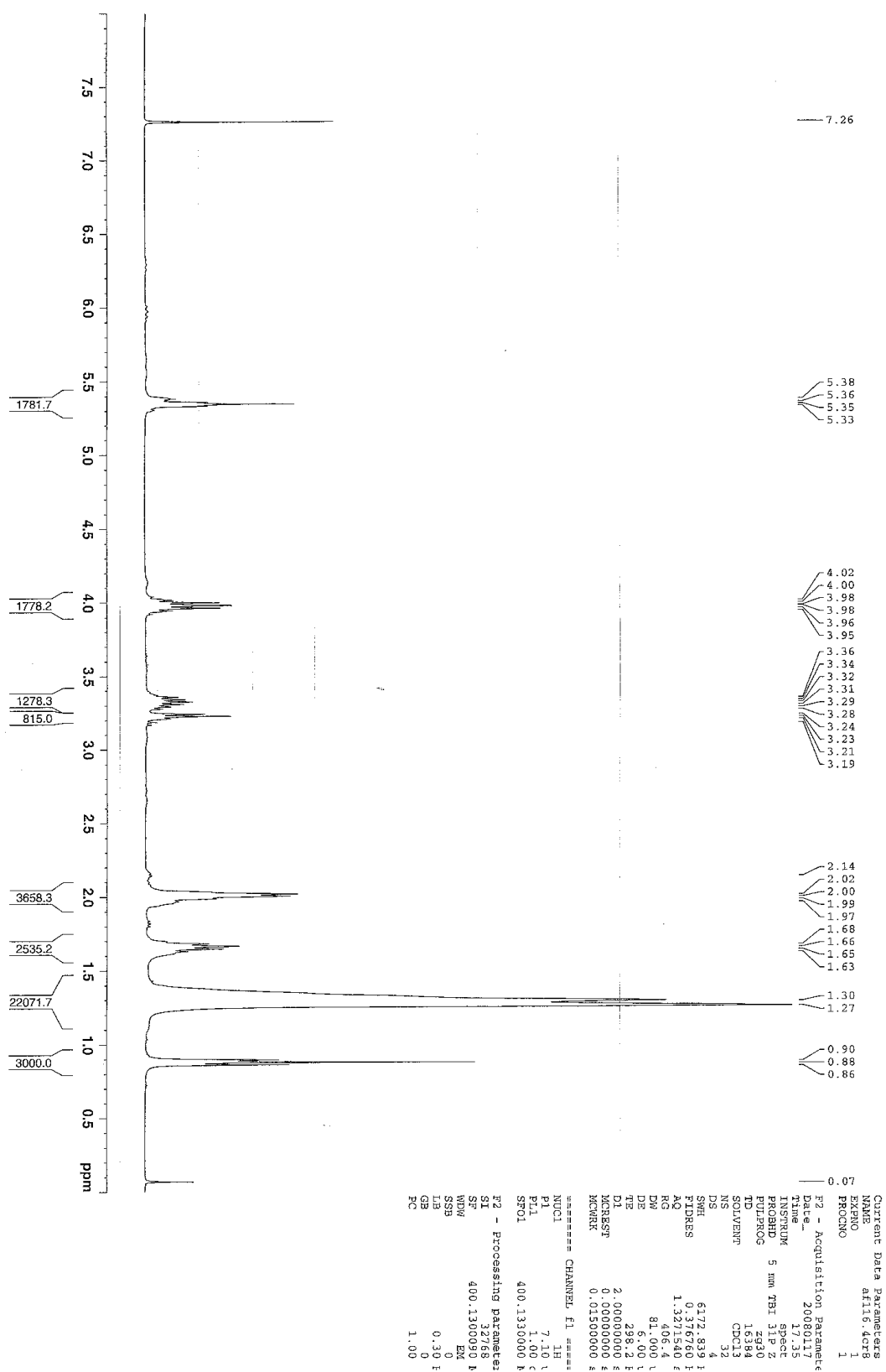


Figure S4-7: ¹H NMR (CDCl₃) spectrum of compound **5a**.

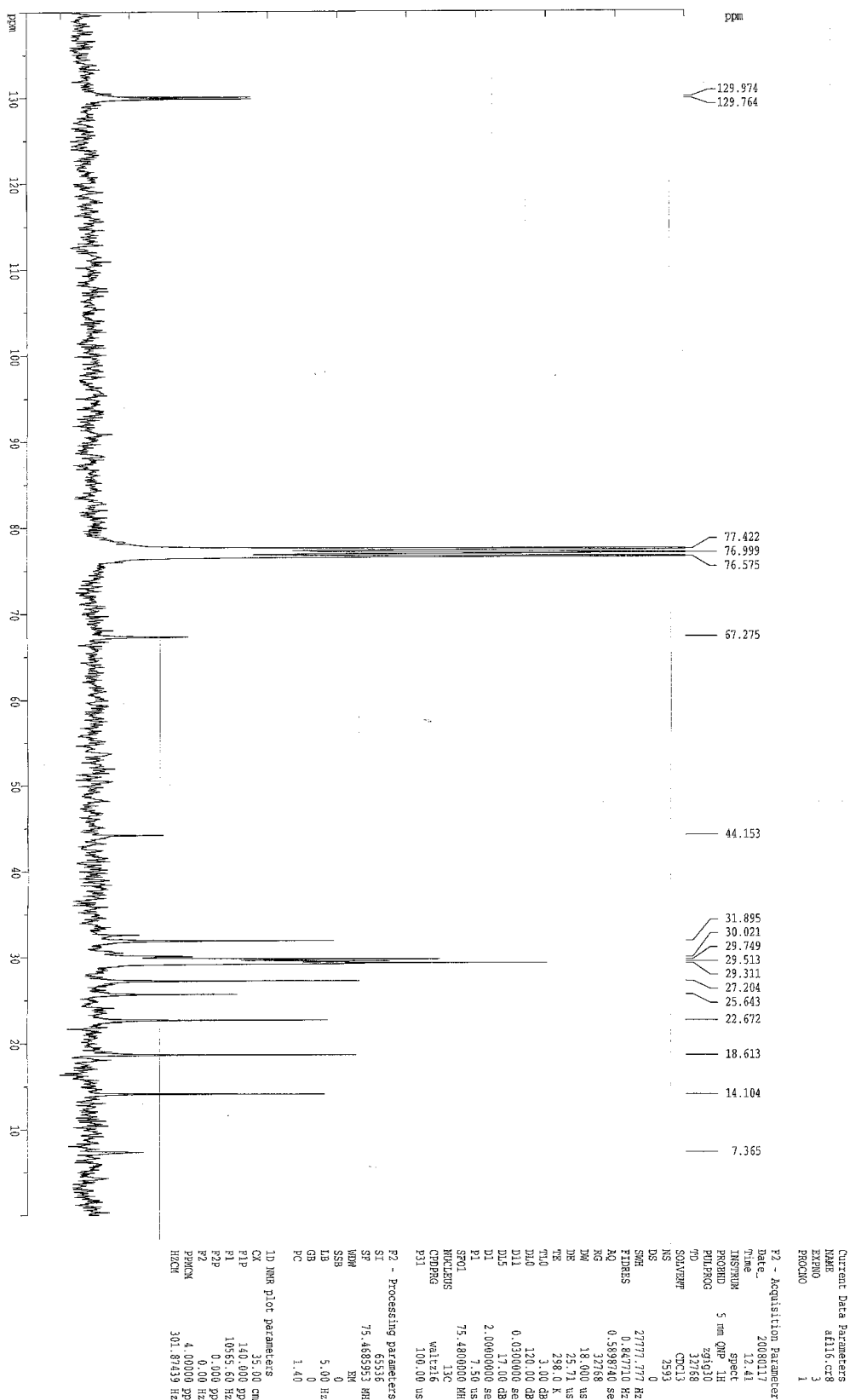


Figure S4-8: ^{13}C NMR (CDCl_3) spectrum of compound 5a.

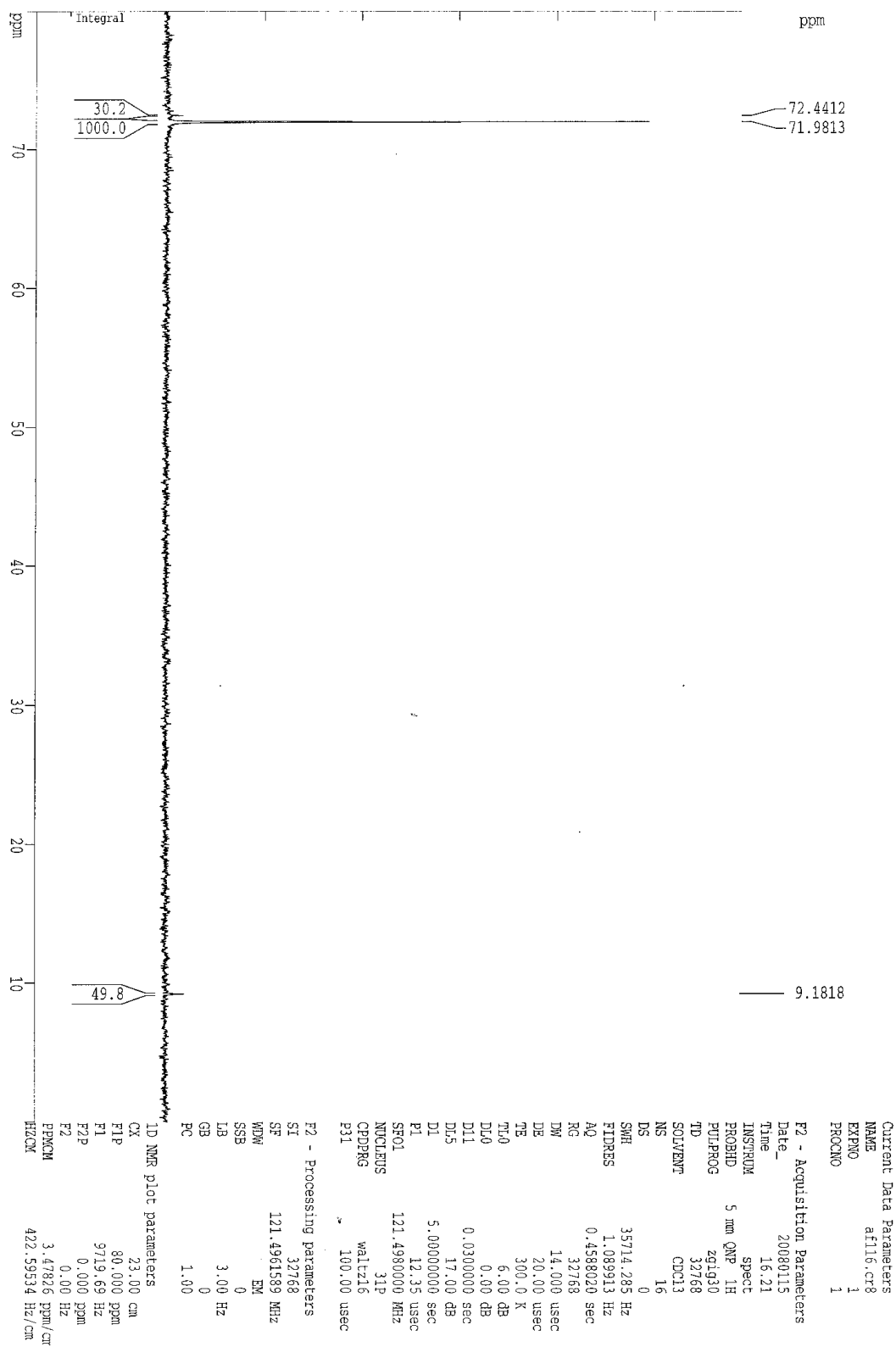


Figure S4-9: ^{31}P NMR (CDCl_3) spectrum of compound 5a.

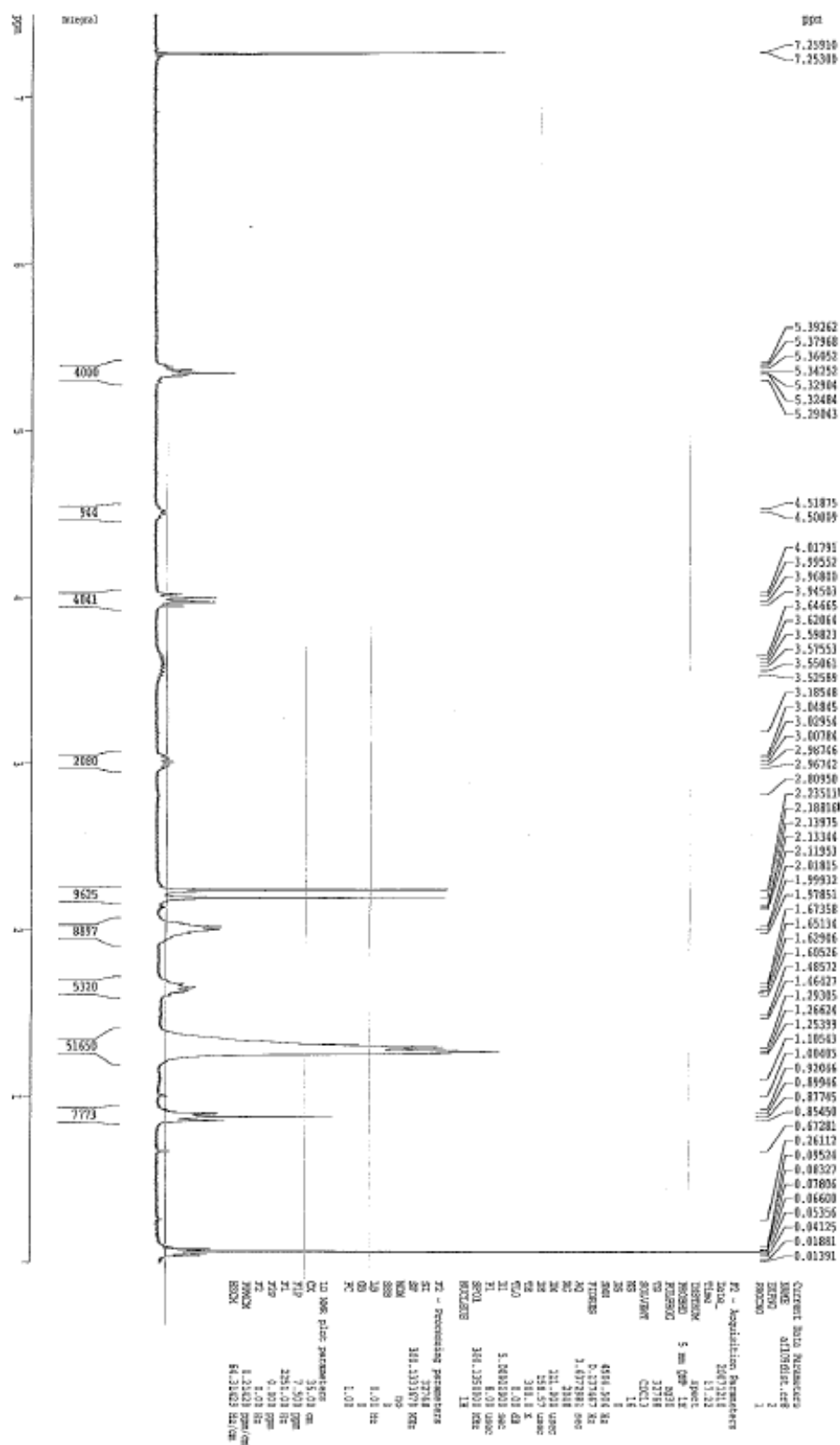


Figure S4-10: ^1H NMR (CDCl_3) spectrum of compound **6a** (BSV14).

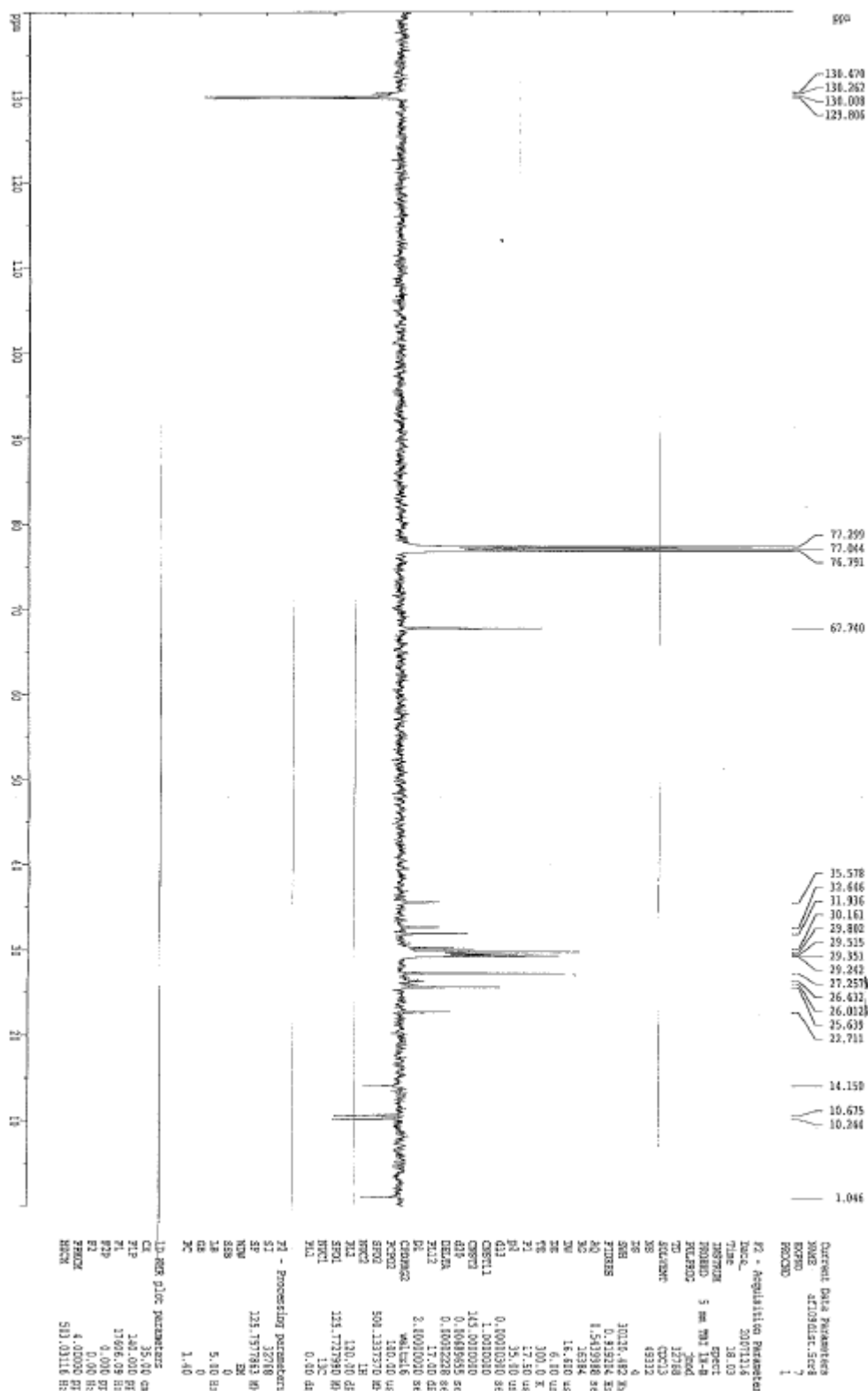


Figure S4-11: ¹³C jmod NMR (CDCl₃) spectrum of compound 6a (BSV14).

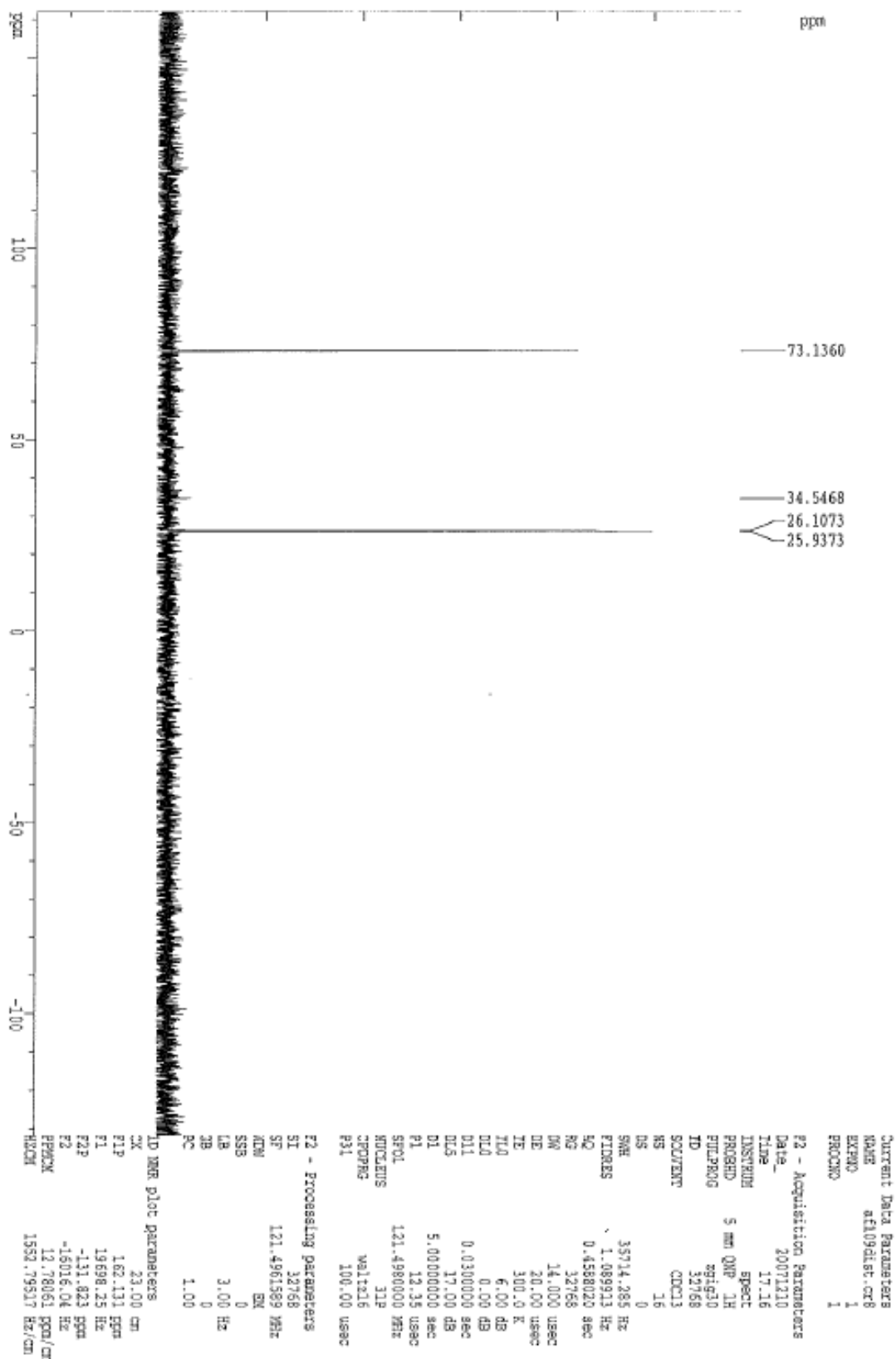


Figure S4-12: ^{31}P NMR (CDCl_3) spectrum of compound **6a** (BSV14).

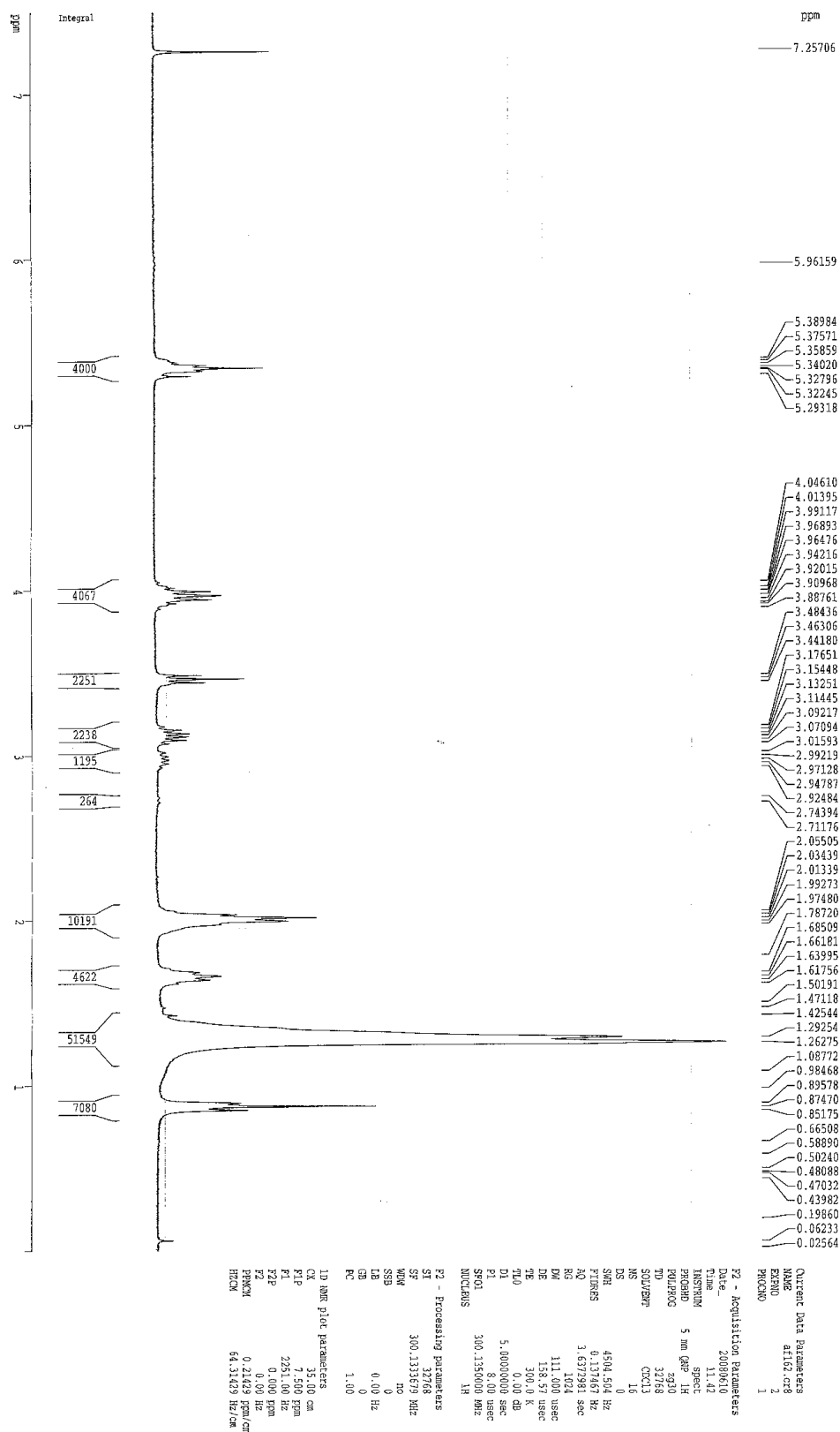


Figure S4-13: ¹H NMR (CDCl₃) spectrum of compound 4b.

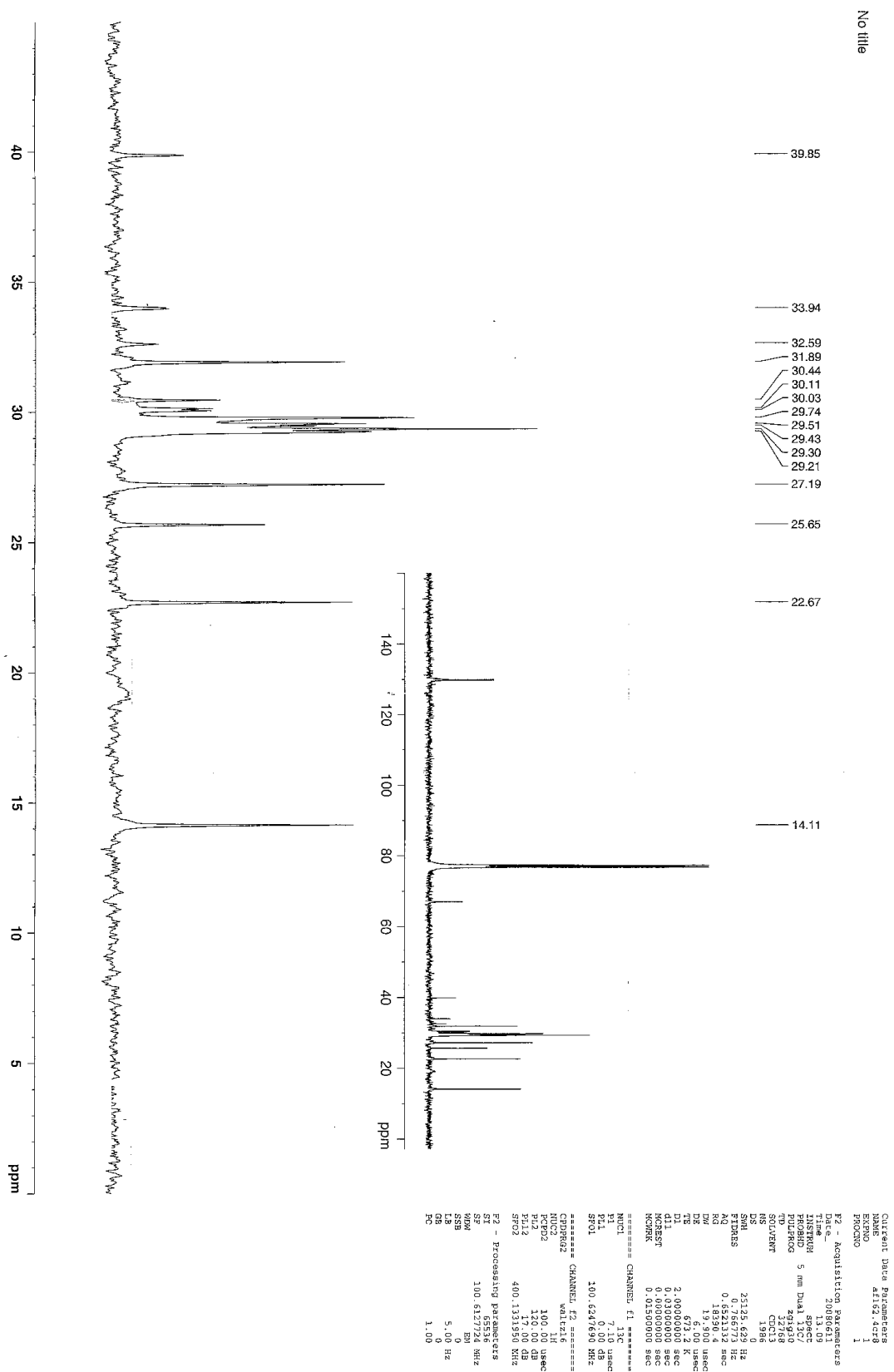


Figure S4-14: ¹³C NMR (CDCl₃) spectrum of compound 4b.

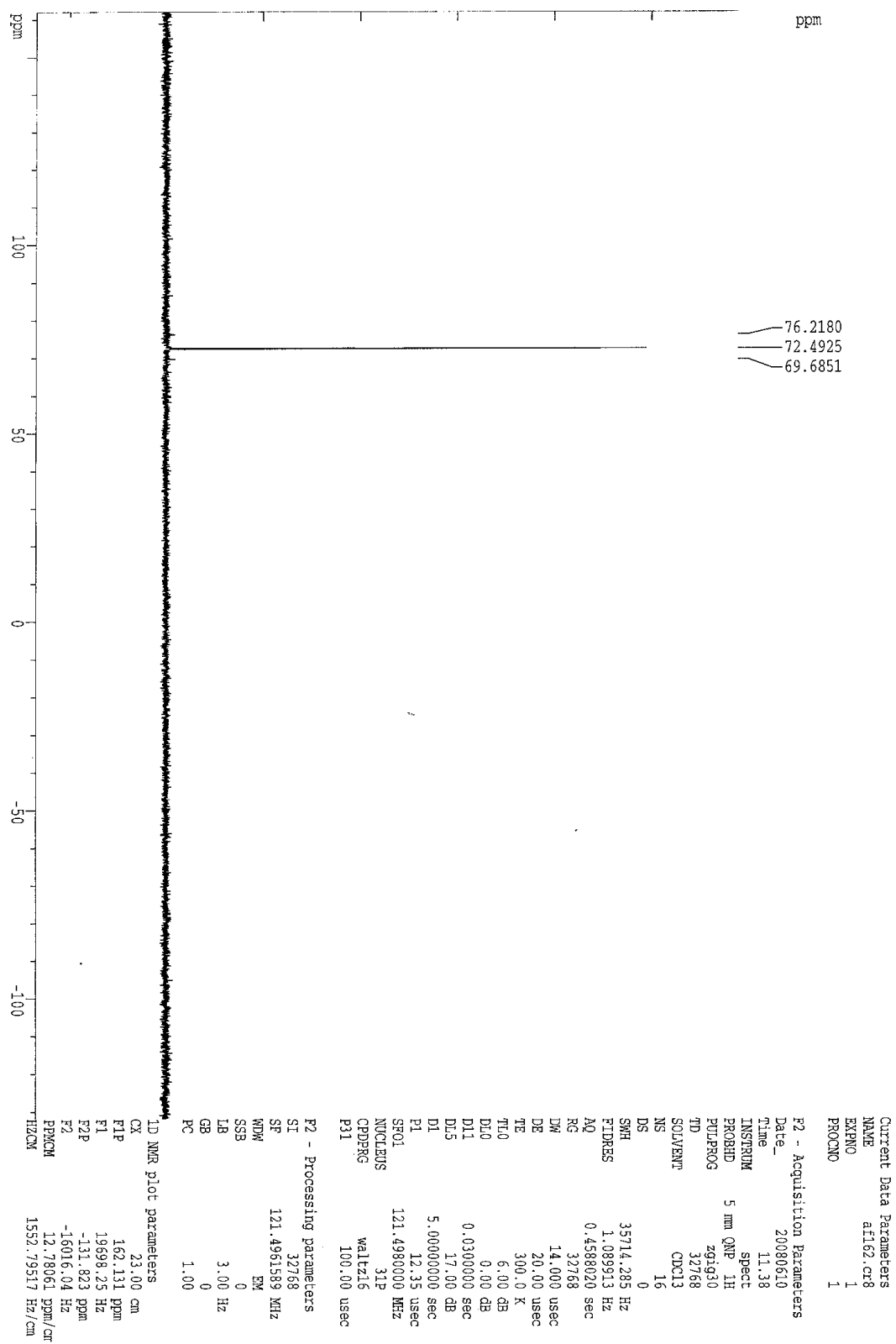


Figure S4-15: ^{31}P NMR (CDCl_3) spectrum of compound **4b**.

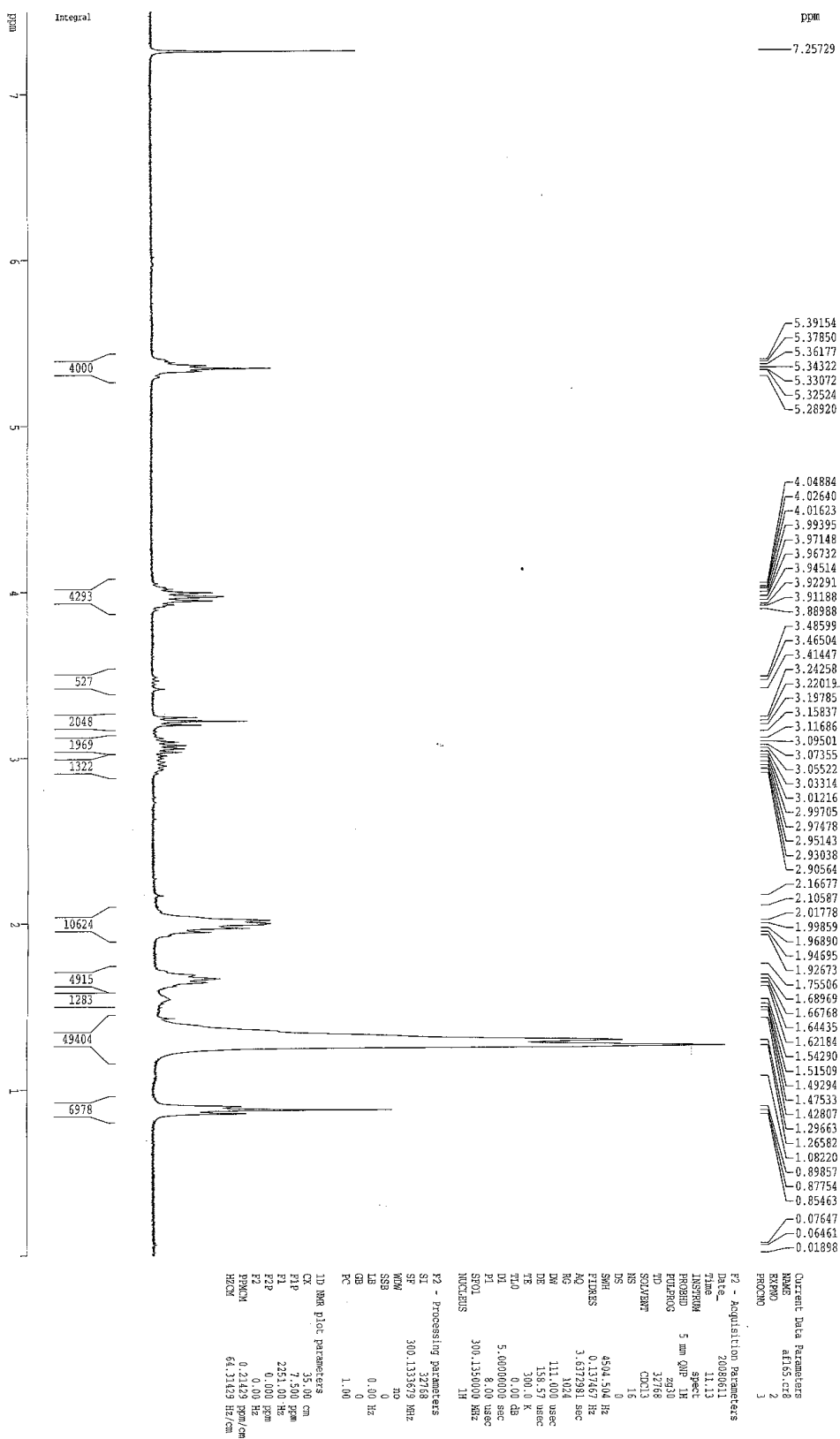


Figure S4-16: ¹H NMR (CDCl₃) spectrum of compound **5b**.

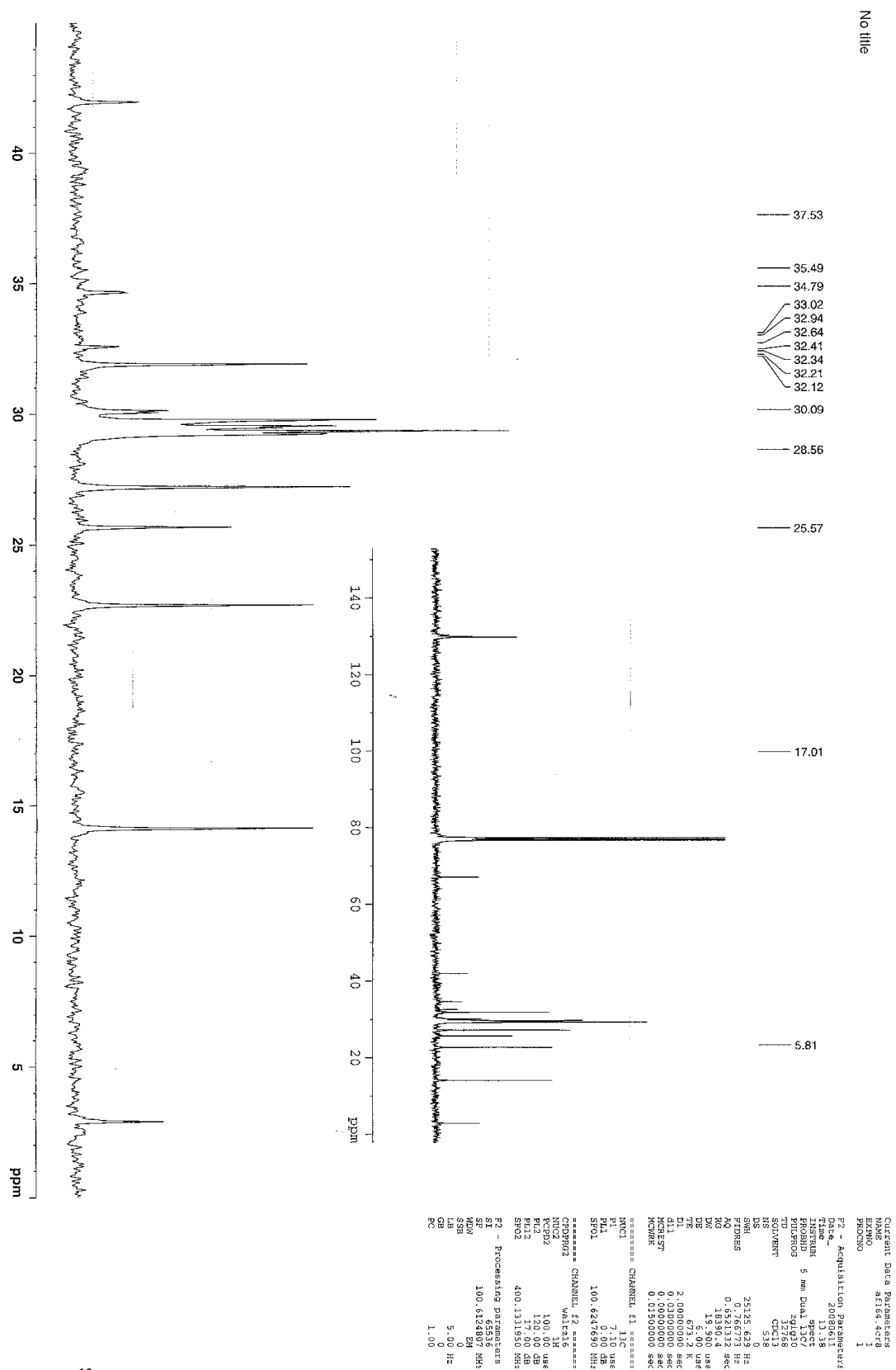


Figure S4-17: ¹³C NMR (CDCl₃) spectrum of compound 5b.

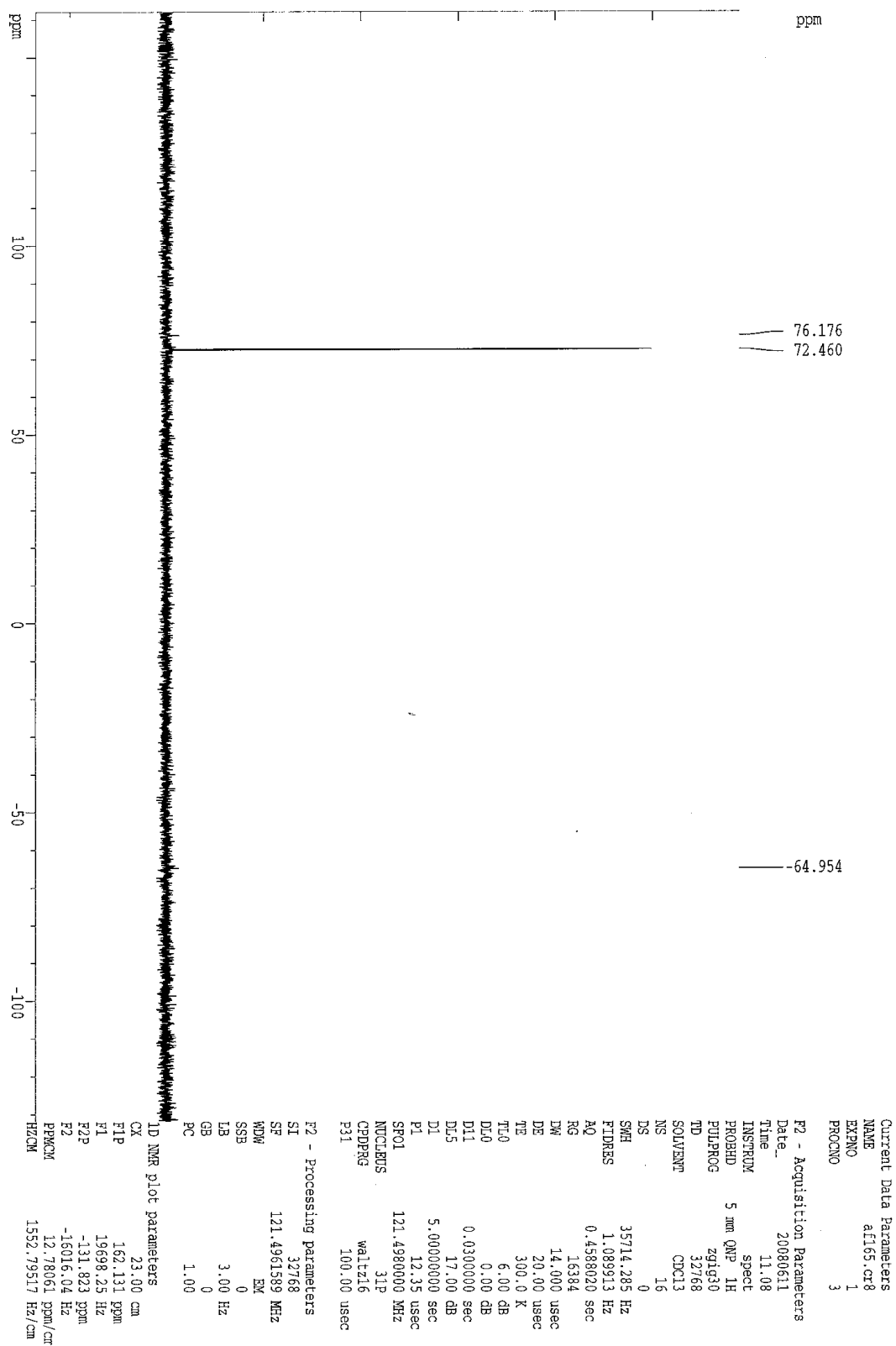


Figure S4-18: ^{31}P NMR (CDCl_3) spectrum of compound **5b**.

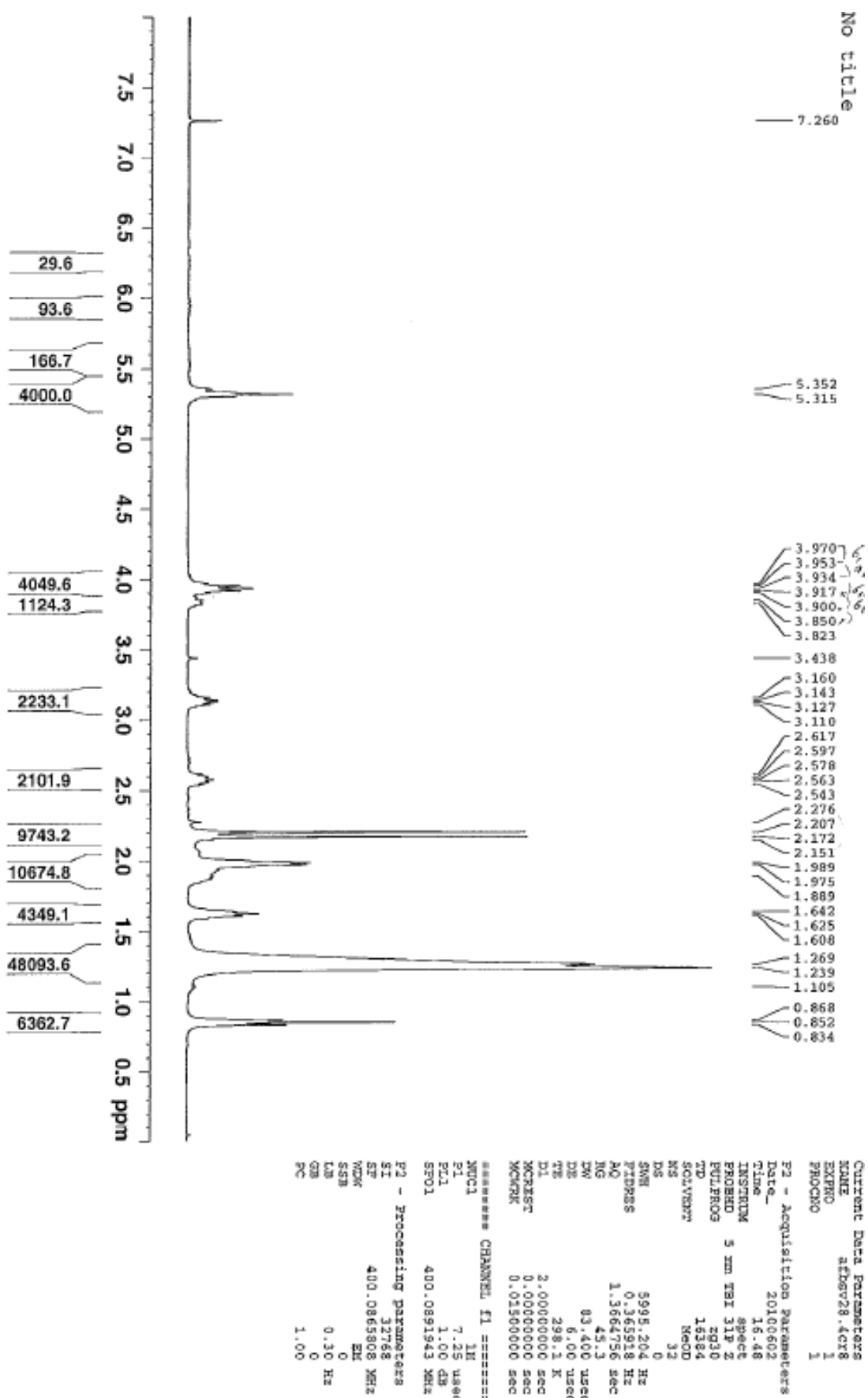


Figure S4-19: ^1H NMR (CDCl_3) spectrum of compound **6b** (BSV28).

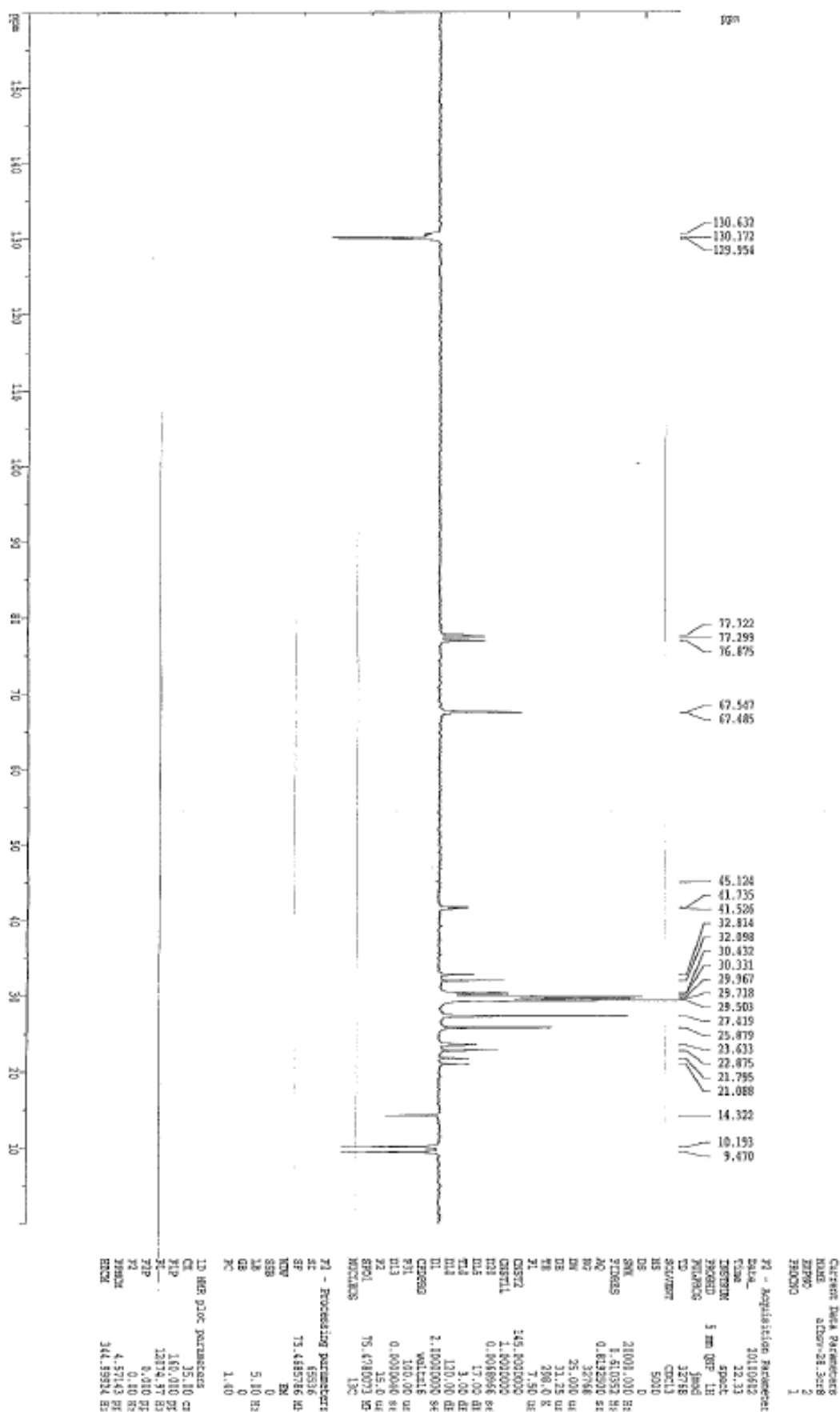


Figure S4-20: ¹³C jmod NMR (CDCl₃) spectrum of compound **6b** (BSV28).

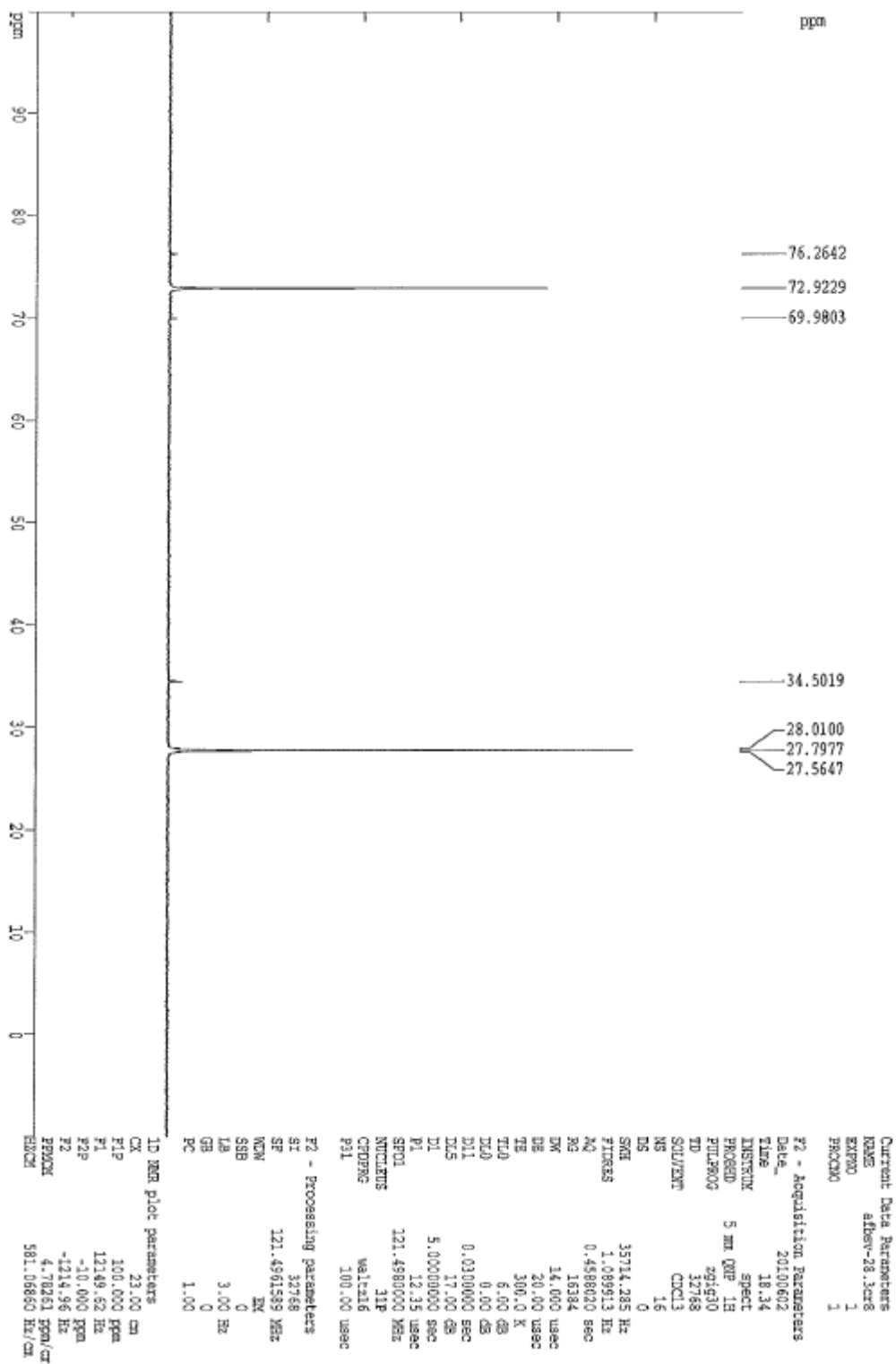


Figure S4-21: ^{31}P NMR (CDCl_3) spectrum of compound **6b** (BSV28).

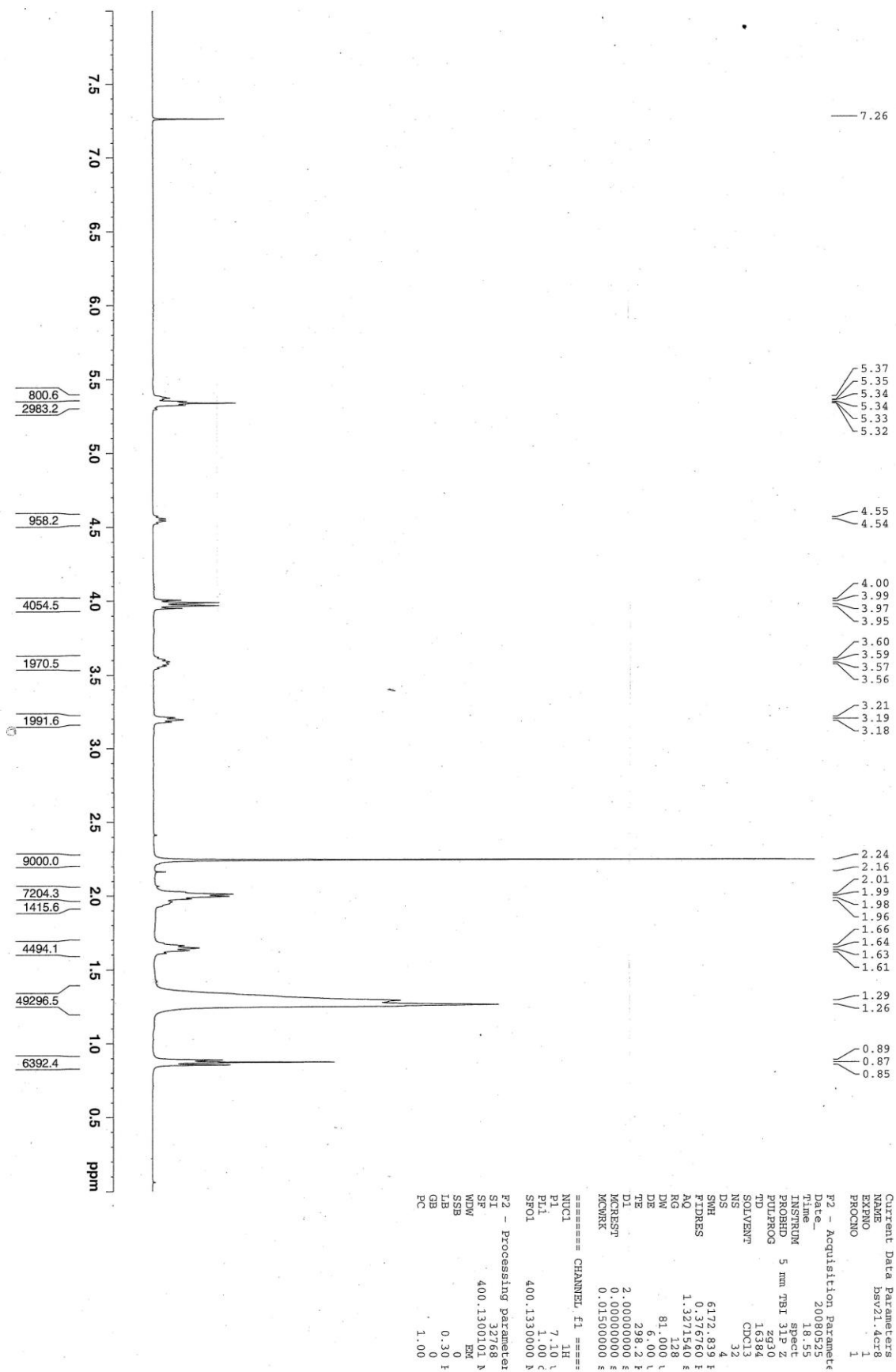


Figure S4-22: ¹H NMR (CDCl₃) spectrum of compound 7 (BSV21).

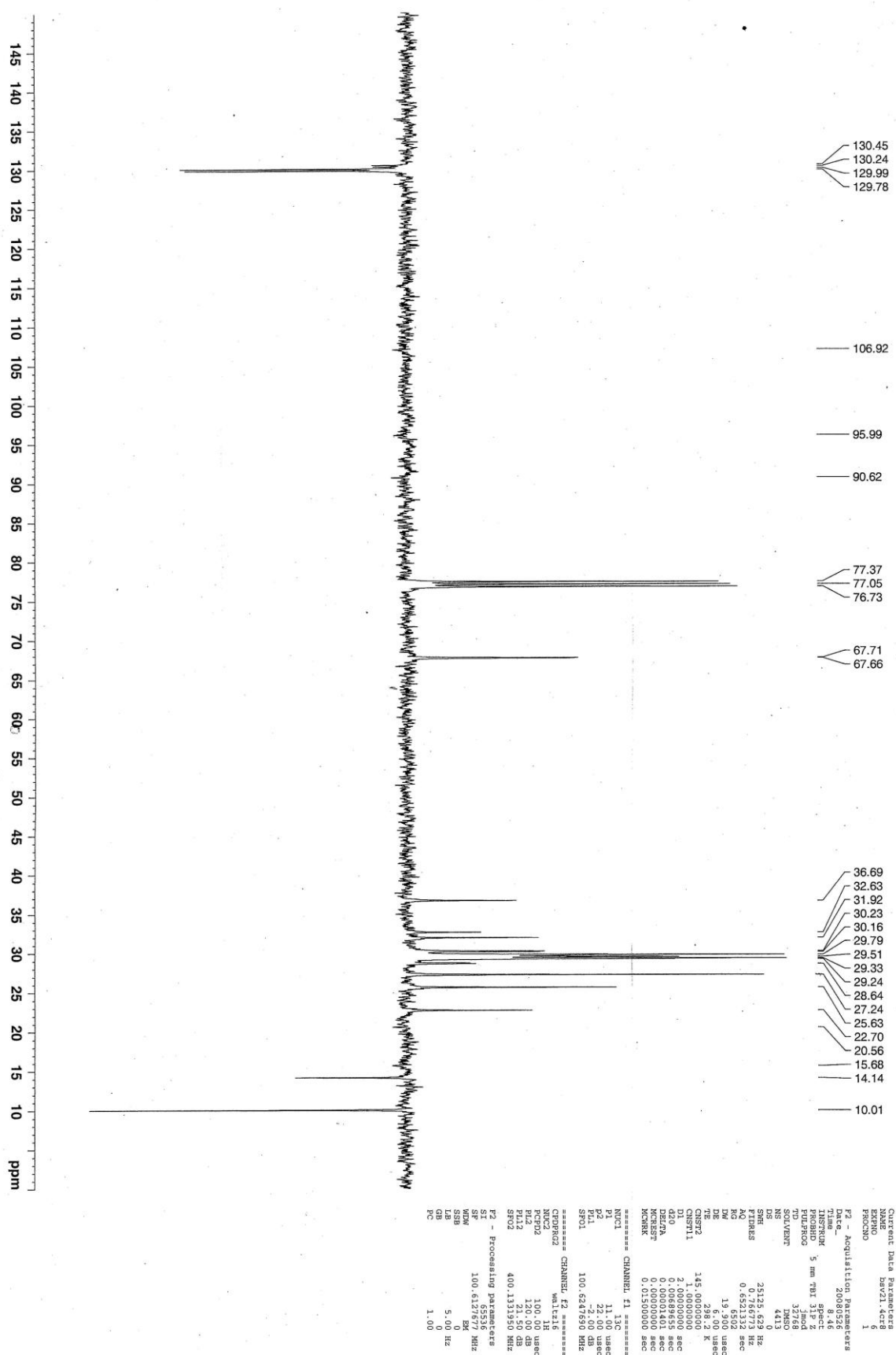


Figure S4-23: ¹³C jmod NMR (CDCl₃) spectrum of compound 7 (BSV21).

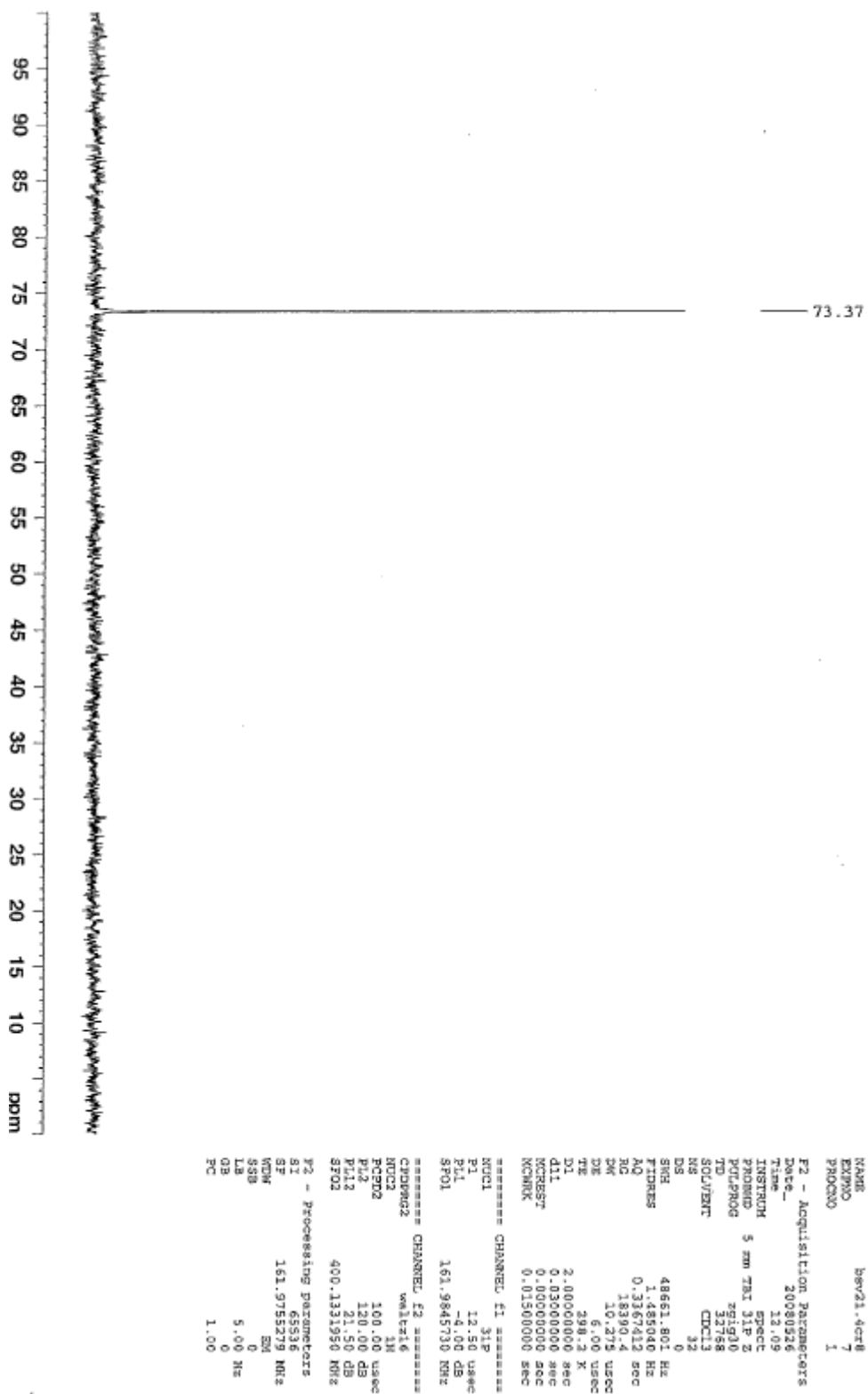


Figure S4-24: ³¹P NMR (CDCl₃) spectrum of compound 7 (BSV21).