Electronic Supplementary Materials

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

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Summary

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 µmol.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 dropewise 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 ± 5 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)

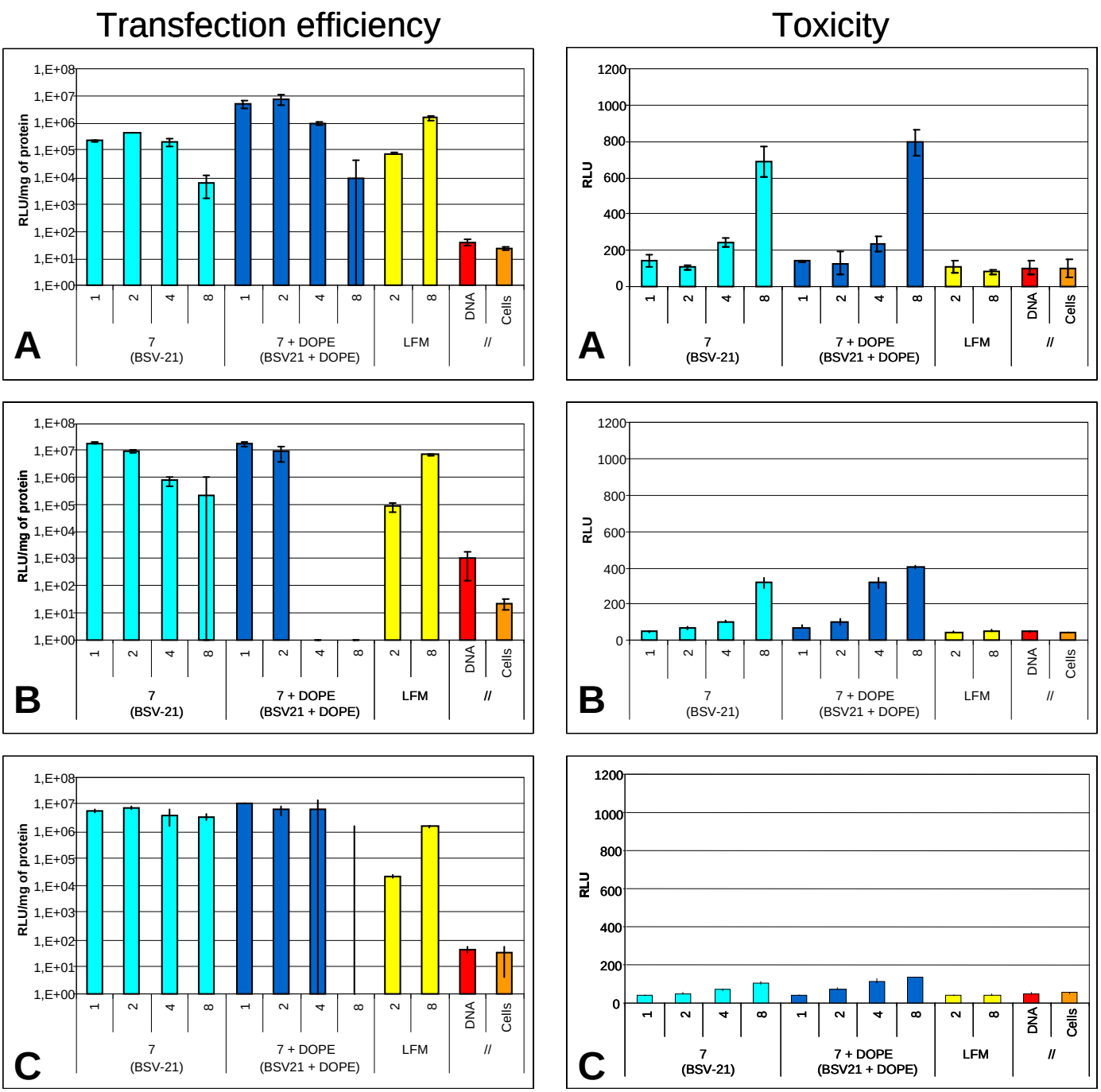

Figure S3-1: *In vitro* transfection efficiency and early toxicity of **7** (BSV21), **7** + DOPE (BSV21 + DOPE) and Lipofectamine as reference. **A**-HeLa cell line. **B**- A549 cell line; **C**- 16HBE14o(-).

ESI-4 NMR Data

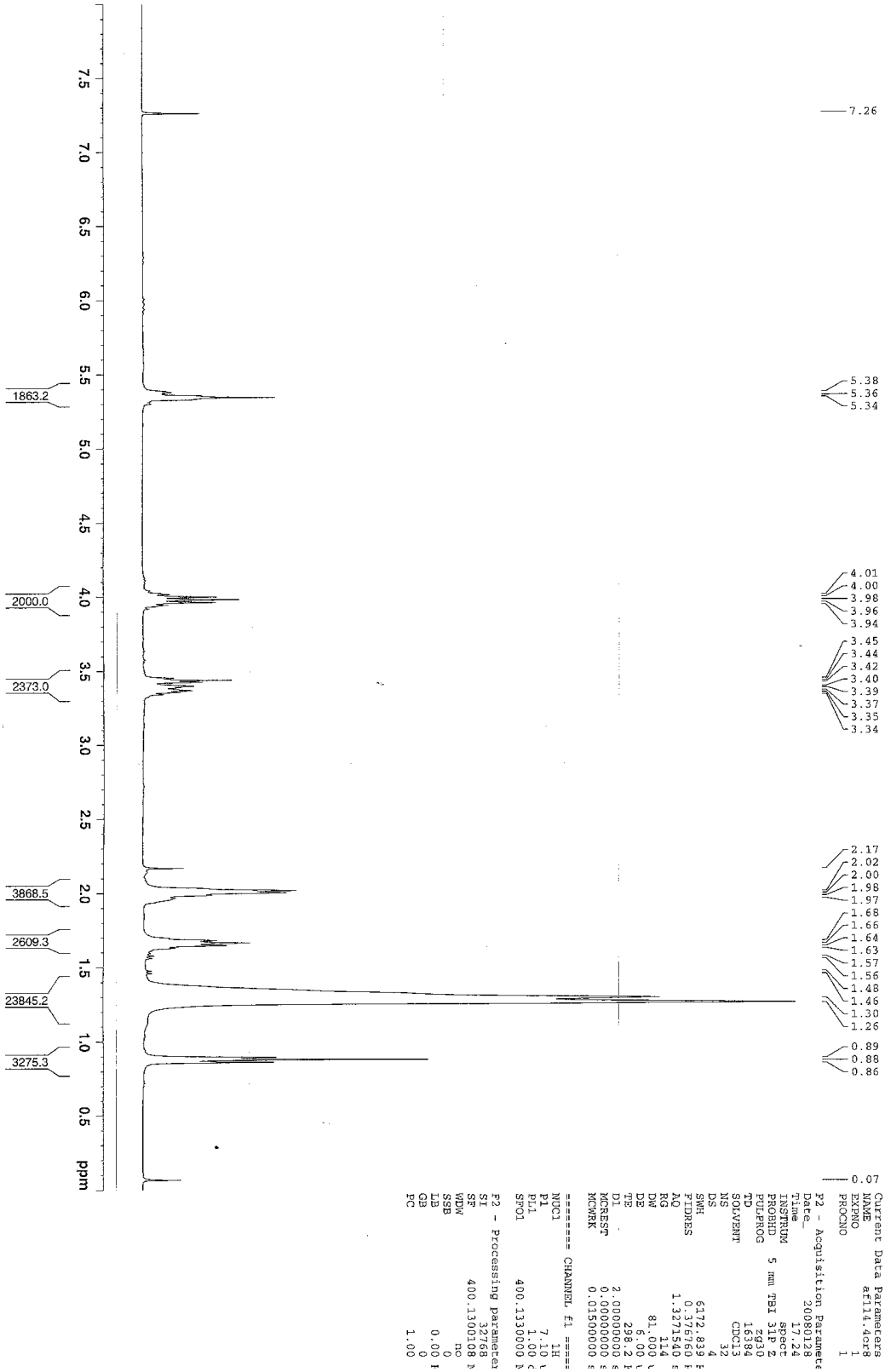

Figure S4-1: ^{1}H NMR ($CDCl_3$) spectrum of compound **4a**.

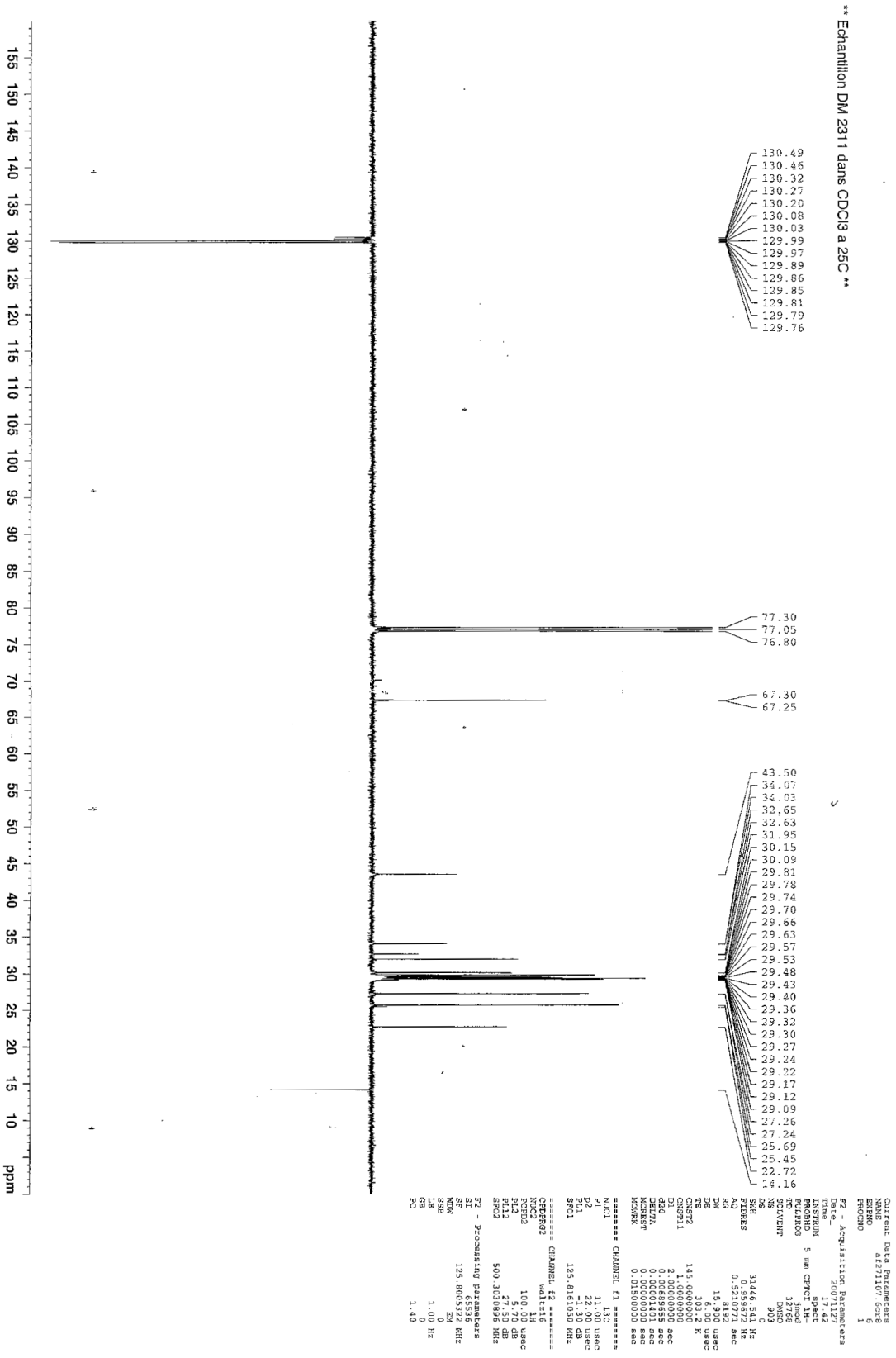

Figure S4-2: ^{13}C NMR ($CDCl_3$) spectrum of compound **4a**.

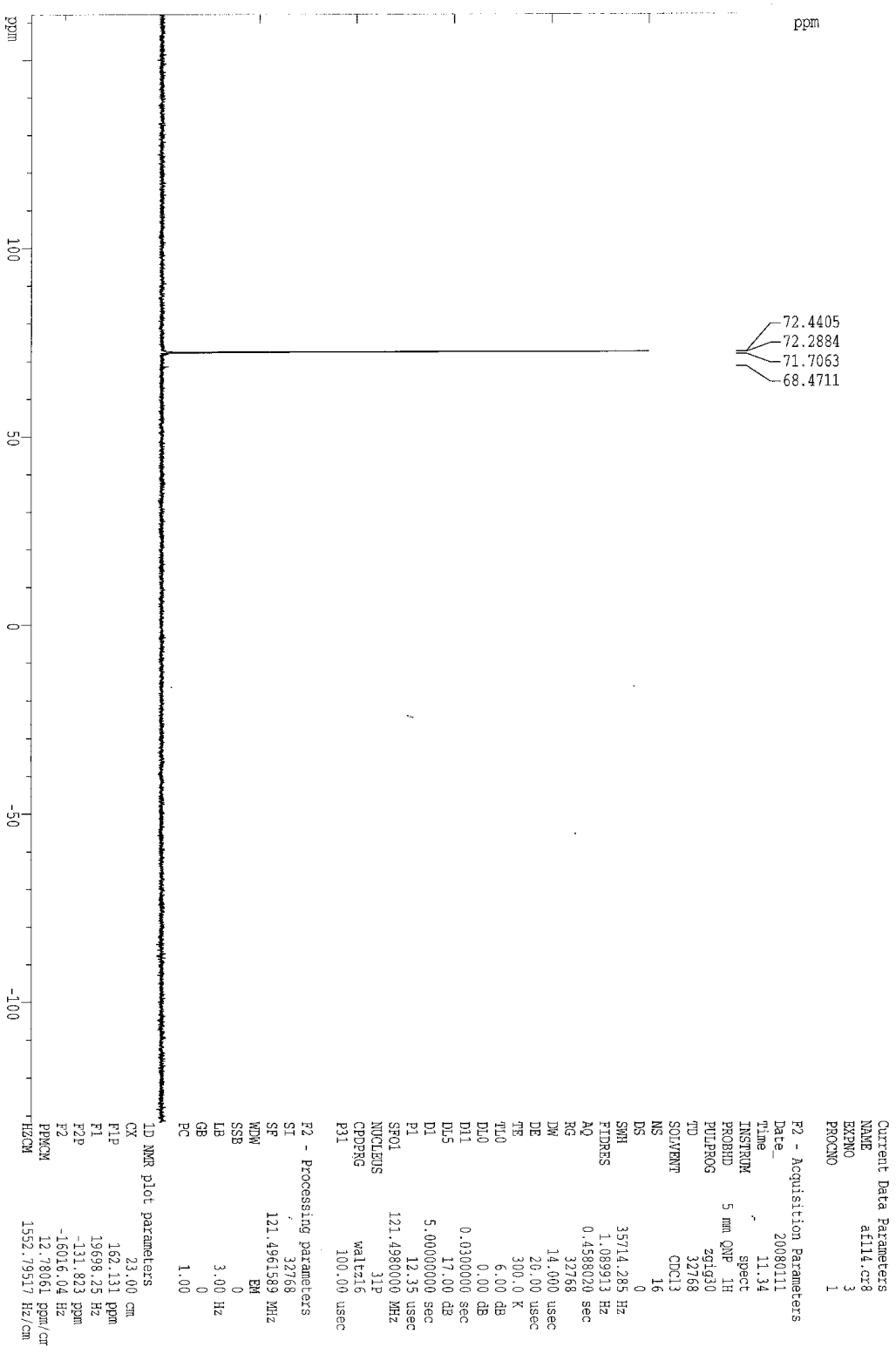

Figure S4-3: ^{31}P NMR ($CDCl_3$) spectrum of compound **4a**.

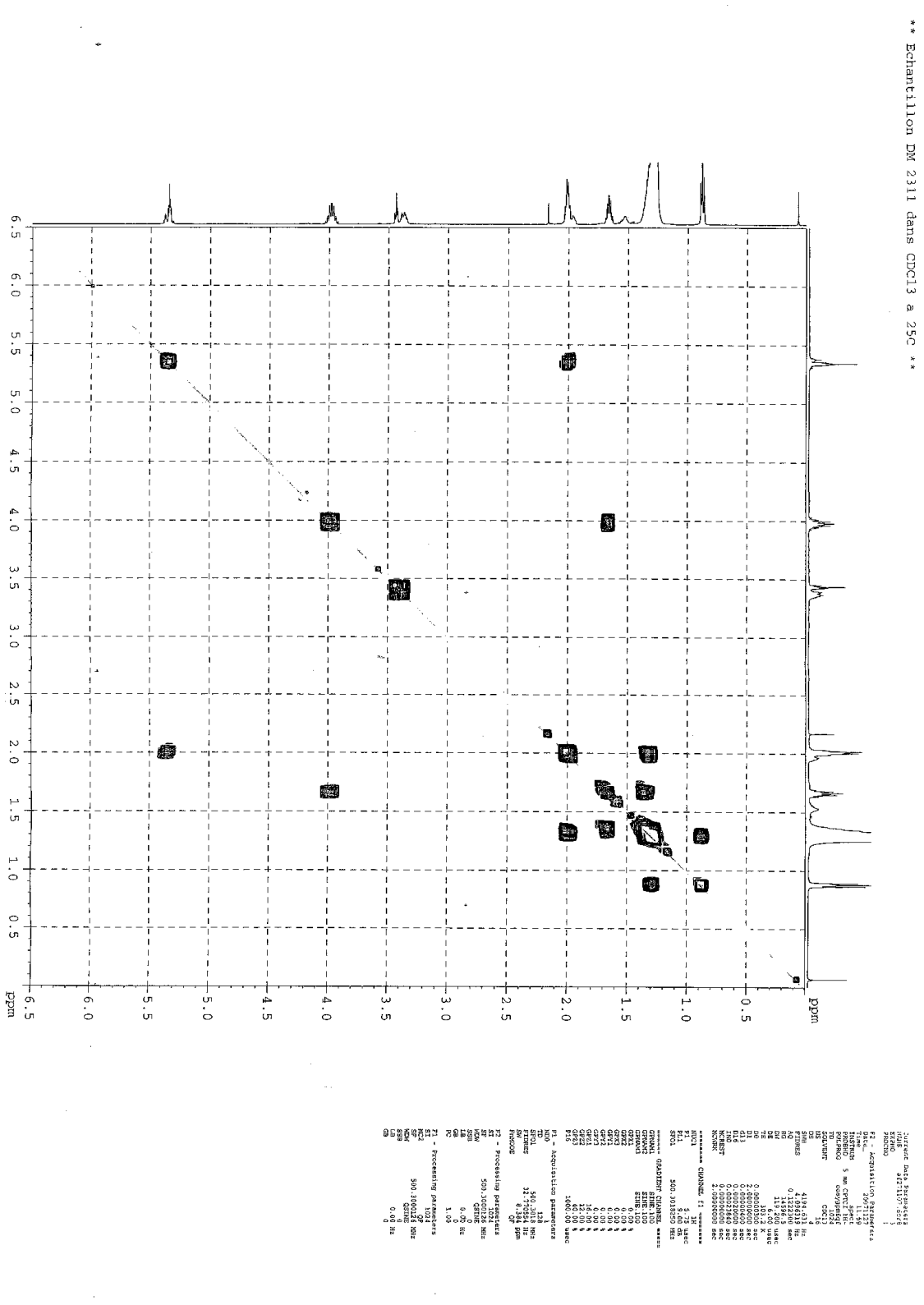

Figure S4-4: 2D Cosy NMR ($CDCl_3$) spectrum of compound **4a**.

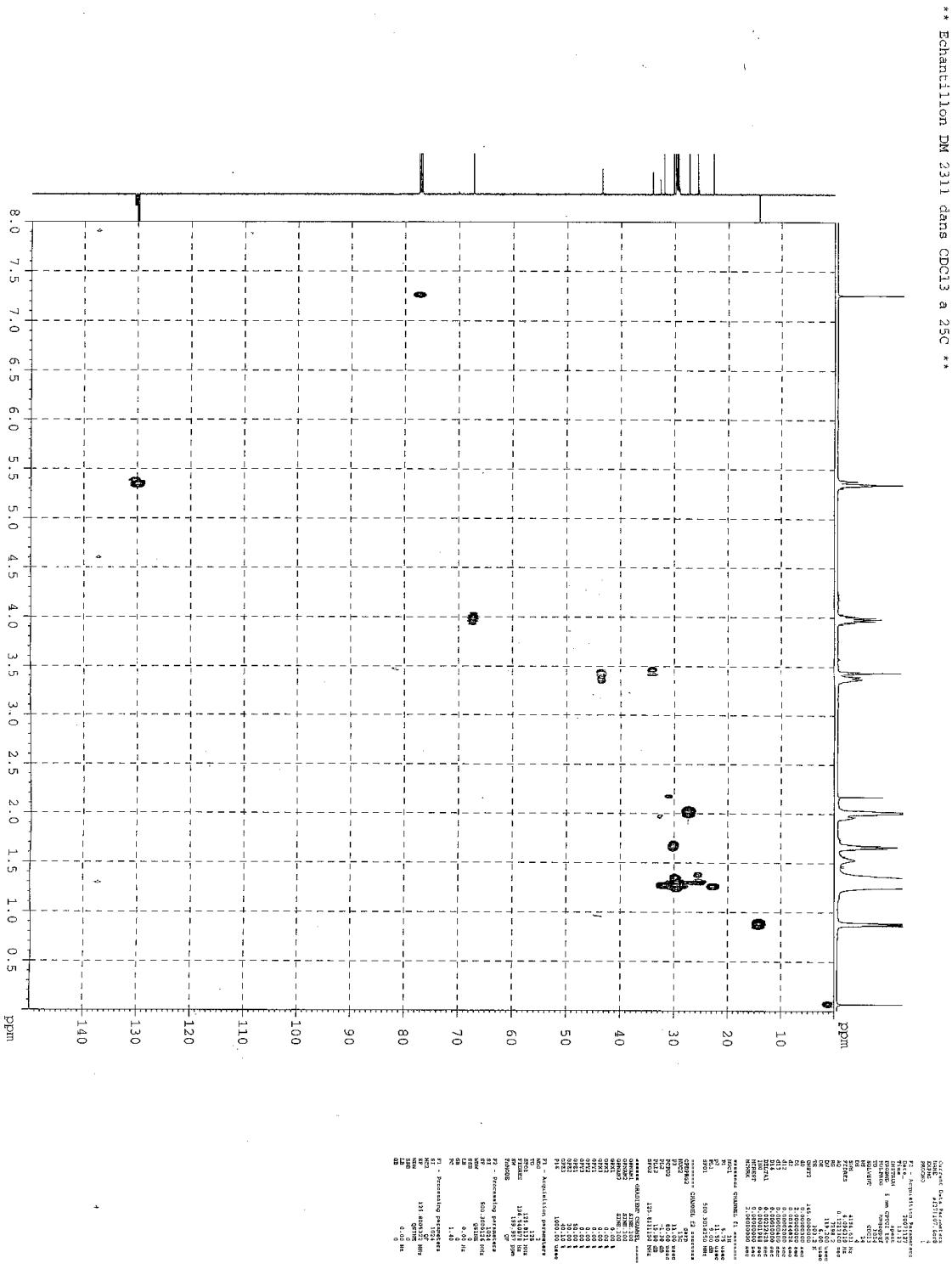

Figure S-4-5: 2D HMQC NMR ($CDCl_3$) spectrum of compound **4a.**

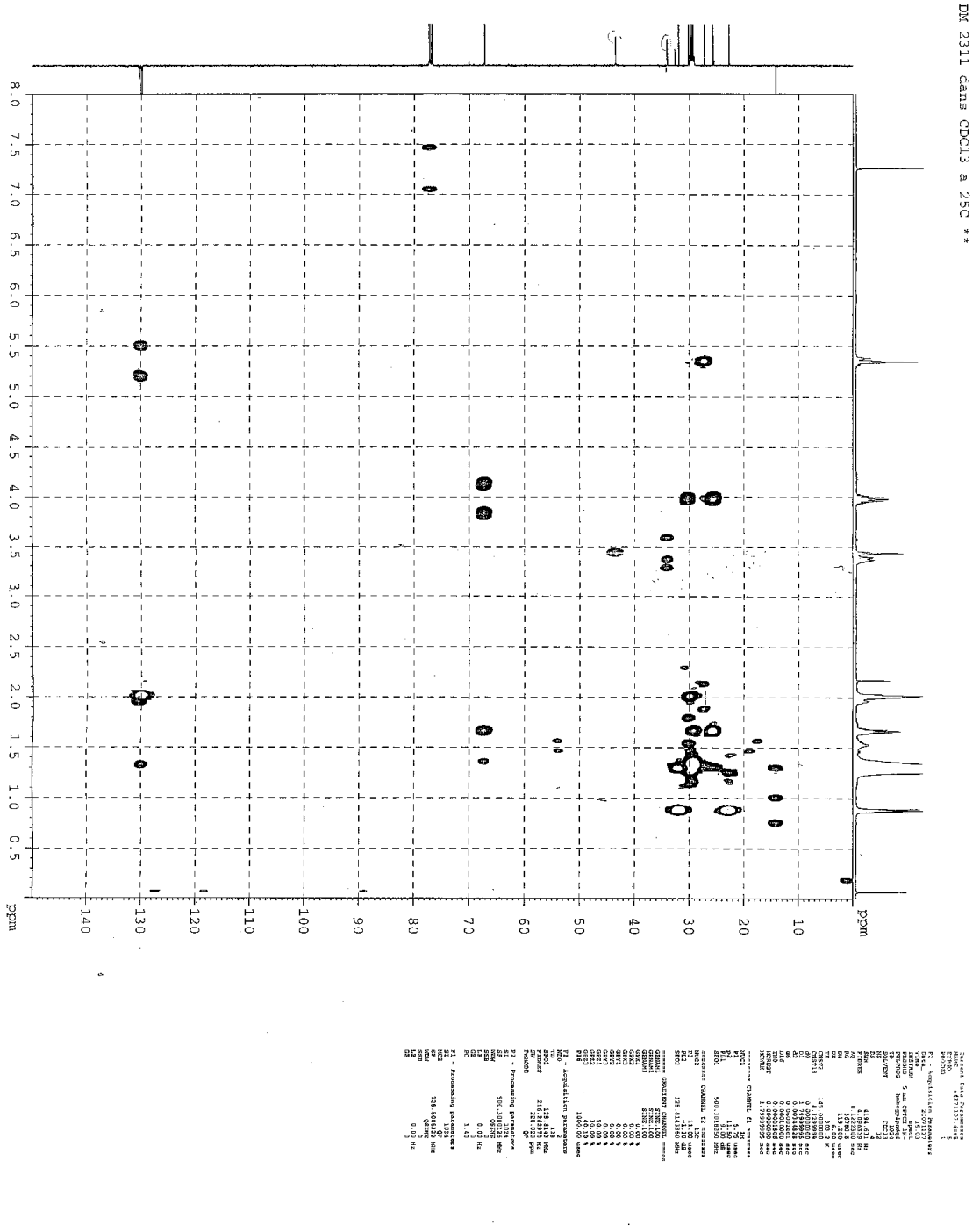

Figure S-4-6: 2D HMBC NMR ($CDCl_3$) spectrum of compound **4a.**

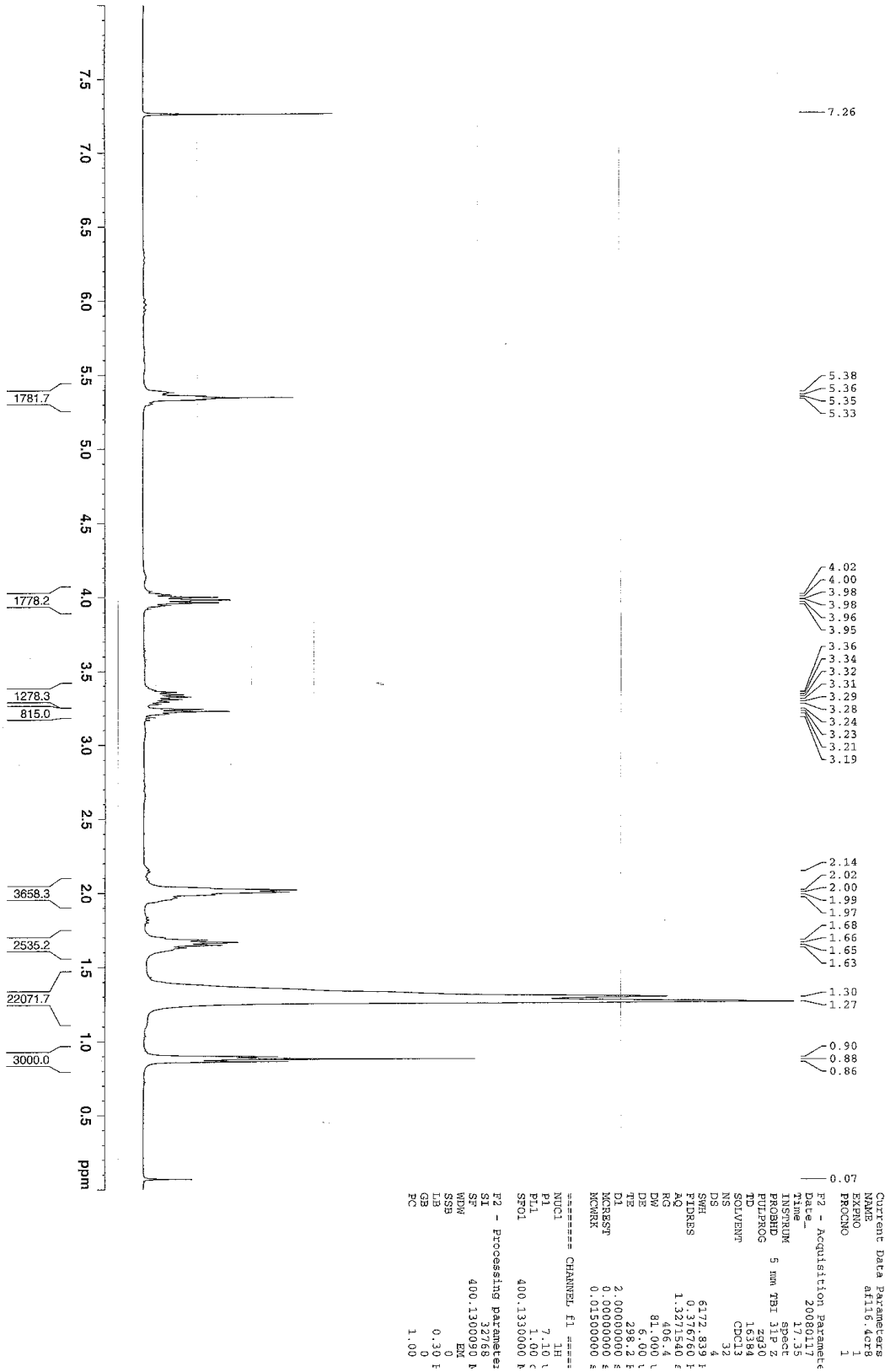

Figure S4-7: ^{1}H NMR ($CDCl_3$) spectrum of compound **5a**.

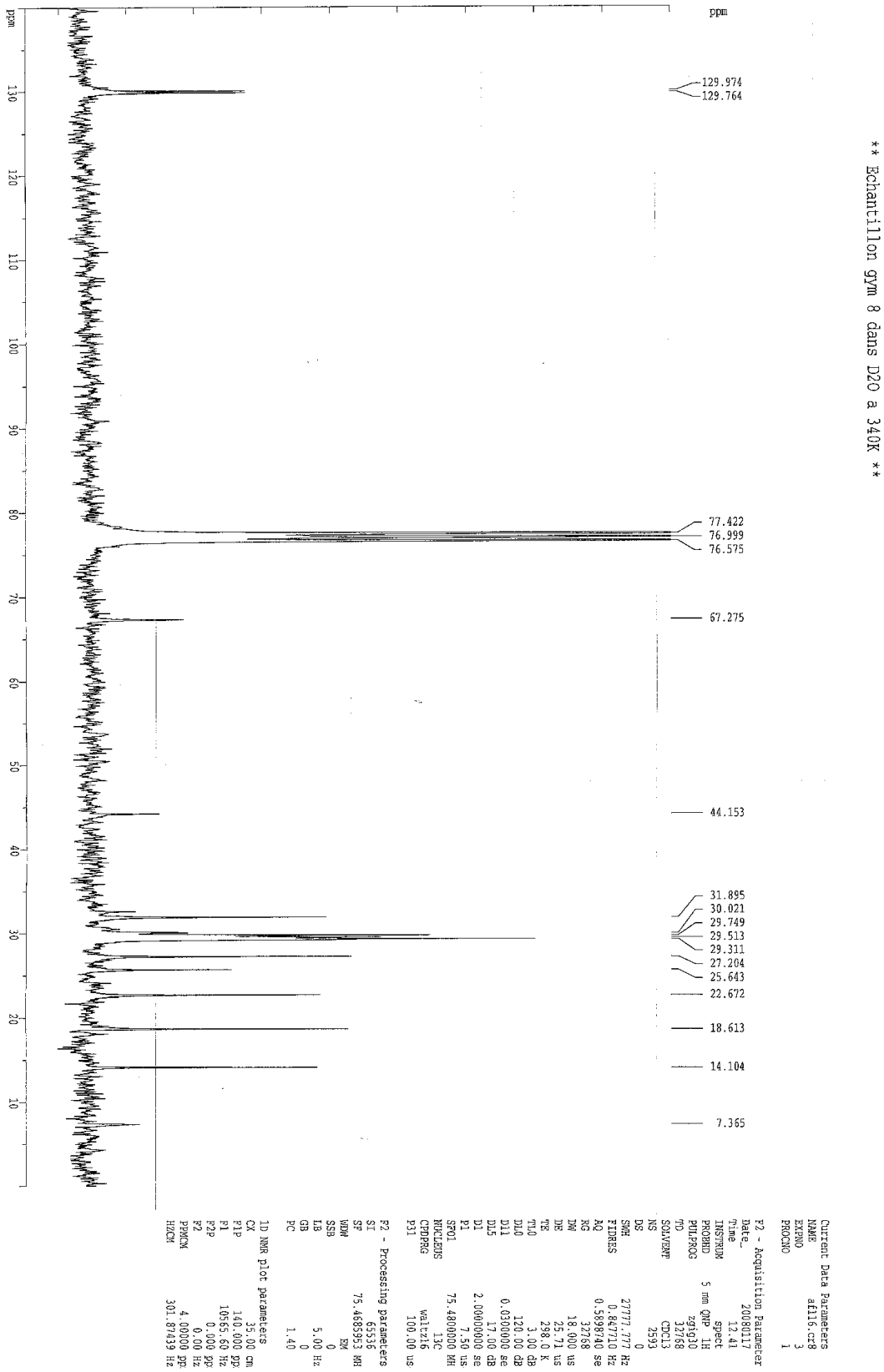

Figure S4-8: ^{13}C NMR ($CDCl_3$) spectrum of compound **5a**.

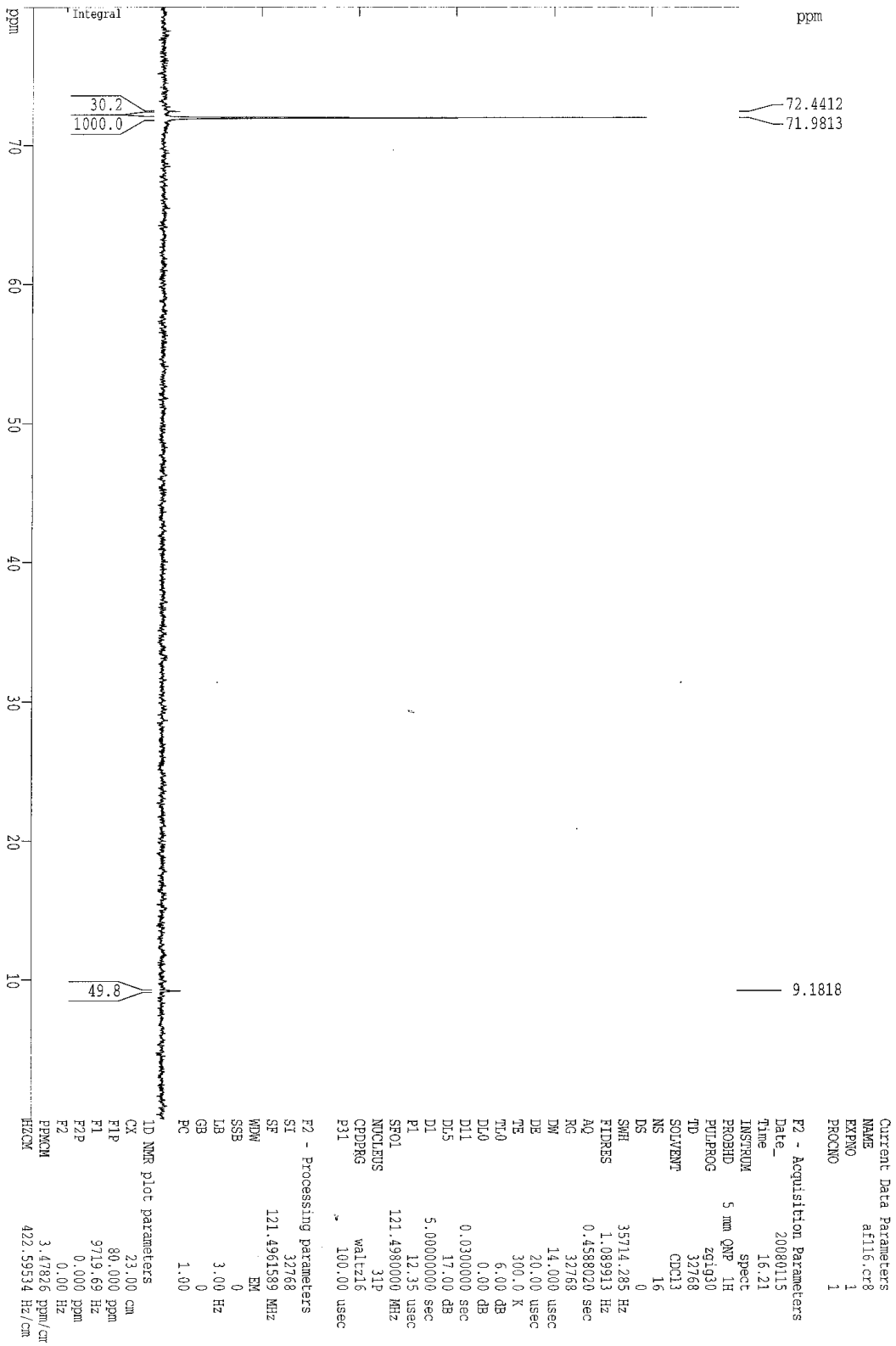

Figure S4-9: ^{31}P NMR ($CDCl_3$) spectrum of compound **5a**.

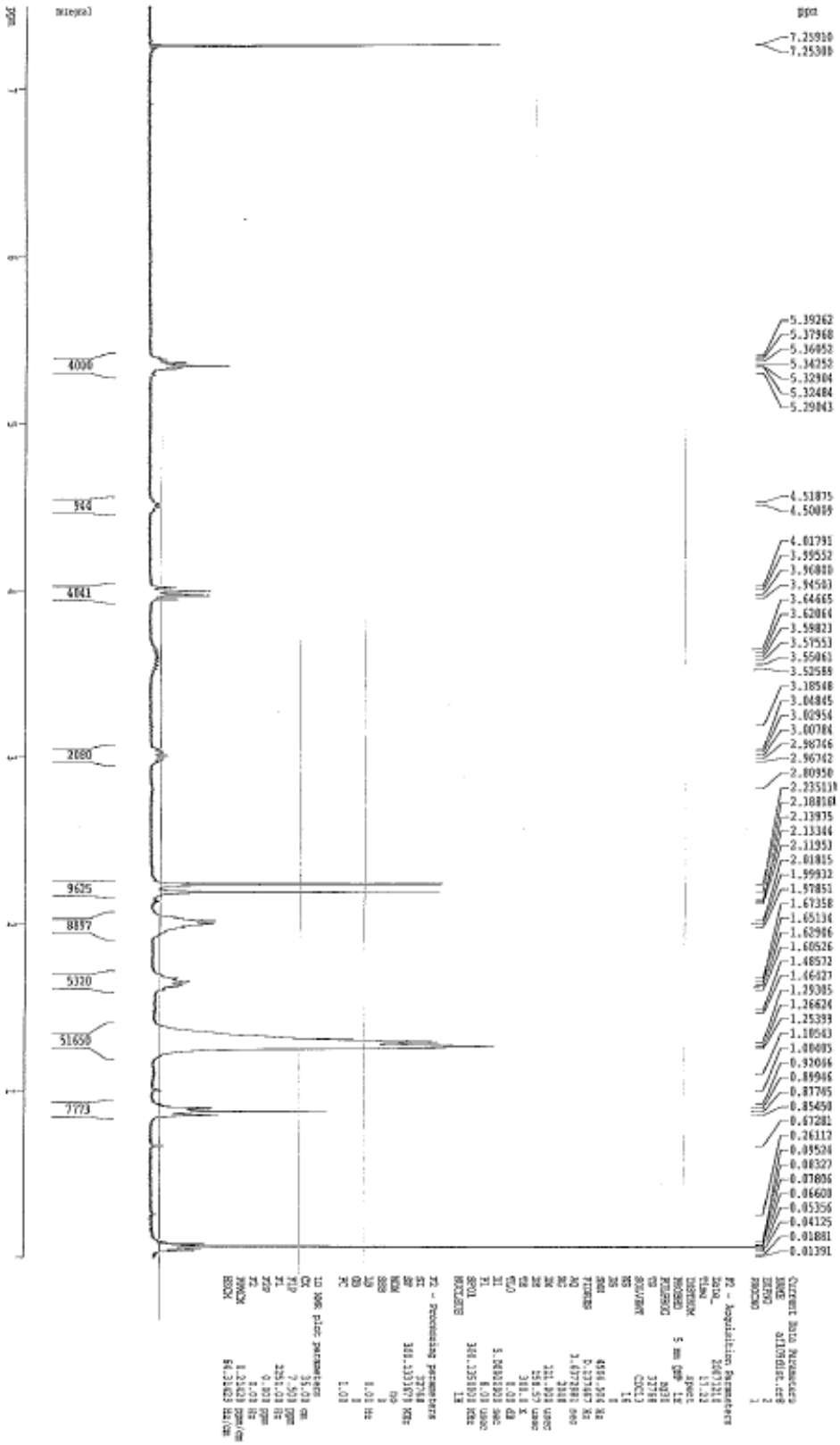

Figure S4-10: ^{1}H NMR ($CDCl_3$) spectrum of compound **6a** (BSV14).

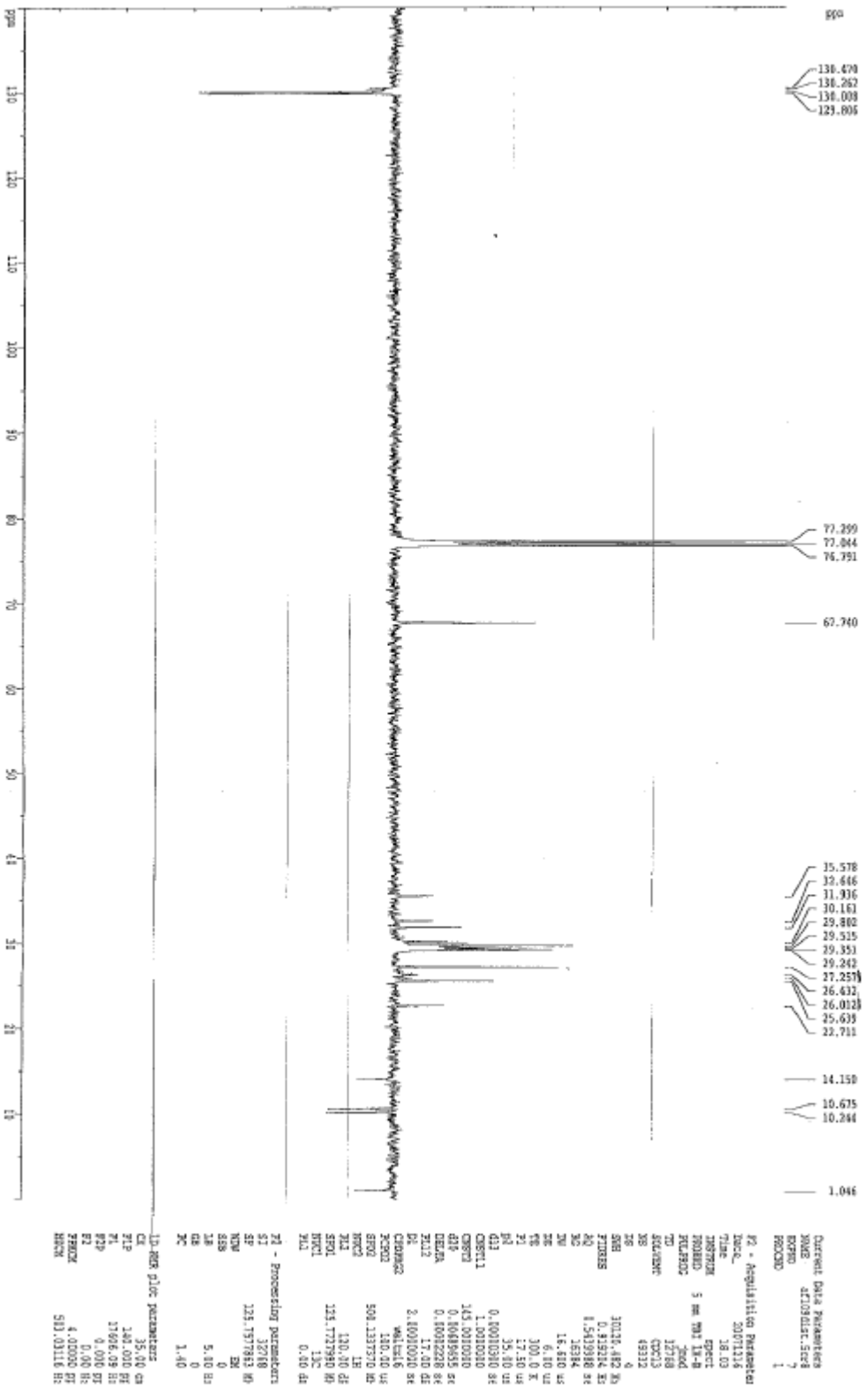

Figure S4-11: ^{13}C jmod NMR ($CDCl_3$) spectrum of compound **6a** (BSV14).

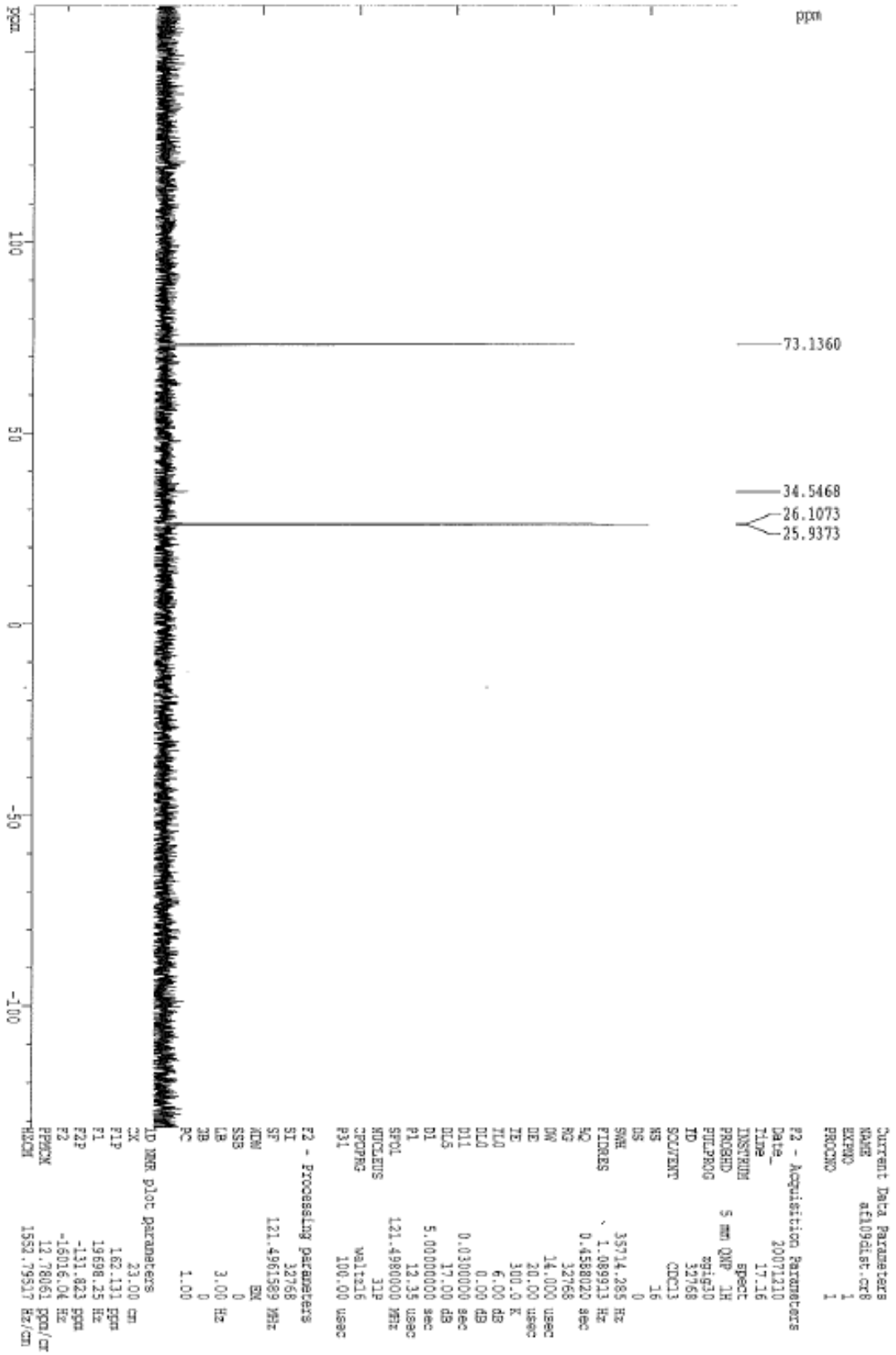

Figure S4-12: ^{31}P NMR ($CDCl_3$) spectrum of compound **6a** (BSV14).

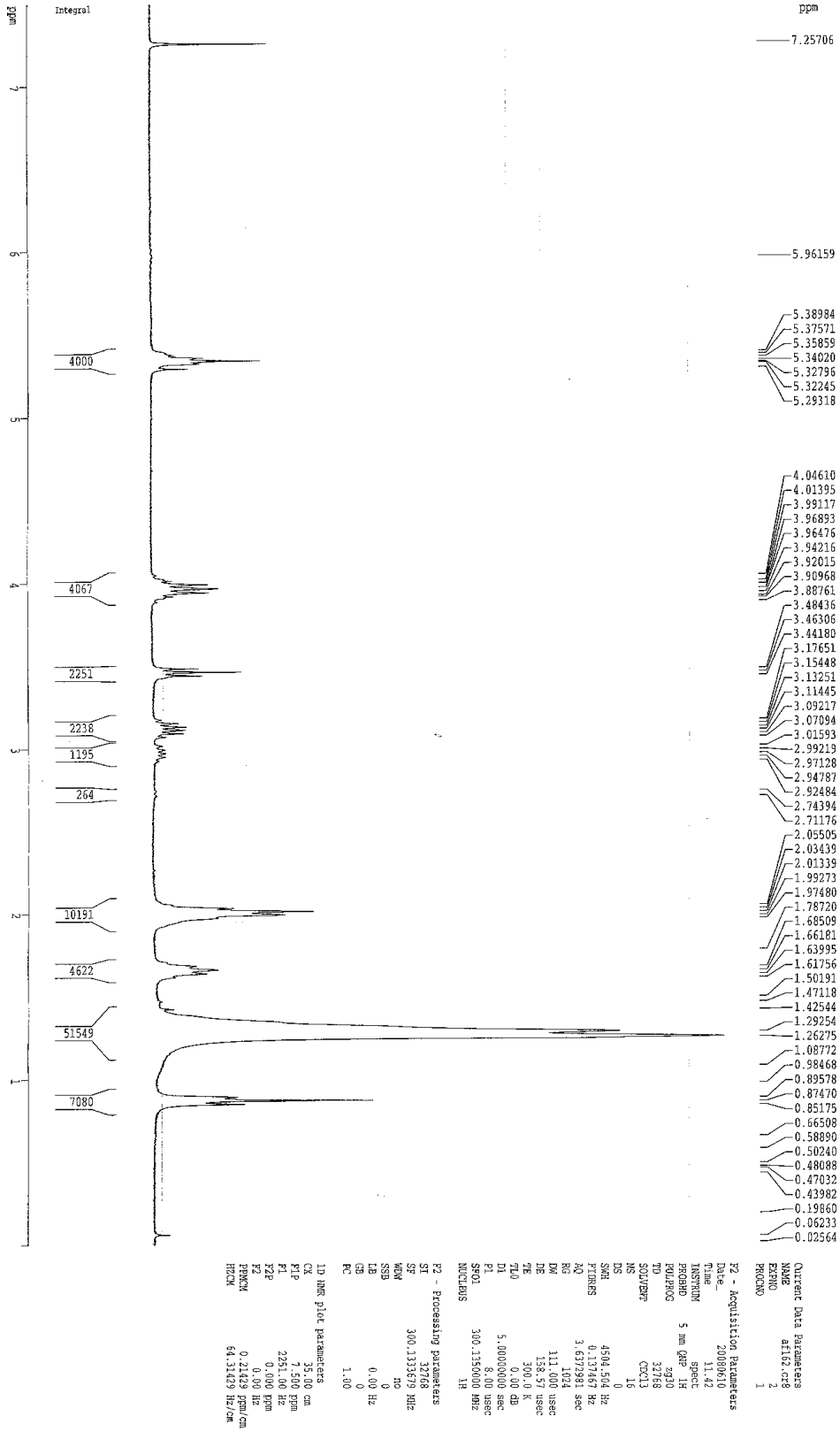

Figure S4-13: ^{1}H NMR ($CDCl_3$) spectrum of compound **4b**.

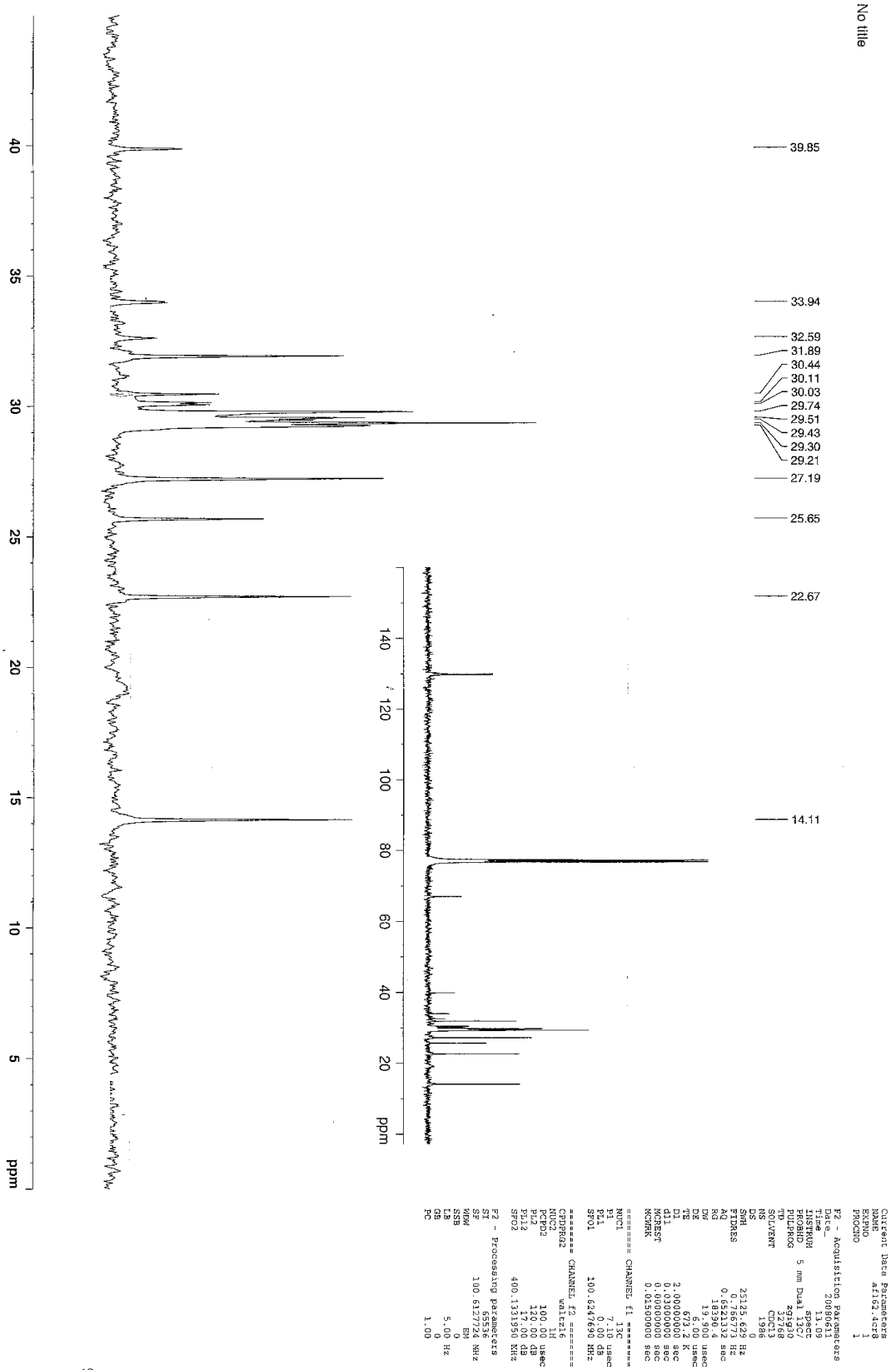

Figure S4-14: ^{13}C NMR ($CDCl_3$) spectrum of compound **4b**.

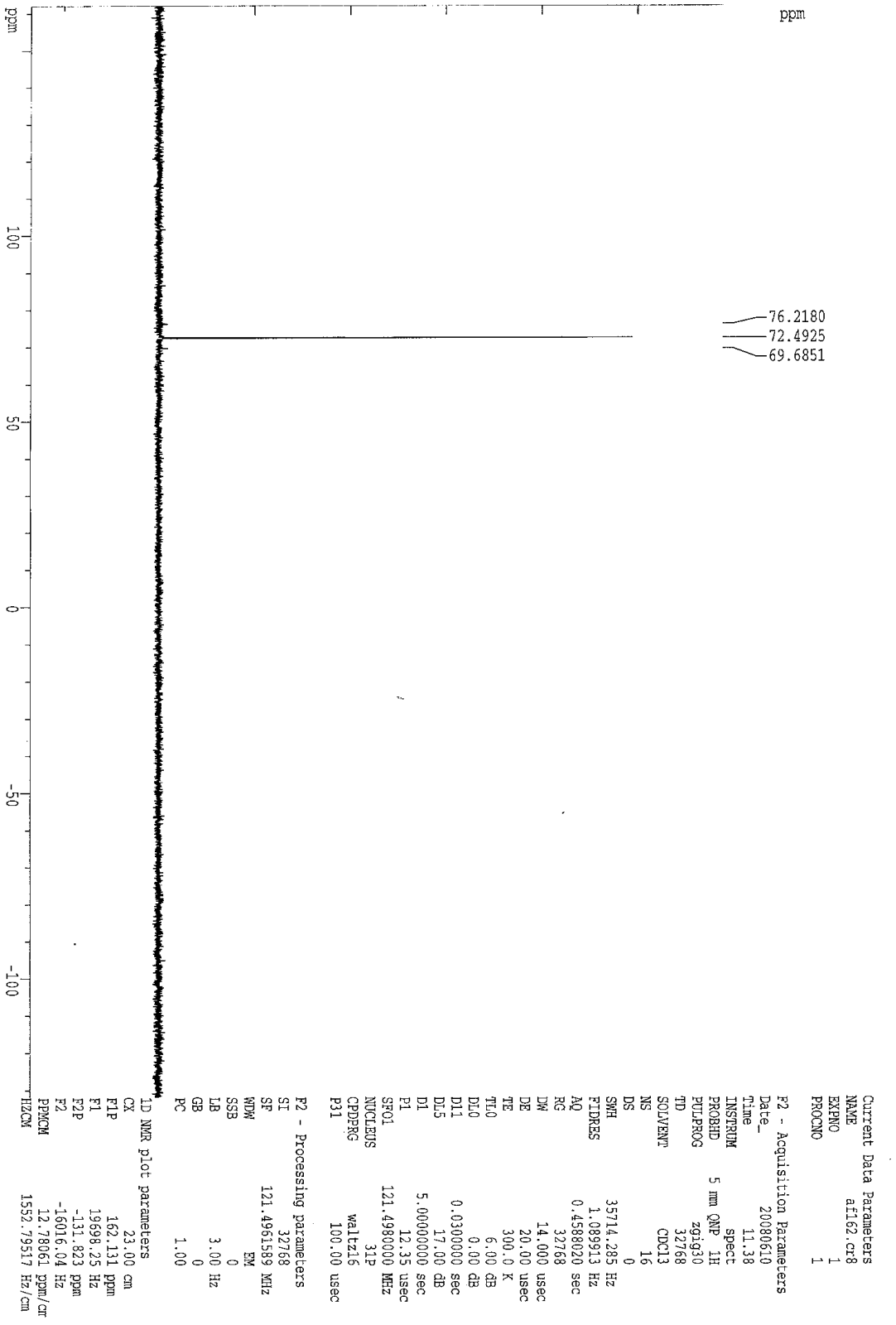

Figure S4-15: ^{31}P NMR ($CDCl_3$) spectrum of compound **4b**.

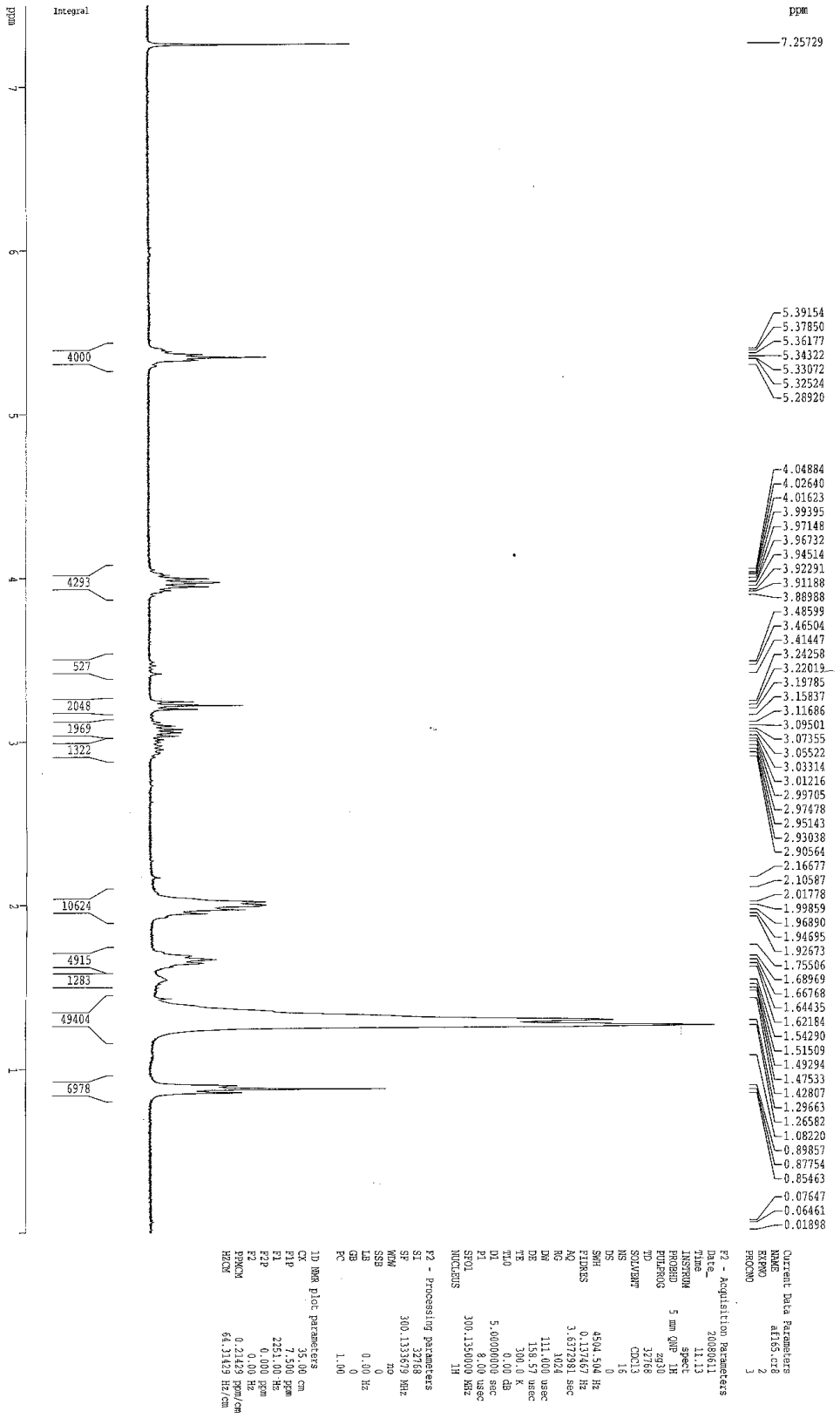

Figure S4-16: ^{1}H NMR ($CDCl_3$) spectrum of compound **5b**.

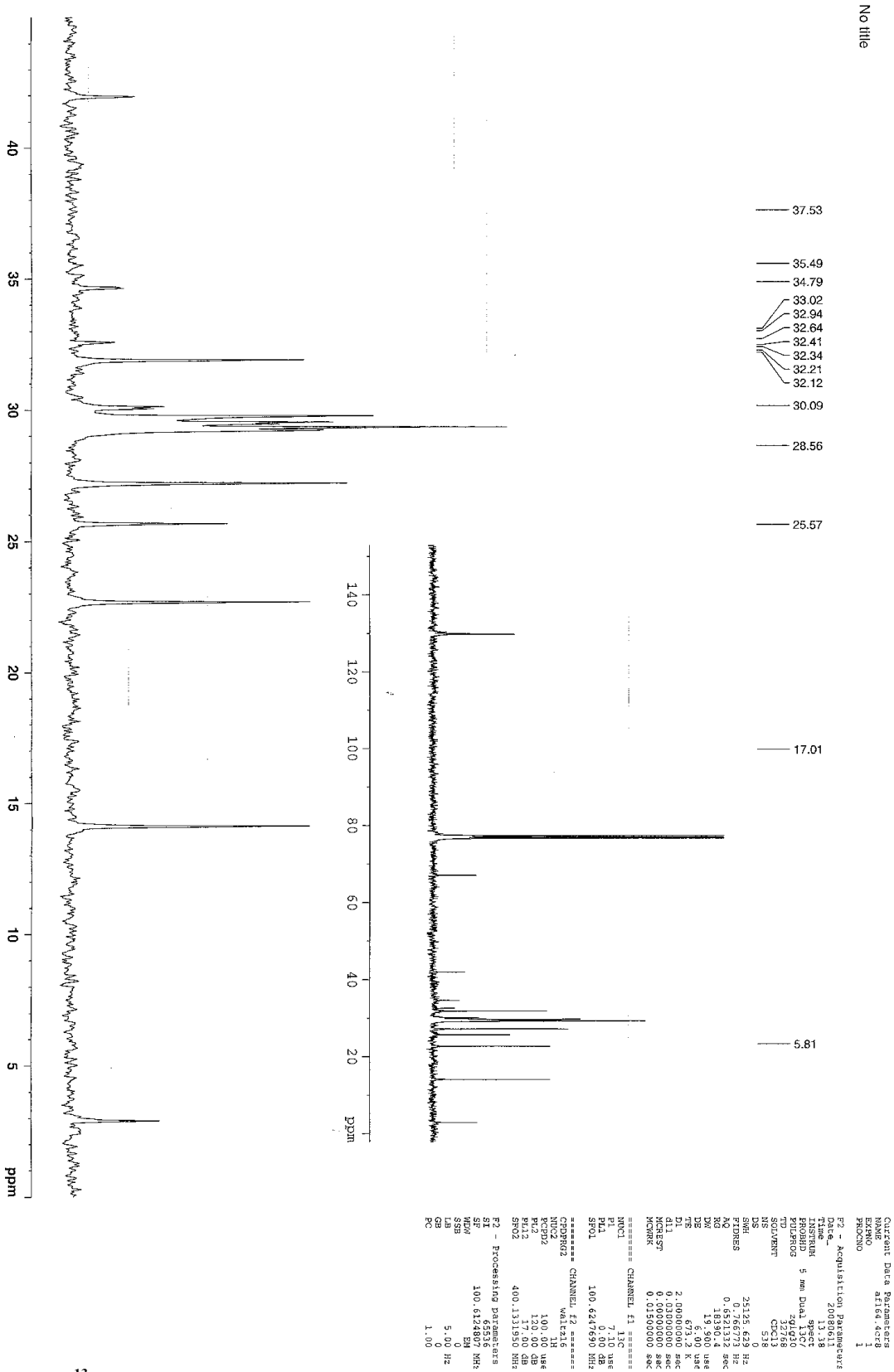

Figure S4-17: ^{13}C NMR ($CDCl_3$) spectrum of compound 5b.

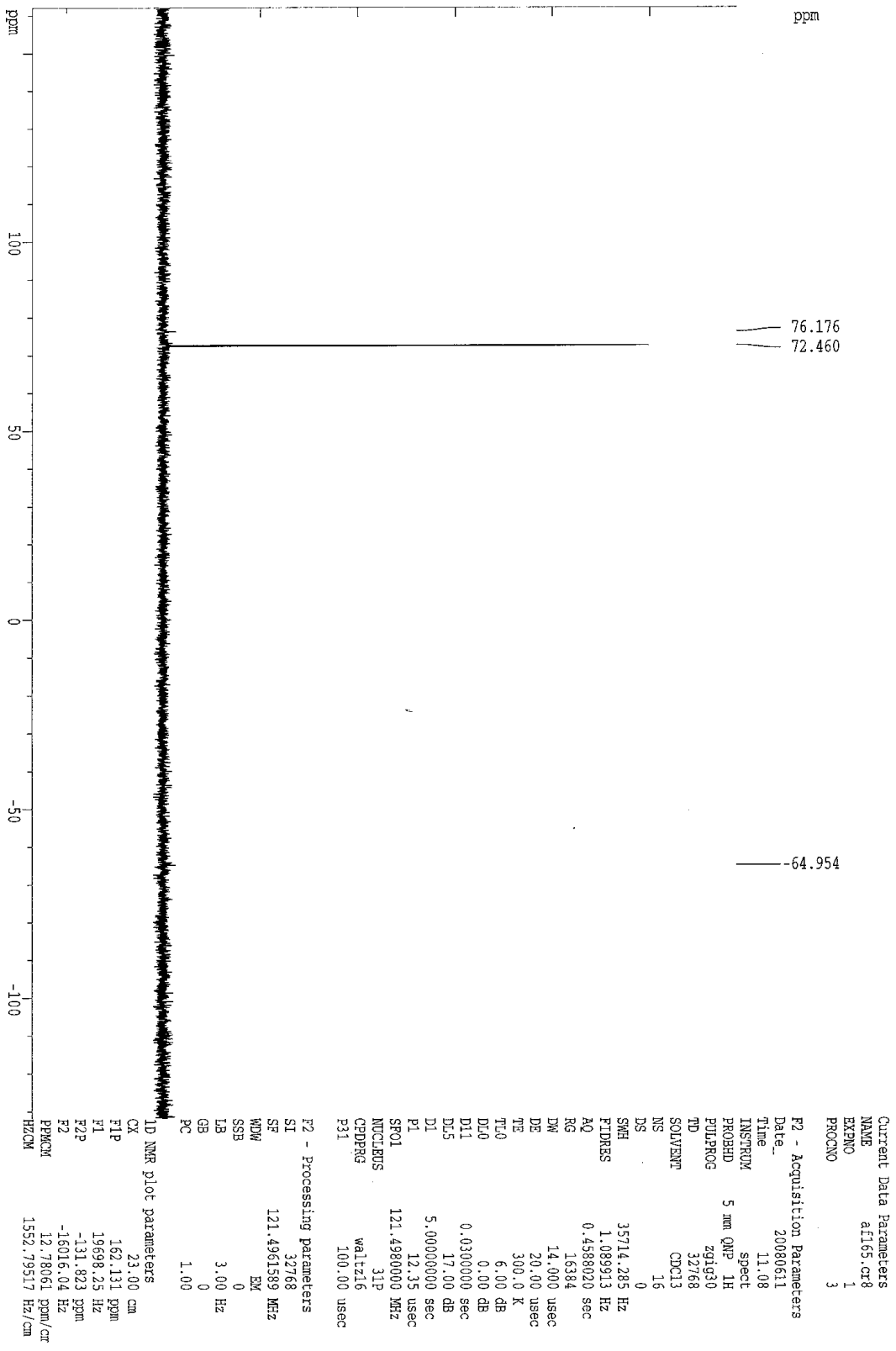

Figure S4-18: ^{31}P NMR ($CDCl_3$) spectrum of compound **5b**.

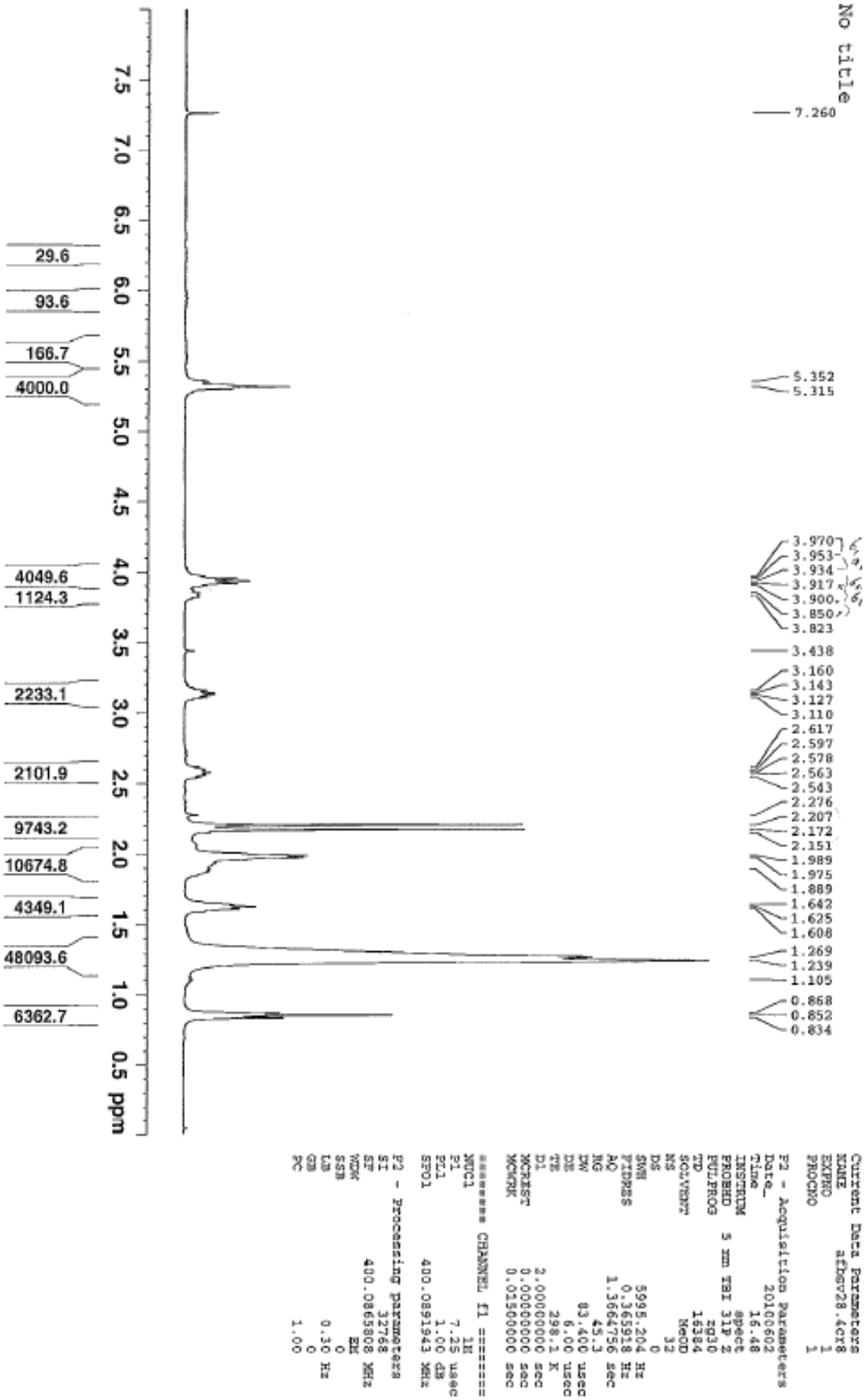

Figure S4-19: ^{1}H NMR ($CDCl_3$) spectrum of compound **6b** (BSV28).

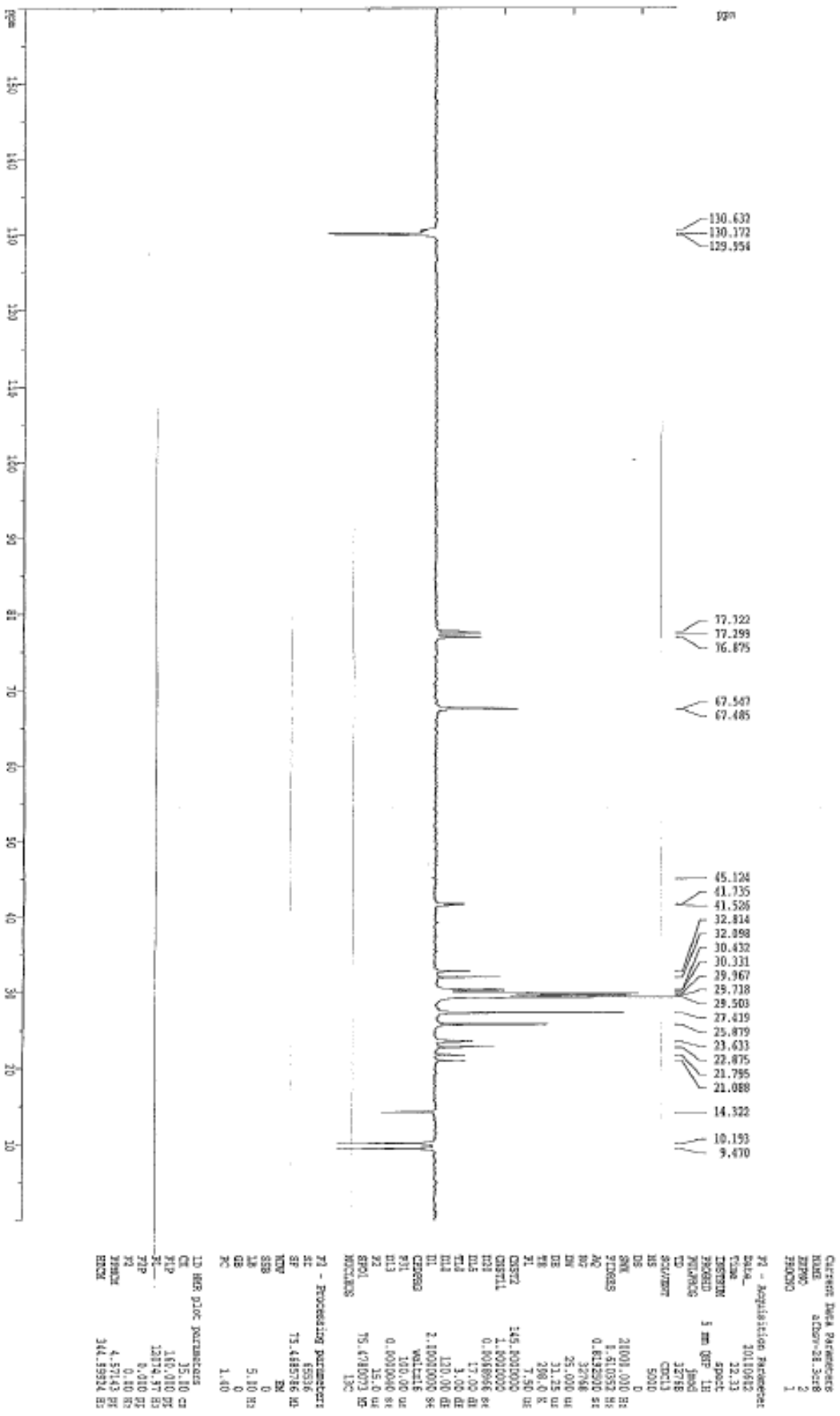

Figure S4-20: ^{13}C jmod NMR ($CDCl_3$) spectrum of compound **6b** (BSV28).

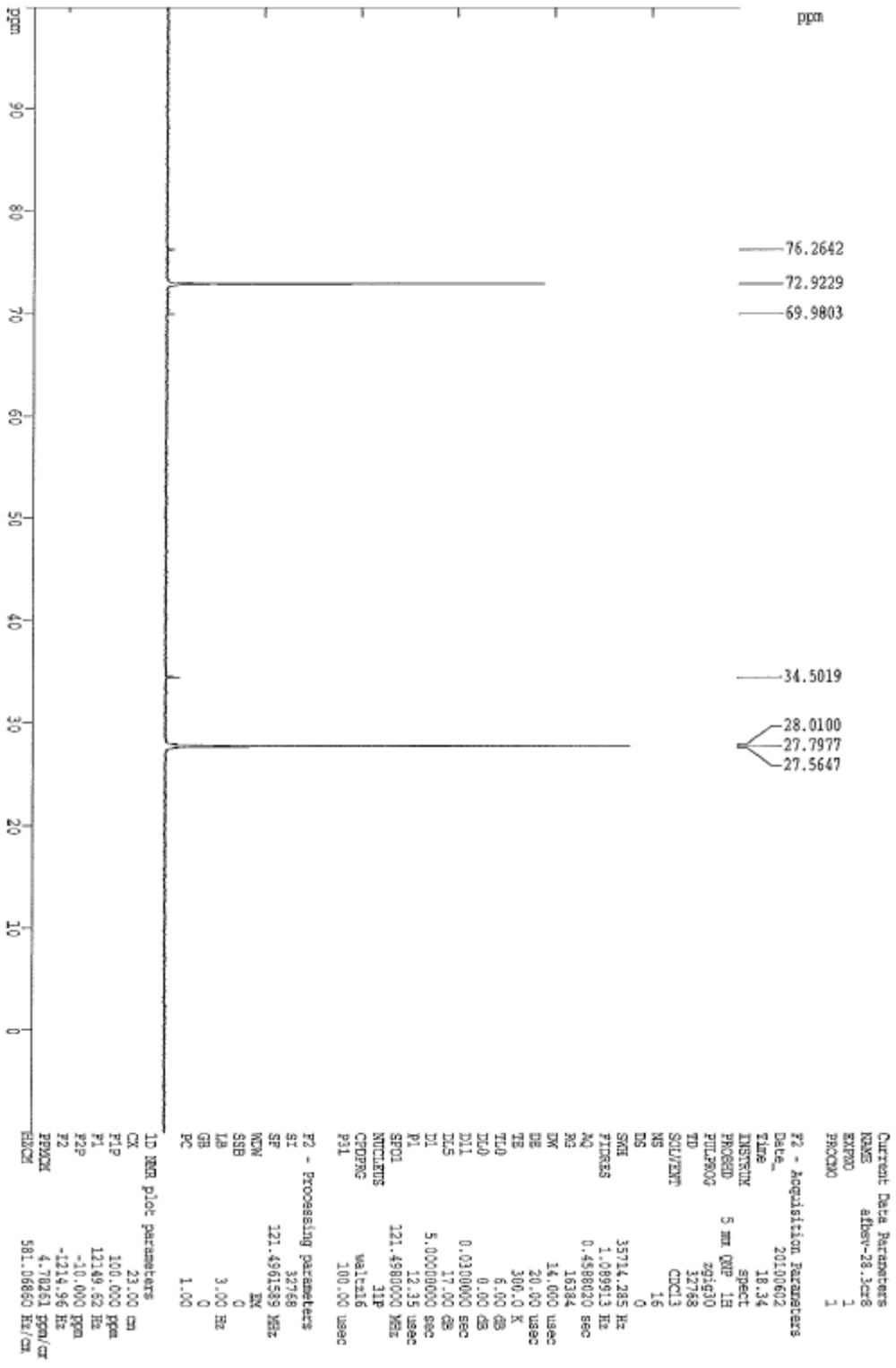

Figure S4-21: ^{31}P NMR ($CDCl_3$) spectrum of compound **6b** (BSV28).

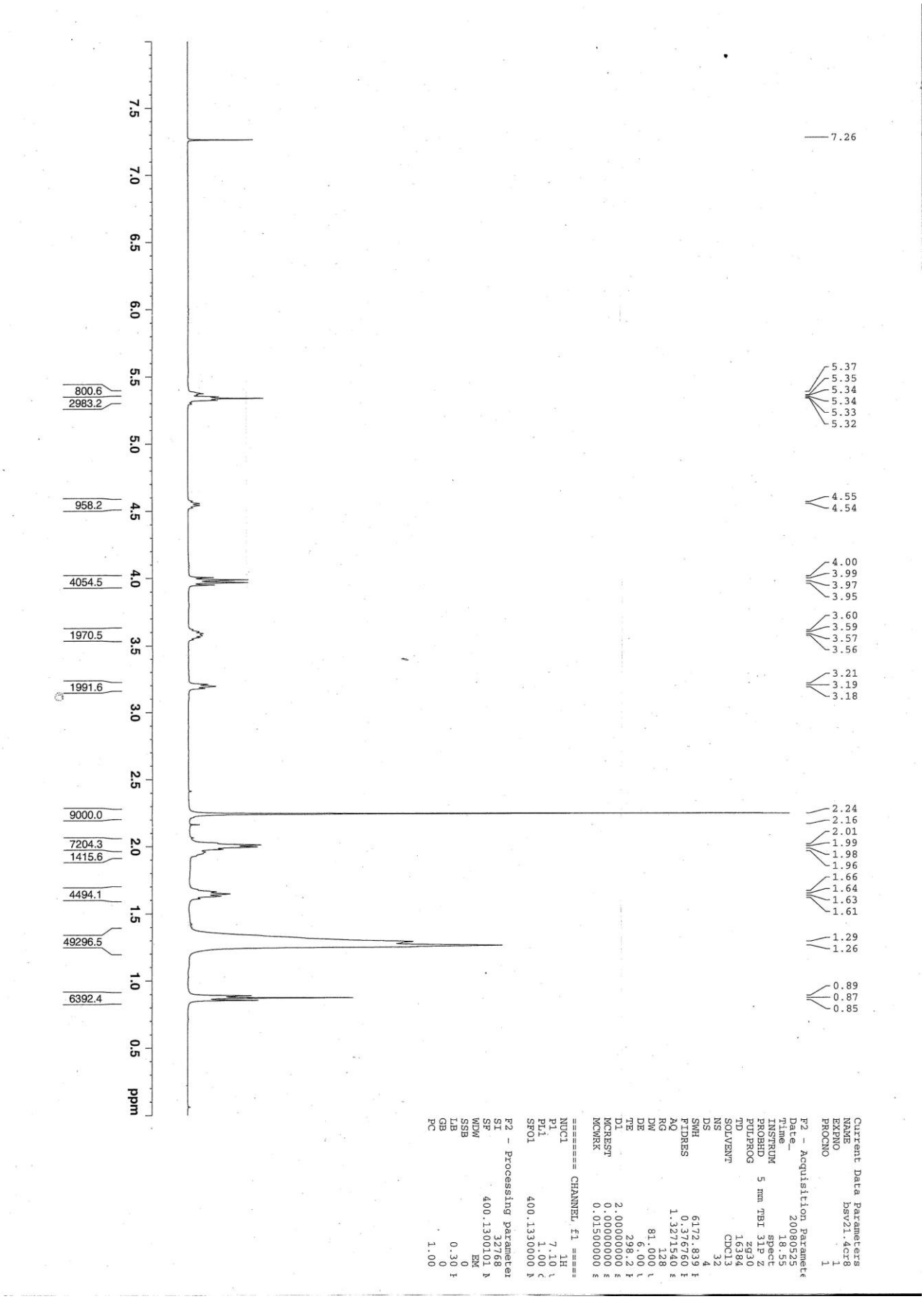

Figure S4-22: ^{1}H NMR ($CDCl_3$) spectrum of compound **7** (BSV21).

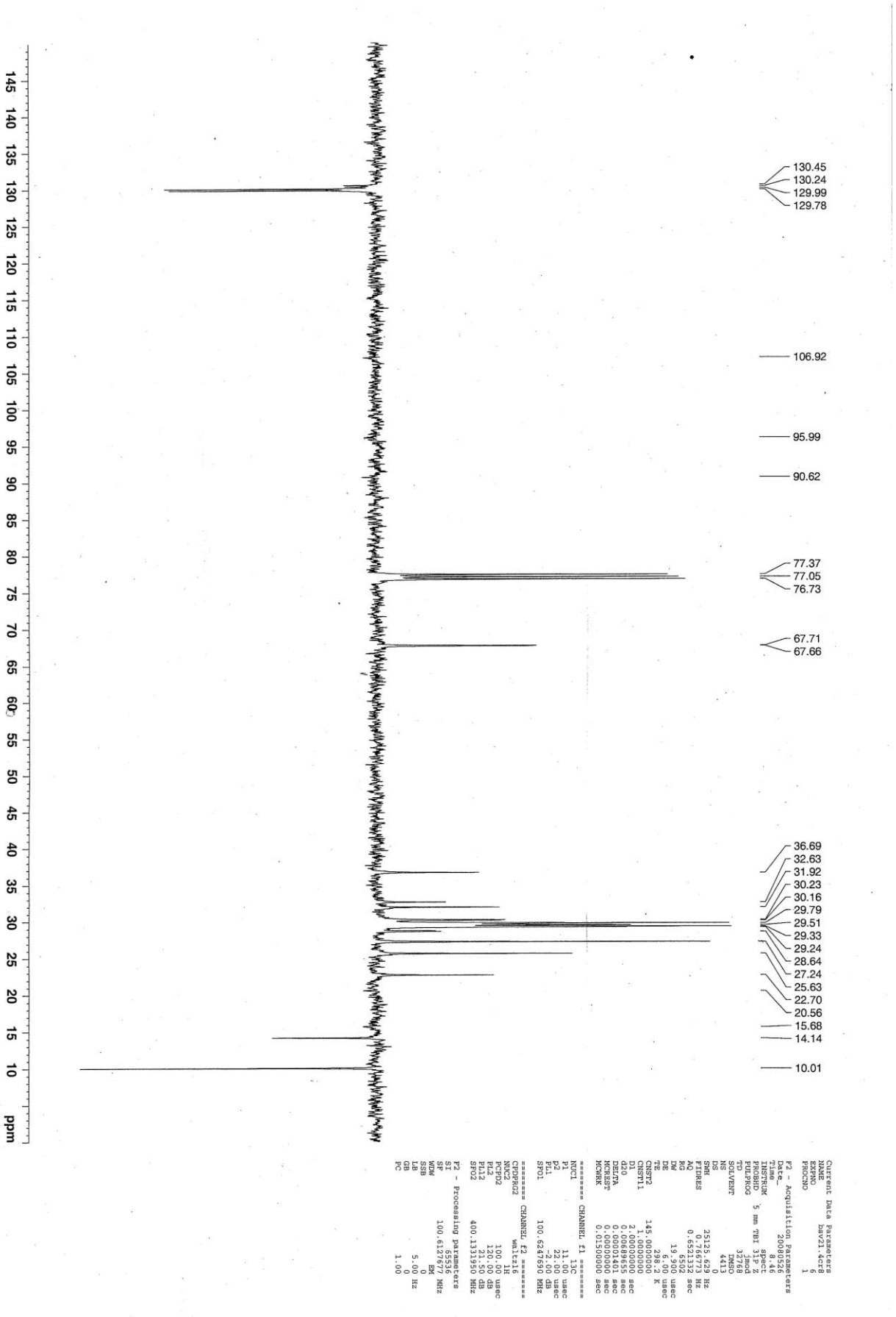

Figure S4-23: ^{13}C jmod NMR ($CDCl_3$) spectrum of compound **7** (BSV21).

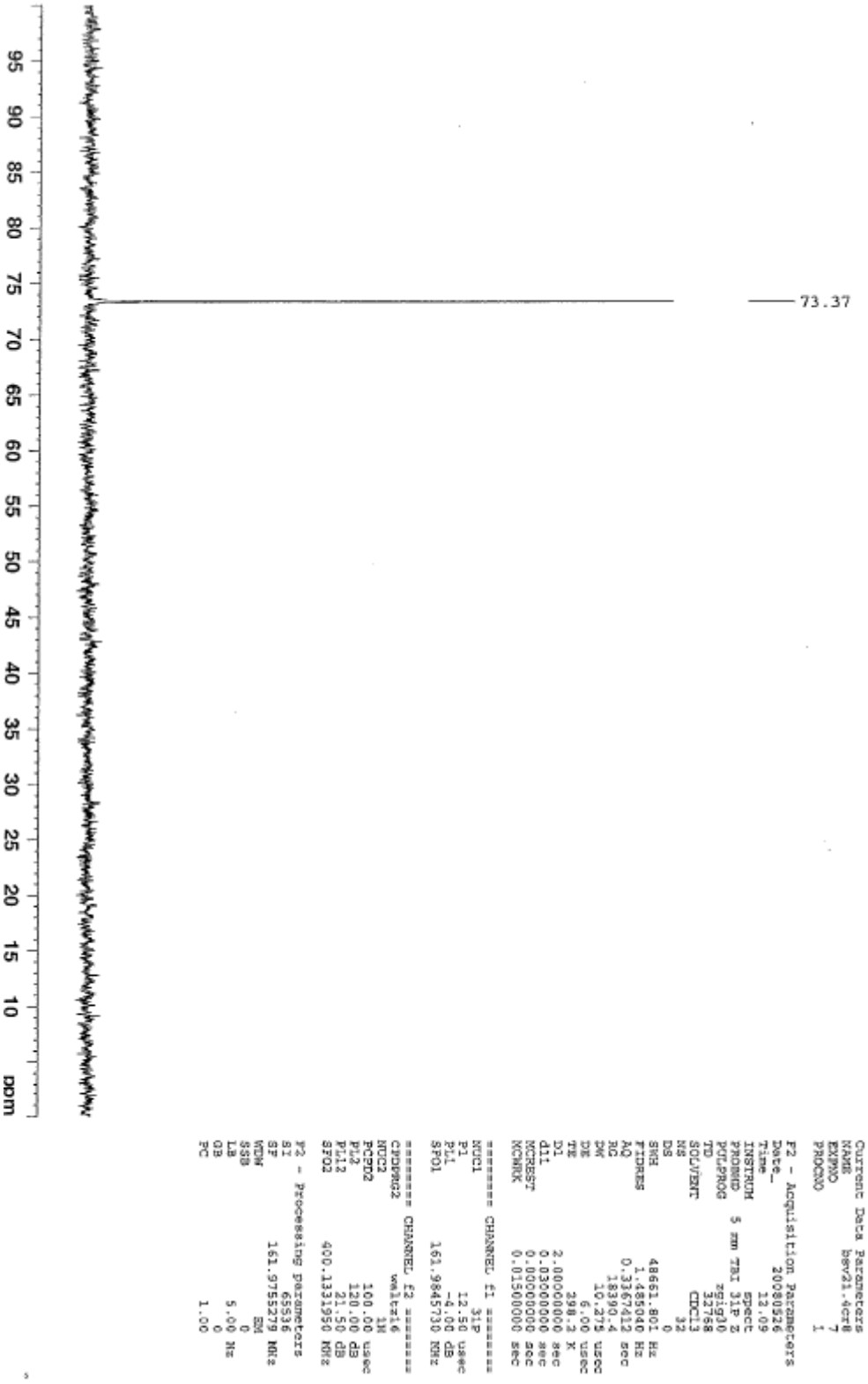

Figure S4-24: ^{31}P NMR ($CDCl_3$) spectrum of compound **7** (BSV21).