

Glycerol Nucleoside Triphosphates: Synthesis and Polymerase Substrate Activities

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Supporting Information

Table of Contents:

1) General Experimental Procedures	3
2) General Phosphorylation and Purification Procedures	3
3) (S)-gTTP	4
4) (S)-gGTP	6
5) (S)-gCTP	8
6) (S)-gATP	10
7) Polymerase Experiments	11
8) NMR Spectra	13

1) General Experimental

Reagents were purchased from Sigma, Aldrich, Acros, MP, and Lancaster. Flash Chromatography was performed using Sorbent Technologies (Atlanta, GA) 60Å Silica Gel with TLC monitoring on EMD (Gibbstown, NJ) Silica Gel 60 F254 plates. UV spectra were acquired using a Beckman (Fullerton, CA) DU650 spectrophotometer. High and low resolution mass spectroscopy was performed by Boston College mass spectroscopy facility. NMR spectra were gathered on either a Varian Gemini 400 or Unity 300 MHz with TMS as internal H¹ standard, solvent peaks for C¹³, and external phosphoric acid for P³¹ spectra. HPLC purification was performed on a Waters (Milford, MA) Delta 600 system with a 2487 dual wavelength detector. DNA oligomers were prepared using an Applied Biosystems 394 DNA/RNA synthesizer using standard solid phase phosphoramidite techniques and reagents purchased from Glenn Research (Sterling, VA). RNA oligomers were purchased from Dharmacon (Lafayette, CO). PAG Electrophoresis was performed using Bio-Rad (Hercules, CA) Sequigen-GT or Hoefer Scientific (San Francisco, CA) apparatus. Gel imaging was performed on a Bio-Rad FXpro Molecular Imager using Kodak-K radioisotope screens.

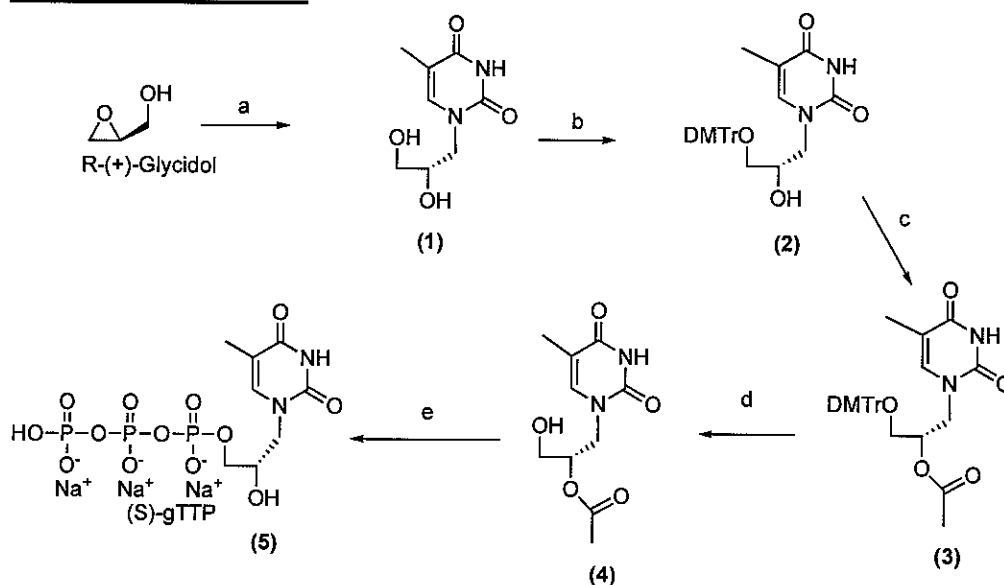
Abbreviations: DCM = Dichloromethane, MeOH = Methanol, TEA = Triethylamine, DCAA = Dichloroacetic Acid, PNK = Polynucleotide Kinase, DMF = Dimethylformamide, TEAB = Triethylammonium Bicarbonate, TEAA = Triethylammonium acetate, gNTPs = glycerol nucleoside triphosphates, DMTr = dimethoxytrityl

2) General Phosphorylation and Purification Procedure

Triphosphate synthesis was carried out according to method developed by Eckstein et. al. All solvents were freshly distilled. Compounds were dried using two coevaporations with freshly distilled pyridine followed by remaining 2 hr under vacuum over P₂O₅ after which time an argon atmosphere was introduced and maintained throughout the reaction. Compounds were dissolved in pyridine and dioxane (A, T, and C) or pyridine and DMF (G) then treated with 1 equivalent of 2-chloro-4H-1,3,2-dioxaphosphorin-4-one (1M in dioxane) and allowed to stir at ambient temperature for 15 minutes. 1.5 eq. of tributylammonium pyrophosphate in DMF and tributylamine were added and allowed to stir for 15 min. 1% I₂ in pyridine/ water 98/2 was added and allowed to react for 15 min. Solvents were removed and two diethyl ether washes were performed to remove excess iodine. Water layers were then treated with concentrated NH₄OH for 8 hours at 55° C for A, C, and G, and 1 h at ambient temperature for T followed by ammonia removal under vacuum.

Ion exchange chromatography was performed using a Mono Q HR 10/10 column eluting with a linear gradient from 0 to 1 M TEAB pH 7.5. Reverse phase chromatography was performed using a 250 × 10 mm divinyl benzene column eluting with 100% 0.1 M TEAA pH 7.0 to 50:50 methanol: TEAA. Cation exchange was performed using Dowex 50WX8-100 resin in the Na⁺ form. ³¹P NMR was carried out in D₂O.

3) (S)-gTTP Synthesis



(a) Thymine/ 0.1 eq. K₂CO₃/DMF/ 85 °C/ 12h; (b) 1.1 eq. DMTr-Cl/ pyridine/ 8h/ rt; (c) 4 eq. Ac₂O/ pyridine/ 3 h/ rt; (d) Dichloroacetic acid/ DCM/ 20 min/ rt (e) *i.* 1 eq. 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one/ dioxane/ pyridine/ 15 min/ rt; *ii.* Tributylammonium pyrophosphate/ DMF/ tributylamine/ 15 min/ rt; *iii.* 1% I₂ in 98/2 pyridine/ H₂O/ 15 min/ rt; *iv.* NH₄OH/ 1 h/ rt; *v.* Dowex 50WX8 Na⁺

Compound (1)

Thymine was dissolved in DMF at 85° C and 0.2 eq. of K₂CO₃ were added followed by 1 eq. (R)-+glycidol and stirred for 24 hours. Product eluted from silica gel in 7% MeOH:DCM at 45% yield as a white powder.

R_f: 0.3 (DCM: MeOH; 90:10, v/v)

¹H NMR (DMSO-d₆) δ: 1.77(d, 3H, J=0.8 Hz), 3.31-3.41(m, 3H), 3.70(dd, 1H, J=3.2 Hz), 3.91(dd, 1H, J₁=3 Hz, J₂=14 Hz), 4.71(t, 1H, J=6 Hz), 5.00(d, 1H, J=5 Hz), 7.41(d, 1H, J=1.2 Hz), 11.21(s, 1H). ¹³C NMR (DMSO-d₆) δ: 12.1, 50.9, 63.7, 69.1, 107.3, 142.7, 150.8, 164.1

LRMS (EI) m/z 222.6 [M+Na⁺; calcd for C₈H₁₂N₂O₄Na: 223.1]

λ_{max}(MeOH): 270nm

Compound (2)

Compound 1 and 1.2 eq. of DMTr-Cl were dissolved in pyridine and stirred for 8 hr at ambient temperature. Solvent was removed under vacuum followed by two coevaporations with toluene. Product eluted by flash chromatography in 98:2:0.01 DCM:MeOH:TEA in 83% yield as a white foam.

R_f: 0.3 (DCM: MeOH; 95:5, v/v)

¹H NMR (DMSO-d₆) δ: 1.59(s, 3H), 2.80(dq, 2H, J₁=4 Hz, J₂=37 Hz), 3.35(q, 1H, J₁=5 Hz, J₂=9 Hz), 3.64(s, 6H), 3.79(m, 2H), 5.15(d, 1H, J=6 Hz), 6.78-7.32 (m, 14H, aromatic), 11.10(s, 1H). ¹³C NMR (DMSO-d₆) δ: 12.0, 51.2, 55.0, 65.6, 67.4, 85.3, 107.4, 113.0, 126.4, 127.5, 127.6, 129.5, 135.5, 142.4, 144.7, 150.8, 157.8, 164.1

LRMS (EI) m/z 525.2 [M+Na⁺; calcd for C₂₉H₃₀N₂O₆Na: 525.2]

λ_{\max} (DCM): 233, 272 nm

Compound (3)

Compound 2 was dissolved in pyridine and treated with 4 eq. of acetic anhydride for 2 h at ambient temperature. Solvent was removed and coevaporated twice with toluene and purified by flash chromatography eluting with 99:1:0.01 DCM:MeOH:TEA in 95% yield as a white foam.

R_f: 0.4 (DCM: MeOH; 97:3, v/v)

¹H NMR (DMSO-d₆) δ : 1.59(s, 3H), 1.93(s, 3H), 2.94(dd, 1H, J₁=3 Hz, J₂=10 Hz), 3.01(dd, 1H, J₁=4 Hz, J₂=10 Hz), 3.64(s, 6H), 3.71(dd, 1H, J₁=8 Hz, J₂=14 Hz), 3.83(dd, 1H, J₁=4 Hz, J₂=14 Hz), 5.12(m, 1H), 6.8-7.3(m, 14H, aromatic), 11.15(s, 1H).

¹³C NMR (DMSO-d₆) δ : 12.5, 21.1, 48.6, 55.4, 62.3, 70.6, 86.5, 110.3, 113.3, 127.0, 127.94, 129.9, 135.4, 140.8, 144.4, 151.0, 158.6, 164.2, 169.8

LRMS (EI) m/z 567.2 [M+Na⁺; calcd for C₃₁H₃₂N₂O₇Na: 567.2]

λ_{\max} : DCM 237, 270 nm

Compound (4)

Compound 3 was dissolved in dry DCM and treated with 3% DCAA in DCM for 15 min until starting material disappeared by TLC. Solution was loaded directly over silica, washed with 3 column volumes DCM and eluted in 97:3 DCM:MeOH in 93% yield as a white powder.

R_f: 0.3 (DCM: MeOH; 95:5, v/v)

¹H NMR (DMSO-d₆) δ : 1.74(s, 3H), 1.95(s, 3H), 3.50(m, 2H), 3.65(dd, 1H, J₁=9 Hz, J₂=14 Hz), 3.97(dd, 1H, J₁=3 Hz, J₂=14 Hz), 4.99-5.04(bm, 2H), 7.43(s, 1H), 11.25(s, 1H). ¹³C NMR (DMSO-d₆) δ : 11.9, 20.8, 48.0, 60.3, 71.9, 108.1, 141.5, 150.7, 164.0, 169.6

HRMS (EI) m/z 265.0791 [M+Na⁺; calcd for C₁₀H₁₄N₂O₅Na: 265.0793]

λ_{\max} (MeOH): 268 nm

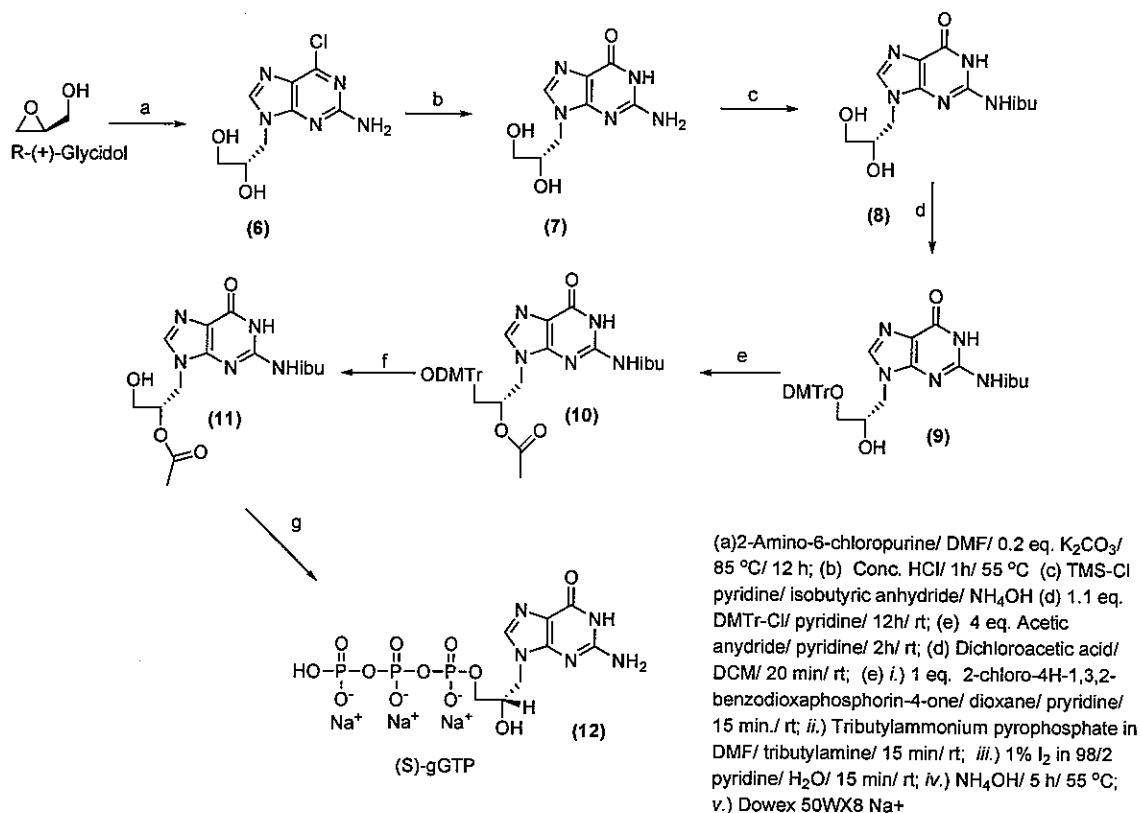
Compound (5)

Compound 5 was prepared from 4 using the general phosphorylation and purification procedure as described¹.

³²P NMR (D₂O) δ : -21.7(t, J=19 Hz), -9.8(d, J=19 Hz), -8.8(d, J=19 Hz)

(1) Ludwig, J.; Eckstein, F.; *J. Org. Chem.* **1989**, *54*, 631

4) gGTP Synthesis



Compound (6)

2-Amino-6-chloropurine was dissolved in DMF and 0.2 eq. of K_2CO_3 were added. 1 eq. of R-(+)-glycidol was added slowly and reaction heated to 85 °C for 24 h. Solvent was removed and compound was purified on silica eluting with 85:15 DCM:MeOH in 82% yield as yellow powder.

R_f: 0.6 (DCM: MeOH; 80:20, v/v)

1H NMR (DMSO- d_6) δ : 3.41(m, 2H), 3.85(m, 1H), 3.94(dd, 1H, J₁=9 Hz, J₂=14 Hz), 4.22(dd, 1H, J₁=3 Hz, J₂=14 Hz), 4.88(bs, 1H), 5.12(bs, 1H), 6.92(s, 2H), 8.05(s, 1H).

^{13}C NMR (DMSO- d_6) δ : 46.5, 63.5, 69.2, 123.1, 143.9, 148.9, 154.0, 159.4

LRMS (EI) m/z 244.1 [$M+H^+$; calcd for $C_8H_{10}ClN_5O_2H$: 244.1]

λ_{max} (MeOH): 222, 247, 310 nm

Compound (7)

Compound 6 was dissolved in concentrated HCl and heated to 55° C for 1 hr followed by drying under vacuum to yield 7 in quantitative yield as a yellow powder.

R_f: 0.54(DCM: MeOH; 50:50, v/v)

1H NMR (DMSO- d_6) δ : 3.43(m, 2H), 3.87(m, 1H), 4.01(dd, 1H, J₁=9.2 Hz, J₂=14 Hz), 4.31(dd, 1H, J₁=3 Hz, J₂=14 Hz), 7.55(s, 2H), 9.20(s, 1H), 11.99(s, 1H). ^{13}C NMR (DMSO- d_6) δ : 48.1, 63.3, 68.6, 107.3, 137.6, 149.5, 153.1, 155.4

LRMS (EI) m/z 226.08 [$M+H^+$; calcd for $C_8H_{11}N_5O_3H$: 226.1]

λ_{max} : MeOH 254, 283 nm

Compound (8)

Compound **8** was prepared by transient amine protection of **7** using procedures outlined by Gait, M.J.²

Rf: 0.4 (DCM: MeOH; 90:10, v/v)

¹H NMR (DMSO-d₆) δ: 1.13(s, 3H), 1.15(s, 3H), 2.81(m, 1H, J=7 Hz), 3.40(m, 2H), 3.82(m, 1H), 3.96(dd, 1H, J1=9 Hz, J2=14 Hz), 4.24(dd, 1H, J1=4 Hz, J2=14 Hz), 4.92(t, 1H, J=6 Hz), 5.18(d, 1H, J=6 Hz), 7.93(s, 1H), 11.72(s, 1H), 12.08(s, 1H).

LRMS (EI) m/z 296.16 [M+H⁺; calcd for C₁₂H₁₇N₅O₄H: 296.13]

λ_{max}(MeOH): 256, 281

Compound (9)

Compound **8** was dissolved in pyridine and reacted with 1.1 eq of DMTr-Cl for 12 h at ambient temperature. Solvent was removed and coevaporated twice with toluene followed by silica gel chromatography eluting with 97:3:0.01 DCM:MeOH:TEA in 86% yield as a white foam.

Rf: 0.3 (DCM: MeOH; 97:3, v/v)

¹H NMR (CDCl₃) δ: 1.23(q, 6H, J1=4, J2=7 Hz), 2.78(s, 1H, J=7 Hz), 3.18(m, 2H), 3.74(s, 6H), 4.07(dd, 1H, J1=9 Hz, J2=14 Hz), 4.20(dd, 1H, J1=3 Hz, J2=14 Hz), 4.32(bm, 1H), 6.7-7.4(aromatic, 14H), 11.68(s, 1H)

LRMS (EI) m/z 598.1 [M+H⁺; calcd for C₃₃H₃₅N₅O₆H: 598.3]

λ_{max}(DCM): 238, 282 nm

Compound (10)

Compound **9** was dissolved in pyridine and treated with 4 eq. of acetic anhydride for 2 h at ambient temperature. Pyridine was removed under vacuum and coevaporated twice with toluene the purified on silica gel eluting with 99:1:0.01 DCM:MeOH:TEA in 85% yield as a white foam.

Rf: 0.7 (DCM: MeOH; 90:10, v/v)

¹H NMR (CDCl₃) δ: 1.18(d, 3H, J=1.6 Hz), 1.20(d, 3H, J=1.2 Hz), 2.03(s, 3H), 2.61(m, 1H), 3.08(dq, 2H, J1=5 Hz, J2=10 Hz, J3=32 Hz), 3.37(s, 6H), 4.28(d, 2H, J=5 Hz), 5.20(t, 1H, J= Hz), 6.7-7.3(m, 13H, aromatic), 8.57(s, 1H), 9.77(s, 1H), 12.00(bs, 1H).

¹³C NMR (CDCl₃) δ: 19.2, 21.1, 36.3, 43.7, 55.4, 62.0, 70.6, 86.5, 113.2, 120.7, 127.0, 127.9, 129.8, 135.2, 135.3, 139.3, 144.2, 147.7, 148.7, 155.6, 158.5, 169.7, 179.1

LRMS (EI) m/z 640.3 [M+H⁺; calcd for C₃₅H₃₇N₅O₇H: 640.3]

λ_{max}(DCM): 238, 282

Compound (11)

Compound **10** was dissolved in dry DCM and treated with 3% DCAA at ambient temperature for 20 min followed by loading over silica and washing with three column volumes of DCM. Product eluted in 97:3 DCM:MeOH in 95% yield as a white powder.

Rf: 0.5 (DCM: MeOH; 90:10, v/v)

² Gait, M.J., ed. *Oligonucleotide Synthesis: A Practical Approach*. New York: Oxford University Press, 1990

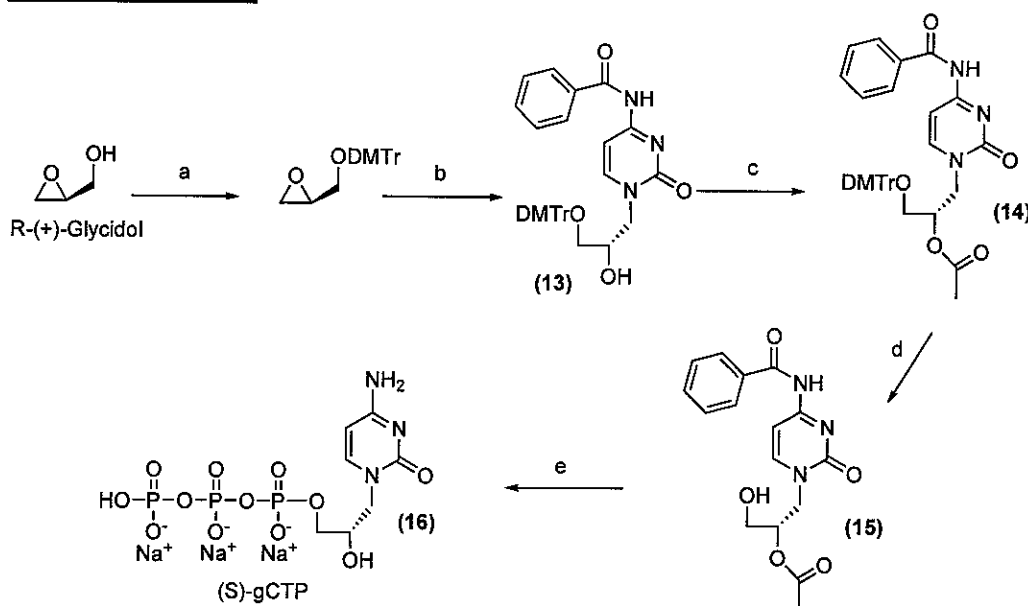
^1H NMR (DMSO- d_6) δ : 1.12(d, 6H, $J=7$ Hz), 1.92(s, 3H), 2.79(m, 1H), 3.52(bm, 2H), 4.29(dq, 2H), 5.08(m, 1H), 5.13(t, 1H, $J=6$ Hz), 7.94(s, 1H), 11.71(s, 1H), 12.09(s, 1H).
 ^{13}C NMR (DMSO- d_6) δ : 18.9, 20.9, 34.7, 43.6, 60.1, 72.3, 119.7, 139.9, 147.6, 148.7, 154.6, 169.4, 179.9
 HRMS (EI) m/z 338.1459 [$M+H^+$; calcd for $\text{C}_{14}\text{H}_{19}\text{N}_5\text{O}_5\text{H}$: 338.1465]
 λ_{max} (MeOH): 254, 259, 284 nm

Compound (12)

Compound **12** was prepared using the general phosphorylation and purification procedures as described¹.

^{32}P NMR (D_2O) δ : -21.4(t, $J=19.5$ Hz), -9.8(d, $J=19.4$ Hz), =7.7(d, $J=19.7$ Hz).

3) gCTP Synthesis



(a) DMTr-Cl/ R-(+)-Glycidol/ DCM/ TEA/ 12 h/ rt; (b) Benzoyl cytosine/ DMF/ 0.2 eq. NaH/ 120 °C/ 4 h; (c) 4 eq. Ac₂O/ pyridine/ 3 h/ rt; (d) Dichloroacetic acid/ DCM/ 20 min/ rt; (e) i.) 1 eq. 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one/ dioxane/ pyridine/ 15 min/ rt; ii.) Tributylammonium pyrophosphate in DMF/ tributylamine/ 15 min/ rt; iii.) 1% **12** in 98/2 pyridine/ H₂O/ 15 min./ rt; iv.) NH₄OH/ 8 h/ 55 °C; v.) Dowex 50WX8 Na⁺

Compound (13)

N₄-Benzoyl cytosine was dissolved in DMF at 120 °C and reacted with 0.1 eq. of NaH for 1 hr followed by addition of 1 eq. of O-DMTr-R-(+)-glycidol and reacted for 4 h at ambient temperature. Solvent was removed and reaction was purified on silica gel eluting with 98:2:0.01 DCM:MeOH:TEA giving a white foam in 35% yield.

^1H NMR (CDCl_3) δ : 3.12(dd, 1H, $J_1=6$, $J_2=10$ Hz), 3.26(dd, 1H, $J_1=5.3$ Hz, $J_2=10$ Hz), 3.84(q, 1H, $J_1=7$ Hz, $J_2=14$ Hz), 4.22(bs, 1H), 4.35(dd, 2H, $J_1=3$ Hz, $J_2=14$ Hz), 6.8-8.0(aromatic, 20H), 8.73(bs, 1H)

^{13}C NMR (CDCl_3) δ : (54.6, 55.3, 64.9, 68.6, 86.3, 96.6, 113.2, 126.8, 127.76, 127.84, 127.99, 128.78, 129.9, 132.9, 133.0, 135.6, 144.6, 150.5, 156.7, 158.4, 163.4, 169.0

LRMS (EI) m/z 592.3 [$M+H^+$; calcd. for $C_{35}H_{33}N_3O_6H$: 592.3]

Compound (14)

Compound **13** was dissolved in pyridine and reacted with 4 eq. of acetic anhydride for 3 h followed by solvent removal and two coevaporations with toluene. Silica gel chromatography eluting in 99:1:0.01 DCM:MeOH:TEA gave product as a white foam in 92% yield.

R_f: 0.6 (DCM: MeOH; 95:5, v/v)

¹H NMR (CDCl₃) δ: (1.99(s, 3H), 3.09(dd, 1H, J₁=4 Hz, J₂=16 Hz), 3.27(dd, 1H, J₁=4 Hz, J₂=10 Hz), 3.69(s, 6H), 3.91(dd, 1H, J₁=8 Hz, J₂=14 Hz), 4.37(dd, 1H, J₁=4 Hz, J₂=13 Hz), 5.26(m, 1H), 6.7-8.0(m, 18H, aromatic). ¹³C NMR (CDCl₃) δ: 21.0, 50.1, 53.6, 55.2, 62.3, 70.4, 86.3, 96.4, 113.2, 126.9, 127.6, 127.8, 128.8, 129.78, 129.82, 132.9, 133.1, 135.2, 135.3, 144.3, 155.3, 158.5, 162.4, 167.0, 169.6

LRMS (EI) m/z 656.2 [$M+Na^+$; calcd for $C_{37}H_{35}N_3O_7Na$: 656.2]

λ_{max}(DCM): 238, 258, 313

Compound (15)

Compound **14** was dissolved in dry DCM and treated with 3% DCAA for 20 minute and loaded directly onto a silica gel column and washed with three column volumes of DCM and eluted in 97:3 DCM:MeOH giving 15 in 95% yield as a white powder.

R_f: 0.7 (DCM: MeOH; 90:10, v/v)

¹H NMR (CDCl₃) δ: 2.10(s, 3H), 3.67(dd, 2H, J₁=4, J₂=13 Hz), 4.25(d, 2H, J=5 Hz), 5.16(m, 1H, J=5 Hz), 7.5-7.7(m, 5H, aromatic), 7.94(d, 2H, J=8Hz). ¹³C NMR (CDCl₃) δ: 21.3, 50.1, 60.5, 72.4, 97.6, 127.8, 129.0, 132.9, 132.3, 133.3, 150.0, 156.7, 163.0, 166.8, 170.3

HRMS (EI) m/z 332.1254 [$M+Na^+$; calcd for $C_{16}H_{17}N_3O_5Na$: 332.1247]

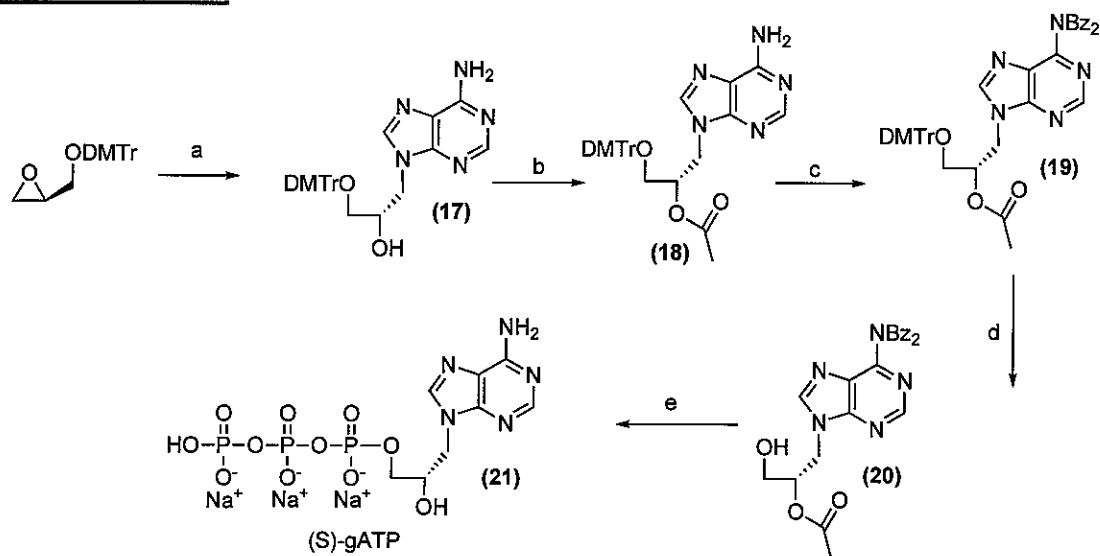
λ_{max}: DCM 258, 310 nm

Compound (16)

Compound **16** was prepared using the general phosphorylation and purification methods as previously described¹.

³²P NMR (D₂O) δ: -21.7(t, J=19 Hz), -9.8(d, J=19 Hz), -8.4(d, J=20 Hz)

4) gATP Synthesis



(a) Adenine/ DMF/ 0.2 eq. NaH/ 120 °C/ 6 h; (b) 1.2 eq. Ac₂O/ pyridine/ 1 h/ rt; (c) 3 eq. Benzoyl-Cl pyridine/ 3 h/ rt; (d) Dichloroacetic acid/ DCM/ 20 min/ rt; (e) *i.*) 1 eq. 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one/ dioxane/ pyridine/ 15 min/ rt; *ii.*) Tributylammonium pyrophosphate/ DMF/ tributylamine/ 15 min/ rt; *iii.*) 1% I₂ in 98/2 pyridine/ H₂O/ 15 min/ rt; *iv.*) NH₄OH/ 5 h/ 55 °C; *v.*) Dowex 50WX8 Na⁺

Compound (17)

Adenine was dissolved in 85° C DMF and reacted with 0.1 eq. NaH for 2 h followed by addition of O-DMTr-R-(+)-glycidol. After 24 h solvent removed and the reaction product purified on silica gel eluting with 94:4:0.01 DCM:MeOH giving white foam in 45% yield.

R_f: 0.6 (DCM: MeOH; 90:10, v/v)

¹H NMR (CDCl₃) δ: 2.95(dd, 1H, J₁=9 Hz, J₂=9 Hz), 3.16(dd, 1H, J₁=6 Hz, J₂=10 Hz), 3.67(s, 6H), 4.10(bm, 1H), 4.18(dd, 1H, J₁=7 Hz, J₂=14 Hz), 4.32(dd, 1H, J₁=2 Hz, J₂=14 Hz), 5.18(bs, 1H), 5.80(bs, 2H), 6.7-7.3(m, 13H, aromatic), 7.66(s, 1H), 8.16(s, 1H). ¹³C NMR (CDCl₃) δ: 48.2, 55.3, 64.9, 69.3, 86.4, 113.2, 119.1, 126.8, 127.8, 128.0, 129.9, 135.6, 135.7, 141.4, 144.5, 149.8, 152.4, 155.5, 158.4

LRMS (EI) m/z 512.0 [M+H⁺; calcd for C₂₉H₂₉N₅O₄H: 512.2]

λ_{max}(DCM): 237, 264 nm

Compound (18)

Compound 17 was dissolved in pyridine and treated with 1.2 eq. of acetic acid which reacted primarily to give the acetylated hydroxyl product which after solvent removal and toluene coevaporations eluted from a silica gel column using 98:2:0.01 DCM:MeOH:TEA. Product was a white foam in 65% yield.

R_f: 0.3 (DCM: MeOH; 95:5, v/v)

R_f: 0.3 (DCM: MeOH; 95:5, v/v)

¹H NMR (CDCl₃) δ: 2.03(s, 3H), 3.25(m, 2H), 3.8(s, 6H), 4.54(qd, 1H, J₁=6 Hz, J₂=15 Hz, J₃=22 Hz), 5.34(m, 1H), 5.91(bs, 2H), 6.8-7.4(m, 13H, aromatic), 7.73(s, 1H), 8.32(s, 1H). ¹³C NMR (CDCl₃) δ: 21.2, 43.9, 55.4, 62.2, 71.1, 86.5, 113.3, 119.6, 127.0, 127.9, 129.88, 129.91, 135.38, 135.41, 140.7, 144.3, 150.2, 153.0, 155.6, 158.6, 169.8

LRMS (EI) m/z 554.0 [M+H⁺; calcd for C₃₁H₃₁N₅O₅H: 554.2]
 λ_{\max} (DCM): 238, 264 nm

Compound (19)

Compound **18** was dissolved in pyridine and treated with 4 eq. of benzoyl-Cl for 4 h at ambient temperature. Solvent was removed and coevaporated twice with toluene before purifying by silica column using 99:1:0.01 TEA giving a white foam in 96% yield.

R_f: 0.5 (DCM: MeOH; 97:3, v/v)

¹H NMR (CDCl₃) δ : 1.99(s, 3H), 3.24(d, 2H, J=5 Hz), 3.78(s, 6H), 4.53(dd, 1H, J₁=7 Hz, J₂=15 Hz), 4.66(dd, 1H, J₁=4 Hz, J₂=15 Hz), 5.30(bm, 1H), 6.8-7.3(aromatic, 23H), 8.03(s, 1H), 8.62(s, 1H). ¹³C NMR (CDCl₃) δ : 21.2, 44.9, 55.5, 62.3, 70.9, 86.8, 113.4, 127.9, 128.0, 128.7, 129.4, 129.9, 130.0, 132.9, 134.2, 135.3, 144.4, 145.3, 151.5, 152.1, 153.4, 158.7, 169.7, 172.1

LRMS (EI) m/z 784/4 [M+Na⁺; calcd for C₄₅H₃₉N₅O₇Na: 784.3]

λ_{\max} (DCM): 238, 276 nm

Compound (20)

Compound **19** was dissolved in dry DCM and treated with 3% DCAA for 20 min followed by loading over silica gel column and washing with three column volumes of DCM. Product eluted in 97:3 DCM:MeOH giving a white powder in 94% yield.

R_f: 0.4 (DCM: MeOH; 95:5, v/v)

¹H NMR (CDCl₃) δ : 2.06(s, 3H), 3.45(dd, 1H, J₁=7 Hz, J₂=12.4 Hz), 3.66(dd, 1H, J₁=5 Hz, J₂=12.8 Hz), 4.58(qd, 2H, J₁=4.4 Hz, J₂=15 Hz), 5.17(bm, 1H), 7.3-7.9(aromatic, 10H), 8.07(s, 1H), 8.66(s, 1H). ¹³C NMR (CDCl₃) δ : 21.2, 43.4, 60.1, 71.9, 127.0, 128.8, 129.5, 133.1, 134.0, 145.7, 152.1, 153.4, 169.9, 172.2

HRMS (EI) m/z 460.1597 [M+H⁺; calcd for C₂₄H₂₁N₅O₅H: 460.1622]

λ_{\max} : DCM 250, 276

Compound (21)

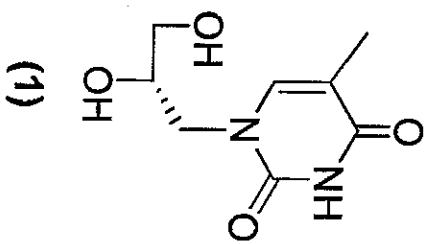
Compound **21** was synthesized using the general phosphorylation and purification methods described previously¹.

³²P NMR (D₂O) δ : -9.93(d, J=19 Hz), -9.75(d, J=19 Hz), -21.5(t, J=19 Hz)

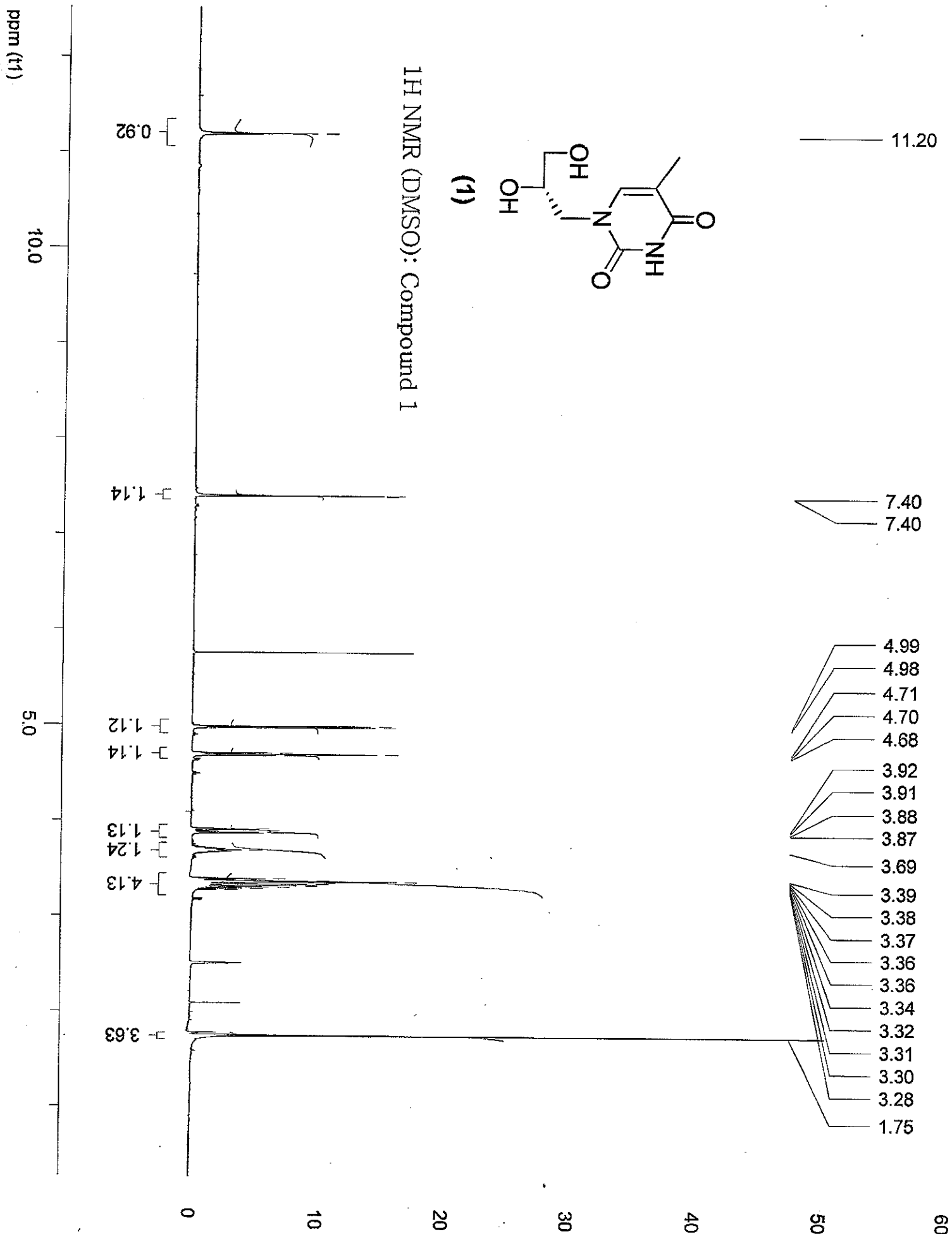
7) Polymerase Experiments

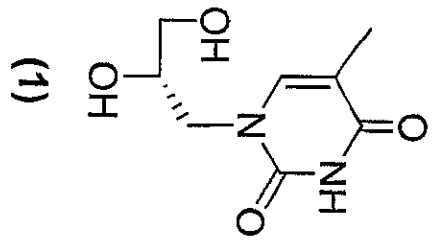
DNA oligomers for gNTP polymerase experiments were synthesized using by standard solid phase procedures. After ammonia treatment, DMTr-on oligomers were purified on a RP Pours column followed by 10% acetic acid treatment and 2 water/ether extractions to remove trityl residue. Oligomers were further purified by 15% denaturing PAGE followed by electroelution and ethanol precipitation. To test purity, the oligomers were ³²P-end-labeled and analyzed by 20% denaturing PAGE. Typical primer extension reactions were carried out in 20 μ l volume in a heated lid thermocycler. Buffers conditions, triphosphate concentrations, and temperatures are all listed in the figure captions.

Oligonucleotide primers were ^{32}P -end-labeled using T4 PNK (New England Biolabs, Beverley, MA) and $\gamma\text{P}^{32}\text{ATP}$ (MP, Irvine, CA) for 1 h at 37°C followed by heat denaturing at 95°C for 15 min. Excess $\gamma\text{P}^{32}\text{ATP}$ was removed using Micro-Spin G-25 size exclusion columns (Amersham-Bioscience, Buckinghamshire, England) and quantitated by UV. Primer and template were annealed by heating to 95°C followed by slow cooling to room temperature. Polymerase reactions each contained 250 nM duplex DNA. Reactions were initiated after 5 min of preheating by the addition of a 2X NTP solution to the annealed primer/template and polymerase. Reactions were quenched using stop buffer (8M urea, 100 mM EDTA, Bromophenol Blue, and Xylene Cyanol) and analyzed by 20% (19:1) denaturing PAGE. Gel imaging was done using Kodak-K radioisotope screen and a Bio-Rad FX molecular imager.

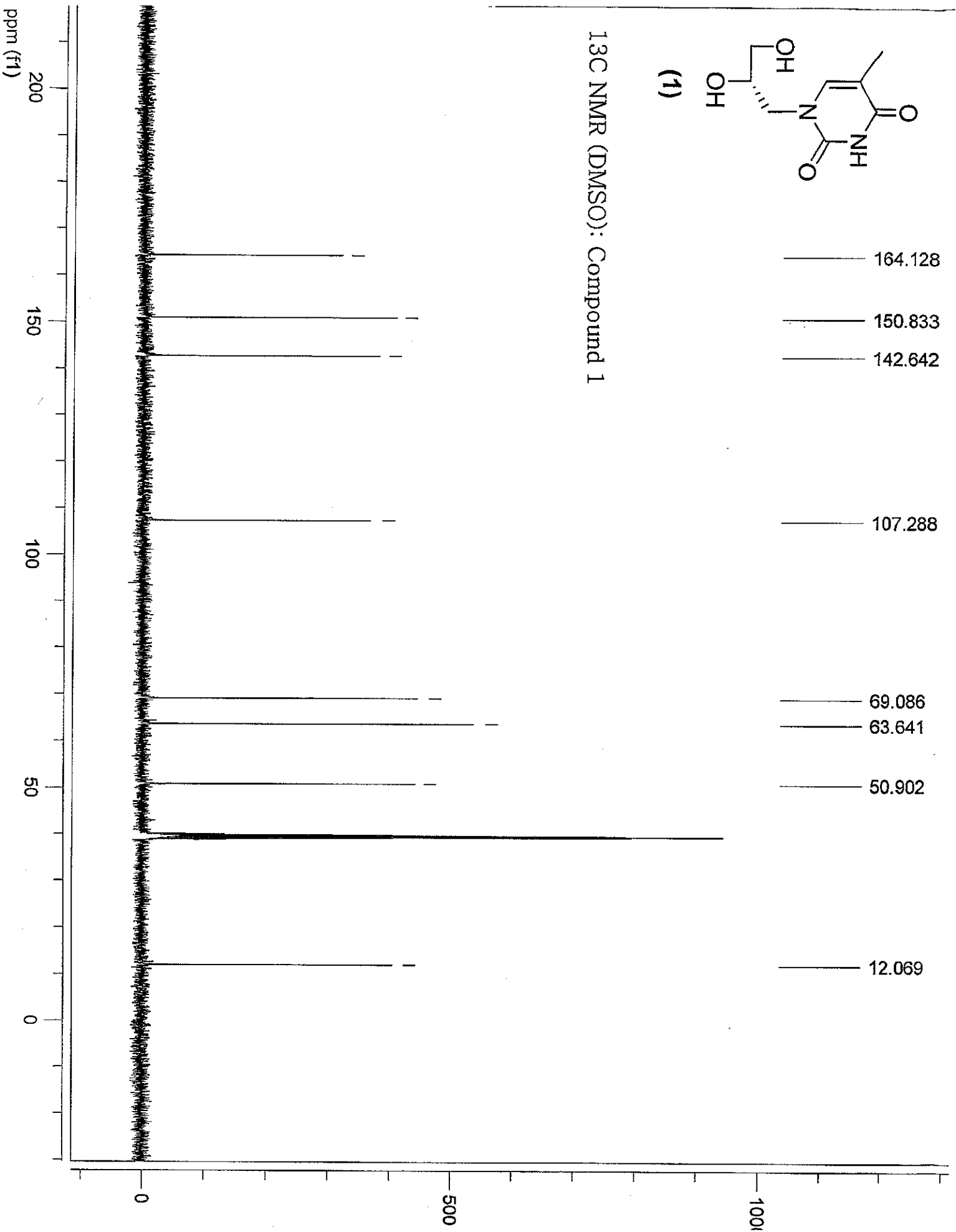


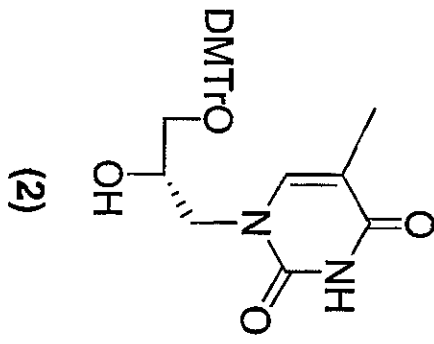
¹H NMR (DMSO): Compound 1



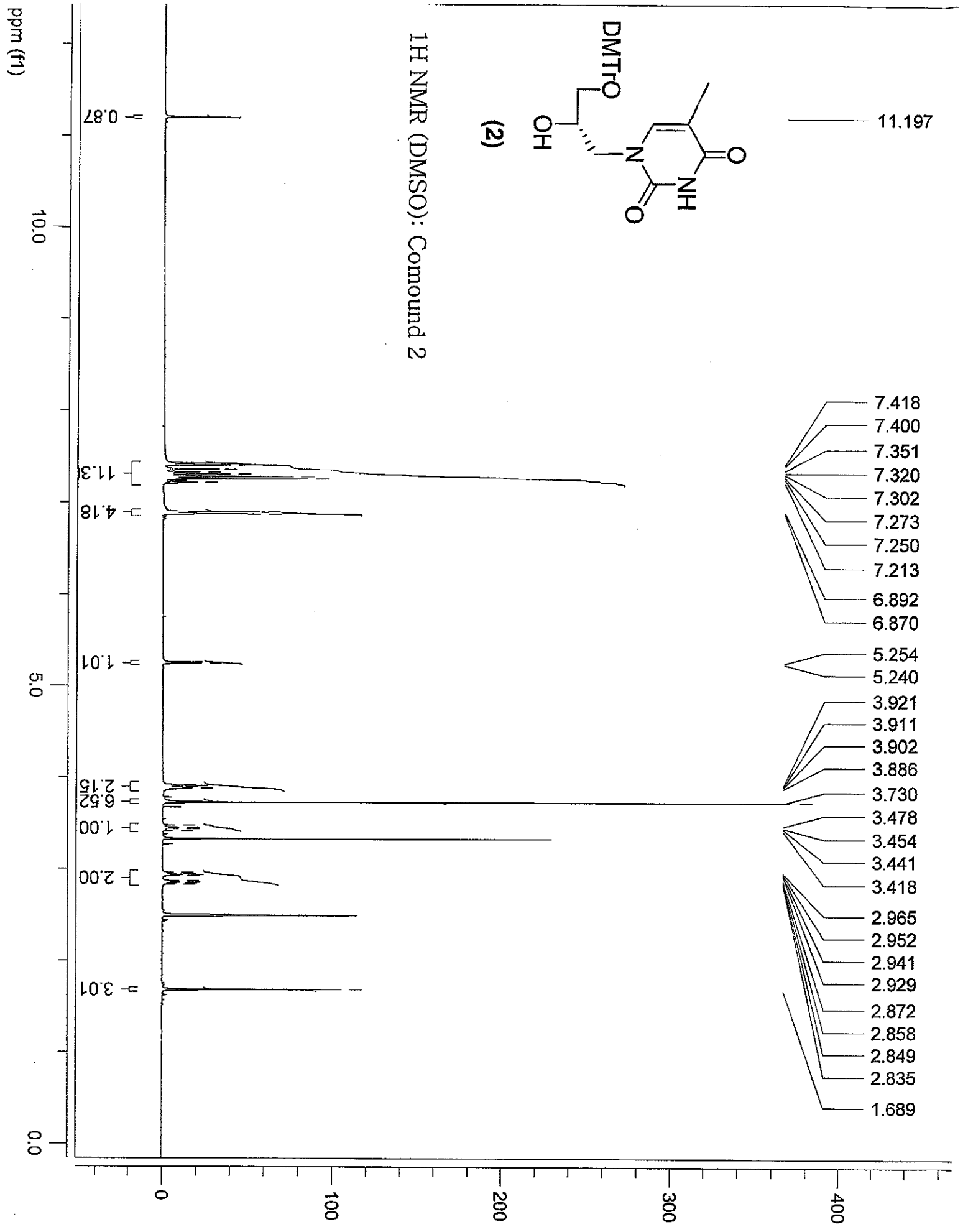


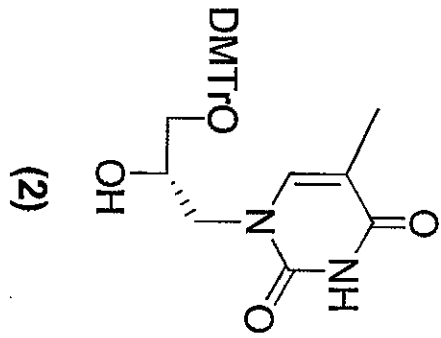
¹³C NMR (DMSO): Compound 1



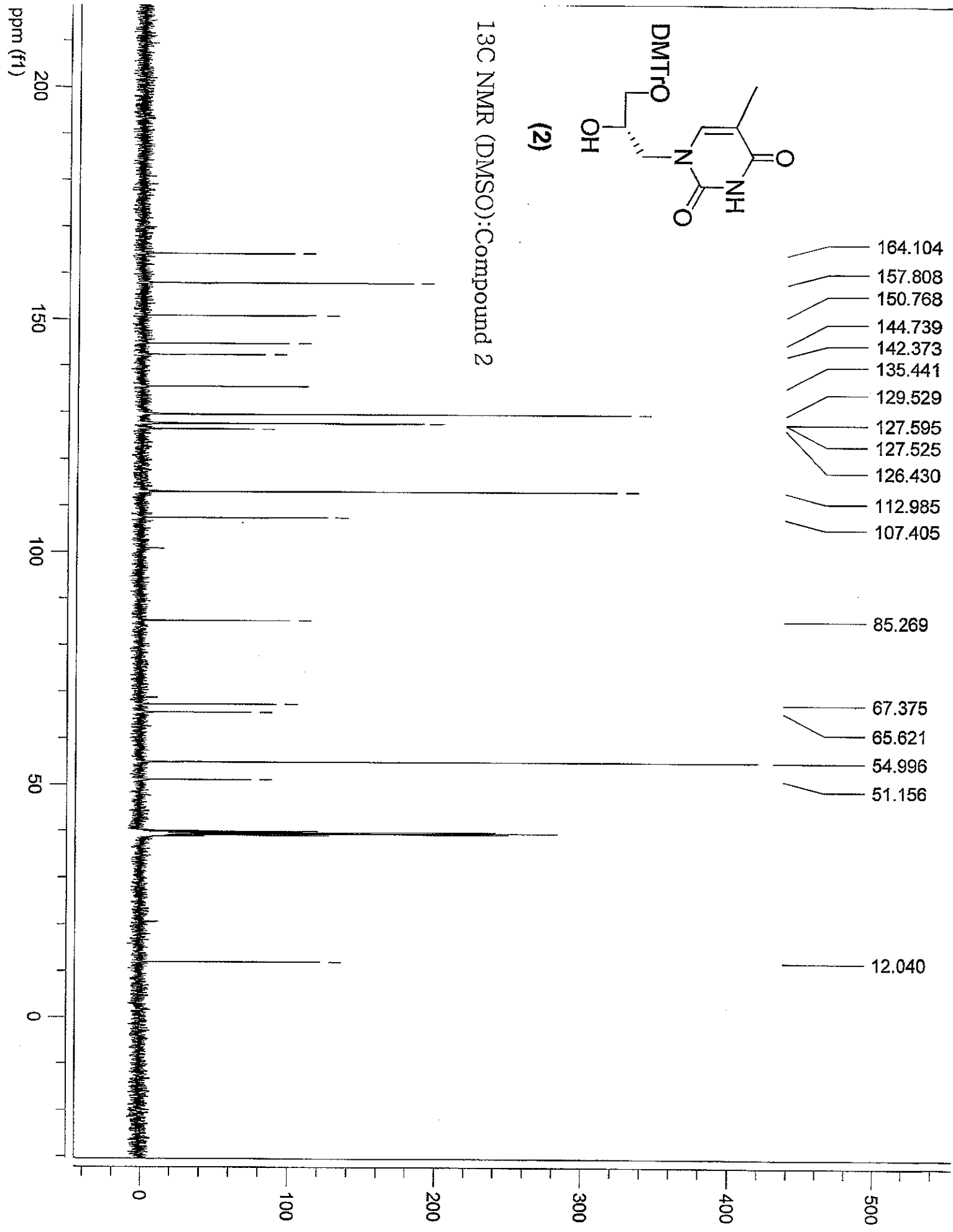


¹H NMR (DMSO): Compound 2





¹³C NMR (DMSO):Compound 2



ppm (f1)

10.0

5.0

0.0

0.84

3.87
3.84
4.19

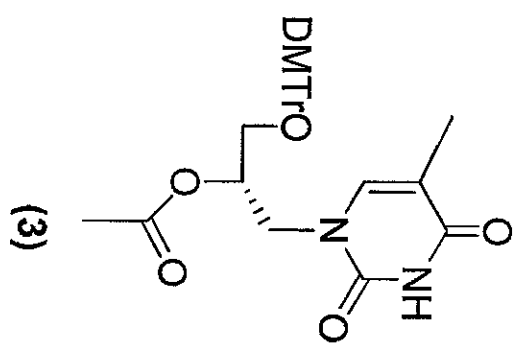
0.98

1.04
6.58

3.32
2.03

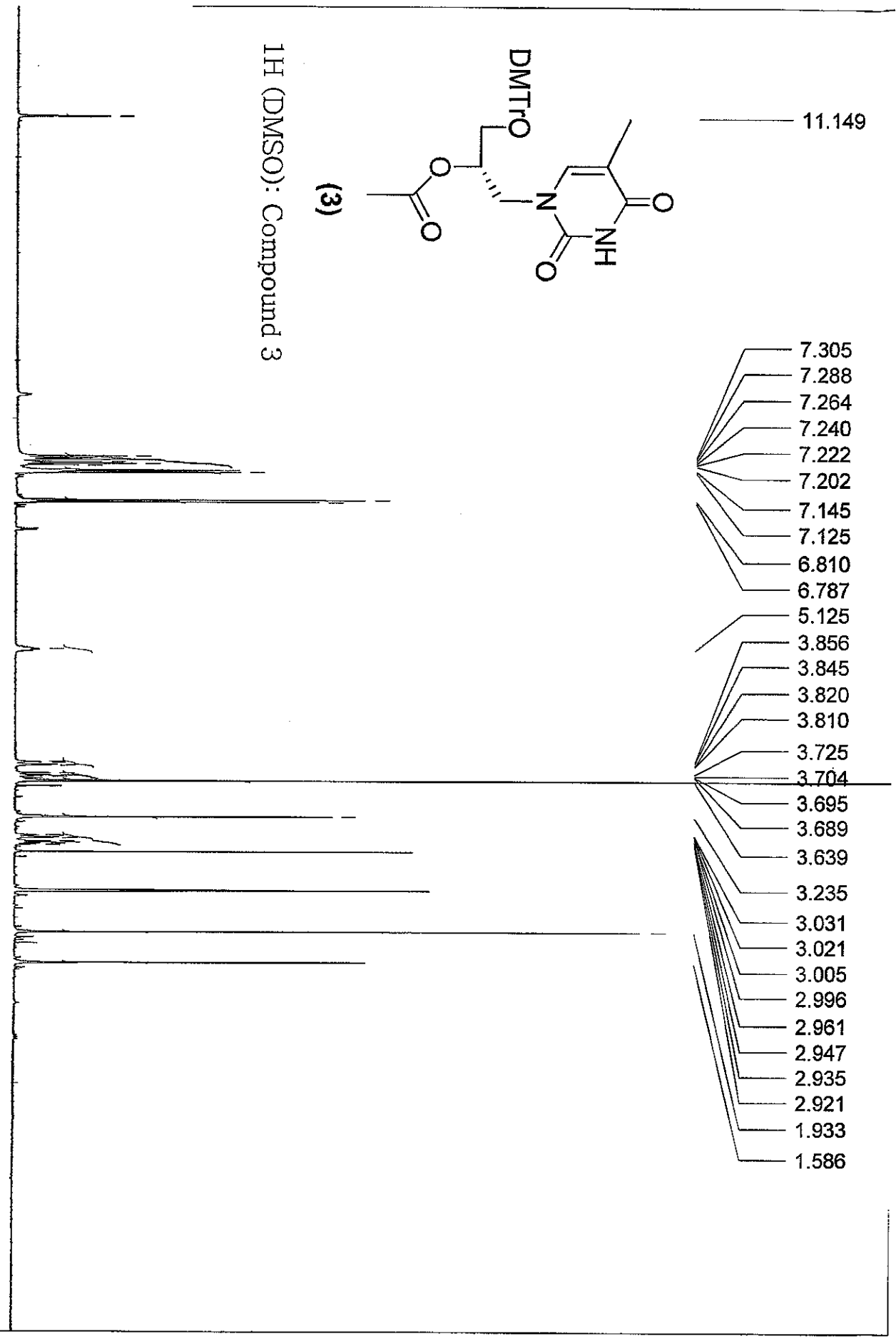
3.00
3.00

¹H (DMSO): Compound 3



11.149

- 7.305
- 7.288
- 7.264
- 7.240
- 7.222
- 7.202
- 7.145
- 7.125
- 6.810
- 6.787
- 5.125
- 3.856
- 3.845
- 3.820
- 3.810
- 3.725
- 3.704
- 3.695
- 3.689
- 3.639
- 3.235
- 3.031
- 3.021
- 3.005
- 2.996
- 2.961
- 2.947
- 2.935
- 2.921
- 1.933
- 1.586

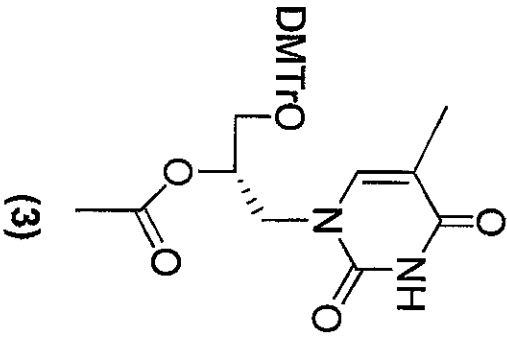


50

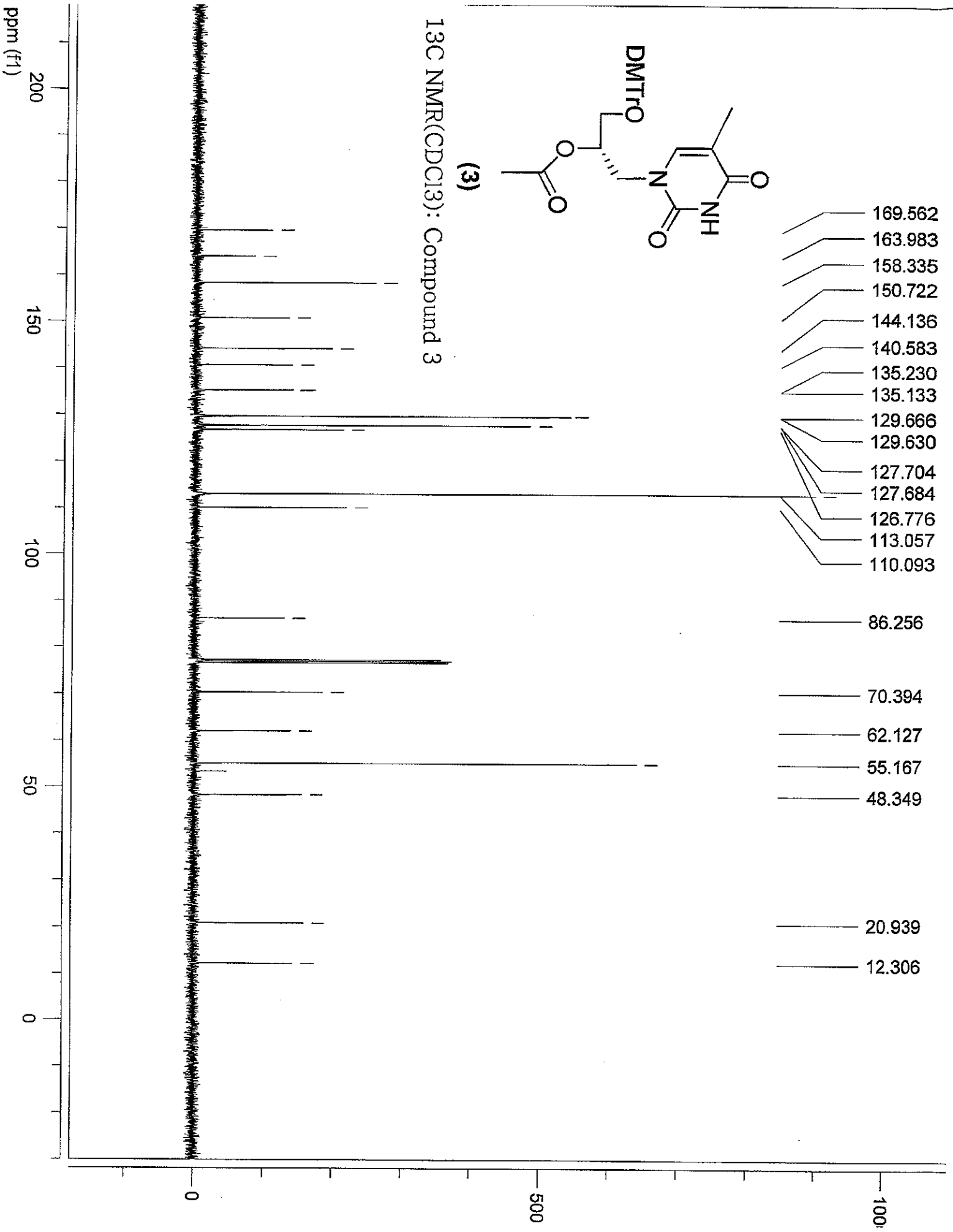
100

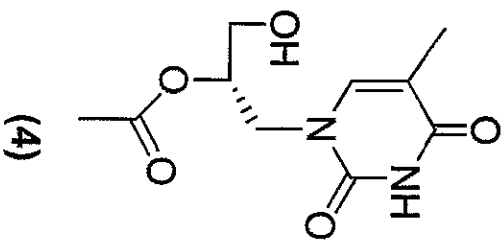
150

200

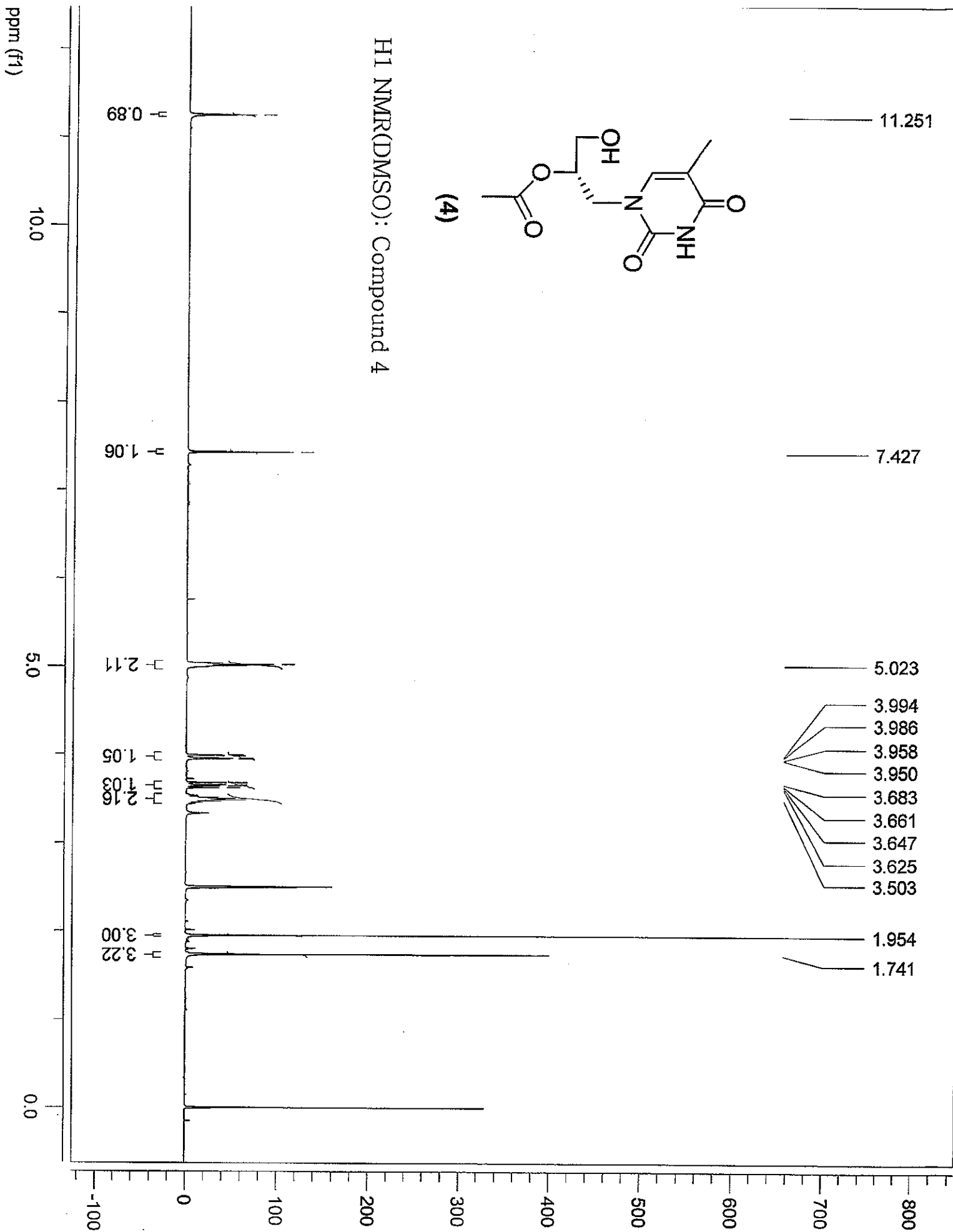


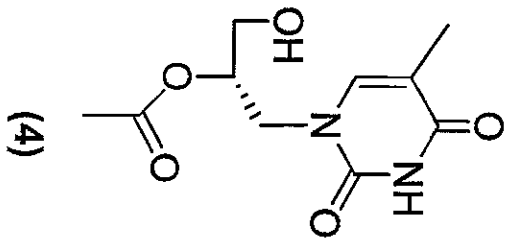
^{13}C NMR(CDCl_3): Compound 3



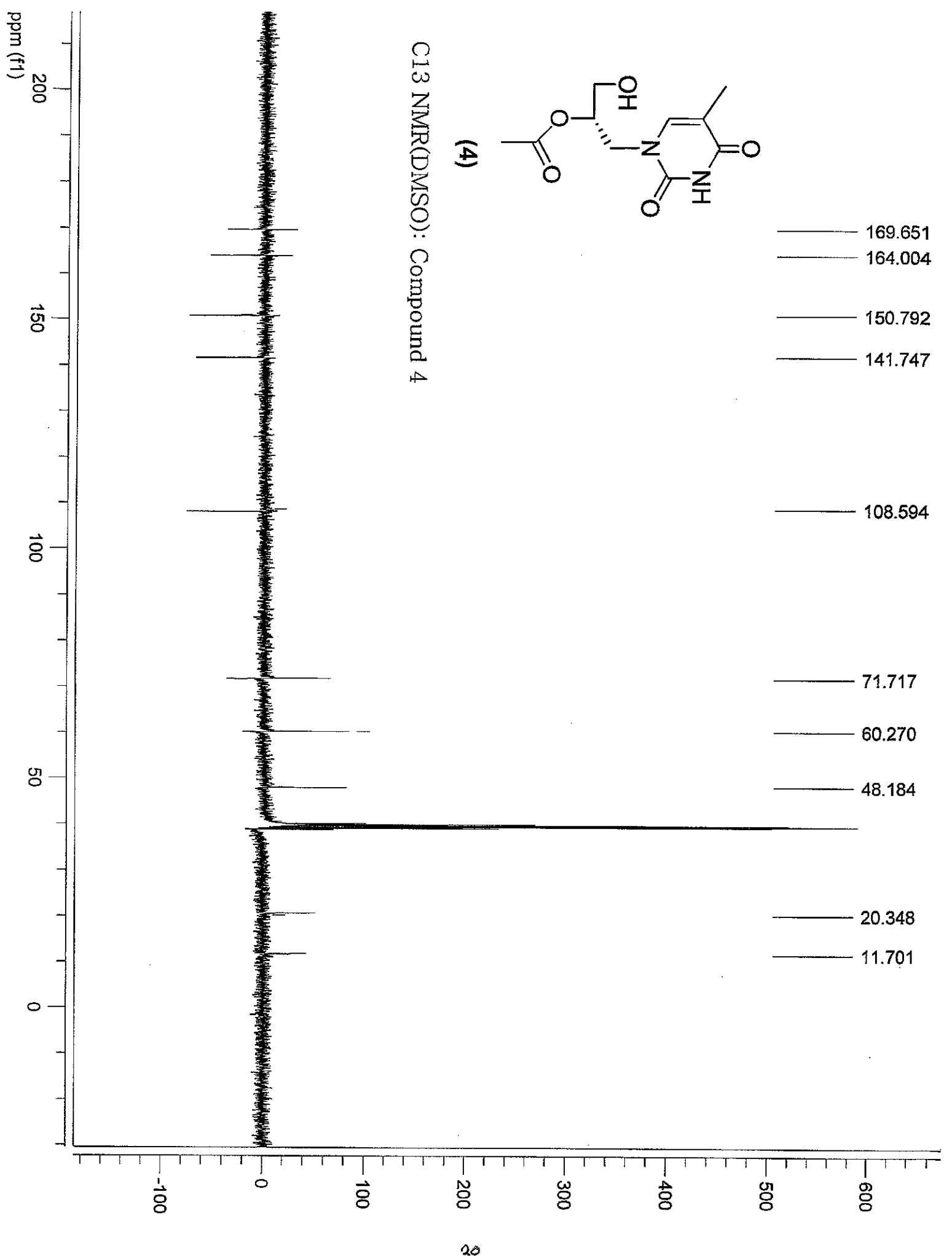


H1 NMR(DMSO): Compound 4



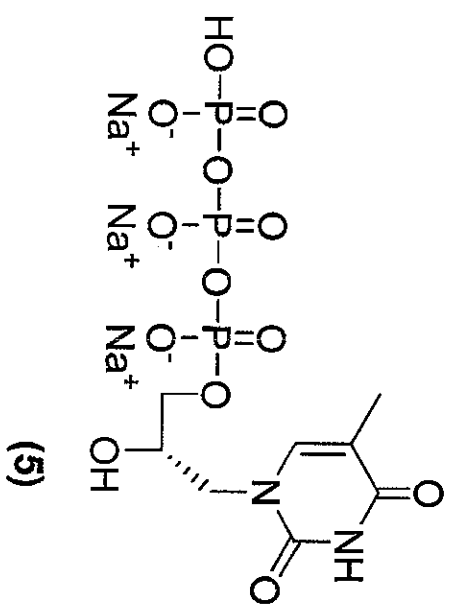


C13 NMR(DMSO): Compound 4

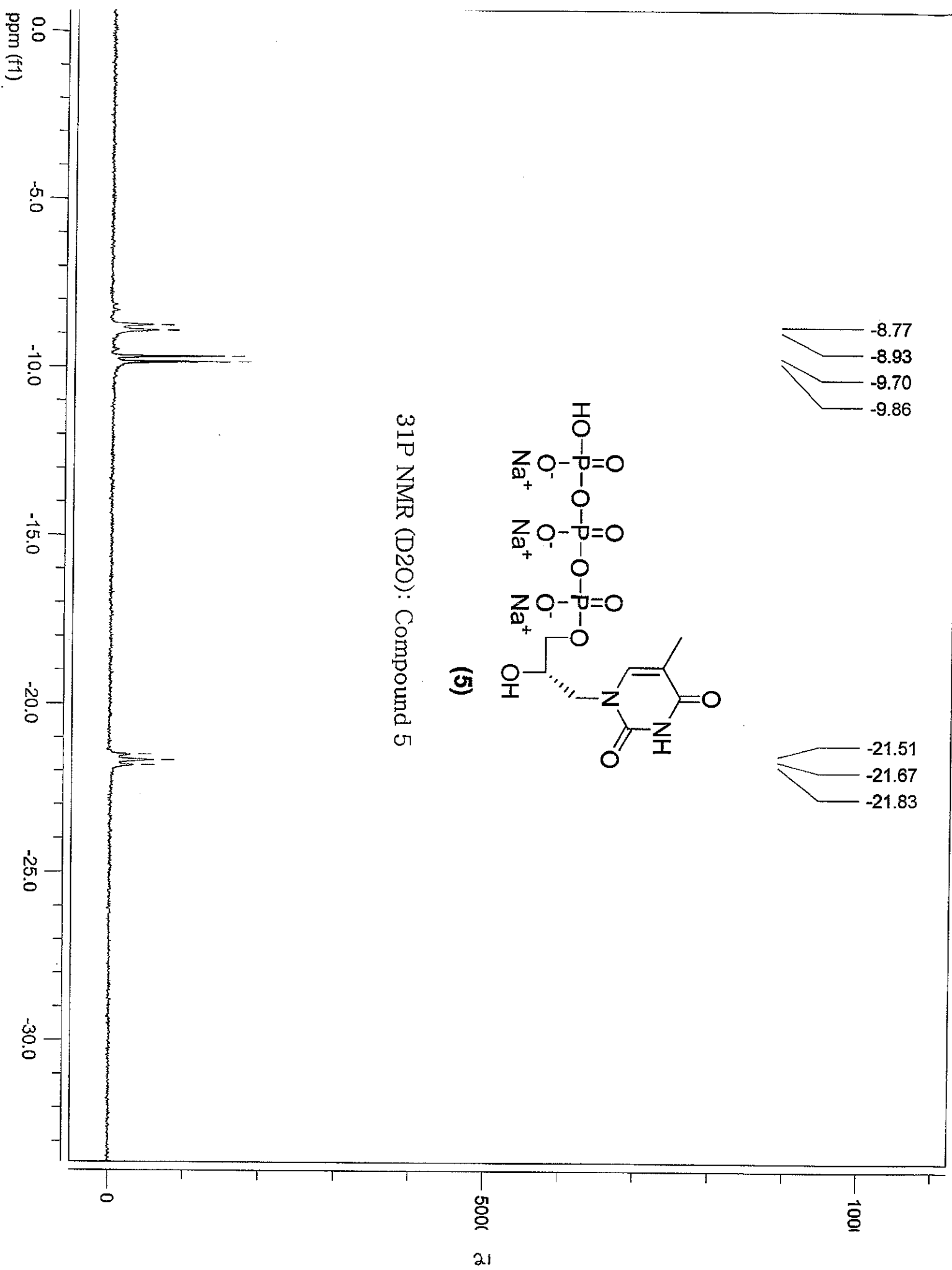


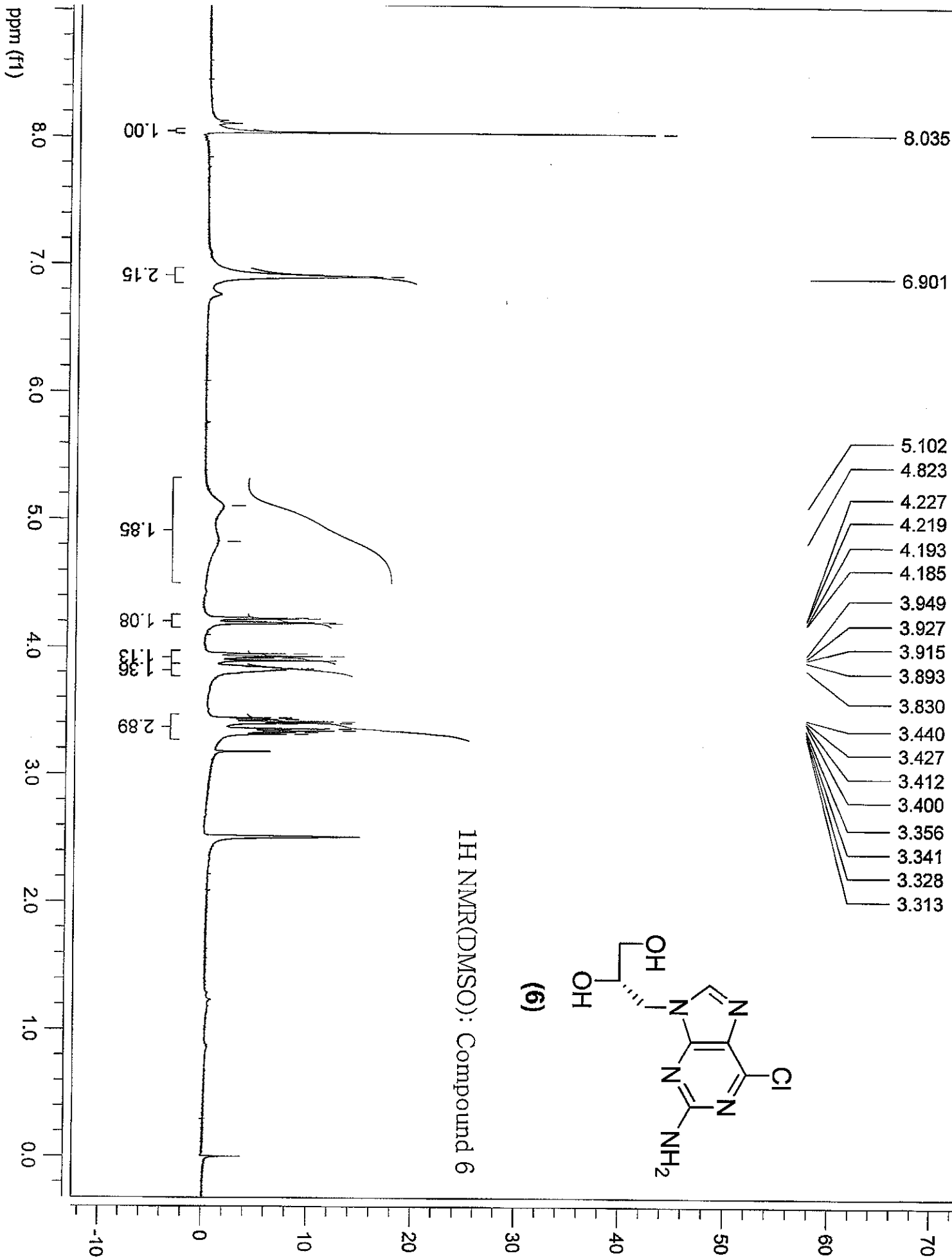
-8.77
-8.93
-9.70
-9.86

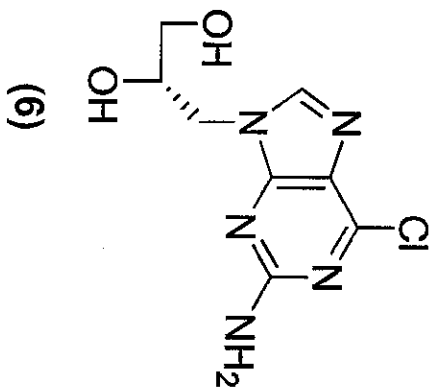
-21.51
-21.67
-21.83



31P NMR (D2O): Compound 5

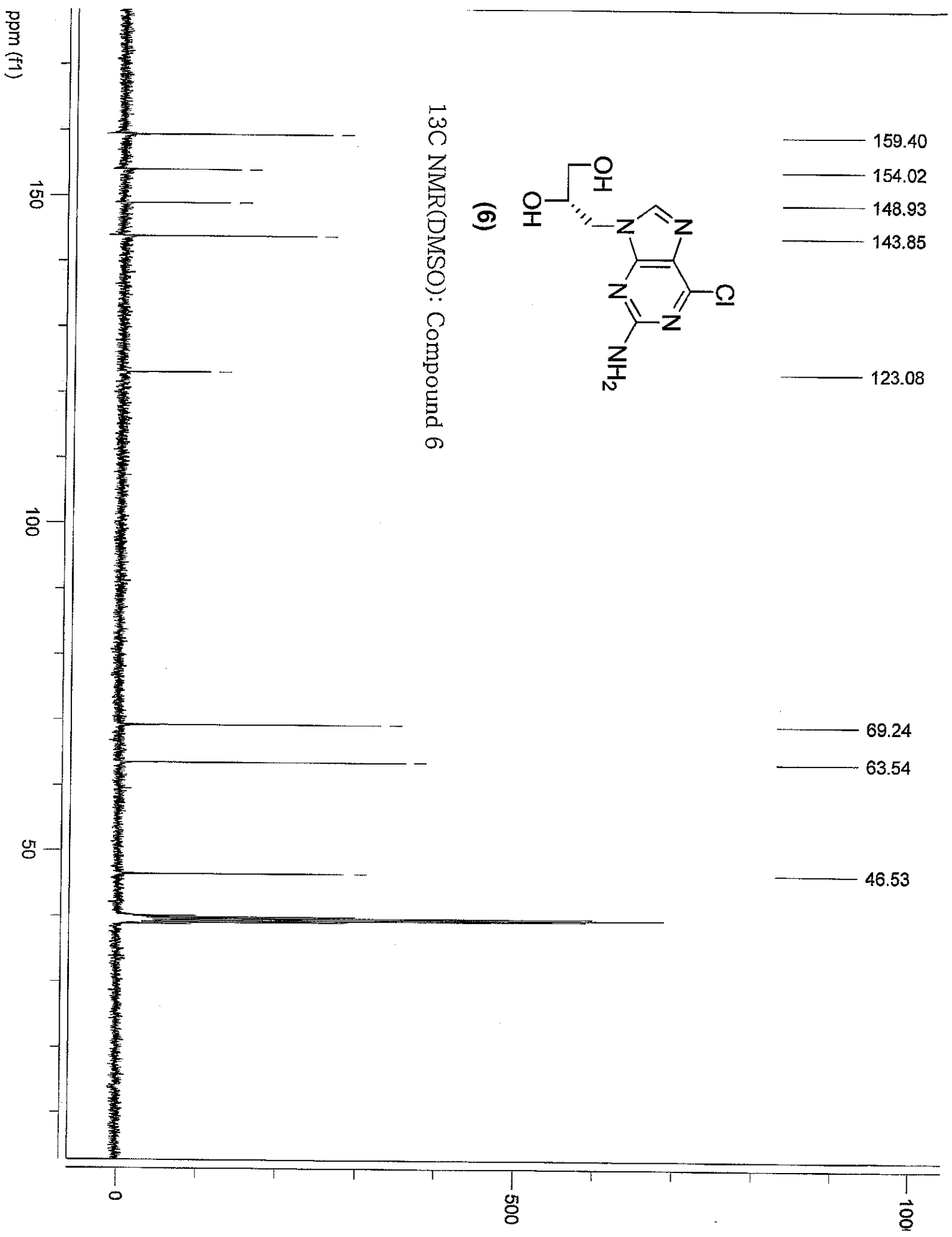


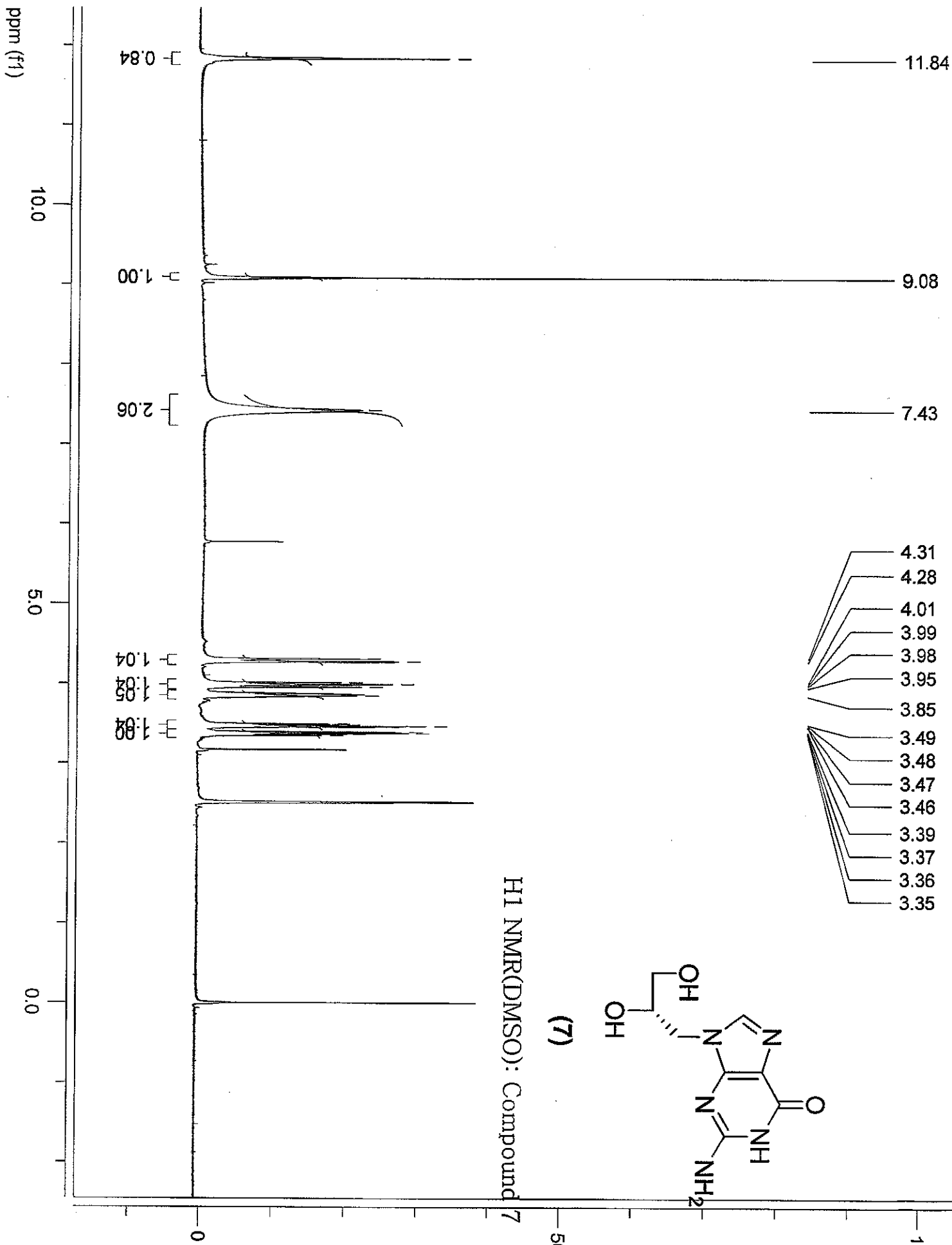




- 159.40
- 154.02
- 148.93
- 143.85
- 123.08
- 69.24
- 63.54
- 46.53

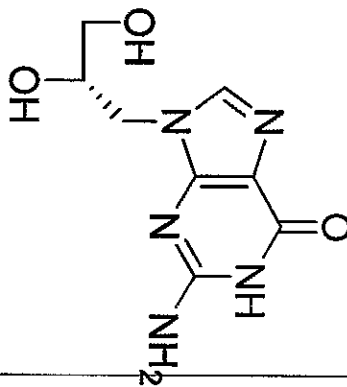
¹³C NMR(DMSO): Compound 6

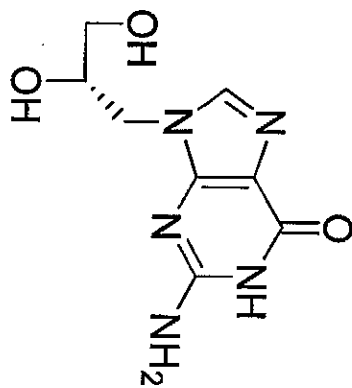




¹H NMR(DMSO): Compound 7

(7)





(7)

C13 NMR(DMSO): Compound 7

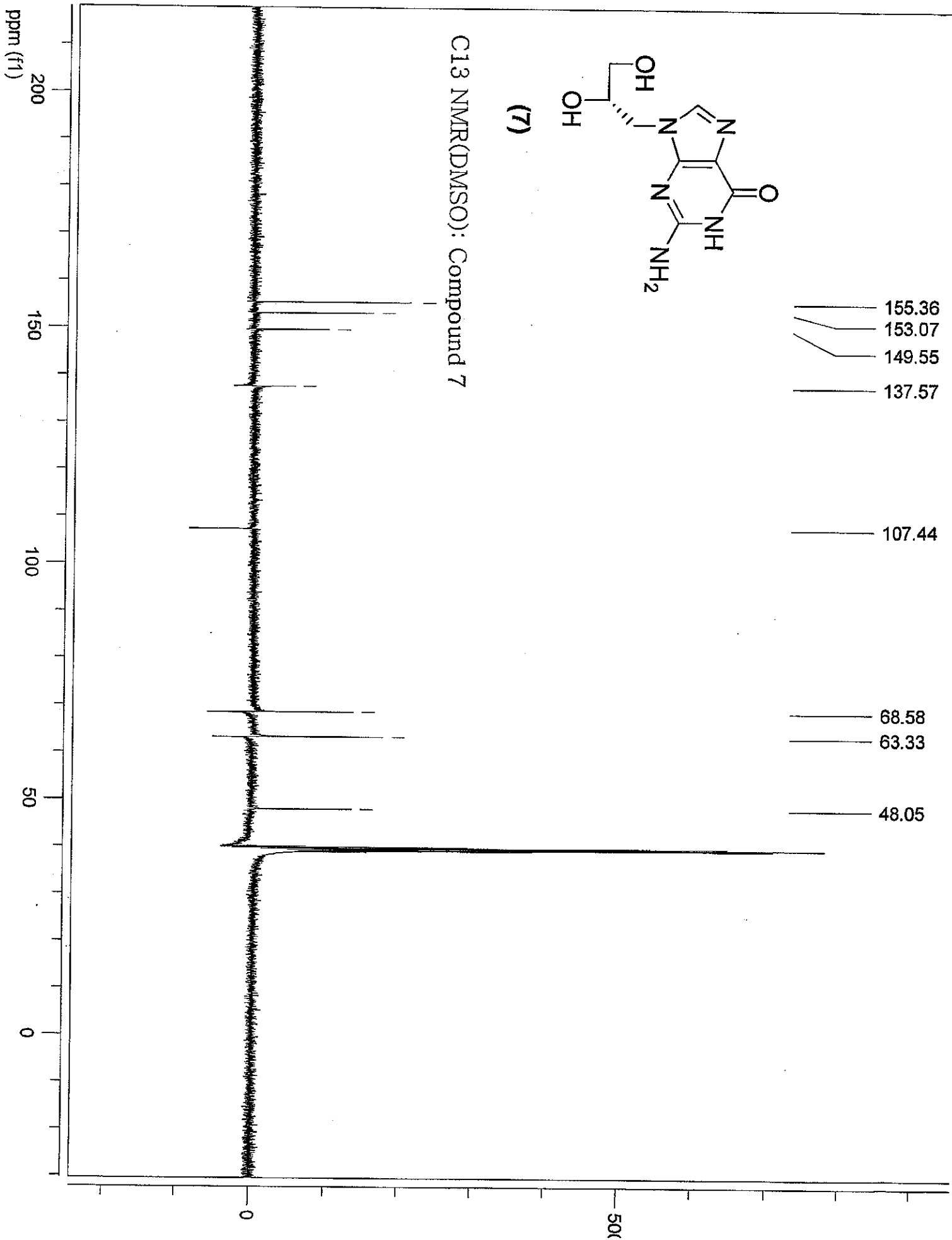
155.36
153.07
149.55
137.57

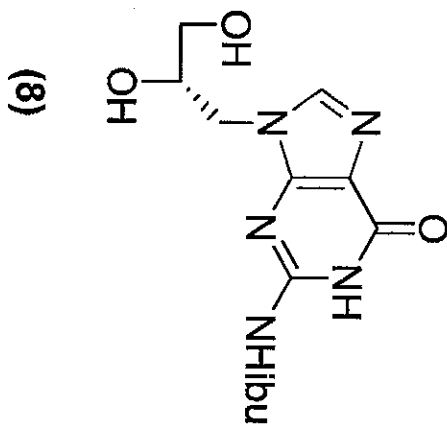
107.44

68.58

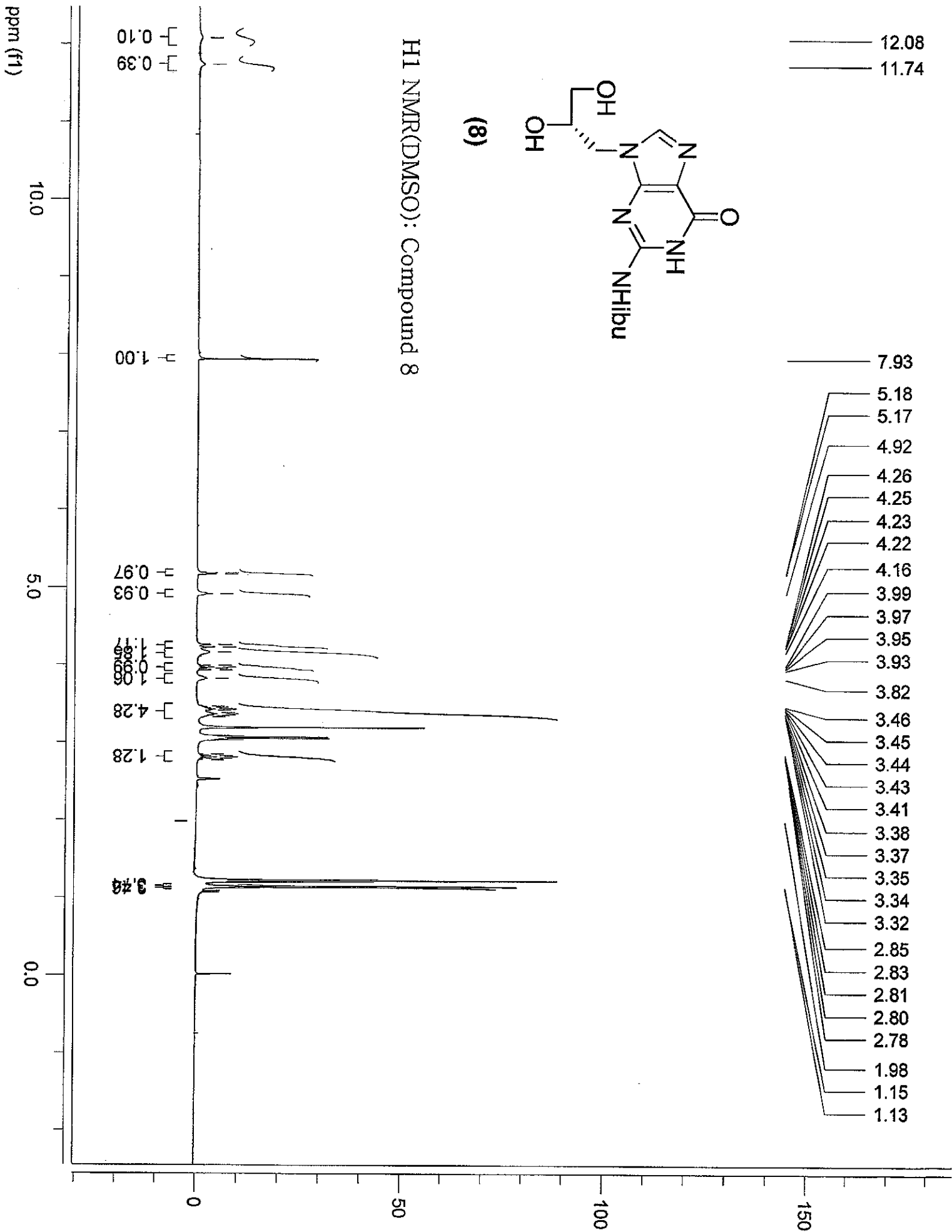
63.33

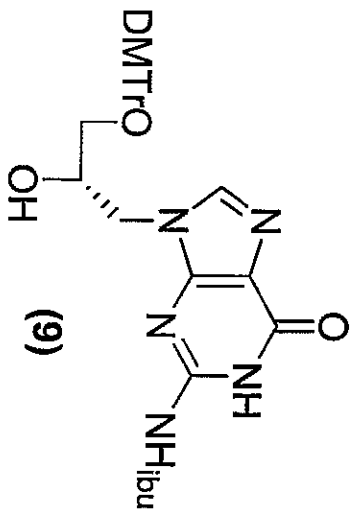
48.05



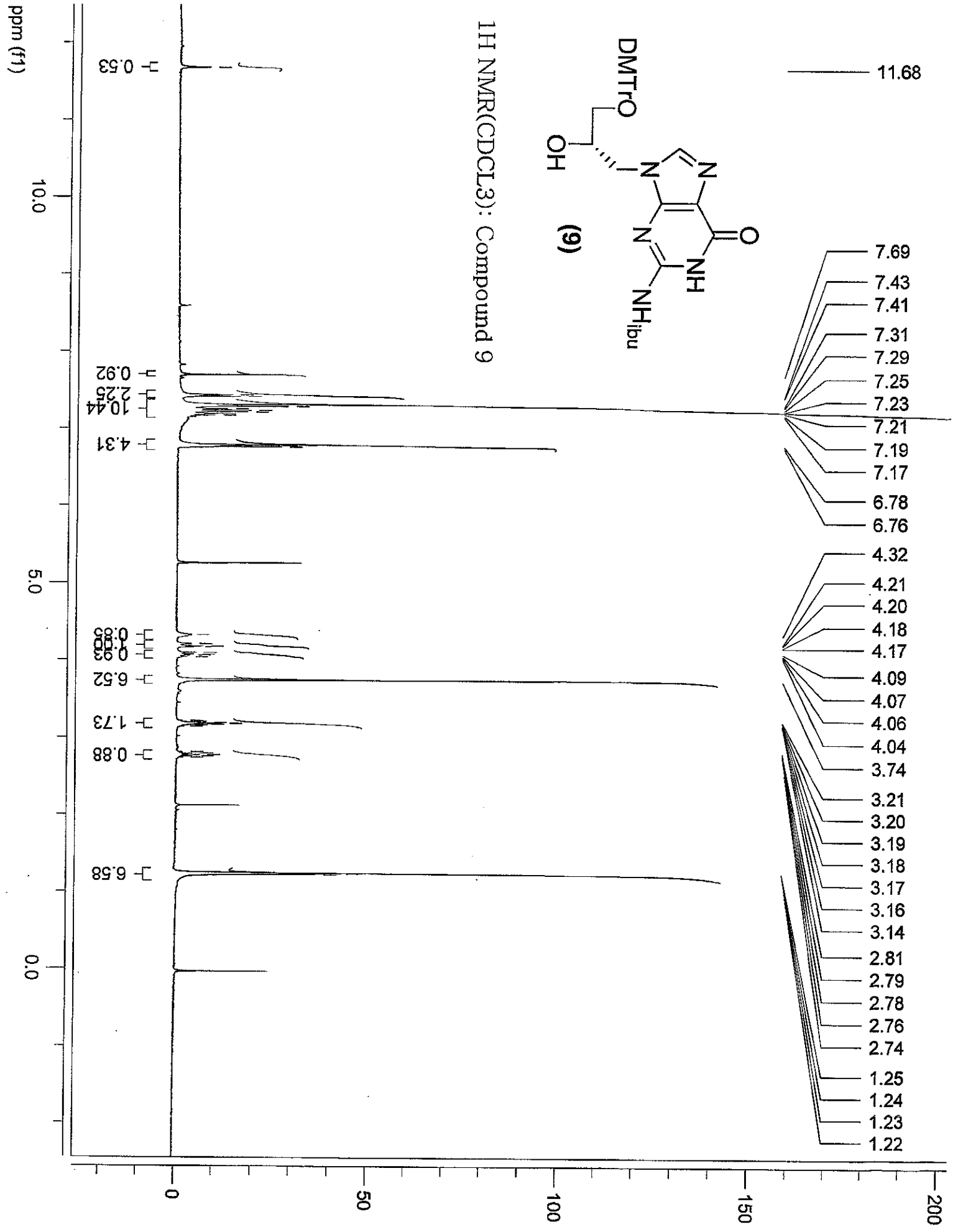


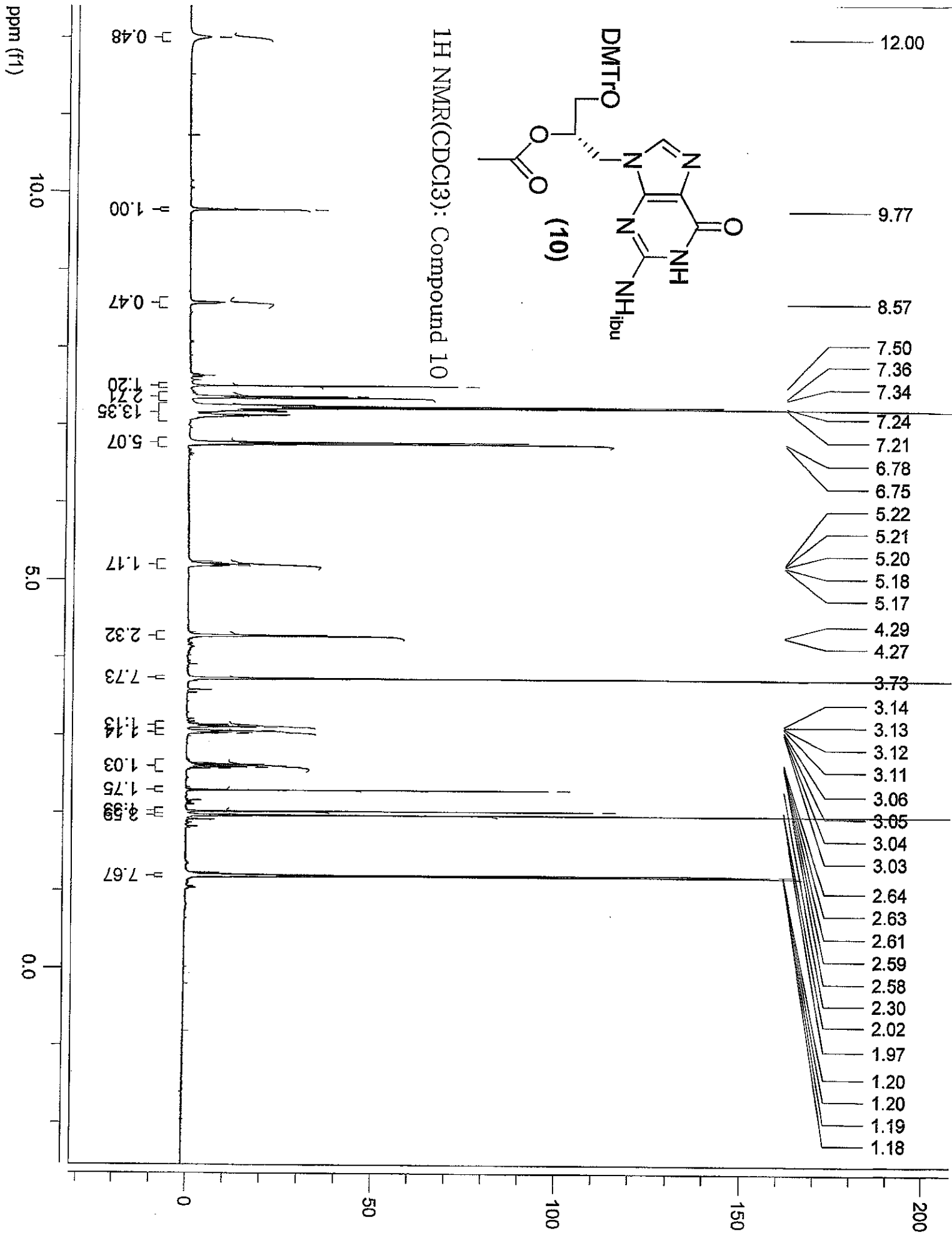
H1 NMR(DMSO): Compound 8

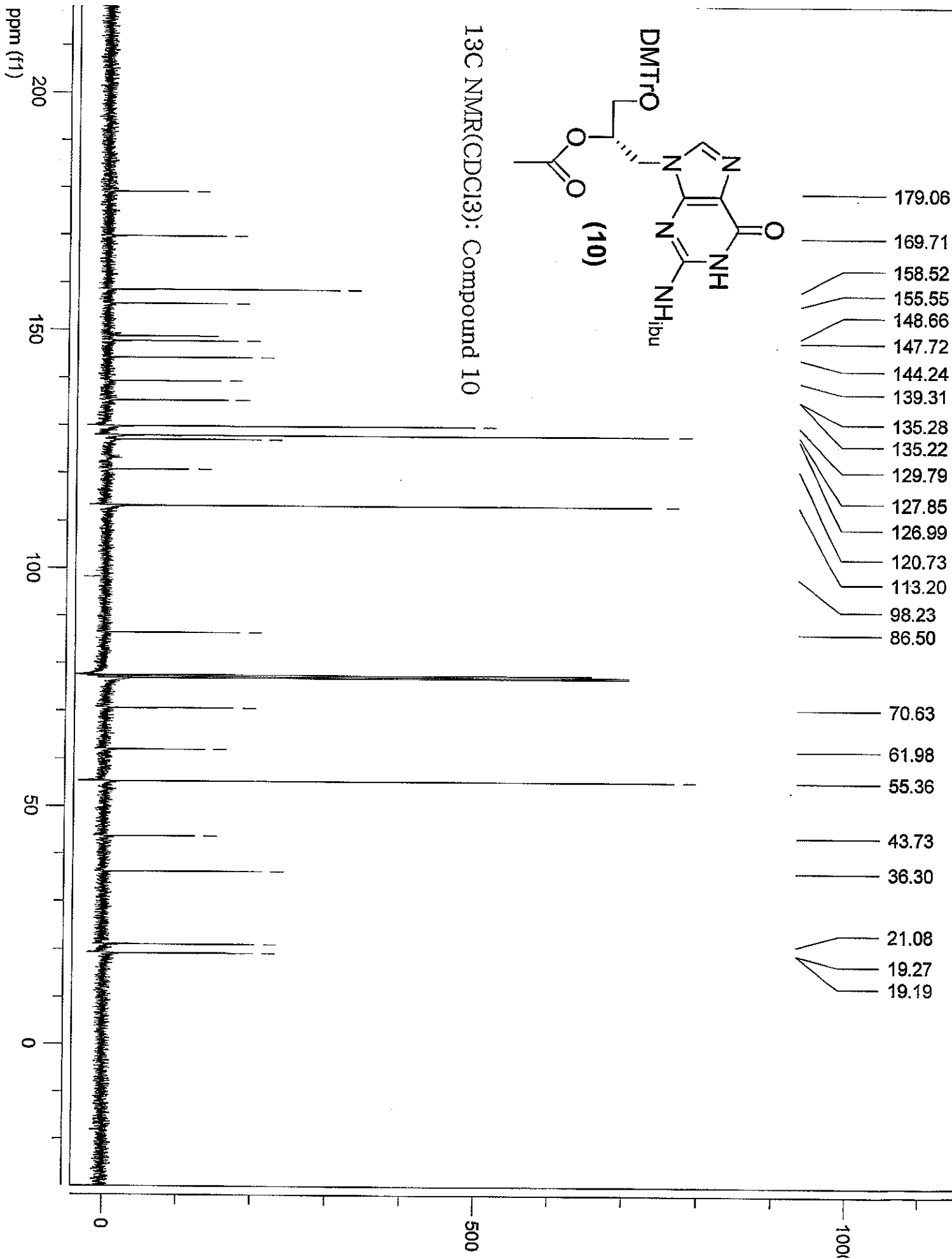


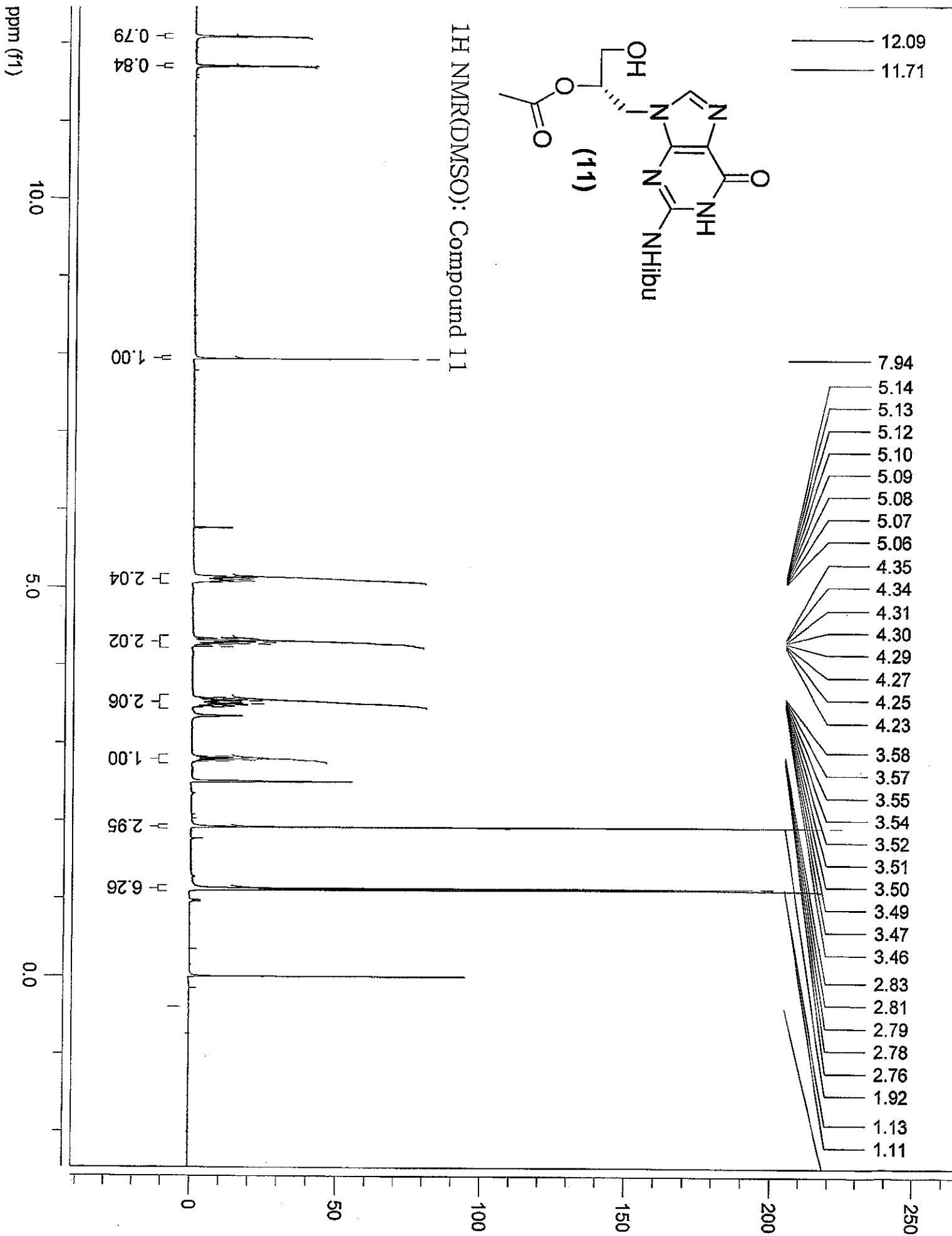


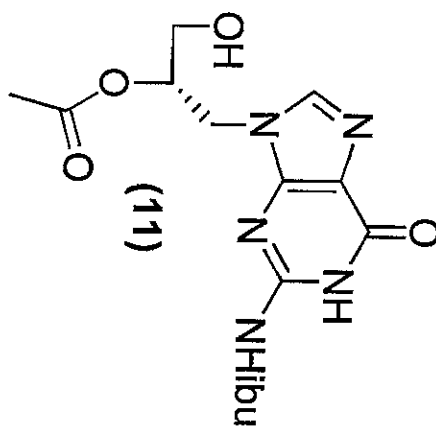
¹H NMR(CDCl₃): Compound 9



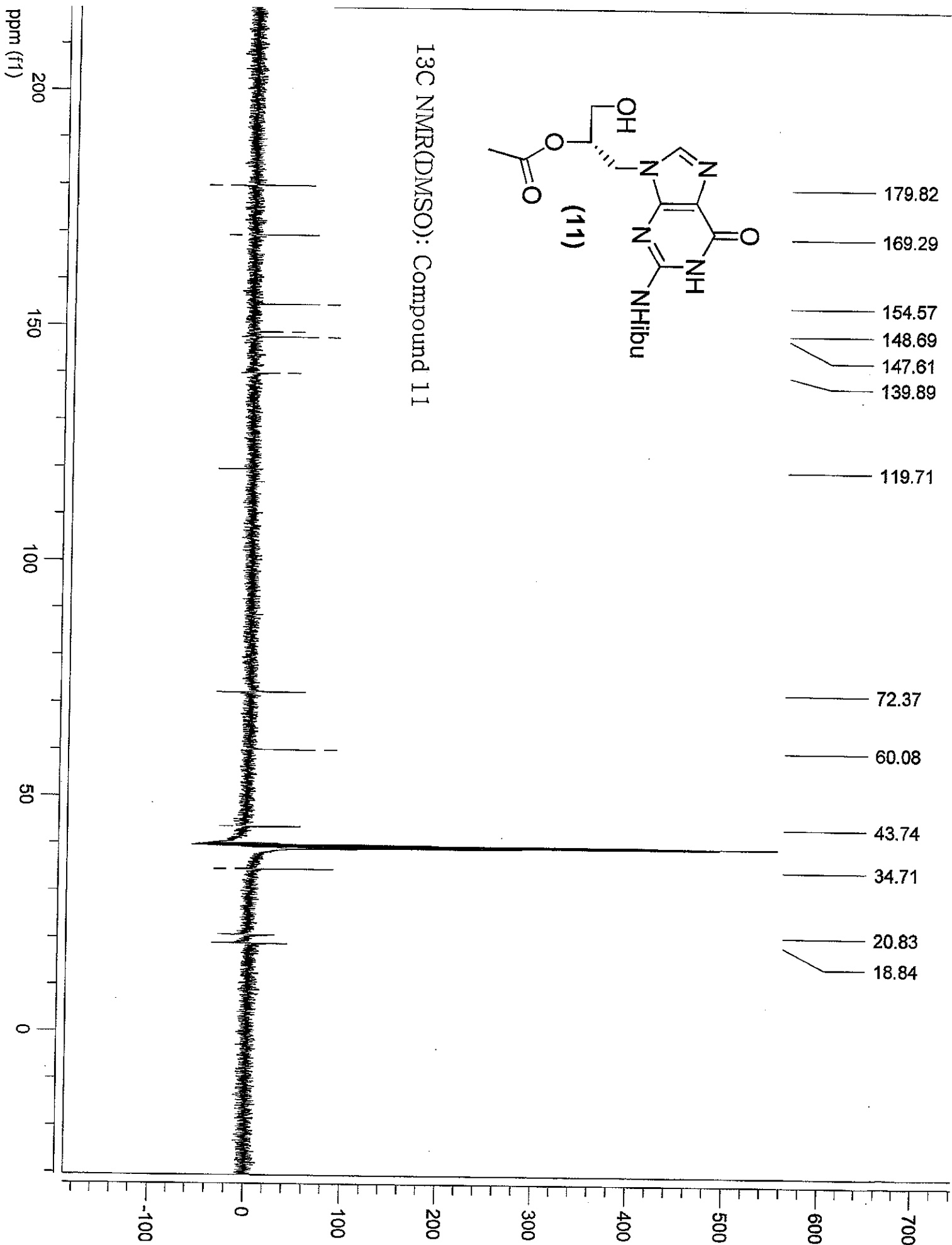


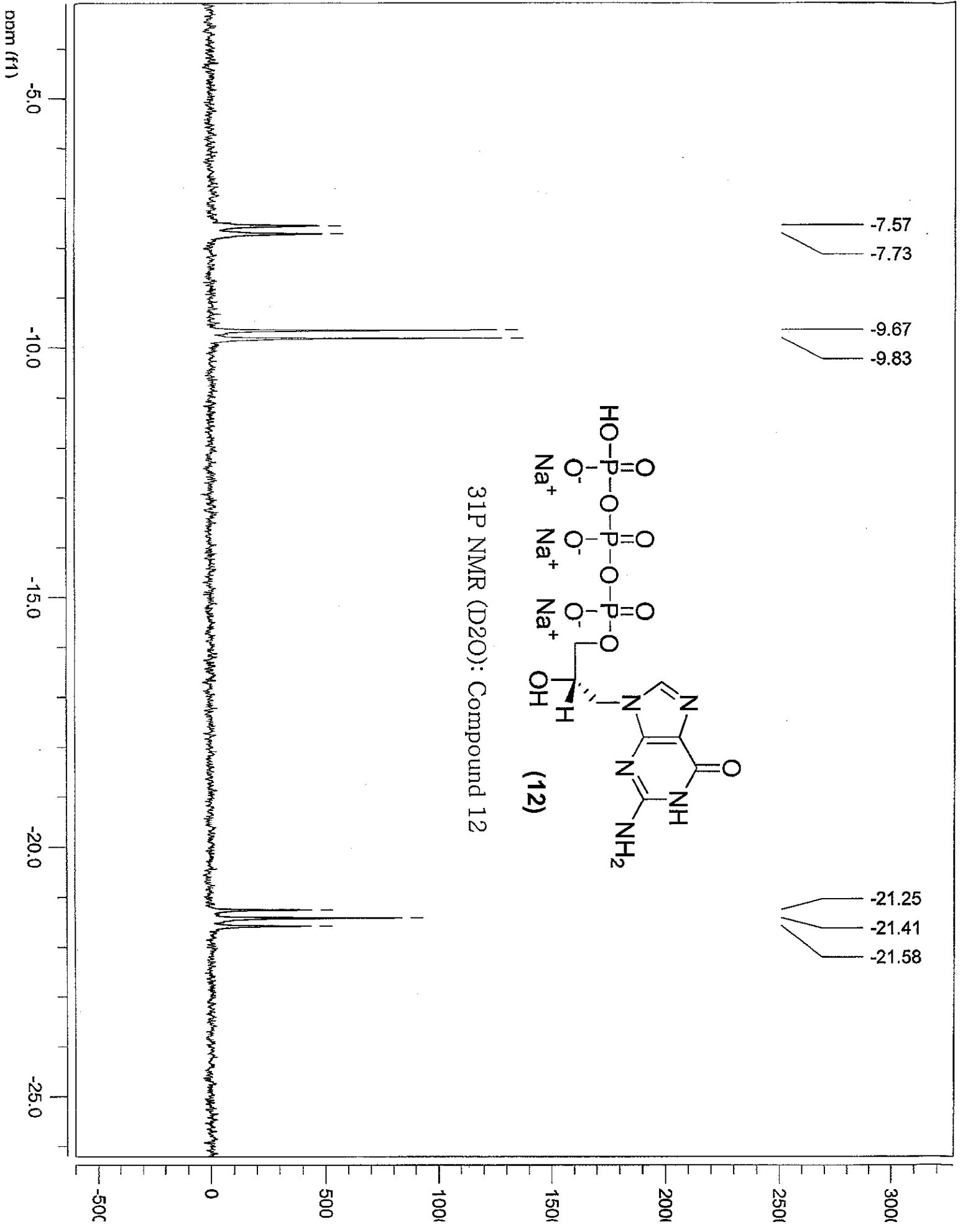






¹³C NMR(DMSO): Compound 11





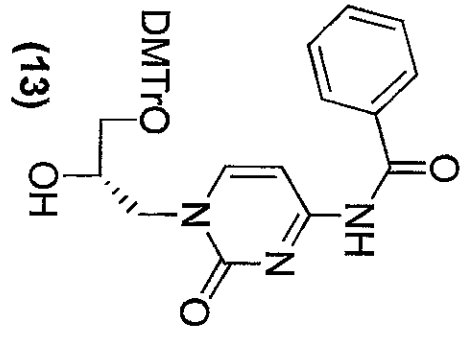
ppm (f1)

5.0

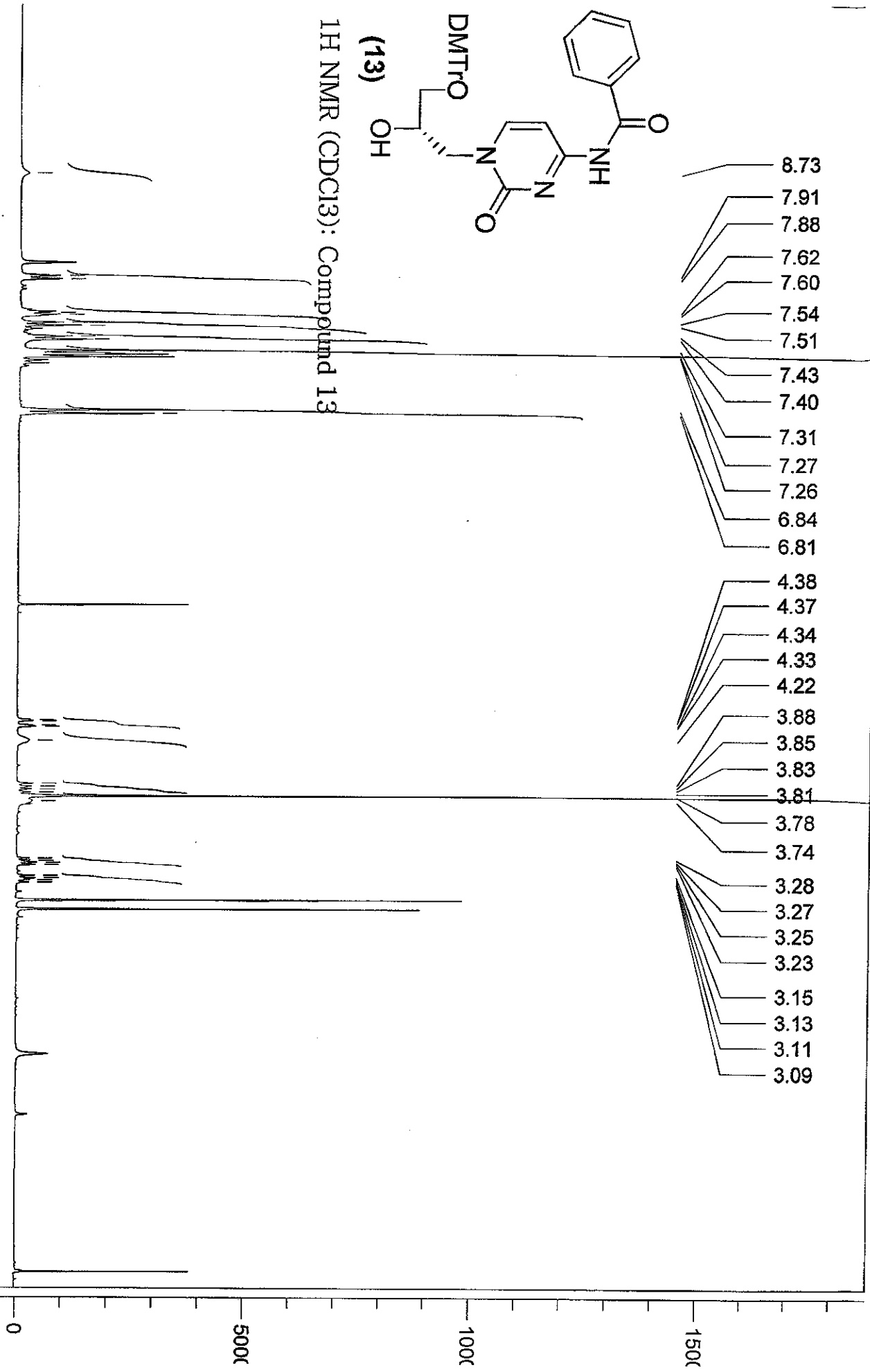
0.0

0.72
2.11
2.26
2.33
2.50
2.54
8.94
4.49
1.06
1.06
1.07
8.85
1.02
1.03

1H NMR (CDCl3): Compound 13

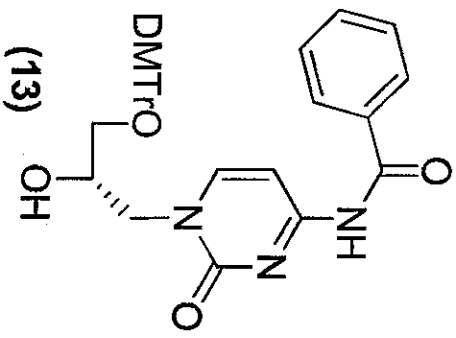


8.73
7.91
7.88
7.62
7.60
7.54
7.51
7.43
7.40
7.31
7.27
7.26
6.84
6.81
4.38
4.37
4.34
4.33
4.22
3.88
3.85
3.83
3.81
3.78
3.74
3.28
3.27
3.25
3.23
3.15
3.13
3.11
3.09



ppm (t1)

¹³C NMR (CDCl₃): Compound 13



- 166.43
- 162.19
- 158.15
- 156.46
- 150.20
- 144.32
- 135.40
- 132.78
- 132.66
- 129.70
- 128.54
- 127.74
- 127.59
- 127.51
- 126.56
- 112.93
- 96.31
- 86.03
- 68.38
- 64.62
- 55.06
- 54.38
- 53.38

150

100

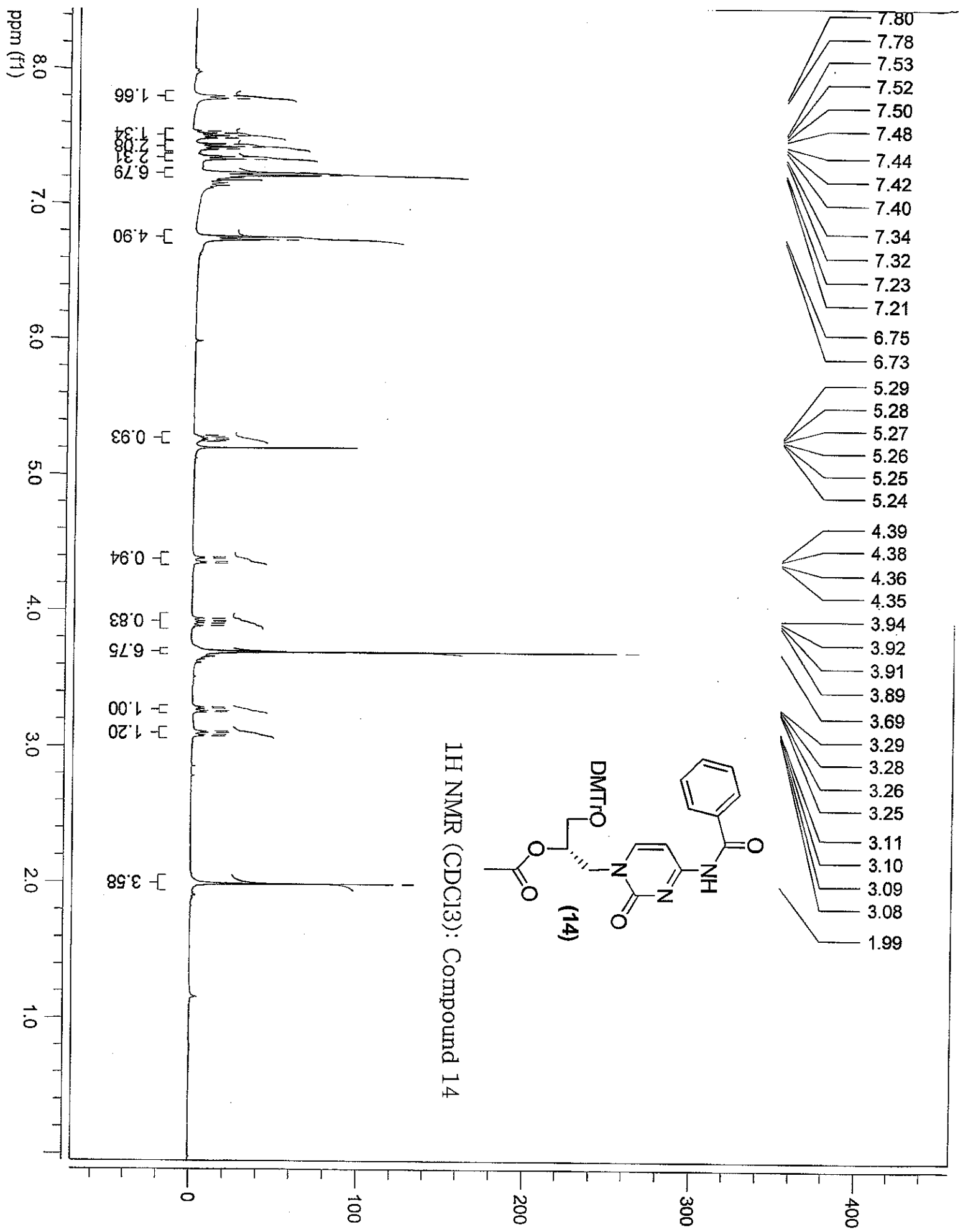
50

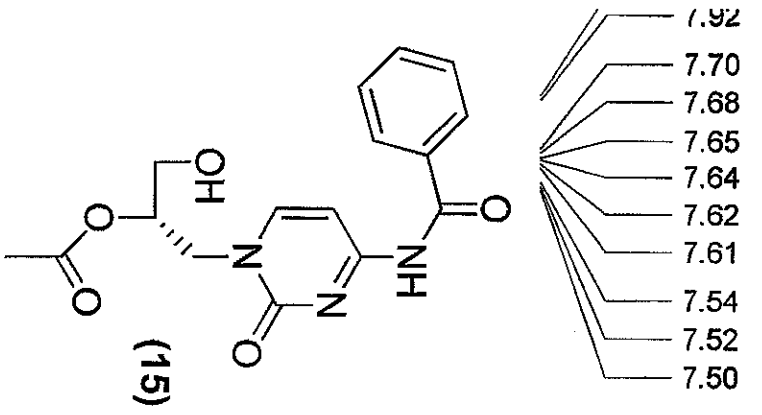
0

500

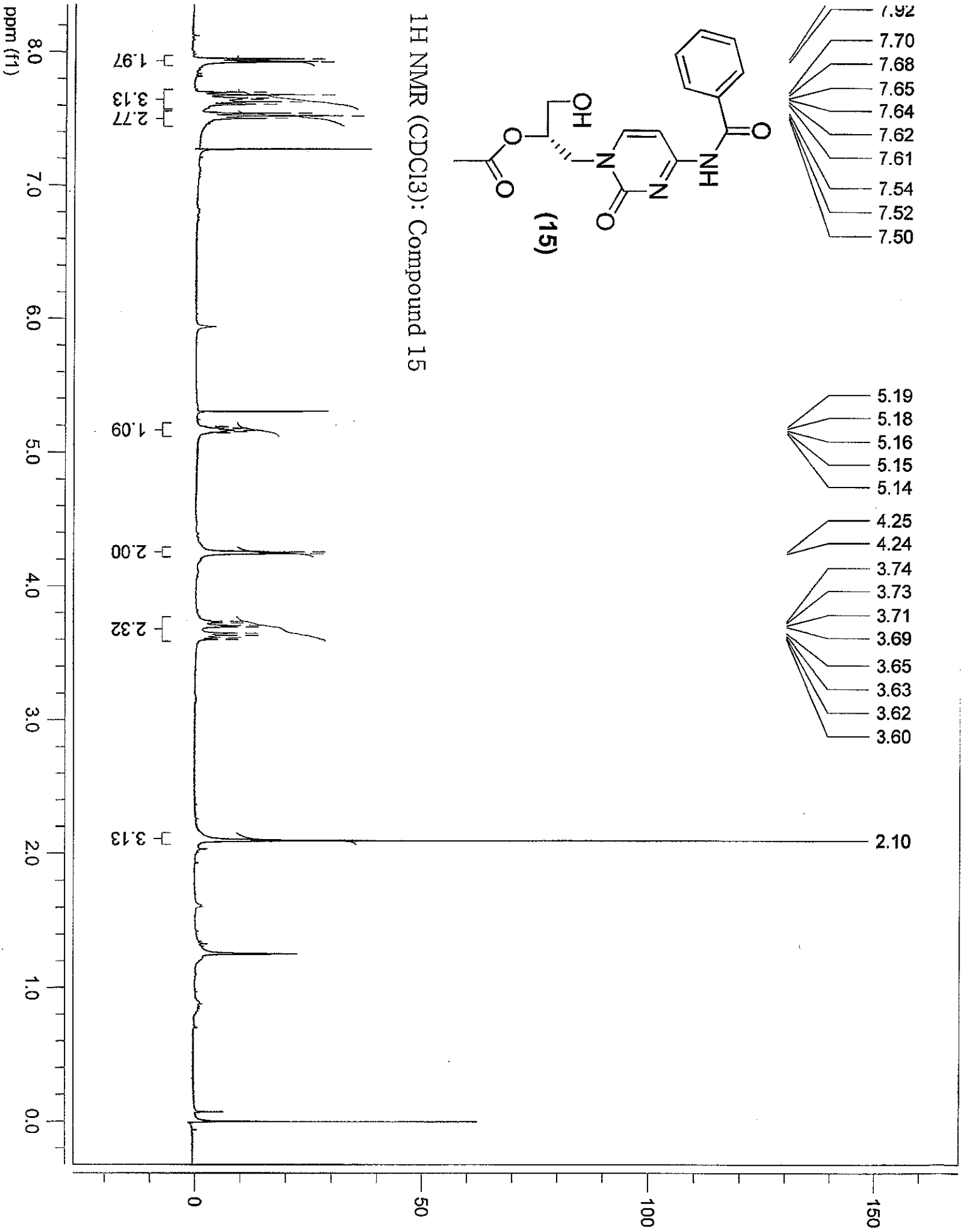
1000

1500





¹H NMR (CDCl₃): Compound 15



ethylated benzoyl c glycidol s
pl stable

SAMPLE NAME DEC-4-VT 180.088
1c Hcr 21 2986 dfrq do H1
1 VENT 1 QDC13 do H1
ACQUISITION Exp 100.895 dm 0.8
1 013 dm JYY V
59966 dm PROCESSING 1.06
23081.8 18
13880 WFT18 not used
7.0 WFT 79
4.099 WFT not used
1000 WFT
150 WFT
no ph 1.009

DISPLAY

1778.1

1378.1

811

408

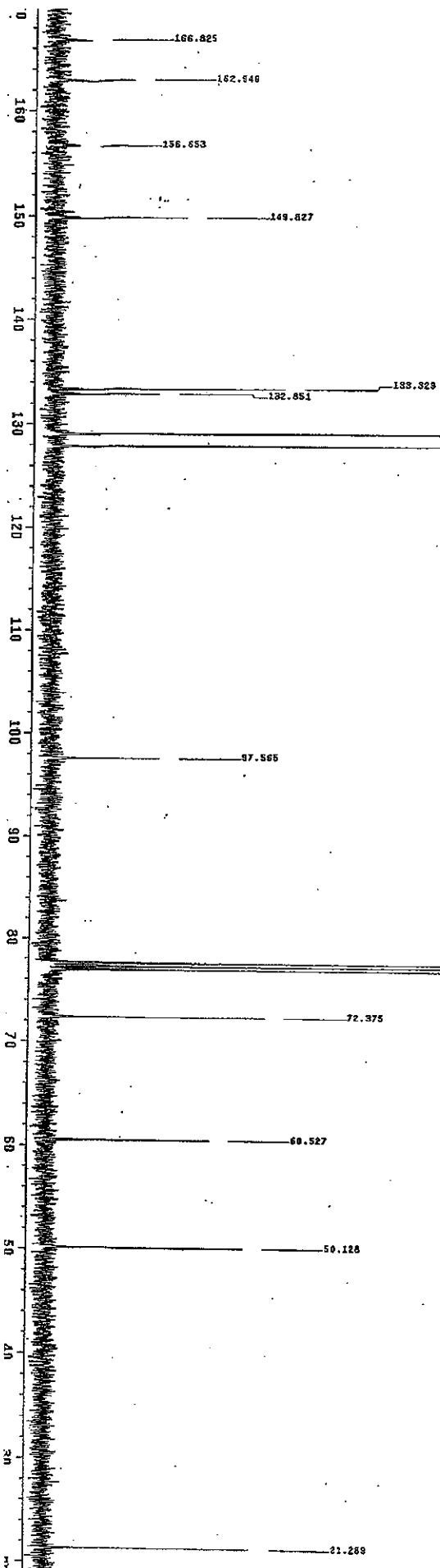
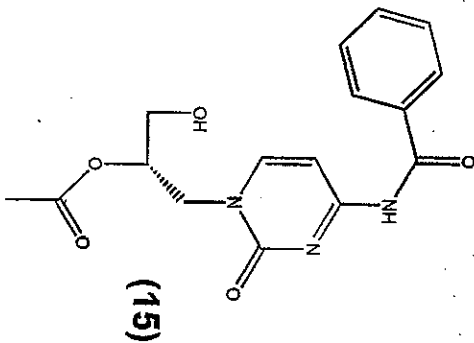
588.05

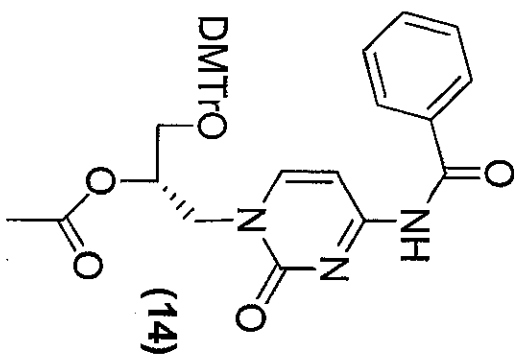
14789.8

7771.8

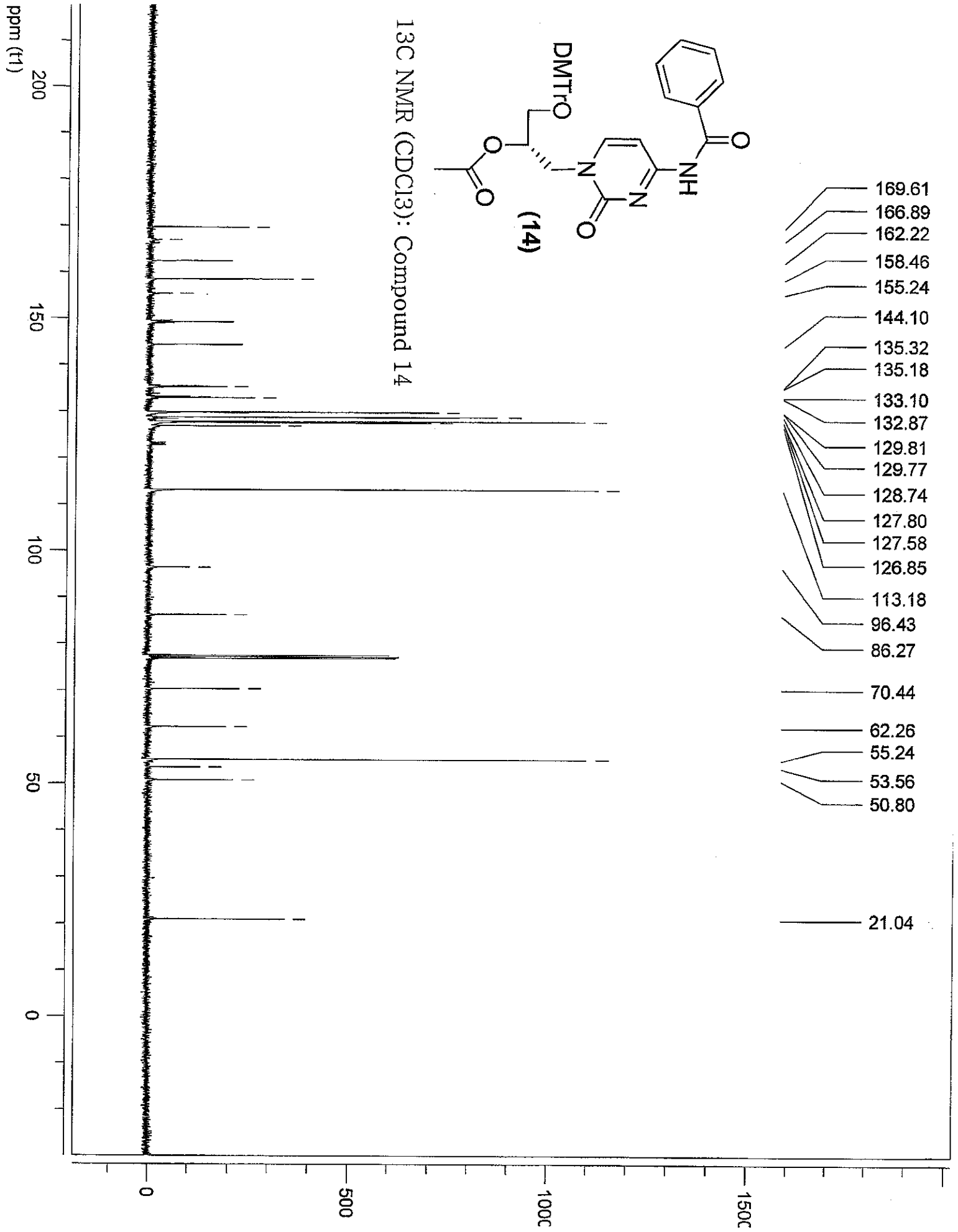
no ph 1.009

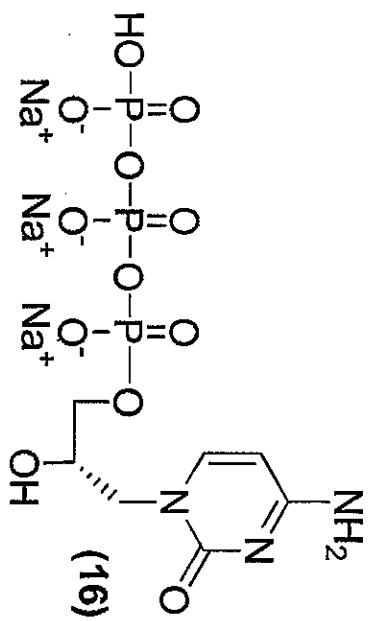
¹H NMR (CDCl₃): Compound 15



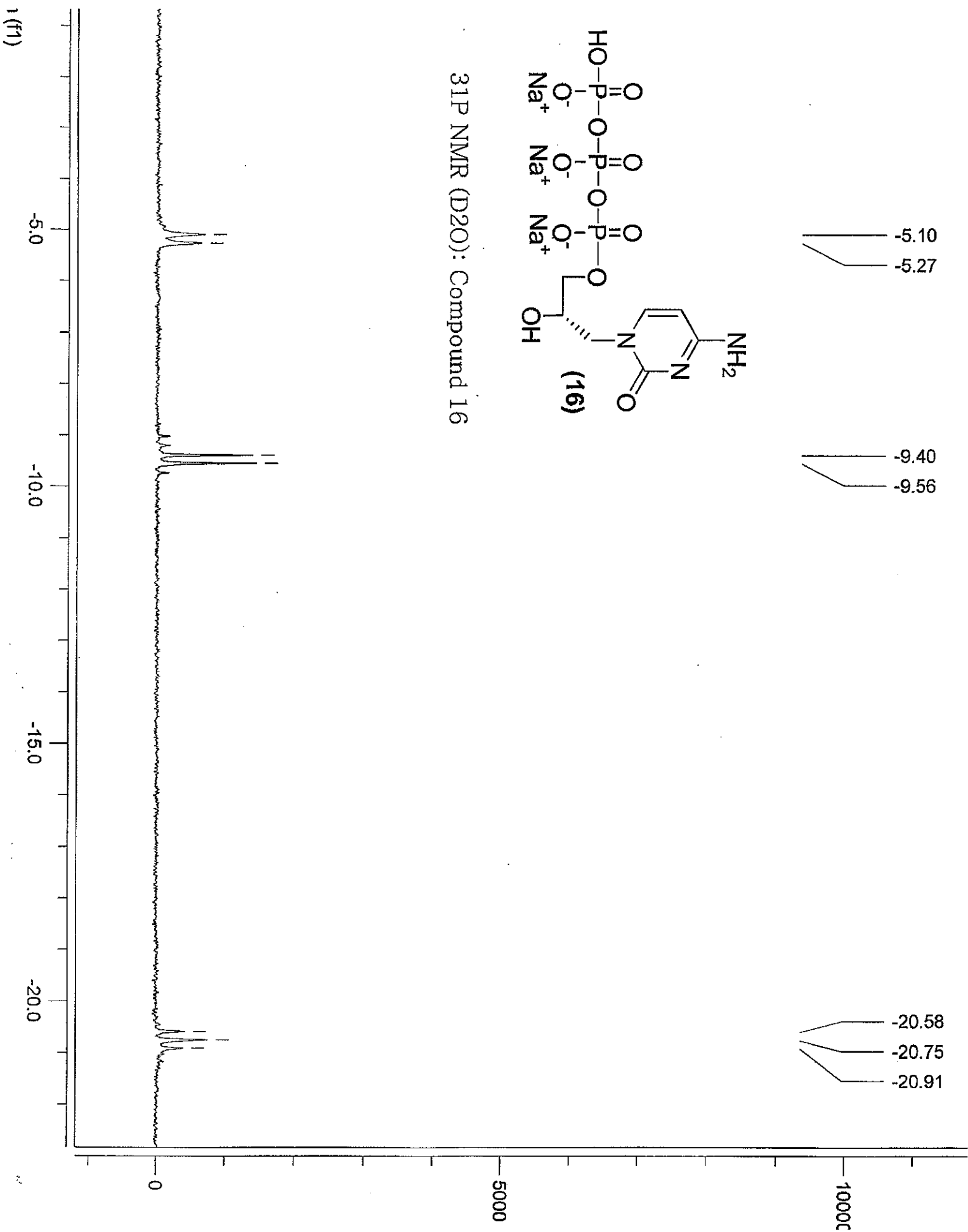


¹³C NMR (CDCl₃): Compound 14



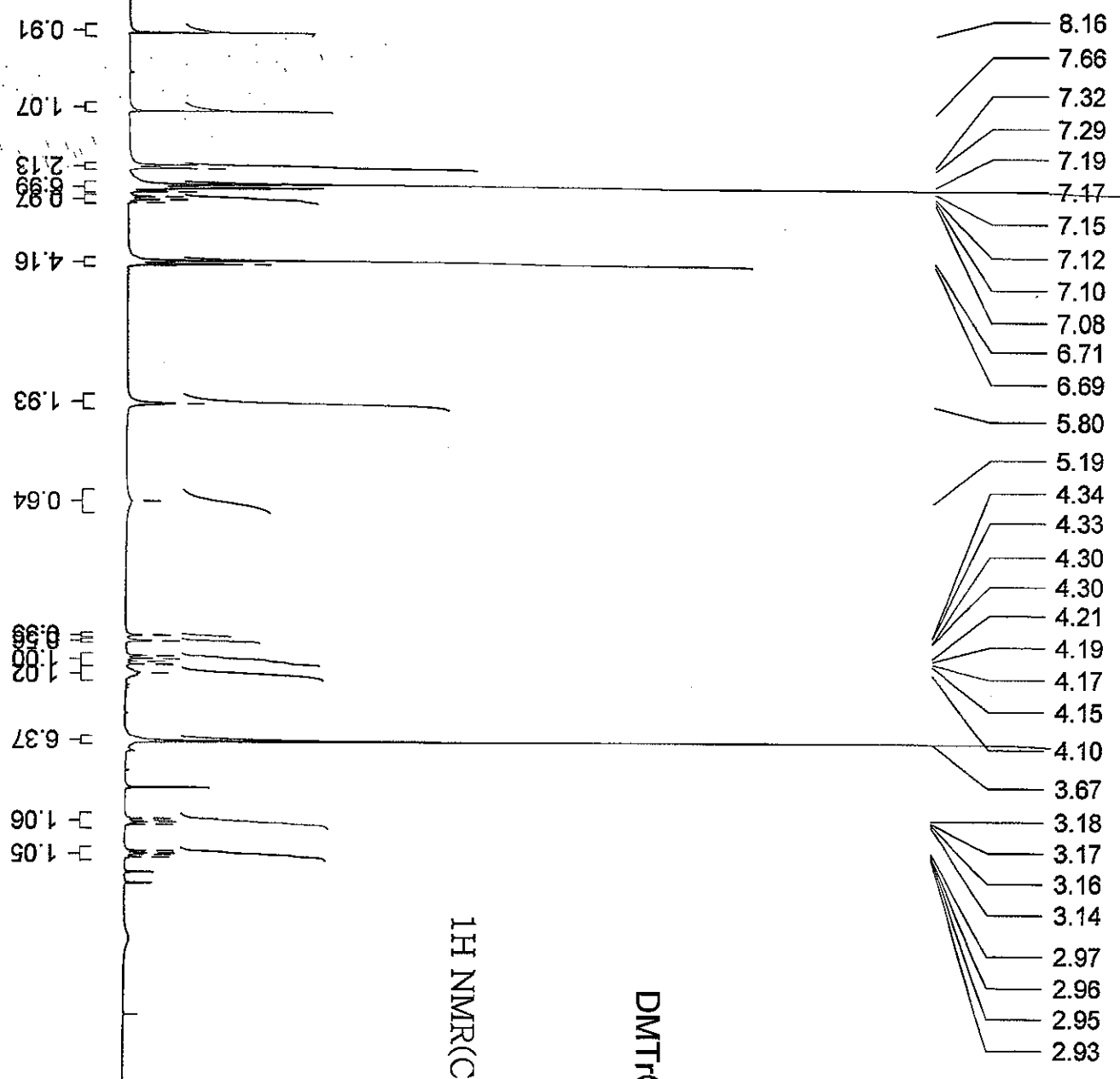


^{31}P NMR (D_2O): Compound 16

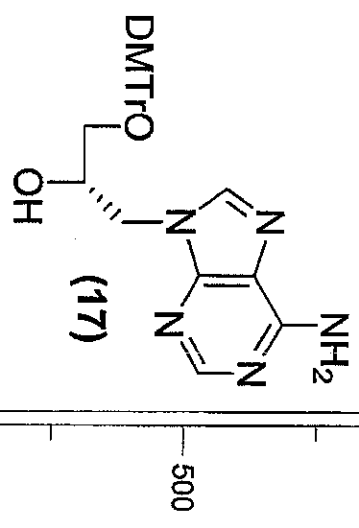


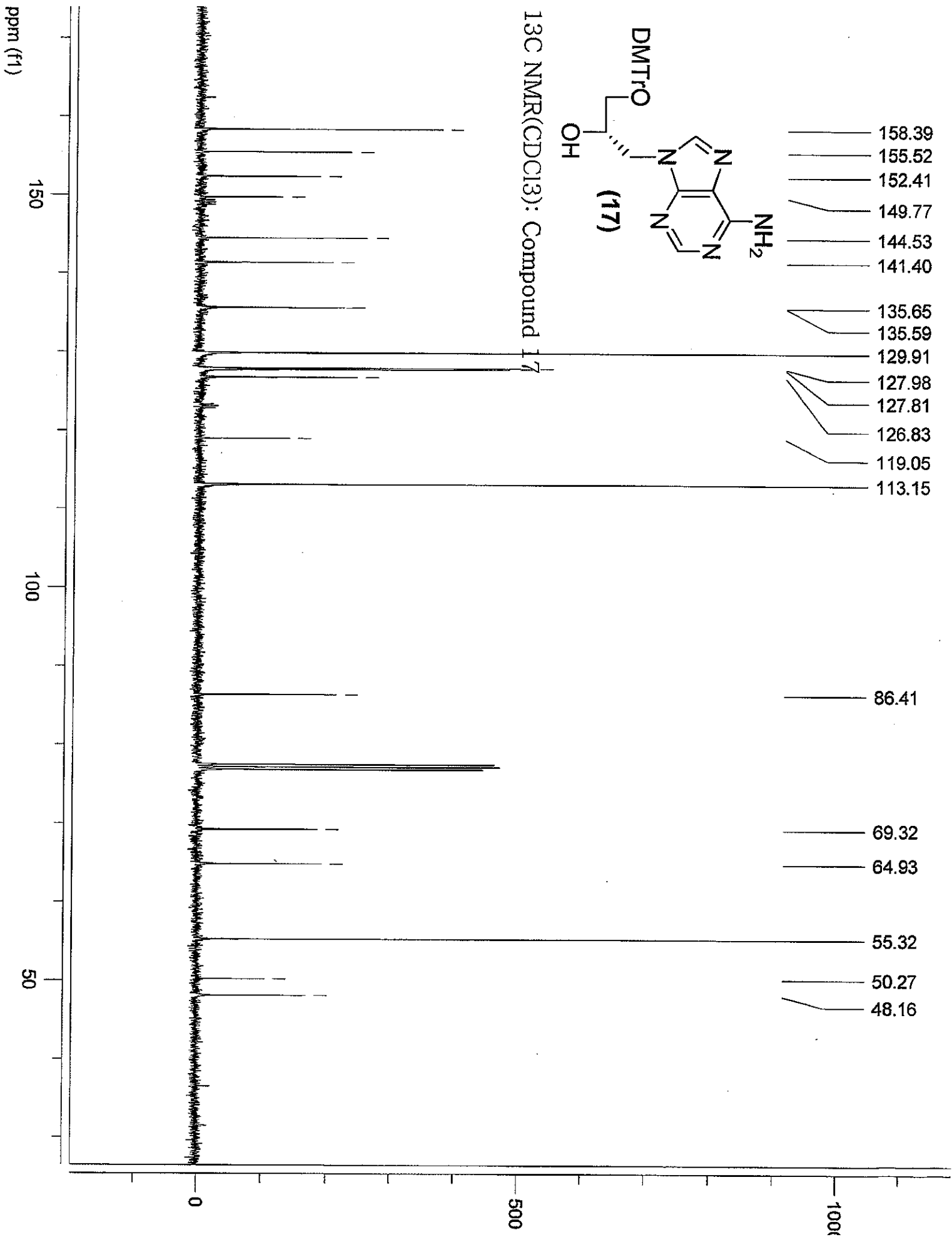
(f1)

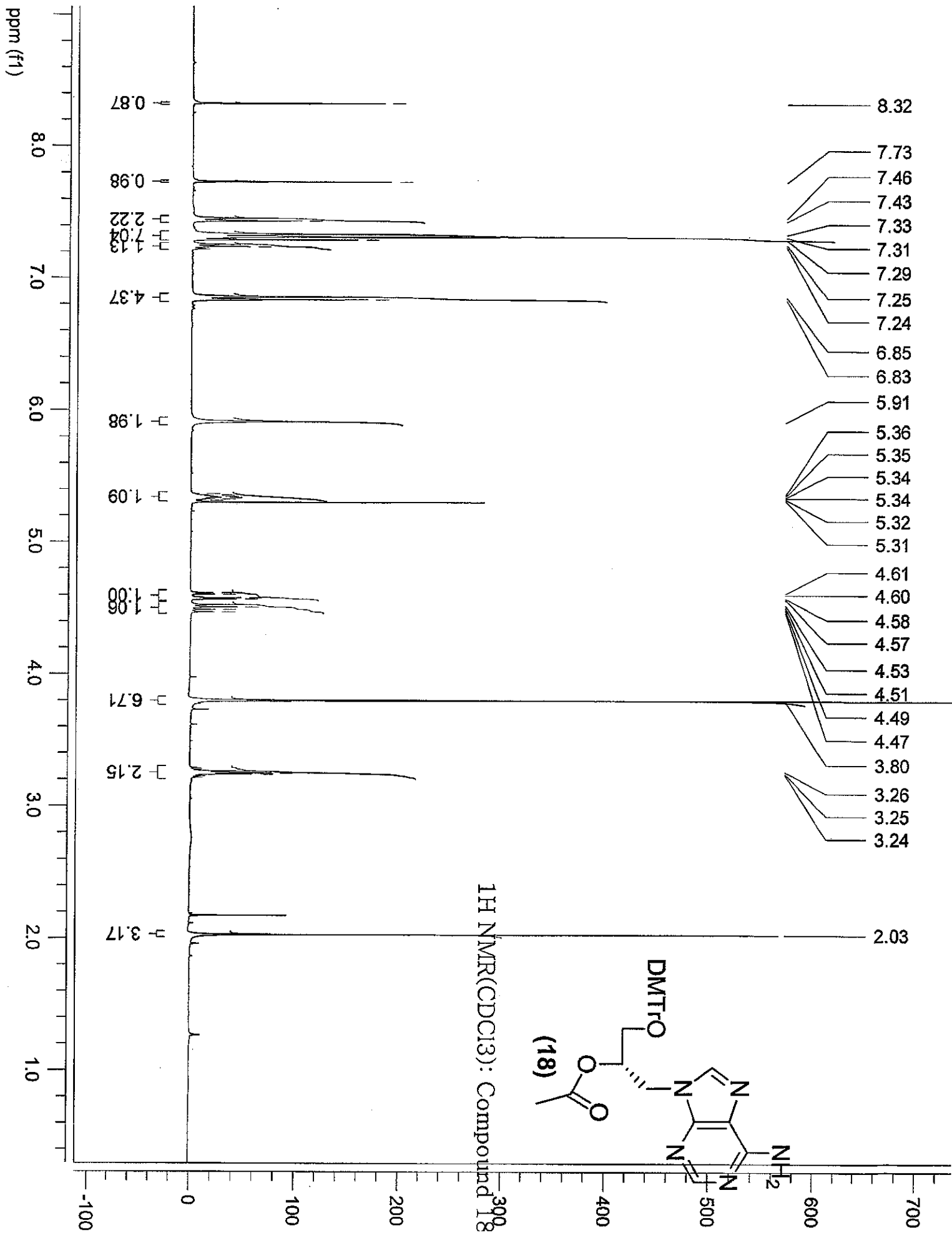
5.0

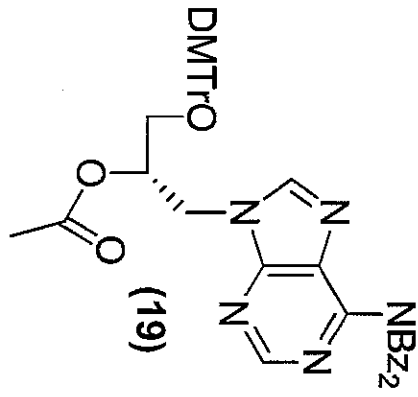


¹H NMR(CDCl₃): Compound 17

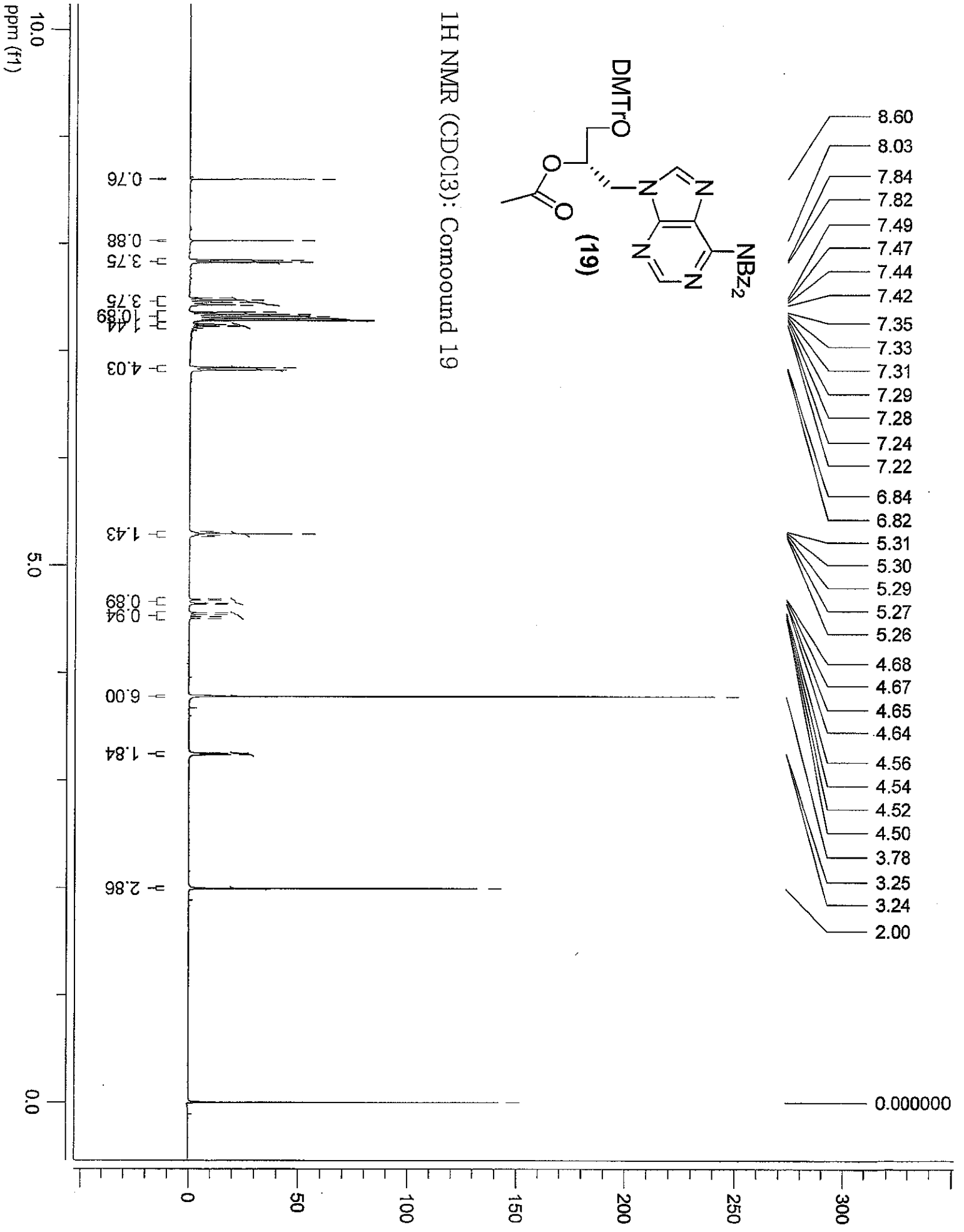


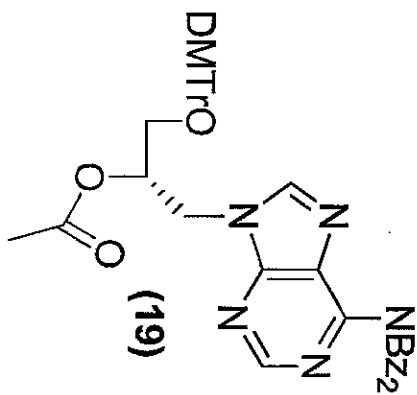




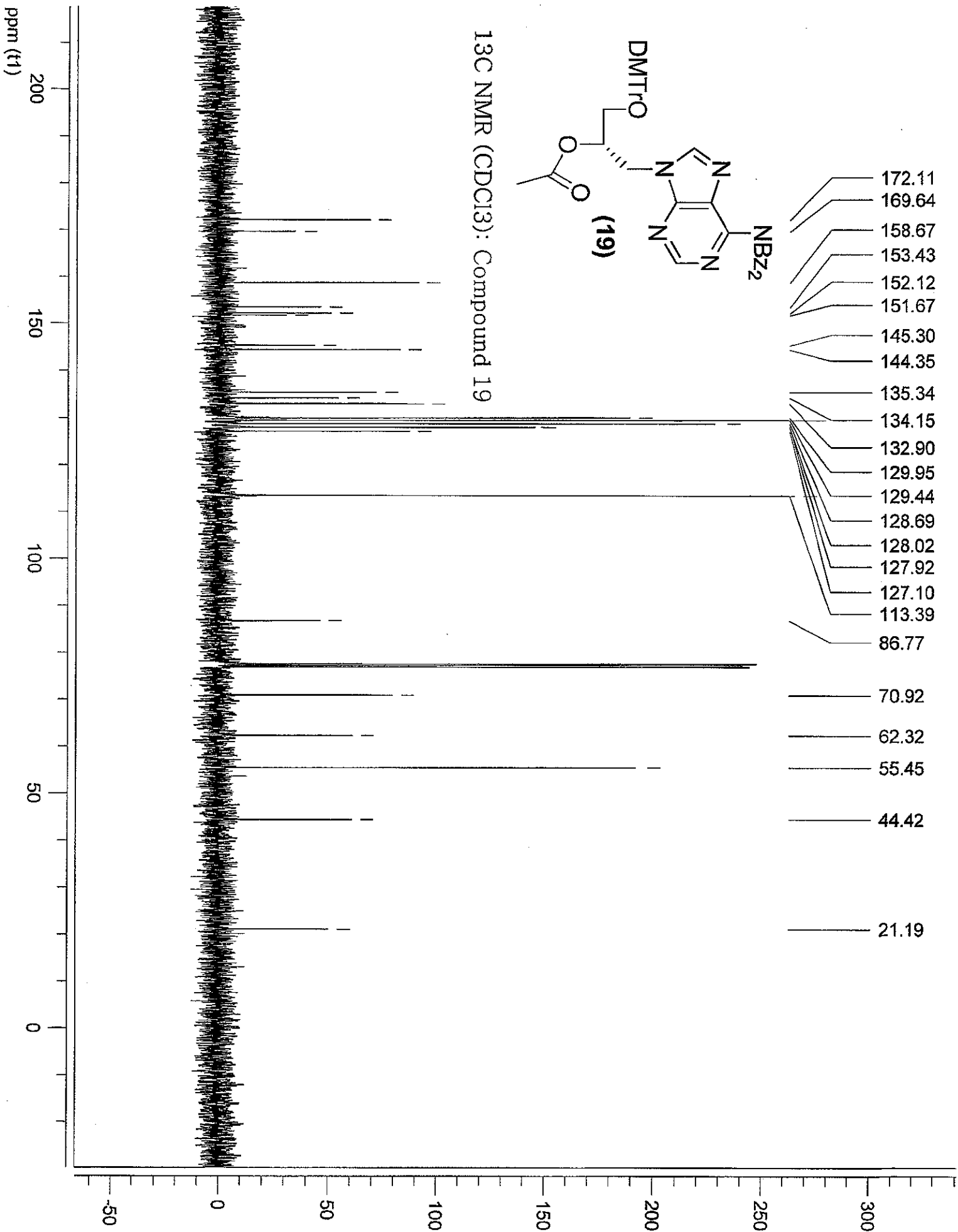


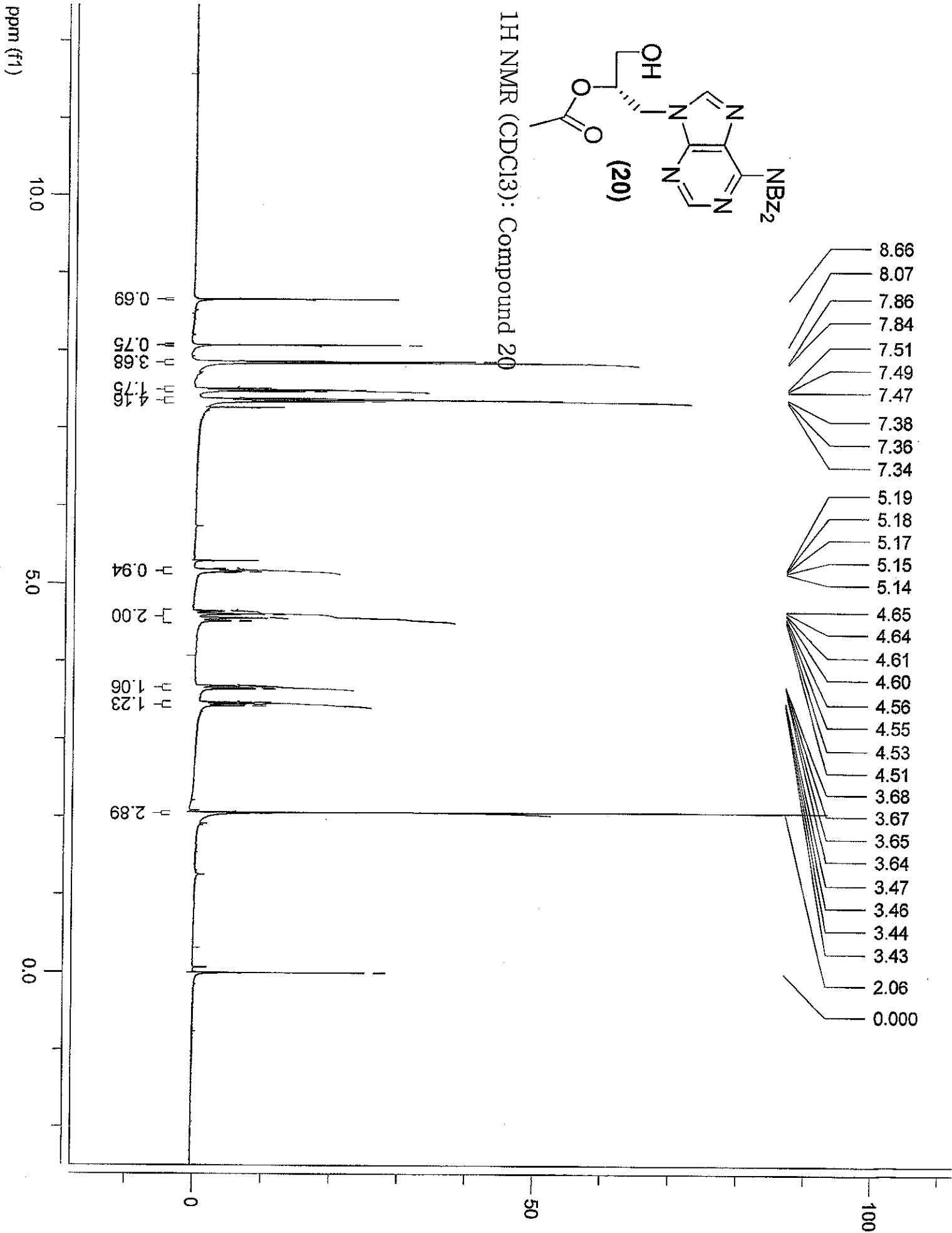
¹H NMR (CDCl₃): Compound 19



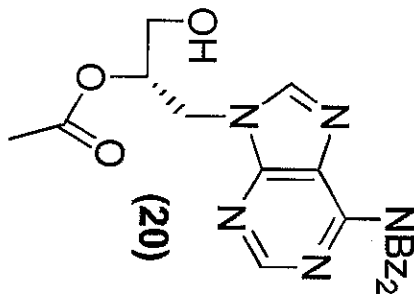


¹³C NMR (CDCl₃): Compound 19

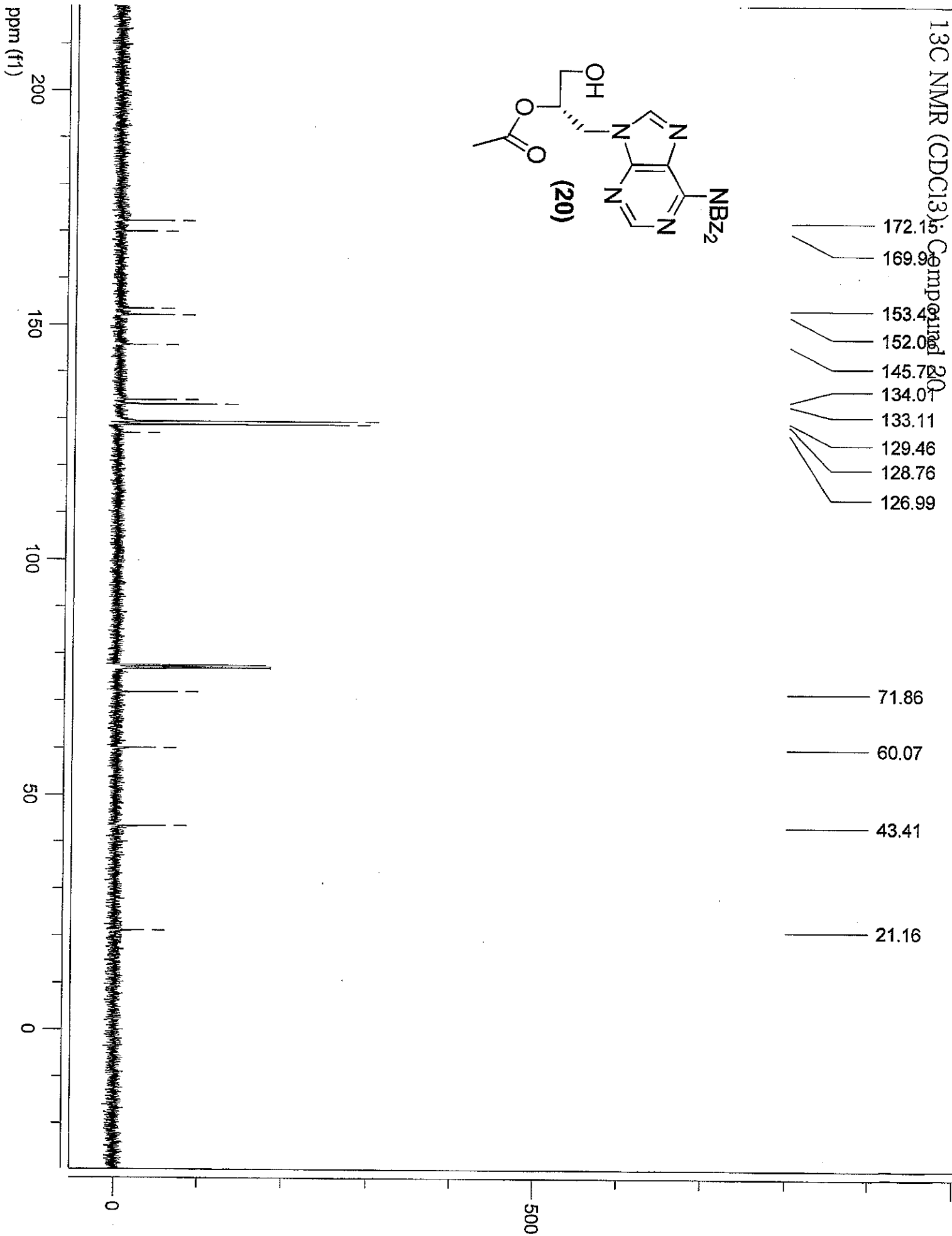


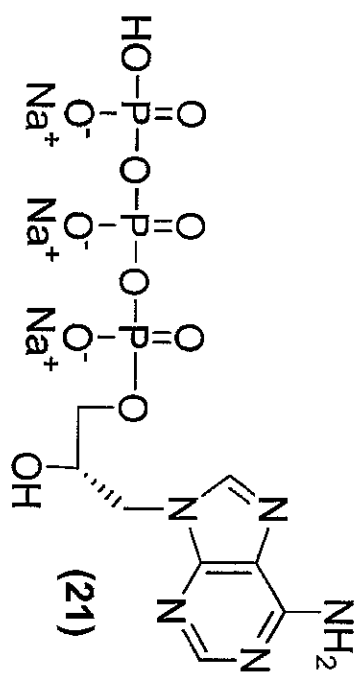


13C NMR (CDCl3) of Compound 20



- 172.1
- 169.9
- 153.4
- 152.0
- 145.7
- 134.0
- 133.1
- 129.4
- 128.7
- 126.9
- 71.86
- 60.07
- 43.41
- 21.16





31P NMR (D2O): Compound 21

