Glycerol Nucleoside Triphosphates: Synthesis and Polymerase Substrate Activities

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Supporting Information

Table of Contents:

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 aquired 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 interal H^1 standard, solvent peaks for C^{13}, and external phosphoric acid for P^{31} 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 Fransisco, CA) apparatus. Gel imaging was performed on a Bio-Rad FXpro Molecular Imager using Kodak-K radioisotope screens.

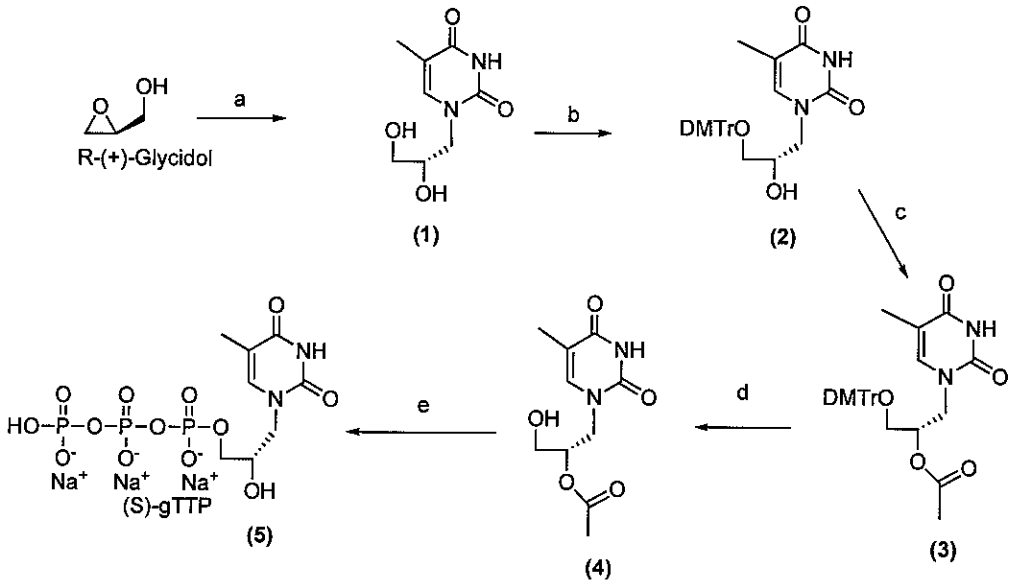
Abbreviations: DCM = Dichloromethane, MeOH = Methanol, TEA = Triethylamine, DCAA = Dichloroacetic Acid, PNK = Polynucleotide Kinase, DMF = Dimethylformamide, TEAB = Triethylammonium Bicarbonate, TEAA = Triethylammonium acetate, gNTPs = glycerol nucleoside triphosphates, DMTr = dimethoxytrityl

2) General Phosphorylation and Purification Procedure

Triphosphate syntheis 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 P_2O_5 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% I_2 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 NH_4OH 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. ^{31}P NMR was carried out in D_2O.

3) (S)-gTTP Synthesis



(a) Thymine/ 0.1 eq. K_2CO_3/DMF/ 85 °C/ 12h; (b) 1.1 eq. DMTr-Cl/ pyridine/ 8h/ rt; (c) 4 eq. Ac_2O/ 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/ pryridine/ 15 min/ rt; *ii.*) Tributylammonium pyrophosphate/ DMF/ tributylamine/ 15 min/ rt; *iii.*) 1% I_2 in 98/2 pyridine/ H_2O/ 15 min/ rt; *iv.*) NH_4OH/ 1 h/ rt; v.) Dowex 50WX8 Na^+

Compound (1)

Thymine was dissolved in DMF at 85° C and 0.2 eq. of K_2CO_3 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.

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

^{1}H NMR (DMSO-d_6) 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). ^{13}C NMR (DMSO-d_6) 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 $C_8H_{12}N_2O_4Na$: 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.

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

^{1}H NMR (DMSO-d_6) δ:1.59(s, 3H), 2.80(dq, 2H, J_1=4 Hz, J_2=37 Hz), 3.35(q, 1H, J_1=5 Hz, J_2=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). ^{13}C NMR (DMSO-d_6) 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 $C_{29}H_{30}N_2O_6Na$: 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.

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

^{1}H NMR (DMSO-d_6) 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).

^{13}C NMR (DMSO-d_6) 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 $C_{31}H_{32}N_2O_7Na$: 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.

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

^{1}H NMR (DMSO-d_6) 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). ^{13}C NMR (DMSO-d_6) 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 $C_{10}H_{14}N_2O_5Na$: 265.0793]

λ_{max}(MeOH): 268 nm

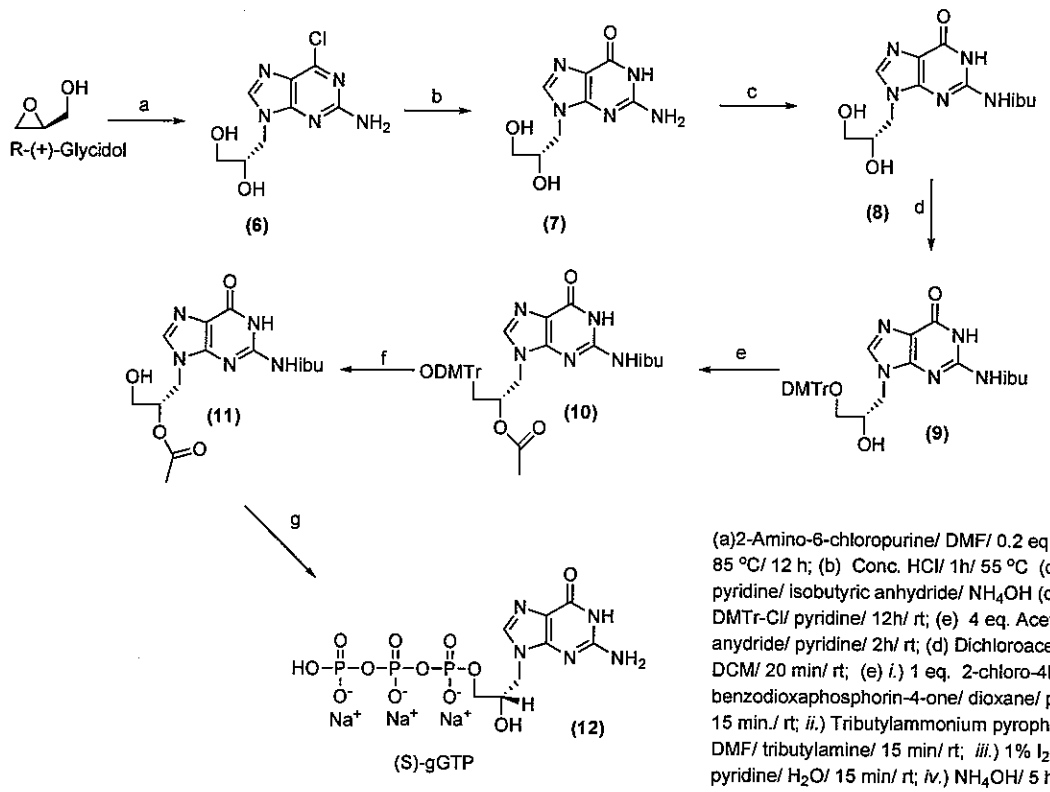
Compound (5)

Compound **5** was prepared from **4** using the general phosphorylation and purification procedure as described[1].

^{32}P NMR (D_2O) 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



(a)2-Amino-6-chloropurine/ DMF/ 0.2 eq. K_2CO_3/ 85 °C/ 12 h; (b) Conc. HCl/ 1h/ 55 °C (c) TMS-Cl pyridine/ isobutyric anhydride/ NH_4OH (d) 1.1 eq. DMTr-Cl/ pyridine/ 12h/ rt; (e) 4 eq. Acetic anydride/ pyridine/ 2h/ rt; (d) Dichloroacetic acid/ DCM/ 20 min/ rt; (e) *i.*) 1 eq. 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one/ dioxane/ pryridine/ 15 min./ rt; *ii.*) Tributylammonium pyrophosphate in DMF/ tributylamine/ 15 min/ rt; *iii.*) 1% I_2 in 98/2 pyridine/ H_2O/ 15 min/ rt; *iv.*) NH_4OH/ 5 h/ 55 °C; *v.*) Dowex 50WX8 Na+

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.

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

^{1}H 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.

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

^{1}H NMR (DMSO-d_6) δ: 3.43(m, 2H), 3.87(m, 1H), 4.01(dd, 1H, J1=9.2 Hz, J=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)

^{1}H NMR (DMSO-d_6) 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 $C_{12}H_{17}N_5O_4H$: 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)

^{1}H NMR ($CDCl_3$) δ: 1.23(q, 6H, J1=4, J2=7 Hz), 2.78(5, 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 $C_{33}H_{35}N_5O_6H$: 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)

^{1}H NMR ($CDCl_3$) 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).

^{13}C NMR ($CDCL_3$) 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 $C_{35}H_{37}N_5O_7H$: 640.3]

λ_{max}(DCM): 238, 282

Comound (11)

Compound **10** was dissolved in dry DCM and treated with 3% DCAA at ambient temperature for 20 min foll0wed 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)

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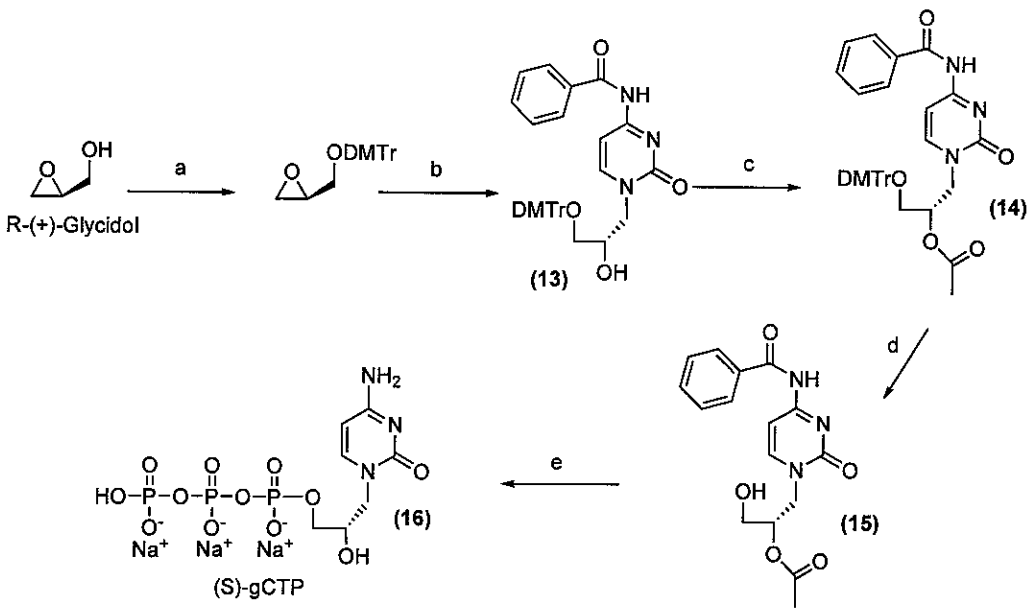
[2] Gait, MJ., ed. Oligonucleotide Synthesis: A Practical Approach. New York: Oxford University Press, 1990

^{1}H 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 $C_{14}H_{19}N_5O_5H$: 338.1465]
λ_{max}(MeOH): 254, 259, 284 nm

Comound (12)

Compound **12** was prepared using the general phosphorylation and purification procedures as described[1].
^{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. Ac_2O/ 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/ pryridine/ 15 min/ rt; *ii.*) Tributylammonium pyrophosphate in DMF/ tributylamine/ 15 min/ rt; *iii.*) 1% I2 in 98/2 pyridine/ H2O/ 15 min./ rt; *iv.*) NH_4OH/ 8 h/ 55 °C; *v.*) Dowex 50WX8 Na^+

Compound (13)

N_4-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.
^{1}H NMR ($CDCl_3$) δ: 3.12(dd, 1H, J1=6, J2=10 Hz), 3.26(dd, 1H, J1=5.3 Hz, J2=10 Hz), 3.84(q, 1H, J1=7 Hz, J2=14 Hz), 4.22(bs, 1H), 4.35(dd, 2H, J1=3 Hz, J2=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)

^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCL_3$) 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

Comound (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)

^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCL_3$) 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]

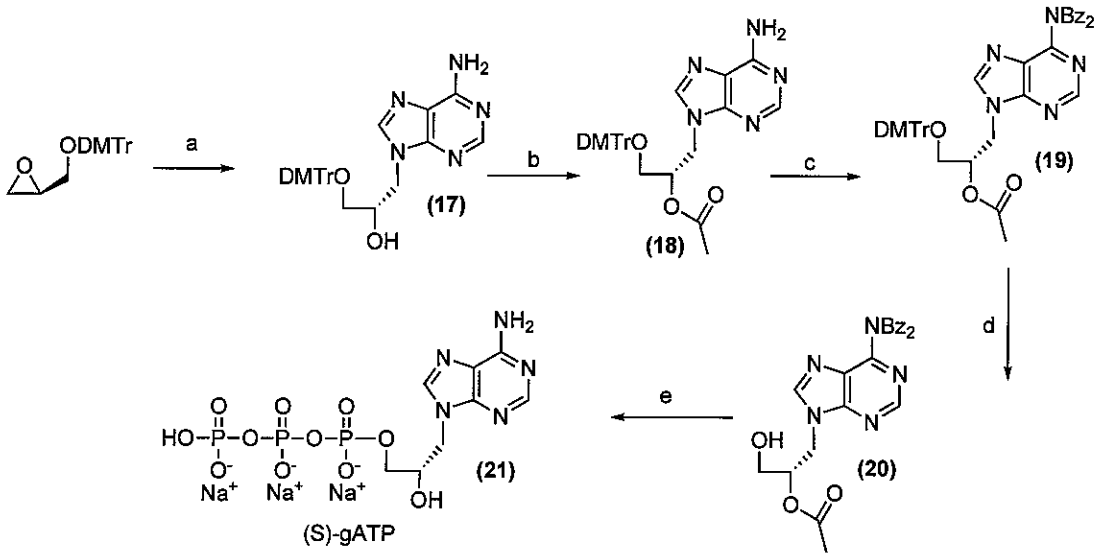
λ_{max}: DCM 258, 310 nm

Compound (16)

Compound **16** was prepared using the general phosphorylation and purification methods as previously described[1].

^{32}P NMR (D_2O) δ: -21.7(t, J=19 Hz), -9.8(d, J=19 Hz), -8.4(d, J=20 Hz)

4) gATP Sythesis



(a) Adenine/ DMF/ 0.2 eq. NaH/ 120 °C/ 6 h; (b) 1.2 eq. Ac_2O/ 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% I_2 in 98/2 pyridine/ H_2O/ 15 min/ rt; *iv.*) NH_4OH/ 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.

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

^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCL_3$) 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 $C_{29}H_{29}N_5O_4H$: 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.

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

^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCL_3$) 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 $C_{31}H_{31}N_5O_5H$: 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.
Rf: 0.5 (DCM: MeOH; 97:3, v/v)
^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCL_3$) 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 $C_{45}H_{39}N_5O_7Na$: 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.
Rf: 0.4 (DCM: MeOH; 95:5, v/v)
^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCL_3$) 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 $C_{24}H_{21}N_5O_5H$: 460.1622]
λ_{max}: DCM 250, 276

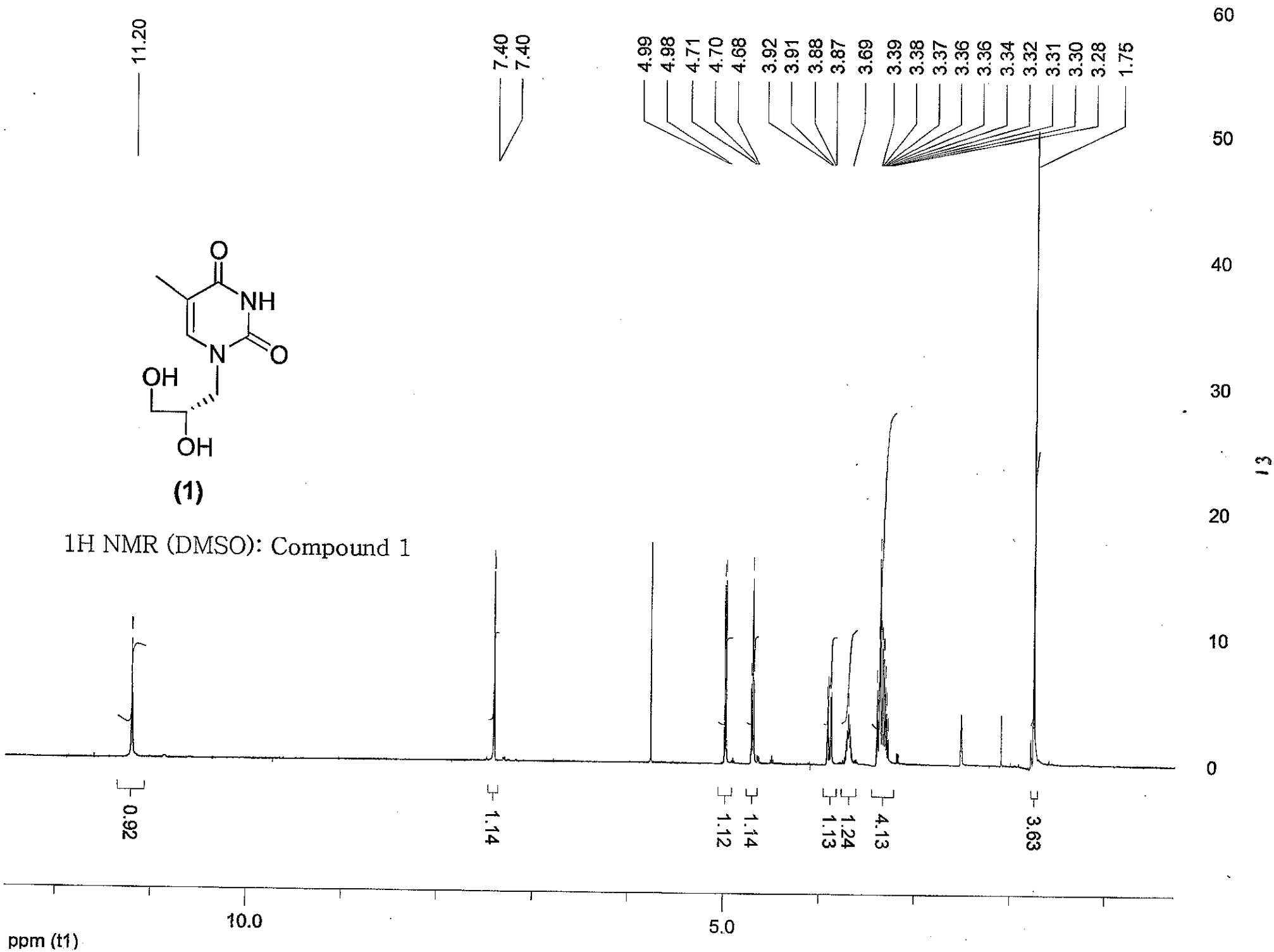
Compound (21)

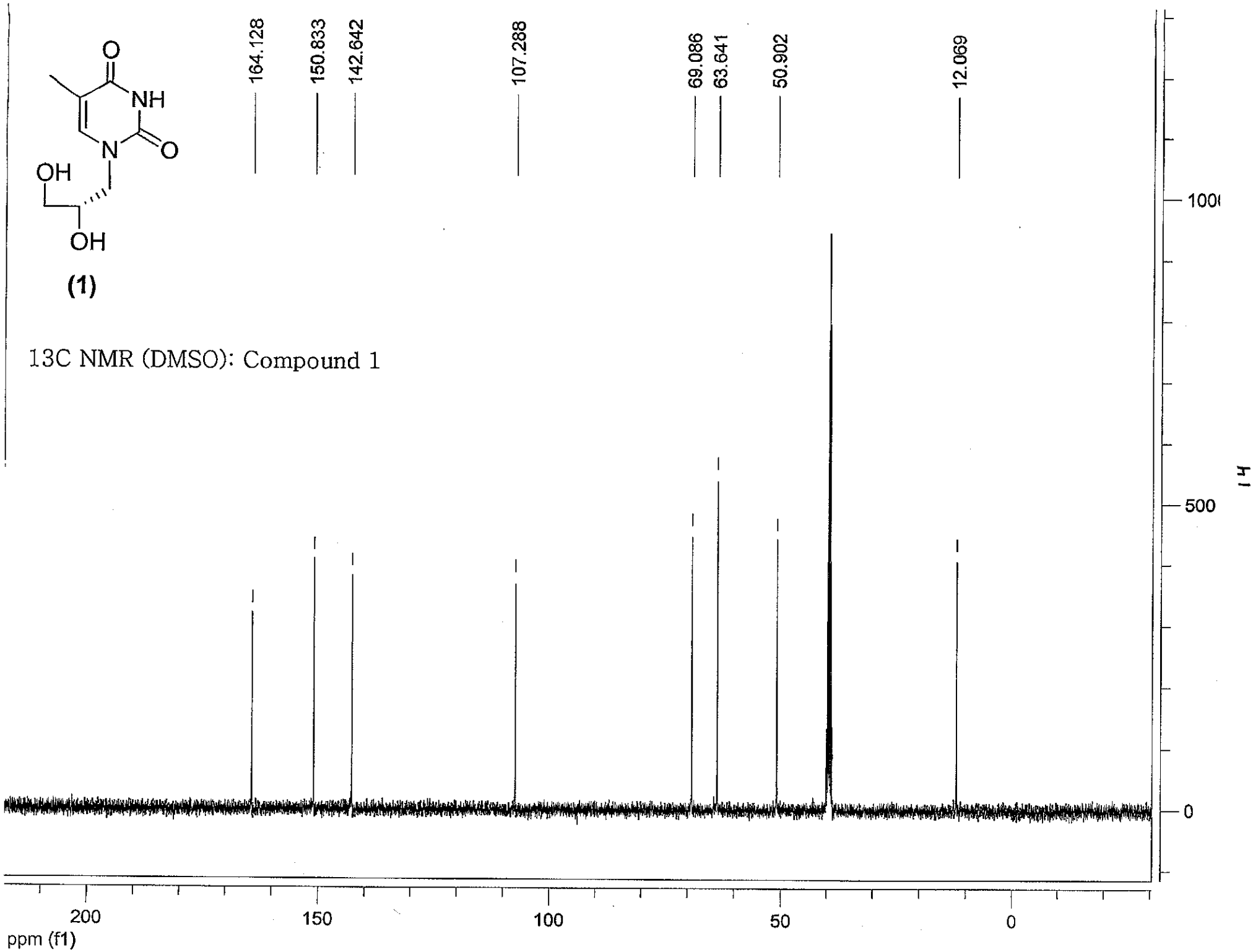
Compound **21**was synthesized using the general phosphorylation and purification methods described previously[1].
^{32}P NMR (D_2O) δ: -9.93(d, J=19 Hz), -9.75(d, J=19 Hz), -21.5(t, J=19 Hz)

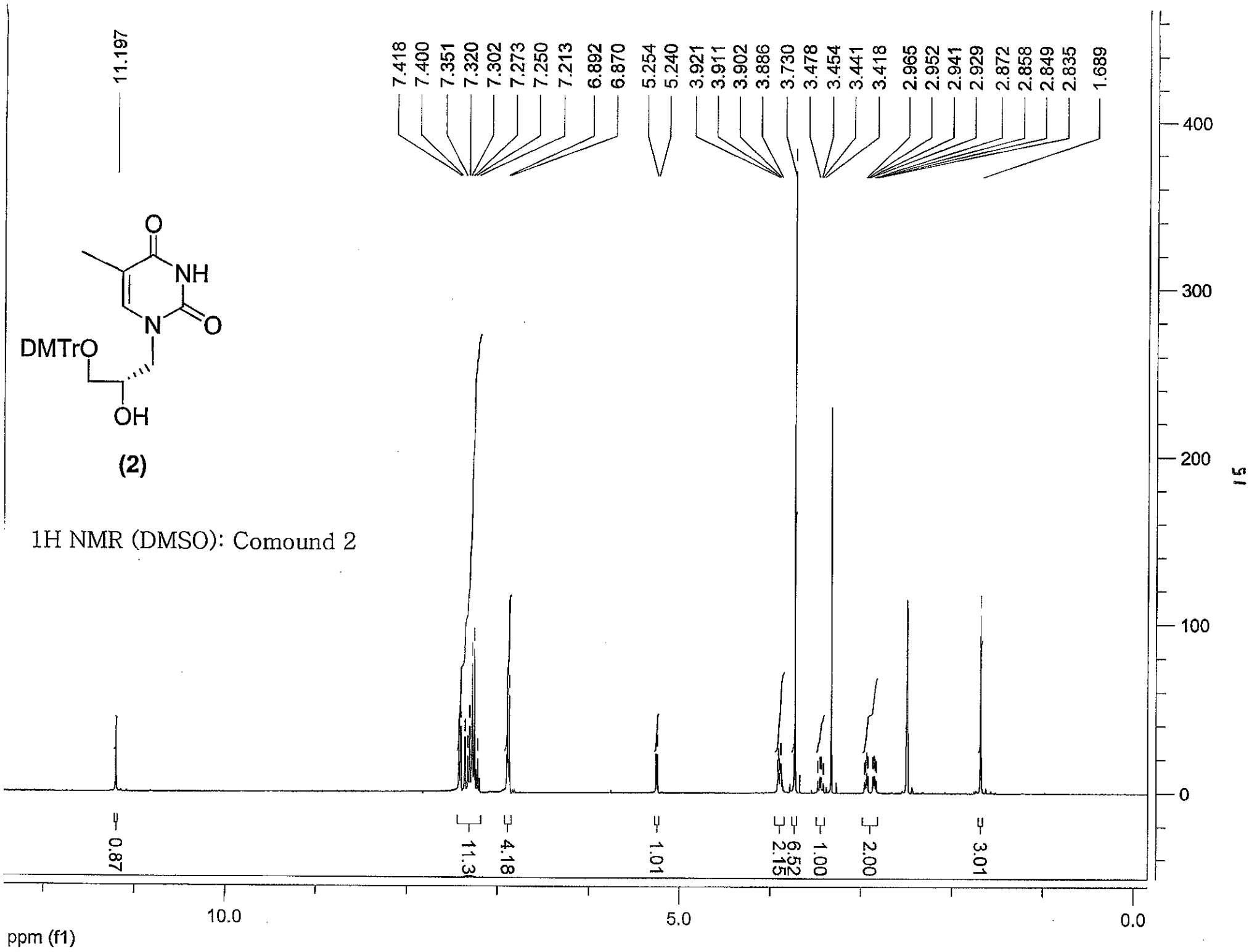
7) Polymerase Experiments

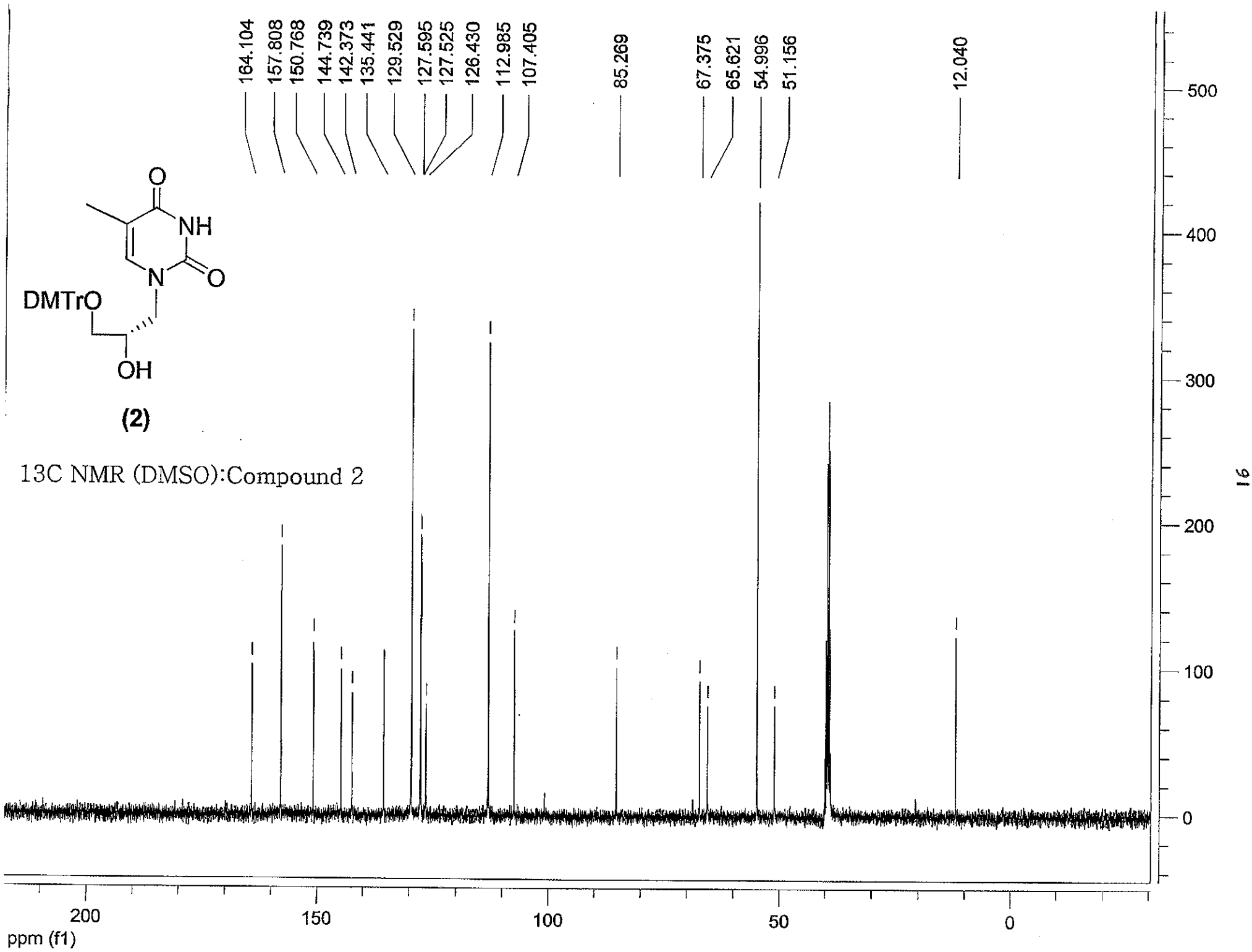
DNA oligomers for gNTP polymerase experiments were synthesized using by standard solid phase procedures. After ammonia treatment, DMTr-on oligomers were purified on a RP Pourus column followed by 10% acetic acid treatment and 2 water/ether extractions to remove trityl residue. Oligomers were further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

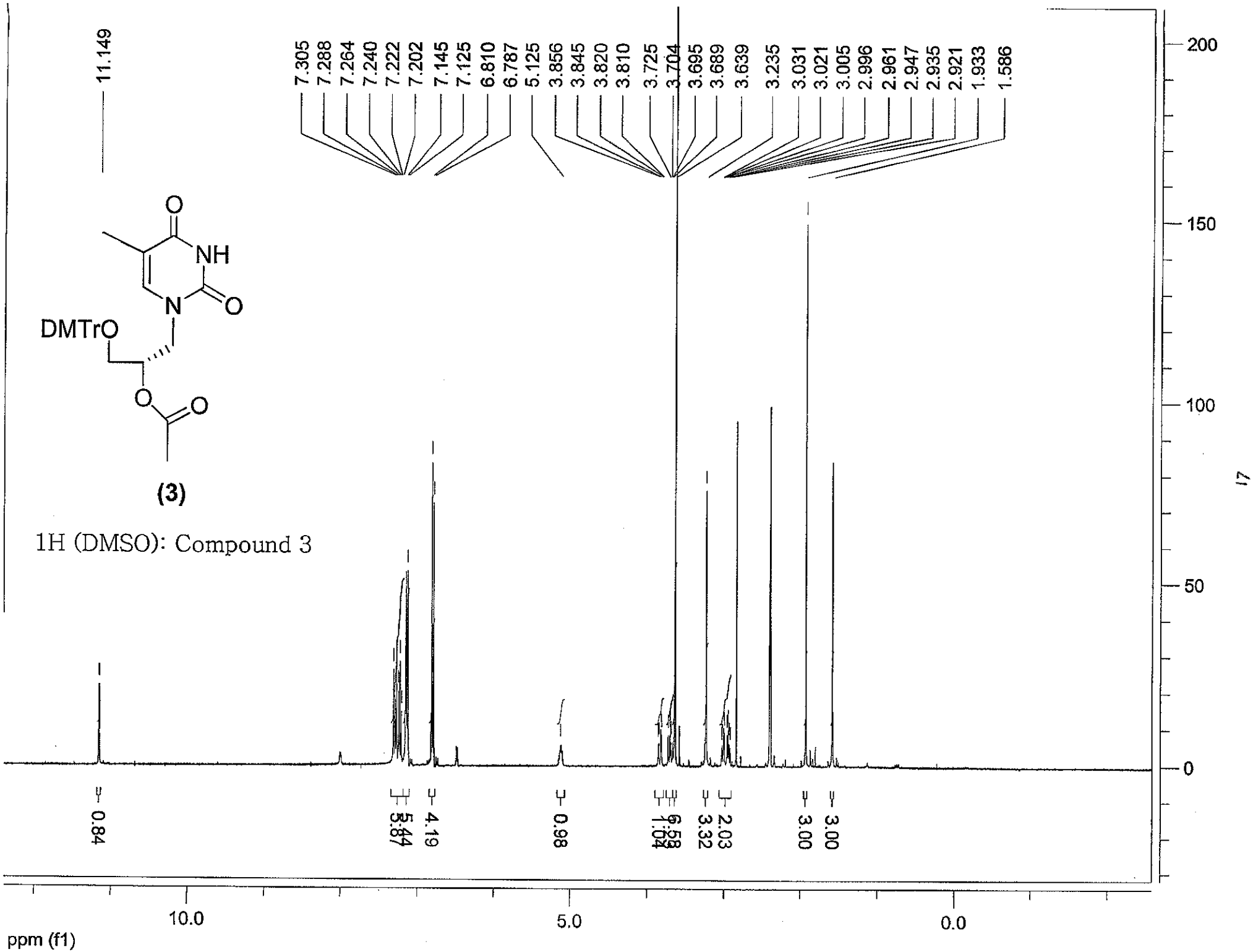
Oligonucleotide primers were ^{32}P-end-labeled using T4 PNK (New England Biolabs, Beverley, MA) and γP32 ATP (MP, Irvine, CA) for 1 h at 37° C followed by heat denaturing at 95° C for 15 min. Excess γ^{32}P ATP was removed using Micro-Spin G-25 size exclusion columns (Amersham-Bioscience, Buckinghamshire, 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.

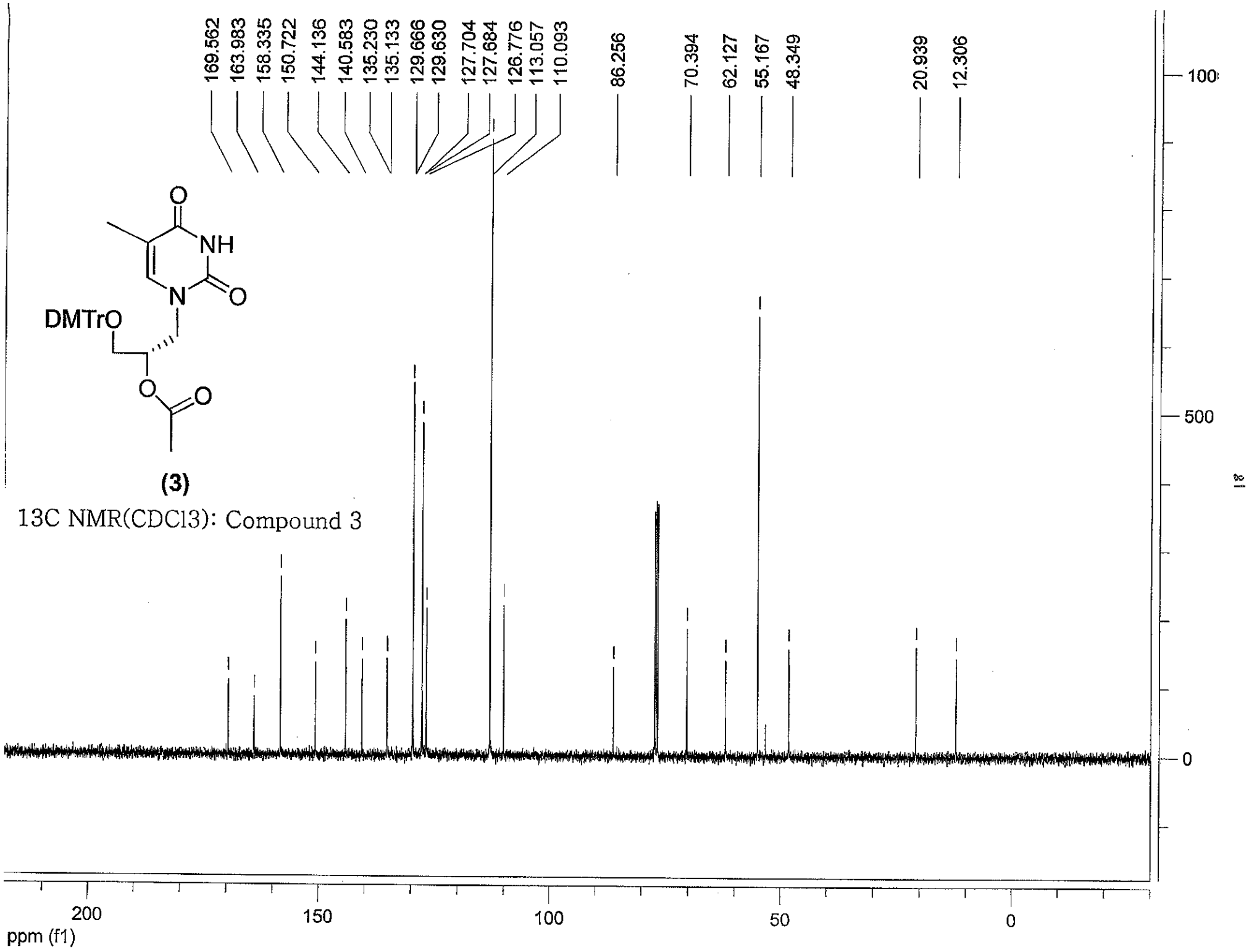

11.20
7.40
7.40
4.99
4.98
4.71
4.70
4.68
3.92
3.91
3.88
3.87
3.69
3.39
3.38
3.37
3.36
3.36
3.34
3.32
3.31
3.30
3.28
1.75
O
NH
N
O
OH
OH
(1)
1H NMR (DMSO): Compound 1
0.92
1.14
1.12
1.14
1.13
1.24
4.13
3.63
10.0
5.0
ppm (t1)
60
50
40
30
20
10
0

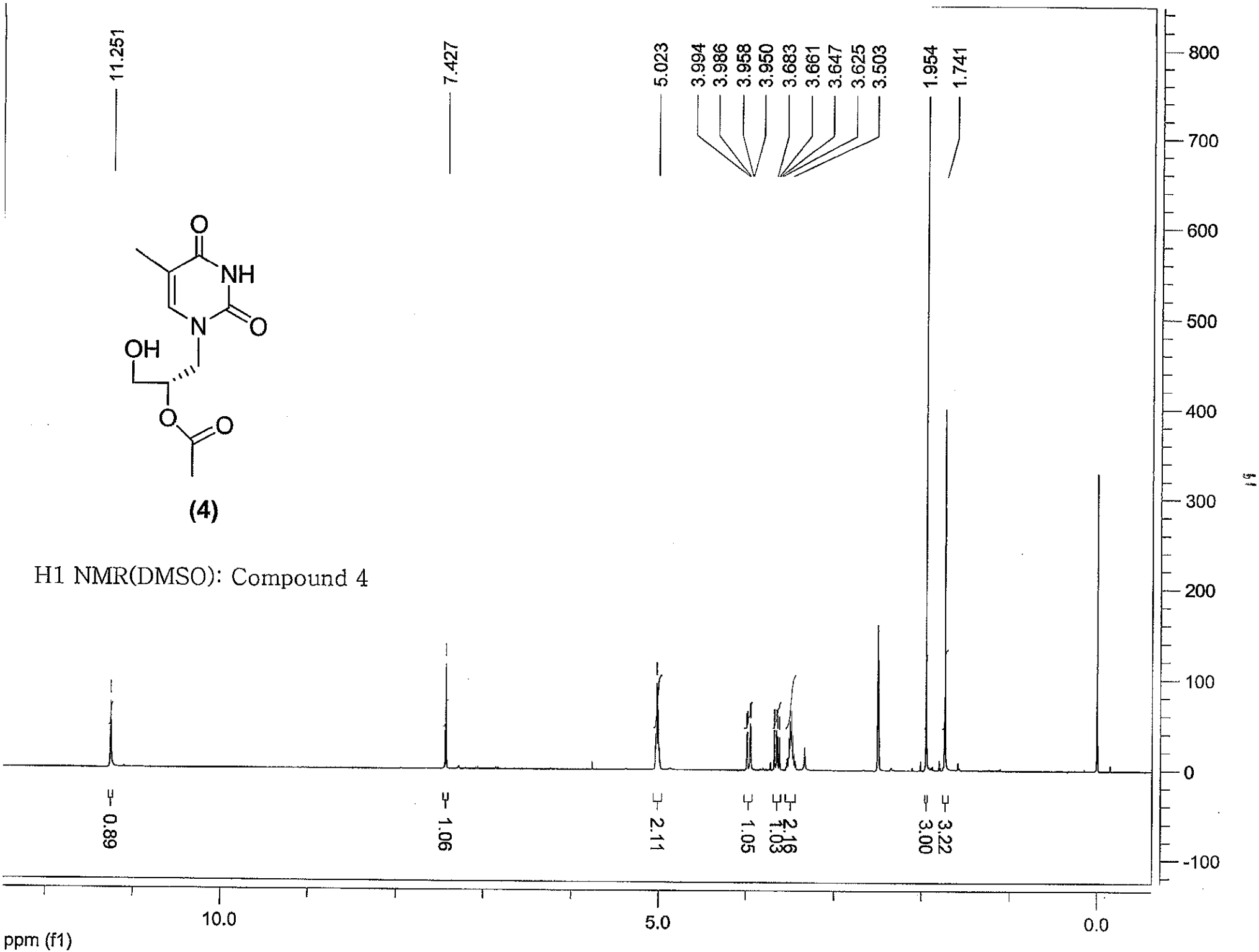

O
NH
N
O
OH
OH
(1)
164.128
150.833
142.642
107.288
69.086
63.641
50.902
12.069
13C NMR (DMSO): Compound 1
100
500
0
H1
200
150
100
50
0
ppm (f1)

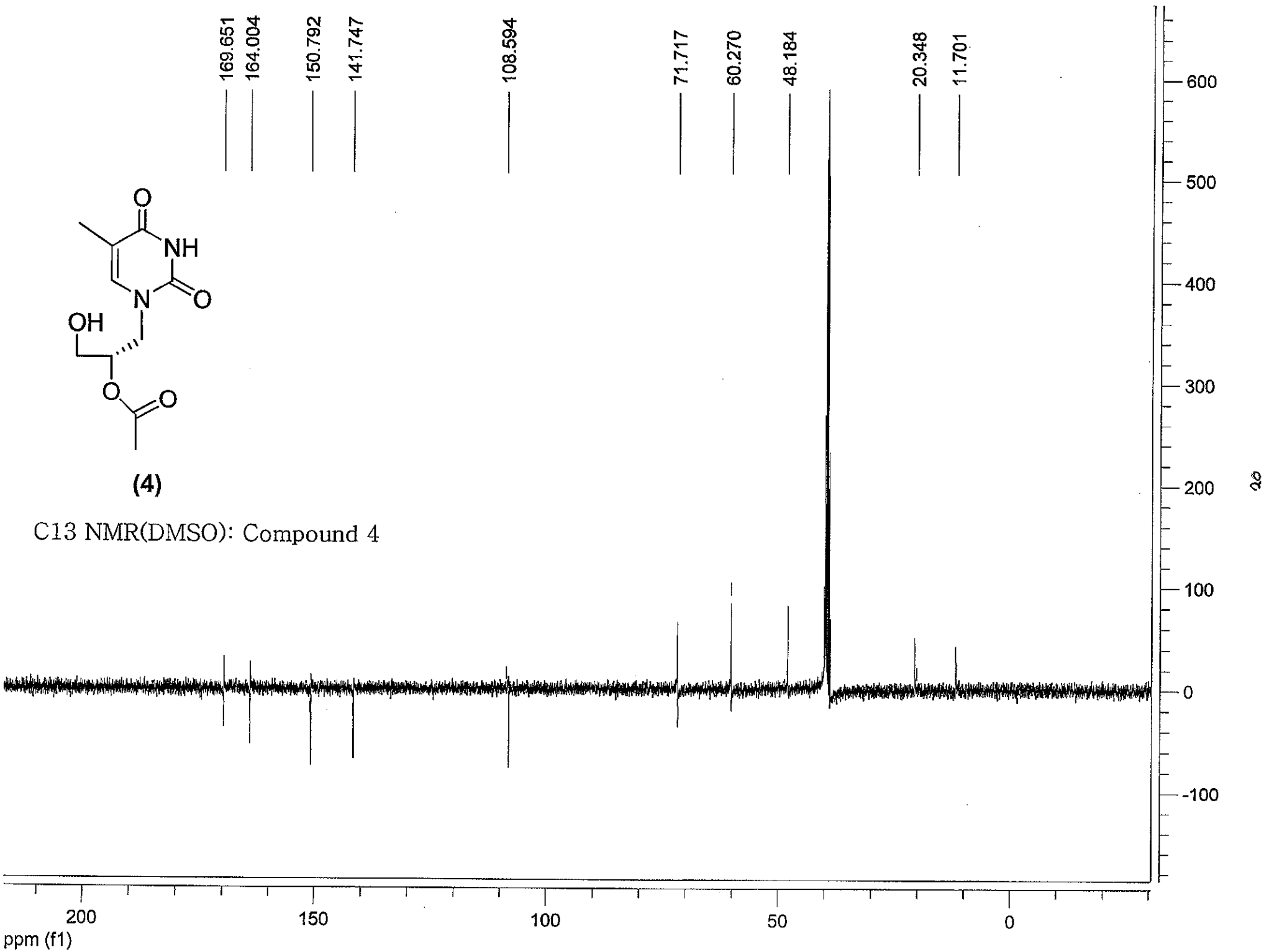

11.197
7.418
7.400
7.351
7.320
7.302
7.273
7.250
7.213
6.892
6.870
5.254
5.240
3.921
3.911
3.902
3.886
3.730
3.478
3.454
3.441
3.418
2.965
2.952
2.941
2.929
2.872
2.858
2.849
2.835
1.689
NH
O
N
O
DMTrO
OH
(2)
1H NMR (DMSO): Comound 2
0.87
11.3
4.18
1.01
2.15
6.52
1.00
2.00
3.01
400
300
200
100
0
10.0
5.0
0.0
ppm (f1)


164.104
157.808
150.768
144.739
142.373
135.441
129.529
127.595
127.525
126.430
112.985
107.405
85.269
67.375
65.621
54.996
51.156
12.040
O
NH
N
O
DMTrO
OH
(2)
13C NMR (DMSO):Compound 2
500
400
300
200
100
0
200
150
100
50
0
ppm (f1)


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
O
NH
N
O
DMTrO
O
O
(3)
1H (DMSO): Compound 3
0.84
5.87
4.19
0.98
1.04
3.32
2.03
3.00
3.00
200
150
100
50
0
10.0
5.0
0.0
ppm (f1)

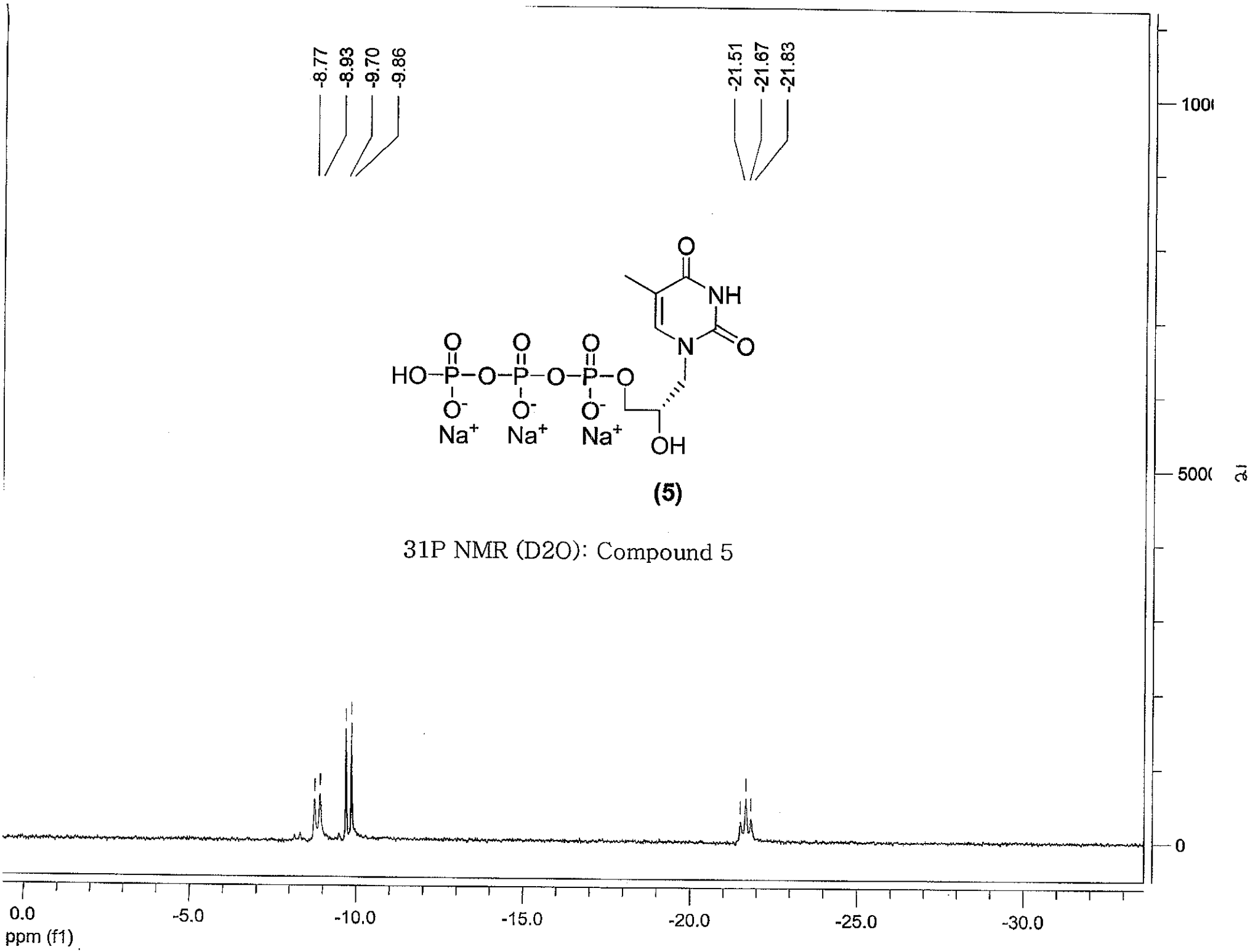

169.562
163.983
158.335
150.722
144.136
140.583
135.230
135.133
129.666
129.630
127.704
127.684
126.776
113.057
110.093
86.256
70.394
62.127
55.167
48.349
20.939
12.306
O
NH
N
O
DMTrO
O
O
(3)
13C NMR(CDCl3): Compound 3
100
500
0
200
150
100
50
0
ppm (f1)


11.251
7.427
5.023
3.994
3.986
3.958
3.950
3.683
3.661
3.647
3.625
3.503
1.954
1.741
O
NH
N
O
OH
O
O
(4)
H1 NMR(DMSO): Compound 4
0.89
1.06
2.11
1.05
1.03
2.16
3.00
3.22
800
700
600
500
400
300
200
100
0
-100
10.0
5.0
0.0
ppm (f1)

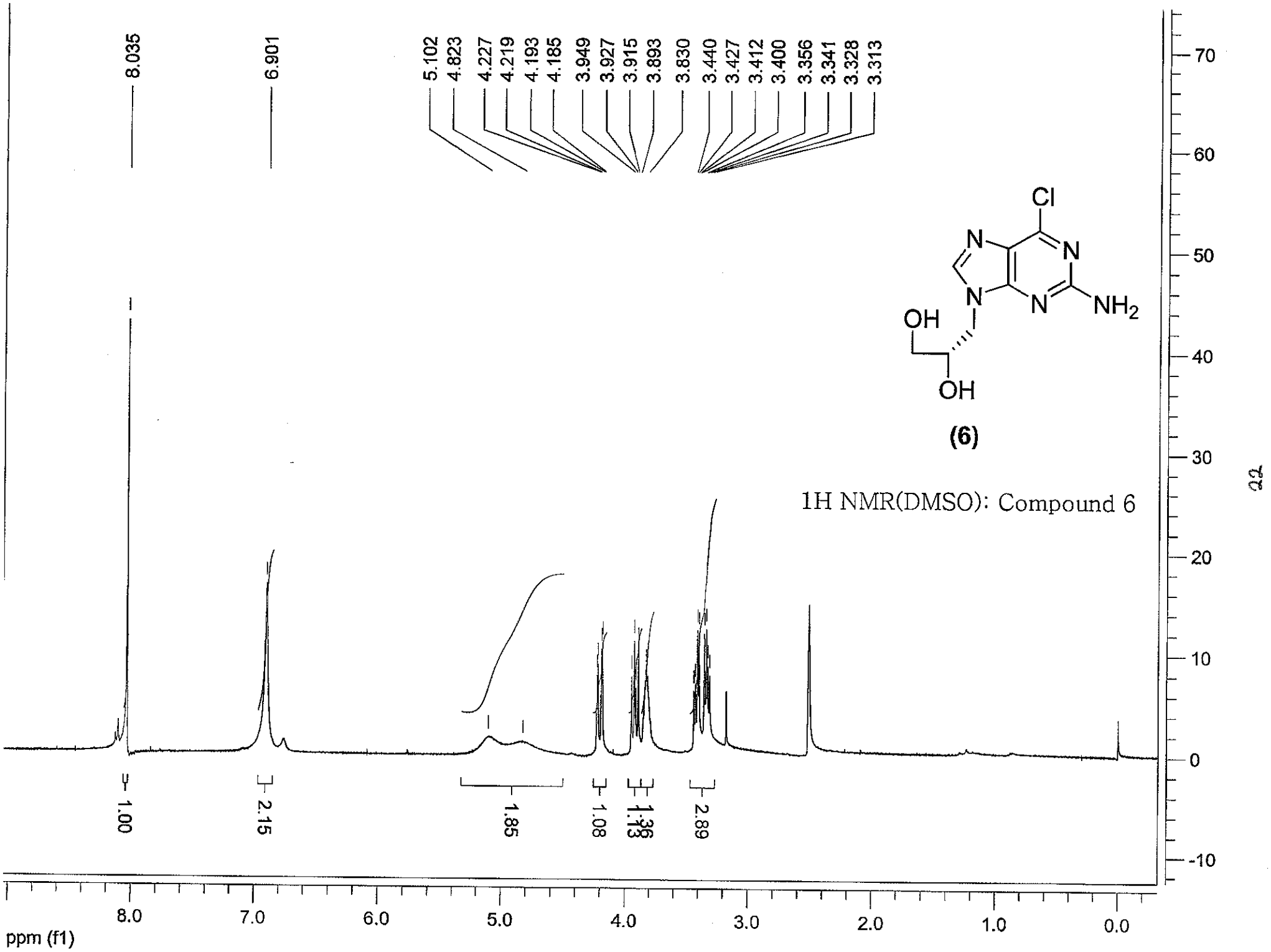


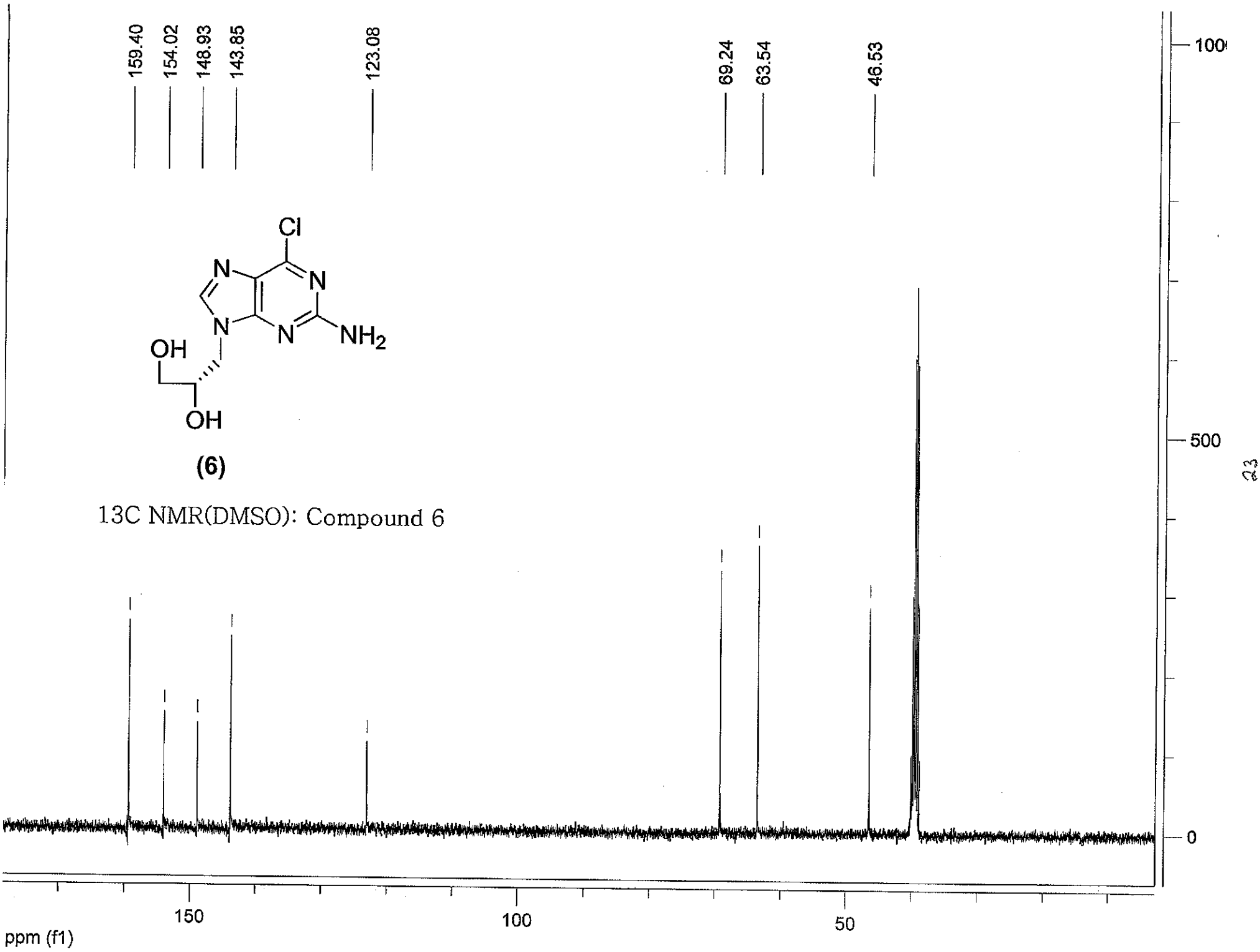
(4)

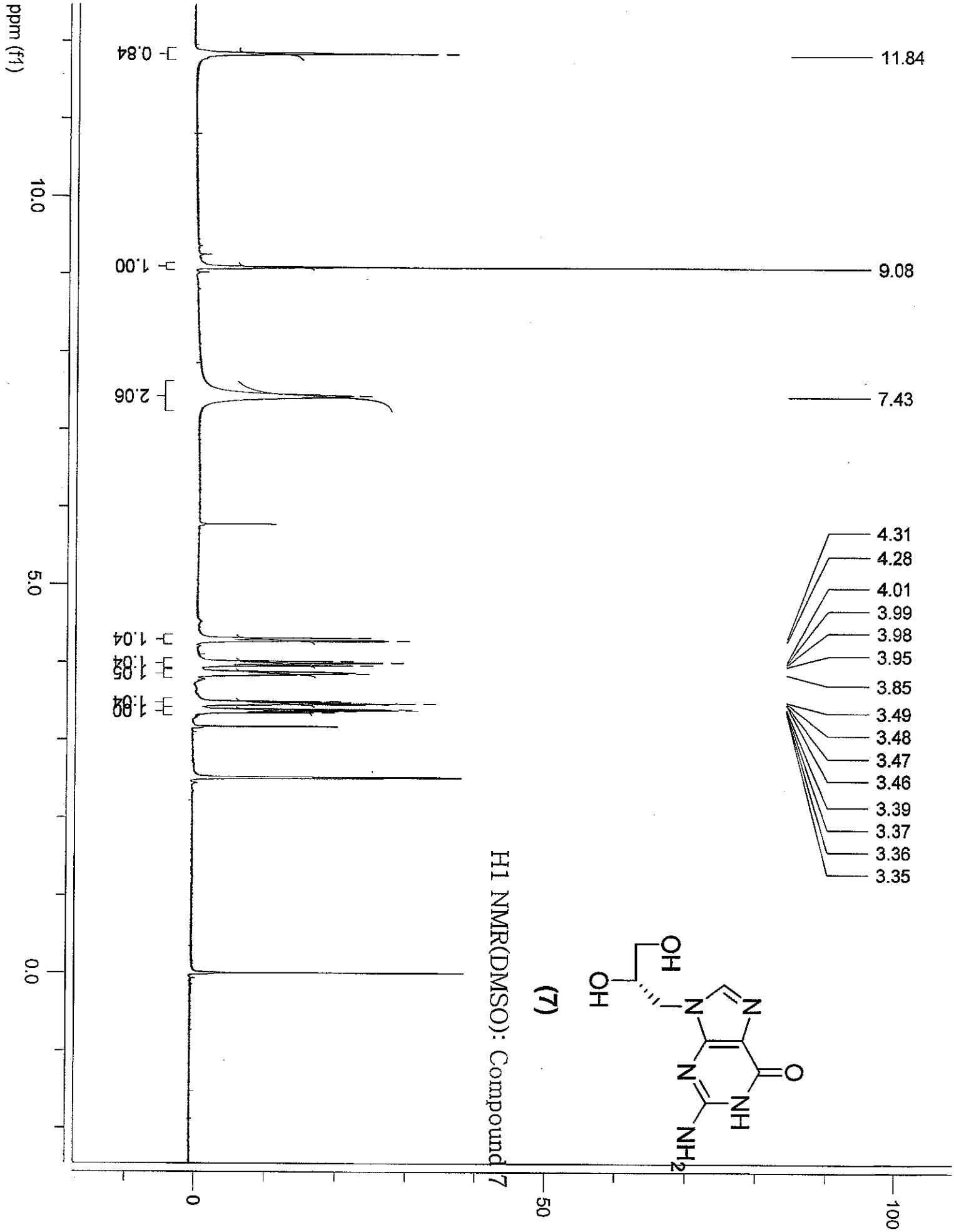
C13 NMR(DMSO): Compound 4



31P NMR (D2O): Compound 5

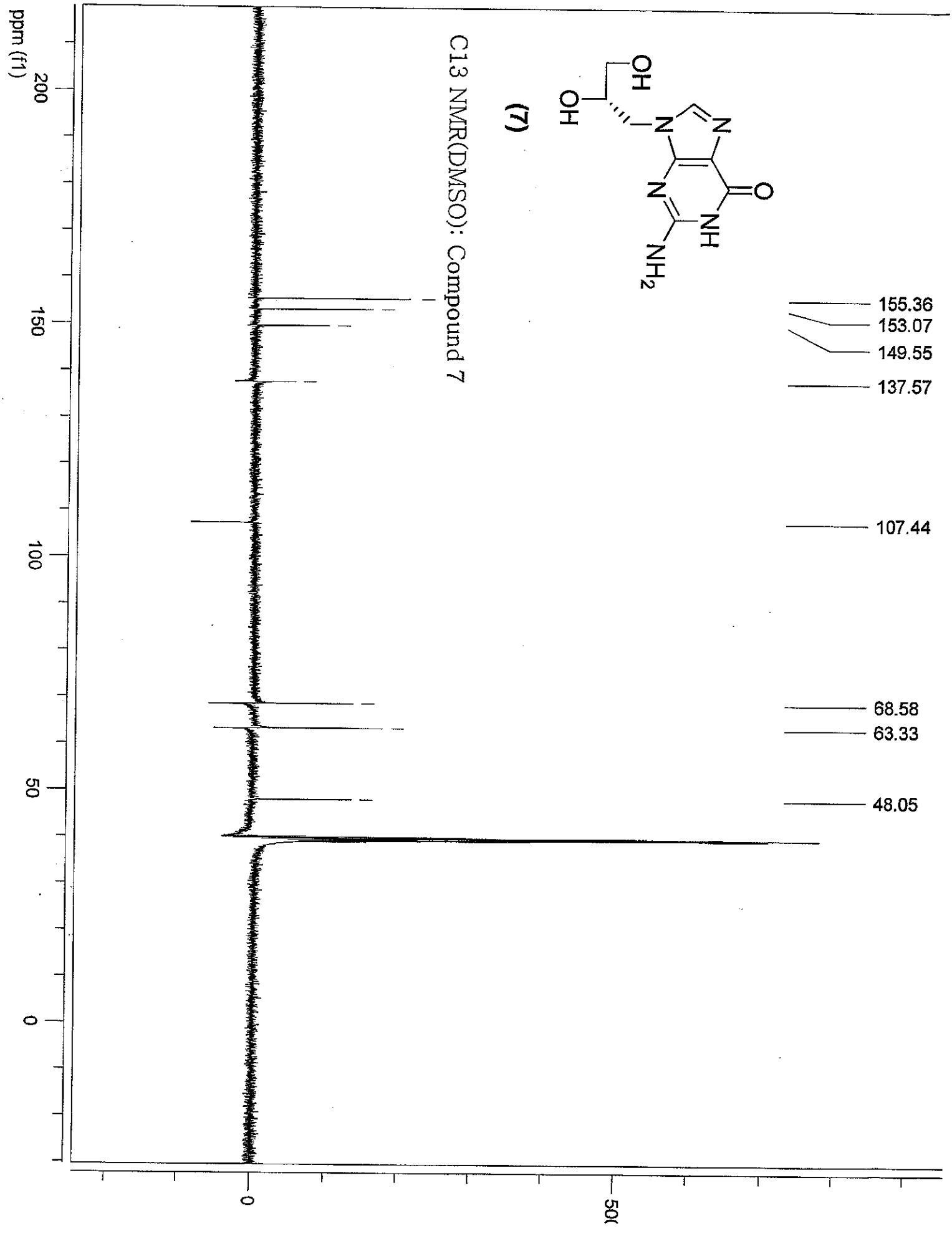

8.035
6.901
5.102
4.823
4.227
4.219
4.193
4.185
3.949
3.927
3.915
3.893
3.830
3.440
3.427
3.412
3.400
3.356
3.341
3.328
3.313
Cl
N
N
N
N
NH2
OH
OH
(6)
1H NMR(DMSO): Compound 6
1.00
2.15
1.85
1.08
1.13
2.89
ppm (f1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
70
60
50
40
30
20
10
0
-10

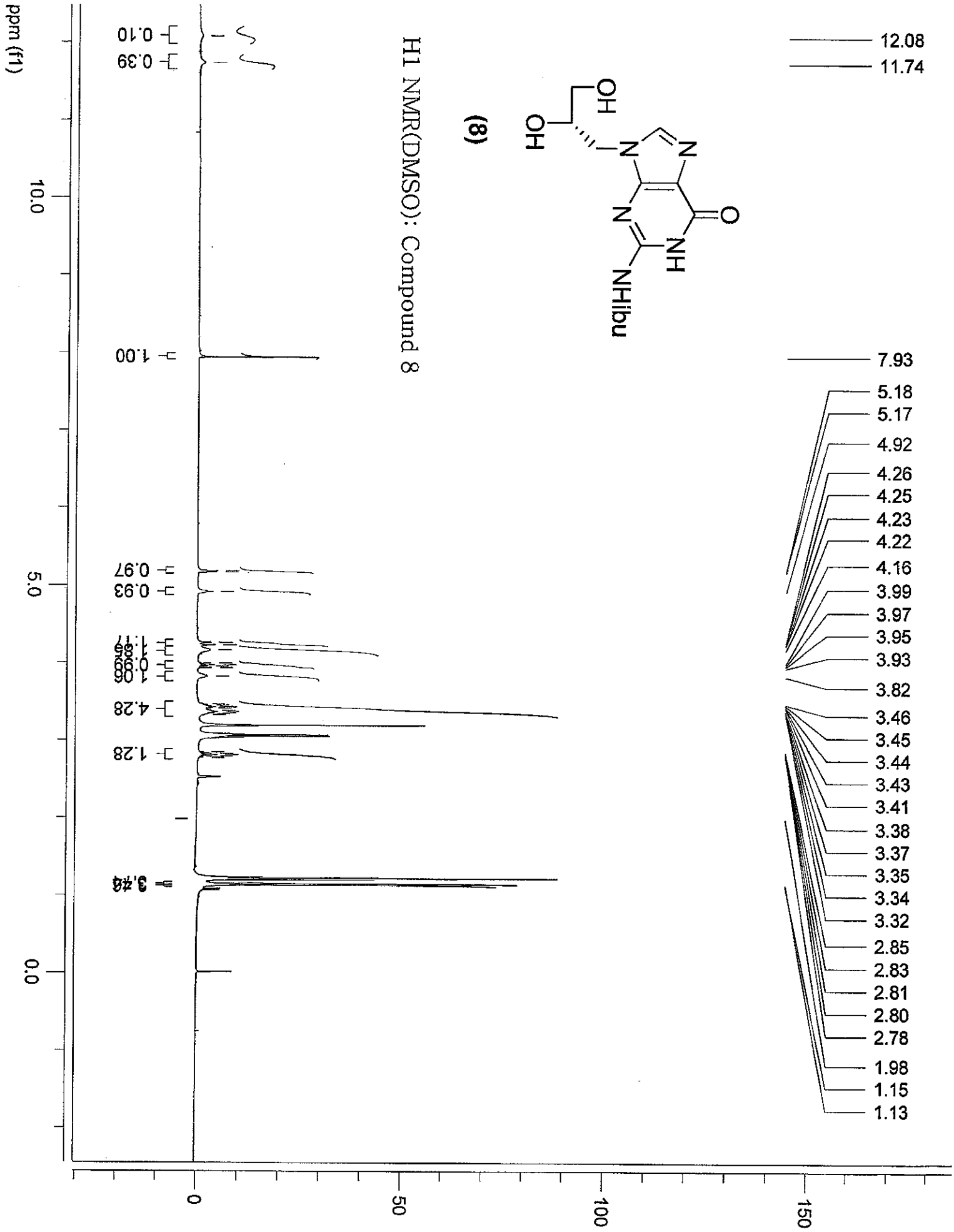

159.40
154.02
148.93
143.85
123.08
69.24
63.54
46.53
Cl
N
N
N
N
NH2
OH
OH
(6)
13C NMR(DMSO): Compound 6
100
500
0
150
100
50
ppm (f1)

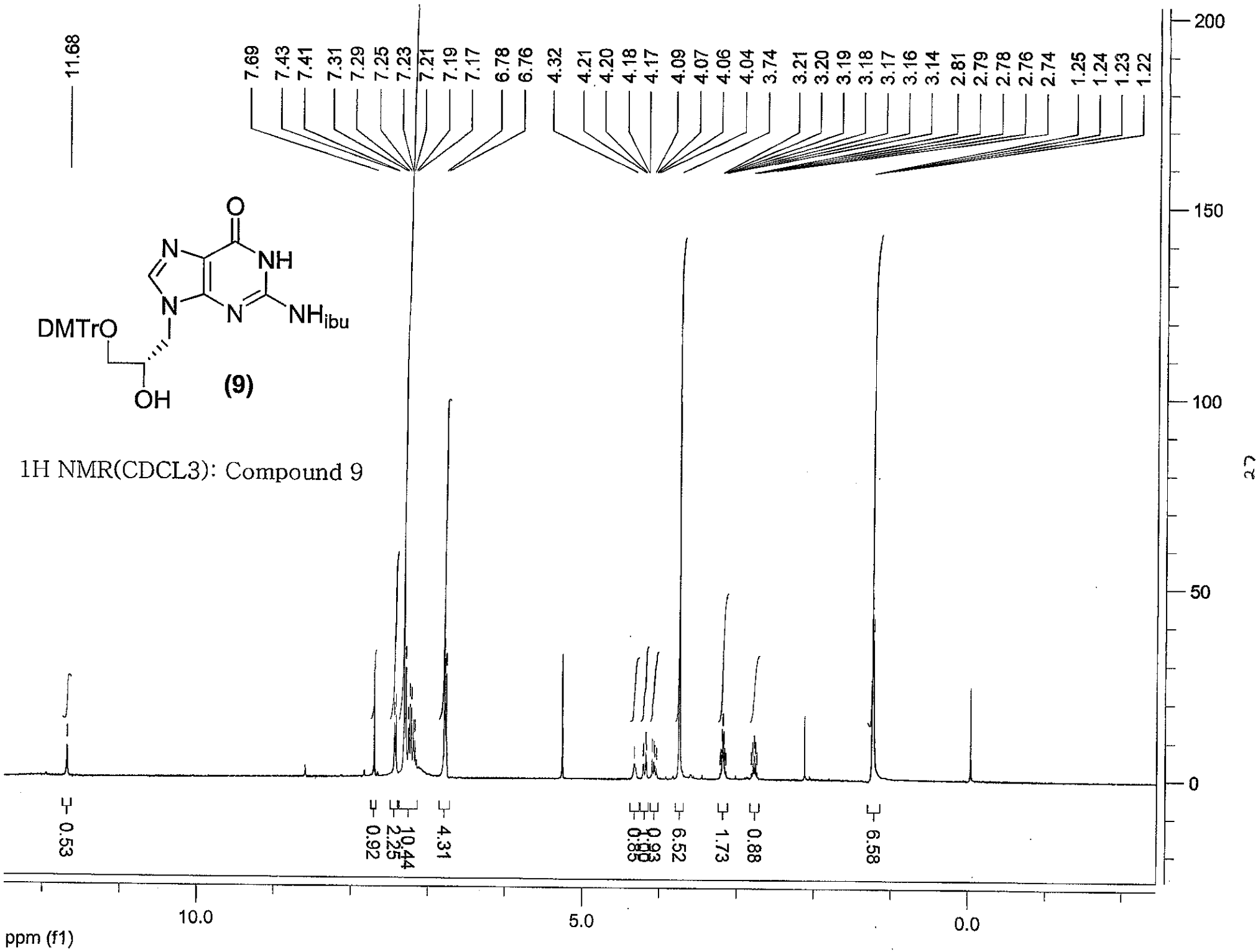


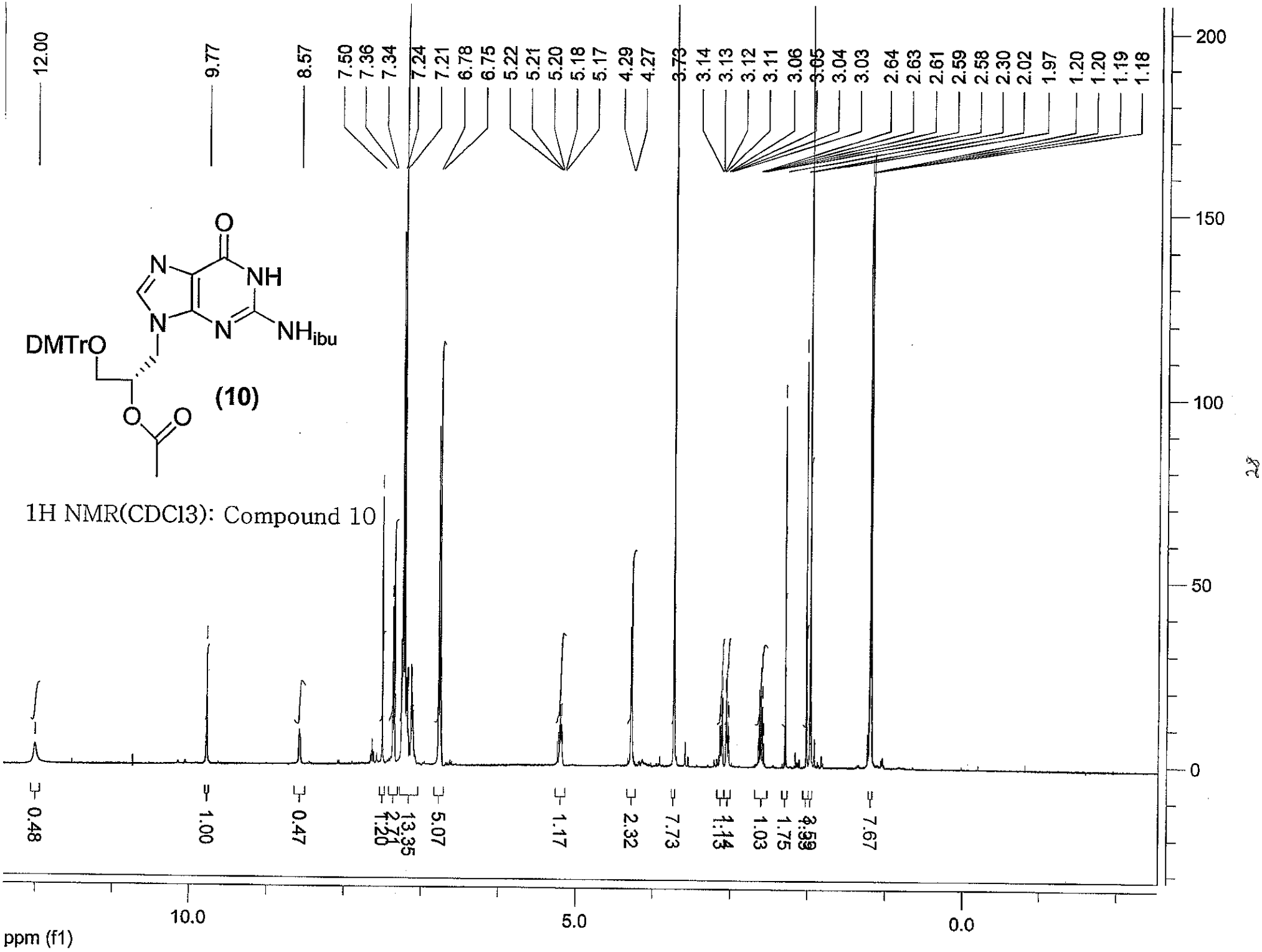
H1 NMR(DMSO): Compound 7

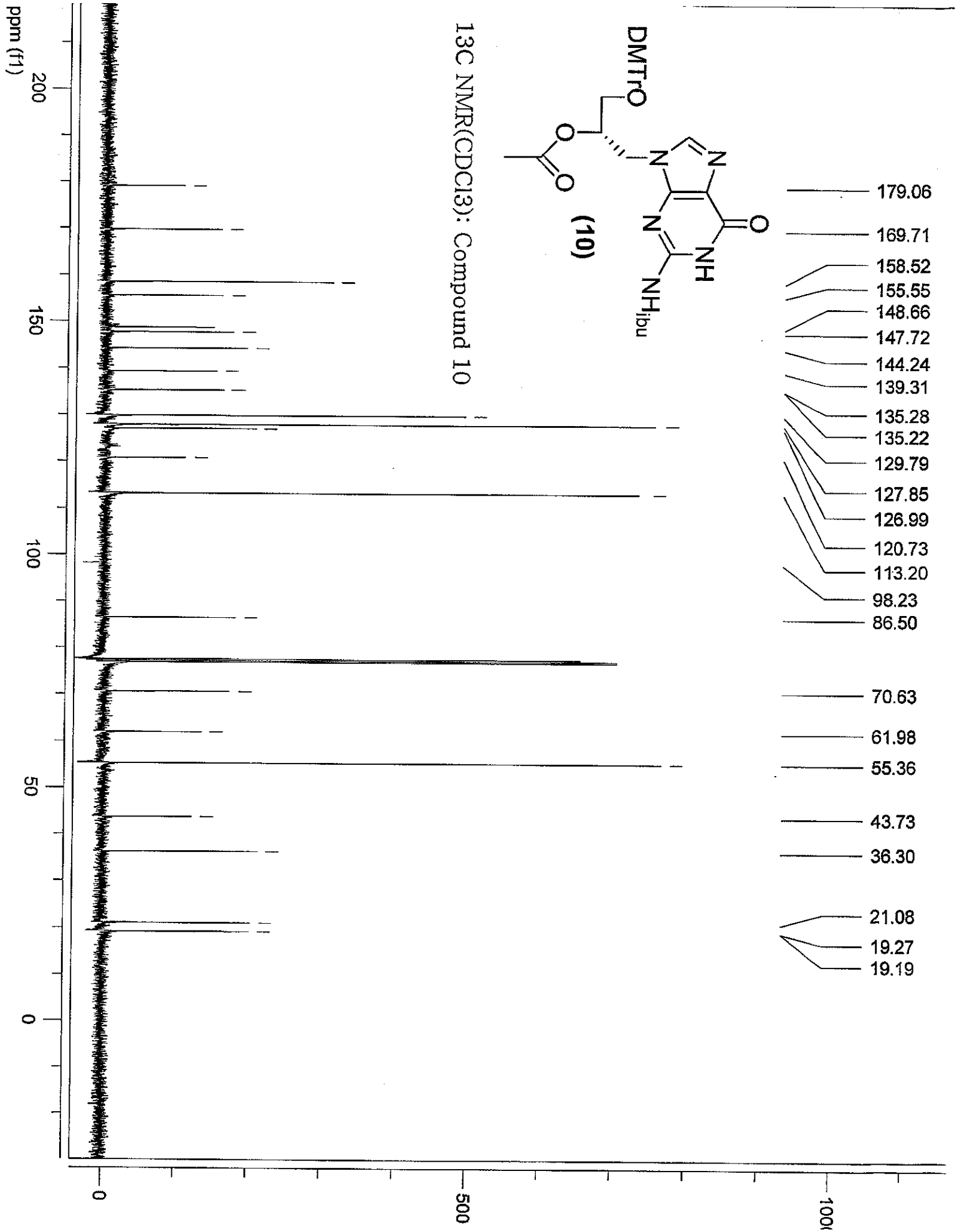
(7)

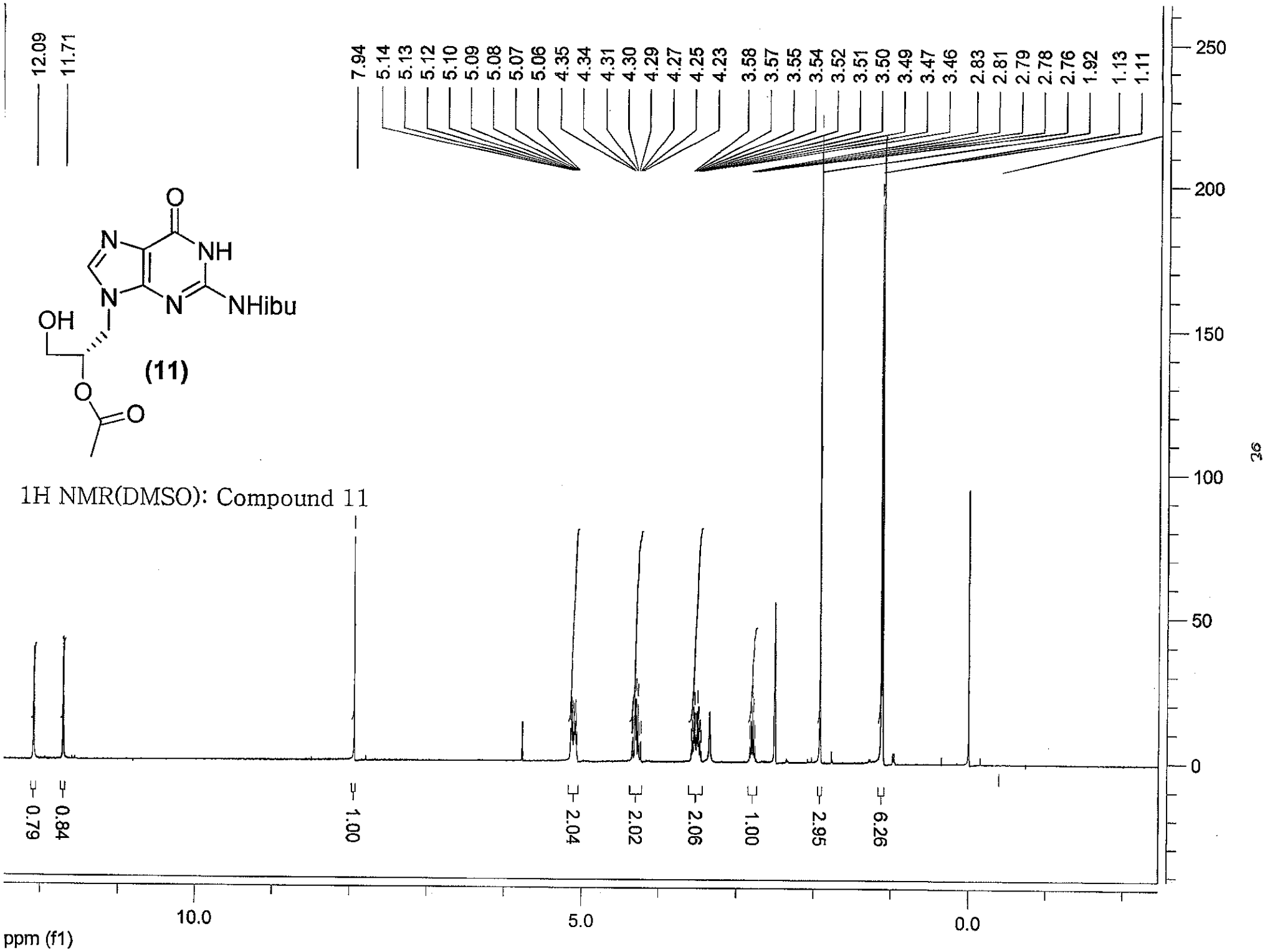

C13 NMR(DMSO): Compound 7
(7)
OH
OH
N
N
N
N
O
NH
NH2
155.36
153.07
149.55
137.57
107.44
68.58
63.33
48.05
ppm (f1)
200
150
100
50
0
0
50

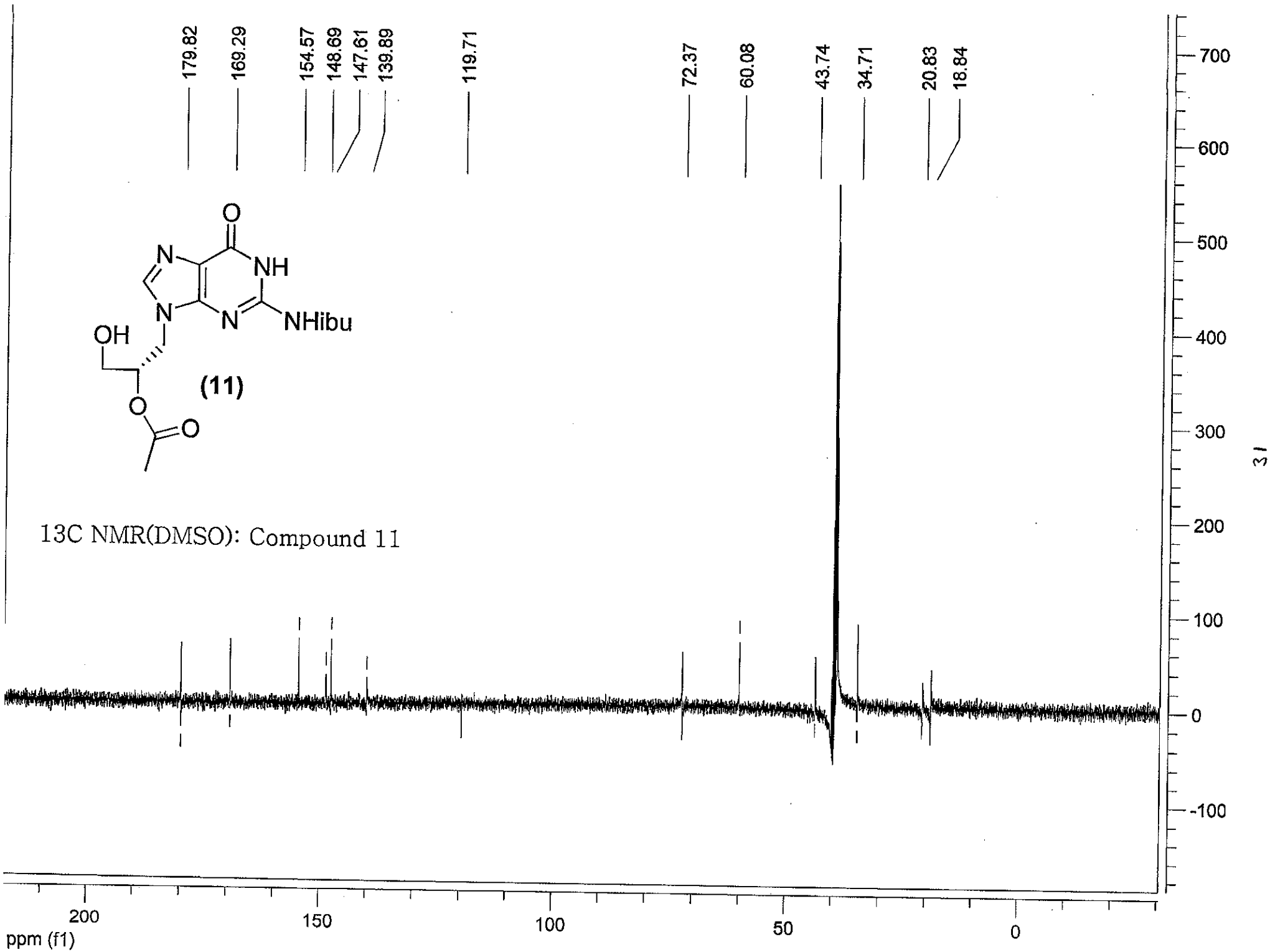

H1 NMR(DMSO): Compound 8
(8)
NHibu
12.08
11.74
7.93
5.18
5.17
4.92
4.26
4.25
4.23
4.22
4.16
3.99
3.97
3.95
3.93
3.82
3.46
3.45
3.44
3.43
3.41
3.38
3.37
3.35
3.34
3.32
2.85
2.83
2.81
2.80
2.78
1.98
1.15
1.13
ppm (f1)

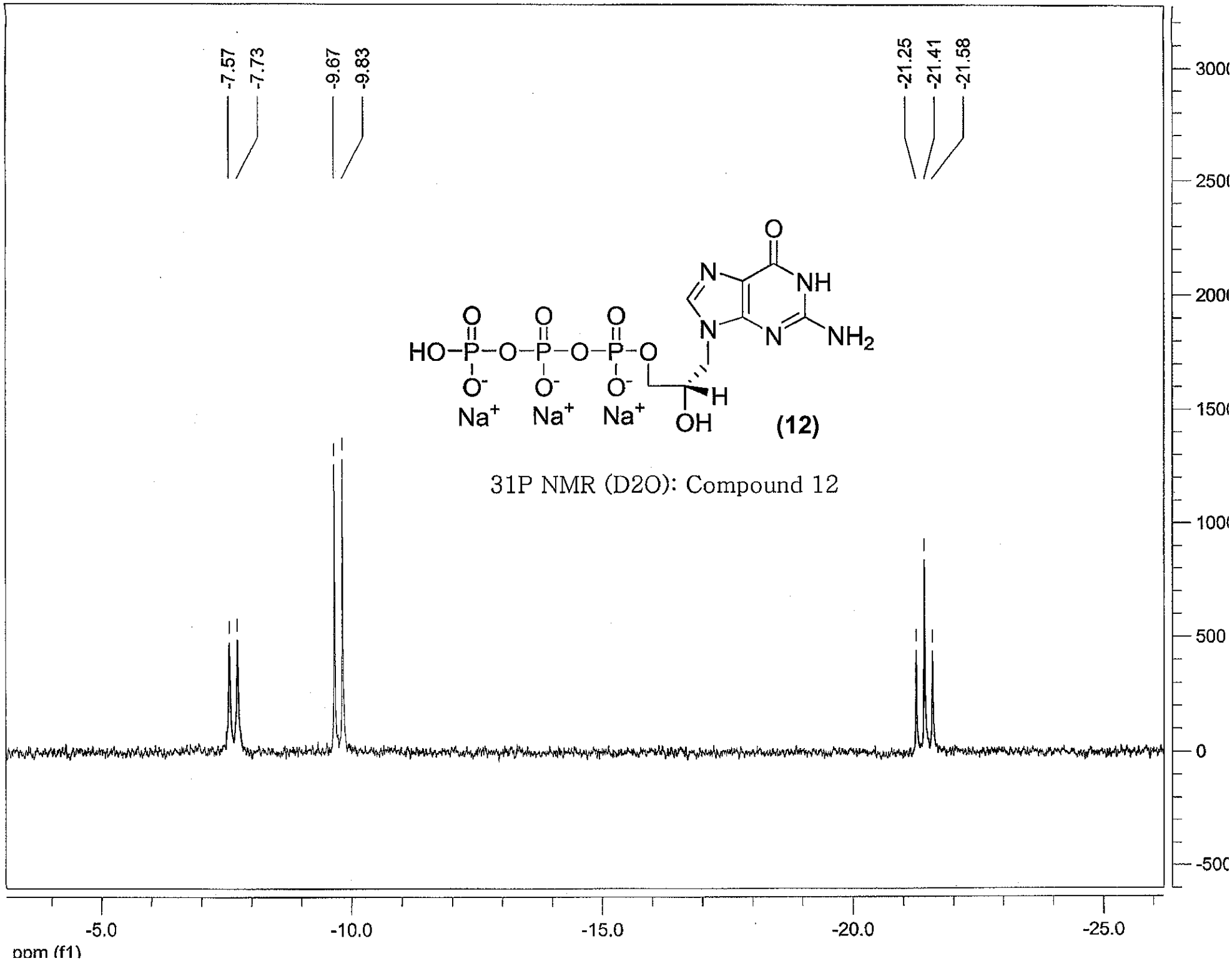

11.68
7.69
7.43
7.41
7.31
7.29
7.25
7.23
7.21
7.19
7.17
6.78
6.76
4.32
4.21
4.20
4.18
4.17
4.09
4.07
4.06
4.04
3.74
3.21
3.20
3.19
3.18
3.17
3.16
3.14
2.81
2.79
2.78
2.76
2.74
1.25
1.24
1.23
1.22
O
NH
N
N
N
NH$_{ibu}$
DMTrO
OH
(9)
1H NMR(CDCL3): Compound 9
0.53
0.92
2.25
10.44
4.31
0.85
0.93
6.52
1.73
0.88
6.58
200
150
100
50
0
10.0
5.0
0.0
ppm (f1)

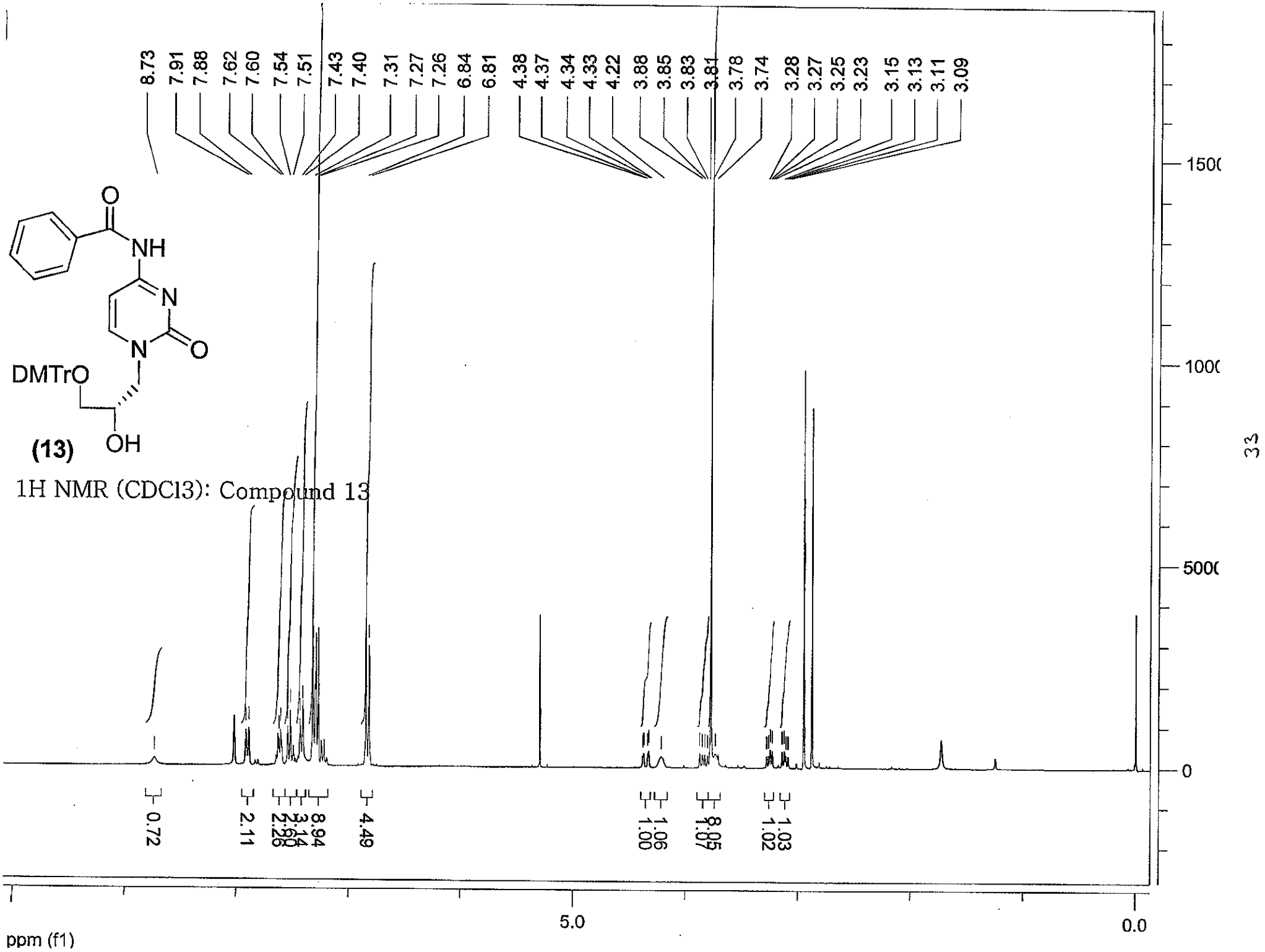

12.00
9.77
8.57
7.50
7.36
7.34
7.24
7.21
6.78
6.75
5.22
5.21
5.20
5.18
5.17
4.29
4.27
3.73
3.14
3.13
3.12
3.11
3.06
3.05
3.04
3.03
2.64
2.63
2.61
2.59
2.58
2.30
2.02
1.97
1.20
1.20
1.19
1.18
DMTrO
NH
NH$_{ibu}$
(10)
1H NMR(CDCl3): Compound 10
0.48
1.00
0.47
1.20
2.71
13.35
5.07
1.17
2.32
7.73
1.14
1.03
1.75
7.67
200
150
100
50
0
10.0
5.0
0.0
ppm (f1)

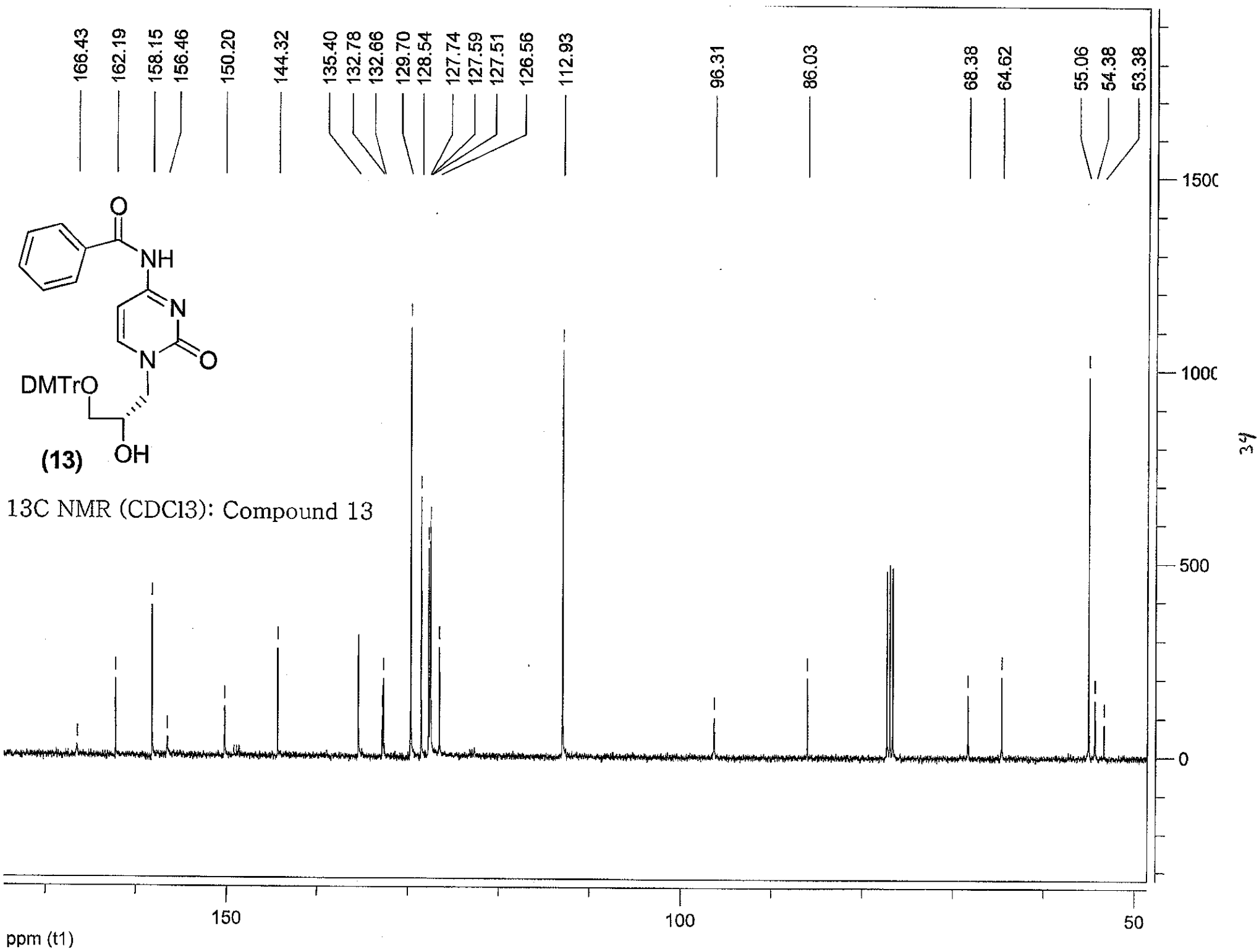

13C NMR(CDCl3): Compound 10
DMTrO
O
O
(10)
N
N
N
NH
O
NHIbu
179.06
169.71
158.52
155.55
148.66
147.72
144.24
139.31
135.28
135.22
129.79
127.85
126.99
120.73
113.20
98.23
86.50
70.63
61.98
55.36
43.73
36.30
21.08
19.27
19.19
ppm (f1)
200
150
100
50
0
0
500
100

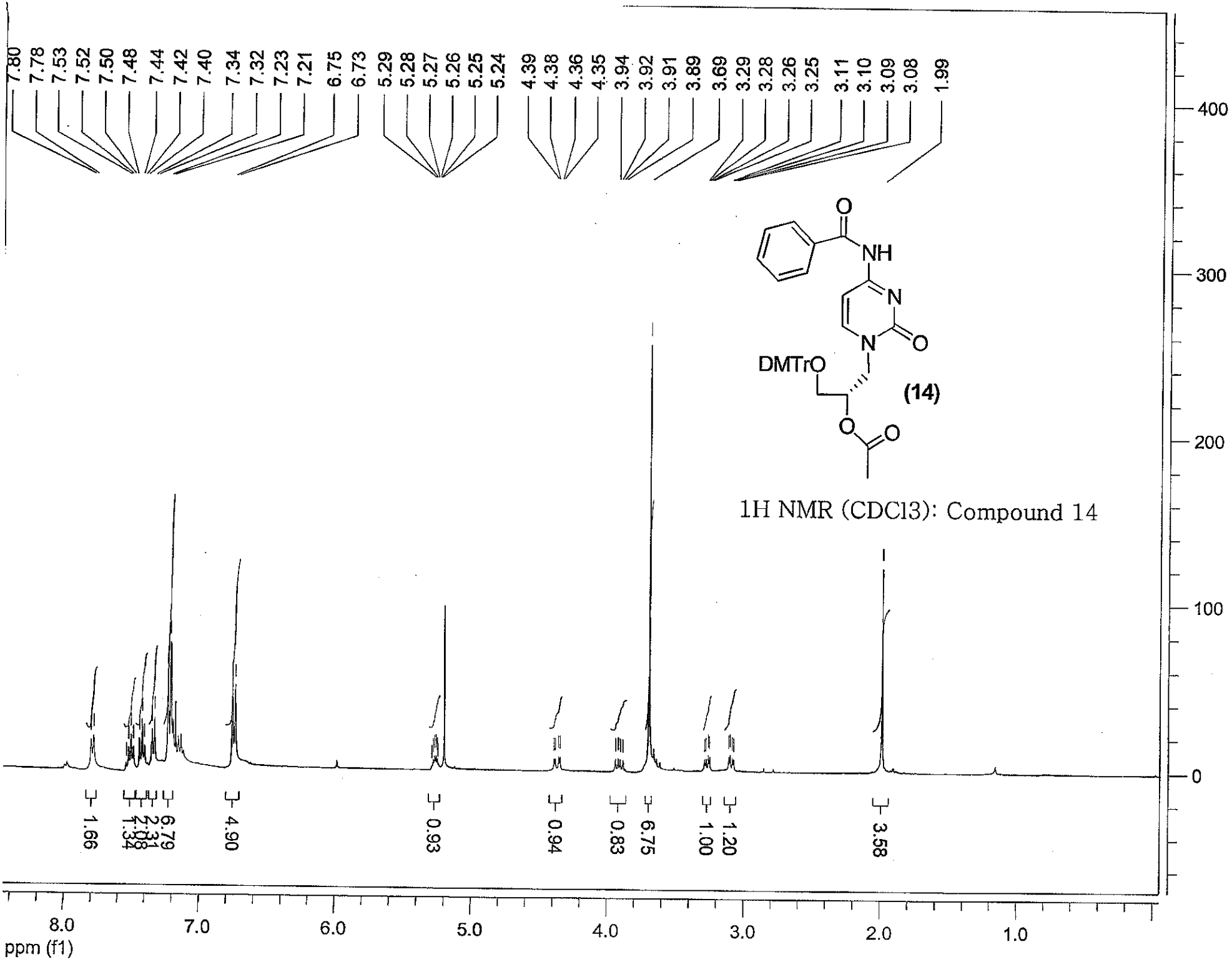

12.09
11.71
7.94
5.14
5.13
5.12
5.10
5.09
5.08
5.07
5.06
4.35
4.34
4.31
4.30
4.29
4.27
4.25
4.23
3.58
3.57
3.55
3.54
3.52
3.51
3.50
3.49
3.47
3.46
2.83
2.81
2.79
2.78
2.76
1.92
1.13
1.11
O
N
NH
N
N
NHibu
OH
(11)
O
O
1H NMR(DMSO): Compound 11
0.79
0.84
1.00
2.04
2.02
2.06
1.00
2.95
6.29
250
200
150
100
50
0
10.0
5.0
0.0
ppm (f1)


179.82
169.29
154.57
148.69
147.61
139.89
119.71
72.37
60.08
43.74
34.71
20.83
18.84
O
N
NH
N
N
NHibu
OH
O
O
O
(11)
13C NMR(DMSO): Compound 11
700
600
500
400
300
200
100
0
-100
200
150
100
50
0
ppm (f1)

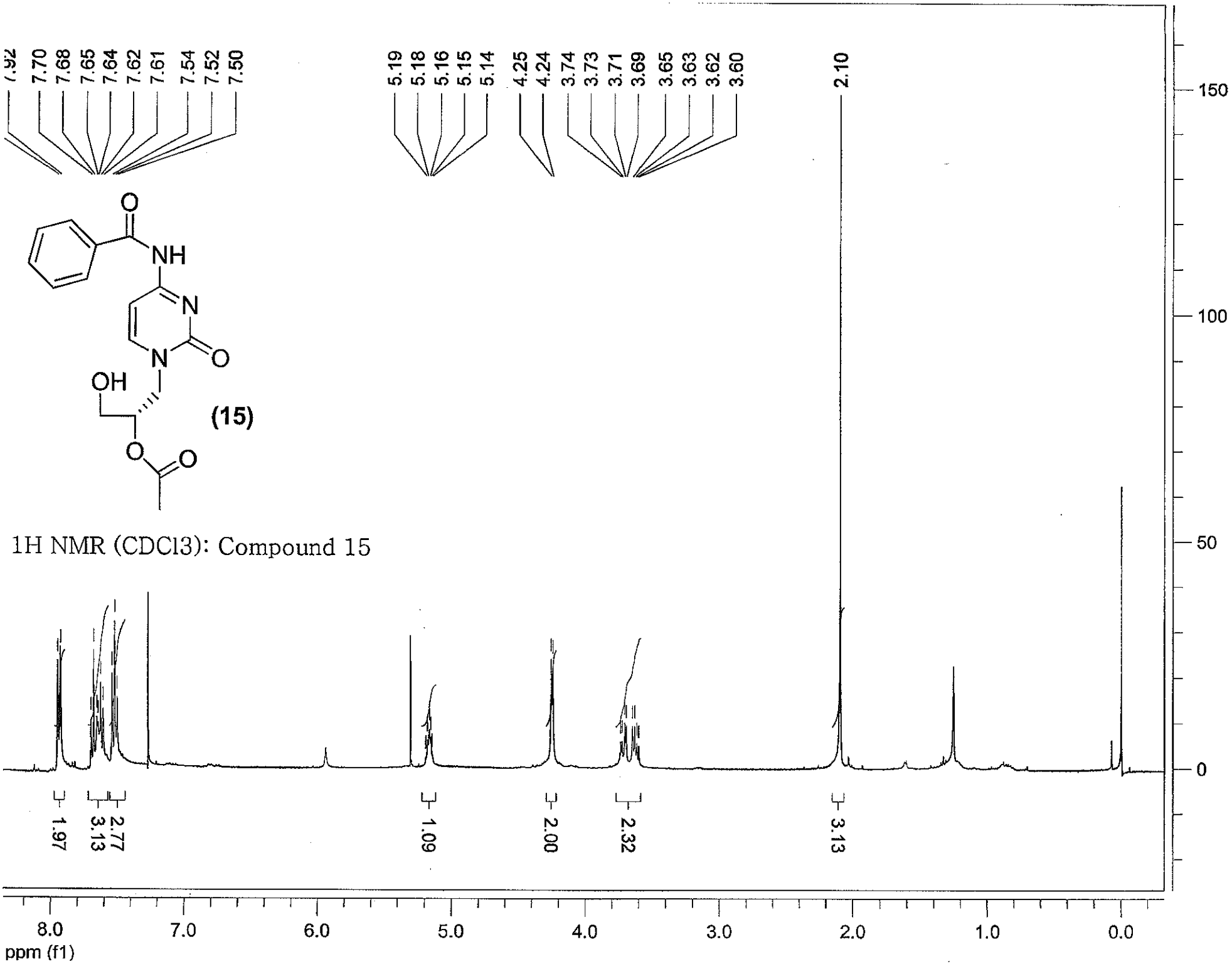

-7.57
-7.73
-9.67
-9.83
-21.25
-21.41
-21.58
HO–P–O–P–O–P–O
Na+ Na+ Na+
OH
H
NH
NH2
(12)
31P NMR (D2O): Compound 12
-5.0
-10.0
-15.0
-20.0
-25.0
ppm (f1)


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
O
NH
N
N
O
DMTrO
OH
(13)
1H NMR (CDCl3): Compound 13
0.72
2.11
2.26
3.14
8.94
4.49
1.00
1.06
1.07
8.05
1.02
1.03
1500
1000
500
0
33
5.0
0.0
ppm (f1)

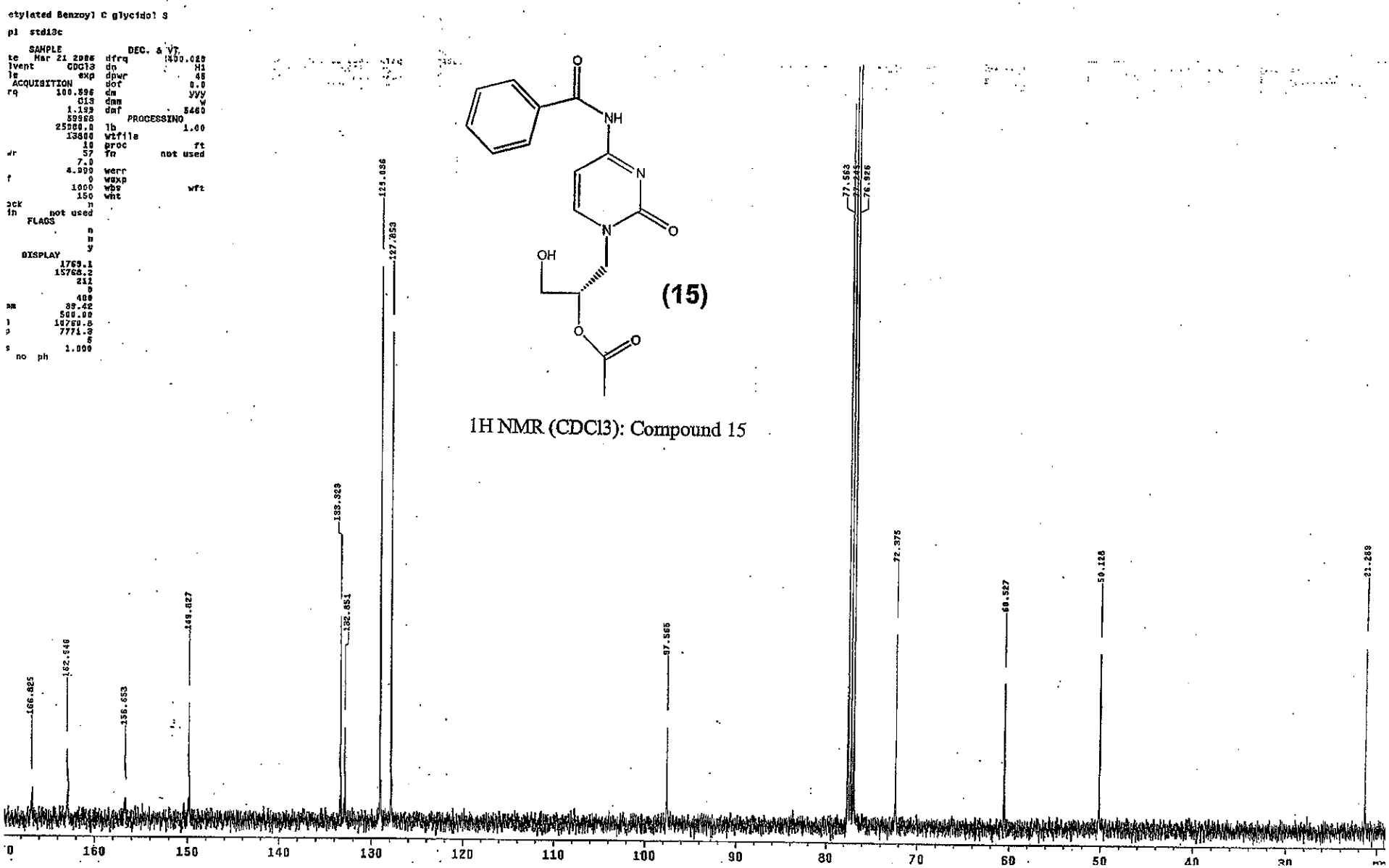

166.43
162.19
158.15
156.46
150.20
144.32
135.40
132.78
132.66
129.70
128.54
127.74
127.59
127.51
126.56
112.93
96.31
86.03
68.38
64.62
55.06
54.38
53.38
O
NH
N
N
O
DMTrO
OH
(13)
13C NMR (CDCl3): Compound 13
1500
1000
500
0
150
100
50
ppm (t1)

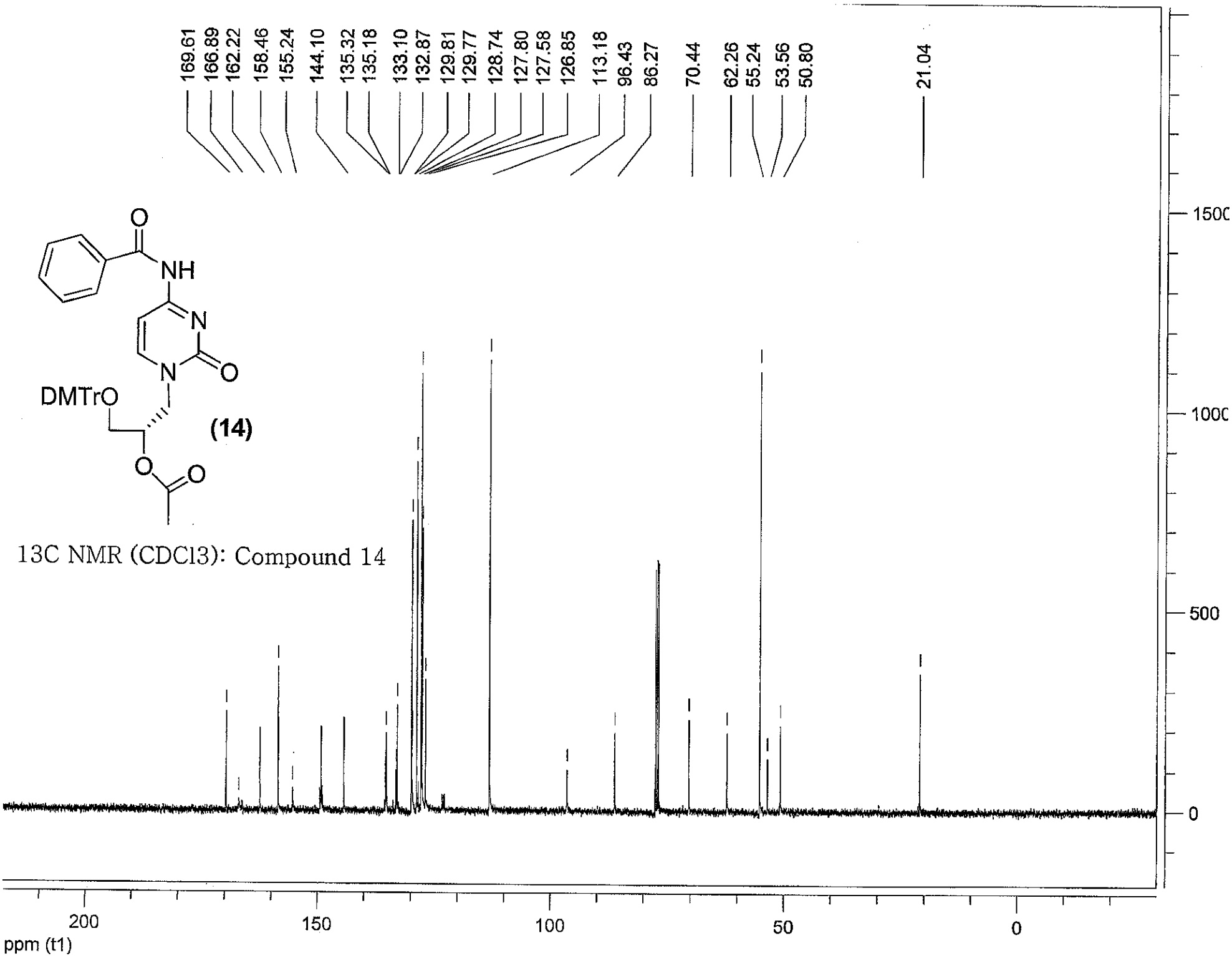


1H NMR (CDCl3): Compound 14

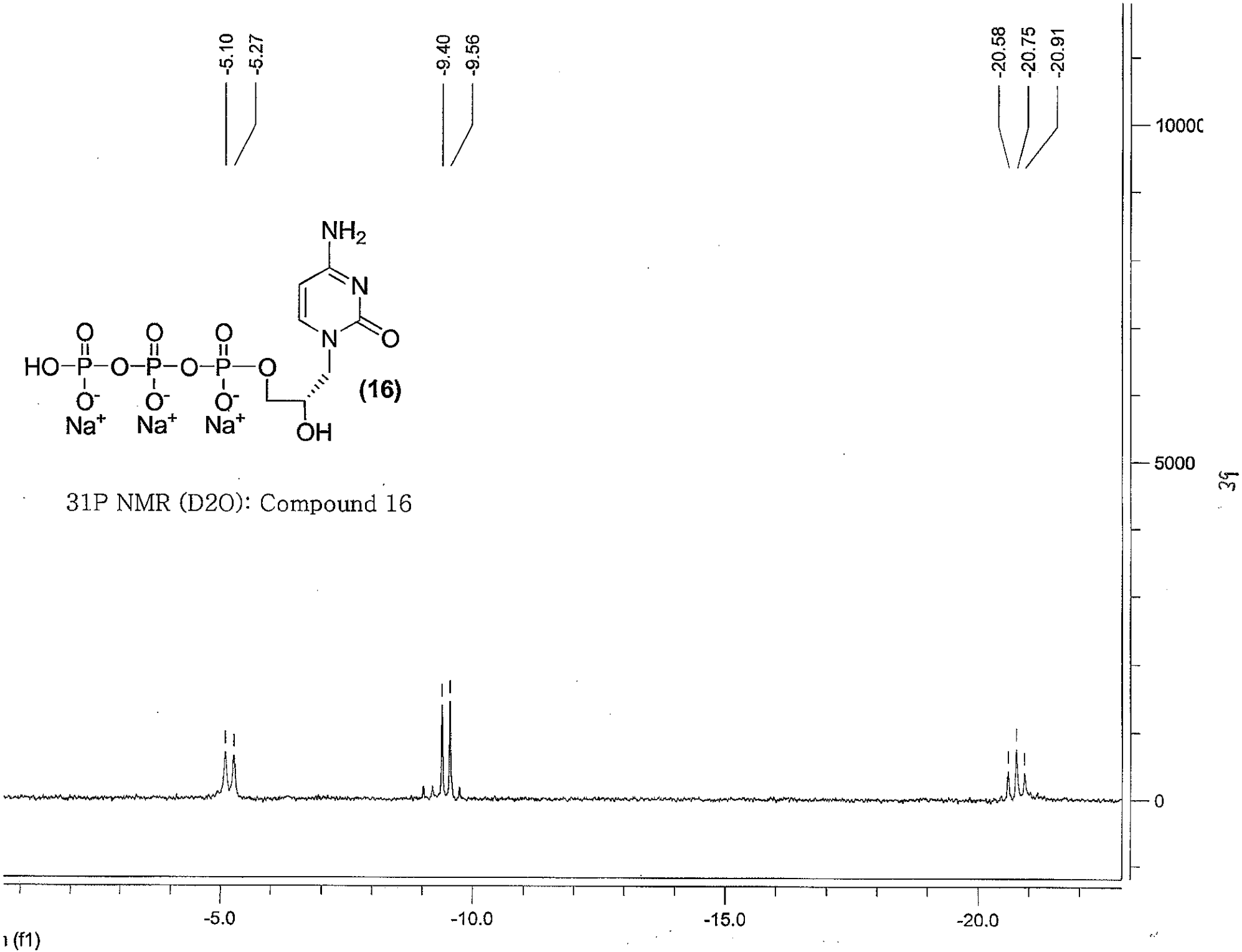


1H NMR (CDCl3): Compound 15

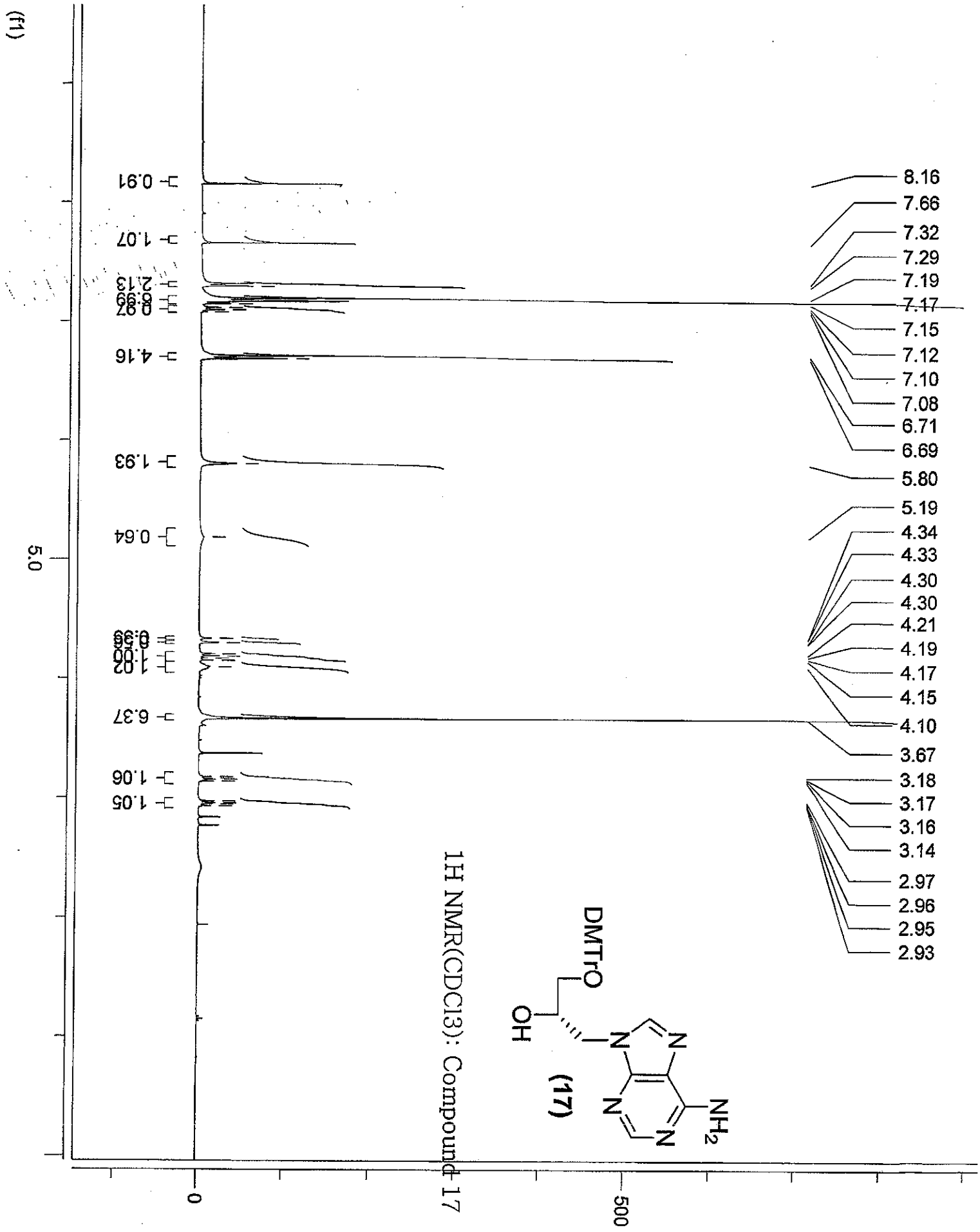

ctylated Benzoyl C glycidol S
pl std13c
SAMPLE
te Mar 21 2006
lvent CDCl3
le exp
ACQUISITION
rq 100.596
D13
1.199
59966
25000.0
13800
16
sr 57
7.0
4.999
f 0
1000
150
ack n
in not used
FLAGS
n
n
y
DISPLAY
1769.1
15768.2
211
0
488
ss 39.42
500.00
l 10759.8
p 7771.9
s
s 1.000
no ph
DEC. & VT
dfrq 400.028
dn H1
dpwr 48
dof 0.0
dm yyy
dmm w
dmf 5460
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp
wbs wft
wnt
NH
N
N
O
O
OH
O
O
(15)
1H NMR (CDCl3): Compound 15
166.825
162.548
156.653
149.827
133.329
132.851
129.036
127.859
97.565
77.563
77.245
76.926
72.375
60.527
50.128
21.259
0
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm



13C NMR (CDCl3): Compound 14

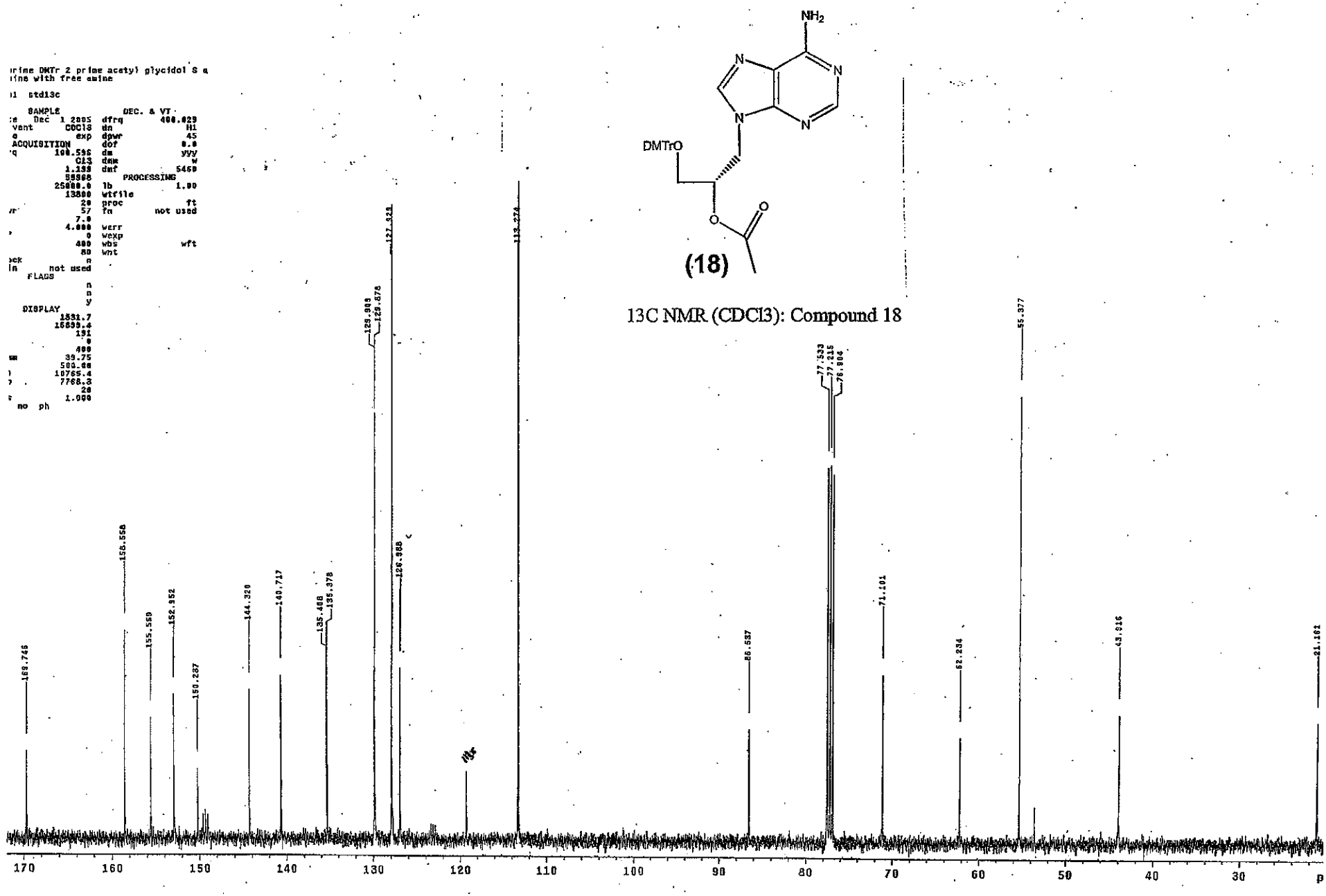


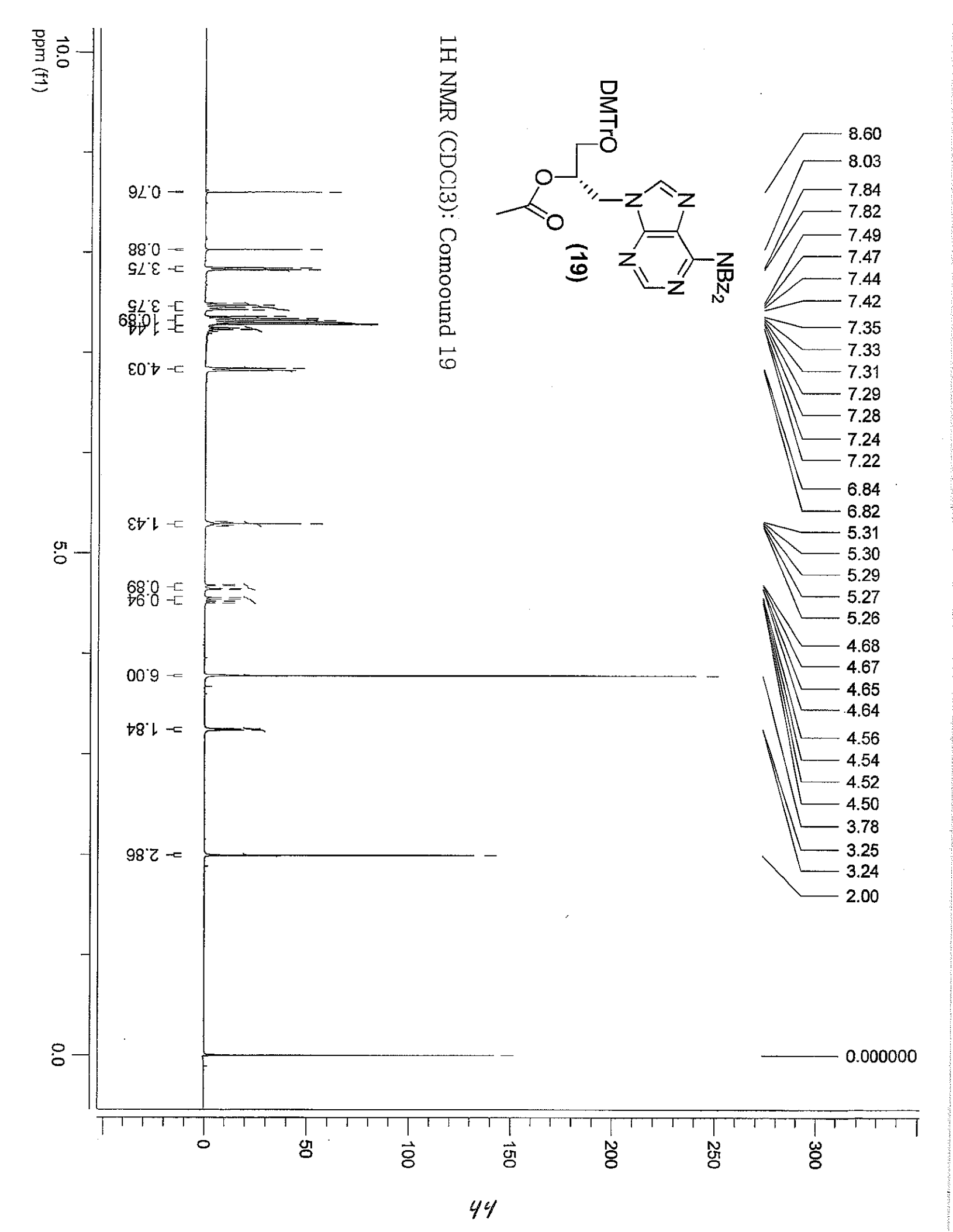
31P NMR (D2O): Compound 16

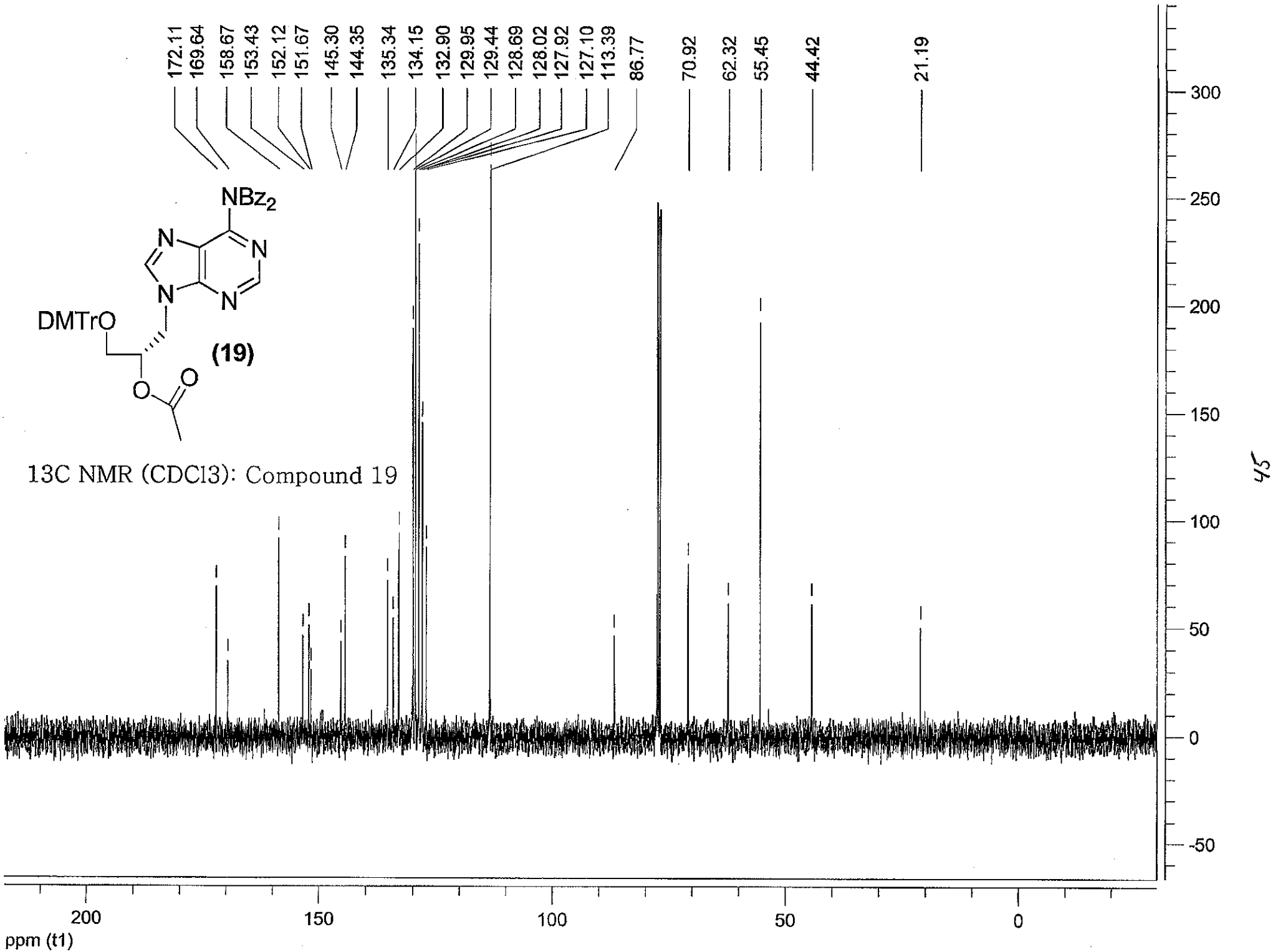

(f1)
5.0
0
500
1H NMR(CDCl3): Compound 17
DMTrO
OH
(17)
NH2
N
8.16
7.66
7.32
7.29
7.19
7.17
7.15
7.12
7.10
7.08
6.71
6.69
5.80
5.19
4.34
4.33
4.30
4.30
4.21
4.19
4.17
4.15
4.10
3.67
3.18
3.17
3.16
3.14
2.97
2.96
2.95
2.93
0.91
1.07
2.13
0.97
4.16
1.93
0.64
1.02
6.37
1.06
1.05

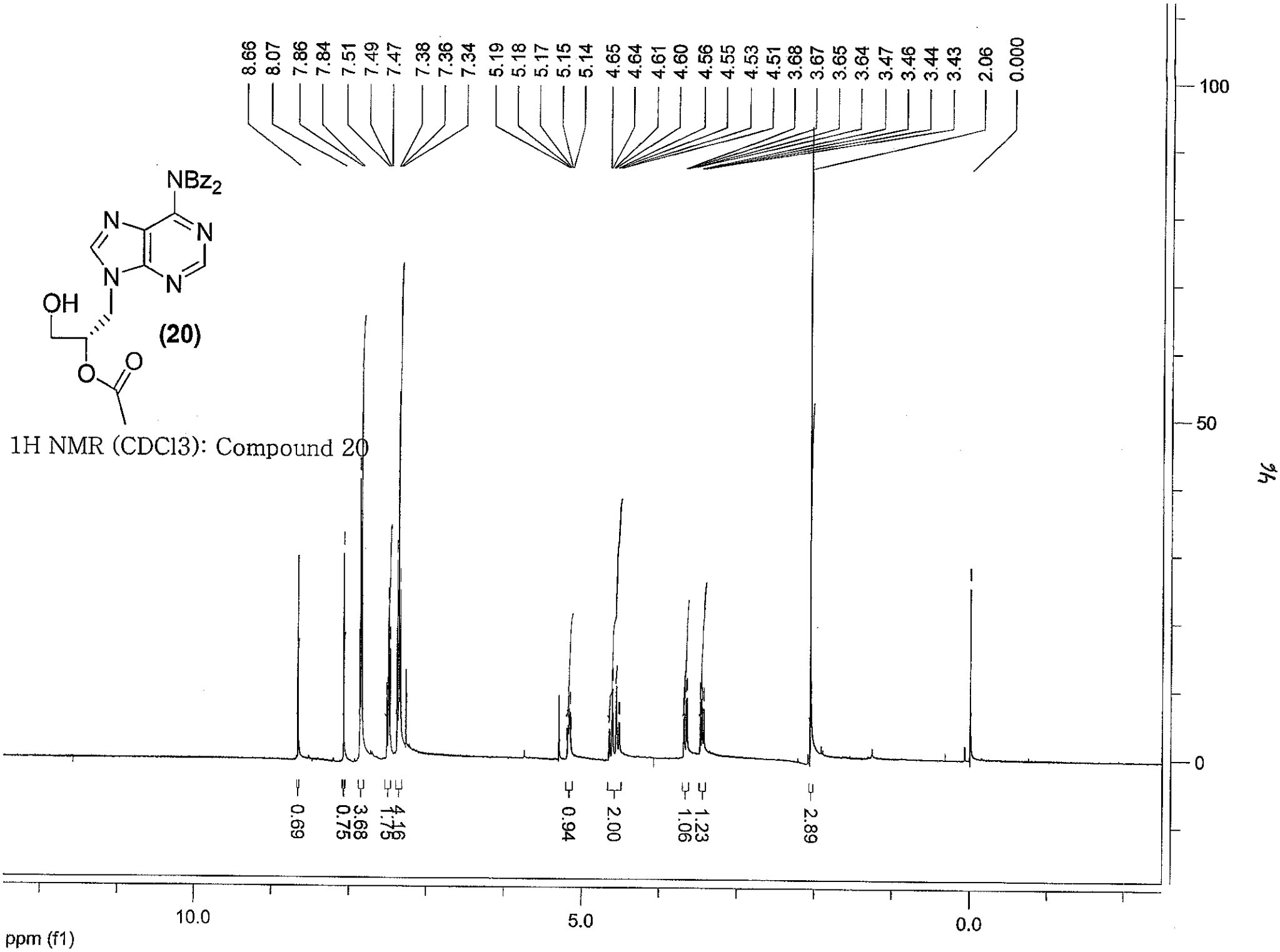
13C NMR(CDCl3): Compound 17
DMTrO
OH
(17)
NH2
158.39
155.52
152.41
149.77
144.53
141.40
135.65
135.59
129.91
127.98
127.81
126.83
119.05
113.15
86.41
69.32
64.93
55.32
50.27
48.16
ppm (f1)
150
100
50
0
500
100

ppm (f1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.87
0.98
2.22
7.04
1.13
4.37
1.98
1.09
1.06
1.00
6.71
2.15
3.17
8.32
7.73
7.46
7.43
7.33
7.31
7.29
7.25
7.24
6.85
6.83
5.91
5.36
5.35
5.34
5.34
5.32
5.31
4.61
4.60
4.58
4.57
4.53
4.51
4.49
4.47
3.80
3.26
3.25
3.24
2.03
-100
0
100
200
300
400
500
600
700
1H NMR(CDCl3): Compound 18
(18)
DMTrO
O
O
N
N
N
N
NH2


rime DMTr 2 prime acetyl glycidol S a
rine with free amine
1 std13c
SAMPLE
Dec 1 2005
vent CDCl3
exp
ACQUISITION
108.59s
C13
1.13s
88565
25000.0
13800
28
57
7.0
4.000
0
480
80
n
not used
FLAGS
n
n
y
DISPLAY
1531.7
15839.4
191
0
400
39.75
500.08
10765.4
7768.2
28
1.000
no ph
DEC. & VT
dfrq 400.029
dn H1
dpwr 45
dof 0.0
dm yyy
dmm w
dmf 5460
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp
wbs wft
wnt
NH2
N
N
N
N
DMTrO
O
O
(18)
13C NMR (CDCl3): Compound 18
158.558
155.569
152.952
150.287
144.320
140.717
135.488
135.378
129.968
129.678
127.523
126.988
118.6
113.274
86.537
77.533
77.215
76.904
71.101
62.234
55.377
43.816
21.162
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
p

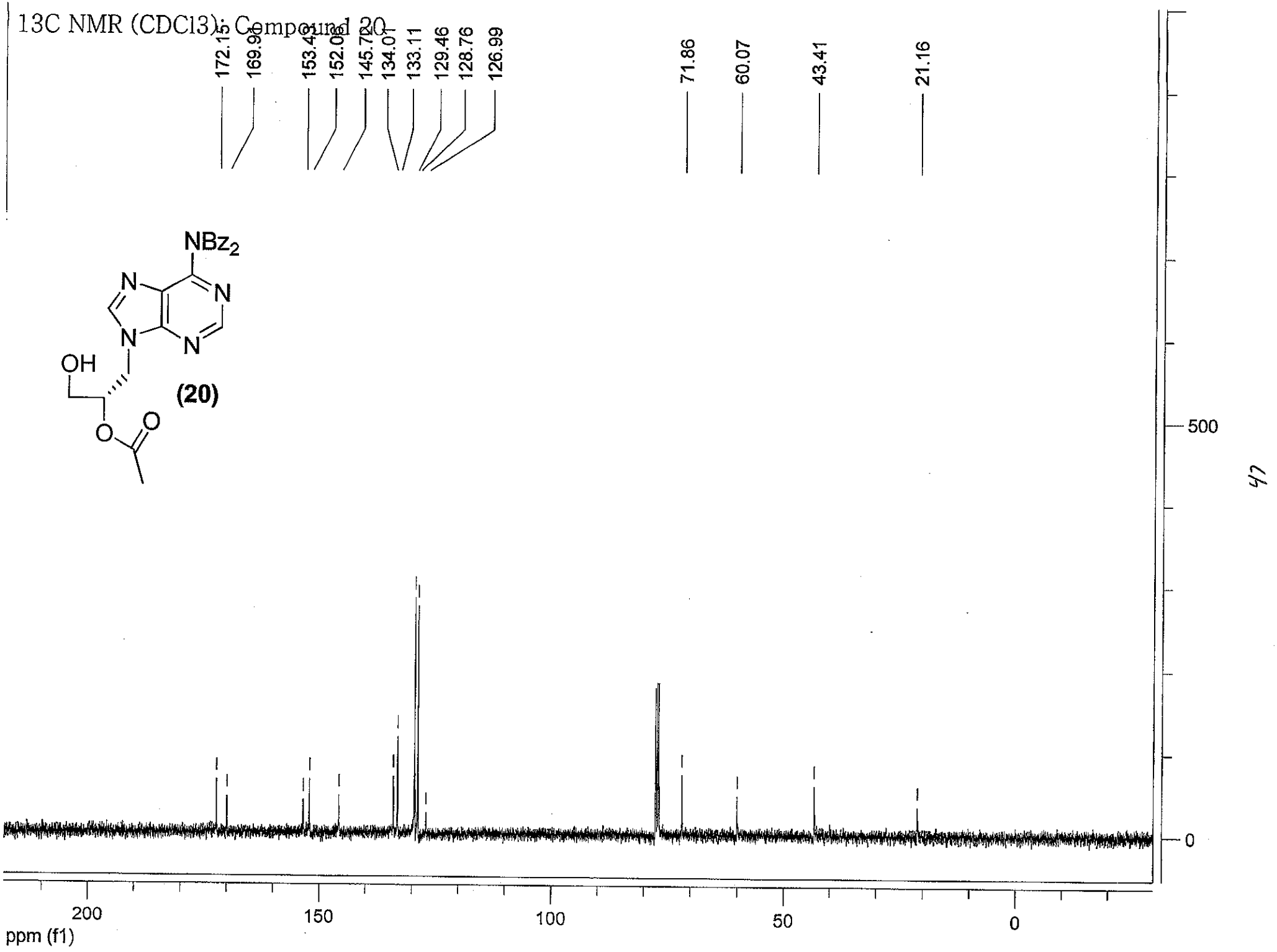

1H NMR (CDCl3): Comoound 19
DMTrO
O
O
(19)
N
N
N
N
NBz2
8.60
8.03
7.84
7.82
7.49
7.47
7.44
7.42
7.35
7.33
7.31
7.29
7.28
7.24
7.22
6.84
6.82
5.31
5.30
5.29
5.27
5.26
4.68
4.67
4.65
4.64
4.56
4.54
4.52
4.50
3.78
3.25
3.24
2.00
0.000000
0.76
0.88
3.75
3.75
0.89
1.44
4.03
1.43
0.89
0.94
6.00
1.84
2.86
10.0
5.0
0.0
ppm (f1)
0
50
100
150
200
250
300


172.11
169.64
158.67
153.43
152.12
151.67
145.30
144.35
135.34
134.15
132.90
129.95
129.44
128.69
128.02
127.92
127.10
113.39
86.77
70.92
62.32
55.45
44.42
21.19
NBz2
N
N
N
N
DMTrO
O
O
(19)
13C NMR (CDCl3): Compound 19
300
250
200
150
100
50
0
-50
200
150
100
50
0
ppm (t1)

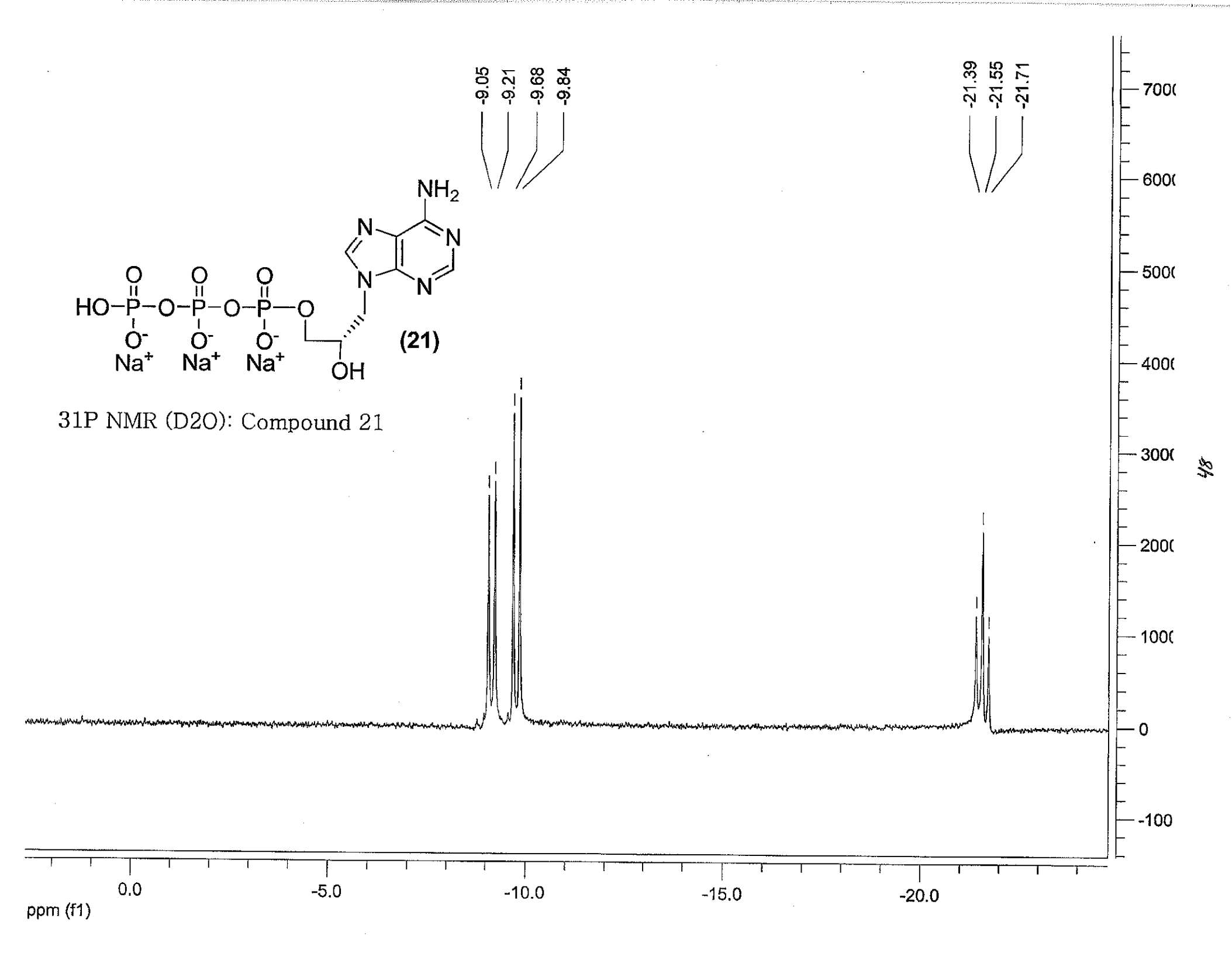


1H NMR (CDCl3): Compound 20

13C NMR (CDCl3): Compound 20



47


-9.05
-9.21
-9.68
-9.84
-21.39
-21.55
-21.71
NH2
HO–P–O–P–O–P–O
O⁻ Na⁺
OH
(21)
31P NMR (D2O): Compound 21
7000
6000
5000
4000
3000
2000
1000
0
-100
0.0
-5.0
-10.0
-15.0
-20.0
ppm (f1)