**Supporting Information**

for

# Design Synthesis and Properties of 2'4'-BNA$^{NC}$: A Bridged Nucleic Acid Analogue

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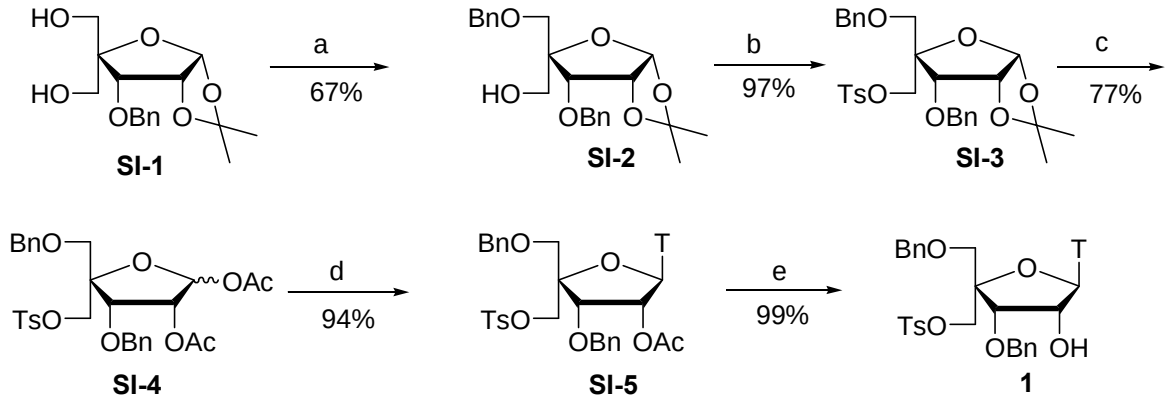
**Contents**

**1. Synthesis of the starting material 1:**

Compound **1** (Scheme SI-1 and Scheme-1 in text also) was synthesized from compound **SI-1** by modification of the procedures described in the literatures.[1,2] Regioselective benzylation of diol **SI-1** was accomplished as described in the literature[1] to afford the dibenzyl derivative **SI-2**. Tosylation of **SI-2** using triethylamine and DMAP instead of pyridine[2] improved the yield to 97%. Acetolysis in the presence of sulphuric acid gave **SI-4** in 77% yield as an anomeric mixture. Incorporation of thymine by the usual method followed by decaetylation by aqueous methylamine (instead of ammonia)[2] provided compound **1** in improved and excellent yields. Full synthetic procedures are described below (experimental procedures):

**Scheme SI-1**: Synthesis of the Starting Material **1**.




*Reagents and conditions*: a) NaH, BnBr, DMF, 0 °C to rt, 4 h; b) $Et_3N$, TsCl, DMAP, $CH_2Cl_2$, 0 °C to rt, 1 h; c) AcOH, $Ac_2O$, $H_2SO_4$, rt, 1.5 h; d) thymine, BSA, TMSOTf, $CH_3CN$, reflux, 13 h; e) 40% aq. $MeNH_2$, THF, rt, 3 h.

**2. Experimenatal procedures for the synthesis of 1:**

**3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-1,2,-*O*-isopropylidene-α-D-ribofuranose (SI-2).** To a stirred solution of **SI-1**[1] (20 g, 64 mmol) in DMF at 0 °C was added NaH (3.3 g, 83 mmol), and benzyl bromide (8.3 ml, 70 mmol). The reaction mixture was then stirred at room temperature for 4 h, then cooled to 0 °C, quenched by the addition

of MeOH and water, and extracted with AcOEt. The organic phase was washed with water and brine, dried ($Na_2SO_4$) and concentrated. The resultant residue was purified by column chromatography (hexane/AcOEt = 2/1) to afford **SI-2** (17.25 g, 67%) as a colorless oil. All analytical data were identical to those previously reported.[1]

**3,5-Di-*O*-benzyl-4-*C*-(*p*-toluenesulphonyloxymethyl)-1,2,-*O*-isopropylidene-α-D-ribofuranose (SI-3).** To a stirred solution of **SI-2** (18.27 g, 45.6 mmol) in $CH_2Cl_2$ (91 ml) at 0 °C was added triethylamine (40 ml, 287 mmol) and DMAP (0.83 g, 6.8 mmol). *p*-Toluenesulphonyl chloride (13.9 g, 73 mmol) was then added at 0 °C and the reaction mixture was stirred at room temperature for 1 h. Saturated $NaHCO_3$ (aq.) solution was added and the mixture was extracted with $CH_2Cl_2$, washed with water and brine, dried ($Na_2SO_4$), and concentrated. The concentrate was purified by column chromatography to give the compound **SI-3** (24.5 g, 97%) as yellow oil.[2]

**1,2-Di-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-(*p*-toluenesulphonyloxymethyl)-α-D-ribofuranose (SI-4).** To a stirred solution of **SI-3** (24.5 g, 44.2 mmol) in acetic acid (49 ml) was added acetic anhydride (25 ml, 265 mmol) and concentrated $H_2SO_4$ (0.46 ml, 8.8 mmol) and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was cooled to 0 °C and quenched by saturated $NaHCO_3$ (aq.) solution. The resultant mixture was extracted with AcOEt, the organic phase was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 3/1) to give an anomeric mixture of the compound **SI-4** (20.4 g, 77%) as colorless oil which showed spectral data identical to that reported earlier.[2]

**1(2-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-(*p*-toluenesulphonyloxymethyl)-α-D-ribofuranosyl)thymine (SI-5).** To a stirred solution of **SI-4** (15.9 g, 27 mmol) and thymine (5.17 g, 41 mmol) in $CH_3CN$ (100 ml) was added *N,O*-bis(trimethylsilyl)acetamide (BSA) (16.81 ml, 68 mmol) and the mixture was refluxed for 3 h. After cooling

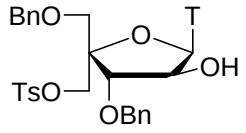
the reaction mixture to 0 °C, trimethylsilyltriflate (5 ml, 28 mmol) was added and the reaction mixture was refluxed overnight. Saturated $NaHCO_3$ (aq.) solution was added and the mixture was extracted with AcOEt, the organic phase was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by column chromatography (hexane/ACOEt = 1/1) to afford the compound **SI-5** (16.9 g, 94%) as colorless solid.

**1(3,5-Di-*O*-benzyl-4-*C*-(*p*-toluenesulphonyloxymethyl)-α-D-ribofuranosyl)thymine (SI-5).** To a stirred solution of **SI-5** (49 mg, 0.07 mmol) in THF was added 40% aqueous methylamine solution (0.11 ml, 1.5 mmol), and the whole was stirred at room temperature for 3 h. The reaction mixture was concentrated under vacuum and extracted with AcOEt. The organic phase was washed with water and brine, dried ($Na_2SO_4$) and concentrated. The resultant residue was purified by column chromatography (hexane/AcOEt = 1/1) to afford **1** (45 mg, 99%) as a white powder. The analytical data of this compound was identical to that reported.[2]

**3. Experimental procedures for the synthesis and characterization data for compounds 2-22:**

**Special notes:** Full experimental procedures and complete characterization data of compounds **9-11, 13, 15, 16, 18, 19** and **21** were described in Supporting Information of our preliminary reports.[3,4] If required, readers can get the data free of charge from the respective site via internet. Only new experimental procedures and data for the new compounds are described below:

**3',5'-Di-*O*-benzyl-5-methyl-4'-(*p*-toluenesulphonyloxymethyl)-*arabino*-uridine (2).** To a stirred solution of **1**

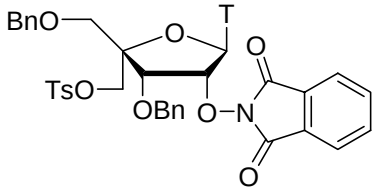



(14 g, 22.48 mmol) in pyridine (67 ml) was added methanesulfonyl chloride (5.2 ml, 67.18 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was diluted with water, and the whole was extracted with AcOEt. The organic phase was washed with saturated

$NaHCO_3$ (aq.) solution, water and brine, dried ($Na_2SO_4$), and concentrated to give the crude mesylate **3** (19.5 g) as a yellow syrup which was used for the next reaction without purification.

The crude mesylate was dissolved in $H_2O$/ethanol (1:2, 450 ml) and 1M NaOH solution (67.5 ml, 67.5 mmol) was added to the above solution and the mixture was stirred at room temperature for 1 h. After being neutralized with 10% HCl, the reaction mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over $Na_2SO_4$, and concentrated. The resultant residue was purified by column chromatography (hexane/AcOEt = 1/1) to provide alcohol **2** (13.75 g, 98% from **1**) as a white powder: Mp 88-89 °C; $[\alpha]_D^{25}$ = +48.3 (*c* = 0.91, $CHCl_3$); IR (KBr): 3359, 3034, 1701, 1665, 1475, 1451, 1363, 1286, 1178, 1101 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.57 (s, 3H), 2.34 (s, 3H), 3.62 (d, *J* = 9 Hz, 1H), 3.69 (d, *J* = 9 Hz, 1H), 4.10 (s, 1H), 4.21 (d, *J* = 10 Hz, 1H), 4.25 (d, *J* = 10 Hz, 1H), 4.39 (d, *J* = 12 Hz, 1H), 4.44 (d, *J* = 12 Hz, 1H), 4.46 (d, *J* = 12 Hz, 1H), 4.60 (d, *J* = 12 Hz, 1H), 4.73 (br s, 1H), 4.85 (d, *J* = 4 Hz, 1H), 6.01 (d, *J* = 4 Hz, 1H), 7.17-7.32 (m, 13H), 7.72 (d, *J* = 8 Hz, 2H), 10.63 (s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 12.2, 21.6, 68.3, 68.9, 72.0, 73.4, 73.5, 83.8, 84.5, 86.7, 107.8, 127.4, 127.7, 127.8, 127.8, 127.8, 128.3, 129.6, 132.5, 136.9, 137.3, 138.6, 144.6, 150.3, 165.4; MS (FAB) *m/z* 623 ($M+H^+$). *Anal.* Calcd for $C_{32}H_{34}N_2O_9S$: C, 61.72; H, 5.50; N, 4.50. Found: C, 61.51; H, 5.55; N, 4.39.

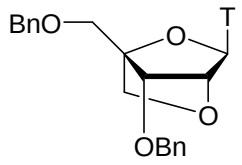
**3',5'-Di-*O*-benzyl-2'-*O*-(1,3-Dihydro-1,3-dioxo-*2H*-isoindol-2-yl)-5-methyl-4'-(*p*-toluenesulphonyloxymethyl)uridine (3).** To a stirred solution of **2** (2.01 g, 3.23 mmol) and 4-dimethylaminopyridine (79 mg, 0.65 mmol) in pyridine (25 ml) was added trifluoromethanesulfonic anhydride (1.63 ml. 9.69 mmol) at 0 °C and the reaction mixture was stirred at room



temperature for 4 h. The mixture was diluted with water and extracted with $CH_2Cl_2$. The organic phase was washed with saturated $NaHCO_3$ solution and brine, dried ($Na_2SO_4$), and concentrated to afford crude triflate (2.4 g), which was subjected to the next reaction without purification.

The crude triflate was dissolved in acetonitrile (30 ml). *N*-Hydroxyphthalimide (0.74 g, 4.52 mmol) and DBU (0.68 ml, 4.52 mmol) were added to the above solution and the mixture was then stirred at room temperature for 4 h. The reaction mixture was diluted with $CH_2Cl_2$, washed with water and brine, dried over $Na_2SO_4$, and concentrated. The resultant residue was purified by column chromatography (hexane/AcOEt = 2:1 to 1:1) to give **3** (1.59 g, 64%) as a light yellow powder: Mp 101-103 °C; $[\alpha]_D^{27}$ = +46.5 (*c* = 1.19, $CHCl_3$); IR (KBr): 3189, 3065, 2926, 1793, 1738, 1696, 1468, 1455, 1362, 1271, 1190, 978 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.34 (s, 3H), 2.41 (s, 3H), 3.66 (d, *J* = 11 Hz, 1H), 4.02 (d, *J* = 11 Hz, 1H), 4.34 (d, *J* = 12 Hz, 1H), 4.46 (d, *J* = 12 Hz, 1H), 4.53 (d, *J* = 12 Hz, 1H), 4.56 (d, *J* = 6 Hz, 1H), 4.71 (d, *J* = 12 Hz, 1H), 4.85 (dd, *J* = 6, 2 Hz, 1H), 5.10 (d, *J* = 12 Hz, 1H), 6.22 (d, *J* = 2 Hz, 1H), 7.11-7.13 (m, 2H), 7.25-7.27 (m, 3H), 7.30-7.39 (m, 7H), 7.47 (s, 1H), 7.75-7.78 (m, 2H), 7.82-7.85 (m, 4H), 8.15 (s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 11.7, 21.6, 69.1, 70.1, 73.1, 73.6, 74.7, 85.9, 87.4, 88.3, 110.6, 123.7, 127.6, 128.1, 128.1, 128.1, 128.2, 128.5, 128.6, 129.9, 132.6, 134.7, 135.4, 136.8, 137.0, 144.8, 149.7, 163.1, 163.3; MS (FAB) *m/z* 768 ($M+H^+$). HRMS (FAB) Calcd for $C_{40}H_{38}N_3O_{11}S$ ($M+H^+$): 768.2227. Found: 768.2221.

**(1S,3R,4R,7S)-7-benzyloxy-1-benzyloxymethyl-3-(thymine-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (4).**[1,5]

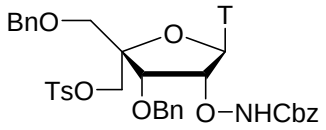



Hydrazine monohydrate (32 μl, 0.66 mmol) was added to a stirred solution of **3** (300 mg, 0.39 mmol) in ethanol (9 ml) and the mixture was stirred at room temperature for

10 min. The mixture was filtered to separate the precipitate, and the filtrate was concentrated to remove ethanol. The concentrated mixture was then diluted with AcOEt, washed with water and brine, dried over $Na_2SO_4$ and concentrated again to give crude amine (249 mg), a portion of which was employed for the next reaction without further purification.

60% NaH (4 mg, 0.094 mmol) was added to a stirred solution of **3** (20 mg, 0.031 mmol) in THF (1 ml) at 0 °C. The mixture was then stirred at room temperature for 17 h. the reaction mixture was cooled to 0 °C and quenched by the addition of MeOH acid solution and extracted with AcOEt, washed with water and brine, dried over $Na_2SO_4$, and concentrated. The concentrate was purified by column chromatography (hexane/AcOEt = 2/1) to give **4** (9.9 mg, 71%) as a white powder which showed identical spectral data of that reported for the 2',4'-BNA (LNA) precursor **4**.[1,5]

**2'-*O*-(*N*-Benzyloxycarbonylamino)-3',5'-di-*O*-benzyl-5-methyl-4'-(*p*-toluenesulphonyloxymethyl)uridine (5).** To a solution of the crude amine (93 mg) (obtained from the compound **3** as described above) in $CH_2Cl_2$ (1.5



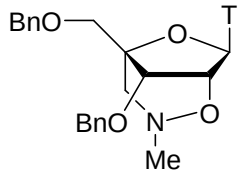

ml) were added an aqueous solution of $NaHCO_3$ (0.6 ml, ca.0.65 mmol) and benzyl chloroformate (28 μl, 0.195 mmol) under ice cooling, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was then poured into saturated $NaHCO_3$ solution and extracted with AcOEt. The organic phase was washed with water and brine, dried over $Na_2SO_4$ and concentrated. The resultant residue was purified by column chromatography (hexane/AcOEt = 1/2) to give **5** (82.0 mg, 82%) as a white powder: Mp 137-139 °C; $[\alpha]_D^{27}$ = +35.1 (*c* = 1.25, $CHCl_3$); IR (KBr): 3179, 3034, 2955, 1762, 1692, 1457, 1364, 1259, 1105, 980, 750 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.52 (s, 3H), 2.41 (s, 3H),

3.58 (d, *J* = 10 Hz, 1H), 3.77 (d, *J* = 10 Hz, 1H), 4.15 (d, *J* = 11 Hz, 1H), 4.25 (d, *J* = 11 Hz, 1H), 4.41 (d, *J* = 6 Hz, 1H), 4.46 (d, *J* = 12 Hz, 1H), 4.50 (d, *J* = 12 Hz, 1H), 4.55 (d, *J* = 12 Hz, 1H), 4.58 (d, *J* = 6 Hz, 1H), 4.78 (d, *J* = 12 Hz, 1H), 5.11 (d, *J* = 12 Hz, 1H), 5.16 (d, *J* = 12 Hz, 1H), 5.89 (d, *J* = 5 Hz, 1H), 7.18-7.32 (m, 17H), 7.73 (d, *J* = 8 Hz, 2H), 7.74 (s, 1H), 8.18 (br s, 1H); $^{13}$C NMR ($CDCl_3$) δ 11.9, 21.6, 67.8, 69.2, 70.4, 73.7, 73.9, 85.2, 86.2, 88.0, 111.3, 123.5, 127.6, 128.0. 128.1, 128.2, 128.3, 128.5, 128.6, 128.6, 128.6, 129.8, 132.5, 134.2, 135.2, 136.1, 137.0, 145.0, 150.1, 157.1, 163.4; MS (FAB) *m/z* 772 ($M+H^+$). HRMS (FAB) Calcd for $C_{40}H_{42}N_3O_{11}S$ ($M+H^+$): 772.2540. Found: 772.2537.

**2'-*O*,4'-*C*-Aminomethylene-3',5'-di-*O*-benzyl-5-methyluridine (6).** To a stirred suspension of 60% NaH (7.8 mg, 0.194 mmol) in THF (0.2 ml) was added a solution of **5** (50 mg, 0.065 mmol) in THF (0.5 ml) at 0 °C. The mixture was then stirred at room temperature for 18 h. the reaction mixture was cooled to 0 °C and quenched by the addition of saturated oxalic acid solution and extracted with AcOEt, washed with water and brine, dried over $Na_2SO_4$, and concentrated. The concentrate was purified by column chromatography (hexane/AcOEt = 2/1 to 1/1) to afford **6** (42.3 mg, 70%) as a white powder: Mp 93-95 °C; $[\alpha]_D^{27}$ = +89.9 (*c* = 1.60, $CHCl_3$); IR (KBr): 3064, 2939, 2874, 1690, 1459, 1273, 1104, 747 $cm^{-1}$; $^1$H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.55 (d, *J* = 13 Hz, 1H), 3.59 (d, *J* = 11 Hz, 1H), 3.73 (d, *J* = 11 Hz, 1H), 3.75 (d, *J* = 13 Hz, 1H), 4.00 (d, *J* = 3 Hz, 1H), 4.46 (br s, 1H), 4.52 (d, *J* = 12 Hz, 1H), 4.54 (d, *J* = 11 Hz, 1H), 4.58 (d, *J* = 11 Hz, 1H), 4.76 (d, *J* = 12 Hz, 1H), 6.29 (s, 1H), 7.25-7.35 (m, 10H), 7.79 (s, 1H), 9.07 (br s, 1H); $^{13}$C NMR ($CDCl_3$) δ 12.0, 49.3, 67.7, 70.5, 71.9, 73.7, 78.6, 82.0, 85.7, 110.4, 127.8, 127.9, 128.1, 128.2, 128.5, 128.7, 135.0, 137.1, 137.1, 149.9, 163.8; MS (FAB) *m/z* 466 ($M+H^+$). HRMS (FAB) Calcd for

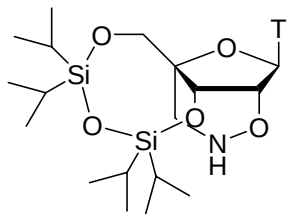
$C_{25}H_{28}N_3O_6$ (M+H$^+$): 466.1978. Found: 466.2001.

**3',5'-Di-*O*-benzyl-5-methyl-2'-*O*,4'-*C*-(*N*-methylaminomethylene)uridine (7).** A 20% solution of formalin (7




μl, 0.0473 mmol) was added at room temperature to a stirred solution of **6** (20 mg, 0.043 mmol) in methanolic solution of pyridinium *p*-toluenesulfonate (1 M, 0.43 ml, 0.43 mmol) and the mixture was stirred for 10 min. The mixture was then cooled to 0 °C and sodium cyanoborohydride (5 mg, 0.086 mmol) was added to the reaction mixture. After stirring at 0 °C for 1 h, the reaction mixture was diluted with AcOEt, washed periodically with water, saturated $NaHCO_3$ solution and brine and dried over $Na_2SO_4$ and concentrated. The resultant residue was purified by column chromatography (hexane/ EtOAc = 2/1) to give compound **7** (19.8 g, 96%) as a white powder: Mp 73-74 °C; $[\alpha]_D^{27}$ = +54.4 (*c* = 0.80, $CHCl_3$); IR (KBr): 3187, 3064, 2886, 1694, 1454, 1362, 1266, 1078, 911, 744 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 1.50 (s, 3H), 2.67 (d, *J* = 11 Hz, 1H), 2.75 (s, 3H), 3.61 (d, *J* = 11 Hz, 1H), 3.75 (d, *J* = 11 Hz, 1H), 3.84 (d, *J* = 3 Hz, 1H), 4.43 (d, *J* = 3 Hz, 1H), 4.50 (d, *J* = 12 Hz, 1H), 4.55 (d, *J* = 12 Hz, 1H), 4.59 (d, *J* = 12 Hz, 1H), 4.72 (d, *J* = 12 Hz, 1H), 6.32 (s, 1H), 7.26-7.36 (m, 10H), 7.72 (s, 1H), 8.85 (s, 1H); $^{13}$C NMR ($CDCl_3$) δ 12.0, 45.5, 58.0, 68.0, 70.5, 71.5, 73.6, 78.1, 82.7, 86.2, 109.3, 127.7, 127.7, 128.0, 128.1, 128.4, 128.6, 135.5, 137.3, 137.4, 149.8, 163.8; MS (FAB) *m/z* 480 (M+H$^+$). HRMS (FAB) Calcd for $C_{26}H_{30}N_3O_6$ (M+H$^+$): 480.2135. Found: 480.2128.

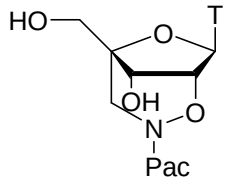
**2'-*O*,4'-*C*-Aminomethylene-5-methyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (12).** Hydrazine




(0.04 ml, 1.3 mmol) was added to a stirred solution of phthalimide derivative **11** (1.0 g, 1.2 mmol) in pyridine (20 ml) at room temperature. After 1 h,

DABCO (260 mg, 2.4 mmol) was added and the mixture was stirred at room temperature for 2 d, then water was added to the reaction mixture and excess pyridine was evaporated. The reaction mixture was then extracted with AcOEt. The organic phase was washed with water and brine, dried over $Na_2SO_4$, and concentrated. The concentrate was purified by column chromatography (hexane/AcOEt = 4/1 to 1/2) to afford **12** (0.47 g, 74%) as a white powder: Mp 113-115 °C; $[\alpha]_D^{26}$ = -6.1 (*c* = 0.93, $CHCl_3$); IR (KBr): 3479, 3262, 3071, 2946, 2868, 1698, 1464, 1388, 1363, 1272, 1229, 1165, 1143 $cm^{-1}$; $^1H$ NMR ($CHCl_3$) δ 0.94-1.13 (m, 28H), 1.93 (d, *J* = 1 Hz, 3H), 2.53 (d, *J* = 13 Hz, 1H), 3.67 (d, *J* = 13 Hz, 1H), 3.68 (d, *J* = 13 Hz, 1H), 4.06 (d, *J* = 13 Hz, 1H), 4.10 (d, *J* = 3 Hz, 1H), 4.35 (d, *J* = 3 Hz, 1H), 6.16 (s, 1H), 7.73 (d, *J* = 1 Hz, 1H), 8.31 (br s, 1H); $^{13}C$ NMR ($CDCl_3$) d 12.2, 12.8, 13.0, 13.4, 16.9, 17.0, 17.1, 17.1, 17.1, 17.3, 17.3, 17.5, 48.6, 60.0, 64.4, 81.0, 82.5, 85.4, 110.3, 134.3, 150.0, 164.2; MS (FAB) *m/z* 528 ($M+H^+$). *Anal.* Calcd for $C_{239}H_{41}N_3O_7Si_2 \cdot 1/10H_2O$: C, 52.17; H, 7.84; N, 7.93. Found: C, 52.02; H, 7.78; N, 7.89.

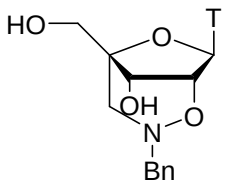
**5-Methyl-2'-*O*,4'-*C*-(*N*-phenoxyacetylaminomethylene)uridine (13).** Triethylamine (32 μl, 0.23 mmol) and




phenoxyacetyl chloride (29 μl, 0.21 mmol) were added to a stirred solution of **12** (100 mg, 0.19 mmol) in dicholoromethane (2 ml) at 0 °C and the mixture was stirred at 0 °C (ice cooling) for 1 h. Saturated $NaHCO_3$ (aq.) was added and the reaction mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over $Na_2SO_4$ and concentrated. The resultant residue was purified by column chromatography ($CHCl_3$/MeOH = 50/1) to give the *N*-phenoxyacetyl derivative of **12** (justified by spectral data)[3] which was deprotected (using the procedure described earlier)[3] to afford monomer **13** (for full spectral data see Supporting Information of

reference 3).

**5-Methyl-2'-*O*,4'-*C*-(*N*-benzylaminomethylene)uridine (14).** Triethylamine (0.4 ml, 2.82 mmol) and benzyl

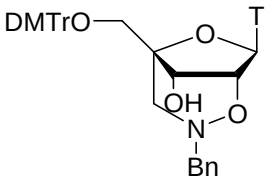



bromide (0.15 ml) were added at 0 °C to a stirred solution of **12** (0.5 g, 0.95 mmol) in dichloromethane (5 ml). The reaction mixture was stirred at room temperature for 9 h, then quenched with saturated $NaHCO_3$ solution and extracted with AcOEt. The organic phase was washed with water and brine, and then concentrated. The resultant residue was purified by column chromatography (hexane/AcOEt = 1/4 to 2/1) to give the benzylated compound (5-methyl-2'-*O*,4'-*C*-(*N*-benzylaminomethylene)-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine) (0.46 g, 79%) as a white powder: Mp 101-103 °C; $[\alpha]_D^{27}$ = -27.0 (*c* = 0.71, $CHCl_3$); IR (KBr): 3186, 2941, 2668, 1193, 1461, 1387, 1264, 1164, 1039, 875,; $^1$H NMR ($CHCl_3$) δ 0.88-1.10 (m, 28H), 1.92 (s, 3H), 2.55 (d, *J* = 11 Hz, 1H), 2.90 (d, *J* = 11 Hz, 1H), 3.63 (d, *J* = 13 Hz, 1H), 3.89 (d, *J* = 13 Hz, 1H), 3.96 (d, *J* = 3 Hz, 1H), 3.98 (d, *J* = 13 Hz, 1H), 4.28 (d, *J* = 13 Hz, 1H), 4.36 (d, *J* = 3 Hz, 1H), 6.37 (s, 1H), 7.28 (d, *J* = 8 Hz, 1H), 7.33 (t, *J* = 8 Hz, 2H), 7.36 (d, *J* = 8 Hz, 2H), 7.12 (s, 1H), 8.44 (br s, 1H); $^{13}$C NMR ($CDCl_3$) δ 12.4, 12.7, 12.8, 12.8, 13.4, 17.0, 17.2, 17.2, 17.3, 17.5, 54.6, 60.2, 62.2, 64.6, 80.8, 83.8, 86.1, 110.0, 127.5, 128.4, 129.0, 135.1, 135.8, 149.7, 163.7; MS (FAB) *m/z* 618 ($M+H^+$). HRMS (FAB) Calcd for $C_{30}H_{48}N_3O_7Si_2$ ($M+H^+$): 618.3031. Found: 618.3025.

Tetrabutylammonium fluoride (1M in THF, 1.26 ml, 1.26 mmol) was added at room temperature to the above benzylated derivative (392 mg, 0.634 mmol) in THF (7.5 ml). After stirring the mixture at room temperature for 10 min, the reaction mixture was concentrated and chromatographed on silica gel (AcOEt/MeOH = 20/1) to

afford the monomer **14** (260 mg, quant.) as a powder: Mp 107-109 °C; $[\alpha]_D^{27}$ = -57.3 (*c* = 0.59, $CHCl_3$); IR (KBr): 3423, 2926, 1690, 1464, 1263, 1054, 732 $cm^{-1}$; $^1H$ NMR ($CD_3OD$) δ 1.87 (s, 3H), 2.72 (d, *J* = 11 Hz, 1H), 2.98 (d, *J* = 11 Hz, 1H), 3.69 (d, *J* = 13 Hz, 1H), 3.73 (d, *J* = 13 Hz, 1H), 3.90 (d, *J* = 13 Hz, 1H), 3.97 (d, *J* = 3 Hz, 1H), 4.14 (d, *J* = 13 Hz, 1H), 4.23 (d, *J* = 3 Hz, 1H), 6.35 (s, 1H), 7.24 (t, *J* = 8 Hz, 1H), 7.30 (t, *J* = 8 Hz, 2H), 7.38 (d, *J* = 7 Hz, 2H), 7.90 (s, 1H), 7.99 (s, 1H); $^{13}C$ NMR ($CD_3OD$) δ 12.7, 56.5, 61.3, 63.2, 65.5, 82.8, 85.0, 86.9, 110.7, 128.3, 129.3, 130.0, 137.7, 138.3, 152.1, 166.6; MS (FAB) *m/z* 376 ($M+H^+$). HRMS (FAB) Calcd for $C_{18}H_{22}N_3O_6$ ($M+H^+$): 376.1509. Found: 376.1505.

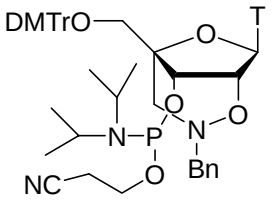
**5'-*O*-(4,4'-dimethoxytrityl)-5-methyl-2'-*O*,4'-*C*-(*N*-benzylaminomethylene)uridine (17).**




4,4'-dimethoxytrityl chloride (57 mg. 0.17 mmol) was added at room temperature to a stirred solution of **14** (31 mg, 0.083 mmol) in pyridine (1.5 ml) and the mixture was stirred at room temperature for 3.5 h. Saturated $NaHCO_3$ (aq.) solution was added and the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried ($Na_2SO_4$) and concentrated. The concentrate was purified by column chromatography (1% triethylamine in hexane/AcOEt = 1/2 to AcOEt/MeOH = 50/1) to afford 52 mg (92%) of **17** as a white powder: Mp 142-144 °C; $[\alpha]_D^{27}$ = -46.7 (*c* = 1.41, $CHCl_3$); IR (KBr): 3534, 2939, 2837, 1694, 1506, 1456, 1253, 1073, 832, 735 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.43 (s, 1H), 2.63 (d, *J* = 8 Hz, 1H), 2.68 (d, *J* = 12 Hz, 1H), 2.77 (d, *J* = 12 Hz, 1H), 3.28 (d, *J* = 11 Hz, 1H), 3.32 (d, *J* = 11 Hz, 1H), 3.78 (s, 6H), 3.88 (d, *J* = 14 Hz, 1H), 4.22 (d, *J* = 14 Hz, 1H), 4.30 (dd, *J* = 3, 8 Hz, 1H), 4.40 (d, *J* = 3 Hz, 1H), 6.46 (s, 1H), 6.83 (d, *J* = 9 Hz, 4H), 7.21-7.31 (m, 12H), 7.42 (dd, *J* = 1, 9 Hz, 2H), 7.79 (br s, 1H), 8.72 (br s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 12.1, 55.2, 55.5, 61.4, 62.2, 65.5, 81.3, 83.3,

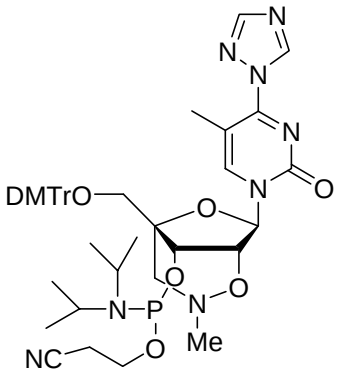
85.4, 86.6, 110.4, 113.3, 127.1, 127.7, 128.0, 128.5, 129.0, 130.0, 135.2, 135.3, 144.2, 149.8, 158.7, 163.8; MS (FAB) *m/z* 700 ($M+Na^+$), 677 ($M+H^+$). HRMS (FAB) Calcd for $C_{39}H_{39}N_3O_8Na$ ($M+Na^+$): 700.2629. Found: 700.2633.

**3'-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-5-methyl-2'-*O*,4'-*C*-(*N*-benzylaminomethylene)uridine (20).** Next, 4,5-dicyanoimidazole (7.5 mg. 0.014 mmol) and 2-cyanoethyl




*N,N,N',N'*-tetraisopropylphosphorodiamidite (0.023 ml, 0.072 mmol) were added to a stirred solution of **17** (40 mg, 0.059 mmol) in acetonitrile (0.5 ml) at room temperature, and the mixture was stirred at room temperature for 7 h. After addition of saturated $NaHCO_3$ (aq.) solution, the reaction mixture was extracted with AcOEt. The organic phase was washed sequentially with saturated $NaHCO_3$ solution, water and brine, then dried over $Na_2SO_4$ and concentrated. Purification by column chromatography (1% triethylamine in hexane/AcOEt = 1/1 to 1/2) afforded a white mass, which was further purified by precipitation by pouring a concentrated solution of the mass in AcOEt to excess hexane under vigorous stirring. The precipitate was a white powder composed of an enantiomeric mixture of **20** (37 mg, 71%): Mp 115-117 °C; $^{31}P$ NMR ($CDCl_3$) δ 148.96; MS (FAB) *m/z* 878 ($M+H^+$). HRMS (FAB) Calcd for $C_{48}H_{57}N_5O_9P$ ($M+H^+$): 878.3894. Found: 878.3864.

**1-[3'-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*,4'-*C*-(*N*-methylaminomethylene)-β-D-ribo-furanosyl]-5-methy-4-(1,2,4-traizol-1-yl)-2-pyrimidinone (22).** Phosphoryl chloride (86 μl, 0.92 mmol) was added to a stirred suspension of 1,2,4-triazole (278 mg, 4.03 mmol) in acetonitrile (9 ml) at 0 °C,[6] and the whole was stirred at 0 °C for 10 min. Triethylamine (0.64 ml, 4.62 mmol) was added and the




reaction mixture was stirred at 0 °C for an additional 35 min. A solution of compound **19** (95 mg, 0.12 mmol) in acetonitrile (3 ml) was added to the mixture and stirring was continued at 0 °C for 5.5 h, and then at room temperature for 2.5 h. The reaction mixture was poured into saturated $NaHCO_3$ (aq.) solution and extracted with AcOEt. The organic phase was washed with water and brine, dried over $Na_2SO_4$ and concentrated. Purification by column chromatography (hexane/AcOEt = 1/2) afforded a white powder, which was further purified by precipitation to give **22** (83 mg, 83%) as a white powder; Mp 107-109 °C; $^{31}P$ NMR (acetone-$d_6$) δ 148.24, 149.91; MS (FAB) *m/z* 853 ($M+H^+$). HRMS (FAB) Calcd for $C_{44}H_{54}N_8O_8P$ ($M+H^+$): 853.3802. Found: 853.3766.

**4. Optimization of reaction conditions for the synthesis of key intermediate 12:**

Compound **12** was synthesized from compound **11** directly via one pot deprotection and cyclization to form a perhydro-1,2-oxazine ring. Optimization of the conditions for this reaction is summarized in Table SI-1. When the reaction was conducted for a very short period with hydrazine monohydrate, the free aminoxy derivative of **11** was obtained exclusively. However, increased amounts of hydrazine monohydrate and prolonged reaction time (entry 2) resulted in the formation of cyclized product **12**. The use of anhydrous hydrazine improved the yield slightly, but a substantial amount of hydrazine was necessary. Employment of MeCN, THF or dichloromethane (entries 3 to 5 respectively) did not improve the yield. In contrast, **12** was obtained in good yield in pyridine (entry 6). In the presence of DBN or DABCO (entries 7-9), hydrazine was reduced to 1 equiv. to obtain the desired cyclized product in very good yield. Product **12** can also be obtained by using hydrazine

monohydrate (entry 10) instead of anhydrous hydrazine (which is toxic), albeit in lower yield

**Table SI-1**: Optimization of Reaction Condition for the Synthesis of Key Intermediate **5**.

| entry | reagent (s) (eq.) | solvent | conditions | yield |
|---|---|---|---|---|
| 1 | $NH_2NH_2$•$H_2O$ (1.7 eq.) | EtOH | rt, 15 min | 0%[a] |
| 2 | $NH_2NH_2$•$H_2O$ (20 eq.)[b] | EtOH | rt, 24 h | 38% |
| 2 | $NH_2NH_2$ (10 eq)[b] | EtOH | rt, 34 h | 44% |
| 3 | $NH_2NH_2$ (10 eq)[b] | $CH_3CN$ | rt, 36 h | 40% |
| 4 | $NH_2NH_2$ (20 eq)[b] | THF | rt, 34 h | 38% |
| 5 | $NH_2NH_2$ (20 eq) | $CH_2Cl_2$ | rt, 28 h | 35% |
| 6 | $NH_2NH_2$ (20 eq) | pyridine | rt, 35 h | 64% |
| 7 | $NH_2NH_2$ (5 eq), DBN (0.2 eq.) | pyridine | rt, 40 h | 74% |
| 8 | $NH_2NH_2$ (1.1 eq), DBN (1.0 eq) | pyridine | rt, 28 h | 66% |
| 9 | $NH_2NH_2$ (1.1 eq), DABCO (2 eq.)[c] | pyridine | rt, 40 h | 78% |
| 10 | $NH_2NH_2$•$H_2O$ (1.1 eq), DABCO (2 eq.)[c] | pyridine | rt, 24 h | 43% |

[a]Only deprotection of the phthalimide moiety occurred, giving only the free aminoxy derivative;[3] [b]Reaction was started with a lower amount of hydrazine which was increased portionwise because a substantial amount of free aminoxy intermediate did not undergo cyclization. DBN = 1,5-diazabicyclo[4.3.0]non-5-ene; DABCO = 1,4-diazabicyclo[2.2.2]octane. [c]Employment of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) gave lower yield.

**5. References:**


(1) Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.

(2) Koshkin, A. A.; Rajwanshi, V. K; Wengel, J. *Tetrahedron Lett*. **1998**, *39*, 4381.

(3) Rahman, S. M. A.; Seki, S. Obika, S.; Haitani, S.; Miyashita, K.; Imanishi, T. *Angew.Chem Int. Ed.* **2007**, *46*, 4306.

(4) Miyashita, K.; Rahman, S. M. A.; Seki, S. Obika, S.; Imanishi, T. *Chem. Commun.* **2007**, 3765.

(5) Sing, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455.

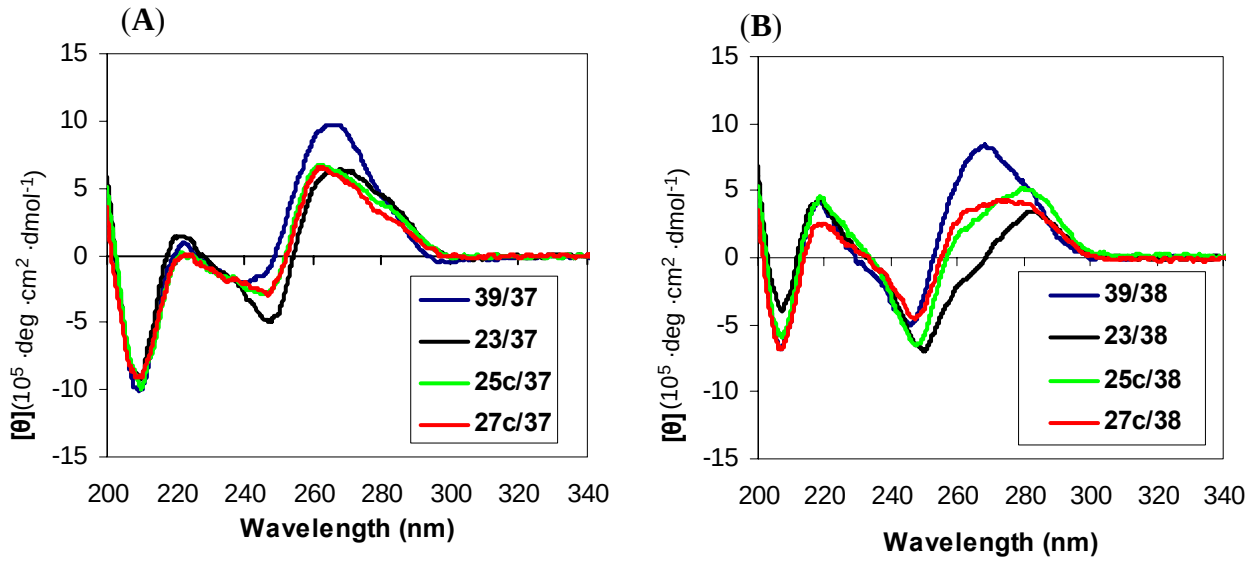
(6) Scerr, M.; Klebba, C.; Hanner, A.; Ganser, A.; Engels, J. W. *Biorg. Med. Chem. Lett.* **1997**, *7*, 1751.

**6. Complete reference of the reference 32 (in text):**

Wahlestedt, C.; Salmi, P.; Good, L.; Kela, J.; Johnsson, T.; Hökfelt, T.; Broberger, C.; Porreca, F.; Lai, J.; Ren, K.; Ossipov, M.; Koshkin, A.; Jakobsen, M.; Skouv, J.; Oerum, H.; Jacobsen, M. H.; Wengel, J. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5633.

***Table S-2***: Yield and MALDI-TOF Mass Data of 2',4'-BNA$^{NC}$[NH], [NMe], [NBn] and 2',4'-BNA (LNA)-Modified Oligonucleotides.

| Oligonucleotides | 2'4'-BNA$^{NC}$[NH] | | 2'4'-BNA$^{NC}$[NMe] | | 2'4'-BNA$^{NC}$[NBn] | | 2'4'-BNA (LNA) | |
|---|---|---|---|---|---|---|---|---|
| | Yield (%) | Mass [M-H]$^-$ (found/calcd) | Yield (%) | Mass [M-H]$^-$ (found/calcd) | Yield (%) | Mass [M-H]$^-$ (found/calcd) | Yield (%) | Mass [M-H]$^-$ (found/calcd) |
| 5'-d(GCGTT**T**TTTGCT)-3' (**24a -24d**) | 26 | 3675.8/3675.4 | 48 | 3688.5/3689.5 | 52 | 3765.4/3765.5 | 22 | 3660.8/3660.4 |
| 5'-d(GCG**T**T**T**T**T**TGCT)-3' (**25a -25d**) | 19 | 3762.6/3761.5 | 63 | 3804.9/3803.6 | 26 | 4031.4/4031.8 | 38 | 3016.9/3016.4 |
| 5'-d(GCGTT**TTT**TGCT)-3' (**26a -26d**) | 8 | 3761.2/3761.5 | 34 | 3803.2/3803.6 | 19 | 4032.0/4031.8 | 35 | 3716.5/3716.4 |
| 5'-d(GCG**TTTTTT**GCT)-3' (**27a -27d**) | 12 | 3890.6/3890.6 | 50 | 3975.3/3974.7 | 44 | 4430.7/4431.2 | 31 | 3800.7/3800.4 |
| 5'-d(TTTTT$^m$CT**T**T$^m$CT$^m$CT$^m$CT)-3' (**29a - 29d**) | 30 | 4539.3/4539.1 | 36 | 4552.7/4553.1 | 44 | 4630.0/4629.1 | 28 | 4523.1/4523.0 |
| 5'-d(TTTT**T**$^m$CT**T**T$^m$C**T**$^m$CT$^m$CT)-3' (**30a - 30d**) | 13 | 4625.7/4625.1 | 31 | 4667.4/4667.2 | 34 | 4894.8/4895.4 | 17 | 4580.8/4580.0 |
| 5'-d(TTTTT$^m$C**TTT**$^m$CT$^m$CT$^m$CT)-3' (**31a – 31d**) | 28 | 4625.7/4625.1 | 30 | 4666.9/4667.2 | 28 | 4895.1/4895.4 | 26 | 4580.1/4580.0 |
| 5'-d(**T**TTT**T**$^m$CTT**T**$^m$CT$^m$C**T**$^m$CT)-3' (**32a, b, d**) | 19 | 4668.5/4668.1 | 41 | 4723.7/4724.3 | | — | 18 | 4608.8/4608.0 |
| 5'-d(T**T**TT**T**$^m$CT**T**T$^m$C**T**$^m$CT$^{\underline{m}}$**C**T)-3' (**33a, b, d**) | 25 | 4710.7/4710.1 | 13 | 4781.2/4781.3 | | — | 18 | 4637.1/4636.0 |
| 5'-d(**T**T**T**T**T**$^m$C**T**TT**T**$^m$C**T**$^m$C**T**$^m$CT)-3' (**34a, b, d**) | 17 | 4797.8/4797.2 | 21 | 4896.4/4895.4 | | — | 19 | 4691.3/4692.1 |
| 5'-d(**TTTTT**$^{\underline{m}}$**CTTT**$^{\underline{m}}$**CT**$^{\underline{m}}$**CT**$^{\underline{m}}$**CT**)-3'(**35a, b, d**) | 4 | 5141.9/5141.4 | 13 | 5352.0/5351.8 | | — | 11 | 4916.0/4916.1 |
| 5'-d(TTTTTTTT**T**T)-3' (**36a – 36d**) | 28 | 3021.9/3022.0 | 50 | 3036.4/3036.1 | 51 | 3111.5/3112.1 | 57 | 3007.1/3007.0 |




**39**; 5'-r(GCGUUUUUUGCT)-3'
**37**; 3'-r(CGCAAAAAACGA)-5'

**23**; 5'-d(GCGTTTTTTGCT)-3'
**37**; 3'-r(CGCAAAAAACGA)-5'

**25c**;5'-d(GCG**T**T**T**T**T**TGCT)-3'
**37**; 3'-r(CGCAAAAAACGA)-5'

**27c**; 5-d(GCG**TTTTTT**GCT)-3'
**37**; 3'-r(CGCAAAAAACGA)-5'

**39**; 5-r(GCGUUUUUUGCT)-3'
**38**; 3-d(CGCAAAAAACGA)-5'

**23**; 5-d(GCGTTTTTTGCT)-3'
**38**; 3-d(CGCAAAAAACGA)-5'

**25c**;5-d(GCG**T**T**T**T**T**TGCT)-3'
**38**; 3-d(CGCAAAAAACGA)-5'

**27c**;5-d(GCG**TTTTTT**GCT)-3'
**33**; 3-d(CGCAAAAAACGA)-5'

**T** = 2',4'-BNA$^{NC}$[N-Bn]-T

**Figure SI-1**. CD Spectra of duplexes formed by 2',4'-BNA$^{NC}$[NBn]-modified oligonucleotides **25c** and **27c** with complementary RNA (**37**, Figure A) and DNA (**38**, Figure B).The spectra were compared with those of natural RNA duplex (**39**/**37**), DNA/RNA duplex (**23**/**37**), RNA/DNA duplex (**39**/**38**) and DNA duplex (**23**/**38**). Duplex concentration: 4μM in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl.

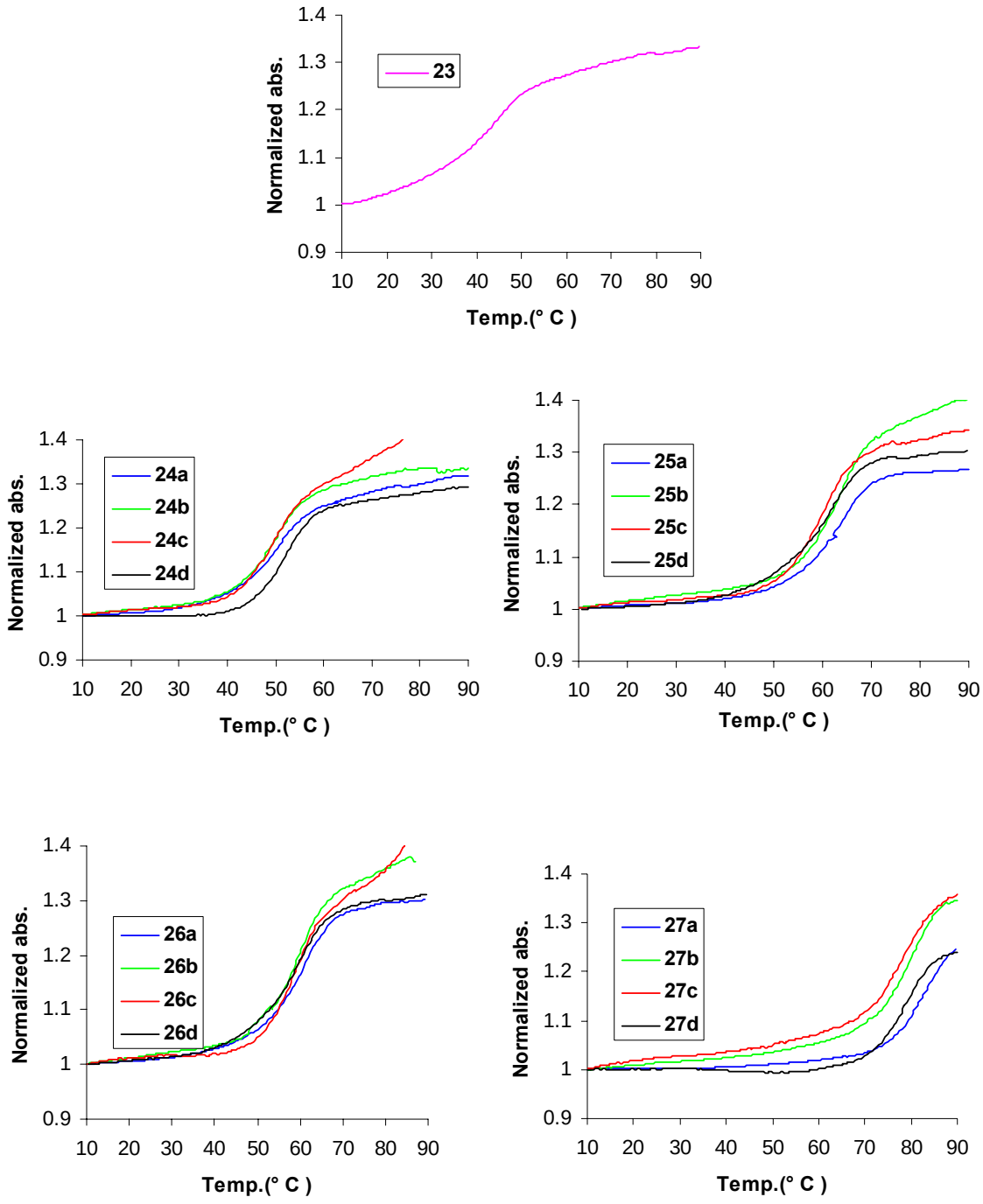



**Figure SI-2**. UV melting curves ($T_m$ curves) for the duplexes formed by natural oligonucleotide **23** and 2',4'-BNA$^{NC}$[NH]-, [NMe]- [NBn]- and 2',4'-BNA (LNA)–modified oligonucleotides **24a-d** to **27a-d** against the complementary ssRNA **37**.

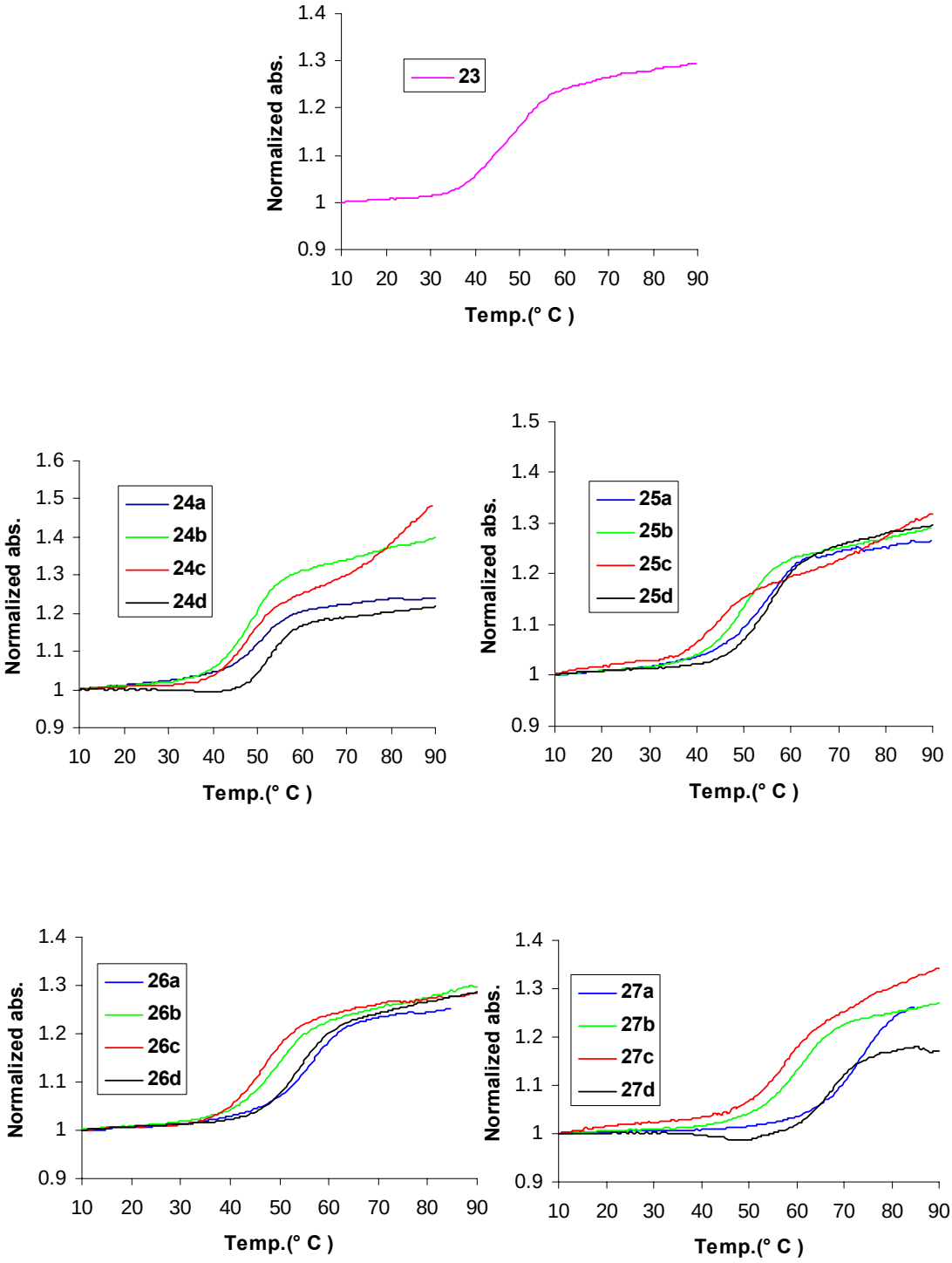



**Figure SI-3**. UV melting curves ($T_m$ curves) for the duplexes formed by natural oligonucleotide **23** and 2',4'-BNA$^{NC}$[NH]-, [NMe]- [NBn]- and 2',4'-BNA (LNA)–modified oligonucleotides **24a-d** to **27a-d** respectively, against the complementary DNA **38**.

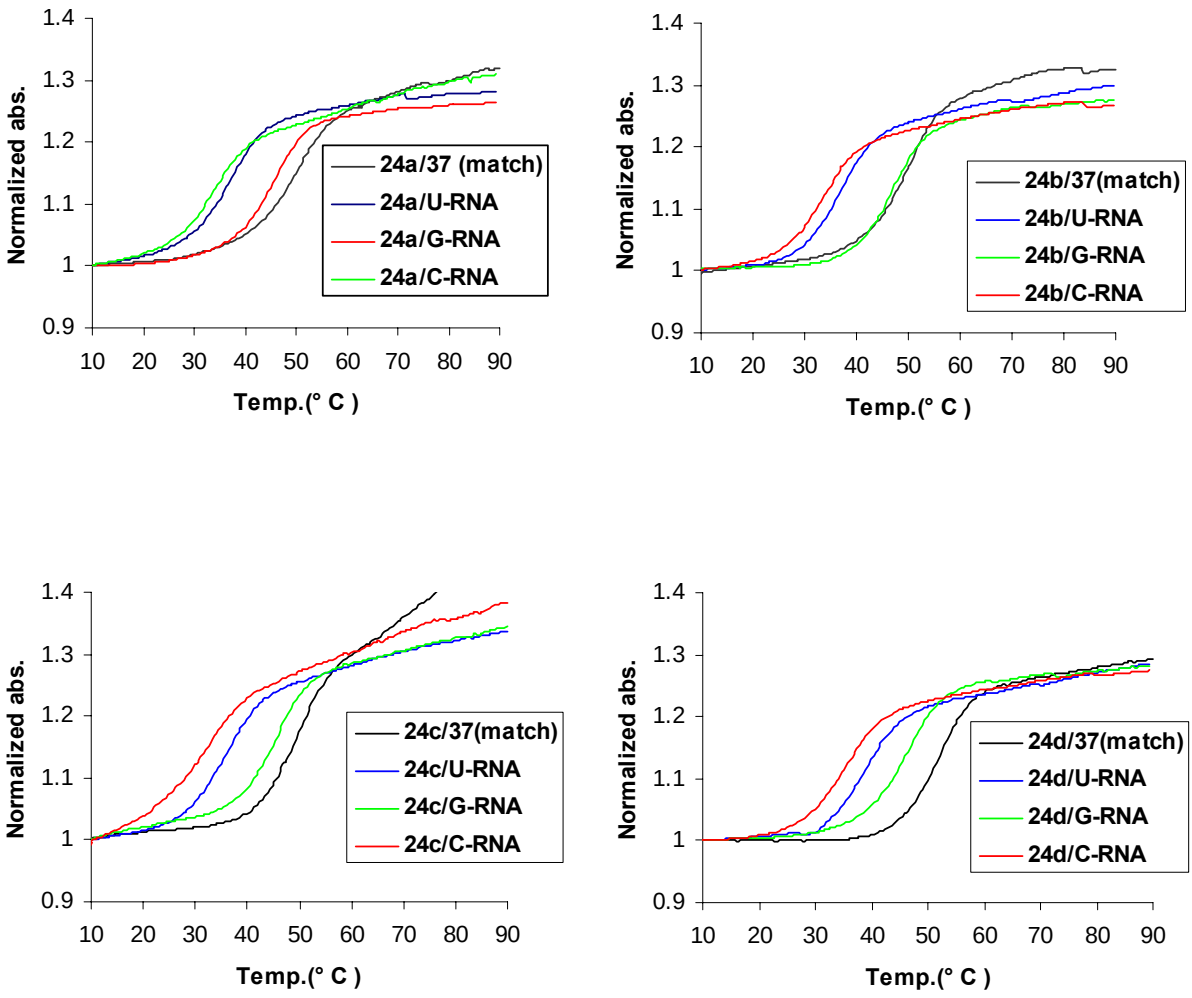



**Figure SI-4**. Mismatch discriminating studies. UV melting curves ($T_m$ curves) for the duplexes formed by 2',4'-BNA$^{NC}$[NH]-, [NMe]- [NBn]- and 2',4'-BNA (LNA)–modified oligonucleotides **24a-d** against match RNA target **37** and the mismatches RNAs; target RNAs = 5'-(AGCAA**X**AAACGC0-3'; **X** = A **(37)**, **X** = U **(U-RNA)**, **X** = G **(G-RNA)**, **X** = C **(C-RNA)**.

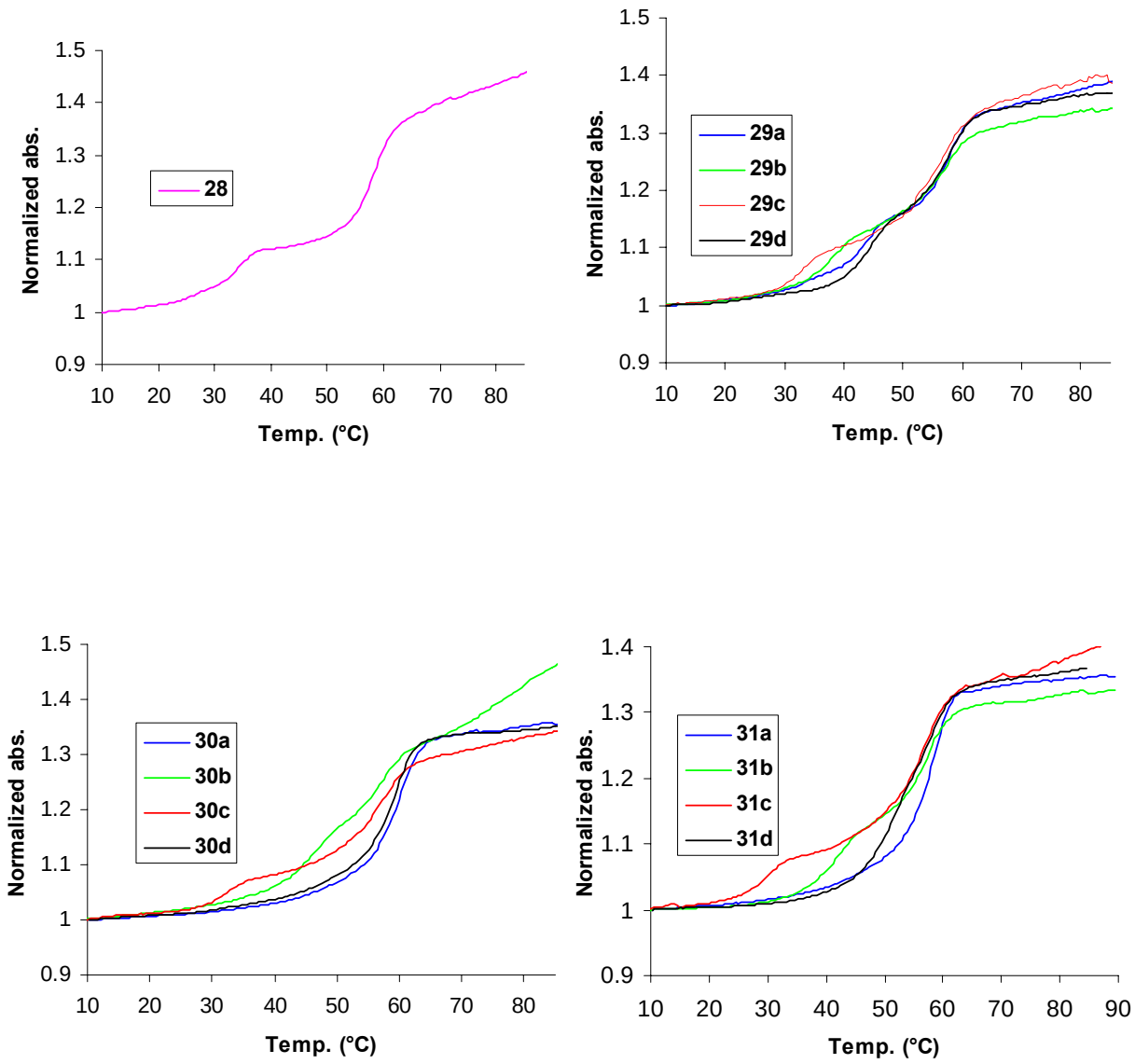



**Figure SI-5**. UV melting curves ($T_m$ curves) for the triplexes formed by natural TFO **28** and 2',4'-BNA$^{NC}$[NH]-, [NMe]- [NBn]- and 2',4'-BNA (LNA)-modified TFOs **29a-d** to **31a-d,** respectively**,** against 21 mer dsDNA in the absence of $Mg^{2+}$. Target dsDNA = 5'd-(GCTAAAAAGAAAGAGAGATCG)-3 / 3'-d(CGATTTTTCTTTCTCTCTAGC)-5'; conditions: 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl; strand concentration = 1.5 μM.

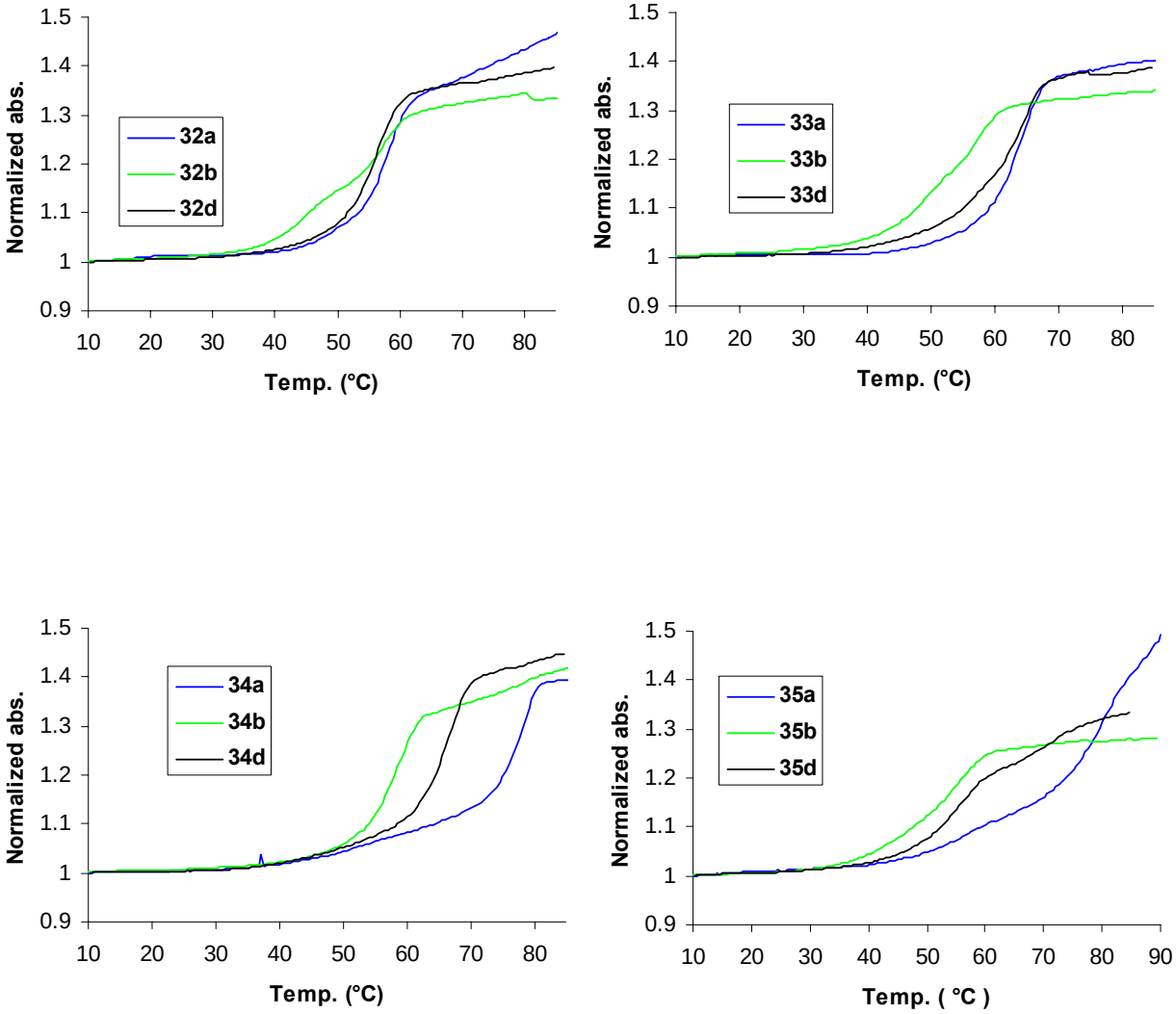



**Figure SI-6**. UV melting curves ($T_m$ curves) for the triplexes formed by 2',4'-BNA$^{NC}$[NH]-, [NMe]- and 2',4'-BNA (LNA)-modified TFOs **32a,b,d** to **35a,b,d,** respectively**,** against 21 mer dsDNA in the absence of $Mg^{2+}$. Target dsDNA = 5'd-(GCTAAAAAGAAAGAGAGATCG)-3 / 3'-d(CGATTTTTCTTTCTCTCTAGC)-5'; conditions: 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl; strand concentration = 1.5 μM.

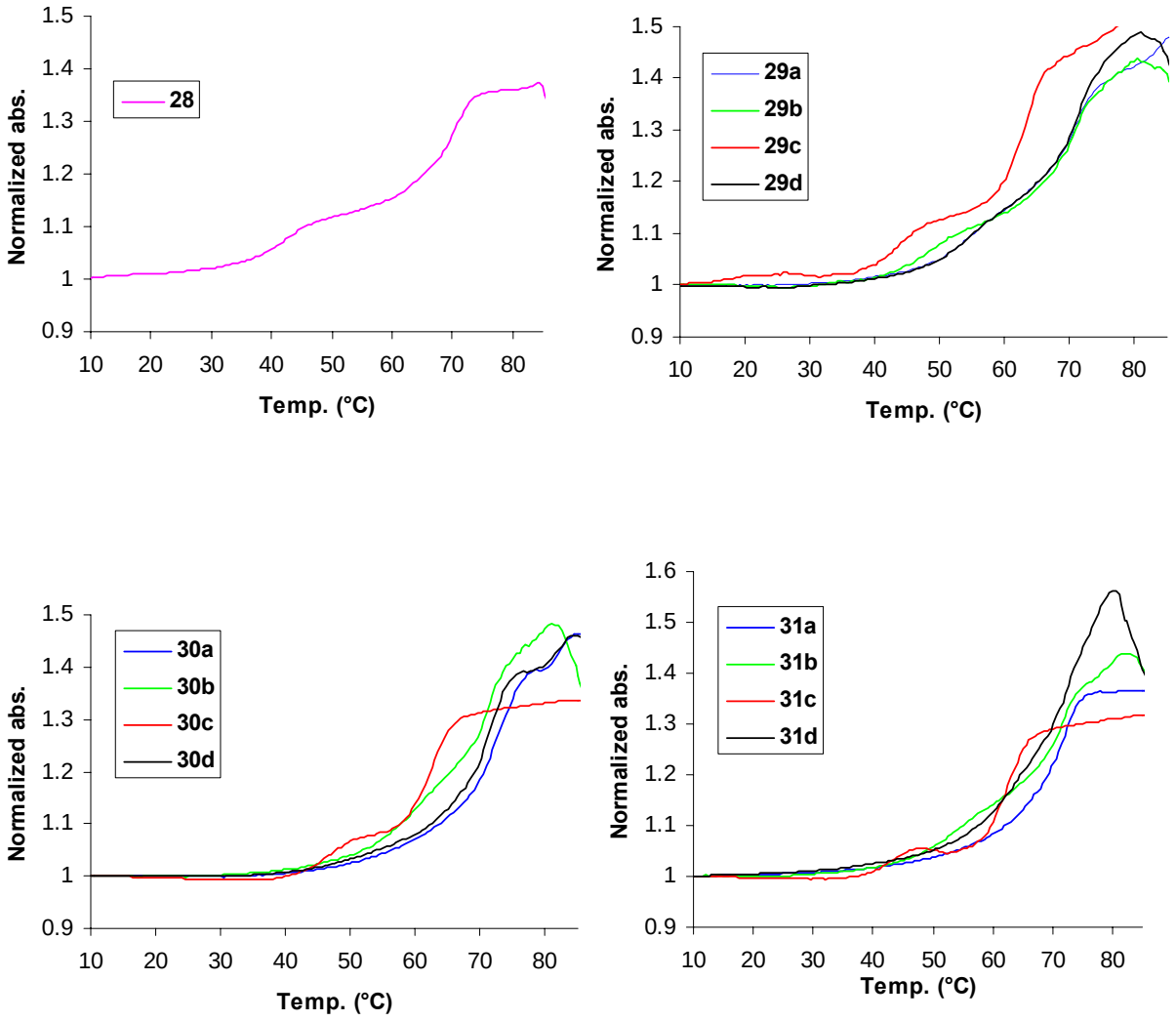



**Figure SI-7**. UV melting curves ($T_m$ curves) for the triplexes formed by natural TFO 28 and 2',4'-BNA$^{NC}$[NH]-, [NMe]-, [NBn]- and 2',4'-BNA (LNA)-modified TFOs **29a-d** to **31a-d,** respectively**,** against 21 mer dsDNA in the presence of $Mg^{2+}$. Target dsDNA = 5'd-(GCTAAAAAGAAAGAGAGATCG)-3 / 3'-d(CGATTTTTCTTTCTCTCTAGC)-5'; conditions: 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$; strand concentration = 1.5 μM.

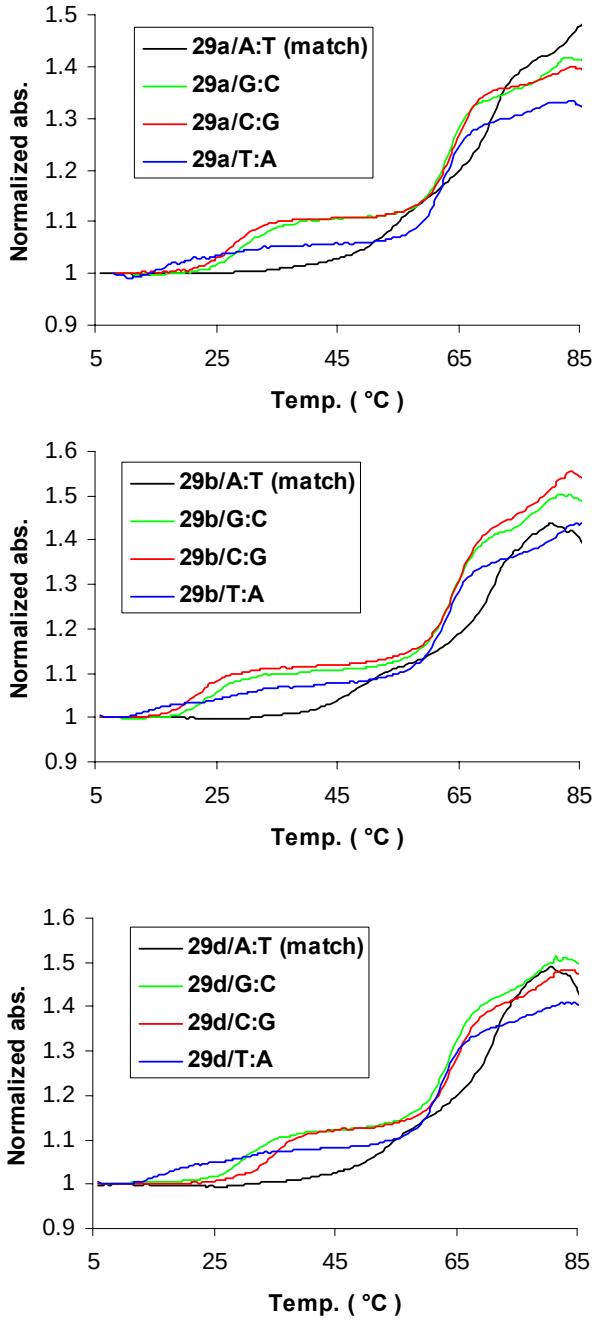



**Figure SI-8**. Mismatch discrimination studies. UV melting curves ($T_m$ curves) for the triplexes formed by 2',4'-BNA$^{NC}$[NH]-, [NMe]- and 2',4'-BNA (LNA)-modified TFOs **29a, 29b** and **29d,** respectively**,** against 21 mer dsDNAs having various arrangements at the center. Target dsDNA = 5'd-(GCTAAAAAGA**X**AGAGAGATCG)-3 / 3'-d(CGATTTTTCT**Y**TCTCTCTAGC)-5'; **X:Y** = A:T (match target), **X:Y** = G:C, C:G or T:A (mismatched targets). Conditions: 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$; strand concentration = 1.5 μM.

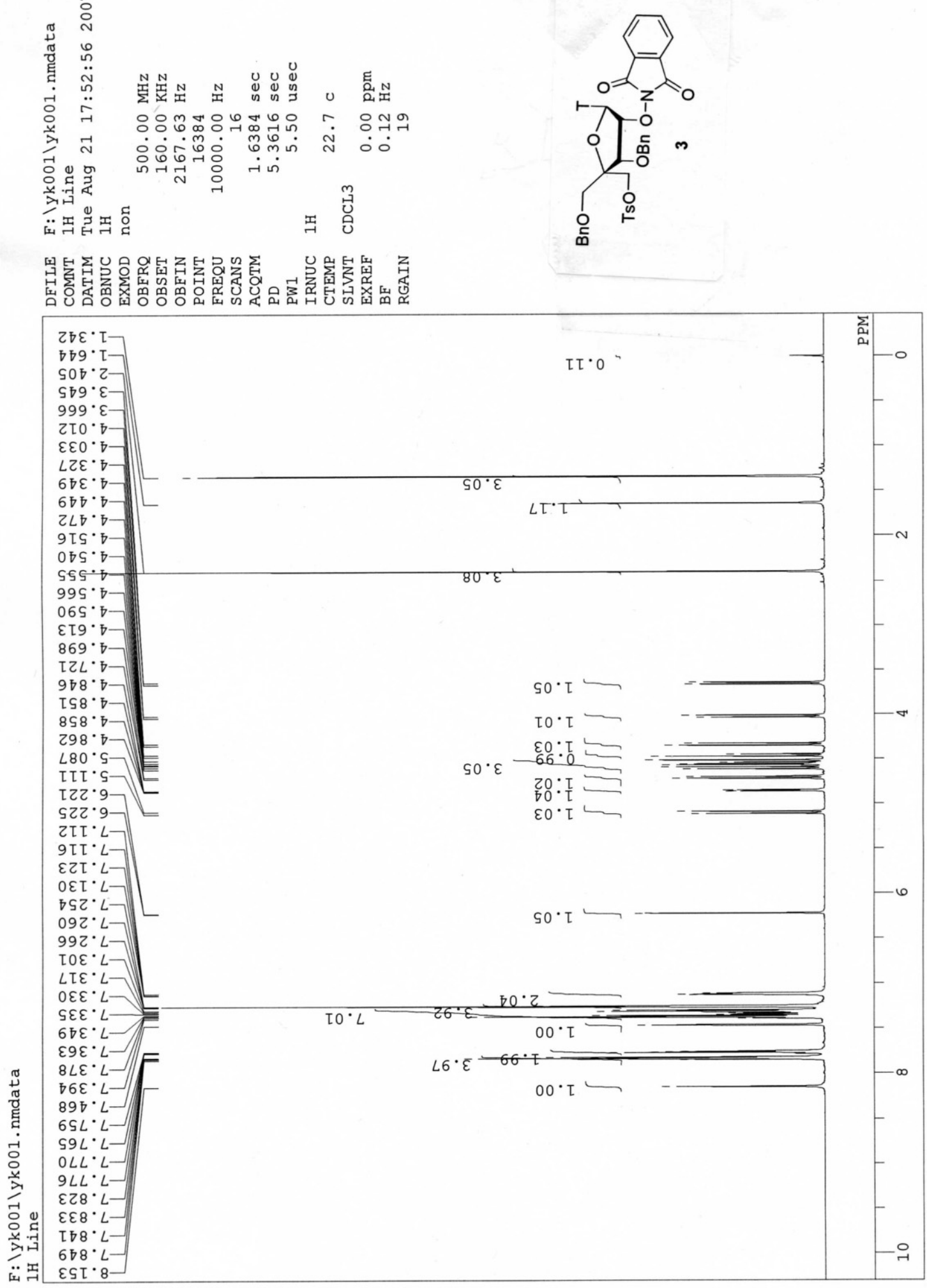
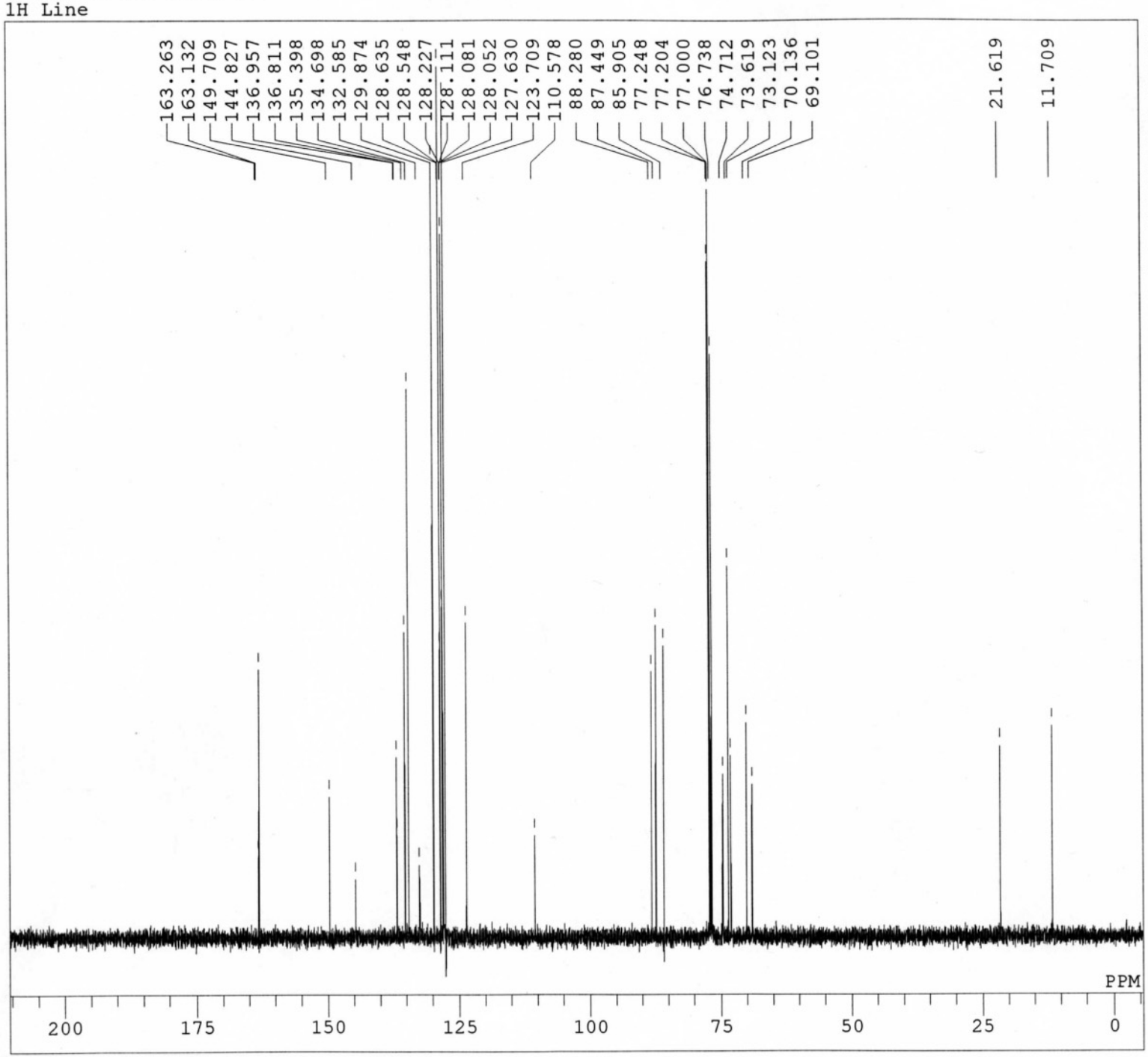
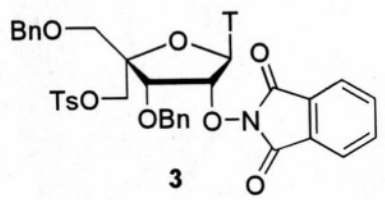



**Figure SI-9:** $^{1}$H-NMR spectrum of compound **3**

**Figure SI-10:** $^{13}$C-NMR spectrum of compound **3**




DFILE F:\bn913c\yk002.nmdata
COMNT 1H Line
DATIM Tue Aug 21 18:51:35 2007
OBNUC 13C
EXMOD bcm
OBFRQ 125.65 MHz
OBSET 120.00 KHz
OBFIN 7958.00 Hz
POINT 16384
FREQU 30030.03 Hz
SCANS 1000
ACQTM 0.5456 sec
PD 2.4544 sec
PW1 8.00 usec
IRNUC 1H
CTEMP 25.2 c
SLVNT CDCL3
EXREF 77.00 ppm
BF 0.12 Hz
RGAIN 29




**3**

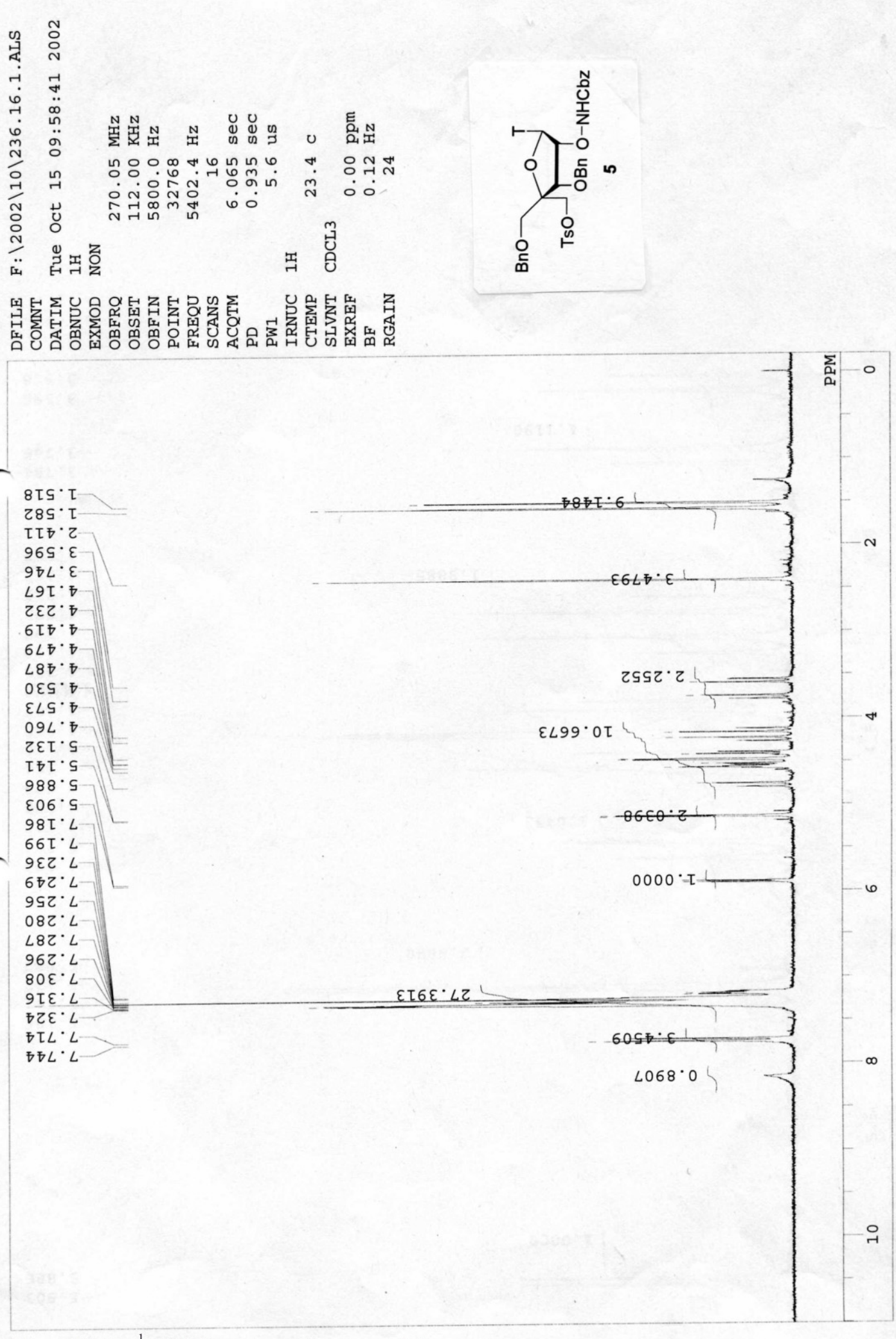



**Figure SI-11:** $^{1}$H-NMR spectrum of compound **5**

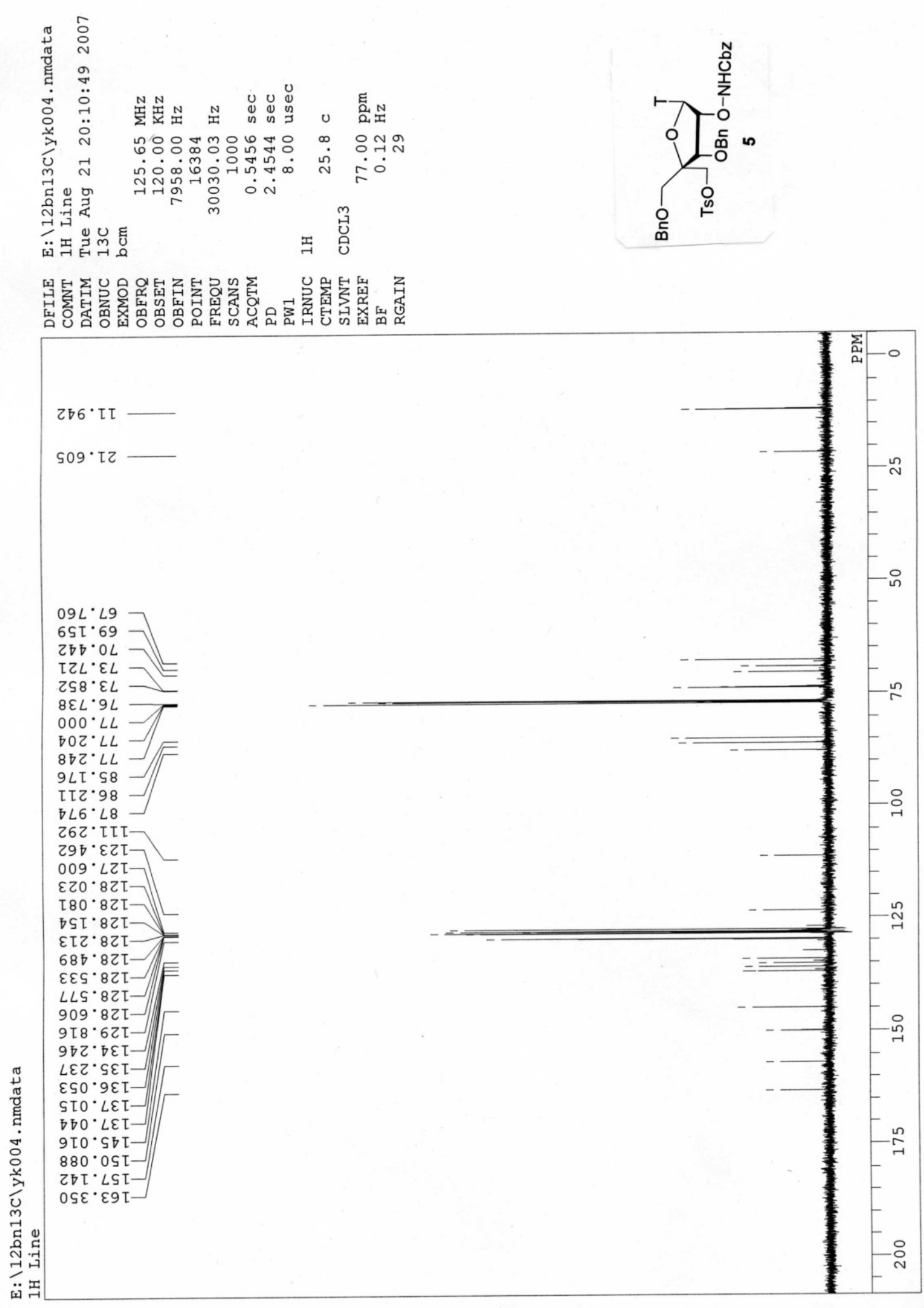
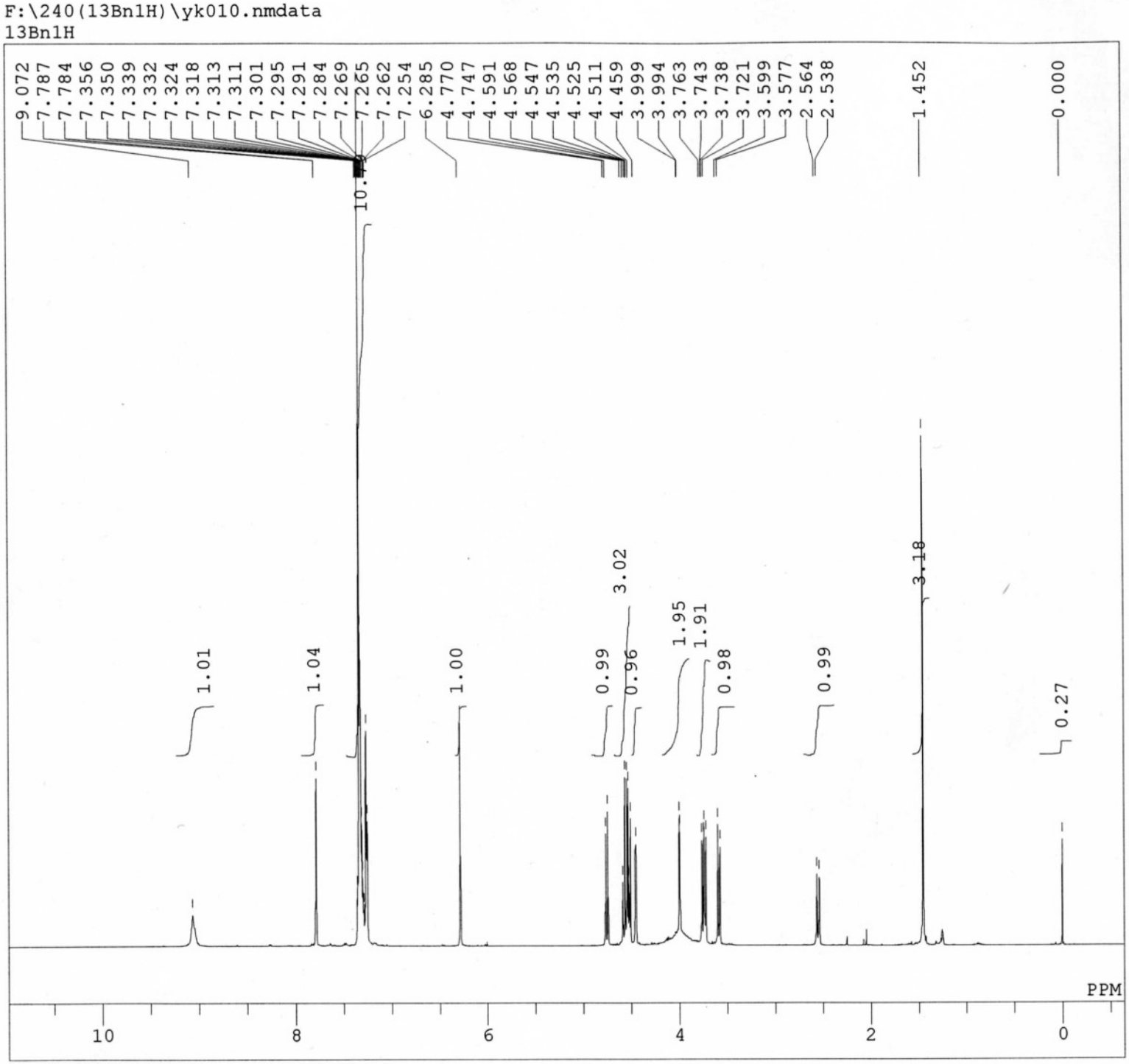
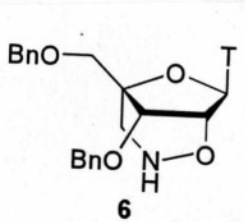



**Figure SI-12:**[13] C-NMR spectrum of compound **5**




DFILE F:\240(13Bn1H)\yk010.nmdata
COMNT 13Bn1H
DATIM Thu Aug 30 11:47:37 2007
OBNUC 1H
EXMOD non
OBFRQ 500.00 MHz
OBSET 160.00 KHz
OBFIN 2167.63 Hz
POINT 16384
FREQU 10000.00 Hz
SCANS 32
ACQTM 1.6384 sec
PD 5.3616 sec
PW1 5.50 usec
IRNUC 1H
CTEMP 22.6 c
SLVNT CDCL3
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 17




**6**

**Figure SI-13:** $^{1}$H-NMR spectrum of compound **6**

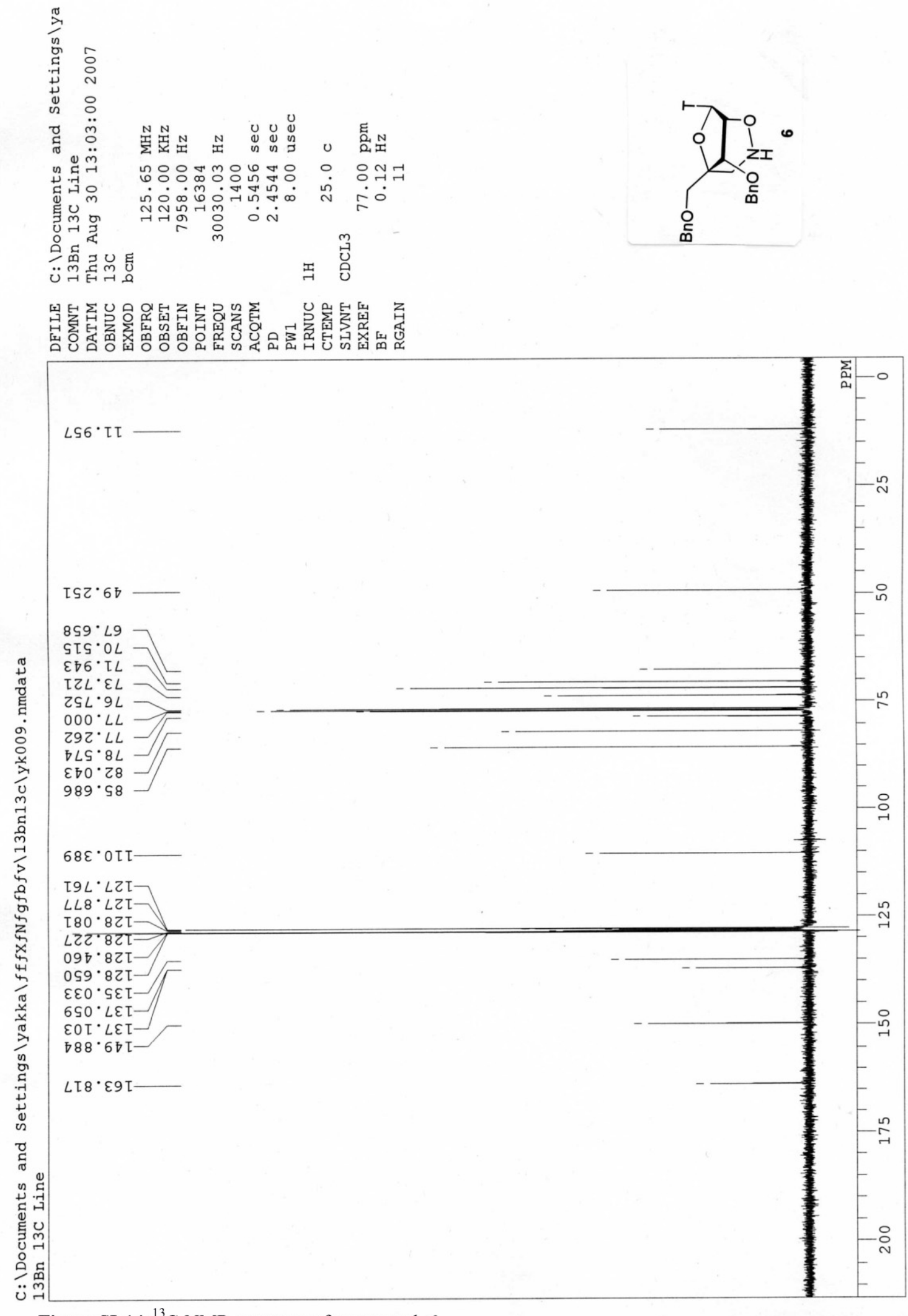



**Figure SI-14:** $^{13}$C-NMR spectrum of compound **6**

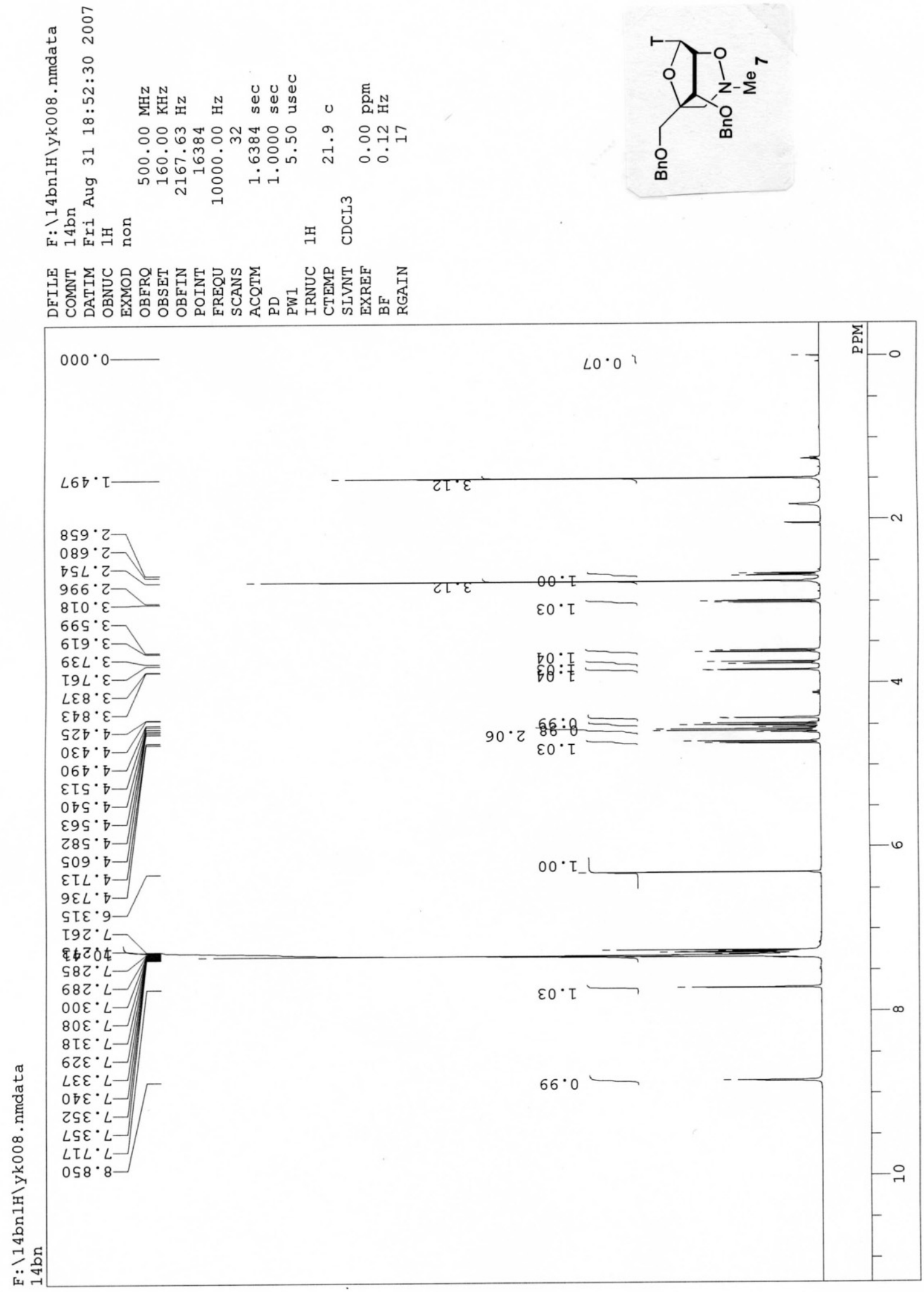



**Figure SI-15:** $^{1}$H-NMR spectrum of compound **7**

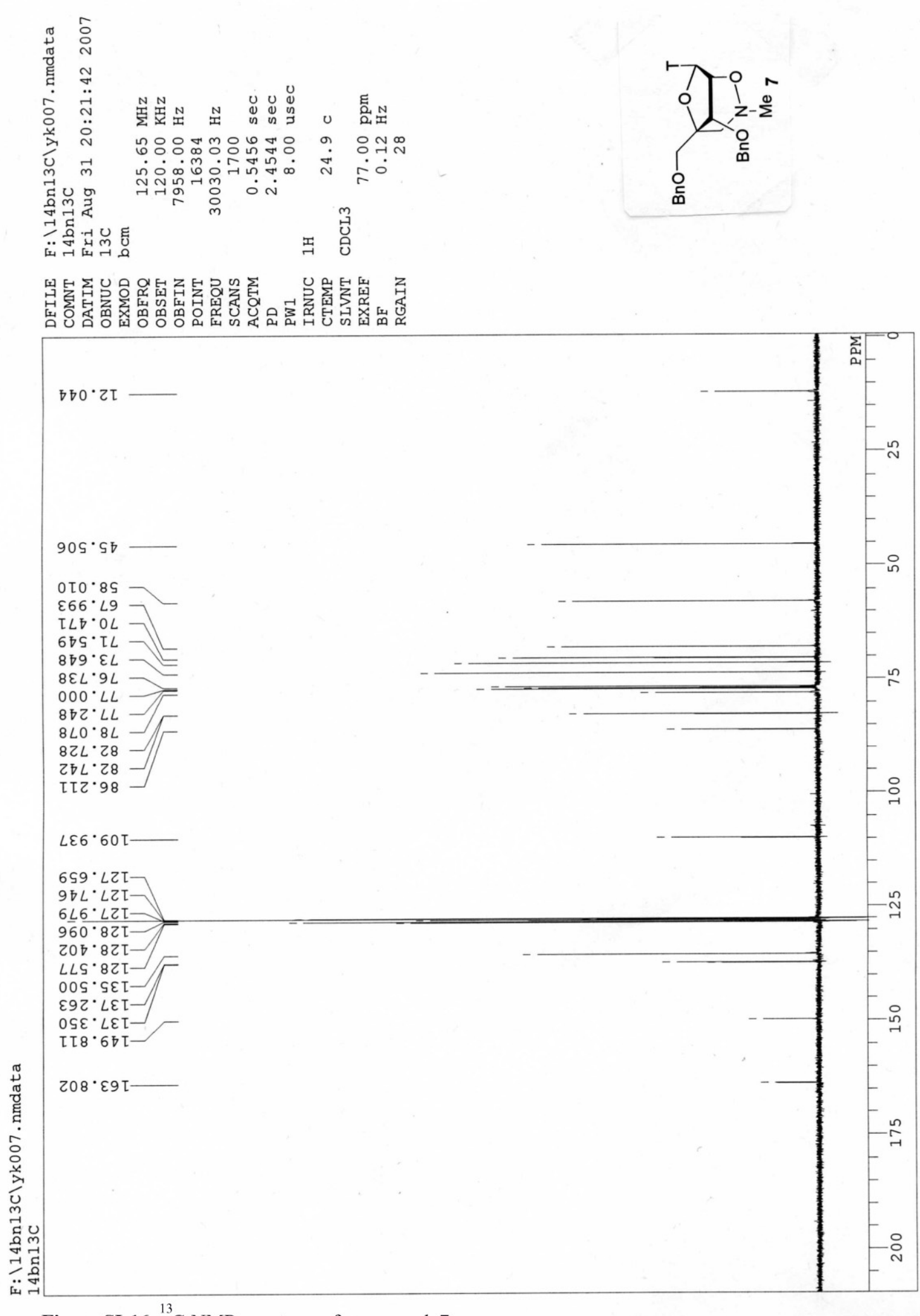



**Figure SI-16:** $^{13}$C-NMR spectrum of compound **7**

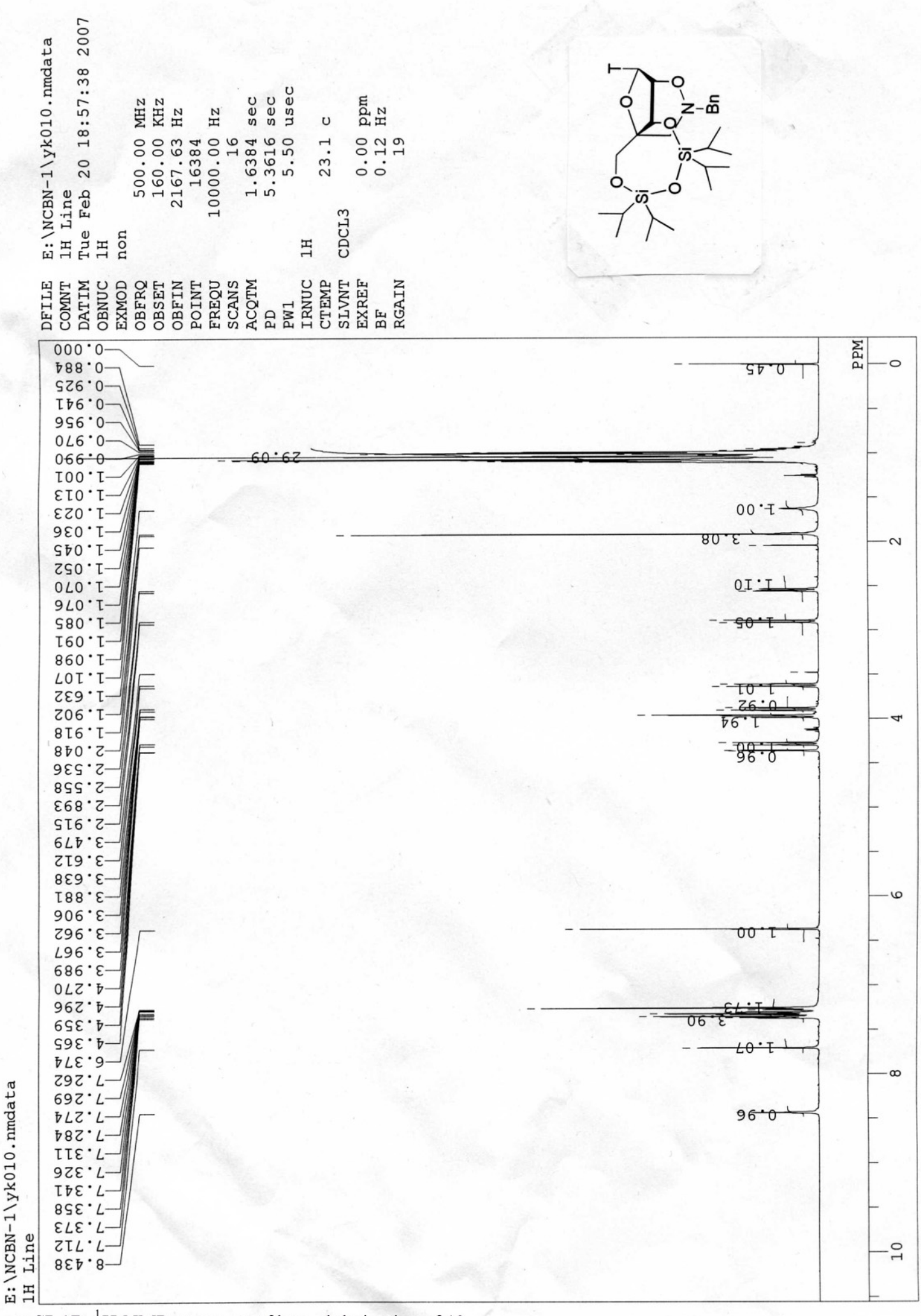



**Figure SI-17:** $^{1}$H-NMR spectrum of benzyl derivative of **12**

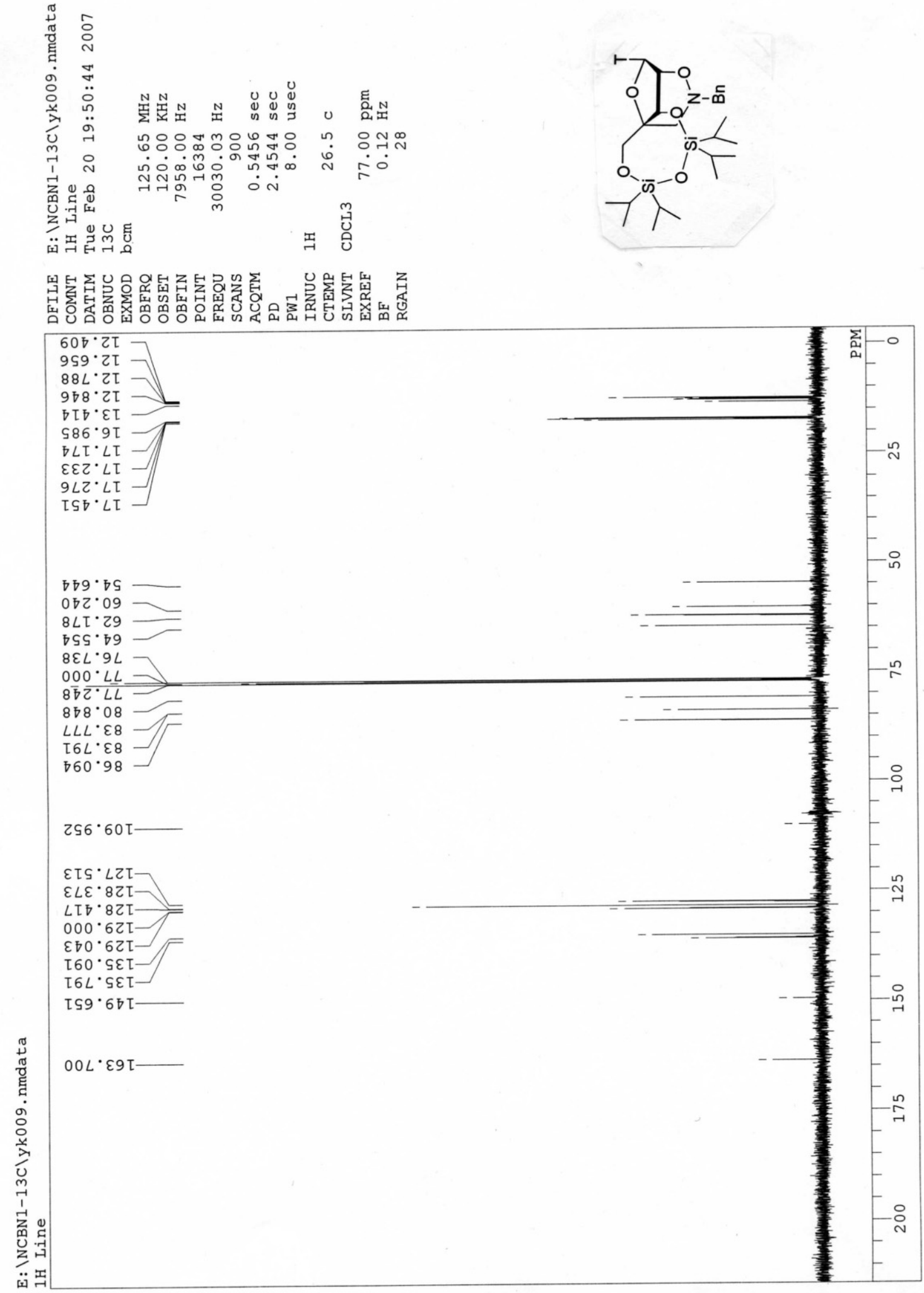
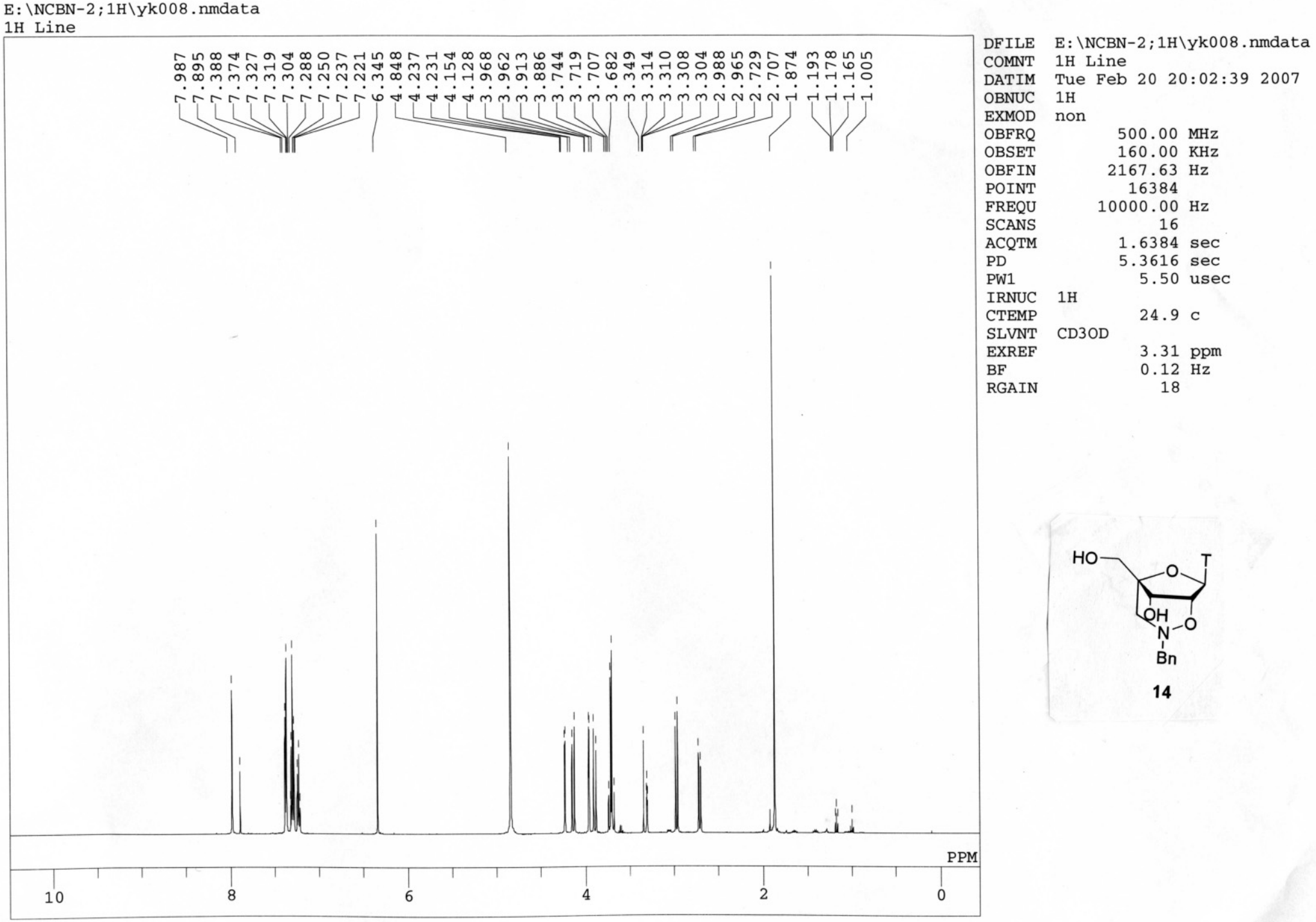



**Figure SI-18:** $^{13}$C-NMR spectrum of benzyl derivative of **12**

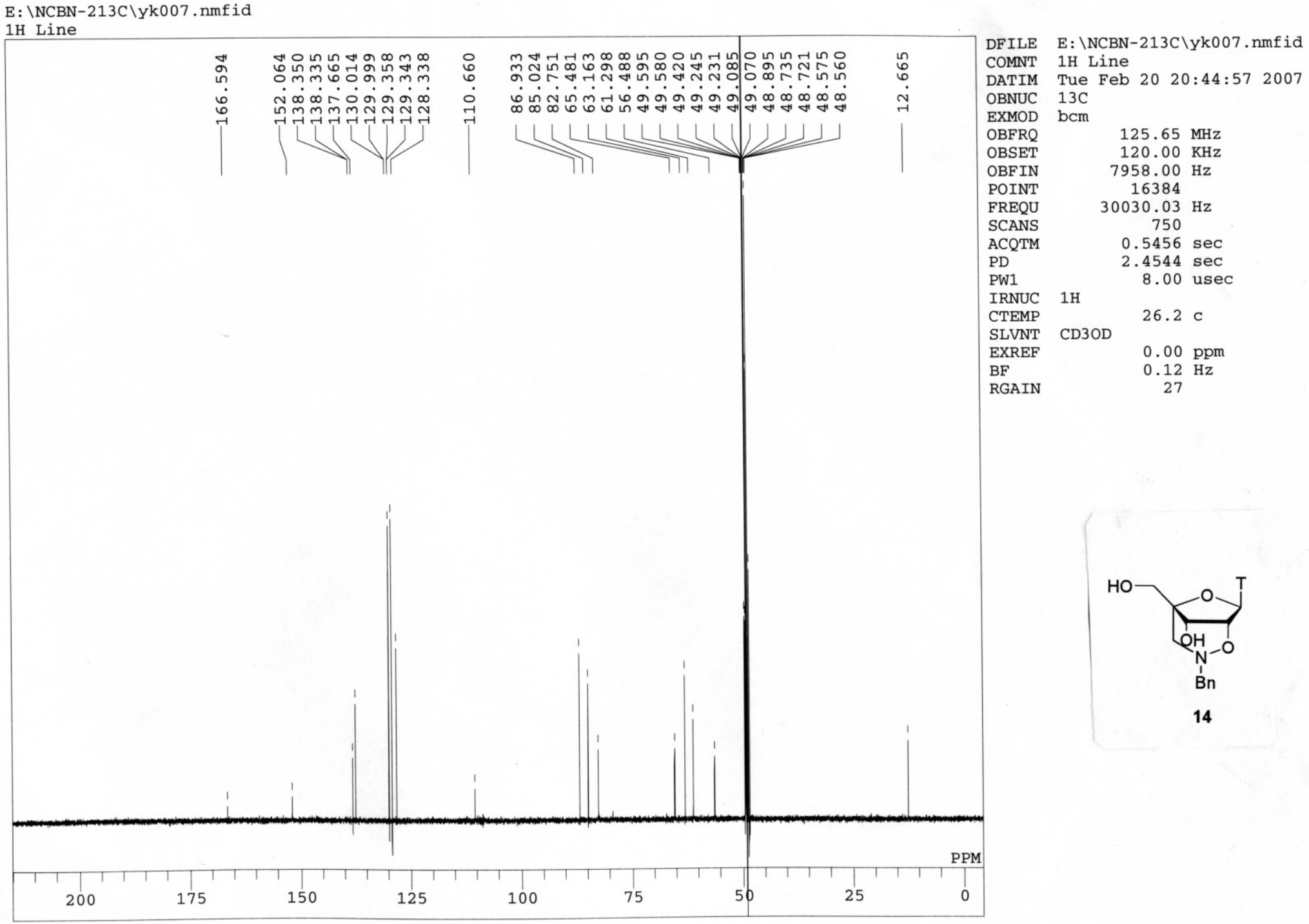



DFILE E:\NCBN-2;1H\yk008.nmdata
COMNT 1H Line
DATIM Tue Feb 20 20:02:39 2007
OBNUC 1H
EXMOD non
OBFRQ 500.00 MHz
OBSET 160.00 KHz
OBFIN 2167.63 Hz
POINT 16384
FREQU 10000.00 Hz
SCANS 16
ACQTM 1.6384 sec
PD 5.3616 sec
PW1 5.50 usec
IRNUC 1H
CTEMP 24.9 c
SLVNT CD3OD
EXREF 3.31 ppm
BF 0.12 Hz
RGAIN 18




**14**

**Figure SI-19:** $^{1}$H-NMR spectrum of compound **14**




DFILE E:\NCBN-213C\yk007.nmfid
COMNT 1H Line
DATIM Tue Feb 20 20:44:57 2007
OBNUC 13C
EXMOD bcm
OBFRQ 125.65 MHz
OBSET 120.00 KHz
OBFIN 7958.00 Hz
POINT 16384
FREQU 30030.03 Hz
SCANS 750
ACQTM 0.5456 sec
PD 2.4544 sec
PW1 8.00 usec
IRNUC 1H
CTEMP 26.2 c
SLVNT CD3OD
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 27




**14**

**Figure SI-20:** $^{13}$C-NMR spectrum of compound **14**

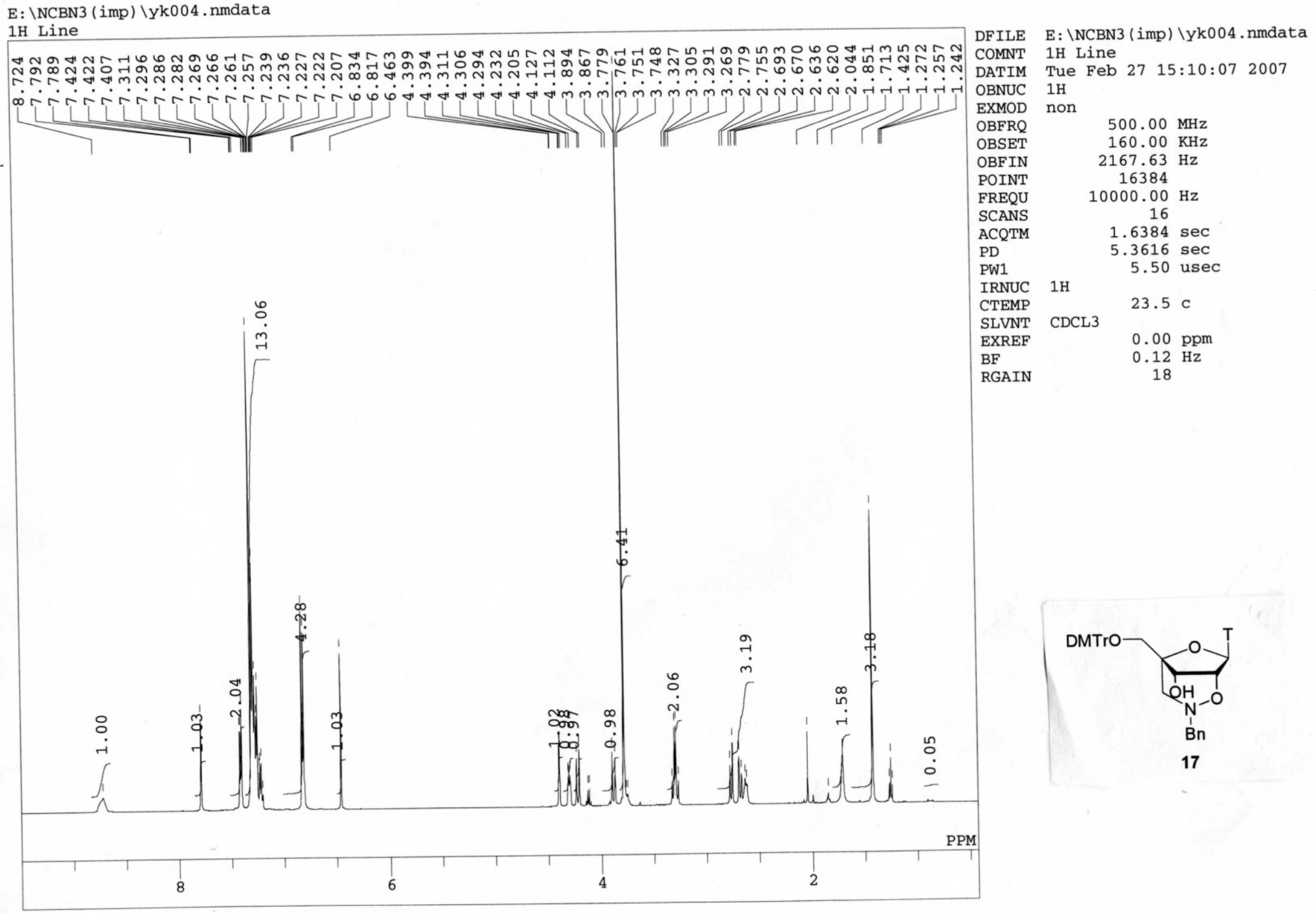
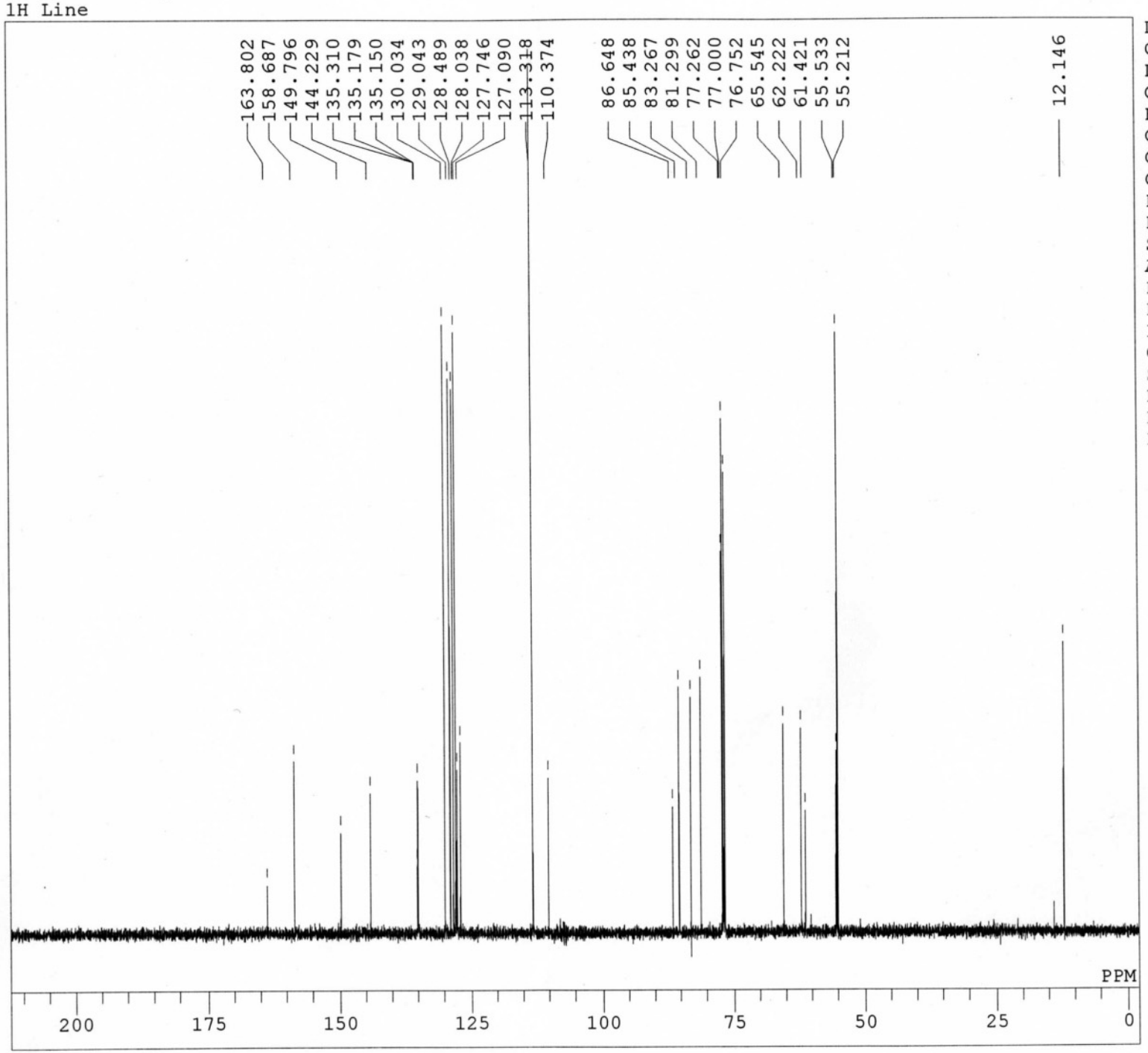



**Figure SI-21:** $^{1}$H-NMR spectrum of compound **17**




DFILE E:\NCBN3-13C\yk003.nmdata
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DATIM Tue Feb 27 16:34:09 2007
OBNUC 13C
EXMOD bcm
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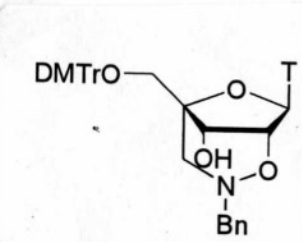
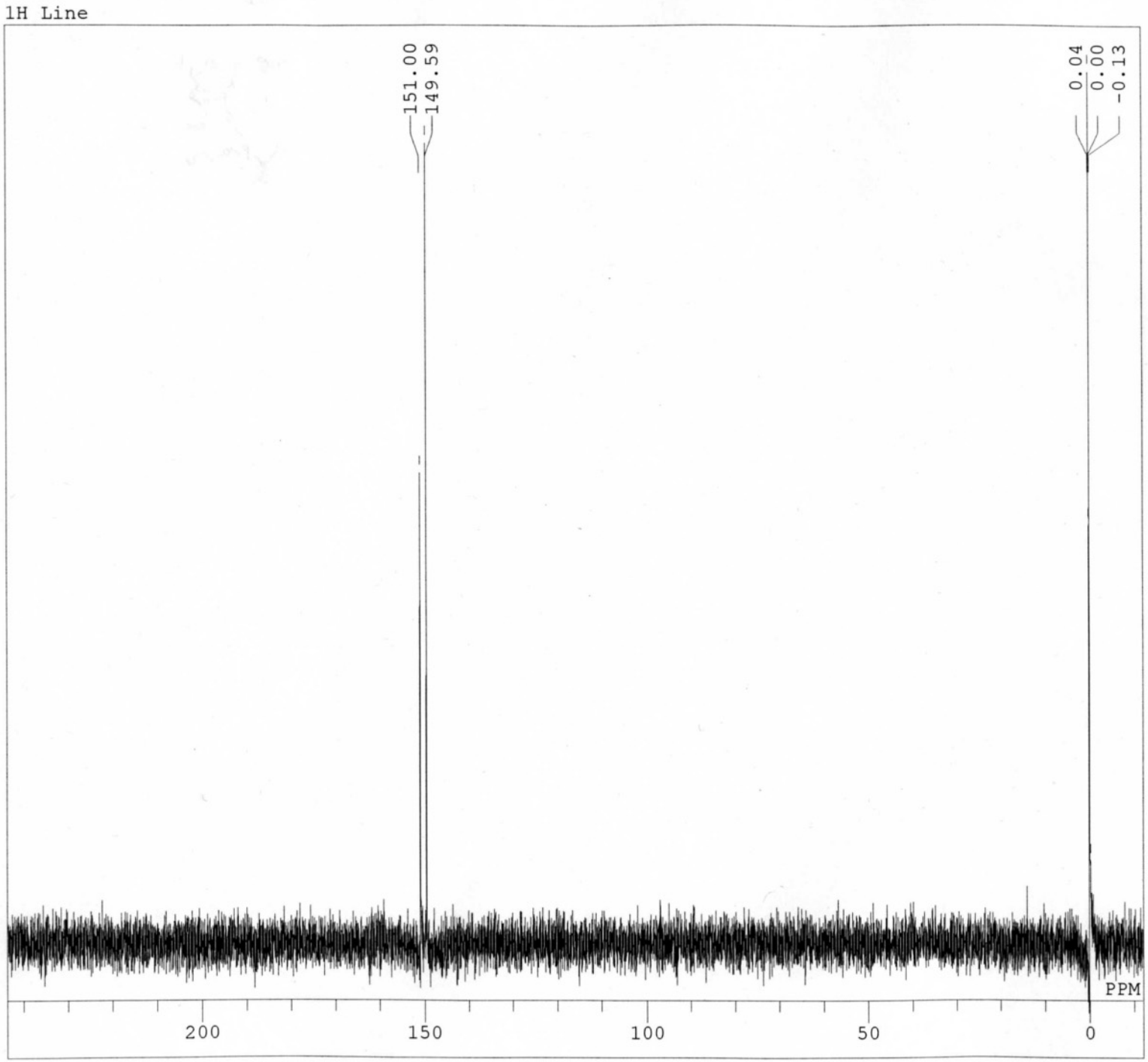
OBSET 120.00 KHz
OBFIN 7958.00 Hz
POINT 16384
FREQU 30030.03 Hz
SCANS 1500
ACQTM 0.5456 sec
PD 2.4544 sec
PW1 8.00 usec
IRNUC 1H
CTEMP 25.3 c
SLVNT CDCL3
EXREF 77.00 ppm
BF 0.12 Hz
RGAIN 30




**17**

**Figure SI-22:** $^{13}$C-NMR spectrum of compound **17**

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EXMOD   bcm
OBFRQ         202.35 MHz
OBSET         140.00 KHz
OBFIN        6820.00 Hz
POINT          32768
FREQU      121951.23 Hz
SCANS            100
ACQTM         0.2687 sec
PD            2.7313 sec
PW1             6.00 usec
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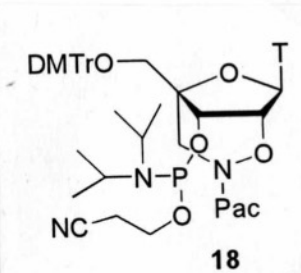
CTEMP           23.8 c
SLVNT   CDCL3
EXREF           0.00 ppm
BF              0.12 Hz
RGAIN             27
```




**18**

**Figure SI-23:** $^{31}$P-NMR spectrum of compound **18**

**Figure SI-24:** $^{31}$P-NMR spectrum of compound **19**

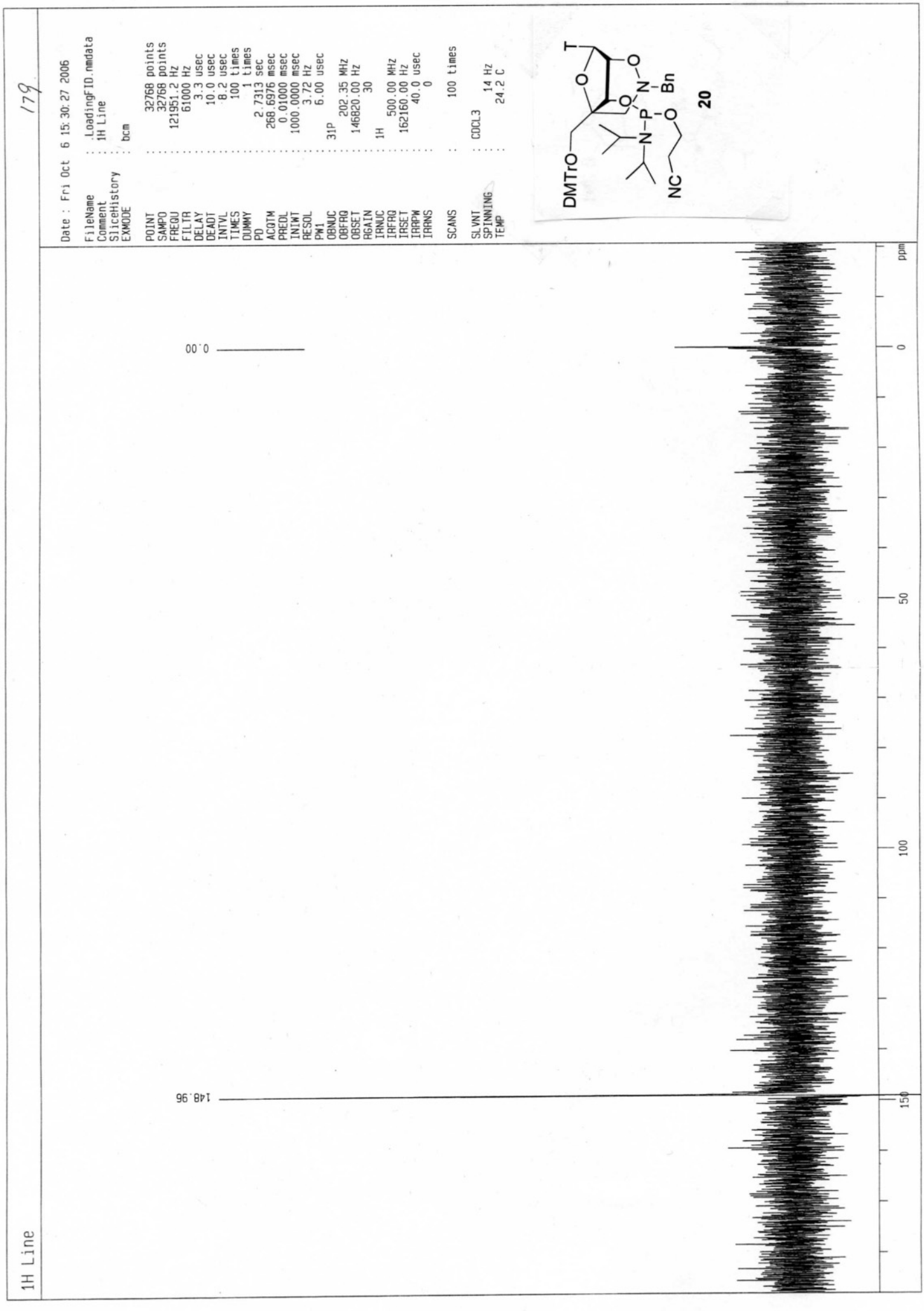



**Figure SI-25:** $^{31}$P-NMR spectrum of compound **20**

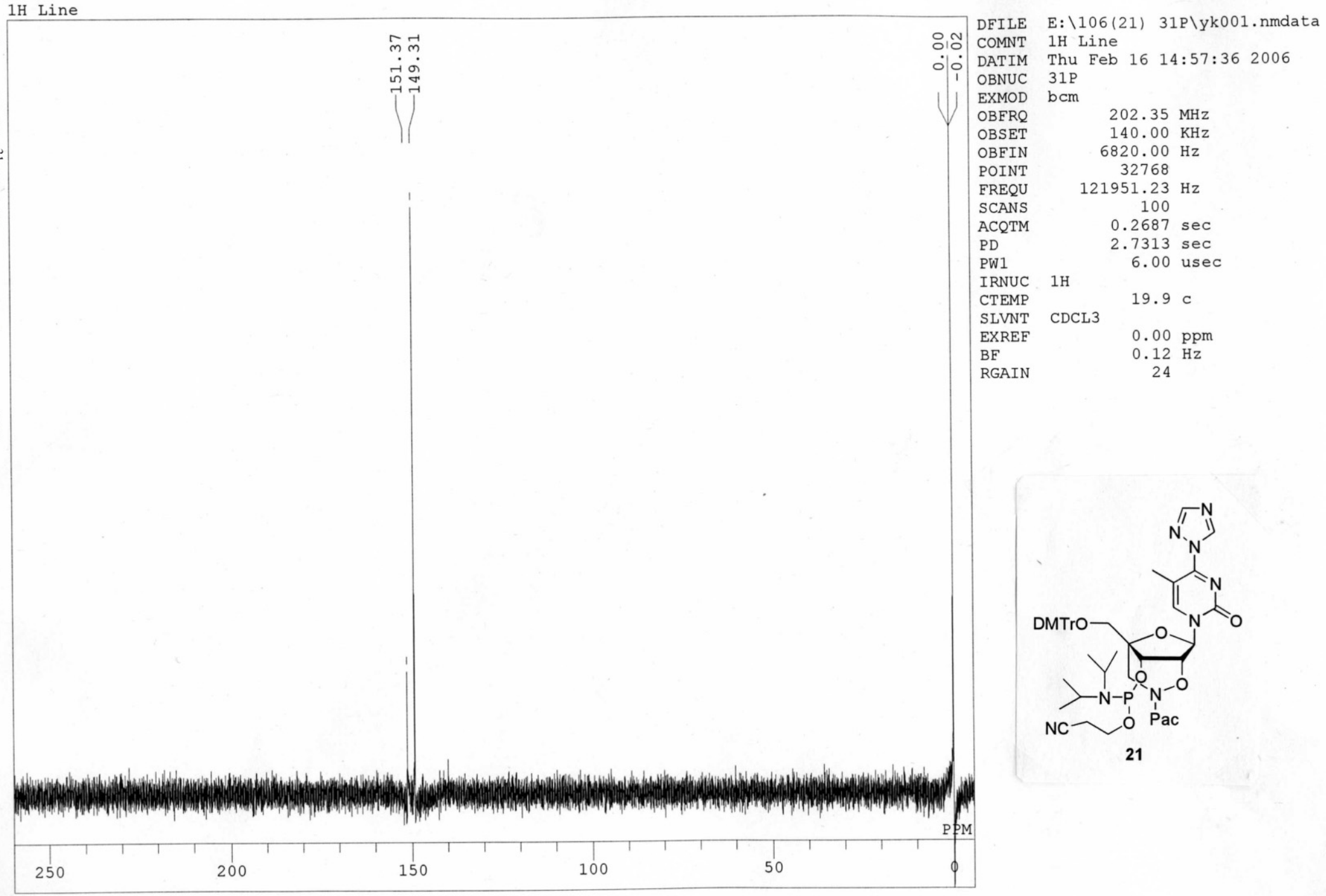



**Figure SI-26:** $^{31}$P-NMR spectrum of compound **21**

**Figure SI-27:** $^{31}$P-NMR spectrum of compound **22**

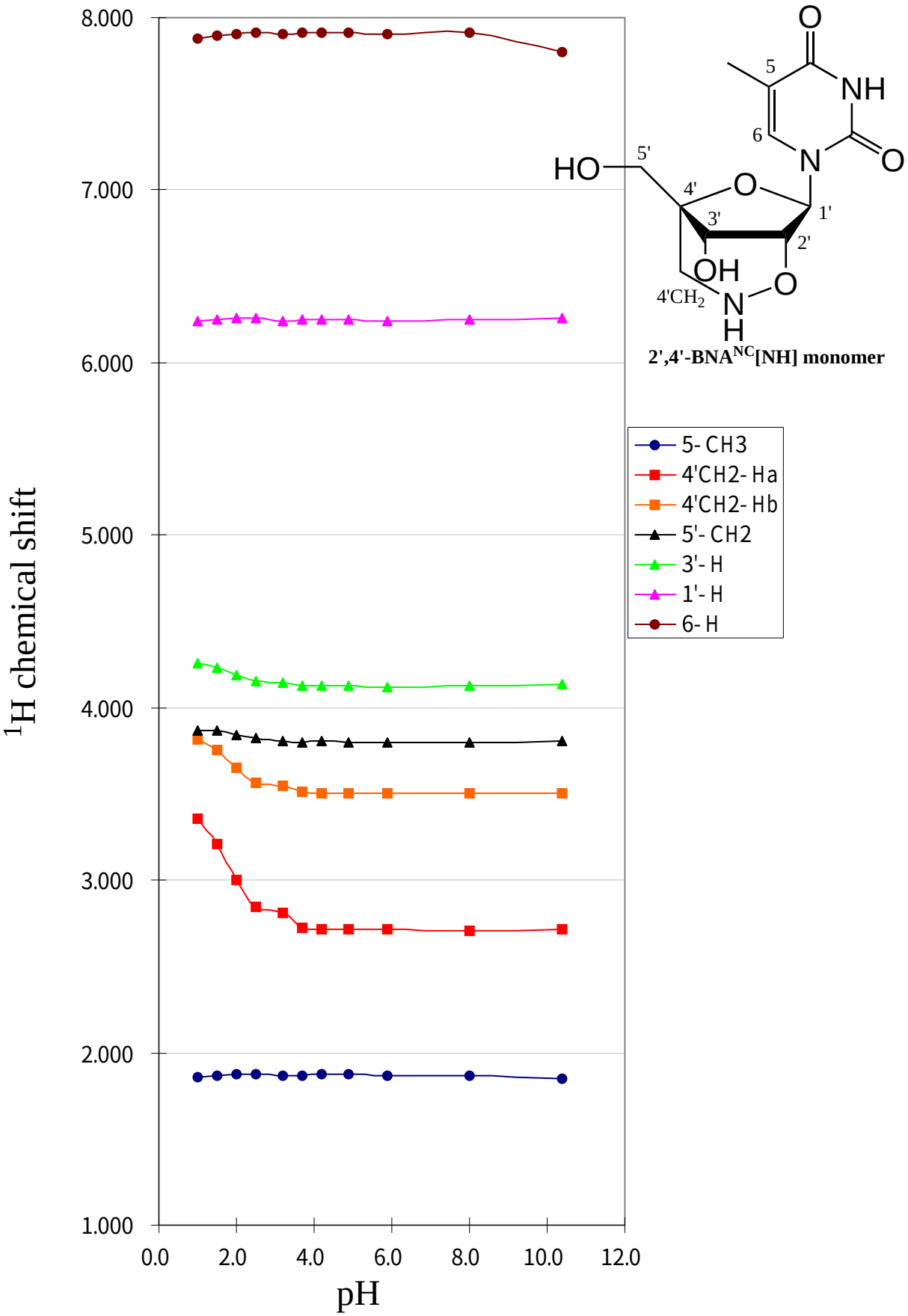



**Figure SI-28.** pH Titration curve-1: dependence of $^{1}$H chemical shifts of 2',4'-BNA$^{NC}$[NH] monomer upon changes of pH.

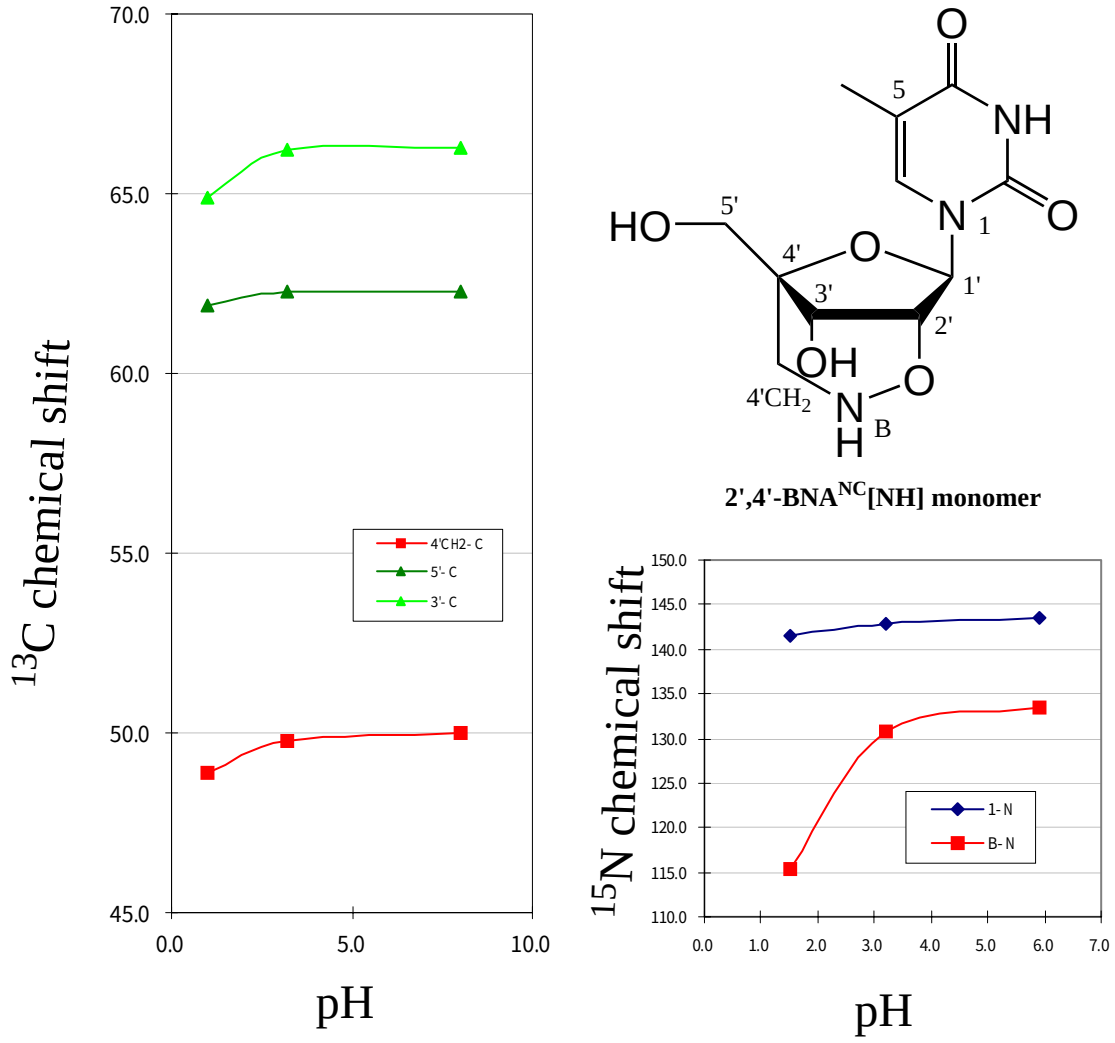



**Figure SI-29**. pH Titration curve-2: dependence of $^{13}C$ and $^{15}N$ chemical shifts of 2',4'-BNA$^{NC}$[NH] monomer upon changes of pH