CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

An Inverse Substrate Orientation for the Regioselective Acylation of 3',5'-Diaminonucleosides Catalyzed by Candida antarctica lipase B?

Iván Lavandera, Susana Fernández, Julia Magdalena, Miguel Ferrero, Romas J. Kazlauskas,* and Vicente Gotora*

Table of Contents

General Spectroscopic and Experimental Data

Candida antarctica lipase B (CAL-B, Novozym 435, 7300 PLU/g) was purchased from suppliers. Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on an Infrared Fourier Transform spectrophotometer using KBr pellets. Flash chromatography was performed using silica gel 60 (230-400 mesh). ^{1}H-, ^{13}C-NMR, and DEPT were obtained using AC-200 (^{1}H, 200.13 MHz and ^{13}C, 50.3 MHz), and AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers for routine experiments. An AMX-400 spectrometer operating at 400.13 and 100.61 MHz for ^{1}H and ^{13}C, respectively, was used for the acquisition of ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments. The chemical shifts are given in delta (δ) values and the coupling constants (*J*) in Hertz (Hz). ESI^{+} was used to record mass spectra (MS). Microanalyses were performed on a Perkin-Elmer model 2400 instrument.

Molecular Modeling

The crystal structure of the CAL-B was relaxed before addition of the substrate to the experimental structure. The PDB file 1lbs was opened with all atoms of the crystal structure, including water molecules. Hydrogen atoms were added to the structure, tested for partial charge balance and corrected for atom types within the AMBER force field. The pH of the catalytic histidine was adjusted to pH 4 to protonate the residue and the structure was then relaxed. Geometry optimizations were carried out in two steps. First, the steepest descent algorithm corrected major high energy structural problems and got the molecule to a local minima with a RMS deviation of about 0.02 Å mol^{-1}. Second, conjugate gradient algorithm gave a faster and more precise minimization near the local minima, obtaining a RMS value of 0.005 Å mol^{-1} or lower. The entire structure was relaxed by a systematic manner that avoided large structural changes. First only the water molecules were optimized after which the side chains of amino acids were released and optimized as well. The entire complex of enzyme and solvent molecules was then released for the final optimization. For all further geometry optimizations in this study the solvent molecules were never fixed. This was to ensure that water molecules were mobile and that their position did not hinder the nucleoside or the enzyme. Energy refers to the potential energy of the enzyme-tetrahedral intermediate complex.

Geometry Optimization of Phosphon(amid)ate Core. A phosphon(amid)ate core of the tetrahedral intermediate mimicking butanoylation of ethanol (X= O; R= Pr) or methylation of ethylamine (X= NH; R= Me) was assembled in the crystal structure. The partial charges on the intermediate atoms were set to those of the tetrahedral intermediate (carbon, not phosphorus), the values of which were obtained by semi-empirical calculation performed using Chem3D using the AM1 parameters.

For the optimization of the phosphon(amid)ate core a systematic approach of releasing different motifs was applied. Initially, the intermediate was allowed to adjust to the enzyme active site by keeping the entire enzyme fixed during the geometry optimization. The side chains of the lipase were then released and allowed to adjust and finally the entire complex was allowed to adjust. This approach avoided drastic changes in the lipase structure caused by nonoptimal conformations of the intermediate. A hydrogen bond calculation was performed after each minimization in order to assure the presence of the critical hydrogen bonds around the catalytic site.[1]

Phosphon(amid)ates including thymidinyl group. After obtaining a catalytically productive intermediate core, the remainder of the nucleoside was added and different conformations were produced by manual adjustment of dihedral angles identified in Figure S1. Geometry optimizations were again performed using the same approach used to optimize the phosphon(amid)ate core with systematic releasing of the substrate, amino acid side chains and the entire enzyme complex. Again, minimizations were carried out with both the steepest descent and conjugate gradients until an RMS value of at least 0.005 Å mol^{-1} was obtained.

The conformational search involving reaction at the 3’ position involved 120° rotations of two dihedral angles identified in Figure S1. With these angle adjustments all possible staggered conformations for both dihedral angles were then optimized. For structures of the 5’ position, however, one extra dihedral angle was available for adjustment with the 5’ nucleophile. Due to the long and flexible structure of this intermediate small adjustments of the dihedral angles caused large changes in the orientation of the thymine moiety. As well, interaction of substrate conformations with the convoluted structure of the enzyme produced more local minima than a nucleoside in free space. Therefore, manual adjustments of 120° did not prove to be

[1] To identify hydrogen bonds, a donor atom to acceptor atom distance of less than 3.20 Å and a donor atom - hydrogen - acceptor atom angle of 120° or greater are required.

sufficient to find all possible local minima. For the 5’ intermediates a more extensive searching process was required. Adjustments of approximately 10-20° were performed for both dihedral angles adjacent to that position. Structures with obvious steric problems were ignored. Additional manual adjustments were done to orient substrate moieties into visible pockets.

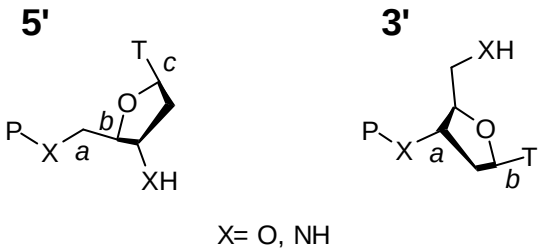


Figure S1. *Manipulated dihedral angles for 5’ and 3’ structures. Dihedral angles corresponding to all the bonds labeled above were adjusted during manual conformational searching. For most angles, adjustments of 120° were made of each dihedral angle to produce possible conformations. Angle a of the 5’ structure was manipulated using smaller adjustments to account for large changes in substrate conformations caused by these adjustments. In addition, different conformations of the ribose ring were explored.*

For aminolysis reaction, phosphonamidates with the diaminonucleoside placed in the large hydrophobic pocket, and the methyl group fitting into the medium pocket were built (inverse structures), following the same procedure shown for the normal binding.

Amine-Protonated Intermediates for Aminolysis Reaction. To obtain these structures, the 5’-amino group was replaced by an ammonium fragment. For the optimization of the phosphonamidate core, the same systematic approach of releasing was applied. First, only the intermediate was allowed to adjust to the enzyme active site. Second, the side chains of the aminoacids were released too, and finally the entire complex was allowed to adjust.

Acyl Chain Effects in Aminolysis Reaction. The last models above correspond to the reaction of a methyl as acyl group. The methyl chain was oriented to fit into the medium hydrophobic pocket. Two other acyl groups were modeled for the CAL-B catalyzed reaction at the 5’-amino group: propyl, and methoxyacetyl. The two acyl moieties were added to the amine-protonated model, and the structures were released in the same manner as shown above.

Experimental Procedures and Spectroscopical Data

Oxime acetyl,[2] butanoyl,[3] decanoyl,[2] and benzoyl[4] have been previously described. **Oxime methoxyacetate.** In a round-bottomed flask fitted with magnetic stirrer and addition funnel, chloride methoxyacetate (1.1 mL, 12 mmol) was added dropwise to a solution of acetone oxime (0.7 g, 10 mmol) in pyridine (1.8 mL, 20 mmol) at 0 °C. After processes finished, CH_2Cl_2 was added and organic phase is separated, washed, and dried (Na_2SO_4). The solvent was evaporated and the crude products were purified by vacuum distilation (10^{-2}-10^{-5} mmHg), obtaining an incolor liquid. R_f (EtOAc) 0.79; IR (NaCl) υ 1776 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) d 1.94 (s, 3H, $H_{3'}$), 1.98 (s, 3H, $H_{3'}$), 3.41 (s, 3H, H_4), and 4.11 (s, 2H, H_2); ^{13}C-NMR ($CDCl_3$, 75.5 MHz) d 16.7 ($C_{3'}$), 21.6 ($C_{3'}$), 59.2 (C_4), 68.8 (C_2), 164.3 (C=N), and 168.1 (C=O); MS (ESI$^+$, *m/z*) 184 [$(M+K)^+$, 3%], and 168 [$(M+Na)^+$, 100]; Anal Calcd (%) for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.6; H, 7.5; N, 9.9.

*Enzymatic Acylation of Thymidine (**1**).* Oxime ester (1.25 mmol) and THF (5 mL) were added to a mixture of **1** (121 mg, 0.50 mmol), CAL-B (125 mg), and 3',5'-di-*O*-TMS-2'-deoxyuridine (25 mg, as internal standard) under nitrogen atmosphere in a 50-mL Erlenmeyer flask. The suspension was shaken at 250 rpm at 30 °C until high or total conversions were achieved, or failing that, until no further reaction was apparent. Periodically, samples were taken from the reaction mixture, enzyme was filtered and the solvent was evaporated under reduced pressure and silylated as described by Sweeley *et al.*[5] The samples were analyzed by high performance liquid chromatography (HPLC) for the acetylation reaction on a Spherisorb W column (250 mm × 4.6 mm × 5 μm), with 3% MeOH/EtOAc as eluent, 0.9 mL/min at 40 ºC. 3',5'-Di-*O*-TMS-2'-deoxyuridine (as internal standard) appeared at 3.33 min; 3',5'-di-*O*-TMS-thymidine at 7.45 min; 3'-*O*-acetyl-5'-*O*-TMS-thymidine at 4.53 min; 5'-*O*-acetyl-3'-*O*-TMS-thymidine at 5.10 min; 3',5'-di-*O*-acetylthymidine at 3.96 min. The other acylations were followed by gas chromatography (GC) on a TRACSIL TRB-5A capillary column (30 m × 0.32 mm × 0.50 μm), with nitrogen as carrier gas and a flame ionization detector. Injector and detector temperatures were set at 300 °C,

[2] Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 833-837.
[3] Gotor, V.; Pulido, R. *J. Chem. Soc., Perkin. Trans. 1* **1991**, 491-492.
[4] Exner, O.; Horak, M.; Pliva, J. *Chem. Ind. (London)* **1958**, 1174-1175.
[5] Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497-2507.

head column pressure at 14 psi and split 90:1, column initial temperature 220 °C (3 min), rate 5 °C/min until 260 °C, then 15 min at 260 °C, followed by heating rate 5 °C/min until 300 °C, column final temperature was 300 °C (10 min). 3',5'-Di-*O*-TMS-2'-deoxyuridine (as internal standard) appeared at 11.8 min; 3',5'-di-*O*-TMS-thymidine at 11.0 min; 3'-*O*-butyryl-5'-*O*-TMS-thymidine at 16.30 min; 5'-*O*-butyryl-3'-*O*-TMS-thymidine at 15.88 min; 3',5'-di-*O*-butyrylthymidine at 23.28 min; 3'-*O*-decanoyl-5'-*O*-TMS-thymidine at 35.69 min; 5'-*O*-decanoyl-3'-*O*-TMS-thymidine at 34.62 min; 5'-*O*-benzoyl-3'-*O*-TMS-thymidine at 30.90 min; 5'-*O*-phenylacetyl-3'-*O*-TMS-thymidine at 32.2 min. Conversion of the reaction was calculated by disappearance of the starting material with respect to internal standard. 3'-*O*-Acetyl-hymidine,[6] 5'-*O*-acetylthymidine,[7] 3',5'-di-*O*-acetylthymidine,[7] 3'-*O*-butyrylthymi-ine,[6] 5'-*O*-butyrylthymidine,[7] 3',5'-di-*O*-butyrylthymidine,[7] 3'-*O*-decanoylthymidine,[6] 5'-*O*-decanoylthymidine,[7] and 5'-*O*-benzoylthymidine,[7] were synthesized as has been previously reported.

5'-*O*-Phenylacetylthymidine. Oxime phenylacetyl (240 mg, 1.25 mmol) and THF (5 mL) were added to a mixture of **1** (121 mg, 0.50 mmol), and CAL-B (125 mg) under nitrogen atmosphere in a 50-mL Erlenmeyer flask. The suspension was shaken at 250 rpm at 60 °C during 37 h. Then, enzyme was filtered, washed with MeOH, and the solvent was evaporated under reduced pressure, and the crude products were purified by chromatography column (gradient eluent 60% EtOAc/Hexane-EtOAc), obtaining a white solid. R_f (EtOAc) 0.3; mp 138-140 °C; IR (KBr) 1717 (C=O) cm^{-1}; ^1H NMR (MeOH-d_4, 200 MHz) 1.92 (s, 3H, H_7), 2.44 (m, 2H, $H_{2'}$), 4.27 (m, 1H, $H_{4'}$), 4.57 (m, 2H, $H_{5'}$), 5.28 (m, 3H, $H_{3'}$+$H_{2''}$), 6.48 (t, 1H, $H_{1'}$, $^3J_{HH}$ 6.8 Hz), 7.55 (m, 5H, H_{ar}), and 7.73 (s, 1H, H_6); ^{13}C NMR (MeOH-d_4, 75.5 MHz) 12.5 (C_7), 40.7 ($C_{2'}$), 68.3 ($C_{5'}$), 71.0 ($C_{2''}$), 71.9 ($C_{3'}$), 85.7 ($C_{4'}$), 86.2 ($C_{1'}$), 111.8 (C_5), 129.5 (CH), 129.6 (2 CH), 136.8 (C_i), 137.5 (C_6), 152.3 (C_2), 156.3 (C=O), and 166.3 (C_4); MS (ESI$^+$, *m/z*) 399 [$(M+K)^+$, 70%], 383 [$(M+Na)^+$, 100], and 361 [$(M+H)^+$, 10]; Anal Calcd (%) for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.8; H, 5.4; N, 7.6; $[a]_D^{20}$= +12.6 (c 0.20, DMSO).

5'-*O*-Methoxyacetylthymidine (3b). Oxime methoxyacetyl (145 mg, 1 mmol) and THF (4 mL) were added to a mixture of **1** (97 mg, 1 mmol), and CAL-B (100 mg)

[6] Gotor, V.; Morís, F. *Synthesis* **1992**, 626-628.
[7] Morís, F.; Gotor, V. *J. Org. Chem.* **1993**, *58*, 653-660.

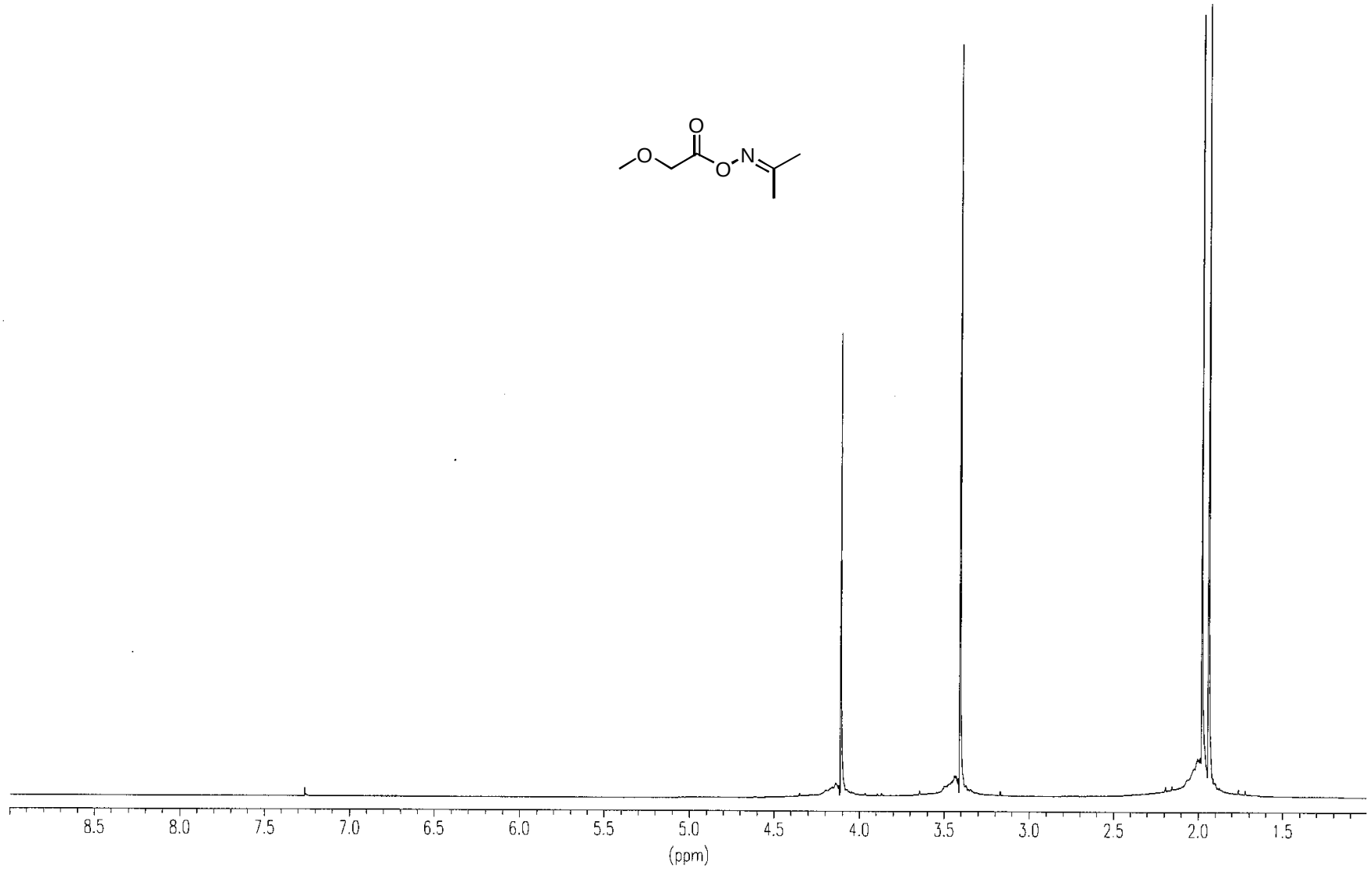
under nitrogen atmosphere in a 50-mL Erlenmeyer flask. The suspension was shaken at 250 rpm at 30 °C during 4 h. Then, enzyme was filtered, washed with MeOH, and the solvent was evaporated under reduced pressure, and the crude products were purified by chromatography column (gradient eluent 60% EtOAc/Hexane-EtOAc), obtaining a white solid. R_f (EtOAc): 0.33; mp 132-134 °C; IR (KBr) 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) d 1.90 (s, 3H, H_7), 2.17 (m, 1H, $H_{2'}$), 2.42 (m, 1H, $H_{2'}$), 2.76 (br s, 1H, OH), 3.43 (s, 3H, $H_{4''}$), 4.09 (m, 2H, $H_{2''}$), 4.19 (m, 1H, $H_{4'}$), 4.30-4.50 (m, 3H, $H_{3'}$+$H_{5'}$), 6.32 (dd, 1H, $H_{1'}$, $^3J_{HH}$ 7.2 Hz), 7.34 (s, 1H, H_6), and 9.88 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50.3 MHz) d 12.3 (C_7), 39.8 ($C_{2'}$), 59.2 ($C_{4''}$), 64.3 ($C_{5'}$), 69.6 ($C_{2''}$), 71.5 ($C_{3'}$), 84.0 (CH), 85.1 (CH), 111.2 (C_5), 135.6 (C_6), 150.7 (C_2), 164.2 (C_4), and 170.0 (C=O); MS (ESI$^+$, *m/z*) 337 [$(M+Na)^+$, 100%]; Anal Calcd (%) for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.91. Found: C, 49.9; H, 5.7; N, 8.8; $[a]_D^{20}$= +4.7 (c 0.22, MeOH).

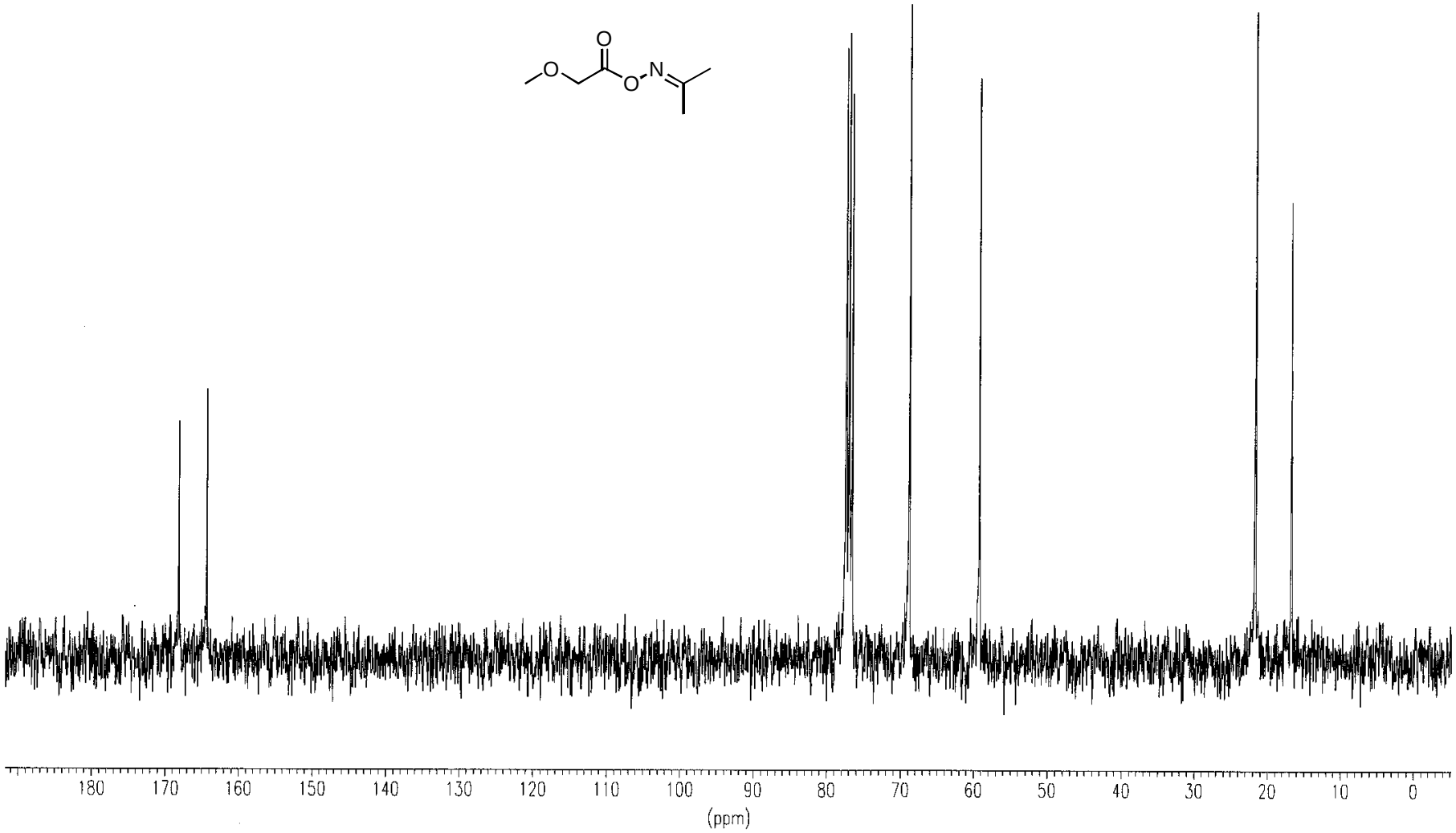
3'-*O*-Methoxyacetylthymidine (4b). This compound was obtained as byproduct in the enzymatic acylation of **1** with oxime methoxyacetyl as a white hygroscopic solid. R_f (EtOAc): 0.45; mp hygroscopic solid; IR (KBr) 1753 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) d 1.94 (s, 3H, H_7), 2.45 (m, 2H, $H_{2'}$), 3.48 (s, 3H, $H_{4''}$), 3.95 (d, 2H, $H_{5'}$, $^3J_{HH}$ 2.3 Hz), 4.10 (s, 2H, $H_{2''}$), 4.12 (m, 1H, $H_{4'}$), 5.48 (m, 1H, $H_{3'}$), 6.26 (dd, 1H, $H_{1'}$, $^3J_{HH}$ 8.2 $^3J_{HH}$ 5.9 Hz), 7.52 (s, 1H, H_6), and 8.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz) d 12.5 (C_7), 37.1 ($C_{2'}$), 59.4 ($C_{4''}$), 62.5 ($C_{5'}$), 69.6 ($C_{2''}$), 75.2 ($C_{3'}$), 84.9 (CH), 86.0 (CH), 111.4 (C_5), 136.2 (C_6), 150.3 (C_2), 163.4 (C_4), and 169.9 (C=O); MS (ESI$^+$, *m/z*) 337 [$(M+Na)^+$, 100%], and 315 [$(M+H)^+$, 16]; Anal Calcd (%) for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.91. Found: C, 49.8; H, 6.0; N, 8.7; $[a]_D^{20}$= -8.7 (c 0.10, MeOH).

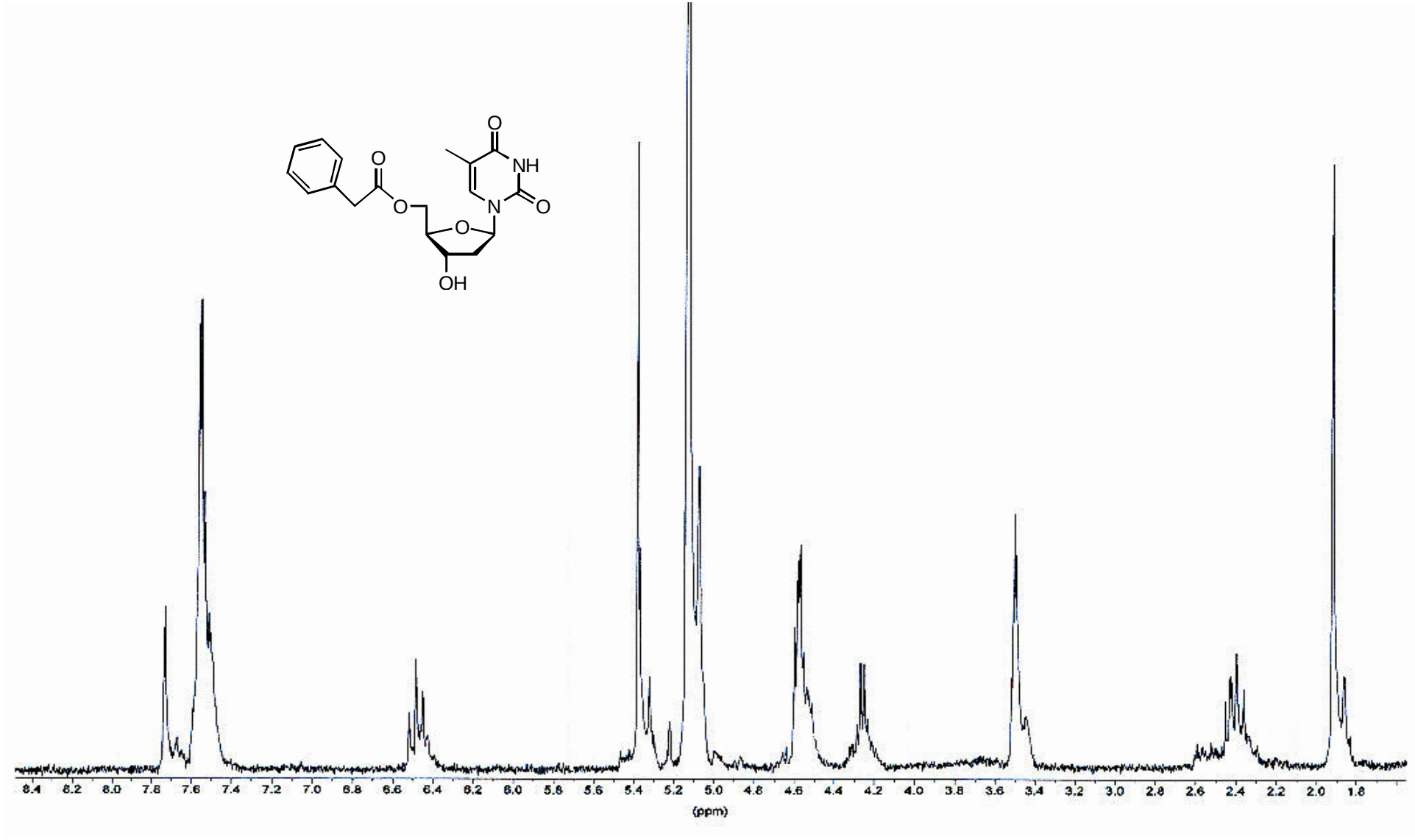
3',5'-Di-*O*-methoxyacetylthymidine (5b). This compound was obtained as byproduct in the enzymatic acylation of **1** with oxime methoxyacetyl as a white hygroscopic solid. R_f (EtOAc): 0.60; mp hygroscopic solid; IR (KBr) 1754 (C=O), and 1701 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) d 1.92 (s, 3H, H_7), 2.23-2.49 (m, 2H, $H_{2'}$), 3.43 (s, 3H, $H_{4''}$), 3.46 (s, 3H, $H_{4''}$), 4.09 (m, 2H, $H_{2''}$), 4.27 (m, 1H, $H_{4'}$), 4.35-4.54 (m, 2H, $H_{5'}$), 5.32 (m, 1H, $H_{3'}$), 6.36 (dd, 1H, $H_{1'}$, $^3J_{HH}$ 8.7 $^3J_{HH}$ 6.0 Hz), 7.34 (s, 1H, H_6), and 9.63 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz) d 12.3 (C_7), 36.8 ($C_{2'}$), 59.3 ($C_{4''}$), 59.4 ($C_{4''}$), 64.1 ($C_{5'}$), 69.4 ($C_{2''}$), 69.8 ($C_{2''}$), 74.8 ($C_{3'}$), 81.7 (CH), 84.5 (CH), 111.7 (C_5), 134.7 (C_6), 150.5 (C_2), 163.7 (C_4), 169.4 (C=O), and 169.7 (C=O); MS

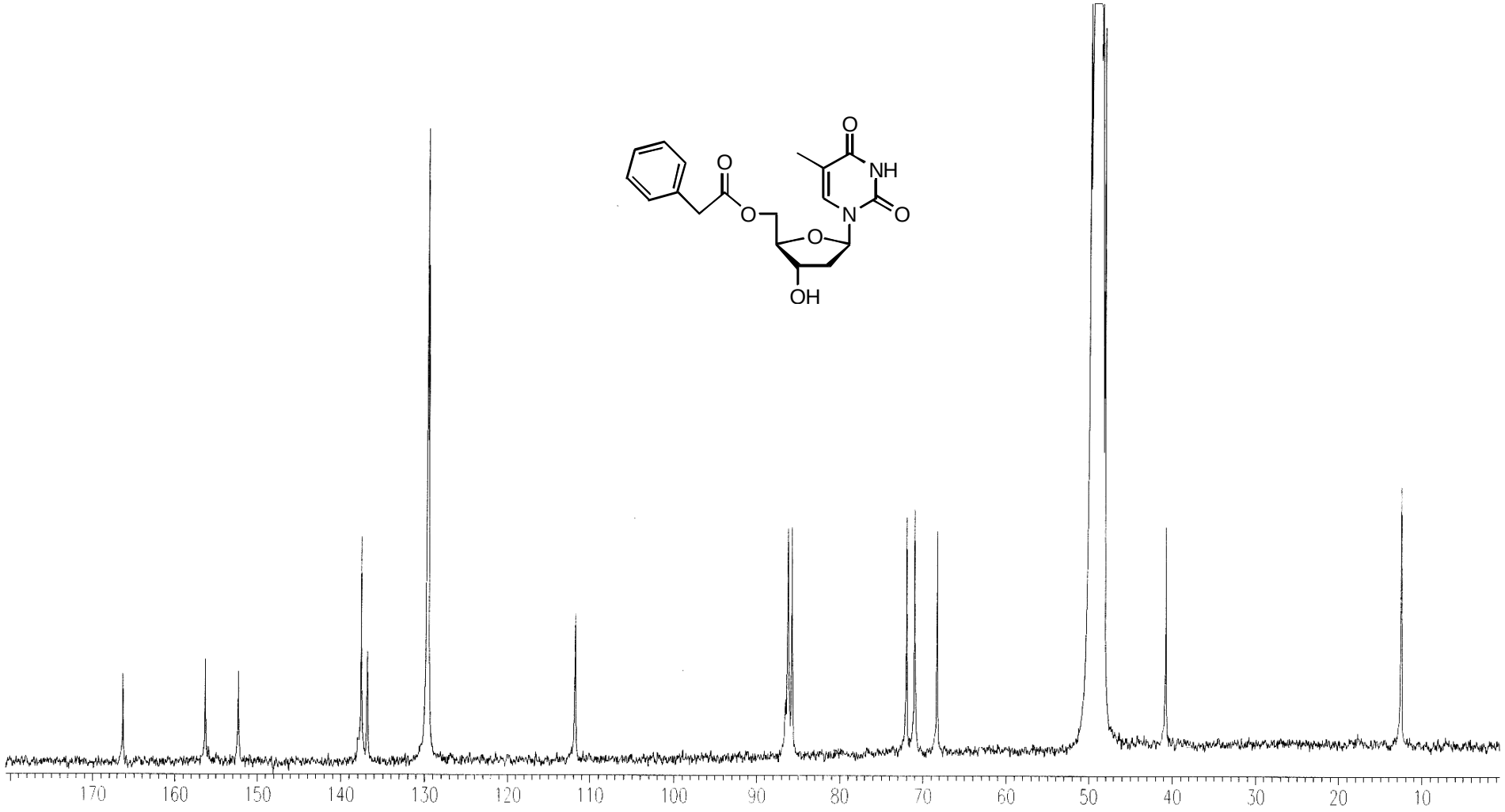
(ESI$^+$, *m/z*) 409 [(M+Na)$^+$, 100%]; Anal Calcd (%) for $C_{16}H_{22}N_2O_9$: C, 49.68; H, 5.77; N, 8.91. Found: C, 49.8; H, 6.0; N, 8.7; $[a]_D^{20}$= -11.8 (c 0.18, MeOH).

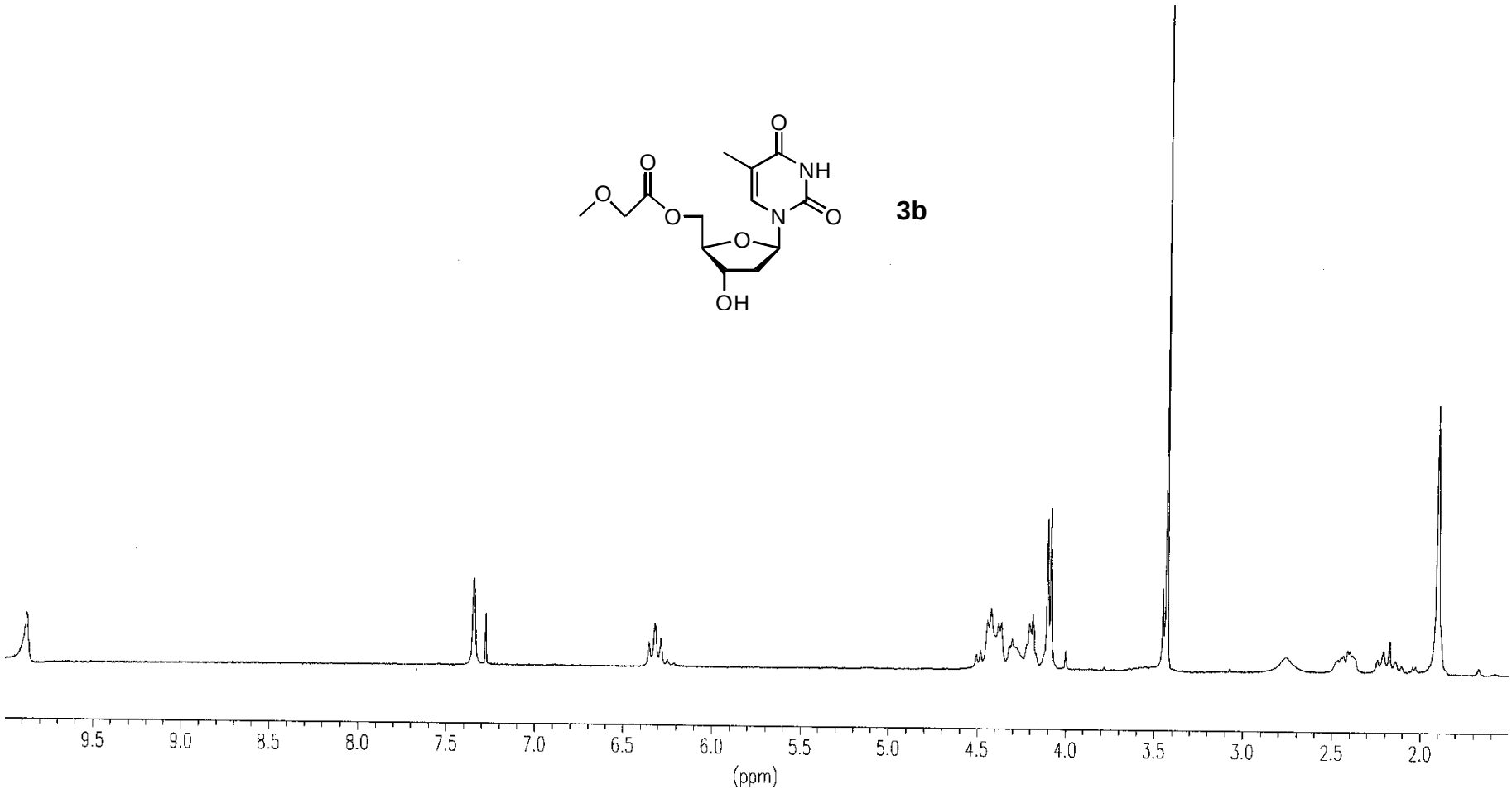
3'-Amino-5'-methoxyacetylamino-3',5'-dideoxythymidine (6b). Methyl methoxyacetyl (66 μL) was added to a suspension of **2** (20 mg, 0.08 mmol), CAL-B (10 mg), and molecular sieves 4Å (20 mg) in dry THF (4.5 mL) under nitrogen, and the mixture was stirred at 250 rpm at 40 ºC during 2 h. Then, the enzyme and molecular sieves were filtered off and washed with MeOH (3×5 mL). The filtrate was evaporated to dryness and the crude residue was purified by flash chromatography column (gradient eluent 10% MeOH/EtOAc–MeOH), yielding a white solid. R_f (5% NH_3(aq)/MeOH) 0.56; mp 155-157 °C; IR (KBr) 1682 (C=O) cm^{-1}; ^{1}H NMR (MeOH-d_4, 300 MHz) d 2.10 (s, 3H, H_7), 2.33-2.54 (m, 2H, $H_{2'}$), 3.52 (m, 1H, $H_{3'}$), 3.61 (s, 3H, $H_{4''}$), 3.70-3.97 (m, 3H, $H_{4'}$+$H_{5'}$), 4.12 (m, 2H, $H_{2''}$), 6.30 (dd, 1H, $H_{1'}$, $^3J_{HH}$ 7.4, $^3J_{HH}$ 4.5 Hz), and 7.69 (s, 1H, H_6); ^{13}C NMR (MeOH-d_4, 75.5 MHz) d 12.4 (C_7), 40.7 ($C_{2'}$), 41.0 ($C_{5'}$), 53.1 ($C_{3'}$), 59.6 ($C_{4''}$), 72.6 ($C_{2''}$), 86.0 (CH), 86.6 (CH), 111.6 (C_5), 138.0 (C_6), 152.4 (C_2), 166.7 (C_4), and 173.1 (C=O); MS (ESI$^+$, *m/z*) 351 [(M+K)$^+$, 3%], 335 [(M+Na)$^+$, 100], and 313 [(M+H)$^+$, 26]; Anal Calcd (%) for $C_{13}H_{20}N_4O_5$: C, 49.99; H, 6.45; N, 17.94. Found: C, 50.2; H, 6.4; N, 17.9; $[a]_D^{20}$= +10.1 (c 0.10, MeOH).

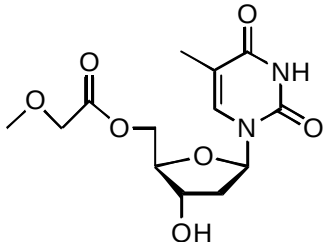

O
O
N
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
(ppm)


O
O
N
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


O
NH
O
N
O
O
O
OH
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
(ppm)

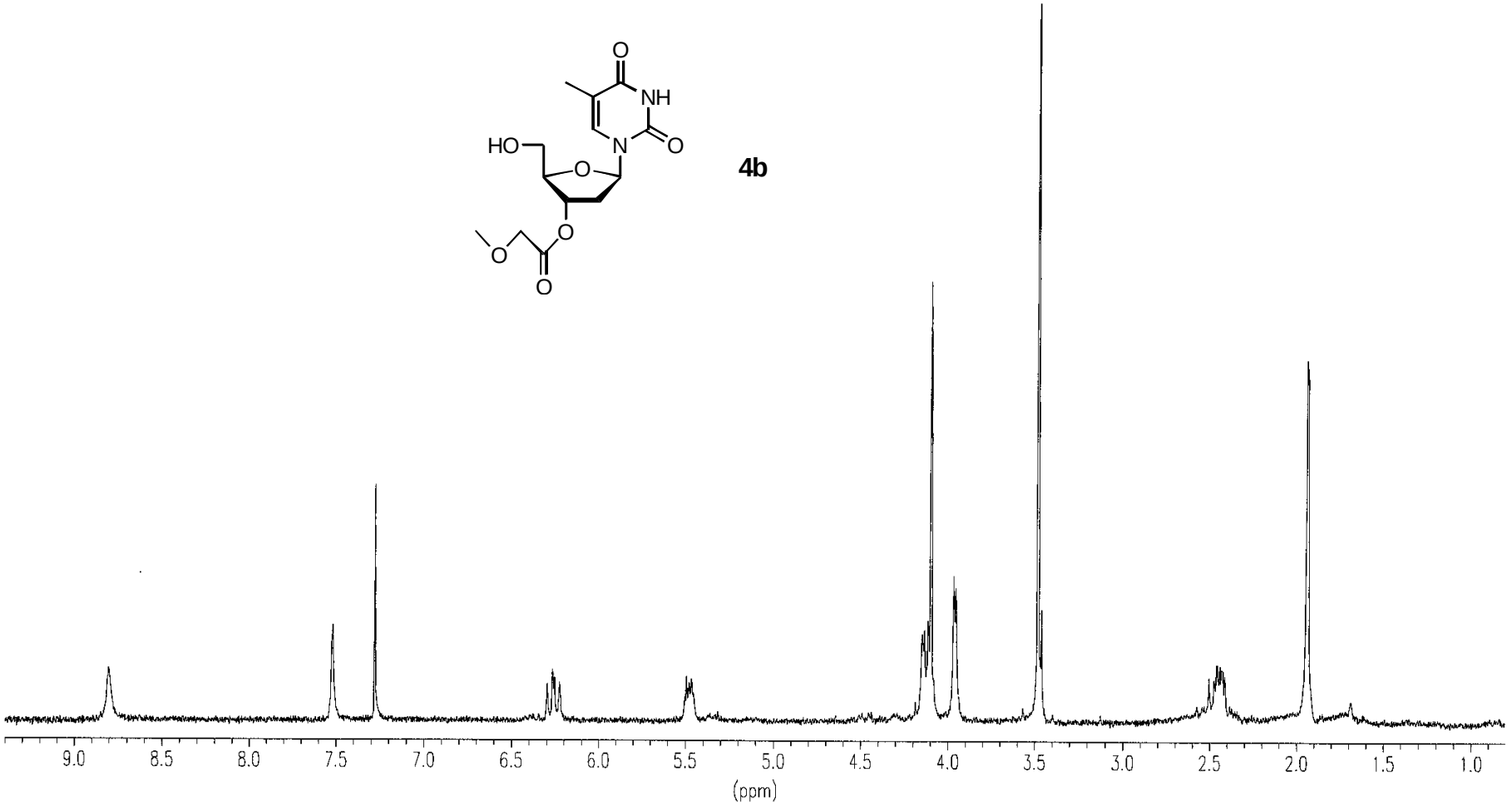

O
NH
O
N
O
O
O
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

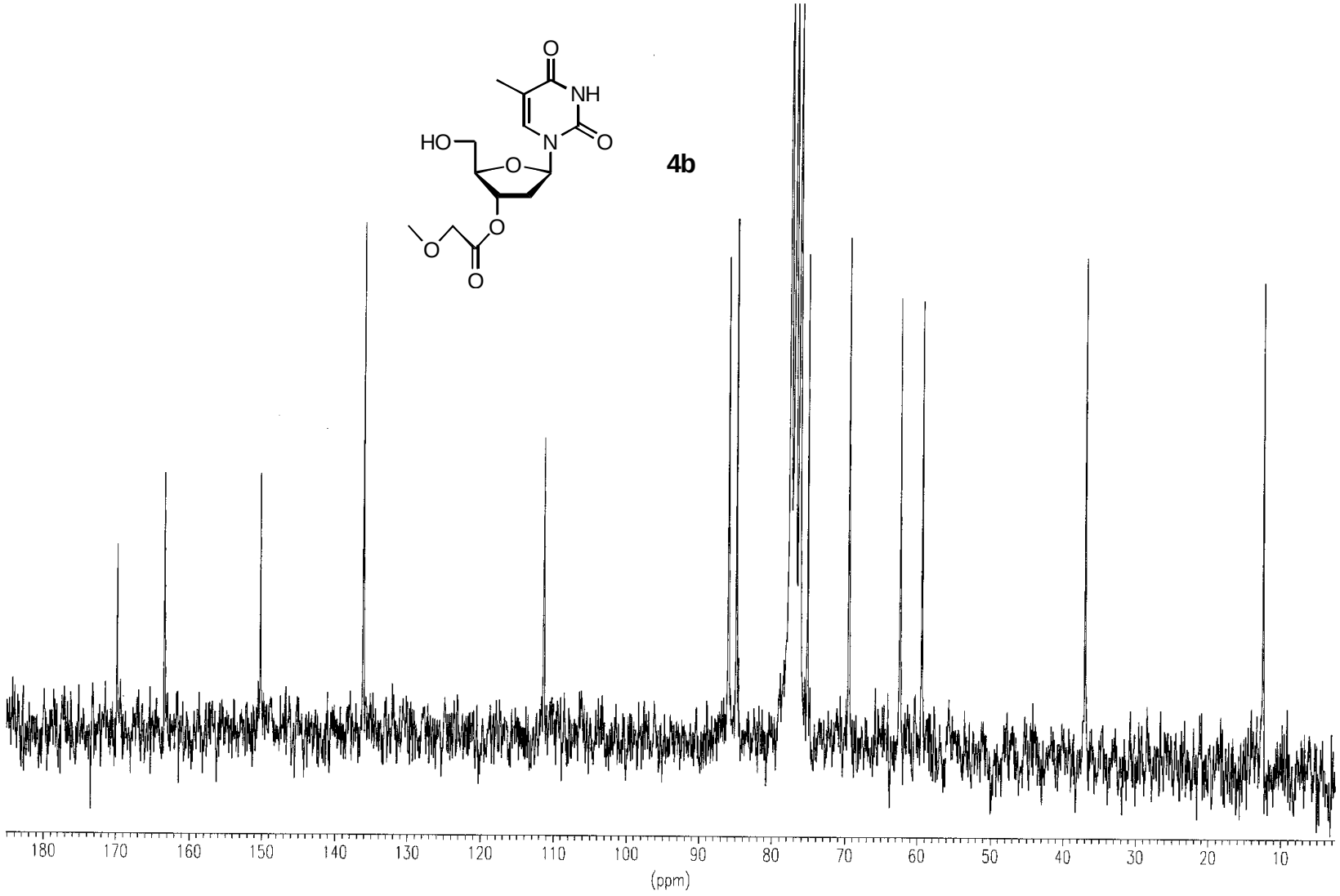

O
NH
O
O
O
O
N
O
O
OH
3b
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
(ppm)

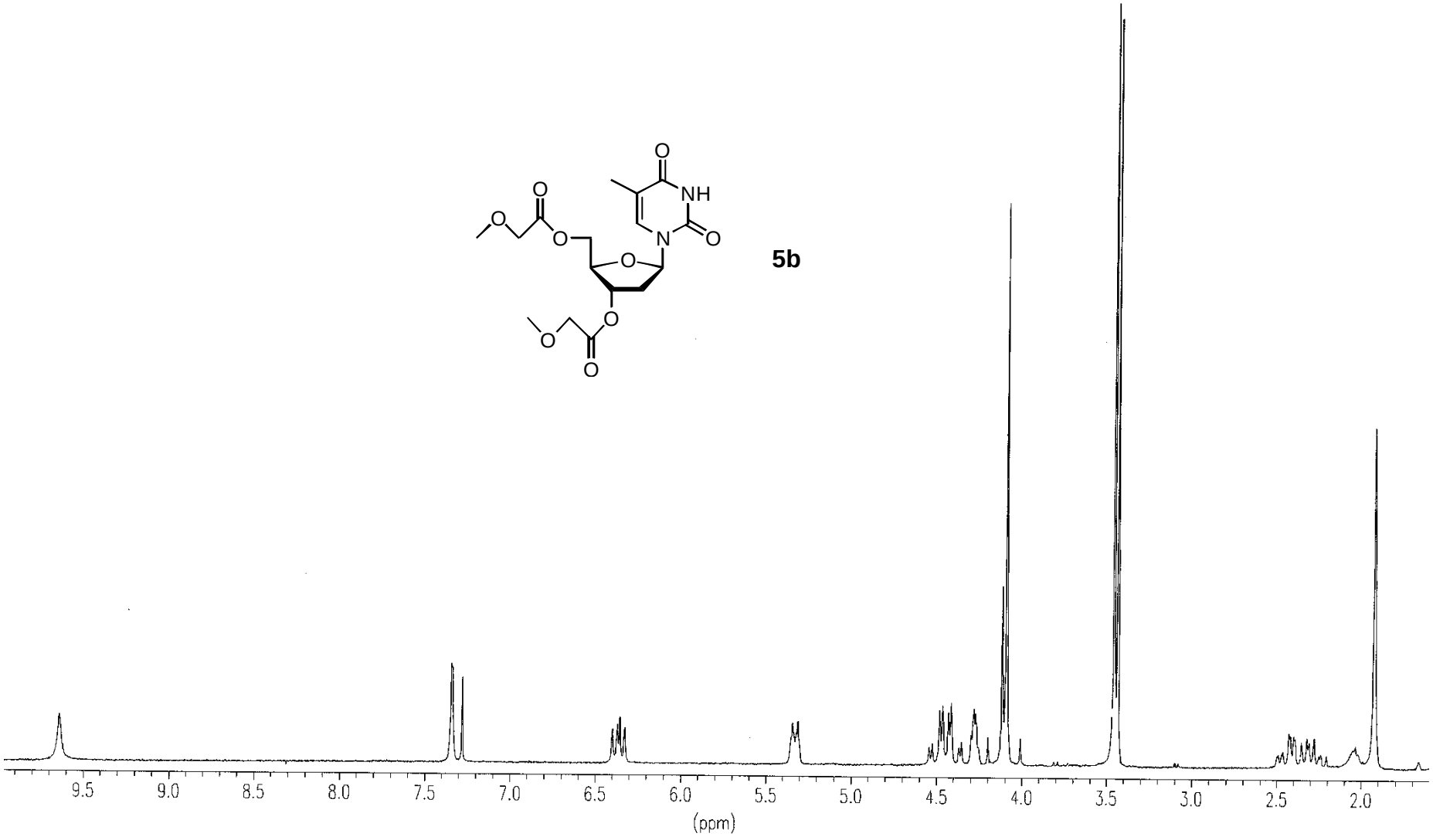

O
NH
O
N
O
O
O
O
OH

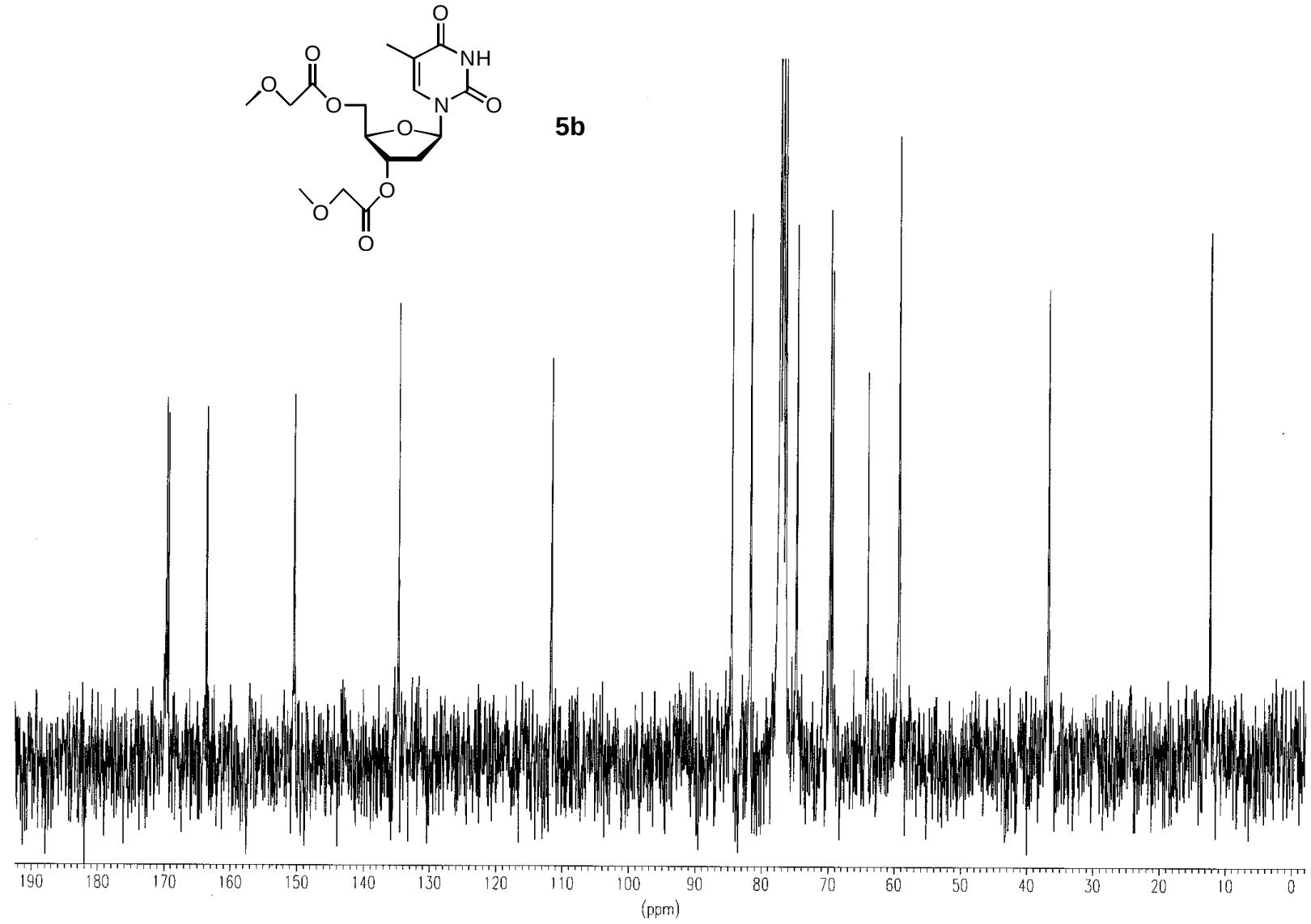
3b

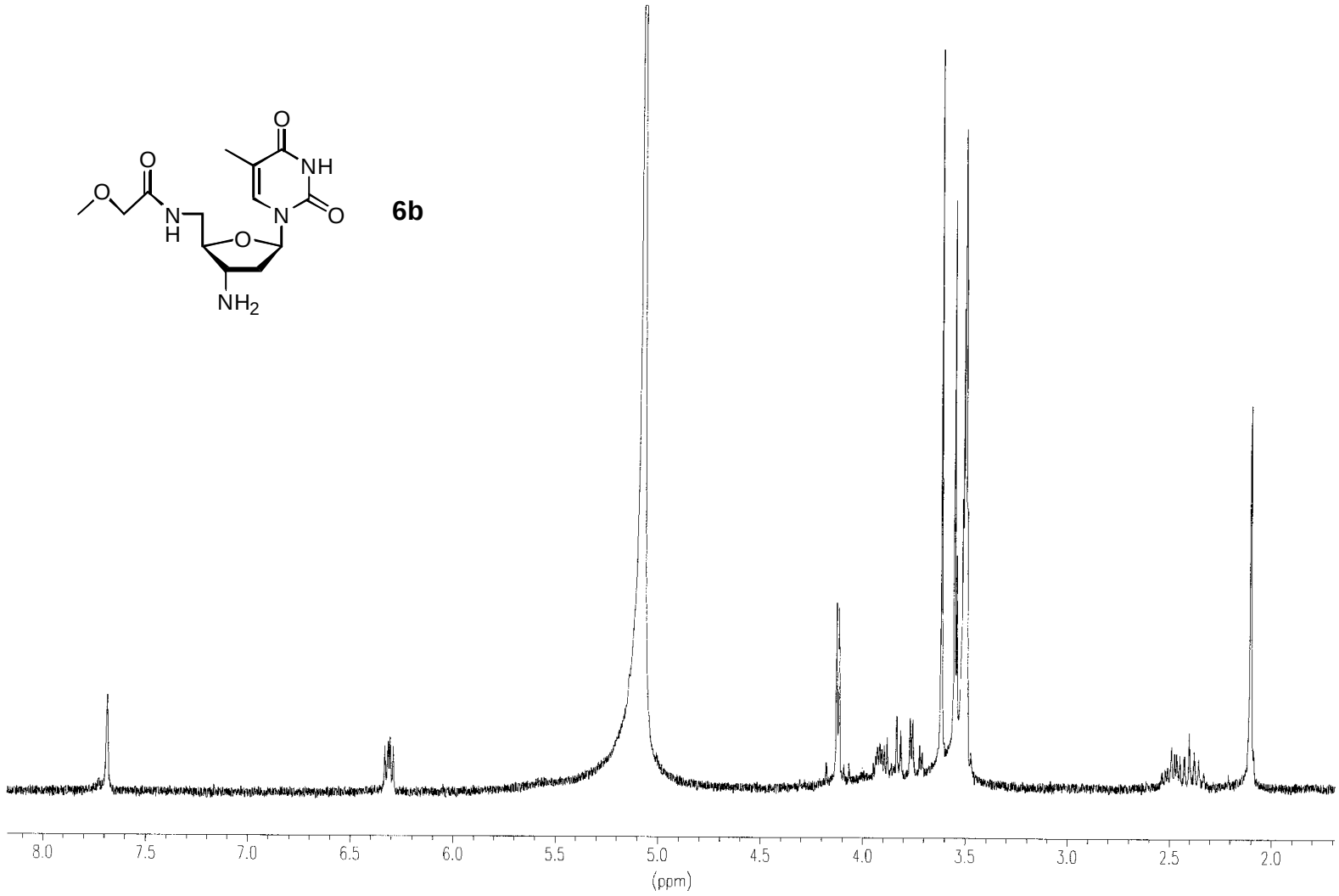
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


O
NH
HO
N
O
O
O
O
O
4b
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(ppm)

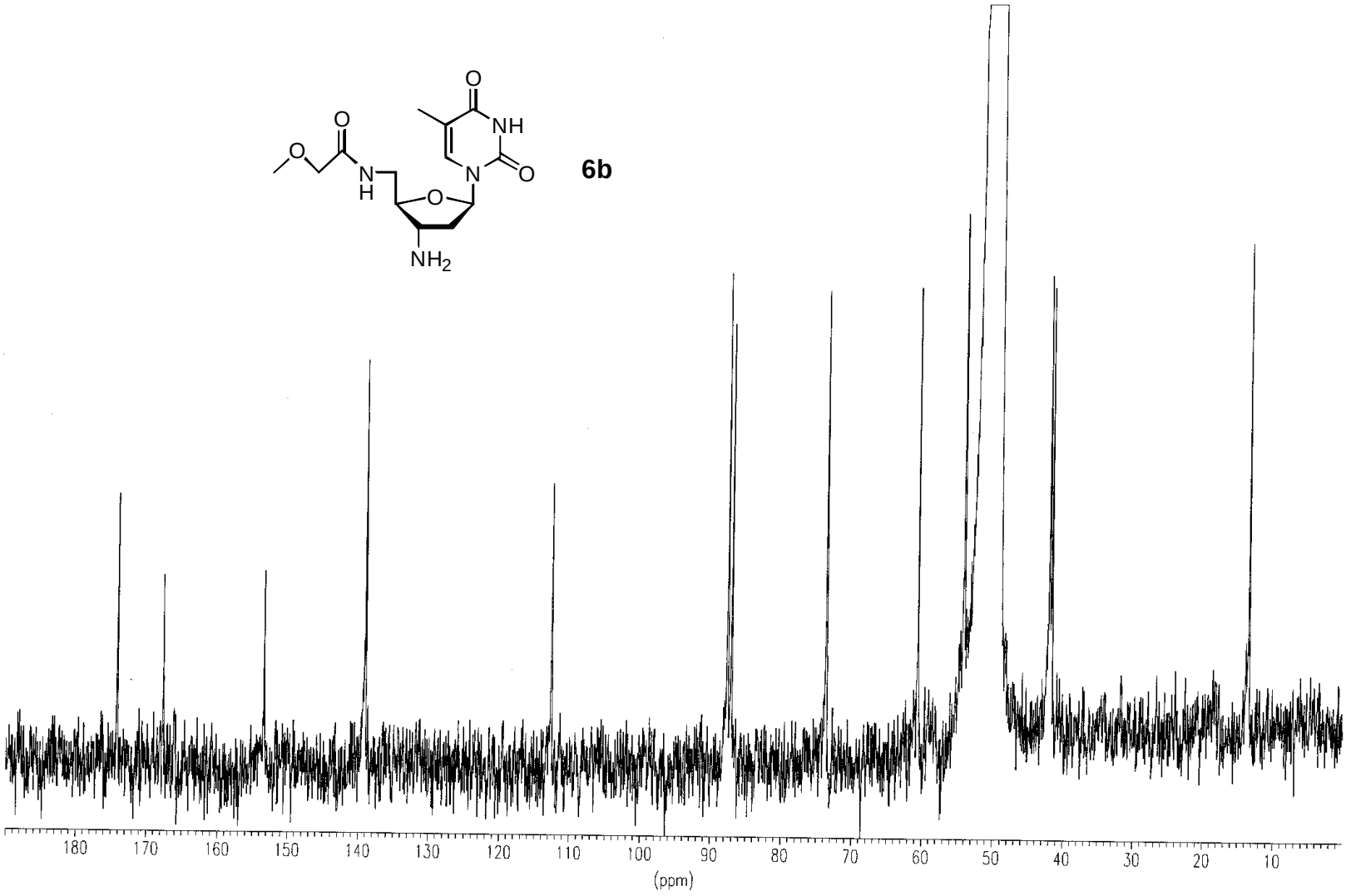

O
NH
HO
N
O
O
O
O
O
4b
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

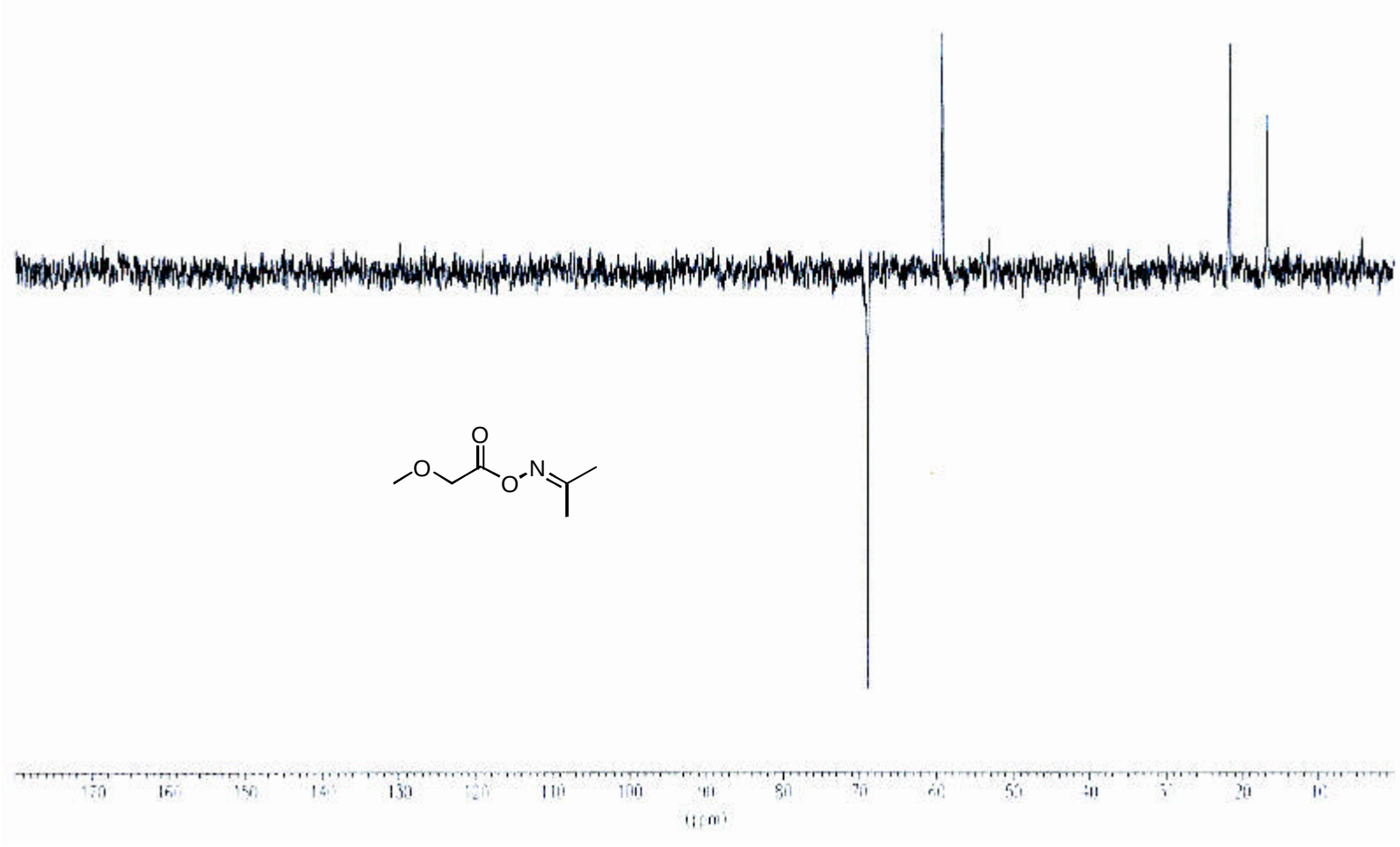

O
NH
O
N
O
O
O
O
O
O
O
O
5b
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
(ppm)

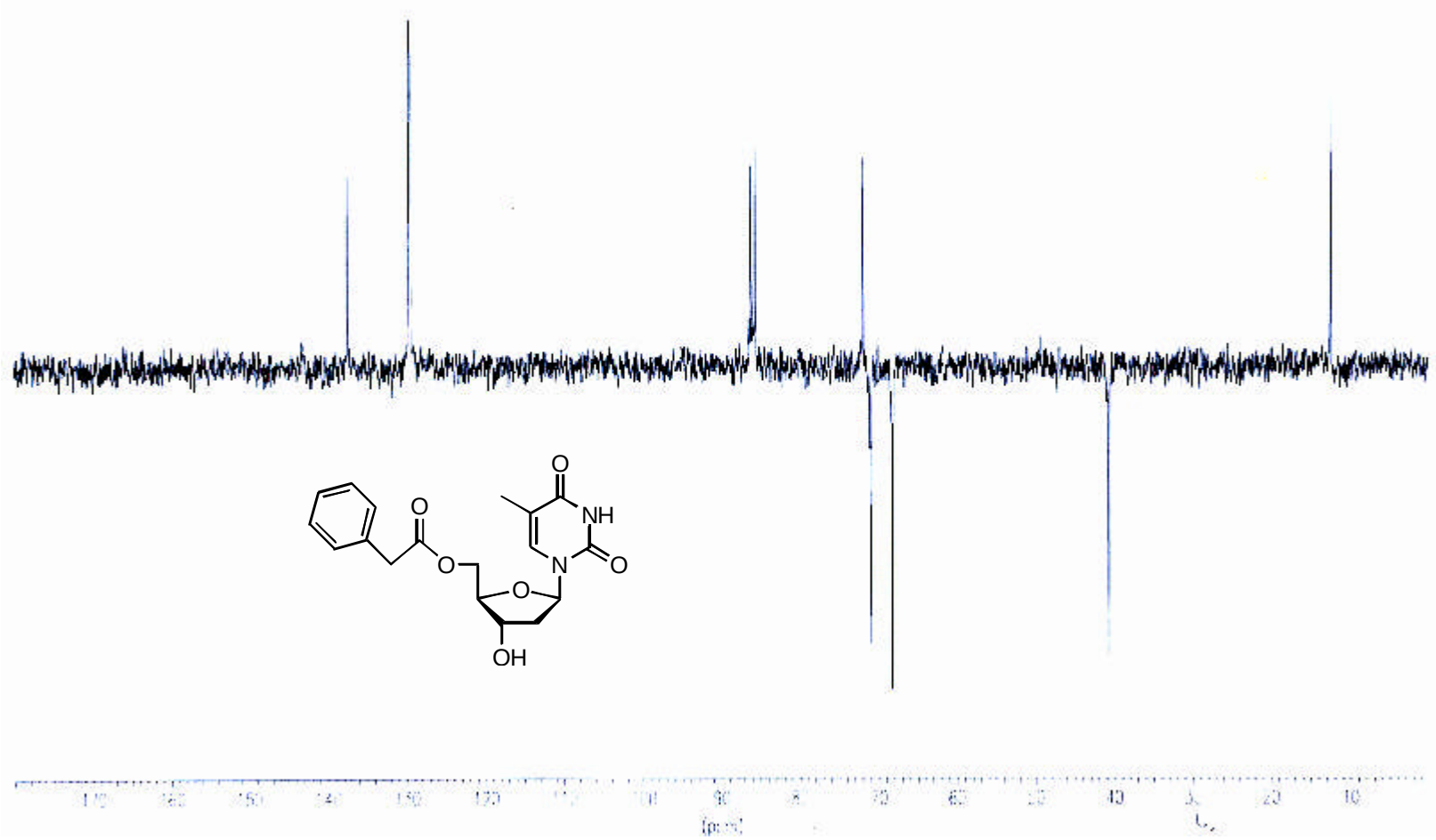

O
NH
O
N
O
O
O
O
O
O
O
5b
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

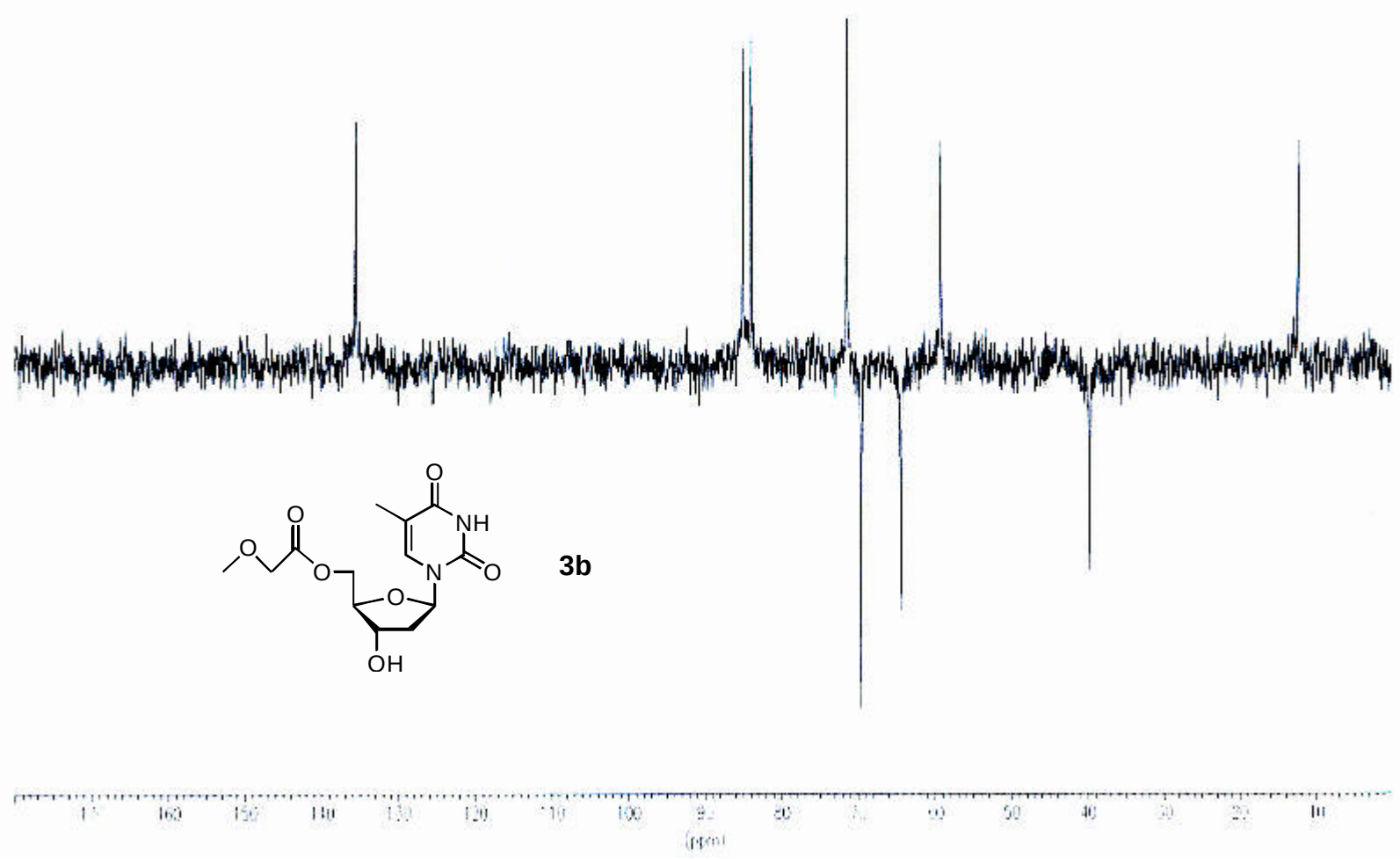


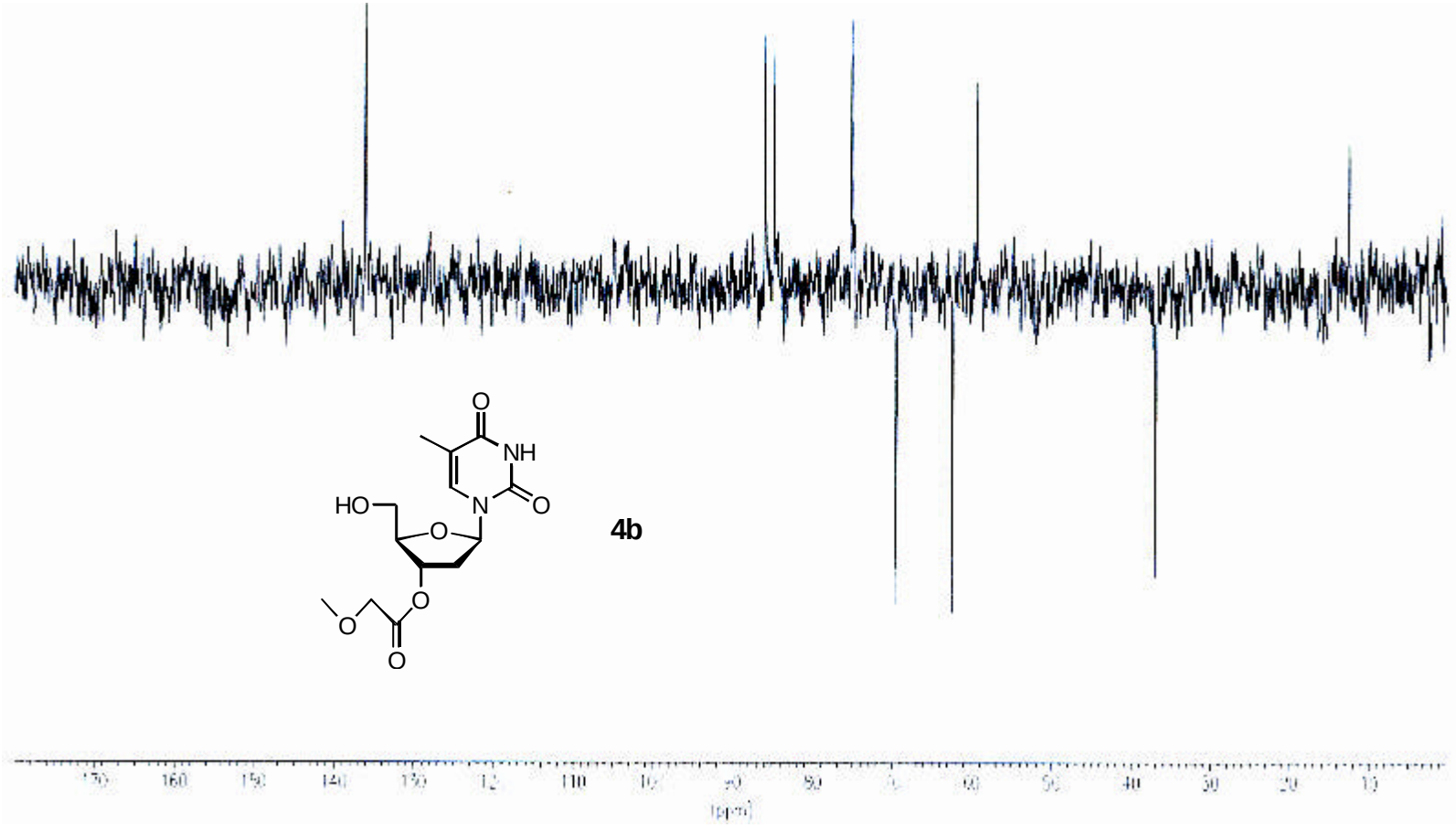
6b

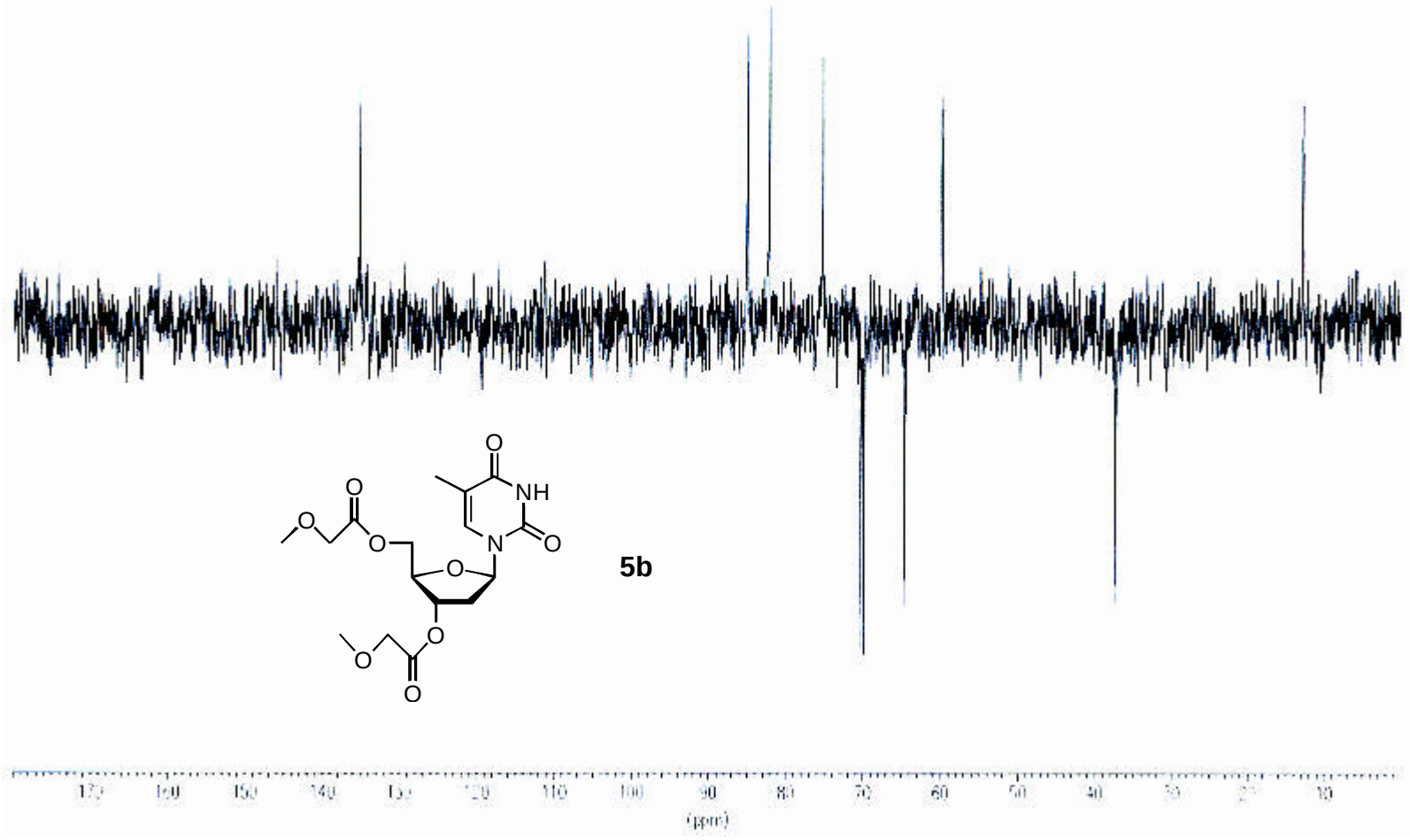

O
NH
O
N
O
N
H
O
NH2
6b
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

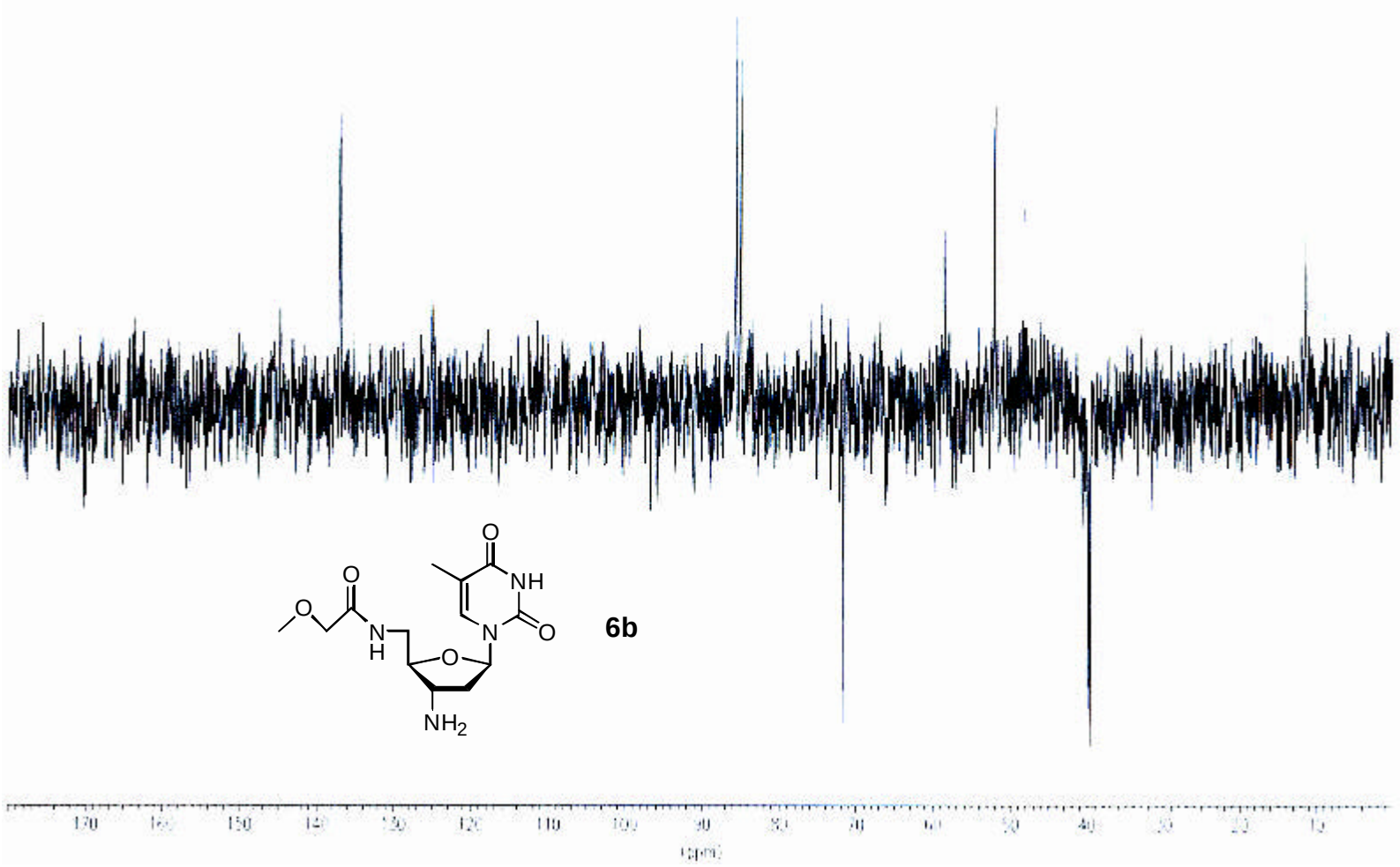

O
O
O
N

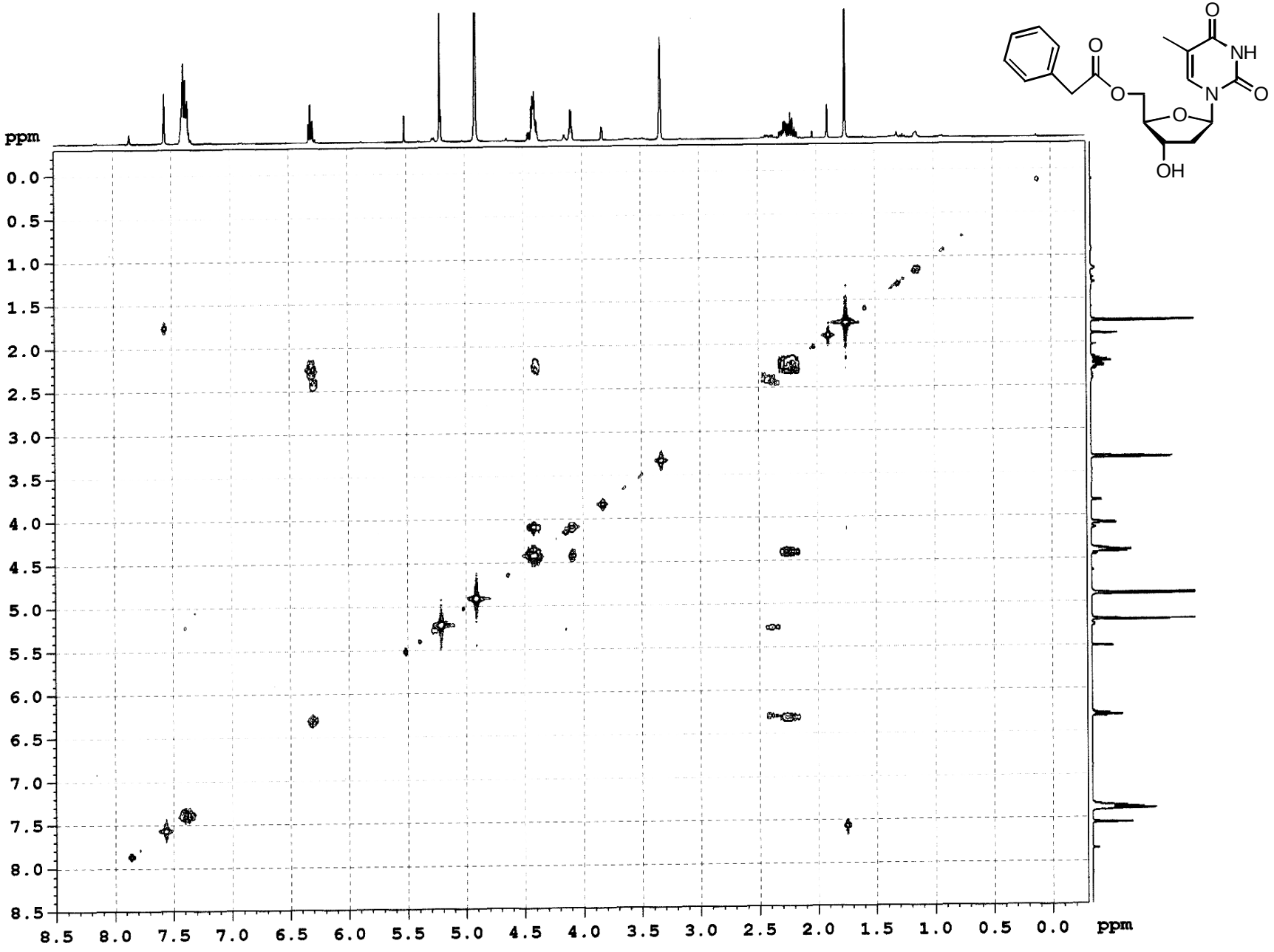

O
NH
N
O
O
O
O
OH

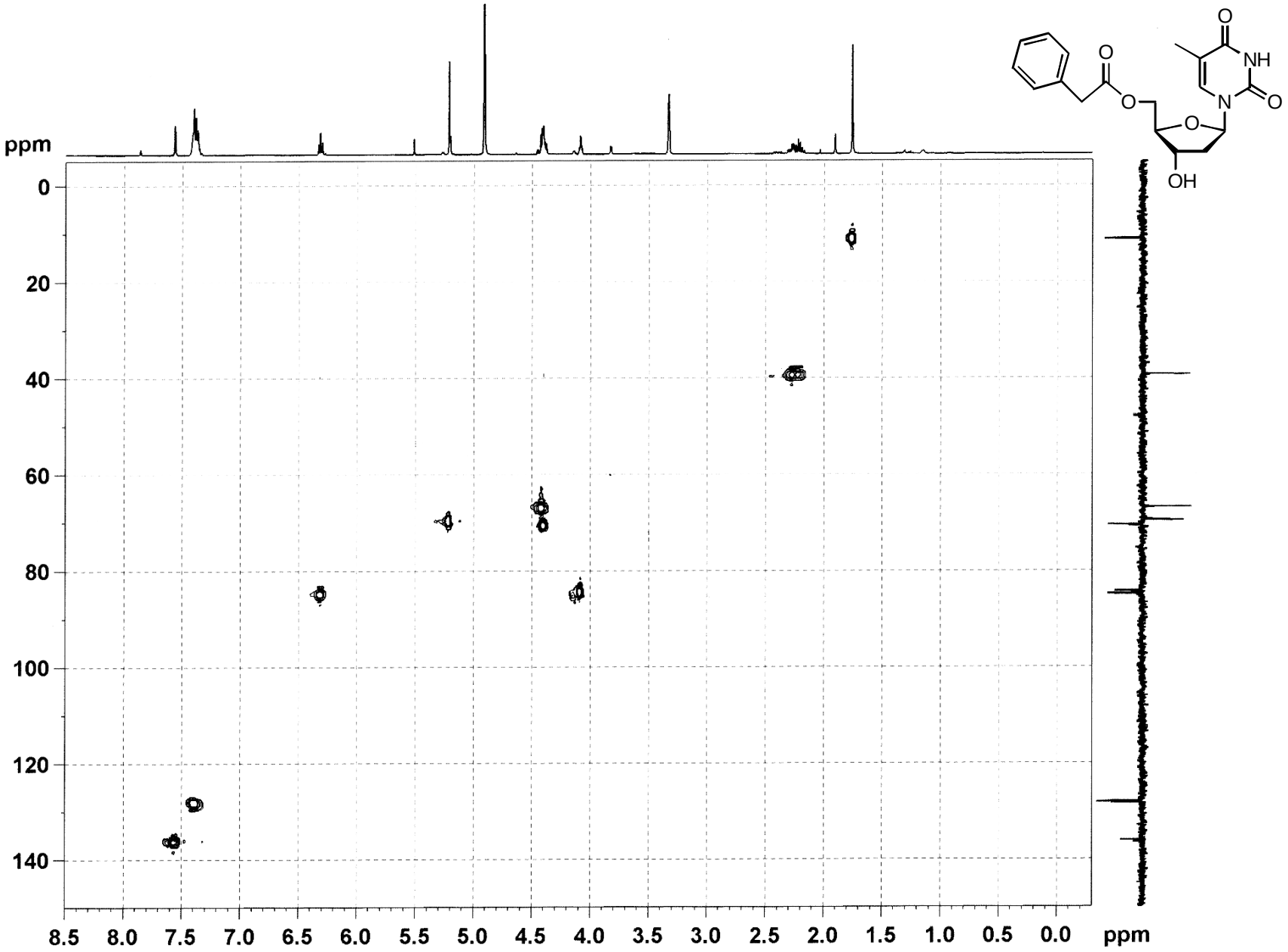

O
NH
N
O
O
O
O
O
O
OH
3b

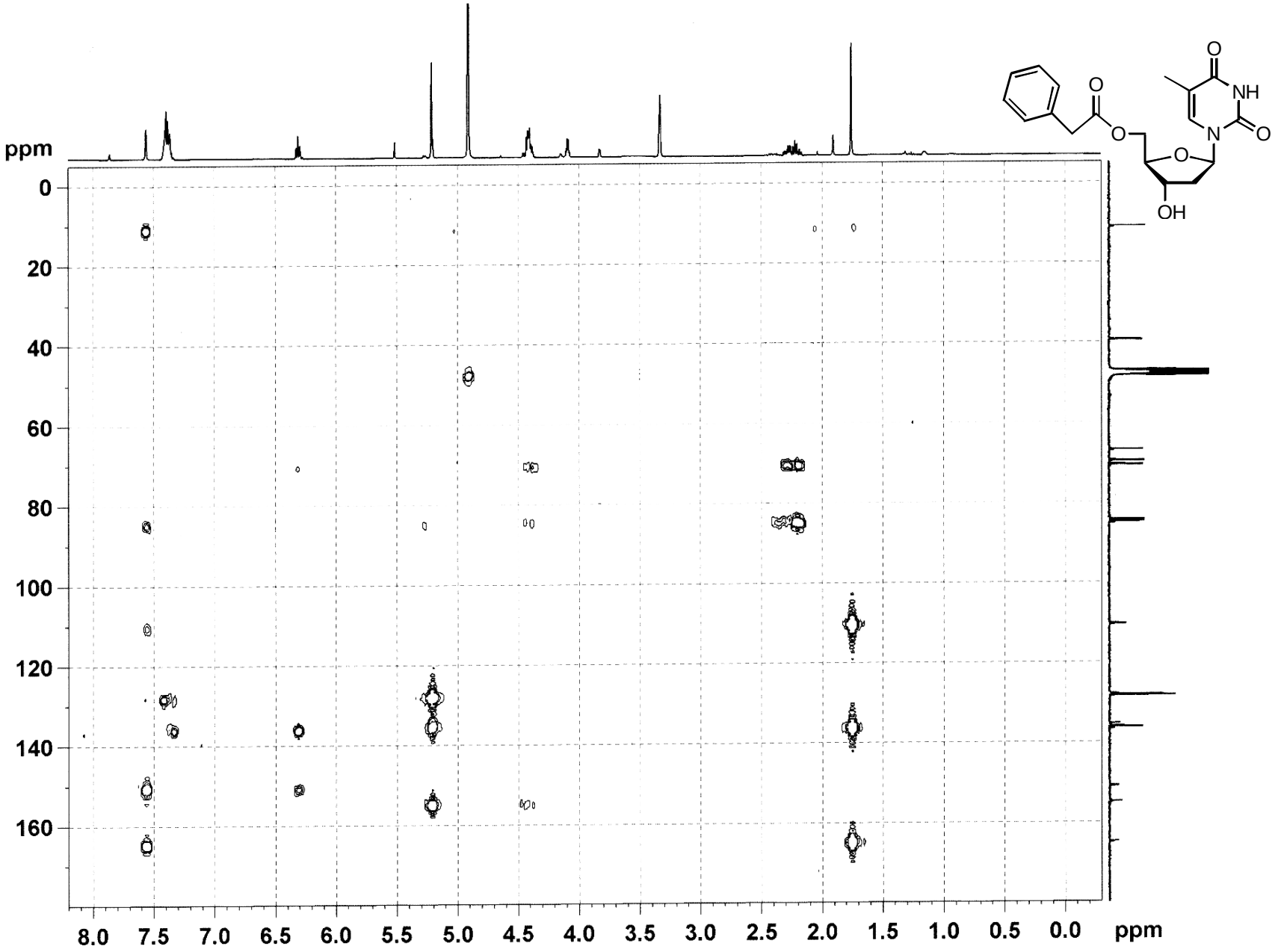

O
NH
HO
N
O
O
O
O
O
4b


O
NH
N
O
O
O
O
O
O
O
O
5b
(ppm)


O
NH
N
O
O
N
H
O
NH2
6b


ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
O
NH
O
N
O
O
O
OH


ppm
0
20
40
60
80
100
120
140
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
O
O
O
NH
N
O
O
OH


ppm
0
20
40
60
80
100
120
140
160
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
O
O
NH
O
N
O
O
OH

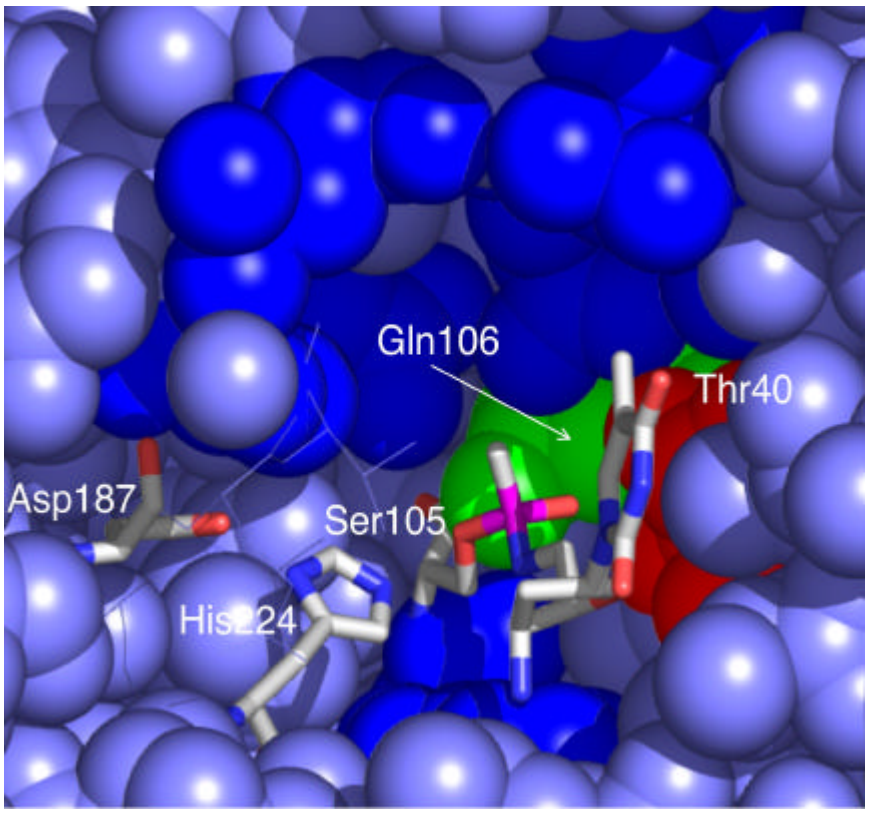


Figure S2. *Phosphonamidate for the 5'-acetylation of* ***2*** *in the normal orientation.*

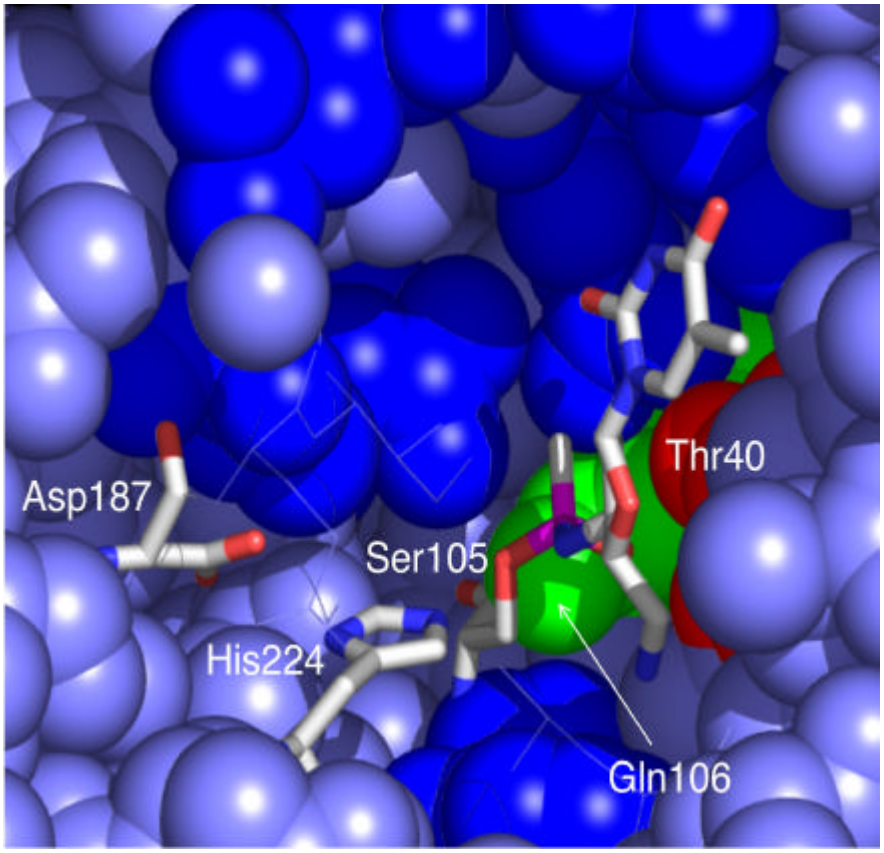


Figure S3. *Phosphonamidate for the 3'-acetylation of* ***2*** *in the normal orientation.*

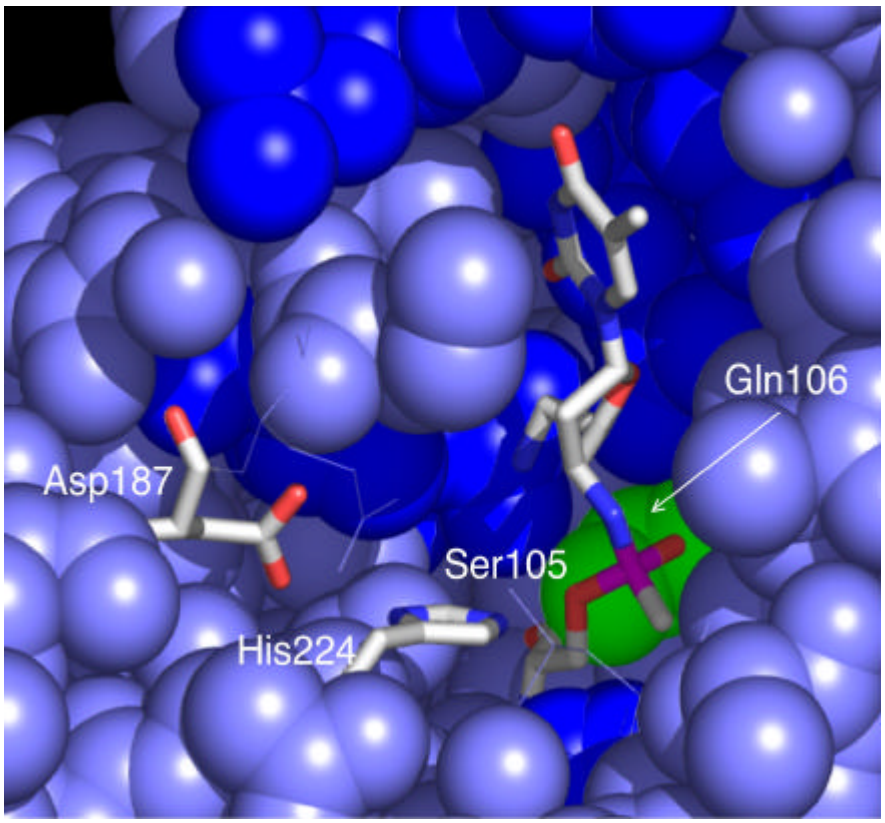


Figure S4. *Phosphonamidate for the 3'-acetylation of* ***2*** *in the inverse orientation. Compare to Figure 3A in the main article.*

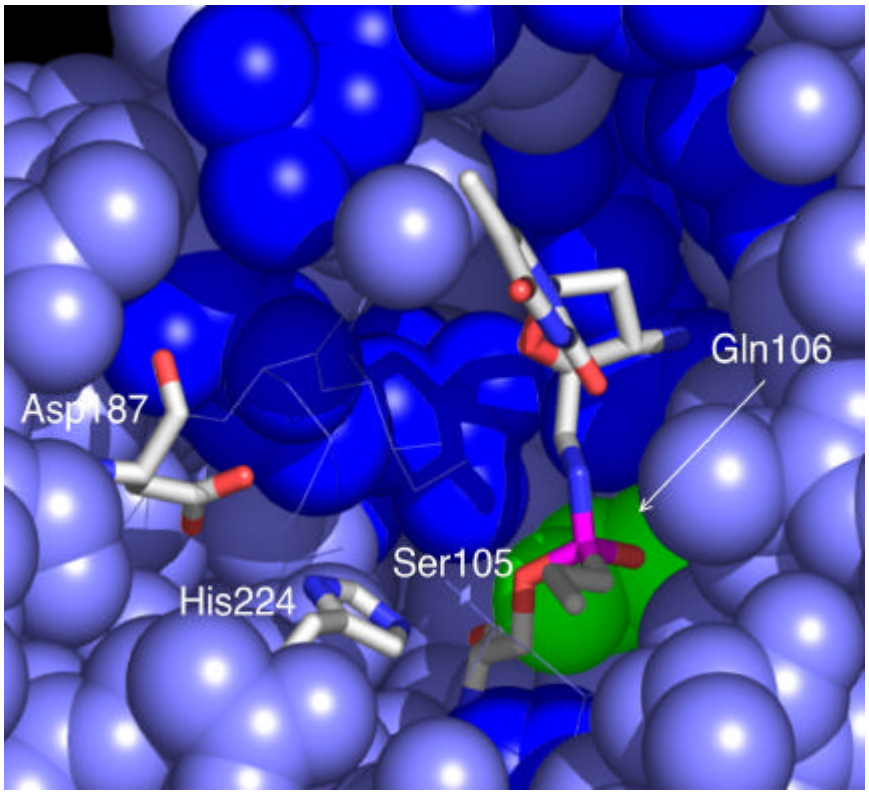


Figure S5. *Phosphonamidate for the 5'-butanoylation of* ***2*** *in the inverse orientation. Compare to Figure 3C in the main article.*