Supplementary Materials: Selective Acetylation of S mall Biomolecules and their Derivatives Catalyzed by $Er(OTf)_3$

Monica Nardi, Maria Luisa Di Gioia, Paola Costanzo, Antonio De Nino, Loredana Maiuolo, Man uela Oliverio, Fabrizio Olivito and Antonio Procopio

Table of Contents

- Sample 15a
- Sample 16a

EI/MS Spectra

- Sample 17a
- Sample 19a
- Sample 20a

1. Experimental Section

All chemicals and solvents were purchased from common commercial sources and were used a s received without any further purification. All reactions were monitored by TLC on silica Merck 60 F254 pre-coated aluminum plates and were developed by spraying with sulfuric acid in ethanol sol ution when possible. The tautomeric forms of acetyl cytosine was purified by semipreparative RP-H PLC chromatography [Phenomenex Jupter C18, 250 × 10 mm, 10 μm, UV 272 nm, 4.0 mL/min, (H_2O 100%). Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Brüker spectromet er at 300 MHz. Chemical shifts are reported in δ units (ppm) with TMS as reference (δ 0.00). All cou pling constants (J) are reported in Hertz. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Brüker at 75 MHz. Chemical shifts are reported in δ units (ppm) r elative to $CDCl_3$ (δ 77.0).

MW-assisted reactions were performed on a Synthos 3000 instrument from Anton Paar, equipp ed with a 4×24MG5 Rotor and an IR probe used for external temperature control.

LC-MS analysis were carried using an Agilent 6540 UHD Accurate - Mass Q-TOF LC–MS (Agil ent, Santa Clara, CA) fitted with a electrospray ionisation source (Dual AJS ESI) operating in positiv e ion mode. Chromatographic separation was achieved using a C18 RP analytical column (Poroshel l 120, SB-C18, 50 × 2.1 mm, 2.7 μm) at 30°C with a elution gradient from 5% to 95% of B over 13 min., A being H2O (0.1% FA) and B CH_3CN (0.1% FA). Flow rate was 0.4 ml/min.

2. General MW-assisted protocol for acetylation of substrates

To water solution (3 mL) of substrate (0.8 mmol) into a 3 mL glass vials to 1-acetylimidazole (2. 4 mmol) and $Er(OTf)_3$ (10 mol %) was added. The mixture was reacted for 30 min in a Synthos 3000 microwave instrument, fixed on the temperature value of 60 °C (IR Limit). The appearance of protec ted product was controlled by TLC and, after, completion, water was removed under reduced press ure and the resulting crude was purified by flash chromatography (CH_2Cl_2/MeOH 9.5:0.5).

Methyl 6-*O*-acetyl α-D-glucopyranoside (**1a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz).[45]

Methyl 6-*O*-acetyl α-D-mannopyranoside (**2a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz). [46]

Methyl 6-*O*-acetyl α-D-galattopyranoside (**3a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz). [47]

Methyl 6-*O*-acetyl β -D-glucopyranoside (**4a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz).[2a]

Methyl 6-*O*-acetyl β-D-mannopyranoside (**5a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz).[46]

Methyl 6-*O*-acetyl β-D-galattopyranoside (**6a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz).[48]

Phenyl 6-*O*-acetyl α-D-glucopyranoside (**7a**): ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 MH z).[49]

Phenyl 6-*O*-acetyl β-D-glucopyranoside (**8a**) : ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 M Hz).[49]

N-acetyl-5'-O-acetyl adenosine (**9a**): ^1H NMR ($CDCl_3$, 300 MHz): δ 2.02 (s, 3H, CH_3COO), 2.18 (s, 3H, CH_3CON), 3.85 (d, 1H, H5', J=13.15), 3.98 (d, 1H, H5', J_{gem}=13.15 Hz), 4.37 (s, 1H, H3'), 5.69 (s, 1H, H 2'), 6.01-6.03 (m, 4H, H4', H1' 2OH), 7.84 (s, 1H, H8), 8.34 (s, 1H, H2). ^{13}C NMR ($CDCl_3$, 75.5 MHz). HRMS (ESI) for ($C_{14}H_{17}N_5O_7$)+ Na]$^+$: calcd 390.1026, found 390.1019 (M+Na)$^+$

5'-O-acetyl thymidine (**10a**): ^1H NMR (DMSO, 300 MHz): δ 1.93 (s, 3H, CH_3), 2.09 (s, 1H, H2'), 2.13 (s, 3H, CH_3COO), 2.18 (s, 1H, H2'), 2.46-2.44 (br, 1H, OH), 4.19 (t, 1H, H3', *J*= 3.40 Hz), 4.27 (t, 1H, H 4', *J*= 3.29 Hz), 4.30 (dd, H5', *J*=11.84 Hz, *J*=3.29 Hz), 4.36-4.40 (m, 1H, H5'), 3.98 (d, 1H, H5', J_{gem}=13. 15 Hz), 6.30 (t, 1H, H1', J= 6.47 Hz), 7.32 (s, 1H, H6), 9.78 (s, 1H, NH). 13C NMR (DMSO, 75.5 MHz): 19.8, 21.5, 41.0, 63.9, 69.3, 82.7, 86.6, 109.8, 137.2, 150.6, 163.8, 170.3. HRMS (ESI) for ($C_{12}H_{16}N_2O_6$)+ N a]$^+$: calcd 307.0906, found 307.0892 (M+Na)$^+$

5'-O-acetyl-2'-deoxyguanosine (**11a**): ^{1}H NMR (DMSO, 300 MHz): δ 2.07 (s, 3H, CH_3COO), 2.36 (m, 1H, H2'), 2.77 (m, 1H, H2'), 3.57-3.58 (m br, 2H, H3' OH), 3.99- 4.02 (m, 2H, H5'), 5.28 (m, 1H, H4'), 6.10 (m, 1H, H1'), 6.72 (s, 2H, NH2), 7.94 (s, 1H, H8). ^{13}C NMR (DMSO, 75.5 MHz): 21.0, 40.7, 61.8, 7 5.3, 82.9, 85.2, 116.9, 135.4, 150.1, 154.2, 157.1, 170.3. HRMS (ESI) for ($C_{12}H_{15}N_5O_2$)+ Na]$^+$: calcd 332.0 971, found 332.0965 (M+Na)$^+$

2'-deoxy-3',5'-di-O-acetylguanosine (**11b**): ^{1}H NMR (DMSO, 300 MHz): δ 2.03 (s, 3H, CH_3COO), 2.0 8 (s, 3H, CH_3COO), 2.41 (1H, ddd, ^{2}J = 14.26 Hz, $^{3}J_1$ = 5.47 Hz, $^{3}J_2$ = 2.1 Hz, H-2b') (m, 1H, H2'), 2.92 (1H, ddd, ^{2}J = 14.27 Hz, ^{3} J_1 = 8.7 Hz, $^{3}J_2$ = 6.4 Hz, H-2a'), 4.15-4.30 (m, 3H, H5', H3'), 5.28 (d, 1H, H4', *J*=6.83), 6.08-6.15 (m, 1H, H1'), 6.52 (2H, s, NH_2), 7.92 (1H, s, H8), 10.68 (1H, s, NH, ^{13}C NMR (DMSO, 75.5 MHz): 20.4, 20.7, 35.4, 63.6, 74.4, 81.4, 82.9, 116.7, 151.0, 153.7, 156.9, 169.9, 170.1.45. HRMS (ESI) for ($C_{14}H_{17}N_5O_6$)+ Na]$^+$: calcd 374.1077, found 374.1071 (M+Na)$^+$

N-acetyl-cytidine (**12a**), 5'-O-acetyl-cytidine (**12b**), 6-O-acetyl-cytidine (**12c**): ^{1}H NMR (DMSO, 300 MHz): δ 2.05 (s, 3H, CH_3A), 2.08 (s, 6H, CH_3B + CH_3C), 3.51-3.71 (m, 6H, H5'A, H5'C, H3'C, , H2'C), 3.82-4.01 (m, 3H, , H5'A, H5'C, H4'A), 4.11-4.29 (m, 4H, H3'A, H4'A, H4'C, H3'B), 5.03-5.07 (m, 3H, H5A, H5B, H5'B), 5.19 (t, 1H, H2'B, *J*= 4.81 Hz), 5.27 (t, 1H, H2'A, *J*= 5.04 Hz), 5.45 (d, H4'B, *J*=5.04 Hz), 5.48 (d, 1H, H5'B), 5.65 (d, 1H, H1'C, *J* = 6.19 Hz), 5.76 (m, 2H, H6C,H5C), 5.83 (d, 1H, H1'A, *J* = 5.96), 5.97 (d, 1H, H1'B), 7.60 (d, 1H, H6B, *J*=7.56 Hz), 7.83 (d, 1H, H6A, *J*=7.56 Hz). ^{13}C NMR (DMSO, 75.5 MHz): 60.5, 60.7, 68.1, 69.6, 71.8, 72.4, 73.2, 75.3, 79.1, 80.4, 81.9, 84.7, 86.7, 88.7, 90.0, 94.2, 94.3, 9 4.4, 141.3, 155.2, 155.4, 155.4, 165.5, 165.5, 169.6, 169.7, 178.3. HRMS (ESI) for ($C_{11}H_{18}N_3O_6$)+ Na]$^+$: cal cd 311.1093, found 311.1093 (M+Na)$^+$, 311.1095 (M+Na)$^+$, 311.1086 (M+Na)$^+$.

4-[2-(Acetyloxy)ethyl]phenyl acetate (**13a**): ^{1}H NMR (DMSO, 300 MHz): δ 1.98 (s, 3H, CH_3), 2.25 (s, 3 H, CH_3), 2.88 (t, 2H, CH_2, *J*= 7.50 Hz), 4.20 (t, 2H, CH_2, *J*= 7.11 Hz), 7.05 (d, 2H, Har, *J*= 8.49 Hz), 7.28 (d, 2H, Har, *J*= 8.46 Hz), ^{13}C NMR (DMSO, 75.5 MHz): Spectroscopic data compared to those reporte d in the literature.[50]

3,4-dihydroxyphenethyl acetate (**14a**): ^{1}H NMR (DMSO, 300 MHz): δ 1.97 (s, 3H, CH_3), 2.68 (t, 2H, C H_2, *J*= 6.87 Hz), 4.10 (t, 2H, CH_2, *J*= 7.11 Hz), 6.45-6.48 (m, 1H, Har), 6.48-6.62 (m, 2H, Har). ^{13}C NMR (DMSO, 75.5 MHz): 20.4, 34.3, 65.2, 115.0, 116.9, 121.1, 128.9, 144.2, 145.6, 170.6. HRMS (ESI) for (C_{10} $H_{12}O_4$)+ H]$^+$: calcd 197.0814, found 197.0818, 219.0635 (M+Na)$^+$

4-hydroxy-3-methoxyphenethyl acetate (**15a**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.99 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.85 (t, 2H, CH_2, *J* = 7.50 Hz), 3.73 (s, 3H, CH_3), 4.33 (t, 2H, CH_2, *J* = 7.11 Hz), 7.03 (d, 2H, Har, *J* = 8.43 Hz), 7.25 (d, 2H, Har, *J* = 8.43 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): 20.4, 34.8, 56.9, 64.8, 113. 7, 151.8, 121.5, 133.1, 142.9, 151.1, 170.0. HRMS (ESI) for ($C_{11}H_{14}O_4$)+ H]$^+$: calcd 211.0970, found 211.0 978, 233.0781 (M+Na)$^+$

Acetyl-3,5-Dihydroxybenzyl alcohol (**16a**): ^{1}H NMR (DMSO, 300 MHz): 2.05 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 6.14-6.18 (m, 3H, Har). ^{13}C NMR (DMSO, 75.5 MHz): 20.6, 35.1, 64.8, 121.5, 128.3, 134.1, 143.2, 169.1, 170.0. ^{13}C NMR (DMSO, 75.5 MHz): 20.6, 65.3, 101.9, 105.6, 105.6, 137.9, 158.3, 170.0. HRMS (E SI) for ($C_9H_{11}O_4$)+ H]$^+$: calcd 183.0657, found 183.0655, 205.0480 (M+Na)$^+$

Glycerol 1,3 diacetate (**17a**): ^{1}H-NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75.5 MHz).Spectroscopic data compared to those reported in the literature.[51]

n-butyl acetate (**18a**): ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 MHz).[52]

1, 4-Butanediol, diacetate (**19a**): ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 MHz). Spectrosc opic data compared to those of the pure product. GC-MS (EI): 114.0 (11), 73.0 (20), 71.0 (30), 54.0 (45), 43.0 (100).[53]

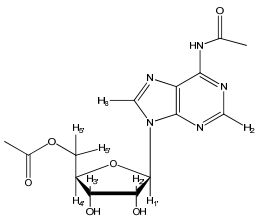
n-octyl acetate (**20a**): ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 MHz). Spectroscopic data c ompared to those of the pure product. GC-MS (EI): 112.0 (10), 84.0 (30), 70.0 (36), 56.0 (30), 43.0 (100). [53]

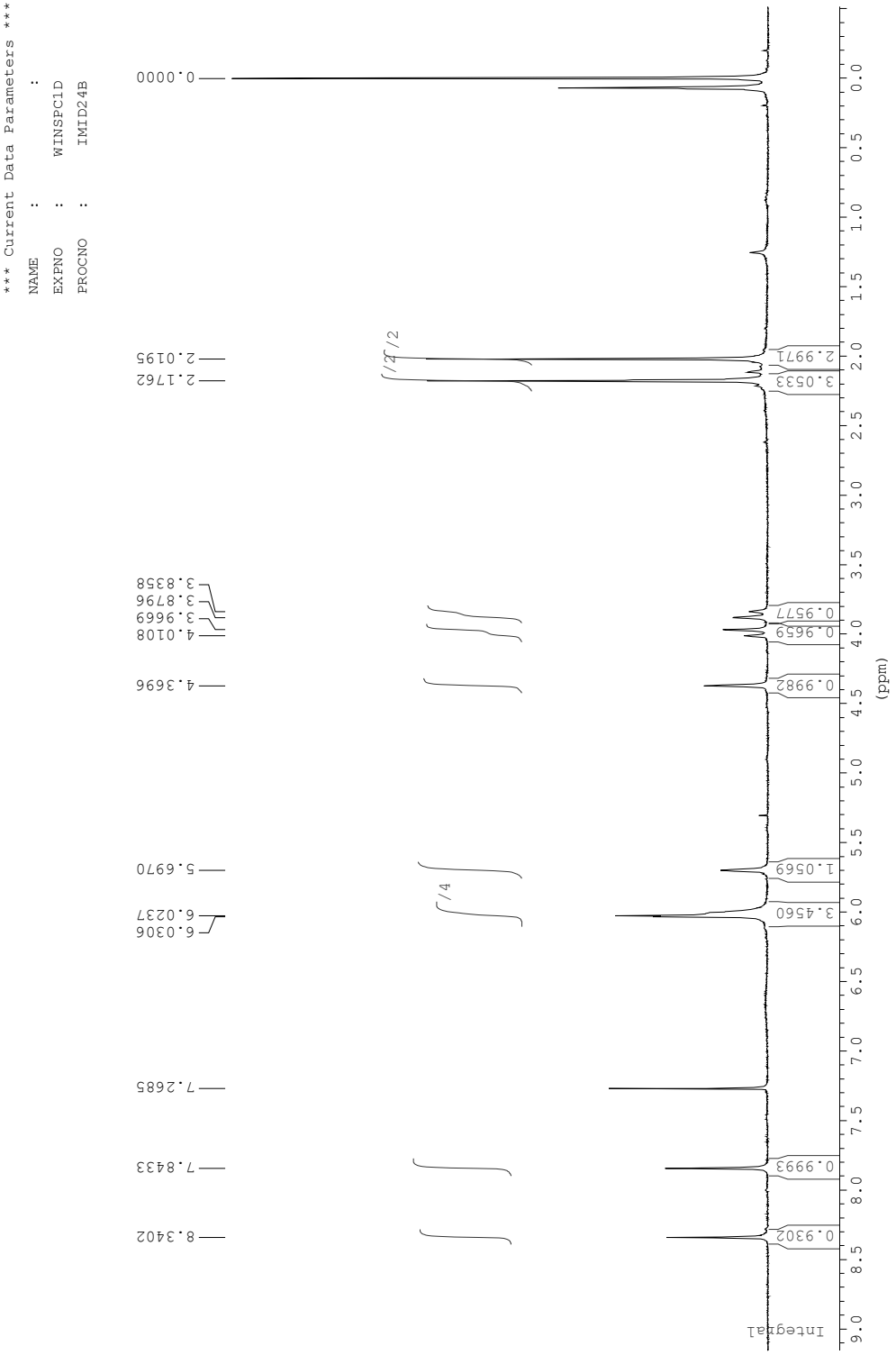
Fmoc-Ser(Ac)-OH **(21a**): ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 MHz). Spectroscopic da ta compared to those of the pure product.

Fmoc-Lys(Ac)-OH (22a): ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C-NMR ($CDCl_3$, 75.5 MHz). Spectroscopic da ta compared to those of the pure product.

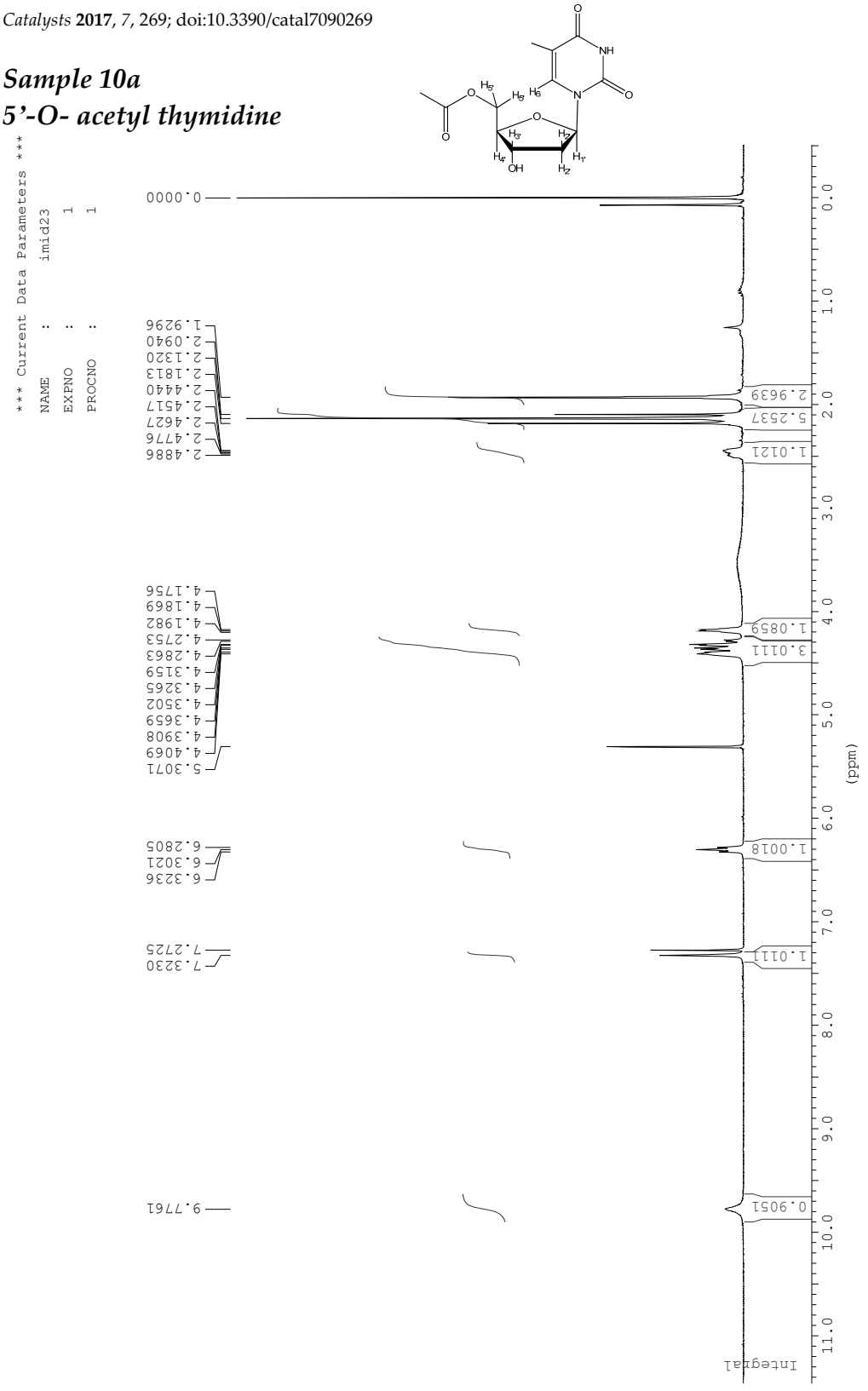
[1]H-NMR Spectra

Sample 9a
N-acetyl-5'-O-acetyl adenosine

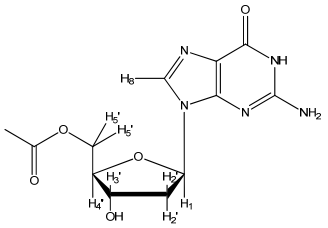


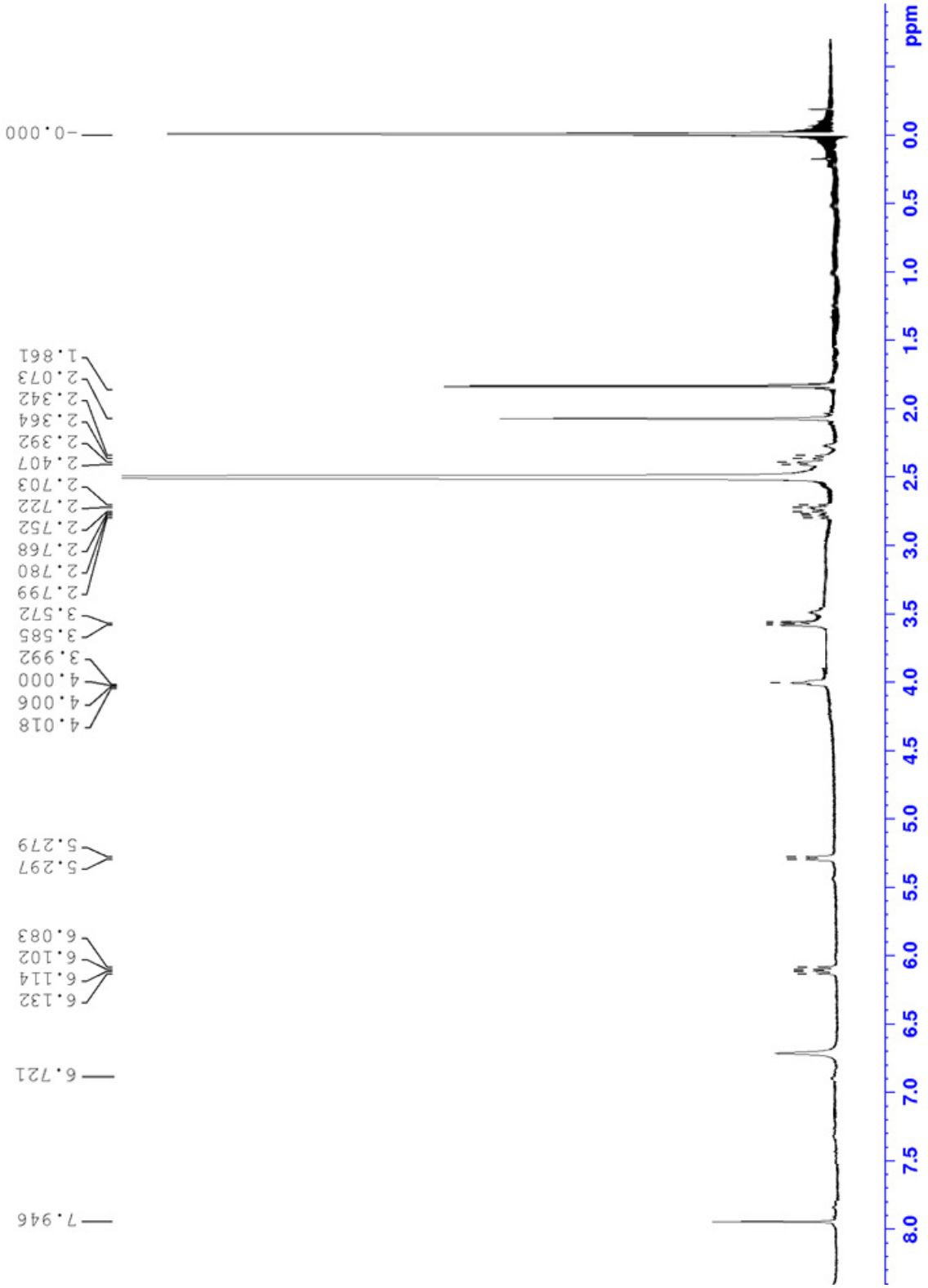


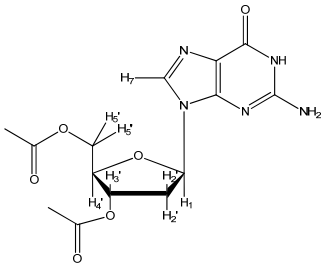
Sample 10a
5'-O- acetyl thymidine



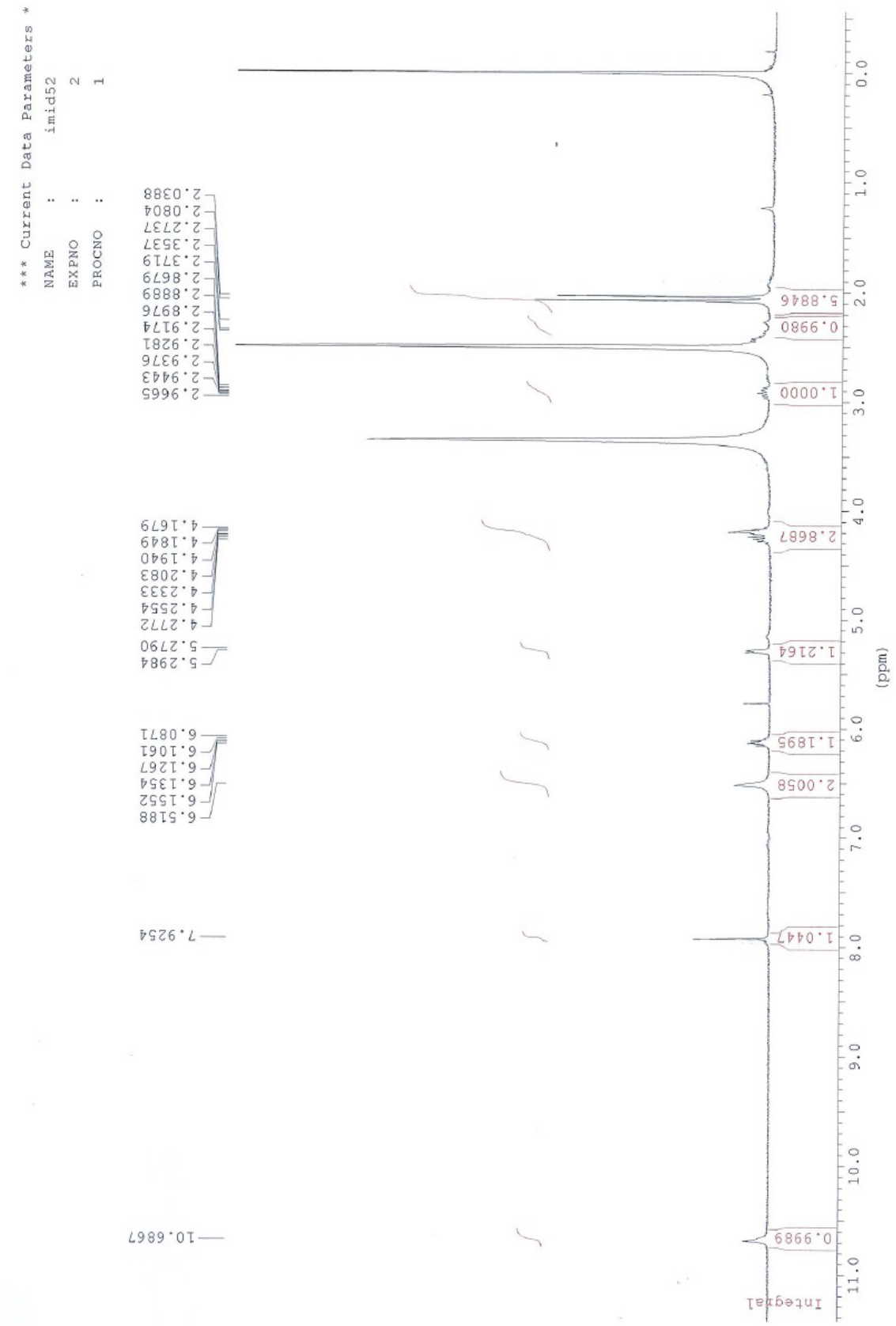
Sample 11a
5'-O-acetyl-2'-deoxyguanosine







Sample 11b
2'-deoxy-3',5'-di-*O*-acetylguanosine

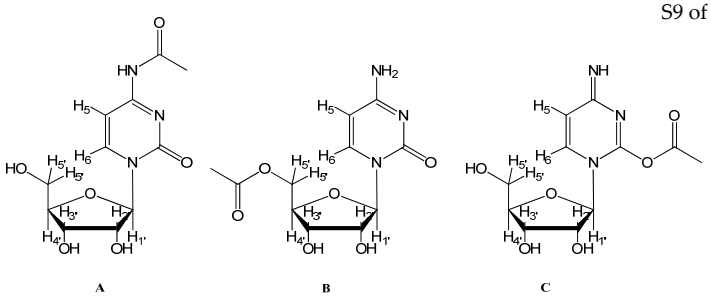


Sample 12°, 12b, 12c

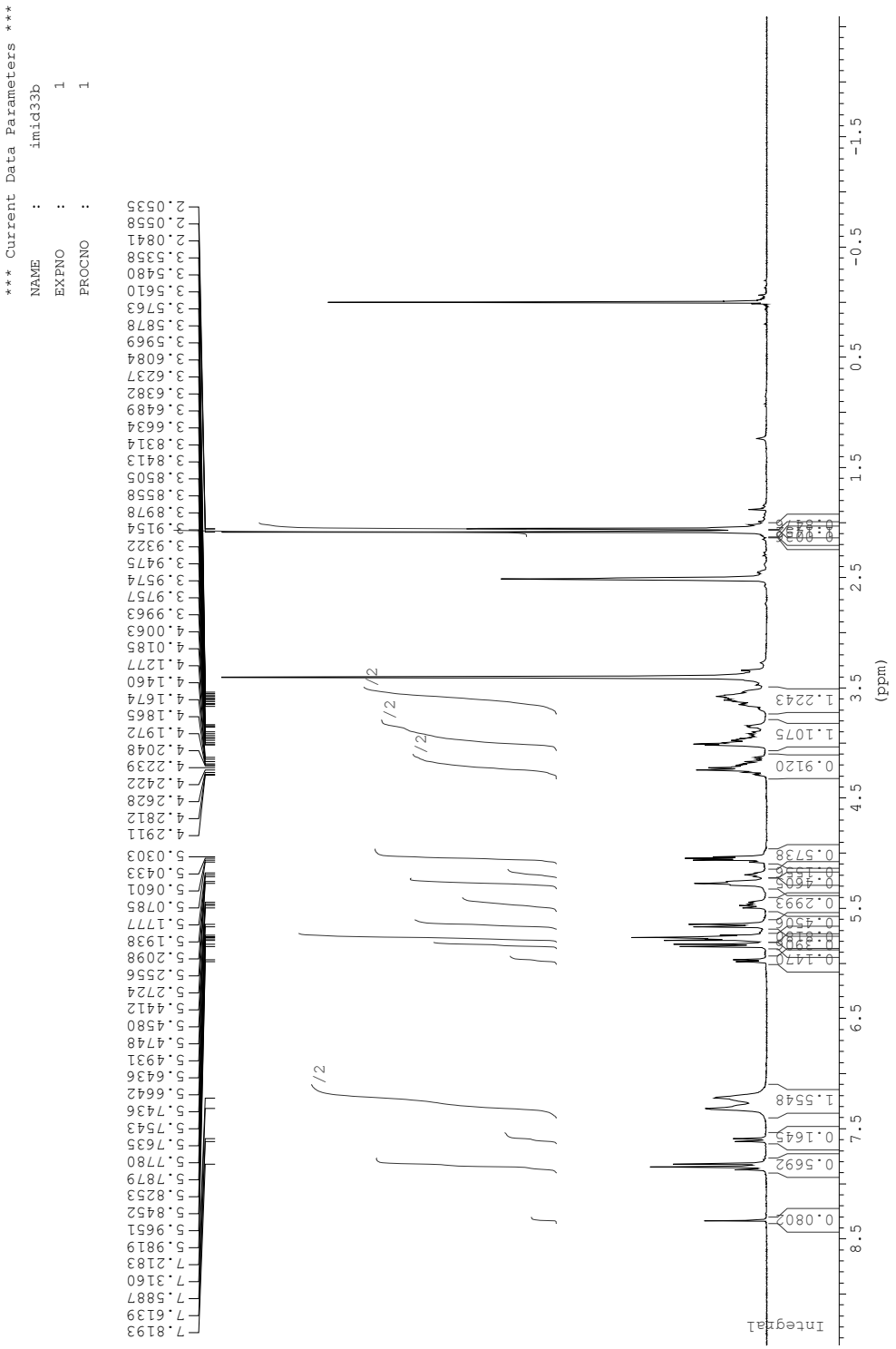
N-acetyl-cytidine (12a),

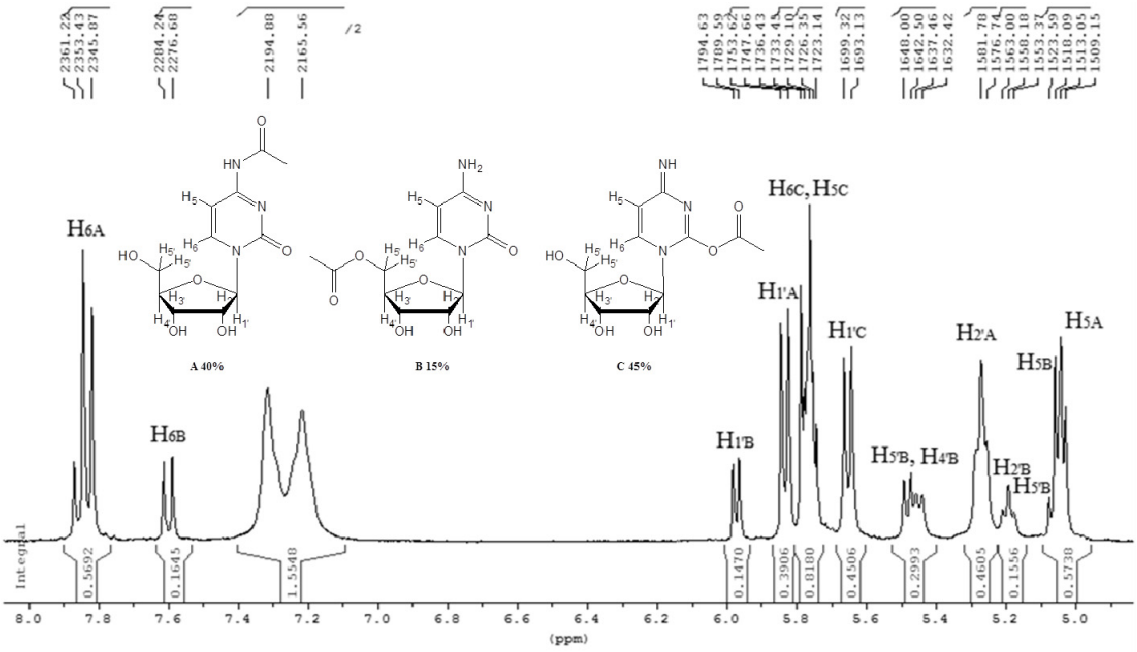
5'-O-acetyl-cytidine (12b),

6-O-acetyl-cytidine (12c):



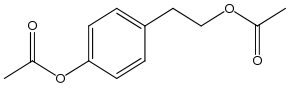
1.2 equiv. di 1-acetilimidazolo **A**= 45.6%; **B**= 14,7%; **C**=39.7%

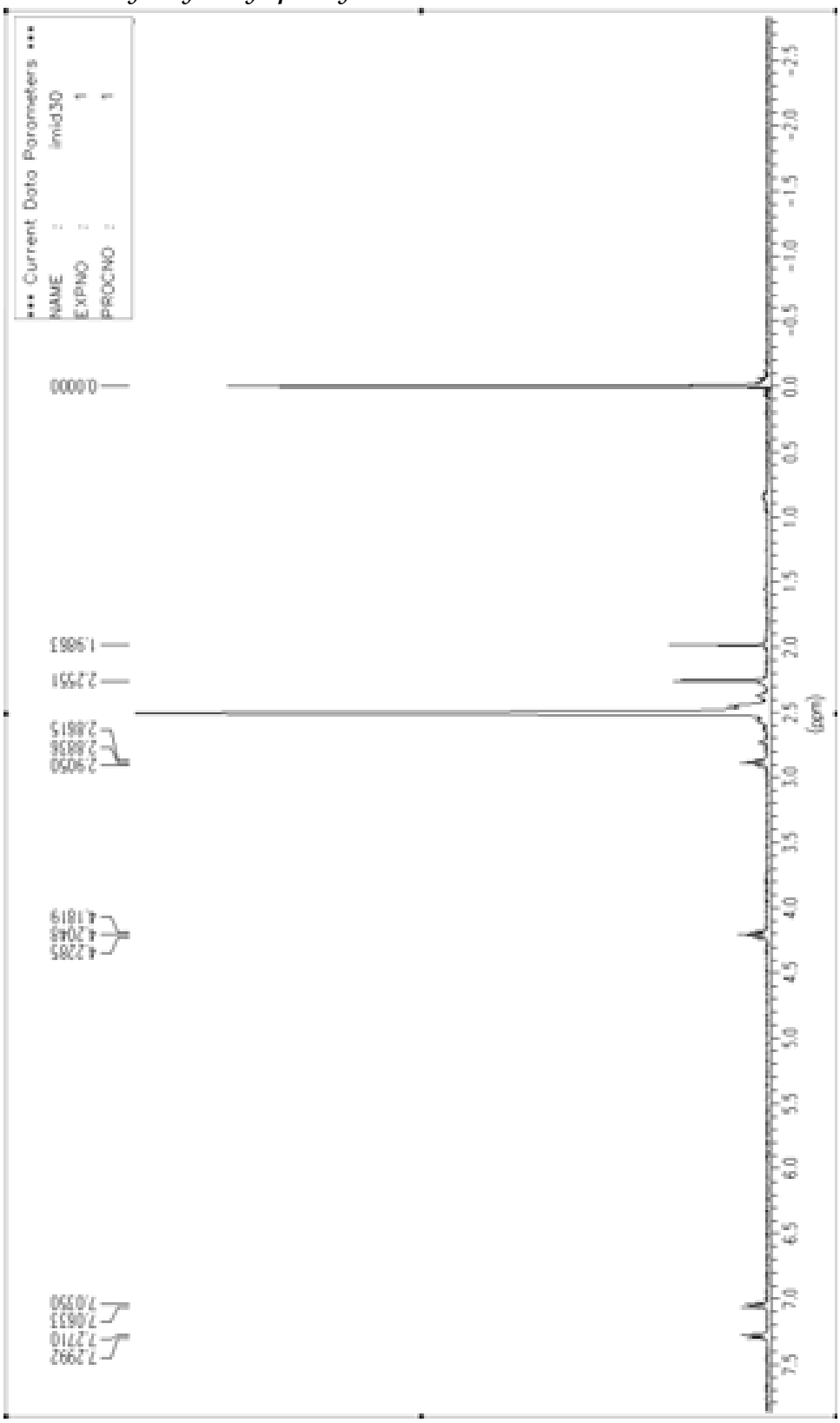




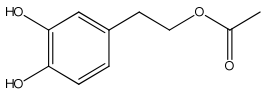
3 e quiv. di 1-acetilimidazolo **A**= 40.0%; **B**= 15,0; **C**=40.0%

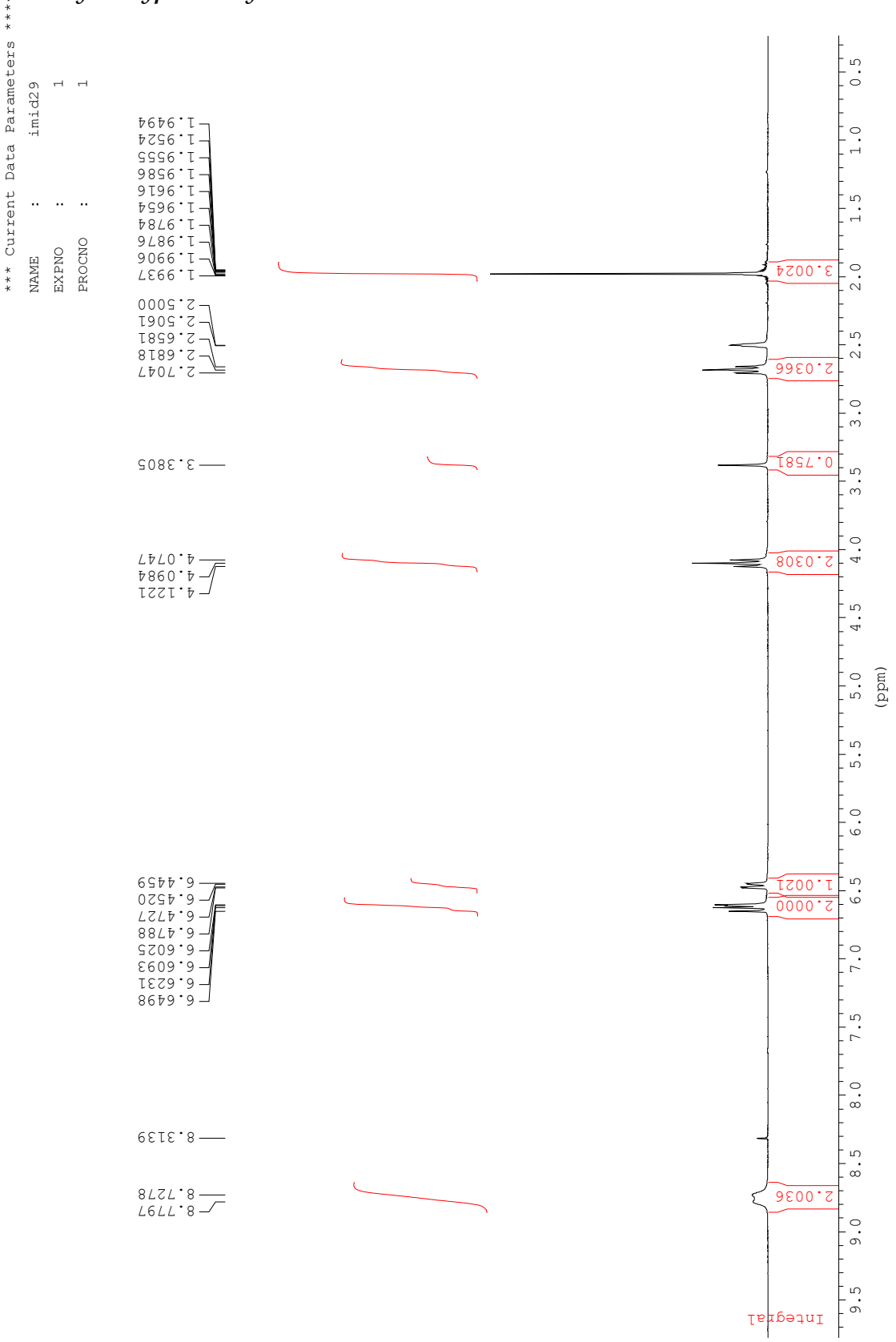
Sample 13a
4-[2-(Acetyloxy)ethyl]phenyl acetate





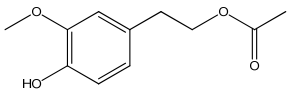
Sample 14a
3,4-dihydroxyphenethyl acetate

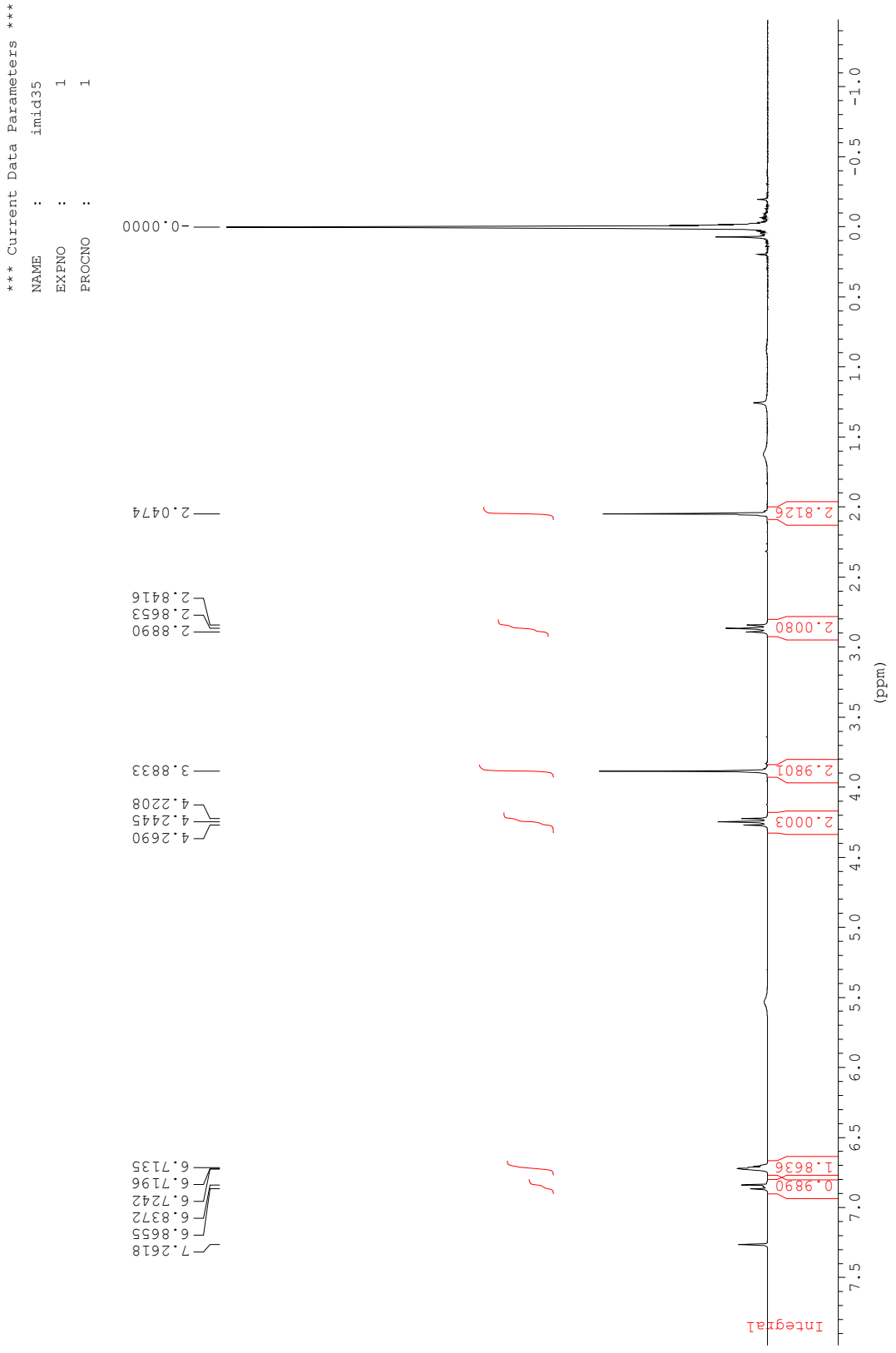




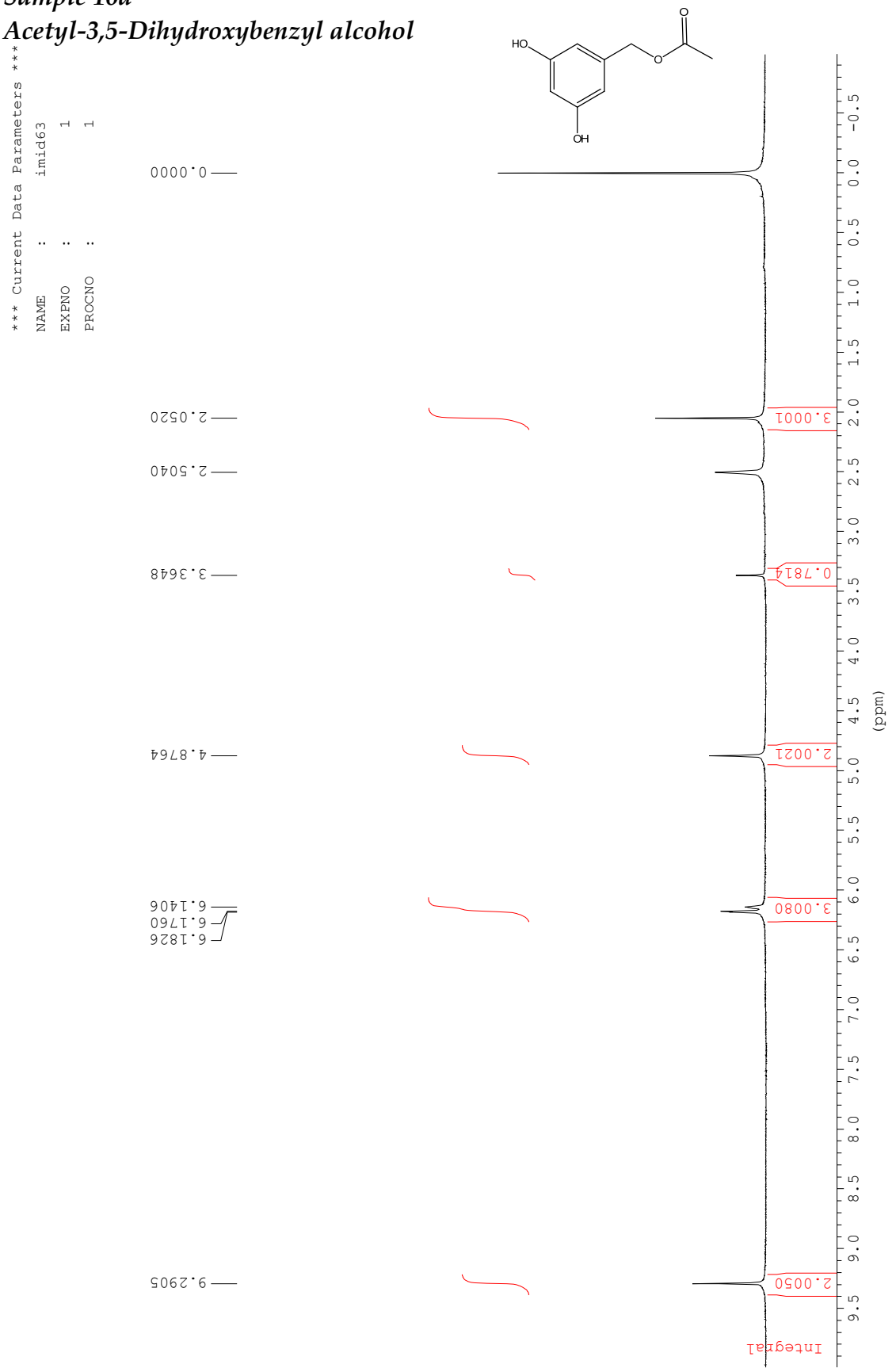
Sample 15a

4-hydroxy-3-methoxyphenethyl acetate

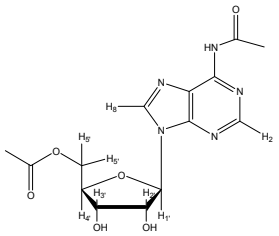


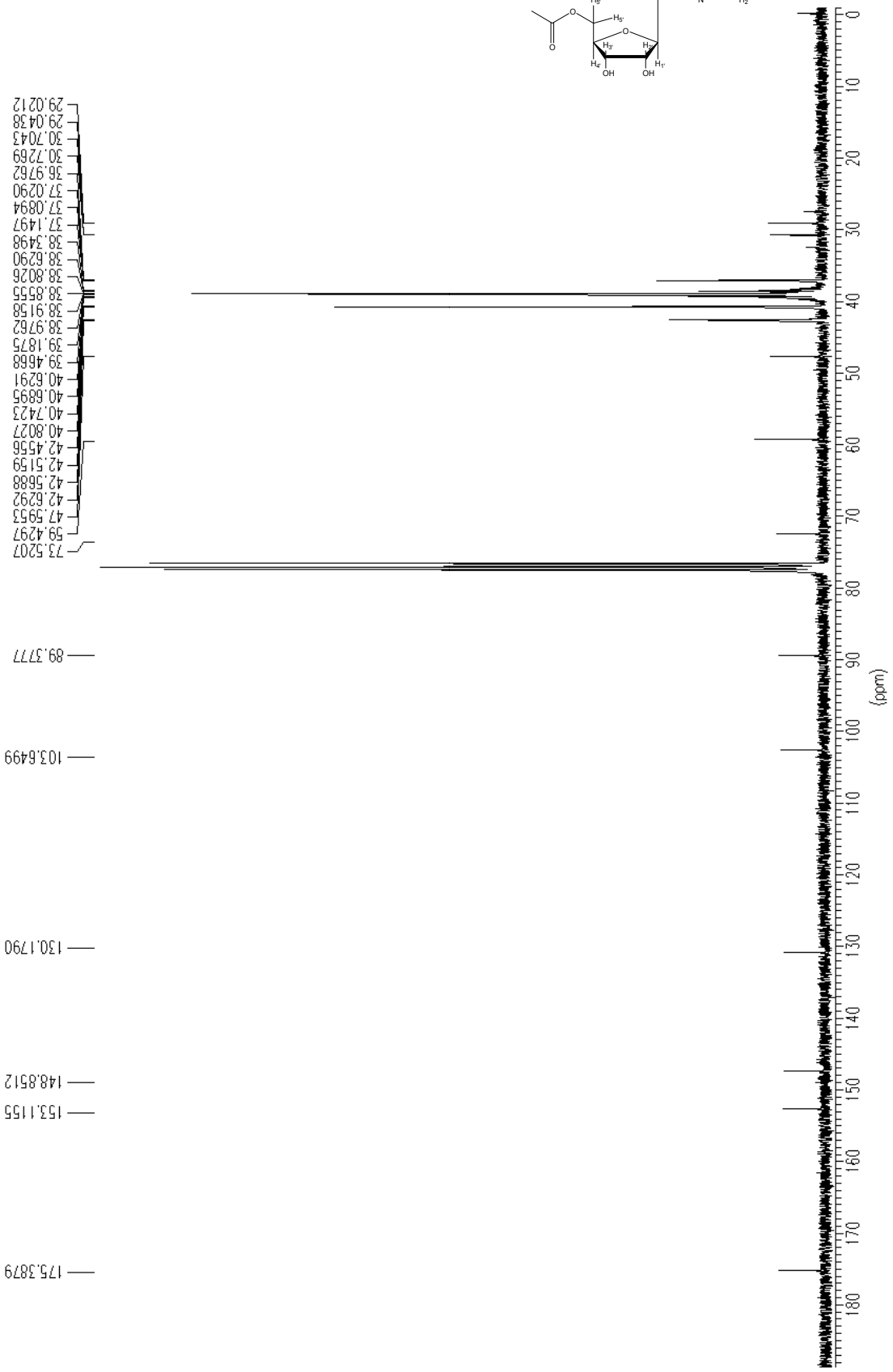


Sample 16a
Acetyl-3,5-Dihydroxybenzyl alcohol

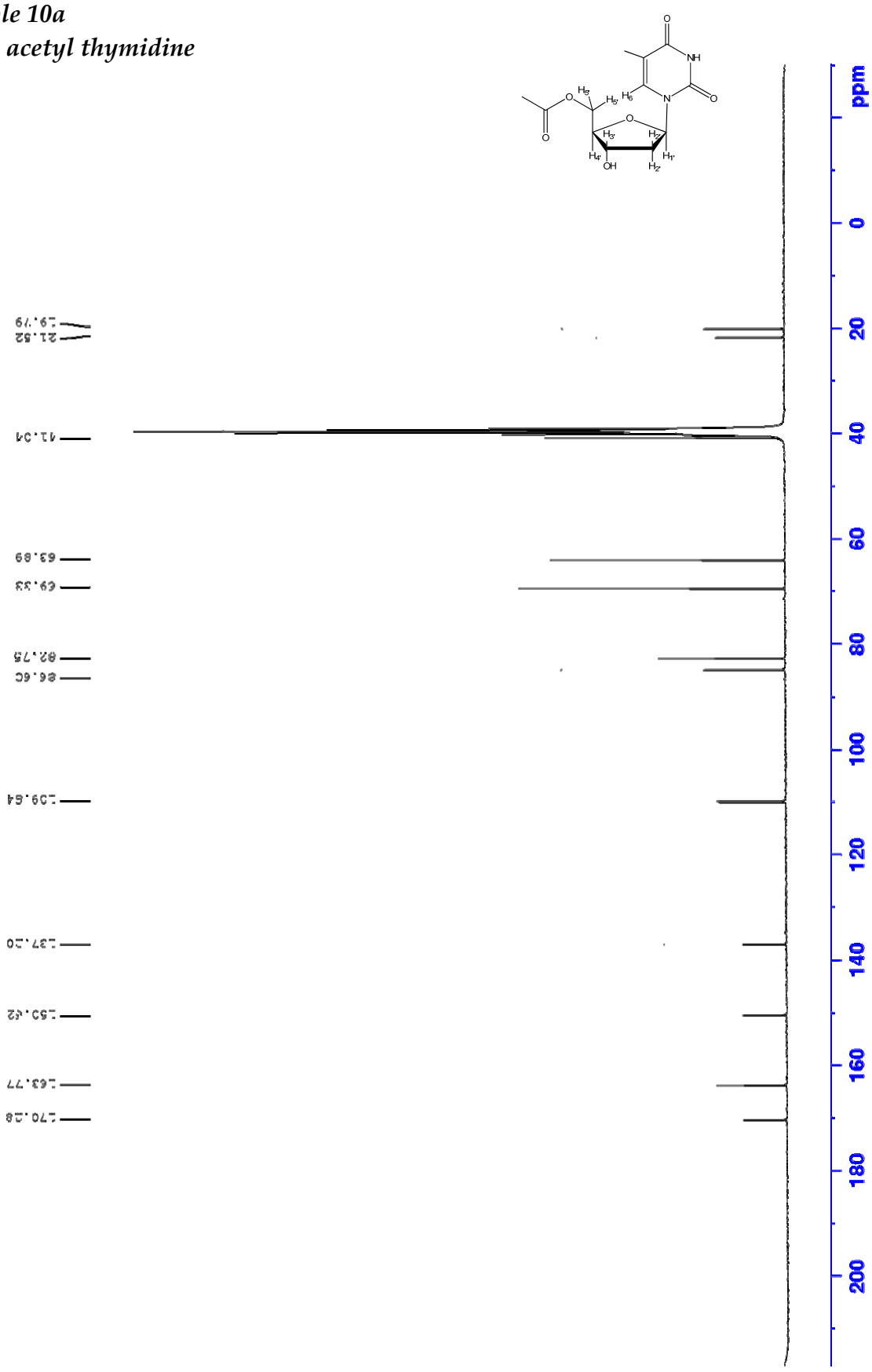


^{13}C-NMR Spectra
Sample 9a
N-acetyl-5'-O-acetyl adenosine

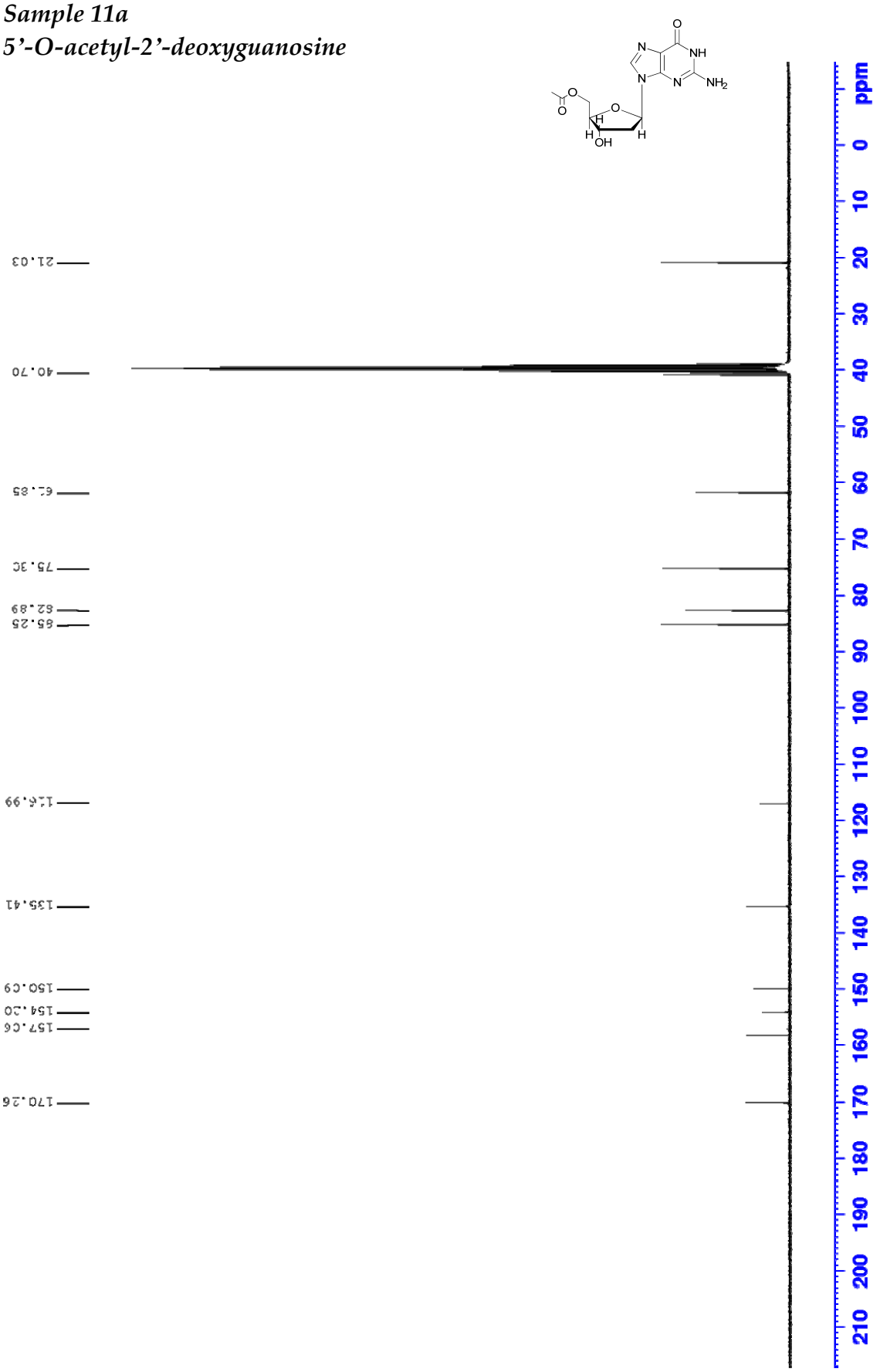




Sample 10a
5'-O- acetyl thymidine

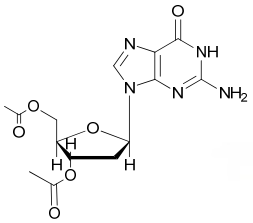


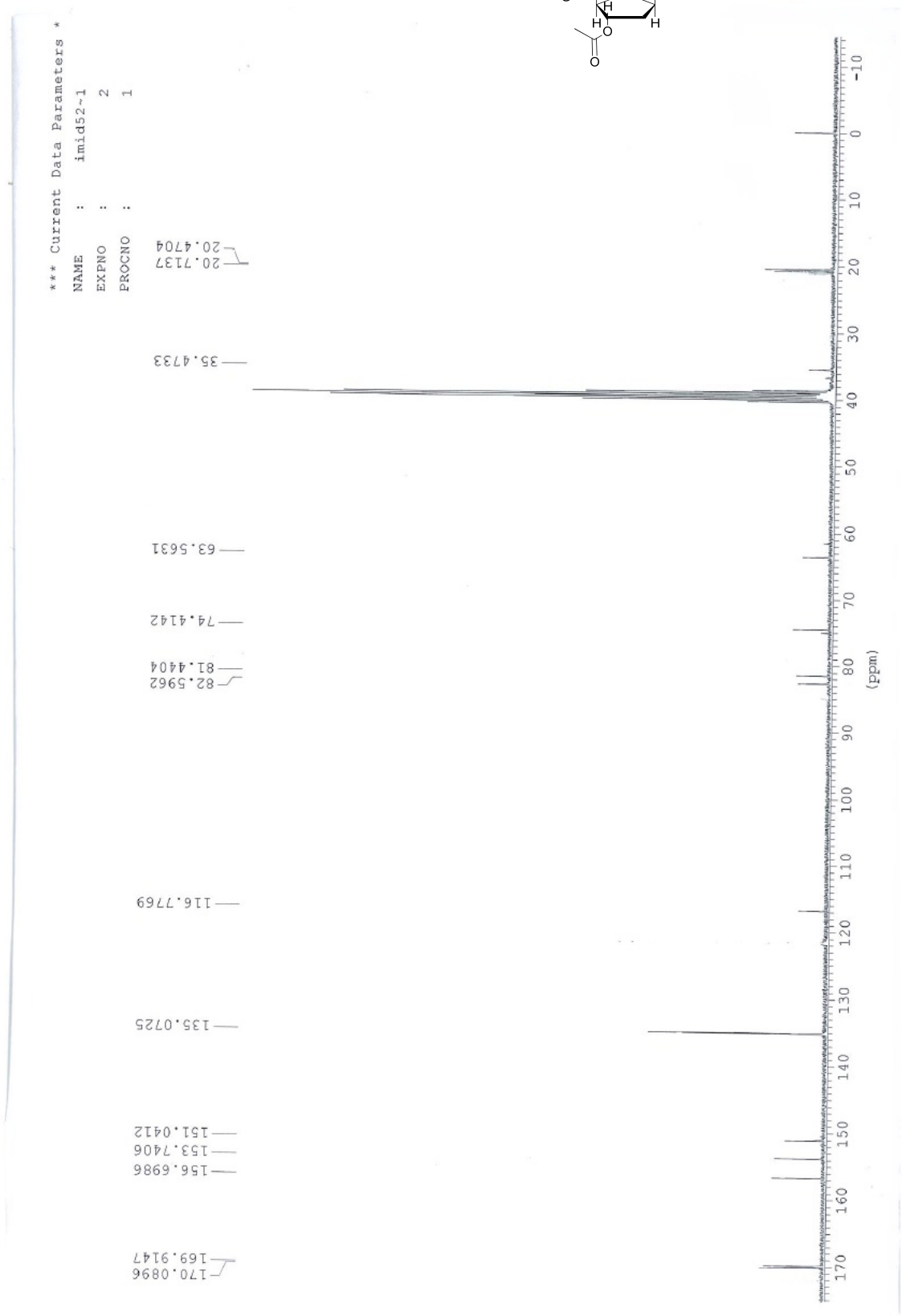
Sample 11a
5'-O-acetyl-2'-deoxyguanosine



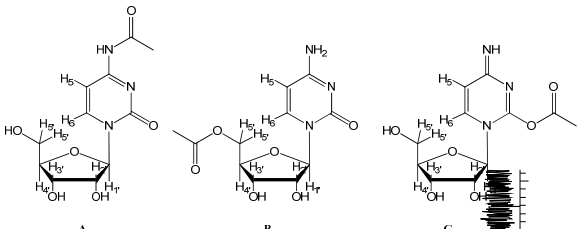
Sample 11b

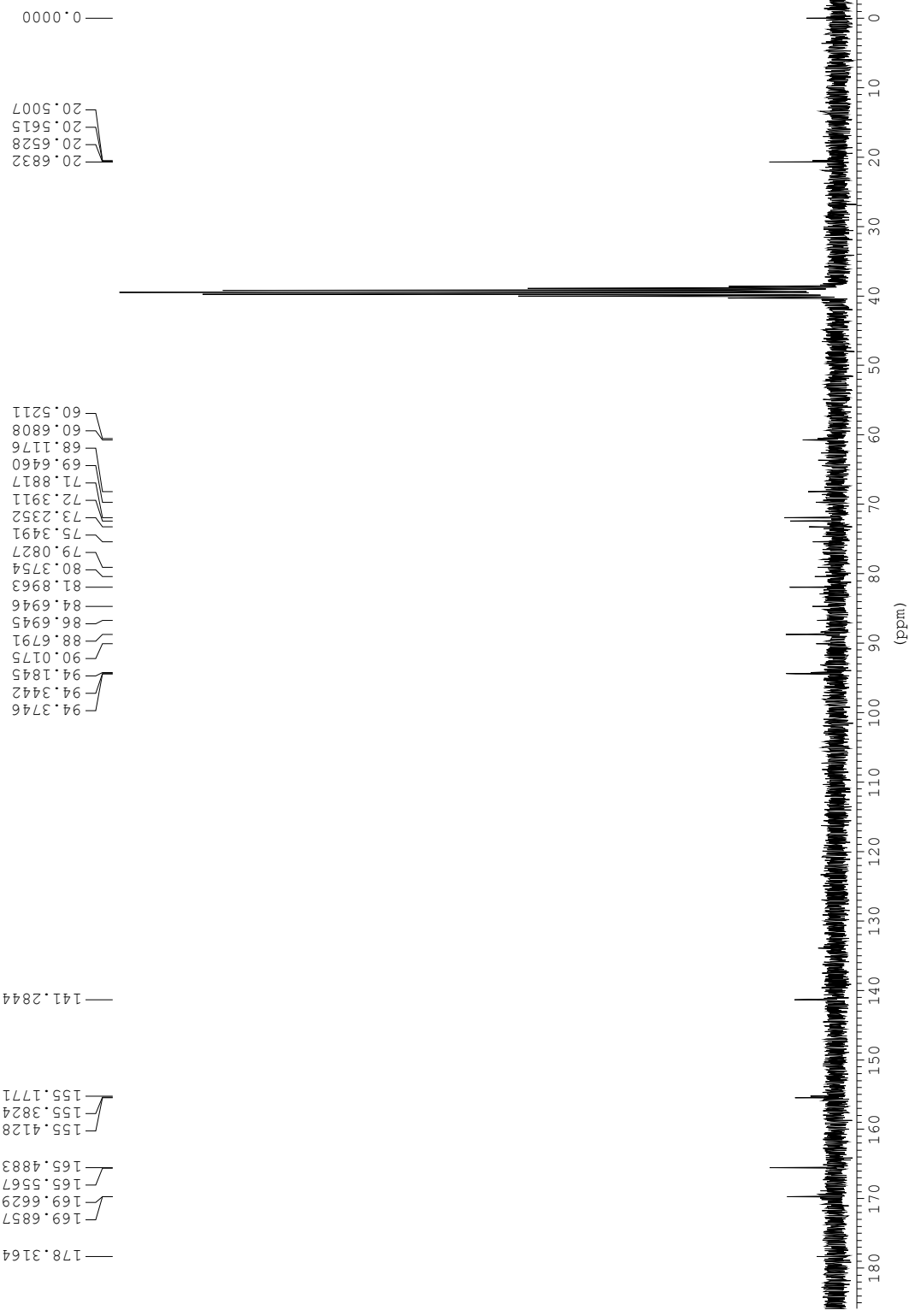
2'-deoxy-3',5'-di-O-acetylguanosine:



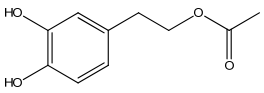


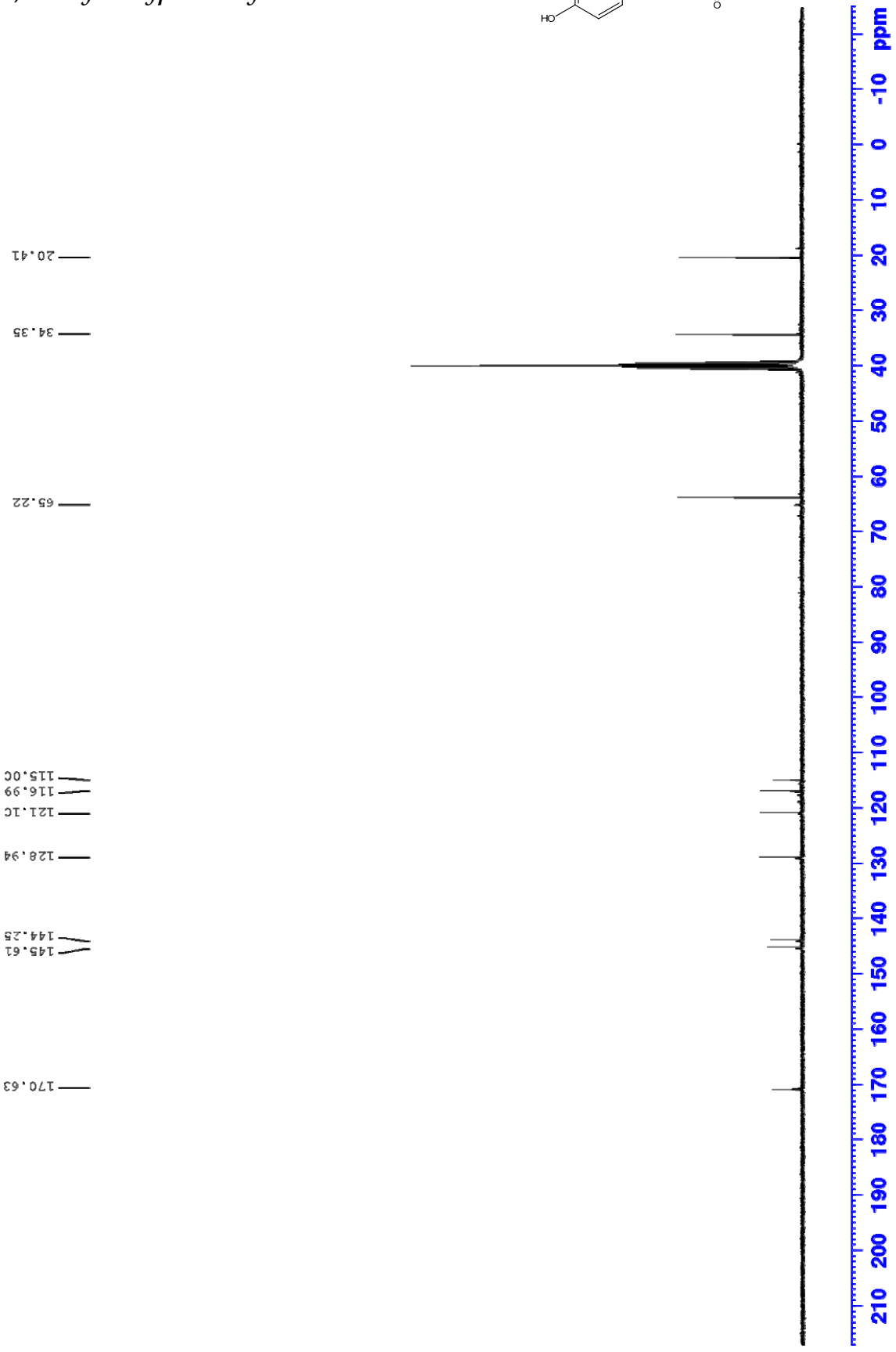
Sample 12a, 12b, 12c
N-acetyl-cytidine (12a),
5'-O-acetyl-cytidine (12b),
6-O-acetyl-cytidine (12c):





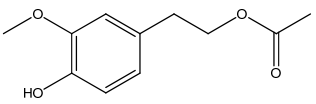
Sample 14a
3,4-dihydroxyphenethyl acetate

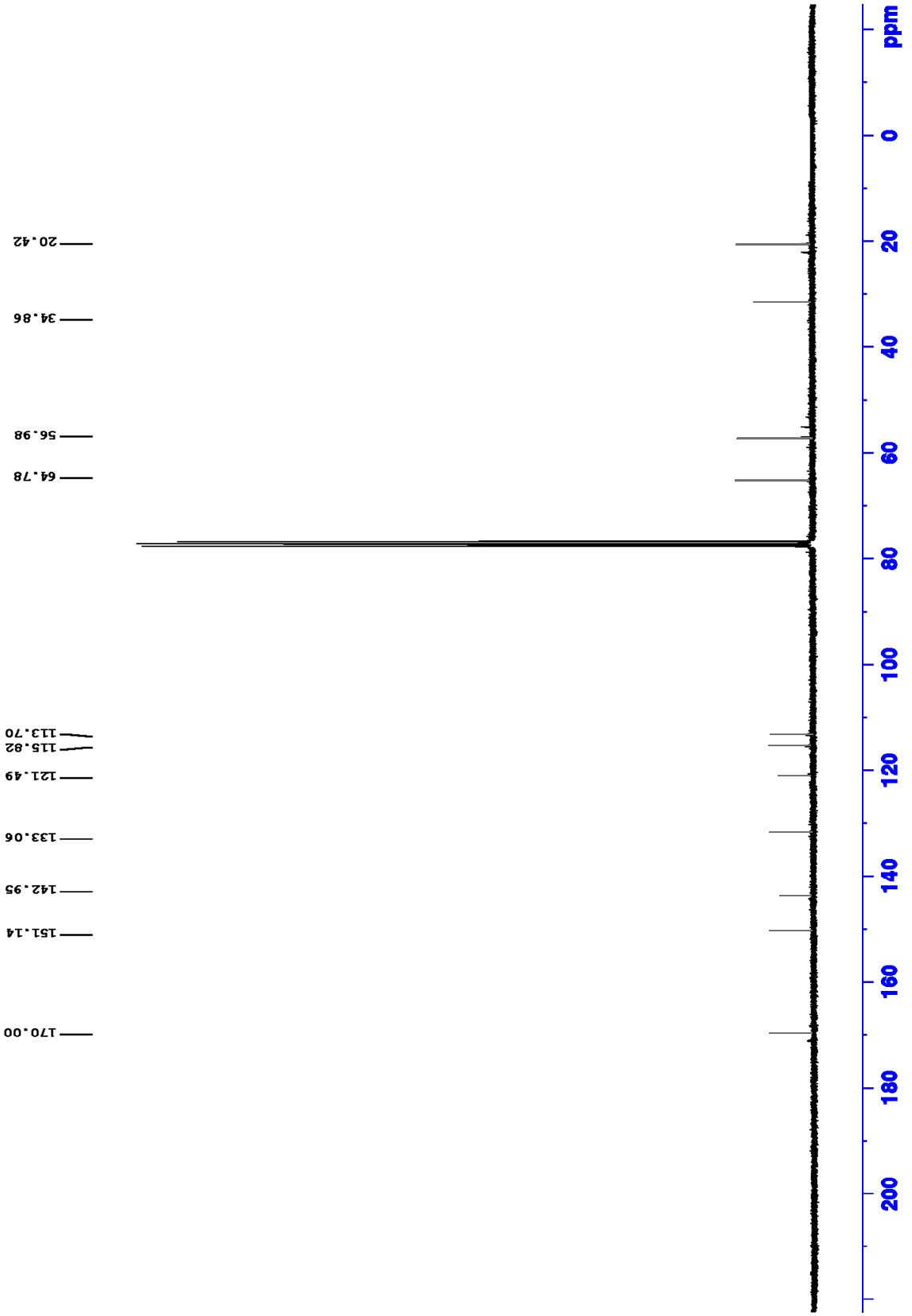




Sample 15a

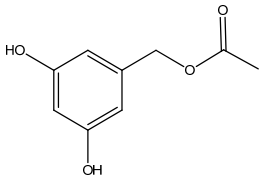
4-hydroxy-3-methoxyphenethyl acetate

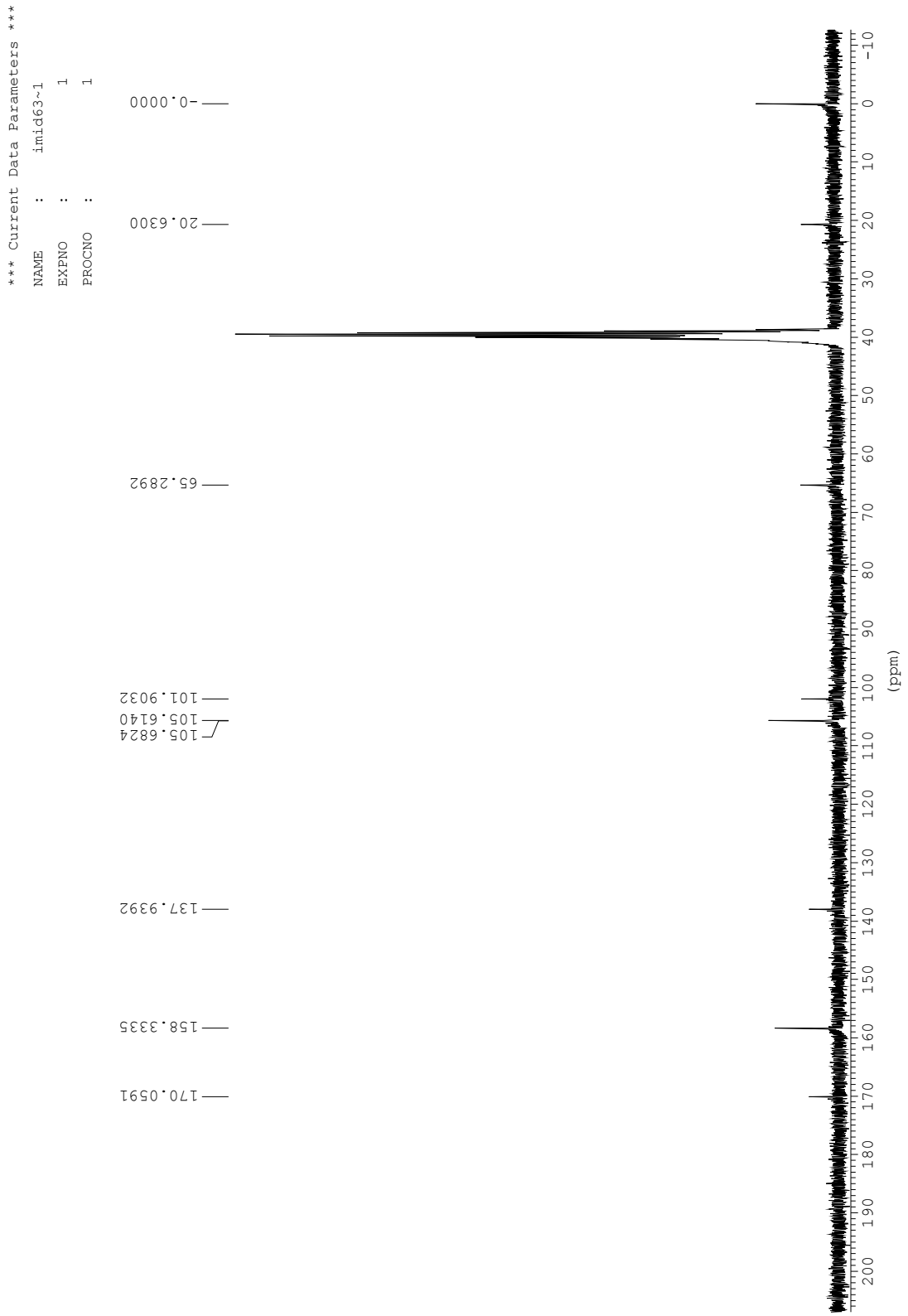




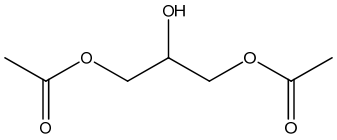
Sample 16a

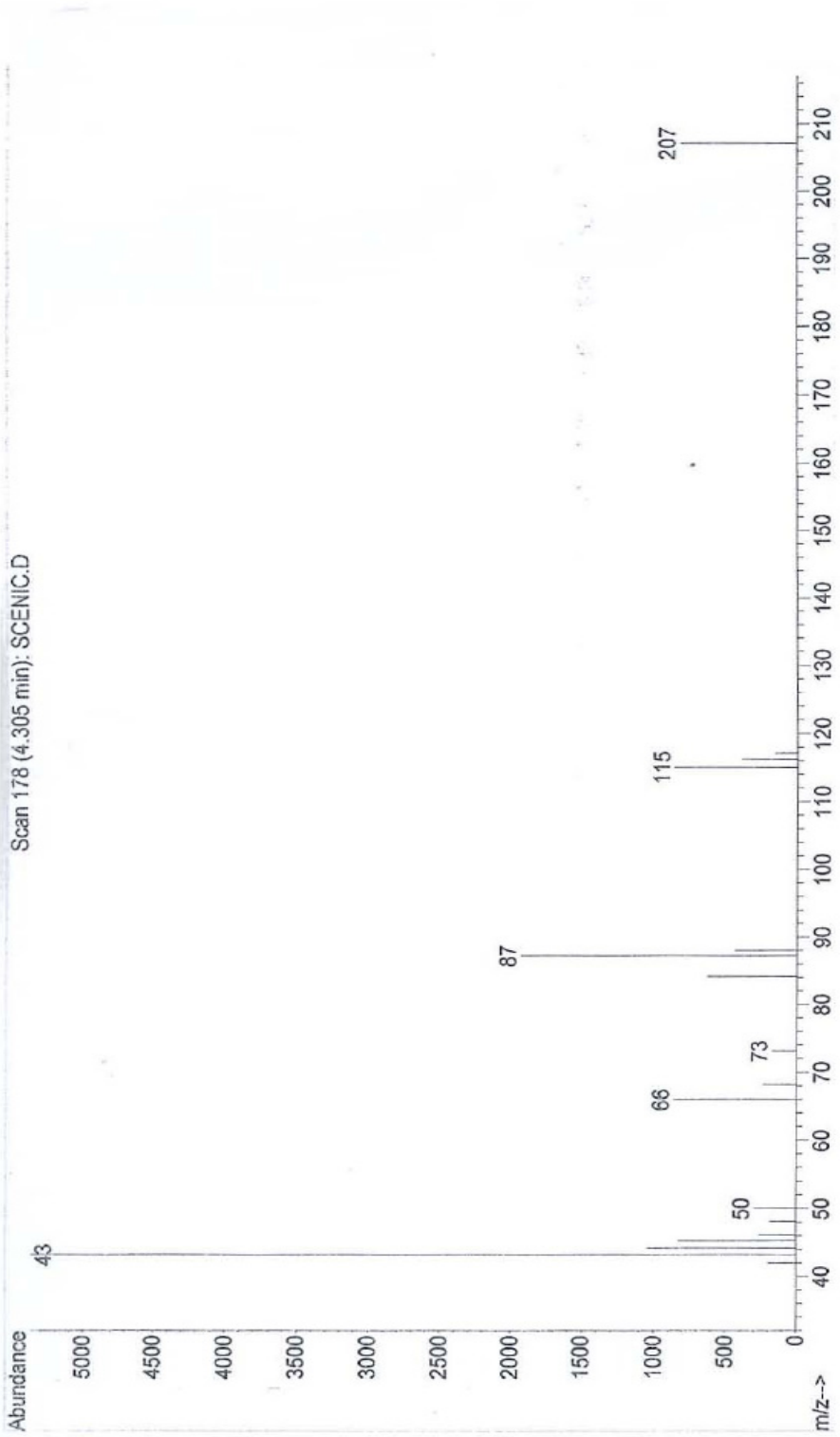
Acetyl-3,5-Dihydroxybenzyl alcohol



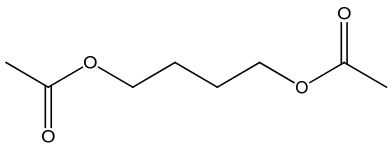


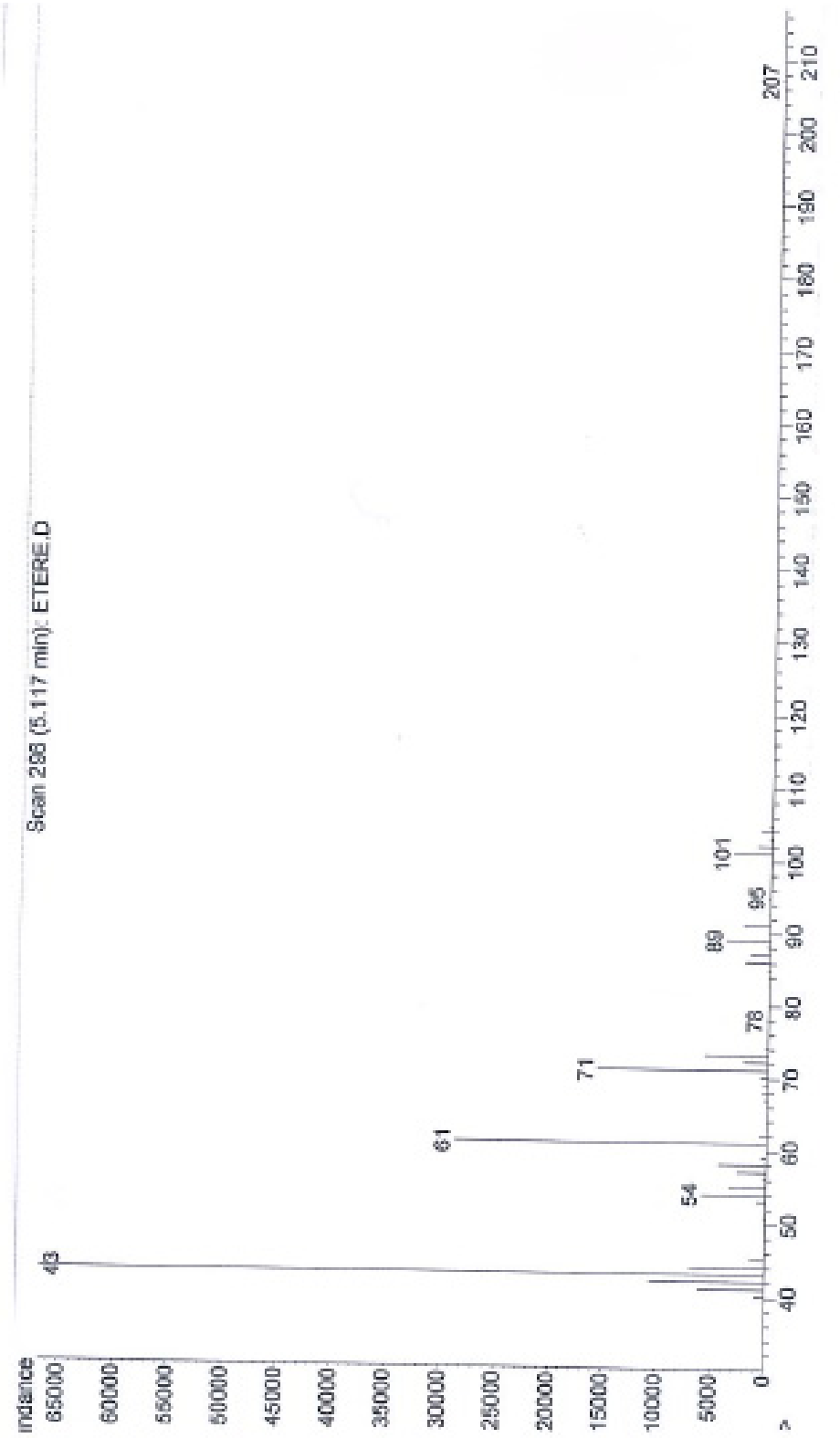
EI/MS Spectra
Sample 17a
Glycerol 1,3 diacetate





EI/MS Spectra
Sample 19a
Butanediol 1,4 diacetate





EI/MS Spectra
Sample 20a
n-octyl acetate