

Supporting Information

Generation of an abasic site in oligonucleotide by using acid-labile 1-deaza-2'-deoxyguanosine and its application to post-synthetic modification

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MATERIALS AND METHODS

General information.

Physical data were measured as follows: ^1H and ^{13}C NMR spectra were recorded on a JEOL FT-NMR spectrometer in $\text{DMSO}-d_6$ as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were detected by disappearance on the addition of D_2O . Mass spectra were recorded on JEOL JMS-FABmate (FAB-MS) at Center for Instrumental Analysis, Hokkaido University. TLC was done on Merck silica gel 60F₂₅₄ precoated plates (Merck, Germany). Silica gels used for column chromatography was ICN Silica 60Å (particle size 63–200 μm) (ICN Biomedicals, Belgium).

3-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-5-(*N,N*-di-*tert*-butyl-formamidino)amino-7-(*N,N*-diphenylcarbamoyl)oxymidazo[4,5-*b*]pyridine (**4**).

A mixture of 3-(2'-deoxy- β -D-ribofuranosyl)-5-(*N,N*-di-*tert*-butylformamidino)-amino-7-(*N,N*-diphenylcarbamoyl)oxymidazo[4,5-*b*]pyridine (**3**) (490 mg, 0.82 mmol) and dimethoxytrityl chloride (560 mg, 1.64 mmol) in pyridine (15 ml) was stirred at room temperature for 1 h. After EtOH (3.0 ml) was added to the mixture, the solvent was removed in vacuo. The residue was dissolved in AcOEt (120 ml), which was washed with aqueous NaHCO_3 (saturated, 40 ml), H_2O (40 ml \times 2), brine (40 ml), then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (3.0 \times 12 cm, 70% AcOEt in hexane) to give **4** (606 mg, 82%) as a pale yellow foam.

FAB-LRMS m/z 903 (MH^+).

FAB-HRMS Calcd. for $\text{C}_{54}\text{H}_{59}\text{N}_6\text{O}_7$ (MH^+) 903.4445, found 903.4445.

^1H NMR (270 MHz, $\text{DMSO}-d_6$) δ : 8.36 (s, 1 H), 8.29 (s, 1 H), 6.64 (s, 1 H), 7.50–7.40 (m, 8 H), 7.33–7.26 (m, 4 H), 7.22–7.14 (m, 7 H), 6.80–6.72 (m, 4 H), 6.45 (t, 1 H, J = 6.6 Hz), 5.35 (d, 1 H, J = 4.6 Hz), 4.43 (ddt, 1 H, J = 4.3, 4.6, 6.6 Hz), 3.95 (ddd, 1 H, J = 3.3, 4.3, 6.6 Hz), 3.67 (s, 3 H), 3.65 (s, 3 H), 3.43 (t, 2 H, J = 7.3 Hz), 3.25 (t, 2 H, J = 7.2 Hz), 3.22 (dd, 1 H, J = 6.6, 9.9 Hz), 3.11 (dd, 1 H, J = 3.3, 9.9 Hz), 2.85 (dt, 1 H, J = 6.6, 13.2 Hz), 2.35 (ddd, 1 H, J = 4.6, 6.6, 13.2 Hz), 1.62–1.43 (m, 4 H), 1.37–1.17 (m, 4 H), 0.92 (t, 3 H, J = 7.4 Hz), 0.85 (t, 3 H, J = 7.2 Hz).

^{13}C NMR (67.8 MHz, $\text{DMSO}-d_6$) δ : 159.52 (C), 157.59 (C), 157.54 (C), 154.42 (CH), 151.05 (C),

149.42 (C), 146.77 (C), 144.54 (C), 141.79 (C), 140.69 (CH), 135.20 (C), 135.14 (C), 129.31 (CH), 129.21 (CH), 128.88 (CH), 127.38 (CH), 127.27 (CH), 126.72 (CH), 126.48 (CH), 126.23 (CH), 123.68 (C), 112.76 (CH), 105.90 (CH), 85.37 (CH), 85.12 (C), 82.53 (CH), 70.54 (CH), 64.13 (CH₂), 54.73 (CH₃), 54.68 (CH₃), 50.43 (CH₂), 44.13 (CH₂), 38.79 (CH₂), 30.51 (CH₂), 28.57 (CH₂), 19.57 (CH₂), 19.05 (CH₂), 13.67 (CH₃), 13.46 (CH₃).

3-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-3'-O-succinyl-β-D-ribofuranosyl]-5-(N,N-di-tert-butylformamidino)amino-7-(N,N-diphenylcarbamoyl)oxyimidazo[4,5-*b*]pyridine (5**).**

A mixture of **4** (220 mg, 0.24 mmol), succinic anhydride (72 mg, 0.72 mmol), and DMAP (29 mg, 0.24 mmol) in pyridine (4.0 ml) was stirred at room temperature for 2 days. After H₂O (0.5 ml) was added, the mixture was concentrated in vacuo. The residue was dissolved in CHCl₃ (70 ml), which was washed with aqueous KH₂PO₄ (saturated, 25 ml × 2), brine (25 ml), then dried (Na₂SO₄) and concentrated in vacuo. The residue was purified by silica gel column chromatography (2.5 × 10 cm, 5% EtOH in CHCl₃) to give **5** (219 mg, 92%) as a pale yellow foam.

FAB-LRMS *m/z* 1003 (MH⁺).

FAB-HRMS Calcd. for C₅₈H₆₃N₆O₁₀ (MH⁺) 1003.4606, found 1003.4605.

¹H NMR (270 MHz, DMSO-*d*₆) δ: 8.44 (s, 1 H), 8.31 (s, 1 H), 8.30 (s, 1 H), 7.51–7.40 (m, 8 H), 7.31–7.26 (m, 4 H), 7.21–7.13 (m, 7 H), 6.78–6.72 (m, 4 H), 6.65 (s, 1 H), 6.43 (t, 1 H, *J* = 6.5 Hz), 5.57 (m, 1 H), 4.11 (ddd, 1 H, *J* = 3.6, 4.0, 6.3 Hz), 3.67 (s, 3 H), 3.65 (s, 3 H), 3.43 (t, 2 H, *J* = 7.3 Hz), 3.24 (t, 2 H, *J* = 7.2 Hz), 3.31 (dd, 1 H, *J* = 6.3, 10.2 Hz), 3.16 (dd, 1 H, *J* = 3.6, 10.2 Hz), 2.55–2.47 (m, 6 H), 1.59–1.41 (m, 4 H), 1.28 (m, 2 H), 1.18 (m, 2 H), 0.90 (t, 3 H, *J* = 7.3 Hz), 0.82 (t, 3 H, *J* = 7.3 Hz).

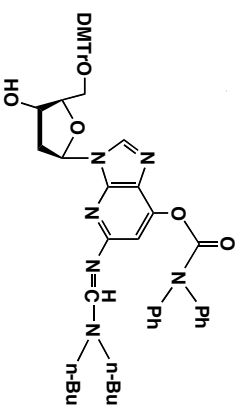
¹³C NMR (67.8 MHz, DMSO-*d*₆) δ: 172.92 (C), 171.48 (C), 159.51 (C), 157.61 (CH), 157.57 (C), 154.37 (C), 151.03 (C), 149.50 (C), 146.48 (C), 144.35 (C), 141.79 (C), 141.42 (CH), 135.10 (C), 135.02 (C), 129.22 (CH), 129.12 (CH), 128.88 (CH), 127.37 (CH), 127.20 (CH), 126.74 (CH), 126.48 (CH), 126.25 (CH), 123.93 (C), 112.75 (CH), 106.24 (CH), 85.25 (C), 83.07 (CH), 82.46 (CH), 78.94 (CH), 73.97 (CH), 63.41 (CH₂), 54.71 (CH₃), 54.68 (CH₃), 50.35 (CH₂), 44.10 (CH₂), 35.32 (CH₂), 30.51 (CH₂), 28.67 (CH₂), 28.56 (CH₂), 28.54 (CH₂), 19.57 (CH₂), 19.01 (CH₂), 13.65 (CH₃), 13.43 (CH₃).

3-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-5-(N,N-di-tert-butylformamidino)amino-7-(N,N-diphenylcarbamoyl)oxyimidazo[4,5-*b*]pyridine 3'-CPG units (6**).**

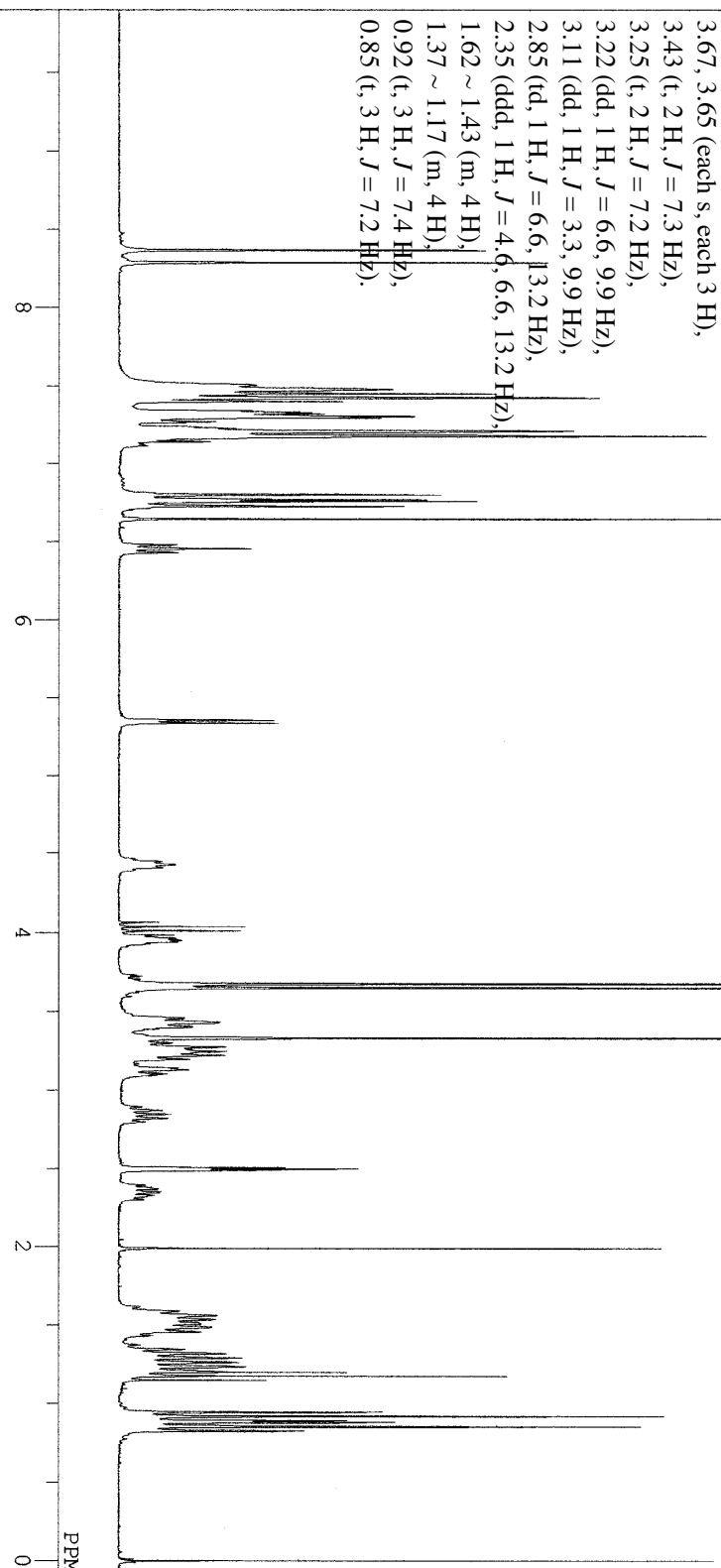
To a solution of **5** (140 mg, 0.14 mmol) and 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC, 27 mg, 0.14 mmol) in DMF (5 ml) was added CPG (CPG Inc., 400 mg) and the mixture was agitated at room temperature for 5 days. The resin was washed with DMF (5 ml × 3), pyridine (5 ml × 3), EtOH (5 ml × 3), and CH₂Cl₂ (5 ml × 3), and then dried under vacuum. To the resin was added capping solution (0.1 M DMAP in pyridine/acetic anhydride 9:1, 5 ml) and the mixture was agitated at room temperature for 2 h. The resin was washed with pyridine (5 ml × 2), EtOH (5 ml × 3), and acetone (5 ml × 3) to give **6** (32.5 μmol/g).

¹H NMR (DMSO-*d*₆) δ:

8.36, 8.29, 6.64 (each s, each 1 H),
7.50 ~ 7.40 (m, 8 H),
7.33 ~ 7.26 (m, 4 H),
7.22 ~ 7.14 (m, 7 H),
6.80 ~ 6.72 (m, 4 H),
6.45 (t, 1 H, *J* = 6.6 Hz),
5.35 (d, 1 H, *J* = 4.6 Hz),
4.43 (tdd, 1 H, *J* = 4.3, 4.6, 6.6 Hz),
3.95 (ddd, 1 H, *J* = 3.3, 4.3, 6.6 Hz),
3.67, 3.65 (each s, each 3 H),
3.43 (t, 2 H, *J* = 7.3 Hz),
3.25 (t, 2 H, *J* = 7.2 Hz),
3.22 (dd, 1 H, *J* = 6.6, 9.9 Hz),
3.11 (dd, 1 H, *J* = 3.3, 9.9 Hz),
2.85 (td, 1 H, *J* = 6.6, 13.2 Hz),
2.35 (ddd, 1 H, *J* = 4.6, 6.6, 13.2 Hz),
1.62 ~ 1.43 (m, 4 H),
1.37 ~ 1.17 (m, 4 H),
0.92 (t, 3 H, *J* = 7.4 Hz),
0.85 (t, 3 H, *J* = 7.2 Hz).

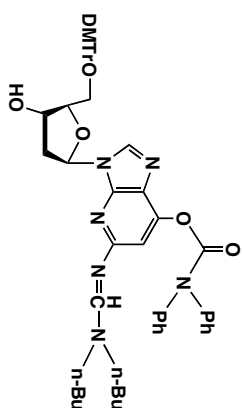


Compound 4

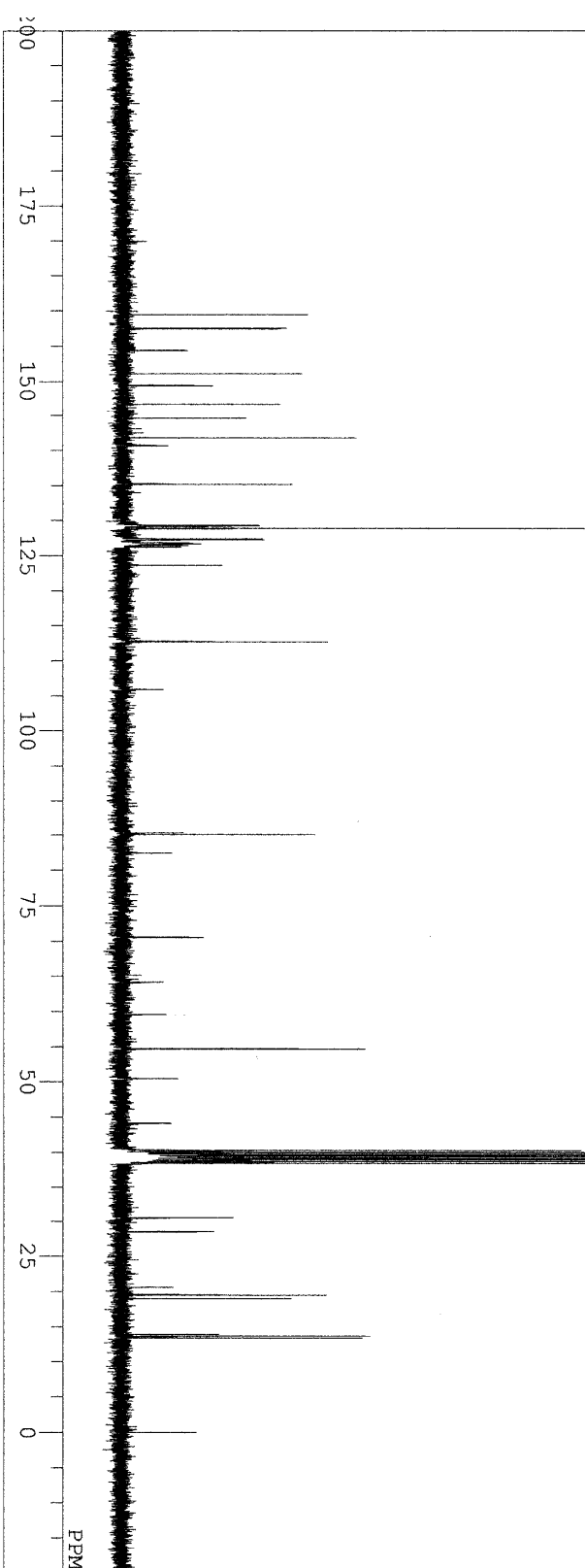


¹³C NMR (DMSO-*d*₆) δ:

159.52 (C), 157.59 (C), 157.54 (C), 154.42 (CH), 151.05 (C), 149.42 (C), 146.77 (C), 144.54 (C), 141.79 (C), 140.69 (CH), 135.20 (C), 135.14 (C), 129.31 (CH), 129.21 (CH), 128.88 (CH), 127.38 (CH), 127.27 (CH), 126.72 (CH), 126.48 (CH), 126.23 (CH), 123.68 (C), 112.76 (CH), 105.90 (CH), 85.37 (CH), 85.12 (C), 82.53 (CH), 70.54 (CH), 64.13 (CH₂), 54.73 (CH₃), 54.68 (CH₃), 50.43 (CH₂), 44.13 (CH₂), 38.79 (CH₂), 30.51 (CH₂), 28.57 (CH₂), 19.57 (CH₂), 19.05 (CH₂), 13.67 (CH₃), 13.46 (CH₃).

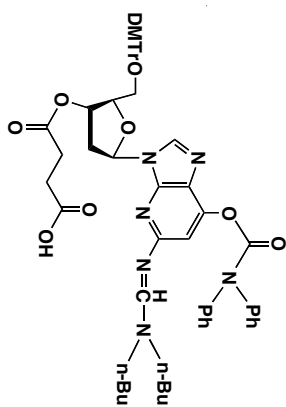


Compound 4

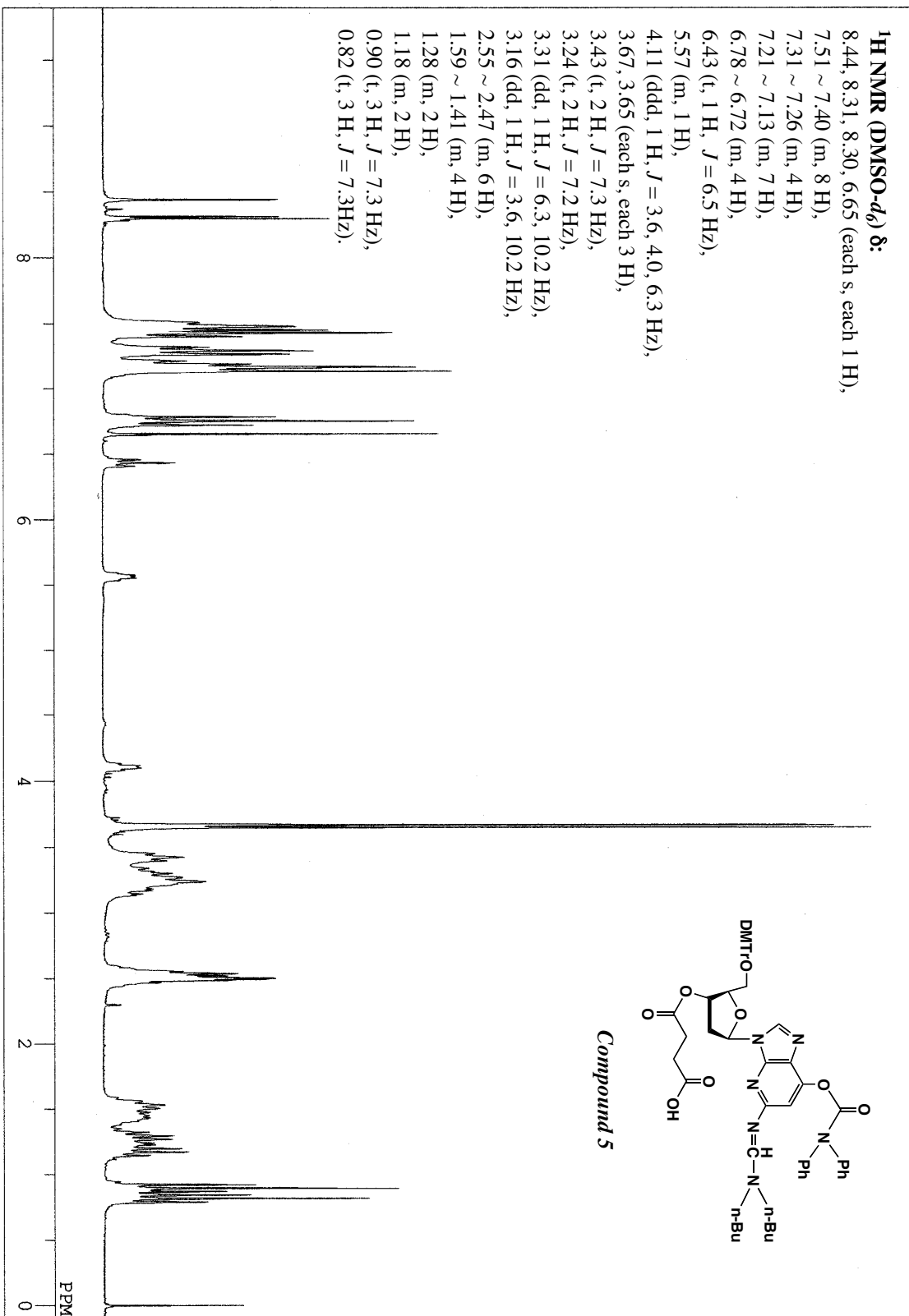


¹H NMR (DMSO-*d*₆) δ:

8.44, 8.31, 8.30, 6.65 (each s, each 1 H),
7.51 ~ 7.40 (m, 8 H),
7.31 ~ 7.26 (m, 4 H),
7.21 ~ 7.13 (m, 7 H),
6.78 ~ 6.72 (m, 4 H),
6.43 (t, 1 H, *J* = 6.5 Hz),
5.57 (m, 1 H),
4.11 (ddd, 1 H, *J* = 3.6, 4.0, 6.3 Hz),
3.67, 3.65 (each s, each 3 H),
3.43 (t, 2 H, *J* = 7.3 Hz),
3.24 (t, 2 H, *J* = 7.2 Hz),
3.31 (dd, 1 H, *J* = 6.3, 10.2 Hz),
3.16 (dd, 1 H, *J* = 3.6, 10.2 Hz),
2.55 ~ 2.47 (m, 6 H),
1.59 ~ 1.41 (m, 4 H),
1.28 (m, 2 H),
1.18 (m, 2 H),
0.90 (t, 3 H, *J* = 7.3 Hz),
0.82 (t, 3 H, *J* = 7.3 Hz).



Compound 5



¹³C NMR (DMSO-*d*₆) δ:

172.92 (C), 171.48 (C), 159.51 (C), 157.61 (CH), 157.57 (C), 154.37 (C), 151.03 (C), 149.50 (C), 146.48 (C), 144.35 (C), 141.79 (C), 141.42 (CH), 135.10 (C), 135.02 (C), 129.22 (CH), 129.12 (CH), 128.88 (CH), 127.37 (CH), 127.20 (CH), 126.74 (CH), 126.48 (CH), 126.25 (CH), 123.93 (C), 112.75 (CH), 106.24 (CH), 85.25 (C), 83.07 (CH), 82.46 (CH), 78.94 (CH), 73.97 (CH), 63.41 (CH₂), 54.71 (CH₃), 54.68 (CH₃), 50.35 (CH₂), 44.10 (CH₂), 35.32 (CH₂), 30.51 (CH₂), 28.67 (CH₂), 28.56 (CH₂), 28.54 (CH₂), 19.57 (CH₂), 19.01 (CH₂), 13.65 (CH₃), 13.43 (CH₃).

