SUPPLEMENTARY INFORMATION

Synthesis and Biological Evaluation of Unprecedented Ring-Expanded Nucleosides (RENs) Containing an Imidazo[4,5-*d*][1,2,6]oxadiazepine Ring System

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General Methods

All the reagents were obtained from commercial sources (Sigma-Aldrich) and were used without further purification. ^{1}H and ^{13}C-NMR spectra were acquired on a Varian Mercury Plus 400 MHz and on a Varian Unit Inova 700 MHz in CD_3OD or $CDCl_3$. Chemical shifts are reported in parts per million (δ) relative to the residual solvent signals: CD_2HOD 3.31 and $CHCl_3$ 7.27 for ^{1}H-NMR; CD_2HOD 49.0 and $CHCl_3$ 77.0 for ^{13}C-NMR. ^{1}H-NMR chemical shifts were assigned by 2D NMR experiments. The abbreviations s, bs, d, dd and m stand for singlet, broad singlet, doublet, doublet of doublets and multiplet, respectively. HPLC analyses and purifications were carried out on a Jasco UP-2075 Plus pump equipped with a Jasco UV-2075 Plus UV detector using a 4.60 x 150 mm LUNA (Phenomenex) silica column (particle size 5 µm) eluted with a linear gradient of MeOH in AcOEt (from 0 to 5% in 15 min, flow 1.0 mL min^{-1}, system A), with a linear gradient of AcOEt in *n*-hexane (from 0 to 100% in 30 min, flow 1.0 mL min^{-1}, system B) or using a 4.8 x 150 mm C-18 reverse-phase column (particle size 5 µm) eluted with a linear gradient of MeOH in H_2O (from 0 to 100% in 30 min, flow 1.3 mL min^{-1}, system C). UV spectra were recorded on a Jasco V-530 UV spectrophotometer. High Resolution MS spectra were recorded on a Bruker APEX II FT-ICR mass spectrometer using electrospray ionization (ESI) technique in positive mode. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 CHN analyser. IR spectra were recorded on a Jasco FT-IR 430 spectrophotometer. Optical rotations were determined on a Jasco polarimeter using a 1 dm cell at 25 °C; concentrations are in g/100 mL. Preparative PLC chromatography was performed using F254 silica gel plates (0.5 mm thick, Merck). Analytical TLC analyses were performed using F254 silica gel plates (0.2 mm thick, Merck). TLC spots were detected under UV light (254 nm). For MTS assays the UV absorbance at 490 nm was read using a Beckman Anthos 96 well Microplate Reader.

Experimental Procedures

General procedure for the preparation of compounds **3a-c**

See ref. 15 in main text.

General procedure for the preparation of compounds **4a-c**

The mixtures of diastereomers **2a-c** (0.030 mmol), prepared as previously described (ref 15a in main text) were dissolved in pyridine (0.5 mL) and stirred at 70 °C for 18 h. Pyridine was evaporated under reduced pressure and the crudes were purified on silica gel plates (developing

system: AcOEt/MeOH, 95:5). The bands were scratched from the plates and the products were eluted with AcOEt/MeOH, 7:3 (50 mL), affording compounds **4a-c**, the purity of which was checked by HPLC (system A, see General Methods).

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-ethyl nebularine N-1 oxide **4a**

Oil (15.0 mg, 0.023 mmol, 78%). $[\alpha]_D$ -14.0 (*c* =0.9 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.98 (s, 1H, H-2), 8.80 (s, 1H, H-8), 6.11 (d, *J* = 5.0 Hz, 1H, H-1'), 4.80-4.75 (m, 1H, H-2'), 4.46-4.42 (m, H-3'), 4.20-4.15 (m, 1H, H-4'), 4.05 (dd, *J* = 11.5, 4.3 Hz, H-5'$_a$), 3.87 (dd, *J* = 11.5, 2.9 Hz, H-5'$_b$), 3.37 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 1.14 (t, *J* = 7.5 Hz, 3H, CH_2CH_3), 0.97 (s, 9H, $C(CH_3)_3$), 0.96 (s, 9H, $C(CH_3)_3$), 0.81 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.15 (s, 6H, 2 x CH_3), 0.020 (s, 3H, CH_3), -0.21 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) ppm 154.5, 145.6 (two C), 141.6, 132.9, 88.3, 86.0, 76.1, 72.0, 62.5, 26.1, 25.8, 25.6, 19.8, 18.5, 18.0, 17.8, 10.3, -4.4, -4.6, -4.7, -5.2, -5.4. *m/z* 661.3620 (HRESIMS) ($[M+Na]^+$, $C_{30}H_{58}N_4NaO_5Si_3$, requires 661.3613). IR (neat) ν_{max} 2923, 1470, 1251, 1122, 834, 781 cm^{-1}; UV (MeOH) λ_{max} 226, 325 nm, shoulders 240, 267 nm.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-methyl nebularine N-1 oxide **4b**

Amorphous white solid (14.4 mg, 0.023 mmol, 78%). $[\alpha]_D$ -19.0 (*c* =0.2 ,$CHCl_3$). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.99 (s, 1H, H-2), 8.80 (s, 1H, H-8), 6.11 (d, *J* = 5.0 Hz, 1H, H-1'), 4.78-4.74 (m, 1H, H-2'), 4.45-4.40 (m, H-3'), 4.19-4.15 (m, 1H, H-4'), 4.05 (dd, *J* = 11.5, 4.3 Hz, H-5'$_a$), 3.86 (dd, *J* = 11.5, 2.8 Hz, H-5'$_b$), 2.85 (s, 3H, CH_3), 0.96 (s, 9H, $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 0.81 (s, 9H, $C(CH_3)_3$), 0.16 (s, 3H, CH_3), 0.15 (s, 6H, 2 x CH_3), 0.14 (s, 3H, CH_3), 0.020 (s, 3H, CH_3), -0.21 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 152.0, 148.5, 146.2, 144.0, 134.5, 90.0, 87.2, 77.2, 73.2, 63.5, 26.5, 26.4, 26.2, 19.3, 18.9, 18.7, 12.3, -4.1, -4.3, -4.8, -5.2, -5.3 *m/z* (HRESIMS) 647.3463 ($[M+Na]^+$, $C_{29}H_{56}N_4NaO_5Si_3$, requires 647.3456). IR (neat) ν_{max} 2927, 1475, 1248, 1126, 831, 787 cm^{-1}; UV (MeOH) λ_{max} 240, 328 nm, shoulders 226, 266 nm.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-phenyl nebularine N-1 oxide **4c**

Pale yellow oil (15.0 mg, 0.022 mmol, 75%). $[\alpha]_D$ -38.2 (*c* =1.9, CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 9.01 (s, 1H, H-2), 8.81 (s, 1H, H-8), 8.20-8.16 (m, 2H, HPh), 7.65-7.60 (m, 3H, HPh), 6.15 (d, *J* = 4.9 Hz,1H, H-1'), 4.81-4.76 (m, 1H, H-2'), 4.48-4.43 (m, 1H, H-3'), 4.21-4.16 (m, 1H, H-4'), 4.07 (dd, *J* = 11.5, 4.2 Hz, 1H, H-5'$_a$), 3.87 (dd, *J* = 11.5, 2.7 Hz, 1H, H-5'$_b$), 0.98 (s, 9H, $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 0.84 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, CH_3), 0.15 (s, 9H, 3 x CH_3), 0.050 (s, 3H, CH_3), -0.15 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 152.4, 149.3, 148.9,

147.5, 145.3, 133.6, 132.6, 132.2 (two C), 129.2 (two C), 127.8, 90.0, 87.1, 77.3, 73.1, 63.4, 26.5, 26.3, 26.2, 19.3, 18.9, 18.7, -4.1, -4.3, -4.8, -5.2, -5.3. *m/z* (HRESIMS) 709.3624 ($[M+Na]^+$, $C_{34}H_{58}N_4NaO_5Si_3$, requires 709.3613). IR (neat) ν_{max} 2950, 2928, 2851, 1253, 1157, 836, 776 cm^{-1}; UV (MeOH) λ_{max} 258, 346 nm, shoulder 300 nm.

General procedure for the preparation of compounds **6a-c**

In a flamed round bottom flask charged with dry nitrogen, **4a-c** (0.020 mmol), dissolved in dry THF (0.5 mL), were added via cannula. To the flasks, fresh Grignard reagents (0.080 mmol) were quickly added in one portion and the mixtures were stirred for 2 h (TLC monitoring: AcOEt/MeOH, 95:5) at room temperature. The reactions were quenched by addition of a 1 M solution of NH_4Cl (1 mL), diluted with AcOEt (10 mL) and extracted with brine (2 x 10 mL). The organic layers were separated, dried (Na_2SO_4), filtered and concentrated under evaporation. Compound **5a** was purified as indicated for **4a-c**, while compounds **5b-c** were used for the next reaction step without further purification. **5a-c** were dissolved CCl_4 (0.5 mL) and then ^{t}BuOOH (10 equiv.) was added in one portion. The systems were gently refluxed for 1 h (TLC monitoring: AcOEt/MeOH, 95:5) and then evaporated under reduced pressure. The crudes were purified by HPLC (system A for **6a-b**, system B for **6c**, see General Methods) affording compounds **6a-c**.

5-(*E,Z*)-Ethylideneamino-1-[2,3,5-tri-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3H-imidazo-4-(*E,Z*)-propanoxime **5a (major isomer)**

Oil (7.8 mg, 0.012 mmol, 60%). Element. Anal. Calcd. for $C_{31}H_{62}N_4O_5Si_3$ C, 56.84; H, 9.54; N, 8.55; found C, 56.81; H, 9.57; N, 8.59; ^{1}H-NMR (500 MHz; pyridine-d_5): δ 12.80 (s, 1H, NOH), 8.91 (q, J = 4.8 Hz, 1H, N=C*H*CH_3), 8.31 (s, 1H, H-2), 6.25 (d, J = 5.0 Hz, 1H, H-1'), 4.81-4.77 (m, 1H, H-2'), 4.56-4.52 (m, 1H, H-3'), 4.33-4.29 (m, 1H, H-4'), 4.07 (dd, J = 11.3, 4.1 Hz, 1H, H-5'$_a$), 3.91 (dd, J = 11.3, 2.7 Hz, 1H, H-5'$_b$), 3.35 (q, J = 7.4 Hz, 2H, CH_2CH_3), 1.93 (d, J = 4.8 Hz, 3H, N=CHC*H*$_3$), 1.38 (t, J = 7.4 Hz, 3H, CH_2CH_3), 0.98 (s, 18H, 2 x $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 0.19 (s, 3H, CH_3), 0.18 (s, 3H, CH_3), 0.17 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.13 (s, 3H, CH_3), 0.06 (s, 3H, CH_3). ^{13}C-NMR (175 MHz; pyridine-d_5) ppm 168.0, 157.4, 139.5, 133.5, 125.9, 88.8, 85.6, 77.2, 73.0, 63.6, 26.60 (two C), 26.58, 26.43 (four C), 26.38 (two C), 23.6, 21.3, 19.0, 18.8, 18.6, 12.0, -3.8, -4.1, -4.2, -4.3, -4.89, -4.93. *m/z* 654.4032 (HRESIMS) ($[M+Na]^+$, $C_{31}H_{62}N_4NaO_5Si_3$, requires 654.4028). UV (MeOH) λ_{max} 232 nm, shoulder 264 nm.

8-Ethyl-5-methyl-3-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine **6a**

Amorphous white solid (7.1 mg, 0.011 mmol, 92%). Element. Anal. Calcd. for $C_{31}H_{60}N_4O_5Si_3$ C,

57.01; H, 9.26; N, 8.58; found C, 57.02; H, 9.28; N, 8.60. $[\alpha]_D$ -37.7 (*c* = 0.3 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.72 (s, 1H, H-2), 6.10 (d, *J* = 4.9 Hz, H-1'), 4.80-4.76 (m, 1H, H-2'), 4.45-4.42 (m, 1H, H-3'), 4.19-4-14 (m, 1H, H-4'), 4.07 (dd, *J* = 11.9, 5.4 Hz, 1H, H-5'$_a$), 3.86 (dd, *J* = 11.9, 2.6 Hz, 1H, H-5'$_b$), 3.37 (q, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 2.82 (s, 3H, CH_3), 1.41 (t, *J* = 7.6 Hz, 3H, CH$_2$C*H*$_3$), 0.97 (s, 9H, C(CH$_3$)$_3$), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.82 (s, 9H, C(CH$_3$)$_3$), 0.17 (s, 3H, CH_3), 0.15 (s, 9H, 3 x CH_3), 0.019 (s, 3H, CH_3), -0.20 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 157.3, 156.2, 147.8, 143.7, 133.0, 90.3, 87.2, 77.4, 73.3, 63.7, 26.8, 26.6, 26.4, 21.3 (two C), 19.6, 19.1, 18.9, 11.1, -3.8, -4.2, -4.6, -4.9, -5.1 . *m/z* 675.3764 (HRESIMS) ($[M+Na]^+$, $C_{31}H_{60}N_4NaO_5Si_3$, requires 675.3769). IR (neat) ν_{max} 2923, 1470, 1251, 1119, 834, 773 cm^{-1}. UV (MeOH) λ_{max} 226, 324 nm.

5-Ethyl-8-methyl-3-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine **6b**

Amorphous white solid (7.2 mg, 0.011 mmol, 55% over two steps). Element. Anal. Calcd. for $C_{31}H_{60}N_4O_5Si_3$ C, 57.01; H, 9.26; N, 8.58; found C, 57.04; H, 9.29; N, 8.61. [□]$_D$ -40.2 (*c* = 0.1 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.74 (s, 1H, H-2), 6.12 (d, *J* = 5.4 Hz, H-1'), 4.86-4.81 (m, 1H, H-2'), 4.44-4.39 (m, 1H, H-3'), 4.19-4-14 (m, 1H, H-4'), 4.06 (dd, *J* = 11.5, 5.3 Hz, 1H, H-5'$_a$), 3.87 (dd, *J* = 11.5, 3.0 Hz, 1H, H-5'$_b$), 3.23 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 2.85 (s, 3H, CH_3), 1.43 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 0.97 (s, 9H, C(CH$_3$)$_3$), 0.96 (s, 9H, C(CH$_3$)$_3$), 0.80 (s, 9H, C(CH$_3$)$_3$), 0.17 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.15 (s, 3H, CH_3), 0.14 (s, 3H, CH_3), 0.0040 (s, 3H, CH_3), -0.23 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 160.2, 151.6, 147.6, 143.1, 132.9, 89.6, 86.9, 76.6, 73.2, 63.6, 27.1, 26.3, 26.2, 25.9, 19.1, 18.7, 18.5, 12.7, 10.4, -4.3, -4.5, -4.9, -5.3, -5.5. *m/z* (HRESIMS) 675.3760 ($[M+Na]^+$, $C_{31}H_{60}N_4NaO_5Si_3$, requires 675.3769). IR (neat) 2925, 1472, 1254, 1118, 835, 773□ ν_{max} cm^{-1}. UV (MeOH) λ_{max} 226, 320 nm

5-Ethyl-8-phenyl-3-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine **6c**

Oil (7.7 mg, 0.011 mmol, 54% over two steps). Element. Anal. Calcd. for $C_{36}H_{62}N_4O_5Si_3$ C, 60.46; H, 8.74; N, 7.83; found C, 60.48; H, 8.72; N, 7.81. $[\alpha]_D$ -26.3 (*c* = 0.2 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.73 (s, 1H, H-2), 8.08-8.04 (m, 2H, HPh), 7.63-7.59 (m, 3H, HPh), 6.17 (d, *J* = 5.3 Hz, H-1'), 4.87-4.84 (m, 1H, H-2'), 4.45-4.41 (m, 1H, H-3'), 4.20-4.16 (m, 1H, H-4'), 4.07 (dd, *J* = 11.3, 4.9 Hz, 1H, H-5'$_a$), 3.88 (dd, *J* = 11.3, 3.0 Hz, 1H, H-5'$_b$), 3.27 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 1.47 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 0.98 (s, 9H, C(CH$_3$)$_3$), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.82 (s, 9H, C(CH$_3$)$_3$), 0.18 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.15 (s, 6H, 2 x CH_3), 0.030 (s, 3H, CH_3), -0.18

(s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 161.4, 149.5, 148.3, 144.4, 132.3 (two C), 132.1 (two C), 129.2 (two C), 128.6, 89.8, 87.1, 76.9, 73.4, 63.7, 27.6, 26.5, 26.4, 26.2, 19.4, 18.9, 18.8, 10.7, -4.1, -4.3, -4.7, -5.1, -5.2. *m/z* 737.3942 (HRESIMS) ($[M+Na]^+$, $C_{36}H_{62}N_4NaO_5Si_3$, requires 737.3926). IR (neat) ν_{max} 2955, 2931, 2858, 1255, 1160, 1124, 1075, 836, 777 cm^{-1}.UV (MeOH) λ_{max} 252, 296, shoulder 346 nm.

General procedure for the desilylation of compounds **6a-c**. Synthesis of **7a-c**

Compounds **6a-c** (0.010 mmol) were dissolved in 1.0 mL of MeOH, and then NH_4F (0.10 mmol) was added in one portion. The systems were refluxed for 5 h (TLC monitoring: AcOEt/MeOH, 7:3). The solvent was removed under reduced pressure and the crudes were dissolved in water, filtered and then purified by HPLC (system C, see General Methods) affording compounds **7a-c**.

8-Ethyl-5-methyl-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine **7a**

Amorphous white solid (3.0 mg, 0.0097 mmol, 97%). Element. Anal. Calcd. for $C_{13}H_{18}N_4O_5$ C, 50.32; H, 5.85; N, 18.06; found C, 50.30; H, 5.83; N, 18.05. $[\alpha]_D$ -3.2 (*c* = 0.1 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.78 (s, 1H, H-2), 6.09 (d, *J* = 5.1 Hz, 1H, H-1'), 4.70-4.65 (m, 1H, H-2'), 4.38-4.34 (m, 1H, H-3'), 4.17-4.11 (m, 1H, H-4'), 3.88 (dd, *J* = 12.2, 3.0 Hz, 1H, H-5'$_a$), 3.77 (dd, *J* = 12.2, 3.4 Hz, 1H, H-5'$_b$), 3.36 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 2.81 (s, 3H, CH_3), 1.41 (t, *J* = 7.5 Hz, 3H, CH_2CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 157.1, 155.8, 148.0, 143.7, 132.8, 90.4, 87.3, 75.9, 71.8, 62.6, 21.0 (two C), 10.9. *m/z* 333.1170 (HRESIMS) ($[M+Na]^+$, $C_{13}H_{18}N_4NaO_5$, requires 333.1175). IR (neat) ν_{max} 3329, 2917, 2848, 1245, 1117 cm^{-1}. UV (MeOH) λ_{max} 226, 325 nm.

5-Ethyl-8-methyl-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine **7b**

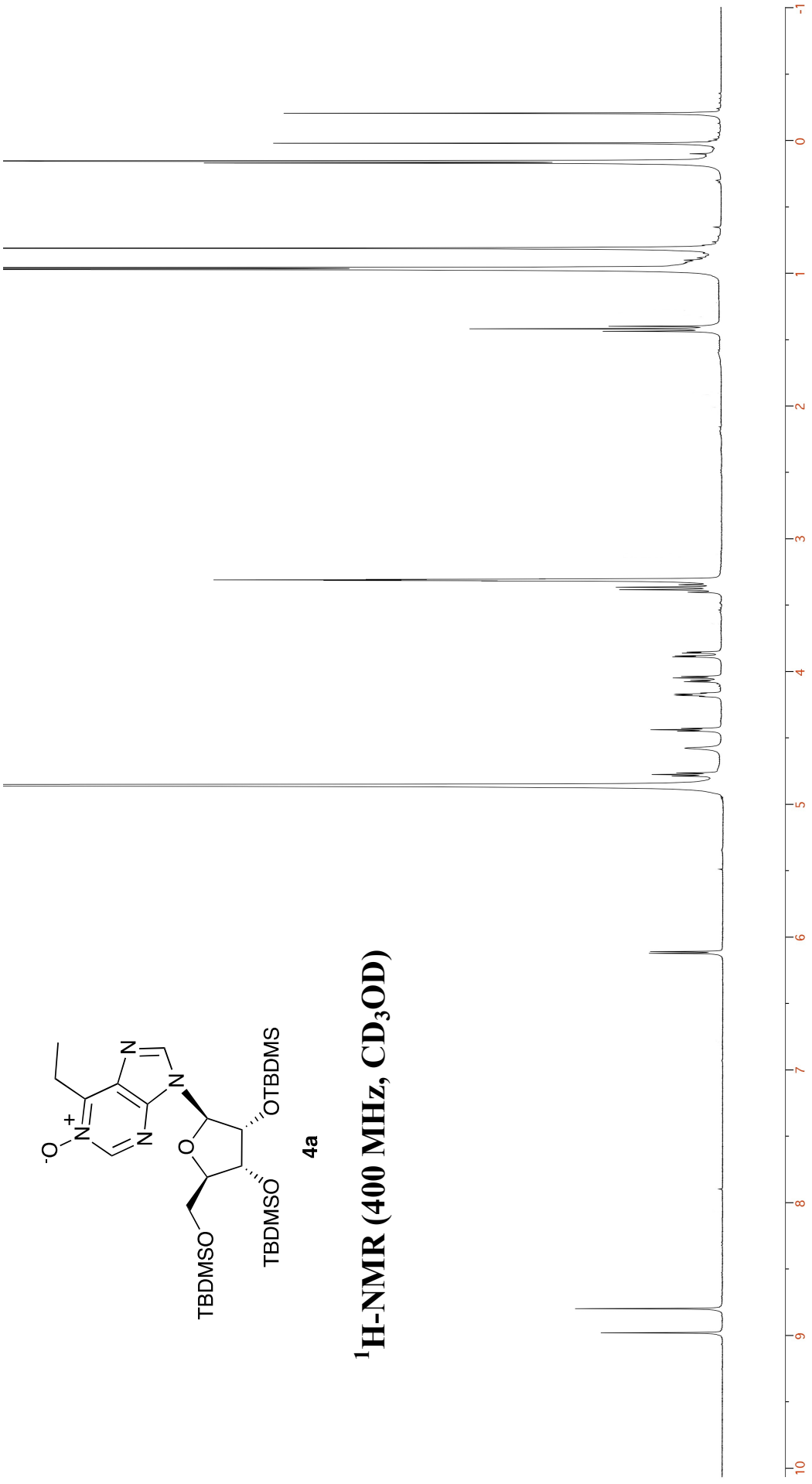
Amorphous white solid (3.0 mg, 0.0096 mmol, 96%). Element. Anal. Calcd. for $C_{13}H_{18}N_4O_5$ C, 50.32; H, 5.85; N, 18.06; found C, 50.33; H, 5.82; N, 18.04. $[\alpha]_D$ -7.9 (*c* = 0.1, CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.76 (s, 1H, H-2), 6.11 (d, *J* = 5.1 Hz, 1H, H-1'), 4.77-4.73 (m, 1H, H-2'), 4.42-4.38 (m, 1H, H-3'), 4.15-4.11 (m, 1H, H-4'), 3.87 (dd, *J* = 12.2, 3.3 Hz, 1H, H-5'$_a$), 3.77 (dd, *J* = 12.2, 3.9 Hz, 1H, H-5'$_b$), 3.21 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 2.84 (s, 3H, CH_3), 1.43 (t, *J* = 7.4 Hz, 3H, CH_2CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 160.3, 151.7, 148.1, 143.5, 133.0, 90.3, 87.1, 75.7, 71.8, 62.7, 27.2, 12.9, 10.3. *m/z* 333.1187 (HRESIMS) ($[M+Na]^+$, $C_{13}H_{18}N_4NaO_5$, requires 333.1175). IR (neat) ν_{max} 3331, 2920, 2852, 1246, 1118 cm^{-1}. UV (MeOH) λ_{max} 225, 320 nm

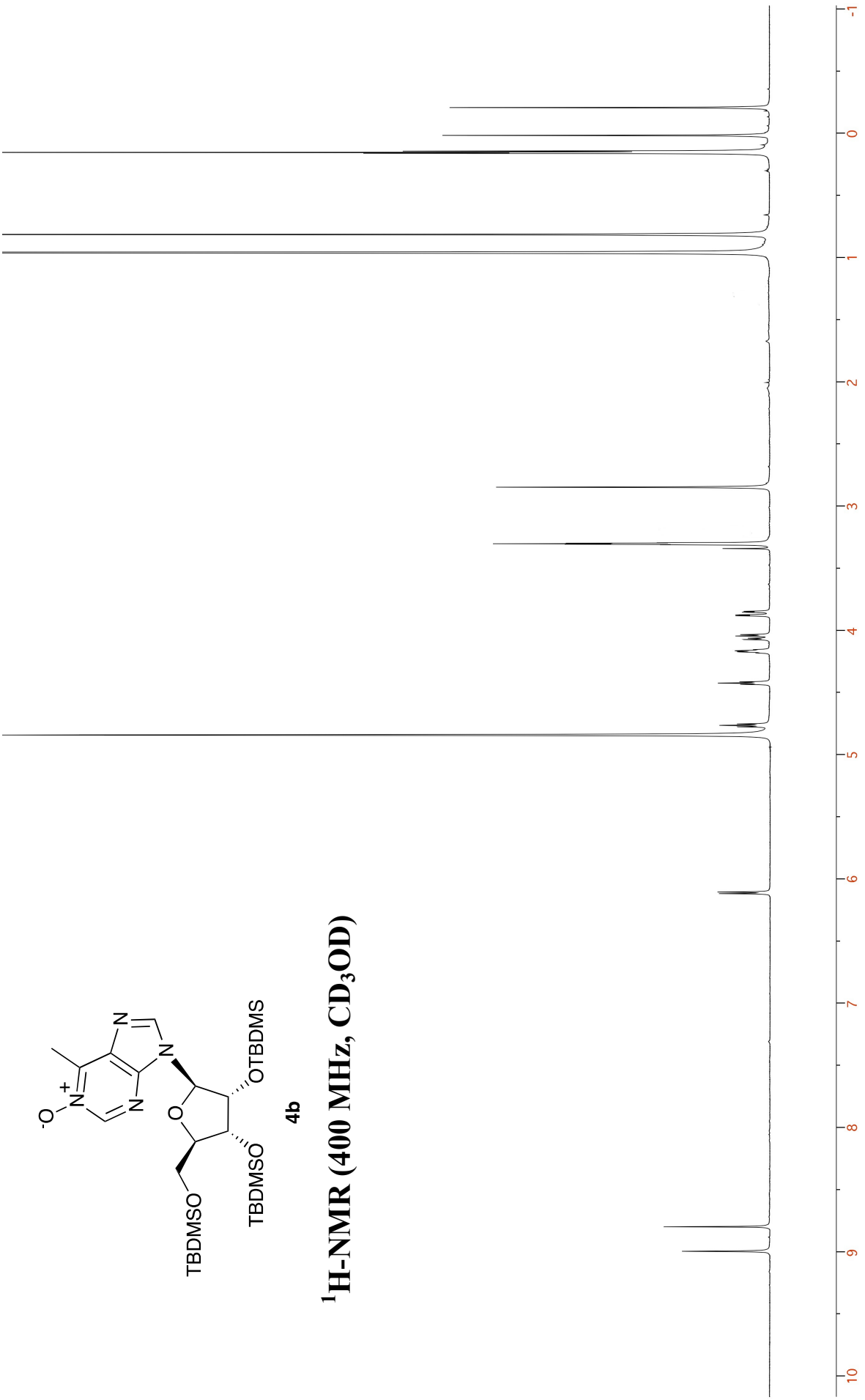
5-Ethyl-8-phenyl-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine **7c**

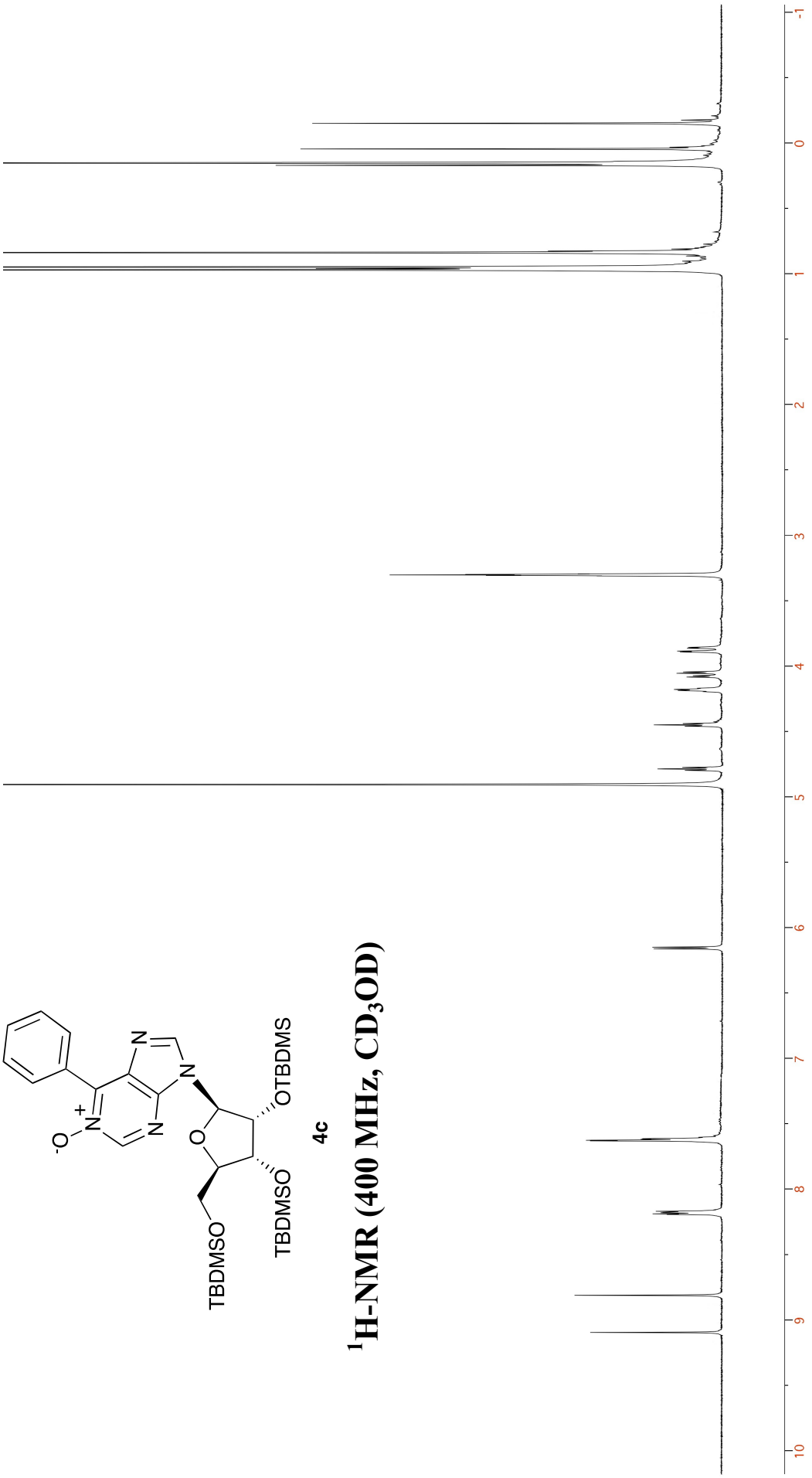
Amorphous white solid (3.5 mg, 0.0094 mmol, 94%). Element. Anal. Calcd. for $C_{18}H_{20}N_4O_5$ C, 58.06; H, 5.41; N, 15.05; found C, 58.03; H, 5.40; N, 15.07. $[\alpha]_D$ = -10.4 (*c* = 0.1 ,CH_3OH). ^{1}H-NMR (700 MHz, CD_3OD) ppm 8.78 (s, 1H, H-2), 8.08-8.05 (m, 2H, HPh), 7.61-7.59 (m, 3H, HPh), 6.16 (d, *J* = 5.0 Hz, 1H, H-1'), 4.80-4.77 (m, 1H, H-2'), 4.43-4.41 (m, 1H, H-3'), 4.16-4.13 (m, 1H, H-4'), 3.88 (dd, *J* = 12.1, 3.3 Hz, 1H, H-5'$_a$), 3.79 (dd, *J* = 12.1, 3.8 Hz, 1H, H-5'$_b$), 3.26 (q, *J* = 7.4 Hz, 2H, CH_2CH_3), 1.47 (t, *J* = 7.4 Hz, 3H, CH_2CH_3). ^{13}C-NMR (175 MHz, CD_3OD) ppm 161.3, 149.4, 148.7, 144.7, 132.3, 133.2, 132.1 (two C), 129.2 (two C), 128.8, 94.4, 87.1, 75.8, 71.9, 62.7, 27.5, 10.4. *m*/*z* 395.1323 (HRESIMS) ($[M+Na]^+$, $C_{18}H_{20}N_4NaO_5$, requires 395.1331). IR (neat) ν_{max} 3335, 2914, 2846, 1245, 1124 cm^{-1}. UV (MeOH) λ_{max} 252, 296, 342 nm.

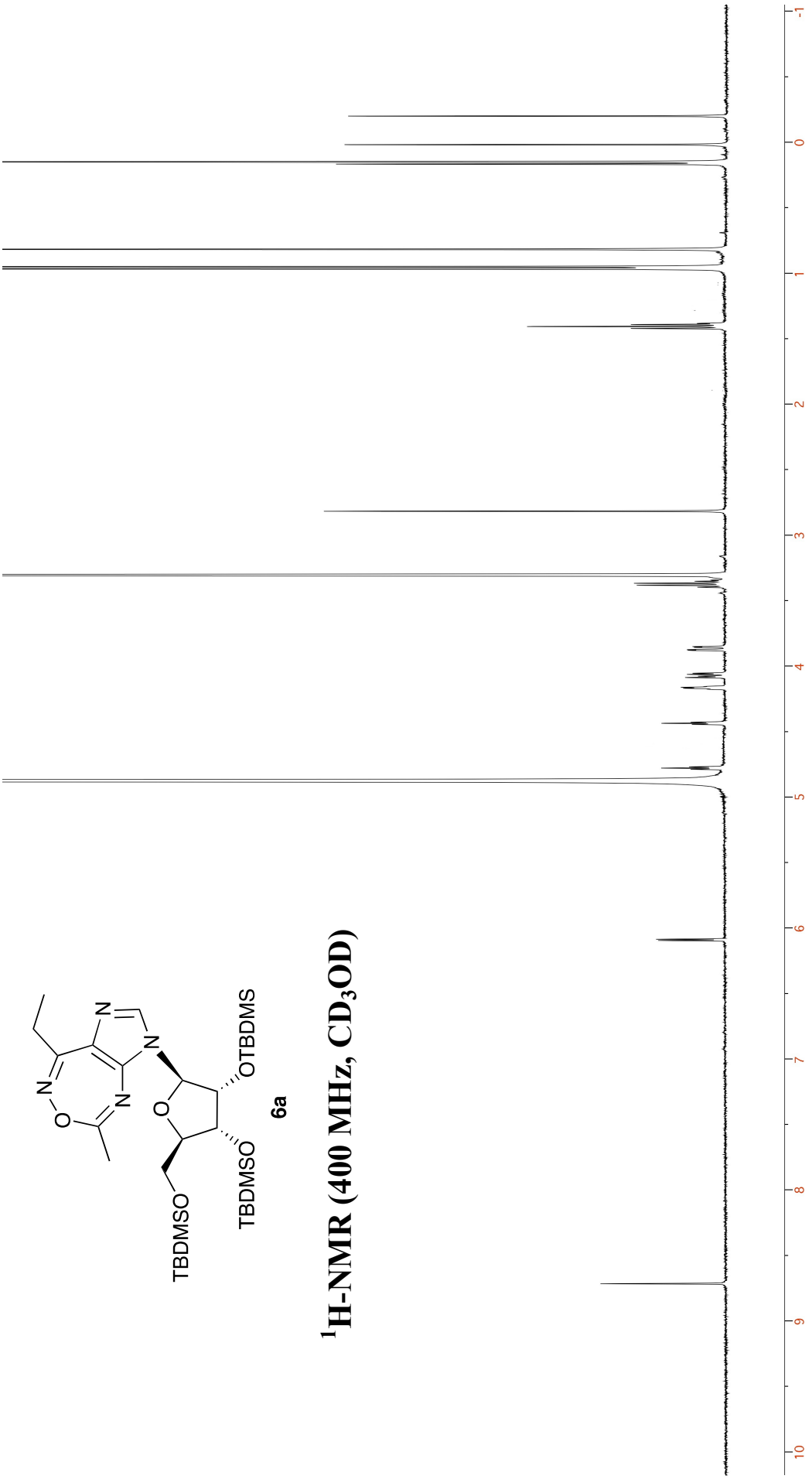
Procedures used for the MTS assays

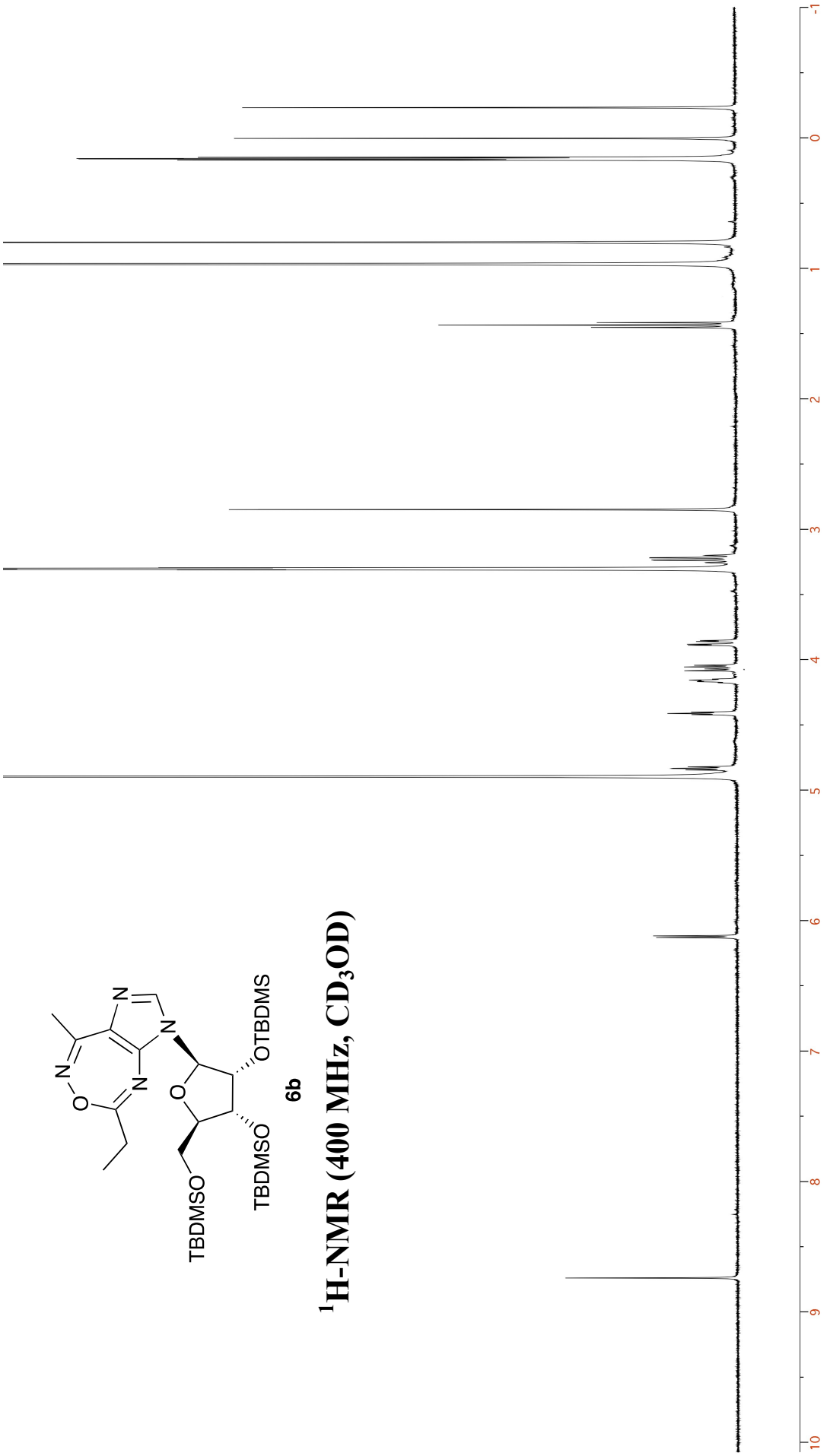
Cell viability was assessed by MTS assay as described elsewhere.[3] Breast (MCF-7 cell line) and lung (A549 cell line) cancer cells were seeded at the concentration of 0.5×10^4 cells per well on 96-well plate and maintained overnight under appropriate condition (1% sodium pyruvate MEM or DMEM, respectively, completed with 10% FCS, 2 mmol L-glutamine and 100 units/mL of penicillin. Cells in quadruplicated were than cultured in 5% FCS media containing different concentrations of tested compounds (0.1 µM, 1 µM, 10 µM and 100 µM) except for control wells that only received 5% FCS media. Every second day cells were washed with PBS and media were replaced. At the indicated time point cell viability was assessed reading the absorbance of treated and control cells at 490 nm using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega), i.e., the 3-(4,5,dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay.

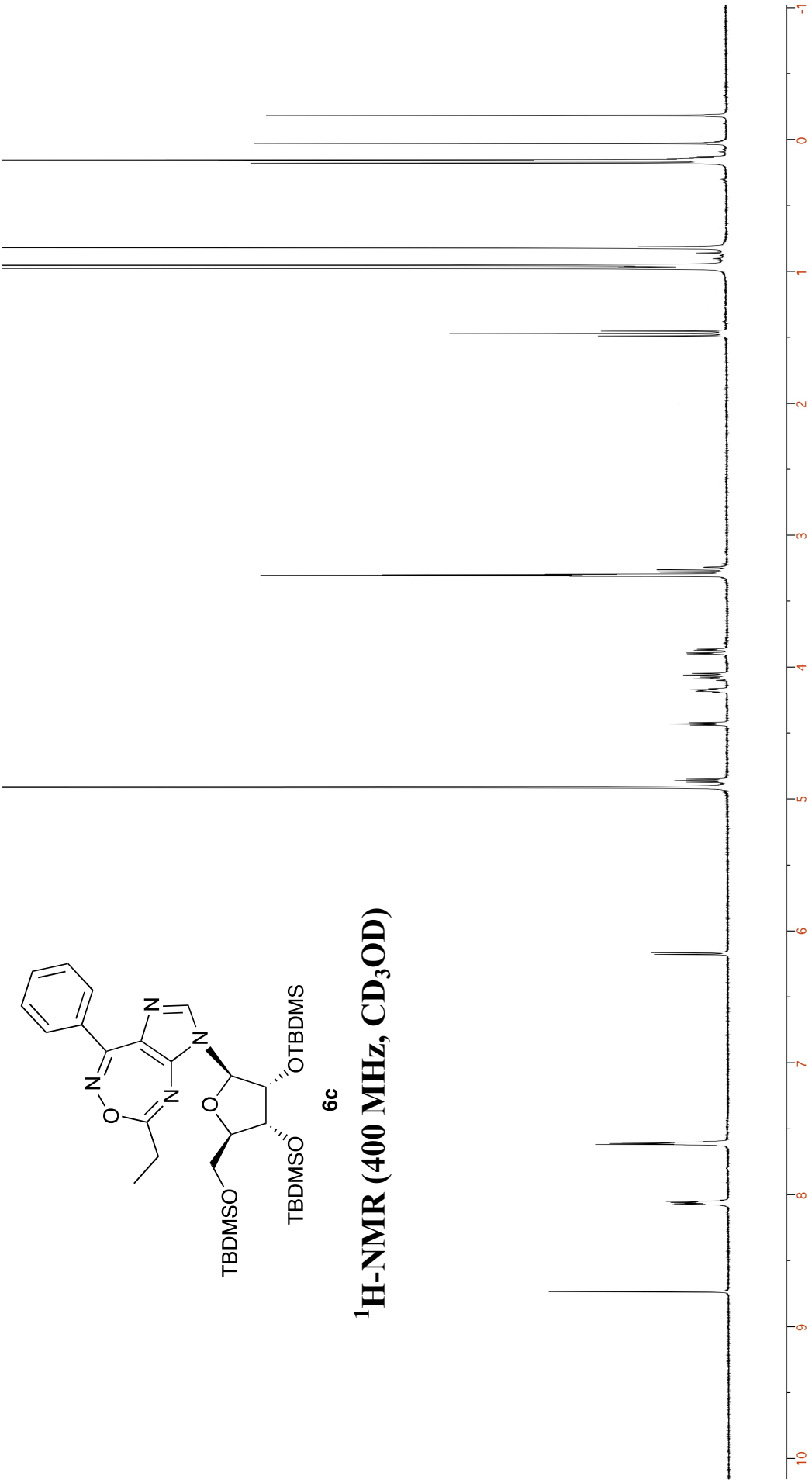

TBDMSO
TBDMSO
OTBDMS
4a
^{1}H-NMR (400 MHz, CD_3OD)

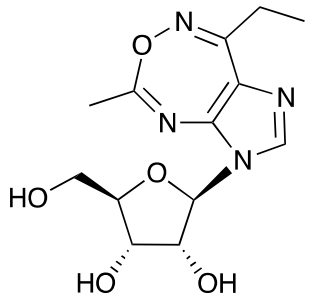

TBDMSO
TBDMSO
OTBDMS
4b
^{1}H-NMR (400 MHz, CD_3OD)


TBDMSO
TBDMSO
OTBDMS
4c
^{1}H-NMR (400 MHz, CD_3OD)


TBDMSO
TBDMSO
OTBDMS
6a
^{1}H-NMR (400 MHz, CD_3OD)

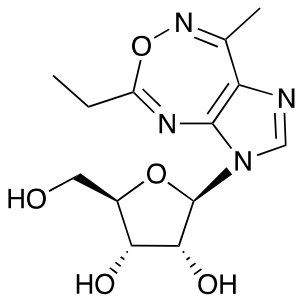

TBDMSO
TBDMSO
OTBDMS
6b
^{1}H-NMR (400 MHz, CD_3OD)


TBDMSO
OTBDMS
TBDMSO
6c
^{1}H-NMR (400 MHz, CD_3OD)



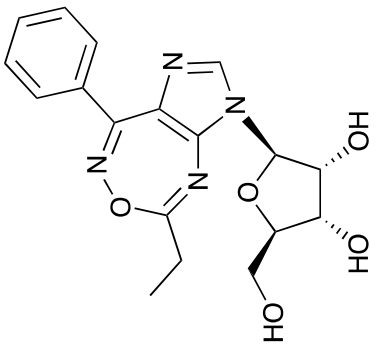
7a

^{1}H-NMR (400 MHz, CD_3OD)



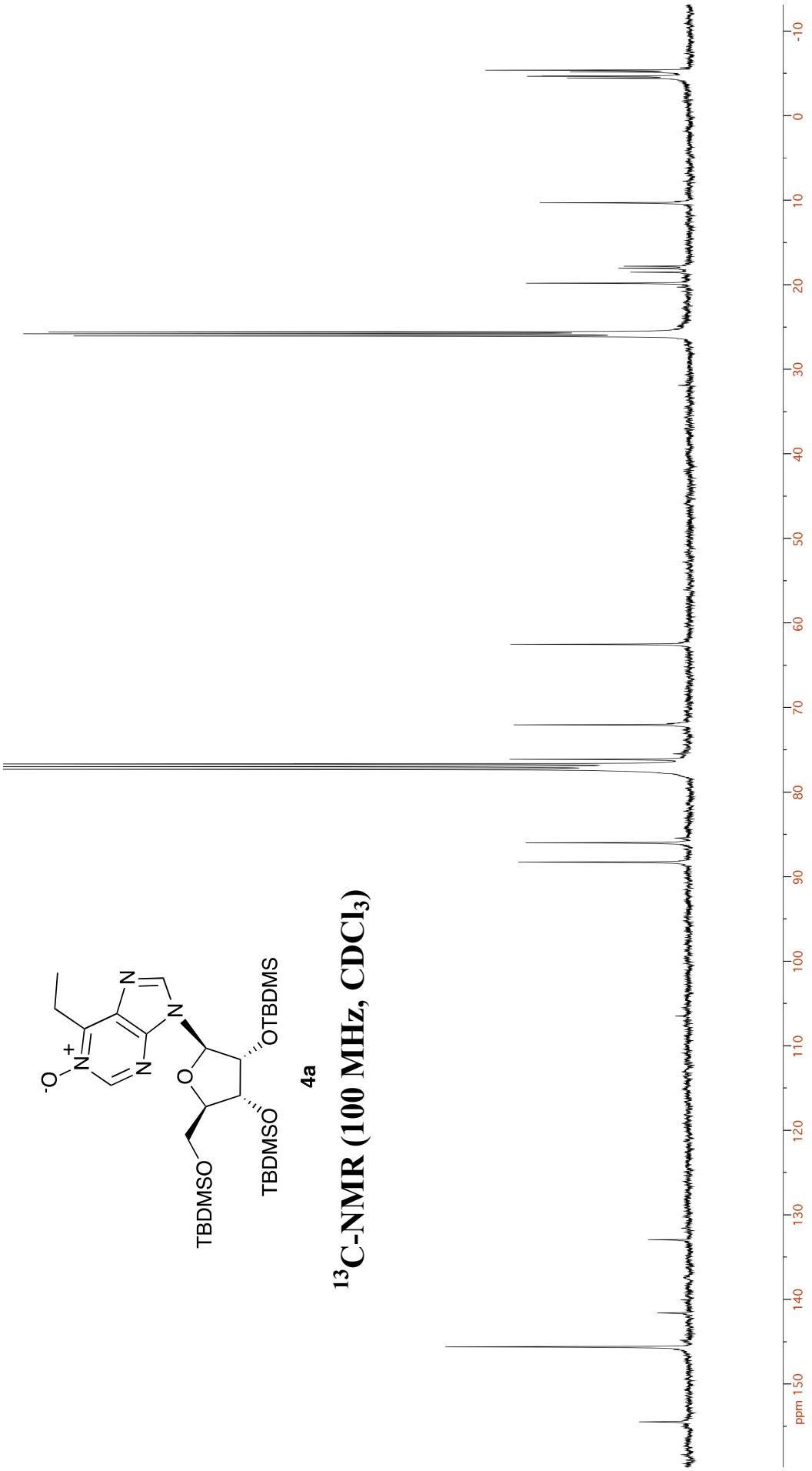
7b

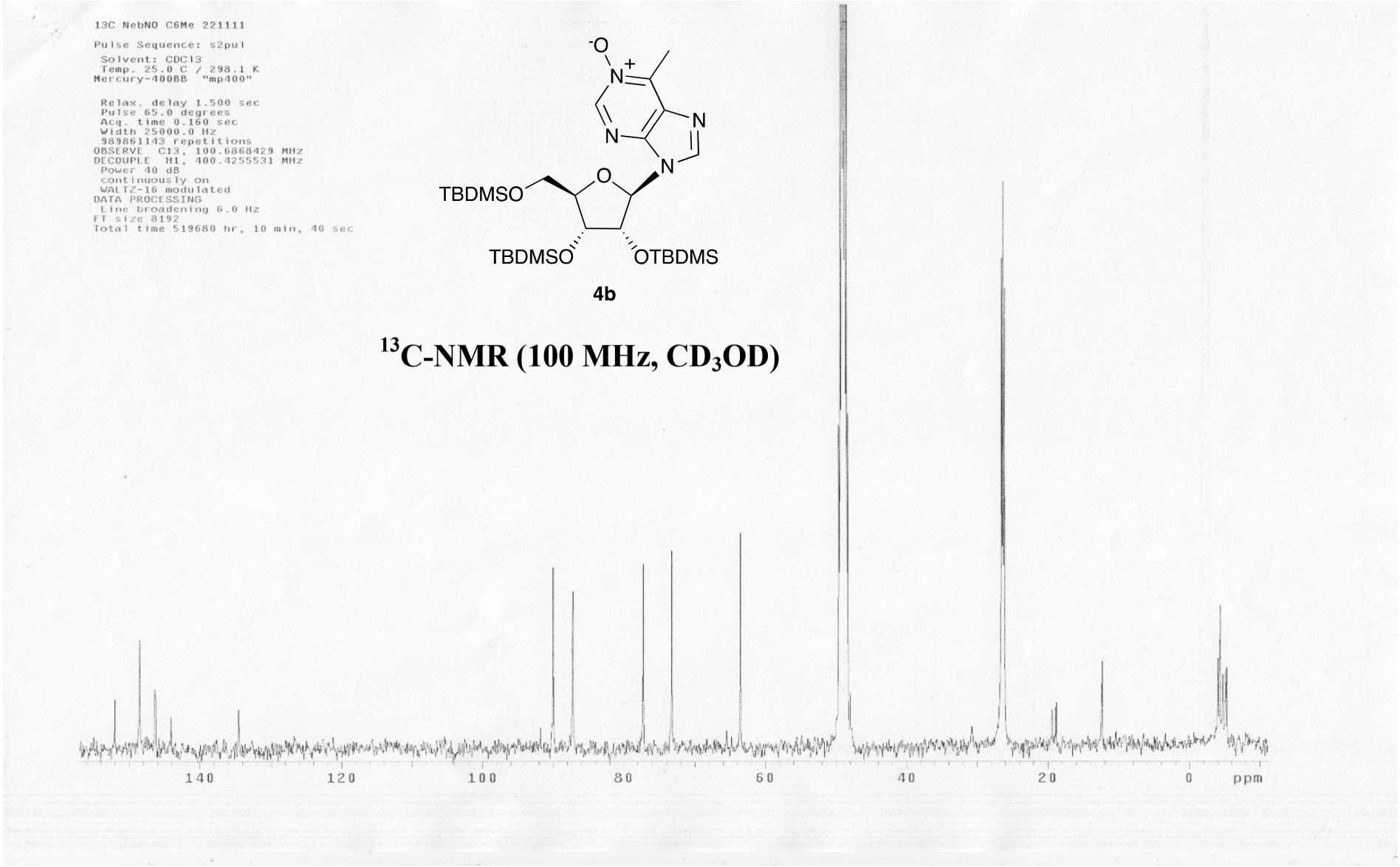
^{1}H-NMR (400 MHz, CD_3OD)

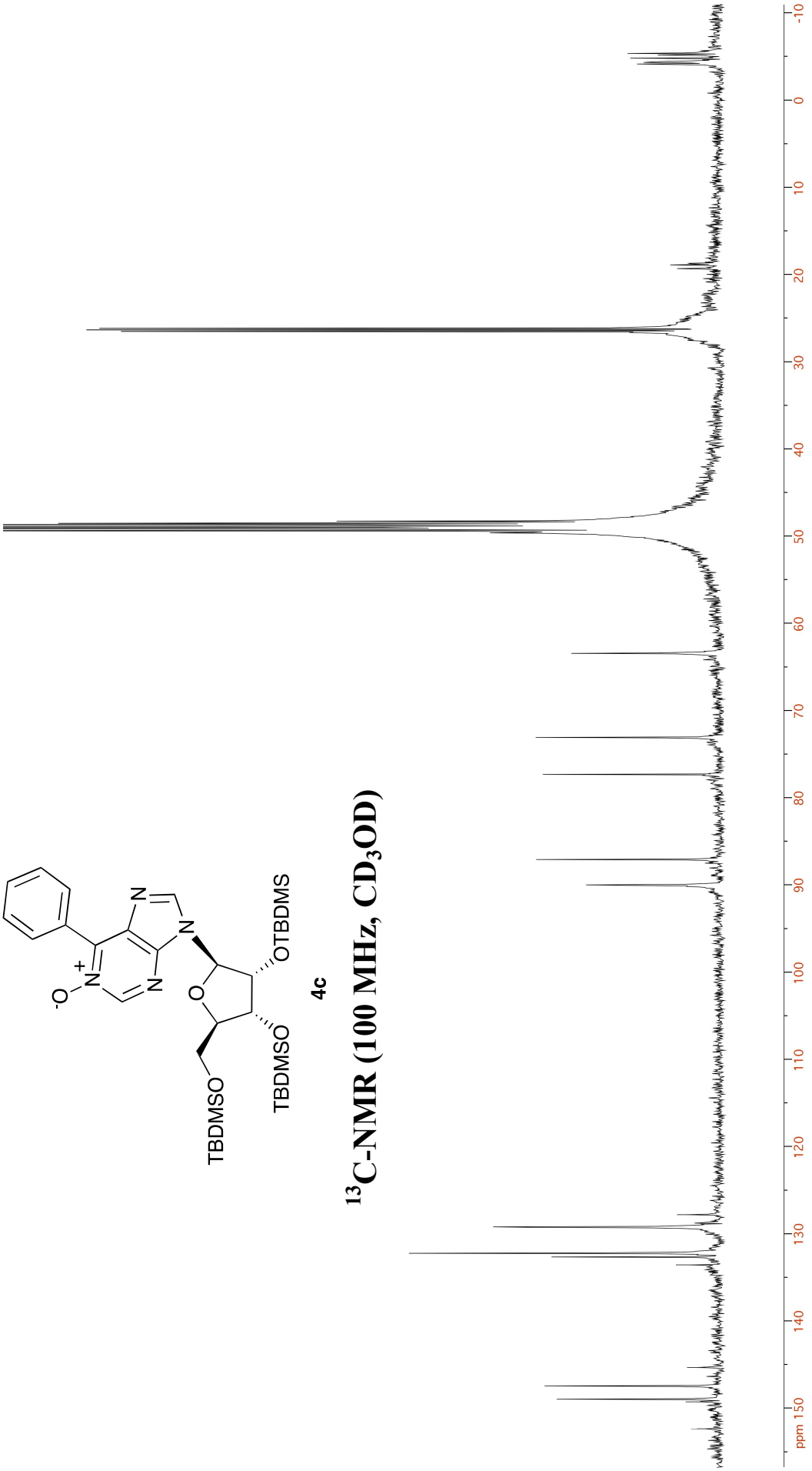


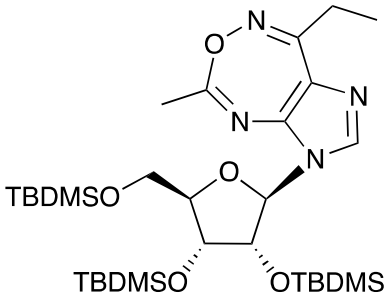
7c

^{1}H-NMR (700 MHz, CD_3OD)


TBDMSO
TBDMSO
OTBDMS
4a
^{13}C-NMR (100 MHz, CDCl$_3$)
ppm 150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

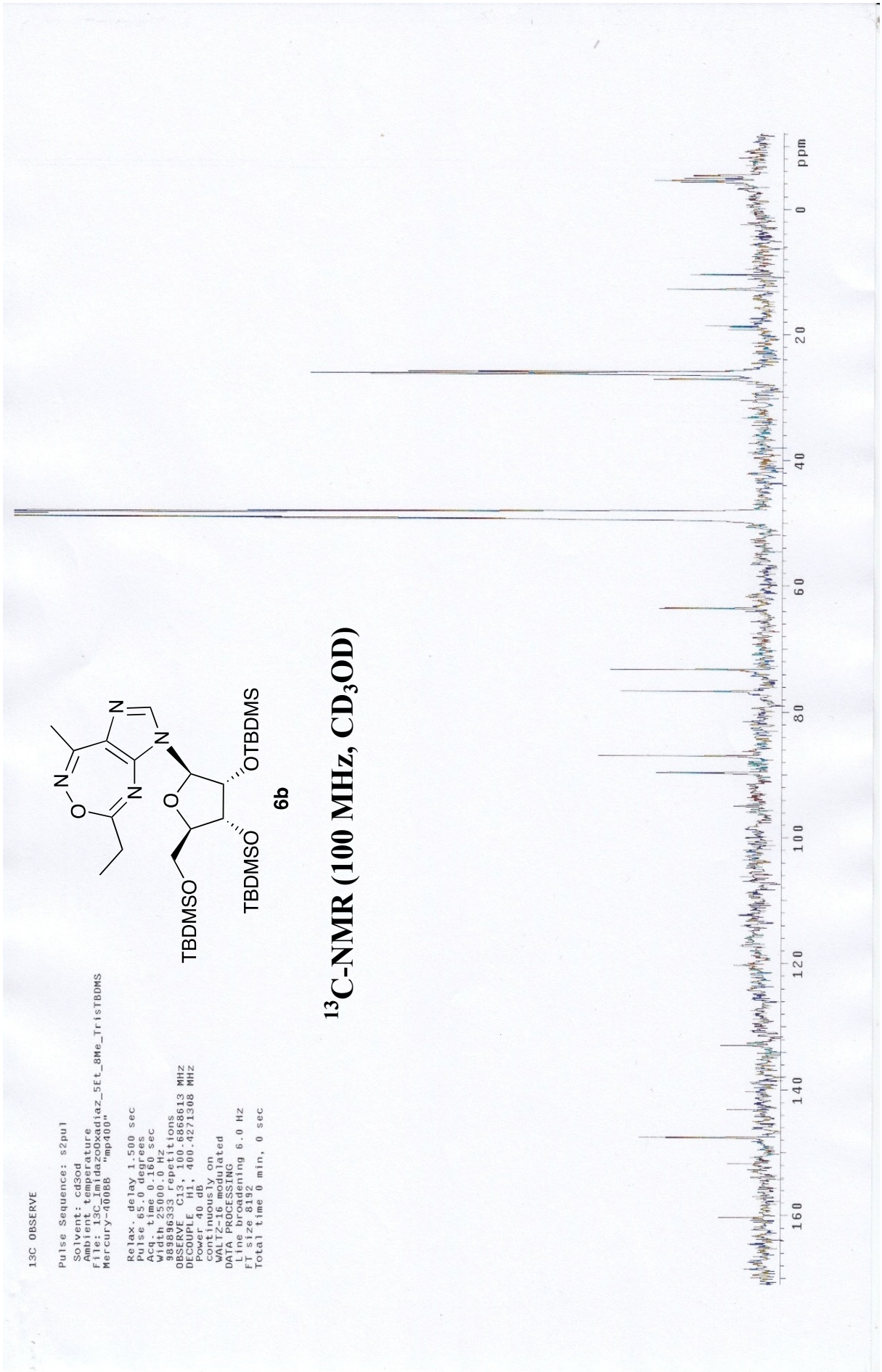


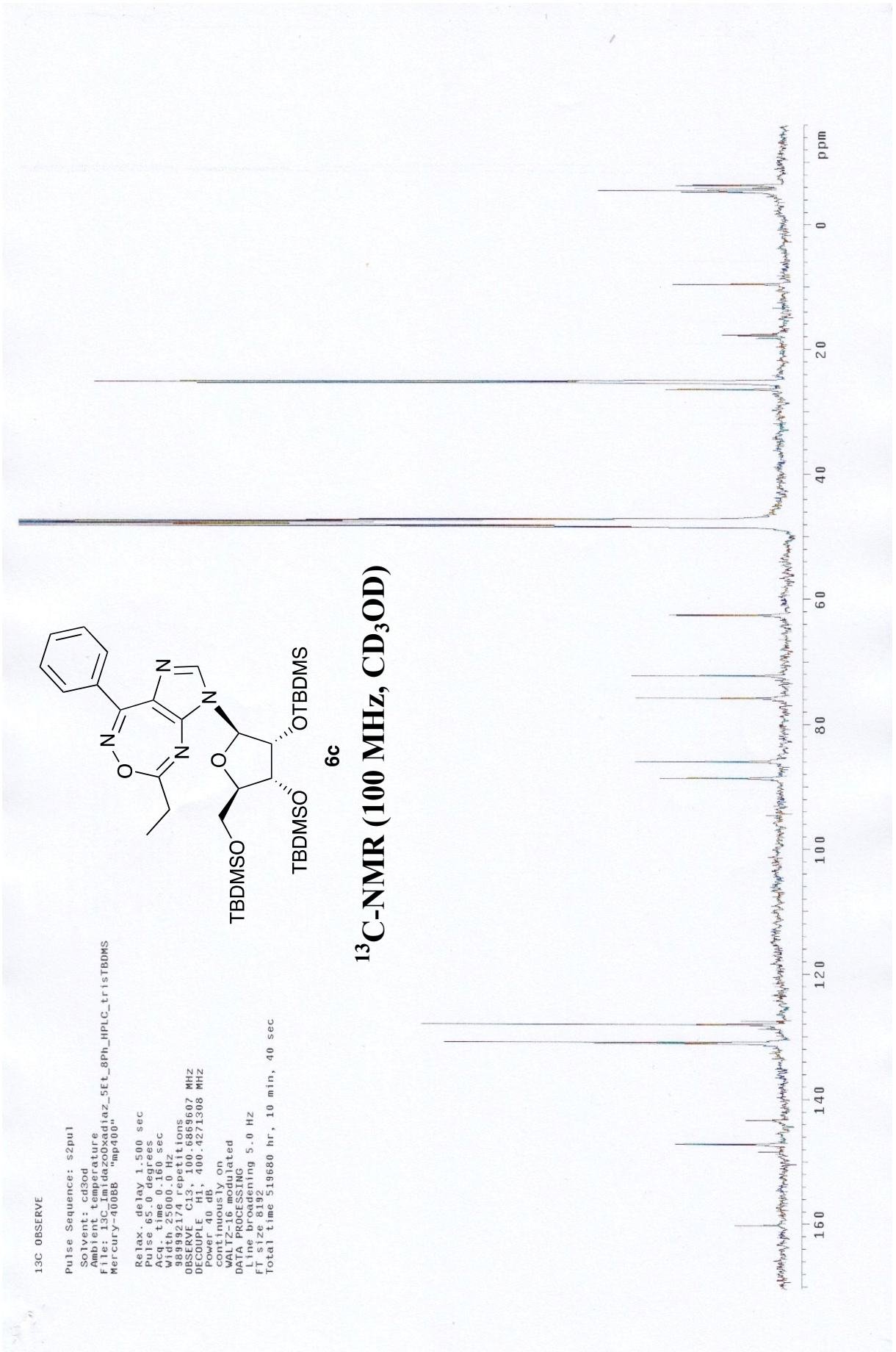

TBDMSO
TBDMSO
OTBDMS
4c
^{13}C-NMR (100 MHz, CD_3OD)
ppm 150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

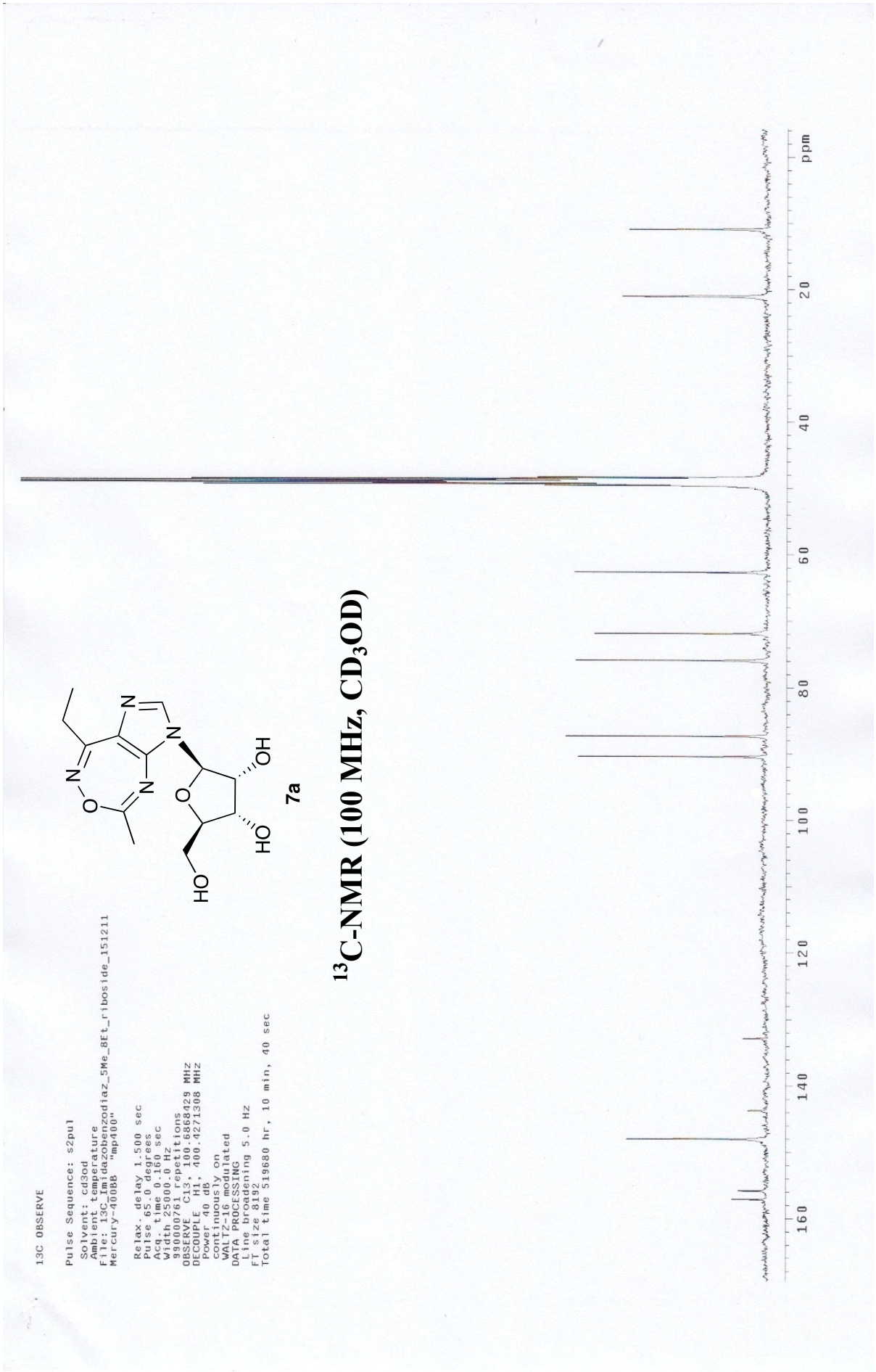


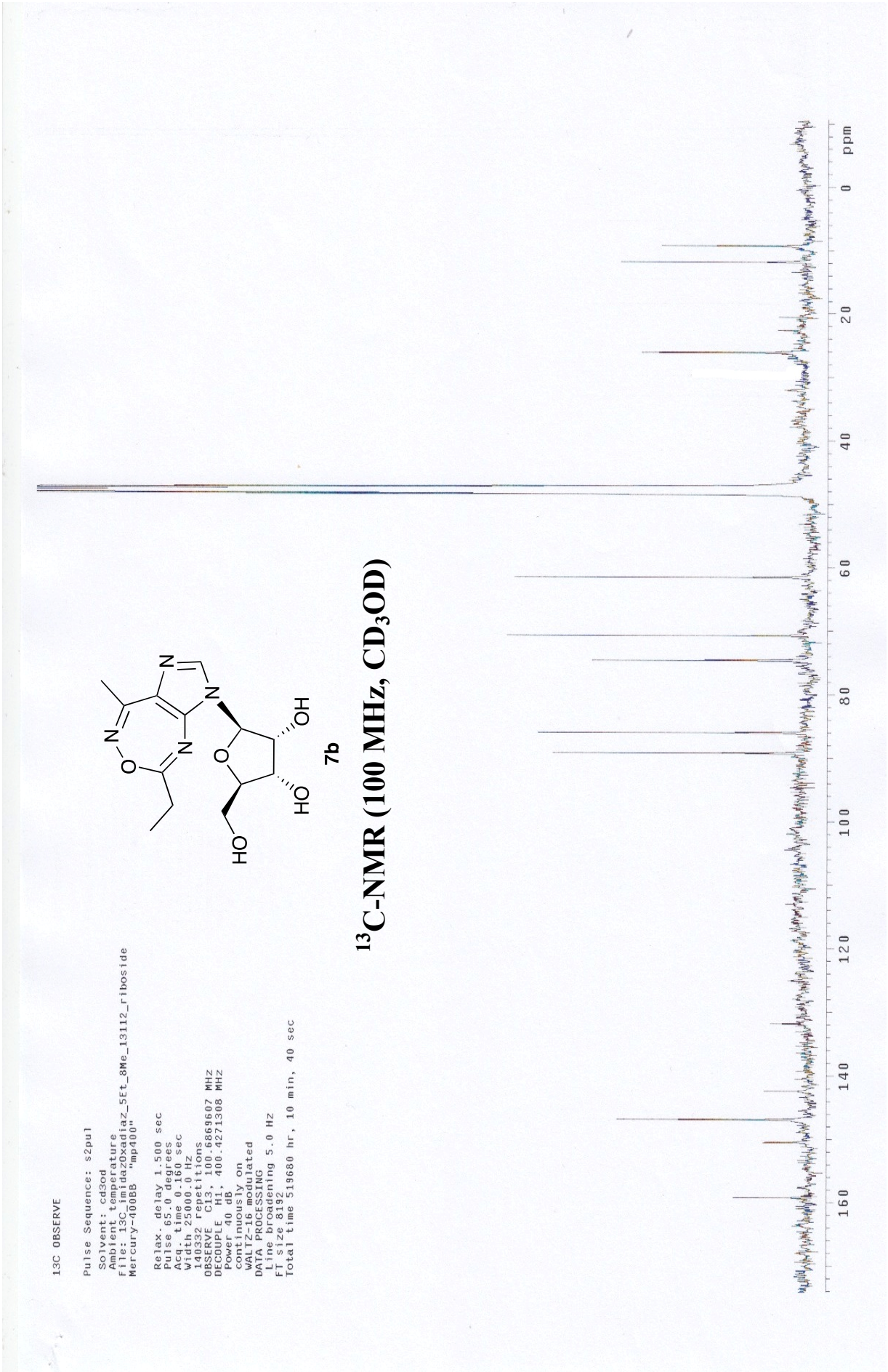
6a

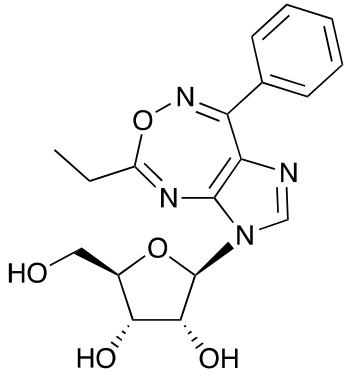
^{13}C-NMR (100 MHz, CD_3OD)


13C OBSERVE
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
File: 13C_Imidazo2Xadiaz_5Et_8Me_TrisTBDMS
Mercury-400BB "mp400"
Relax. delay 1.500 sec
Pulse 65.0 degrees
Acq. time 0.160 sec
Width 25000.0 Hz
98896333 repetitions
OBSERVE C13, 100.6868613 MHz
DECOUPLE H1, 400.4271308 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 6.0 Hz
FT size 8192
Total time 0 min, 0 sec
TBDMSO
OTBDMS
TBDMSO
6b
13C-NMR (100 MHz, CD3OD)
160
140
120
100
80
60
40
20
0
ppm


13C OBSERVE
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
File: 13C_imidazoOxadiaz_5Ft_8Ph_HPLC_trisTBDMS
Mercury-400BB "mp400"
Relax. delay 1.500 sec
Pulse 65.0 degrees
Acq. time 0.160 sec
Width 25000.0 Hz
89992174 repetitions
OBSERVE C13, 100.6809607 MHz
DECOUPLE H1, 400.4271308 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 5.0 Hz
FT size 8192
Total time 519680 hr, 10 min, 40 sec
TBDMSO
TBDMSO
OTBDMS
6c
13C-NMR (100 MHz, CD3OD)
160
140
120
100
80
60
40
20
0
ppm

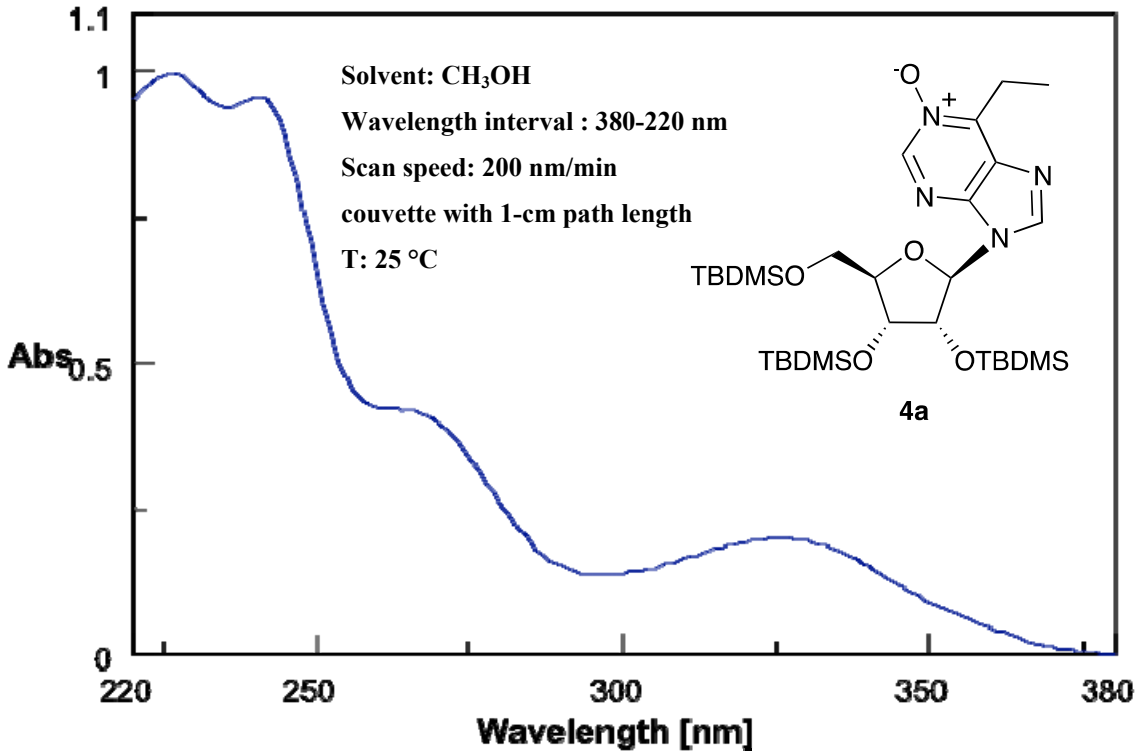

13C OBSERVE
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
File: 13C_Imidazobenzodiaz_5Me_8Et_riboside_151211
Mercury-400BB "mp400"
Relax. delay 1.500 sec
Pulse 65.0 degrees
Acq. time 0.160 sec
Width 25000.0 Hz
99000761 repetitions
OBSERVE C13, 100.6868429 MHz
DECOUPLE H1, 400.4271308 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 5.0 Hz
FT size 8192
Total time 519680 hr, 10 min, 40 sec
7a
13C-NMR (100 MHz, CD3OD)
160
140
120
100
80
60
40
20
ppm

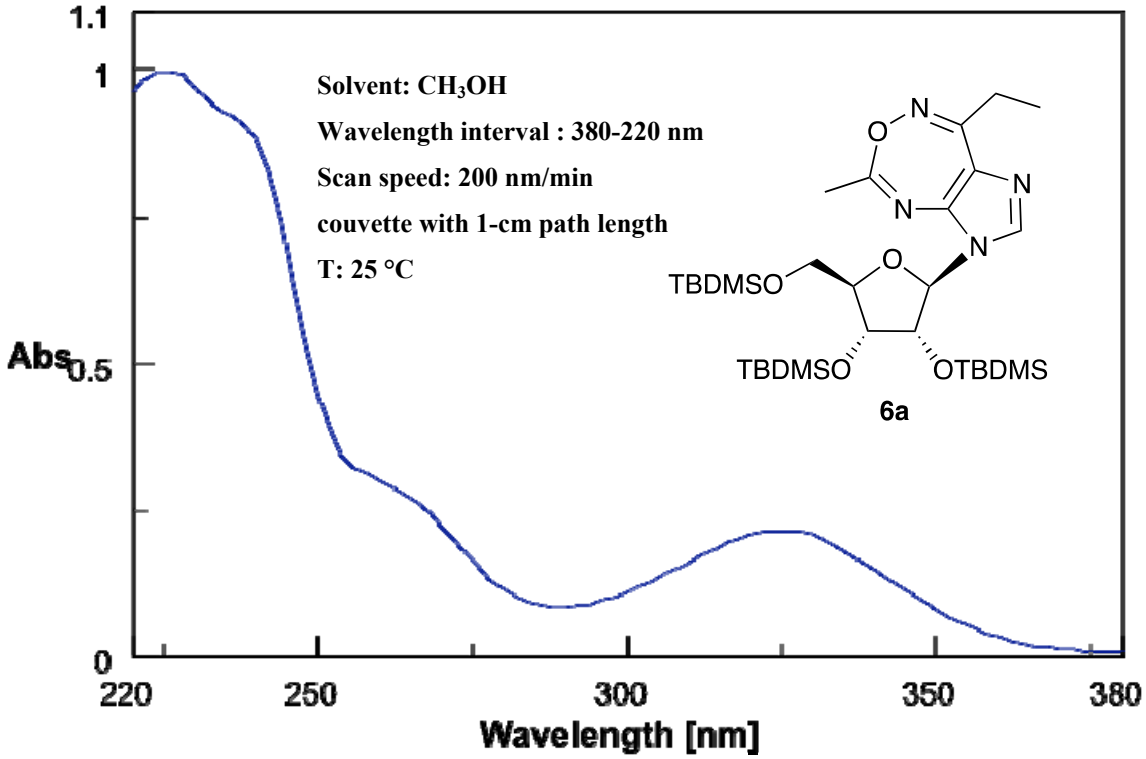

13C OBSERVE
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
File: 13C_imidazOxadiaz_5Et_8Me_13112_riboside
Mercury-400BB "mp400"
Relax. delay 1.500 sec
Pulse 65.0 degrees
Acq. time 0.160 sec
Width 25000.0 Hz
140332 repetitions
OBSERVE C13, 100.6869607 MHz
DECOUPLE H1, 400.4271308 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 5.0 Hz
FT size 8192
Total time 519680 hr, 10 min, 40 sec
N
N
N
O
N
O
HO
HO
OH
7b
13C-NMR (100 MHz, CD3OD)
160
140
120
100
80
60
40
20
0
ppm

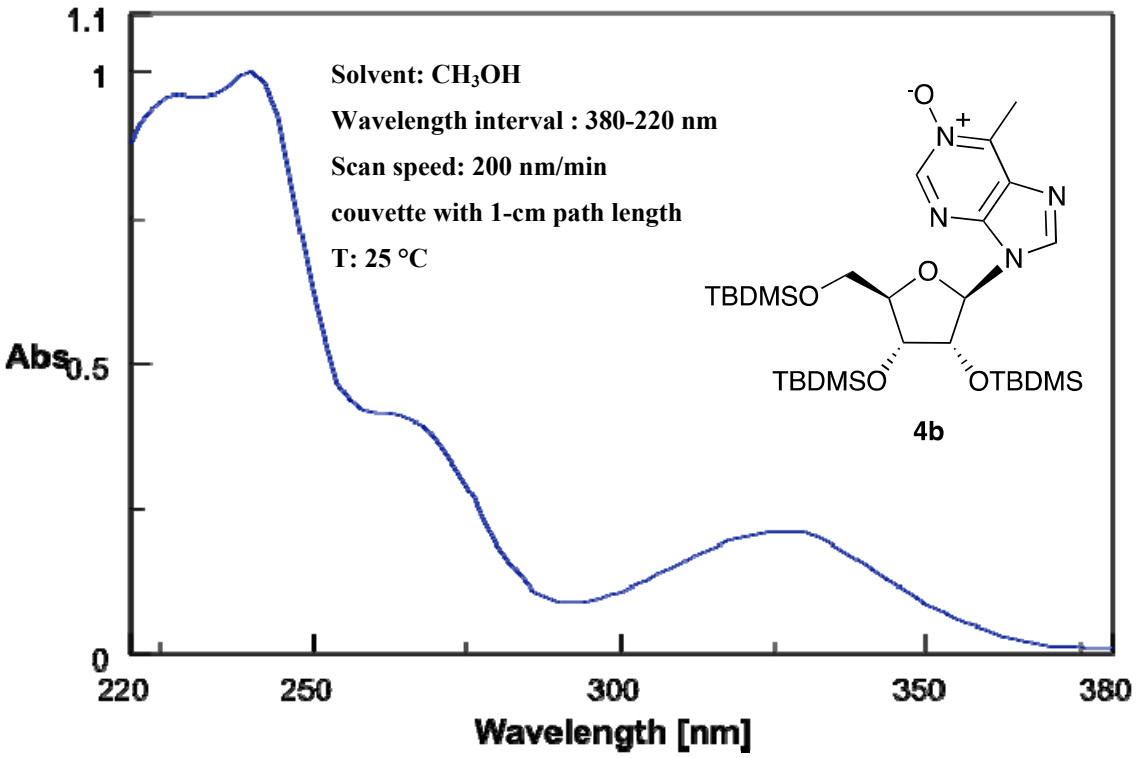


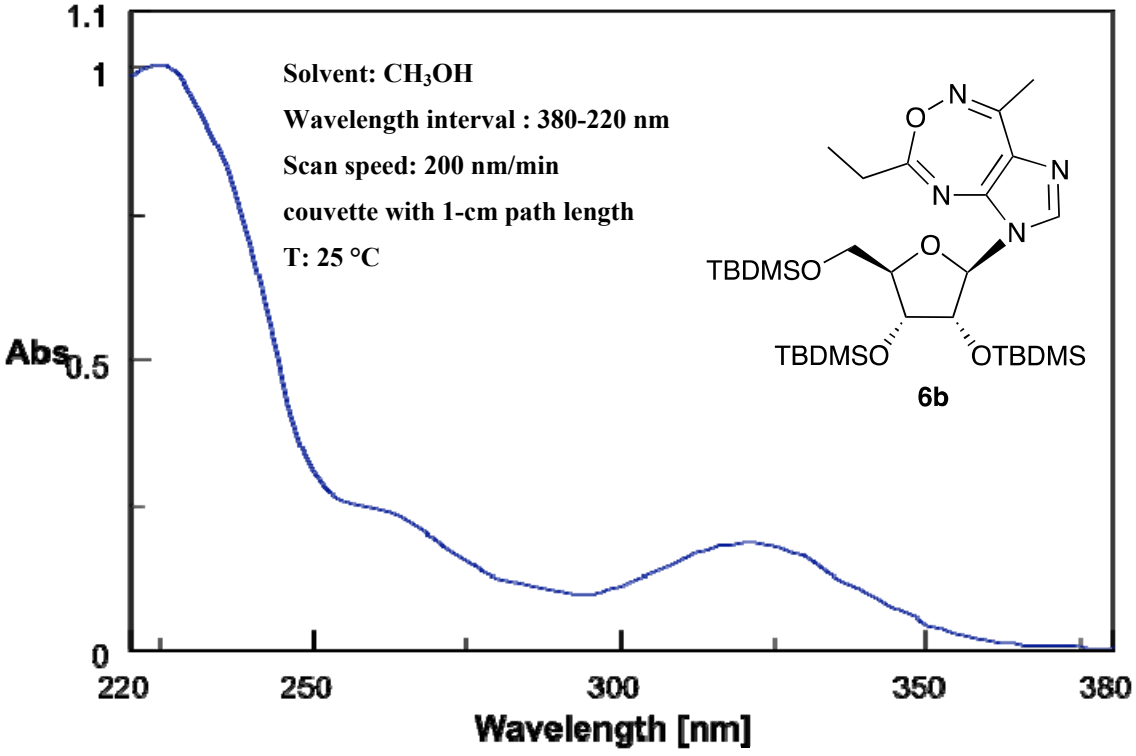
7c

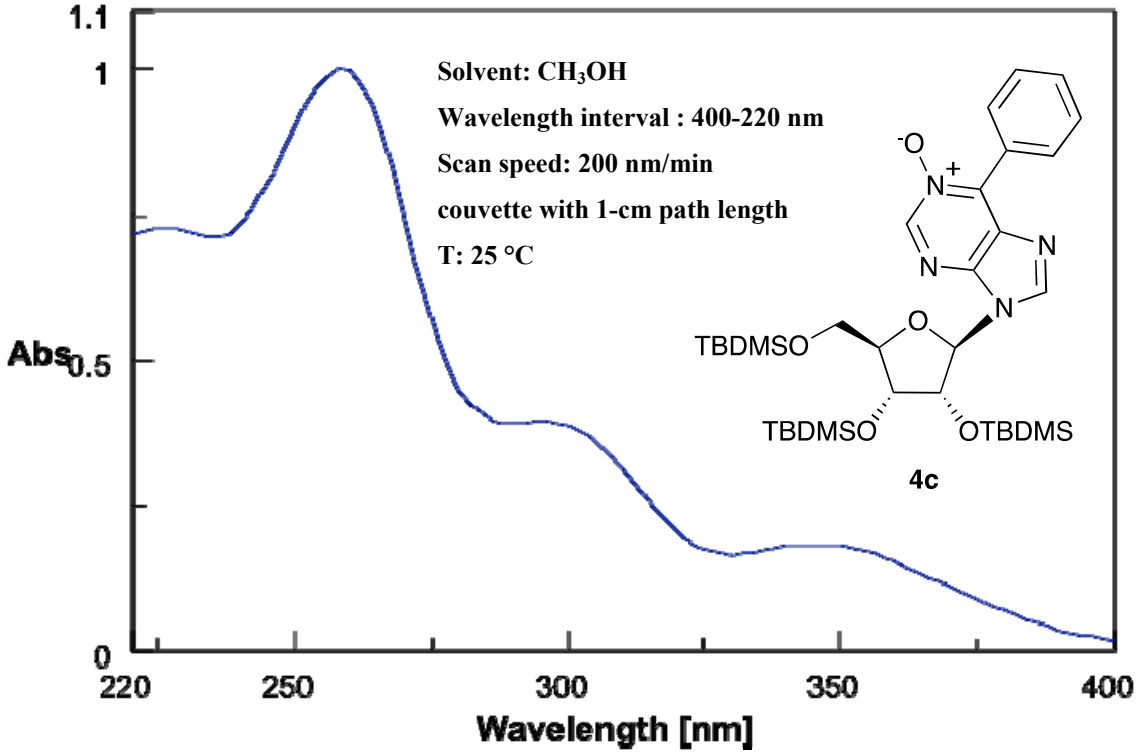
^{13}C-NMR (175 MHz, CD_3OD)

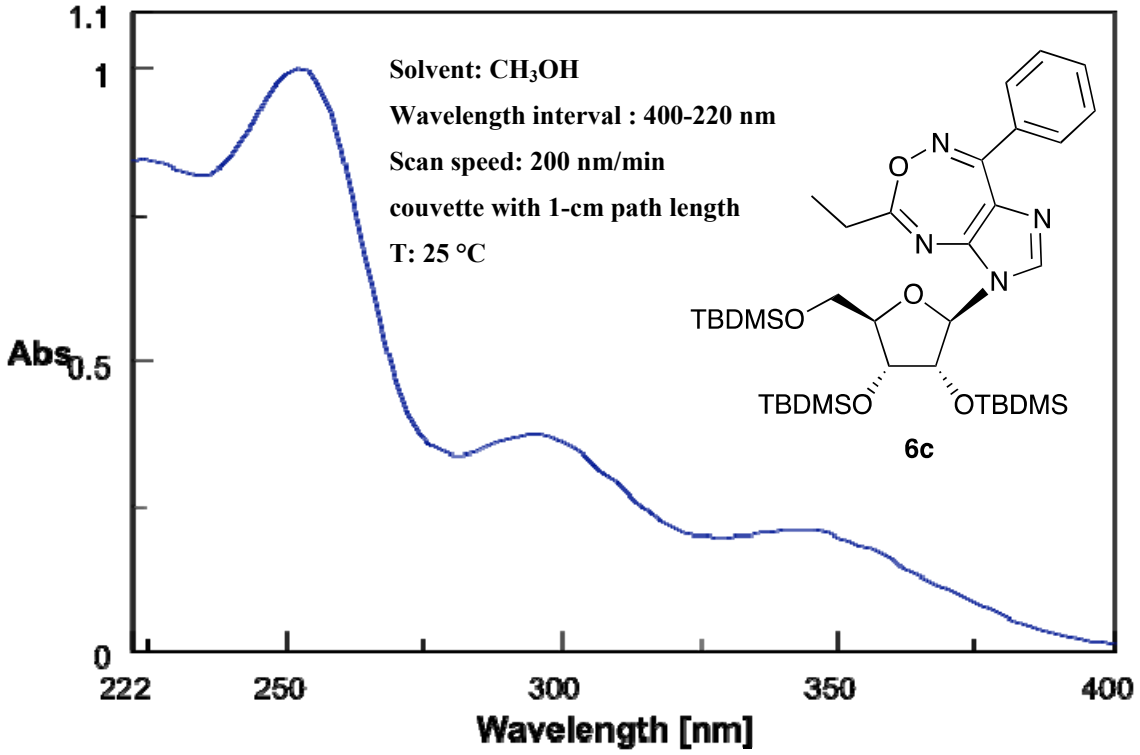





Solvent: CH3OH
Wavelength interval : 380-220 nm
Scan speed: 200 nm/min
couvette with 1-cm path length
T: 25 °C
Abs
1.1
1
0.5
0
220
250
300
350
380
Wavelength [nm]
TBDMSO
TBDMSO
OTBDMS
4b


Solvent: CH3OH
Wavelength interval : 380-220 nm
Scan speed: 200 nm/min
couvette with 1-cm path length
T: 25 °C
Abs
1.1
1
0.5
0
220
250
300
350
380
Wavelength [nm]
TBDMSO
TBDMSO
OTBDMS
6b


Solvent: CH3OH
Wavelength interval : 400-220 nm
Scan speed: 200 nm/min
couvette with 1-cm path length
T: 25 °C
Abs
1.1
1
0.5
0
220
250
300
350
400
Wavelength [nm]
TBDMSO
TBDMSO
OTBDMS
4c


Solvent: CH3OH
Wavelength interval : 400-220 nm
Scan speed: 200 nm/min
couvette with 1-cm path length
T: 25 °C
Abs
1.1
1
0.5
0
222
250
300
350
400
Wavelength [nm]
TBDMSO
TBDMSO
OTBDMS
6c

Antitumor activity of compounds 7a-c

MCF-7 breast cancer cell line and A549 lung cancer cell line were treated with different concentrations (0.1 μM, 1 μM, 10 μM and 100 μM) of novel RENs **7a-c**. Cisplatin was used as control since its activity in these cell lines has been extensively studied.[1,2] Cell viability was assessed by measuring the mitochondrial activity at day 1, 3 or 7 (MTS assay) *in vitro*.[3] We found that all tested RENs increased significantly the mitochondrial activity of treated MCF-7 cells at day 1 and 3, with the exception of **7b** that resulted inactive at day 1 (Fig. S1, panels A and B). This phenomenon is often reported as "starving state" leading up to cell death at later time points and/or at higher concentrations. Accordingly, at day 7 RENs **7b** and **7c** increased the mitochondrial activity at concentrations up to 10 μM, while at 100 μM they showed 38% and 40% cell death, respectively (Fig. 1, panel C). At the same time point **7a** increased the mitochondrial activity at 0.1 μM and 1 μM, but showed 16% cell killing effect already at 10 μM and 55% cell death at 100 μM. It is noteworthy that at day 7 at 10 μM compound **7a** proved to be as cytotoxic as cisplatin (16% and 15% cell death, respectively). **7a-c** resulted less active in A549 cell line at almost all tested conditions (Fig. 2, panels A-C) and, in agreement with the "starving state" hypothesis, we did not observe any increased mitochondrial activity at day 3 and 7. The most active compound in A549 cell line was **7a** that showed 21% cell death at day 7 at 10 μM. All together these data showed that the new synthesized compounds possess some degree of cytotoxic activity, whose manifestation in some cases was slower than the control. Further and more specific experiments would have to be performed in order to fully clarify the reason for such phenomenon. However, at this point we can speculate that only active metabolites formed around day 7 were capable to kill cells.

[1] C. W. Yde, O. G. Issinger, *Int. J. Oncol.*, 2006, **29(6)**, 1397.
[2] W. G. Telford, J. Bradford, W. Godfrey, R. W. Robey, S. E. Bates, *Stem Cells*, 2007, **24**, 1029.
[3] A. H. Cory, T. C. Owen, J. A. Barltrop, J. G. Cory, *Cancer Commun.*, 1991, **3**, 207.

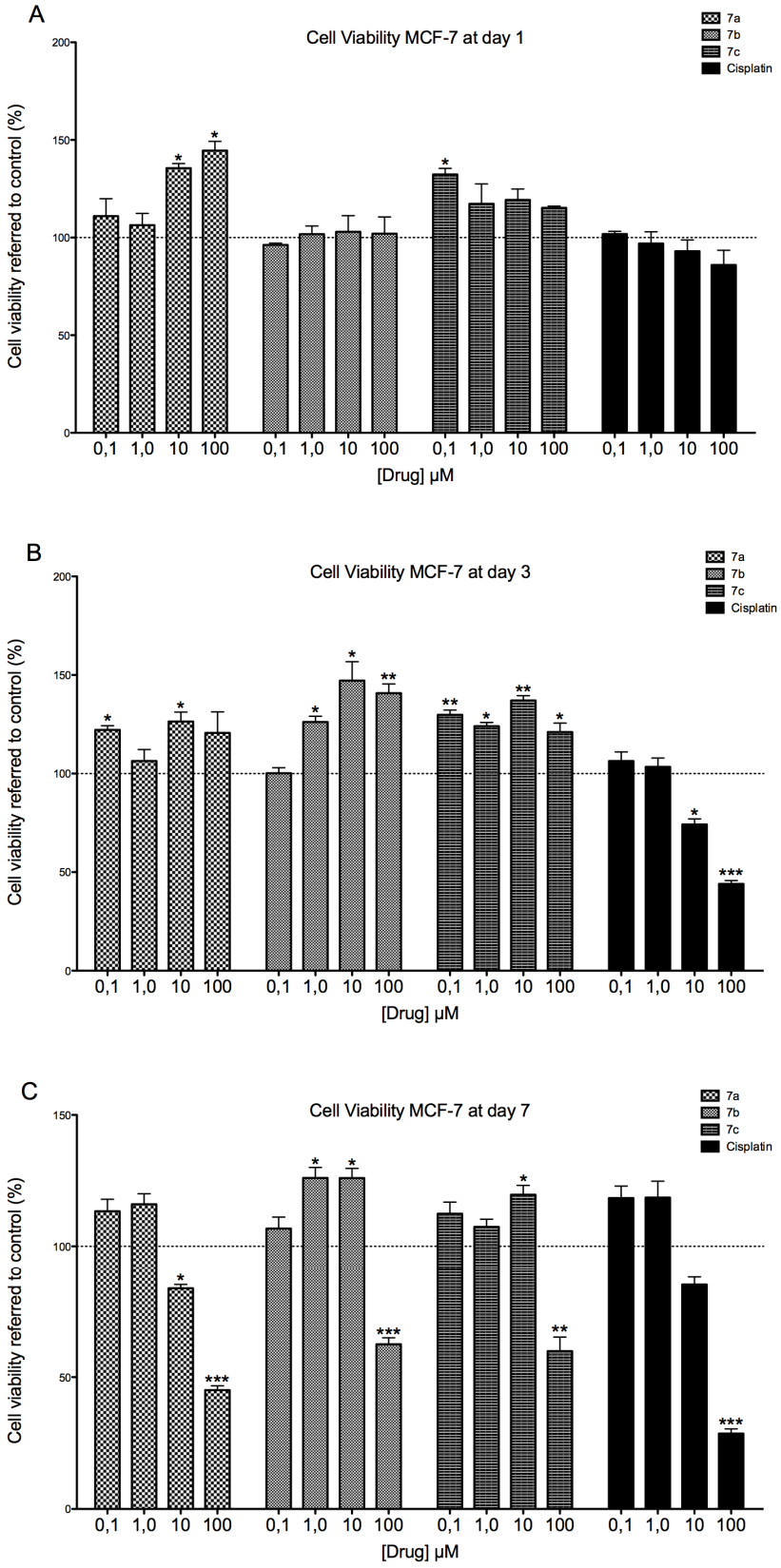


Fig. 1 Cytotoxic effect of RENs 7a-c and cisplatin in MCF-7 breast cancer-derived cell line. MCF-7 cell line was treated with four different concentrations (0.1 µM, 1 µM, 10 µM and 100 µM) of compound **7a-c** and cisplatin. Cell viability (% referred to untreated cells) was assessed at day 1 (Panel A), day 3 (Panel B) and day 7 (Panel C). Columns, mean of quadruplicates; bars, SE (*$p < 0.05$; **$p < 0.01$;***$p < 0.001$).

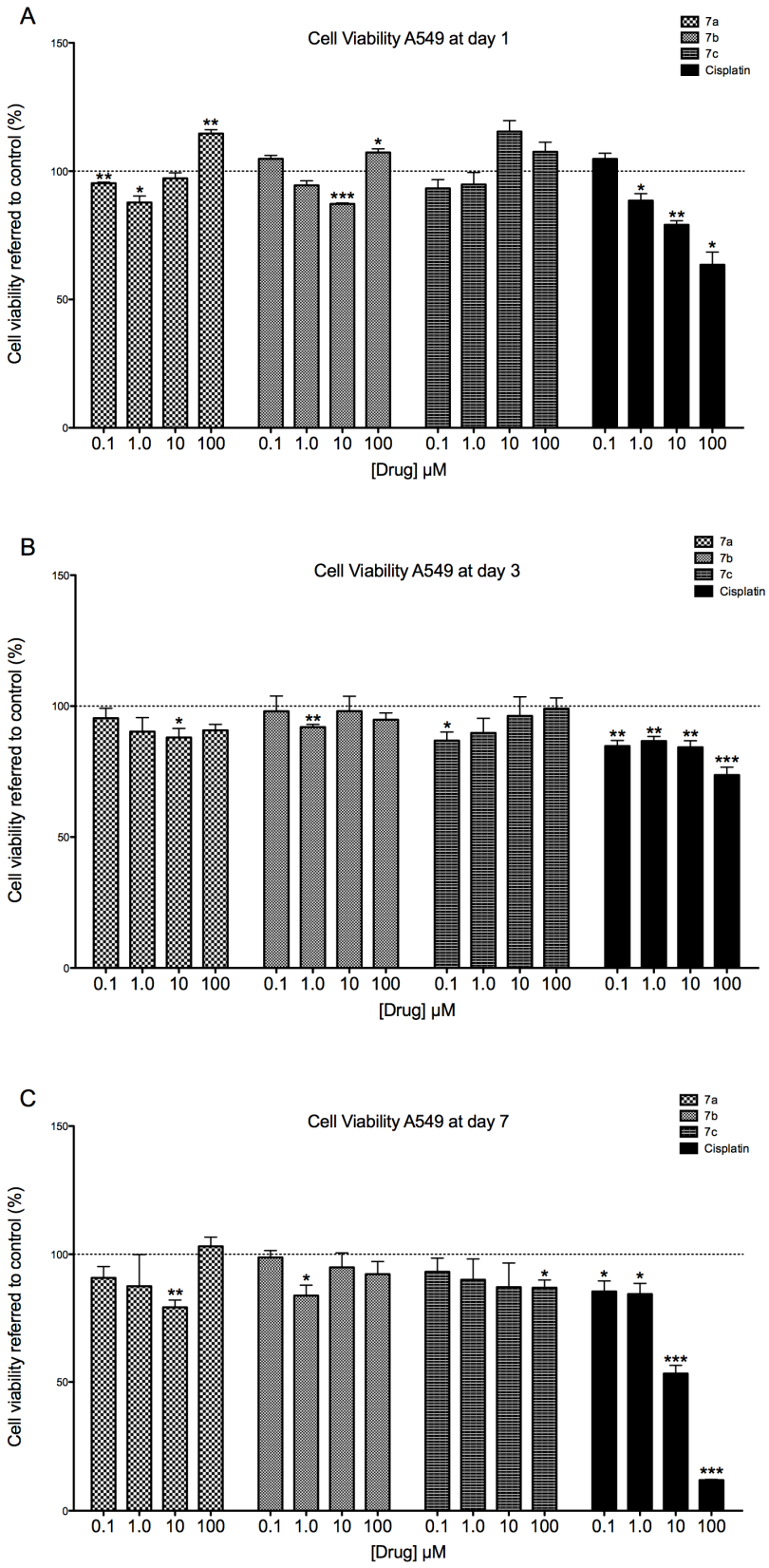


Fig. 2 Cytotoxic effect of RENs 7a-c and cisplatin in A549 lung cancer-derived cell line. A549 cell line was treated with four different concentrations (0.1 μM, 1 μM, 10 μM and 100 μM) of compound **7a-c** and cisplatin. Cell viability (% referred to untreated cells) was assessed at day 1 (Panel A), day 3 (Panel B) and day 7 (Panel C). Columns, mean of quadruplicates; bars, SE (*$p < 0.05$; **$p < 0.01$;***$p < 0.001$)