Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Synthesis and Electrochemical Studies of an Anthraquinone-Conjugated Nucleoside and Derived Oligonucleotides

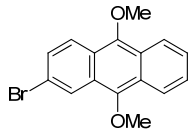
Mikkel F. Jacobsen, Elena E. Ferapontova and Kurt V. Gothelf*

Centre for DNA Nanotechnology at iNANO and Department of Chemistry, University of Aarhus,

Langelandsgade 140, 8000 Aarhus C, Denmark

General Experimental Methods. Unless otherwise stated, all reactions were carried out under nitrogen or argon. Removing O_2 (degassing) from solutions/suspensions was performed by bobbling N_2-gas through them. Reactions were monitored by thin-layer chromatography (TLC) analysis on Merck® silica gel 60 F_{254} TLC plates. Spots were visualized by exposure to ultraviolet (UV) light (254 nm), or by staining with a 5% solution of phosphormolybdenic acid (PMA) in ethanol or basic aqueous potassium permanganate ($KMnO_4$) and then heating. Preparative thin layer chromatography (PTLC) was performed on Merck precoated silica gel $60_{F\text{-}254}$ glass-supported plates with 1.0 or 2.0 mm thickness. Flash chromatography was carried out using Merck® silica gel 60 (230-400 mesh). Acetonitrile (MeCN) and dichloromethane (CH_2Cl_2) were dried by distillation from CaH_2. Tetrahydrofuran (THF) was dried by distillation from Na/benzophenone. All other solvents were of HPLC grade quality or better and used as such. All reagents were purchased at the highest commercial quality and used without further purification. NMR spectra were recorded at 400 MHz (^{1}H NMR) and at 100 MHz (^{13}C NMR), and calibrated to TMS or to the residual solvent peak. The following abbreviations are used for NMR data: s, singlet; d, doublet; q, quartet; t, triplet; m, multiplet; br, broad; app, apparent. Coupling constants are rounded to nearest 0.5 Hz. All compounds synthesized were determined to be >95% pure by ^{1}H NMR. Melting points are uncorrected.

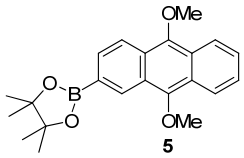
Compound **2**[1] was prepared according to a literature procedure.



4

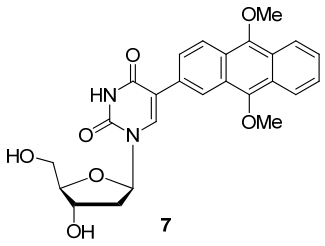
2-Bromo-9,10-dimethoxyanthracene (4).[2] To a suspension of **2** (5.742 g, 20.0 mmol) in THF (68 mL) and water (28 mL) was added TBABr (800 mg, 2.48 mmol) followed by $Na_2S_2O_4$ (20.9 g, 120 mmol) at rt. The mixture was stirred for 20h at rt. KOH (13.5 g, 240 mmol) was added. While the mixture was cooled in an ice-water bath, Me_2SO_4 (18.9 mL, 200 mmol) was added drop wise. The mixture was stirred for further 2h at rt. The bulk of THF was removed by evaporation *in vacuo*, and CH_2Cl_2 (100 mL) was added to the aq. residue, and the mixture was stirred vigorously for 5 min and filtered through a pad of Celite©. The organic phase was separated and washed once with water, dried over $MgSO_4$ and evaporated to dryness. The residue was subjected to column chromatography (*n*-hexane-CH_2Cl_2, 4:1→3:1) to afford **4** (4.289 g, 68%) as a yellow, fluorescent solid: Mp 170-171°C; R_f = 0.4 (*n*-hexane-CH_2Cl_2, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45-8.46 (app dd, *J* = 0.5, 2.0 Hz, 1H), 8.26-8.31 (m, 2H), 8.17 (dd, *J* = 0.5, 9.0 Hz, 1H), 7.51-7.55 (m, 3H), 4.11 (s, 3H), 4.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9, 147.6, 128.9, 126.1, 125.8, 125.7, 125.6, 125.2, 124.7 (2C), 123.1, 122.7, 122.7, 120.1, 63.5, 63.4. The ^{1}H NMR spectral data are in excellent agreement with that previously published.[2]

[1] R.S. Coleman, M. A. Mortensen, *Tet. Lett.,* 2003, **44**, 1215-1219.
[2] J. F. Keana, S. P. Vaikunth, S. Ohmiya, C. E. Klopfenstein, *J. Org. Chem.,* 1986, **51**, 3456-3462.



5

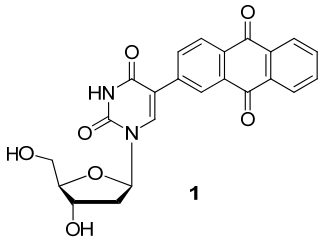
2-(9,10-Dimethoxyanthracen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5). To a solution of **4** (1.269 g, 4.00 mmol) in THF (25 mL) was added dropwise *n*-BuLi (3.1 mL, 5.00 mmol) at -78°C. The mixture was stirred at -78°C for 15 min. During this period the initial clear red color had fainted to orange and a precipitate had formed. Trimethylborate (1.35 mL, 12.0 mL) was added in one flush, and the mixture was stirred for 20 min at -78°C and allowed to warm slowly (30 min) to 0°C. Aq. HCl-solution (10 mL, 20% w/w) was slowly added at 0°C, and the mixture was stirred for 30 min at 0°C and allowed to warm to rt in the cooling bath. The bulk of THF was removed by evaporation *in vacuo*, and the aq. residue was extracted with CH_2Cl_2 several times. The combined organic extracts were dried over $MgSO_4$, and evaporated to dryness to leave the crude boronic acid. The boronic acid was dissolved in THF (20 mL) and molecular sieves (~5 g, 3Å) were added together with pinacol (1.418 g, 12.0 mmol). The mixture was stirred at rt for 1 h, and filtered through a pad of Celite©, and evaporated to dryness *in vacuo*. The residue was subjected to column chromatography (*n*-hexane-CH_2Cl_2, 3:1 with 5% v/v TEA, then CH_2Cl_2 followed by CH_2Cl_2:Et_2O, 20:1→2:1) to yield **5** with an impurity (pinacol). The impure product was dissolved in CH_2Cl_2, and the solution was washed three times with water, dried over $MgSO_4$ and evaporated to dryness *in vacuo* to afford pure **5** (1.031, 71%) as a yellow, stiff foam: R_f = ~0.3 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.27-8.34 (m, 3H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.51 (m, 2H), 4.18 (s, 3H), 4.12 (s, 3H), 1.42 (s, 12H).



7

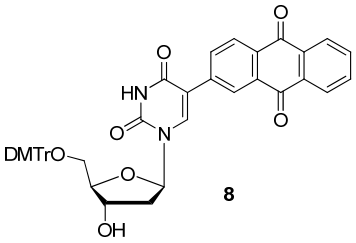
5-(9,10-Dimethoxyanthracen-2-yl)-1-((2*R*,4*R*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (7). To a degassed suspension of **6** (655 mg, 1.85 mmol) in water (75 mL) and MeOH (60 mL) was a degassed solution of **5** in THF (20 mL). Additional THF (80 mL), $Pd(PPh_3)_4$ (214 mg, 0.185 mmol) and solid NaOH (1.48 g, 37.0 mmol) was added and the mixture was refluxed for 17 h. The resulting dark brown solution was cooled to rt, and solid NH_4Cl (10 g) and water (50 mL) was added. The mixture was stirred vigorously for 5 min, and CH_2Cl_2 (50 mL) was added. The aq. phase was separated and further extracted with EtOAc (2x100 mL). The combined organic phases were dried over $MgSO_4$ and filtered. Silica gel (~6 g) was added and the mixture was evaporated to dryness *in vacuo*. The resulting powder was loaded onto a silica gel column, and subjected to column chromatography (CH_2Cl_2-MeOH, 20:1→10:1→5:1) affording **7** (447 mg, 52%) as a yellow solid: Mp >220°C (decomp.); R_f = 0.4 (CH_2Cl_2-MeOH, 10:1); ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (dd, *J* = 0.5, 1.5 Hz, 1H), 8.56 (s, 1H), 8.26-8.31 (m, 3H), 7.71 (dd, *J* = 1.5, 9.0 Hz, 1H), 7.53 (t, *J* = 3.0 Hz, 1H), 7.51 (t, *J* = 3.0 Hz, 1H), 6.43 (t, *J* = 6.5 Hz, 1H), 4.50 (dt, *J* = 3.0, 5.0 Hz, 1H), 4.13 (s, 3H), 4.11 (s, 3H), 4.00 (app q, *J* = 3.0 Hz, 1H), 3.86 (dd, *J* = 3.0, 12.0 Hz, 1H), 3.78 (dd, *J* = 3.0, 12.0 Hz, 1H), 2.38-2.41 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 149.8,

148.1, 147.7, 138.7, 130.4, 126.1, 125.9, 125.8, 124.6, 124.4, 124.2, 123.2, 122.3 (2C), 122.2, 120.7, 113.2, 87.8, 84.8, 70.3, 63.2, 63.2, 61.0, 40.3; HRMS (ES) *m/z*: $[M]^+$ calcd for $C_{25}H_{24}N_2NaO_7$, 464.1584; found, 464.1595.



1

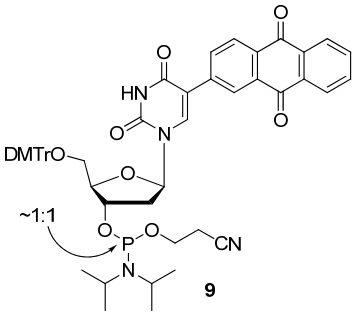
5-(9,10-Dioxo-9,10-dihydroanthracen-2-yl)-1-((2*R*,4*R*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (1). To a suspension of **7** (372 mg, 0.80 mmol) in water-MeOH (4.4 mL, 10:1 v/v) was added [bis(trifluoroacetoxy)iodo]benzene (BTI) (1.376 g, 3.20 mmol) at rt. The mixture was stirred vigorously at rt for 24 h. MeOH (10 mL) was added, and the mixture was concentrated *in vacuo*. The residue was evaporated several times with PhMe until a dry, bright yellow solid residue remained. The residue was washed several times with PhMe and CH_2Cl_2. This yielded pure **1** (342 mg, 98%) as a yellow solid: Mp >220°C (decomp.); R_f = ~0.4 (CH_2Cl_2-MeOH, 10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.7 (br s, 1H), 8.58 (s, 1H), 8.55 (d, *J* = 1.5 Hz, 1H), 8.20-8.24 (m, 2H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.06 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.92-7.97 (m, 2H), 6.23 (t, *J* = 6.5 Hz, 1H), 5.31 (d, *J* = 4.5 Hz, 1H), 5.22 (t, *J* = 9.0 Hz, 1H), 4.29-4.35 (m, 1H), 3.85 (q, *J* = 3.0 Hz, 1H), 3.60-3.70 (m, 2H), 2.28-2.35 (m, 1H), 2.18-2.24 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4, 182.0, 161.8, 149.7, 140.1, 139.5, 134.5, 134.5, 133.1, 133.1, 132.8, 132.7, 131.3, 126.9, 126.7, 126.7, 125.8, 111.3, 87.6, 85.0, 69.9, 60.7; 40.4
HRMS (ES) *m/z*: $[M+Na]^+$ calcd for $C_{23}H_{18}N_2NaO_7$, 457.1012; found, 457.1010.



8

1-((2*R*,4*R*,5*R*)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-(9,10-dioxo-9,10-dihydroanthracen-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (8). Compound **1** (123 mg, 0.284 mmol) was dissolved in pyridine (5 mL) and evaporated to dryness *in vacuo*, and redissolved in pyridine (5 mL). A solution of DMTrCl (125 mg, 0.369 mmol) in pyridine (5 mL) was added over a period of 2 h, and the mixture was stirred for 15 h. MeOH (1 mL) was added, and the mixture was evaporated to dryness. The residue was evaporated with PhMe several times, and subjected to column chromatography (CH_2Cl_2-EtOAc, 1:1) to yield **8** (93 mg, 44%) as a yellow stiff foam. Data for **8**: R_f = ~0.4 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (br s, 1H), 8.26-8.30 (m, 3H), 8.02 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.76-7.81 (m, 2H), 7.59 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.27-7.29 (m, 2H), 7.07-7.17 (m, 7H), 6.40 (t, *J* = 6.5 Hz, 1H), 4.54-4.57 (m, 1H), 4.11 (q, *J* = 3.5 Hz, 1H), 3.62 (s, 3H), 3.61 (s, 3H), 3.44 (dd, *J* = 3.0, 10.5 Hz, 1H), 3.56 (dd, *J* = 3.5, 10.5 Hz, 1H), 2.57-2.62 (m, 1H), 2.46 (d, *J* = 3.5 Hz, 1H), 2.37-2.46 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.7, 182.6, 161.8, 158.5, 158.4, 150.2, 144.2, 138.5, 138.4, 135.7, 135.5, 134.1 (2C), 133.6 (2C), 133.4,

133.3, 132.3, 129.9, 129.8, 128.1, 127.9, 127.4, 127.2 (2C), 127.1, 126.7, 114.0, 113.2, 113.1, 86.8, 86.6, 86.0, 72.1, 63.6, 60.5, 55.1, 41.6; HRMS (ES) *m/z*: $[M+Na]^+$ calcd for $C_{44}H_{36}N_2NaO_9$, 759.2319; found, 759.2361.



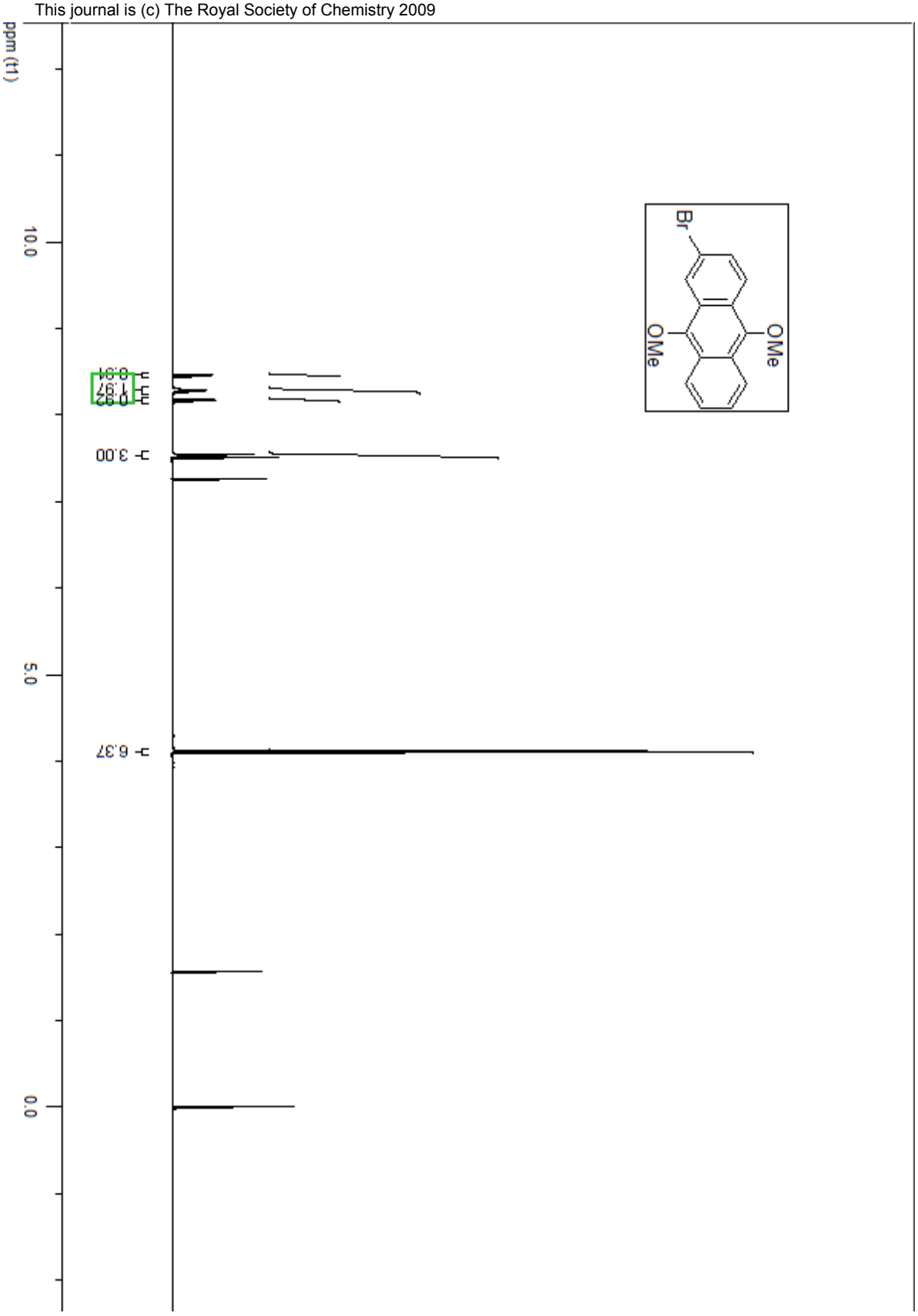
(2*R*,3*R*,5*R*)-2-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(5-(9,10-dioxo-9,10-dihydroanthracen-2-yl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl 2-cyanoethyl diisopropylphosphoramidite (9). To a solution of **8** (59 mg, 80 μmol) in CH_2Cl_2 (1.0 mL) was added DIPEA (35 μL, 0.20 mmol) followed by 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (27 μL, 120 μmol) at rt. The mixture was stirred for 30 min, and evaporated to dryness *in vacuo*. The residue was subjected to column chromatography (CH_2Cl_2-EtOAc, 1:1) to afford **9** (61 mg, 81%) as a yellow stiff foam and as a ~1:1 diastereoisomeric mixture: R_f = 0.65(CH_2Cl_2-EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (br s, 1H), 8.27-8.32 (m, 6H), 8.07 (s, 1H), 8.03 (s, 1H), 7.76-7.82 (m, 6H), 7.51 (t, *J* = 1.5 Hz, 1H), 7.49 (t, *J* = 1.5 Hz, 1H), 7.28-7.31 (m, 4H), 7.10-7.19 (m, 14H), 6.59-6.64 (m, 6H), 6.37-6.42 (m, 2H), 4.59-4.66 (m, 2H), 4.19-4.24 (m, 2H), 3.43-3.87 (m, 10H), 3.62 (s, 6H), 3.62 (s, 3H), 3.61 (s, 3H), 3.27-3.33 (m, 2H), 2.60-2.73 (m, 4H), 2.38-2.45 (m, 4H), 1.15-1.18 (m, 18H), 1.05 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, benzene--d_6) δ 182.6, 182.3, 161.6 (2C), 159.1, 159.0, 150.2, 150.0, 144.9 (2C), 139.0 (2C), 138.1 (2C), 136.1 (2C), 136.0, 135.9, 134.1 (2C), 133.7 (2C), 133.6, 133.5, 132.6 (2C), 130.3, 128.6 (2C), 128.2 (2C), 127.4, 127.2, 127.1 (2C), 127.0 (2C), 117.8, 117.6, 114.0, 113.9, 113.5 (2C), 87.1 (2C), 86.3 (2C), 86.0 (2C), 85.8 (2C), 73.6 (*J* = 16.5 Hz), 73.3 (*J* = 15.0 Hz), 63.5, 63.4, 58.7 (*J* = 6.5 Hz), 58.5 (*J* = 7.0 Hz), 54.7 (4C), 43.6 (2C), 43.5 (2C), 40.9, 40.5 (*J* = 4.5 Hz), 24.6 (2C), 20.2 (*J* = 7.0 Hz), 20.0 (*J* = 7.0 Hz); HRMS (ES) *m/z*: $[M+Na]^+$ calcd for $C_{53}H_{53}N_4NaO_{10}P$, 959.3397; found, 959.3506.

Automated DNA synthesis of oligonucleotides O1-O7. DNA sequences were synthesized by the DNA Technology, Risskov, Denmark. The phosphoroamidite **9** (mixture phosphoramidite epimers) was incorporated via standard procedures. Oligonucleotides **O1** and **O6** were synthesized with at C6-disulfide (HO-$(CH_2)_6$-S-S-$(CH_2)_6$-) modification (Glen Research) at the 5′-end. The synthesized oligonucleotides were all purified by reverse phase HPLC, their concentration determined by UV-Vis spectroscopy and their identity confirmed by MALDI-TOF mass spectrometry before use (**O1**: calc. exact mass: 5094.917, found 5094.822; **O2**: calc. exact mass: 4766.824, found 4767.919; **O6**: calc. exact mass: 9647.607, found 9650.665).

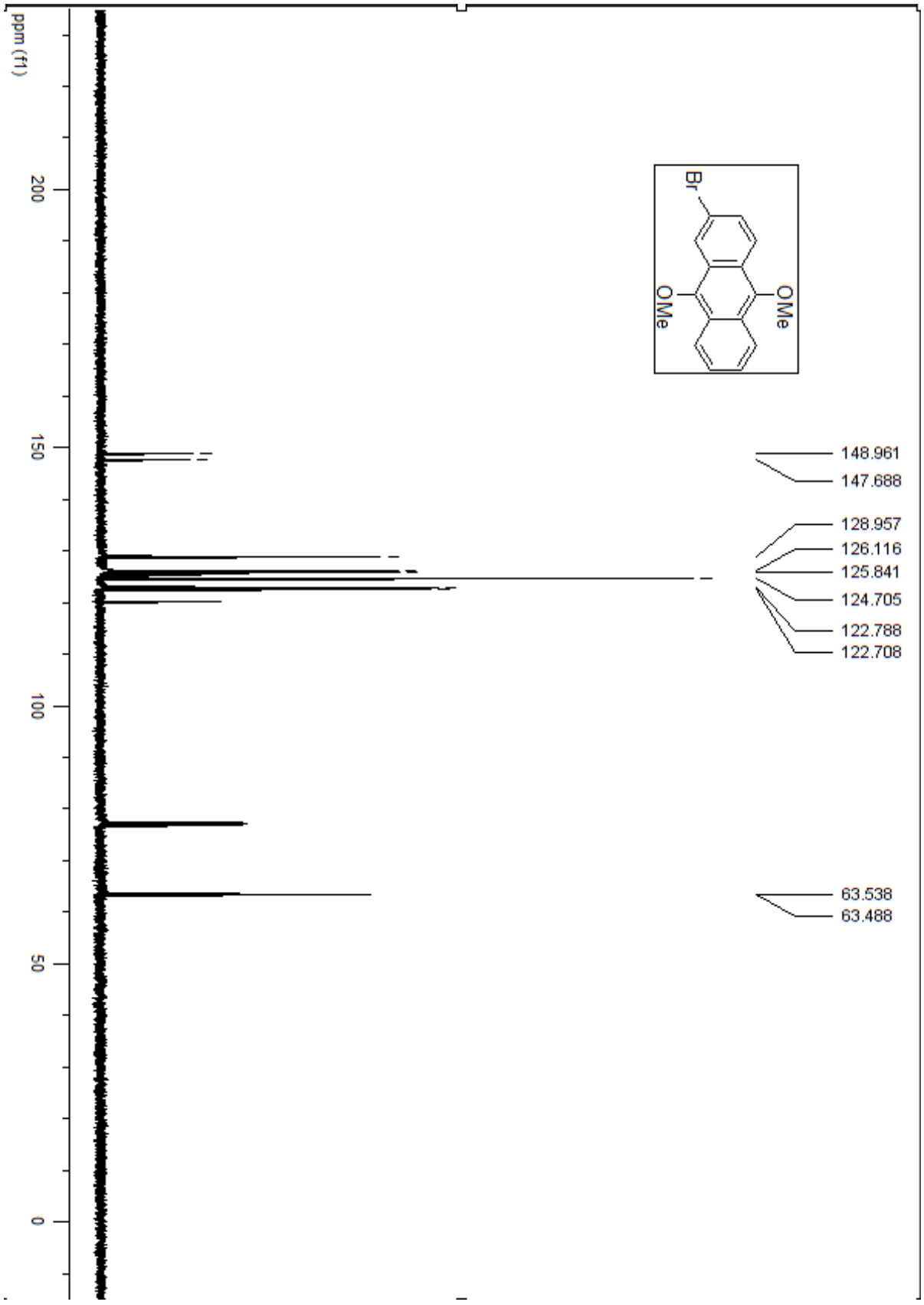
Electrode modification with AQ-DNA. Gold disk electrodes (∅ 0.2 cm) were mechanically polished to a mirror luster stepwise in 1 μm diamond- and in 0.1 μm alumna slurry (Struers, Denmark) on microcloth (Buehler, Germany), ultrasonicated in ethanol/water bath for 5 min, electrochemically polished by cycling in 1 M H_2SO_4 as described elsewhere,[2] and further kept in absolute ethanol for 30 min. Modification of these electrodes with **O1** and **O6** disulfides was performed by 24 h adsorption from the 17 μM DNA solution. Alternatively, the clean gold electrodes were modified with hybridized duplexes. After modification, the electrodes, carefully rinsed with a buffer solution, were treated with 0.01% solution of 1-mercaptohexanol in 0.1 M PBS, pH 7.2, for 1 h. The modified electrodes were buffer washed and immediately used in electrochemical experiments. For surface hybridization, DNA-modified electrodes were exposed to 20 μM solutions of either complementary or mismatched DNA (**O3**, **O5**, or **O7**, respectively) in 0.1 M PBS, pH 7.2, containing 0.15 M NaCl and 5 mM $MgCl_2$, for 1 h at room temperature, under gentle shaking. After hybridization, the electrodes were carefully washed with the hybridization buffer solution and used in further electrochemical experiments.

Instrumentation and procedure. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were done with a three-electrode potentiostat AUTOLAB PGSTAT 30 (Eco Chemie B.V., Utrecht, Netherlands) equipped with GPES 4.97 software. A SCE electrode was the reference and a Pt wire was the auxiliary electrode. For de-aeration, the solutions were purged with Ar for 1 h before the experiments. Electrolyte was aqueous 0.1 M PBS, pH 6.2, containing 0.15 M NaCl and 5 mM $MgCl_2$.

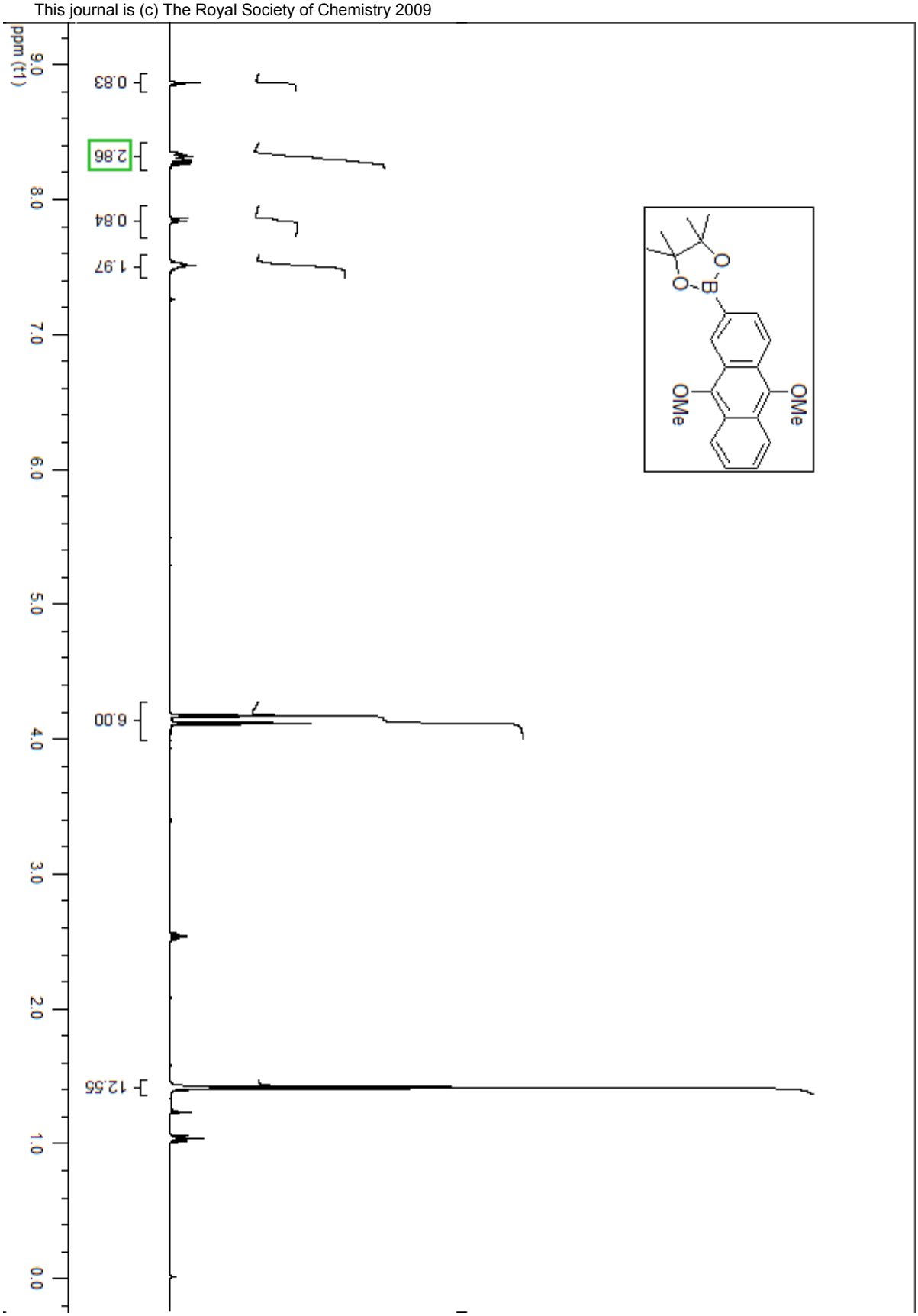
[2] E. Ferapontova, T. Ruzgas, L. Gorton, *Anal. Chem.,* 2003, **75**, 4841.



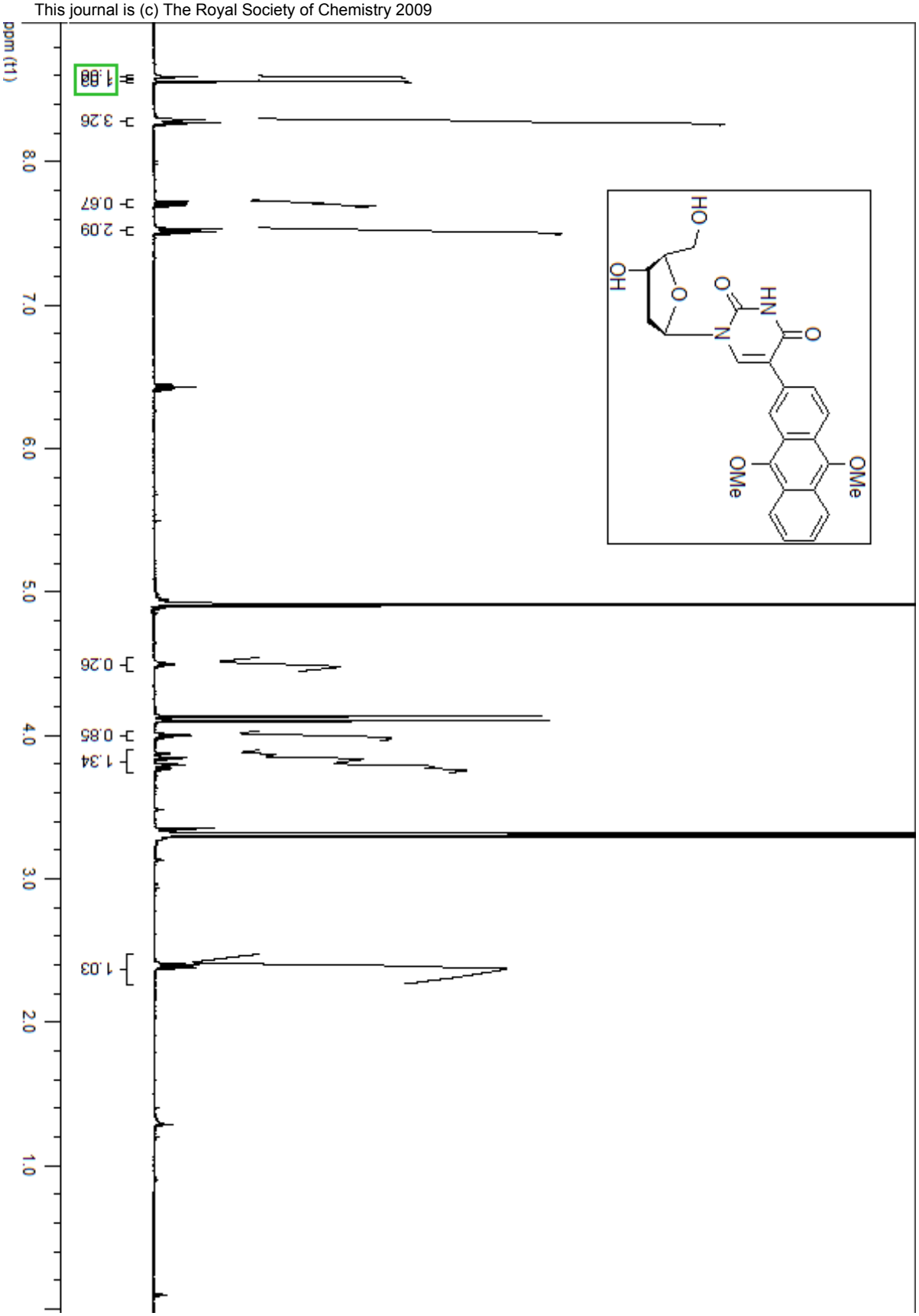
^{1}H NMR (400 MHz) of **4** in $CDCl_3$



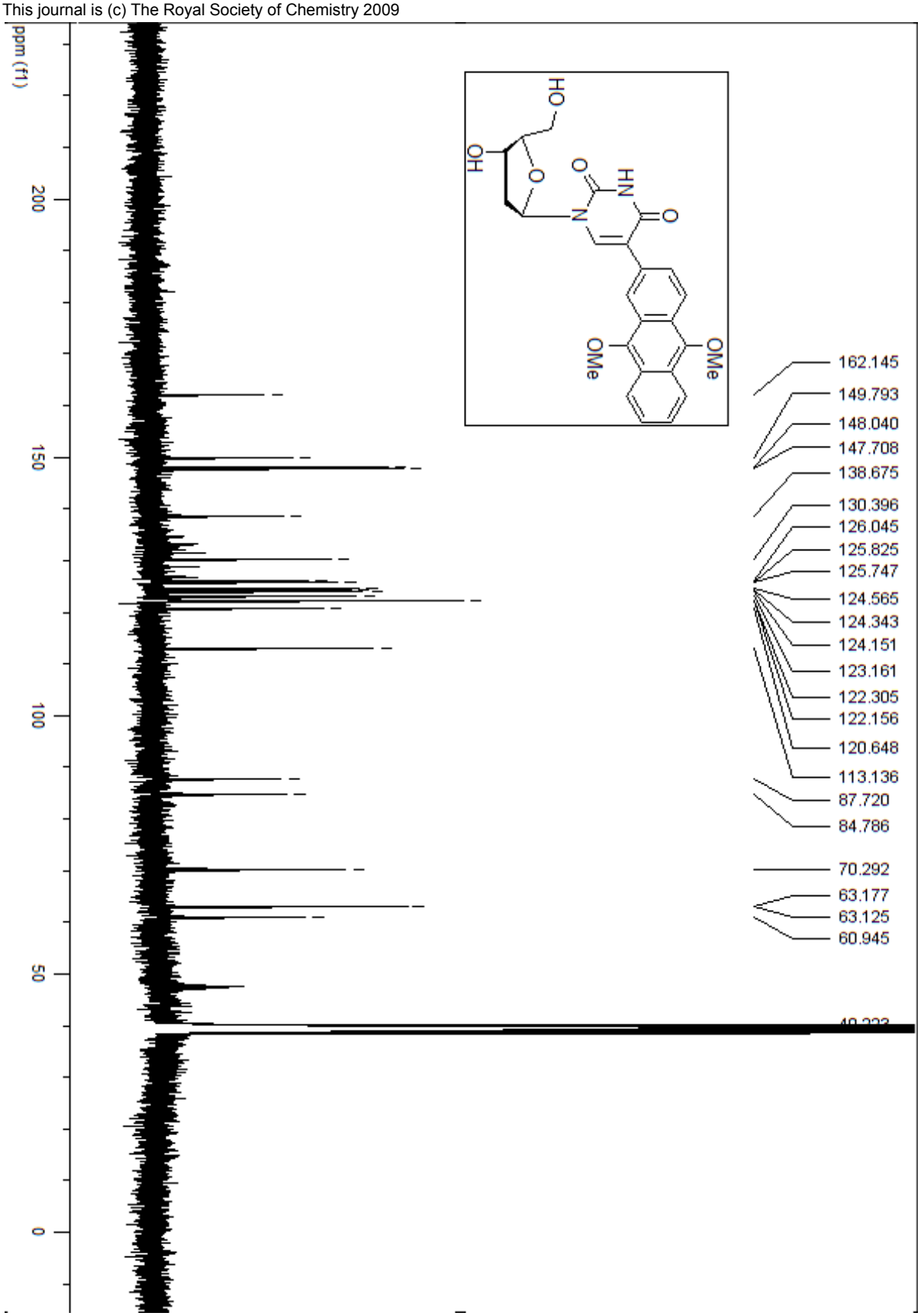
^{13}C NMR (100 MHz) of **4** in $CDCl_3$



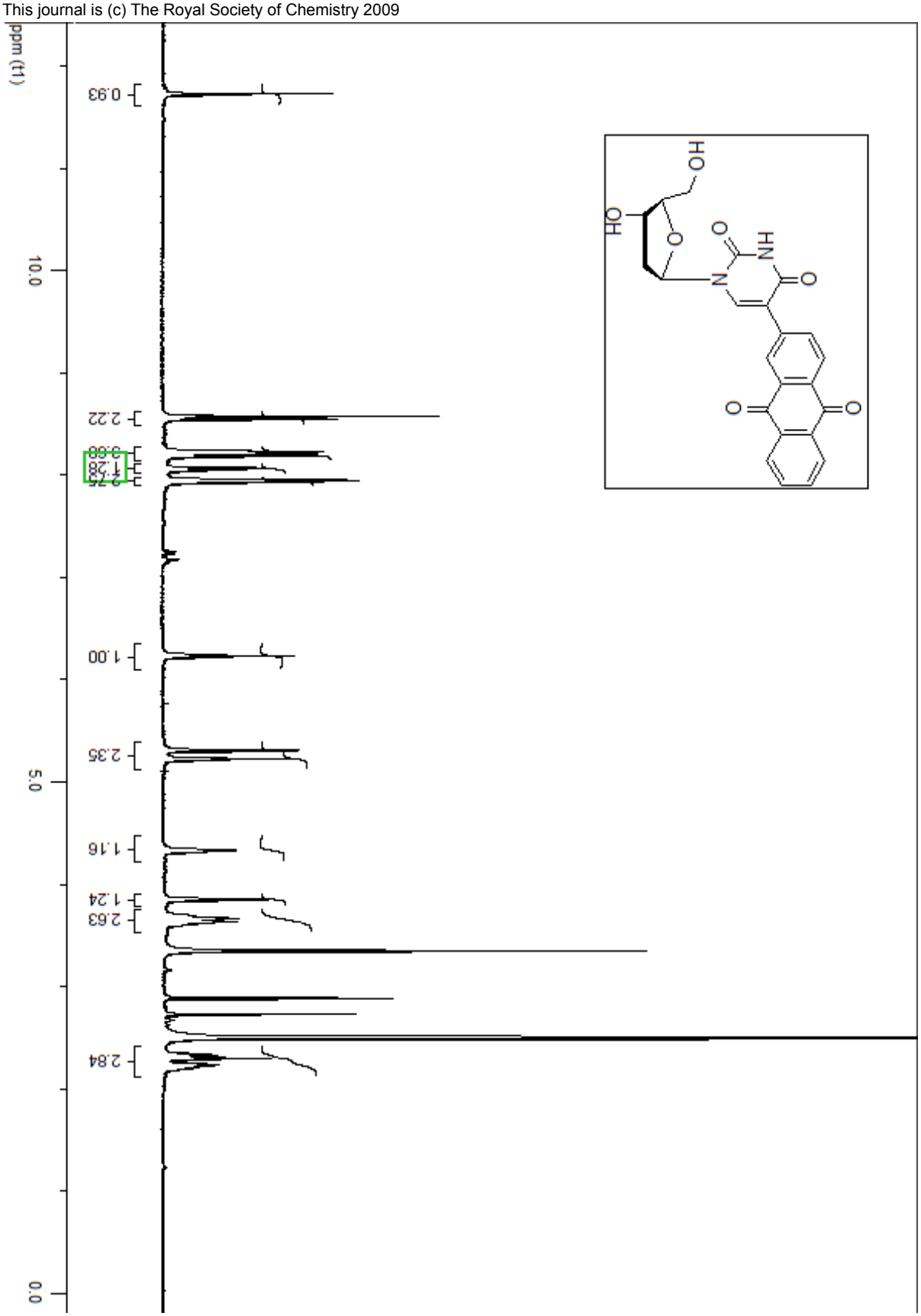
^{1}H NMR (400 MHz) of **5** in $CDCl_3$



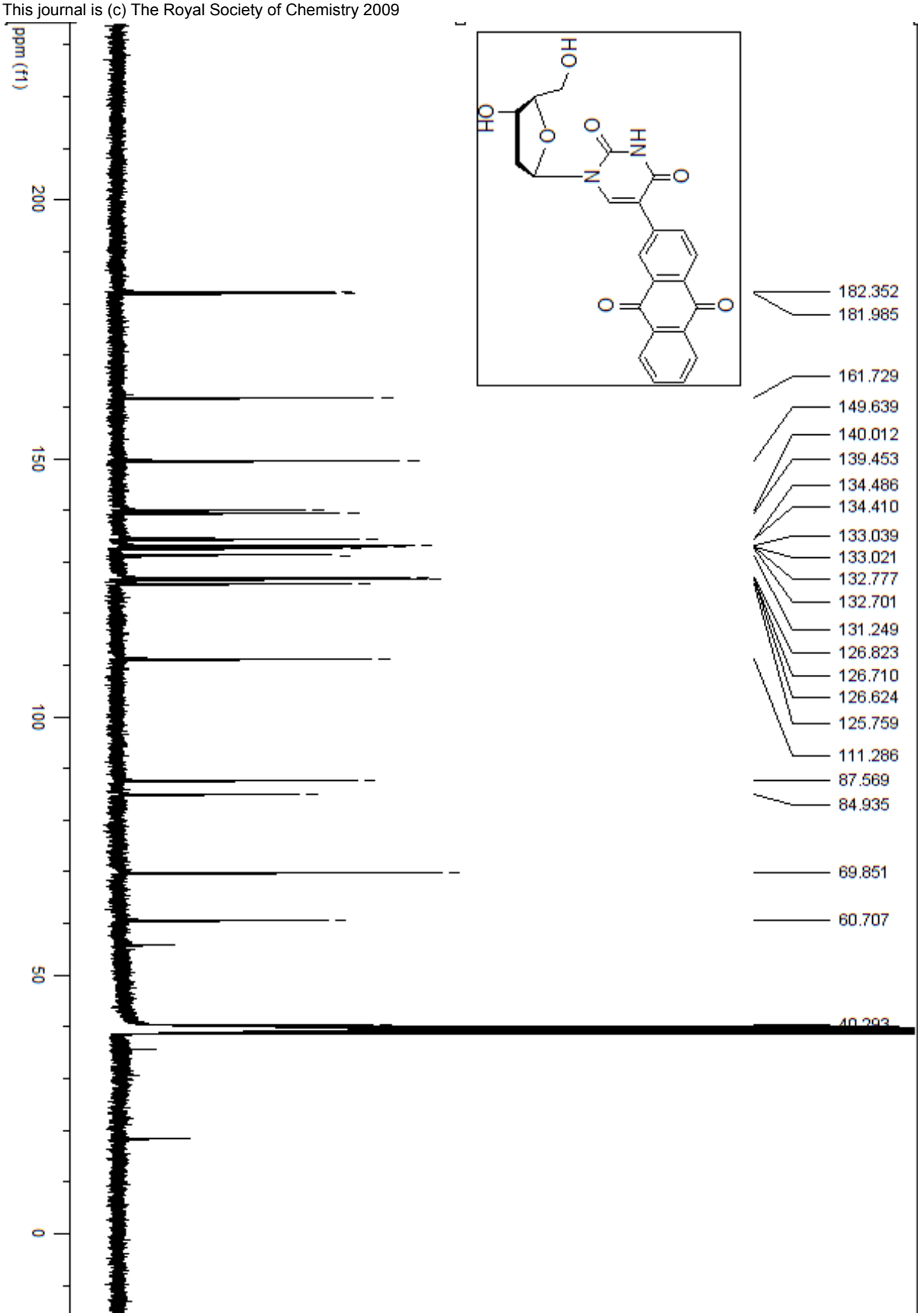
^{1}H NMR (400 MHz) of **7** in CD_3OD



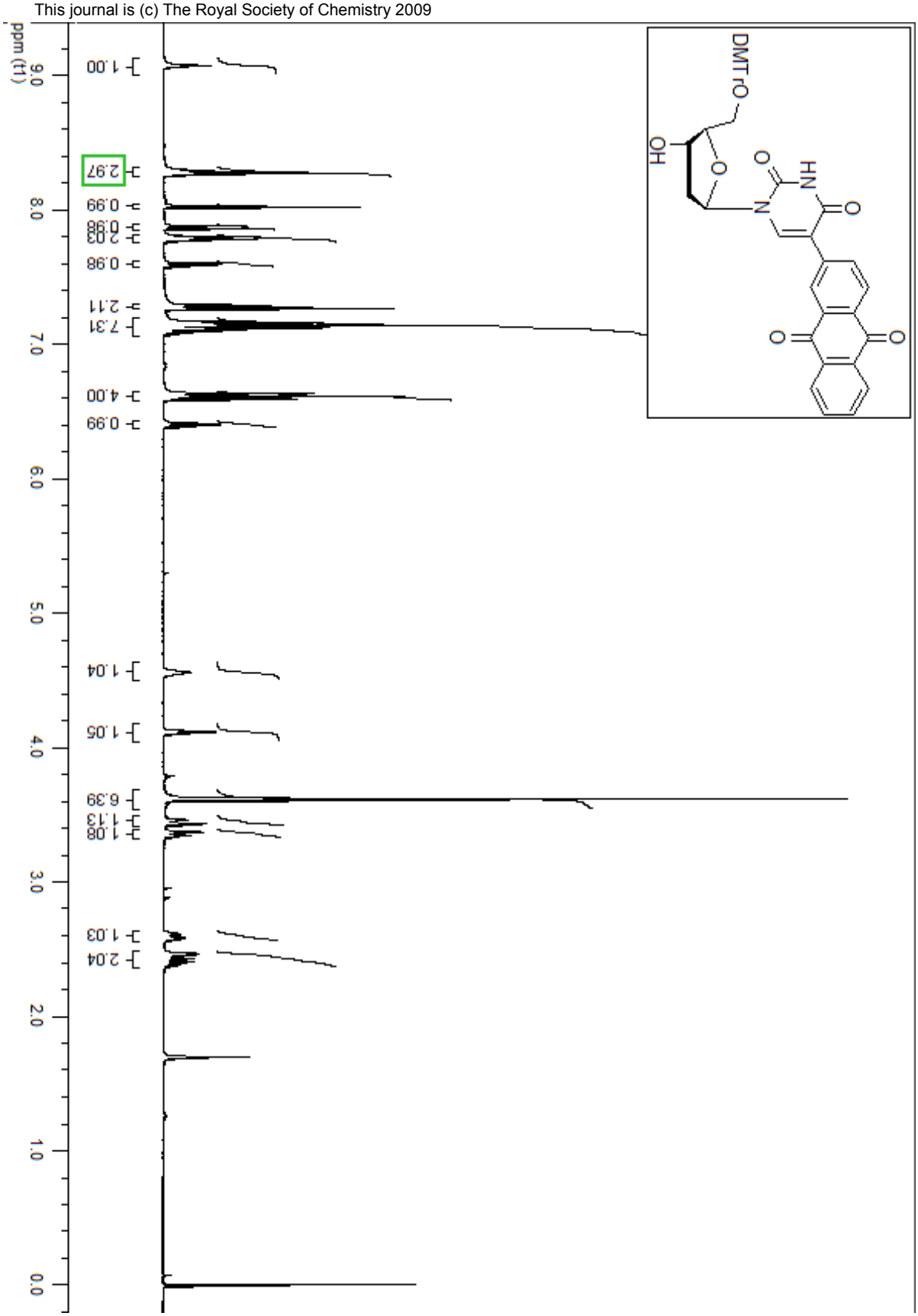
^{13}C NMR (100 MHz) of **7** in DMSO-d_6



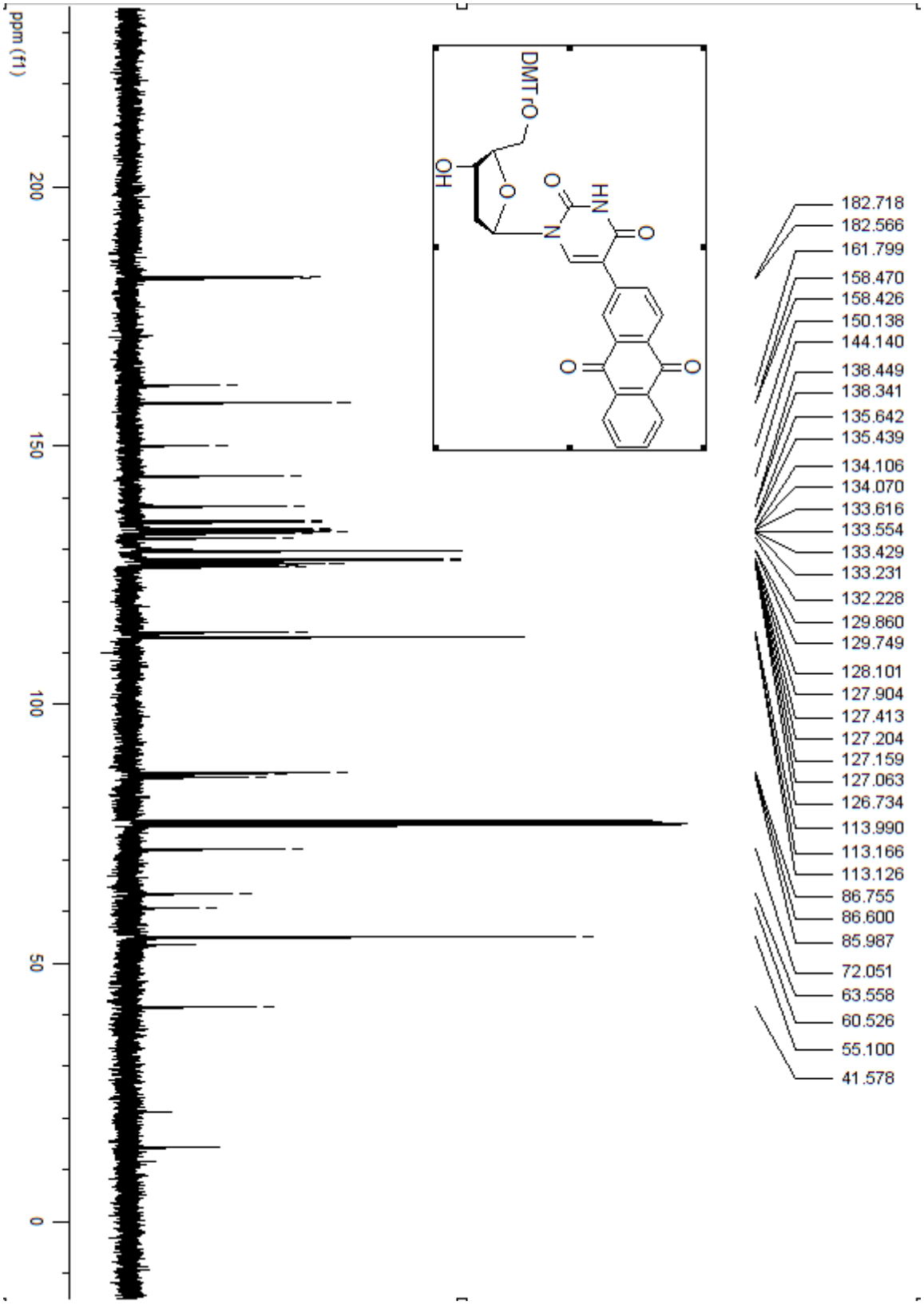
^{1}H NMR (400 MHz) of **1** in DMSO-d_6



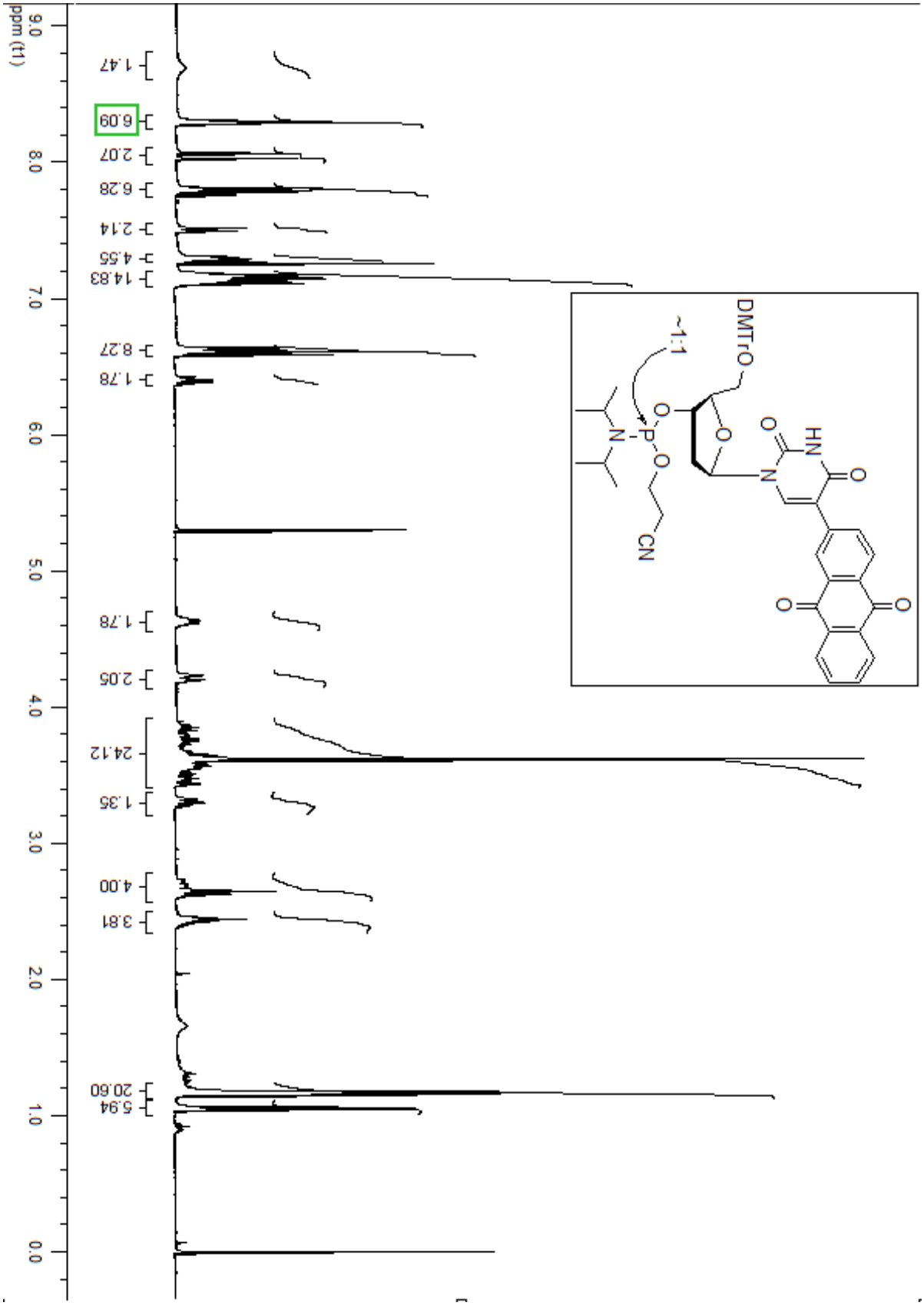
^{13}C NMR (100 MHz) of **1** in DMSO-d_6



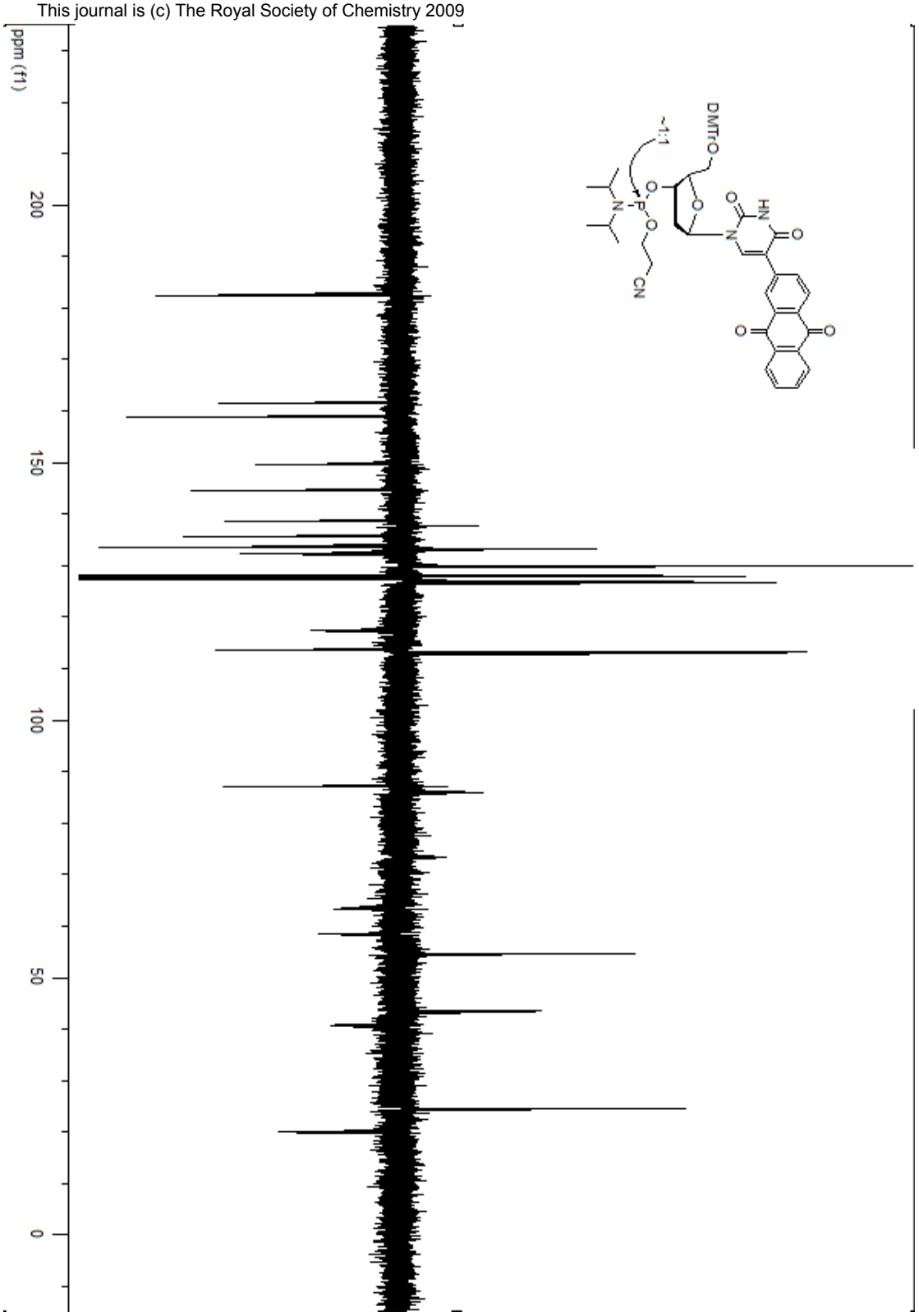
^{1}H NMR (400 MHz) of **8** in $CDCl_3$



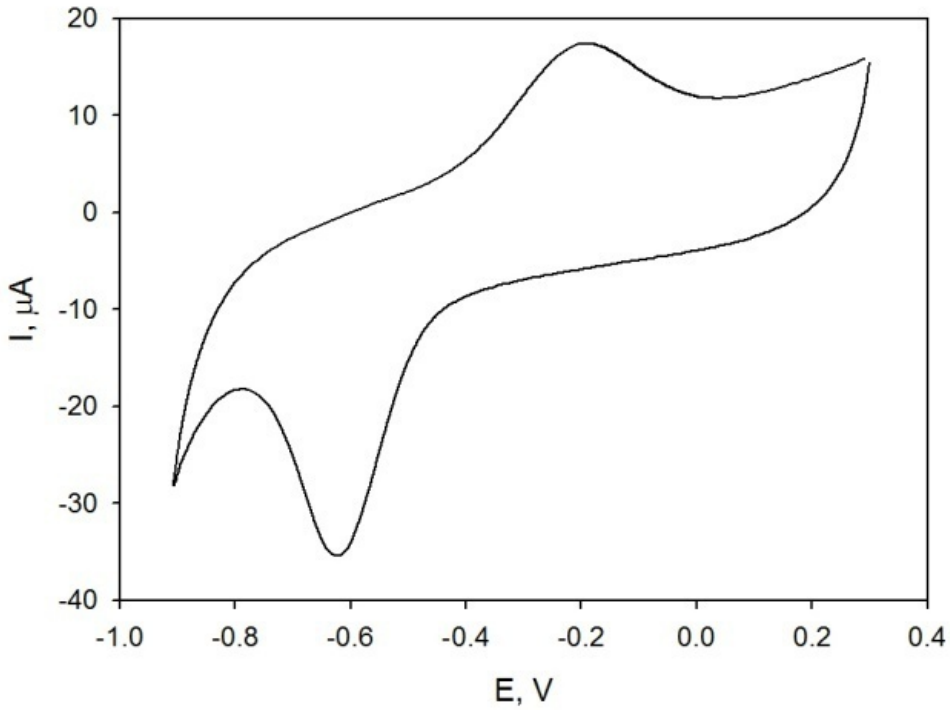
^{13}C NMR (100 MHz) of **8** in $CDCl_3$



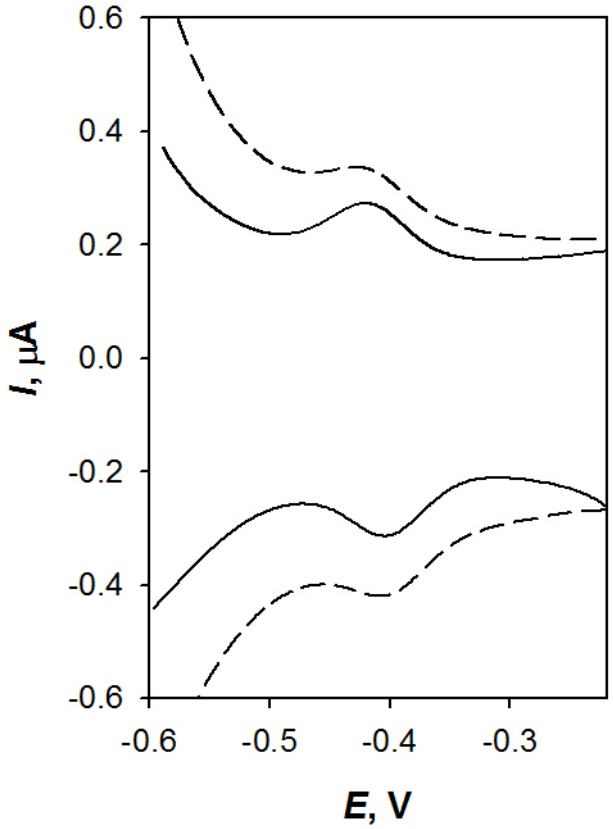
^{1}H NMR (400 MHz) of **9** in $CDCl_3$



APT ^{13}C NMR (100 MHz) of **9** in benzene-d_6



Cyclic voltammogram of 0.4 mM AQ-dU (**1**) in 20 mM PBS/0.15 M NaCl, pH 6.2, scan rate is 4 V s^{-1}.



Differential pulse voltammograms of a gold electrode modified with **O1**, before (solid line) and after hybridization with a complementary strand **O3** (dash-dotted line). Effective scan rate is 10 mV s^{-1}.