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SUPPORTING INFORMATION

Harnessing an emissive guanine surrogate to design small-molecule fluorescent chemosensors of *O*^{6}-methyl-guanine-DNA-methyltransferase (MGMT)

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Experimental Part

Chemical synthesis

General remarks: All commercially available chemicals were reagent grade and used without further purification. NMR spectra were acquired on a Bruker Avance 300 spectrometer (^{1}H: 300 MHz, ^{13}C: 75 MHz) at 25 °C; chemical shifts are given in ppm (δ) values. Multiplicities of ^{13}C NMR signals were determined from APT experiments. The purity of final compounds was assessed by LC/MS analysis (Waters Alliance 2695 equipped with a Phenomenex Luna C18(2) 1.6 µm column and a photodiode array detector; eluent A: water with 0.1% formic acid, eluent B: MeCN with 0.1% formic acid, gradient elution with 0 to 20% of eluent B). Mass spectra (MS, ESI in the positive-ion mode) were recorded with a Waters ZQ instrument (cone voltage: 30 V). 2-Aminothieno[3,4-*d*]pyrimidin-4(3*H*)-one (thienoguanine, th**G**$_N$) was prepared as described by Y. Tor et al.[1]

1-(2-Aminothieno[3,4-*d*]pyrimidin-4-yl)-4-(dimethylamino)pyridinium chloride (1): th**G**$_N$ (1.11 g, 6.70 mmol, 1.0 equiv.), 2-mesitylenesulfonyl chloride (2.30 g, 10.1 mmol, 1.5 equiv.), and 4-dimethylaminopyridine (982 mg, 8.04 mmol, 1.2 equiv.) were suspended in anhydrous MeCN (67 mL) under inert atmosphere. DBU (1.60 mL, 1.63 g, 10.1 mmol, 1.5 equiv.) was added dropwise. The thick grey mixture turned orange and was stirred overnight at 50 °C. The reaction mixture was filtered, and the yellow solid was washed with MeCN (3 × 15 mL), Et_2O

(3 × 30 mL), and dried under vacuum, to give **1** (1.56 g, 75%) as a yellow powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 8.79 (d, J = 7.7 Hz, 2H, H2′, H6′), 8.44 (d, J = 3.0 Hz, 1H, H7), 7.50 (d, J = 3.0 Hz, 1H, H9), 7.28 (d, J = 7.7 Hz, 2H, H3′, H5′), 7.02 (s, 2H, NH_2), 3.36 (s, 6H, NMe_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 157.9 (C_q), 157.2 (C_q), 154.9 (C_q), 154.4 (C_q), 139.7 (CH), 124.1 (CH), 117.0 (C_q), 109.6 (CH), 107.7 (CH), 40.5 (CH_3); MS (ESI$^+$): m/z (%) = 272.3 (100) [M]$^+$, 168.2 (8), 136.8 (47); purity (LC): 93%.

4-(Benzyloxy)thieno[3,4-*d*]pyrimidin-2-amine ($^{th}G_B$): *t*-BuOK (269 mg, 2.40 mmol, 4.0 equiv.) was dissolved in anhydrous pyridine (4.1 mL) and degassed through three vacuum-argon cycles. Benzyl alcohol (0.31 mL, 324 mg, 3.00 mmol, 5.0 equiv.) was added to the mixture, followed by compound **1** (180 mg, 0.60 mmol, 1.0 equiv.). The mixture was stirred for 2 h at room temperature and then concentrated under reduced pressure. The residue was dissolved in DCM and washed with water (3X). The organic phases were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (SiO_2; gradient elution: 100% DCM to 90:10 DCM / MeOH), to give **$^{th}G_B$** (42 mg, 27%) as a light green powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.93 (d, J = 3.3 Hz, 1H, H7), 7.47 (m, 2H), 7.43–7.36 (m, 3H), 7.09 (d, J = 3.3 Hz, 1H, H9), 5.52 (s, 2H, CH_2), 4.95 (s, 2H, NH_2); ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ (ppm) = 164.4 (C_q), 159.2 (C_q), 152.9 (C_q), 136.1 (C_q), 128.7 (CH), 128.5 (CH), 128.4 (CH), 121.8 (CH), 118.6 (C_q), 108.8 (CH), 68.3 (CH_2); MS (ESI$^+$): m/z (%) = 258.0 (100) [M + H]$^+$; purity (LC): 100%.

4-((3-Iodobenzyl)oxy)thieno[3,4-*d*]pyrimidin-2-amine ($^{th}G_I$): *t*-BuOK (269 mg, 2.40 mmol, 4.0 equiv.) was dissolved in anhydrous pyridine (4 mL) and degassed through three vacuum-argon cycles. (3-Iodophenyl)methanol (380 µL, 702 mg, 3.00 mmol, 5.0 equiv.) was added to the mixture, followed by compound **1** (185 mg, 0.60 mmol, 1.0 equiv.). The mixture was stirred overnight at room temperature. Then, the reaction mixture was concentrated under reduced pressure and dissolved in DCM, washed 3 times with water, dried over $MgSO_4$ and finally filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2; gradient elution: 100% DCM to 90:10 DCM/MeOH), to give **$^{th}G_I$** (68 mg, 30%) as a light green powder; ^{1}H NMR (300 MHz, $CDCl_3$): 7.95 (d, J = 3.3 Hz, 1H, H7), 7.84 (s, 1H, H2′), 7.70 (d, J = 7.9 Hz, 1H), 7.44 (d, J = 7.9 Hz, 1H), 7.16–7.10 (m, 2H, H9 and H5′), 5.46 (s, 2H, CH_2), 4.85 (br s, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 164.2 (C_q), 159.0 (C_q), 152.8 (C_q), 138.4 (C_q), 137.5 (CH), 137.3 (CH), 130.5 (CH), 127.5 (CH), 121.9 (CH), 118.4 (C_q), 109.0 (CH), 94.5 (C_q), 67.2 (CH_2); MS (ESI$^+$): m/z (%): 385.3 (100) [M + H]$^+$, 217.1 (8) [$C_7H_6I^+$]; purity (LC): 95%.

4-((4-Bromothiophen-2-yl)methoxy)thieno[3,4-*d*]pyrimidin-2-amine ($^{th}G_{th}$): *t*-BuOK (269 mg, 2.40 mmol, 4.0 equiv.) was dissolved in anhydrous pyridine (4 mL) and degassed through

three vacuum-argon cycles. (4-Bromothiophen-2-yl)methanol (579 mg, 3.00 mmol, 5.0 equiv.) was added to the mixture, followed by compound **1** (180 mg, 0.60 mmol, 1.0 equiv.). The mixture was stirred overnight at room temperature. Then, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in DCM, washed 3 times with water, dried over Na_2SO_4 and finally filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (DCM / MeOH, 95:5) to give $^{th}\mathbf{G_{th}}$ (69 mg, 34%) as light green powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.90 (d, J = 2.9 Hz, 1H, H7), 7.23 (s, 1H), 7.11 (s, 1H), 7.09 (d, J = 3.0 Hz, 1H, H9), 5.61 (s, 2H, CH_2), 5.04 (br s, 2H, NH_2); ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ (ppm) = 163.8 (C_q), 158.8 (C_q), 152.8 (C_q), 139.4 (C_q), 131.1 (CH), 124.5 (CH), 121.9 (CH), 118.2 (C_q), 109.5 (C_q), 108.9 (CH), 62.0 (CH_2); MS (ESI$^+$): *m*/*z* (%) = 342.1 (92), 344.0 (100) [*M* + H]$^+$, 177.0 (60), 174.9 (55) [$C_5H_5BrS^+$]; purity (LC): 100%.

4-Ethoxythieno[3,4-*d*]pyrimidin-2-amine ($^{th}\mathbf{G_{Et}}$): Compound **1** (180 mg, 0.6 mmol, 1.0 equiv.) was suspended in absolute EtOH (6 mL) and the mixture was refluxed for 2 h, while turning from deep yellow to dark-brown. The solvent was removed under reduced pressure and the product was purified by flash chromatography (SiO_2; eluent: DCM / MeOH, 95:5), to give $^{th}\mathbf{G_{Et}}$ (68 mg, 58%) as a yellow powder; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 7.90 (d, J = 3.2 Hz, 1H, H7), 7.27 (d, J = 3.2 Hz, 1H, H9), 5.02 (s, 2H, NH_2), 4.52 (q, J = 7.1 Hz, 2H, O*C*H_2CH$_3$), 1.46 (t, J = 7.1 Hz, 3H, OCH$_2$*C*H_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 164.6 (C_q), 159.5 (C_q), 152.8 (C_q), 121.6 (CH), 118.8 (C_q), 108.5 (CH), 62.7 (CH_2), 14.4 (CH_3); MS (ESI$^+$): *m*/*z* (%) = 196.1 (100) [*M* + H]$^+$, 168.1 (48) [*M* − Et + H]$^+$; purity (LC): 100%.

Photophysical and enzymatic experiments

General: Stock solutions of $^{th}\mathbf{G_N}$, $^{th}\mathbf{G_B}$, $^{th}\mathbf{G_i}$, $^{th}\mathbf{G_{th}}$, and $^{th}\mathbf{G_{Et}}$ were prepared in DMSO at a concentration of 2 to 10 mM. Absorption spectra were measured on a UV Hitachi 2900 spectrophotometer using the slit width at 1.5 nm, resolution of 0.5 nm and a scan speed of 100 nm min^{-1}.

Quantum yields measurements: Solutions of compounds (in water) and of quantum yield standard (quinine sulfate, 0.5 M in H_2SO_4, Φ = 0.546)[2] were prepared in quartz cells with a cross-section of 1 × 1 cm, in a final volume of 3 mL and an optical density of OD ≤ 0.1 at the excitation wavelength (325 nm). Absorption at 325 nm was measured, and fluorescence spectra were recorded at room temperature on a FluoroMax-3 spectrofluorimeter using λ_{ex} = 325 nm, λ_{em} = 335 to 700 nm, and slit widths of 2.5 nm. After the measurement, 1 mL of the solution was removed from the cell and substituted with 1 mL of water (or 0.5 M in H_2SO_4 for

quinine sulfate), and absorption and emission spectra of the resulting solution were recorded again. The procedure was repeated four times in order to obtain a total of five data points. The integrated areas of emission spectra (*y*) were plotted as a function of the absorbance at 325 nm (*x*) and fitted to a linear model with zero intercept ($y = B(x)$). The quantum yield (Φ) for each sample was calculated by using Equation (1):

$$\Phi = \Phi_{STD} \frac{B}{B_{STD}} \frac{n^2}{n_{STD}^2}, \quad (1)$$

where Φ_{STD} is the fluorescence quantum yield of the standard, B and B_{STD} are the slopes of the plots described above, and n and n_{STD} the solvent refractive indexes of the sample and the standard solutions, respectively.

Real-time monitoring of reaction of thG derivatives with MGMT: Recombinant, His-tagged human MGMT was purchased from Novus Biologicals (NBC1-18534, 0.5 mg mL^{-1} solution) and the actual protein concentration (9.8-10.1 µM) was determined by OD_{280} measurement (NanoDrop) using ε_{280} = 39,300 cm^{-1} M^{-1} as reported by Fried et al.[3] Working solution of compounds (100 or 200 nM) were prepared in 10 mM Tris-HCl, 100 mM NaCl, 1 mM DTT buffer (pH 8.5) by dilution of intermediate solutions of $^{th}\mathbf{G}_{\mathbf{I}}$ or $^{th}\mathbf{G}_{\mathbf{th}}$ in DMSO (2 µM, final DMSO concentration: 5% v/v), and placed in semi-micro quartz fluorescence cells (1 × 0.2 cm, working volume: 400 µL). Time runs of fluorescence emission intensity were recorded on an Agilent Cary Eclipse Bio fluorimeter equipped with a thermostatic sample holder set at 37 °C, using λ_{ex} = 314 nm, λ_{em} = 400 nm, slit widths of 10 nm, PMT voltage of 800 V, and integration time of 1.5 s. After incubation for approximately 3 min in the absence of the enzyme, an aliquot of MGMT was rapidly added to the cell, the solution was mixed, and fluorescence intensity was recorded every 16 s for a total time of 3000 s. Data were time-adjusted to set the enzyme addition point to t = 0.

In the experiments with inhibitor, Lomeguatrib (400 to 0.4 µM solution in DMSO, final concentration: 10 nM to 1 µM) was added to the solution of the $^{th}\mathbf{G}_{\mathbf{th}}$ probe (prepared from a 4 µM DMSO solution, to the keep final DMSO concentration of 5% v/v) prior to addition of the enzyme. The experiments were performed as described above.

References

(1) Shin, D.; Sinkeldam, R. W.; Tor, Y. *J. Am. Chem. Soc.* **2011**, *133* (38), 14912–14915.

(2) Brouwer, A. M. *Pure Appl. Chem.* **2011**, *83* (12), 2213–2228.

(3) Rasimas, J. J.; Pegg, A. E.; Fried, M. G. *J. Biol. Chem.* **2003**, *278* (10), 7973–7980.

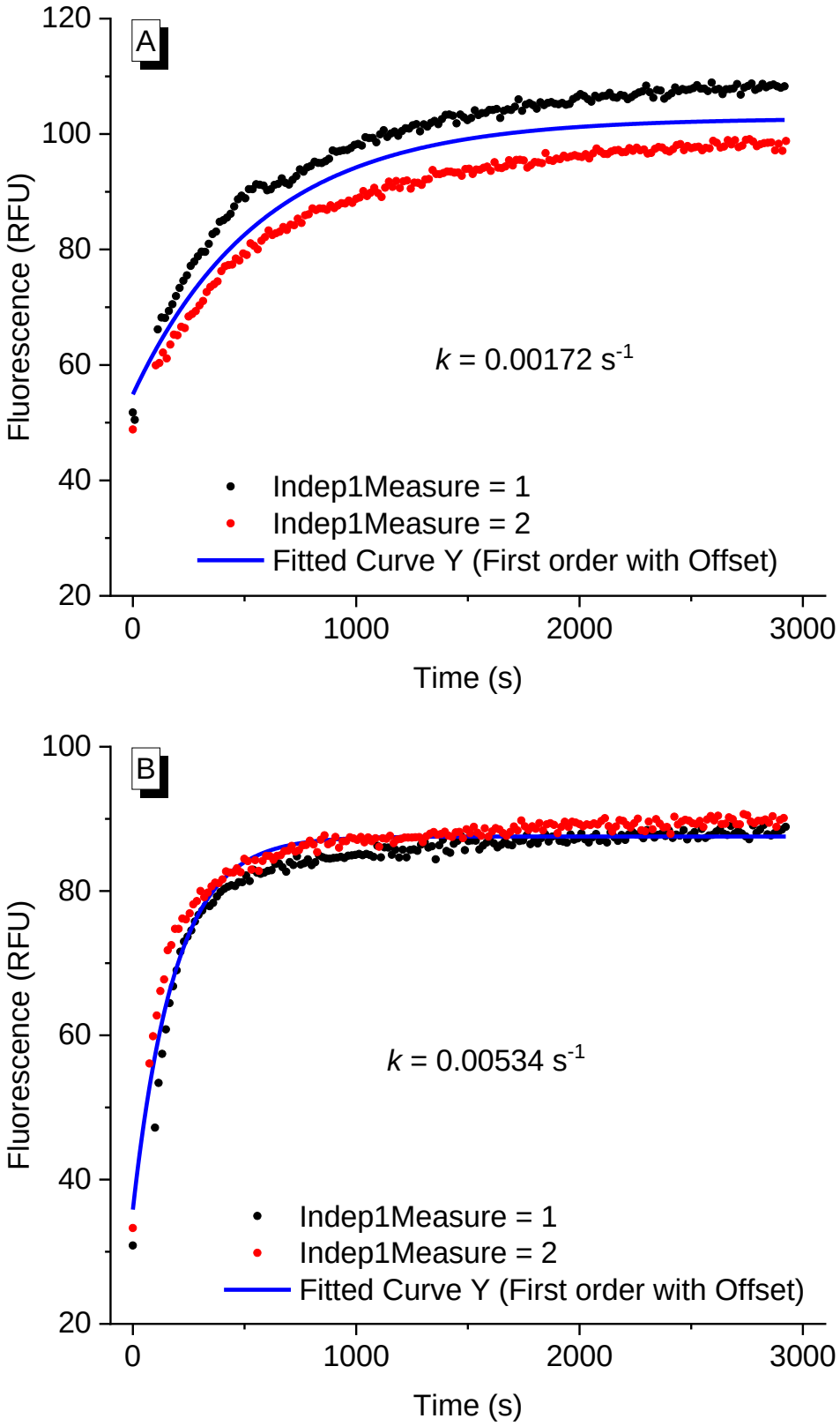
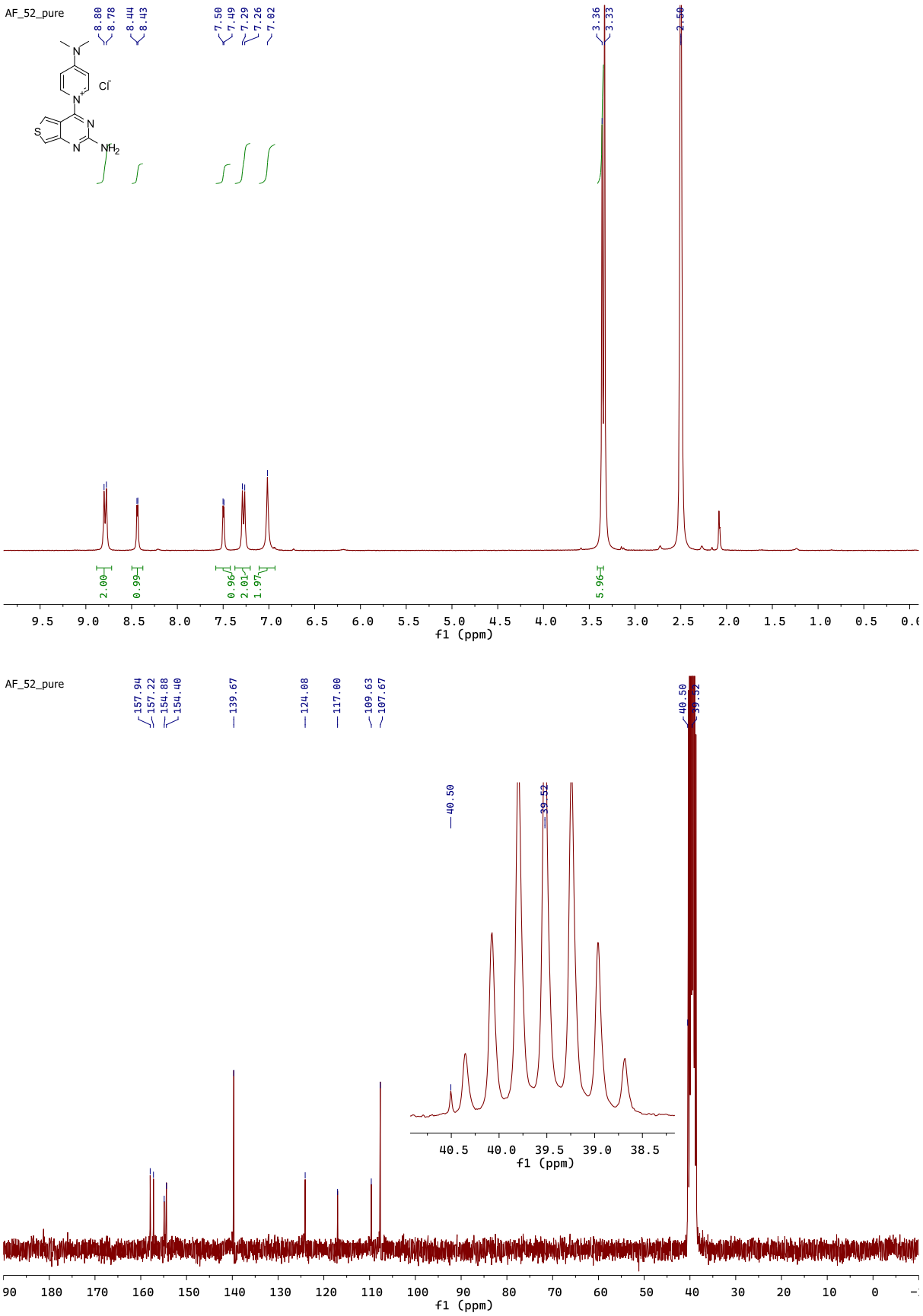
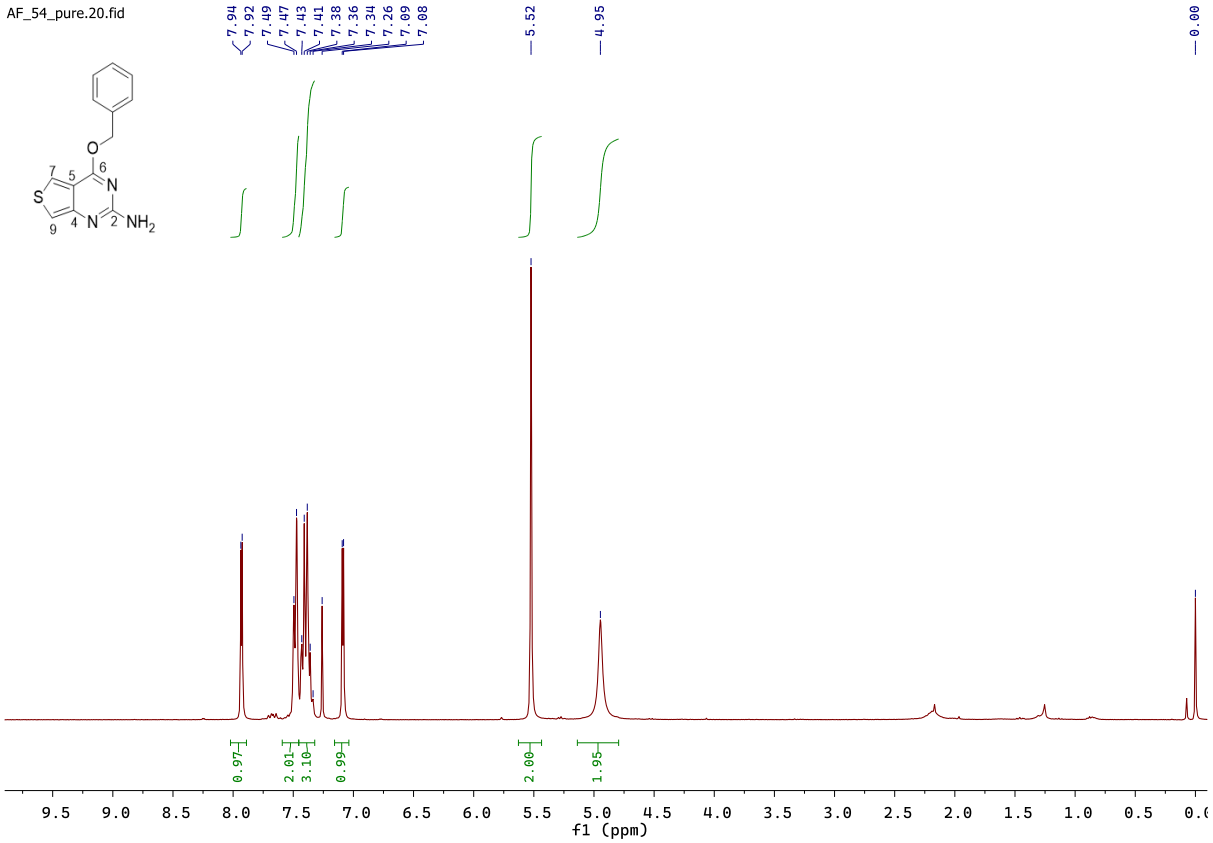
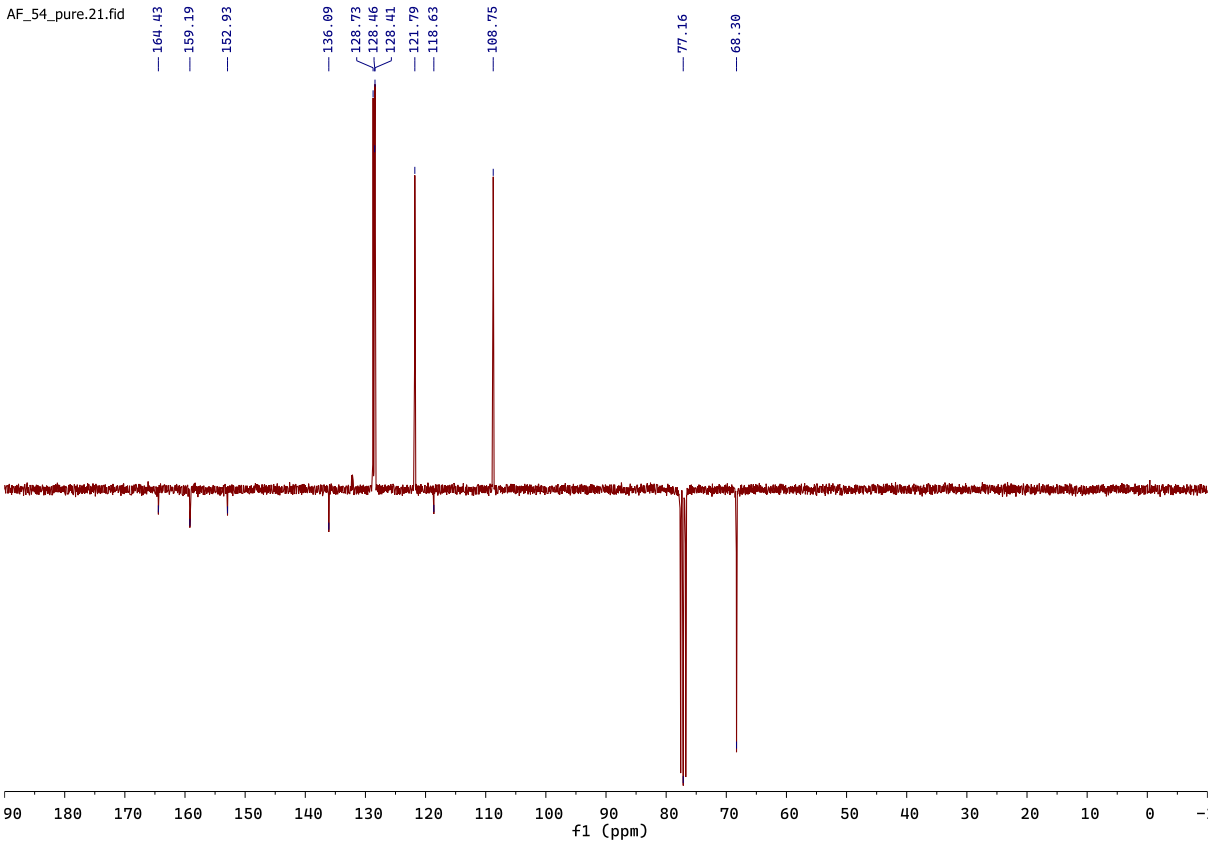


Figure S1. Fluorescence increase upon addition of MGMT (20 nM) to solutions of A) $^{th}\mathbf{G}_{I}$ and B) $^{th}\mathbf{G}_{th}$ (200 nM in 10 mM Tris-HCl, 100 mM NaCl, 10 mM DTT buffer, pH 8.5). In each panel, data from two independent experiments were globally fitted with pseudo-first-order reaction model, with the rate constant (*k*) values indicated in the plots.

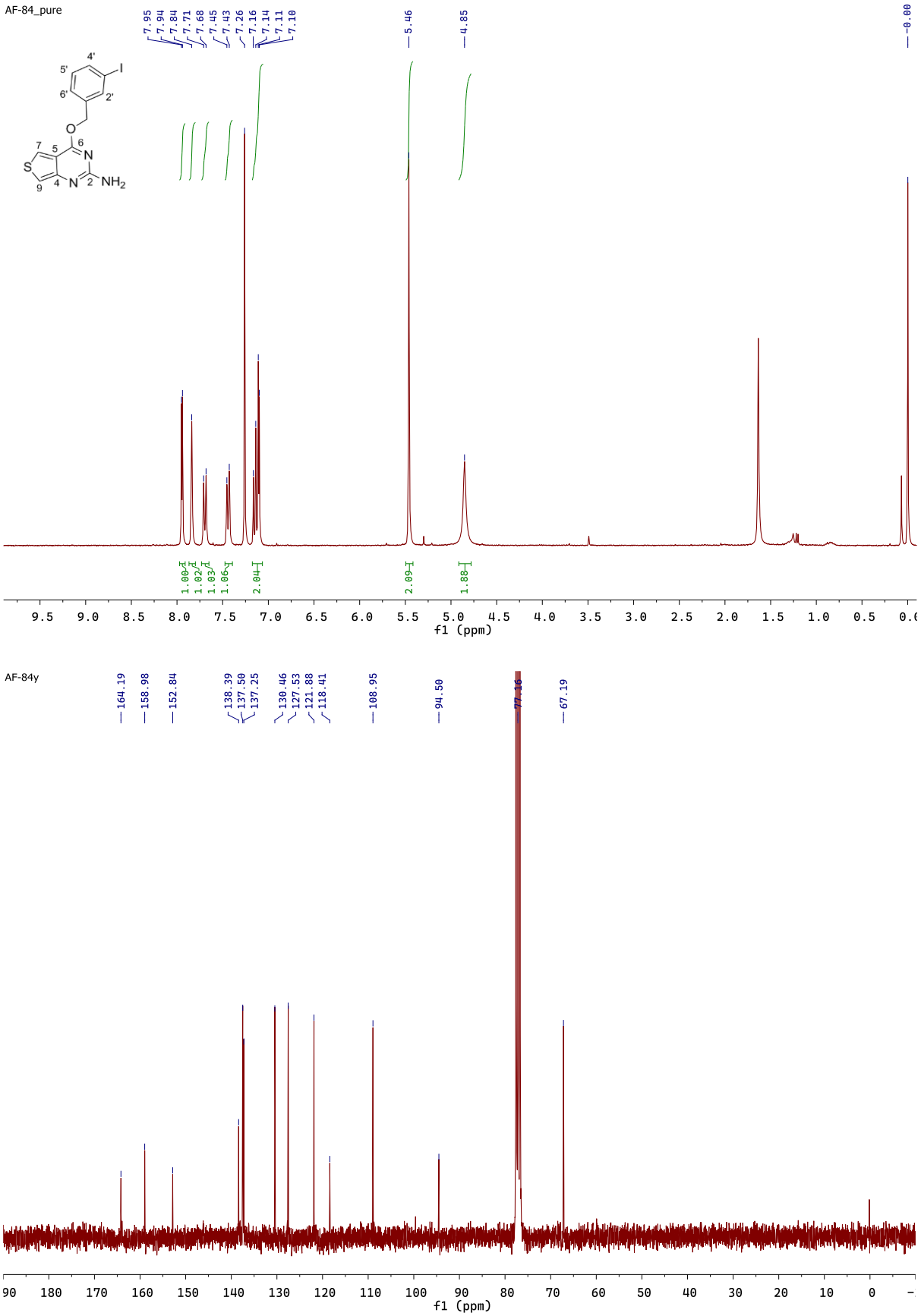


^{1}H (top) and ^{13}C (bottom) spectra of compound **1** in DMSO-d_6.

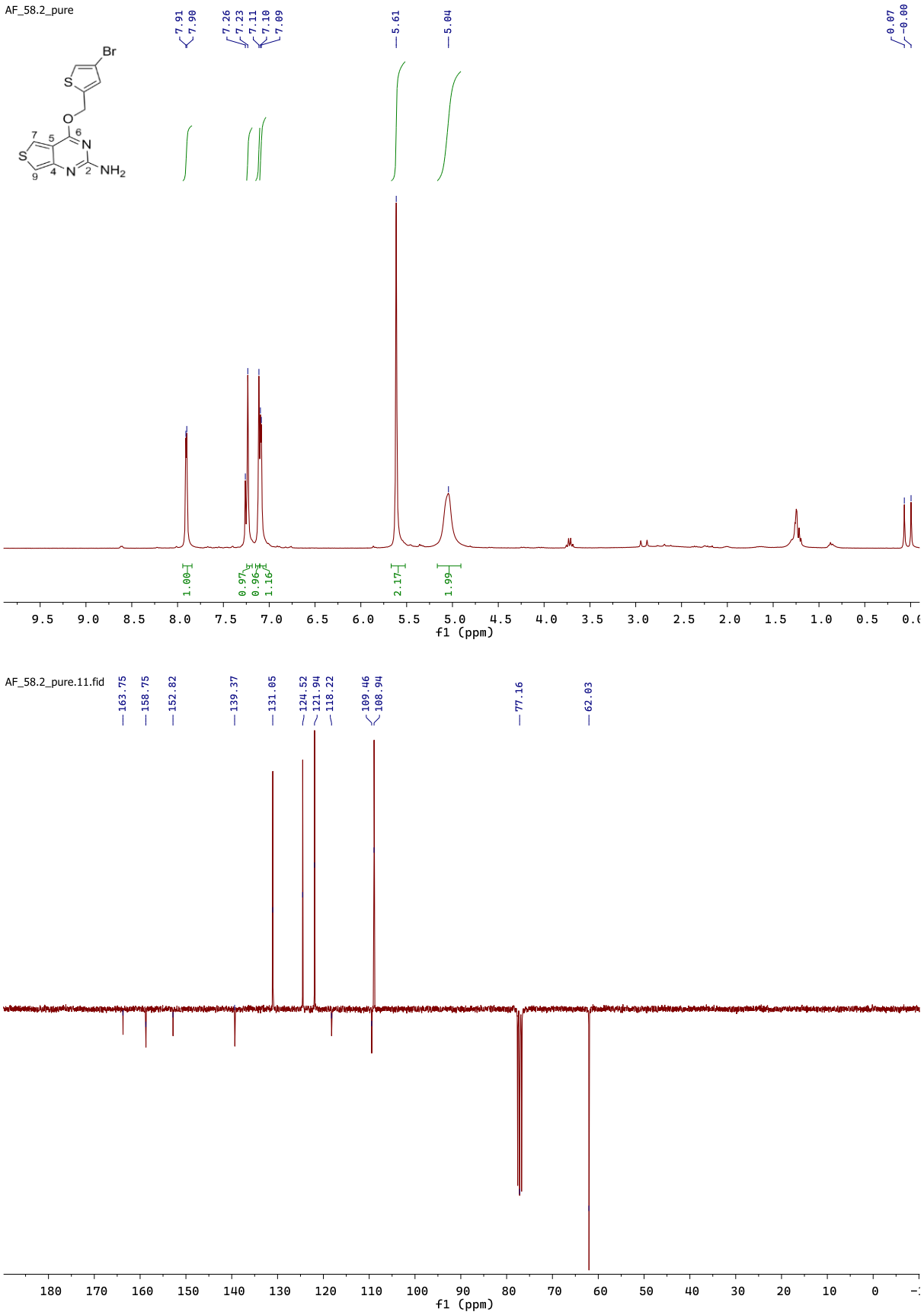




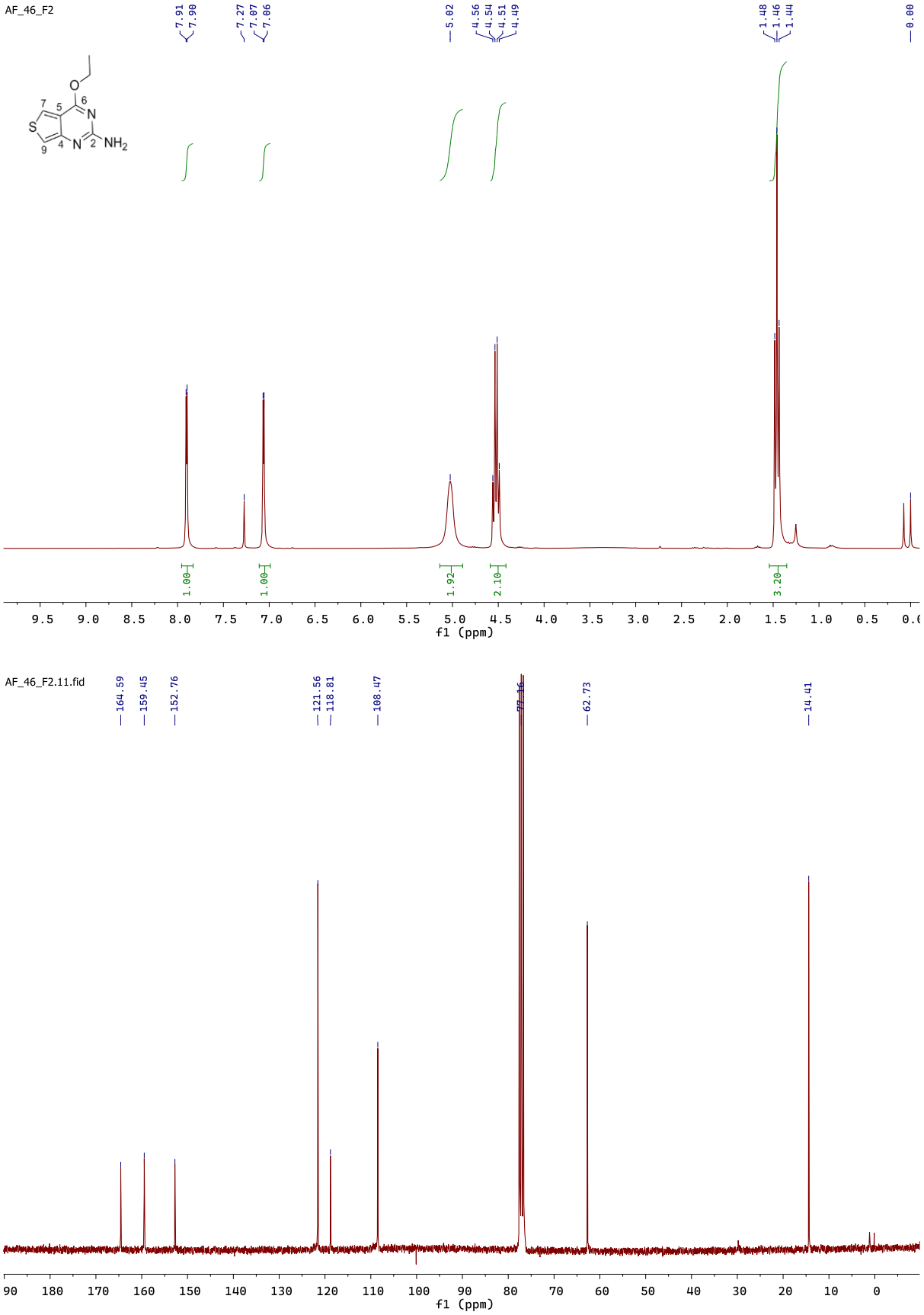
^{1}H (top) and ^{13}C APT (bottom) spectra of $^{th}\mathbf{G_B}$ in $CDCl_3$.



^{1}H (top) and ^{13}C (bottom) spectra of $^{th}\mathbf{G}_{I}$ in $CDCl_3$.



^{1}H (top) and ^{13}C APT (bottom) spectra of $^{th}\mathbf{G}_{th}$ in $CDCl_3$.



^{1}H (top) and ^{13}C (bottom) spectra of $^{th}\mathbf{G}_{\mathbf{Et}}$ in $CDCl_3$.