Supplementary Information

1. Materials and Methods

Reagents were purchased from Sigma-Aldrich, TCI, Spectrum, Acros, Fisher Scientific, and VWR and were used without further purification unless otherwise specified. Methyl 4-amino thiophene 3-carboxylate hydrochloride was purchased from Matrix Scientific. Azetidine hydrochloride and difluoroazetidine hydrochloride were purchased from Synthonix. Solvents were purchased from Sigma-Aldrich and Fisher Scientific, and dried by standard techniques. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254). Column chromatography was carried out with Teledyne ISCO Combiflash Rf with silica gel particle size 40-63 µm. NMR spectra were obtained on Jeol ECA 500 MHz and Varian VX 500 MHz at room temperature, except for compounds **6a** (taken at 80 °C) and **11** (taken at 60 °C) . Mass spectra were obtained on an Agilent 6230 HR-ESI-TOF MS at the Molecular Mass Spectrometry Facility at the UCSD Chemistry and Biochemistry Department.

1.1. Abbreviations

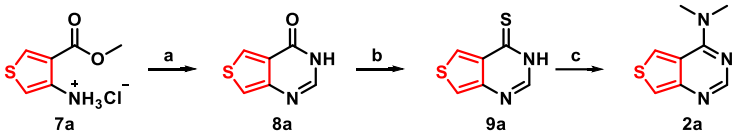
ACN: acetonitrile; DCM: dichloromethane; DIPEA: Diisopropylethylamine; DMSO: dimethyl sulfoxide; EtOH: ethanol; HCl: Hdyrochloric acid; $HNMe_2$: dimethylamine; K_2CO_3: potassium carbonate, MeOH: methanol; mQ: Milliq water; NaOH: sodium hydroxide; P_2O_5: phosphorous pentoxide; P_2S_5: phosphorous pentasulfide; rt: room temperature; TFA: trifluoroacetic acid.

2. Synthetic procedures

Starting precursors **7b** and **10** were synthesized based on previously published procedures.[1,2]

2.1. Synthesis of dimethyladenine analogue 2a

Scheme S1. Synthesis of thieno- derivative **2a**



(a) Formamidine acetate, EtOH, reflux, ON, 78%. (b) P_2S_5, Pyridine, 110 °C, 2 h, 83%. (c) 2M $HNMe_2$ in MeOH, 60 °C, ON, 64%.

Thieno[3,4-*d*]pyrimidin-4(3*H*)-one (8a)

To a 1 L round-bottom flask was added methyl 4-amino thiophene 3-carboxylate hydrochloride **7a** (50 g, 256 mmol) and formamidine acetate (80.7 g, 775 mmol, 3 eq). EtOH (500 ml) was added, and the solution was refluxed at 105 °C overnight. A precipitate gradually forms and following reaction completion the solution is cooled and filtered. The solid is washed with water and EtOH, then dried over P_2O_5 to yield the desired product as an off-greenish fluffy solid (30.7 g, 78%).^{1}H NMR (500 MHz, DMSO-d_6) δ 11.66 (s, 1H), 8.47 (d, J = 3.2 Hz, 1H), 7.80 – 7.75 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.26, 148.96, 144.13, 128.32, 126.84, 118.73. ESI-HRMS calculated for $C_6H_5N_2OS$ $[M+H]^+$ 153.0117, found 153.0114.

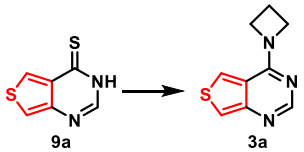
Thieno[3,4-*d*]pyrimidine-4(*3H*)-thione (9a).

To a flame-dried 100 ml round bottom flask purged with argon was added compound **8a** (15 g, 98.7 mmol) and phosphorous pentasulfide (65.8 g, 296 mmol, 3 eq). Anhydrous pyridine (750 mL) was added and the heterogenous solution was stirred at room temperature for 10 minutes, then heated to 105 °C and stirred for 3 hours. If the reaction did not reach completion within the allotted time, half an equivalent of phosphorous pentasulfide was added every half hour until the starting material was fully consumed. Once finished, the reaction was cooled to room temperature and evaporated to dryness. 750 ml of water was added to the crude to form an orange suspension. A solution of 40% NaOH in water was then added dropwise until the pH was 12-13 and the suspension cleared. 37% HCl was then added dropwise until the solution was neutral and a yellowish precipitate formed that was filtered off and dried over P_2O_5 for 2 hours (14.5g, 83%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.20 (s, 2H), 8.55 (d, J = 3.3 Hz, 2H), 7.91 (d, J = 3.3 Hz, 1H), 7.82 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.92, 143.85, 141.92, 133.31, 130.74, 120.12. ESI-HRMS calculated for $C_6H_4N_2S_2$ $[M+H]^+$ 168.9889, found 168.9887.

***N*,*N*-dimethylthieno[3,4-*d*]pyrimidin-4-amine (2a)**

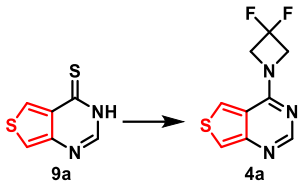
The thionated substate **9a** (0.15 g, 0.9 mmol, 1 eq) was placed in a heavy wall cylindrical pressure vessel along with a solution of 2M dimethylamine in MeOH (7 ml, 14 mmol, 15 eq). The vessel was sealed, and the suspension was stirred at 65 °C overnight. The solution gradually turns clear, and following reaction completion the vessel was then cooled, and the solution was evaporated to dryness. Column chromatography of the crude residue with a gradient of 0-5% MeOH in DCM yielded a cream-colored solid (0.1 g, 64%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.46 (d, *J* = 3.0 Hz, 1H), 8.34 – 8.31 (m, 1H), 8.29 (d, *J* = 3.4 Hz, 1H), 3.84 (d, *J* = 3.6 Hz, 3H), 3.64 (d, *J* = 3.6 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 158.51, 149.04, 138.52, 127.44, 118.32, 112.39, 42.22, 41.66. ESI-HRMS calculated for $C_8H_{10}N_3S$ $[M+H]^+$ 180.0590, found 180.0591.

2.2. Synthesis of azetidine-analogues 3a–6a



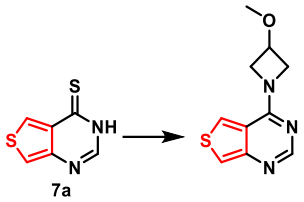
4-(azetidin-1-yl)thieno[3,4-*d*]pyrimidine (3a)

Compound **9a** (100 mg, 0.6 mmol, 1 eq) was placed in a 25 ml round-bottom flask and MeOH (5 ml) was added creating a suspension. Azetidine hydrochloride (0.94 g, 10 mmol, 17 eq) was added followed by DIPEA (1.9 g, 15 mmol, 2.6 ml, 25 eq). The solution was allowed to stir at 70 °C overnight. The reaction was cooled to room temperature and evaporated to dryness. The crude was dissolved in DCM and extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-5% MeOH in DCM yielded the desired compound **3a** as a yellowish solid (40 mg, 38%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, *J* = 3.1 Hz, 1H), 8.02 (s, 1H), 7.68 (d, *J* = 3.1 Hz, 1H), 4.63 (s, 2H), 4.19 (s, 2H), 2.41 (q, *J* = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.84, 154.22, 151.06, 123.18, 119.47, 114.66, 54.17 – 52.15 (m), 16.92. ESI-HRMS calculated for $C_9H_{10}N_3S$ $[M+H]^+$ 192.0590, found 192.0591.



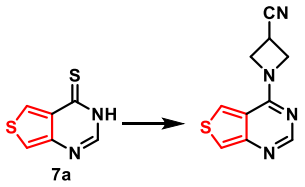
4-(3,3-difluoroazetidin-1-yl)thieno[3,4-d]pyrimidine (4a)

Compound **9a** (0.11 g, 0.7 mmol) was placed in a 25 ml round-bottom flask and MeOH (5 ml) was added creating a suspension. 2,2-Difluoroazetidine hydrochloride (1.3 g, 10 mmol, 20 eq) was added followed by DIPEA (1.9 g, 2.6 ml, 15 mmol, 23 eq). The solution was allowed to stir at 70 °C overnight. The reaction was cooled to room temperature and evaporated to dryness. The crude was dissolved in DCM and extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-4% MeOH in DCM yielded the desired compound **4a** as a yellowish solid (78 mg, 53%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (d, J = 3.1 Hz, 1H), 8.17 (s, 1H), 7.86 (d, J = 3.2 Hz, 1H), 4.97 – 4.77 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.30 (t, J = 4.3 Hz), 153.66, 150.94, 123.26, 119.43, 119.37, 117.27, 116.00, 115.11. ESI-HRMS calculated for $C_9H_8F_2N_3S$ $[M+H]^+$ 228.0402, found 228.0403.



4-(3-methoxyazetidin-1-yl)thieno[3,4-*d*]pyrimidine (5a)

Compound **7a** (100 mg, 0.6 mmol, 1 eq) was placed in a 25 ml round-bottom flask and MeOH (5 ml) was added creating a suspension. 3-methoxyazetidine hydrochloride (1.5 g, 12 mmol, 20 eq) was added followed by DIPEA (2.3 g, 18 mmol, 3.1 ml, 30 eq). The solution was allowed to stir at 70 °C overnight. The reaction was cooled to room temperature and evaporated to dryness. The crude was dissolved in DCM and extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-5% MeOH in DCM yielded the desired compound **5a** as a yellowish solid (40 mg, 30%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (d, J = 3.1 Hz, 1H), 8.05 (s, 1H), 7.73 (d, J = 3.1 Hz, 1H), 4.81 (s, 1H), 4.38 (m, 3H), 3.99 (s, 1H), 3.26 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.99, 154.11, 151.03, 123.24, 119.51, 114.99, 70.22, 56.03. ESI-HRMS calculated for $C_{10}H_{12}N_3OS$ $[M+H]_+$ 222.0696, found 222.0695.

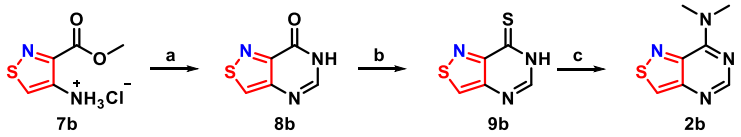


1-(thieno[3,4-*d*]pyrimidin-4-yl)azetidine-3-carbonitrile (6a)

Compound **7a** (100 mg, 0.6 mmol, 1 eq) was placed in a 25 ml round-bottom flask and MeOH (5 ml) was added creating a suspension. 3-azetidine carbonitrile hydrochloride (1.41 g, 12 mmol, 20 eq) was added followed by DIPEA (2.3 g, 18 mmol, 3.1 ml, 30 eq). The solution was allowed to stir at 70 °C overnight. The reaction was cooled to room temperature and evaporated to dryness. The crude was dissolved in DCM and extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-5% MeOH in DCM yielded the desired compound **6a** as a yellowish solid (43 mg, 33%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 (d, J = 3.1 Hz, 1H), 8.11 (s, 1H), 7.75 (d, J = 3.1 Hz, 1H), 4.72 (t, J = 9.1 Hz, 2H), 4.64 – 4.54 (m, 2H), 4.04 – 3.94 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.29, 153.88, 151.05, 123.23, 121.07, 115.61, 18.93. ESI-HRMS calculated for $C_{10}H_9N_4S$ $[M+H]^+$ 219.0542, found 219.0539.

2.3. Synthesis of isothiazolo[4,3-*d*] based dimethyladenine analogue 2b

Scheme S2. Synthesis of isothiazolo- derivative **2b**



(a) Formamidine acetate, EtOH, reflux, ON, 76%. (b) Lawesson's reagent, Pyridine, 110 °C, 2 h, 92%. (c) 2M $HNMe_2$ in MeOH, 60 °C, ON, 69%.

Isothiazolo[4,3-*d*]pyrimidin-7(6*H*)-one (8b)

To a 250 ml round-bottom flask was added **7b** (1.2 g, 6.4 mmol) and formamidine acetate (2 g, 19.2 mmol, 3 eq). 200 proof ethanol (14 ml) was added, and the solution was refluxed at 105 °C overnight. A precipitate gradually forms and following reaction completion the solution is cooled and filtered. The precipitate was filtered off and washed with cold EtOH yielding a yellowish solid that was dried over P_2O_5 (0.74 g, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.15 (s, 1H), 9.47 (s, 1H), 7.98 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.44, 151.68, 148.89, 146.38, 145.27. ESI-HRMS calculated for $C_5H_3N_3OS$ $[M+Na]^+$ 175.9889, found 175.9891.

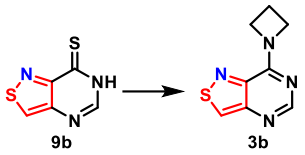
Isothiazolo[4,3-*d*]pyrimidine-7(6*H*)-thione (9b)

To a 100 ml round-bottom flask that was flame-dried and argon purged was added compound **8b** (0.88 g, 5.7 mmol) and anhydrous pyridine (25 ml), Lawesson's reagent (4.6 g, 11.5 mmol, 2 eq) was added and the solution was stirred at 110 °C for 2 hours. Upon reaction completion the solution was cooled in an ice bath and concentrated to dryness. The crude was resuspended in water (6 ml) and placed in an ice bath. 40% NaOH in water was added dropwise until the solution was sufficiently clear and the suspension had cleared. After stirring an additional 5 minutes, the solution was solution by adding 37% HCl dropwise. The resulting precipitate was filtered off and washed with water, yielding the desired product **9b** as a desired solid (0.89 g, 92%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.16, 155.59, 148.43, 144.12, 143.32. ESI-HRMS calculated for $C_9H_8F_2N_3S$ $[M-H]^-$ 167.9696, found 167.9696.

***N*,*N*-dimethylisothiazolo[4,3-*d*]pyrimidin-7-amine (2b)**

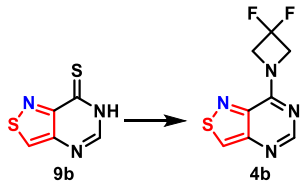
The thionated substrate **9b** (0.11 g, 0.6 mmol) was placed in a 50 ml round-bottom flask. A solution of 2M $HNMe_2$ in MeOH (15 ml, 30 mmol, 50 eq) was added and the reaction was stirred at 60 °C overnight. The solution was then evaporated to dryness and the crude was subjected to column chromatography using a gradient of 0-5% MeOH in DCM to yield an orange-colored solid (80 mg, 69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 0H), 8.26 (s, 0H), 3.91 (s, 1H), 3.29 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.32 (d, J = 1.9 Hz), 154.55, 154.28, 151.32 (d, J = 2.3 Hz), 146.62, 143.87 – 143.67 (m). ESI-HRMS calculated for $C_7H_9N_4S$ $[M+H]^+$ 181.0543, found 181.0542.

2.4. Synthesis of azetidine analogues 3b–6b



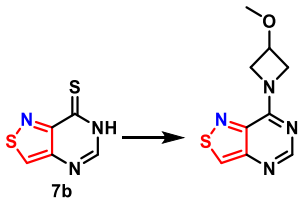
7-(azetidin-1-yl)isothiazolo[4,3-*d*]pyrimidine (3b)

The thionated substrate **9b** (0.1 g, 0.6 mmol) was placed in a 25 ml round-bottom flask and MeOH (5 ml) was added to create a suspension. Azetidine hydrochloride (1.1 g, 12 mmol, 20 eq) was added followed by DIPEA (2.3 g, 18 mmol, 3.1 ml, 30 eq). The solution was allowed to stir at 60 °C overnight. Following reaction completion, the solution was cooled to room temperature and evaporated to dryness. The crude was then extracted between DCM and saturated sodium bicarbonate solution. The aqueous phase was washed 3 times with DCM and the organic phases were combined, dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude residue using a gradient of 0-3% MeOH in DCM yielded the desired product **3b** as a yellowish solid (68 mg, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.18 (s, 1H), 4.75 (t, J = 7.7 Hz, 3H), 4.23 (t, J = 7.7 Hz, 3H), 2.44 (dd, J = 15.6, 7.9 Hz, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.00, 154.46, 149.86, 146.15, 143.68, 54.57, 50.81, 17.43. ESI-HRMS calculated for $C_7H_9N_4S$ $[M+H]^+$ 193.0542, found 193.0541.



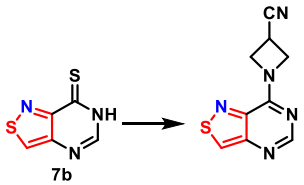
7-(3,3-difluoroazetidin-1-yl)isothiazolo[4,3-*d*]pyrimidine (4b)

Compound **9b** (0.1 g 0.6 mmol) was placed in a 25 ml round-bottom flask and MeOH (5 ml) was added to create a suspension. 2,2-difluoroazetidine hydrochloride (1.5 g, 12 mmol, 20 eq) was added followed by DIPEA (2.3 g, 18 mmol, 3.1 ml, 30 eq). The reaction was allowed to stir at 60 °C overnight. Following reaction completion, the solution was evaporated to dryness. Following reaction completion, the solution was cooled to room temperature and evaporated to dryness. The crude was then extracted between DCM and saturated sodium bicarbonate solution. The aqueous phase was washed 3 times with DCM and the organic phases were combined, dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude residue using a gradient of 0-3% MeOH in DCM yielded the desired product **4b** as a yellowish solid (94 mg, 70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.33 (s, 1H), 5.15 (s, 3H), 4.68 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.92 (t, J = 4.5 Hz), 154.56, 149.84, 145.82, 145.27, 119.58, 117.42, 115.26. ^{19}F NMR (471 MHz, DMSO-d_6) δ -99.57 (p, J = 12.5 Hz). ESI-HRMS calculated for $C_8H_6F_2N_4S$ $[M+H]^+$ 229.0354, found 229.0355.



4-(3-methoxyazetidin-1-yl)thieno[3,4-*d*]pyrimidine (5b)

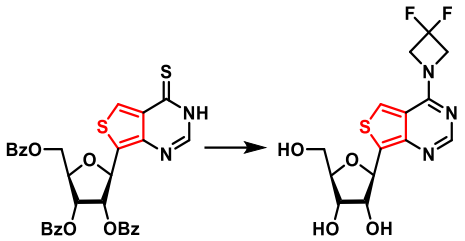
Compound **7b** (61 mg, 0.36 mmol, 1 eq) was placed in a 25 ml round-bottom flask and MeOH (3 ml) was added creating a suspension. 3-methoxyazetidine hydrochloride (0.9 g, 7.3 mmol, 20 eq) was added followed by DIPEA (1.4 g, 11 mmol, 1.9 ml, 30 eq). The solution was allowed to stir at 70 °C overnight. The reaction was cooled to room temperature and evaporated to dryness. The crude was dissolved in DCM and extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-5% MeOH in DCM yielded the desired compound **5b** as a yellowish solid (27 mg, 33%) following recrystallization from DCM/hexanes. ^{1}H NMR (500 MHz, DMSO- d_6) δ 9.44 (s, 1H), 8.21 (s, 1H), 4.96 (dd, J = 10.4, 6.1 Hz, 1H), 4.51 (d, J = 11.0 Hz, 1H), 4.45 – 4.37 (m, 2H), 4.03 (d, J = 10.6 Hz, 1H), 3.26 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.92, 154.64, 149.87, 146.11, 144.12, 70.55, 61.28, 57.69, 56.04. ESI-HRMS calculated for $C_9H_{11}N_4OS$ $[M+H]^+$ 223.0648, found 223.0649.



1-(isothiazolo[4,3-d]pyrimidin-7-yl)azetidine-3-carbonitrile (6b)

Compound **7b** (81 mg, 0.5 mmol, 1 eq) was placed in a 25 ml round-bottom flask and MeOH (4 ml) was added creating a suspension. 3-azetidine carbonitrile hydrochloride (1.14 g, 10 mmol, 20 eq) was added followed by DIPEA (1.86 g, 14 mmol, 2.5 ml, 30 eq). The solution was allowed to stir at 70 °C overnight. The reaction was cooled to room temperature and evaporated to dryness. The crude was dissolved in DCM and extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-5% MeOH in DCM yielded the desired compound **6b** as a yellowish solid (43 mg, 41%) following recrystallization from Et_2O/hexanes. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.27 (s, 1H), 5.07 – 4.85 (m, 2H), 4.55 – 4.36 (m, 2H), 4.02 (tt, J = 9.0, 6.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.73 (s), 154.69 (s), 149.82 (s), 145.72 (s), 144.70 (s), 120.95 (s), 57.30 (s), 53.83 (s), 19.38 (s). ESI-HRMS calculated for $C_9H_8N_5S$ $[M+H]^+$ 218.0495, found 218.0497.

2.5. Synthesis of nucleoside 11



10

(*2R*,*5R*)-2-(4-(3,3-difluoroazetidin-1-yl)thieno[3,4-*d*]pyrimidin-7-yl)-5-(hydroxymethyl) tetrahydrofuran-3,4-diol (11)

Compound **10** (150 mg, 0.24 mmol, 1 eq) was placed in a 25 ml round-bottom flask. 5 ml of a 1:1 mixture of satd. NH_4OH: satd. $MeNH_2$ was added. The solution was allowed to stir at room temperature for 15 minutes, then evaporated to dryness. The reaction was cooled to room temperature and evaporated to dryness. The crude riboside was coevaporated with MeOH three times followed by DCM twice. Once dried, the crude was dissolved in MeOH and 3,3-dilfuoroazetidine HCl (632 mg, 4.9 mmol, 20 eq) was added followed by DIPEA (950 mg, 7.35 mmol, 1.3 ml, 30 eq). The reaction was allowed to stir at 60 °C overnight. The solution was evaporated to dryness and the crude was resuspended in MeOH. 1.5 g K_2CO_3 was added, and the heterogeneous mixture was stirred for 15 minutes, then filtered. The filtrate was evaporated and loaded onto silica. Column chromatography using a gradient of 0-7% MeOH in DCM yielded the desired product as a gold solid (65 mg, extracted with saturated sodium bicarbonate followed by brine. The organic phase was dried with sodium sulfate, filtered, and evaporated to dryness. Column chromatography of the crude product with a gradient of 0-5% MeOH in DCM yielded the desired compound **5b** as a yellowish solid (54 mg, 61%). The compound was then triturated with ethanol yielding a white solid (34 mg, 24%) suitable in purity for photophysical studies. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.15 (s, 1H), 5.35 (d, *J* = 6.5 Hz, 1H), 5.05 (s, 1H), 4.87 (t, *J* = 12.2 Hz, 4H), 4.75 (s, 1H), 4.13 (dd, *J* = 6.5, 5.1 Hz, 1H), 4.01 – 3.92 (m, 1H), 3.85 (q, *J* = 4.1 Hz, 1H), 3.56 (dd, *J* = 11.7, 4.0 Hz, 1H), 3.50 (dd, *J* = 11.7, 4.5 Hz, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 157.43 (t, *J* = 4.6 Hz), 153.24, 147.22, 133.32, 121.52, 119.95, 117.35, 85.74, 77.98, 77.86, 72.29, 62.79. ESI-HRMS calculated for $C_{14}H_{16}F_2N_3O_4S$ $[M+H]^+$ 360.0824, found 360.0828.

3. Structural analysis

3.1. Experimental summary

The single crystal X-ray diffraction studies were carried out on a Bruker APEX II Ultra CCD diffractometer equipped with Cu K_α radiation (λ = 1.54178). A 0.170 x 0.105 x 0.025 mm orange crystal was mounted on a Cryoloop with Paratone oil.

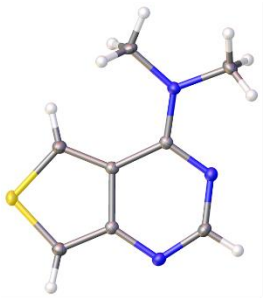
Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ϖ scans. Crystal-to-detector distance was 45 mm using exposure 1.0s s with a scan width of 0.75°. Data collection was 100.0% complete to 25.242° in θ.

A total of 6956 reflections were collected covering the indices, -22<=h<=23, -5<=k<4, -17<=l<=17. 2074 reflections were found to be symmetry independent, with a R_{int} of 0.0663. Indexing and unit cell refinement indicated a **P**rimitive, **Orthorhombic** lattice. The space group was found to be **$Pca2_1$**. The data were integrated using the Bruker SAINT Software program and scaled using the SADABS software program. Solution by direct methods (SHELXT) produced a complete phasing model consistent with the proposed structure.

All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-2014). All carbon bonded hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-2014. All other hydrogen atoms (H-bonding) were located in the difference map (N-H, O-H). There positions were refined using "riding" model. Crystallographic data are summarized in Table 1.

3.2. X-ray crystal structures

Table S1. Crystal data and structure refinement for compound **2a**

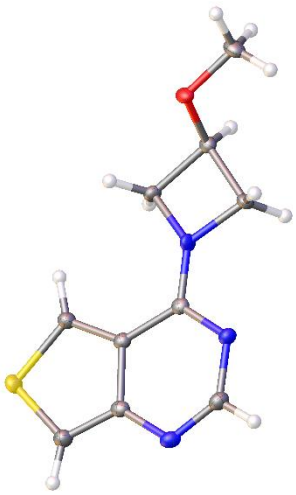


Report date	2018-11-13	
Identification code	tor_kh1027_3	
Empirical formula	C8 H9 N3 S	
Molecular formula	C8 H9 N3 S	
Formula weight	179.24	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 7.1286(4) Å	α= 92.0730(10)°.
	b = 7.4500(4) Å	β= 103.7770(10)°.
	c = 7.8894(4) Å	γ = 102.2880(10)°.
Volume	395.94(4) $Å^3$	
Z	2	
Density (calculated)	1.503 Mg/m^3	
Absorption coefficient	0.348 mm^{-1}	
F(000)	188	
Crystal size	0.18 x 0.04 x 0.04 mm^3	
Crystal color, habit	colorless plank	
Theta range for data collection	2.669 to 26.368°.	
Index ranges	-8<=h<=8, -9<=k<=9, -8<=l<=9	
Reflections collected	4560	
Independent reflections	1625 [R(int) = 0.0281]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.5628 and 0.5205	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1625 / 0 / 111	
Goodness-of-fit on F^2	1.052	
Final R indices [I>2sigma(I)]	R1 = 0.0293, wR2 = 0.0782	
R indices (all data)	R1 = 0.0308, wR2 = 0.0797	
Largest diff. peak and hole	0.519 and -0.195 e.$Å^-$	

Table S2. Crystal data and structure refinement for compound **2b**

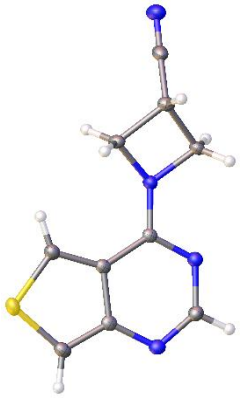
Report date	2019-08-08	
Identification code	tor_mmetza	
Empirical formula	C7 H8 N4 S	
Molecular formula	C7 H8 N4 S	
Formula weight	180.23	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 7.6743(3) Å	α= 95.9810(10)°.
	b = 7.7814(3) Å	β= 98.6310(10)°.
	c = 13.3599(6) Å	γ = 91.5710(10)°.
Volume	783.73(6) $Å^3$	
Z	4	
Density (calculated)	1.527 Mg/m^3	
Absorption coefficient	0.355 mm^{-1}	
F(000)	376	
Crystal size	0.125 x 0.08 x 0.06 mm^3	
Crystal color, habit	light yellow block	
Theta range for data collection	1.551 to 26.369°.	
Index ranges	-9<=h<=9, -9<=k<=9, -16<=l<=16	
Reflections collected	14003	
Independent reflections	3217 [R(int) = 0.0400]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7454 and 0.6839	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3217 / 0 / 221	
Goodness-of-fit on F^2	1.051	
Final R indices [I>2sigma(I)]	R1 = 0.0329, wR2 = 0.0814	
R indices (all data)	R1 = 0.0370, wR2 = 0.0841	
Largest diff. peak and hole	0.399 and -0.266 e.$Å^{-3}$	

Table S3. Crystal data and structure refinement for compound **5a**



Report date	2022-01-10	
Identification code	tor157	
Empirical formula	C10 H11 N3 O S	
Molecular formula	C10 H11 N3 O S	
Formula weight	221.28	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$Pca2_1$	
Unit cell dimensions	a = 18.4848(10) Å	α= 90°.
	b = 3.9998(2) Å	β= 90°.
	c = 13.4934(8) Å	γ = 90°.
Volume	997.64(9) $Å^3$	
Z	4	
Density (calculated)	1.473 Mg/m^3	
Absorption coefficient	0.299 mm^{-1}	
F(000)	464	
Crystal size	0.175 x 0.12 x 0.1 mm^3	
Crystal color, habit	light yellow plank	
Theta range for data collection	2.204 to 26.736°.	
Index ranges	-22<=h<=23, -5<=k<=4, -17<=l<=17	
Reflections collected	6956	
Independent reflections	2074 [R(int) = 0.0663]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.6947 and 0.6593	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2074 / 1 / 137	
Goodness-of-fit on F^2	1.063	
Final R indices [I>2sigma(I)]	R1 = 0.0350, wR2 = 0.0901	
R indices (all data)	R1 = 0.0362, wR2 = 0.0913	
Absolute structure parameter	0.04(7)	
Largest diff. peak and hole	0.240 and -0.199 $e.Å^{-3}$	

Table S4. Crystal data and structure refinement for compound **6a**



Report date	2022-01-10	
Identification code	tor158	
Empirical formula	C10 H8 N4 S	
Molecular formula	C10 H8 N4 S	
Formula weight	216.26	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 7.6117(7) Å	α= 78.693(2)°.
	b = 8.1553(8) Å	β= 73.317(2)°.
	c = 8.2163(7) Å	γ = 84.818(2)°.
Volume	478.77(8) $Å^3$	
Z	2	
Density (calculated)	1.500 Mg/m^3	
Absorption coefficient	0.305 mm^{-1}	
F(000)	224	
Crystal size	0.14 x 0.125 x 0.1 mm^3	
Crystal color, habit	colorless block	
Theta range for data collection	2.549 to 26.024°.	
Index ranges	-9<=h<=9, -10<=k<=10, -10<=l<=10	
Reflections collected	5661	
Independent reflections	1884 [R(int) = 0.0316]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.5626 and 0.5337	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1884 / 0 / 136	
Goodness-of-fit on F^2	1.046	
Final R indices [I>2sigma(I)]	R1 = 0.0311, wR2 = 0.0817	
R indices (all data)	R1 = 0.0333, wR2 = 0.0834	
Largest diff. peak and hole	0.371 and -0.187 $e.Å^{-3}$	

4. Absorption and emission spectroscopy assays and data

Table S1. Photophysical and chemical properties of thieno[3,4-*d*]pyrimidine and isothiazolo[4,3-*d*]pyrimidine nucleobase and nucleoside analogs

	solvent	λ_{abs} (ε)[a]	λ_{em} (Φ)[a]	Φε	Stokes shift[a]	polarity sensitivity[b]
2a	water	346 (1.03±0.01)	411 (0.0005±0.0002)	5	3.80±0.13	-0.69±5.5
	dioxane	353 (0.94±0.02)	416 (0.47±0.01)	4392	4.33±0.06	
	D_2O	346 (0.98±0.01)	416 (0.0015±0.0001)	14	4.77±0.10	
3a	water	344 (1.11±0.04)	407 (0.06±0.01)	715	4.40±0.09	53±9
	dioxane	351 (1.27±0.01)	412 (0.75±0.01)	9484	4.23±0.02	
	D_2O	345 (1.00±0.01)	406 (0.07±0.01)	687	4.41±0.12	
4a	water	344 (0.90±0.01)	419 (0.64±0.03)	5766	5.14±0.03	81±17
	dioxane	343 (0.85±0.02)	419 (0.77±0.04)	6587	5.26±0.08	
	D_2O	343 (0.84±0.02)	418 (0.57±0.01)	4826	5.29±0.05	
5a	water	344 (1.12±0.01)	415 (0.13±0.01)	1421	4.99±0.14	80±19
	dioxane	349 (1.03±0.02)	411 (0.70±0.01)	7218	4.28±0.05	
	D_2O	344 (1.11±0.01)	416 (0.15±0.01)	1728	5.00±0.10	
6a	water	345 (1.30±0.01)	421 (0.23±0.01)	2951	5.17±0.04	110±23
	dioxane	345 (1.19±0.01)	409 (0.64±0.01)	7579	4.52±0.12	
	D_2O	344 (1.27±0.01)	416 (0.33±0.01)	4164	5.03±0.07	
2b	water	350 (1.17±0.26)	412 (0.0001±0.0001)	1.6	n/a	n/a
	dioxane	358 (1.03±0.02)	418 (0.0010±0.0001)	14	n/a	
	D_2O	349 (1.11±0.02)	417 (0.0001±0.0001)	1.1	n/a	
3b	water	352 (1.22±0.05)	416 (0.0030±0.0001)	33	4.38±0.10	-17±4
	dioxane	358 (1.05±0.49)	415 (0.13±0.01)	1192	3.84±0.13	
	D_2O	349 (1.23±0.01)	415 (0.0030±0.0001)	41	4.51±0.05	
4b	water	341 (1.21±0.01)	403 (0.064±0.001)	770	4.54±0.06	11±8
	dioxane	348 (1.03±0.01)	407 (0.020±0.001)	173	4.19±0.08	
	D_2O	341 (1.22±0.04)	403 (0.073±0.001)	884	4.49±0.06	
5b	water	346 (1.11±0.02)	405 (0.007±0.001)	77	4.12±0.05	-2±8
	dioxane	356 (0.96±0.01)	413 (0.14±0.01)	821	3.84±0.12	
	D_2O	346 (1.06±0.01)	408 (0.010±0.01)	102	4.47±0.05	

6b	water	344 (1.11±0.01)	406 (0.03±0.01)	340	4.45±0.09	34±4
	dioxane	352 (0.94±0.01)	410 (0.06±0.01)	607	4.07±0.04	
	D_2O	343 (1.07±0.01)	406 (0.03±0.01)	293	4.49±0.12	
11	water	352 (0.94±0.03)	435 (0.30±0.04)	2838	5.41±0.19	
	dioxane	357 (0.94±0.01)	420 (0.17±0.01)	1444	4.21±0.01	
	D_2O	352 (0.95±0.01)	44 (0.47±0.01)	4486	5.23±0.05	

[a] λ_{abs}, ε, λ_{em} and Stokes shift are reported in nm, 10^3 M^{-1} cm^{-1}, nm and 10^3 cm^{-1} respectively. All the photophysical values reflect the average over three independent measurements. [b] Sensitivity to solvent polarity reported in cm^{-1}/ (kcal mol^{-1}) is equal to the slope of the linear fit in Figure S20.

4.1. General

Spectroscopic grade DMSO, dioxane, ethylene glycol, glycerol, and methanol were obtained from Sigma-Aldrich and aqueous solutions were prepared with milliq water. All measurements were carried out in a 1 cm four-sided quartz cuvette from Helma.

Absorption spectra were measured on a Shimadzu UV-2450 spectrophotometer setting the slit at 1 nm and using a resolution of 0.5 nm. All the spectra were corrected for the blank. Emission and excitation spectra were measured on a Horiba Fluoromax-4 equipped with a cuvette holder with a stirring system setting the excitation slit at 1 nm and the emission slit at 3 nm, the resolution at 1 nm and the integration time 0.1 s. Emission spectra were measured exciting at 350 nm and excitation spectra were taken at 420 nm unless otherwise specified. Both instruments were equipped with a thermostat-controlled ethylene glycol-water bath fitted to specially designed cuvette holder and the temperature was kept at 25.00 ± 0.10 °C.

The compounds were dissolved in DMSO to prepare highly concentrated stock solutions (10X): **2a** (31.55 mM), **2b** (23.77 mM), **3a** (34.03 mM), **3b** (24.61 mM), **4a** (25.77 mM), **4b** (27.19 mM), **5a** (26.69 mM), **5b** (30.62 mM), **6a** (25.92 mM), **6b** (32.94 mM), and **11** (31.84 mM). For the determination of extinction coefficient and quantum yield, these 10X stock solutions were used. For these experiments, aliquots (10 μl) of the 10X solution were diluted with air-saturated solvents (2.990 ml). The solutions were then diluted by a factor of 2 in the corresponding solvent and remeasured. This dilution was repeated four times total, with the most dilute solution being used to measure the emission spectra and subsequent quantum yield. The absorbance values were corrected for the blank and the extinction coefficient was extrapolated for each wavelength using a linear best fit. For all other experiments, 1X concentrated stock solutions were utilized by dilution of the 10X stock solutions into spectroscopic grade DMSO. In a typical experiment, aliquots (10 μl) of the 1X solution were diluted with air-saturated solvents (2.990 ml). The solution were mixed witha pipette for 10 seconds and placed in the cuvette holder at 25.00 ± 0.10 °C for 3 minutes before spectra were recorded. All sample contain 0.3 v/v % of DMSO. For solutions in higher viscosity solvents (ethylene glycol, glycerol) the samples were checked under a 360 nm UV lamp to ensure the solutions were properly mixed.

4.2. Fluorescence quantum yield

The samples' concentration were adjusted to have an optical density lower than 0.1 at the excitation wavelength (λ_{ex}). The fluorescence quantum yield (Φ) were evaluated based on an external standard, quinine sulfate in 0.5 M H_2SO_4 (0.546, λ_{ex} 350 nm) by using the following equation.

$$\Phi = \Phi_{STD} \frac{I}{I_{STD}} \frac{OD_{STD}}{OD} \frac{n^2}{n_{STD}^2}$$

Where Φ_{STD} is the fluorescence quantum yield of the standard, I and I_{STD} are the integrated area of the emission band of the sample and the standard respectively, OD and OD_{STD} are the optical density at the excitation wavelength for the sample and the standard respectively and n and n_{STD} are the solvent refractive index of the sample and the standard solutions respectively.

4.3. Absorption, emission, and excitation spectra of compounds 2–6a/b

Absorption, emission spectra and the corresponding excitation spectra were also recorded in water and dioxane varying the excitation wavelength or the emission wavelength of interest. Solutions were prepared as described above. The emission spectra were recorded upon excitation every 10 nm covering the main absorption band between 260 and 380 nm. The emission spectra were corrected for either the corresponding optical density of the sample or normalized to unit in intensity at the emission maxima. The excitation spectra were recorded by fixing the emission wavelength every 10 nm between 380 and 500 nm. The excitation spectra were then normalized to unit in intensity at the excitation maxima.

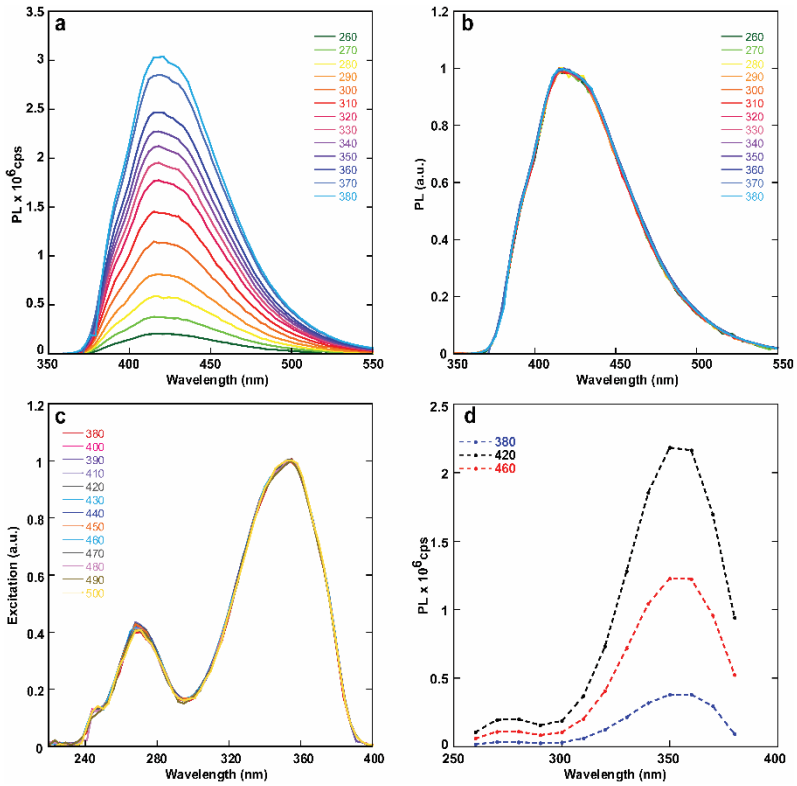


Figure S1. Emission spectra in dioxane of compound **2a** recorded upon excitation every 10 nm between 260-380 nm. The spectra were normalized for (a) the corresponding absorbance intensity or (b) to unit in intensity. (c) Excitation spectra of compound **2a** in dioxane recorded at select emission wavelengths, covering the emission band. (d) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **2a** in dioxane.

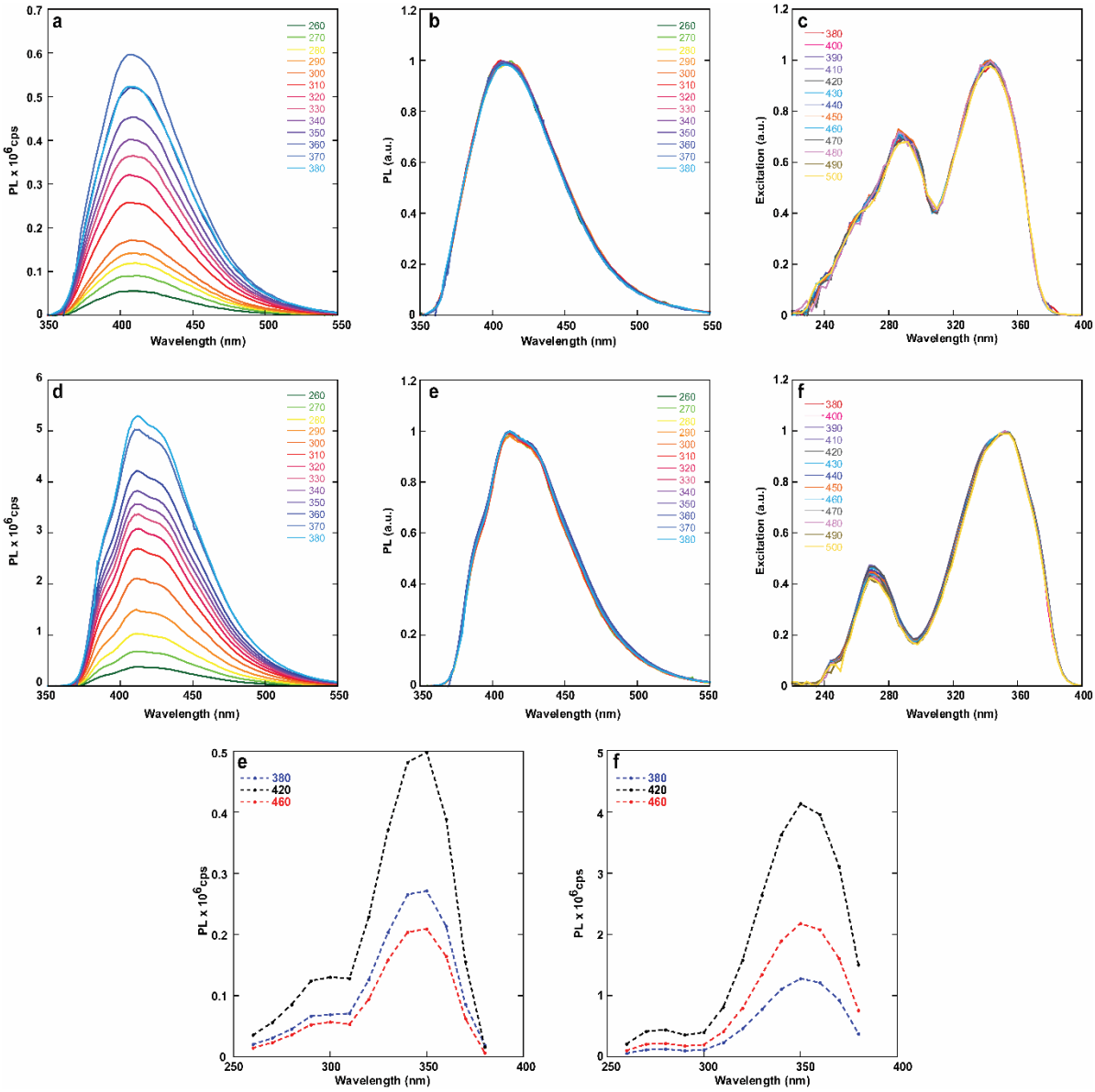


Figure S2. Spectra in of compound **3a** recorded upon excitation every 10 nm between 260-380 nm in water (a-c) and dioxane (d-f). The spectra were recorded in and normalized for (a,d) the corresponding absorbance intensity or (b,e) to unit in intensity. (c) Excitation spectra of compound **3a** in water and (f) dioxane recorded at select emission wavelengths, covering the emission band. (e) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **3a** in water and (f) dioxane.

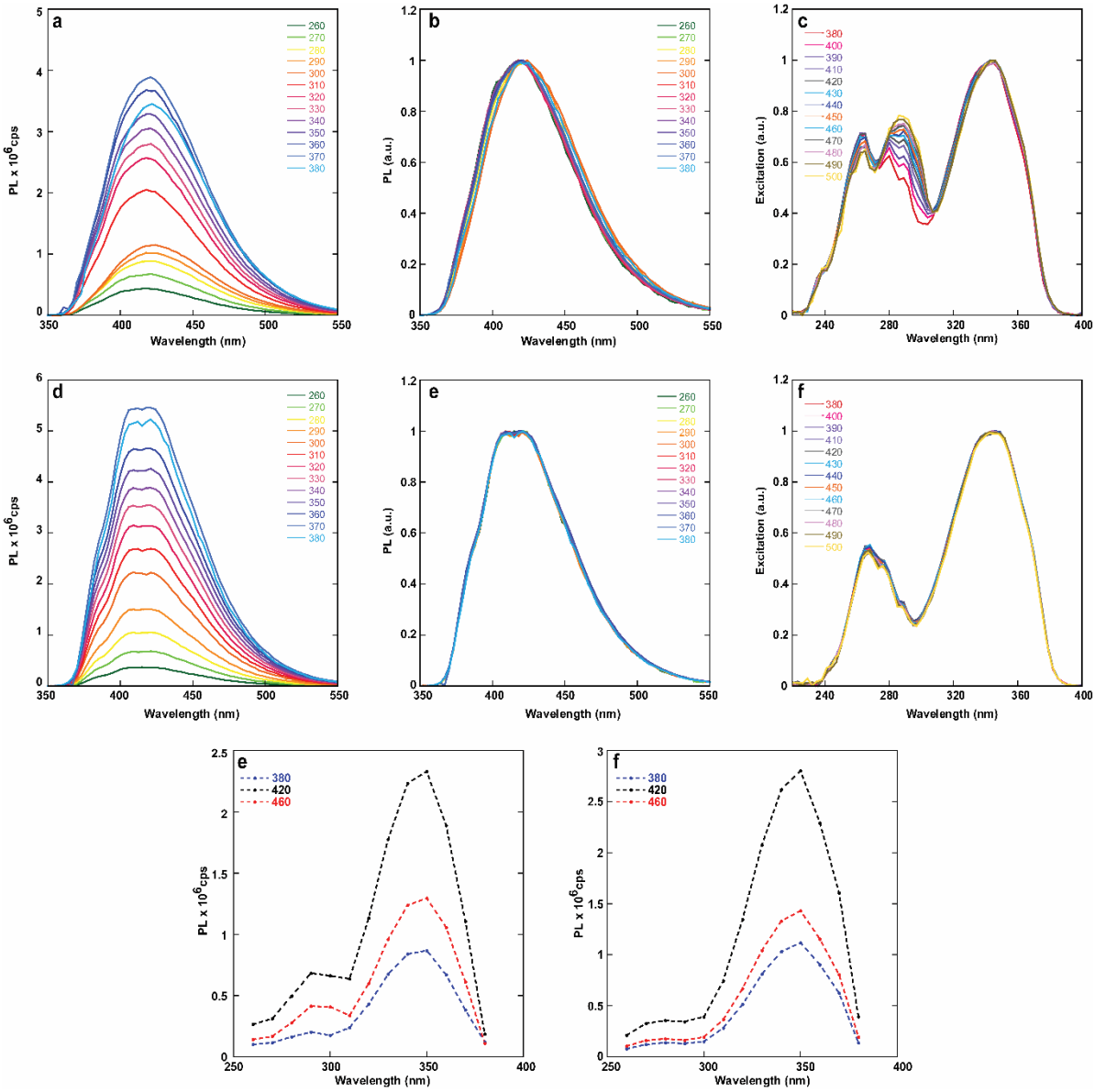


Figure S3. Spectra in of compound **4a** recorded upon excitation every 10 nm between 260-380 nm in water (a-c) and dioxane (d-f). The spectra were recorded in and normalized for (a,d) the corresponding absorbance intensity or (b,e) to unit in intensity. (c) Excitation spectra of compound **4a** in water and (f) dioxane recorded at select emission wavelengths, covering the emission band. (e) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **4a** in water and (f) dioxane.

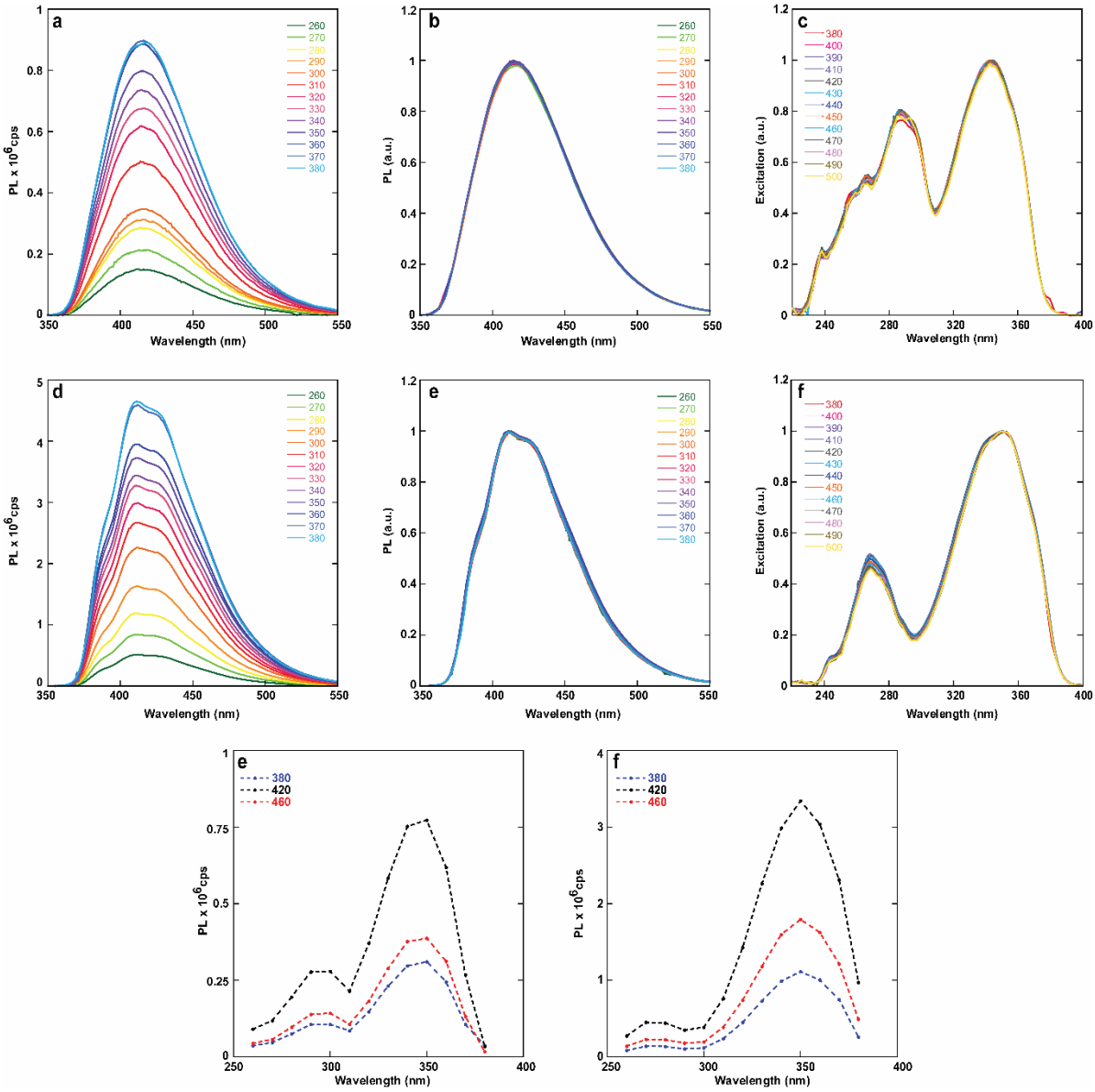


Figure S4. Spectra in of compound **5a** recorded upon excitation every 10 nm between 260-380 nm in water (a-c) and dioxane (d-f). The spectra were recorded in and normalized for (a,d) the corresponding absorbance intensity or (b,e) to unit in intensity. (c) Excitation spectra of compound **5a** in water and (f) dioxane recorded at select emission wavelengths, covering the emission band. (e) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **5a** in water and (f) dioxane.

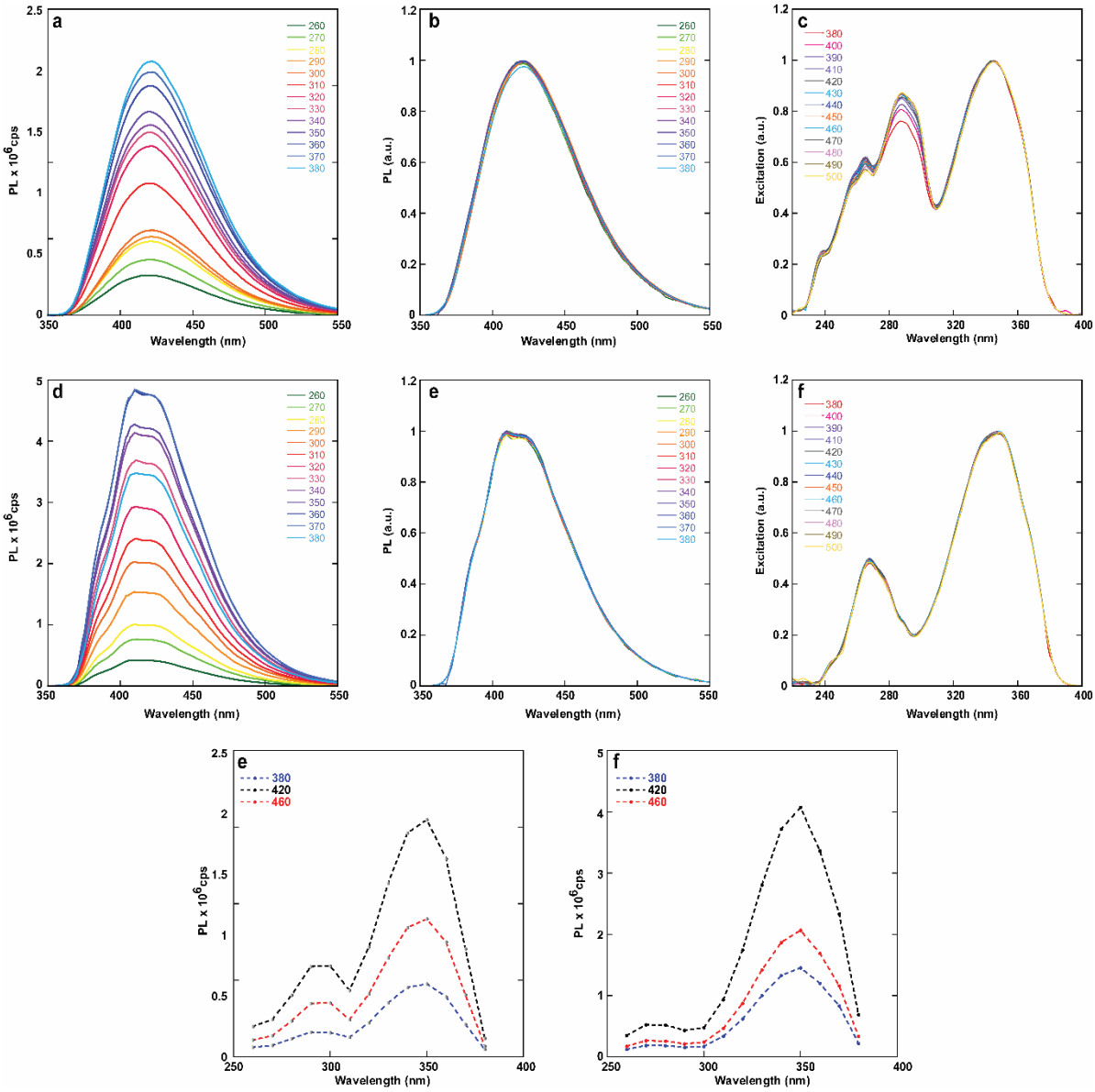


Figure S5. Spectra in of compound **6a** recorded upon excitation every 10 nm between 260-380 nm in water (a-c) and dioxane (d-f). The spectra were recorded in and normalized for (a,d) the corresponding absorbance intensity or (b,e) to unit in intensity. (c) Excitation spectra of compound **6a** in water and (f) dioxane recorded at select emission wavelengths, covering the emission band. (e) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **6a** in water and (f) dioxane.

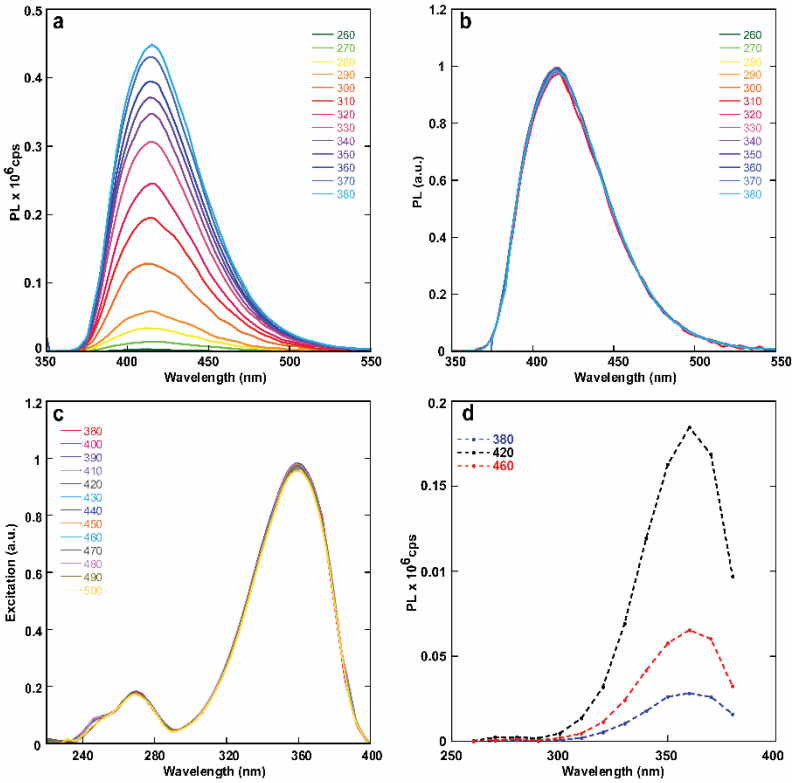


Figure S6. Emission spectra in dioxane of compound **3b** recorded upon excitation every 10 nm between 260-380 nm. The spectra were normalized for (a) the corresponding absorbance intensity or (b) to unit in intensity. (c) Excitation spectra of compound **3b** in dioxane recorded at select emission wavelengths, covering the emission band. (d) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **3b** in dioxane.

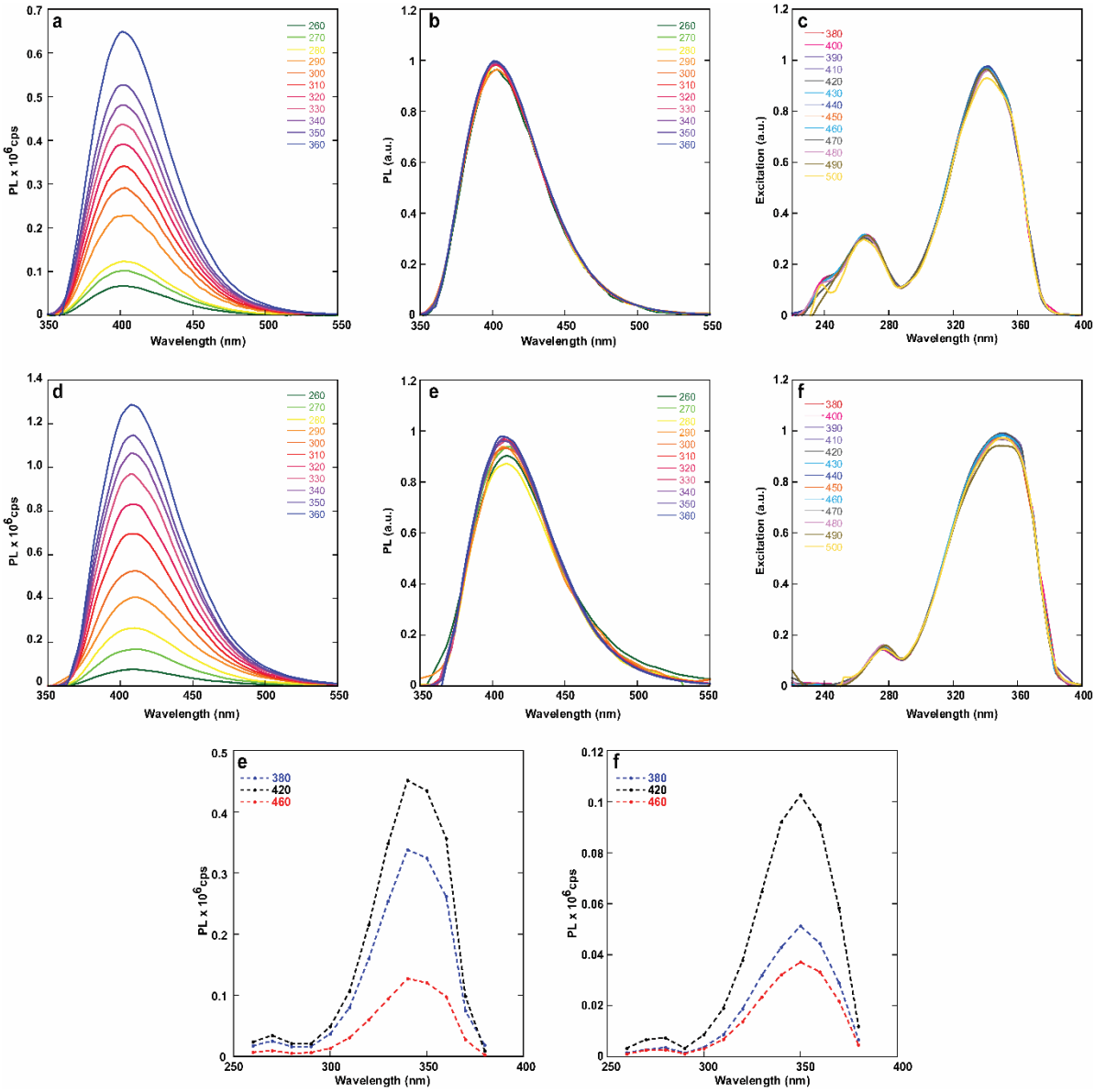


Figure S7. Emission spectra in dioxane of compound **4b** recorded upon excitation every 10 nm between 260-380 nm. The spectra were normalized for (a) the corresponding absorbance intensity or (b) to unit in intensity. (c) Excitation spectra of compound **4b** in dioxane recorded at select emission wavelengths, covering the emission band. (d) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **4b** in dioxane.

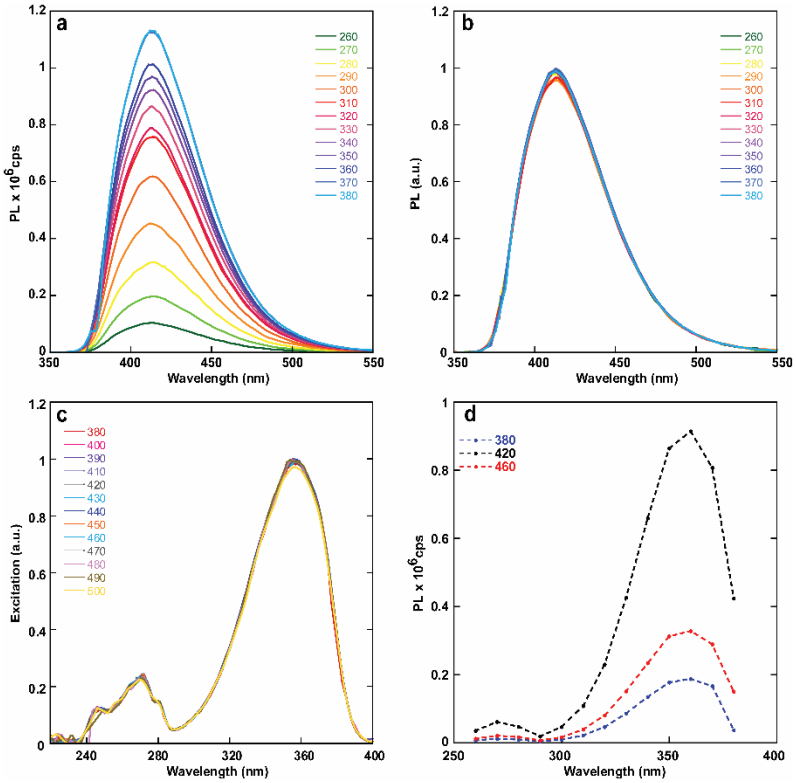


Figure S8. Emission spectra in dioxane of compound **5b** recorded upon excitation every 10 nm between 260-380 nm. The spectra were normalized for (a) the corresponding absorbance intensity or (b) to unit in intensity. (c) Excitation spectra of compound **5b** in dioxane recorded at select emission wavelengths, covering the emission band. (d) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **5b** in dioxane.

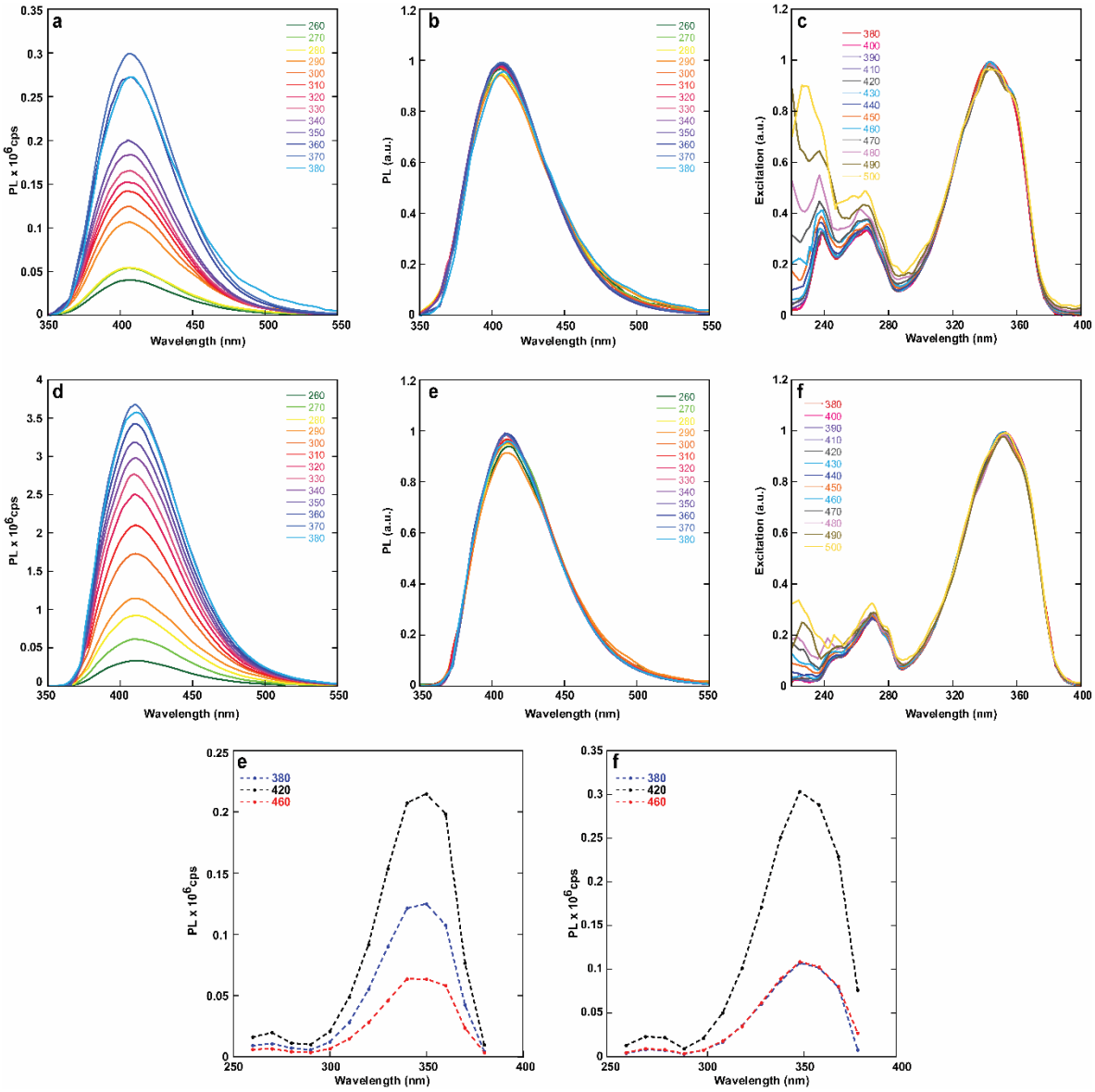


Figure S9. Emission spectra in dioxane of compound **6b** recorded upon excitation every 10 nm between 260-380 nm. The spectra were normalized for (a) the corresponding absorbance intensity or (b) to unit in intensity. (c) Excitation spectra of compound **6b** in dioxane recorded at select emission wavelengths, covering the emission band. (d) Extrapolated excitation spectra by plotting the emission spectra intensities at selected wavelengths upon excitation at different wavelengths of compound **6b** in dioxane.

4.4. Sensitivity to viscosity

Stock solutions (100 ml) of 20%, 40%, 60%, and 80% glycerol in methanol were prepared by mixing spectroscopic grade glycerol and methanol in a volumetric flask. The solutions were mixed for several days to allow for proper mixing. 1X stock solutions of the nucleobases in DMSO (10 µl) were then diluted in 2990 µl of MeOH, glycerol, or MeOH-glycerol mixtures and measured at 25 °C. For 80% glycerol in methanol and pure glycerol solutions, the samples were gently heated to induce mixing and allowed to cool for 30 minutes before taking spectra. The sample viscosity values were determined using the following equation:[3,4]

$$\ln \eta_{mix} = \sum_{i=1}^{2} w_i \cdot \ln \eta_i$$

Where η_{mix} and η_i stand for the viscosity of the mixture and the viscosity of component i respectively. w_i stands for the weight fraction of component i. Values for pure MeOH and glycerol at 25 °C were taken from the literature.[5–7] Viscosity values for the methanol-glycerol mixtures are reported in Table S2.

Table S2. Viscosity values for MeOH-glycerol mixtures.

Glycerol % in MeOH	η [cP]	Log (η)
0	0.63	-0.20
20	4.74	0.67
40	24.8	1.4
60	98.9	2.0
80	319.7	2.5
100	875.00	2.9

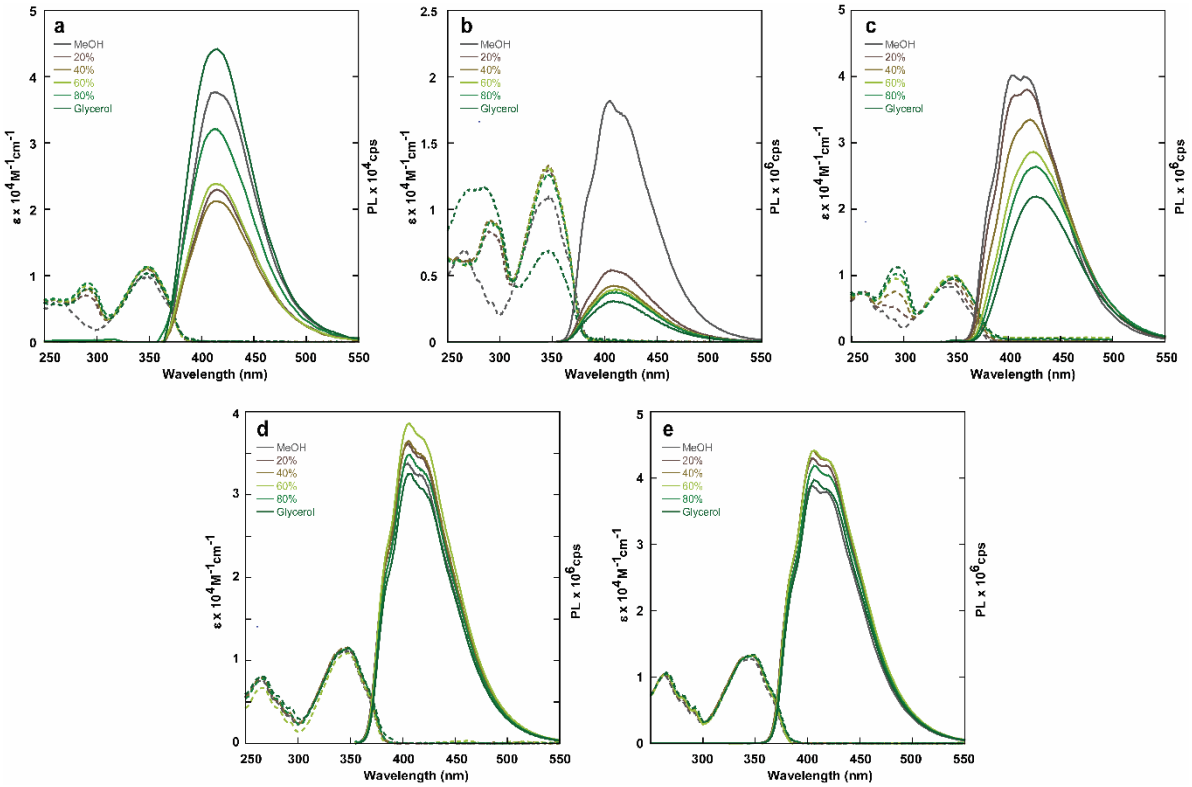


Figure S10. Absorption (dashed lines) and emission (solid lines) traces in mixtures of methanol and glycerol at 25 °C for (a) **2a**, (b) **3a**, (c) **4a**, (d) **5a**, and (e) **6a**. The emission spectra were normalized to 0.1 intensity at the excitation wavelength.

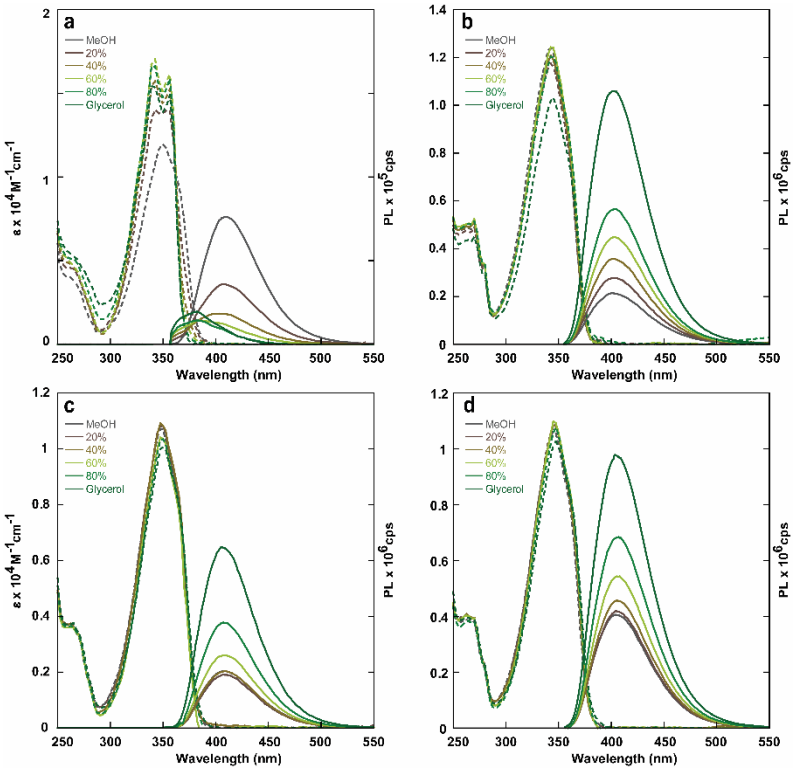


Figure S11. Absorption (dashed lines) and emission (solid lines) traces in mixtures of methanol and glycerol at 25 °C for (b) **3b**, (b) **4b**, (c) **5b**, and (d) **6b**. The emission spectra were normalized to 0.1 intensity at the excitation wavelength.

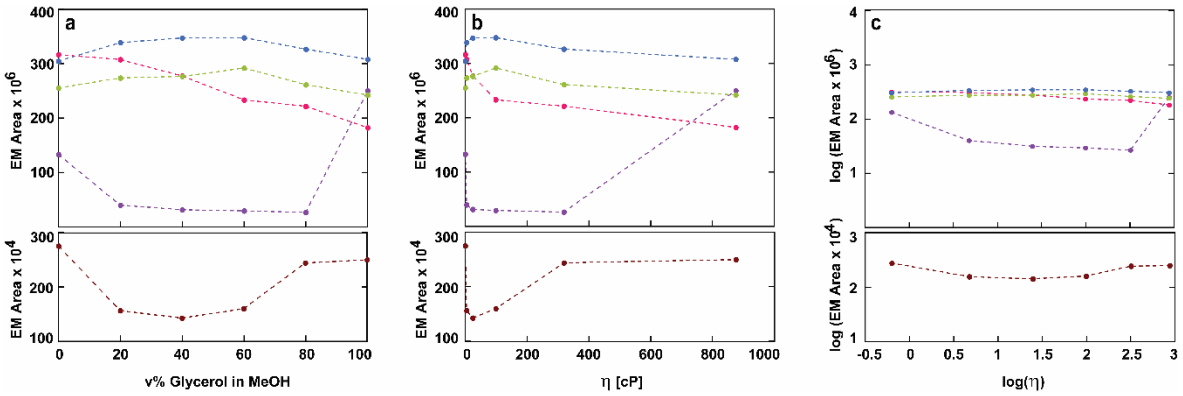


Figure S12. Area under the emission band of **2a** (red), **3a** (purple), **4a** (pink), **5a** (light green) and **6a** (blue) plotted against (a) v% glycerol in MeOH and (b) viscosity coefficients for MeOH-glycerol mixtures. (c) logarithm of the area under the emission bands of specified compounds plotted against the logarithm of the viscosity coefficients.

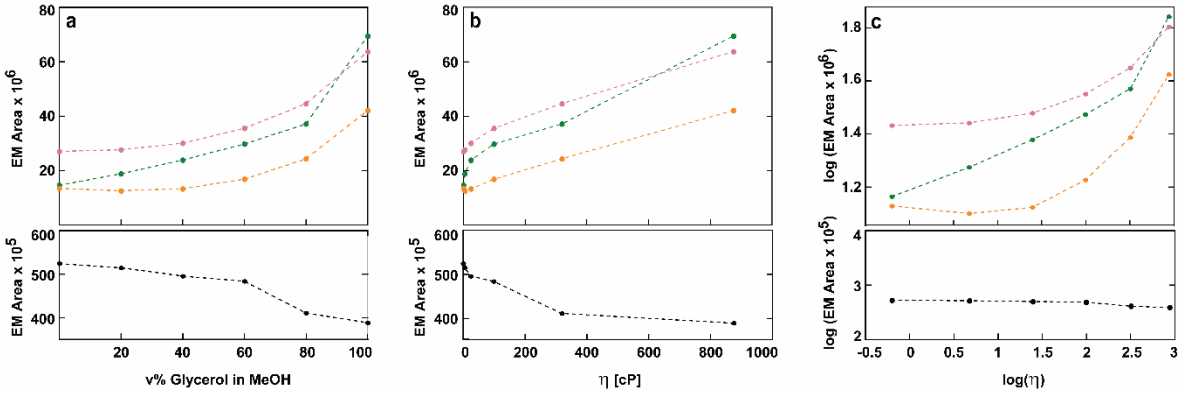


Figure S13. Area under the emission band of **3b** (black), **4b** (dark green), **5b** (orange) and **6b** (magenta) plotted against (a) v% glycerol in MeOH and (b) viscosity coefficients for MeOH-glycerol mixtures. (c) logarithm of the area under the emission bands of specified compounds plotted against the logarithm of the viscosity coefficients.

4.5. Sensitivity to temperature-induced viscosity

For further analysis of viscosity-dependent fluorescence of compounds **2-6a** and **3-6b**, 10 µl of 1X nucleobase stock solutions in DMSO were diluted in 2990 µl of spectroscopic grade ethylene glycol. The solutions were allowed to equilibrate to the appropriate temperature for 30 minutes before measuring the absorption, emission, and excitation spectra of the samples.

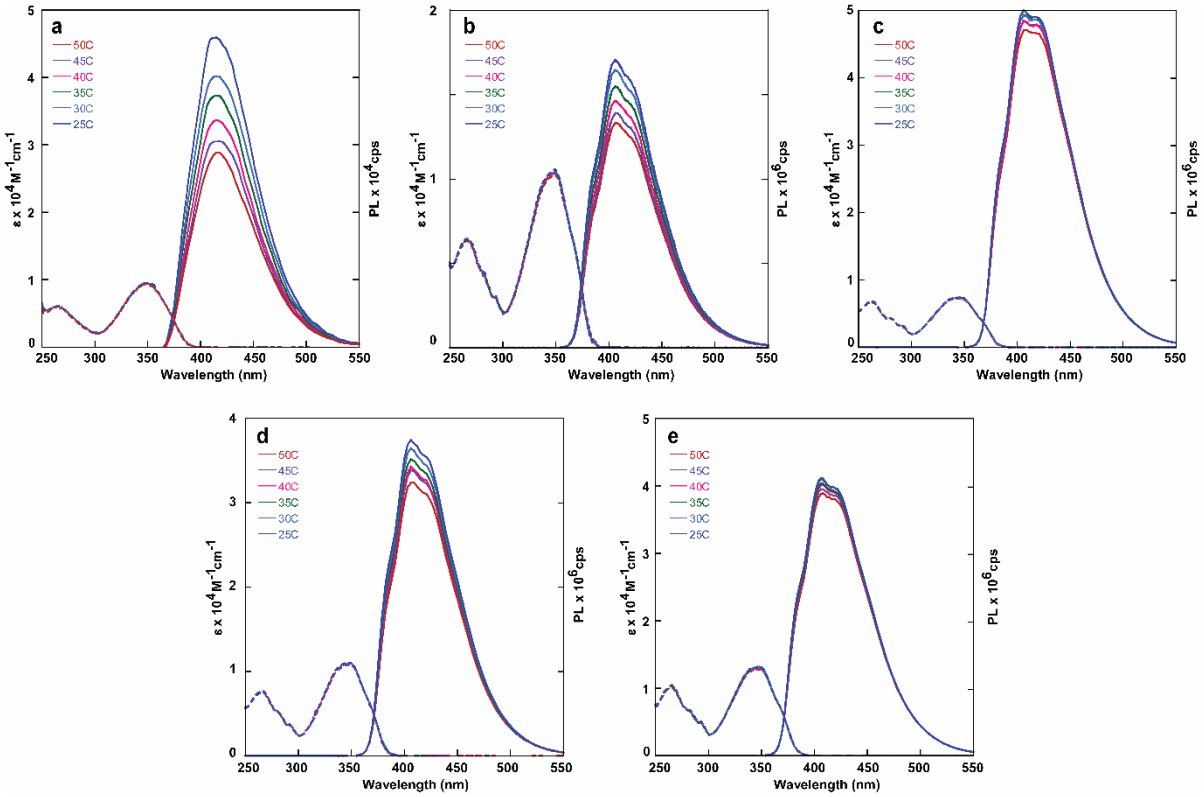


Figure S14. Absorption (dashed lines) and emission (solid lines) traces in ethylene glycol for (a) **2a**, (b) **3a**, (c) **4a**, (d) **5a**, and (e) **6a** between 25 and 50 °C. The emission spectra were normalized to 0.1 intensity at the excitation wavelength.

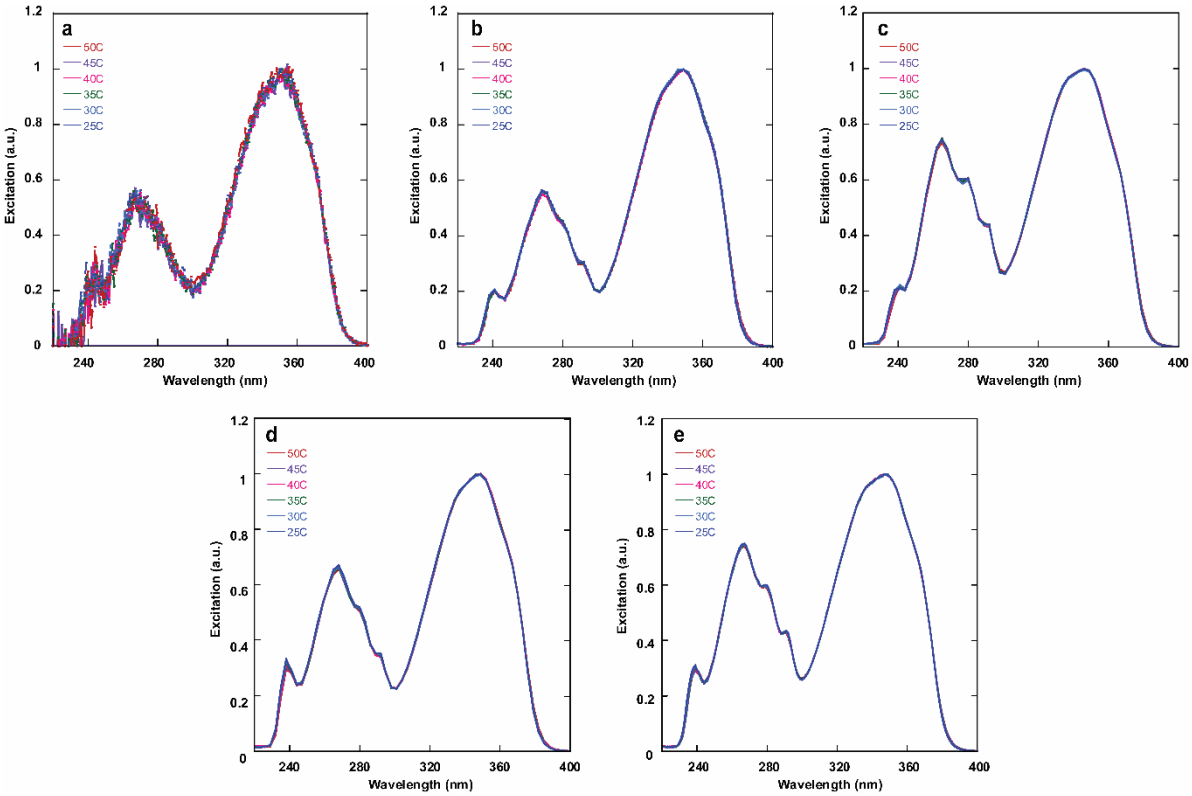


Figure S15. Excitation spectra in ethylene glycol for (a) **2a**, (b) **3a**, (c) **4a**, (d) **5a**, and (e) **6a** between 25 and 50 °C. The emission spectra were normalized to the maximum value.

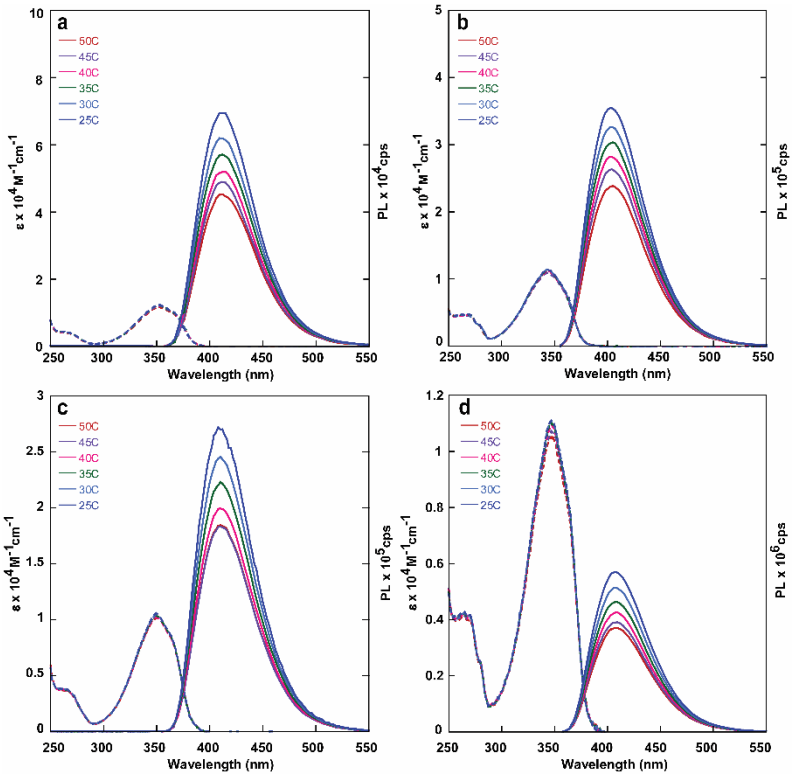


Figure S16. Absorption (dashed lines) and emission (solid lines) traces in ethylene glycol for (a) **3b**, (b) **4b**, (c) **5b**, and **(**d) **6b** between 25 and 50 °C. The emission spectra were normalized to 0.1 intensity at the excitation wavelength.

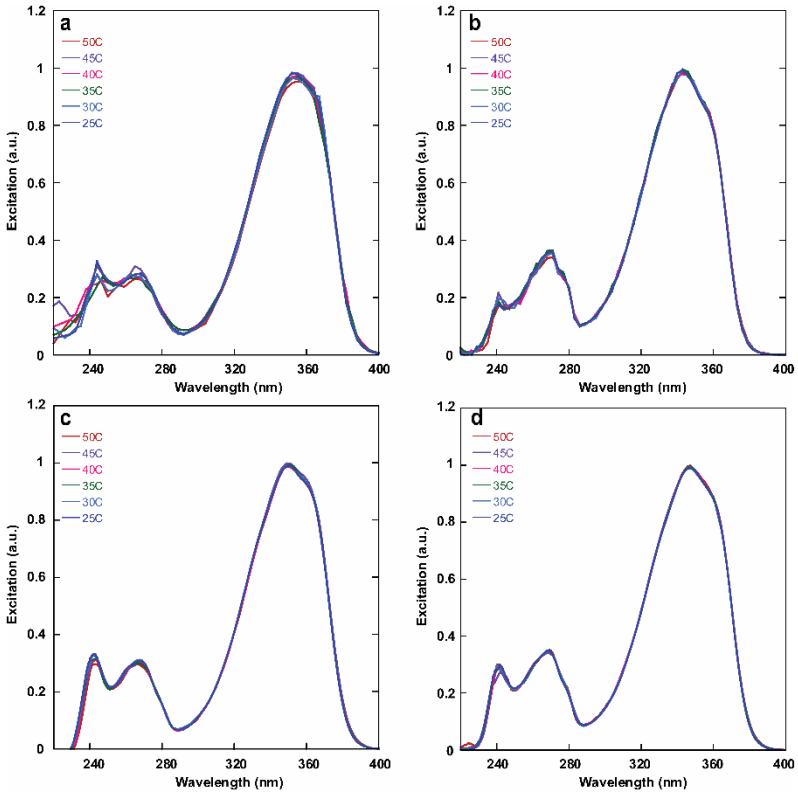


Figure S17. Excitation spectra in ethylene glycol for (a) **3b**, (b) **4b**, (c) **5b**, and (d) **6b** between 25 and 50 °C. The excitation spectra were normalized to the maximum value.

4.6. Sensitivity to polarity

Experiments evaluating the effect of polarity were performed in water, dioxane and their mixtures (20, 40, 60 and 80 v/v % water in dioxane). Solutions were prepared in advance and allowed to mix overnight. The sample $E_T(30)$ values were determined by dissolving a small amount of Reichardt's dye in the mixture of the same solvent used to dilute the nucleoside's DMSO sample. The observed long wavelength absorption maximum (λ_{abs}^{max}) was converted to the $E_T(30)$ values with the following equation.

$$E_T(30) = \frac{28591}{\lambda_{abs}^{max}}$$

Table S3. $E_T(30)$ experimental values for water/dioxane mixtures

Water % in dioxane	Reported $E_T(30)$[a] (Kcal mol^{-1})	Experimental $E_T(30)$ (Kcal mol^{-1})
0	36.4	36.5
20	48.3	48.3
40	51.6	52.4
60	55.0	55.5
80	57.5	58.4
100	63.1	-

[a] Literature values.[8] Due to solubility limitation the water $E_T(30)$ value was not calculated.

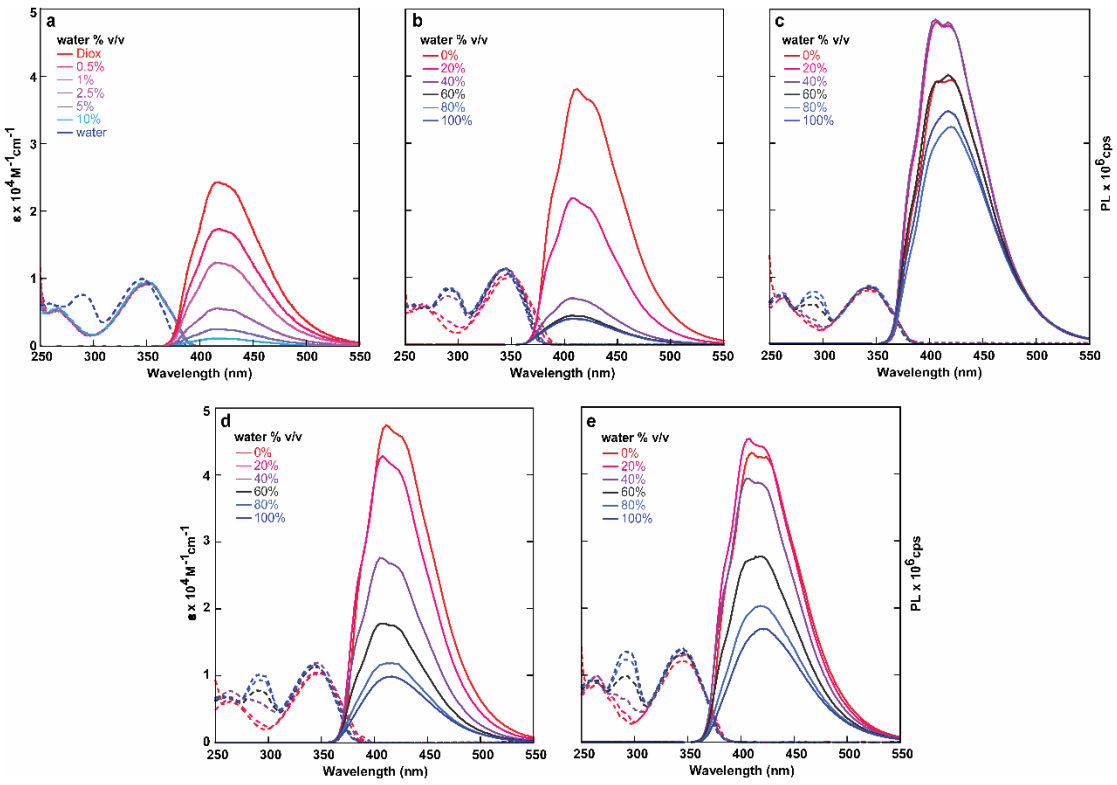


Figure S18. Absorption (dashed lines) and emission (solid lines) traces in water, dioxane and mixture thereof for **2a** (a), **3a** (b), **4a** (c), **5a** (d) and **6a** (e). The emission spectra were normalized to 0.1 intensity at the excitation wavelength.

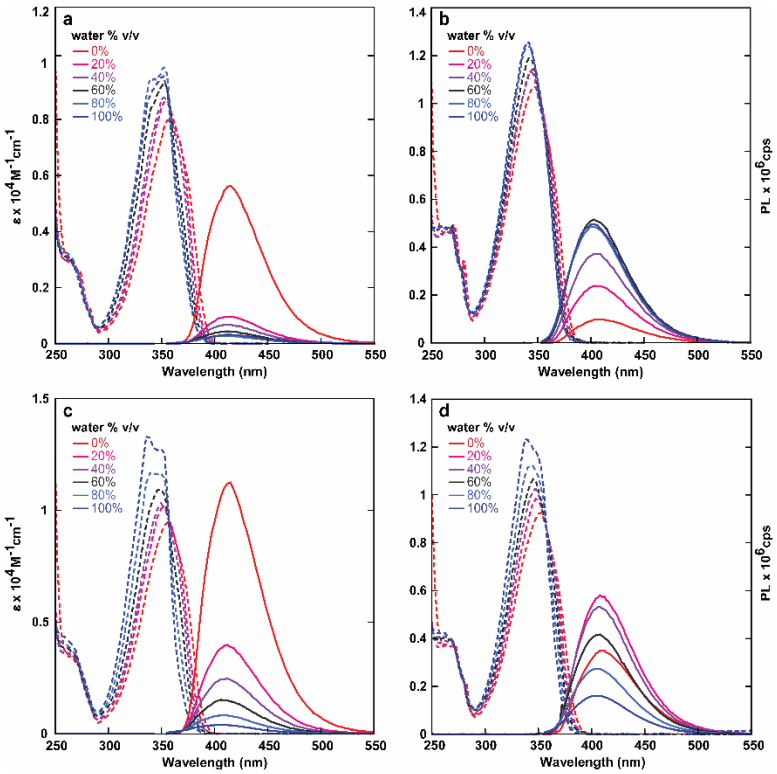


Figure S19. Absorption (dashed lines) and emission (solid lines) traces in water, dioxane and mixture thereof for **3b** (a), **4b** (b), **5b** (c), and **6b** (d). The emission spectra were normalized to 0.1 intensity at the excitation wavelength.

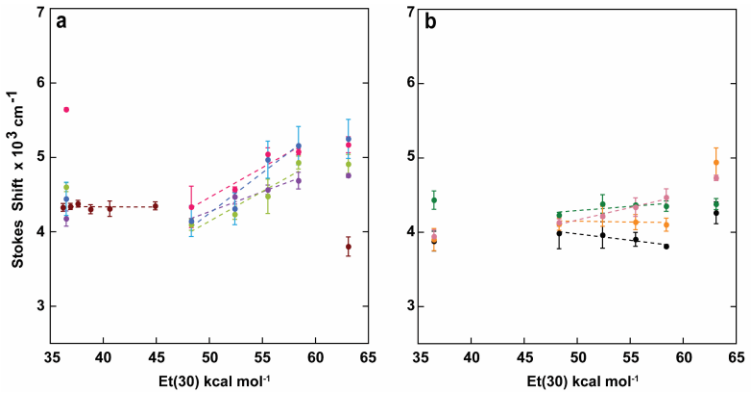


Figure S20. Stokes shift vs. solvent polarity correlation ($E_T(30)$) of water/dioxane mixtures for (a) **2a** (red), **3a** (purple), **4a** (pink), **5a** (light green), and **6a** (blue) as well as (b) **3b** (black), **4b** (dark green), **5b** (orange), and **6b** (magenta).

5. HPLC analysis of compounds 2–6a/b

To validate the photophysical experiments described above, the stock solutions were checked for purity using HPLC before running photophysical experiments. 20 μl of 1X stock solutions (compounds **2a**, **3**–**6a/b**) or 10X stock solutions (compounds **2b** and **11**) were diluted with 0.1% TFA in water (200 μl). HPLC analysis was carried out with an Agilent 1260 series system with a Sepax Bio-C18, 5μm, 300 Å, 10 × 250 mm column. 0.1% TFA stock solutions were prepared by dissolving 1 ml of TFA (Sigma-Aldrich, 99%) in 999 ml MilliQ water and filtered using Millipore type GNWP 0.2 μm filters before use. Each injection (100 μl) was subjected to a gradient (20 minutes, from 0.5 to 40% acetonitrile 0.1% TFA in water 0.1% TFA) followed by a flush (10 minutes). A flow rate of 3 mL / minute was used and the run was carried out at 25.00 ± 0.10 °C. Each run was monitored at 260, 280 and 380 nm with calibrated references at 600 nm and slit set at 1 nm.

6. Supplementary figures

6.1. HPLC Traces of stock solutions for compounds 2–6a/b and 11

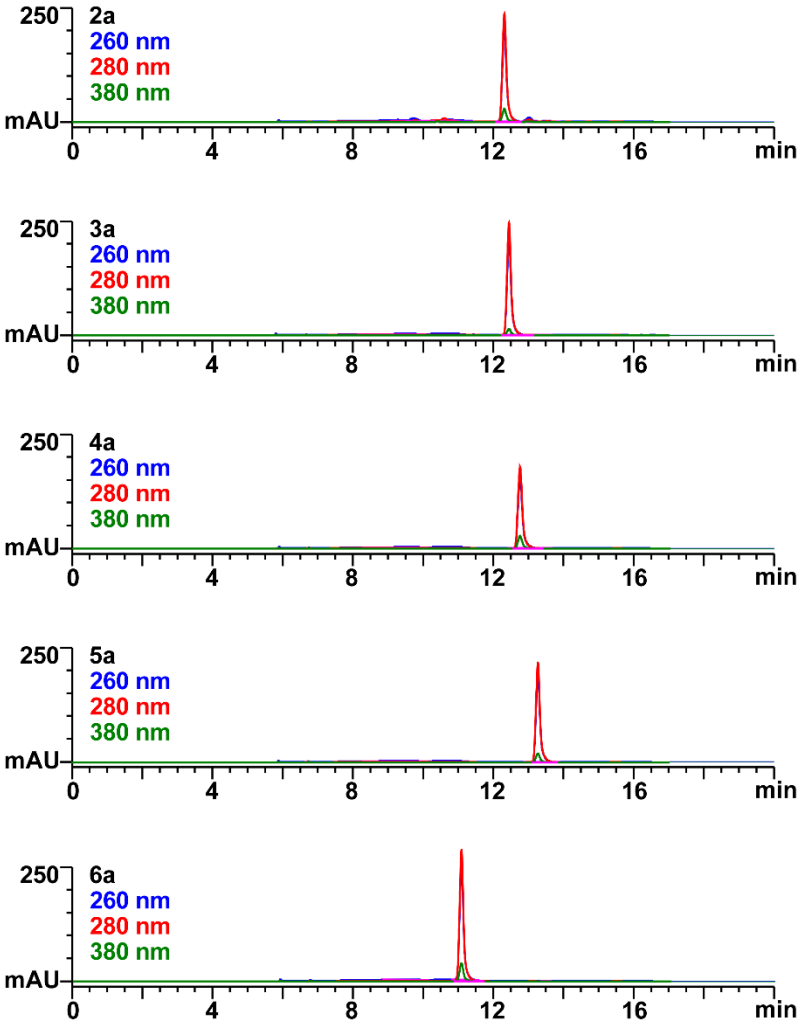


Figure S21. Raw HPLC traces of stock solutions for compounds **2–6a**

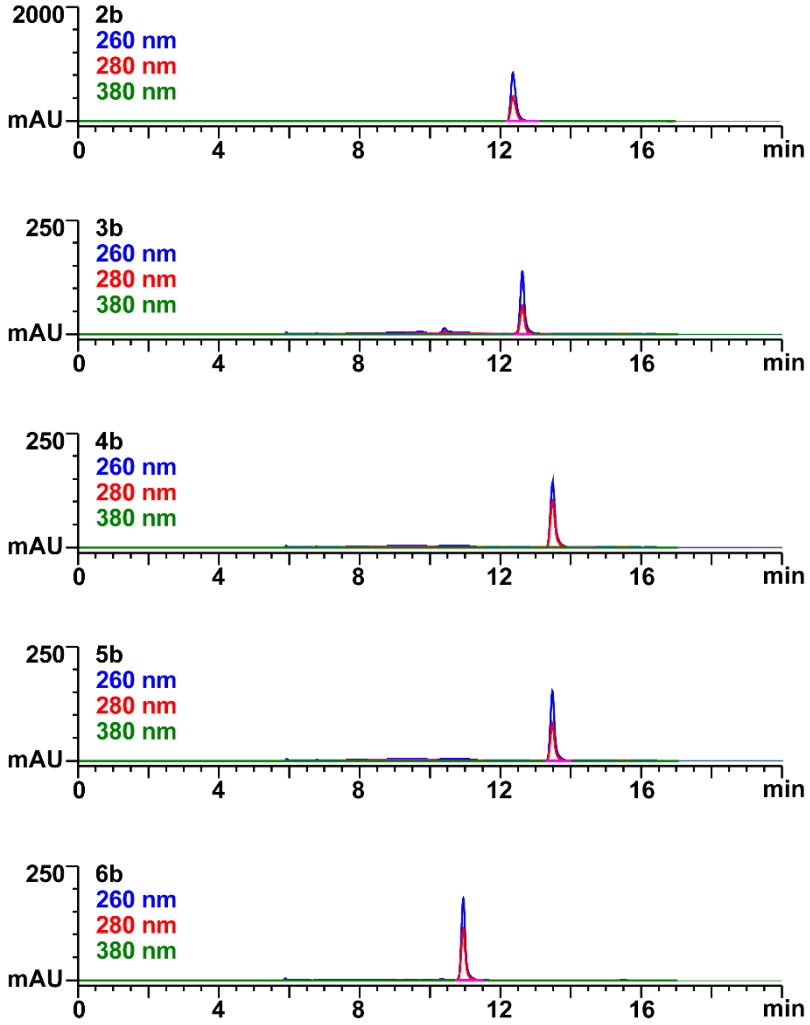


Figure S22. Raw HPLC traces for compounds **2**–**6b**.

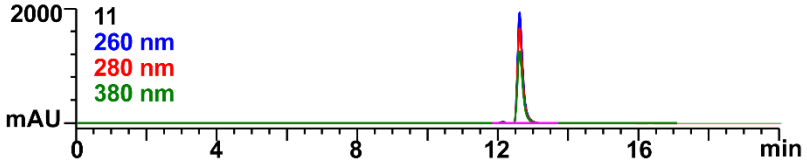


Figure S23. Raw HPLC traces for compound **11**.

6.2. NMR spectroscopy

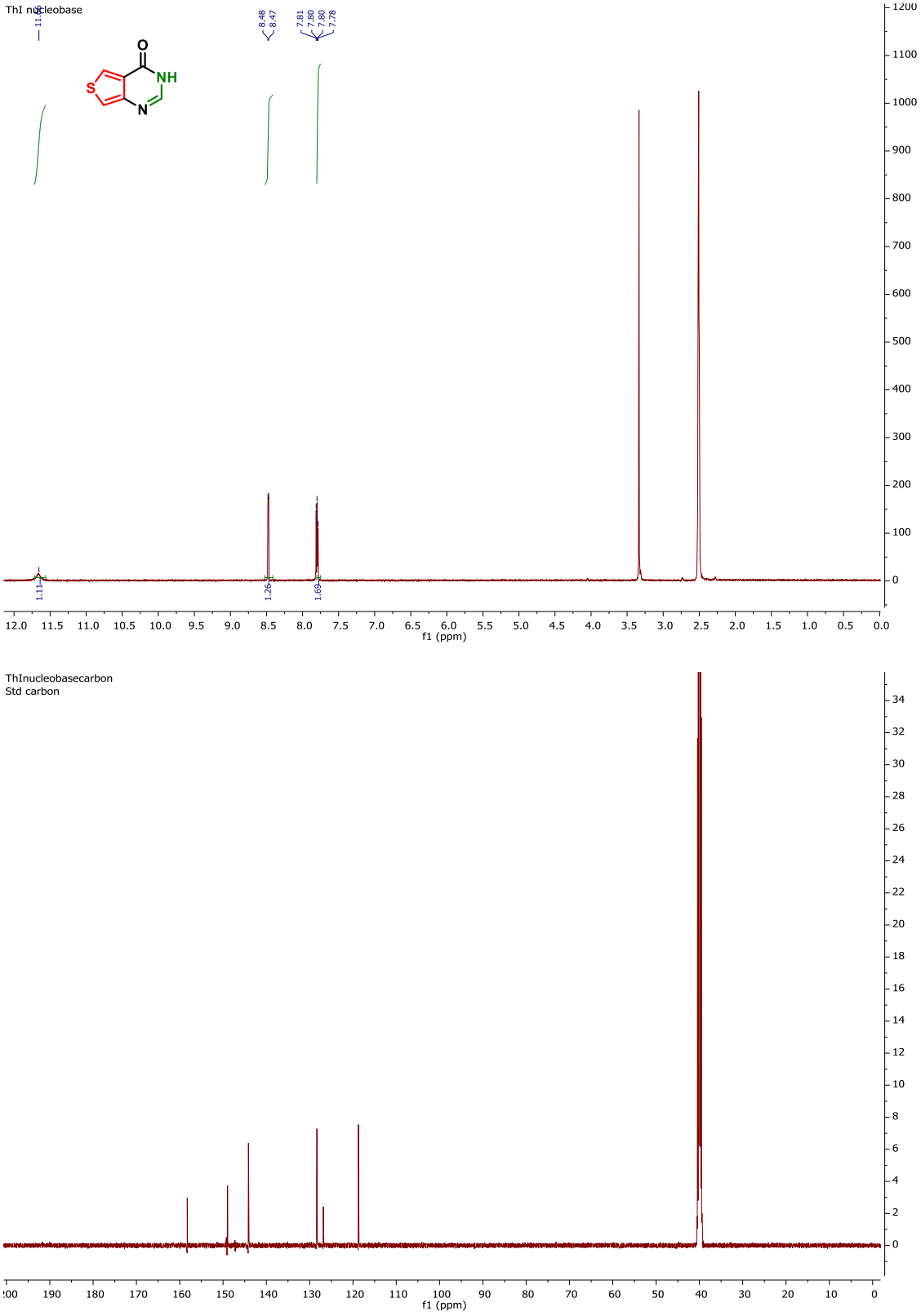


Figure S24. ^{1}H and ^{13}C NMR spectra of **6a**.

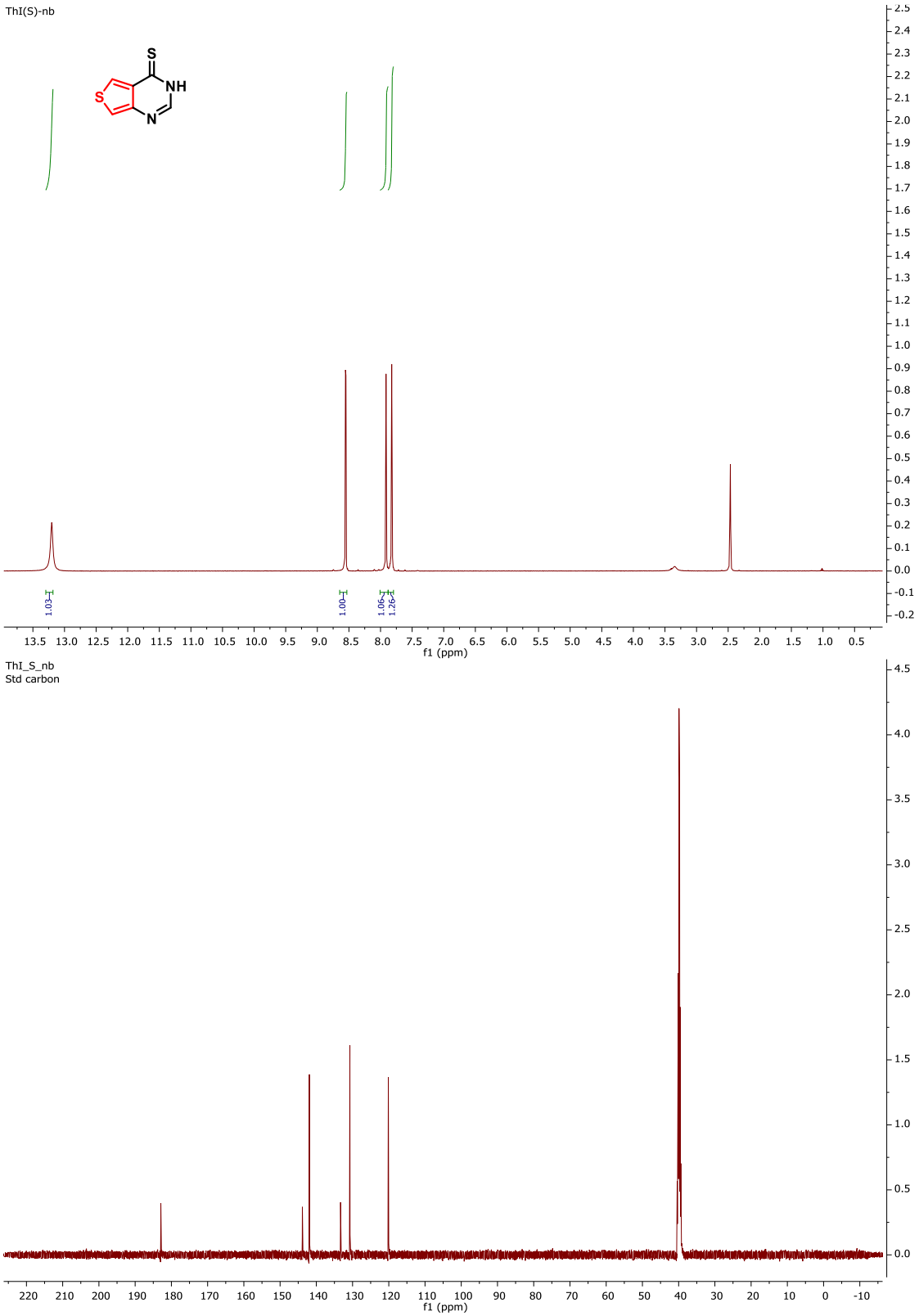


Figure S25 ^{1}H and ^{13}C NMR spectra of **7a**.

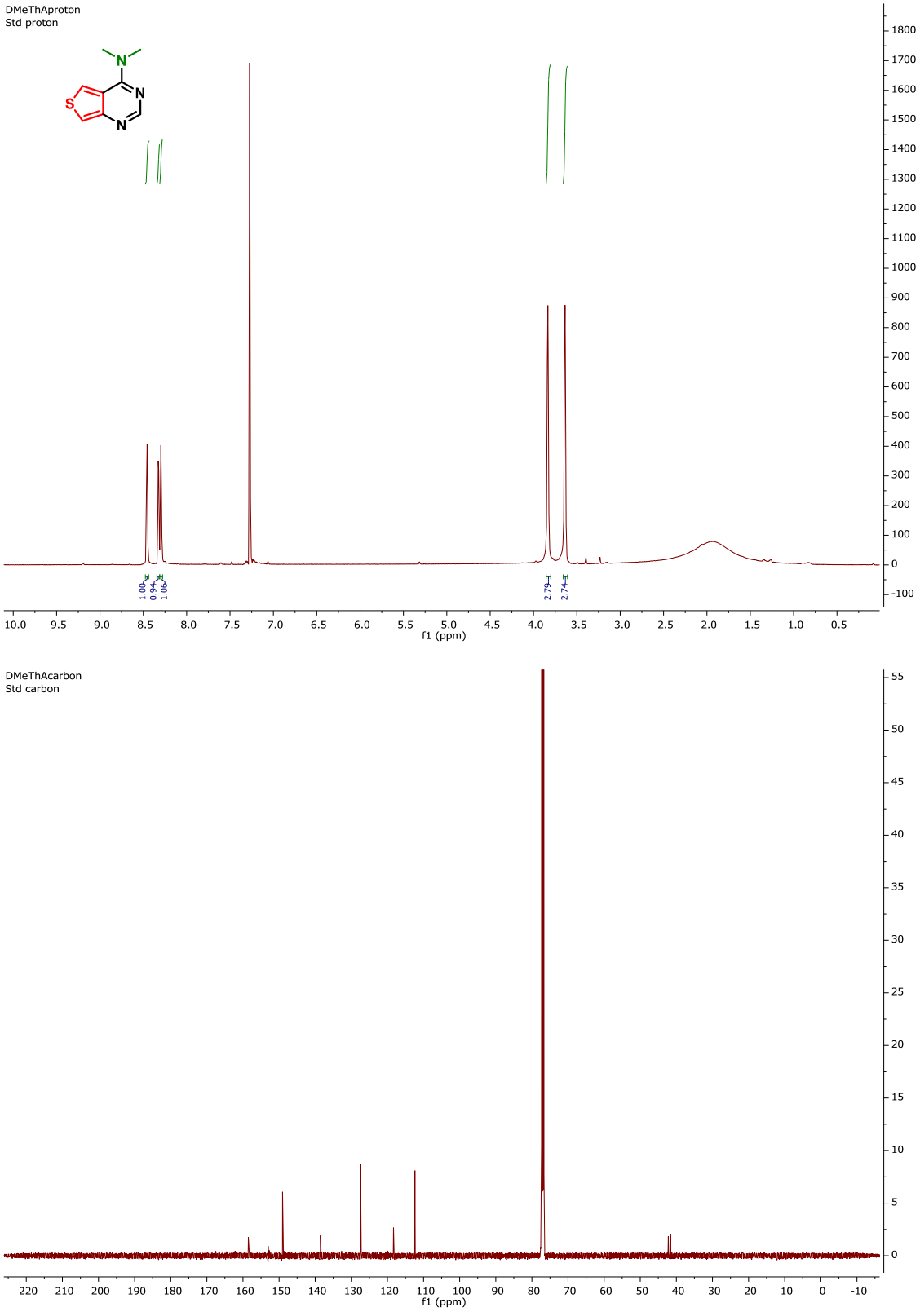


Figure S26. ^{1}H and ^{13}C NMR spectra of **2a**.

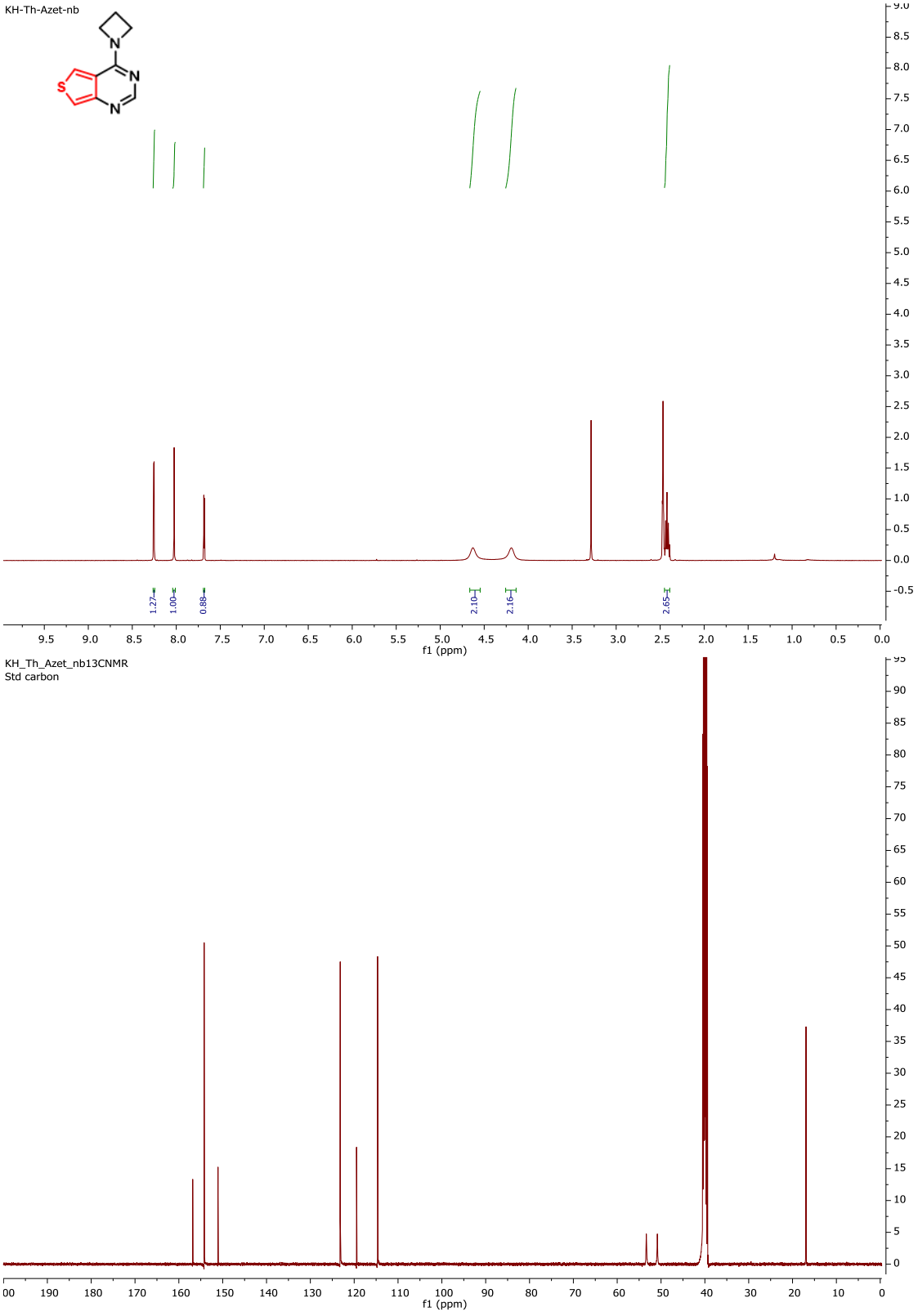


Figure S27. ^{1}H and ^{13}C NMR spectra of **3a**.

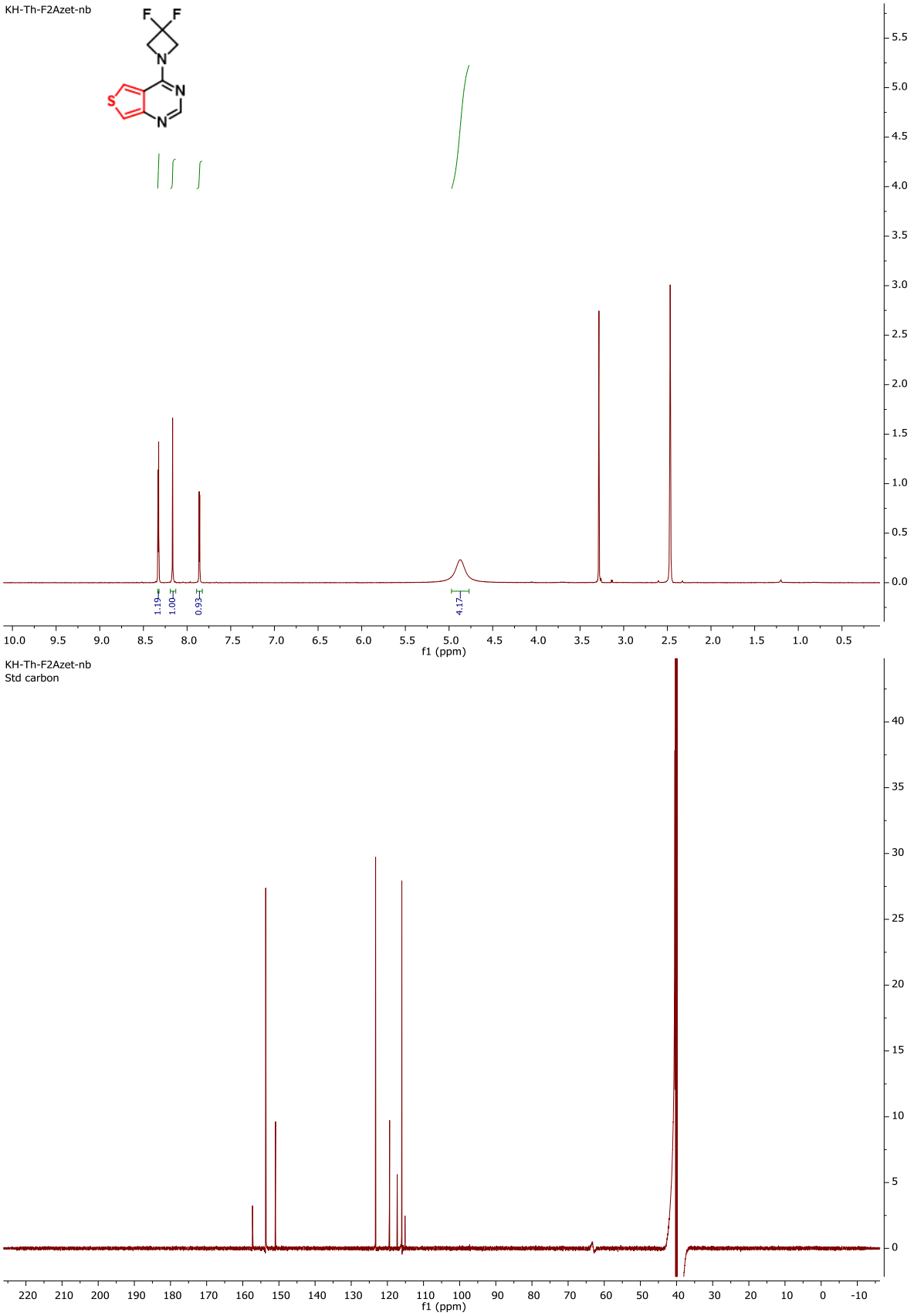


Figure S28. ^{1}H and ^{13}C NMR spectra of **4a**.

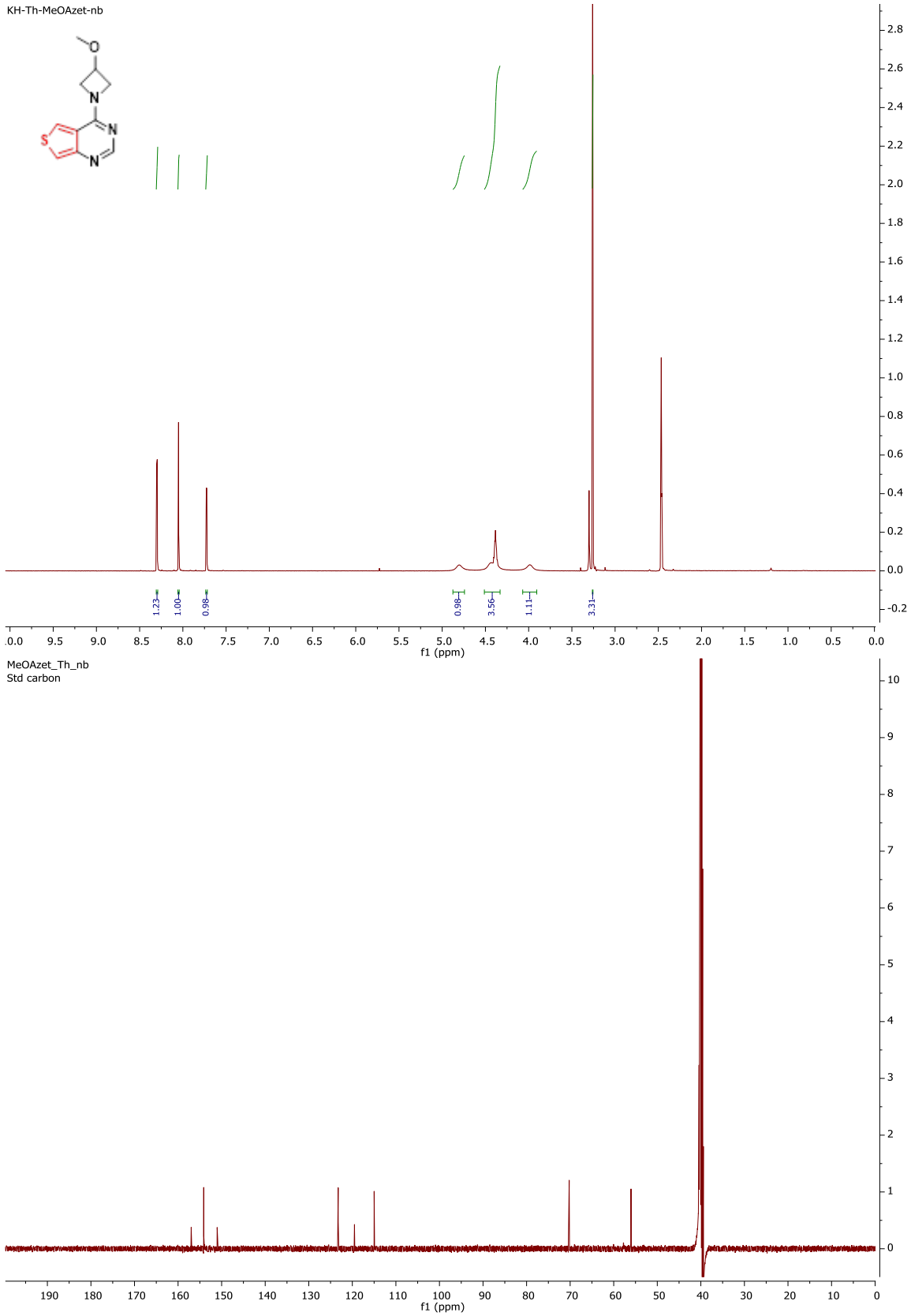


Figure S29. ^{1}H and ^{13}C NMR spectra of **5a**.

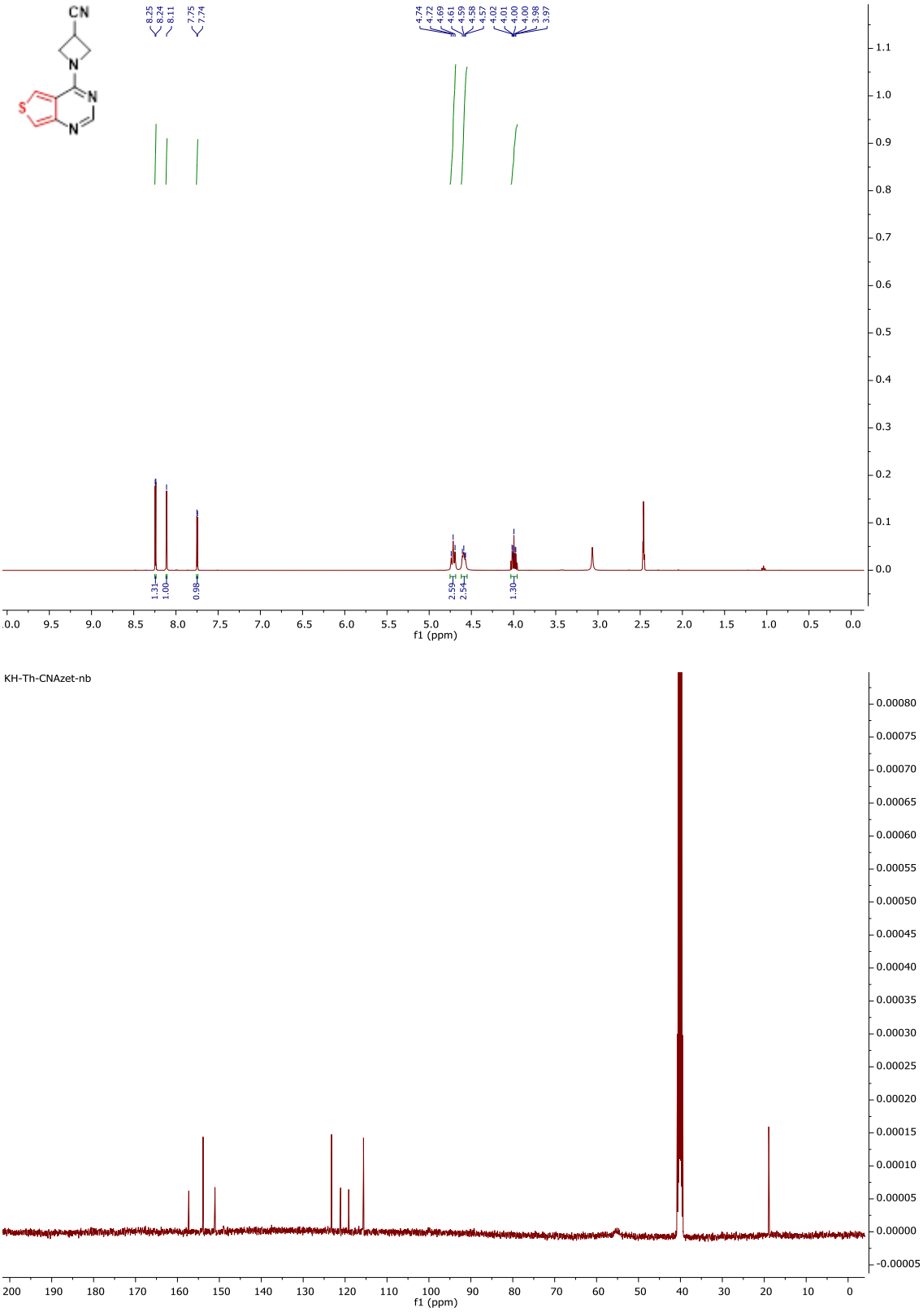


Figure S30. ^{1}H and ^{13}C NMR spectra of **6a**.

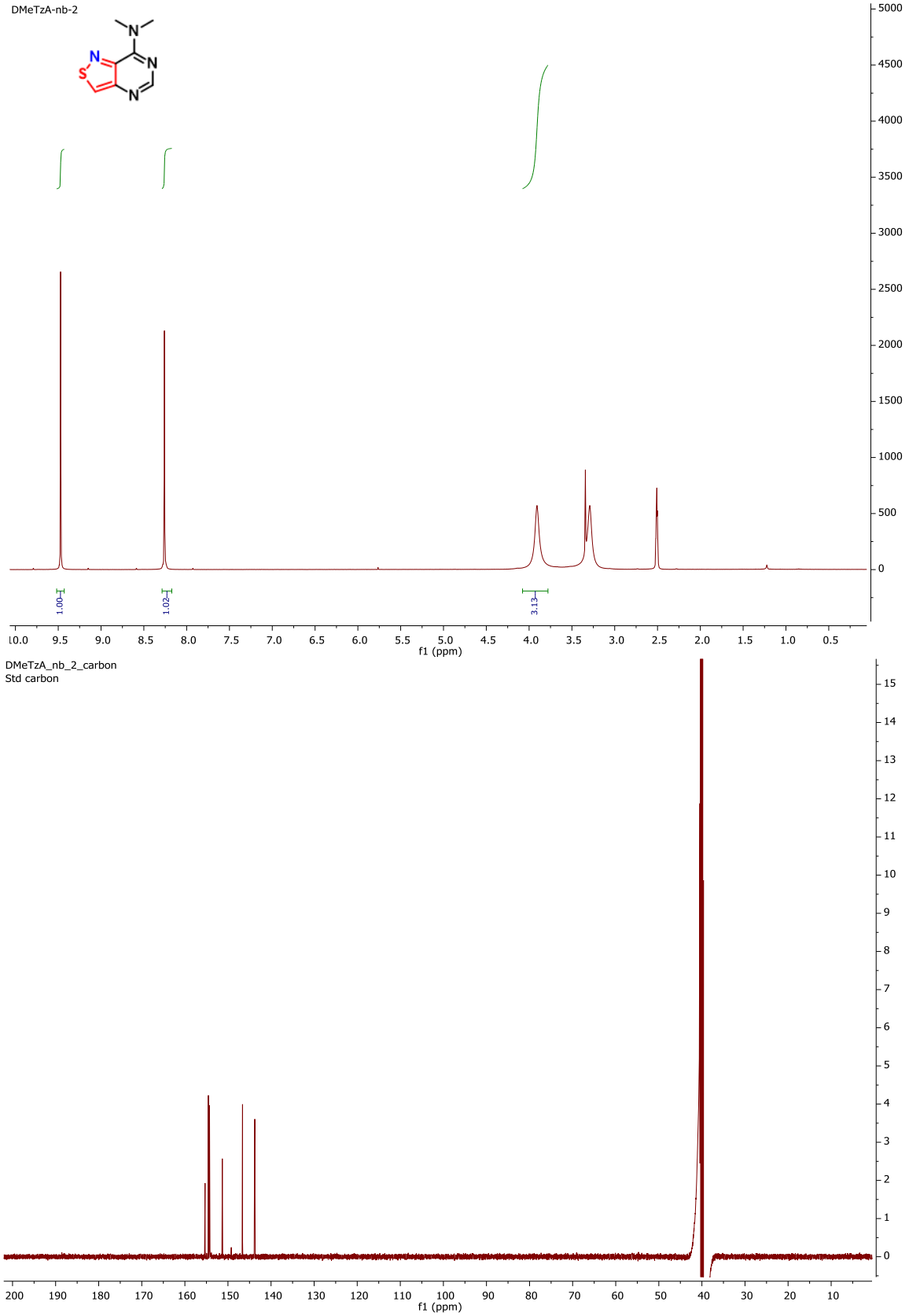


Figure S31. ^{1}H and ^{13}C NMR spectra of **2b**.

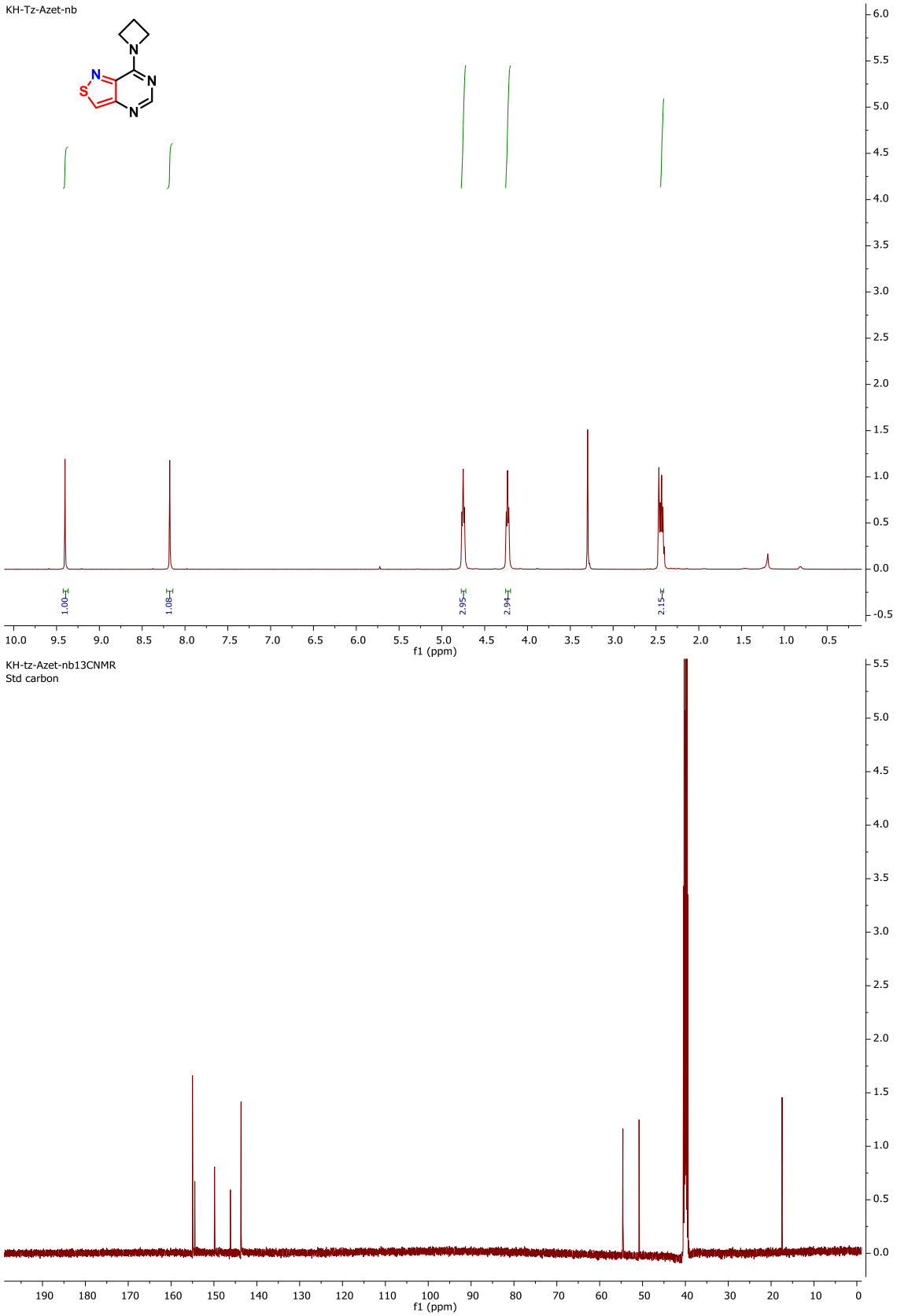


Figure S32. ^{1}H and ^{13}C NMR spectra of **3b**.

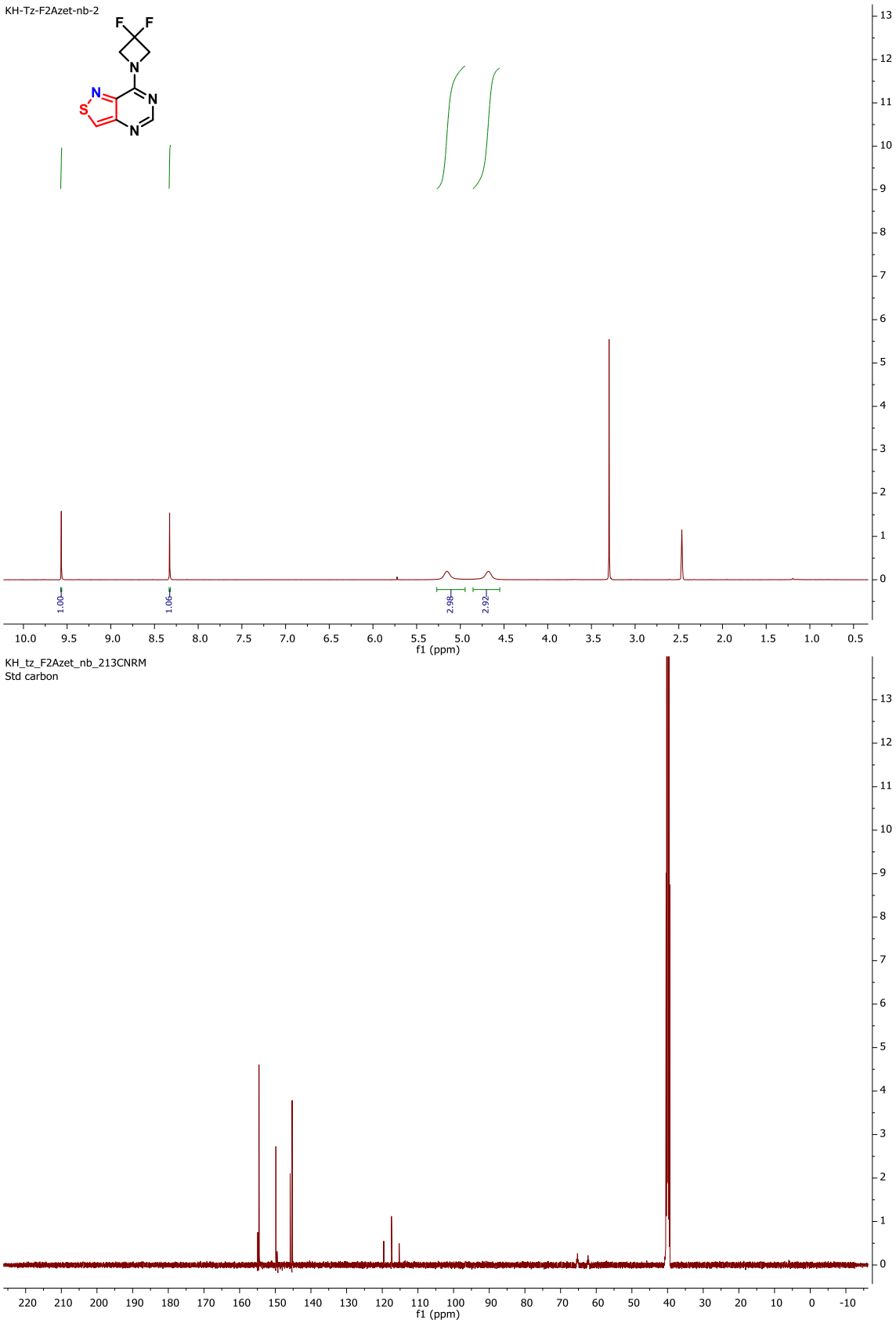


Figure S33. ^{1}H and ^{13}C NMR spectra of **4b**.

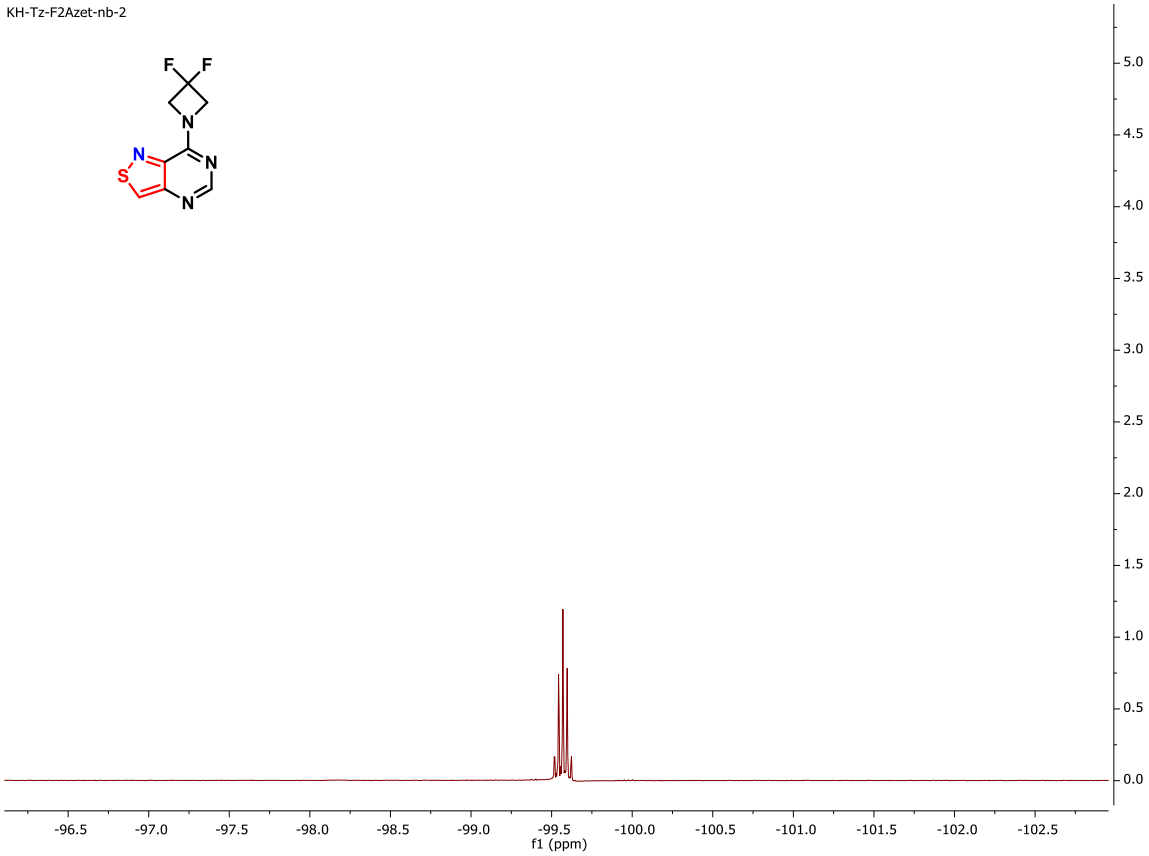


Figure S34. ^{19}F NMR spectra of **4b**.

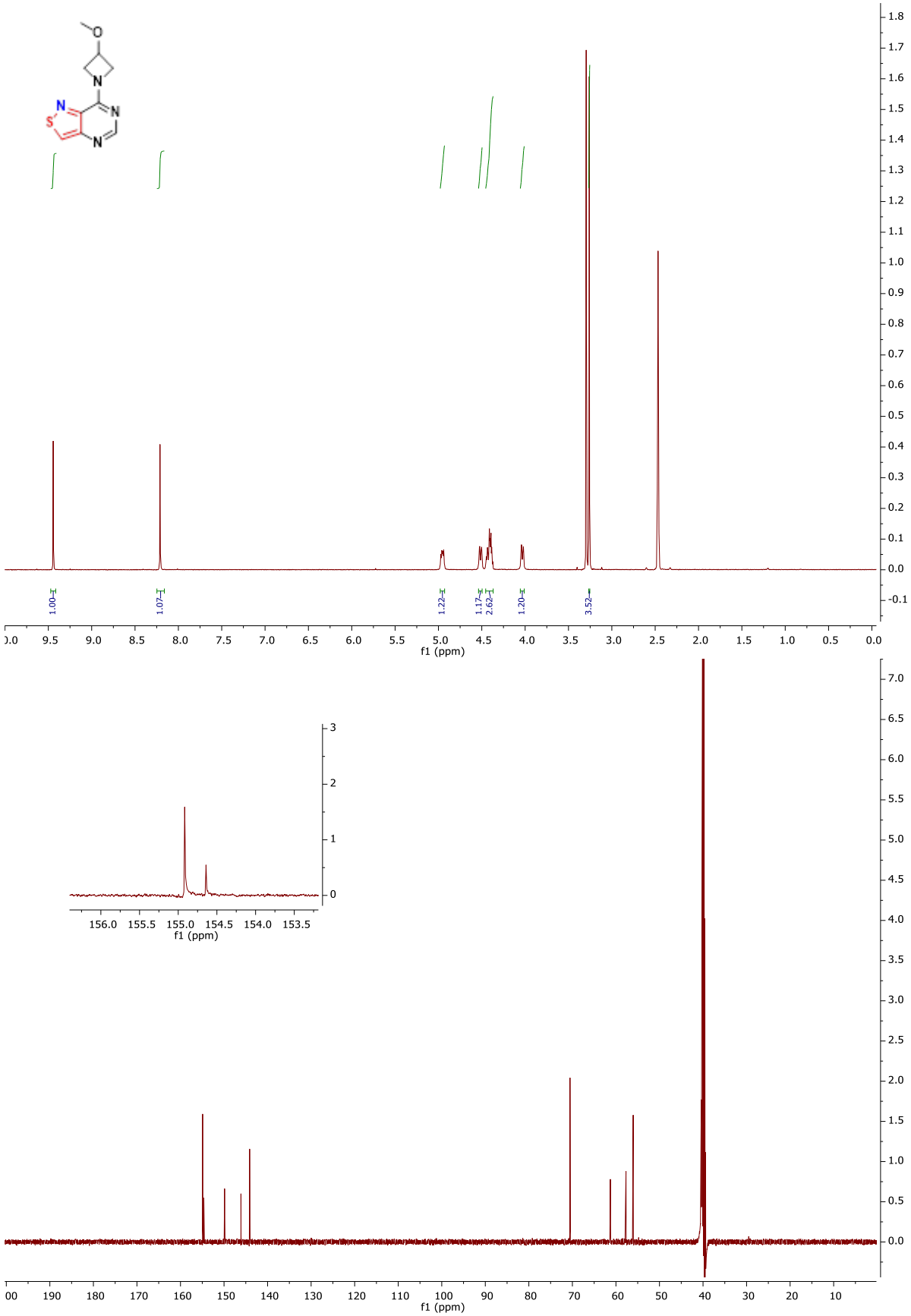


Figure S35. ^{1}H and ^{13}C NMR spectra of **5b**.

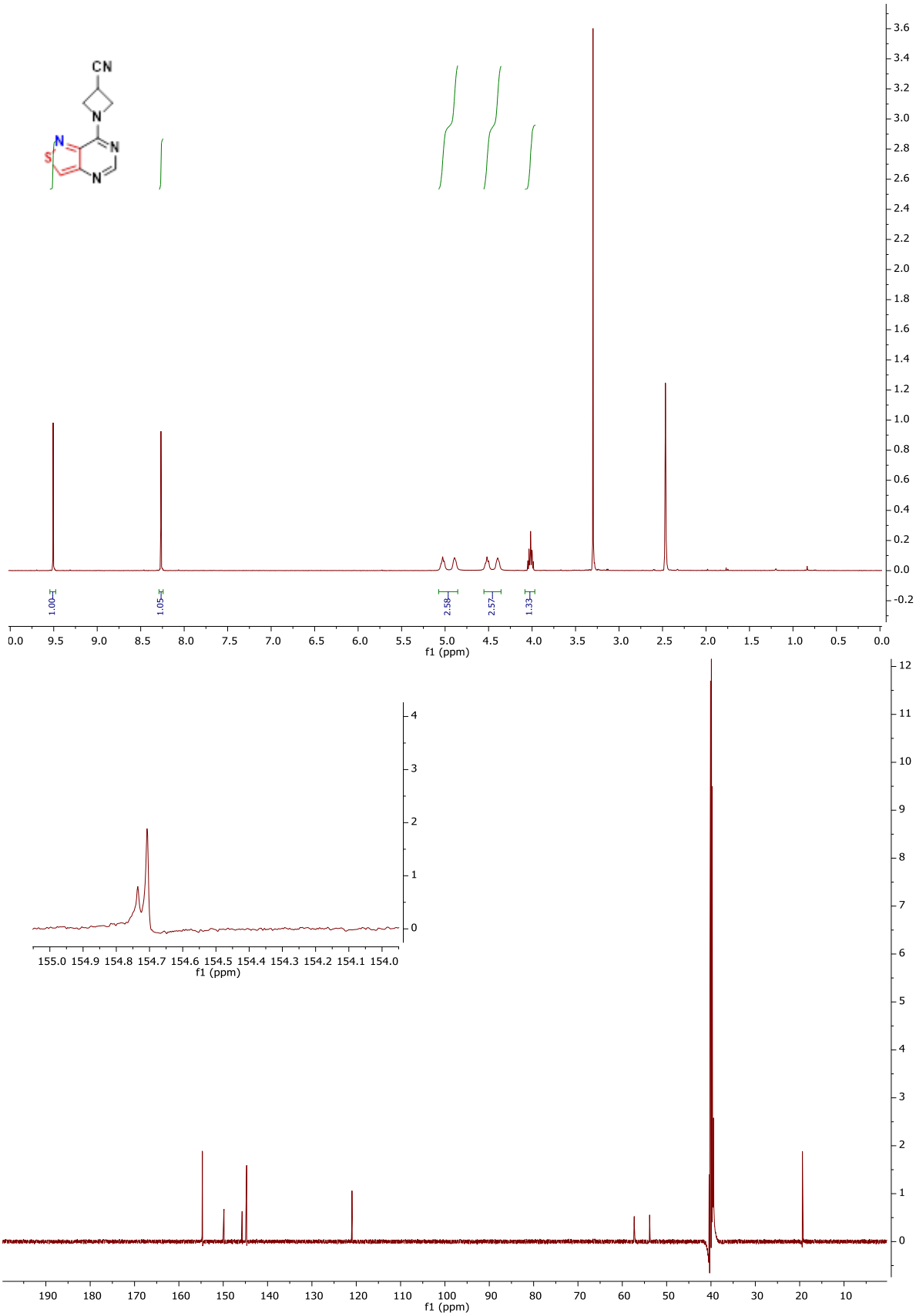


Figure S36. ^{1}H and ^{13}C NMR spectra of **6b**.

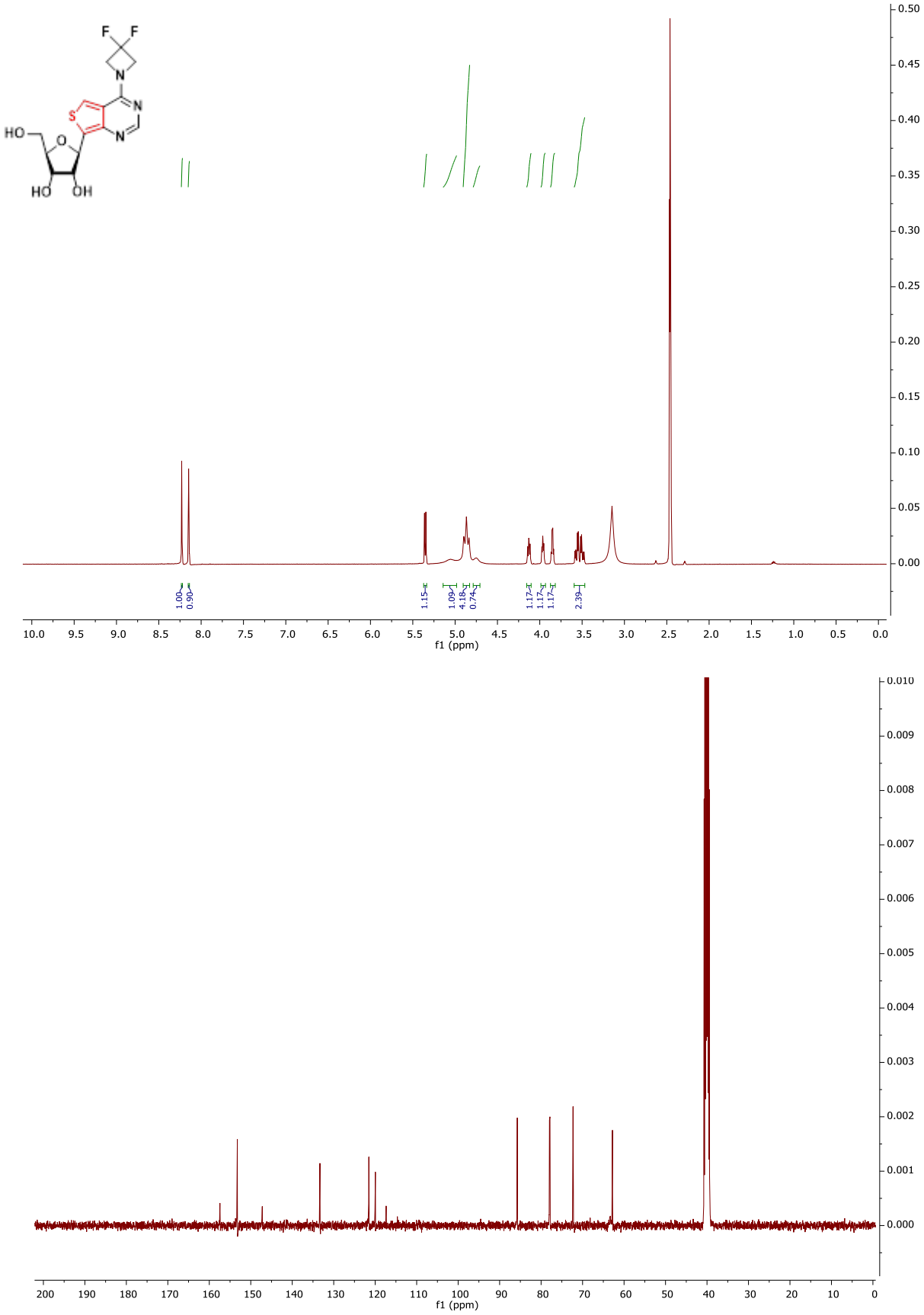


Figure S37. ^{1}H and ^{13}C NMR spectra of **11**.

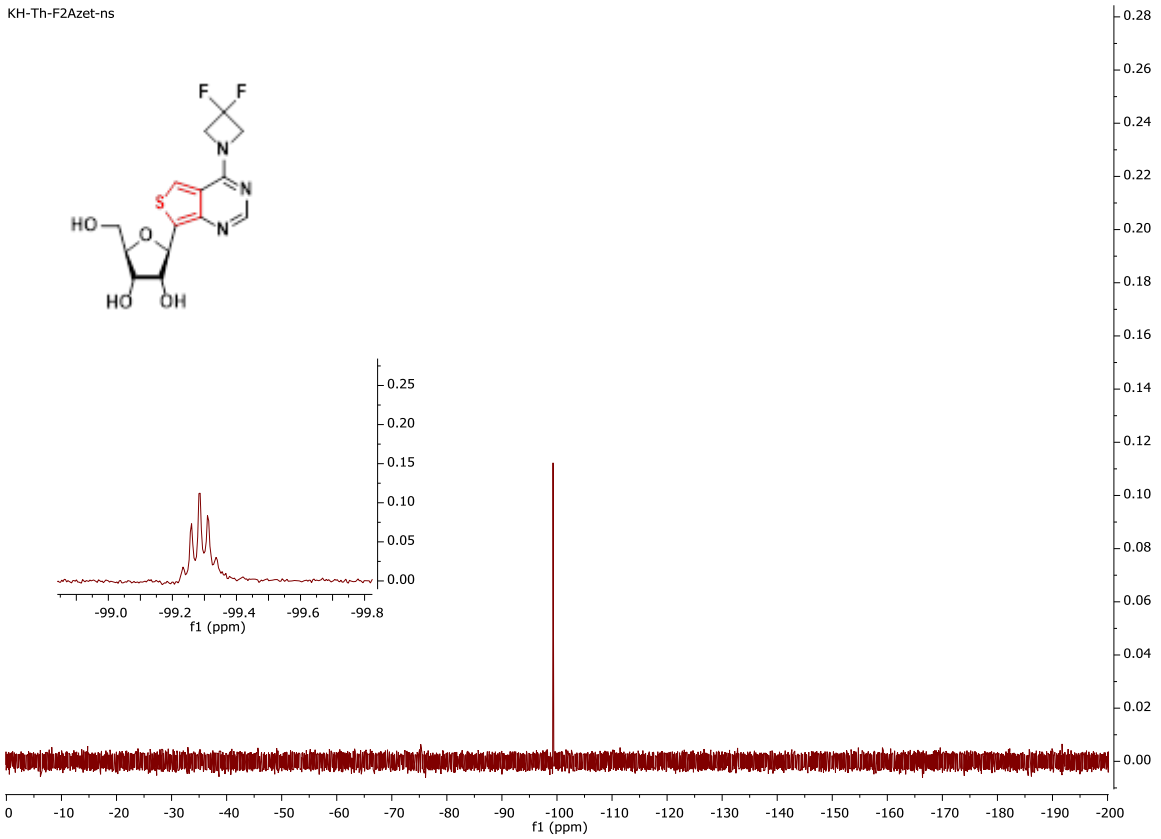


Figure S3. ^{19}F NMR spectra of **11**.

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