Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Development of subtype-selective covalent ligands for the adenosine A_{2B} receptor by tuning the reactive group

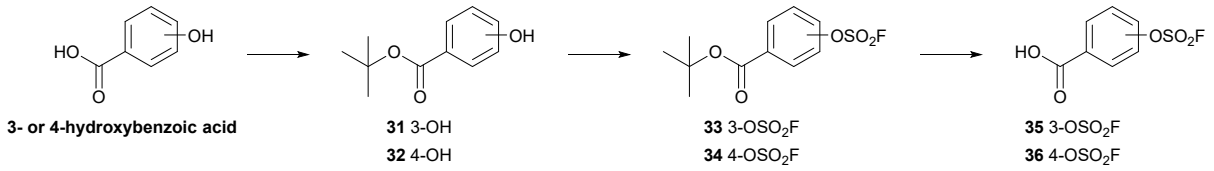
Table of contents

General Chemistry

All commercially available reagents and solvents were obtained from Sigma Aldrich, Fisher Scientific, VWR chemicals, Biosolve and J&K Scientific. All reactions were carried out under an N_2 atmosphere, unless noted otherwise. Thin layer chromatography was performed on TLC Silica gel 60 F254 (Merck) and visualized using UV irradiation at a wavelength of 254 or 366 nM. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a Bruker AV-400 (400 MHz), Bruker AV-500 spectrometer (500 MHz) or Bruker AV-600 spectrometer (600 MHz). Chemical shift values are reported in ppm (δ) using tetramethylsilane or solvent resonance as the internal standard. Coupling constants (J) are reported in Hz. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), h (hextet) or m (multiplet) followed by the number of represented hydrogen atoms. Compound purity was determined by LC-MS, using a LCMS-2020 system (Shimadzu) coupled to a Gemini® 3 µm C18 110Å column (50 x 3 mm). In brief, compounds were dissolved in H2O:MeCN:t-BuOH 1:1:1, injected onto the column and eluted with a linear gradient of H2O:MeCN 90:10 + 0.1% formic acid to H2O:MeCN 10:90 + 0.1% formic acid over the course of 15 minutes.

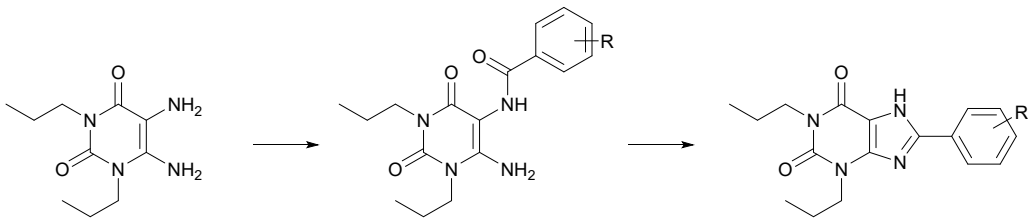
Synthetic Procedures

Dipropyl-substituted xanthines



3- or 4-hydroxybenzoic acid | **31** 3-OH, **32** 4-OH | **33** 3-OSO_2F, **34** 4-OSO_2F | **35** 3-OSO_2F, **36** 4-OSO_2F

Scheme 1 Synthesis of 3- and 4-fluorosulfonate benzoic acid.

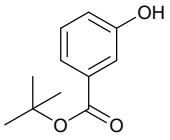


1

2 R = 3-SO_2F
3 R = 4-SO_2F
4 R = 3-OSO_2F
5 R = 4-OSO_2F
6 R = 3-NHBoc
7 R = 4-NHBoc

8 R = 3-SO_2F
9 R = 4-SO_2F
10 R = 3-OSO_2F
11 R = 4-OSO_2F
12 R = 3-NHBoc
d → **13** R = 3-NCS
14 R = 4-NHBoc
d → **15** R = 4-NCS

Scheme 2 Synthesis of the N^1, N^3-dipropyl-substituted xanthines.



***tert*-Butyl 3-hydroxybenzoate** (**31**)[1]

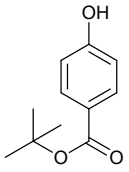
3-Hydroxybenzoic acid (849 mg, 6.15 mmol, 1.0 eq) was suspended in dry benzene (100 mL) and refluxed. N,N-Dimethylformamide di-*tert*-butyl acetal (5.0 g, 24.59 mmol, 4.0 eq) was added dropwise over 20 minutes and the mixture was refluxed an additional 30 minutes. The mixture was then cooled, washed with water (50 mL), a saturated $NaHCO_3$ solution (2 x 50 mL) and brine (50 mL). The organic

layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (Pentane:EtOAc 5:1) to yield **31** as a colorless oil (440 mg, 2.27 mmol, 37%).

TLC (Pentane:EtOAc 5:1) R_f = 0.43.

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.64 (dd, J = 2.7, 1.6 Hz, 1H), 7.54 (dt, J = 7.7, 1.3 Hz, 1H), 7.48 (s, 1H), 7.27 (t, J = 7.9 Hz, 1H), 7.08 (ddd, J = 8.1, 2.6, 1.0 Hz, 1H), 1.58 (s, 9H).

HPLC 99%, RT 9.686 min. **LC-MS** $[ESI - H]^+$: 193.00.

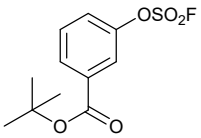


***tert*-Butyl 4-hydroxybenzoate** (**32**)[2]

tert-Butanol (49.5 mL, 521 mmol, 36.0 eq) was added to a solution of 4-hydroxybenzoic acid (2.0 g, 14.48 mmol, 1.0 eq) and DMAP (88 mg, 0.72 mmol, 0.05 eq) in dry THF (50 mL). A solution of DCC (3.0 g, 14.48 mmol, 1.0 eq) in dry THF (50 mL) was added dropwise over 30 minutes. The mixture was stirred overnight at rt. The formed side-product was removed by filtration and the filtrate was concentrated, dissolved in DCM. A saturated $NaHCO_3$ solution was added and the aqueous layer was extracted with DCM (3 x), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (Pentane:EtOAc 5:1) to yield **32** as a white solid (1.4 g, 7.41 mmol, 51%).

TLC (Pentane:EtOAc 5:1) R_f = 0.50.

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.89 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 1.59 (s, 9H).



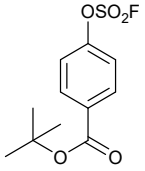
***tert*-Butyl 3-((fluorosulfonyl)oxy)benzoate** (**33**)[3]

[4-(Acetylamino)phenyl]imidosulfuryl difluoride (AISF) (854 mg, 2.72 mmol, 1.2 eq) was added to a solution of **31** (440 mg, 2.27 mmol, 1.0 eq) in dry THF (10 mL). DBU (751 µL, 4.98 mmol, 2.2 eq) was added and the mixture was stirred at rt for 1 h. EtOAc (50 mL) and 0.5 M HCl (50 mL) were added and the mixture was extracted with EtOAc (3 x 50 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (Pentane:EtOAc 9:1) to yield **33** as a colorless oil (571 mg, 2.07 mmol, 91%).

TLC (Pentane:EtOAc 9:1) R_f = 0.90.

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 8.05 (dt, J = 7.4, 1.5 Hz, 1H), 7.96 – 7.92 (m, 1H), 7.58 – 7.53 (m, 1H), 7.53 – 7.48 (m, 1H), 1.61 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ [ppm] = 163.7, 150.0, 134.9, 130.4, 129.7, 124.9, 122.1, 82.5, 28.2.

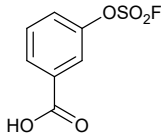


***tert*-Butyl 4-((fluorosulfonyl)oxy)benzoate** (**34**)

AISF (777 mg, 2.47 mmol, 1.2 eq) was added to a solution of **32** (400 mg, 2.06 mmol, 1.0 eq) in dry THF (10 mL). DBU (683 µL, 4.53 mmol, 2.2 eq) was added and the mixture was stirred at rt for 1 h. EtOAc (50 mL) and 0.5 M HCl (50 mL) were added and the aqueous layer was extracted with EtOAc (3 x 50 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (Pentane:EtOAc 9:1) to yield **34** as a colorless oil (494 mg, 1.79 mmol, 87%).

TLC (DCM:MeOH 98:2) R_f = 0.83.

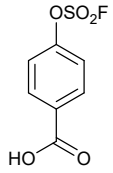
^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 8.12 (d, J = 8.9 Hz, 2H), 7.39 (dd, J = 9.0, 0.9 Hz, 2H), 1.60 (s, 9H).



3-((Fluorosulfonyl)oxy)benzoic acid (**35**)

TFA (8.27 mL, 107 mmol, 60.0 eq) was added to a solution of **33** (571 mg, 2.07 mmol, 1.0 eq) in DCM (8 mL). The mixture was stirred for 3 h at rt, after which the reaction showed full completion. The solvents were evaporated under reduced pressure to yield **35** as a white solid (455 mg, 2.07 mmol, quant).

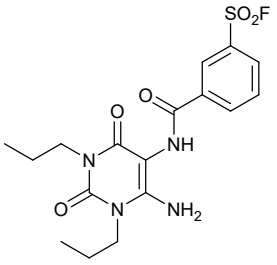
^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 11.83 (s, 1H), 8.23 – 8.17 (m, 1H), 8.10 (s, 1H), 7.68 – 7.60 (m, 2H).



4-((Fluorosulfonyl)oxy)benzoic acid (**36**)

TFA (8.27 mL, 107 mmol, 60.0 eq) was added to a solution of **34** (494 mg, 1.79 mmol, 1.0 eq) in DCM (8 mL). The mixture was stirred for 3 h at rt, after which the reaction showed full completion. The solvents were evaporated under reduced pressure to yield **36** as a white solid (394 mg, 1.79 mmol, quant).

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 8.26 (d, J = 9.0 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H).

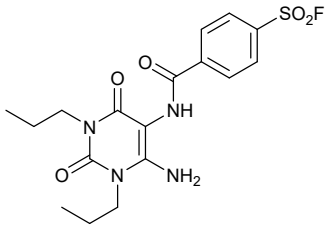


3-((6-Amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)benzenesulfonyl fluoride (**2**)

EDC·HCl (933 mg, 4.86 mmol, 1.1 eq) and DIPEA (770 µL, 4.42 mmol, 1.0 eq) were added to a solution of 5,6-diamino-1,3-dipropyluracil (**1**) hydrochloride (1511 mg, 5.75 mmol, 1.3 eq) and 3-(fluorosulfonyl)benzoic acid (903 mg, 4.42 mmol, 1.0 eq) in dry DMF (21 mL). The mixture was stirred overnight at rt. EtOAc (15 mL) was added and the organic layer was washed with water (400 mL) and brine (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 99:1 → 97:3) to yield **2** as yellow solid (1085 mg, 2.63 mmol, 60%).

TLC (DCM:MeOH 98:2) R_f = 0.27

^{1}H NMR (400 MHz, CD_3OD) δ [ppm] = 8,75 (s, J = 1.8 Hz, 1H), 8.51 (d, J = 7.9, 1.4 Hz, 1H), 8.28 (d, J = 8.1, 1.3Hz, 1H), 7,89 (t, J = 7.9 Hz, 1H), 3.98 – 3.92 (t, 2H), 3.92 – 3.86 (t, 2H), 1.79 – 1.70 (d, 2H), 1.70 – 1.61 (d, 2H), 1.02 (t, J = 7.4 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H).

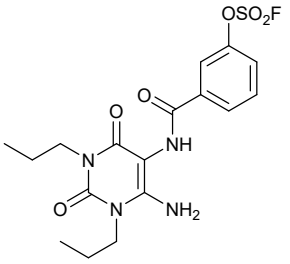


4-((6-Amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)benzenesulfonyl fluoride (**3**)

EDC·HCl (932 mg, 4.86 mmol, 1.1 eq) and DIPEA (752 µL, 4.32 mmol, 1.0 eq) were added to a solution of 5,6-diamino-1,3-dipropyluracil (**1**) hydrochloride (1161 mg, 4.42 mmol, 1.0 eq) and 4-(fluorosulfonyl)benzoic acid (902 mg, 4.42 mmol, 1.0 eq) in dry DMF (20 mL). The mixture was stirred for 2.5 h at rt. EtOAc (150 mL) was then added and the organic layer was washed with water (150 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL). The organic layers were combined, washed with water (3 x 100 mL), brine (100 mL), combined, dried over $MgSO_4$ and concentrated under reduced pressure to yield **3** as an off-white solid (749 mg, 1.82 mmol, 41%).

TLC (Pentane:EtOAc 1:1) R_f = 0.49.

^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ [ppm] = 8.34 (d, J = 8.3 Hz, 2H), 8.19 (d, J = 8.5 Hz, 2H), 6.46 (s, 1H), 3.97 (t, J = 7.8 Hz, 2H), 3.82 (t, J = 7.3 Hz, 2H), 1.72 (h, J = 7.4 Hz, 2H), 1.58 (h, J = 7.5 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 7.5 Hz, 3H).



3-((6-Amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl sulfurofluoridate (**4**)

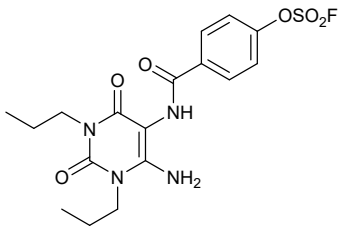
EDC·HCl (420 mg, 2.19 mmol, 1.1 eq) was added to a solution of **35** (439 mg, 1.99 mmol, 1.0 eq) in dry DMF (10 mL). 5,6-Diamino 1,3-dipropyl uracil (**1**) hydrochloride (524 mg, 1.99 mmol, 1.0 eq) was added and the mixture became a pink solution. DIPEA (693 µL, 3.98 mmol, 2.0 eq) was added and to form a clear orange solution. The mixture was then stirred for 4 h, upon which no starting material was detected anymore by LCMS. EtOAc (50 mL) was added and the organic mixture was washed with brine (3 x 50 mL). The aqueous layers were combined and back-extracted with EtOAc (3 x 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica column chromatography to yield **4** as yellow substance (329 mg, 0.77 mmol, 39%).

TLC (DCM:MeOH 96:4): Rf = 0.41

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 9.17 (s, 1H), 8.16 – 8.10 (m, 2H), 7.80 (dd, J = 8.3, 1.4 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 6.84 (s, 2H), 3.85 (t, J = 8.1, 7.2 Hz, 2H), 3.73 (t, J = 7.2 Hz, 2H), 1.60 – 1.54 (m, 2H), 1.53 – 1.47 (m, 2H), 0.89 (t, J = 7.4 Hz, 3H), 0.83 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 164.6, 159.0, 151.7, 150.4, 149.4, 137.3, 130.7, 128.7, 123.8, 120.5, 86.8, 43.7, 41.9, 20.9, 20.8, 11.2, 10.7.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ 39.0.



4-((6-Amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl sulfurofluoridate (**5**)

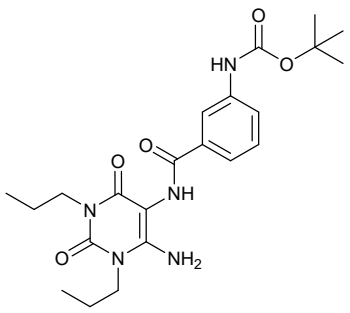
EDC·HCl (312 mg, 1.63 mmol, 0.9 eq) was added to a solution of **36** (376 mg, 1.71 mmol, 0.9 eq) in dry DMF (7.5 mL). The mixture was stirred for 30 min and then 5,6-diamino 1,3-dipropyl uracil (**5**) hydrochloride (476 mg, 1.81 mmol, 1.0 eq) and DIPEA (257 µL, 1.48 mmol, 1.0 eq) were added. The mixture was stirred for 5 h, upon which DIPEA (257 µL, 1.48 mmol, 1.0 eq) was added. The mixture was then stirred overnight. EtOAc (25 mL) was added and the organic layer was washed with water (2 x 25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (PE:EtOAc 7:3 → 0:1) to yield **5** as an orange solid (387 mg, 0.90 mmol, 50%).

TLC (DCM:MeOH 98:2) R_f = 0.53.

^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ [ppm] = 9.13 (s, 1H), 8.18 (d, J = 8.9 Hz, 2H), 7.44 (d, J = 8.7 Hz, 2H), 6.63 (s, 2H), 3.88 (t, J = 7.8 Hz, 2H), 3.77 (t, J = 7.2 Hz, 2H), 1.65 (h, J = 7.2 Hz, 2H), 1.52 (h, J = 7.5 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H), 0.82 (t, J = 7.5 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2CO$) δ [ppm] = 165.1, 160.5, 152.1, 151.6, 150.2, 134.6, 130.4, 120.5, 88.0, 44.2, 42.3, 21.0, 21.0, 10.6, 10.2.

^{19}F NMR (471 MHz, $(CD_3)_2CO$) δ [ppm] = 38.2.

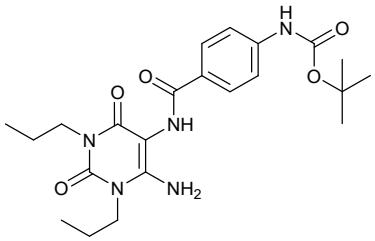


***tert*-Butyl (3-((6-amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl)carbamate** (**6**)

3-(Boc-amino)benzoic acid (474 mg, 2.00 mmol, 1.0 eq), EDC·HCl (422 mg, 2.20 mmol, 1.1 eq) and DIPEA (350 µL, 2.00 mmol, 1.0 eq) were added to a solution of 5,6-diamino 1,3-dipropyl uracil (**1**) hydrochloride (525 mg, 2.00 mmol, 1.0 eq) in dry DMF (10 mL). The mixture was stirred for 3 h, after which another 2.0 equivalents of DIPEA were added (350 µL, 2.00 mmol, 1.0 eq). The mixture was stirred for another hour and then diluted with EtOAc (80 mL). The organic layer was washed with water (3 x 80 mL), brine (80 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 90:10 → 95:5) to yield **6** as an off-white solid (446 mg, 1.00 mmol, 50%).

TLC (DCM:MeOH 97:3) R_f = 0.42

^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ [ppm] = 8.05 (t, J = 2.0 Hz, 1H), 7.70 (dd, J = 8.0, 1.5 Hz, 1H), 7.62 (ddd, J = 7.8, 1.8, 1.1 Hz, 1H), 7.36 (t, J = 7.9 Hz, 1H), 3.94 (t, J = 7.8 Hz, 2H), 3.80 (t, J = 7.3 Hz, 2H), 1.72 (h, J = 7.1 Hz, 2H), 1.65 – 1.51 (m, 2H), 1.49 (s, 9H), 0.94 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 7.5 Hz, 3H).



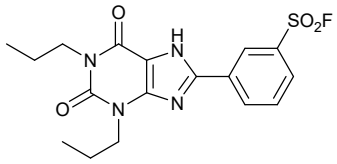
***tert*-Butyl (4-((6-amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl)carbamate** (**7**)

4-(Boc-amino)benzoic acid (237 mg, 1.00 mmol, 1.0 eq), EDC·HCl (211 mg, 1.10 mmol, 1.1 eq) and DIPEA (174 µL, 1.00 mmol, 1.0 eq) were added to a solution of 5,6-diamino 1,3-dipropyl uracil (**1**) hydrochloride (263 mg, 1.00 mmol, 1.0 eq) in dry DMF (5 mL). The mixture was stirred for 4 h, after which LCMS indicated full consumption of starting material. EtOAc (50 mL) was added and the organic layer was washed with water (2 x 50 mL) and brine (50 mL). The aqueous layers were combined and back-extracted with EtOAc (2 x 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 99:1 → 90:10) to yield **7** as an off-white solid (302 mg, 0.68 mmol, 68%).

TLC (DCM:MeOH 95:5) R_f = 0.27.

^{1}H NMR (400 MHz, CD_3OD) δ [ppm] = 7.93 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H), 3.88 – 3.81 (m, 2H), 3.81 – 3.75 (m, 2H), 1.68 – 1.56 (m, 4H), 1.54 (s, 9H), 0.96 (t, J = 7.4 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, MeOD) δ [ppm] = 170.3, 162.1, 154.7, 154.2, 152.2, 144.4, 130.0, 128.4, 118.4, 89.0, 81.2, 45.7, 44.0, 28.7, 22.2, 22.0, 11.6, 11.2.



3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)benzenesulfonyl fluoride (**8**)

PPSE (approximately 5 mL) was added to **2** (1085 mg, 2.63 mmol, 1.0 eq). The mixture was refluxed for 4 h at 170 °C and afterwards cooled down to rt overnight. MeOH (50 mL) was added and the formed residue was collected by filtration and subsequently purified by silica column chromatography (DCM:MeOH 99.5:0.5 → 85:15) to yield **8** as an off-white solid (281 mg, 0.71 mmol, 27%).

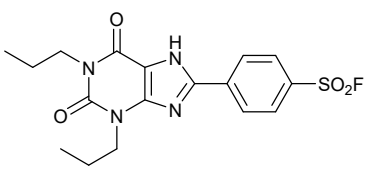
TLC (DCM:MeOH 99.5:0.5) R_f = 0.60.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 14.32 (s, 1H), 8.83 (t, J = 1.7 Hz, 1H), 8.61 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 8.9 Hz, 1H), 7.93 (t, J = 8.0 Hz, 1H), 4.02 (t, J = 7.4 Hz, 3H), 3.86 (t, J = 7.4 Hz, 2H), 1.74 (hept, J = 7.4 Hz, 2H), 1.58 (hept, J = 7.5 Hz, 2H), 0.91 (d, J = 7.4 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ [ppm] = 155.1, 151.5, 149.0, 147.9, 134.8, 133.6 (d, J = 24.1 Hz), 132.4, 131.7. 130.3, 126.4, 109.6, 45.5, 43.2, 21.8, 21.8, 12.1, 12.0.

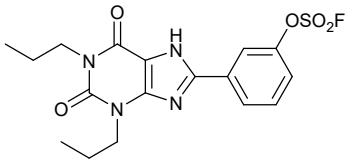
^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 66.5.

HPLC 100%, RT 11.292 min. **LC-MS** $[ESI + H]^+$: 395.05.

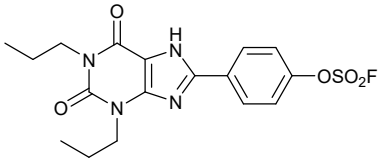


4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)benzenesulfonyl fluoride (**9**)

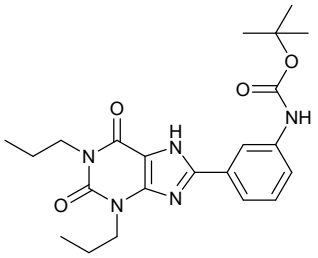
PPSE (approximately 2 mL) was added to **3** (500 mg, 1.21 mmol, 1.0 eq), refluxed for 1 h at 170 °C and afterwards cooled down to rt. MeOH was added and the product was allowed to crystallize overnight. The residue was collected and purified by silica column chromatography (DCM:MeOH 99.5:0.5 → 98.5:1.5) to yield **9** as an off-white solid (46 mg, 0.12 mmol, 10%).
TLC (DCM:MeOH 98:2) R_f = 0.40.
^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 14.41 (s, 1H), 8.47 (d, J = 8.6 Hz, 2H), 8.29 (d, J = 8.7 Hz, 2H), 4.03 (t, J = 7.2 Hz, 2H), 3.88 (t, J = 7.3 Hz, 2H), 1.75 (h, J = 7.4 Hz, 2H), 1.59 (h, J = 7.5 Hz, 2H), 0.91 (t, J = 7.5 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H).
^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 66.8.
HPLC: 97%, RT 11.395 min. LC-MS [ESI + H]+: 395.05.



3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl sulfurofluoridate (**10**)
PPSE (2 mL) was added to **4** (329 mg, 0.77 mmol, 1.0 eq) and refluxed at 170 °C. The mixture was stirred for 4 h and afterwards cooled down to rt. Water (50 mL) was added and the aqueous mixture was extracted with DCM (3 x 50 mL). The organic layers were combined, washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 99.5:0.5 → 98:2) to yield **10** as a white solid (186 mg, 0.45 mmol, 59%).
TLC (DCM:MeOH 99.5:0.5): Rf = 0.44.
^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 14.11 (s, 1H), 8.28 – 8.20 (m, 2H), 7.77 – 7.68 (m, 2H), 4.01 (t, J = 7.2 Hz, 2H), 3.86 (t, J = 7.4 Hz, 2H), 1.74 (h, J = 7.4 Hz, 2H), 1.58 (h, J = 7.4 Hz, 2H), 0.89 (dt, J = 12.1, 7.4 Hz, 6H).
^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 154.2, 150.6, 150.0, 148.1, 147.5, 131.7, 131.4, 127.0, 122.5, 118.5, 108.4, 44.5, 42.2, 20.8, 11.2, 11.0.
^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ 39.6.



4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl sulfurofluoridate (**11**)
PPSE (2 mL) was added to **5** (387 mg, 0.90 mmol, 1.0 eq) and refluxed at 170 °C. The mixture was stirred for 4 h and afterwards cooled down to rt. Water (50 mL) was added and the aqueous layer was extracted with DCM (3 x 25 mL). The organic layers were combined, washed with brine (50 mL) and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 99.75:0.25 → 99.5:0.5) to yield **11** as a white solid (198 mg, 0.48 mmol, 53%).
TLC (DCM:MeOH 98:2) R_f = 0.68.
^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 14.06 (s, 1H), 8.32 – 8.26 (m, 2H), 7.76 (d, J = 8.9 Hz, 2H), 4.01 (t, J = 6.9 Hz, 2H), 3.90 – 3.83 (m, 2H), 1.74 (h, J = 7.4 Hz, 2H), 1.58 (h, J = 7.5 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 7.5 Hz, 3H).
^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.2, 150.6, 150.3, 148.2, 148.0, 129.6, 128.8, 121.9, 108.4, 44.5, 42.2, 20.8, 20.8, 11.2, 11.0.
^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 39.0.
HPLC 98%, RT 11.524 min. **LC-MS** [ESI + H]$^+$: 411.10.

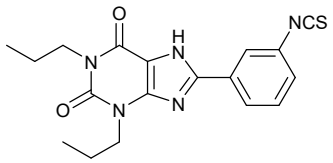


***tert*-Butyl (3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl)carbamate** (**12**)

2 M NaOH (5 mL) was added to a solution of **6** (446 mg, 1.00 mmol, 1.0 eq) in dioxane (5 mL). The mixture was refluxed for 3 h at 120 °C. The reaction was then cooled down to rt and water (65 mL) was added. The aqueous layer was washed with EtOAc (4 x 80 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 99:1 → 97:3) to yield **12** (170 mg, 0.40 mmol, 40%) and Boc-deprotected **12** (65 mg, 0.20 mmol, 20%), both as an off-white powder. The products were combined and used directly in the next reaction (0.60 mmol, 60%).

TLC (DCM:MeOH 98:2) R_f = 0.42.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 13.88 (s, 1H), 9.57 (s, 1H), 8.27 (s, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 4.06 (t, J = 6.9 Hz, 2H), 3.90 (t, J = 7.5 Hz, 2H), 1.85 – 1.72 (m, 2H), 1.71 – 1.55 (m, 2H), 1.53 (s, 9H), 0.95 (t, J = 6.2 Hz, 3H), 0.91 (t, J = 6.4 Hz, 3H).



8-(3-Isothiocyanatophenyl)-1,3-dipropyl-3,7-dihydro-1H-purine-2,6-dione (**13**)

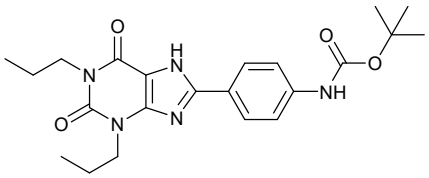
TFA (3 mL) was added to a suspension of **12** (0.60 mmol, 1.0 eq) in DCM (7 mL) and the mixture was stirred for 1 h at rt. TLC indicated full consumption of starting material and therefore 2 M NaOH (65 mL) was added to neutralize the reaction. The aqueous layer was extracted with EtOAc (4 x 60 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated to yield the respective amine. 3 M HCl (32 mL) was added to the crude amine to form a suspension. Thiophosgene (450 µL, 5.87 mmol, 9.8 eq) was added and the mixture was stirred for 2 h at rt. The mixture was then diluted with water (75 mL) and the aqueous layer was extracted with EtOAc (4 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 98:2 → 90:10) to yield **13** as an off-white powder (173 mg, 0.46 mmol, 77% over two steps).

TLC (DCM:MeOH 9:1) R_f = 0.26.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 13.99 (s, 1H), 8.13 (s, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.51 (d, J = 9.0 Hz, 1H), 4.01 (t, J = 6.8 Hz, 2H), 3.86 (t, J = 7.3 Hz, 2H), 1.74 (h, J = 7.4 Hz, 2H), 1.58 (h, J = 7.5 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 3.6 Hz, 3H).

^{13}C NMR (101 MHz, $(CD_3)_2SO$): δ [ppm] = 154.1 , 150.6 , 148.1 , 148.0, 134.9, 131.0, 130.7, 130.4, 127.1, 125.6, 123.7, 108.2, 44.5, 42.2, 20.9, 20.9, 11.2, 11.1.

HPLC 97%, RT 12.229 min. **LC-MS** $[ESI + H]^+$: 370.10.



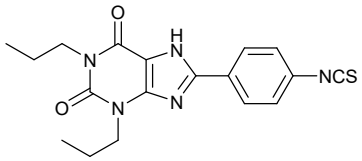
***tert*-Butyl (4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl)carbamate** (**14**)

2 M NaOH (10 mL) was added to a solution of **7** (302 mg, 0,68 mmol, 1.0 eq) in dioxane (10 ml). The mixture was refluxed at 120 °C for 2 h and afterwards allowed to cool down to rt. Water (50 mL) was then added and the aqueous layer was extracted with EtOAc (3 x 50 mL). The organic layers were combined, dried with $MgSO_4$, filtered and concentrated under reduced pressure to yield **14** as an off-white solid (242 mg, 0,566 mmol, 84 % yield).

TLC (DCM:MeOH 98:2): Rf = 0.42.

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 13.11 (s, 1H), 8.22 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 8.3 Hz, 2H), 4.16 (t, J = 7.4 Hz, 2H), 4.06 (t, J = 7.6 Hz, 2H), 1.93 – 1.81 (m, 2H), 1.78 – 1.69 (m, 2H), 1.54 (s, 9H), 1.02 – 0.93 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ [ppm] = 155.8, 152.7, 151.8, 151.2, 149.9, 140.8, 128.1, 123.5, 118.3, 107.9, 67.2, 45.4, 43.4, 28.5, 21.5, 21.5, 11.6, 11.3.



8-(4-Isothiocyanatophenyl)-1,3-dipropyl-3,7-dihydro-1H-purine-2,6-dione (**15**)

TFA (6 mL) was added to a suspension of **14** (242 mg, 0.57 mmol, 1.0 eq) in DCM (12 mL). The mixture immediately became a clear solution. The mixture was stirred for 1 h, upon which TLC showed full consumption of starting material. 2 M NaOH (50 mL) was added and the aqueous phase was extracted with EtOAc (3 x 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. 3 M HCl (30 mL) was added to the crude amine to form a suspension. Thiophosgene (437 µL, 5.70 mmol, 10.0 eq) was added and the mixture was stirred for 2 h at rt. Water (70 mL) was then added and the aqueous layer was extracted with EtOAc (4 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 99.75:0.25 → 99:1) to yield **15** as a white powder (144 mg, 0.39 mmol, 68% over two steps).

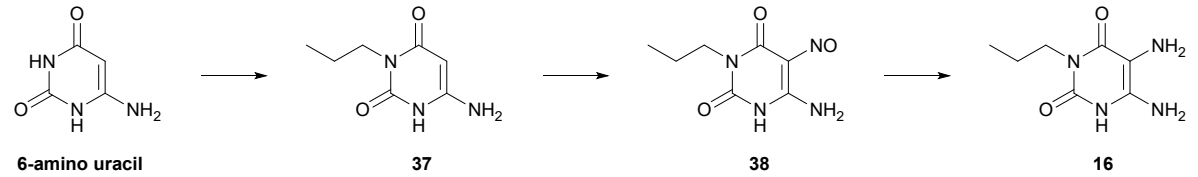
TLC (DCM:MeOH 9:1) R_f = 0.30.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 13.95 (s, 1H), 8.16 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 4.01 (t, J = 7.5, 7.0 Hz, 2H), 3.86 (t, J = 7.3 Hz, 2H), 1.74 (h, J = 7.3 Hz, 2H), 1.58 (h, J = 7.4 Hz, 2H), 0.93 – 0.85 (m, 6H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.1, 150.6, 148.5, 148.3, 134.7, 131.3, 127.9, 127.7, 126.6, 108.2, 44.4, 42.2, 20.8 (2C), 11.2, 11.0.

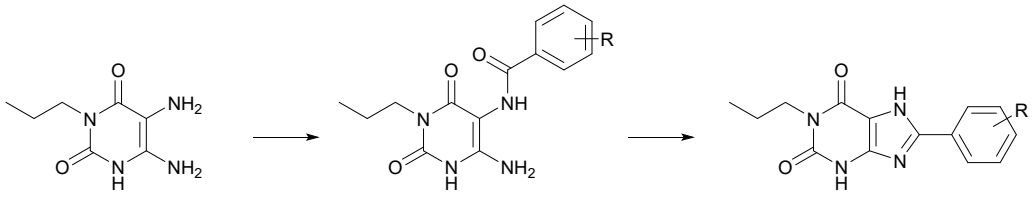
HPLC 98%, RT 12.194 min. **LC-MS** $[ESI + H]^+$: 370.10.

Monopropyl-substituted xanthines



6-amino uracil **37** **38** **16**

Scheme 3 Synthesis of 1-propyl 5-6-diamino uracil.

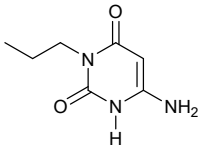


16

17 R = 3-SO_2F
18 R = 4-SO_2F
19 R = 3-OSO_2F
20 R = 4-OSO_2F
21 R = 3-NHFmoc
22 R = 4-NHFmoc

23 R = 3-SO_2F
24 R = 4-SO_2F
25 R = 3-OSO_2F
26 R = 4-OSO_2F
27 R = 3-NHFmoc
g → **28** R = 3-NCS
29 R = 4-NHFmoc
g → **30** R = 4-NCS

Scheme 4 Synthesis of the *N*[1]-propyl-substituted xanthines.



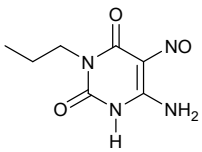
6-Amino-3-propylpyrimidine-2,4(1H,3H)-dione (**37**)[4]

6-aminouracil (20.00 g, 157 mmol, 1.0 eq) and ammonium sulfate (500 mg, 3.78 mmol) were added to a three-neck flask. HMDS (99 ml, 472 mmol, 3.0 eq) was added and the suspension was refluxed at 200 °C. After 2 h the suspension became a clear solution. The solution was cooled down to 80 °C and the HMDS was distilled off by boiling at 200 °C for 8 h. The solution was then cooled to 70 °C and iodine (150 mg, 0.591 mmol, cat) and 1-bromopropane (29 mL, 314 mmol, 2.0 eq) were added. The mixture was refluxed at 70 °C overnight. Extra 1-bromopropane (14.5 mL, 157 mmol, 1.0 eq) was added and the mixture was refluxed at 120 °C for 8 h, following by stirring at 70 °C overnight. Full conversion of starting material was observed and the mixture was put on ice. A saturated bicarb solution (400 mL) was gradually added. The dirty pink suspension was filtered over a glass filter and subsequently washed with water (100 mL), toluene (100 mL) and diethyl ether (100 mL). This yielded pure **37** as an orange/brown solid (21.439 g, 127 mmol, 81 % yield).

TLC (DCM:MeOH 9:1): R_f = 0.46.

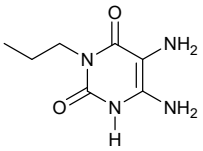
^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 10.31 (s, 1H), 6.16 (s, 2H), 4.53 (d, J = 1.9 Hz, 1H), 3.71 – 3.50 (m, 2H), 1.46 (h, J = 7.5 Hz, 2H), 0.81 (t, J = 7.5 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 162.9, 153.5, 151.0, 74.1, 40.2, 21.0, 11.2.

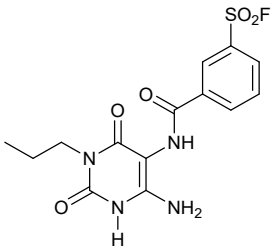


6-Amino-5-nitroso-3-propylpyrimidine-2,4(1H,3H)-dione (**38**)[4]

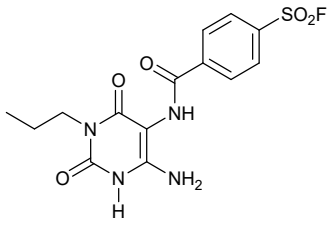
37 (21.439 g, 127 mmol, 1.0 eq) was dissolved in 300 mL H_2O:AcOH 1:1 at 65 °C. Sodium nitrite (10.97 g, 159 mmol, 1.3 eq) was added in parts to the stirring solution. An immediate color change was observed from brown to purple and back to brown. After approximately 30 minutes brown vapors started to form (NO_x). At this point the reaction was cooled on ice, filtered over a glass filter and washed thoroughly with water. This yielded **38** as a brown solid (18.429 g, 93 mmol, 73 % yield).
TLC (DCM:MeOH 9:1): R_f = 0.29.
^{1}H NMR (400 MHz, $(CH_3)_2SO$) δ [ppm] = 11.40 (s, 1H), 8.03 (s, 1H), 3.79 (t, J = 7.4 Hz, 2H), 1.59 (h, J = 7.5 Hz, 2H), 0.89 (t, J = 7.5 Hz, 3H).
^{13}C NMR (101 MHz, $(CH_3)_2SO$) δ [ppm] = 161.2, 149.2, 144.5, 139.7, 41.4, 20.8, 11.2.



5,6-Diamino-3-propylpyrimidine-2,4(1H,3H)-dione (**16**)[5]
38 (1000 mg, 5.05 mmol, 1.0 eq) was dissolved in MeOH (40 ml) and brought under an N_2 atmosphere. platinum(IV) oxide (20 mg, cat) was added and the mixture was flushed two times with H_2 (g). The mixture was stirred for 1 h under H_2(g), after which a white/grey precipitate had formed. DCM (180 mL) was added and the mixture was filtered over Celite. The Celite was washed with 10% MeOH in DCM (100 mL) and the filtrate was concentrated under reduced pressure. This yielded **16** as an orange/brown solid (806 mg, 4.38 mmol, 87 % yield). This was used in the next steps without further purification.
^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 5.56 (s, 2H), 3.70 – 3.59 (m, 2H), 1.48 (m, 2H), 0.81 (t, J = 7.5 Hz, 3H).



3-((6-Amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)benzenesulfonyl fluoride (**17**)
EDC·HCl (688 mg, 3.59 mmol, 1.20 eq) was added to a solution of crude **16** (551 mg, 2.99 mmol, 1.0 eq) and 3-(fluorosulfonyl)benzoic acid (641 mg, 3.14 mmol, 1.05 eq) in dry DMF (10 mL). The mixture was stirred overnight at rt. Water (80 mL) was then added and the aqueous layer was extracted with EtOAc (13x). The organic layers were combined, washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 96:4 → 90:10) to yield **17** as a white solid (598 mg, 2.99 mmol, 54%).
TLC (DCM:MeOH 9:1): R_f = 0.48.
^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 10.55 (s, 1H), 9.37 (s, 1H), 8.66 (t, J = 1.8 Hz, 1H), 8.45 (dt, J = 7.9, 1.4 Hz, 1H), 8.31 (ddd, J = 8.0, 2.0, 1.1 Hz, 1H), 7.92 (t, J = 7.9 Hz, 1H), 6.28 (s, 2H), 3.66 (t, J = 7.2 Hz, 2H), 1.51 (h, J = 7.5 Hz, 2H), 0.84 (t, J = 7.5 Hz, 3H).
^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 164.3, 160.6, 150.6, 149.9, 136.6, 135.8, 131.6 (d, J = 23.6 Hz), 130.7, 130.6, 127.6, 86.3, 40.9, 21.0, 11.2.
^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 66.1.
HPLC RT 7.393 min. **LC-MS** $[ESI + H]^+$: 371.00.



4-((6-Amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)benzenesulfonyl fluoride (**18**)

EDC·HCl (513 mg, 2.67 mmol, 1.3 eq) was added to a solution of crude **16** (379 mg, 2.06 mmol, 1.0 eq) and 4-(fluorosulfonyl)benzoic acid (462 mg, 2.26 mmol, 1.1 eq) in dry DMF (8 mL). The mixture was stirred at rt overnight. Water (80 mL) was then added and the aqueous layers was extracted with EtOAc (7x). The organic layers were combined, washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 95:5 → 90:10) to yield **18** as a yellow solid (100 mg, 0.27 mmol, 13%).

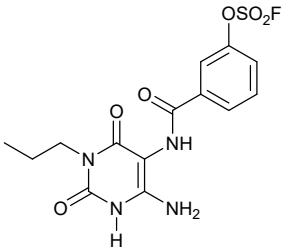
TLC (DCM:MeOH 9:1): R_f = 0.48.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 10.55 (s, 1H), 9.30 (s, 1H), 8.27 (s, 4H), 6.26 (s, 2H), 3.66 (t, J = 7.4 Hz, 2H), 1.55 – 1.46 (m, 2H), 0.84 (t, J = 7.5 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 164.8, 160.5, 150.5, 149.9, 141.8, 133.4 (d, J = 23.7 Hz), 129.7, 128.3, 86.3, 40.9, 21.0, 11.2.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 66.0.

HPLC RT 7.370 min. **LC-MS** $[ESI + H]^+$: 371.00.



3-((6-amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl sulfurofluoridate (**19**)

EDC·HCl (428 mg, 2.24 mmol, 1.2 eq) was added to a solution of crude **16** (343 mg, 1.86 mmol, 1.0 eq) and **35** (430 mg, 1.96 mmol, 1.05 eq) in dry DMF (10 mL). The mixture was stirred overnight at rt. TLC and LCMS showed full conversion of starting material and therefore water (80 mL) was added. The formed precipitate was collected and the filtrate was extracted with 5% MeOH in hot $CHCl_3$. The organic layer was concentrated and both residues were purified by column chromatography ($CHCl_3$:MeOH 98:2 → 92.5:7.5), combined and re-crystallized in MeOH to yield **19** as a white solid (237 mg, 0.61 mmol, 33%).

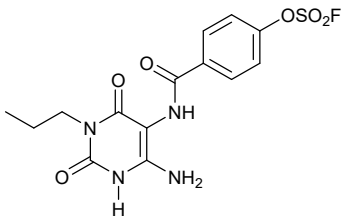
TLC ($CHCl_3$:MeOH 95:5) R_f = 0.32.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 10.54 (s, 1H), 9.17 (s, 1H), 8.11 (d, J = 7.6 Hz, 2H), 7.86 – 7.76 (m, 1H), 7.72 (t, J = 8.0 Hz, 1H), 6.24 (s, 2H), 3.66 (t, J = 7.3 Hz, 2H), 1.50 (h, J = 7.5 Hz, 2H), 0.83 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 164.5, 160.6, 150.6, 150.0, 149.4, 137.3, 130.8, 128.6, 123.8, 120.5, 86.4, 41.0, 21.0, 11.2.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 38.9.

HPLC 100%, RT 7.817 min. **LC-MS** $[ESI + H]^+$: 386.95.



4-((6-Amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl sulfurofluoridate (**20**)

EDC·HCl (446 mg, 2.33 mmol, 1.3 eq) was added to a solution of crude **16** (330 mg, 1.79 mmol, 1.0 eq) and **36** (394 mg, 1.79 mmol, 1.0 eq) in dry DMF (10 mL). The mixture was stirred overnight at rt. TLC and LCMS showed full conversion of starting material and therefore water (80 mL) was added. The aqueous layer was extracted with EtOAc (4 x). The organic layers were combined, washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM:MeOH 94:6 → 90:10) and re-crystallized in MeOH to yield **20** as a white solid (104 mg, 0.27 mmol, 15%).

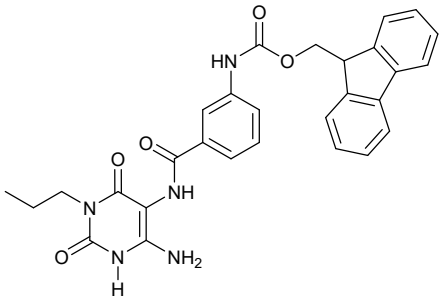
TLC ($CHCl_3$:MeOH 94:6) R_f = 0.32.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 10.51 (s, 1H), 9.09 (s, 1H), 8.14 (d, J = 8.5 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 6.19 (s, 2H), 3.66 (t, J = 7.2 Hz, 2H), 1.50 (h, J = 7.4 Hz, 2H), 0.84 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 164.0, 159.6, 150.2, 149.6, 149.0, 134.4, 129.6, 119.9, 85.5, 39.9, 20.0, 10.2.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 38.9.

HPLC 100%, RT 7.875 min. **LC-MS** $[ESI + H]^+$: 386.95.

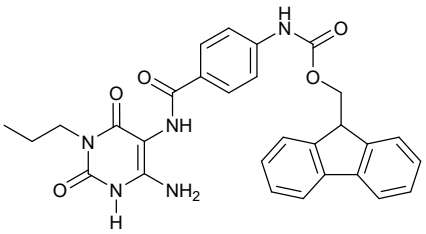


(9H-Fluoren-9-yl)methyl (3-((6-amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl)carbamate (**21**)

3-(Fmoc-amino)benzoic acid (1.34 g, 3.74 mmol, 1.05 eq) and EDC·HCl (819 mg, 4.27 mmol, 1.2 eq) were added to a solution of crude **16** (656 mg, 3.56 mmol, 1.0 eq) in dry DMF (20 mL). The mixture was stirred for 2 days, after which LCMS and TLC showed full conversion of starting material. The DMF was removed by heating under reduced pressure and water (100 mL) was added. The mixture was extracted with EtOAc (4 x 100 mL), washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was collected, washed several times with MeOH and acetone and further purified by column chromatography ($CHCl_3$:MeOH 95:5) to yield **21** as an off-white solid (397 mg, 0.76 mmol, 21%).

TLC ($CHCl_3$:MeOH 95:5) R_f = 0.20.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 10.47 (s, 1H), 9.89 (s, 1H), 8.84 (s, 1H), 7.99 (s, 1H), 7.91 (d, J = 7.5 Hz, 2H), 7.77 (d, J = 7.4 Hz, 2H), 7.65 (d, J = 8.1 Hz, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.35 (td, J = 7.4, 1.3 Hz, 3H), 6.07 (s, 2H), 4.47 (d, J = 6.8 Hz, 2H), 4.32 (t, J = 6.8 Hz, 1H), 3.66 (t, J = 7.6, 7.1 Hz, 2H), 1.50 (h, J = 7.5 Hz, 2H), 0.84 (t, J = 7.5 Hz, 3H).



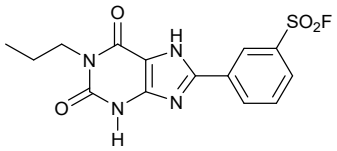
(9H-fluoren-9-yl)methyl (4-((6-amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbamoyl)phenyl)carbamate (**22**)

4-(Fmoc-amino)benzoic acid (1.89 g, 5.25 mmol, 1.2 eq) and EDC·HCl (1.09 g, 5.70 mmol, 1.3 eq) were added to a solution of **16** (806 mg, 4.38 mmol, 1.0 eq) in dry DMF (16 mL). The mixture was stirred for 1 h, after which LCMS showed completion of the reaction. EtOAc (250 mL) was added and the organic layer was washed with brine (3 x 100 mL). Upon addition of brine, a precipitate formed in the organic layer. The precipitate was collected and recrystallized in MeOH to yield **22** as yellow/green solid (1.17 g, 2.22 mmol, 51%).

TLC (DCM:MeOH 98:2): Rf = 0.44.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 10.62 (s, 1H), 10.01 (s, 1H), 8.76 (s, 1H), 7.92 (dd, J = 13.8, 7.9 Hz, 4H), 7.78 (d, J = 7.5 Hz, 2H), 7.54 (s, 2H), 7.45 (t, J = 7.4 Hz, 2H), 7.37 (t, J = 6.8 Hz, 2H), 6.20 (s, 2H), 4.53 (d, J = 6.7 Hz, 2H), 4.34 (t, J = 6.6 Hz, 1H), 3.66 (t, J = 7.5 Hz, 2H), 1.51 (h, J = 7.1 Hz, 2H), 0.84 (t, J = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ [ppm] = 165.8, 160.7, 153.3, 150.6, 149.9, 143.7, 141.7, 140.8, 128.8, 128.3, 127.7, 127.2, 125.2, 120.2, 117.1, 87.0, 65.8, 46.6, 40.9, 21.0, 11.2.



3-(2,6-Dioxo-1-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)benzenesulfonyl fluoride (**23**)

Trimethylsilyl polyphosphate (PPSE) (2.50 g, 13.72 mmol, 9.0 eq) was added to **17** (568 mg, 1.53 mmol, 1.0 eq). The mixture was refluxed at 150 °C for 3.5 h under an N_2 atmosphere. LCMS measurements indicated full conversion of the reaction. The mixture was cooled on ice and MeOH (25 mL) was added. The mixture was stirred for 15 minutes and filtrated. The filtrate was stirred in MeOH (15 mL) and filtrated again. The residues were combined, washed with cold MeOH and dried *in vacuo* to yield **23** as a white powder (467 mg, 1.33 mmol, 86%).

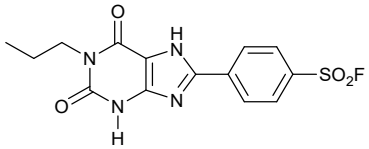
TLC (DCM:MeOH 98:2) R_f = 0.44.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 14.11 (s, 1H), 11.95 (s, 1H), 8.78 (s, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 7.89 (t, J = 7.9 Hz, 1H), 3.80 (t, J = 7.4 Hz, 2H), 1.56 (h, J = 7.5 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.9, 150.9, 147.4, 147.0, 133.5, 132.6 (d, J = 24.0 Hz), 131.4, 130.8, 129.1, 125.5, 108.5, 41.5, 20.9, 11.2.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 66.0.

HPLC: 100%, RT 9.095 min **LC-MS** $[ESI + H]^+$: 353.00.



4-(2,6-Dioxo-1-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)benzenesulfonyl fluoride (**24**) **(LUF7982)**

PPSE (430 mg, 2.36 mmol, 9.0 eq) was added to **18** (97 mg, 0.26 mmol, 1.0 eq) and refluxed at 150 °C for 7 h under an N_2 atmosphere. More PPSE (430 mg, 2.36 mmol, 9.0 eq) was added and the mixture was refluxed for another 4 h, upon which LCMS measurements indicated full conversion of starting material. The mixture was cooled on ice, MeOH (25 mL) was added and the formed crystals were filtrated. The filtrate was stirred in MeOH (15 mL) for 15 minutes and filtrated again. The residues were combined, washed with cold MeOH en dried *in vacuo* to yield **24** (LUF7982) as a white powder (68 mg, 0.19 mmol, 74%).

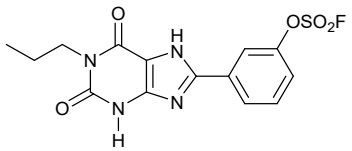
TLC (DCM:MeOH 98:2) R_f = 0.44.

^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ [ppm] = 14.24 (s, 1H), 12.05 (s, 1H), 8.43 (d, J = 8.3 Hz, 2H), 8.28 (d, J = 8.3 Hz, 2H), 3.83 (t, J = 7.5 Hz, 2H), 1.69 – 1.47 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.98, 150.92, 147.68, 146.98, 135.92, 131.67 (d, J = 23.9 Hz), 129.23, 128.02, 109.08, 41.54, 20.85, 11.19.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 66.8.

HPLC 99%, RT 9.124 min. **LC-MS** $[ESI + H]^+$: 353.00.



3-(2,6-Dioxo-1-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl sulfurofluoridate (**25**)

PPSE (2.2 g, 12.07 mmol, 22.2 eq) was added to **19** (210 mg, 0.54 mmol, 1.0 eq) and refluxed at 150 °C. LCMS showed full conversion of starting material after 3h. Therefore the mixture was cooled on ice and MeOH (50 mL) was added. The formed residue was collected and washed with cold MeOH. Recrystallization in $CHCl_3$/MeOH yielded **25** as a white solid (161 mg, 0.44 mmol, 80%).

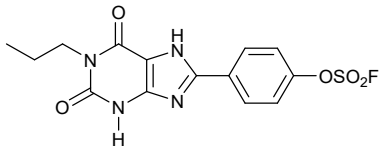
TLC (DCM:MeOH 95:5) R_f = 0.63.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 14.00 (s, 1H), 11.98 (s, 1H), 8.22 (dd, J = 6.3, 2.0 Hz, 2H), 7.79 – 7.67 (m, 2H), 3.82 (t, J = 7.5 Hz, 2H), 1.57 (h, J = 7.5 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.9, 150.9, 150.0, 147.6, 147.5, 131.7, 131.5, 126.7, 122.9, 118.4, 108.3, 41.5, 20.9, 11.2.

^{19}F NMR (471 MHz, $CD_3)_2SO$) δ [ppm] = 38.9.

HPLC 97%, RT 9.332 min. **LC-MS** $[ESI + H]^+$: 368.95.



4-(2,6-Dioxo-1-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl sulfurofluoridate (**26**) **(LUF7993)**

PPSE (1.2 g, 6.59 mmol, 25.7 eq) was added to **20** and refluxed at 150 °C. The mixture was refluxed for 2 h, after which LCMS indicated full conversion of starting material. The mixture was cooled on ice and MeOH (25 mL) was added. The formed precipitate was collected, washed with cold MeOH and recrystallized in MeOH (15 mL). This yielded **26** (LUF7993) as a white powder (83 mg, 0.23 mmol, 88%).

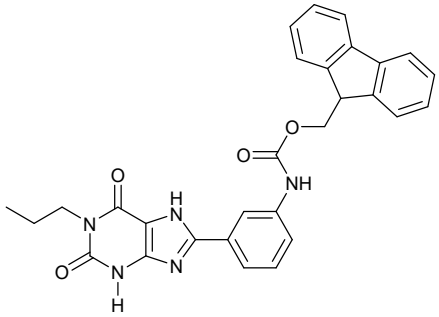
TLC (DCM:MeOH 95:5) R_f = 0.55.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 13.90 (s, 1H), 11.94 (s, 1H), 8.26 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 3.81 (t, J = 7.4 Hz, 2H), 1.57 (h, J = 7.4 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.9, 151.0, 150.3, 148.1, 147.6, 129.8, 128.7, 121.9, 108.2, 41.5, 20.9, 11.2.

^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ [ppm] = 38.94.

HPLC 99%, RT 9.327 min. **LC-MS** [ESI + H]$^+$: 369.00.



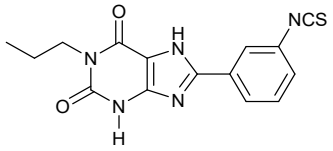
(9H-Fluoren-9-yl)methyl (3-(2,6-dioxo-1-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl)carbamate (**27**)

PPSE (3.44 g, 18.88 mmol, 25.0 eq) was added to **21** (397 mg, 0.76 mmol, 1.0 eq) and the mixture was refluxed at 150 °C. After 3 h of stirring, TLC showed full conversion of the reaction. The mixture was cooled down to rt and water (40 mL) and brine (10 mL) were added. The aqueous layer was extracted by EtOAc (6 x 80 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. This yielded **27** (269 mg, 0.53 mmol, 70%) as an off-white solid.

TLC ($CHCl_3$:MeOH 95:5) R_f = 0.55.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] = 13.71 (s, 1H), 11.92 (s, 1H), 9.94 (s, 1H), 8.33 (s, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.76 (t, J = 7.1 Hz, 3H), 7.43 (t, J = 7.5 Hz, 3H), 7.40 – 7.32 (m, 3H), 4.49 (d, J = 6.8 Hz, 2H), 4.33 (t, J = 6.8 Hz, 1H), 3.82 (t, J = 7.5 Hz, 2H), 1.57 (h, J = 7.1 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H).

HPLC 95%, RT 11.075 min. **LC-MS** [ESI + H]$^+$: 508.10.



8-(3-Isothiocyanatophenyl)-1-propyl-3,7-dihydro-1H-purine-2,6-dione (**28**)

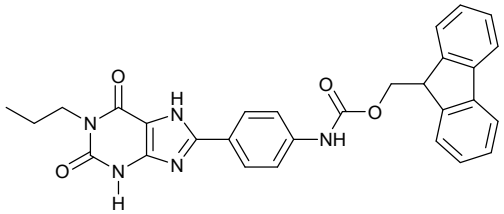
20% Piperidine in DMF (6 mL) was added to remove the Fmoc group of **27** (265 mg, 0.52 mmol, 1.0 eq). The deprotection was complete after 5 min of stirring at rt. The solvents were then removed under reduced pressure and the residue was washed with MeOH to remove the remaining N-Fmoc-piperidine. 3 M HCl (25 mL) was added to the crude amine to form a suspension. Thiophosgene (333 µL, 4.35 mmol, 8.4 eq) was added and the mixture was stirred for 4 h at rt. The aqueous suspension was filtered and the precipitate was washed thoroughly with EtOAc to yield **28** as a white solid (129 mg, 0.39 mmol, 75% over two steps).

TLC (DCM:MeOH 95:5) R_f = 0.62.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 14.01 – 13.74 (s, 1H), 11.94 (s, 1H), 8.12 (t, J = 1.9 Hz, 1H), 8.06 (dt, J = 7.9, 1.3 Hz, 1H), 7.57 (t, J = 7.9 Hz, 1H), 7.48 (dd, J = 8.0, 1.0 Hz, 1H), 3.80 (t, J = 7.3 Hz, 2H), 1.56 (h, J = 7.3 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ [ppm] = 154.8, 150.9, 148.1, 147.4, 135.3, 131.0, 130.7, 130.5, 126.8, 125.5, 123.6, 108.1, 41.4, 20.8, 11.2.

HPLC 99%, RT 9.825 min. **LC-MS** [ESI - H]$^-$: 326.95.

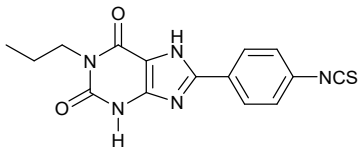


(9H-Fluoren-9-yl)methyl (4-(2,6-dioxo-1-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenyl)carbamate (**29**)

PPSE (22 mL) was added to **22** (1.17 g, 2.22 mmol, 1.0 eq) and refluxed at 170 °C. After 5 h of stirring, the mixture was cooled down to rt and water (250 mL) was added. The formed precipitate was collected and purified by column chromatography (DCM:MeOH 99.5:0.5 → 95:5) to yield **29** as an off-white solid (618 mg, 1.22 mmol, 55%).

TLC (DCM:MeOH 98:2) R_f = 0.38.

1H NMR (400 MHz, $(CD_3)_2SO$) δ [ppm] 13.50 (s, 1H), 11.87 (s, 1H), 9.98 (s, 1H), 8.01 (dd, J = 16.6, 8.5 Hz, 2H), 7.92 (d, J = 7.4 Hz, 2H), 7.76 (d, J = 7.4 Hz, 2H), 7.63 – 7.51 (m, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H), 4.54 (d, J = 6.6 Hz, 2H), 4.33 (t, J = 6.5 Hz, 1H), 3.81 (t, J = 7.5 Hz, 2H), 1.57 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H).



8-(4-Isothiocyanatophenyl)-1-propyl-3,7-dihydro-1H-purine-2,6-dione (**30**) **(LUF8002)**

20% Piperidine in DMF (6 mL) was added to remove the Fmoc group of **29** (85 mg, 0.17 mmol, 1.0 eq). The deprotection was complete after 5 min of stirring at rt. The solvents were then removed under reduced pressure and the residue was washed with MeOH to remove the remaining N-Fmoc-piperidine. 3 M HCl (3 mL) was then added to the crude amine to form a suspension. Thiophosgene (150 µL, 1.96 mmol, 11.9 eq) was added and the mixture was stirred for 2 h at rt. LCMS indicated full conversion of starting material, therefore water (50 mL) was added. The formed precipitate was collected and washed with DCM (50 mL) and EtOAc (50 mL). The residue was dried under reduced pressure to yield **30** (LUF8002) as an off-white powder (40 mg, 0.12 mmol, 71% over two steps).

TLC (DCM:MeOH 95:5) R_f = 0.55.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ [ppm] = 13.81 (s, 1H), 11.94 (s, 1H), 8.13 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 3.82 (t, J = 7.5 Hz, 2H), 1.57 (h, J = 7.3 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H).

13C NMR (151 MHz, $(CD_3)_2SO$) δ [ppm] = 154.9, 151.0, 148.6, 147.6, 134.7, 131.3, 128.1, 127.7, 126.7, 108.1, 41.5, 20.9, 11.2.

HPLC 95%, RT 9.834 min. **LC-MS** $[ESI + H]^+$: 327.95.

Biological procedures

Cell lines

CHO-spap cells stably expressing the human A_{2B} receptor (CHO-spap-hA_{2B}AR) were kindly provided by S.J. Dowell (Glaxo Smith Kline, UK). Chinese hamster ovary (CHO) cells stably expressing the human adenosine A_1 receptor (CHOhA_1AR) were kindly provided by Prof. S.J. Hill (University of Nottingham, UK). Human embryonic kidney 293 cells stably expressing the human adenosine A_{2A} receptor (HEK293hA_{2A}AR) were kindly provided by Dr. J. Wang (Biogen/IDEC, Cambridge, MA). CHO cells stably expressing the human adenosine A_3 receptor (CHOhA_3AR) were a kindly provided by Dr. K.N. Klotz (University of Würzburg, Germany).

Radioligands

[^{3}H]8-(4-(4-(4-Chlorophenyl)piperazide-1-sulfonyl)phenyl)-1-propylxanthine ([^{3}H]PSB-603, specific activity 79 Ci/mmol) was purchased from Quotient Bioresearch. [^{3}H]1,3-dipropyl-8-cyclopentyl-xanthine ([^{3}H]DPCPX, specific activity 137 Ci/mmol) was purchased from ARC, Inc. [^{3}H]4-(-2-[7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-ylamino)ethyl) phenol ([^{3}H]-ZM241385, specific activity 50 Ci/mmol) was purchased from ARC, Inc. [^{3}H]8-Ethyl-4-methyl-2-phenyl-(8R)-4,5,7,8-tetrahydro-1H-imidazo[2,1-i]-purin-5-one ([^{3}H]PSB-11, specific activity 56 Ci/mmol) was a gift from Prof. C.E. Müller (University of Bonn, Germany).

Chemicals

5'-N-ethylcarboxamidoadenosine (NECA), N^6-Cyclopentyladenosine (CPA) and adenosine deaminase (ADA) were purchased from Sigma Aldrich. ZM241385 was kindly donated by Dr. S.M. Poucher (Astra Zeneca, Manchester, UK). CGS21680 was purchased from Ascent Scientific. PSB 1115 potassium salt was purchased from Tocris Bioscience. All other chemicals were of analytical grade and obtained from standard commercial sources.

Cell culture and membrane preparation

CHO-spap-hA_{2B}R cells, CHOhA_1AR cells, HEK293hA_{2A}AR cells and CHOhA_3AR were cultured and membranes were prepared as previously reported.[6]

Radioligand displacement assays

Single point radioligand displacement assays on CHOhA_1AR cells, HEK293hA_{2A}AR cells and CHOhA_3AR cells were performed as previously reported.[6] Full curve radioligand displacement assays were performed using ChO-spap-hA_{2B}AR membranes and a concentration rage of competing ligand. 30 µg of protein in a total volume of 100 µL assay buffer (0.1% CHAPS in 50 mM Tris-HCl pH 7.4) was taken and pre-incubated for either 0 or 4 h with the competing ligand. ~1.5 nM [^{3}H]PSB-603 was then added and the membranes were co-incubated for 30 min at 25 °C. Nonspecific binding was determined in the presence of 10 µM ZM241385. Incubations were terminated by vacuum filtration to separate the bound and free radioligand through prewetted 96-well GF/C filter plates using a Filtermate-harvester (PerkinElmer). Filters were subsequently washed 5 times with ice-cold wash buffer (0.1% BSA in 50 mM Tris-HCl pH 7.4). The plates were dried at 55 °C after which 25 µL of MicroscintTM-20 cocktail (PerkinElmer) was added to each well. After 3 h the filter-bound radioactivity was determined by scintillation spectrometry using a 2450 MicroBeta^2 Microplate Counter (PerkinElmer).

Wash-out assays

100 µL of assay buffer (0.1% CHAPS in 50 mM TrisHCl pH 7.4) containing 1 µM of competing ligand (10 µM in case of PSB 1115) and 200 µL of assay buffer were added to 100 µL of ChO-spap-hA_{2B}AR membrane suspension (80 µg of protein) in a 2 mL Eppendorf tube. The tubes were incubated 2 h at 25 °C while shaking. The 'washed' group of samples was centrifuged (5 min, 13 200 rpm, 4 °C), the supernatant was removed, the pellet was resuspended in 1 mL of assay buffer and incubated for 10 min at 25 °C while shaking at 900 rpm. The washing steps were repeated three times. After the last washing step, the membrane pellets were resuspended in 300 µL of assay buffer to determine radioligand displacement. Both washed and unwashed samples were transferred to test tubes and incubated together with 100 µL of 1.5 nM [^{3}H]PSB-603 for 2 h at 25 °C. Nonspecific binding was determined in the presence of 10 µM ZM241385. The incubation was terminated by vacuum filtration through prewetted 96-well GF/C filter plates using a Brandol M24 Scintillation harvester. Filters were subsequently washed 3 times with ice-cold wash buffer (0.1% BSA in 50 mM Tris-HCl pH 7.4). The filter-bound radioactivity was determined using a Tri-Carb 2900TR Liquid Scintillation Analyzer (PerkinElmer).

Data Analysis

All data from radioligand displacement and wash-out assays were analyzed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA). IC_{50} values were converted to K_i values using the Cheng-Prusoff equation.[7] The K_D values of [^{3}H]PSB603 at CHO-spap-hA_{2B}AR membranes (1.7 nM) was taken from in-house determinations.

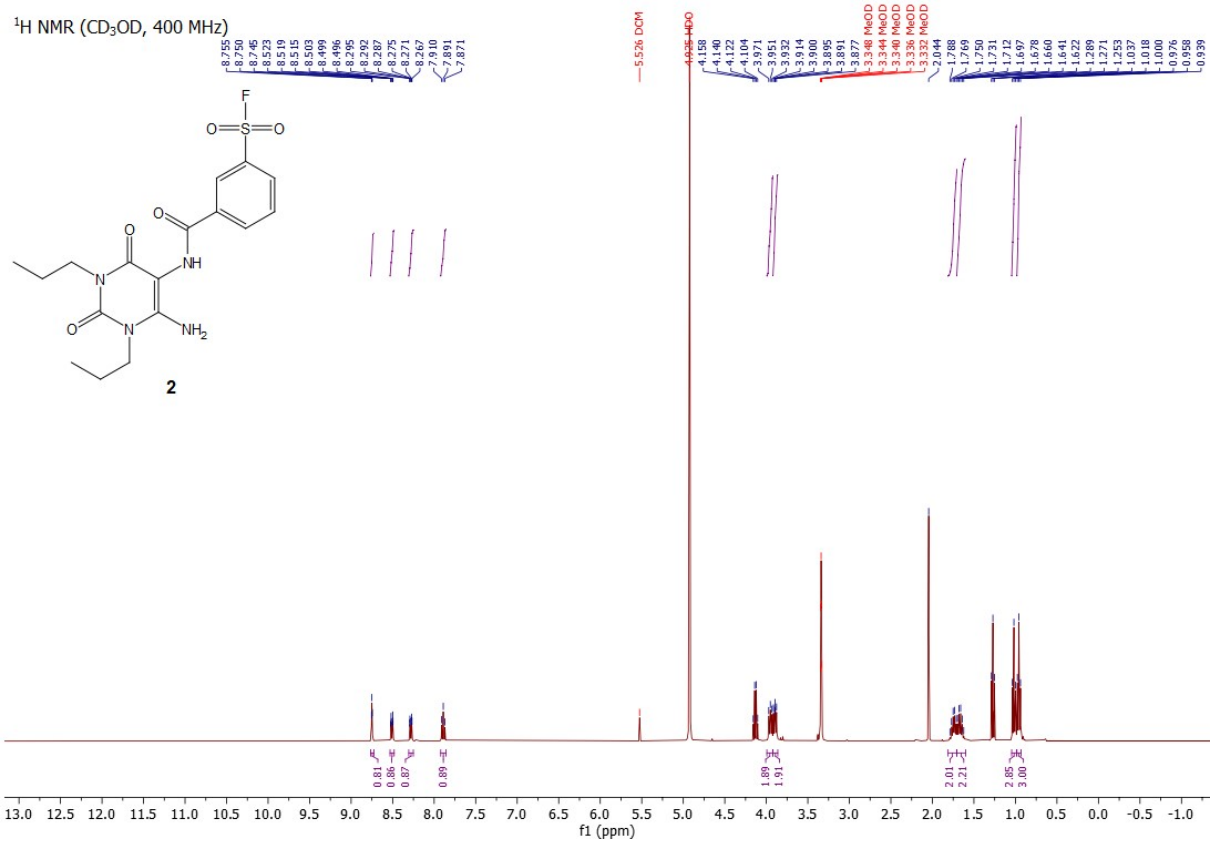
Computational Procedures

Docking of LUF7982 in the adenosine A_{2B} receptor

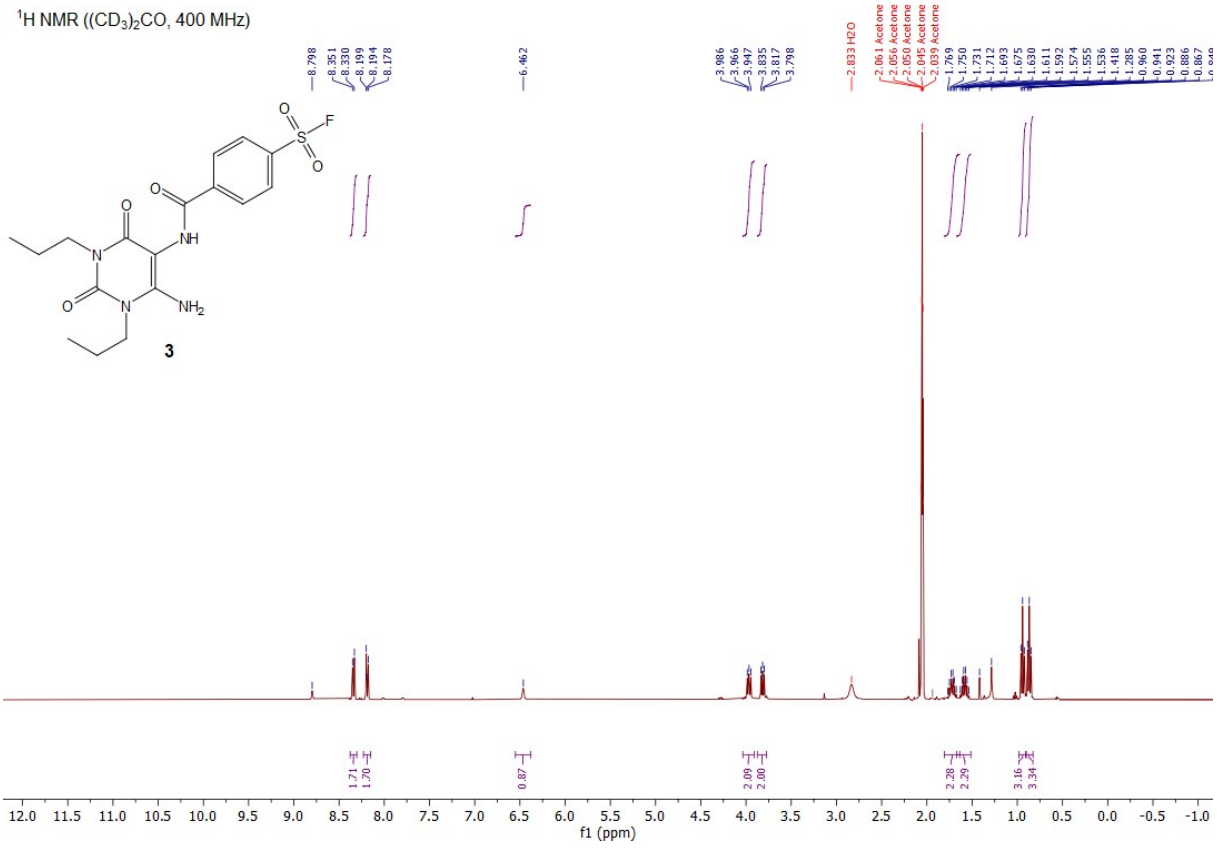
The A_{2B}AR homology model was retrieved from the GPCRdb.[8] Several orientations of the extracellular loop 3 (EL3) loop region (between residue numbers 258 and 270) were generated using MODELLER,[9] to obtain viable orientations of the Lysine residues in the binding site. 3D coordinates of LUF7982 were generated using rdkit.[10] Thereafter, LUF7982 was manually docked in the receptor model using PyMOL,[11] and subsequently minimized using the all-atom minimization tool in ICM Pro.[12] Residue numbers are presented with their Ballosteros-Weinstein numbering scheme.[13] Distances given in Å are the distances calculated between the N-atom of the lysine residue and the S-atom of the sulfonyl fluoride warhead.

NMR Spectra

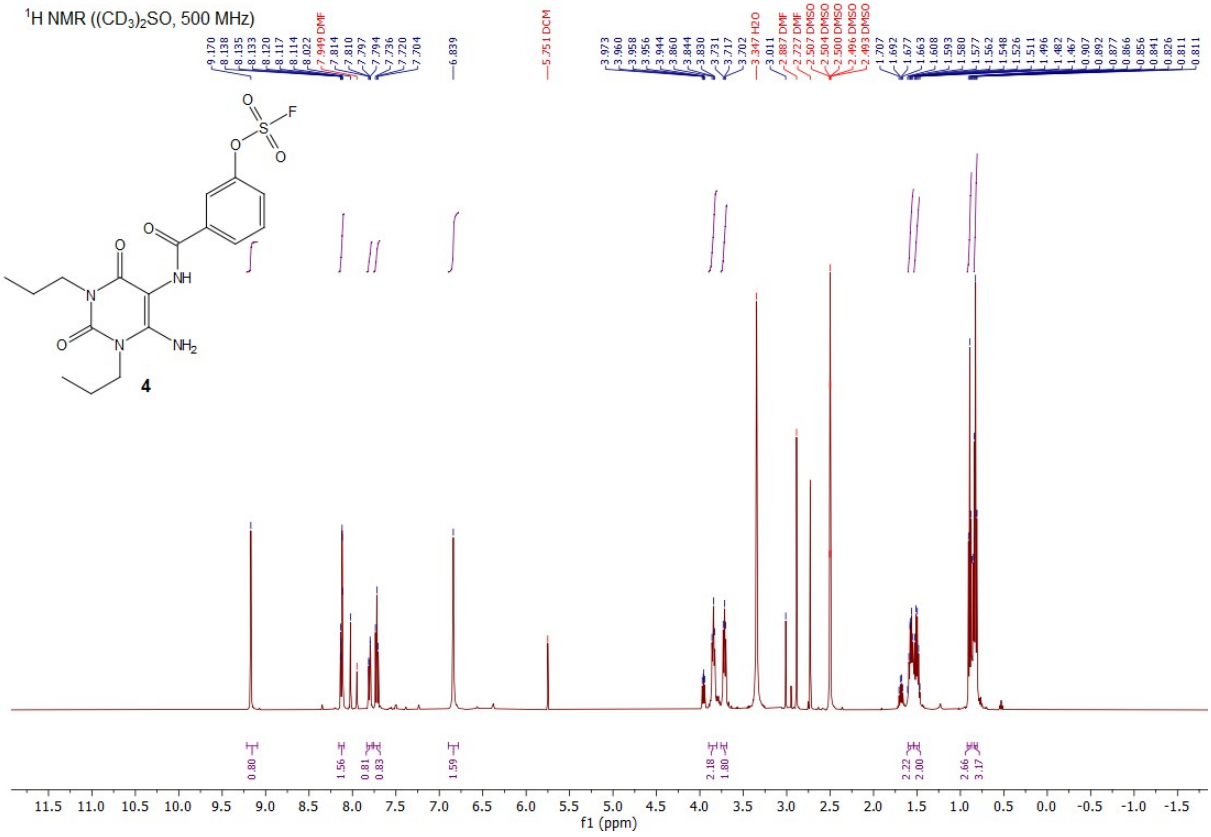
^{1}H NMR (CD_3OD, 400 MHz)



^{1}H NMR ($(CD_3)_2CO$, 400 MHz)

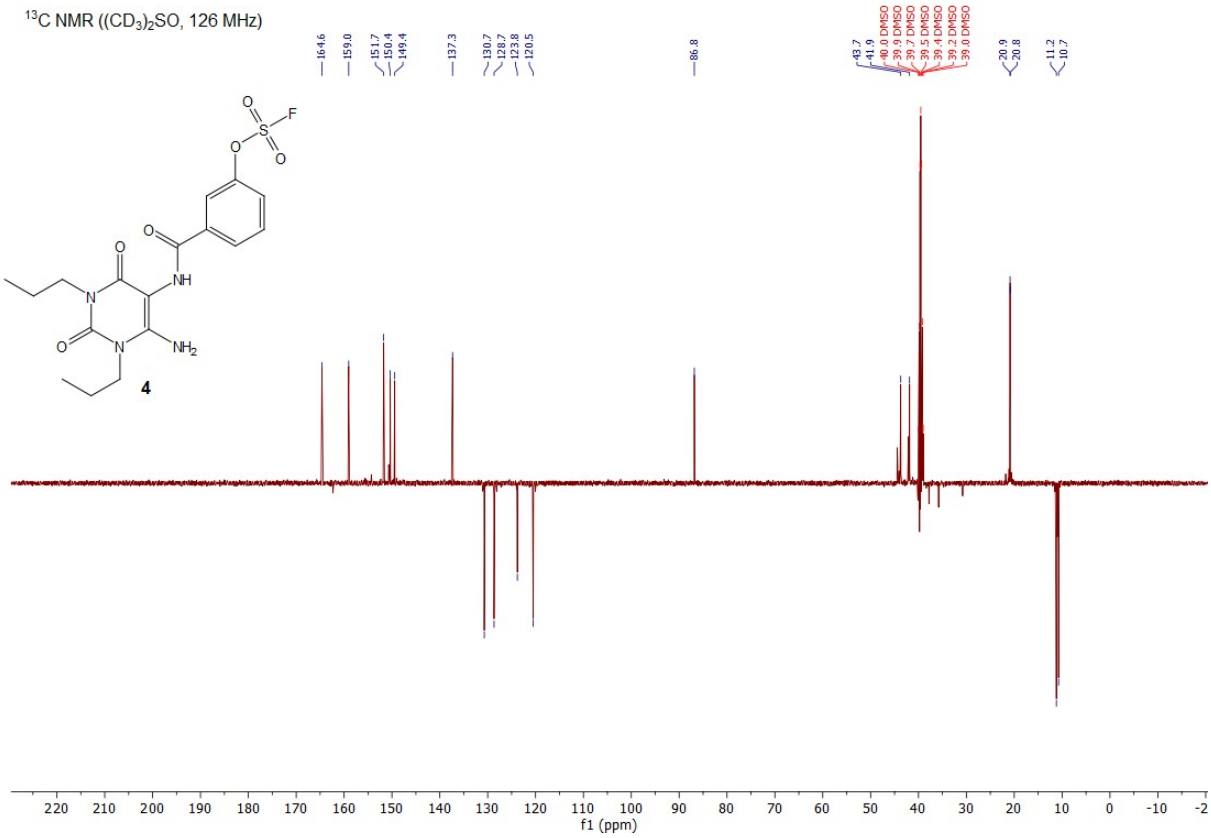


^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



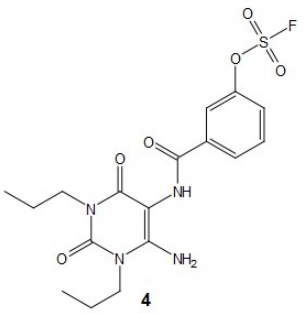
4

^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



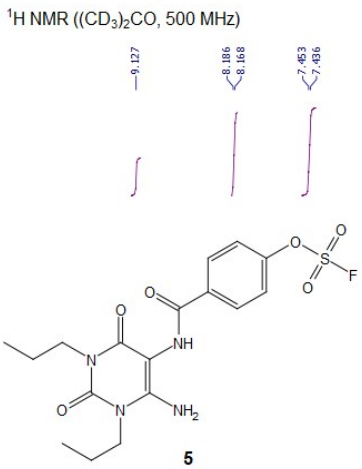
4

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



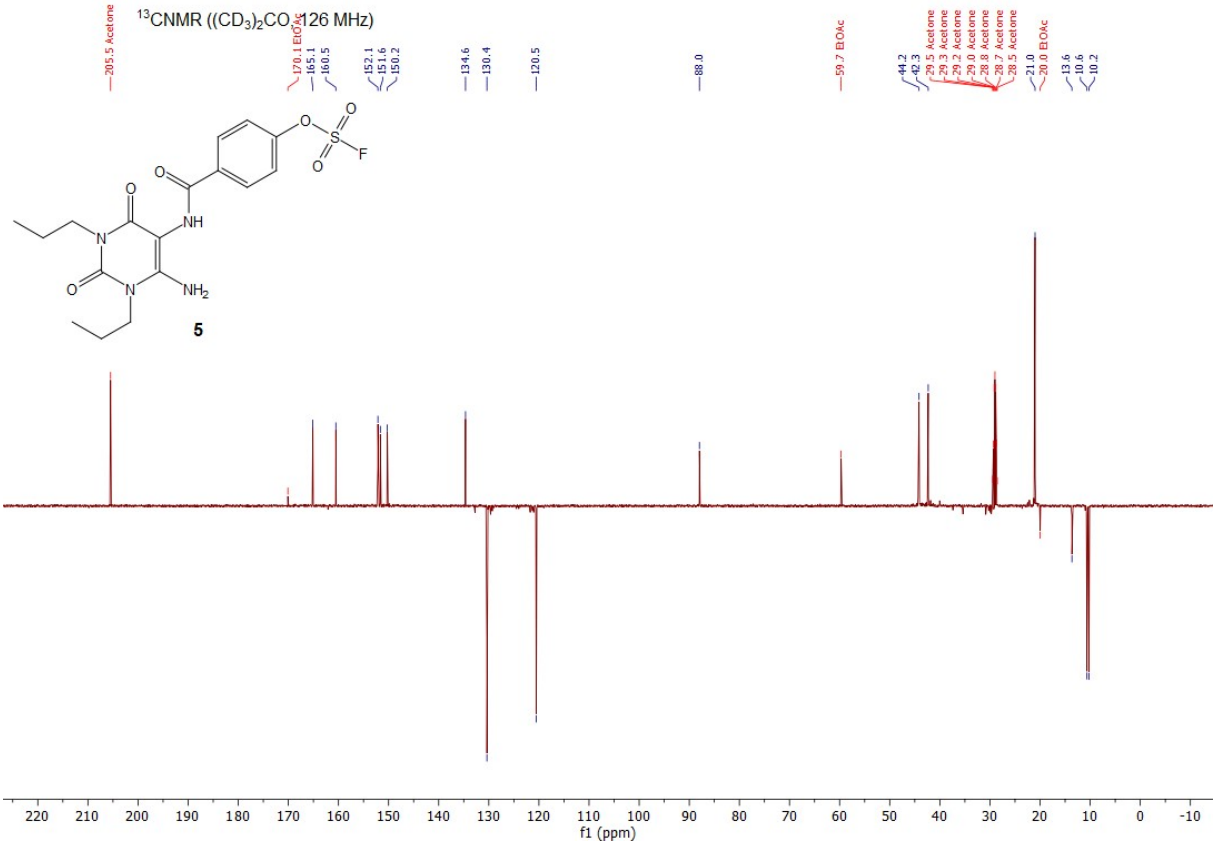
4

^{1}H NMR ((CD$_3$)$_2$CO, 500 MHz)



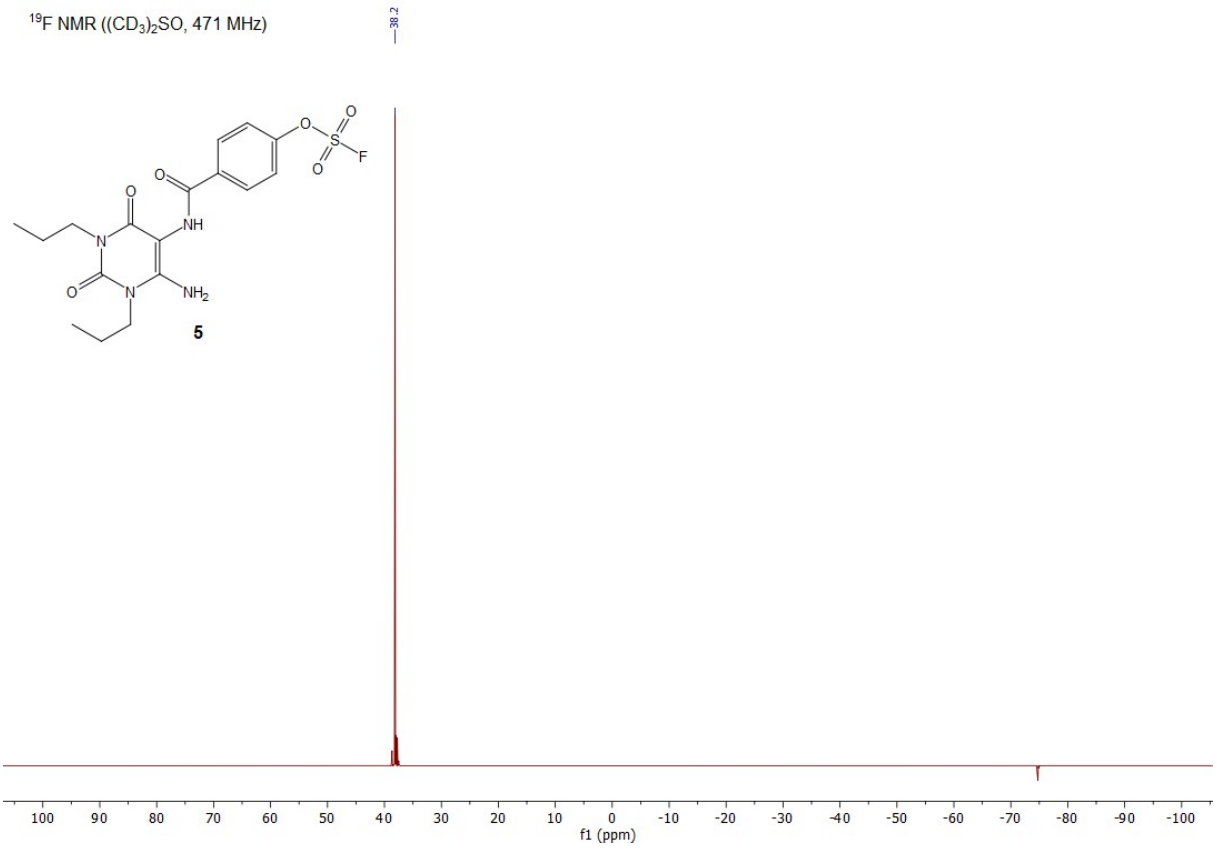
5

^{13}CNMR ($(CD_3)_2CO$, 126 MHz)



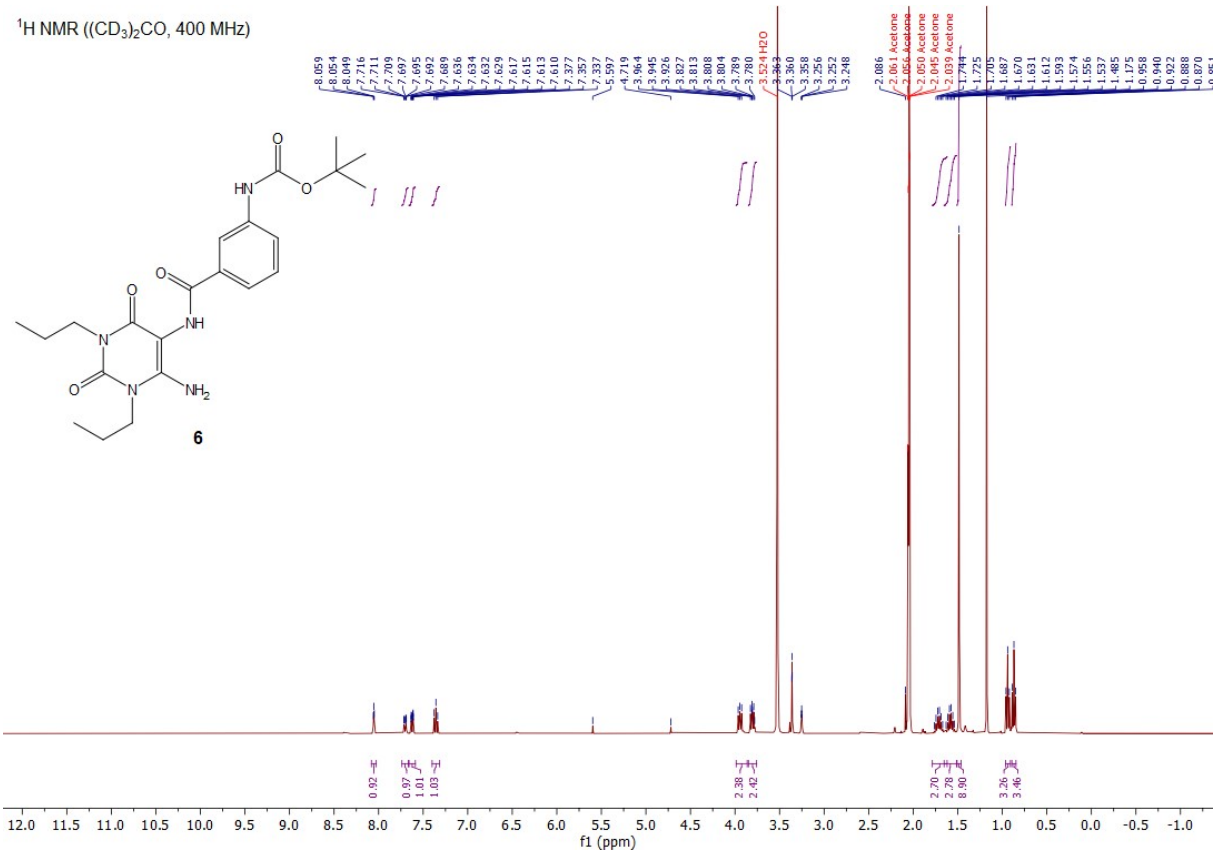
5

^{19}F NMR ($(CD_3)_2SO$, 471 MHz)

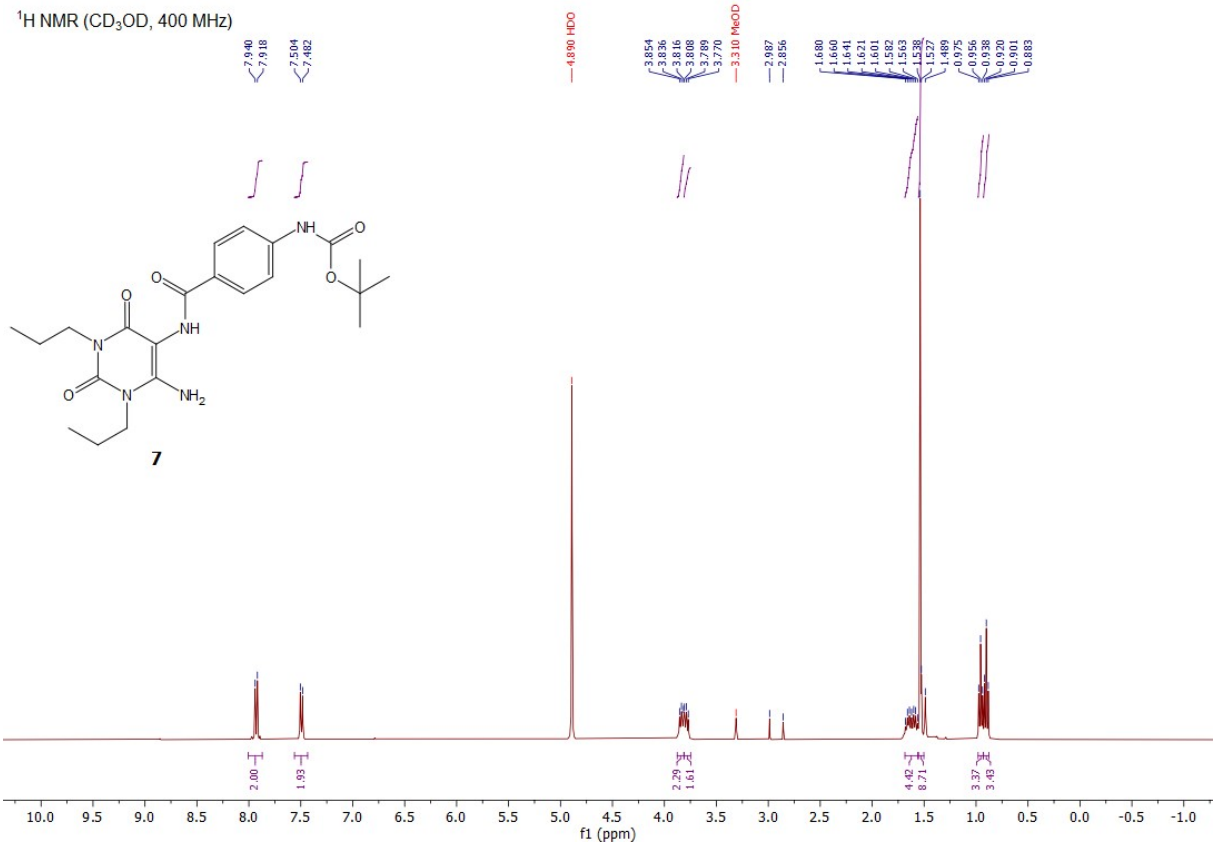


5

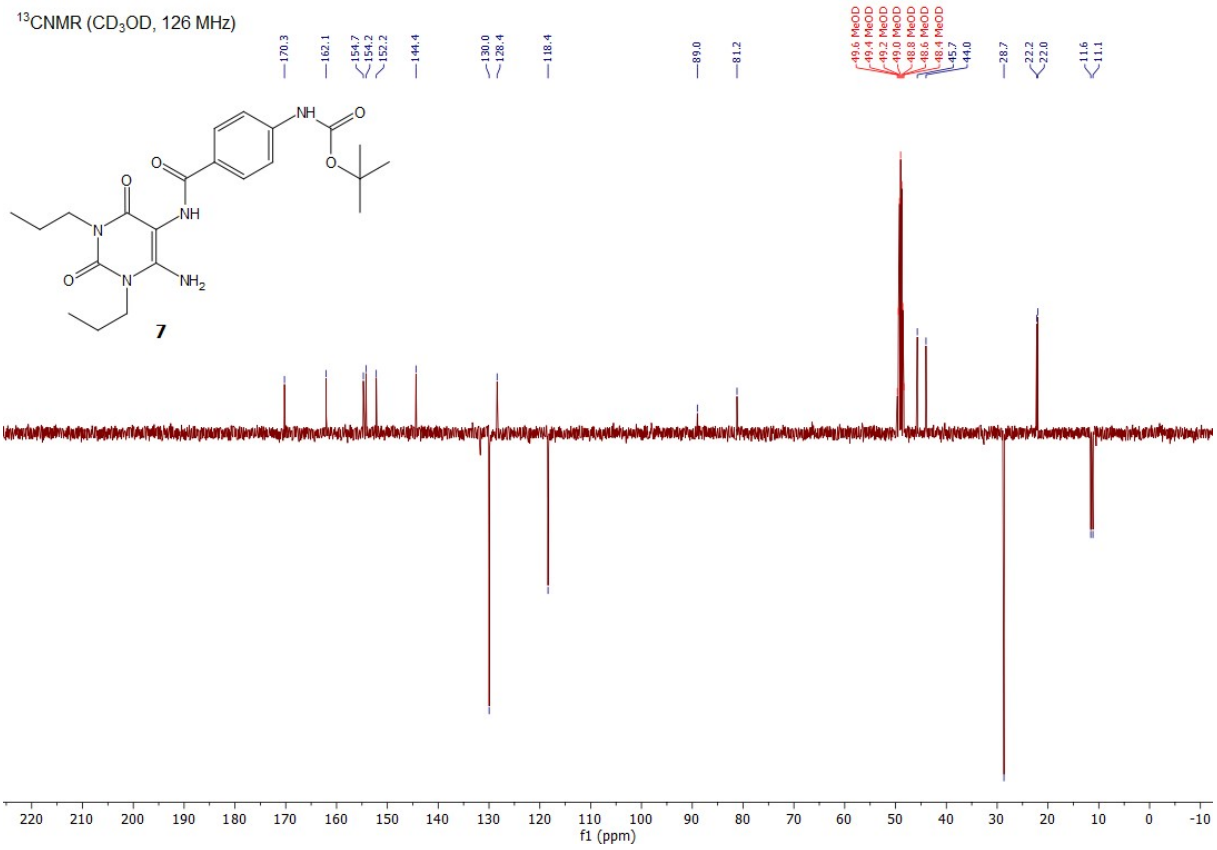
^{1}H NMR ($(CD_3)_2CO$, 400 MHz)



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

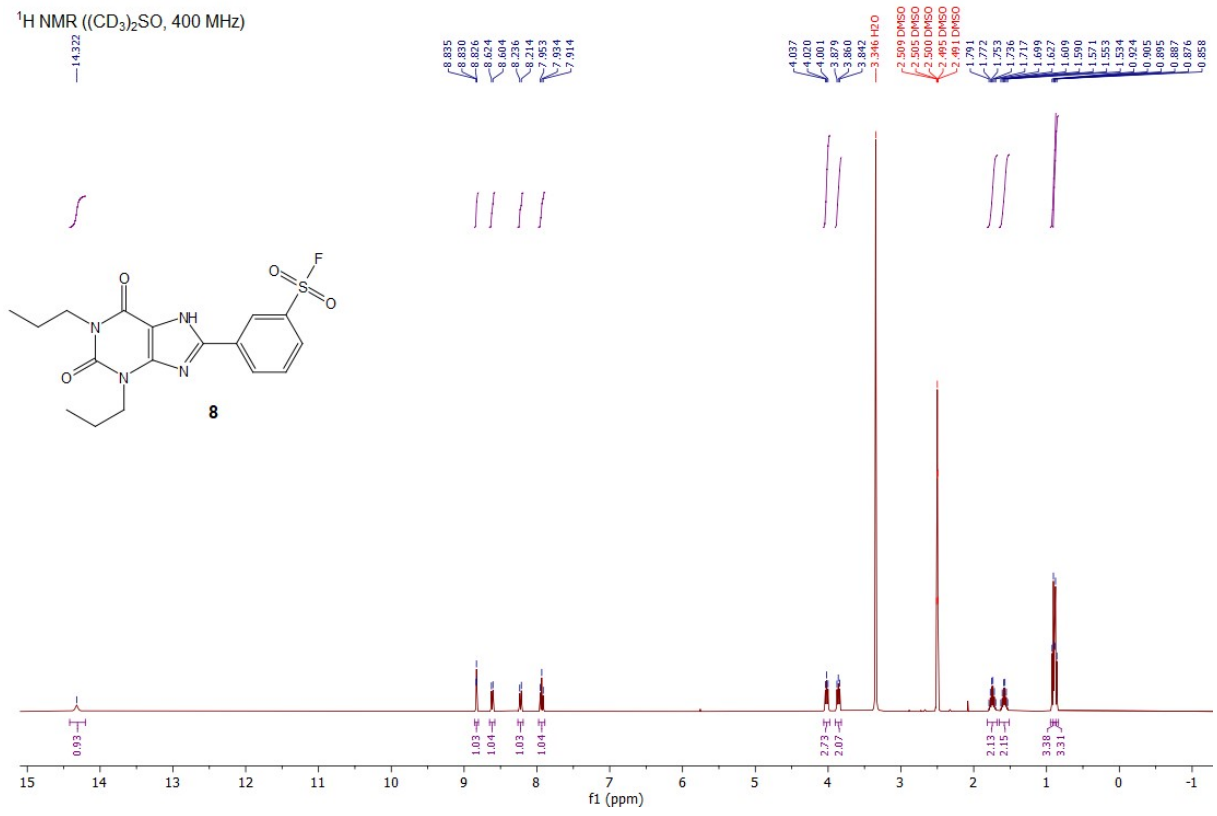


^{13}CNMR (CD_3OD, 126 MHz)



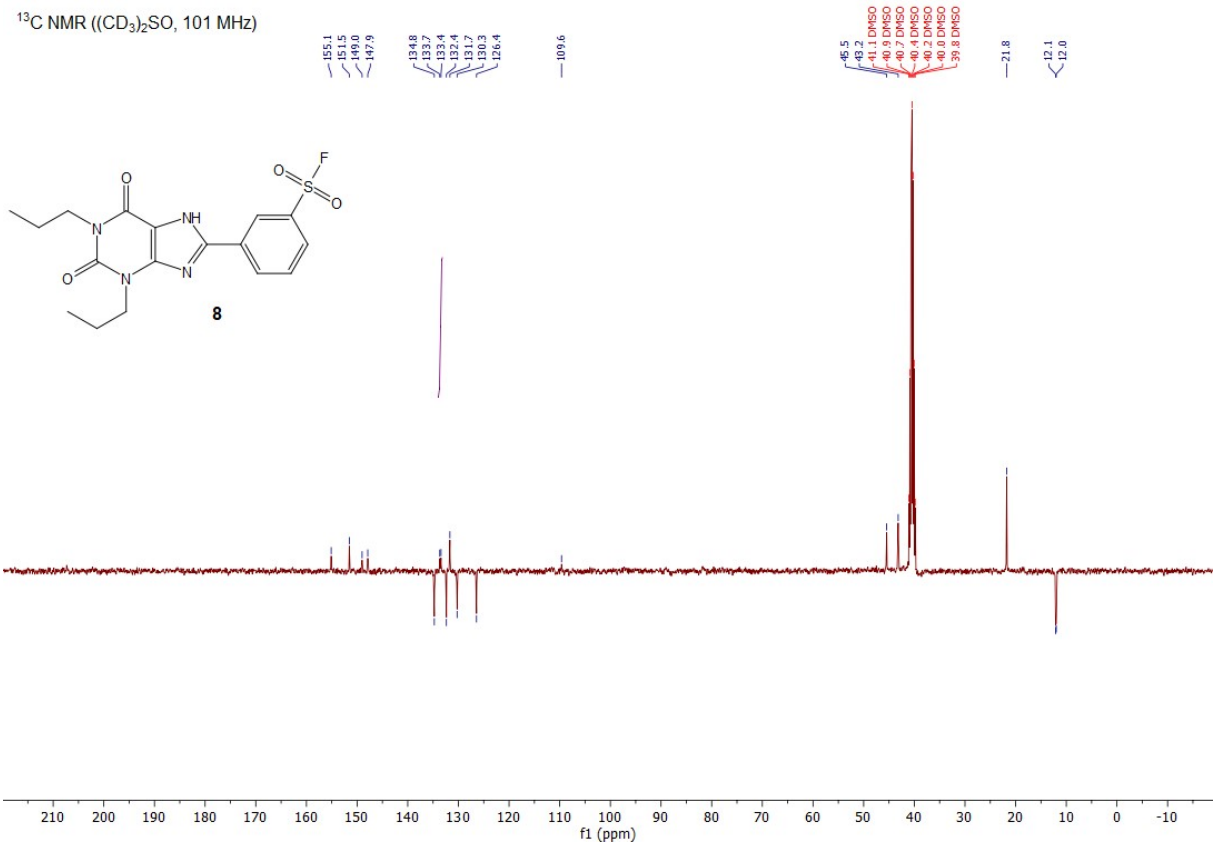
7

^{1}H NMR ($(CD_3)_2SO$, 400 MHz)



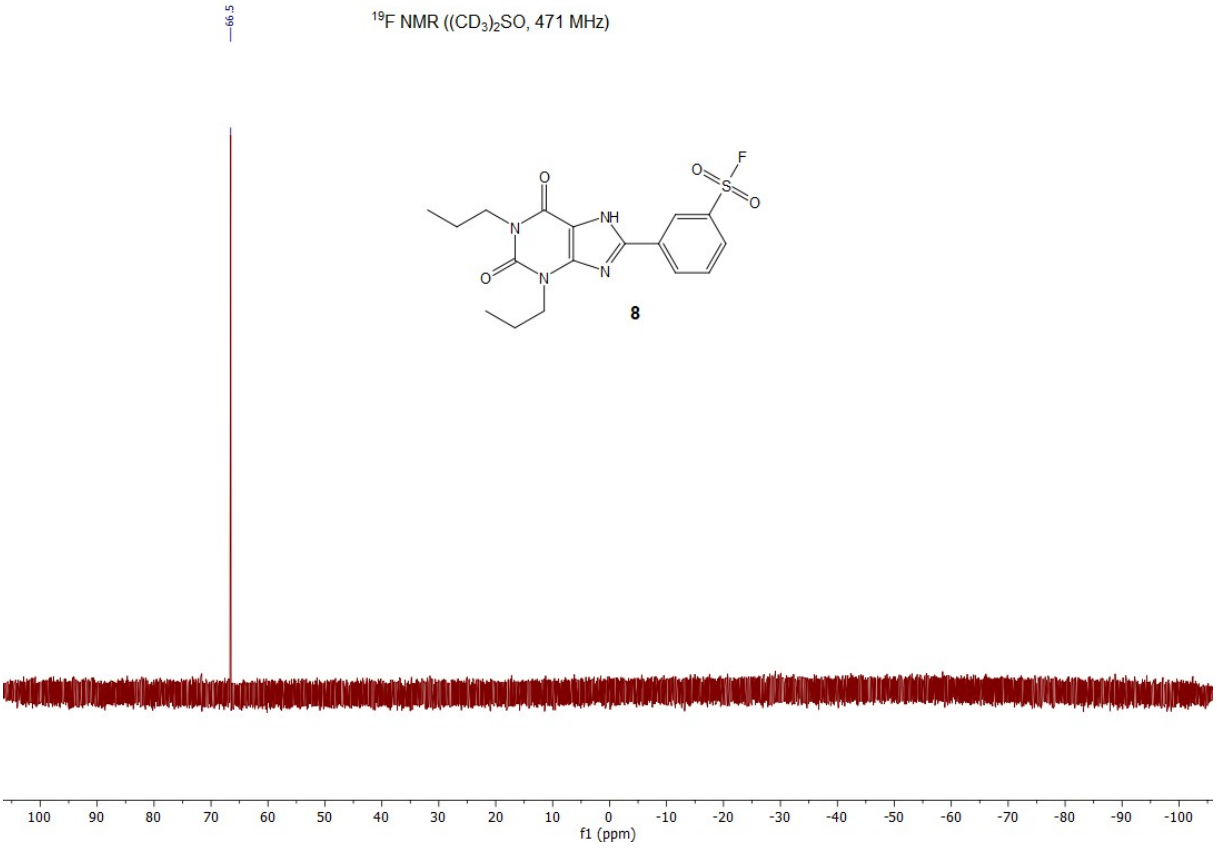
8

^{13}C NMR ((CD$_3$)$_2$SO, 101 MHz)



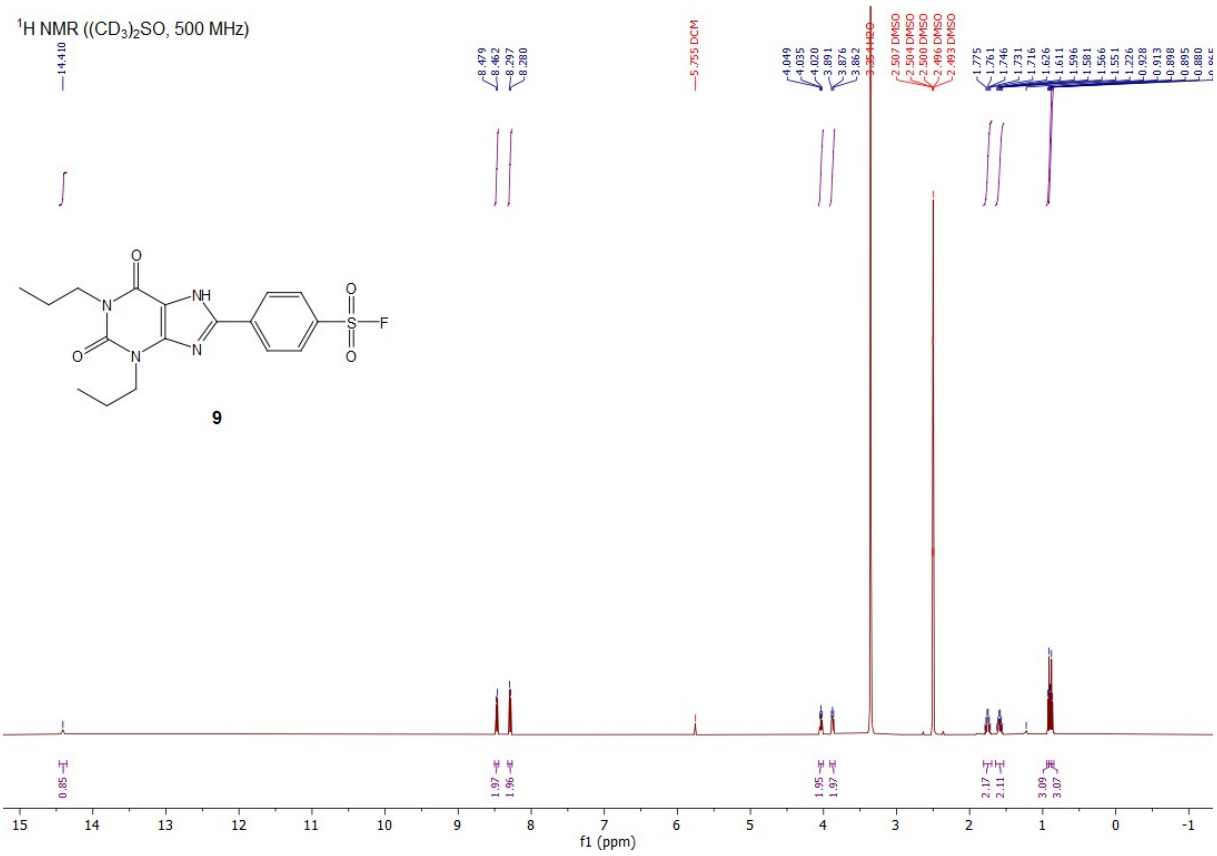
8

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



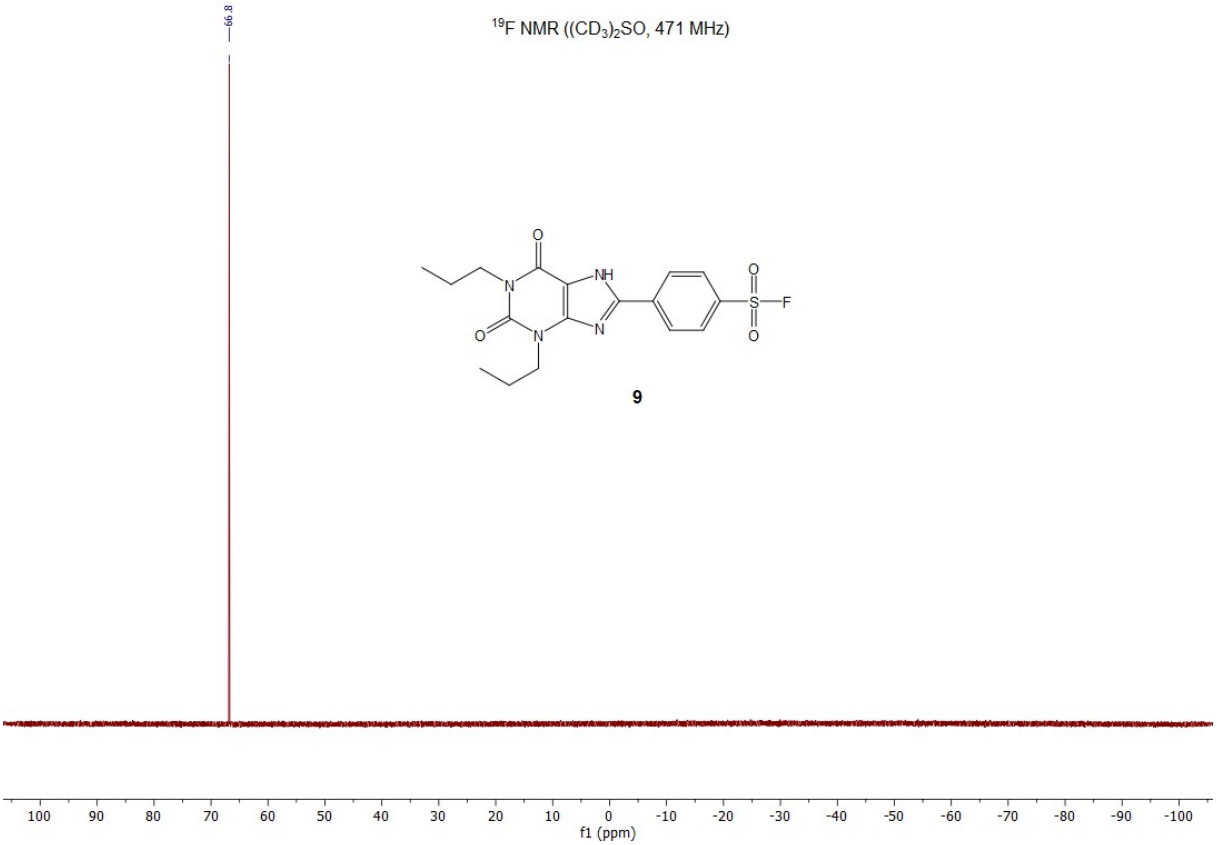
8

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



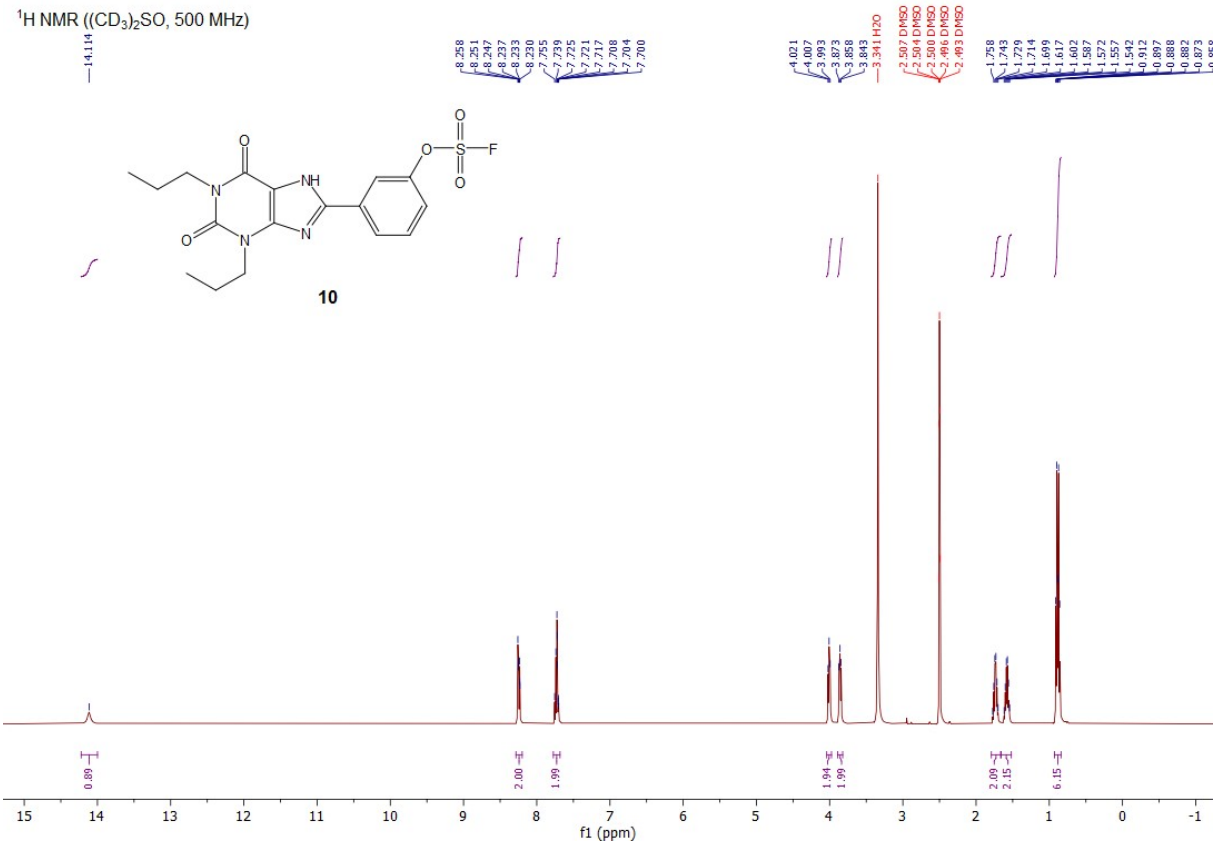
9

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



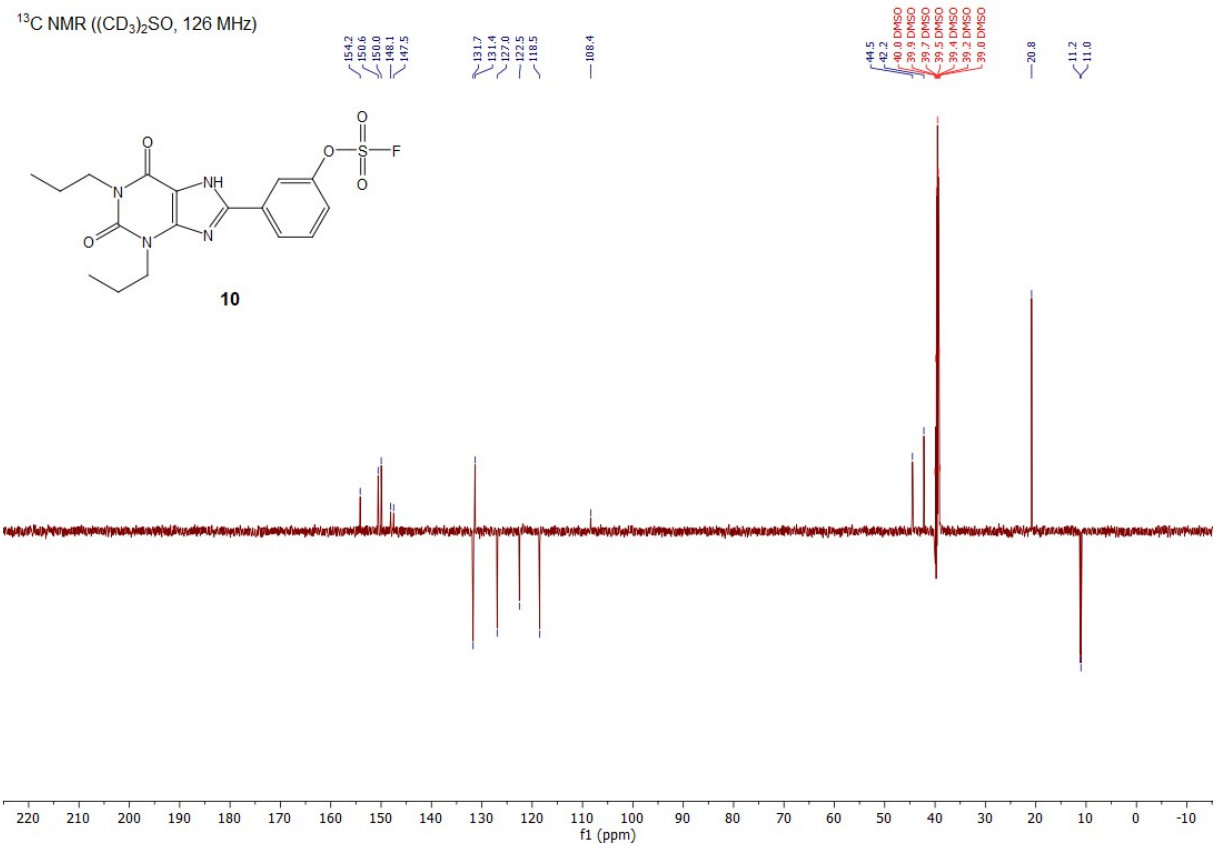
9

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



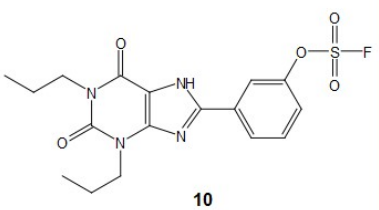
10

^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



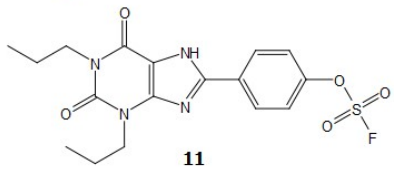
10

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



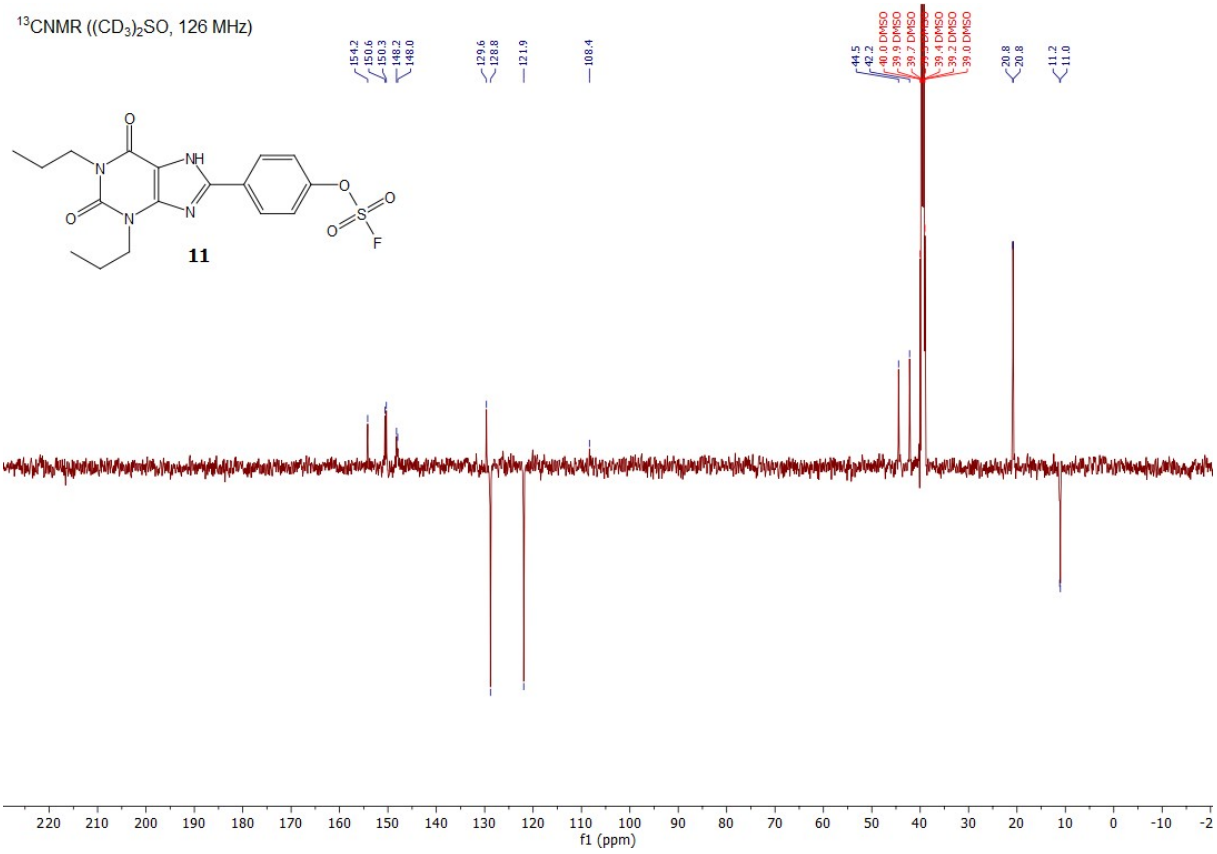
10

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



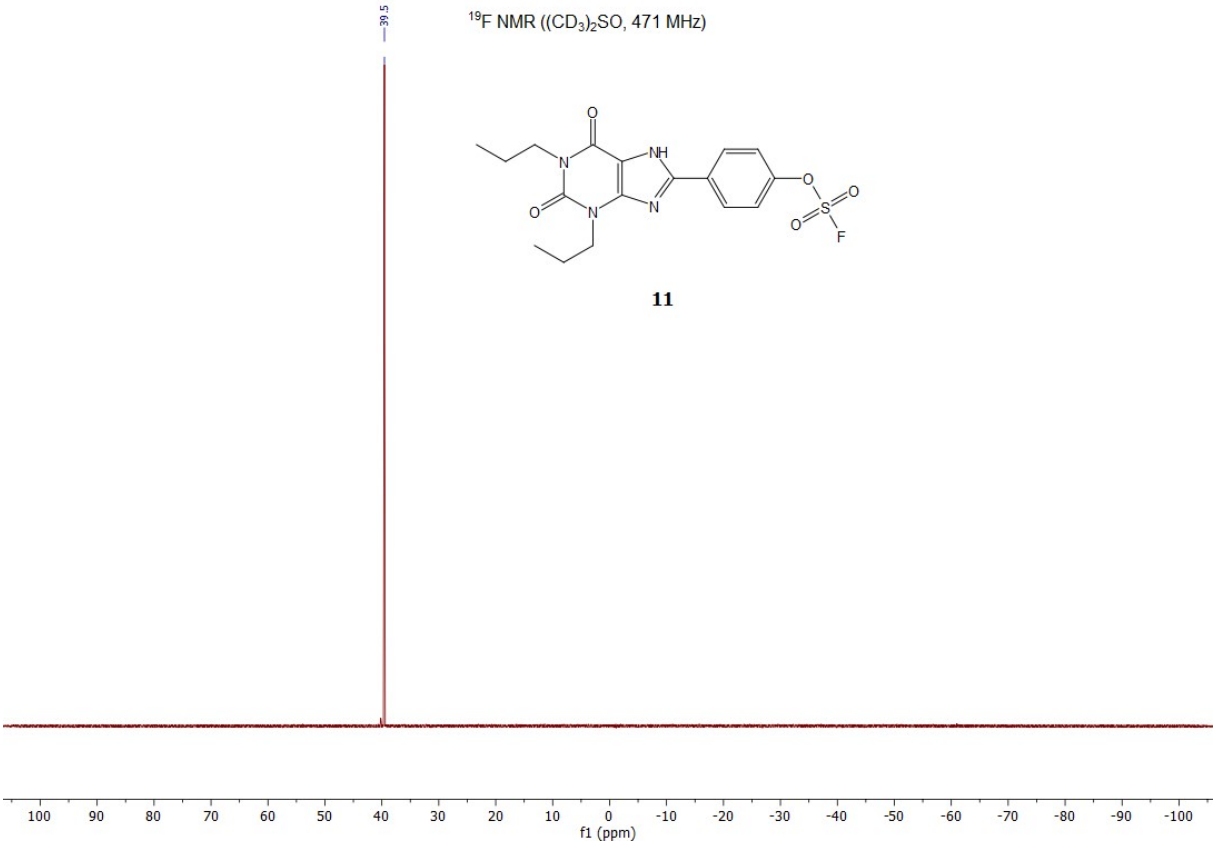
11

^{13}CNMR ((CD$_3$)$_2$SO, 126 MHz)



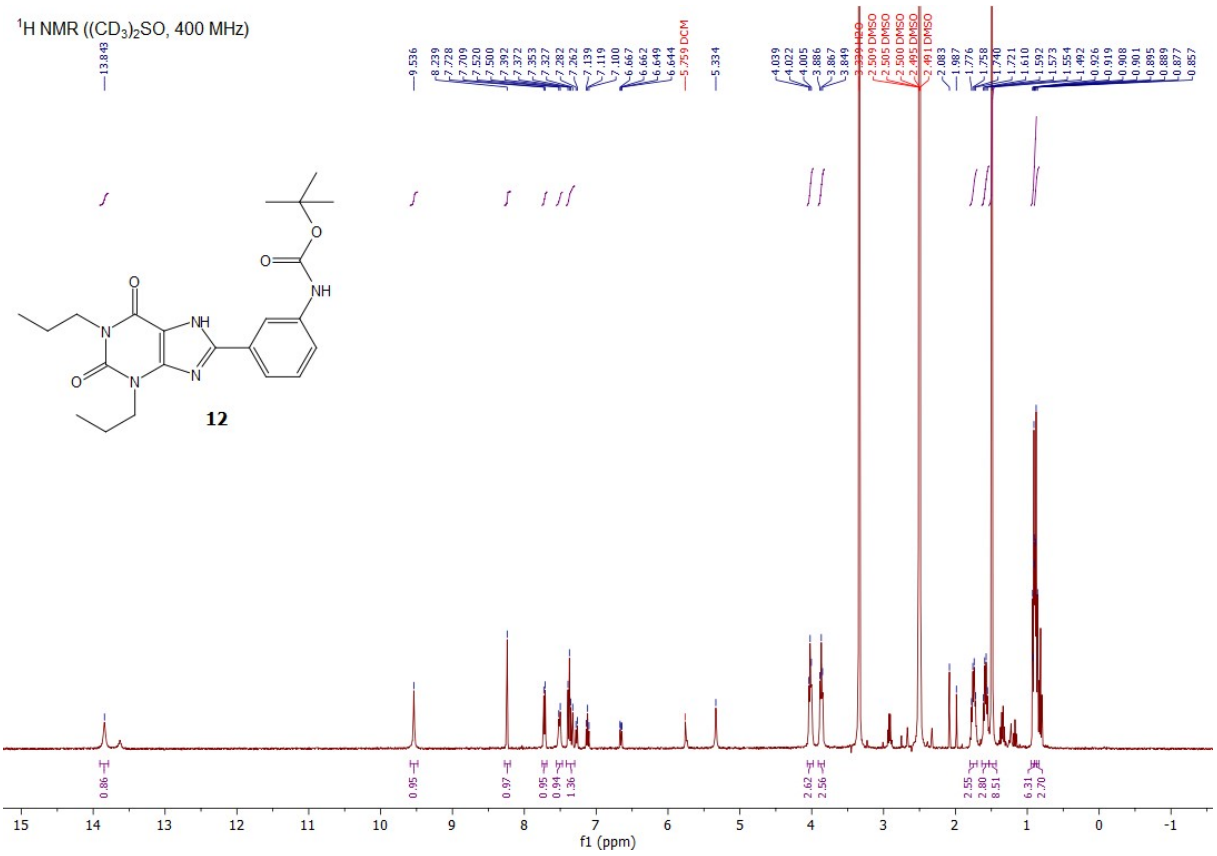
11

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



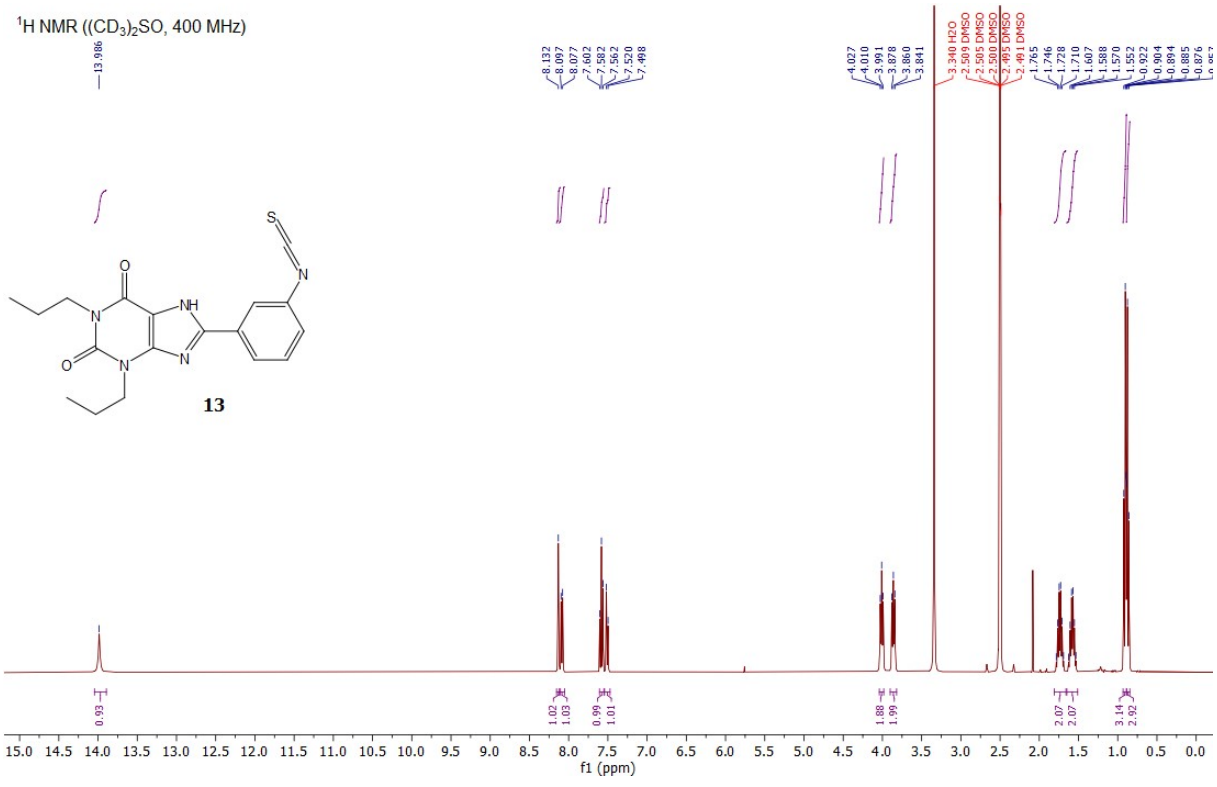
11

^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz)



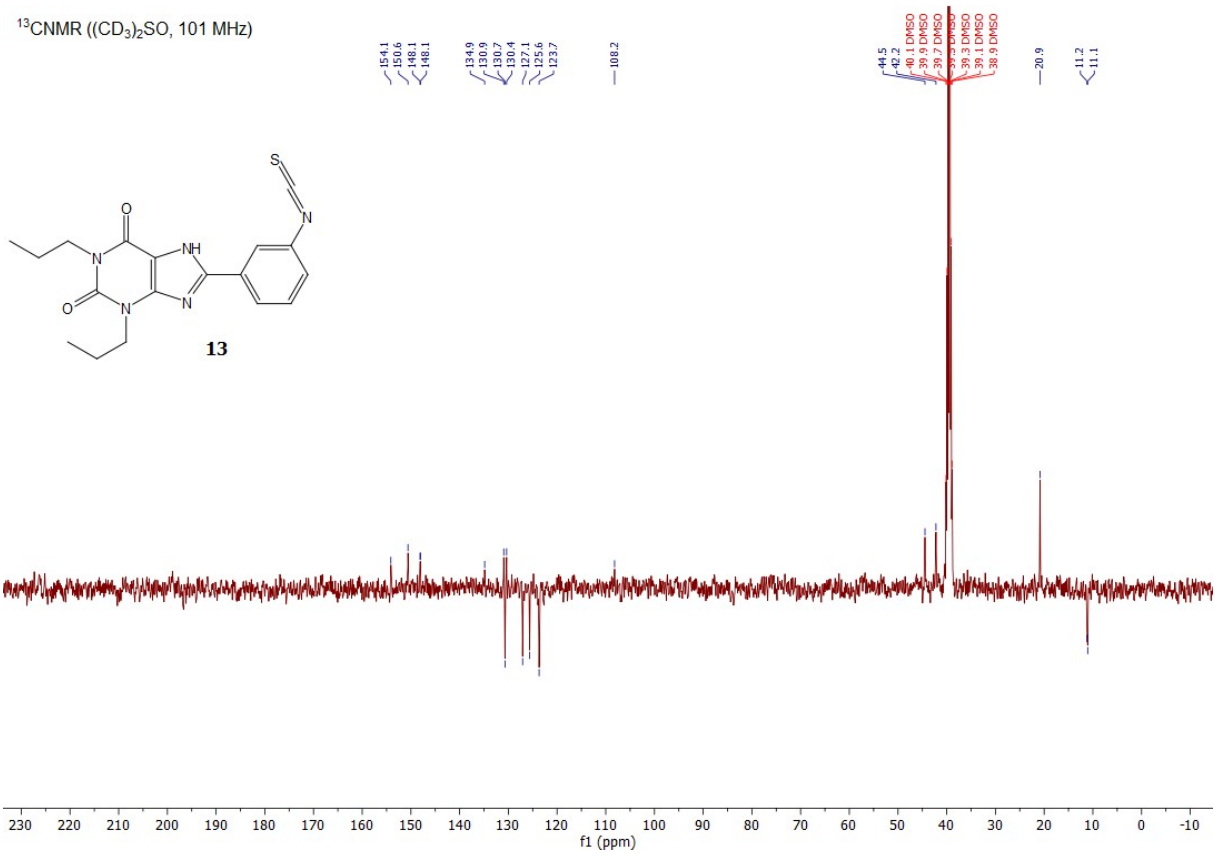
12

^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz)



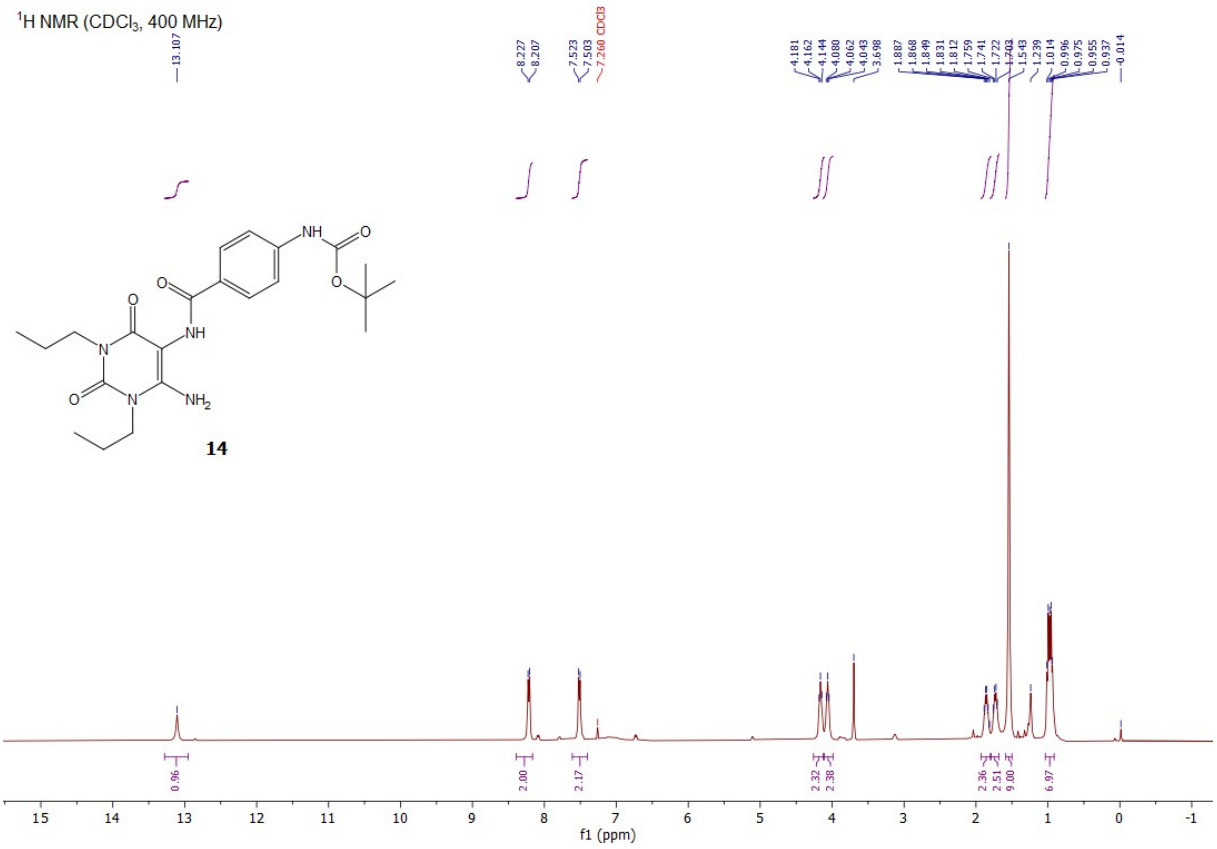
13

^{13}CNMR ($(CD_3)_2SO$, 101 MHz)



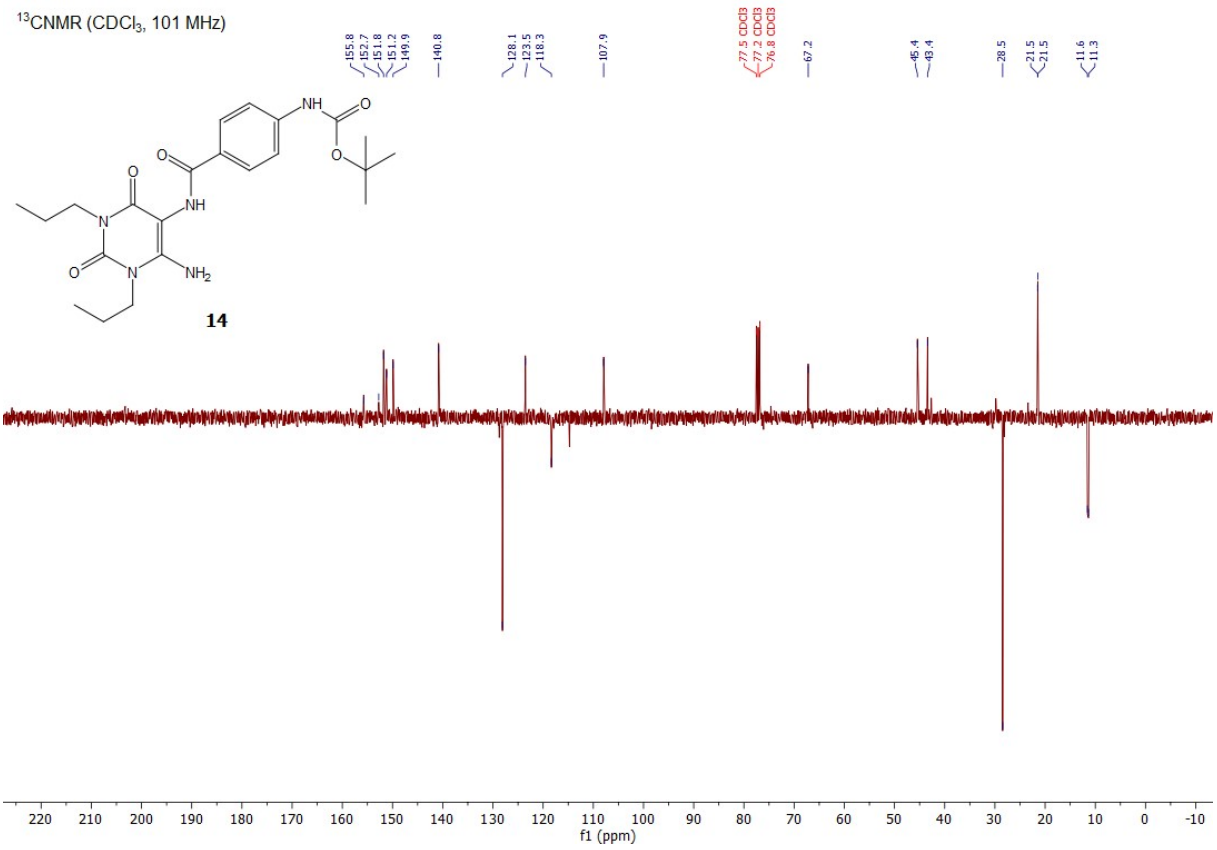
13

^{1}H NMR ($CDCl_3$, 400 MHz)



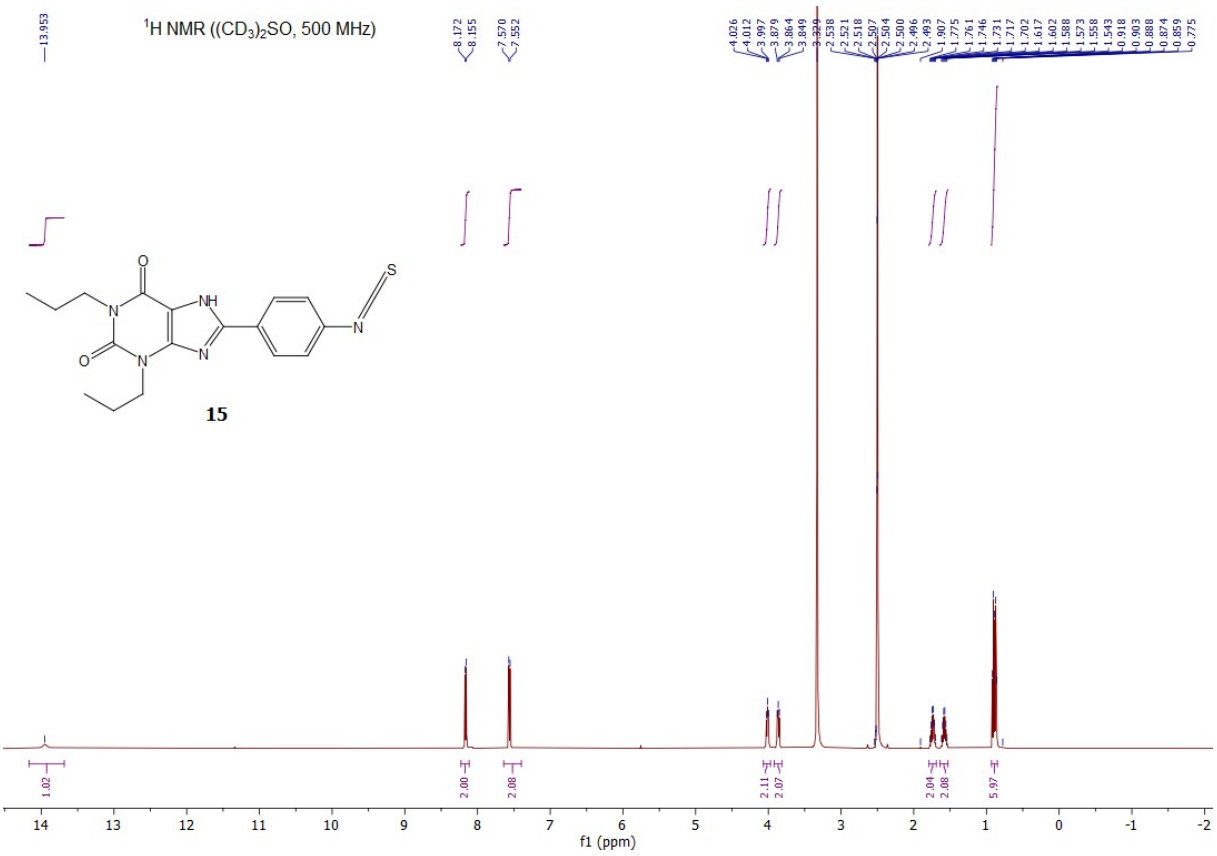
14

^{13}CNMR ($CDCl_3$, 101 MHz)



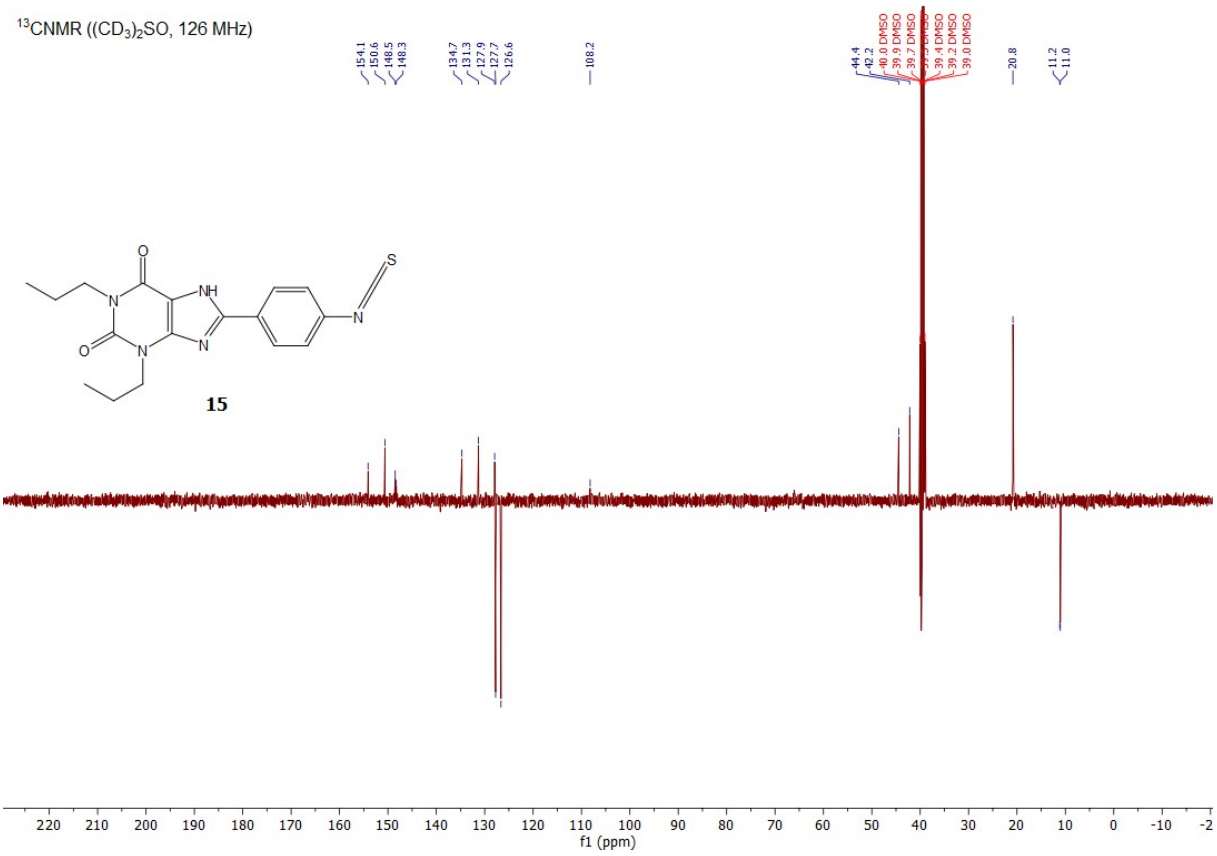
14

^{1}H NMR ($(CD_3)_2SO$, 500 MHz)



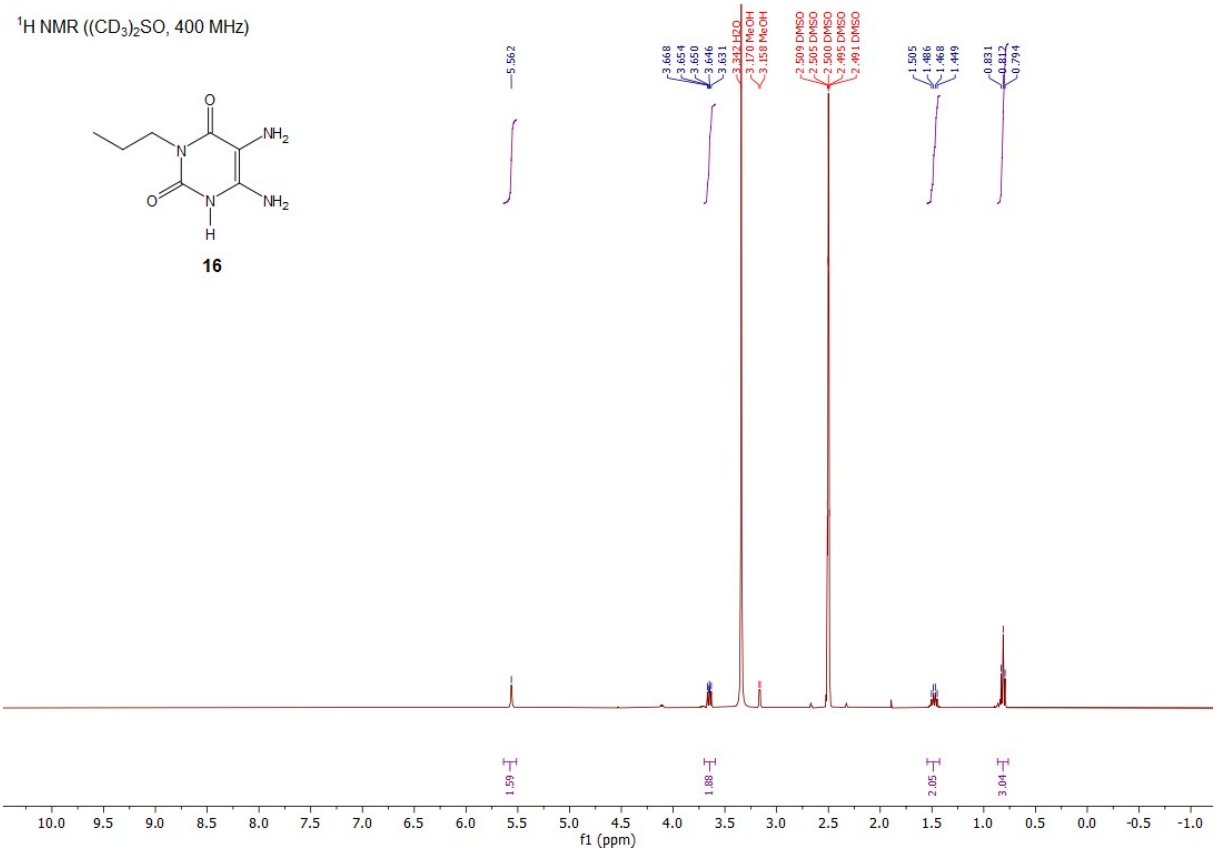
15

^{13}CNMR ($(CD_3)_2SO$, 126 MHz)



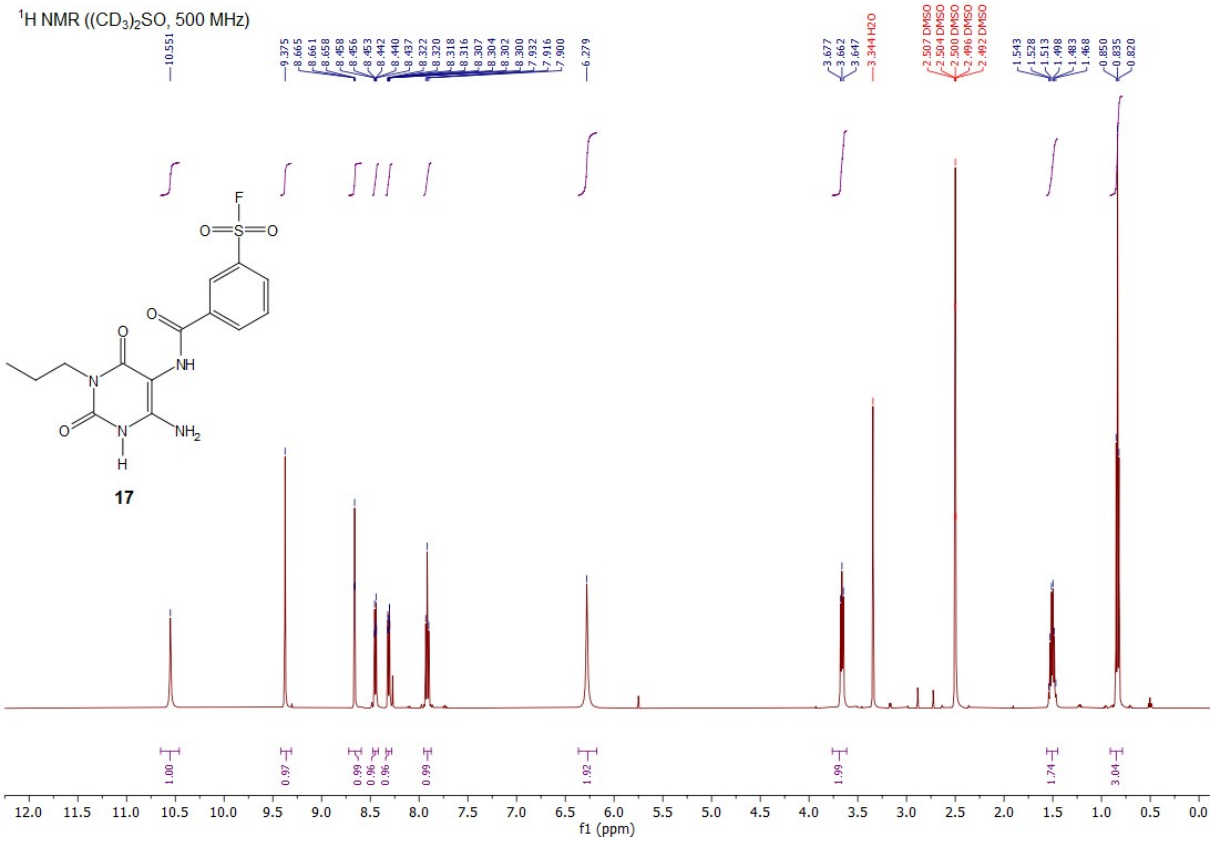
15

^{1}H NMR ($(CD_3)_2SO$, 400 MHz)



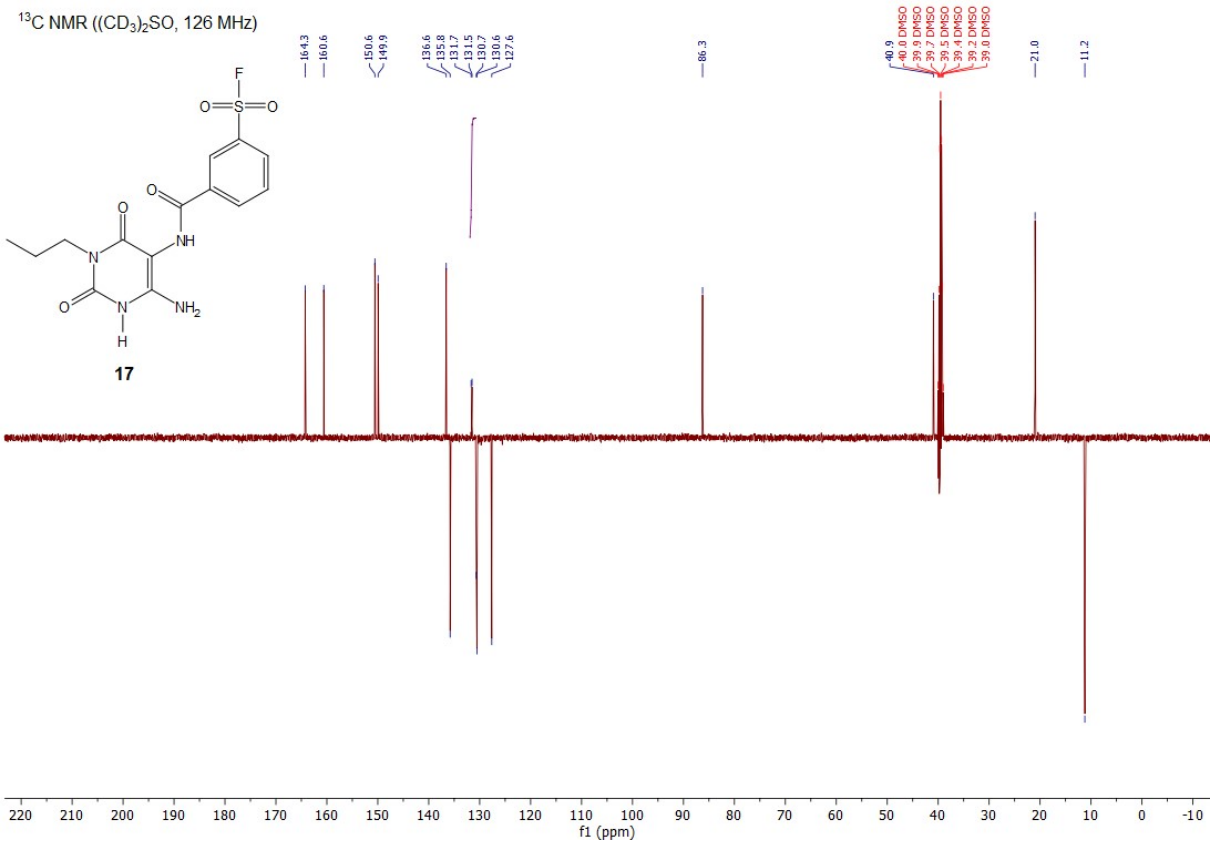
16

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



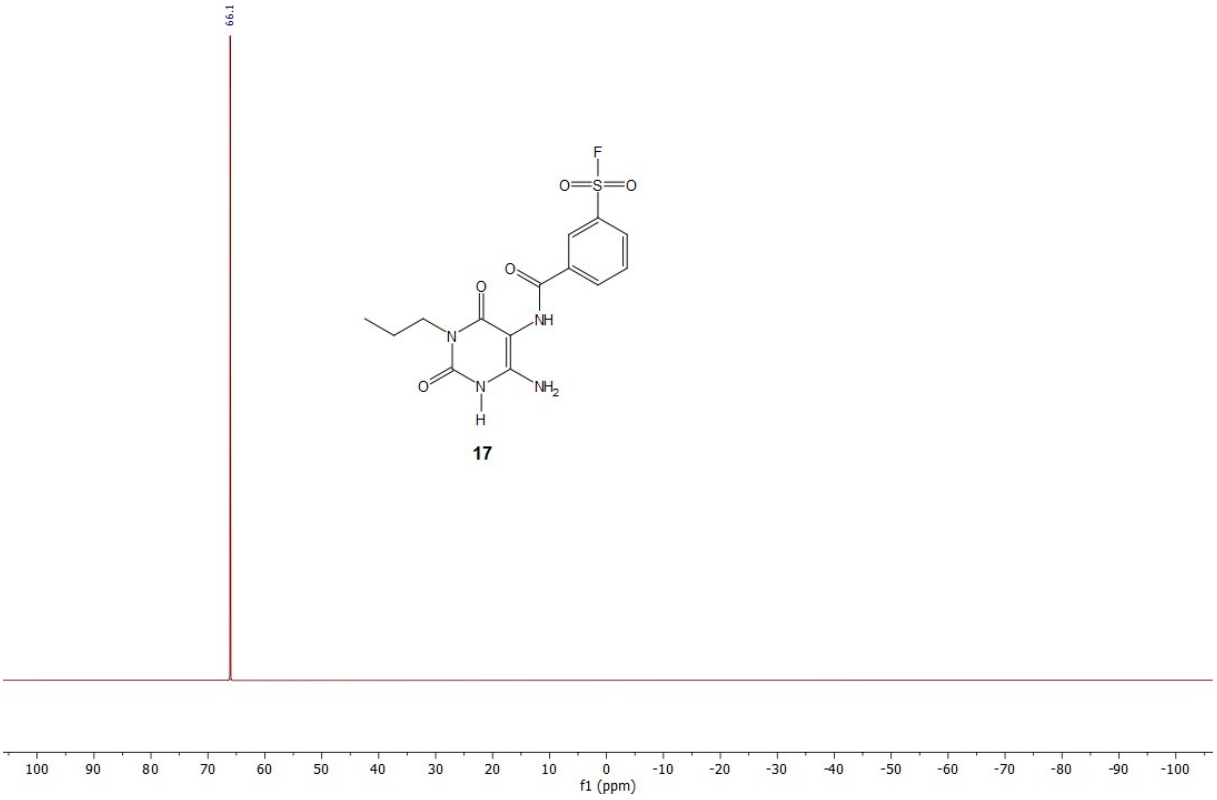
17

^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



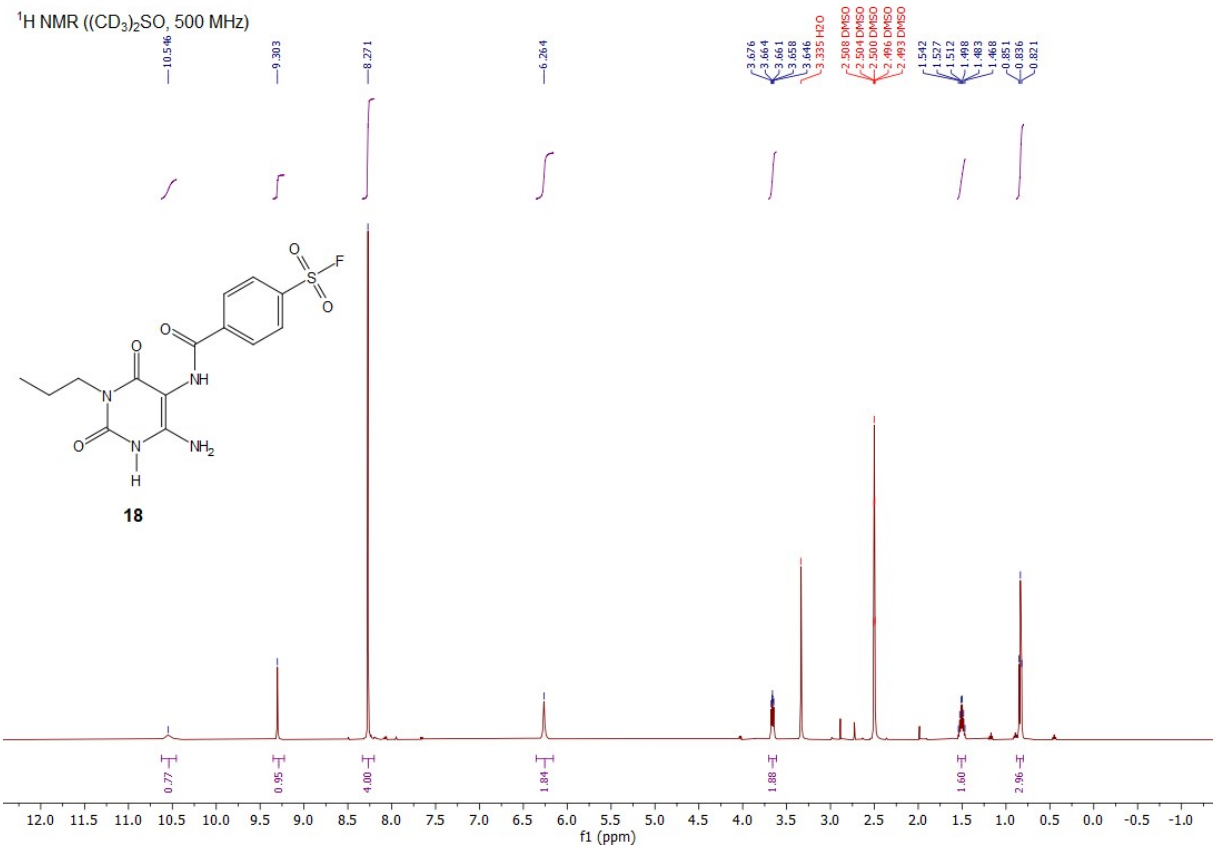
17

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



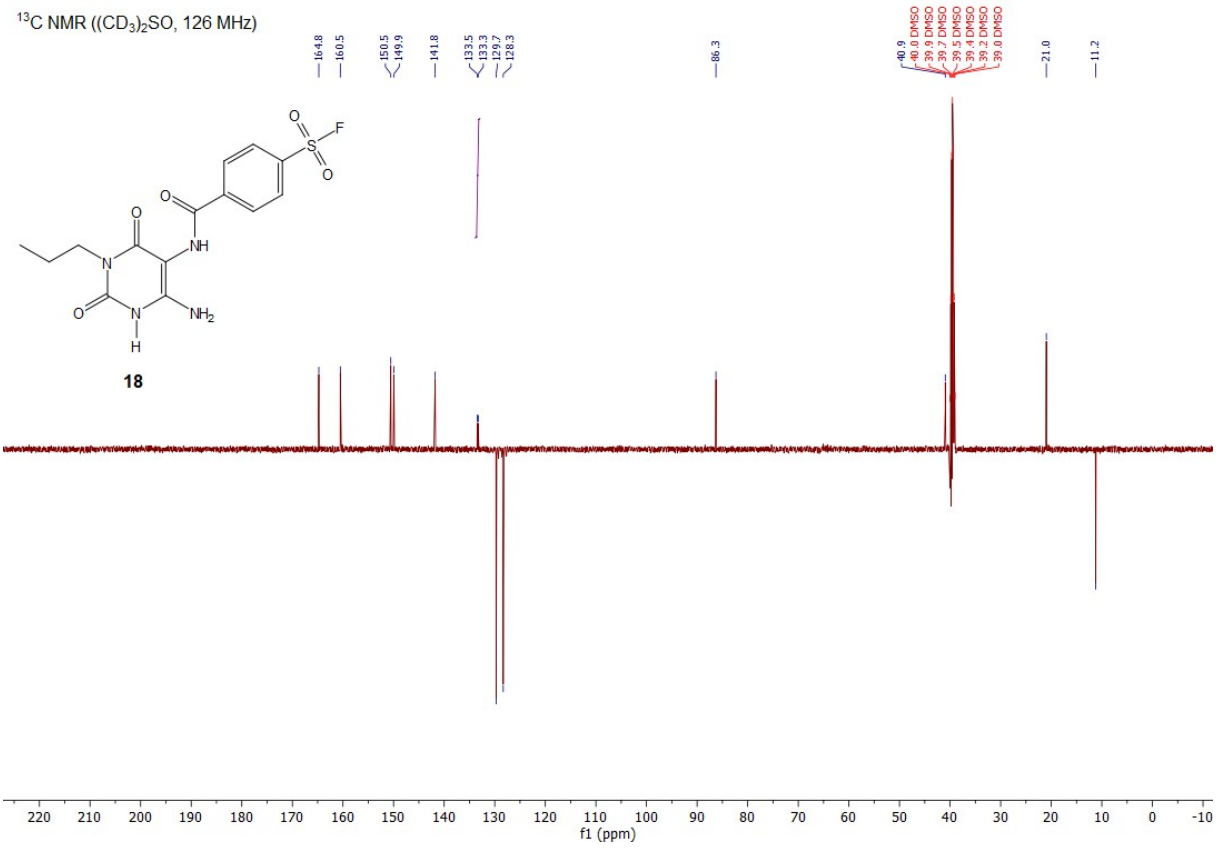
17

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)

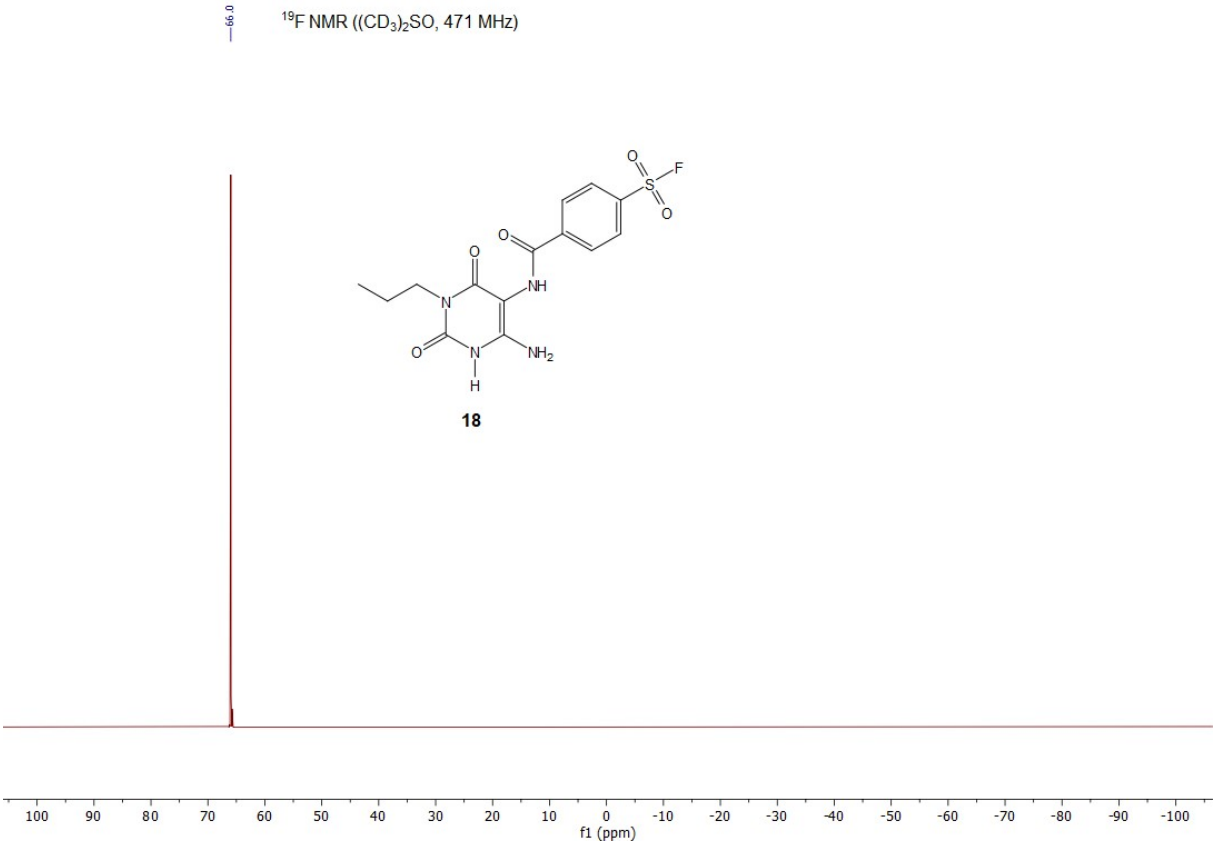


18

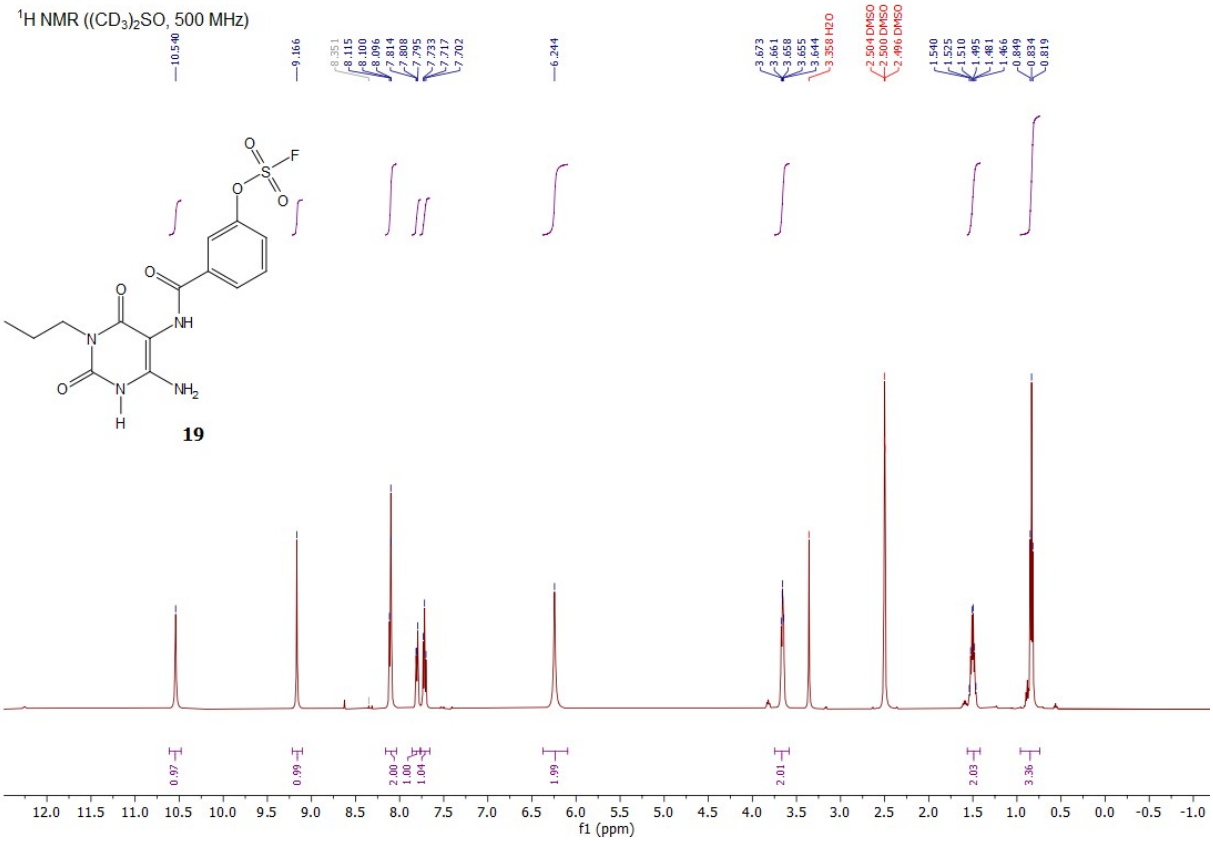
^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)

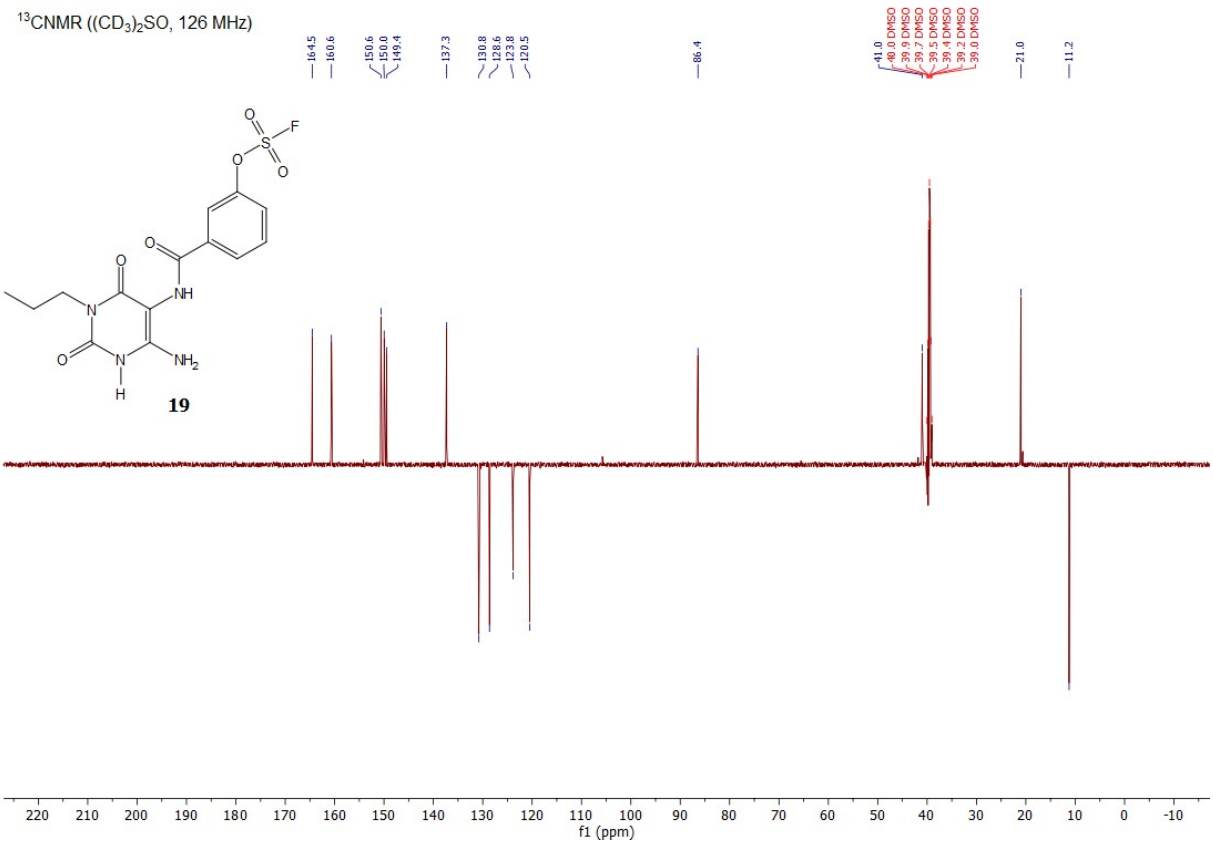


^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



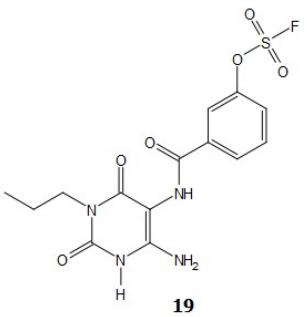
19

^{13}CNMR ((CD$_3$)$_2$SO, 126 MHz)



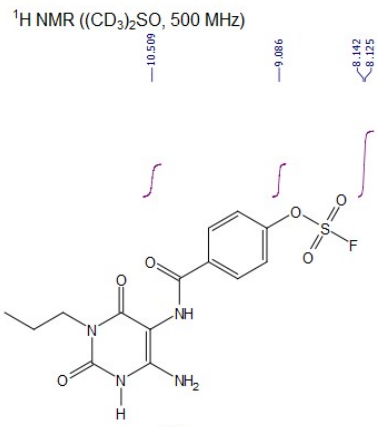
19

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



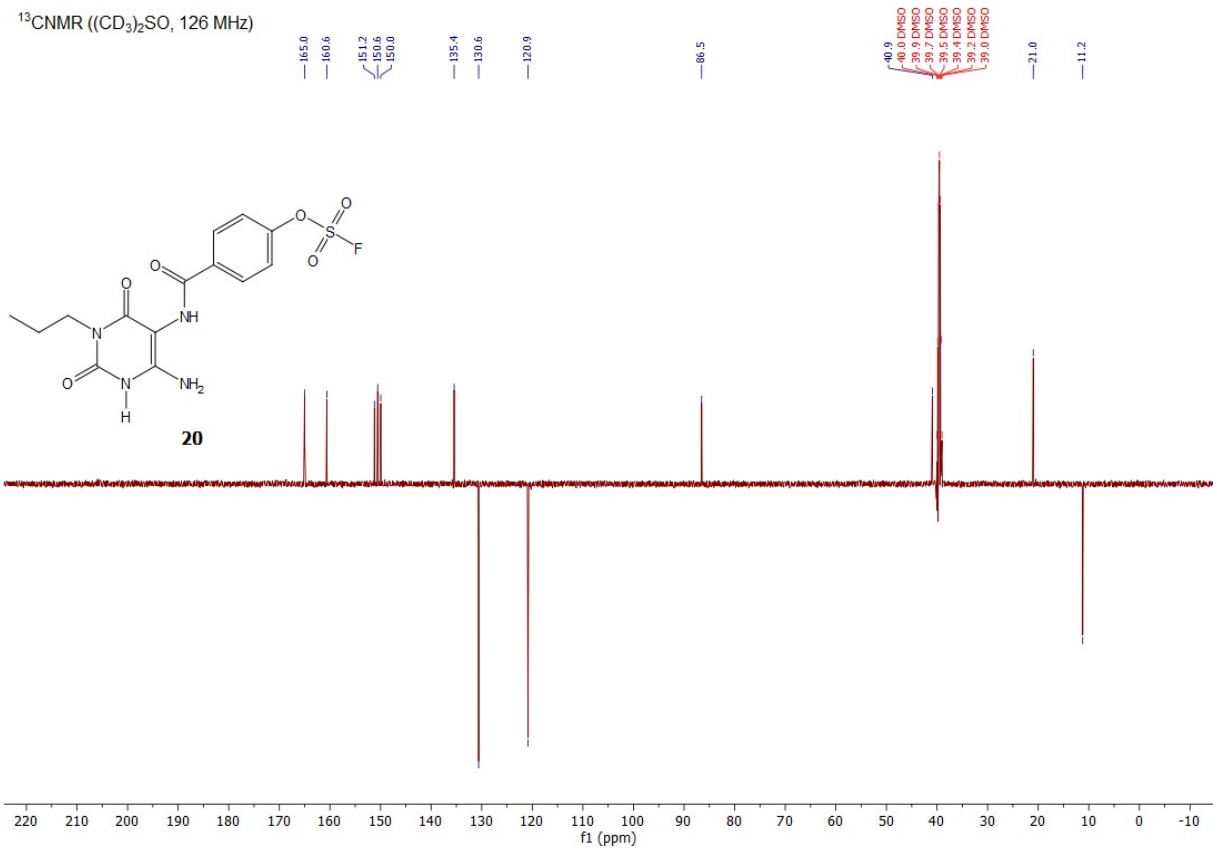
19

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



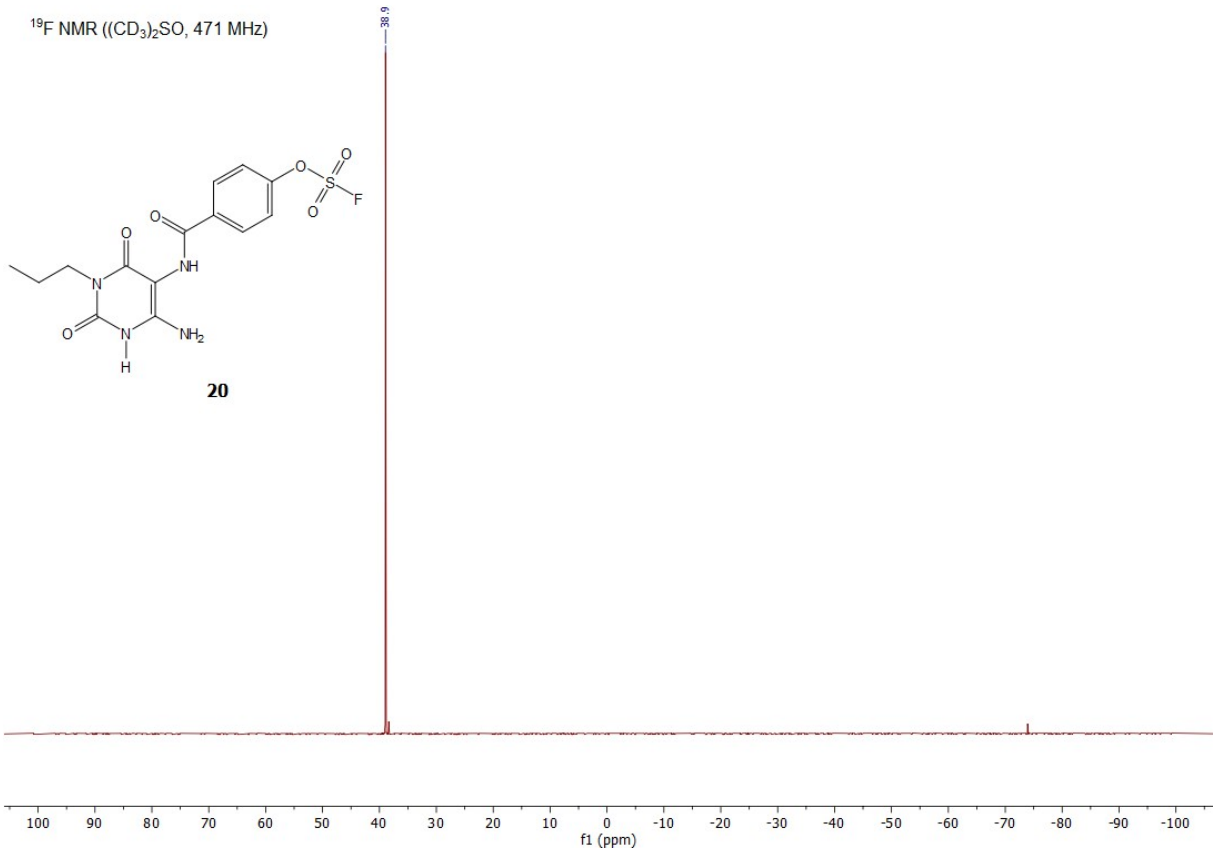
20

^{13}CNMR ($(CD_3)_2SO$, 126 MHz)

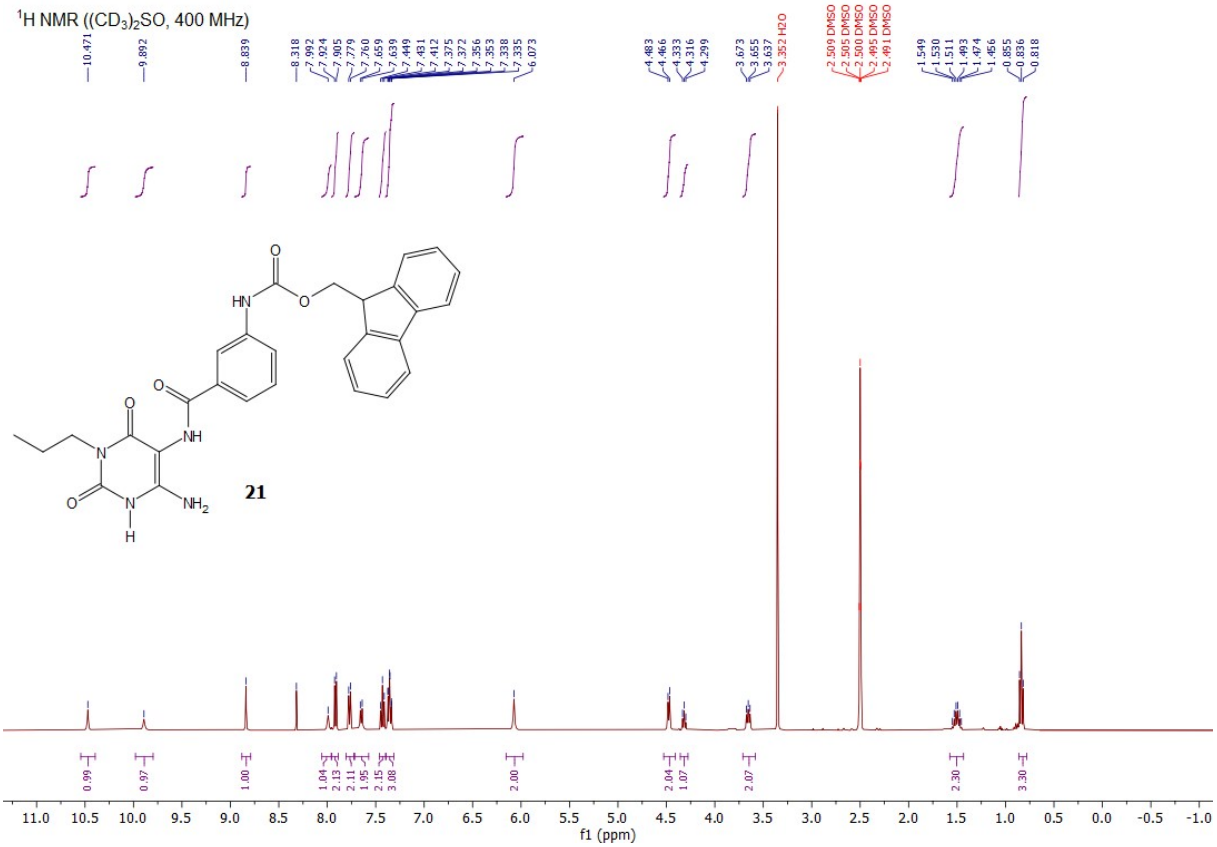


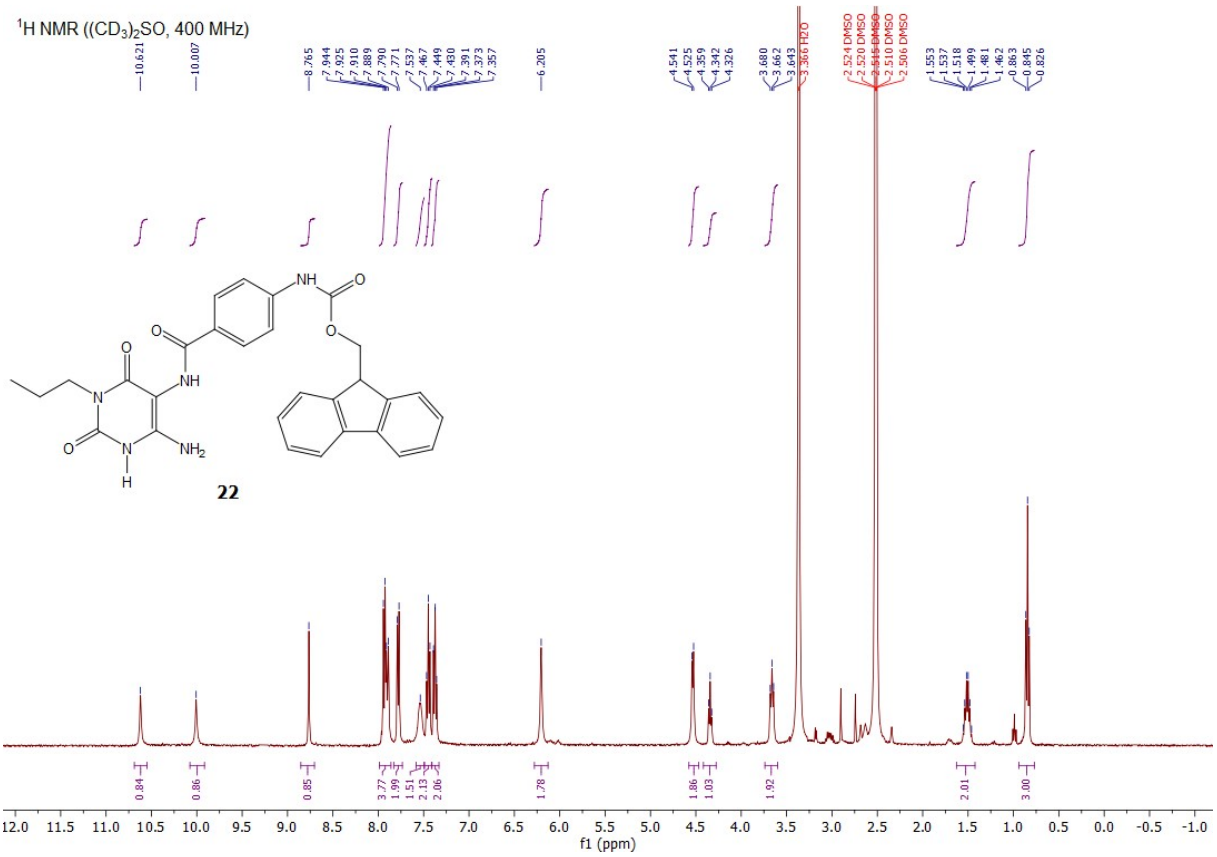
20

^{19}F NMR ($(CD_3)_2SO$, 471 MHz)

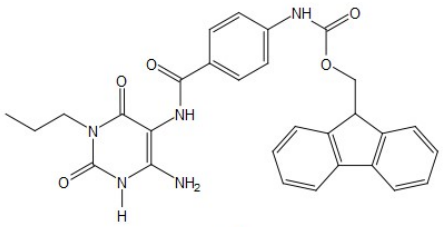


20


1H NMR ((CD3)2SO, 400 MHz)
10.471
9.892
8.839
8.318
7.992
7.924
7.905
7.779
7.760
7.659
7.639
7.449
7.431
7.412
7.375
7.372
7.356
7.353
7.338
7.335
6.073
4.483
4.466
4.333
4.316
4.299
3.673
3.655
3.637
3.352 H2O
2.509 DMSO
2.505 DMSO
2.500 DMSO
2.495 DMSO
2.491 DMSO
1.549
1.530
1.511
1.493
1.474
1.456
0.855
0.836
0.818
HN
O
NH
N
NH2
H
21
0.99
0.97
1.00
1.04
2.13
1.95
2.15
3.08
2.00
2.04
1.07
2.07
2.30
3.30
f1 (ppm)

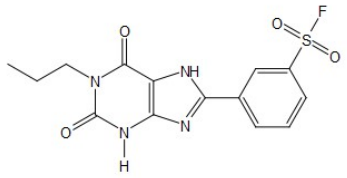

1H NMR ((CD3)2SO, 400 MHz)
10.621
10.007
8.765
7.944
7.925
7.910
7.889
7.790
7.771
7.537
7.467
7.449
7.430
7.391
7.373
7.357
6.205
4.541
4.525
4.359
4.342
4.326
3.680
3.662
3.643
3.366 H2O
2.524 DMSO
2.520 DMSO
2.510 DMSO
2.506 DMSO
1.553
1.537
1.518
1.499
1.481
1.462
0.863
0.845
0.826
NH
O
NH2
H
22
0.84
0.86
0.85
3.77
1.99
1.51
2.13
2.06
1.78
1.86
1.03
1.92
2.01
3.00
f1 (ppm)

^{13}CNMR ((CD$_3$)$_2$SO, 101 MHz)



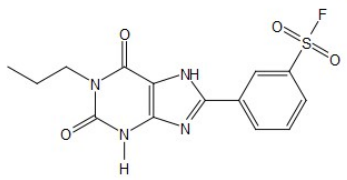
22

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



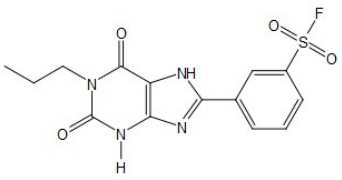
23

^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



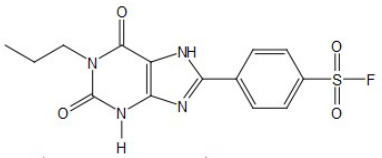
23

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



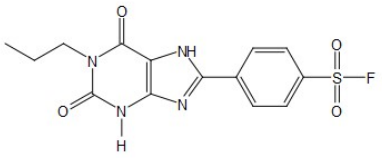
23

^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz)



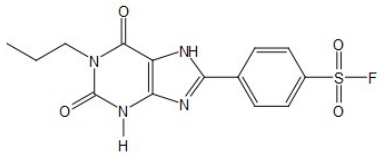
24 (LUF7982)

^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



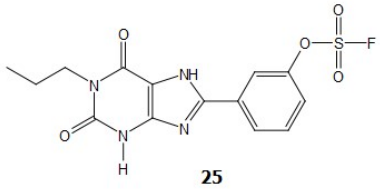
24 (LUF7982)

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



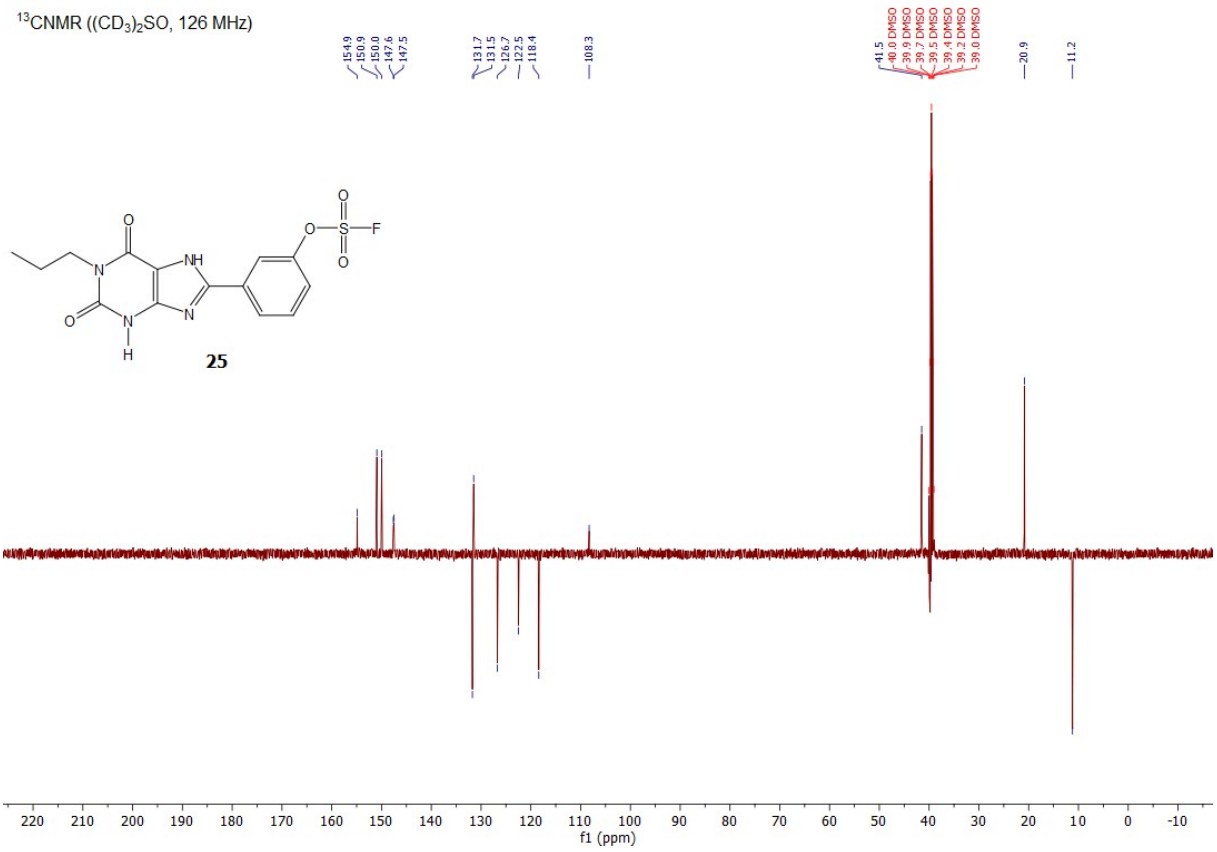
24 (LUF7982)

^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz)



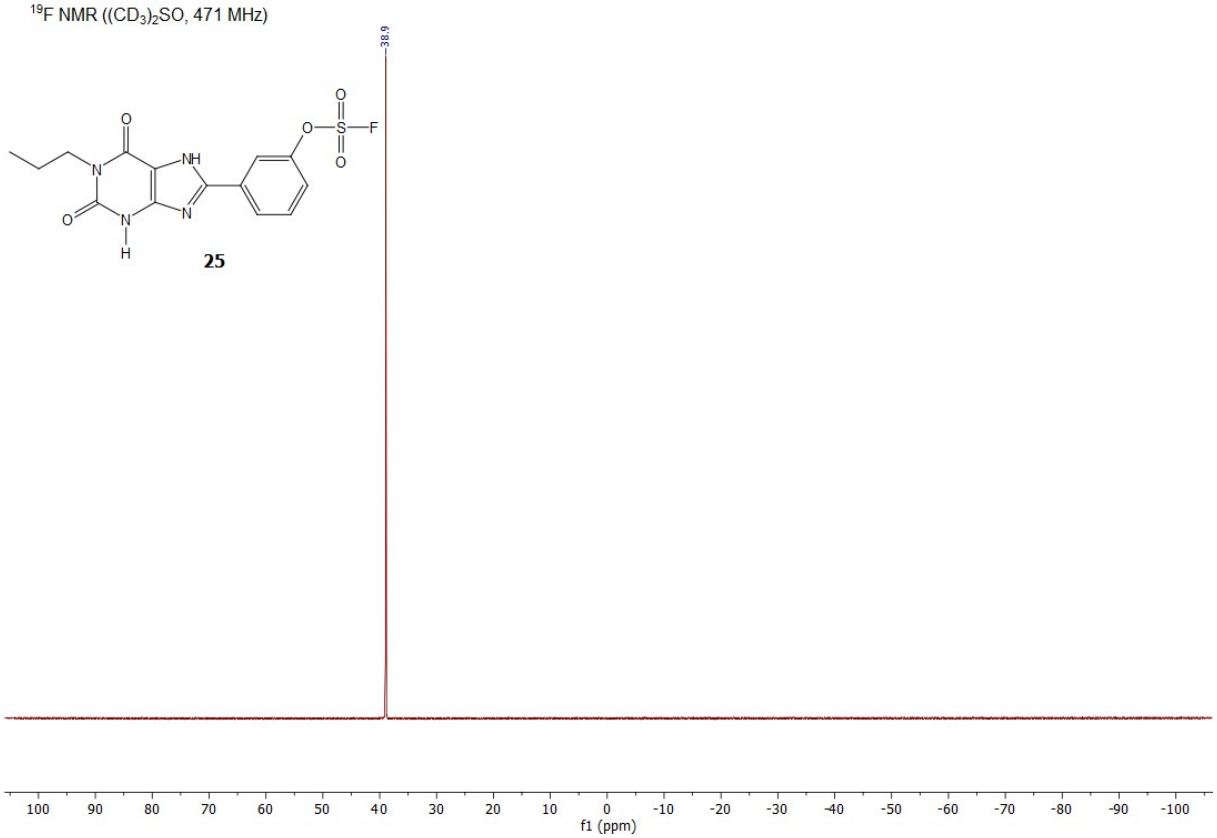
25

^{13}CNMR ($(CD_3)_2SO$, 126 MHz)



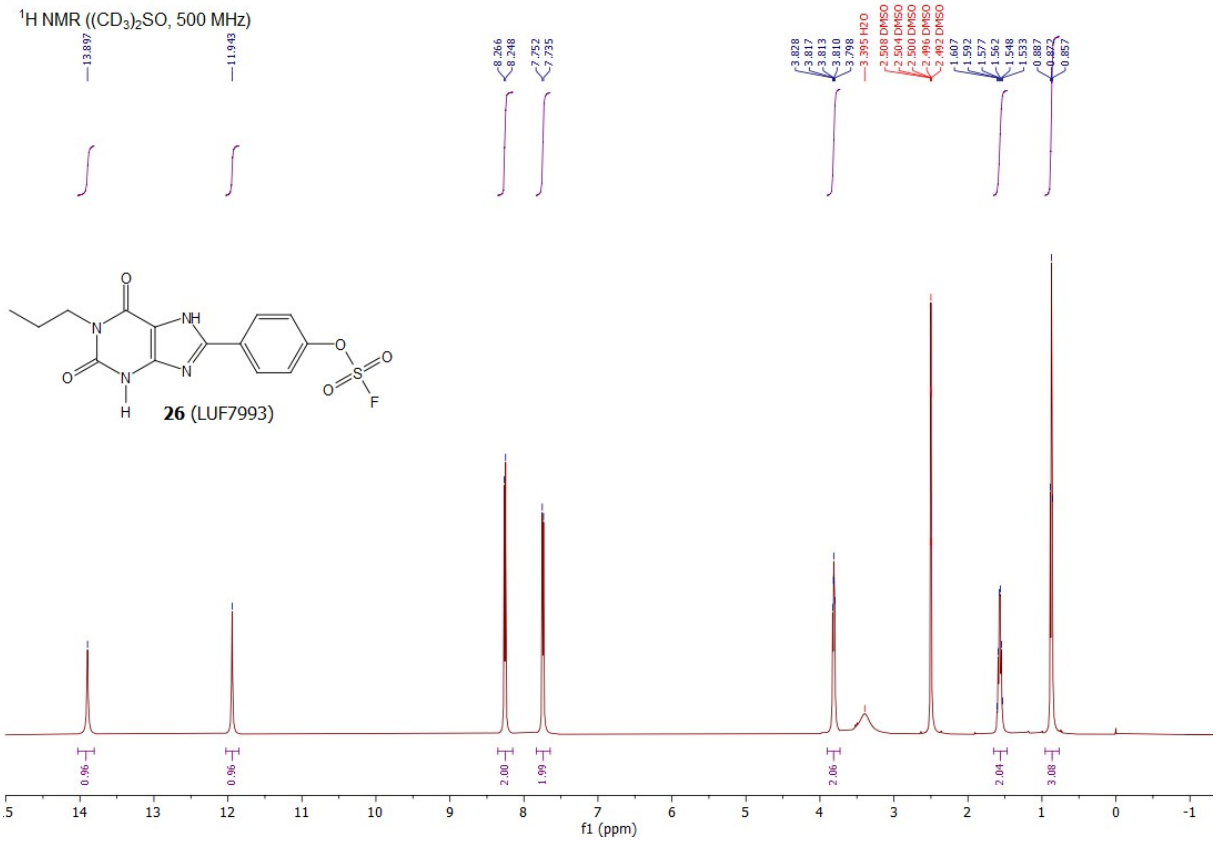
25

^{19}F NMR ($(CD_3)_2SO$, 471 MHz)



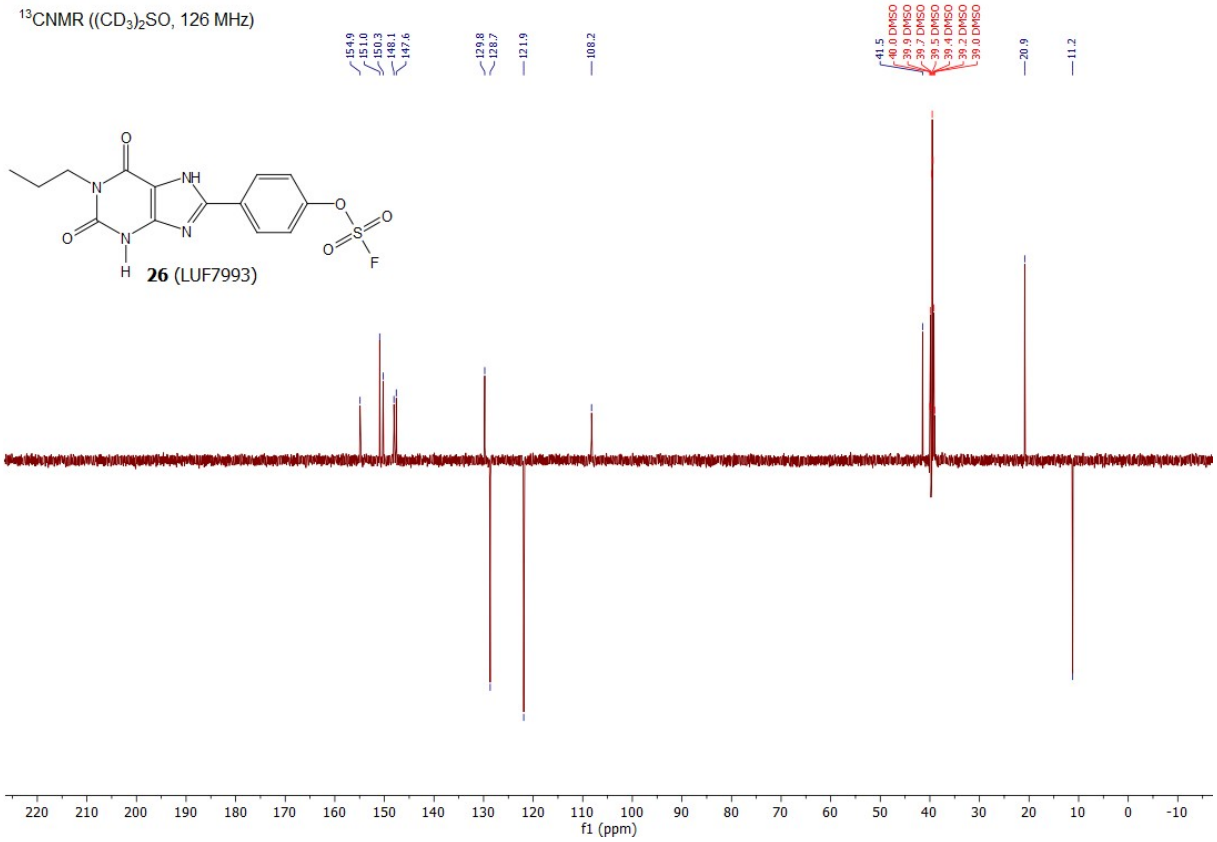
25

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



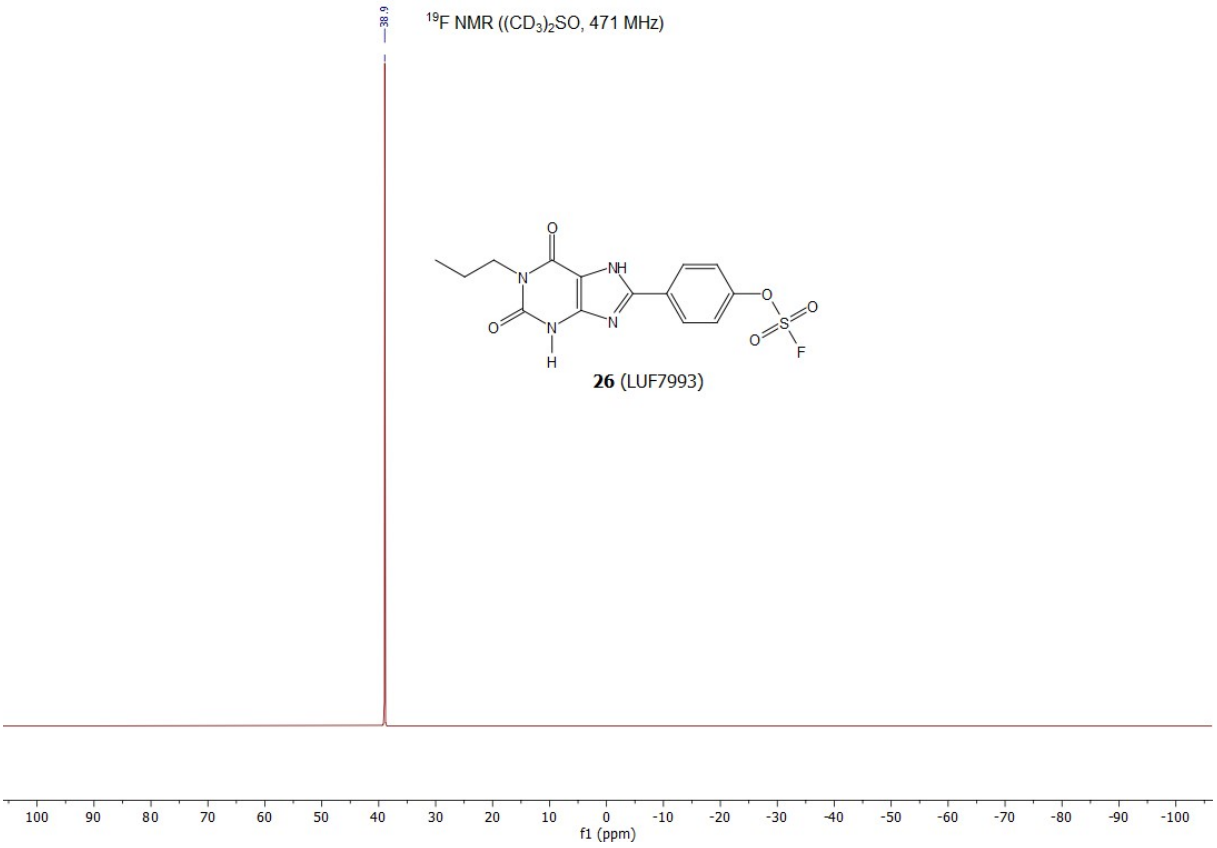
26 (LUF7993)

^{13}CNMR ((CD$_3$)$_2$SO, 126 MHz)



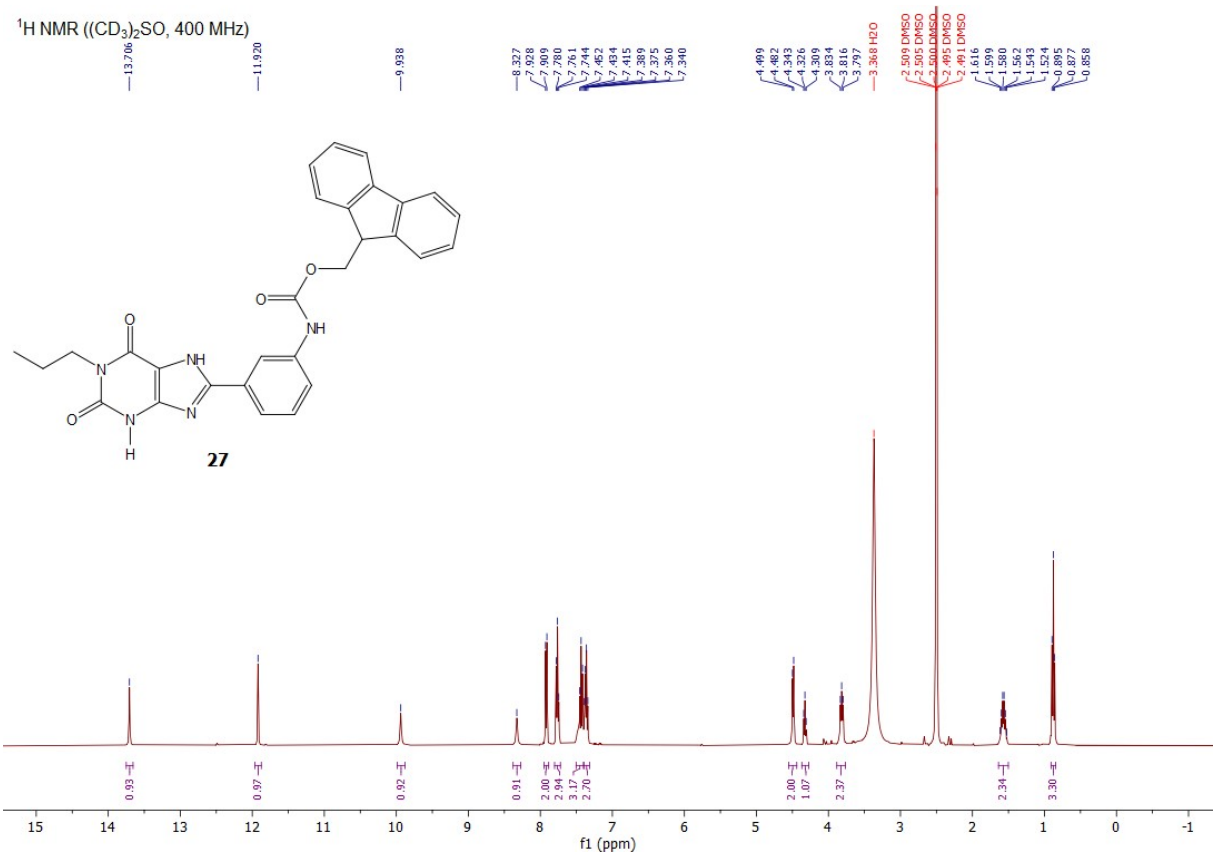
26 (LUF7993)

^{19}F NMR ((CD$_3$)$_2$SO, 471 MHz)



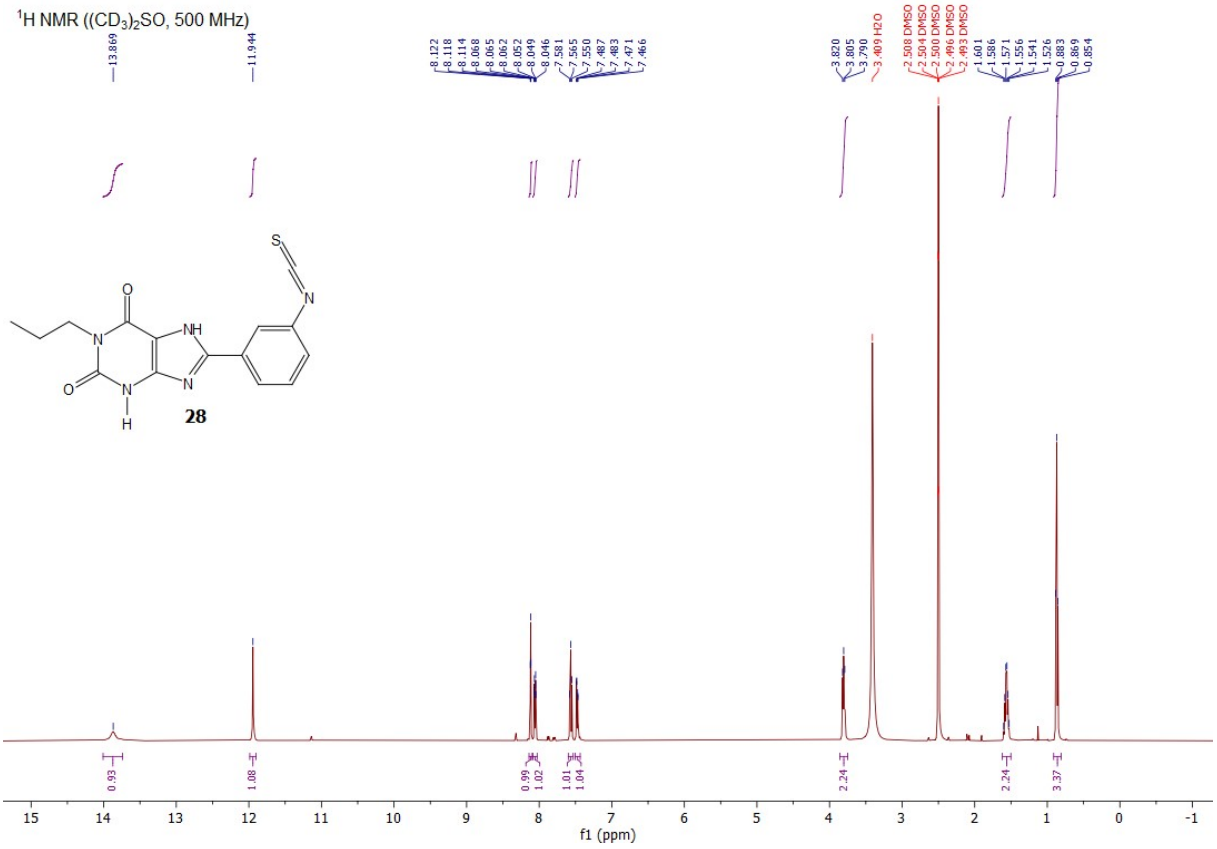
26 (LUF7993)

^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz)



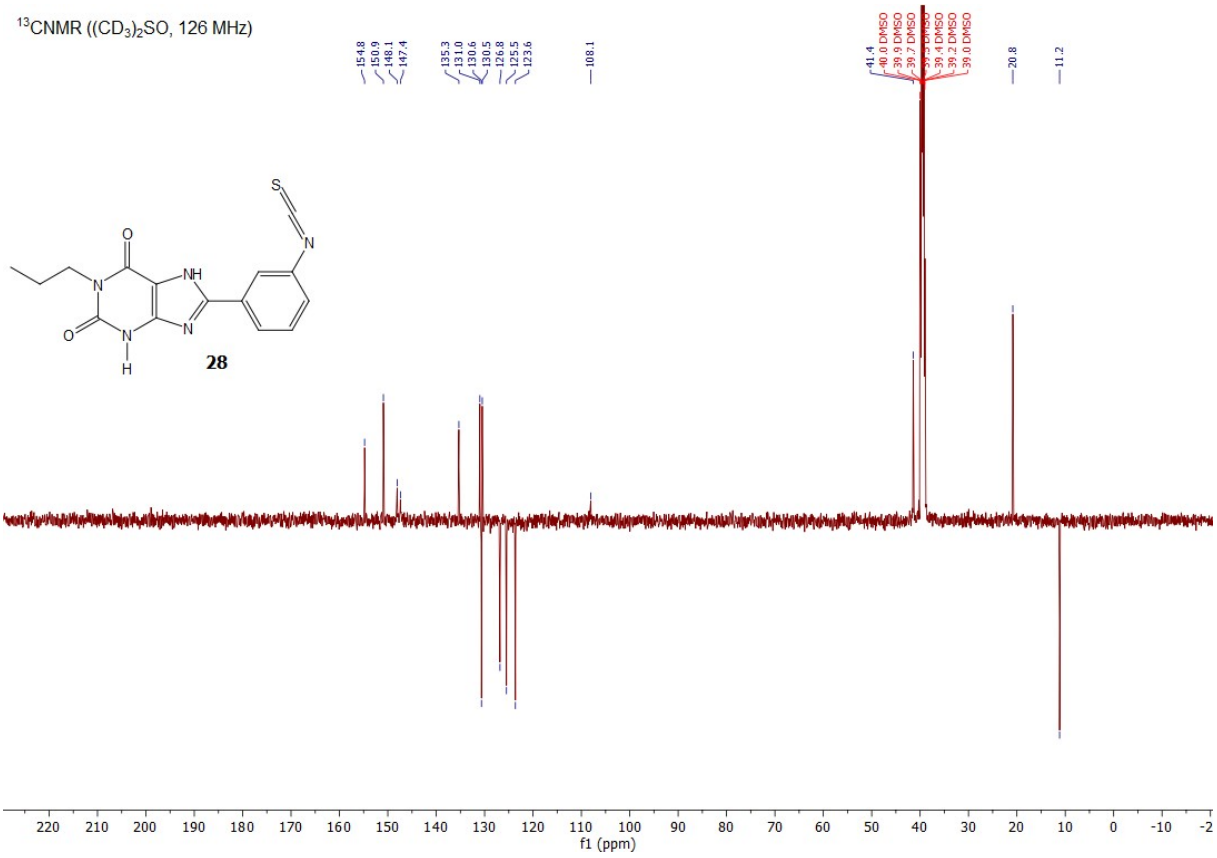
27

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)

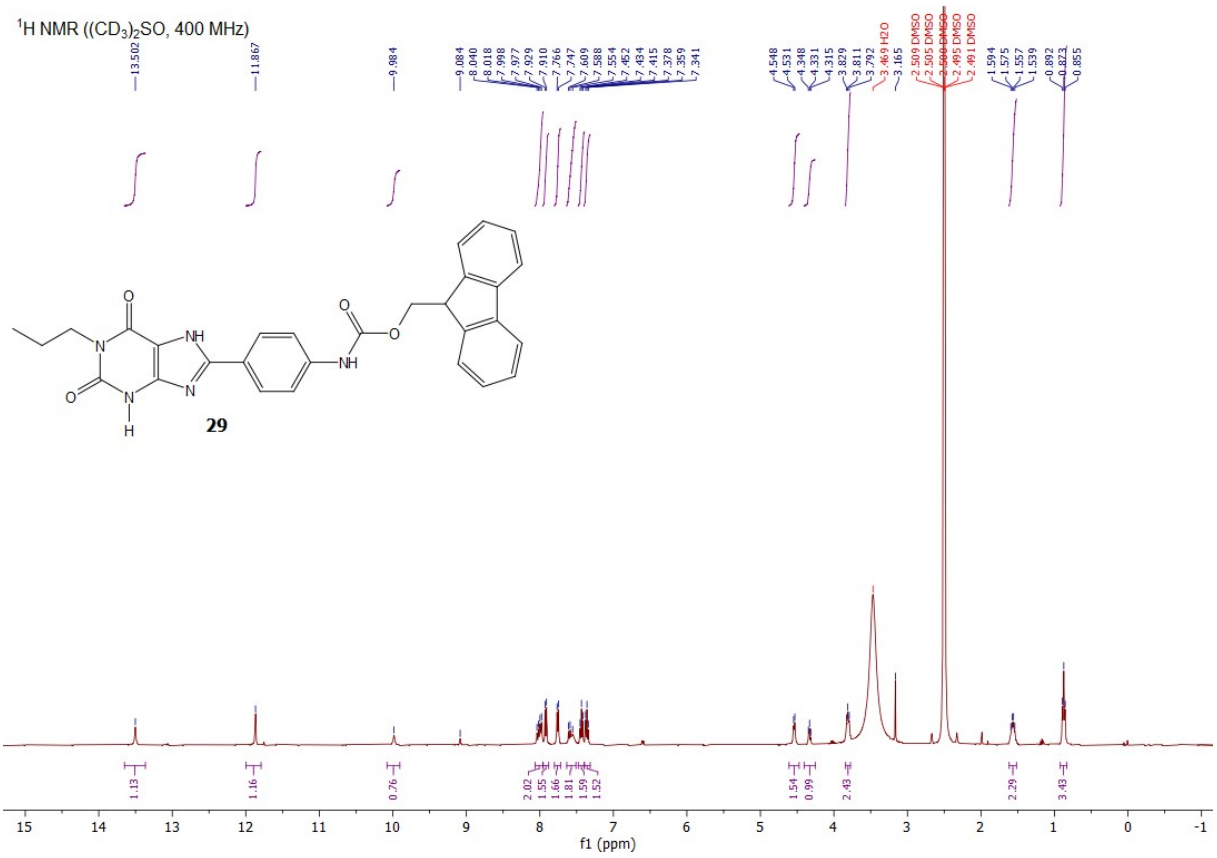


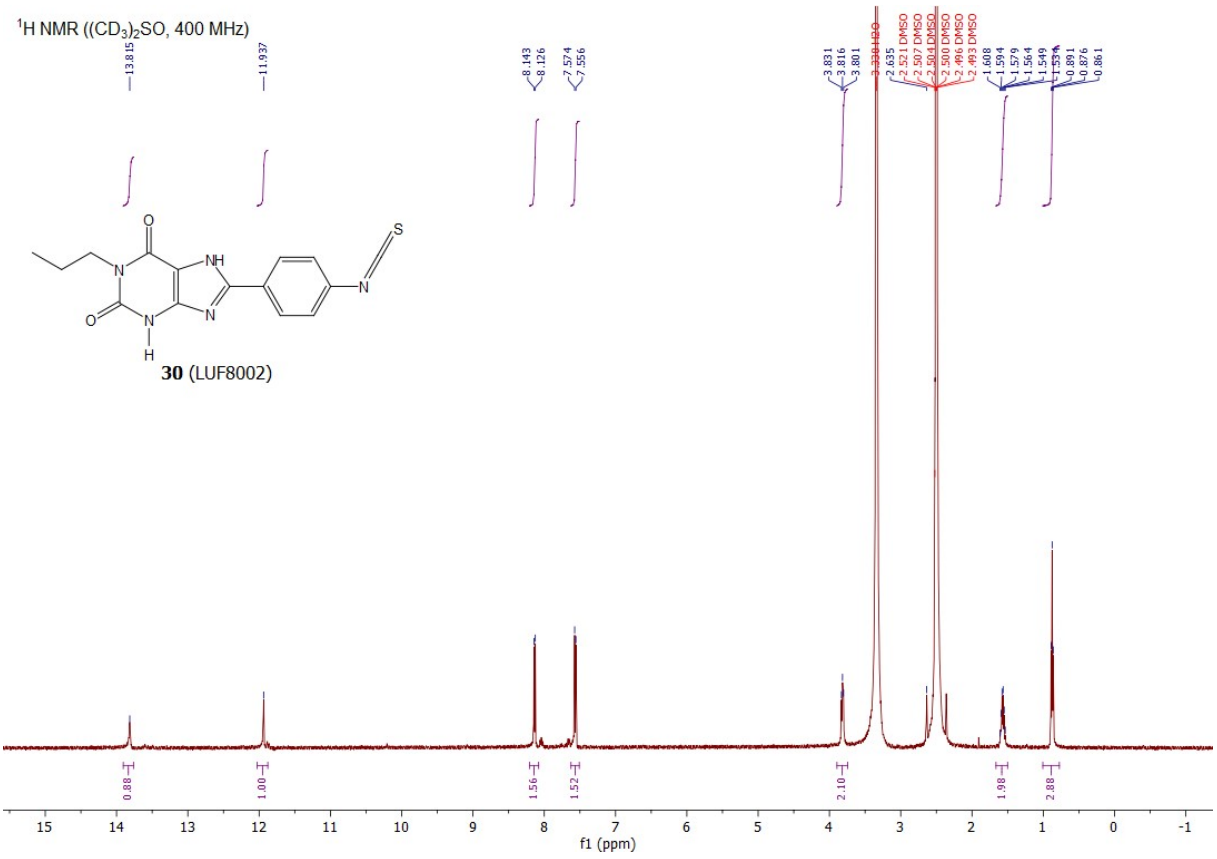
28

^{13}CNMR ((CD$_3$)$_2$SO, 126 MHz)

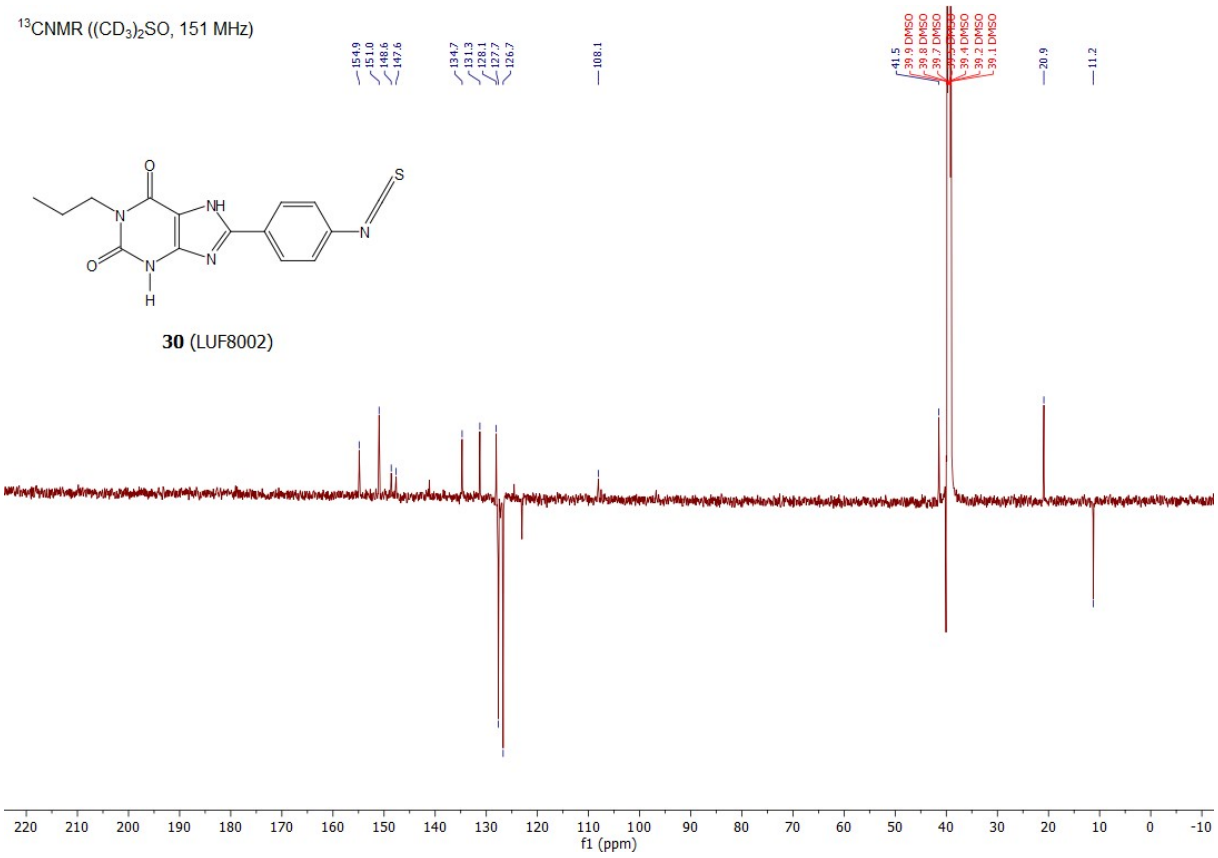


28


1H NMR ((CD3)2SO, 400 MHz)
29
f1 (ppm)

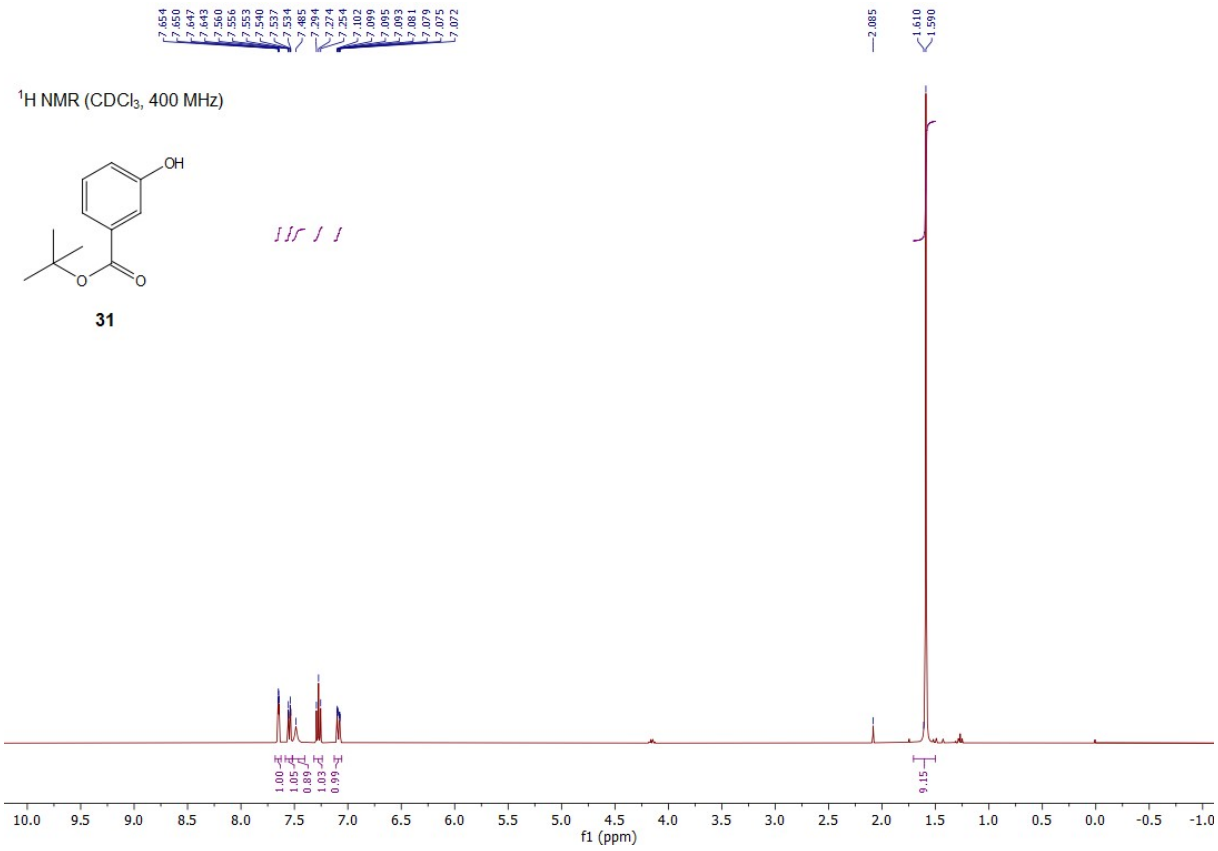

1H NMR ((CD3)2SO, 400 MHz)
30 (LUF8002)
f1 (ppm)

^{13}CNMR ($(CD_3)_2SO$, 151 MHz)



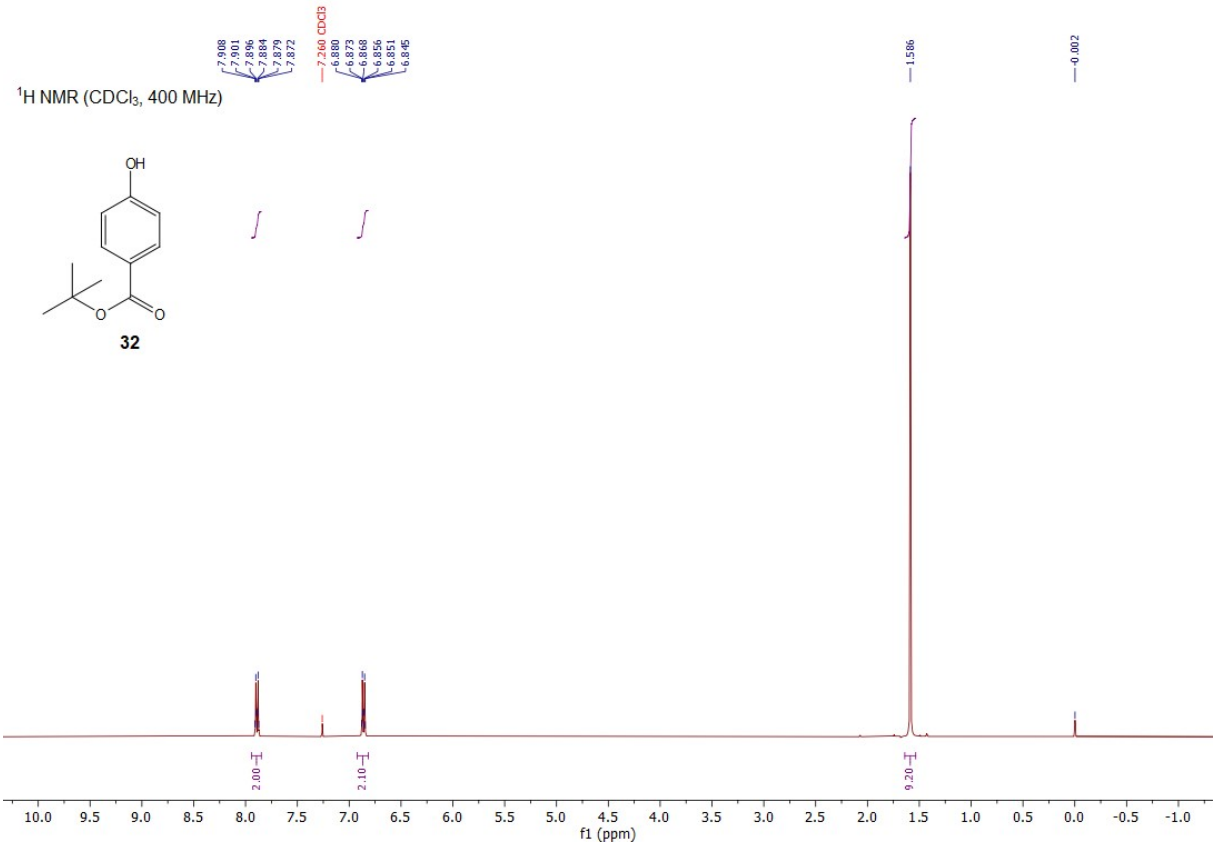
30 (LUF8002)

^{1}H NMR ($CDCl_3$, 400 MHz)



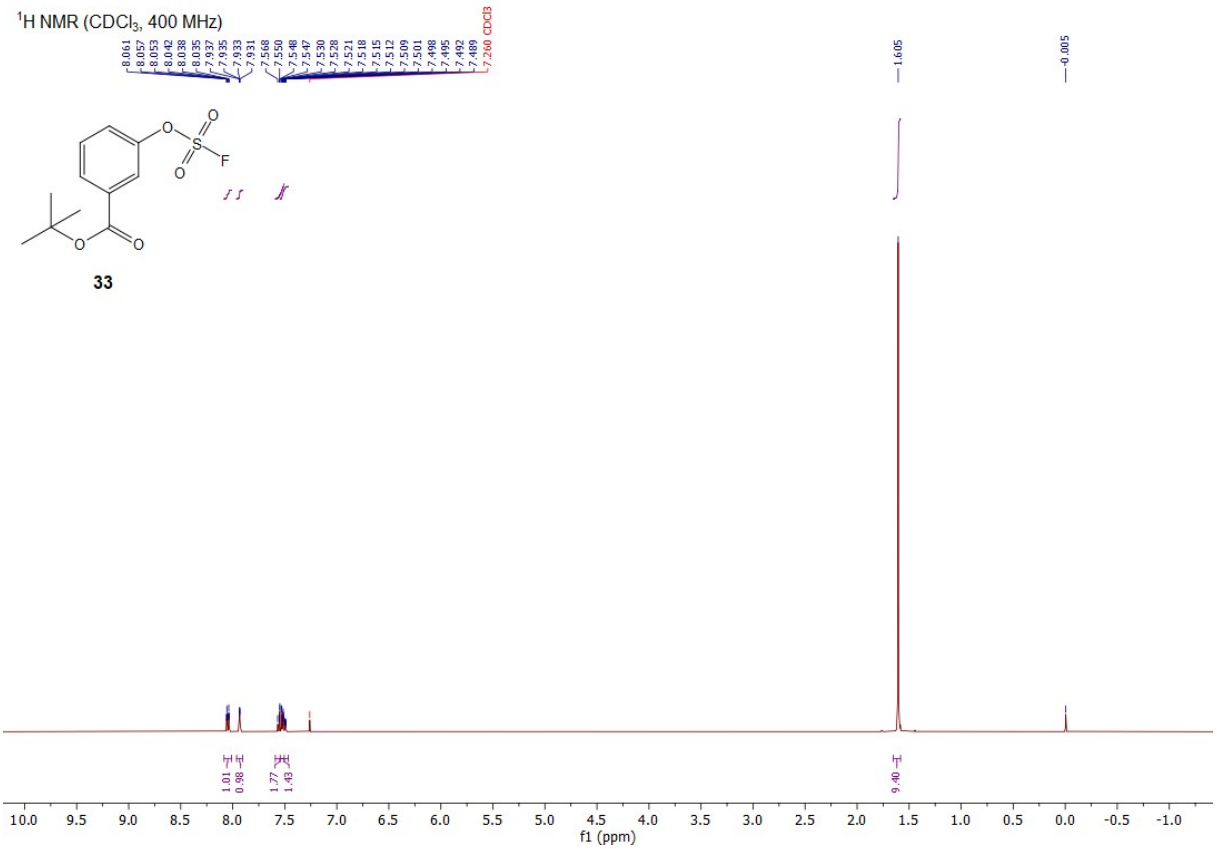
31

^{1}H NMR ($CDCl_3$, 400 MHz)



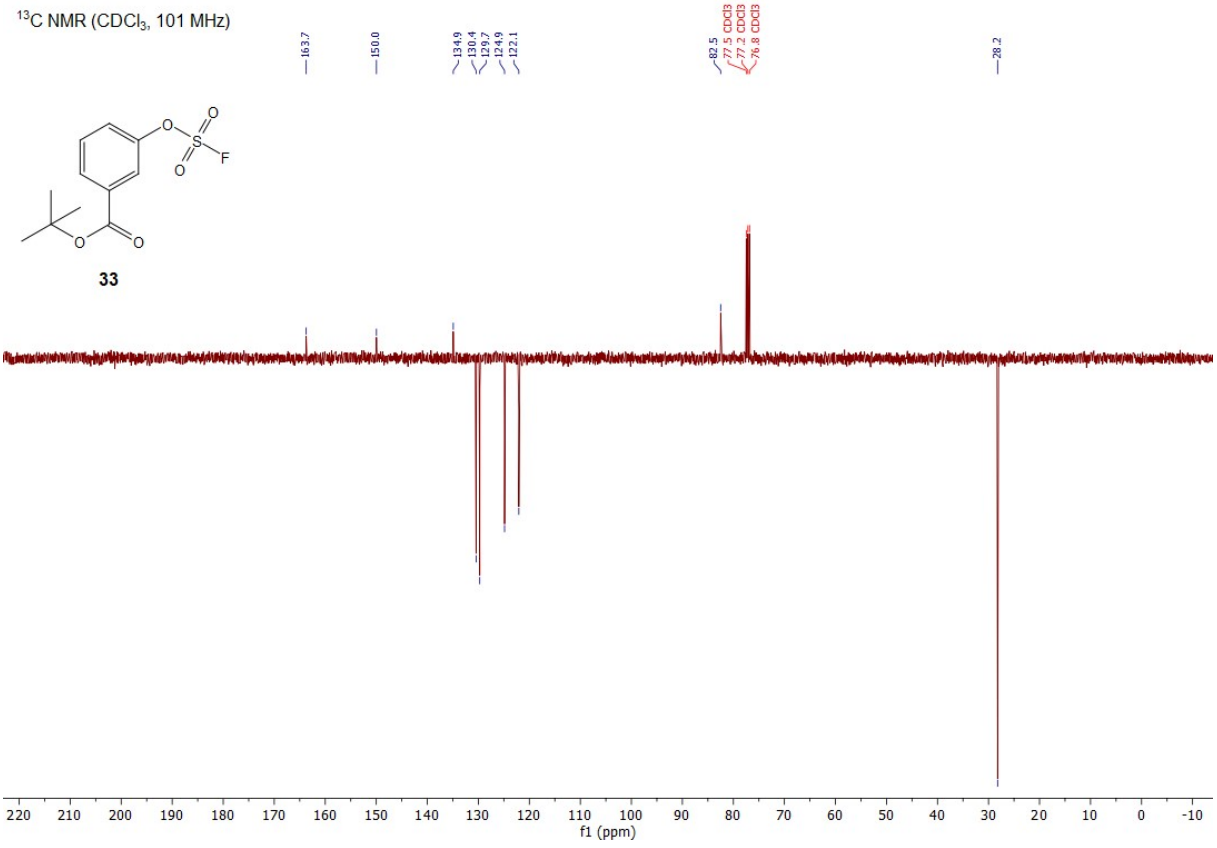
32

^{1}H NMR ($CDCl_3$, 400 MHz)



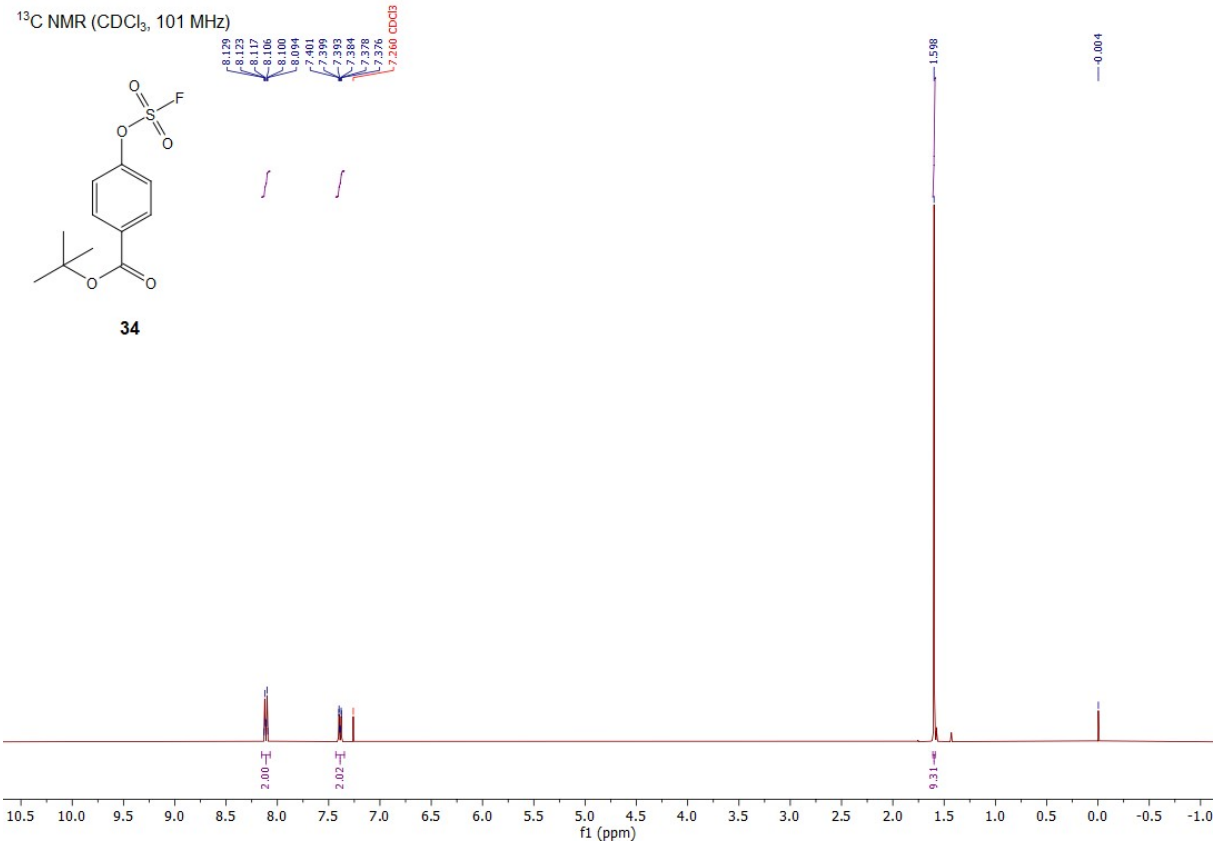
33

^{13}C NMR ($CDCl_3$, 101 MHz)



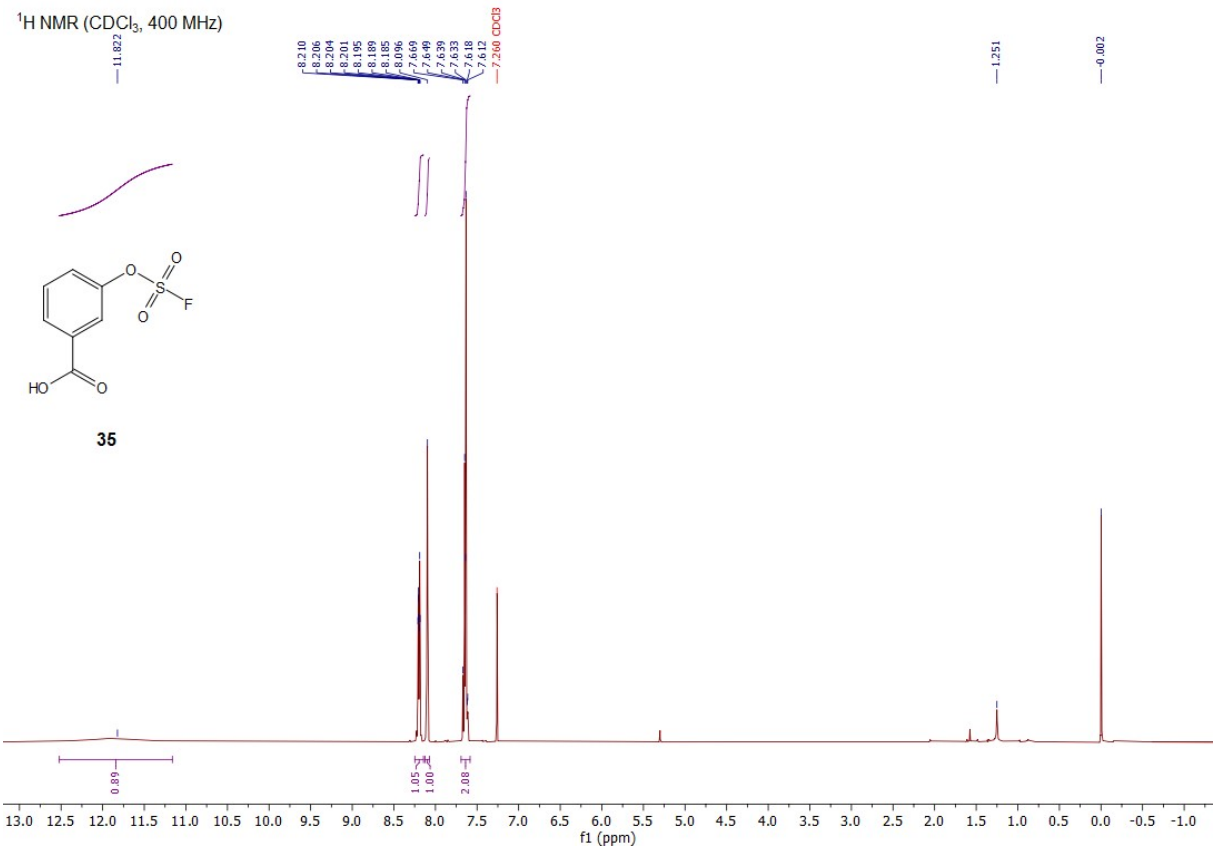
33

^{13}C NMR ($CDCl_3$, 101 MHz)



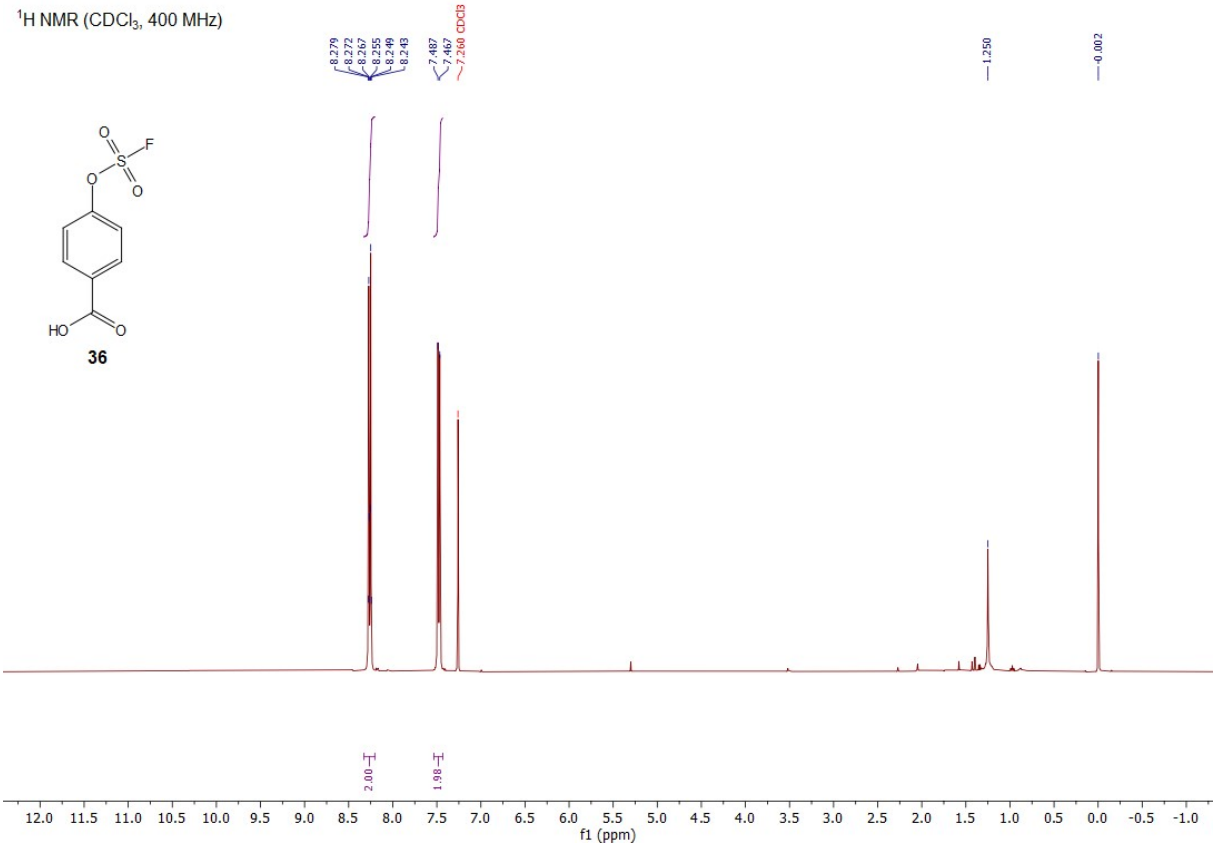
34

^{1}H NMR ($CDCl_3$, 400 MHz)



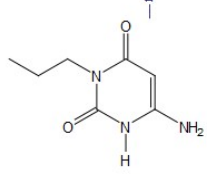
35

^{1}H NMR ($CDCl_3$, 400 MHz)



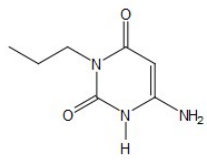
36

^{1}H NMR ((CD$_3$)$_2$SO, 500 MHz)



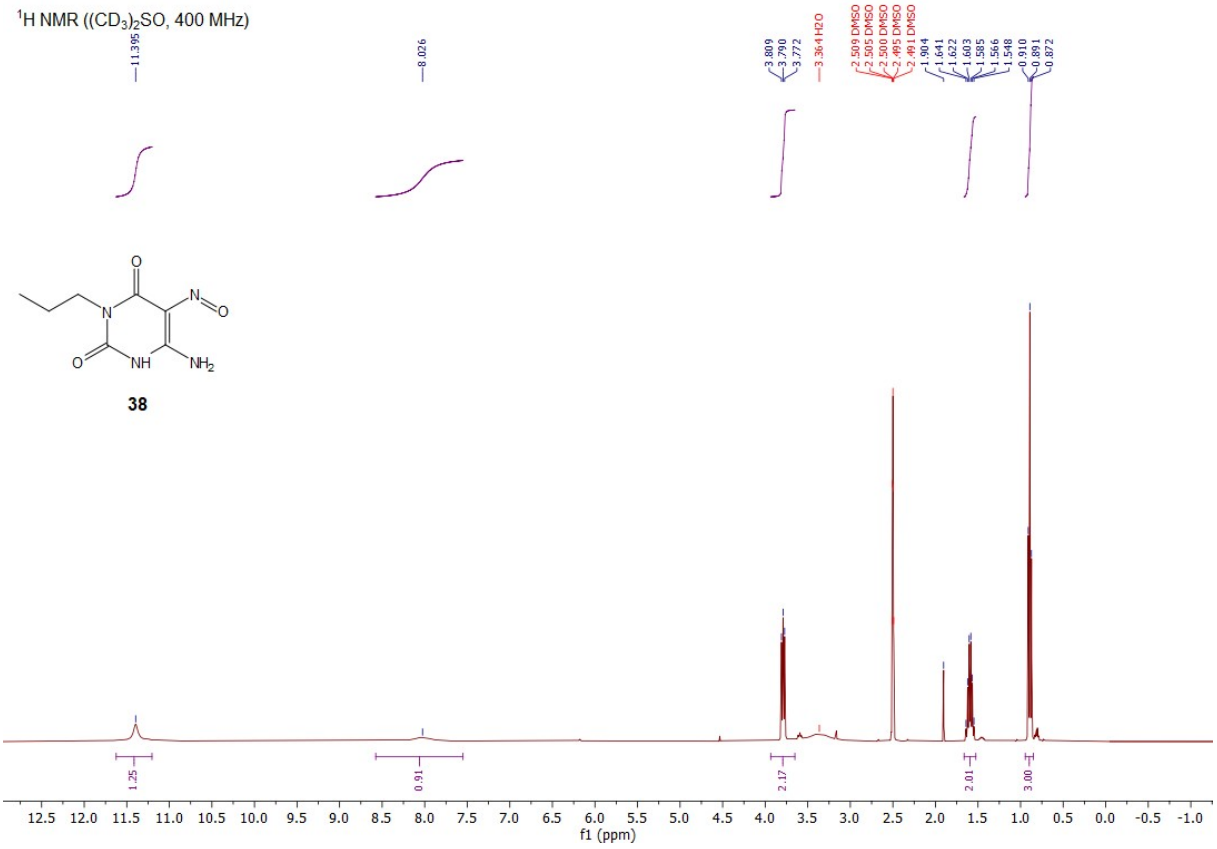
37

^{13}C NMR ((CD$_3$)$_2$SO, 126 MHz)



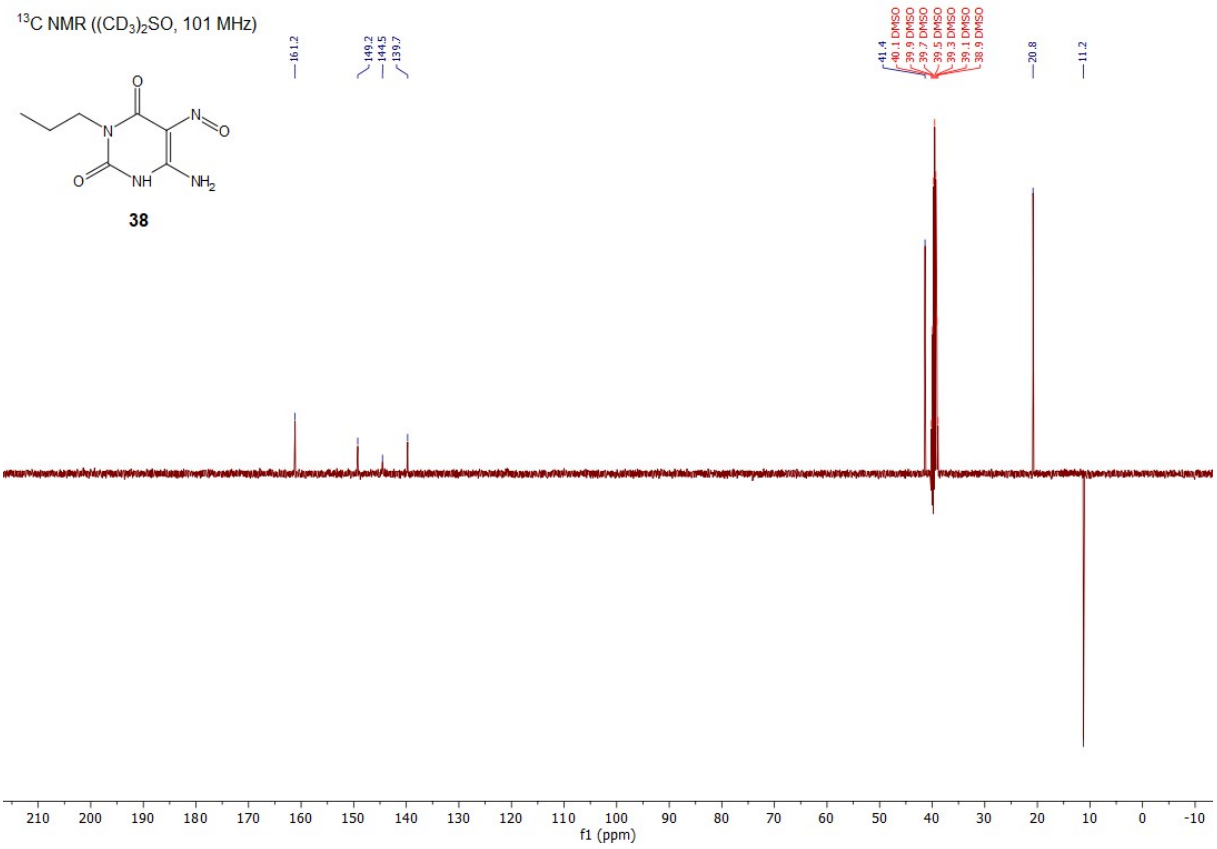
37

^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz)



38

^{13}C NMR ((CD$_3$)$_2$SO, 101 MHz)



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