Azo-Tagged C4N4 Fluorophore: Unusual Overcrowded Structures and Their Applications to Fluorescent Imaging

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Table of Contents

1. General Methods

1-1. Reactions and purifications

Unless otherwise noted, all reactions were carried out under an inert atmosphere and were stirred with Teflon-coated magnetic stir bars. All work-up and purification procedures were carried out with reagent-grade solvents under ambient atmosphere. Thin layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm) pre-coated with silica gel 60 F254 and visualized by UV quenching. Flash column chromatography was performed on a Biotage Isolera Spektra One.

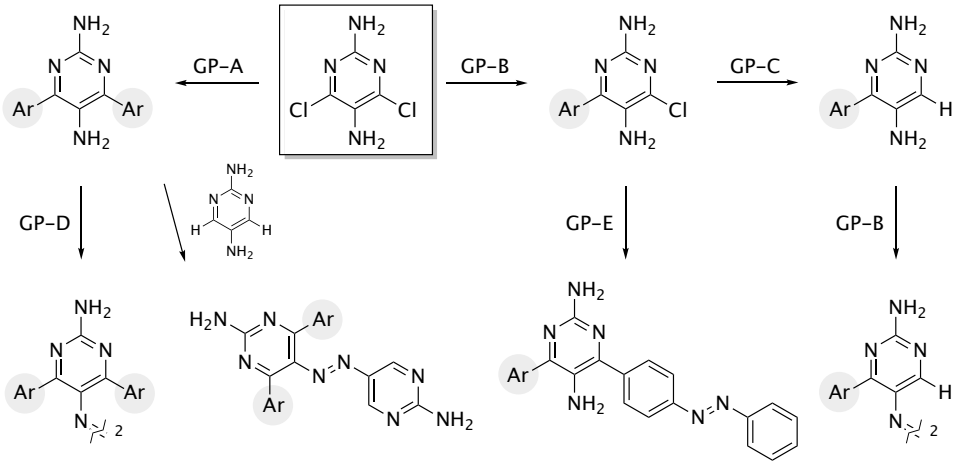
1-2. Characterizations

Infrared (IR) spectra were recorded on a JASCO FT/IR-4700. NMR spectra were recorded on a Bruker AVANCE 500 or a Bruker AVANCE NEO 600. Chemical shifts (δ) are given in ppm relative to residual solvent peaks[1]. Data for ^{1}H NMR are reported as follows: chemical shift (multiplicity, coupling constants where applicable, number of hydrogens). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublet), dt (doublet of triplet), ddd (doublet of doublet of doublet), q (quartet), m (multiplet), brs (broad). High resolution mass spectra (ESI Orbitrap (+)) were measured on JEOL JMS-T100LP. Melting points were measured on a Yanagimoto Seisakusho Micro Melting Point Apparatus. Cyclic voltammogram was recorded on ALS potentiostat 615E. Single-crystal X-ray data were collected on a Rigaku R-AXIS RAPID II imaging plate area detector with graphite-monochromated Cu-K**α** radiation. UV-Visible absorption spectra were recorded using a 0.1 dm cell on a Jasco V-670 spectrophotometer. Fluorescence emission spectra were recorded using a 0.1 dm cell on a Jasco FP-8600 spectrofluorometer, with which absolute fluorescence quantum yields were measured with a Jasco ILF-835 unit (100 mm integrating sphere).

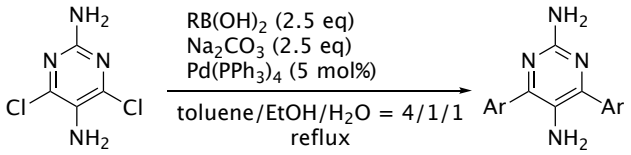
1-3. Solvents and reagents

Unless otherwise noted, materials were purchased from commercial suppliers and were used without purification. Dry THF, toluene, and CH_2Cl_2 were dispensed from a solvent supply system (Kanto Chemical). Anhydrous 1,2-dichloroethane, methanol, and DMF were purchased from Sigma-Aldrich or Kanto Chemical. 2,5-Diamino-4,6-dichloropyrimidine was purchased from Tokyo Chemical Industry. 2,6-Dimethylphenylboronic was purchased from Combi-Blocks. $(Ir[dF(CF_3)ppy]_2(dtbbpy))PF_6$ was purchased from Sigma-Aldrich.

2. Synthesis of Compounds

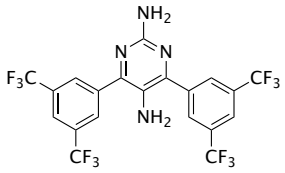


2-1. Synthesis of C4N4 compounds



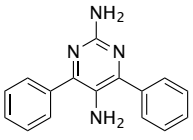
GP-A: To a suspension of 2,5-diamino-4,6-dichloropyrimidine (1.0 eq) in toluene (0.1 M), EtOH (0.4 M) and H_2O (0.4 M) were added boronic acid (2.5 eq), Na_2CO_3 (2.5 eq), $Pd(PPh_3)_4$ (5 mol%). The resulting solution was stirred under reflux for 24 h, and cooled to room temperature. After the addition of H_2O, aqueous phase was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. Volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc).

4,6-Bis(3,5-(trifluoromethyl)phenyl)pyrimidine-2,5-diamine (1a)



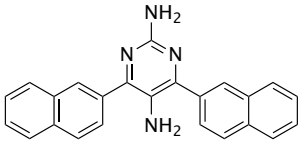
Prepared by GP-A with a slight modification from 2,5-diamino-4,6-dichloropyrimidine (332 mg, 1.9 mmol, 1.0 eq), 3,5-(trifluoromethyl)phenylboronic acid (1.18 g, 4.6 mmol, 2.5 eq), Na_2CO_3 (1.00 g, 9.4 mmol, 5.1 eq), and $Pd(PPh_3)_4$ (227 mg, 0.2 mmol, 10 mol%), and obtained as a yellow solid (437 mg, 78%). Spectroscopic data matched with those reported.[1]

4,6-Biphenylpyrimidine-2,5-diamine (1b)



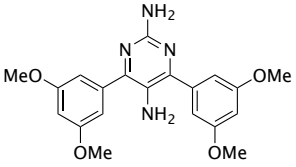
Prepared by GP-A with a slight modification from 2,5-diamino-4,6-dichloropyrimidine (716 mg, 4.0 mmol, 1.0 eq), phenylboronic acid (1.21 g, 10.0 mmol, 2.5 eq), Na_2CO_3 (1.06 g, 10.0 mmol, 5.0 eq), and $Pd(PPh_3)_4$ (231 mg, 0.2 mmol, 5 mol%), and obtained as a yellow solid (966 mg, 92%). Spectroscopic data matched with those reported.[1]

4,6-Bis(naphthalen-2-yl)pyrimidine-2,5-diamine (1c)



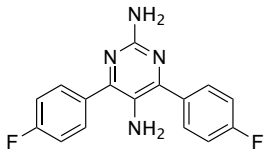
Prepared by GP-A from 2,5-diamino-4,6-dichloropyrimidine (143 mg, 0.8 mmol, 1.0 eq), naphthalen-2-ylboronic acid (172 mg, 2.0 mmol, 2.5 eq), Na_2CO_3 (106 mg, 2.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (46.1 mg, 0.04 mmol, 5 mol%), and obtained as a yellow solid (96.4 mg, 66%). **M.p.** 189–192 °C; **IR** (KBr) 3280, 3141, 2395, 2351, 2164, 1618, 1552, 1442, 1379, 1263 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.33 (s, 2H), 8.00 (d, J = 8.4 Hz, 2H), 7.93–7.95 (m, 2H), 7.89–7.91 (m, 4H), 7.55–7.56 (m, 4H), 4.76 (brs, 2H), 3.66 (brs, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 157.23, 154.14, 134.39, 133.62, 133.21, 128.83, 128.59, 128.33, 128.25, 127.87, 127.06, 126.66, 126.01.; **HRMS (ESI)** *m/z* calc'd for $C_{24}H_{19}N_4$ $[M+H]^+$: 363.1610, found: 363.1600.

4,6-Bis(3,5-methoxyphenyl)pyrimidine-2,5-diamine (1d)



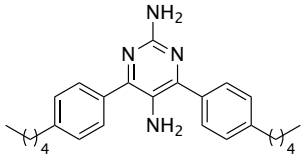
Prepared by GP-A from 2,5-diamino-4,6-dichloropyrimidine (71.6 mg, 0.4 mmol, 1.0 eq), 3,5-dimethoxyphenylboronic acid (182 mg, 1.0 mmol, 2.5 eq), Na_2CO_3 (53.0 mg, 1.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (23.0 mg, 0.02 mmol, 5 mol%), and obtained as a yellow solid (149 mg, 97%). Spectroscopic data matched with those reported.[1]

4,6-Bis(4-fluorophenyl)pyrimidine-2,5-diamine (1e)



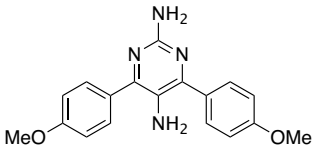
Prepared by GP-A from 2,5-diamino-4,6-dichloropyrimidine (143 mg, 0.8 mmol, 1.0 eq), 4-fluorophenylboronic acid (350 mg, 2.0 mmol, 2.5 eq), Na_2CO_3 (212 mg, 2.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (46.1 mg, 0.04 mmol, 5 mol%), and obtained as a yellow solid (261 mg, 81%). **M.p.** 226–227 °C; IR (KBr) 3475, 3296, 2289, 2092, 1595, 1501, 1432, 1374, 1209, 839 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.78–7.81 (m, 4H), 7.18–7.22 (m, 4H), 4.75 (brs, 2H), 3.44 (brs, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 163.44 (d, ^1J = 249.9 Hz), 156.90, 153.37, 132.89, 130.75 (d, ^3J = 8.6 Hz), 127.90, 116.11 (d, ^2J = 21.5 Hz). **^{19}F NMR** (471 MHz, $CDCl_3$) δ –110.72.; **HRMS** (ESI) *m/z* calc'd for $C_{16}H_{13}F_2N_4$ $[M+H]^+$: 299.1108, found: 299.1099.

4,6-Bis(4-pentylphenyl)pyrimidine-2,5-diamine (1f)



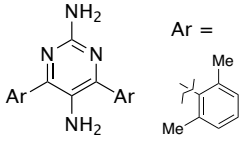
Prepared by GP-A from 2,5-diamino-4,6-dichloropyrimidine (71.6 mg, 0.4 mmol, 1.0 eq), 4-*n*-pentylphenylboronic acid (192 mg, 1.0 mmol, 2.5 eq), Na_2CO_3 (106 mg, 2.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (23.1 mg, 0.04 mmol, 5 mol%), and obtained as a yellow solid (157 mg, 97%). **M.p.** 69–70 °C; **IR** (KBr) 3306, 3196, 2921, 2856, 1610, 1547, 1442, 1372, 1206, 1117, 1175 cm^{-1}; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.68 (dd, J = 2.1, 10.2 Hz, 4H), 7.31 (d, J = 8.4 Hz, 4H), 4.71 (brs, 2H), 3.52 (brs, 2H), 2.66 (t, J = 7.2 Hz, 4H), 1.64 (dt, J = 7.5 Hz, 4H), 1.31–1.37 (m, 8H), 0.90 (t, J = 7.0 Hz, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 157.05, 154.02, 144.25, 134.35, 128.85, 128.39, 127.63, 35.80, 31.46, 31.04, 22.55, 14.05.; **HRMS (ESI)** *m/z* calc'd for $C_{26}H_{35}N_4$ $[M+H]^+$: 403.2862, found: 403.2852.

4,6-Bis(4-methoxyphenyl)pyrimidine-2,5-diamine (1g)



Prepared by GP-A from 2,5-diamino-4,6-dichloropyrimidine (143 mg, 0.8 mmol, 1.0 eq), 4-methoxyphenylboronic acid (303 mg, 2.0 mmol, 2.5 eq), Na_2CO_3 (212 mg, 2.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (46.1 mg, 0.04 mmol, 5 mol%), and obtained as a yellow solid (174 mg, 68%). **M.p.** 248–250 °C; **IR** (KBr) 3469, 3372, 3065, 2924, 1600, 1549, 1506, 1432, 1305, 1248, 1175, 1112 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 4H), 7.02 (d, J = 8.8 Hz, 4H), 4.65 (brs, 2H), 3.87 (s, 6H), 3.49 (brs, 2H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 160.55, 156.91, 153.78, 130.20, 129.53, 127.95, 114.37, 55.55.; **HRMS (ESI)** *m/z* calc'd for $C_{18}H_{19}N_4O_2$ $[M+H]^+$: 323.1508, found: 323.1510.

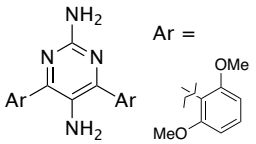
4,6-Bis(2,6-dimethylphenyl)pyrimidine-2,5-diamine (1h)



Prepared GP-A from 2,5-diamino-4,6-dichloropyrimidine (71.6 mg, 0.4 mmol, 1.0 eq), 2,6-dimethylphenylboronic acid (150 mg, 1.0 mmol, 2.5 eq), Na_2CO_3 (106 mg, 1.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (23.1 mg, 0.02 mmol, 5 mol%), and obtained as a pink solid (50.4 mg, 40%). **M.p.** 148–150 °C; **IR** (KBr) 3468, 3282, 3154, 2925, 2855, 2360, 1610, 1550, 1445, 1371, 1207, 1042, 1023 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.22 (t, J = 7.6 Hz, 2H), 7.14 (d, J = 7.6 Hz, 4H), 4.80 (brs,

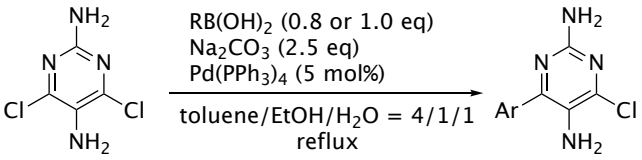
2H), 3.78 (s, 12H), 2.81 (brs, 2H).; **^{13}C NMR** (126 MHz, CD_3OD) δ 158.30, 156.10, 137.02, 136.28, 130.49, 130.08, 129.00, 19.53.; **HRMS (ESI)** *m/z* calc'd for $C_{20}H_{23}N_4$ $[M+H]^+$: 319.1923, found: 319.1916.

4,6-Bis(2,6-dimethoxyphenyl)pyrimidine-2,5-diamine (1i)



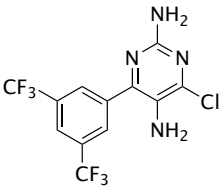
Prepared by GP-A from 2,5-diamino-4,6-dichloropyrimidine (143 mg, 0.8 mmol, 1.0 eq), 2,6-dimethoxyphenylboronic acid (183 mg, 2.0 mmol, 2.5 eq), Na_2CO_3 (106 mg, 2.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (46.1 mg, 0.04 mmol, 5 mol%), and obtained as an orange solid (115 mg, 75%). **M.p.** 249–252 °C; **IR** (KBr) 3467, 3281, 3153, 2924, 2855, 1611, 1550, 1446, 1371, 1207, 1041, 1023 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 (t, J = 8.4 Hz, 2H), 6.65 (d, J = 8.4 Hz, 4H), 4.72 (brs, 2H), 3.01 (brs, 2H), 2.16 (s, 12H); **^{13}C NMR** (126 MHz, CD_3Cl_3) δ 158.12, 156.84, 150.98, 131.70, 130.65, 114.26, 104.67, 56.25; **HRMS (ESI)** *m/z* calc'd for $C_{20}H_{23}N_4O_4$ $[M+H]^+$: 383.1719, found: 383.1707.

2-2. Synthesis of C4N4-Cl compounds



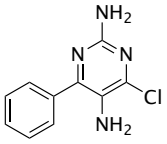
GP-B: To a suspension of 2,5-diamino-4,6-dichloropyrimidine (1.0 eq) in toluene (0.1 M), EtOH (0.4 M) and H_2O (0.4 M) were added boronic acid (0.8 or 1.0 eq), Na_2CO_3 (2.5 eq), $Pd(PPh_3)_4$ (5 mol%). The resulting solution was stirred under reflux for 24 h, and cooled to room temperature. After the addition of H_2O, aqueous phase was extracted with dichloromethane (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. Volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc).

4-Chloro-6-(3,5-bis(trifluoromethyl)phenyl)pyrimidine-2,5-diamine (1a')



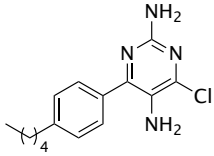
Prepared by GP-C from 2,5-diamino-4,6-dichloropyrimidine (1.79 g, 10.0 mmol, 1.0 eq), 3,5-bis(trifluoromethyl)phenylboronic acid (2.58 g, 10.0 mmol, 1.0 eq), Na_2CO_3 (2.65 g, 25.0 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (578 mg, 0.50 mmol, 5.0 mol%), and obtained as a pink solid (523 mg, 15%). **M.p.** 121–122 °C; **IR** (KBr) 3324, 3197, 1641, 1475, 1346, 1273, 1124 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.30 (s, 2H), 7.96 (s, 1H), 4.84 (brs, 2H), 3.66 (brs, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 156.09, 150.07, 149.99, 138.64, 132.47 (q, ^2J = 33.7 Hz), 128.91 (q, ^3J = 3.7 Hz), 123.45 (sept, ^3J = 3.8 Hz), 123.19 (q, ^1J = 272.9 Hz). **^{19}F NMR** (471 MHz, $CDCl_3$) δ –62.89; **HRMS (ESI)** *m/z* calc'd for $C_{12}H_6ClF_6N_4$ $[M–H]^-$: 355.0185, found: 355.0180.

4-Chloro-6-phenylpyrimidine-2,5-diamine (1b')



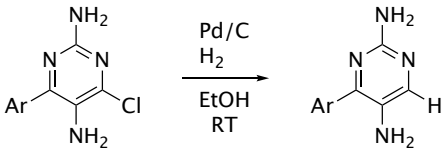
Prepared by GP-C from 2,5-diamino-4,6-dichloropyrimidine (17.9 mg, 0.10 mmol, 1.0 eq), phenylboronic acid (12.2 mg, 0.10 mmol, 1.0 eq), Na_2CO_3 (26.5 mg, 0.25 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (5.8 mg, 5.0 μmol, 5 mol%), and obtained as an yellow solid (11.8 mg, 53%). Spectroscopic data matched with those reported.[1]

4-Chloro-6-(4-*n*-pentylphenyl)pyrimidine-2,5-diamine (1n')



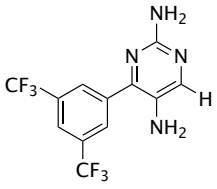
Prepared by GP-C from 2,5-diamino-4,6-dichloropyrimidine (268 mg, 1.50 mmol, 1.5 eq), 4-*n*-pentylphenylboronic acid (192 mg, 1.00 mmol, 1.0 eq), Na_2CO_3 (266 mg, 2.50 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (57.8 mg, 0.05 mmol, 5.0 mol%), and obtained as an orange solid (161 mg, 37%). **M.p.** 65–66 °C; **IR** (KBr) 3295, 3170, 2928, 2360, 1611, 1451, 1352, 1208, 1135, 1053, 839 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.62 (dd, J = 2.0, 6.5 Hz, 2H), 7.30 (d, J = 6.5 Hz, 2H), 4.71 (brs, 2H), 3.70 (brs, 2H), 2.66 (t, J = 7.5 Hz, 2H), 1.64 (m, J = 7.5 Hz, 2H), 1.31–1.34 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.87, 154.14, 147.96, 144.98, 133.59, 129.03, 128.14, 127.16, 35.85, 31.49, 31.04, 22.58, 14.09.; **HRMS (ESI)** *m/z* calc'd for $C_{15}H_{20}ClN_4$ $[M+H]^+$: 291.1377, found: 291.1365.

2-3. Synthesis of C4N4-H compounds



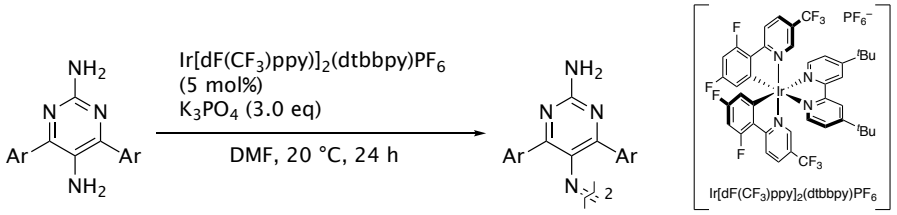
GP-C: To a solution of C4N4-Cl (1.0 eq) in EtOH (0.1 M) were added 10% Pd/C and NaOAc (2.0 eq). The suspension was stirred under a hydrogen atmosphere for 16 h at room temperature. After N_2 was flushed into the flask, the mixture was filtered through a pad of celite®, washed with DCM, and volatiles were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc).

4-(3,5-Bis(trifluoromethyl)phenyl)pyrimidine-2,5-diamine (1k)



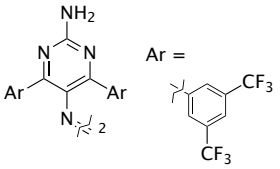
Prepared by GP-D from **3a** (214mg, 0.60 mmol), 10% Pd/C (10.8 mg) and NaOAc (98.4 mg, 1.20 mmol, 2.0 eq) and obtained as a yellow solid (148 mg, 69%). **M.p.** 220–223 °C; **IR** (KBr) 3410, 3189, 2352, 1612, 1461, 1276, 1118 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.34 (m, 2H), 8.06 (s, 1H), 7.94 (s, 1H), 4.87 (brs, 2H), 3.03 (brs, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 158.08, 149.23, 149.08, 139.06, 135.88, 132.31 (q, ^2J = 33.6 Hz), 130.16, 128.95, 128.88 (q, ^3J = 3.7 Hz), 128.17, 123.06 31 (sept, ^3J = 3.8 Hz), 122.61 (q, ^1J = 272.8 Hz). **^{19}F NMR** (471 MHz, $CDCl_3$) δ –62.87; **HRMS (ESI)** *m/z* calc'd for $C_{12}H_9F_6N_4$ $[M+H]^+$: 323.0731, found: 323.0719.0

2-4. Synthesis of Azo-C4N4 compounds



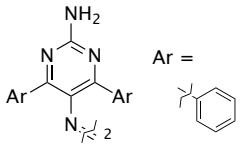
GP- D: To a cloudy solution of C4N4 (1.0 eq), K_3PO_4 (3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5 mol%) in DMF was stirred at 20 °C with a LED light (PER-AMP, Techno Sigma, 448 nm). The reaction was filtered through a pad of celite® and the residue was rinsed with CH_2Cl_2 (50–100 mL) to dissolve azo products. Volatiles in filtrates were removed under reduced pressure and the resulting residue was triturated with CH_2Cl_2 (ca. 1 mL). The resulting suspension was filtered to collect *E*-isomer. Glasswares were rinsed with CH_2Cl_2/MeOH = 1/1 (10–20 mL), and volatiles were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc).

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-bis(trifluoromethyl)phenyl)pyrimidine-2-amine) (2a)



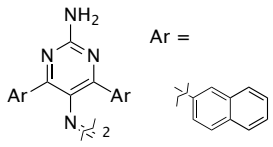
Prepared by GP-B from C4N4 **1a** (213 mg, 0.2 mmol, 1.0 eq), K_3PO_4 (255 mg, 0.6 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (22.4 mg, 10.0 μmol, 5 mol%), and obtained as an orange solid (18.3 mg, 9%). **M.p.** 285–287 °C; **IR** (KBr) 3421, 2930, 2868, 1603, 1532, 1463, 1355, 1277, 1121, 1056, 1023 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.72 (d, J = 1.6 Hz, 8H), 7.68 (t, J = 1.6 Hz, 4H), 5.60 (brs, 4H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 161.37, 159.84, 139.80, 134.27, 130.00 (d, ^3J = 1.3 Hz), 129.45 (q, ^2J = 33.2 Hz), 122.91 (q, ^1J = 272.8 Hz), 122.26 (d, ^3J = 1.9 Hz). **^{19}F NMR** (471 MHz, $CDCl_3$) δ –62.95; **HRMS (ESI)** *m/z* calc'd for $C_{40}H_{15}F_{24}N_8$ $[M–H]^-$: 1063.1036, found: 1063.1056.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-bisphenyl)pyrimidine-2-amine) (2b)



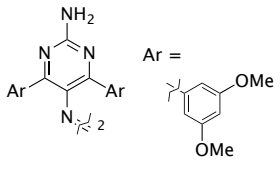
Prepared by GP-B with a slight modification from C4N4 **1b** (13.1 mg, 0.05 mmol, 1.0 eq), K_3PO_4 (31.8 mg, 0.15 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (2.8 mg, 2.5 μmol, 5 mol%), and obtained as a red solid (11.1 mg, 85%). **M.p.** 289–290 °C; **IR** (KBr) 3299, 3150, 2922, 1624, 1512, 1462, 1405, 1232 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.30–7.26 (m, 12H), 7.11–7.13 (m, 8H), 5.25 (brs, 4H); **^{13}C NMR** (126 MHz, DMSO-d_6) 162.20, 161.47, 138.05, 135.11, 129.19, 128.72, 127.64. **HRMS (ESI)** *m/z* calc'd for $C_{32}H_{25}N_8$ $[M+H]^+$: 521.2202, found: 521.2206.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(naphthalen-2-yl)pyrimidine-2-amine) (2c)



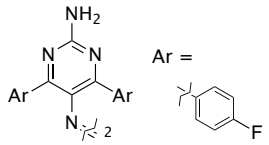
Prepared by GP-B from C4N4 **1c** (18.1 mg, 0.05 mmol, 1.0 eq), K_3PO_4 (31.8 mg, 0.15 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (2.8 mg, 2.5 μmol, 5 mol%), and obtained as an orange solid (9.5 mg, 53%). **M.p.** 269–271 °C; **IR** (KBr) 3307, 3187, 2926, 1616, 1513, 1454, 1034 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.82 (s, 4H), 7.79 (d, J = 8.0 Hz, 4H), 7.67 (d, J = 8.0 Hz, 4H), 7.52–7.45 (m, 12H), 7.07 (d, J = 1.5, 8.5 Hz, 4H), 5.40 (brs, 4H).; **^{13}C NMR** (126 MHz, DMSO-d_6) (162.37, 161.71, 135.84, 135.70, 133.45, 133.00, 132.54, 128.61, 128.47, 127.80, 127.15, 126.78, 126.68.; **HRMS (ESI)** *m/z* calc'd for $C_{48}H_{33}N_8$ $[M+H]^+$: 721.2828, found: 721.2833.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-dimethoxyphenyl)pyrimidine-2-amine) (2d)



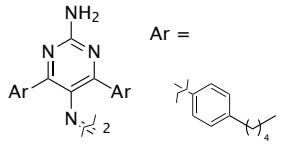
Prepared by GP-B from C4N4 **1d** (38.2 mg, 0.10 mmol, 1.0 eq), K_3PO_4 (63.7 mg, 0.30 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5.6 mg, 5.0 μmol, 5 mol%), and obtained as an orange solid (22.6 mg, 59%). **M.p.** >300 °C (no change); **IR** (KBr) 3347, 3196, 2923, 2856, 1604, 1526, 1447, 1369, 1286, 1201, 1149 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.43 (d, J = 2.3 Hz, 8H), 6.32 (t, J = 2.3 Hz, 4H), 5.26 (brs, 4H), 3.71 (s, 24H); **^{13}C NMR** (151 MHz, DMSO-d_6) 161.31, 161.25, 159.41, 139.28, 134.97, 107.51, 100.34, 55.04.; **HRMS (ESI)** *m/z* calc'd for $C_{40}H_{41}N_8O_8$ $[M+H]^+$: 761.3047, found: 761.3047.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(4-fluorophenyl)pyrimidine-2-amine) (2e)



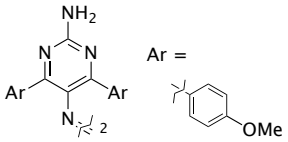
Prepared by GP-B from C4N4 **1e** (29.8 mg, 0.10 mmol, 1.0 eq), K_3PO_4 (63.6 mg, 0.30 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5.6 mg, 5.0 µmol, 5 mol%), and obtained as a red solid (13.6 mg, 46%). **M.p.** 285–287 °C; **IR** (KBr) 3473, 3302, 1595, 1501, 1429, 1371, 1210 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.24–7.21 (m, 8H), 6.98 (t, J = 5.4 Hz, 8H), 5.30 (brs, 4H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 162.29 (d, ^1J = 283.3 Hz), 161.42 (d, ^3J = 8.6 Hz), 134.85, 134.34, 134.32, 131.37 (d, ^3J = 8.3 Hz), 114.64 (d, ^2J = 21.7 Hz).; **^{19}F NMR** (471 MHz, DMSO-d_6) δ –112.37.; **HRMS (ESI)** *m/z* calc'd for $C_{32}H_{21}F_4N_8$ $[M+H]^+$: 593.1825, found: 593.1840

5,5'-(Diazene-1,2-diyl)bis(4,6-bis(4-pentylphenyl)pyrimidine-2-amine) (2f)



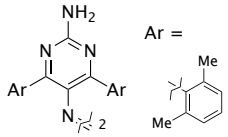
Prepared by GP-B from C4N4 **1f** (40.3 mg, 0.10 mmol, 1.0 eq), K_3PO_4 (63.6 mg, 0.30 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5.6 mg, 5.0 µmol, 5 mol%), and obtained as an orange solid (18.0 mg, 45%). **M.p.** 269–271 °C; **IR** (KBr) 3291, 3167, 2924, 2855, 1613, 1507, 1447, 1216, 1056, 1022, 840, 790 cm^{-1}; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.34 (s, 16H), 5.43 (brs, 4H), 2.72–2.74 (m, 8H), 1.72–1.74 (m, 8H), 1.40–1.43 (m, 16H), 0.95–0.99 (m, 12H).; **^{13}C NMR** (126 MHz, $CDCl_3$) 163.04, 160.95, 144.02, 136.75, 135.61, 129.43, 127.98, 35.94, 31.62, 31.19, 22.68, 14.17.; **HRMS (ESI)** *m/z* calc'd for $C_{52}H_{65}N_8$ $[M+H]^+$: 801.5332, found: 801.5329.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(4-methoxyphenyl)pyrimidine-2-amine) (2g)



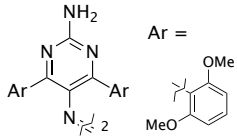
Prepared by GP-B from C4N4 **1g** (32.2 mg, 0.10 mmol, 1.0 eq), K_3PO_4 (63.7 mg, 0.30 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5.6 mg, 5.0 µmol, 5 mol%), and obtained as a red solid (23.1 mg, 72%). **M.p.** 269–271 °C; **IR** (KBr) 3463, 3357, 1605, 1500, 1239, 1173, 1026 cm^{-1}; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.75 (m, 8H), 7.02 (m, 8H), 4.65 (brs, 4H), 3.87 (s, 12H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 161.34, 161.26, 159.94, 135.25, 131.06, 130.39, 113.01, 55.24.; **HRMS (ESI)** *m/z* calc'd for $C_{36}H_{33}N_8O_4$ $[M+H]^+$: 641.2625, found: 641.2627.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(2,6-dimethylphenyl)pyrimidine-2-amine) (2h)



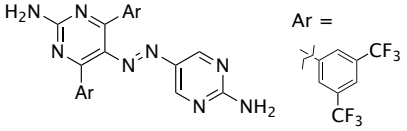
Prepared by GP-B from C4N4 **1h** (25.5 mg, 0.08 mmol, 1.0 eq), K_3PO_4 (50.9 mg, 0.24 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (4.5 mg, 4.0 µmol, 5 mol%), and obtained as an orange solid (9.0 mg, 35%). **M.p.** >300 °C (no change); **IR** (KBr) 3271, 3147, 1766, 1609, 1527, 1431, 1196, 1033, 769 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.12 (t, J = 7.5 Hz, 4H), 6.94 (d, J = 7.5 Hz, 8H), 5.27 (brs, 4H), 1.60 (s, 24H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 162.37, 161.71, 135.84, 135.70, 133.45, 133.00, 132.54, 128.61, 128.47, 127.80, 127.15, 126.78, 126.68.; **HRMS (ESI)** *m/z* calc'd for $C_{40}H_{41}N_8$ $[M+H]^+$: 633.3454, found: 633.3474.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(2,6-dimethoxyphenyl)pyrimidine-2-amine) (2i)



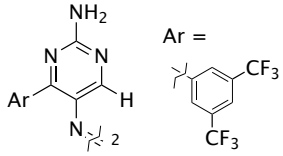
Prepared by GP-B from C4N4 **1i** (38.2 mg, 0.10 mmol, 1.0 eq), K_3PO_4 (63.6 mg, 0.30 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5.6 mg, 5.0 µmol, 5 mol%), and obtained as an orange solid (3.5 mg, 9%). **M.p.** 269–271 °C; **IR** (KBr) 3451, 3271, 2929, 2836, 2298, 2109, 1591, 1462, 1247, 1102, 1024 cm^{-1}; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 7.14 (t, J = 8.3 Hz, 4H), 6.69 (brs, 4H), 6.40 (d, J = 8.4 Hz, 8H), 3.41 (s, 24H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 161.16, 157.74, 156.79, 138.80, 128.23, 118.00, 104.08, 55.36.; **HRMS (ESI)** *m/z* calc'd for $C_{40}H_{41}N_8O_8$ $[M+H]^+$: 761.3047, found: 761.3028.

5-((2-Aminopyrimidin-5-yl)diazenyl)-4,6-bis(3,5-bis(trifluoromsethyl)phenyl)pyrimidin-2-amine (2j)



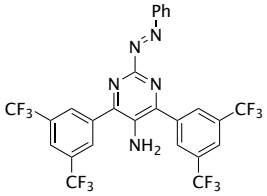
Prepared by GP-B with a slight modification from **1a** (53.4 mg, 0.10 mmol, 2.0 eq), 2,5-diaminopyrimidine (5.5 mg, 0.05 mmol, 1.0 eq), K_3PO_4 (31.8 mg, 0.15 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (2.8 mg, 2.5 µmol, 5 mol%), and obtained as an orange solid (2.5 mg, 8%). **M.p.** 227–230 °C; **IR** (KBr) 3491, 3299, 3162, 2922, 2108, 1631, 1542, 1470, 1356, 1274, 1120 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.31 (s, 2H), 8.12 (s, 4H), 7.94 (s, 2H), 5.54 (brs, 2H), 5.47 (brs, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 163.85, 161.35, 159.73, 153.60, 151.76, 139.32, 138.89, 136.69, 131.57 (q, ^2J = 23.7 Hz), 130.40 (q, ^3J = 3.0 Hz), 123.26 (q, ^1J = 272.7 Hz), 123.06 (q, ^3J = 3.2 Hz). **^{19}F NMR** (471 MHz, $CDCl_3$) δ –62.83.; **HRMS (ESI)** *m/z* calc'd for $C_{24}H_{11}F_{12}N_8$ $[M–H]^–$: 639.0915, found: 639.0938.

5,5'-(Diazene-1,2-diyl)bis(4-(3,5-bis(trifluoromethyl)phenyl)pyrimidin-2-amine) (2k)



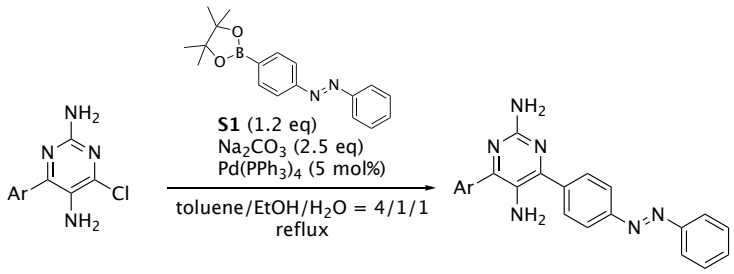
Prepared by GP-B from **1k** (80.6 mg, 0.25 mmol, 1.0 eq), K_3PO_4 (158 mg, 0.75 mmol, 3.0 eq), and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (14.0 mg, 12.5 µmol, 5 mol%), and obtained as an orange solid (43.2 mg, 54%). **M.p.** 268–270 °C; **IR** (KBr) 3318, 3173, 2171, 1594, 1326, 1274, 1173, 1125 cm^{-1}; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.63 (s, 4H), 8.57 (s, 2H), 8.31 (s, 2H), 7.78 (brs, 4H).; **^{13}C NMR** δ (126 MHz, $CDCl_3$) 175.04, 162.93, 162.84, 140.51, 131.34 (q, ^2J = 33.6 Hz), 129.92, 129.36 (q, ^3J = 3.8 Hz), 124.48 (q, ^3J = 3.8 Hz), 123.53 (q, ^1J = 272.6 Hz). **^{19}F NMR** (376 MHz, $CDCl_3$) δ –62.77; **HRMS (ESI)** *m/z* calc'd for $C_{24}H_{11}F_{12}N_8$ $[M–H]^–$: 639.0915, found: 639.0898.

5,5'-(Diazene-1,2-diyl)bis(4-(3,5-bis(4-pentylphenyl)pyrimidin-2-amine) (2l)



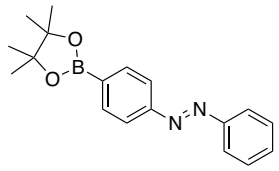
To a solution of **1a** (53.4 mg, 0.10 mmol, 1.0 eq) in acetic acid (200 µL) were added nitrosobenzene (12.9 mg, 0.12 mmol, 1.2 eq). The resulting solution was stirred under 60 °C for 3 days, and cooled to room temperature. Volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc), affording **2k** (22.7 mg, 36%) as an orange solid. **M.p.** 144–146 °C; **IR** (KBr) 3307, 3202, 2931, 1626, 1534, 1356, 1276, 1173, 1116, 898, 677 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.15 (s, 4H), 7.93 (s, 2H), 7.26–7.43 (m, 5H), 5.54 (brs, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 162.14, 159.77, 151.47, 140.37, 134.08, 131.22, 130.57 (q, ^3J = 3.0 Hz), 129.74 (q, ^2J = 23.1 Hz), 129.21, 129.01, 123.27 (q, ^1J = 272.9 Hz), 122.56, 121.62, 119.43. **^{19}F NMR** (471 MHz, $CDCl_3$) δ –62.88.; **HRMS (ESI)** *m/z* calc'd for $C_{26}H_{14}F_{12}N_5$ $[M+H]^+$: 624.1058, found: 624.1058.

2-5. Synthesis of C4N4 with an azobenzene unit



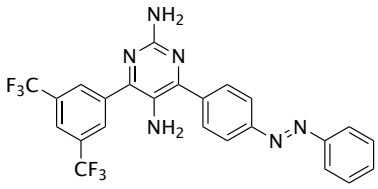
GP-E: To a suspension of C4N4-Cl (1.0 eq) in toluene (0.1 M), EtOH (0.4 M) and H_2O (0.4 M) were added pinacol boronate **S1** (1.2 eq), Na_2CO_3 (2.5 eq), $Pd(PPh_3)_4$ (5 mol%). The resulting solution was stirred under reflux for 24 h, and cooled to room temperature. After the addition of H_2O, aqueous phase was extracted with dichloromethane (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. Volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc).

1-Phenyl-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (S1)



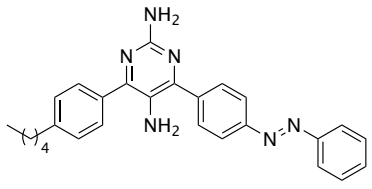
Prepared by following the reported procedure.[2] To a suspension of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (219 mg, 1.0 mmol), nitrosobenzene (214 mg, 2.0 mmol, 2.0 eq) were added acetic acid (16 mL). The solution was stirred at room temperature for 5 h. After the addition of H_2O, aqueous phase was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. Volatiles were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc), and isolated as an orange solid (189 mg, 61%). Spectroscopic data matched with those reported.[2]

4-(3,5-Bis(trifluoromethyl)phenyl)-6-(4-(phenyldiazenyl)phenyl)pyrimidine-2,5-diamine (2m)



Prepared by GP-E with a slight modification from **2a** (53.5 mg, 0.15 mmol, 1.0 eq), pinacol boronate **S1** (92.5 mg, 0.30 mmol, 2.0 eq), Na_2CO_3 (39.8 mg, 0.38 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (8.7 mg, 7.5 μmol, 5.0 mol%), and obtained as an orange solid (61.4 mg, 81%). **M.p.** 220–223 °C; **IR** (KBr) 3466, 3357, 2927, 1600, 1513, 1450, 1350, 1174, 1123, 832, 671 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.38 (s, 2H), 8.07 (d, J = 8.5 Hz, 2H), 7.94–7.97 (m, 5H), 7.50–7.57 (m, 3H), 4.80 (brs, 2H), 3.52 (brs, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 157.31, 154.94, 153.14, 152.69, 150.68, 139.25, 138.69, 132.33 (q, ^2J = 33.4 Hz), 131.62, 129.61, 129.29, 129.23 (q, ^3J = 4.1 Hz), 129.04, 128.07, 123.50, 123.29 (q, ^1J = 273.0 Hz), 123.18, 123.13 (q, ^3J = 3.7 Hz). **^{19}F NMR** (471 MHz, $CDCl_3$) δ –62.82.; **HRMS (ESI)** *m/z* calc'd for $C_{24}H_{15}F_6N_6$ $[M–H]^-$: 501.1262, found: 501.1265.

4-(4-*n*-Pentylphenyl)-6-(4-(phenyldiazenyl)phenyl)pyrimidine-2,5-diamine (2n)



Prepared by GP-E from **1n′** (116 mg, 0.40 mmol, 1.0 eq), pinacol boronate **S1** (148 mg, 0.48 mmol, 1.2 eq), Na_2CO_3 (106 mg, 1.00 mmol, 2.5 eq), and $Pd(PPh_3)_4$ (23.1 mg, 0.02 mmol, 5.0 mol%), and obtained as an orange solid (115 mg, 66%). **M.p.** 39–41 °C; **IR** (KBr) 3298, 3174, 2924, 2857, 2290, 2086, 1548, 1439, 1374, 1209 849 763 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.05 (d, J = 8.5 Hz, 2H), 7.97 (t, J = 8.3 Hz, 2H), 7.69 (d, J = 8.1 Hz, 2H), 7.94–7.97 (m, 5H), 7.33 (d, J = 8.3 Hz, 2H), 4.76 (brs, 2H), 3.57 (brs, 2H), 2.67 (t, J = 7.6 Hz, 2H), 1.64-1.67 (m, 2H), 1.33-1.35 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) 156.73, 152.97, 152.75, 144.89, 139.34, 133.88, 131.49, 129.67, 129.28, 129.17, 128.51, 128.17, 123.41, 123.15, 35.94, 31.56, 31.15, 22.67, 14.17.; **HRMS (ESI)** *m/z* calc'd for $C_{27}H_{29}N_6$ $[M+H]^+$: 437.2454, found: 437.2442.

3. Condition Screening of Azo Formation

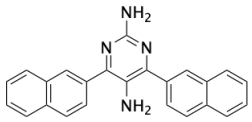
Supplementary Table 1. Transformation of **1b** to **2b**.

Entry	Base	Solvent	Temperature (°C)	Yield
1	K_3PO_4	MeCN	–20	44%
2	K_3PO_4	MeCN	0	41%[b]
3	K_3PO_4	MeCN	20	61%[b]
4	K_3PO_4	Toluene	20	34%
5	K_3PO_4	DMF	20	82%[b]
6	NaO^{t}Bu	DMF	20	-

[a] **1b** (0.25 mM) was stirred in the presence of base (3.0 eq) and Ir[dF(CF_3)ppy)]$_2$(dtbbpy)PF_6 (5.0 mol%) for 24 h.
[b] ^{1}H NMR yields were shown using 1,1,2,2-tetrachloroethane as an internal standard.

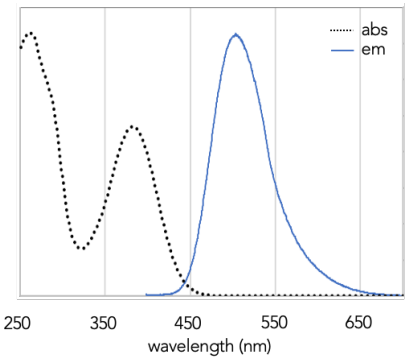
4. Absorption/Emission Spectra

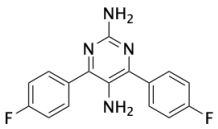
All spectra were recorded using 0.2 mM solution for absorption, and 0.02 mM for emission in $CHCl_3$.



1c

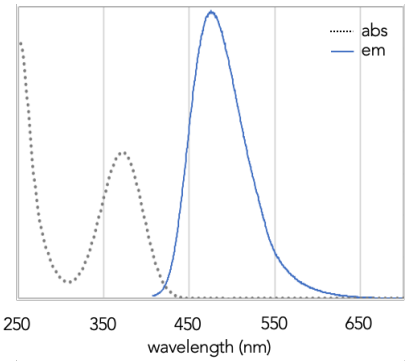
λ_{abs}: 382 (nm)
λ_{em}: 504 (nm)
φ_F = 0.43

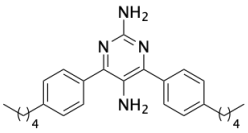




1e

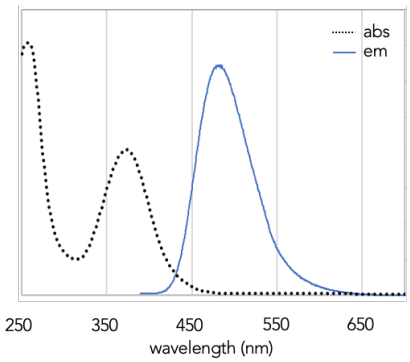
λ_{abs}: 372 (nm)
λ_{em}: 476 (nm)
φ_F = 0.55

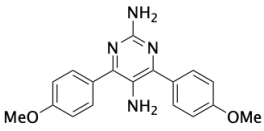




1f

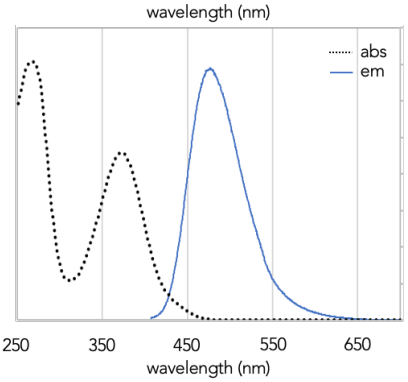
λ_{abs}: 372 (nm)
λ_{em}: 480 (nm)
φ_F = 0.55

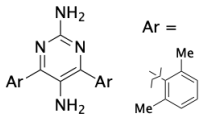




1g

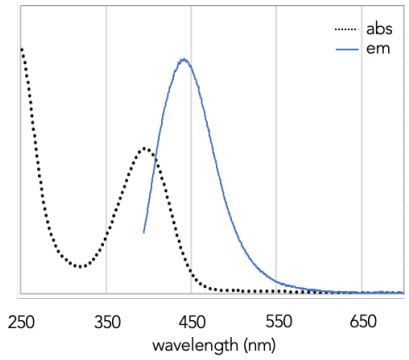
λ_{abs}: 372 (nm)
λ_{em}: 477 (nm)
φ_F = 0.50

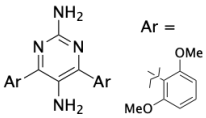




1h

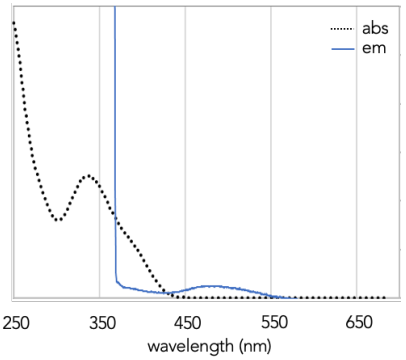
λ_{abs}: 390 (nm)
λ_{em}: 442 (nm)
φ_F = 0.55

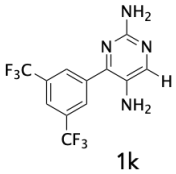




1i

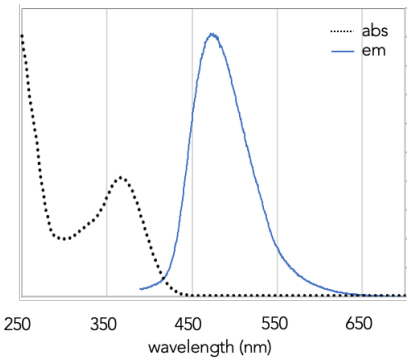
λ_{abs}: 337 (nm)
λ_{em}: 482 (nm)
φ_F = 0.02

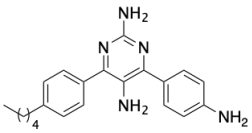




1k

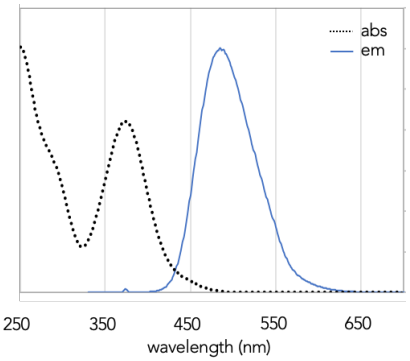
λ_{abs}: 369 (nm)
λ_{em}: 472 (nm)
φ_F = 0.50





2n-reduced

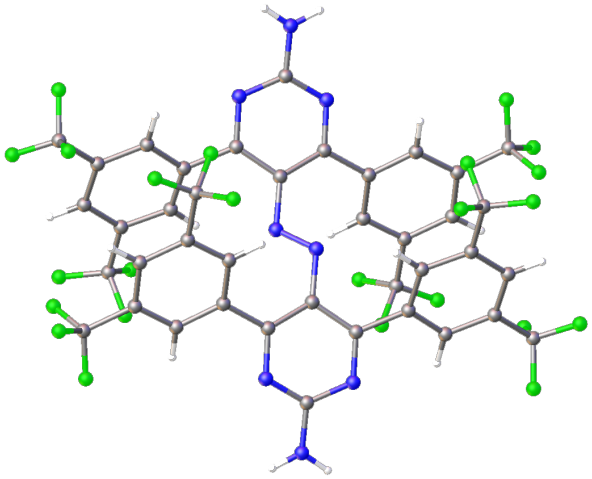
λ_{abs}: 380 (nm)
λ_{em}: 491 (nm)
φ_F = 0.26



5. X-ray Crystallographic Analysis

5-1. Crystal structure of **2a**

Single crystals of **2a** were obtained by slow evaporation from solution in dichloromethane at room temperature. A suitable crystal was selected and the sample was measured on a Rigaku R-AXIS RAPIS II diffractometer using multilayer mirror monochromated Cu-Kα radiation (λ = 1.54184 nm). The data were collected at 93.15 K. Refined structure and crystallographic parameters are summarized in Supplementary Table 1 and Supplementary Fig 1. CCDC 2234930 contains the supplementary crystallographic data.



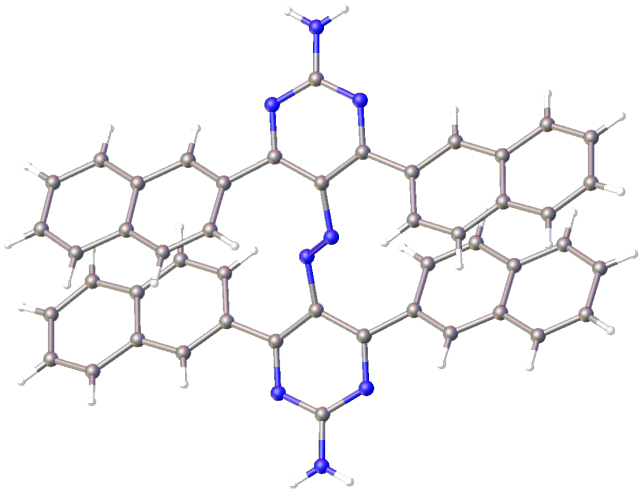
Supplementary Fig 1. ORTEP diagram of **2a**
Ellipsoids are set at 50% probability.

Supplementary Table 2. Selected crystal data of **2a**

Empirical Formula	$C_{40}H_{16}F_{24}N_8$
Formula Weight	1064.59
Crystal Dimensions	0.3 × 0.2 × 0.2 mm
Crystal System	monoclinic
Space group	$P2_1/n$
Lattice Parameters	
a	7.93703(15) Å
b	15.3896(3) Å
c	16.2323(3) Å
V	1942.33(8) Å^3
α	90 °
β	101.588(7) °
γ	90 °
Z value	4
R_1	0.0547
wR_2	0.1548
D_{calc}	1.820 g/cm^3
F_{000}	1056.0

5-2. Crystal structure of **2c**

Single crystals of **2c** were obtained by slow evaporation from solution in dichloromethane at room temperature. A suitable crystal was selected and the sample was measured on a Rigaku R-AXIS RAPIS II diffractometer using multilayer mirror monochromated Cu-Kα radiation (λ = 1.54184 nm). The data were collected at 93.15 K. Refined structure and crystallographic parameters are summarized in Supplementary Table 2 and Supplementary Fig 2. CCDC 2234929 contains the supplementary crystallographic data.



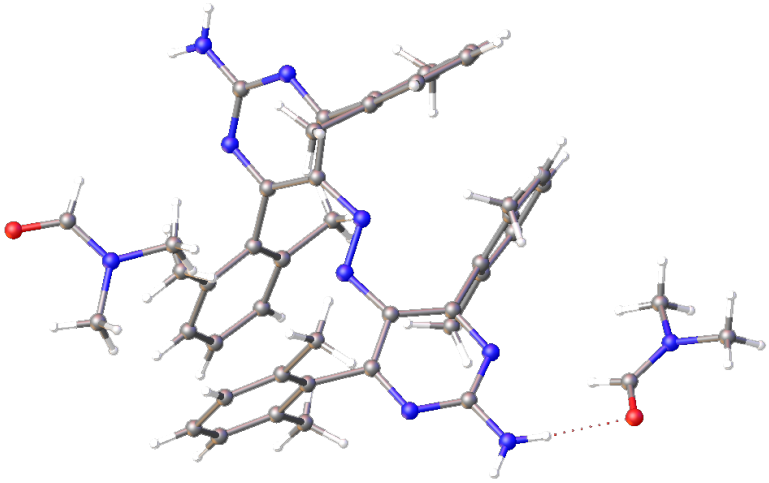
Supplementary Fig 2. ORTEP diagram of **2c**
Ellipsoids are set at 50% probability.

Supplementary Table 3. Selected crystal data of **2c**

Empirical Formula	$C_{48}H_{32}N_8$
Formula Weight	720.84
Crystal Dimensions	0.2 × 0.1 × 0.02 mm
Crystal System	orthorhombic
Space group	$Pna2_1$
Lattice Parameters	
a	22.7717(3) Å
b	10.3216(2) Å
c	30.7588(5) Å
V	7229.6(2) Å^3
Z value	4
R_1	0.0453
wR_2	0.1211
D_{calc}	1.324 g/cm^3
F_{000}	3008.0

5-3. Crystal structure of **2h**

Single crystals of **2h** were obtained by slow evaporation from solution in DMF at room temperature. A suitable crystal was selected and the sample was measured on a Rigaku R-AXIS RAPIS II diffractometer using multilayer mirror monochromated Cu-Kα radiation (λ = 1.54184 nm). The data were collected at 93.15 K. Refined structure and crystallographic parameters are summarized in Supplementary Table 3 and Supplementary Fig 3. CCDC 2234933 contains the supplementary crystallographic data.



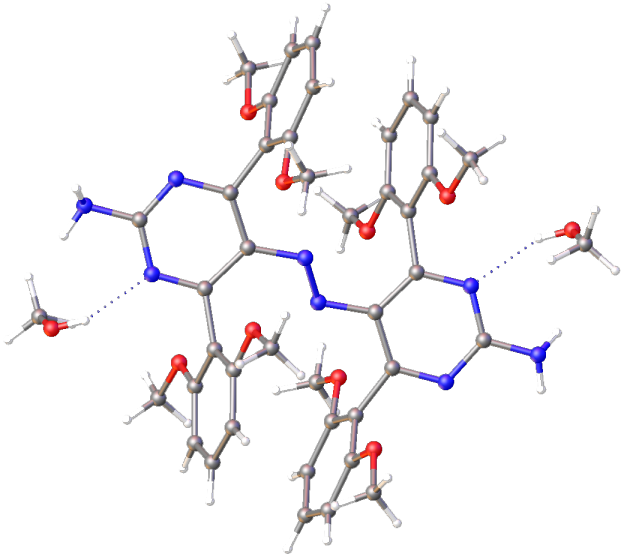
Supplementary Fig 3. ORTEP diagram of **2h**
Ellipsoids are set at 50% probability.

Supplementary Table 4. Selected crystal data of **2h**

Empirical Formula	$C_{40}H_{40}N_8$
Formula Weight	632.82
Crystal Dimensions	0.2 × 0.08 × 0.03 mm
Crystal System	monoclinic
Space group	$P2_1/n$
Lattice Parameters	
a	11.83720(10) Å
b	23.7847(2) Å
c	15.47280(10) Å
V	4250.65(6) Å^3
α	90 °
β	102.6430(10) °
γ	90 °
Z value	4
R_1	0.0483
wR_2	0.1345
D_{calc}	1.217 g/cm^3
F_{000}	1664.0

5-4. Crystal structure of **2i**

Single crystals of **2i** were obtained by slow evaporation from solution in Methanol at room temperature. A suitable crystal was selected and the sample was measured on a Rigaku R-AXIS RAPIS II diffractometer using multilayer mirror monochromated Cu-Kα radiation (λ = 1.54184 nm). The data were collected at 93.15 K. Refined structure and crystallographic parameters are summarized in Supplementary Table 4 and Supplementary Fig 4. CCDC 2234931 contains the supplementary crystallographic data.



Supplementary Fig 4. ORTEP diagram of **2i**
Ellipsoids are set at 50% probability.

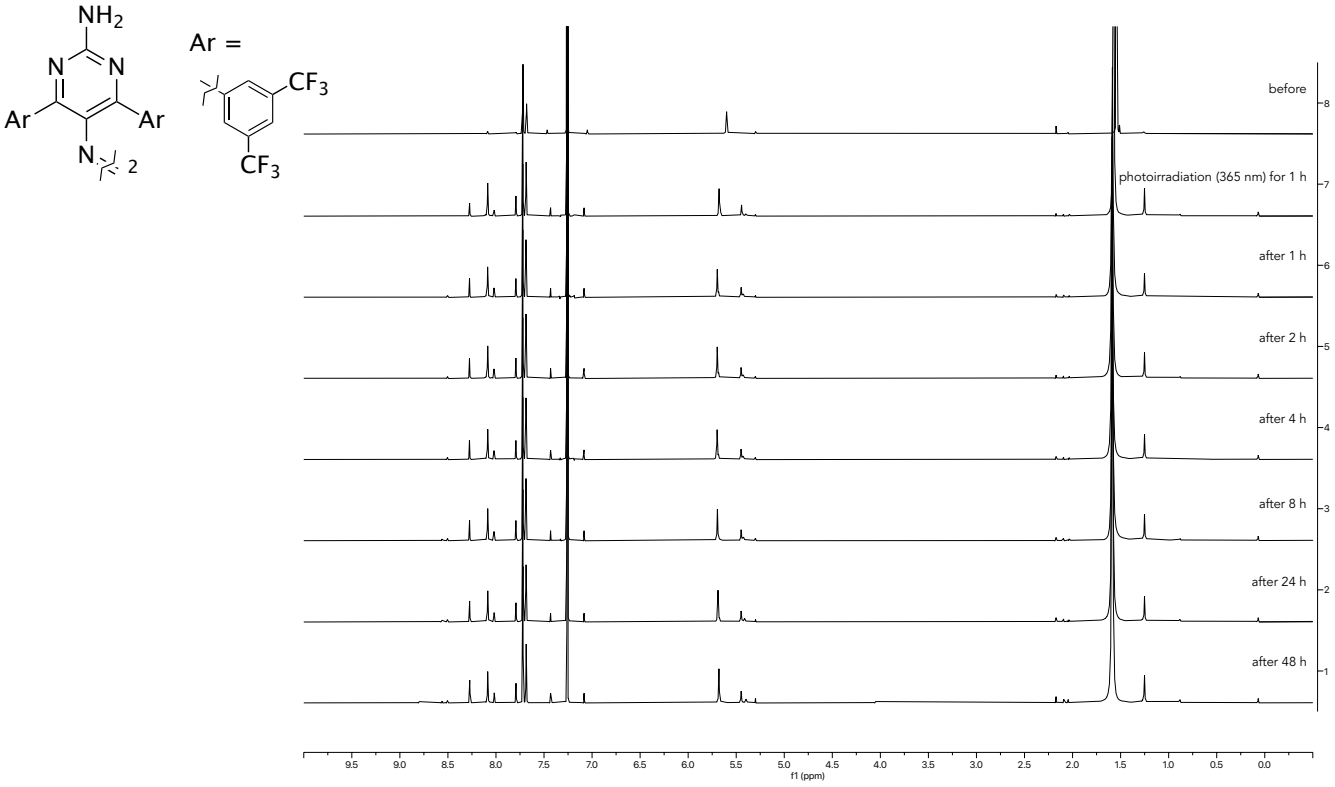
Supplementary Table 5. Selected crystal data of **2i**

Empirical Formula	$C_{40}H_{40}N_8O_8$
Formula Weight	760.81
Crystal Dimensions	0.1 × 0.1 × 0.02 mm
Crystal System	monoclinic
Space group	$P2_1$
Lattice Parameters	
a	9.1606(2) Å
b	24.5485(6) Å
c	9.1691(2) Å
V	2029.14(8) Å^3
α	90 °
β	100.233(2) °
γ	90 °
Z value	1
R_1	0.1602
wR_2	0.4890
D_{calc}	1.350 g/cm^3
F_{000}	872.0

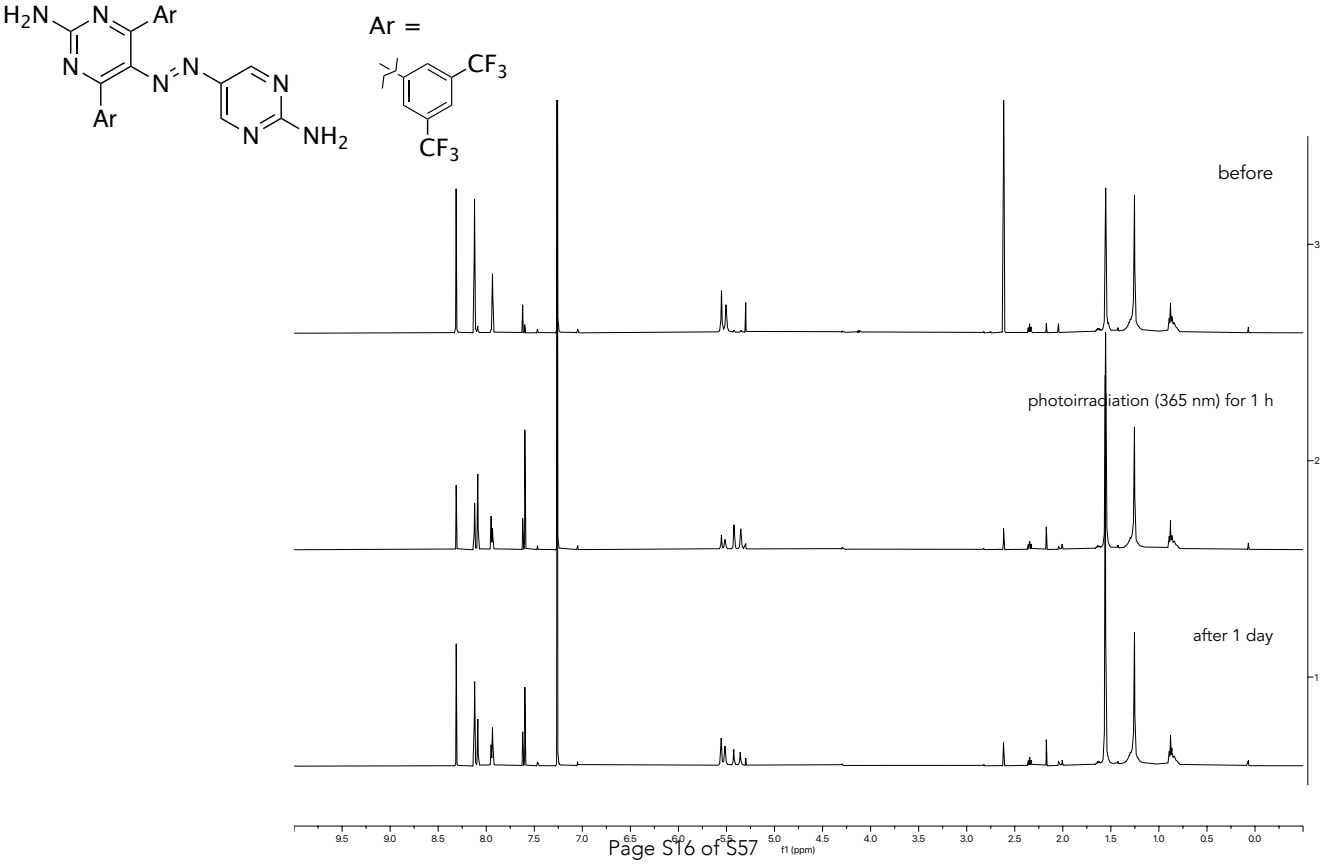
6. *Cis-Trans* Photoisomerization

The solution of compounds, **2b** and **2j** in quartz NMR sample tubes were irradiated at the wavelength of 365 nm (3 W cm^{-2}) for 24 h, and ^1H NMR analysis was measured.

5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-bis(trifluoromethyl)phenyl)pyrimidine-2-amine) (2b)

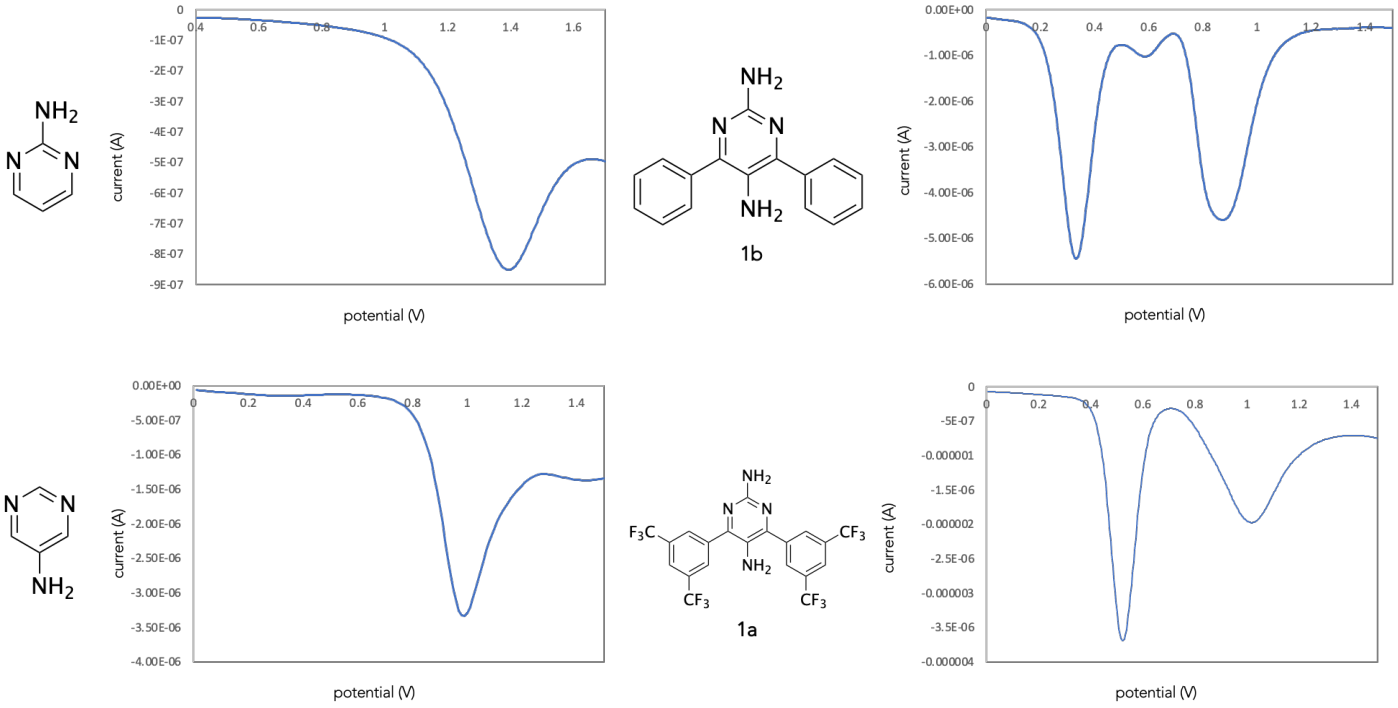


5-((2-Aminopyrimidin-5-yl)diazenyl)-4,6-bis(3,5-bis(trifluoromethyl)phenyl)pyrimidin-2-amine)(2j)



7. Differential Pulse Voltammetry (DPV) Analysis

Differential pulse voltammograms of 2-aminopyrimidine, 5-aminopyrimidine, **1a**, and **1b** (1 mM each) were recorded at 100 mV/s in a 0.1 M acetonitrile solution of $[^{n}Bu_4N](ClO_4)$. Glassy carbon working electrode and platinum counter electrode were used. Potentials are reported versus the saturated calomel reference electrode.



8. Chemical Reduction of Azo Compounds.

8-1. Reduction of azobenzene

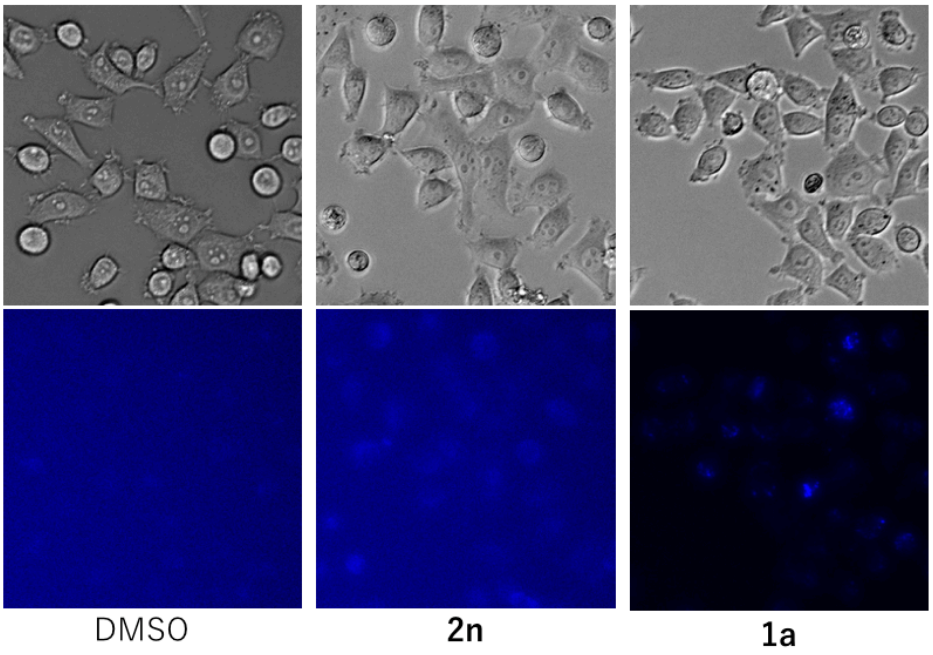
To a 50 mL flask charged with azobenzene (18.2 mg, 0.1 mmol) and dry THF (10 mL), was added Pd/C (1.8 mg) at room temperature. The resulting suspension was stirred under H_2 atmosphere (1 atm) for 30 min. The mixture was filtered through a pad of celite®, and ^{1}H NMR analysis of the concentrated filtrate proved all of azobenzene was reduced to aniline.

8-2. Reduction of azo compound **2h** with 2,6-xylyl groups

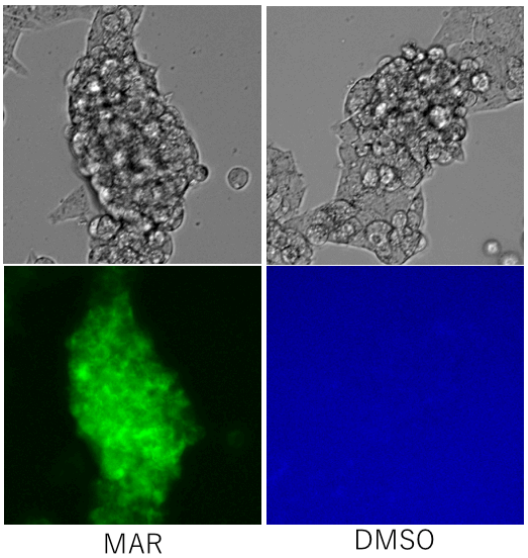
To a 10 mL flask charged with azobenzene (12.6 mg, 0.02 mmol) and dry THF (2 mL), was added Pd/C (1.3 mg) at room temperature. The resulting suspension was stirred under H_2 atmosphere (1 atm) for 48 min. The mixture was filtered through a pad of celite®, and ^{1}H NMR analysis of the concentrated filtrate proved **2h** remained unchanged.

9. Hypoxia Cell Imaging

HepG2 cells were cultured in Dulbecco's modified Eagle's medium with high glucose (FUJIFILM Wako Chemicals, Osaka, Japan) containing 10% fetal bovine serum and penicillin/streptomycin (Nacalai Tesque, Kyoto, Japan), and HeLa cells were cultured in Eagle's minimal essential medium containing 10% fetal bovine serum and penicillin/streptomycin. HepG2 or HeLa cells were seeded into a 12-well plate and incubated at 37 ˚C for 16 h. The cells were treated with compound (final 1 μM) or DMSO at 37 ˚C for 4 h. Specimens were observed under a THUNDER 3D Live cell system (Leica Microsystems, Wetzlar, Germany). The excitation light for fluorescence observation was 395 nm and the emission filter was 535 nm.



Supplementary Fig 5. Confocal micrographs of 3D-cultured spheroids of HeLa cells co-incubated with C4N4 azo compounds **2n** or C4N4 fluorescent compound **1a** (1 μM, 37 °C, 4 h). Upper: DIC (differential interference contrast); lower: Fluorescent micrographs.



Supplementary Fig 6. Positive and negative control of Hypoxic cell imaging of HepG2 cells using MAR[3] (Positive control) and DMSO (negative control). Confocal micrographs of 3D-cultured spheroids of HeLa cells co-incubated with C4N4 azo compounds **2n** or C4N4 fluorescent compound **1a** (1 μM, 37 °C, 4 h). Upper: DIC (differential interference contrast); lower: Fluorescent micrographs.

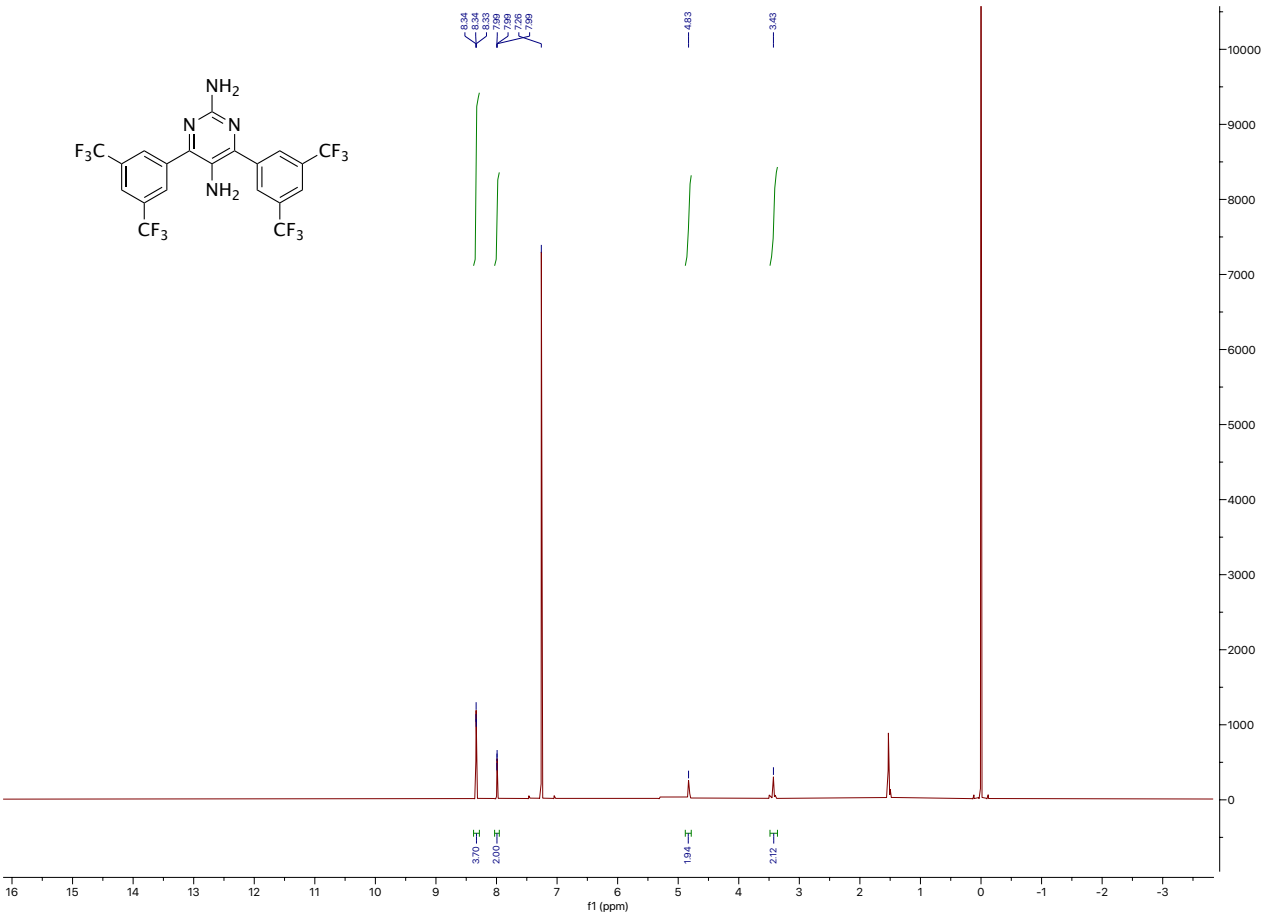
10. References

1. Noda, H.; Asada, Y.; Maruyama, T.; Takizawa, N.; Noda, N. N.; Shibasaki, M.; Kumagai, N. *Chem. Eur. J.* **2019**, *25*, 4299–4304.
2. Teders, M.; Pogodaev, A. A.; Bojanov, G.; Huck, W. T. S. *J. Am. Chem. Soc.* **2021**, *143*, 5709–5716.
3. Piao, W.; Tsuda, S.; Tanaka, Y.; Maeda, S.; Liu, F.; Takahashi, S.; Kushida, Y.; Komatsu, T.; Ueno,T.; Terai, T.; Nakazawa, T.; Uchiyama, M.; Morokuma, K.; Nagano, T.; Hanaoka, K. *Angew. Chem. Int. Ed.* **2013**, *52*, 13028–13032.

11. Spectra

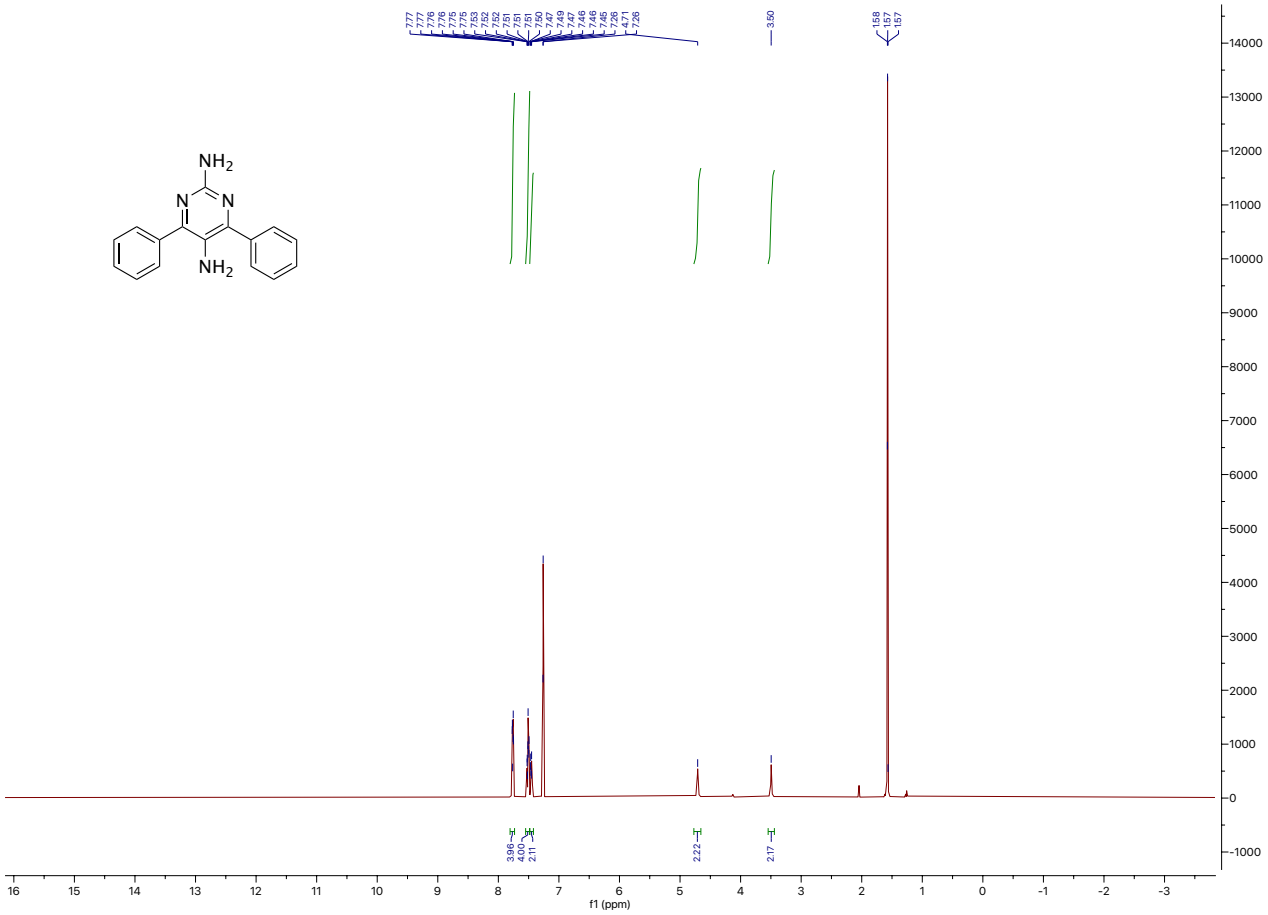
4,6-Bis(3,5-(trifluoromethyl)phenyl)pyrimidine-2,5-diamine (1a)

^{1}H NMR (500 MHz, $CDCl_3$)



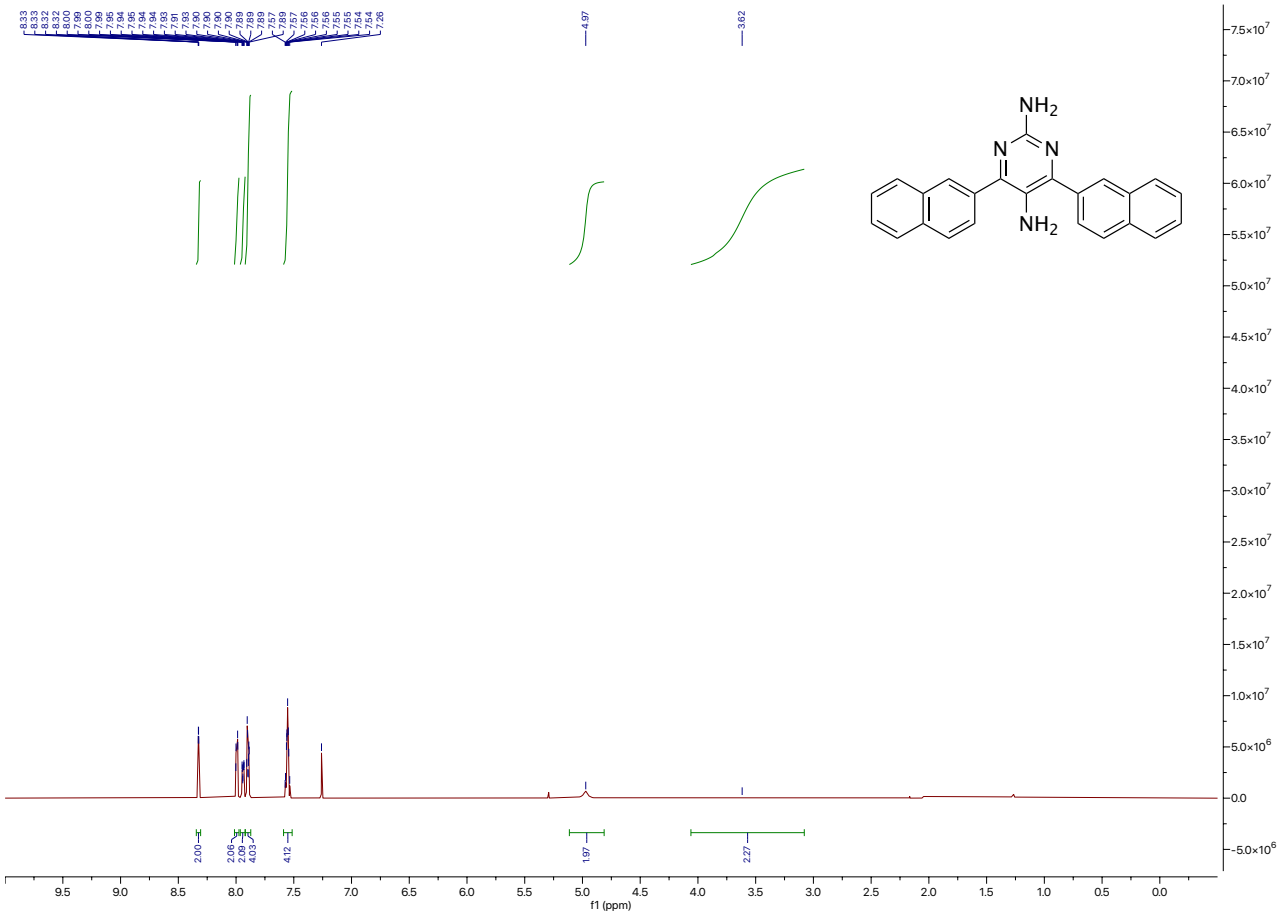
4,6-Biphenylpyrimidine-2,5-diamine (1b)

^{1}H NMR (500 MHz, $CDCl_3$)

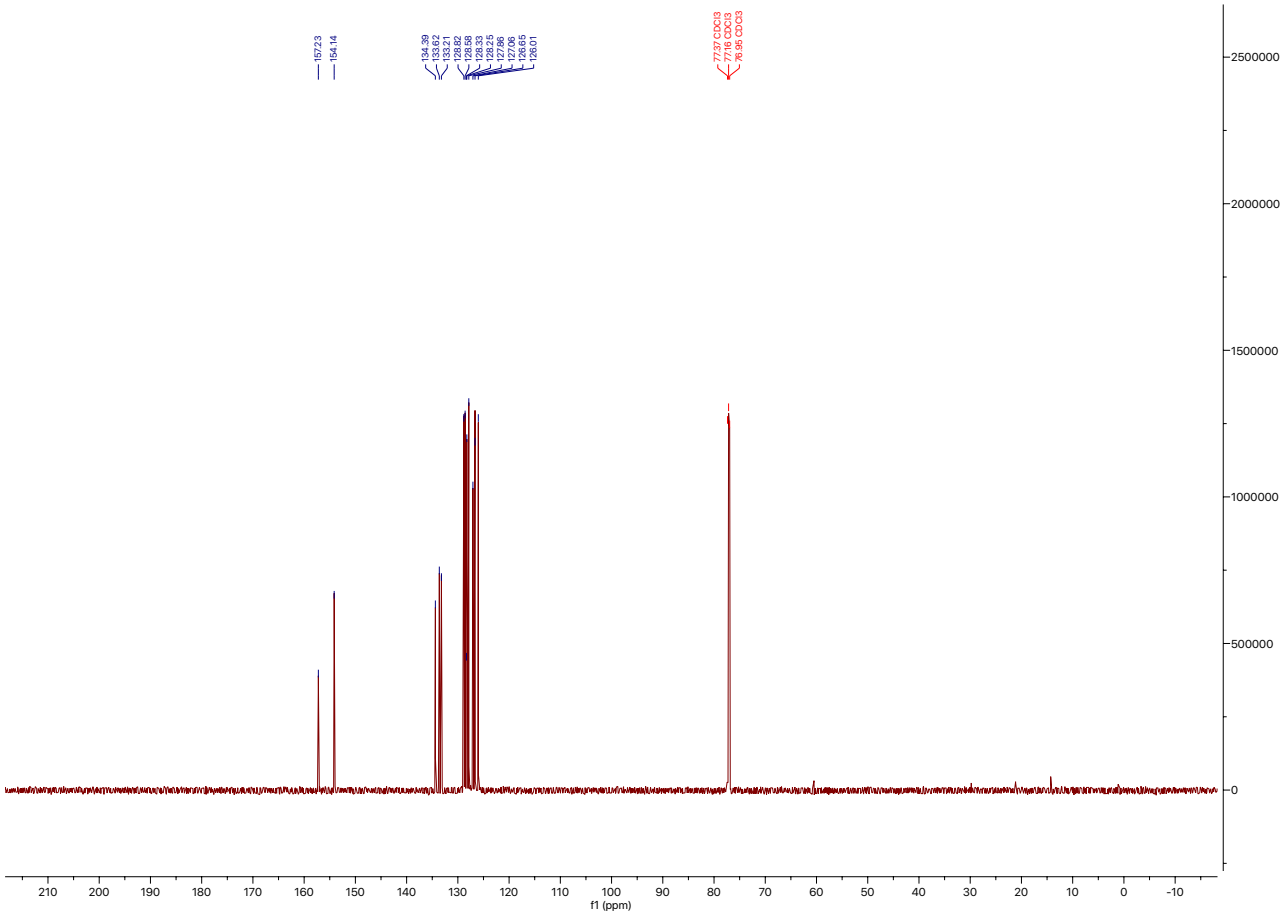


4,6-Bis(naphthalen-2-yl)pyrimidine-2,5-diamine (1c)

^{1}H NMR (500 MHz, $CDCl_3$)

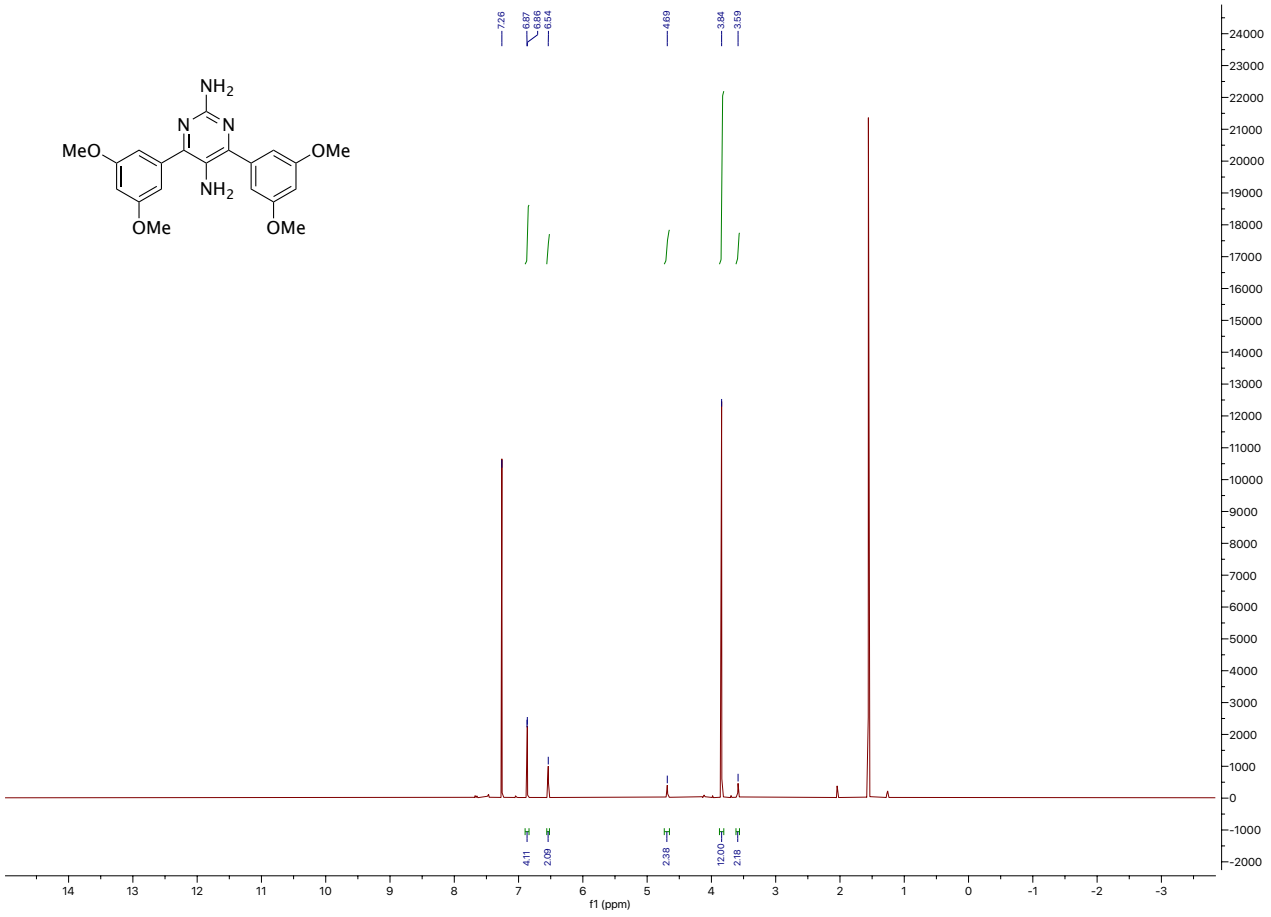


^{13}C NMR (151 MHz, $CDCl_3$)



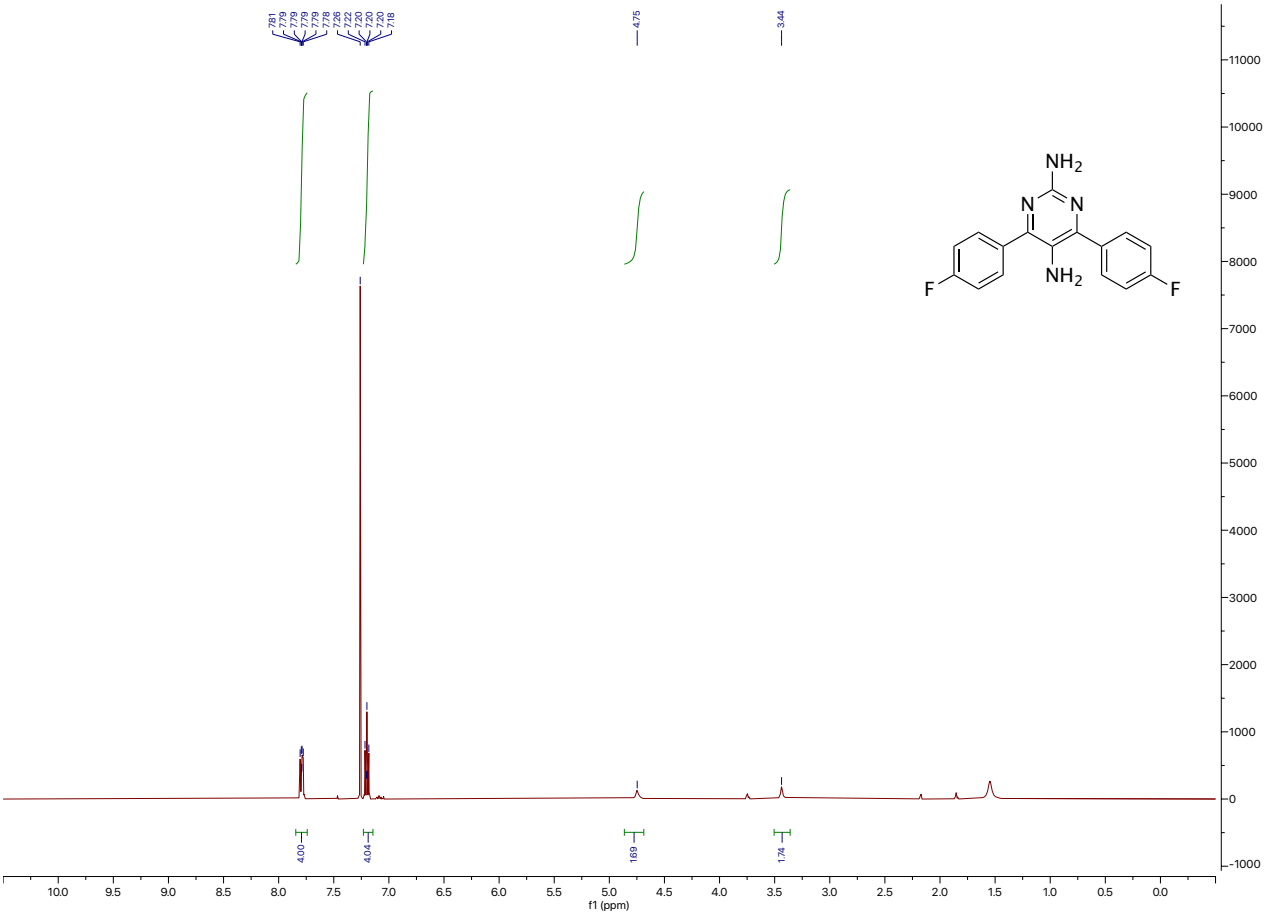
4,6-Bis(3,5-methoxyphenyl)pyrimidine-2,5-diamine (1d)

^{1}H NMR (500 MHz, $CDCl_3$)

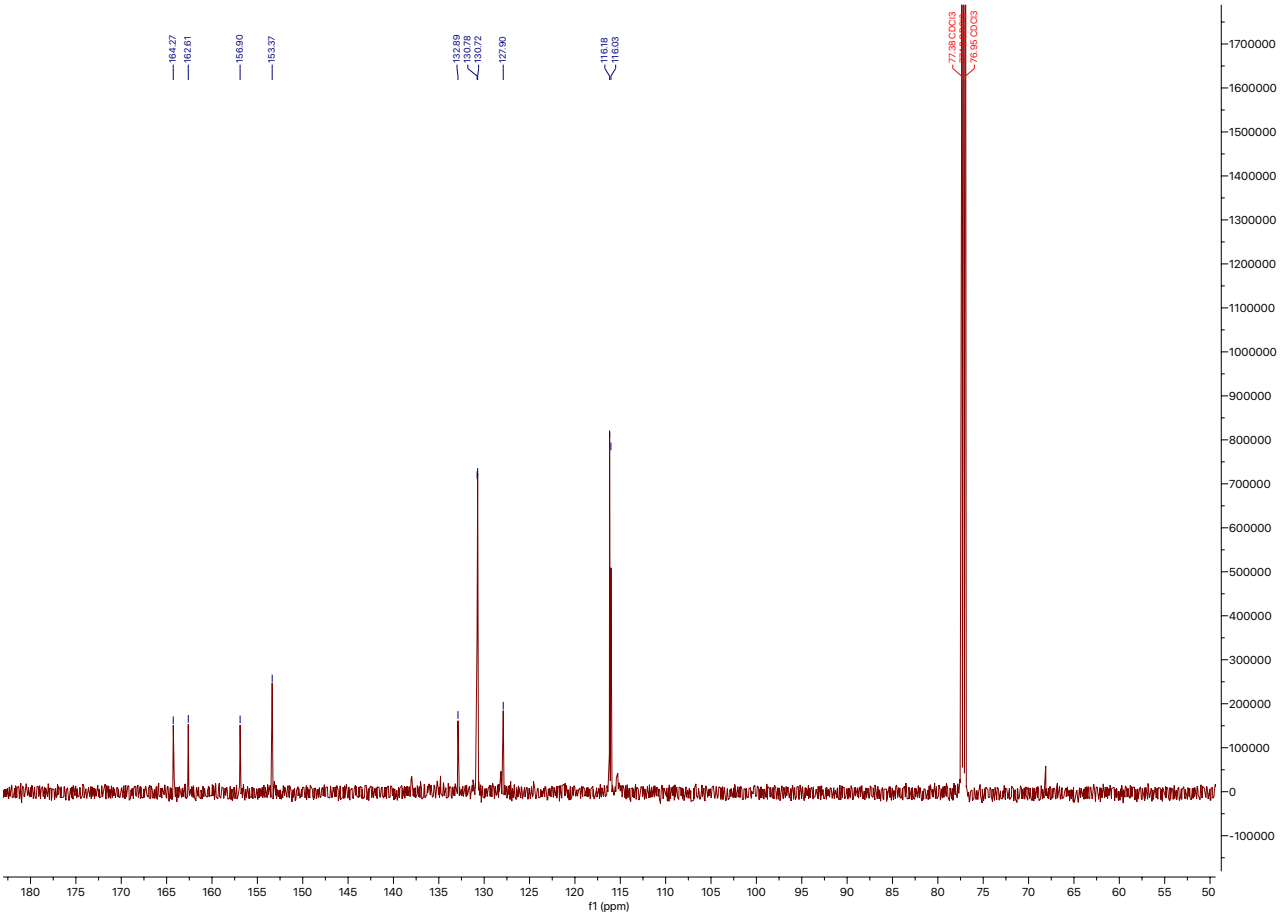


4,6-Bis(4-fluorophenyl)pyrimidine-2,5-diamine (1e)

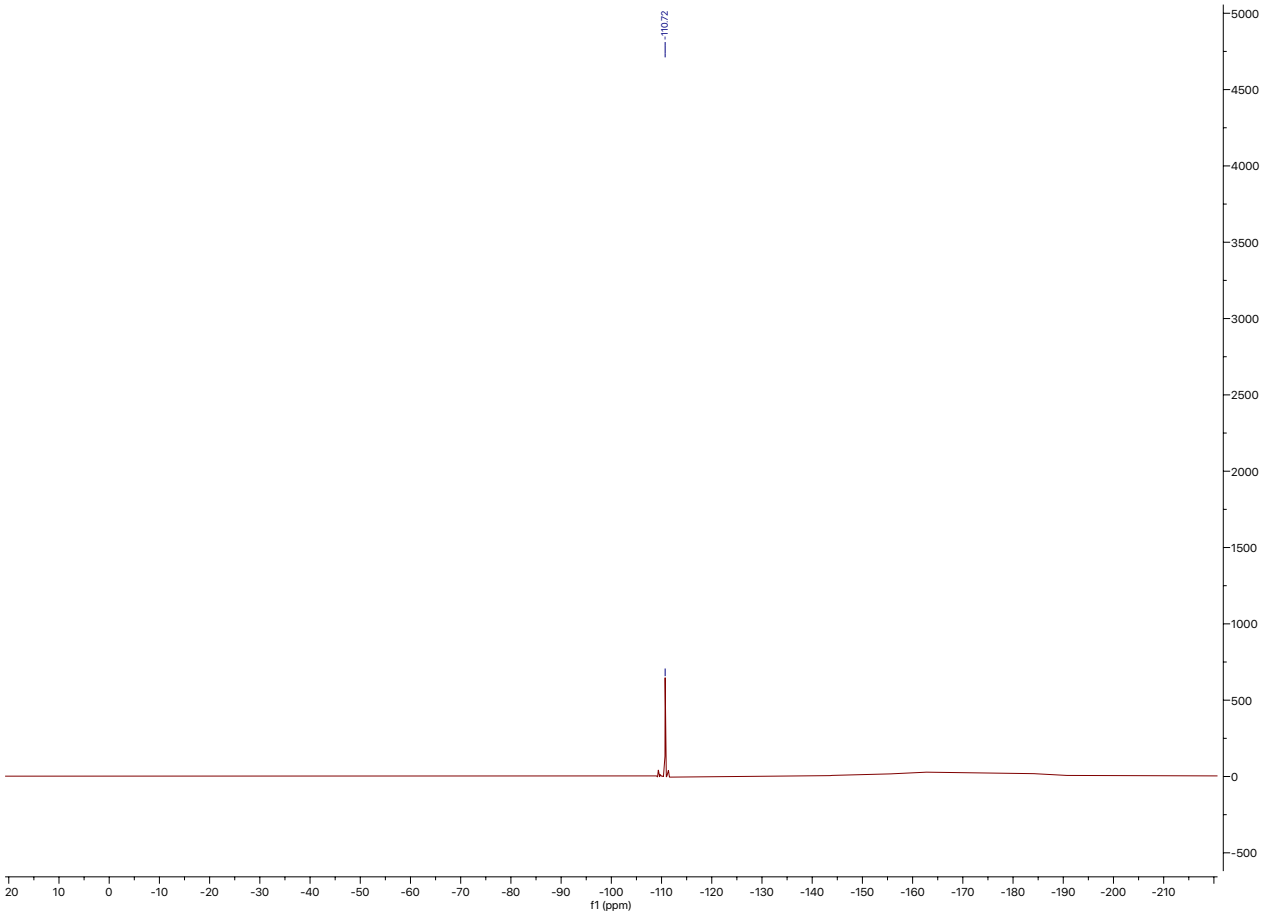
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

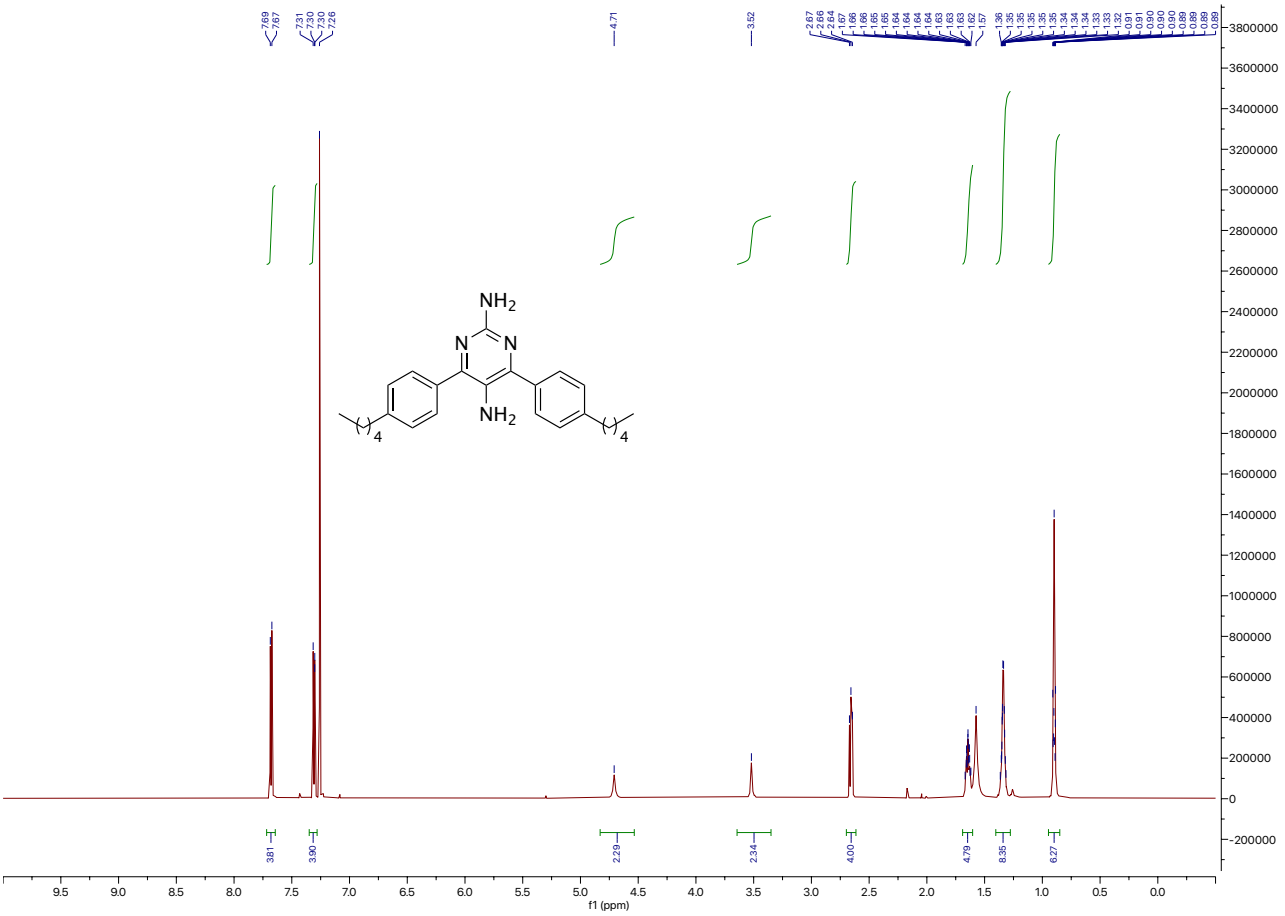


^{19}F NMR (471 MHz, $CDCl_3$)

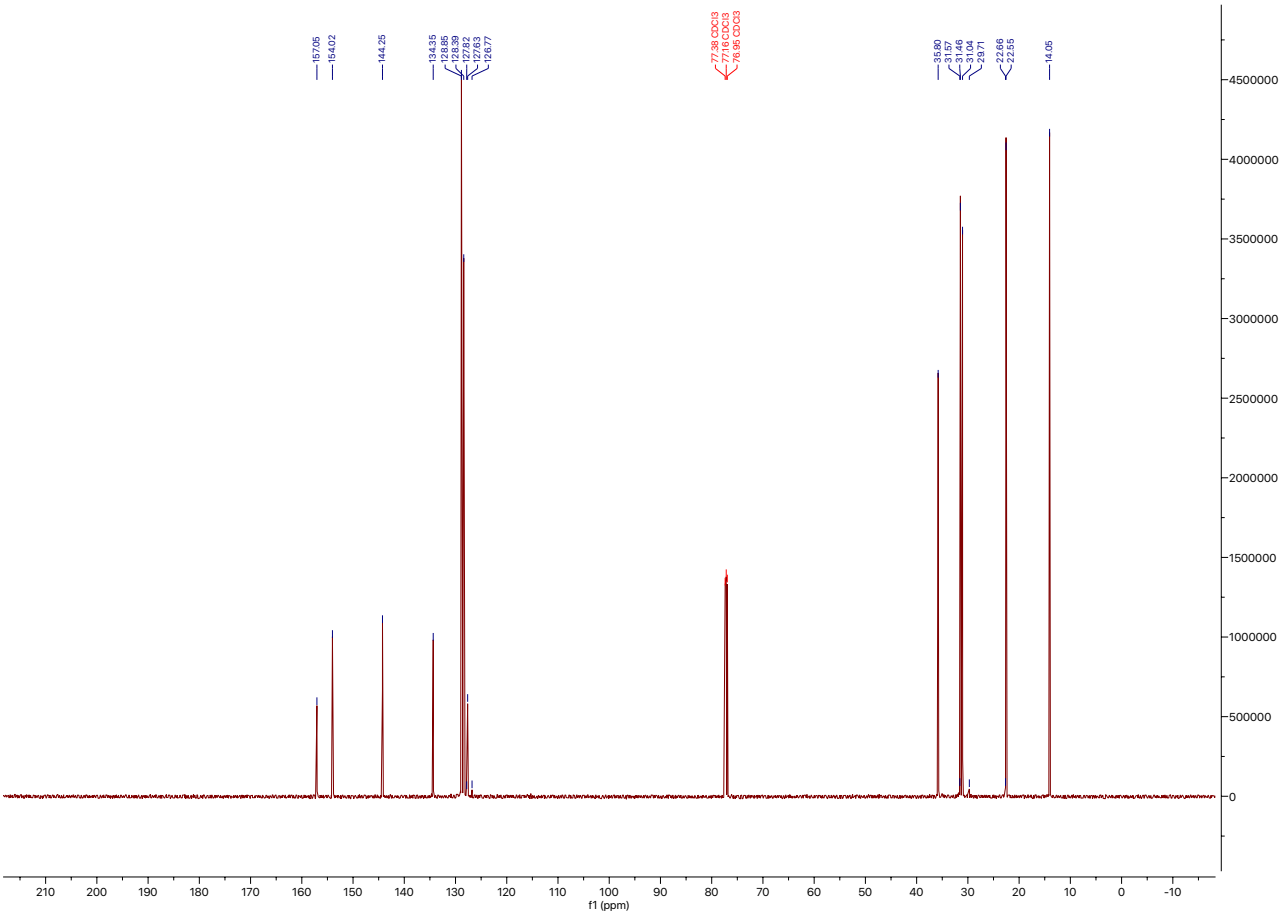


4,6-Bis(4-pentylphenyl)pyrimidine-2,5-diamine (1f)

^{1}H NMR (600 MHz, $CDCl_3$)

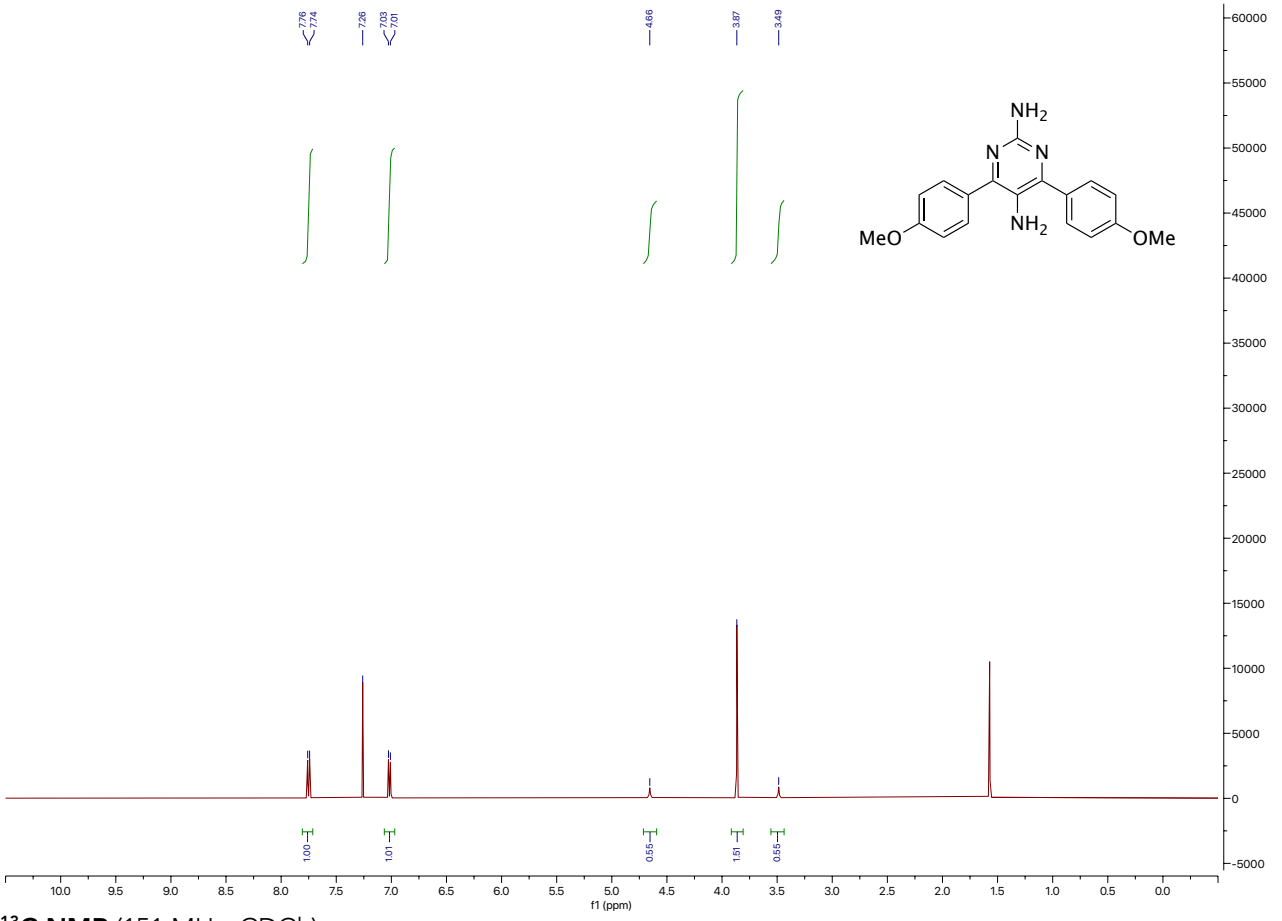


^{13}C NMR (151 MHz, $CDCl_3$)

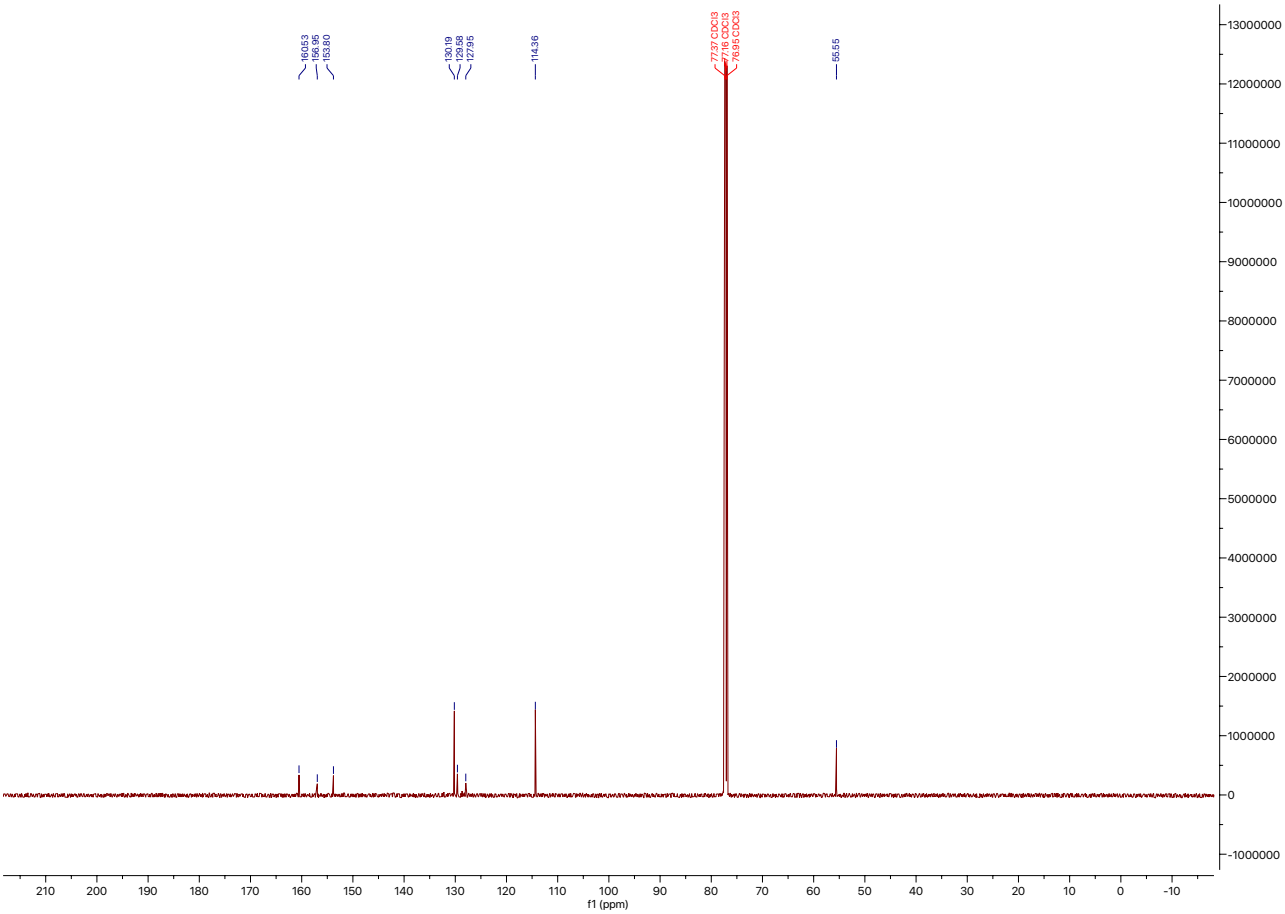


4,6-Bis(4-methoxyphenyl)pyrimidine-2,5-diamine (1g)

^{1}H NMR (500 MHz, $CDCl_3$)

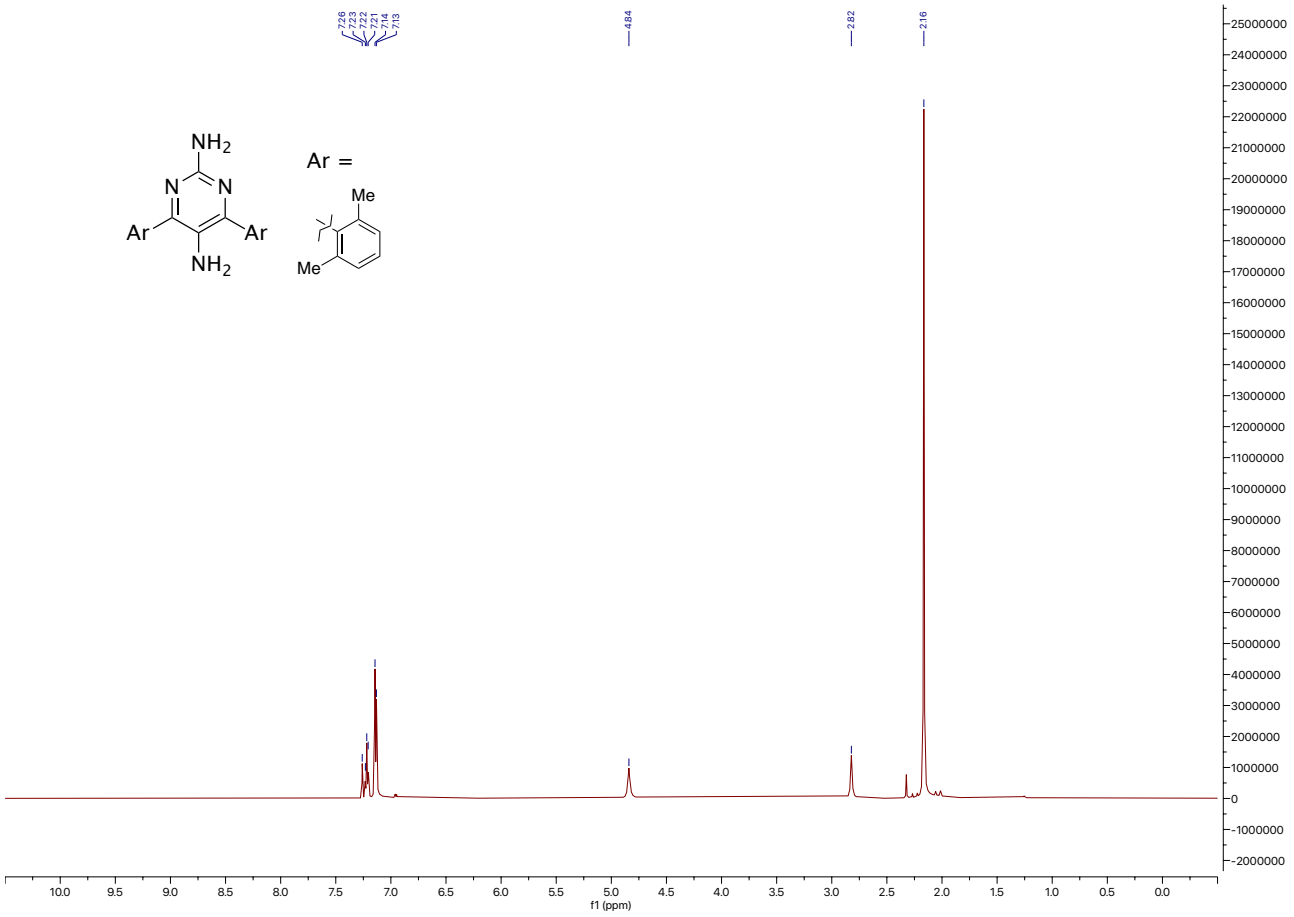


^{13}C NMR (151 MHz, $CDCl_3$)

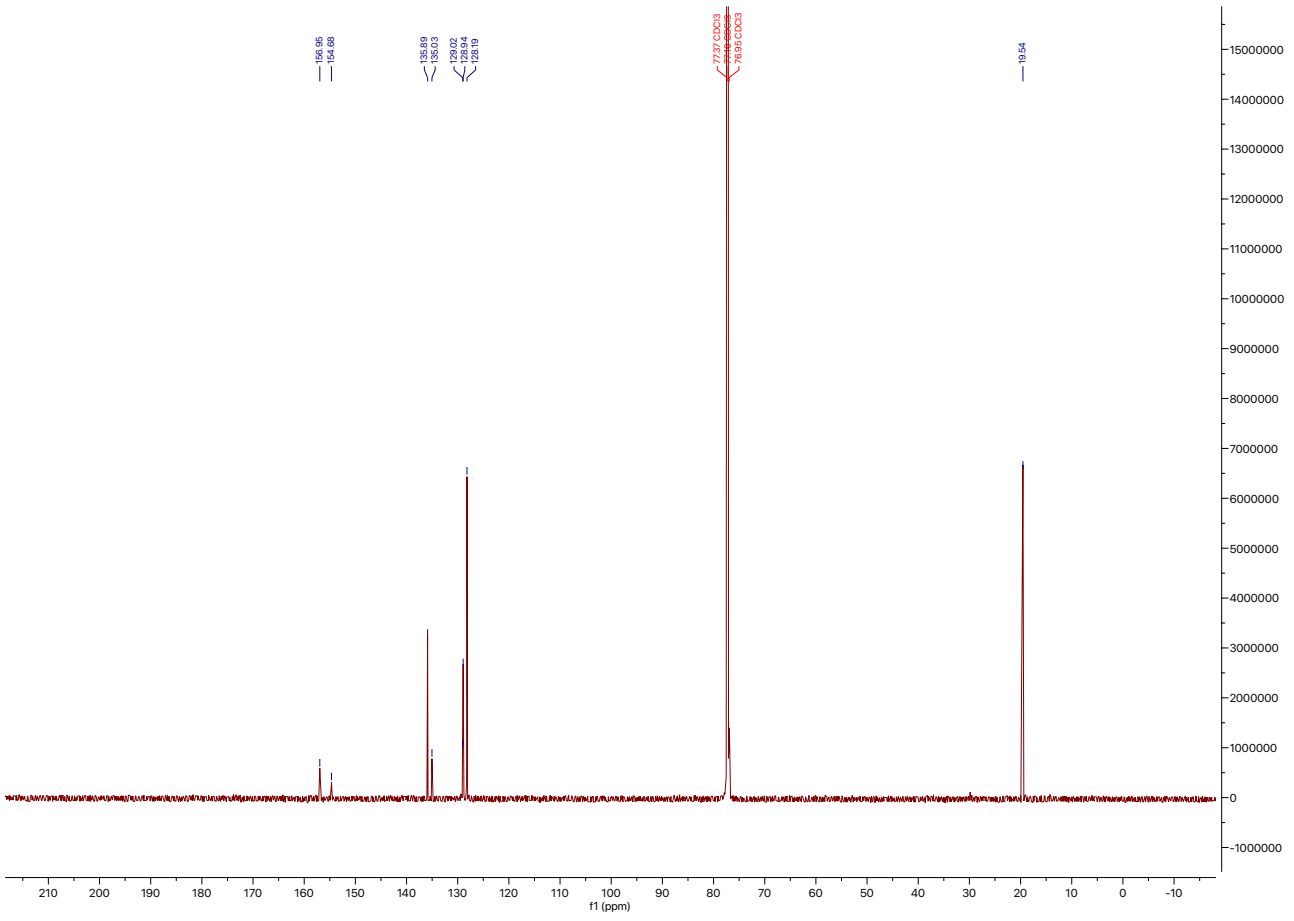


4,6-Bis(2,6-dimethylphenyl)pyrimidine-2,5-diamine (1h)

^{1}H NMR (500 MHz, $CDCl_3$)

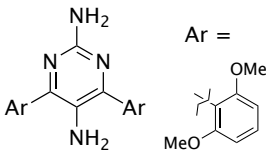


^{13}C NMR (151 MHz, $CDCl_3$)

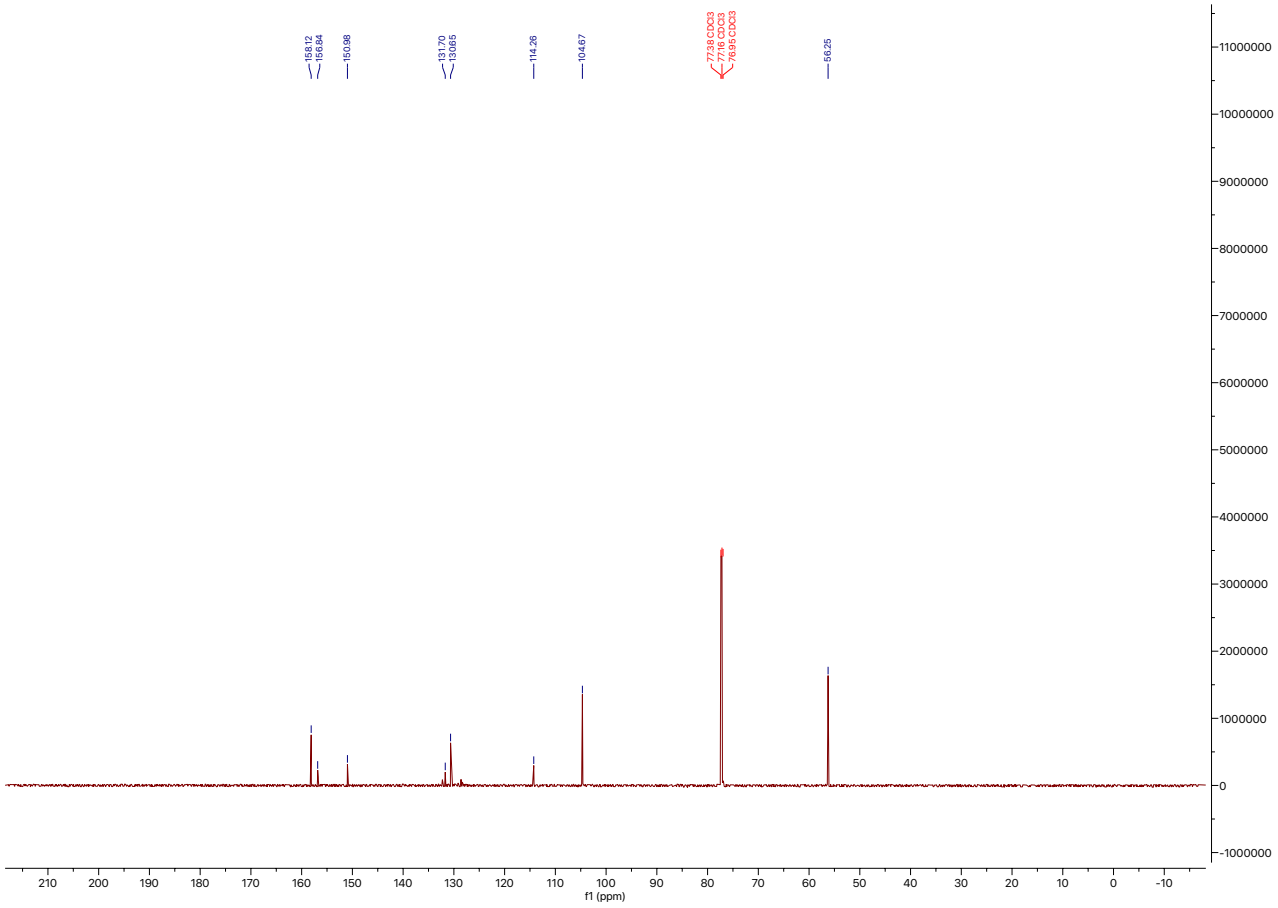


4,6-Bis(2,6-dimethoxyphenyl)pyrimidine-2,5-diamine (1i)

^{1}H NMR (500 MHz, $CDCl_3$)

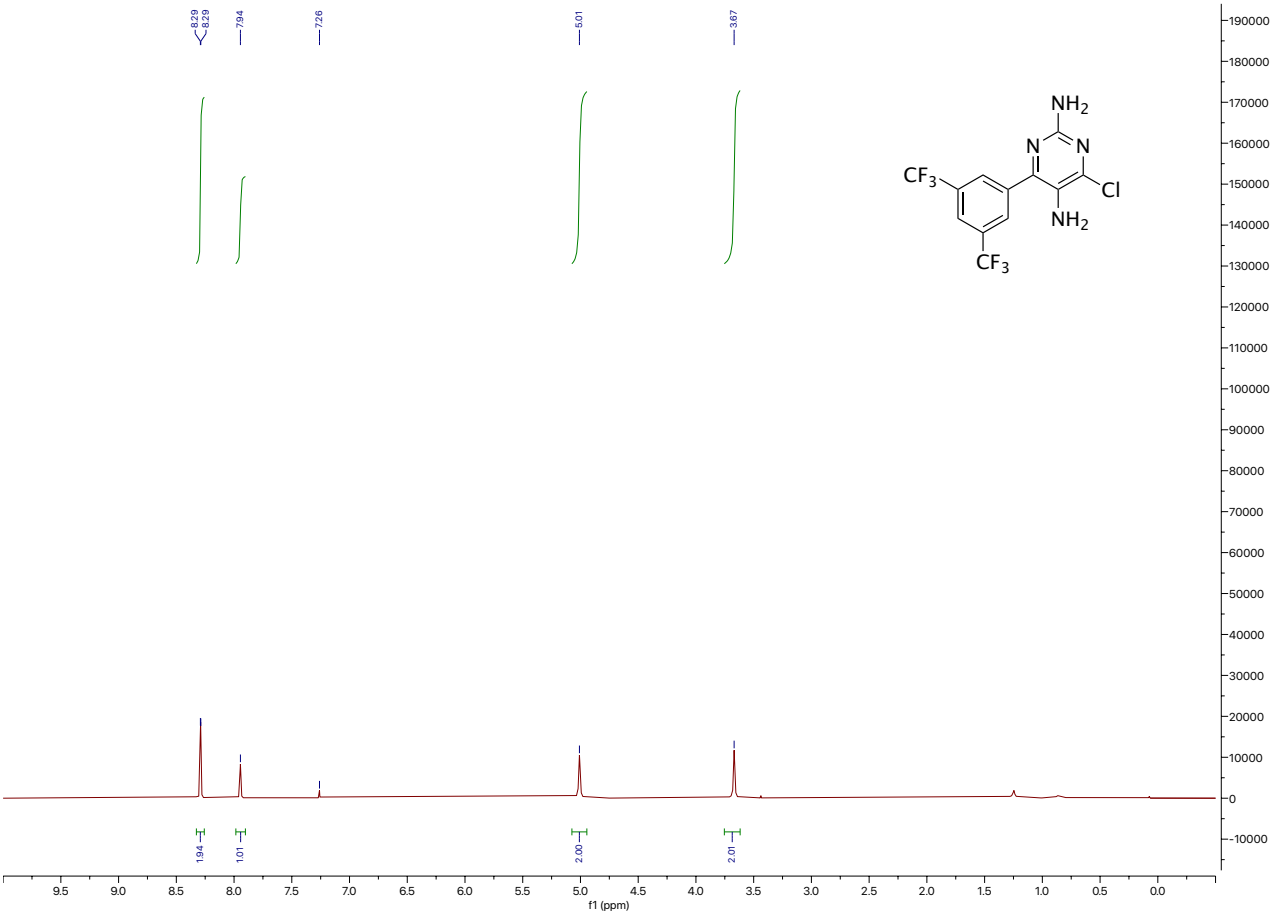


^{13}C NMR (151 MHz, $CDCl_3$)

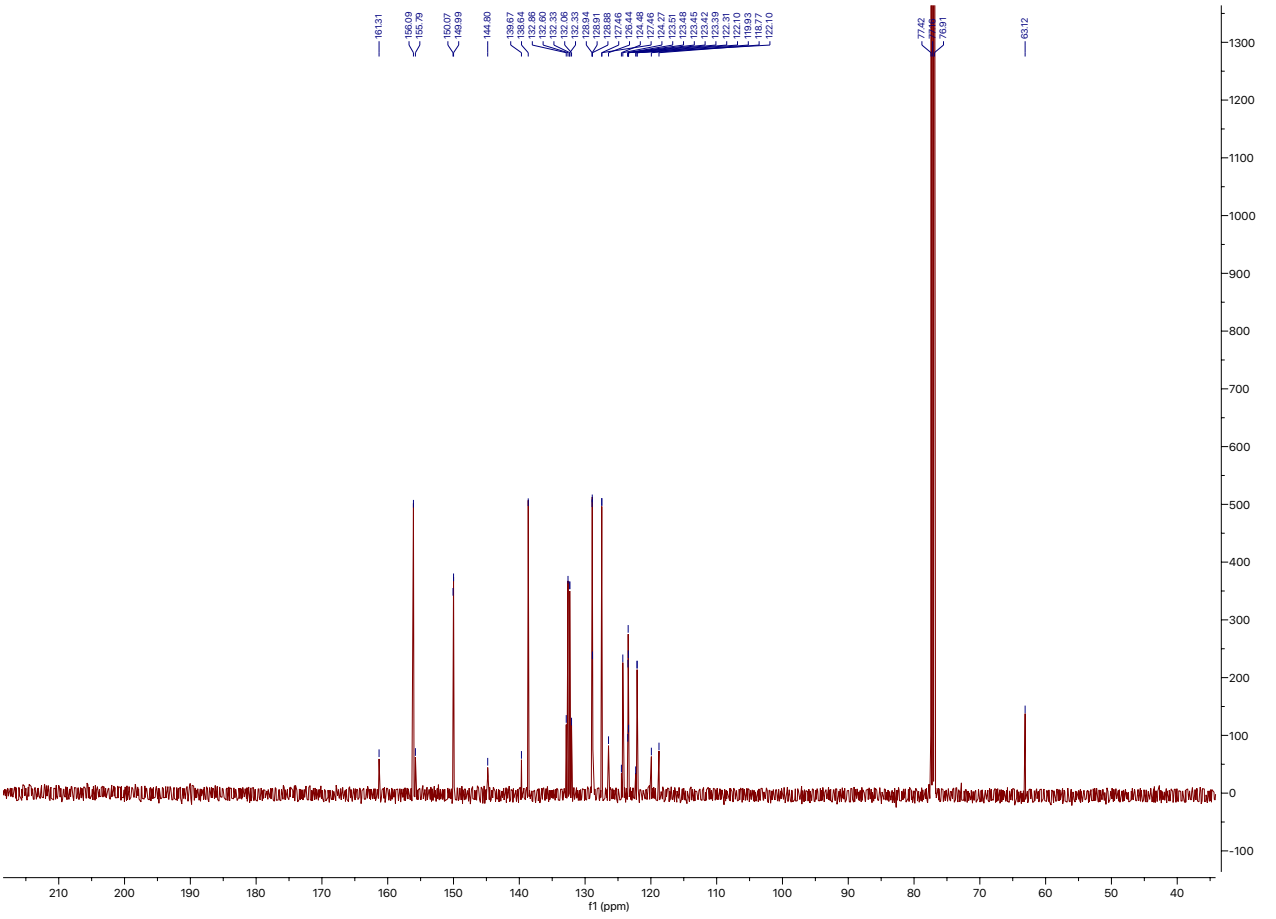


4-Chloro-6-(3,5-bis(trifluoromethyl)phenyl)pyrimidine-2,5-diamine (1a')

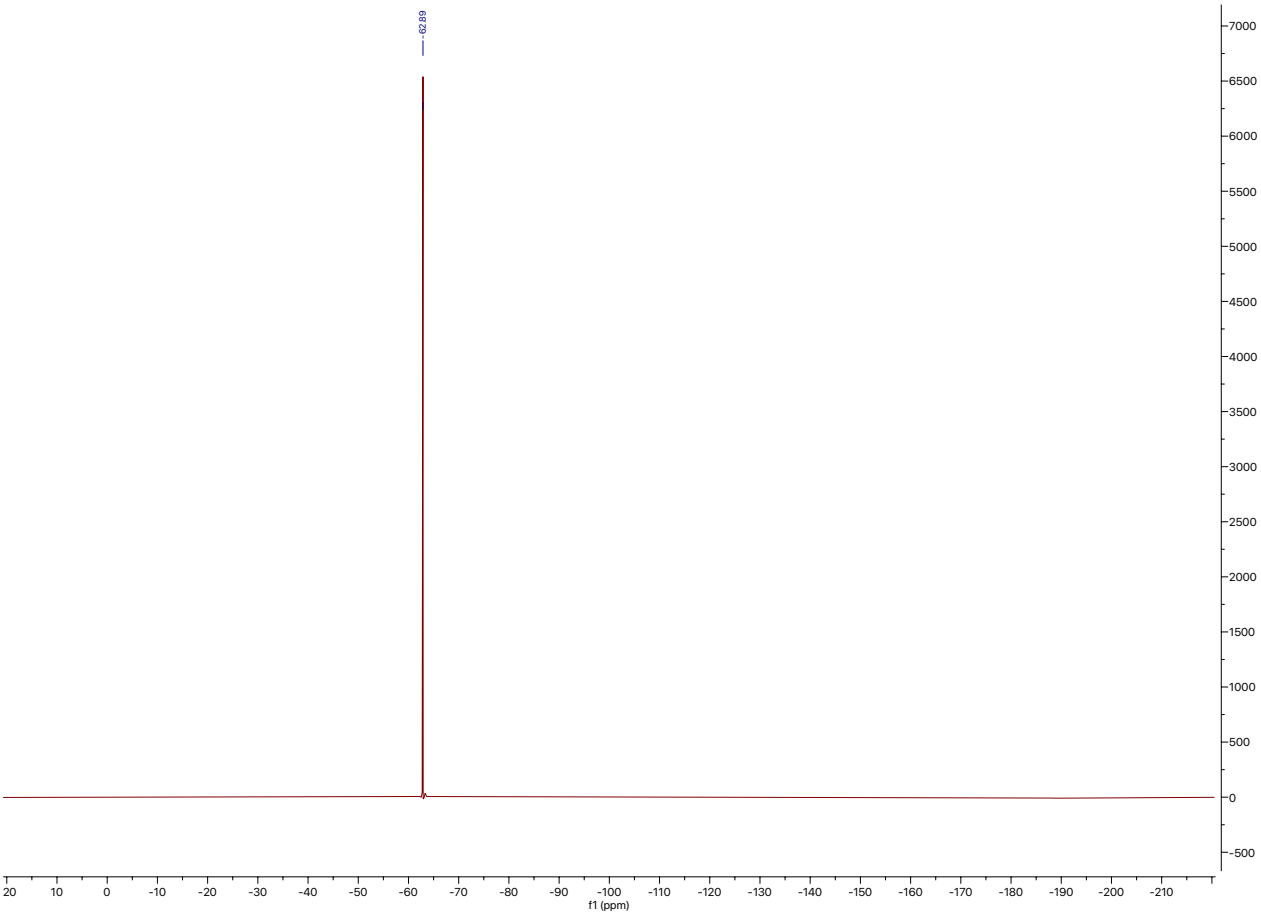
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)

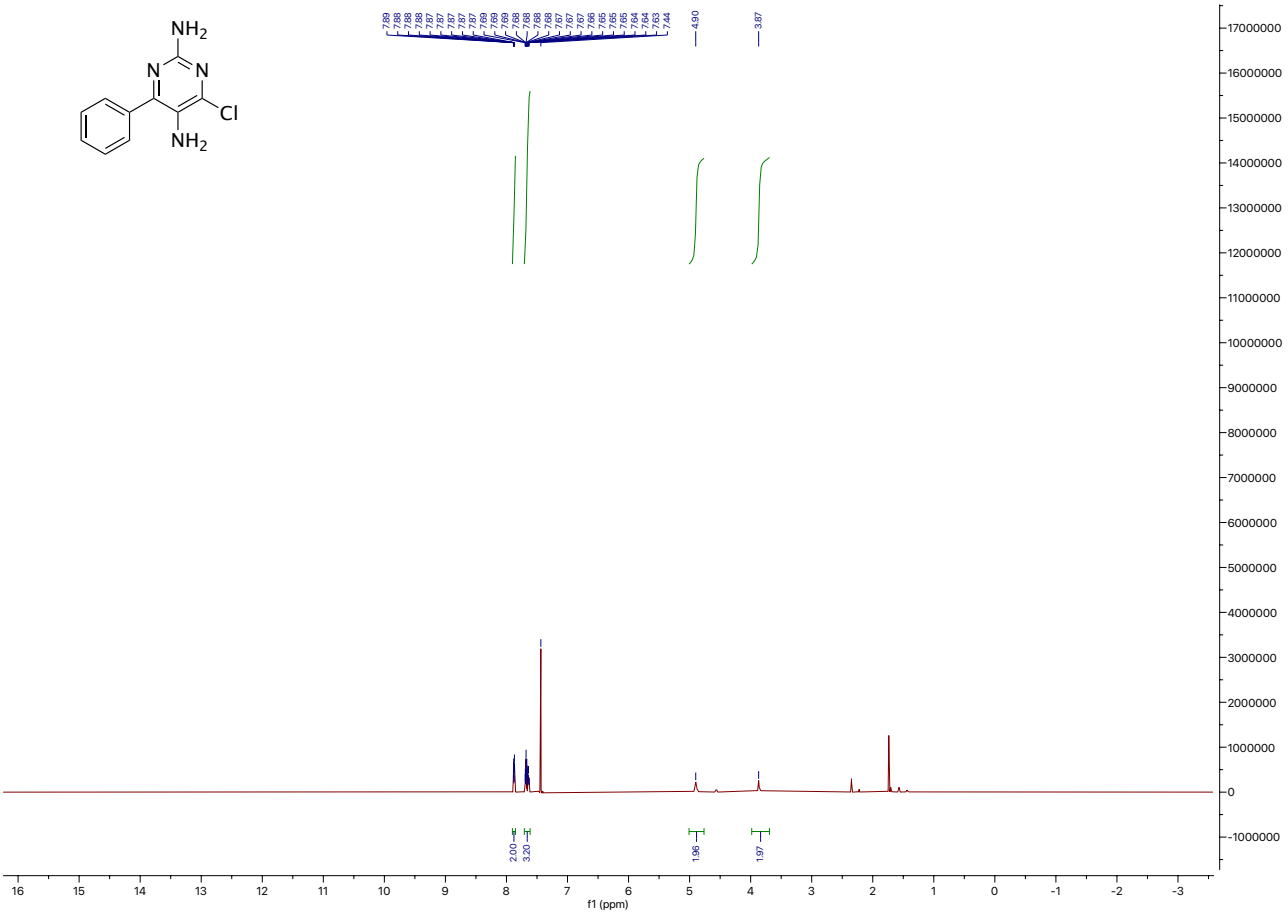


^{19}F NMR (471 MHz, $CDCl_3$)



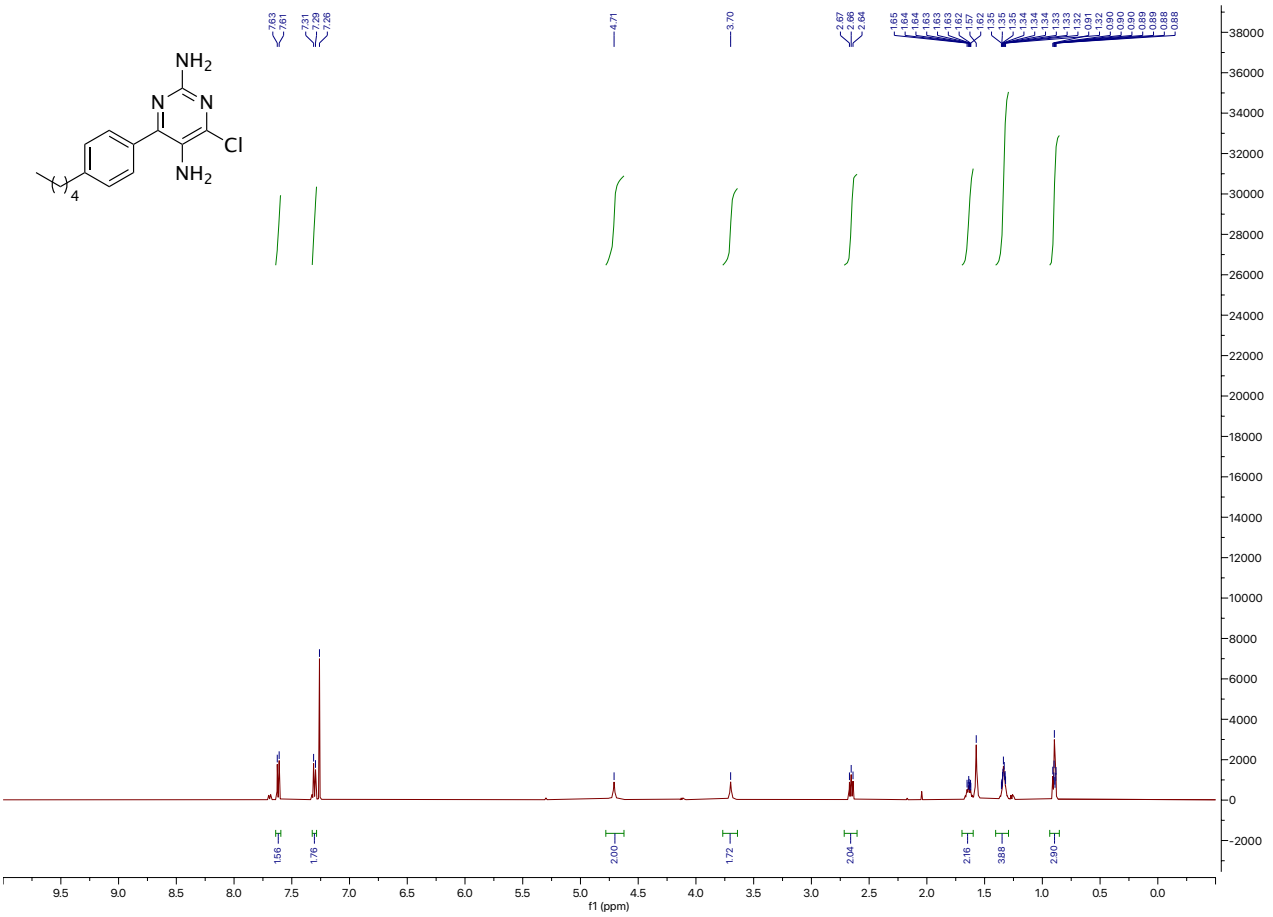
4-Chloro-6-phenylpyrimidine-2,5-diamine (1b')

^{1}H NMR (600 MHz, $CDCl_3$)

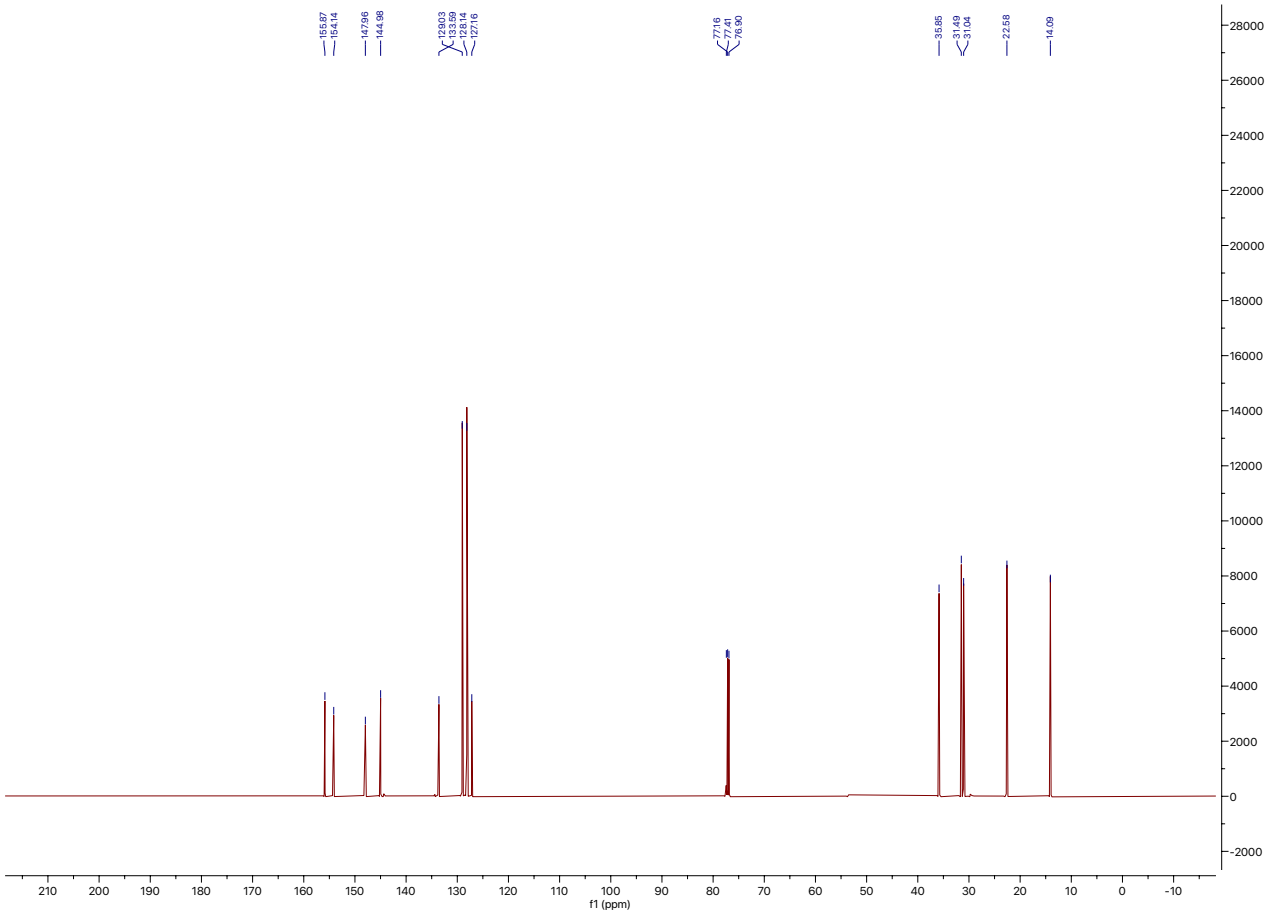


4-Chloro-6-(4-*n*-pentylphenyl)pyrimidine-2,5-diamine (1n')

^{1}H NMR (500 MHz, $CDCl_3$)

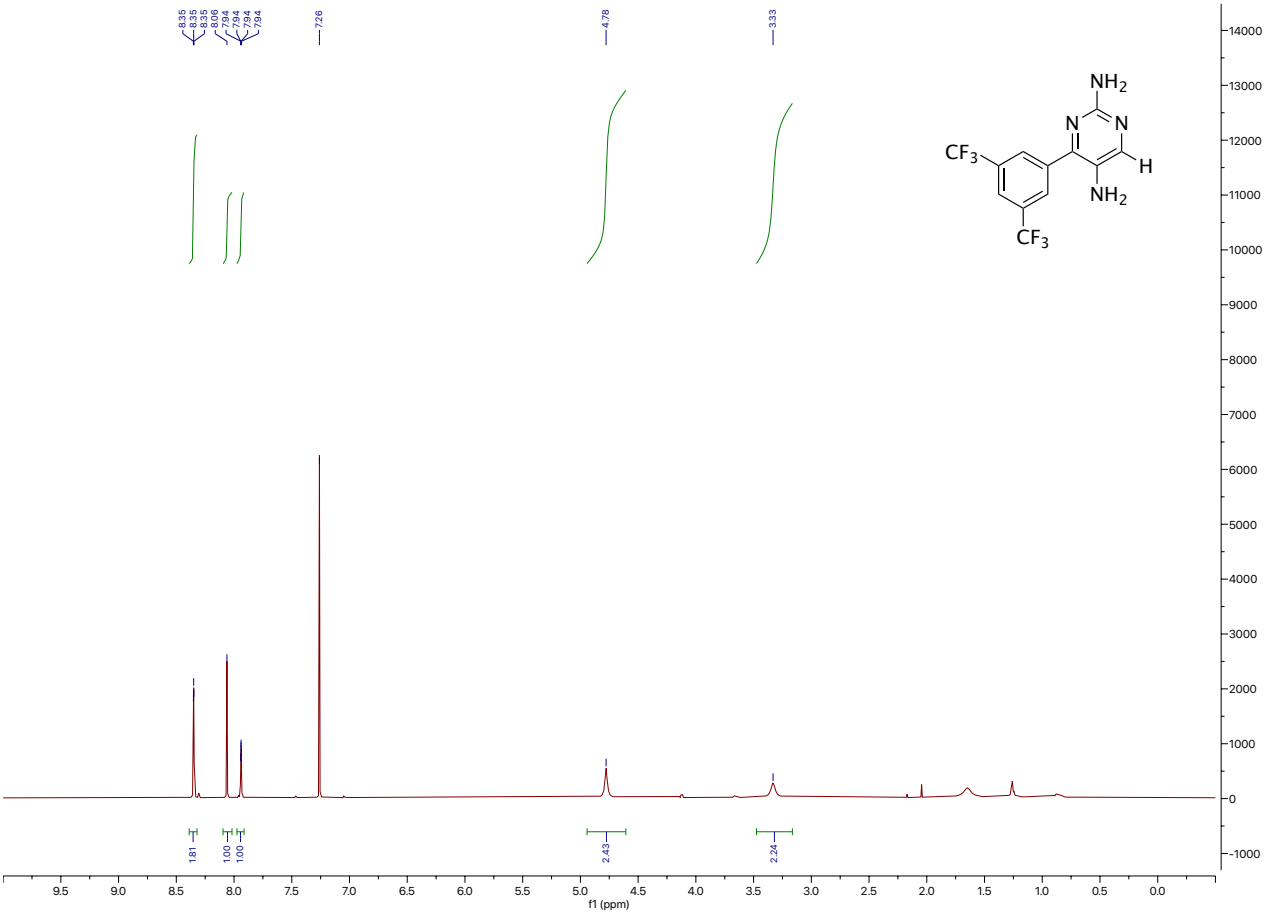


^{13}C NMR (151 MHz, $CDCl_3$)

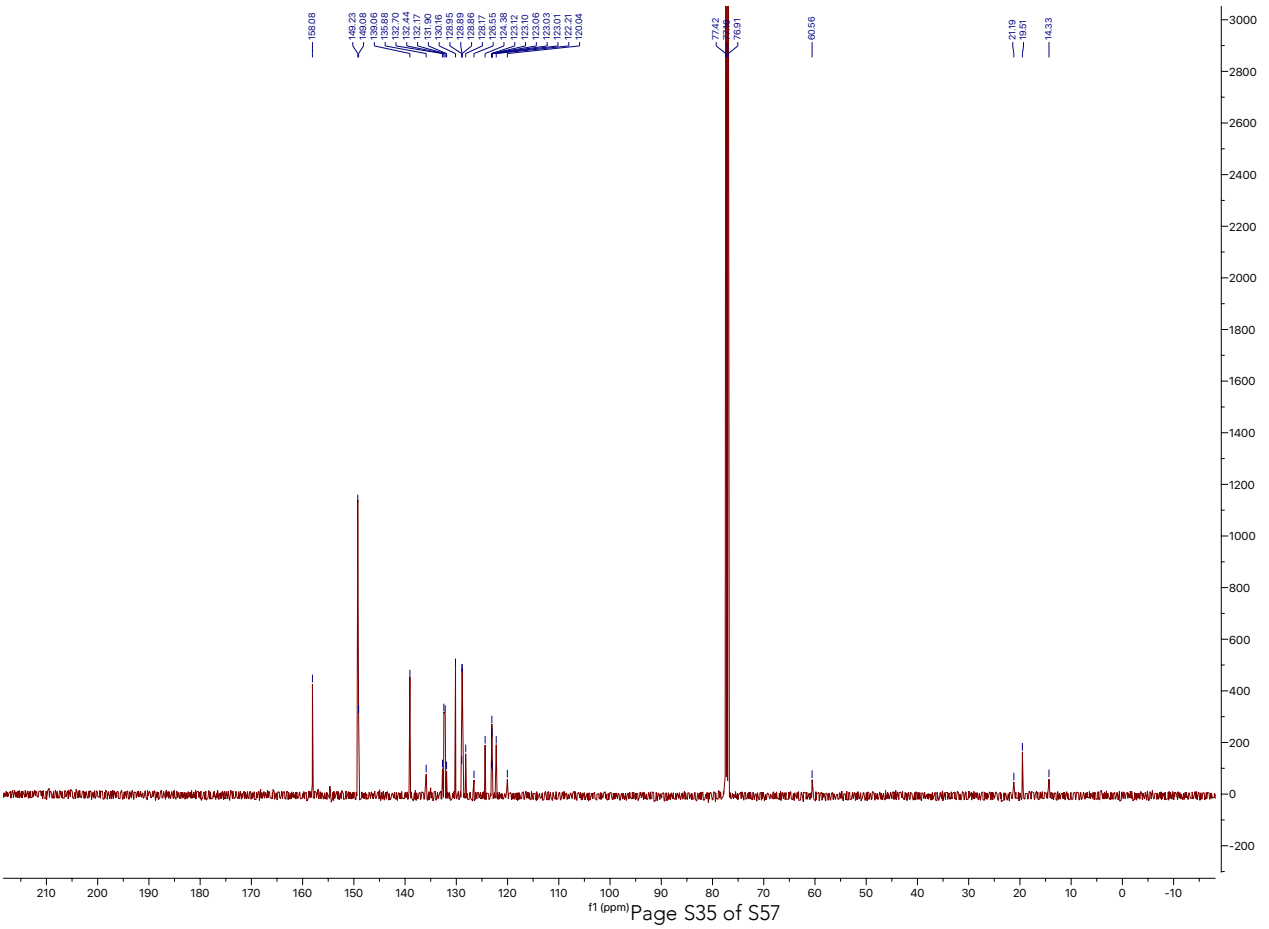


4-(3,5-Bis(trifluoromethyl)phenyl)pyrimidine-2,5-diamine (1k)

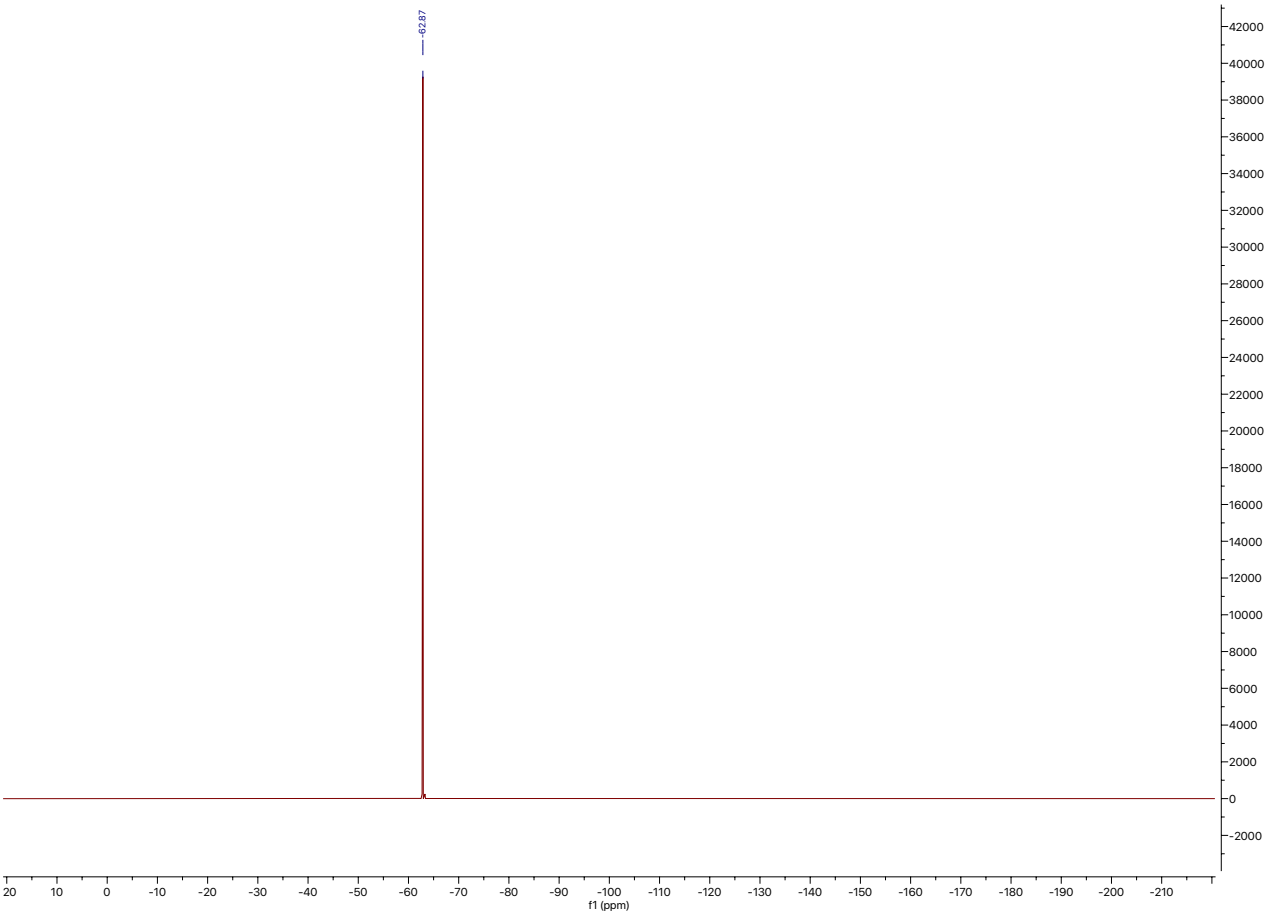
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)

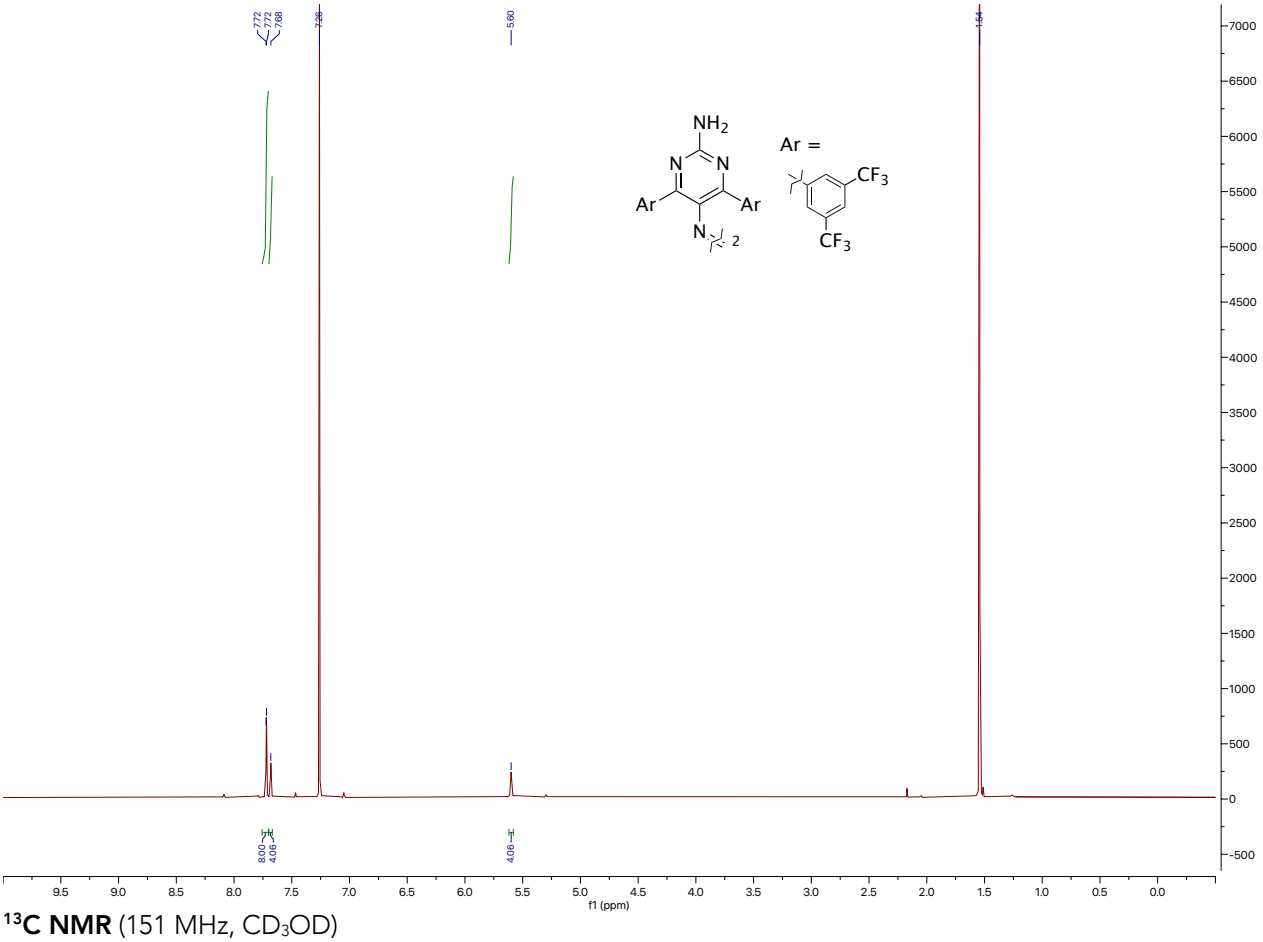


^{19}F NMR (471 MHz, $CDCl_3$)

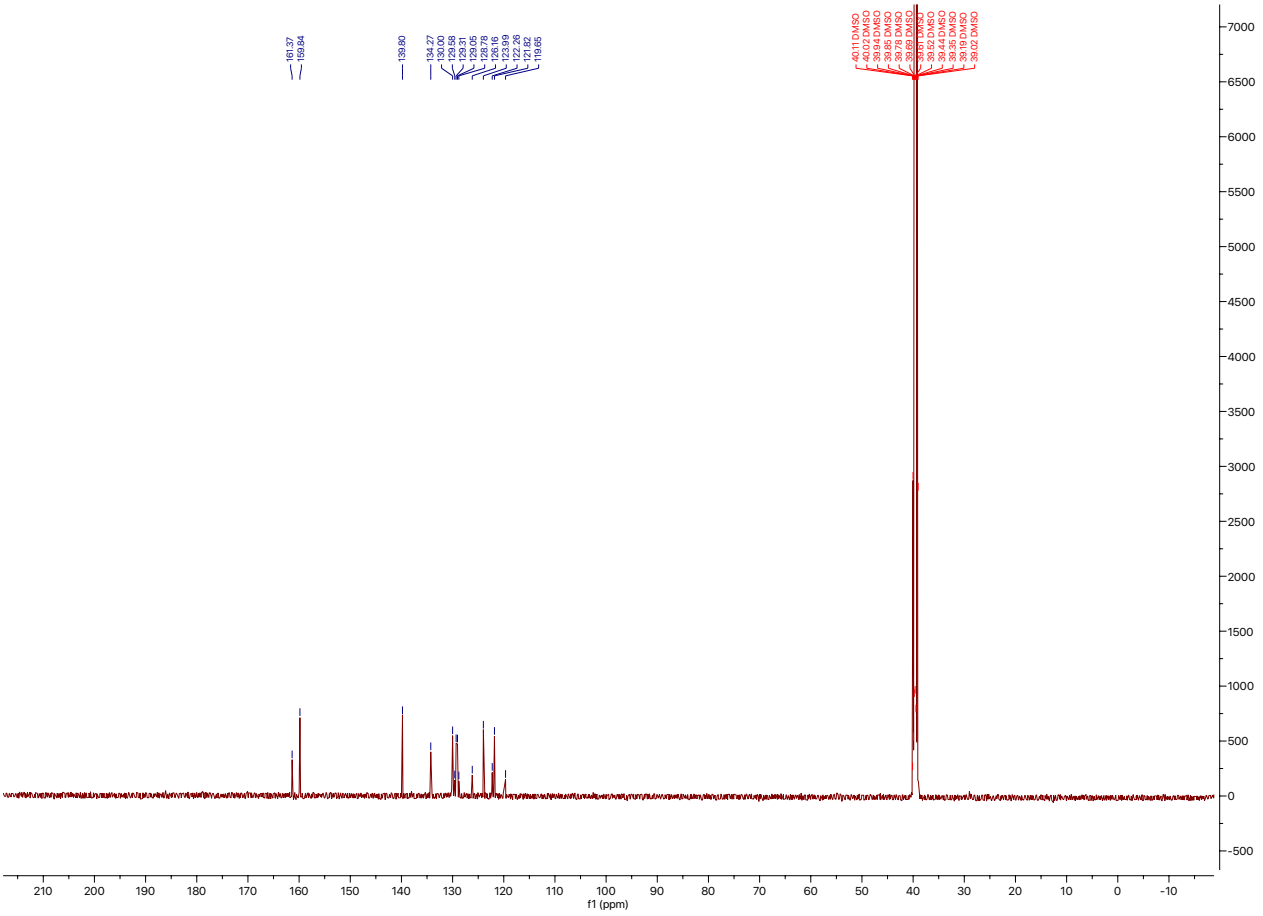


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-bis(trifluoromethyl)phenyl)pyrimidine-2-amine) (2a)

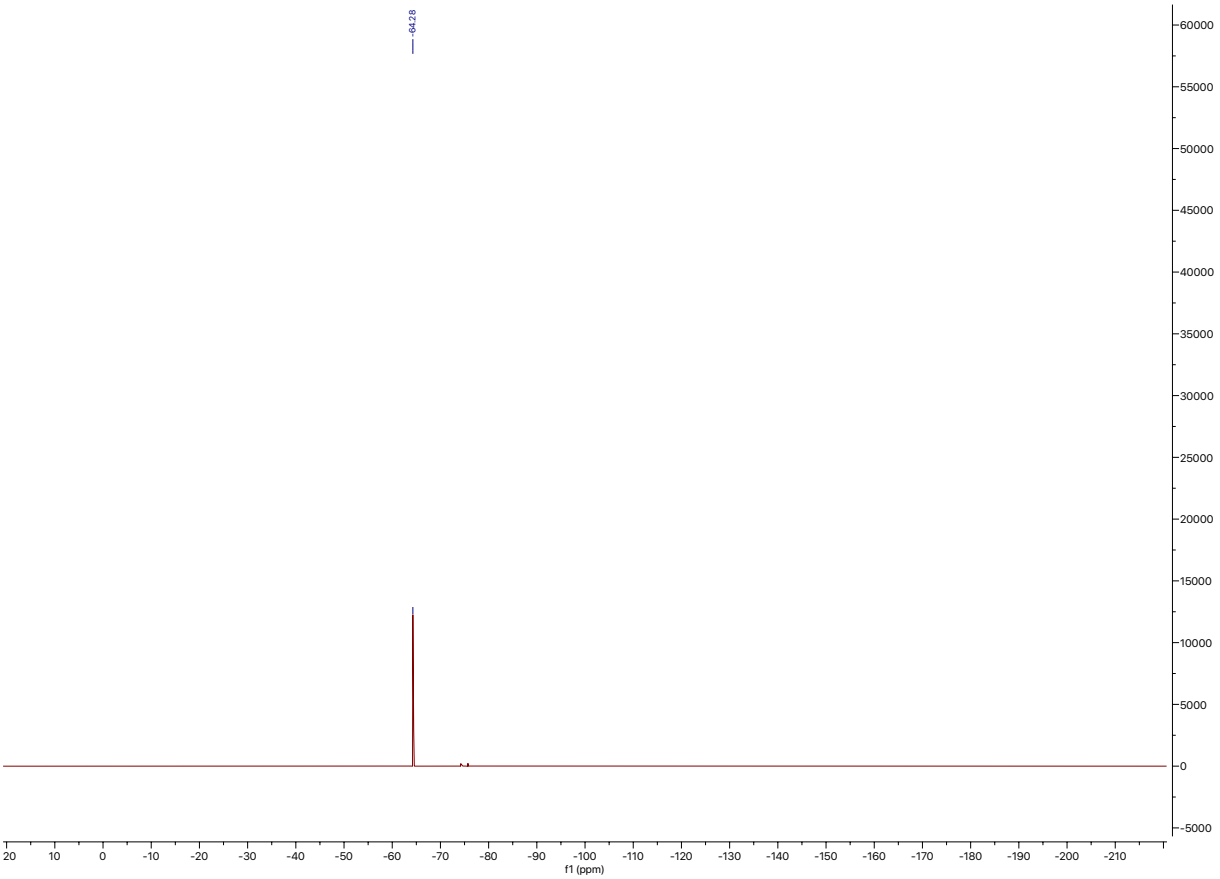
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, CD_3OD)

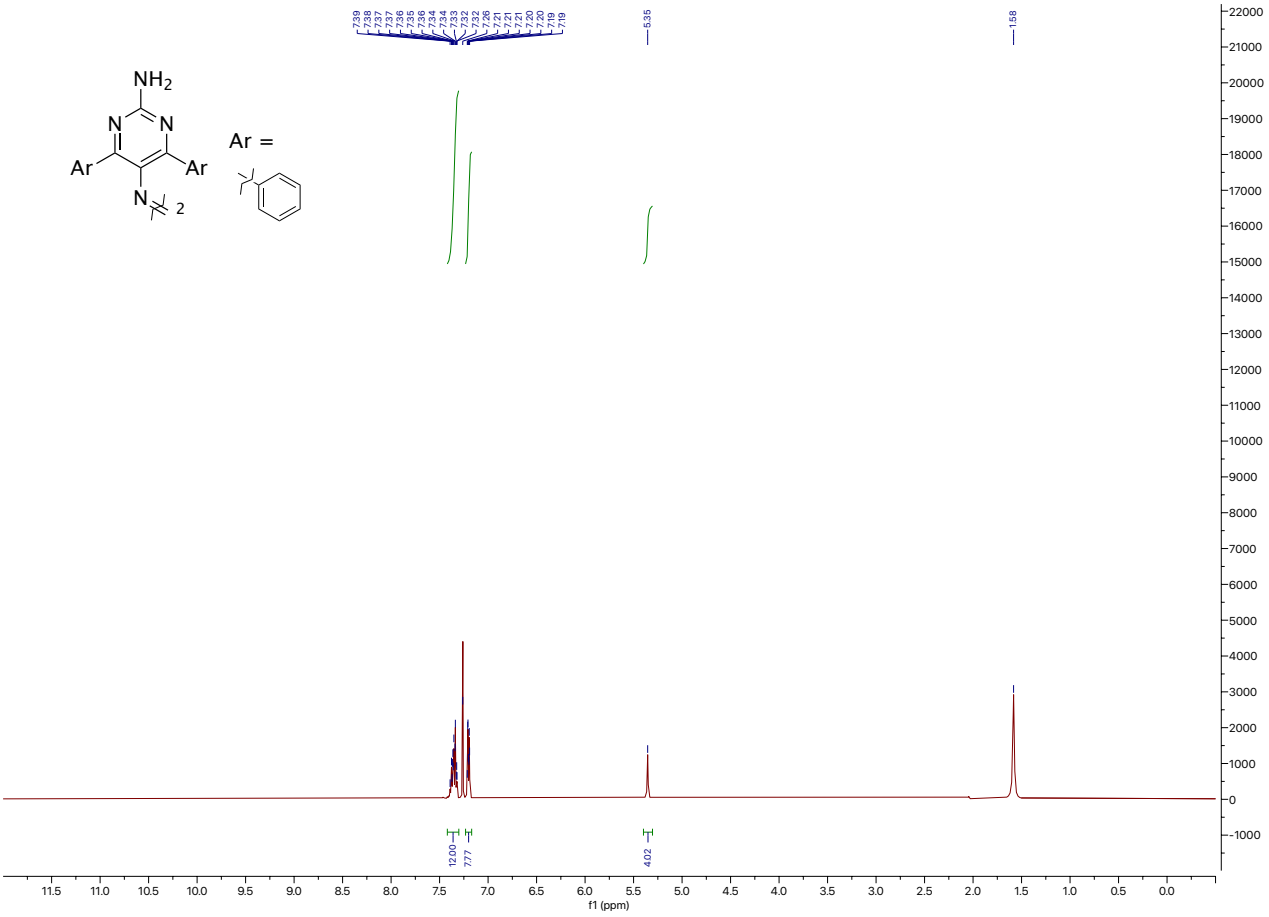


^{19}F NMR (471 MHz, $CDCl_3$)

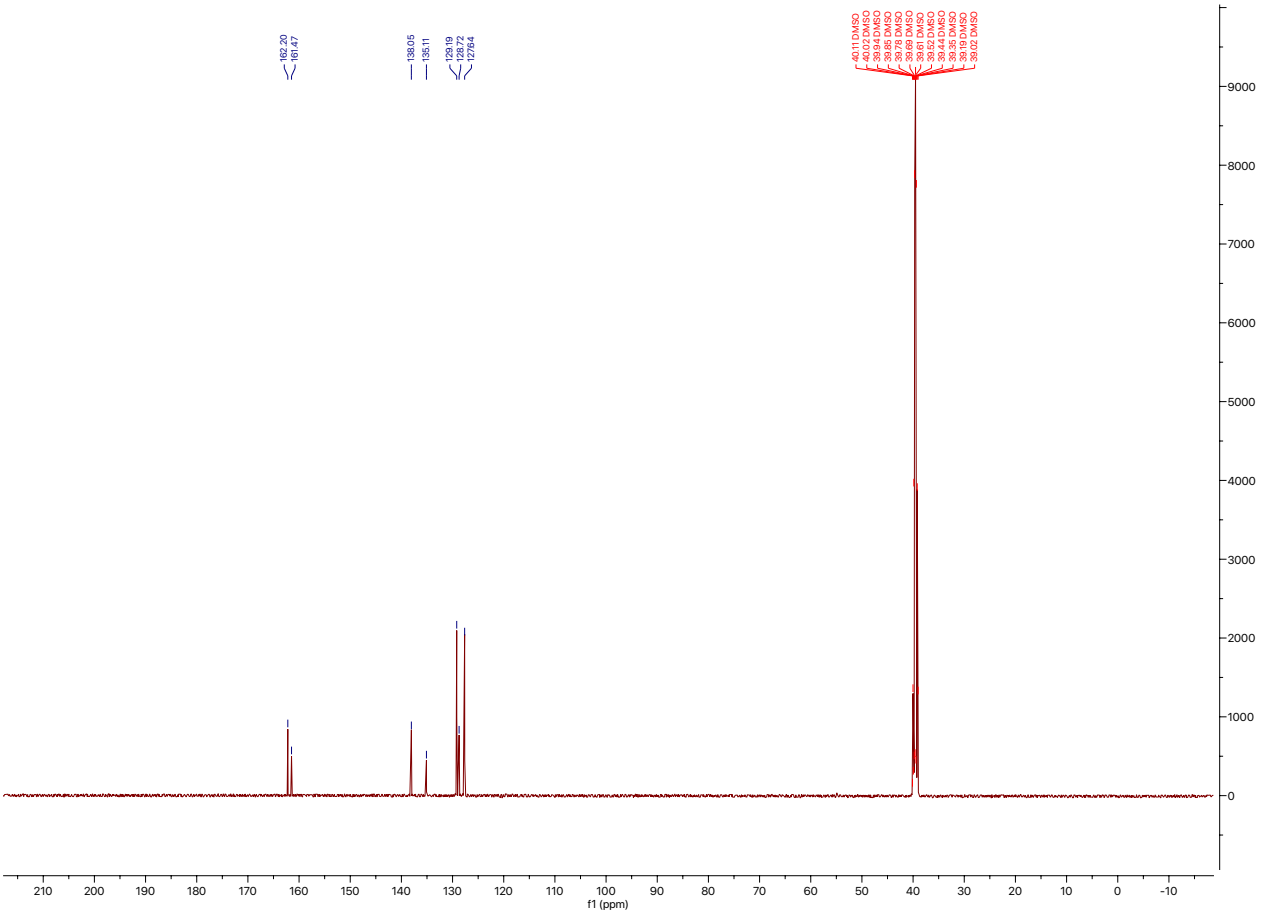


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-bisphenyl)pyrimidine-2-amine) (2b)

^{1}H NMR (500 MHz, $CDCl_3$)

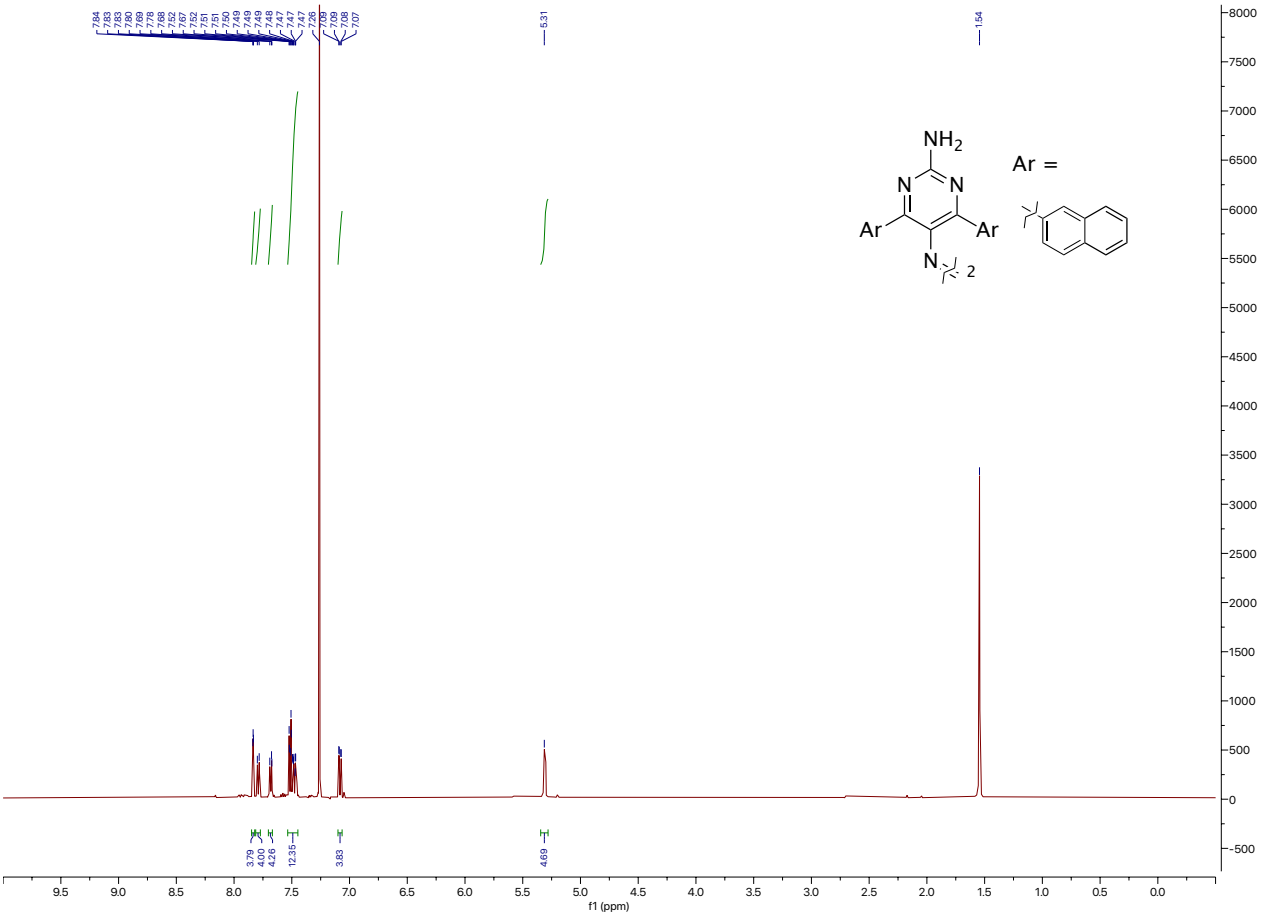


^{13}C NMR (151 MHz, $CDCl_3$)

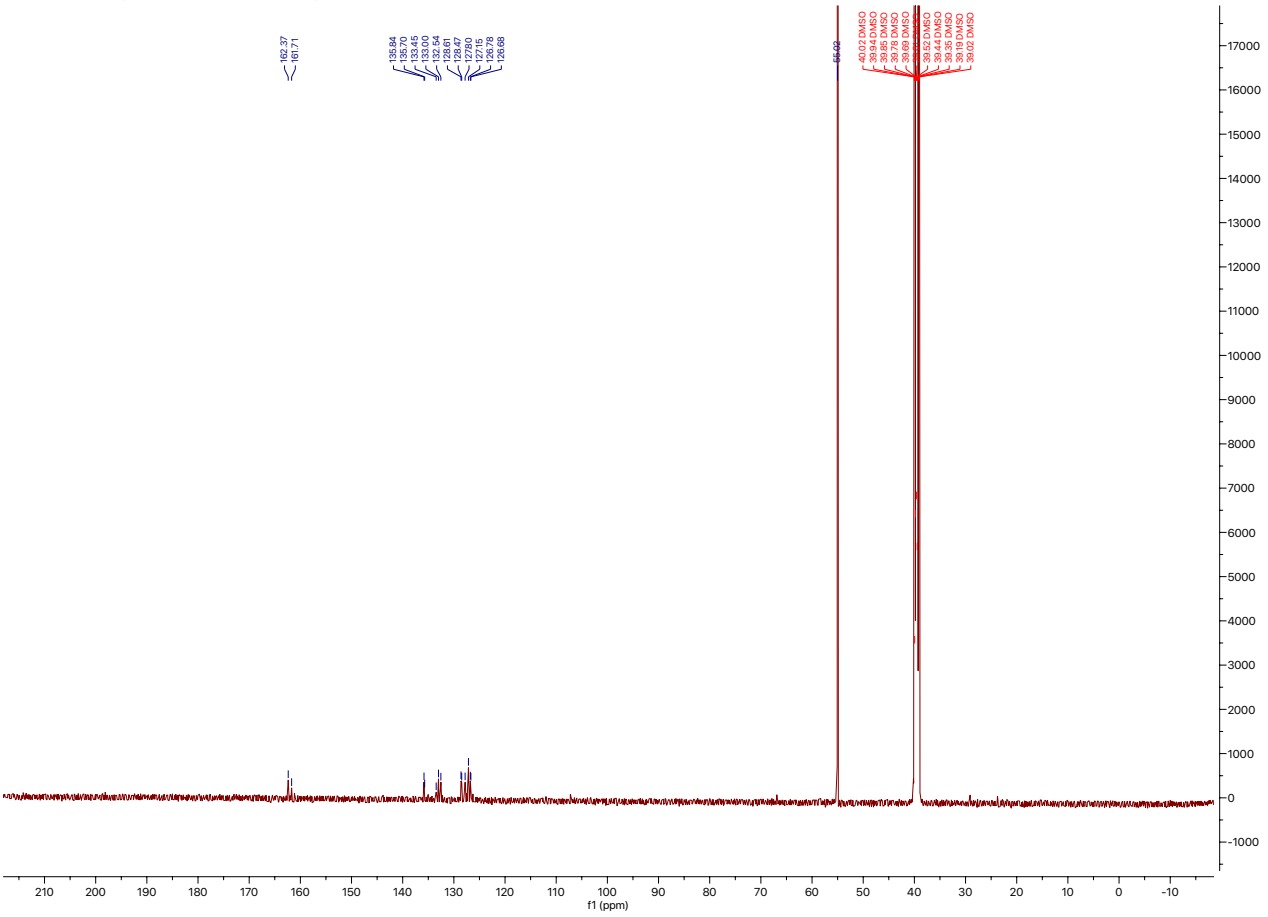


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(naphthalen-2-yl)pyrimidine-2-amine) (2c)

^{1}H NMR (500 MHz, $CDCl_3$)

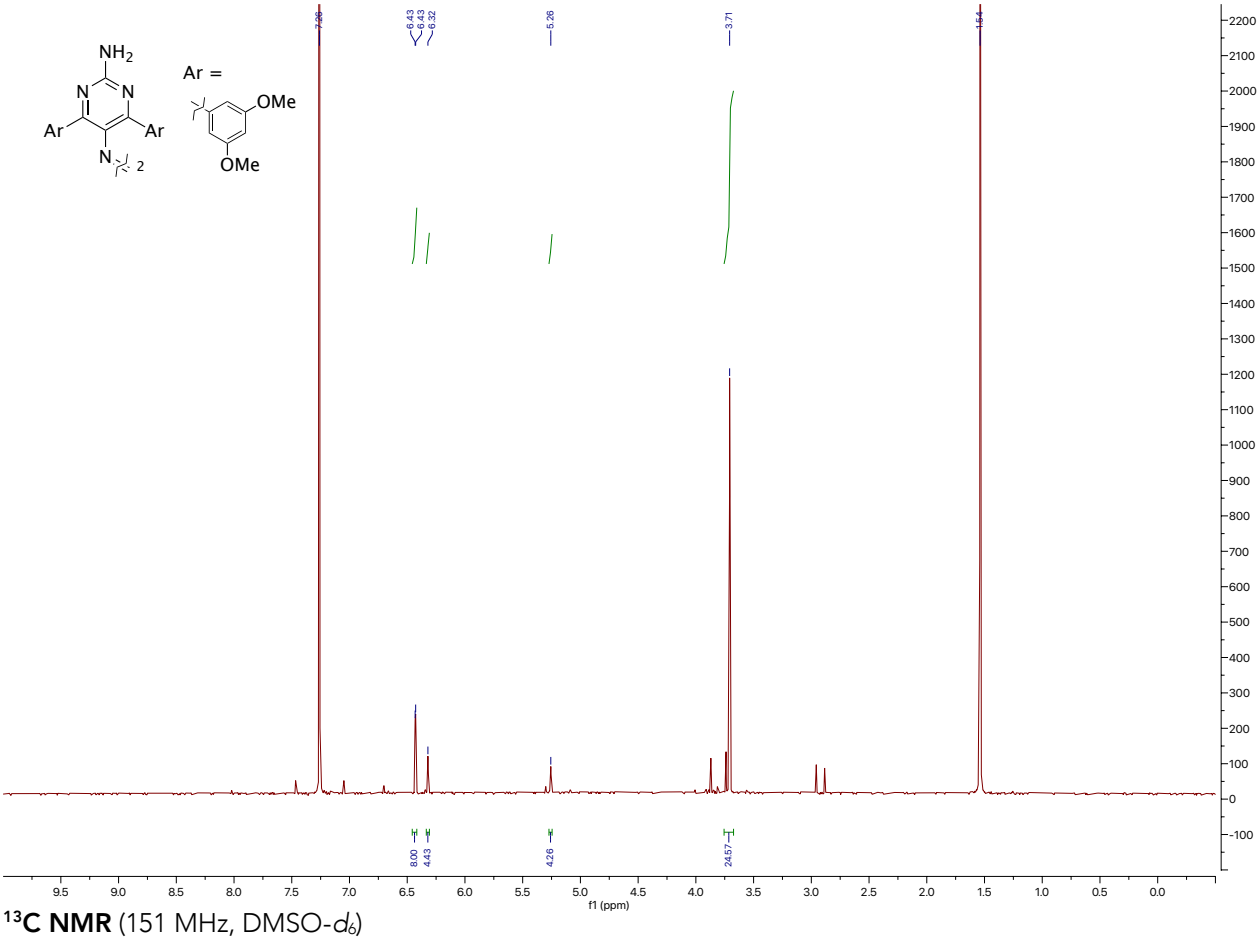


^{13}C NMR (151 MHz, $CDCl_3$)

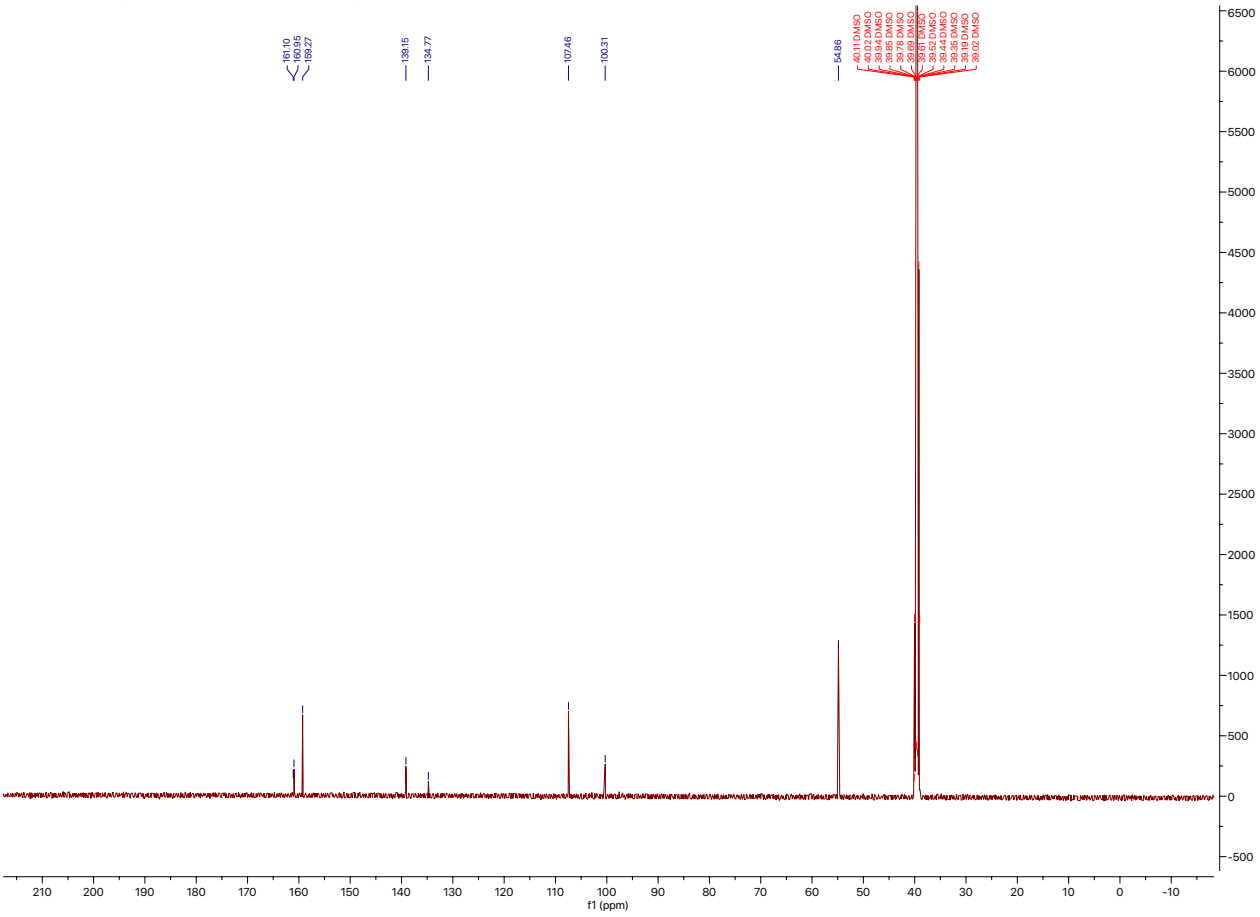


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(3,5-dimethoxyphenyl)pyrimidine-2-amine) (2d)

^{1}H NMR (500 MHz, $CDCl_3$)

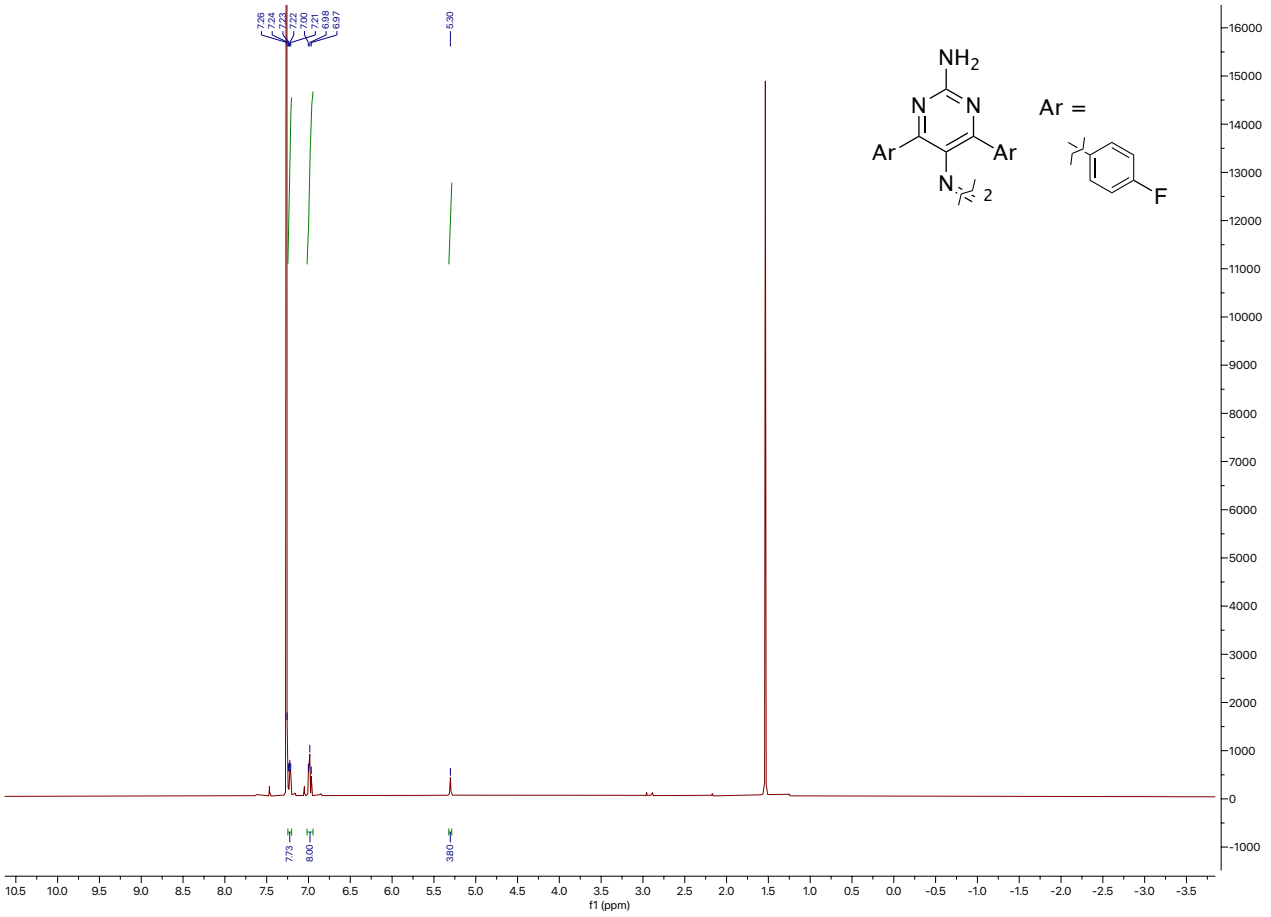


^{13}C NMR (151 MHz, DMSO-d_6)

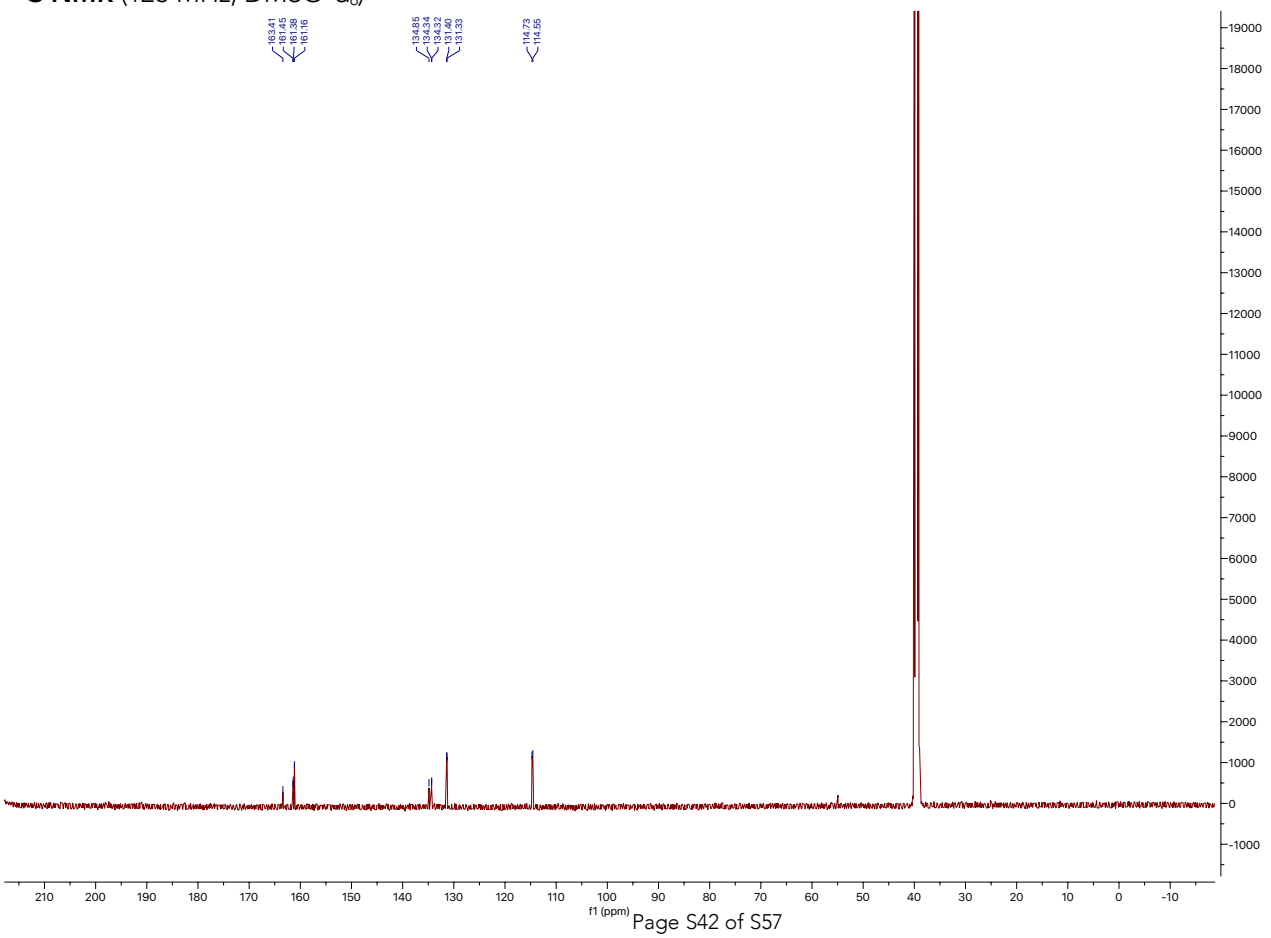


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(4-fluorophenyl)pyrimidine-2-amine) (2e)

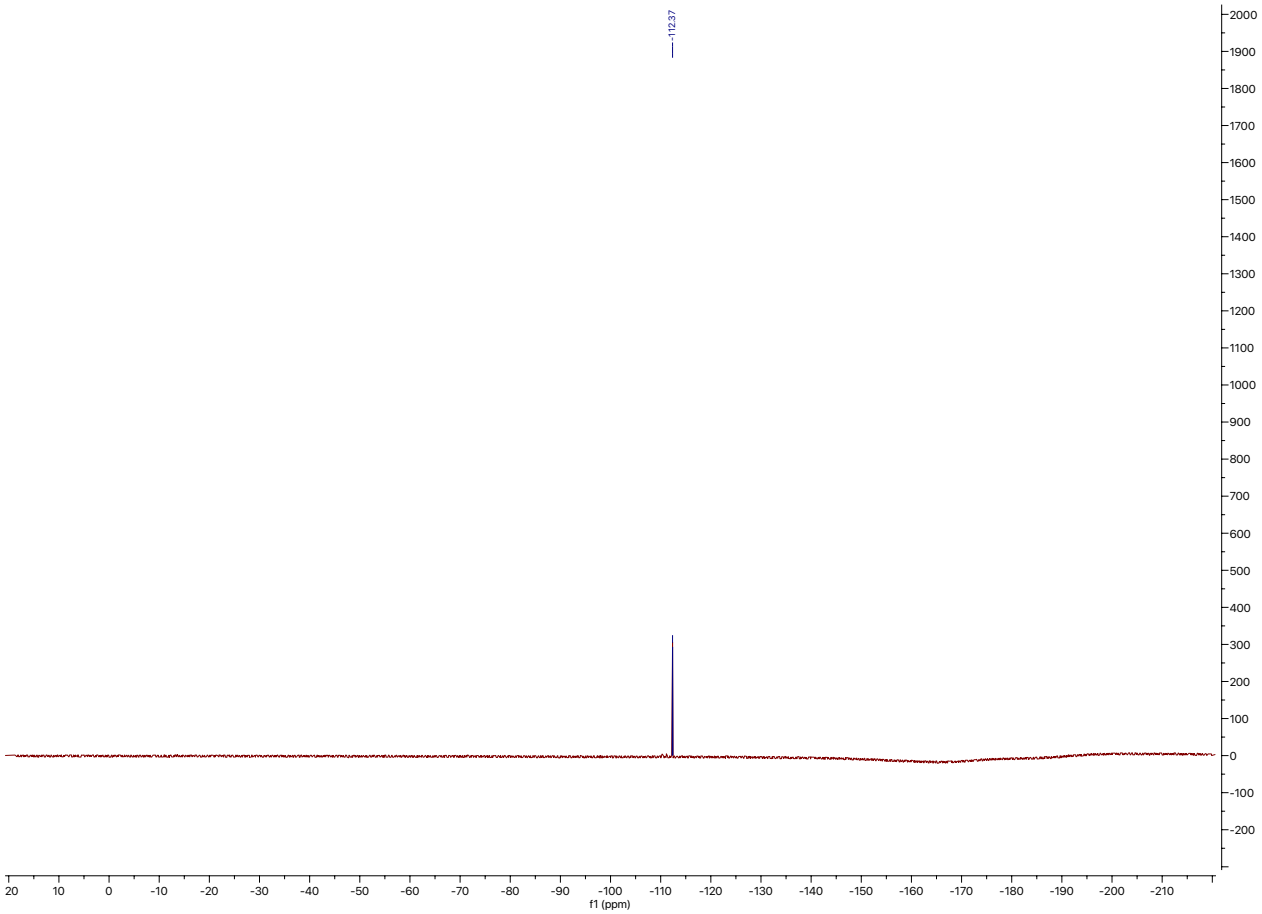
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, DMSO-d_6)

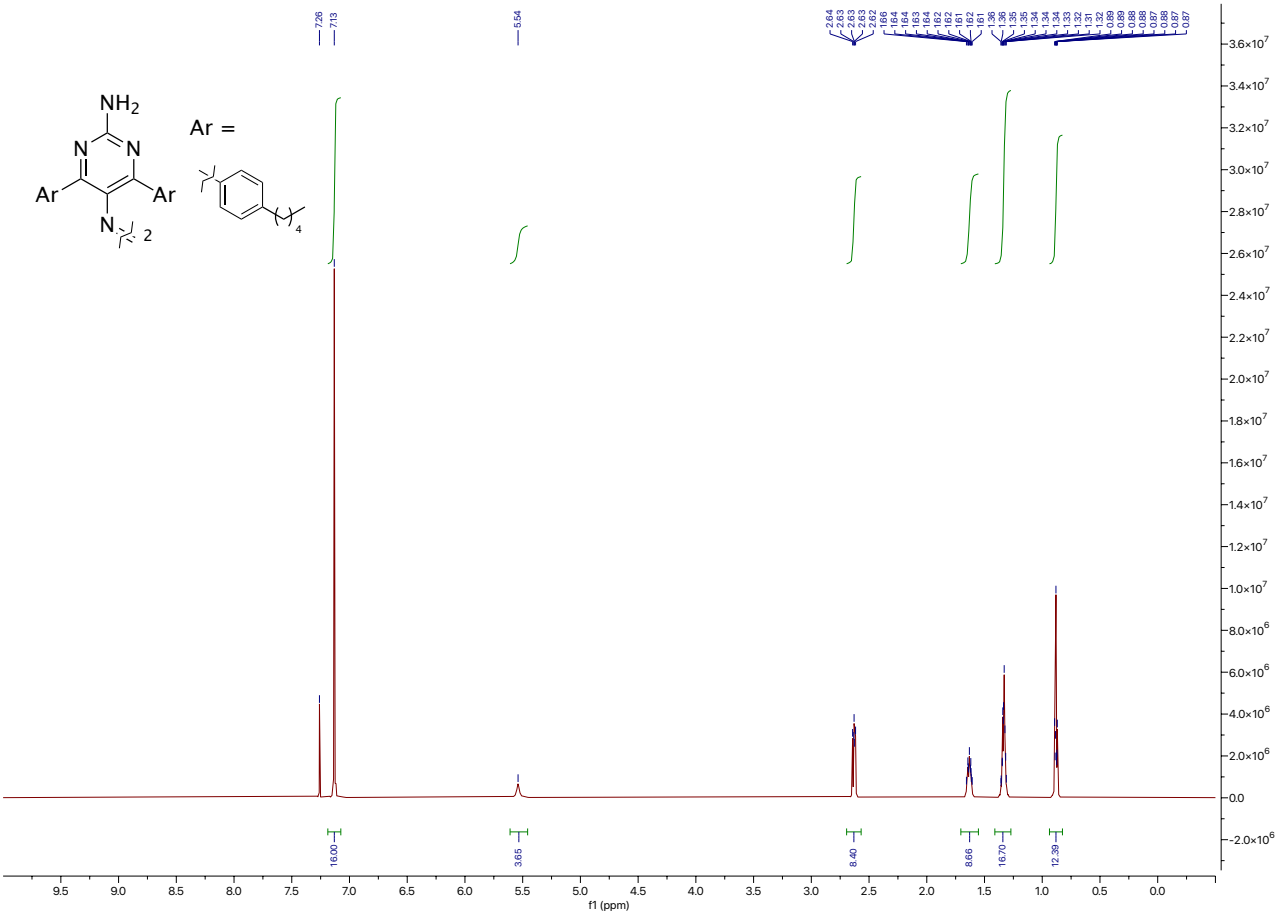


^{19}F NMR (471 MHz, DMSO-d_6)

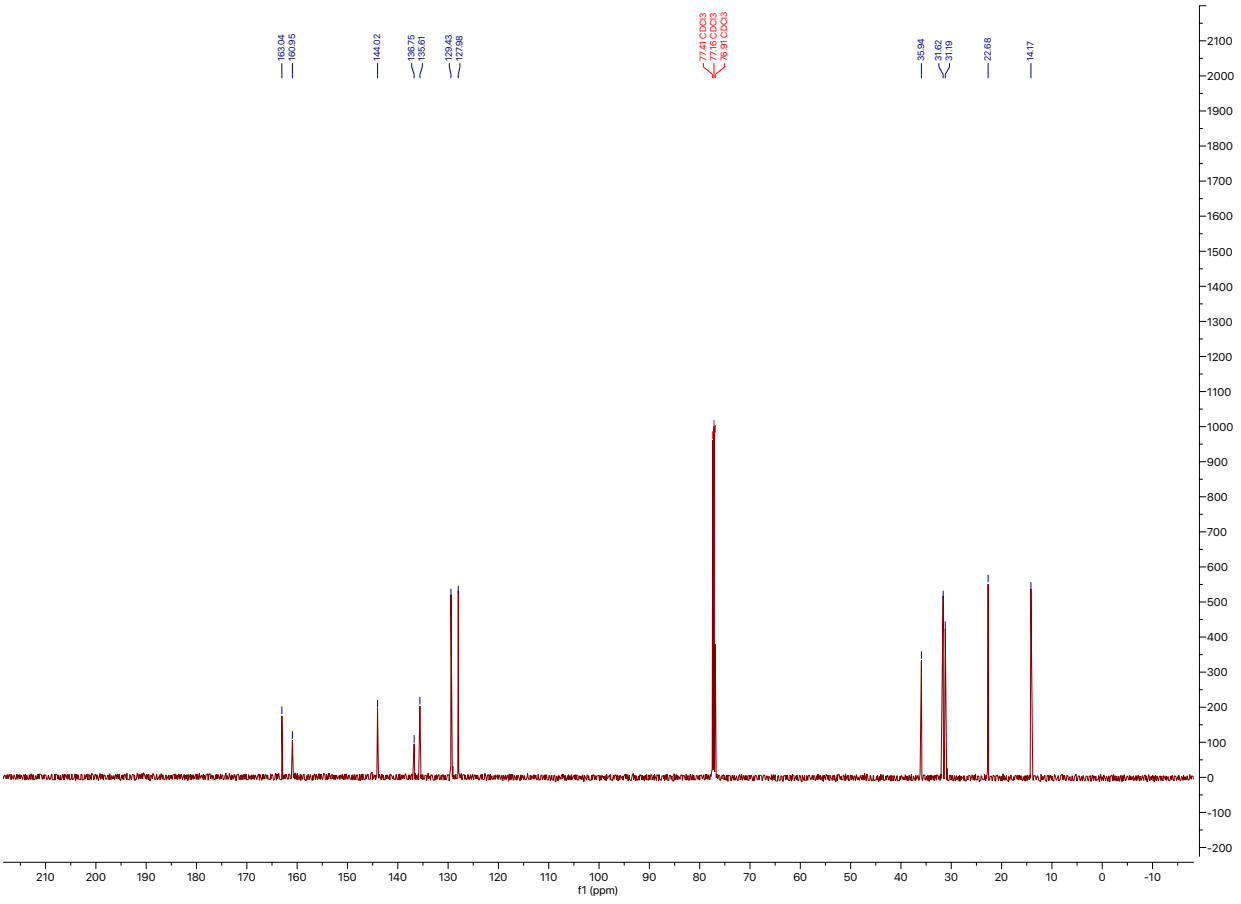


5,5'-(Diazene-1,2-diyl)bis(4,6-bis(4-pentylphenyl)pyrimidine-2-amine) (2f)

^{1}H NMR (600 MHz, $CDCl_3$)

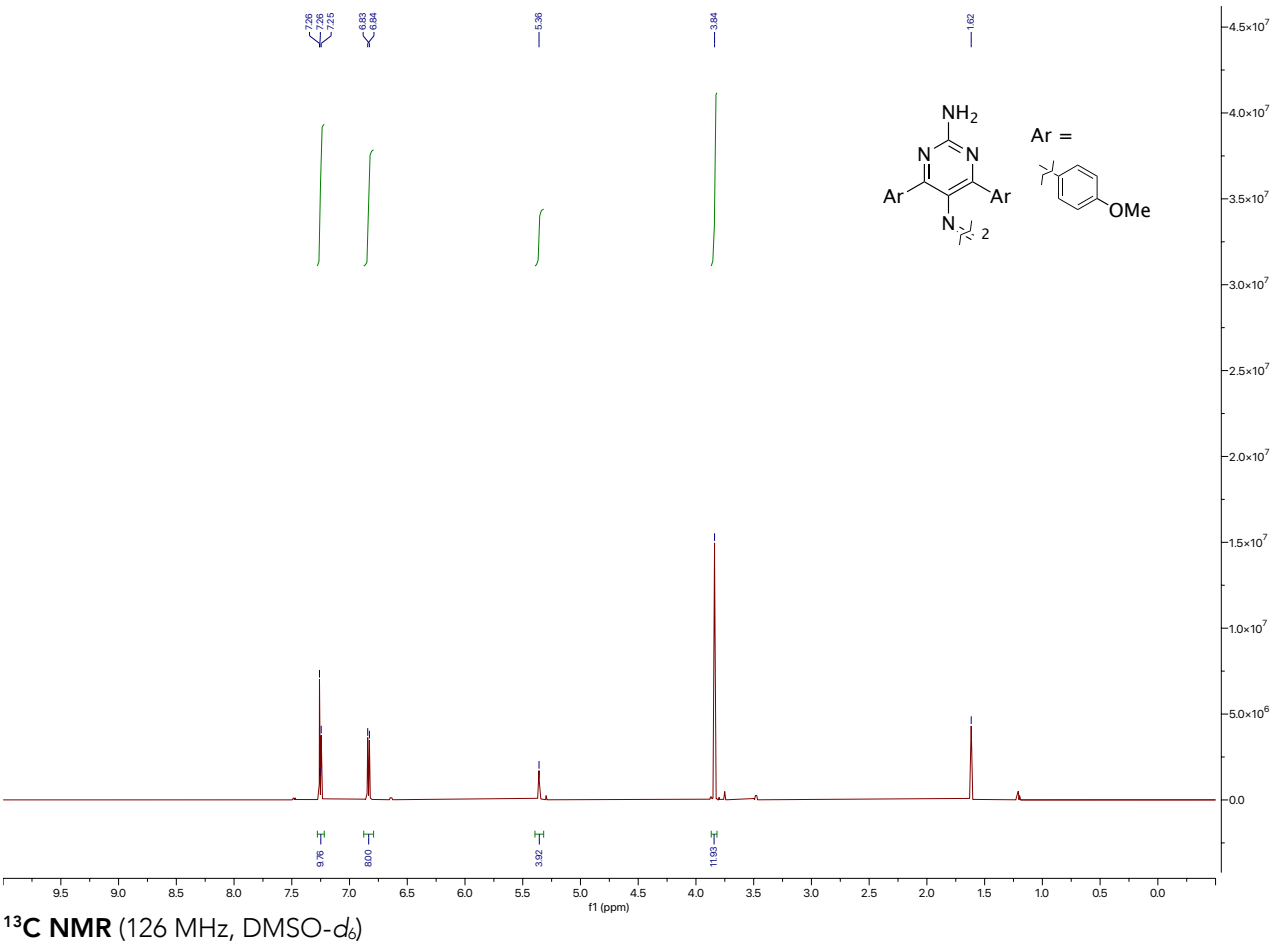


^{13}C NMR (126 MHz, $CDCl_3$)

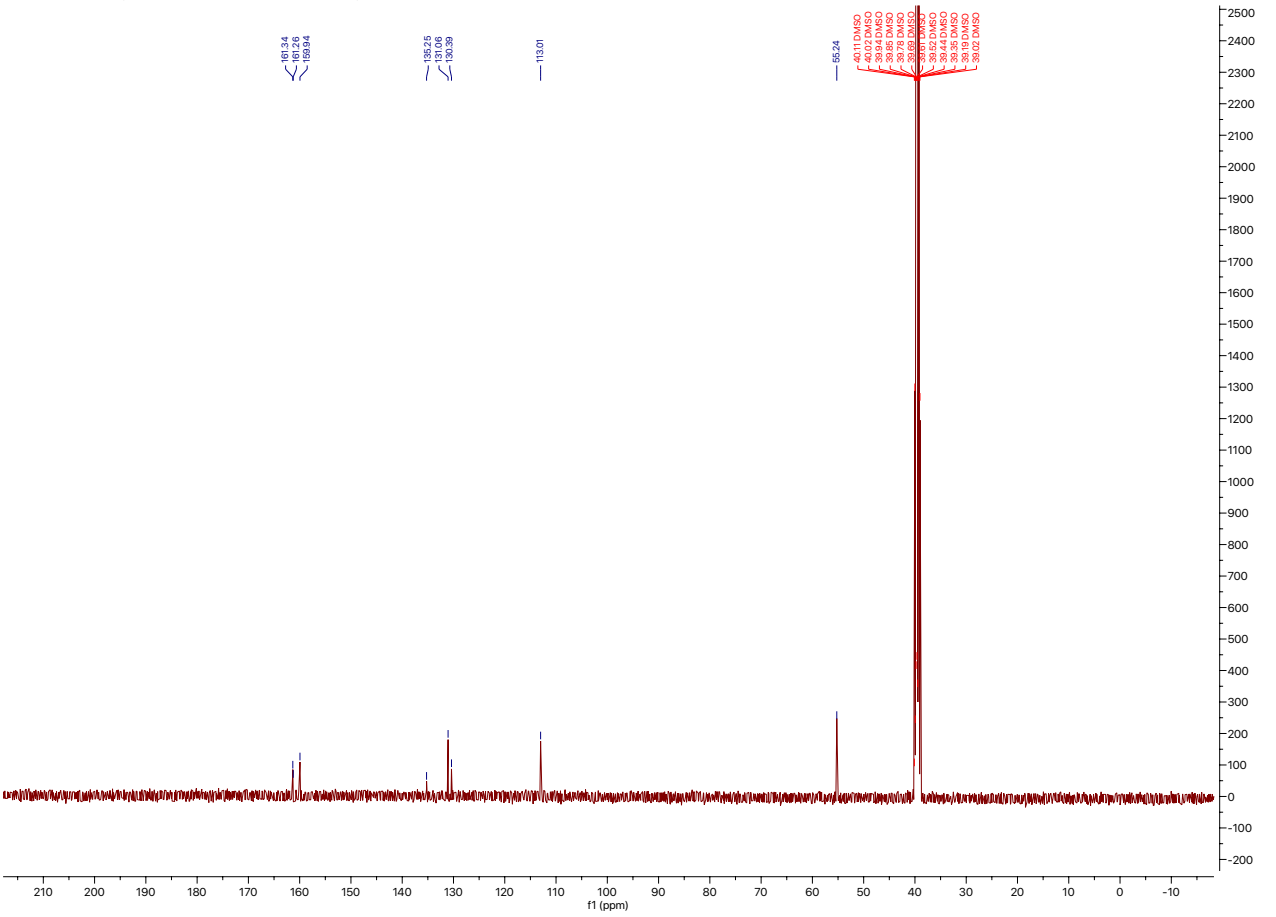


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(4-methoxyphenyl)pyrimidine-2-amine) (2g)

^{1}H NMR (600 MHz, $CDCl_3$)

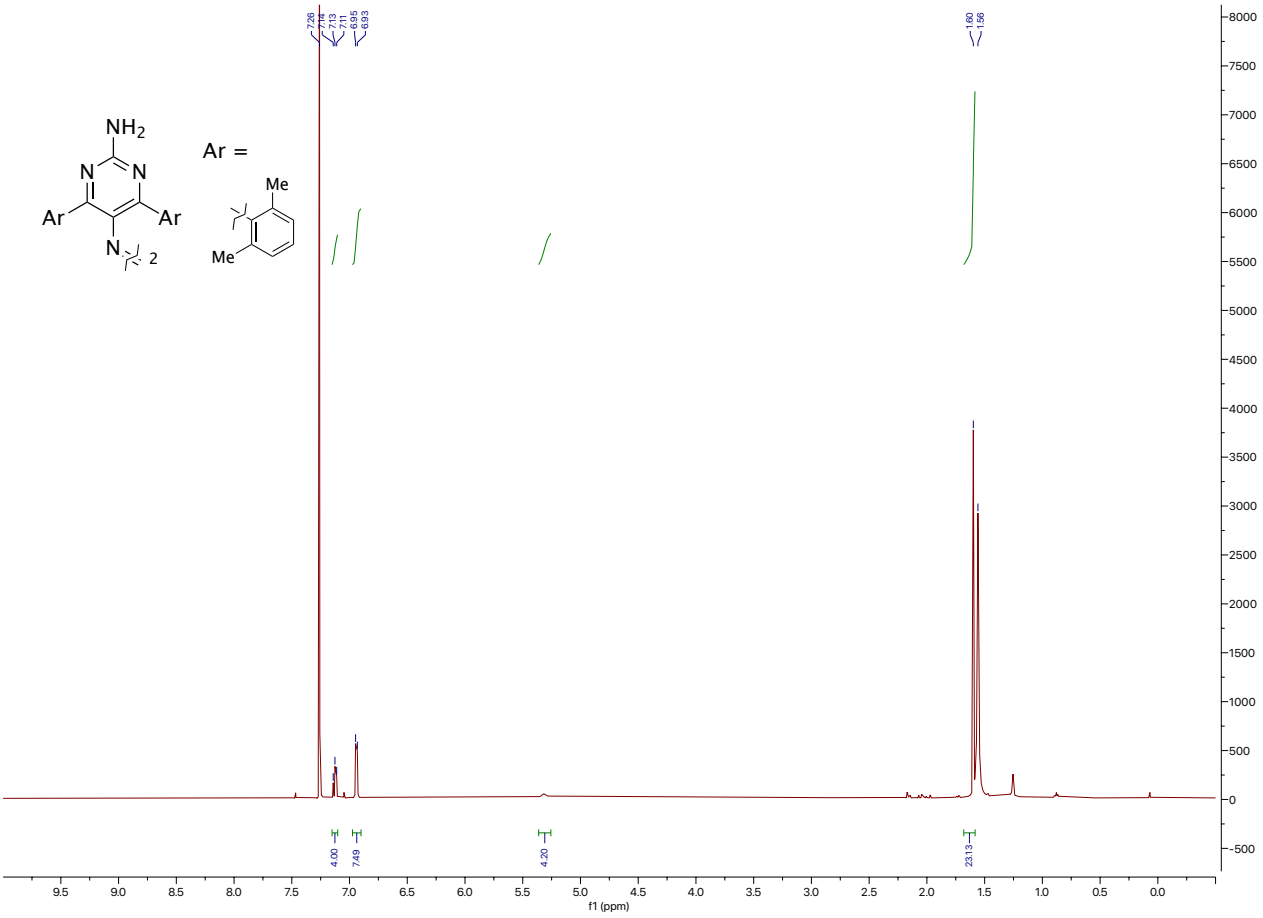


^{13}C NMR (126 MHz, DMSO-d_6)

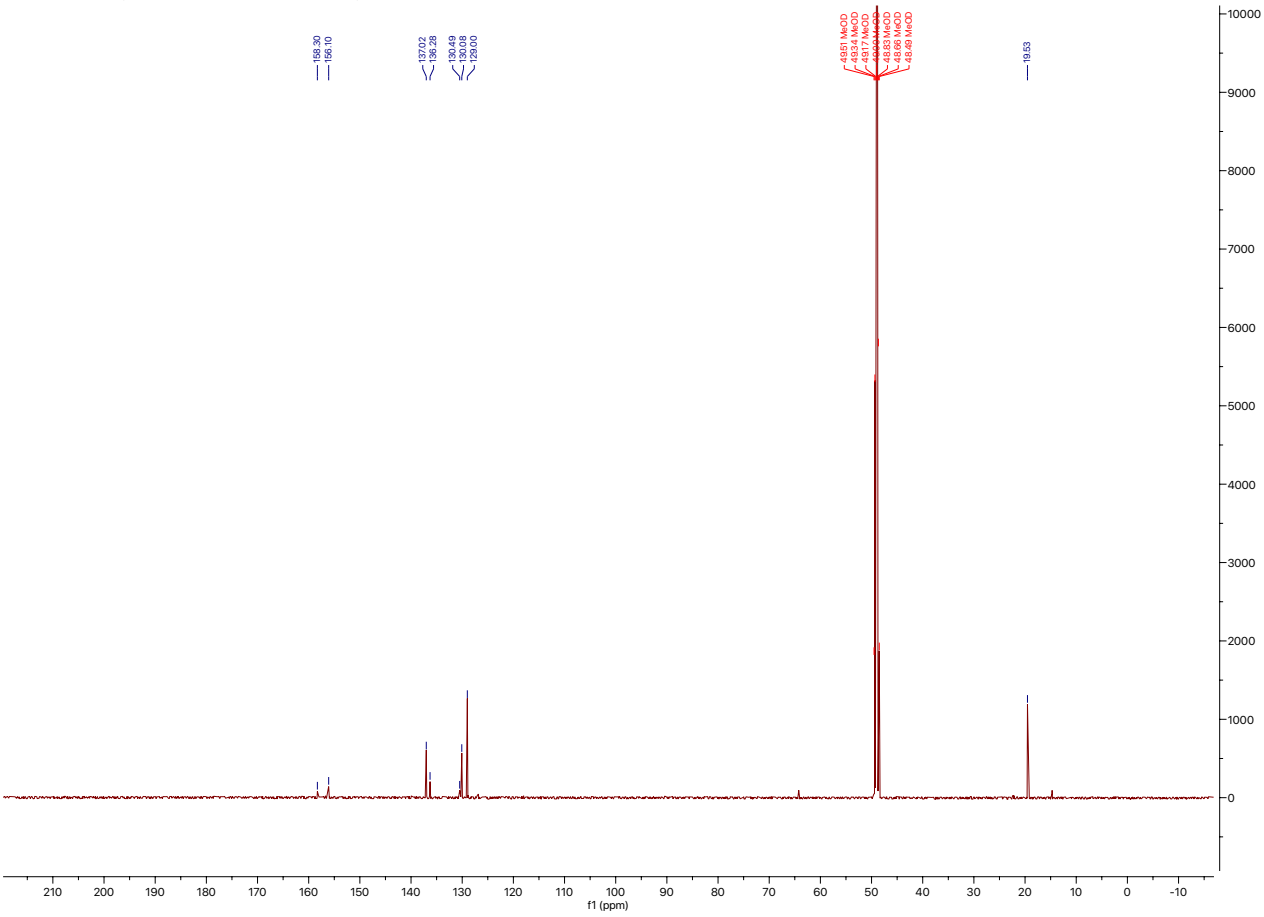


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(2,6-dimethylphenyl)pyrimidine-2-amine) (2h)

^{1}H NMR (500 MHz, $CDCl_3$)

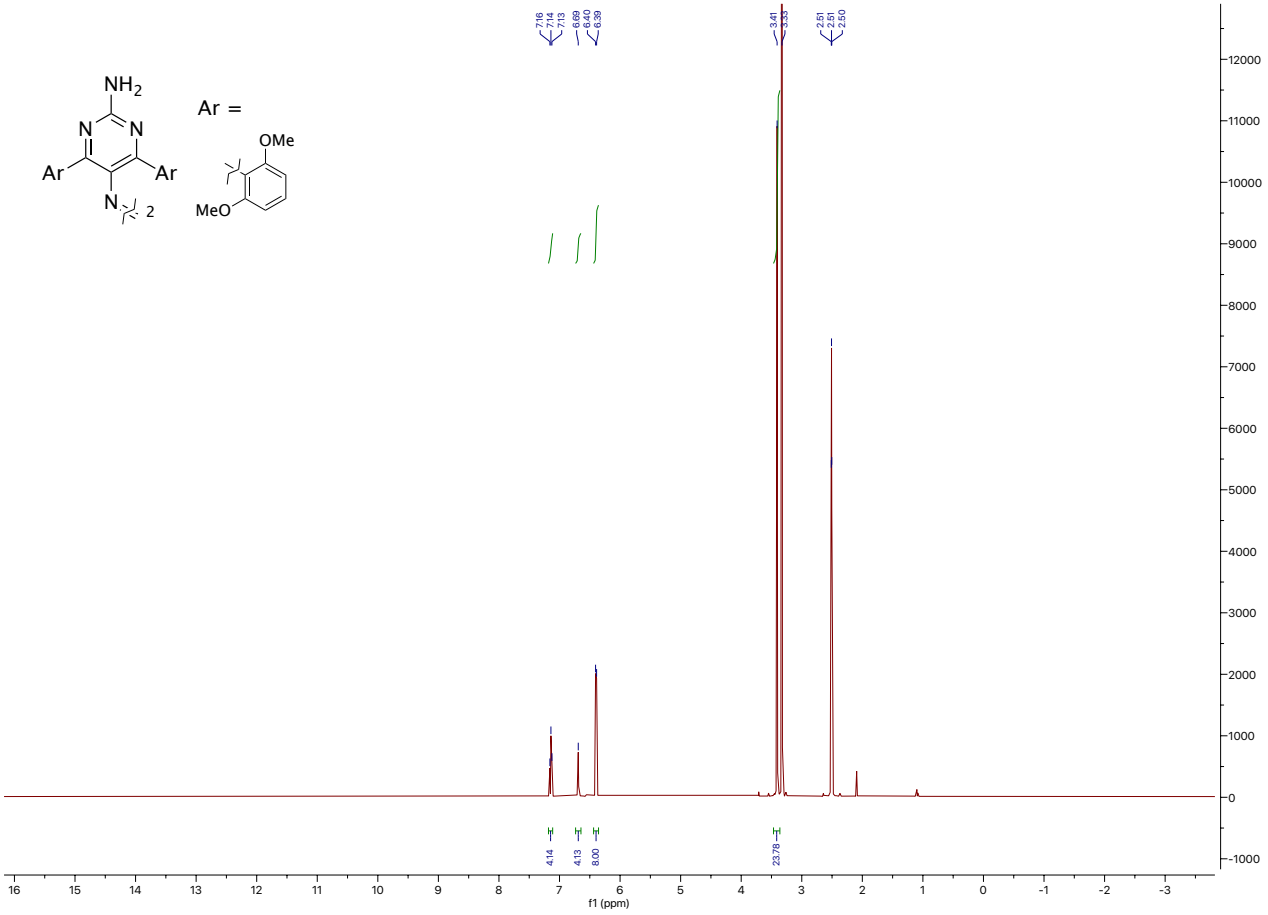


^{13}C NMR (126 MHz, DMSO-d_6)

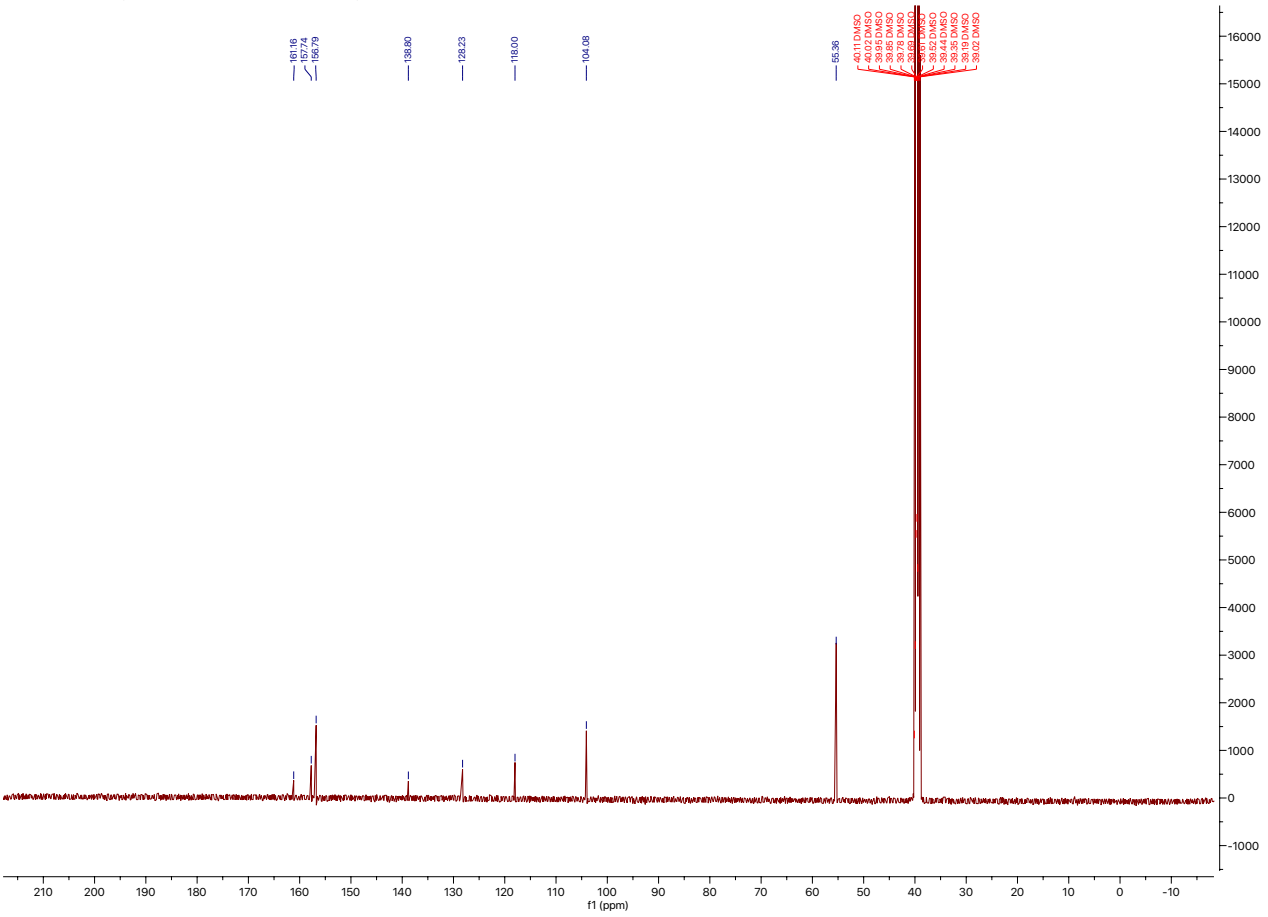


5,5'-(Diazene-1,2-diyl)bis(4,6-Bis(2,6-dimethoxyphenyl)pyrimidine-2-amine) (2i)

^{1}H NMR (500 MHz, DMSO-d_6)

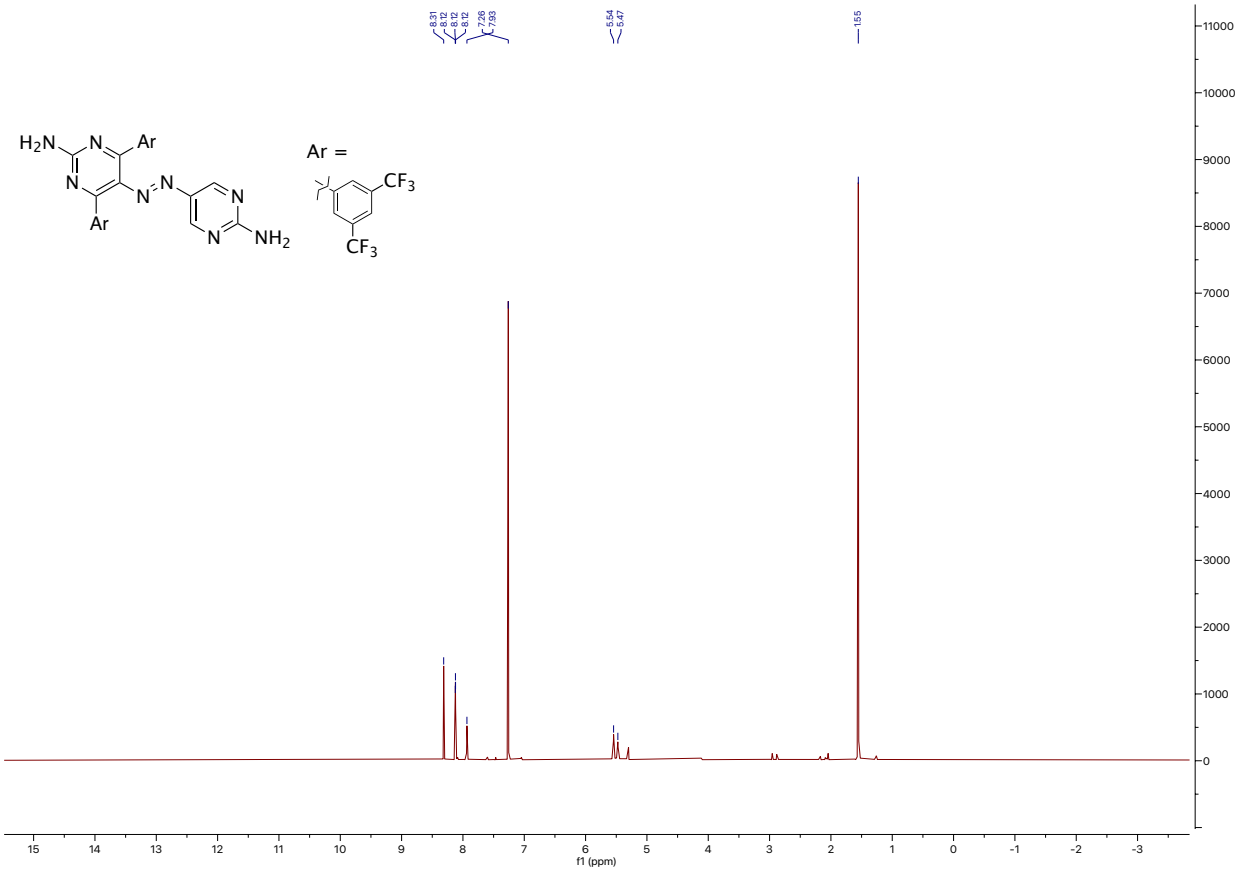


^{13}C NMR (126 MHz, DMSO-d_6)

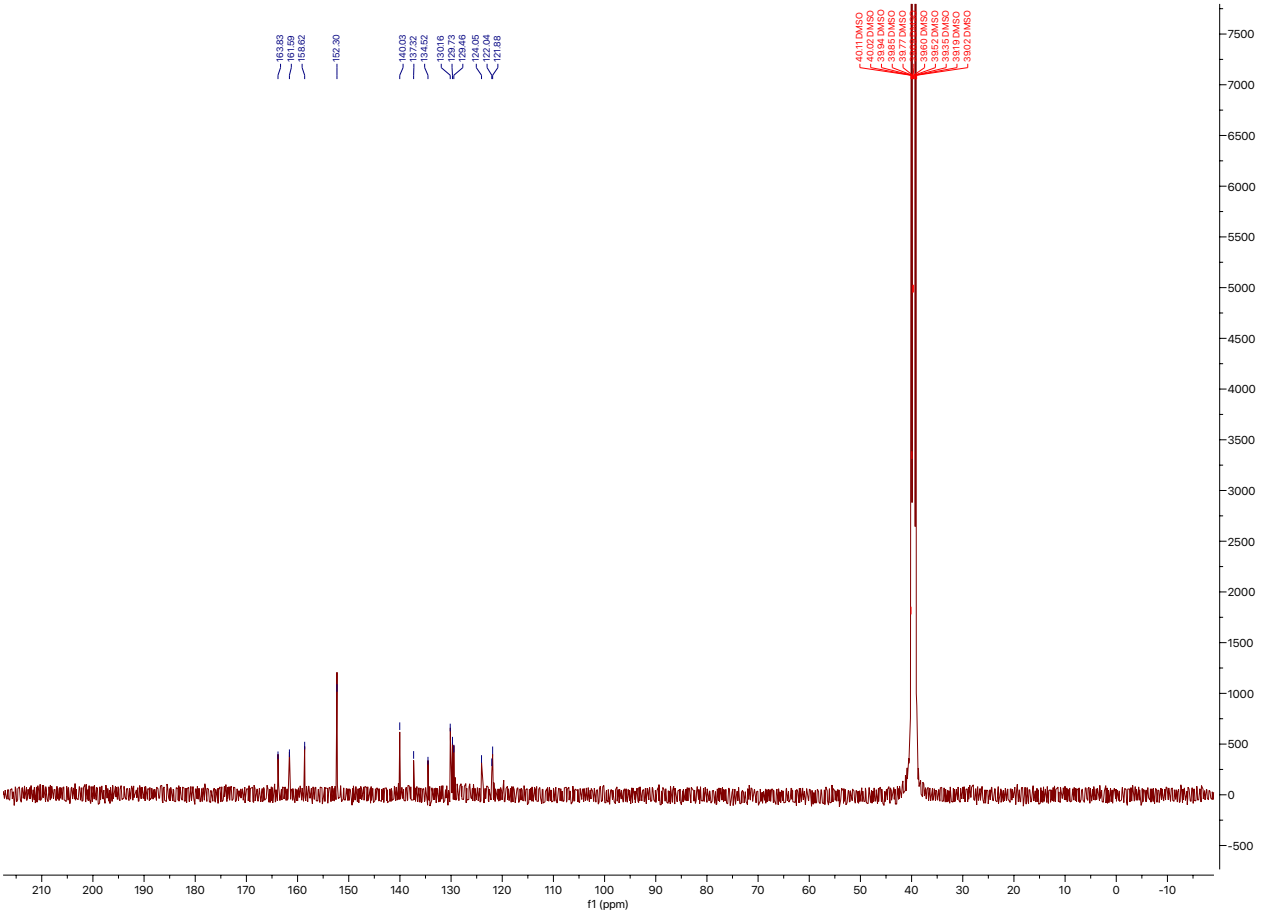


5-((2-Aminopyrimidin-5-yl)diazenyl)-4,6-bis(3,5-bis(trifluoromsethyl)phenyl)pyrimidin-2-amine)(2j)

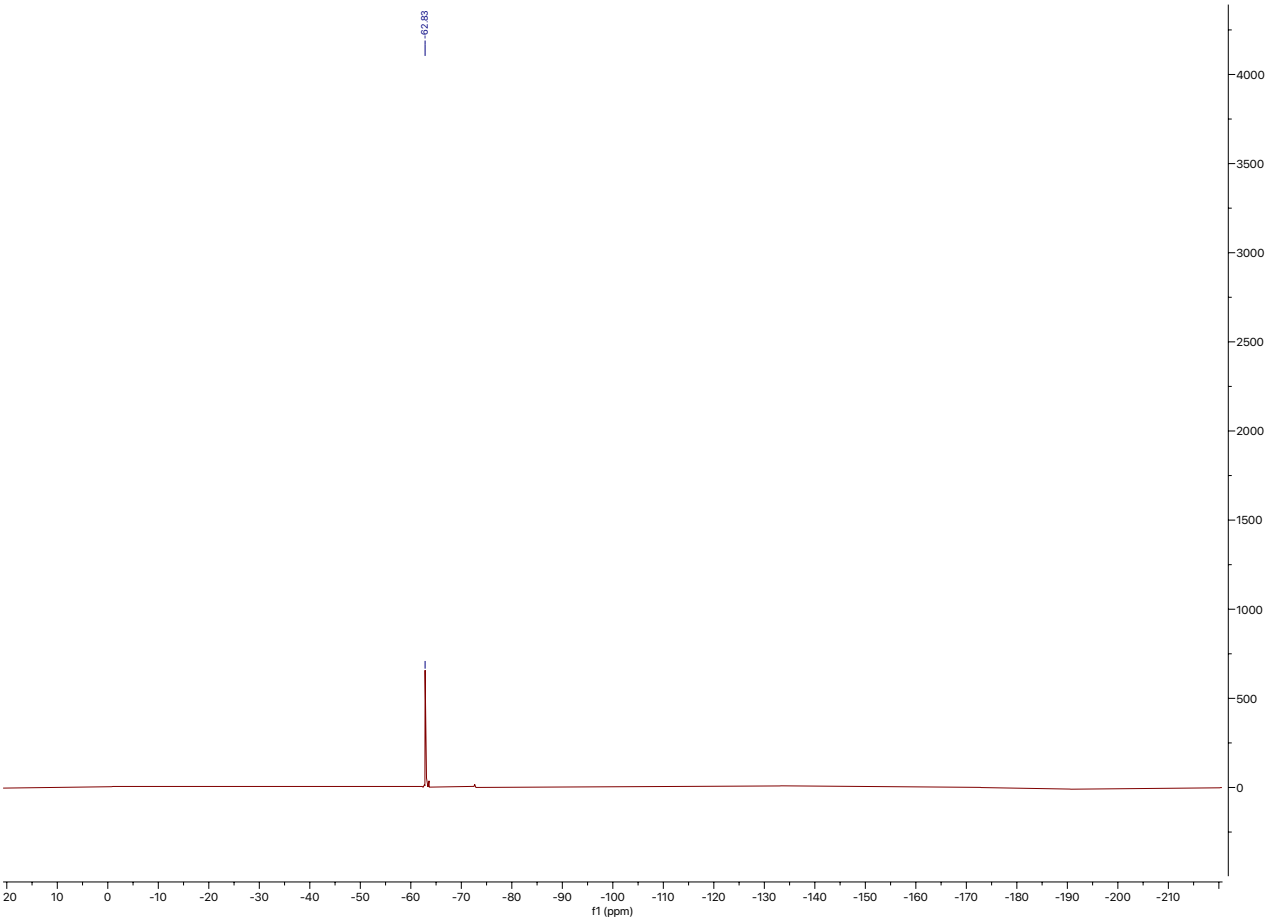
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

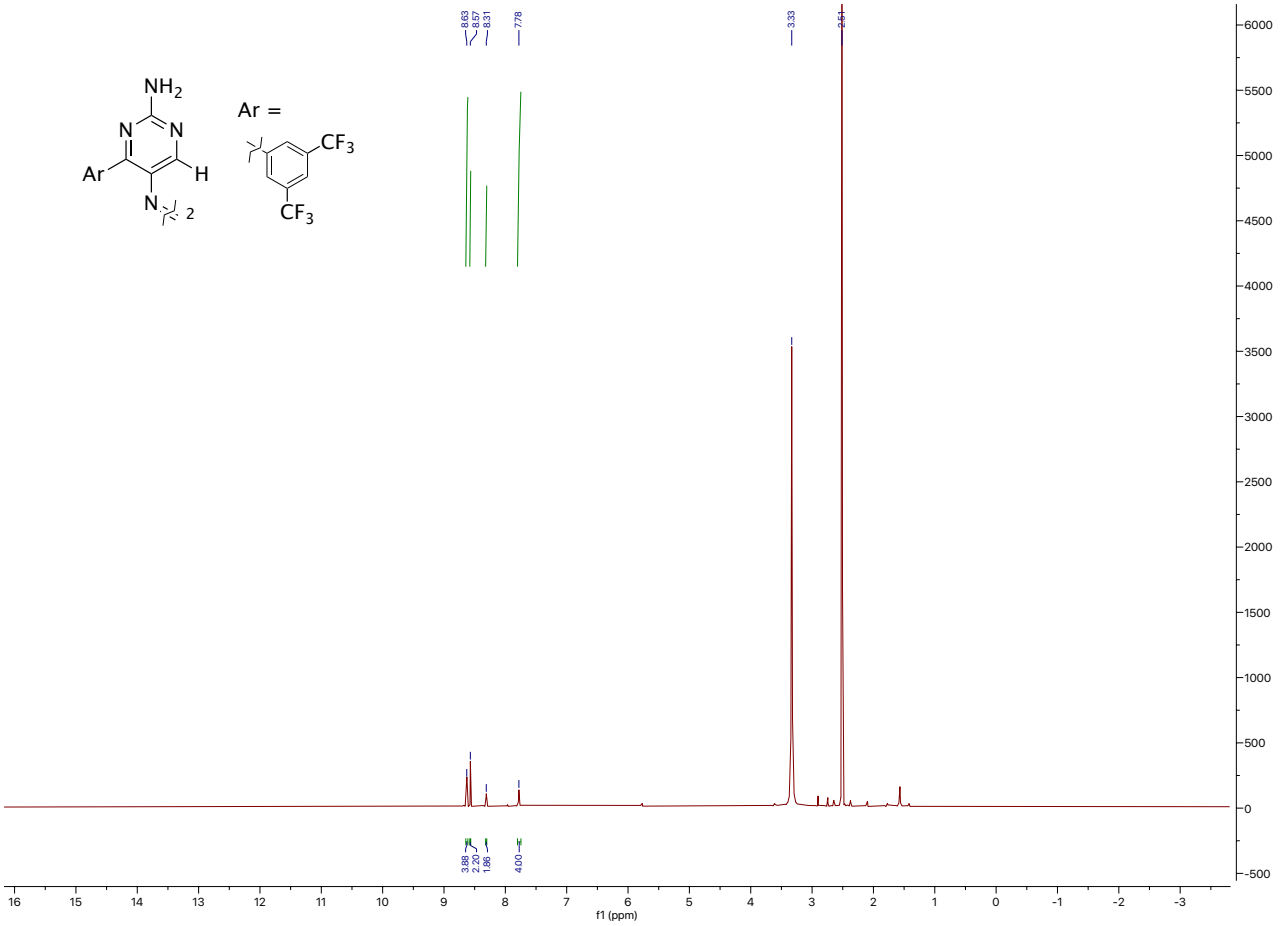


^{19}F NMR (376 MHz, $CDCl_3$)

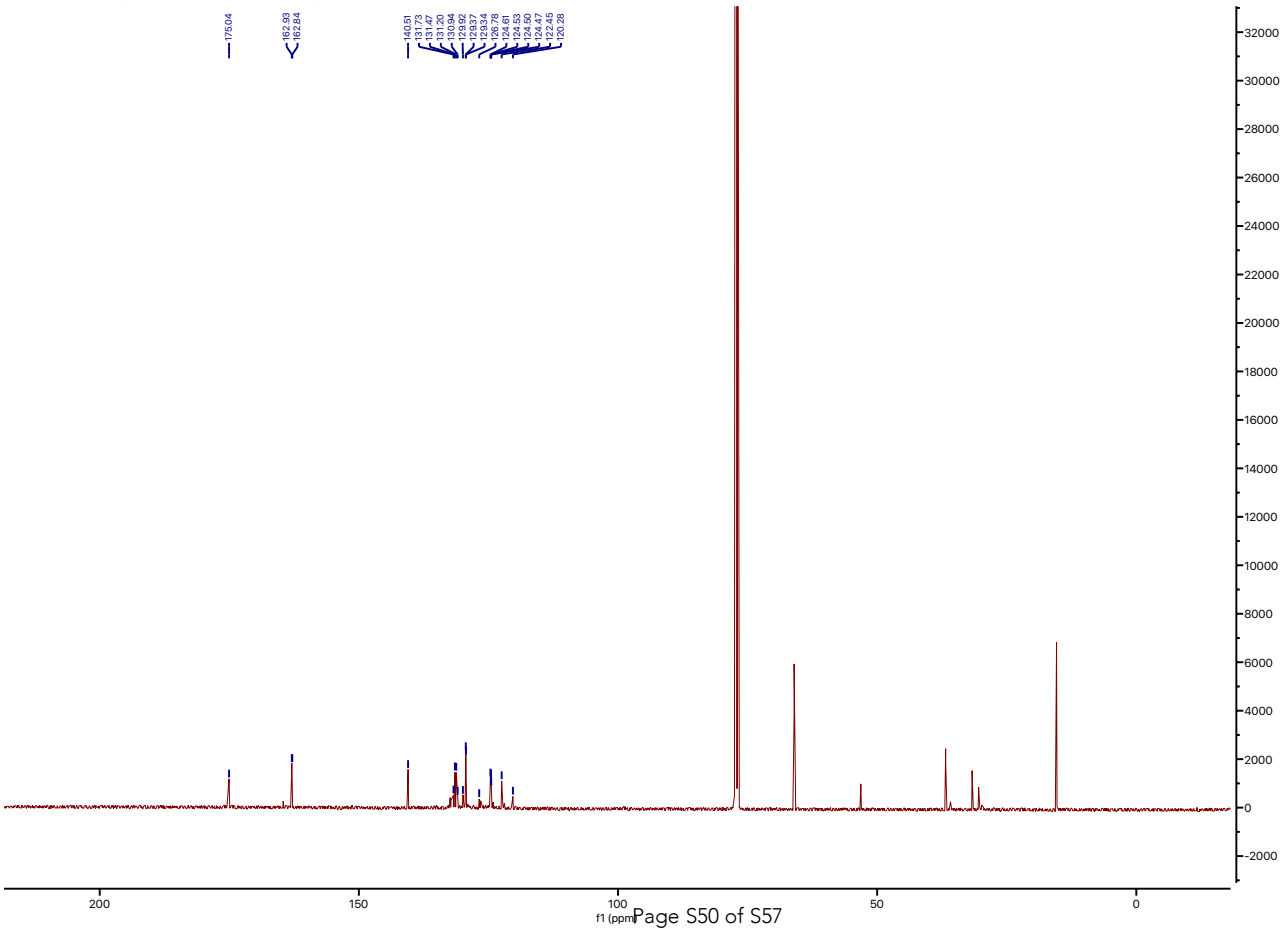


5,5'-(Diazene-1,2-diyl)bis(4-(3,5-bis(trifluoromethyl)phenyl)pyrimidin-2-amine) (2k)

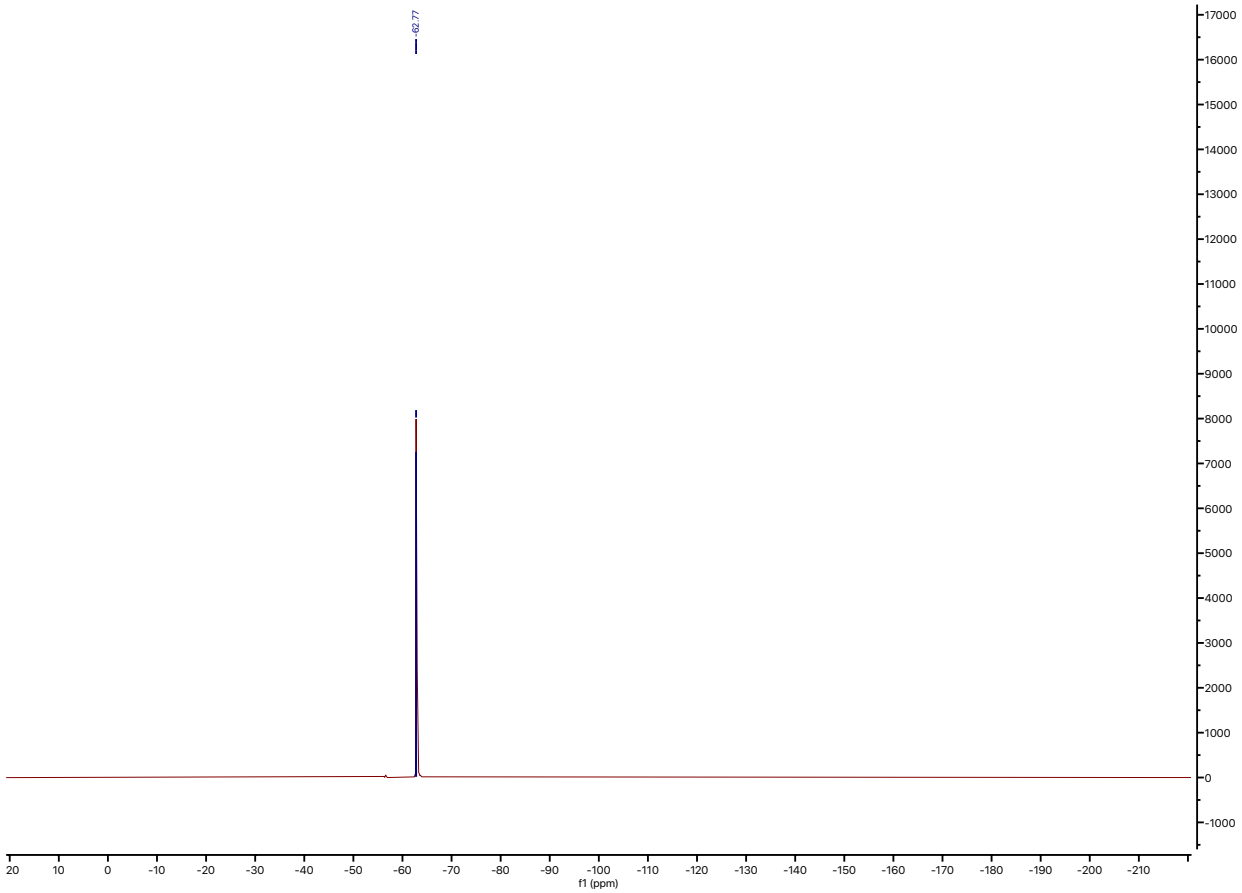
^{1}H NMR (500 MHz, DMSO-d_6)



^{13}C NMR (126 MHz, $CDCl_3$)

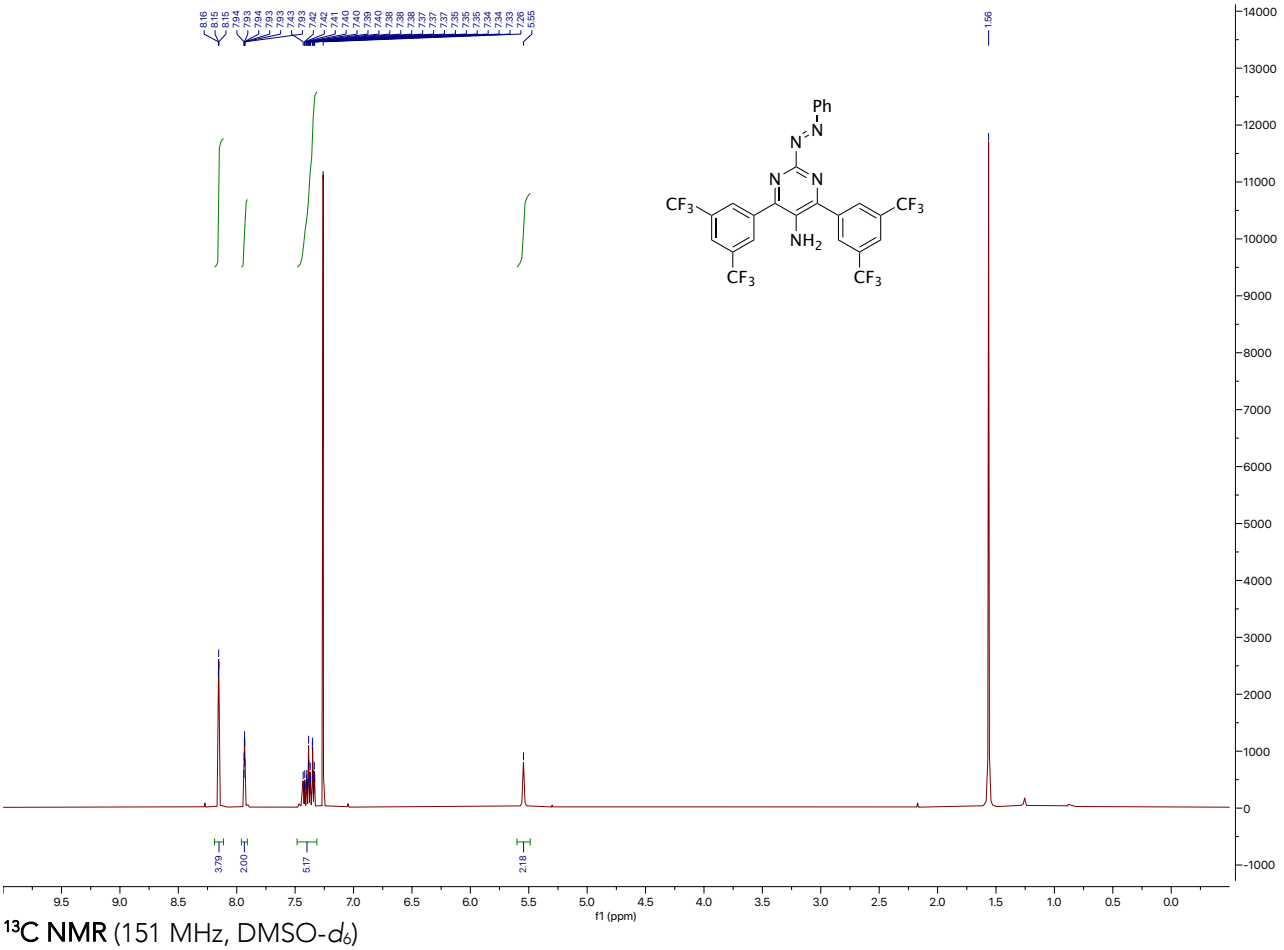


^{19}F NMR (376 MHz, $CDCl_3$)

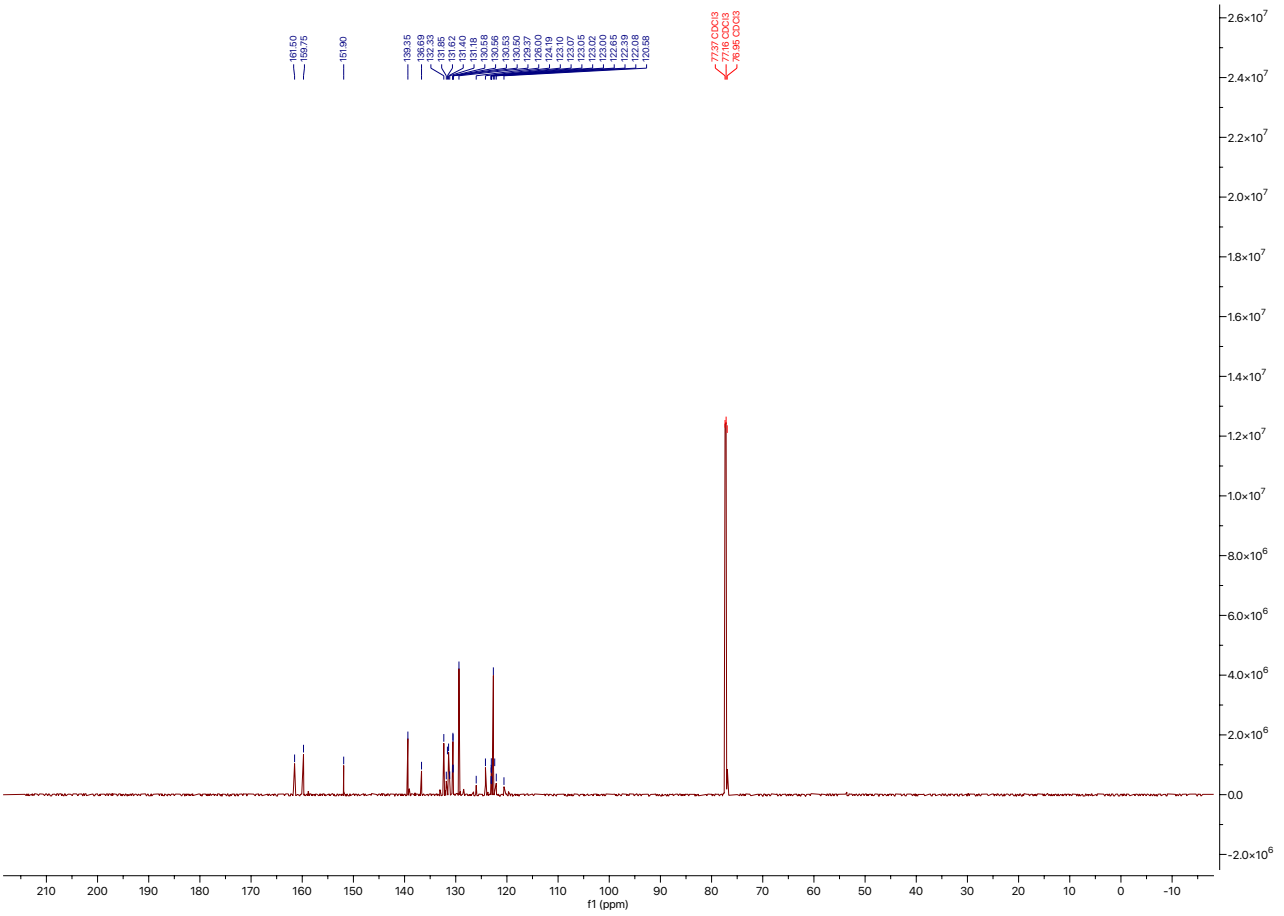


5,5'-(Diazene-1,2-diyl)bis(4-(3,5-bis(4-pentylphenyl)pyrimidin-2-amine)(2l)

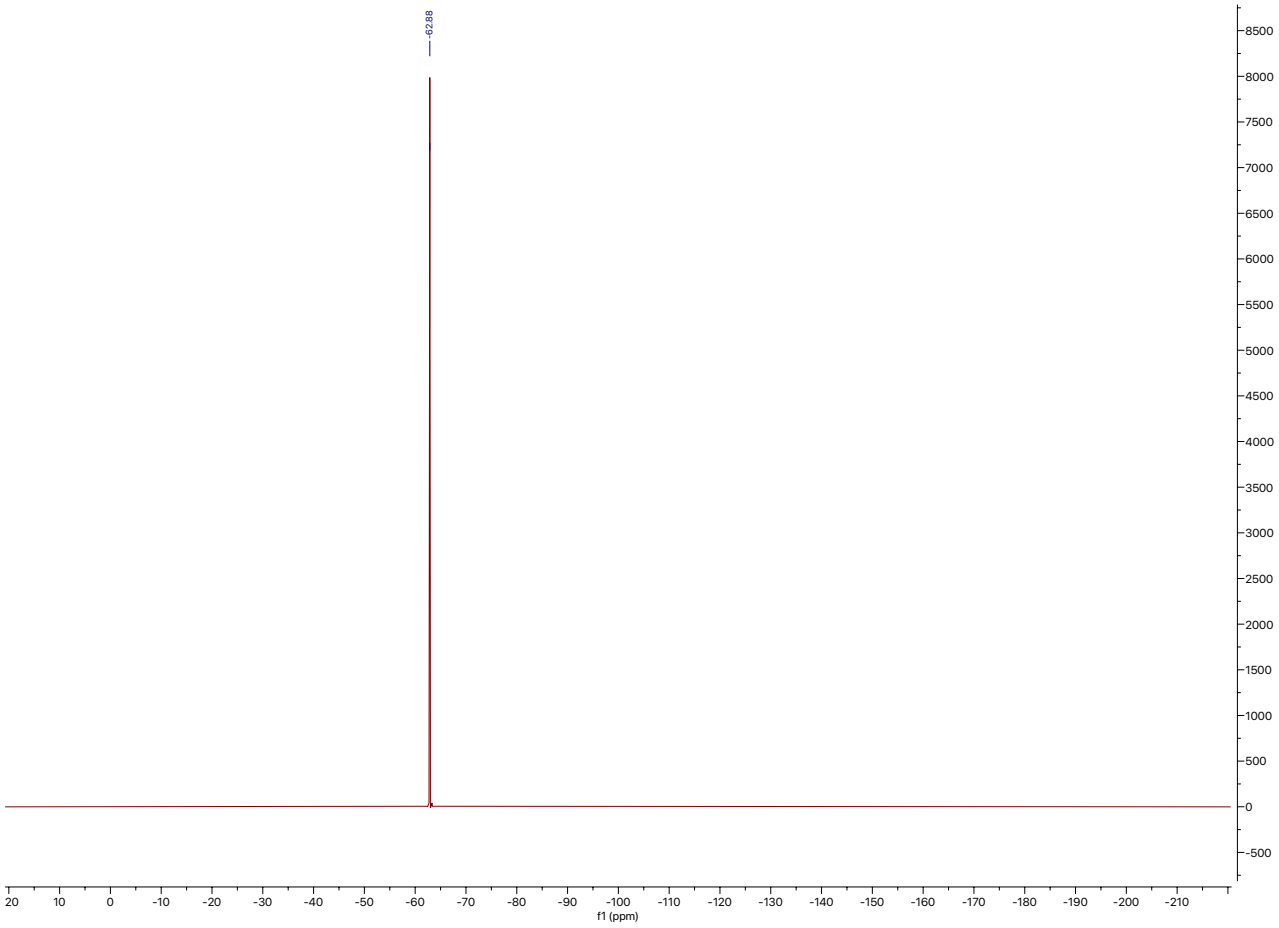
^{1}H NMR (500 MHz, $CDCl_3$)



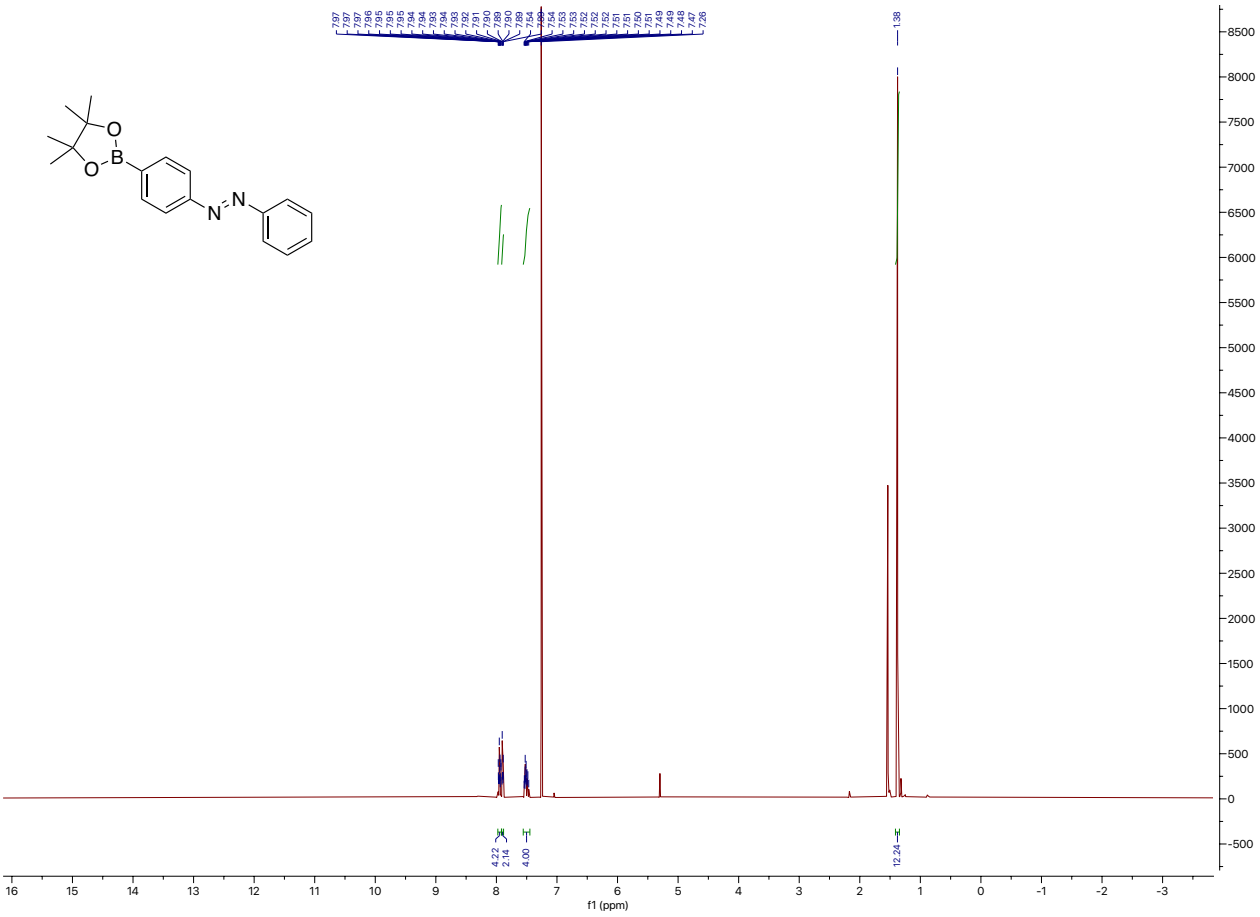
^{13}C NMR (151 MHz, DMSO-d_6)



^{19}F NMR (471 MHz, $CDCl_3$)

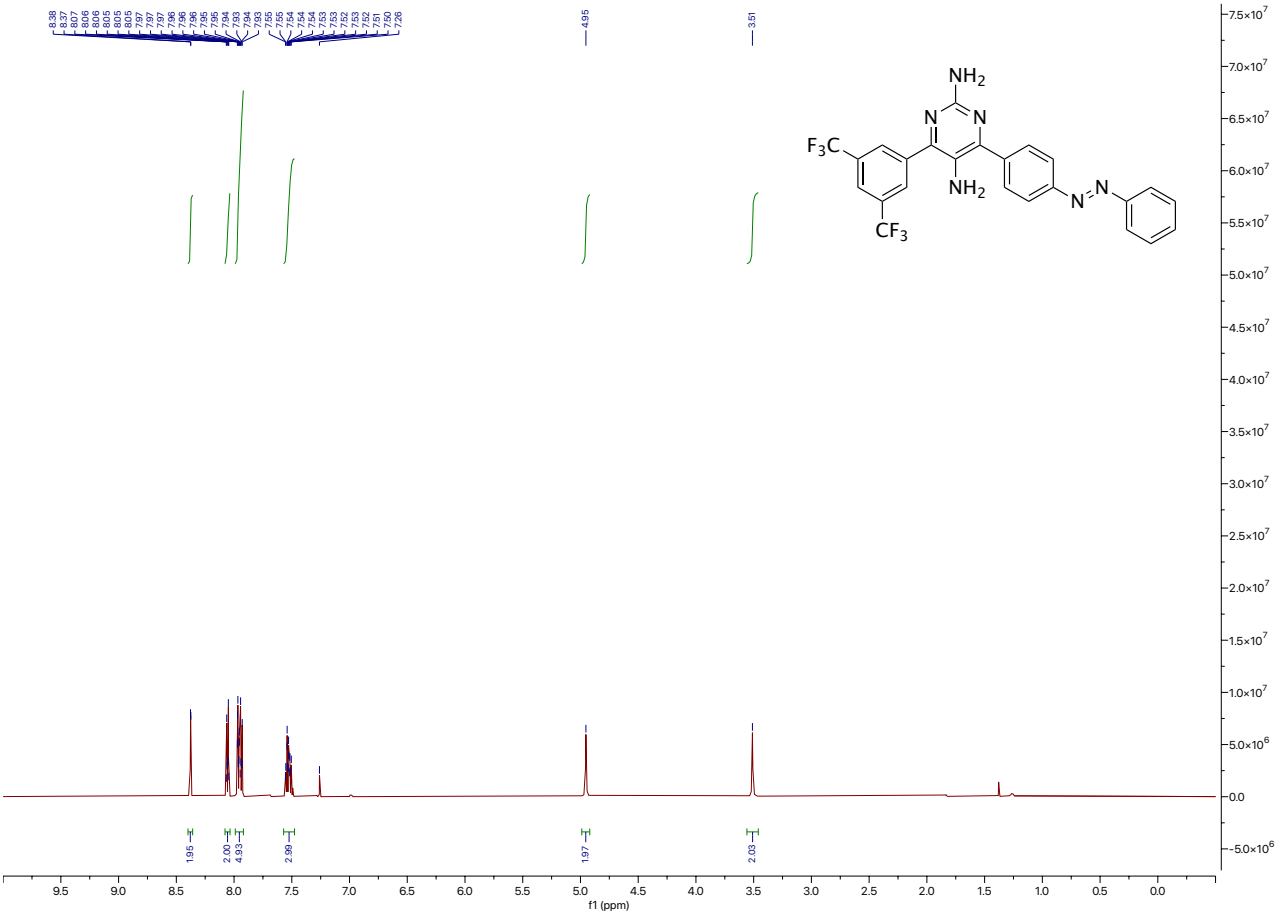


1-Phenyl-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (S1)

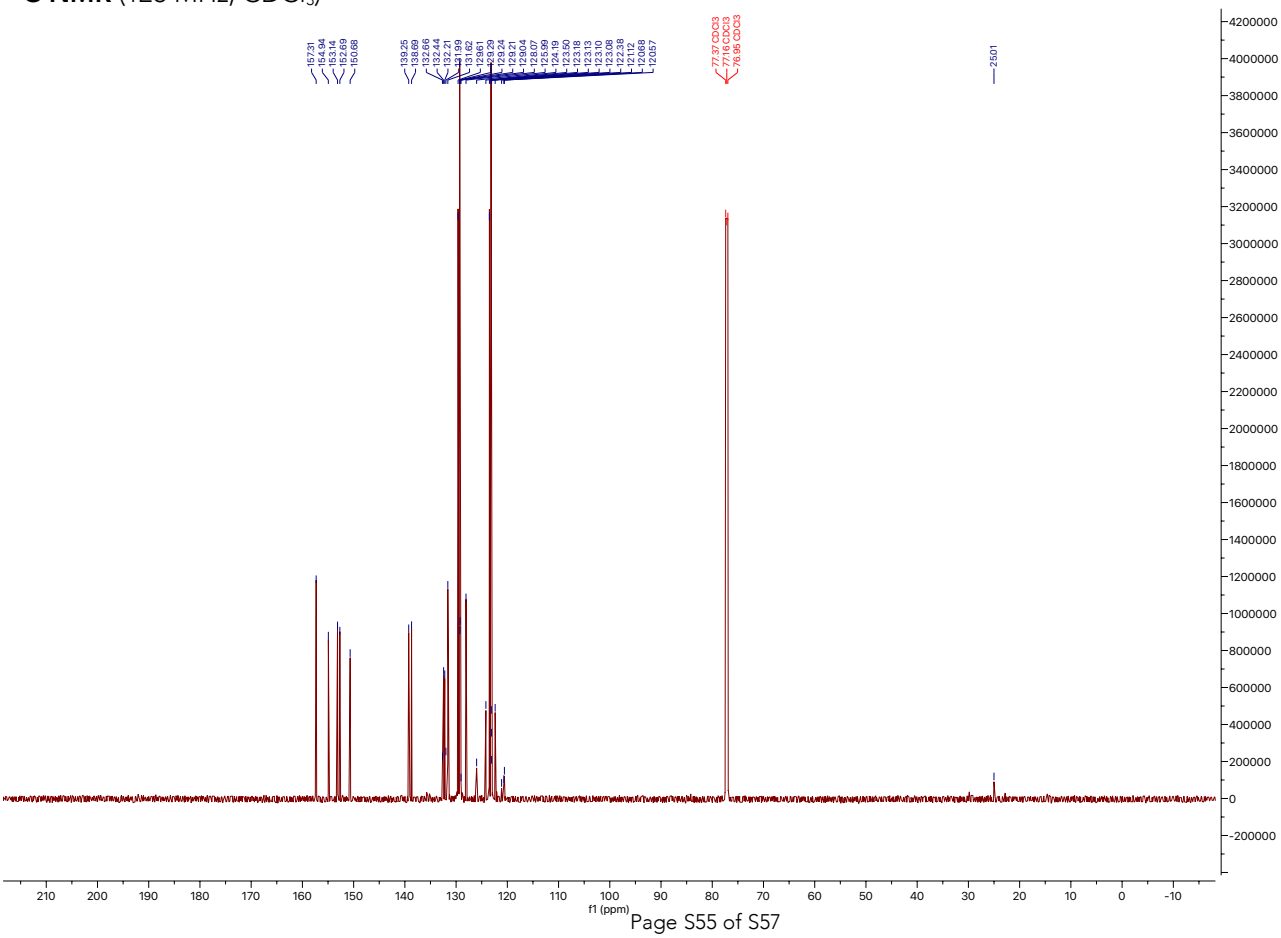


4-(3,5-Bis(trifluoromethyl)phenyl)-6-(4-(phenyldiazenyl)phenyl)pyrimidine-2,5-diamine (2m)

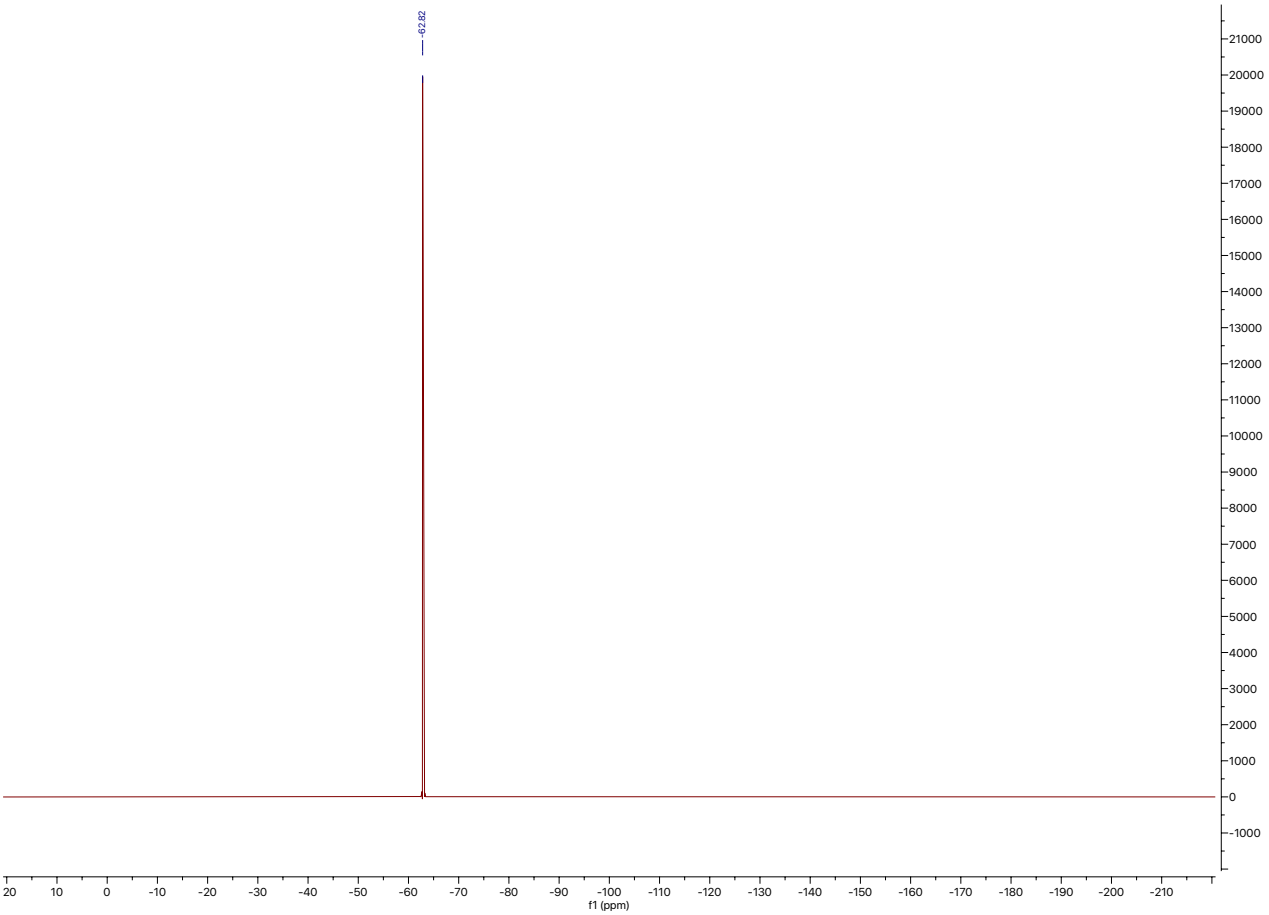
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)

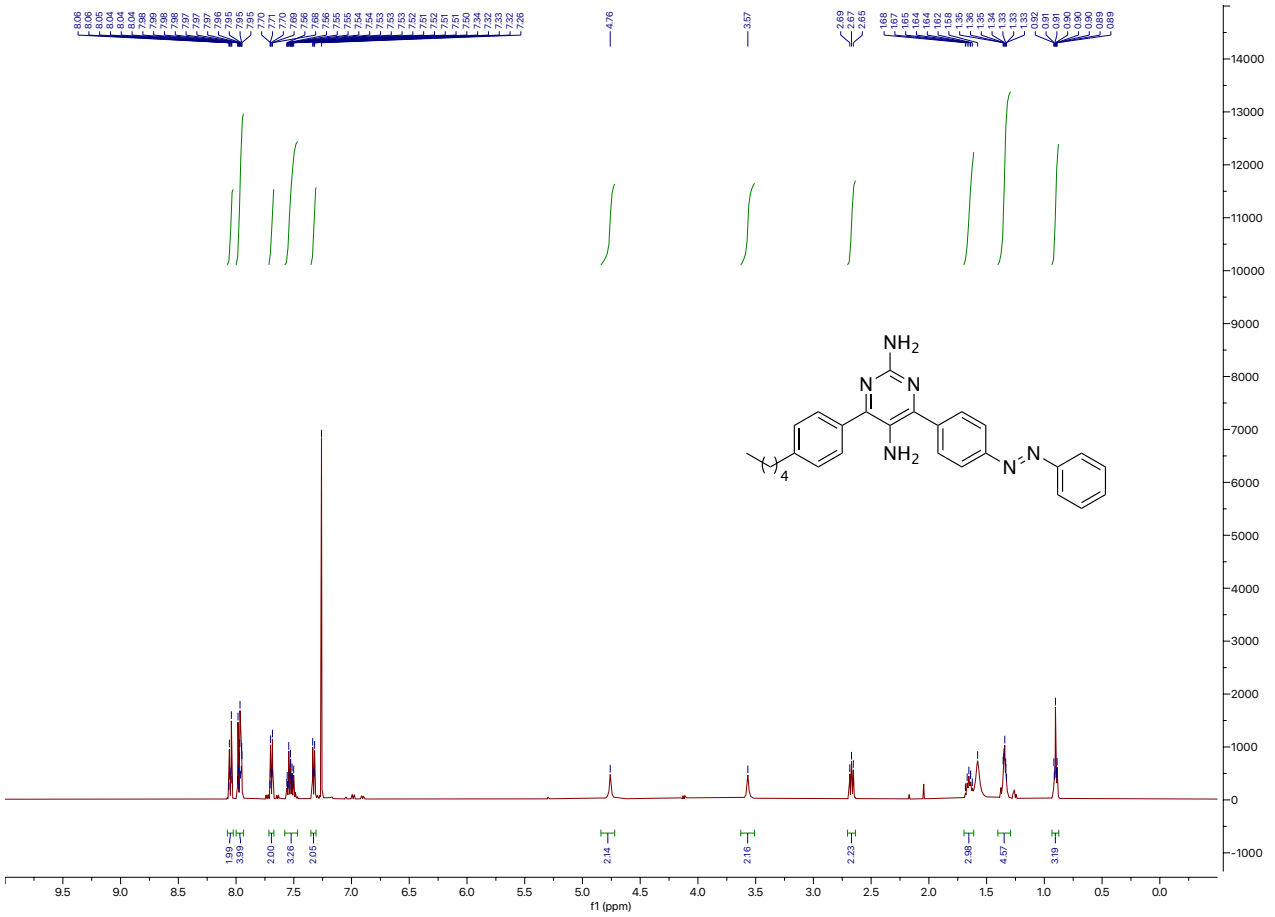


^{19}F NMR (471 MHz, $CDCl_3$)



4-(4-*n*-Pentylphenyl)-6-(4-(phenyldiazenyl)phenyl)pyrimidine-2,5-diamine (2n)

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)