# Indoloxytriazines as Binding Molecules for the JAK2JH2 Pseudokinase Domain and Its V617F Variant

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**Supporting Information**

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## 1. Experimental Details

### 1.1 Database

The drug-like subset from ZINC12 database [1] containing more than 10 million compounds (10,637,968 – 2012-08-22) was used for the virtual screening. The compounds were processed with LigPrep (v 2.5) to generate three-dimensional structures with multiple conformers [2].

### 1.2 Virtual Screening

The database was employed in the docking calculations using the Glide 5.7.109 standard precision (SP) mode [3] to the crystal structure of JAK2 JH2 mutant V617F (PDBID:4FVR) [4]. Three hydrogen bond constraints with GLN626, GLU627 and VAL629 were added to increase binding affinity to the hinge area. The best ranked ca. 6400 were redocked with Glide XP [5], and 1K best complexes were visualized. The selection was based on the docking scores, on the favourable interactions with the receptor and also on the predictions of physical properties from QikProp [6]. Molecules containing unwanted structural features were removed (readily hydrolysable and/ or containing highly electrophilic functional groups).

### 1.3 Purchased Compounds

The identities of all purchased compounds were confirmed by $^{1}$H NMR and high-resolution mass spectrometry; purity was normally > 95% as shown by high-performance liquid chromatography.

NMR spectra were recorded on Agilent DD2 600 (600 MHz), and DD2 400 (400 MHz) instruments. Mass determinations were performed using electrospray ionization on water Micromass ZQ (LC- MS) and on an Agilent Technologies 6890N (GC-MS). HRMS (ESI-TOF) analyses were performed on Waters Xevo QTOF equipped with Z-spray electrospray ionization source. The purity (≥ 95%) of all final synthesized compounds was determined by reverse phase HPLC, using a Waters 2487 dual λ absorbance detector with a Waters 1525 binary pump and a Phenomenex Luna 5μ C18(2) 250 x 4.6 mm column. Samples were run at 1 mL/min using gradient mixtures of 5-100% of water with 0.1% trifluoroacetic acid (TFA) (A) and 10:1 acetonitrile:water with 0.1% TFA (B) for 22 min followed by 3 min at 100% B.

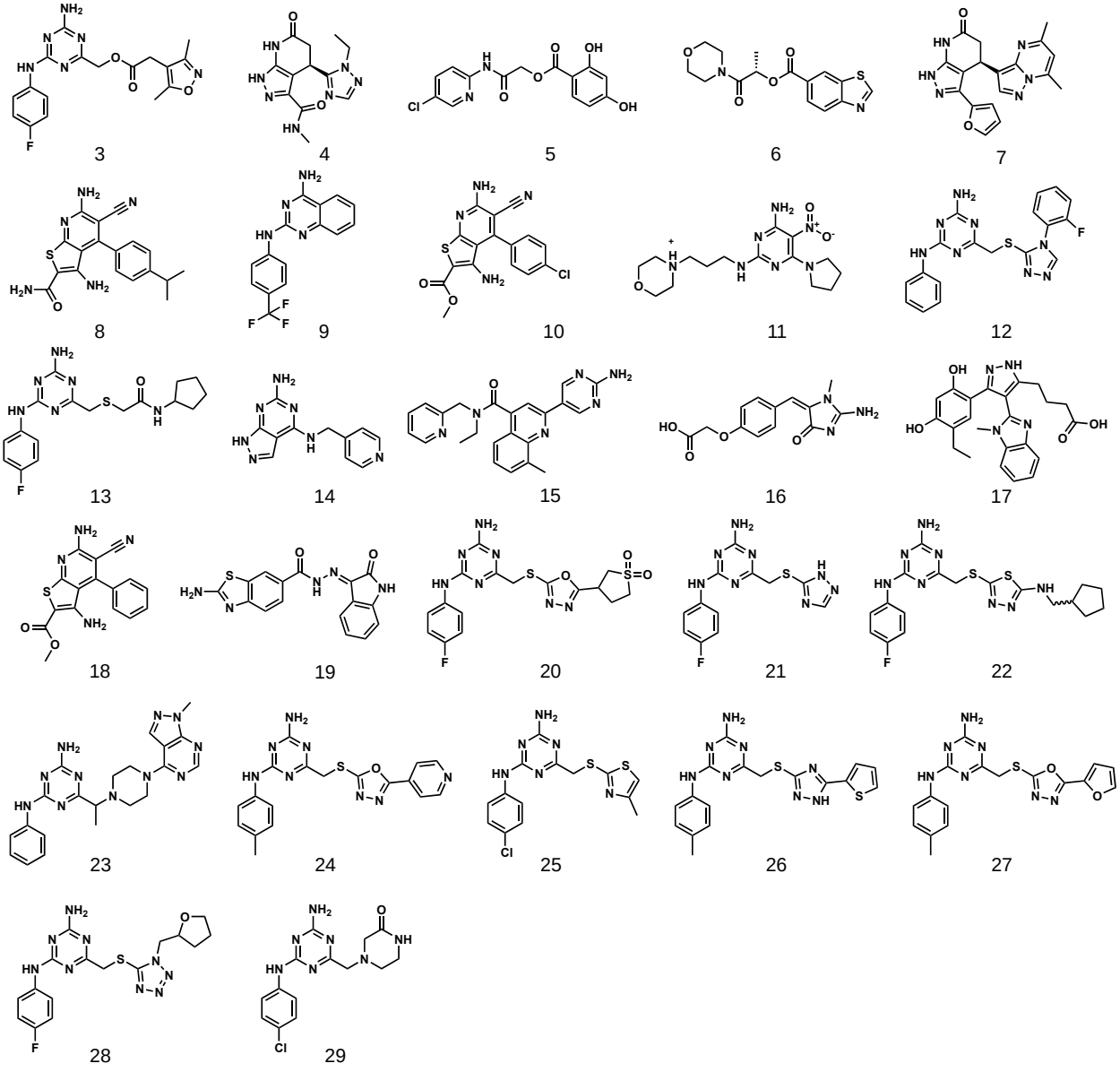



**Figure ES1.** Structures of the purchased (**3**-**19**) obtained from the virtual screening. Compounds **20**-**29** are analogues of **4**, **12** and **15**, found through SciFinder substructure-search, were also purchased and tested.

**Table ES1.** Experimentally binding affinities ($k_d$) using fluorescence polarization (FP) [7] against JAK2 JH1 WT, JAK2 JH2 WT and JAK2 JH2 VF, docking score and ZINC ID for the active compounds obtained from the virtual screening.

| | $K_d$ (μM) | | | Glide SP (kcal/mol) | ZINC ID |
|---|---|---|---|---|---|
| | JAK2 - WT | | JAK2-VF | | |
| Compound | JH1 | JH2 | JH2 | | |
| **3** | NA | NA | 250 | -10.02 | 16675328 |
| **4** | NA | 300 | NA | -9.96 | 65372817 |
| **5** | 115 | NA | NA | -9.98 | 06080826 |
| **6** | NA | NA | 300 | -9.81 | 46804407 |
| **9** | 200 | 150 | 130 | -9.59 | 32617730 |
| **11** | NA | NA | 250 | -9.41 | 05111325 |
| **12** | NA | 92 | 40 | -9.76 | 05702514 |
| **13** | NA | 300 | 300 | -9.69 | 15495946 |
| **14** | NA | NA | 300 | -9.15 | 32511757 |
| **15** | 250 | 119 | 55 | -9.71 | 15495946 |
| **19** | NA | NA | 300 | -9.47 | 05706308 |
| **20** | **NA** | **292** | **75** | | |
| **21** | NA | 108 | 50 | | |
| **22** | **NA** | **149** | **65** | | |
| **23** | NA | 300 | 200 | | |
| **25** | 150 | 41 | 35 | | |
| **26** | 215 | 147 | 45 | | |
| **27** | 300 | 200 | 95 | | |
| **28** | NA | 85 | 45 | | |
| **29** | NA | 150 | 80 | | |

1.4 Fluorescence Polarization Assay

1.4.1 Determination of Tracer Affinity with JAK2

In a flat black bottom 96 well plate (Corning), the buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 20% Glycerol, 0.5 mM TCEP, 0.01% Tween 20) is added - 200 µL to column 1 (blank), 295 µL to column 2, 150 µL to columns 3-12. 5 µL of protein (179.0 µM JAK2-JH2-WT, 154.7 µM JAK2-JH2-V617F, 126.3 µM JAK2-JH1) were added to column 2. 150 µL was transferred, using a multichannel pipette, from column 2 to 3, 3 to 4, 4 to 5, until reaching the last column to make a serial dilution (1:2). 50 µl of 24.0 nM tracer were added from columns 2-12 and fluorescence polarization was measured at $\lambda_{exc}$ = 485 ± 20 nm, $\lambda_{em}$ = 535 ± 25 nm using an Infinite F500 plate reader until no FP variation was observed. From the lowest and highest FP values (tracer free and tracer fully bound to JAK) fraction of ligand bound to the protein to ligand total ($L_b/L_t$) was calculated for each concentration of the JAK2-JH2-WT, JAK2-JH2-V617F, and JAK2-JH1 (Figure S2B). Experiments were carried out by quadruplicates in three independent experiments. The data provided a typical saturation-binding curve and $K_d$ was calculated fitting the results to the Hill equation using Prism 7.

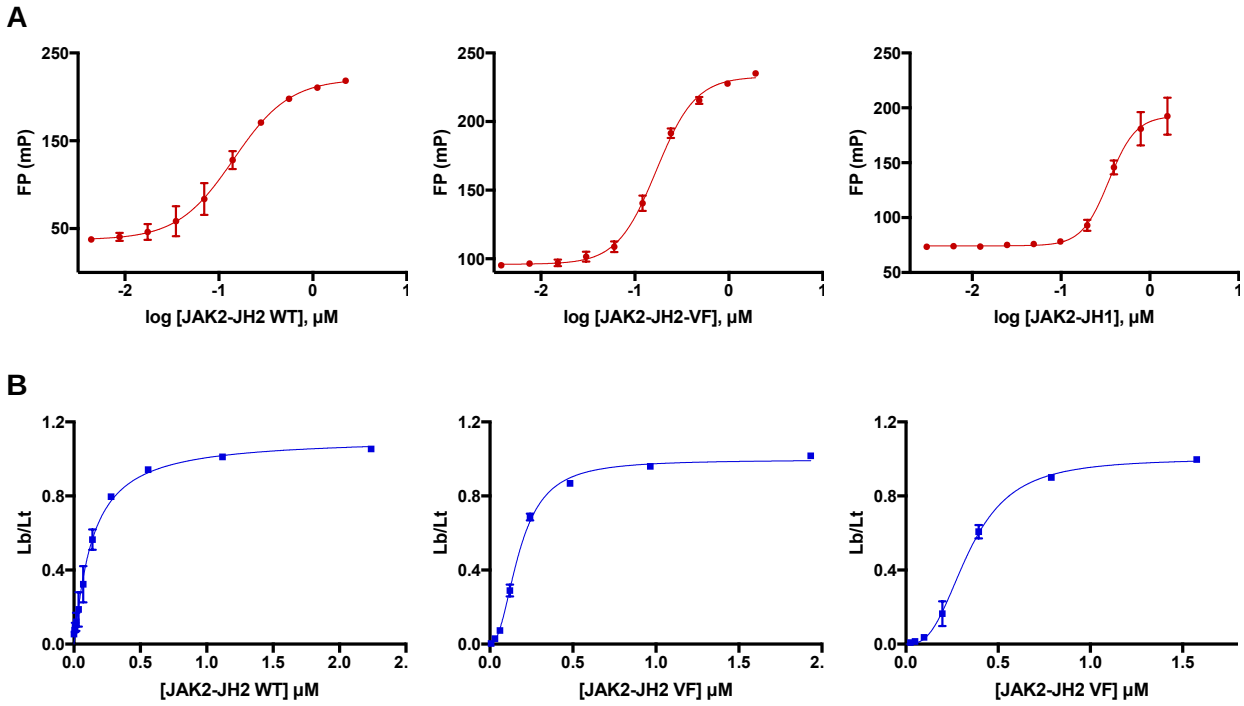



**Figure ES2**. Determination of binding affinities for tracer (6 nM) through saturation experiments. (A) Variation of FP values as a function of JAK2-JH2-WT, JAK2-JH2-V617F, and JAK2-JH1 concentration. (B) $K_d$ determination for JAK2-JH2-WT, JAK2-JH2-VF, and JAK2-JH1. Lb/Lt = ratio of ligand bound to the total. Data from quadruplicate experiments in three independent assays. Mean ± SEM plotted for all data.

### 1.4.2 Competitive FP Assay Protocol [8]

In a flat black bottom 96 well plate (Corning), 200 µL of FP buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 20% Glycerol, 0.5 mM TCEP, 0.01% Tween 20) were added to column 1 (blank), 150 µL to column 2, and 140 µL to columns 3-12. 10 µL of 2.96 µM of JAK2-JH2 WT (3.52 µM for JAK2-JH2-VF, and 6.93 µM for JAK2-JH1), were added to columns 3-12, followed by the addition of 2 µL of DMSO to columns 1-3. 2 µL of inhibitor in DMSO at different concentrations were added from column 4 to 12. 50 µL of 24 nM of tracer were added to columns 2-12. Fluorescence polarization was measured at $\lambda_{exc}$ = 485 ± 20 nm, $\lambda_{em}$ = 535 ± 25 nm for 1 hour. Experiments were carried out in quadruplicate with three independent experiments. Data were analyzed by a least-squares non-linear fit, generated using Prism 7 in order to determine the compound's $IC_{50}$. $K_d$ values for each inhibitor were calculated using the following equation based on the $IC_{50}$, $K_d$ of the tracer ($K_d^t$), total ($L_t$) and bound ($L_b$) tracer, as well as total protein concentration ($P_t$) [9].

$$K_d^I = \frac{L_b IC_{50} K_d^t}{P_t L_t + L_b(P_t - L_t + L_b - K_d^t)}$$

## 2. General Procedure for Preparation of Compound 33 Analogues

### 2.1 General Information

Reagents and solvents were obtained from commercial supplies and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using Merck pre-coated silica gel plates (analytical, $SiO_2$-60, $F_{254}$). TLC plates were visualized under U.V. light (254 nm). Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Microwave reactions were performed using a Biotage® Initiator$^+$ microwave synthesizer using the standard settings. Flash column chromatography was performed on a Combiflash® Rf+ (Teledyne Isco, Lincoln, NE) with RediSep RF GOLD® (silica gel, particle size 20-40 μm) prepared cartridges. Preparative reverse phase HPLC was utilized for the purification of **33i-j**, **33l**, and **33n-o** using an Agilent 1260 Infinity II system equipped with G7161A Preparative Binary Pump., G7115A Diode Array Detector WR., G7157A Preparative Autosampler, G7159B Agilent Preparative Open-Bed Fraction Collector and an Agilent 5 Prep-C18 21× 100 mm column with a gradient of 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile as the mobile phase. Purity assessment for **33i-j**, **33l**, and **33n-o** was conducted with the same system, using an Agilent Prep-C18 4.6 × 100 mm, 5μM particle size, scaler column with a gradient of 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile as the mobile phase.

Nuclear magnetic resonance (NMR) spectra were recorded either on an Agilent $DD_2$ 400 ($^1$H NMR, $^{13}$C NMR recorded at 400, and 101 MHz, respectively), an Agilent $DD_2$ 500 ($^1$H NMR, $^{13}$C NMR recorded at 500, and 126 MHz, respectively), or an Agilent $DD_2$ 600 ($^1$H NMR, $^{13}$C NMR recorded at 600, and 151 MHz, respectively). All spectra were recorded at room temperature, 62 ºC or 80 ºC as noted. Chemical shifts are reported in ppm relative to deuterated solvent as an internal standard ($\delta_H$ DMSO-$d_6$ 2.50 ppm, $\delta_C$ DMSO-$d_6$ 39.52 ppm; $\delta_H$ Methanol-$d_6$ 3.31 ppm, $\delta_C$ Methanol-$d_6$ 49.00 ppm $\delta_H$ Acetone-$d_6$ 2.05 ppm, $\delta_C$ Acetone-$d_6$ 29.84 ppm, 206.26 ppm; $\delta_H$ Chloroform-*d* 7.26 ppm, $\delta_C$ Chloroform-*d* 77.16 ppm) with the following convention for describing multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad signal, dd = doublet of doublets, etc). High resolution mass spectroscopy (HRMS) measurements of assayed compounds were recorded using a Waters Acquity UPLC® coupled to a Waters Xevo® QTOF mass spectrometer equipped with a Waters ZSpray™ electrospray ionization source.

Mass spectrometric measurements for compounds **35**-**40**, **33i-j**, **33l**, and **33n-o** were performed with a Shimadzu Scientific Instruments QToF 9030 LC-MS system, equipped with a Nexera LC-40D xs UHPLC, consisting of a CBM-40 Lite system controller, a DGU-405 Degasser Unit, two LC-40D XS UHPLC pumps, a SIL-40C XS autosampler and a Column Oven CTO-40S. UV data was collected with a Shimadzu Nexera HPLC/UHPLC Photodiode Array Detector SPD M-40 in the range of 190 - 800nm. Mass spectra were subsequently recorded with the quadrupole time-of-flight (QToF) 9030 mass spectrometer. The samples were held at 4 deg C in the autosampler compartment. 0.3uL of each spiked solution were injected into a sample loop and separated on a

Shim-pack Scepter C18-120, 1.9um, 2.1x100mm Column, equilibrated at 40 deg C in a column oven. A binary gradient was used:

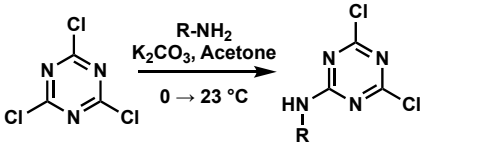
Solvent A: Water, HPLC grade Chromasolv, with 0.1% Formic Acid

Solvent B: Acetonitrile, HPLC grade Chromasolv, with 0.1% Formic Acid

The ionization source was run in "ESI" mode, with the electrospray needle held at +4.5kV. Nebulizer Gas was at 2 L/min, Heating Gas Flow at 10 L/min and the Interface at 300 deg C. Dry Gas was at 10 L/min, the Desolvation Line at 250 deg C and the heating block at 400 deg C. Mass spectra were recorded in the range of 50 to 2000 m/z in positive ion mode. Measurements and data post-processing were performed with LabSolutions 5.97 Realtime Analysis and PostRun.

2.2 Synthesis of *Triazine* derivatives

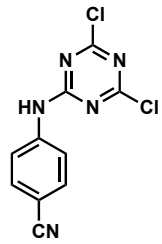
**Scheme ES1.** Synthesis of Intermediates **30a-b**.




**30a** (R = 4-PhCN)
**30b** (R = 4-PhSO$_2$NH$_2$)

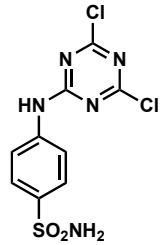
**General Procedure A:** Synthesis of Intermediates **30a-b.**

To a solution of cyanuric chloride (2.710 mmol, 1.0 eq.) in acetone (10 ml) at 0 $^{o}$C was added potassium carbonate (5.420 mmol, 2.0 eq.) and the solution was stirred 20 minutes. The amino derivative (2.710 mmol, 1.0 eq.) was added portion wise and the reaction was allowed to warm at room temperature and stir overnight. The solvent was removed under reduced pressure. The product was collected by filtration, washed with $H_2O$ and dried under vacuum.




**30 a**

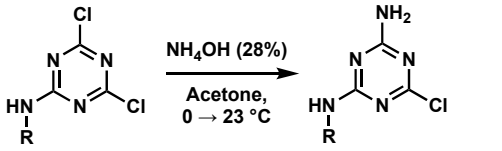
**4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)benzonitrile (30a)** (0.578 g, 81% yield) $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 7.87 (d, $J$ = 8.8 Hz, 2H), 7.75 (d, $J$ = 8.9 Hz, 2H). HRMS (ESI) calcd for $[M+H]^+$ $C_{10}H_6Cl_2N_5$ 266.0001, found 266.0006.



30 b

**4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)benzenesulfonamide (30b)** (2.720 g, 31% yield) $^1$H NMR (500 MHz, DMSO-$d_6$) δ 11.42 (s, 1H), 7.86 – 7.82 (m, 2H), 7.79 – 7.75 (m, 2H), 7.33 (s, 2H). HRMS (ESI) calcd for $[M+H]^+$ $C_9H_8Cl_2N_5O_2S$ 319.9776, found 319.9771.

**Scheme ES2.** Synthesis of Intermediates **31a-b**.

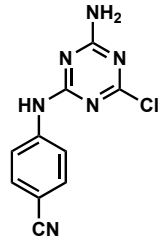



**30a** (R = 4-PhCN)
**30b** (R = 4-PhSO$_2$NH$_2$)

**31a** (R = 4-PhCN)
**31b** (R = 4-PhSO$_2$NH$_2$)

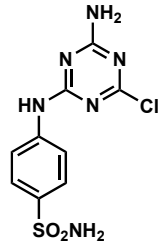
**<u>General Procedure B</u>**: <u>Synthesis of Intermediates **31a-b**</u>.

To a solution of **30** (14.5 mmol) in acetone (100 ml) at 0$^o$C was added $NH_4OH$ 28% (1 ml). The reaction was allowed to warm at room temperature and stir overnight. The precipitated product was collected by filtration and washed with $H_2O$.



31 a

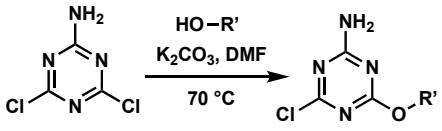
**4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzonitrile (31a)** (3.034 g, 85% yield) $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.25 (s, 1H), 8.06 (d, $J$ = 8.6 Hz, 2H), 7.71 (d, $J$ = 8.7 Hz, 2H), 7.15 (s, 1H), 7.00 (s, 1H). HRMS (ESI) calcd for $[M+H]^+$ $C_{10}H_8ClN_6$ 247.0501, found 247.0504.



31 b

**4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzenesulfonamide (31b)** (0.131 g, 70% yield) $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.30 (s, 1H), 7.89 (d, $J$ = 8.5 Hz, 2H), 7.80 – 7.67 (m, 4H), 7.25 (s, 2H). HRMS (ESI) calcd for $[M+H]^+$ $C_9H_{10}ClN_6O_2S$ 301.0274, found 301.0269.

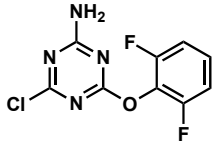
**Scheme ES3.** Synthesis of Aromatic Ether Intermediates **32a-c**.




**32a-c** (R'= e'-f', j')

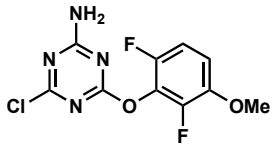
**General Procedure C**: Synthesis of Aromatic Ether Intermediates **32a-c**.

A solution of 4,6-dichloro-1,3,5-triazin-2-amine (1.212 mmol, 1.0 eq.), functionalized phenol with (1.212 mmol, 1.0 eq.) and $K_2CO_3$ (3.636 mmol, 3.0 eq.) in DMF (2 mL) was stirred at 70°C for 18 hours. The reaction was cooled to room temperature, poured into $H_2O$ and extracted with EtOAc. The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude mixture was loaded on silica to give the desired aromatic ether derivative after column chromatography.



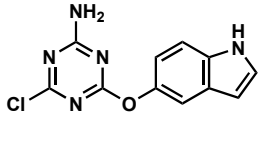

**32 a**

**4-chloro-6-(2,6-difluorophenoxy)-1,3,5-triazin-2-amine (32a)** (0.213 g, 68% yield) $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.37 (bs, 1H), 8.30 (bs, 1H), 7.46 – 7.22 (m, 3H). HRMS (ESI) calcd for $[M+H]^+$ $C_9H_6ClF_2N_4O$ 259.0198, found 259.0193.




**32 b**

**4-chloro-6-(2,6-difluoro-3-methoxyphenoxy)-1,3,5-triazin-2-amine (32b)** (0.164 g, 47% yield) $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.38 (bs, 1H), 8.32 (bs, 1H), 7.25 (td, $J$ = 9.7, 2.1 Hz, 1H), 7.14 (td, $J$ = 9.3, 4.9 Hz, 1H), 3.87 (s, 3H). HRMS (ESI) calcd for $[M+H]^+$ $C_{10}H_8ClF_2N_4O_2$ 289.0304, found 289.0299.

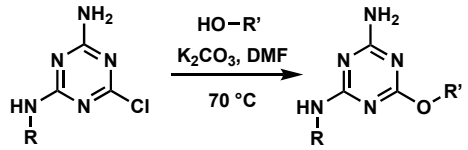



**32 c**

**4-((1*H*-indol-5-yl)oxy)-6-chloro-1,3,5-triazin-2-amine (32c)** (0.214 g, 68%) $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.19 (s, 1H), 8.01 (bs, 1H), 7.99 (bs, 1H), 7.46 – 7.37 (m, 2H), 7.33 (d, $J$ = 2.3 Hz, 1H), 6.91 (dd, $J$ = 8.7, 2.3 Hz, 1H), 6.43 (t, $J$ = 2.4 Hz, 1H). HRMS (ESI) calcd for $[M+H]^+$ $C_{11}H_9ClN_5O$ 262.0496, found 262.0498.

## 2.3 General Method for the Synthesis of the Aromatic Ether Analogs

**Scheme ES4.** Synthesis of Aromatic Ether Analogs **33a-d** and **33g-o**.

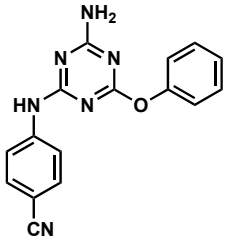



**31a** (R = 4-PhCN)
**31b** (R = 4-PhSO$_2$NH$_2$)

**33a-d; 33g-o**

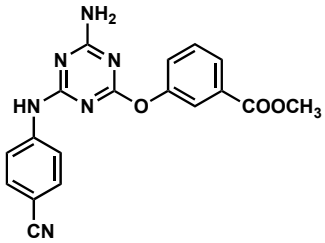
**General Procedure C**: Synthesis of Aromatic Ether Analogs **32a-d** and **33g-o**.

A solution of 4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino) derivative (0.20 mmol), functionalized phenol (0.30 mmol) and $K_2CO_3$ (0.60 mmol) in DMF (2 mL) was stirred at 70 ºC for 18 hours. The reaction was cooled to room temperature, poured into $H_2O$ and extracted with EtOAc. The organic layer was dried over $Na_2SO_4$ and the solvent removed under reduced pressure. The crude mixture was loaded on silica to give the desired aromatic ether derivative after column chromatography.



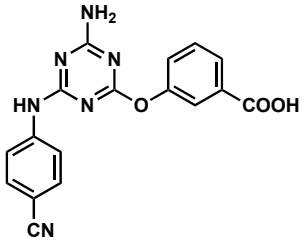
**33 a**

**4-((4-amino-6-phenoxy-1,3,5-triazin-2-yl)amino)benzonitrile (33a)** (62 % yield) $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.01 (s, 1H), 7.94 (d, $J$ = 7.2 Hz, 2H), 7.58 (d, $J$ = 8.3 Hz, 2H), 7.47 (t, $J$ = 7.5 Hz, 2H), 7.31 (t, $J$ = 7.4 Hz, 1H), 7.20 (d, $J$ = 7.9 Hz, 2H), 6.67 (d, $J$ = 16.3 Hz, 2H). $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 170.02, 166.83, 153.66, 145.04, 133.49, 130.21, 126.09, 122.98, 120.43, 119.73, 105.43. HRMS (ESI) calcd. For $[M+H]^+$ $C_{16}H_{13}N_6O$ 305.1151, found 305.1155.



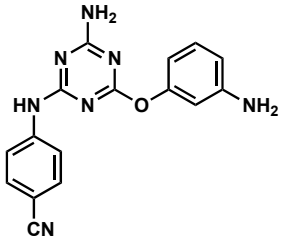
**33 b'**

**Methyl 3-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)benzoate (33b')** (59 % yield) $^1$H NMR (600 MHz, Acetone-$d_6$) δ 9.04 (s, 1H), 7.95 (d, $J$ = 7.6 Hz, 3H), 7.79 (s, 1H), 7.62 (t, $J$ = 7.9 Hz, 1H), 7.58 (d, $J$ = 7.6 Hz, 2H), 7.49 (d, $J$ = 7.7 Hz, 1H), 6.74 (d, $J$ = 50.9 Hz, 1H), 3.90 (s, 3H). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 169.94, 166.58, 153.67, 133.51, 132.56, 130.58, 127.84, 127.03, 123.85, 120.53, 120.46, 119.70, 105.61, 52.60. HRMS (ESI) calcd. For $[M+H]^+$ $C_{18}H_{15}N_6O_3$ 363.1206, found 363.1203.



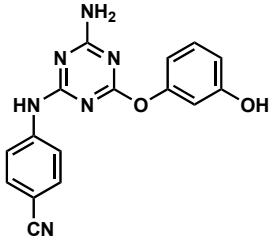

**33 b**

**3-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)benzoic acid (33b)** This compound was obtained by hydrolysis of **33b'** (45 % yield) $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 13.19 (s, 1H), 10.02 (s, 1H), 7.84 (d, $J$ = 7.7 Hz, 3H), 7.68 (s, 1H), 7.62 (d, $J$ = 7.0 Hz, 2H), 7.57 (t, $J$ = 7.8 Hz, 1H), 7.49 (d, $J$ = 7.4 Hz, 1H), 7.38 (s, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.67, 168.21, 166.66, 165.32, 152.17, 144.20, 132.70, 129.88, 126.19, 122.71, 119.45, 119.35, 103.33. HRMS (ESI) calcd for $[M+H]^+$ $C_{17}H_{13}N_6O_3$ 349.1049, found 349.1043.



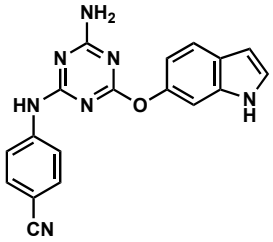

**33 c**

**4-((4-amino-6-(3-aminophenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (33c)** (26 % yield) $^{1}$H NMR (600 MHz, Acetone-$d_6$) δ 9.00 (s, 1H), 7.97 (s, 2H), 7.59 (d, $J$ = 8.1 Hz, 2H), 7.10 (t, $J$ = 8.0 Hz, 1H), 6.60 (d, $J$ = 7.8 Hz, 3H), 6.48 (s, 1H), 6.37 (d, $J$ = 7.8 Hz, 1H), 4.84 (s, 2H). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 170.09, 166.85, 154.63, 150.73, 145.15, 133.51, 130.37, 120.44, 119.80, 112.15, 110.75, 108.76, 105.33. HRMS (ESI) calcd for $[M+H]^+$ $C_{16}H_{14}N_7O$ 320.1260, found 320.1256.



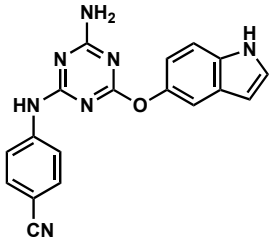

**33 d**

**4-((4-amino-6-(3-hydroxyphenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (33d)** (23 % yield). $^{1}$H NMR (600 MHz, Acetone-$d_6$) δ 9.03 (s, 1H), 8.68 (s, 1H), 7.97 (s, 2H), 7.59 (d, $J$ = 7.8 Hz, 2H), 7.26 (t, $J$ = 7.9 Hz, 1H), 6.78 (d, $J$ = 7.8 Hz, 1H), 6.66 (d, $J$ = 8.9 Hz, 3H). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 172.39, 170.04, 166.84, 159.23, 154.60, 145.08, 133.50, 130.62, 120.45, 119.76, 113.91, 110.34, 105.41. HRMS (ESI) calcd. For $[M+H]^+$ $C_{16}H_{13}N_6O_2$ 321.1100, found 321.1095.



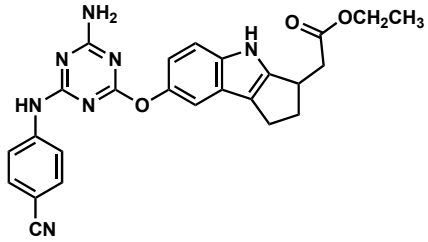

**33 g**

**4-((4-((1H-indol-6-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)benzonitrile (33g)** (54 % yield) $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ 10.36 (s, 1H), 8.99 (s, 1H), 7.94 (s, 1H), 7.60 (d, $J$ = 8.5 Hz, 1H), 7.49 (d, $J$ = 7.4 Hz, 2H), 7.40 (s, 1H), 7.23 (s, 1H), 6.86 (d, $J$ = 8.5 Hz, 1H), 6.63 (s, 2H), 6.55 (s, 1H). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 170.09, 166.86, 148.96, 145.16, 137.17, 133.44, 126.73, 126.32, 121.15, 120.38, 119.76, 115.15, 105.51, 105.27, 102.40. HRMS (ESI) calcd for $[M+H]^+$ $C_{18}H_{14}N_7O$ 344.1260, found 344.1265.



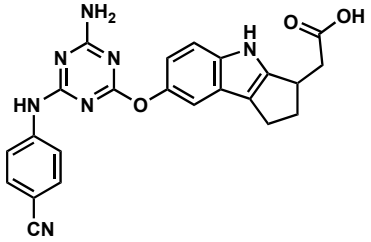
33 h

**4-((4-((1H-indol-5-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)benzonitrile (33h)** (36 % yield) $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ 10.40 (s, 1H), 8.96 (s, 1H), 8.02 – 7.83 (m, 2H), 7.47 (d, *J* = 8.5 Hz, 3H), 7.43 (s, 1H), 7.34 (d, *J* = 2.2 Hz, 1H), 6.92 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.60 (s, 2H), 6.51 (t, *J* = 2.5 Hz, 1H). $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 170.09, 166.86, 146.95, 145.18, 134.88, 133.41, 129.26, 127.00, 120.39, 119.77, 117.05, 113.40, 112.33, 105.21, 102.64. HRMS (ESI) calcd. for $[M+H]^+$ $C_{18}H_{14}N_7O$ 344.1260, found 344.1258.



33 i'

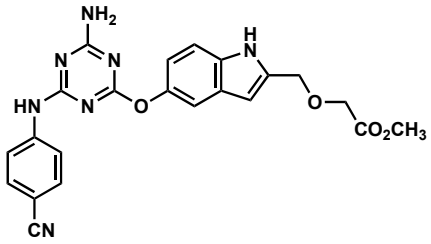
**Ethyl 2-(7-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (33i')** (52 % yield) To a flame dried flask equipped with a stir bar was added 51.9 mg of 4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzonitrile **(31a)** (0.210 mmol), 55.9 mg of ethyl 2-(7-hydroxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3- yl) acetate (0.216 mmol, 1.02 eq., commercially available) and 88.4 mg of potassium carbonate (0.640 mmol, 3.04 eq.). The flask was flushed with nitrogen, at which point 3 mL of DMF were added and the solution was heated to 70 °C overnight for 15 hours. The mixture was then concentrated in vacuo and purified via column chromatography (0-100% EtOAc in Hexanes), yielding 50.4 mg of ethyl 2-(7-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (**33i'**) (0.107 mmol , 51.6% yield) as a colorless oil. $^{1}$H NMR (500 MHz, Methanol-$d_4$) δ 7.66 (br s, 2H), 7.40 – 7.25 (m, 3H), 7.10 (d, *J* = 2.3 Hz, 1H), 6.81 (dd, *J* = 8.7, 2.3 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.60 (p, *J* = 6.9 Hz, 1H), 2.84 – 2.67 (m, 4H), 2.56 (dd, *J* = 15.9, 7.6 Hz, 1H), 2.22 – 2.12 (m, 1H), 1.25 (t, *J* = 7.1 Hz, 3H). Note: H of the COOH moiety was not observed. $^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 174.34, 173.42, 169.98, 166.88, 148.49, 146.79, 145.32, 140.48, 133.65, 125.84, 120.80, 120.17, 119.67, 115.63, 112.93, 111.92, 105.48, 61.66, 40.53, 36.91, 36.77, 24.13, 14.56. MS (ESI) calcd for $[M+H]^+$ $C_{25}H_{24}N_7O_3$ 470.2, found 470.1; $[M+Na]^+$ 492.2, found 492.1; $[M-H]^+$ 468.2, found 468.2.



33 i

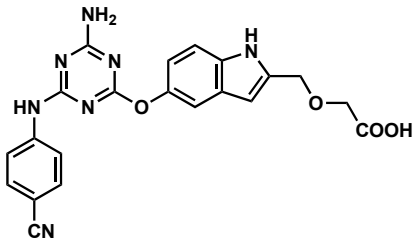
**2-(7-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid (33i)** (55 % yield) To a flask equipped with a stir bar was added 35.9 mg of **33i'** (0.0765 mmol), 3 mL of THF, and 3 mL of EtOH. 270 µL of 2M NaOH (aq.) (0.540 mmol, 7.06 eq.) was then added and the solution was allowed to stir under ambient atmosphere and

temperatures overnight for 15.5 hours. The solution was quenched with 2 M HCl (aq.) to pH 4 and concentrated in vacuo. The residue was then purified by reverse-phase HPLC Retention Time=13.396 min (5 % B- 100 % B over 20 minutes), yielding 18.6 mg of compound **33i** (0.0421 mmol, 55.1 % yield) as a white solid. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.71 (s, 1H), 9.89 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.22 (br s, 1H), 7.19 (br s, 1H), 7.06 (d, *J* = 2.2 Hz, 1H), 6.77 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.50 (p, *J* = 7.0, 6.5 Hz, 1H), 2.77 – 2.58 (m, 4H), 2.39 (dd, *J* = 16.0, 8.7 Hz, 1H), 2.14 – 2.03 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ $^{13}$C NMR (126 MHz, dmso) δ 173.49, 171.65, 168.31, 165.44, 147.64, 145.15, 144.43, 138.47, 132.65, 124.11, 119.39, 117.37, 114.43, 112.12, 110.58, 103.07, 48.60, 35.60, 35.09, 23.11. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{20}N_7O_3$ 442.1628, found 442.1643.




**33 j'**

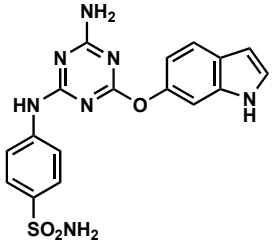
**Methyl 2-((5-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1*H*-indol-2-yl)methoxy)acetate** (**33j'**) (60 % yield) Methyl 2-((5-hydroxy-1*H*-indol-2-yl)methoxy)acetate (**34**) (37.2 mg, 0.158 mmol, 1 eq.) was combined with 0.8 mL anhydrous dimethylformamide and potassium carbonate (69.7 mg, 0.5 mmol, 3.2 eq.) in a flame-dried pressure vial equipped with a rubber septum. The mixture was stirred at rt for 5-10min, then was heated at 60 °C and stirred for another 5-10 min. 4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzonitrile **(31a)** (41.4 mg, 0.168 mmol 1.1eq.) was added, and the reaction stirred at 60° C for 11h. Afterwards, solvent was evaporated *in vacuo* and the residue was purified with normal-phase column chromatography (Dichloromethane/Methanol). Methyl 2-((5-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1*H*-indol-2-yl)methoxy)acetate (**33j'**) was isolated as a white solid. Yield: 42.5 mg, 60%. The corresponding carboxylic acid **33j** was also identified as one of the reaction products, and was isolated as a pale white solid. Yield: 15 mg, 22%. $^{1}$H NMR (600 MHz, DMSO-d6) δ 11.27 (s, 1H), 9.92 (s, 1H), 7.87 (brs, 2H), 7.55 (brs, 2H), 7.36 (d, J = 8.7 Hz, 1H), 7.28 (d, J = 2.2 Hz, 1H), 7.23 (brs, 2H), 6.90 (dd, J = 8.7, 2.3 Hz, 1H), 6.41 (d, 1H), 4.69 (s, 2H), 4.17 (s, 2H), 3.66 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 171.63, 170.44, 168.33, 165.44, 145.39, 144.42, 136.21, 134.27, 133.01, 132.67, 127.79, 119.39, 116.26, 112.35, 111.68, 103.09, 101.75, 66.30, 65.39, 51.50. [M+H]: 446.1571, found 446.1586, and 468.1405 $[M+Na^+]$.




**33 j**

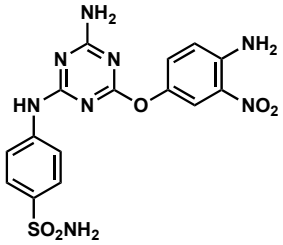
**2-((5-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1*H*-indol-2-yl)methoxy)acetic acid (33j)** (27 % yield) Methyl 2-((5-((4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)oxy)-1*H*-indol-2-yl)methoxy)acetate **(33j')** (20 mg, 0.045 mmol, 1.0 eq.) was suspended in 4.5 mL dioxane. Sodium hydroxide (0.58 mL of a 2M aqueous solution, 1.16 mmol, 25.8

eq.) was added dropwise, and the reaction was allowed to stir at rt for 1h, when TLC indicated completion. Subsequently the reaction was concentrated to dryness, and 1.5mL water was added. The pH was adjusted to ~4, the precipitate was collected with centrifugation and removal of the supernatant water, followed by drying under a nitrogen stream. Then the residue was purified using a preparatory HPLC column with a gradient of acetonitrile with 0.1% formic acid /water with 0.1% formic acid, to afford **33j** as a pale-white solid. Yield 5.3 mg, 27%. Purity 99%. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 11.77 (s, 1H), 9.92 (s, 1H), 7.90 (d, $J$ = 5.2 Hz, 2H), 7.57 (d, $J$ = 6.9 Hz, 2H), 7.37 (d, $J$ = 8.6 Hz, 1H), 7.30 – 7.15 (m, 3H), 6.86 (dd, $J$ = 8.6, 2.3 Hz, 1H), 6.33 (s, 1H), 4.67 (s, 2H), 3.84 (s, 2H). Note: H of the COOH moiety was not observed. $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 172.58, 171.63, 168.32, 165.47, 145.21, 144.45, 137.90, 134.14, 132.70, 127.95, 119.43, 119.40, 115.76, 112.12, 111.62, 103.08, 100.49, 68.93, 65.33. [M+H]:  432.1415, found 432.1429, and 454.1247 [M+Na$^+$].



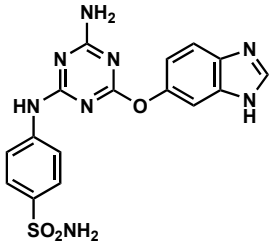

**33 k**

**4-((4-((1H-indol-6-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)benzenesulfonamide (33k)** (38 % yield) $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ 10.35 (s, 1H), 8.89 (s, 1H), 7.91 (s, 2H), 7.67 (d, $J$ = 7.0 Hz, 2H), 7.59 (d, $J$ = 8.4 Hz, 1H), 7.38 (s, 1H), 7.22 (s, 1H), 6.86 (d, $J$ = 8.3 Hz, 1H), 6.58 (s, 2H), 6.54 (s, 1H), 6.41 (s, 2H). $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 170.05, 166.96, 148.96, 144.16, 138.00, 137.15, 127.61, 126.67, 126.28, 121.08, 119.85, 115.16, 105.44, 102.40. HRMS (ESI) calcd for [M+H]$^+$ $C_{17}H_{17}N_7O_3S$ 398.1035, found 398.1040.



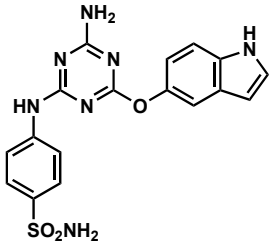

**33 l'**

**4-((4-amino-6-(4-amino-3-nitrophenoxy)-1,3,5-triazin-2-yl)amino)benzenesulfonamide** (**33l'**) (63 % yield) To a flame dried flask equipped with a stir bar 4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**31b**) was added (301.9 mg,1.00 mmol), 4-amino-3-nitrophenol (154.4 mg, 1.00 mmol, 1.00 eq.) and potassium carbonate (416.7 mg, 3.02 mmol, 3.00 eq.). The flask was flushed with nitrogen, at which point 4 mL of DMF were added and the solution was heated to 70 °C overnight for 16 hours. The mixture was then concentrated in vacuo, suspended in acetone, filtered, and washed with acetone. The supernatant was then collected, concentrated in vacuo, and suspended in dichloromethane. This mixture was then filtered, washed with dichloromethane, and the precipitate was isolated, yielding 263.1 mg (0.629 mmol) of (**33l'**) as a burnt yellow solid. $^{1}$H NMR (500 MHz, Acetone-$d_6$) δ 9.00 (s, 1H), 7.91 (br s, 2H), 7.84 (s, 1H), 7.74 (d, $J$ = 8.5 Hz, 2H), 7.35 (d, $J$ = 8.5 Hz, 1H), 7.16 (d, $J$ = 9.1 Hz, 1H), 7.14 (s, 2H), 6.72 (br s, 2H), 6.48 (br s, 2H). $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 172.42, 169.87, 166.78, 144.77, 143.89, 142.52, 138.20, 132.24, 131.10, 127.60, 120.51, 120.02, 118.34. MS (ESI) calcd for [M+H]$^+$ $C_{15}H_{15}N_8O_5S$ 419.1, found 419.1.



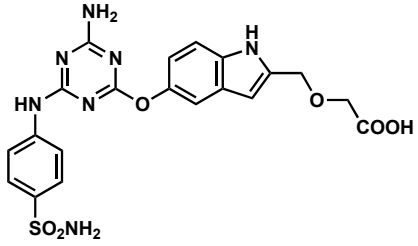
33 l

**4-((4-((1H-benzo[d]imidazol-5-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)benzenesulfonamide (33l)** (29 % yield) [10] To a flame dried microwave vessel equipped with a stir bar, 4-((4-amino-6-(4-amino-3-nitrophenoxy)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (25.9 mg, 0.0779 mmol) was added $SnCl_2$ (38.8 mg, 0.205 mmol, 3.31 eq.). The vessel was purged with nitrogen and 2 mL of formic acid were added. The vessel was then microwaved for 15 minutes at 130 °C. The contents of the flask were then concentrated in vacuo and purified on reverse phase HPLC, yielding 7.1 mg of 4-((4-((1H-benzo[d]imidazol-5-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)benzenesulfonamide (0.0178 mmol, 28.8 % yield) as a white solid. $^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.81 (s, 1H), 8.31 (s, 1H), 7.80 (s, 2H), 7.62 (d, $J$ = 8.6 Hz, 1H), 7.57 (s, 2H), 7.41 (d, $J$ = 2.3 Hz, 1H), 7.24 (s, 1H), 7.17 (s, 3H), 7.06 (dd, $J$ = 8.6, 2.3 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 171.36, 168.26, 165.43, 163.01, 147.61, 142.91, 142.81, 136.80, 126.16, 118.91, 116.96. HRMS (ESI) calcd for $[M+H]^+$ $C_{16}H_{15}N_8O_3S$ 399.0988, found 399.0981.



33 m

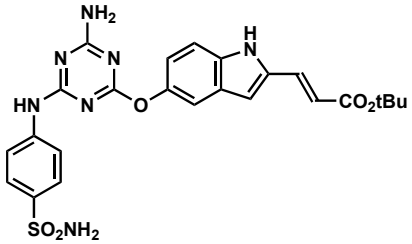
**4-((4-((1H-indol-5-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)benzenesulfonamide (33m)** (44 % yield) $^1$H NMR (600 MHz, Acetone-$d_6$) δ 10.39 (s, 1H), 8.86 (s, 1H), 7.90 (s, 2H), 7.66 (s, 2H), 7.46 (d, $J$ = 8.5 Hz, 1H), 7.42 (s, 1H), 7.34 (s, 1H), 6.93 (d, $J$ = 8.0 Hz, 1H), 6.54 (s, 2H), 6.51 (s, 1H), 6.40 (s, 2H). $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 170.22, 167.12, 147.11, 144.37, 138.10, 135.01, 129.41, 127.78, 127.15, 120.01, 117.24, 113.50, 112.46, 102.80. HRMS (ESI) calcd for $[M+H]^+$ $C_{17}H_{17}N_7O_3S$ 398.1035, found 398.1029.



33 n

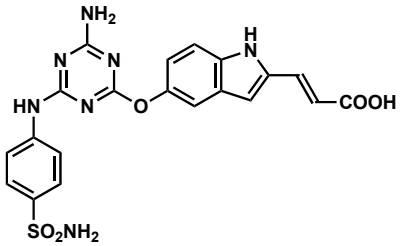
**2-((5-((4-amino-6-((4-sulfamoylphenyl)amino)-1,3,5-triazin-2-yl)oxy)-1*H*-indol-2-yl)methoxy)acetic** (12.5 % yield) Methyl 2-((5-hydroxy-1*H*-indol-2-yl)methoxy)acetate **(34)** (34 mg, 0.14 mmol, 1.0 eq.) was combined with 0.7 mL anhydrous dimethylformamide and potassium carbonate (58 mg, 0.42 mmol, 3.0 eq.) in a flame-dried pressure vial equipped with a rubber septum. The mixture was stirred at rt for 5-10min, then was heated at 60°C and stirred for another 5-10min. 4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**31b**) (42.1 mg, 0.14 mmol 1.0 eq.) was added, and the reaction stirred at 60 °C for 37h. The mixture was concentrated and was subjected to normal-phase column chromatography (Dichloromethane/Methanol). The fractions containing the title product were collected and dried. The residue

was further processed: 1 mL water was added and the pH was adjusted to ~4. The precipitate was collected with centrifugation and removal of the supernatant water, followed by drying under a nitrogen stream. Then the residue was purified using a preparatory HPLC column with a gradient of acetonitrile with 0.1% formic acid /water with 0.1% formic acid, to afford the title compound as a beige solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 11.28 (s, 1H), 9.78 (s, 1H), 7.83 (s, 2H), 7.58 (d, 2H), 7.36 (d, $J$ = 8.6 Hz, 1H), 7.27 (d, $J$ = 2.2 Hz, 1H), 7.24 – 7.07 (m, 4H), 6.89 (dd, $J$ = 8.6, 2.1 Hz, 1H), 6.39 (s, 1H), 4.68 (s, 2H), 4.05 (s, 2H). Note: H of the COOH moiety was not observed. $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 171.61, 171.60, 168.33, 165.52, 145.38, 143.05, 136.76, 136.60, 134.20, 127.84, 126.23, 118.94, 116.15, 112.25, 111.61, 101.42, 66.55, 65.29. HRMS (ESI) calcd for $[M+H]^+$ $C_{20}H_{20}N_7O_6S$ 486.1196, found 486.1218, and 508.1037 $[M+Na^+]$, 95.5 % pure.




**33 o'**

**(E)-(4-amino-6-((2-(3-(tert-butoxy)-3-oxoprop-1-en-1-yl)-1H-indol-5-yl)oxy)-1,3,5-triazin-2-yl)(4-sulfamoylphenyl)amide (33o')** (62 % yield) To a flask dried in a vacuum dessicator equipped with a stir bar and containing 17.2 mg (0.0663 mmol) of tert-butyl (E)-3-(5-hydroxy-1H-indol-2-yl)acrylate **(35)** was added 21.1 mg (0.070 mmol, 1.06 eq.) of 4-((4-amino-6-chloro-)1,3,5-triazin-2-yl)amino)benzenesulfonamide **(31b)** and 28.9 mg (0.209 mmol, 3.15 eq.) of potassium carbonate. The flask was purged with nitrogen, at which point 3 mL of DMF was added. The solution was then heated to 70 °C and stirred overnight. The solution was then concentrated in vacuo, partitioned between EtOAc and water, and extracted with EtOAc. The organic layer was dried with $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified via column chromatography (10:1 $CH_2Cl_2$:MeOH) yielding 21.4 mg (0.0410 mmol, 61.7 % yield) of (**33o'**) as a white solid. $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 7.73 (d, $J$ = 5.6 Hz, 2H), 7.64 (d, $J$ = 5.6 Hz, 2H), 7.57 (d, $J$ = 16.0 Hz, 1H), 7.40 (d, $J$ = 8.8 Hz, 1H), 7.34 (d, $J$ = 2.2 Hz, 1H), 7.02 (dd, $J$ = 8.8, 2.3 Hz, 1H), 6.79 (s, 1H), 6.36 (d, $J$ = 16.0 Hz, 1H), 1.55 (s, 9H). $^{13}$C NMR (101 MHz, Methanol-$d_4$) δ 173.21, 169.99, 168.26, 166.99, 147.46, 144.44, 137.86, 137.58, 136.56, 135.15, 129.97, 127.84, 120.31, 119.98, 118.72, 114.25, 112.77, 109.06, 81.71, 28.47. MS (ESI) calcd. For $[M-H]^-$ $C_{24}H_{24}N_7O_5S$ 522.2, found 522.2.

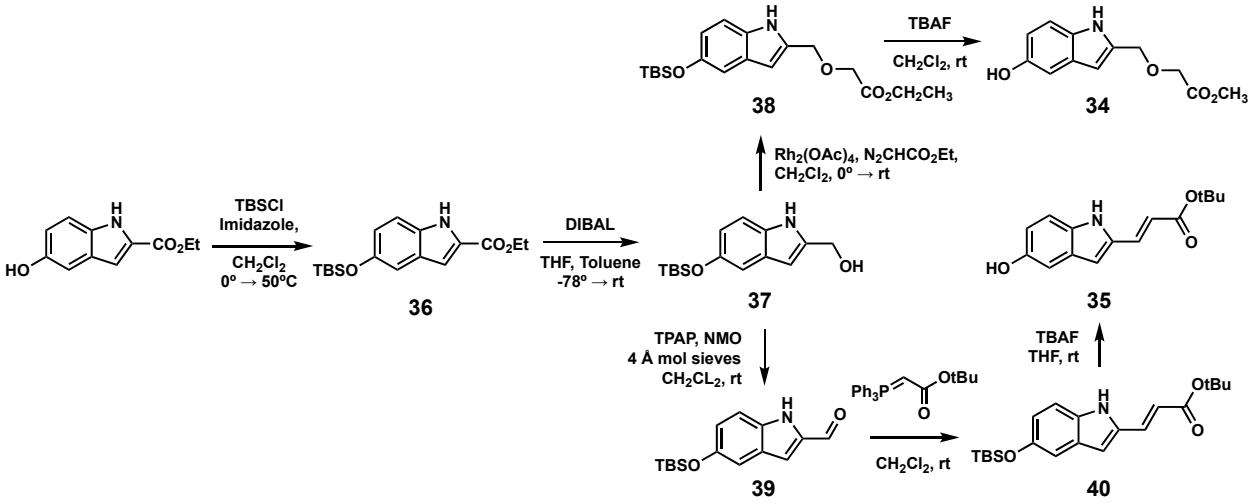



**33 o**

**(E)-3-(5-((4-amino-6-((4-sulfamoylphenyl)amino)-1,3,5-triazin-2-yl)oxy)-1H-indol-2-yl)acrylic acid (33o)** (2 46 % yield) To a flask equipped with a stir bar and 18.0 mg (0.0344 mmol) of (E)-(4-amino-6-((2-(3-(tert-butoxy)-3-oxoprop-1-en-1-yl)-1H-indol-5-yl)oxy)-1,3,5-triazin-2-yl)(4-sulfamoylphenyl)amide (**33o'**) was added 2 mL of $CH_2Cl_2$ and 2 mL of TFA. The solution was stirred at room temperature

for 1 hour, at which point the mixture was concentrated in vacuo. The residue was then purified by reverse phase HPLC to yield the title compound **(33o)** as a white solid. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 11.66 (s, 1H), 9.82 (s, 1H), 7.84 (d, *J* = 5.4 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 15.9 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 2.3 Hz, 1H), 7.24 (br s, 1H), 7.19 (br s, 3H), 7.00 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.81 (s, 1H), 6.48 (d, *J* = 16.1 Hz, 1H). Note: H of the COOH moiety was not observed. $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 171.49, 168.29, 165.50, 145.76, 143.02, 136.79, 135.56, 135.43, 128.17, 126.24, 118.94, 118.69, 112.89, 111.82, 107.15. HRMS (ESI) calcd. For $[M+H]^+$ $C_{20}H_{18}N_7O_5S$ 468.1085, found 468.1075. Retention Time=10.530 min (5 % B-100 % B over 20 minutes), 96.4 % pure.

**Scheme ES5**. Synthesis of Indole Intermediates **34** and **35**.




**Ethyl 5-((*tert*-butyldimethylsilyl)oxy)-1*H*-indole-2-carboxylate (36)** (0.733 g, 94 % yield) To a flame-dried flask, imidazole (299 mg, 4.39 mmol, 1.8 eq) was combined with anhydrous tetrahydrofuran (6 mL). Upon complete dissolution, ethyl 5-hydroxy-1*H*-indole-2-carboxylate (500 mg, 2.44 mmol, 1.0 eq) was added, the mixture stirred for 10 min, and was cooled to 0° C. Subsequently *tert*-butyldimethylsilyl chloride (551 mg, 3.66 mmol, 1.5 eq) was added portion-wise. The reaction was warmed to rt and then heated at 50 ° C for 12.5 h. Afterwards solvent was evaporated, dichloromethane (60mL) was added to the residue, and the organic layer was washed 3 times with 30mL water, 2 times with 30mL BRINE, and dried over sodium sulfate. After filtering and solvent evaporation, the title product was isolated as a pale-white solid. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 11.71 (s, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.03 (dd, *J* = 11.2, 1.8 Hz, 2H), 6.83 (dd, *J* = 8.8, 2.3 Hz, 1H), 4.32 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 0.96 (s, 9H), 0.17 (s, 6H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 161.21, 148.88, 133.21, 127.83, 127.29, 119.50, 113.22, 110.08, 107.12, 60.29, 25.62, 17.91, 14.29, -4.53. HRMS (ESI) calcd for $[M+H]^+$ $C_{17}H_{26}NO_3Si$ 320.1682, found 320.1685.

**(5-((tert-butyldimethylsilyl)oxy)-1*H*-indol-2-yl)methanol (37) (**1.60 g, 74 % yield) In a flame-dried flask, ethyl 5-((*tert*-butyldimethylsilyl)oxy)-1*H*-indole-2-carboxylate **(36)** (2.5 g, 7.83 mmol, 1 eq.) was dissolved in anhydrous tetrahydrofuran (50 mL). The mixture was brought to -78° C, followed by dropwise, slow addition of diisobutylaluminum(III) hydride (26.1 mL of 1.2M solution in toluene, 31.32 mmol, 4eq.) for over 1h. At the end of the addition, the reaction was warmed to rt, and was stopped at 1.5 h, when TLC indicated consumption of the starting material. Subsequently, the reaction was quenched with excess methanol until bubbling was ceased, and the solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate, was washed 2 times with sodium bicarbonate, 2 times with BRINE, and was dried over sodium sulfate. After filtering and solvent evaporation, the residue was further purified with normal-phase column chromatography (Hexanes /Ethyl Acetate) to afford the title product as a yellow oil. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 10.82 (s, 1H), 7.17 (d, $J$ = 8.6 Hz, 1H), 6.87 (d, $J$ = 1.8 Hz, 1H), 6.58 (dd, $J$ = 8.6, 2.2 Hz, 1H), 6.15 (s, 1H), 5.18 (t, $J$ = 5.5 Hz, 1H), 4.56 (d, $J$ = 5.5 Hz, 2H), 0.96 (s, 9H), 0.15 (s, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 147.95, 140.96, 131.86, 128.51, 114.40, 111.41, 108.92, 98.25, 56.92, 17.97, 14.10, -4.44. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{24}NO_2Si$ 278.1576, found 278.1584.

**Ethyl 2-((5-((*tert*-butyldimethylsilyl)oxy)-1*H*-indol-2-yl)methoxy)acetate (38)** (0.249 g, 31 % yield) (5-((*tert*-butyldimethylsilyl)oxy)-1*H*-indol-2-yl)methanol **(37)** (0.605 g, 2.18 mmol, 1 eq.), was combined with anhydrous dichloromethane (3mL) in a flame-dried pressure vial equipped with a rubber septum. The mixture was cooled to 0°C and rhodium acetate dimer was added (0.034 g, 0.077 mmol, 0.035 eq.) The reaction was purged with nitrogen and was allowed to stir for 5-10 min at 0° C. Subsequently ethyl diazoacetate (395 µL, 87% wt solution in dichloromethane, 3.27 mmol, 1.5 eq.) was added slowly. After the addition was complete, the reaction was stirred for 10 min at at 0° C, then warmed to rt and was allowed to run for 21h. The crude mixture was filtered through celite and washed with copious amounts of dichloromethane. The organic phase was washed 7 times with water, until discoloration, one time with BRINE, and was dried over sodium sulfate. Salts were filtered and solvent was evaporated under reduced pressure. The residue was further purified with normal-phase column chromatography (Hexanes /Ethyl Acetate) to afford the title product as a bright yellow waxy solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 11.01 (s, 1H), 7.19 (d, $J$ = 8.6 Hz, 1H), 6.90 (d, $J$ = 2.3 Hz, 1H), 6.62 (dd, $J$ = 8.6, 2.3 Hz, 1H), 6.27 (d, $J$ = 1.6 Hz, 1H), 4.63 (s, 2H), 4.13 – 4.10 (m, 4H, overlapping terminal $CH_2$ of ether, and $CH_2$ of ethyl ester), 1.21 – 1.18 (m, 3H), 0.96 (s, 9H), 0.15 (s, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.94, 148.12, 135.56, 132.23, 128.13, 115.30, 111.68, 109.12, 101.22, 66.41, 65.42, 60.20, 25.70, 17.96, 14.06, -4.46. HRMS (ESI) calcd for $[M+H]^+$ $C_{19}H_{30}NO_4Si$ 364.1944, found 364.1943.

**Methyl 2-((5-hydroxy-1*H*-indol-2-yl)methoxy)acetate (34)** (0.075 g, 48 % yield) Ethyl 2-((5-((*tert*-butyldimethylsilyl)oxy)-1*H*-indol-2-yl)methoxy)acetate **(38)** (0.242 g, 0.67 mmol, 1.0 eq.) was dissolved in dichloromethane (8 mL) and tetrabutylammonium fluoride (0.8 mL, 1M solution in THF, 1.2 eq.) was added

dropwise. After the addition was complete, the reaction stirred at rt for 3 min and subsequently dried in vacuo. The residue was purified with normal-phase column chromatography (Dichloromethane/Acetonitrile/Methanol). Transesterification occurred during the process, converting the ethyl to methyl ester, thus affording the title product as a white solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 9.39 (d, J = 17.0 Hz, 1H), 8.00 (d, J = 8.7 Hz, 1H), 6.92 (s, 1H), 6.76 (dd, J = 8.7, 2.4 Hz, 1H), 6.44 (s, 1H), 4.89 (s, 2H), 4.47 (s, 2H), 3.38 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 165.75, 154.52, 135.45, 130.51, 127.26, 115.81, 113.02, 105.88, 103.03, 67.83, 67.43, 62.14.

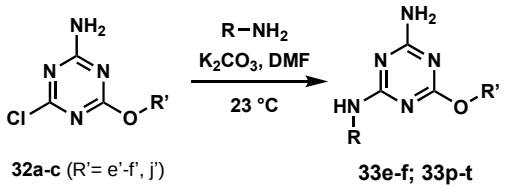
**5-[tert-butyl(dimethyl)silyl]oxy-1*H*-indole-2-carbaldehyde (39)** (0.67 g, 69 % yield) To a flask dried in a vacuum desiccator equipped with a stir bar and containing 0.098 mg, 0.354 mmol of [5-[tert-butyl(dimethyl)silyl]oxy-1*H*-indol-2-yl]methanol **(37)** was added 8.5 mg (0.024 mmol, 0.068 eq) of TPAP, 55.8 mg (0.484 mmol, 1.37 eq) of NMO, and 182.3 mg (500 mg/mmol) of 4 Å molecular sieves. The flask was purged under nitrogen, at which point 3 mL of $CH_2Cl_2$ was added and the solution was stirred for 23 hours at room temperature. The solution was concentrated in vacuo, and purified by column chromatography (0 to 66 % EtOAc in hexanes). $^{1}$H NMR (500 MHz, Chloroform-*d*) δ 9.81 (s, 1H), 9.06 (s, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.16 (d, *J* = 1.1 Hz, 1H), 7.13 (d, *J* = 2.2 Hz, 1H), 6.98 (dd, *J* = 8.9, 2.3 Hz, 1H), 1.01 (s, 9H), 0.21 (s, 6H). $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 182.07, 150.37, 136.61, 133.99, 128.11, 122.88, 114.31, 113.09, 111.64, 25.88, 18.37, -4.28. MS (ESI) calcd. For $[M+H]^+$ $C_{15}H_{22}NO_2Si$ 276.1, found 276.2.

**tert-butyl (E)-3-(5-((tert-butyldimethylsilyl)oxy)-1*H*-indol-2-yl)acrylate (40)** (0.066 mg, 50 % yield) To a flask dried in a vacuum desiccator equipped with a stir bar and containing 97.7 mg (0.355 mmol) of 5-[tert-butyl(dimethyl)silyl]oxy-1*H*-indole-2-carbaldehyde **(39)** was added 133.5 mg (0.355 mmol, 1.00 eq) of tert-butyl 2-(triphenyl-$\lambda^5$-phosphaneylidene)acetate. The flask was purged with nitrogen, and 3 mL $CH_2Cl_2$ was added. The solution was stirred overnight at room temperature, at which point the solution was concentrated in vacuo. The residue was purified using column chromatography (10 % EtOAc in hexanes). $^{1}$H NMR (400 MHz, Chloroform-*d*) δ 8.75 (s, 1H), 7.56 (d, *J* = 16.0 Hz, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 7.03 (d, *J* = 2.3 Hz, 1H), 6.83 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.67 (s, 1H), 6.24 (d, *J* = 16.0 Hz, 1H), 1.57 (s, 9H), 1.02 (s, 9H), 0.21 (s, 6H). $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 166.72, 149.81, 134.45, 133.81, 133.69, 129.24, 119.19, 117.39, 111.67, 110.47, 108.01, 80.86, 28.40, 25.91, 18.36, -4.29. MS (ESI) calcd. For $[M+H]^+$ $C_{21}H_{32}NO_3Si$ 374.2, found 374.2.

**tert-butyl (E)-3-(5-hydroxy-1H-indol-2-yl)acrylate (35)** (0.036 g, 85 % yield) To a flask dried in a vacuum desiccator equipped with a stir bar, purged with nitrogen, and 61.8 mg (0.165 mmol) of tert-butyl (E)-3-(5-((tert-butyldimethylsilyl)oxy)-1*H*-indol-2-yl)acrylate **(40)** was added 3 mL of THF and 0.35 mL (0.35 mmol, 0.212 eq) of 1M TBAF in THF. The solution was stirred for 2 hours at room temperature, at which point the solution was concentrated in vacuo. The residue was purified via column chromatography (0 to 100 % EtOAc in hexanes). $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 7.51 (d, *J* = 15.9 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.76 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.59 (s, 1H), 6.27 (d, *J* = 15.9 Hz, 1H), 1.52 (s, 9H). $^{13}$C NMR (101 MHz,

Methanol-$d_4$) δ 168.55, 152.04, 135.57, 134.93, 130.45, 117.39, 115.73, 112.79, 108.43, 105.49, 81.51, 28.47. MS (ESI) calcd. For $[M+Na]^+$ $C_{15}H_{17}NNaO_3$ 282.1, found 282.2.

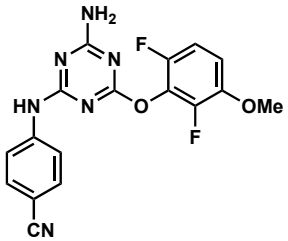
**Scheme ES6.** Synthesis of Aniline Analogs **33e-f** and **33p-t**.




**32a-c** (R'= e'-f', j') **33e-f; 33p-t**

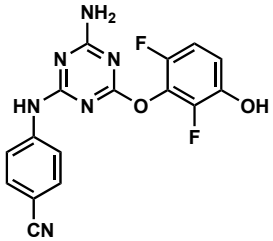
**General Procedure E**: Synthesis of Aniline Analogs **33e-f** and **33p-t**.

A solution of 4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino) derivative (0.20 mmol), functionalized aniline (0.30 mmol) and $K_2CO_3$ (0.60 mmol) in DMF (2 mL) was stirred at 70 °C for 18 hours. The reaction was cooled to room temperature, poured into $H_2O$ and extracted with EtOAc. The organic layer was dried over $Na_2SO_4$ and the solvent removed under reduced pressure. The crude mixture was loaded on silica to give the desired aromatic ether derivative after column chromatography.




**33 e**

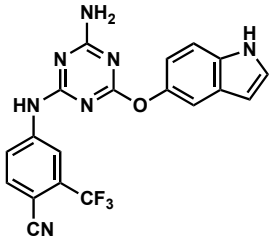
**4-((4-amino-6-(2,6-difluoro-3-methoxyphenoxy)-1,3,5-triazin-2-yl)amino) benzonitrile (33e)** (86%) $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.13 (s, 1H), 8.03 – 7.78 (m, 2H), 7.58 (d, $J$ = 8.4 Hz, 2H), 7.10 (d, $J$ = 7.5 Hz, 2H), 6.85 (d, $J$ = 30.5 Hz, 2H), 3.92 (s, 3H). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 171.27, 170.05, 166.88, 151.10 (d, $J$ = 2.4 Hz), 149.50 (d, $J$ = 2.5 Hz), 147.23 (d, $J$ = 4.2 Hz), 146.07 (dd, $J$ = 9.1, 2.8 Hz), 145.58 (d, $J$ = 4.3 Hz), 144.72, 120.65, 119.66, 113.48 – 109.28 (m), 105.86, 57.19. HRMS (ESI) calcd. For $[M+H]^+$ $C_{17}H_{13}F_2N_6O_2$ 371.1068, found 371.1066.




**33 f**

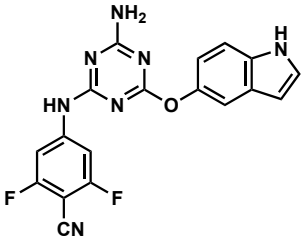
**4-((4-amino-6-(2,6-difluoro-3-hydroxyphenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (33f)** obtained by demethylation of **33e** (54%) To a solution of 4-((4-amino-6-(2,6-difluoro-3-methoxyphenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (60 mg, 0.16 mmol) in $CH_2Cl_2$ (0.5 mL) at 0°C was added phenylmethanethiol (0.37 mL) and $AlCl_3$ (32 mg, 0.24 mmol). The reaction was stirred for 30 minutes and a second portion of $AlCl_3$ was added. The reaction was allowed to warm at room temperature and stirred for other 90 minutes. The reaction was quenched with a slow addition of $H_2O$ and a saturated solution of $NaHCO_3$. The aqueous phase was extracted with EtOAc and the

organic layer washed with brine and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the crude mixture loaded on silica to give the free alcohol after column chromatography. $^1$H NMR (600 MHz, Acetone-$d_6$) δ 9.14 (s, 1H), 8.92 (s, 1H), 7.93 (s, 2H), 7.61 (d, $J$ = 8.3 Hz, 2H), 7.06 – 6.94 (m, 2H), 6.85 (d, $J$ = 42.9 Hz, 2H). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 171.35, 170.02, 166.89, 150.18, 148.59, 146.54 (d, $J$ = 4.2 Hz), 144.92 (d, $J$ = 4.3 Hz), 144.73 (d, $J$ = 14.6 Hz), 143.34 (d, $J$ = 11.0 Hz), 133.53, 120.64, 119.68, 111.24 (dd, $J$ = 19.9, 3.9 Hz), 105.81. HRMS (ESI) calcd for $[M+H]^+$ $C_{16}H_{11}F_2N_6O_2$ 357.0912, found 357.0907.



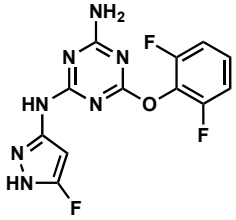
**33 p**

**4-((4-((1*H*-indol-5-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)-2-(trifluoromethyl)benzonitrile (33p)** (0.032 g, 14% yield) $^1$H NMR (600 MHz, DMSO-$d_6$) δ 11.16 (s, 1H), 10.26 (s, 1H), 8.24 (s, 2H), 7.90 – 7.77 (m, 1H), 7.45 – 7.36 (m, 3H), 7.32 (d, $J$ = 2.3 Hz, 2H), 6.90 (dd, $J$ = 8.7, 2.3 Hz, 1H), 6.42 (t, $J$ = 2.5 Hz, 1H). $^{13}$C NMR (151 MHz, dmso) δ 171.62, 168.26, 165.45, 145.20, 144.86, 135.87, 133.64, 131.46 (d, $J$ = 31.6 Hz), 127.89, 126.62, 123.40, 121.88, 116.99 – 116.43 (m), 116.20, 115.71, 112.16, 111.78, 101.27, 99.40. HRMS (ESI) calcd for $[M+H]^+$ $C_{19}H_{13}F_3N_7O$ 412.1134, found 412.1127.



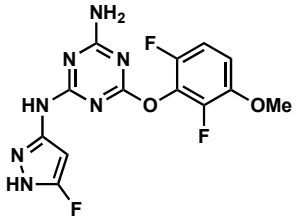
**33 q**

**4-((4-((1*H*-indol-5-yl)oxy)-6-amino-1,3,5-triazin-2-yl)amino)-2,6-difluorobenzonitrile (33q)** (0.021 g, 10% yield) $^1$H NMR (600 MHz, DMSO-*d6*) δ 11.16 (s, 1H), 10.32 (s, 1H), 7.64 (bs, 2H), 7.44 (bs, 2H), 7.41 (s, 1H), 7.40 (s, 1H), 7.31 (d, $J$ = 2.3 Hz, 1H), 6.90 (dd, $J$ = 8.8, 2.3 Hz, 1H), 6.42 (t, $J$ = 2.5 Hz, 1H). $^{13}$C NMR (151 MHz, dmso) δ 171.64, 168.29, 165.27, 163.41 (d, $J$ = 7.2 Hz), 161.73 (d, $J$ = 7.4 Hz), 145.16, 133.68, 129.68, 127.90, 126.66, 115.66, 112.10, 111.84, 110.33, 102.10, 101.94, 101.28. HRMS (ESI) calcd for $[M+H]^+$ $C_{18}H_{12}F2N_7O$ 380.1071, found 380.1065.



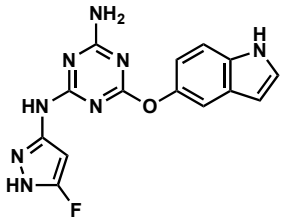
**33 r**

**6-(2,6-difluorophenoxy)-$N^2$-(5-fluoro-1*H*-pyrazol-3-yl)-1,3,5-triazine-2,4-diamine (33r)** (0.057 g, 23%) $^1$H NMR (500 MHz, DMSO-$d_6$) δ 12.08 (s, 1H), 10.56 (s, 1H), 7.60 (d, $J$ = 41.1 Hz, 2H), 7.40 – 7.22 (m, 3H), 5.49 (s, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 169.73, 168.12, 164.10, 163.23, 161.36, 155.88 (d, $J$ = 4.0 Hz), 153.91 (d, $J$ = 4.2 Hz), 140.03, 128.15, 126.74, 112.63 (dd, $J$ = 17.6, 4.2 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{12}H_9F_3N_7O$ 324.0821, found 324.0816.



**33 s**

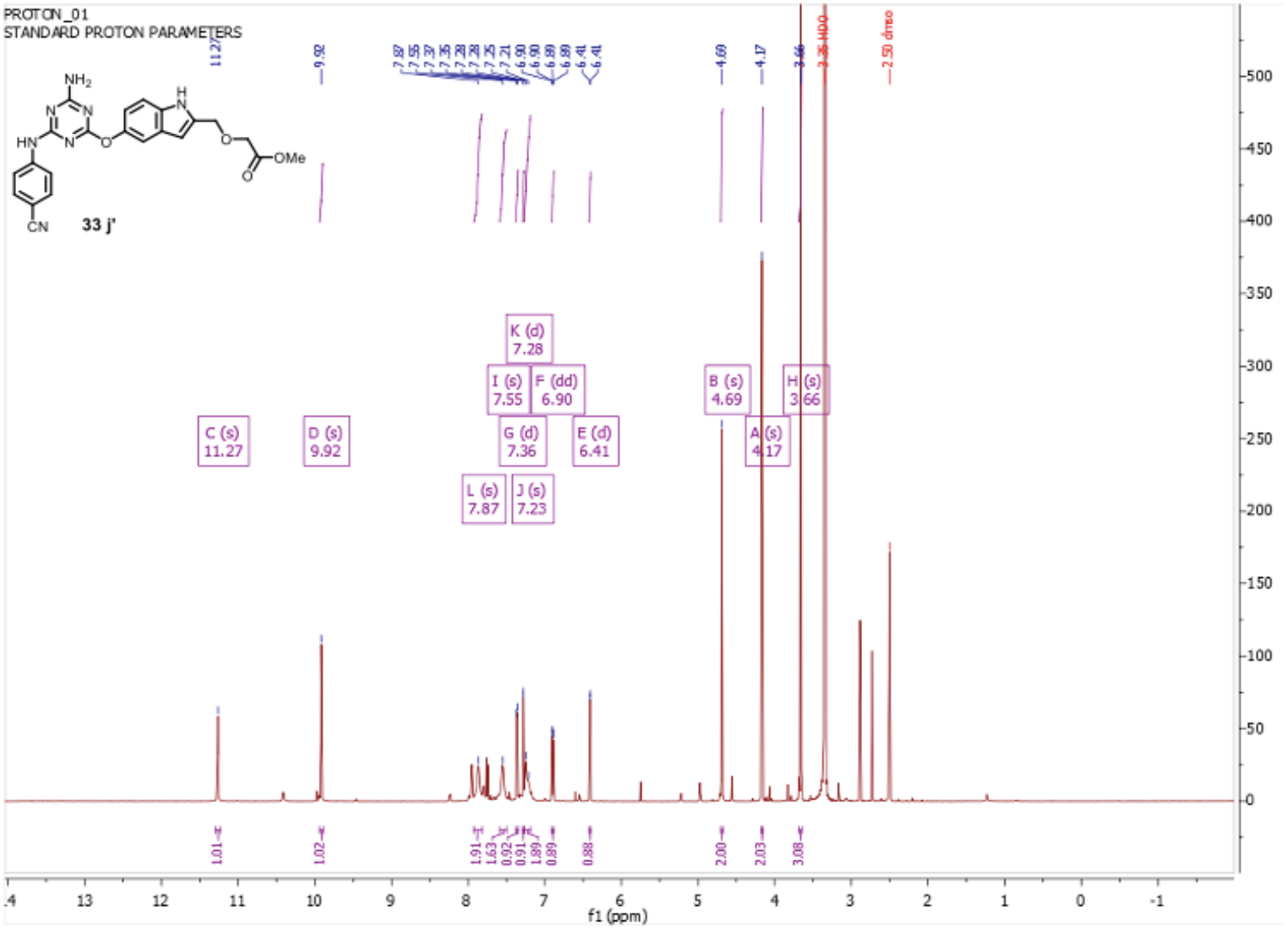
**6-(2,6-difluoro-3-methoxyphenoxy)-$N^2$-(5-fluoro-1*H*-pyrazol-3-yl)-1,3,5-triazine-2,4-diamine (33s)** (0.060 g, 31%) $^1$H NMR (600 MHz, DMSO-$d_6$) δ 12.09 (bs, 1H), 10.58 (bs, 1H), 7.66 (bs, 1H), 7.56 (bs, 1H), 7.21 (td, $J$ = 9.6, 1.9 Hz, 1H), 7.09 (td, $J$ = 9.1, 4.6 Hz, 1H), 5.49 (bs, 1H), 3.86 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.71, 168.15, 163.10, 161.55, 149.43, 147.84, 146.21 – 143.27 (m), 140.62, 128.94 – 128.75 (m), 110.64 – 110.49 (m), 109.87, 103.50 (d, $J$ = 17.8 Hz), 56.61. HRMS (ESI) calcd for $[M+H]^+$ $C_{13}H_{11}F_3N_7O_2$ 354.0926, found 354.0932.

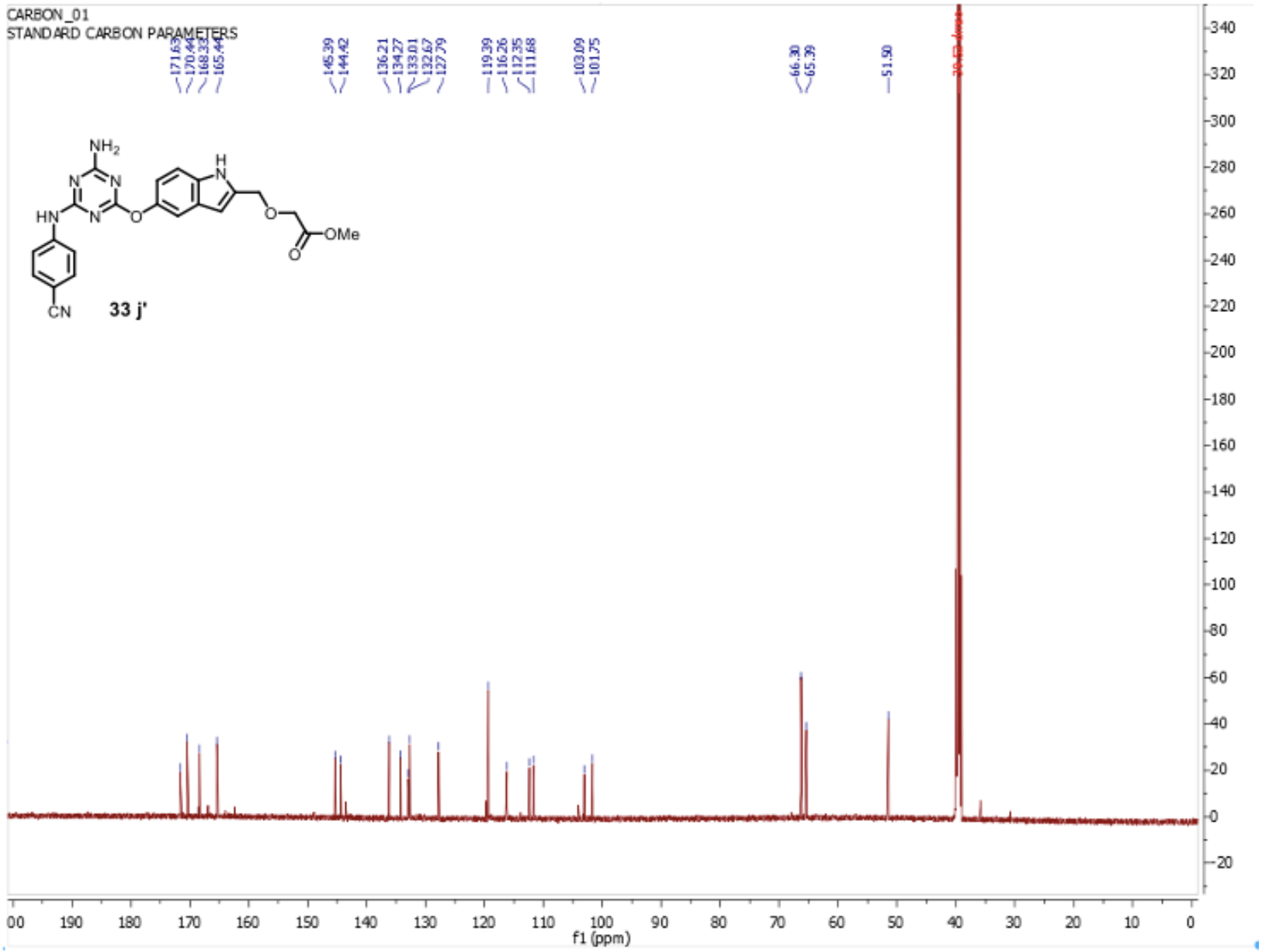


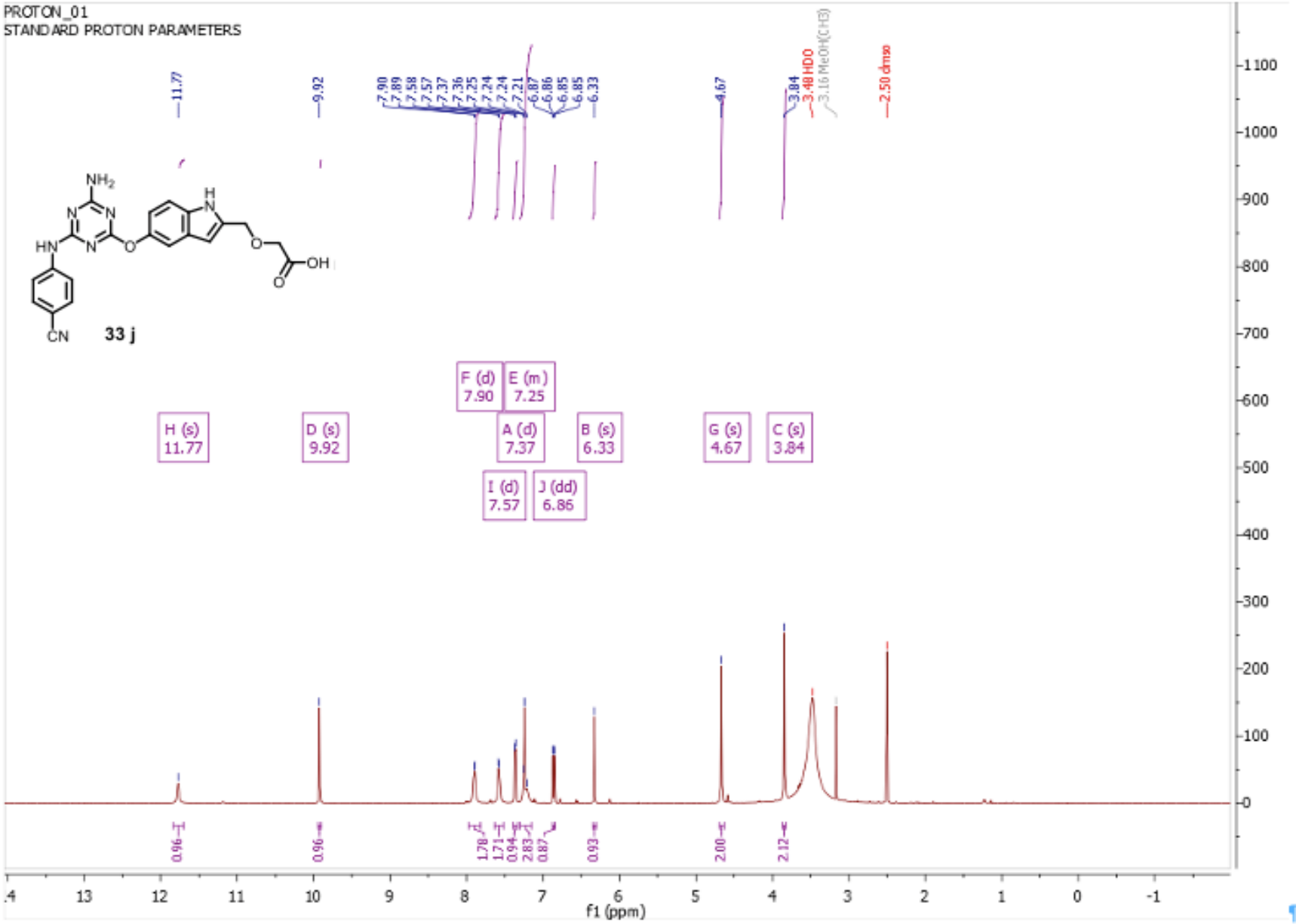
**33 t**

**6-((1*H*-indol-5-yl)oxy)-$N^2$-(5-fluoro-1*H*-pyrazol-3-yl)-1,3,5-triazine-2,4-diamine (33t)** (0.017 g, 10%) $^1$H NMR (500 MHz, DMSO-$d_6$) δ 12.07 (s, 1H), 10.38 (s, 1H), 7.96 (d, $J$ = 7.1 Hz, 1H), 7.43 – 7.25 (m, 5H), 6.95 – 6.75 (m, 2H), 6.45 – 6.39 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 171.66, 170.08, 168.18, 164.29, 163.27, 161.39, 145.18, 133.56, 127.87, 126.59, 112.15, 111.74, 101.23. HRMS (ESI) calcd for $[M+H]^+$ $C_{14}H_{12}FN_8O$ 327.1118, found 327.1123.

**3. $^{1}H$ and $^{13}C$ NMR Spectra**




CARBON_01
STANDARD CARBON PARAMETERS
171.63
170.44
168.33
165.44
146.39
144.42
136.21
134.27
133.01
132.67
127.79
119.39
116.26
112.35
111.88
103.09
101.75
66.30
65.39
51.90
NH2
HN
N
O
H
OMe
CN
33 j'
f1 (ppm)


PROTON_01
STANDARD PROTON PARAMETERS
11.77
9.92
7.90
7.89
7.58
7.57
7.37
7.36
7.25
7.24
7.24
7.21
6.87
6.86
6.85
6.85
6.33
4.67
3.84
3.48 HDO
3.16 MeOH(CH3)
2.50 dmso
NH2
HN
N
N
N
O
H
N
O
O
OH
CN
33 j
F (d) 7.90
E (m) 7.25
H (s) 11.77
D (s) 9.92
A (d) 7.37
B (s) 6.33
G (s) 4.67
C (s) 3.84
I (d) 7.57
J (dd) 6.86
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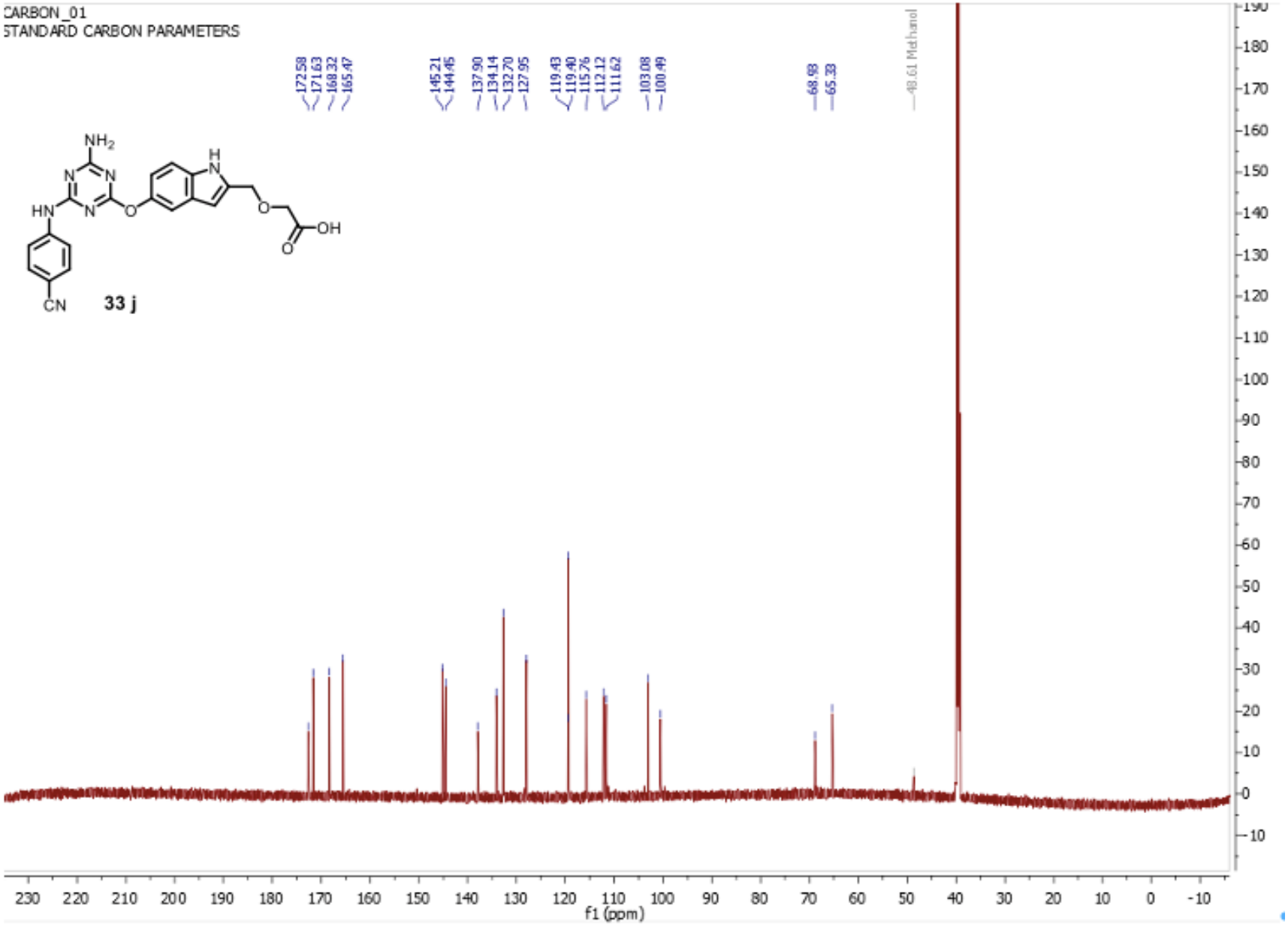
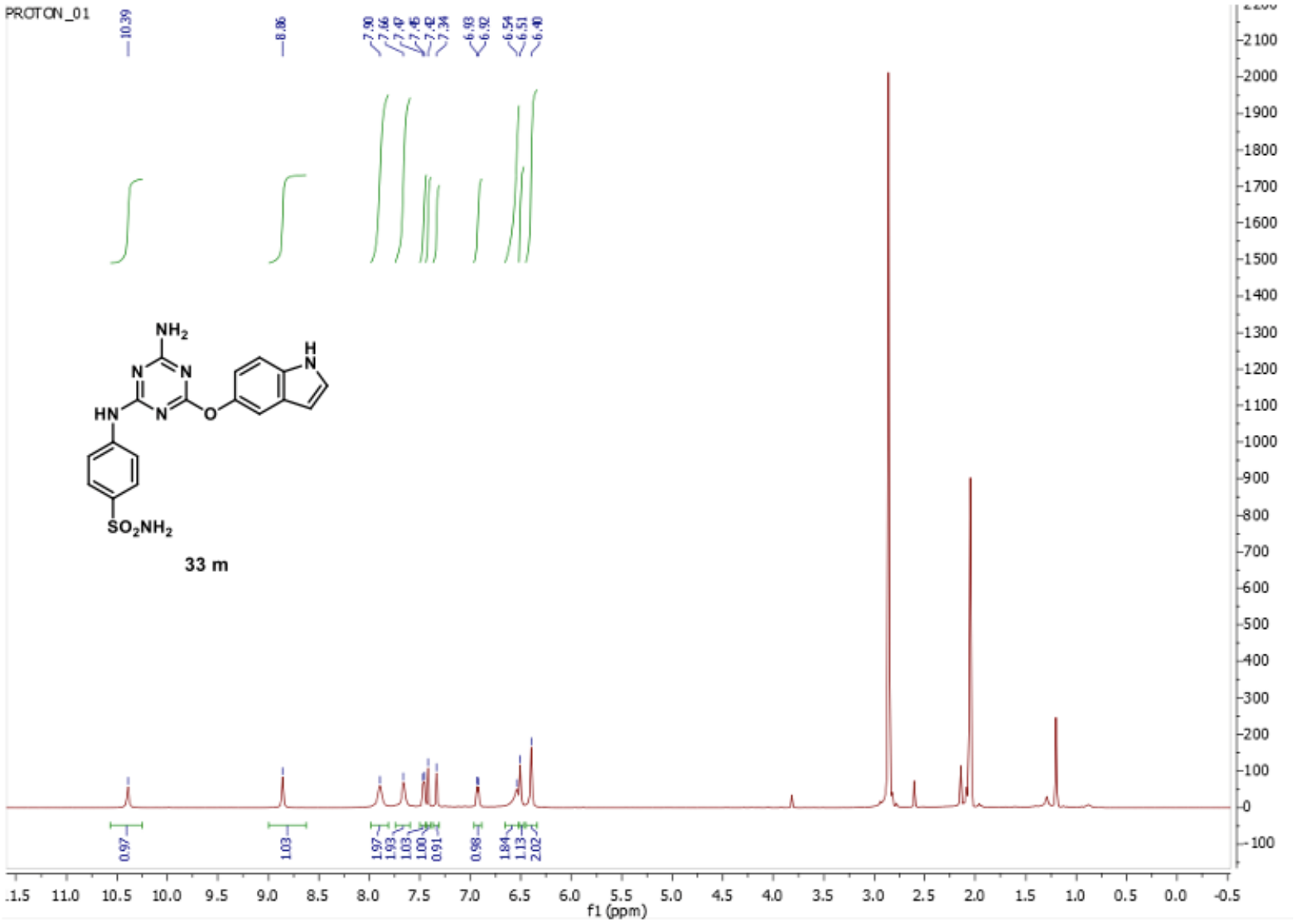
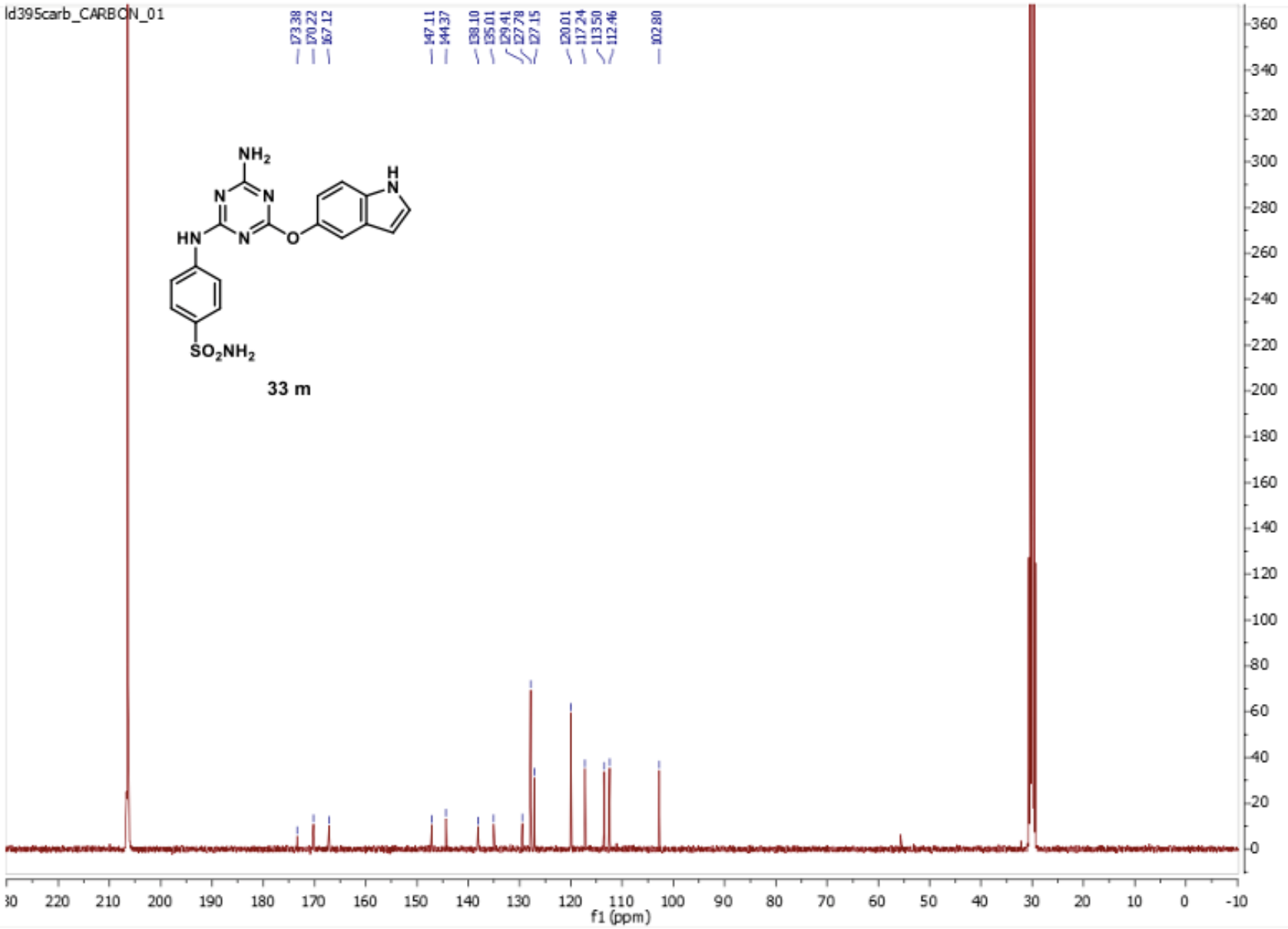
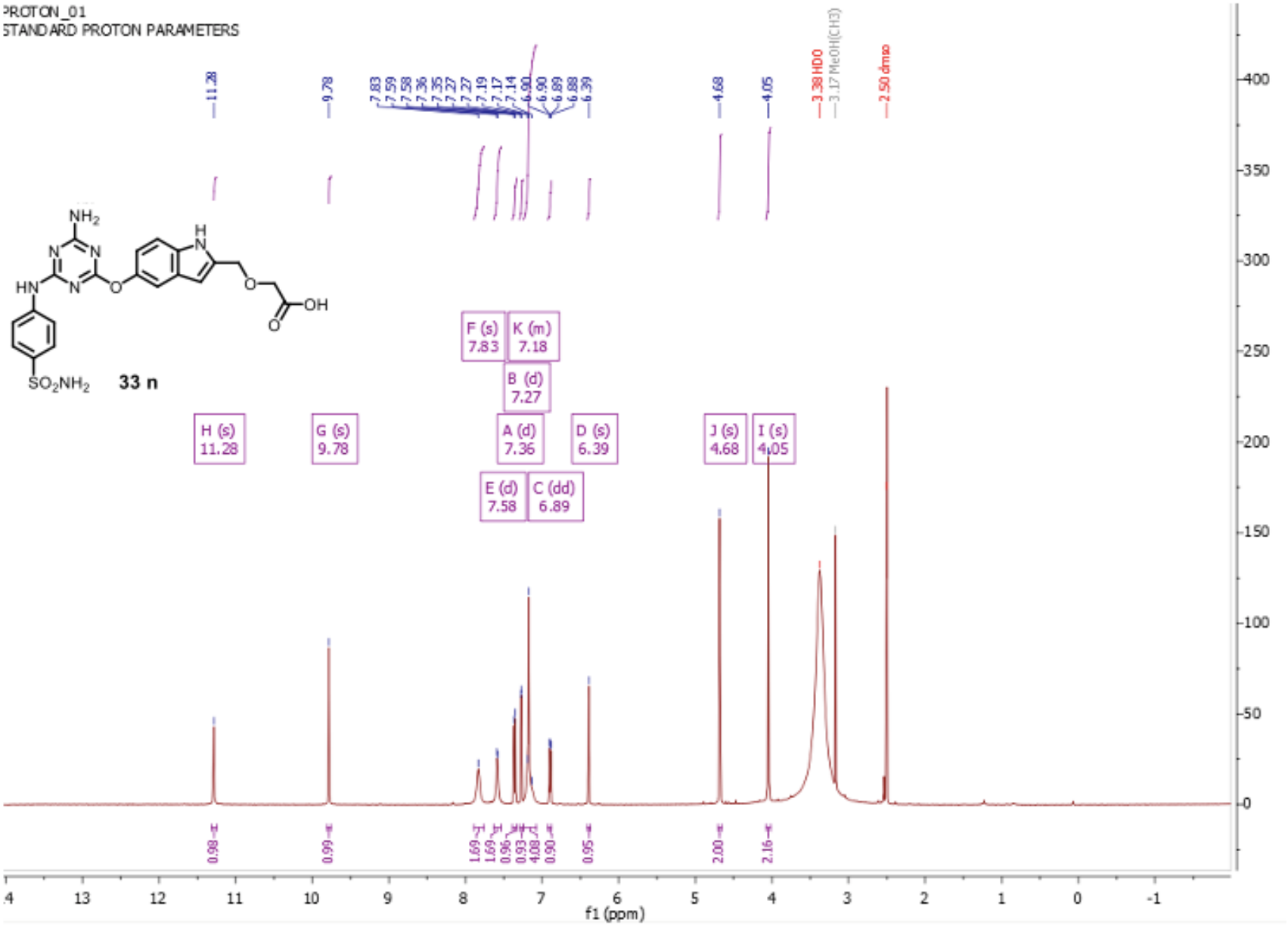
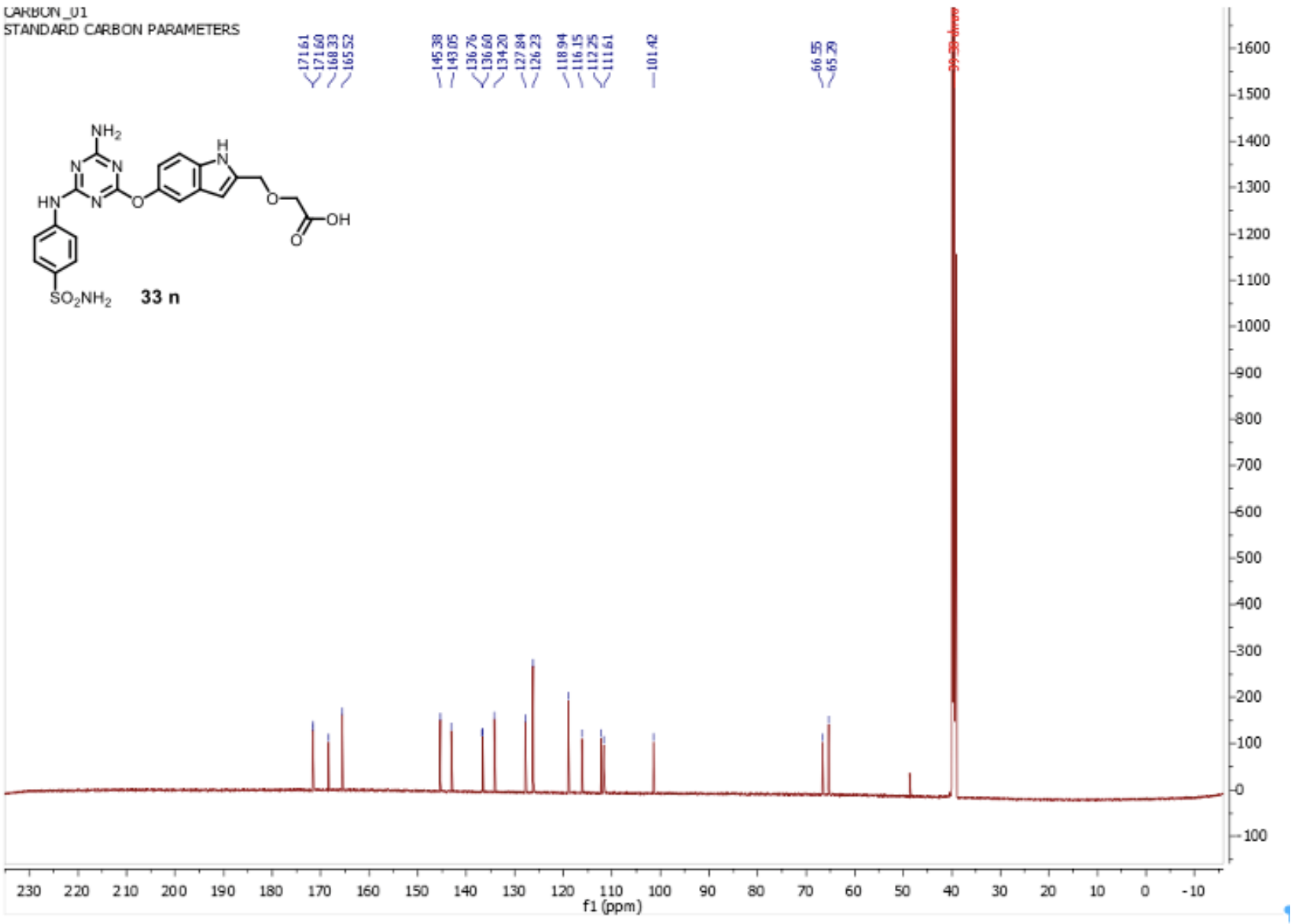
0.96
1.78
1.71
0.94
2.83
0.87
0.93
2.00
2.12
f1 (ppm)

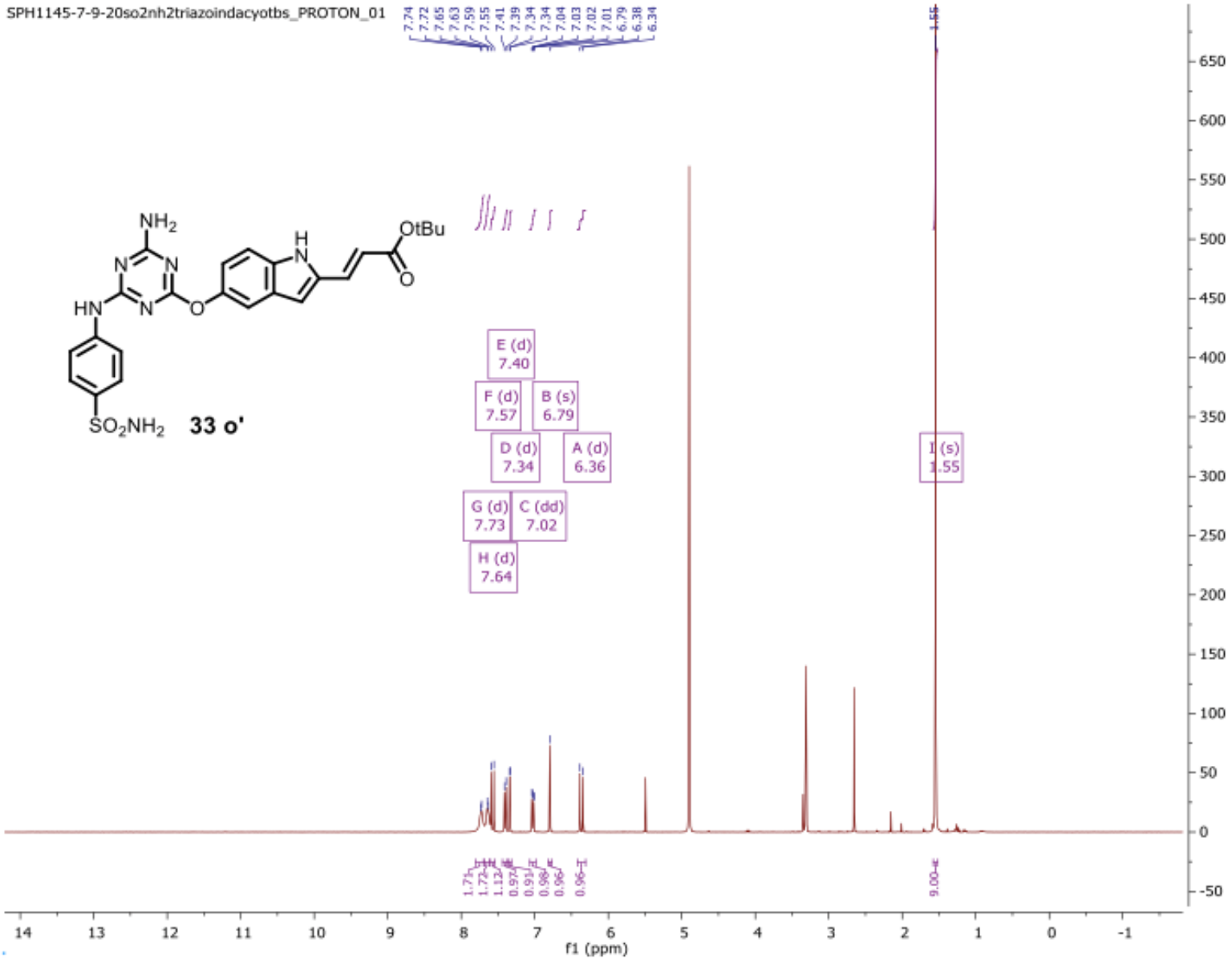

CARBON_01
STANDARD CARBON PARAMETERS
172.58
171.63
168.32
165.47
145.21
144.45
137.90
134.14
132.70
127.95
119.43
119.40
115.76
112.12
111.62
103.08
100.49
68.98
65.33
48.61 Methanol
NH2
HN
N
O
H
OH
CN
33 j
f1 (ppm)

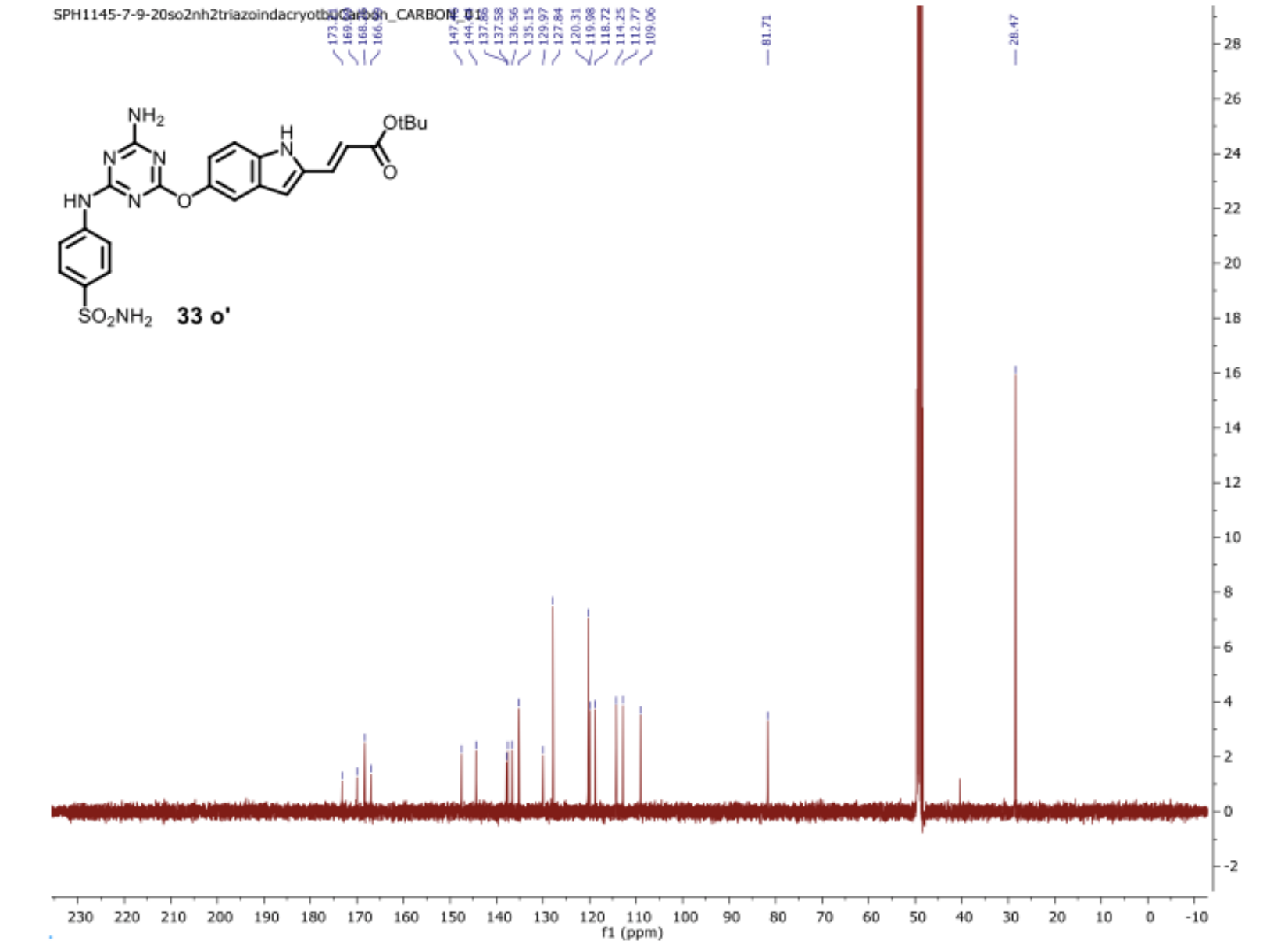

PROTON_01
10.39
8.86
7.90
7.86
7.47
7.45
7.42
7.34
6.93
6.92
6.54
6.51
6.40
NH2
HN
N
N
N
O
H
N
SO2NH2
33 m
0.97
1.03
1.97
1.93
1.03
1.00
0.91
0.98
1.84
1.13
2.02
f1 (ppm)


ld395carb_CARBON_01
173.38
170.22
167.12
147.11
144.37
138.10
135.01
129.41
127.78
127.15
120.01
117.24
113.50
112.46
102.80
NH2
N
N
N
HN
O
H
N
SO2NH2
33 m
f1 (ppm)


PROTON_01
STANDARD PROTON PARAMETERS
11.28
9.78
7.83
7.59
7.58
7.36
7.35
7.27
7.27
7.19
7.17
7.14
6.90
6.89
6.88
6.39
4.68
4.05
3.38 HDO
3.17 MeOH(CH3)
2.50 dmso
NH2
HN
N
N
N
O
H
N
O
O
OH
SO2NH2
33 n
F (s) 7.83
K (m) 7.18
B (d) 7.27
H (s) 11.28
G (s) 9.78
A (d) 7.36
D (s) 6.39
J (s) 4.68
I (s) 4.05
E (d) 7.58
C (dd) 6.89
0.98
0.99
1.69
1.69
0.96
0.93
4.08
0.90
0.95
2.00
2.16
f1 (ppm)


CARBON_01
STANDARD CARBON PARAMETERS
171.61
171.60
168.33
165.52
145.38
143.05
136.76
136.60
134.20
127.84
126.23
118.94
116.15
112.25
111.61
101.42
66.55
65.29
39.50
NH2
HN
OH
SO2NH2
33 n
f1 (ppm)


SPH1145-7-9-20so2nh2triazoindacyotbs_PROTON_01
7.74
7.72
7.65
7.63
7.59
7.55
7.41
7.39
7.34
7.34
7.04
7.03
7.02
7.01
6.79
6.38
6.34
1.55
NH2
N
N
N
HN
O
H
N
OtBu
O
SO2NH2
33 o'
E (d)
7.40
F (d)
7.57
B (s)
6.79
D (d)
7.34
A (d)
6.36
G (d)
7.73
C (dd)
7.02
H (d)
7.64
I (s)
1.55
1.71
1.72
1.12
0.97
0.91
0.98
0.96
0.96
9.00
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)


SPH1145-7-9-20so2nh2triazoindacryotbuCarbon_CARBON_01
NH2
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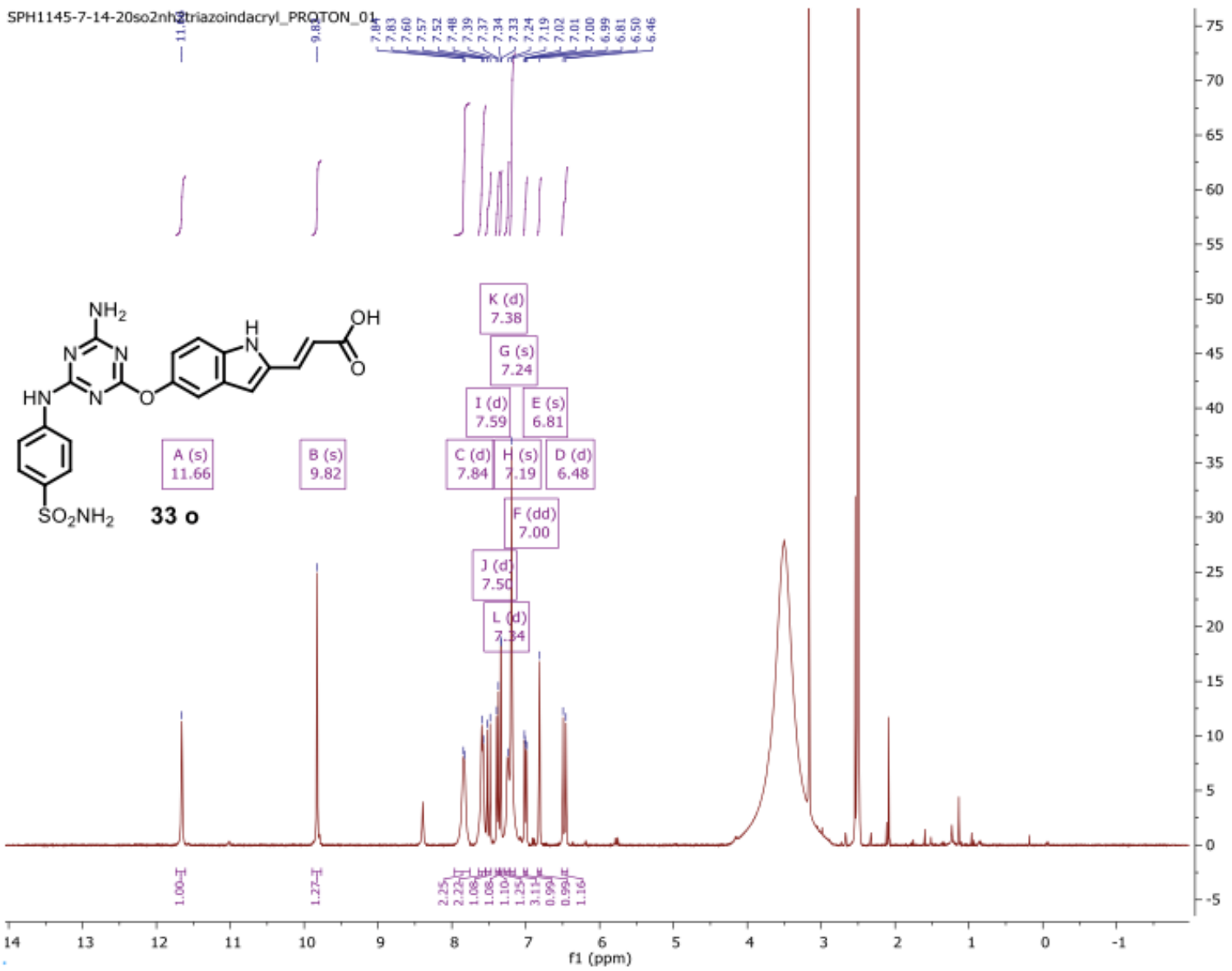
N
HN
O
H
N
OtBu
O
SO2NH2
33 o'
81.71
28.47
f1 (ppm)


SPH1145-7-14-20so2nh2triazoindacryl_PROTON_01
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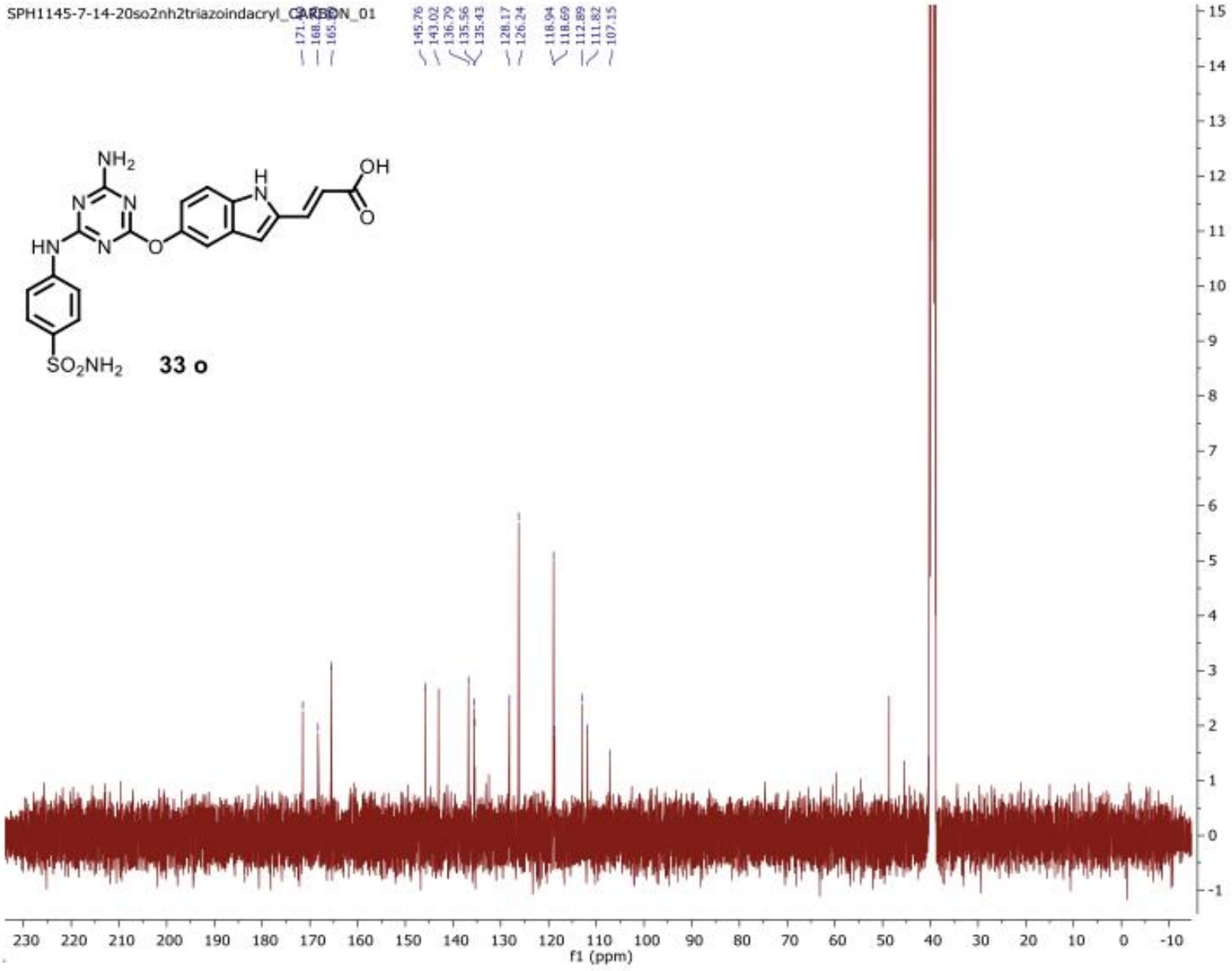
N
HN
O
H
N
OH
O
SO2NH2
33 o
A (s)
11.66
B (s)
9.82
K (d)
7.38
G (s)
7.24
I (d)
7.59
E (s)
6.81
C (d)
7.84
H (s)
7.19
D (d)
6.48
F (dd)
7.00
J (d)
7.50
L (d)
7.34
f1 (ppm)


SPH1145-7-14-20so2nh2triazoindacryl_CARBON_01
171.
168.
165.
145.76
143.02
136.79
135.56
135.43
128.17
126.24
118.94
118.69
112.89
111.82
107.15
NH2
N
N
N
HN
O
H
N
OH
O
SO2NH2
33 o
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

**4. Structure Determination by X-ray Crystallography**

Protein expression and purification was performed identical to a previously described protocol [11]. JAK2 JH2 protein at a concentration of 6.0 mg/mL in a buffer consisting of 20 mM Tris pH 8.0, 100 mM NaCl, 10% glycerol, and 1 mM TCEP was crystallized by hanging drop vapor diffusion (room temperature) using reservoir solution consisting of 0.1 M Tris pH 8.0, 0.2 M sodium acetate, 12−24% PEG4000, and 1 mM TCEP. Crystal growth was initiated by streak seeding. Protein-ligand complexes were prepared by transferring empty JAK2 JH2 crystals into reservoir solution containing 4 mM small-molecule ligand and 8% DMSO for 24 h. Prior to freezing, crystals were cryo-protected by briefly exposing them to reservoir solution containing 20% glycerol.

Diffraction data were collected in-house on Rigaku MicroMax-007HF X-ray generator (λ =1.54 Å) equipped with a Dectris Pilatus 200K detector. Datasets were indexed, integrated, and scaled using HKL2000 [12]. Phases were calculated by molecular replacement (search model: 4FVQ) using Phaser [13]. Structure refinement was performed using Phenix version 1.11.1-2575 [14]. Cartesian simulated annealing was performed as initial step of refinement, followed by refinement of XYZ coordinates, occupancies, and individual B factors. Manual model building was performed using Coot [15]. Small-molecule ligands' restraints were prepared using Phenix eLBOW [16]. Crystallographic data collection and refinements statistics are shown in Table ES2.

**Table ES2. Crystallographic data collection and refinement statistics**

| | **small-molecule ligand** | | |
|---|---|---|---|
| | **22** | **33b** | **33m** |
| PDB ID | 7JYQ | 7JYO | 6XJK |
| space group | $P2_1$ | $P2_1$ | $P2_1$ |
| unit cell parameters: | | | |
| *a*, *b*, *c* (Å)<br>*α, β, γ* (°) | 44.4, 57.3, 61.0,<br>90.0, 110.6 | 44.6, 57.4, 61.1,<br>90.0, 111.0, 90.0 | 45.0, 57.6, 61.2,<br>90.0, 111.5, 90.0 |
| Matthews coefficient (Å$^3$/Da)[a] | 47.0 | 47.0 | 47.0 |
| solvent content (%)[a] | 2.3 | 2.3 | 2.3 |
| **Diffraction data** | | | |
| resolution range (Å) | 50.00–1.86 (1.93–1.86) | 50.00–2.16 (2.24–2.16) | 50.00–2.02 (2.05–2.02) |
| unique reflections | 24049 (2297) | 15316 (1383) | 18548 (922) |
| $R_{sym}$ (%) | 5.8 (22.6) | 5.7 (18.3) | 11.5 (54.5) |
| *CC*1/2 | 0.970 (0.920) | 0.979 (0.930) | 0.930 (0.734) |
| $\langle I/\sigma(I)\rangle$ | 19.7 (3.3) | 15.2 (3.0) | 8.7 (2.1) |

| | | | |
|---|---|---|---|
| completeness (%) | 99.3 (96.5) | 98.5 (89.5) | 96.9 (96.8) |
| redundancy | 3.0 (2.6) | 3.0 (2.1) | 2.9 (2.6) |
| Wilson *B* factor (Å$^2$) | 21.2 | 24.5 | 23.8 |
| **Refinement** | | | |
| resolution range (Å) | 29.11–1.86 | 40.44–2.16 | 40.50–2.02 |
| reflections used (work/free) | 22852/1182 | 14548/751 | 17419/923 |
| $R_{work}/R_{free}$ (%) | 17.9/22.1 | 18.5/24.6 | 22.7/27.3 |
| protein residues | 272 | 270 | 265 |
| inhibitor atoms | 23 | 26 | 29 |
| water molecules | 155 | 72 | 62 |
| RMSD from ideality: | | | |
| bond lengths (Å) | 0.006 | 0.007 | 0.007 |
| bond angles (°) | 0.8 | 0.8 | 0.8 |
| Ramachandran plot:[b] | | | |
| Ramachandran favored (%) | 98.9 | 96.6 | 97.7 |
| Ramachandran outliers (%) | 0.0 | 0.0 | 0.0 |
| Mean B factors (Å$^2$): | | | |
| protein | 22.4 | 29.8 | 28.5 |
| inhibitor | 31.4 | 25.3 | 29.0 |
| water molecules | 28.7 | 29.1 | 28.3 |

Values in parenthesis describe the highest resolution shell. [a]Computed with *CCP4 Matthews_coef* [17]. [b]Computed with *MolProbity* [18].

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