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6-Bromoindirubin-3'-oxime Derivatives are Highly Active Colistin Adjuvants against *Klebsiella pneumoniae*

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Table S1. Initial screen of indirubin analogues for colistin resistance suppression

	KP B9			KP B9	
Compound	**MIC(μM)**	**Colistin MIC (μg/mL) (fold reduction)**	**Compound**	**MIC(μM)**	**Colistin MIC (μg/mL) (fold reduction)**
-	-	512		-	512
5	>200	1(512)	**28**	>200	2(256)
12	>200	>16	**29**	>200	2(256)
13	>200	>16	**30**	>200	>16
14	>200	>16	**31**	>200	>16
15	>200	>16	**32**	>200	>16
16	>200	4(128)	**33**	>200	>16
17	>200	8(64)	**34**	>200	4(128)
18	>200	>16	**35**	>200	8(64)
19	>200	>16	**36**	>200	2(256)
20	>200	>16	**37**	>200	>16
21	>200	>16	**38**	>200	>16
22	>200	4(128)	**39**	>200	1(512)
23	>200	8(64)	**40**	>200	>16
24	>200	1(512)	**41**	>200	>16
25	>200	2(256)	**42**	>200	>16
26	>200	0.5(1024)	**43**	>200	>16
27	>200	1(512)			

All compounds tested at 5 μM

Table S2. Dose response data for active analogues against KPB9

Compound	Concentration (µM)	Colistin MIC (µg/mL) (fold reduction)
5	5	1(512)
	3	32(16)
	1	>64[a]
24	5	1(512)
	3	2(256)
	1	32(16)
25	5	2(256)
	3	16(32)
	1	>64[a]
26	5	0.5(1024)
	3	1/2(512/256)
	1	16(32)
27	5	1(512)
	3	2(256)
	1	32(16)
28	5	2(256)
	3	32(16)
	1	>64[a]
29	5	2(256)
	3	2(256)
	1	>64[a]
36	5	2(256)
	3	4(128)
	1	64 (8)
39	5	1(512)
	3	4(128)
	1	>64

[a]Highest concentration tested

Table S3. Compound MICs against colistin resistant isolate panel (μM)

Compound	**KP C3**	**KP F9**	**KP I2**	**KP F2210291*mcr-1***	**AB 3941**	**EC25922 *mcr-1***	**TRPA162**
-	128	256	512	16	1024	16	1024
5	>200	>200	>200	>200	>200	>200	>200
24	>200	>200	>200	>200	>200	>200	>200
26	>200	>200	>200	>200	>200	>200	>200
27	>200	>200	>200	>200	>200	>200	>200
29	>200	>200	>200	>200	>200	>200	>200

Table S4. Colistin potentiation activity of lead compounds from initial screen against colistin resistant isolate panel

Compound	**KP C3**	**KP F9**	**KP I2**	**KP F2210291 *mcr-1***	**AB 3941**	**EC25 922 *mcr-1***	**TRP A162**
-	128	256	512	16	1024	16	1024
5	4(32)	4(64)	8(64)	2(8)	>16	2(8)	>16
24	4(32)	4(64)	4(128)	2/4(8/4)	>16	1(16)	>16
26	2/1(64/128)	2(64)	2(256)	1(16)	>16	2(8)	>16
27	4(32)	4(64)	4(128)	2(8)	>16	2(8)	>16
29	4(32)	4(64)	4/8(128/64)	2(8)	>16	2(8)	>16

All compounds were tested at 5μM

Table S5. Percent hemolysis compared to 1% Triton x-100

	Hemolysis%				
Compound	25 μM	50 μM	100 μM	200 μM	400 μM
26	0.2% ± 0.004	1.1% ±0.008	3.1% ±0.02	0.0% ± 0.0	2.7% ± 0.007

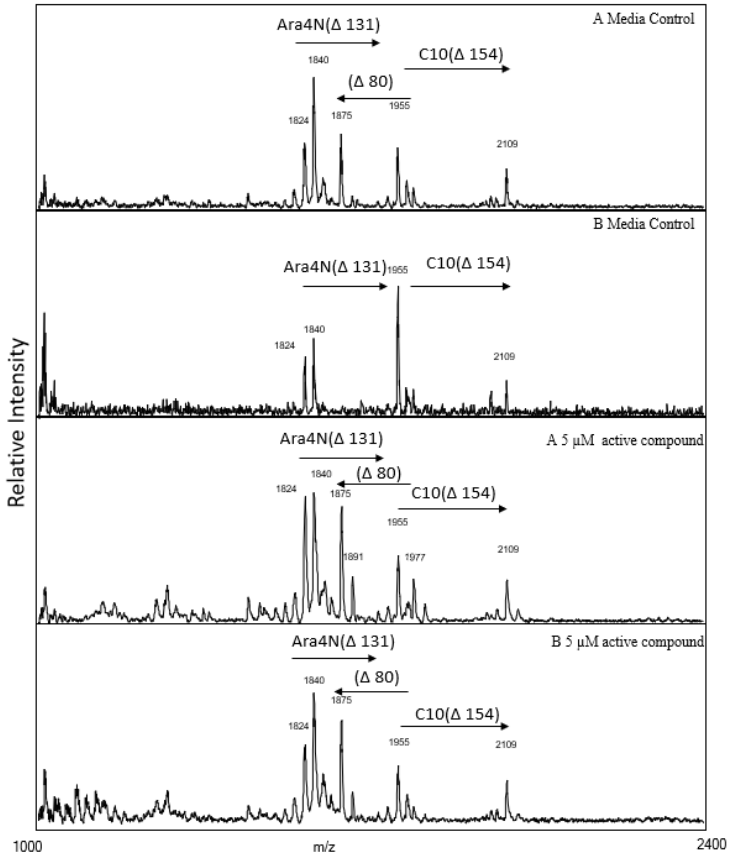

Figure S1. MALDI-TOF analysis of lipid A composition of untreated cells and treated with 5 μM compound **26**

Biological Procedures

Bacterial strains and growth conditions

Acinetobacter baumannii and *Klebsiella pneumoniae* strains were routinely grown on LB Lennox agar plates from glycerol stocks at least every three weeks. For use in biological assays, strains were grown in cation-adjusted Mueller Hinton broth (CAMHB, BD). *A. baumannii* strain 3941 was obtained from Walter Reed Army Institute for Research (WRAIR). *K. pneumoniae* strains B9, C3, EC25922$^{mcr\text{-}1}$ and F2210291^{mcr-1} were from Professor Robert Ernst's strain collection at The University of Maryland, Baltimore. *K. pneumoniae* strains F9 and I2, and *P. aeruginosa* strains TRPA 162 were obtained from Professor Yohei Doi at the University of Pittsburgh. Stock cultures were stored in 25 % glycerol and maintained at −80 °C. LB Lennox and agar were purchased from Fisher Bioreagents (Cat# BP14272) and Thermo Fisher Scientific (Cat# A10752.36), respectively. CAMHB was purchased from BD Diagnostics (Cat# 297963). Colistin sulfate salt was purchased from Sigma Aldrich (Cat# C4461). OD_{600} was recorded on the Thermo GENESYS^{TM} 10UV UV-Vis Spectrophotometer. All water used in biological assays is purified by MilliporeSigma™ Milli-Q™ Ultrapure Water Systems Accessory. All assays were run in duplicate and repeated at least two separate times.

Broth microdilution method for the determination of minimum inhibitory concentrations

Bacteria were cultured for 6 hours in CAMHB and then subcultured to 5×10^5 CFU/mL in fresh CAMHB. To aliquots (1 mL) was added compound from stock solutions in DMSO, such that the compound concentration equaled the highest concentration tested. Samples were then dispensed (200 µL) into the first row of a 96-well microtiter plate in which all but the final row of subsequent wells were prefilled with 100 µL of the untreated bacterial subculture. The final row was filled with media to act as a sterility control and blank. Row one wells were mixed 6-7 times, then, 100 µL was withdrawn and transferred to row two. Row two wells were mixed 6-7 times followed by a 100 µL transfer from row two to row three. This procedure was used to serially dilute the rest of the rows of the microtiter plate, excluding the last prefilled row, which was used to measure growth in the absence of compound. Plates were then sealed with GLAD Press'n Seal and incubated under stationary conditions at 37 °C. After 16 hours, the plates were removed, and MIC values were measured by recording the OD_{600} of each well. OD_{600} was monitored by a SYNERGY HTX multi-mode reader. Growth that registered as <10% in comparison to untreated control on the multi-mode reader was found to correspond to clear wells, thus MIC values were recorded as the lowest concentration required to achieve 90% growth inhibition compared to growth in untreated wells as assessed by the SYNERGY HTX multi-mode reader.

Broth microdilution method for measurement of colistin potentiation

Bacteria were cultured for 6 hours in CAMHB and diluted to 5×10^5 CFU/mL in fresh CAMHB. To aliquots (4 mL) was added compound from stock solutions in DMSO. One aliquot was not dosed to allow measurement of the colistin MIC in the absence of compound. A 1 mL aliquot of each sample was dosed with colistin, and from this 200 µL was dispensed into the first row of a 96-well microtiter plate in which all but the final row of subsequent wells was prefilled with 100 µL of the corresponding compound dosed bacterial suspension The final row was filled with media to act as a sterility control and blank. Row one wells were mixed 6-7 times, then, 100 µL was withdrawn and transferred to row two. Row two wells were mixed 6-7 times followed by a 100 µL transfer from row two to row three. This procedure was used to serially dilute the rest of the rows of the microtiter plate, excluding the last prefilled row, which was used to measure growth in the presence of compound alone. Plates were then sealed with GLAD Press'n Seal and incubated under stationary conditions at 37 °C. After 16 hours, the plates were removed, and MIC values were measured by recording the OD_{600} of each well. MIC values were determined as the minimum concentration required to achieve 90% growth inhibition compared to growth in untreated wells.

Hemolysis

To obtain the sheep red blood cells ready for hemolysis assay, defibrinated sheep blood (Hemostat Labs: DSB30) (1.5 mL) was placed into a microcentrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was then removed, and the cells were resuspended in 1 mL of phosphate-buffered saline (PBS) (Alfa Aesar Cat# J60465). The suspension was centrifuged as above and the supernatant was then removed. This was repeated an additional two times. After the final time, cells were suspended in 10 mL PBS, then aliquoted (1 mL), and test compounds added. PBS was used as a negative control and a zero-hemolysis marker. Triton X-100 (1%) (Acros Organics Cat# AC327371000) was used as a positive control serving as the 100% lysis marker. Samples were then placed in an incubator at 37 ˚C while being shaken at 200 rpm for one hour. After one hour, the samples were transferred to microcentrifuge tubes and centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in water. The absorbance of the supernatant was then measured with a UV spectrometer (Thermo GENESYS^{TM} 10UV-Vis Spectrophotometer) at a 540 nm wavelength.

Time–kill Curves

Strains were cultured overnight in CAMHB and subcultured to 5×10^5 CFU/mL in fresh CAMHB. The subculture was then transferred to culture tubes in 4 mL aliquots, which were dosed with carrier only (control), adjuvant, colistin, or adjuvant plus colistin. All samples were then incubated at 37 °C with shaking. At 2, 4, 6, 8, and 24-hour time points, 100 µL was taken from each sample and ten-fold diluted in CAMHB up to 7 times. 100 µL of diluted culture was plated on LB (Lennox) agar and incubated at 37 °C overnight. The total number of bacterial colonies on each plate was determined using a SphereFlash® colony counter (Neutec Inc).

Lipid A Analysis Procedure

K. pneumoniae B9 was cultured in 5 mL of CAMHB for 18 h at 37 °C and then subcultured to 1×10^6 CFU in 120 mL of fresh CAMHB. Subcultures were split and left untreated or dosed with compound **26** at the minimum effective concentration (5 µM) and incubated for 8 h. Treatment was completed in duplicate. Cells were pelleted for 15 min at 4000 rpm and 4 °C. The supernatant was discarded, and the pellet was washed with 5 mL of endotoxin free water. The cells were pelleted again, and the supernatant was discarded. Pellets were stored at −80 °C until shipped on dry ice to the Ernst lab for analysis.

Processing & Analysis: 1µL of sample pellet was scraped and plated directly to a steel re-usable MALDI plate. 1 µL of 70% citric acid extraction buffer was spotted on top of the plated bacteria. The steel MALDI plate was added to chamber with water on bottom and placed in 110 ºC oven for 30 minutes. After, the plate was rinsed with water and air dried. 1 µL of Norharmane matrix was spotted on each sample. Samples were analyzed in negative ion mode on a Bruker Microflex in negative ion mode. Data were processed with flexAnalysis software.

General chemistry experimental:

General procedure of characterization and purification

General: All reagents were purchased from commercially available sources without further purification. Flash chromatography was performed using 60 Å mesh standard grade silica gel from Sorbetch (Cat# 10734). NMR solvents were obtained from Cambridge Isotope Labs and used without further purification/drying. All ^{1}H NMR (400 MHz) were recorded at 25°C on a Bruker Avance spectrometer. All ^{13}C NMR (101 MHz or 126 MHz) spectra were also recorded at 25°C on Bruker Avance spectrometers. Chemical shifts (δ) are given in parts per million (ppm) relative to the respective NMR solvent peak; coupling constants (J) are in hertz (Hz). Abbreviations used are s, singlet; d, doublet; dd, doublet of doublets; td, triplet of doublets; m, multiplet. All high-resolution mass spectrometry measurements were made by the Mass Spectrometry and Proteomics Facility at the University of Notre Dame. Infrared spectra were obtained on a FT/IR-4100 spectrophotometer (ν_{max} in cm^{-1}). UV absorbance was recorded on a Genesys 10 scanning UV/visible spectrophotometer (λ_{max} in nm). HPLC data was obtained from Advion A-2030 Scientific Instruments LC system.

HPLC general procedure

Samples were analyzed using reverse phase liquid chromatography coupled with low resolution tandem mass spectrometry (LC-MS/MS). An Advion A-2030 Scientific Instruments LC system was used. Separation was achieved using a Phenomenex C^{18} reversed-phase column (5 μm, 150 x 4.6 mm). HPLC grade water was purchased from Macro Fine Chemicals. HPLC grade acetonitrile was purchased from VWR chemicals BDH. For analysis 20 µL of 1 mg/mL sample was injected. Two methods were used in this paper. Method A is a general method for all compounds. Method B is used for compounds **41** and **43**. Method A: Solvent flow of 1.000 ml/min while the initial eluent (70% water:30% acetonitrile with 0.1% formic acid) was held constant for 1 minute. From minutes 1 to 12, the mobile phase composition increased linearly to 1% water: 99% acetonitrile with 0.1% formic acid. From 12 to 14 minutes, the mobile phase composition was held constant at 1% water: 99% acetonitrile with 0.1% formic acid. Finally, from 14 to 16 minutes, the column was re-equilibrated with 70% water: 30% acetonitrile with 0.1% formic acid. Method B: Solvent flow of 1.000 ml/min while the initial eluent (95% water: 5% acetonitrile with 0.1% formic acid) were held constant for 1 minute. From 1 to 12 minutes, the mobile phase composition increased linearly to 35% water: 65% acetonitrile with 0.1% formic acid. From 12 to 14 minutes, the mobile phase composition was held constant at 1% water: 99% acetonitrile with 0.1% formic acid. From 14 to 16 min, the column was re-equilibrated with 95% water: 5% acetonitrile with 0.1% formic acid. HPLC data acquisition and analysis were performed using Advion LC Data Express software. All compounds showed >95% purity.

Synthetic procedures:

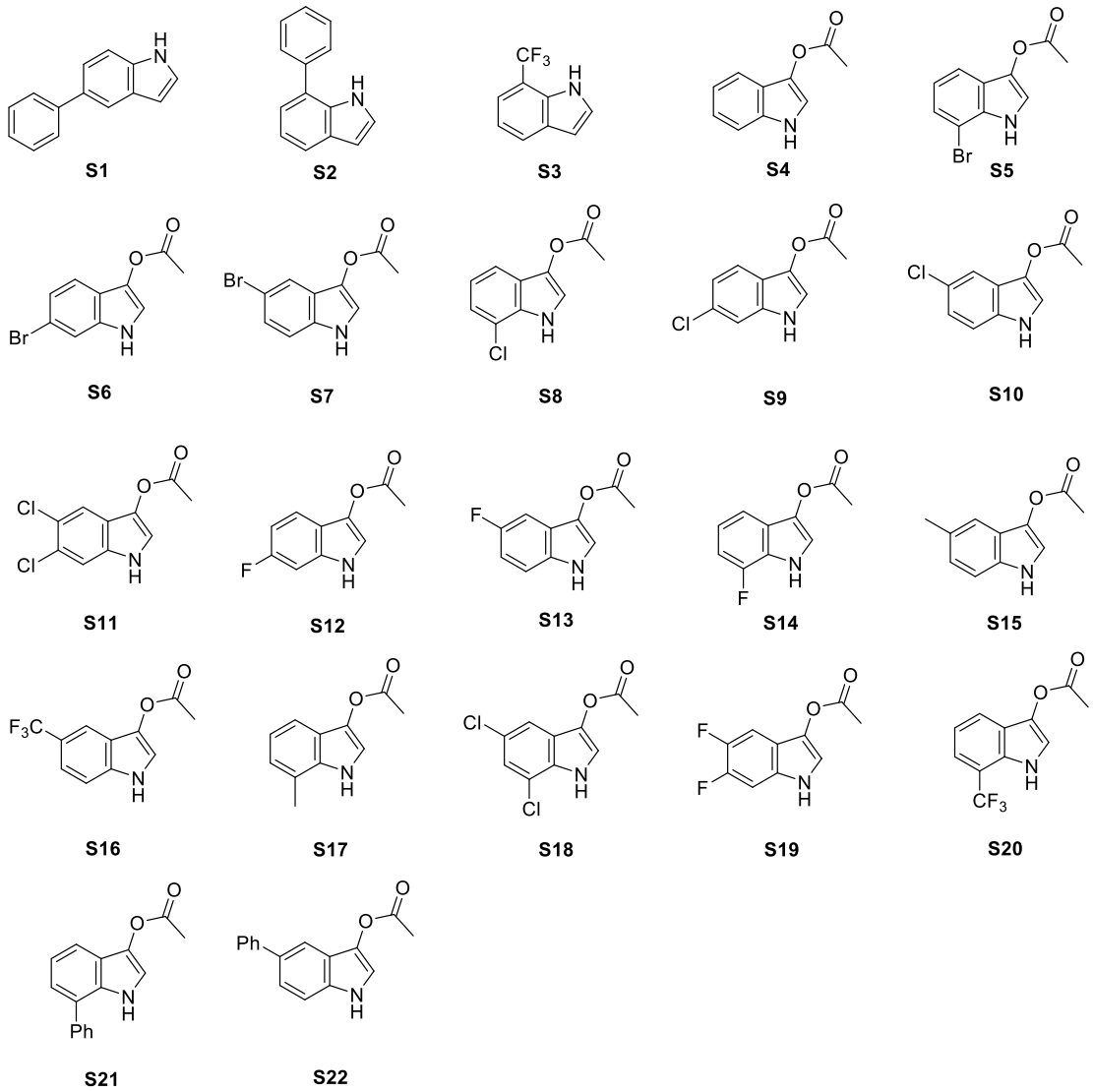

Figure S2. Structures of intermediates.

General method for synthesis of phenylindole: The phenylindole product was made following a previously reported synthetic procedure.[1] To a stirred solution of corresponding indole (1.0 g, 1.0 eq, 5.1 mmol) in 10 mL toluene: ethanol: water (8:1:0.5) was added Pd $(PPh_3)_4$ (0.88g, 0.15 equiv, 0.77 mmol), and phenol boronic acids (1.1 g, 1.8 equiv, 9.2 mmol) were added, then K_2CO_3 (1.1 g, 2.0 equiv, 10 mmol) was added. The resulting reaction mixture was heated at 80 °C. After consumption of starting material (monitored by TLC), the reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (20 mL) and brine (20 mL). Then the organic layer was dried over Na_2SO_4. The residue was purified by silica gel column chromatograph, to obtain following compounds.

5-Phenyl-1*H*-indole (S1): The title compound was synthesized following the general procedure for synthesis of phenylindole to afford **S1** (Figure 2) as a white solid (780 mg, 79%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.18 (s, 1H), 7.87 (s, 1H), 7.66 (d, *J* = 7.1 Hz, 2H), 7.50 – 7.40 (m, 4H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.26 – 7.24 (m, 1H), 6.64 – 6.60 (m, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 142.53, 135.28, 133.43, 128.63, 128.37, 127.39, 126.30, 124.81, 121.93, 119.26, 111.20, 103.05. HRMS (ESI): calcd for ($C_{14}H_{12}N$) $[M+H]^+$: 194.0964, found: 194.0960; Characterization is consistent with literature.[1]

7-Phenyl-1*H*-indole (S2): The title compound was synthesized following the general procedure for synthesis of phenylindole to afford **S2** (Figure 2) as a white solid (850 mg, 86%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.42 (s, 1H), 7.72 – 7.59 (m, 3H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.25 – 7.18 (m, 3H), 6.64 (dd, *J* = 3.2, 2.1 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 139.26, 133.69, 129.14, 128.24, 128.22, 127.40, 125.59, 124.32, 121.88, 120.30, 120.03, 103.07. HRMS (ESI): calcd for ($C_{14}H_{12}N$) $[M+H]^+$: 194.0964, found: 194.0962; Characterization is consistent with literature.[2]

7-(Trifluoromethyl)-1*H*-indole (S3): To a stirred mixture of 7-trifluoromethyl-1H-indole-2,3-dione (1.00 g, 1.0 equiv, 4.65 mmol) in THF (20 mL) was added boron trifluoride etherate (1.98 g, 3 equiv, 13.9 mmol) followed by sodium borohydride (615 mg, 3.5 equiv, 16.3 mmol). The resulting mixture was stirred at 0 °C for 2 hrs, then water (5 mL) was added and the mixture was stirred at 0 °C for 10 min. The solution was acidified to pH = 1 with 2N HCl, warmed to r.t. and stirred at r.t. for 20 min prior to extraction with ethyl acetate. The extracts were dried over Na_2SO_4, concentrated in vacuo and the residue purified by chromatography over silica gel eluting with hexane to give 7-trifluoromethyl-1*H*-indole **S3** (Figure 2) as product. (580 mg, 67.4%) ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.55 (s, 1H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 2.8 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.65 (dd, *J* = 3.0, 2.2 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 131.28, 129.41, 125.33, 124.75, 123.72 (q, *J* = 271.4 Hz), 119.53 (q, *J* = 4.6 Hz), 119.18, 113.34 (q, *J* = 32.6 Hz), 103.11. HRMS (ESI): calcd for ($C_9H_7F_3N$) $[M+H]^+$: 186.0525, found: 186.0527; Characterization is consistent with literature.[3]

General Method for synthesis of acetoxyindole (S4-S20): The acetoxyindole products were prepared as previously outlined.[4]

Step 1: Into a round bottom flask, was placed a solution of 1*H*-indole (1.5 g or 1.0 g, 1 equiv) in MeOH/H_2O (150/30 mL). To this was added potassium iodide (1.1 equiv), followed by sodium hydroxide (1.1 equiv). To the above was added I_2 (1.1 equiv). The resulting solution was stirred for 3 hours at room temperature. The reaction mixture was then quenched by the addition of 5% sodium sulfite (100 mL). 100 mL ethyl acetate was added and the organic layer was washed with water (100mL) followed by brine (100 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and the solvent removed via rotary evaporation. The crude product was then dried under reduced pressure for 2 hrs.

Step 2: Into a round bottom flask was placed a solution of crude product from step 1 in acetic acid (150 mL). To the mixture was added CH_3COOAg (1.5 equiv). The resulting solution was stirred for 2.5 hrs at 90 °C. The resulting reaction mixture was filtered with filter paper on a Buchner funnel and the filtrate was concentrated by evaporation under vacuum using a rotary evaporator. The resulting solution was dissolved with 100 mL of ethyl acetate. The resulting mixture was washed 2 times with brine (100 mL). The residue was purified by chromatography over silica gel using hexanes and ethyl acetate (5:1) to give the corresponding acetoxyindole.

1*H*-Indol-3-yl acetate (S4): The title compound was synthesized following the general procedure to afford **S4** (Figure 2) as a white solid (7.0 g, 50%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.84 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 2.7 Hz, 1H), 7.36 – 7.30 (m, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.14 (t, *J* = 7.0 Hz, 1H), 2.37 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_9NNaO_2$) $[M+Na]^+$: 198.0525, found: 198.0525; Characterization was consistent with previously published literature.[4]

7-Bromo-1*H*-indol-3-yl acetate (S5): The title compound was synthesized following the general procedure to afford **S5** (Figure 2) as a light purple solid (400 mg, 31%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.05 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 2.6 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.02 (t, *J* = 7.8 Hz, 1H), 2.37 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8BrNNaO_2$)

$[M+Na]^+$: 275.9631, found: 275.9634; Characterization was consistent with previously published literature.[4,5]

6-Bromo-1*H*-indol-3-yl acetate (S6): The title compound was synthesized following the general procedure to afford **S6** (Figure 2) as a light purple solid (500 mg, 39%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.86 (s, 1H), 7.49 (s, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.7 Hz, 1H), 7.24 (dd, *J* = 8.5, 1.6 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8BrNNaO_2$) $[M+Na]^+$: 275.9631, found: 275.9637; Characterization was consistent previously published literature.[6]

5-Bromo-1*H*-indol-3-yl acetate (S7): The title compound was synthesized following the general procedure to afford **S7** (Figure 2) as a light purple solid (550 mg, 42%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.89 (s, 1H), 7.70 (s, 1H), 7.41 – 7.34 (m, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.21 (d, *J* = 8.6 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8BrNNaO_2$) $[M+Na]^+$: 275.9631, found: 275.9636; Characterization was consistent previously with published literature.[7]

7-Chloro-1*H*-indol-3-yl acetate (S8): The title compound was synthesized following the general procedure to afford **S8** (Figure 2) as a light purple solid (415mg, 20^{1}H NMR (400 MHz, chloroform-*d*) δ 8.08 (s, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 2.6 Hz, 1H), 7.23 (d, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 2.37 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8ClNNaO_2$) $[M+Na]^+$: 232.0136, found: 232.0140; Characterization was consistent with previously published literature.[5]

6-Chloro-1*H*-indol-3-yl acetate (S9): The title compound was synthesized following the general procedure to afford **S9** (Figure 2) as a light purple solid (810 mg, 39%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.87 (s, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.39 (d, *J* = 2.7 Hz, 1H), 7.24 (d, *J* = 8 Hz, 1H), 7.17 (dd, *J* = 8.7, 2.0 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8ClNNaO_2$) $[M+Na]^+$: 232.0136, found: 232.0138; Characterization was consistent with previously published literature.[8]

5-Chloro-1*H*-indol-3-yl acetate (S10): The title compound was synthesized following the general procedure to afford **S10** (Figure 2) as a light purple solid (798 mg, 38%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.85 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.7 Hz, 1H), 7.32 (d, *J* = 1.8 Hz, 1H), 7.11 (dd, *J* = 8.5, 1.8 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8ClNNaO_2$) $[M+Na]^+$: 232.0136, found: 232.0138; Characterization was consistent with previously published literature.[9]

5,6-Dichloro-1*H*-indol-3-yl acetate (S11): The title compound was synthesized following the general procedure to afford **S11** (Figure 2) as a light purple solid (655 mg, 50%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.89 (s, 1H), 7.64 (s, 1H), 7.44 (s, 1H), 7.38 (s, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_7Cl_2NNaO_2$) $[M+Na]^+$: 265.9746, found: 265.9749; Characterization was consistent previously published literature.[10]

6-Fluoro-1*H*-indol-3-yl acetate (S12): The title compound was synthesized following the general procedure to afford **S12** (Figure 2) as a light purple solid (446 mg, 31%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.84 (s, 1H), 7.47 (dd, *J* = 8.7, 5.3 Hz, 1H), 7.33 (d, *J* = 2.6 Hz, 1H), 7.01 (dd, *J* = 9.5, 2.2 Hz, 1H), 6.91 (ddd, *J* = 9.5, 8.8, 2.2 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8FNNaO_2$) $[M+Na]^+$: 216.0431, found: 216.0436; Characterization was consistent with previously published literature.[11]

5-Fluoro-1*H*-indol-3-yl acetate (S13): The title compound was synthesized following the general procedure to afford **S13** (Figure 2) as a light purple solid (340 mg, 24%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.85 (s, 1H), 7.40 (d, *J* = 2.8 Hz, 1H), 7.24 (d, *J* = 4.2 Hz, 1H), 7.19 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.96 (dt, *J* = 9.1, 2.5 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for

($C_{10}H_8FNNaO_2$) $[M+Na]^+$: 216.0431, found: 216.0437; Characterization was consistent with previously published literature.[12]

7-Fluoro-1H-indol-3-yl acetate (S14): The title compound was synthesized following the general procedure to afford **S14** (Figure 2) as a light purple solid (478 mg, 33%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.02 (s, 1H), 7.42 (d, *J* = 2.6 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.04 (dt, *J* = 7.9, 4.6 Hz, 1H), 6.93 (ddd, *J* = 11.2, 7.9, 0.9 Hz, 1H), 2.37 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_8FNNaO_2$) $[M+Na]^+$: 216.0431, found: 216.0429; Characterization was consistent with previously published literature.[13]

5-Methyl-1*H*-indol-3-yl acetate (S15): The title compound was synthesized following the general procedure to afford **S15** (Figure 2) as a light purple solid (450 mg, 21%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.77 (s, 1H), 7.35 (s, 1H), 7.32 (d, *J* = 2.2 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.07 (dd, *J* = 8.4, 1.7 Hz, 1H), 2.47 (s, 3H), 2.39 (s, 3H). HRMS (ESI): calcd for ($C_{11}H_{12}NO_2$) $[M+H]^+$: 190.0863, found: 190.0866; Characterization was consistent previously with published literature.[7]

5-(Trifluoromethyl)-1*H*-indol-3-yl acetate (S16): The title compound was synthesized following the general procedure to afford **S16** (Figure 2) as a light purple solid (847 mg, 43%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.15 (s, 1H), 7.90 (s, 1H), 7.50 – 7.43 (m, 2H), 7.43 – 7.37 (m, 1H), 2.42 (s, 3H). HRMS (ESI): calcd for ($C_{11}H_8F_3NNaO_2$) $[M+Na]^+$: 266.0399, found: 266.0396; Characterization was consistent with previously published literature.[4]

7-methyl-1*H*-indol-3-yl acetate (S17): The title compound was synthesized following the general procedure to afford **S17** (Figure 2) as a light purple solid (228 mg, 16%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.78 (s, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 2.7 Hz, 1H), 7.10 – 6.92 (m, 2H), 2.44 (s, 3H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{11}H_{12}NO_2$) $[M+H]^+$: 190.0863, found: 190.0867; Characterization was consistent with previously published literature.[14]

5,7-Dichloro-1*H*-indol-3-yl acetate (S18): The title compound was synthesized following the general procedure to afford **S18** (Figure 2) as a light purple solid (766 mg, 58%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.10 (s, 1H), 7.47 (s, 1H), 7.46 (s, 1H), 7.23 (d, *J* = 1.8 Hz, 1H), 2.36 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_7Cl_2NNaO_2$) $[M+Na]^+$: 265.9746, found: 265.9746; Characterization was consistent with previously published literature.[15]

5,6-Difluoro-1*H*-indol-3-yl acetate (S19): The title compound was synthesized following the general procedure to afford **S19** (Figure 2) as a light purple solid (556 mg, 40%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.88 (s, 1H), 7.39 (d, *J* = 2.7 Hz, 1H), 7.34 – 7.29 (m, 1H), 7.13 (dd, *J* = 10.3, 6.4 Hz, 1H), 2.38 (s, 3H). HRMS (ESI): calcd for ($C_{10}H_7F_2NNaO_2$) $[M+Na]^+$: 234.0337, found: 234.0333; Characterization was consistent with previously published literature.[16]

7-(Trifluoromethyl)-1H-indol-3-yl acetate (S20): The title compound was synthesized following the general procedure to afford **S20** (Figure 2) as a light purple solid (359 mg, 46.8%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.24 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.21 (t, *J* = 7.8 Hz, 1H), 2.38 (s, 3H). HRMS (ESI): calcd for ($C_{11}H_8F_3NNaO_2$) $[M+Na]^+$: 266.0399, found: 266.0395; Characterization was consistent with previously published literature.[17]

7-Phenyl-1H-indol-3-yl acetate (S21): The title compound was synthesized following the general procedure to afford **S21** (Figure 2) as a light purple solid (700 mg, 53.8%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.09 (s, 1H), 7.64 – 7.60 (m, 2H), 7.56 – 7.49 (m, 3H), 7.45 – 7.38 (m, 1H), 7.37 (d, *J* = 2.7 Hz, 1H), 7.25 – 7.20 (m, 2H), 2.38 (s, 3H). HRMS (ESI): calcd for ($C_{16}H_{14}NO_2$) $[M+H]^+$: 252.1019, found: 252.1022; Characterization was consistent with previously published literature.[4]

5-Phenyl-1H-indol-3-yl acetate (S22): The title compound was synthesized following the general procedure to afford **S22** (Figure 2) as a light purple solid (800 mg, 41.0%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.88 (s, 1H), 7.75 (s, 1H), 7.65 (d, *J* = 7.4 Hz, 2H), 7.48 – 7.41 (m, 3H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 2.38 (s, 3H). HRMS (ESI): calcd for ($C_{16}H_{14}NO_2$) $[M+H]^+$: 252.1019, found: 252.1022; Characterization was consistent with previously published literature.[4]

General method for synthesis of indirubin-3'-oxime derivatives:
Step1**:** Methanol (10 mL) was vigorously stirred under argon for 20 mins, then the corresponding isatin (200 mg or 100 mg, 1 equiv) and 3-acetoxyindole (1.0 equiv) were added, and stirring was continued for 5 min. Anhydrous Na_2CO_3 (2.0 equiv) was added, and the stirring was continued for 3 h. The resulting dark precipitate was filtered and washed with aqueous methanol (1:1, 10 mL) to give corresponding indirubin product.

Step2: The appropriate indirubin derivative was dissolved in pyridine (10 mL). While stirring, hydroxylamine hydrochloride (10 equiv) was added and the mixture was heated under reflux (120 °C) for 3 hrs. The solvent was then removed by rotary evaporation under reduced pressure, then, the mixture was dissolved in ethyl acetate (35 mL) and washed with 1N HCl (35 mL) and brine (20 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and then concentrated under vacuum. The residue was purified by chromatography on a silica gel column using hexanes and ethyl acetate (4:1) to give the corresponding oxime product.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (5): The title compound was synthesized following the general procedure to afford **5** as a red solid (180 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.63 (s, 1H), 11.76 (s, 1H), 10.87 (s, 1H), 8.56 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 7.47 – 7.36 (m, 2H), 7.10 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.08 – 7.00 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.14, 151.82, 146.44, 145.24, 140.12, 132.60, 128.39, 124.71, 123.15, 122.47, 122.29, 118.30, 116.89, 112.28, 111.95, 98.18. UV(λ_{max}): 290 nm; IR (cm^{-1}): 3160, 1650, 1606, 1560; HRMS (ESI): calcd for ($C_{16}H_{11}BrN_3O_2$) $[M+H]^+$: 356.0029, found: 356.0033; HPLC trace: 97.0%. Characterization was consistent with previously published literature.[18]

(2Z,3E)-3-(Hydroxyimino)-[2,3'-biindolinylidene]-2'-one (12): The title compound was synthesized following the general procedure to afford **12** as a red solid (84 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.49 (s, 1H), 11.74 (s, 1H), 10.74 (s, 1H), 8.65 (dd, *J* = 7.9, 1.1 Hz, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 3.7 Hz, 1H), 7.13 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.07 – 7.00 (m, 1H), 6.95 (dt, *J* = 7.7, 1.2 Hz, 1H), 6.90 (dd, *J* = 7.7, 1.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.41, 151.80, 145.73, 145.34, 138.79, 132.52, 128.44, 126.40, 123.46, 123.14, 121.94, 120.85, 116.97, 112.02, 109.31, 99.36. UV(λ_{max}): 500 nm; IR (cm^{-1}): 3466, 3402, 3180, 1668, 1607, 1562; HRMS (ESI): calcd for ($C_{16}H_{12}N_3O_2$) $[M+H]^+$: 278.0924, found: 278.0930; HPLC trace: 98.9%. Characterization was consistent previously with published literature.[19]

(2Z,3E)-6'-Fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (13): The title compound was synthesized following the general procedure to afford **13** as a red solid (140 mg, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.52 (s, 1H), 11.64 (s, 1H), 10.88 (s, 1H), 8.71 – 8.54 (m, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 7.46 – 7.35 (m, 2H), 7.03 (ddd, *J* = 8.2, 5.3, 3.1 Hz, 1H), 6.76 (ddd, *J* = 9.7, 8.7, 2.6 Hz, 1H), 6.71 (dd, *J* = 9.2, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.71, 162.49, 160.09, 151.76, 145.40 (d, *J* = 3.3 Hz), 140.22 (d, *J* = 11.7 Hz), 132.56, 128.42, 124.60 (d, *J* = 8.6 Hz), 121.99, 119.62 (d, *J* = 2.3 Hz), 116.94, 112.11, 106.86 (d, *J* = 21.4 Hz), 98.50, 97.19 (d, *J* = 26.7 Hz). UV(λ_{max}): 290 nm; IR (cm^{-1}): 3533, 3397, 3184, 1667, 1606, 1563; HRMS (ESI): calcd for ($C_{16}H_{11}FN_3O_2$) $[M+H]^+$: 296.0833, found: 296.0830; HPLC trace: 95.3%. Characterization was consistent with previously published literature.[18]

(2Z,3E)-3-(Hydroxyimino)-6'-methoxy-[2,3'-biindolinylidene]-2'-one (14): The title compound was synthesized following the general procedure to afford **14** as a red solid (80 mg,

46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 1H), 11.47 (s, 1H), 10.70 (s, 1H), 8.57 (d, *J* = 8.5 Hz, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.42 – 7.34 (m, 2H), 6.99 (ddd, *J* = 8.1, 5.7, 2.7 Hz, 1H), 6.54 – 6.46 (m, 2H), 2.50 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 171.96, 158.92, 151.77, 145.53, 143.51, 140.56, 132.40, 128.45, 124.78, 121.46, 117.00, 116.22, 111.79, 106.47, 99.75, 95.76, 55.63. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3272, 1612, 1564; HRMS (ESI): calcd for ($C_{17}H_{14}ClN_3O_3$) $[M+H]^+$: 308.1030, found: 308.1035; HPLC trace: 96.9%. Characterization was consistent with previously published literature.[20]

(2Z,3E)-3-(Hydroxyimino)-6'-methyl-[2,3'-biindolinylidene]-2'-one (15): The title compound was synthesized following the general procedure to afford **15** as a red solid (85 mg, 26%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.40 (s, 1H), 11.62 (s, 1H), 10.67 (s, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.49 – 7.25 (m, 2H), 7.01 (ddd, *J* = 8.2, 5.4, 3.1 Hz, 1H), 6.77 (d, *J* = 7.7 Hz, 1H), 6.72 (d, *J* = 1.6 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 171.68, 151.80, 145.43, 144.75, 139.16, 136.09, 132.46, 128.44, 123.46, 121.70, 121.55, 120.59, 116.98, 111.89, 110.05, 99.67, 21.91. UV(λ_{max}): 502 nm; IR (cm^{-1}): 3160, 1649, 1612, 1553; HRMS (ESI): calcd for ($C_{15}H_8ClF_6N2O$) $[M+H]^+$: 292.1081, found: 292.1079; HPLC trace: 95.8%. Characterization was consistent with previously published literature.[19]

(2Z,3E)-6'-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (16): The title compound was synthesized following the general procedure to afford **16** as a red solid (133 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.62 (s, 1H), 11.74 (s, 1H), 10.88 (s, 1H), 8.62 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 7.47 – 7.34 (m, 2H), 7.12 – 7.01 (m, 1H), 6.97 (d, *J* = 8.3 Hz, 1H), 6.90 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.30, 151.78, 146.29, 145.25, 139.94, 132.59, 130.06, 128.39, 124.37, 122.26, 122.13, 120.32, 116.90, 112.26, 109.20, 98.18. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3404, 1668, 1608, 1567; HRMS (ESI): calcd for ($C_{16}H_{11}ClN_3O_2$) $[M+H]^+$: 312.0534, found: 312.0533; HPLC trace: 98.2%. Characterization was consistent with previously published literature.[18]

(2Z,3E)-3-(Hydroxyimino)-6'-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (17): The title compound was synthesized following the general procedure to afford **17** as a red solid (190 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.80 (s, 1H), 11.96 (s, 1H), 11.01 (s, 1H), 8.77 (d, *J* = 8.2 Hz, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.72 – 7.37 (m, 2H), 7.26 (d, *J* = 8.2 Hz, 1H), 7.12 (s, 1H), 7.09 (dt, *J* = 7.4, 1.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.18, 151.78, 148.08, 145.00, 138.53, 132.64, 128.32, 126.98, 125.19 (q, *J* = 271.3 Hz), 125.56 (q, *J* = 31.7 Hz), 122.94, 122.76, 117.38, 116.90, 112.56, 105.30, 97.46. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3578, 1658, 1614, 1565; HRMS (ESI): calcd for ($C_{17}H_{11}F_3N_3O_2$) $[M+H]^+$: 346.0798, found: 346.0805; HPLC trace: 98.2%. Characterization was consistent with previously published literature.[21]

(2Z,3E)-5'-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (18): The title compound was synthesized following the general procedure to afford **18** as a red solid (147 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.69 (s, 1H), 11.84 (s, 1H), 10.86 (s, 1H), 8.66 (d, *J* = 2.2 Hz, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.49 – 7.32 (m, 2H), 7.15 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.06 (ddd, *J* = 8.1, 6.5, 2.0 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.18, 151.92, 146.83, 145.17, 137.30, 132.64, 128.46, 125.58, 125.15, 124.75, 122.59, 122.39, 116.91, 112.32, 110.36, 98.24. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3245, 1655, 1609, 1562; HRMS (ESI): calcd for ($C_{16}H_{11}ClN_3O_2$) $[M+H]^+$: 312.0534, found: 312.0534; HPLC trace: 96.5%. Characterization was consistent with previously published literature.[22]

(2Z,3E)-5'-Bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (19): The title compound was synthesized following the general procedure to afford **19** as a red solid (81 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.64 (s, 1H), 11.77 (s, 1H), 10.80 (s, 1H), 8.70 (d, *J* = 2.1 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 7.42 – 7.30 (m, 2H), 7.21 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.00 (ddd, *J* = 8.1, 6.4, 2.1 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.04, 151.93, 146.84, 145.15, 137.62, 132.64, 128.48, 128.42, 125.24, 122.40, 116.92, 113.11, 112.33, 110.93, 98.09. UV(λ_{max}): 290 nm; IR (cm^{-1}): 3240, 1655, 1608, 1561; HRMS

(ESI): calcd for ($C_{16}H_{11}BrN_3O_2$) $[M+H]^+$: 356.0029, found: 356.0030; HPLC trace: 96.4%. Characterization was consistent with previously published literature.[20]

(2Z,3E)-7'-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (20): The title compound was synthesized following the general procedure to afford **20** as a red solid (138 mg, 39%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.67 (s, 1H), 11.86 (s, 1H), 11.10 (s, 1H), 8.63 (d, *J* = 7.8 Hz, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.54 – 7.25 (m, 2H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.06 (dt, *J* = 7.3, 1.5 Hz, 1H), 6.97 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.19, 151.76, 147.06, 145.12, 135.76, 132.61, 128.39, 125.66, 125.12, 122.44, 121.84, 121.68, 116.90, 113.72, 112.37, 98.64. UV(λ_{max}): 496 nm; IR (cm^{-1}): 3243, 1657, 1609, 1563; HRMS (ESI): calcd for ($C_{16}H_{11}ClN_3O_2$) $[M+H]^+$: 312.0534, found: 312.0539; HPLC trace: 96.4%. Characterization was consistent with previously published literature.[23]

(2Z,3E)-7'-Bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (21): The title compound was synthesized following the general procedure to afford **21** as a red solid (89 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.65 (s, 1H), 11.86 (s, 1H), 10.94 (s, 1H), 8.66 (d, *J* = 7.8 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 7.61 – 7.36 (m, 2H), 7.29 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.05 (dt, *J* = 7.3, 1.5 Hz, 1H), 6.90 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.09, 151.76, 147.10, 145.11, 137.34, 132.62, 128.60, 128.39, 125.10, 122.45, 122.27, 122.09, 116.90, 112.37, 101.99, 98.80. UV(λ_{max}): 290 nm; IR (cm^{-1}): 3157, 1672, 1610, 1569; HRMS (ESI): calcd for ($C_{16}H_{11}BrN_3O_2$) $[M+H]^+$: 356.0029, found: 356.0036; HPLC trace: 95.2%. Characterization was consistent with previously published literature.[20]

(2Z,3E)-5,6'-Dibromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (22): The title compound was synthesized following the general procedure to afford **22** as a red solid (183 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.88 (s, 1H), 11.78 (s, 1H), 10.89 (s, 1H), 8.53 (d, *J* = 8.4 Hz, 1H), 8.32 (d, *J* = 2.0 Hz, 1H), 7.59 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.10 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 170.99, 150.82, 145.59, 144.46, 140.37, 134.91, 130.28, 124.84, 123.24, 122.27, 118.72, 118.60, 114.35, 113.37, 112.05, 98.98. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3153, 1697, 1662, 1604, 1570; HRMS (ESI): calcd for ($C_{16}H_{10}Br_2N_3O_2$) $[M+H]^+$: 433.9135, found: 433.9134; HPLC trace: 95.0%. Characterization was consistent with previously published literature.[20]

(2Z,3E)-6,6'-Dibromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (23): The title compound was synthesized following the general procedure to afford **23** as a red solid (139 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.75 (s, 1H), 11.77 (s, 1H), 10.91 (s, 1H), 8.55 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.71 (d, *J* = 1.8 Hz, 1H), 7.21 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.10 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 170.97, 151.01, 146.62, 145.83, 140.44, 129.66, 125.53, 124.95, 124.81, 123.26, 122.26, 118.81, 116.08, 115.24, 112.05, 99.24. UV(λ_{max}): 294 nm; IR (cm^{-1}): 3136, 1666, 1604, 1566; HRMS (ESI): calcd for ($C_{16}H_{10}Br_2N_3O_2$) $[M+H]^+$: 433.9134, found: 433.9130; HPLC trace: 99.4%. Characterization was consistent with previously published literature.[20]

(2Z,3E)-6',7-Dibromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (24): The title compound was synthesized following the general procedure to afford **24** as a red solid (68 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.92 (s, 1H), 11.83 (s, 1H), 11.05 (s, 1H), 8.48 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 7.5 Hz, 1H), 7.66 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.13 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.08 – 6.97 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.65, 151.22, 145.43, 143.15, 140.35, 134.66, 127.78, 124.89, 123.99, 123.58, 121.85, 119.19, 118.48, 112.41, 103.41, 99.68. UV(λ_{max}): 294 nm; IR (cm^{-1}): 3154, 1666, 1609, 1563; HRMS (ESI): calcd for ($C_{16}H_{10}Br_2N_3O_2$) $[M+H]^+$: 433.9134, found: 433.9128; HPLC trace: 96.7%.

(2Z,3E)-6'-Bromo-5-chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (25): The title compound was synthesized following the general procedure to afford **25** as a red solid (94 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.88 (s, 1H), 11.78 (s, 1H), 10.89 (s, 1H), 8.53 (d, *J*

= 8.5 Hz, 1H), 8.18 (d, *J* = 1.4 Hz, 1H), 7.47 (d, *J* = 1.4 Hz, 2H), 7.10 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.02 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.98, 150.94, 145.77, 144.12, 140.35, 132.16, 127.50, 125.68, 124.81, 123.23, 122.28, 118.69, 118.08, 113.84, 112.03, 98.97. UV(λ_{max}): 292 nm; IR (cm^{-1}): 3286, 1725, 1654, 1605, 1570; HRMS (ESI): calcd for ($C_{16}H_{10}BrClN_3O_2$) $[M+H]^+$: 389.9639, found: 389.9645; HPLC trace: 97.6%.

(2Z,3E)-6'-Bromo-6-chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (26): The title compound was synthesized following the general procedure to afford **26** as a red solid (96 mg, 52%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.73 (s, 1H), 11.78 (s, 1H), 10.91 (s, 1H), 8.55 (d, *J* = 8.4 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 1.9 Hz, 1H), 7.09 (ddd, *J* = 13.0, 8.3, 2.0 Hz, 2H), 7.03 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.98, 150.91, 146.57, 145.97, 140.45, 136.71, 129.47, 124.96, 123.27, 122.26, 121.93, 118.81, 115.79, 112.38, 112.06, 99.24. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3147, 1677, 1612, 1582; HRMS (ESI): calcd for ($C_{16}H_{10}BrClN_3O_2$) $[M+H]^+$: 389.9639, found: 389.9638; HPLC trace: 95.4%.

(2Z,3E)-6'-Bromo-7-chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (27): The title compound was synthesized following the general procedure to afford **27** as a red solid (78 mg, 21%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.93 (s, 1H), 11.85 (s, 1H), 11.06 (s, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 7.55 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.23 – 6.97 (m, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 171.68, 151.08, 145.56, 141.52, 140.34, 131.85, 127.31, 124.89, 123.69, 123.59, 121.86, 119.20, 118.68, 114.94, 112.42, 99.71. UV(λ_{max}): 290nm; IR (cm^{-1}): 3230, 1657, 1607, 1560; HRMS (ESI): calcd for ($C_{16}H_{10}BrClN_3O_2$) $[M+H]^+$: 389.9639, found: 389.9635; HPLC trace: 95.2%.

(2Z,3E)-6'-Bromo-5-fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (28): The title compound was synthesized following the general procedure to afford **28** as a red solid (166 mg, 43%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.85 (s, 1H), 11.74 (s, 1H), 10.88 (s, 1H), 8.52 (d, *J* = 8.4 Hz, 1H), 7.95 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.46 (dd, *J* = 8.7, 4.5 Hz, 1H), 7.31 (dt, *J* = 9.0, 2.8 Hz, 1H), 7.10 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.97, 158.97, 156.62, 151.43, 146.45, 141.81, 140.18, 123.52 (d, *J* = 228.6 Hz), 123.17, 119.36 (d, *J* = 23.8 Hz), 118.43, 117.25 (d, *J* = 10.0 Hz), 114.66 (d, *J* = 25.8 Hz), 113.31 (d, *J* = 8.0 Hz), 111.98, 98.52. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3291, 1649, 1607, 1572; HRMS (ESI): calcd for ($C_{16}H_{10}BrFN_3O_2$) $[M+H]^+$: 373.9935, found: 373.9928; HPLC trace: 93.9%.

(2Z,3E)-6'-Bromo-7-fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (29): The title compound was synthesized following the general procedure to afford **29** as a red solid (127 mg, 33%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.92 (s, 1H), 11.79 (s, 1H), 11.03 (s, 1H), 8.50 (d, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.41 (dd, *J* = 10.6, 8.3 Hz, 1H), 7.14 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.12 – 7.04 (m, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 171.89, 151.20 (d, *J* = 2.7 Hz), 147.64 (d, *J* = 243.4 Hz), 146.22, 140.43, 132.04 (d, *J* = 13.1 Hz), 125.08, 124.91 (d, *J* = 3.2 Hz), 123.79, 123.56 (d, *J* = 5.4 Hz), 122.14, 120.25 (d, *J* = 3.6 Hz), 119.28, 119.15, 112.61, 99.74. UV(λ_{max}): 284 nm; IR (cm^{-1}): 3146, 1667, 1619, 1567; HRMS (ESI): calcd for ($C_{16}H_{10}BrFN_3O_2$) $[M+H]^+$: 373.9935, found: 373.9928; HPLC trace: 95.3%.

(2Z,3E)-6'-Bromo-6-fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (30): The title compound was synthesized following the general procedure to afford **30** as a red solid (155 mg, 40%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.61 (s, 1H), 11.80 (s, 1H), 10.91 (s, 1H), 8.57 (d, *J* = 8.4 Hz, 1H), 8.23 (dd, *J* = 8.5, 5.8 Hz, 1H), 7.31 (dd, *J* = 9.8, 2.4 Hz, 1H), 7.11 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.03 (d, *J* = 1.9 Hz, 1H), 6.84 (ddd, *J* = 9.6, 8.5, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 171.01, 164.76 (d, *J* = 246.8 Hz), 150.75, 147.31 (d, *J* = 13.7 Hz), 146.41, 140.43, 130.15 (d, *J* = 10.8 Hz), 125.00, 123.26, 122.23, 118.77, 113.72, 112.05, 108.87 (d, *J* = 23.3 Hz), 100.01 (d, *J* = 28.0 Hz), 99.10. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3662, 1665, 1599, 1571; HRMS (ESI): calcd for ($C_{16}H_{10}BrFN_3O_2$) $[M+H]^+$: 373.9935, found: 373.9961; HPLC trace: 96.3%.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-5-phenyl-[2,3'-biindolinylidene]-2'-one (31): The title compound was synthesized following the general procedure to afford **31** as a red solid (188 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.76 (s, 1H), 11.83 (s, 1H), 10.90 (s, 1H), 8.58 (d, J = 8.5 Hz, 1H), 8.49 (d, J = 1.9 Hz, 1H), 7.73 (dd, J = 8.3, 1.9 Hz, 1H), 7.63 (d, J = 7.3 Hz, 3H), 7.54 (d, J = 8.3 Hz, 1H), 7.47 (t, J = 7.7 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 7.11 (dd, J = 8.4, 2.0 Hz, 1H), 7.04 (d, J = 1.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.10, 151.71, 146.45, 144.75, 140.49, 140.21, 134.54, 131.27, 129.48, 127.49, 126.86, 126.46, 124.80, 123.19, 122.44, 118.42, 117.67, 112.77, 111.99, 98.54. UV(λ_{max}): 288nm; IR (cm^{-1}): 3662, 1656, 1613, 1579; HRMS (ESI): calcd for ($C_{22}H_{15}BrN_3O_2$) $[M+H]^+$: 432.0342, found: 432.0345; HPLC trace: 100.0%.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-7-phenyl-[2,3'-biindolinylidene]-2'-one (32): The title compound was synthesized following the general procedure to afford **32** as a red solid (178 mg, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.79 (s, 1H), 12.11 (s, 1H), 10.86 (s, 1H), 8.50 (d, J = 8.4 Hz, 1H), 8.26 (d, J = 7.6 Hz, 1H), 7.67 – 7.55 (m, 4H), 7.52 – 7.43 (m, 2H), 7.18 (t, J = 7.7 Hz, 1H), 7.11 (dd, J = 8.4, 2.0 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.53, 151.48, 146.64, 141.67, 140.02, 136.98, 132.42, 129.93, 128.57, 128.13, 127.95, 124.63, 124.32, 123.42, 123.21, 122.18, 118.56, 117.62, 112.28, 98.38. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3666, 1711, 1661, 1606, 1566; HRMS (ESI): calcd for ($C_{17}H_{15}BrF_4$ N_3O) $[M+H]^+$: 432.0329, found: 432.0327; HPLC trace: 99.6%.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-7-methyl-[2,3'-biindolinylidene]-2'-one (33): The title compound was synthesized following the general procedure to afford **33** as a red solid (120 mg, 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.70 (s, 1H), 11.77 (s, 1H), 11.00 (s, 1H), 8.52 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.31 (d, J = 7.6 Hz, 1H), 7.13 (dd, J = 8.4, 2.0 Hz, 1H), 7.07 (d, J = 1.9 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.70, 151.84, 146.81, 143.23, 139.92, 133.58, 126.25, 124.59, 123.36, 122.60, 122.21, 119.99, 118.41, 116.57, 112.20, 98.19, 15.91. UV(λ_{max}): 292 nm; IR (cm^{-1}): 3149, 1661, 1606, 1573; HRMS (ESI): calcd for ($C_{17}H_{13}BrN_3O_2$) $[M+H]^+$: 370.0186, found: 370.0185; HPLC trace: 96.1%.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-5-methyl-[2,3'-biindolinylidene]-2'-one (34): The title compound was synthesized following the general procedure to afford **34** as a red solid (165 mg, 47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (s, 1H), 11.70 (s, 1H), 10.84 (s, 1H), 8.55 (d, J = 8.4 Hz, 1H), 8.07 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 7.7 Hz, 0H), 7.09 (dd, J = 8.4, 2.0 Hz, 1H), 7.03 (d, J = 1.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.14, 151.87, 146.77, 143.17, 139.96, 133.15, 131.28, 128.67, 124.59, 123.09, 122.52, 118.07, 117.02, 112.00, 111.90, 97.71, 21.15. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3150, 1650, 1613, 1567; HRMS (ESI): calcd for ($C_{17}H_{13}BrN_3O_2$) $[M+H]^+$: 370.0186, found: 370.0191; HPLC trace: 97.4%.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-5-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (35): The title compound was synthesized following the general procedure to afford **35** as a red solid (191 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.95 (s, 1H), 11.91 (s, 1H), 10.93 (s, 1H), 8.55 (d, J = 8.4 Hz, 1H), 8.45 (s, 1H), 7.77 (d, J = 9.6 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.11 (dd, J = 8.4, 2.0 Hz, 1H), 7.03 (d, J = 1.9 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.14, 150.93, 148.26, 145.61, 140.89, 129.92, 125.33, 125.24 (q, J = 271.2 Hz), 125.05 (q, J = 4.2 Hz), 123.56, 122.33, 122.32 (q, J = 31.9 Hz), 119.40, 117.13, 113.01, 112.36, 100.34. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3662, 3164, 1656, 1617, 1568; HRMS (ESI): calcd for ($C_{17}H_{13}BrN_3O_2$) $[M+H]^+$: 370.0186, found: 370.0186; HPLC trace: 99.1%.

(2Z,3E)-6'-Bromo-3-(hydroxyimino)-7-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (36): The title compound was synthesized following the general procedure to afford **36** as a red solid (140 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.44 (s, 1H), 12.60 (s, 1H), 11.48 (s, 1H), 8.90 (dd, J = 8.1, 4.1 Hz, 2H), 8.14 (d, J = 7.9 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 7.55 (dd, J = 8.4, 2.0 Hz, 1H), 7.47 (d, J = 1.9 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.90, 150.11,

145.65, 141.20 (d, *J* = 2.1 Hz), 140.72, 132.70, 128.93 (d, *J* = 4.4 Hz), 125.25, 124.68 (q, *J* = 271.8 Hz), 123.87, 122.65, 121.95, 119.67, 119.03, 112.71, 111.23 (q, *J* = 32.9 Hz), 100.34. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3155, 1708, 1667, 1603, 1567; HRMS (ESI): calcd for ($C_{17}H_{10}BrF_3N_3O_2$) $[M+H]^+$: 423.9903, found: 423.9900; HPLC trace: 96.0%.

(2Z,3E)-6'-Bromo-5,6-dichloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (37): The title compound was synthesized following the general procedure to afford **37** as a red solid (168 mg, 48%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.96 (s, 1H), 11.80 (s, 1H), 10.92 (s, 1H), 8.51 (d, *J* = 8.4 Hz, 1H), 8.32 (s, 1H), 7.73 (s, 1H), 7.10 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.02 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.88, 150.18, 145.32, 144.94, 140.59, 134.44, 128.94, 125.02, 123.45, 123.33, 122.10, 119.10, 116.98, 114.01, 112.13, 99.79. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3503, 3427, 3156, 1671, 1615, 1566; HRMS (ESI): calcd for ($C_{16}H_9BrCl_2N_3O_2$) $[M+H]^+$: 423.9250, found: 423.9252; HPLC trace: 95.2%.

(2Z,3E)-6'-Bromo-5,7-dichloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (38): The title compound was synthesized following the general procedure to afford **38** as a red solid (189 mg, 54%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 14.05 (s, 1H), 11.69 (s, 1H), 10.95 (s, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 8.02 (d, *J* = 1.9 Hz, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.01 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.91 (d, *J* = 1.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 171.53, 150.12, 144.89, 140.50, 140.44, 130.95, 126.60, 126.11, 124.87, 123.58, 121.58, 119.48, 119.11, 115.56, 112.40, 100.30. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3238, 1655, 1614, 1561; HRMS (ESI): calcd for ($C_{16}H_9BrCl_2N_3O_2$) $[M+H]^+$: 423.9250, found: 423.9255; HPLC trace: 96.7%.

(2Z,3E)-6'-Bromo-5,6-difluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (39): The title compound was synthesized following the general procedure to afford **39** as a red solid (191 mg, 92%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.76 (s, 1H), 11.70 (s, 1H), 10.85 (s, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.09 (t, *J* = 9.2 Hz, 1H), 7.46 (dd, *J* = 11.1, 6.8 Hz, 1H), 7.13 – 6.91 (m, 2H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.88, 152.11 (dd, *J* = 249.2, 14.5 Hz), 150.59, 145.95, 145.16 (dd, *J* = 238.2, 13.8 Hz), 142.37 (d, *J* = 11.8 Hz), 140.42, 124.89, 123.24, 122.13, 118.82, 116.55 (d, *J* = 21.2 Hz), 112.61 – 112.21 (m), 112.03, 101.55 (d, *J* = 23.3 Hz), 99.38. UV(λ_{max}): 286 nm; IR (cm^{-1}): 3149, 1671, 1625, 1605; HRMS (ESI): calcd for ($C_{16}H_9BrF_2N_3O_2$) $[M+H]^+$: 391.9841, found: 391.9841; HPLC trace: 95.4%.

(2Z,3E)-7-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (40): The title compound was synthesized following the general procedure to afford **40** as a red solid (68 mg, 38%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.81 (s, 1H), 11.90 (s, 1H), 10.94 (s, 1H), 8.74 – 8.50 (m, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 7.55 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.19 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.08 (t, *J* = 7.9 Hz, 1H), 6.99 (dt, *J* = 7.7, 1.2 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 171.88, 151.08, 144.79, 141.72, 139.09, 131.80, 127.38, 127.24, 123.70, 123.36, 122.55, 121.24, 118.76, 114.78, 109.75, 100.93. UV(λ_{max}): 292 nm; IR (cm^{-1}): 3241, 1669, 1613, 1563; HRMS (ESI): calcd for ($C_{16}H_{11}ClN_3O_2$) $[M+H]^+$: 312.0532, found: 312.0534; HPLC trace: 95.2%.

(2Z,3E)-6'-Bromo-3-((3,4-dihydroxybutoxy)imino)-[2,3'-biindolinylidene]-2'-one (41):
Step1: Dissolve 6-Bromoindirubin-3'-oxime (65mg, 1.0 equiv, 0.18 mmol) in DMF (5 mL), followed by the addition of TEA (24 mg, 1.3 equiv, 0.24 mmol) and 4-(2-bromoethyl)-2,2-dimethyl-1,3-dioxolane (46 mg, 1.2 equiv, 0.24 mmol). The resulting mixture was stirred for 18 hrs (overnight) at room temperature. Ethyl acetate (20 mL) was added to the mixture and the mixture was washed by water (20 mL) and brine (20 ml). The organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The crude product was used in the second step without purification.

Step2: The product from step 1 was dissolved in methanol (5mL) and 1 N HCl (5 mL) and allowed to stir at room temperature overnight. Ethyl acetate (10 mL) was added to the mixture and the mixture was washed with water (10 mL) and brine (10 ml). The organic layer was dried

over anhydrous Na_2SO_4, filtered, and then concentrated under vacuum. The residue was purified by chromatography on a silica gel column with dichloromethane and methanol (100: 5) to give the title compound (25 mg, 49%). (30 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 10.91 (s, 1H), 8.56 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 7.7 Hz, 1H), 7.45 (d, J = 4.1 Hz, 2H), 7.17 (dd, J = 8.4, 2.0 Hz, 1H), 7.09 – 7.01 (m, 2H), 4.78 (d, J = 5.1 Hz, 1H), 4.71 (t, J = 6.2 Hz, 2H), 4.64 (t, J = 5.6 Hz, 1H), 3.77 – 3.61 (m, 1H), 3.41 (dt, J = 10.7, 5.3 Hz, 1H), 2.15 (ddt, J = 14.7, 7.6, 3.8 Hz, 1H), 1.82 (td, J = 8.6, 5.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.08, 151.53, 145.84, 145.15, 140.41, 133.32, 128.64, 125.10, 123.61, 122.27, 122.15, 118.69, 116.60, 112.49, 111.98, 99.27, 74.42, 68.52, 66.42, 33.57. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3190, 1650, 1613, 1561; HRMS (ESI): calcd for ($C_{20}H_{19}BrN_3O_4$) $[M+H]^+$: 444.0553, found: 444.0554; HPLC trace: 95.8%.

(2Z,3E)-6'-Bromo-3-((2-(4-methylpiperazin-1-yl)ethoxy)imino)-[2,3'-biindolinylidene]-2'-one (42):
Step1: To a solution of 6-Bromoindirubin-3'-oxime (100 mg, 1 equiv, 0.28 mmol) in DMF (5 ml) was added triethylamine (36 mg, 1.3 equiv, 0.36ml) and 1,2-dibromoethane (106 mg, 2 equiv, 0.56 mmol) under argon. The resulting mixture was stirred for 17 hrs at room temperature. Water (50 ml) was then added and the resulting precipitate collected by filtration with filter paper on a Buchner funnel and washed with water. The crude product was used in the second step without purification.

Step2: 50 mg of crude product from step1 was dissolved in 5 ml of anhydrous DMF. 1-methylpiperazine (13 mg, 1.2 equiv, 0.13 mmol) was added under stirring and the mixture was then heated at 50 °C for 18 hrs. The reaction mixture was poured into water (30 ml) and the precipitate was filtered and washed with water. The residue was purified by chromatography using hexanes and ethyl acetate (1:2) on a silica gel column to give the title compound (30 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 10.85 (s, 1H), 8.46 (d, J = 8.5 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 4.0 Hz, 2H), 7.08 (dd, J = 8.4, 2.0 Hz, 1H), 7.02 – 6.93 (m, 2H), 4.63 (t, J = 5.9 Hz, 2H), 2.81 (t, J = 5.9 Hz, 2H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.05, 151.81, 145.88, 145.02, 140.45, 133.40, 128.75, 125.04, 123.53, 122.25, 122.11, 118.74, 116.58, 112.50, 112.02, 99.41, 74.70, 56.68, 55.07, 53.13, 45.91. UV(λ_{max}): 288 nm; IR (cm^{-1}): 3659, 1662, 1607, 1556; HRMS (ESI): calcd for ($C_{23}H_{25}BrN_5O_2$) $[M+H]^+$: 482.1186, found: 482.1185; HPLC trace: 97.5%. Characterization was consistent with previously published literature.[24]

(2Z,3E)-6'-Bromo-3-((2-(piperazin-1-yl)ethoxy)imino)-[2,3'-biindolinylidene]-2'-one (43):
Step1: To a solution of 6-Bromoindirubin-3'-oxime (100 mg, 1 equiv, 0.28 mmol) in DMF (5 ml) was added triethylamine (36 mg, 1.3 equiv, 0.36ml) and 1,2-dibromoethane (106 mg, 2 equiv, 0.56 mmol) under argon, and the resulting mixture stirred for 17 hrs at room temperature. Water (50 ml) was then added and the precipitate formed was collected by filtration with filter paper on a Buchner funnel and washed with water (10 mL) and brine (10 mL) once. The crude product was used in the second step without purification.

Step2: 50 mg of crude product from step was dissolved in 5 ml of anhydrous DMF. Piperazine (11 mg, 1.2 equiv, 0.13 mmol) was added under stirring and the mixture was then heated at 50 °C for 18 hrs. The mixture was then poured into water (30 ml) and the precipitate was filtered with filter paper on a Buchner funnel and washed with water (10 mL) and brine (10 mL) once. The residue was purified by chromatography on a silica gel column with dichloromethane and methanol (100: 5) to give the title compound (25 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 10.89 (s, 1H), 8.50 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 7.41 (d, J = 4.2 Hz, 2H), 7.11 (dd, J = 8.5, 2.0 Hz, 1H), 7.05 – 6.98 (m, 2H), 4.67 (t, J = 5.9 Hz, 2H), 2.81 (t, J = 5.9 Hz, 2H), 2.68 (t, J = 4.9 Hz, 4H), 2.48 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.06, 151.75, 145.85, 145.04, 140.44, 133.38, 128.73, 125.03, 123.51, 122.25, 122.11, 118.73,

116.59, 112.49, 112.02, 99.38, 74.64, 57.53, 54.67, 45.98. UV(λ_{max}): 292 nm; IR (cm^{-1}): 3190, 1662, 1606, 1557; HRMS (ESI): calcd for ($C_{22}H_{23}BrN_5O_2$) $[M+H]^+$: 468.1030, found: 468.1026; HPLC trace: 95.3%. Characterization was consistent with previously published literature.[24]

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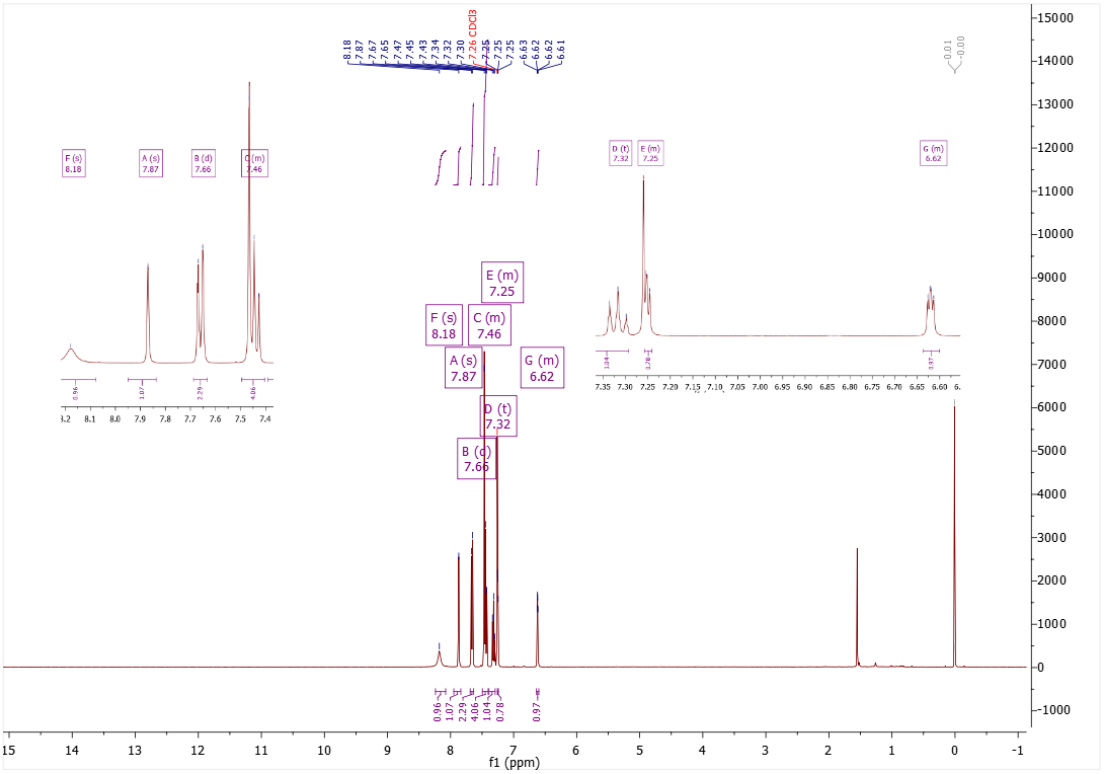
NMR spectra

S1 5-Phenyl-1H-indole



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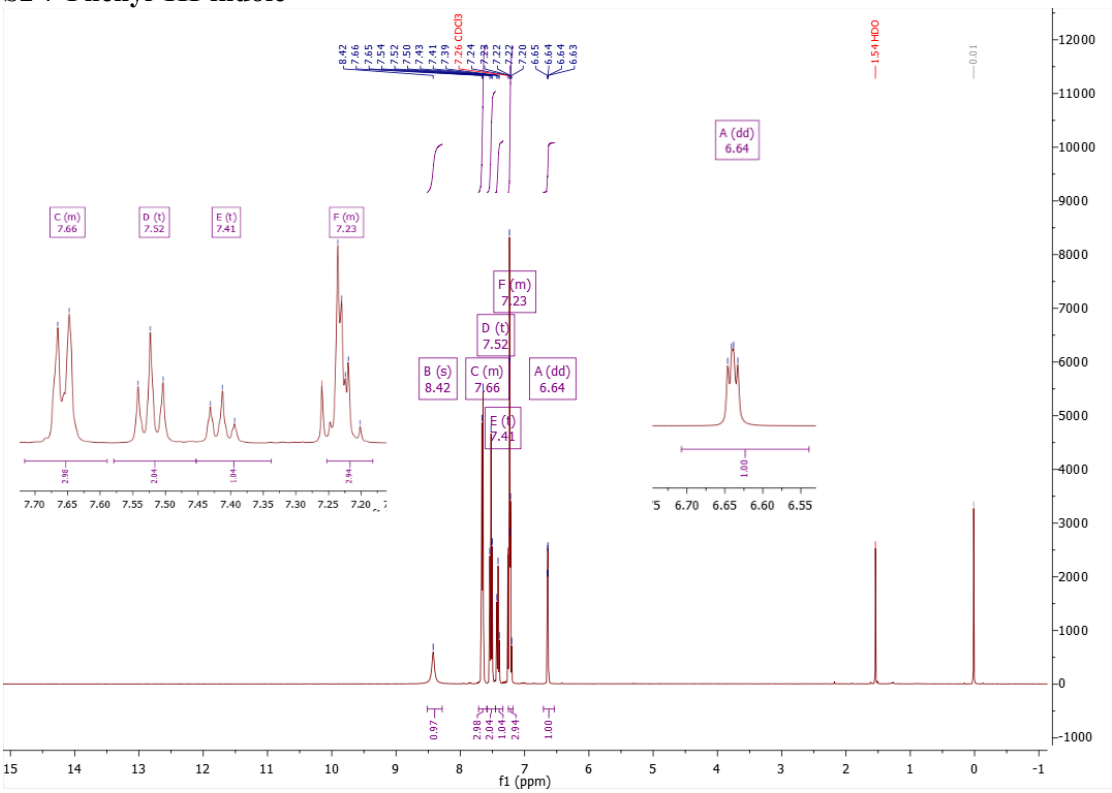
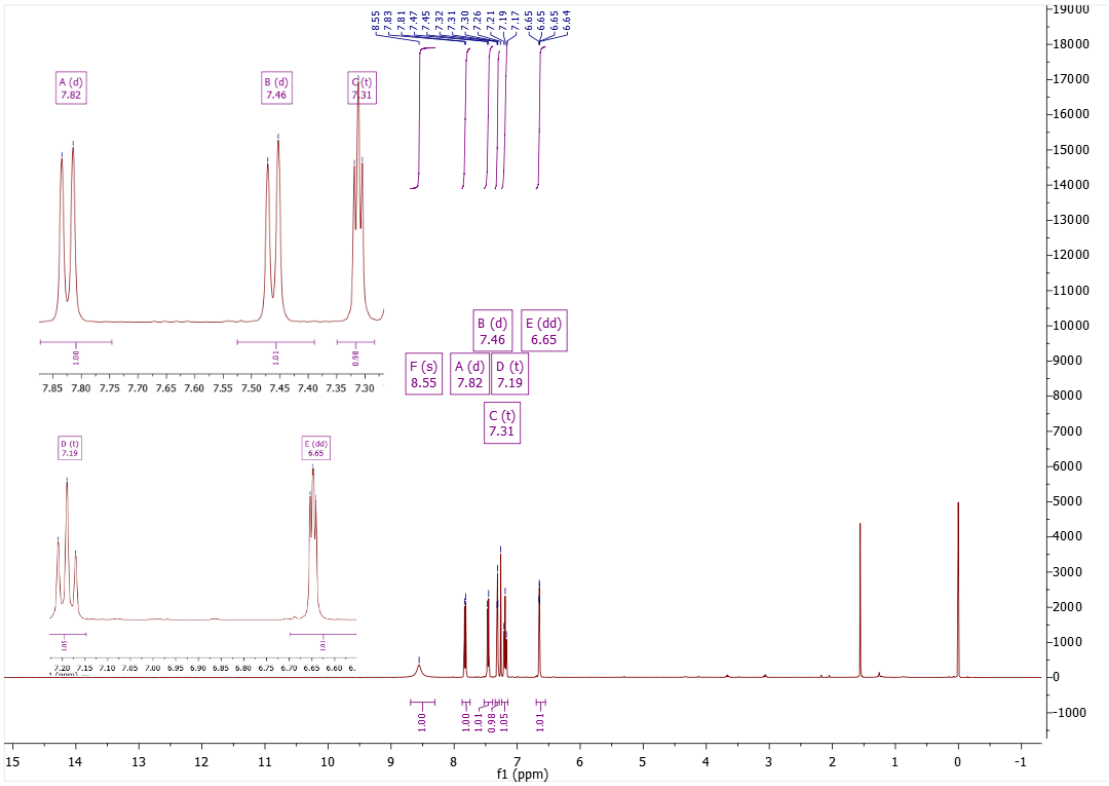
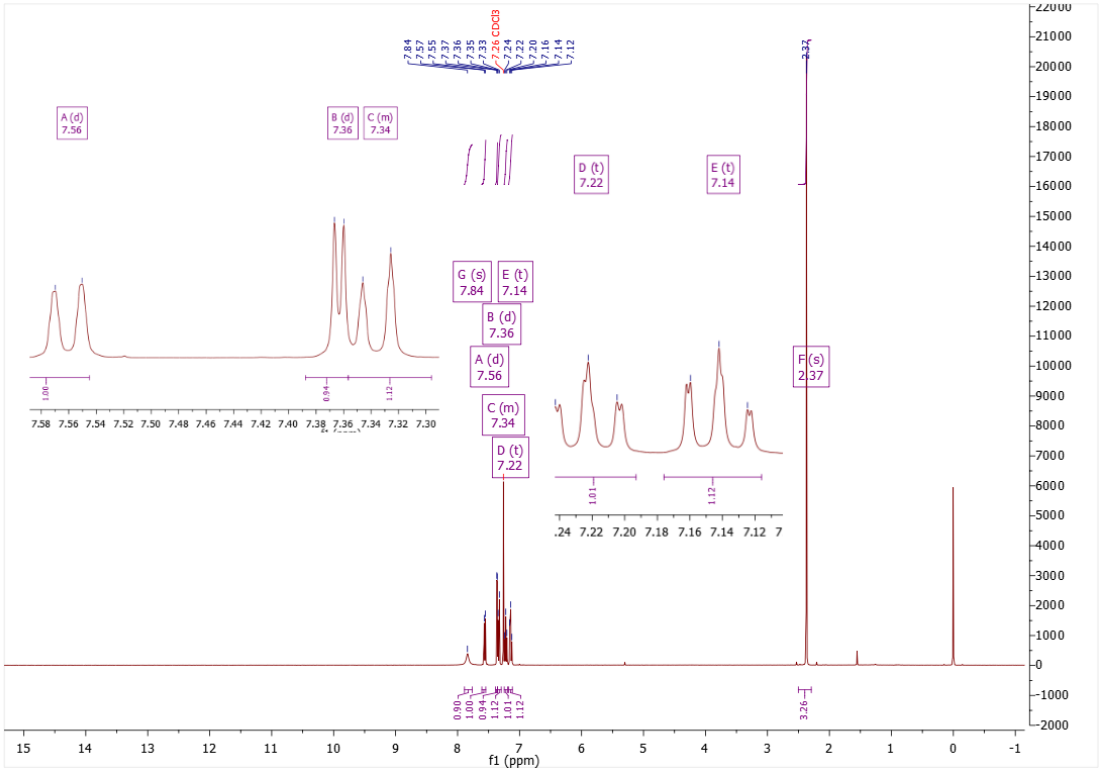


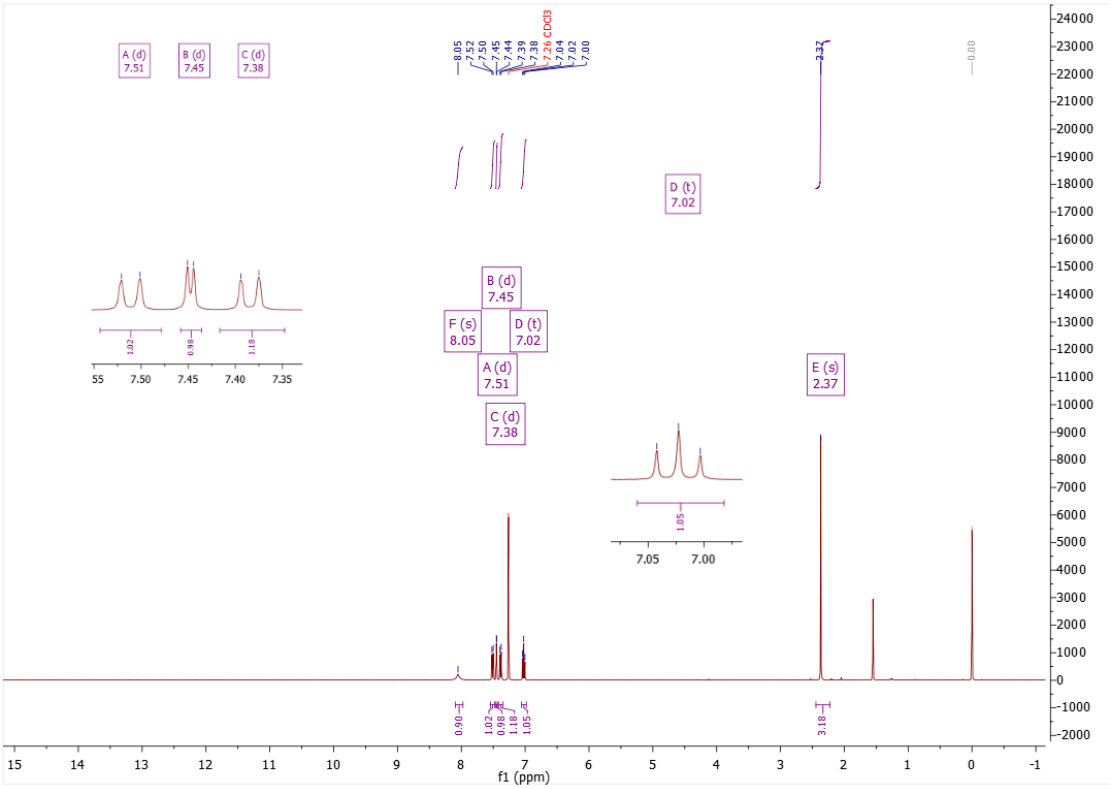
S3 7-(Trifluoromethyl)-1H-indole



S4 1*H*-Indol-3-yl acetate



S5 7-Bromo-1*H*-indol-3-yl acetate



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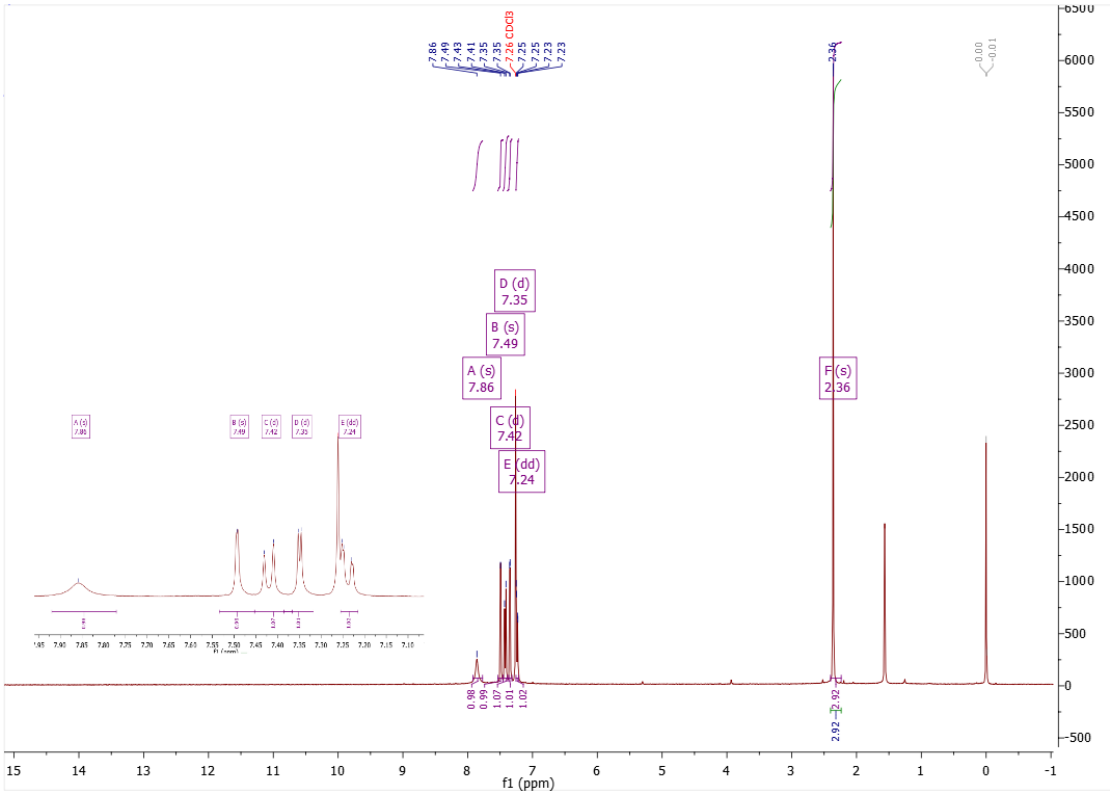

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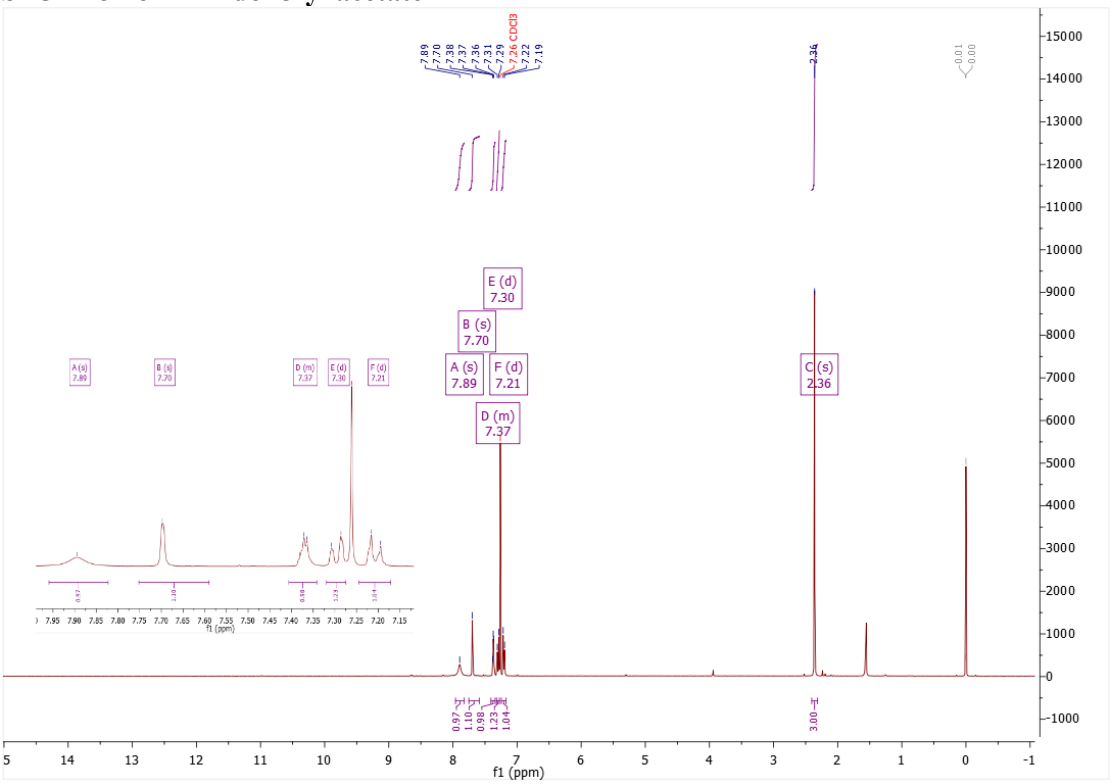

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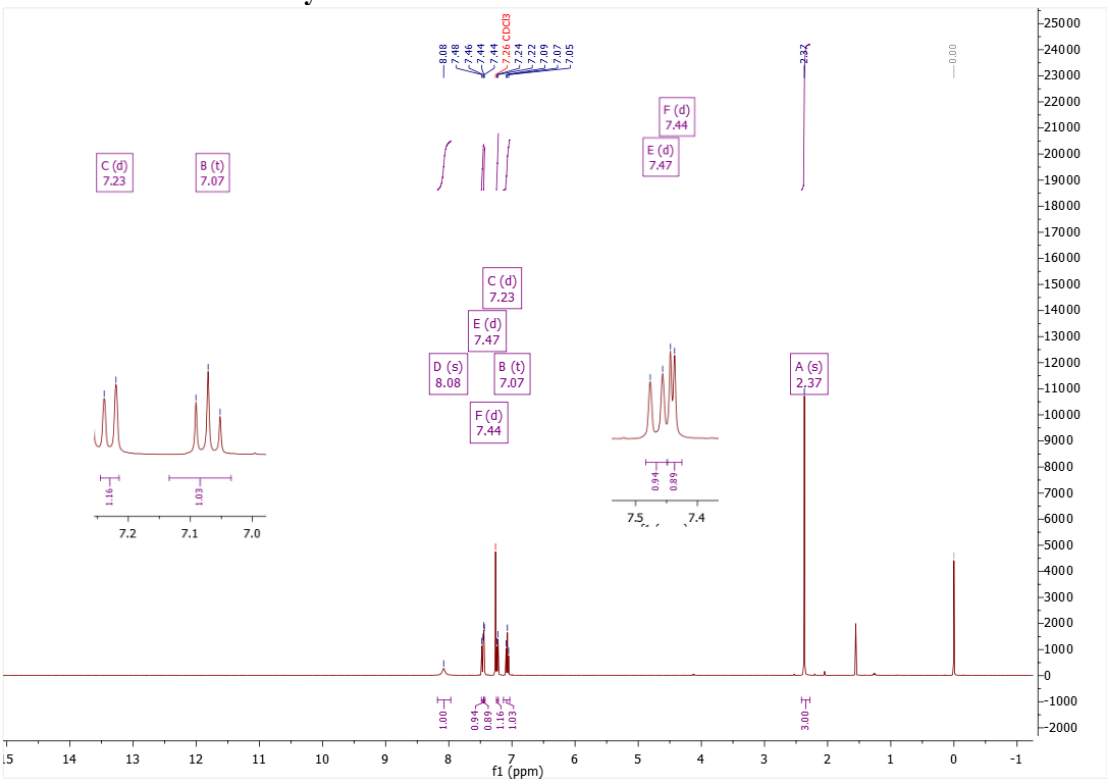

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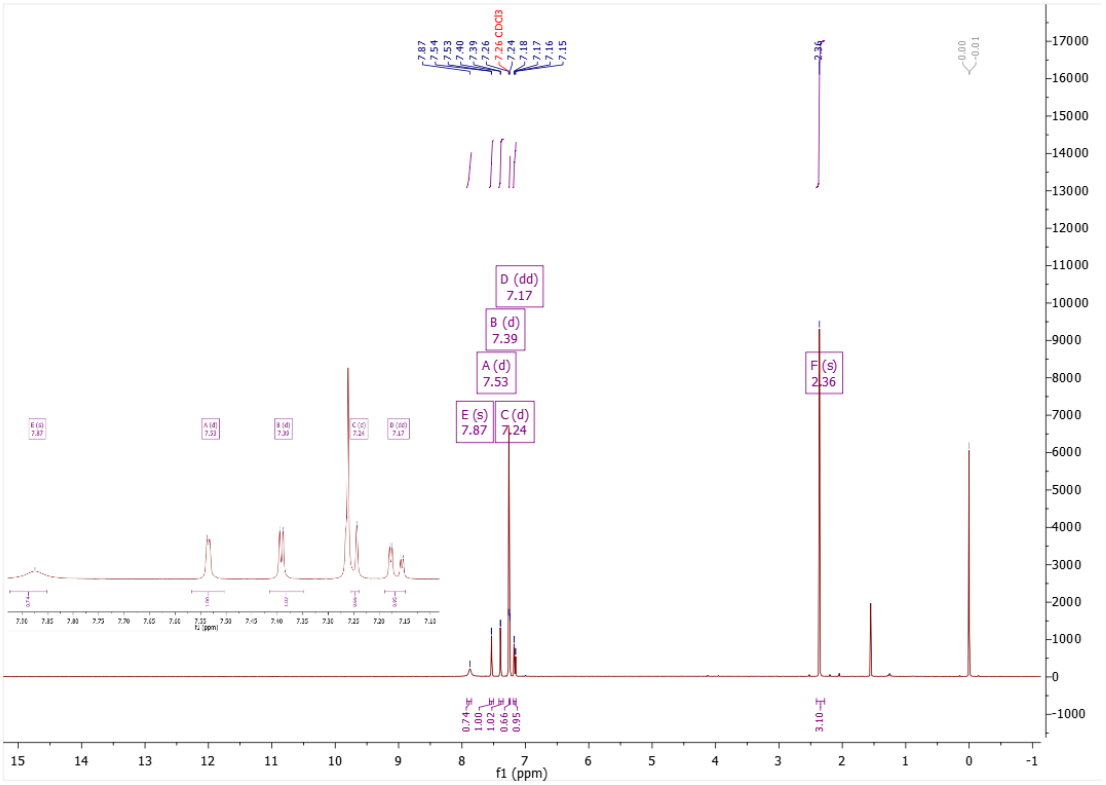

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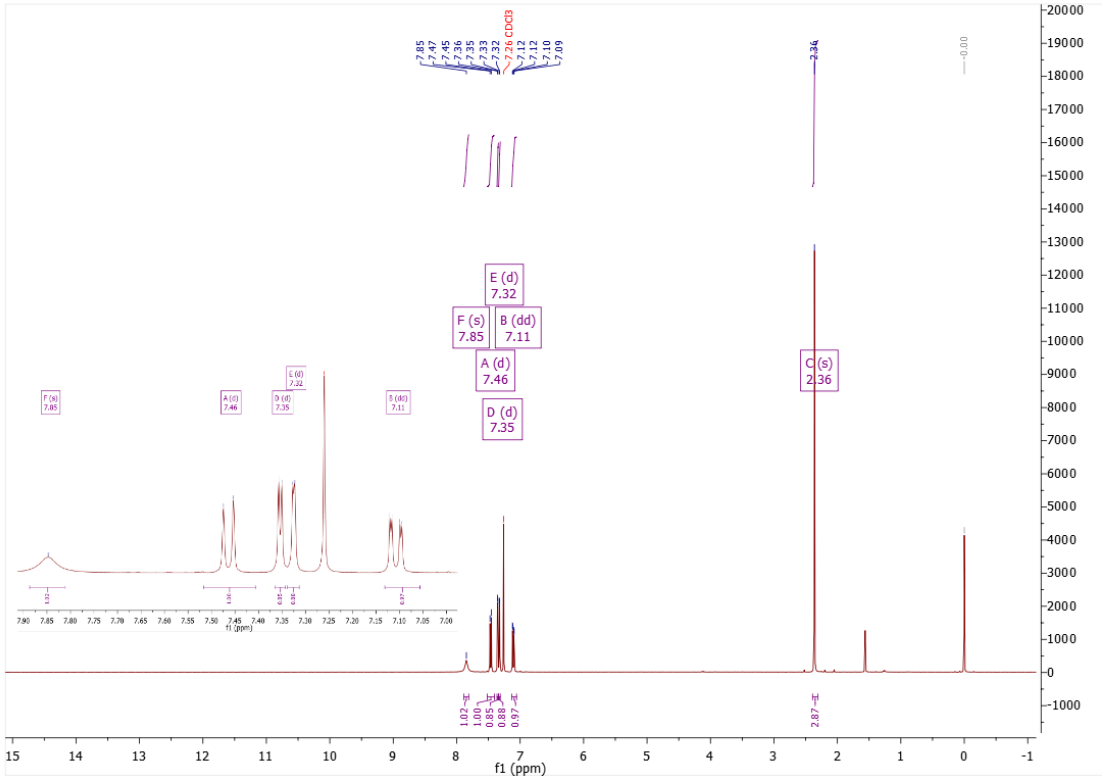

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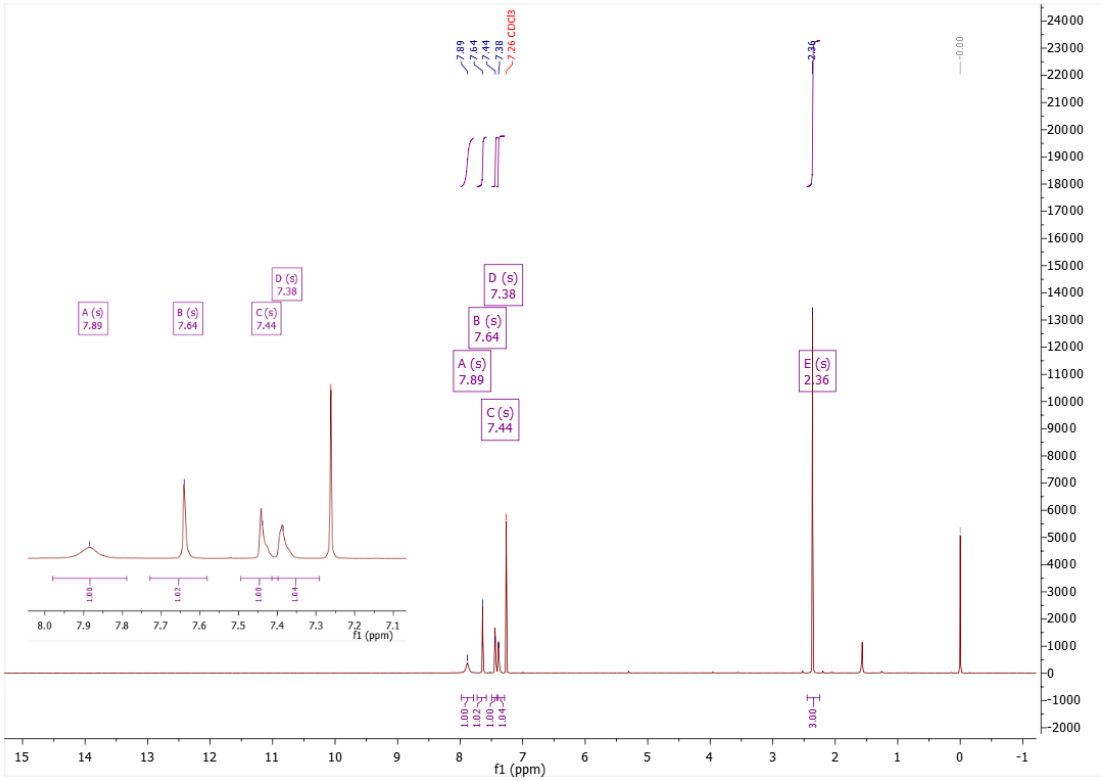

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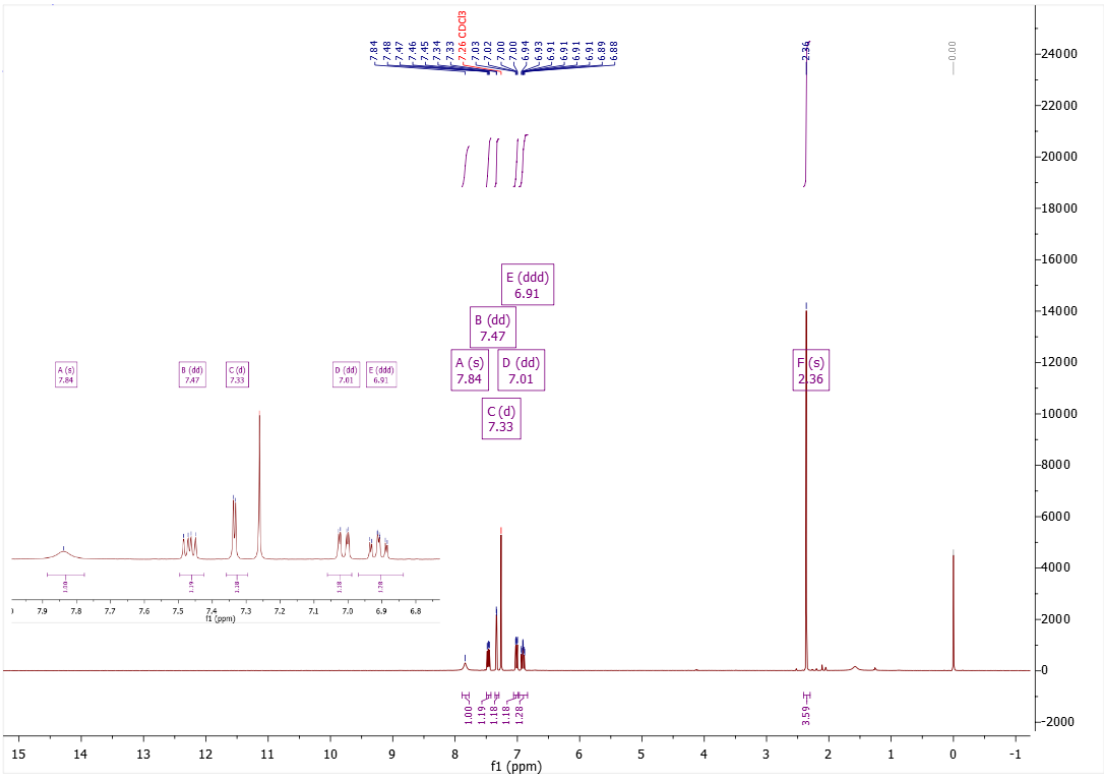

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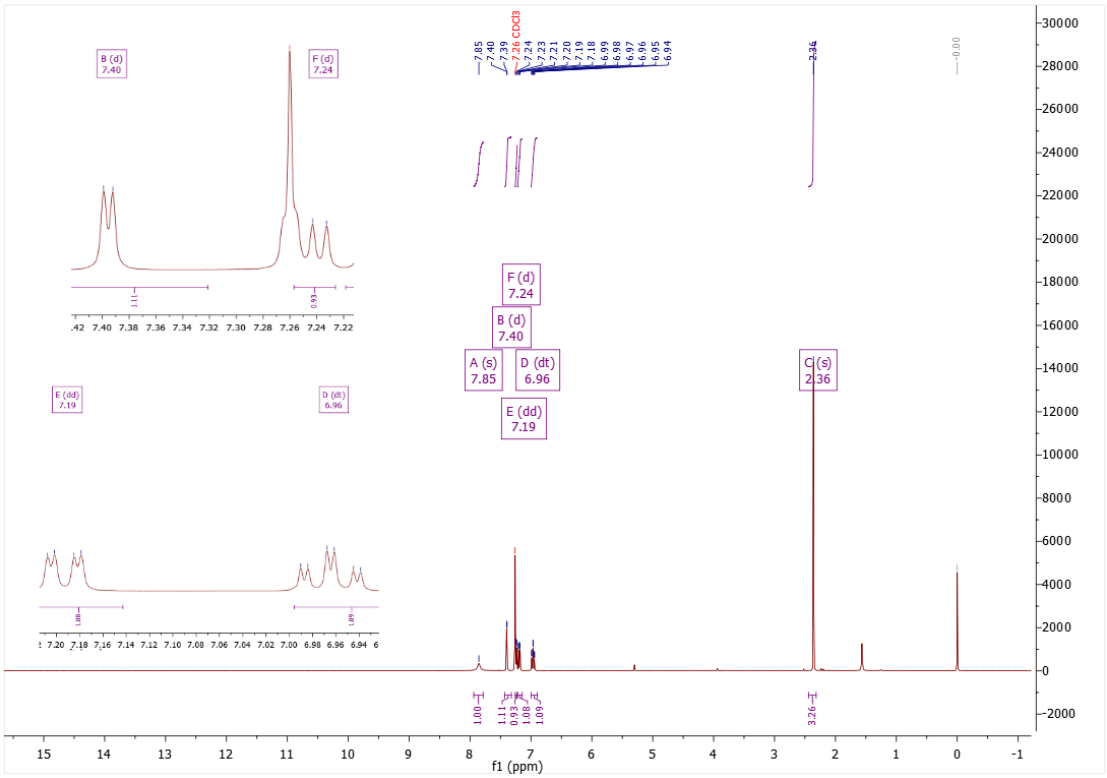

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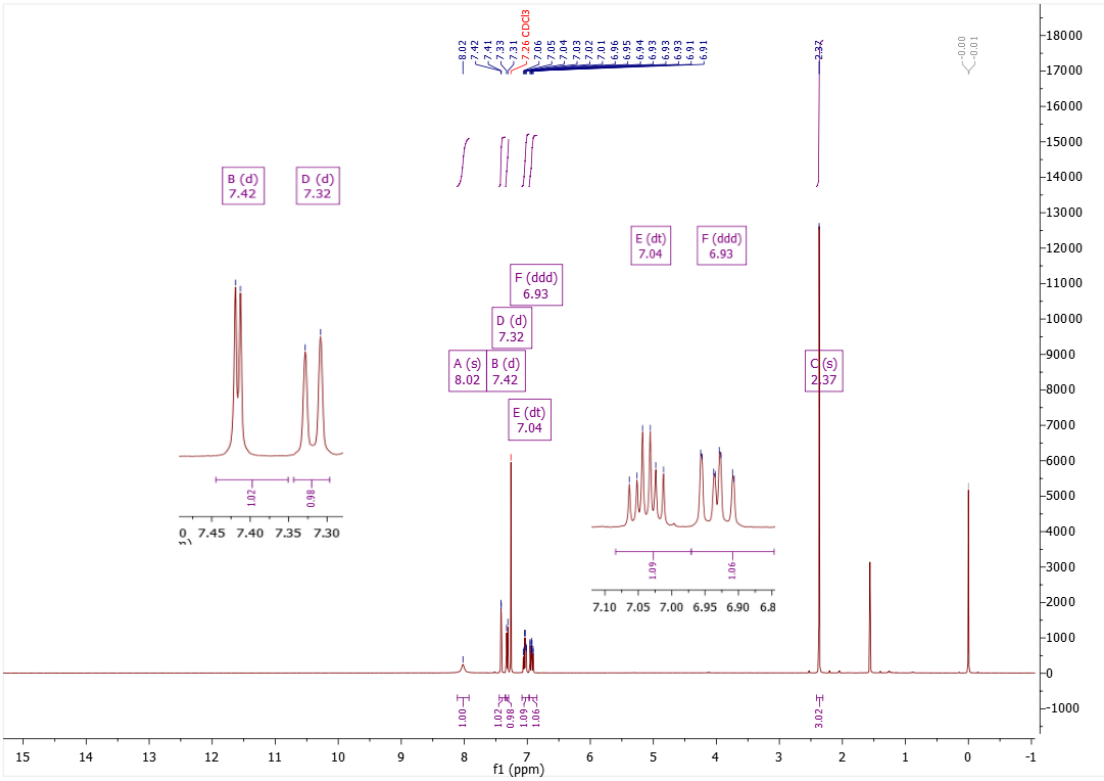

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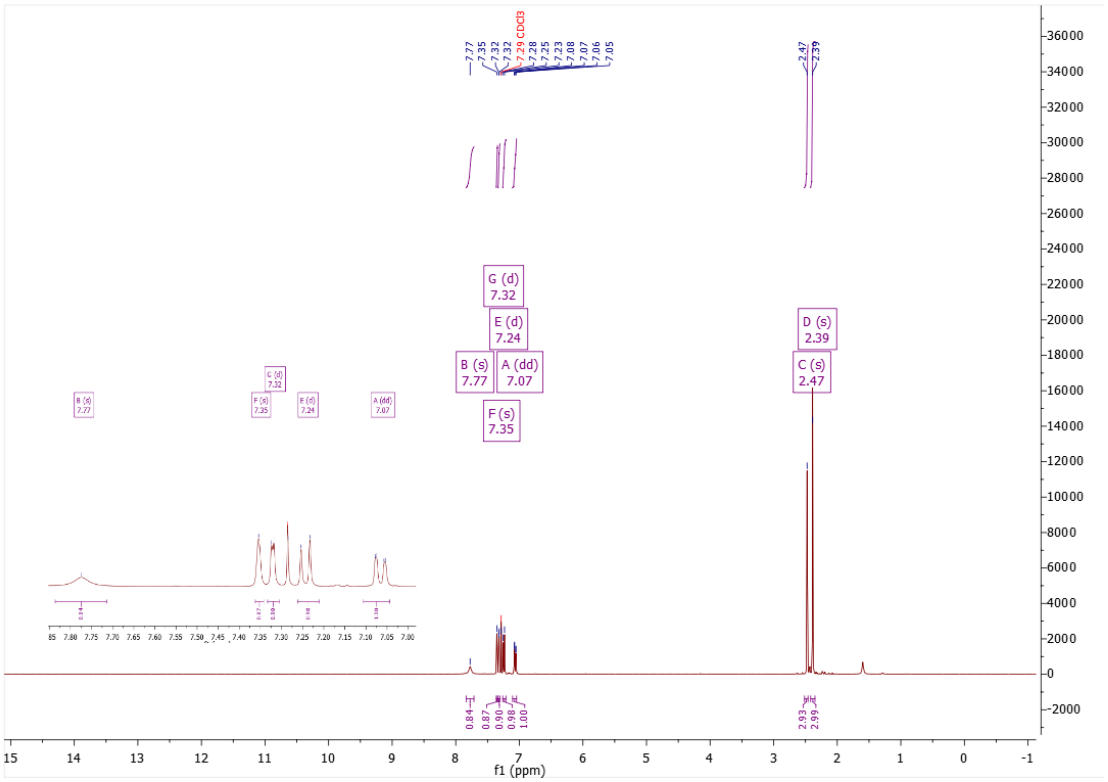

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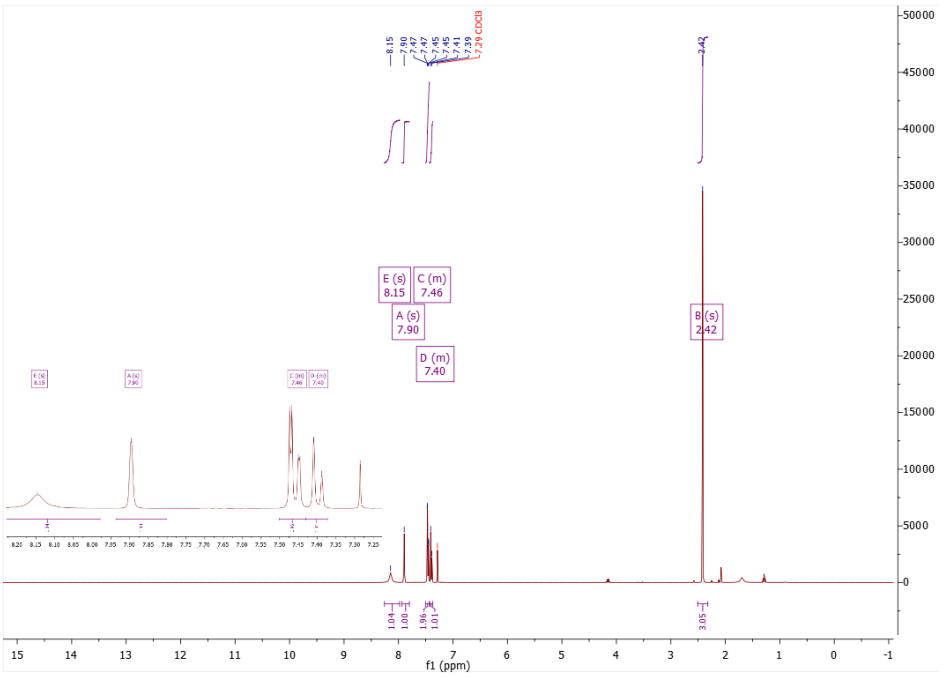

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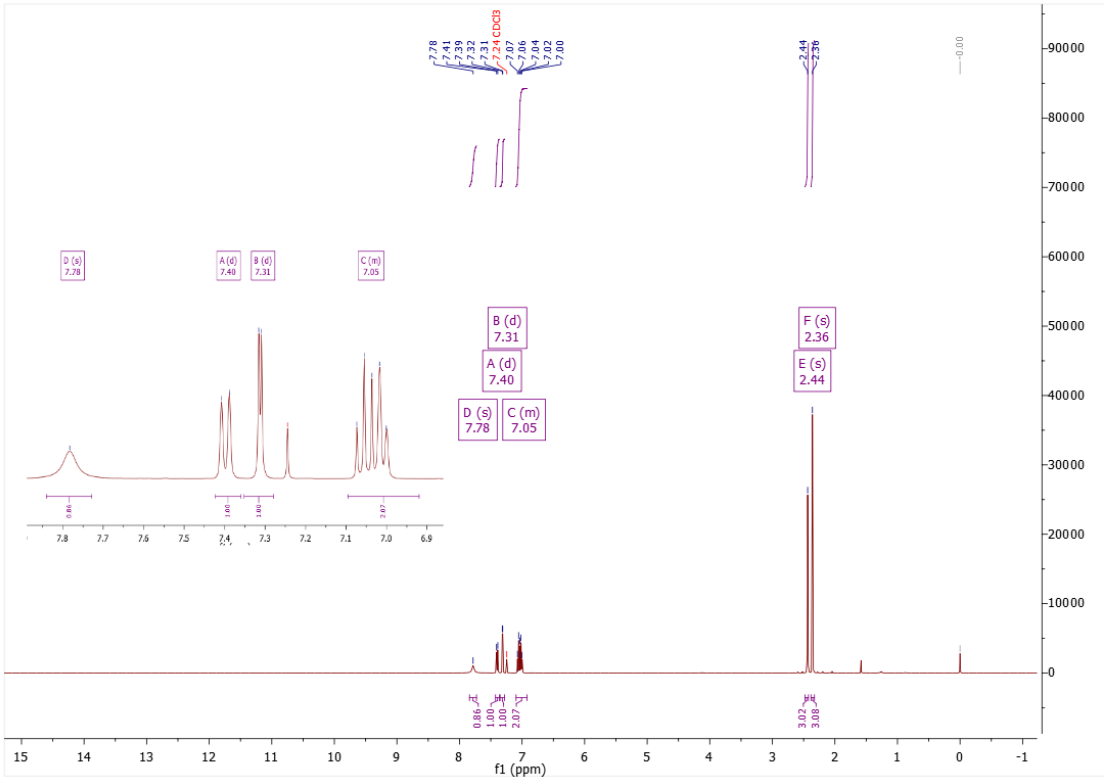

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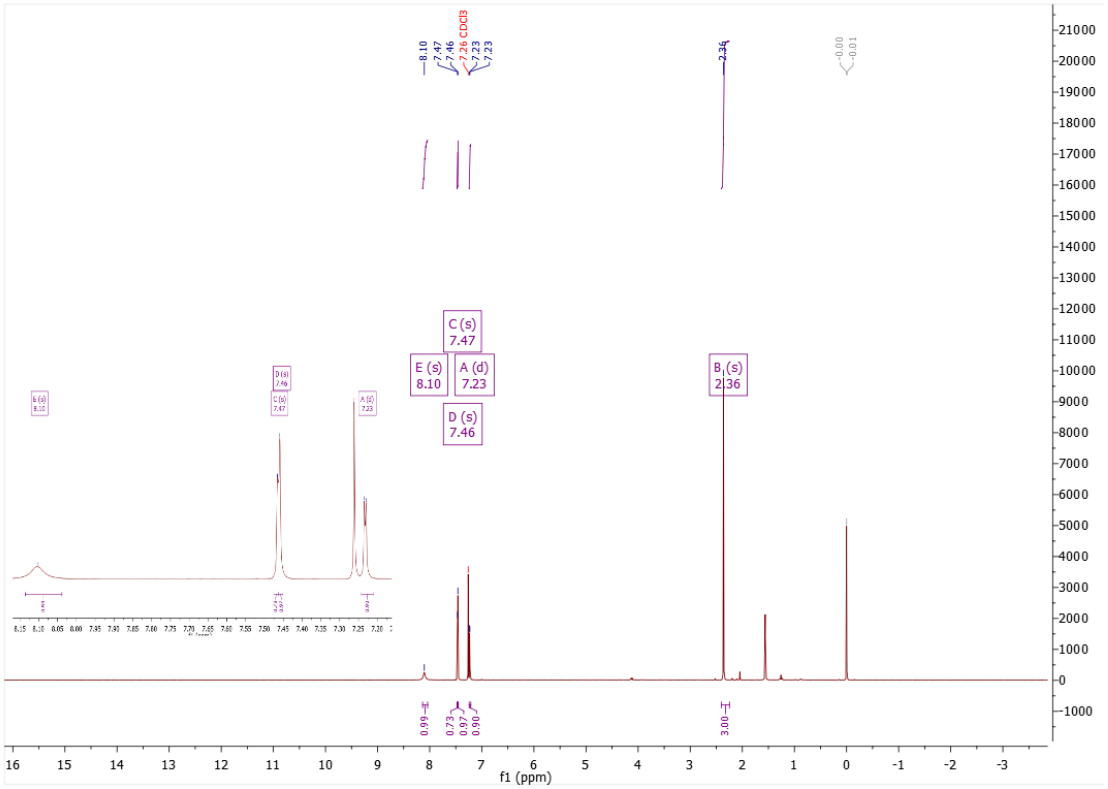

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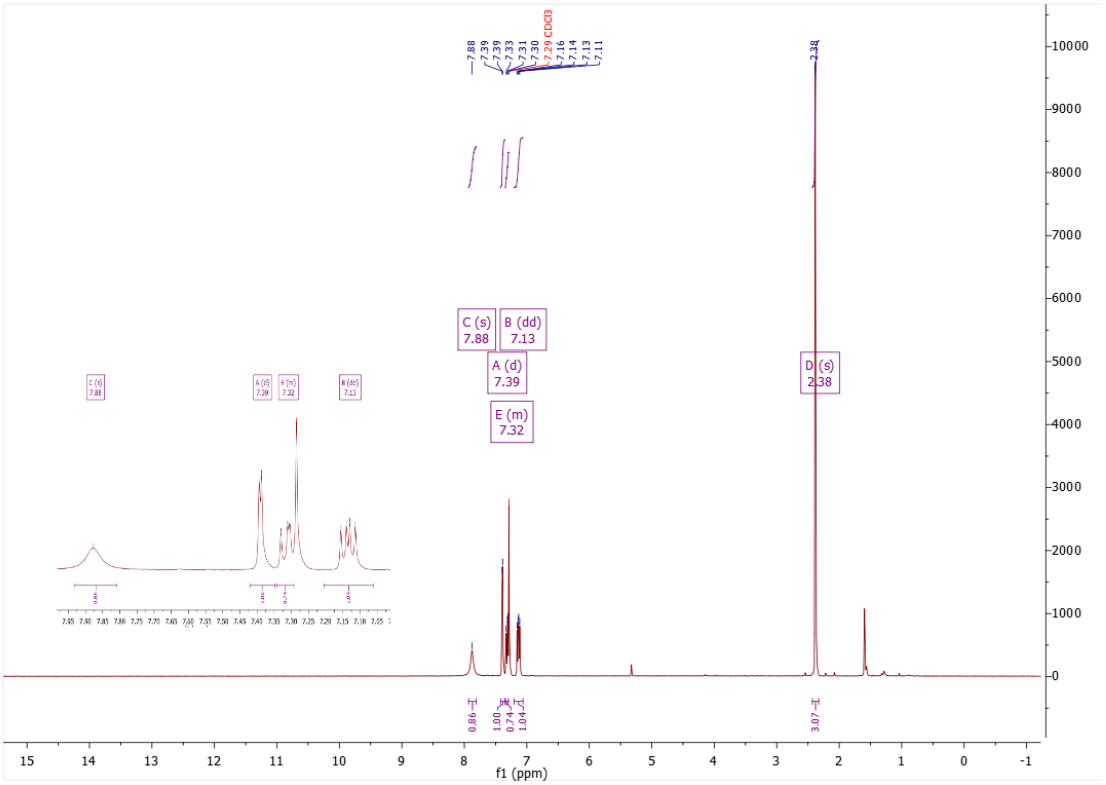
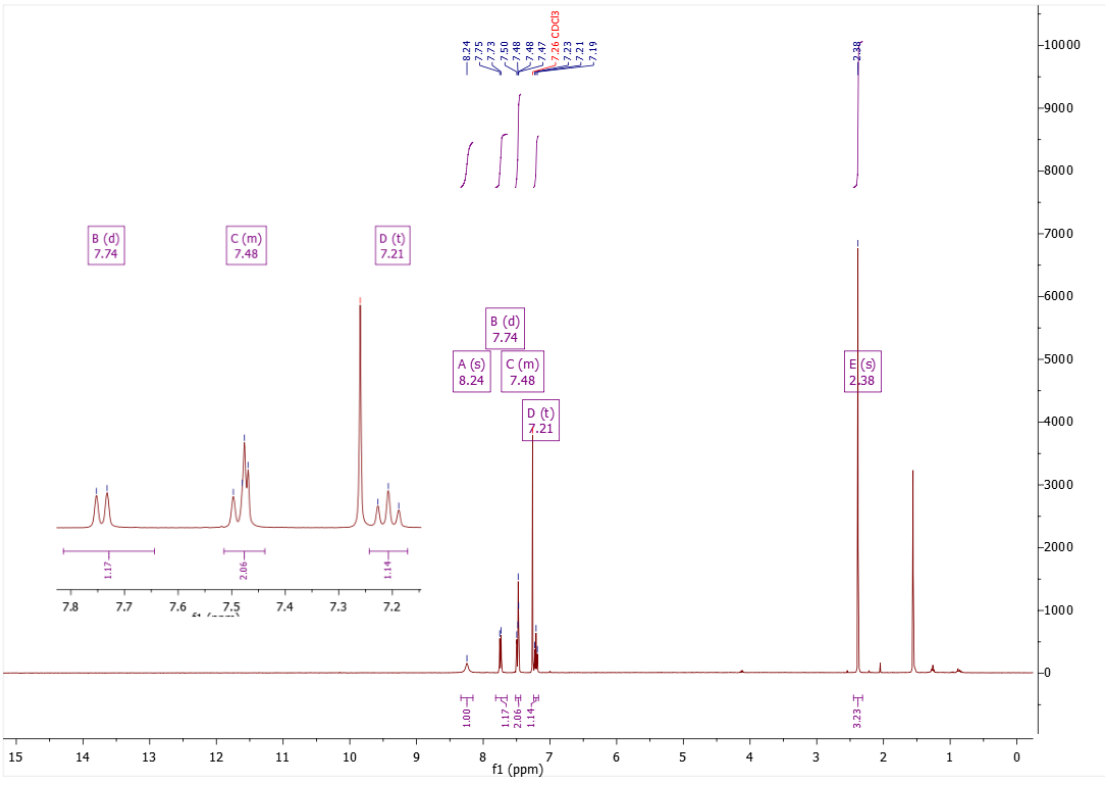
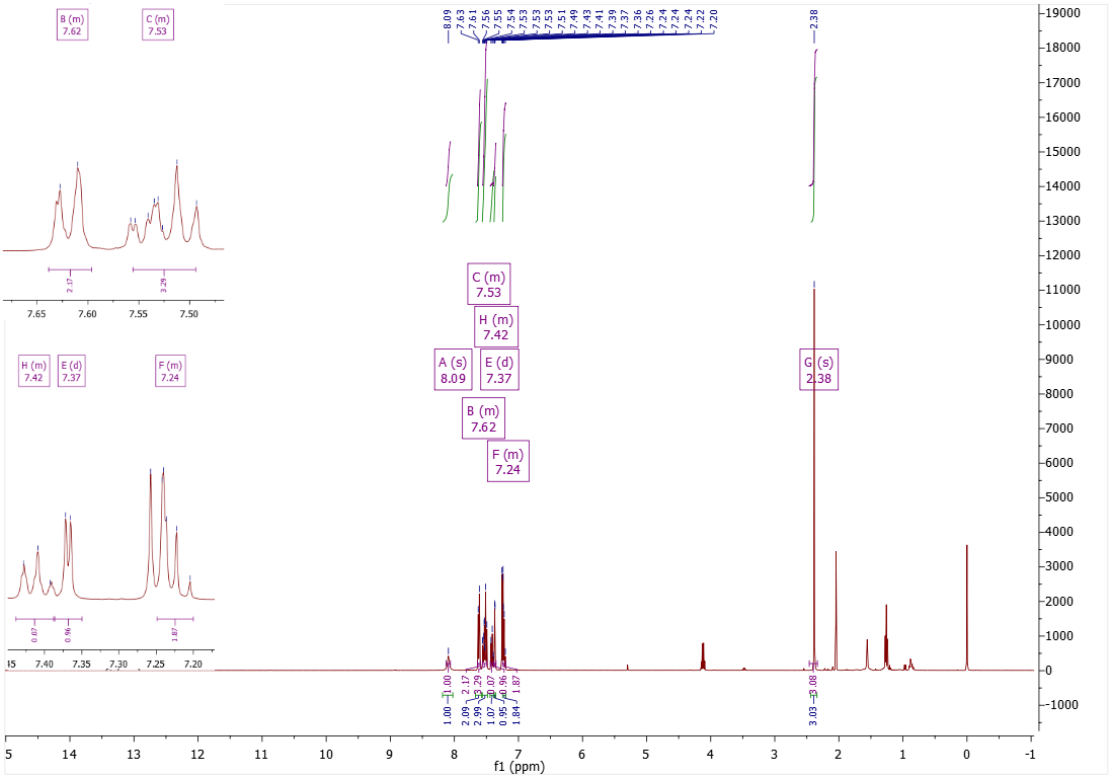


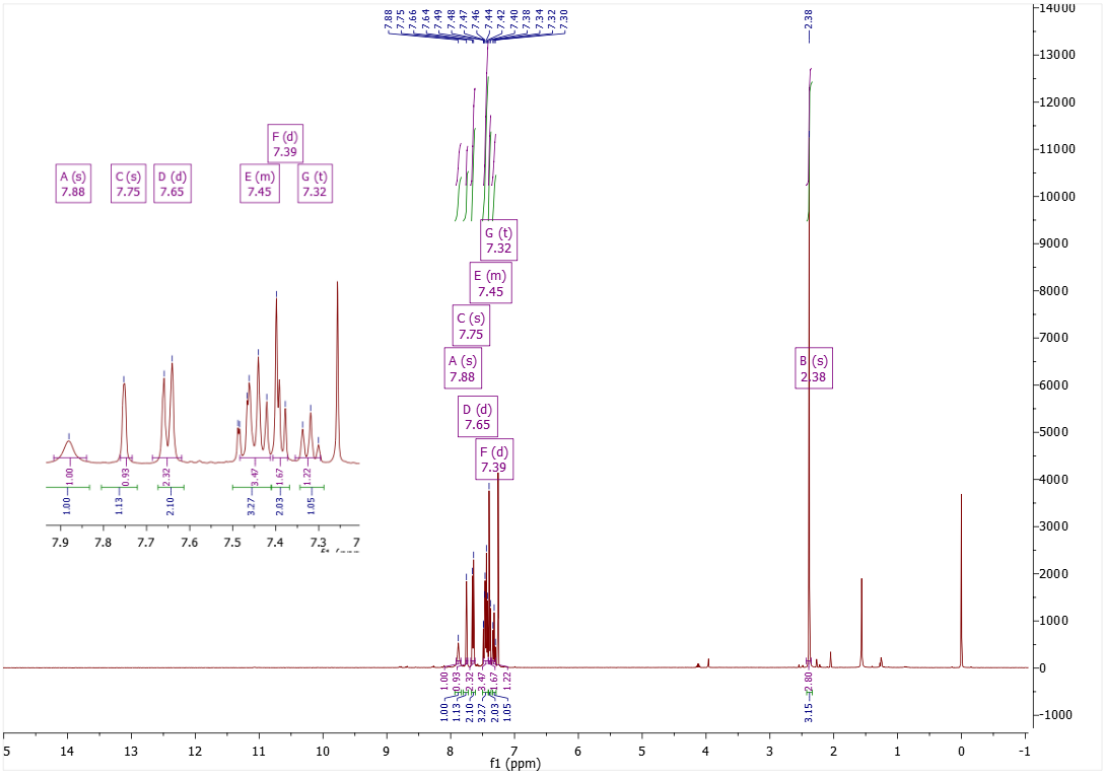
S20 7-(Trifluoromethyl)-1H-indol-3-yl acetate



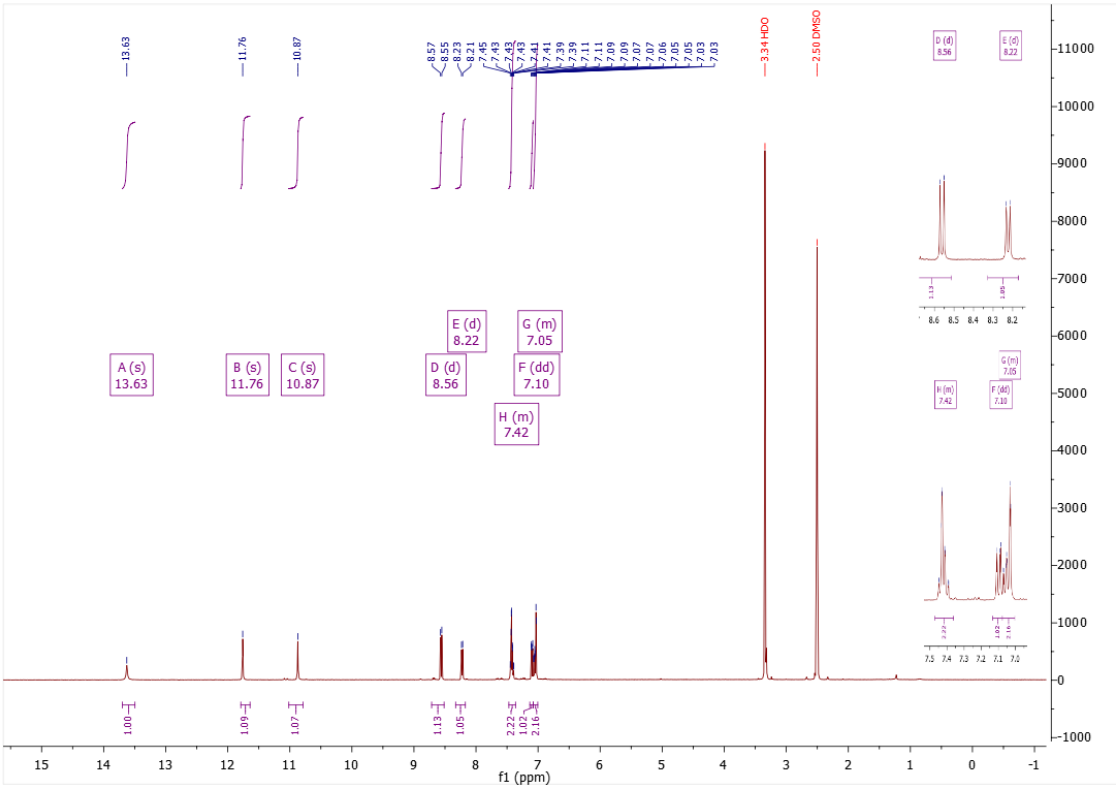
S21 7-Phenyl-1H-indol-3-yl acetate



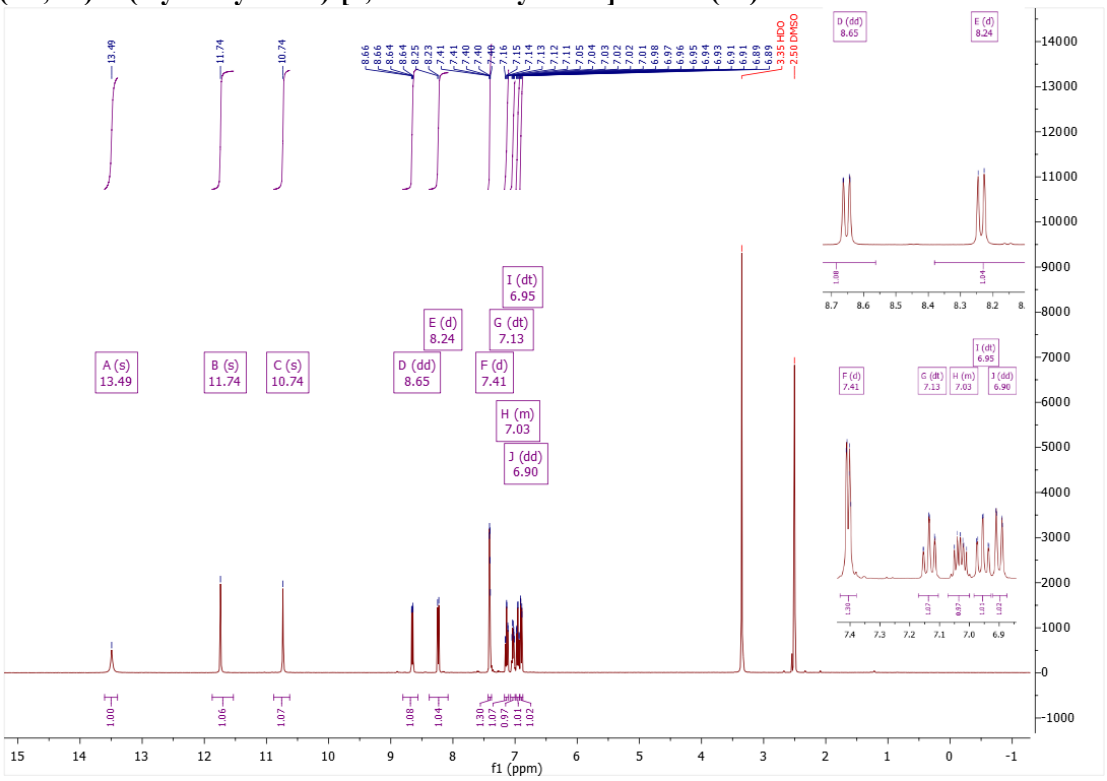
S22 5-Phenyl-1H-indol-3-yl acetate



(2Z,3E)-6'-Bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (5)



(2Z,3E)-3-(Hydroxyimino)-[2,3'-biindolinylidene]-2'-one (12)



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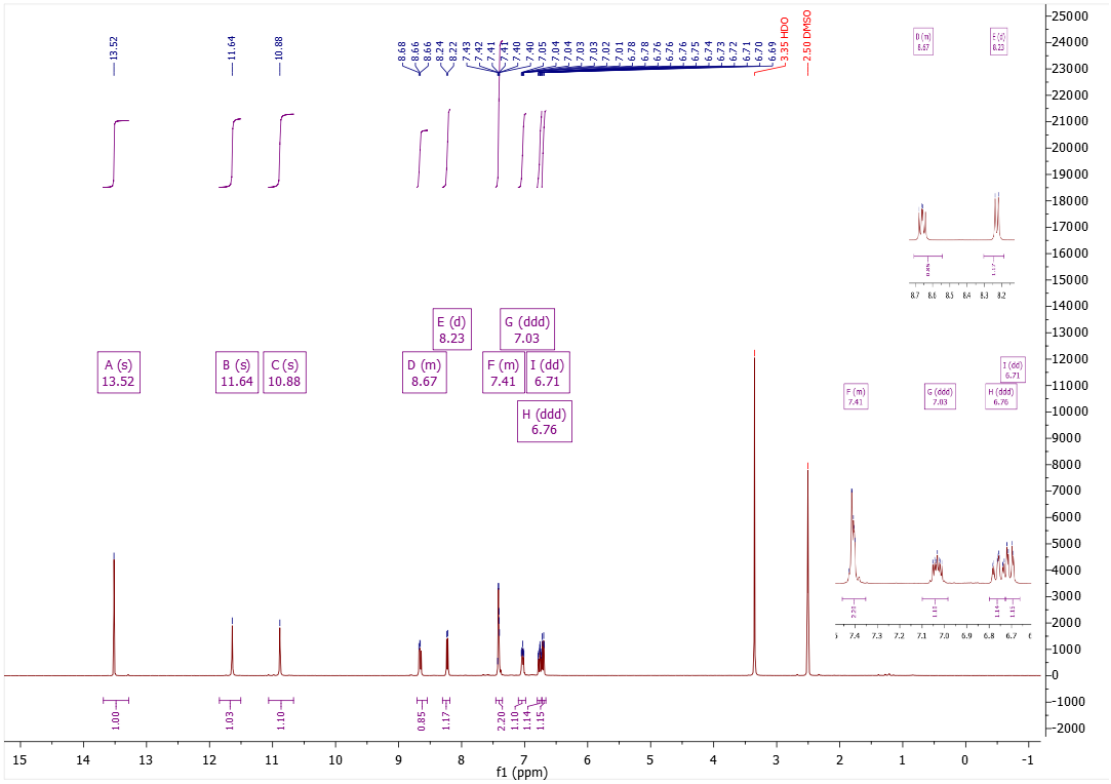

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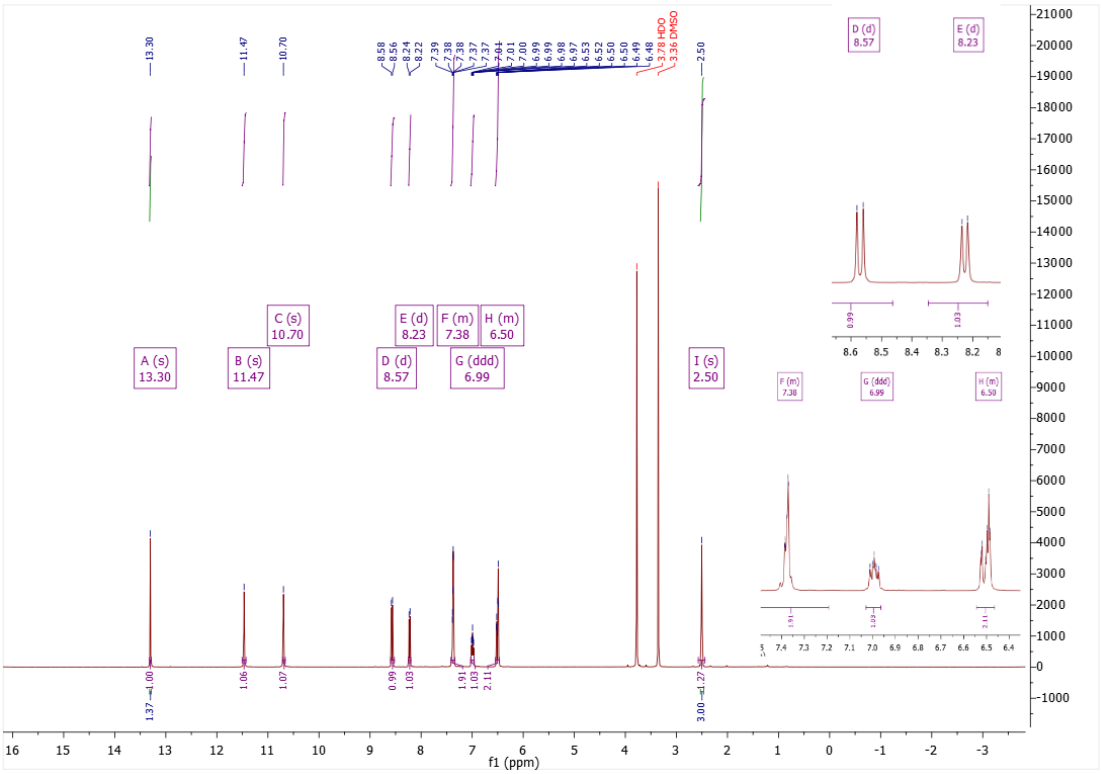

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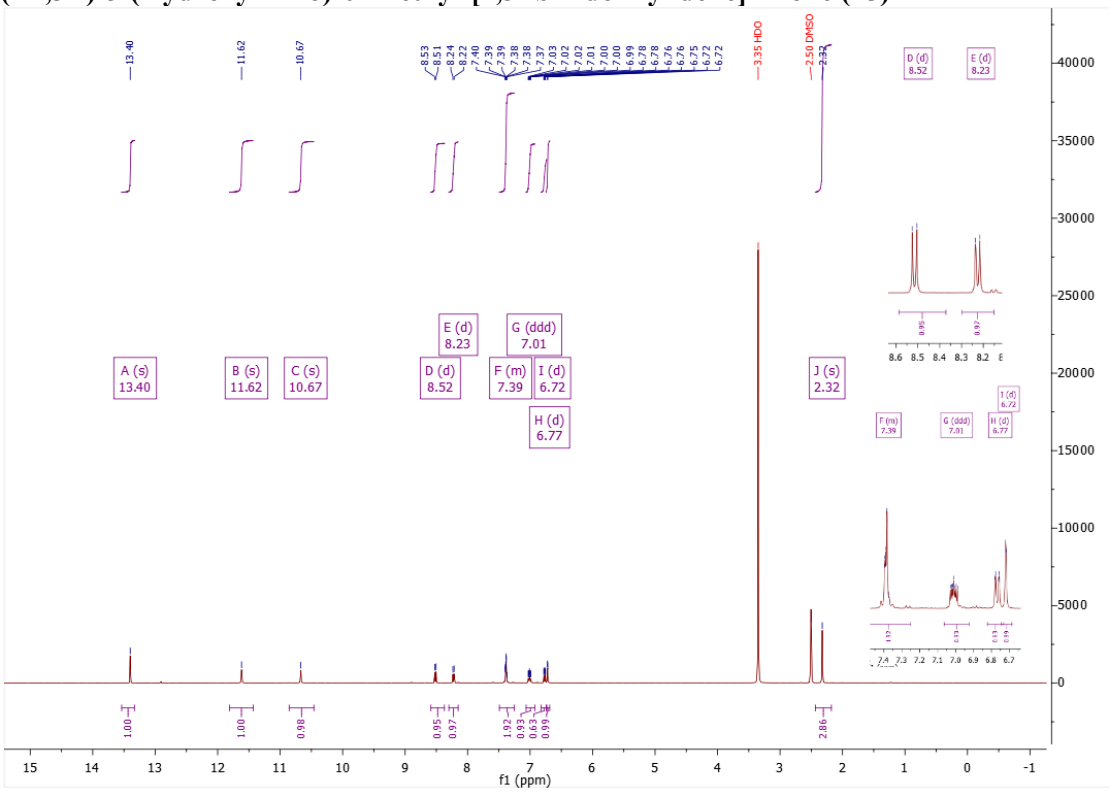
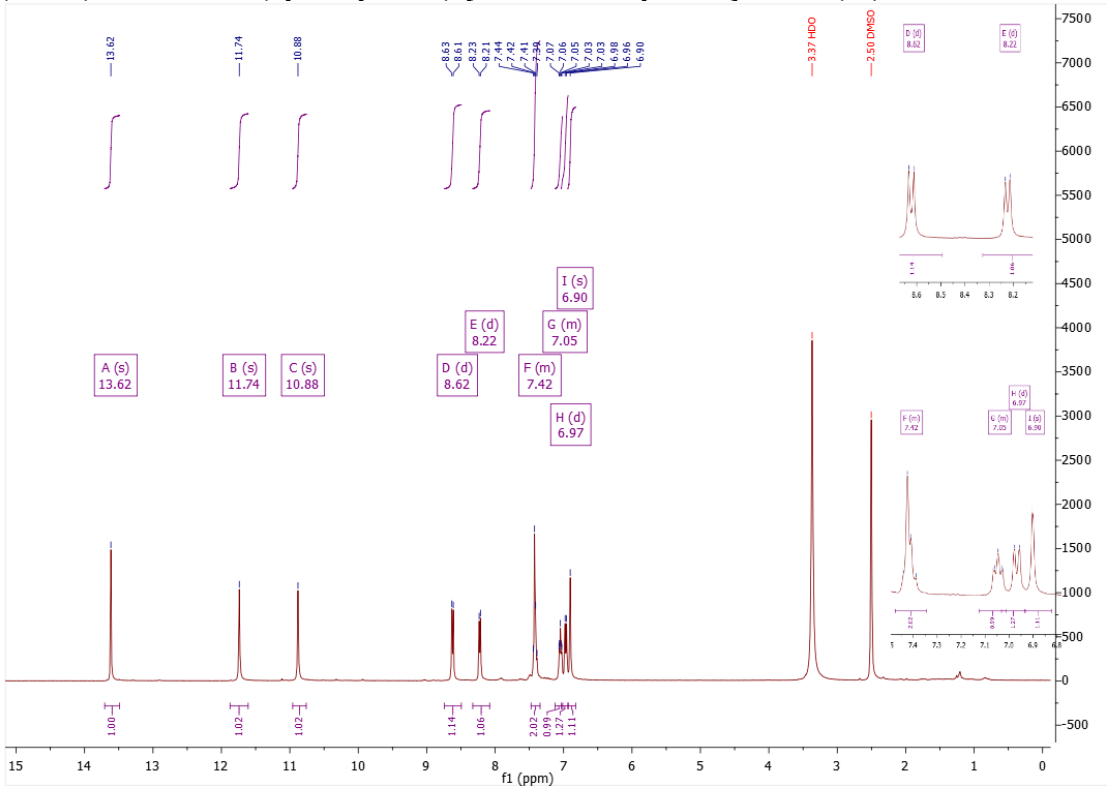


(2Z,3E)-6'-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (16)



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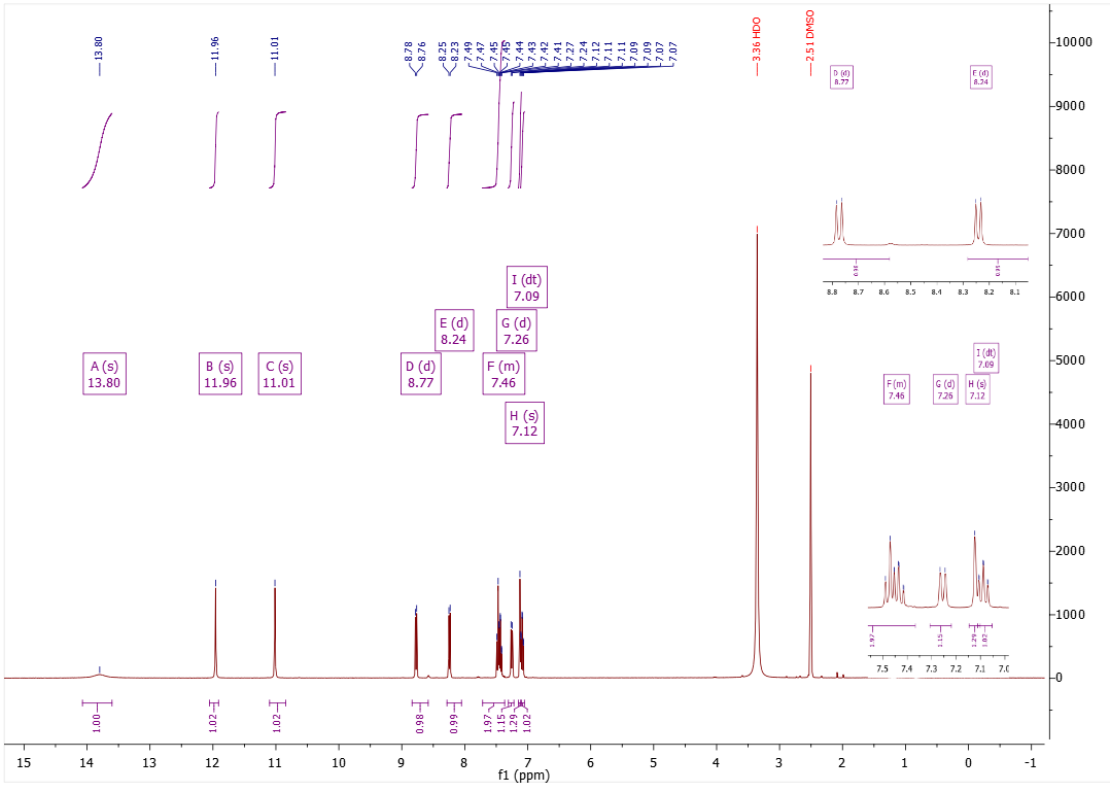

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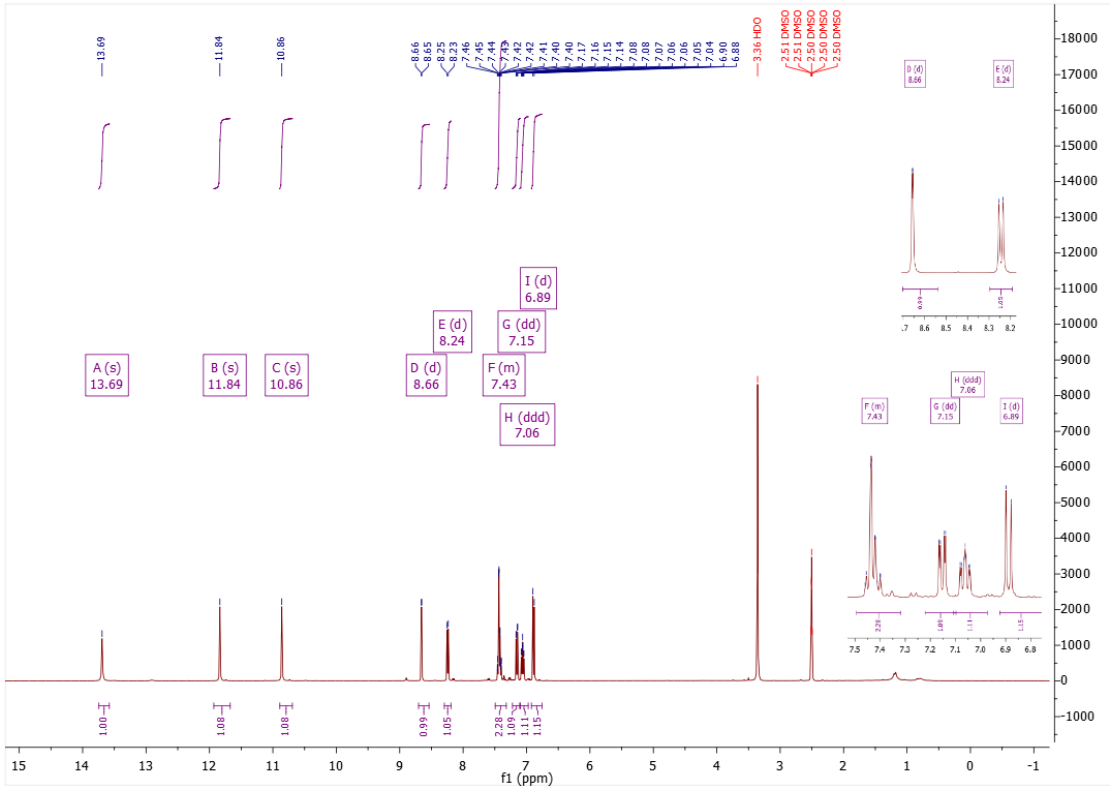
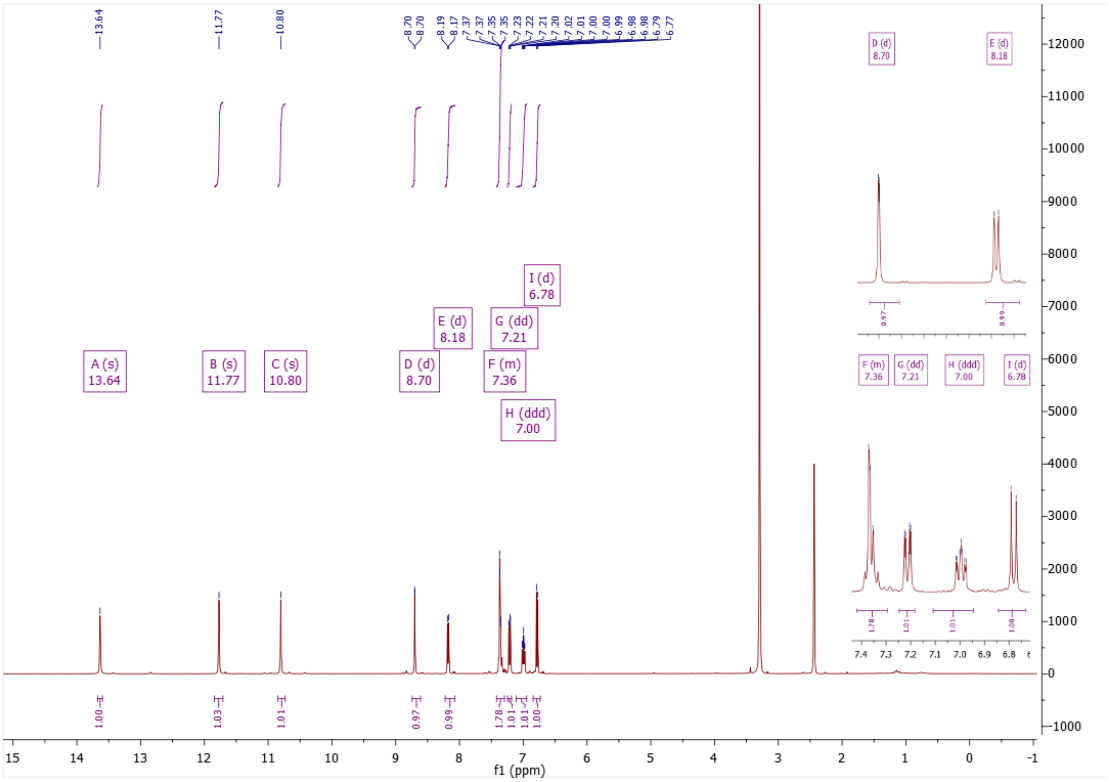
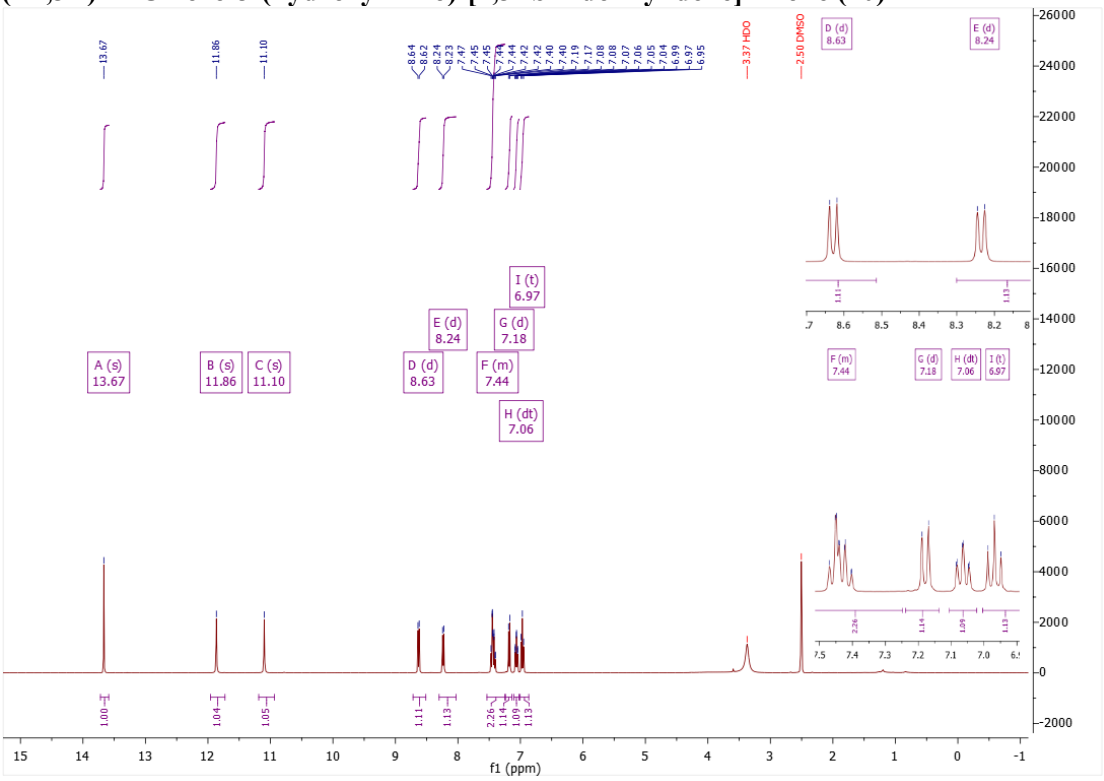


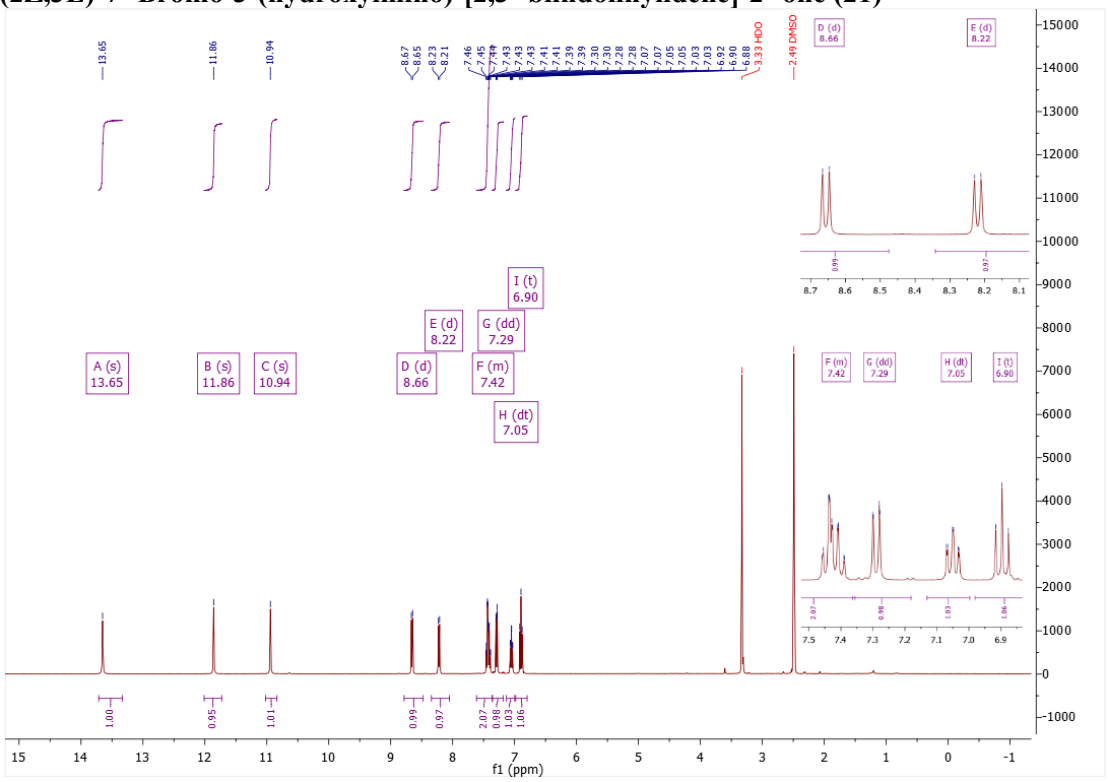
(2Z,3E)-5'-Bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (19)



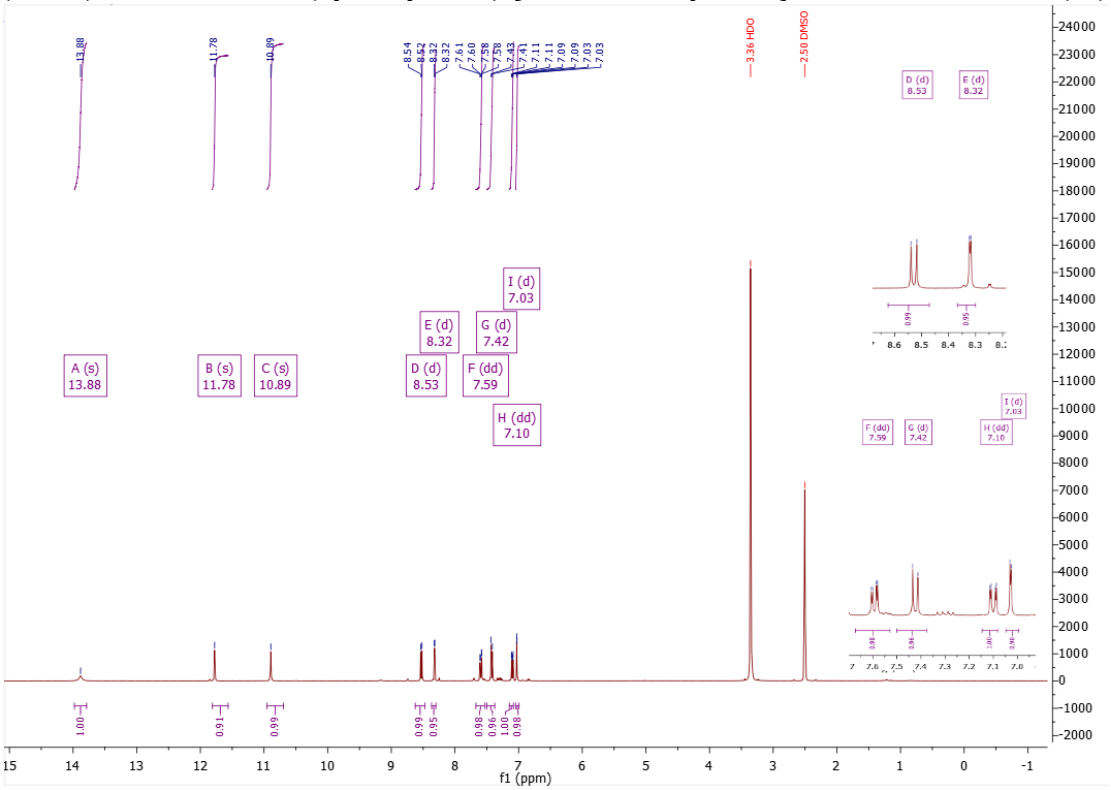
(2Z,3E)-7'-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (20)



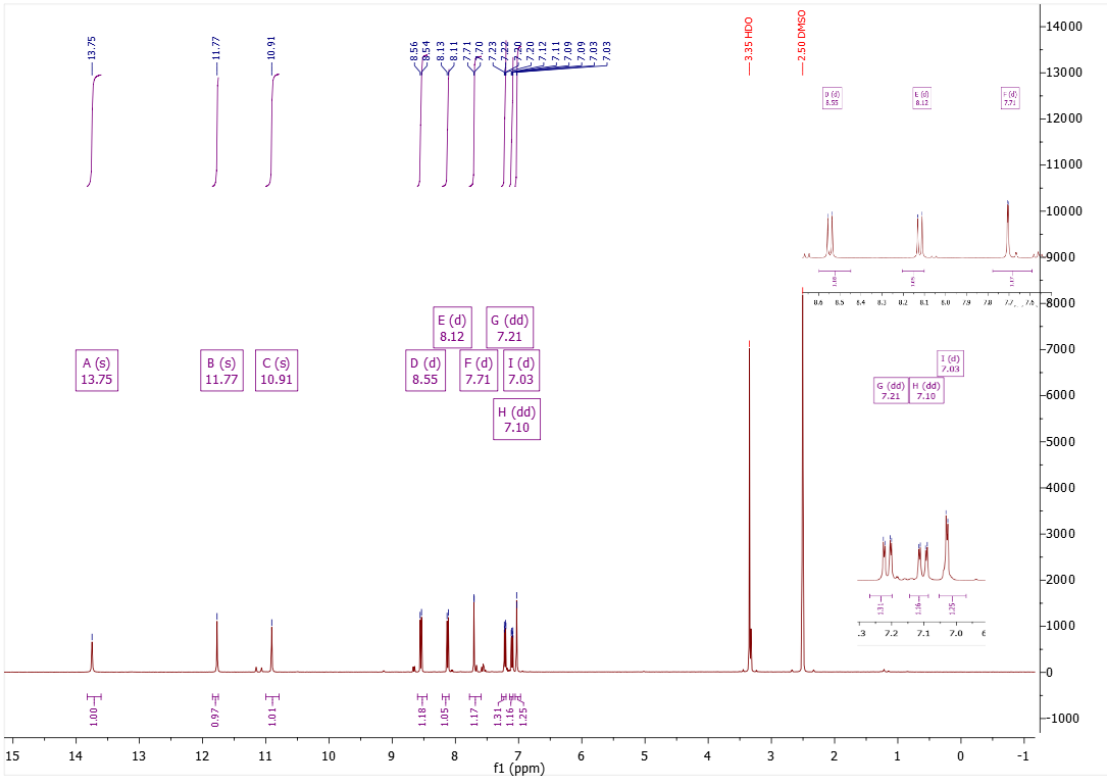
(2Z,3E)-7'-Bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (21)



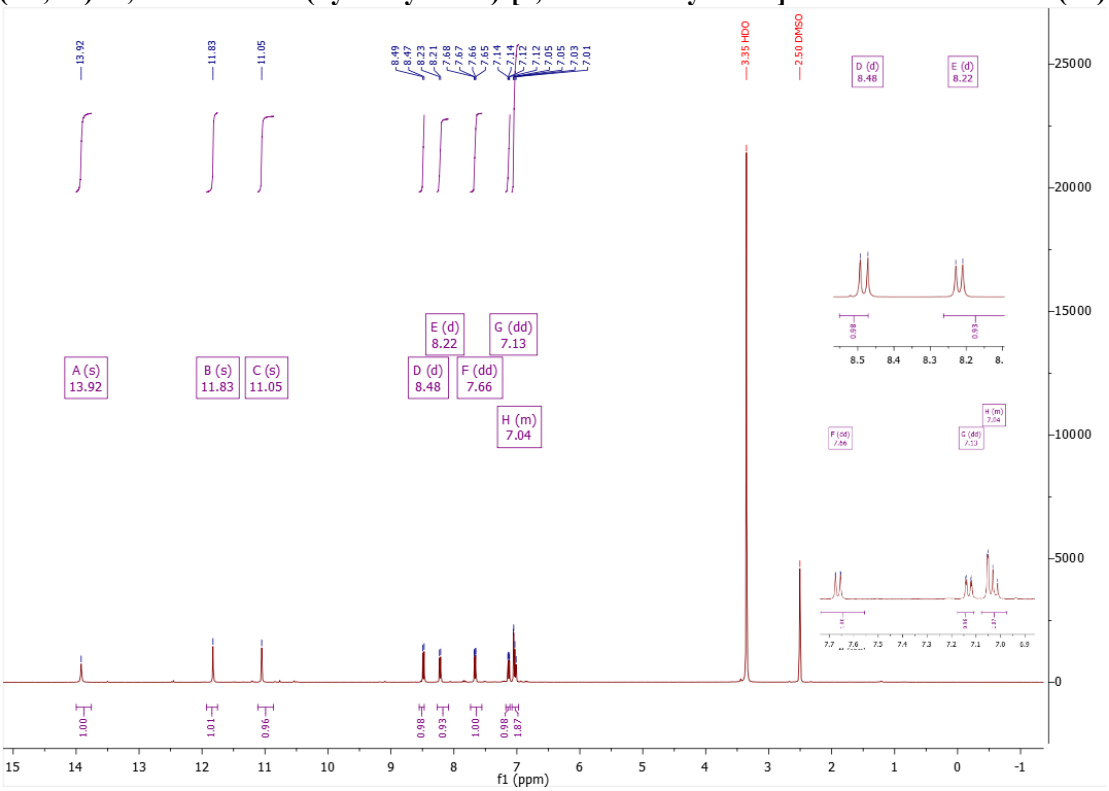
(2Z,3E)-5,6'-Dibromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (22)



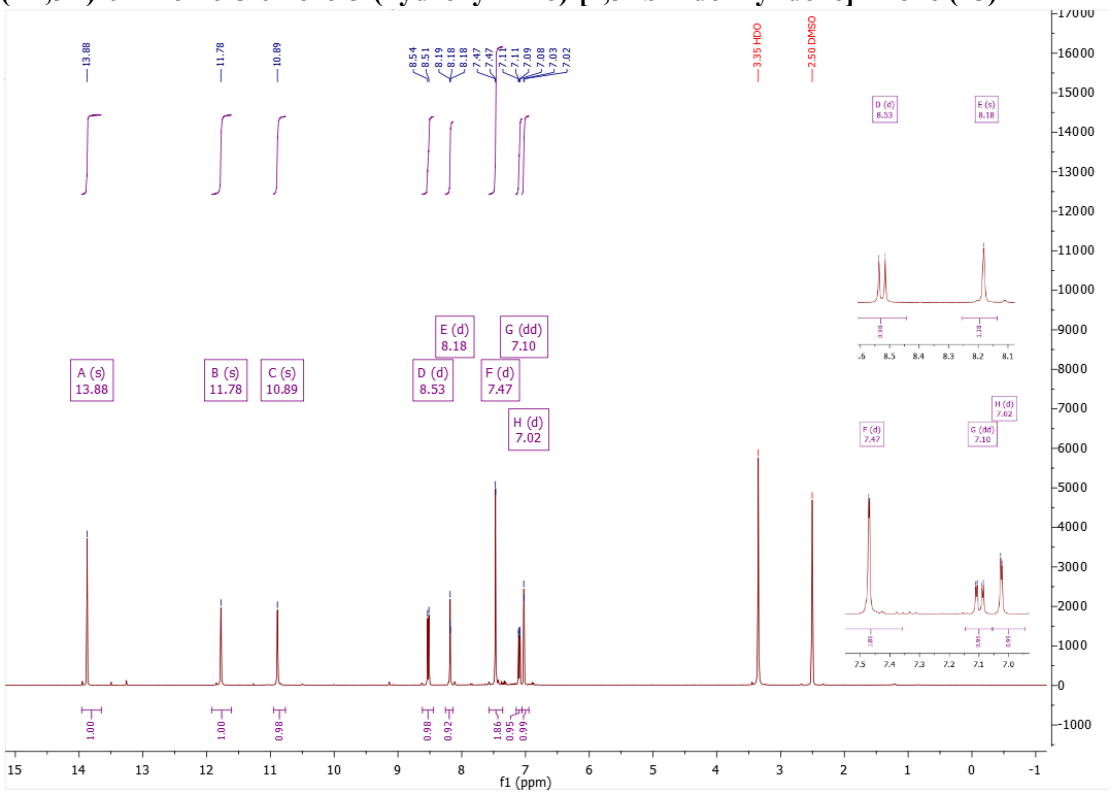
(2Z,3E)-6,6'-Dibromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (23)



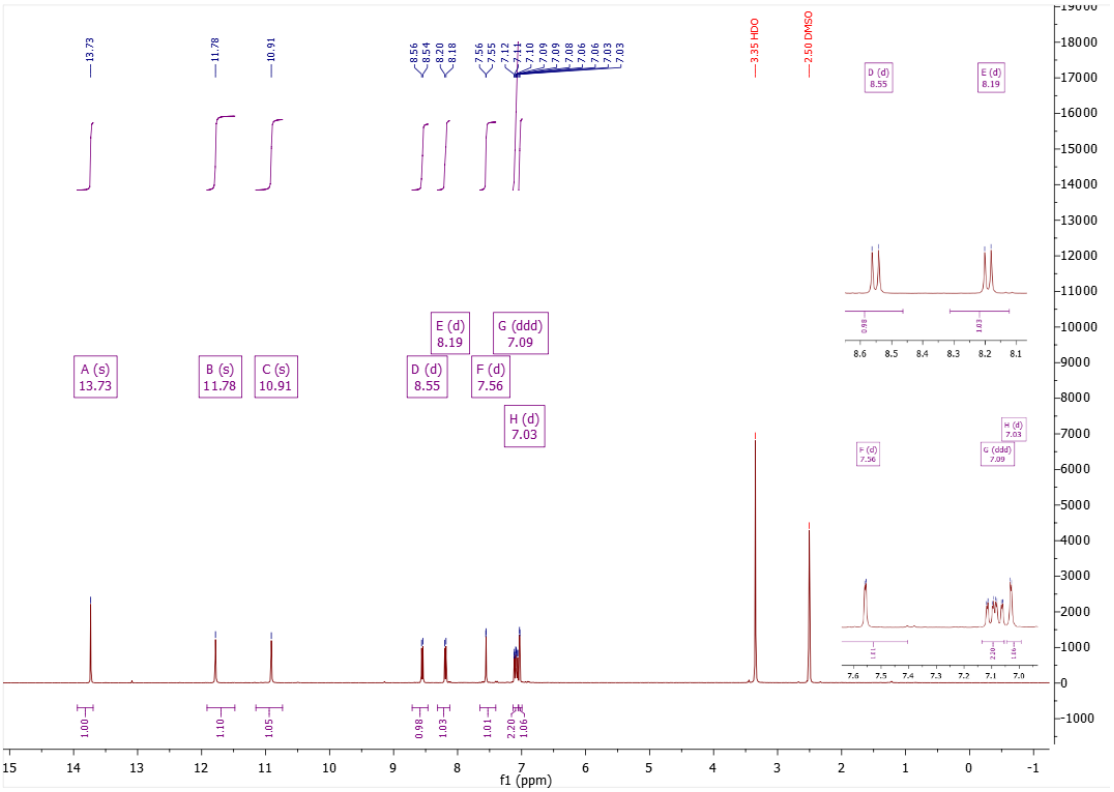
(2Z,3E)-6',7-Dibromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (24)



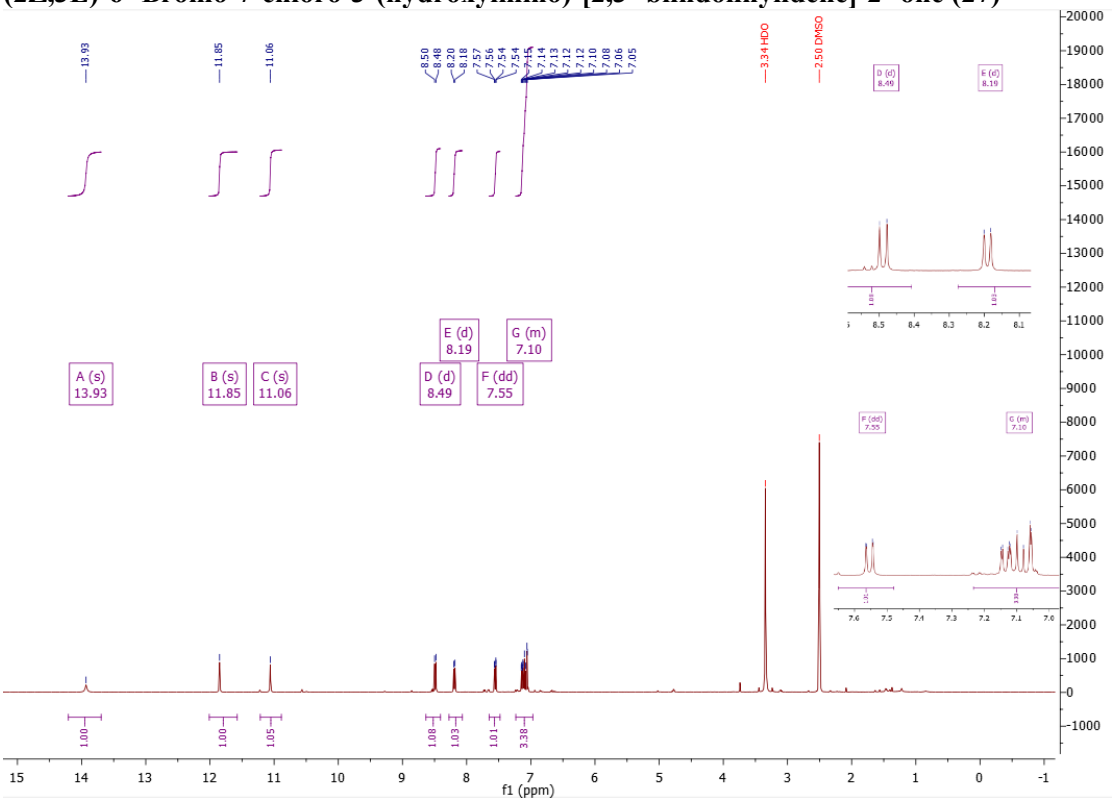
(2Z,3E)-6'-Bromo-5-chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (25)



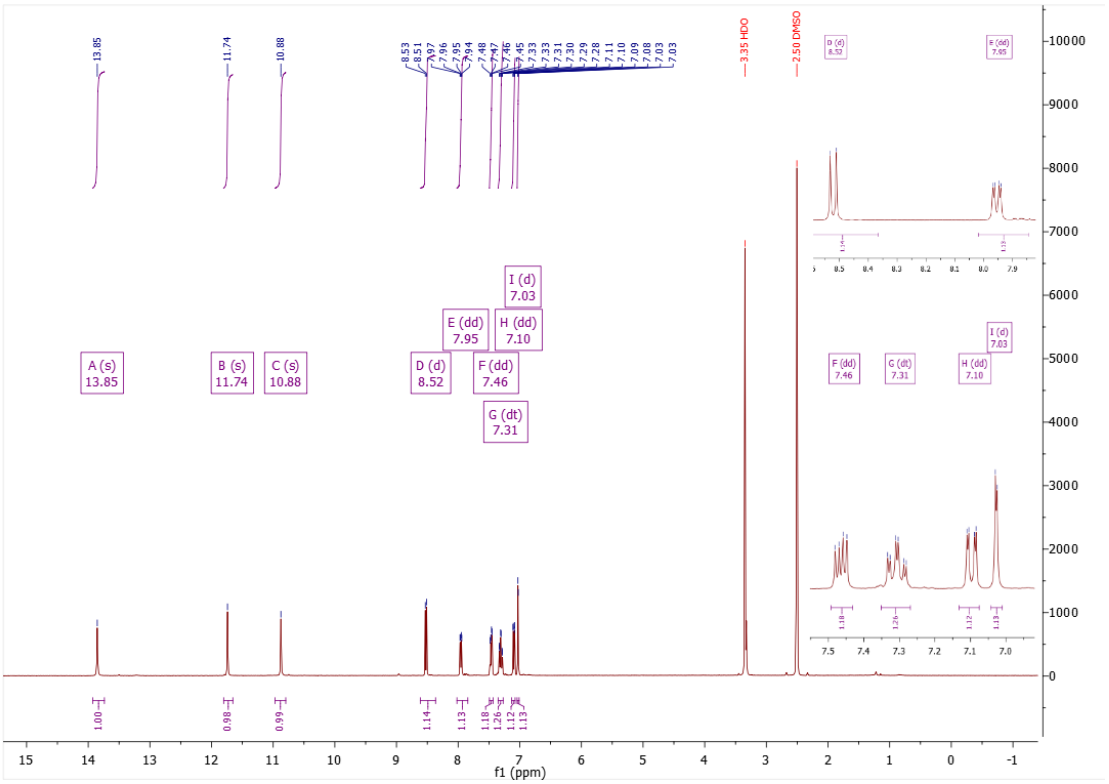
(2Z,3E)-6'-Bromo-6-chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (26)



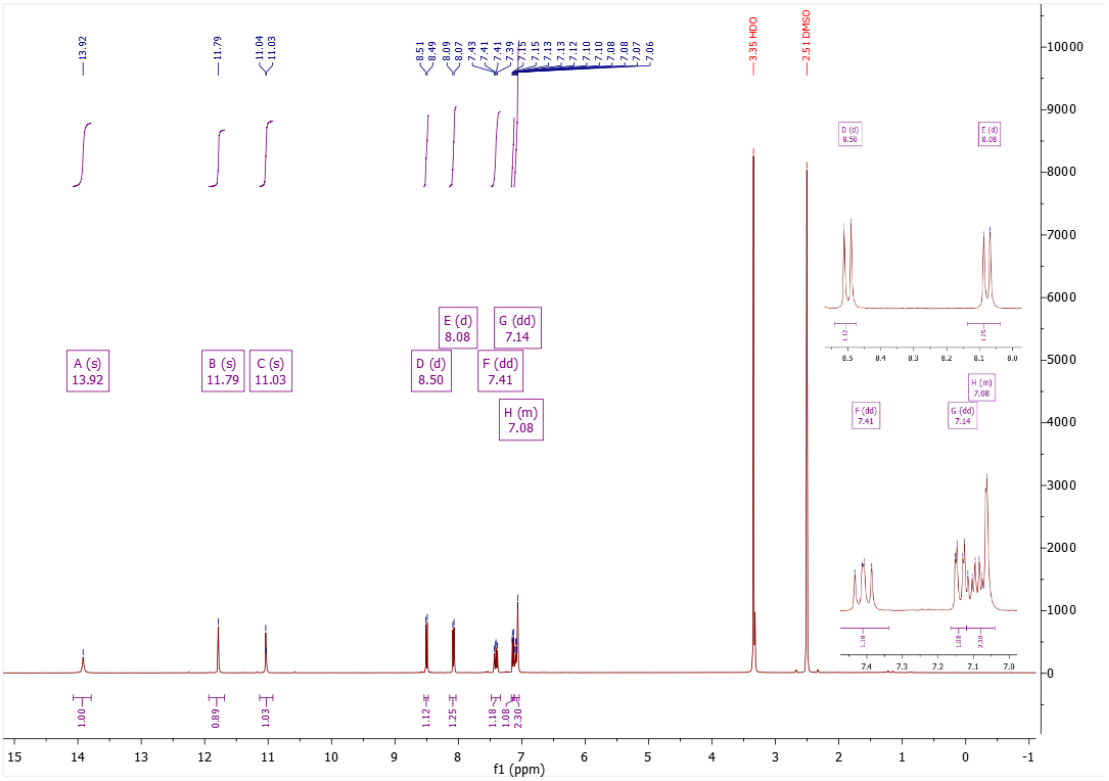
(2Z,3E)-6'-Bromo-7-chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (27)



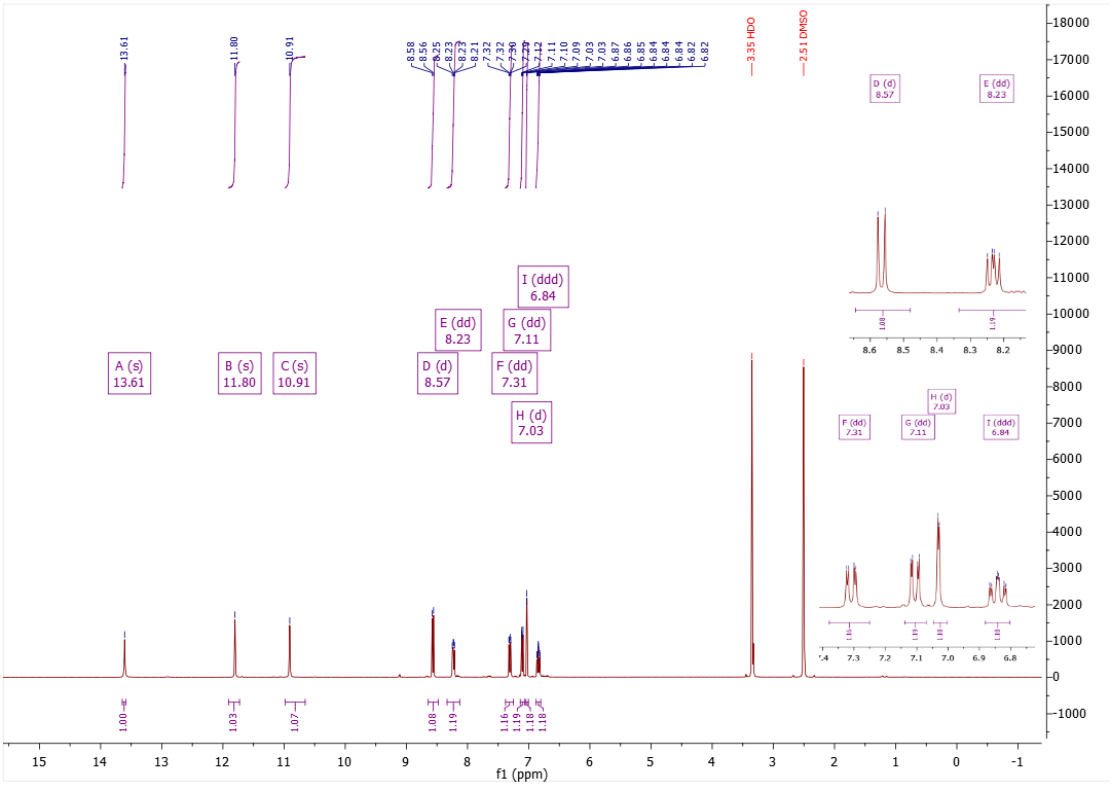
(2Z,3E)-6'-Bromo-5-fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (28)



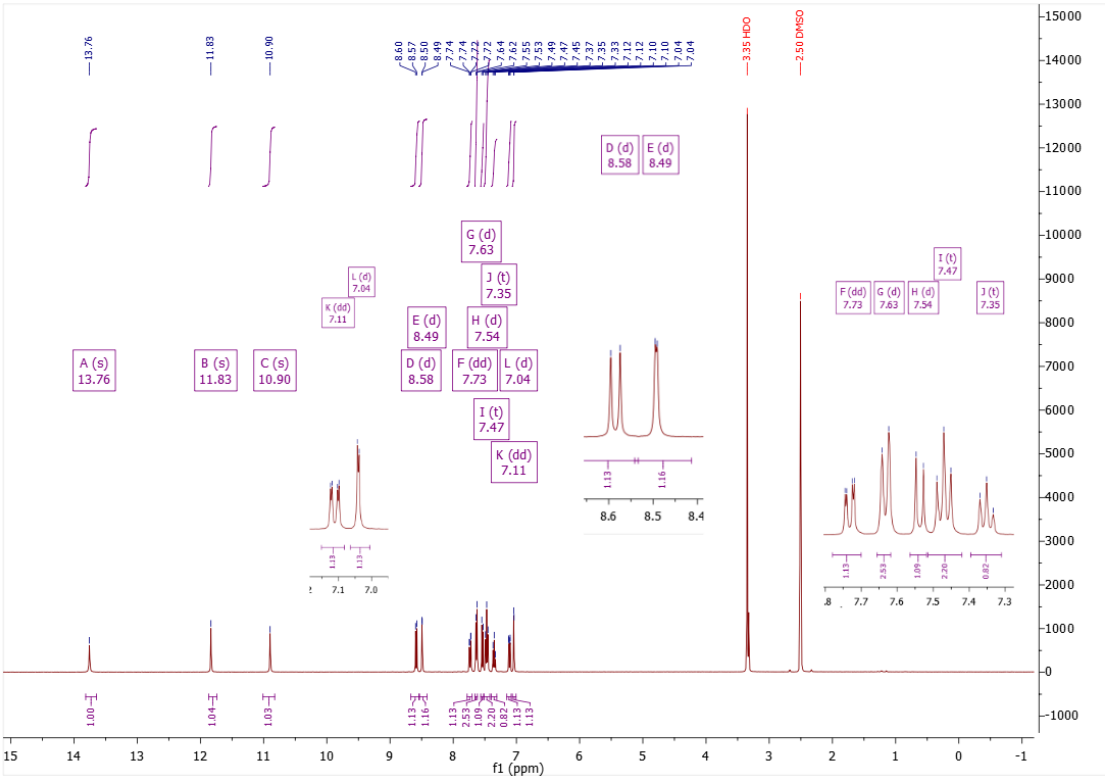
(2Z,3E)-6'-Bromo-7-fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (29)



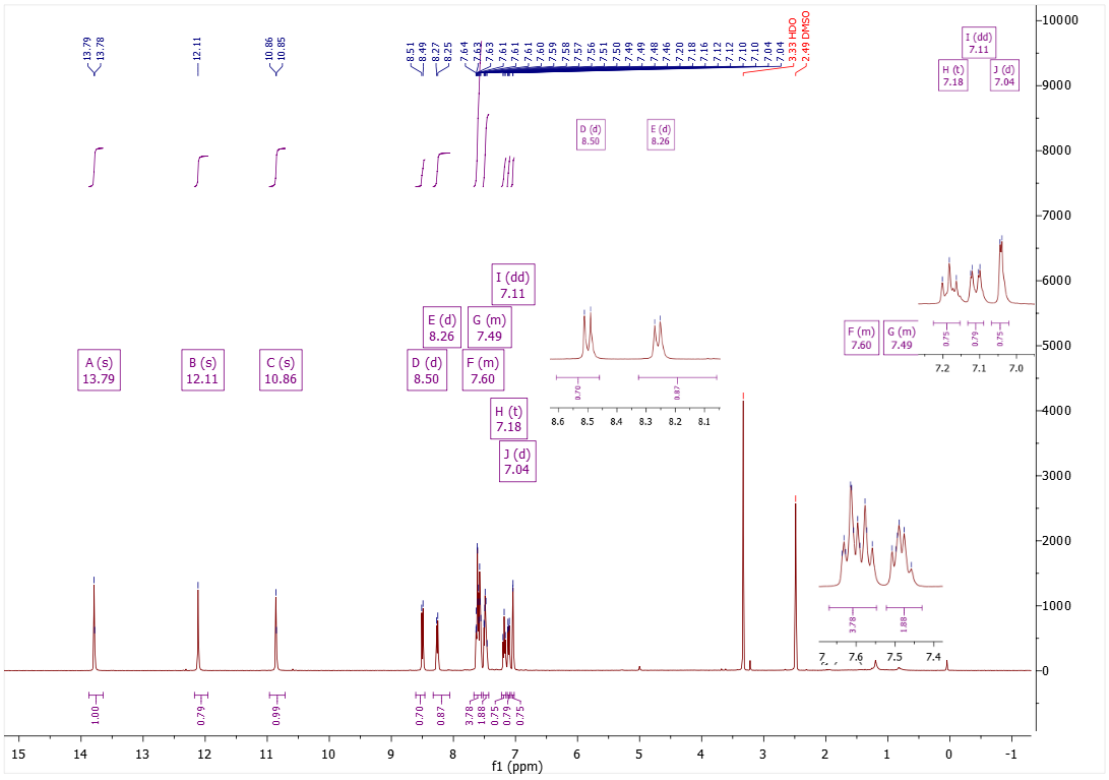
(2Z,3E)-6'-Bromo-6-fluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (30)



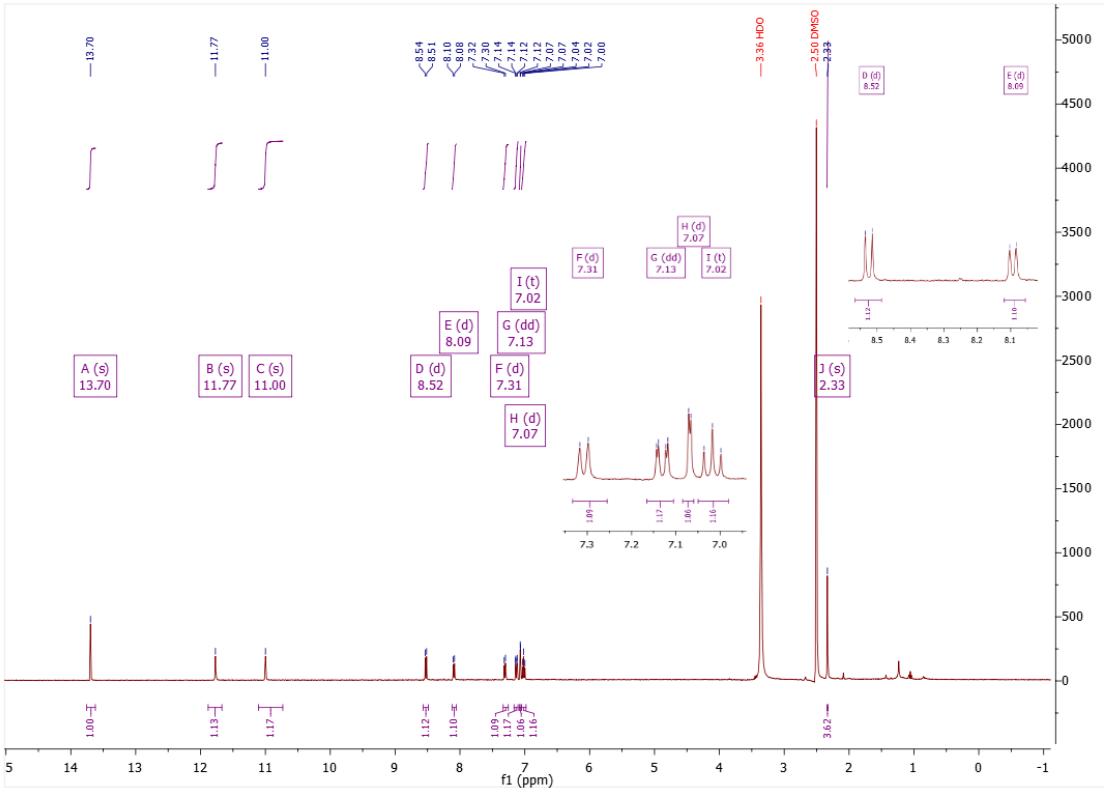
(2Z,3E)-6'-Bromo-3-(hydroxyimino)-5-phenyl-[2,3'-biindolinylidene]-2'-one (31):



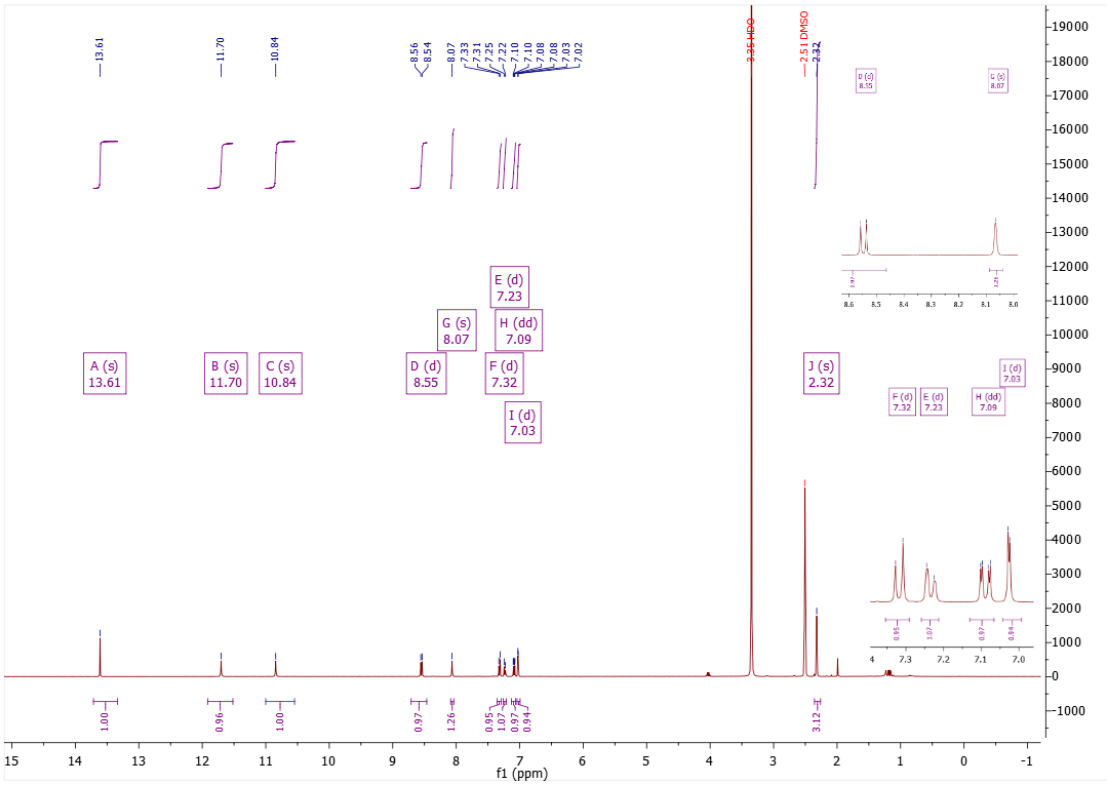
(2Z,3E)-6'-Bromo-3-(hydroxyimino)-7-phenyl-[2,3'-biindolinylidene]-2'-one (32):



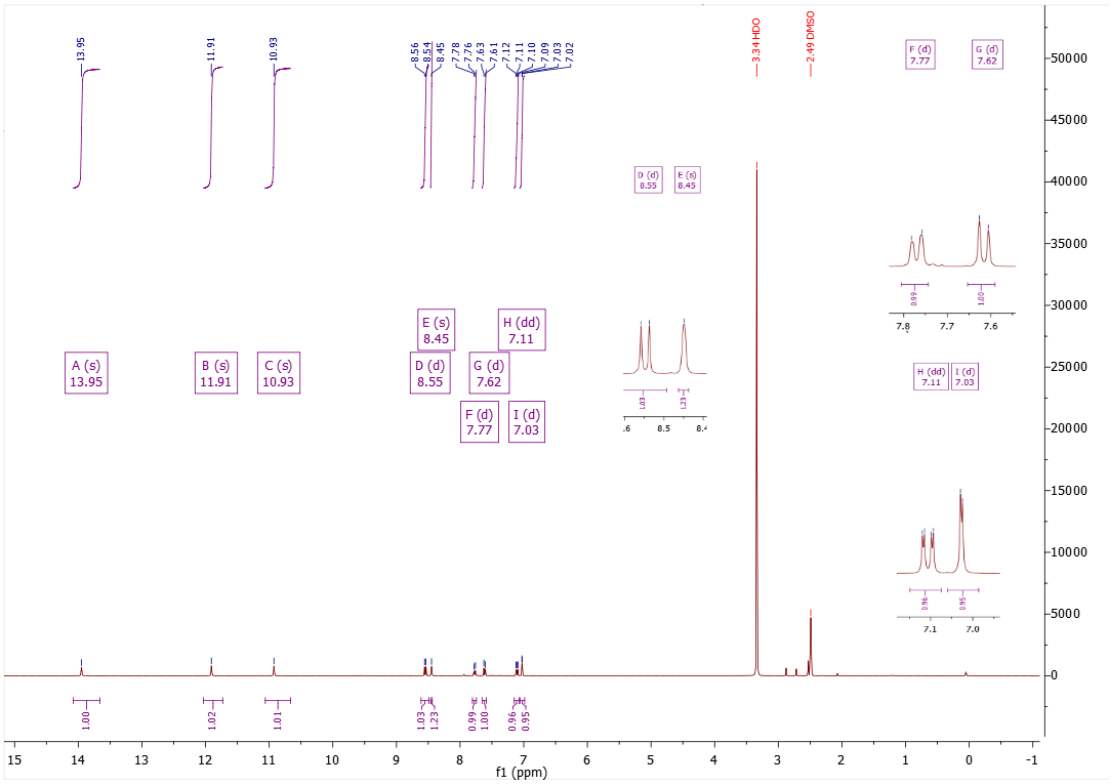
(2Z,3E)-6'-Bromo-3-(hydroxyimino)-7-methyl-[2,3'-biindolinylidene]-2'-one (33):



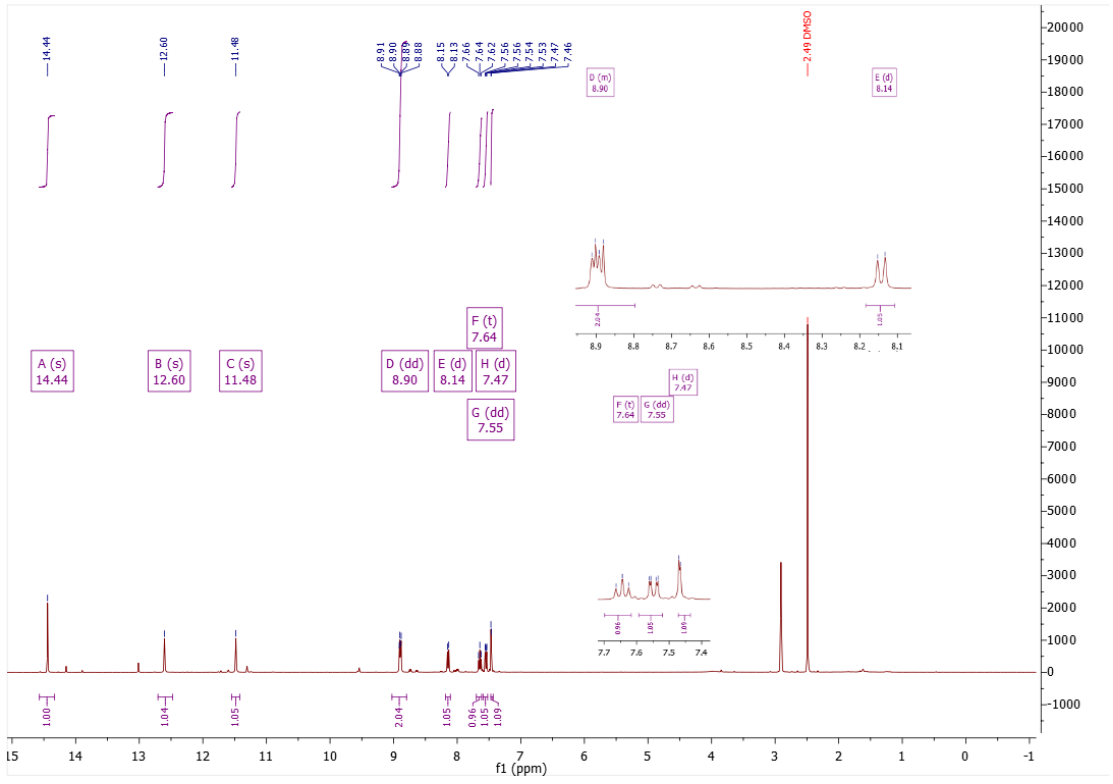
(2Z,3E)-6'-Bromo-3-(hydroxyimino)-5-methyl-[2,3'-biindolinylidene]-2'-one (34):



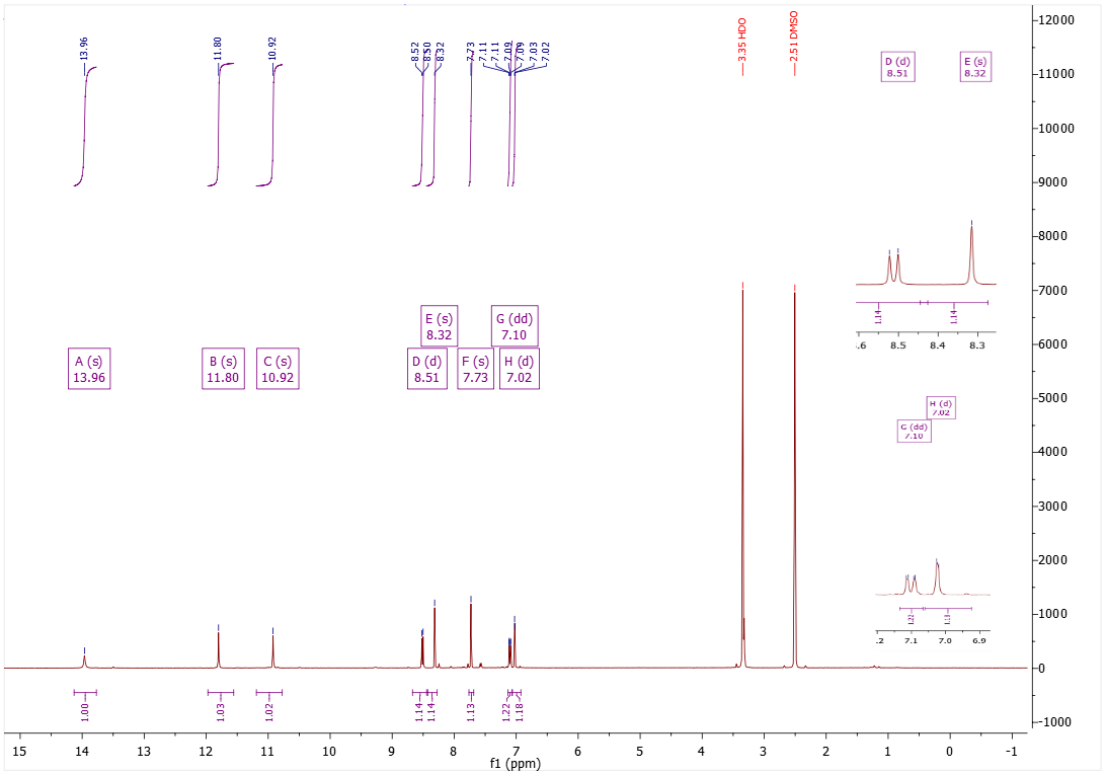
(2Z,3E)-6'-Bromo-3-(hydroxyimino)-5-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (35):



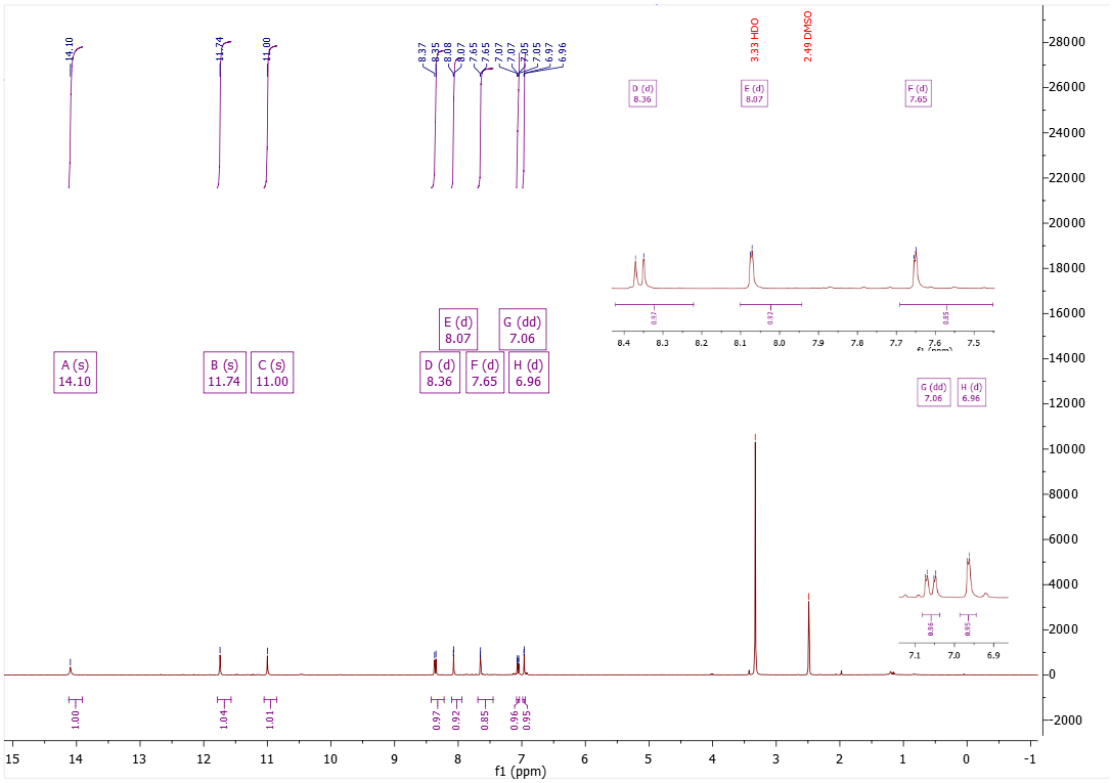
(2Z,3E)-6'-Bromo-3-(hydroxyimino)-7-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (36):



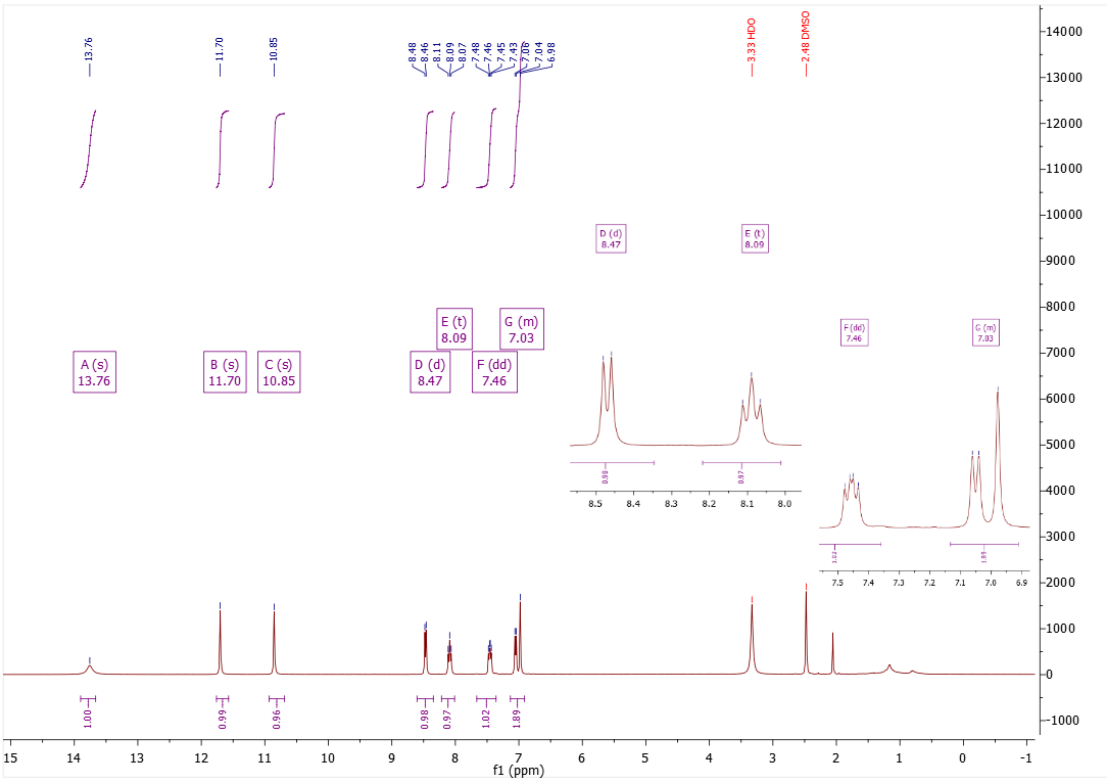
(2Z,3E)-6'-Bromo-5,6-dichloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (37):



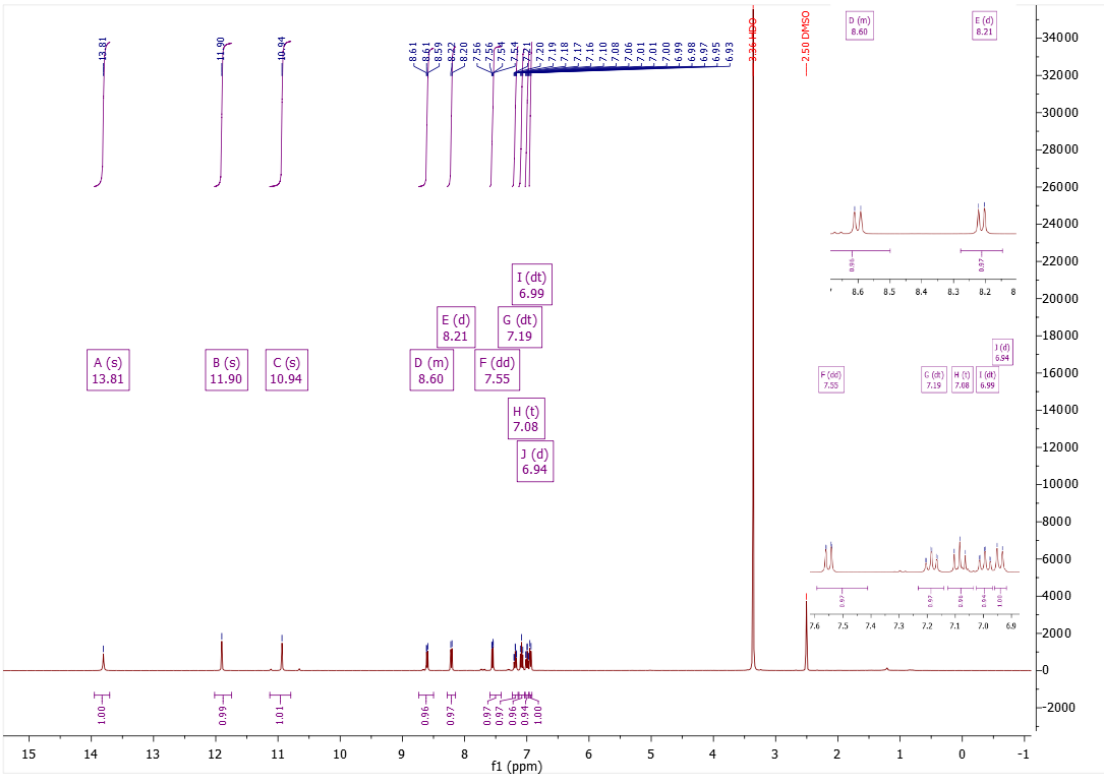
(2Z,3E)-6'-Bromo-5,7-dichloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (38):



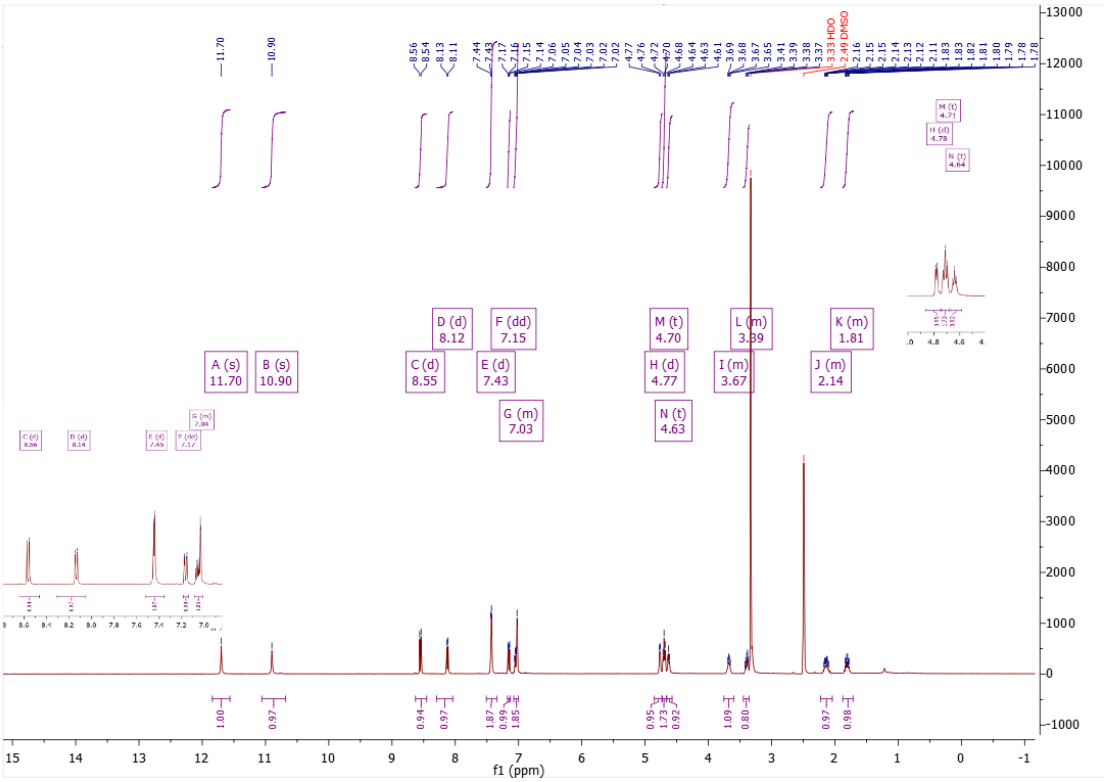
(2Z,3E)-6'-Bromo-5,6-difluoro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (39):



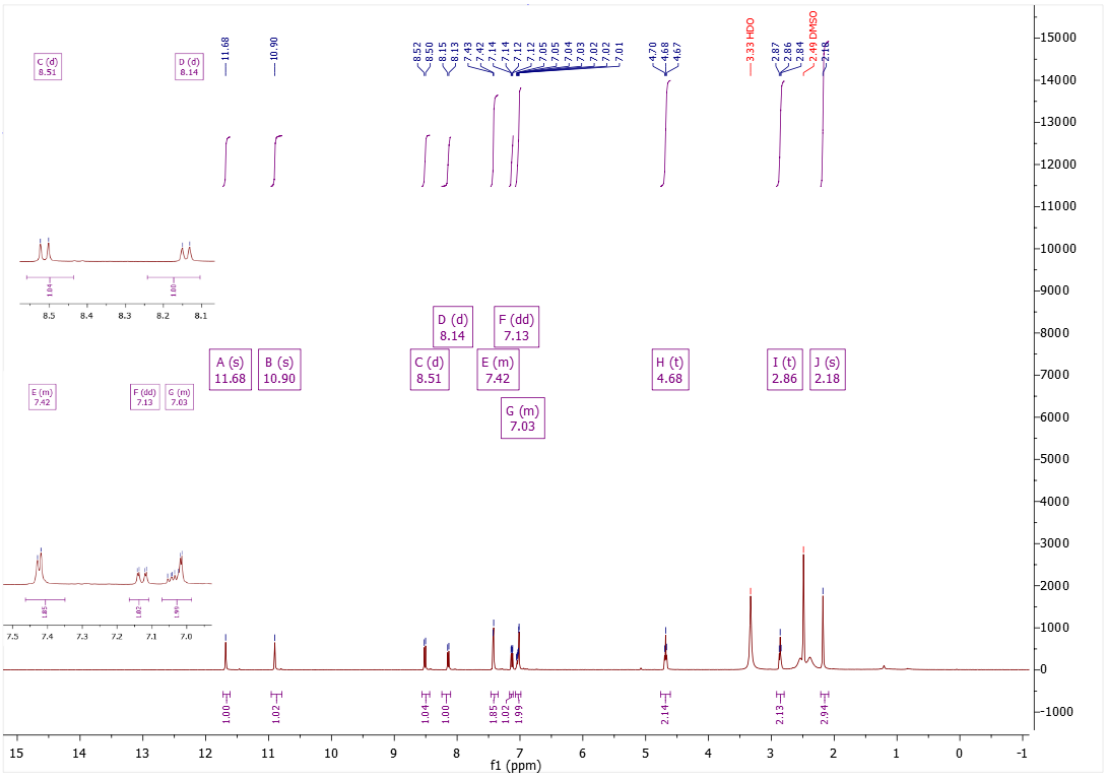
(2Z,3E)-7-Chloro-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one (40)



(2Z,3E)-6'-Bromo-3-((3,4-dihydroxybutoxy)imino)-[2,3'-biindolinylidene]-2'-one (41):



(2Z,3E)-6'-Bromo-3-((2-(4-methylpiperazin-1-yl)ethoxy)imino)-[2,3'-biindolinylidene]-2'-one (42):



(2Z,3E)-6'-Bromo-3-((2-(piperazin-1-yl)ethoxy)imino)-[2,3'-biindolinylidene]-2'-one (43):