Meridianin D Analogues Possess Antibiofilm Activity Against *Mycobacterium smegmatis*

Sara M. Brackett[1#], Karlie E. Cox[1#], Samantha L. Barlock[1], William M. Huggins[2], David F. Ackart[3], Randall J. Bassaraba[3], Roberta J. Melander[1], and Christian Melander[1*]

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame IN 46556
[2]Department of Chemistry, North Carolina State University, Raleigh NC 27695
[3]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins CO 80523

[#]these authors contributed equally to this work

[*]to whom correspondence should be addressed: cmelande@nd.edu

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Biological assay protocols and data

Broth Microdilution Method for MIC Determination of *M. smegmatis*

Cultures (48 h) were standardized to 5 x 10^5 CFU/mL in 7H9 broth supplemented with ADC enrichment. Aliquots (1 mL) were placed in culture tubes, and compound was added from 100 mM stock samples in DMSO, such that the compound concentration equaled the highest concentration tested (200 μM). Samples were then aliquoted (200 μL) into the first row of wells of a 96-well plate, with all remaining wells being filled with 100 μL of initial bacterial subculture. Row one wells were mixed five times before 100 μL was transferred to the following row (row two). Row two was then mixed five times, and 100 μL was transferred to row three. This process provided a serial dilution of the compound and was continued until the final row had been mixed. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 48 hours. After 48 hours, the wells were stained with alamarBlue (Thermo- Fisher) and were incubated under stationary conditions at 37°C for 8 hours. After 8 hours, the wells with viable bacteria were visualized as pink and the wells with no viable bacteria were visualized as blue. MIC values were recorded as the lowest concentration at which no viable bacteria were observed.

Broth Microdilution Method for Antibiotic Resensitization of *M. smegmatis*

Cultures (48 h) were standardized to 5 x 10^5 CFU/mL in 7H9 broth supplemented with ADC enrichment. Aliquots (4 mL) were placed in culture tubes, and compound was added from 100 mM stock samples in DMSO, such that the compound concentration was 30% of the MIC of the compound against the particular bacterial strain. One milliliter of the resulting solution was aliquoted into a separate culture tube and was dosed with antibiotic so that the resulting concentration was the highest desirable concentration to be tested. Bacteria treated with antibiotic alone were used as a control. Row one of a 96-well plate was filled with 200 μL of the antibiotic/ compound solution, and the remaining rows were filled with 100 μL per well of the remaining 4 mL bacterial subculture. Row one wells were mixed five times before 100 μL was transferred to the following row (row two). Row two was then mixed five times, and 100 μL was transferred to row three. This process was repeated until the second to last row had been reached. The last row would have only compound and serve as a negative control. The antibiotic only treated bacteria was plated by aliquoting 200 μL of the treated bacteria into row one and filling the remaining rows with untreated bacteria from the original subculture. The rows were mixed in the same way as described above. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37 °C for 48 hours. After 48 hours, the wells were stained with alamarBlue (Thermo-Fisher) and were incubated under stationary conditions at 37 °C for 8 hours. After 8 hours, the wells with viable bacteria were visualized as pink and the wells with no viable bacteria were visualized as blue. MIC values were recorded as the lowest concentration at which no viable bacteria were observed, which was determined by the blue well at the lowest concentration. Fold reductions were determined by comparison of the compound treated wells with the antibiotic only control well.

General static dispersion assay protocols for *M. smegmatis*

Cultures were incubated for 48 hours and then standardized to 0.01 in Difco M9 minimal salts media supplemented with 20 mL of 20% glucose, 2 mL of 1M $MgSO_4$ and 0.1 mL

1M $CaCl_2$. 100 µL of the subculture was aliquoted into every well in columns 2-11 of a 96-well PVC microtiter plate. Columns 1 and 12 were left empty to serve as control wells. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37 °C for 48 hours. After 48 hours, the media was discarded, and the plates were washed thoroughly with water. 100 µL of fresh media containing the appropriate concentration of compound was added to all of the wells in columns 2-4 and 9-11. 100 µL of sterile media was added to all of the wells in columns 1 and 12 and columns 5-8. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37 °C for 24 hours. After 24 hours, media was discarded, and the plates were washed thoroughly with water. 110 µL of a 0.1% aqueous solution of crystal violet was added to every well, and the plates were left at ambient temperature for 30 minutes. After 30 minutes, the crystal violet was disposed, and the plates were washed thoroughly with water. 200 µL of 95% ethanol was added to each well, and the plates were left at ambient temperature for 10 minutes. 125 µL of the ethanol solution was transferred to a fresh polystyrene microtiter plate, and the plate was quantified by measuring the OD_{540}. The percent dispersion was calculated by comparing the OD_{540} of the treated wells with the OD_{540} of the untreated wells, which contained only media after biofilm growth. The first and last column, which had only sterile media, were used as blanks and those values were subtracted from the OD_{540} obtained in the other columns.

Hemolysis Assay

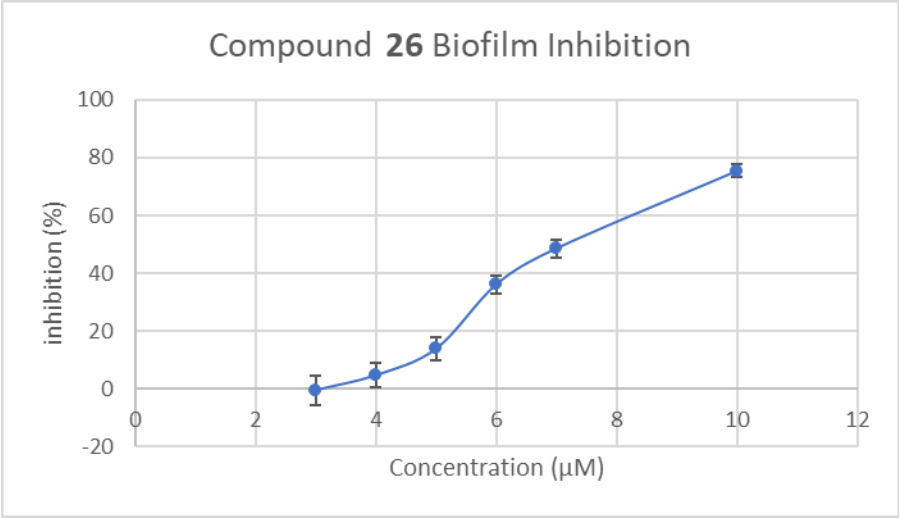
Hemolysis was performed on mechanically difibrinated sheep blood (Hemostat Laboratories: DSB100). Difibrinated blood (1.5 mL) was placed into an Eppendorf tube and centrifuged for 10 min at 10,000 rpm. The supernatant was removed and then the cells were resuspended in 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged as before, the supernatant removed, and the cells were resuspended two more times. The final cell suspension was diluted 10-fold. Compound was added from a DMSO stock solution to aliquots of the 10-fold suspension dilution of blood to give the desired concentrations to be tested. Triton X (1%) was used as a positive control (100% lysis). PBS was used as a negative control (zero hemolysis). Samples were placed in an incubator at 37 °C with shaking at 200 rpm for 1 h. After 1 h, the samples were centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was then measured with a UV spectrometer at 540 nm.

General Information

The *Mycobacterium smegmatis* strain (ATCC 700084, mc_2155) was obtained from ATCC (Manassas, VA). Stock cultures were stored in glycerol stock media (25% v/v glycerol and 7H9, ADC, Tween 80) and maintained at -80 °C. The strain was maintained and cultured in 7H9 or on 7H10 agar (OADC, glycerol) until utilized in the assays outlined above. All assays were run in duplicate and repeated at least two separate times. The biofilm assays were repeated at least three times and standard deviations were calculated for all IC_{50} and EC_{50} values. All compounds were dissolved as their HCl salts in molecular biology grade DMSO as 10 or 100 mM stock solutions and stored at -20 °C.

Dose-response curves for lead compounds 26 and 31

Compound 26


Compound 26 Biofilm Inhibition
inhibition (%)
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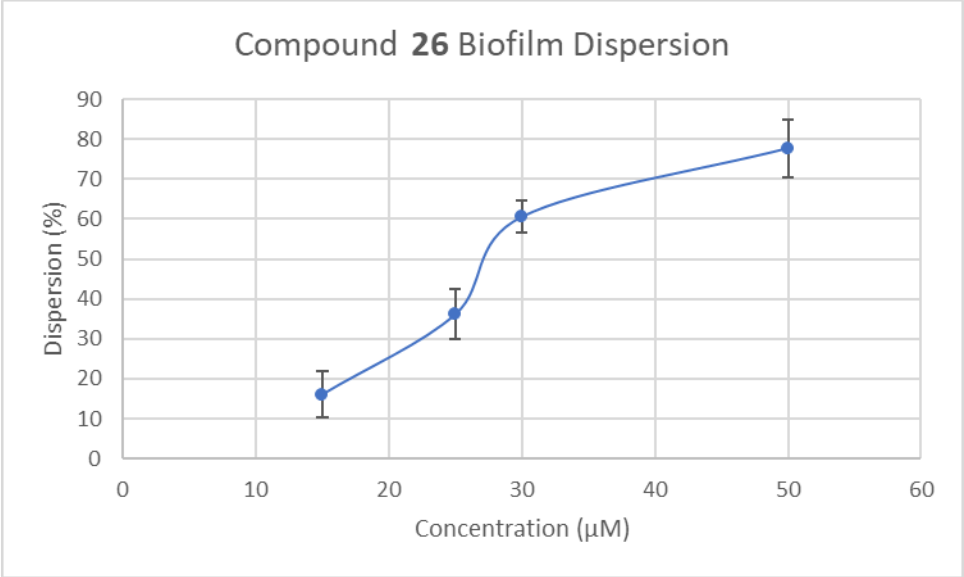
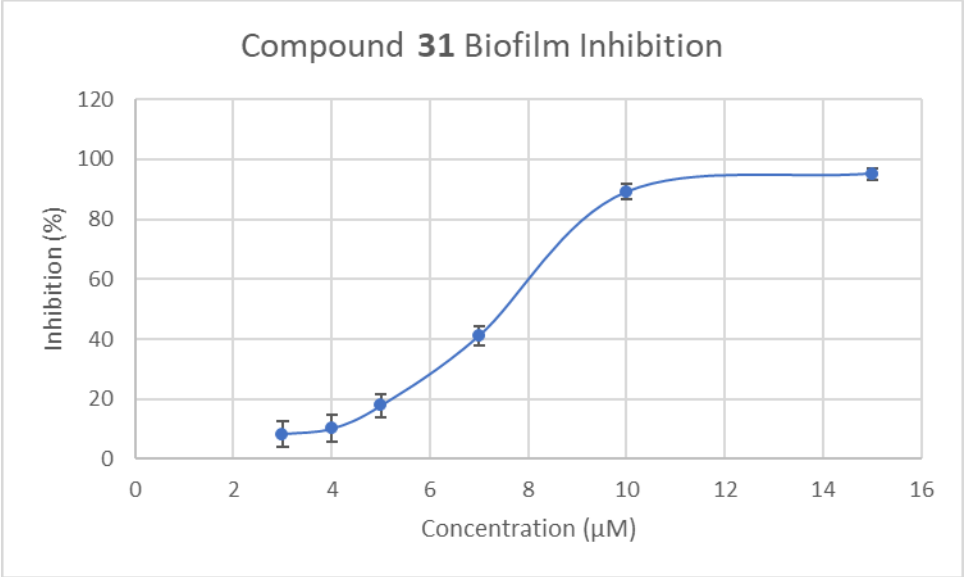
80
60
40
20
0
-20
0
2
4
6
8
10
12
Concentration (µM)


Compound 26 Biofilm Dispersion
Dispersion (%)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Concentration (µM)

Compound 31


Compound 31 Biofilm Inhibition
Inhibition (%)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
Concentration (µM)

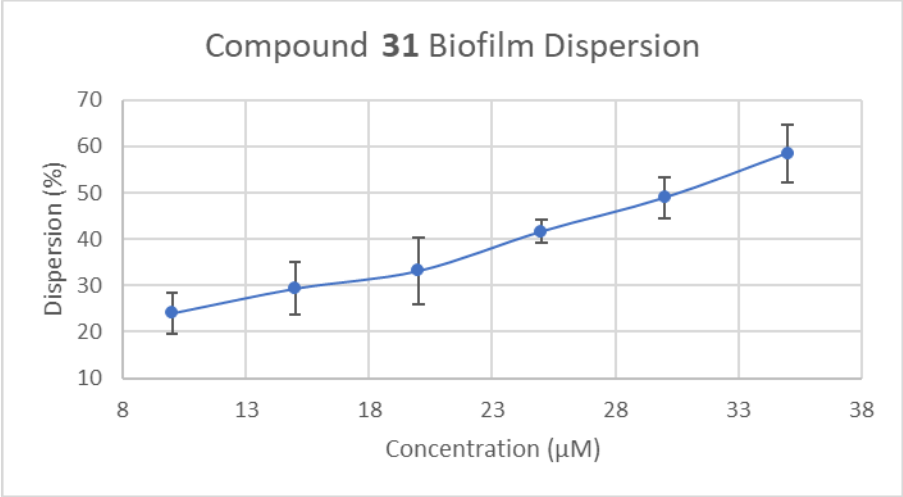

Compound 31 Biofilm Dispersion
Dispersion (%)
70
60
50
40
30
20
10
8
13
18
23
28
33
38
Concentration (µM)

Table S1: Potentiation activity with Meridianin D analogues against *M. smegmatis*. (fold reductions are in parenthesis)

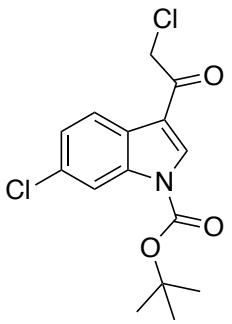
Compound	Concentration tested (µM)	Cefotaxime MIC (µg/mL)	Oxacillin MIC (µg/mL)	Cefoxitin MIC (µg/mL)	Ceftazidime MIC (µg/mL)	Ampicillin MIC (µg/mL)
		128	512	16	512	128
1	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
2	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
3	60	64 (2)	256 (2)	16 (0)	512 (0)	128 (0)
4	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
5	60	64 (2)	512 (0)	8 (2)	512 (0)	128 (0)
6	60	32 (4)	256 (2)	16 (0)	128 (4)	64 (2)
7	60	64 (2)	256 (2)	16 (0)	128 (4)	64 (2)
8	60	64 (2)	512 (0)	16 (0)	512 (0)	128 (0)
9	60	32 (4)	256 (2)	16 (0)	256 (2)	64 (2)
10	60	128 (0)	512 (0)	16 (0)	256 (2)	64 (2)
11	60	32 (4)	256 (2)	16 (0)	512 (0)	64 (2)
12	60	64 (2)	256 (2)	16 (0)	512 (0)	64 (2)
13	60	64 (2)	256 (2)	16 (0)	512 (0)	128 (0)
14	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
15	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
16	60	128 (0)	512 (0)	16 (0)	512 (0)	64 (2)
17	60	64 (2)	256 (2)	16 (0)	512 (0)	128 (0)
18	60	128 (0)	512 (0)	16 (0)	512 (0)	64 (2)
19	60	16 (8)	256 (2)	8 (2)	64 (8)	32 (4)
20	60	64 (2)	512 (0)	16 (0)	512 (0)	128 (0)
21	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
22	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
23	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
24	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
25	30	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
26	30	128 (0)	512 (0)	16 (0)	128 (4)	128 (0)
27	60	64 (2)	256 (2)	16 (0)	512 (0)	64 (2)
28	60	128 (0)	512 (0)	16 (0)	512 (0)	64 (2)
29	60	128 (0)	512 (0)	16 (0)	512 (0)	128 (0)
30	60	128 (0)	512 (0)	16 (0)	512 (0)	64 (2)
31	60	64 (2)	256 (2)	16 (0)	512 (0)	64 (2)

Chemistry experimental and characterization

General remarks. All reagents used for chemical synthesis were purchased from commercially available sources without further purification. Flash chromatography was performed using 60 Å mesh standard grade silica gel from Sorbetch. NMR solvents were obtained from Cambridge Isotope Labs and used as is. All ^{1}H NMR (400 MHz) were recorded at 25°C on a Bruker Avance spectrometer. All ^{13}C NMR (101 MHz) spectra were also recorded at 25°C on a Bruker Avance spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to the respective NMR solvent; 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 in the Mass Spectrometry and Proteomics Facility at the University of Notre Dame. Infrared spectra were obtained on a FT/IR-4100 spectrophotometer (νmax in cm^{-1}). UV absorbance was recorded on a Genesys 10 scanning UV/visible spectrophotometer (λmax in nm).

Compounds 1-31

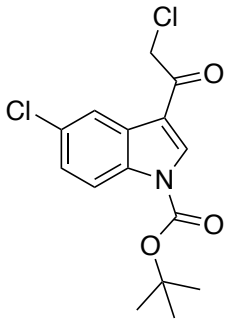
These compounds were previously synthesized and characterized by Huggins et al.[1]



tert-Butyl 6-chloro-3-(2-chloroacetyl)-1*H*-indole-1-carboxylate (33a)

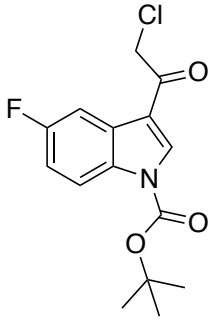
The title compound was synthesized by dissolving 6-chloroindole **(32a)** (33 mmol) in toluene (120 mL) and heating to 60°C. Pyridine (1 eq) was added and then chloroacetyl chloride (1 eq) was added dropwise. The reaction was left stirring under inert atmosphere at 60°C for 2 hours. After 2 hours, the reaction was removed from the heat and 300 mL of water and 50 mL of methanol was added to the reaction. The reaction was stirred at room temperature for 1 hour. After an hour, the reaction was filtered and precipitate was washed with water and then recrystallized with ethanol. The precipitate was left on vacuum overnight. The intermediate (1.5 mmol) was then dissolved in THF (10 mL) with di-*tert*-butyl dicarbonate (1.5 eq) and *N,N*-dimethyl pyridine-4-amine (0.1 eq) and was left to stir for 4 hours at room temperature under inert atmosphere. After 4 hours, the reaction was concentrated under reduced pressure. The resulting product was dissolved in dichloromethane and extracted once with water (20 mL) and once with brine (20 mL). The product was submitted to flash column chromatography 10-30% ethyl acetate: hexanes resulting a white solid in a 23% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 2.2 Hz, 1H), 8.33 (s, 1H), 8.06 (d, J = 8.9 Hz, 1H), 7.37 (dd, J = 8.9, 2.2 Hz, 1H), 4.55 (s, 2H),

1.72 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.88, 148.50, 133.77, 133.20, 130.73, 128.46, 126.29, 122.30, 116.62, 116.11, 86.40, 45.79, 28.08. IR ν_{max} (cm^{-1}): 3154, 3136, 1757, 1739, 1679, 1663, 680. λ_{max}= 292 nm. HRMS (ESI) calcd for $C_{15}H_{16}Cl_2NO_3$ $[M+H]^+$: 328.0502, found 328.0500.



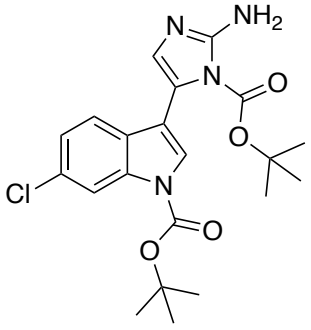
***tert*-Butyl 5-chloro-3-(2-chloroacetyl)-1*H*-indole-1-carboxylate (33b)**

The title compound was synthesized by dissolving 5-chloroindole **32b** (33 mmol) in toluene (120 mL) and heating to 60°C. Pyridine (1 eq) was added and then chloroacetyl chloride (1 eq) was added dropwise. The reaction was left stirring under inert atmosphere at 60°C for 2 hours. After 2 hours, the reaction was removed from the heat and 300 mL of water and 50 mL of methanol was added to the reaction. The reaction was stirred at room temperature for 1 hour. After an hour, the reaction was filtered and precipitate was washed with water and then recrystallized with ethanol. The precipitate was left on vacuum overnight. The intermediate (4.7 mmol) was then dissolved in THF (15 mL) with di-*tert*-butyl dicarbonate (1.5 eq) and *N,N*-dimethyl pyridine-4-amine (0.1 eq) and was left to stir for 4 hours at room temperature under inert atmosphere. After 4 hours, the reaction was concentrated under reduced pressure. The resulting product was dissolved in dichloromethane and extracted once with water (20 mL) and once with brine (20 mL). The product was submitted to flash column chromatography 10-30% ethyl acetate: hexanes resulting a white solid in a 31% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 2.2 Hz, 1H), 8.33 (s, 1H), 8.06 (d, J = 8.9 Hz, 1H), 7.37 (dd, J = 8.9, 2.2 Hz, 1H), 4.55 (s, 2H), 1.72 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.88, 148.50, 133.77, 133.20, 130.73, 128.46, 126.29, 122.30, 116.62, 116.11, 86.40, 45.79, 28.08. IR ν_{max} (cm^{-1}): 3155, 3127, 1758, 1740, 1680, 1664, 680. λ_{max}= 306 nm. HRMS (ESI) calcd for $C_{15}H_{16}Cl_2NO_3$ $[M+H]^+$: 328.0502, found 328.0505.



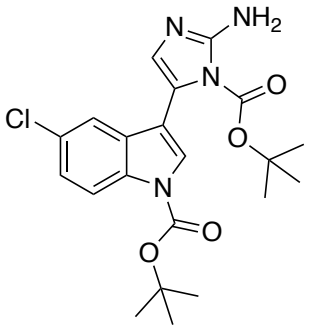
***tert*-Butyl 3- (2-chloroacetyl)-5-fluoro-1*H*-indole-1-carboxylate (33c)**

The title compound was synthesized by dissolving 5-fluoroindole **32c** (37 mmol) in toluene (120 mL) and heating to 60°C. Pyridine (1 eq) was added and then chloroacetyl chloride (1 eq) was added dropwise. The reaction was left stirring under inert atmosphere at 60°C for 2 hours. After 2 hours, the reaction was removed from the heat and 300 mL of water and 50 mL of methanol was added to the reaction. The reaction was stirred at room temperature for 1 hour. After an hour, the reaction was filtered and precipitate was washed with water and then recrystallized with ethanol. The precipitate was left on vacuum overnight. The intermediate (8.6 mmol) was then dissolved in THF (10 mL) with di-*tert*-butyl dicarbonate (1.5 eq) and *N,N*-dimethyl pyridine-4-amine (0.1 eq) and was left to stir for 4 hours at room temperature under inert atmosphere. After 4 hours, the reaction was concentrated under reduced pressure. The resulting product was dissolved in dichloromethane and extracted once with water (20 mL) and once with brine (20 mL). The product was submitted to flash column chromatography 10-30% ethyl acetate: hexanes resulting a white solid in a 20% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.11 – 8.07 (m, 1H), 8.04 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.14 (td, *J* = 9.0, 2.7 Hz, 1H), 4.56 (s, 2H), 1.72 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.90, 161.63, 148.60, 133.43, 131.77, 116.22, 116.13, 114.10, 113.85, 108.56, 108.31, 86.24, 45.78, 28.08. IR ν_{max} (cm^{-1}): 3154, 3127, 1737, 1682, 1659, 685. λ_{max}= 306 nm. HRMS (ESI) calcd for $C_{15}H_{16}ClFNO_3$ $[M+H]^+$: 312.0797, found 312.0788.



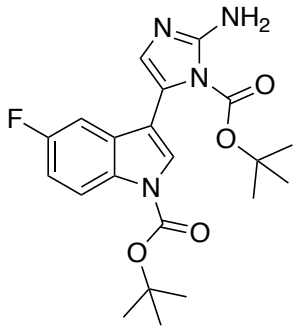
***tert*-Butyl 3- (2-amino-1-(tert-butoxycarbonyl)-1*H*-imidazol-5-yl)-6-chloro-1*H*-indole-1-carboxylate (34a)**

Intermediate **33a** (2.25 mmol), sodium iodide (4 eq), *N*-Boc guanidine (6 eq) was dissolved in dimethylformamide (DMF) (25 mL) and allowed to stir under an inert atmosphere for 48 hours at room temperature. After 48 hours, the reaction was washed with water (75 mL) and the aqueous layer was extracted with ethyl acetate (3x25 mL). The organic extractions were combined and washed with brine (30 mL). The organic layer was dried with magnesium sulfate and concentrated under reduced pressure. The resulting product was submitted to flash column chromatography 10-30% ethyl acetate: hexanes resulting a yellow solid at 7% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.90 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.29 (d, *J* = 2.0 Hz, 1H), 7.12 (s, 1H), 5.69 (s, 2H), 1.66 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.10, 149.26, 131.36, 130.57, 126.34, 123.67, 123.42, 120.87, 115.78, 114.36, 106.89, 85.45, 84.25, 28.18, 28.12, 28.05. IR ν_{max} (cm^{-1}): 3426, 1725, 1651, 1633, 663. λ_{max}= 304 nm. HRMS (ESI) calcd for $C_{21}H_{26}ClN_4O_4$ $[M+H]^+$: 433.1637, found 433.1634.



***tert*-Butyl 3- (2-amino-1-(tert-butoxycarbonyl)-1*H*-imidazol-5-yl)-5-chloro-1*H*-indole-1-carboxylate (34b)**

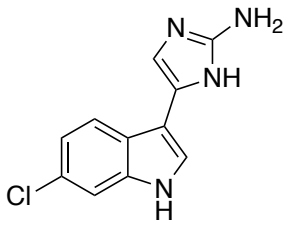
Intermediate **33b** (1.48 mmol), sodium iodide (4 eq), *N*-Boc guanidine (6 eq) was dissolved in DMF (25 mL) and allowed to stir under an inert atmosphere for 48 hours at room temperature. After 48 hours, the reaction was washed with water (75 mL) and the aqueous layer was extracted with ethyl acetate (3x25 mL). The organic extractions were combined and washed with brine (30 mL). The organic layer was dried with magnesium sulfate and concentrated under reduced pressure. The resulting product was submitted to flash column chromatography 10-30% ethyl acetate: hexanes resulting a yellow solid at 15% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.90 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.12 (s, 1H), 5.69 (s, 2H), 1.66 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.10, 149.26, 131.36, 130.57, 126.34, 123.67, 123.42, 120.87, 115.78, 114.36, 106.89, 85.45, 84.25, 28.18, 28.05. IR ν_{max} (cm^{-1}): 3425, 1724, 1650, 1632, 667. λ_{max}= 288 nm. HRMS (ESI) calcd for $C_{21}H_{26}ClN_4O_4$ $[M+H]^+$: 433.1637, found 433.1622.



***tert*-Butyl 3- (2-amino-1-(tert-butoxycarbonyl)-1*H*-imidazol-5-yl)-5-fluoro-1*H*-indole-1-carboxylate (34c)**

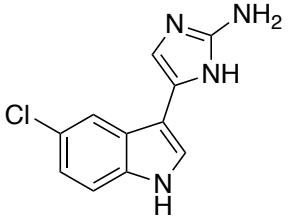
Intermediate **33c** (1.72 mmol), sodium iodide (4 eq), *N*-Boc guanidine (6 eq) was dissolved in DMF (25 mL) and allowed to stir under an inert atmosphere for 48 hours at room temperature. After 48 hours, the reaction was washed with water (75 mL) and the aqueous layer was extracted with ethyl acetate (3x25 mL). The organic extractions were combined and washed with brine (30 mL). The organic layer was dried with magnesium sulfate and concentrated under reduced pressure. The resulting product was submitted to flash column chromatography 10-30% ethyl acetate: hexanes resulting a yellow solid at 24% yield. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.96 (d, *J* = 5.2 Hz, 1H), 7.50 – 7.42 (m, 1H), 7.27 (d, *J* = 5.8 Hz, 1H), 7.13 – 7.07 (m, 1H), 5.72 (s, 2H), 1.66 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.25, 150.10, 149.37, 131.48, 124.66, 116.46, 116.46, 112.35, 112.11, 106.70, 106.70, 85.47, 83.97, 28.23, 28.07. IR ν_{max} (cm^{-1}): 3425, 1726, 1650, 1635, 678. λ_{max}= 288 nm. HRMS (ESI) calcd for $C_{21}H_{26}FN_4O_4$ $[M+H]^+$: 417.1933, found 417.1927.



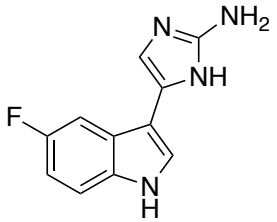
5-(6-Chloro-1*H*-indol-3-yl)-1*H*-imidazol-2-amine (35)

Intermediate **34a** (0.16 mmol) was dissolved in dichloromethane (DCM) (2 mL) and placed on ice, stirring, under an inert atmosphere of argon. TFA (0.5 mL) was added dropwise and the reaction was allowed to warm to room temperature over 16 hours. After 16 hours, the reaction was concentrated under reduced pressure and the resulting product was washed twice with cold DCM. The DCM was removed and the product was dissolved in methanol and hydrochloric acid (HCl) was added to create the HCl salt. ^1H NMR (400 MHz, [D_6] CD_3OD): δ 7.67 (d, 1H, *J*=8 Hz.), 7.59 (s, 1H), 7.46 (d, 1H, *J*=2 Hz.), 7.14 (dd, 1H, *J*= 8 Hz.), 7.02 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 148.5, 138.6, 129.5, 124.8, 124.4, 123.9, 121.9, 121.1, 112.7, 108.1, 105.0. IR ν_{max} (cm^{-1}): 3152, 1664, 787. λ_{max}= 292 nm. HRMS (ESI) calcd for $C_{11}H_{10}ClN_4$ $[M+H]^+$: 233.0589, found 233.0599.



5-(5-Chloro-1*H*-indol-3-yl)-1*H*-imidazol-2-amine (36)

Intermediate **34b** (0.22 mmol) was dissolved in DCM (3.2 mL) and placed on ice, stirring, under an inert atmosphere of argon. TFA (0.8 mL) was added dropwise and the reaction was allowed to warm to room temperature over 16 hours. After 16 hours, the reaction was concentrated under reduced pressure and the resulting product was washed twice with cold DCM. The DCM was removed and the product was dissolved in methanol and HCl was added to create the HCl salt. ^1H NMR (400 MHz, [D_6] CD_3OD): δ 7.69 (dd, 1H, *J*=4 Hz.), 7.60 (s, 1H), 7.42 (dd, 1H, *J*=8 Hz.), 7.19 (dd, 1H, *J*= 8 Hz.), 7.02 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 148.5, 136.6, 127.2, 126.8, 125.5, 123.9, 123.8, 119.4, 114.2, 108.1, 104.5. IR ν_{max} (cm^{-1}): 3144, 3052, 1673, 694. λ_{max}= 298 nm. HRMS (ESI) calcd for $C_{11}H_{10}ClN_4$ $[M+H]^+$: 233.0589, found 233.0588.



5-(5-Fluoro-1*H*-indol-3-yl)-1*H*-imidazol-2-amine (37)

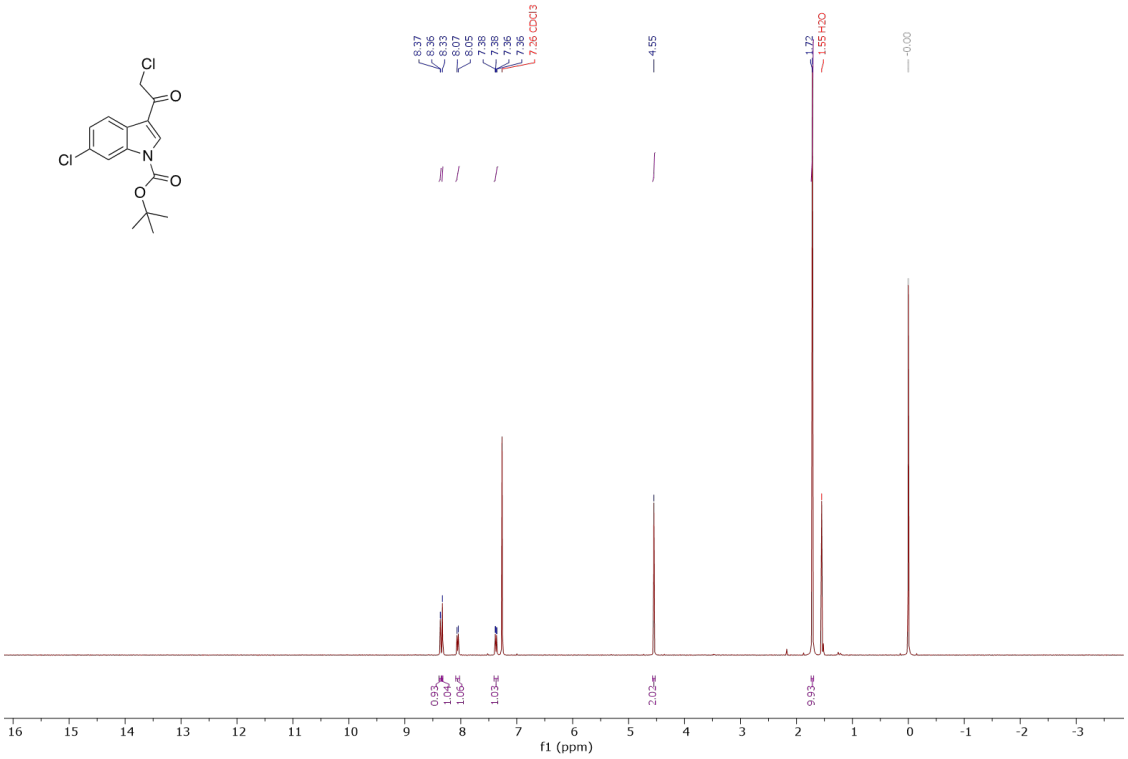
Intermediate **34c** (0.42 mmol) was dissolved in DCM (6.4 mL) and placed on ice, stirring, under an inert atmosphere of argon. TFA (1.6 mL) was added dropwise and the reaction was allowed to warm to room temperature over 16 hours. After 16 hours, the reaction was concentrated under reduced pressure and the resulting product was washed twice with cold DCM. The DCM was removed and the product was dissolved in methanol and HCl was added to create the HCl salt. ^{1}H NMR (400 MHz, [D_6] CD_3OD): δ 7.62 (s, 1H), 7.41 (m, 2H), 7.00 (s, 1H), 6.99 (td, 1H, *J*=12 Hz.). ^{13}C NMR (100 MHz, CD_3OD) δ 161.1 (*J*=230 Hz.), 158.8 (*J*=230 Hz.), 148.7, 135.0, 126.3 (*J*=10 Hz.), 126.2 (*J*=10 Hz.), 125.9, 124.3, 114.2 (*J*=10 Hz.), 114.1 (*J*=10 Hz.), 112.2 (*J*=26 Hz.), 111.9 (*J*=26 Hz.), 108.0, 105.2 (*J*=8 Hz.), 105.1 (*J*=8 Hz.), 104.9. IR ν_{max} (cm^{-1}): 3143, 2978, 1670, 701. λ_{max}= 296 nm. HRMS (ESI) calcd for $C_{11}H_{10}FN_4$ $[M+H]^+$: 217.0884, found 217.0888.

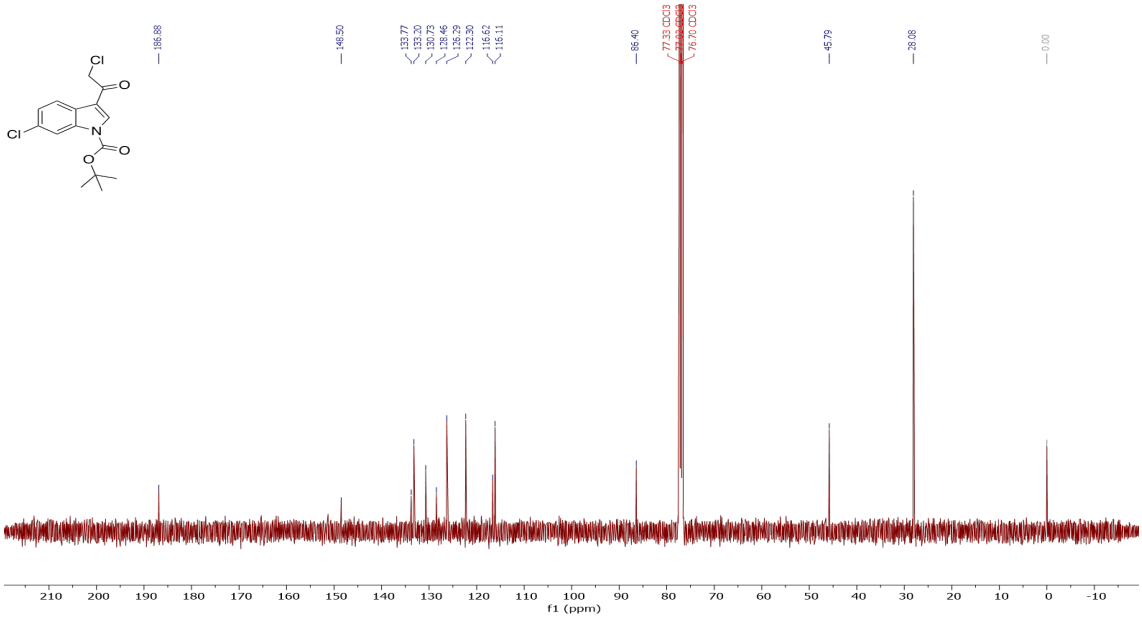
Reference

1. Huggins, W. M.; Barker, W. T.; Baker, J. T.; Hahn, N. A.; Melander, R. J.; Melander, C., Meridianin D Analogues Display Antibiofilm Activity against MRSA and Increase Colistin Efficacy in Gram-Negative Bacteria. *ACS Med Chem Lett* **2018,** *9* (7), 702-707.

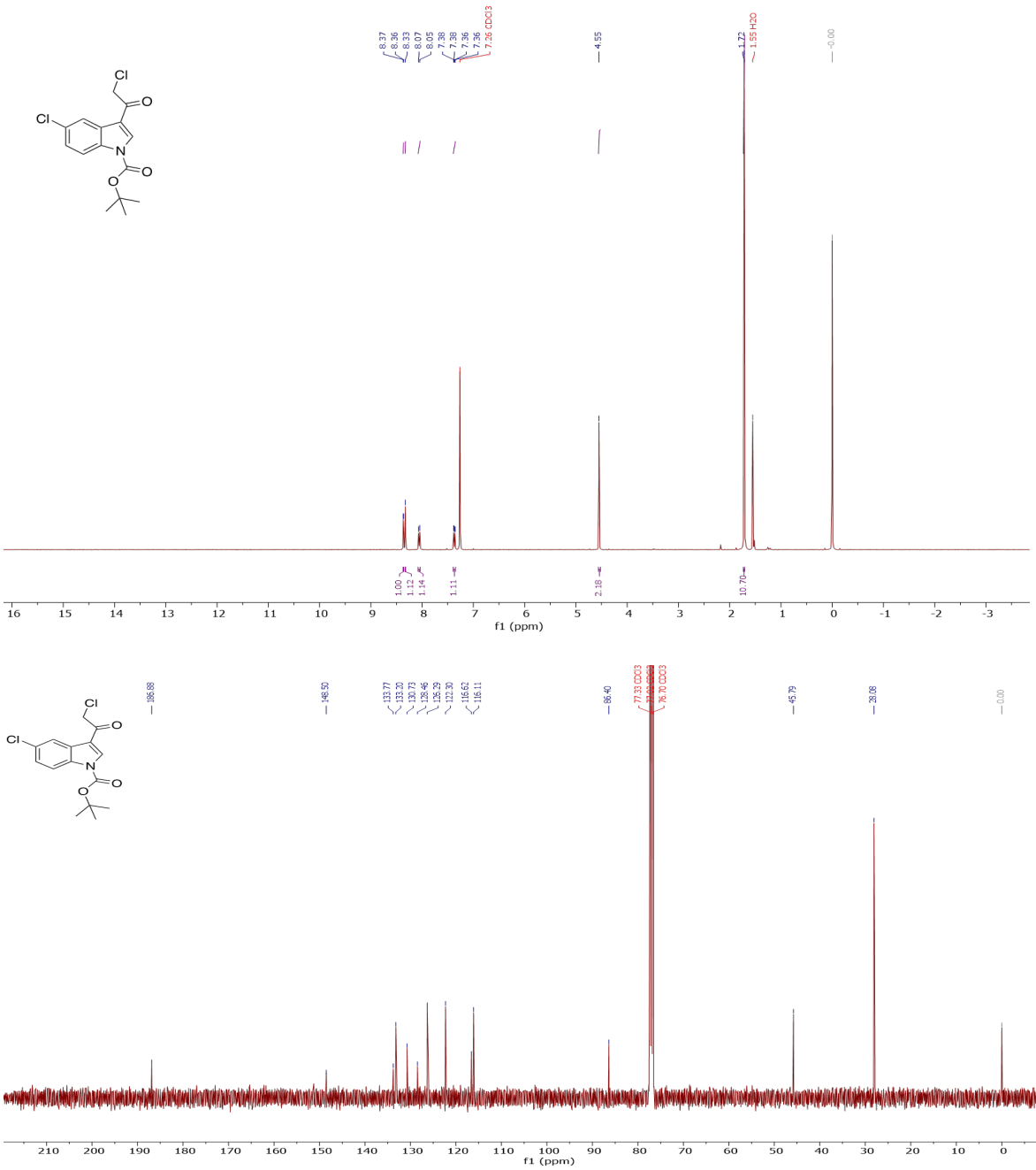
^{1}H and ^{13}C Nuclear Magnetic Resonance Spectra

Compound 33a

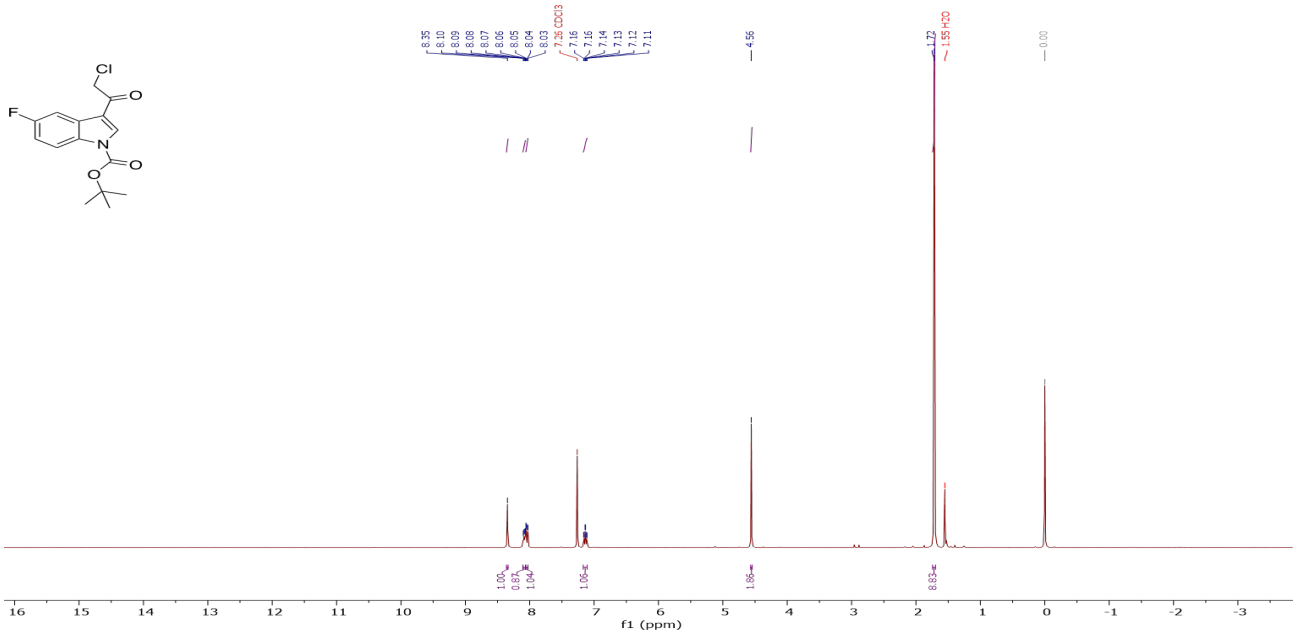


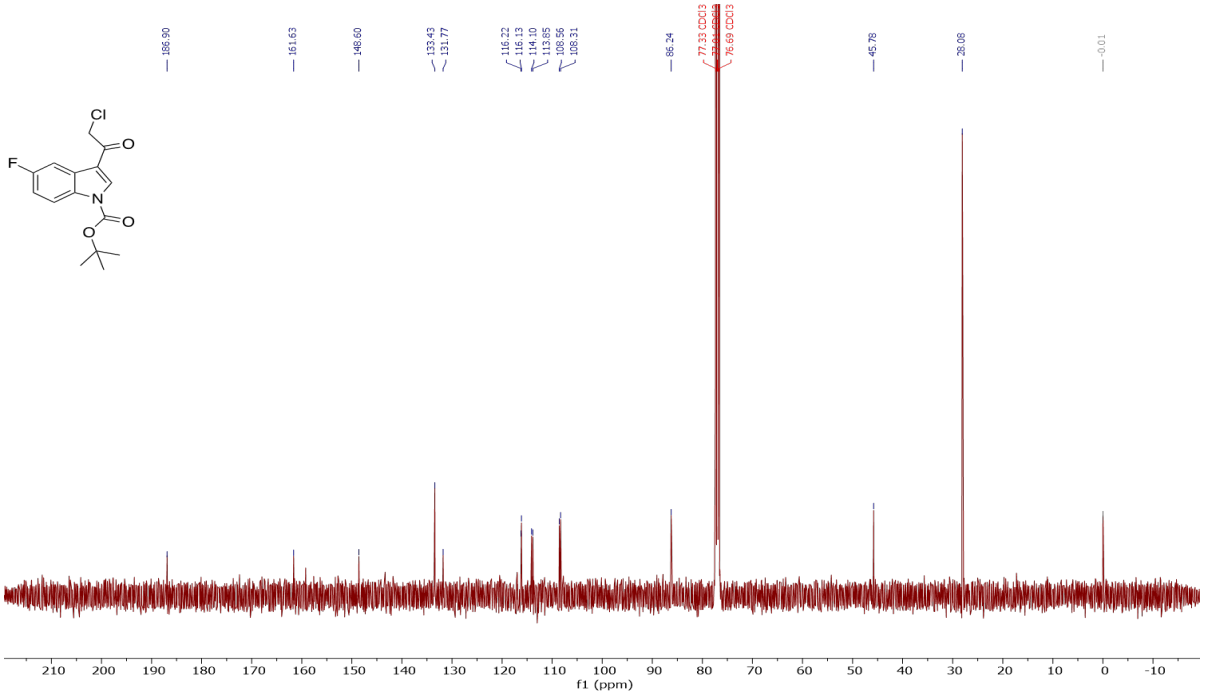


Compound 33b

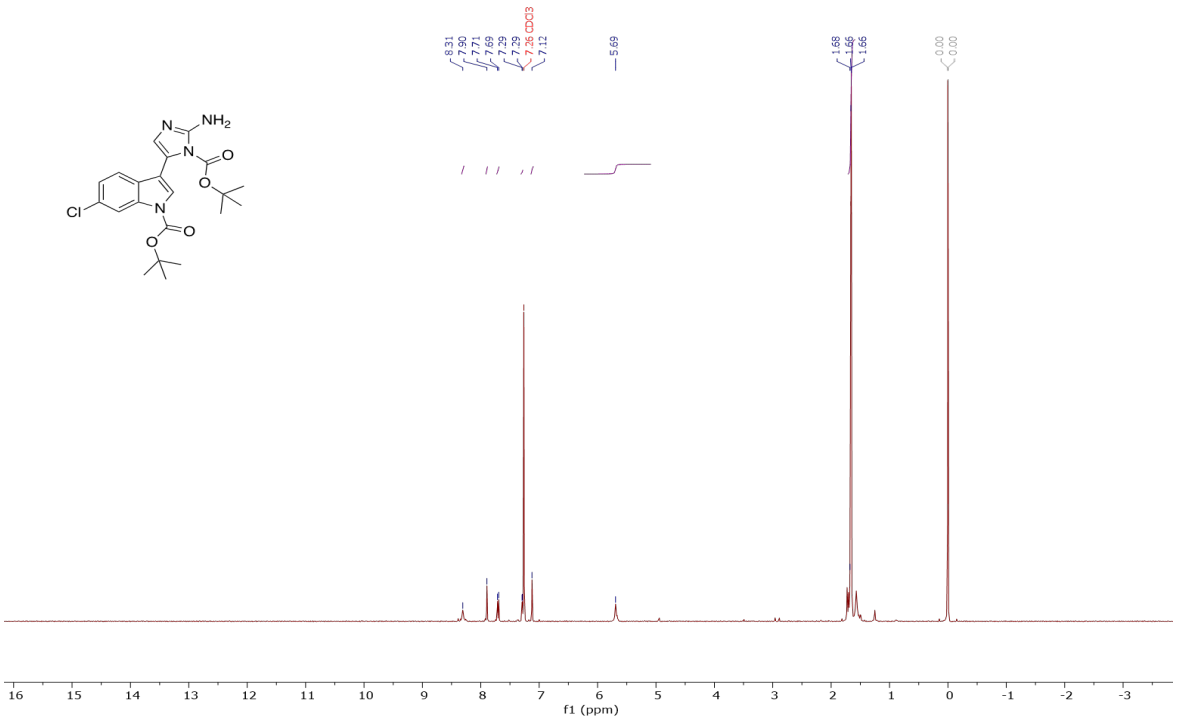


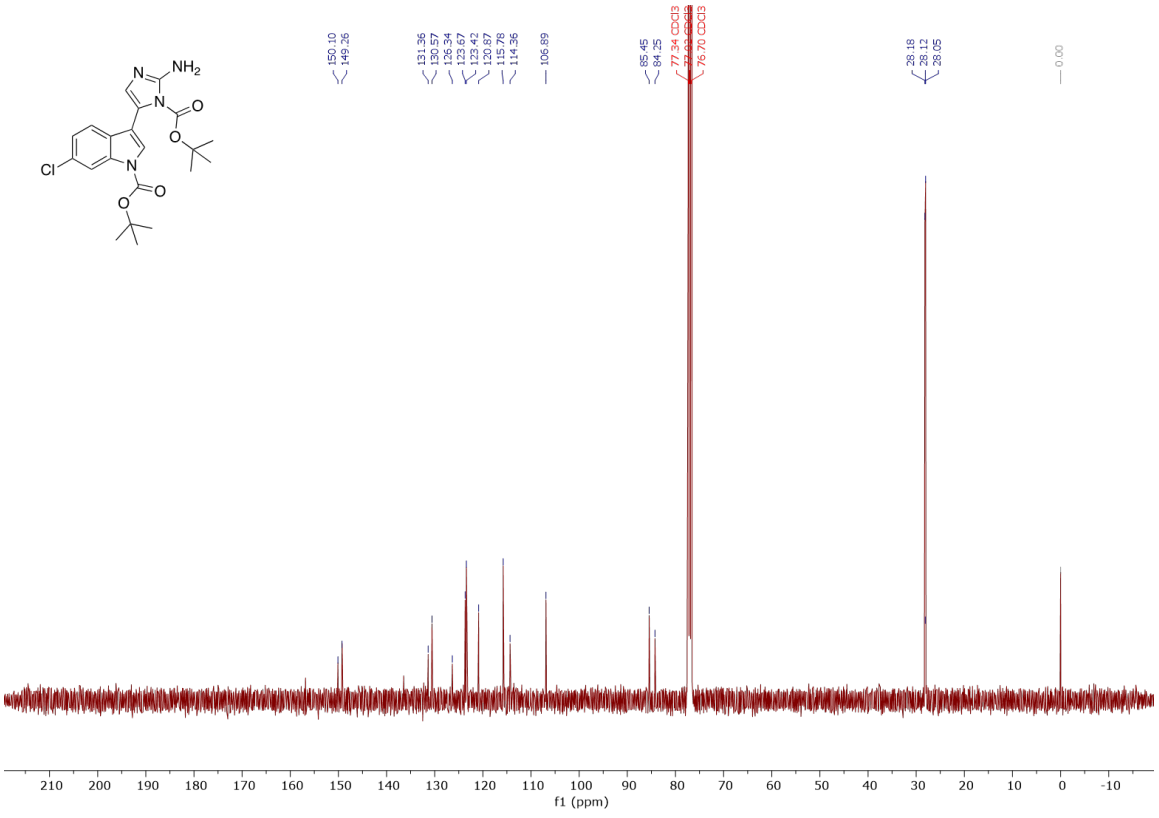
Compound 33c



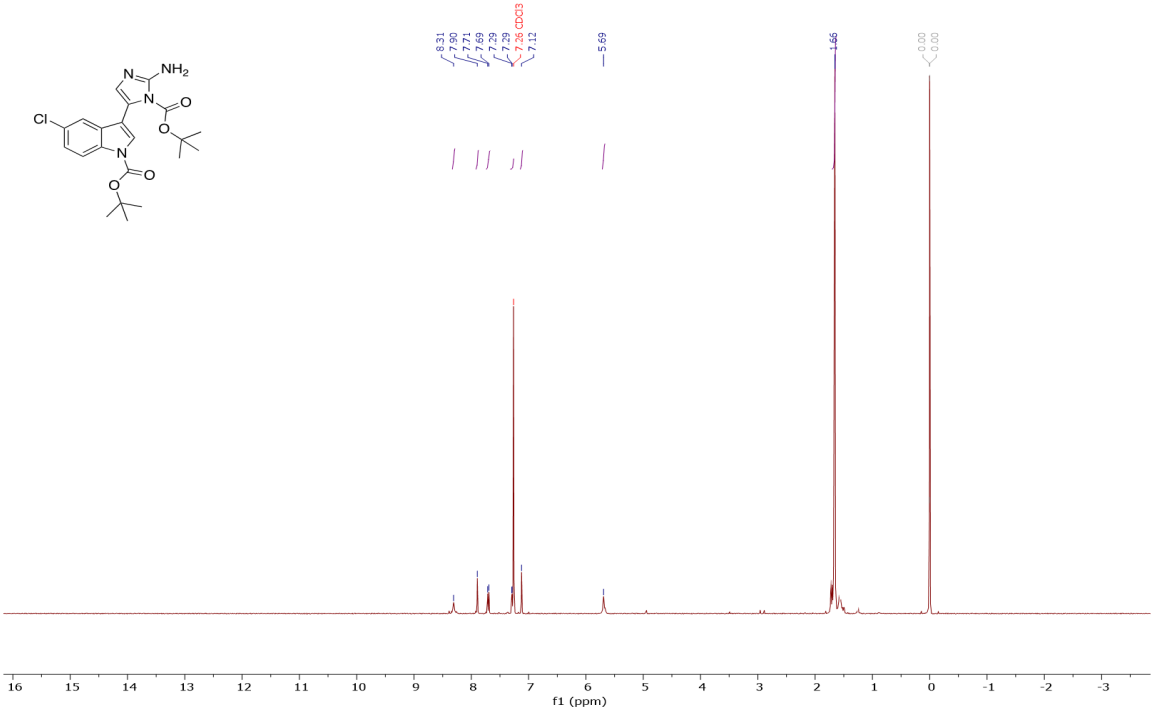


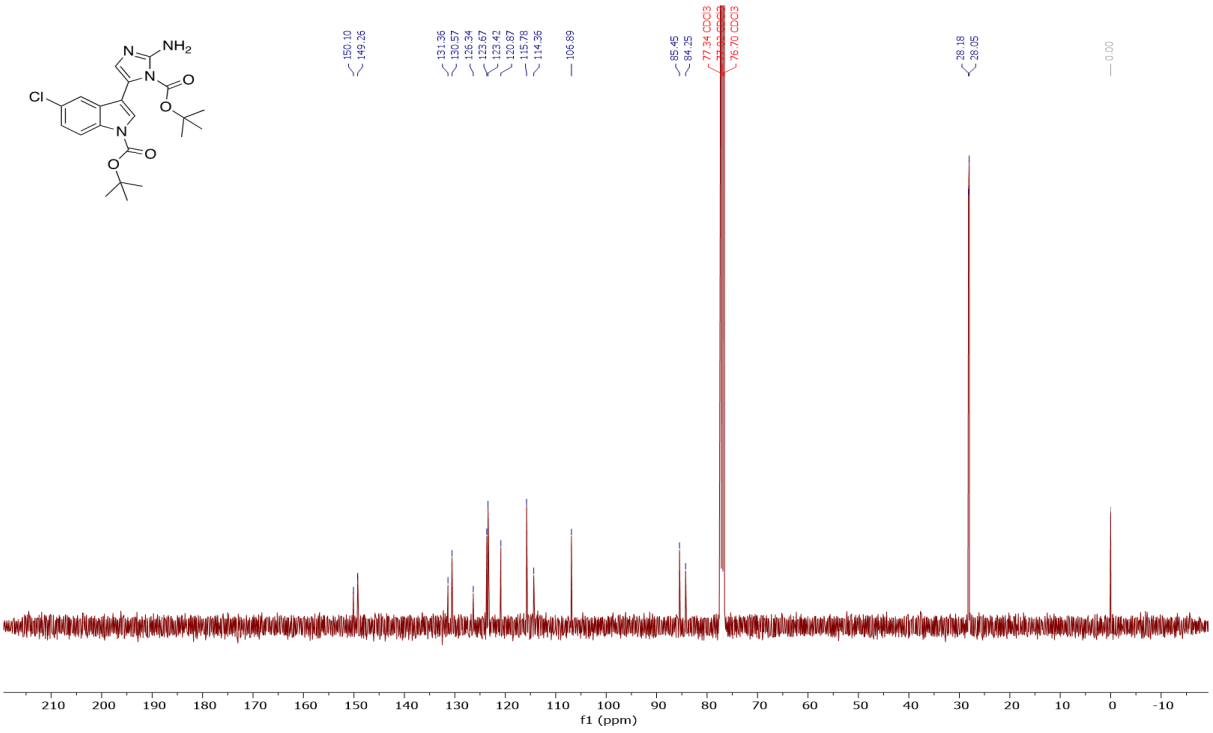
Compound 34a



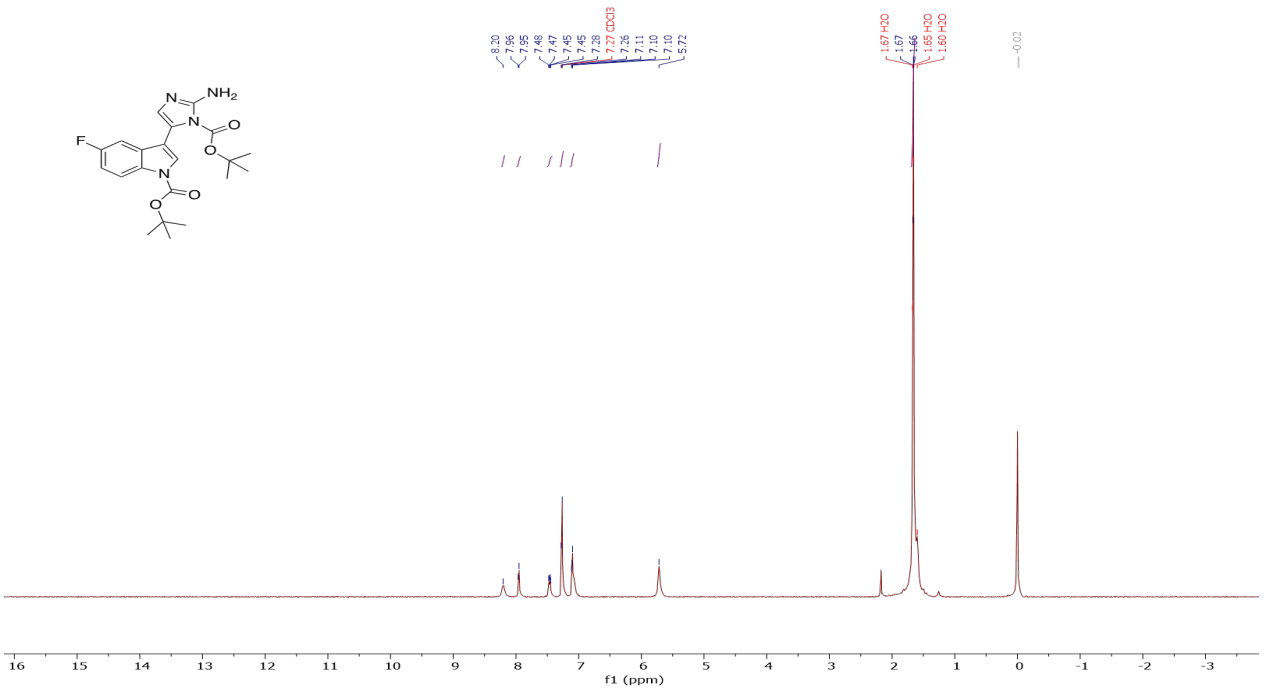


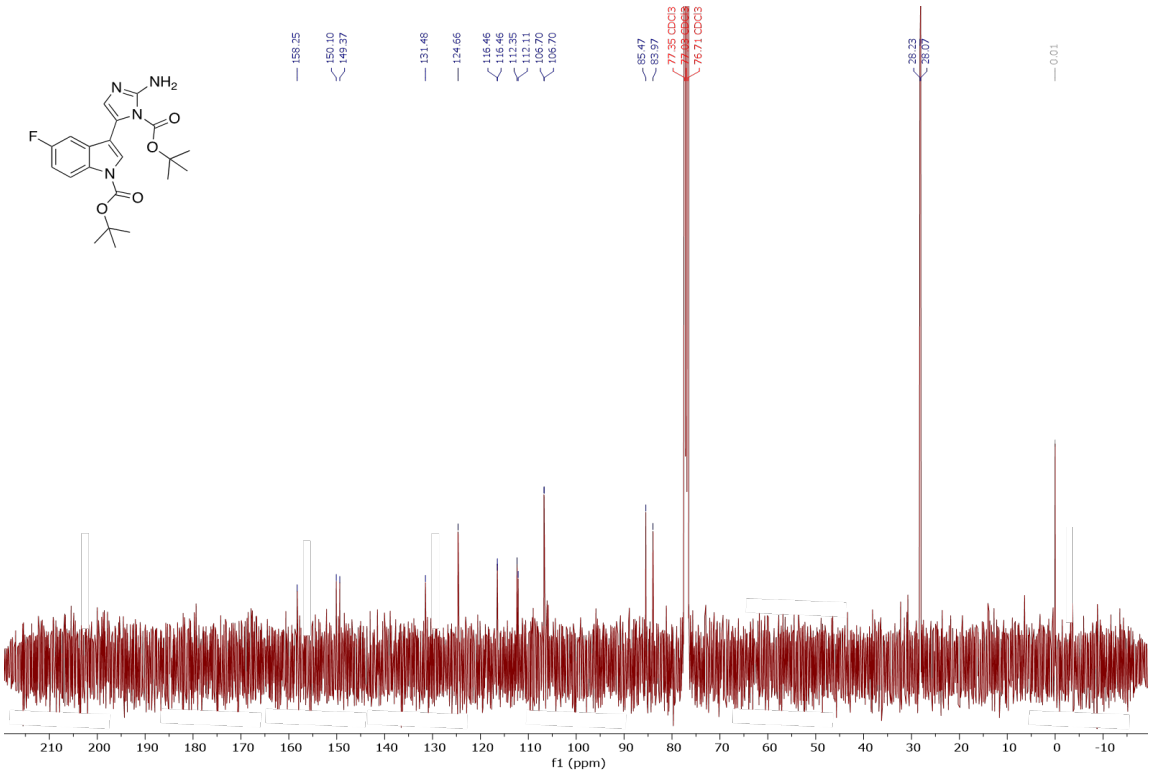
Compound 34b



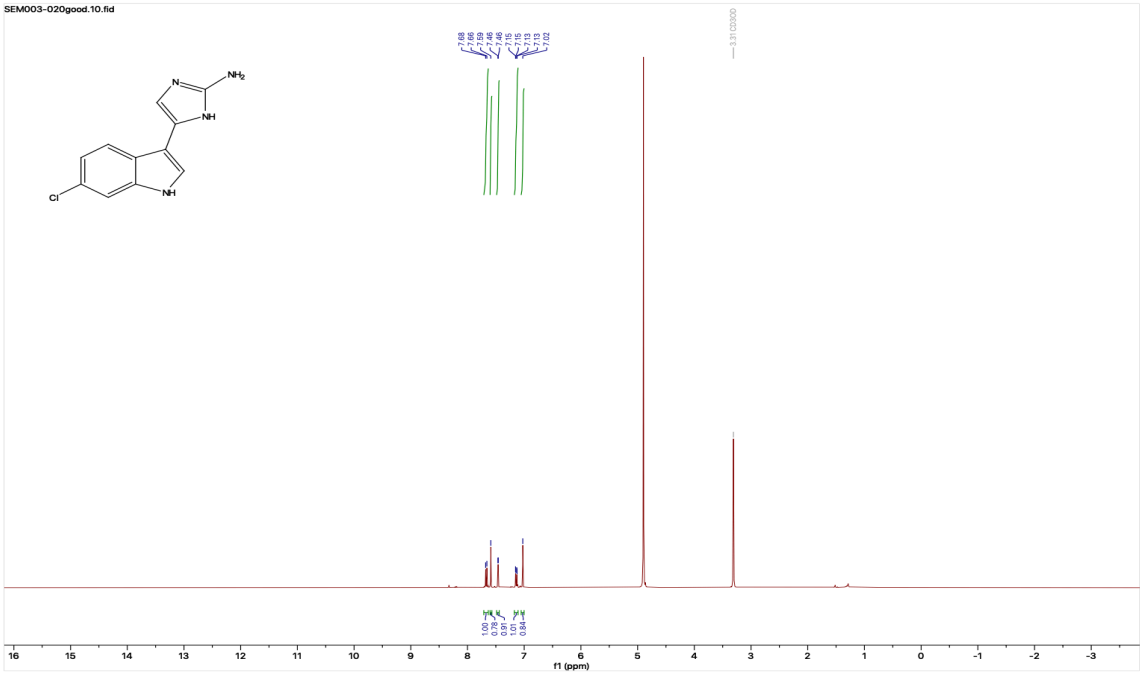


Compound 34c





Compound 35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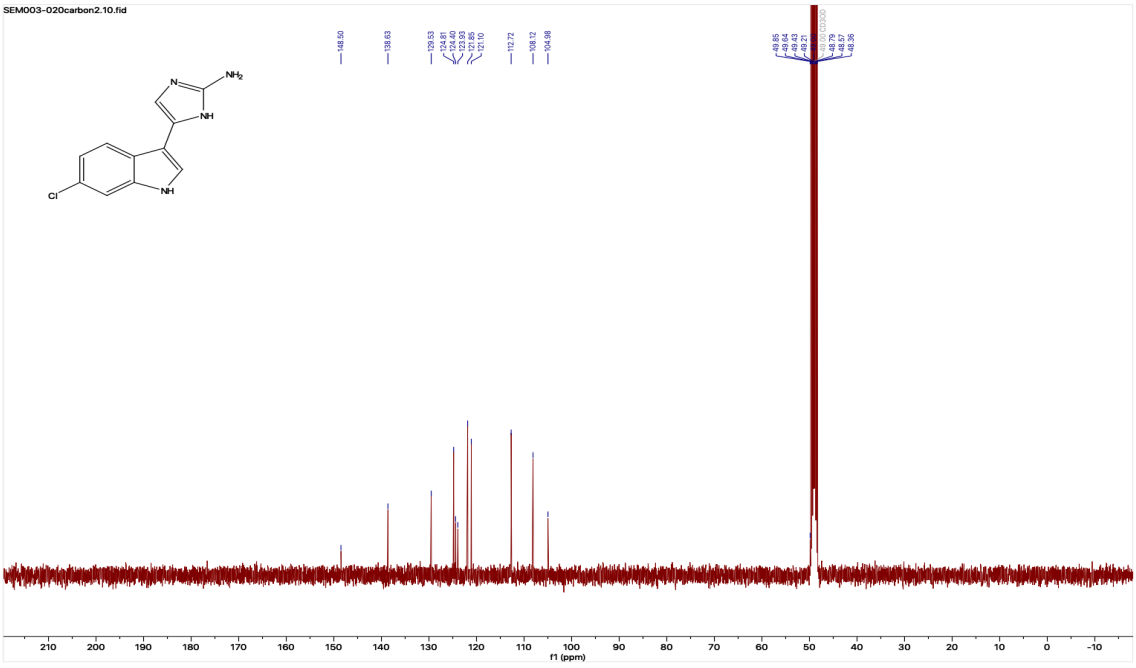
f1 (ppm)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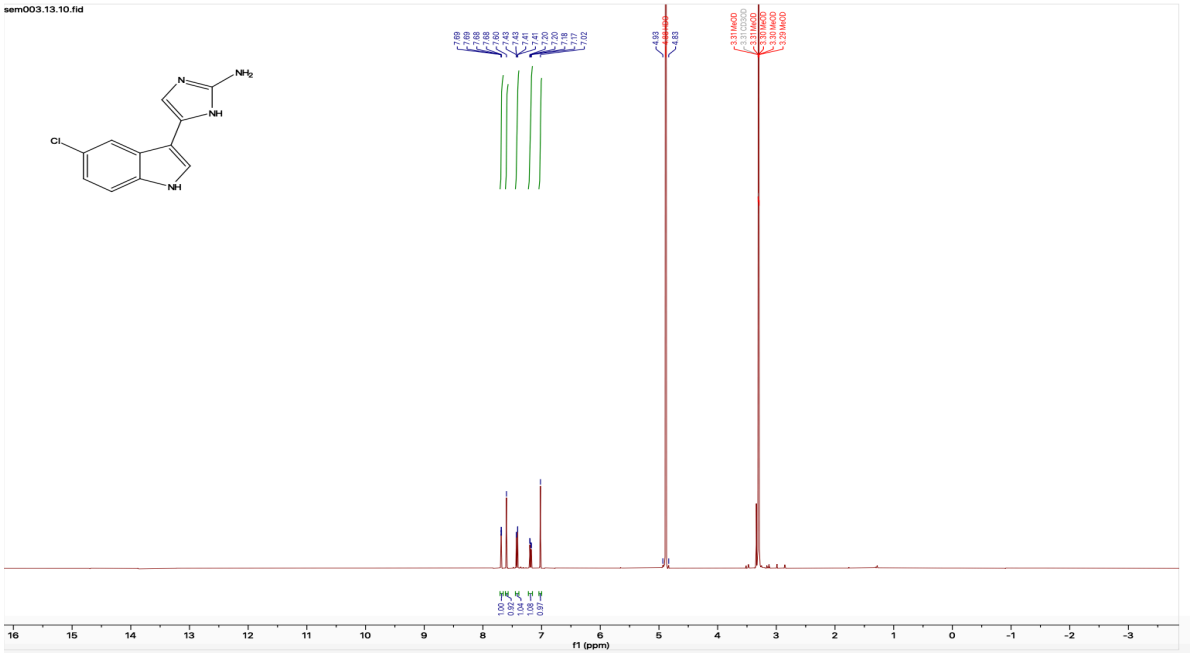
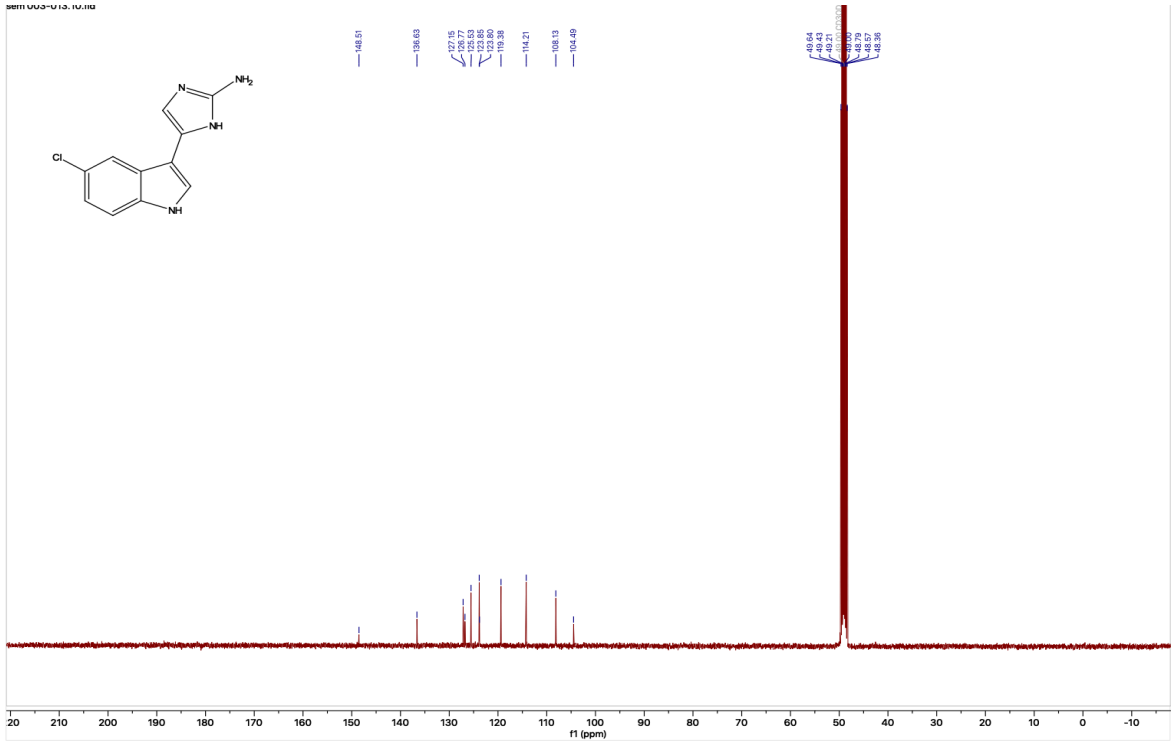
48.36
f1 (ppm)

Compound 36





Compound 37