Supporting information for:

Structure-activity studies with bis-amidines that potentiate Gram-positive specific antibiotics against Gram-negative pathogens

Charlotte M.J. Wesseling,[a] Cornelis J. Slingerland,[a] Shanice Veraar,[a] Samantha Lok,[a] and Nathaniel I. Martin[a,*]

[a]*Biological Chemistry Group, Institute of Biology Leiden, Leiden University, 2333 BE Leiden, The Netherlands.*

*n.i.martin@biology.leidenuniv.nl

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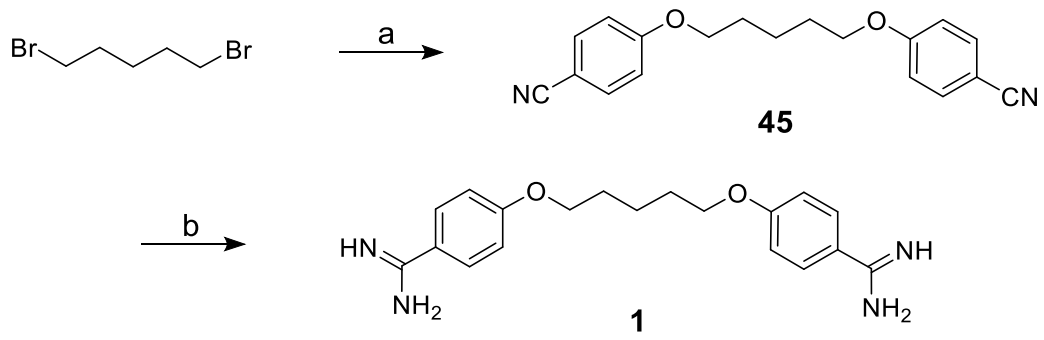
Abbreviations

ACS	American Chemical Society
CBr_4	Carbon tetrabromide
$CDCl_3$	Deuterated chloroform
EtOAc	Ethyl acetate
EtOH	Ethanol
H_2SO_4	Sulfuric acid
HCl	Hydrogen chloride
MeOH	Methanol
MIC	Minimal inhibitory concentration
MSC	Minimal synergistic concentration
Na_2SO_4	Sodium sulfate
PPh_3	Triphenylphosphine

General notes

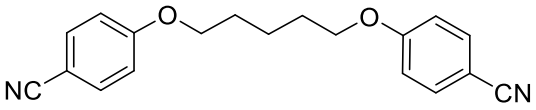
General procedures. All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. For compound characterization, 1H NMR spectra were recorded at 400 MHz with chemical shifts reported in parts per million (ppm) downfield relative to $CHCl_3$ (7.26) or DMSO (δ 2.50). ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), coupling constant (J) in hertz (Hz) and the number of protons. Where appropriate, the multiplicity is preceded by br, indicating that the signal was broad. ^{13}C NMR spectra were recorded at 101 MHz with chemical shifts reported relative to $CDCl_3$ (δ 77.16) or DMSO (δ 39.52). HRMS analysis was performed on a Shimadzu Nexera X2 UHPLC system with a Waters Acquity HSS C18 column (2.1 × 100 mm, 1.8 μm) at 30 °C and equipped with a diode array detector. The following solvent system, at a flow rate of 0.5 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, 0.1 % formic acid in acetonitrile. Gradient elution was as follows: 95:5 (A/B) for 1 min, 95:5 to 15:85 (A/B) over 6 min, 15:85 to 0:100 (A/B) over 1 min, 0:100 (A/B) for 3 min, then reversion back to 95:5 (A/B) for 3 min. This system was connected to a Shimadzu 9030 QTOF mass spectrometer (ESI ionisation) calibrated internally with Agilent's API-TOF reference mass solution kit (5.0 mM purine, 100.0 mM ammonium trifluoroacetate and 2.5 mM hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine) diluted to achieve a mass count of 10000. Compounds **1**, **45**, **47**, and **48** had NMR spectra and mass spectra consistent with the assigned structures in literature.[1–4] Purity of the final compounds **1**, **2**, **3**, **9**, **10**, **11**, **12**, **15**, **16**, **21**, **22**, **23**, **24**, **1b**, **21b**, **22b**, **23b**, **1c**, **21c**, **22c**, **23c**, **37**, **38**, **43**, and **44** was confirmed to be ≥ 95% by analytical RP-HPLC using a Shimadzu Prominence-i LC-2030 system with a Dr. Maisch ReproSil Gold 120 C18 column (4.6 × 250 mm, 5 μm) at 30 °C and equipped with a UV detector monitoring at 214 nm. The following solvent system, at a flow rate of 1 mL/min, was used: solvent A, 0.1 % TFA in water/acetonitrile, 95/5; solvent B, 0.1 % TFA in water/acetonitrile, 5/95. Gradient elution was as follows: 95:5 (A/B) for 2 min, 95:5 to 0:100 (A/B) over 30 min, 0:100 (A/B) for 1 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 3 min. The compounds were purified via preparative HPLC using a BESTA-Technik system with a Dr. Maisch Reprosil Gold 120 C18 column (25 × 250 mm, 10 μm) and equipped with a ECOM Flash UV detector monitoring at 214 nm. The following solvent system, at a flow rate of 12 mL/min, was used: solvent A, 0.1 % TFA in water/acetonitrile 95/5; solvent B, 0.1 % TFA in water/acetonitrile 5/95. Unless stated otherwise in the protocol, the gradient elution was as follows: 100:0 (A/B) to 0:100 (A/B) over 25 min, 0:100 (A/B) for 3 min, then reversion back to 100:0 (A/B) over 1 min, 100:0 (A/B) for 1 min.

Synthesis



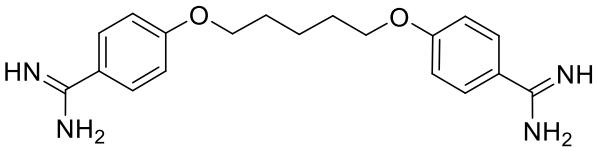
Scheme S1 Synthesis of pentamidine (**1**). Reagents and conditions: (a) 4-Cyanophenol, NaH, DMF, 80°C, 1h (78%); (b) i) LHMDS, THF, 48h, rt, ii) 4M HCl (dioxane), 0°C to rt, overnight (quant.).

4,4'-(pentane-1,5-diylbis(oxy))dibenzonitrile (45)

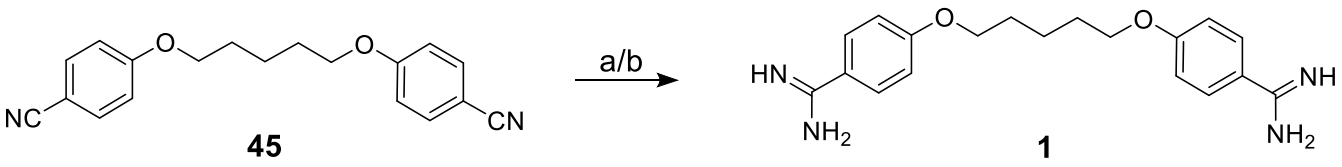


4-cyanophenol (1.14 g, 9.6 mmol, 2.4 eq.) was suspended in dry DMF (12 mL) under argon atmosphere. The suspension was cooled to 0 °C using an ice bath and NaH (384 mg, 60% dispersion in mineral oil, 2.4 eq.) was slowly added. The reaction was stirred until a clear solution appeared, the ice bath was removed and 1,5-dibromopentane (0.92 g, 4.0 mmol) was added. The reaction mixture was heated to 80 °C for 1 hour and then cooled to room temperature. Water (35 mL) was added to the mixture to obtain precipitation. The precipitate was filtered, washed with water and recrystallized from EtOH to give compound **45** as white crystals (0.95 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.9 Hz, 4H), 6.93 (d, *J* = 8.9 Hz, 4H), 4.03 (t, *J* = 6.3 Hz, 4H), 1.93 – 1.84 (m, 4H), 1.72 – 1.61 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.37, 134.09, 119.36, 115.24, 103.91, 68.14, 28.81, 22.73.

4,4'-(pentane-1,5-diylbis(oxy))dibenzimidamide (1)

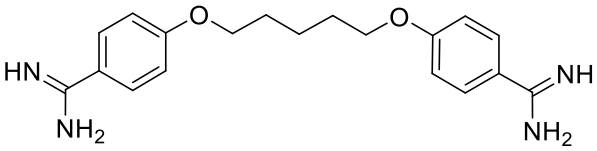


Compound **45** (94 mg, 0.3 mmol) was dissolved in dry THF (2 mL) under argon atmosphere and LHMDS (1.2 mL, 1 M THF solution, 4.0 eq.) was added. The reaction was stirred at room temperature for 48 hours or longer until complete conversion to the bis-amidine (monitored by LCMS). The solution was cooled to 0 °C and quenched with HCl (4.5 mL, 4 M dioxane solution, 60 eq.). The mixture was stirred at room temperature overnight, then diluted with diethyl ether and filtered. The precipitate was purified by preparative HPLC with the gradient 0-100% in 30 minutes. The samples were analyzed and the combined pure fractions were dried to give pentamidine (**1**) (120 mg, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 3H), 9.06 (s, 3H), 7.81 (d, J = 8.9 Hz, 4H), 7.15 (d, J = 8.9 Hz, 4H), 4.12 (t, J = 6.4 Hz, 4H), 1.88 – 1.75 (m, 4H), 1.65 – 1.52 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.70, 163.06, 130.19, 119.50, 114.79, 68.05, 28.21, 22.09. HRMS (ESI): calculated for $C_{19}H_{24}N_4O_2$ $[M+H]^+$ 341.1977, found 341.1977.



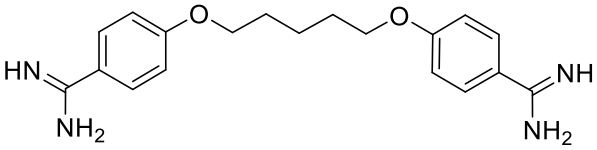
Scheme S2 Exploration of the optimal acidic quench. Reagents and conditions: (a) i) LHMDS, THF, 48h, ii) 2M HCl (aq), 0°C to rt, overnight (68%) or (b) i) LHMDS, THF, 48h, ii) sat. ethanolic HCl, 0°C to rt, overnight (9%).

4,4'-(pentane-1,5-diylbis(oxy))dibenzimidamide (1) using 2M HCl (aq)

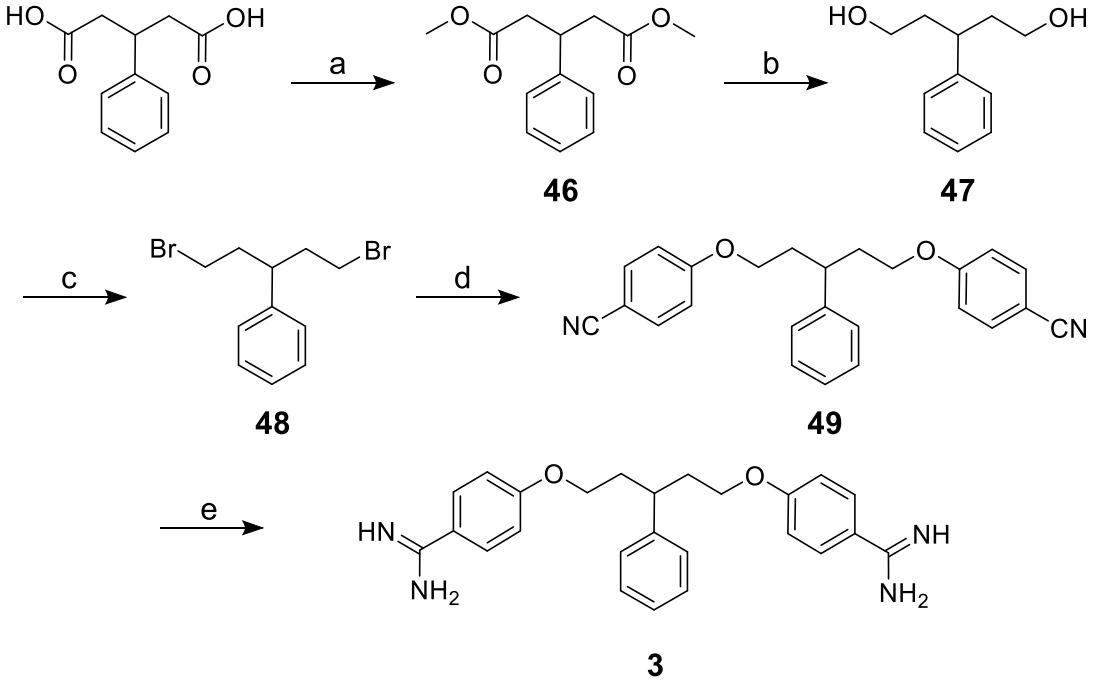


Following the procedure as described for compound **1** except for using 2 M HCl (aq) (20 mL) as acidic quench afforded pentamidine (**1**) (71 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 4H), 9.06 (s, 4H), 7.81 (d, J = 8.9 Hz, 4H), 7.15 (d, J = 8.9 Hz, 4H), 4.12 (t, J = 6.4 Hz, 4H), 1.88 – 1.75 (m, 4H), 1.65 – 1.52 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.70, 163.06, 130.19, 119.50, 114.79, 68.05, 28.21, 22.09. HRMS (ESI): calculated for $C_{19}H_{24}N_4O_2$ $[M+H]^+$ 341.1977, found 341.1977.

4,4'-(pentane-1,5-diylbis(oxy))dibenzimidamide (1) using sat. ethanolic HCl



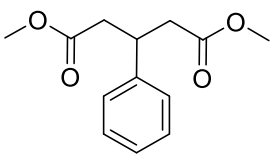
Following the procedure as described for compound **1** except for using freshly prepared sat. ethanolic HCl (20 mL) as acidic quench afforded pentamidine (**1**) (9 mg, 9%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 4H), 9.06 (s, 4H), 7.81 (d, J = 8.9 Hz, 4H), 7.15 (d, J = 8.9 Hz, 4H), 4.12 (t, J = 6.4 Hz, 4H), 1.88 – 1.75 (m, 4H), 1.65 – 1.52 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.70, 163.06, 130.19, 119.50, 114.79, 68.05, 28.21, 22.09. HRMS (ESI): calculated for $C_{19}H_{24}N_4O_2$ $[M+H]^+$ 341.1977, found 341.1977.



Scheme S3 Synthesis of 4,4'-((3-phenylpentane-1,5-diyl)bis(oxy))dibenzimidamide (**3**). Reagents and conditions: (a) H_2SO_4, MeOH, 70°C, overnight (91%); (b) DIBAL-H, DCM, 0°C, 1 hour (quant.); (c)

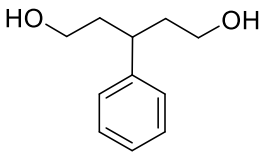
NBS, PPh_3, DCM, 0°C to rt, 2 hours (62%) (d) 4-Cyanophenol, NaH, DMF, 80°C, 1h (89%); (e) i) LHMDS, THF, 48h, ii) 4M HCl (dioxane), 0°C to rt, overnight (23%).

Dimethyl 3-phenylpentanedioate (46)



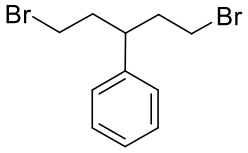
3-phenylpentanedioic acid (1.04 g, 5 mmol) was dissolved in MeOH (20 mL) and a few drops of H_2SO_4 were added to the solution. The reaction mixture was refluxed at 70 °C overnight, concentrated in vacuo and redissolved in DCM (50 mL). The organic layer was washed with water (4 mL) five times. The organic layer was then washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified using column chromatography (petroleum ether/EtOAc = 17:3) to give dimethyl ester **46** (1.08 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 2H), 7.25 – 7.18 (m, 3H), 3.70 – 3.61 (m, 1H), 3.59 (s, 6H), 2.73 (dd, *J* = 15.6, 7.2 Hz, 2H), 2.65 (dd, *J* = 15.6, 7.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.19, 142.67, 128.74, 127.28, 127.10, 51.75, 40.53, 38.36.

3-phenylpentane-1,5-diol (47)



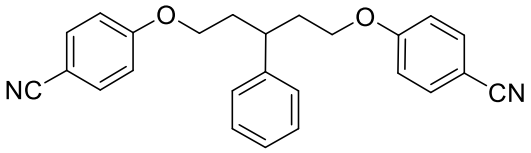
Dimethyl ester **46** (1.07 mg, 4.5 mmol) was dissolved in dry DCM (12.5 mL) under argon atmosphere. The mixture was cooled to 0 °C using an ice bath. DIBAL-H (21.7 mL, 1M dioxane solution, 4.8 eq.) was added dropwise to the cooled solution and stirred for 1 hour. The reaction was quenched with Rochelle salt (30 mL, sat. aq.) and the biphasic mixture was stirred at room temperature overnight. The layers were separated and the aqueous layer was extracted two times with diethyl ether. The organic layers were combined, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The diol **47** (863 mg, quant.) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.27 (m, 2H), 7.24 – 7.15 (m, 3H), 3.62 – 3.52 (m, 2H), 3.52 – 3.43 (m, 2H), 2.98 – 2.84 (m, 1H), 2.01 – 1.79 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.48, 128.80, 127.73, 126.63, 61.04, 39.55, 38.94.

(1,5-dibromopentan-3-yl)benzene (48)



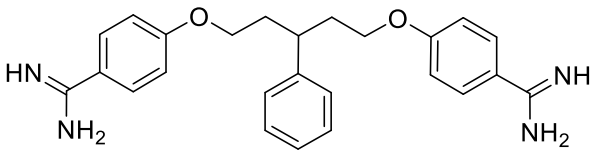
Compound **47** (400 mg, 2.2 mmol) was dissolved in dry DCM (10 mL), PPh_3 (1.46 g, 5.5 mmol, 2.5 eq.) was added, and the mixture under argon was cooled to 0 °C using an ice bath. N-bromosuccinimide (0.65 g, 5.5 mmol, 2.5 eq.) was added portion wise. After the addition, the ice bath was removed and the reaction was stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the crude product was purified by column chromatography (petroleum ether/EtOAc = 99:1) to give compound **48** (415 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.33 (m, 3H), 7.22 – 7.16 (m, 2H), 3.28 (ddd, J = 10.0, 6.6, 5.5 Hz, 2H), 3.21 – 2.94 (m, 3H), 2.20 – 2.13 (m, 4H).. ^{13}C NMR (101 MHz, $CDCl_3$) (including PPh3=O peaks) δ 134.00, 133.81, 132.37, 132.27, 129.09, 129.05, 128.78, 128.74, 128.67, 127.88, 127.23, 42.79, 39.34, 31.54.

4,4'-((3-phenylpentane-1,5-diyl)bis(oxy))dibenzonitrile (49)



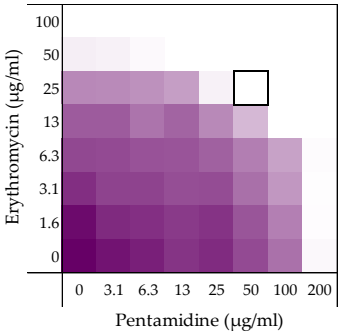
Following the procedure as described above for compound **45**, using compound **48** (500 mg, 1.6 mmol), afforded crude compound **49** (546 mg, 89%). The crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.40 (m, 5H), 7.24 – 7.24 (m, 2H), 7.12 – 7.08 (m, 2H), 6.82 – 6.67 (m, 4H), 3.81 (ddd, J = 9.4, 6.6, 4.9 Hz, 2H), 3.72 (ddd, J = 9.4, 8.1, 6.0 Hz, 2H), 3.11 – 2.96 (m, 1H), 2.24 – 2.12 (m, 2H), 2.09 – 1.98 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.23, 142.69, 137.35, 137.24, 134.08, 133.96, 133.77, 128.84, 128.66, 128.59, 127.69, 127.13, 119.34, 115.25, 104.02, 66.21, 39.00, 36.00, 29.84.

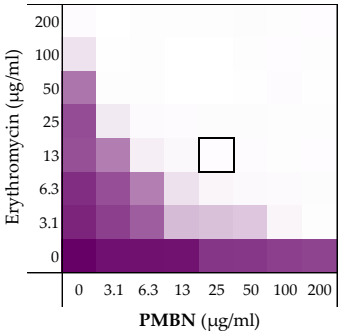
4,4'-((3-phenylpentane-1,5-diyl)bis(oxy))dibenzimidamide (3)

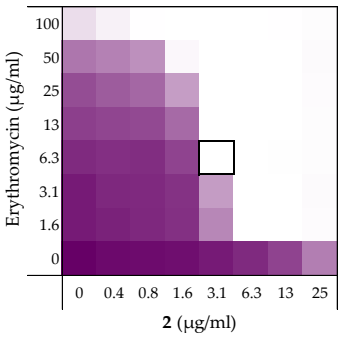


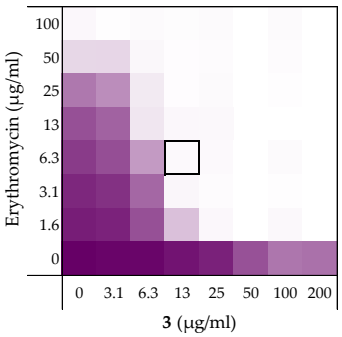
Compound **49** (109 mg, 0.28 mmol) was dissolved in the LHMDS solution (1.1 mL, 1 M THF solution, 4.0 eq.) under argon atmosphere. The reaction was stirred at room temperature for 48 hours or longer until complete conversion to the bis-amidine (monitored by LCMS). The solution was cooled to 0 °C and quenched with HCl (4.5 mL, 4 M dioxane solution, 60 eq.). The mixture was stirred at room temperature overnight, then diluted with diethyl ether and filtered. The precipitate was purified by preparative HPLC with the gradient 20-100% in 30 minutes. The samples were analyzed and the combined pure fractions were dried to give compound **3** (27.4 mg, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (d, J = 12.6 Hz, 8H), 7.77 (d, J = 8.9 Hz, 4H), 7.34 – 7.16 (m, 5H), 7.05 (d, J = 9.0 Hz, 4H), 4.00 – 3.90 (m, 2H), 3.83 (dd, J = 15.0, 8.9 Hz, 2H), 3.14 – 3.04 (m, 1H), 2.29 – 2.16 (m, 2H), 2.13 – 2.00 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.81, 162.92, 143.38, 130.21, 128.62, 127.69, 126.58, 119.64, 66.21, 38.31, 35.10. HRMS (ESI): calculated for $C_{25}H_{28}N_4O_2$ $[M+H]^+$ 417.2291, found 417.2287.

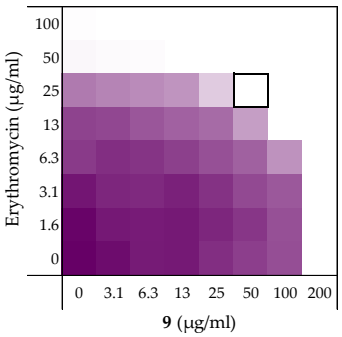
Checkerboard assays and FICI data against *E.coli* BW25113 with erythromycin

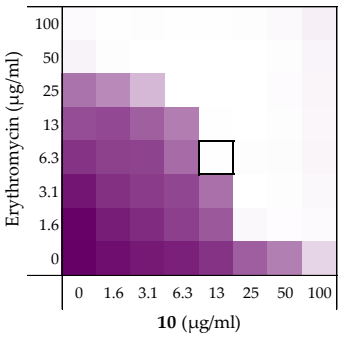


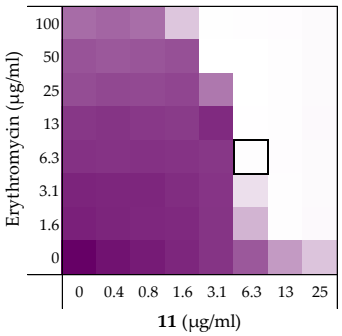


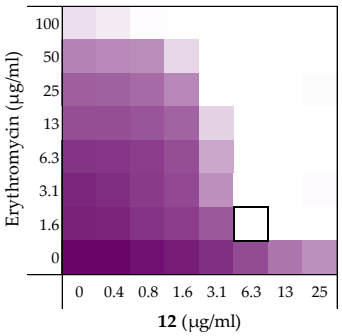


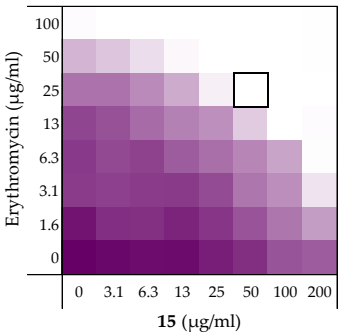


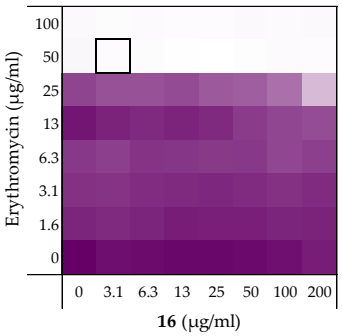


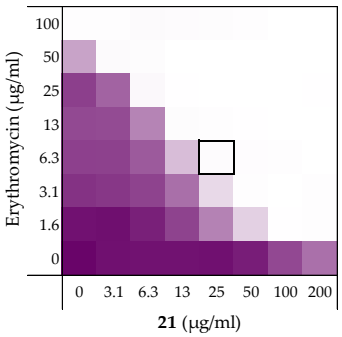


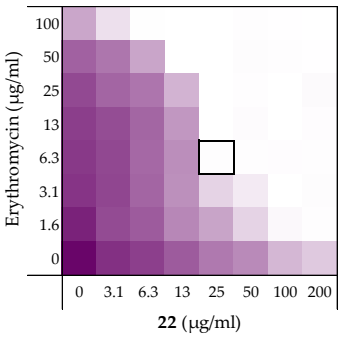


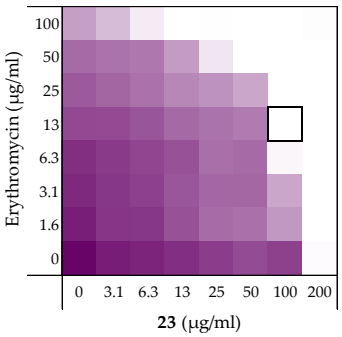


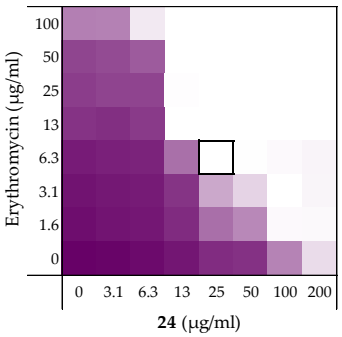


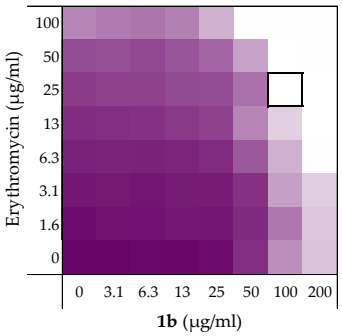


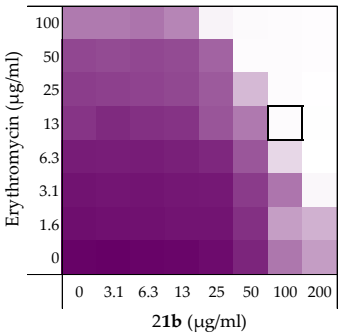


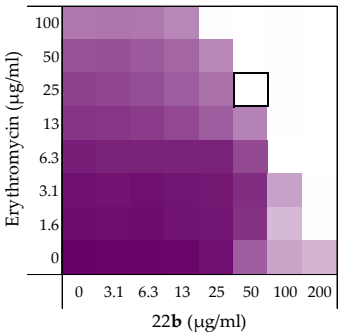


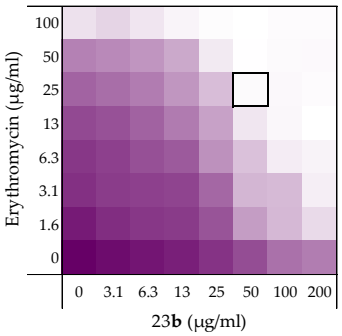












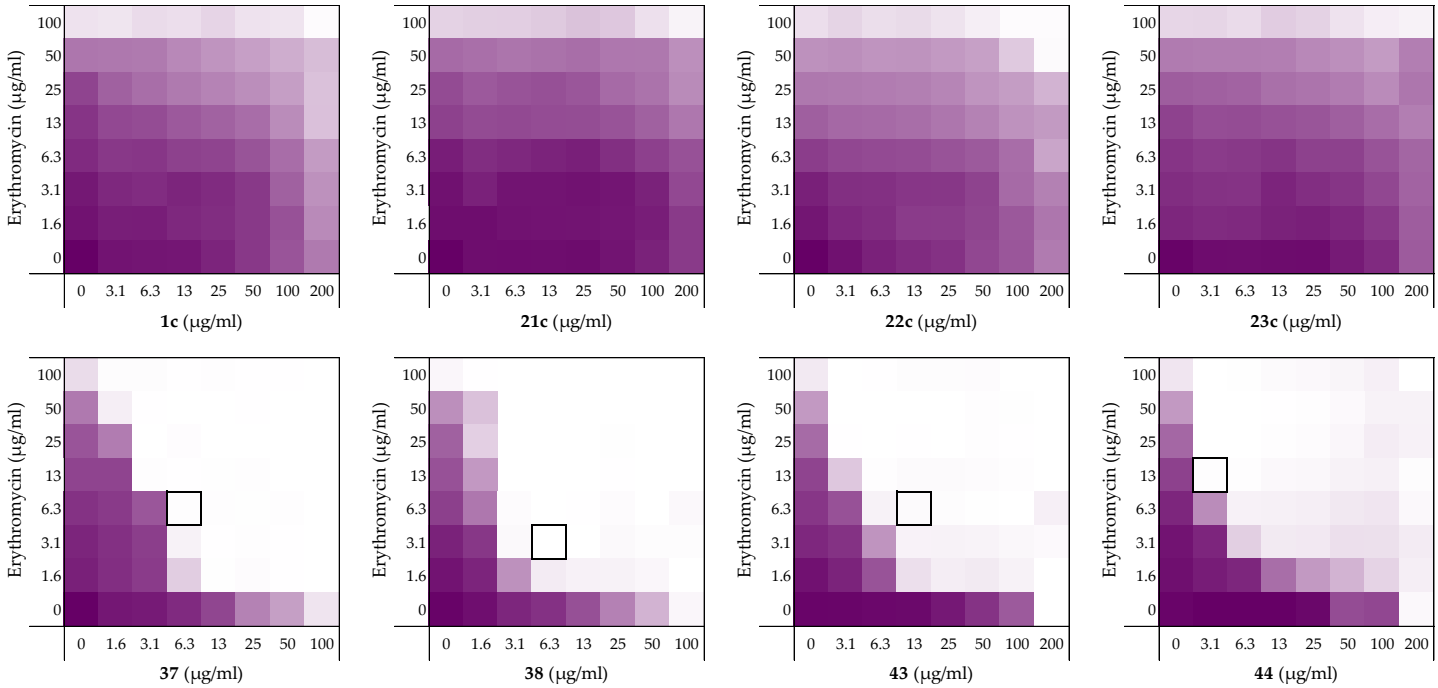
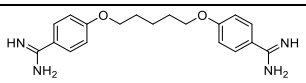
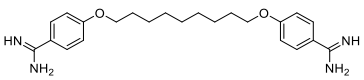
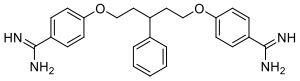
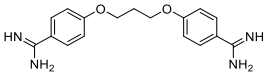
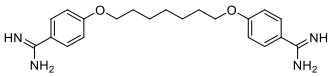
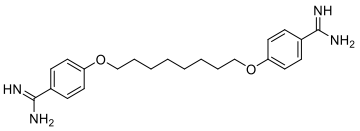
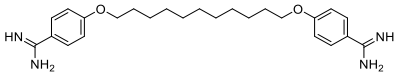
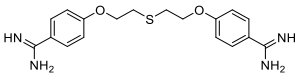
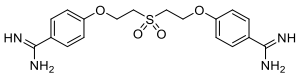
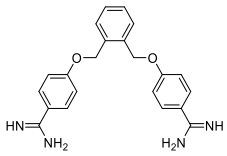
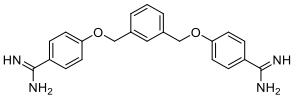
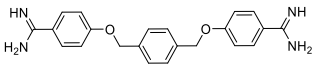
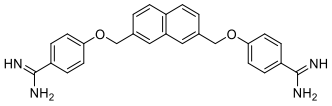
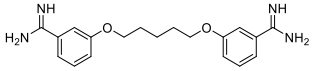
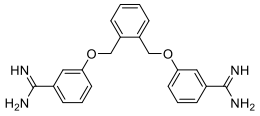
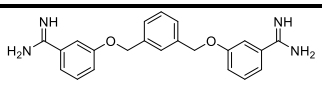
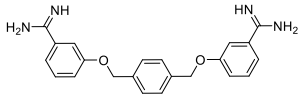
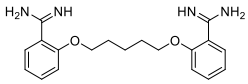
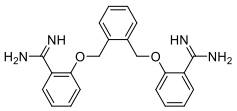
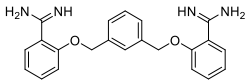
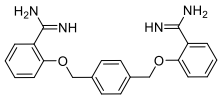
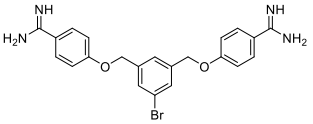
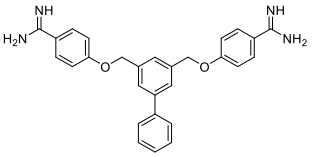
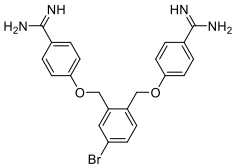
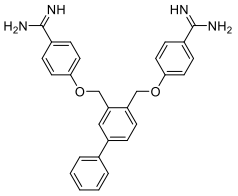


Figure S1 Checkerboard assays of the compounds and PMBN in combination with erythromycin versus *E.coli* BW25113. OD600 values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

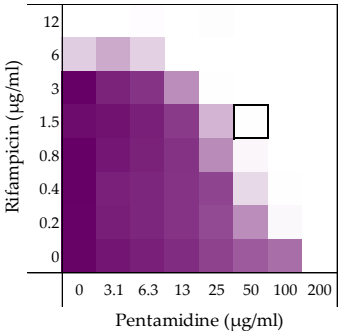
Table S1 Synergistic data of compounds and PMBN of the checkerboard assays with erythromycin as shown in Figure S1. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

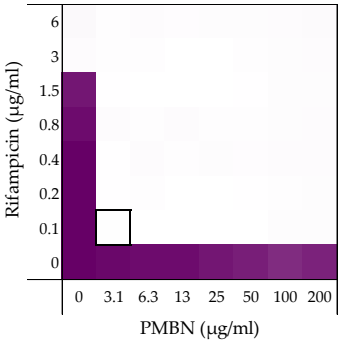
	Structures	MIC	MSC	MIC ery	MSC ery	FICI
1		200	50	100	25	0.500
2		>25	3.13	>100	6.25	≤0.094
3		>200	12.5	>100	6.25	≤0.063
9		200	50	100	25	0.500
10		>100	12.5	100	6.25	≤0.125
11		>25	6.25	>100	6.25	≤0.156
12		>25	6.25	>100	1.56	≤0.133
15		>200	50	100	25	≤0.375
16		>200	3.13	50	50	>0.5[a]
21		>200	25	100	6.25	≤0.125
22		>200	25	>100	6.25	≤0.094
23		>200	100	>100	12.5	≤0.313
24		>200	25	>100	6.25	≤0.094
1b		>200	100	>100	25	≤0.375
21b		>200	100	>100	12.5	≤0.313

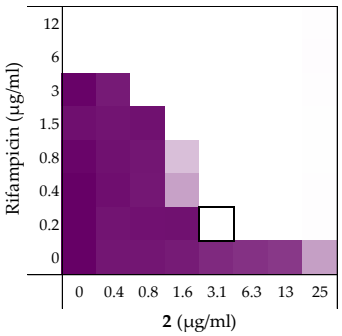
22b		>200	50	>100	25	≤0.250
23b		>200	50	>100	25	≤0.250
1c		>200	nd.	>100	nd.	>0.5[a]
21c		>200	nd.	>100	nd.	>0.5[a]
22c		>200	nd.	>100	nd.	>0.5[a]
23c		>200	nd.	>100	nd.	>0.5[a]
37		>100	6.25	>100	6.25	≤0.063
38		>100	6.25	>100	3.13	≤0.047
43		200	12.5	>100	6.25	≤0.094
44		200	3.13	>100	12.5	≤0.078
PMBN		>200	25	200	12.5	≤0.125

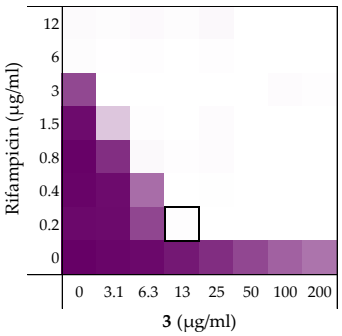
[a] Synergy is defined as FICI ≤ 0.5.[5]

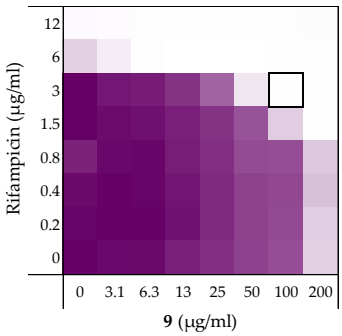
Checkerboard assays and FICI data against *E.coli* BW25113 with rifampicin

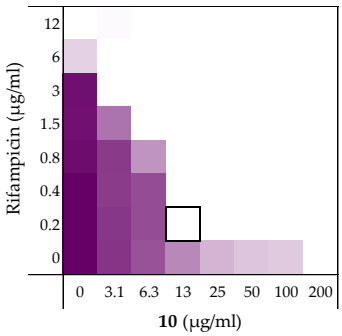


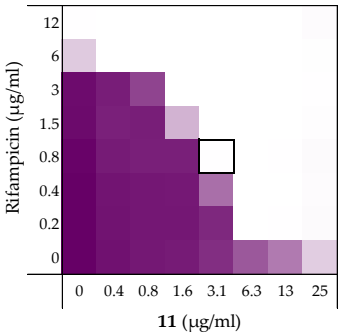


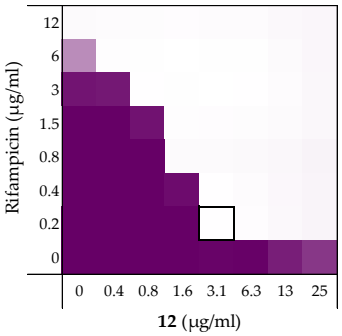


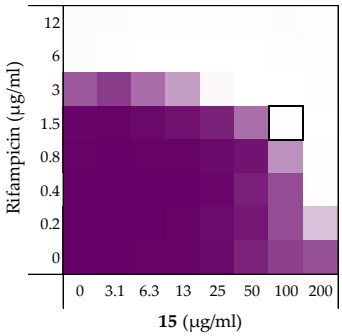


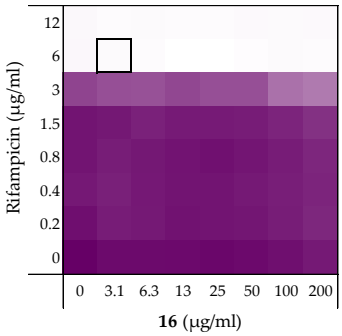


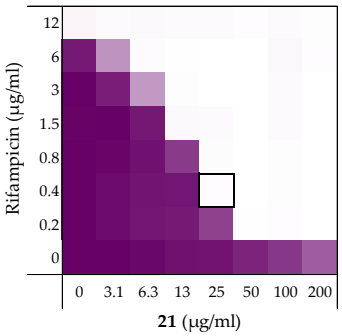


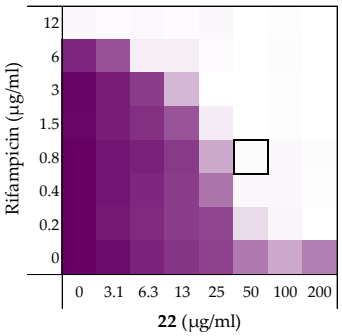


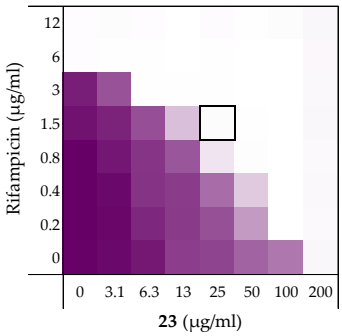


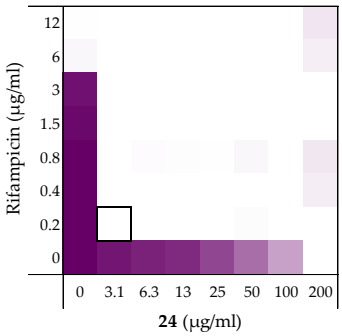


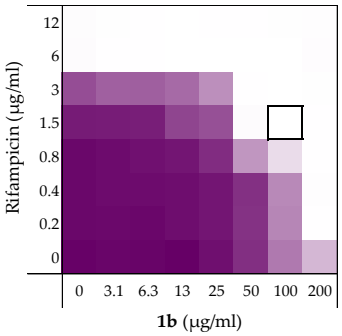


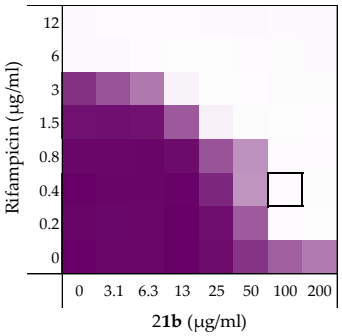


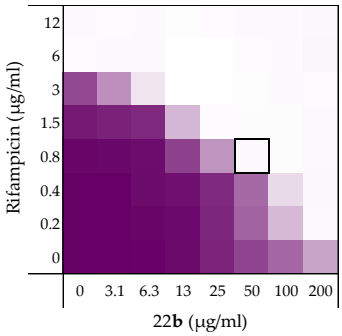


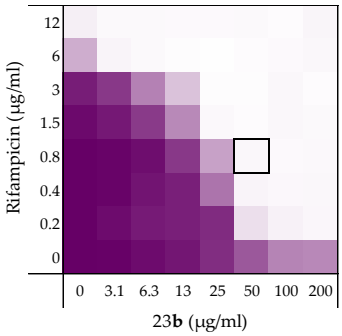












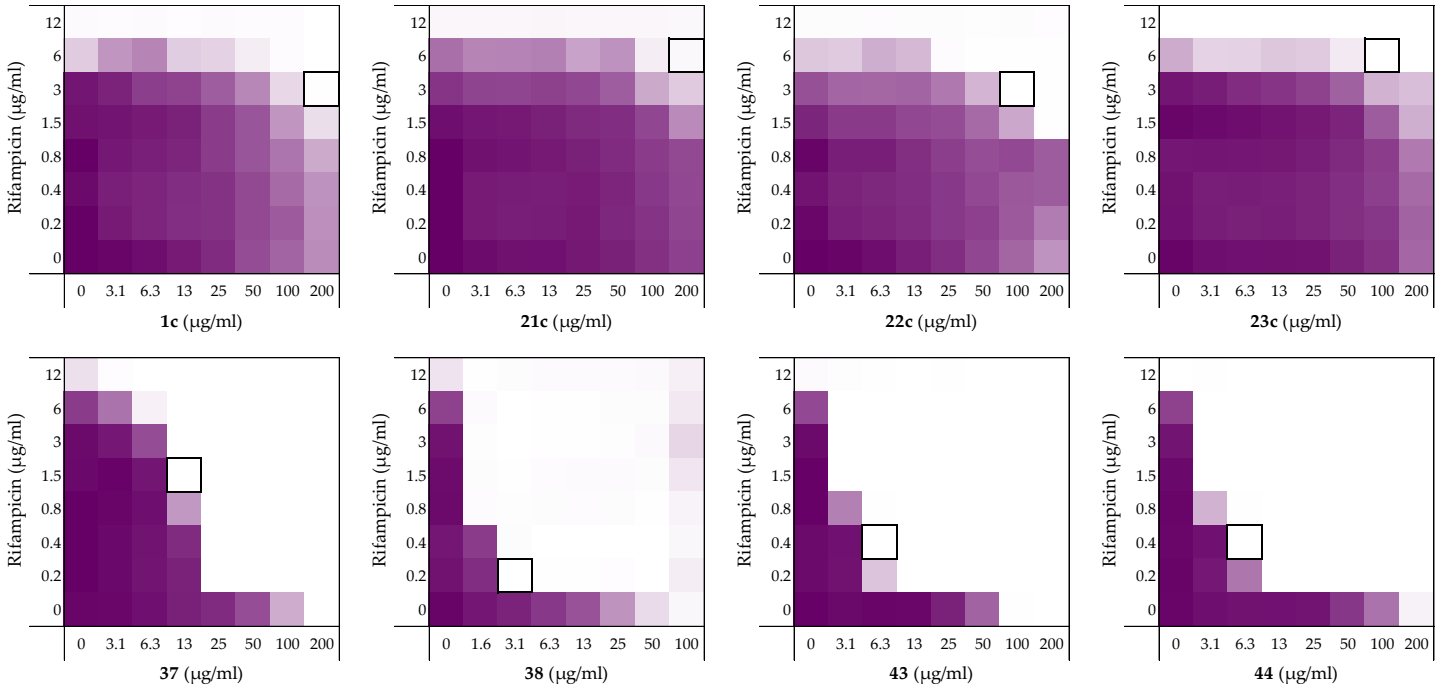
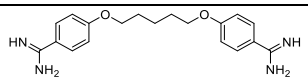
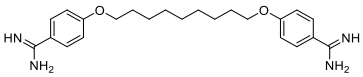
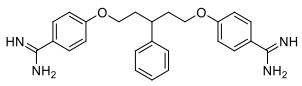
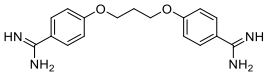
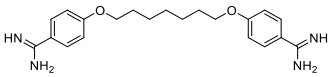
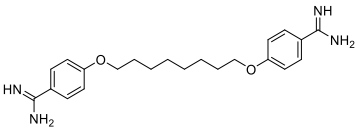
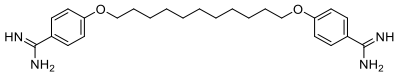
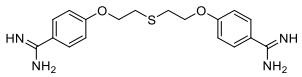
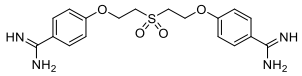
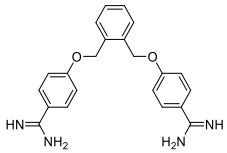
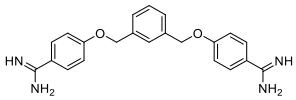
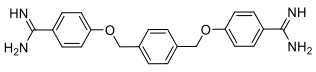
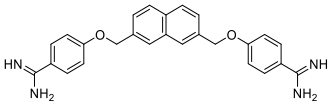
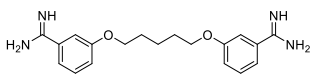
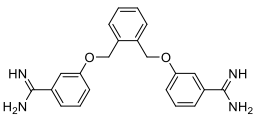
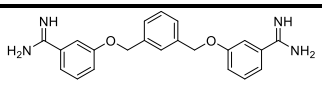
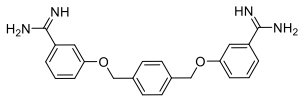
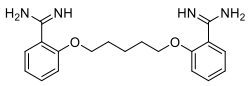
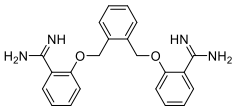
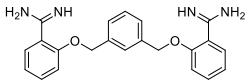
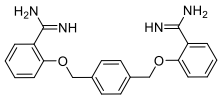
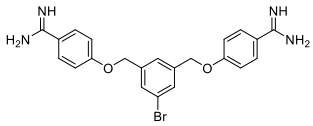
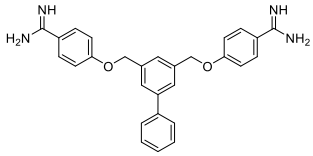
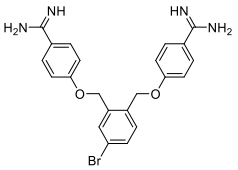
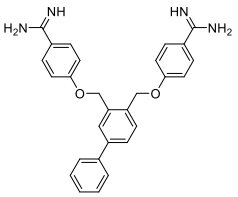


Figure S2 Checkerboard assays of the compounds and PMBN in combination with rifampicin versus *E.coli* BW25113. OD600 values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S2 Synergistic data of compounds and PMBN of the checkerboard assays with rifampicin as shown in Figure S2. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		200	50	12	1.5	0.375
2		>25	3.13	6	0.19	≤0.094
3		>200	12.5	6	0.19	≤0.063
9		>200	100	12	3	≤0.500
10		200	12.5	12	0.19	0.078
11		>25	3.13	12	0.75	≤0.125
12		>25	3.13	12	0.19	≤0.078
15		>200	100	6	1.5	≤0.500
16		>200	3.13	6	6	>0.5[a]
21		>200	25	12	0.38	≤0.094
22		>200	50	12	0.75	≤0.188
23		200	25	6	1.5	0.375
24		200	3.13	12	0.19	0.031
1b		>200	100	12	1.5	≤0.375
21b		>200	100	6	0.38	≤0.313

22b		>200	50	6	0.75	≤0.250
23b		>200	50	>12	0.75	≤0.156
1c		>200	nd.	12	nd.	>0.5[a]
21c		>200	nd.	12	nd.	>0.5[a]
22c		>200	nd.	12	nd.	>0.5[a]
23c		>200	nd.	12	nd.	>0.5[a]
37		200	12.5	>12	1.5	≤0.125
38		100	3.13	>12	0.19	≤0.039
43		100	6.25	12	0.38	0.094
44		>200	6.25	12	0.38	≤0.047
PMBN		>200	3.125	3	0.09	≤0.039

[a] Synergy is defined as FICI ≤ 0.5.[5]

Synergy data of compounds 1, 3, 21, 22, 23b, 37, 38, 43, 44, and PMBN

Checkerboard assays and FICI data against *E.coli* BW25113 with novobiocin

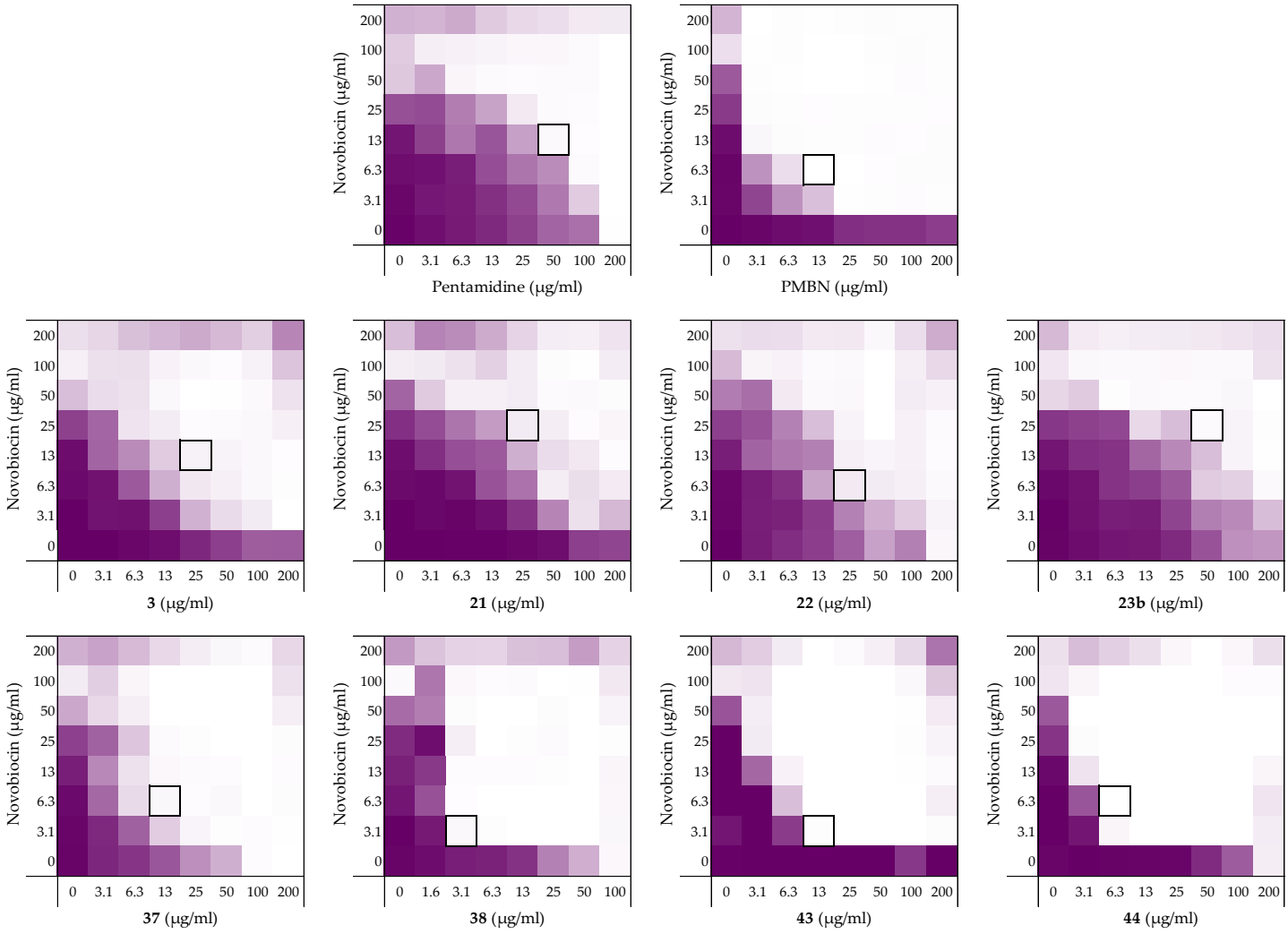
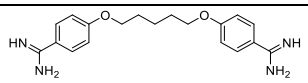
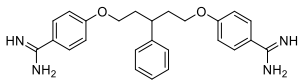
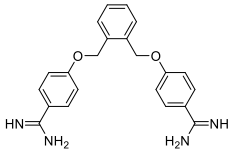
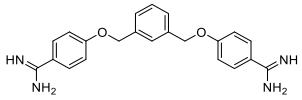
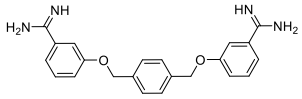
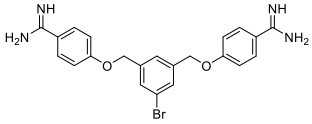
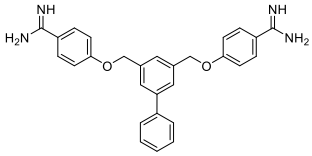
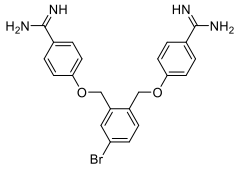
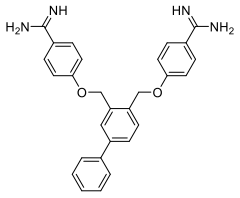


Figure S3 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with novobiocin versus *E.coli* BW25113. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S3 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* BW25113 with novobiocin as shown in Figure S3. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC nov	MSC nov	FICI
1		200	50	>200	12.5	≤0.281
3		>200	25	>200	12.5	≤0.094
21		>200	25	>200	25	≤0.125
22		>200	25	>200	6.25	≤0.078
23b		>200	50	>200	25	≤0.188
37		200	12.5	>200	6.25	≤0.078
38		100	3.13	>200	3.13	≤0.039
43		>200	12.5	>200	3.13	≤0.039
44		>200	6.25	>200	6.25	≤0.031
PMBN		>200	12.5	>200	6.25	≤0.047

Checkerboard assays and FICI data against *E.coli* BW25113 with vancomycin

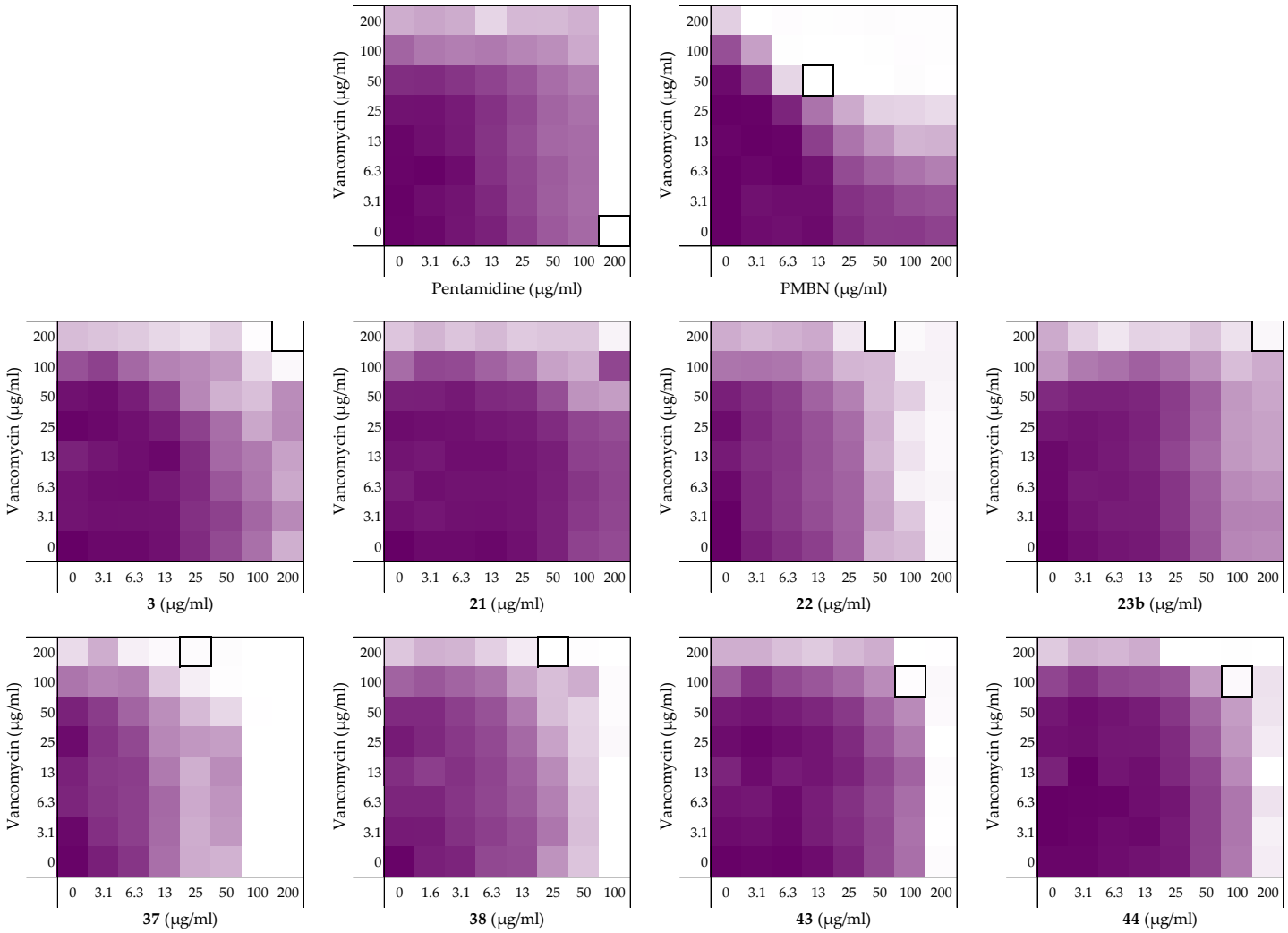
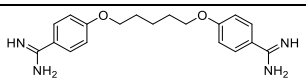
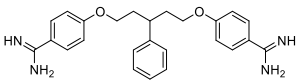
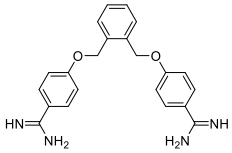
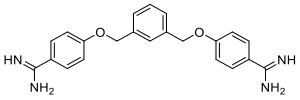
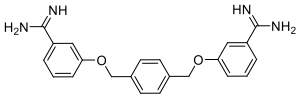
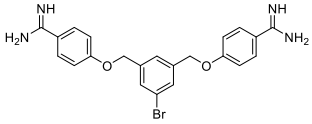
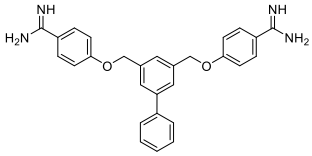
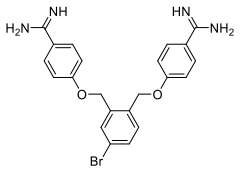
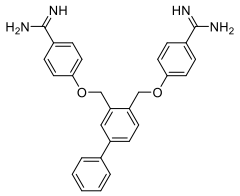


Figure S4 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with vancomycin versus *E.coli* BW25113. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S4 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* BW25113 with vancomycin as shown in Figure S4. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC van	MSC van	FICI
1		200	200	>200	-	>0.5[a]
3		>200	200	>200	200	>0.5[a]
21		>200	>200	>200	>200	>0.5[a]
22		>200	50	>200	200	>0.5[a]
23b		>200	200	>200	200	>0.5[a]
37		100	25	>200	200	>0.5[a]
38		100	25	>200	200	>0.5[a]
43		200	100	>200	100	>0.5[a]
44		>200	100	>200	100	≤0.500
PMBN		>200	12.5	>200	50	≤0.156

[a] Synergy is defined as FICI ≤ 0.5.[5]

Synergy data of compounds 1, 3, 21, 22, 23b, 37, 38, 43, 44, and PMBN with rifampicin

E. coli ATCC25922

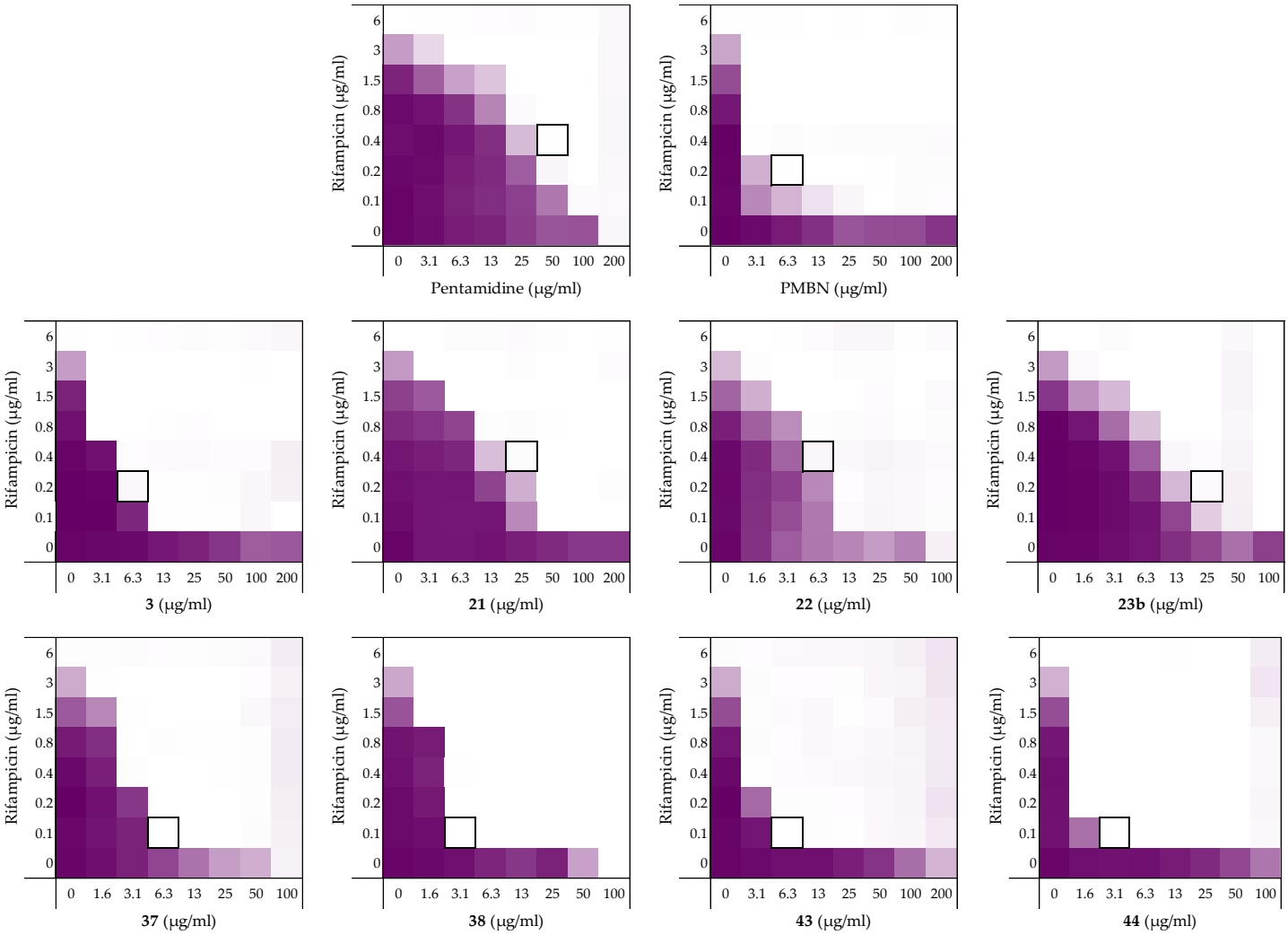
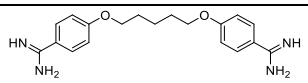
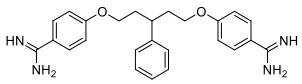
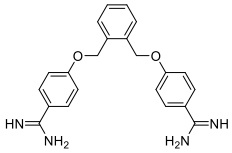
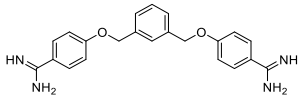
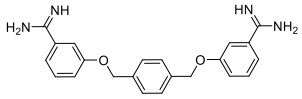
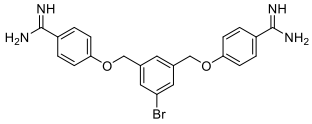
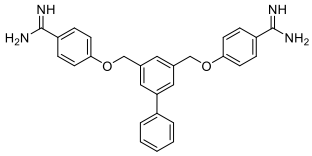
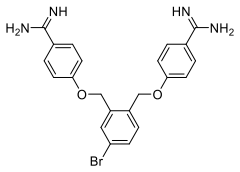
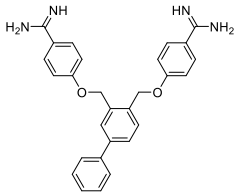


Figure S5 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* ATCC25922. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S5 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* ATCC25922 with rifampicin as shown in Figure S5. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		200	50	6	0.38	0.313
3		>200	6.25	6	0.19	≤0.047
21		>200	25	6	0.38	≤0.125
22		200	6.25	6	0.38	0.094
23b		200	25	6	0.19	0.156
37		100	6.25	6	0.09	0.078
38		100	3.13	6	0.09	0.047
43		>200	6.25	6	0.09	≤0.031
44		200	3.13	6	0.09	0.031
PMBN		>200	6.25	6	0.19	≤0.047

***E. coli* W3110**

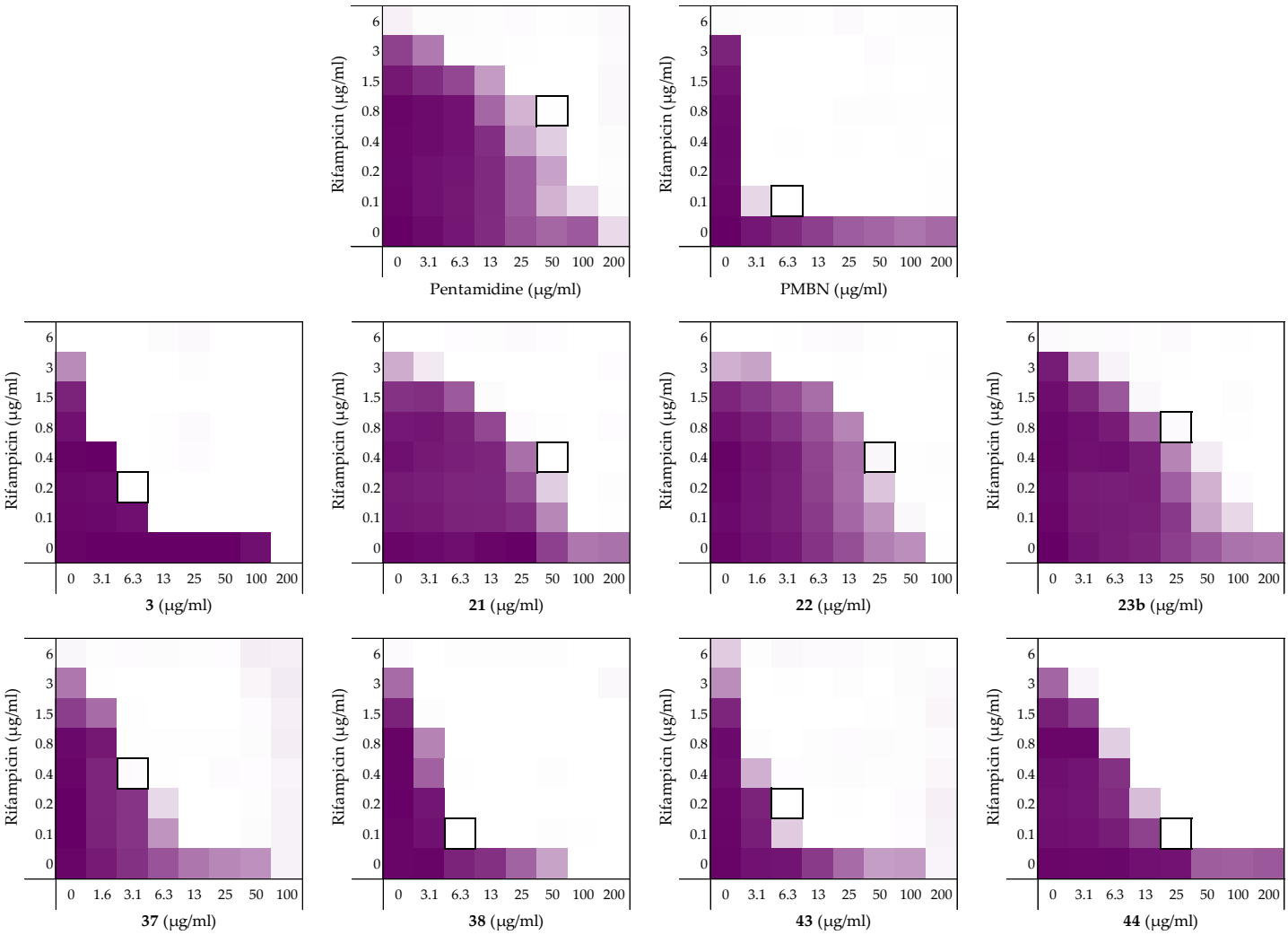
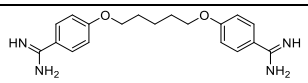
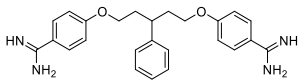
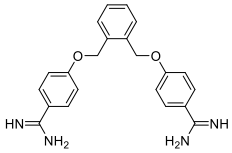
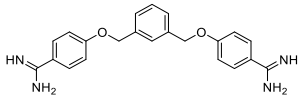
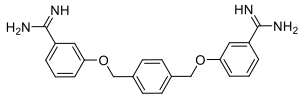
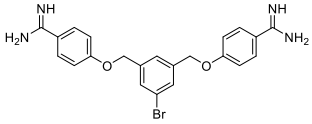
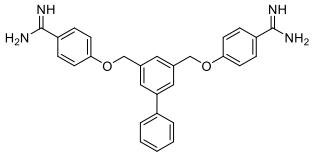
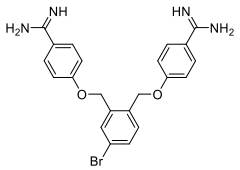
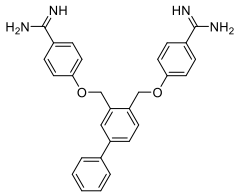


Figure S6 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* W3110. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S6 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* W3110 with rifampicin as shown in Figure S6. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	50	>6	0.75	≤0.188
3		200	6.25	6	0.19	0.063
21		>200	50	6	0.38	≤0.188
22		100	25	6	0.38	0.313
23b		>200	25	6	0.75	≤0.188
37		100	3.13	>6	0.38	≤0.063
38		100	6.25	>6	0.09	≤0.070
43		200	6.25	>6	0.19	≤0.047
44		>200	25	6	0.09	≤0.078
PMBN		>200	6.25	6	0.09	≤0.031

***E. coli* 552060.1**

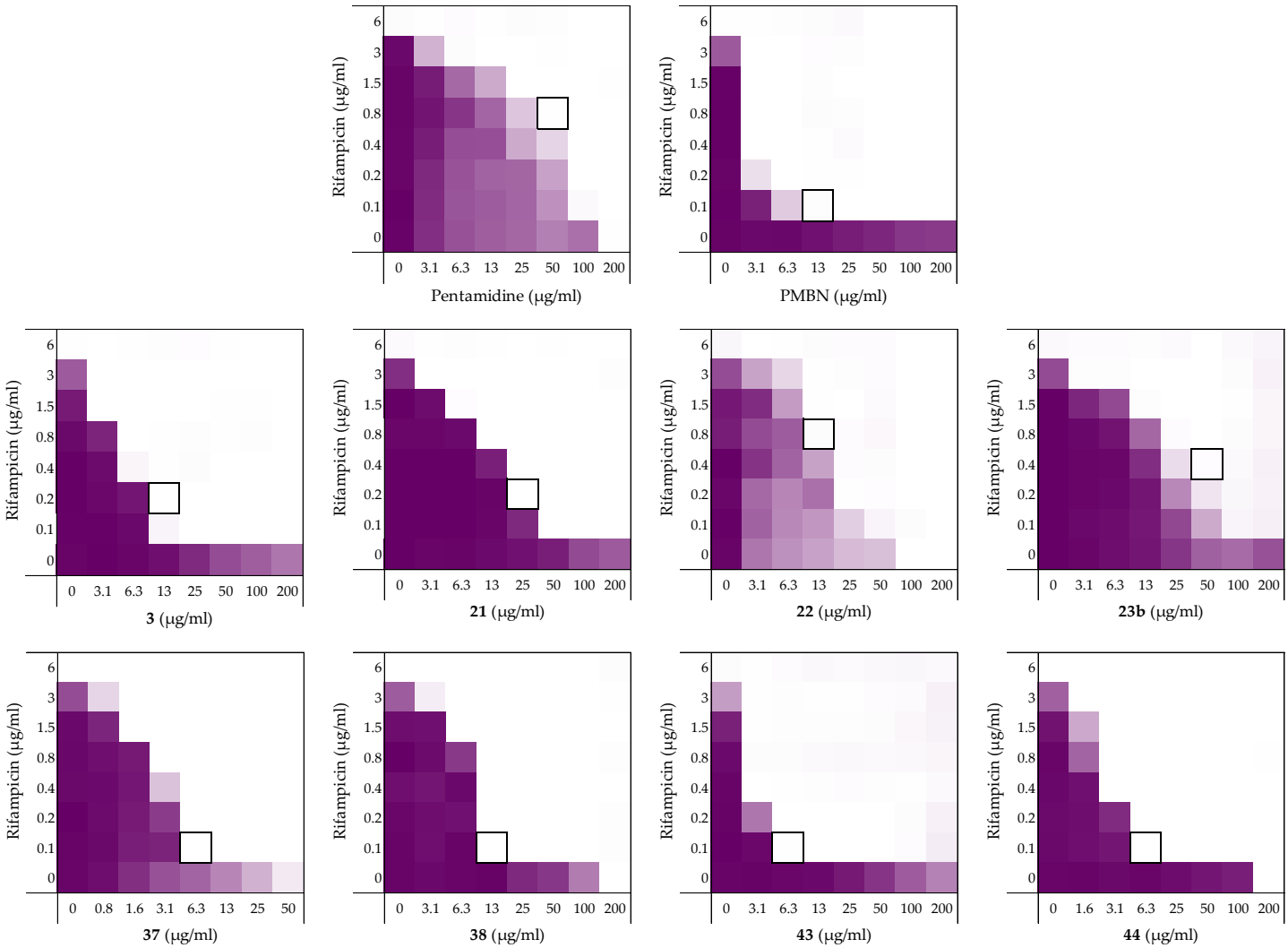
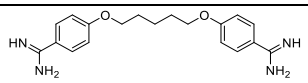
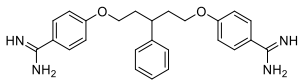
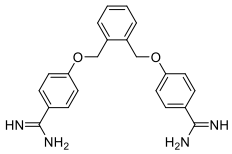
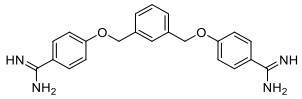
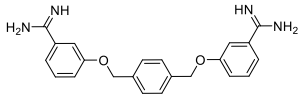
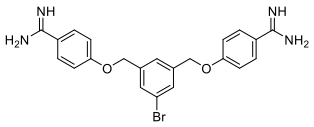
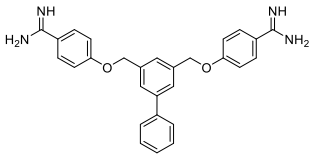
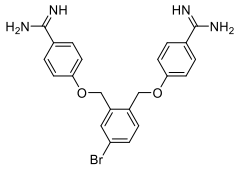
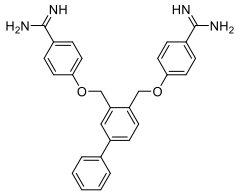


Figure S7 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* 552060.1. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S7 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* 5552060.1 with rifampicin as shown in Figure S7. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		200	50	6	0.75	0.375
3		>200	12.5	6	0.19	≤0.063
21		>200	25	6	0.19	≤0.094
22		100	12.5	6	0.75	0.250
23b		>200	50	6	0.38	≤0.188
37		100	6.25	6	0.09	0.078
38		200	12.5	6	0.09	0.078
43		>200	6.25	6	0.09	≤0.031
44		200	6.25	6	0.09	0.047
PMBN		>200	12.5	6	0.09	≤0.047

E. coli BW25113 mcr-1

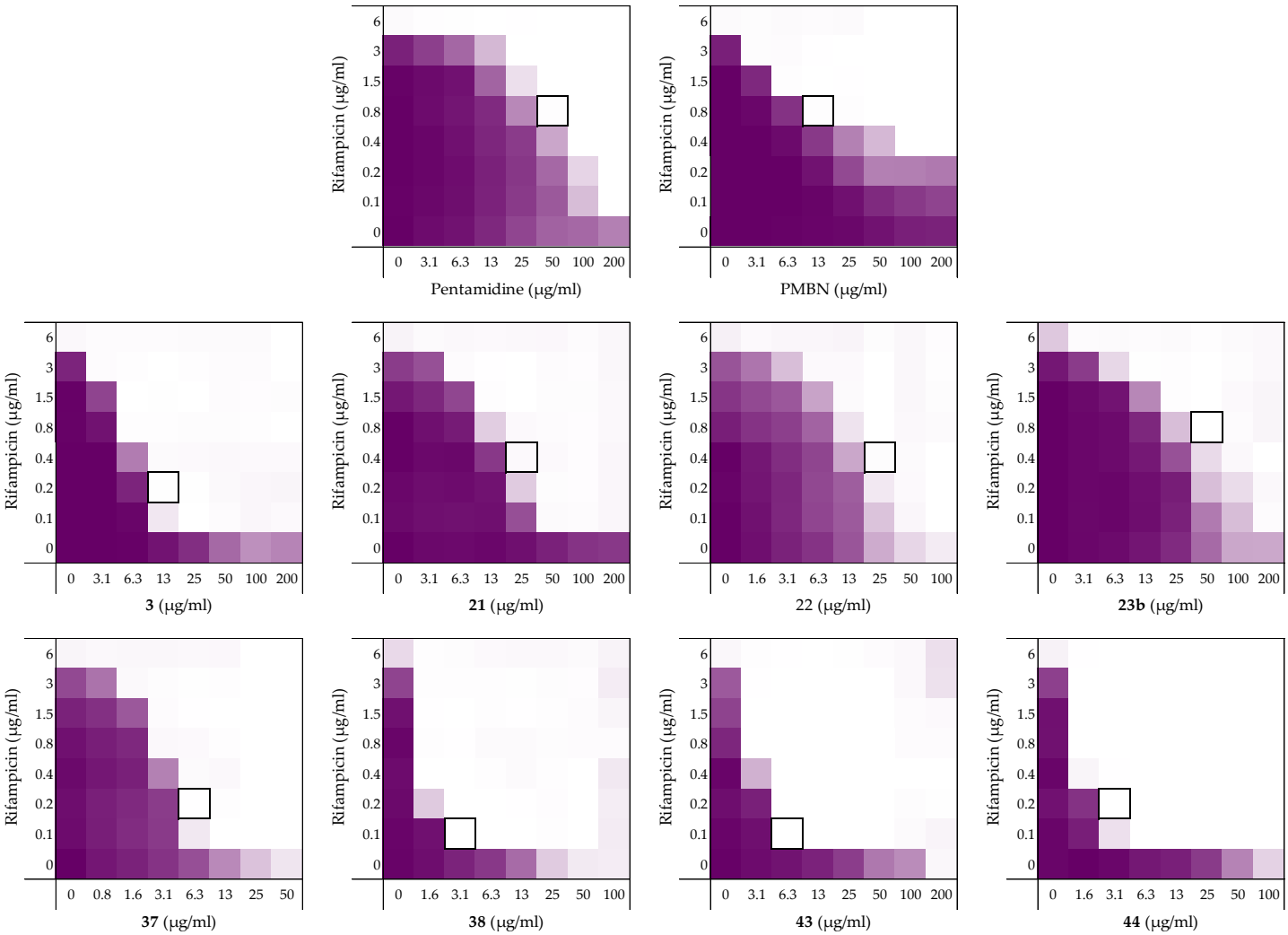
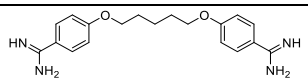
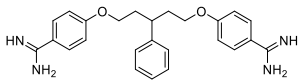
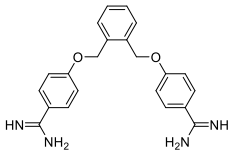
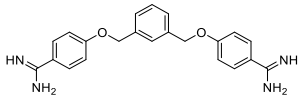
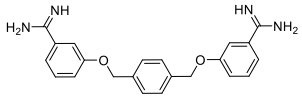
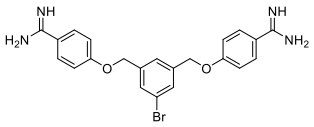
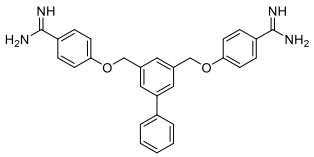
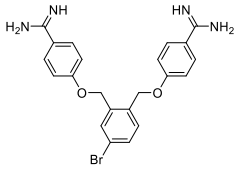
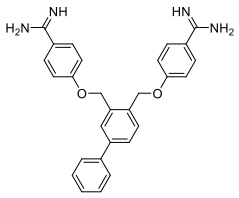


Figure S8 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* BW25113 mcr-1. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S8 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* BW25113 mcr-1 with rifampicin as shown in Figure S8. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	50	6	0.75	≤0.250
3		>200	12.5	6	0.19	≤0.063
21		>200	25	>6	0.38	≤0.094
22		>100	25	>6	0.38	≤0.156
23b		>200	50	>6	0.75	≤0.188
37		>50	6.25	>6	0.19	≤0.078
38		100	3.13	>6	0.09	≤0.039
43		200	6.25	>6	0.09	≤0.039
44		>100	3.13	>6	0.19	≤0.031
PMBN		>200	12.5	6	0.75	≤0.156

E. coli **mcr-1**

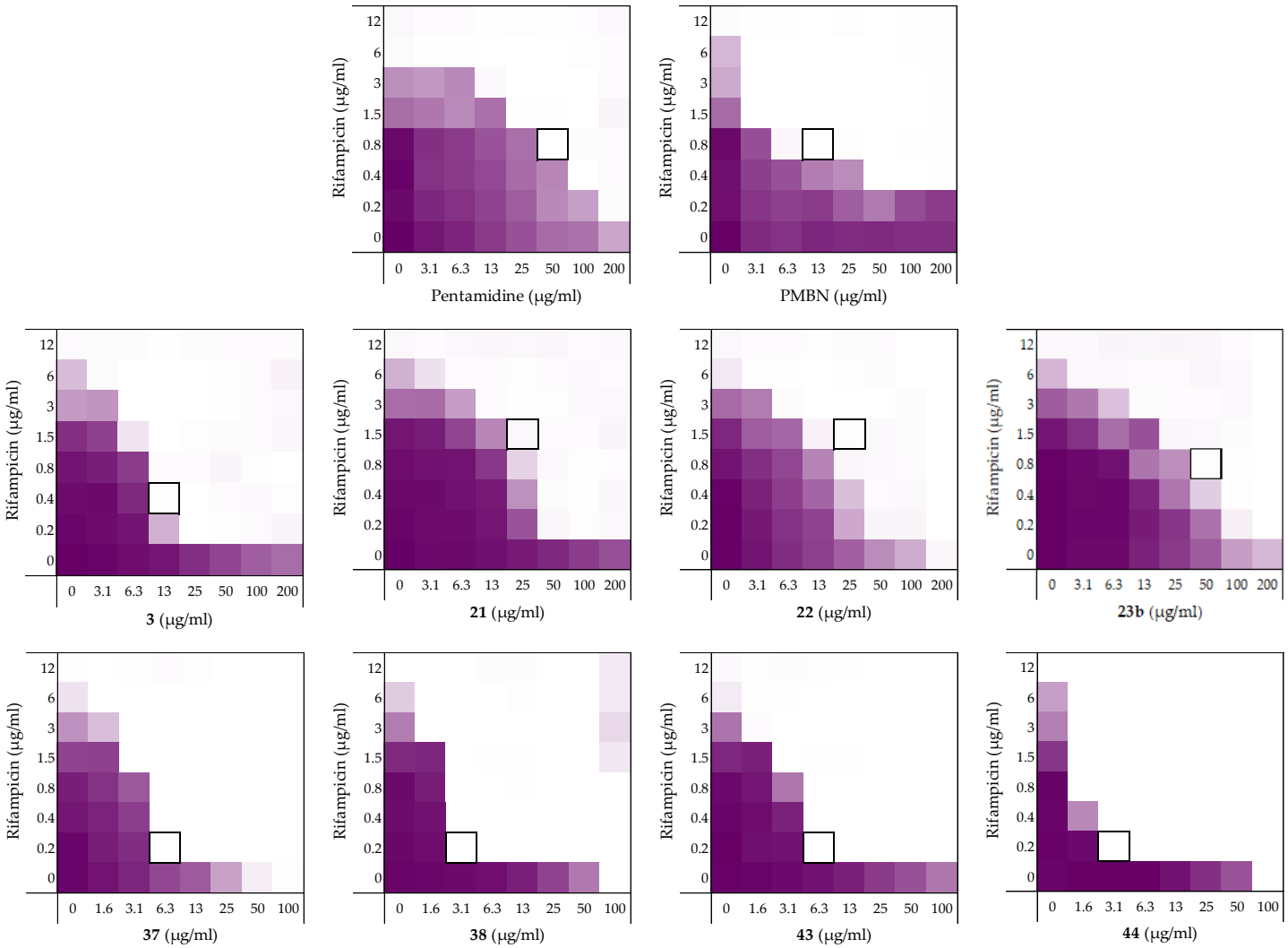
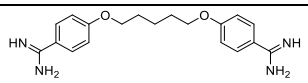
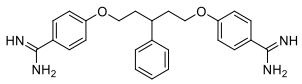
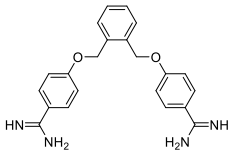
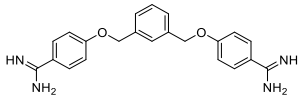
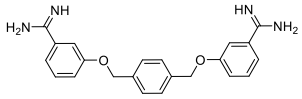
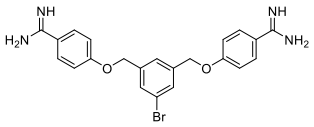
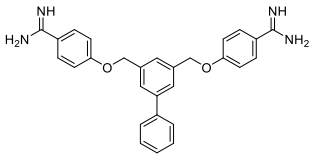
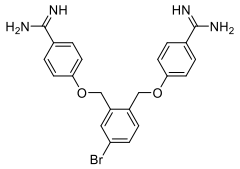
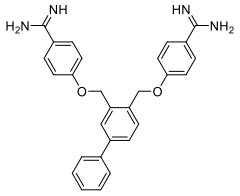


Figure S9 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* mcr-1. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S9 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* mcr-1 with rifampicin as shown in Figure S9. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	50	12	0.75	≤0.188
3		>200	12.5	12	0.38	≤0.063
21		>200	25	12	1.5	≤0.188
22		>200	25	12	1.5	≤0.188
23b		>200	50	12	0.75	≤0.188
37		100	6.25	12	0.19	0.078
38		100	3.13	12	0.19	0.047
43		>100	6.25	12	0.19	≤0.047
44		100	3.13	12	0.19	0.047
PMBN		>200	12.5	12	0.75	≤0.094

E. coli EQASmcr-1/EQAS 2016 412016126

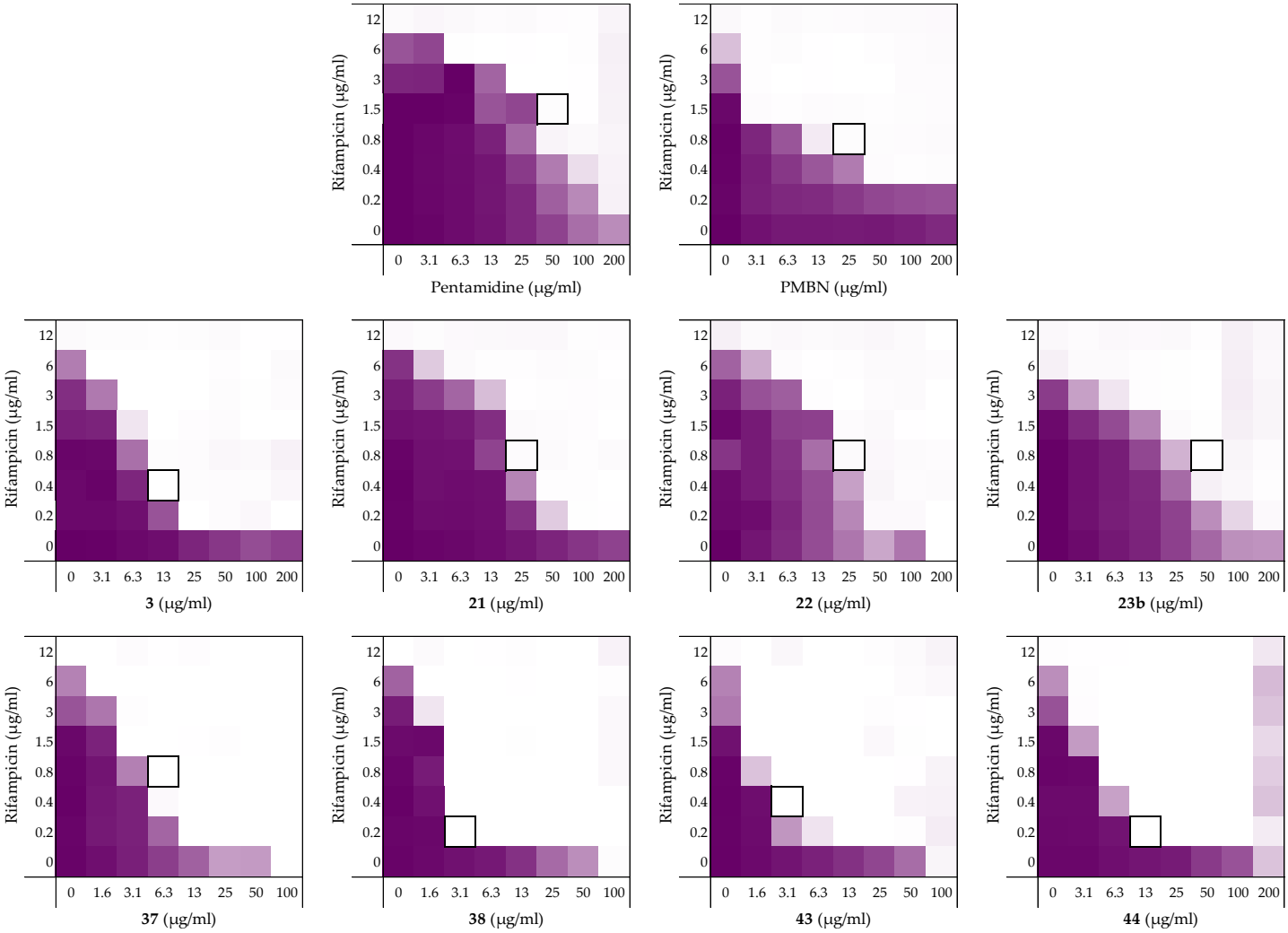
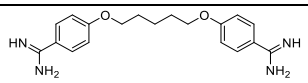
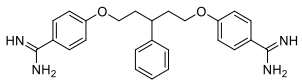
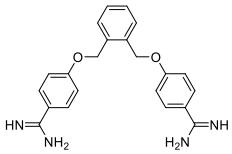
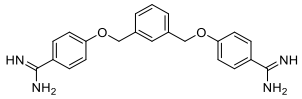
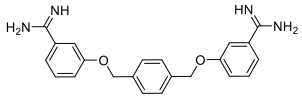
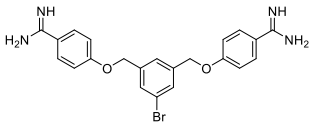
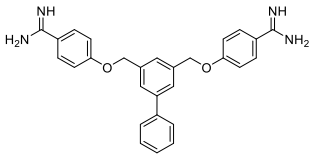
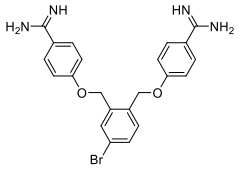
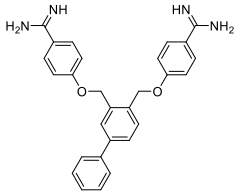


Figure S10 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* EQASmcr-1/EQAS 2016 412016126. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S10 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* EQASmcr-1/EQAS 2016 412016126 with rifampicin as shown in Figure S10. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	50	12	1.5	≤0.250
3		>200	12.5	12	0.38	≤0.063
21		>200	25	12	0.75	≤0.125
22		200	25	12	0.75	0.188
23b		>200	50	12	0.75	≤0.188
37		100	6.25	12	0.75	0.125
38		100	3.13	12	0.19	0.047
43		100	3.13	12	0.38	0.063
44		200	12.5	12	0.19	0.078
PMBN		>200	25	12	0.75	≤0.125

E. coli EQASmcr-2/EQAS 2016 KP37

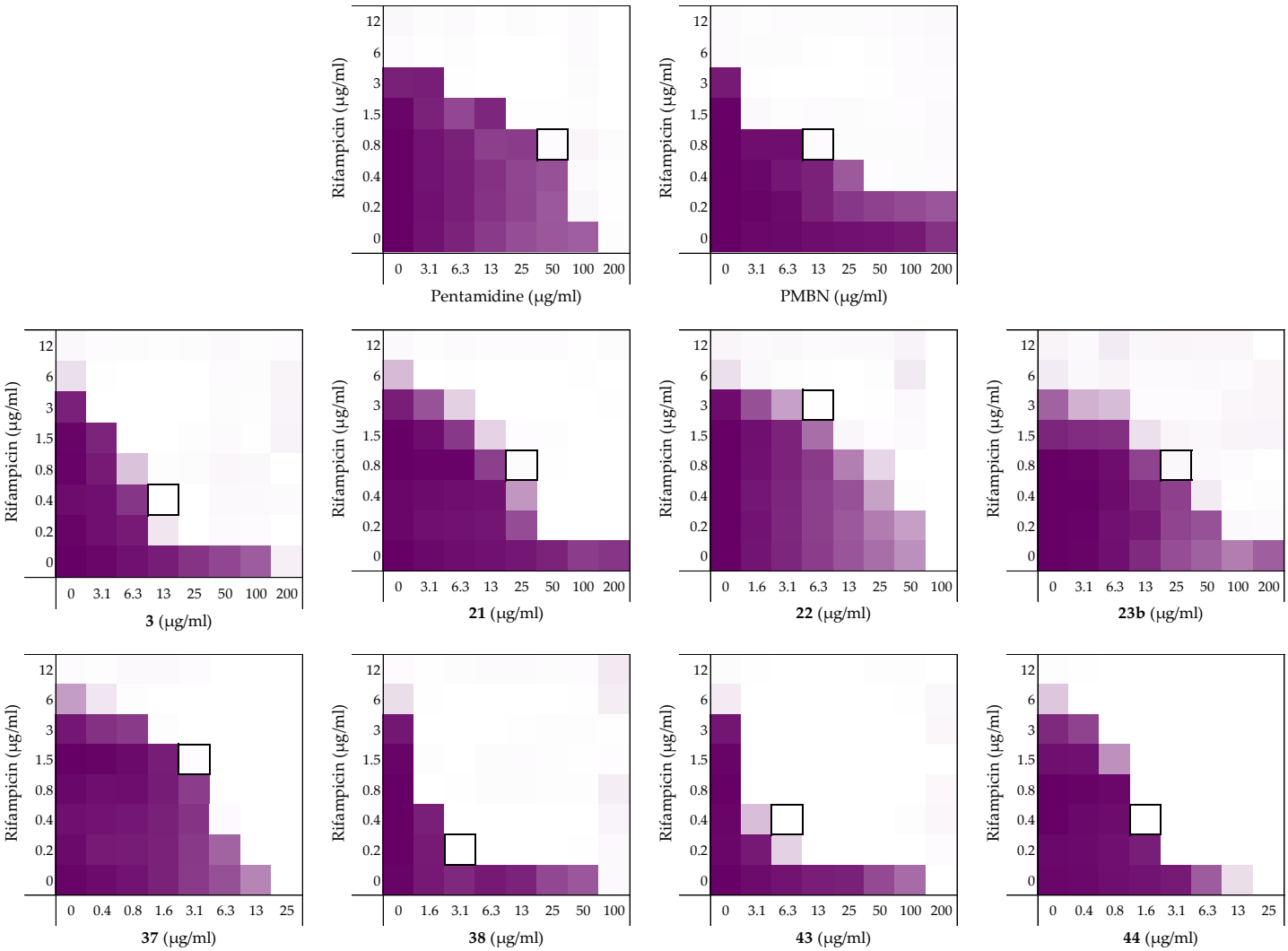
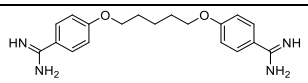
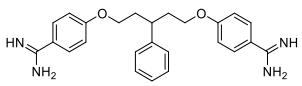
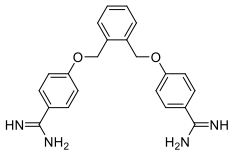
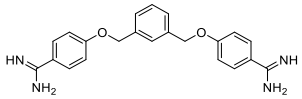
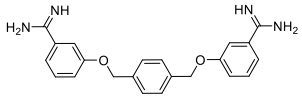
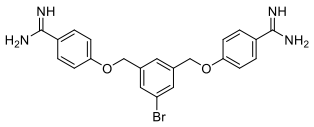
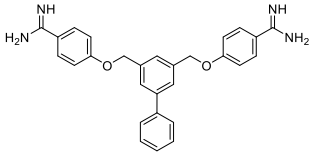
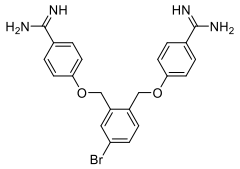
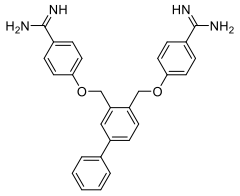


Figure S11 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* EQASmcr-2/EQAS 2016 KP37. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S11 Synergistic data compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* EQASmcr-2/EQAS 2016 KP37 with rifampicin as shown in Figure S11. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		200	50	6	0.75	0.375
3		>200	12.5	12	0.38	≤0.063
21		>200	25	12	0.75	≤0.125
22		100	6.25	12	3	0.313
23b		>200	25	12	0.75	≤0.125
37		25	3.13	12	1.5	0.250
38		100	3.13	12	0.19	0.047
43		200	6.25	12	0.38	0.063
44		25	1.56	12	0.38	0.094
PMBN		>200	12.5	6	0.75	≤0.156

E. coli EQASmcr-3/EQAS 2017 2013-SQ352

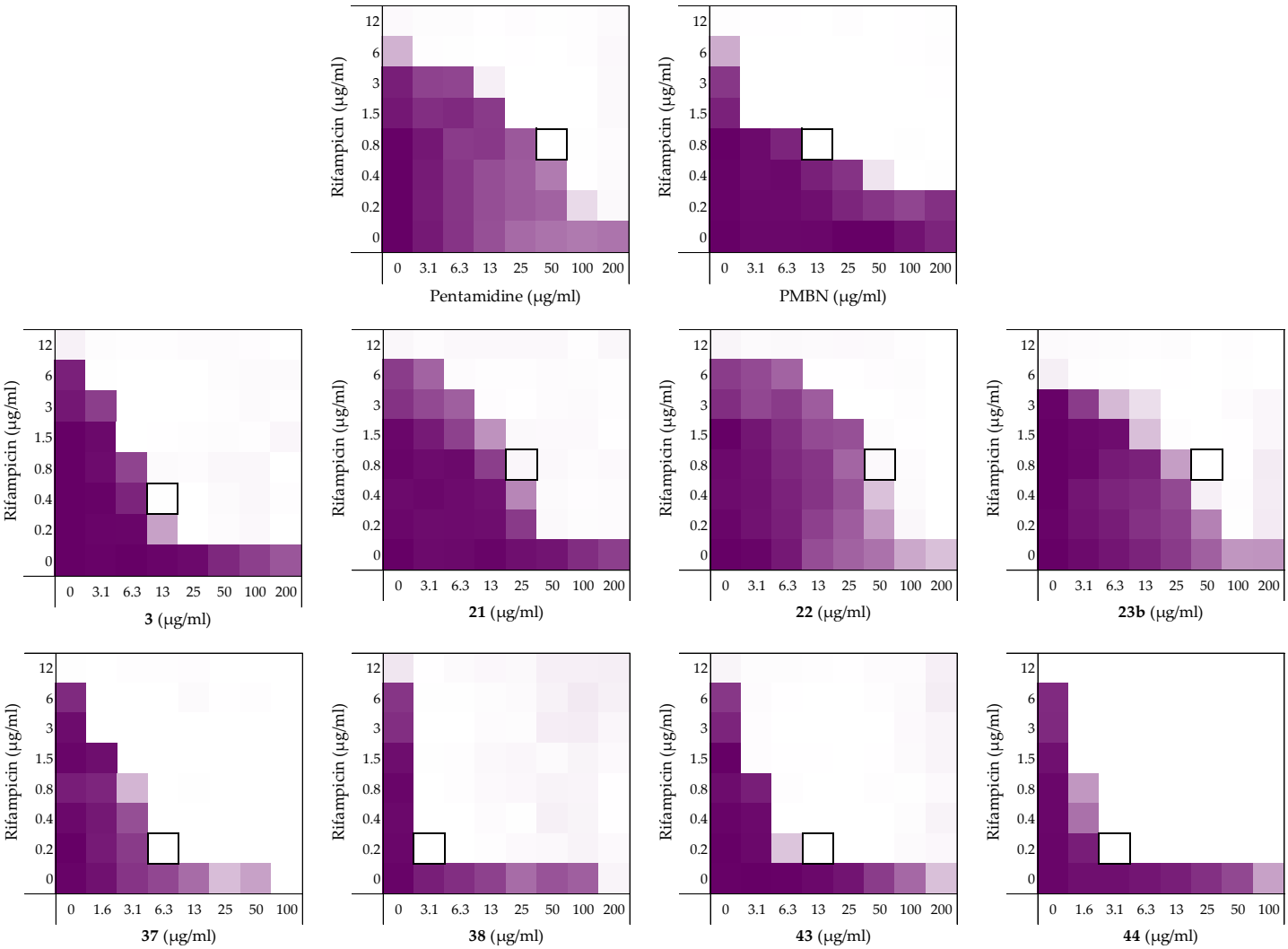
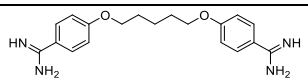
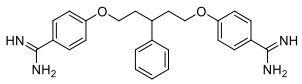
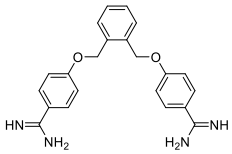
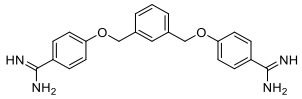
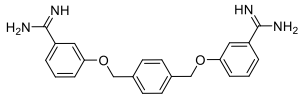
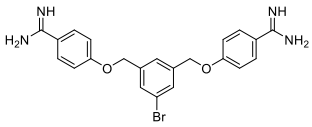
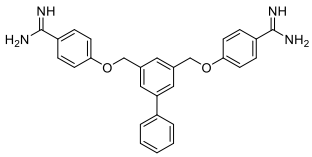
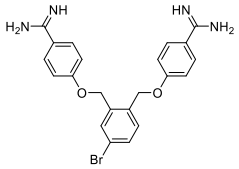
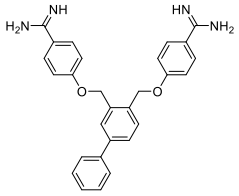


Figure S12 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* EQASmcr-3/EQAS 2017 2013-SQ352. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S12 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* EQASmcr-3/EQAS 2017 2013-SQ352 with rifampicin as shown in Figure S12. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	50	12	0.75	≤0.188
3		>200	12.5	12	0.38	≤0.063
21		>200	25	12	0.75	≤0.125
22		>200	50	12	0.75	≤0.188
23b		>200	50	12	0.75	≤0.188
37		100	6.25	12	0.19	0.078
38		200	3.13	12	0.19	0.031
43		>200	12.5	12	0.19	≤0.047
44		>100	3.13	12	0.19	≤0.031
PMBN		>200	12.5	12	0.75	≤0.094

E. coli RC00089

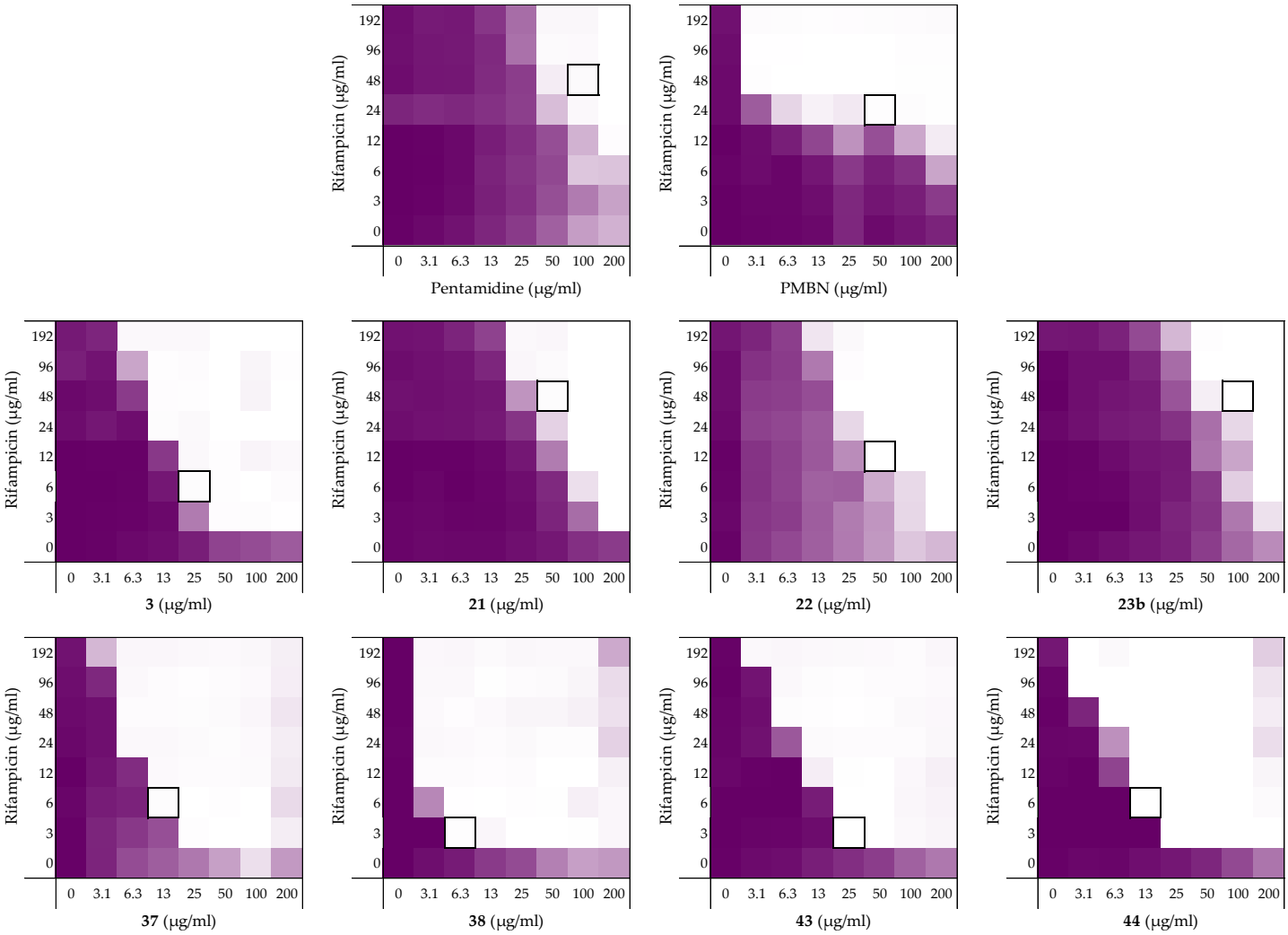
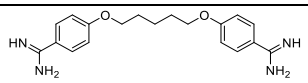
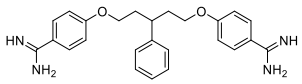
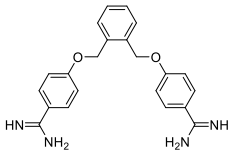
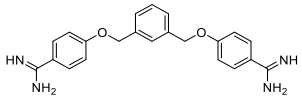
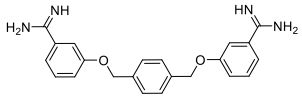
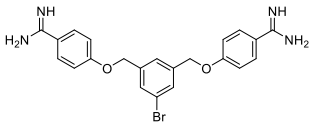
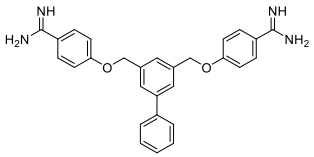
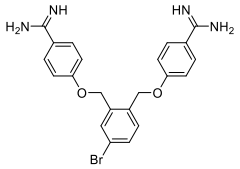
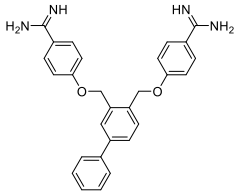


Figure S13 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *E.coli* RC00089. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S13 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *E.coli* RC00089 with rifampicin as shown in Figure S13. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	100	>192	48	≤0.375
3		>200	25	>192	6	≤0.078
21		>200	50	>192	48	≤0.250
22		>200	50	>192	12	≤0.156
23b		>200	100	>192	48	≤0.375
37		>200	12.5	>192	6	≤0.047
38		>200	6.25	>192	3	≤0.023
43		>200	25	>192	3	≤0.070
44		>200	12.5	>192	6	≤0.047
PMBN		>200	50	>192	24	≤0.188

A. baumannii ATCC17978

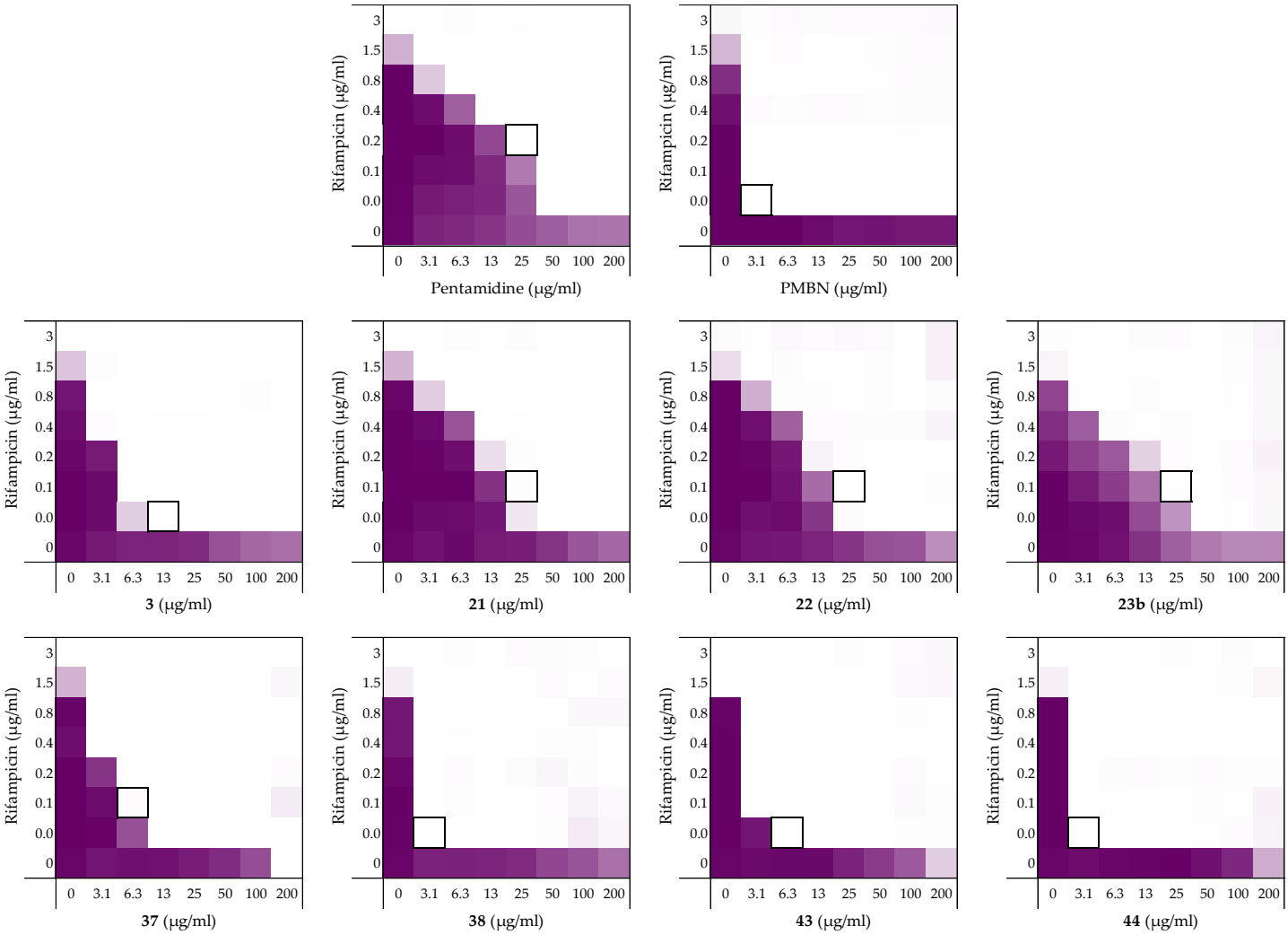
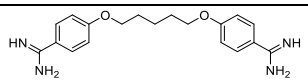
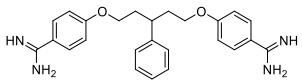
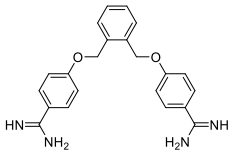
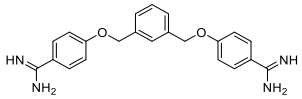
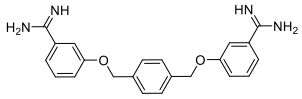
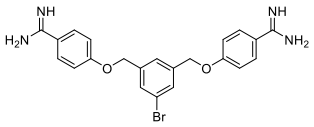
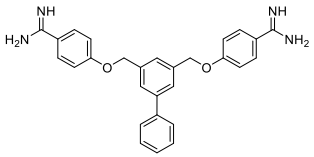
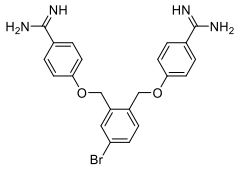
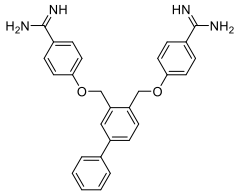


Figure S14 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *A. baumannii* ATCC17978. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S14 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *A. baumannii* ATCC17978 with rifampicin as shown in Figure S14. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	25	3	0.19	≤0.125
3		>200	12.5	3	0.05	≤0.047
21		>200	25	3	0.09	≤0.094
22		>200	25	3	0.09	≤0.094
23b		>200	25	3	0.09	≤0.094
37		>200	6.25	3	0.09	≤0.047
38		>200	3.13	3	0.05	≤0.023
43		>200	6.25	1.5	0.05	≤0.047
44		>200	3.13	3	0.05	≤0.023
PMBN		>200	3.13	3	0.05	≤0.023

K. pneumoniae ATCC13883

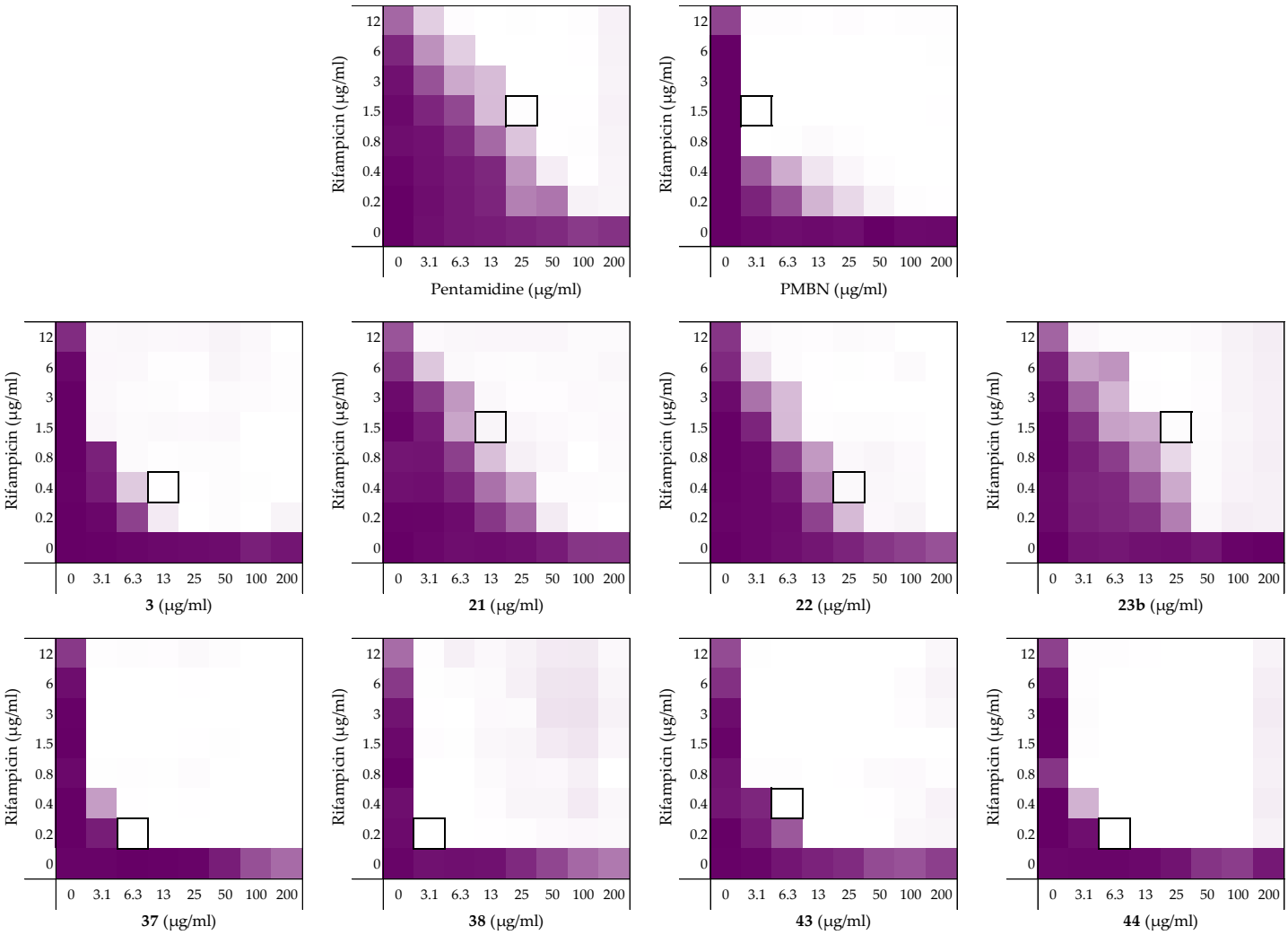
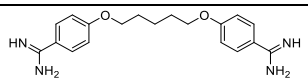
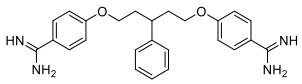
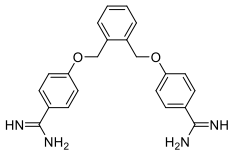
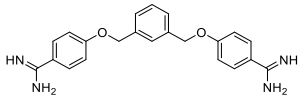
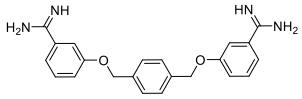
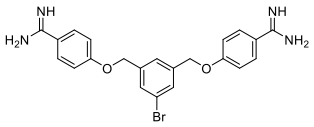
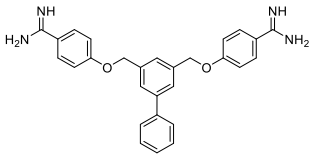
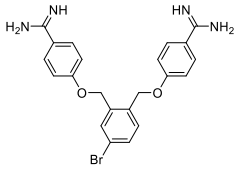
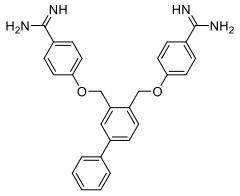


Figure S15 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *K. pneumoniae* ATCC13883. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S15 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *K. pneumoniae* ATCC13883 with rifampicin as shown in Figure S15. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	25	>12	1.5	≤0.125
3		>200	12.5	>12	0.38	≤0.047
21		>200	12.5	>12	1.5	≤0.094
22		>200	25	>12	0.38	≤0.078
23b		>200	25	>12	1.5	≤0.125
37		>200	6.25	>12	0.19	≤0.023
38		>200	3.13	>12	0.19	≤0.016
43		>200	6.25	>12	0.38	≤0.031
44		>200	6.25	>12	0.19	≤0.023
PMBN		>200	3.13	>12	1.5	≤0.070

P. aeruginosa ATCC27853

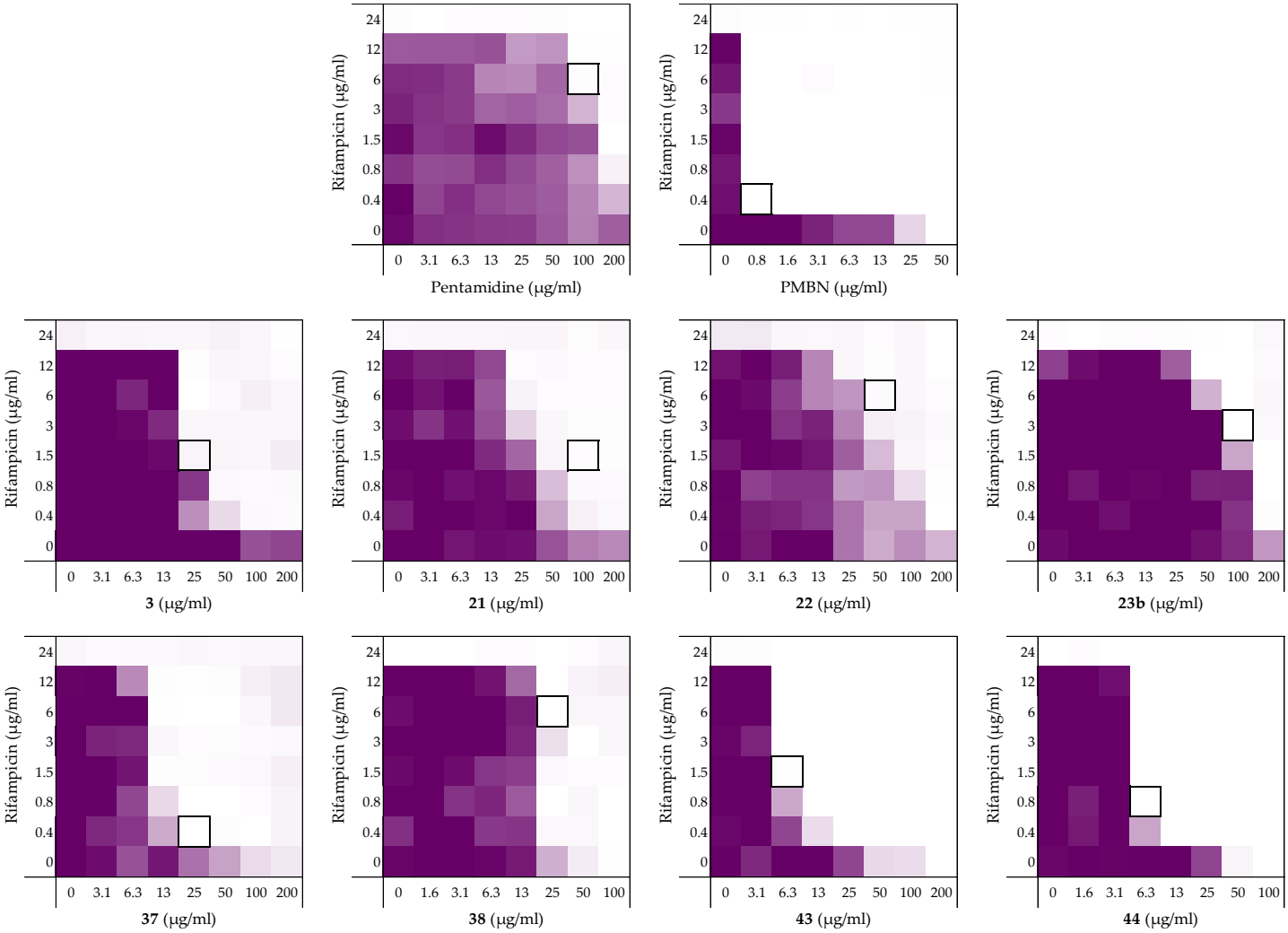
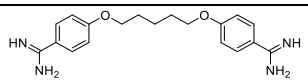
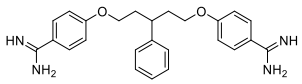
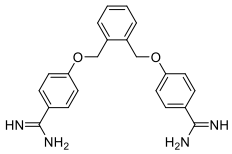
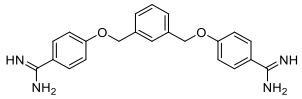
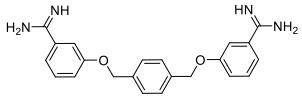
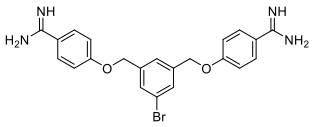
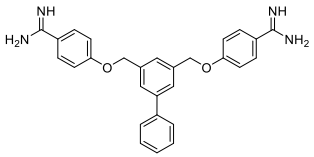
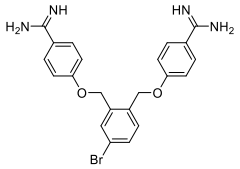
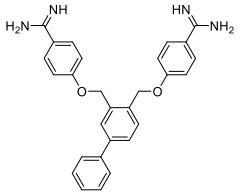


Figure S16 Checkerboard assays of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN in combination with rifampicin versus *P. aeruginosa* ATCC27853. OD_{600} values were measured using a plate reader and transformed to a gradient: purple represents growth, white represents no growth. In each case, the bounded box in the checkerboard assays indicates the minimal synergistic concentration (MSC) of compound and antibiotic resulting in the lowest FICI.

Table S16 Synergistic data of compounds **1**, **3**, **21**, **22**, **23b**, **37**, **38**, **43**, **44**, and PMBN of the checkerboard results for *P. aeruginosa* ATCC27853 with rifampicin as shown in Figure S16. All minimal inhibitory concentrations (MICs) and minimal synergistic concentrations (MSCs) are in μg/mL.

	Structures	MIC	MSC	MIC rif	MSC rif	FICI
1		>200	100	24	6	≤0.500
3		>200	25	24	1.5	≤0.125
21		>200	100	24	1.5	≤0.313
22		>200	50	>24	6	≤0.250
23b		>200	100	24	3	≤0.375
37		>200	25	24	0.38	≤0.078
38		100	25	24	6	0.500
43		200	6.25	24	1.5	0.094
44		100	6.25	24	0.75	0.094
PMBN		50	0.78	24	0.38	0.031

Hemolysis assay

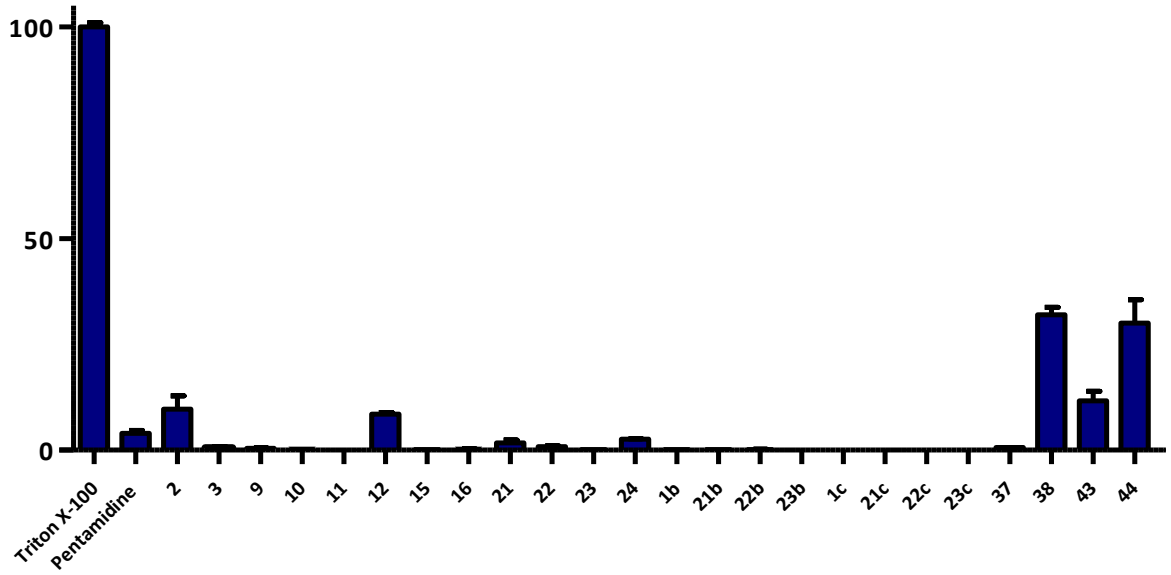


Figure S17 Hemolytic activity of al compounds (200 μg/mL) after 1 hour of incubation. The hemolysis assay was performed as described in materials and methods. Values below 10% were defined as non-hemolytic.[6] Error bars represent the standard deviation based on n=3 technical replicates.

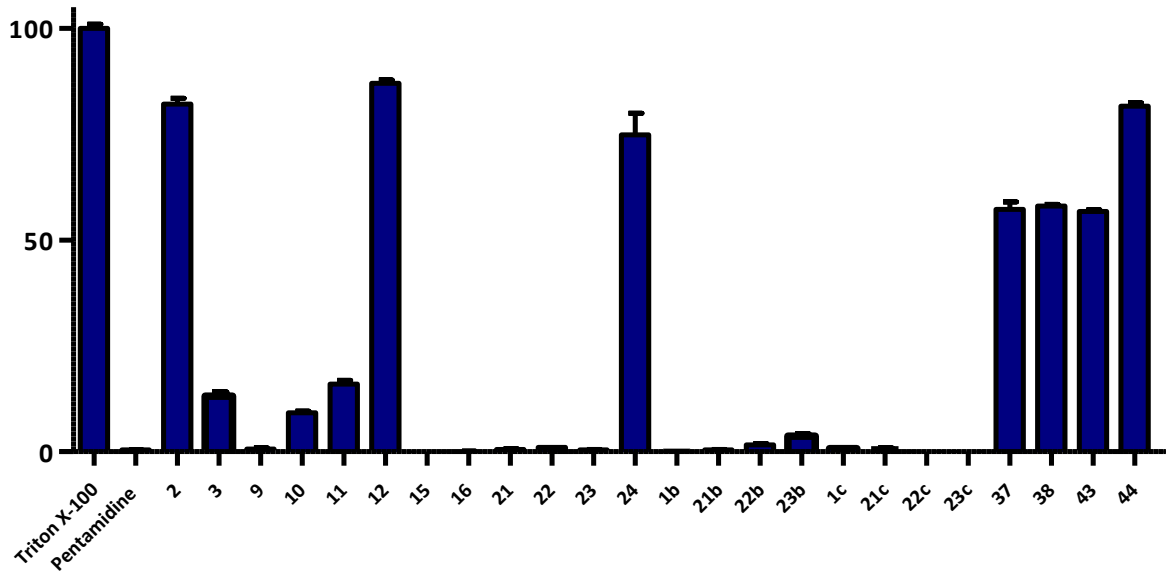
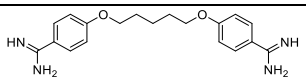
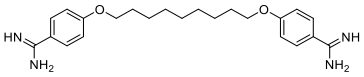
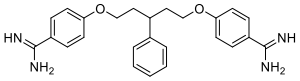
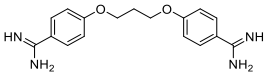
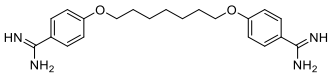
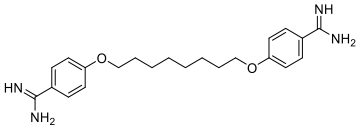
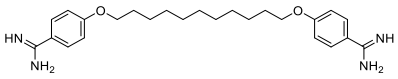
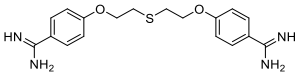
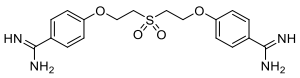
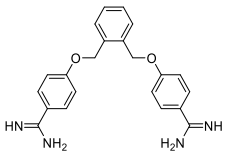
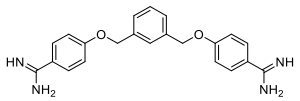
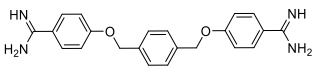
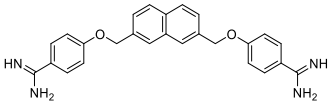
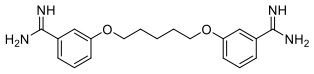
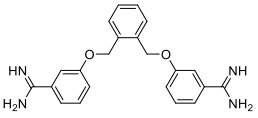
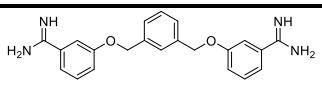
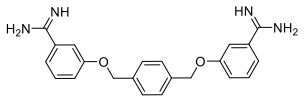
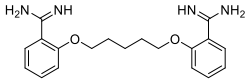
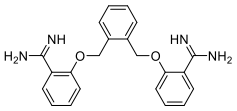
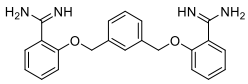
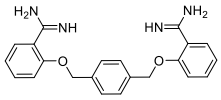
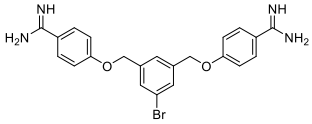
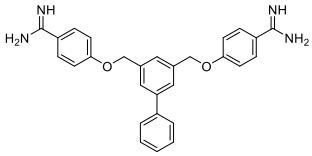
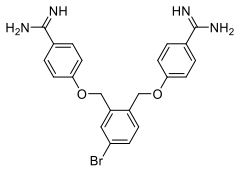
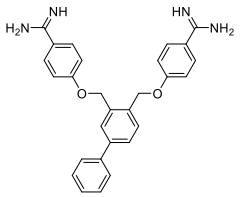


Figure S18 Hemolytic activity of all compounds (200 μg/mL) after 20 hours of incubation. The hemolysis assay was performed as described in materials and methods. Values below 10% were defined as non-hemolytic.[6] Error bars represent the standard deviation based on n=3 technical replicates.

Table S17 Hemolytic activity of all compounds (200 μg/mL). The hemolysis assay was performed as described in materials and methods. Values below 10% were defined as non-hemolytic.[6]

	Structures	Hemolysis 1 hour (%)	Hemolysis 20 hours (%)
1		4.0	0.4
2		9.7	82
3		0.5	13
9		0.4	0.6
10		0.3	9.2
11		0.0	16
12		8.5	87
15		0.2	0.0
16		0.2	0.1
21		1.7	0.5
22		0.9	1.1
23		0.2	0.4
24		2.6	75
1b		0.1	0.1
21b		0.1	0.4

22b		0.2	1.6
23b		0.0	3.7
1c		0.0	0.7
21c		0.0	0.4
22c		0.0	0.0
23c		0.0	0.0
37		0.6	57
38		32	58
43		12	57
44		30	82

Outer membrane permeability assay

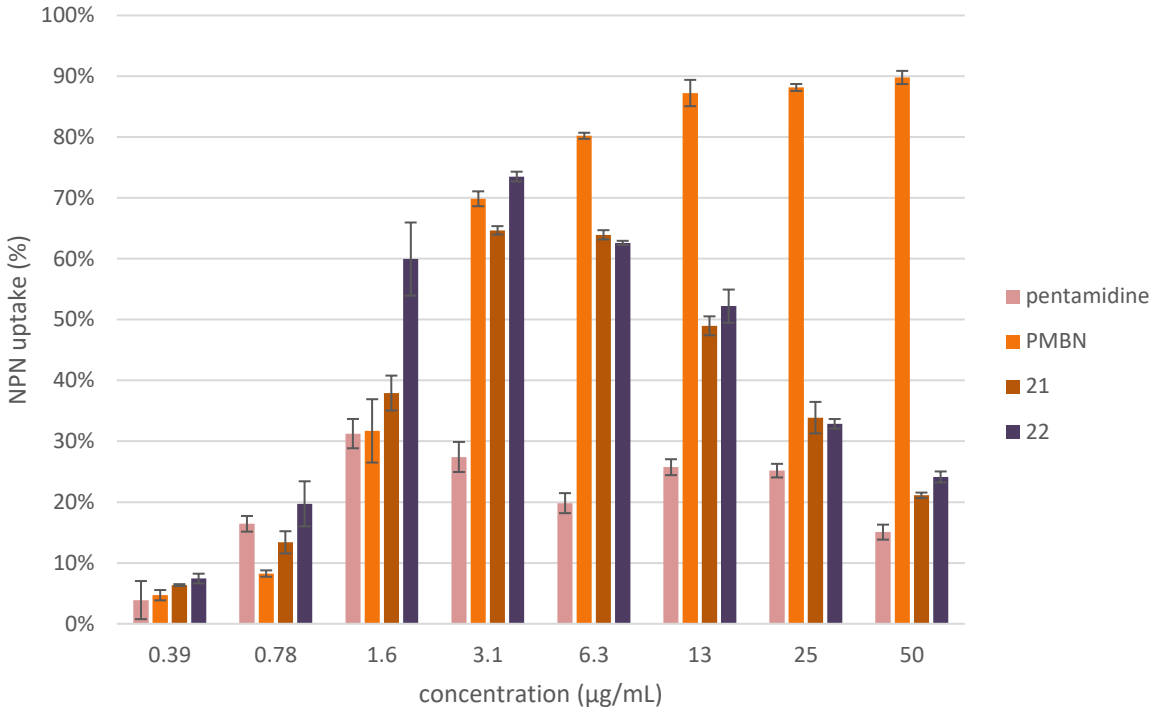
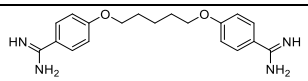
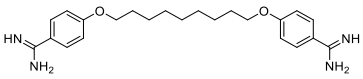
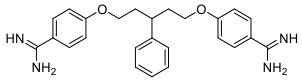
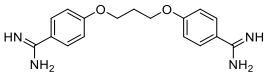
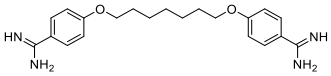
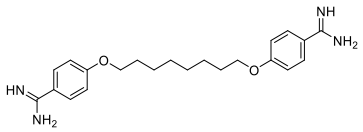
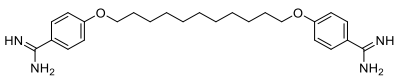
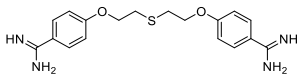
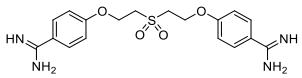
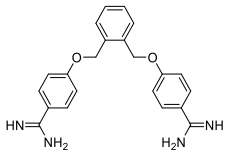
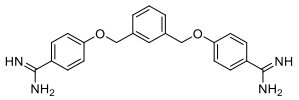
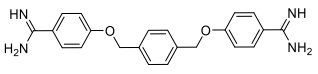
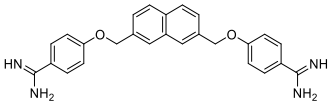


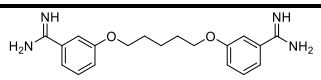
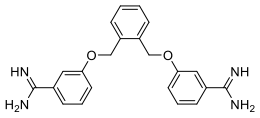
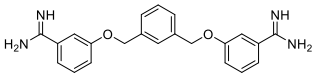
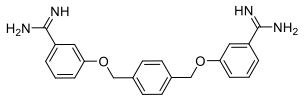
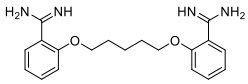
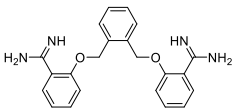
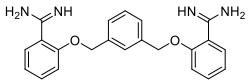
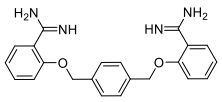
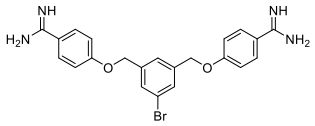
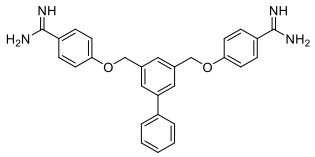
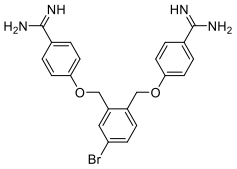
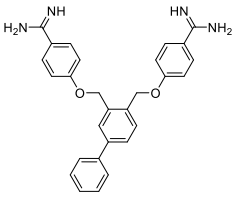
Figure S19 Outer membrane permeabilization assay of compounds **1**, **21**, **22**, and PMBN with *E.coli* BW25113 using *N*-phenyl-napthalen-1-amine (NPN) (at 0.01 mM) as fluorescent probe. The read-out was performed using a plate reader with λ_{ex} 355 nm and λ_{em} 420 nm. The NPN uptake values shown are relative to the uptake signal obtained upon treating the cells with 100 µg/mL colistin as previously reported.[7] Error bars represent the standard deviation based on n=3 technical replicates. Of note is the maximum NPN fluorescence measured for pentamidine and bis-amidines **21** and **22** at 3.1 µg/mL (0.01 mM). At higher bis-amidines concentrations, NPN fluorescence decreases, an effect not observed for PMBN.

Compound characterization and analysis

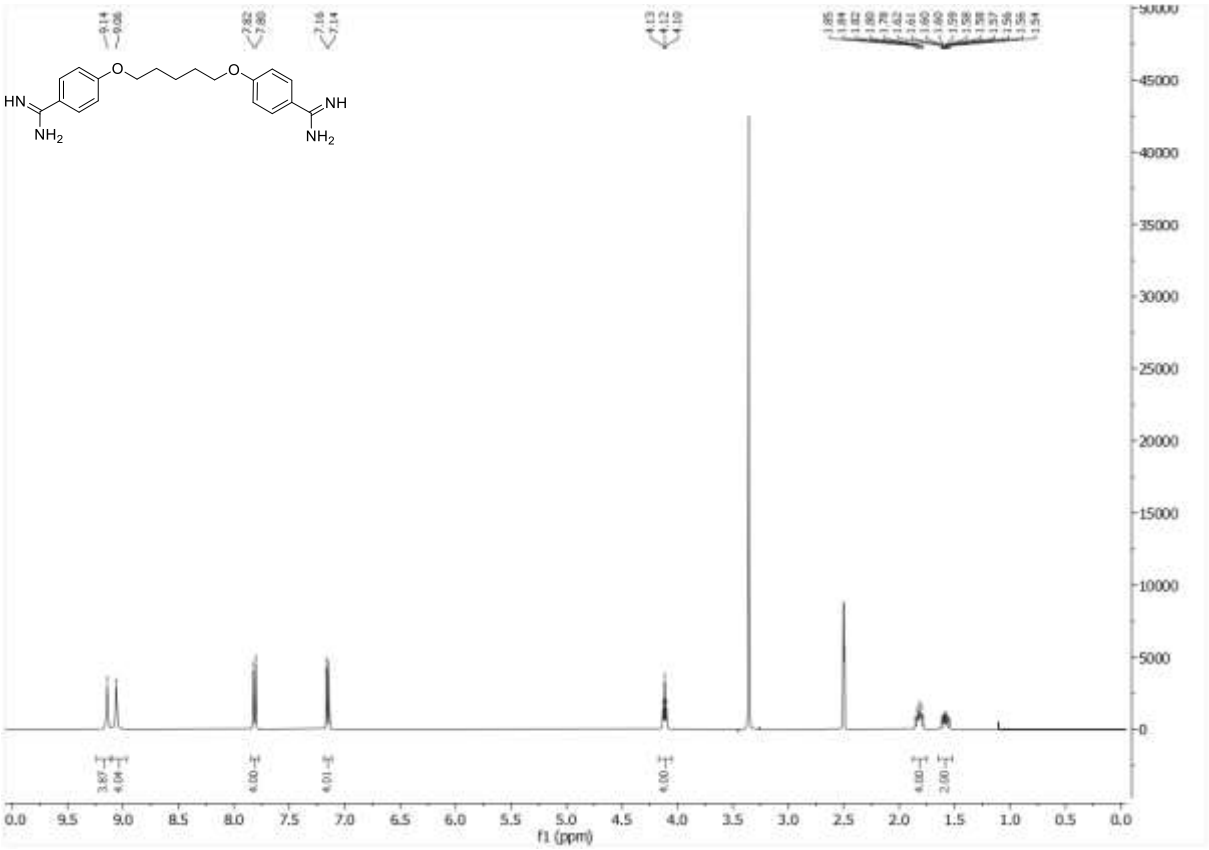
HRMS characterization

Table S18 Overview of the HRMS results obtained using a Shimadzu Nexera X2 UHPLC system with a Waters Acquity HSS C18 column (2.1 × 100 mm, 1.8 µm) at 30 °C and equipped with a diode array detector. This system was connected to a Shimadzu 9030 QTOF mass spectrometer (ESI ionisation) calibrated internally with Agilent's API-TOF reference mass solution kit (5.0 mM purine, 100.0 mM ammonium trifluoroacetate and 2.5 mM hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine) diluted to achieve a mass count of 10000.

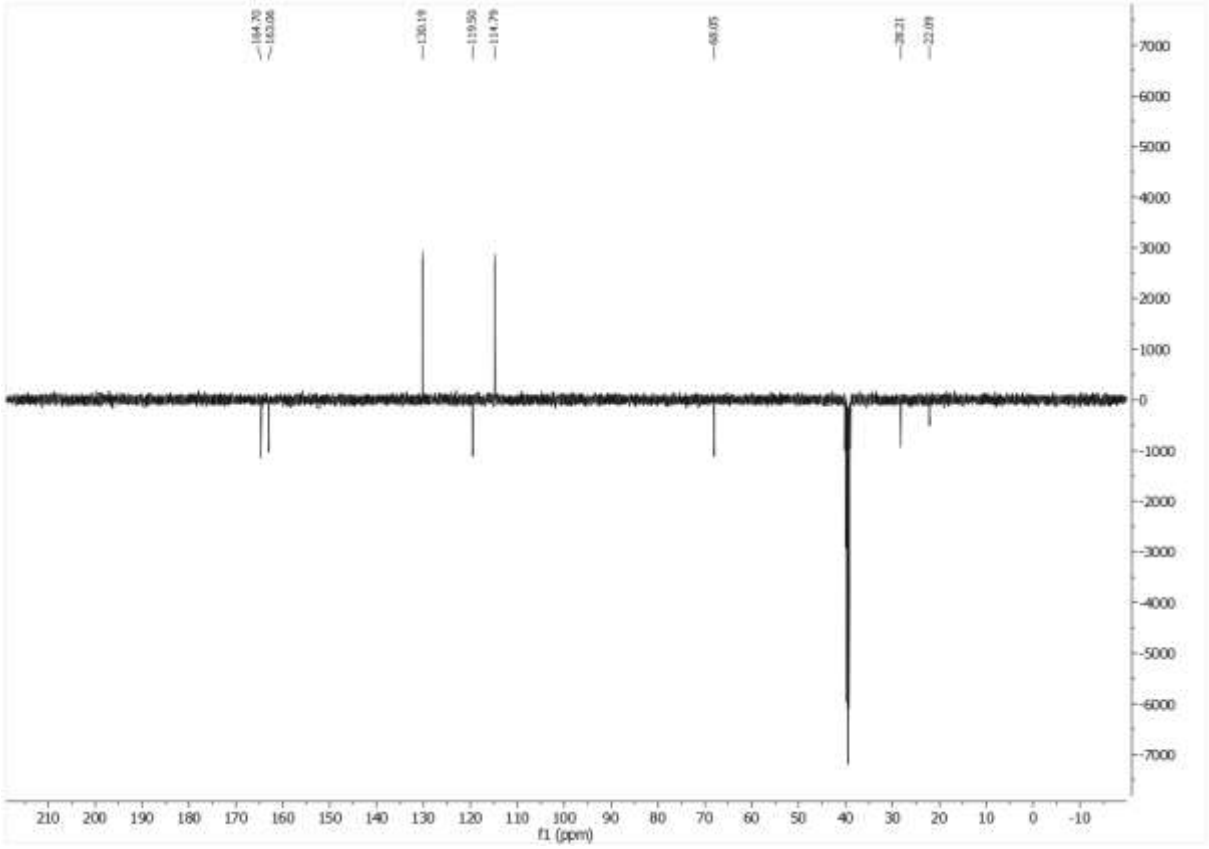
	Structures		$[M+H]^+$ calculated	$[M+H]^+$ found
1		C19H24N4O2	341.1977	341.1977
2		C23H32N4O2	397.2604	397.2597
3		C25H28N4O2	417.2291	417.2287
9		C17H20N4O2	313.1664	313.1662
10		C21H28N4O	369.2290	369.2290
11		C22H30N4O2	383.2447	383.2446
12		C25H36N4O2	425.2916	425.2919
15		C18H22N4O2S	359.1541	359.1541
16		C18H22N4O4S	391.1441	391.1434
21		C22H22N4O2	375.1821	375.1821
22		C22H22N4O2	375.1821	375.1821
23		C22H22N4O2	375.1821	375.1820
24		C26H24N4O2	425.1977	425.1977

1b		C19H24N4O2	341.1977	341.1977
21b		C22H22N4O2	375.1821	375.1821
22b		C22H22N4O2	375.1821	375.1821
23b		C22H22N4O2	375.1821	375.1818
1c		C19H24N4O2	341.1977	341.1972
21c		C22H22N4O2	375.1821	375.1815
22c		C22H22N4O2	375.1821	375.1816
23c		C22H22N4O2	375.1821	375.1816
37		C22H21BrN4O2	453.0926	453.0924
38		C28H26N4O2	451.2135	451.2130
43		C22H21BrN4O2	453.0926	453.0923
44		C28H26N4O2	451.2135	451.2129

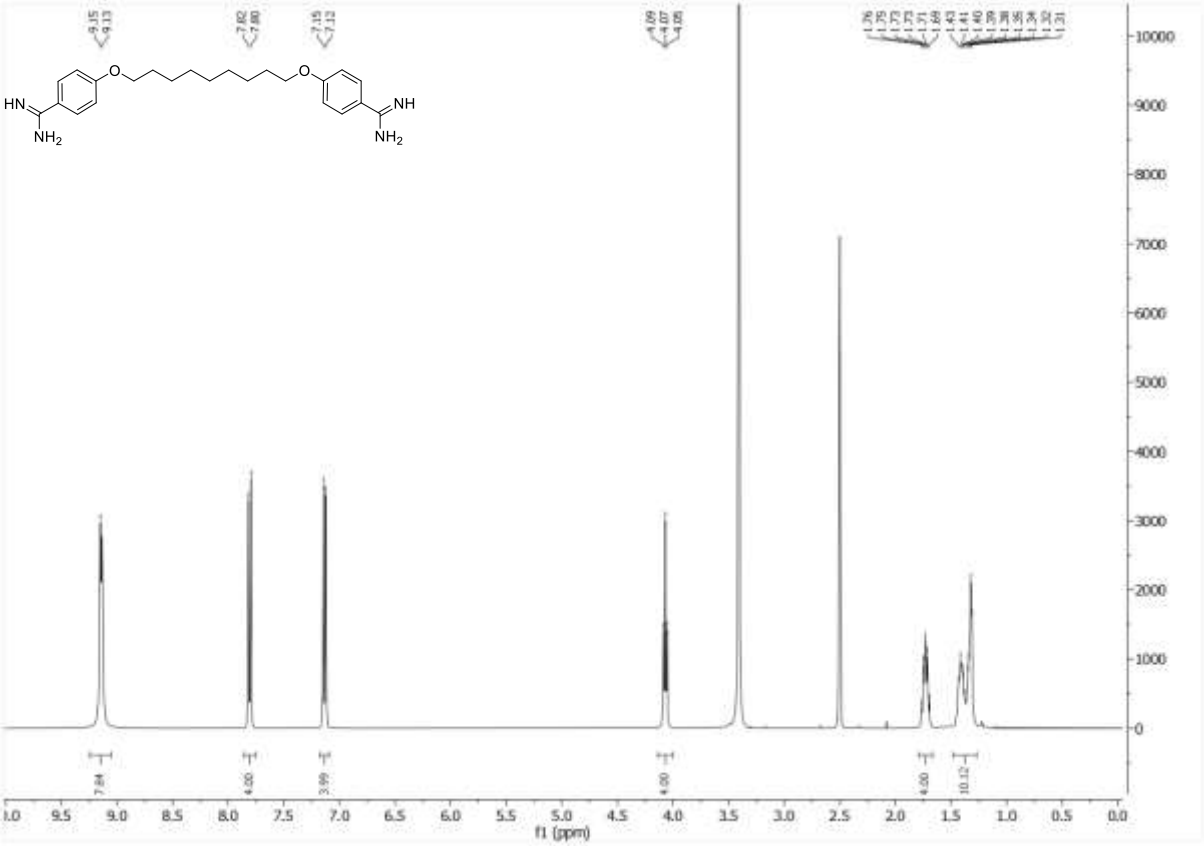
NMR data Compound **1** ^{1}H NMR (DMSO)



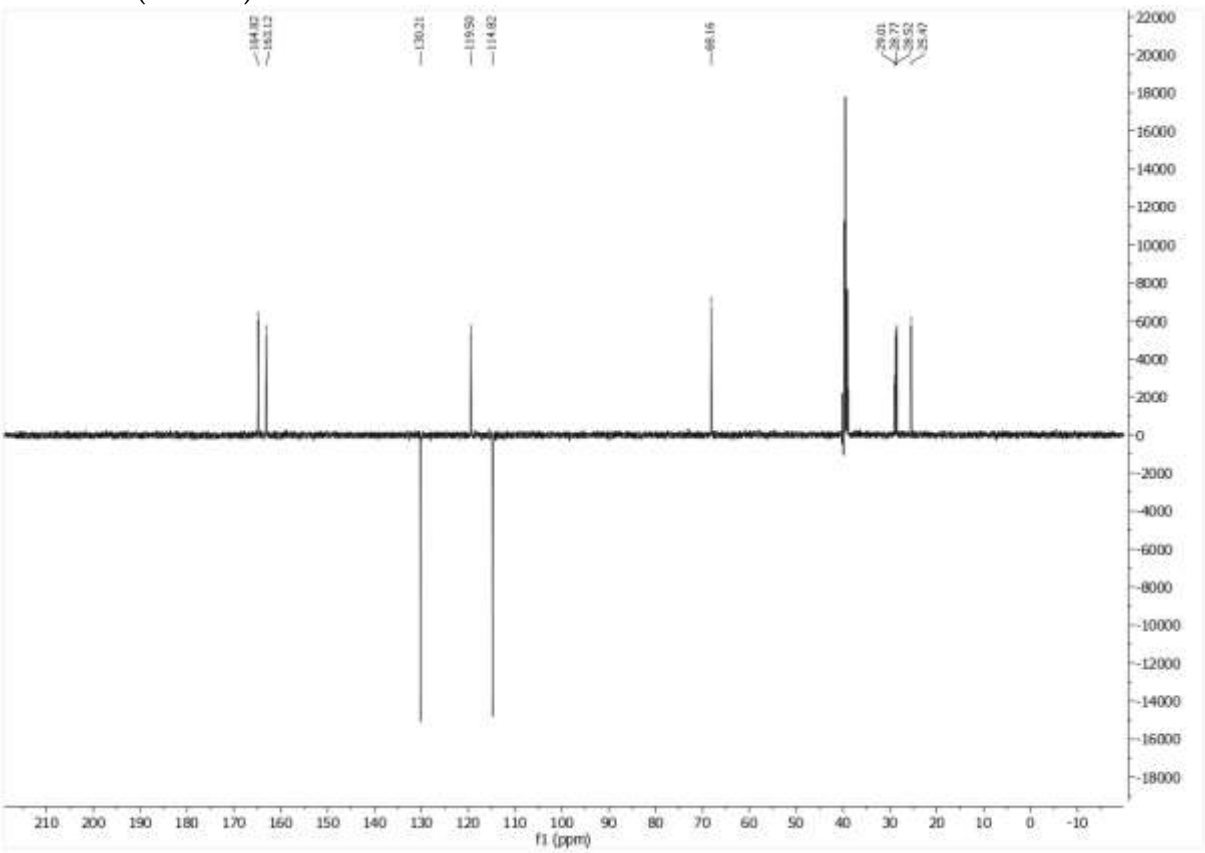
^{13}C NMR (DMSO)



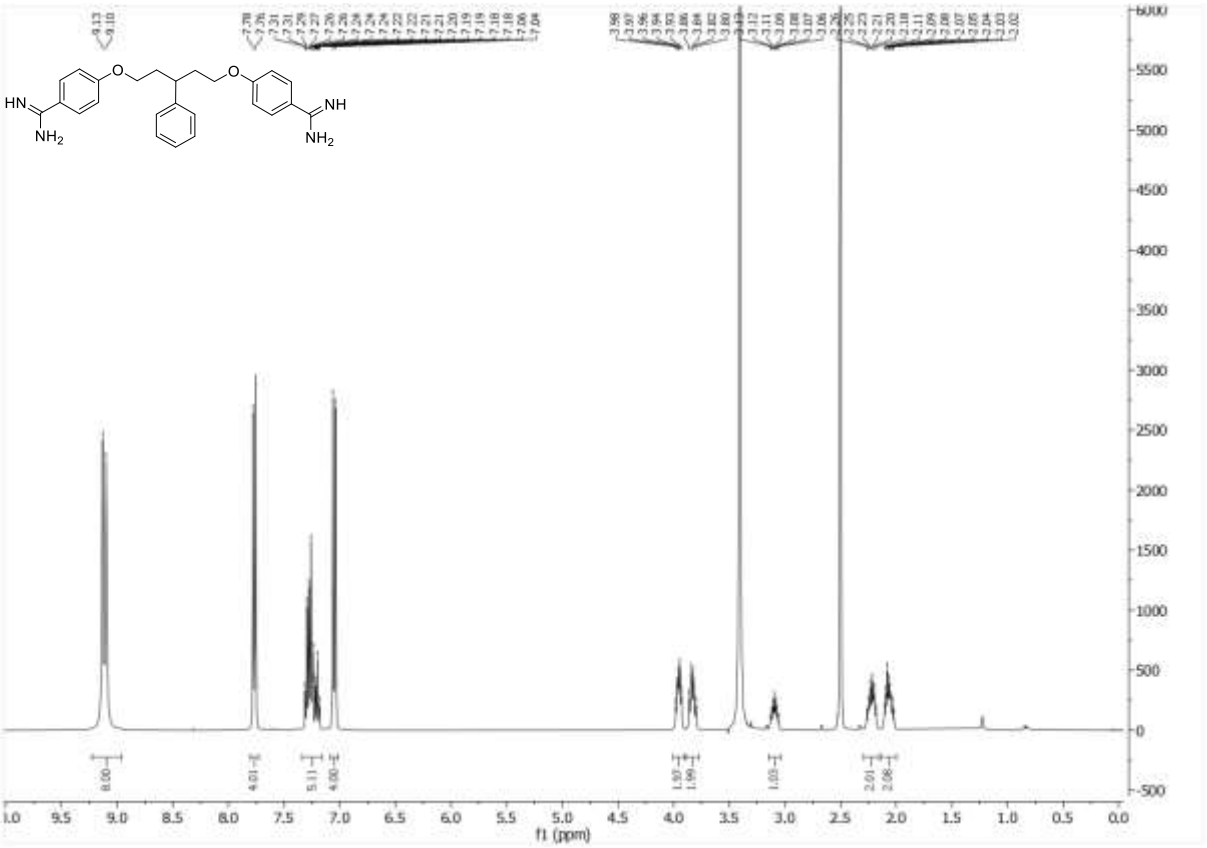
Compound **2** ^{1}H NMR (DMSO)



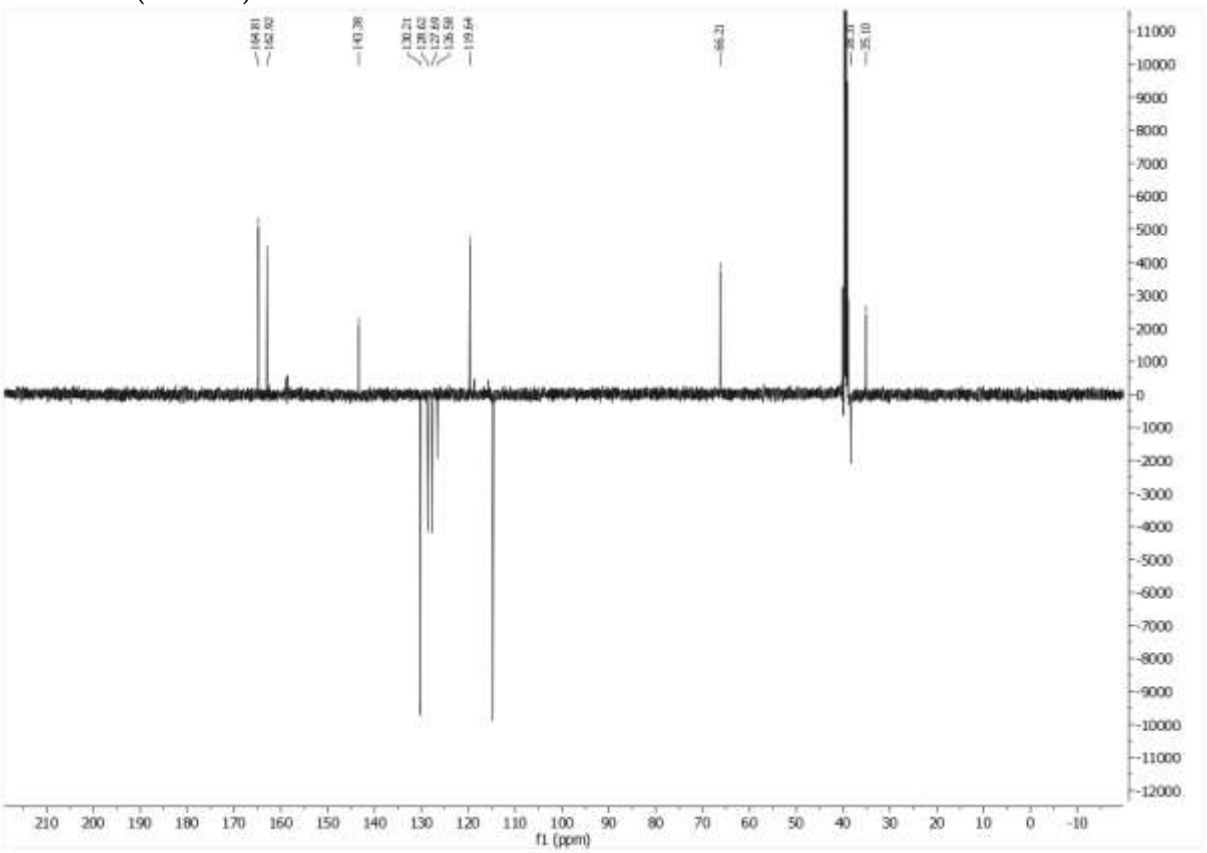
^{13}C NMR (DMSO)



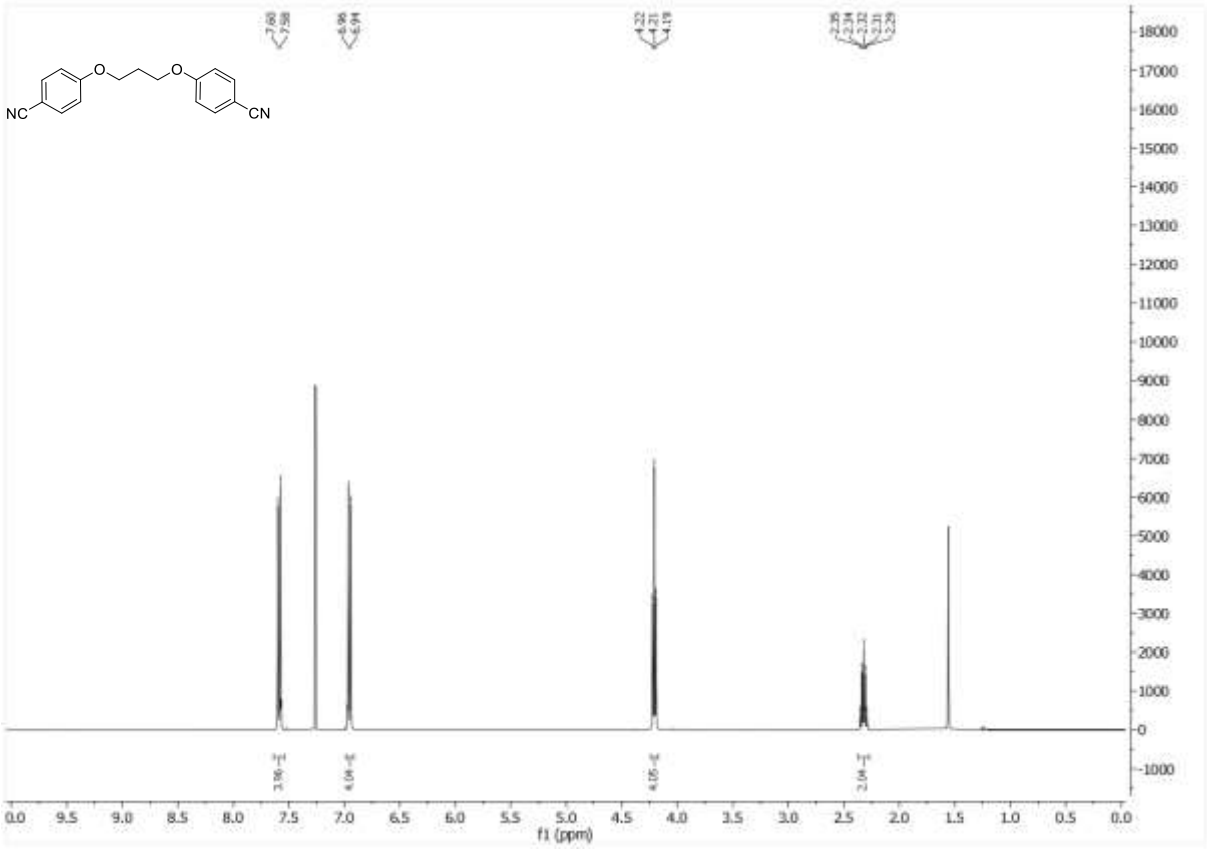
Compound **3** ^{1}H NMR (DMSO)



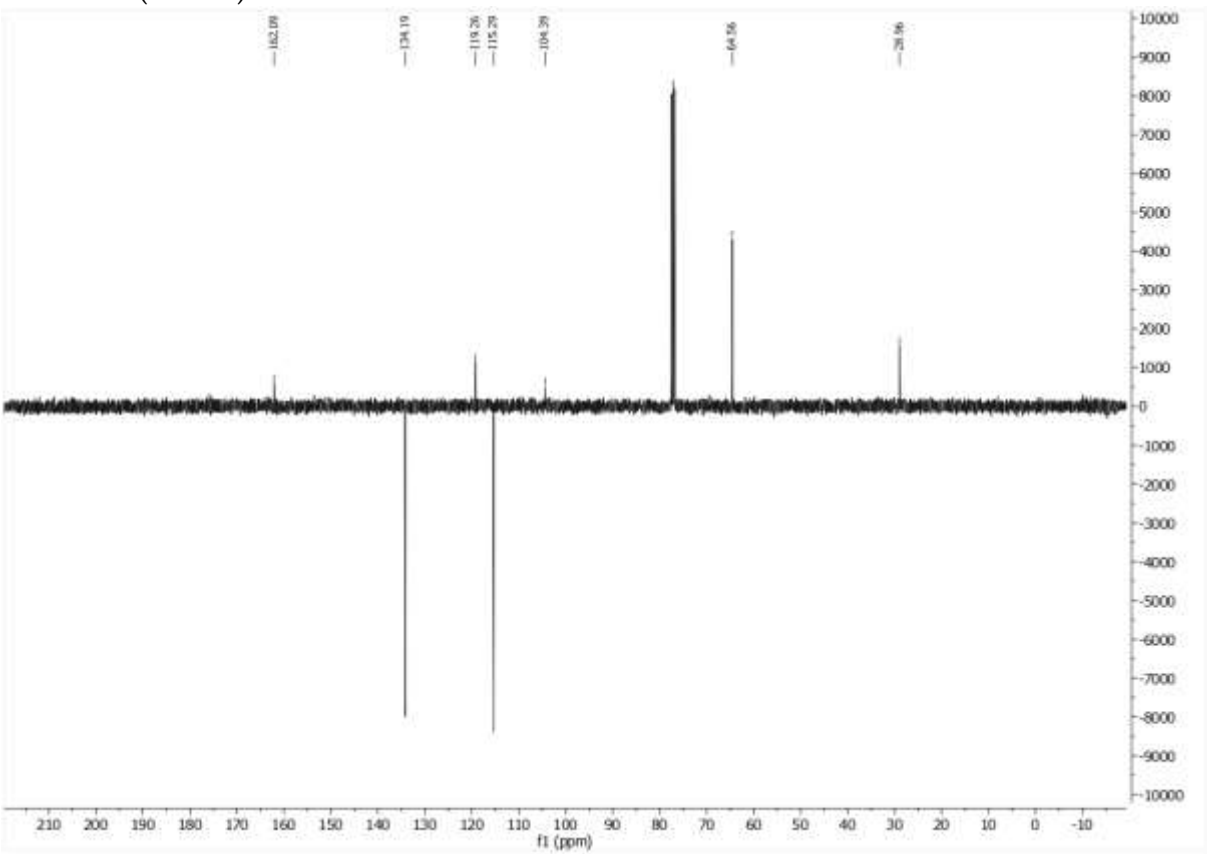
^{13}C NMR (DMSO)



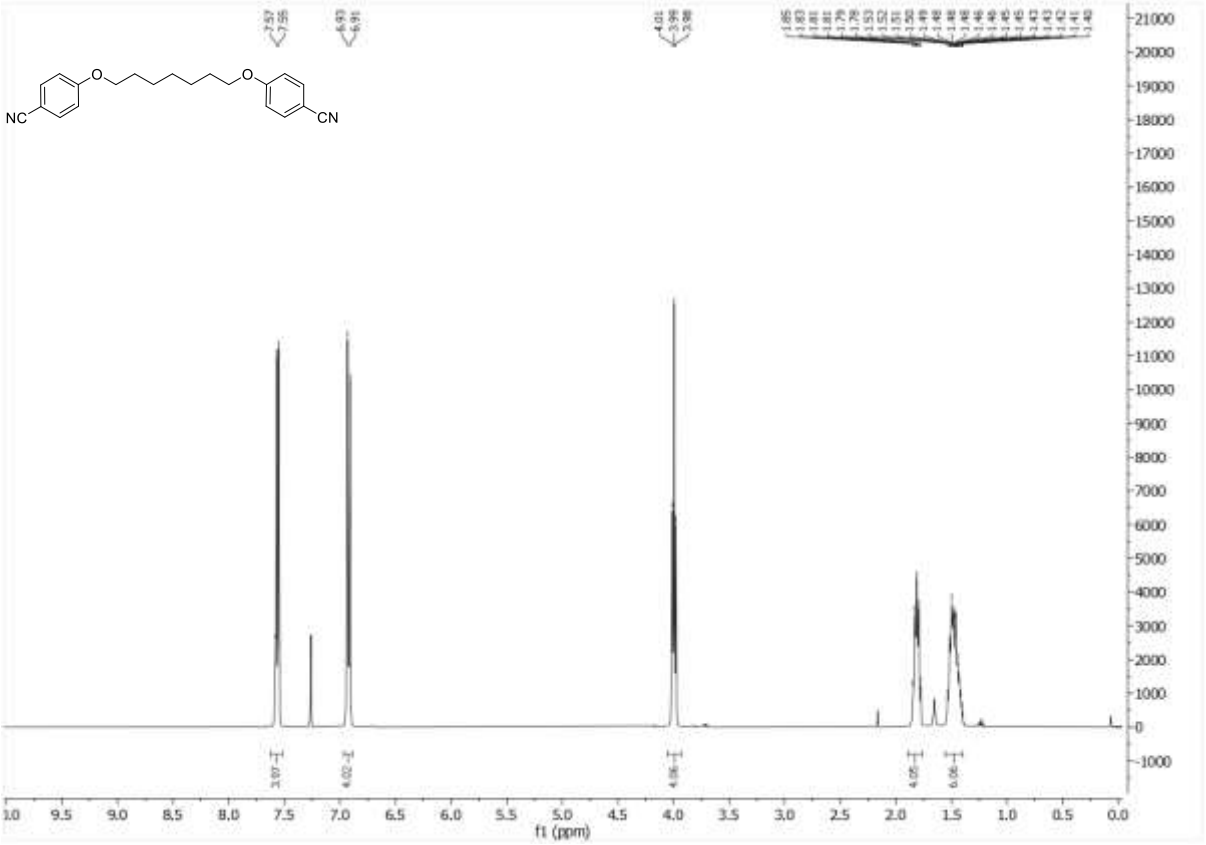
Compound **4** ^{1}H NMR ($CDCl_3$)



^{13}C NMR ($CDCl_3$)



Compound **5** ^{1}H NMR ($CDCl_3$)



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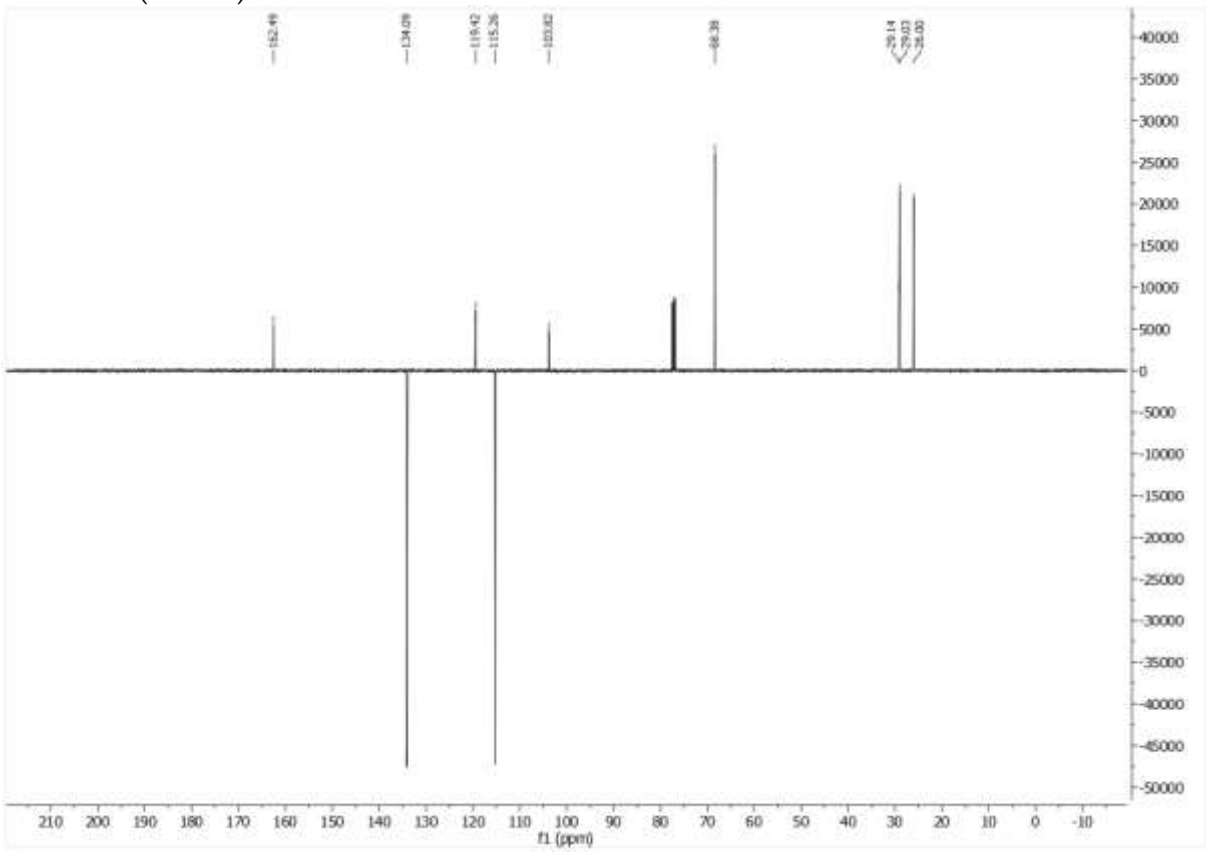

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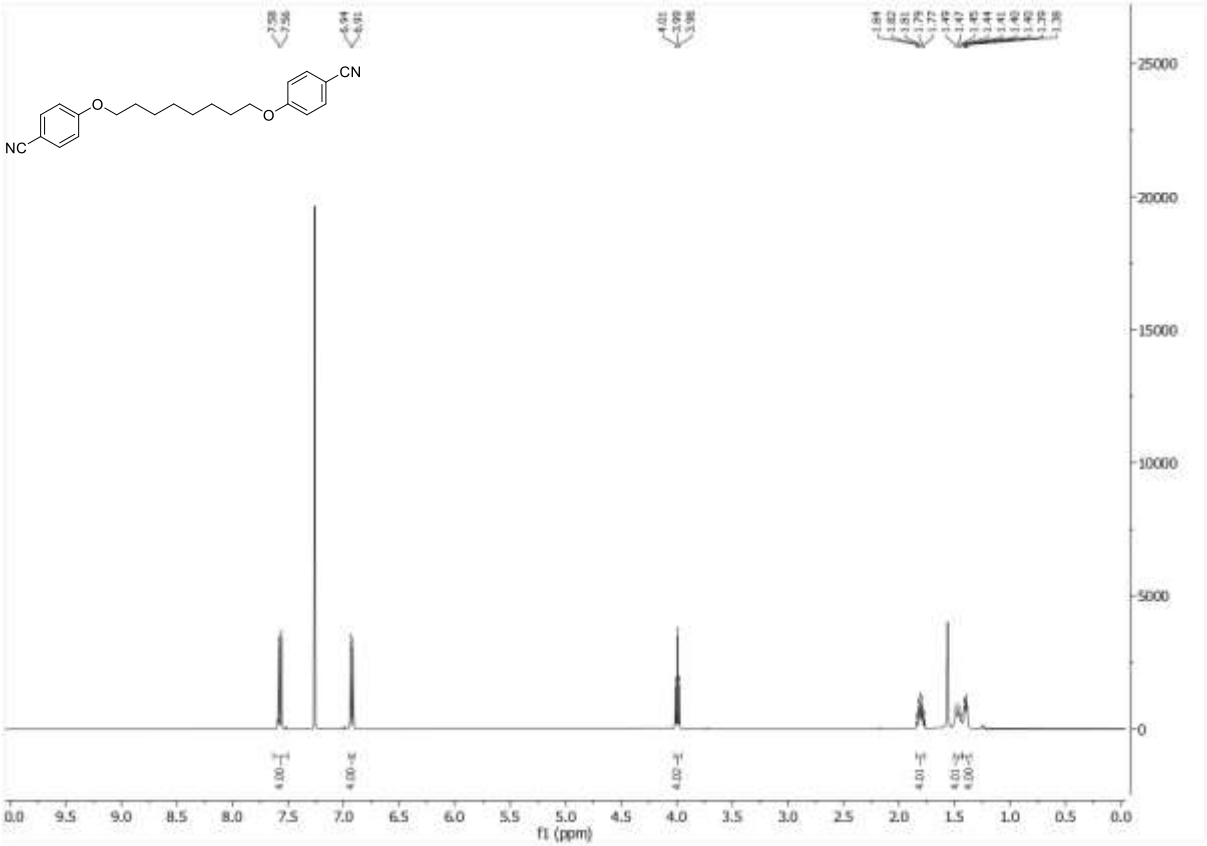
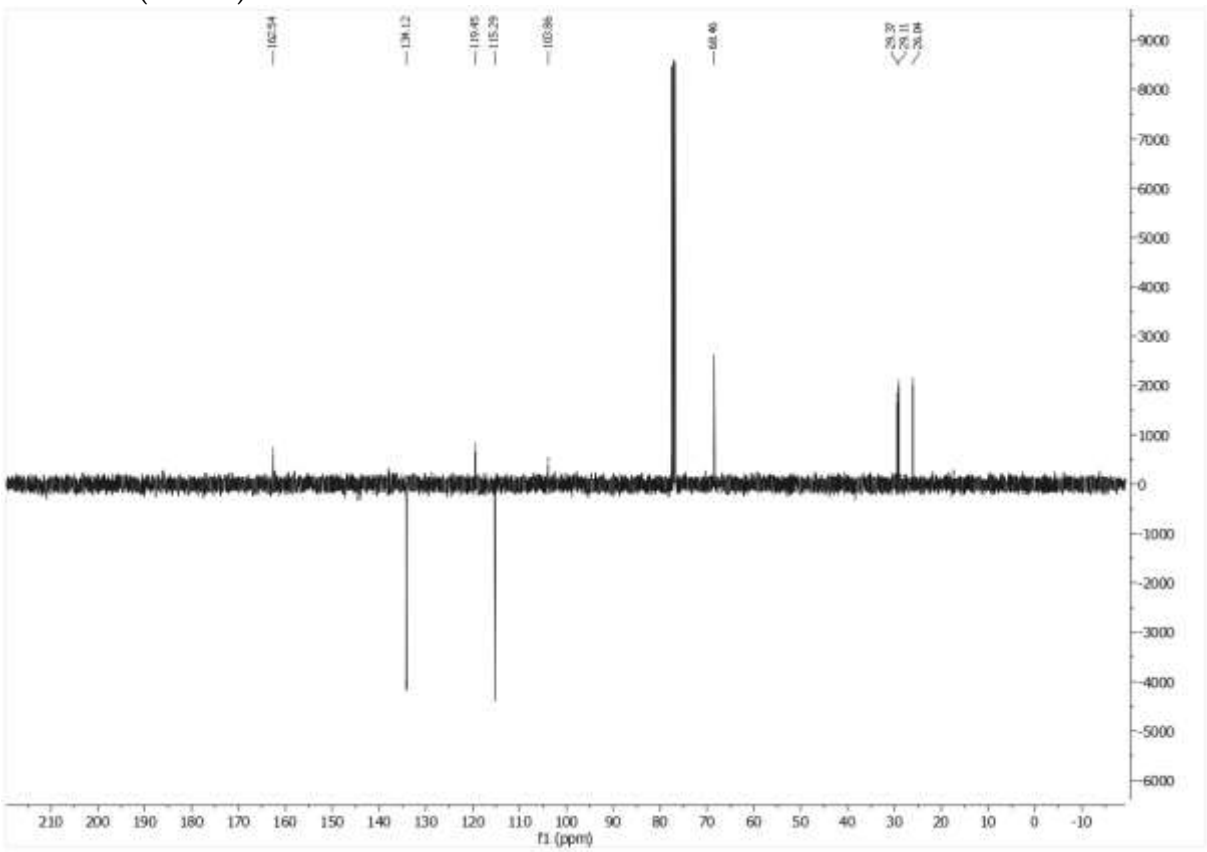
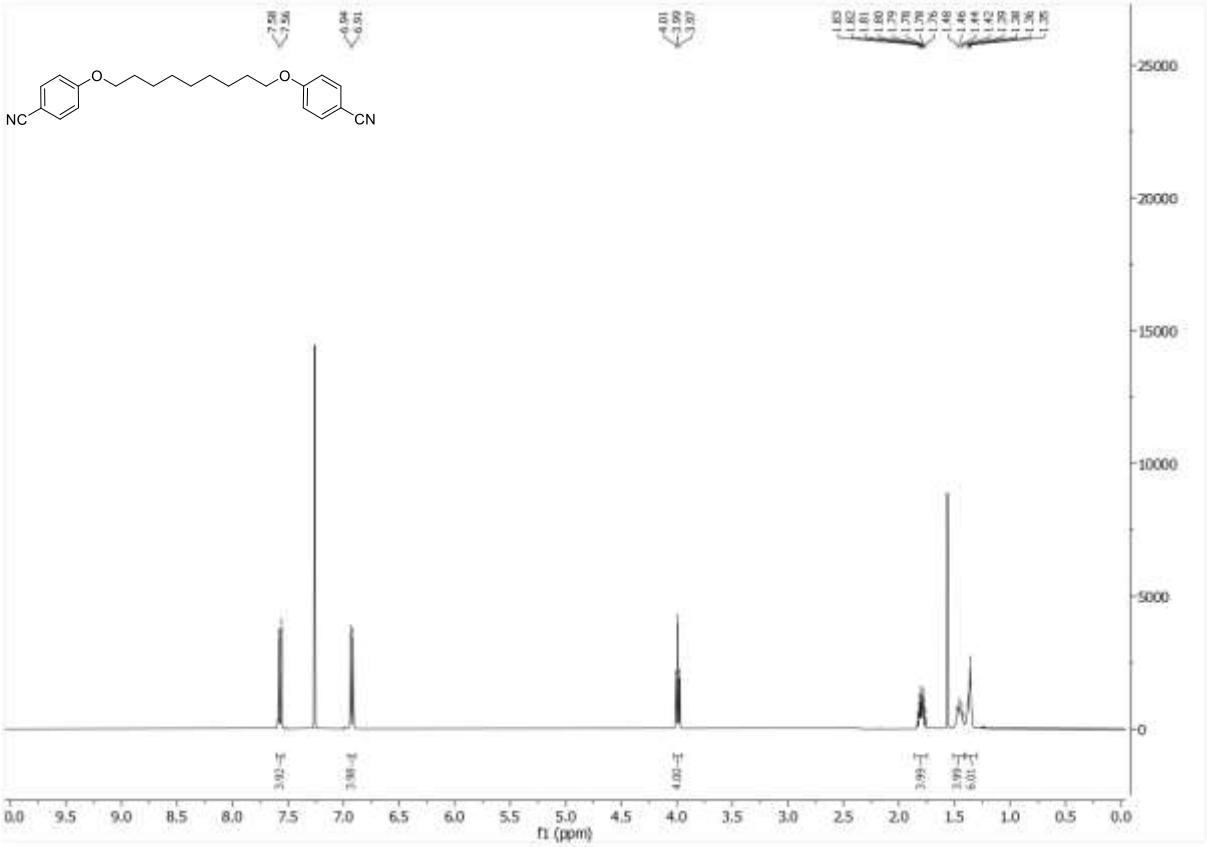


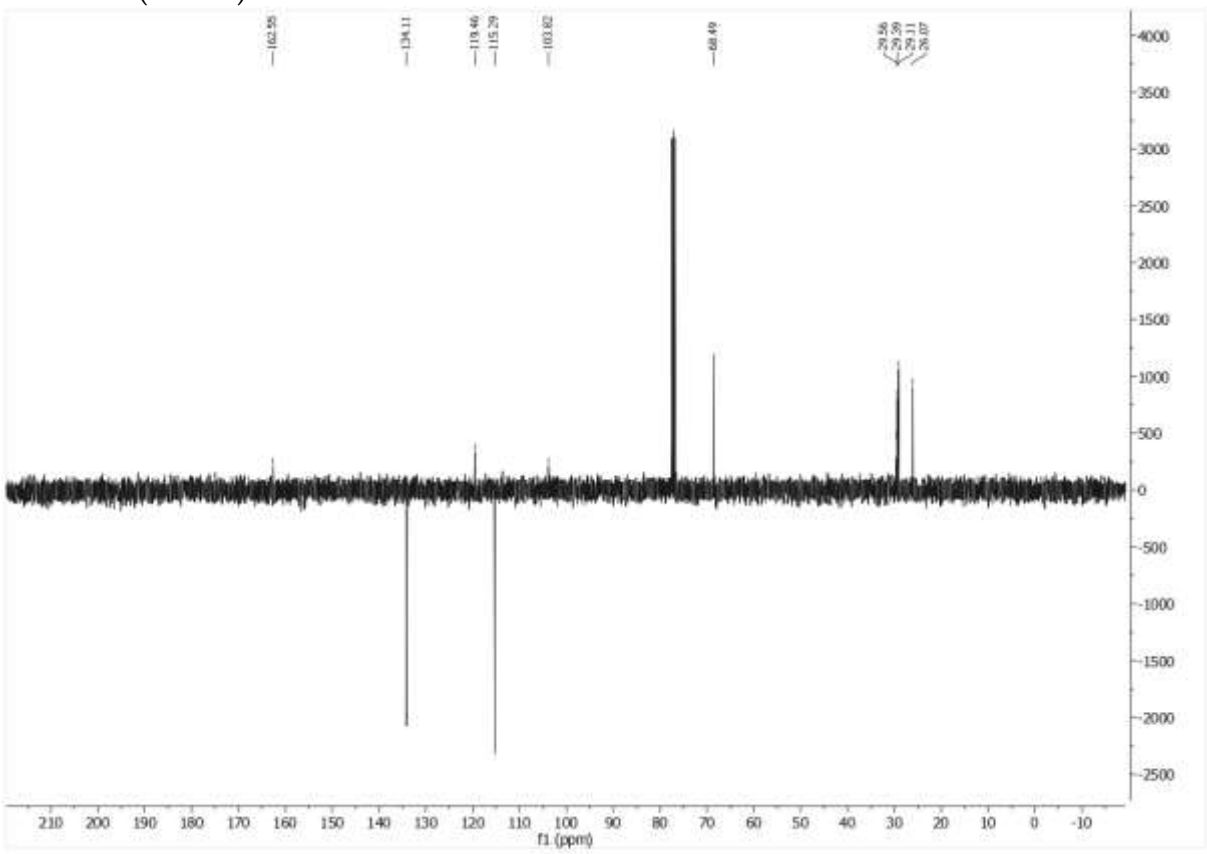
^{13}C NMR ($CDCl_3$)



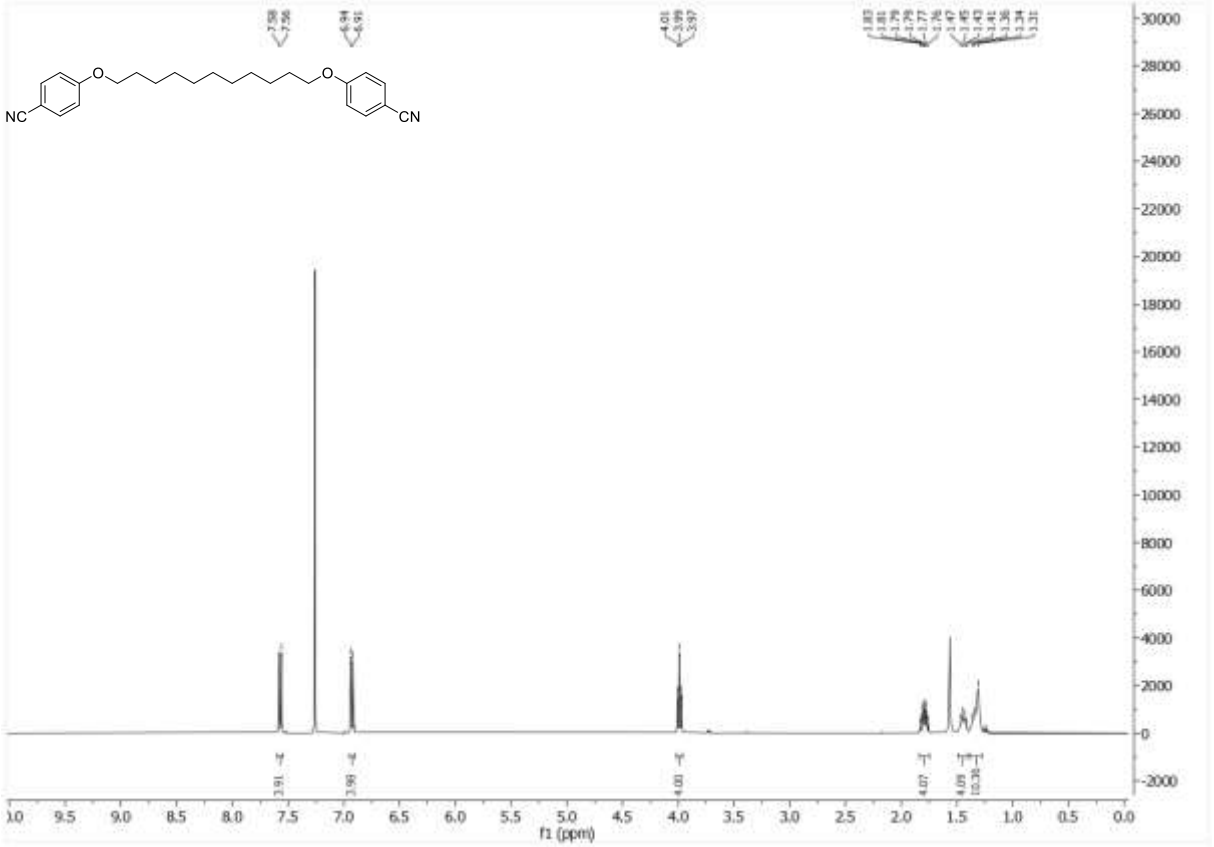
Compound **7** ^{1}H NMR ($CDCl_3$)



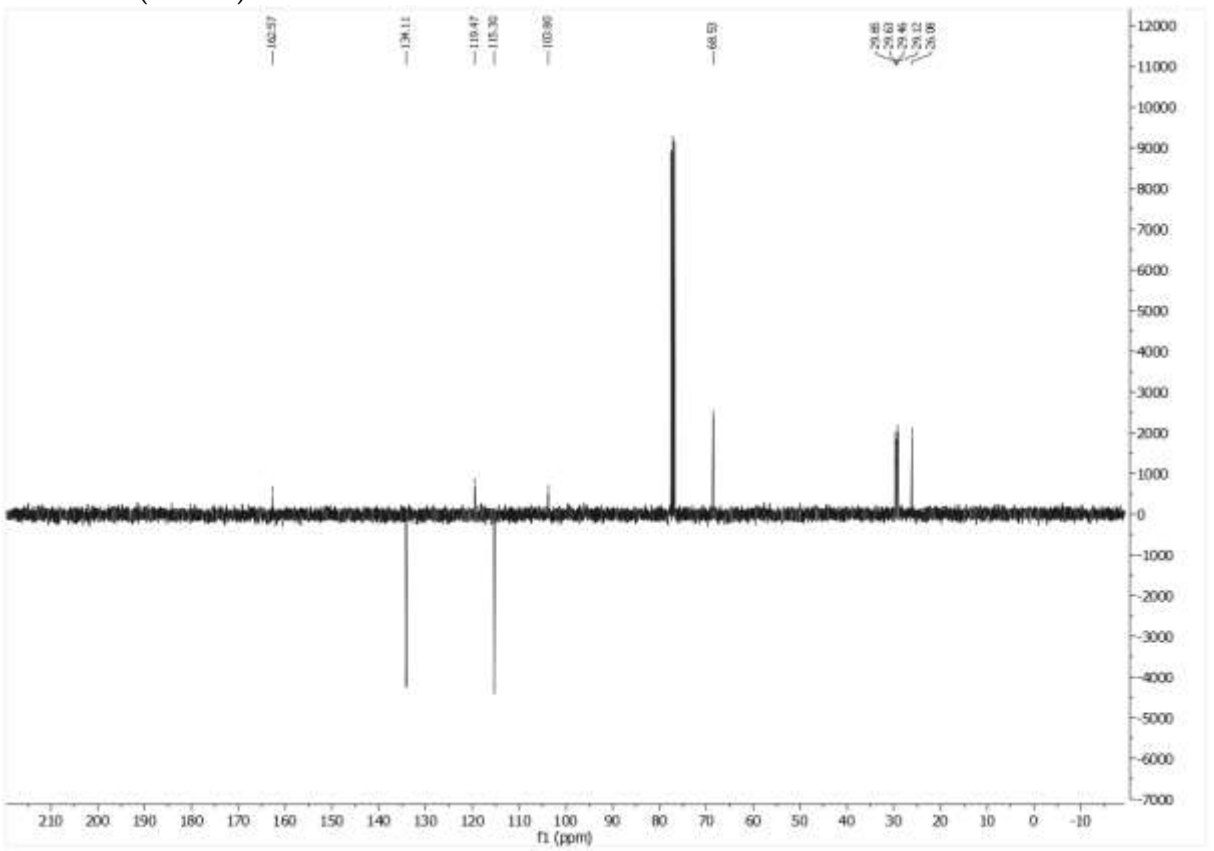
^{13}C NMR ($CDCl_3$)



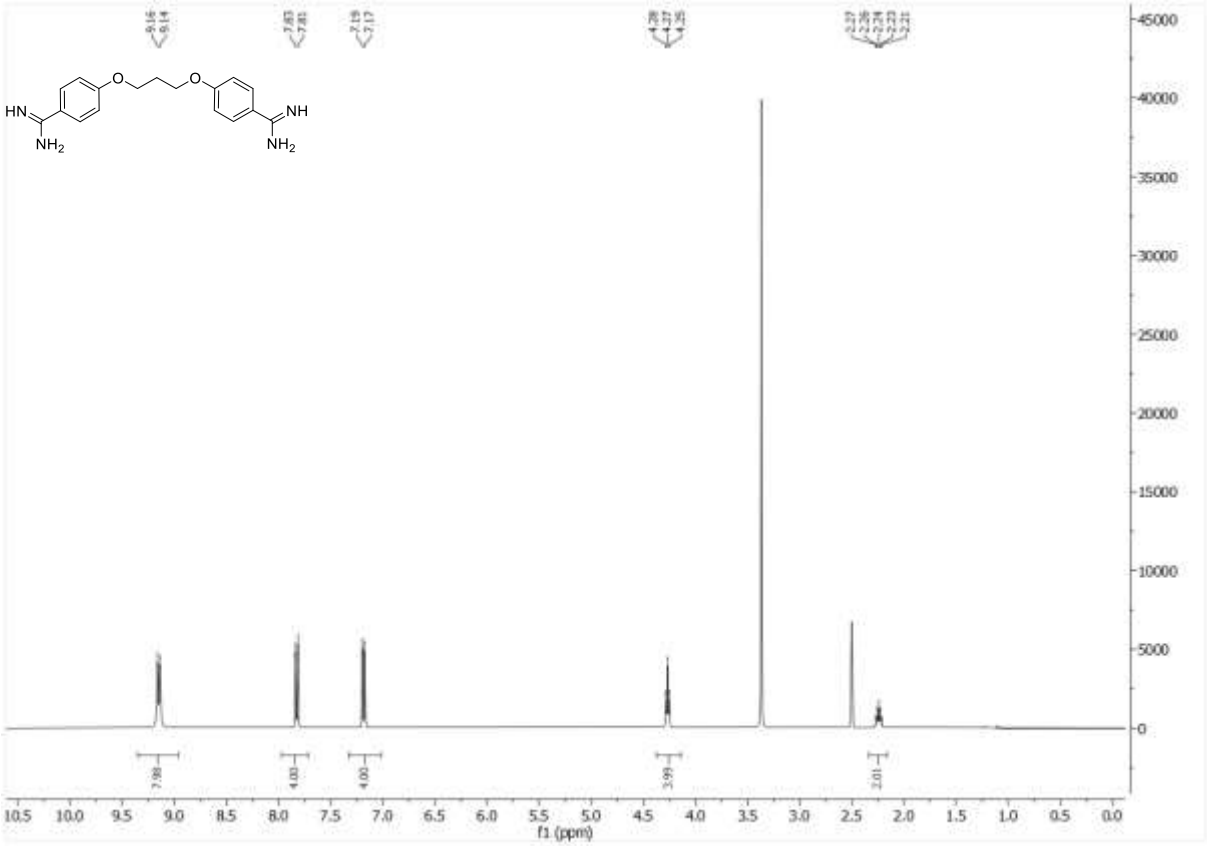
Compound **8** ^{1}H NMR ($CDCl_3$)



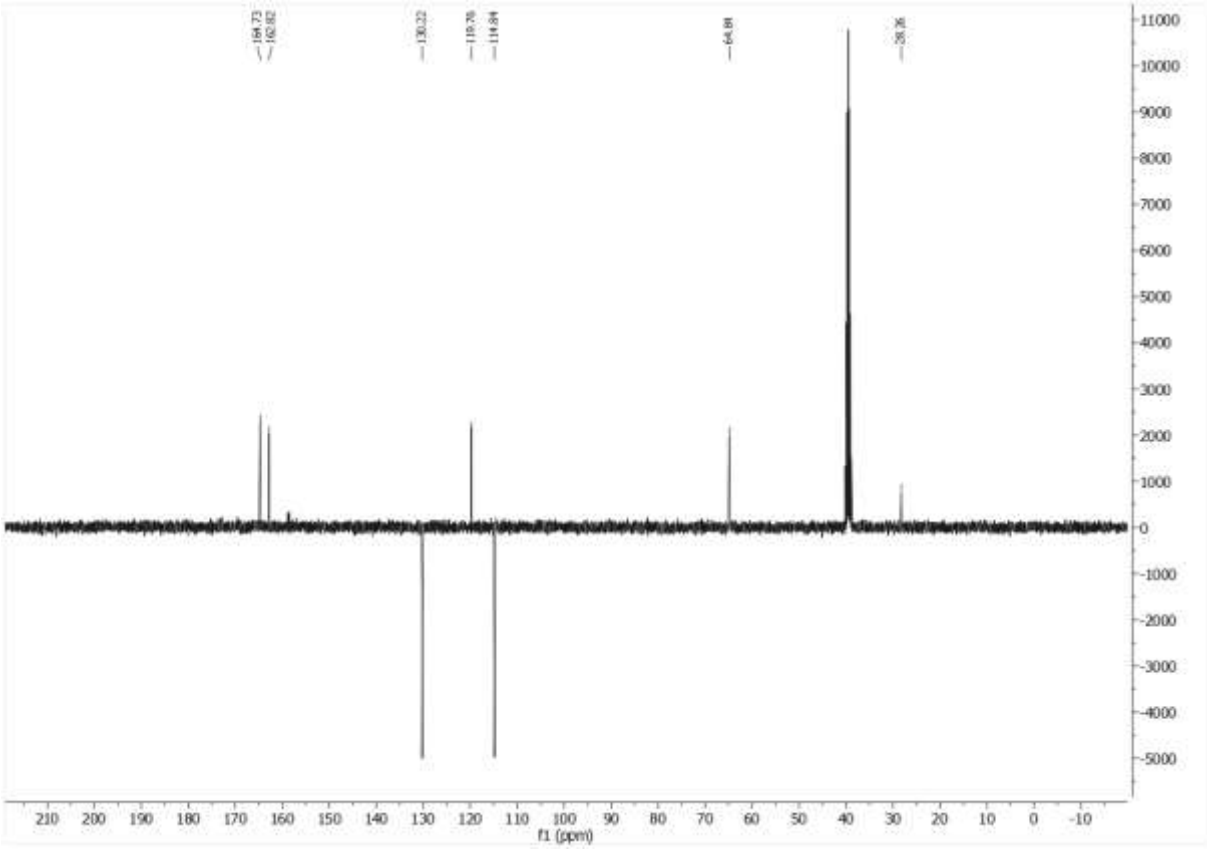
^{13}C NMR ($CDCl_3$)



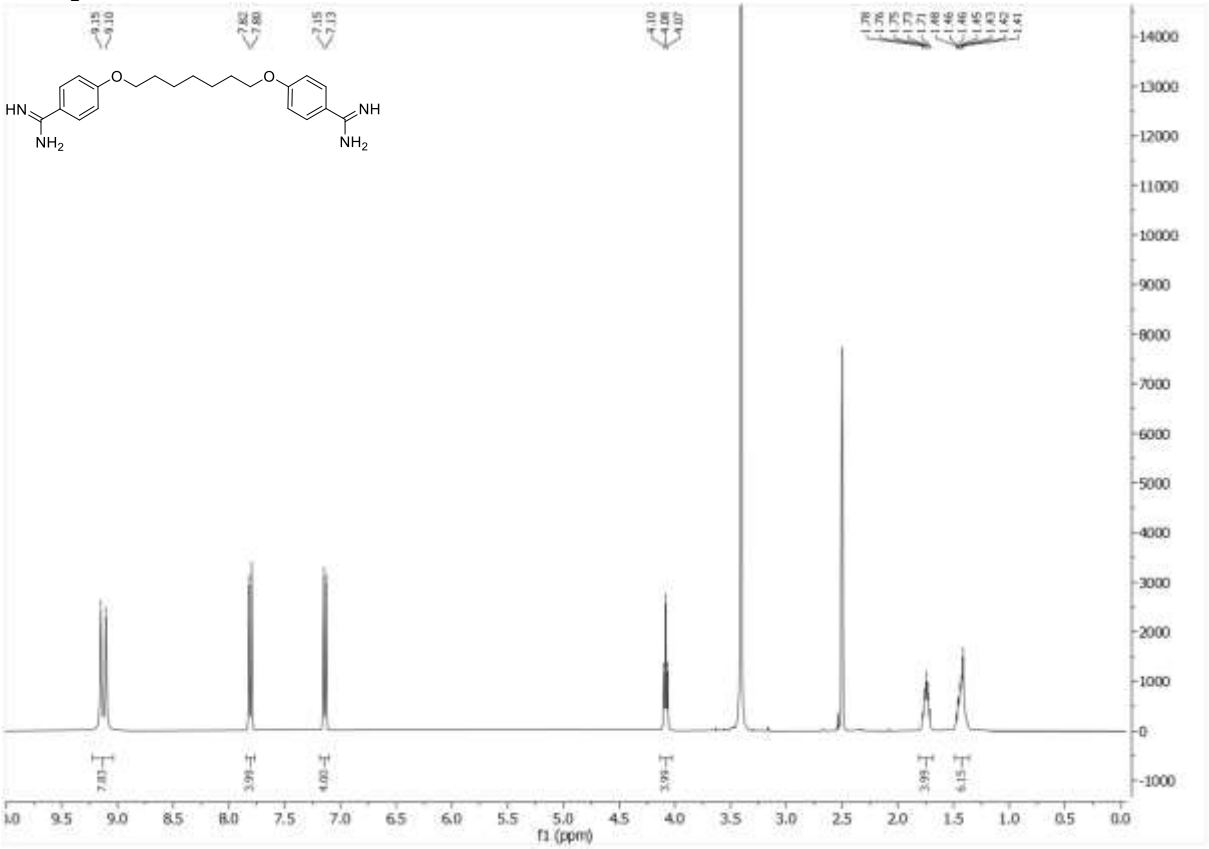
Compound **9** ^{1}H NMR (DMSO)



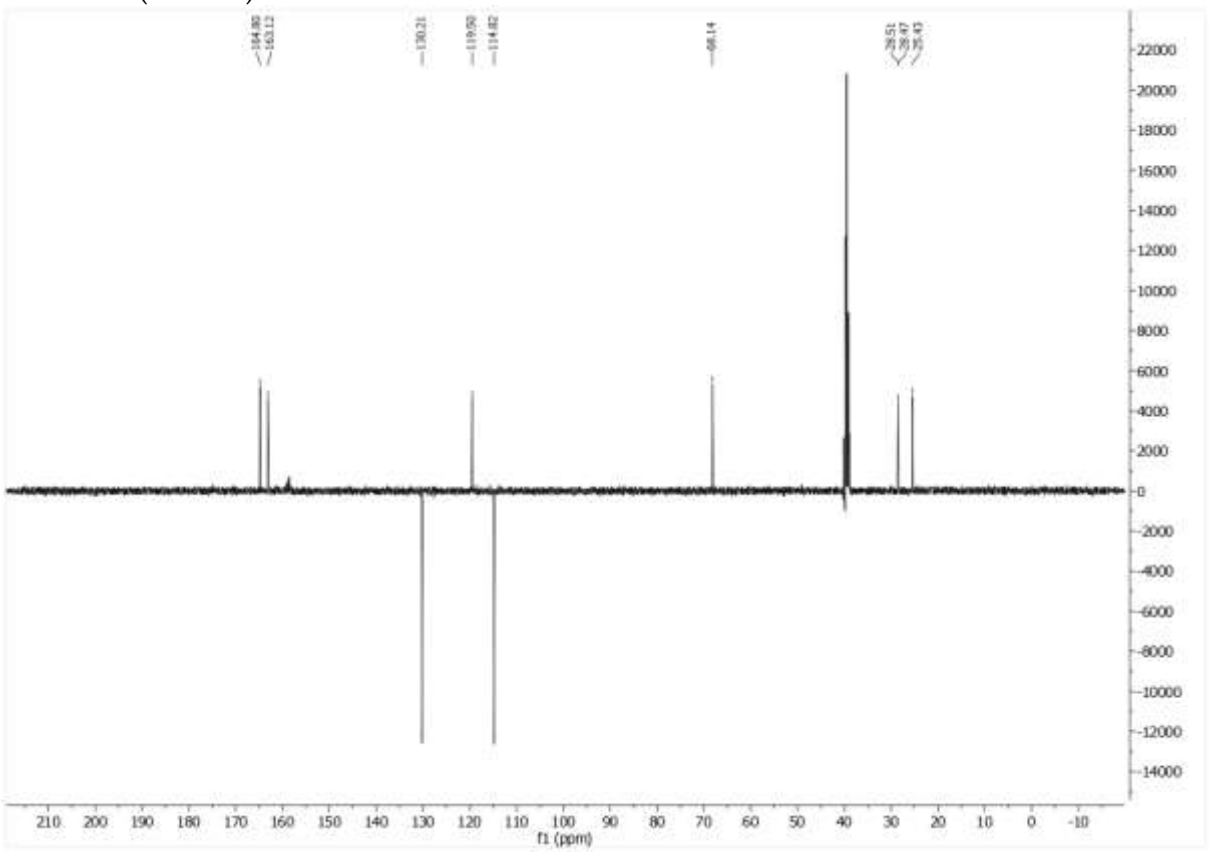
^{13}C NMR (DMSO)



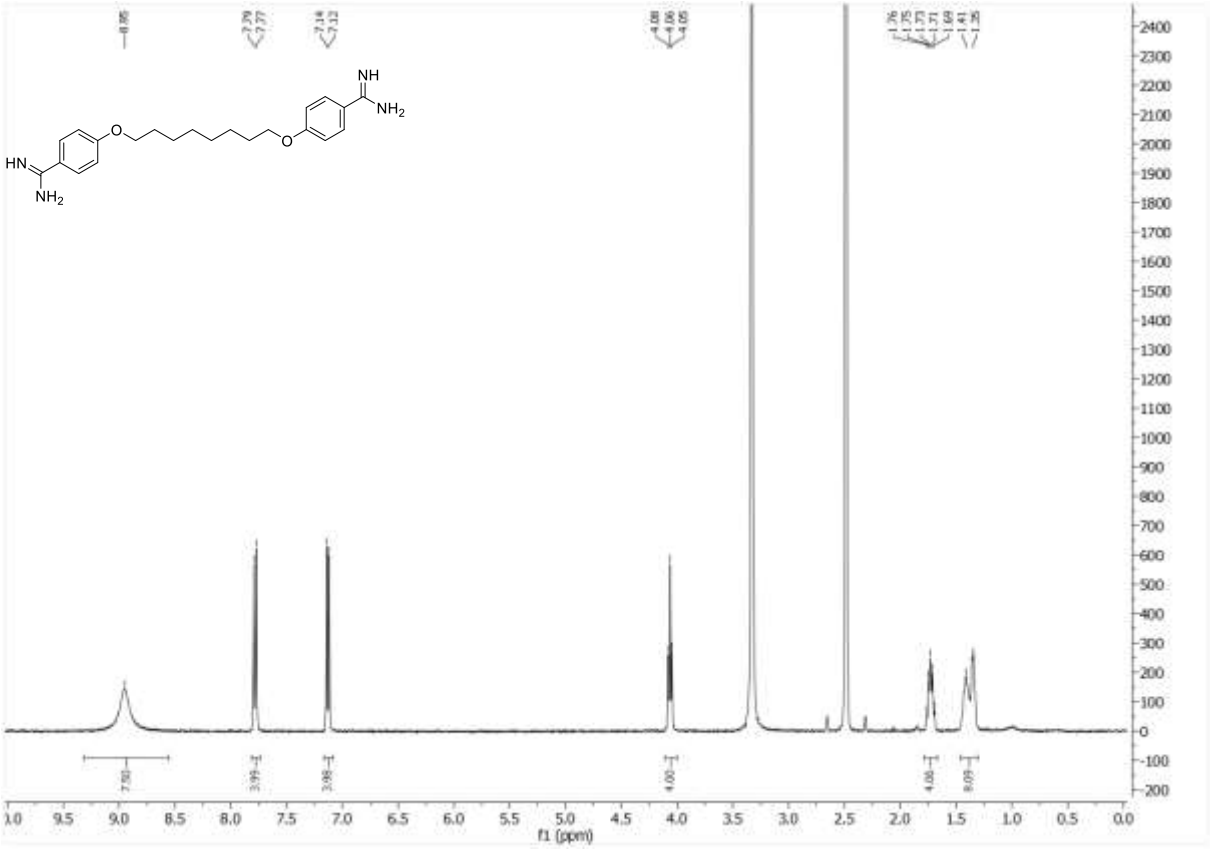
Compound **10** ^{1}H NMR (DMSO)



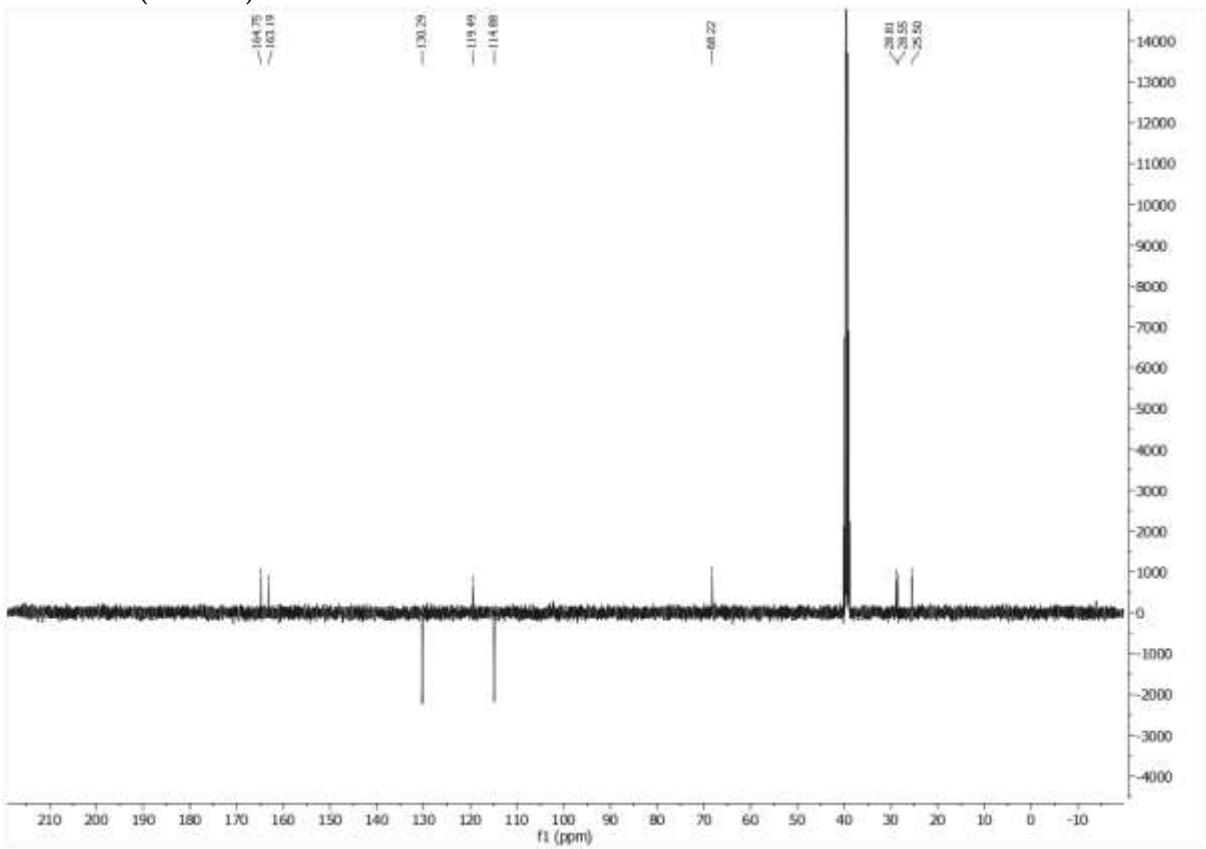
^{13}C NMR (DMSO)



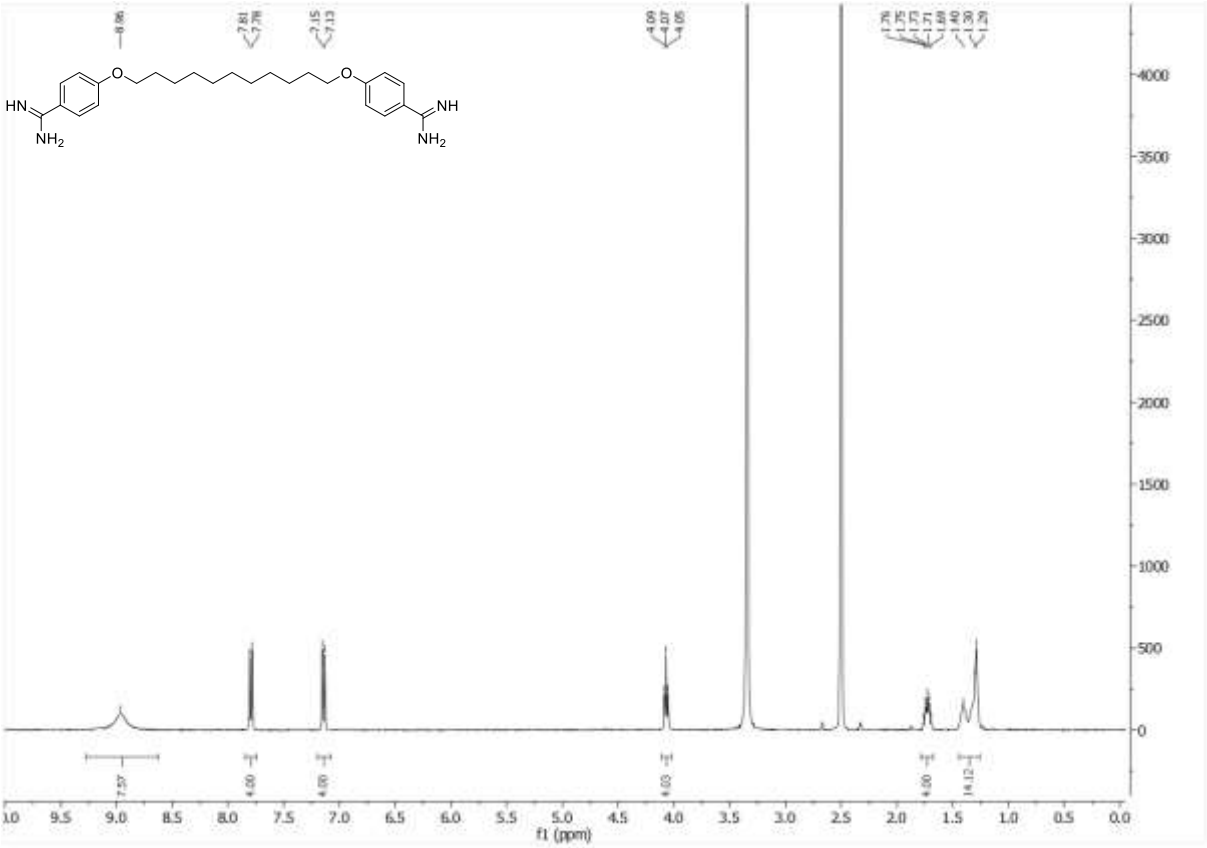
Compound **11** ^{1}H NMR (DMSO)



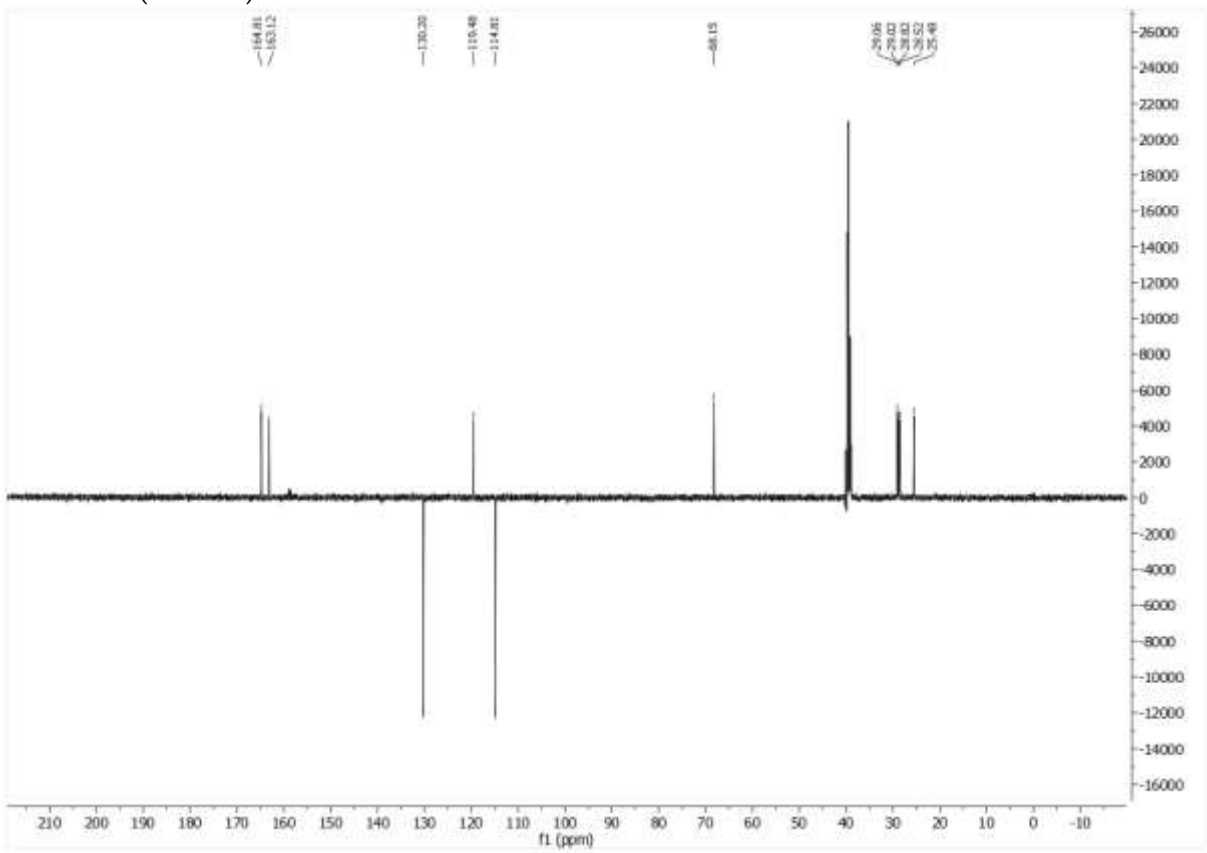
^{13}C NMR (DMSO)



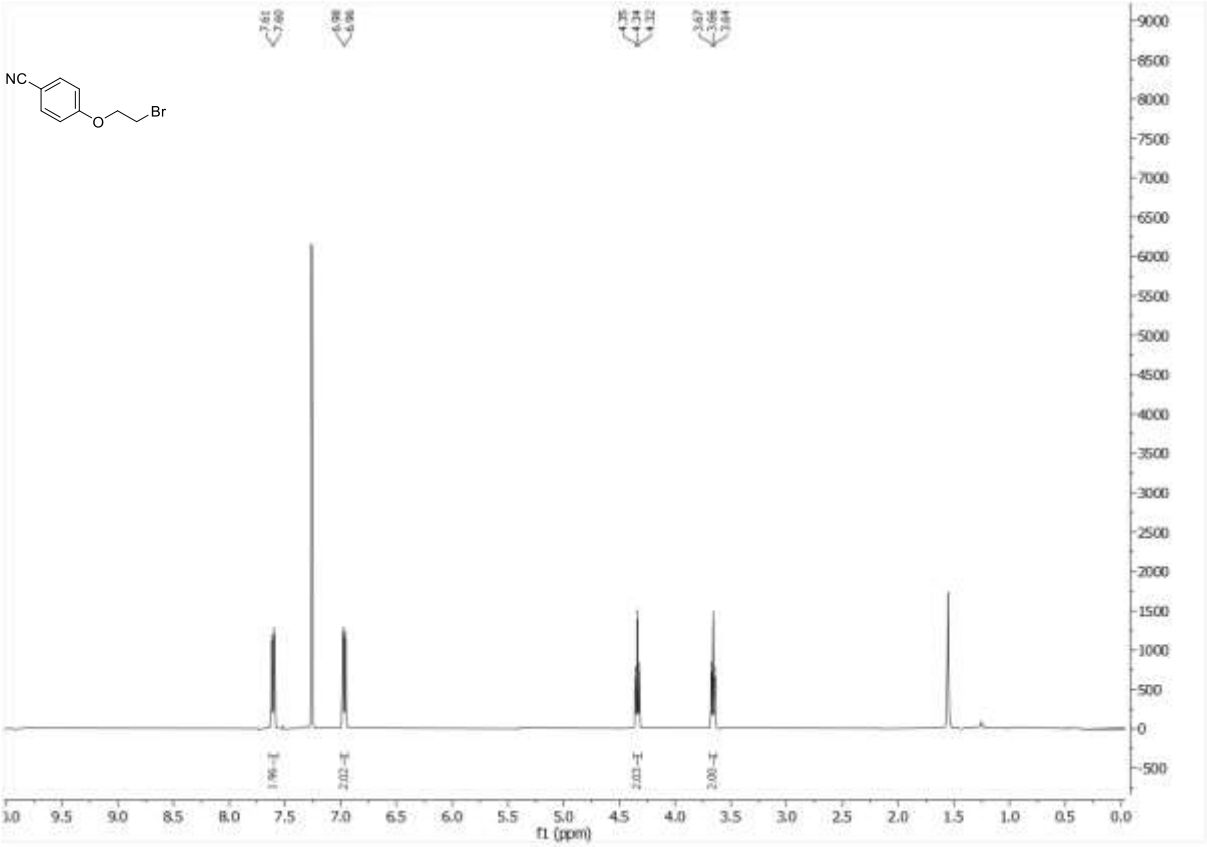
Compound **12** ^{1}H NMR (DMSO)



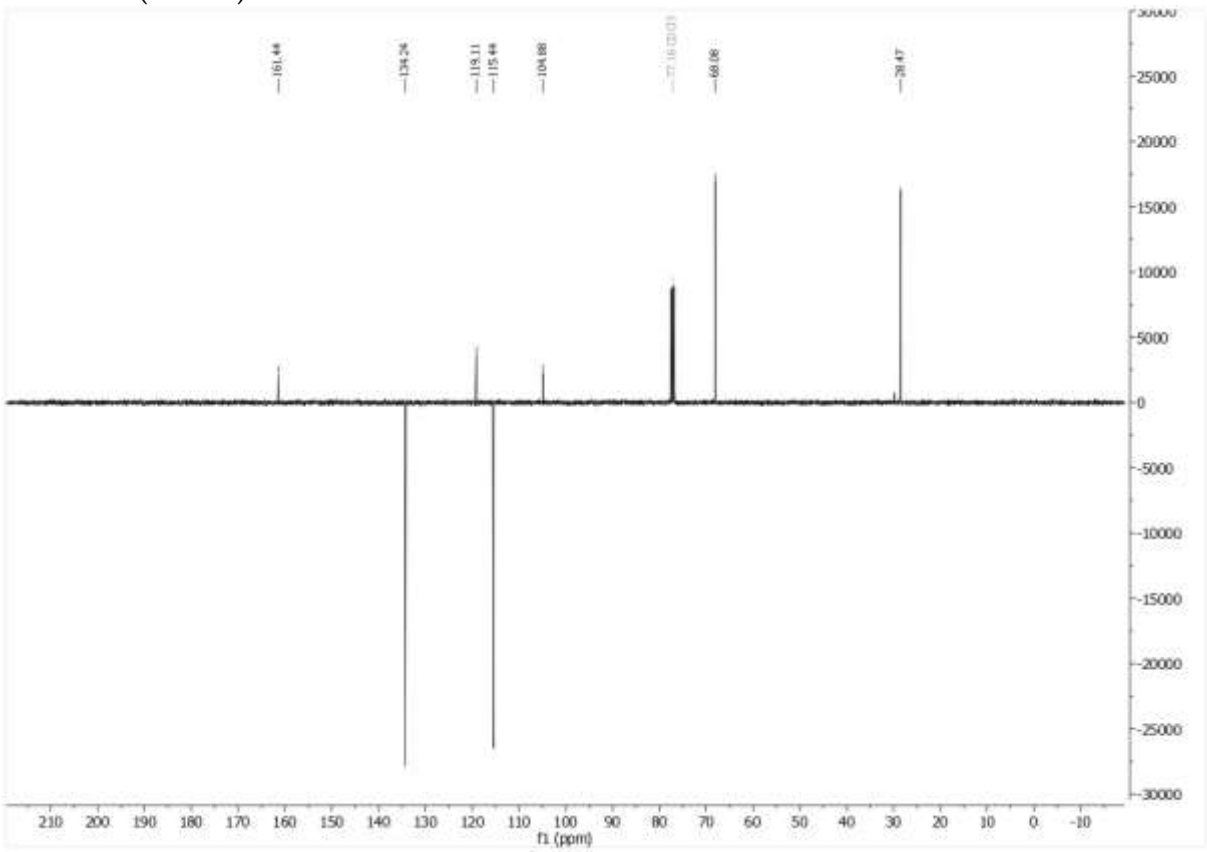
^{13}C NMR (DMSO)



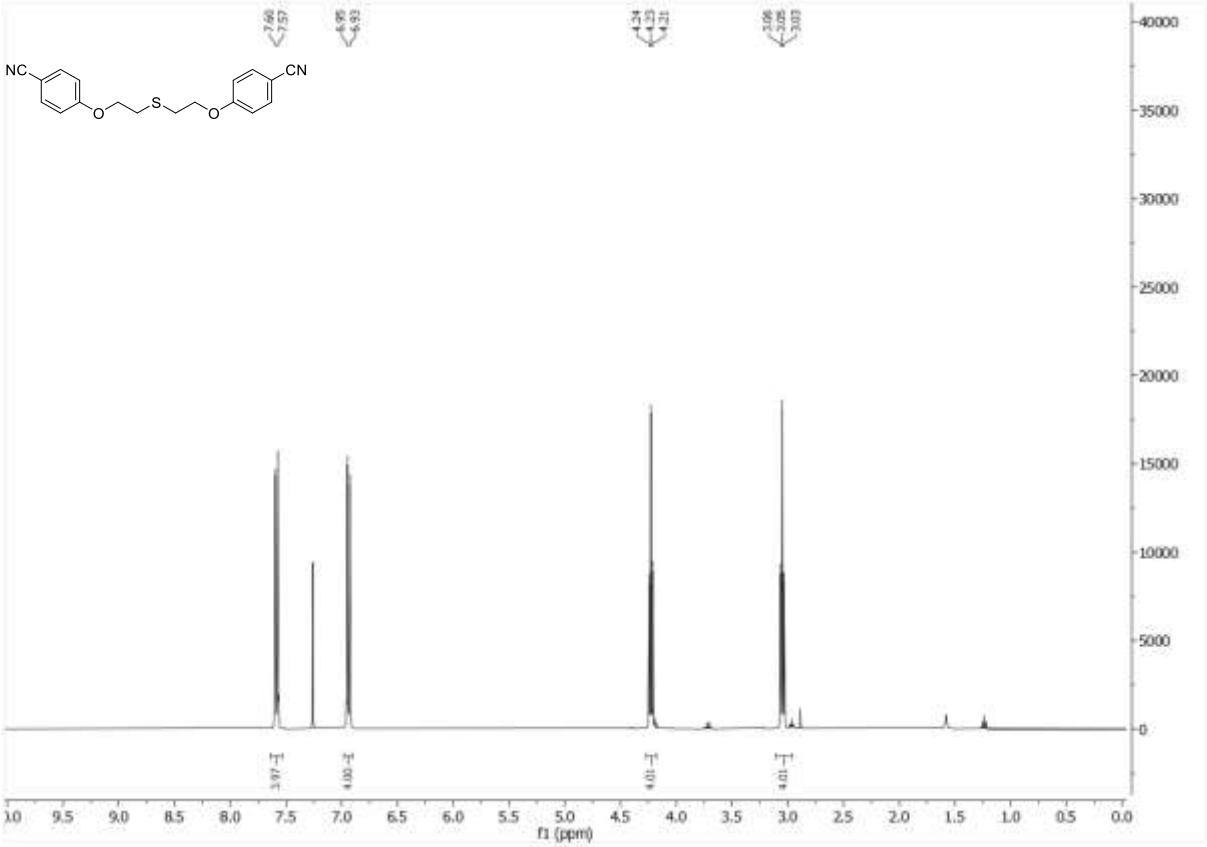
Compound **13** ^{1}H NMR ($CDCl_3$)



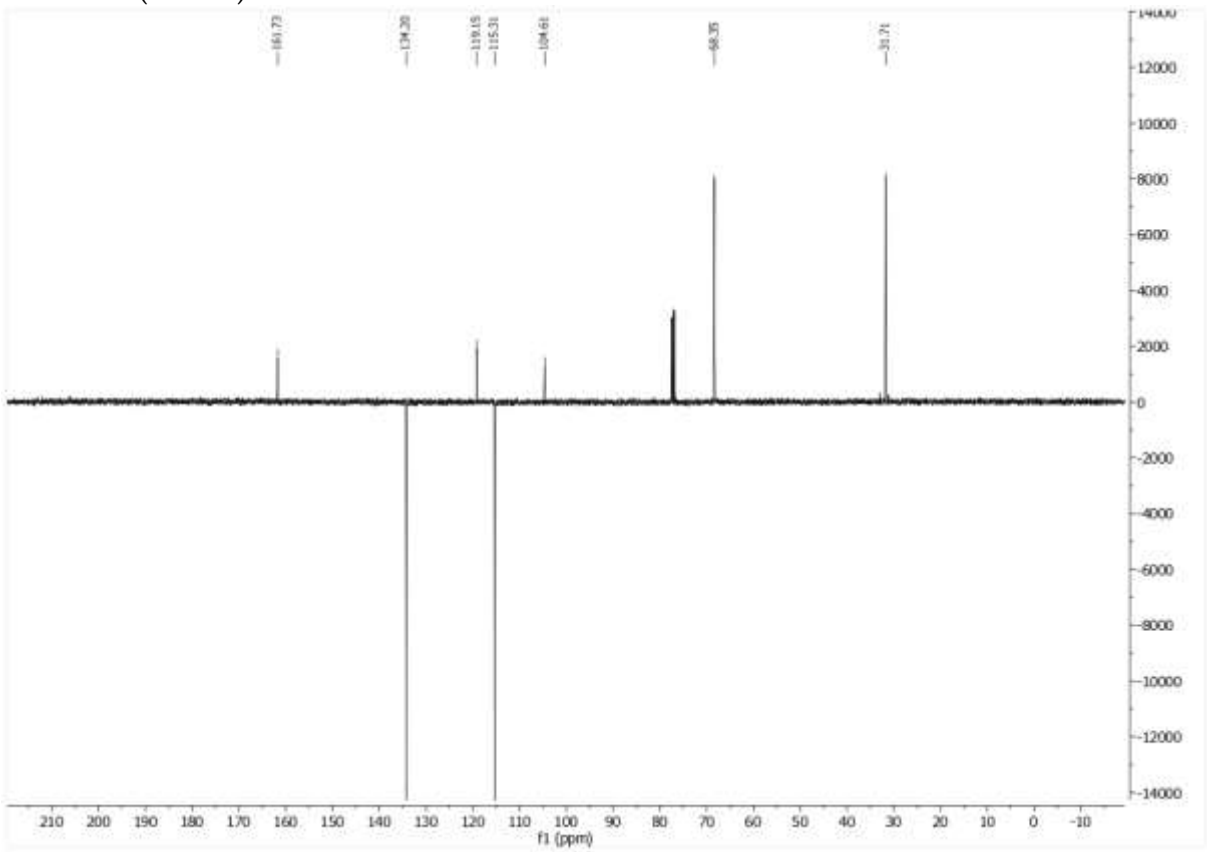
^{13}C NMR ($CDCl_3$)



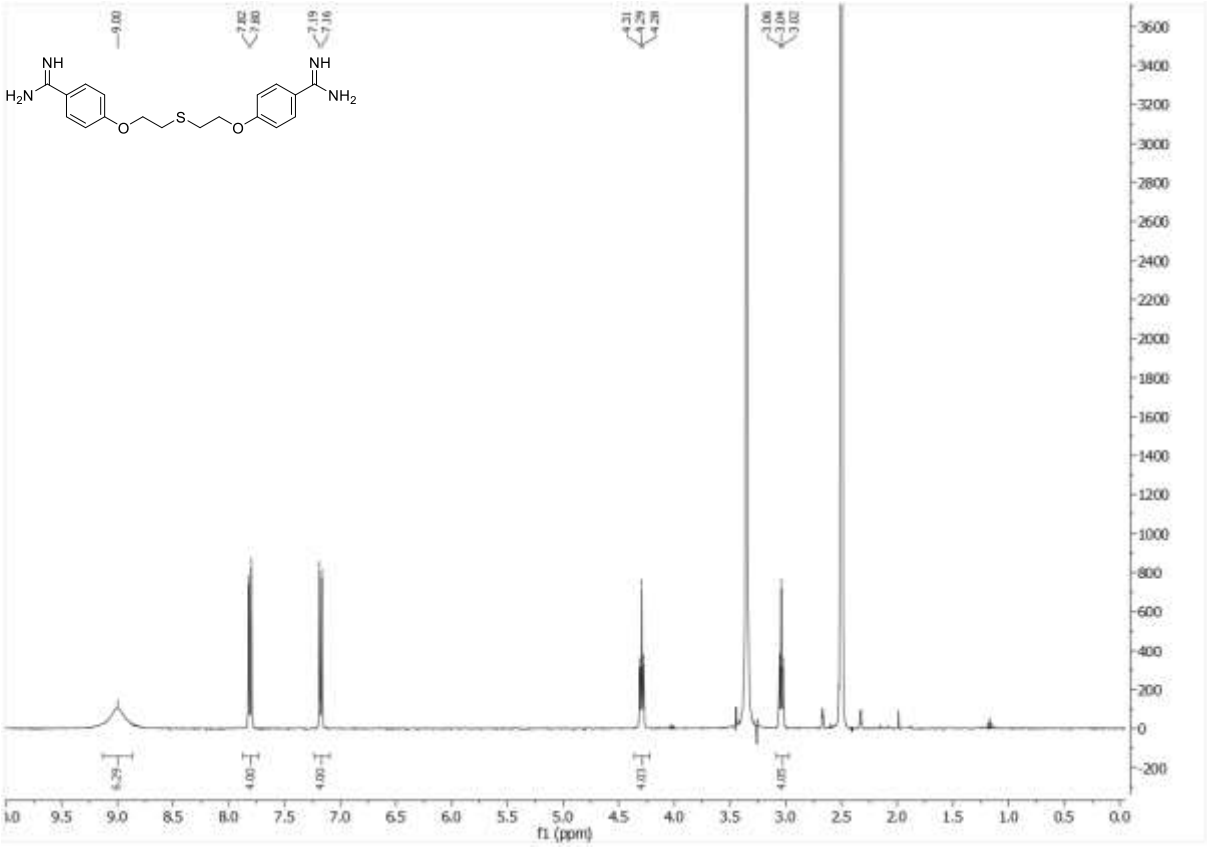
Compound **14** ^{1}H NMR ($CDCl_3$)



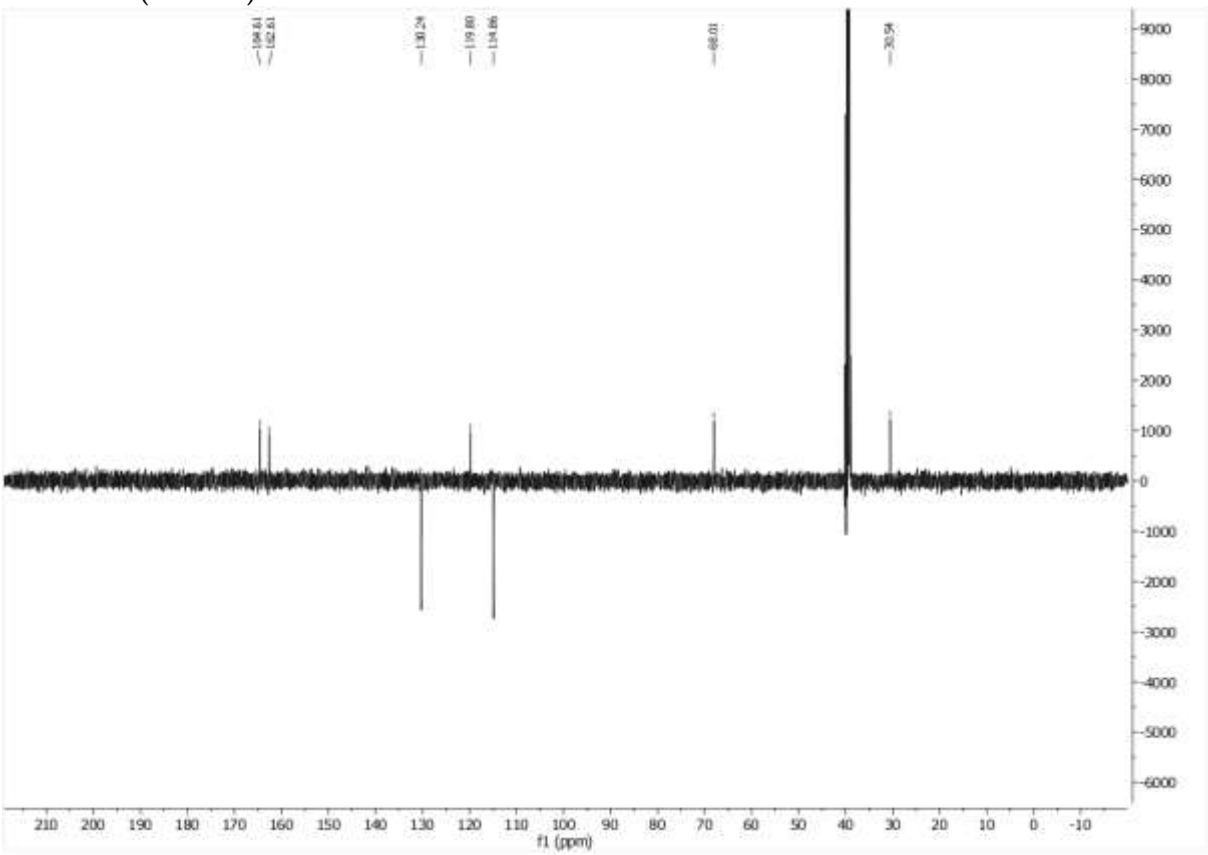
^{13}C NMR ($CDCl_3$)



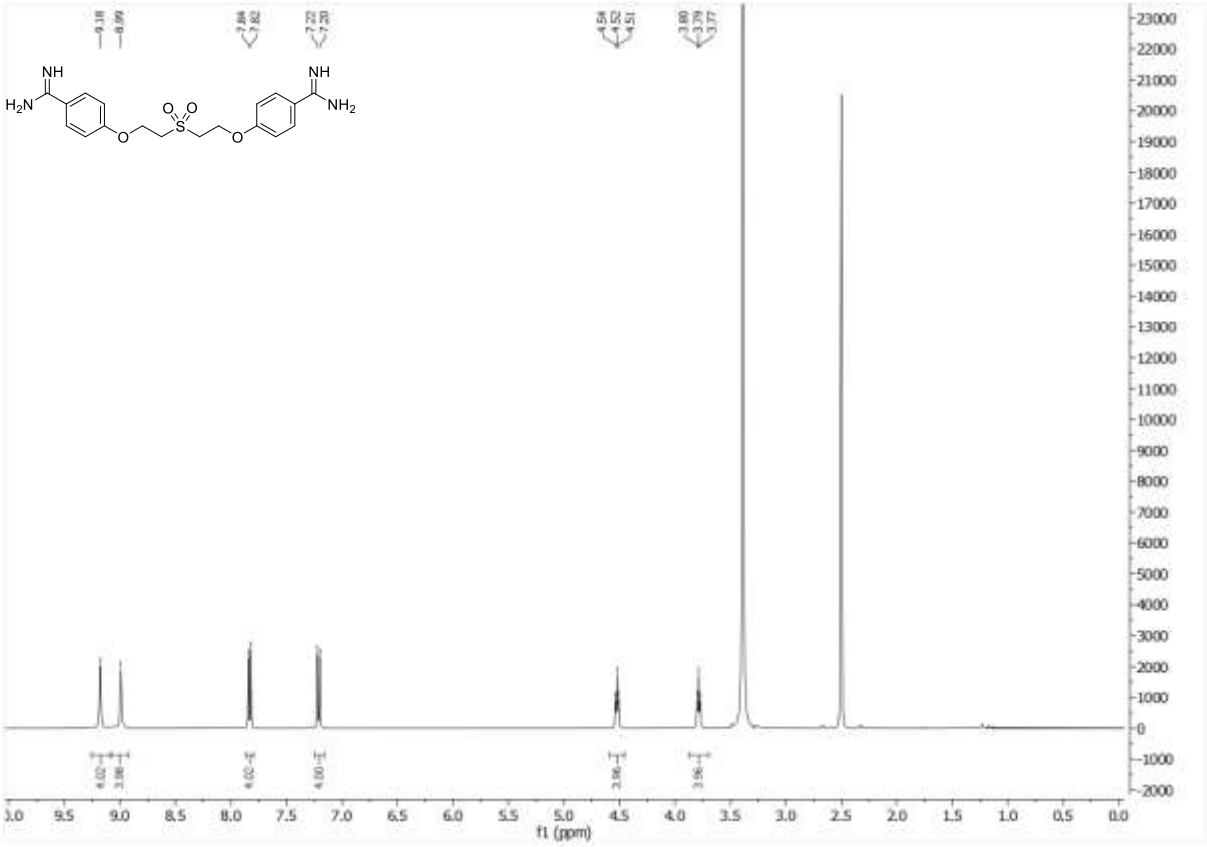
Compound **15** ^{1}H NMR (DMSO)



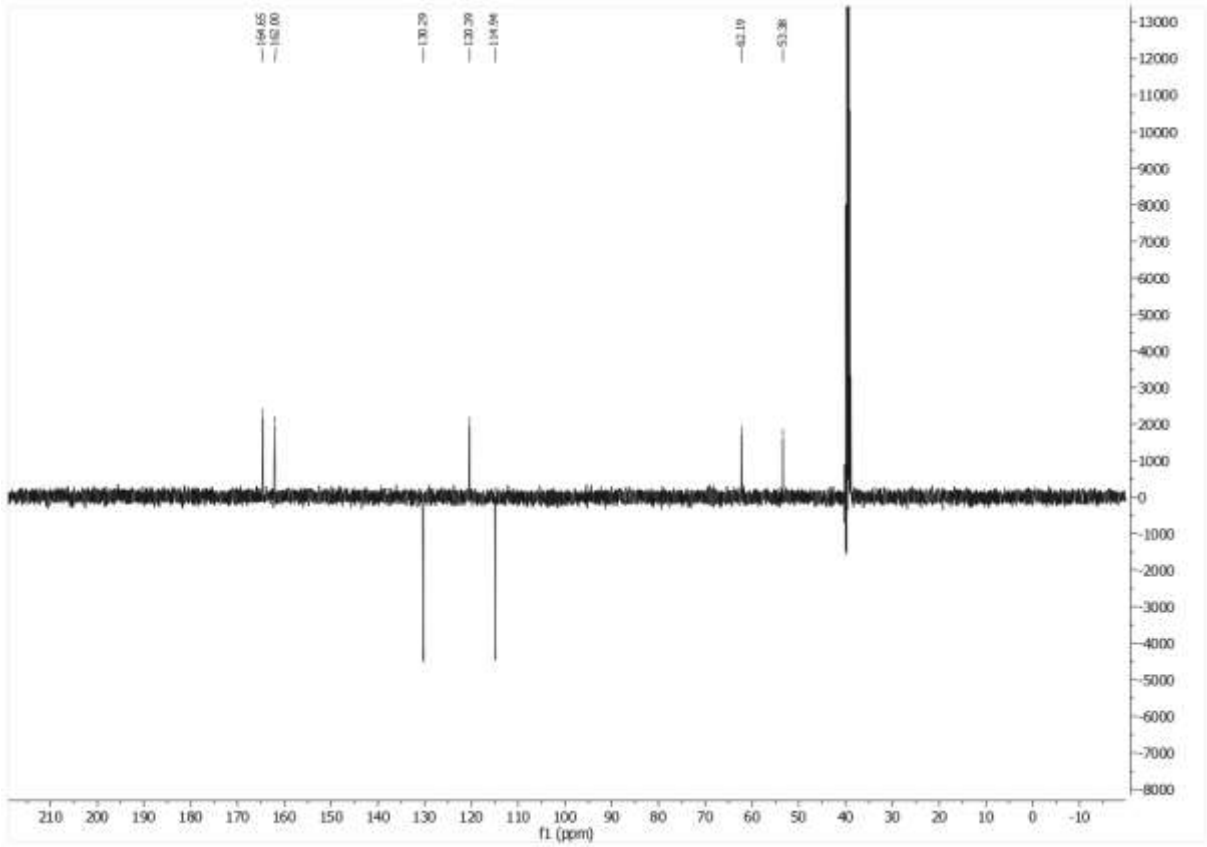
^{13}C NMR (DMSO)



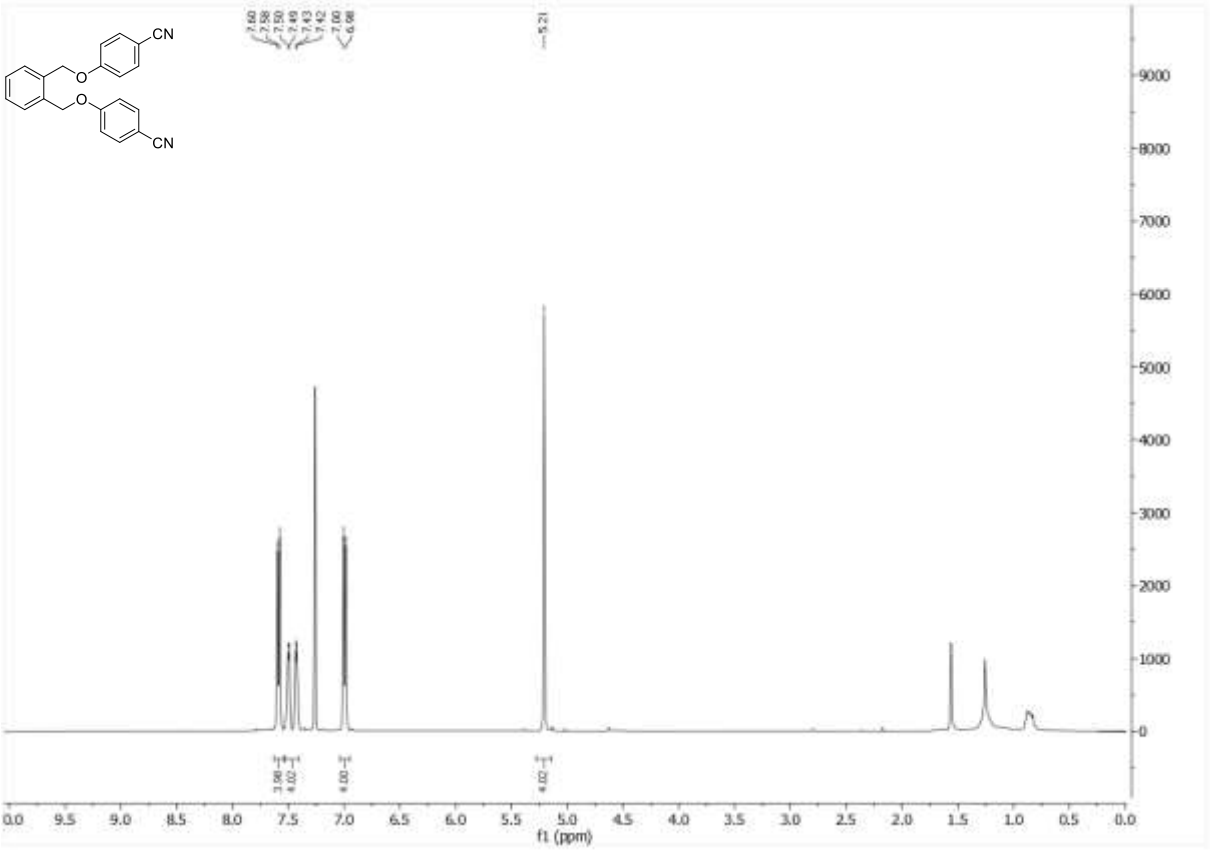
Compound **16** ^{1}H NMR (DMSO)



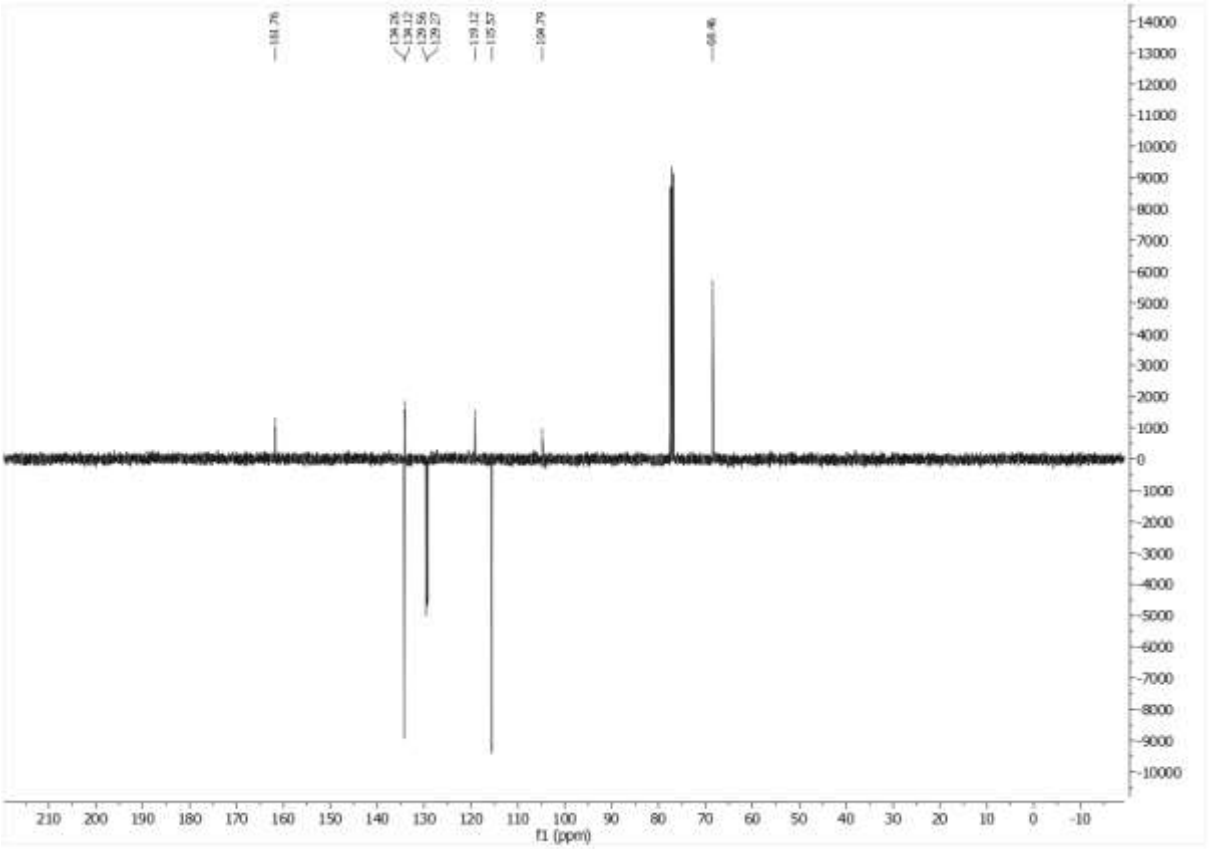
^{13}C NMR (DMSO)



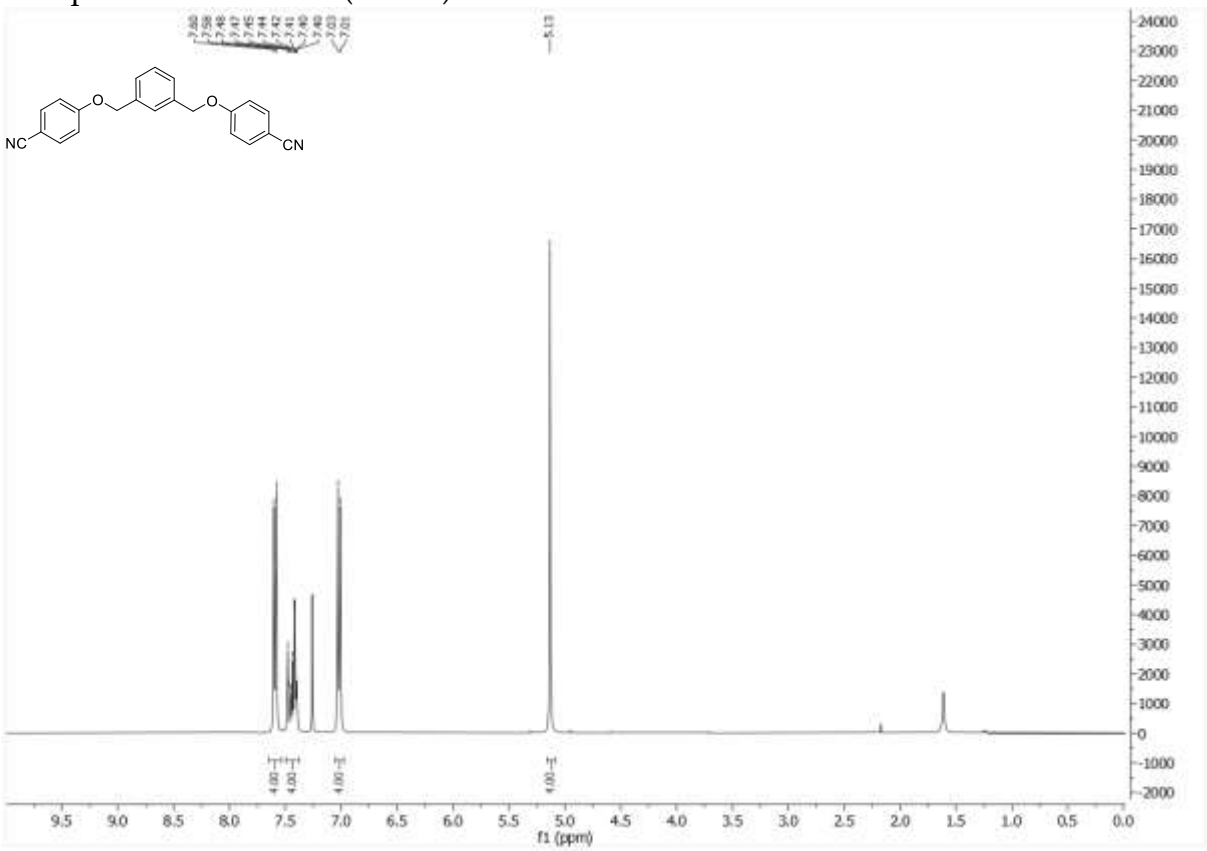
Compound **17** ^{1}H NMR ($CDCl_3$)



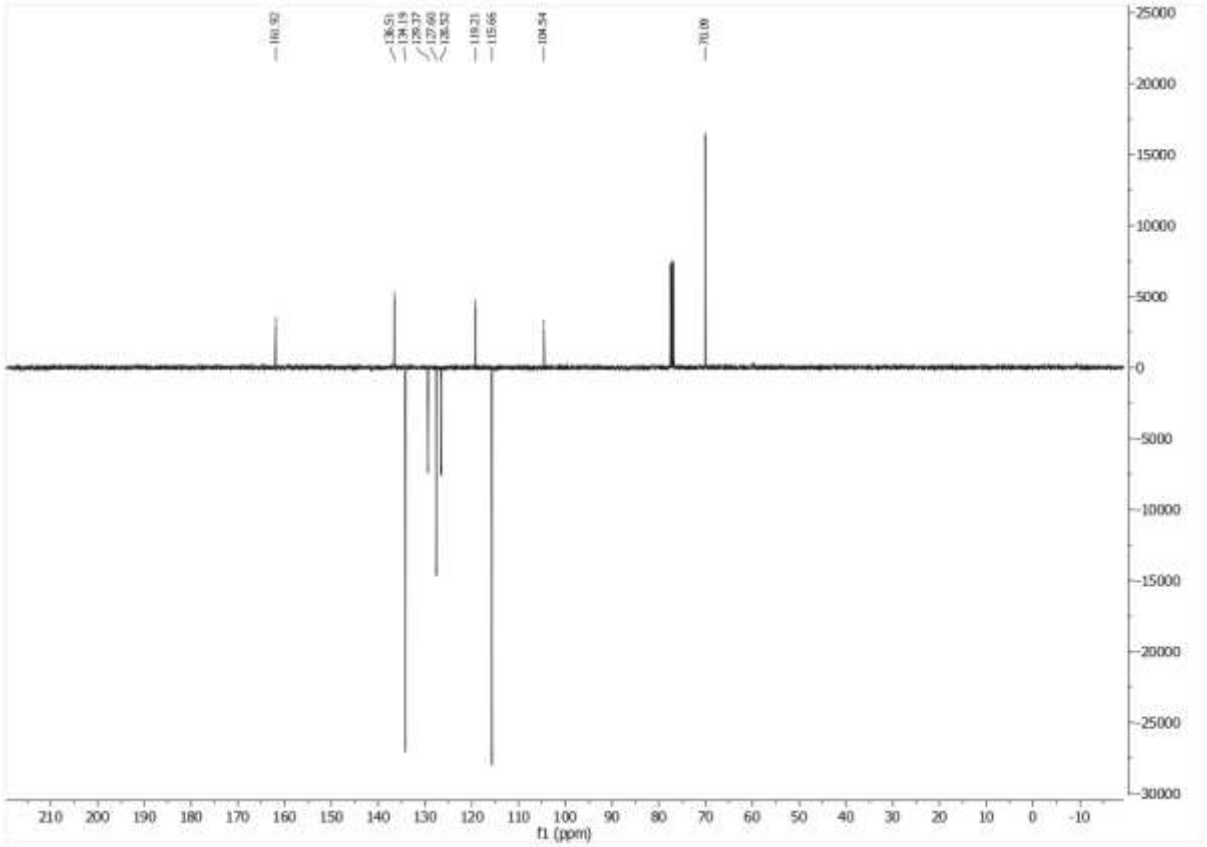
^{13}C NMR ($CDCl_3$)



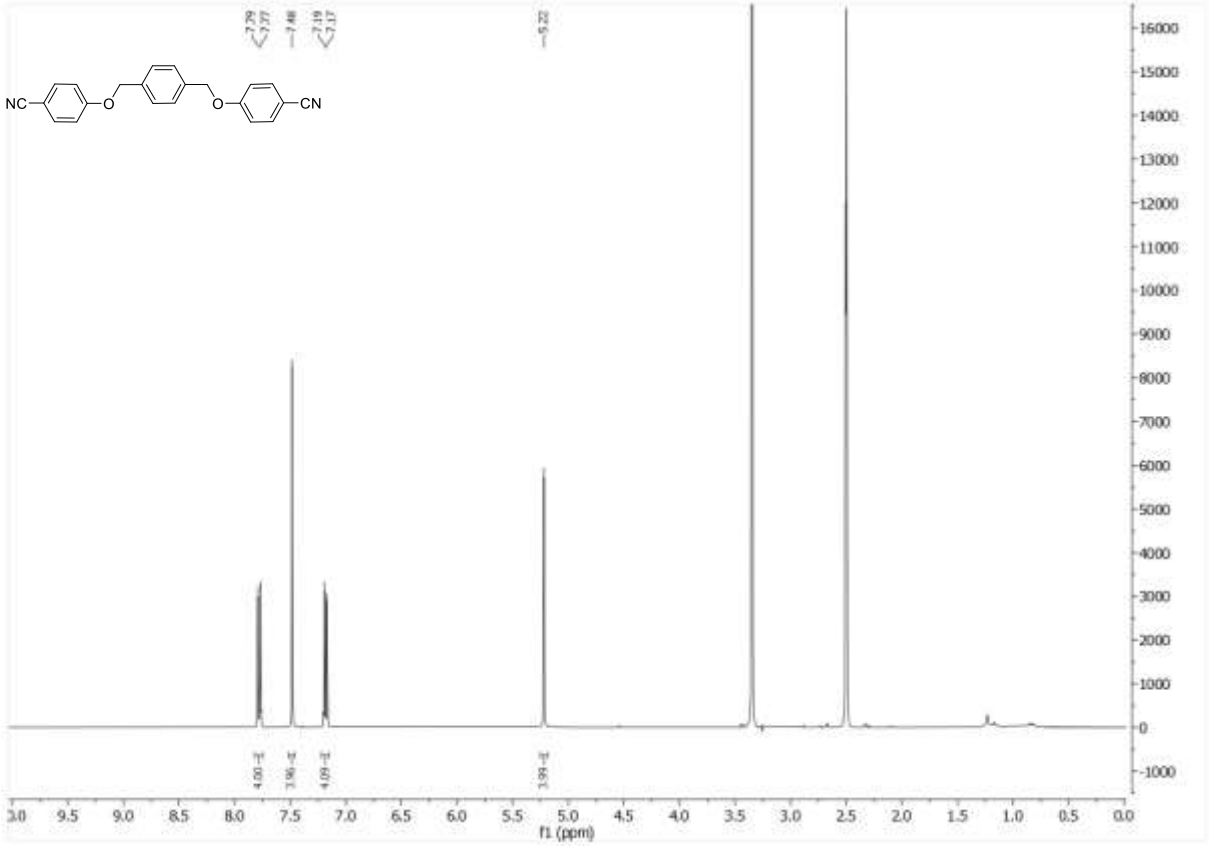
Compound **18** ^{1}H NMR ($CDCl_3$)



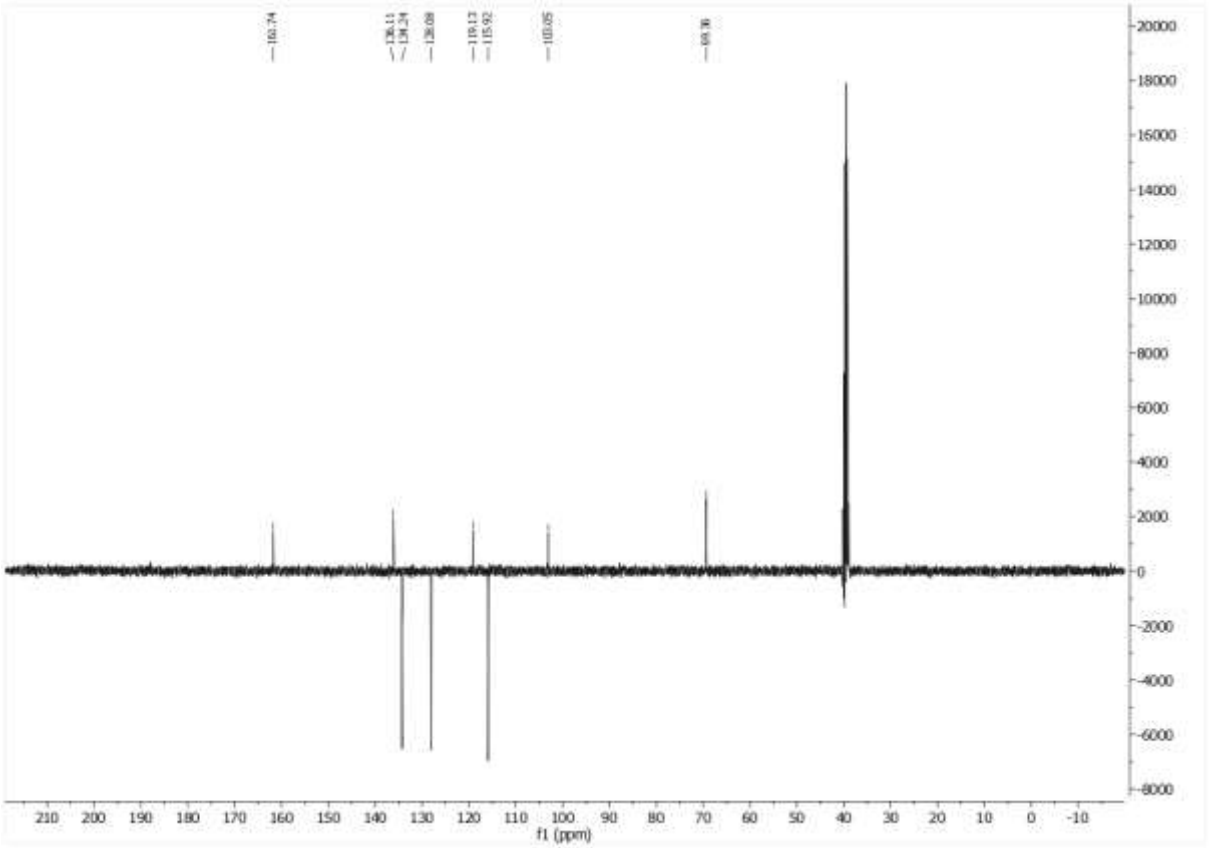
^{13}C NMR ($CDCl_3$)



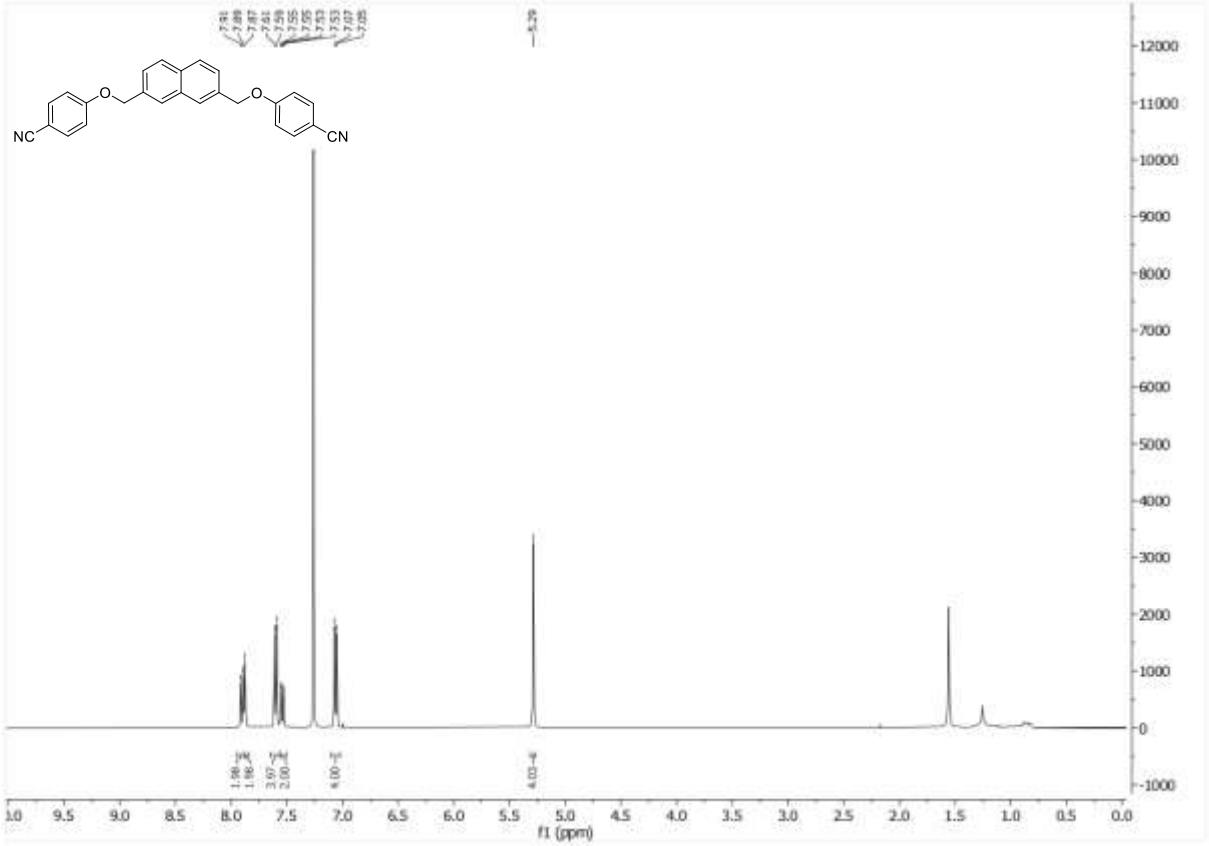
Compound **19** ^{1}H NMR ($CDCl_3$)



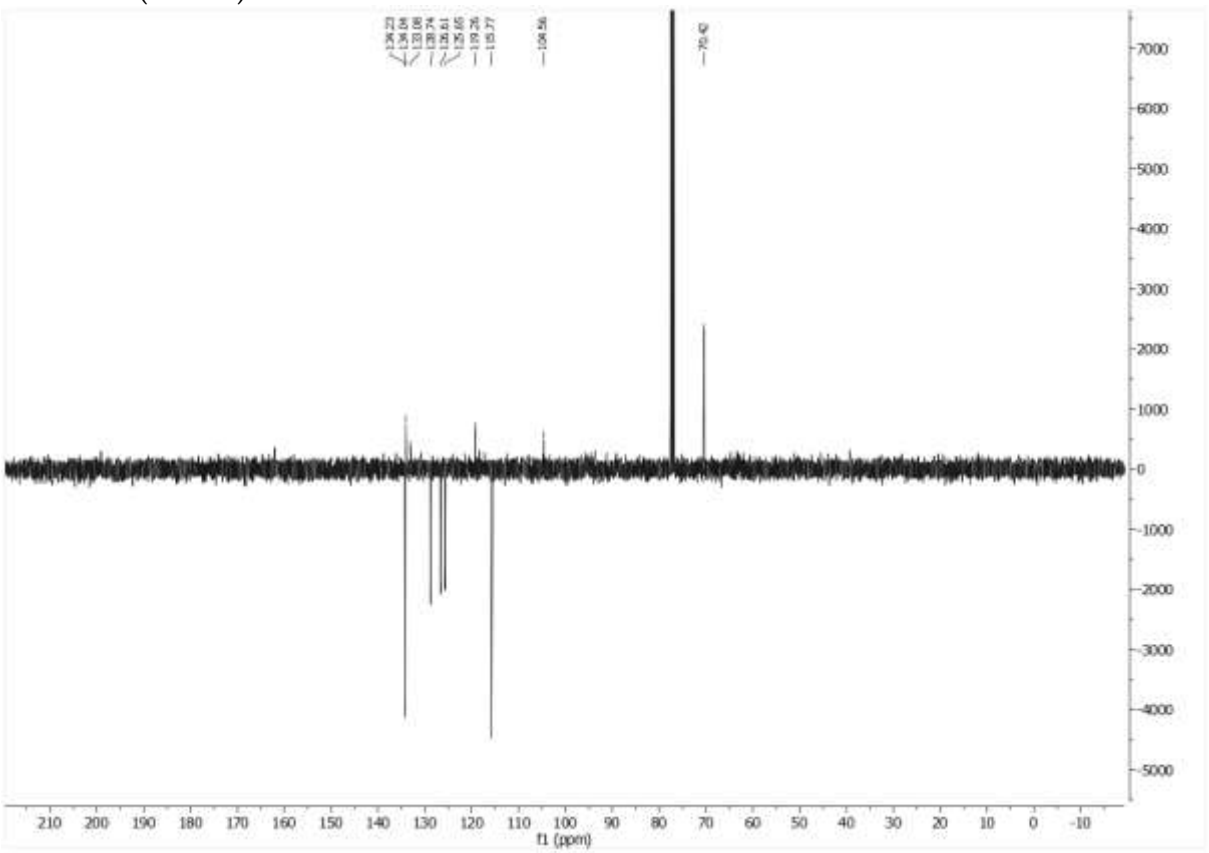
^{13}C NMR ($CDCl_3$)



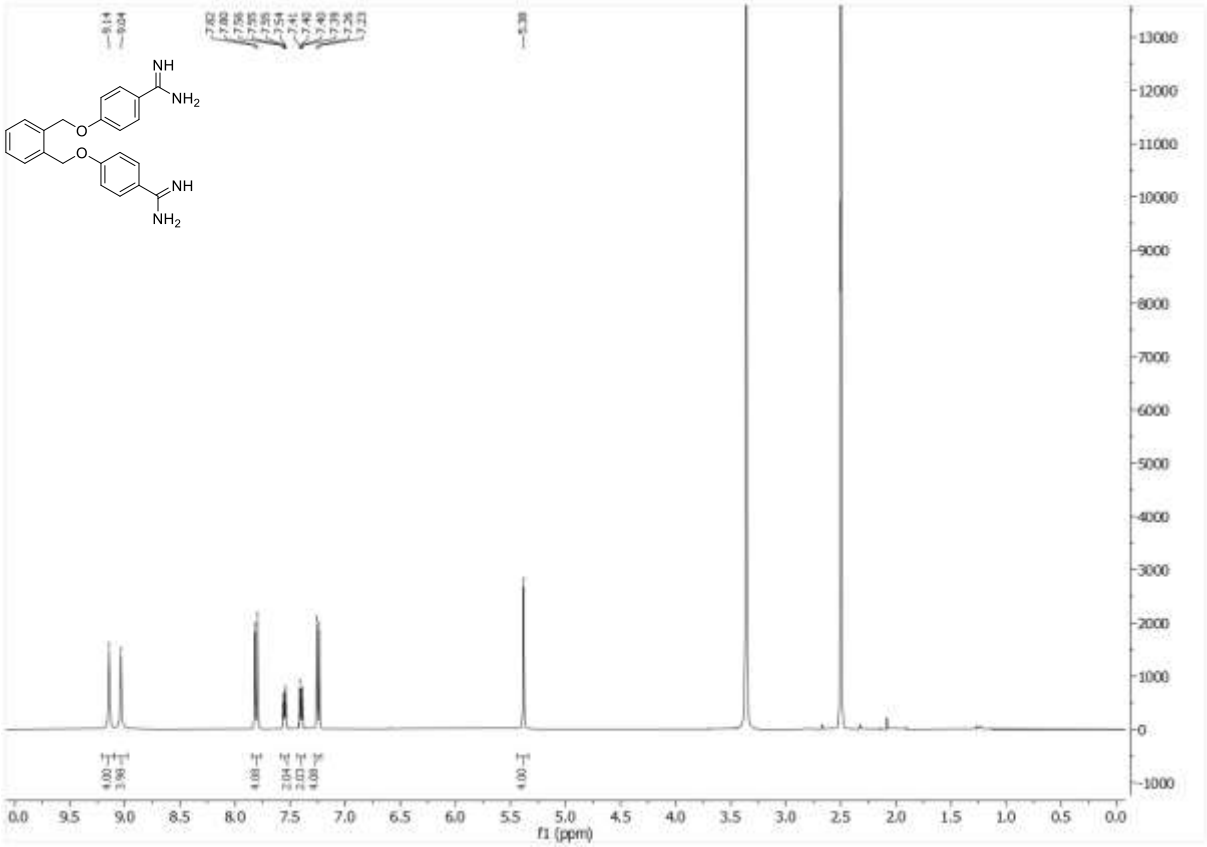
Compound **20** ^{1}H NMR ($CDCl_3$)



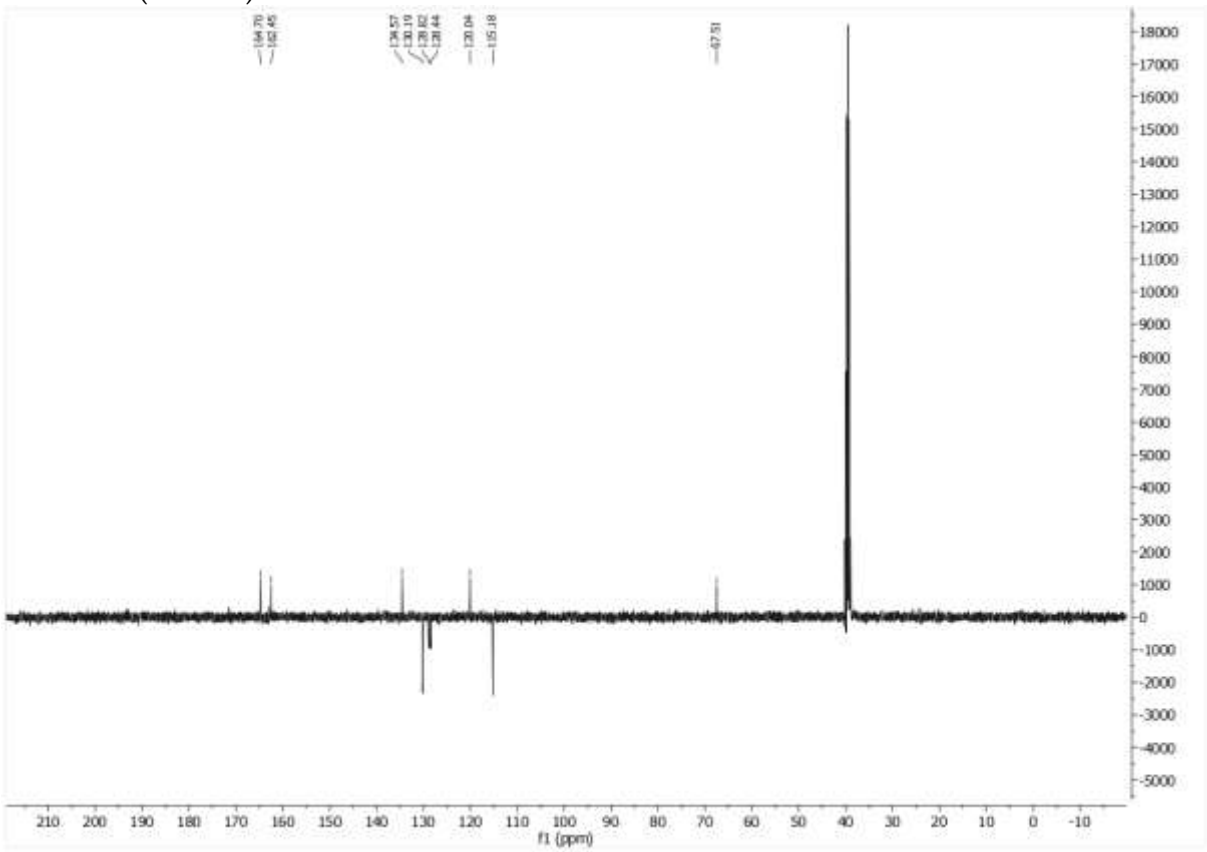
^{13}C NMR ($CDCl_3$)



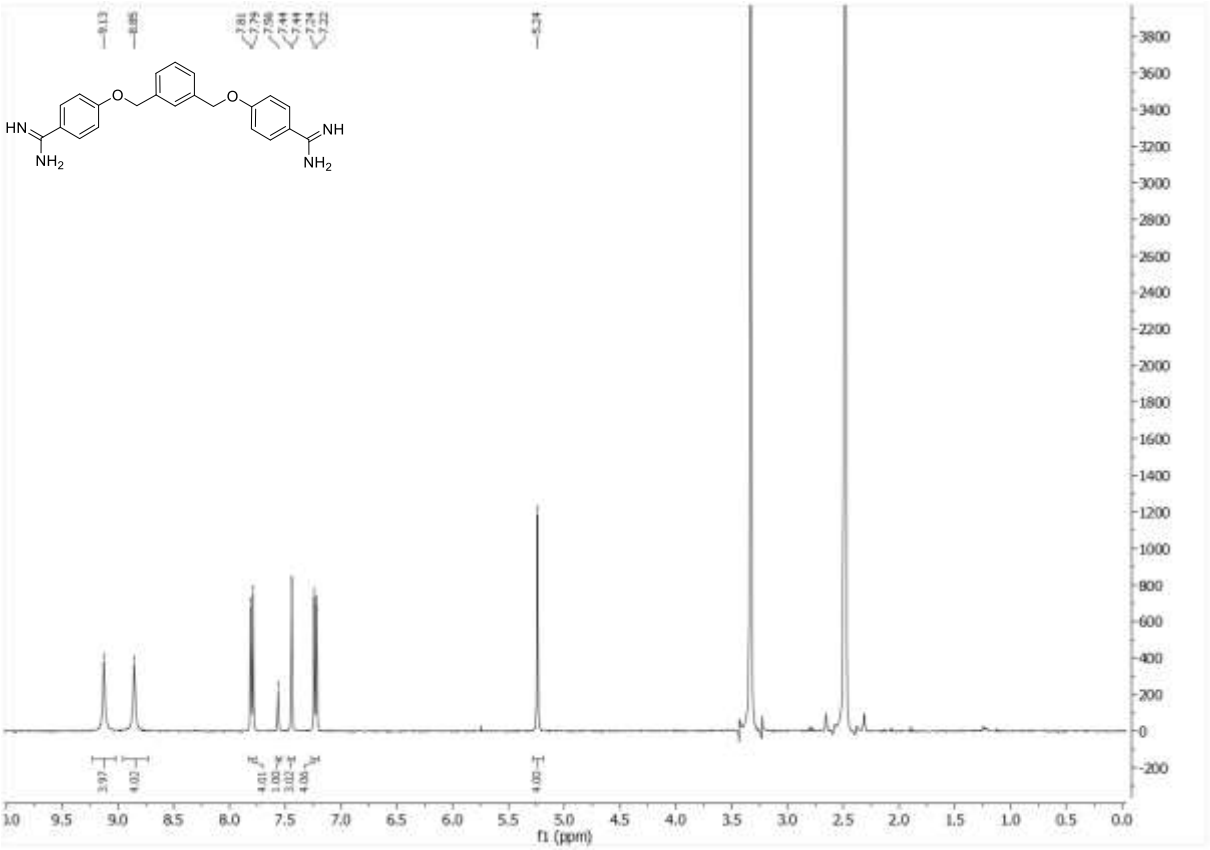
Compound **21** ^{1}H NMR (DMSO)



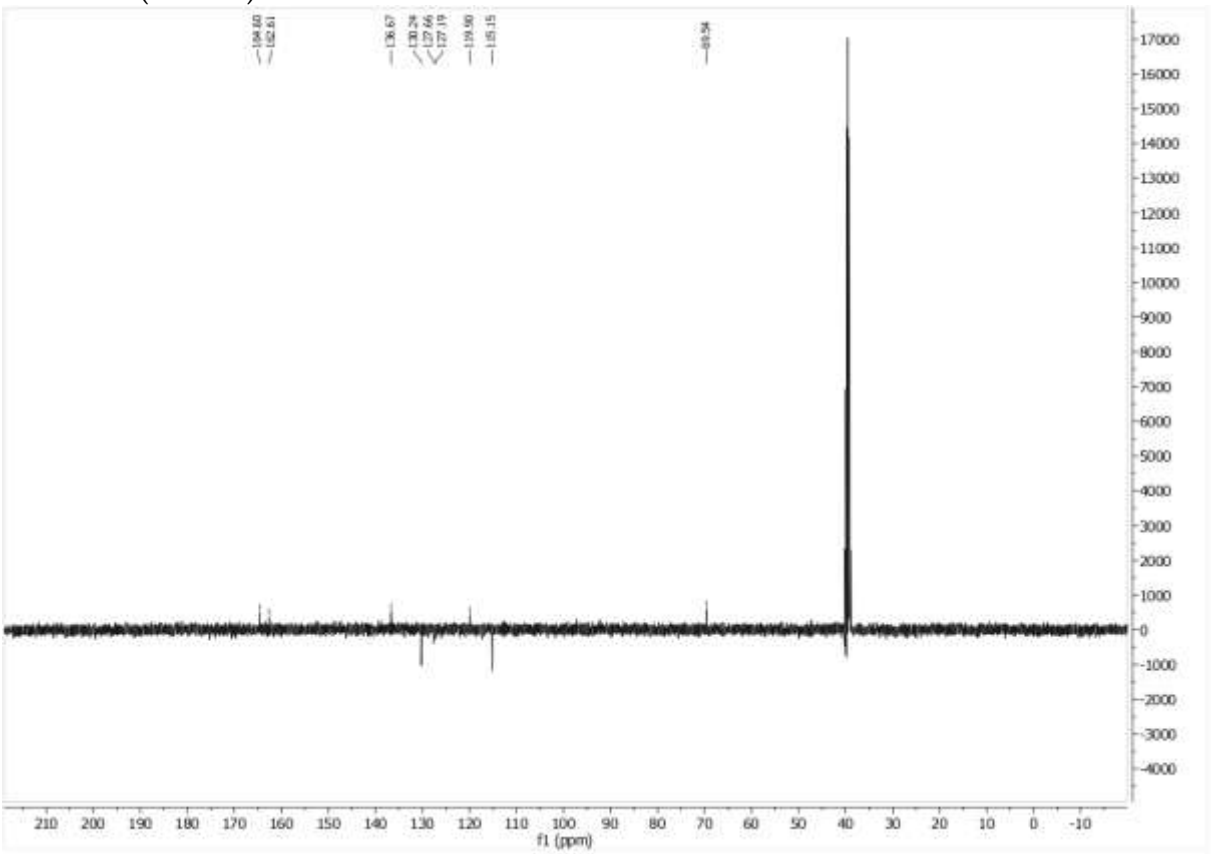
^{13}C NMR (DMSO)



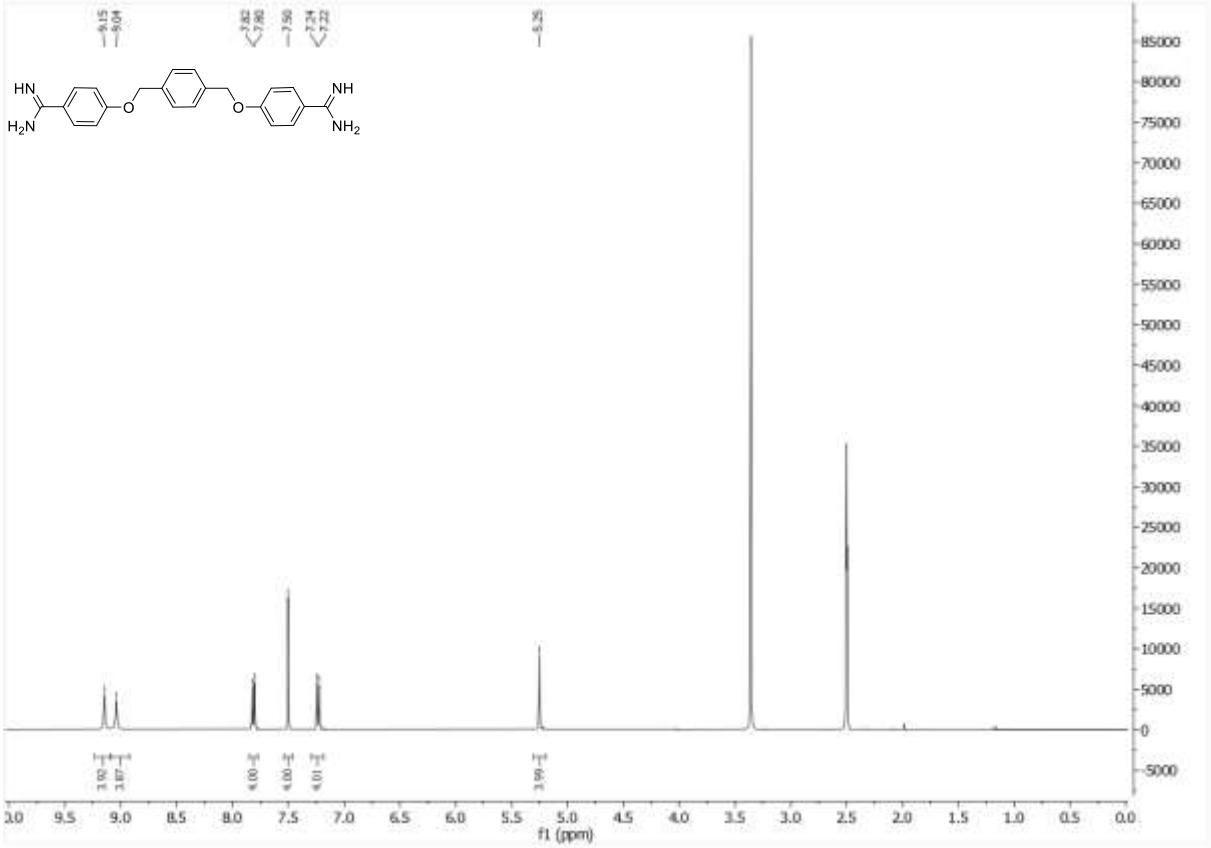
Compound **22** ^{1}H NMR (DMSO)



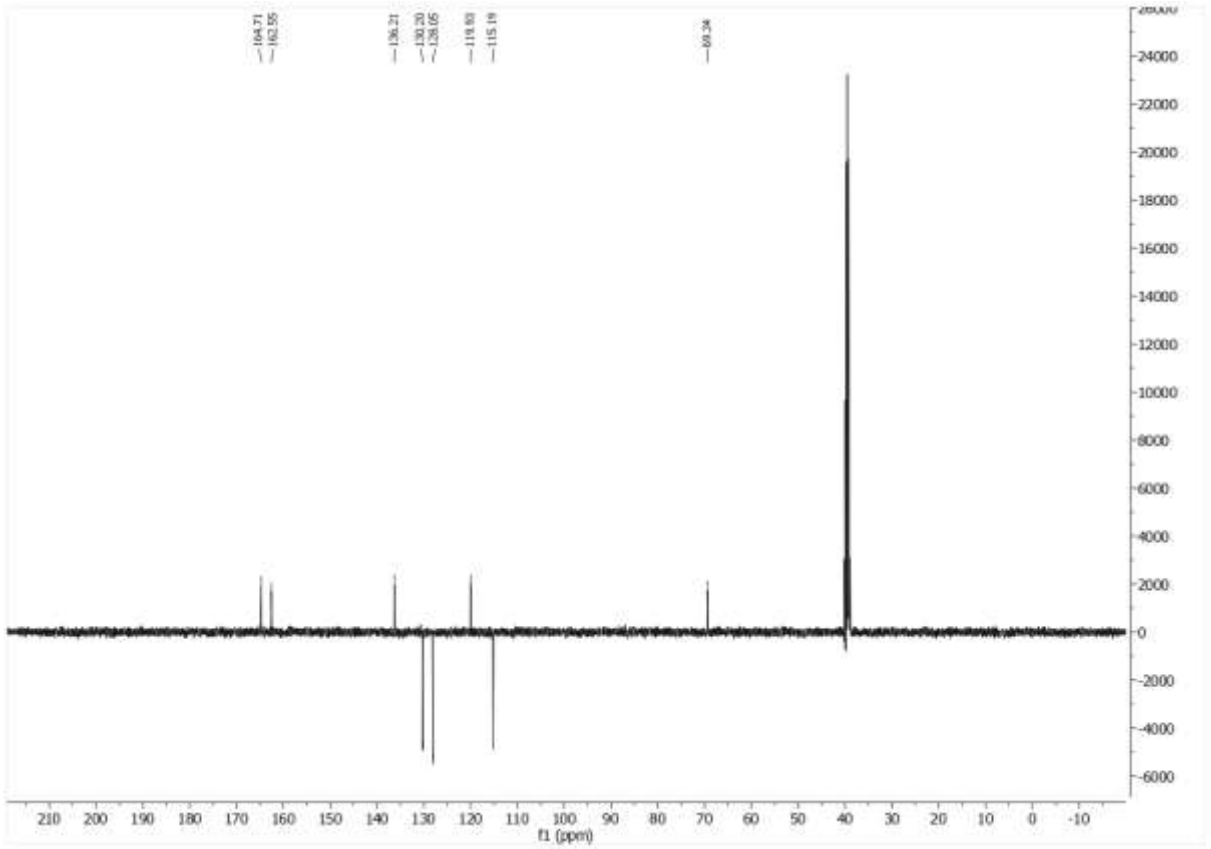
^{13}C NMR (DMSO)



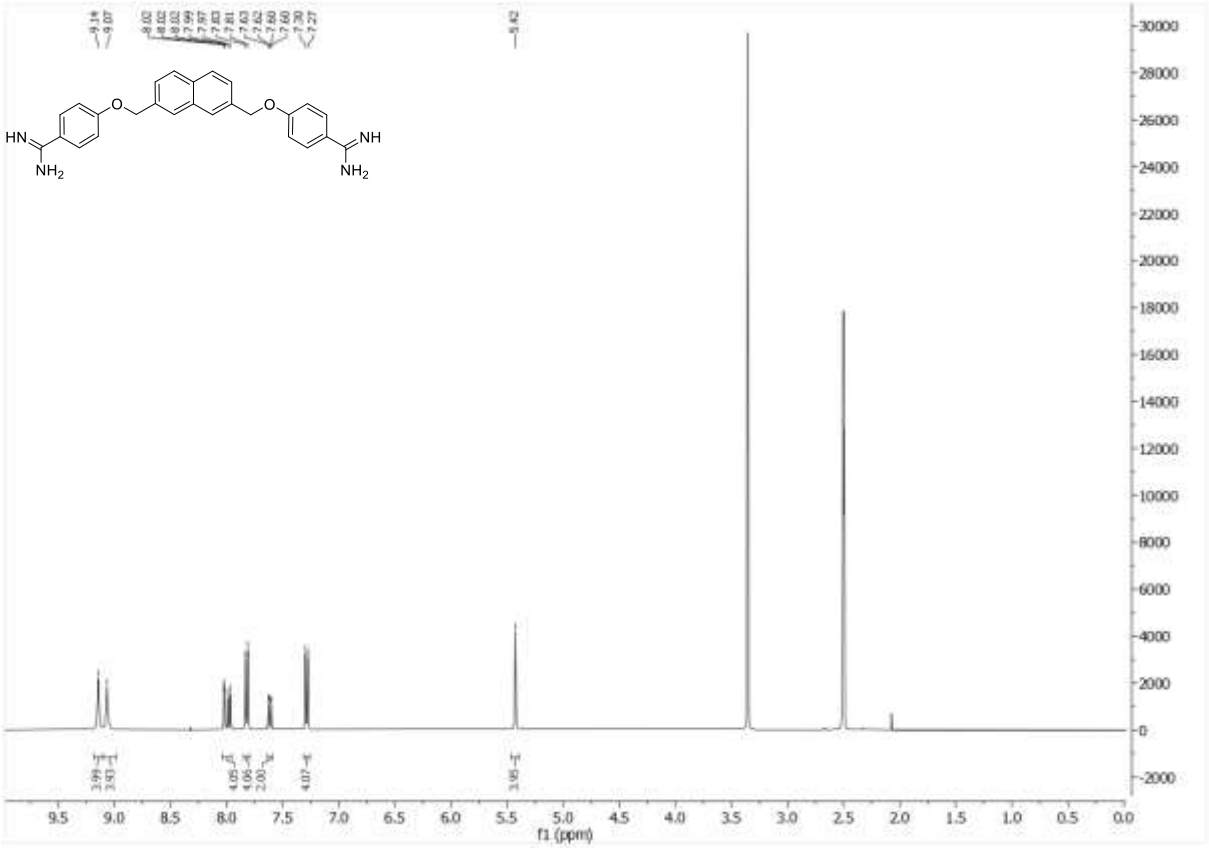
Compound **23** ^{1}H NMR (DMSO)



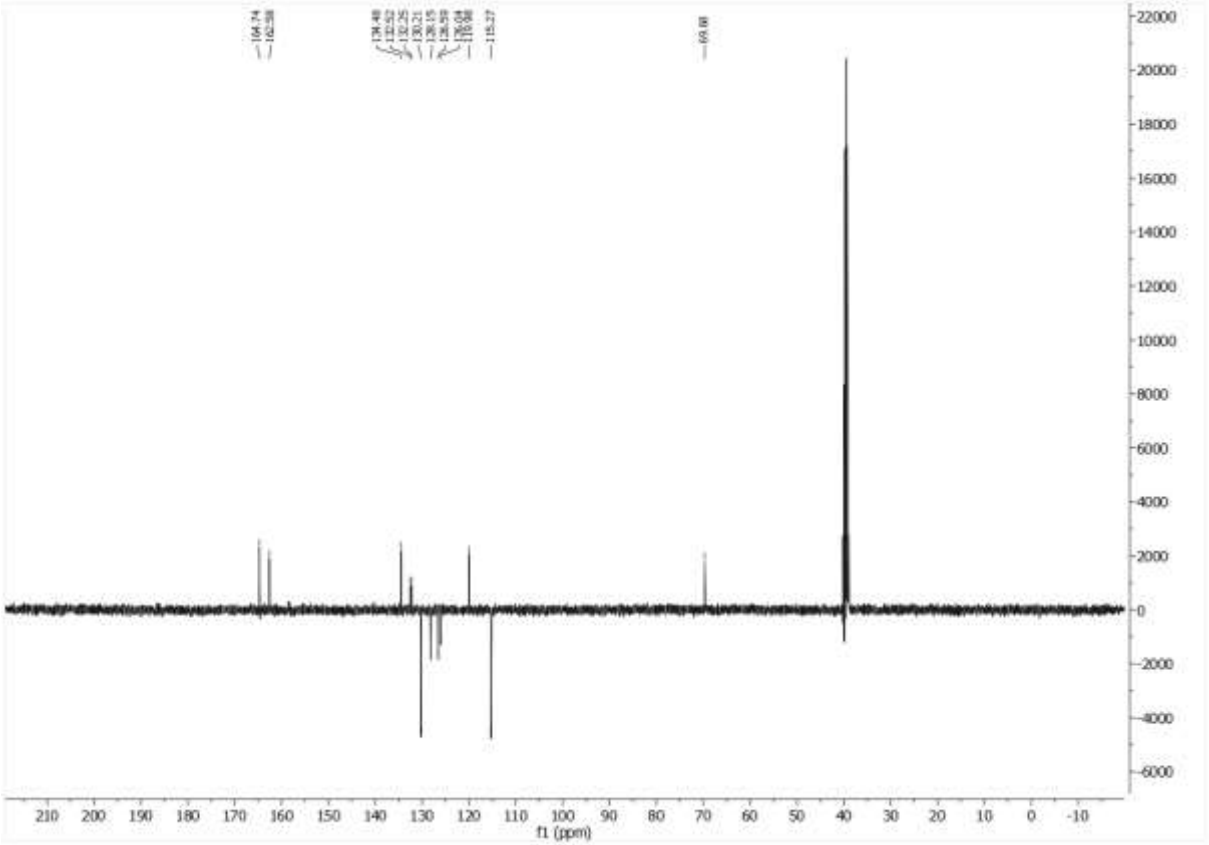
^{13}C NMR (DMSO)



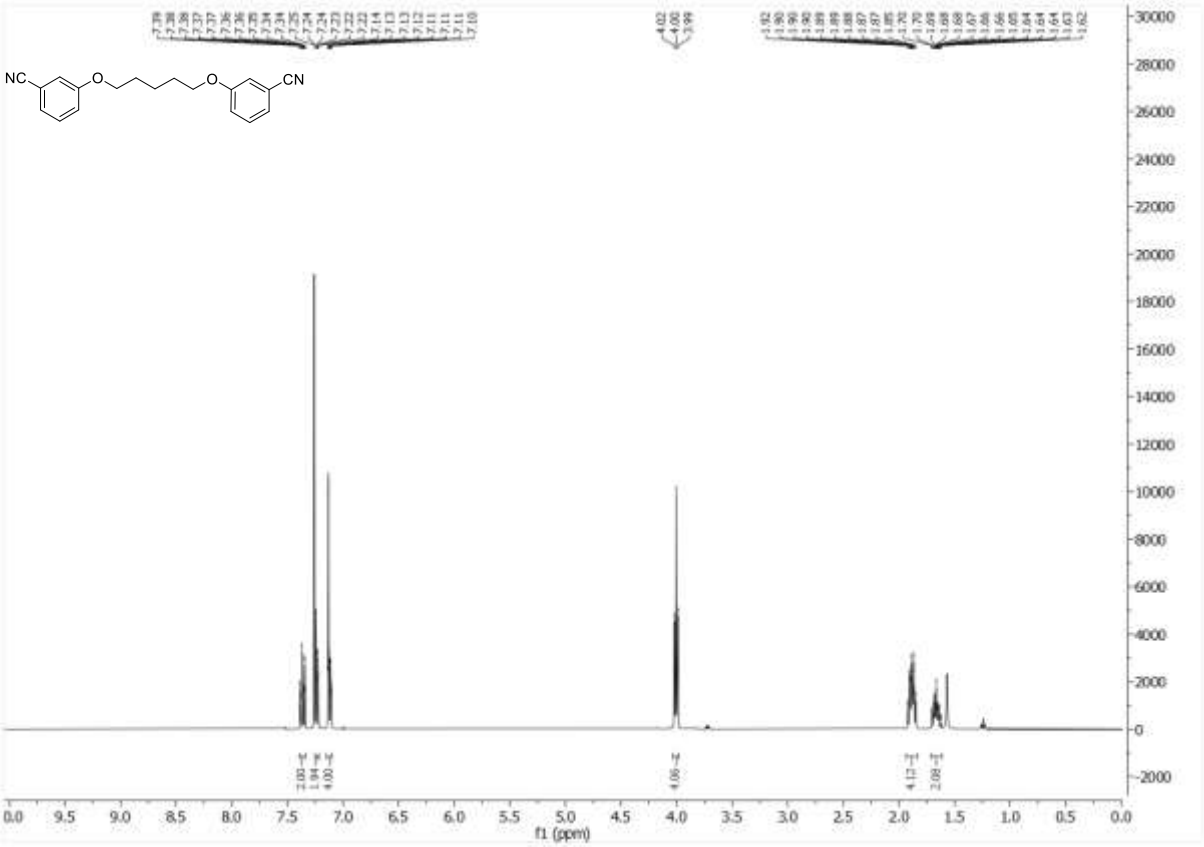
Compound **24** ^{1}H NMR (DMSO)



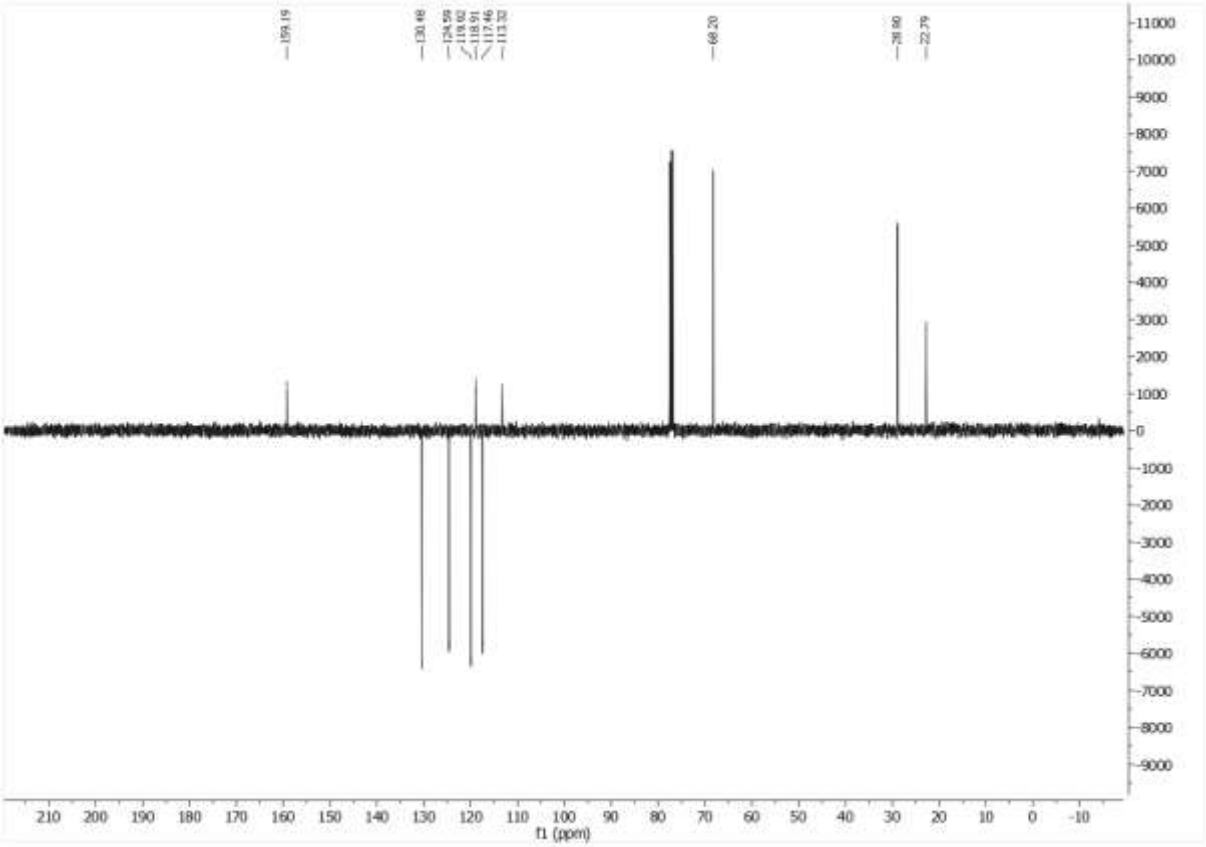
^{13}C NMR (DMSO)



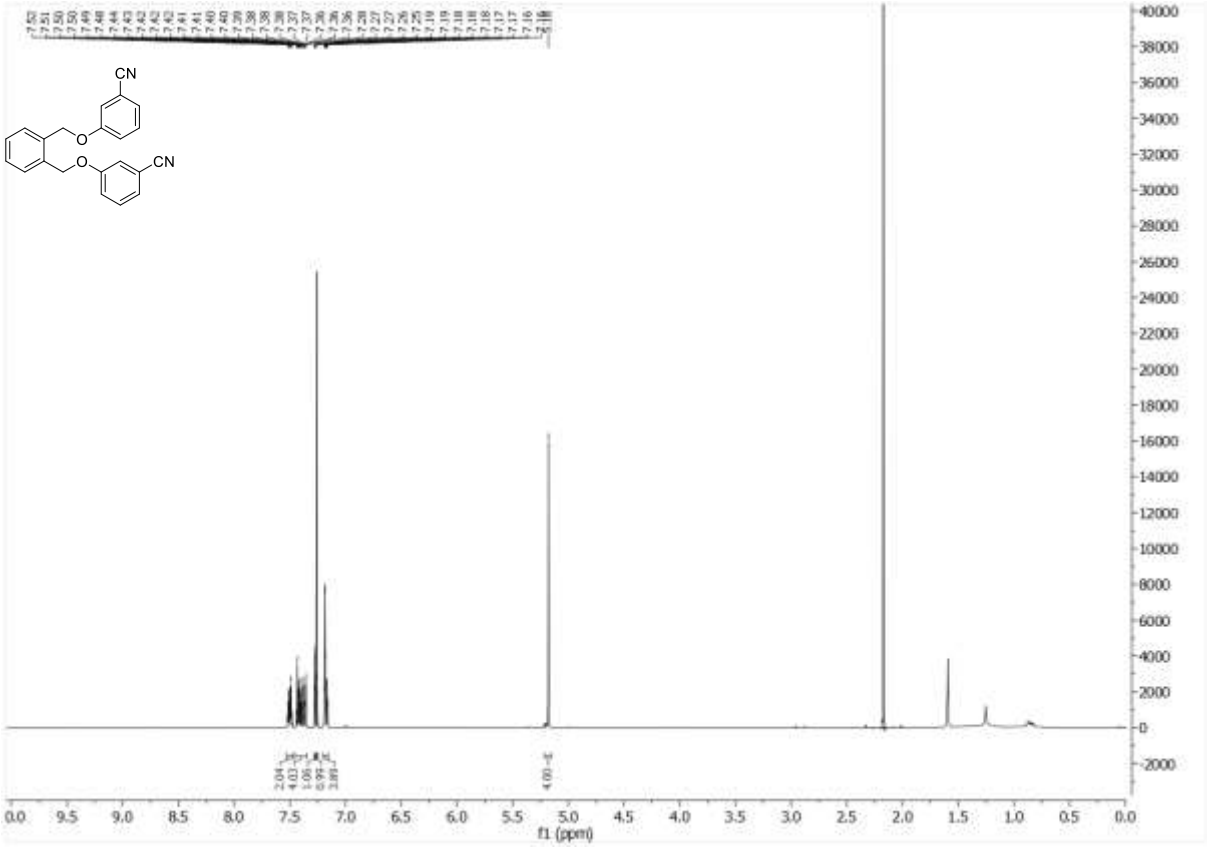
Compound **25** ^{1}H NMR ($CDCl_3$)



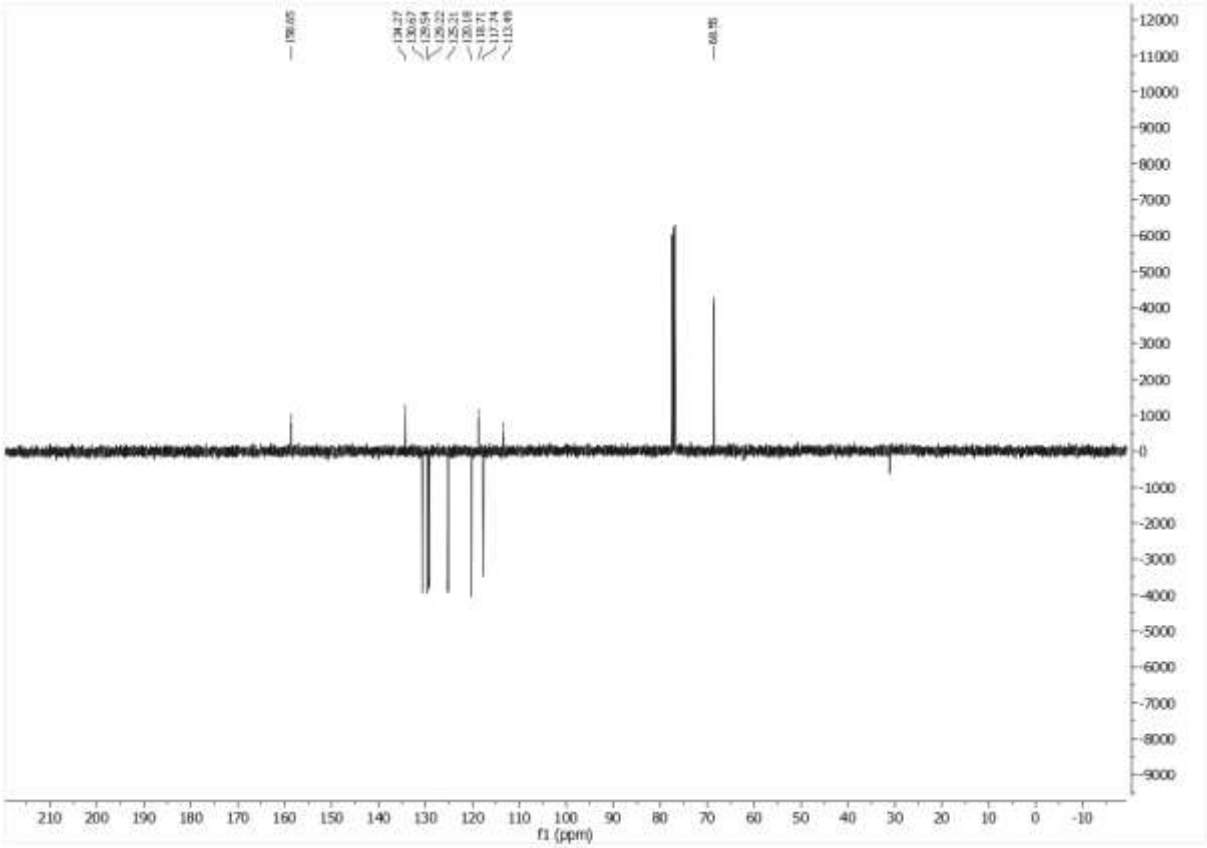
^{13}C NMR ($CDCl_3$)



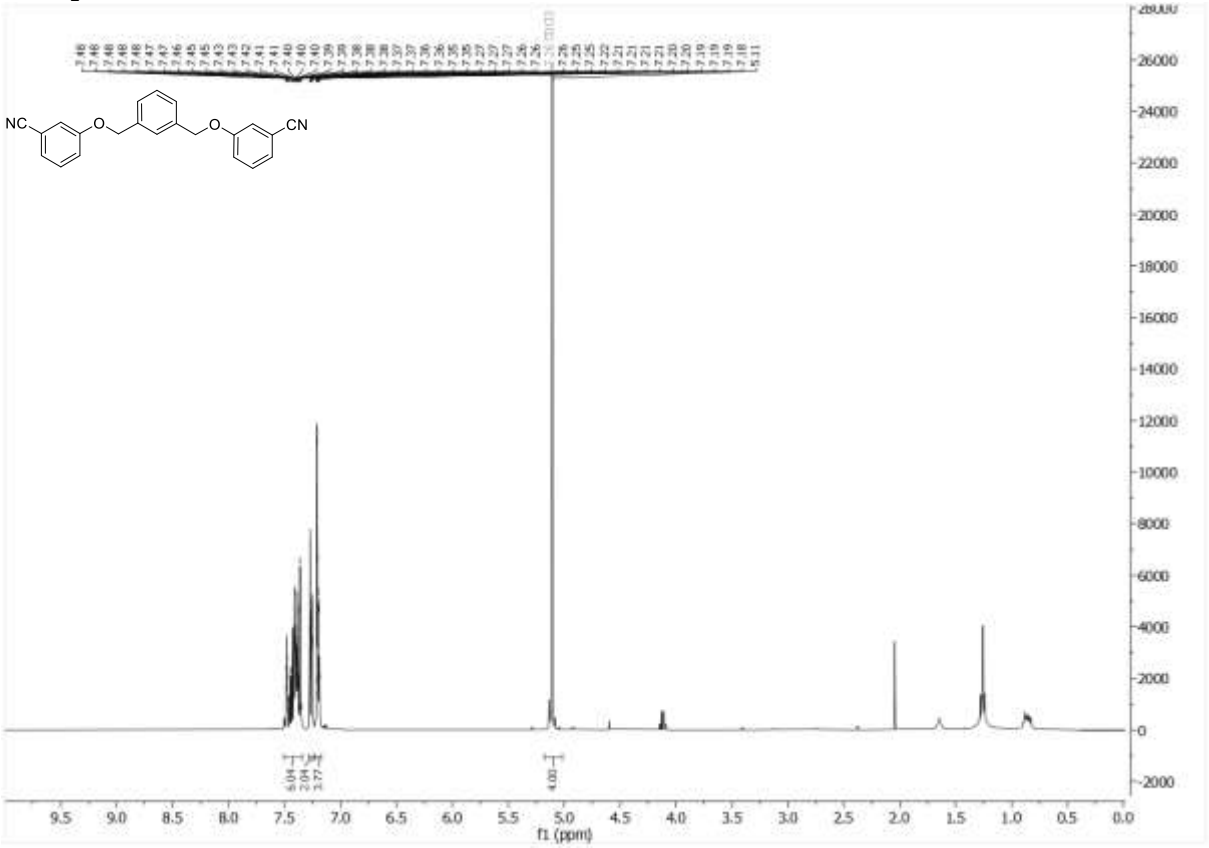
Compound **26** ^{1}H NMR ($CDCl_3$)



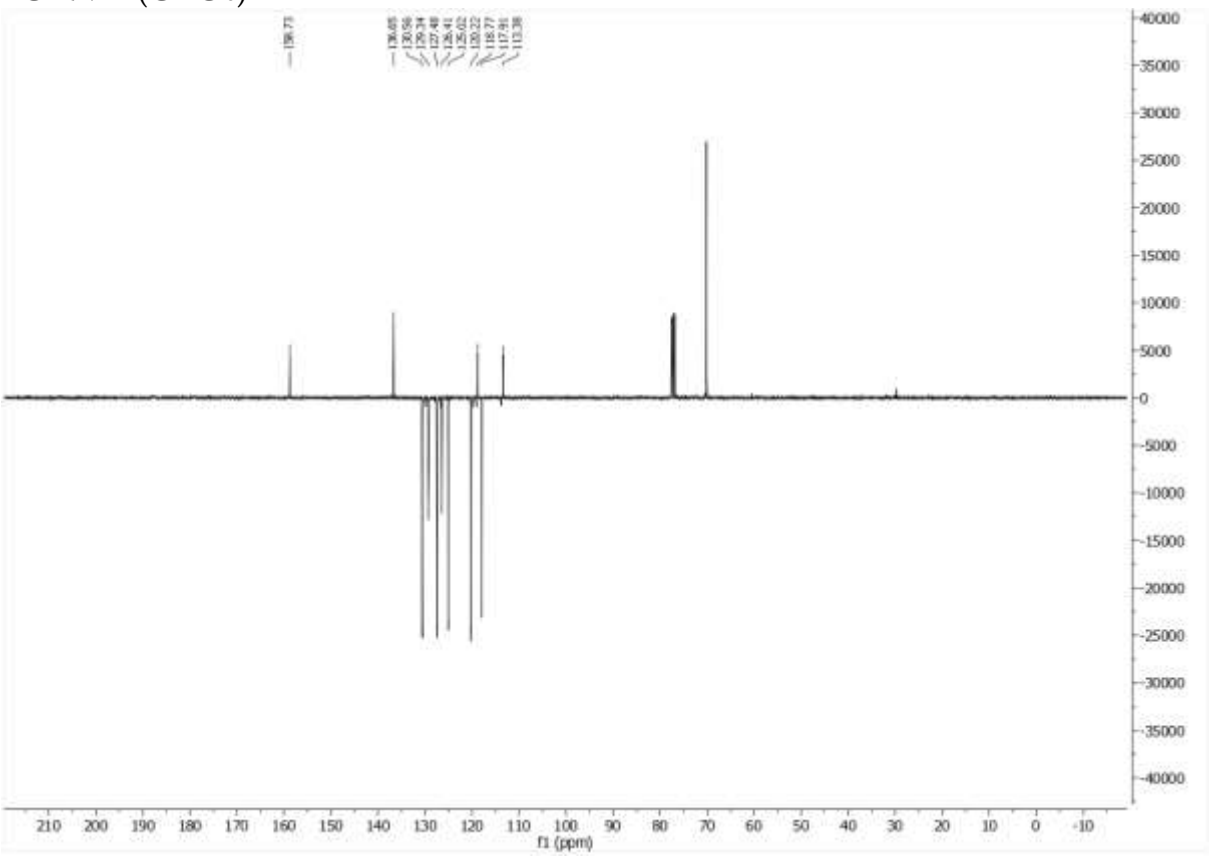
^{13}C NMR ($CDCl_3$)



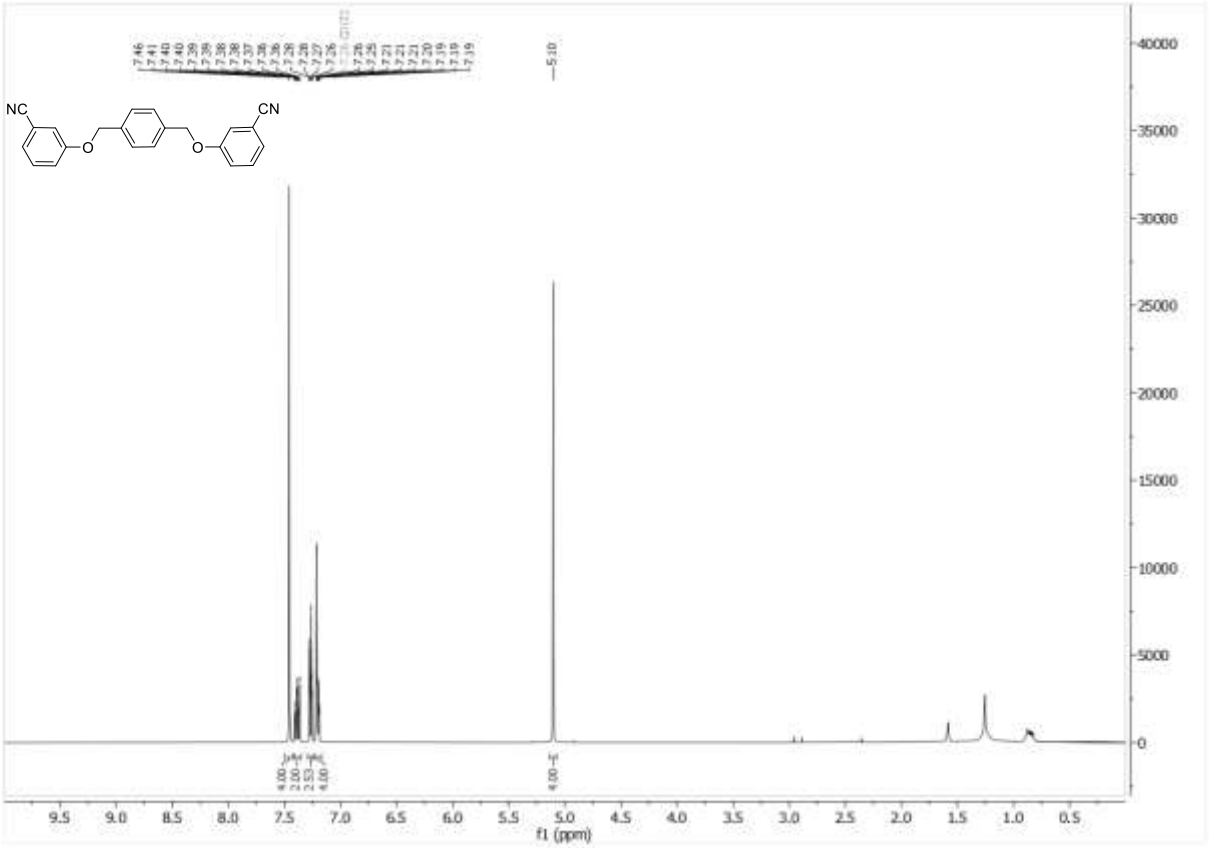
Compound **27** ^{1}H NMR ($CDCl_3$)



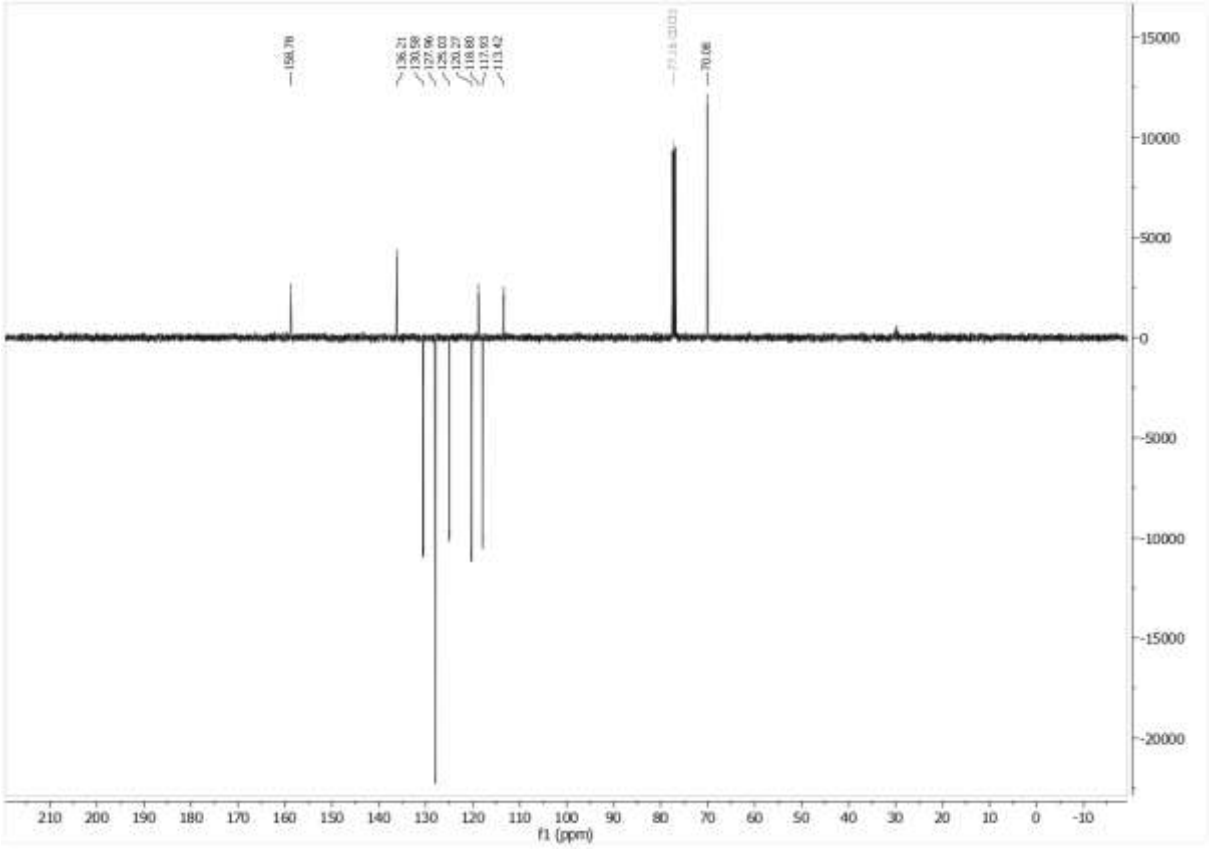
^{13}C NMR ($CDCl_3$)



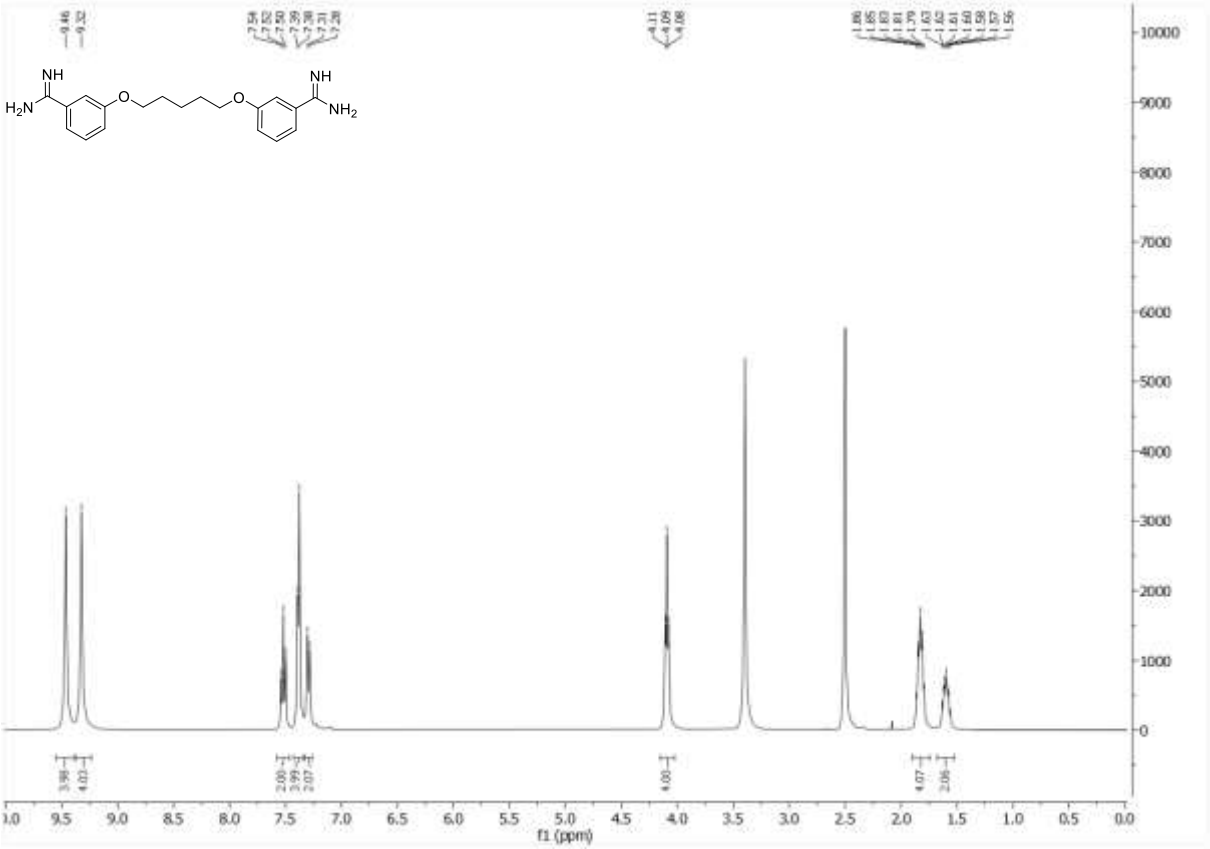
Compound **28** ^{1}H NMR ($CDCl_3$)



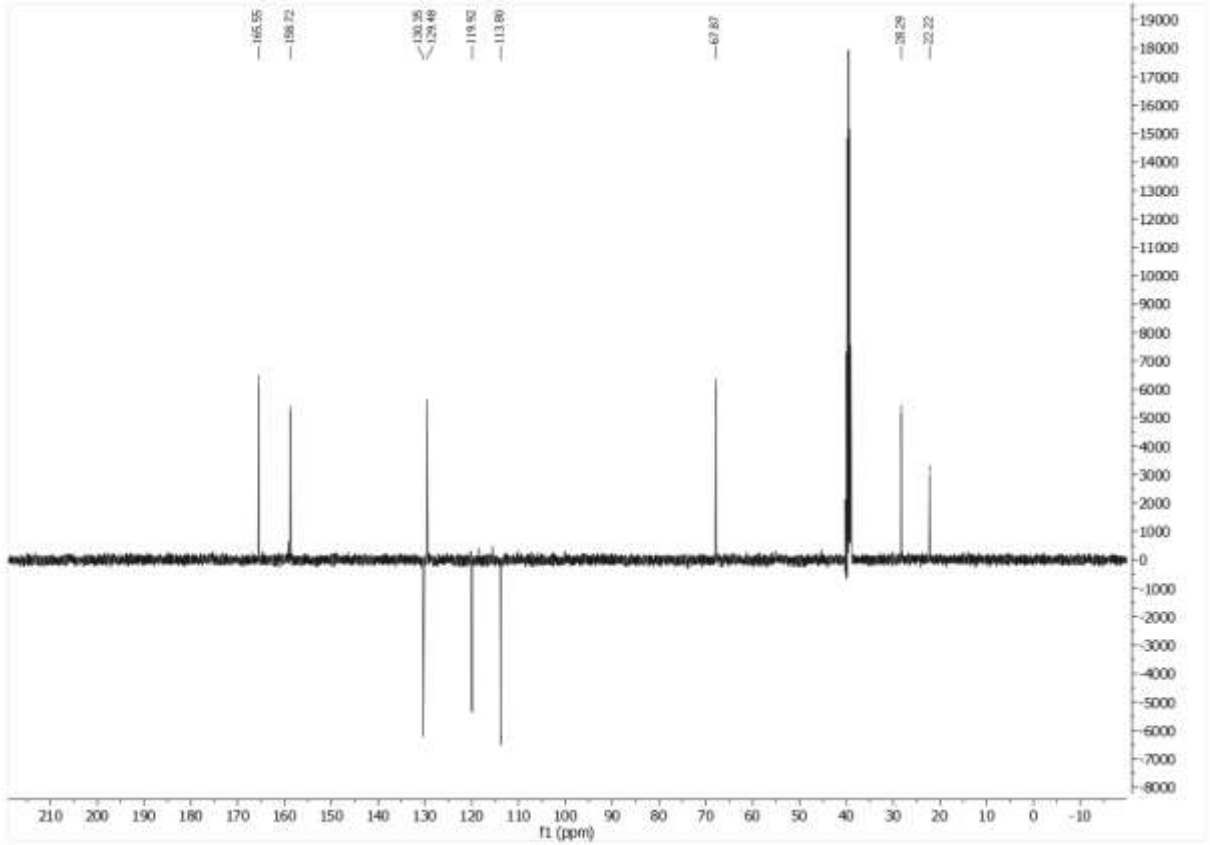
^{13}C NMR ($CDCl_3$)



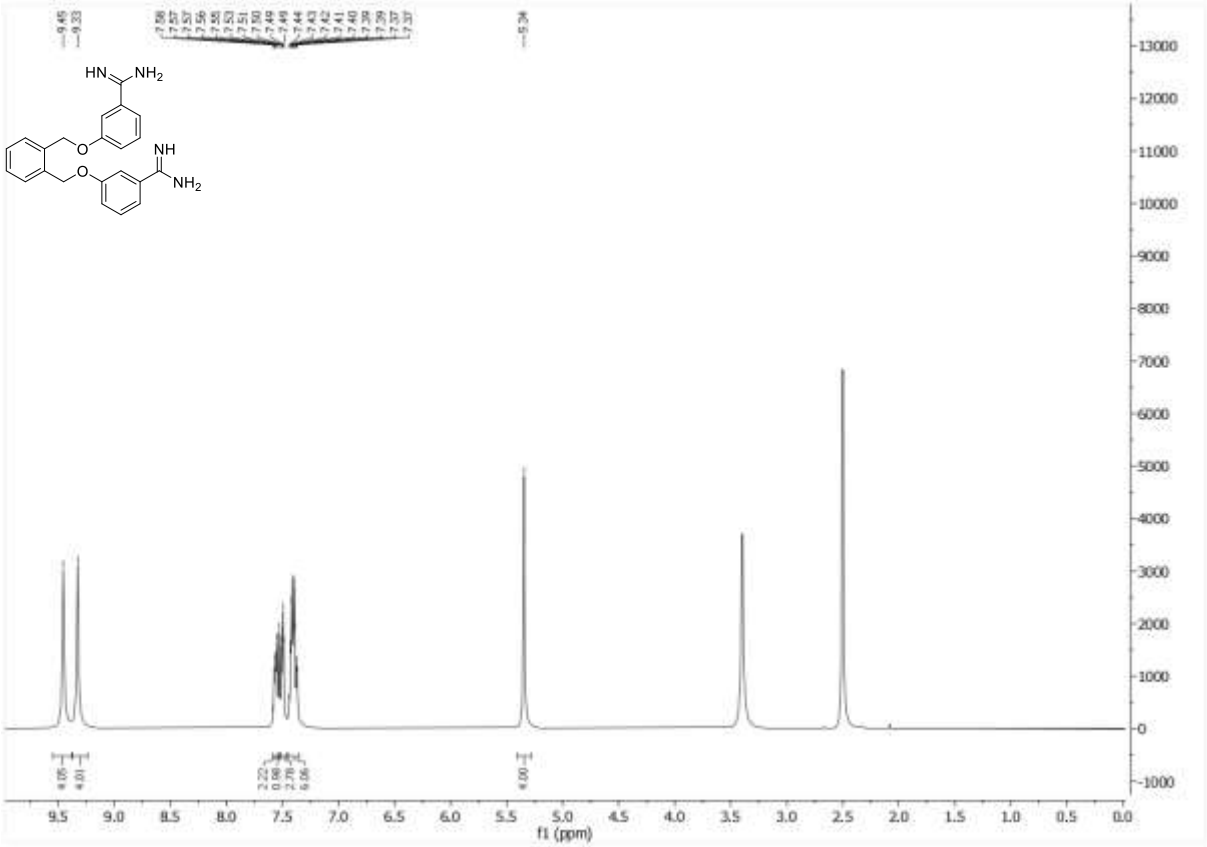
Compound **1b** ^{1}H NMR (DMSO)



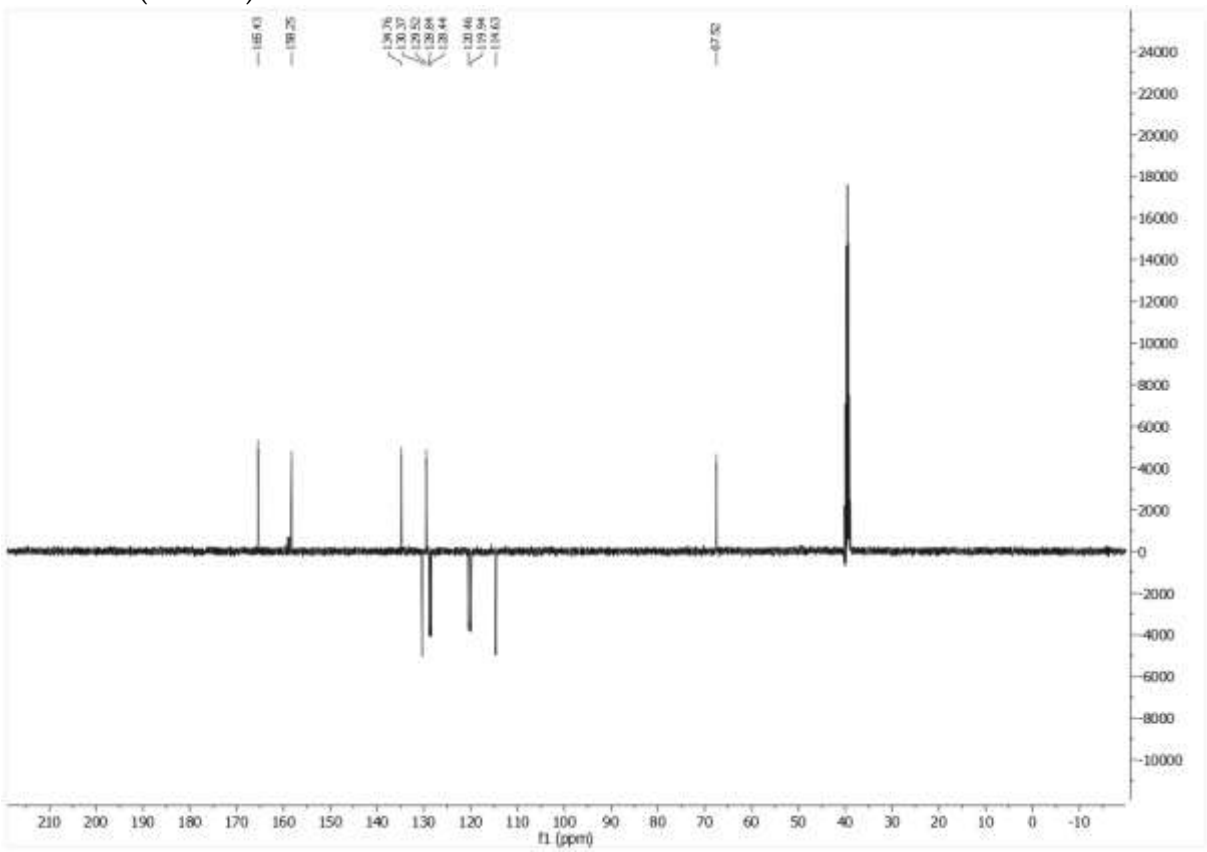
^{13}C NMR (DMSO)



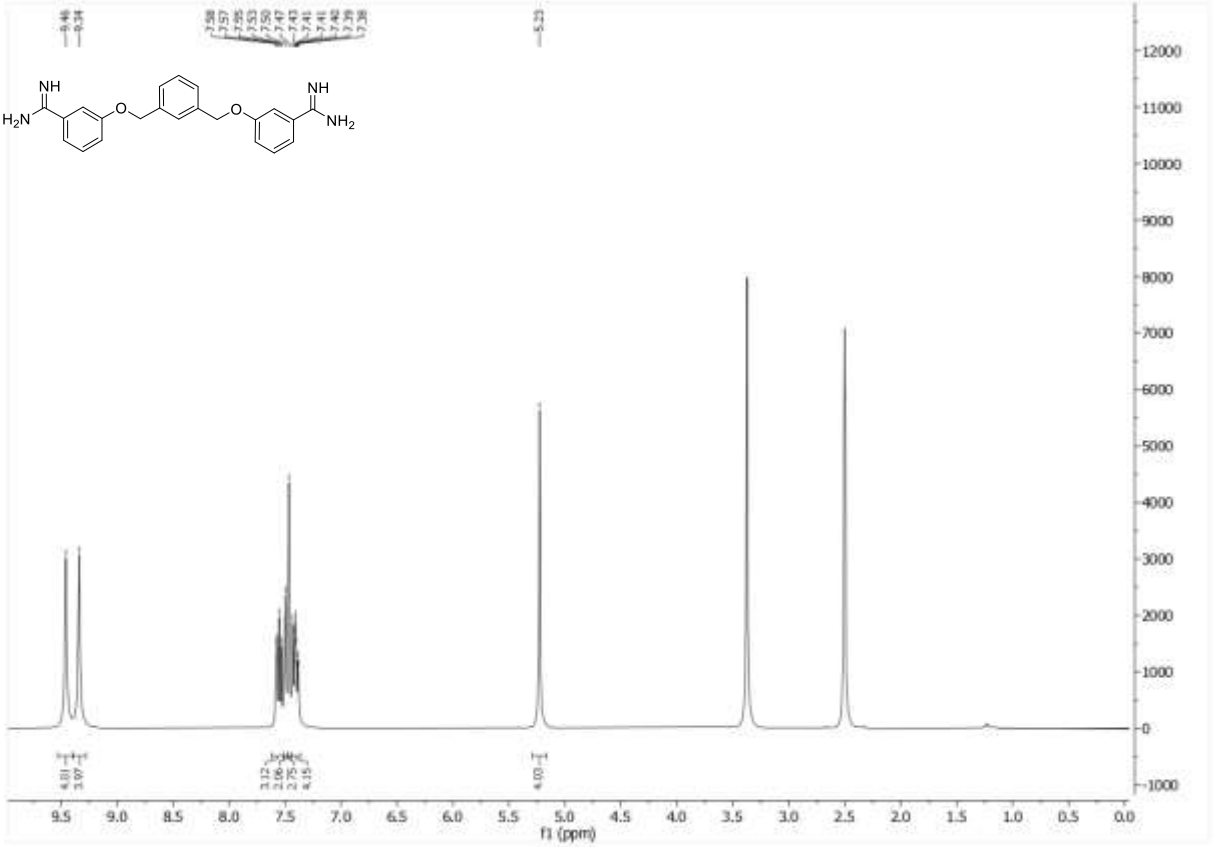
Compound **21b** ^{1}H NMR (DMSO)



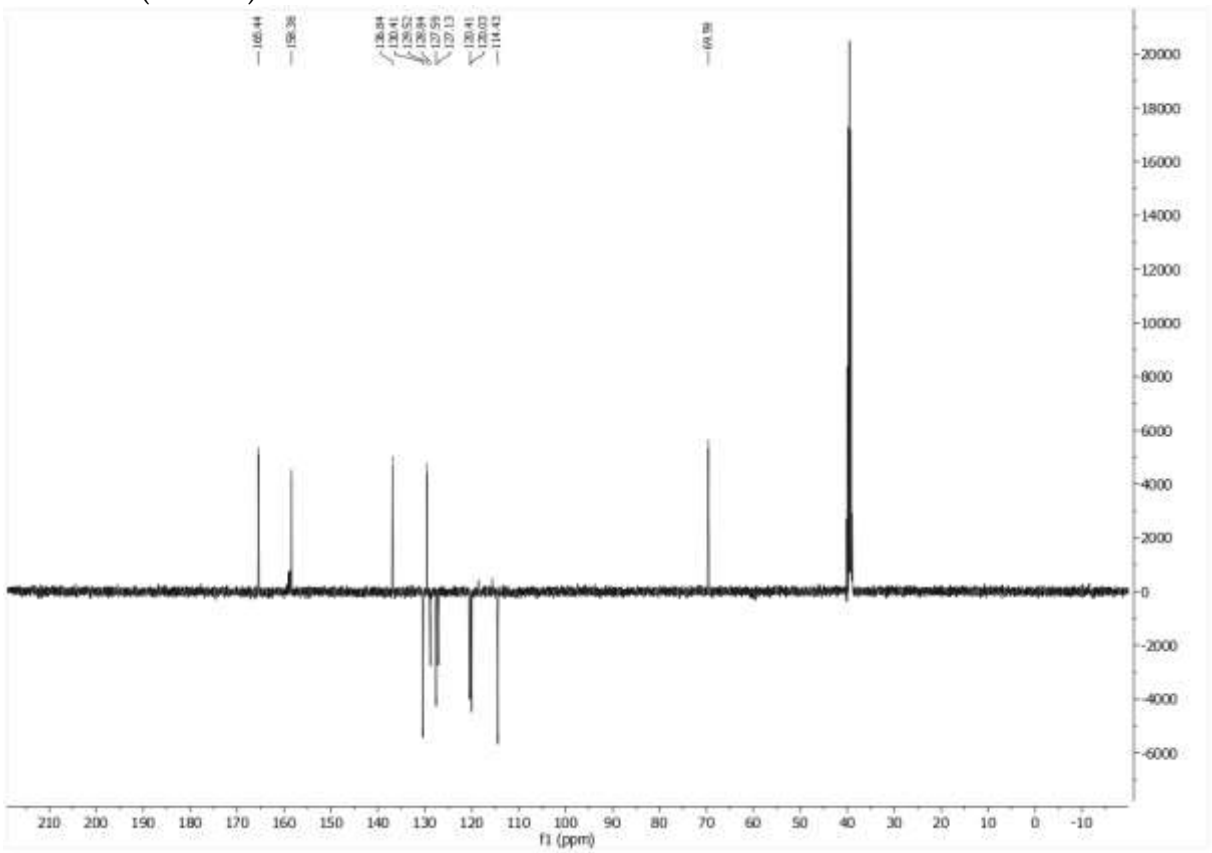
^{13}C NMR (DMSO)



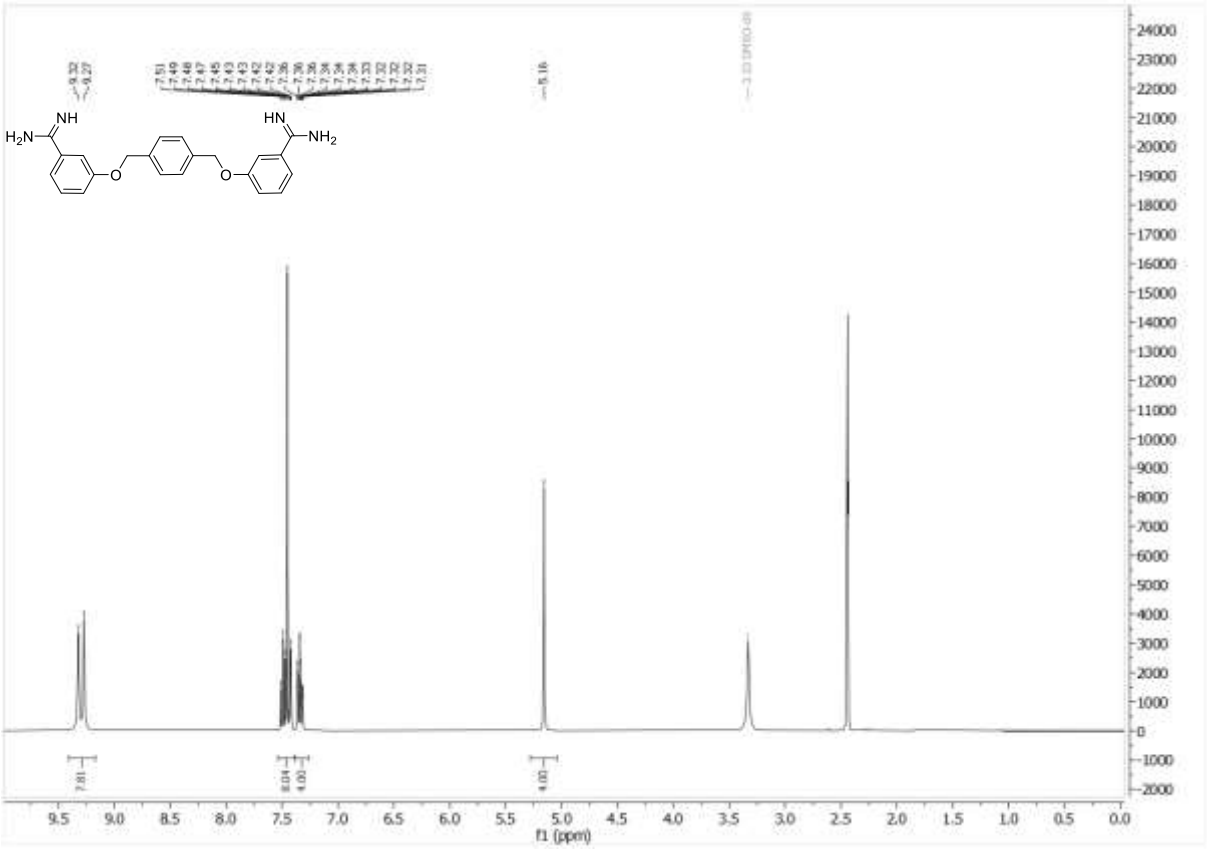
Compound **22b** ^{1}H NMR (DMSO)



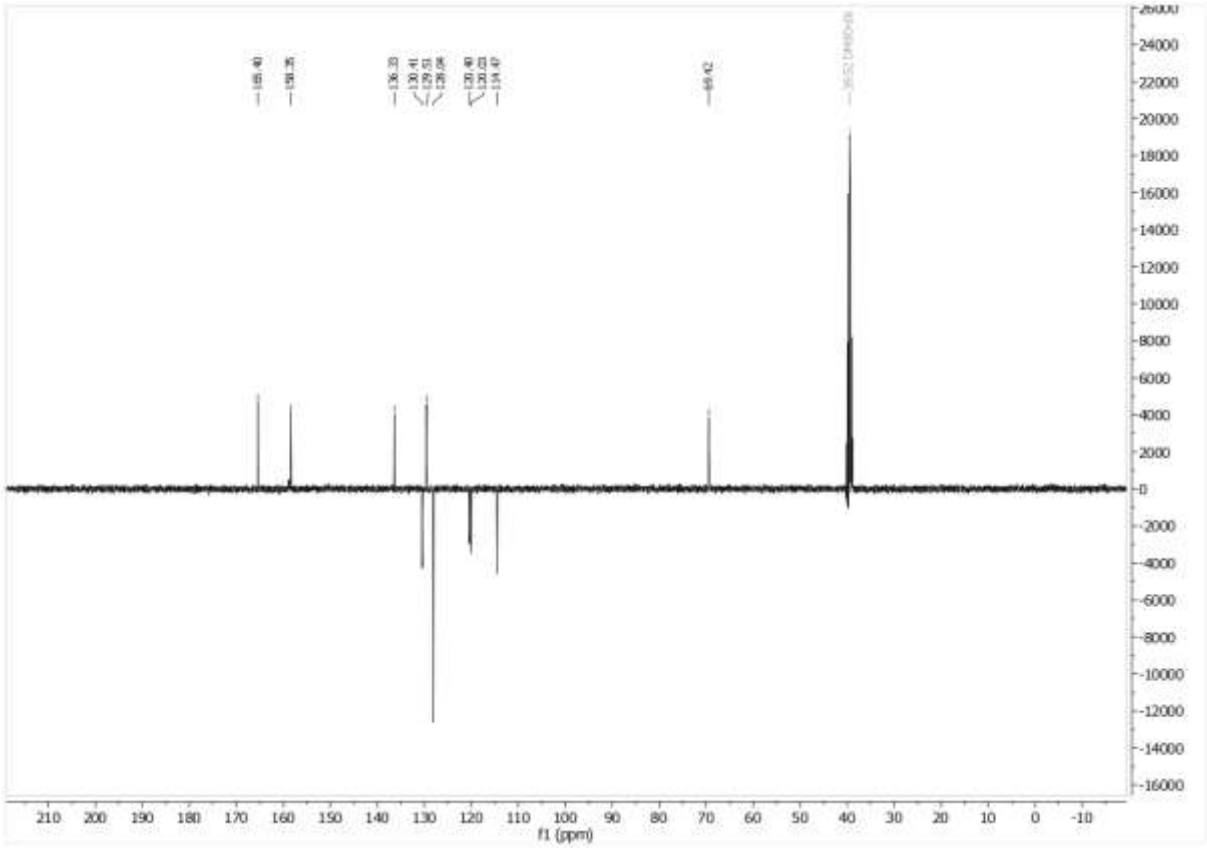
^{13}C NMR (DMSO)



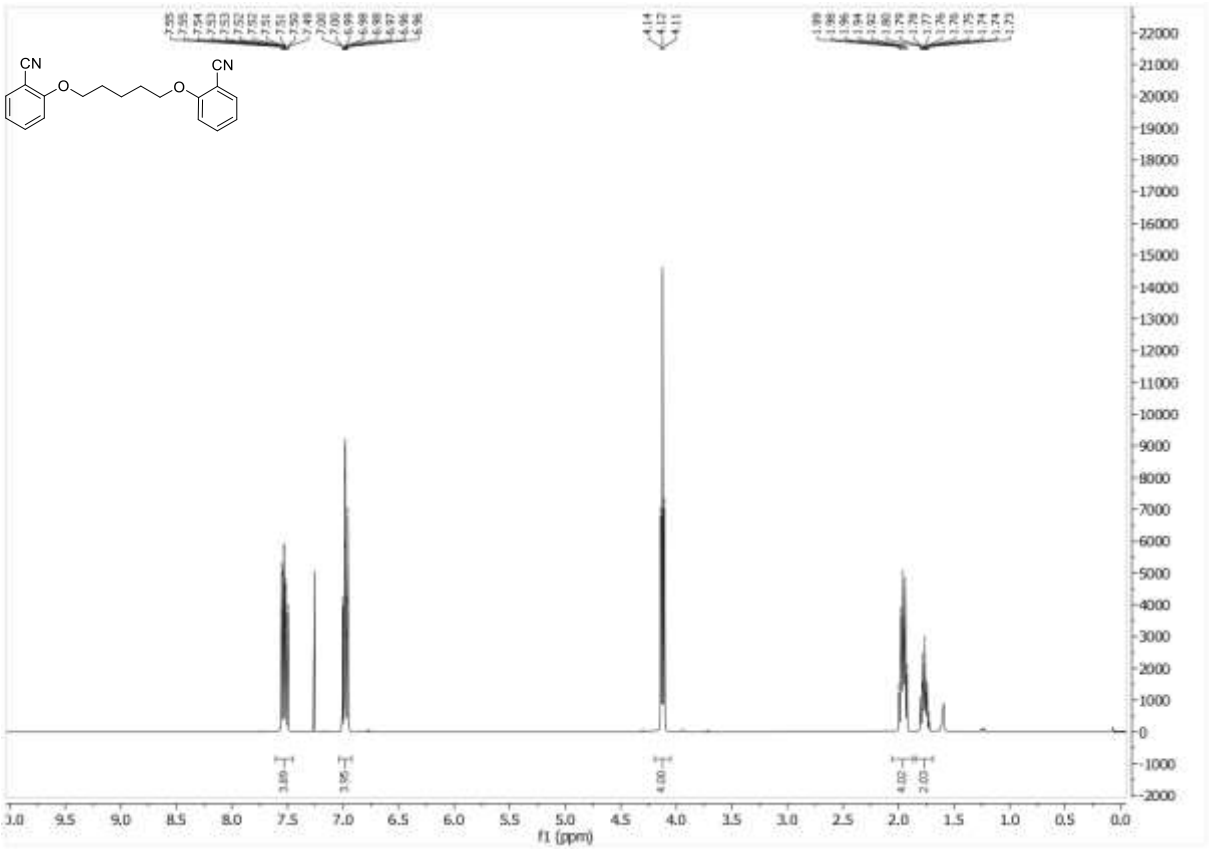
Compound **23b** ^{1}H NMR (DMSO)



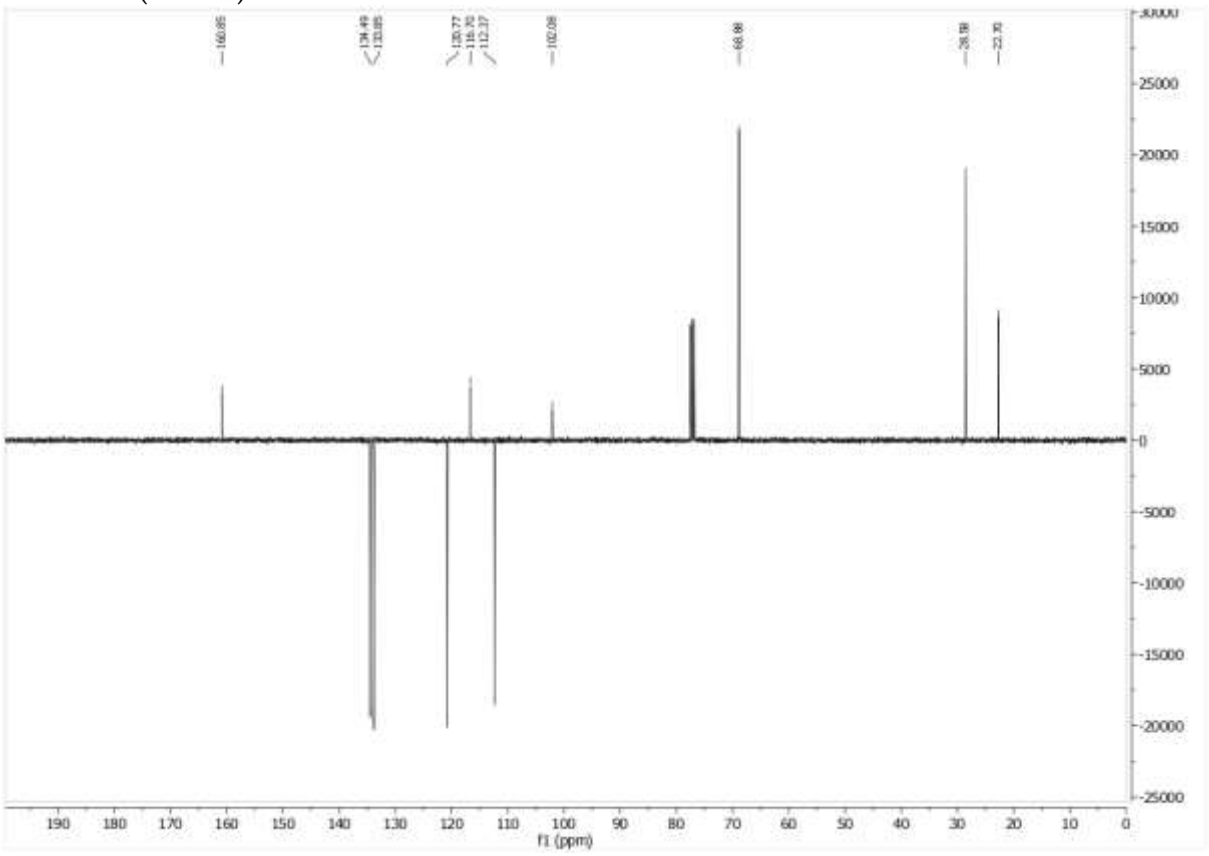
^{13}C NMR (DMSO)



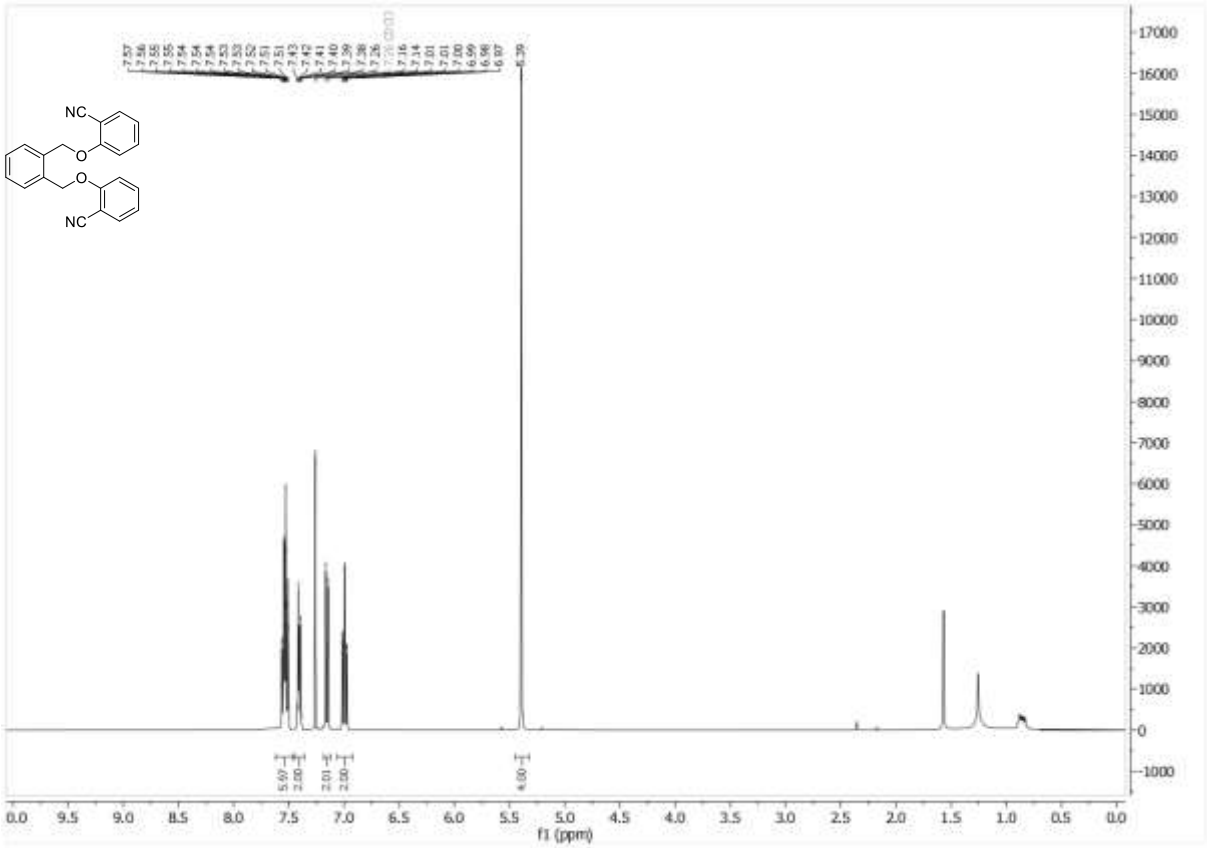
Compound **29** ^{1}H NMR ($CDCl_3$)



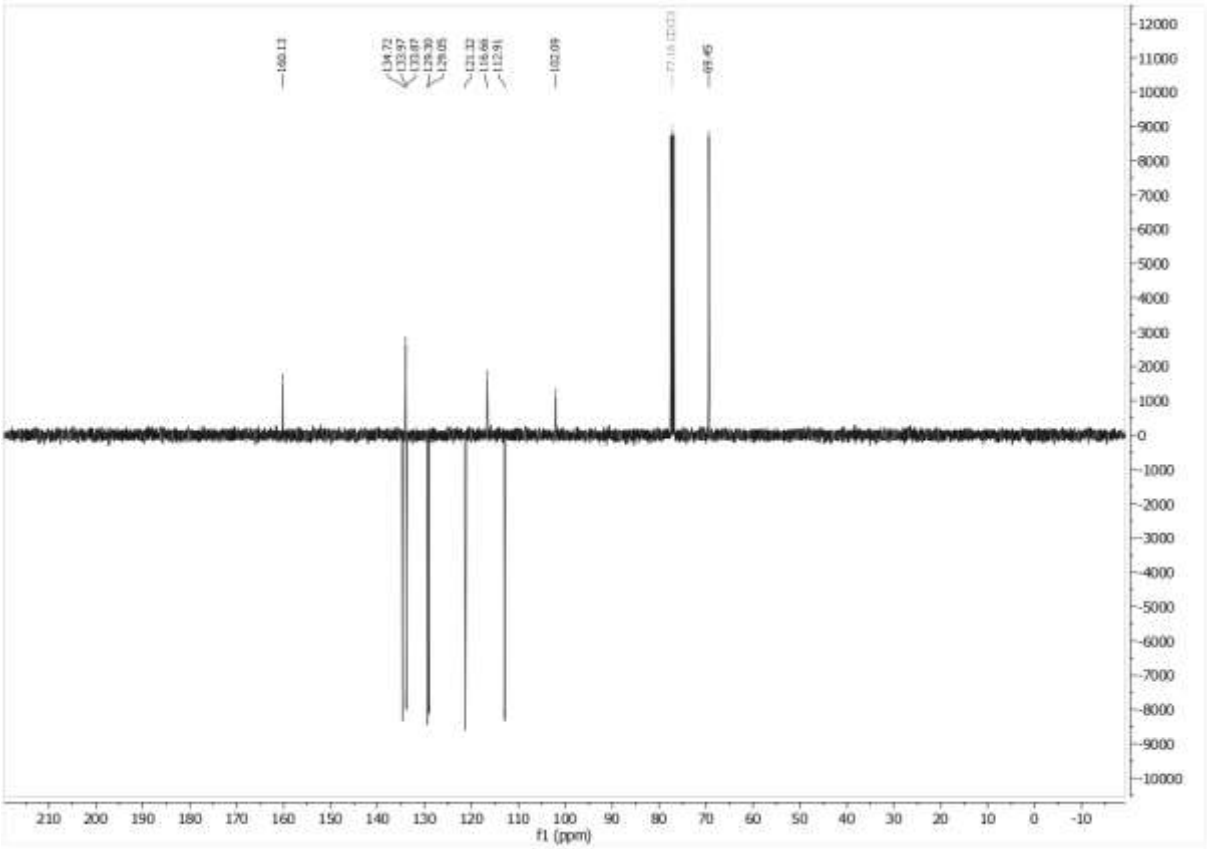
^{13}C NMR ($CDCl_3$)



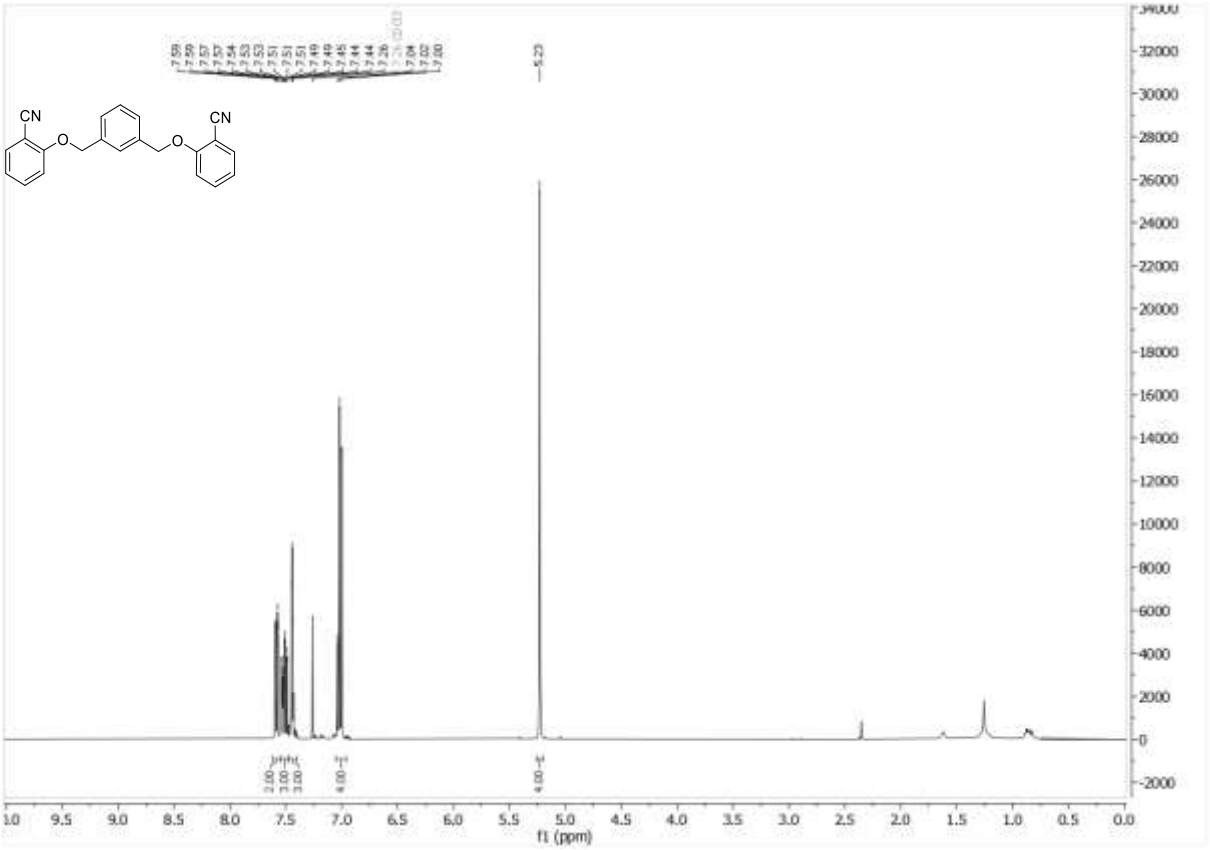
Compound **30** ^{1}H NMR ($CDCl_3$)



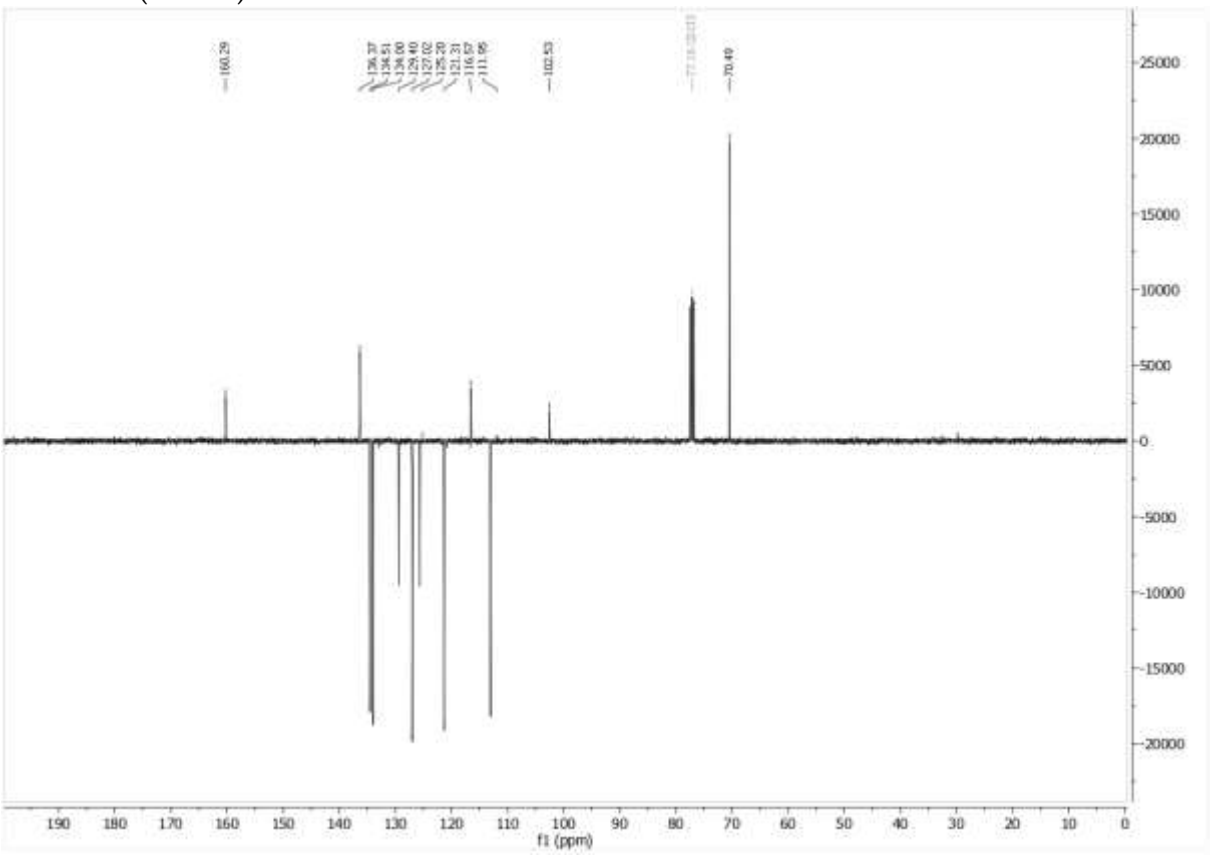
^{13}C NMR ($CDCl_3$)



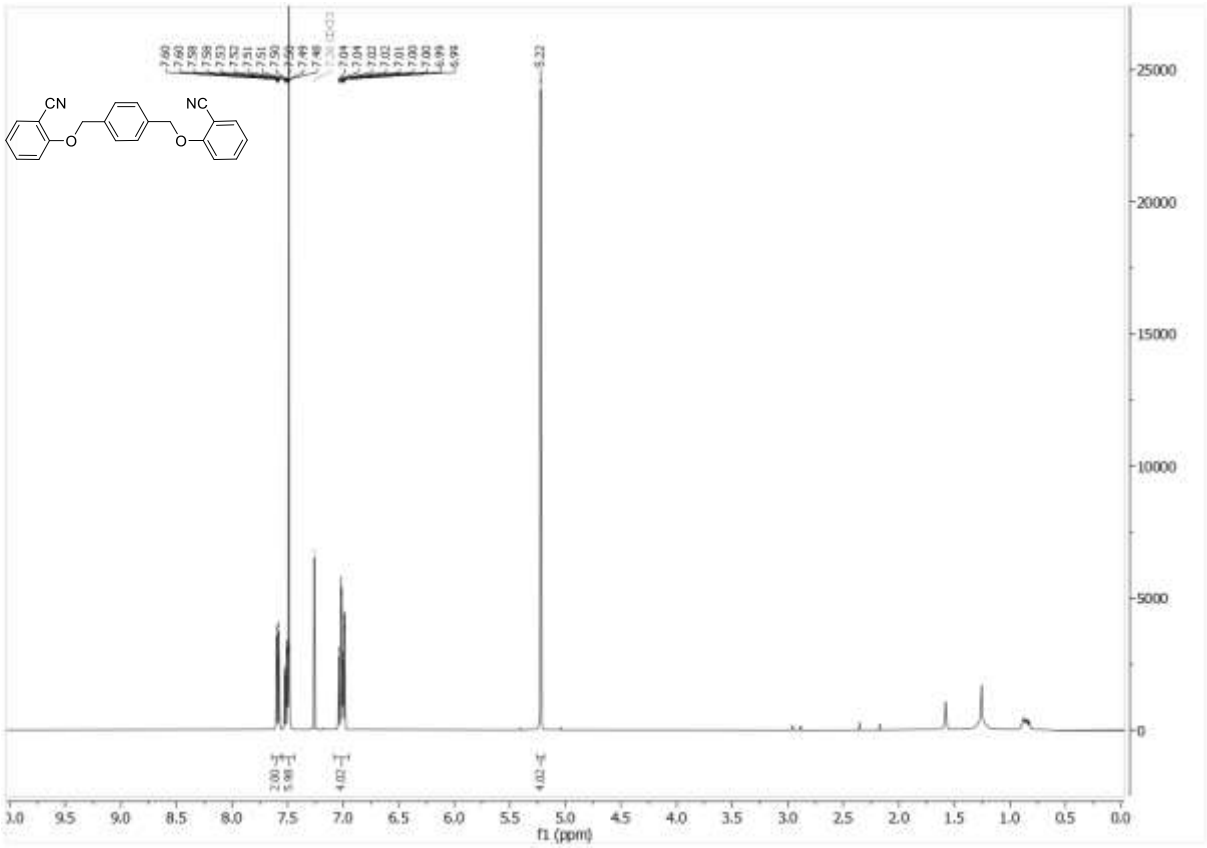
Compound **31** ^{1}H NMR ($CDCl_3$)



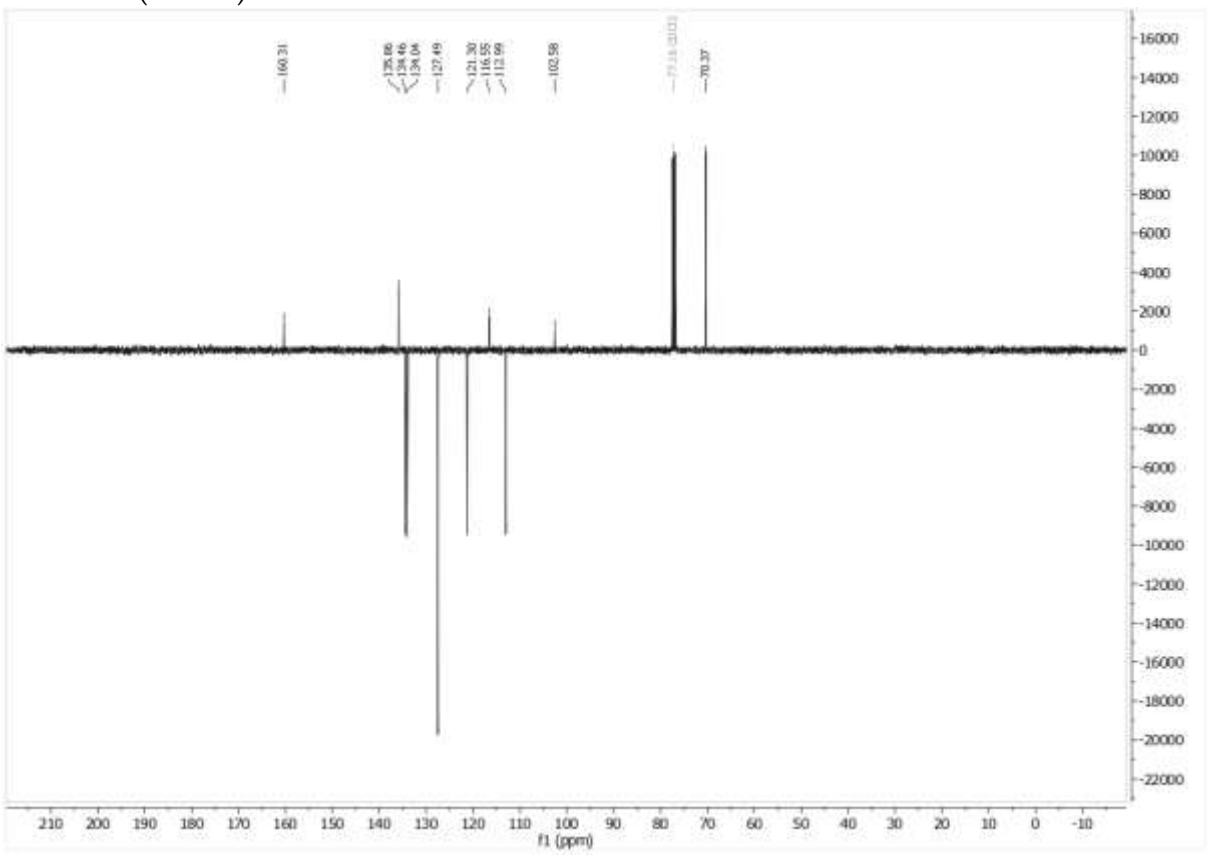
^{13}C NMR ($CDCl_3$)



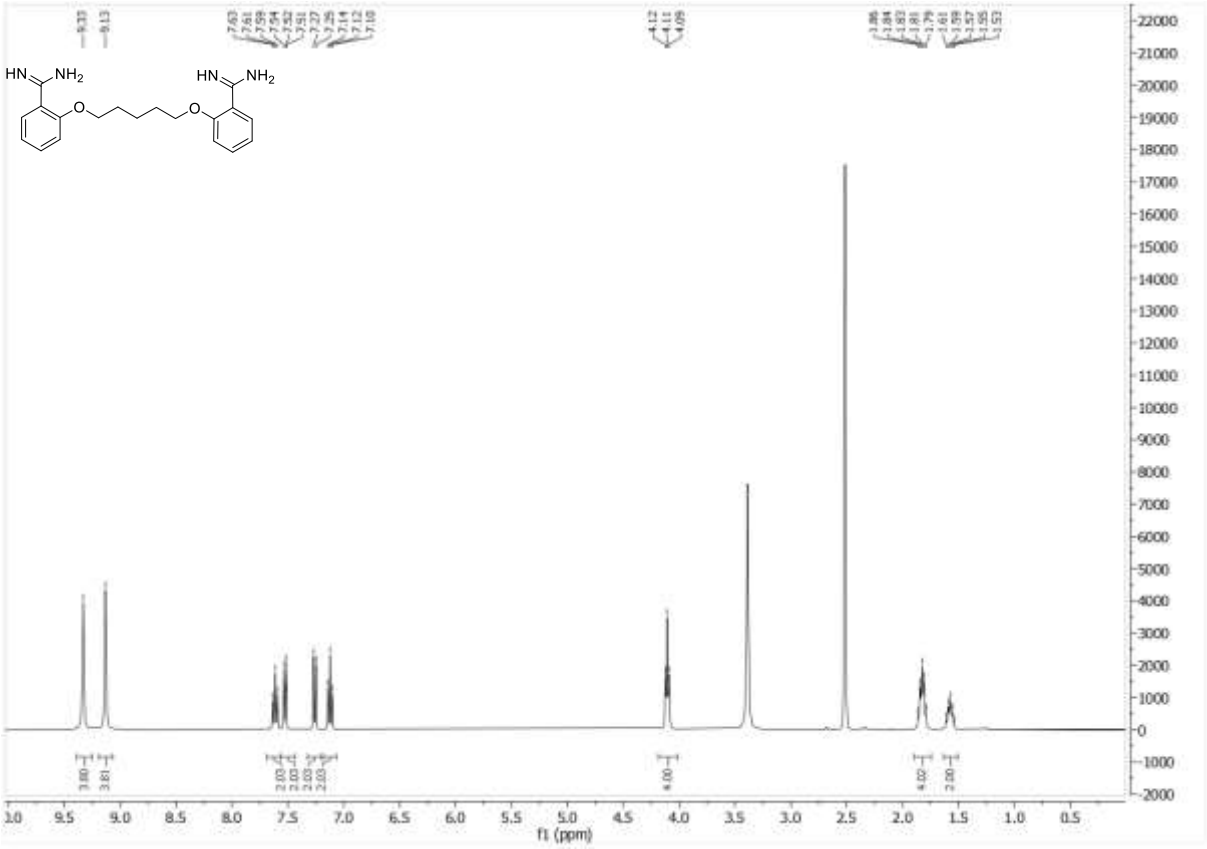
Compound **32** ^{1}H NMR ($CDCl_3$)



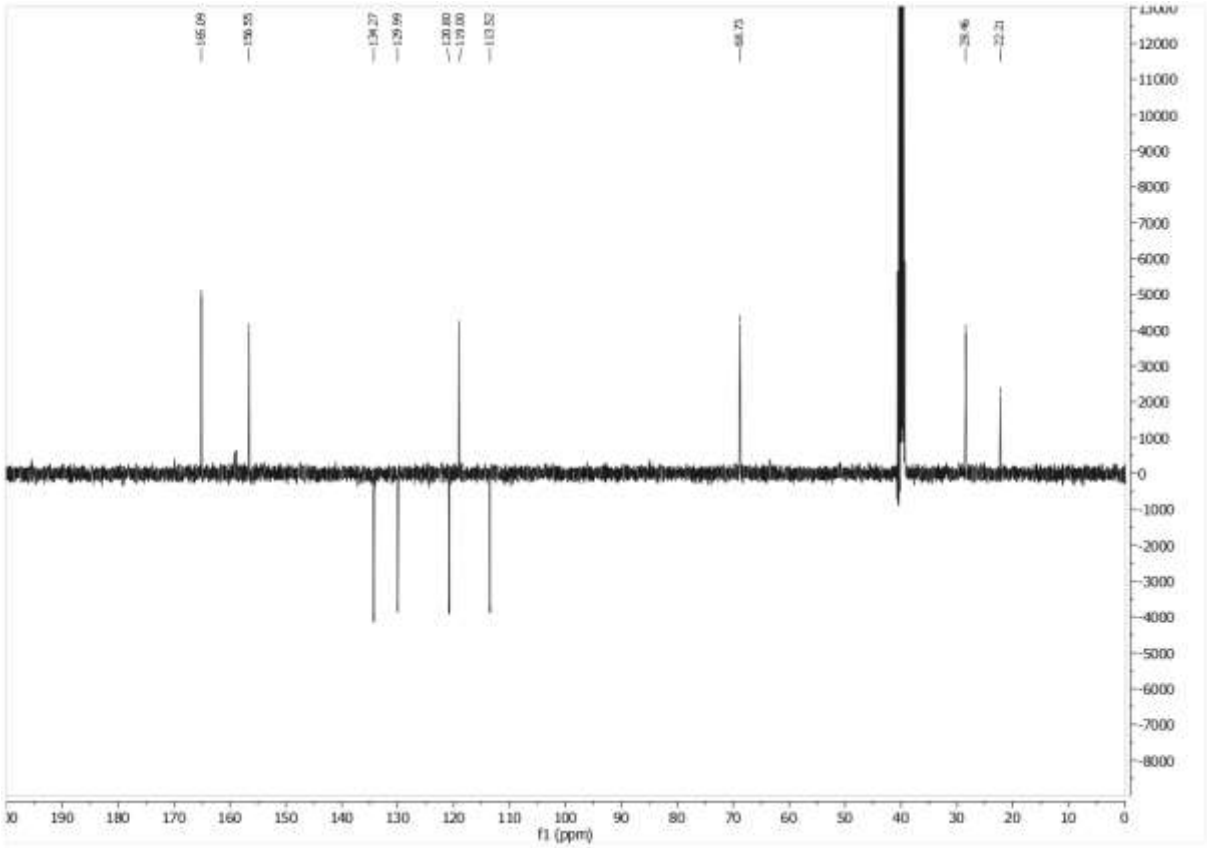
^{13}C NMR ($CDCl_3$)



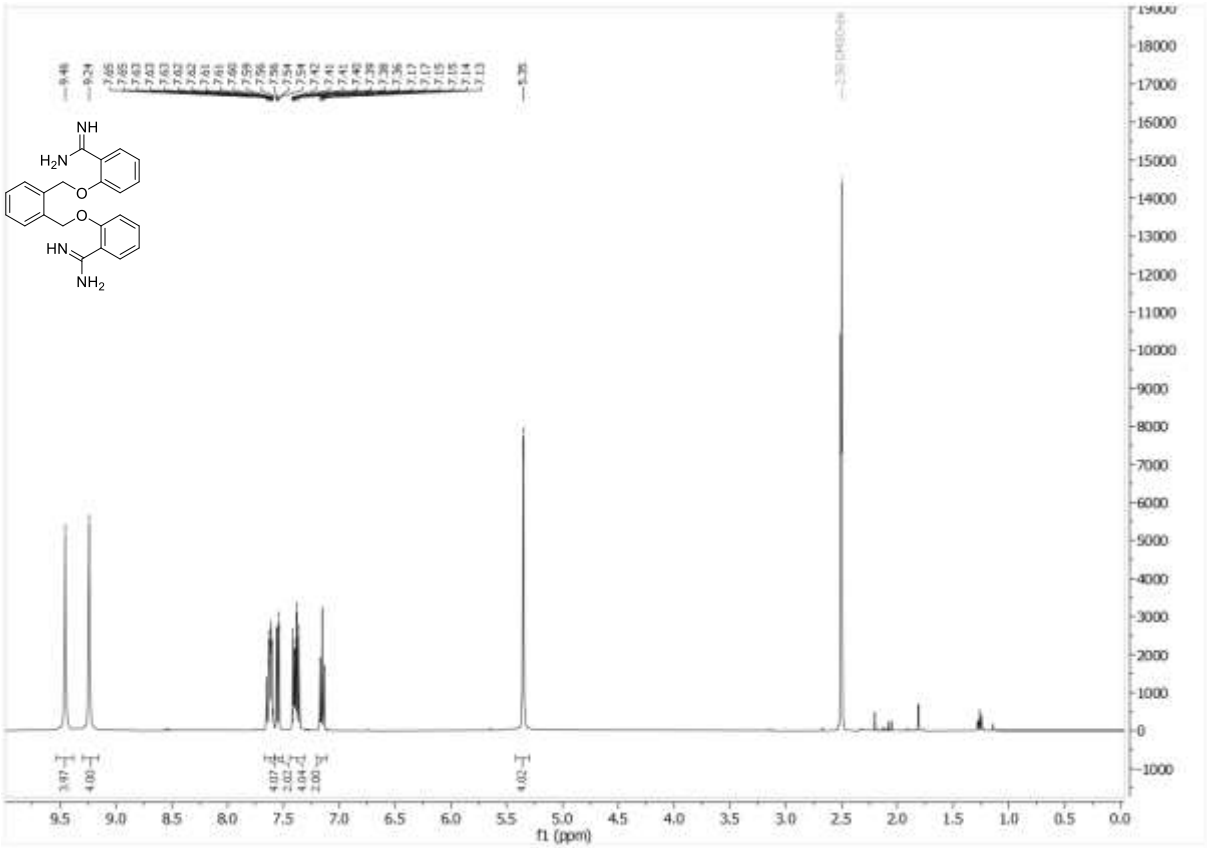
Compound **1c** ^{1}H NMR (DMSO)



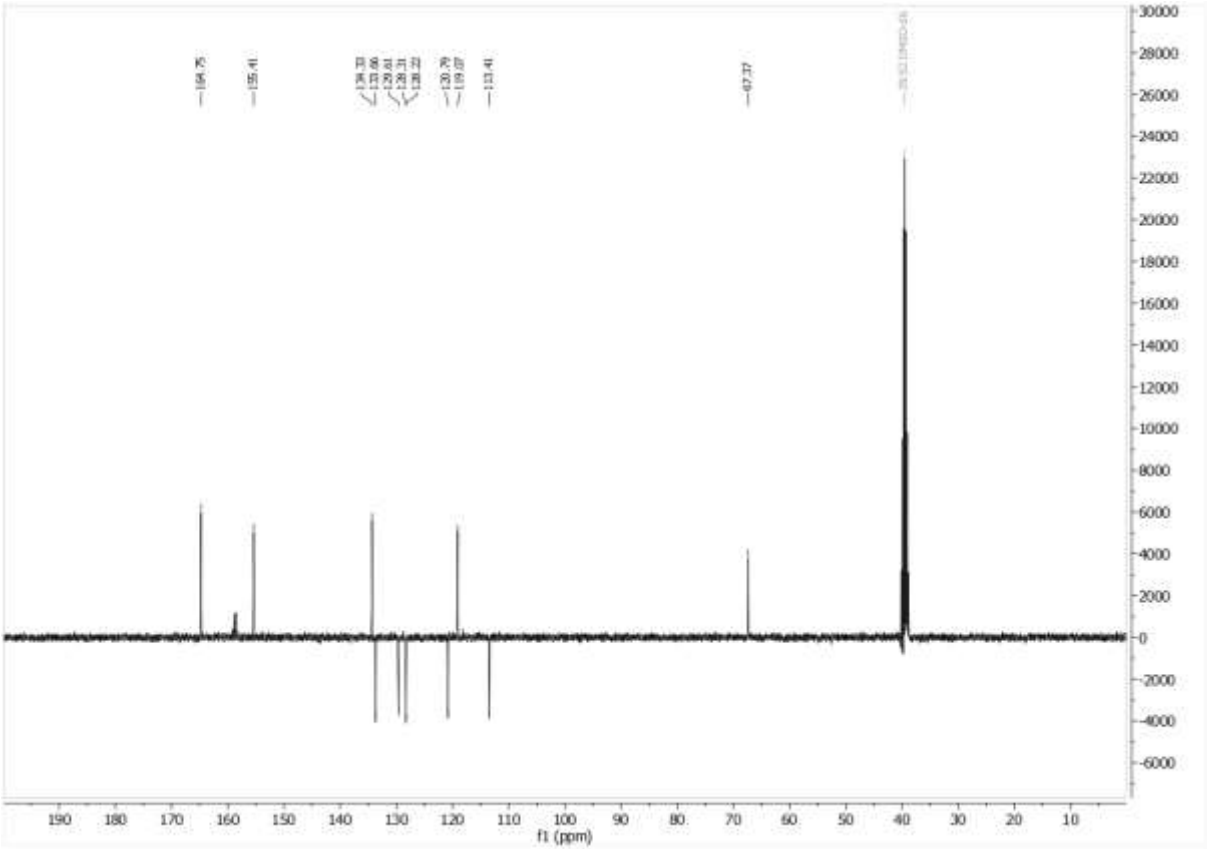
^{13}C NMR (DMSO)



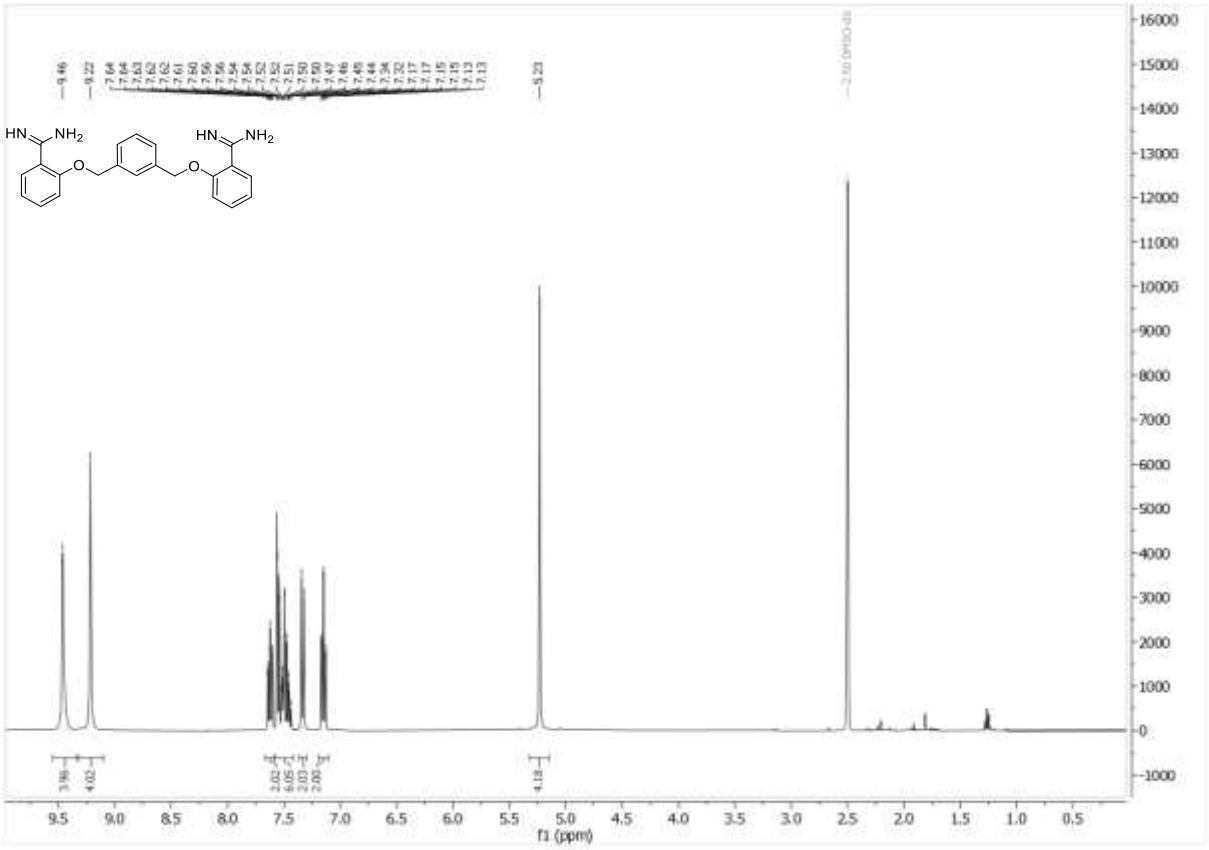
Compound **21c** ^{1}H NMR (DMSO)



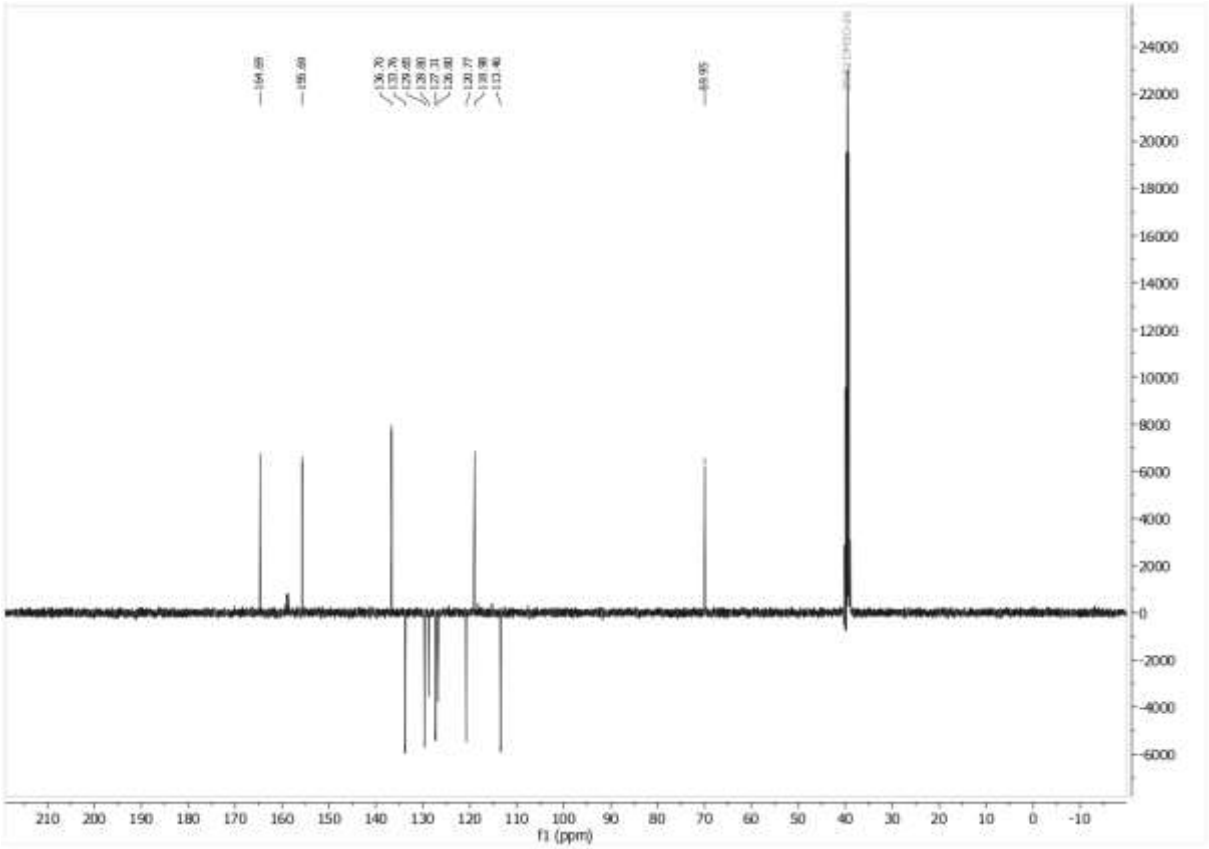
^{13}C NMR (DMSO)



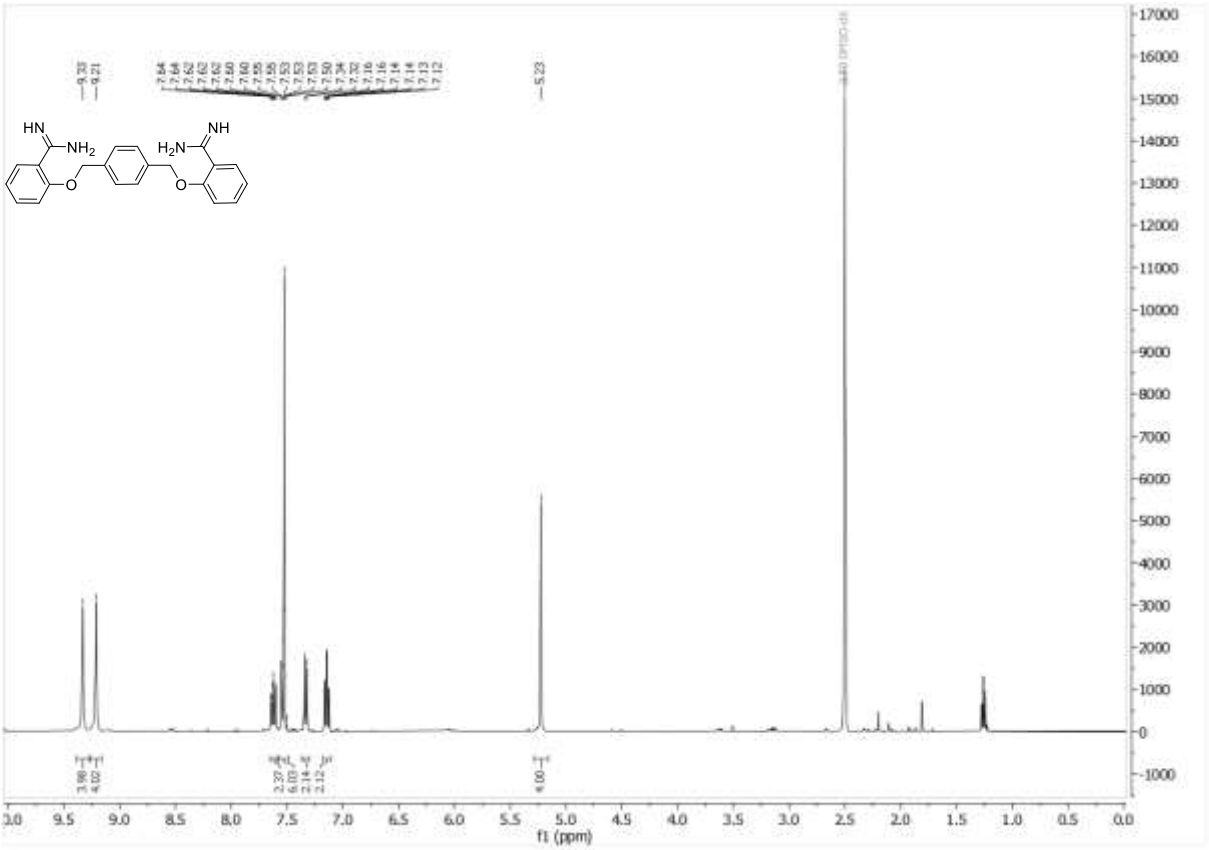
Compound **22c** ^{1}H NMR (DMSO)



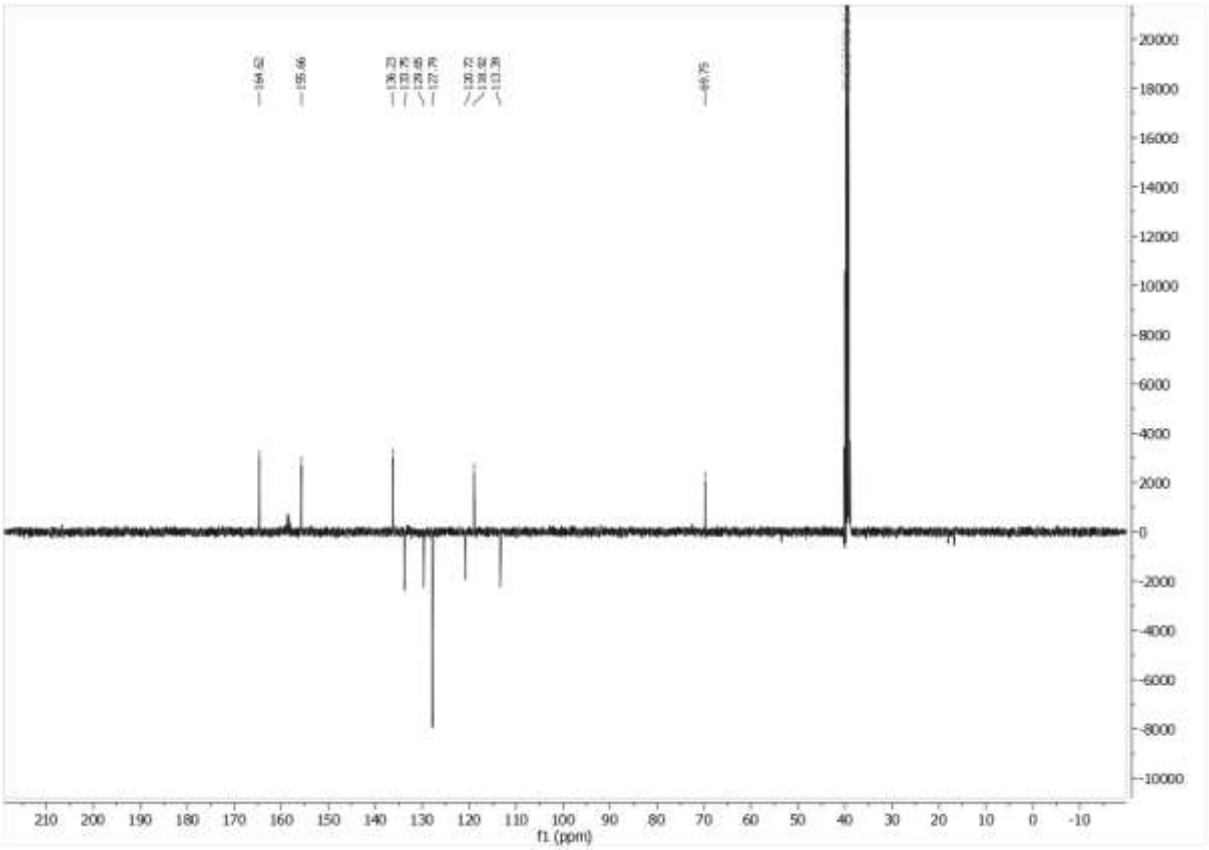
^{13}C NMR (DMSO)



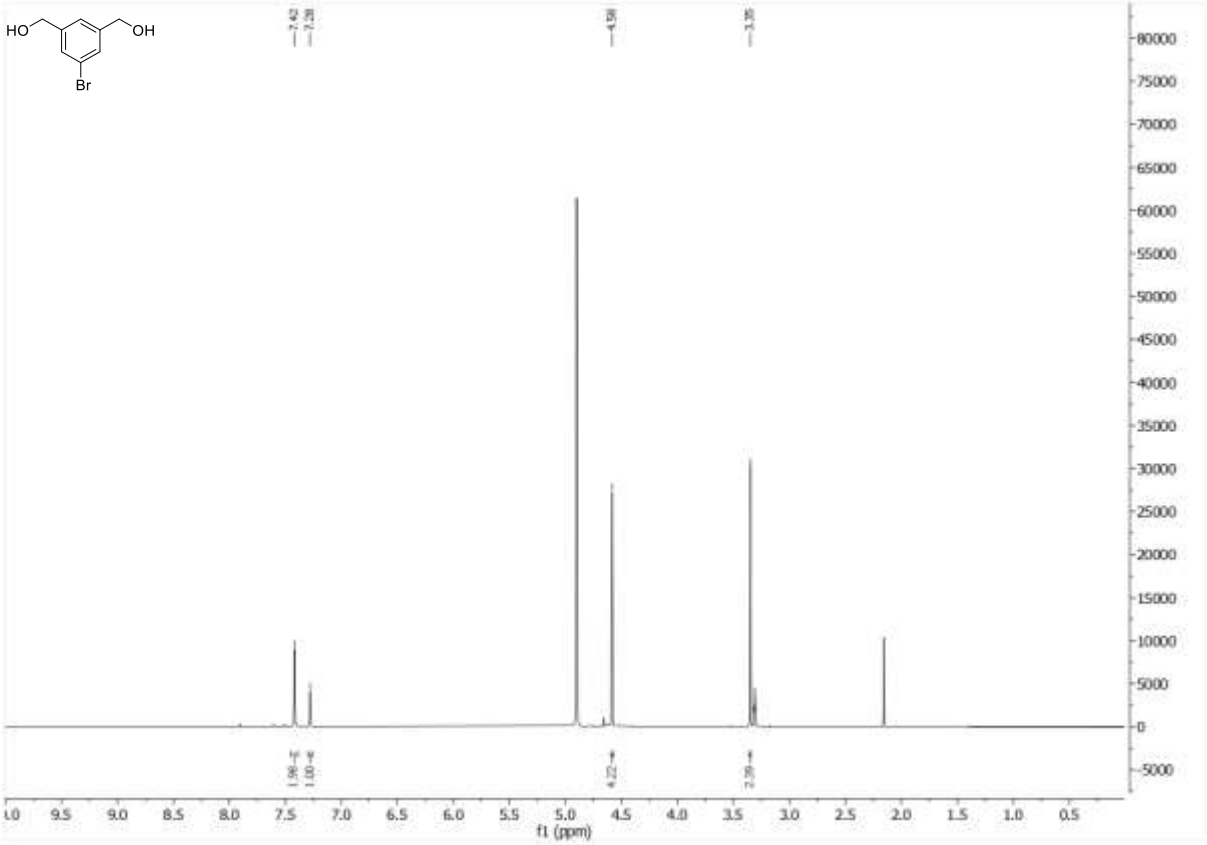
Compound **23c** ^{1}H NMR (DMSO)



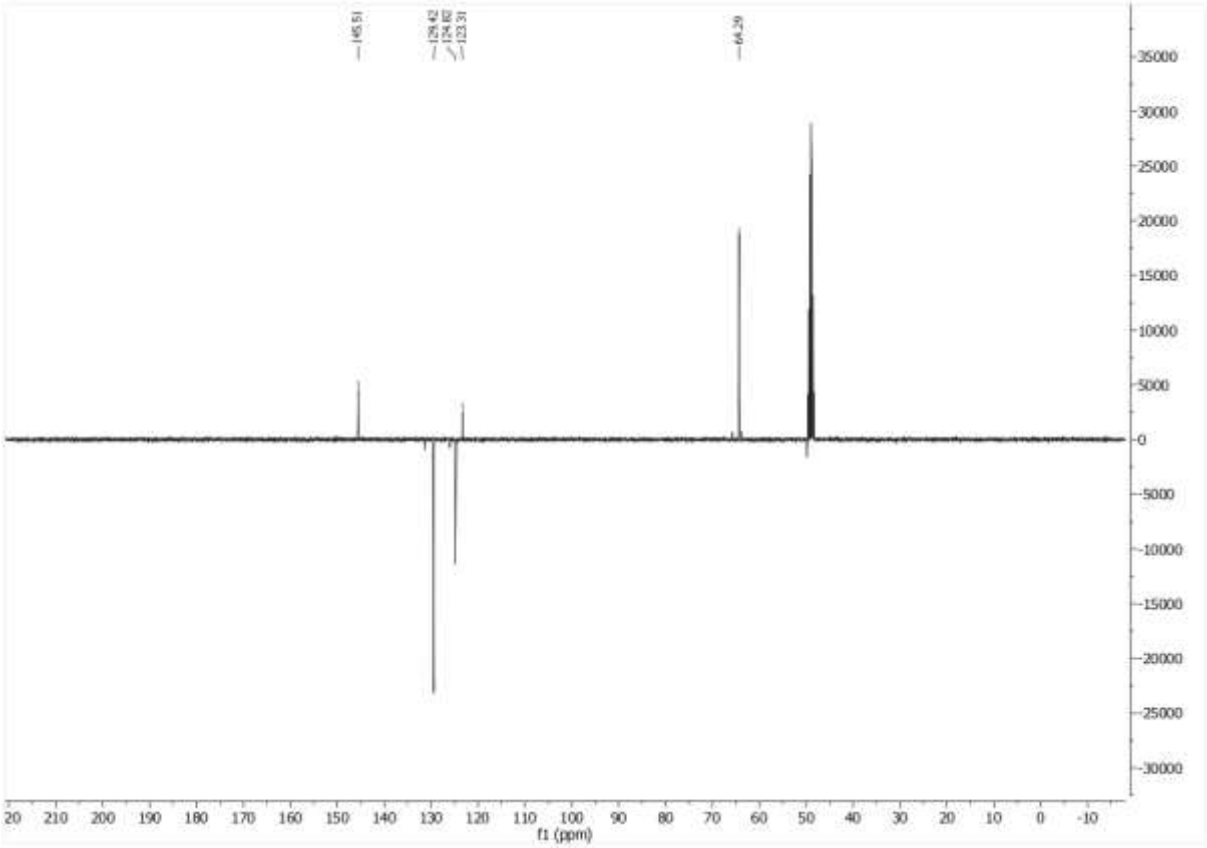
^{13}C NMR (DMSO)



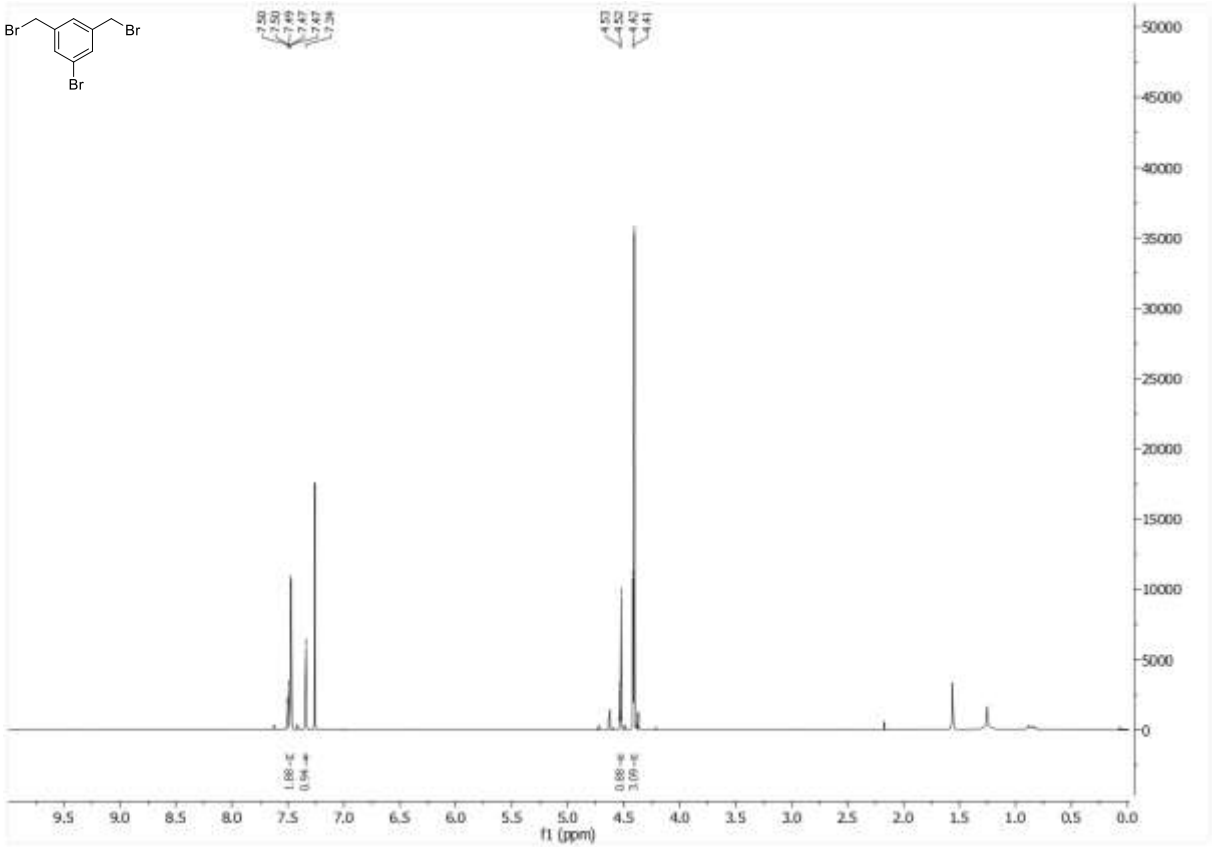
Compound **33** ^{1}H NMR (MeOD)



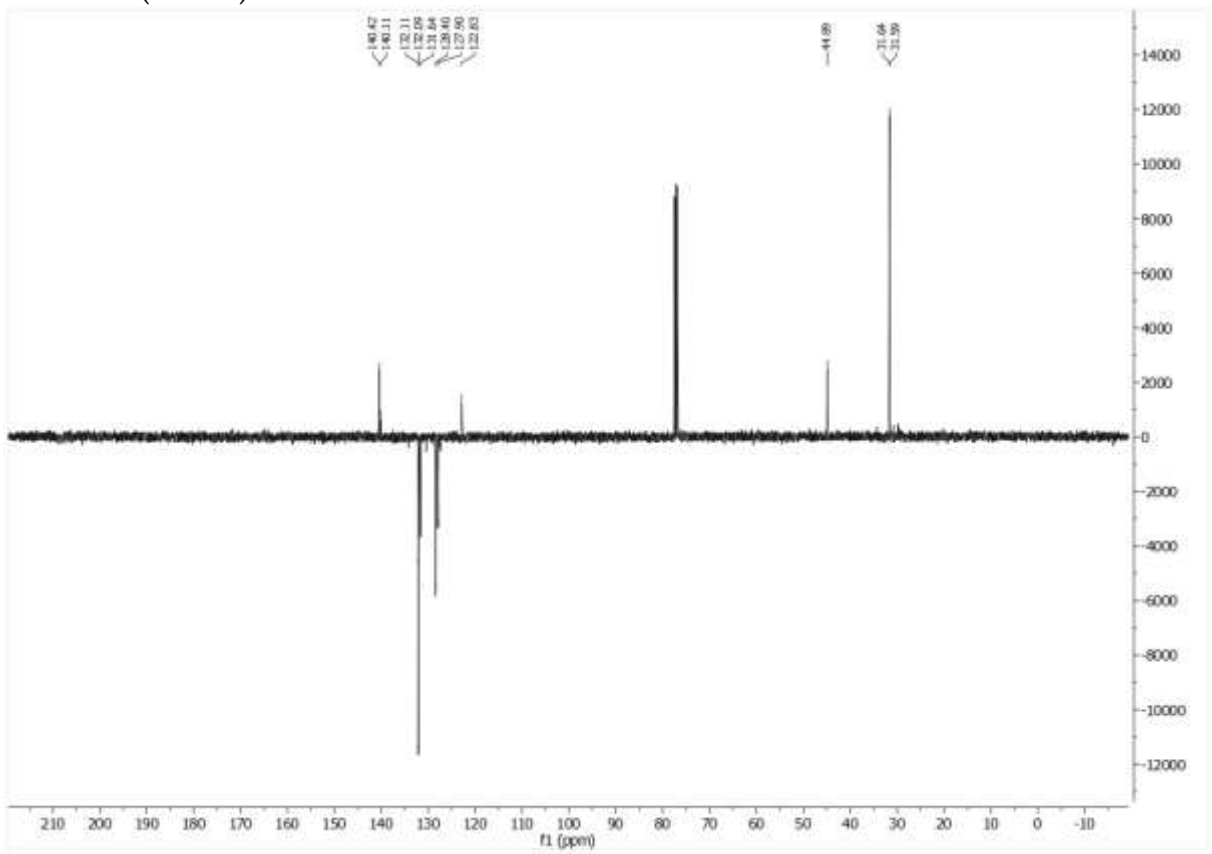
^{13}C NMR (MeOD)



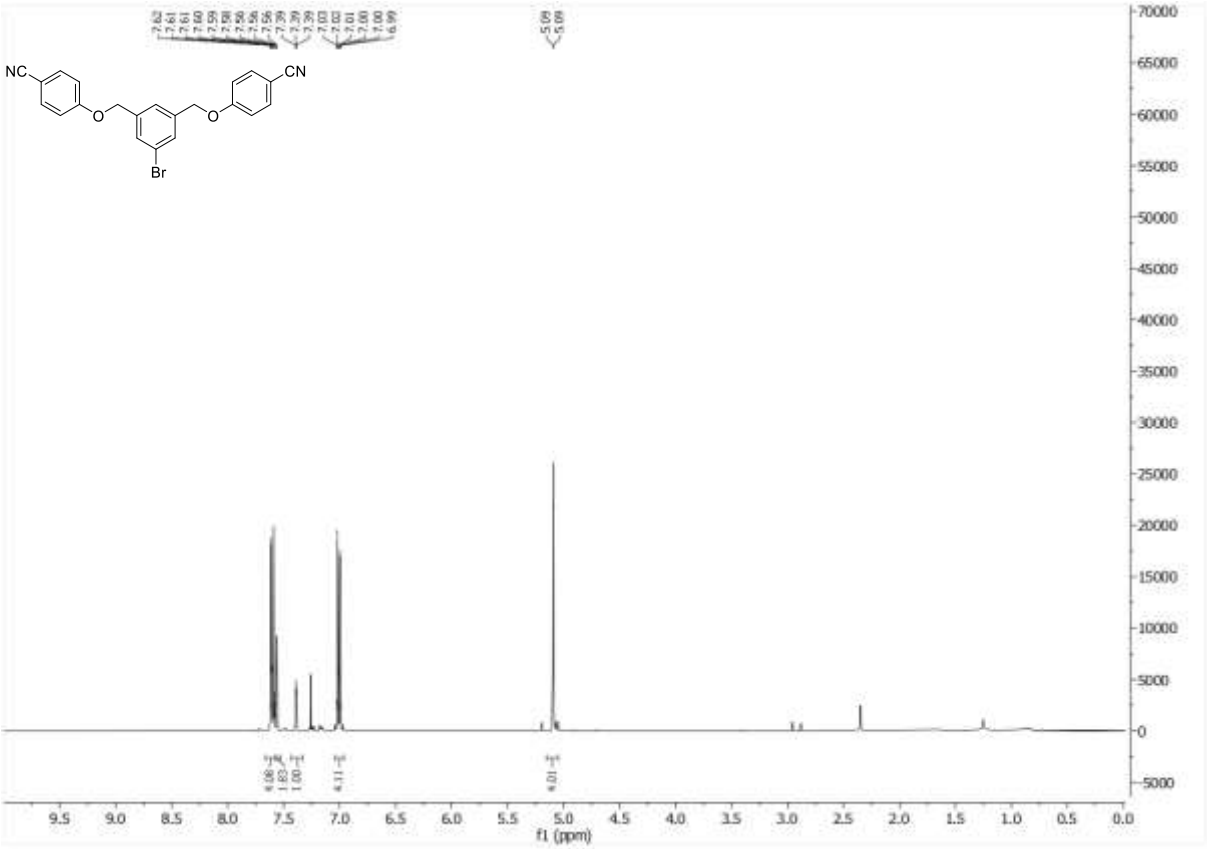
Compound **34** ^{1}H NMR ($CDCl_3$)



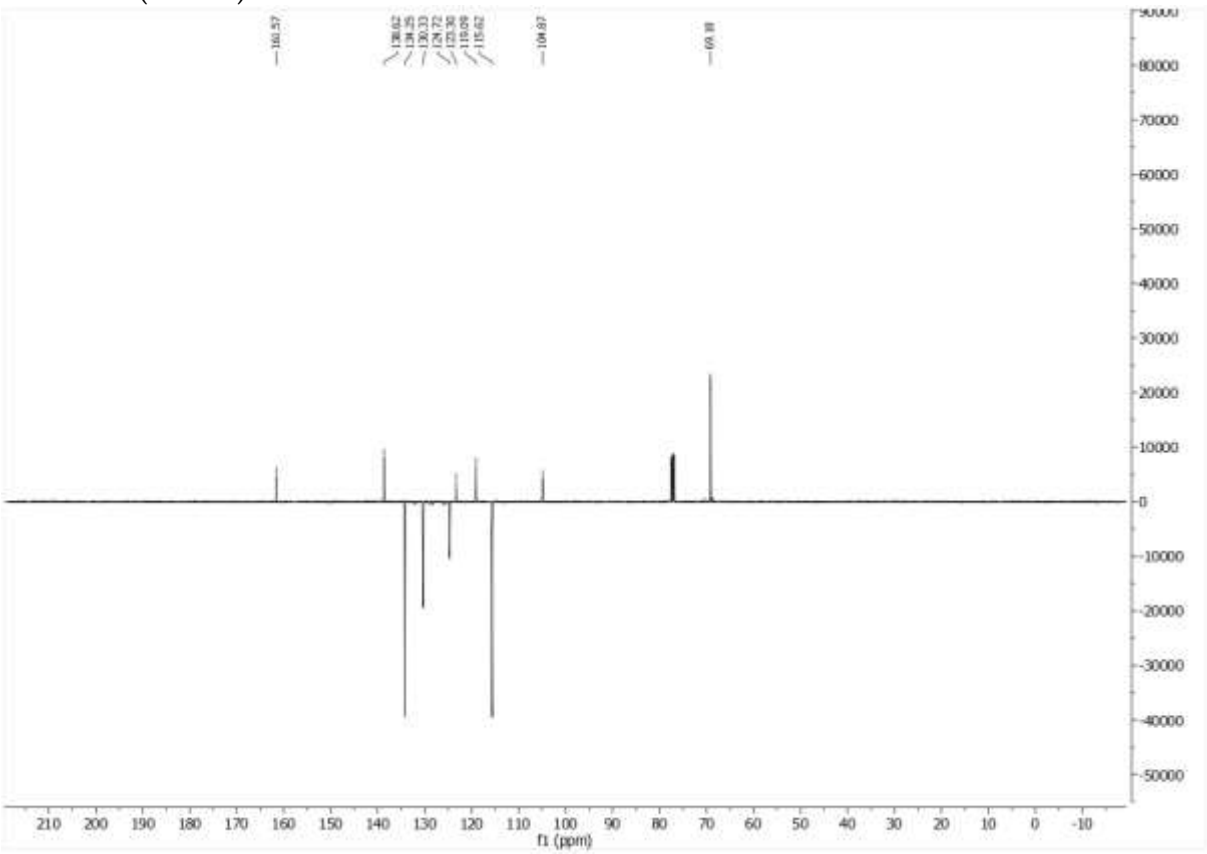
^{13}C NMR ($CDCl_3$)



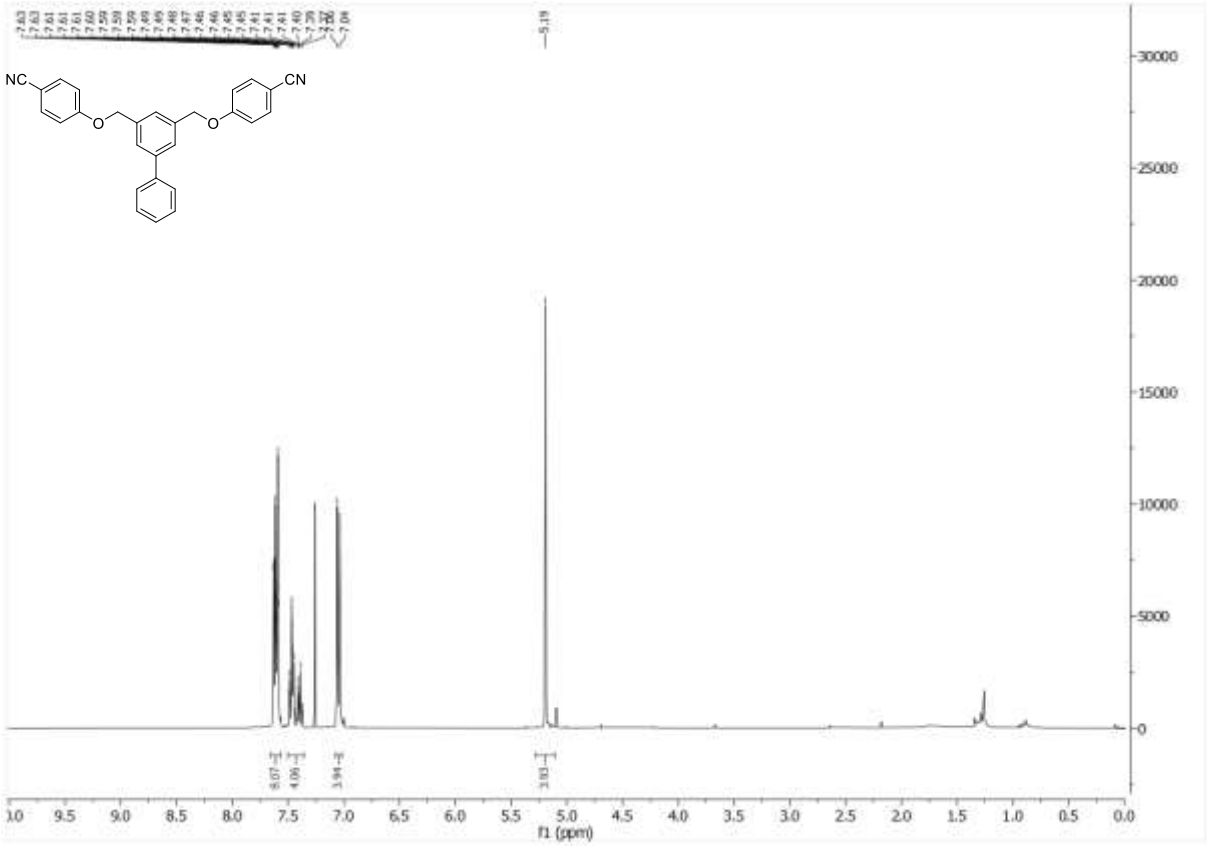
Compound **35** ^{1}H NMR ($CDCl_3$)



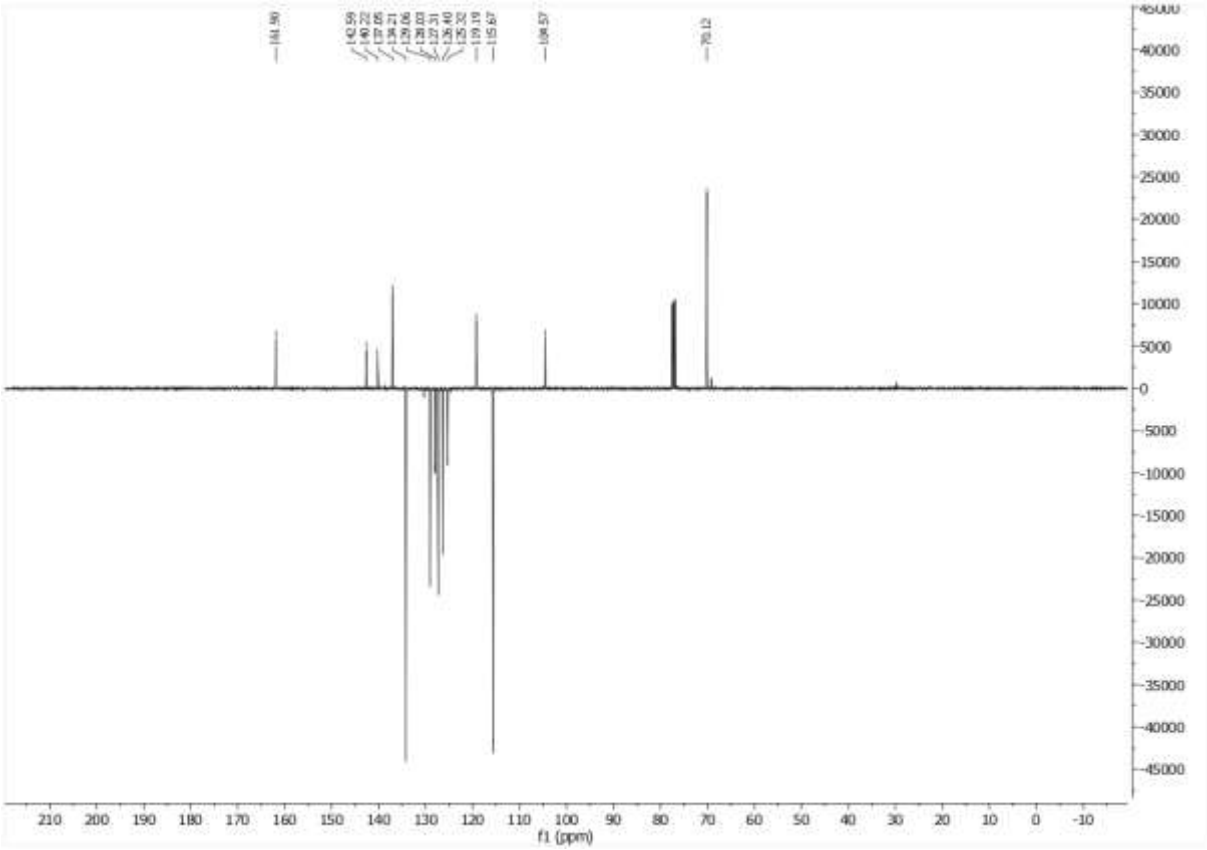
^{13}C NMR ($CDCl_3$)



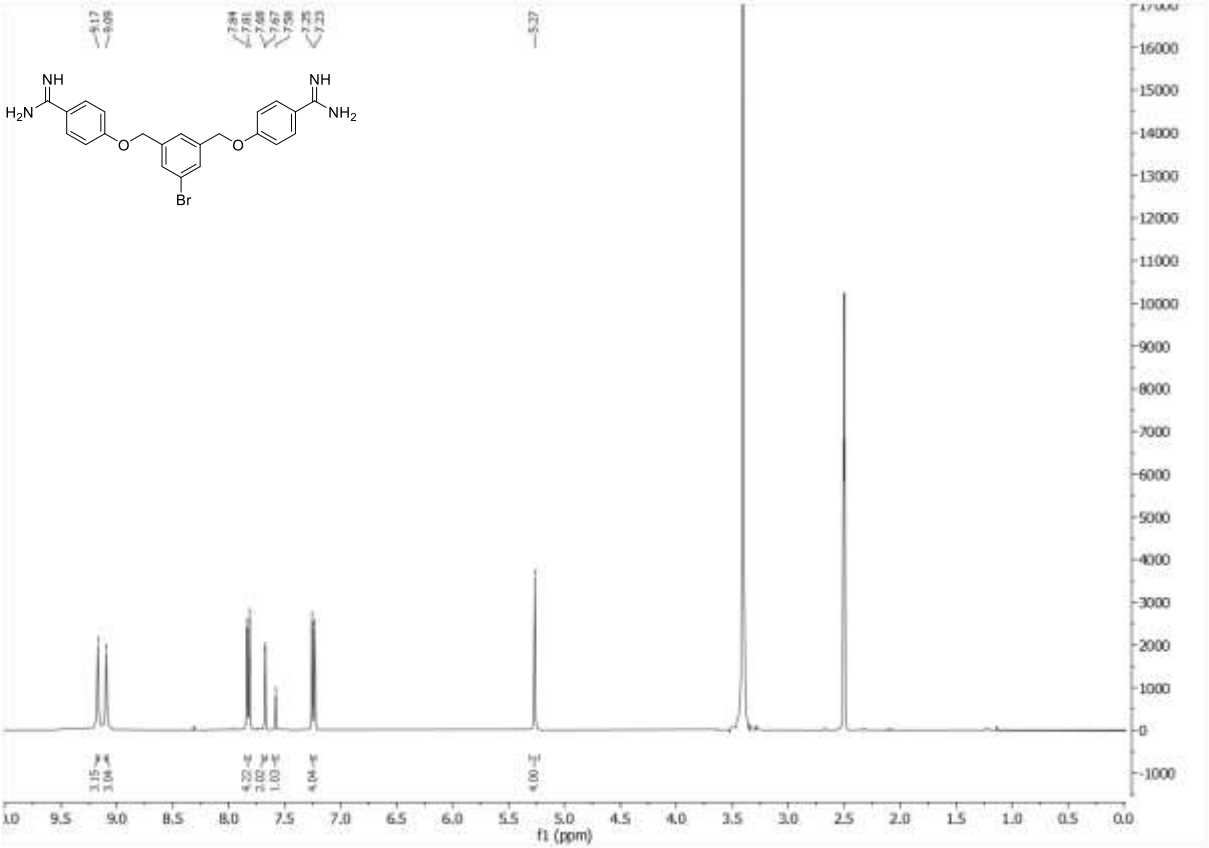
Compound **36** ^{1}H NMR ($CDCl_3$)



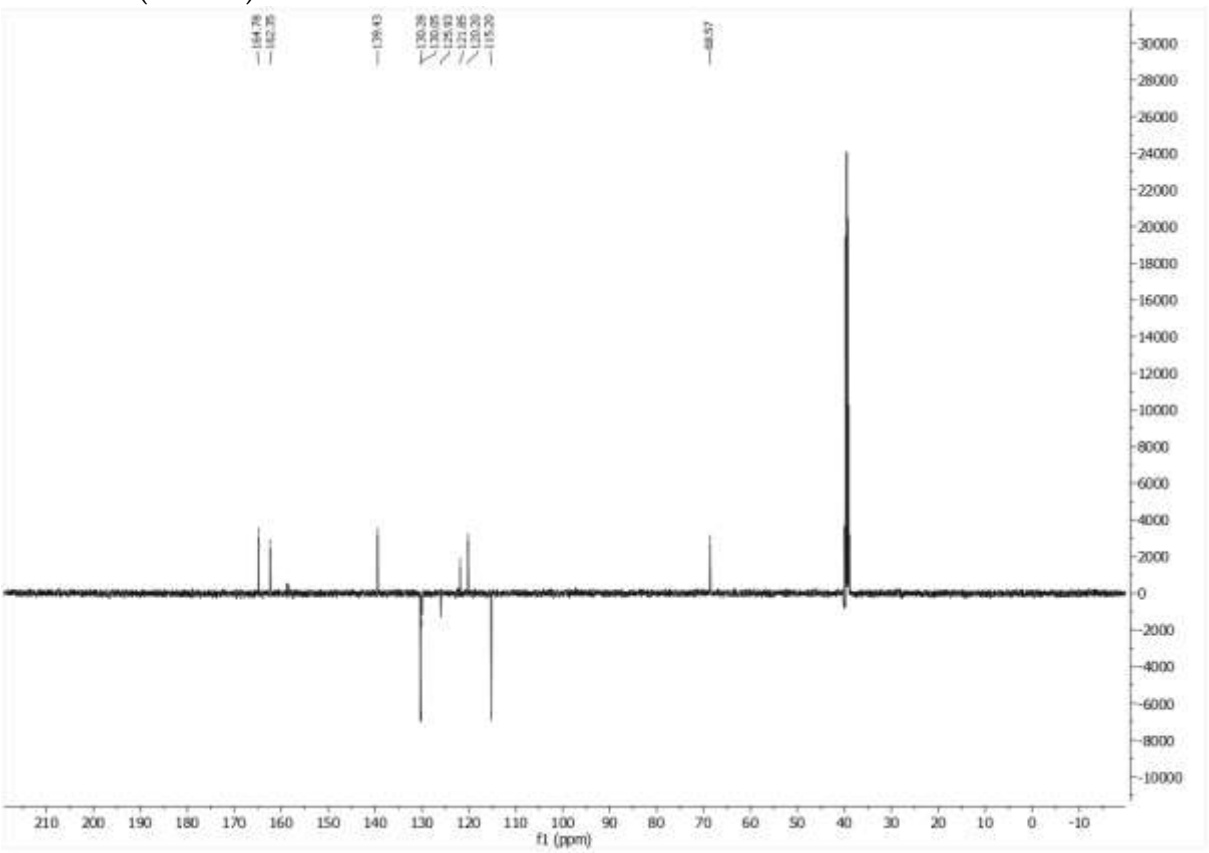
^{13}C NMR ($CDCl_3$)



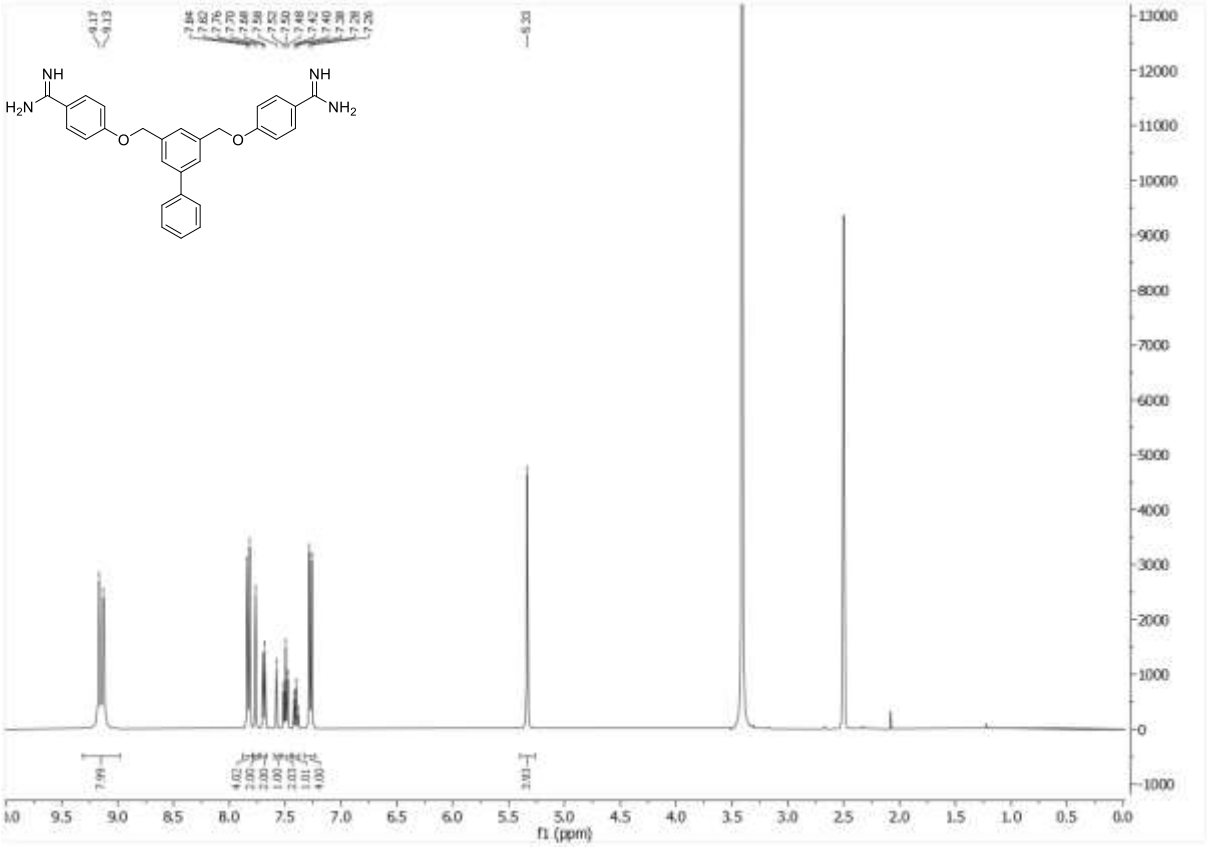
Compound **37** ^{1}H NMR (DMSO)



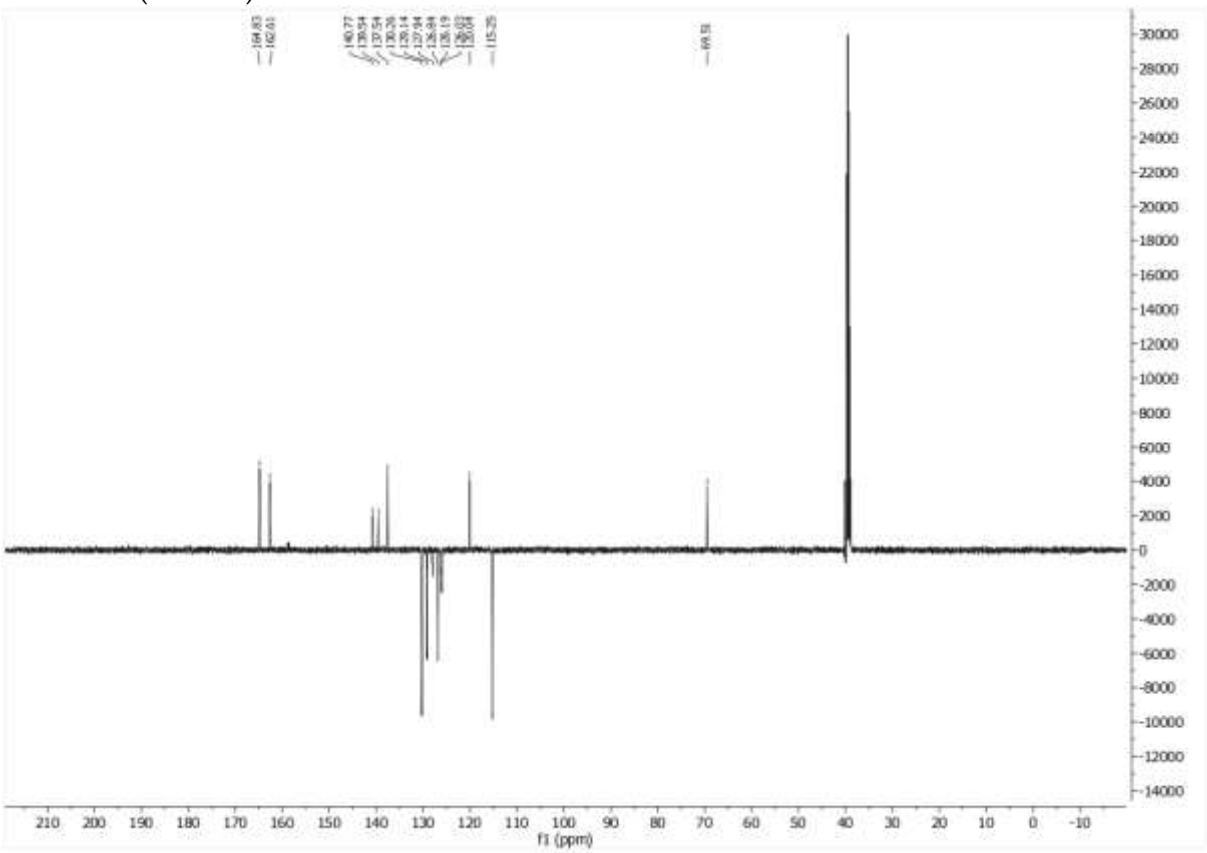
^{13}C NMR (DMSO)



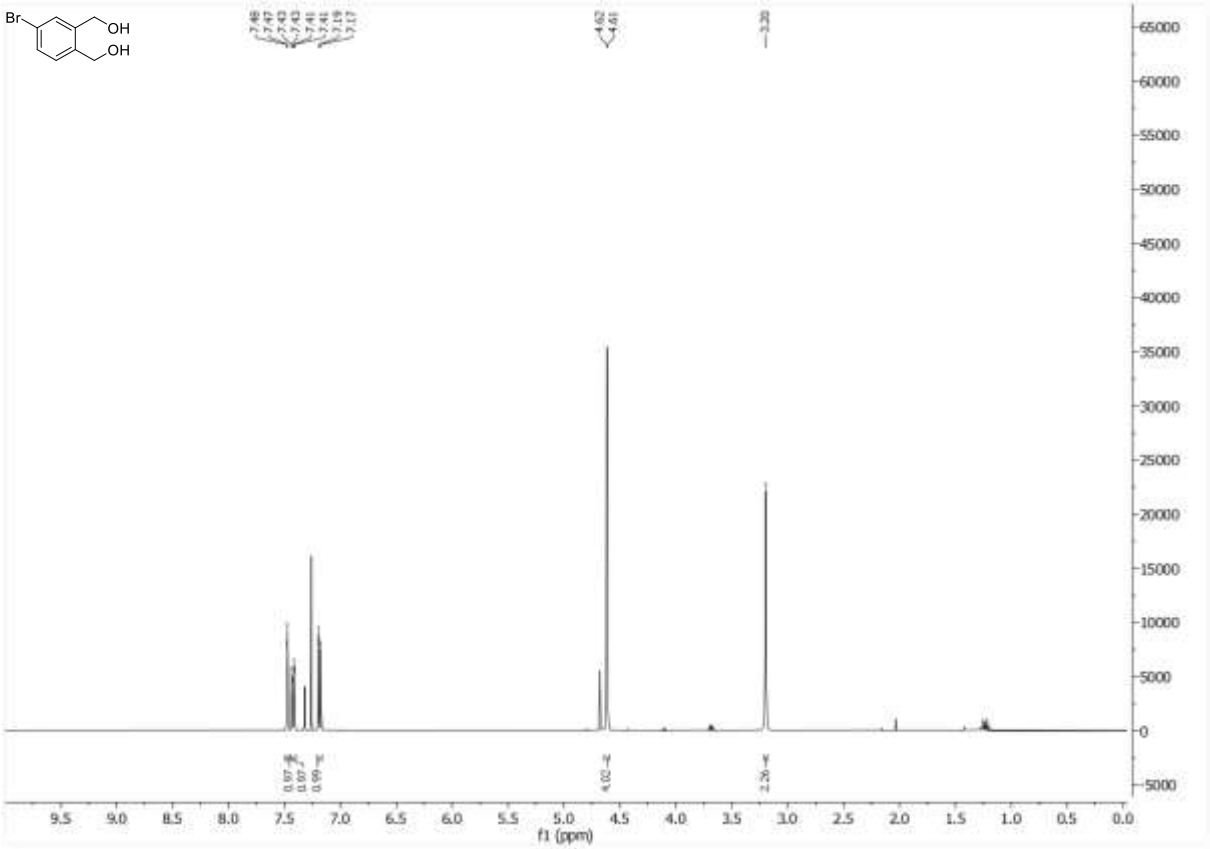
Compound **38** ^{1}H NMR (DMSO)



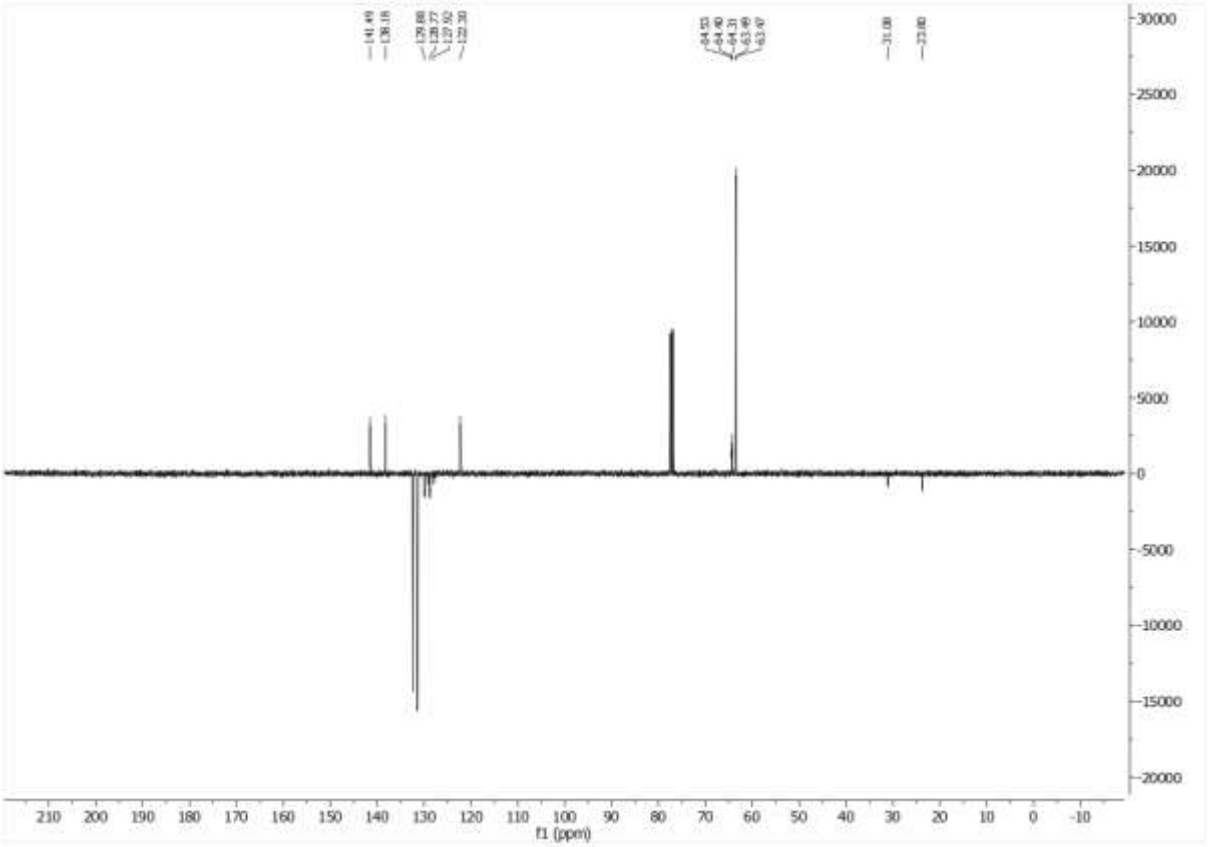
^{13}C NMR (DMSO)



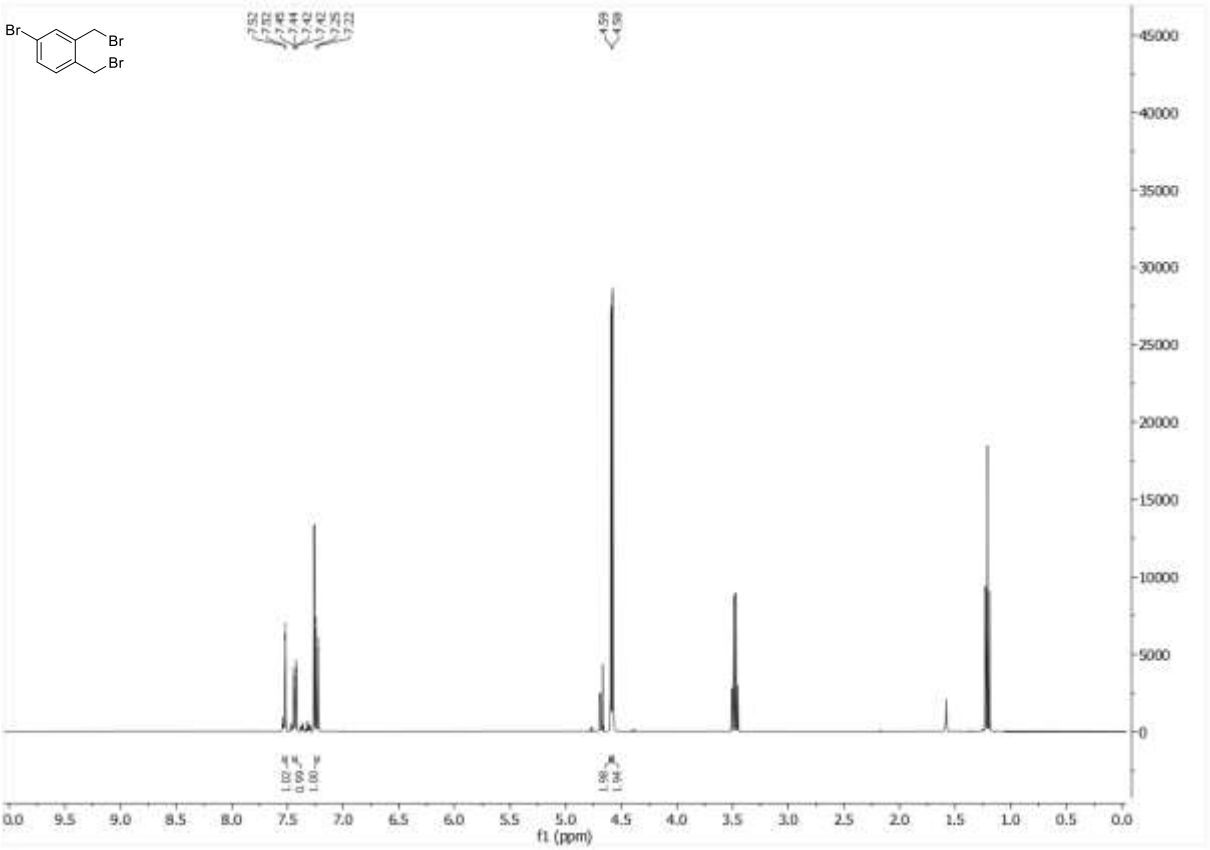
Compound **39** ^{1}H NMR ($CDCl_3$)



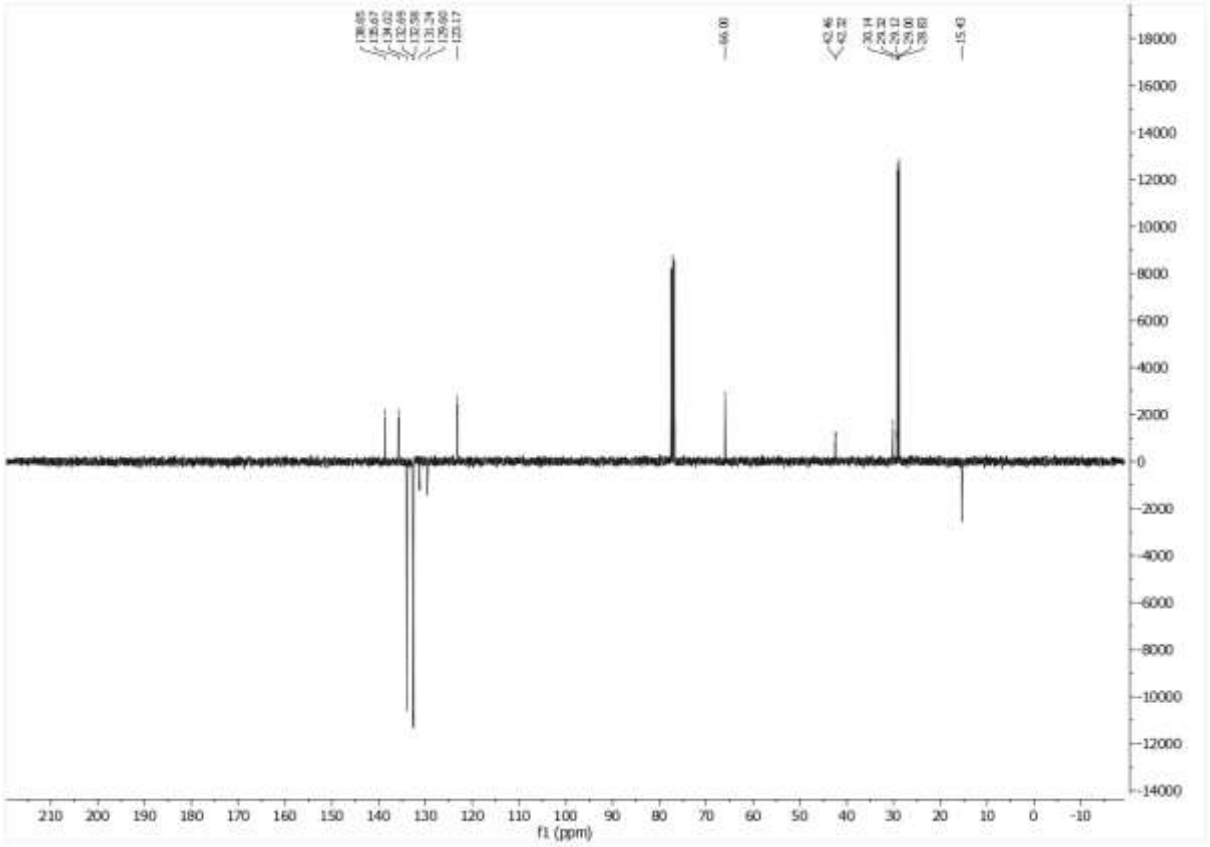
^{13}C NMR ($CDCl_3$)



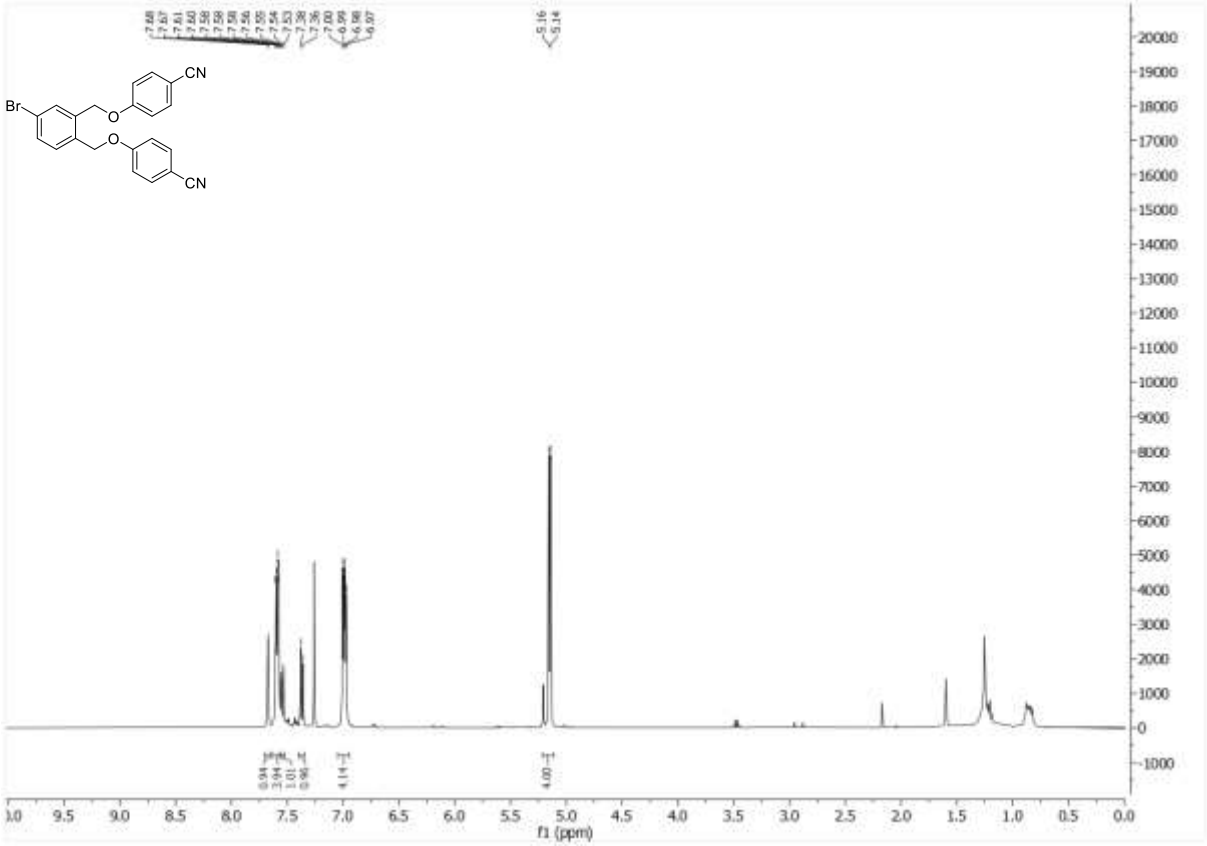
Compound **40** ^{1}H NMR ($CDCl_3$)



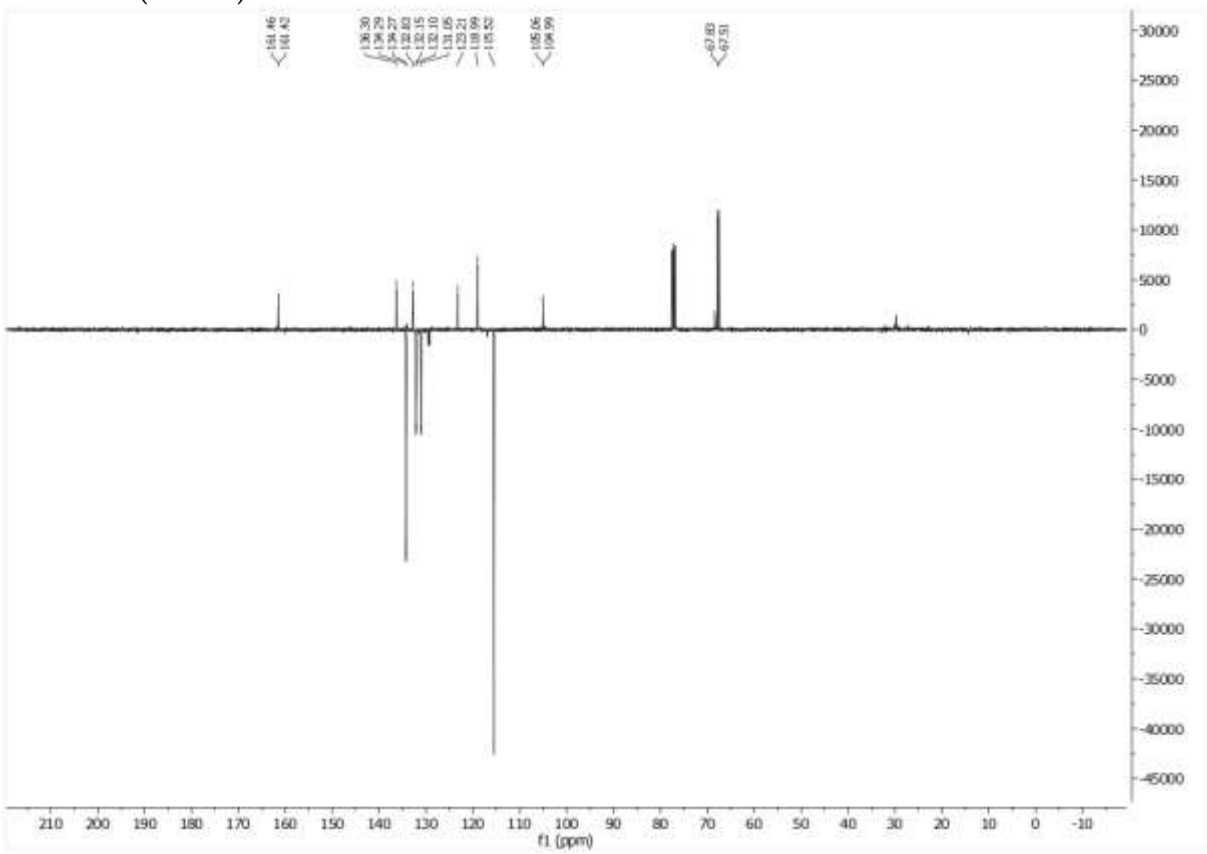
^{13}C NMR ($CDCl_3$)



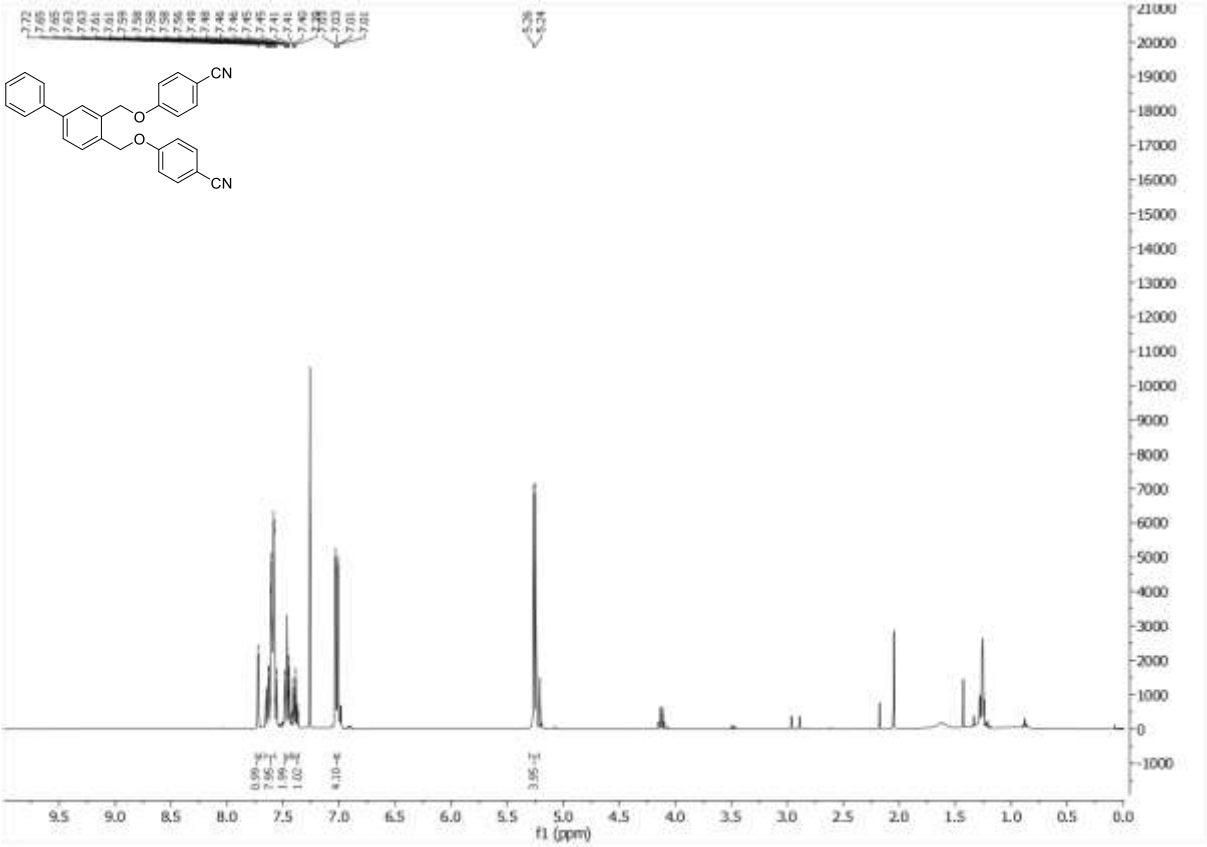
Compound **41** ^{1}H NMR ($CDCl_3$)



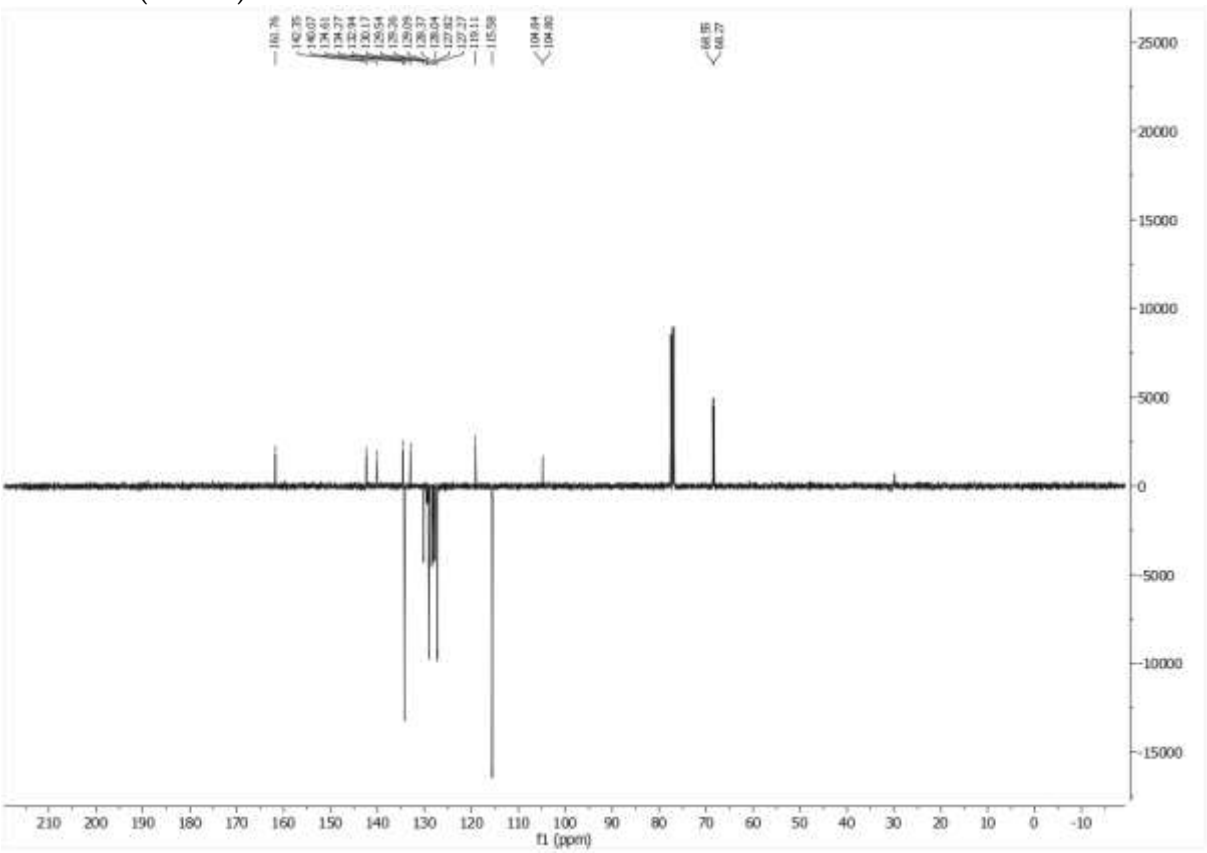
^{13}C NMR ($CDCl_3$)



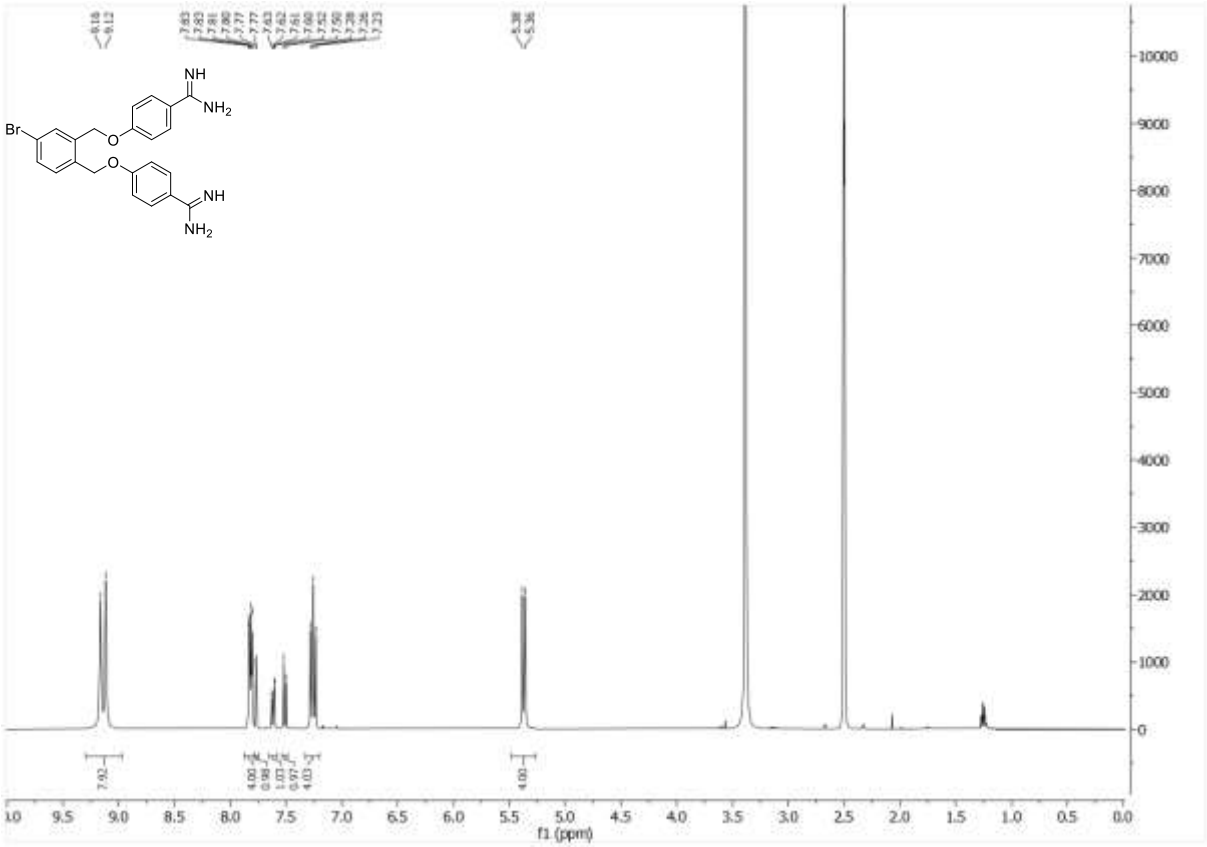
Compound **42** ^{1}H NMR ($CDCl_3$)



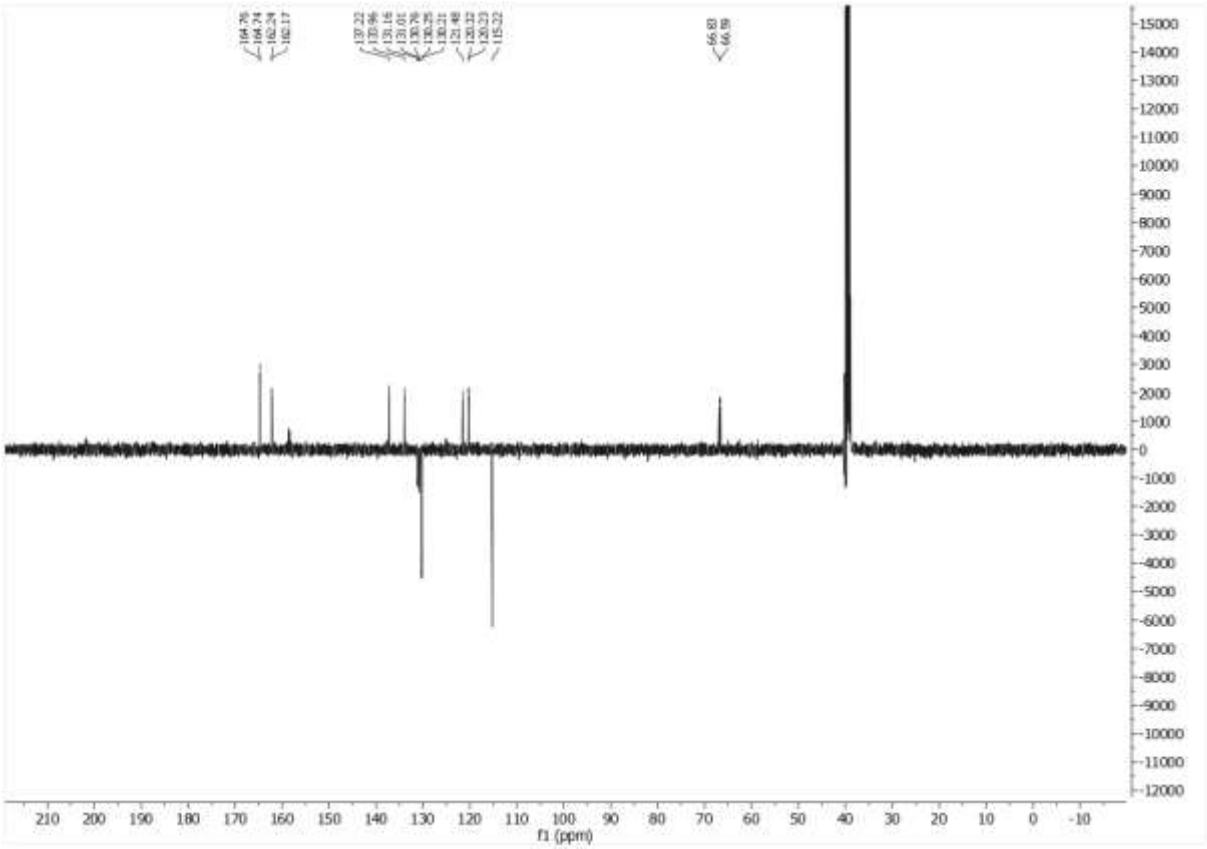
^{13}C NMR ($CDCl_3$)



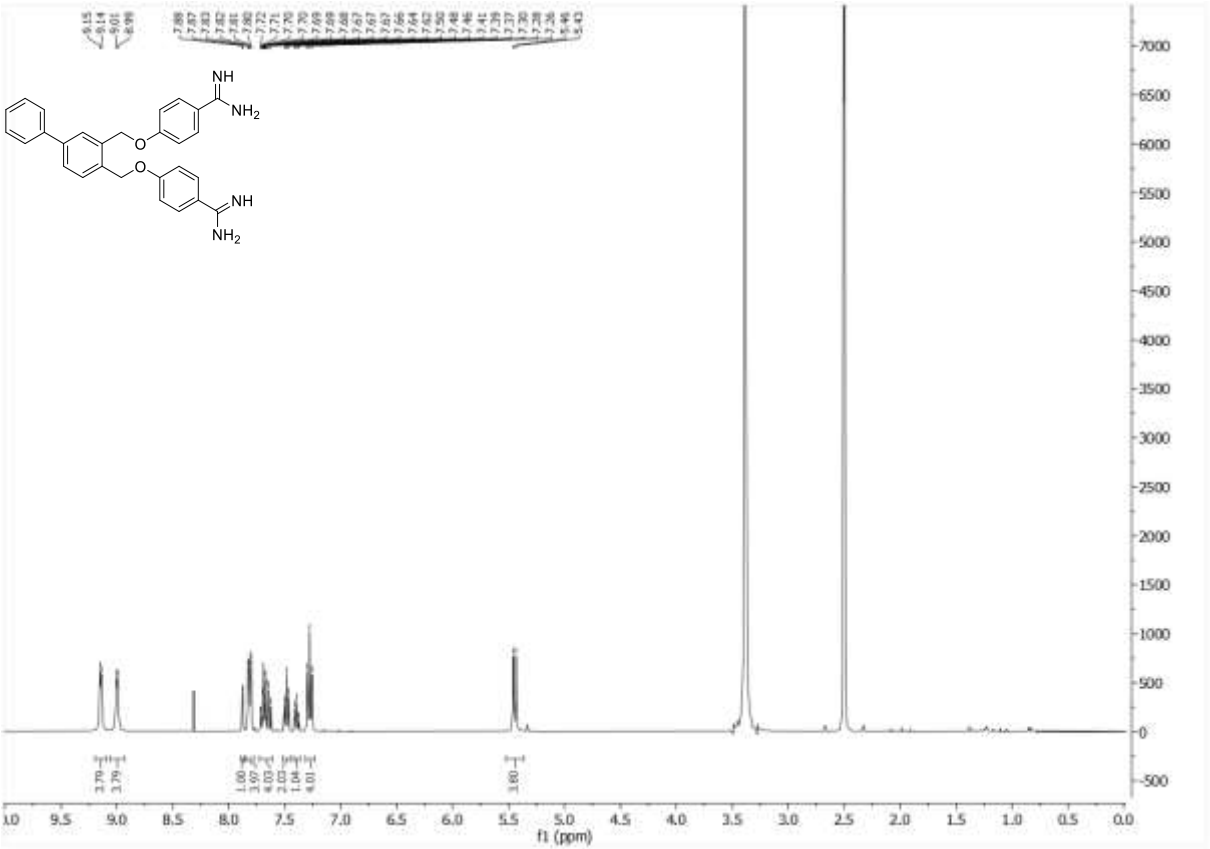
Compound **43** ^{1}H NMR (DMSO)



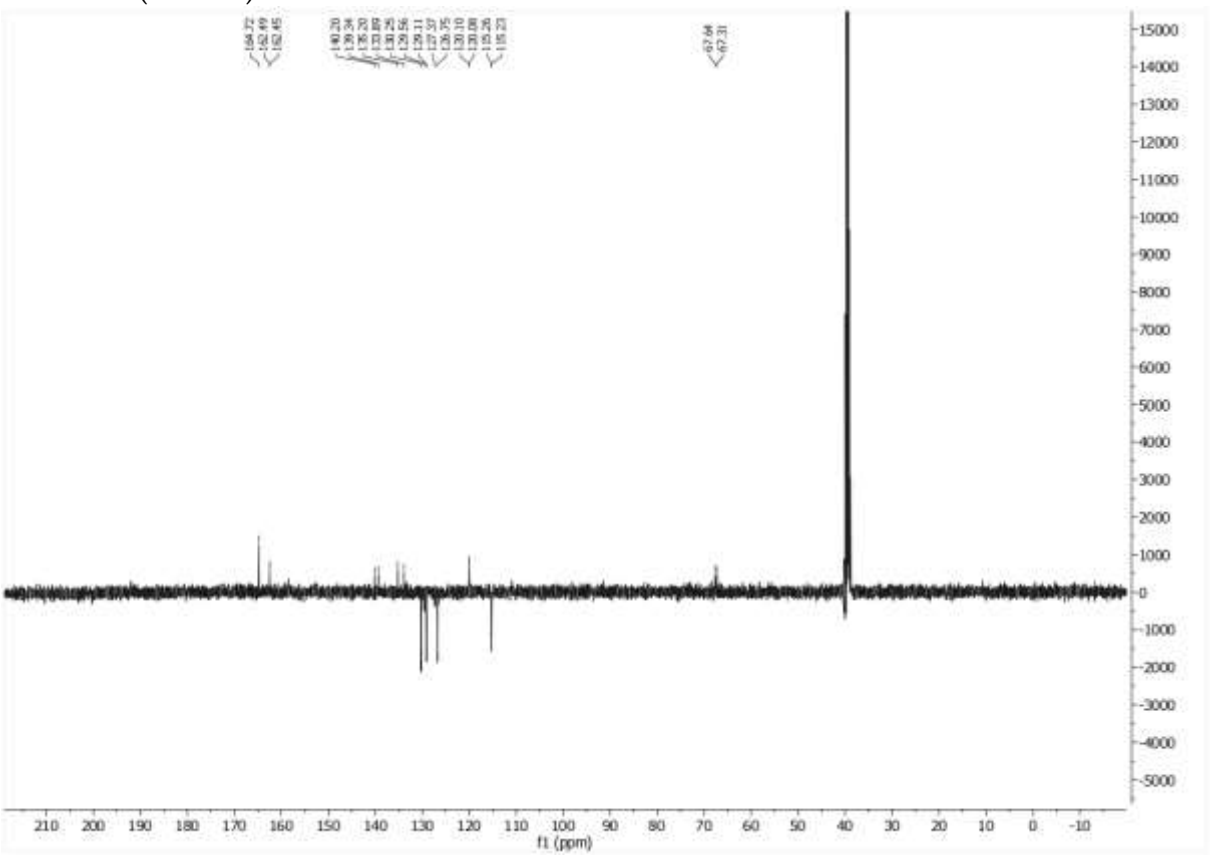
^{13}C NMR (DMSO)



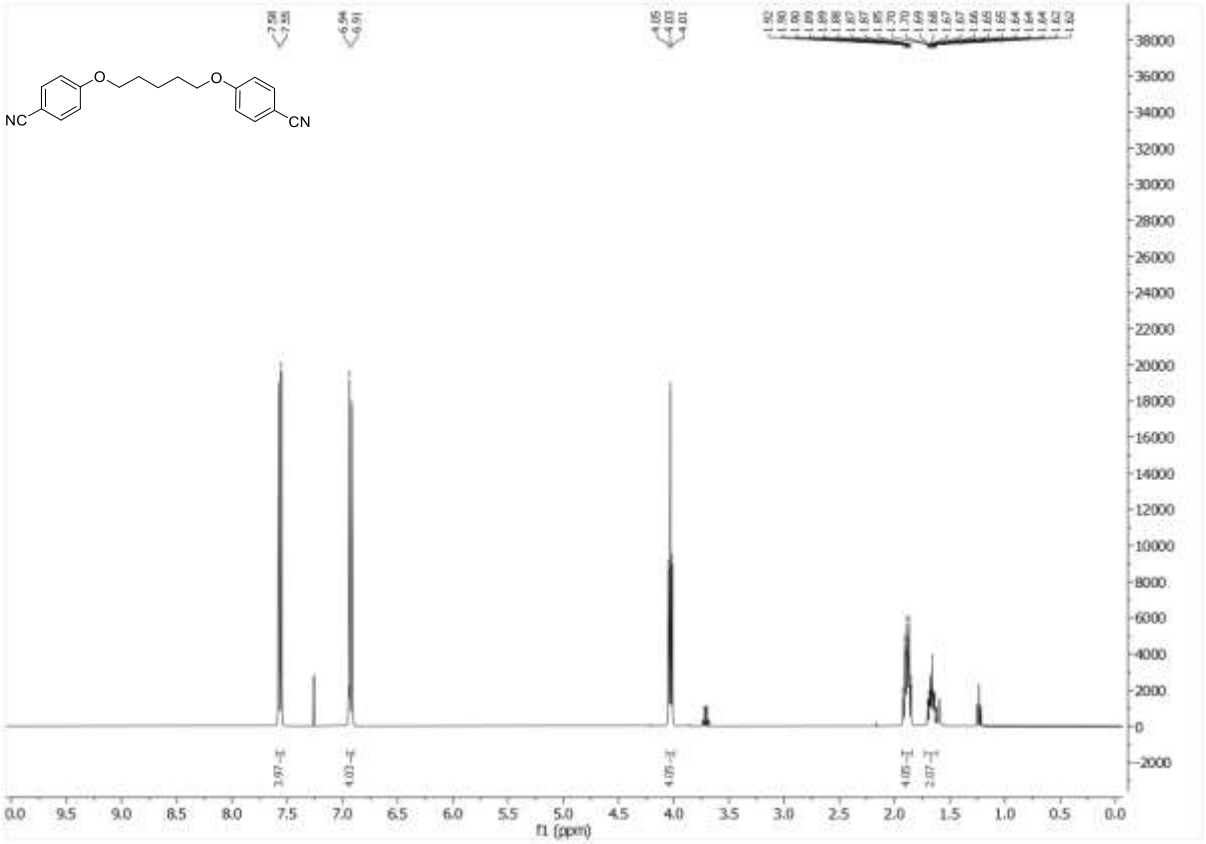
Compound **44** ^{1}H NMR (DMSO)



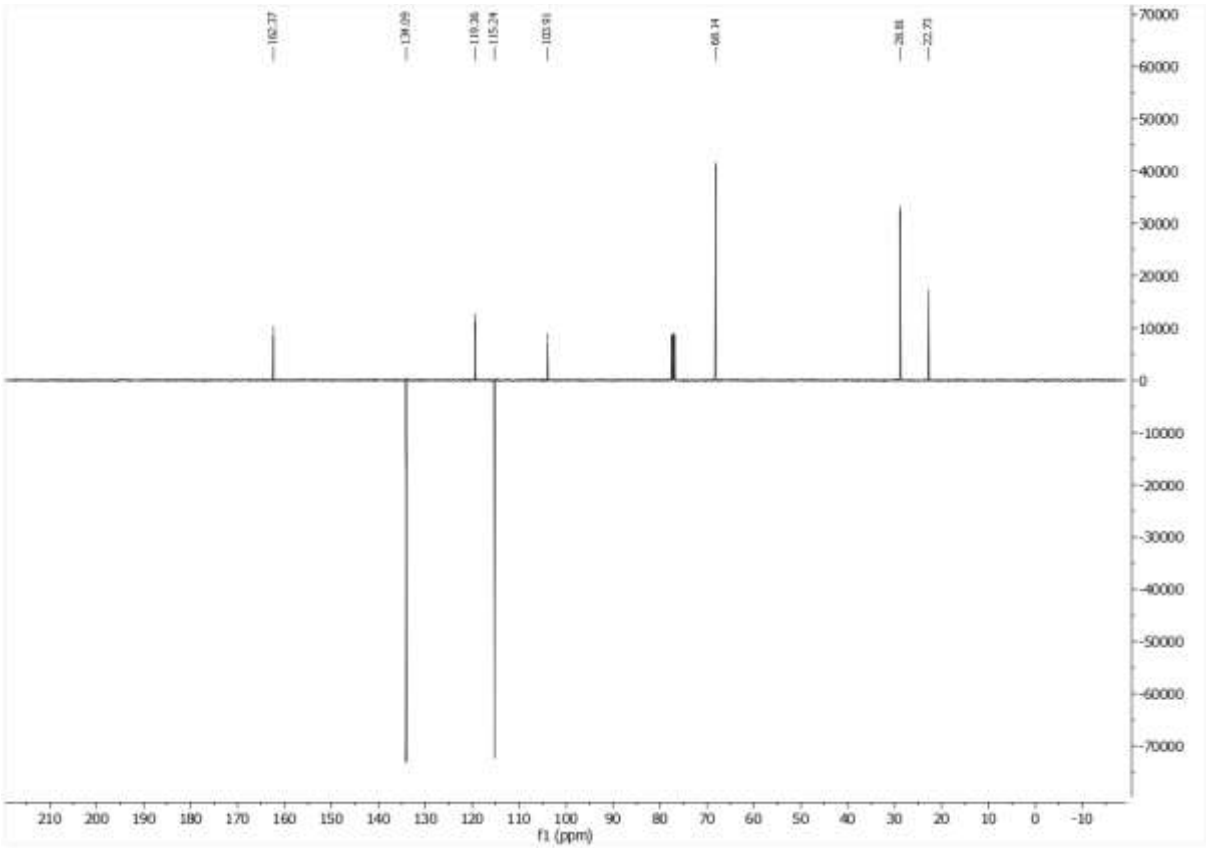
^{13}C NMR (DMSO)



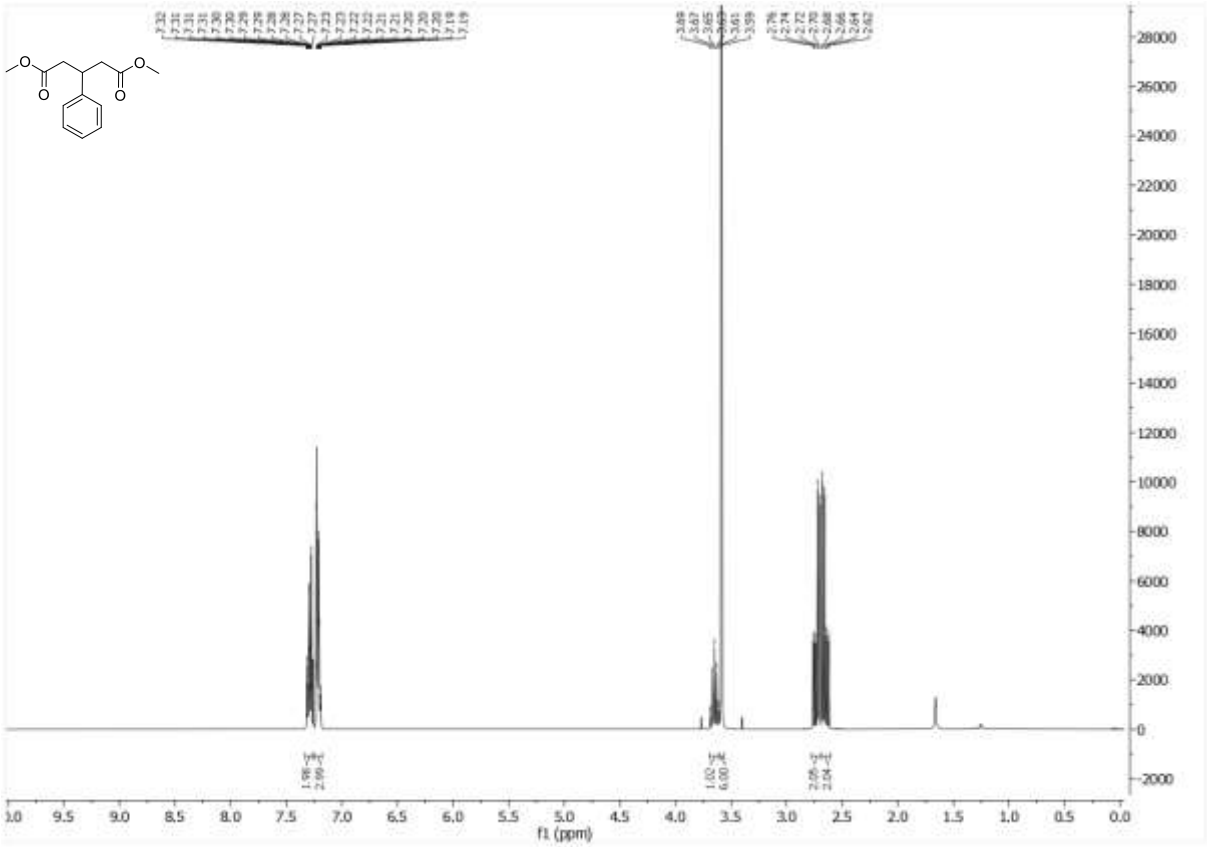
Compound **45** ^{1}H NMR ($CDCl_3$)



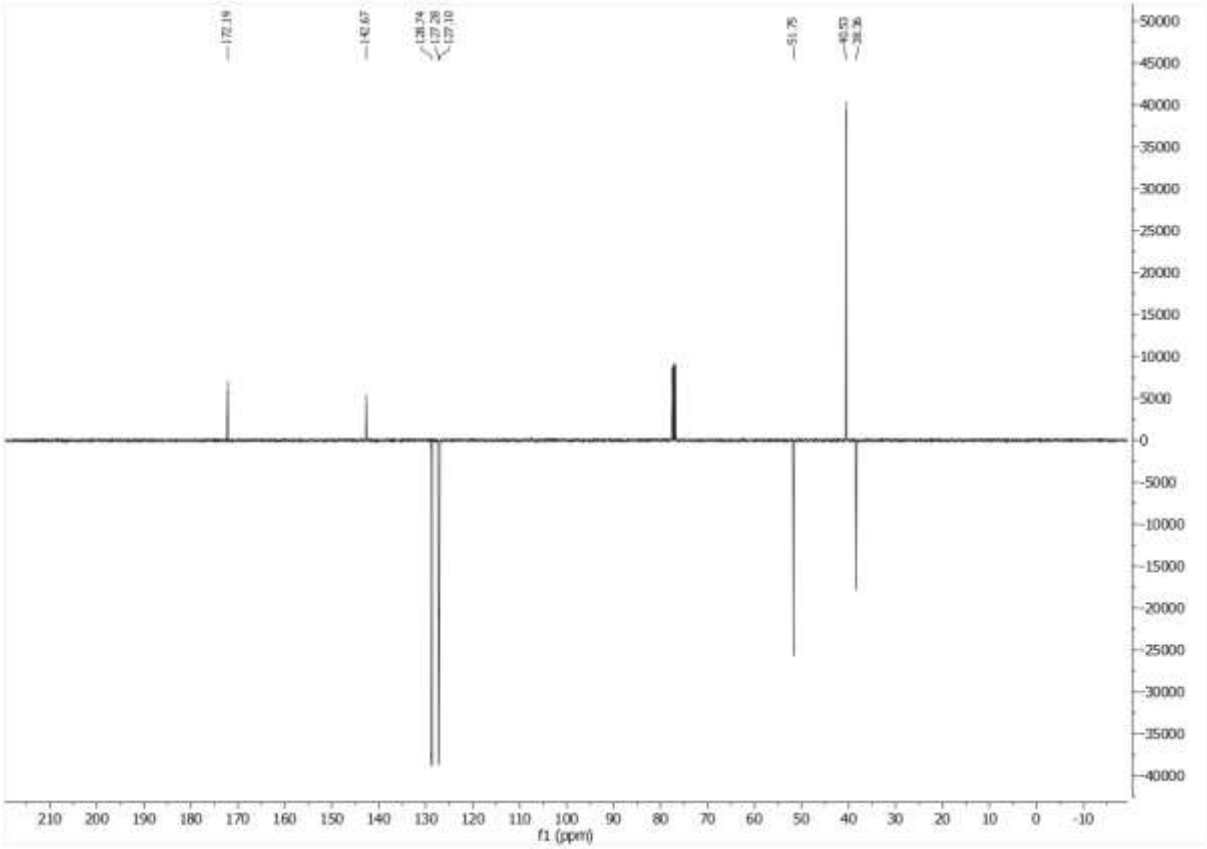
^{13}C NMR ($CDCl_3$)



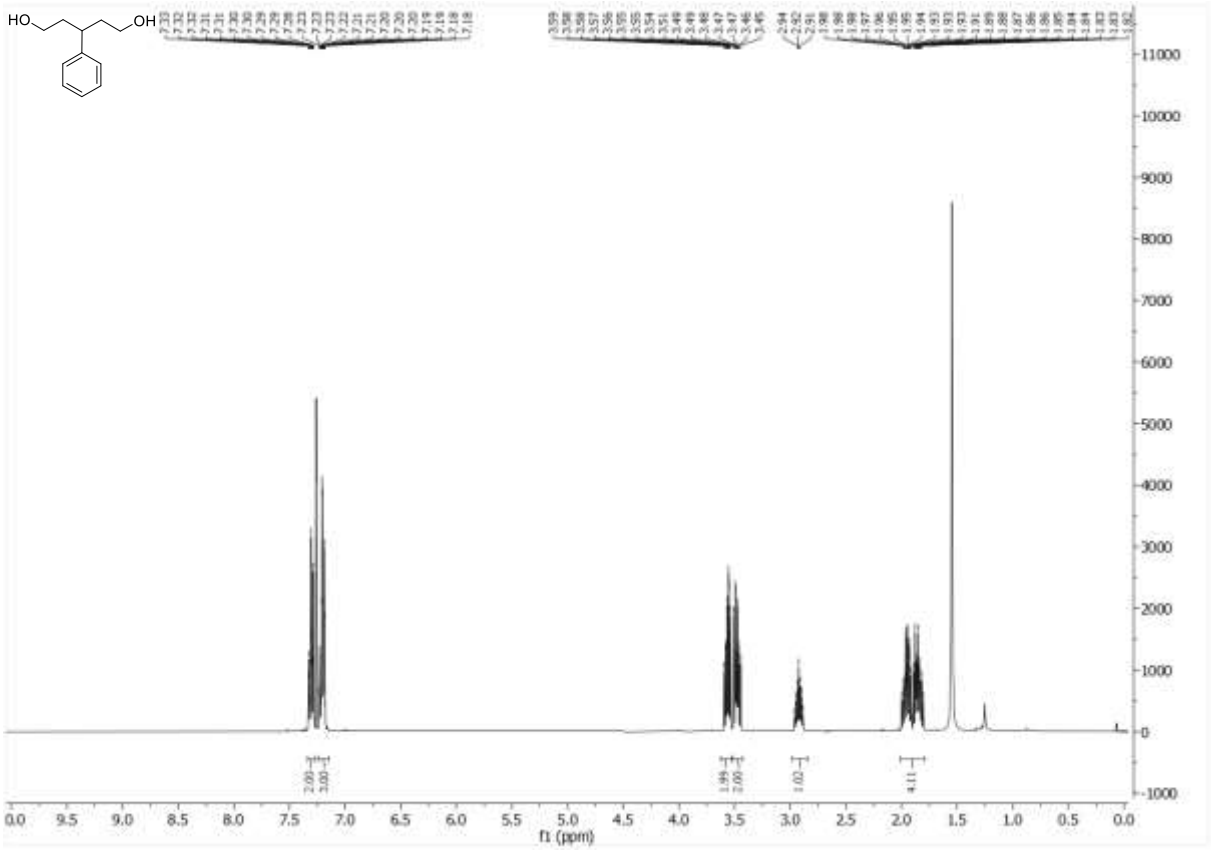
Compound **46** ^{1}H NMR ($CDCl_3$)



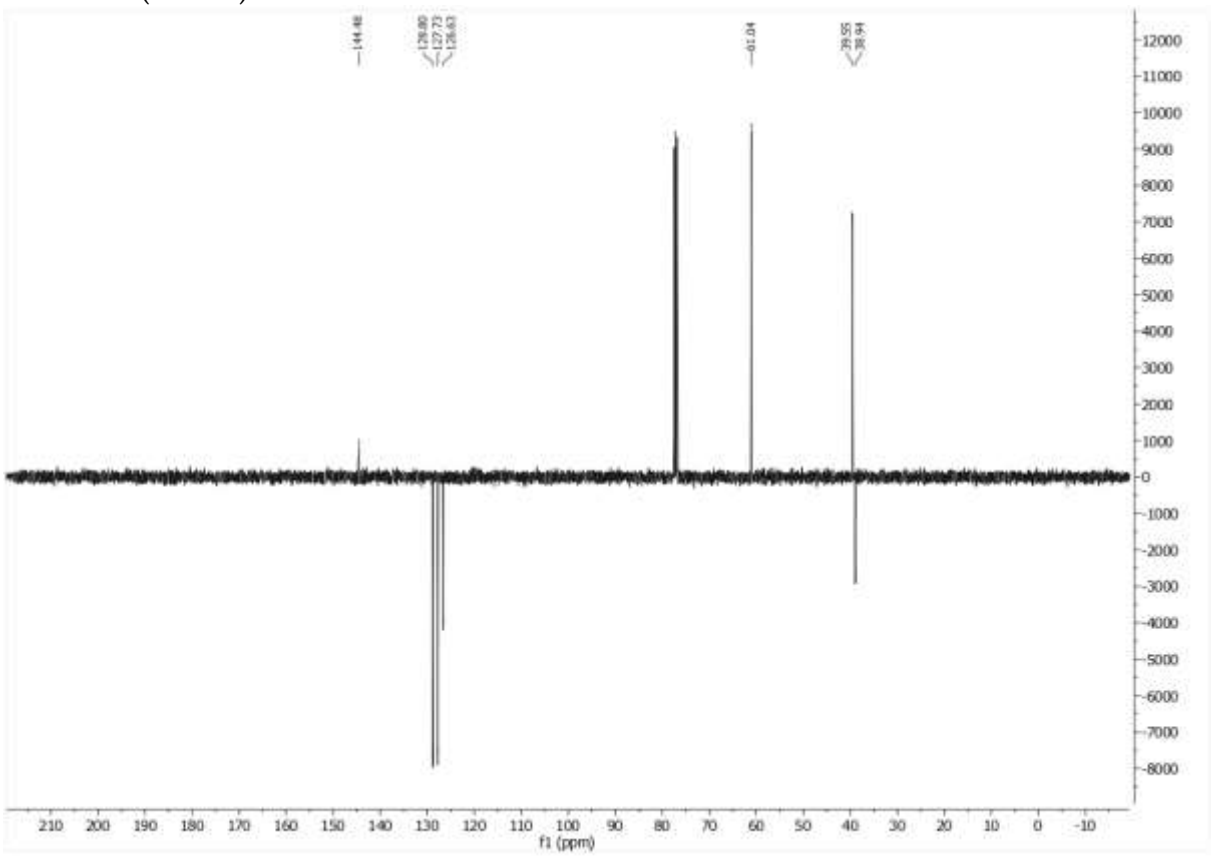
^{13}C NMR ($CDCl_3$)



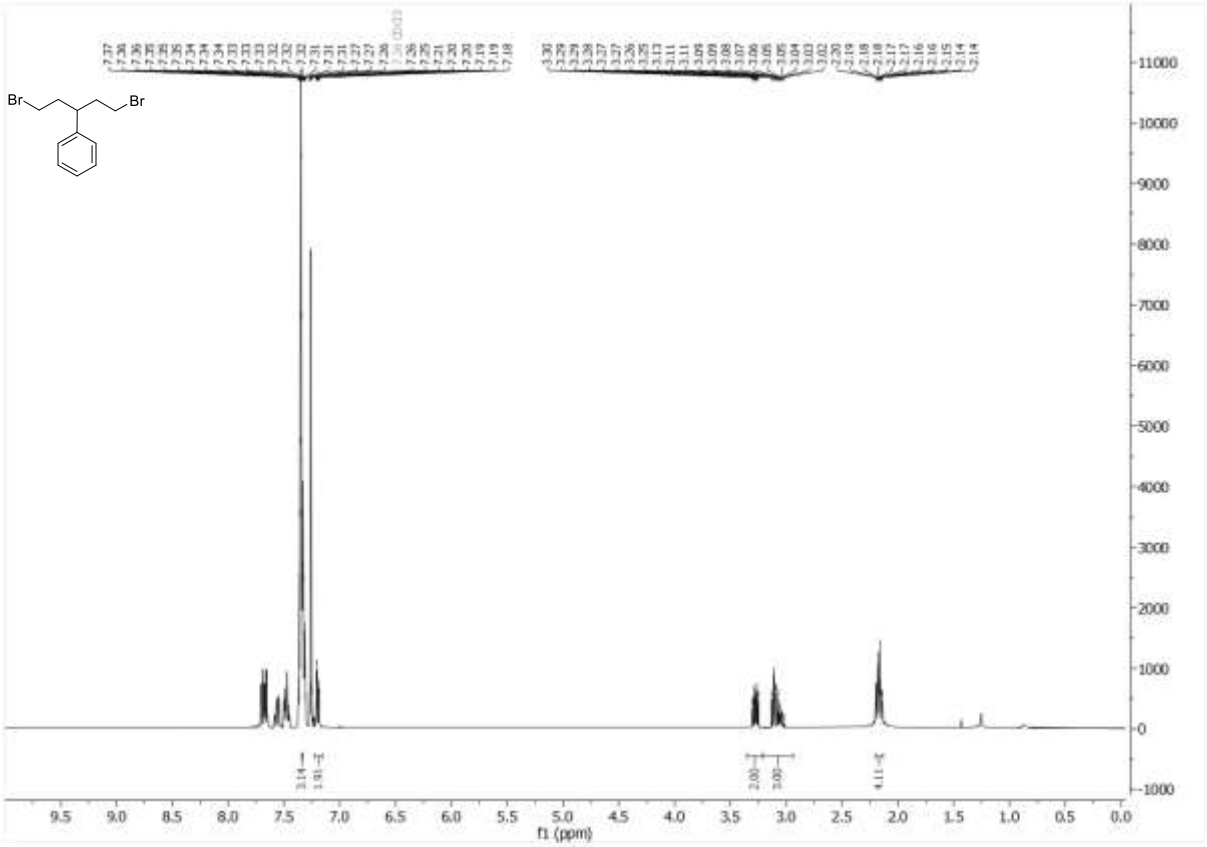
Compound **47** ^{1}H NMR ($CDCl_3$)



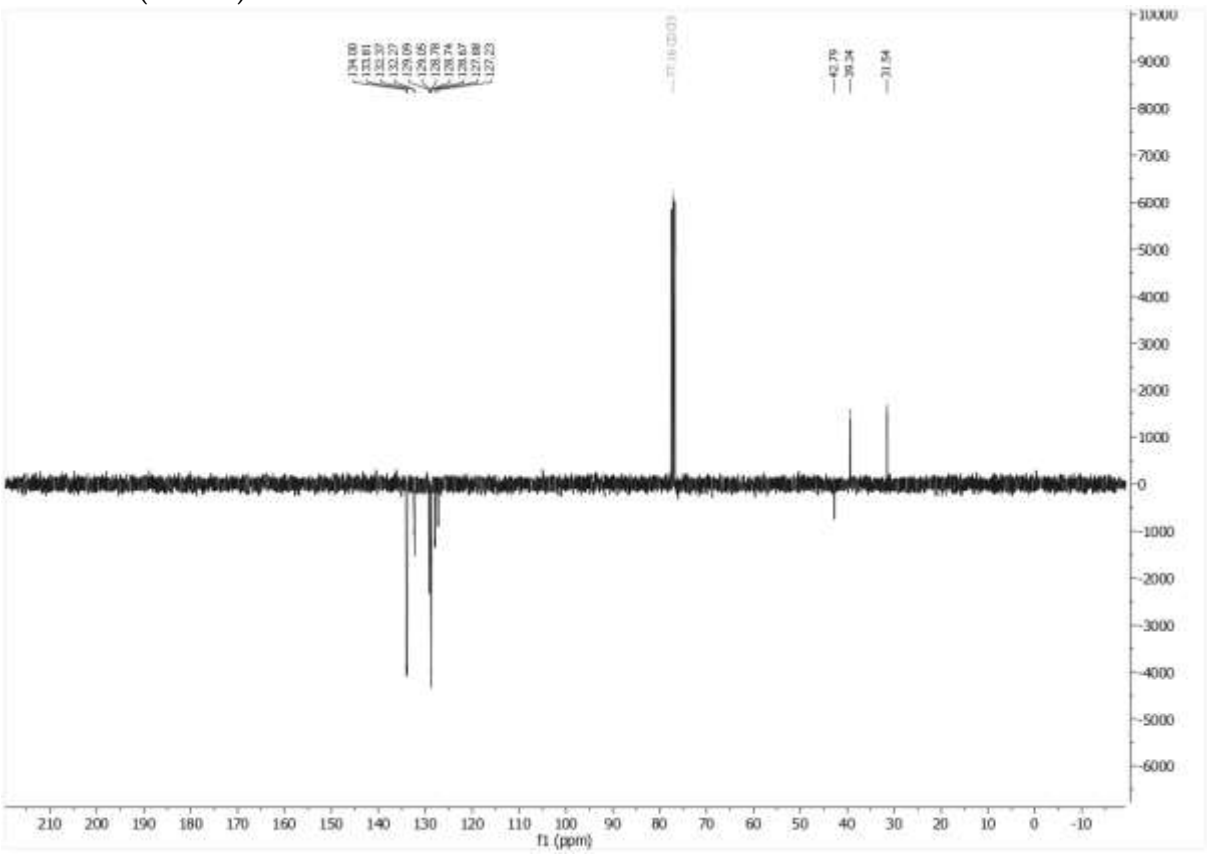
^{13}C NMR ($CDCl_3$)



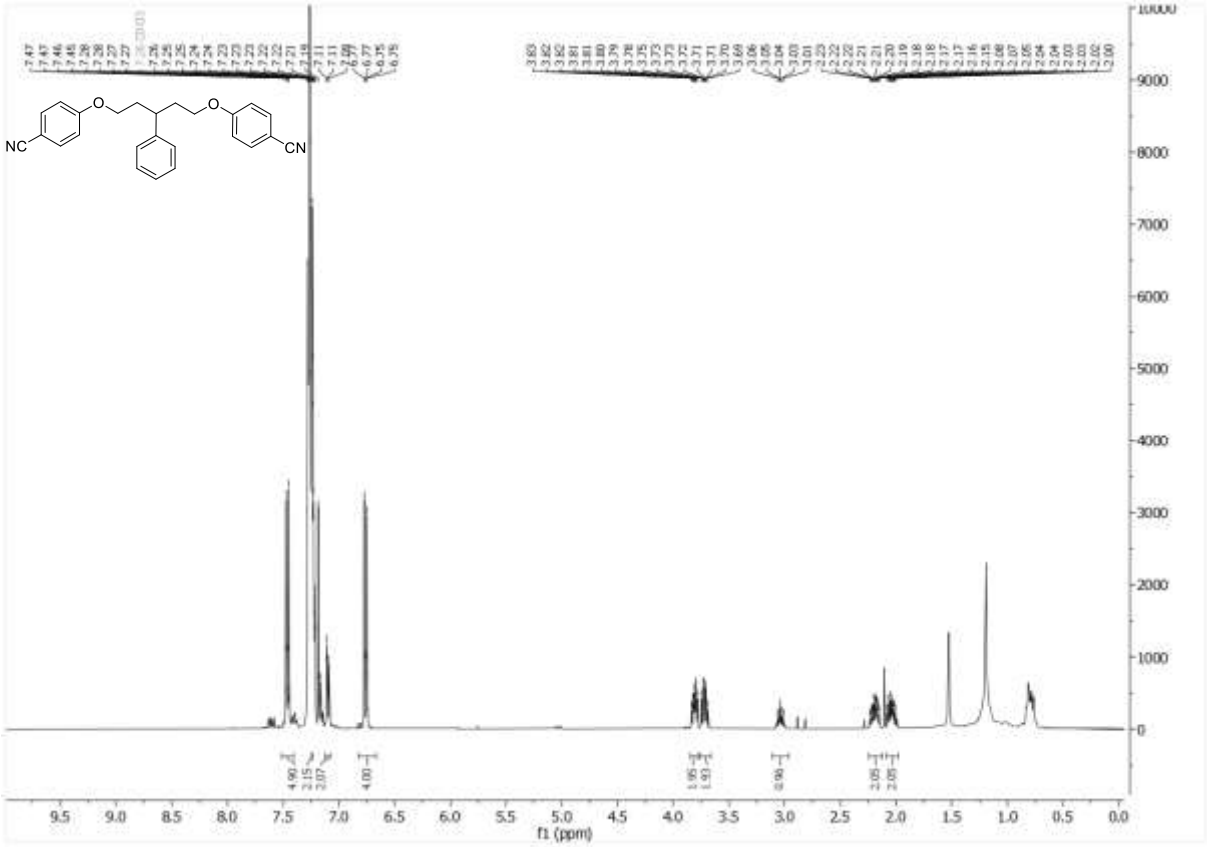
Compound **48** ^{1}H NMR ($CDCl_3$)



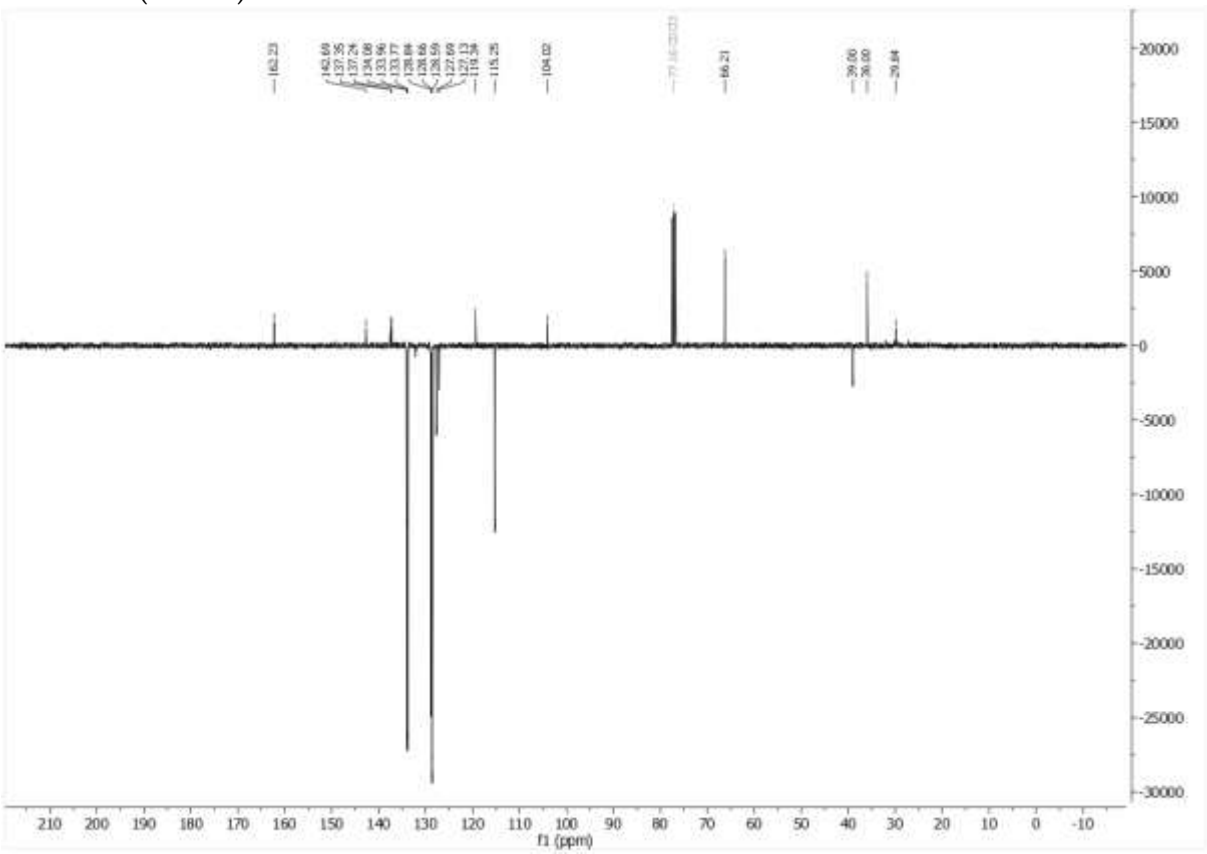
^{13}C NMR ($CDCl_3$)



Compound **49** ^{1}H NMR ($CDCl_3$)

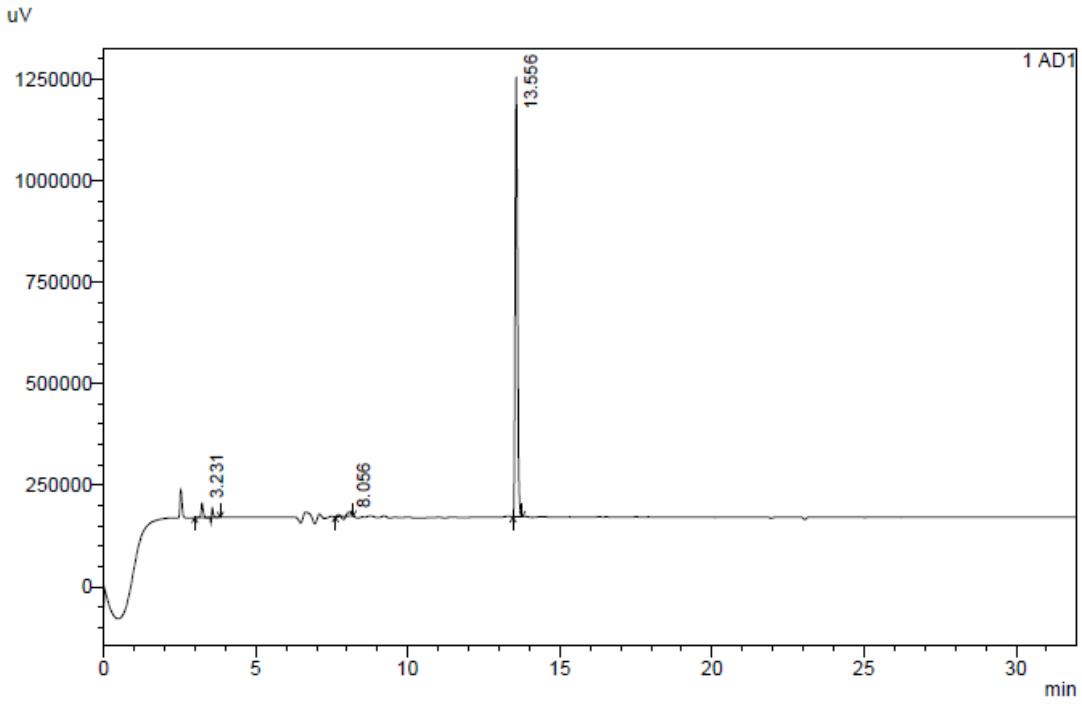


^{13}C NMR ($CDCl_3$)



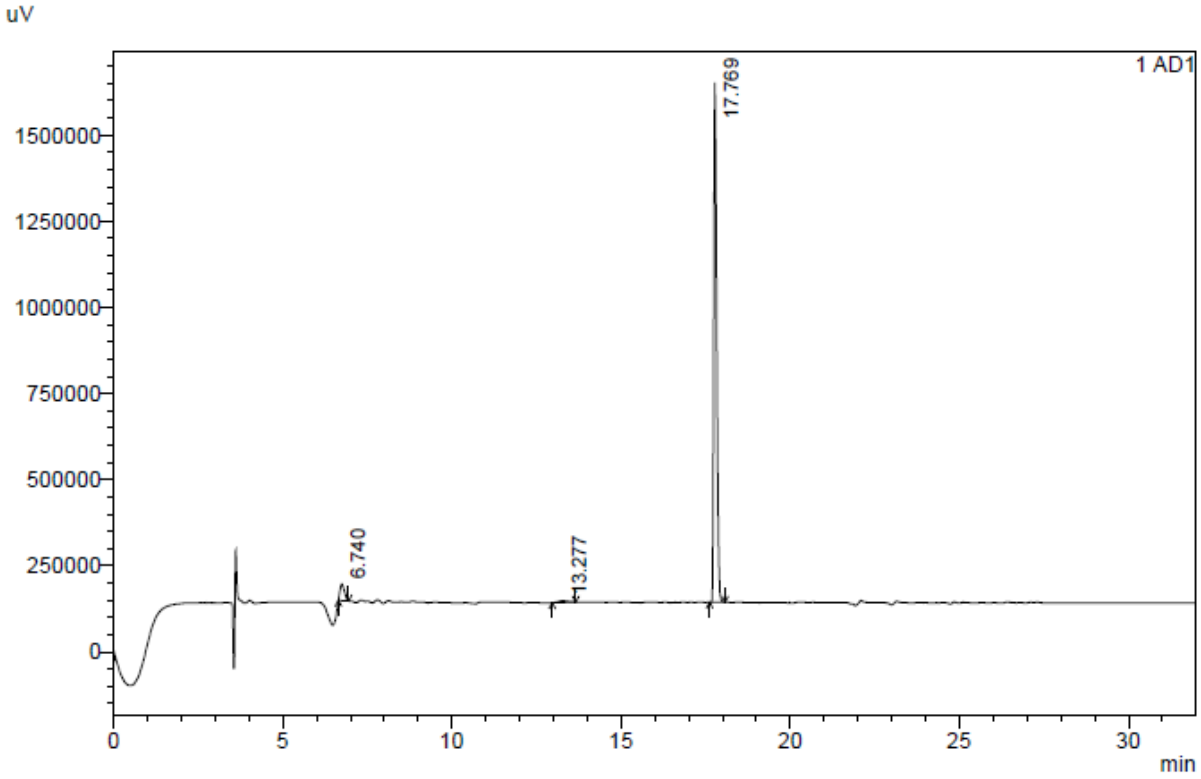
HPLC traces of the final compounds

Compound 1 (pentamidine)



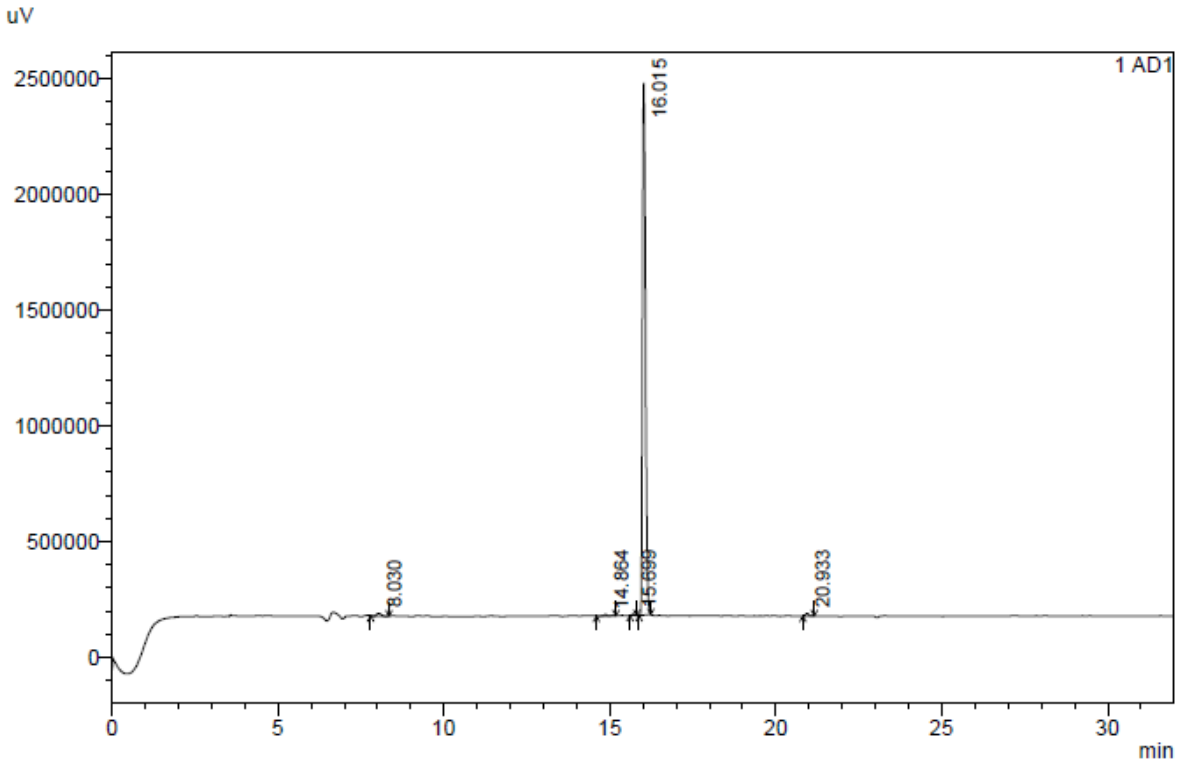
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	3.231	2.992	3.825	190502	35279	5.400	3.229
2	8.056	7.608	8.200	69410	9121	7.610	1.176
3	13.556	13.450	13.758	5640117	1083450	5.206	95.595

Compound 2



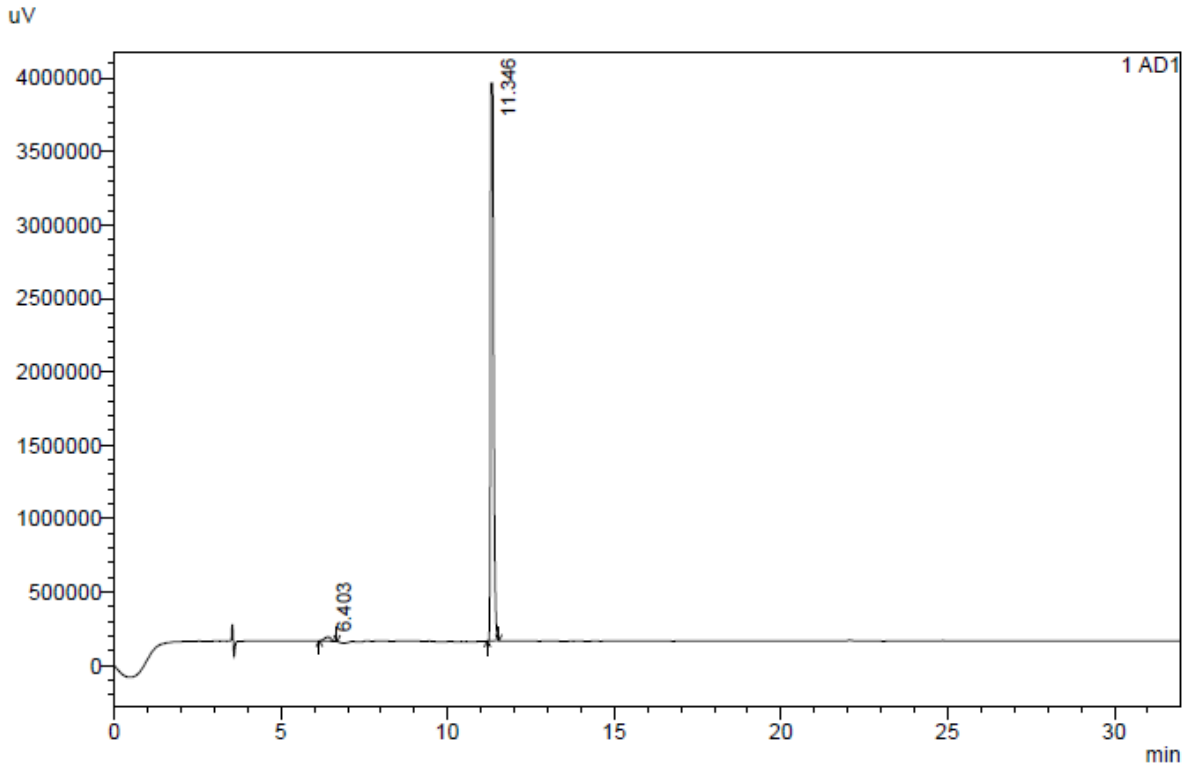
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.740	6.642	6.942	443553	47778	9.284	4.540
2	13.277	12.958	13.658	88491	4715	18.767	0.906
3	17.769	17.617	18.067	9236966	1507950	6.126	94.554

Compound 3



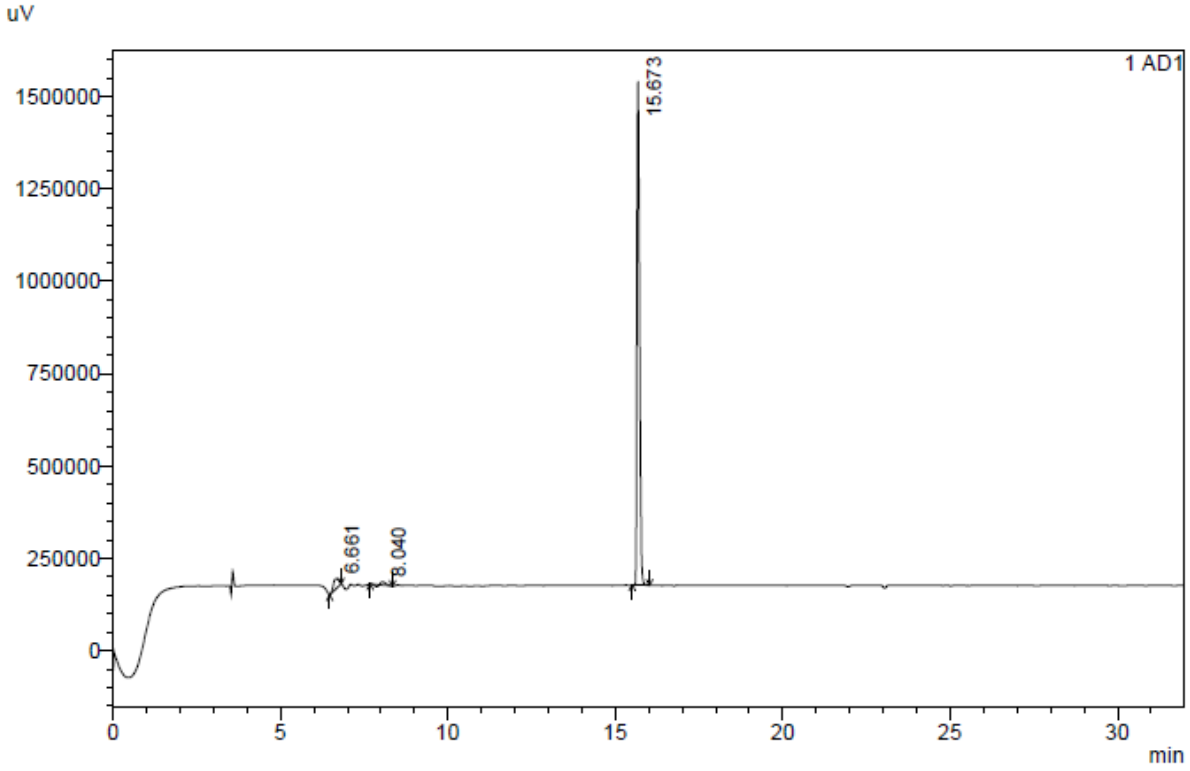
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	8.030	7.783	8.342	60897	11027	5.522	0.414
2	14.864	14.592	15.183	37761	6700	5.636	0.257
3	15.699	15.600	15.808	34487	7583	4.548	0.235
4	16.015	15.875	16.225	14503539	2298347	6.310	98.645
5	20.933	20.808	21.158	66145	11357	5.824	0.450

Compound 9



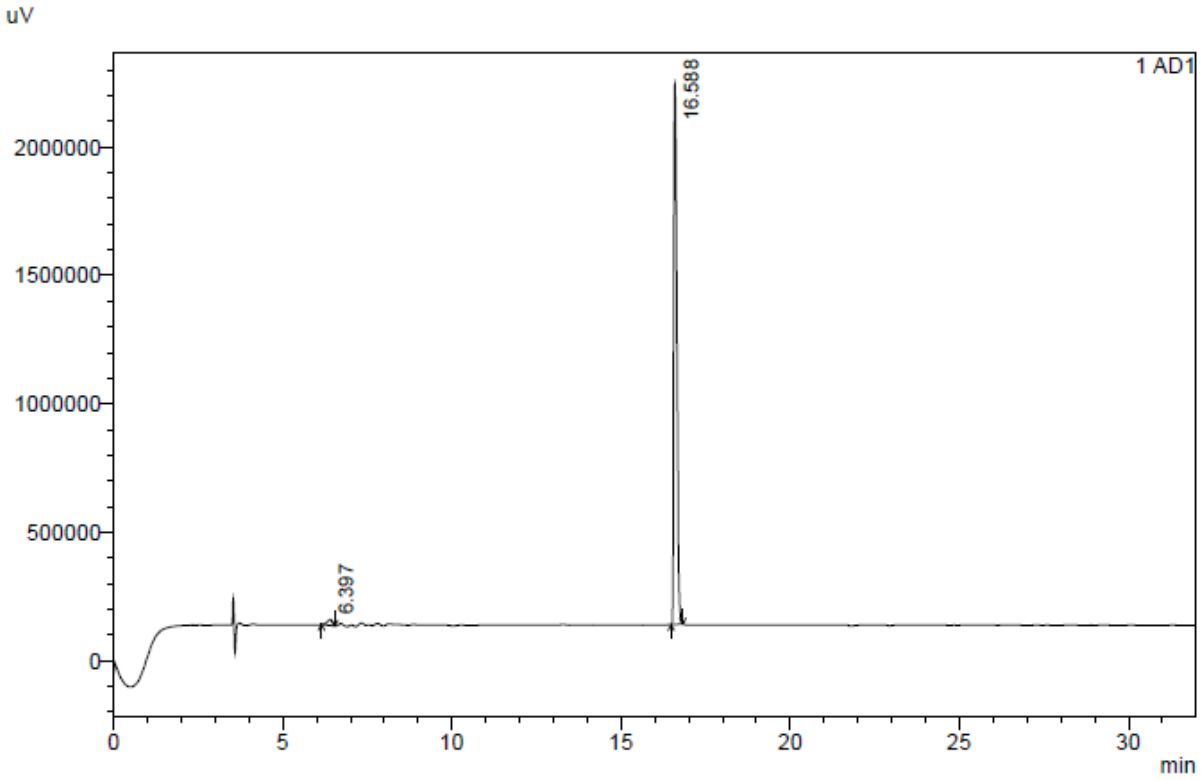
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.403	6.150	6.675	374416	28114	13.318	1.440
2	11.346	11.192	11.533	25632395	3795638	6.753	98.560

Compound 10



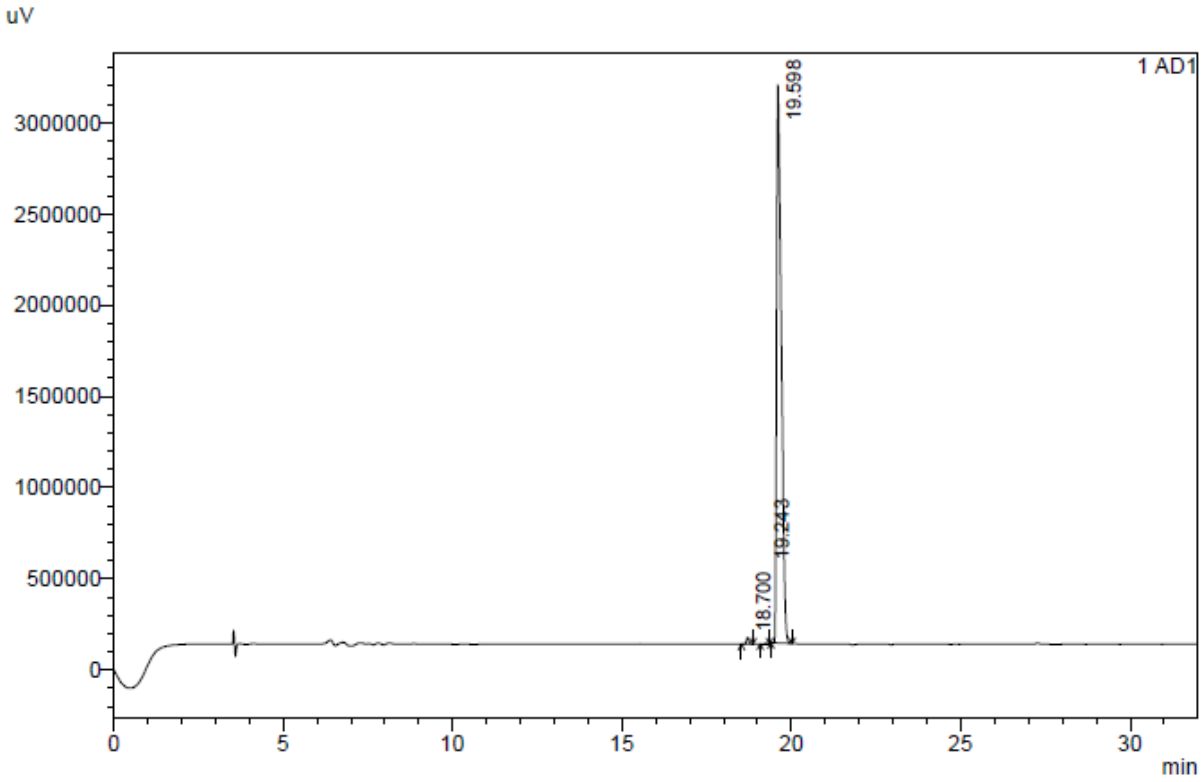
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.661	6.467	6.808	308582	26667	11.572	3.980
2	8.040	7.675	8.342	60636	8554	7.088	0.782
3	15.673	15.500	16.017	7383375	1363595	5.415	95.237

Compound 11



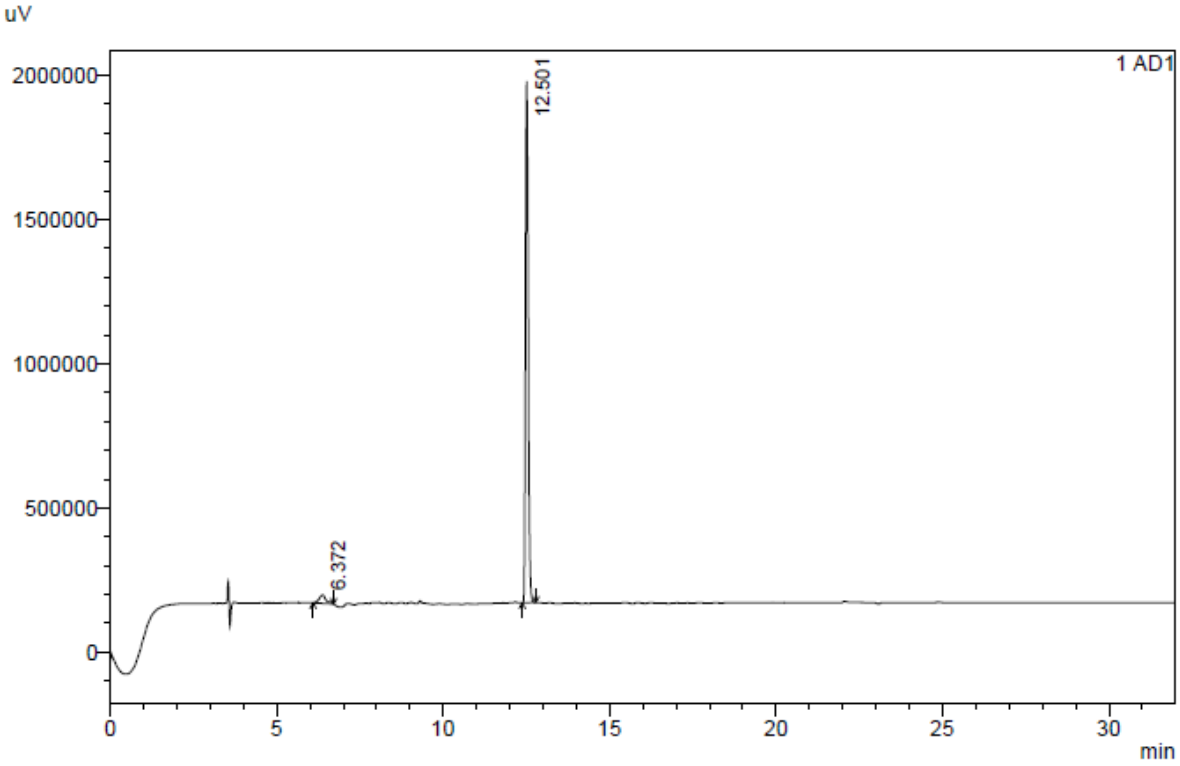
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.397	6.142	6.533	239908	23412	10.247	1.741
2	16.588	16.475	16.817	13541831	2104780	6.434	98.259

Compound 12



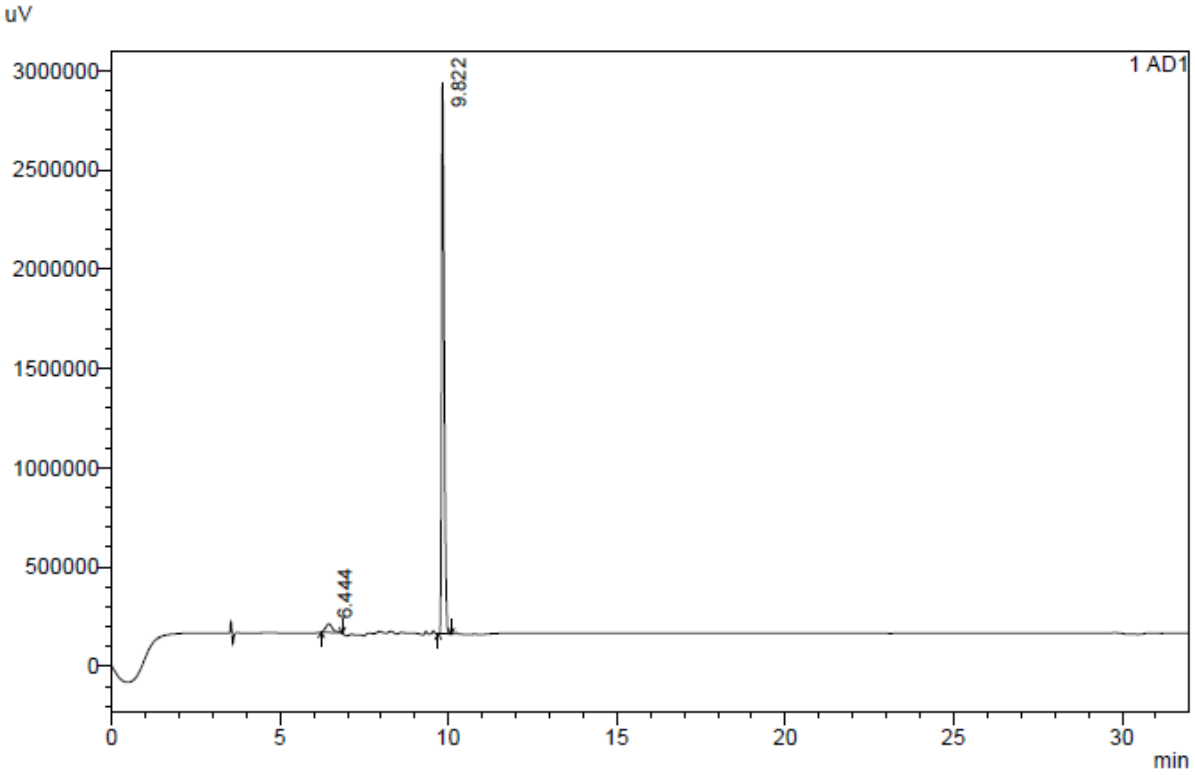
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	18.700	18.517	18.867	175315	34829	5.034	0.585
2	19.243	19.075	19.350	6383	2594	2.461	0.021
3	19.598	19.392	20.017	29799644	3061564	9.733	99.394

Compound 15



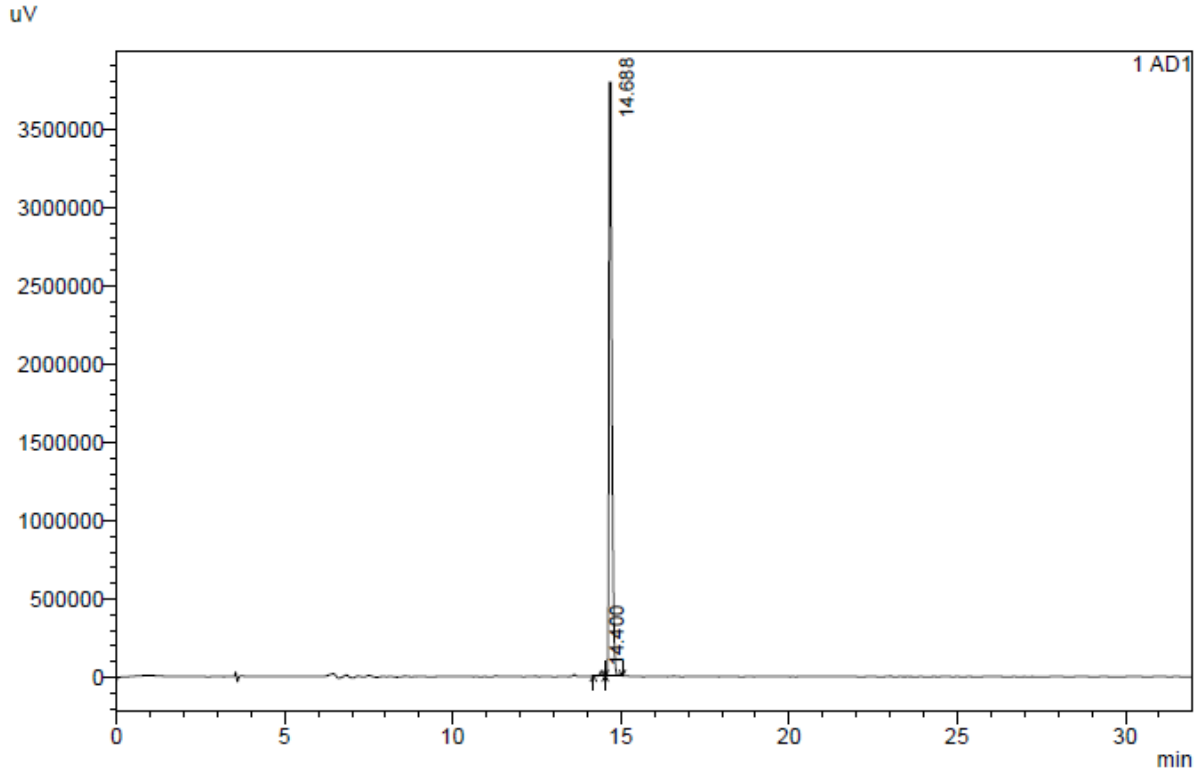
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.372	6.083	6.700	423802	29177	14.525	3.979
2	12.501	12.367	12.783	10226985	1807168	5.659	96.021

Compound 16



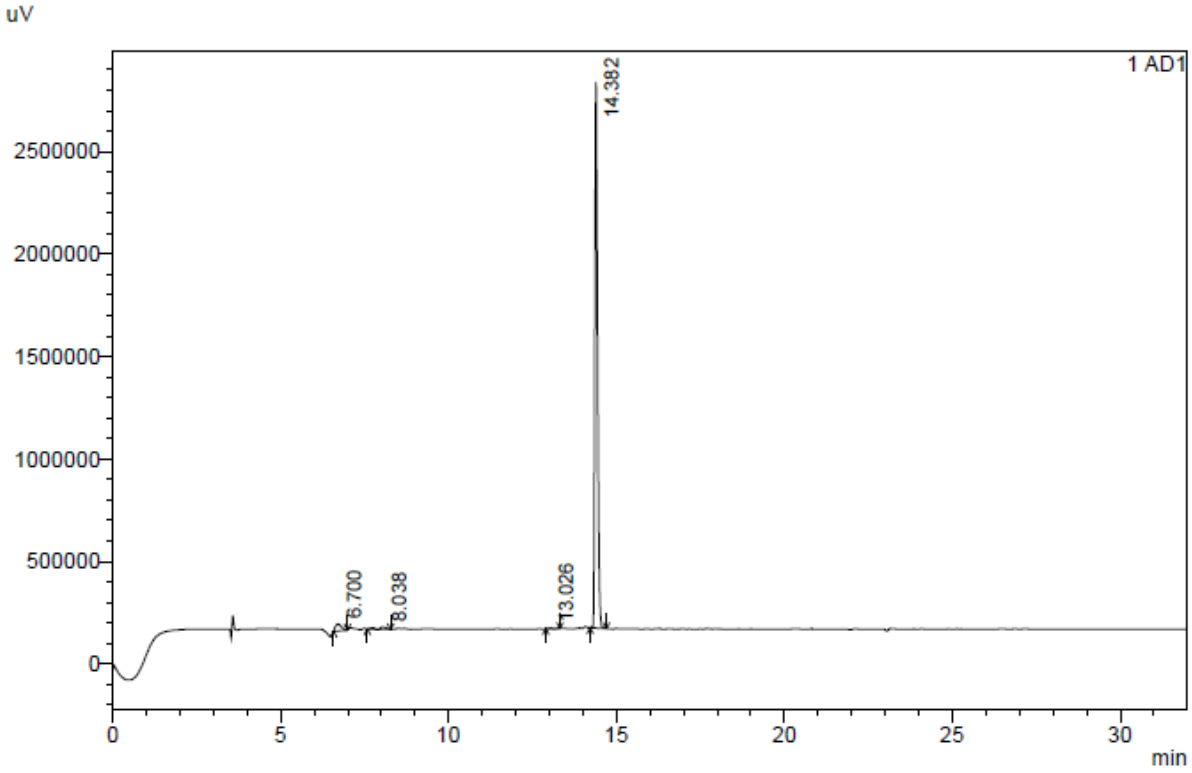
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.444	6.217	6.842	655818	43897	14.940	4.240
2	9.822	9.692	10.075	14811375	2776888	5.334	95.760

Compound 21



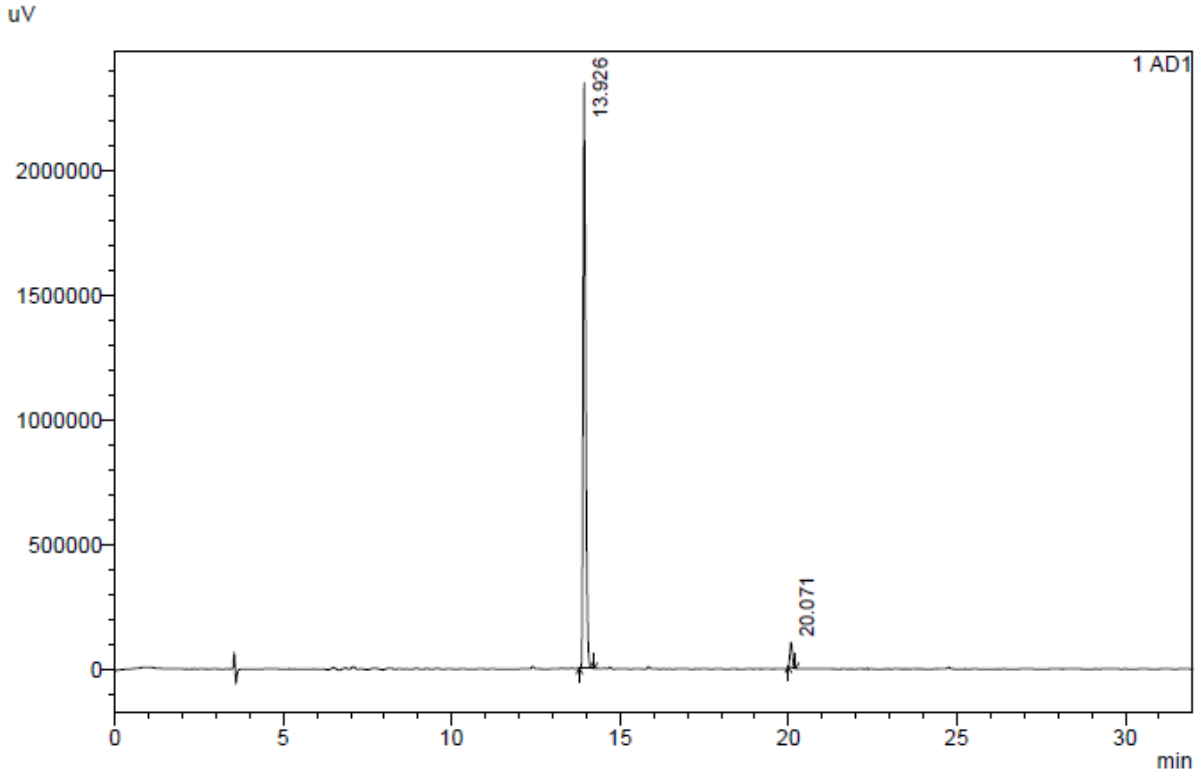
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	14.400	14.175	14.525	142542	29102	4.898	0.593
2	14.688	14.525	15.042	23905797	3788678	6.310	99.407

Compound 22



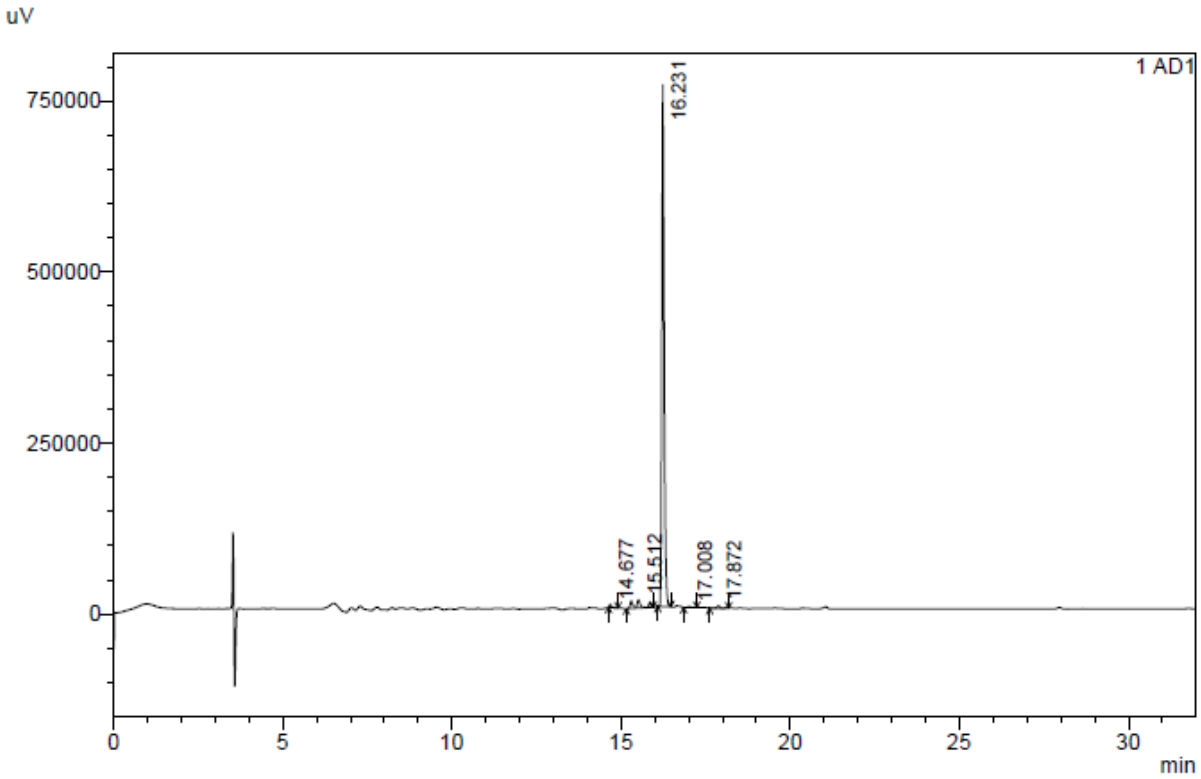
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.700	6.567	6.983	401732	33536	11.979	2.572
2	8.038	7.575	8.267	158535	10627	14.918	1.015
3	13.026	12.892	13.308	473	3816	0.124	0.003
4	14.382	14.208	14.700	15059471	2664649	5.652	96.410

Compound 23



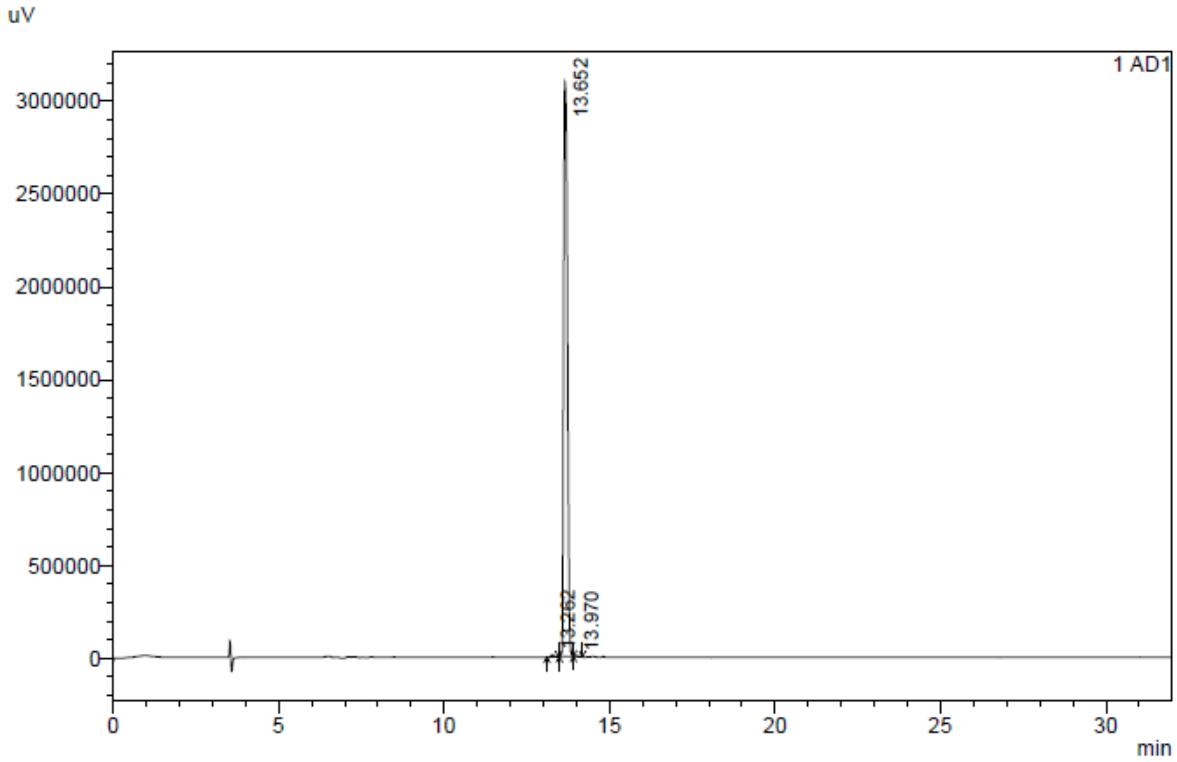
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	13.926	13.792	14.225	12946767	2350277	5.509	95.971
2	20.071	19.992	20.217	543566	101156	5.374	4.029

Compound 24



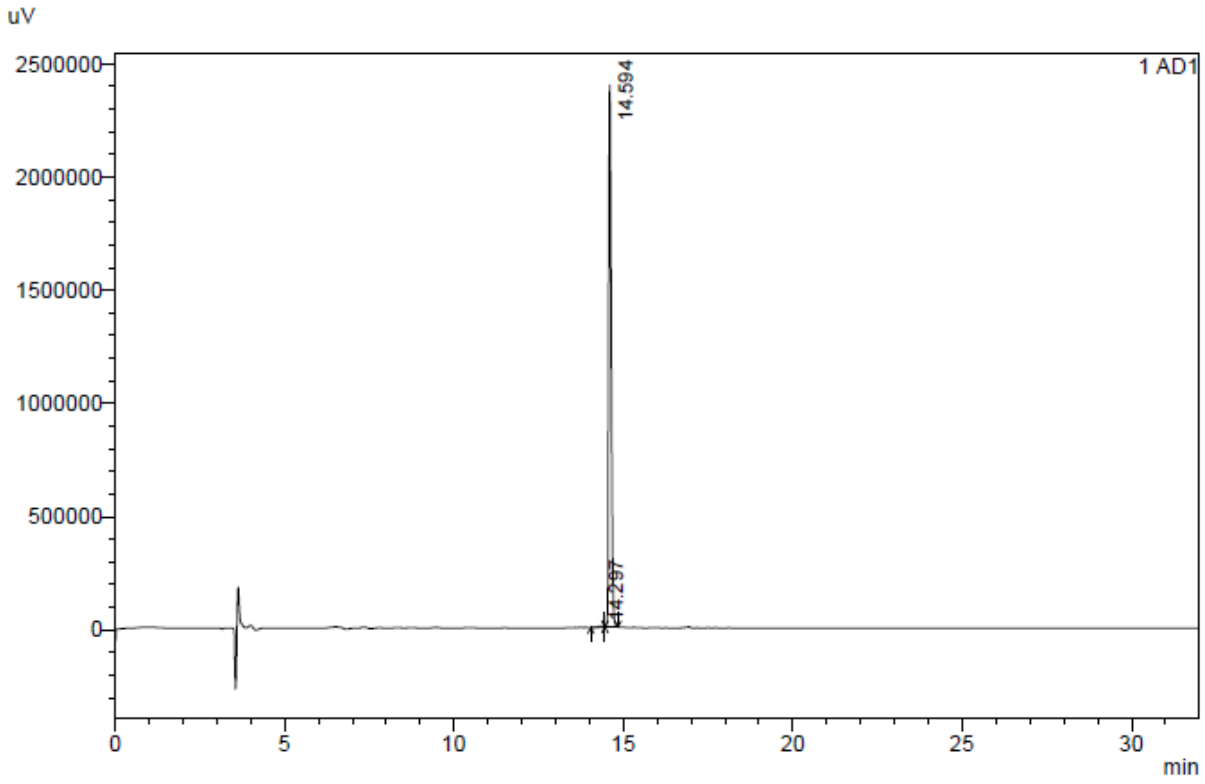
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	14.677	14.625	14.908	12503	4226	2.958	0.314
2	15.512	15.150	15.950	166069	11935	13.915	4.175
3	16.231	16.083	16.467	3768502	763165	4.938	94.742
4	17.008	16.850	17.233	10857	1486	7.309	0.273
5	17.872	17.617	18.175	19730	3456	5.708	0.496

Compound 1b



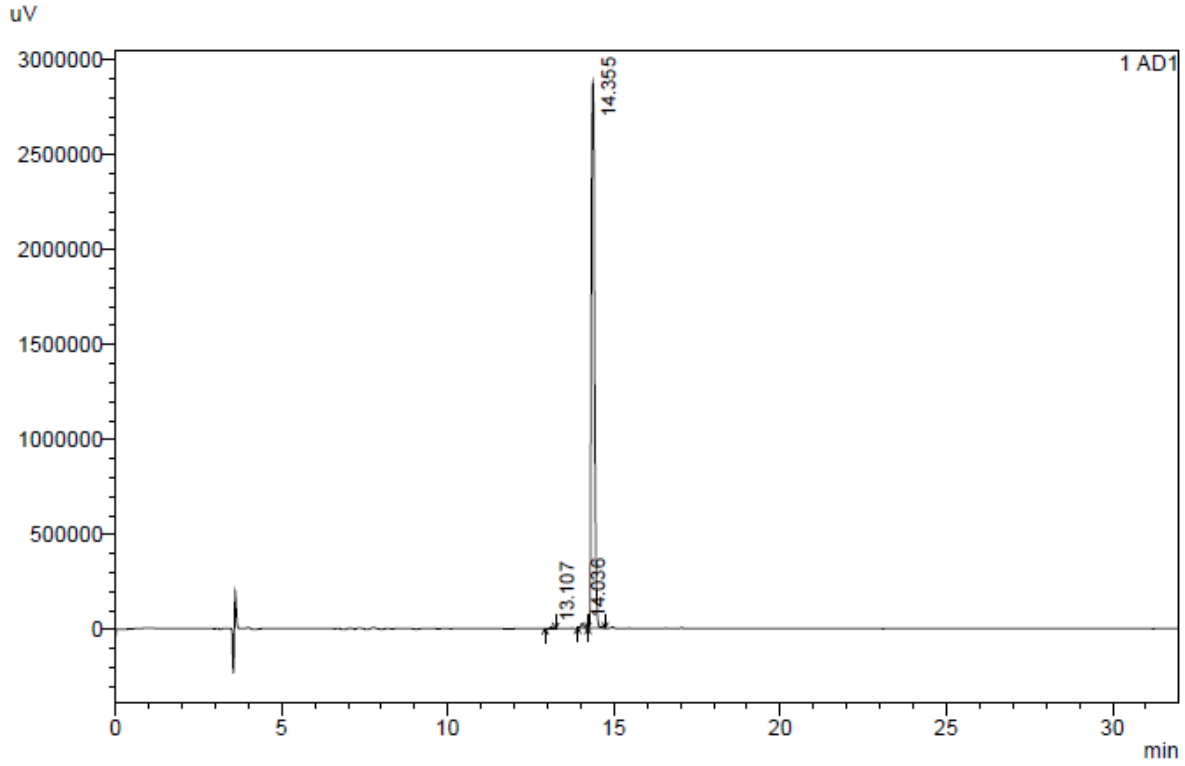
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	13.262	13.100	13.450	83660	14203	5.890	0.331
2	13.652	13.483	13.900	25196598	3093159	8.146	99.595
3	13.970	13.900	14.175	18717	5136	3.644	0.074

Compound 21b



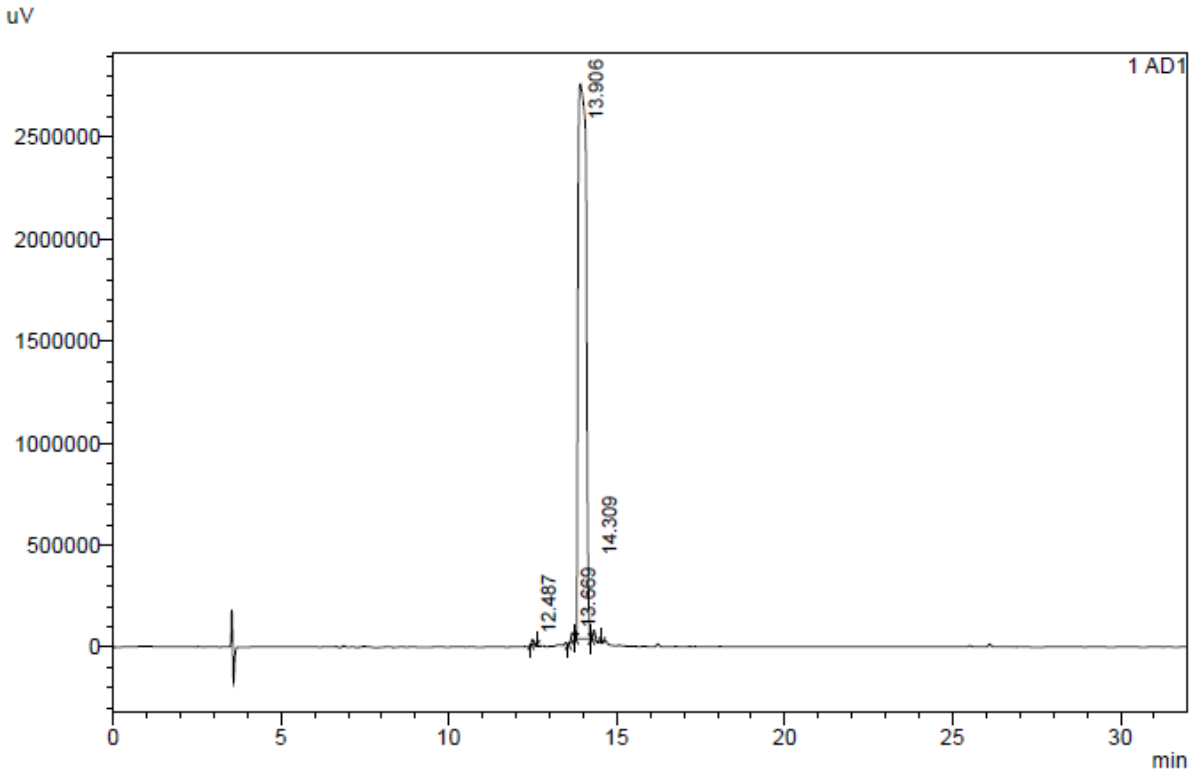
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	14.297	14.033	14.450	22797	4495	5.072	0.184
2	14.594	14.450	14.833	12365991	2395367	5.162	99.816

Compound 22b



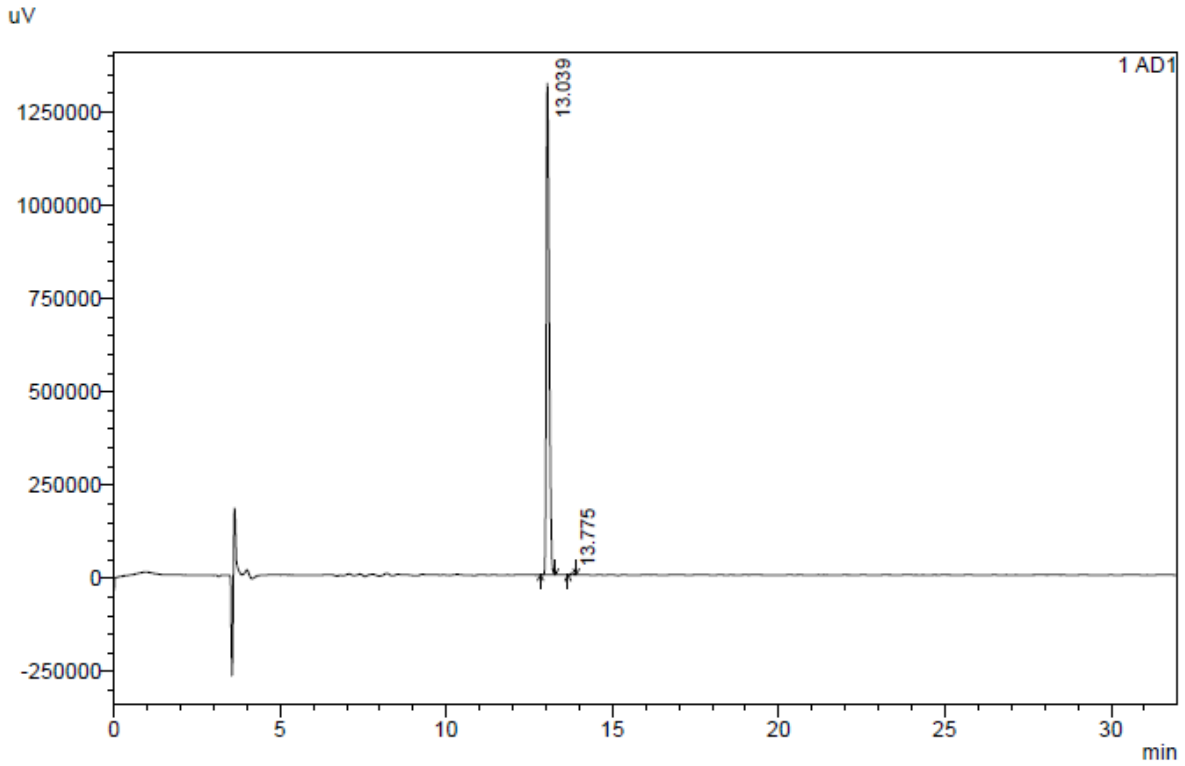
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	13.107	12.925	13.242	45068	8390	5.372	0.228
2	14.036	13.900	14.208	157275	24844	6.330	0.796
3	14.355	14.208	14.733	19561400	2877992	6.797	98.976

Compound 23b



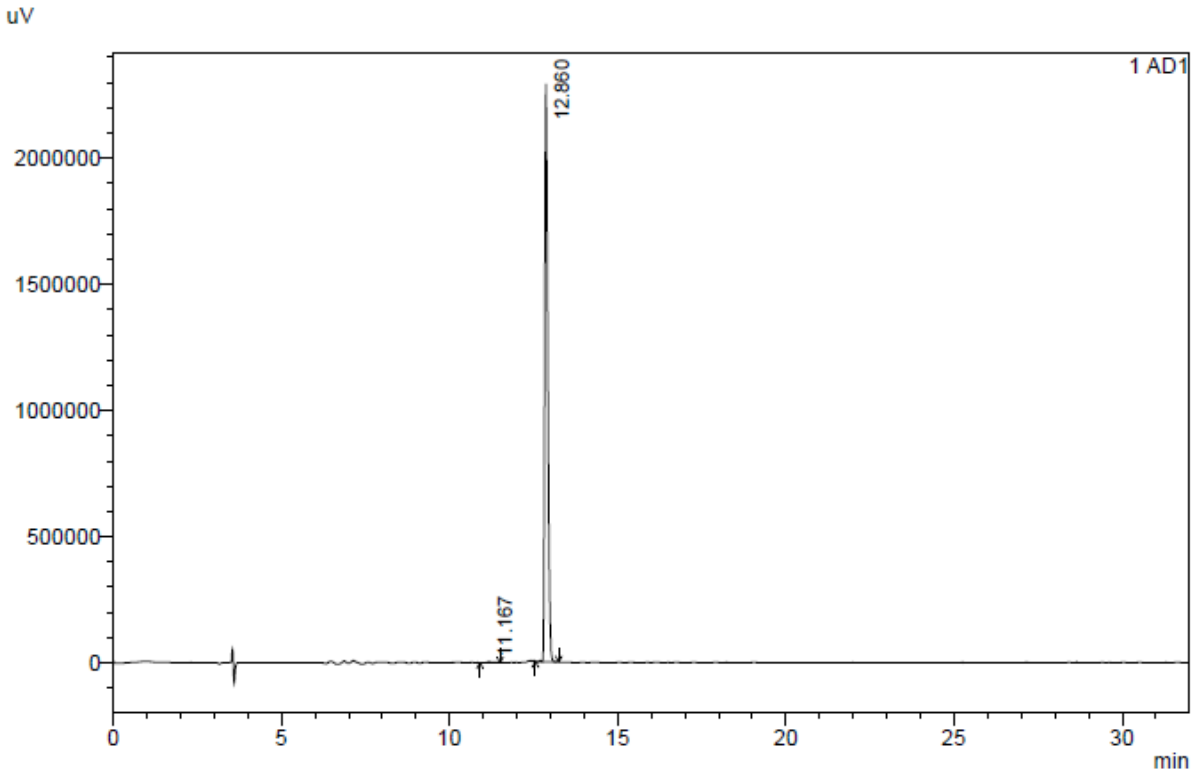
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	12.487	12.442	12.608	101927	23975	4.251	0.227
2	13.669	13.550	13.758	232873	39151	5.948	0.519
3	13.906	13.758	14.242	44303219	2719916	16.288	98.729
4	14.309	14.242	14.558	235457	47432	4.964	0.525

Compound 1c



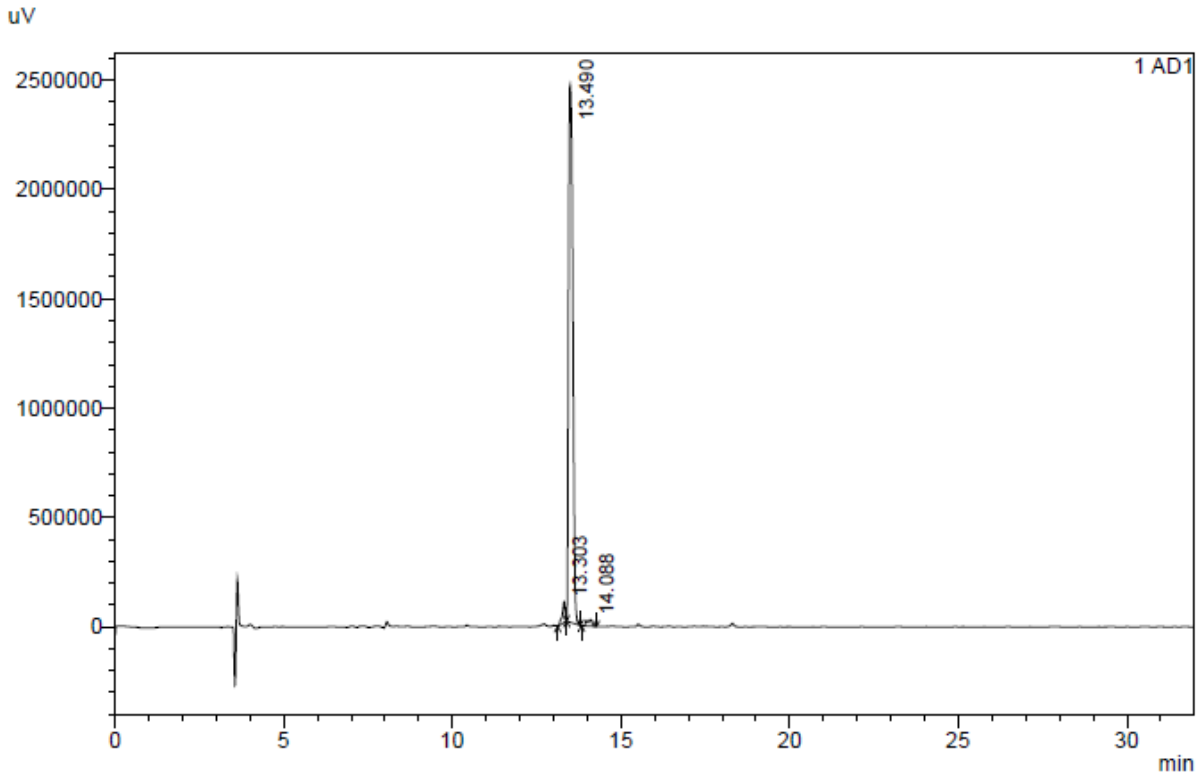
R.Time	I.Time	F.Time	Area	Height	A/H	Conc.	Mark
1	13.039	12.825	13.275	8183496	1318105	6.209	99.663
2	13.775	13.658	13.900	27695	5568	4.974	0.337

Compound 21c



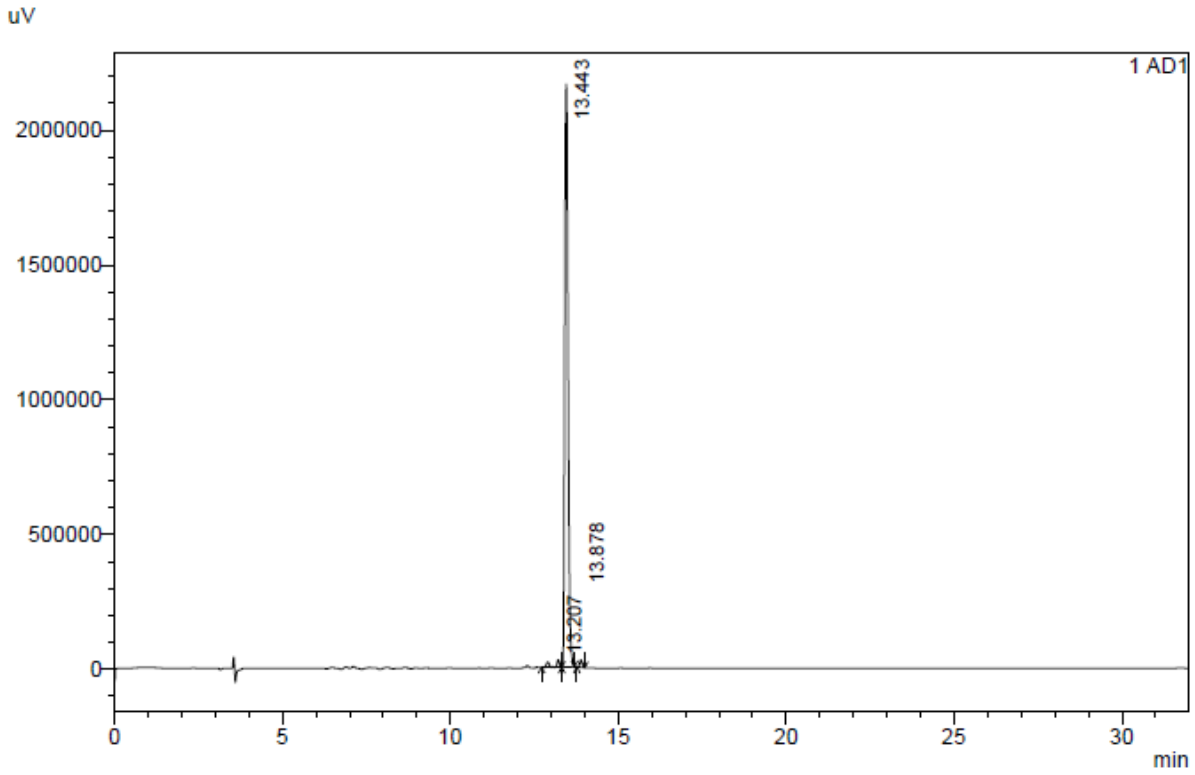
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	11.167	10.900	11.500	35151	4153	8.464	0.237
2	12.860	12.542	13.242	14803062	2289534	6.466	99.763

Compound 22c



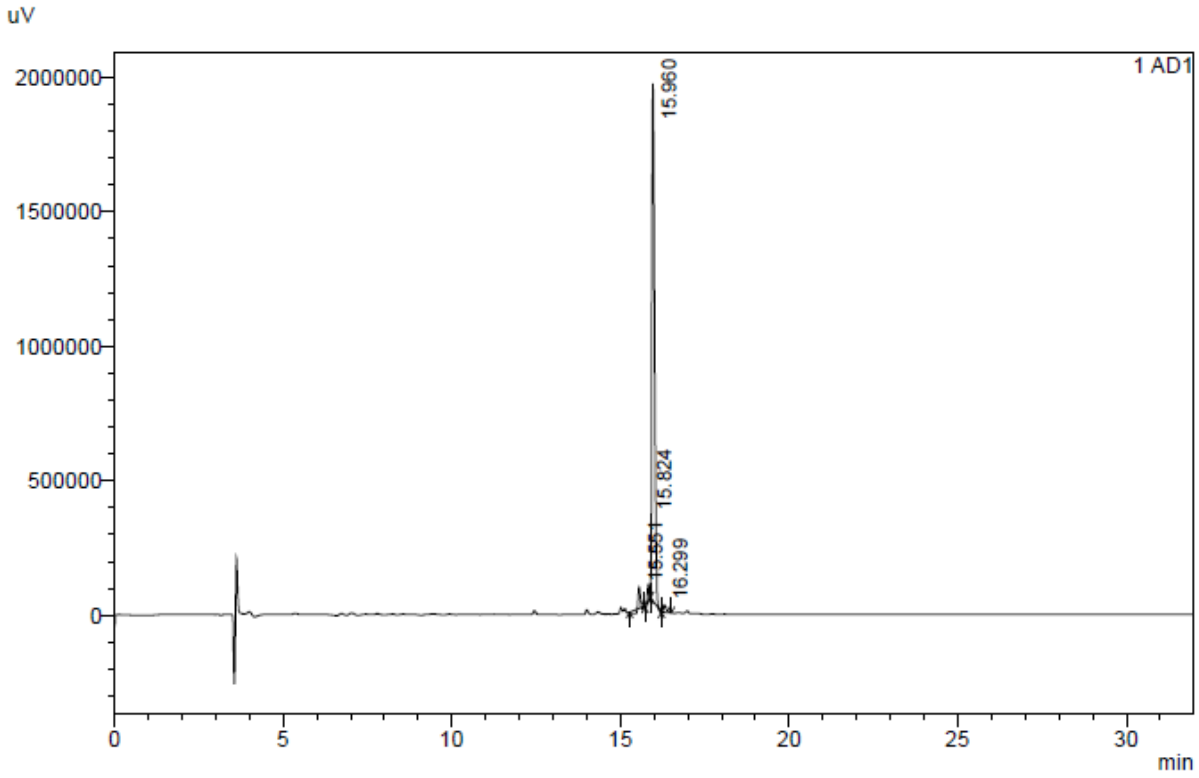
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	13.303	13.117	13.375	552622	96040	5.754	2.454
2	13.490	13.383	13.792	21644706	2461180	8.794	96.112
3	14.088	13.825	14.267	322991	31356	10.301	1.434

Compound 23c



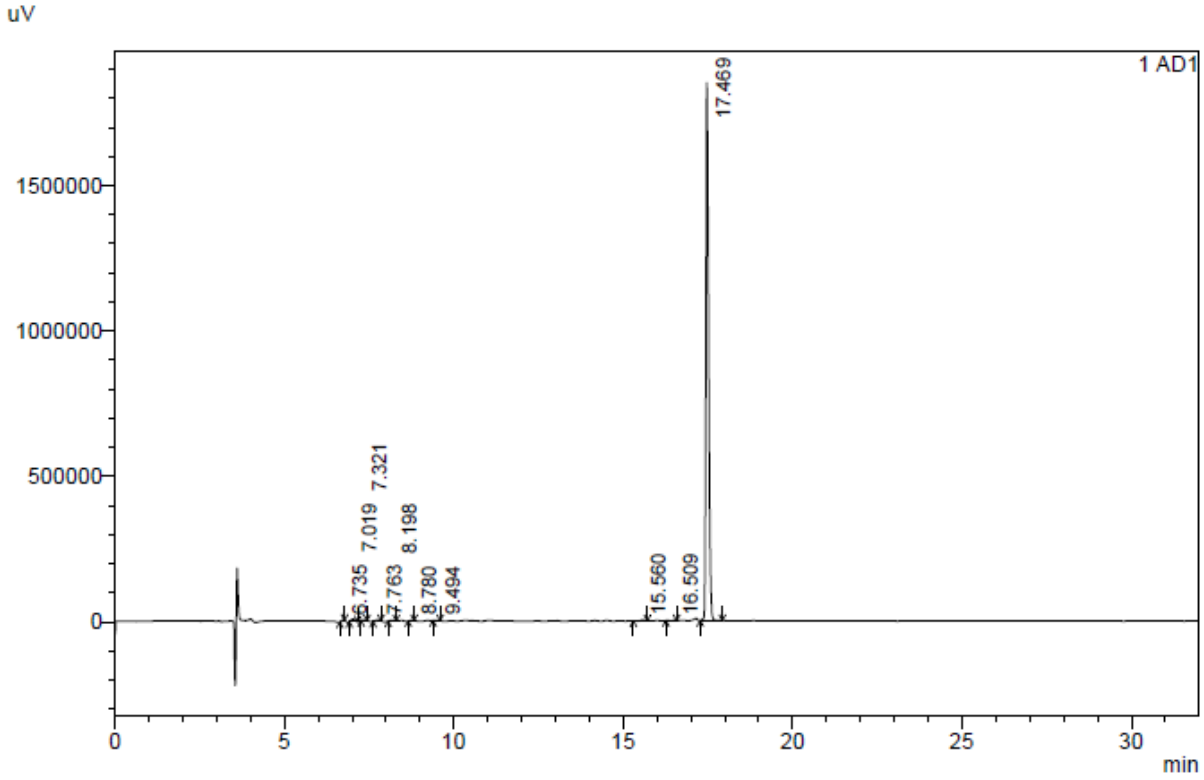
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	13.207	12.717	13.308	248041	27492	9.022	1.597
2	13.443	13.308	13.692	15137905	2164339	6.994	97.492
3	13.878	13.758	14.000	141331	28156	5.020	0.910

Compound 37



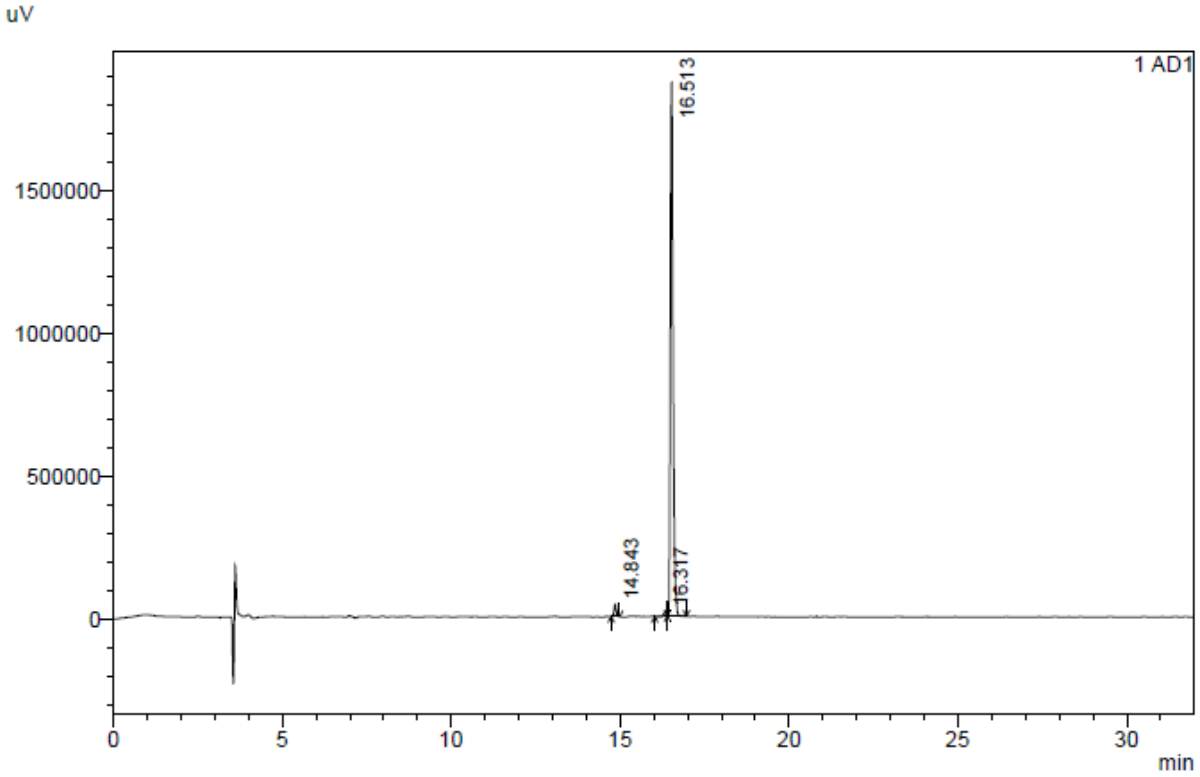
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	15.551	15.267	15.692	151905	79091	1.921	1.378
2	15.824	15.725	15.883	194984	62106	3.140	1.769
3	15.960	15.883	16.208	10507916	1924112	5.461	95.344
4	16.299	16.208	16.500	166284	28251	5.886	1.509

Compound 38



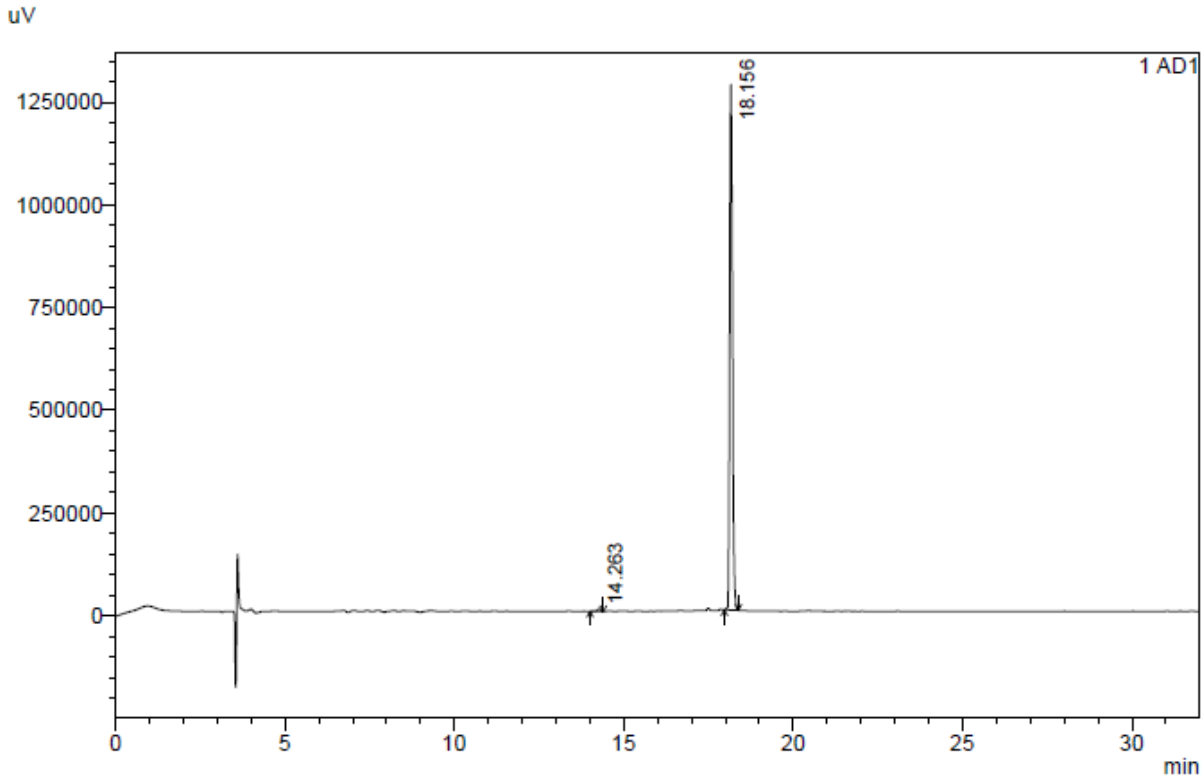
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	6.735	6.633	6.775	20956	3745	5.596	0.197
2	7.019	6.917	7.158	77499	9219	8.406	0.727
3	7.321	7.258	7.433	13771	2063	6.674	0.129
4	7.763	7.608	7.850	36488	4026	9.064	0.342
5	8.198	8.058	8.300	21307	2231	9.549	0.200
6	8.780	8.650	8.825	7368	849	8.679	0.069
7	9.494	9.383	9.592	6954	936	7.431	0.065
8	15.560	15.292	15.708	6774	3350	2.022	0.064
9	16.509	16.258	16.575	9760	2782	3.508	0.092
10	17.469	17.267	17.925	10463720	1849984	5.656	98.116

Compound 43



Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	14.843	14.733	14.975	198400	42476	4.671	1.793
2	16.317	16.017	16.400	50296	13012	3.865	0.455
3	16.513	16.400	16.958	10813807	1870089	5.783	97.752

Compound 44



Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Conc.
1	14.263	14.000	14.383	42465	5853	7.255	0.586
2	18.156	17.967	18.383	7208937	1280203	5.631	99.414

Sources of bacterial strains

Leiden University Medical Center (LUMC), Department of Medical Microbiology, Albinusdreef 2, 2333 ZA Leiden, The Netherlands

A. baumannii ATCC 17978

E.coli ATCC 25922

K. pneumoniae ATCC 13883

P. aeruginosa ATCC 27853

Utrecht University Medical Center (UMC), Microbiology department, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

E.coli BW25113

E.coli 552060.1

Utrecht University Medical Center (UMC), Clinical Microbiology group, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

E.coli RC00089

Wageningen Bioveterinary Research, Bacteriology and Epidemiology, Houtribweg 39, 8221 RA Lelystad, The Netherlands

E. coli EQASmcr-1 (EQAS 2016 412016126)

E. coli EQASmcr-2 (EQAS 2016 KP37)

E. coli EQASmcr-3 (EQAS 2017 2013-SQ352)

References

(1) Gromyko, A. V., Popov, K. V., Mosoleva, A. P., Streltsov, S. A., Grokhovsky, S. L., Oleinikov, V. A., Zhuze, A. L. (2005) DNA sequence-specific ligands: XII. Synthesis and cytological studies of dimeric hoechst 33258 molecules. *Russ. J. Bioorganic Chem.*, 31 (4), 344–351. DOI: 10.1007/s11171-005-0047-z.

(2) Akhtar, W. M., Armstrong, R. J., Frost, J. R., Stevenson, N. G., Donohoe, T. J. (2018) Stereoselective Synthesis of Cyclohexanes via an Iridium Catalyzed (5 + 1) Annulation Strategy. *J. Am. Chem. Soc.*, 140 (38), 11916–11920. DOI: 10.1021/jacs.8b07776.

(3) López-Cortina, S., Medina-Arreguin, A., Hernández-Fernández, E., Berns, S., Guerrero-Alvarez, J., Ordoñez, M., Fernández-Zertuche, M. (2010) Stereochemistry of base-induced cleavage of methoxide ion on cis- and trans-1,4-diphenylphosphorinanium salts. A different behavior with a phenyl substituent. *Tetrahedron*, 66 (32), 6188–6194. DOI: 10.1016/J.TET.2010.05.095.

(4) Berglund, A. J., Bodner, M. J. Aryl diamidines and prodrugs thereof for treating myotonic dystrophy. U.S. patent US 2013/0281462 A1, 2013.

(5) Odds, F. C. (2003) Synergy, antagonism, and what the chequerboard puts between them. *J. Antimicrob. Chemother.*, 52 (1), 1–1. DOI: 10.1093/jac/dkg301.

(6) Amin, K., Dannenfelser, R.-M. (2006) In vitro hemolysis: Guidance for the pharmaceutical scientist. *J. Pharm. Sci.*, 95 (6), 1173–1176. DOI: 10.1002/JPS.20627.

(7) MacNair, C. R., Stokes, J. M., Carfrae, L. A., Fiebig-Comyn, A. A., Coombes, B. K., Mulvey, M. R., Brown, E. D. (2018) Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. *Nat. Commun.*, 9 (2018), 458. DOI: 10.1038/s41467-018-02875-z.