Supporting Information

Triazole-Diindolylmethane Conjugates as New Antitubercular Agents: Synthesis, Bioevaluation and Molecular Docking

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Experimental section

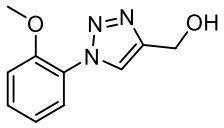
4.1.1. General Procedure for the Preparation of compounds (2a-j)

In a round-bottom flask equipped with a magnetic stirring bar, substituted aniline **1a-j** (8 mmol) was dissolved in stirred solution of 5N HCl (10 mL) in an ice bath. Then $NaNO_2$ (12 mmol in 25 mL of H_2O) was added dropwise. The mixture was stirred for 20-30 min. Sodium azide (16 mmol) dissolved in 25 mL water was added dropwise. Then, the system was stirred for another 2-4 h at rt. The mixture was extracted with ethyl acetate and the organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residual crude product was used directly without purification.

4.1.2. General Procedure for the Preparation of compounds (3a-j)

In a round-bottom flask equipped with a magnetic stirring bar, aromatic azide **2a-j** (0.83 mmol) was added to a solvent of *t*-butanol along with propargyl alcohol (0.83 mmol), $CuSO_4$ pentahydrate (0.083 mmol), sodium ascorbate (0.41 mmol) and H_2O. The reaction mixture was stirred for 48-72 h at rt; the progress of reaction was monitored by TLC. Then, the reaction mixture was poured in to 50 mL ice water and the obtained solid was filtered, dried and recrystallized from ethanol to obtain a solid pure derivatives **3a-j** in yields ranging from 63-82%.

4.1.3. (1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methanol (3h)



The compound **3h** was prepared according to general procedure 4.1.2. in 85% yield. m.p. 115-117 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.42 (td, *J* = 8.1, 1.4 Hz, 1H), 7.09 (t, *J* = 7.9 Hz, 2H), 4.89 (s, 2H), 3.88 (s, 3H).

4.1.4. General Procedure for the Preparation of compounds (4a-j)

In a round-bottom flask equipped with a magnetic stirring bar, Collins reagent (CrO_3:2Py) was prepared in situ by adding (1.2 mmol) CrO_3 to a stirred solution of (2.5 mmol) pyridine in dichloromethane and celite. Chromium trioxide must be added over pyridine, as doing an inverse addition leads to an explosion. Then, 0.5 mmol of synthesized 1,2,3-triazole (**3a-j**) in CH_2Cl_2 was slowly added to Collins reagent (CrO_3:2Py) and stirred at room temperature for 2-6 h. The reaction was monitored with TLC. After completion the reaction mixture was filtered through a pad of silica or celite. The residue was washed with CH_2Cl_2, the organic phases was washed with diluted HCl and the resulting organic solution is dried by Na_2SO_4 and concentrated in vacuo, dried, recrystallized by ethanol to give pure aldehydes (**4a-j**).

Table S1: Antitubercular activity data of compounds **6a-s** against active and dormant *Mtb* H37Ra.

Entry	% Inhibition of *Mtb* H37Ra growth in presence of compounds					
	Active *Mtb*			Dormant *Mtb*		
	30 µg/mL	10 µg/mL	3 µg/mL	30 µg/mL	10 µg/mL	3 µg/mL
6a	66.44	71.04	57.85	60.07	83.23	70.63
6b	85.33	93.41	82.24	85.71	86.97	91.86
6c	53.95	59.90	27.59	77.92	69.96	64.24
6d	72.88	74.37	25.31	85.19	82.05	59.50
6e	-16.42	-8.07	-14.76	9.76	15.69	17.94
6f	76.14	68.86	39.68	80.95	90.86	85.23
6g	66.22	68.78	58.11	68.18	76.53	65.27
6h	73.70	82.81	56.22	85.92	90.27	67.15
6i	45.09	-20.55	-23.55	10.13	4.79	-13.72
6j	80.85	78.85	12.13	88.22	87.23	73.45
6k	-50.82	-11.20	-46.59	-6.77	7.25	-10.57
6l	89.19	94.05	89.61	95.11	91.42	89.71
6m	59.62	58.23	41.98	59.80	75.11	70.87
6n	85.87	90.23	88.06	85.84	89.87	86.56
6o	57.57	66.53	52.86	61.12	58.15	57.08
6p	39.61	38.03	34.88	40.24	36.36	38.78
6q	60.34	76.76	52.88	74.53	86.82	87.18
6r	91.45	89.06	84.89	90.36	90.22	70.34
6s	82.88	76.80	31.86	88.91	90.07	89.32

[The % Inhibition in the presence of test material is calculated by following formula.

% inhibition I = {(Average of Control-Average of Compound)/ (Average of Control-Average of Blank)} X 100

Where control is culture medium with cells and DMSO and blank is culture medium without cells. Compounds were considered inactive if % I <90 at 30 µg/mL. For all samples, each compound concentration was tested in triplicates in a single experiment.]

Table S2: Antibacterial activity data of compounds **6a-s** at 3µg/ml concentration.

Entry	*B.subtilis* (% Inhibition)	*S. aureus* (% Inhibition)	*E.coli* (% Inhibition)	*P.aeroginosa* (% Inhibition)
6a	46.45	10.25	14.71	4.22
6b	**92.28**	23.67	21.96	2.59
6c	56.47	20.82	13.40	5.08
6d	67.47	27.00	23.02	-2.05
6e	35.75	20.14	22.47	7.33
6f	42.84	15.97	23.95	5.98
6g	46.86	11.01	13.86	3.13
6h	**91.94**	20.56	25.10	-0.94
6i	44.53	9.38	14.41	3.75
6j	59.59	18.87	19.26	-2.44
6k	29.48	16.23	14.84	6.46
6l	**97.20**	30.75	20.01	7.24
6m	18.78	19.79	23.79	0.86
6n	46.04	8.20	19.17	6.81
6o	35.52	11.23	25.05	4.73
6p	25.99	10.65	20.09	5.81
6q	42.51	10.48	22.25	4.60
6r	-10.81	**71.77**	-5.13	-25.29
6s	**70.17**	12.01	22.26	2.49

Molecular docking study

Table S3. Quantitative per-residue interaction analysis of the Molecular docking study on DprE1 for the most active triazole-diindolylmethane conjugates.

Entry	*Mtb* H37Ra (µg/mL)		Docking Score	Glide Interaction Energy (kcal/mole)	Per-Residues interactions			
	Active (IC_{50})	Dormant (IC_{50})			Van der Waals (kcal/mol)	Coulombic (kcal/mol)	H-bonds (Å)	π-π Stacking (Å)
6b	2.19	1.55	-8.101	-58.886	Lys418(-3.636), Tyr415(-1.748), Cys387(-2.319), Asn385(-1.46), Phe369(-1.291), Lys367(-2.101), Val365(-3.537), Leu363(-1.784), Gln336(-1.931), Gly321(-1.767), Phe320(-1.983), Leu317(-2.505), His132(-2.092), Ile131(-2.351), Thr118(-2.118), Gly117(-2.018), Pro116(-2.22), Tyr60(-1.915), Arg58(-1.369)	Tyr415(-0.659), Glu322(-1.794), Asp318(-1.133), Leu317(-2.807), Gly117(-3.053), Pro116(-1.257), Arg58(-1.105)	Leu317 (2.05), Arg58(2.02)	His132 (2.81)
6f	14.81	2.96	-7.812	-50.564	Lys418(-1.337), Ala417(-1.117), Tyr415(-1.135), Cys387(-1.715), Lys367(-1.999), Val365(-1.979), Gln336(-1.413), Phe320(-1.126), Leu317(-1.668), Tyr314(-1.779), Ser228(-1.142), Lys134(-1.034), His132(-1.443), Ile131(-1.012), Thr118(-1.093), Gly117(-1.067), Pro116(-1.821), Tyr60(-1.182), Ser59(-2.448), Arg58(-1.03)	Lys418(-2.602), Asp389(-1.331), Asn385(-1.023), Arg58(-1.143),	Lys418 (2.03)	His132 (2.848)
6l	1.52	1	-8.491	-63.731	Lys418(-2.977), Ala417(-1.982), Tyr415(-1.951), Cys387(-2.98), Val365(-4.835), Leu363(-1.985), Gln336(-1.987), Gly321(-1.822), Leu317(-2.965), Pro316(-1.878), Tyr314(-1.836), Lys134(-1.914), Gly133(-1.926), His132(-3.127), Ile131(-1.794), Val121(-1.875), Thr118(-2.222), Gly117(-3.155), Pro116(-3.517), Tyr60(-1.939), Ser59(-1.894), Arg58(-2.145),	Lys418(-7.602), Asp389(-1.878), Asn385(-1.113), Lys134(-1.561), Cys129(-1.41), Arg119(-1.121)	Lys418 (2.27), Tyr60 (1.83)	His132 (2.763)
6n	2.27	1	-8.105	-56.489	Lys418(-2.927), Tyr415(-1.297), Cys387(-2.264), Asn385(-1.372), Phe369(-1.147), Lys367(-1.973), Val365(-3.325), Leu363(-1.669), Gln336(-1.983), Asn324(-1.272), Gly321(-1.642), Phe320(-1.914), Leu317(-2.527), Trp230(-1.055), His132(-2.597), Val121(-1.025), Thr118(-2.11), Gly117(-2.663), Pro116(-2.216), Tyr60(-1.914), Arg58(-1.217)	Lys418(-5.973), Asp318(-1.536), Gly117(-1.742), Pro116(-1.692),	Lys418 (2.22),	His132 (2.848)
6q	2.04	0.37	-8.249	-60.305	Lys418(-3.818), Ala417(-1.954), Tyr415(-1.855), Cys387(-2.561), Asn385(-1.52), Phe369(-1.353), Lys367(-2.102), Phe366(-1.248), Val365(-4.288), Leu363(-1.853), Gln336(-1.955), Gly321(-1.782), Phe320(-2.116), Leu317(-2.112), His132(-3.148), Thr118(-2.134), Gly117(-3.069), Pro116(-2.567), Tyr60(-1.977), Arg58(-2.117)	Glu322(-1.922), Leu317(-3.347), Gly117(-3.043), Pro116(-1.389), Tyr60(-0.633)	Leu317 (1.88), Arg58(2.46)	His132 (2.886)

6r	0.22	0.12	-7.972	-51.088	Lys418(-1.482), Tyr415(-1.133), Cys387(-2.306), Phe369(-1.182), Lys367(-2.078), Val365(-2.769), Leu363(-1.205), Gln336(-1.249), Gly321(-1.531), Phe320(-1.437), Leu317(-1.24), Pro316(-1.006), Ser228(-1.201), Lys134(-1.585), His132(-1.729), Ile131(-1.273), Val121(-1.035), Thr118(-1.122), Gly117(-1.23), Pro116(-2.094), Tyr60(-1.195), Arg58(-1.034)	Lys418(-1.717), Tyr415(-0.727), Asp389(-1.468), Glu322(-1.325), Asp318(-1.447), Lys134(-0.908)	Lys418 (1.92),	His132 (3.124)
6s	6.25	2.56	-7.982	-54.143	Lys418(-2.135), Tyr415(-1.167), Asp389(-1.62), Cys387(-2.531), 386(-1.372), Asn385(-1.296), Phe369(-1.197), Lys367(-1.681), Phe366(-1.331), Val365(-2.791), Leu363(-1.45), Gln336(-1.296), Gln334(-1.138), Asn324(-1.265), Gly321(-1.571), Phe320(-1.727), Leu317(-2.187), His132(-2.023), Ile131(-1.849), Val121(-1.024), Thr118(-1.264), Gly117(-1.008), Pro116(-2.191), Tyr60(-1.551), Arg58(-1.074)	Tyr415(-0.881), Glu322(-1.191), Asp318(-1.312), Leu317(-2.277), Gly117(-1.138), Pro116(-1.41), Tyr60(-0.658)	Leu317 (2.19), Arg58(2.42)	His132 (2.833)

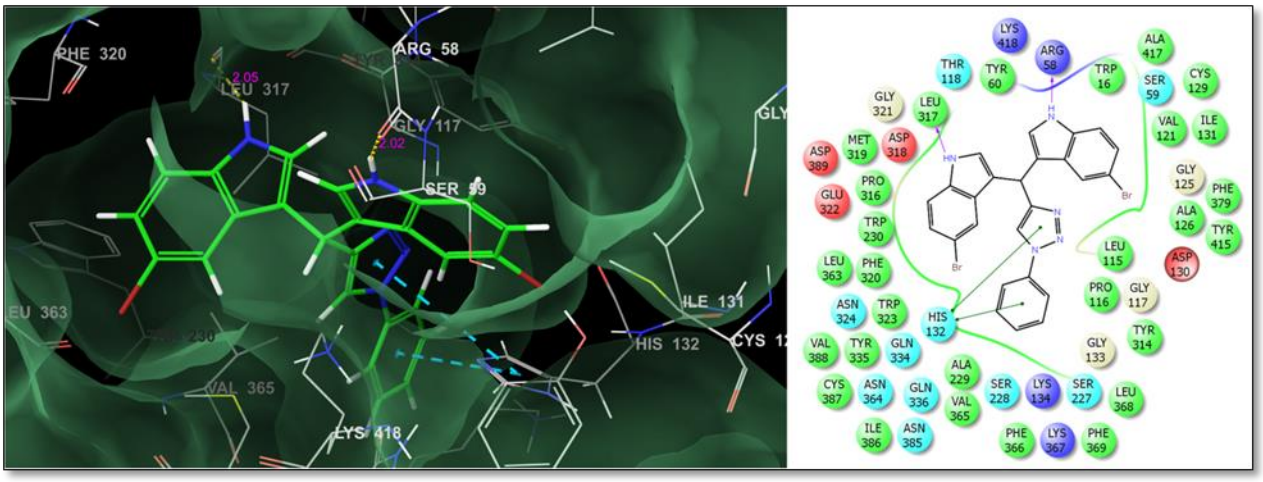


Figure S1: Binding mode of **6b** into the active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions).

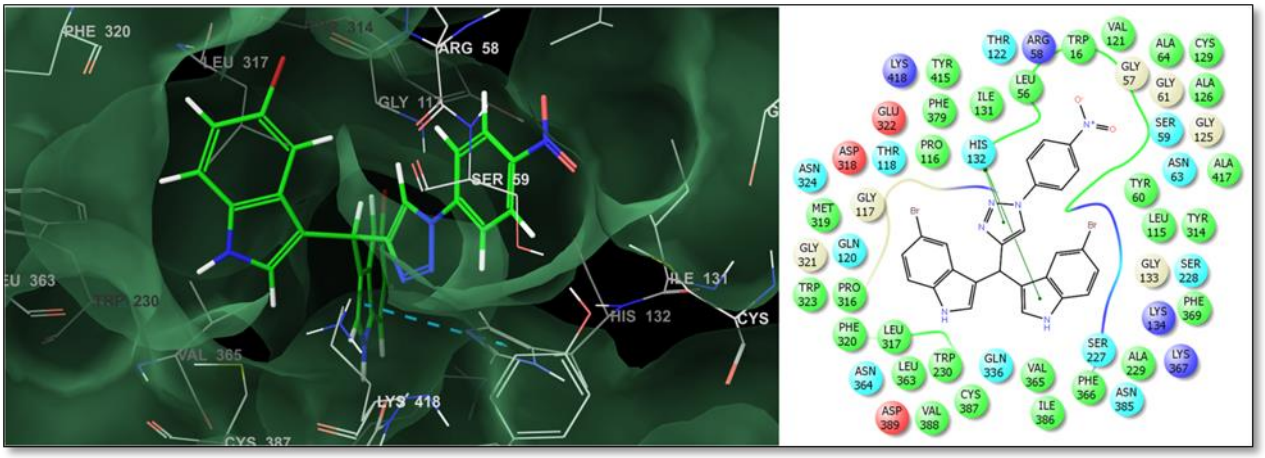


Figure S2: Binding mode of **6f** into the active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions).

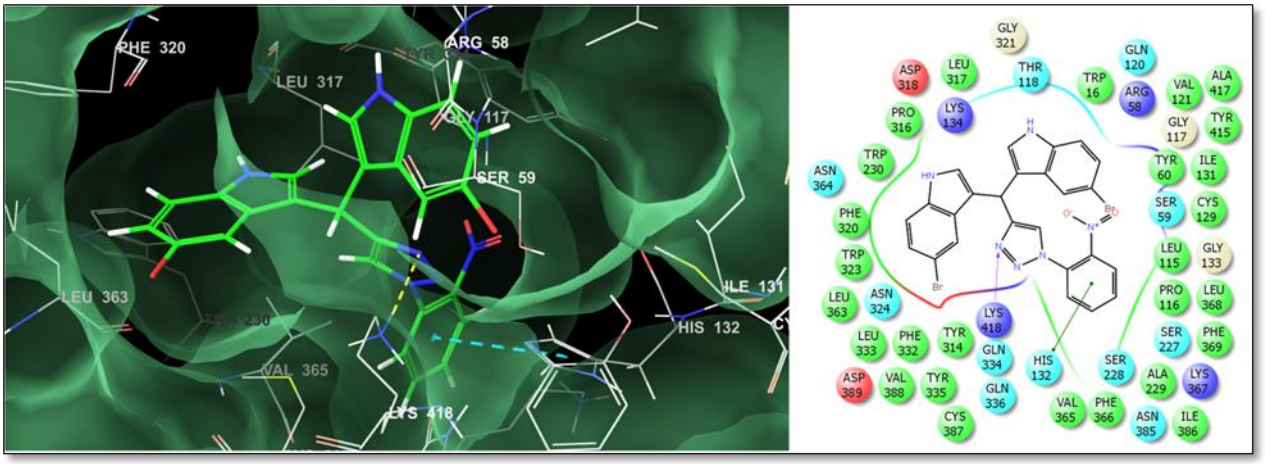


Figure S3: Binding mode of **6n** into the active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions).

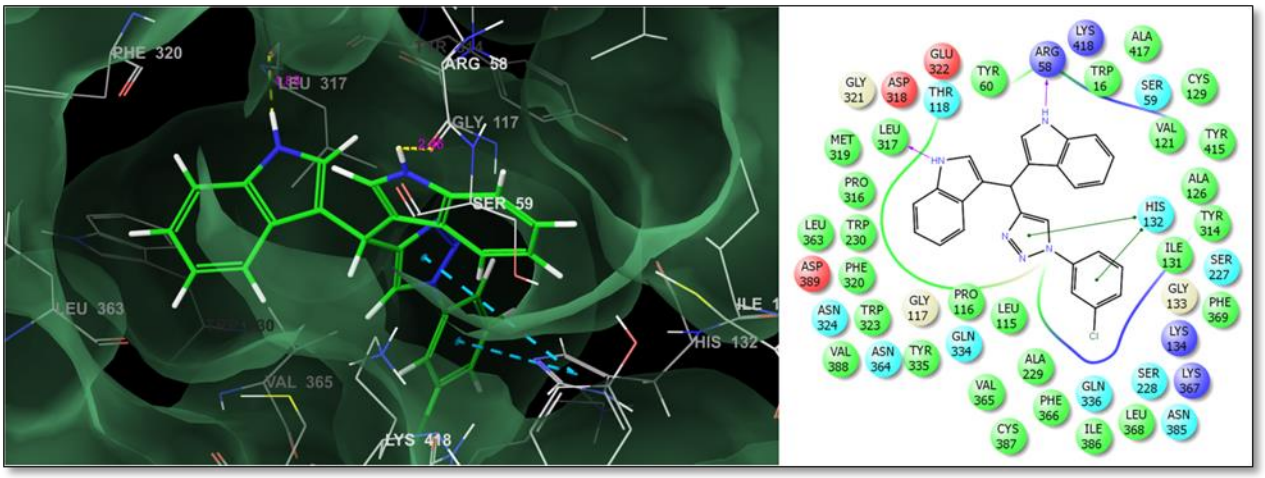


Figure S4: Binding mode of **6q** into the active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions).

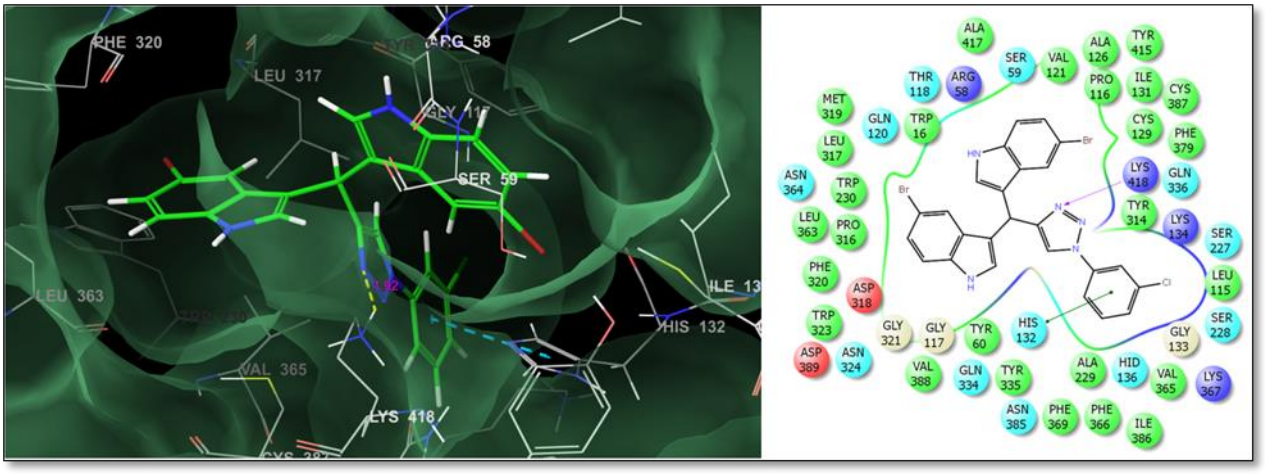


Figure S5: Binding mode of **6r** into the active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions).

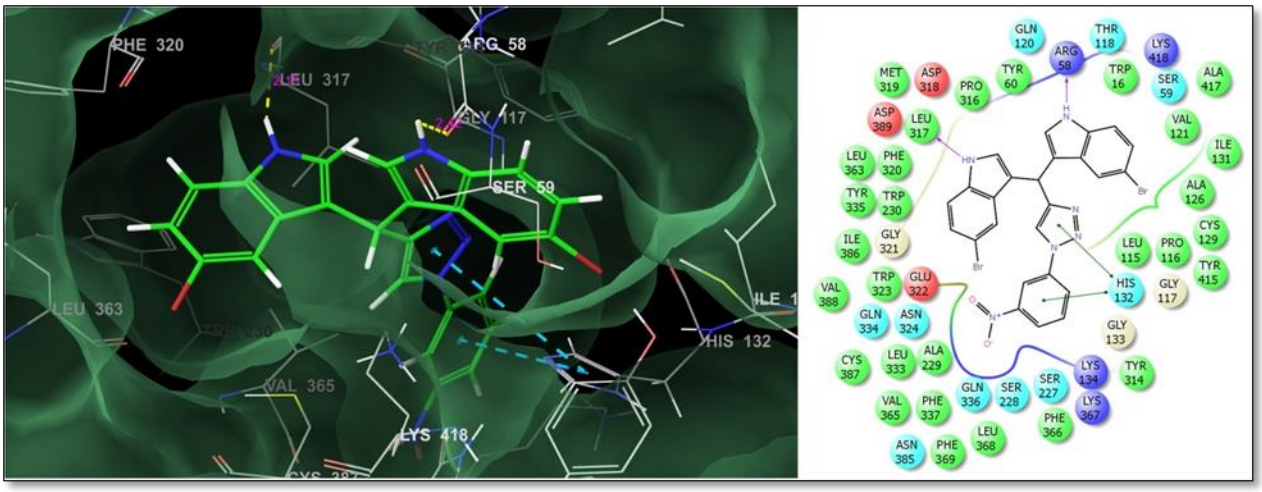
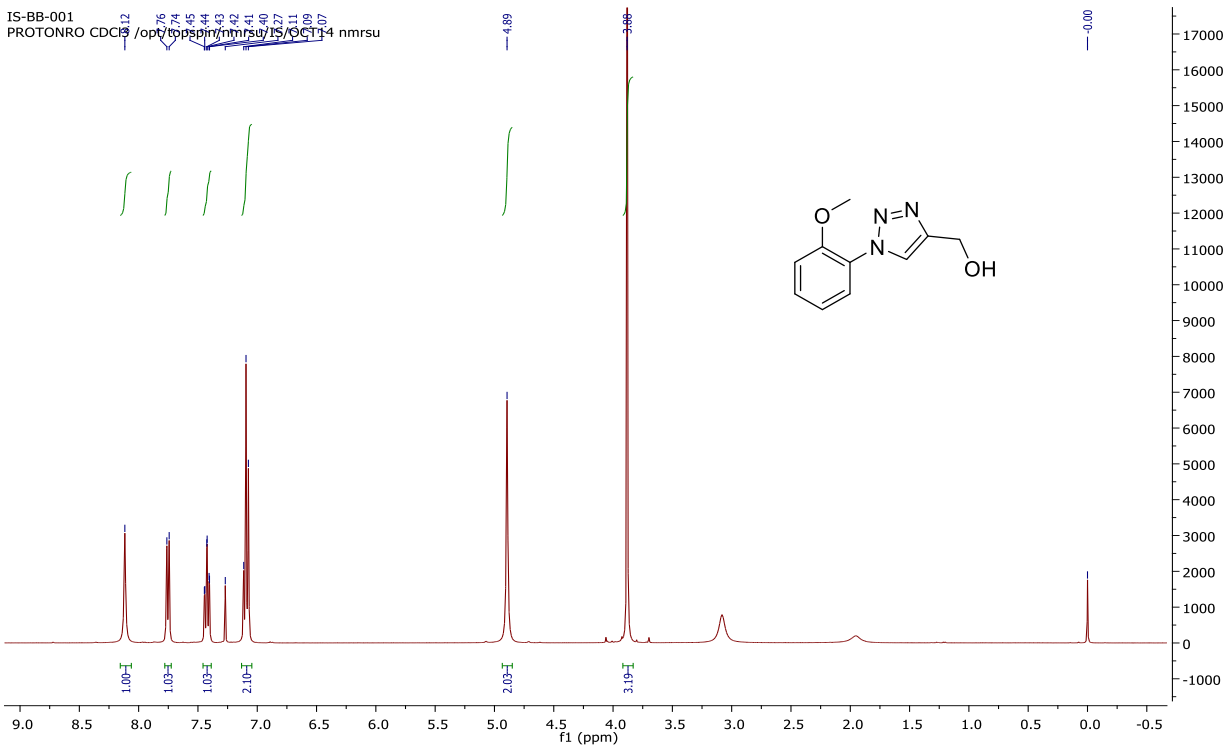


Figure S6: Binding mode of **6s** into the active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions).

The IR, ^{1}H NMR, ^{13}C NMR, DEPT NMR and Mass spectra of target compounds.

3h. ^{1}H NMR, 400 MHz, $CDCl_3$



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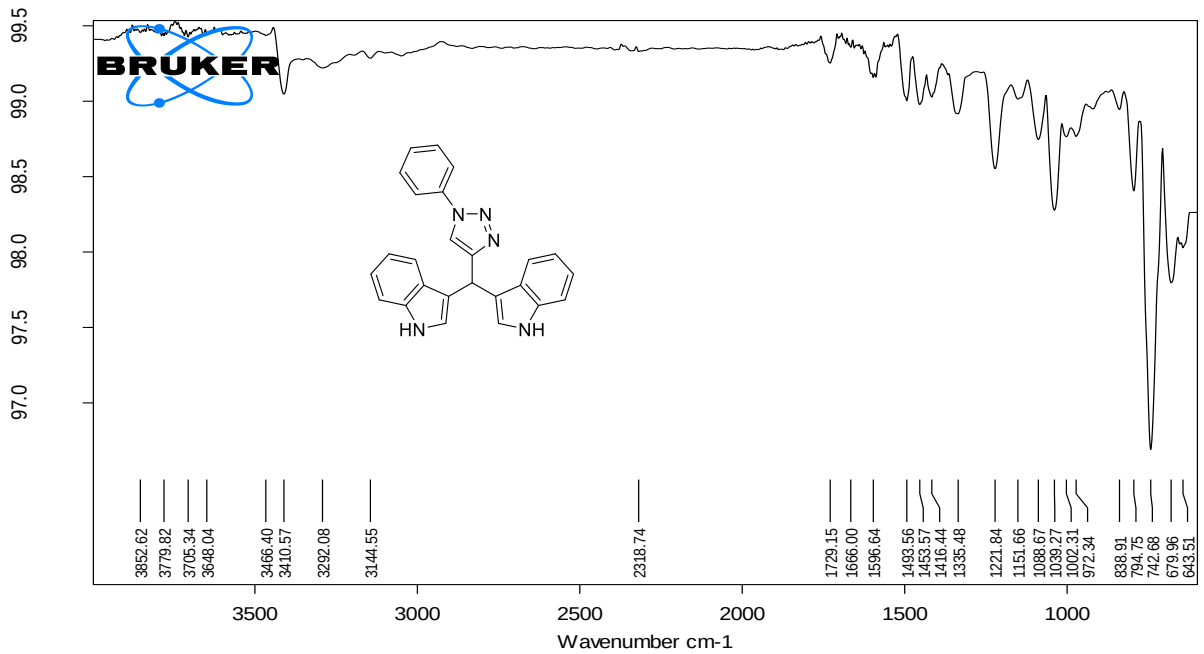

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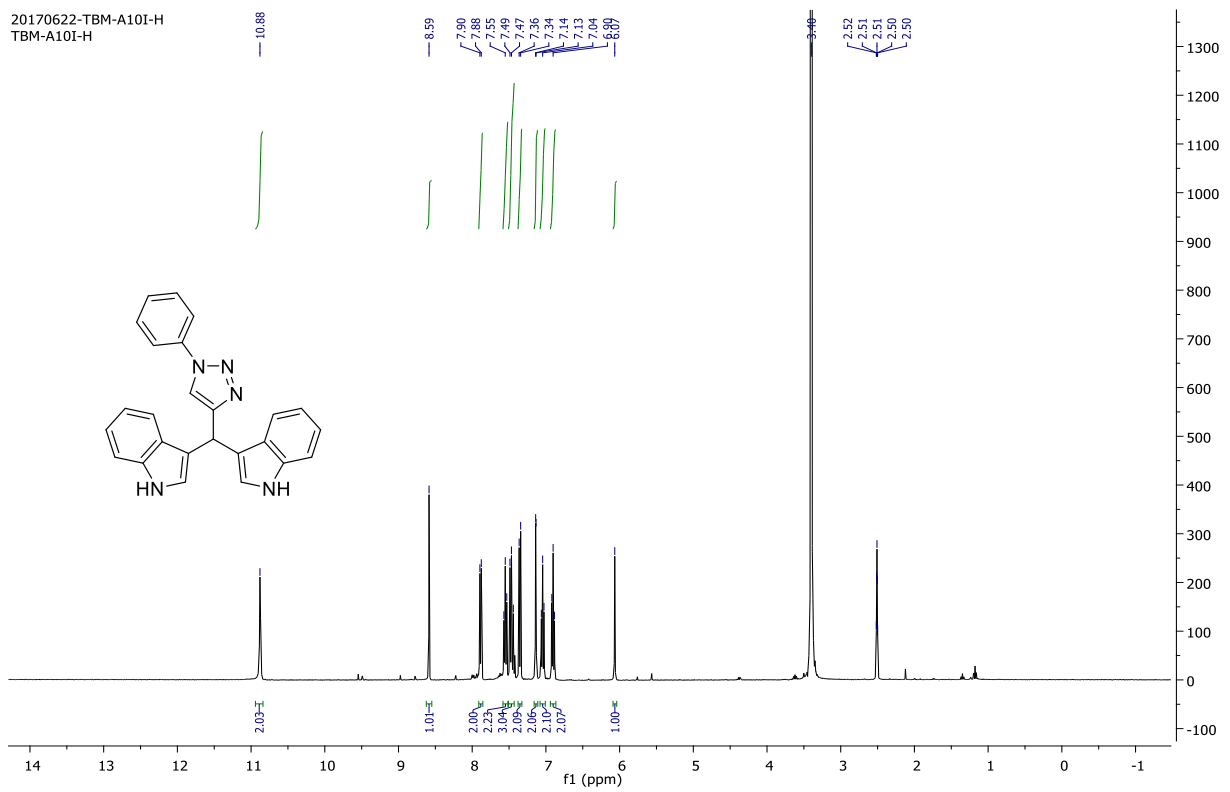
6a. ^{1}H NMR, 400 MHz, DMSO-d_6



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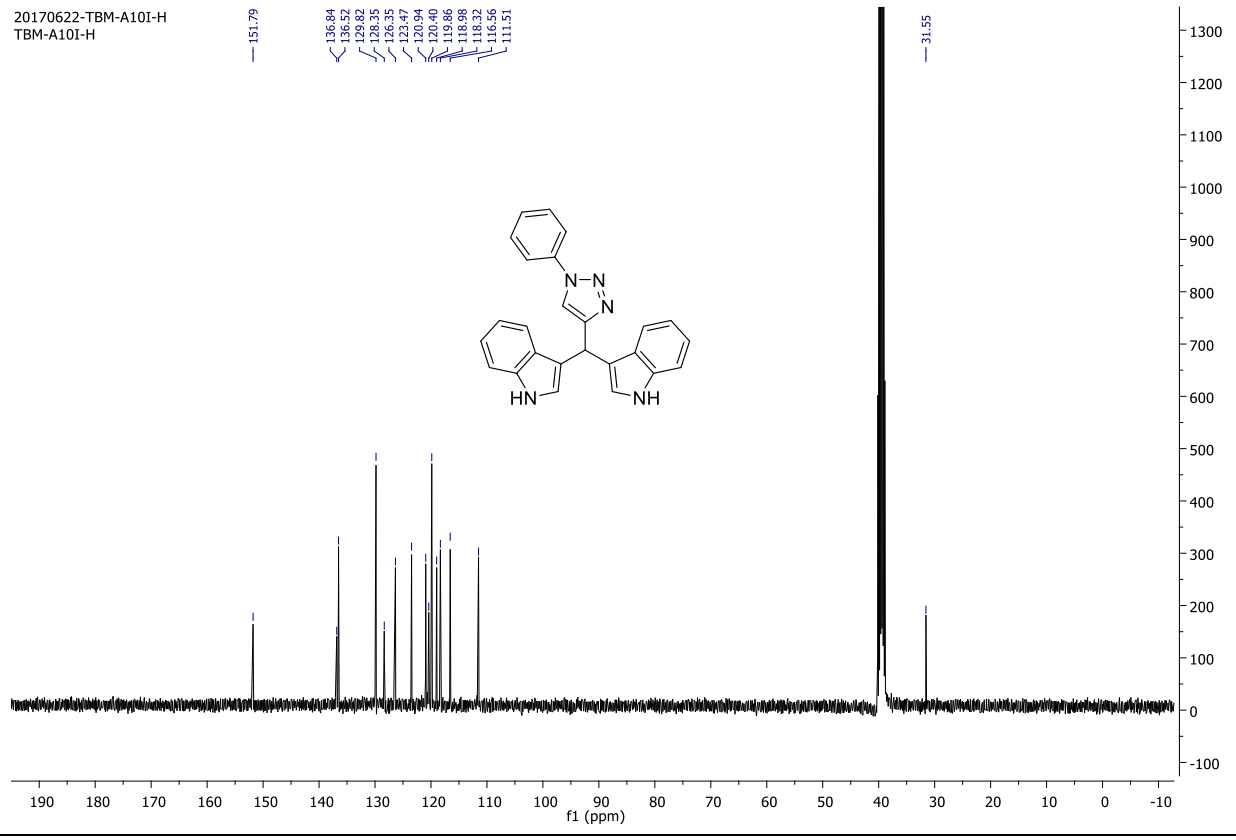

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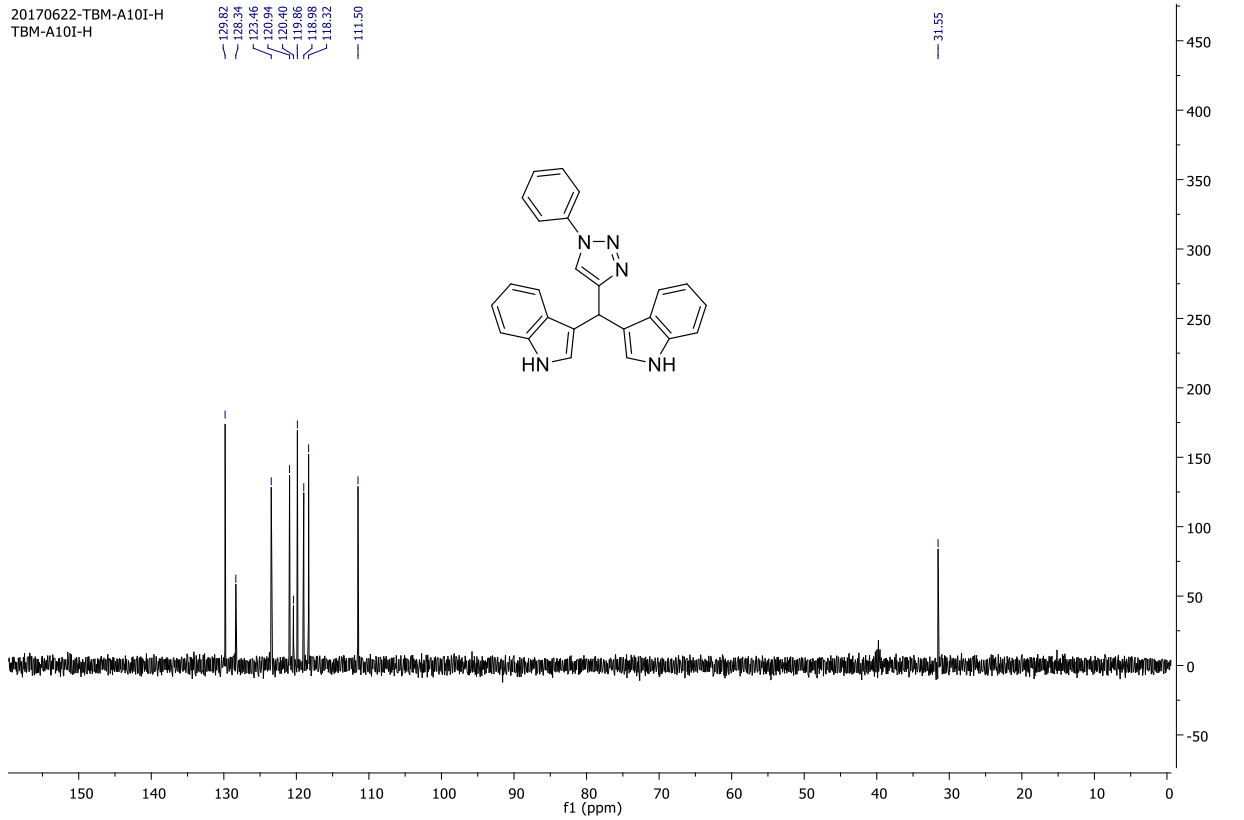

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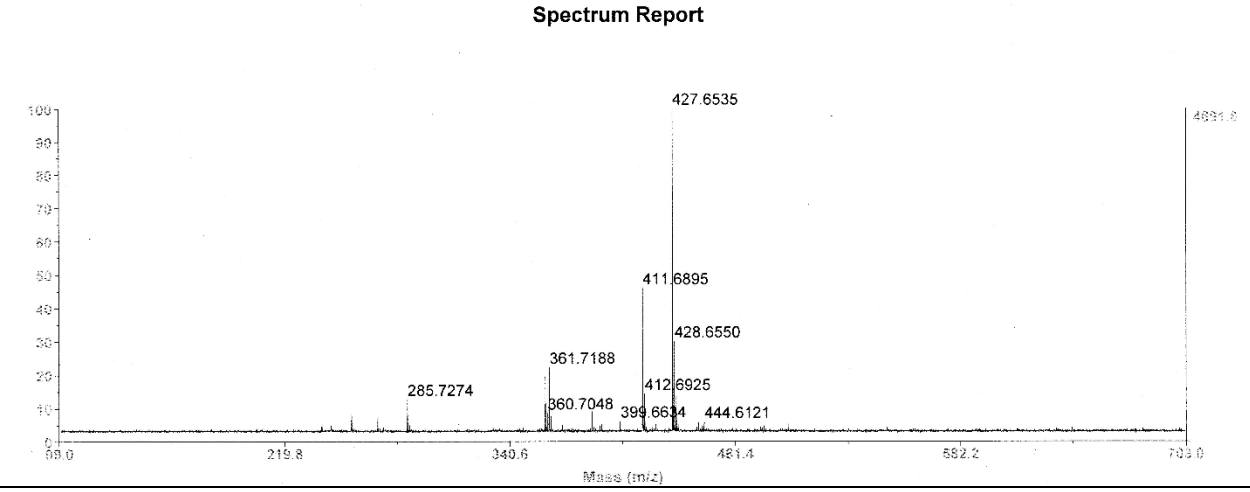
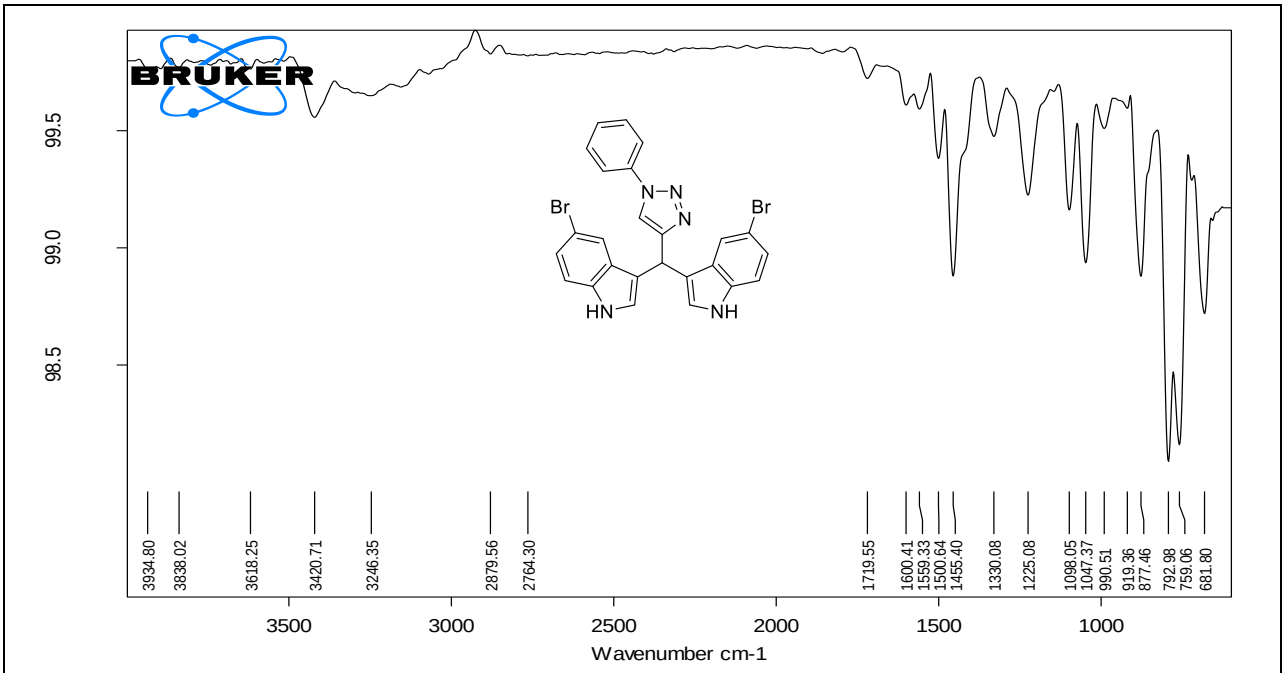


6b. FTIR



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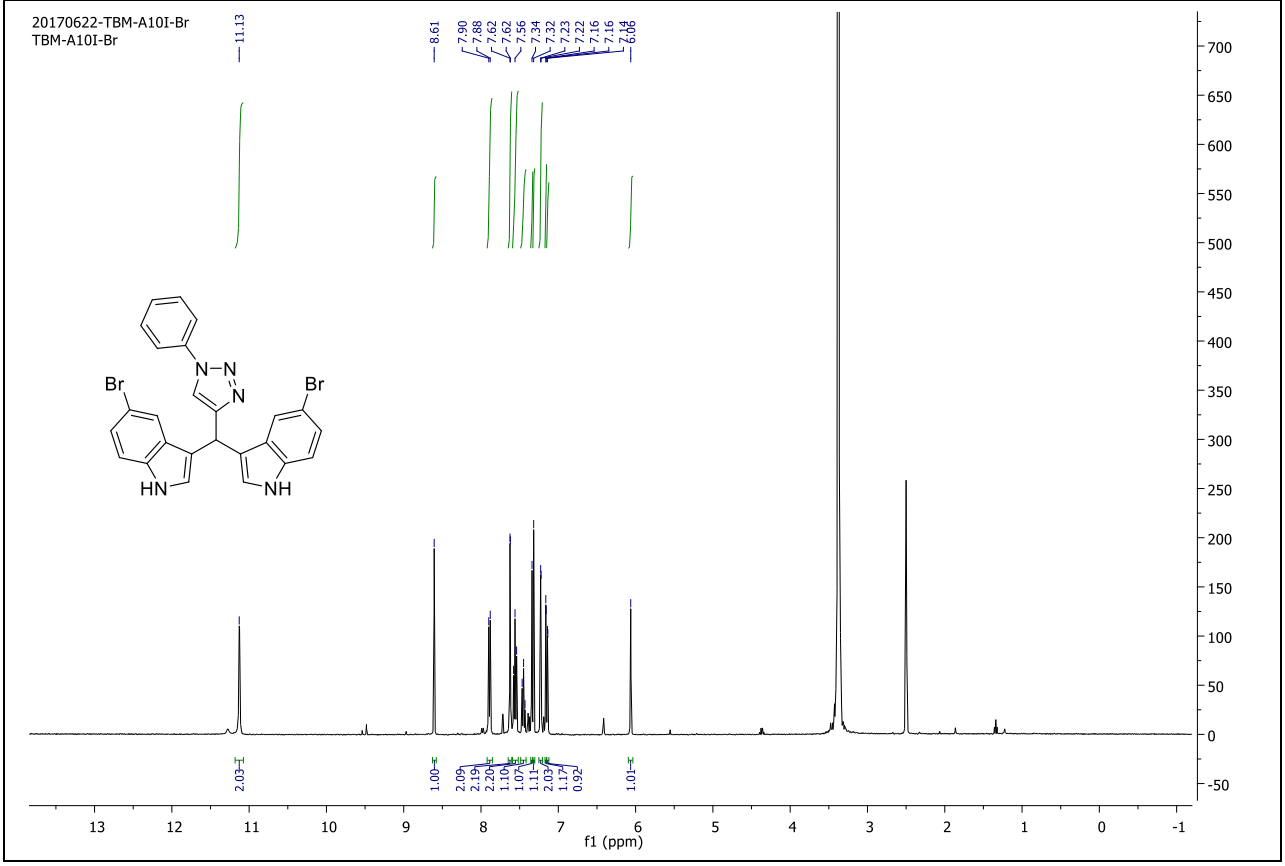
6b. ^{1}H NMR, 400 MHz, DMSO-d_6



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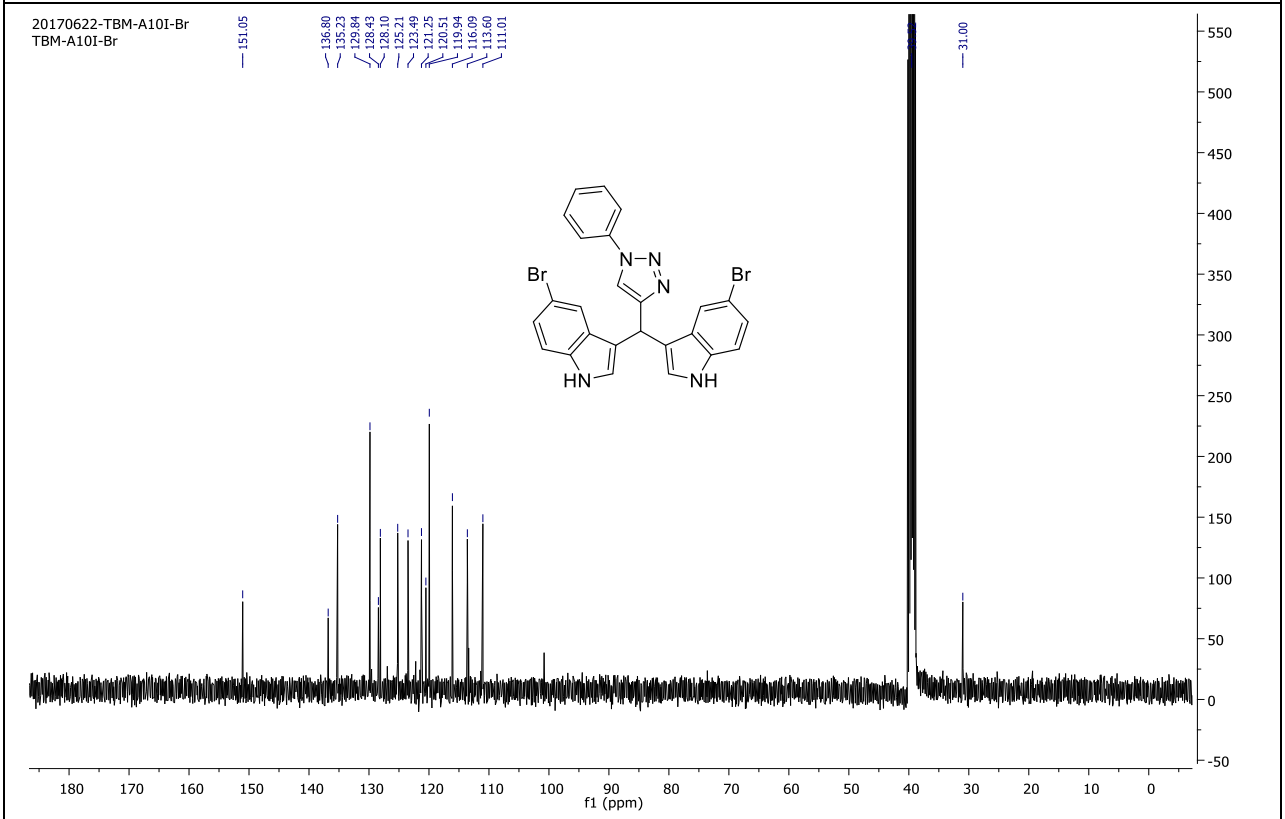

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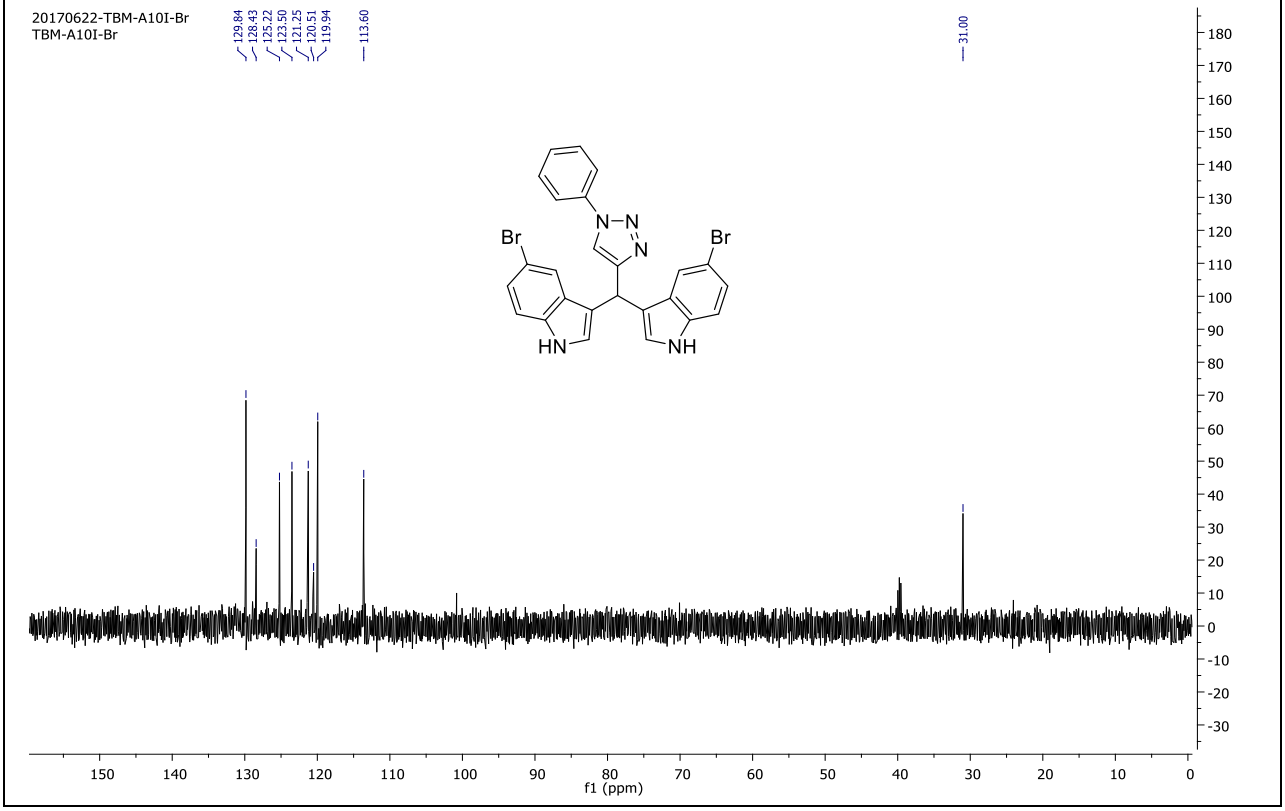

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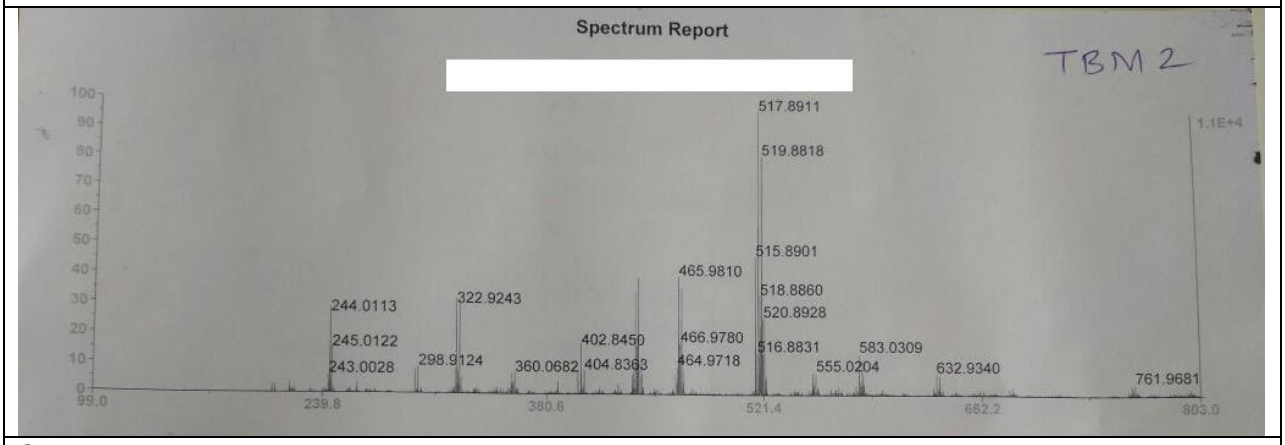
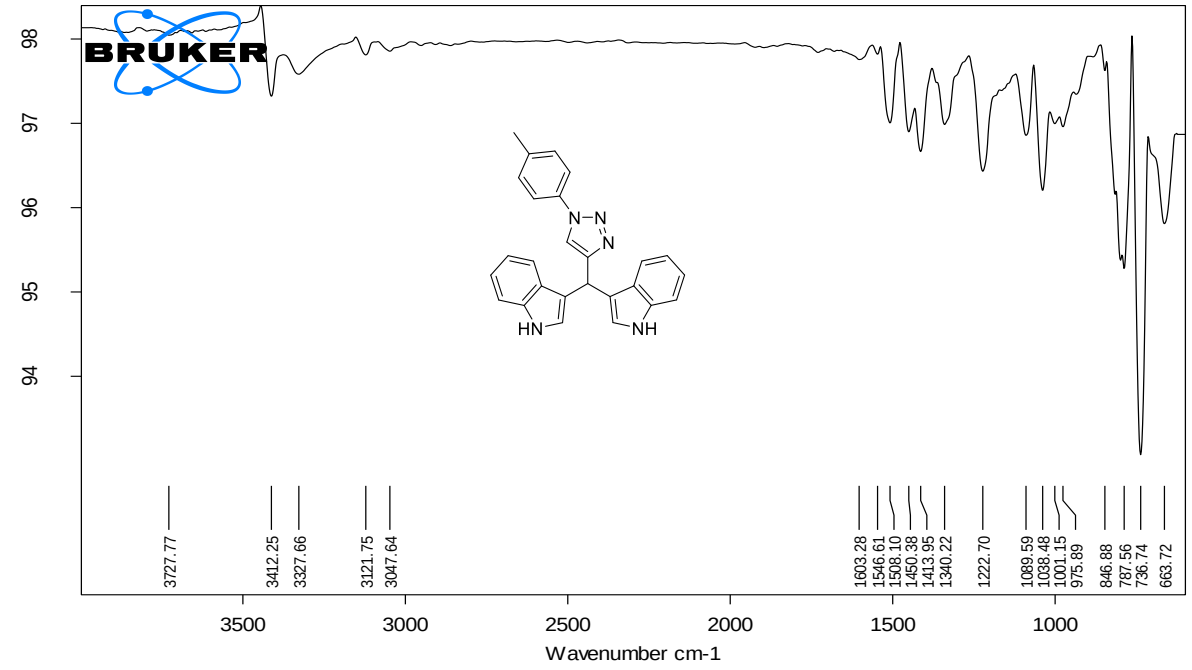


6c. FTIR



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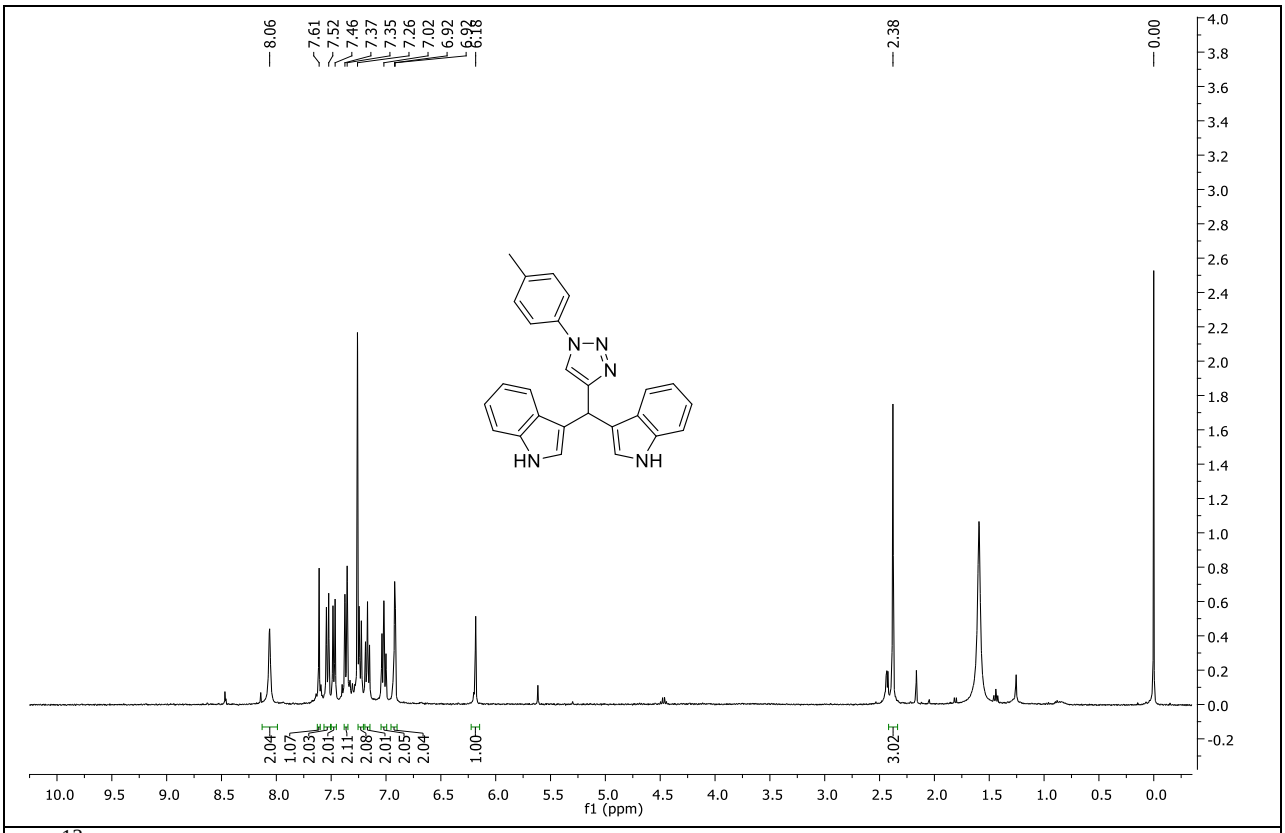
6c. ^{1}H NMR, 400 MHz, $CDCl_3$



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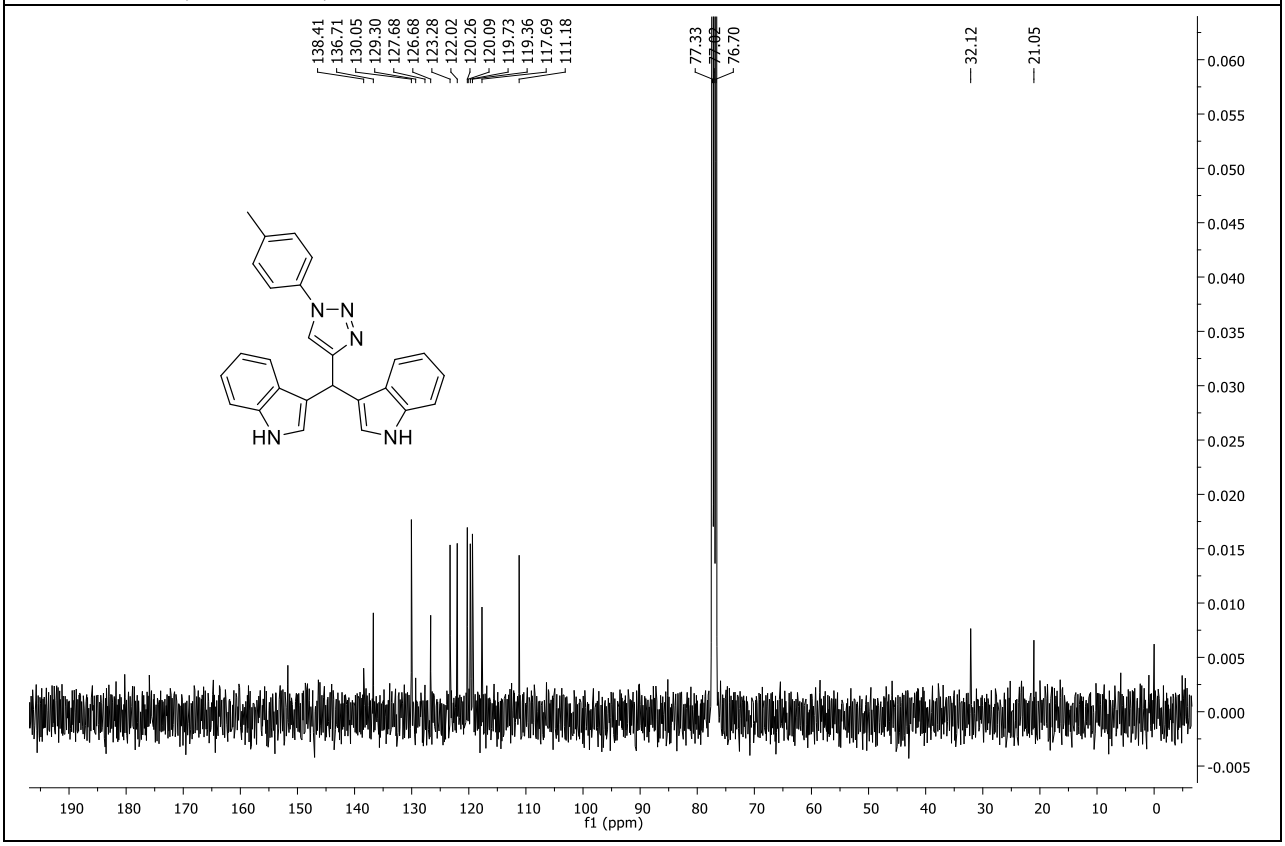

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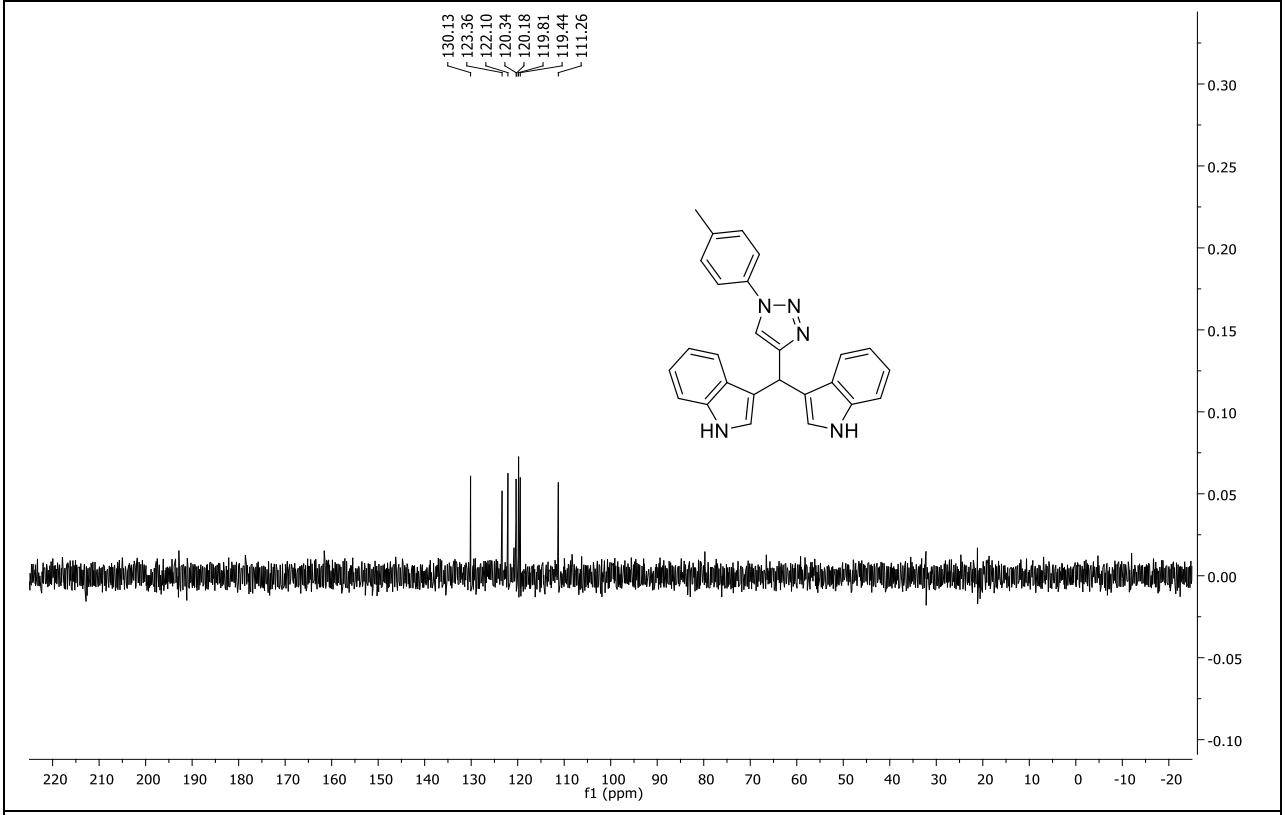
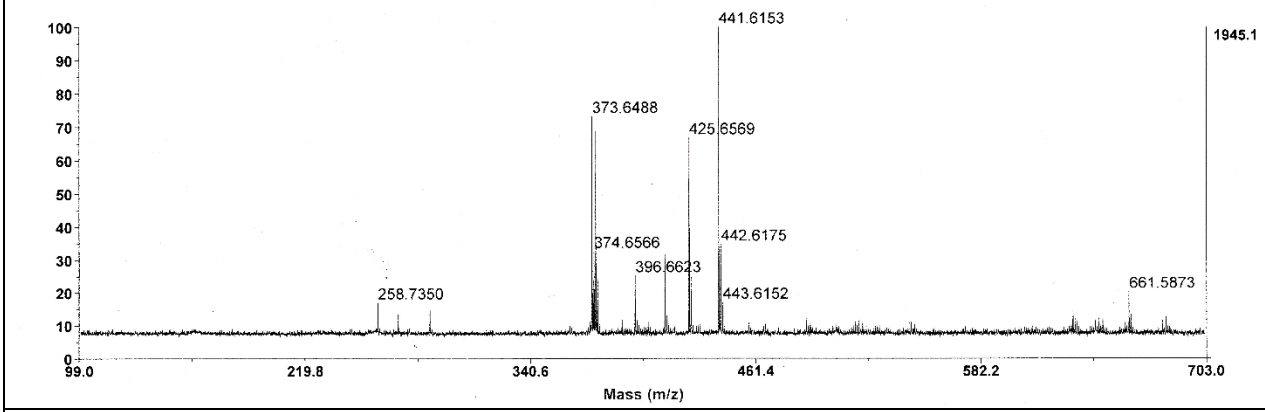


6c. Mass



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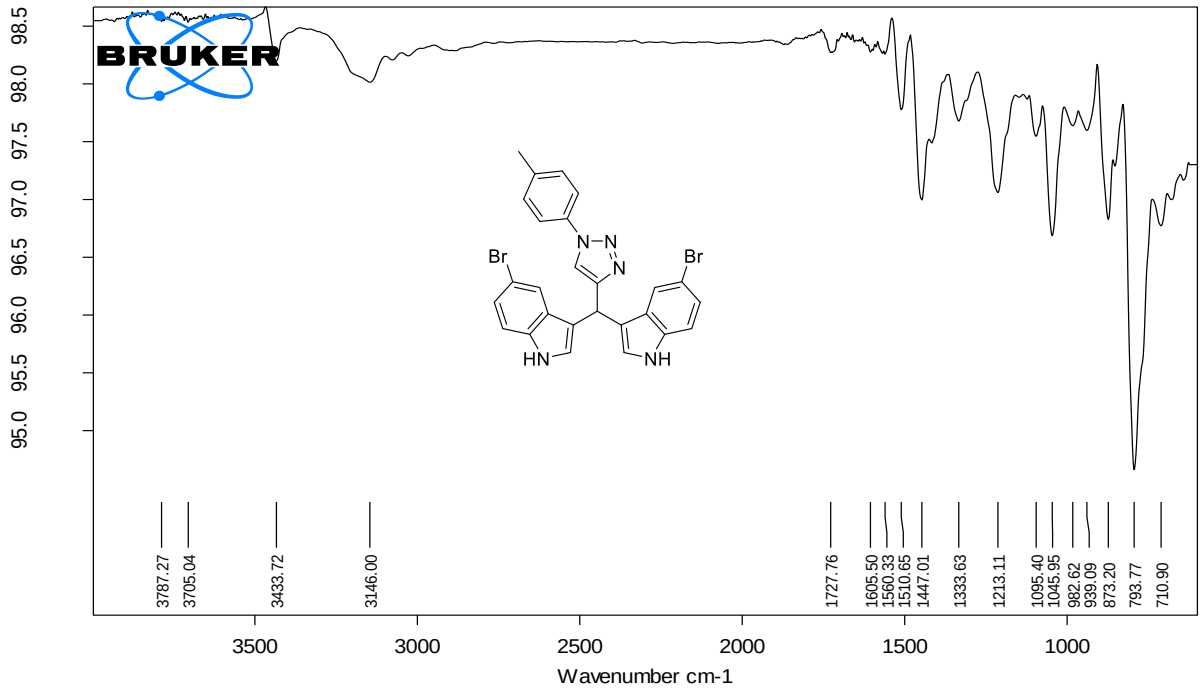

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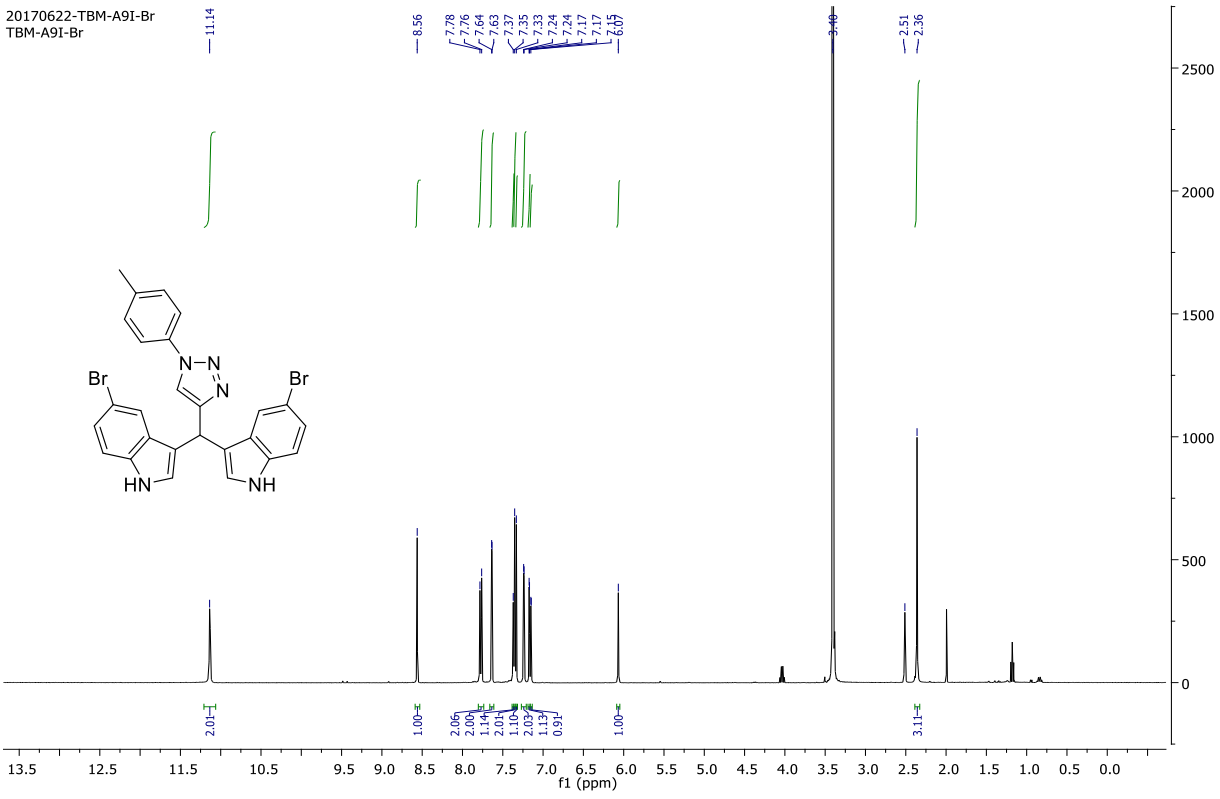
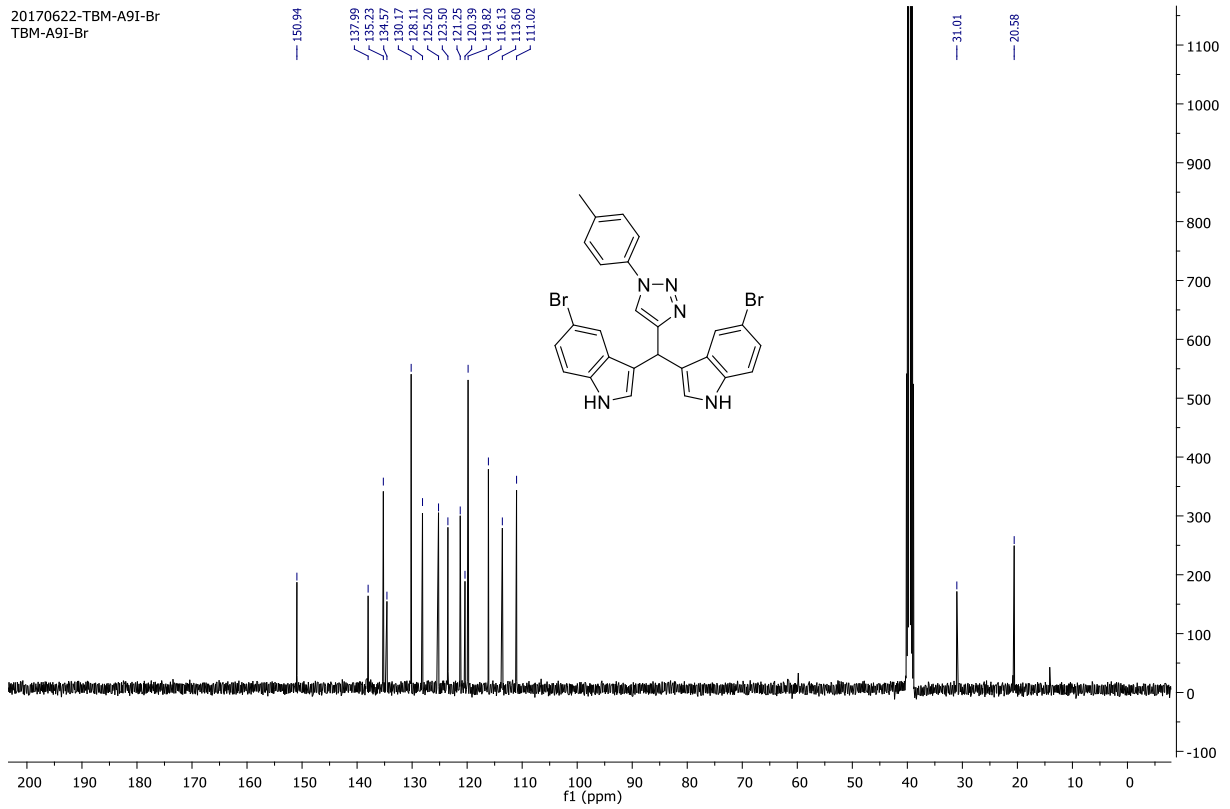
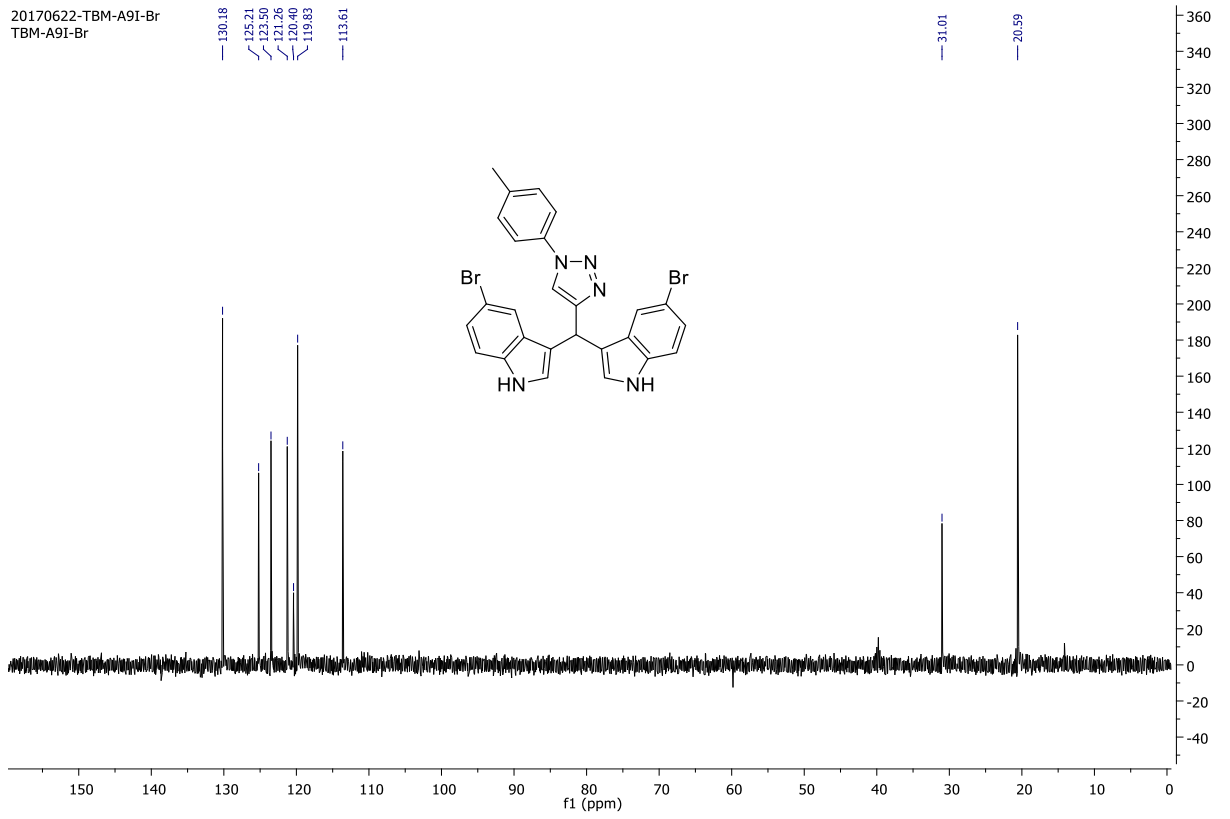
6d. ^{1}H NMR, 400 MHz, DMSO-d_6



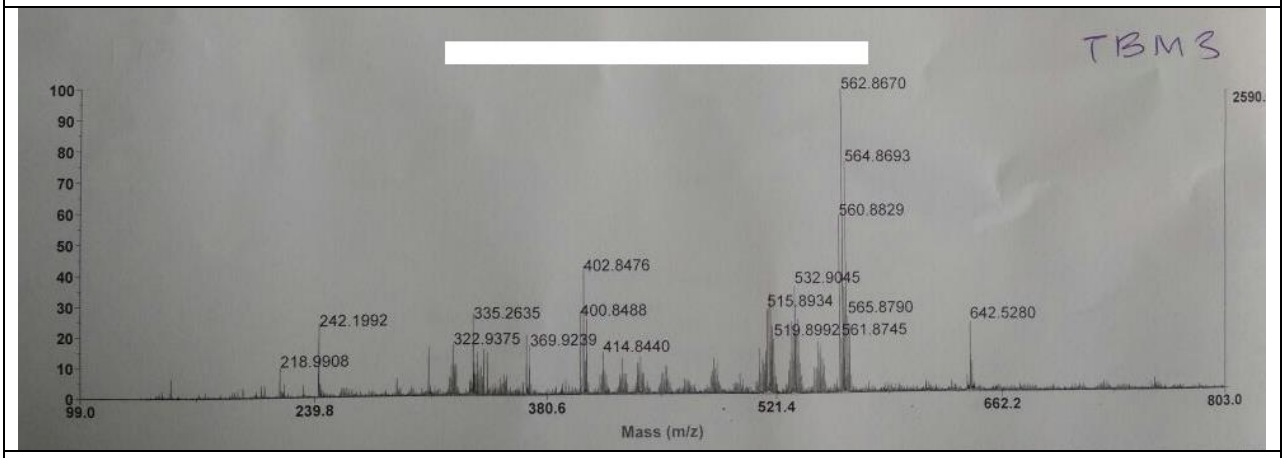
6d. ^{13}C NMR, 100 MHz, DMSO-d_6



6d. DEPT NMR, 100 MHz, DMSO-d_6



6d. Mass



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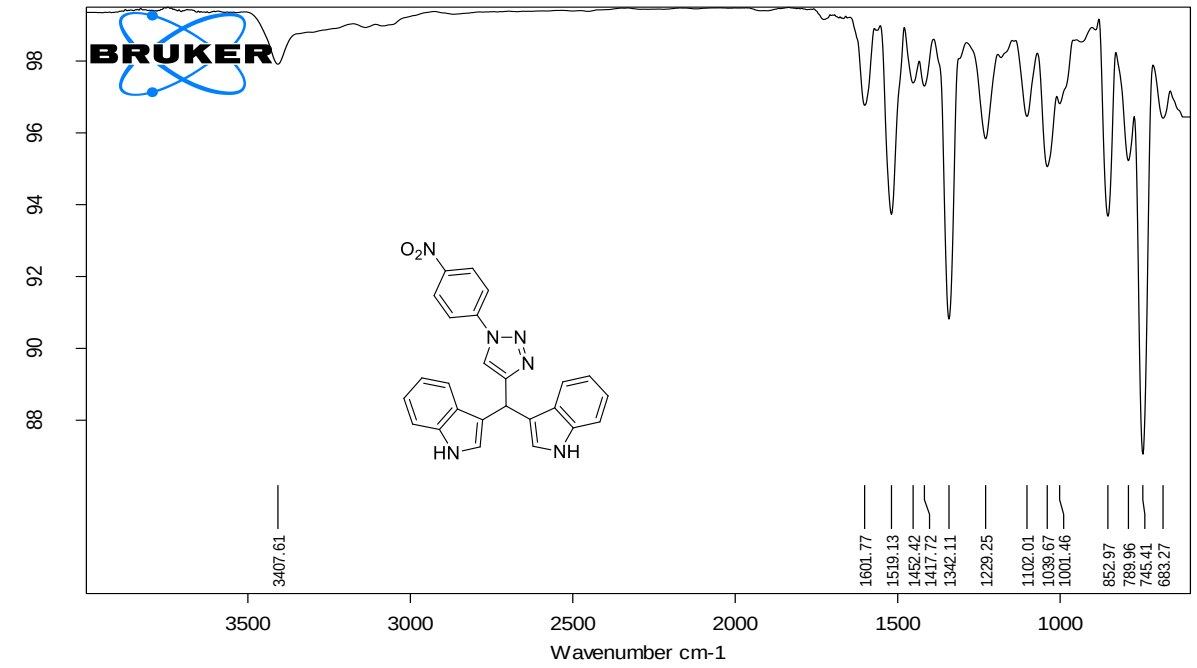

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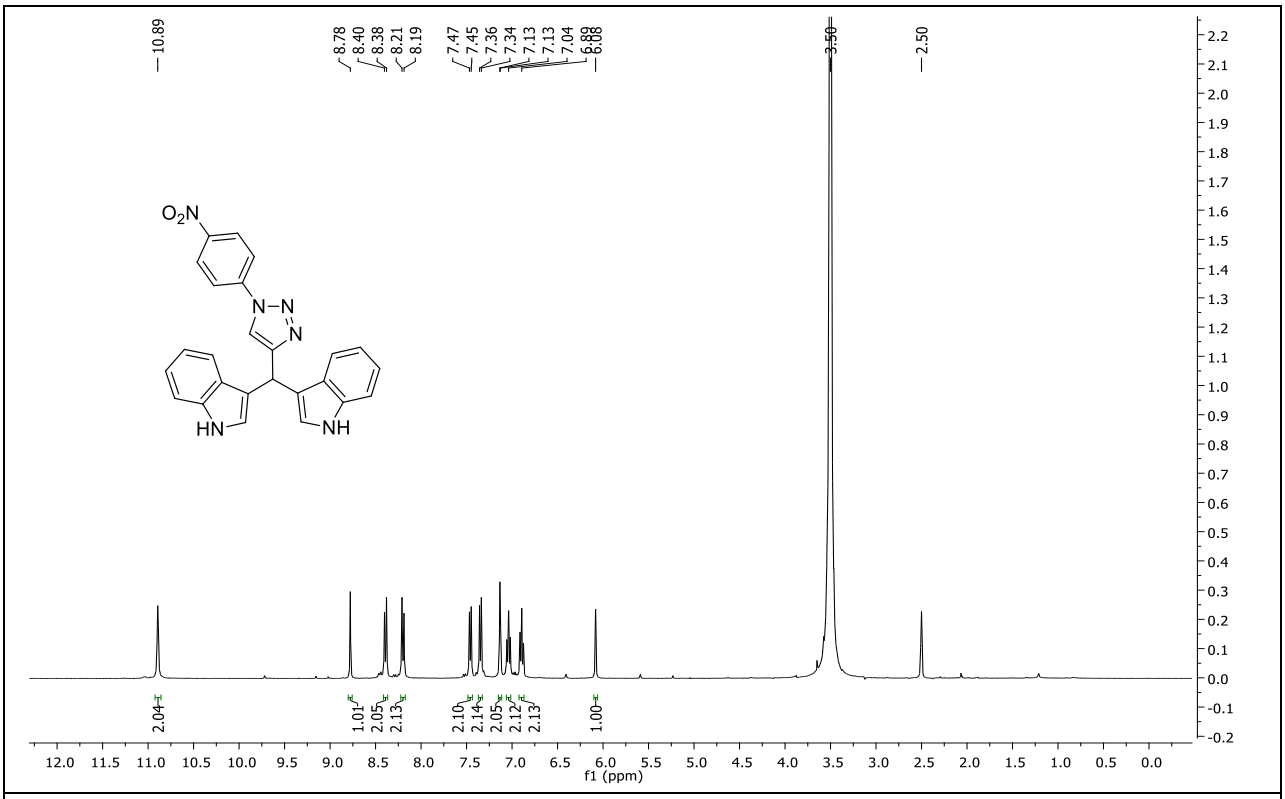
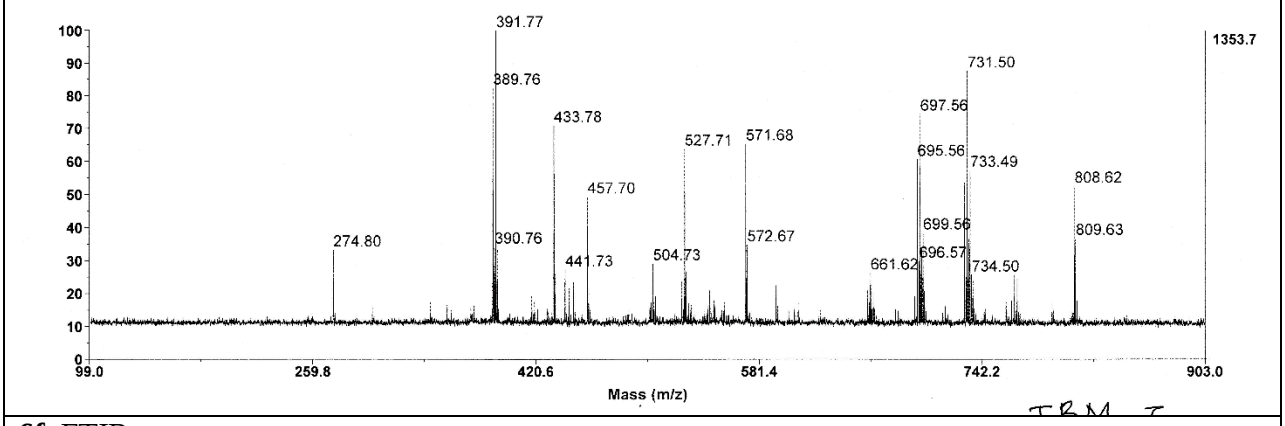
6e. ^{1}H NMR, 400 MHz, DMSO-d_6



6e. Mass



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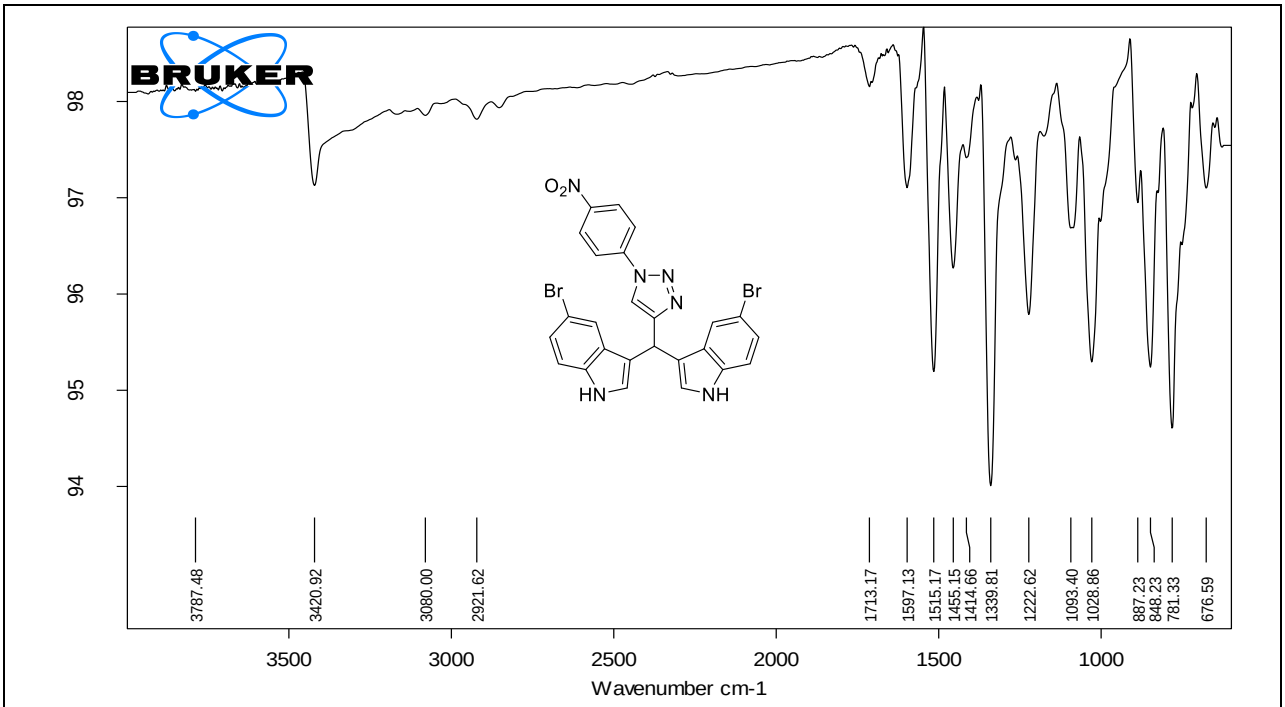

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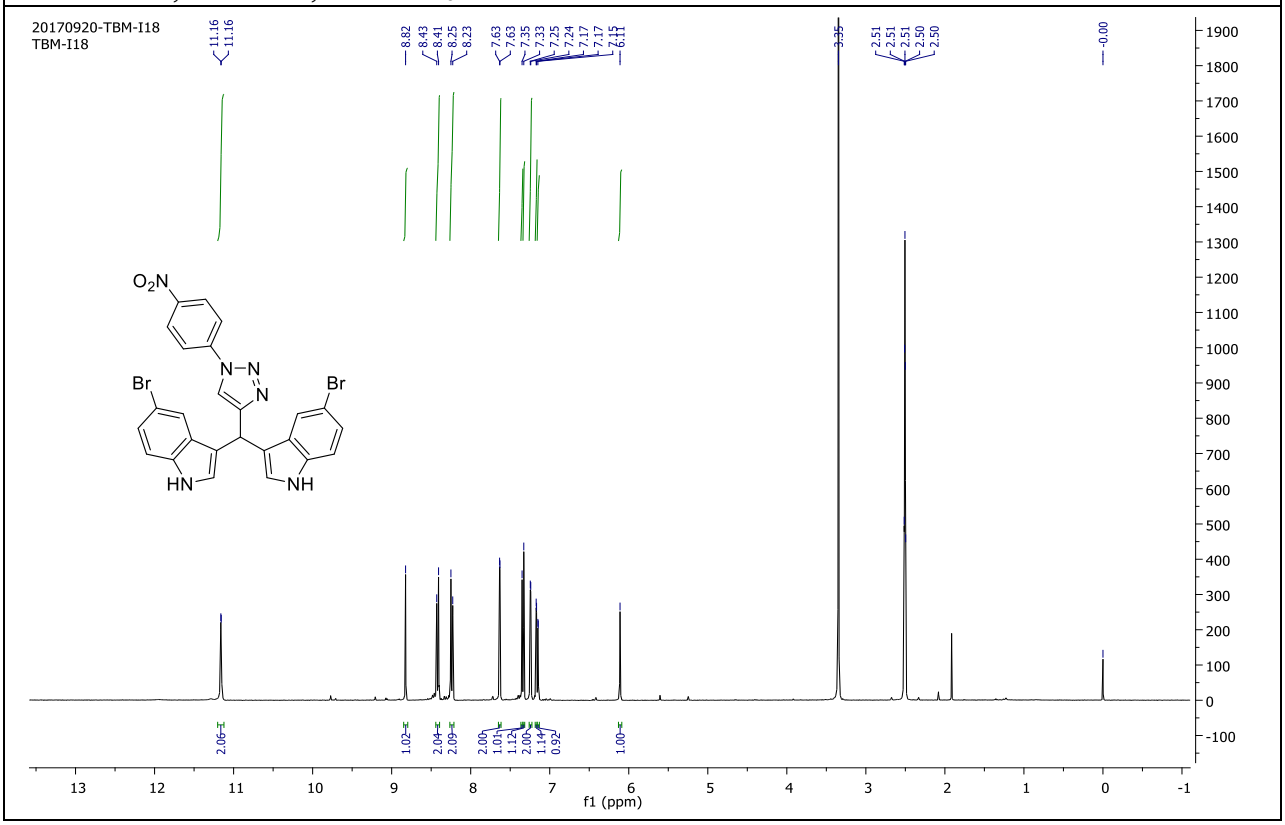
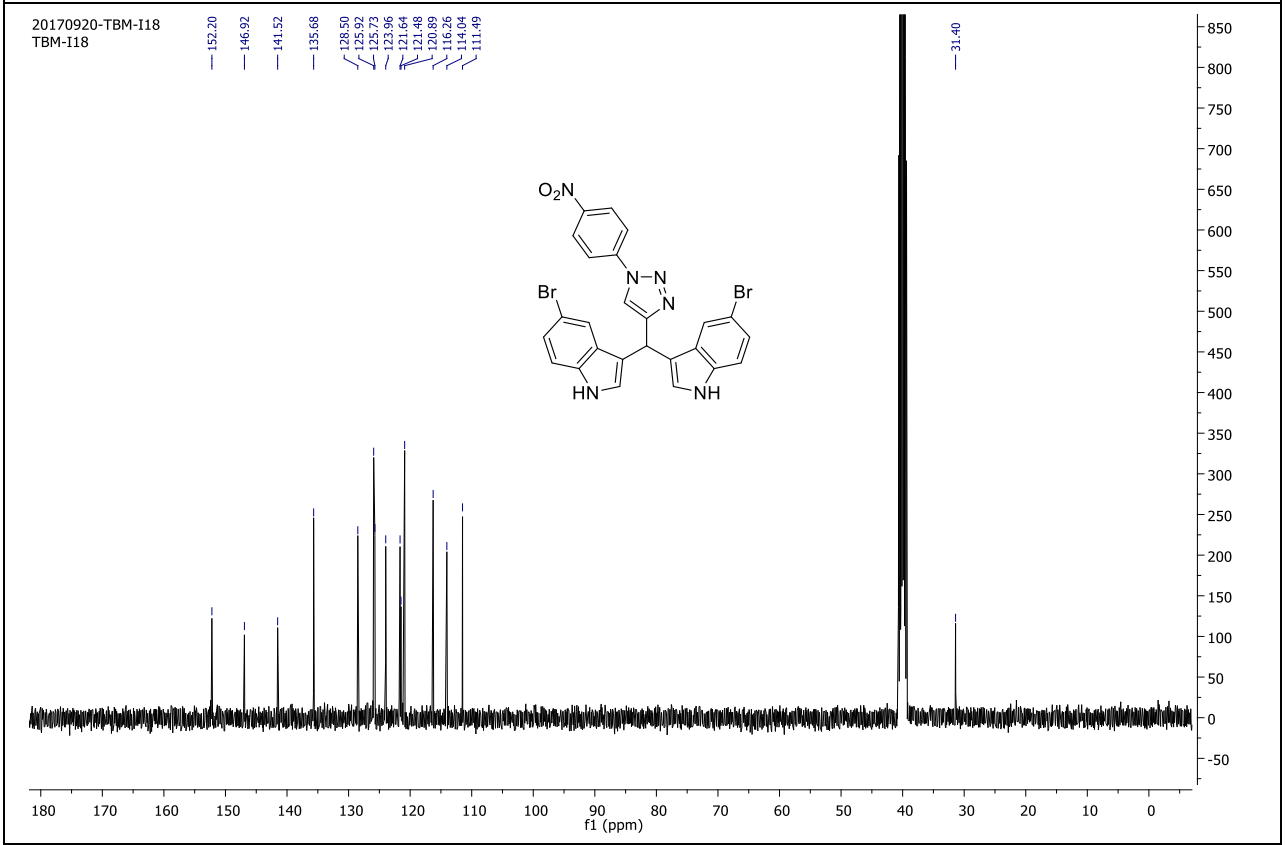
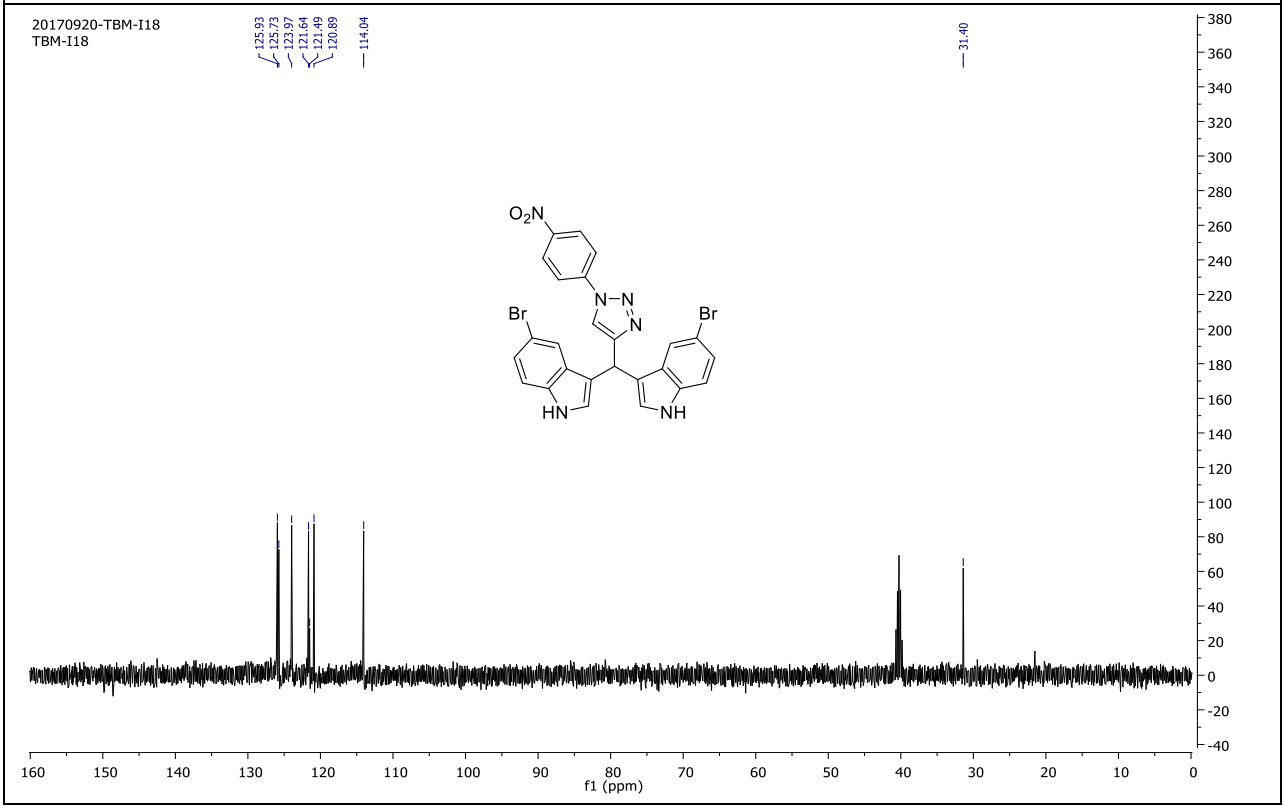
6f. ^{1}H NMR, 400 MHz, DMSO-d_6



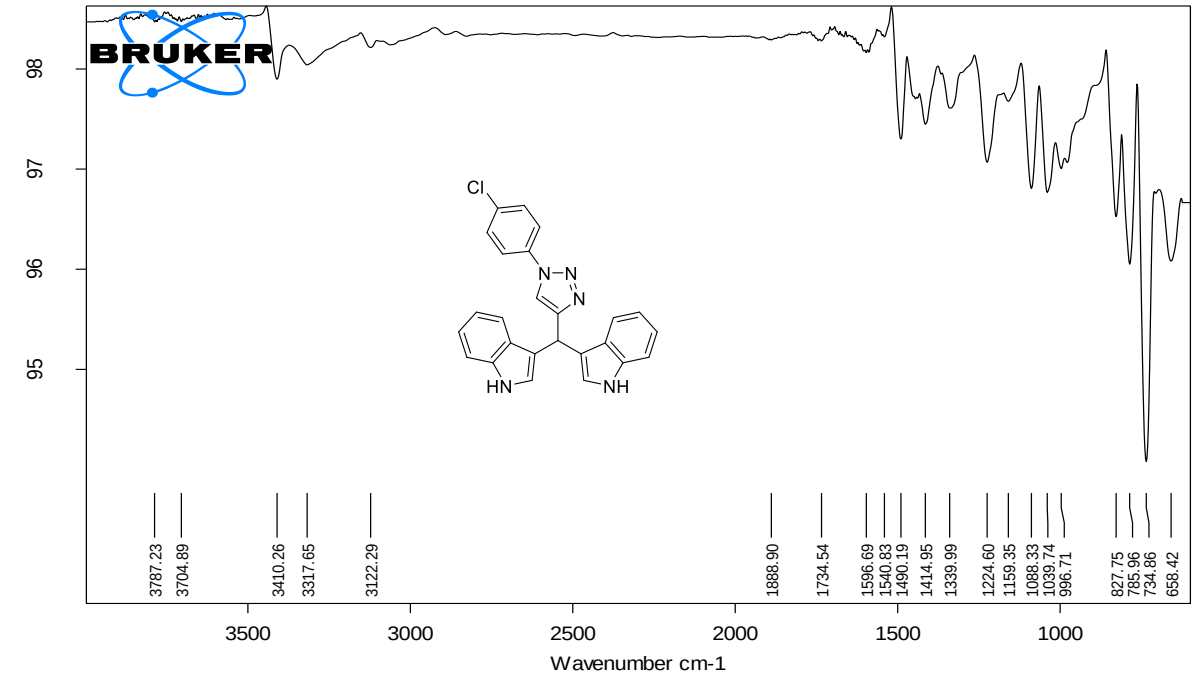
6f. ^{13}C NMR, 100 MHz, DMSO-d_6



6f. DEPT NMR, 100 MHz, DMSO-d_6



6g. FTIR



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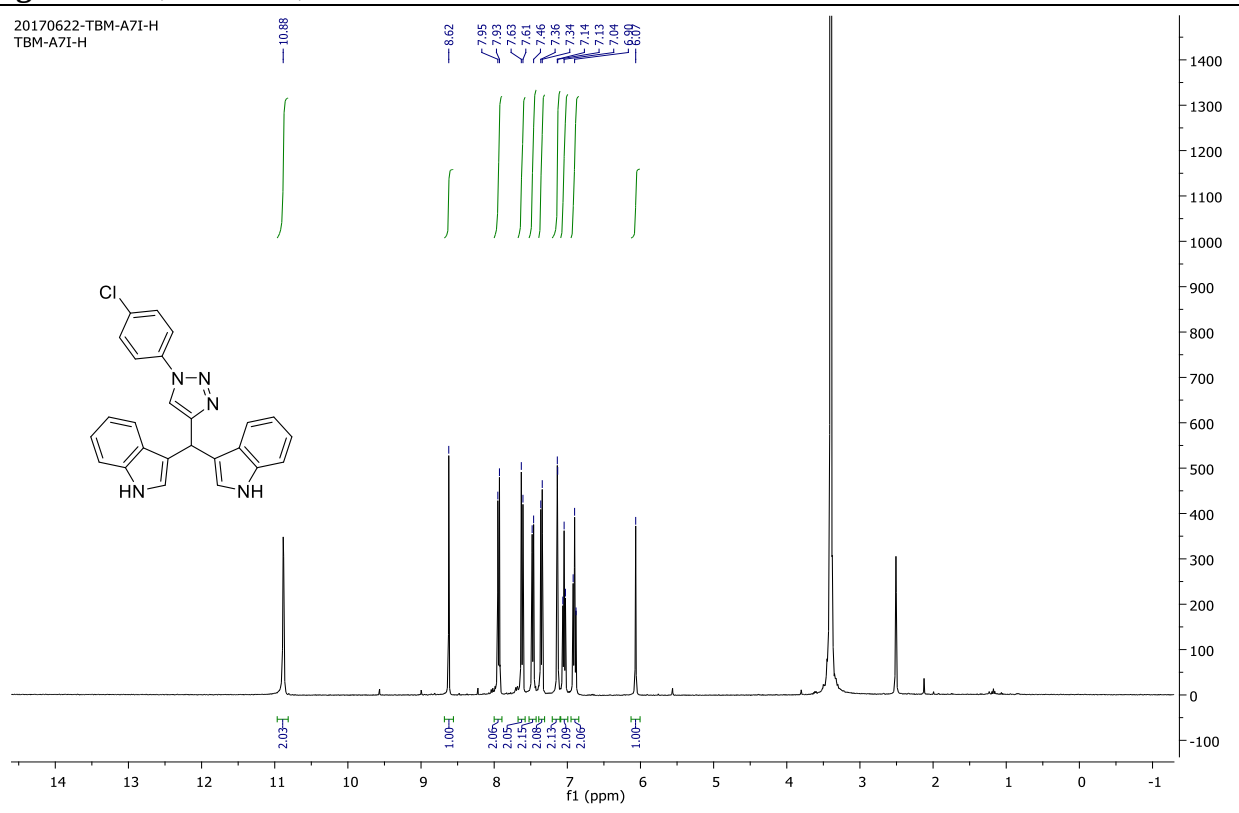
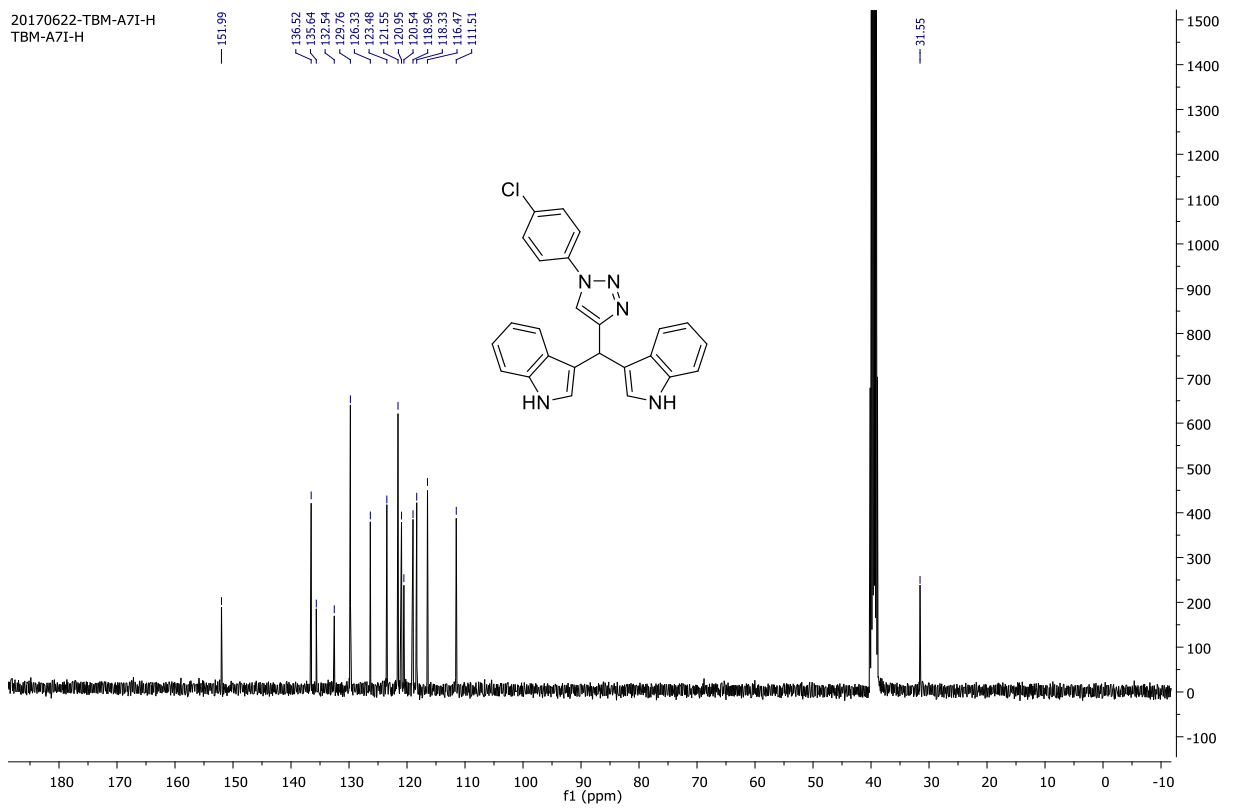
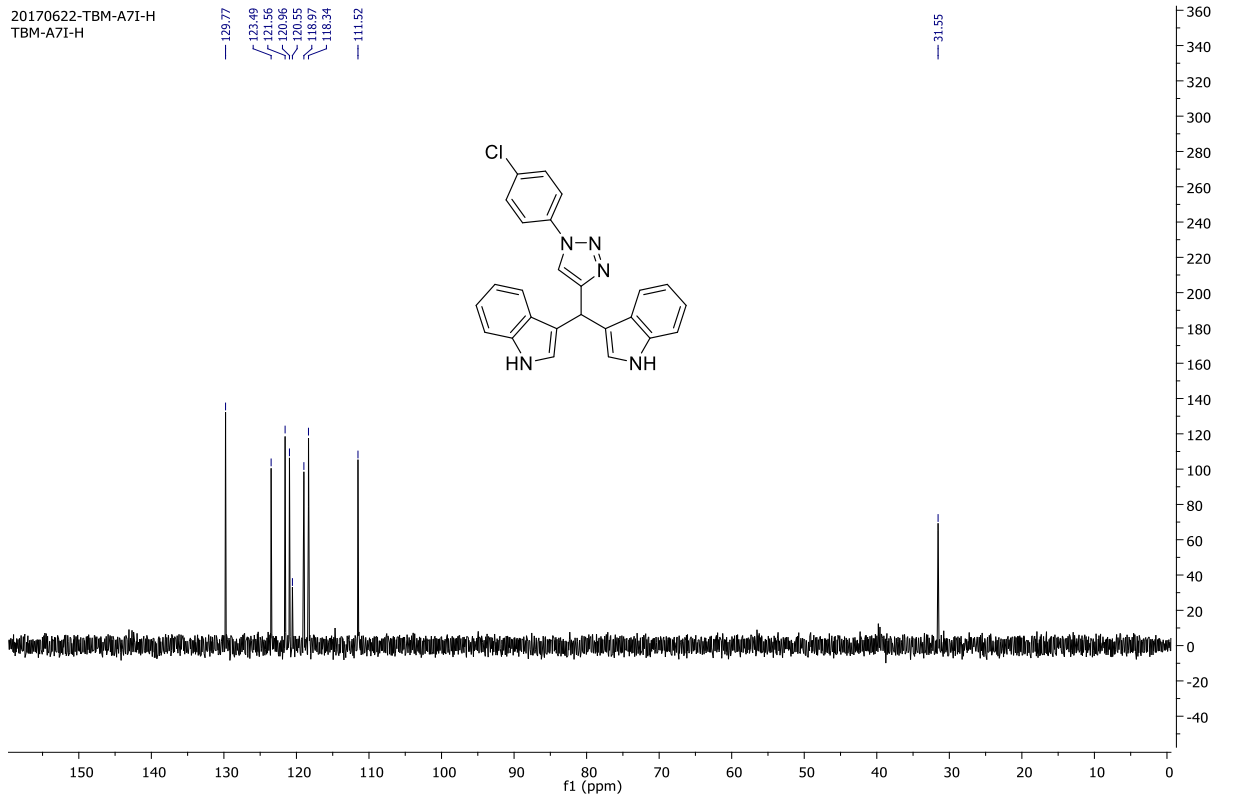
6g. ^{1}H NMR, 400 MHz, DMSO-d_6



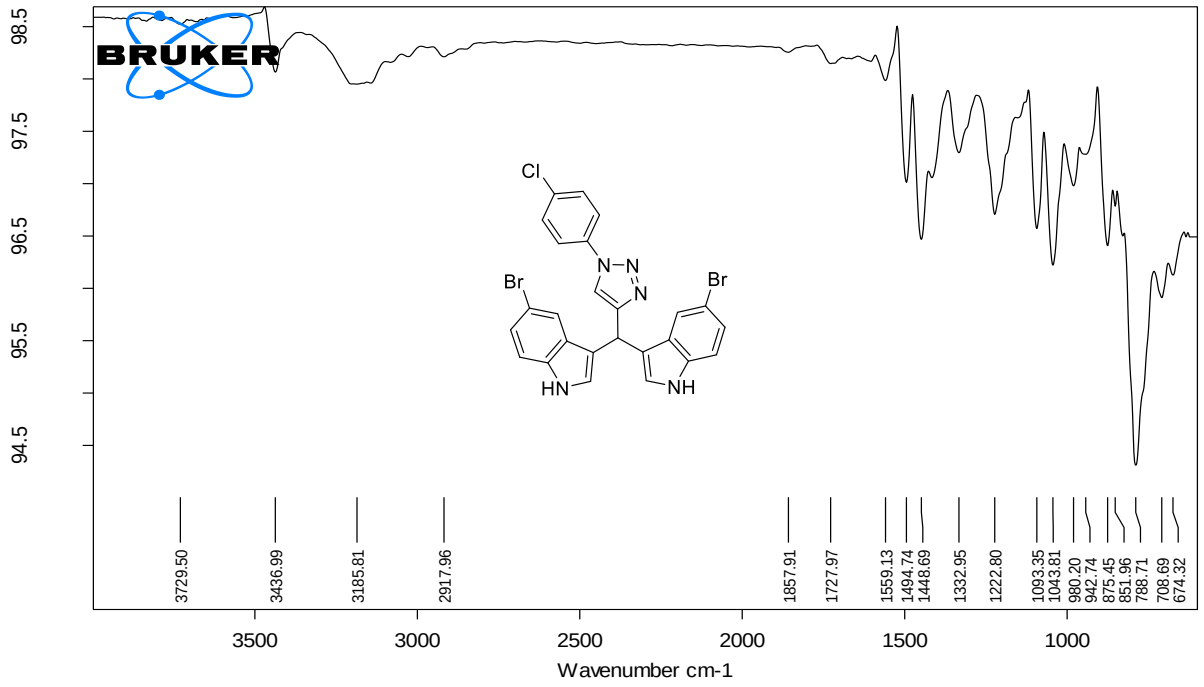
6g. ^{13}C NMR, 100 MHz, DMSO-d_6



6g. DEPT NMR, 100 MHz, DMSO-d_6



6h. FTIR



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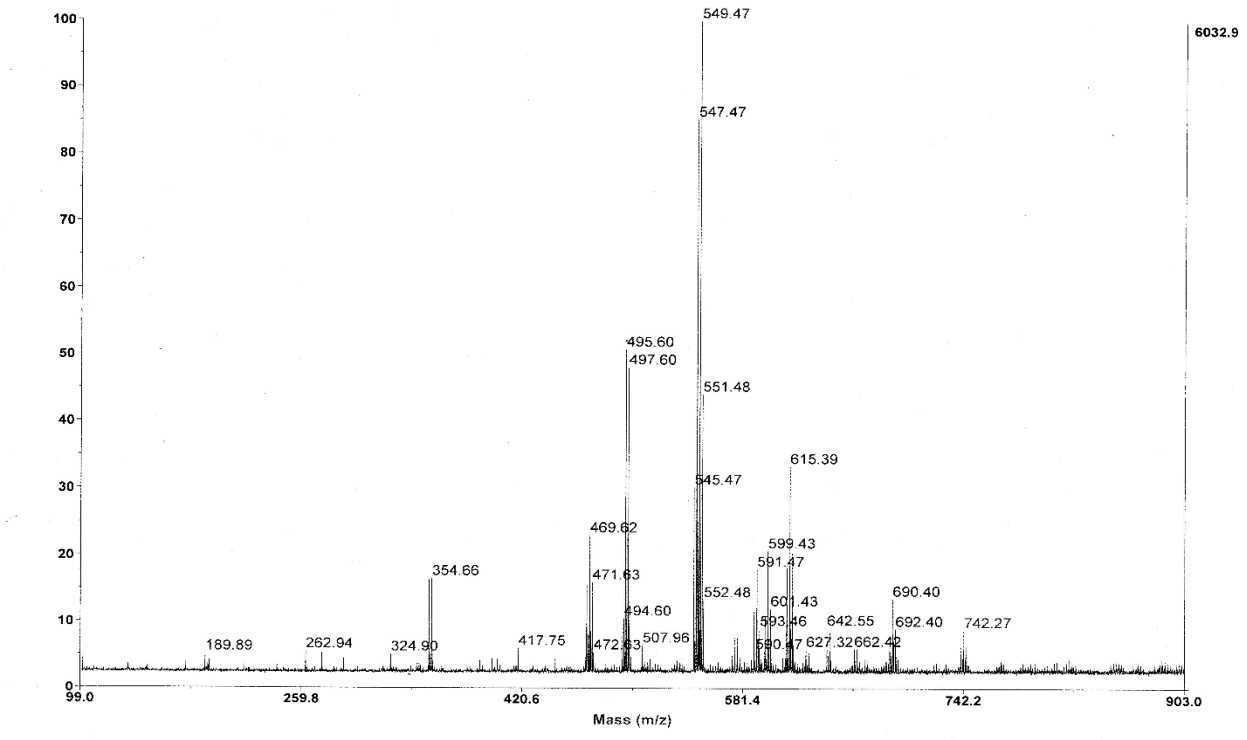
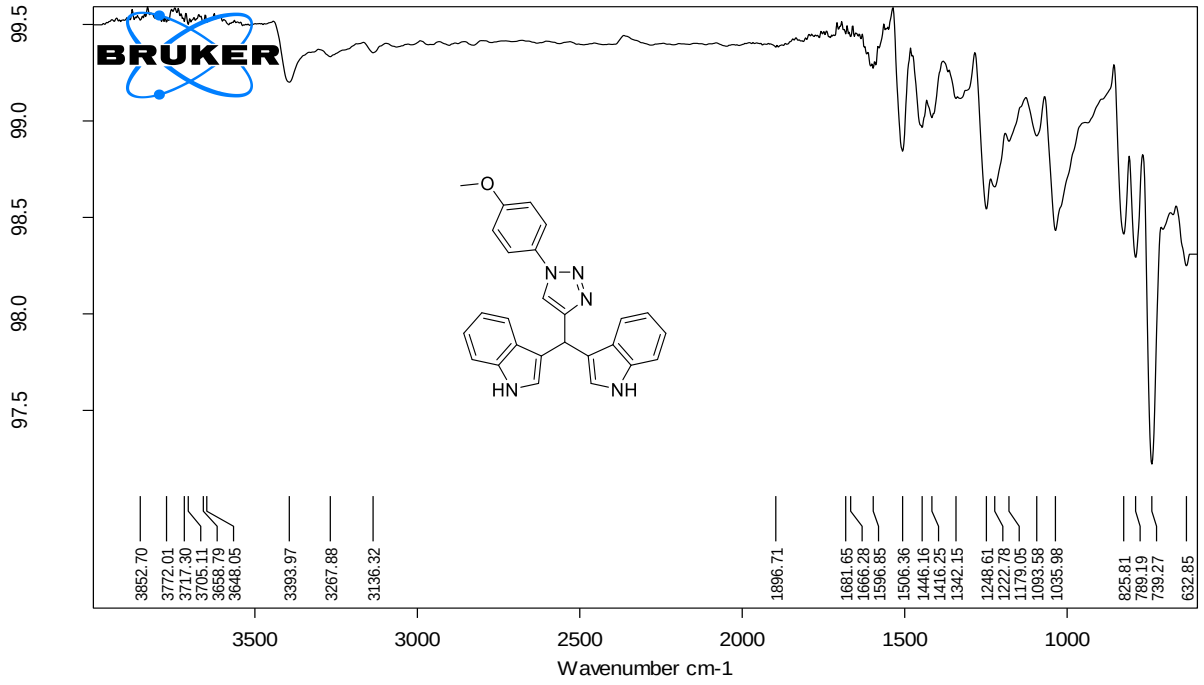
6h. Mass



6i. FTIR



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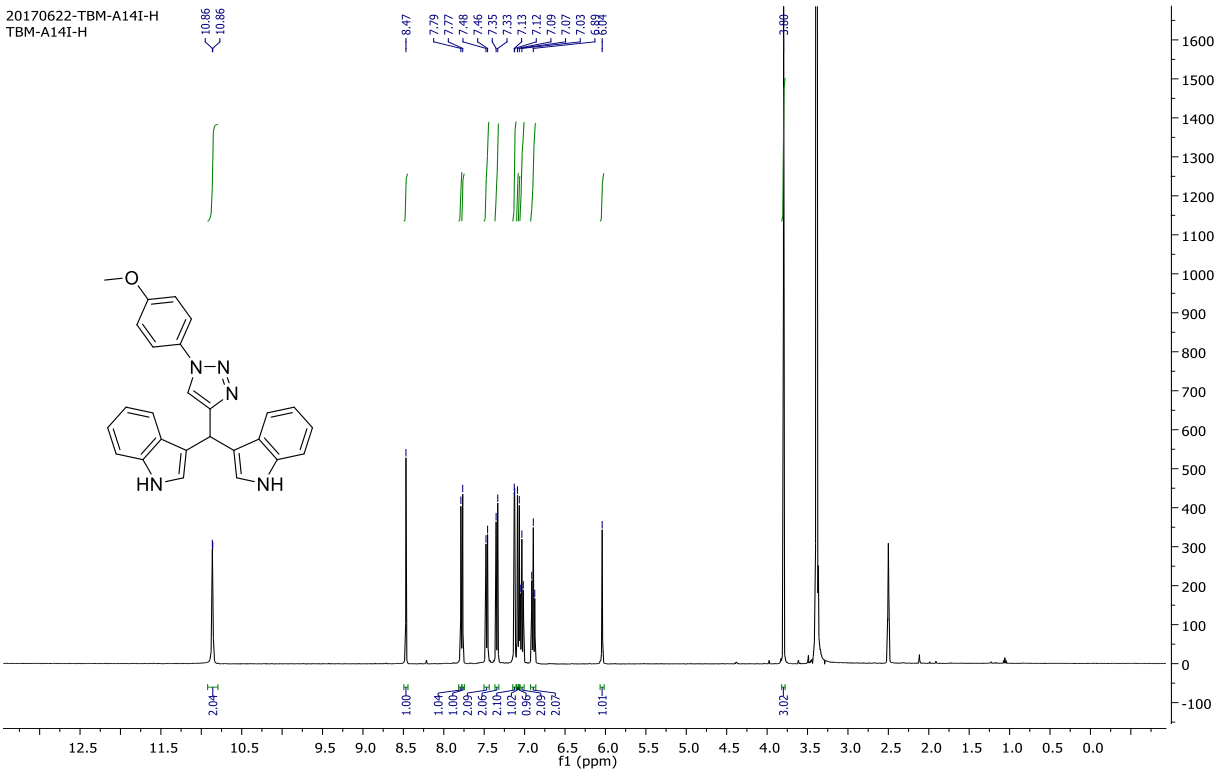
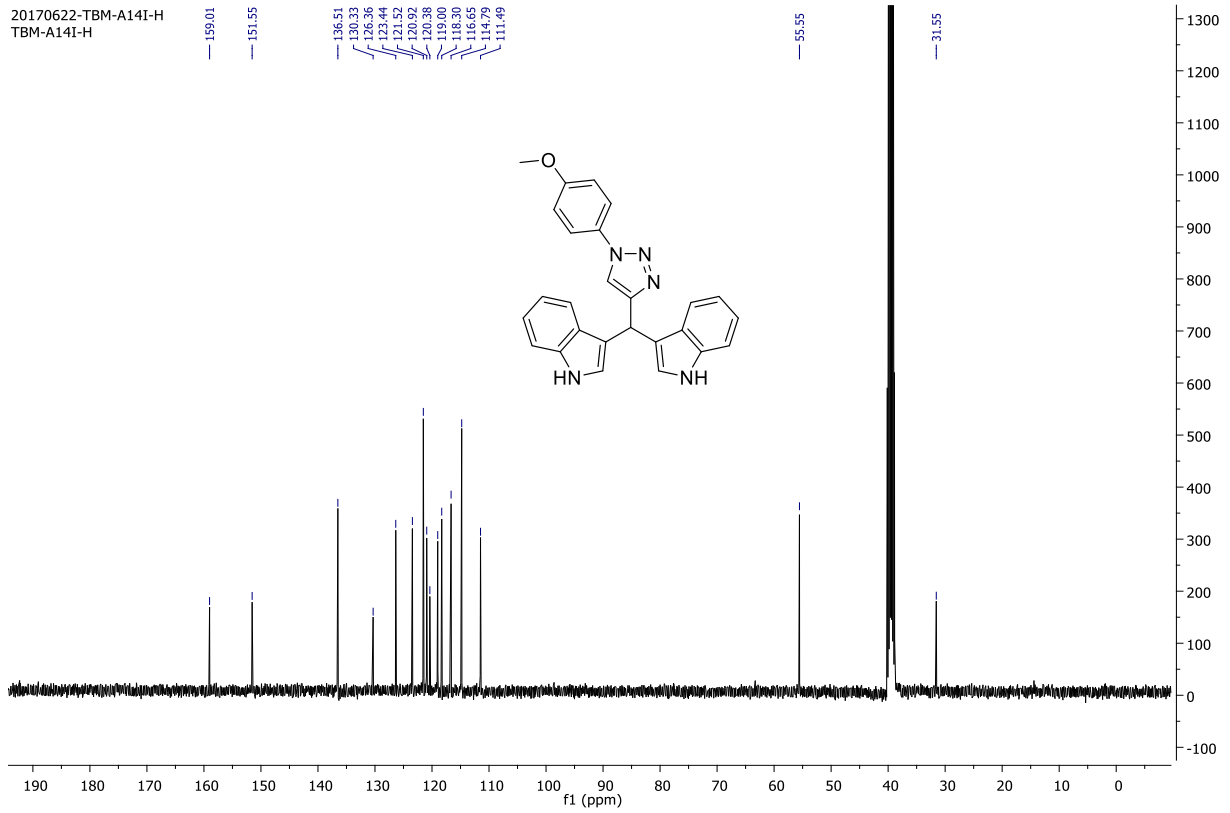
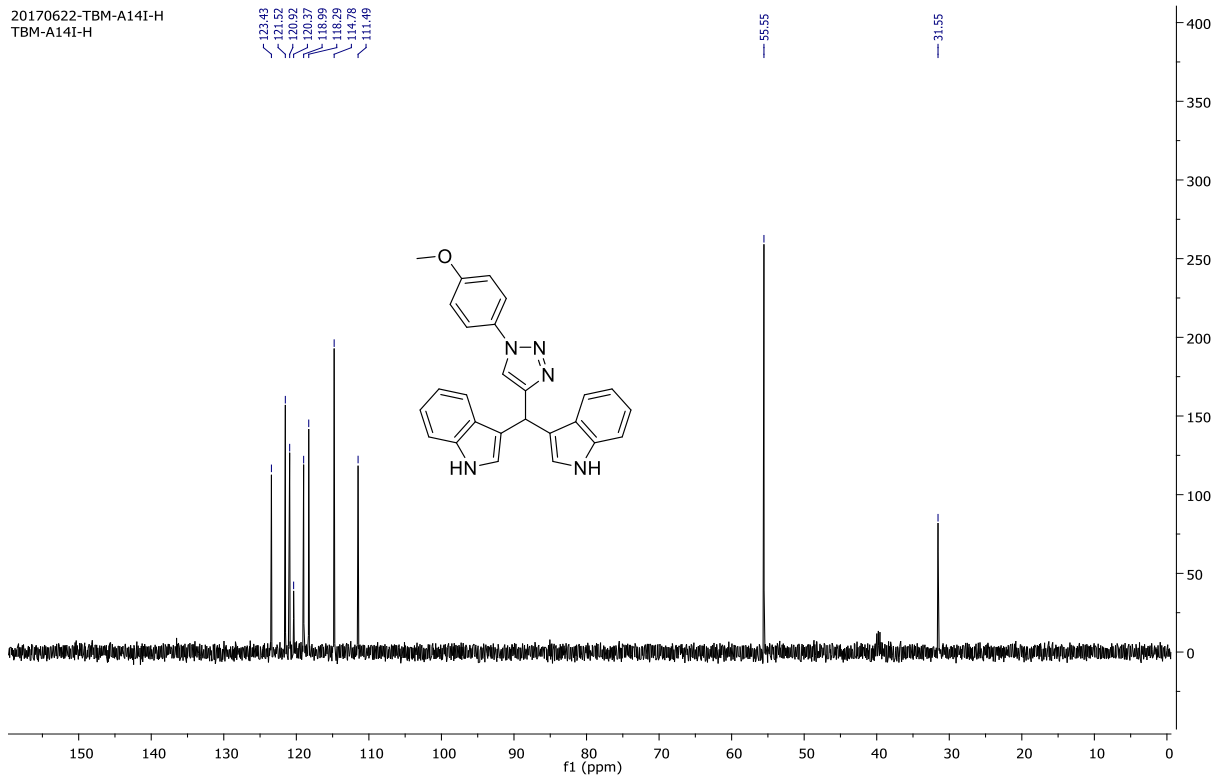
6i. ^{1}H NMR, 400 MHz, DMSO-d_6



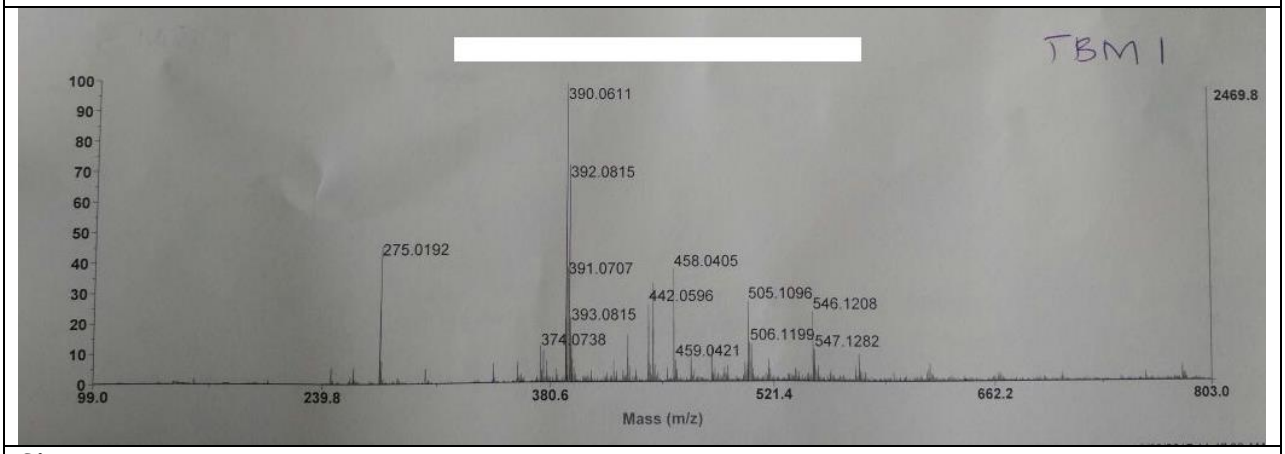
6i. ^{13}C NMR, 100 MHz, DMSO-*d*$_6$



6i. DEPT NMR, 100 MHz, DMSO-*d*$_6$



6i. Mass



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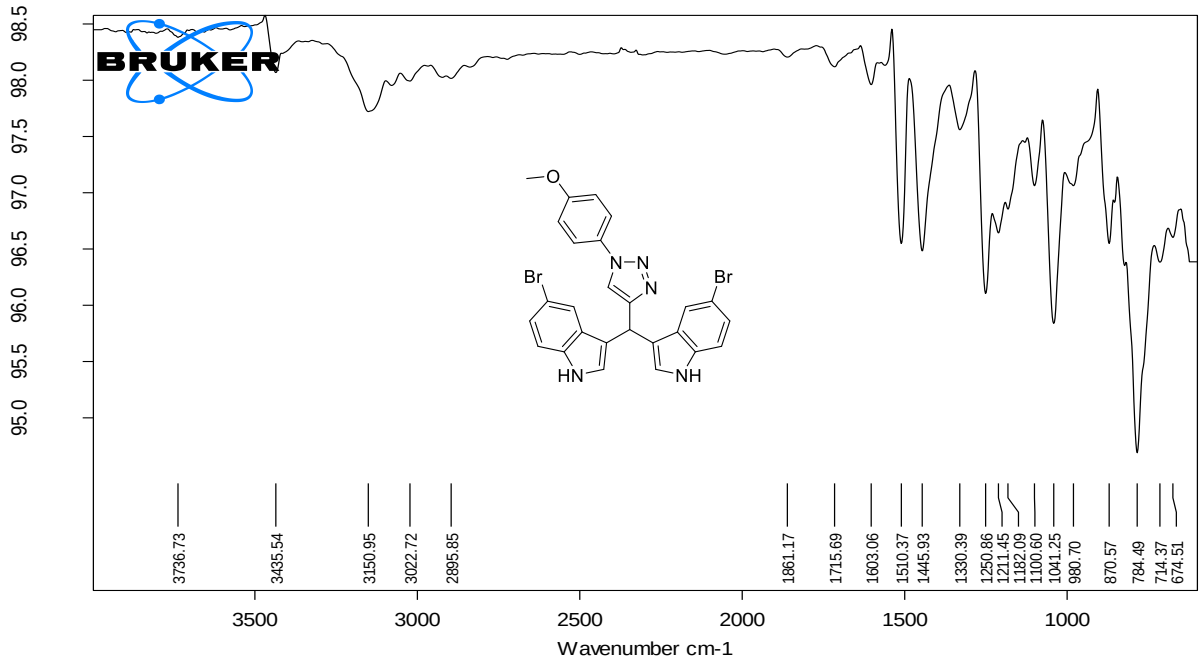

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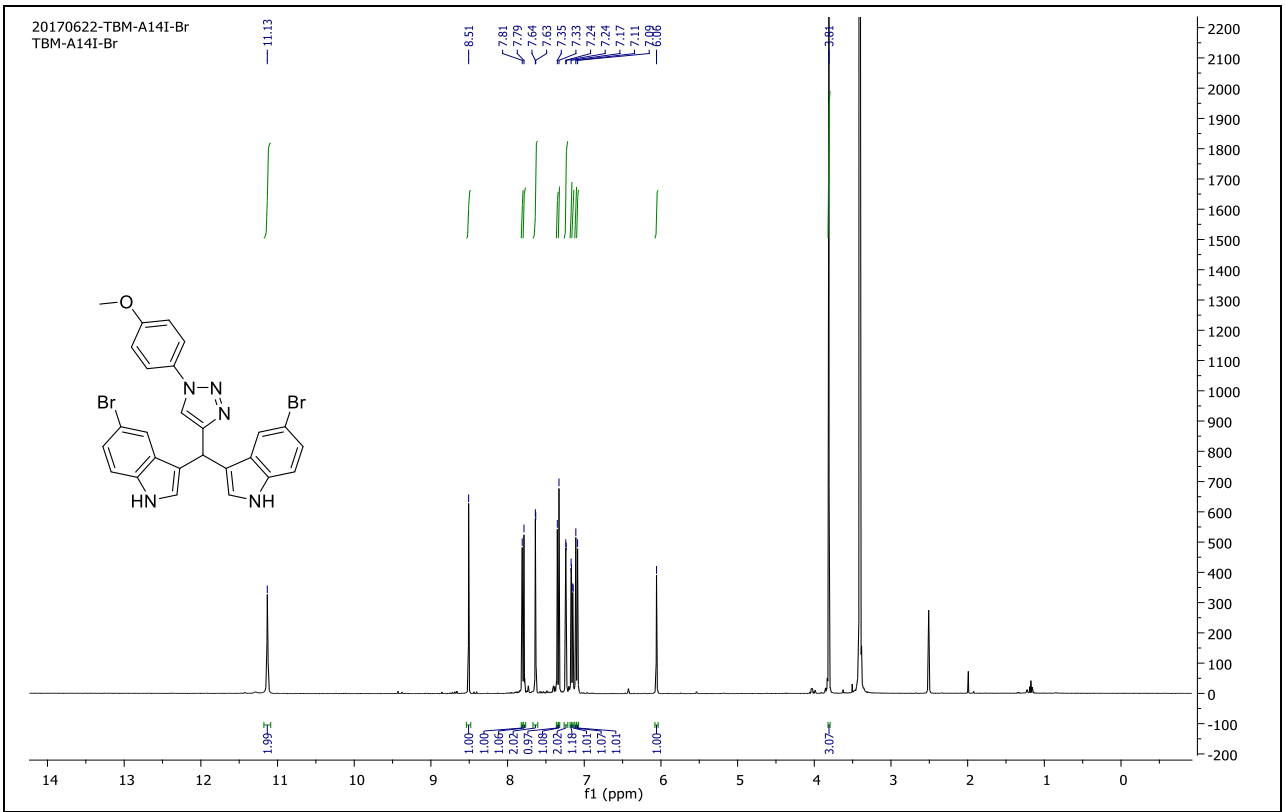
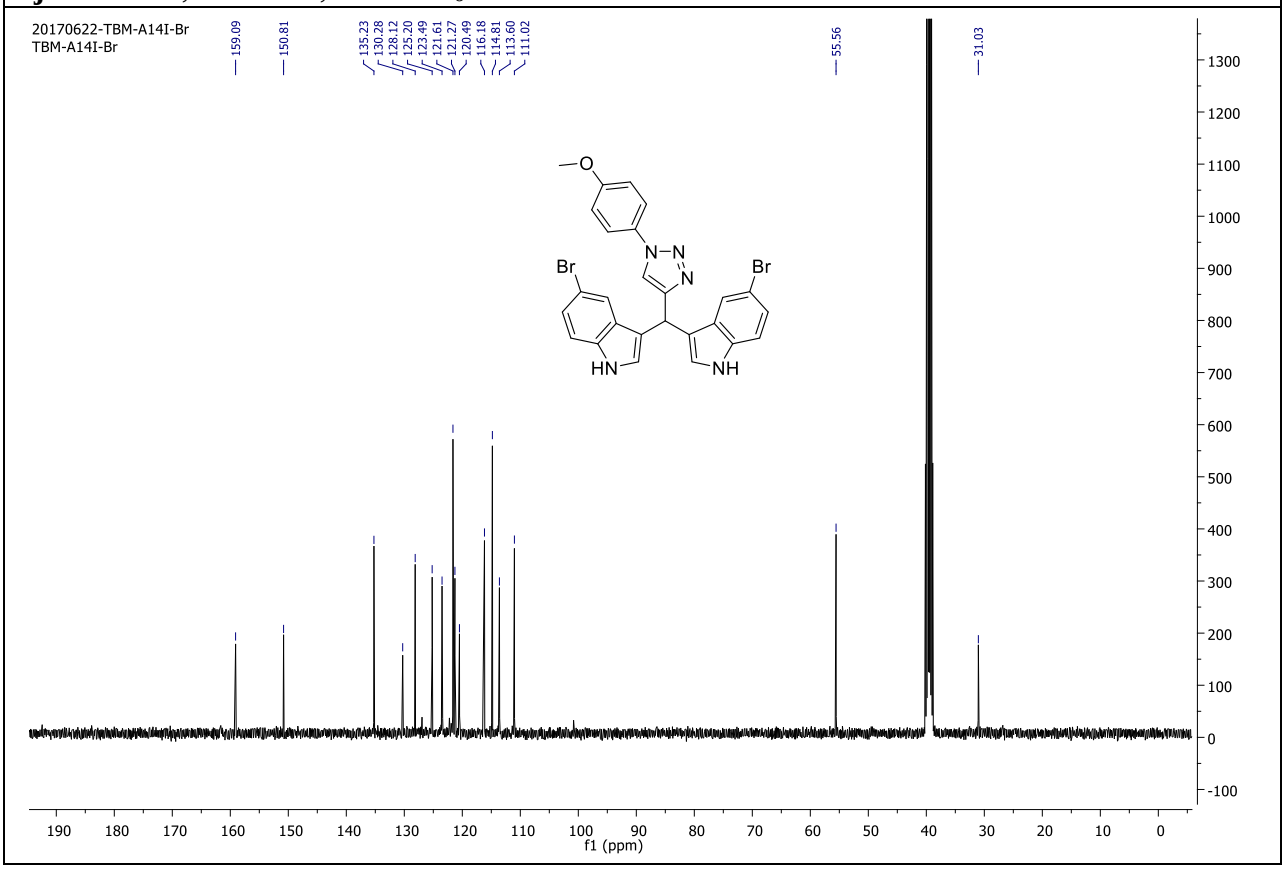
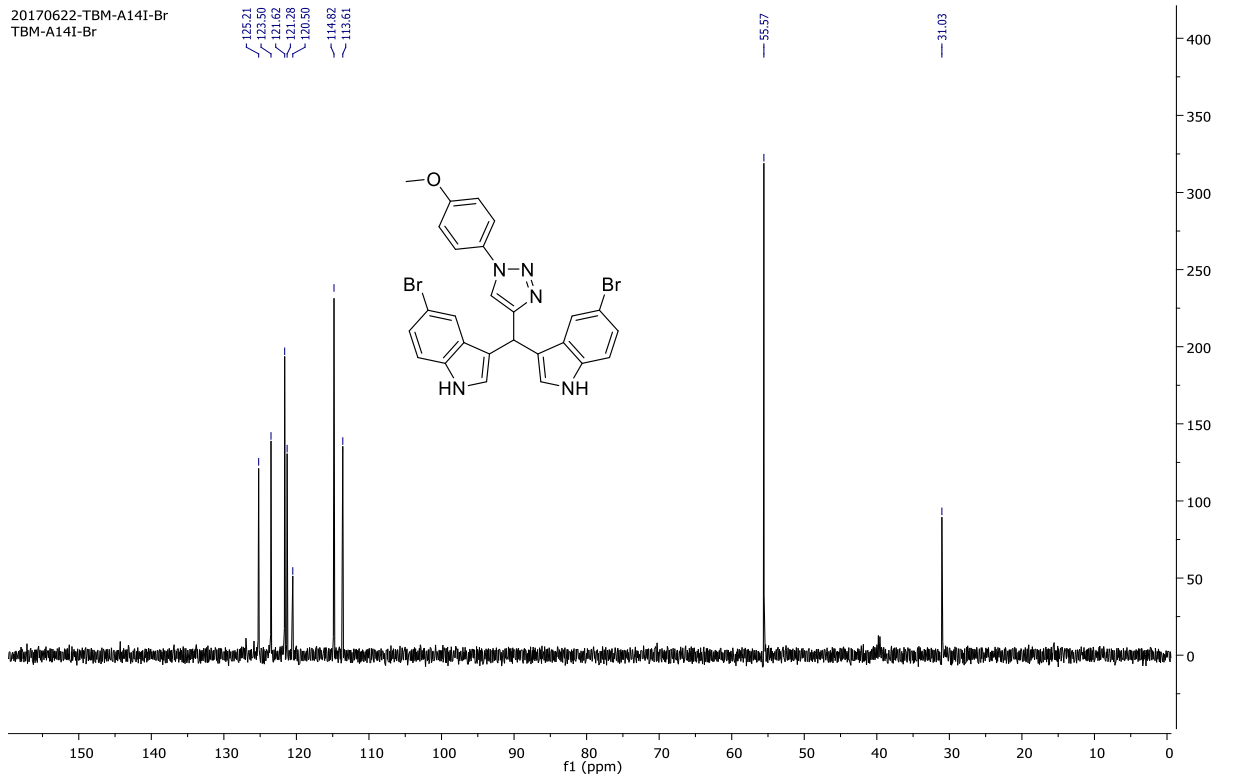
6j. ^{1}H NMR, 400 MHz, DMSO-d_6



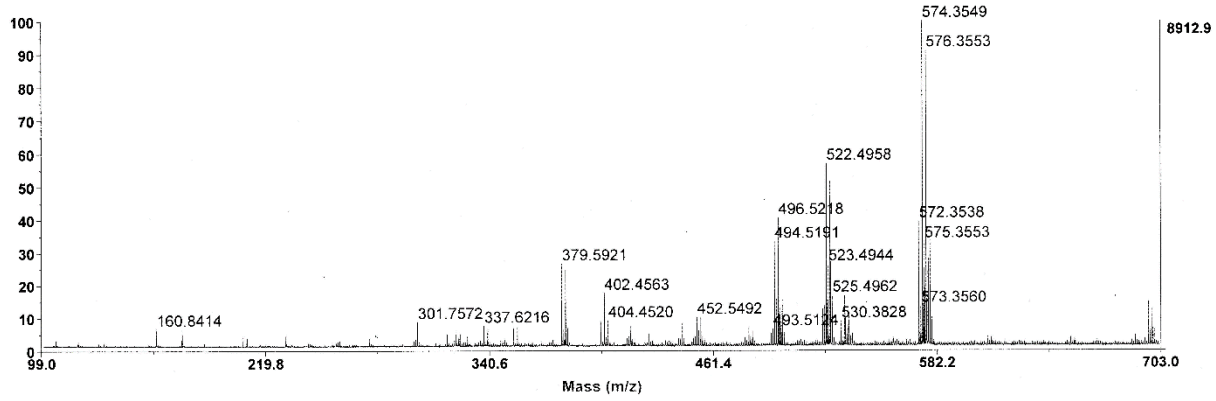
6j. ^{13}C NMR, 100 MHz, DMSO-*d*$_6$



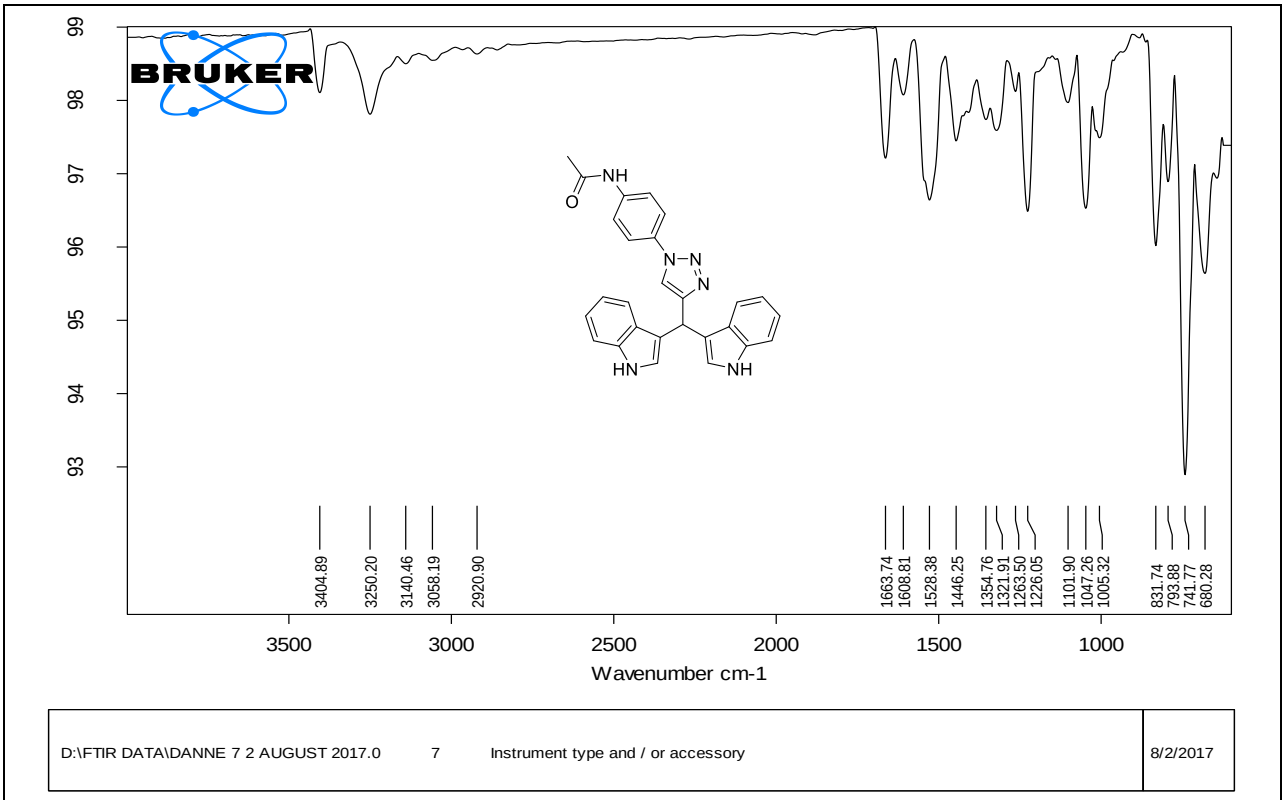
6j. DEPT NMR, 100 MHz, DMSO-d_6



6j. Mass



6k. FTIR



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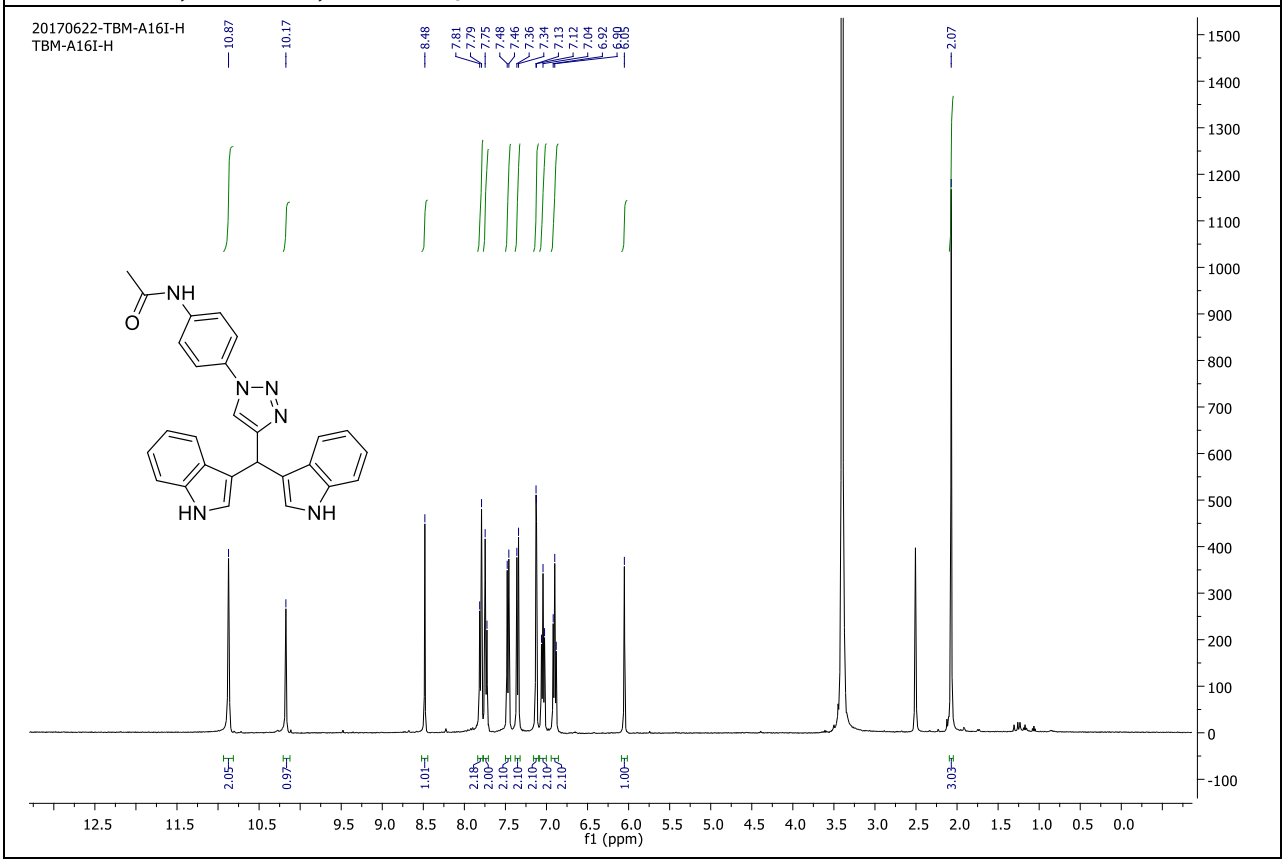
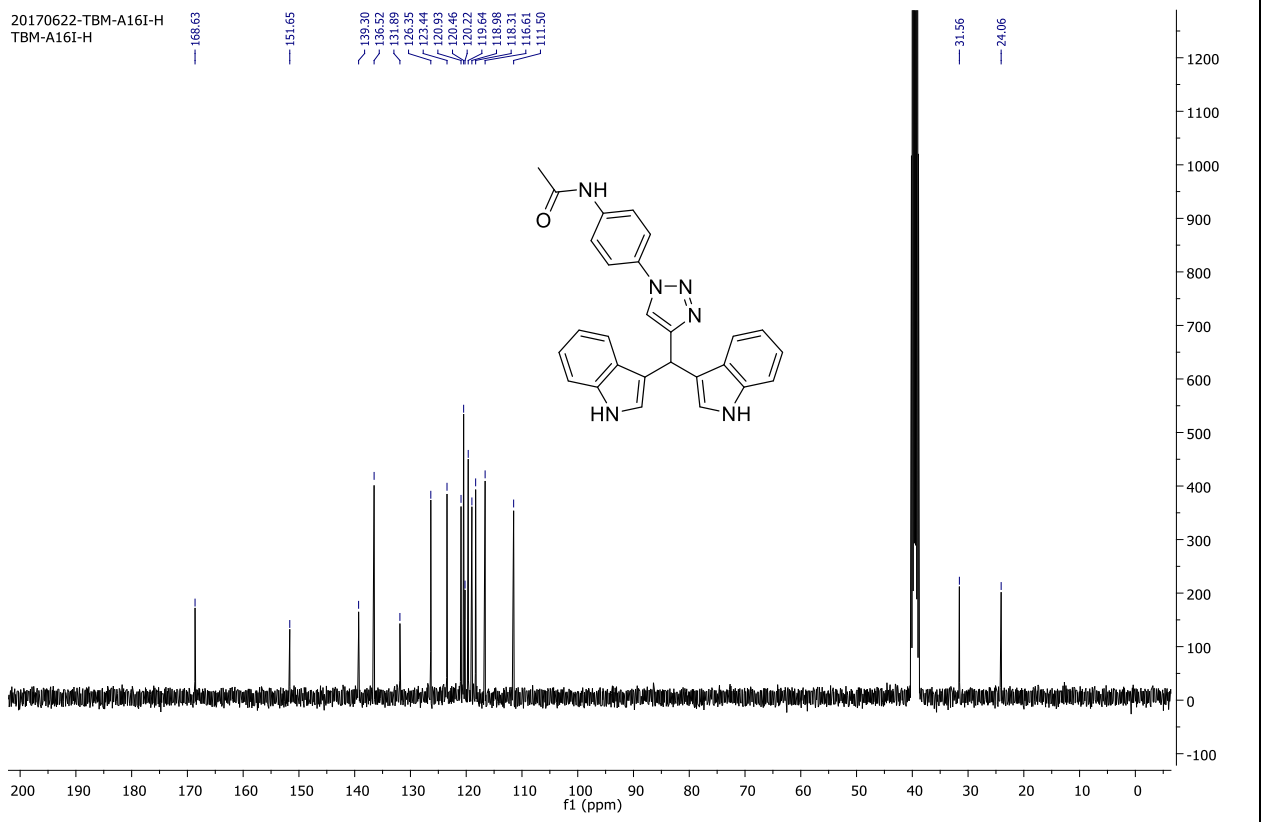
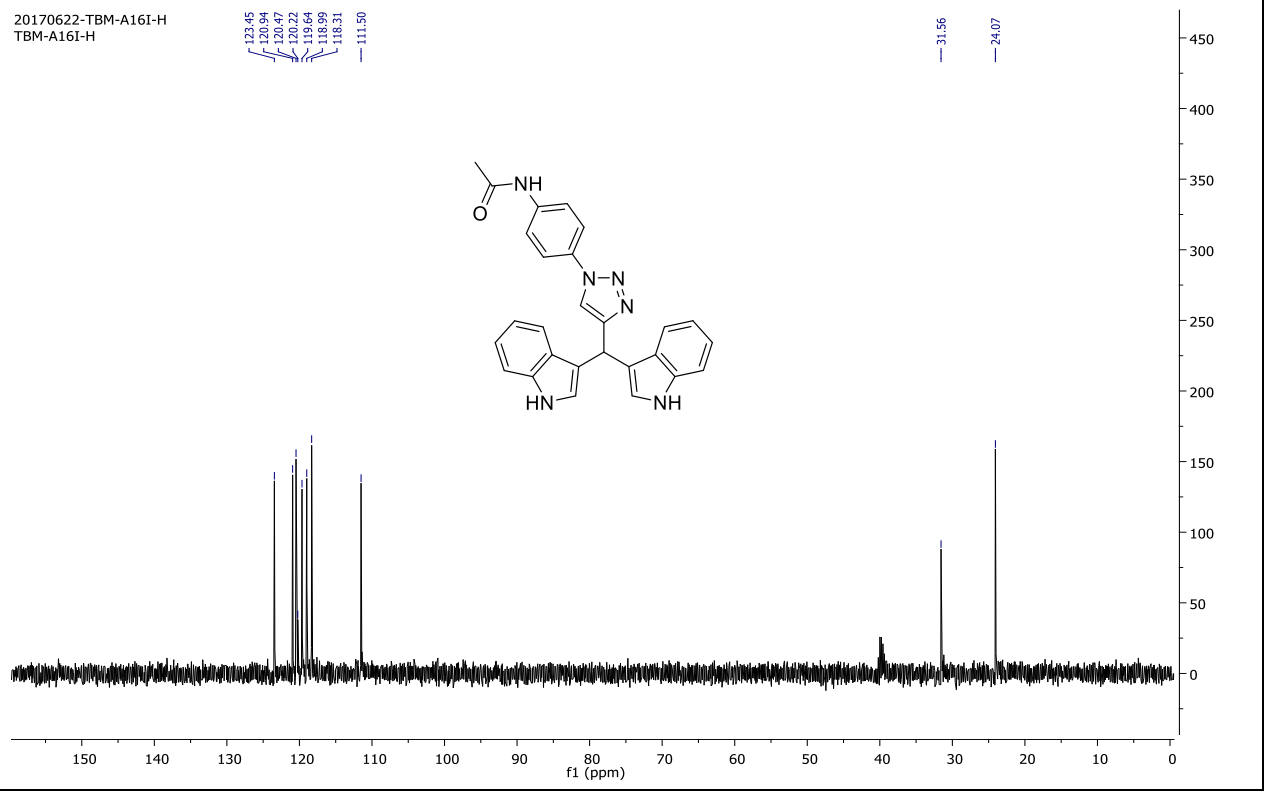
6k. ^{1}H NMR, 400 MHz, DMSO-d_6



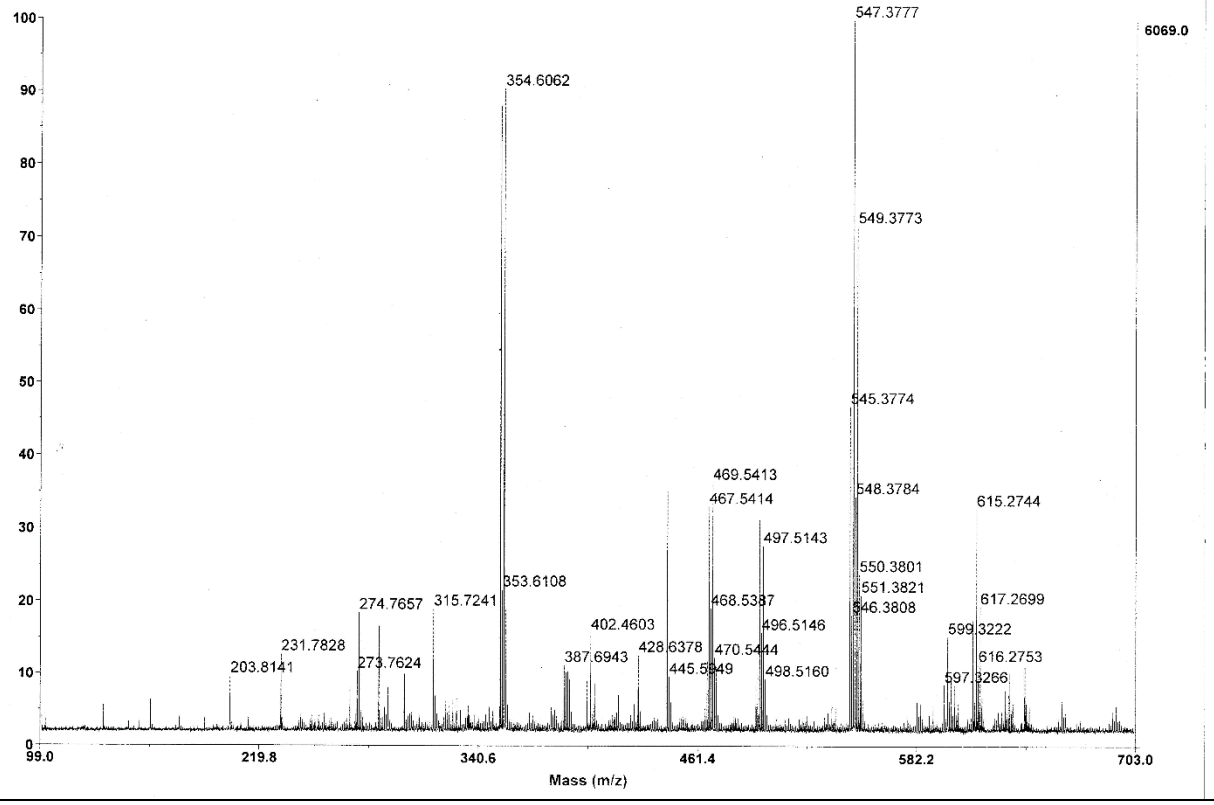
6k. ^{13}C NMR, 100 MHz, DMSO-d_6



6k. DEPT NMR, 100 MHz, DMSO-d_6



6k. Mass



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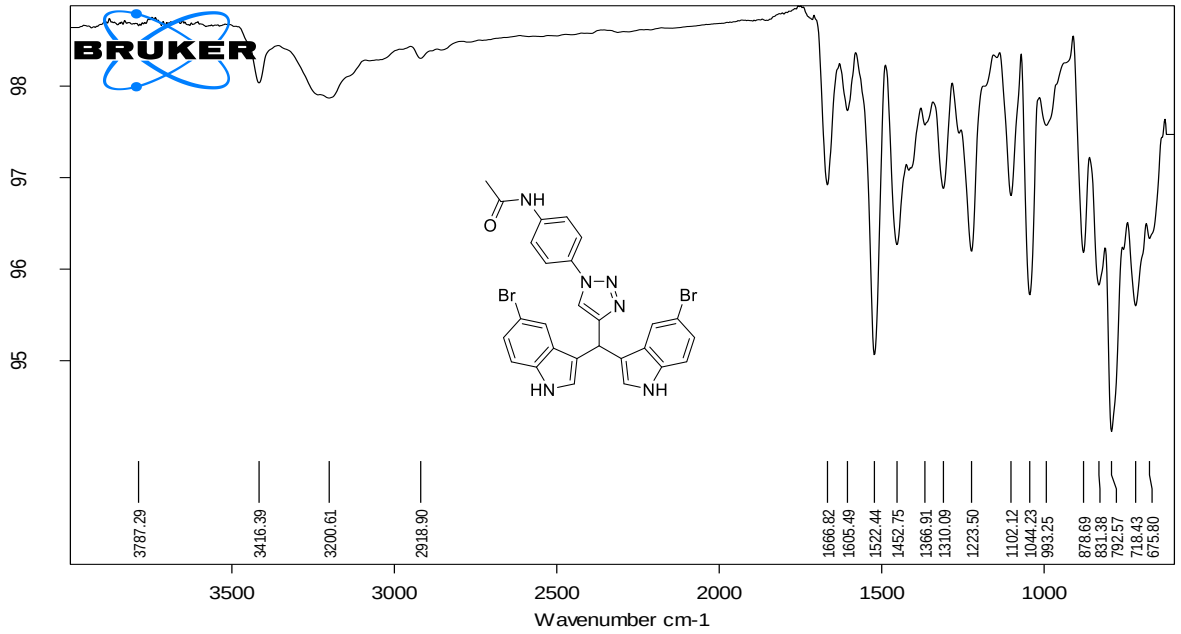

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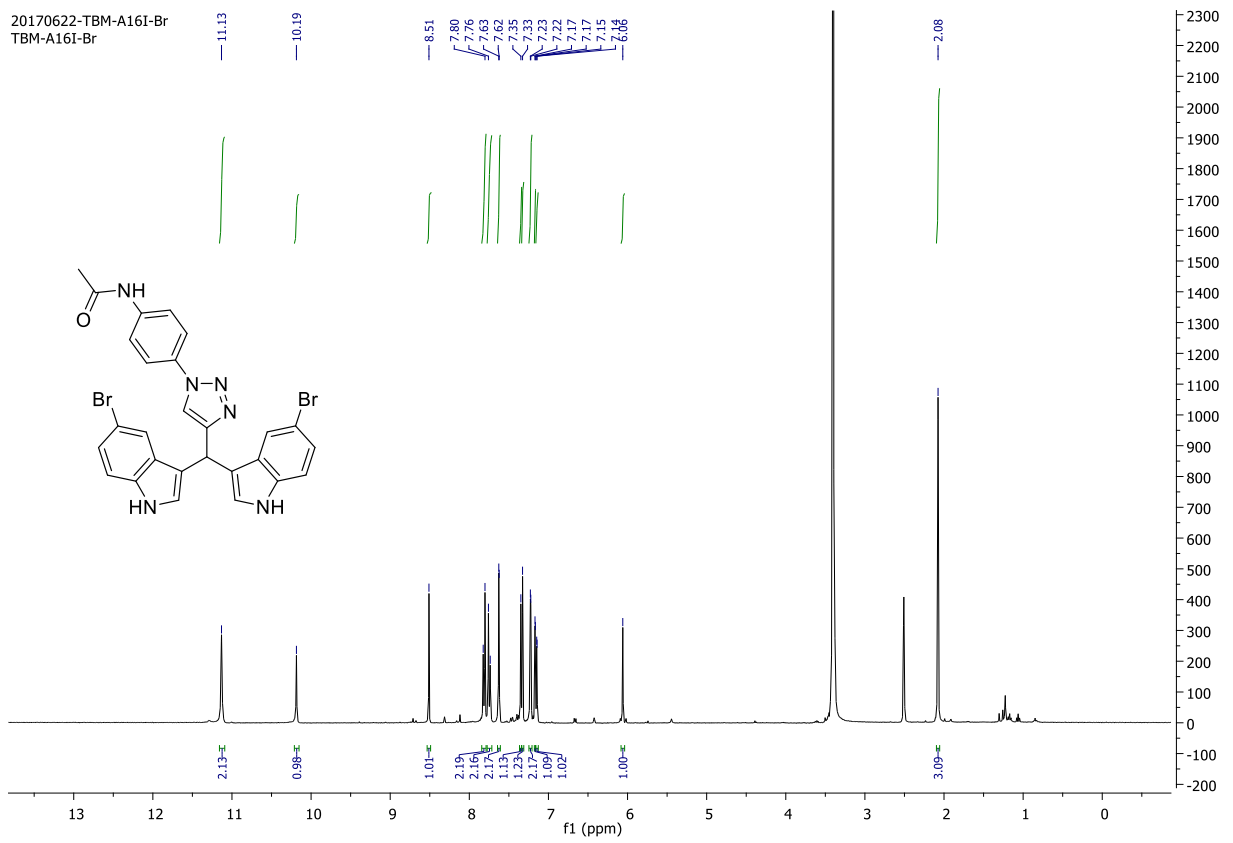
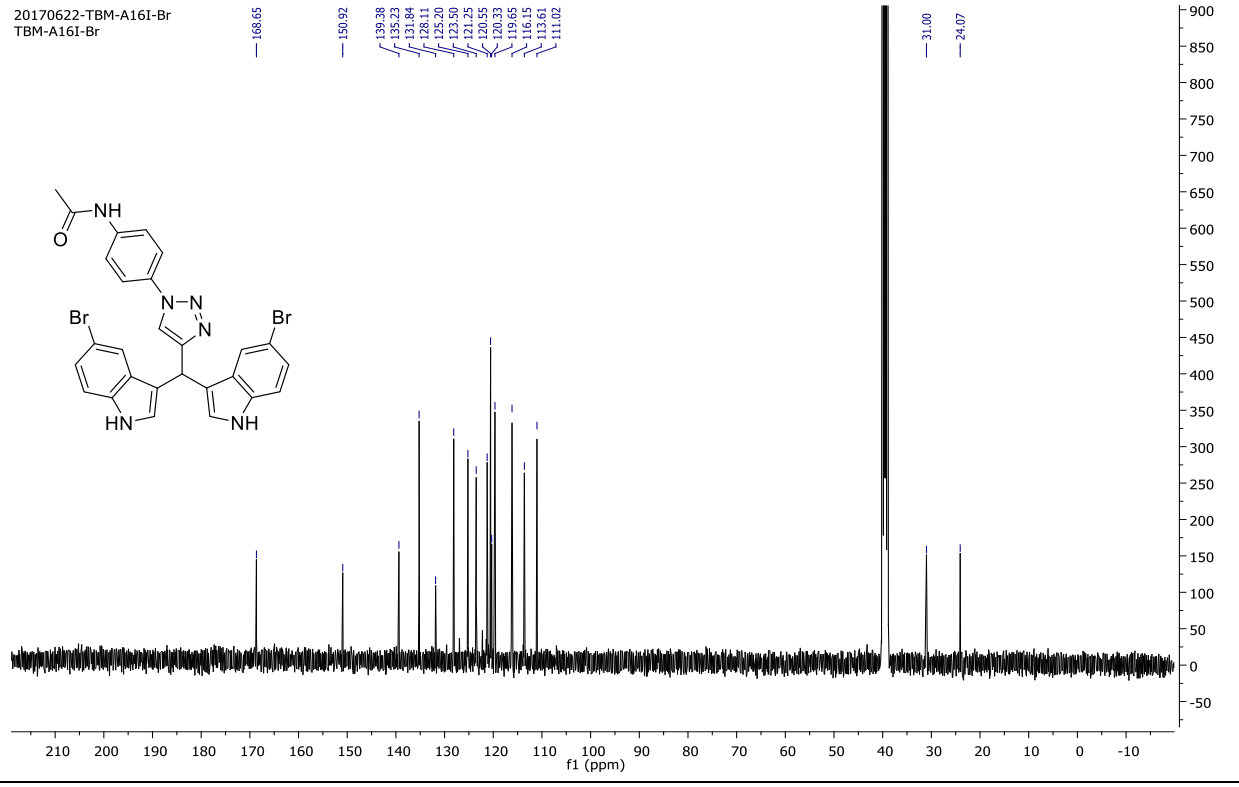
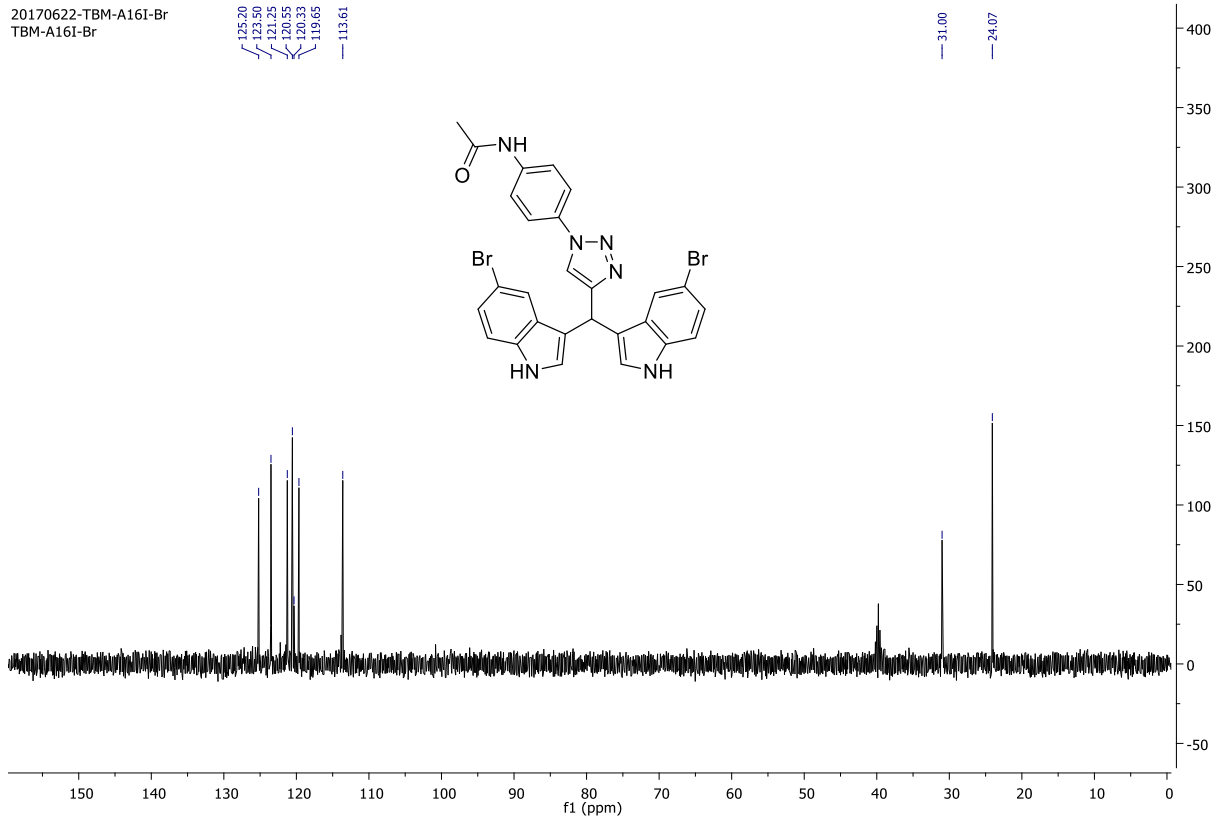
6l. ^{1}H NMR, 400 MHz, DMSO-d_6



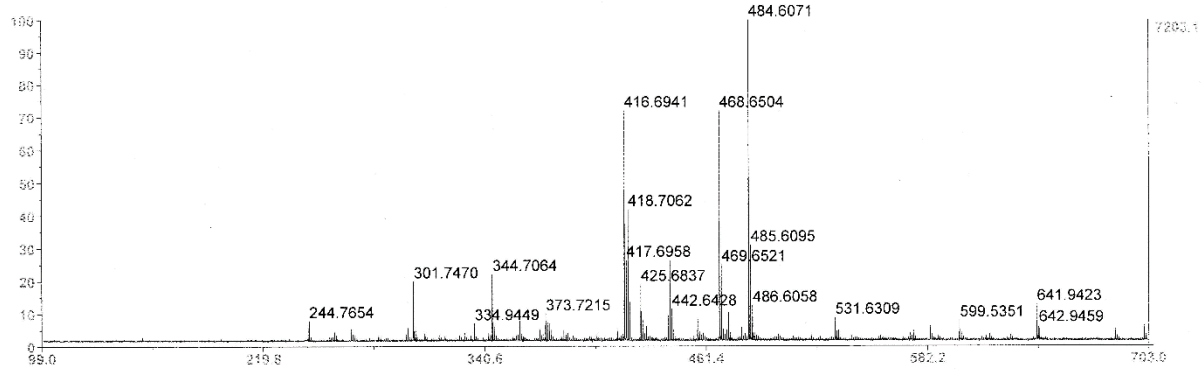
6l. ^{13}C NMR, 100 MHz, DMSO-d_6



6l. DEPT NMR, 100 MHz, DMSO-d_6



6l. Mass



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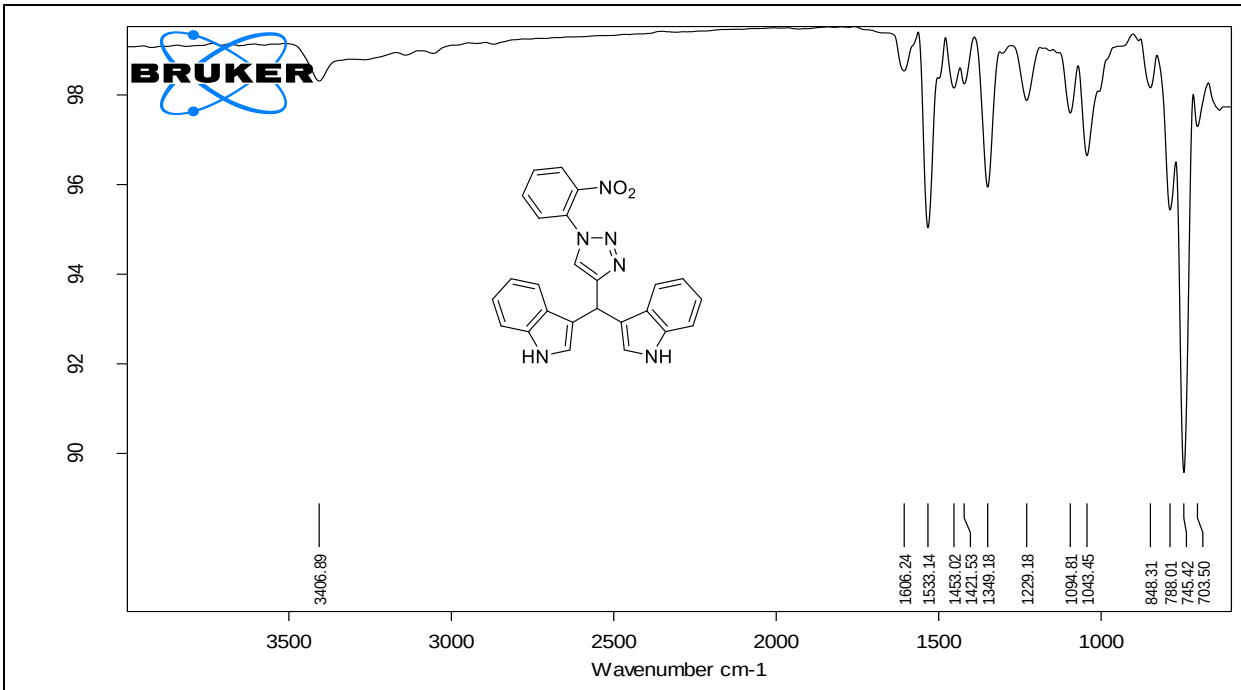

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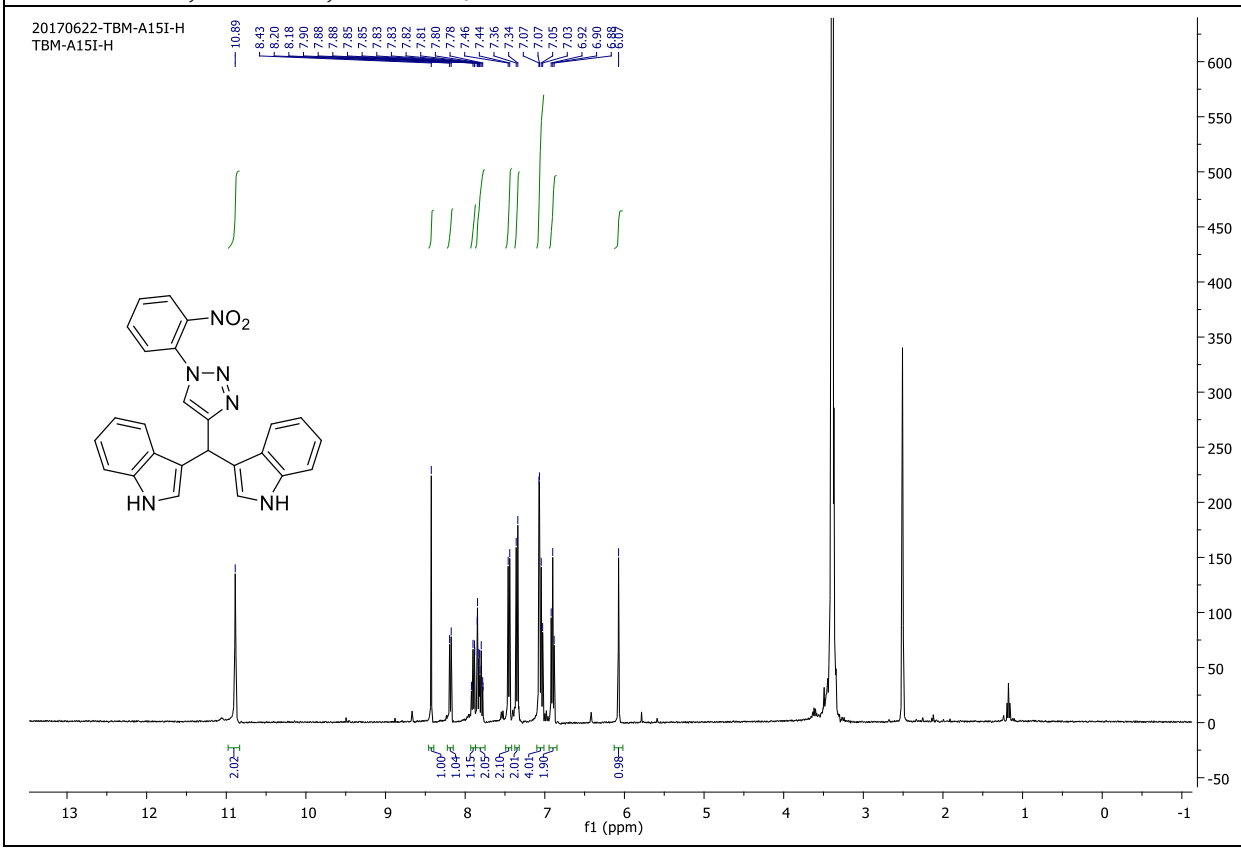
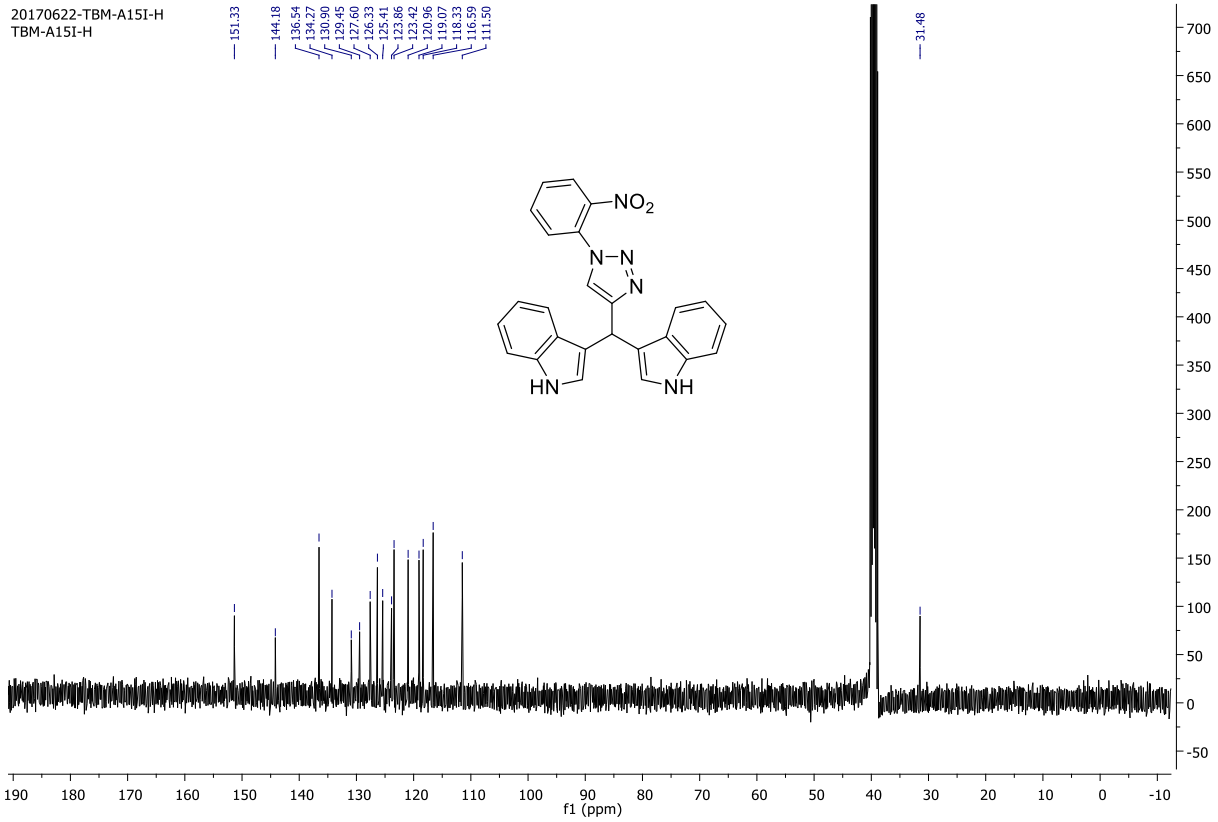
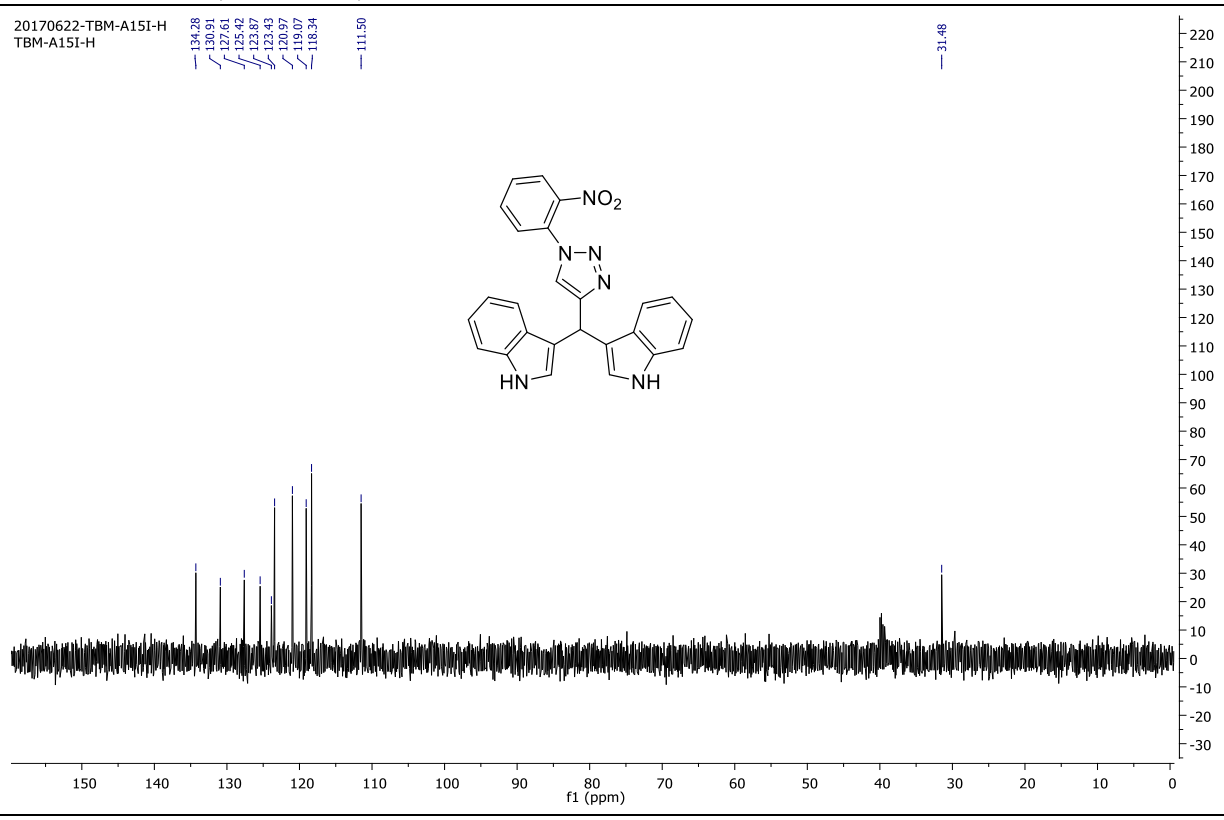
6m. ^{1}H NMR, 400 MHz, DMSO-d_6



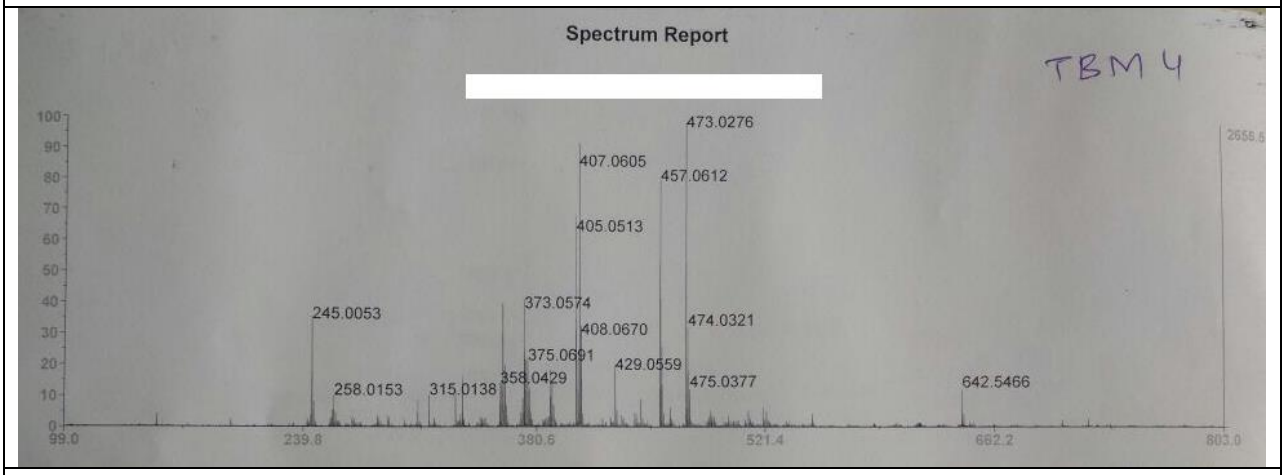
6m. ^{13}C NMR, 100 MHz, DMSO-*d*$_6$



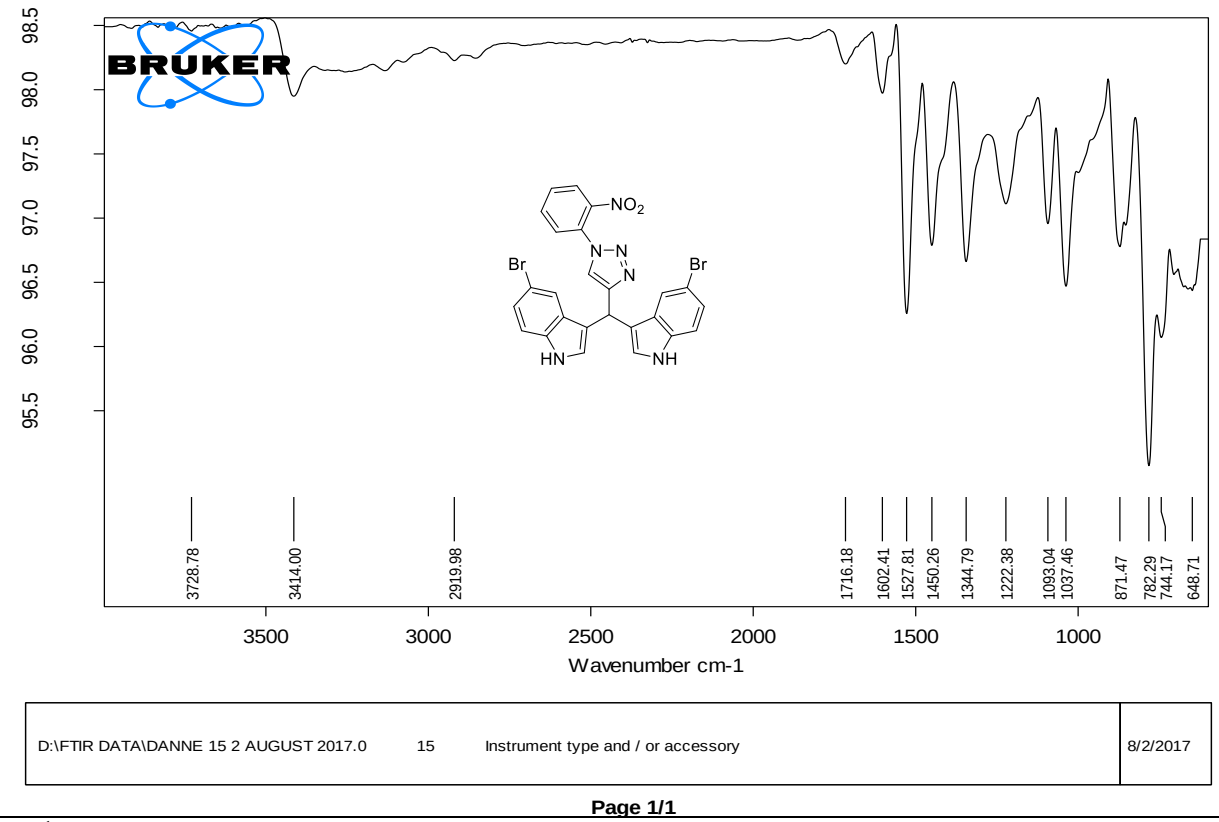
6m. DEPT NMR, 100 MHz, DMSO-*d*$_6$



6m. Mass



6n. FTIR



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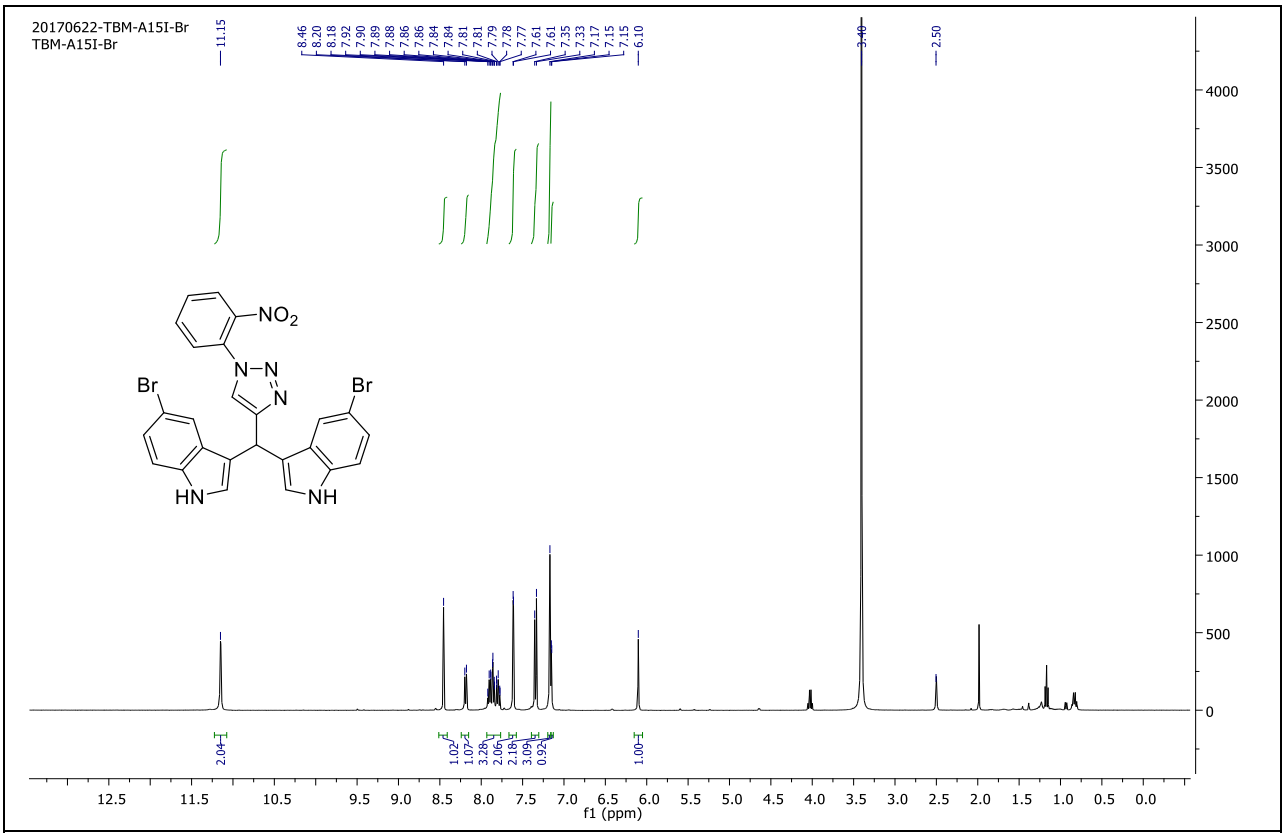
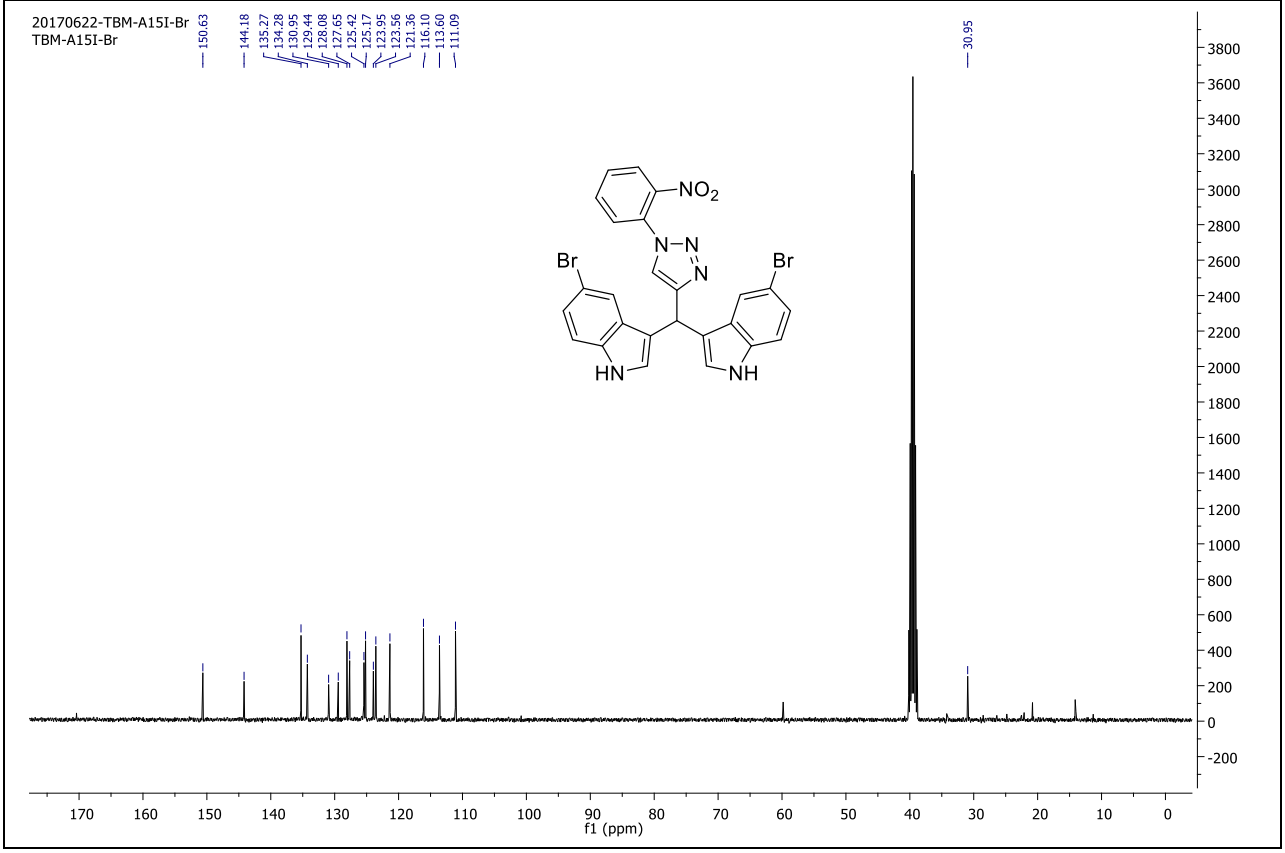
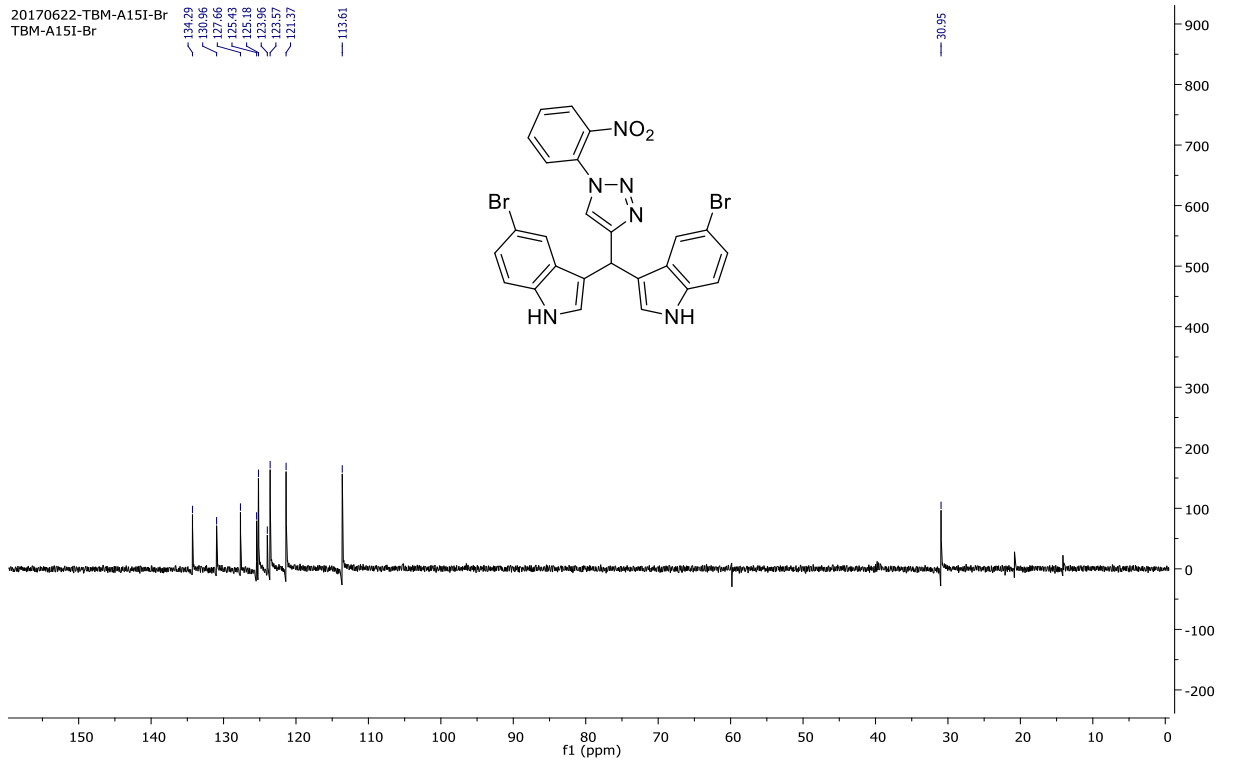
6n. ^{1}H NMR, 400 MHz, DMSO-d_6



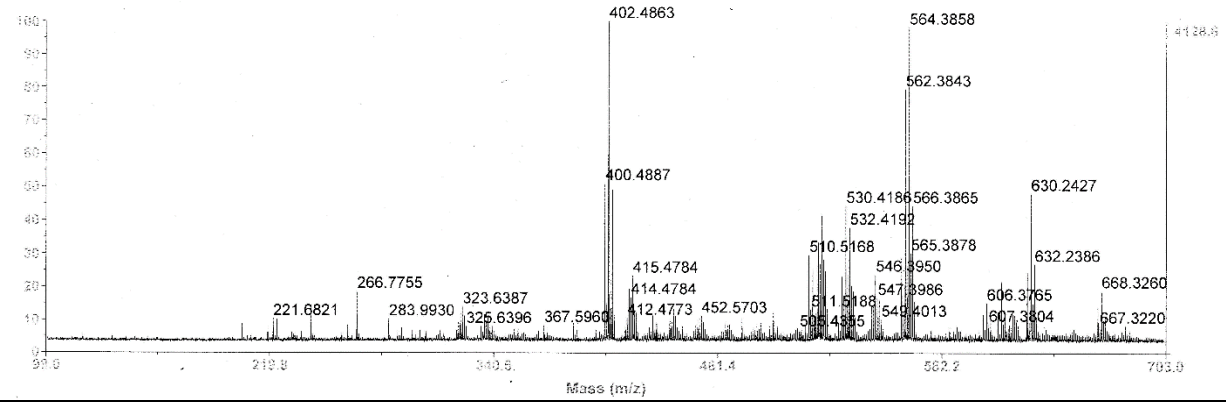
6n. ^{13}C NMR, 100 MHz, DMSO-*d*$_6$



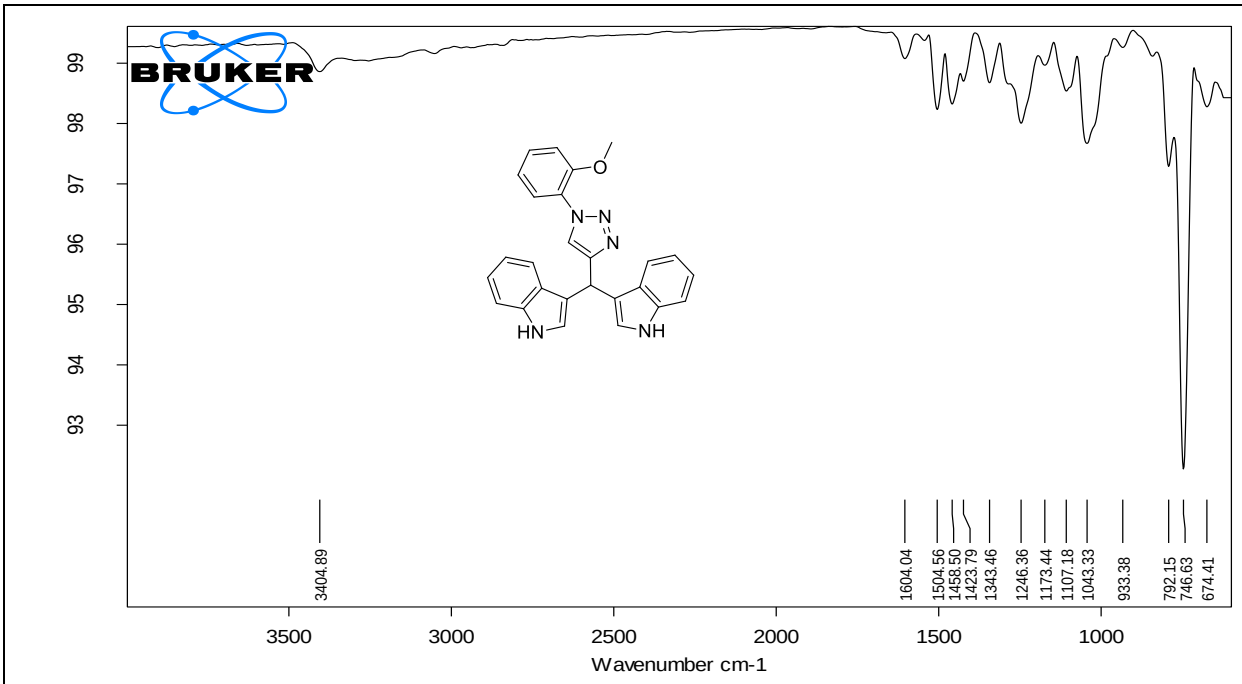
6n. DEPT NMR, 100 MHz, DMSO-d_6



6n. Mass



6o. FTIR



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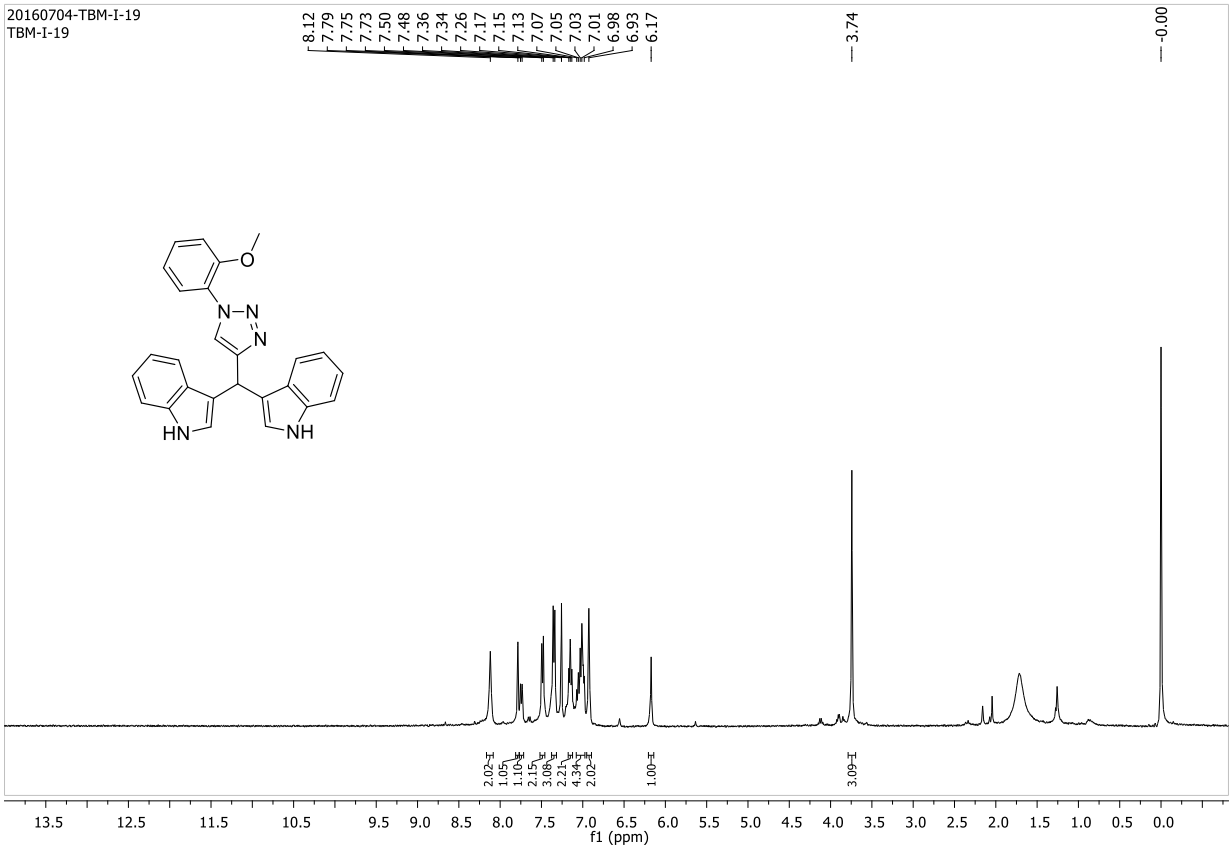
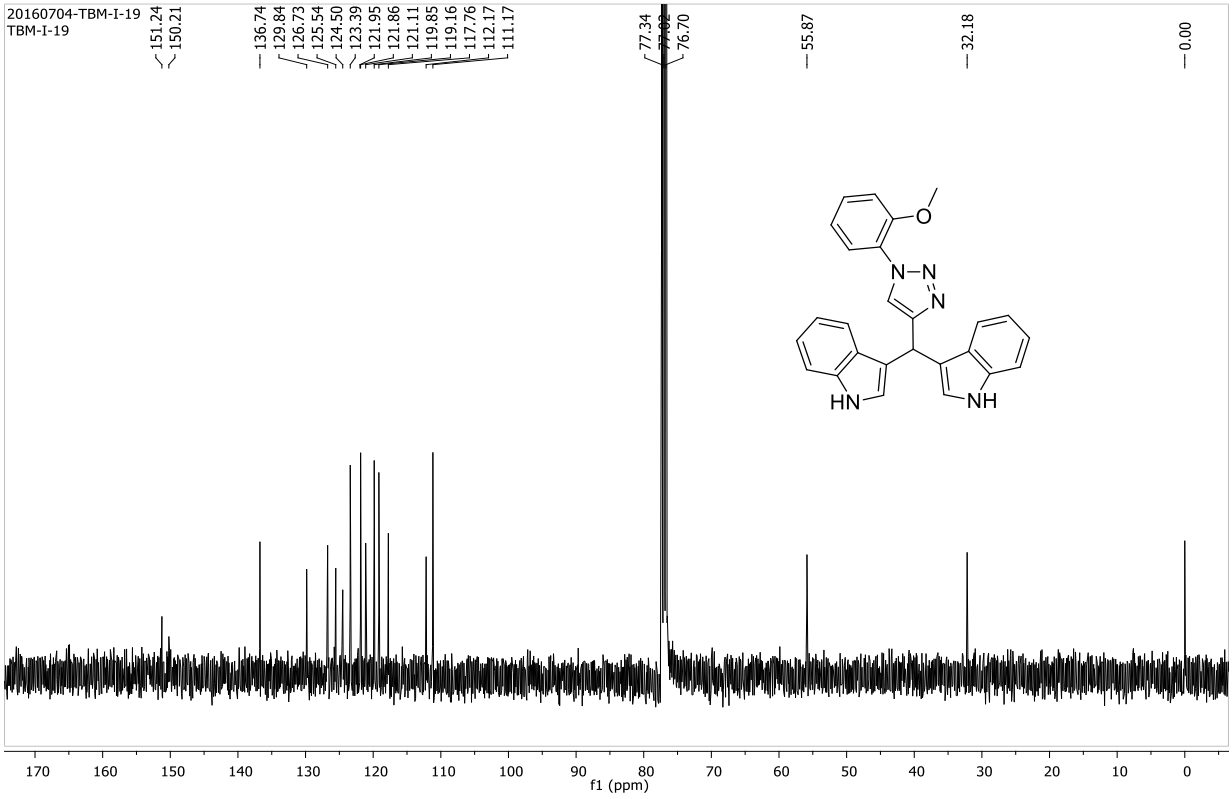
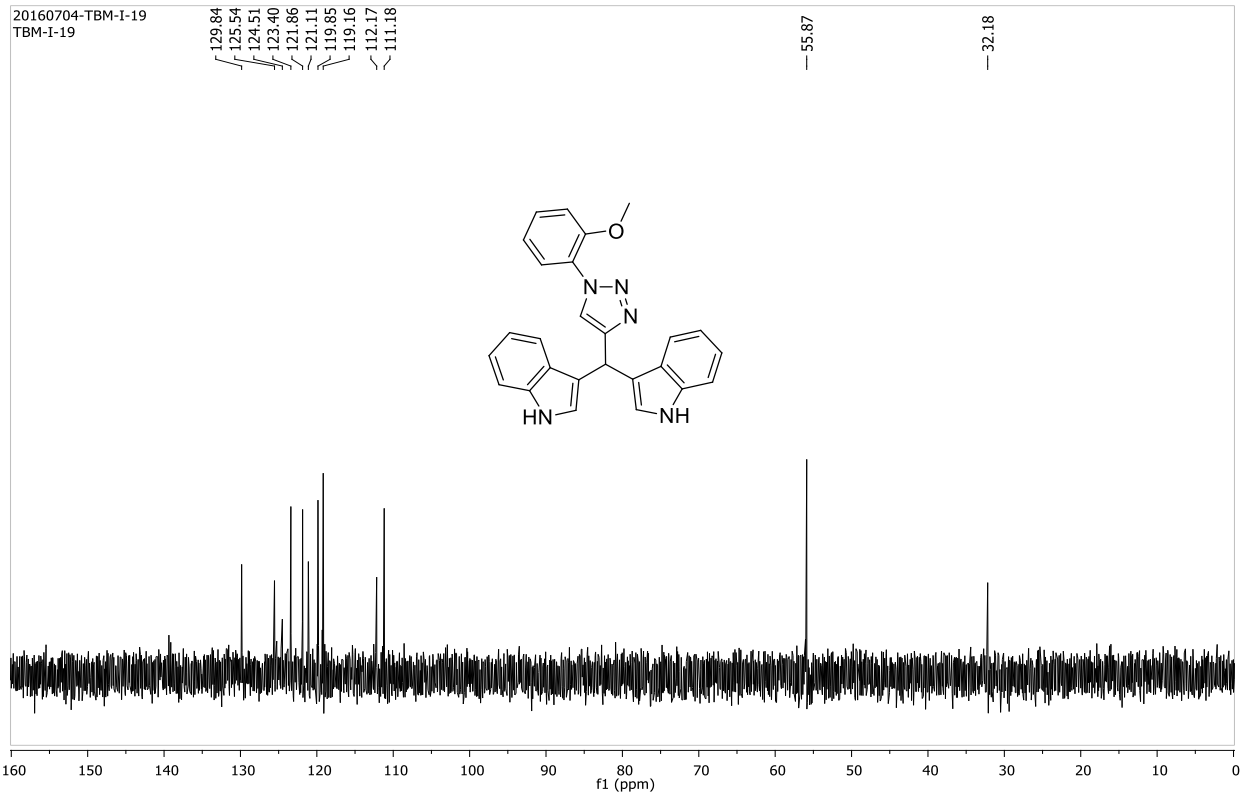
6o. ^{1}H NMR, 400 MHz, $CDCl_3$



6o. ^{13}C NMR, 100 MHz, $CDCl_3$



6o. DEPT NMR, 100 MHz, $CDCl_3$



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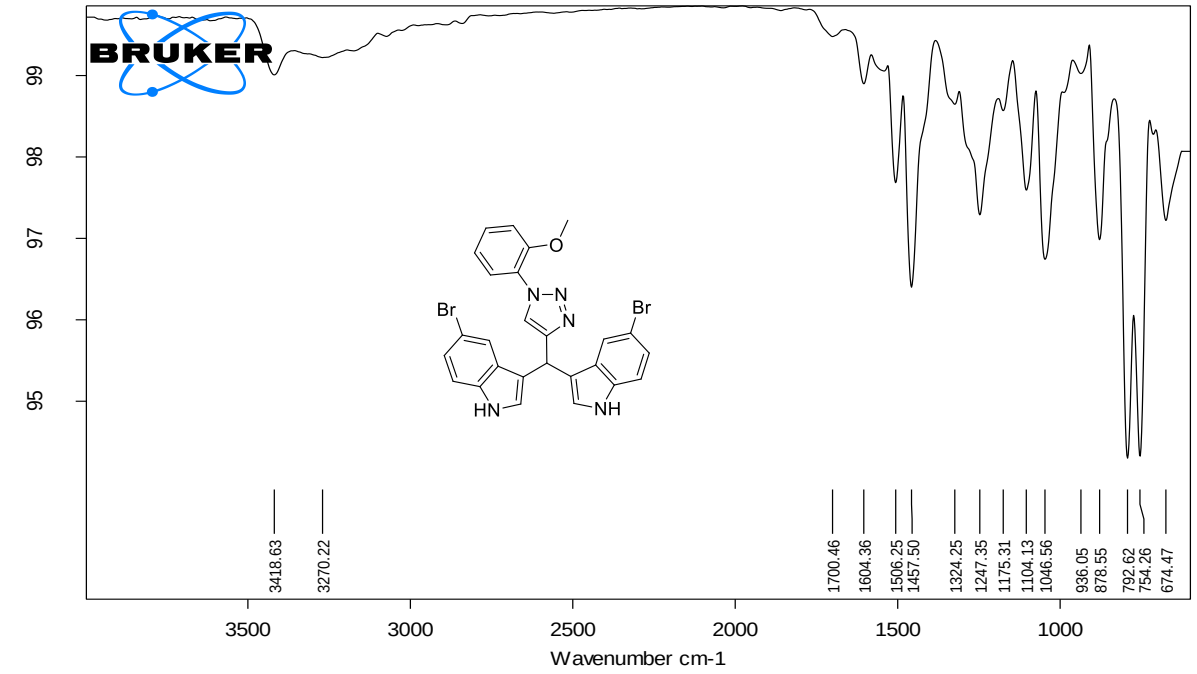

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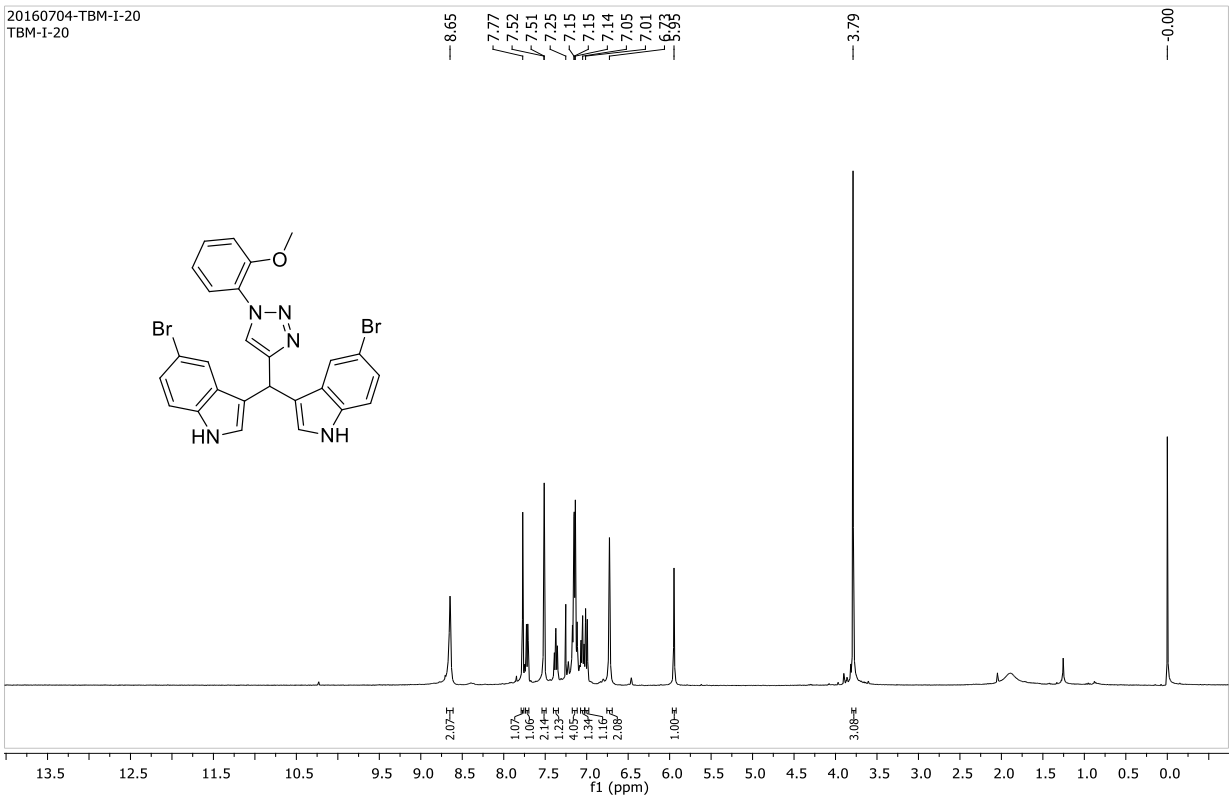
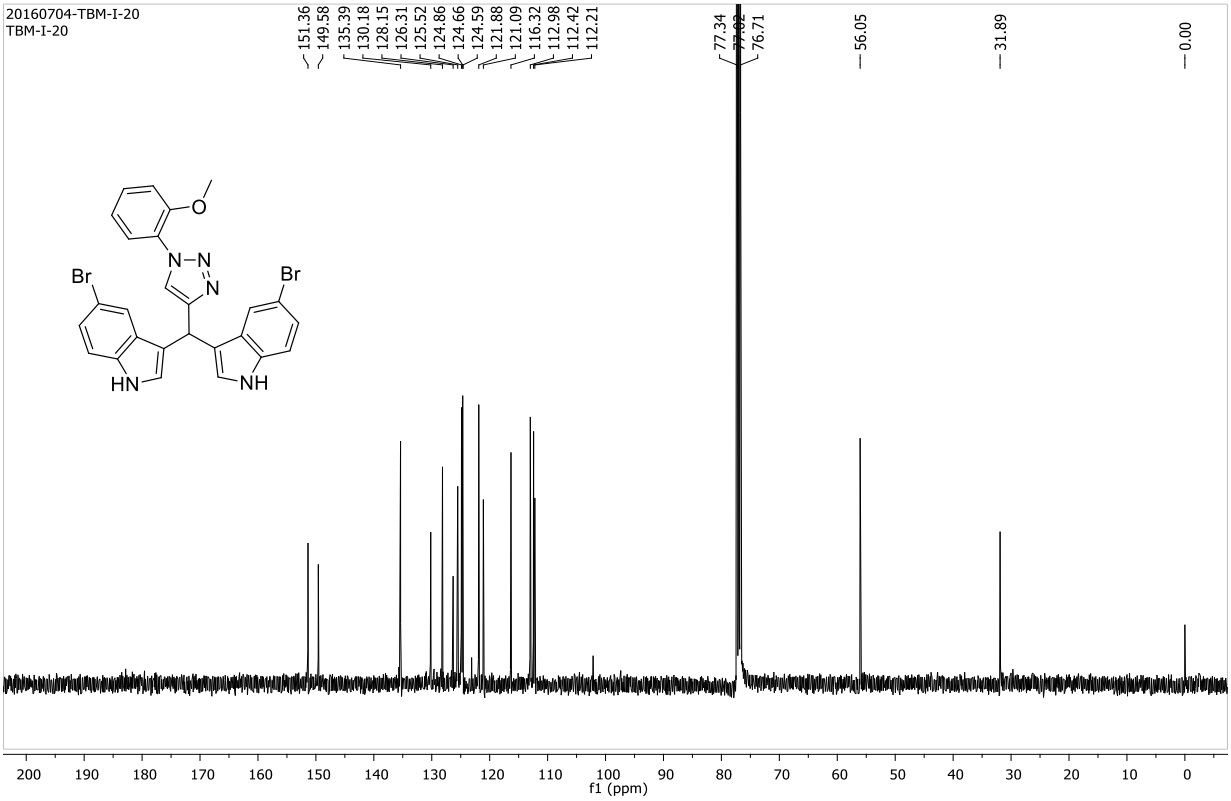
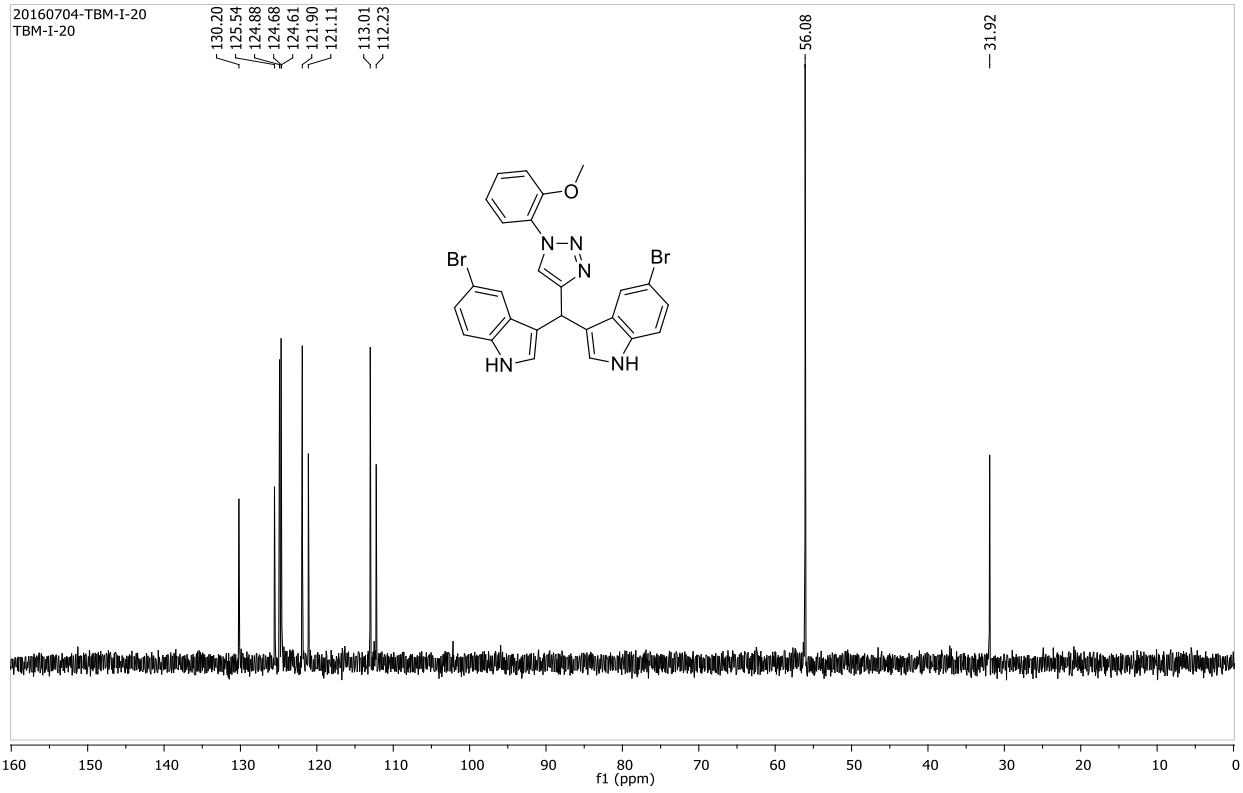
6p. ^{1}H NMR, 400 MHz, $CDCl_3$



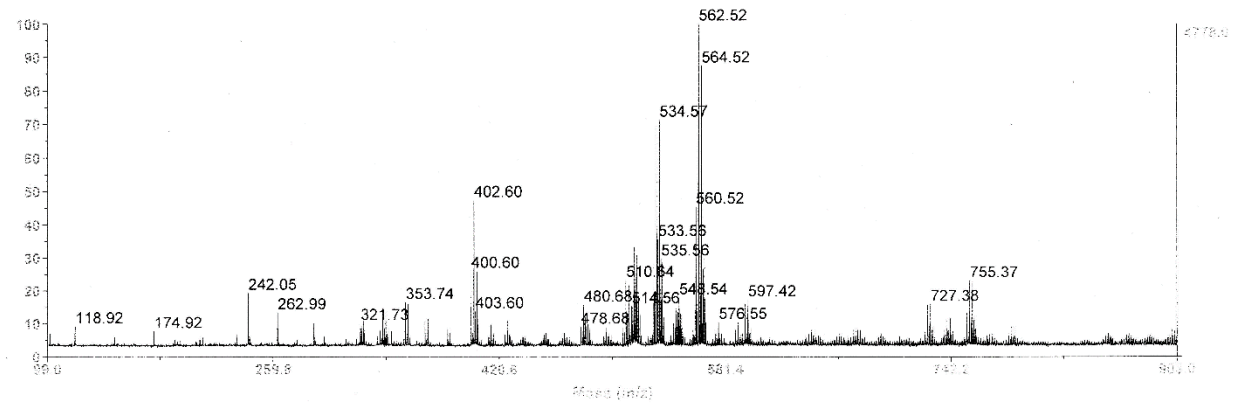
6p. ^{13}C NMR, 100 MHz, $CDCl_3$



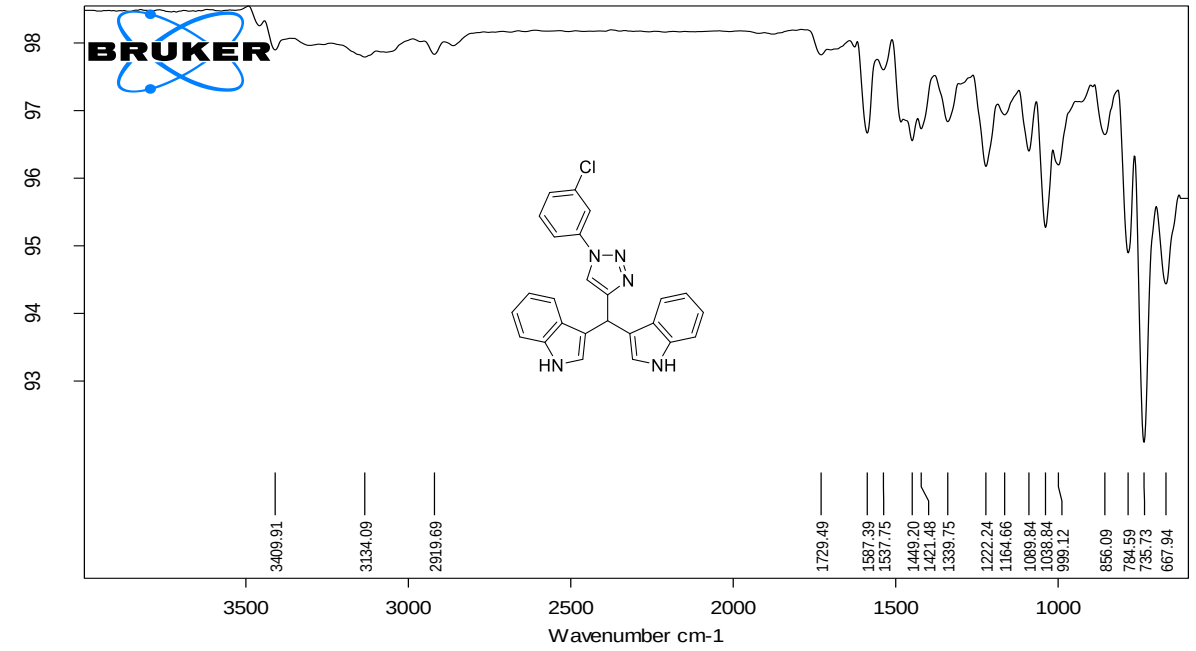
6p. DEPT NMR, 100 MHz, $CDCl_3$



6p. Mass



6q. FTIR



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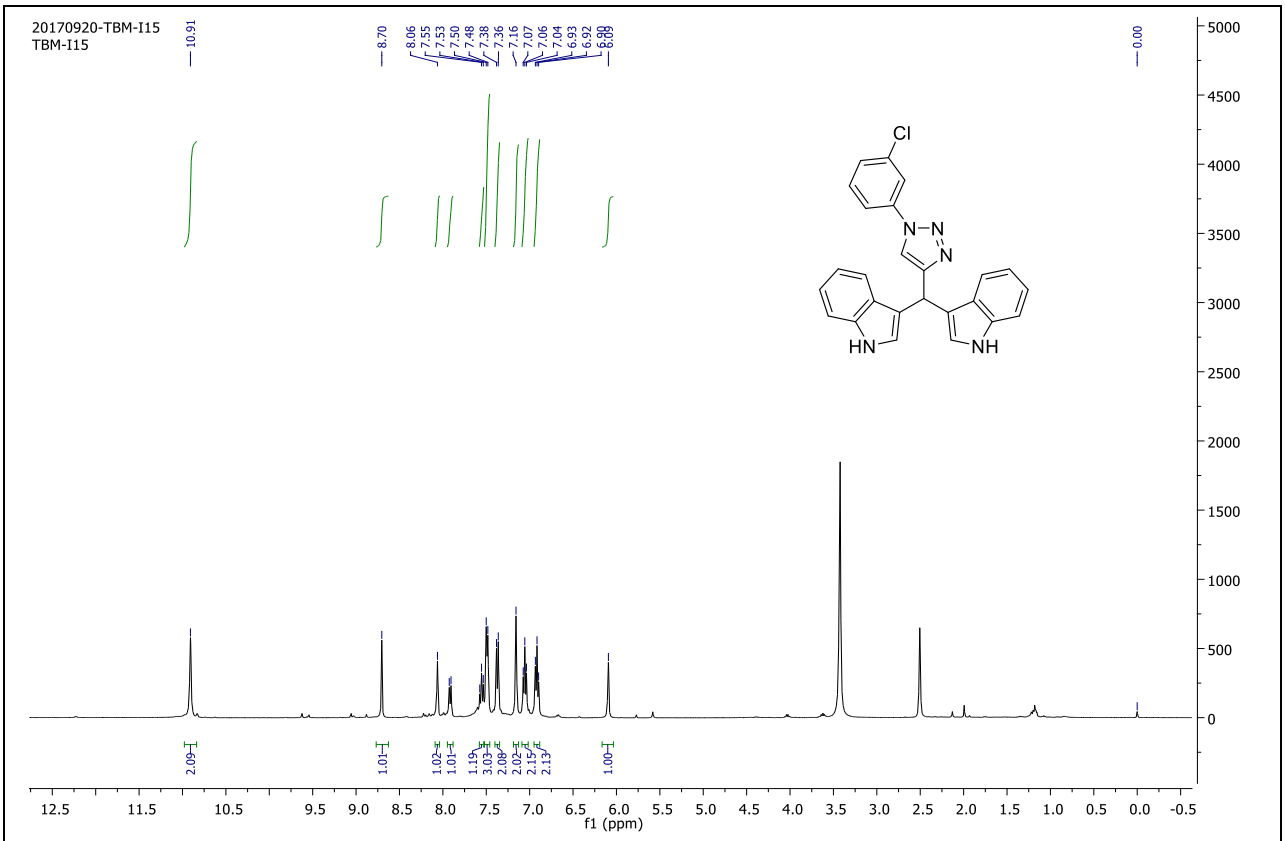
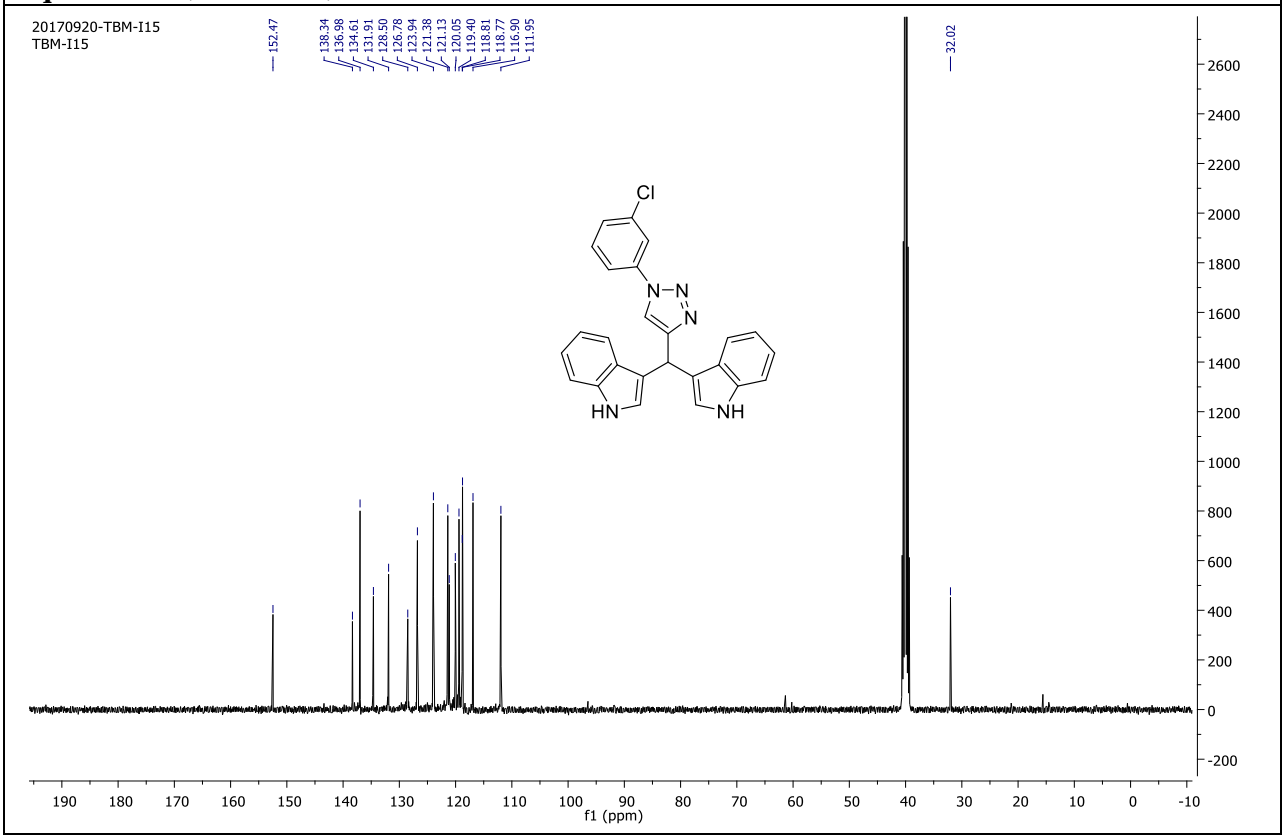
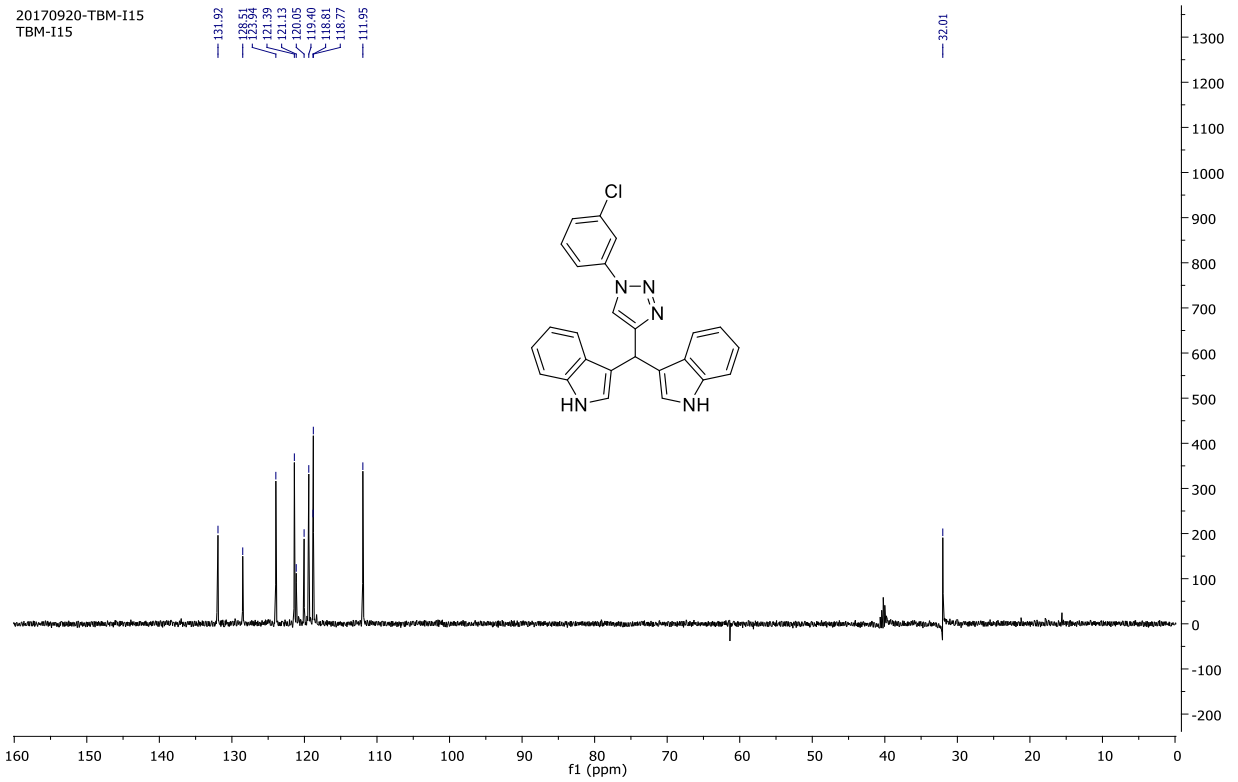
6q. ^{1}H NMR, 400 MHz, DMSO-d_6



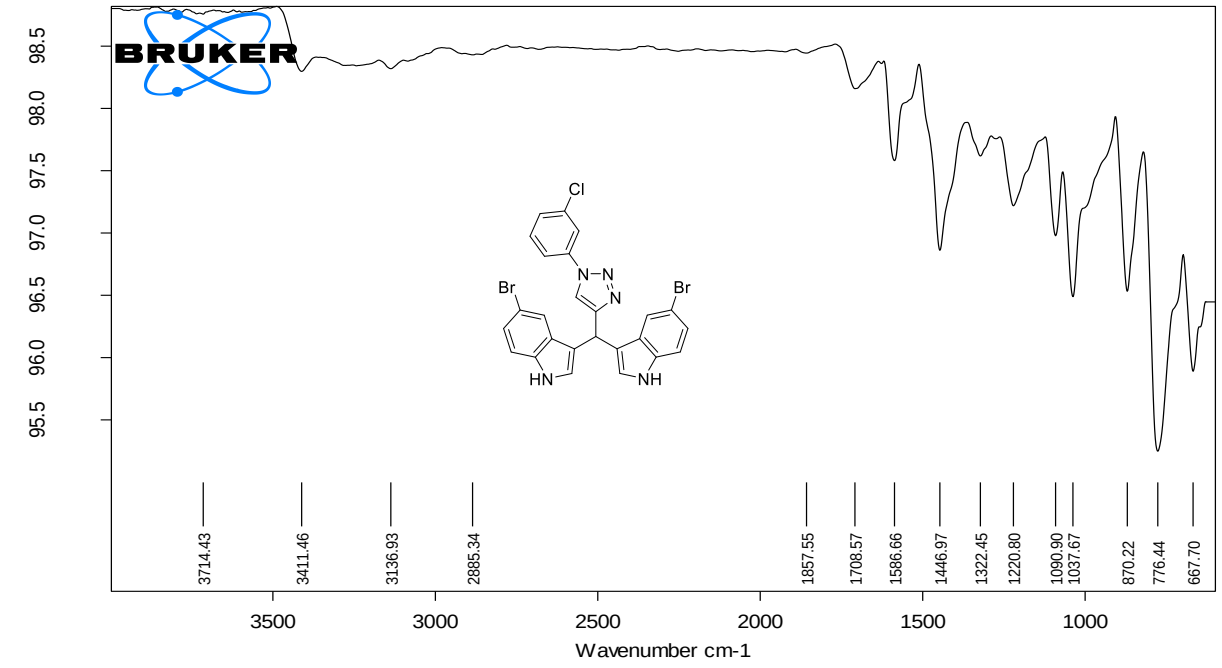
6q. ^{13}C NMR, 100 MHz, DMSO-d_6



6q. DEPT NMR, 100 MHz, DMSO-d_6



6r. FTIR



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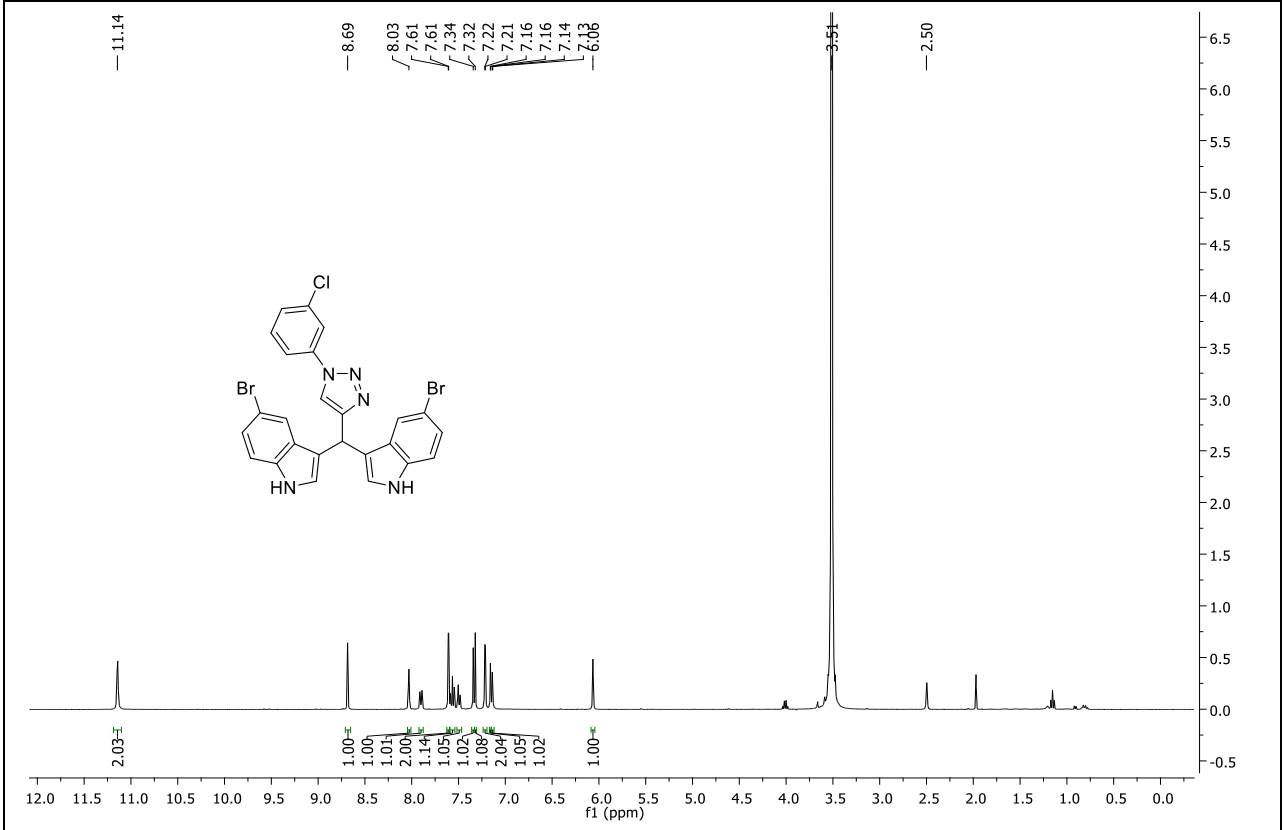
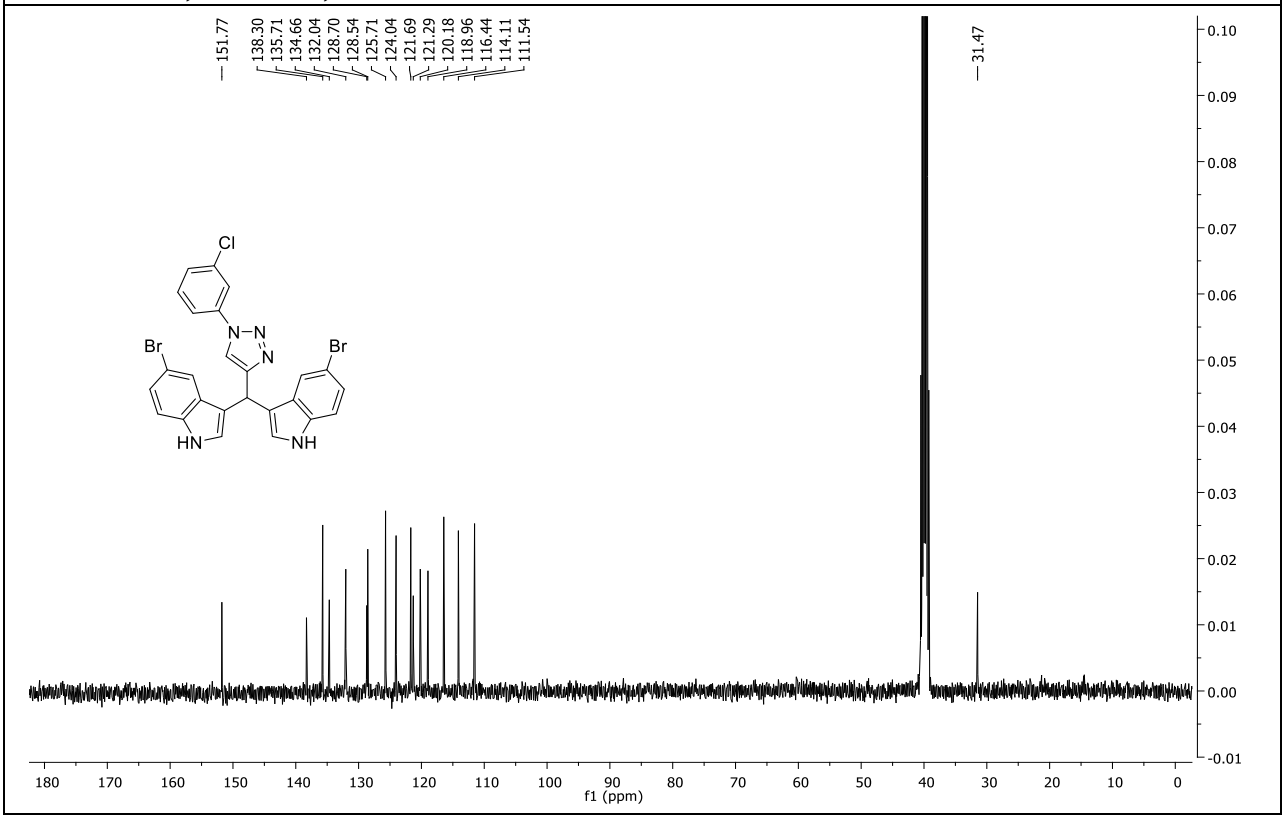
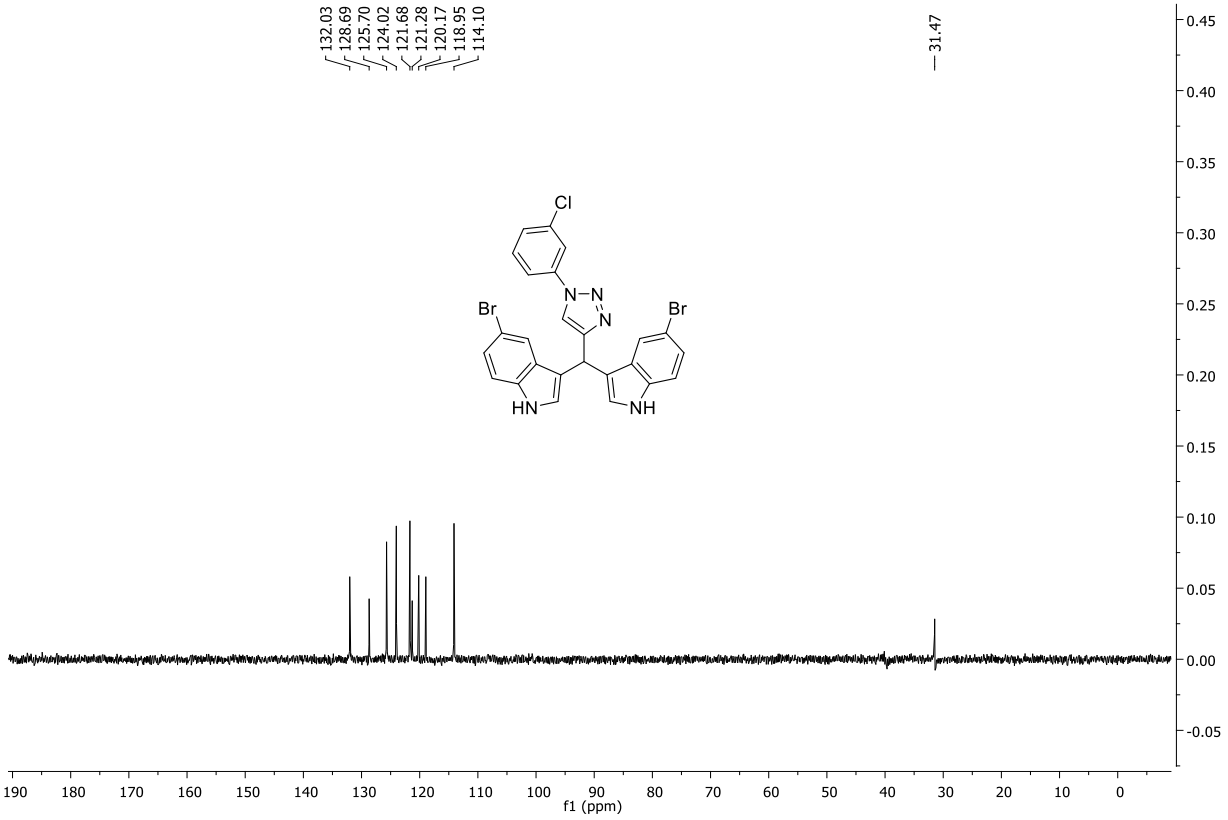
6r. ^{1}H NMR, 400 MHz, DMSO-d_6



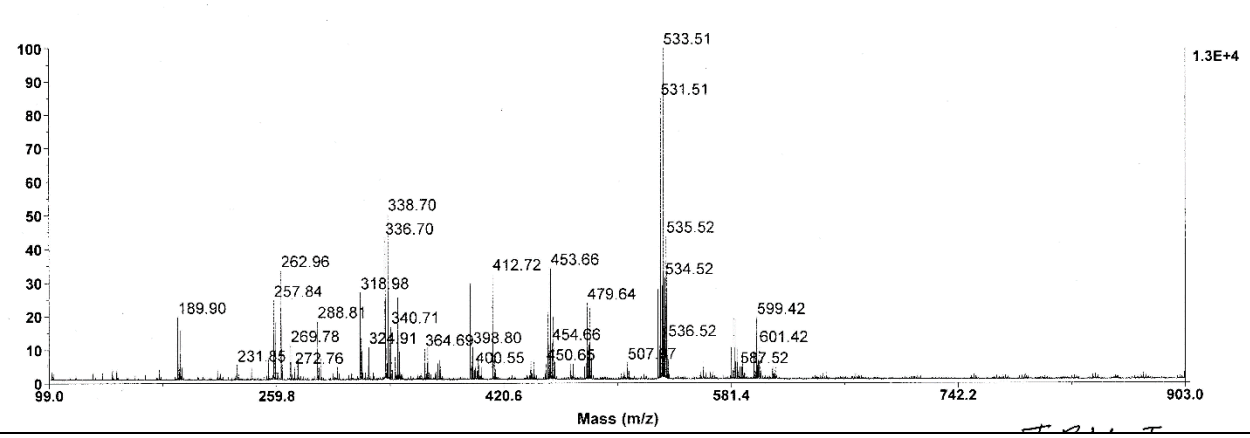
6r. ^{13}C NMR, 100 MHz, DMSO-d_6



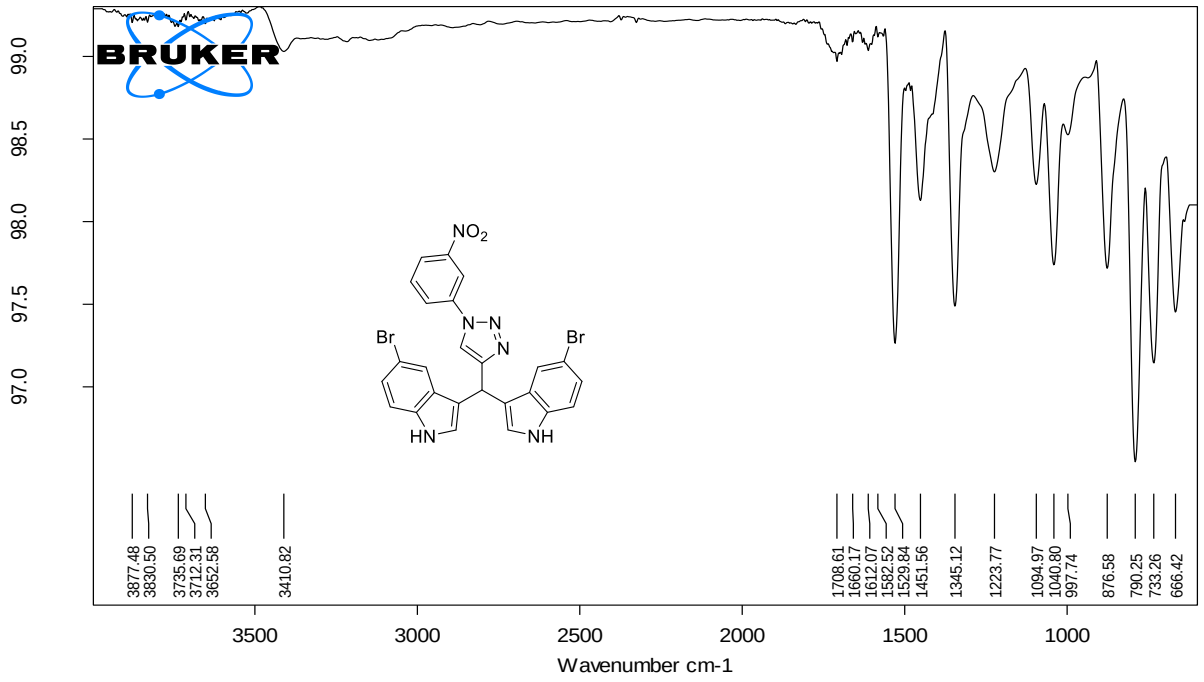
6r. DEPT NMR, 100 MHz, DMSO-d_6



6r. Mass



6s. FTIR



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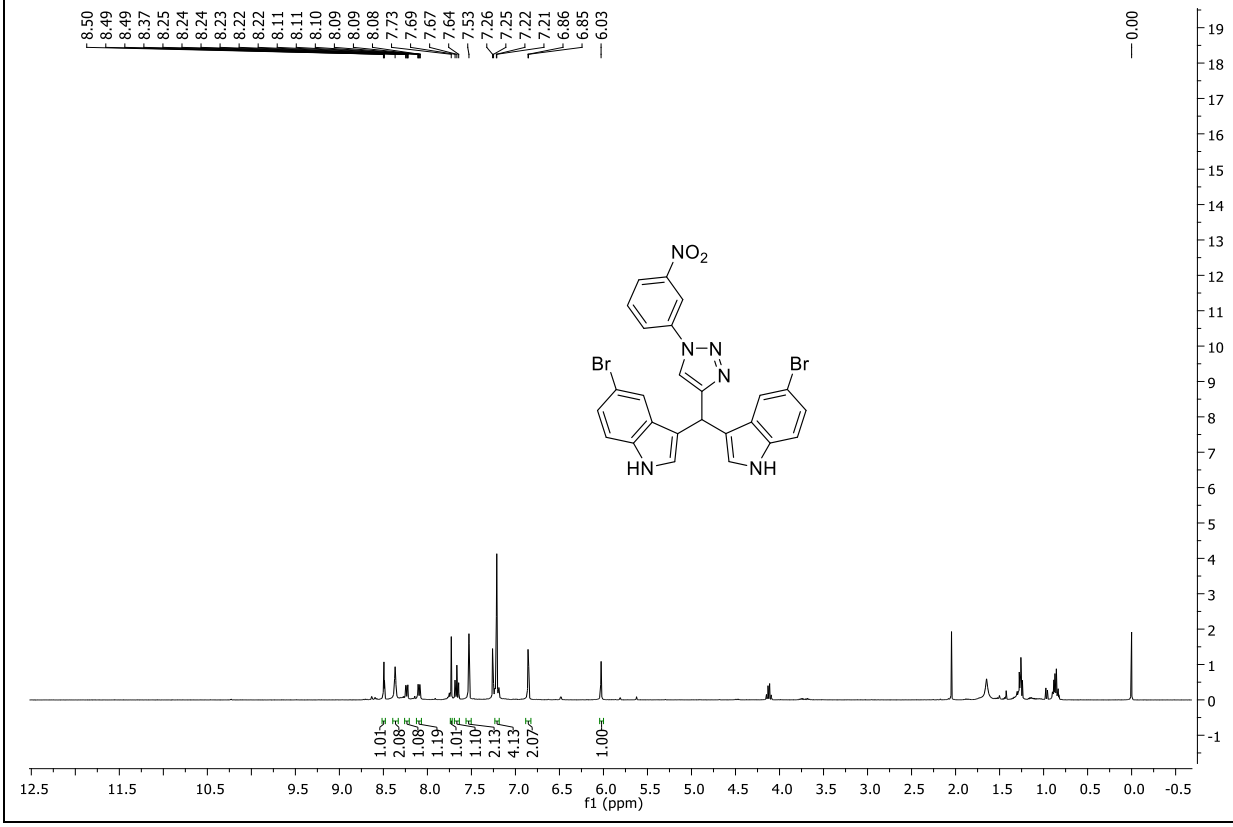
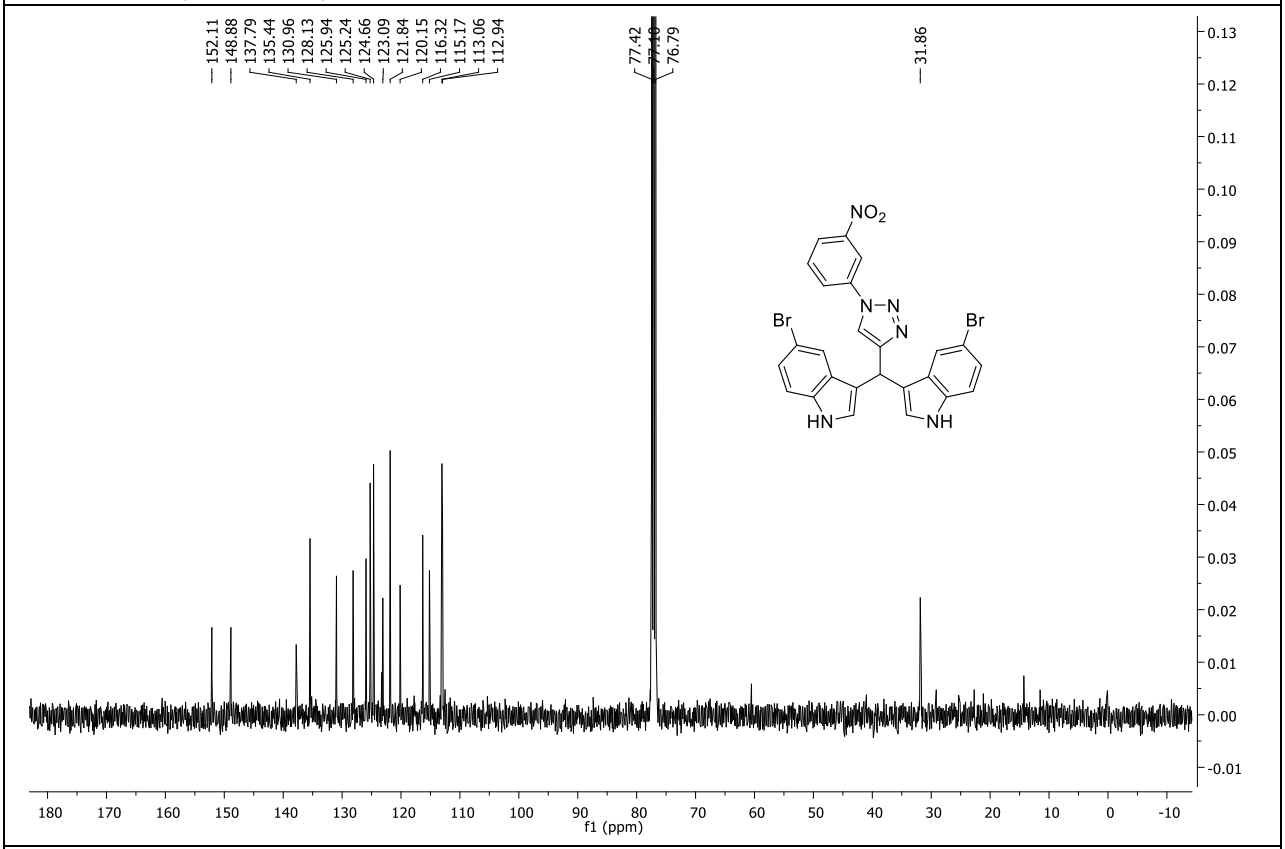
6s. ^{1}H NMR, 400 MHz, $CDCl_3$



6s. ^{13}C NMR, 100 MHz, $CDCl_3$



6s. DEPT NMR, 100 MHz, $CDCl_3$