Supporting Information belonging to the manuscript:

Combining Synthetic Biology with Synthetic Electrochemistry to Expand The Chemical Space of Indolocarbazoles Family

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Figure S1. The indolocarbazoles (ICZs) family. The indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole backbone, are an important class of N-containing heterocycles that have attracted a plethora of attention owing to extraordinary therapeutic potential. The prominent family members are rebeccamycin, staurosporine, AT2433, and k252a, among others.

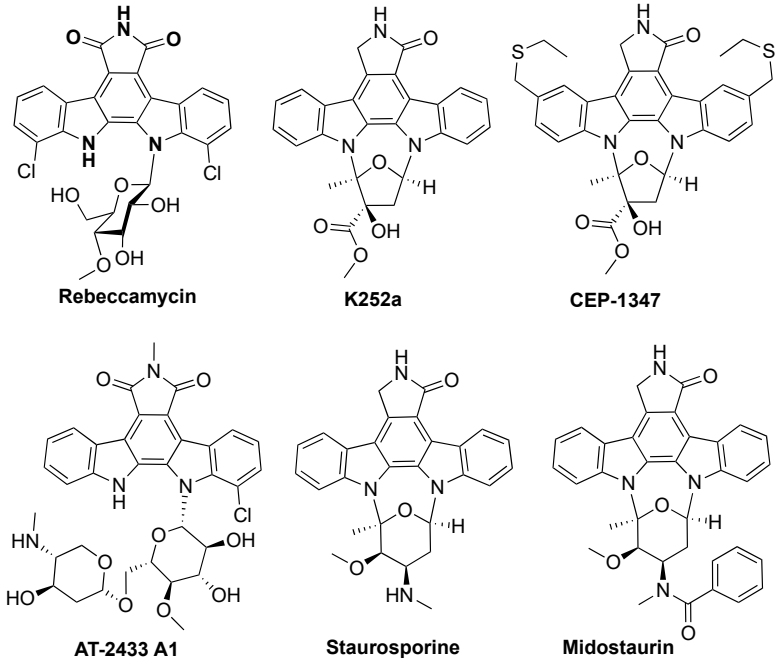


Figure S2. The shunt pathway of k252c. Precursor feeding did not increase K252c production but resulted in dose-dependent accumulation of indole-3-carboxylaldehyde (ICA), which had previously been shown to result from chemical degradation of Im-IPA.

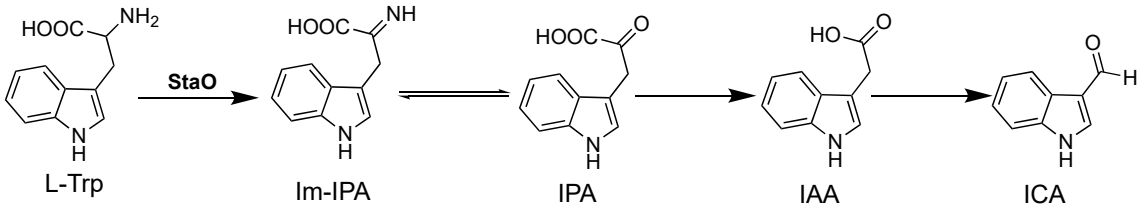


Figure S3. HPLC-DAD profiling of enzymatic glycosylation of k252c and k252d. Both substrates were transformed into complex mixtures by BsGT1. The glycoside products were judged by the characteristic UV spectra of substrates. The peaks labelled with number in the chromatogram were identified glycoside products as presented in Figure 3, whereas those labelled with question marks remained unidentified.

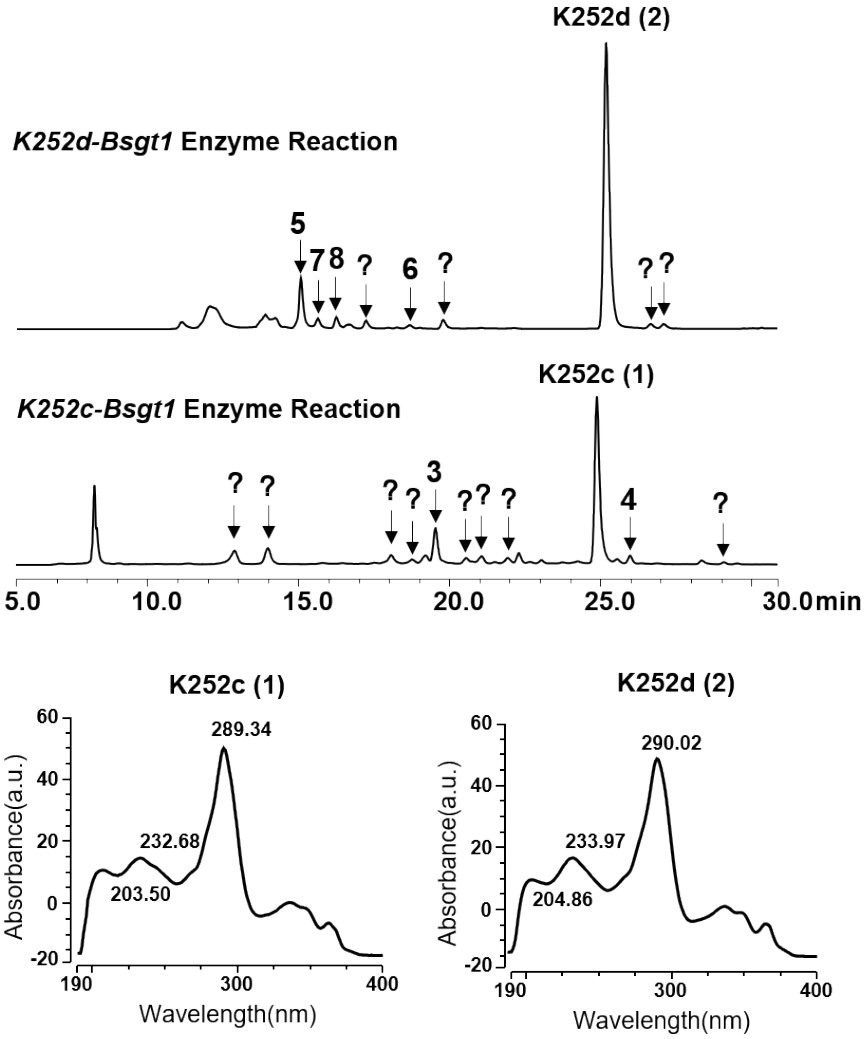


Figure S4. HPLC-DAD profiling of electroorganic reactions.

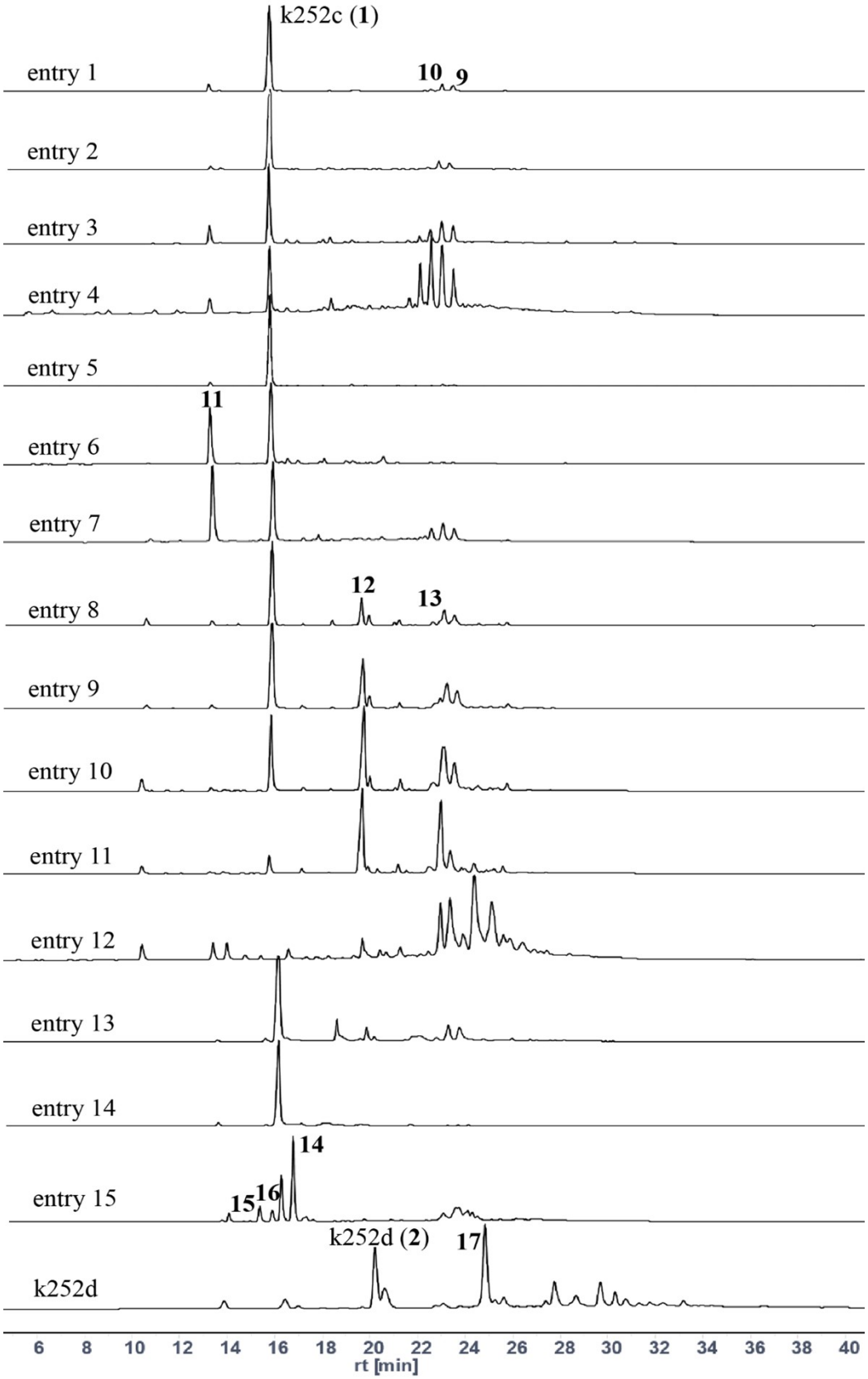


Figure S5. N−N dimer is prone to undergo homolytic cleavage. Compound **10** was dissolved in MeOH at concentration of 0.1 mg/mL, and decomposed in a time-dependent manner.

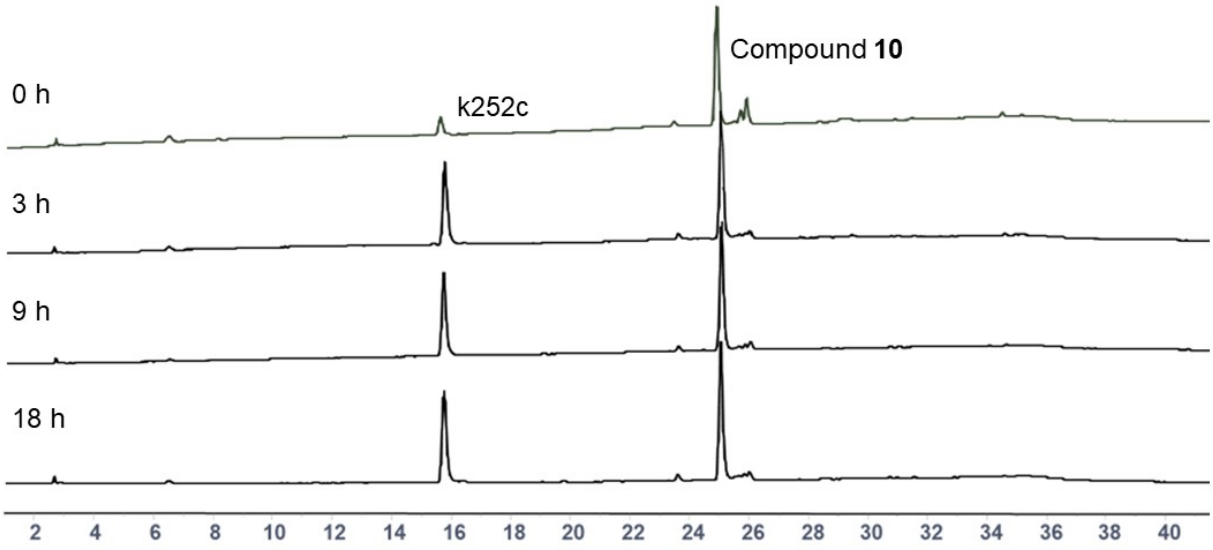


Table S1. Strains and plasmids used in this study.

Organisms/Strains	Description	Source/references
Escherichia coli DH5α	F φ80*lacZ ΔM15 Δ*(*lacZYA-argF*) U169 *recA1 endA1 hsdR17(rk⁻ mk⁺ phoA ΔsupE44 thi-1 gyrA96 relA1* λ-	Life Technologies
Escherichia coli BAP1	BL21(DE3) *ΔprpRBCD::PT7-sfp-PT7-prpE*	(Pfeifer and Admiraal 2001)
Escherichia coli BL21(DE3)	*E. coli* B F⁻*ompT hsdS*$_B$ (*r*$_B$⁻*m*$_B$⁻) *gal dcm* (DE3)	This study
Escherichia coli str. K-12 substr. W3110	F-λ-rph-1 INV(rrnD,rrnE)(DE3)(CamR)	(Gu et al, 2012)
Escherichia coli W3110-60	W3110 *ΔtrpR::FRT*	(Gu et al, 2012)
Escherichia coli W3110-62	W3110 *ΔtrpR::FRT*Δ*tnaA::FRT*Δ*pstG::FRT*	(Gu et al, 2012)
Escherichia coli W3110-63	W3110 *ΔtrpR::FRT*Δ*tnaA::FRT*Δ*pstG::FRT ΔtrpL::FRT*	(Gu et al, 2012)
Escherichia coli W3110-Wu	The T7 RNA polymerase system was inserted into the genome of *Escherichia coli* W3110-63	This study
Escherichia coli W3110-K252	Heterologous expression of pET28a-K252c in *Escherichia coli* W3110-Wu	This study
Escherichia coli W3110-Rha	Heterologous expression of pET28a-Rha in *Escherichia coli* W3110-Wu	This study
Escherichia coli W3110-KR	Heterologous expression of pET28a-K252c and pET28a-Rha in *Escherichia coli* W3110-Wu	This study
pET28a	*E. coli* expression vector, ColEl ori, Km^r	Novagen
pET28a-K252	pET28a derivative containing genes *staODPC,* obtained by commercial DNA synthesis, Km^r	This study
pET28a-Rha	pET28a derivative containing genes *rfbABCD* from *E. coli* K-12, Km^r	This study
p15A-origin	*E. coli* expressionvector, Cm	Addgene
p15A-T7 RNAp	Ligation of the T7pol-lacI-lys from BL21(DE3)、W3110-up-HA(1000bp) and W3110-down-HA(1000bp) to p15A-Kan, Kmr	This study

Table S2. Primers used in this study. (Normal: homologous arms; Bold: core primers; Underlined: Restriction site.)

Primer	Sequence（5’ → 3’）	Function
T7 RNAp-F	CCTTAAACGCCTGGTTGCTACG**GCCATGGTGTCCGACTTATG**	cloning T7pol-lacI-lys from BL21(DE3)
T7 RNAp-R	TAAAAAAACAGGGAGGCTATTA**GGAACTGGCTGTTGTTACCG**	
Cm-F	GATAACAATCATTCCCGAAG**TTGATCGGCACGTAAGAGGTTC**	cloning *Cm* from p15A-origin
Cm-R	CATAAGTCGGACACCATGGC**CGTAGCAACCAGGCGTTTAAGG**	
W3110-UP-F	**GAAGACGAGGTAATGTGAGG**	cloning upstream homologous arm from W3110(DE3) for recombination
W3110-UP-R	**CTTCGGGAATGATTGTTATC**	
W3110-DOWN-F	**TAATAGCCTCCCTGTTTTTTTAG**	cloning downstream homologous arm from W3110(DE3) for recombination
W3110-DOWN-R	**GAAAAACTGGACGCTGAAGG**	
p15A-F	CCTTCAGCGTCCAGTTTTTCCGAGCTCGCTTGGACTCCTG	PCR linearize the plasmid p15A
p15A-R	CCTCACATTACCTCGTCTTCCGTCTCATTTTCGCCAGATATC	
Rfb-F	GTGCCGCGCGGCAGC<u>CAT</u>**ATG**AAAATACTTGTTACTGGTG	cloning *Rfb* from MG1655 for synthetic rhamnose
Rfb-R	TGTCGACGGAGCTC<u>GAATTC</u>**TCATGCAATTAATTTTAATCT**	
StaG-T7-F	GGGTCTTGAGGGGTTTTTTG**CAATTGCGTCCGGCGTAGAGGATCG**	cloning *staG* into plasmid pET28a-staG for gene recombination
StaG-T7-R	GAAGCTTCTCGAGGAATTC**CAAAAAACCCCTCAAGACCCG**	
pET28a-F	**GAATTCGAGCTCCGTCGACAAG**	PCR linearize the plasmid pSET28a
pET28a-R	**ATGGCTGCCGCGCGGCACC**	

Table S3. Antiproliferative assays of ICZs. All the 17 ICZs derivatives in this study were tested inhibition against 20 human cell lines, whereby each compound was applied at 20 μM. The presented inhibitory rates are mean value of three independent experiments. Doxorubicin hydrochloride (Dox) at 10 μM was used as a positive control.

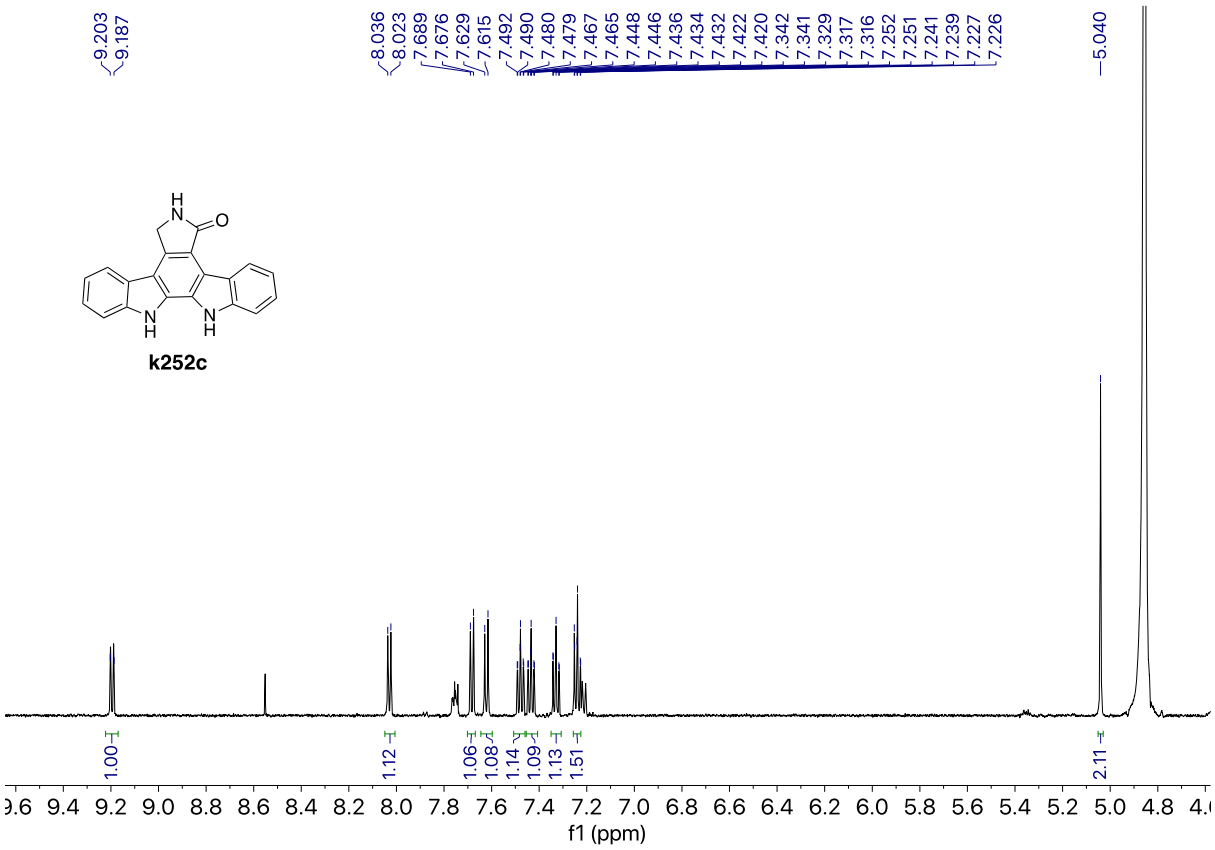
	Cell inhibition±SD（%）				
Compound	A549	MKN-45	HCT 116	HeLa	K-562
1	81.38±0.86%	86.39±0.86%	63.24±3.01%	56.73±3.57%	47.41±2.89%
2	87.36±0.53%	87.69±1.24%	73.10±0.54%	99.18±0.36%	43.87±0.57%
3	1.43±2.14%	15.29±3.04%	3.24±1.01%	27.53±0.81%	9.08±2.99%
4	1.95±2.27%	11.46±2.66%	1.92±3.32%	7.76±2.47%	3.45±5.82%
5	-0.76±2.92%	6.67±2.24%	0.34±3.01%	9.05±2.50%	11.72±1.81%
6	10.01±2.74%	22.81±0.70%	12.52±1.72%	16.41±2.75%	15.80±2.78%
7	1.98±3.24%	6.39±2.47%	0.28±3.18%	9.96±0.86%	-1.00±5.74%
8	9.47±1.32%	14.21±1.20%	10.74±0.76%	10.56±1.71%	11.90±1.96%
9	16.25±2.86%	29.40±1.05%	1.84±1.96%	15.98±2.21%	13.39±2.95%
10	1.67±0.70%	16.16±0.73%	0.83±2.49%	8.52±1.66%	13.63±1.40%
11	89.36±2.69%	93.58±0.35%	93.17±1.06%	99.22±0.43%	86.68±0.37%
12	12.56±2.27%	25.50±3.09%	2.83±1.23%	11.95±2.29%	14.22±1.58%
13	-1.10±4.45%	20.21±3.85%	3.71±3.16%	7.51±2.17%	22.09±5.52%
14	3.73±2.89%	14.49±3.82%	1.57±2.37%	-0.28±3.85%	9.42±1.61%
15	6.21±3.05%	4.21±5.36%	1.13±2.55%	3.91±1.55%	18.05±2.34%
16	2.12±1.28%	14.46±0.92%	-0.47±1.68%	2.25±3.49%	21.46±1.32%
17	33.10±1.84%	67.01±2.34%	35.52±1.08%	42.11±0.95%	42.36±3.01%
Dox	89.02±0.27%	81.59±1.45%	75.19±1.78%	98.91±0.42%	63.85±1.20%
	Cell inhibition±SD（%）				
Compound	786-O	TE-1	5637	GBC-SD	L-02
1	99.13±0.12%	82.90±0.99%	96.47±0.77%	62.92±1.21%	82.76±2.20%
2	96.65±0.28%	92.30±1.33%	98.34±0.38%	67.82±2.84%	88.41±2.17%
3	11.42±1.32%	23.24±0.33%	33.72±2.77%	16.23±2.16%	1.42±2.28%
4	1.20±3.71%	6.07±3.46%	3.39±2.71%	10.74±3.45%	1.39±2.68%
5	1.81±0.90%	-2.14±2.38%	12.34±2.30%	4.16±0.54%	-0.75±1.84%
6	1.25±1.19%	1.05±2.12%	29.80±2.29%	2.35±1.67%	4.01±1.84%
7	3.19±2.24%	-0.58±1.53%	6.93±0.80%	-1.76±4.34%	1.17±3.18%
8	3.60±2.76%	-1.97±2.41%	27.32±0.69%	2.27±2.43%	0.88±3.56%
9	31.70±6.00%	3.78±2.78%	36.61±1.37%	32.84±3.39%	8.61±3.90%
10	4.25±1.39%	0.29±3.47%	1.18±6.29%	8.29±3.54%	2.00±3.20%
11	99.49±0.86%	96.33±0.93%	98.67±0.26%	91.64±0.84%	96.98±0.66%
12	14.28±2.76%	-0.17±4.55%	23.24±2.44%	1.07±6.94%	13.92±1.41%
13	6.86±1.62%	2.34±3.98%	1.45±3.83%	12.44±1.27%	4.21±1.72%
14	3.93±2.11%	4.61±2.68%	1.50±3.64%	1.55±0.68%	12.74±2.55%
15	16.20±3.09%	16.31±2.38%	31.82±1.07%	17.33±2.52%	26.38±0.85%
16	2.72±0.71%	1.32±2.02%	8.72±1.24%	9.78±0.98%	21.07±0.49%
17	70.41±4.79%	15.03±2.27%	74.25±1.51%	69.06±1.90%	33.49±0.89%
Dox	95.82±0.52%	79.10±0.94%	97.59±0.20%	81.45±1.86%	98.78±0.14%
	Cell inhibition±SD（%）				
Compound	MCF7	HepG2	SF126	DU145	CAL-62
1	12.16±1.21%	90.72±0.72%	74.43±1.71%	75.55±1.27%	78.58±1.81%
2	44.49±1.42%	86.22±2.74%	81.36±3.92%	88.19±1.57%	85.91±0.54%
3	9.46±3.12%	4.51±2.65%	31.98±1.46%	17.99±1.35%	7.09±2.82%
4	6.23±4.22%	-1.34±5.35%	21.08±1.84%	11.96±2.48%	5.56±1.71%
5	0.74±2.71%	-0.43±0.52%	3.32±1.83%	7.65±0.82%	3.77±2.18%
6	2.07±2.86%	1.45±4.46%	13.02±1.02%	15.91±3.29%	18.58±3.86%
7	1.33±1.69%	1.69±4.01%	2.44±2.85%	6.50±3.22%	3.32±2.66%
8	1.93±0.55%	-1.56±2.41%	11.49±3.07%	7.36±0.81%	15.17±0.82%
9	-3.99±3.34%	36.70±2.42%	25.22±1.33%	19.83±0.56%	5.30±2.01%

Compound	Cell MCF7	HepG2	SF126	DU145	CAL-62
10	9.98±3.14%	7.69±1.37%	8.16±1.31%	9.52±3.09%	7.09±2.37%
11	88.75±2.14%	98.30±0.77%	98.58±0.50%	93.28±0.47%	95.41±1.33%
12	3.38±4.96%	26.57±3.31%	14.56±3.39%	5.44±3.00%	0.42±2.24%
13	12.79±1.38%	27.21±0.33%	12.14±0.12%	5.56±1.51%	13.52±2.42%
14	35.72±0.66%	79.27±1.11%	60.57±2.61%	43.05±0.58%	18.08±2.63%
15	16.72±2.98%	34.76±3.06%	13.49±2.12%	28.02±4.74%	25.18±0.74%
16	9.91±3.54%	5.53±0.15%	10.75±2.06%	19.08±2.59%	12.96±3.06%
17	9.06±0.32%	45.36±4.79%	41.26±1.85%	58.33±1.23%	52.03±3.22%
Dox	63.82±0.32%	91.62±0.37%	95.64±0.67%	68.65±0.34%	94.70±0.47%

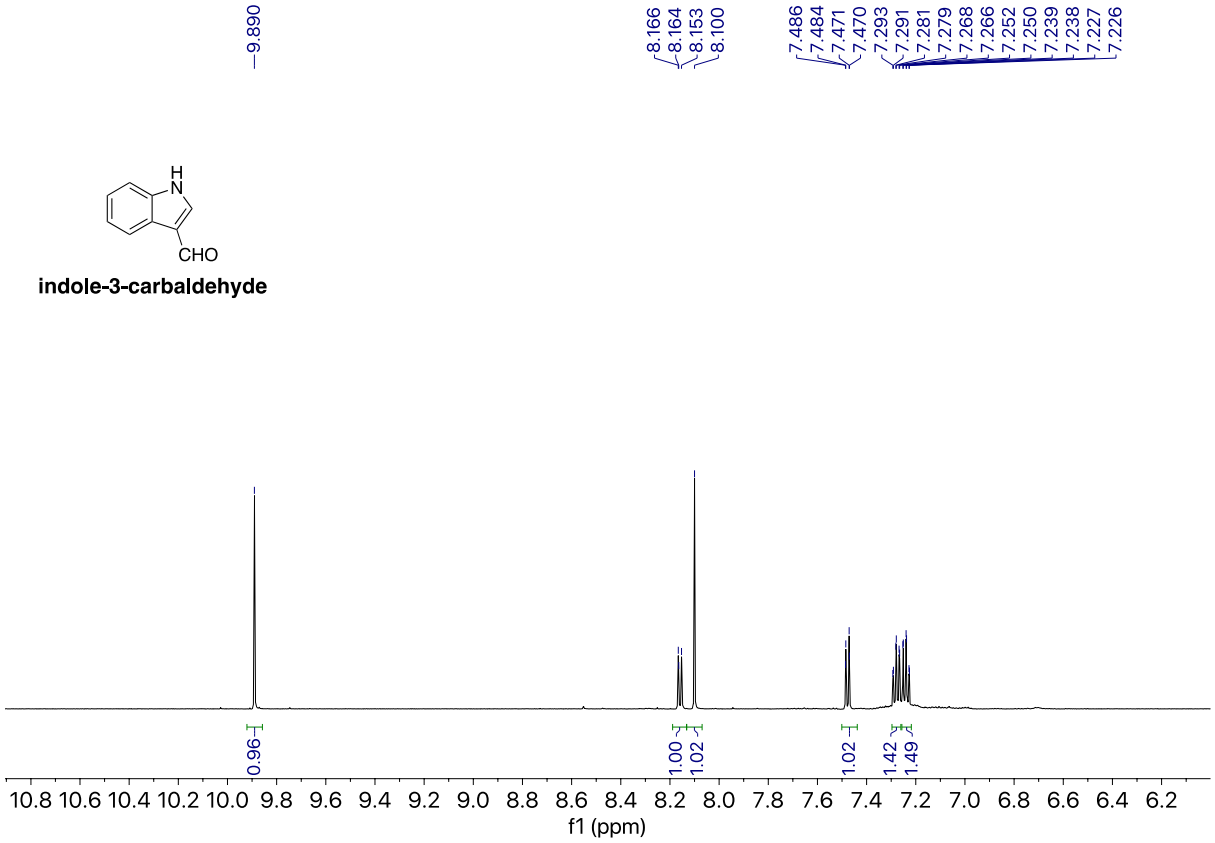
Compound	Cell inhibition±SD（%） PATU8988T	HOS	A-375	A-673	293T
1	50.27±2.71%	75.02±1.26%	47.83±1.66%	87.73±1.96%	68.60±1.64%
2	86.17±0.56%	77.24±3.01%	85.27±4.49%	84.57±1.19%	85.59±0.88%
3	33.16±2.56%	13.33±1.13%	21.48±1.98%	0.00±2.29%	21.45±3.49%
4	10.29±5.43%	10.80±2.13%	12.78±5.17%	11.46±6.72%	14.28±2.01%
5	12.69±4.52%	7.28±1.20%	9.76±2.23%	3.62±3.31%	15.45±1.67%
6	11.39±5.01%	25.02±4.12%	31.25±1.49%	14.02±8.10%	19.17±1.50%
7	8.73±1.77%	10.42±4.13%	12.52±2.26%	-17.02±5.05%	24.68±7.40%
8	16.46±3.72%	18.66±4.43%	34.91±2.91%	-5.51±7.48%	26.80±2.84%
9	1.03±2.27%	14.78±3.33%	26.45±0.73%	31.76±3.84%	43.56±3.35%
10	11.02±1.05%	5.41±1.61%	13.56±1.91%	16.08±2.37%	10.00±2.74%
11	95.94±1.19%	98.77±0.37%	98.02±0.82%	97.68±0.41%	98.78±0.77%
12	1.06±4.23%	10.84±3.91%	-0.64±4.20%	13.22±1.66%	39.60±3.62%
13	16.39±1.46%	13.88±2.49%	19.38±0.58%	18.09±4.16%	34.56±2.95%
14	1.06±1.15%	8.44±2.22%	9.37±0.89%	4.09±1.48%	25.31±3.96%
15	16.89±1.93%	21.27±1.58%	31.35±0.76%	0.95±1.85%	38.60±1.71%
16	-0.53±1.78%	9.77±2.98%	11.98±2.97%	0.05±2.09%	32.69±0.97%
17	17.71±1.55%	72.62±5.86%	93.32±0.36%	93.35±1.11%	83.24±5.75%
Dox	95.72±0.61%	94.56±0.29%	93.36±0.08%	91.95±0.68%	93.30±0.56%

All spectra employed in structure elucidation.

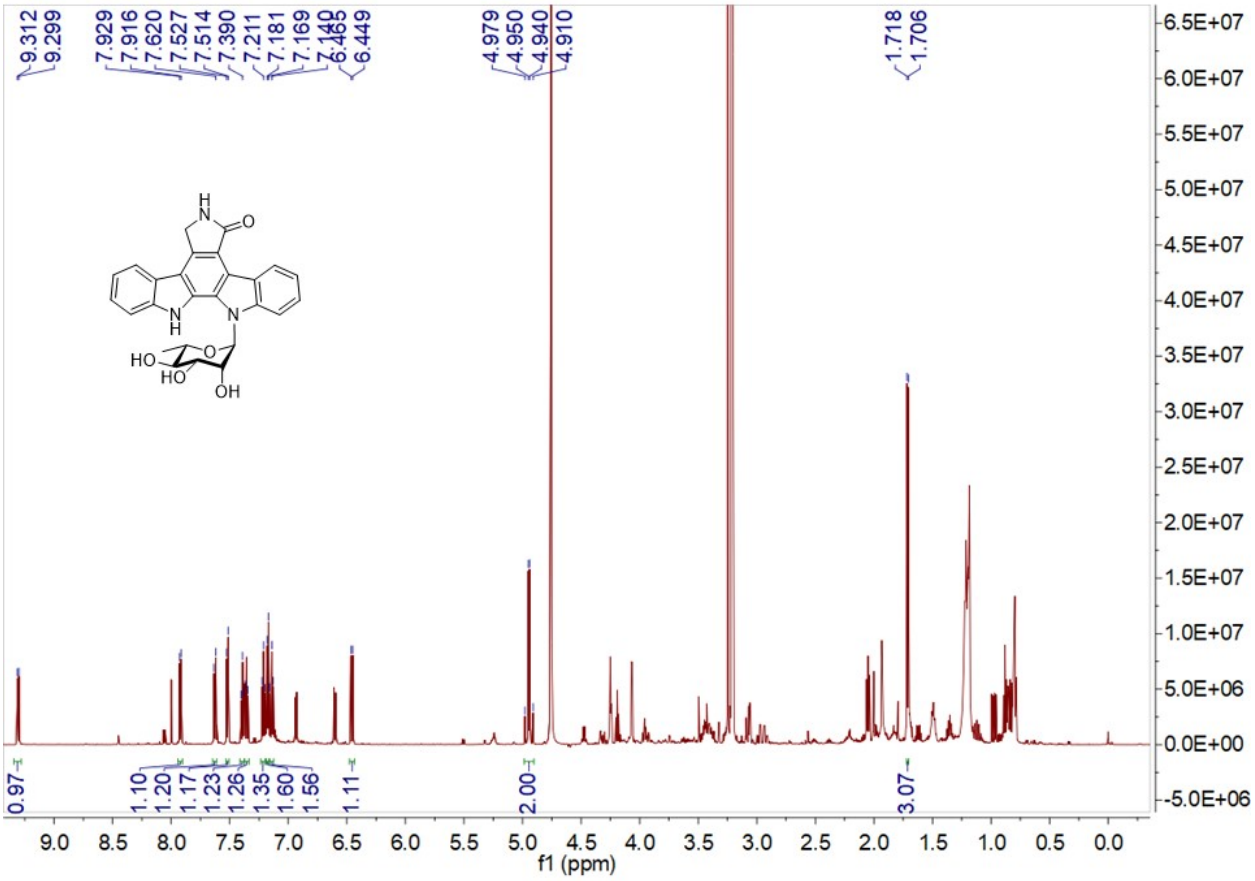
S1. ^{1}H NMR spectrum of k252c (1)



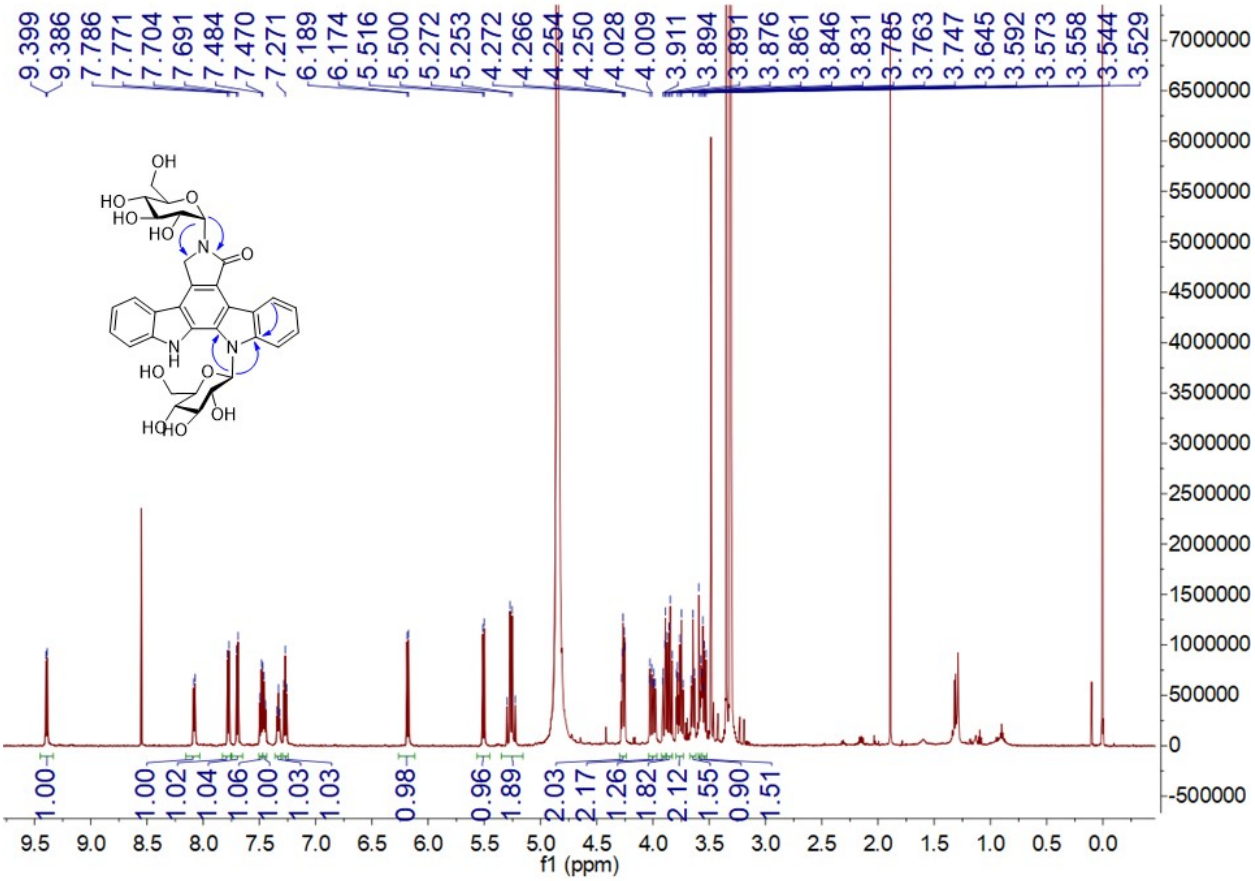
S2. ^{1}H NMR spectrum of indole-3-carbaldehyde.



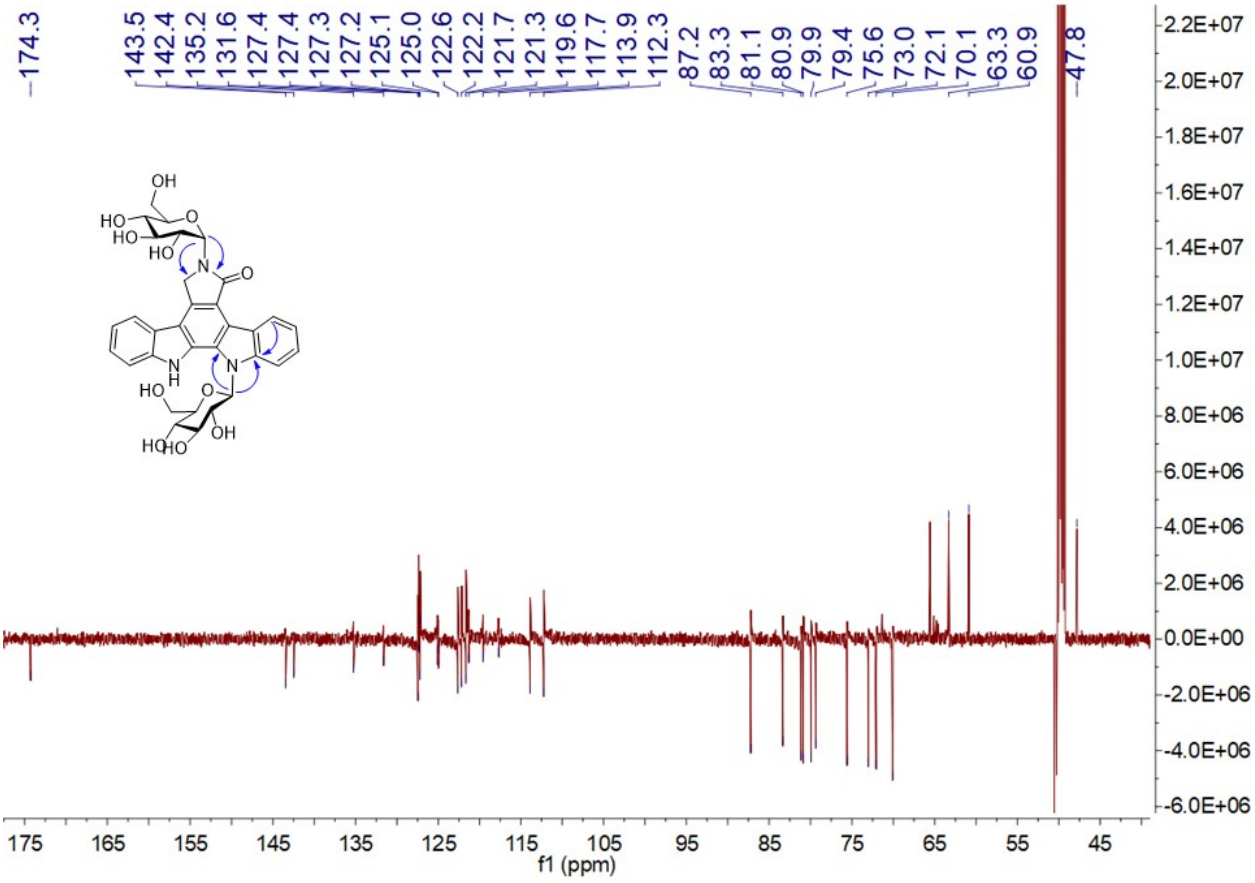
S3. ^{1}H NMR spectrum of k252d (2).



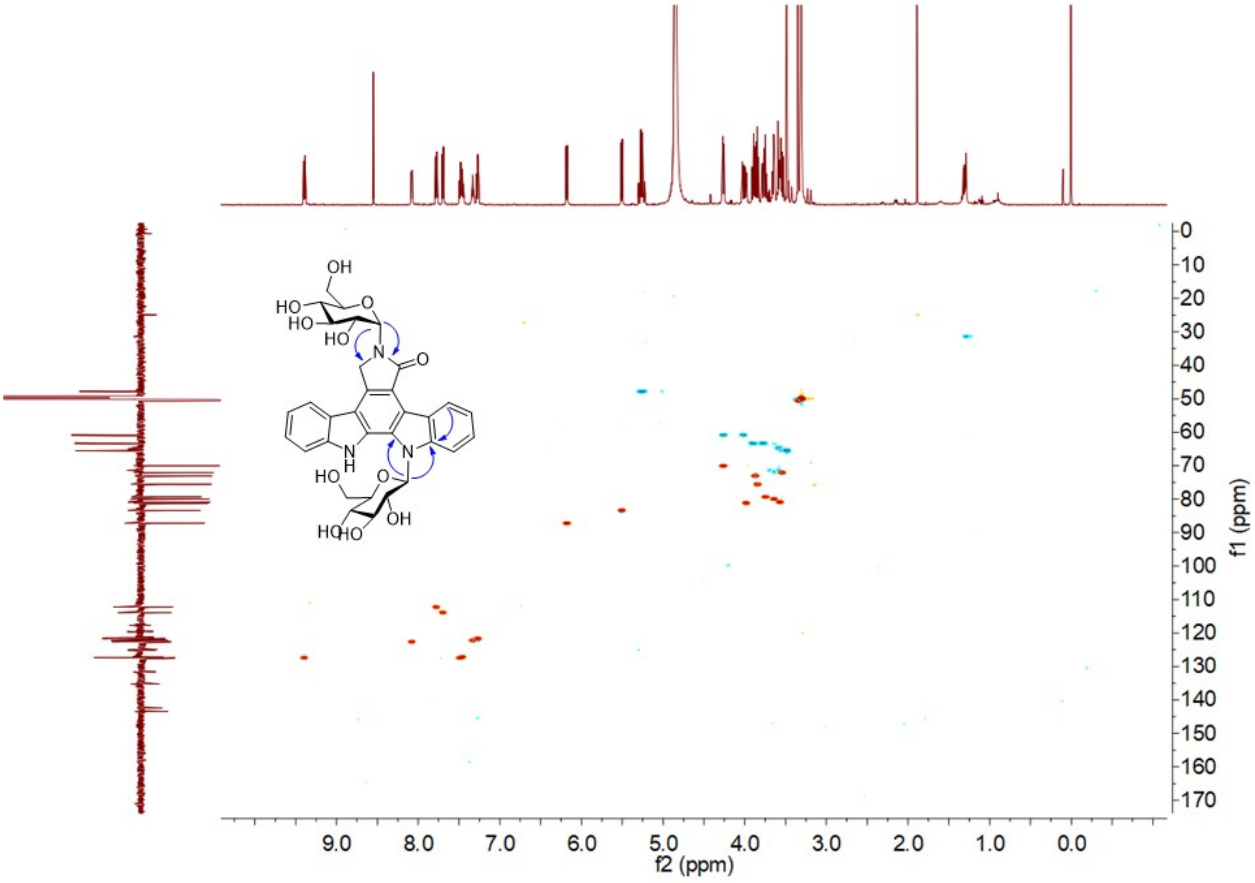
S4. ^{1}H NMR spectrum of (3).



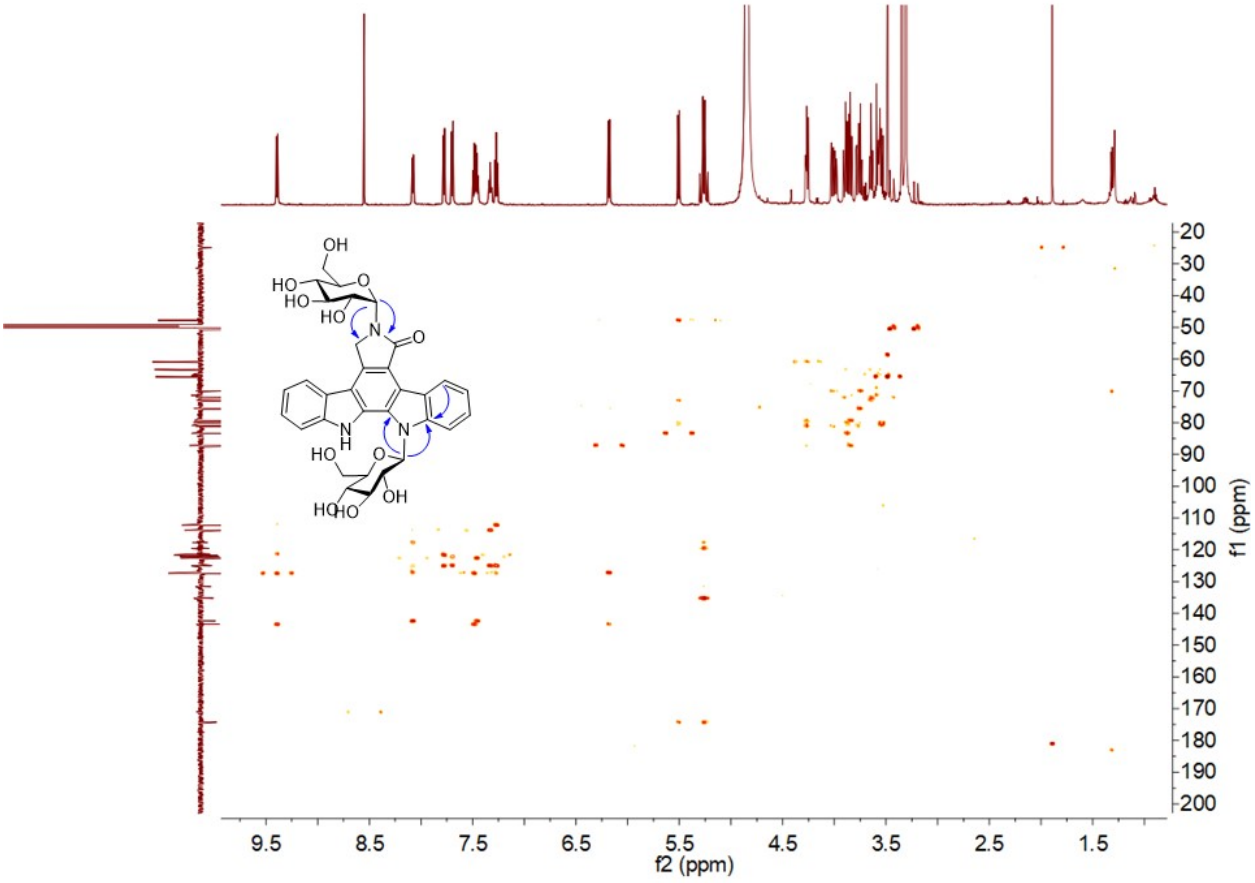
S5. ^{13}C NMR spectrum of (3).



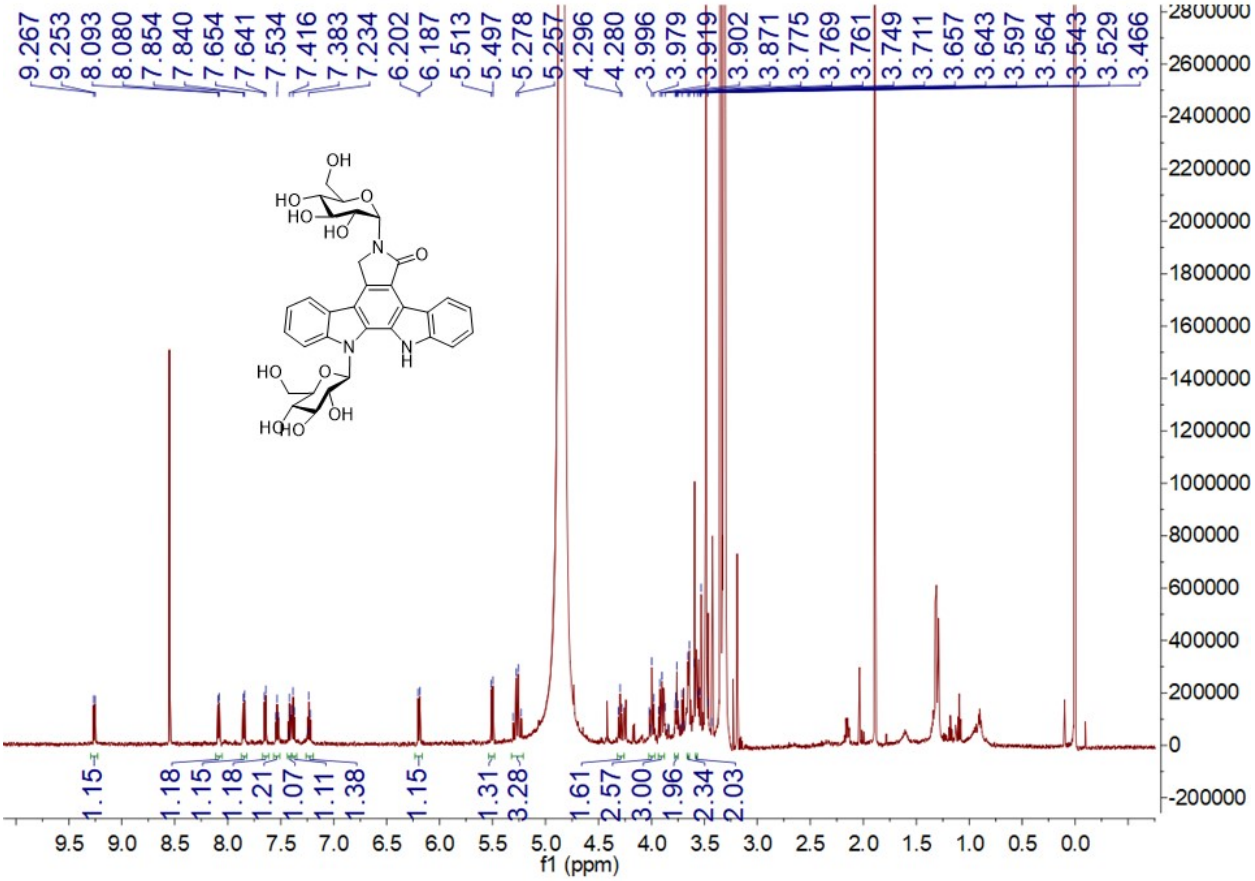
S6. HSQC spectrum of (3).



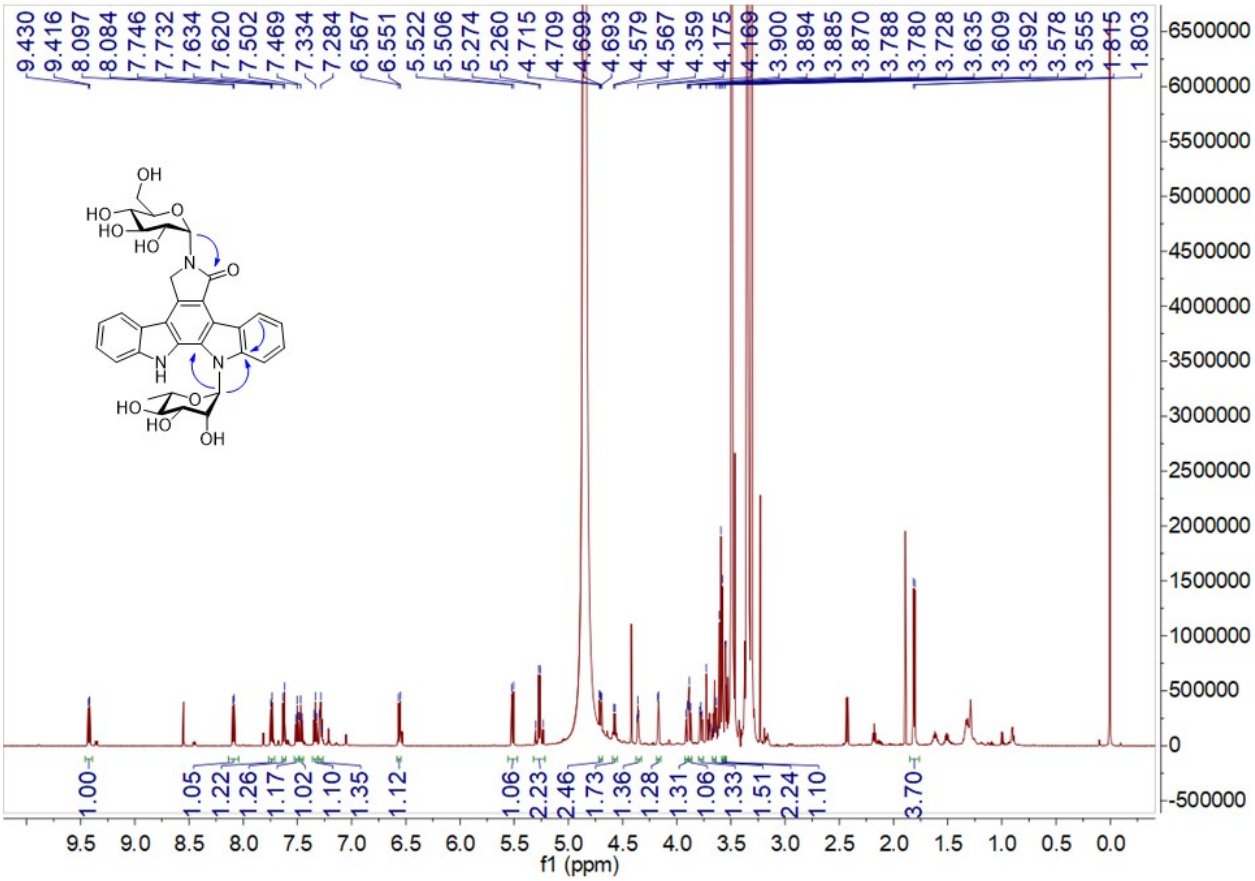
S7. HMBC spectrum of (3).



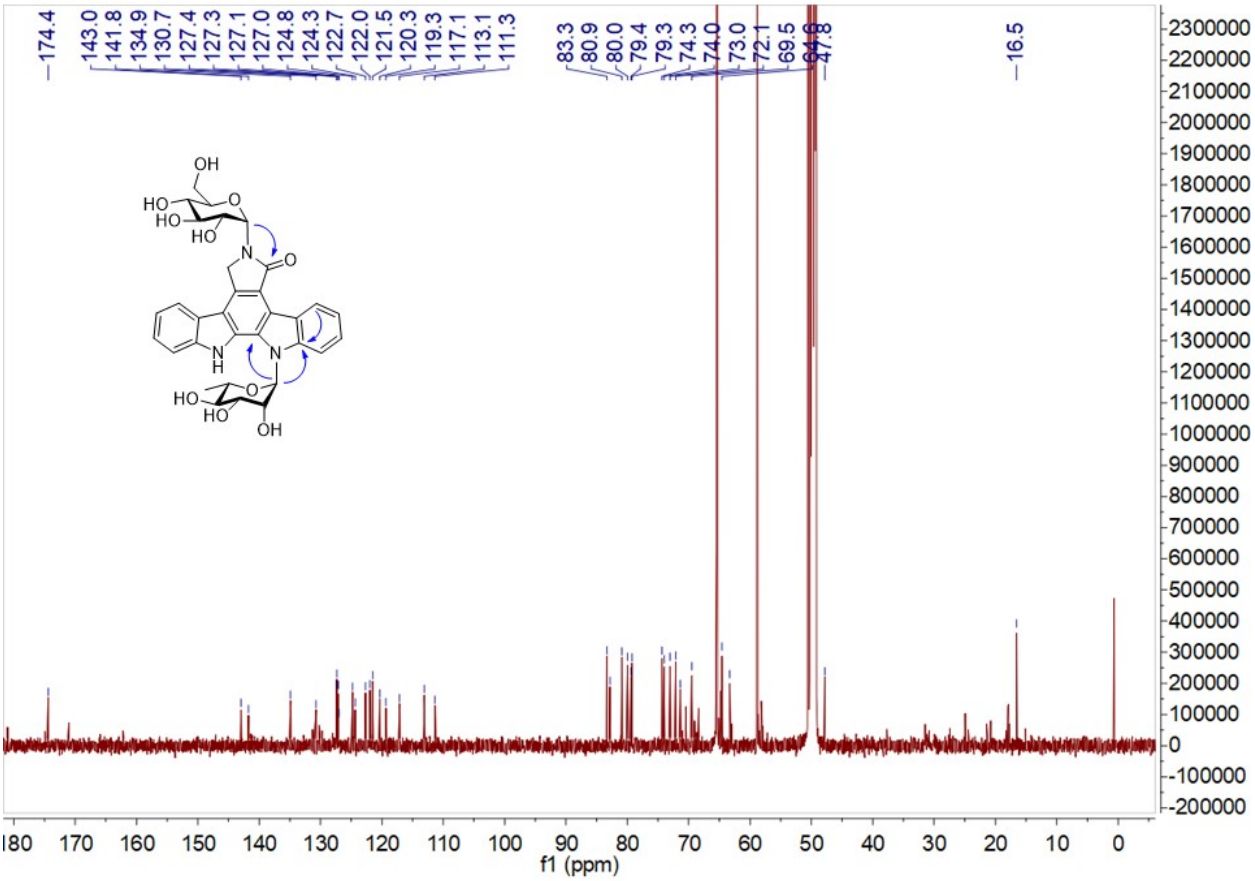
S8. ^{1}H spectrum of (4).



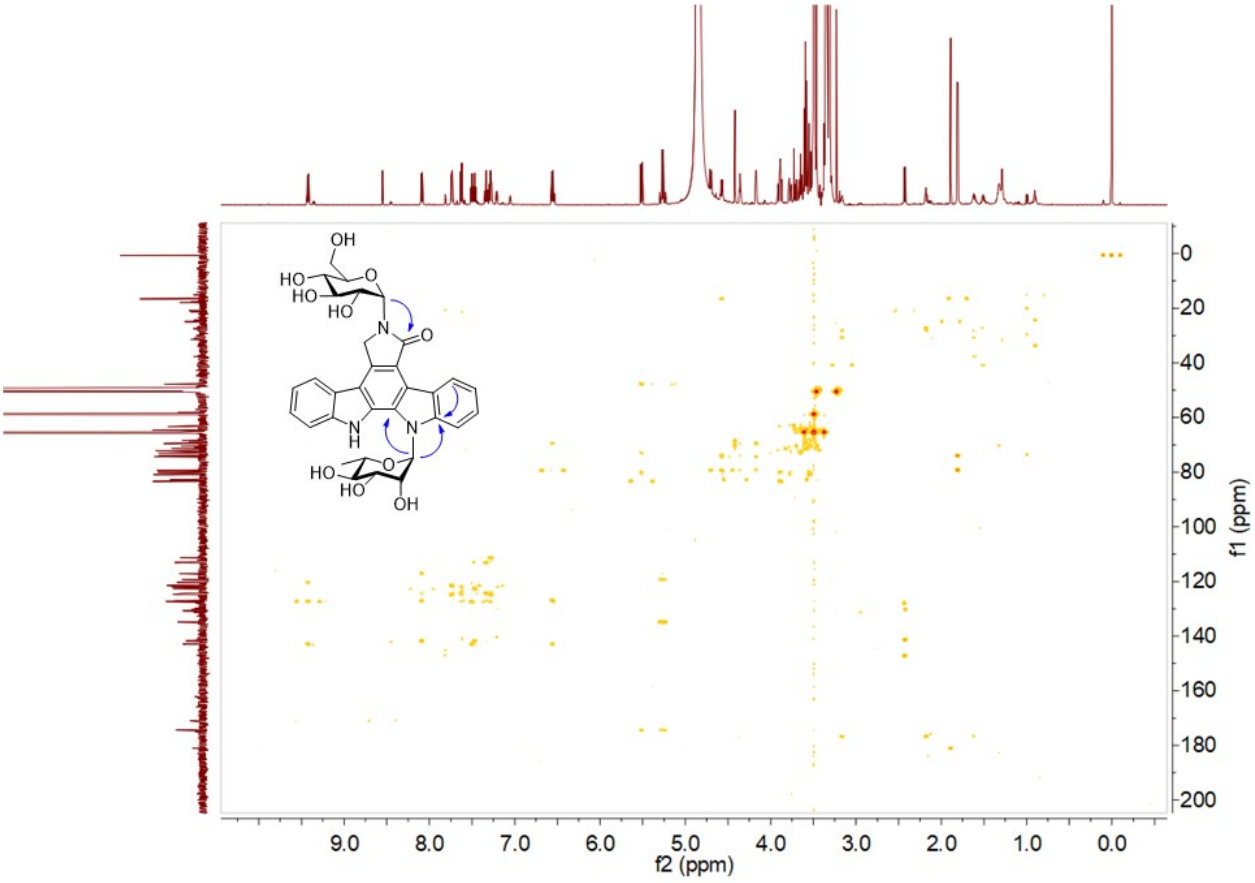
S9. ^{1}H NMR spectrum of (5).



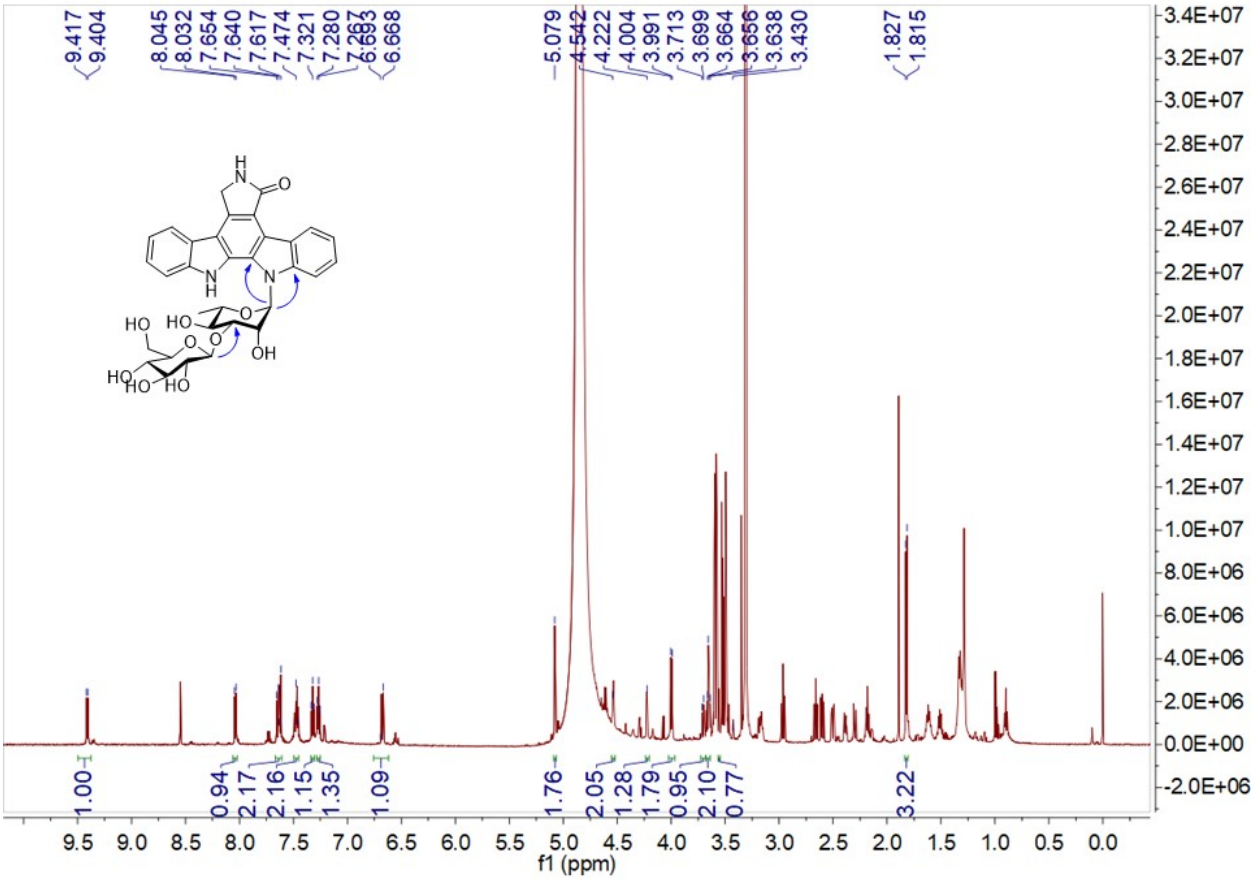
S10. ^{13}C NMR spectrum of (5).



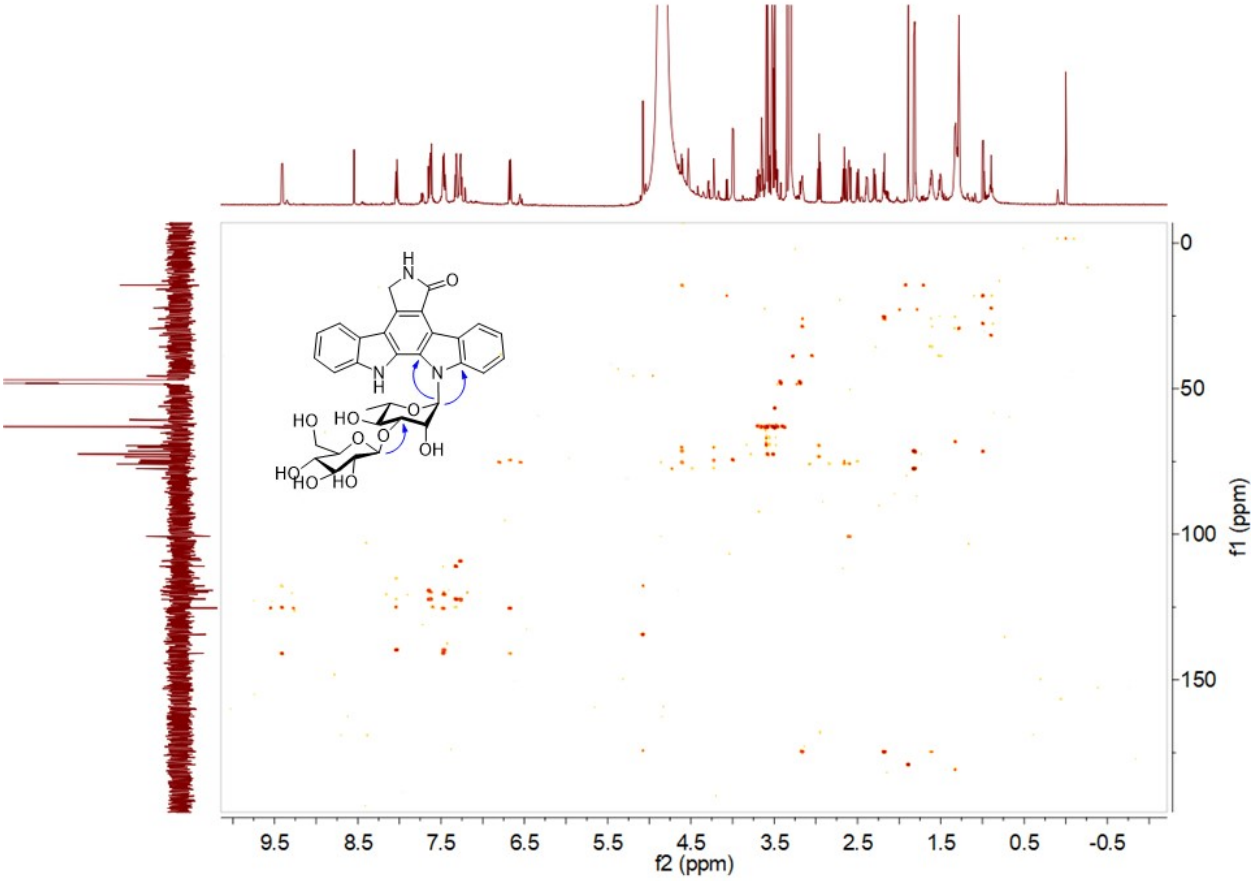
S11. HMBC spectrum of (5).



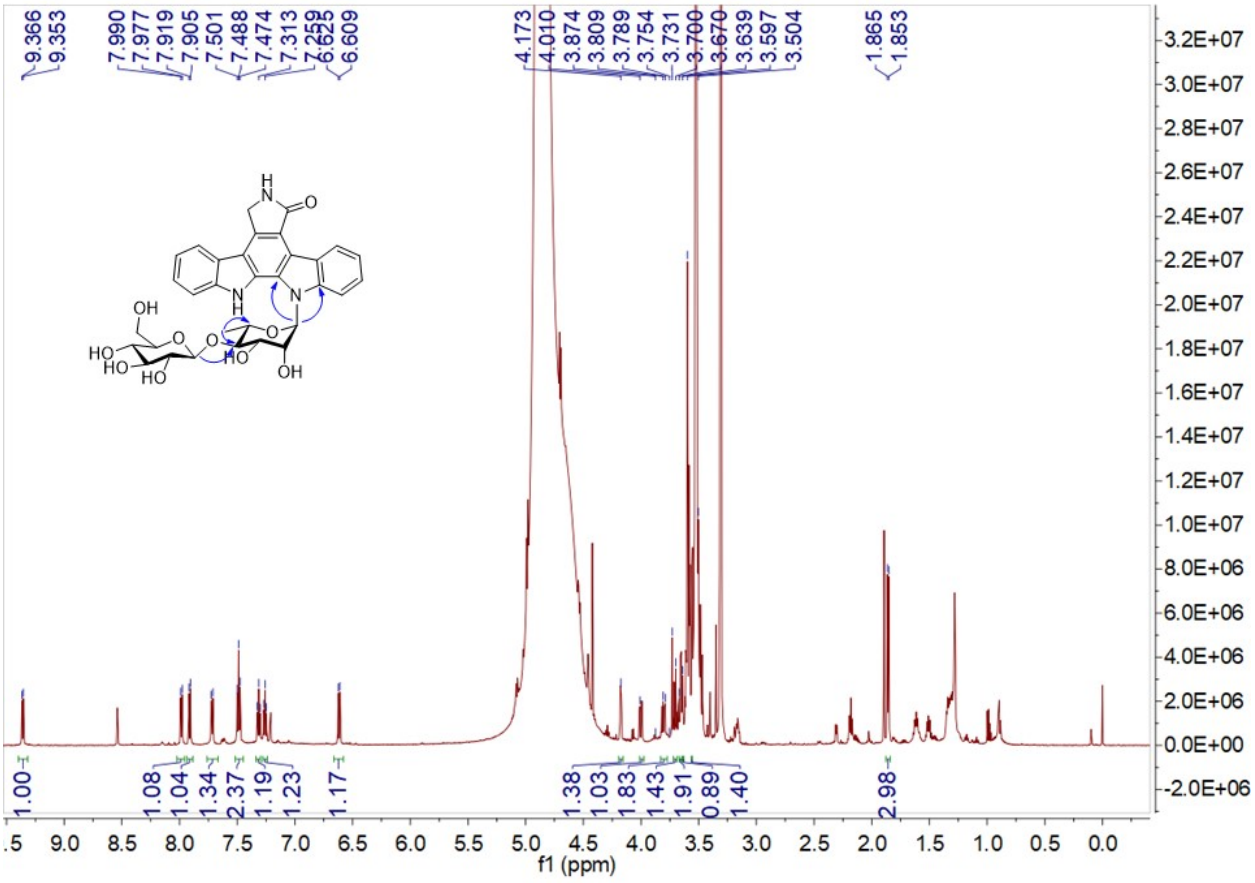
S12. ^{1}H spectrum of (6).



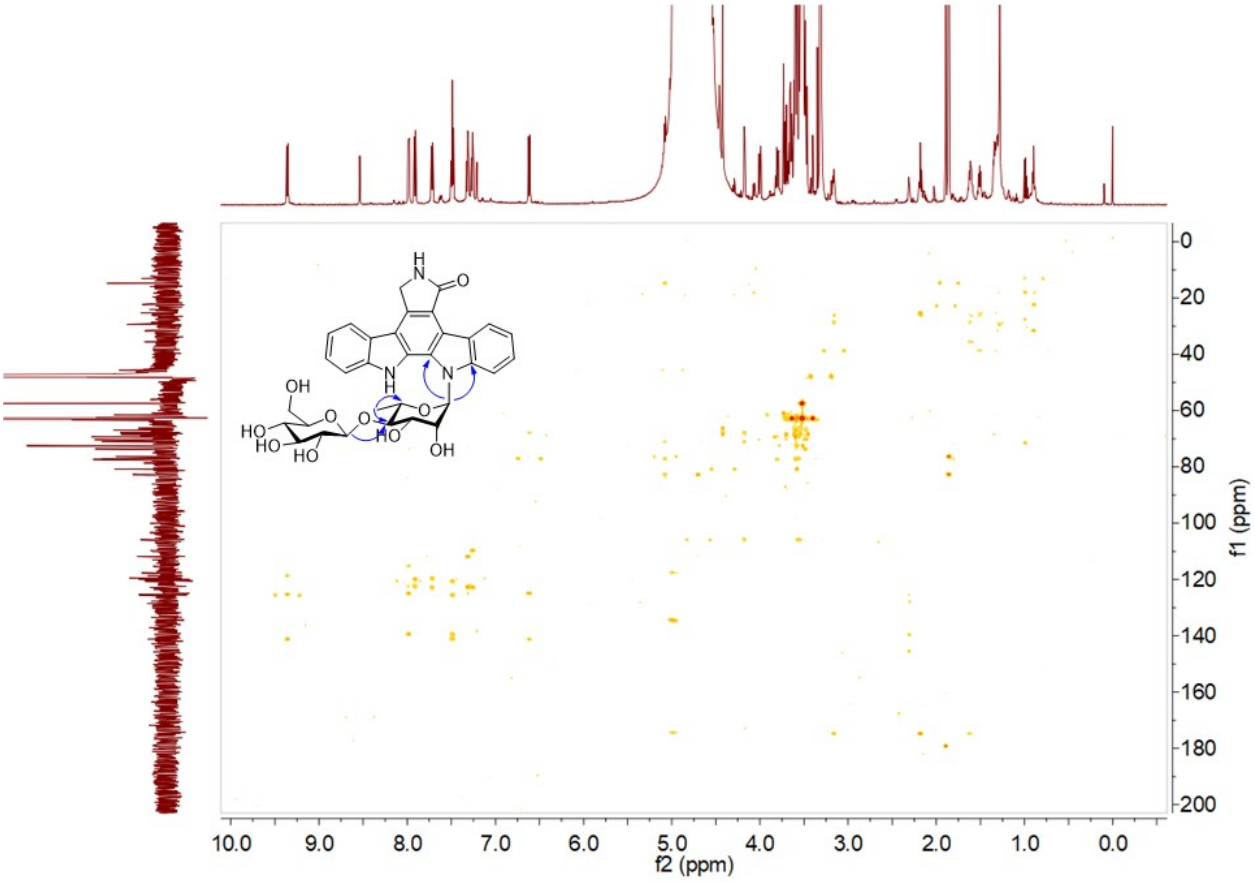
S13. HMBC spectrum of (6).



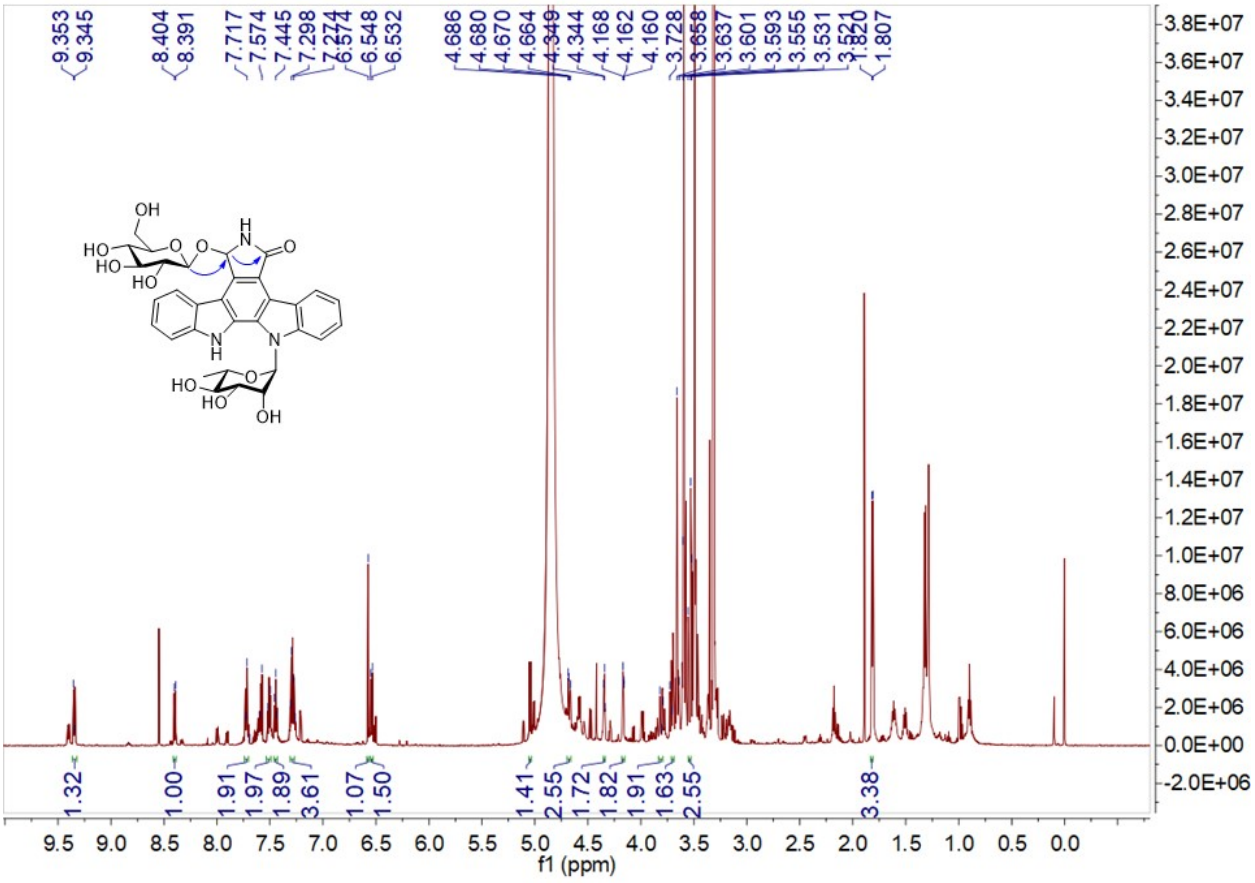
S14. ^{1}H spectrum of (7).



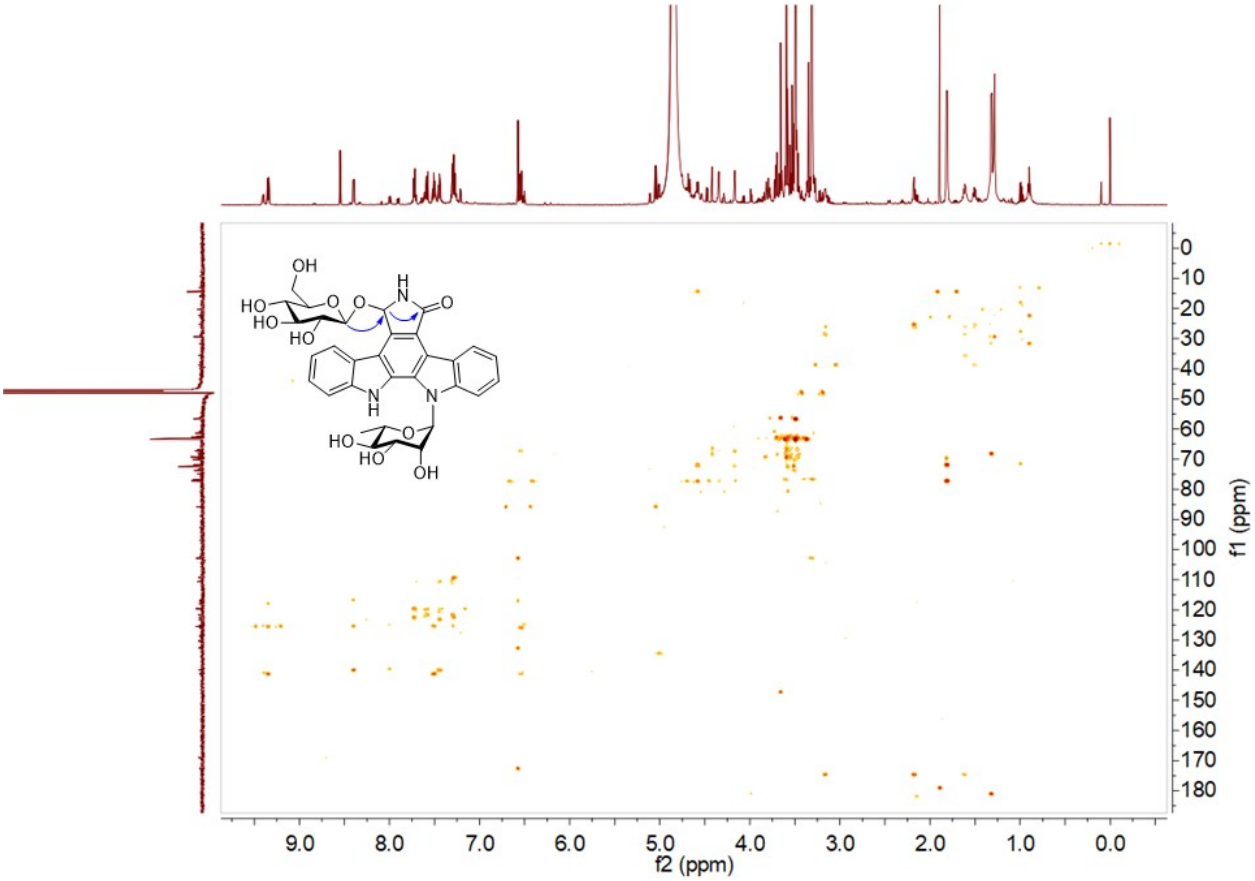
S15. HMBC spectrum of (7).



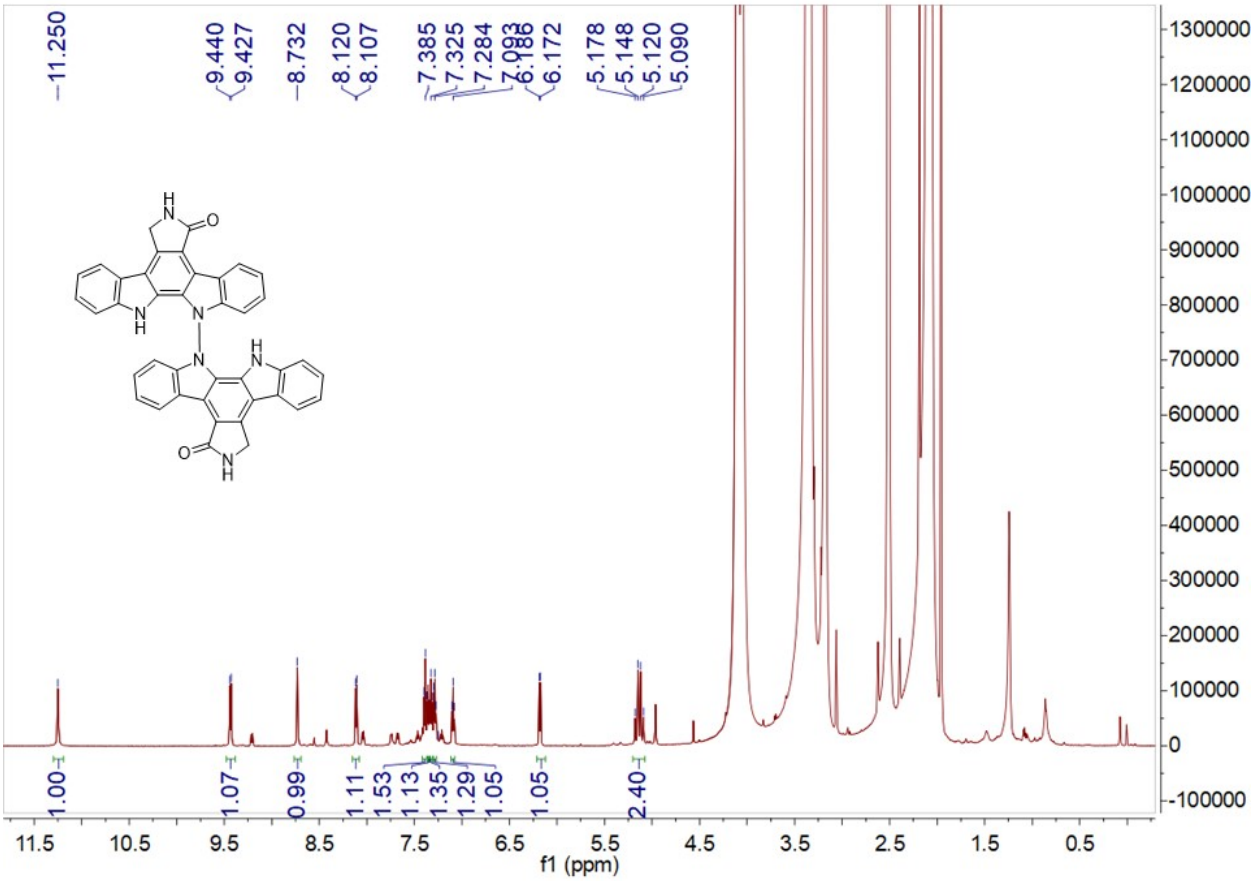
S16. ^{1}H spectrum of (8).



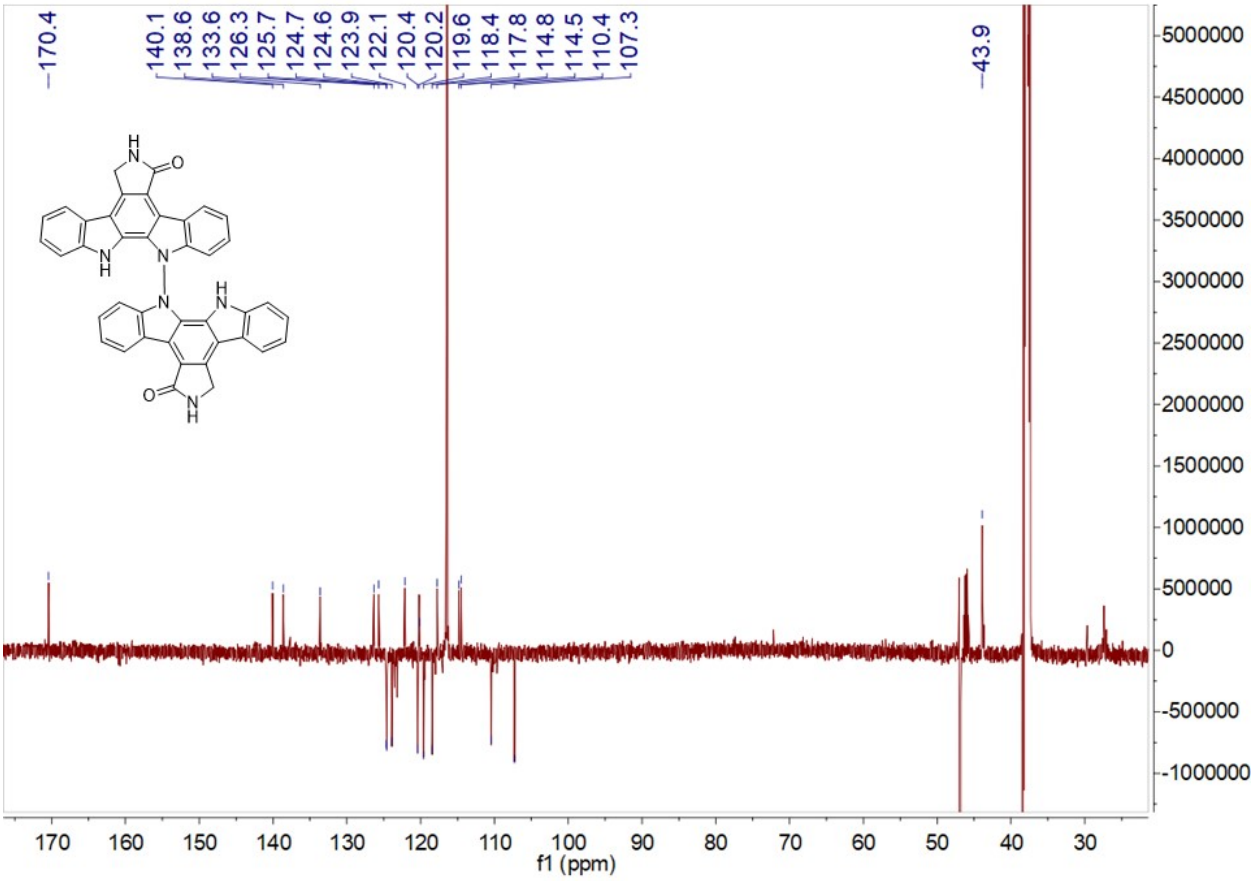
S17. HMBC spectrum of (8).



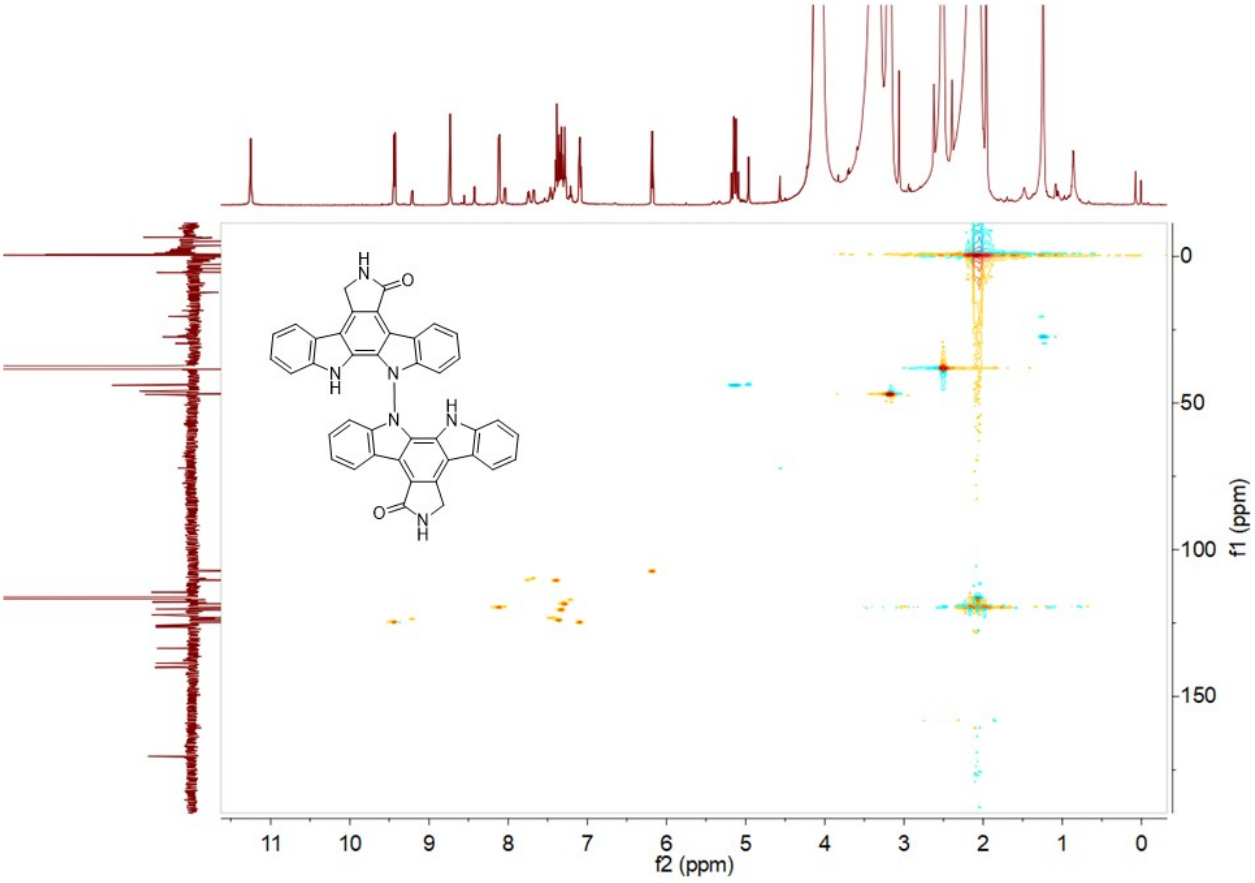
S18. ^{1}H spectrum of (9).



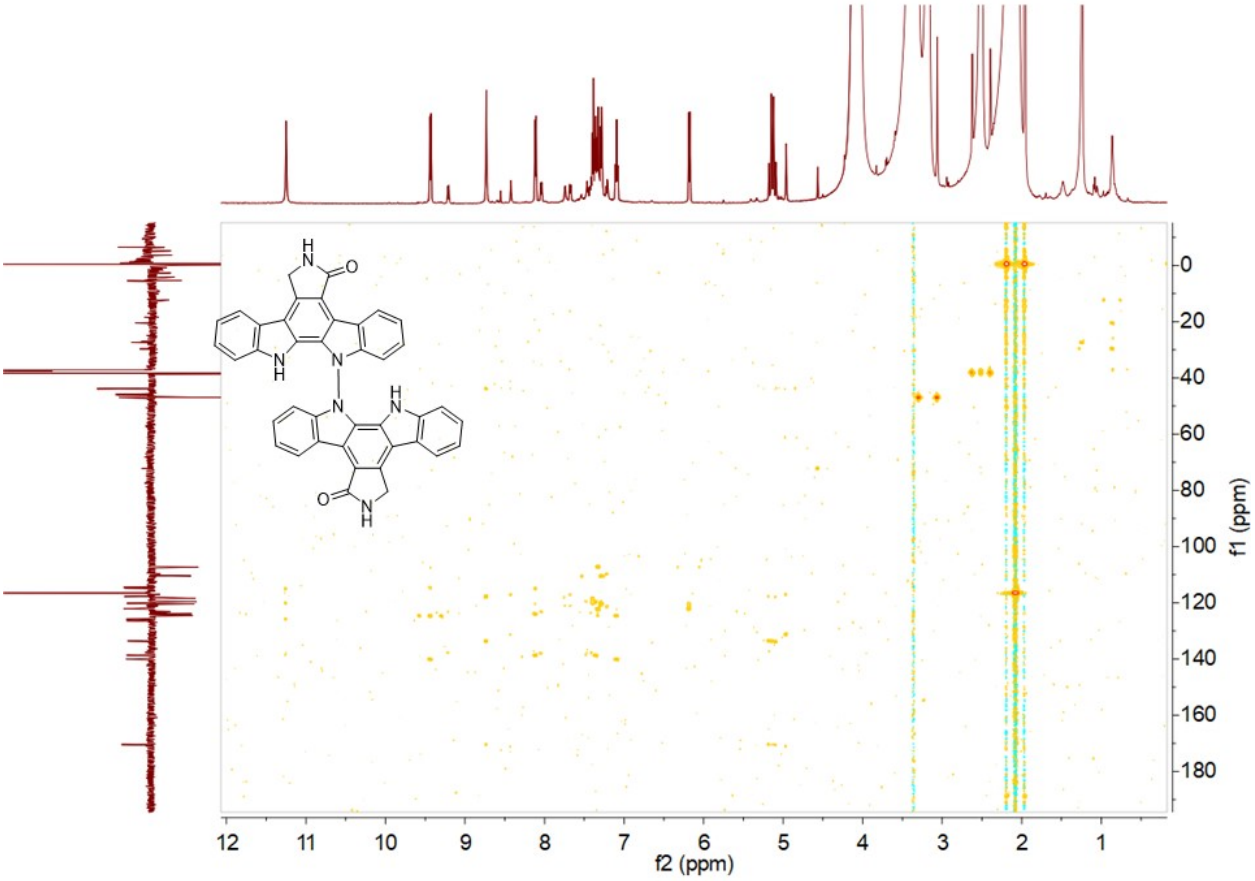
S19. ^{13}C spectrum of (9).



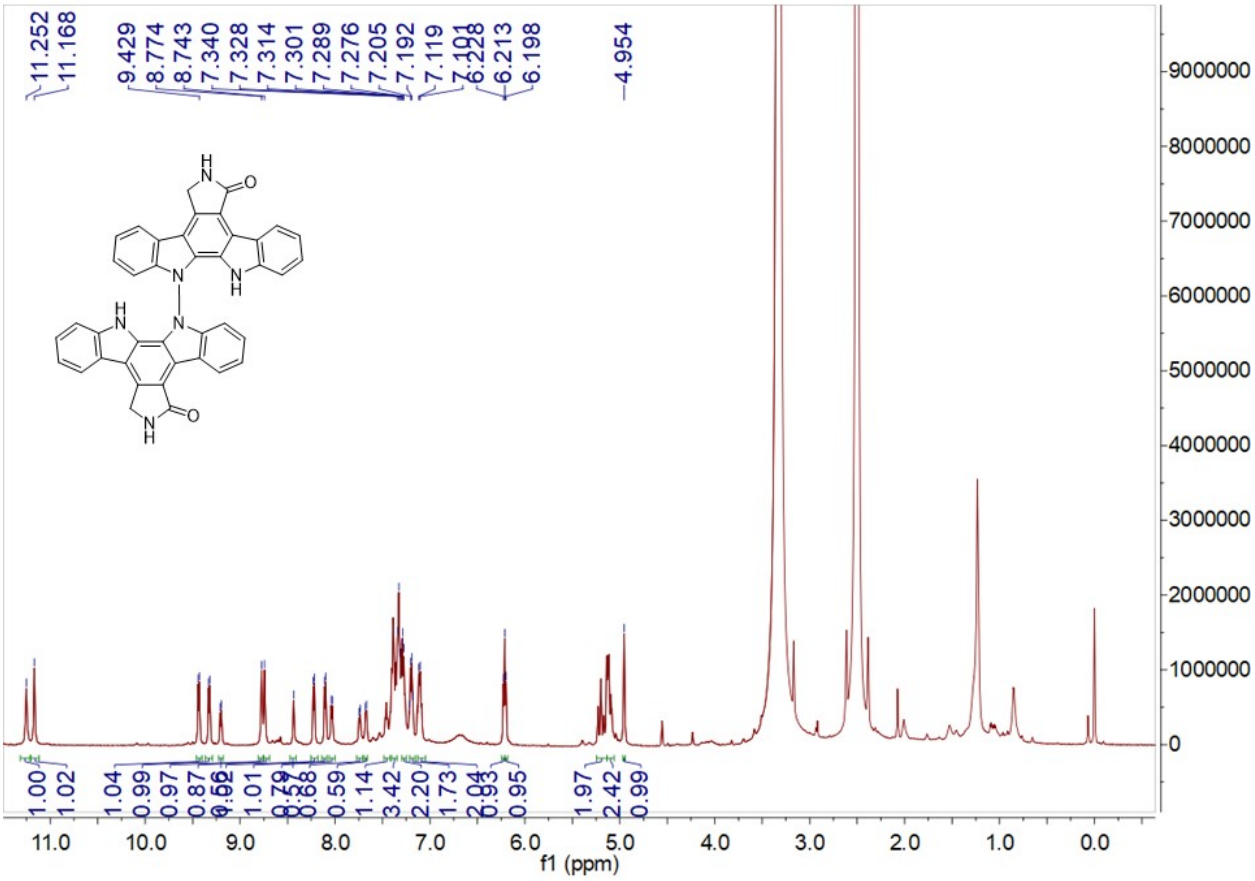
S20. HSQC spectrum of (9).



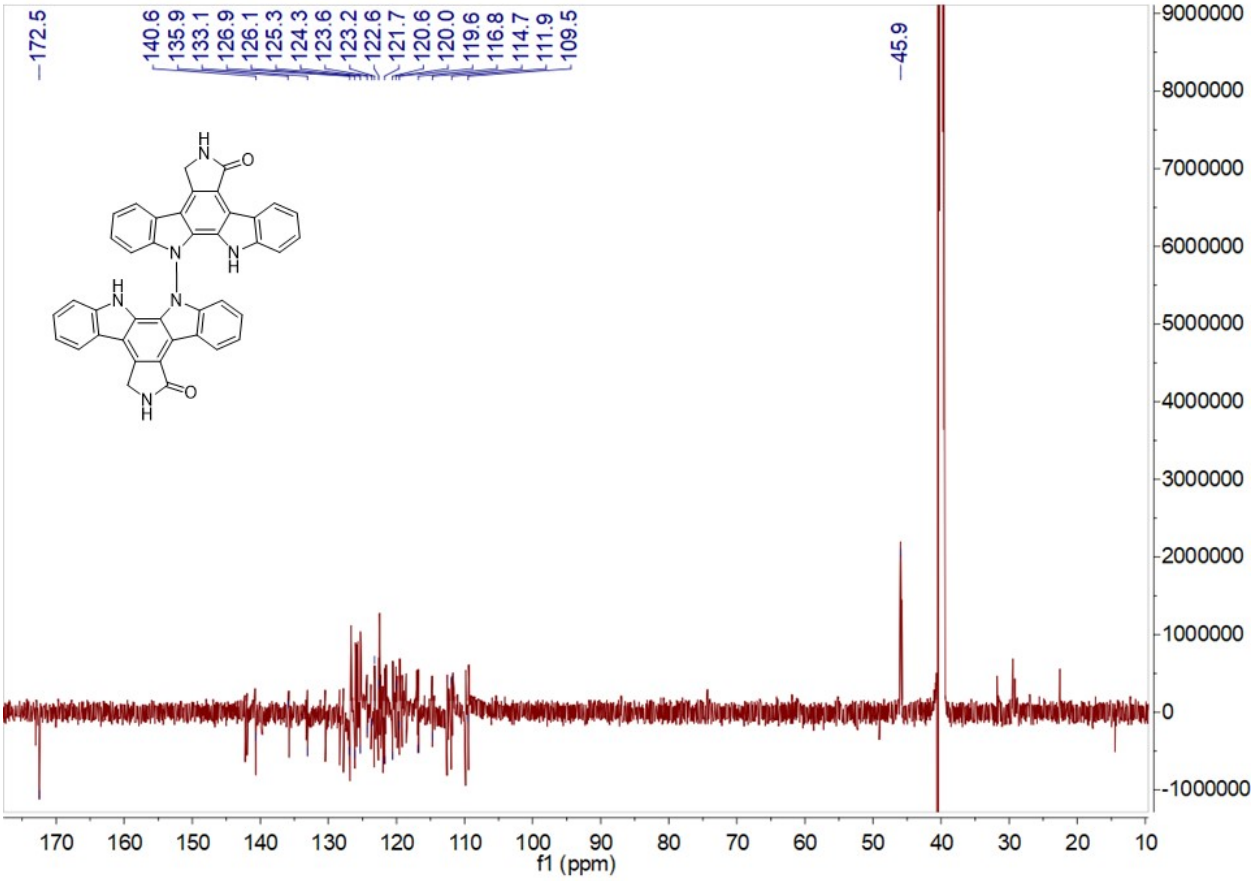
S21. HMBC spectrum of (9).



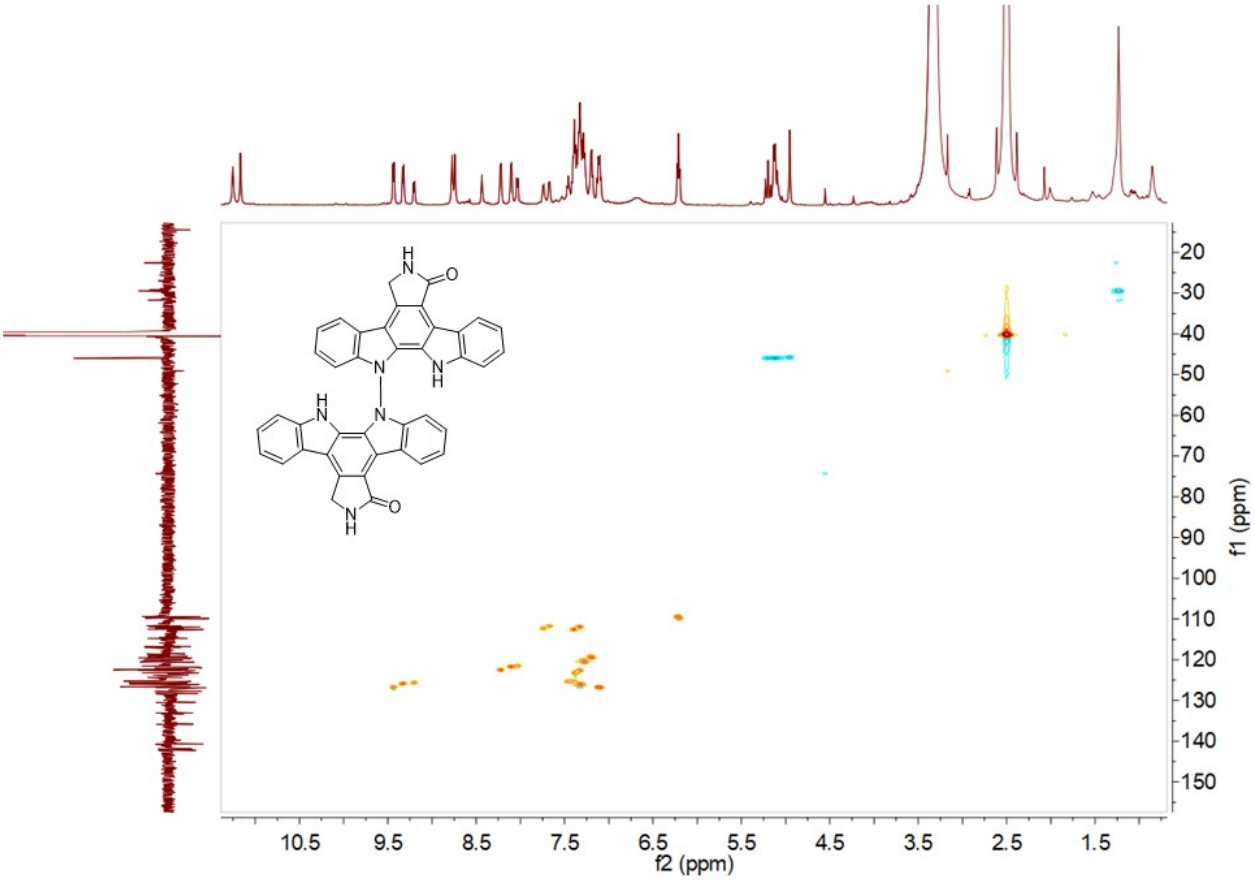
S22. ^{1}H spectrum of (10).



S23. ^{13}C spectrum of (10).

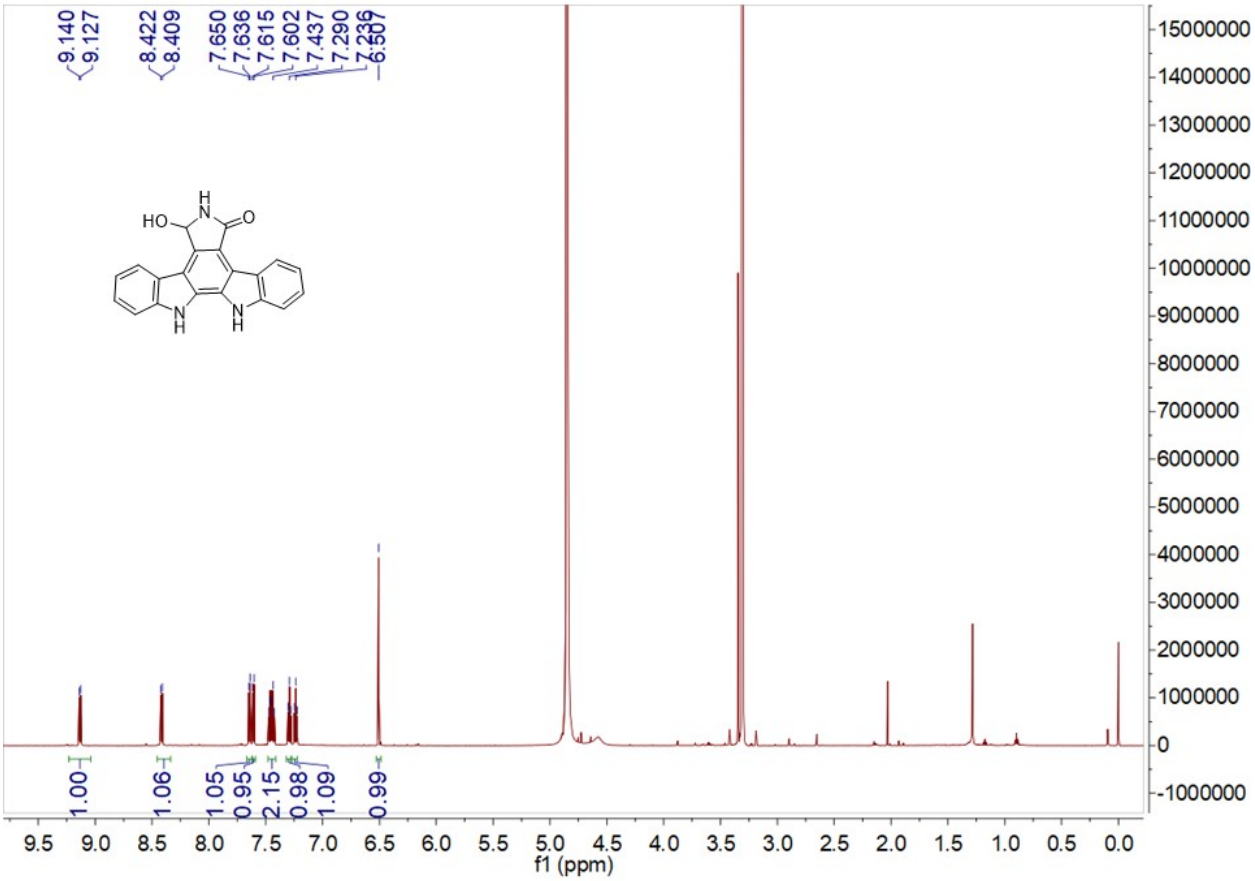


S24. HSQC spectrum of (10).

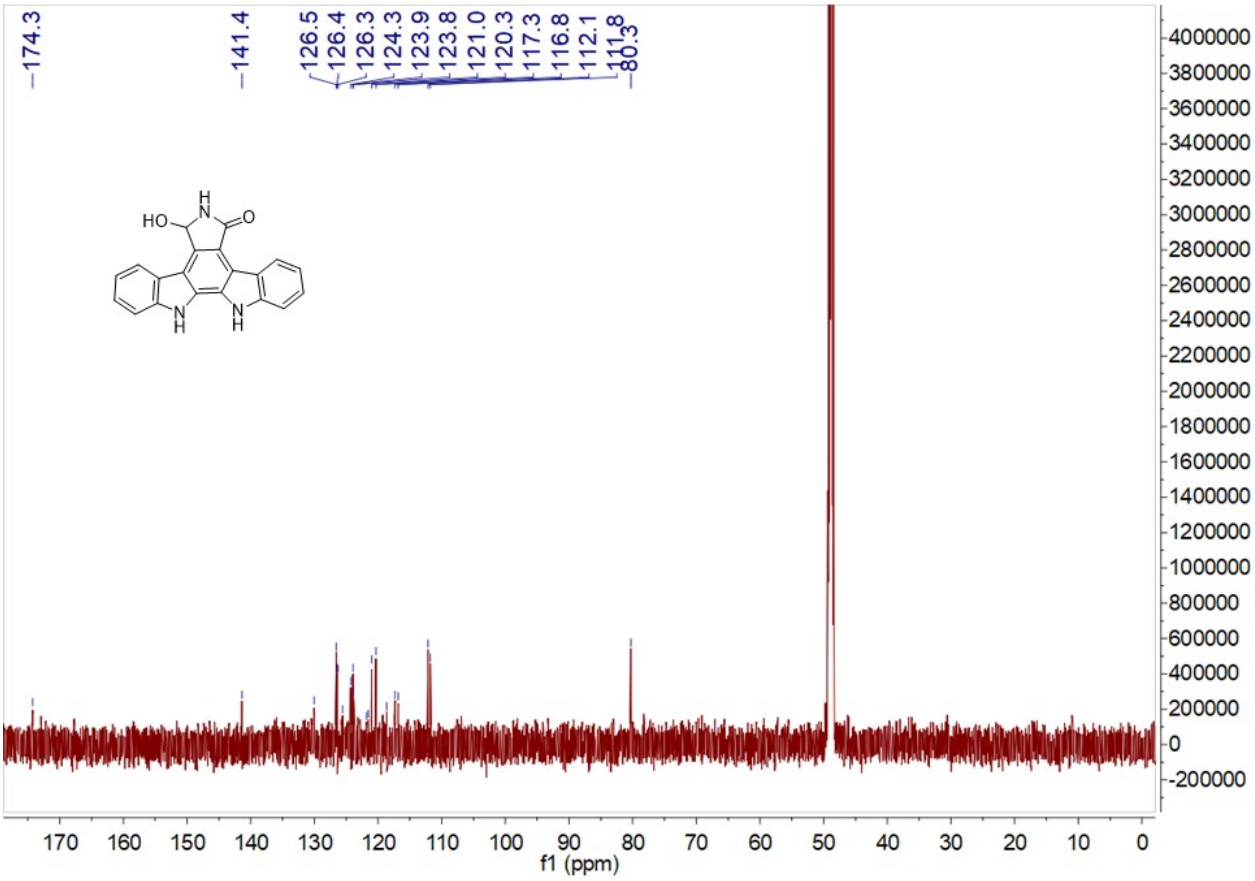


S25. HMBC spectrum of (10).

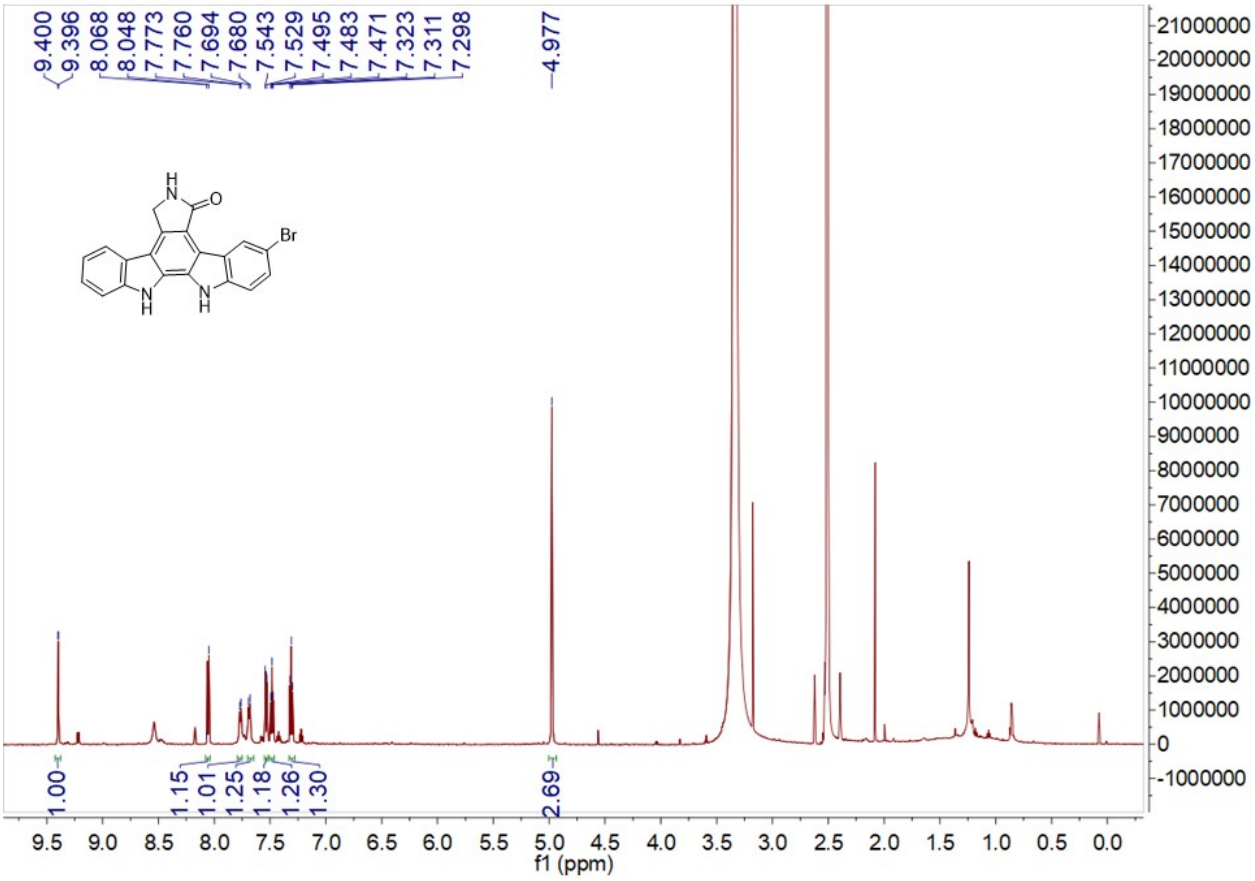
S26. ^{1}H spectrum of (11).



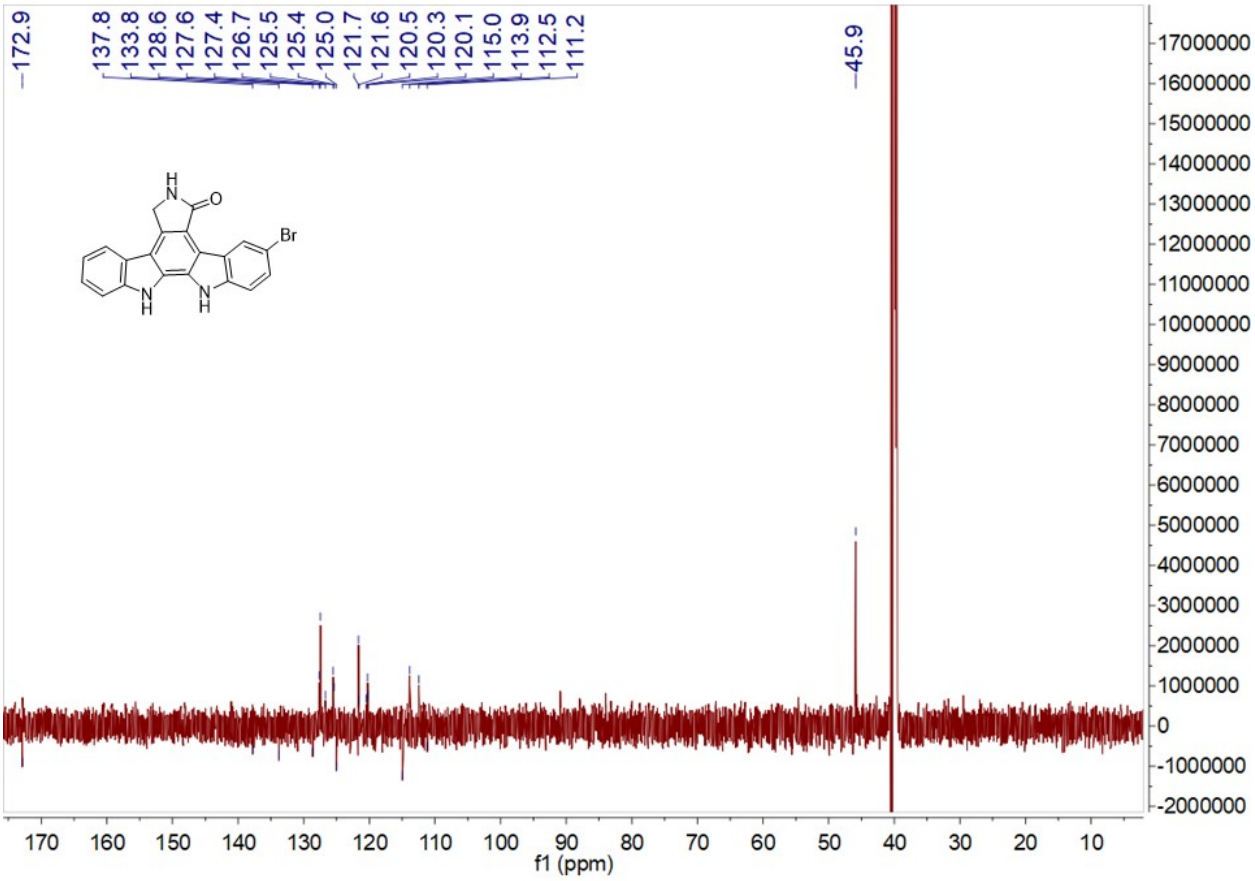
S27. ^{13}C spectrum of (11).



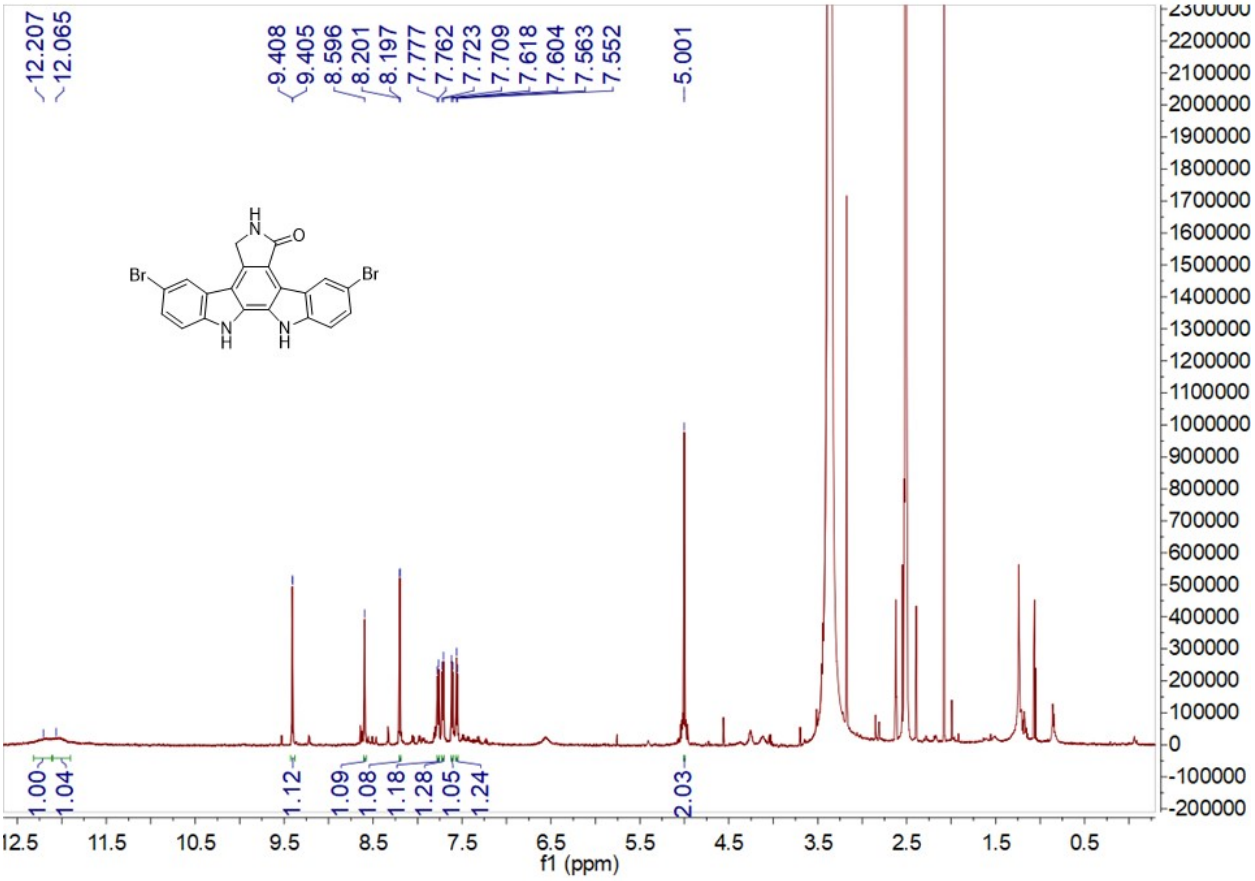
S28. ^{1}H spectrum of (12).



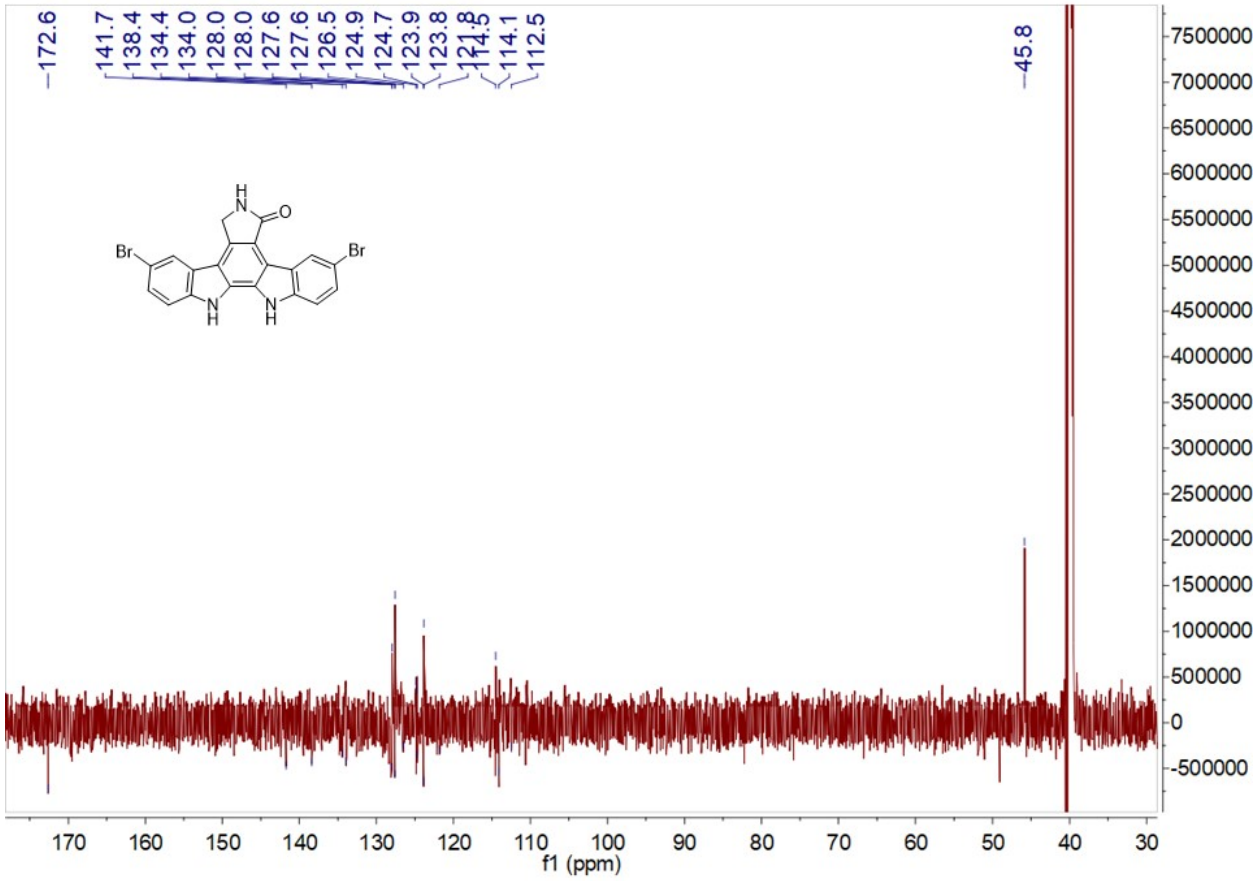
S29. ^{13}C spectrum of (12).



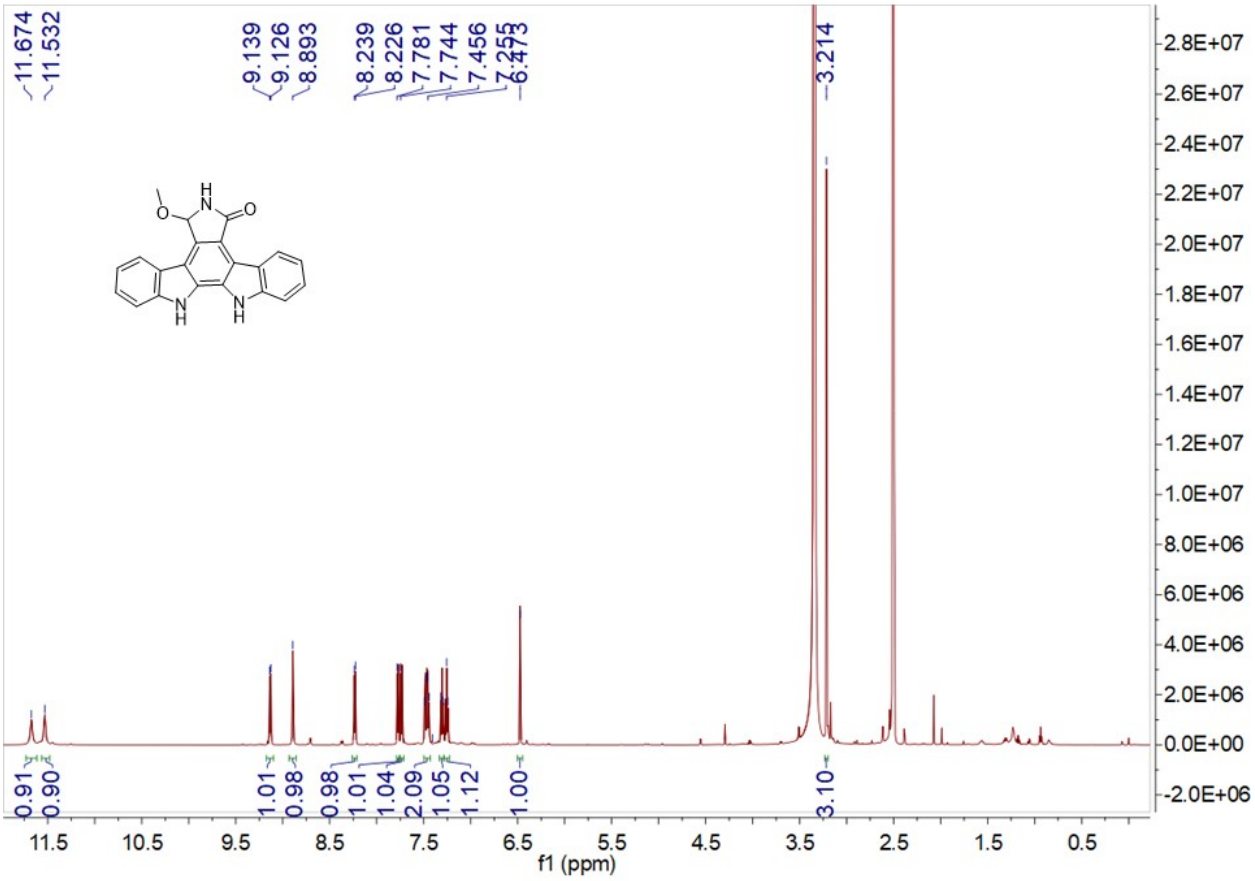
S30. ^{1}H spectrum of (13).



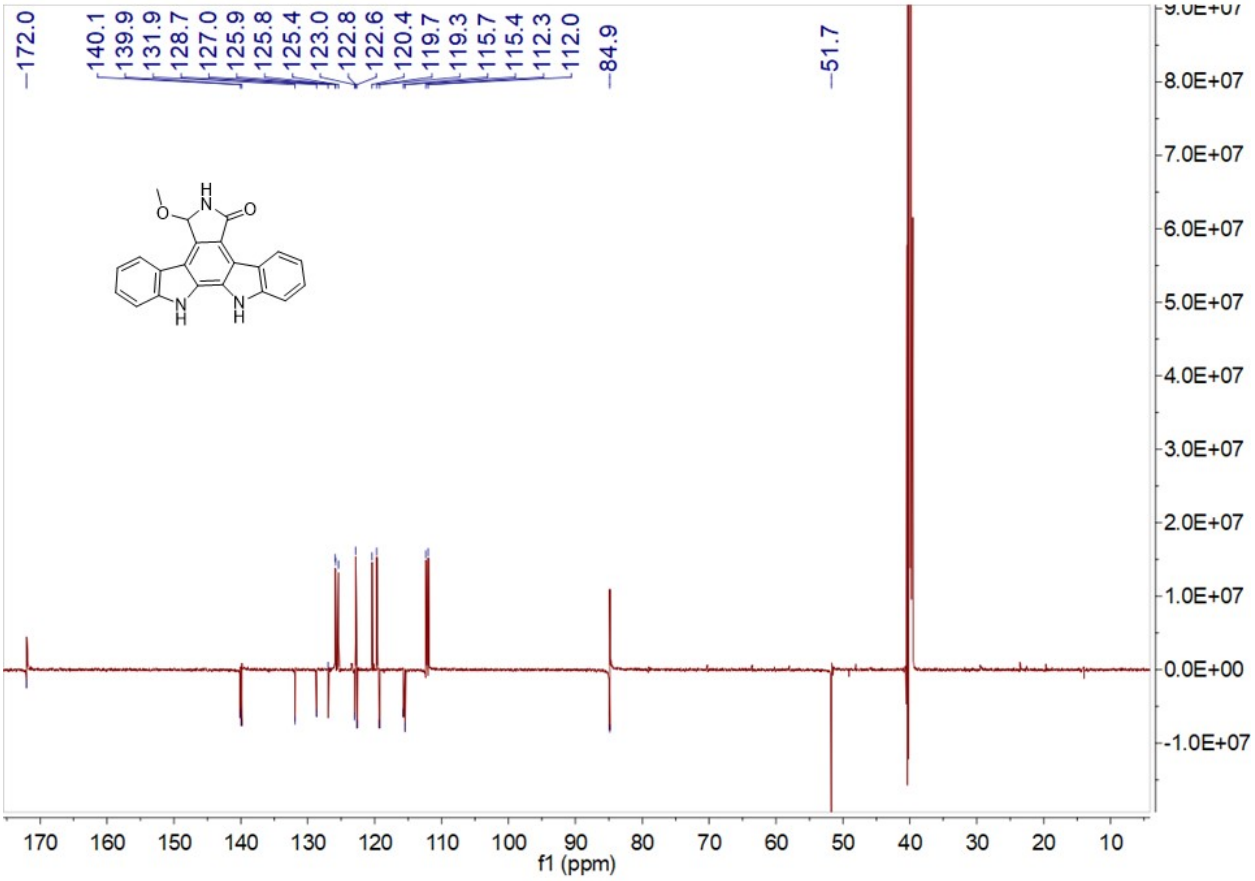
S31. ^{13}C spectrum of (13).



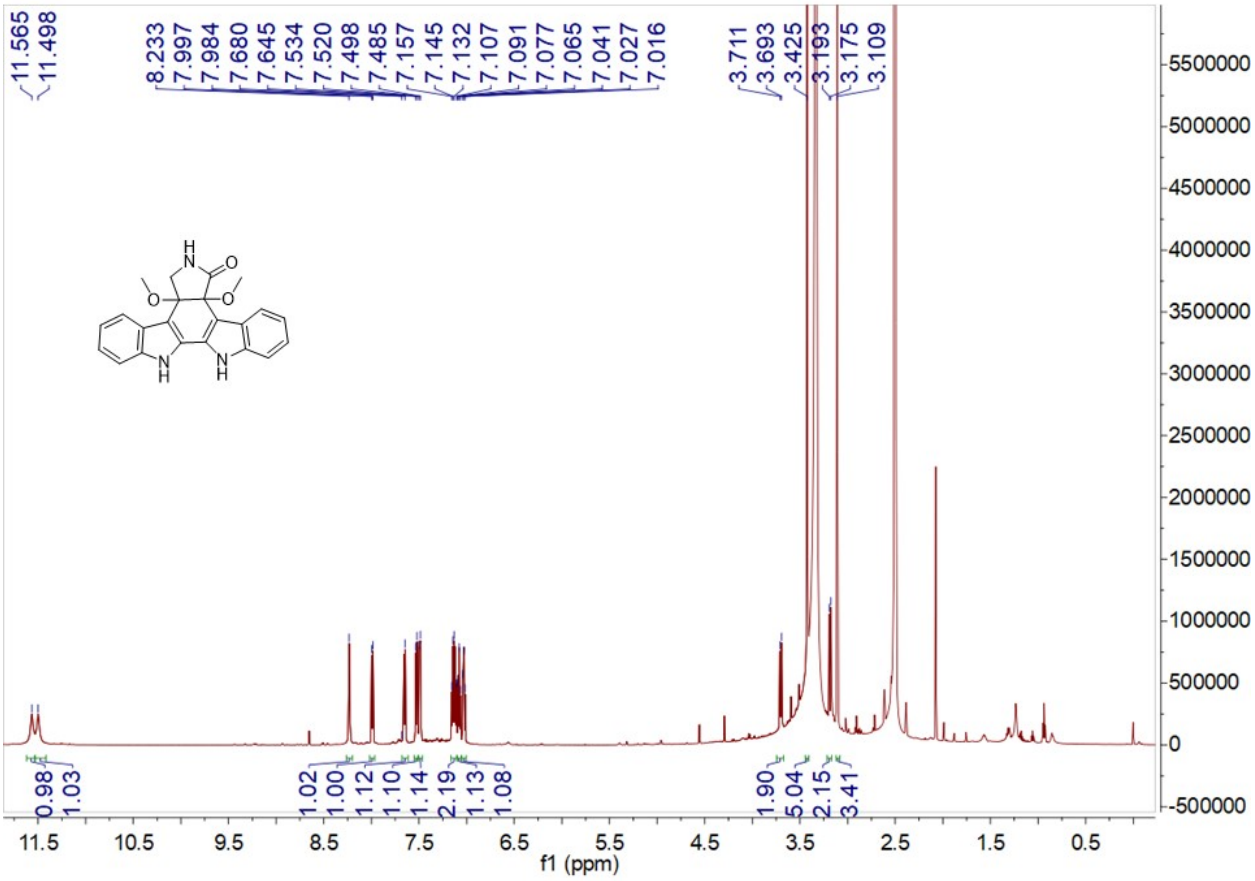
S32. ^{1}H spectrum of (14).



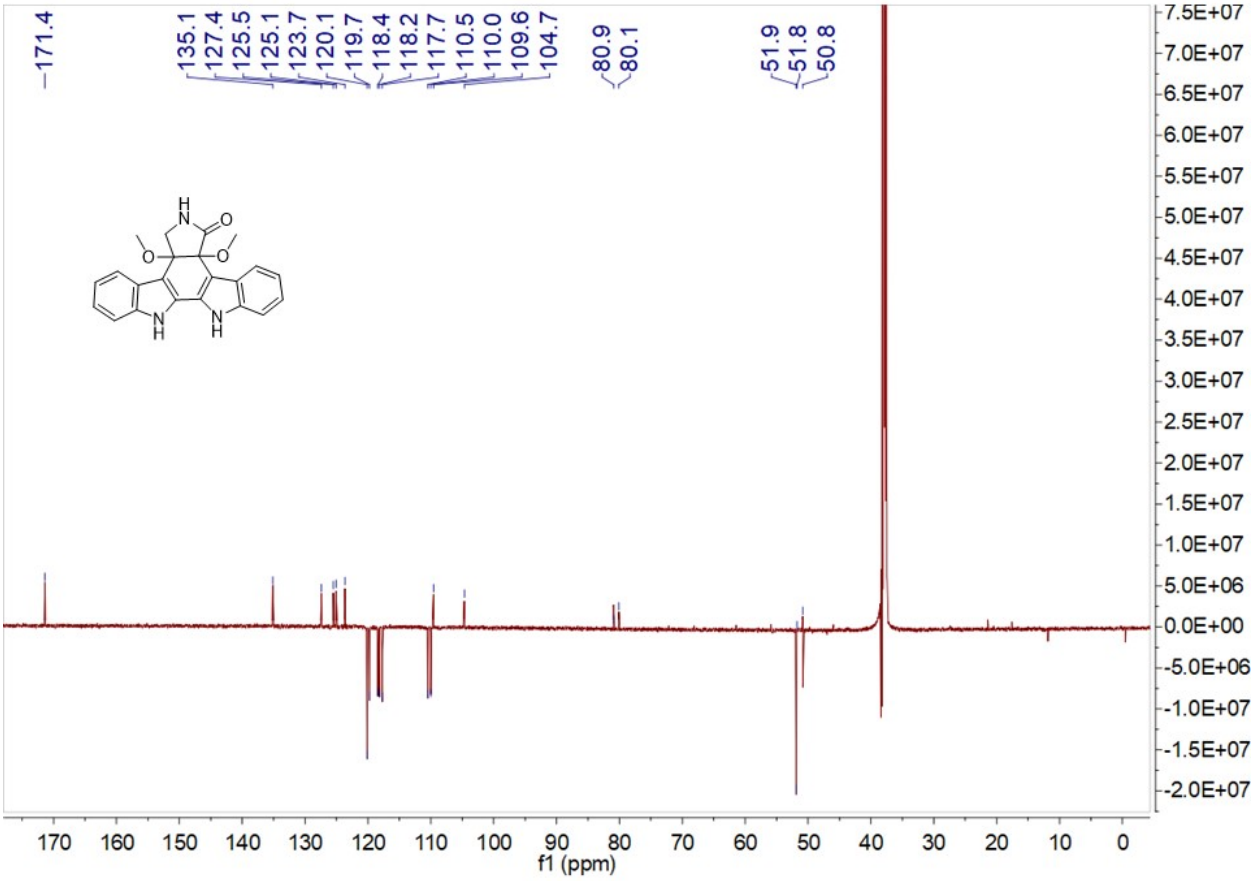
S33. ^{13}C spectrum of (14).



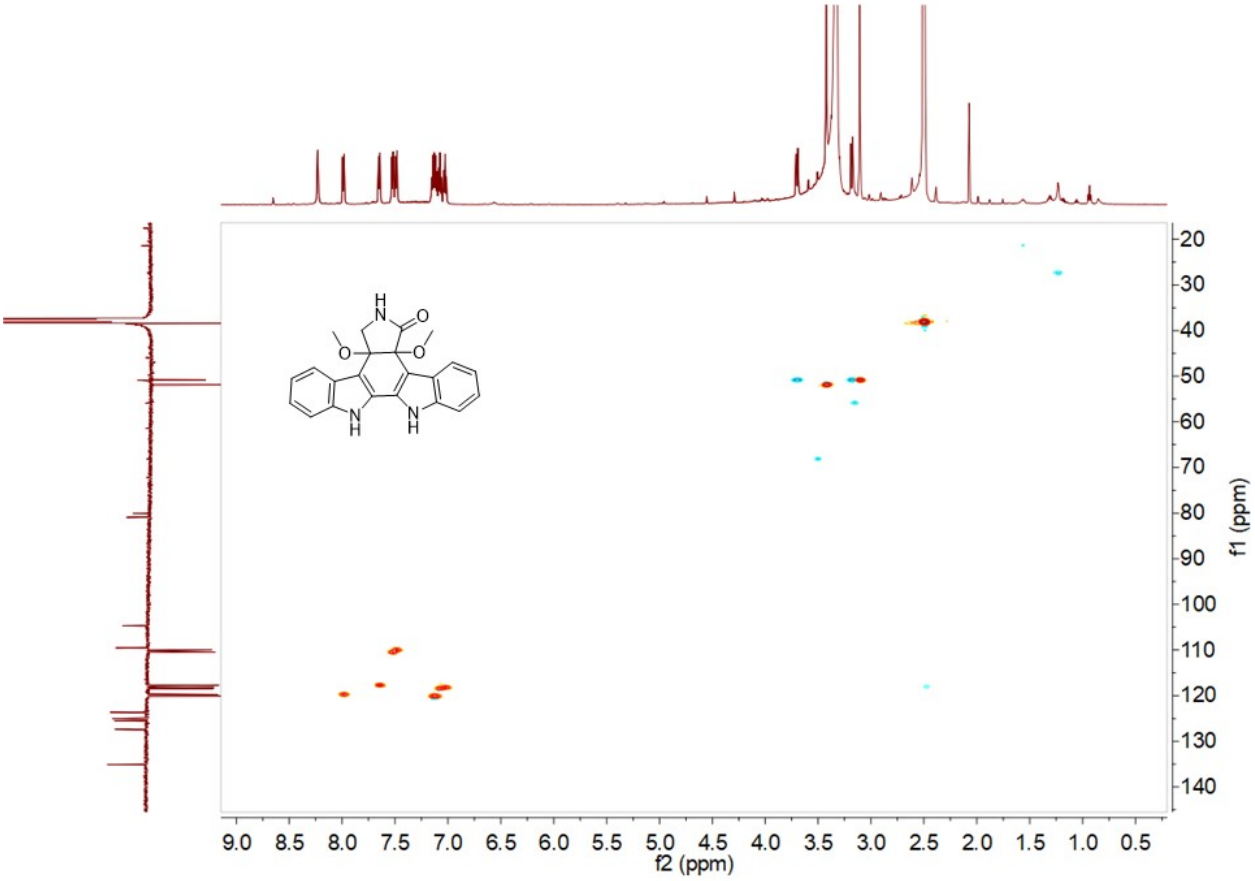
S34. ^{1}H spectrum of (15).



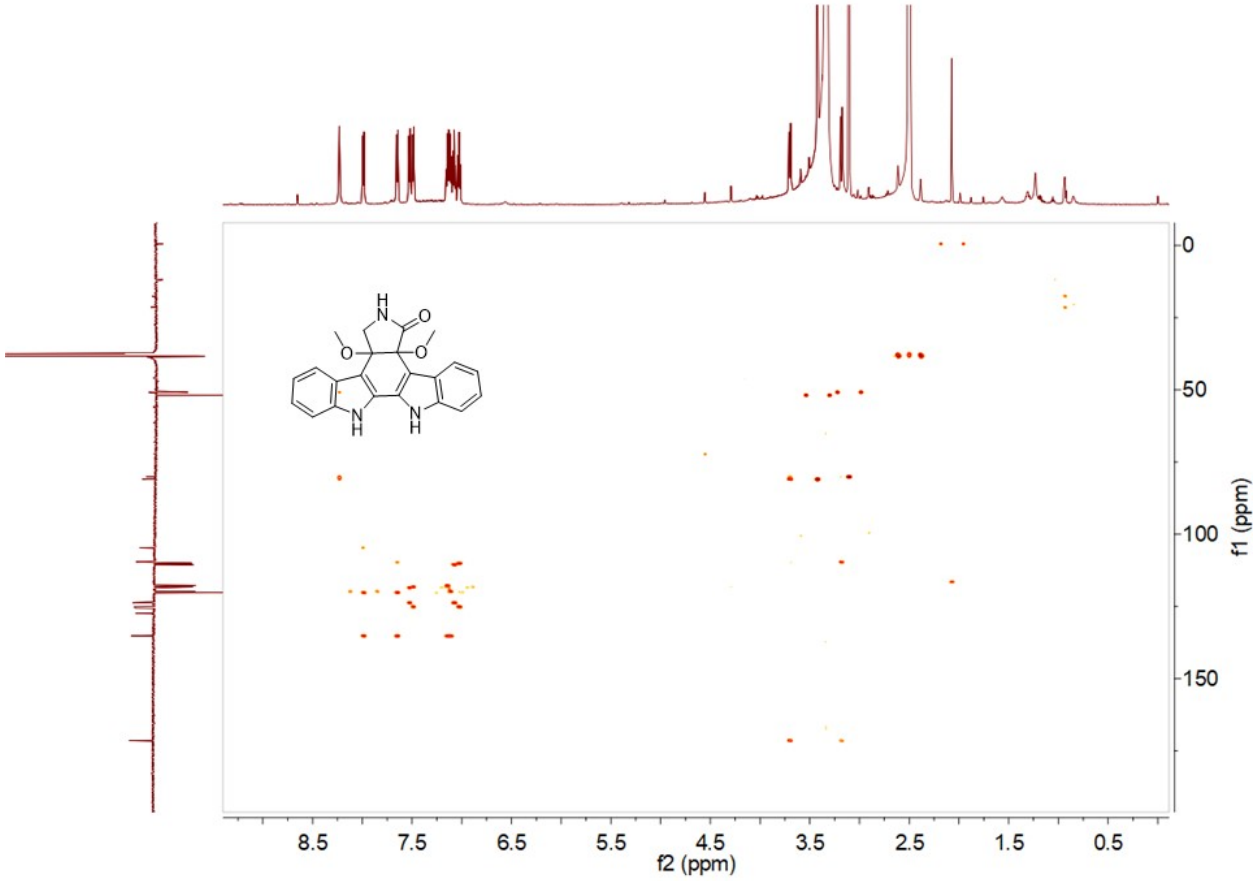
S35. ^{13}C spectrum of (15).



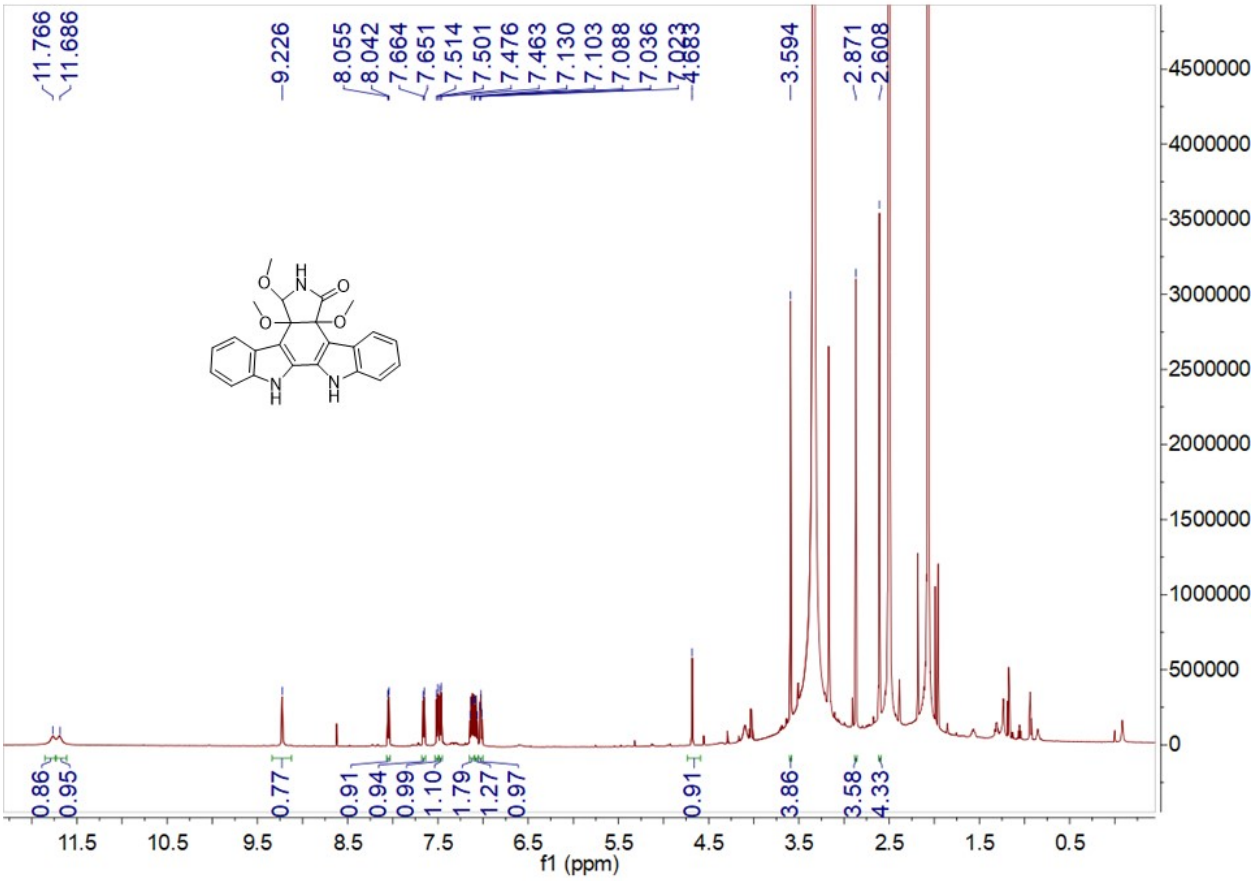
S36. HSQC spectrum of (15).



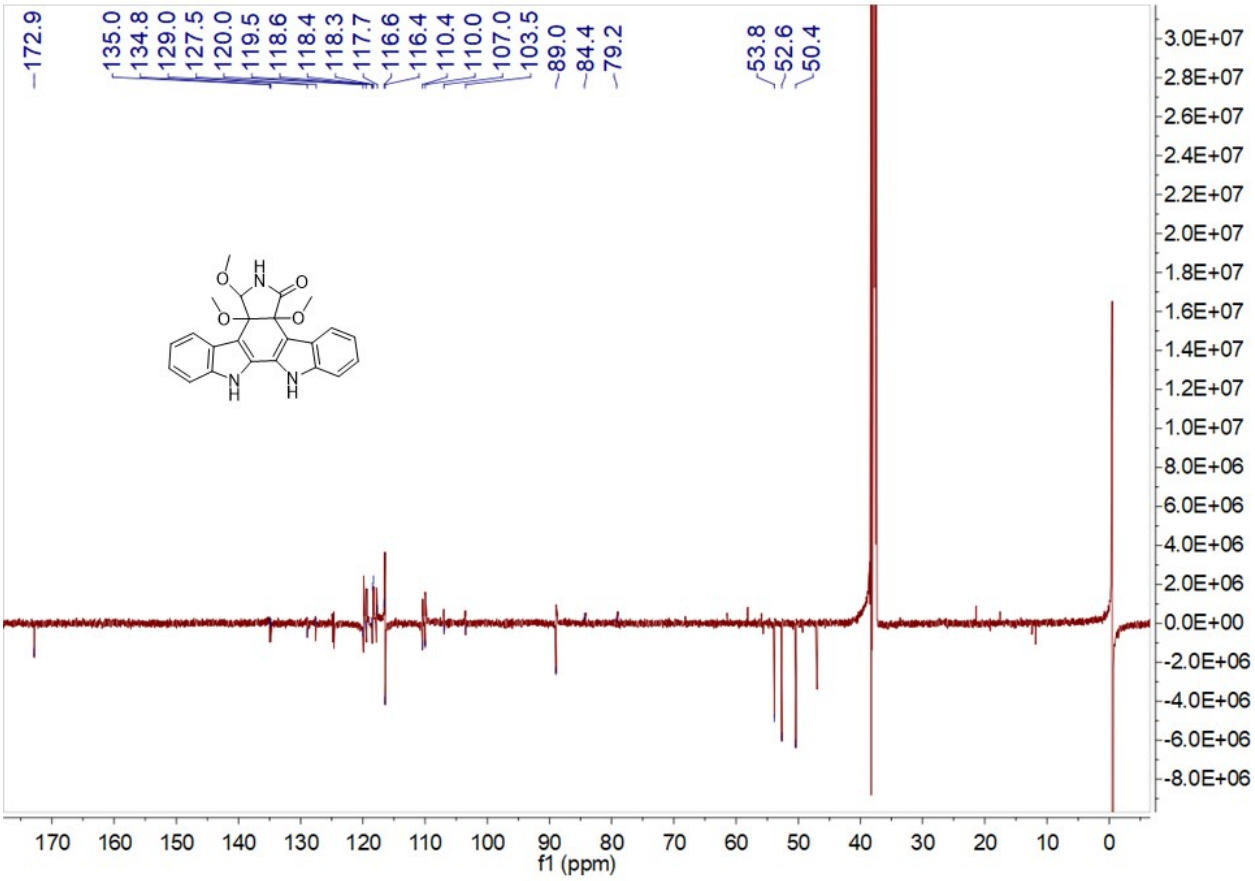
S37. HMBC spectrum of (14).



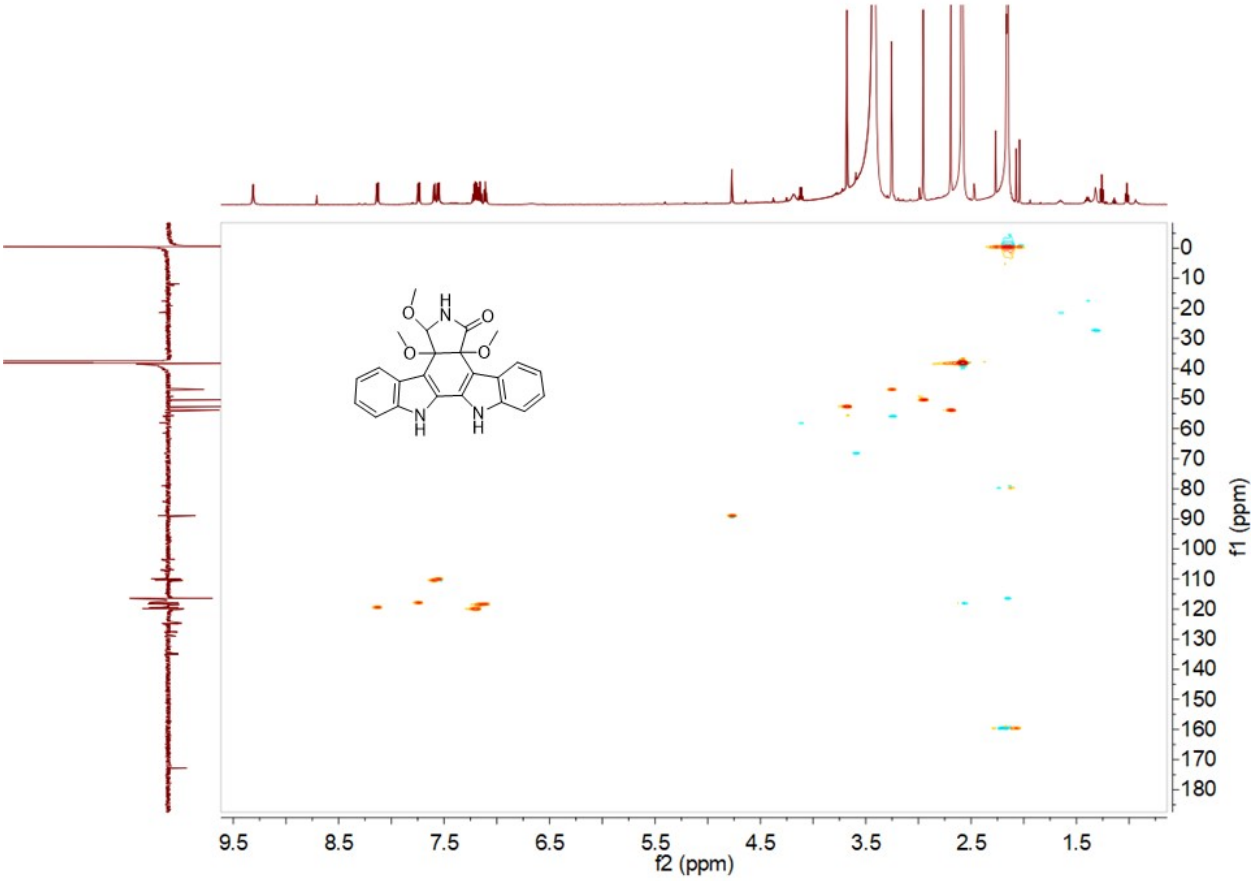
S38. ^{1}H spectrum of (16).



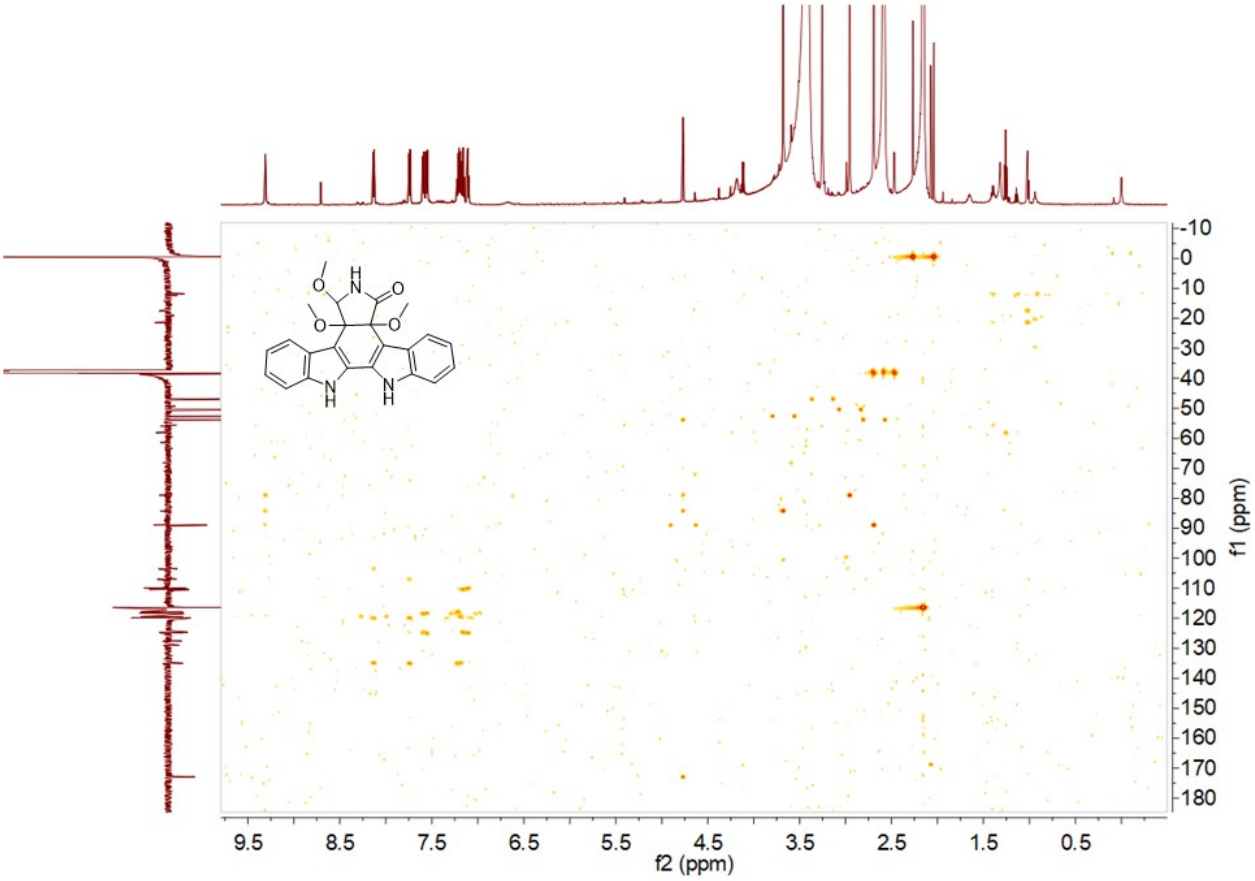
S39. ^{13}C spectrum of (16).



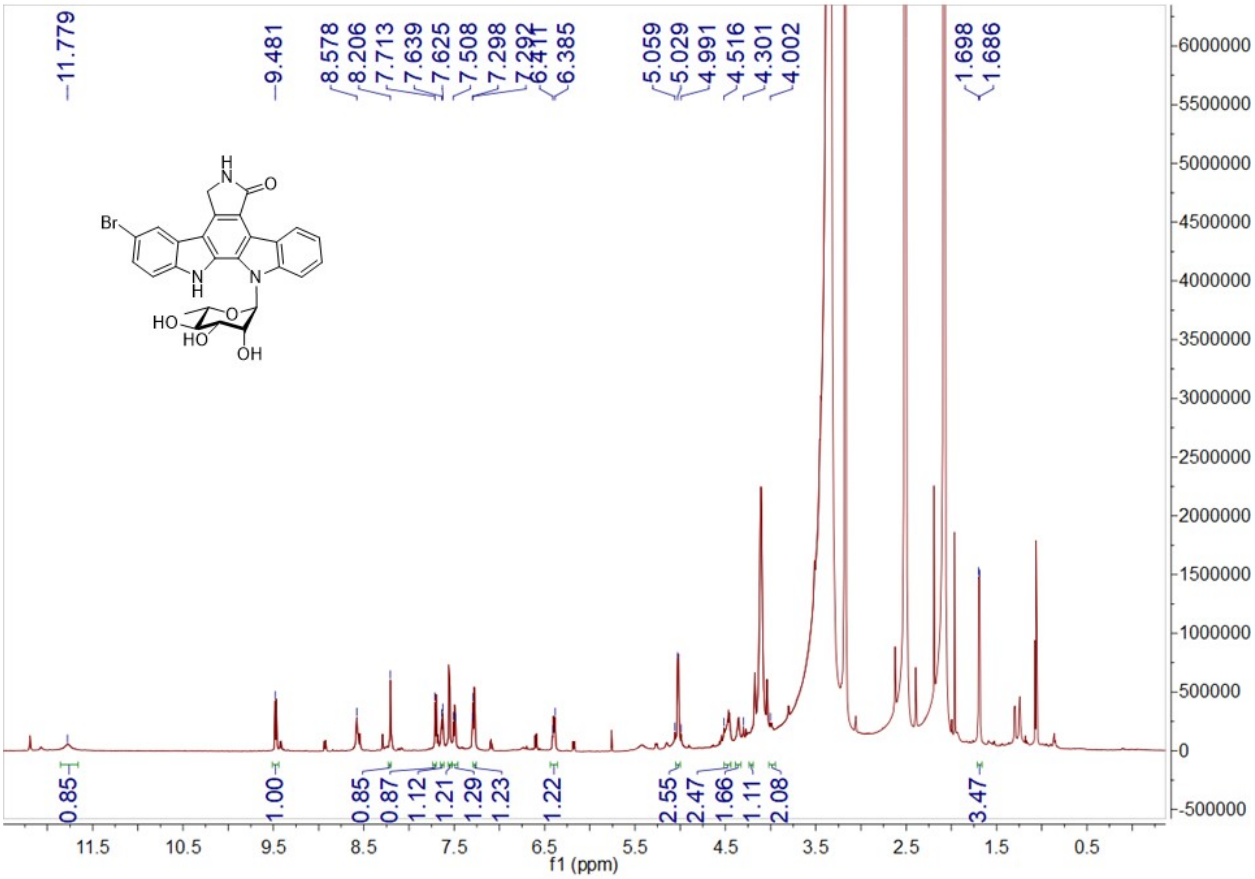
S40. HSQC spectrum of (16).



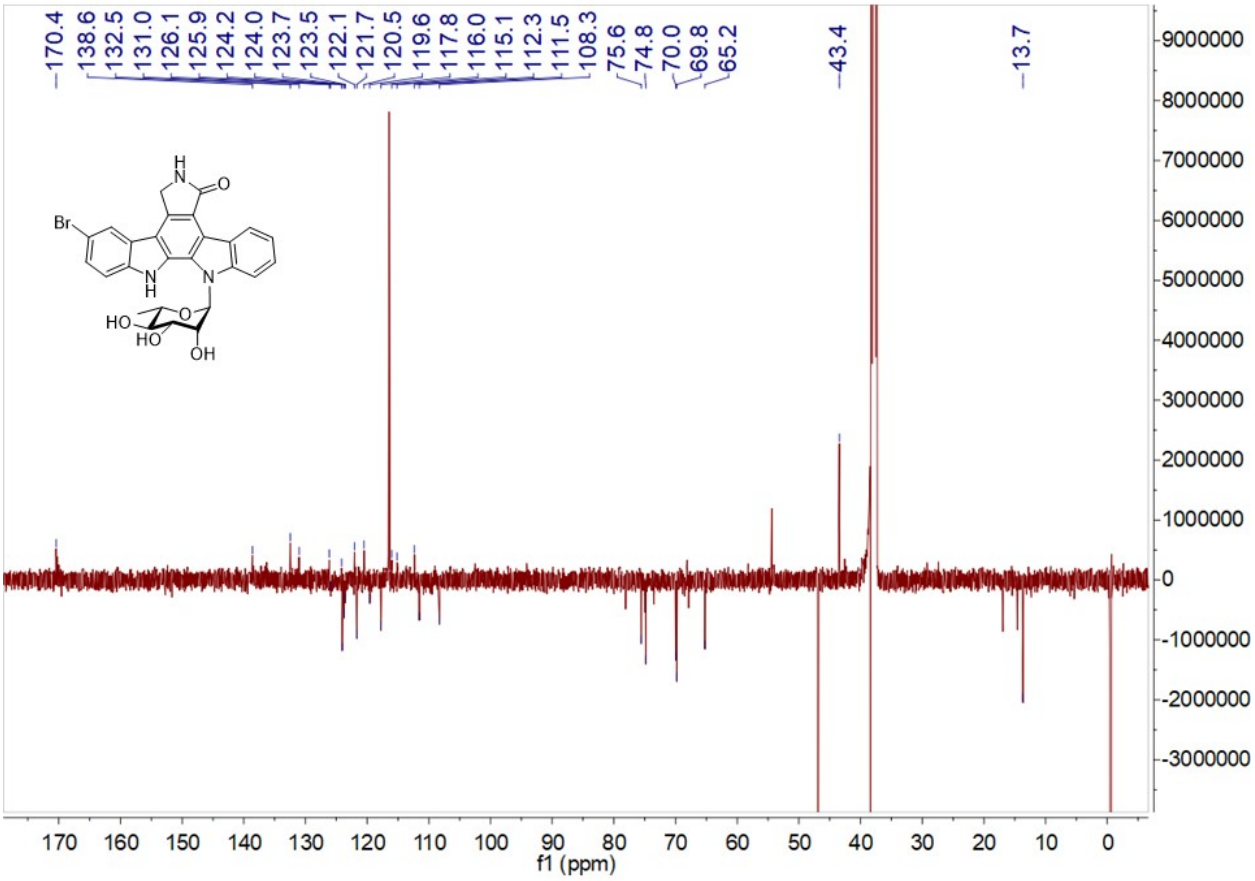
S41. HMBC spectrum of (16).



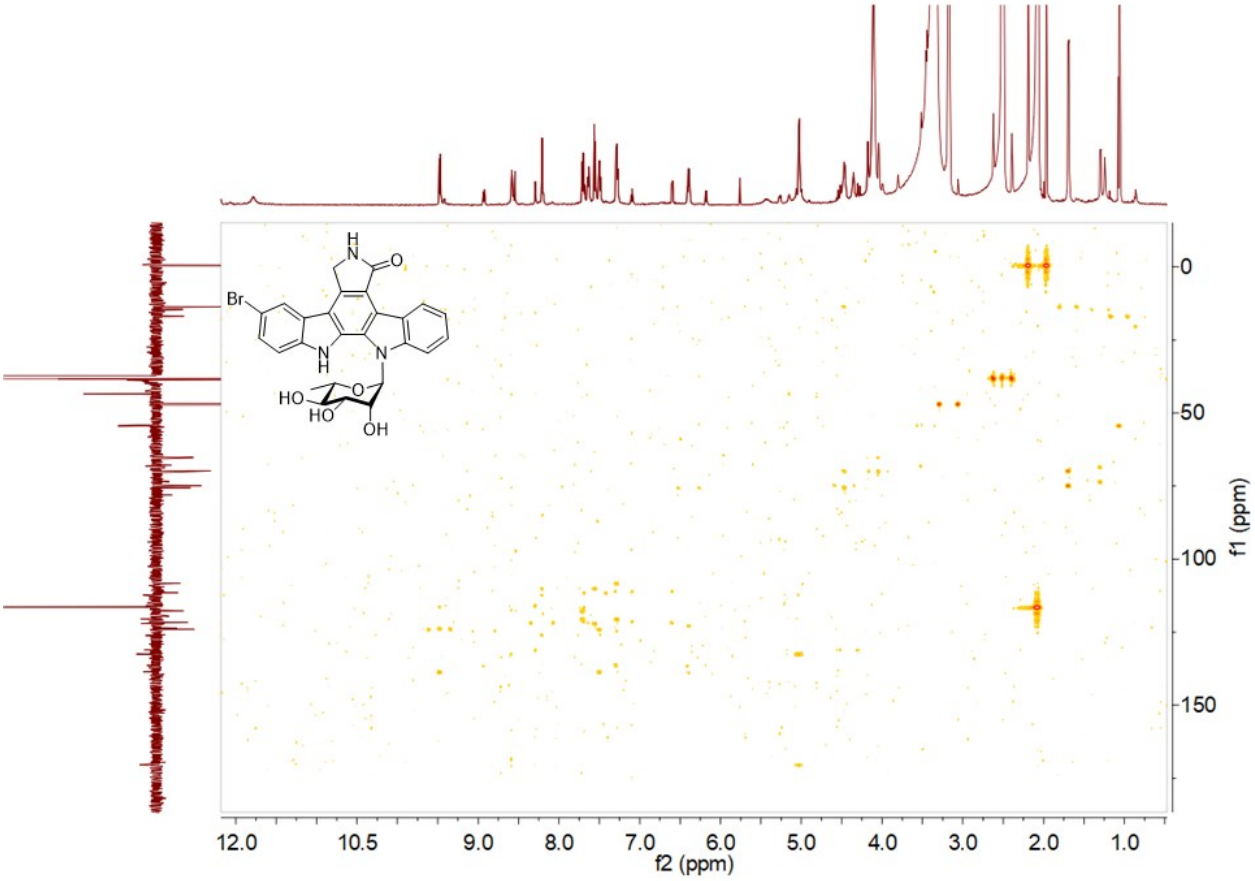
S42. ^{1}H spectrum of (17).



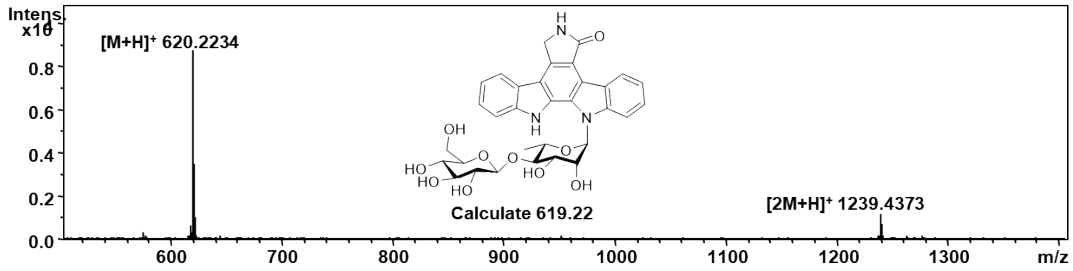
S43. ^{13}C spectrum of (17).

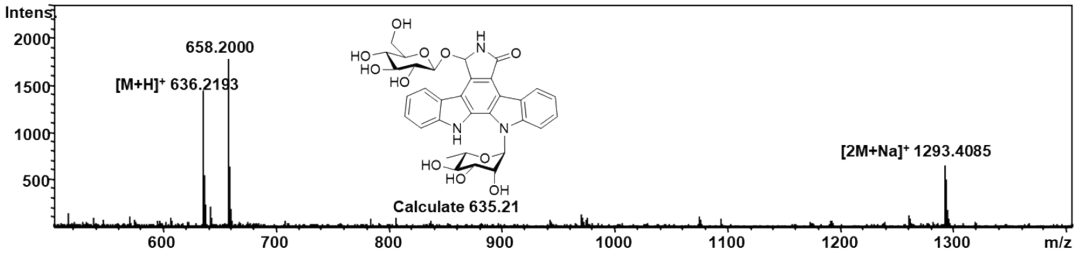


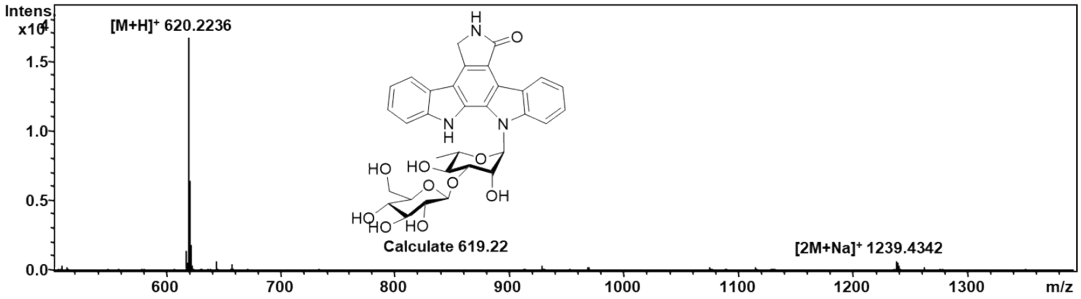
S44. HMBC spectrum of (17).

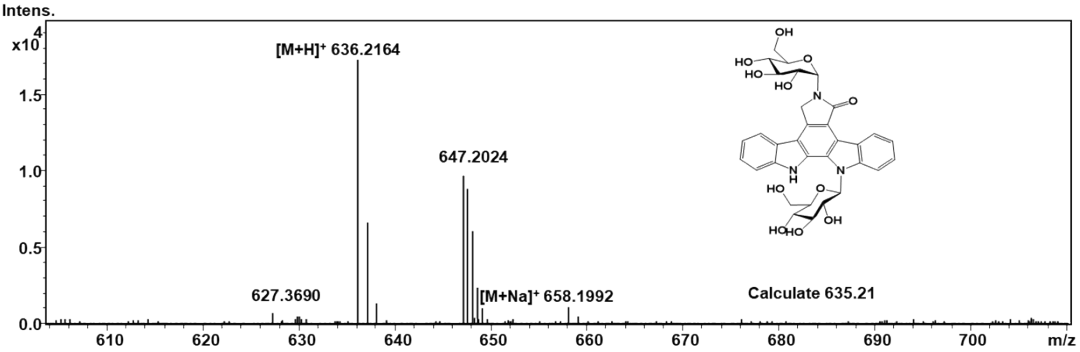


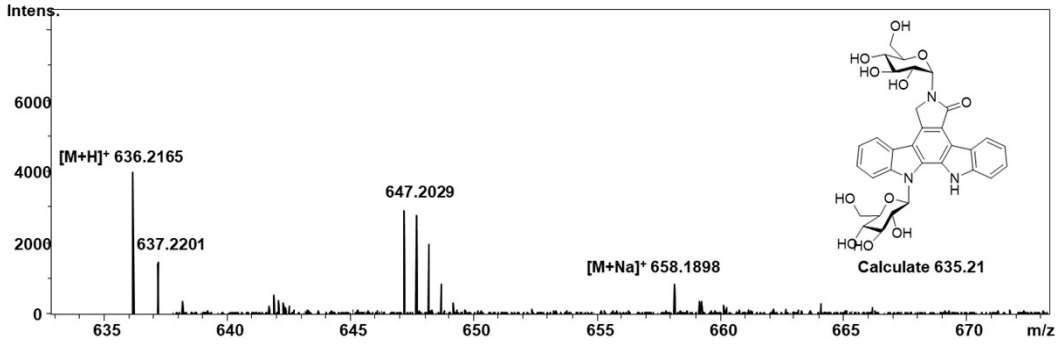
S45. HRESIMS spectrum of compounds.

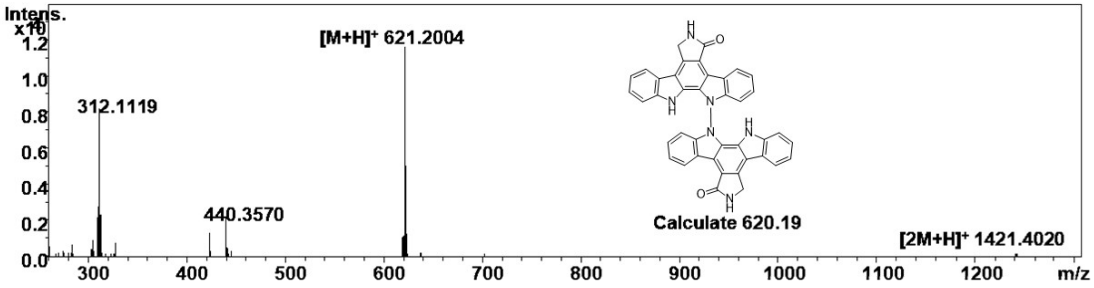


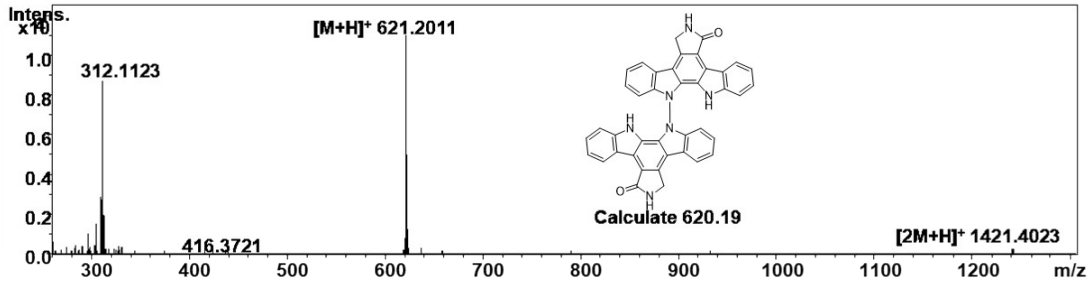


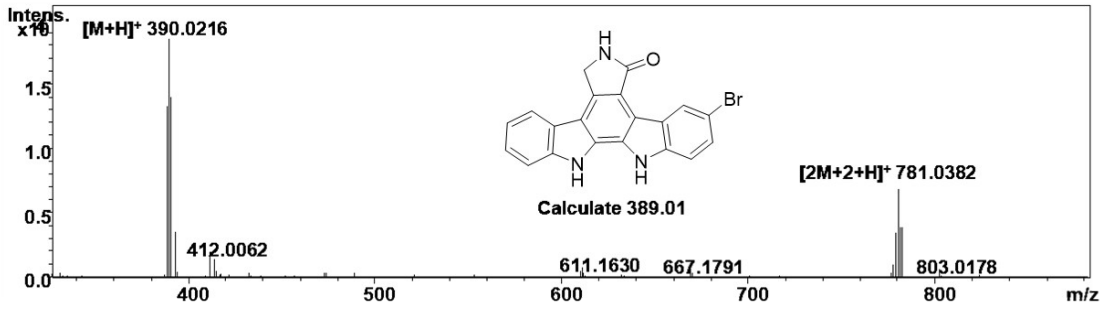


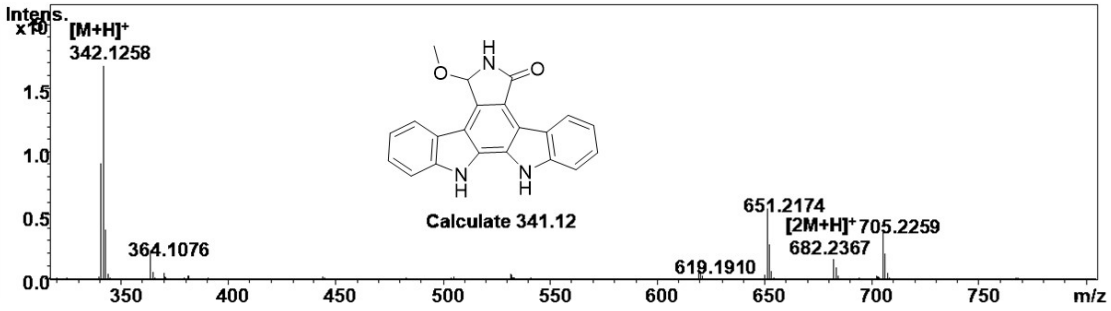


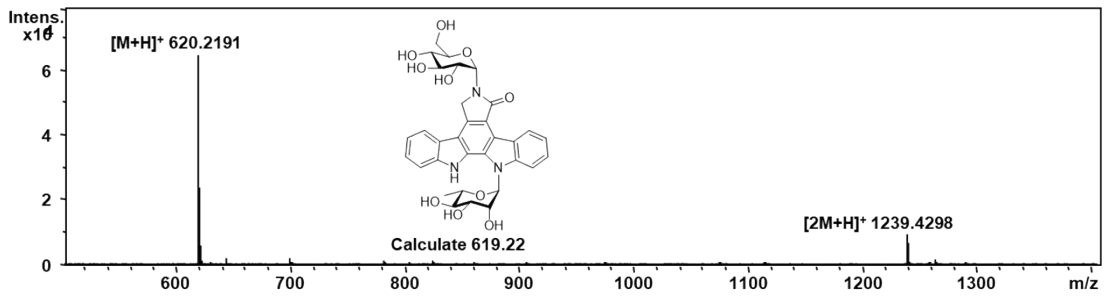


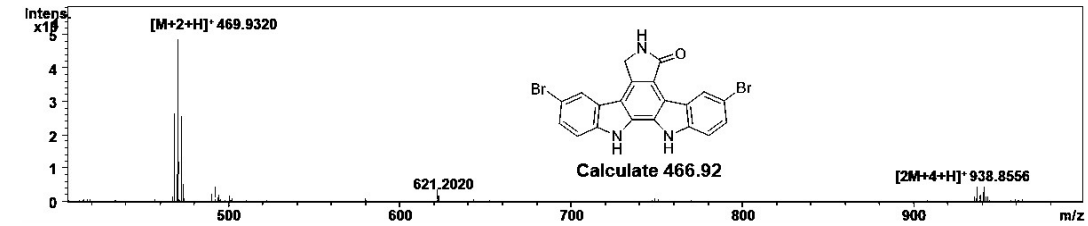

Intens.
x10
[M+H]+ 621.2004
312.1119
440.3570
Calculate 620.19
[2M+H]+ 1421.4020
m/z

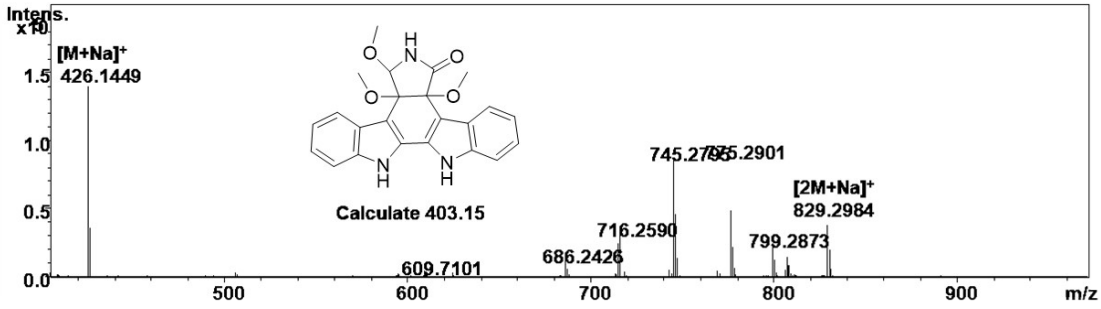

Intens.
x10
[M+H]+ 621.2011
312.1123
416.3721
Calculate 620.19
[2M+H]+ 1421.4023
m/z

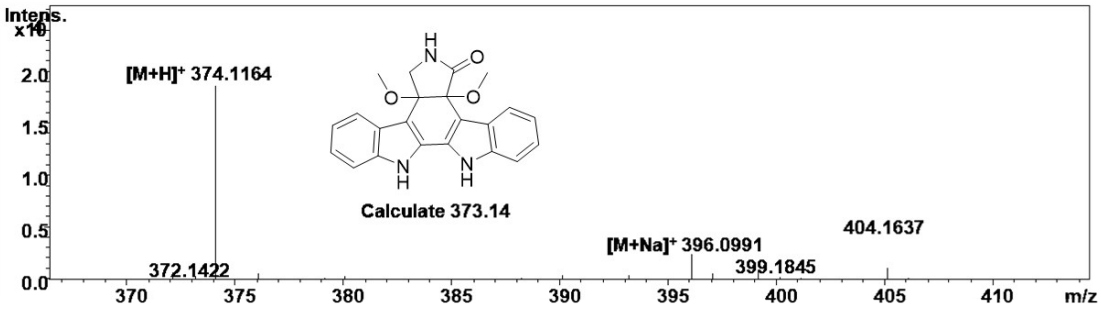

Intens.
x10
[M+H]+ 390.0216
Br
Calculate 389.01
[2M+2+H]+ 781.0382
412.0062
611.1630
667.1791
803.0178
m/z

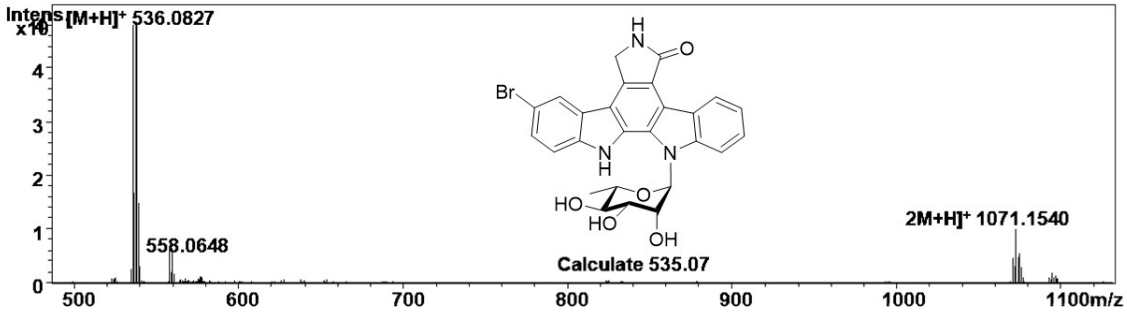

Intens.
x10
[M+H]+
342.1258
Calculate 341.12
651.2174
[2M+H]+ 705.2259
682.2367
364.1076
619.1910
m/z


Intens.
x10
[M+H]+ 620.2191
Calculate 619.22
[2M+H]+ 1239.4298
m/z


Intens.
x10
[M+2+H]+ 469.9320
621.2020
Calculate 466.92
[2M+4+H]+ 938.8556
m/z


Intens.
x10
[M+Na]+
426.1449
Calculate 403.15
745.2795
775.2901
[2M+Na]+
829.2984
716.2590
799.2873
686.2426
609.7101
m/z


Intens.
x10
[M+H]+ 374.1164
Calculate 373.14
404.1637
[M+Na]+ 396.0991
399.1845
372.1422
m/z


Intens.
x10
[M+H]+ 536.0827
Br
HO
HO
OH
2M+H]+ 1071.1540
558.0648
Calculate 535.07
m/z