Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Materials for

Unlocking mild-condition benzene ring contraction using nonheme diiron *N*-oxygenase

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Experimental Procedures

General Experiment procedures: Unless otherwise noted, chemical reagents were purchased commercially and used as received (Sigma-Aldrich, Macklin Lab, and Aladdin Bio-Chem Technology, *etc*.). GDH was purchased from SyncoZymes Co., Ltd (Shanghai). Protein marker and Braford protein concentration quantification kit was purchased from Yeasen Biotech (Shanghai). ^{1}H- and ^{13}C- NMR spectra were recorded on a Bruker UltraShield 400 MHz and 600 MHz spectrometer. High resolution mass spectra were obtained by using Bruker UHR ES TOF MS. Single crystal X-ray diffraction data was collected on SuperNova diffractometer.

AzoC (GenBank Accession Code: AKQ24642.1) sequence:

MSSRAPEELTGVQSPELPAYDPDDQAENAVIARLAGNWHRRAAVKREEPNLADLFELARDDYPERIL
PFRDHPTFRALPPEDRARLLSWAWISYNRTTVLLEGQIVNPAFQLGLDGEFPQPVSELMQRSLAQAMV
DEQYHTLMHLNASAVTRRRRGEAFADAALPKPLVVREHEARLASCANERERRLTTLAFATVAEISIN
AYLNLIADDKEIQPVNSATVRIHNRDEYCHASISAVLAEQVHHTLDDGERRYFLQSLVAGLEAFVGND
FMAWHRIMDEAGIRGGHEMLDDIQHAGGRKRLVQDFSGLRKLVERLDAVDDLDFDWSRSVTGSDA
VSPTR

AzoC-III sequence (the red marked letters represent the mutant amino acid residues):

MSSRAPEELTGVQSPELPAYDPDDQAENAVIARLAGNWHRRAAVKREEPNLADLFELARDDYPERIL
PFRDHPTFRALPPEDRARLLSWAWISYNRT**G**VL**G**EGQIVNPAFQLGLDGEFPQPVSELMQRSLAQAM
VDEQYHTLMHLNASAVTRRRRGEAFADAALPKPLVVREHEARLASCANERERRLTTLAFATVAEISI
NAYLNLIADDKEIQPVNSATVRIHNRDEYCHASISAVLAEQVHHTLDDGERRYFLQSLVAGLEAFVGN
DFMAWHRIMDEAGIRGGHEMLDDIQHAGGRKRLVQDFSGLRKLVERLDAVDDLDFDWSRSVTGSD
AVSPTR

DNA sequence of AzoC coding gene:

ATGTCTTCTCGTGCCCCGGAAGAACTGACAGGTGTTCAGAGTCCGGAATTACCGGCCTATGATCC
GGATGATCAGGCCGAAAATGCAGTTATTGCCCGCTTAGCAGGTAATTGGCATCGTCGCGCCGCCG
TTAAACGCGAAGAACCGAATCTGGCCGATCTGTTTGAATTAGCACGCGATGATTATCCGGAACGC
ATTCTGCCGTTTCGCGATCATCCGACCTTTCGCGCCCTGCCGCCGGAAGATCGCGCACGTTTACTG
TCTTGGGCATGGATTTCTTATAATCGTACCACCGTGCTGCTGGAAGGTCAGATTGTTAATCCGGCC
TTTCAGCTGGGCTTAGATGGTGAATTTCCGCAGCCGGTGAGCGAATTAATGCAGCGCTCACTGGC
ACAGGCAATGGTTGATGAACAGTATCATACCTTAATGCATTTAAATGCAAGCGCCGTGACCCGTC
GTCGTCGCGGCGAAGCCTTTGCCGATGCCGCATTACCGAAACCGCTGGTTGTGCGTGAACATGAA
GCACGCTTAGCAAGTTGTGCCAATGAACGTGAACGTCGCCTGACCACCCTGGCCTTTGCCACCGT
TGCAGAAATTTCTATTAATGCCTATCTGAATCTGATTGCAGATGATAAAGAAATTCAGCCGGTTA
ATAGCGCAACCGTGCGTATTCATAATCGCGATGAATATTGTCATGCCTCAATTAGTGCAGTGTTA
GCAGAACAGGTTCATCATACCCTGGATGATGGCGAACGTCGCTATTTTTTACAGTCACTGGTGGC
AGGCTTAGAAGCCTTTGTGGGCAATGATTTTATGGCATGGCATCGTATTATGGATGAAGCAGGTA
TTCGCGGCGGCCATGAAATGTTAGATGATATTCAGCACGCAGGCGGTCGTAAACGTTTAGTTCAG
GATTTTAGTGGTCTGCGTAAACTGGTGGAACGCTTAGATGCAGTGGATGATCTGGATTTTGATTG
GTCACGTAGTGTGACCGGGAGCGATGCAGTGTCTCCGACCCGC

Protein expression and purification: *Escherichia coli* BL21(DE3) containing the AzoC (or mutants) expressing plasmid pET28a-azoC (or mutants) was incubated into 1 L of TB liquid culture with the supplementation of 50 μg/mL kanamycin, and further cultivated at 37 °C, 220

r.p.m for 5 h, until the OD 600 reached 0.6. Then, the culture was added with 0.1 mM isopropyl β-D-1-thiogalactopyranoside and 1mM Mohr's salt (ammonium ferric sulfate), and further incubated at 25 °C for 24 h. After that, cells were collected and lysed with sonication in buffer A (20 mM Tris, pH 8.0, 250 mM NaCl, and 10 mM imidazole). Then, the cell lysate was centrifuged at 14000 rpm, 4 °C for 15 min, and the supernatant was collected and referred to Ni^{2+}-nitrilotriacetic acid resin column for protein purification. After loading the sample, the Ni^{2+}-nitrilotriacetic acid resin column was first eluted with buffer A solution containing 50mM imidazole to remove the undesired proteins, then was eluted with buffer A solution containing 250mM imidazole to get the target enzyme. The recombinant protein was desalted on a 10-kDa YM-3 column with 20mM Tris buffer (pH 7.5). The resulting enzyme was analyzed with SDS-PAGE. Protein concentration was determined with the Bradford assay, and bovine serum albumin was employed as standard.

Protein engineering method: Mutation experiments were conducted by whole plasmid PCR using Pfu DNA polymerase. The plasmid pET28a-AzoC was used as the template for PCR amplified with corresponding mutagenesis primer pairs. The resulting PCR product was digested with DpnI to eliminate the template. Then, the digested products were transformed into *E.coli* BL21(DE3), and further incubated at 37 °C for the single colony isolation on the LB agar plate with the addition of 50 μg/mL kanamycin. Single colonies grown on the plate were further cultivated for protein purification, and tested for enzymatic activities. The positive mutant was referred to DNA sequencing to identify the mutant site.

GDH Stock solution: NAD^+ (5 mg/mL), Glucose (180 mg/mL), GDH (500 U/mL) dissolved in PBS buffer (pH 8).

Scale-up bioconversion method: *E. coli* BL21(DE3) containing pET28a-azoC were grown in TB liquid culture with the supplementation of with 1mM ammonium ferric sulfate and 50 μg/mL kanamycin and further cultivated at 37 °C, 220 r.p.m for 5 h until the OD 600 reached 1. Then, the culture was added with 0.1 mM isopropyl β-D-1-thiogalactopyranoside and further inducted at 25 °C for 24 h. Later, cells were collected and lysed, and referred to 14000 rpm centrifuge at 4 °C for 15 min. The supernatants were collected and diluted 10 times with HEPES buffer or Tris buffer or PBS buffer, then the solution was added with 100 μM PMS, 100 μM TEMPO, 1mM phloroglucinol and 3 mM amines, and incubated at 25 °C, 200 rpm for 12 h.

HPLC analysis method: HPLC analysis was performed on a Thermol Ultimate 3000 system with VWD Uv detector. A Thermol Hypersil Gold column (250*4.6 mm, 5 μm) was used. Mobile phase A was 0.1% triflouroacetic acid (TFA) in water, and mobile phase B was acetonitrile. Flow rate was 1 mL/min. During the analysis, mobile phase B raised from 10% to 100% in 20 min, and kept at 100% for 5 min.

Isotope replacement experiment: 1 mM phloroglucinol and 2 mM nitrosobenzene were added into NaOD's D2O solution (pH 8), then the solution was incubated at room temperature for 1 h. Then the resulting sample was referred to LC-HRMS analysis. And another reaction sample in NaOH's H2O solution (pH 8) was used as control.

Silica gel chromatography: Silica gel chromatography were performed with silica gel (200-300 meshes). Hexane and ethyl acetate were used as eluents. Scale-up reactions were extracted using ethyl acetate and then vacuum dried, the products were then purified using silica gel chromatography. Compound **3-3a** were purified using Hexane/Ethyl acetate (1:1) as the eluent, and **3b-3o** were purified using Hexane/Ethyl acetate (4:1) as the eluent.

Crystallization of 3a: Purified **3a** was dissolved in methanol to afford crystal for X-ray analysis. Crystallographic data for the reported compound **3a** has been deposited with the Cambridge Crystallographic Data Center (CCDC) under CCDC ID: 2193171.

Table S1. PCR primers

Primer Name	Primer Sequence (5' to 3')	Tm
T98A-F	accttccagcagcacggcggtacgattataagaaa	78.1
T98A-R	tttcttataatcgtaccgccgtgctgctggaaggt	
T98S-F	tccagcagcacgctggtacgattataagaaatcc	78.0
T98S-R	ggatttcttataatcgtaccagcgtgctgctgga	
T98C-F	ctgaccttccagcagcacgcaggtacgattataagaaatc	79.1
T98C-R	gatttcttataatcgtacctgcgtgctgctggaaggtcag	
T98V-F	ctgaccttccagcagcacgacggtacgattataagaaatc	78.1
T98V-R	gatttcttataatcgtaccgtcgtgctgctggaaggtcag	
T98G-F	ctgaccttccagcagcacgccggtacgattataagaaatc	79.1
T98G-R	gatttcttataatcgtaccggcgtgctgctggaaggtcag	
L101A-F	attaacaatctgaccttccgccagcacggtggtacgatta	78.1
L101A-R	taatcgtaccaccgtgctggcggaaggtcagattgttaat	
L101V-F	caatctgaccttccaccagcacggtggtacg	80.3
L101V-R	cgtaccaccgtgctggtggaaggtcagattg	
L101G-F	attaacaatctgaccttcccccagcacggtggtacgatta	78.1
L101G-R	taatcgtaccaccgtgctgggggaaggtcagattgttaat	
T98A/L101A -F	gattaacaatctgaccttccgccagcacggcggtacgattataagaaatcc	79.1
T98A/L101A -R	ggatttcttataatcgtaccgccgtgctggcggaaggtcagattgttaatc	
T98G/L101G-F	ggattaacaatctgaccttcccccagcacgccggtacgattataagaaatcca	79.1
T98G/L101G -R	tggatttcttataatcgtaccggcgtgctgggggaaggtcagattgttaatcc	
T98A/L101G -F	gattaacaatctgaccttcccccagcacggcggtacgattataagaaatcc	80.0
T98A/L101G -R	ggatttcttataatcgtaccgccgtgctgggggaaggtcagattgttaatc	
T98G/L101A-F	ggattaacaatctgaccttccgccagcacgccggtacgattataagaaatcca	79.0
T98G/L101A -R	tggatttcttataatcgtaccggcgtgctggcggaaggtcagattgttaatcc	
Sequencing-F	AGCGCTGCATTAATTCGCTC	58
Sequencing-R	CGATCATCCGACCTTTCGCG	

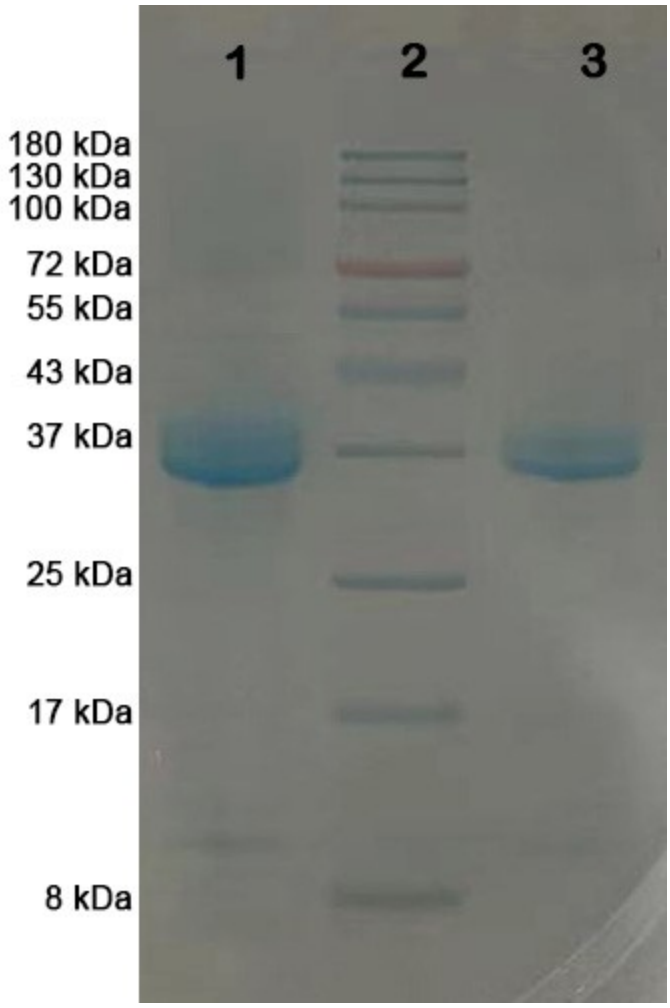

Fig. S1. SDS-PAGE analysis of purified AzoC and AzoC-III.
Lane 1, AzoC;
Lane 2, protein marker;
Lane 3, AzoC-III. Theoretical molecular weight of AzoC mutant was 39 KD.

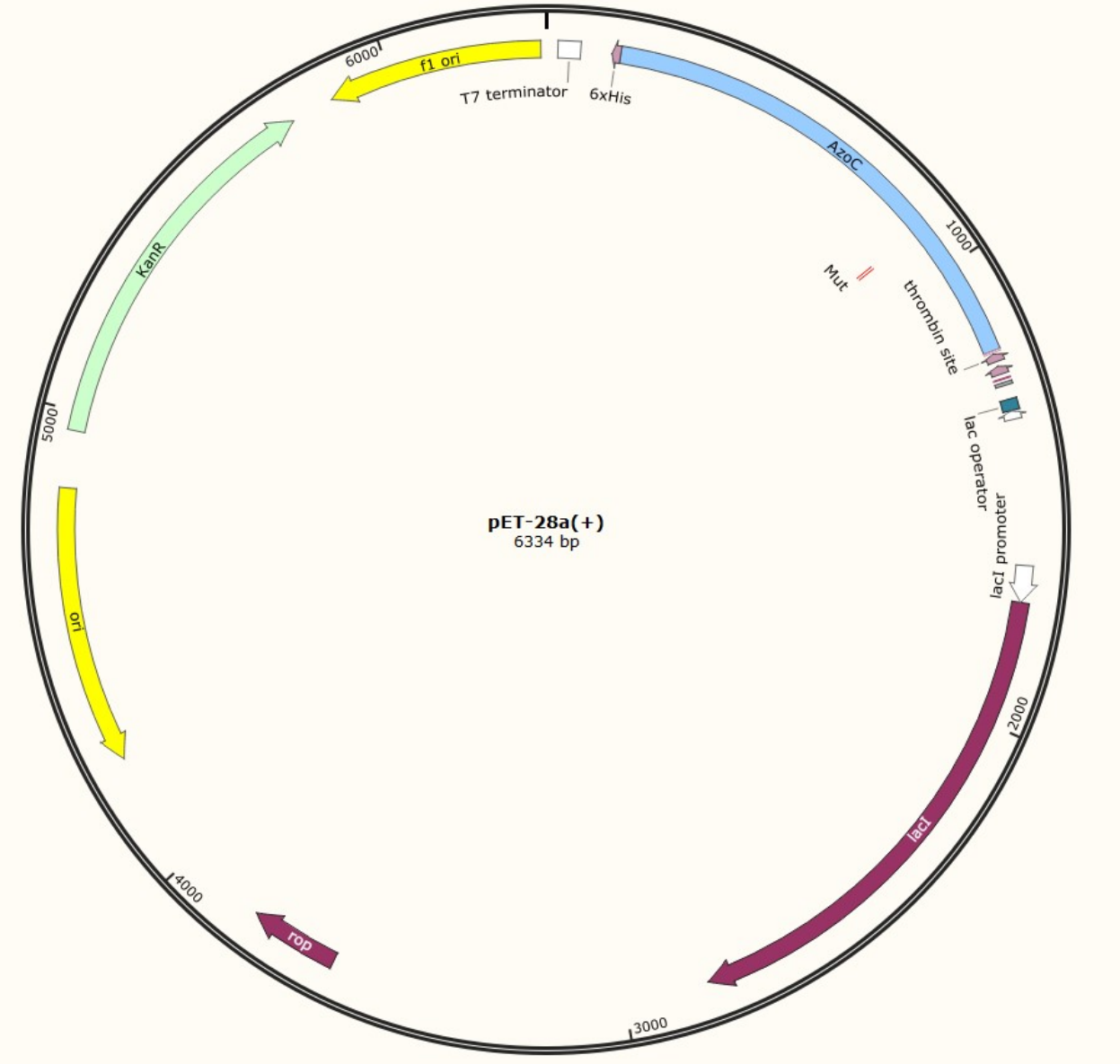

Fig. S2. Plasmid map of pET28a-AzoC.

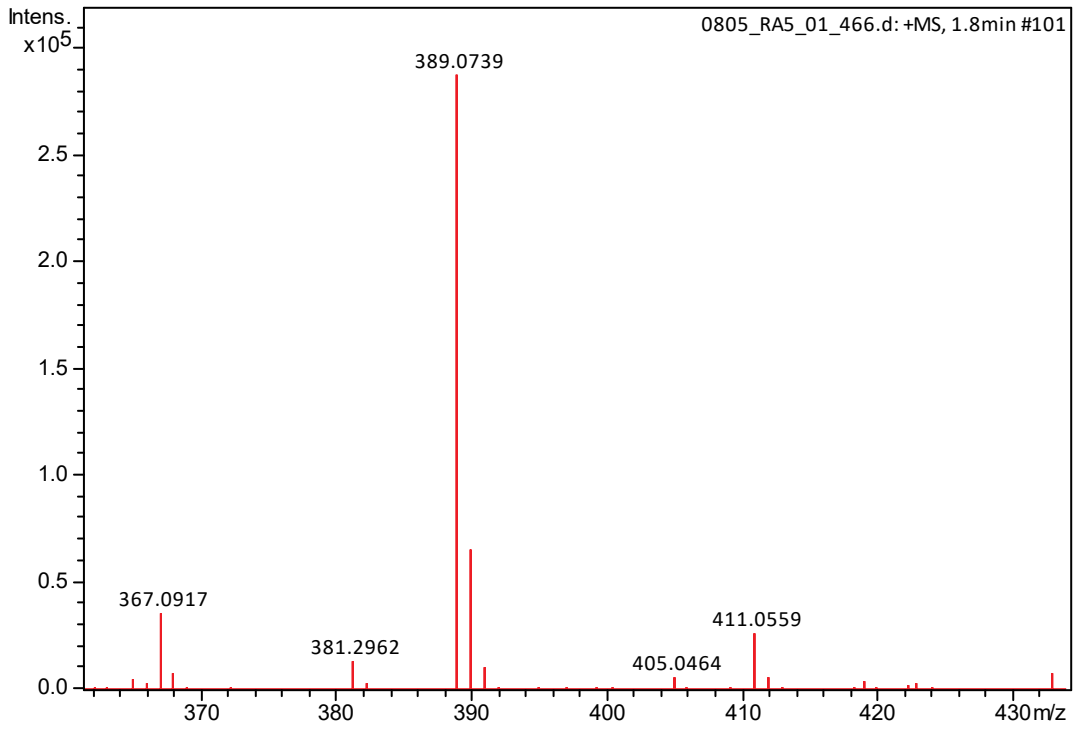

Fig. S3. HRMS of **3**. $[M+H]^+$ calculated for 367.0925, found as 367.0917. $[M+Na]^+$ calculated for 389.0744, found as 389.0739.

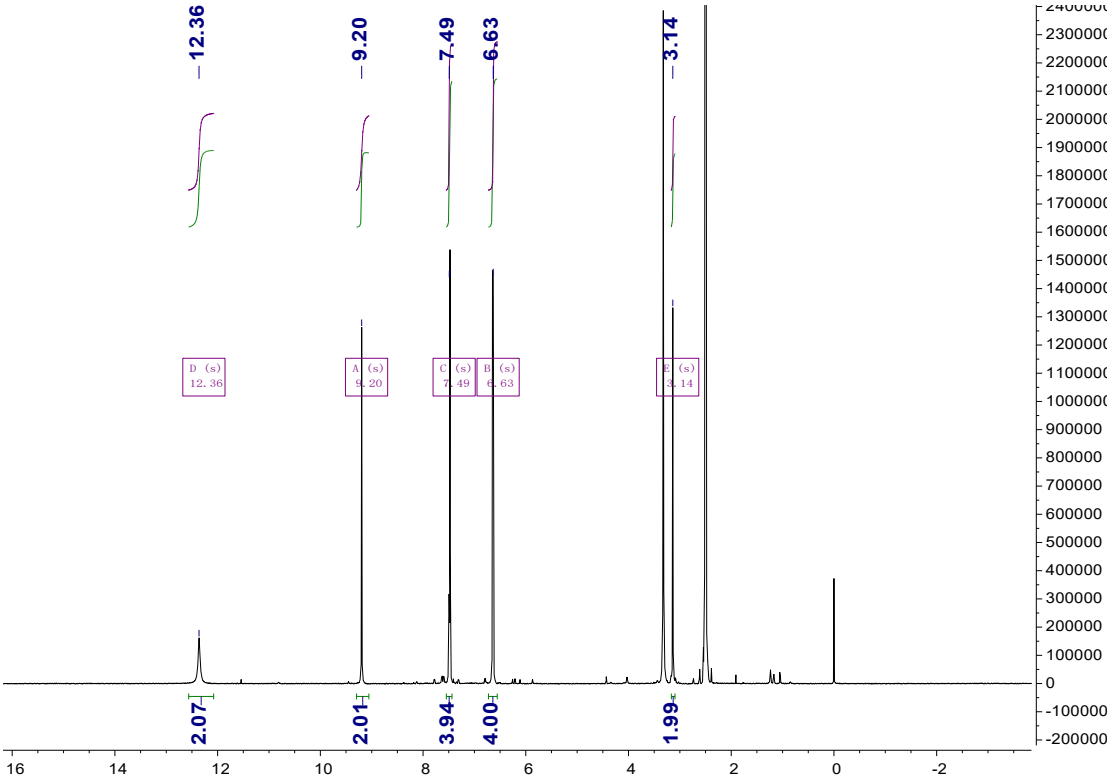

Fig. S4. ^{1}H NMR of **3**. (600 MHz, DMSO-d_6) δ 12.36 (s, 2H), 9.20 (s, 2H), 7.49 (s, 4H), 6.63 (s, 4H), 3.14 (s, 2H).

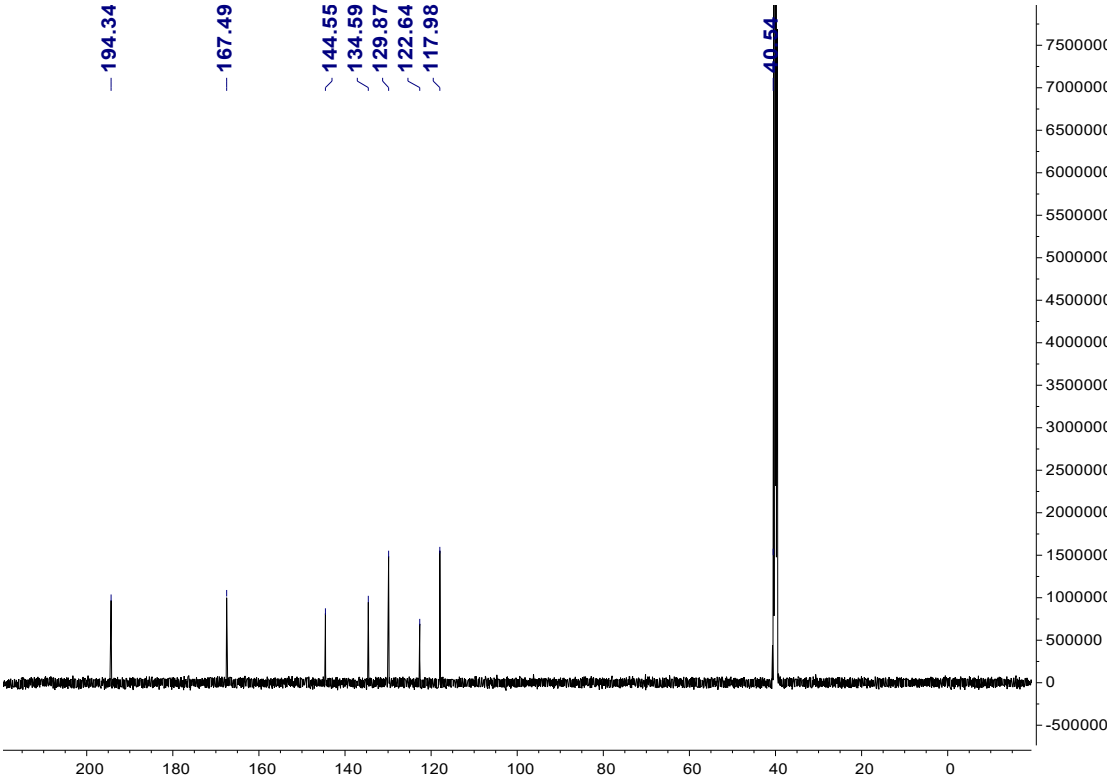

Fig. S5. ^{13}C NMR of 3. (151 MHz, DMSO-d_6) δ 194.34, 167.49, 144.55, 134.59, 129.87, 122.64, 117.98, 40.54.

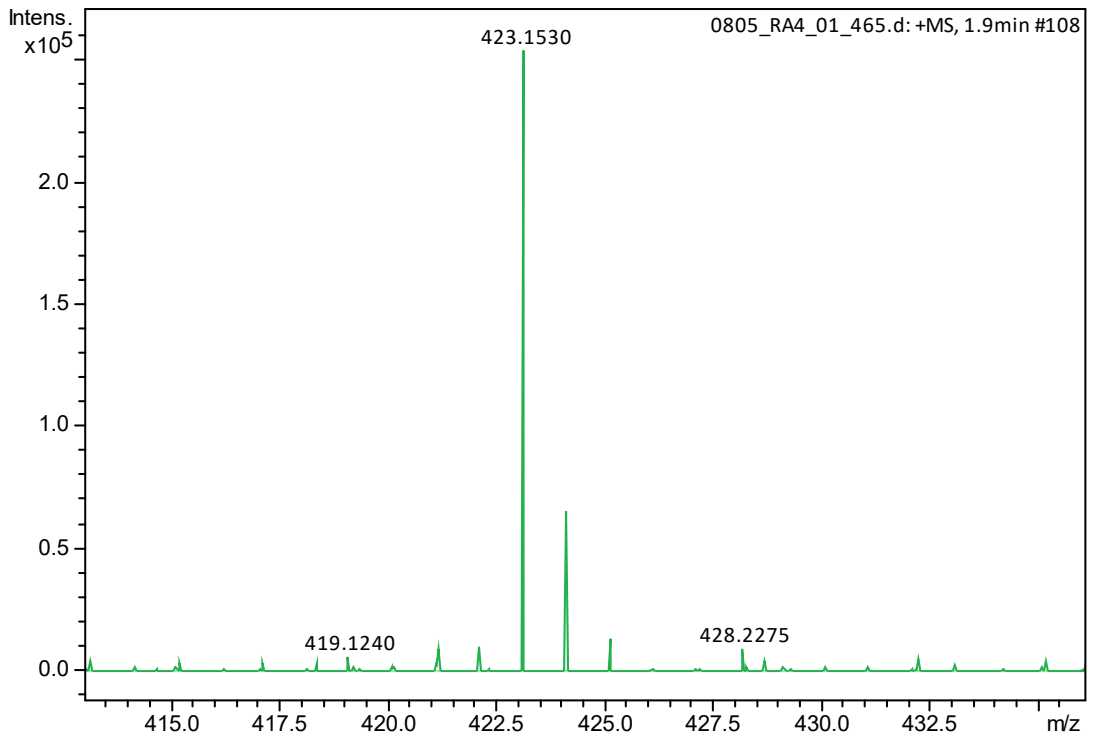

Fig. S6. HRMS of **3a**. $[M+H]^+$ calculated for 423.1556, found as 423.1530.

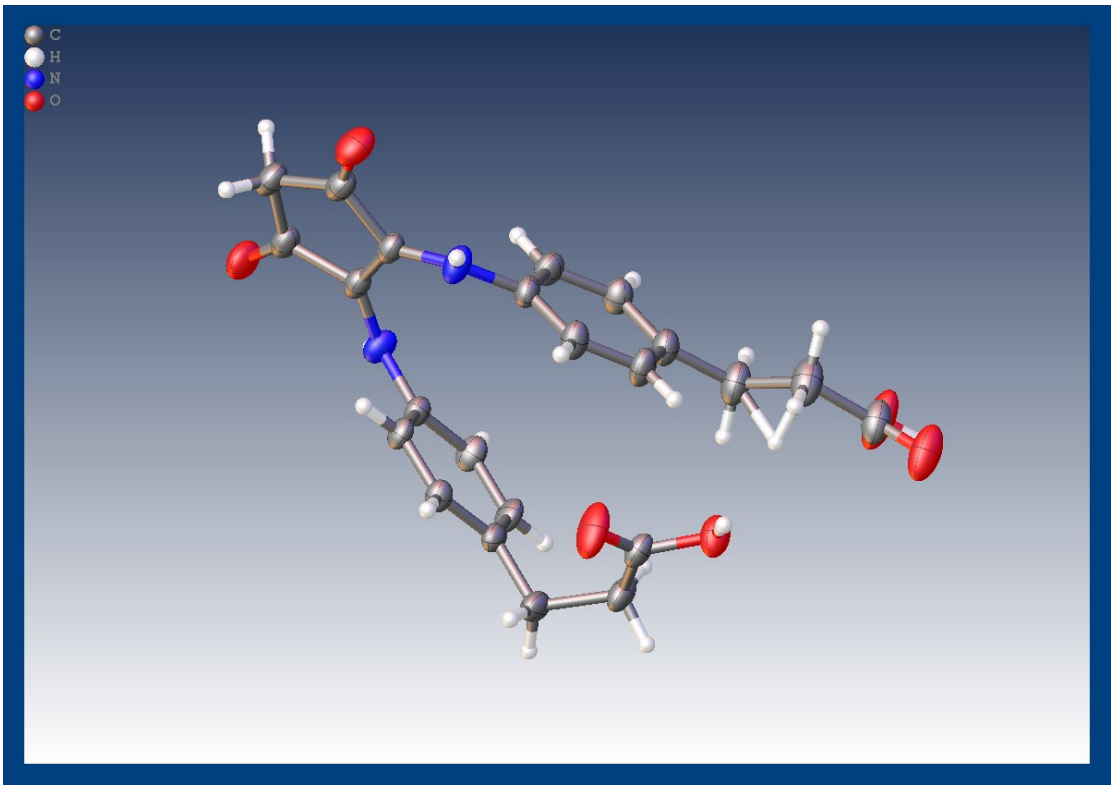

Fig. S7. Crystal structure of **3a**.

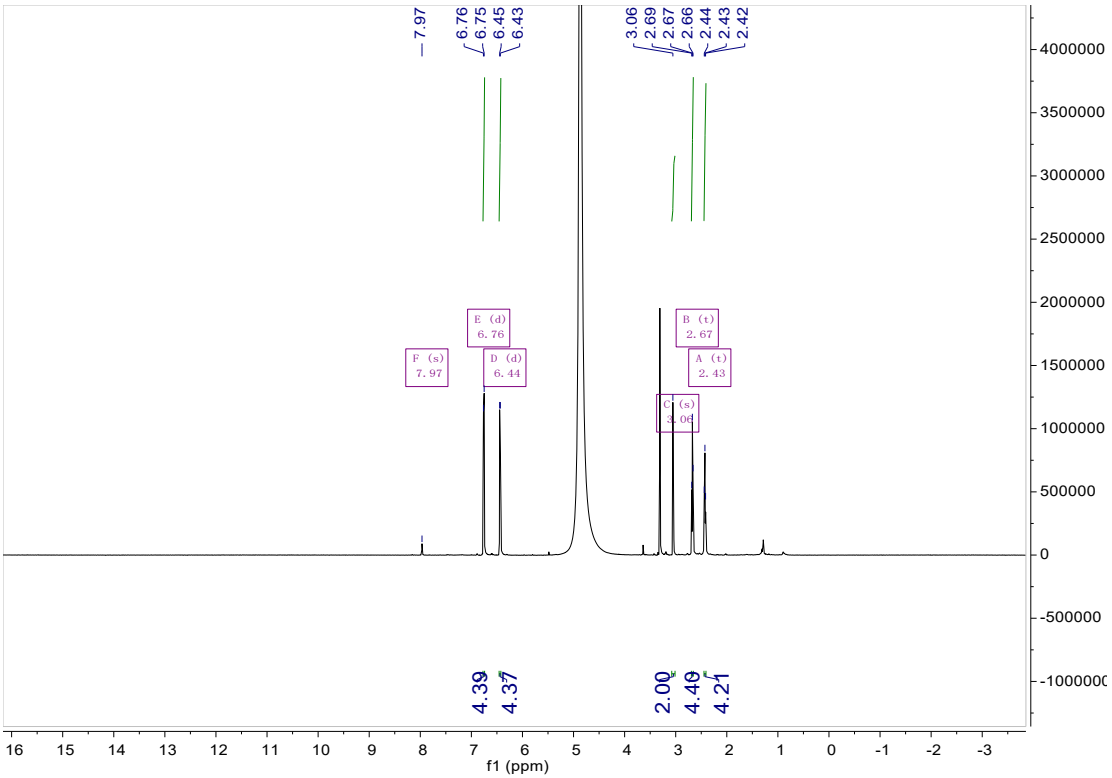

Fig. S8. [1]H NMR of compound **3a**. [1]H NMR (600 MHz, Methanol-d_4) δ 7.97 (s), 6.76 (d, J = 8.3 Hz, 4H), 6.44 (d, J = 8.3 Hz, 4H), 3.06 (s, 2H), 2.67 (t, J = 7.6 Hz, 4H), 2.43 (t, J = 7.6 Hz, 4H).

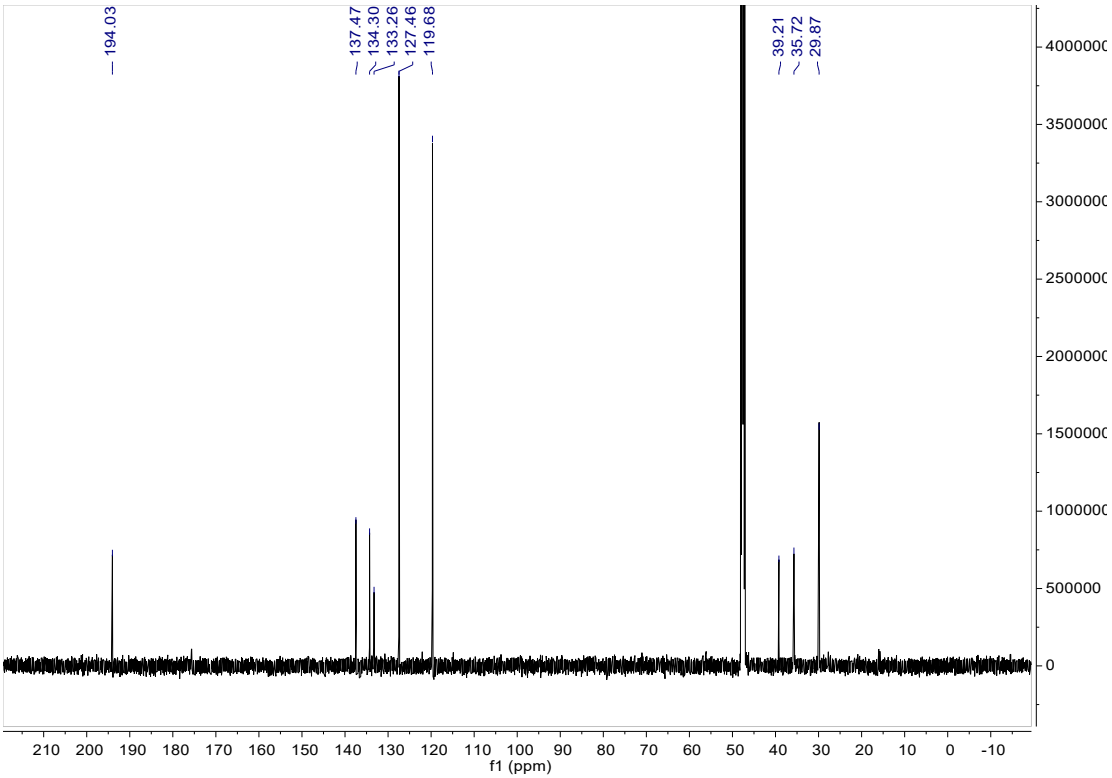

Fig. S9. ^{13}C NMR of compound **3a**.^{13}C NMR (151 MHz, Methanol-d_4) δ 194.03, 137.47, 134.30, 133.26, 127.46, 119.68, 39.21, 35.72, 29.87.

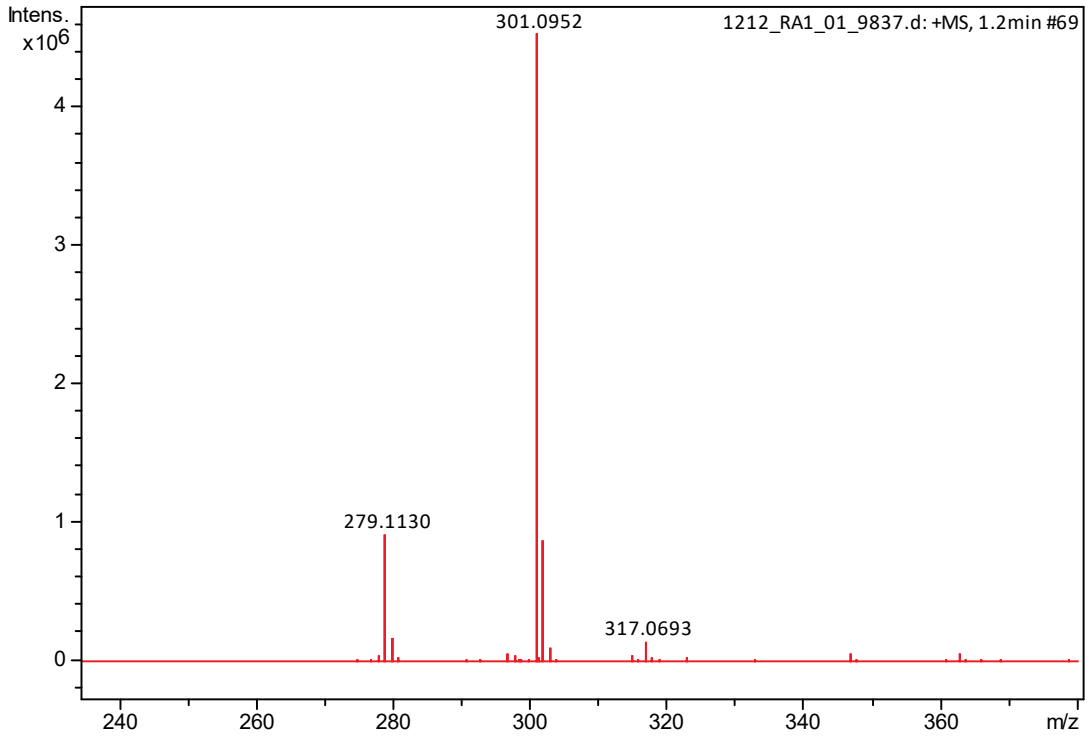

Fig. S10. HRMS of **3b**. $[M+H]^+$ calculated for 279.1134, found as 279.1130. $[M+Na]^+$ calculated for 301.0953, found as 301.0952. $[M+K]^+$ calculated for 317.0693, found as 317.0693.

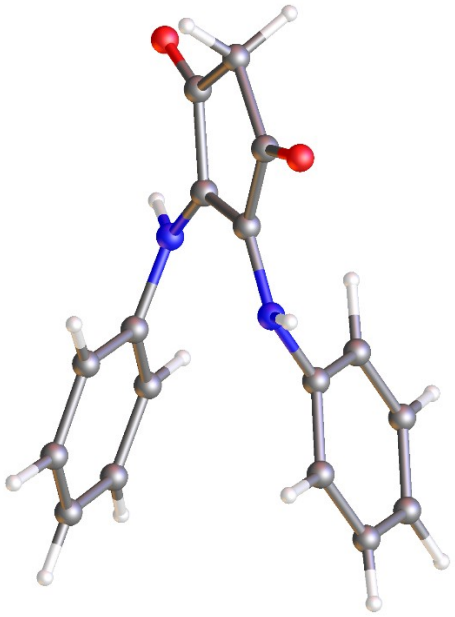

Fig. S11. Crystal structure of **3b**.

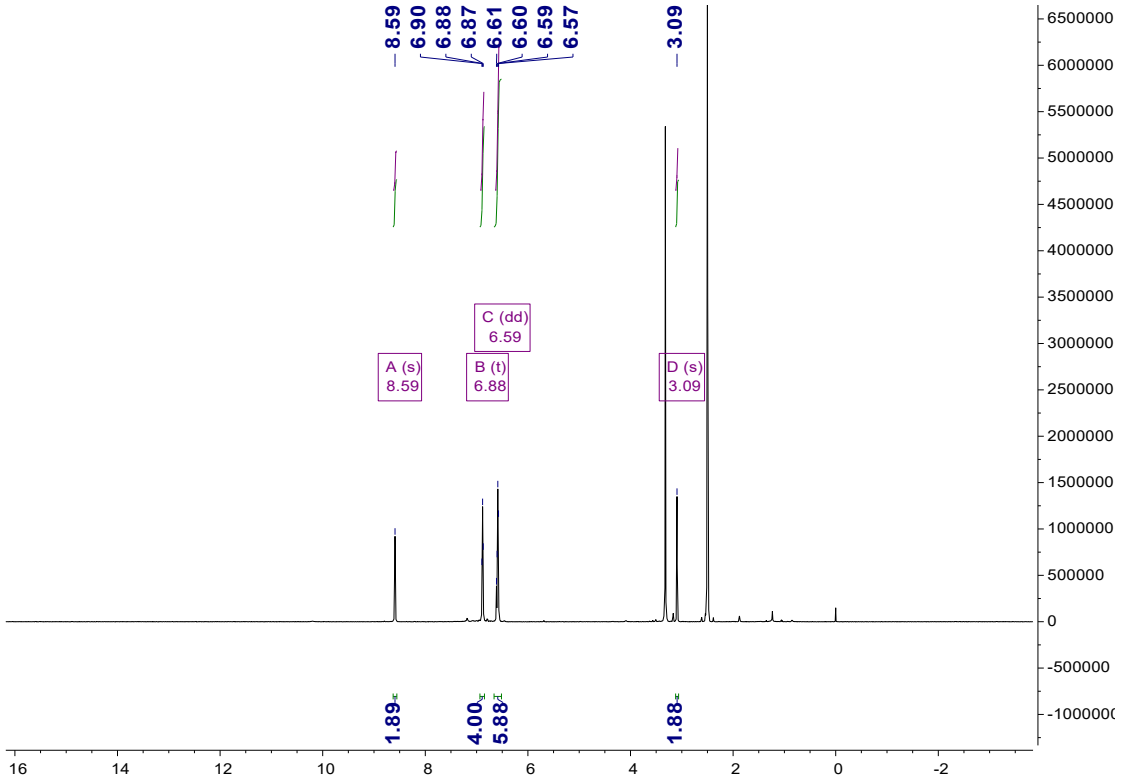

Fig. S12. ^{1}H NMR of compound **3b**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59 (s, 2H), 6.88 (t, J = 7.7 Hz, 4H), 6.59 (dd, J = 16.0, 7.8 Hz, 6H), 3.09 (s, 2H).

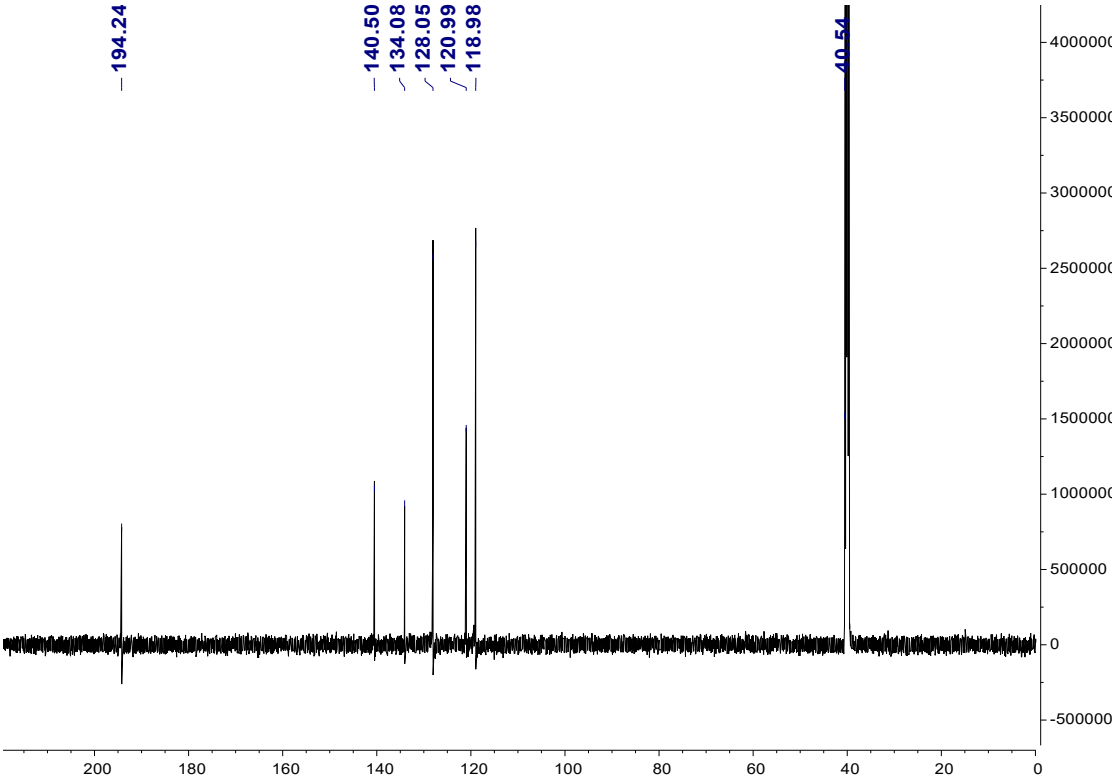

Fig. S13. ^{13}C NMR of compound **3b**. ^{13}C NMR (151 MHz, DMSO-d_6) δ 194.24, 140.50, 134.08, 128.05, 120.99, 118.98, 40.54.

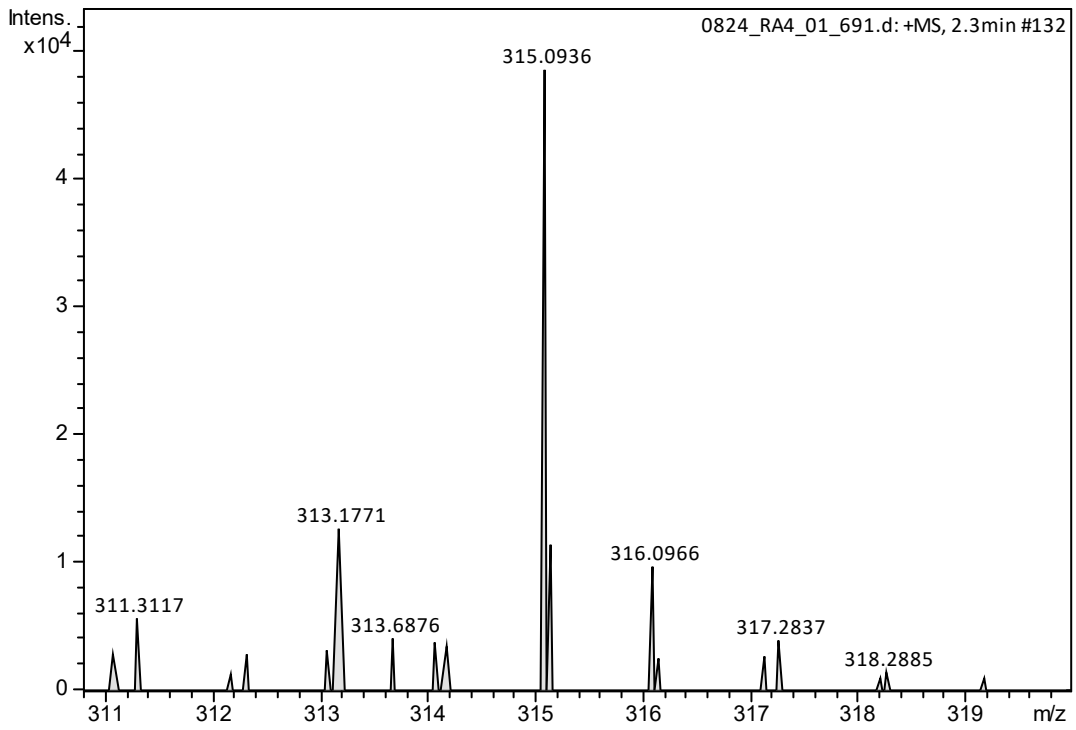

Fig. S14. HRMS of **3c**. $[M+H]^+$ calculated for 315.0940, found as 389.0932.

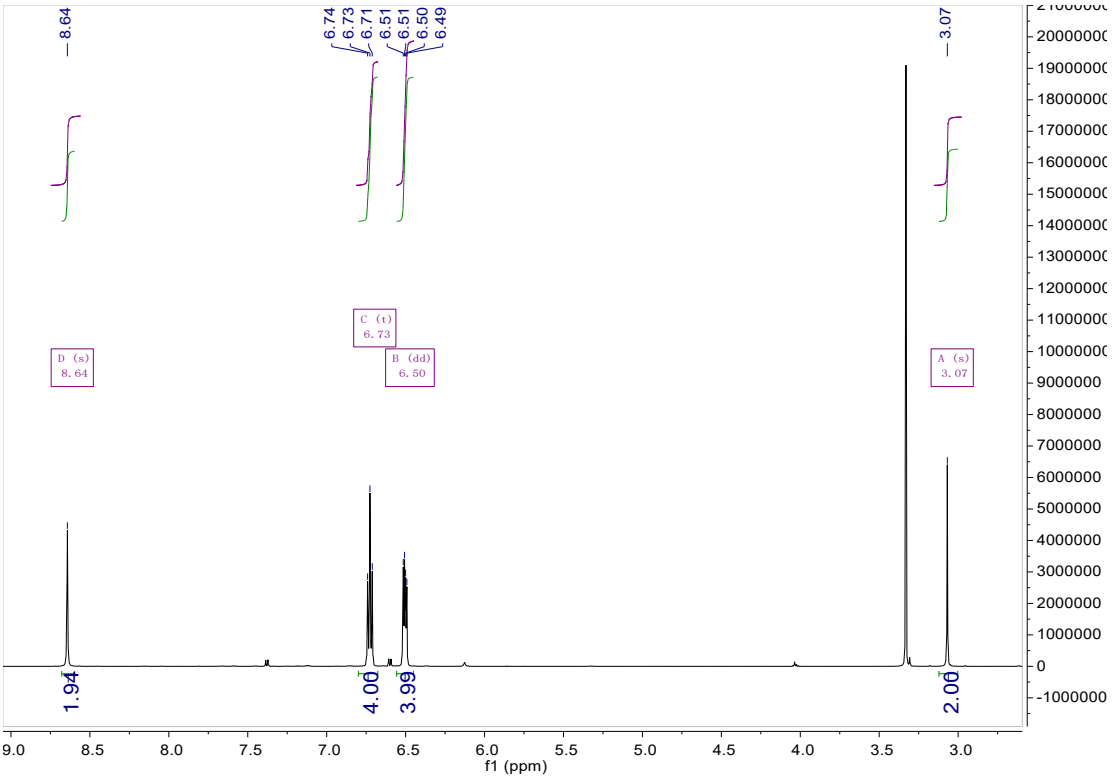

Fig. S15. ^{1}H NMR of **3c**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (s, 2H), 6.73 (t, J = 8.8 Hz, 4H), 6.50 (dd, J = 9.0, 4.8 Hz, 4H), 3.07 (s, 2H).

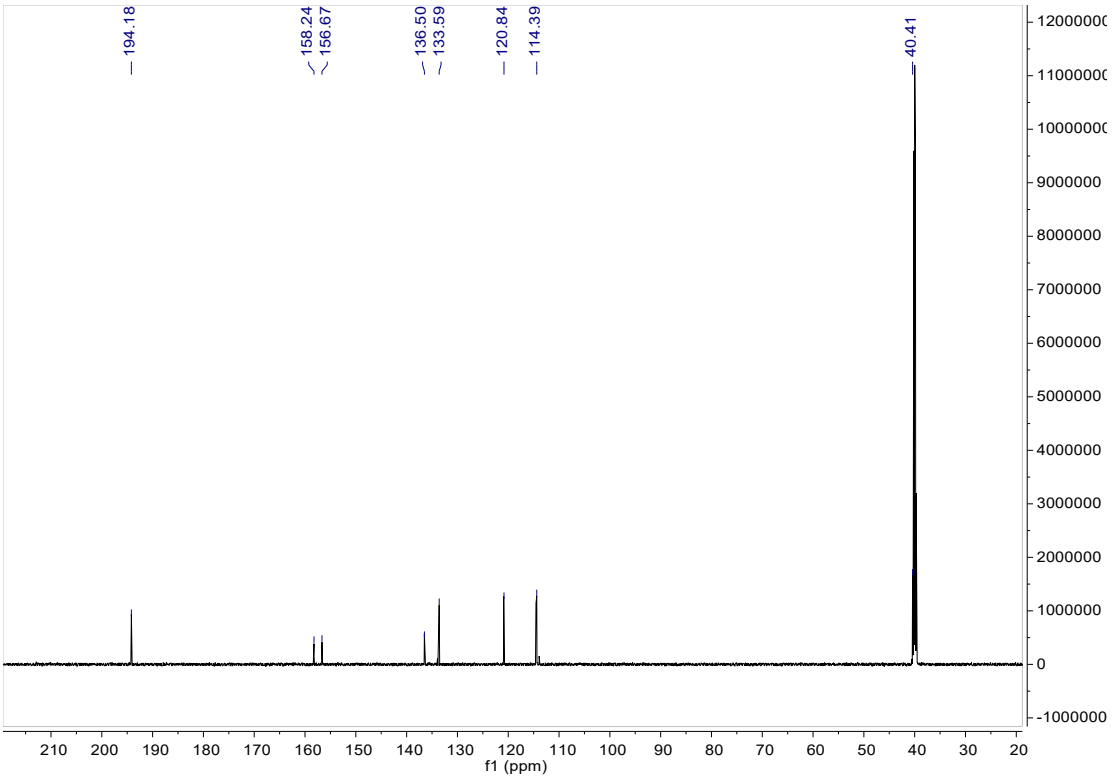

Fig. S16. ^{13}C NMR of **3c**. ^{13}C NMR (151 MHz, DMSO-d_6) δ 194.18, 158.24, 156.67, 136.50, 133.59, 120.84, 114.39, 40.41.

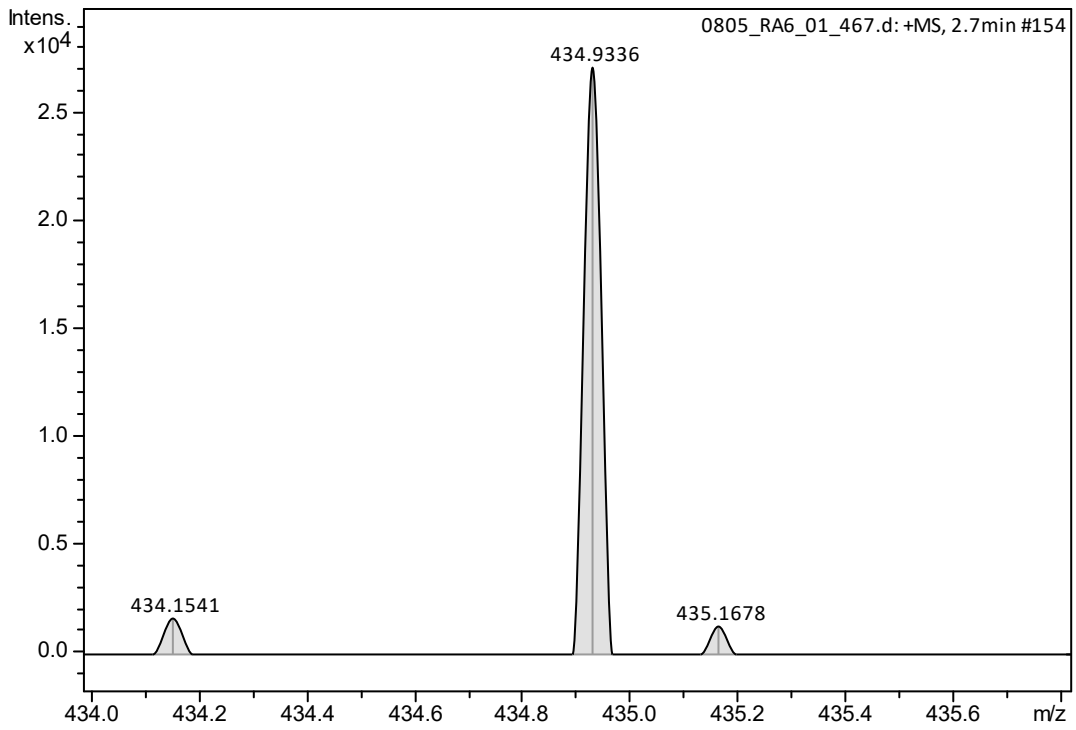

Fig. S17. HRMS of **3d**. $[M+H]^+$ calculated for 434.9338, found as 434.9336.

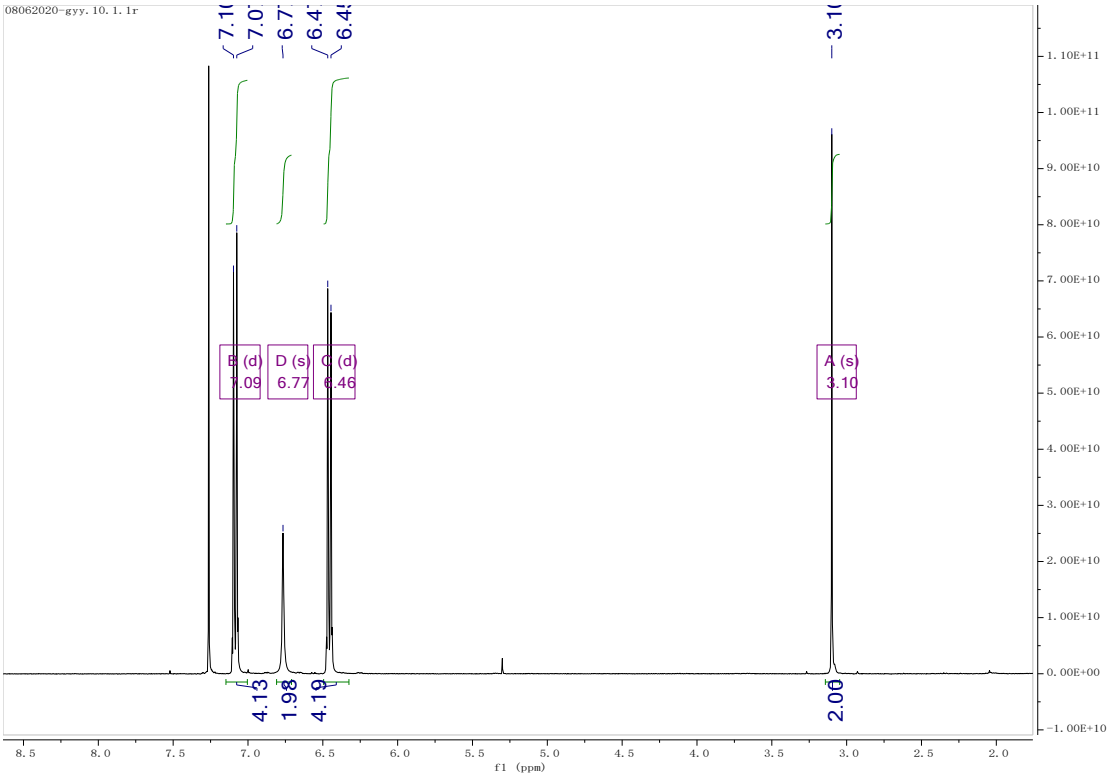

Fig. S18. [1]H NMR of **3d**. [1]H NMR (400 MHz, Chloroform-*d*) δ 7.09 (d, *J* = 8.8 Hz, 4H), 6.77 (s, 2H), 6.46 (d, *J* = 8.7 Hz, 4H), 3.10 (s, 2H).

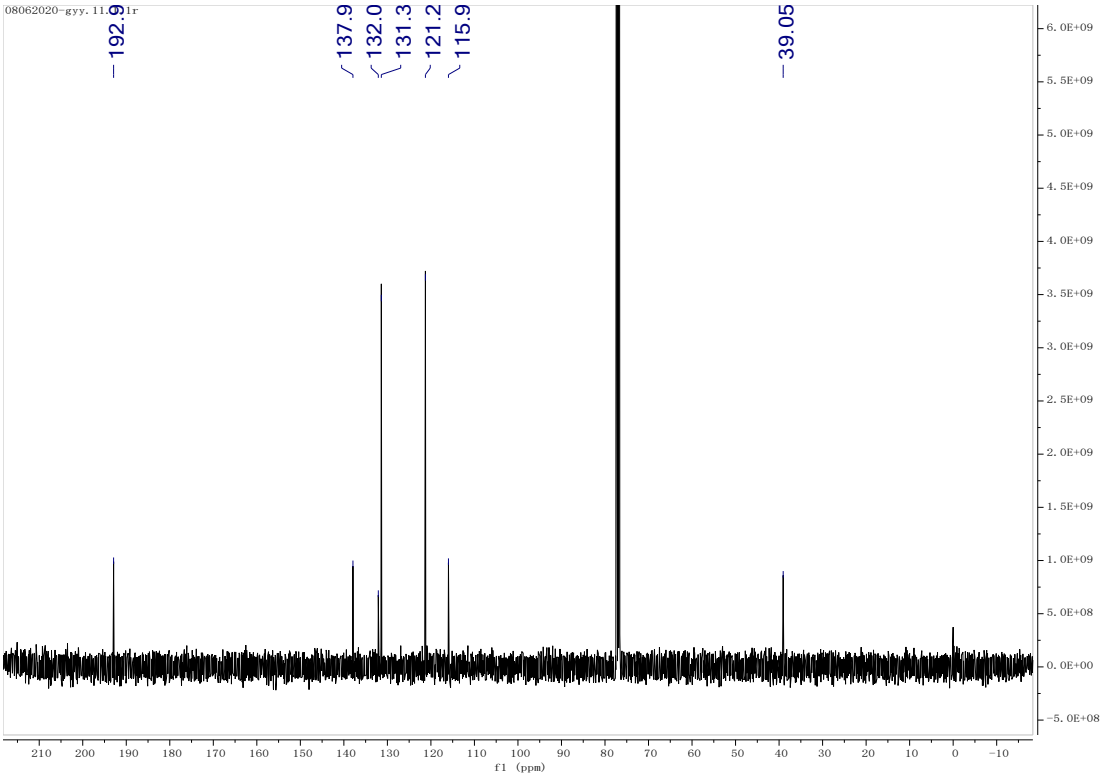

Fig. S19. ^{13}C NMR of **3d**. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 192.91, 137.91, 132.08, 131.39, 121.26, 115.97, 39.05.

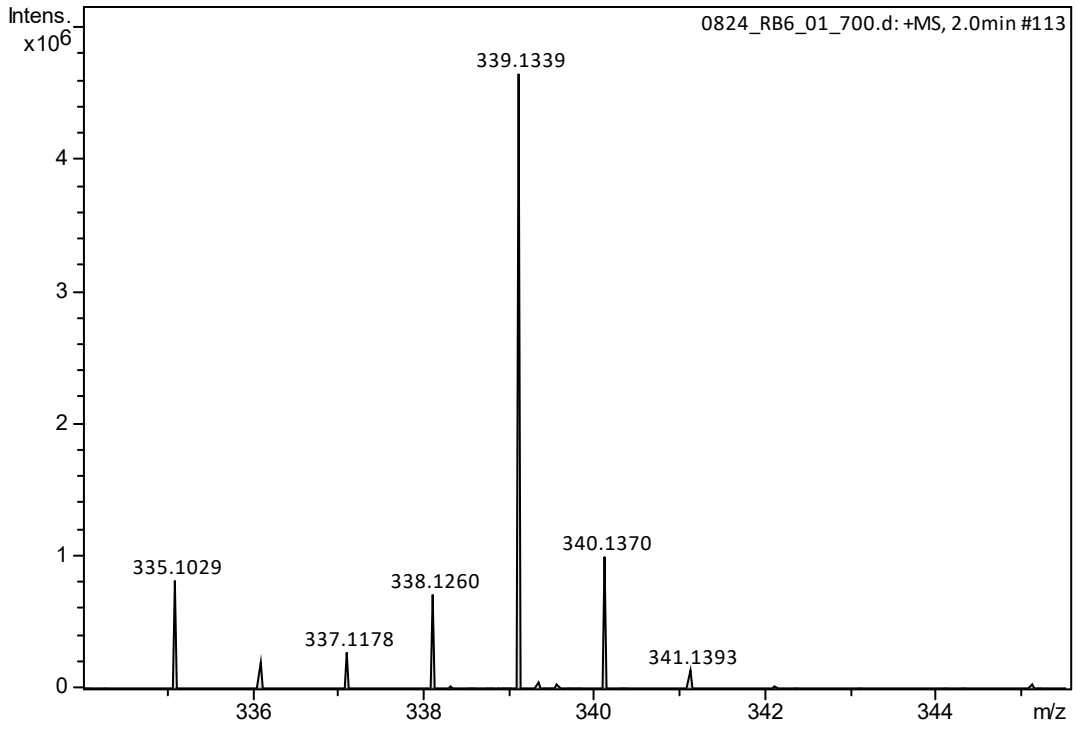

Fig. S20. HRMS of **3e**. $[M+H]^+$ calculated for 339.1339, found as 339.1339.

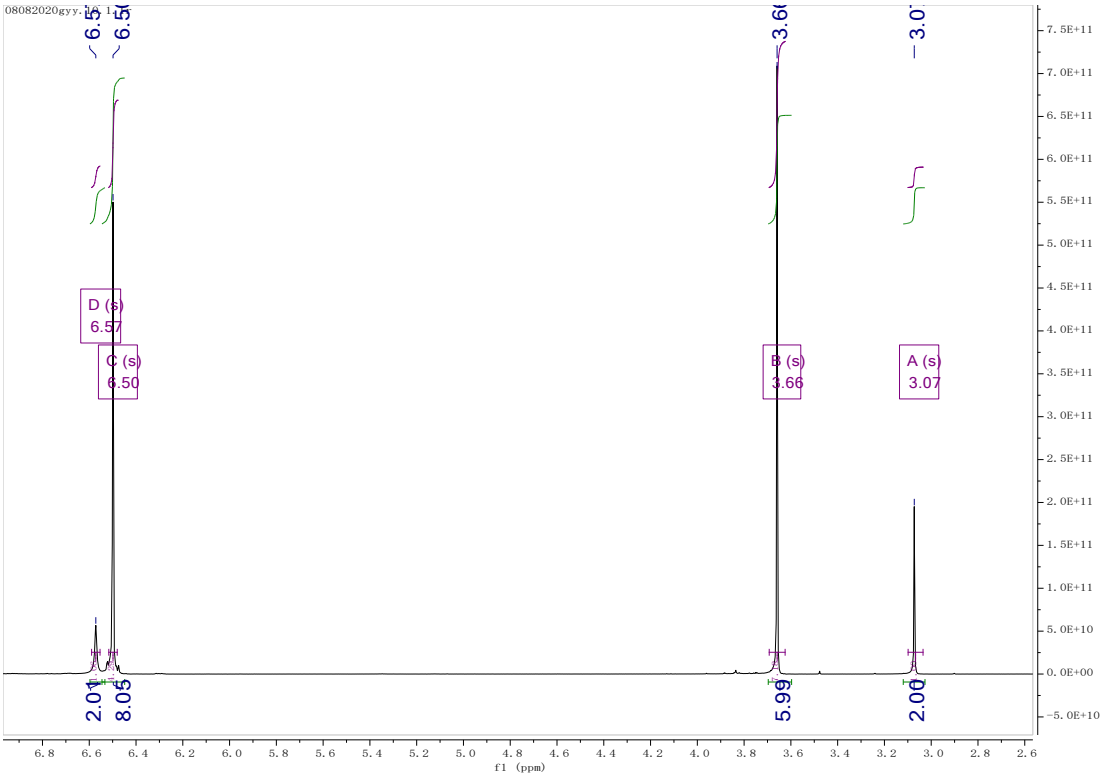

Fig. S21. ^{1}H NMR of **3e**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.57 (s, 2H), 6.50 (s, 8H), 3.66 (s, 6H), 3.07 (s, 2H).

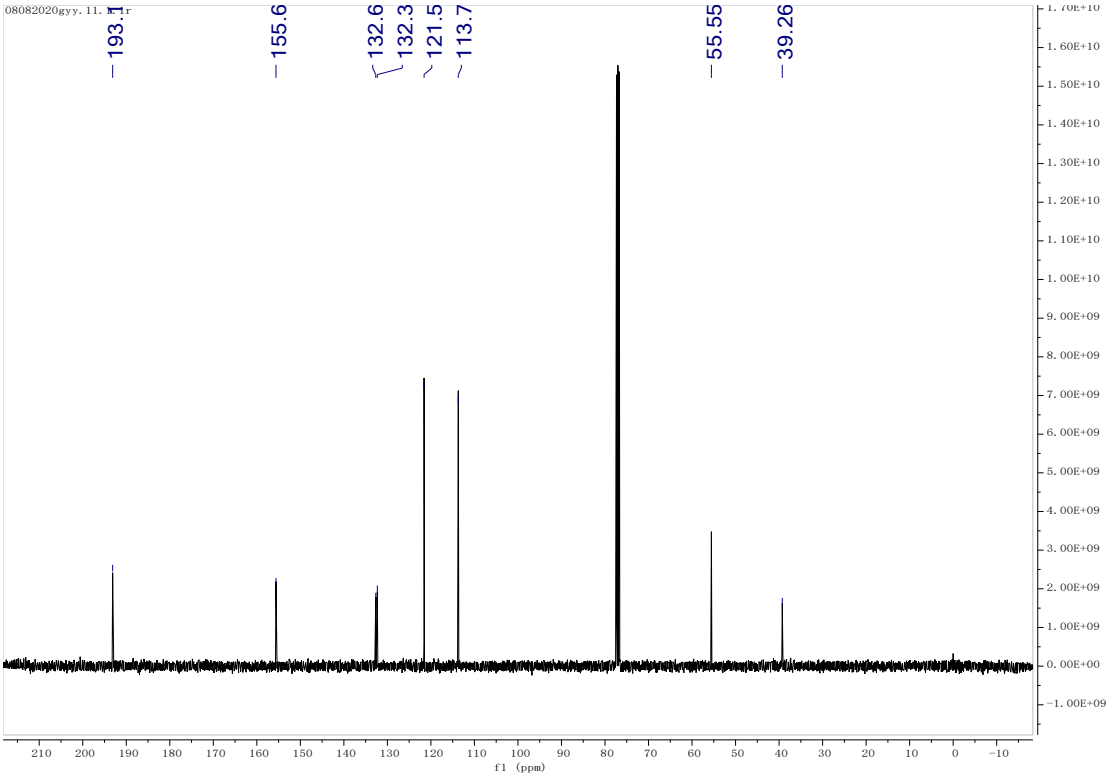

Fig. S22. ^{13}C NMR of **3e**. (101 MHz, Chloroform-*d*) δ 193.13, 155.60, 132.67, 132.31, 121.57, 113.71, 55.55, 39.26.

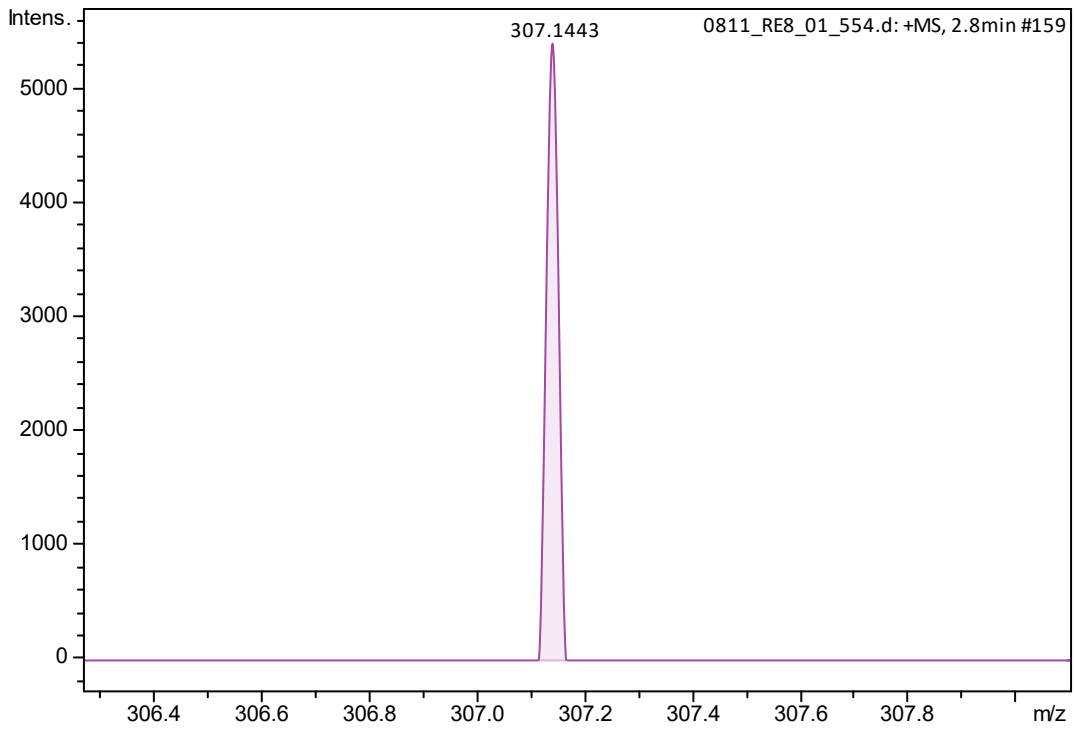

Fig. S23. HRMS of **3f**. $[M+H]^+$ calculated for 307.1441, found as 307.1443.

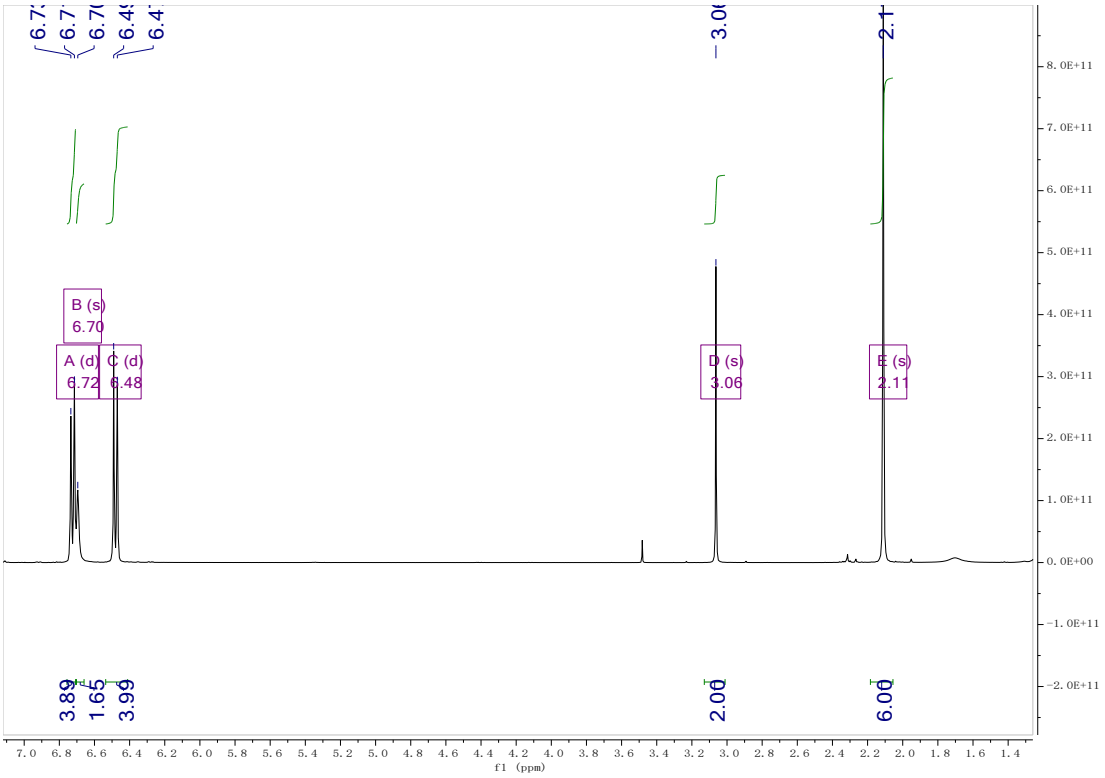

Fig. S24. ^{1}H NMR of **3f**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.72 (d, *J* = 8.2 Hz, 4H), 6.70 (s, 2H), 6.48 (d, *J* = 8.4 Hz, 4H), 3.06 (s, 2H), 2.11 (s, 6H).

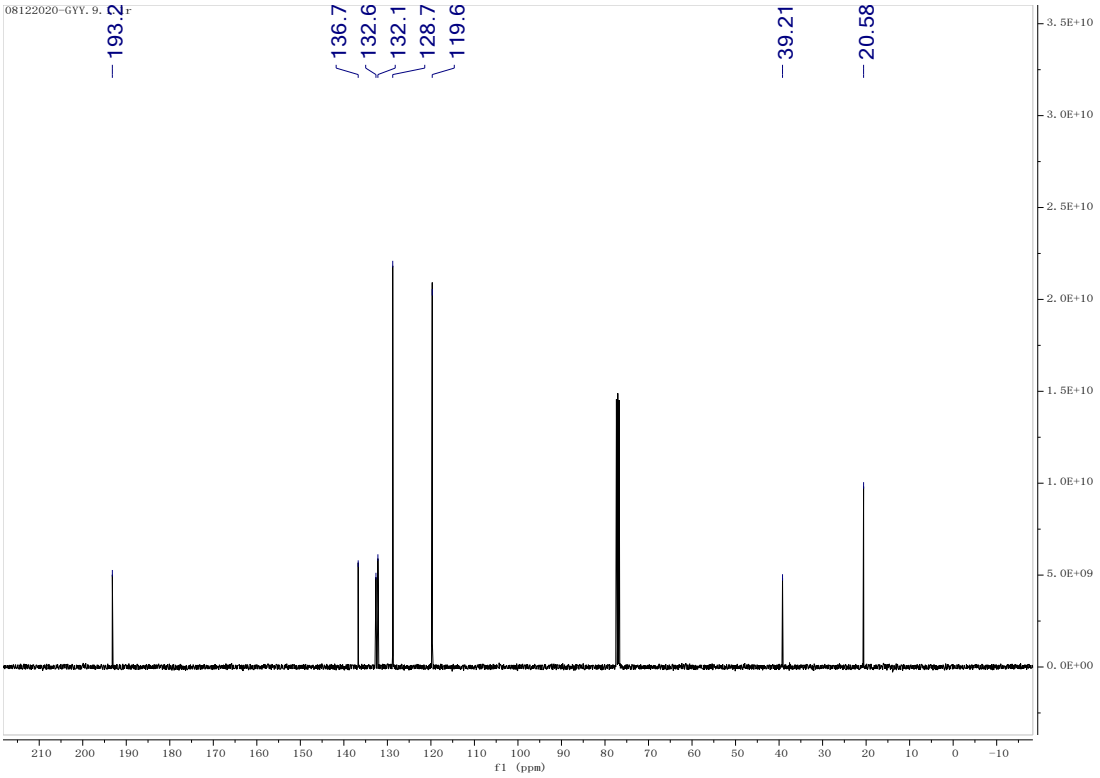

Fig. S25. ^{13}C NMR of **3f**. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 136.71, 132.63, 132.18, 128.77, 119.69, 39.21, 20.58.

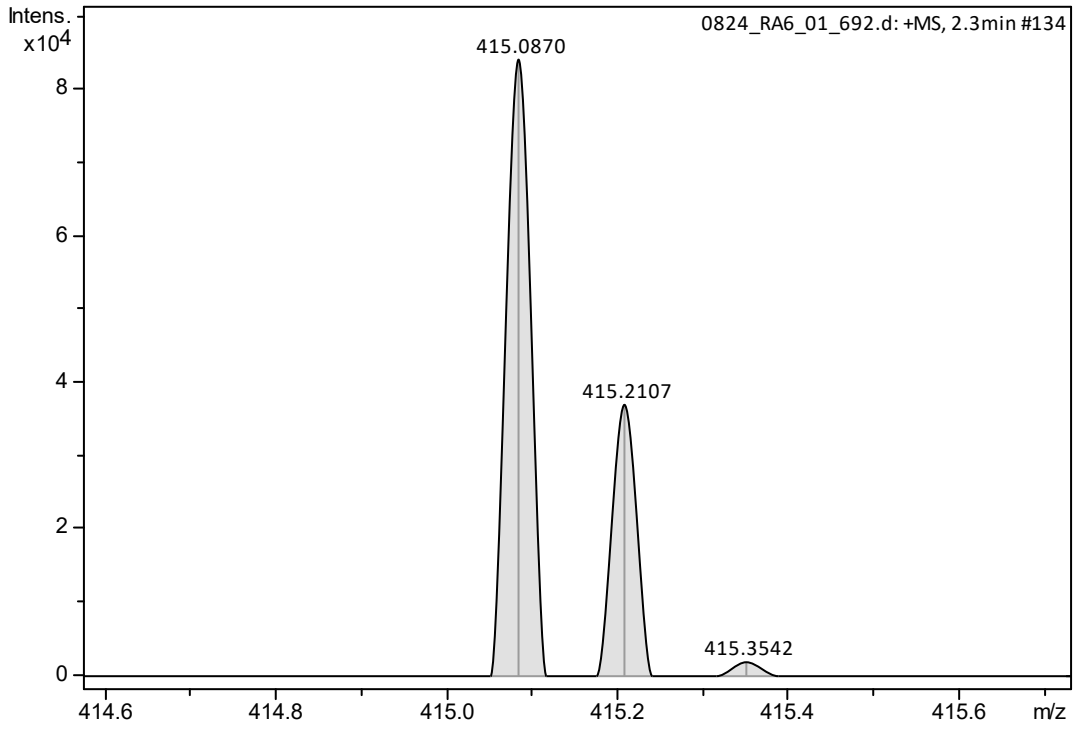

Fig. S26. HRMS of **3g**. $[M+H]^+$ calculated for 415.0876, found as 415.0870.

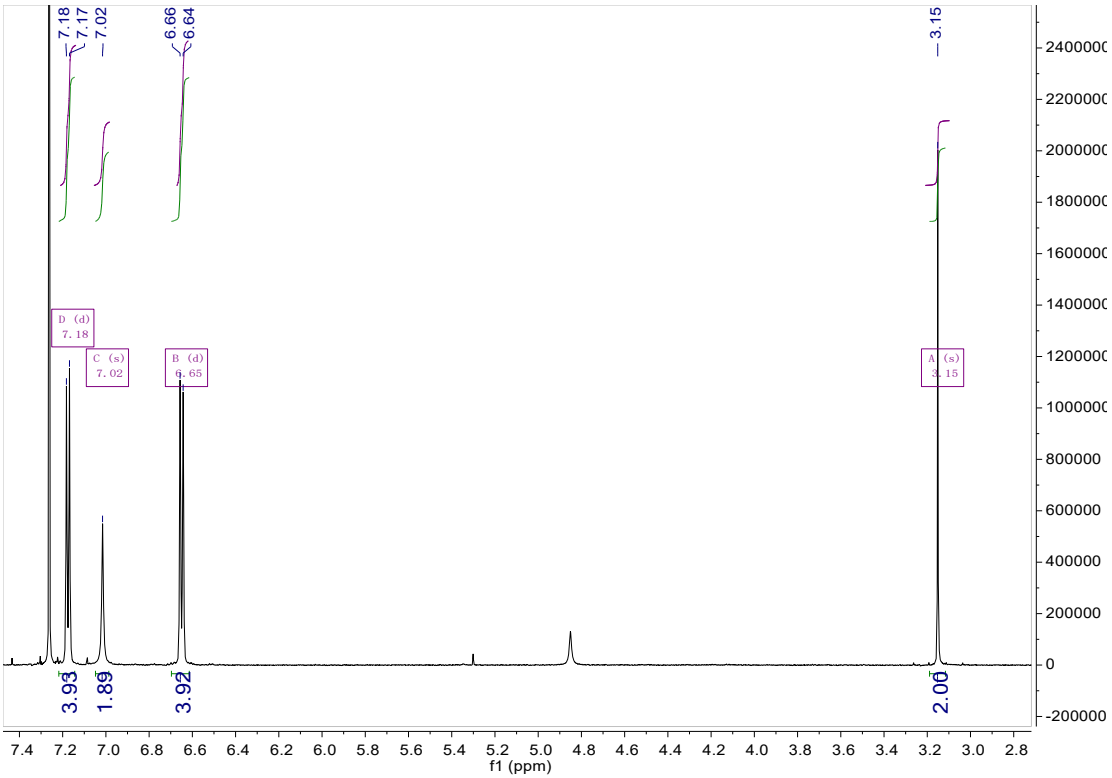

Fig. S27. ^{1}H NMR of **3g**. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.18 (d, *J* = 8.5 Hz, 4H), 7.02 (s, 2H), 6.65 (d, *J* = 8.5 Hz, 4H), 3.15 (s, 2H).

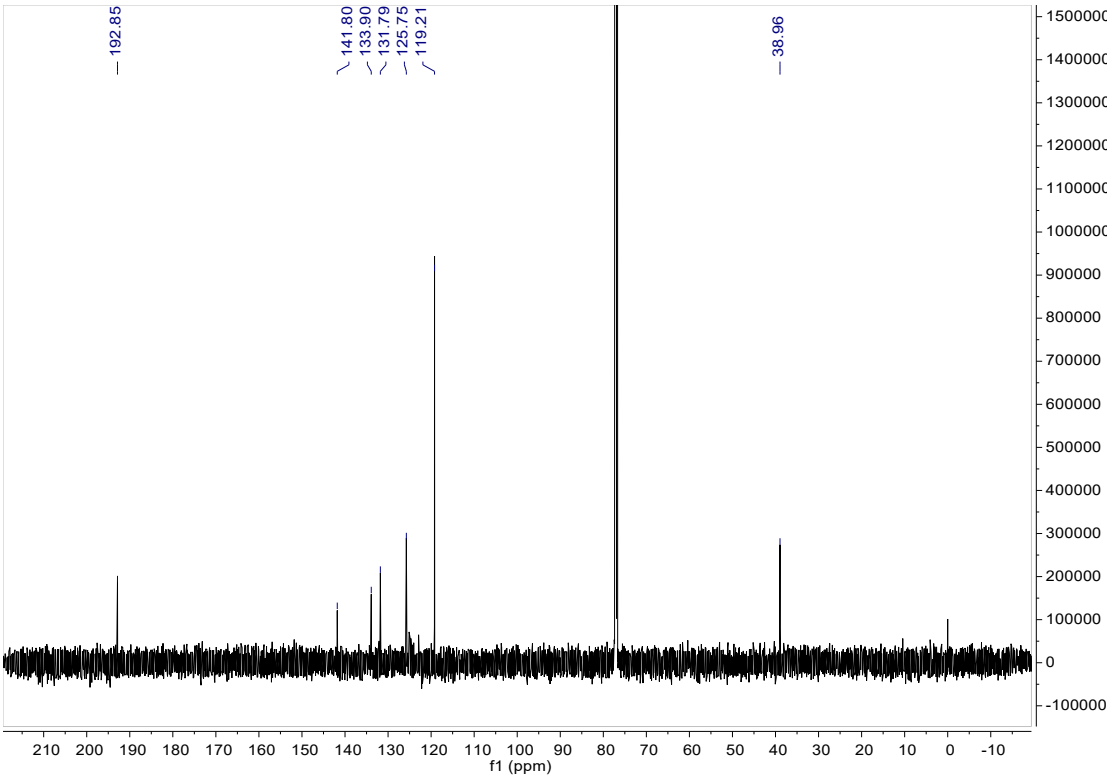

Fig. S28. ^{13}C NMR of **3g**. (151 MHz, Chloroform-*d*) δ 192.85, 141.80, 133.90, 131.79, 125.75, 119.21, 38.96.

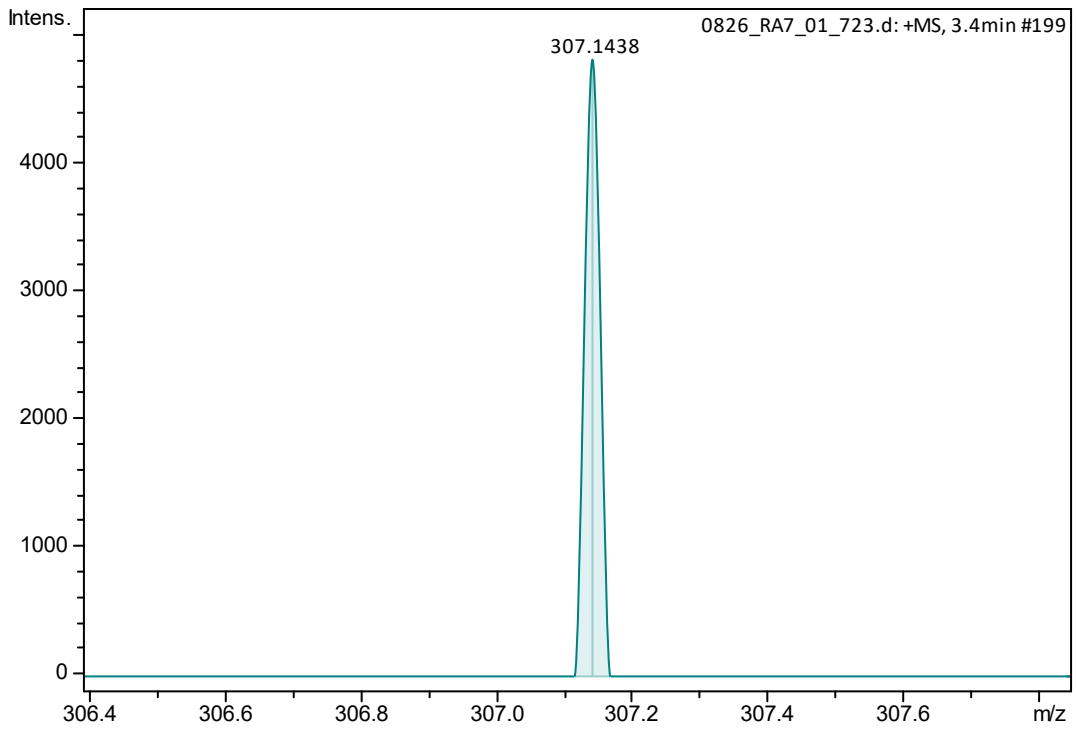

Fig. S29. HRMS of **3h**. $[M+H]^+$ calculated for 307.1441, found as 307.1438.

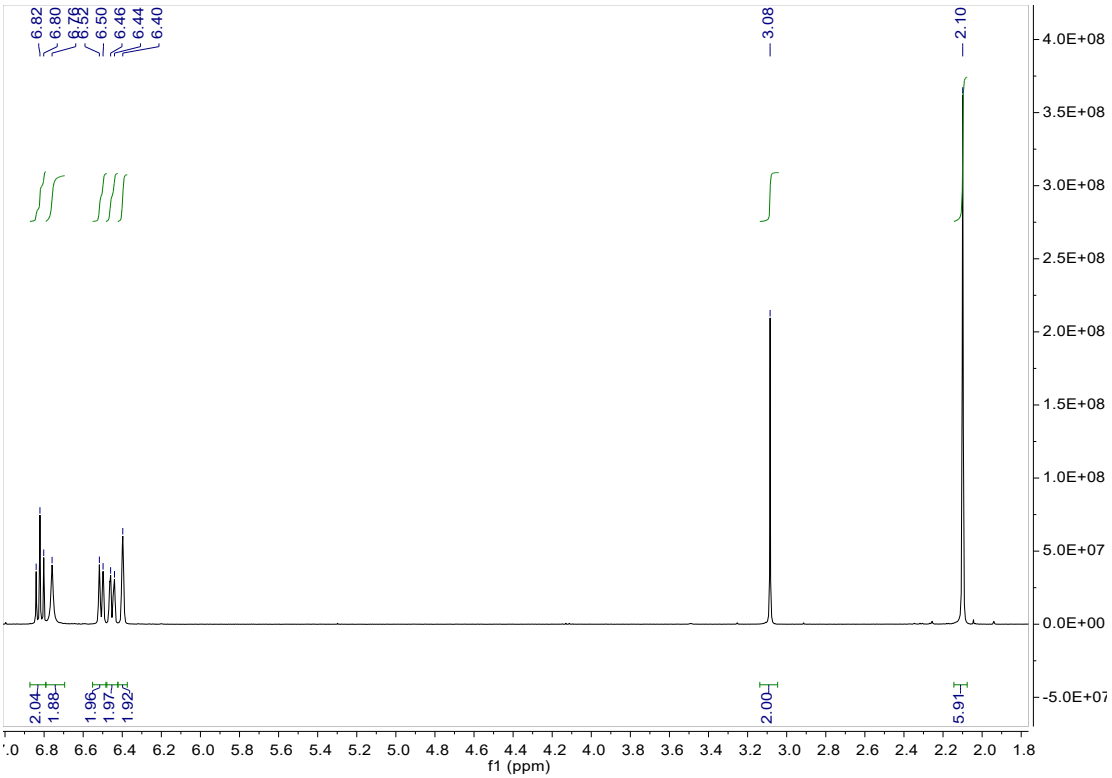

Fig. S30. [1]H NMR of **3h**. (400 MHz, Chloroform-*d*) δ 6.82 (t, *J* = 7.8 Hz, 2H), 6.76 (s, 2H), 6.51 (d, *J* = 7.5 Hz, 2H), 6.45 (d, *J* = 8.0 Hz, 2H), 6.40 (s, 2H), 3.08 (s, 2H), 2.10 (s, 6H).

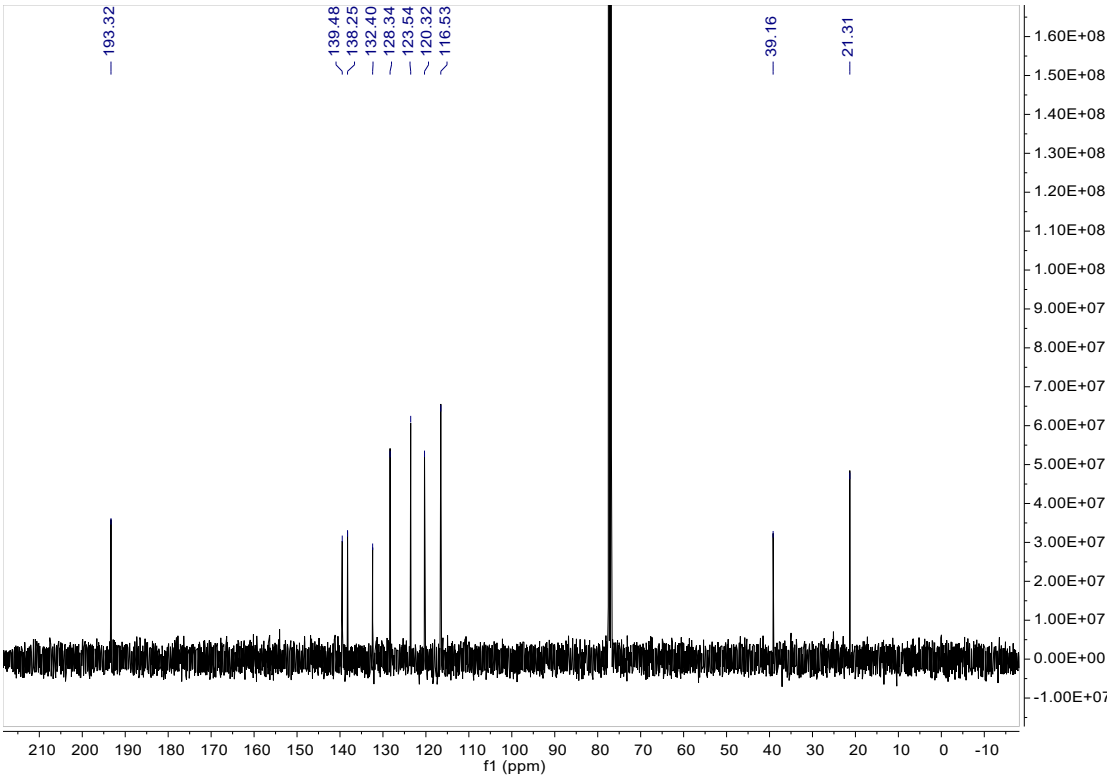

Fig. S31. ^{13}C NMR of **3h**. (101 MHz, Chloroform-*d*) δ 193.32, 139.48, 138.25, 132.40, 128.34, 123.54, 120.32, 116.53, 39.16, 21.31.

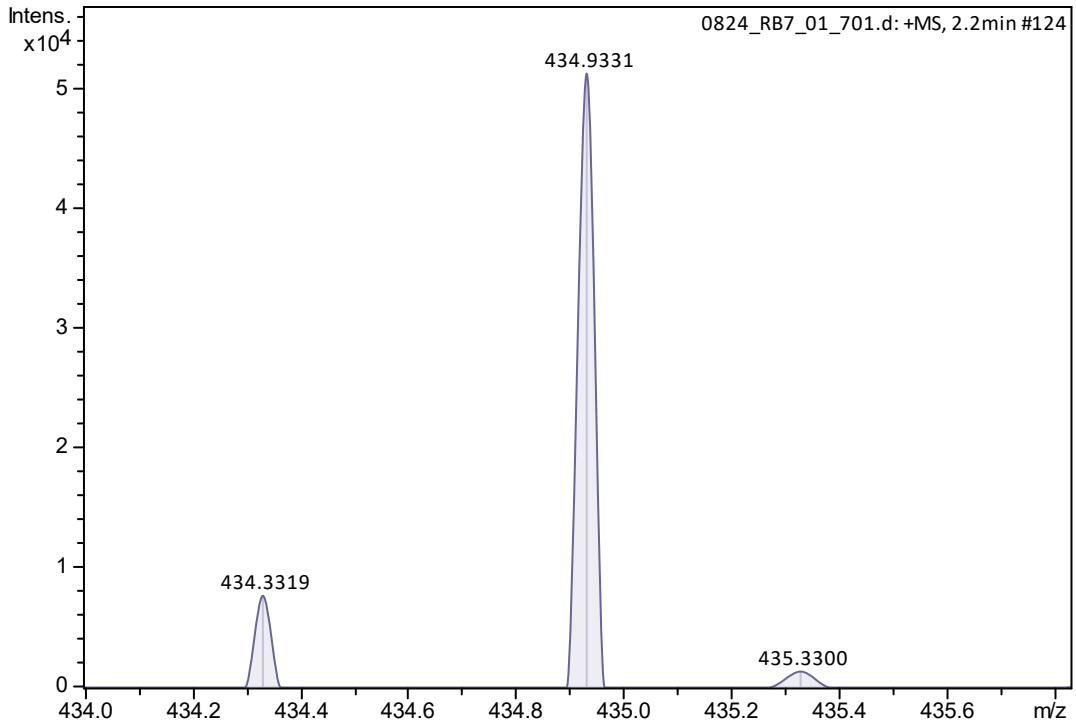

Fig. S32. HRMS of **3i**. $[M+H]^+$ calculated for 434.9338, found as 434.9331.

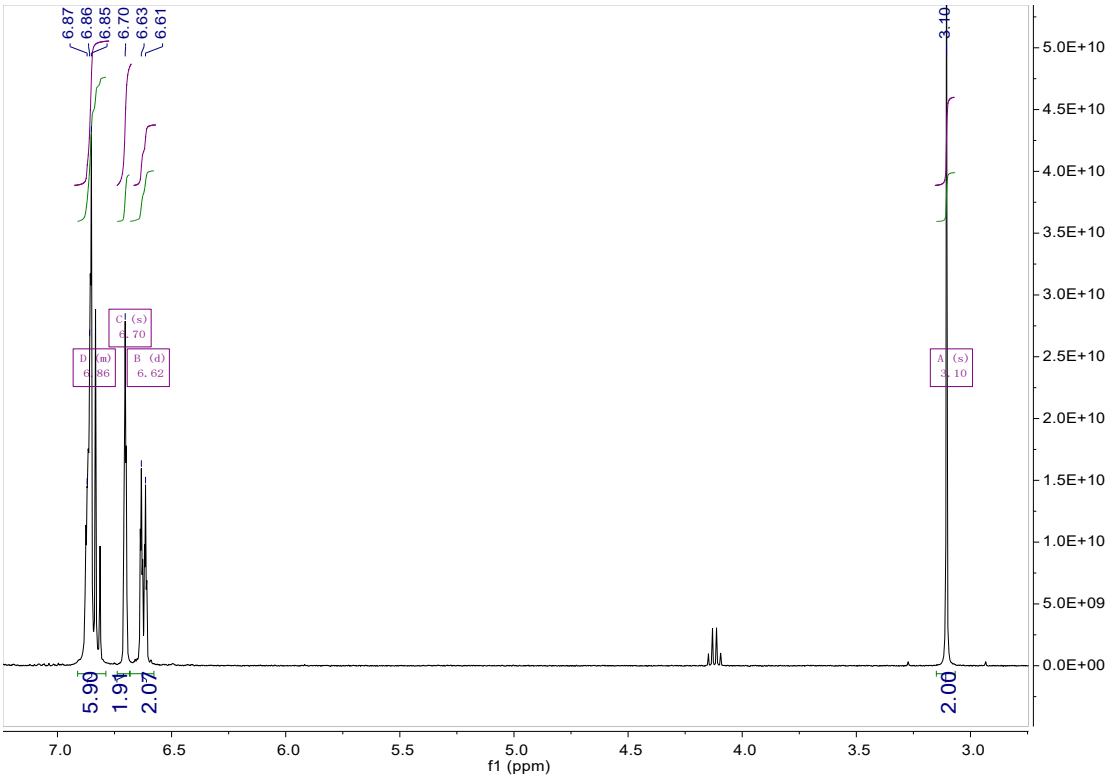

Fig. S33. ^{1}H NMR of **3i**. (400 MHz, Chloroform-*d*) δ 6.92 – 6.78 (m, 6H), 6.70 (s, 2H), 6.62 (d, J = 7.3 Hz, 2H), 3.10 (s, 2H).

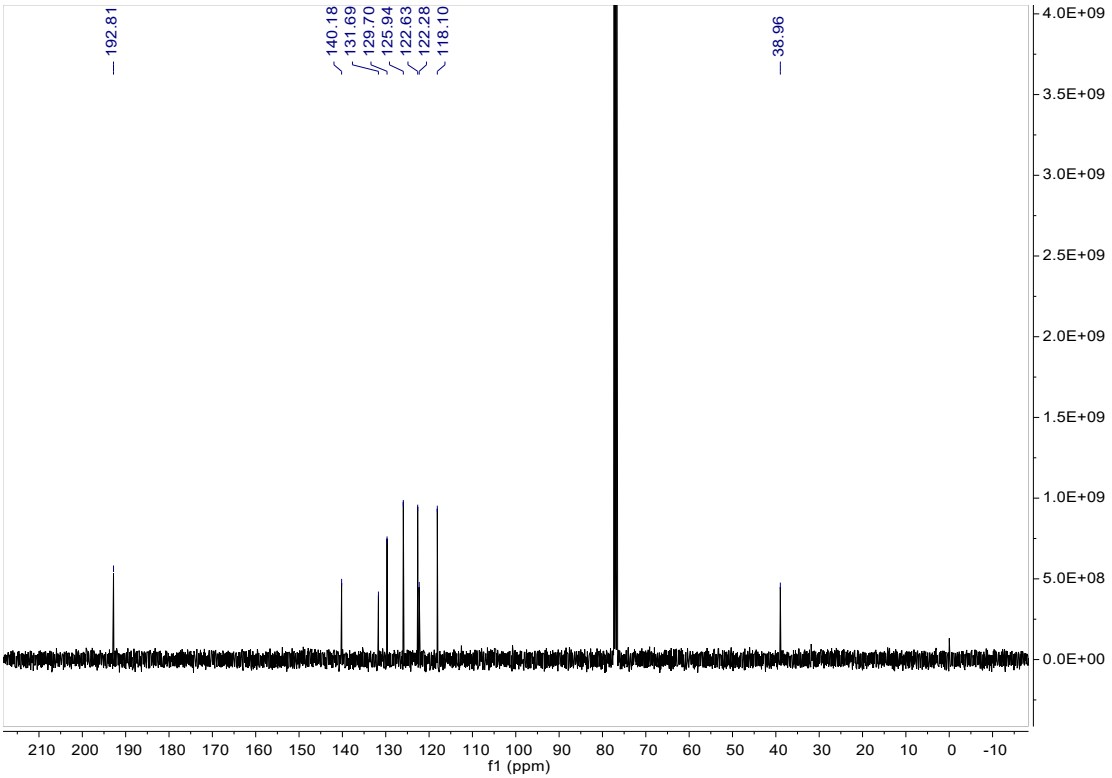

Fig. S34. ^{13}C NMR of **3i**. (101 MHz, Chloroform-*d*) δ 192.81, 140.18, 131.69, 129.70, 125.94, 122.63, 122.28, 118.10, 38.96.

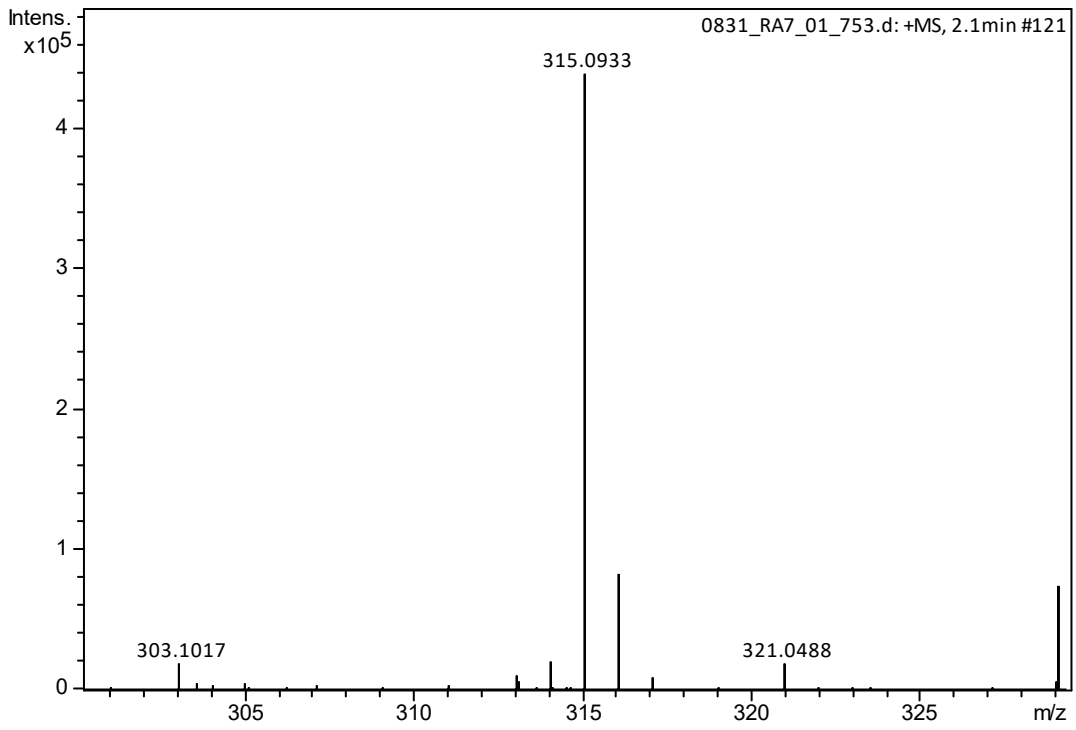

Fig. S35. HRMS of **3j.** $[M+H]^+$ calculated for 315.0939, found as 315.0933.

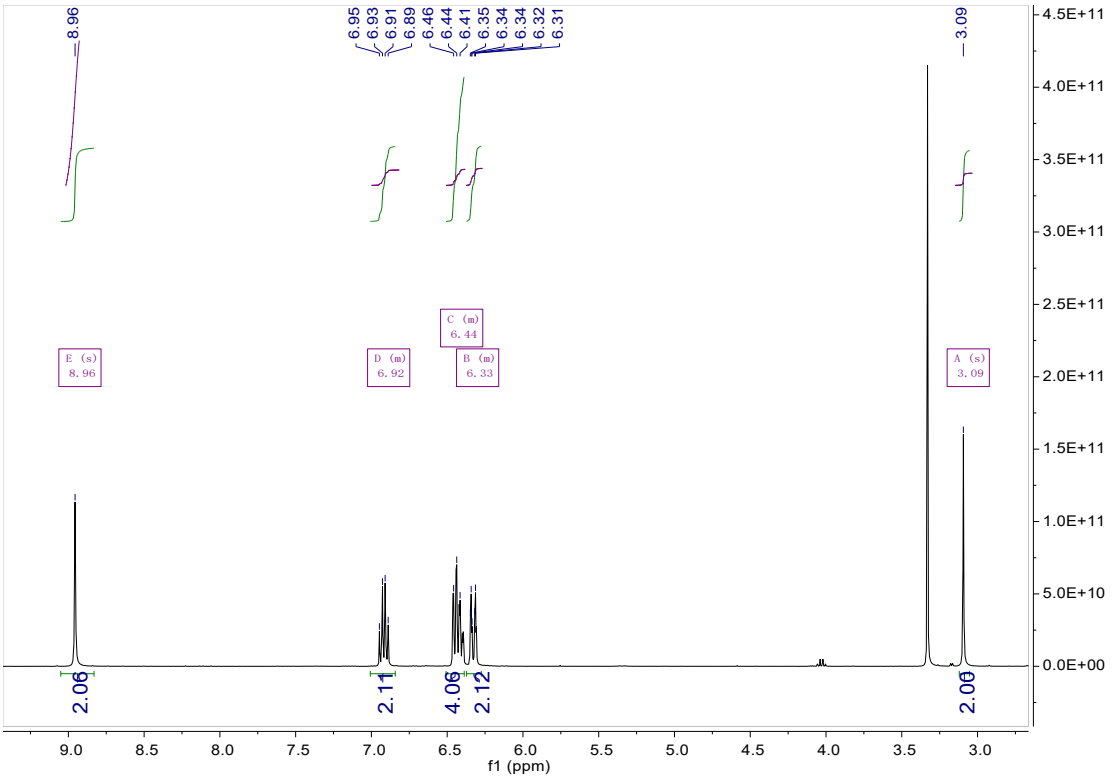

Fig. S36. ^{1}H NMR of **3j**. (400 MHz, DMSO-d_6) δ 8.96 (s, 2H), 7.00 – 6.82 (m, 2H), 6.50 – 6.38 (m, 4H), 6.37 – 6.27 (m, 2H), 3.09 (s, 2H).

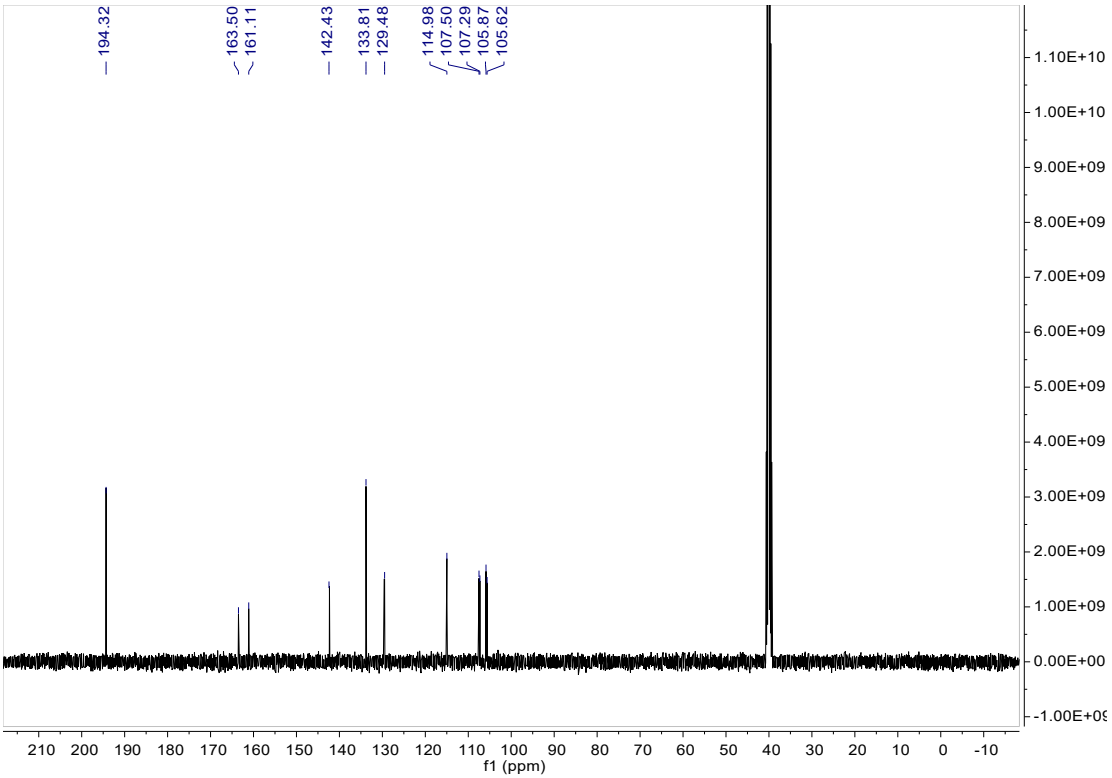

Fig. S37. ^{13}C NMR of **3j**. (101 MHz, DMSO-d_6) δ 194.32, 163.50, 161.11, 142.43, 133.81, 129.48, 114.98, 107.50, 107.29, 105.87, 105.62.

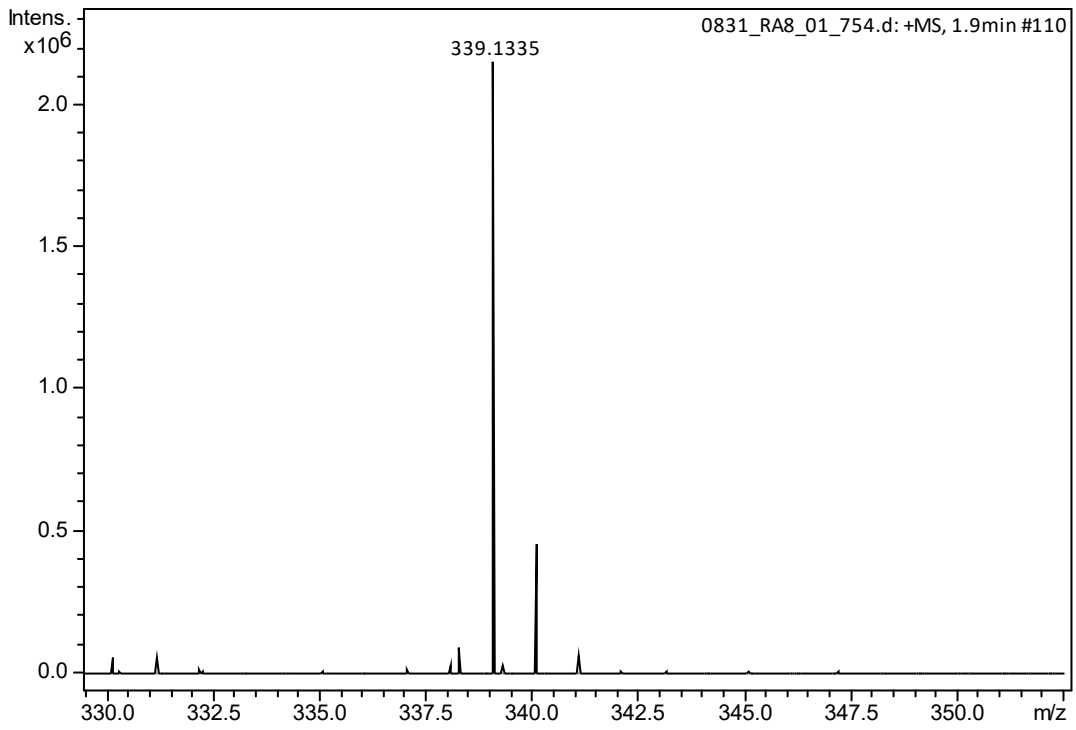

Fig. S38. HRMS of **3k**. $[M+H]^+$ calculated for 339.1339, found as 339.1336. $[M+Na]^+$ calculated for 361.1164, found as 361.1156.

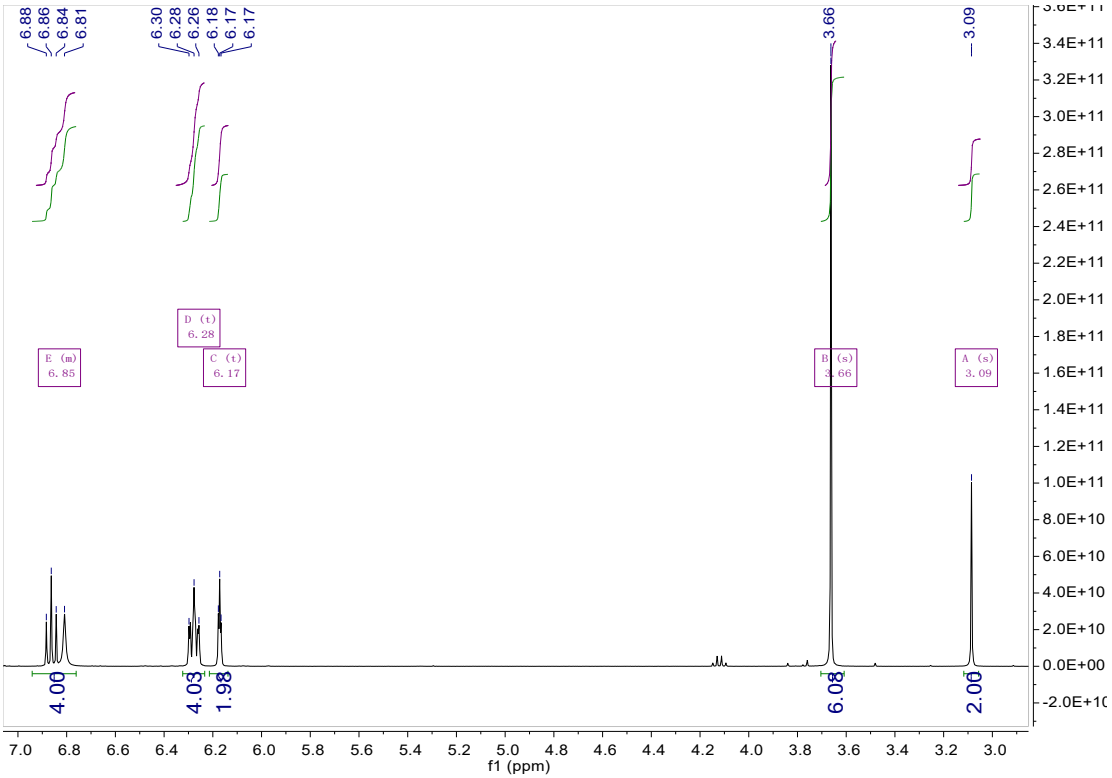

Fig. S39. [1]H NMR of **3k**. (400 MHz, Chloroform-*d*) δ 6.92 – 6.77 (m, 4H), 6.28 (t, *J* = 8.2 Hz, 4H), 6.17 (t, *J* = 2.2 Hz, 4H), 3.66 (s, 6H), 3.09 (s, 2H).

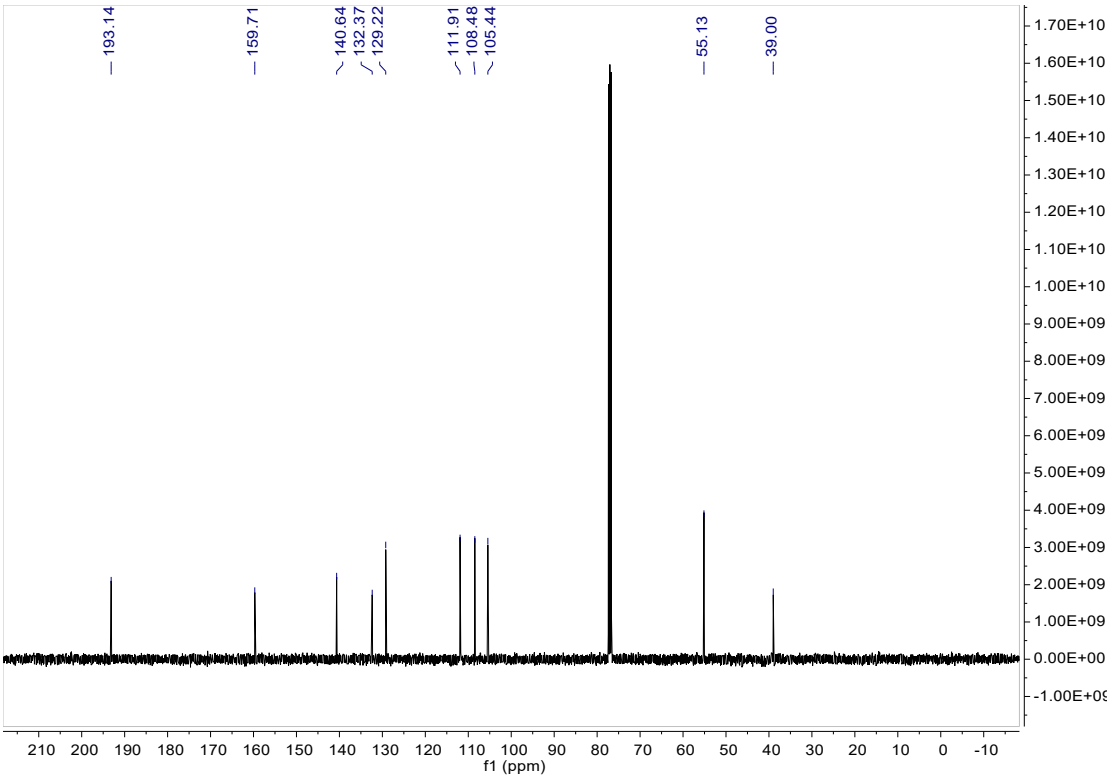

Fig. S40. ^{13}C NMR of **3k**. (101 MHz, Chloroform-*d*) δ 193.14, 159.71, 140.64, 132.37, 129.22, 111.91, 108.48, 105.44, 55.13, 39.00.

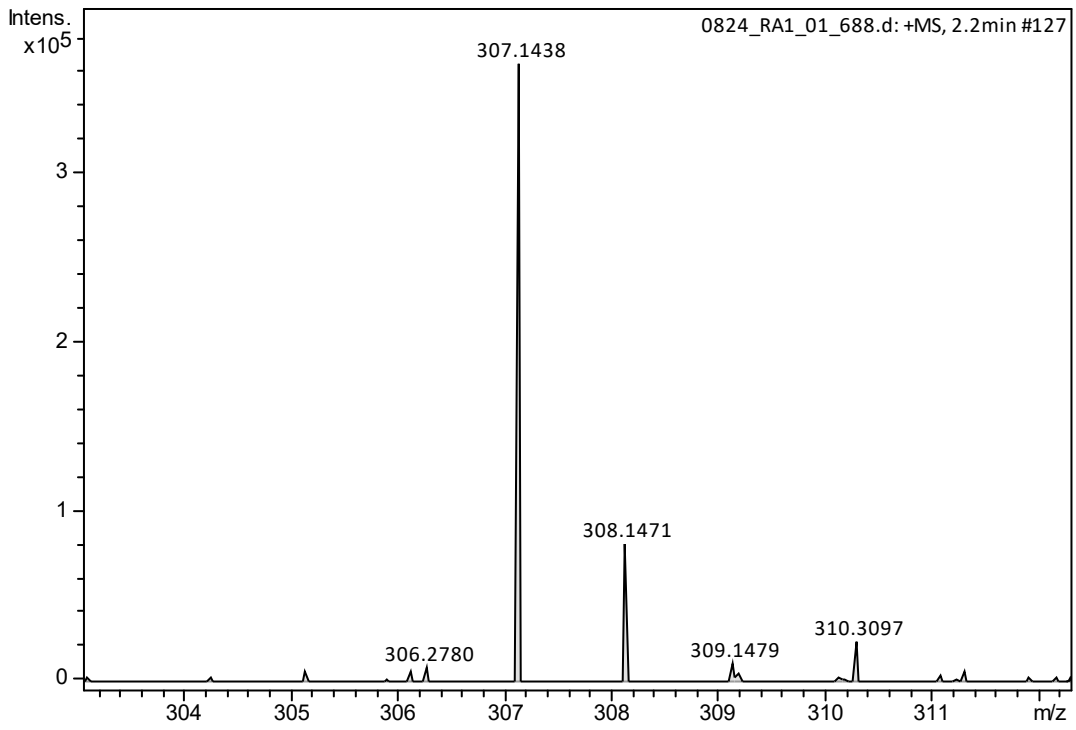

Fig. S41. HRMS of **3l**. $[M+H]^+$ calculated for 307.1441, found as 307.1438.

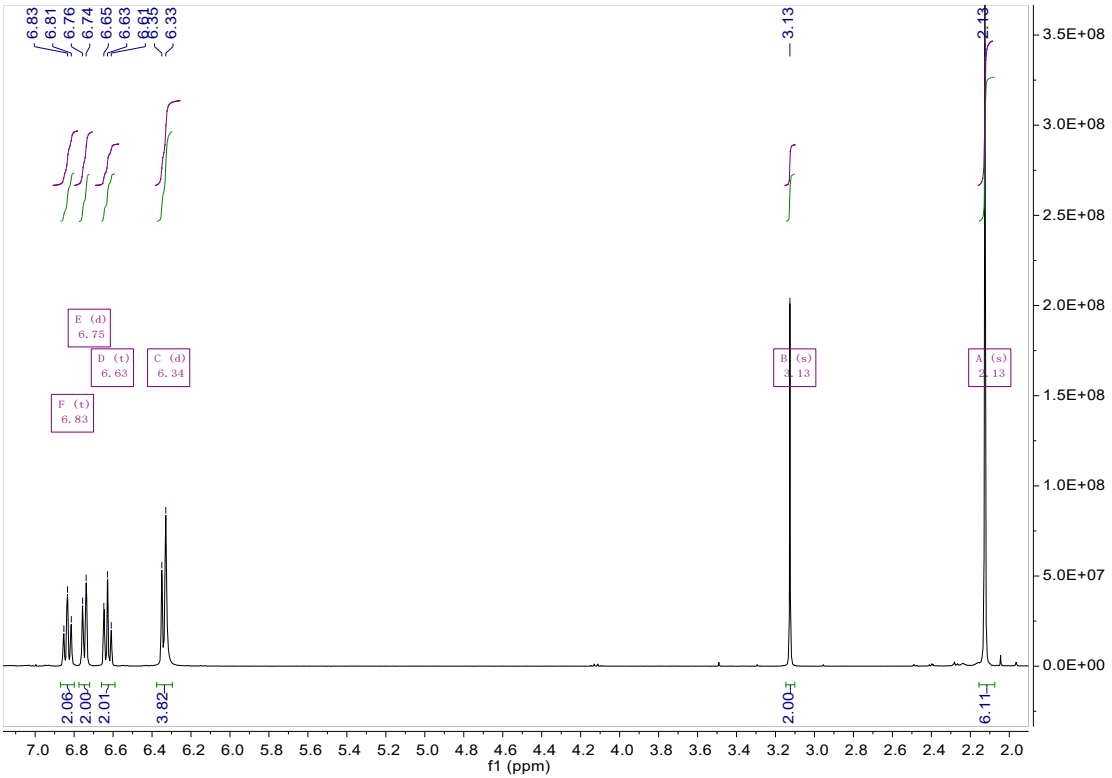

Fig. S42. ^{1}H NMR of **3l**. (400 MHz, Chloroform-*d*) δ 6.83 (t, *J* = 7.6 Hz, 2H), 6.75 (d, *J* = 7.3 Hz, 2H), 6.63 (t, *J* = 7.7 Hz, 2H), 6.34 (d, *J* = 8.2 Hz, 4H), 3.13 (s, 2H), 2.13 (s, 6H).

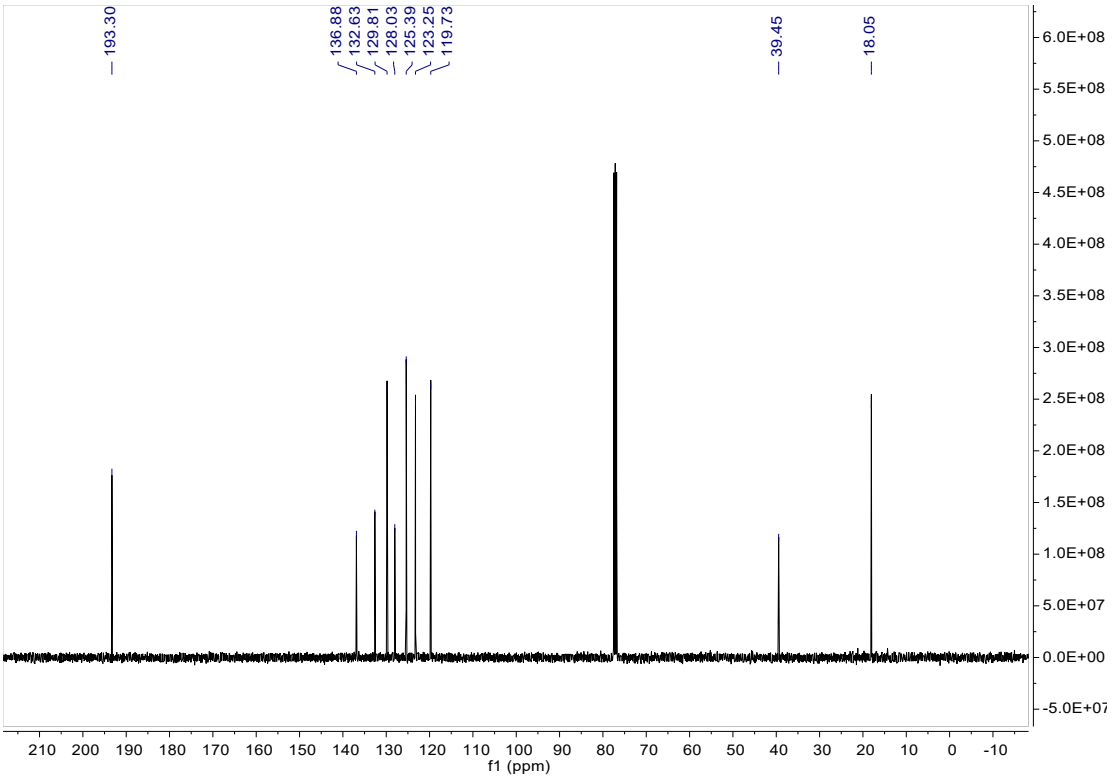

Fig. S43. ^{13}C NMR of **3l**. (101 MHz, Chloroform-*d*) δ 193.30, 136.88, 132.63, 129.81, 128.03, 125.39, 123.25, 119.73, 39.45, 18.05.

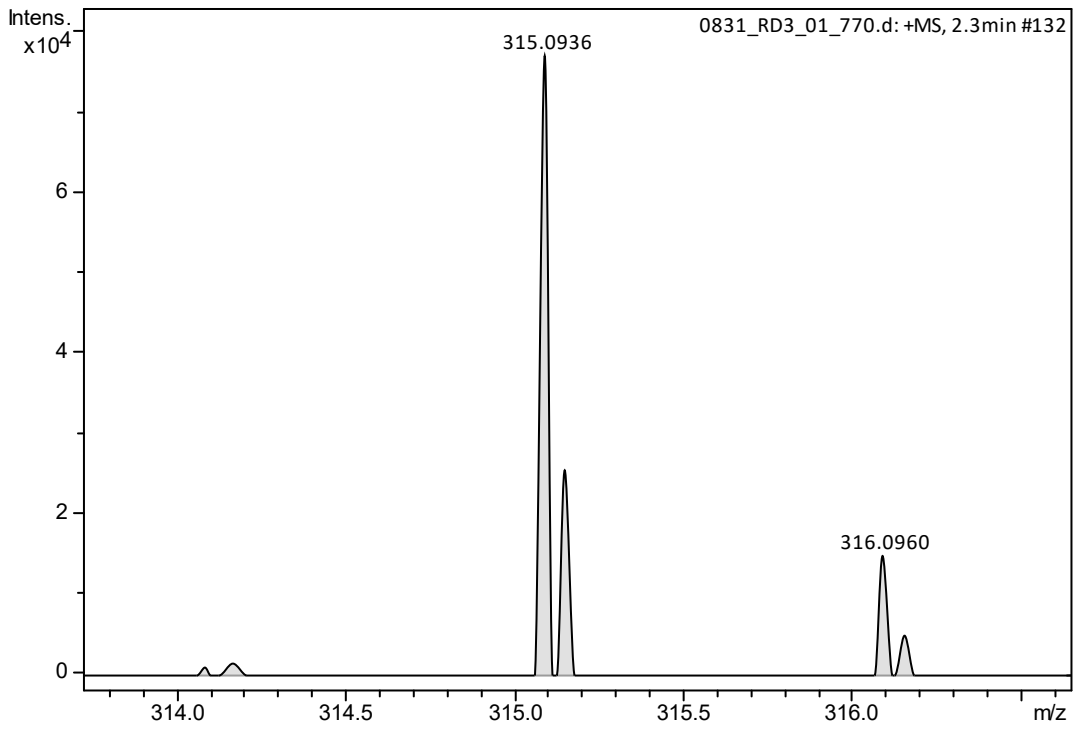

Fig. S44. HRMS of **3m**. $[M+H]^+$ calculated for 315.0939, found as 315.0936.

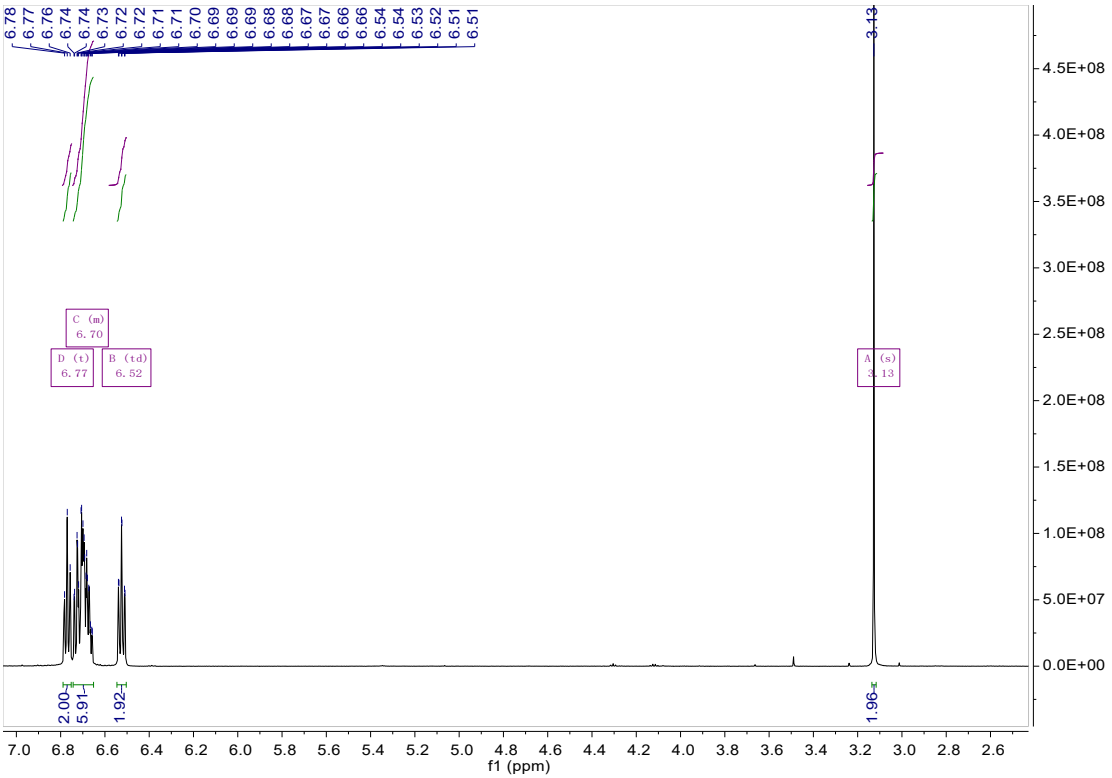

Fig. S45. ^{1}H NMR of **3m**. (600 MHz, Chloroform-*d*) δ 6.77 (t, *J* = 7.6 Hz, 2H), 6.75 – 6.65 (m, 6H), 6.52 (td, *J* = 8.3, 1.4 Hz, 2H), 3.13 (s, 2H).

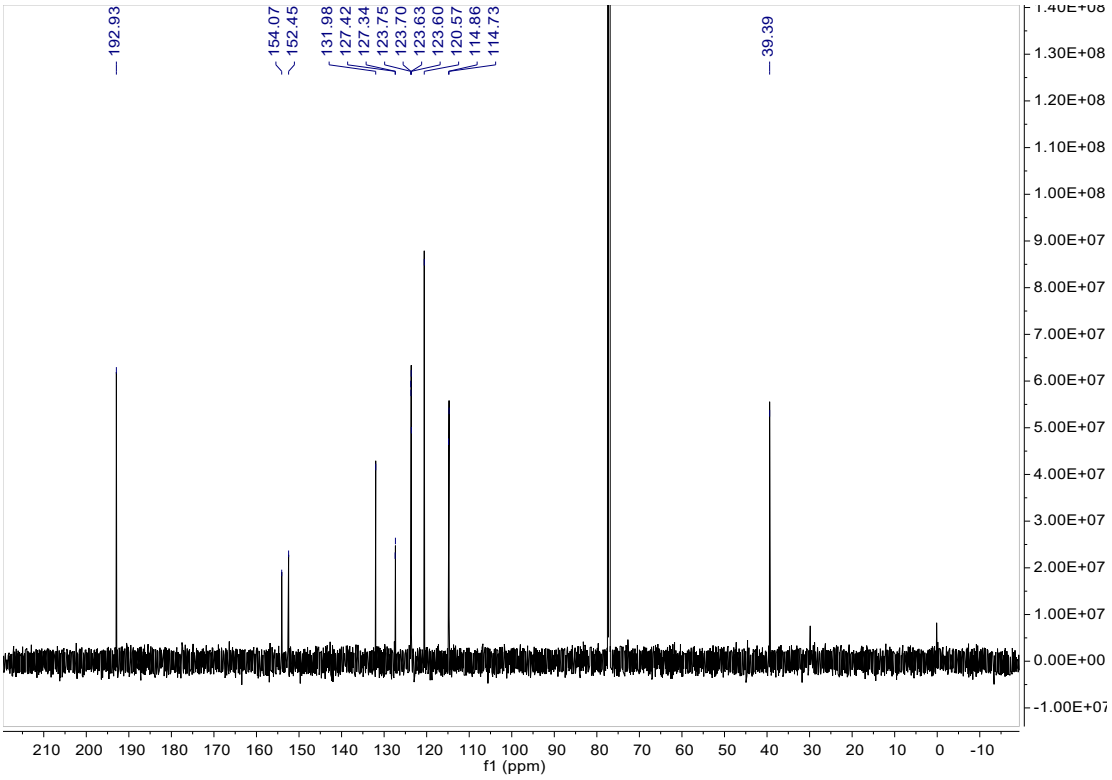

Fig. S46. ^{13}C NMR of **3m**. (151 MHz, Chloroform-*d*) δ 192.93, 154.07, 152.45, 131.98, 127.42, 127.34, 123.75, 123.70, 123.63, 123.60, 120.57, 114.86, 114.73, 77.37, 77.16, 76.95, 39.39.

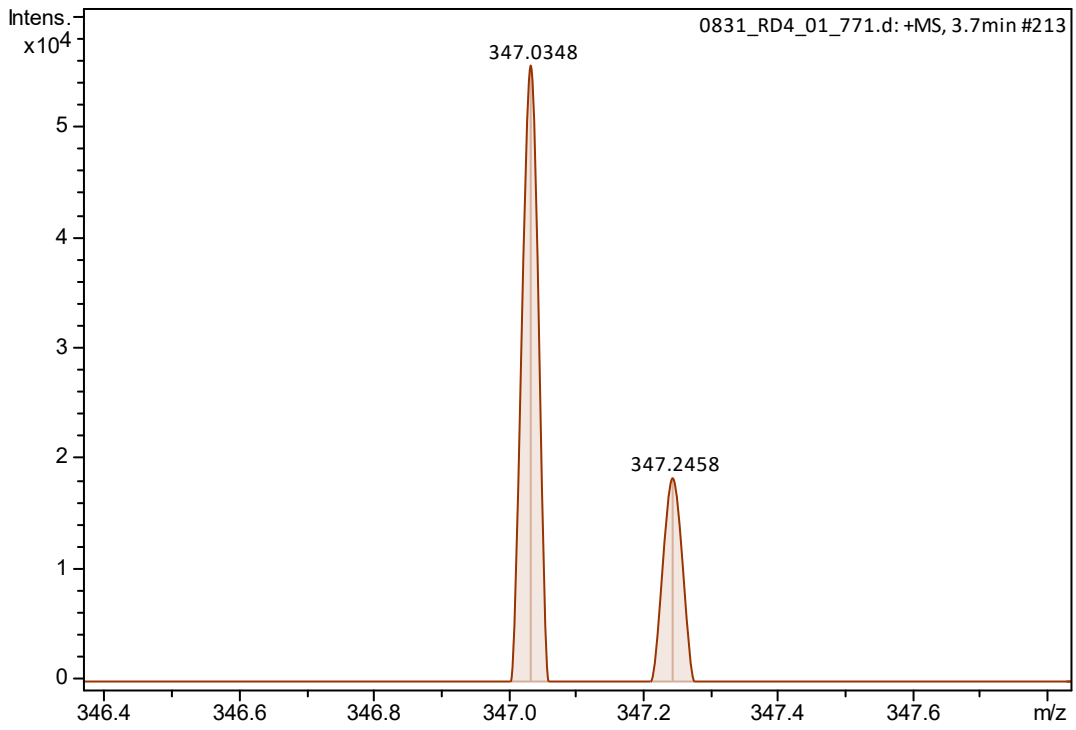

Fig. S47. HRMS of **3n**. $[M+H]^+$ calculated for 347.0349, found as 347.0348.

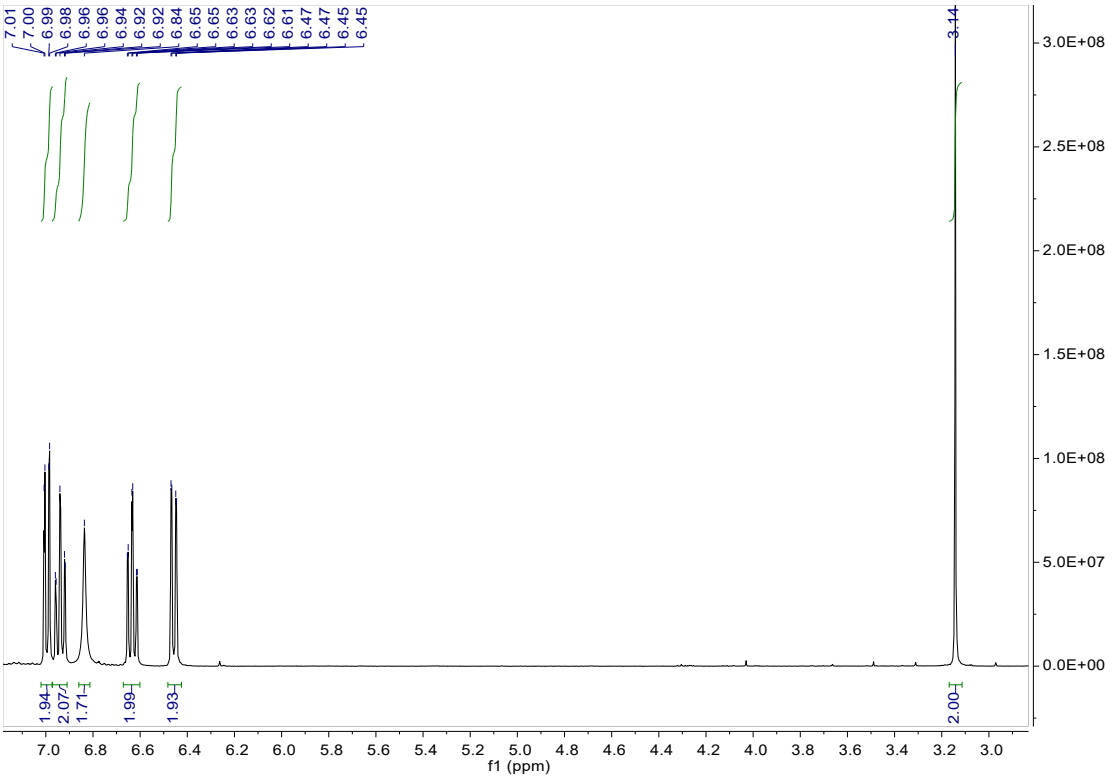

Fig. S48. ^{1}H NMR of **3n**. (400 MHz, Chloroform-*d*) δ 7.00 (dd, *J* = 8.0, 1.4 Hz, 2H), 6.97 – 6.91 (m, 2H), 6.84 (s,2H), 6.63 (td, *J* = 7.7, 1.4 Hz, 2H), 6.46 (dd, *J* = 8.0, 1.3 Hz, 2H), 3.14 (s, 2H).

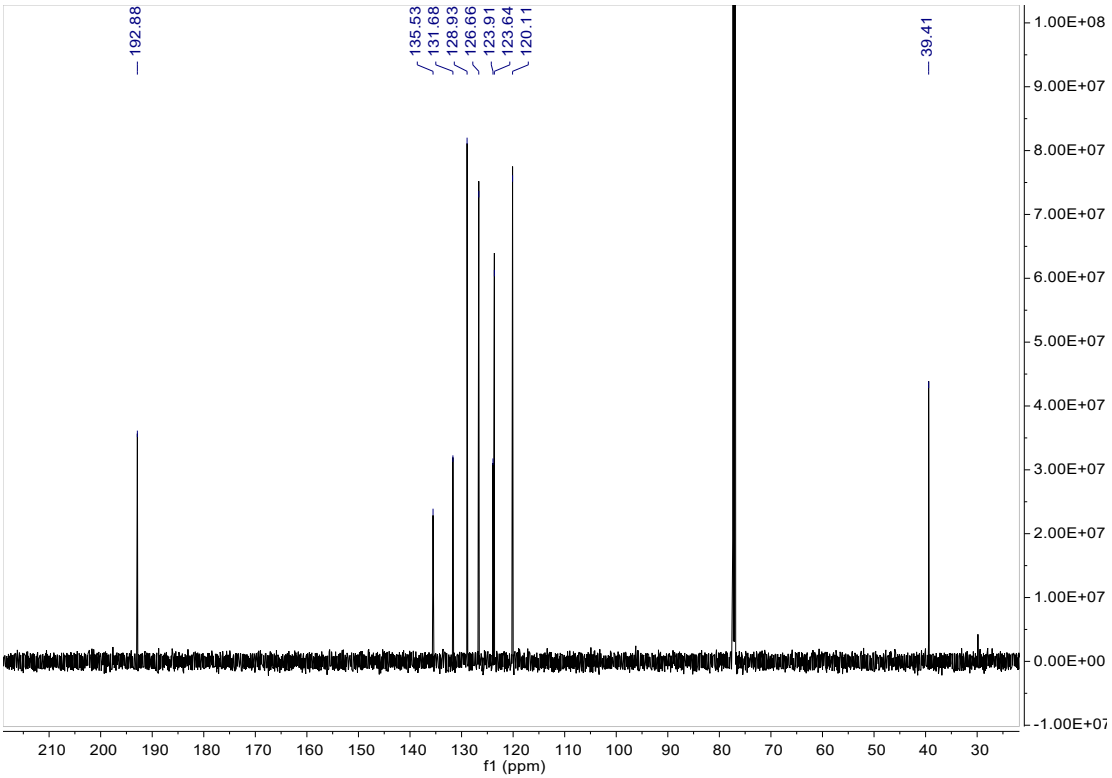

Fig. S49. ^{13}C NMR of **3n**. (151 MHz, Chloroform-*d*) δ 192.88, 135.53, 131.68, 128.93, 126.66, 123.91, 123.64, 120.11, 39.41.

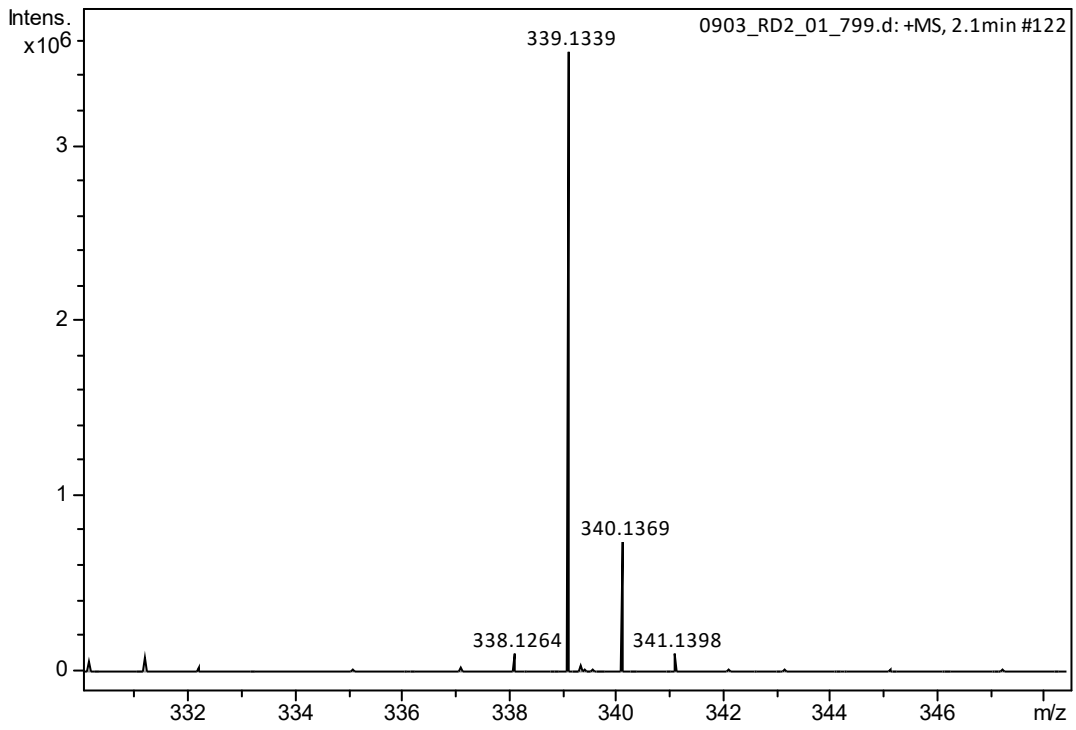

Fig. S50. HRMS of **3o**. $[M+H]^+$ calculated for 339.1339, found as 339.1339.

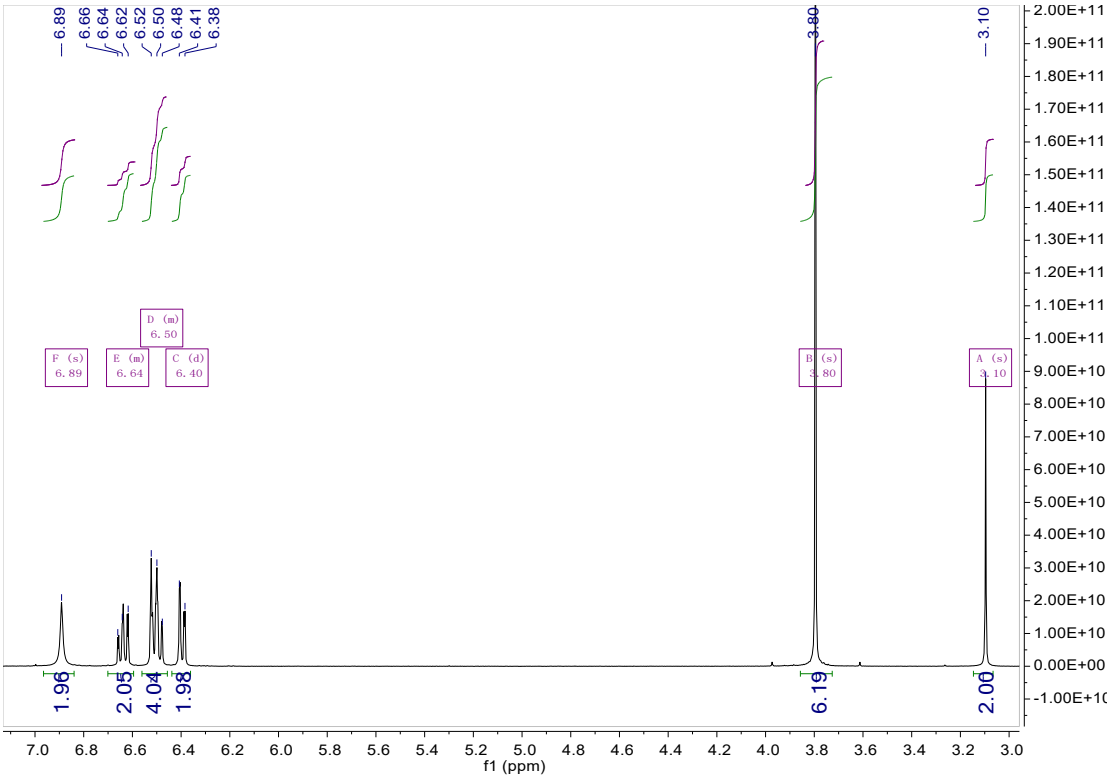

Fig. S51. [1]H NMR of **3o**. (400 MHz, Chloroform-*d*) δ 6.89 (s, 2H), 6.70 – 6.59 (m, 2H), 6.57 – 6.46 (m, 4H), 6.40 (d, *J* = 9.2 Hz, 2H), 3.80 (s, 6H), 3.10 (s, 2H).

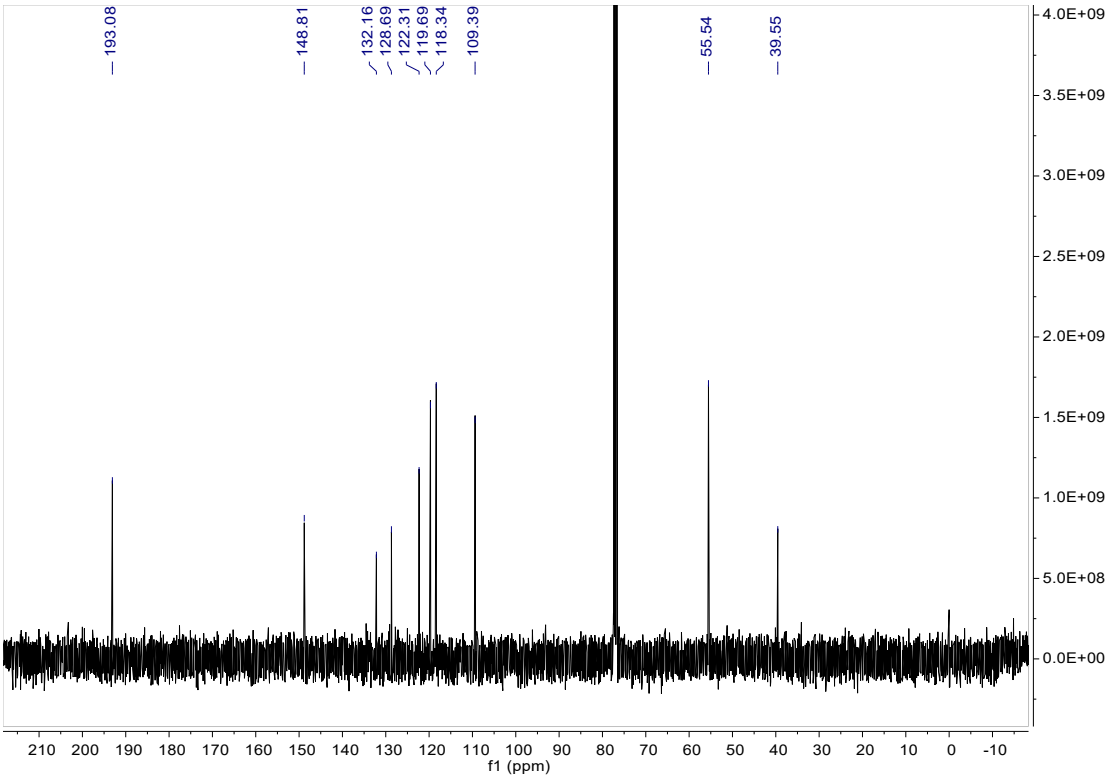

Fig. S52. ^{13}C NMR of **3o**. (101 MHz, Chloroform-*d*) δ 193.08, 148.81, 132.16, 128.69, 122.31, 119.69, 118.34, 109.39, 55.54, 39.55.

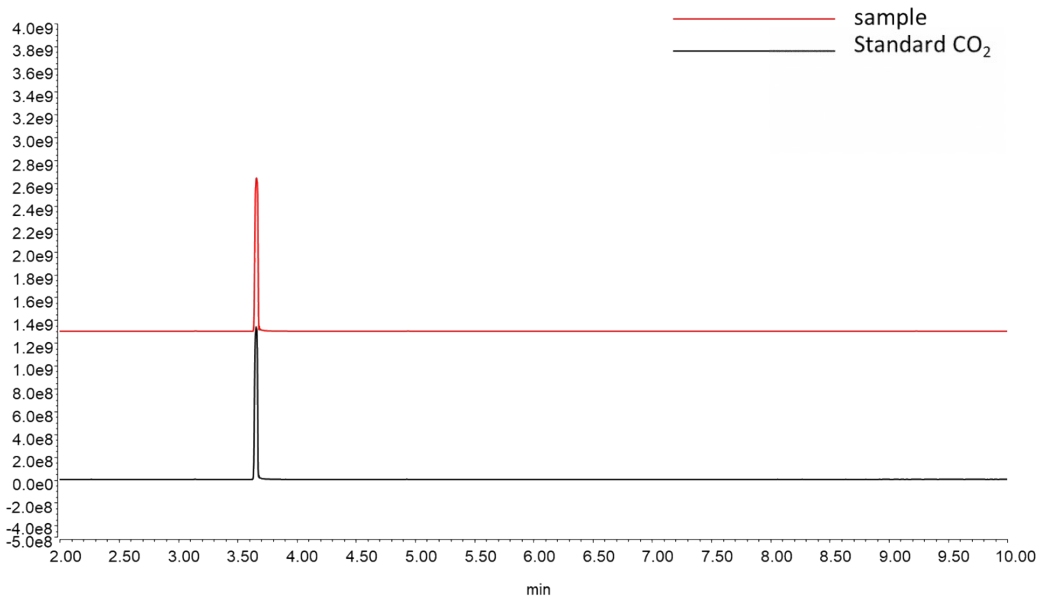

Fig. S53. CO_2 production via GC analysis.

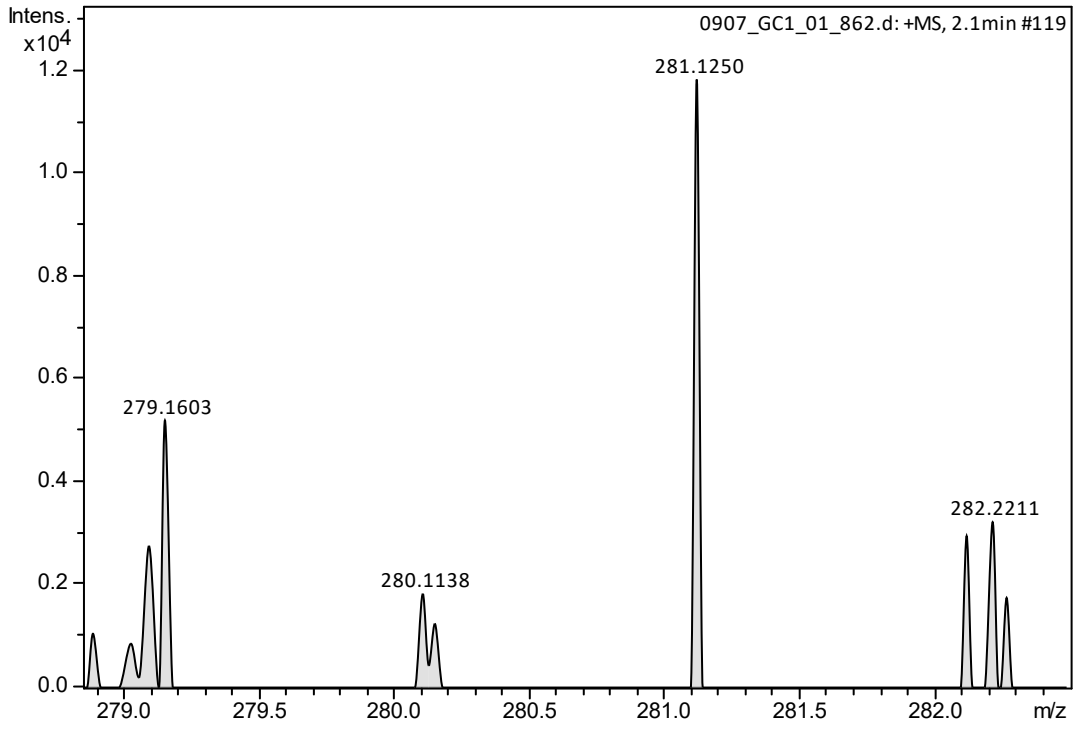

Fig. S54. HRMS of isotope replacement experiment. Molecular formula, $C_{17}H_{12}D_2N_2O_2$, $[M+H]^+$ calculated for 281.1255, found as 281.1250. 1 mM phloroglucinol and 2 mM nitrosobenzene were added into NaOD's D_2O solution (pH 8), then the solution was incubated at room temperature for 1 h. Then the resulting sample was referred to LC-HRMS analysis

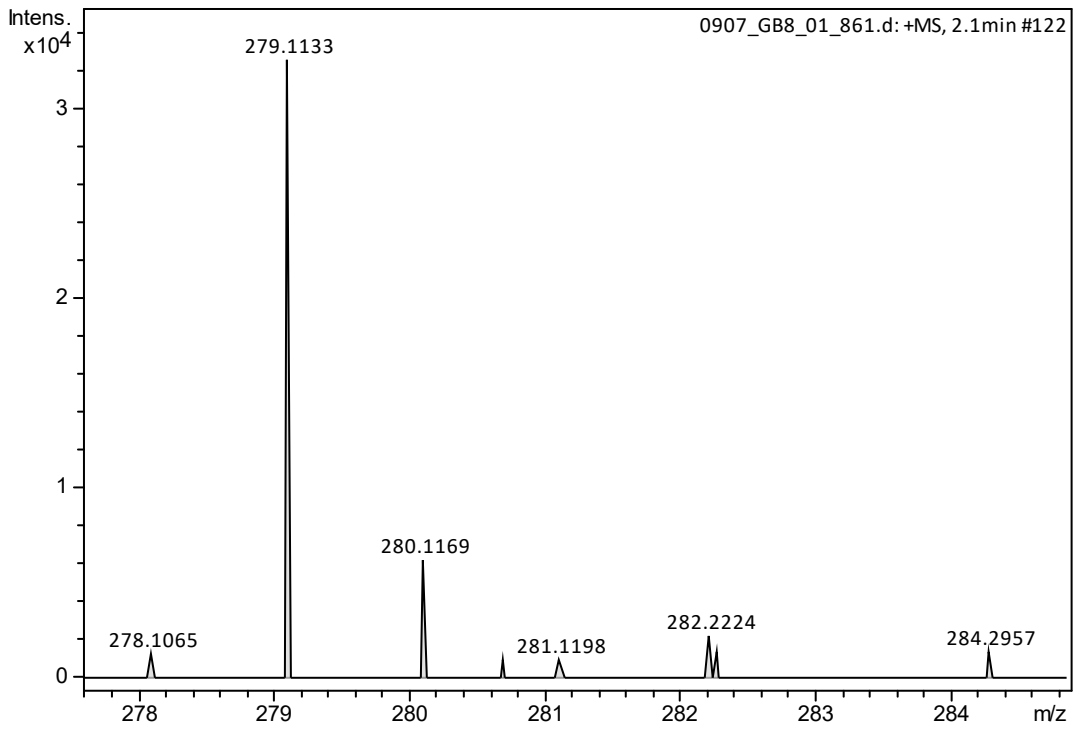

Fig. S55. HRMS of isotope replacement experiment **(control reaction).** Molecular formula, $C_{17}H_{14}N_2O_2$, $[M+H]^+$ calculated for 279.1128, found as 279.1133. 1 mM phloroglucinol and 2 mM nitrosobenzene were added into NaOH's H_2O solution (pH 8), then the solution was incubated at room temperature for 1 h. Then the resulting sample was referred to LC-HRMS analysis.

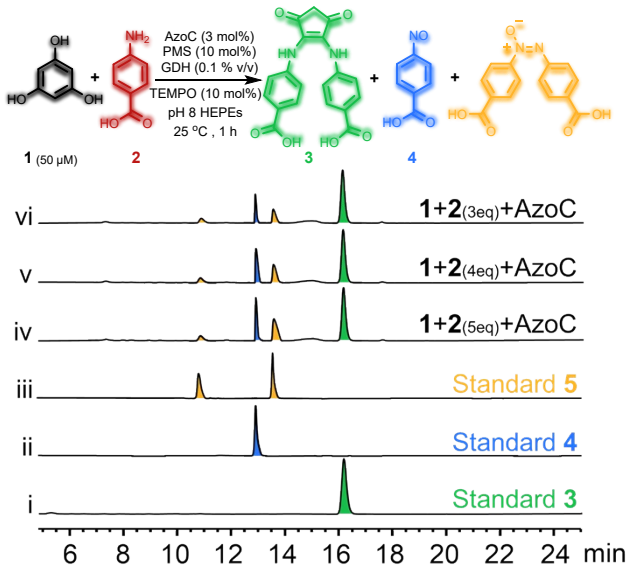

Fig. S56. Influence of **2**/**1** ratio on the production of **3**. (i) standard **3**; (ii) standard **4**; (iii) standard **5**; (iv) 50 μM **1**, 250 μM **2**, with 1.5 μM AzoC; (v) 50 μM **1**, 200 μM **2**, with 1.5 μM AzoC; (vi) 50 μM **1**, 150 μM **2**, with 1.5 μM AzoC.

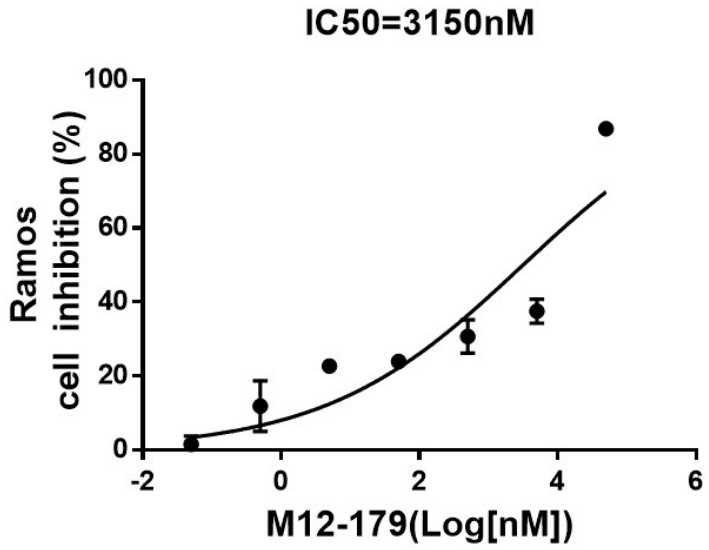

Fig. S57. IC50 of **3a** against lymphoma cell line RAMOS.

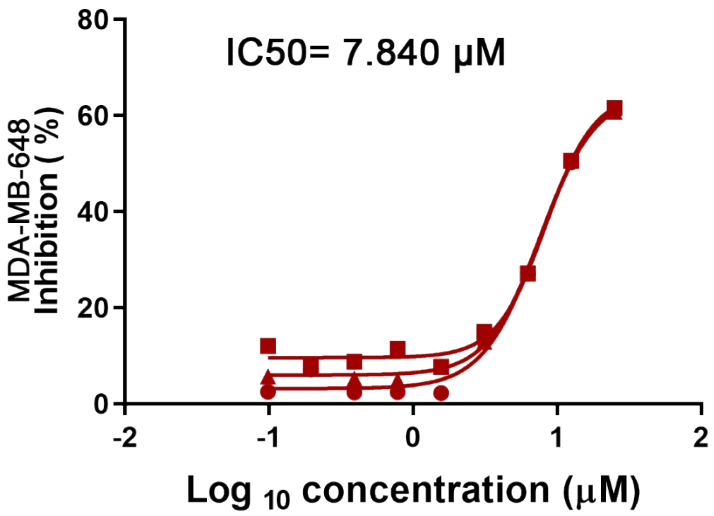

Fig. S58. IC50 of **3g** against breast cancer cell line MDA-MB-648.