Supporting Information

SI Materials and Methods

Construction of Plasmids for Protein Expression in *E. coli*. Individual genes were PCR amplified from genomic DNA and cloned into pET24b, pETDuet, or pCDFDuet-1 by restriction enzyme digestion (Thermo Scientific) and ligation with Quick T4 DNA ligase (New England Biolabs). All primers used are listed in Table S3. Plasmids were isolated using a QIAprep Spin Miniprep Kit (Qiagen) and confirmed by DNA Sequencing (UC Berkeley DNA Sequencing Facility).

Identification of *E. coli-scoA-E* and *E. coli-mmaA-E* metabolites through untargeted, comparative metabolomics. *E. coli* BAP1 strain harnessing plasmids containing either *scoA-E* or *mmaA-E* were grown in triplicate along with a control harnessing empty vectors. 30 mL cultures were grown at 37ºC in LB containing appropriate antibiotics until OD_{600} 0.5 and induced with 0.5 mM IPTG. For *E. coli-mmaA-E*, decenoic acid or dodecenoic acid was fed to a final concentration of 1 mM at the time of induction. After induction, the temperature was decreased to 20ºC, and compound production was allowed to proceed for approximately 48 h. The entire culture (both pellet and supernatant) was extracted with an equal amount of chloroform. The organic extract was dried and redissolved in methanol and analyzed via liquid chromatography-high resolution mass spectroscopy (LC-HRMS). LC-HRMS analysis was normally performed using an Agilent Technologies 6520 Accurate-Mass Q-TOF LC-MS instrument and an Agilent Eclipse Plus C18 column (4.6 x 100 mm). A linear gradient of 2-98% CH_3CN (vol/vol) over 45 min in H_2O with 0.1% formic acid (vol/vol) at a flow rate of 0.5 mL/min was used. XCMS software was used for untargeted metabolomic comparisons of extracts from strains containing *scoA-E* or *mmaA-E* to the empty vector control. In a typical small-scale metabolomics analysis, for compounds **1** and **2**, A linear gradient of 10-50% CH_3CN (vol/vol) over 12 min in H_2O with 0.1% (vol/vol) formic acid at a flow rate of 0.5 mL/min was used. For compounds **6** and **7**, a linear gradient of 50-98% CH_3CN (vol/vol) over 12 min in H_2O with 0.1% (vol/vol) formic acid at a flow rate of 0.5 mL/min, or a linear gradient of 10 to 98% over 30 min followed by 10 min at 98%. HRMS/MS analysis was conducted using targeted MS/MS with collision energy of 5-30V.

Large-scale Production, Purification, and Characterization of 1 and 2. A total of 40 L (40 ×1-L) of the *E. coli-scoA-E* was cultured in LB with appropriate antibiotics. The cultures were inoculated with 10 mL of a seed culture and grown at 37ºC to OD600 ≈ 0.5 – 0.7 before induction with 0.5 mM IPTG. After induction, the temperature was dropped to 20ºC, and compound production was allowed to proceed for approximately two days. The cells were pelleted by centrifugation (6371 × g, 15 min) and compounds **1** and **2** were extracted from the culture medium using two volumes of chloroform. The solvent was removed by rotary evaporation, and the combined residue was re-dissolved in methanol. Purification by HPLC was conducted using an Agilent 1260 HPLC with a C18 Vydac 218TP1022 column 10µm (22 x 250 mm) using a linear gradient of 5-30% CH_3CN (vol/vol) over 50 min in H_2O without formic acid at a flow rate of 5 mL/min and an injection of 1 mL. Fractions were screened using LC-HRMS using an Agilent Eclipse Plus C18 column (4.6 x 100 mm) and a linear gradient of 10-50% CH_3CN (vol/vol) over 12 min in H_2O with 0.1% (vol/vol) formic acid at a flow rate of 0.5 mL/min. Fractions containing **1** were combined and those containing **2** were also combined, separately. Further HPLC purification was conducted using a C18 Vydac 218TP1022 column 10µm (22 x 250 mm) and an isocratic program of 15% CH_3CN (vol/vol) in H_2O at a flow rate of 5 mL/min. Fractions containing **1** and **2** were again determined by LC-HRMS and combined, separately. A final round of HPLC purification was conducted using an Inertsil ODS-4 column (6 mm x 250 mm) using an isocratic program of 23% CH_3CN (vol/vol) in H_2O at a flow rate of 1 mL/min. The resulting 3 mg of purified **1** and 0.5 mg of purified **2** were dried and analyzed by LC-HRMS and NMR. NMR spectra (1D: ^{1}H, ^{13}C and 2D: HSQC, dqf-COSY, HMBC, and ROESY) were recorded on a Bruker Biospin 900 MHz spectrometer with a cryoprobe in DMSO-d6 (Cambridge Isotope Laboratories).

Overexpression and Purification of Proteins. Expression and purification for all proteins with a His6-tag followed the same general procedure and is detailed as follows: cells were grown at 37°C in 700 mL of LB with the appropriate antibiotic to an OD600 of 0.5. The cells were then cooled on ice for 10 min and induced with 0.12 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 16 h at 16°C. Subsequently, the cells were harvested by centrifugation (6371 × g, 15 min, 4°C), resuspended in 30 mL lysis buffer (25 mM HEPES, pH 8, 0.5 M NaCl, 5 mM imidazole), and lysed by homogenization on ice. Cellular debris was removed by centrifugation

(27216 × g, 1 h, 4°C). Ni-NTA agarose resin was added to the supernatant (1.5 mL/L of culture), and the solution was nutated at 4°C for 1 h. The protein-resin mixture was loaded onto a gravity flow column, and proteins were eluted with increasing concentrations of imidazole in Buffer A (20 mM HEPES, pH 8.0). Purified proteins were concentrated and buffer exchanged into Buffer A + 10% glycerol using Amicon Ultra spin filters. Proteins were flash frozen in liquid nitrogen and stored at -80°C. The approximate proteins yields were 8 mg/L for ScoA (158 kDa), 12 mg/L for ScoB (12 kDa), 14 mg/L for ScoC (57 kDa), 40 mg/L for ScoD (19 kDa), 15 mg/L for ScoE (34 kDa), 14 mg/L for MmaA (152kDa), 7 mg/L for MmaB (10 kDa), 30 mg/L for MmaC (58 kDa), 32 mg/L for MmaD (23 kDa), and 18 mg/L for MmaE (34 kDa).

ATP-PP_i Exchange Assays. Assays were performed in 100 μL of reaction buffer (50 mM Tris-HCl/2 mM $MgCl_2$, pH 8) containing 5 mM ATP, 1 mM $Na_4[^{32}P]$-pyrophosphate (PP_i) (~3 x 10^6 cpm/mL), 1 mM TCEP, 5 mM substrate, and 5 μM enzyme. Reactions were incubated at 25°C for 2 h, then quenched by the addition of a charcoal suspension (1.6% w/v activated charcoal, 0.1 M Na_4PP_i, 3.5% $HClO_4$). Free $[^{32}P]PP_i$ was removed by centrifugation of the sample followed by washing twice with wash solution (0.1 M Na_4PP_i and 3.5% $HClO_4$). Charcoal-bound radioactivity was measured on a Beckman LS 6500 scintillation counter.

LC-HRMS Analysis of ScoB and MmaB-bound Biosynthetic Intermediates. Assays were performed in 50 μL of 50 mM HEPES (pH 8.0) containing 5 mM ATP, 2 mM $MgCl_2$, 5 mM acid substrate (C4-C12), 0−50 μM of each protein. The reaction mixture was mixed gently and incubated for two hours at room temp. After incubation, 450 uL of water was added and then the mixture was filtered (0.2 μm). LC-HRMS analysis was normally performed using an Agilent Technologies 6520 Accurate-Mass Q-TOF LC-MS instrument and a Phenomenex Aeris widepore XBC18 column (250 x 21 mm) with a linear gradient of 15 to 98% CH_3CN (v/v) over 21 min in H_2O with 0.1% (v/v) formic acid, at a flow rate of 0.15 mL min^{-1}. The data were analyzed using Agilent MassHunter Qualitative Analysis software using the maximum entropy deconvolution feature.

Product Formation Assays Using ScoABC and MmaABC. Assays were performed in 50 μL of 50 mM HEPES (pH 8.0) containing 5 mM ATP, 2 mM $MgCl_2$, 5 mM acid substrate (C4-

C12), 0−50 μM of each protein, 1 mM TCEP, 2 mM NADPH, and 5 mM Lys. The reaction mixture was mixed gently and incubated for two hours at room temp. After incubation, the reaction was quenched with 50 uL MeOH and protein residues were removed by centrifugation. LC-HRMS analysis was normally performed using an Agilent Eclipse Plus C18 column (4.6 Å~ 100 mm) with a linear gradient of 5 to 98% CH_3CN (v/v) over 30 min in H_2O with 0.1% (v/v) formic acid (for ScoABC), or 15 to 98% over 20 min followed by 10 min at 98% CH_3CN (v/v) in H_2O with 0.1% (v/v) formic acid (for MmaABC), at a flow rate of 0.5 mL min^{-1}.

Biochemical Assays of ScoBCD and MmaBCD. Assays were performed in 50 μL of 50 mM HEPES (pH 8.0) containing 5 mM ATP, 2 mM $MgCl_2$, 5 mM acid substrate (C4-C12), 0−50 μM of each protein, and 200 mM Gly. For labeling experiments, [2-^{13}C]Gly, [^{15}N]Gly, or [2-^{13}C, ^{15}N]Gly was used. The reaction mixture was mixed gently and incubated at room temp. At various time points, the reaction was quenched with 50 uL MeOH and incubated on ice for 10 minutes. The protein residues were pelleted by centrifugation for 10 min at 4ºC and the supernatant was used for LC-HRMS analysis. The protein pellet was then resuspended in 100 uL of MeOH and centrifuged again and the supernatant was discarded (this process was repeated three times). The pellet was then re-dissolved in 50 uL of 100 mM KOH and heated at 70ºC for 10 min. The solution was then neutralized with 50 uL of 100 mM HCl. The solution was spin filtered (Amicon Ultra Centrifugal Filters 3 kDa, 0.5 mL) to remove any particles and the flow-through was used for LC-HRMS analysis, which was normally performed using an Agilent Eclipse Plus C18 column (4.6 Å~ 100 mm). For ScoBCD experiments, a linear gradient of 10 to 50% CH_3CN (v/v) over 12 min in H_2O with 0.1% (v/v) formic acid, at a flow rate of 0.5 mL min^{-1} was used. For MmaBCD experiments, a linear gradient of 20 to 98% CH_3CN (v/v) over 18 min in H_2O with 0.1% (v/v) formic acid, at a flow rate of 0.5 mL min^{-1} was used.

Gene Disruption in *M. marinum* and Mutant Analysis. Deletion of the *Mmar_0256-0260 (mmaA-E)* locus in *Mycobacterium marinum* strain M was performed by cloning 800-bp of the start of *Mmar0260* and end of *Mmar_0256* on either side of the hygromycin resistance gene on pMSG360 and using homologous recombination via transduction with the temperature-sensitive mycobacteriophage phAE87, as described previously. The phage was incubated with *M. marinum* cells at an MOI of 10 for 4 h at 39ºC before plating on hygromycin 7H10 plates and

growing at 30ºC. The *Mmar_0256-0260* deletion was verified by Sanger sequencing.

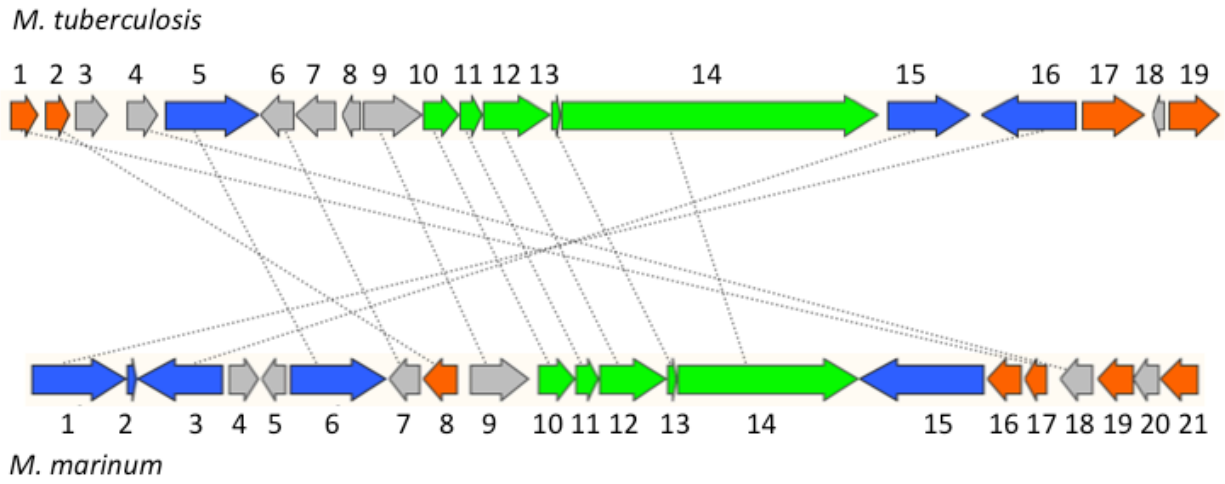
ICP-OES Analyses. Wild-type and *ΔmmaA-E* were grown in 50 mL Sauton's medium at 30ºC and 105 rpm in triplicate. 15 mL of culture was harvested at late exponential phase and washed 3x with cold 1 mM EDTA. Cell pellets were dried at 100ºC overnight and resuspended in 70% trace metal grade nitric acid. The cell suspensions were boiled overnight in acid-washed Pyrex Erlenmeyer flasks. Dried samples were redissolved in 5 mL 2% nitric acid prior to analysis. Standard solutions were dissolved in the same matrix. The concentration of the trace elements was determined using a Perkin Elmer 5300 DV ICP-OES at College of Natural Resources, University of California at Berkeley. The analytical precision of the measurements is better than ±2% RSD.

MS and MS/MS data have been deposited to the EMBL-EBI MetaboLights (1) database (DOI: 10.1093/nar/gks1004. PubMed PMID: 23109552) with the identifier MTBLS454. The complete dataset can be accessed here http://www.ebi.ac.uk/metabolights/MTBLS454

Reference:

1. Haug K, *et al.* (2013) MetaboLights - an open-access general-purpose repository for metabolomics studies and associated meta-data. *Nucleic Acids Res* 41:D781-D786.

SI Figures

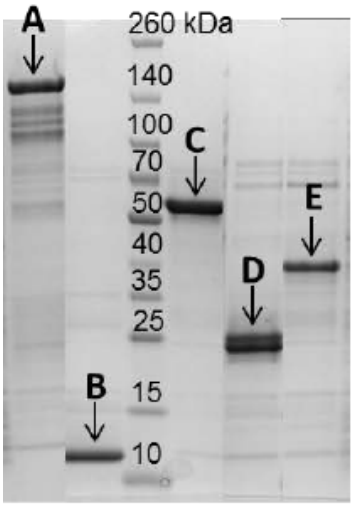


Gene Number	Locus	Putative Function
Mtb 1	Rv0088	Polyketide cyclase/dehydratase
Mtb 2	Rv0089	O-methyltransferase
Mtb 3	Rv0090	Membrane protein
Mtb 4	Rv0091	MTA/SAH nucleosidase
Mtb 5	Rv0092	CtpA Cation-transporting P-type ATPase
Mtb 6	Rv0093	Anti-sigma factor
Mtb 7	Rv0094c	Membrane protein
Mtb 8	Rv0095	Conserved hypothetical protein
Mtb 9	Rv0096	PPE family protein
Mtb 10	Rv0097	Oxidase
Mtb 11	Rv0098	Thioesterase
Mtb 12	Rv0099	Acyl ACP ligase
Mtb 13	Rv0100	Acyl carrier protein
Mtb 14	Rv0101	Non-ribosomal peptide synthetase
Mtb 15	Rv0102	CtaG cytochrome C oxidase assembly factor
Mtb 16	Rv0103c	CtpB Cation-transporting P-type ATPase
Mtb 17	Rv0104	cAMP dependent protein kinase
Mtb 18	Rv0105c	L28P Family of ribosomal proteins
Mtb 19	Rv0106	Nitrile hydratase

Gene Number	Locus	Putative Function
Mma1	Mmar_0269	CtpB Cation-transporting P-type ATPase
Mma2	Mmar_0268	Metal Chaperone
Mma3	Mmar_0267	CtaG cytochrome C oxidase assembly factor
Mma4	Mmar_0266	Secreted protein
Mma5	Mmar_0265	SigC regulatory element
Mma6	Mmar_0264	CtpA Cation-transporting P-type ATPase
Mma7	Mmar_0263	Putative Zinc Finger; anti-sigma factor
Mma8	Mmar_0262	O-methlytransferase
Mma9	Mmar_0261	PPE family protein
Mma10	MmaE	Oxidase
Mma11	MmaD	Thioesterase
Mma12	MmaC	Acyl ACP ligase
Mma13	MmaB	Acyl carrier protein
Mma14	MmaA	Non-ribosomal peptide synthetase
Mma15	Mmar_0255	mmpl5 MFS
Mma16	Mmar_0254	Ketoacyl reductase
Mma17	Mmar_0253	SRPBCC Superfamily (putative polyketide cyclase/dehydratase)
Mma18	Mmar_0251	MTA/SAH nucleosidase
Mma19	Mmar_0250	Desaturase
Mma20	Mmar_0249	TetR transcriptional regulator
Mma21	Mmar_0248	Short-chain type dehydrogenase/reductase

Figure S1. Bioinformatics analysis of *M. marinum* strain M and *M. tuberculosis* H37Rv biosynthetic gene clusters and surrounding genes. ABCDE are colored green, genes putatively related to metal transport are colored blue, putative fatty acid modification genes are colored orange, and genes with other functions are colored gray. The genes are numbered and putative functions are listed in the table. Homologous genes between the two clusters are cross-linked in the figure.

MmaA-E



ScoA-E

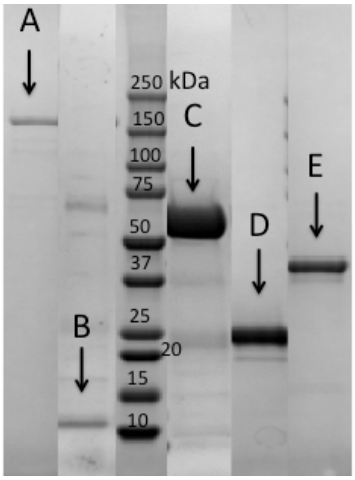
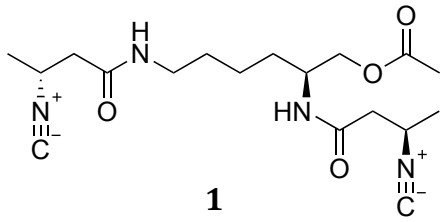
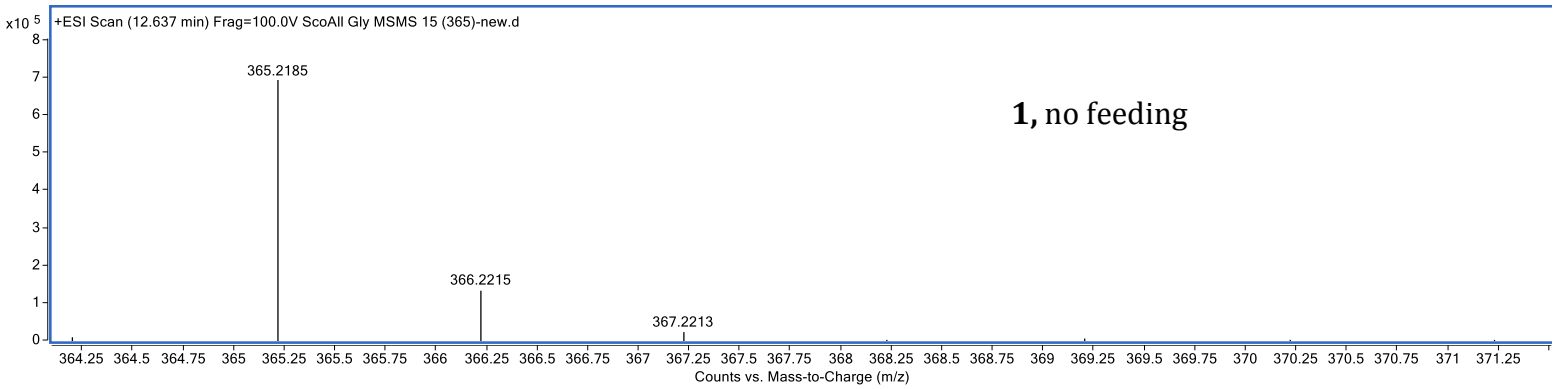


Figure S2. SDS-PAGE analysis of the *E. coli* purified proteins. All proteins have an *N*-terminal hexahistidine tag and were expressed as largely soluble. Criterion Tris-HCl gels (4-15% precast, Biorad) were used.

m/z $[M+H]^+$
Obs. 365.2185
Calc. 365.2183
Error: 0.5 ppm



1



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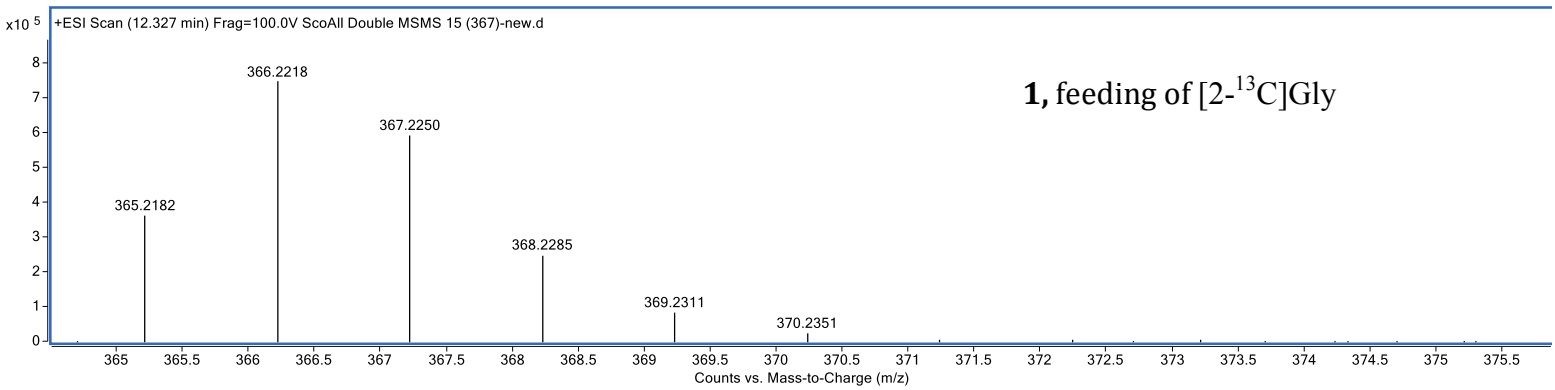
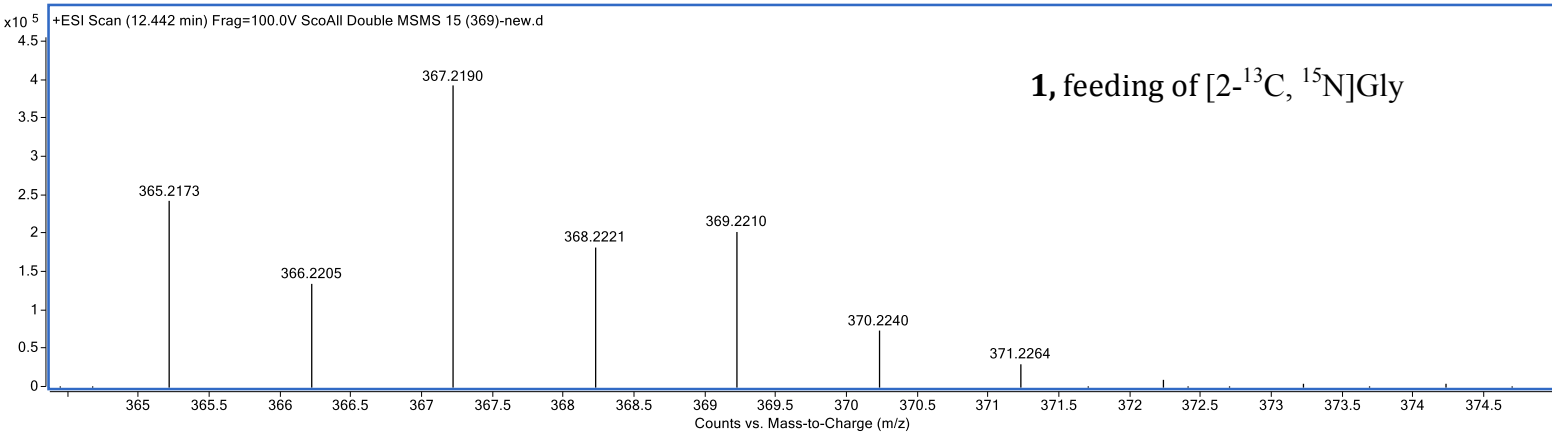


1, feeding of [2-^{13}C]Gly



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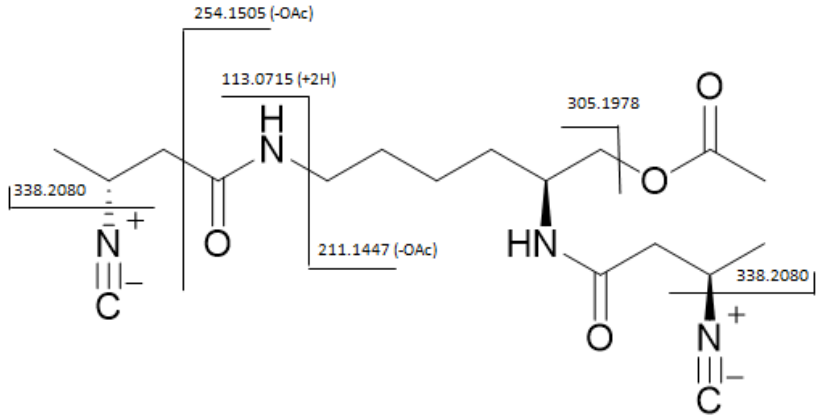
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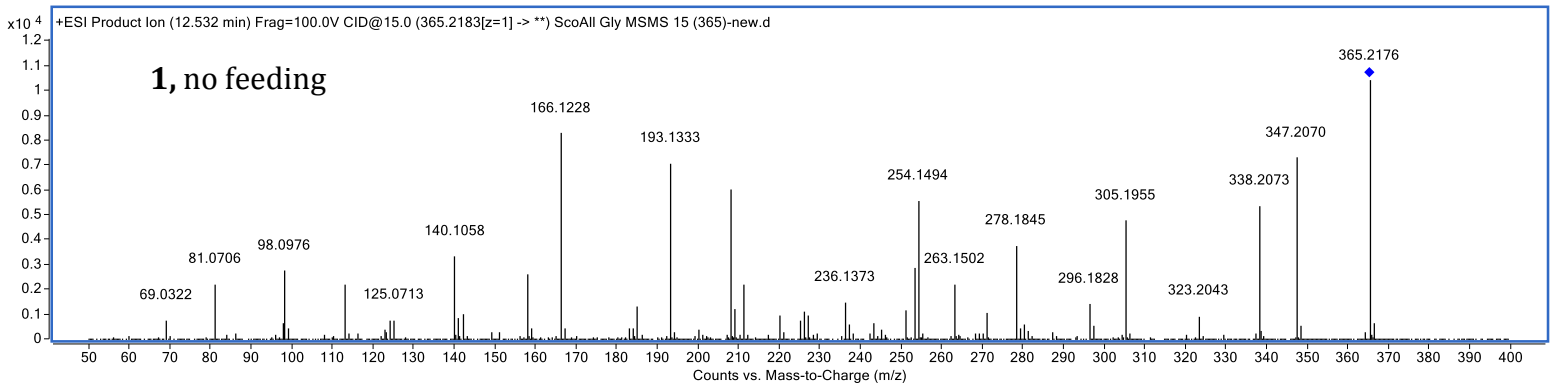
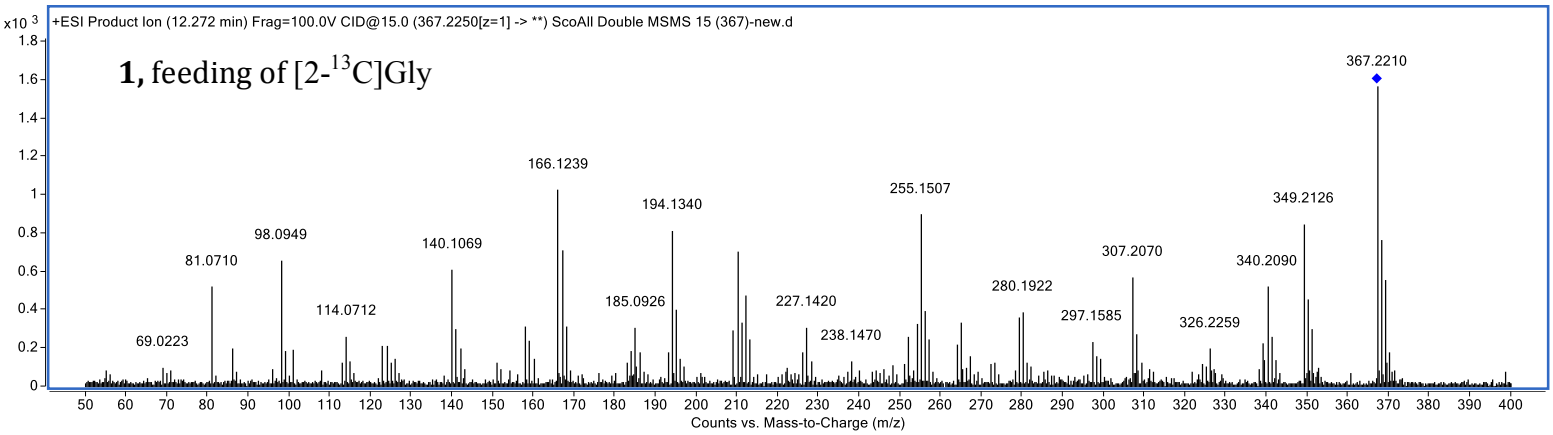
113.0715 (+2H)
305.1978
338.2080
211.1447 (-OAc)
338.2080


+ESI Product Ion (12.532 min) Frag=100.0V CID@15.0 (365.2183[z=1] -> **) ScoAll Gly MSMS 15 (365)-new.d
1, no feeding
365.2176
166.1228
193.1333
347.2070
254.1494
338.2073
305.1955
278.1845
140.1058
98.0976
81.0706
263.1502
236.1373
296.1828
69.0322
125.0713
323.2043
Counts vs. Mass-to-Charge (m/z)


+ESI Product Ion (12.272 min) Frag=100.0V CID@15.0 (367.2250[z=1] -> **) ScoAll Double MSMS 15 (367)-new.d
1, feeding of [2-13C]Gly
367.2210
166.1239
255.1507
194.1340
349.2126
98.0949
140.1069
307.2070
81.0710
340.2090
280.1922
185.0926
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114.0712
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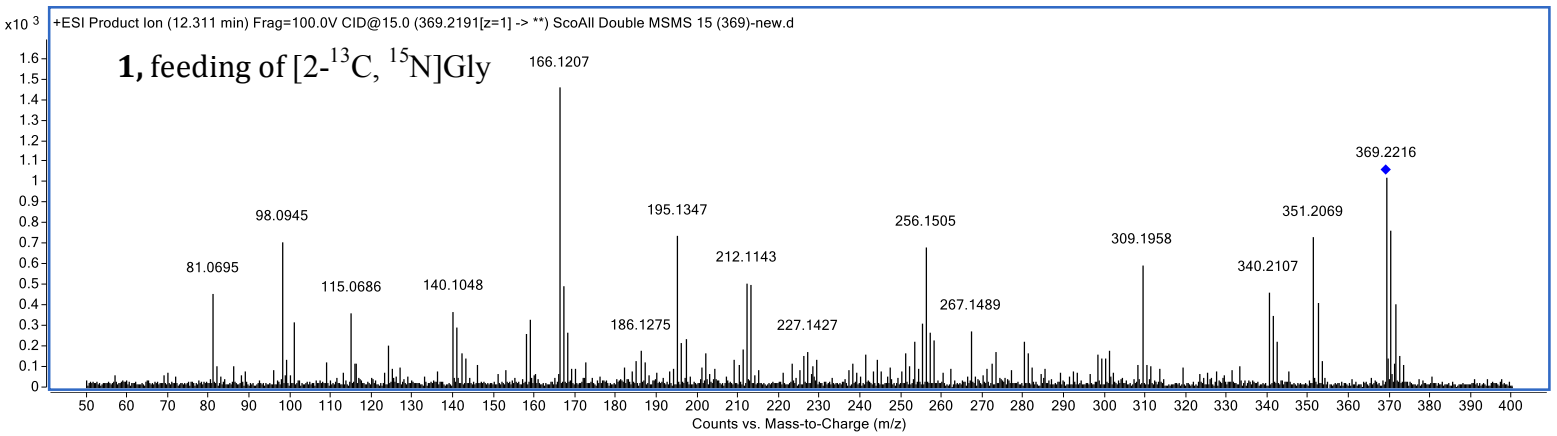
238.1470
326.2259
69.0223
Counts vs. Mass-to-Charge (m/z)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166.1207
369.2216
98.0945
195.1347
256.1505
351.2069
309.1958
212.1143
81.0695
340.2107
115.0686
140.1048
267.1489
186.1275
227.1427
Counts vs. Mass-to-Charge (m/z)

m/z $[M+H]^+$
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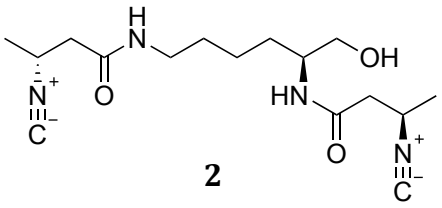
Error: 0.3 ppm



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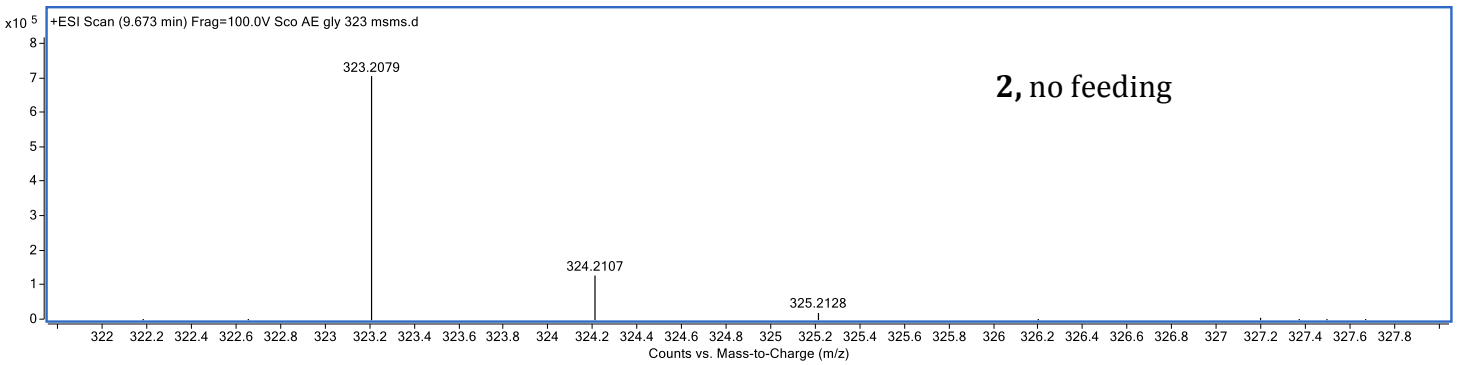

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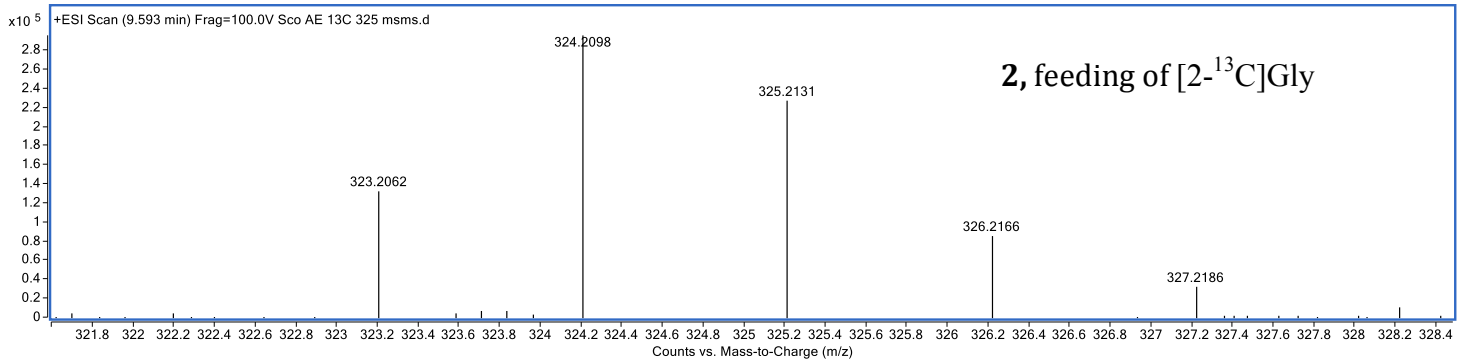

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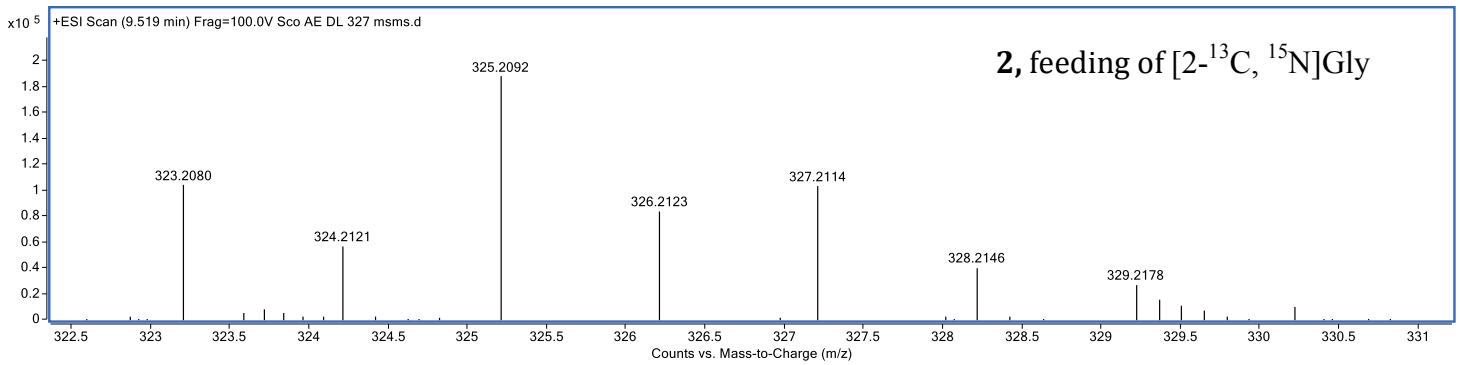

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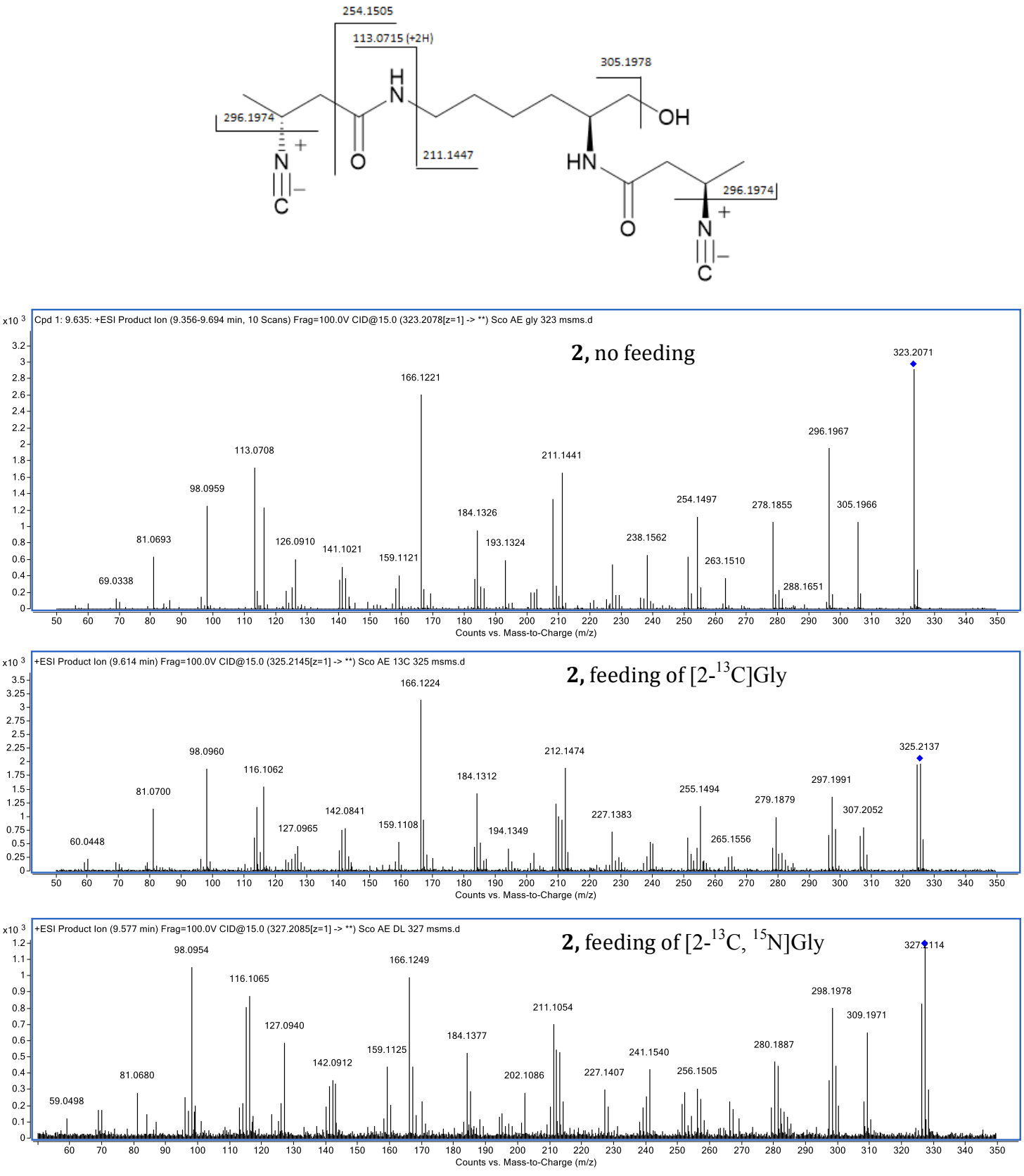
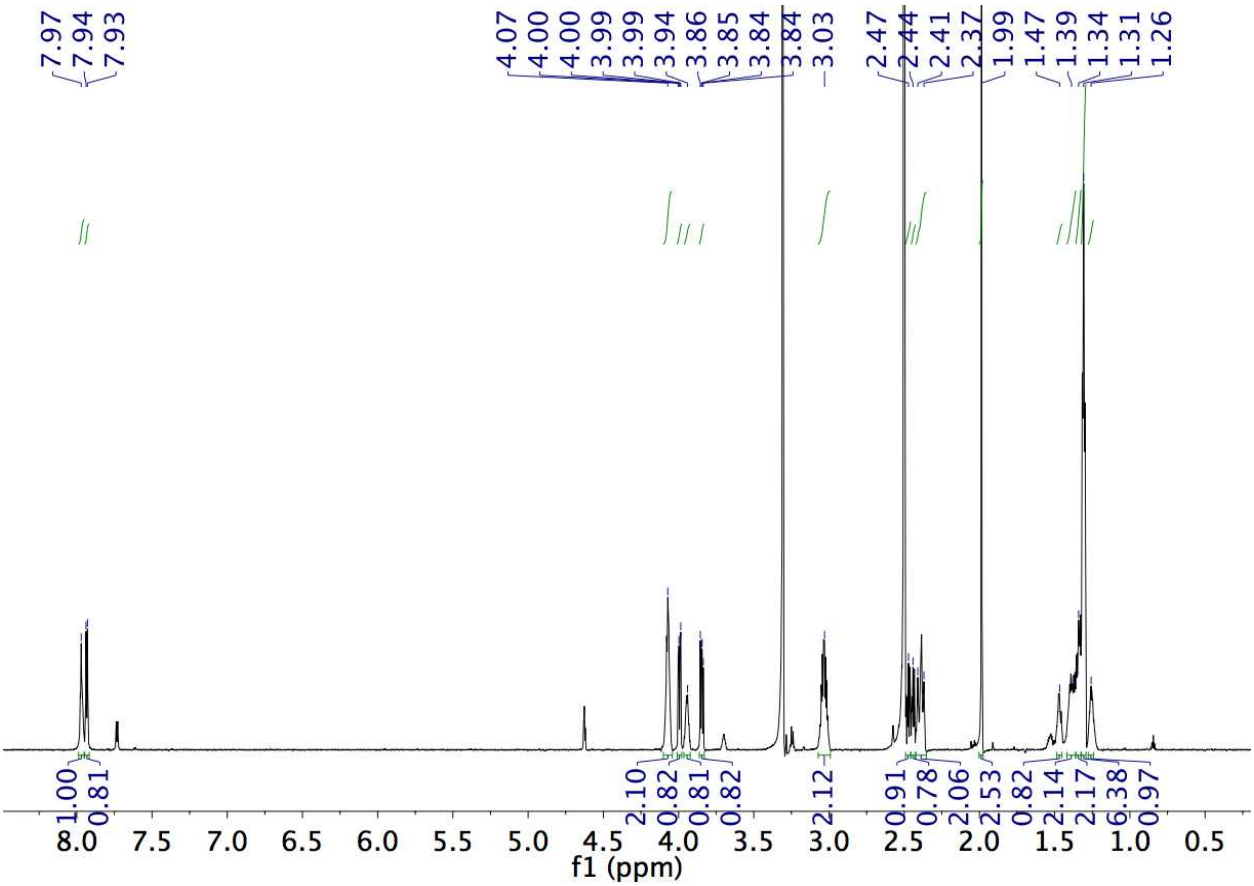
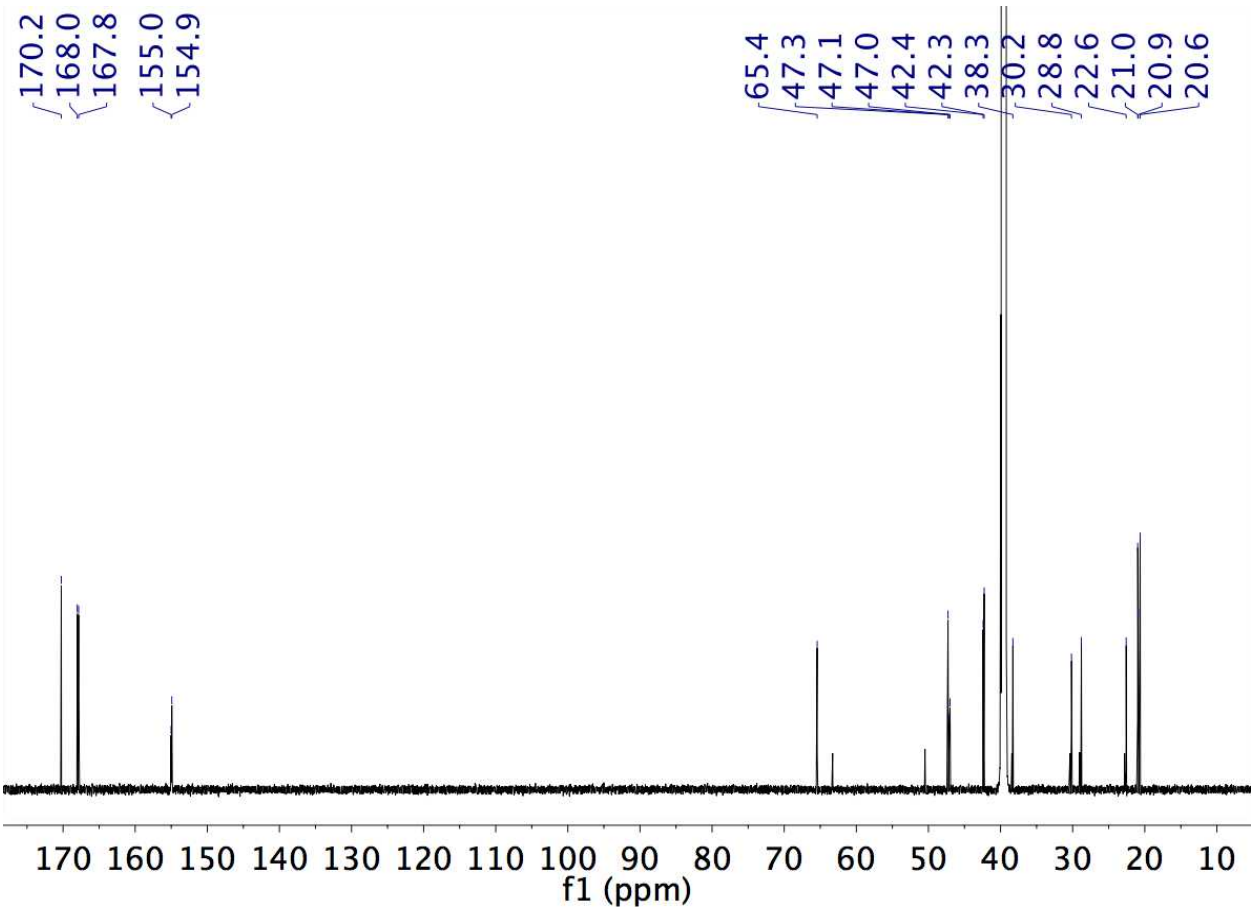


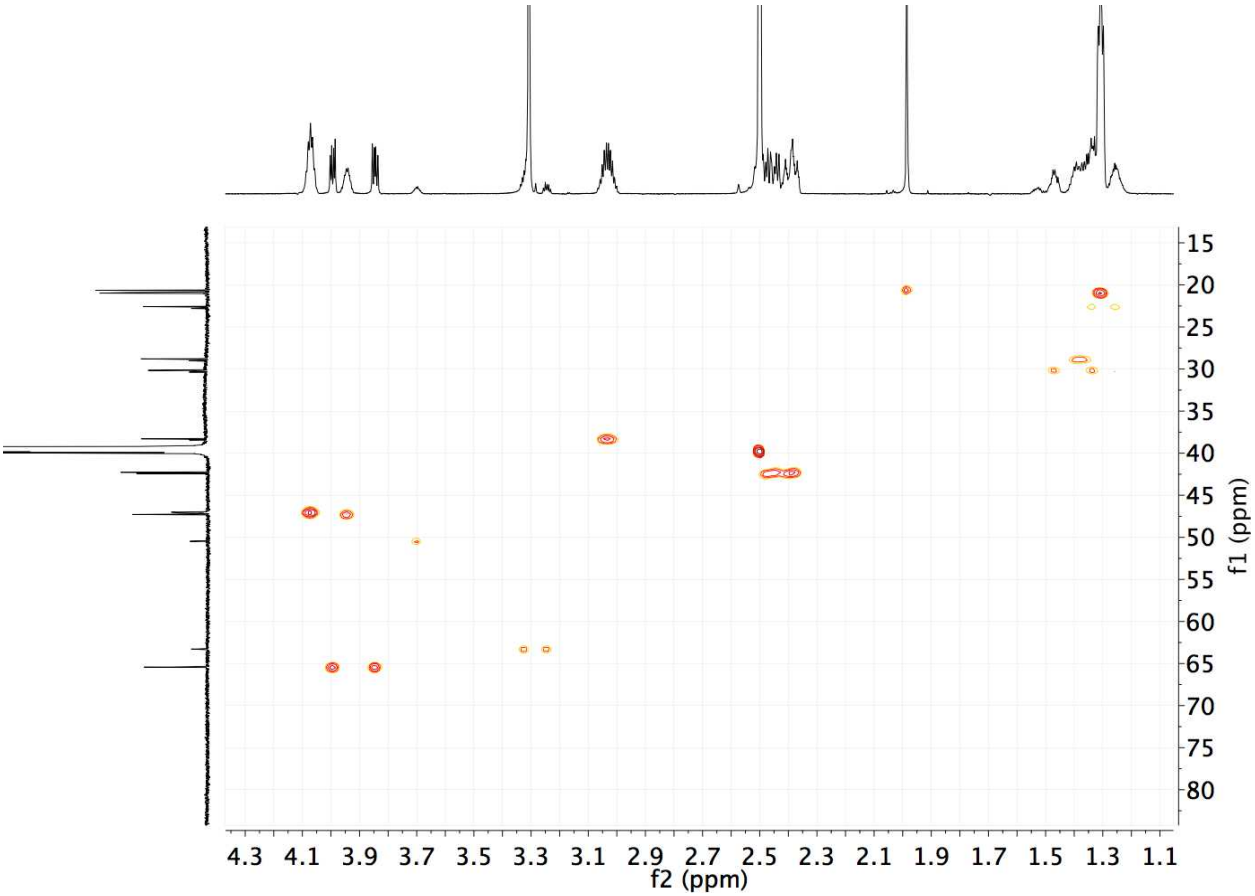
Figure S3. HRMS analysis of **1** and **2**.



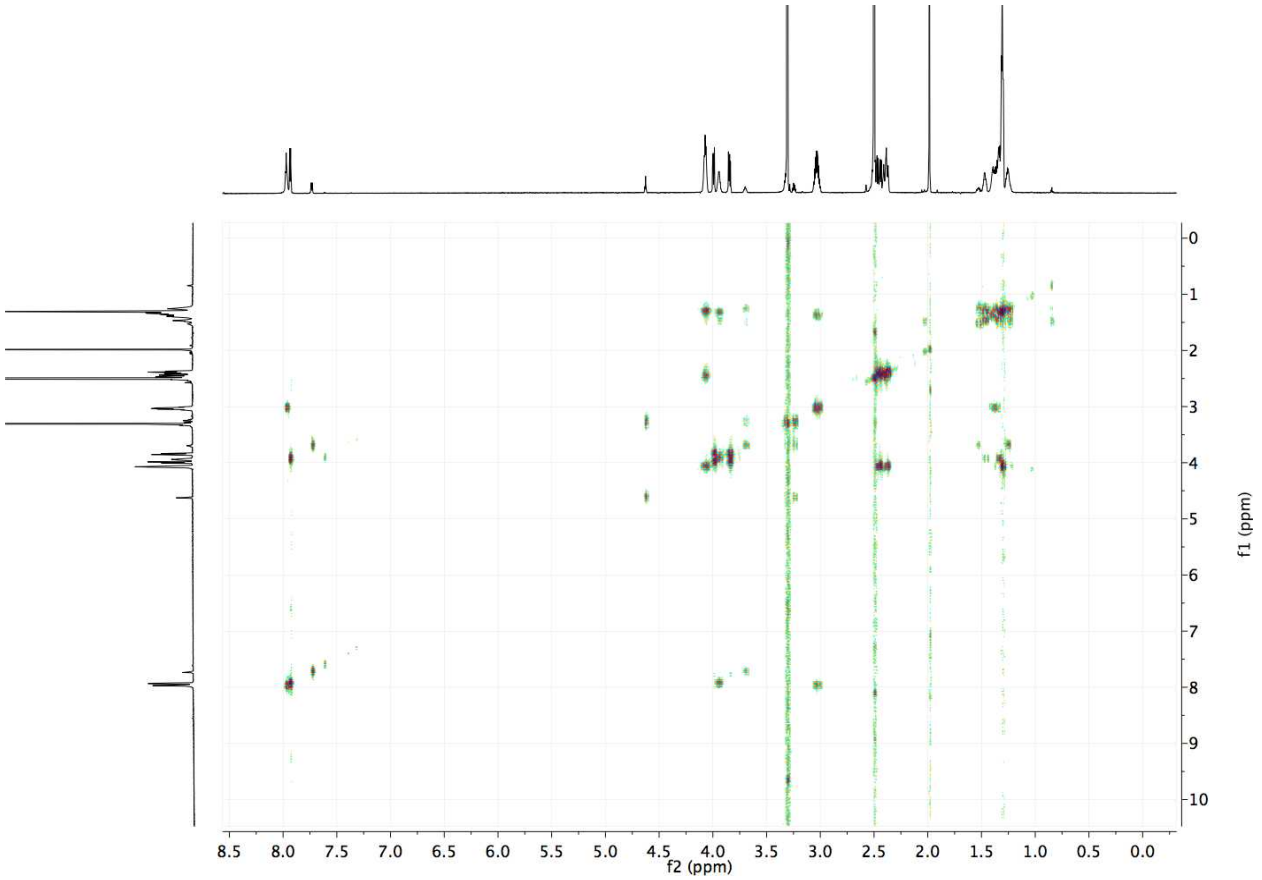
^{1}H NMR spectrum (DMSO-d_6, 900 MHz) of compound **1**.



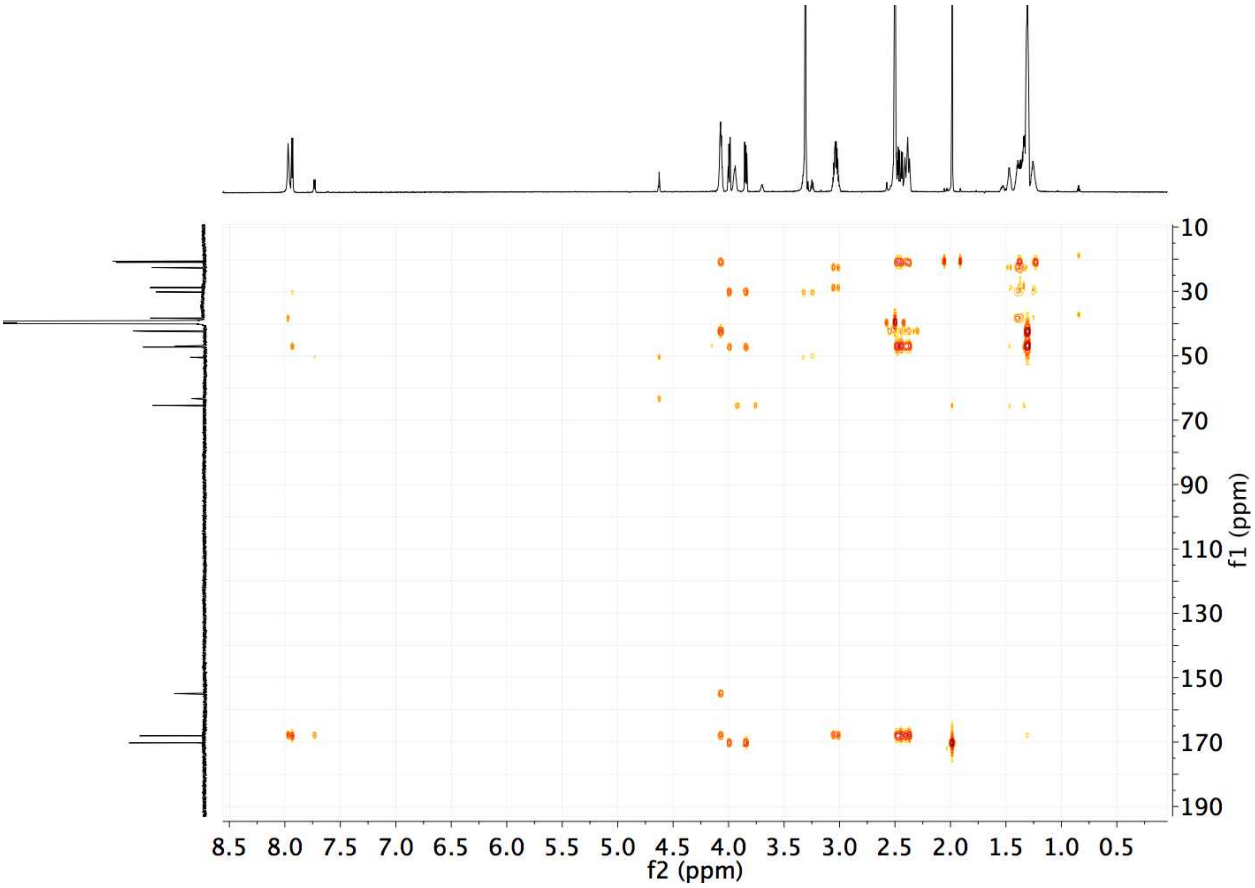
^{13}C NMR spectrum (DMSO-d_6, 225 MHz) of **1**



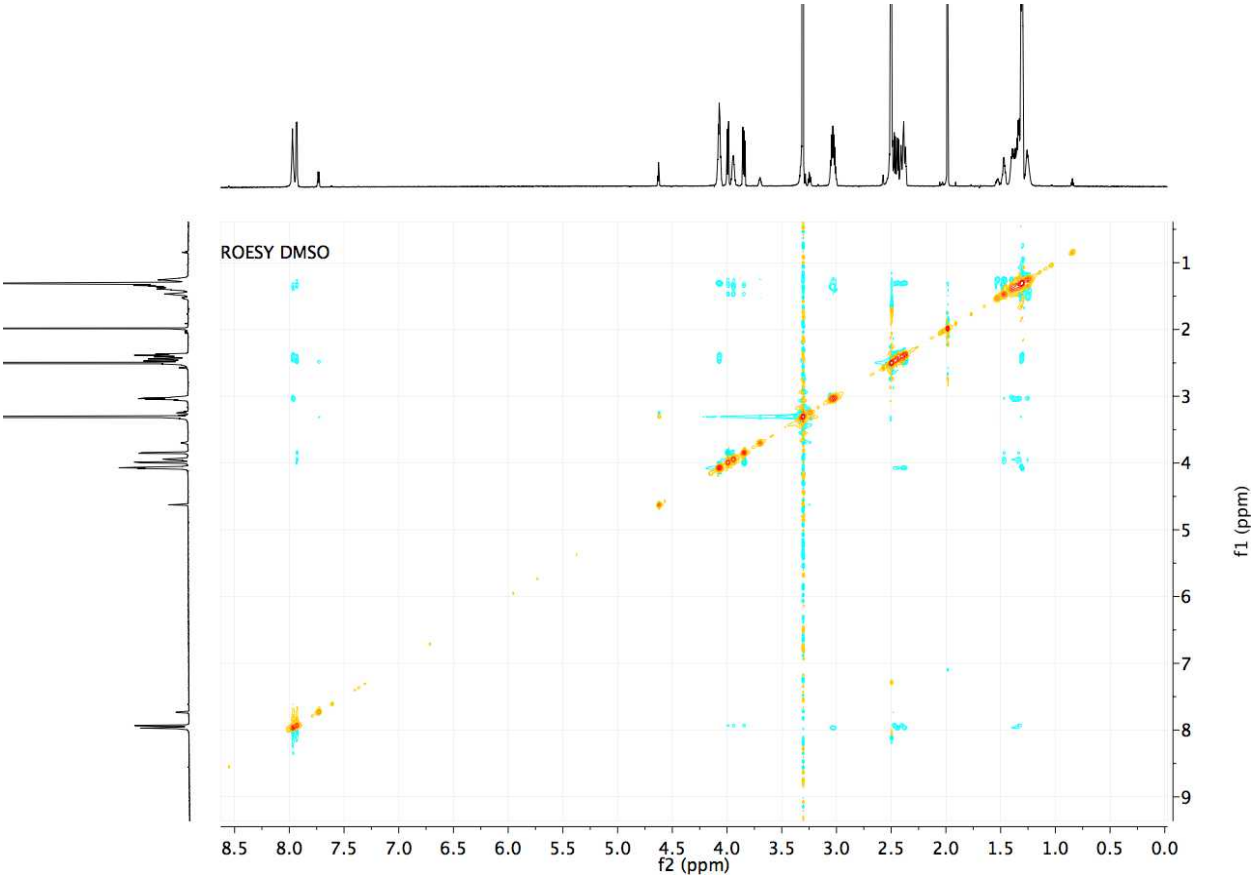
HSQC spectrum (DMSO-d_6, 900 MHz) of **1**.



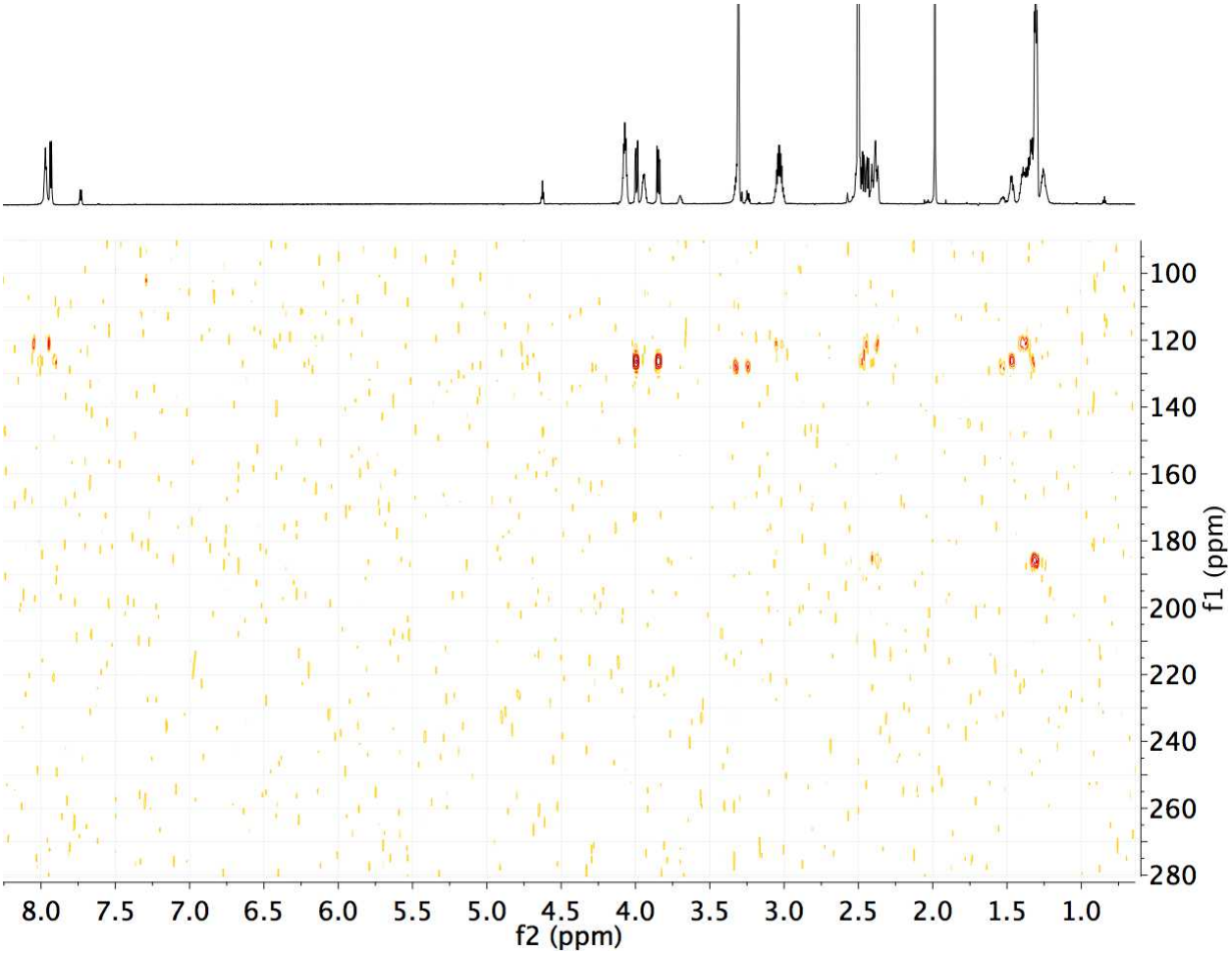
^{1}H, ^{1}H-COSY spectrum (DMSO-d_6, 900 MHz) of **1.**



HMBC spectrum (DMSO-d_6, 900 MHz) of **1**.

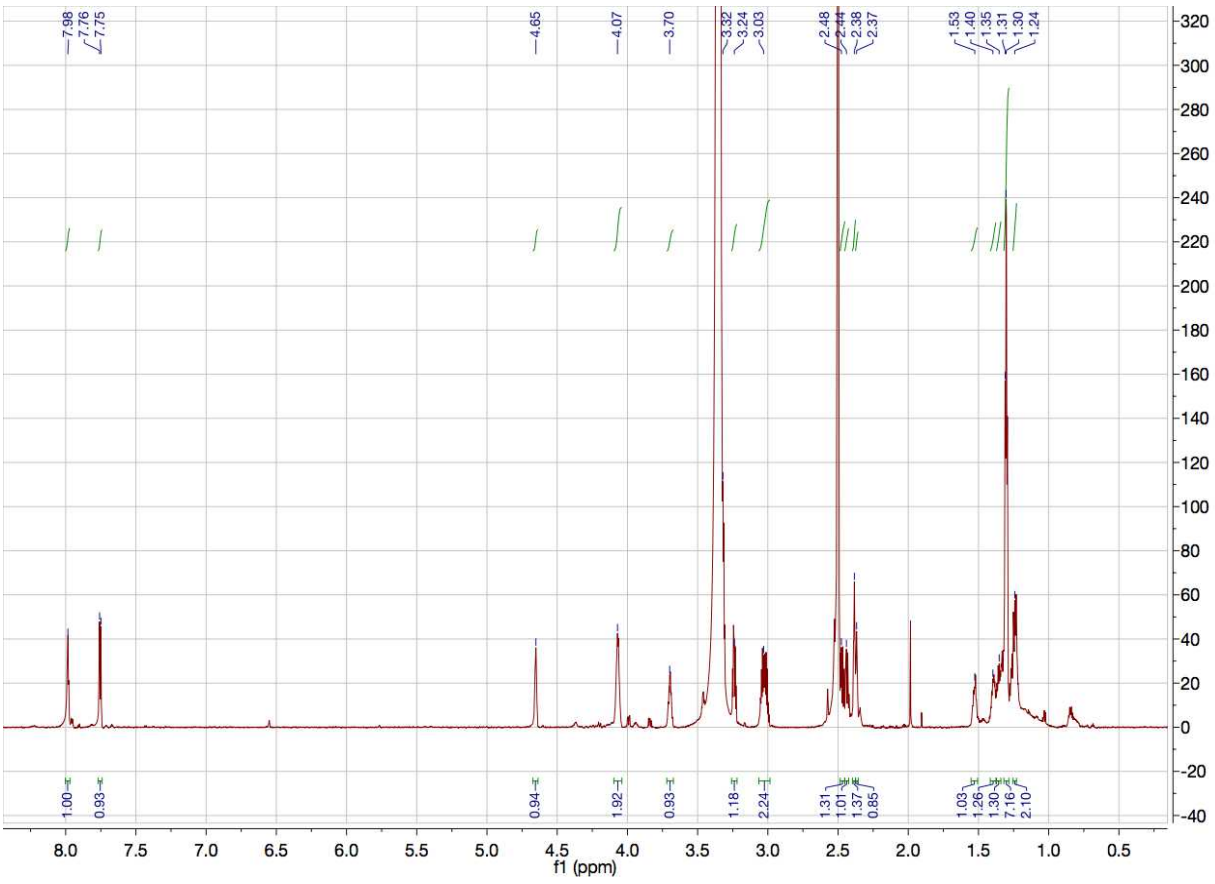


^{1}H, ^{1}H-ROESY spectrum (DMSO-d_6, 900 MHz) of **1.**

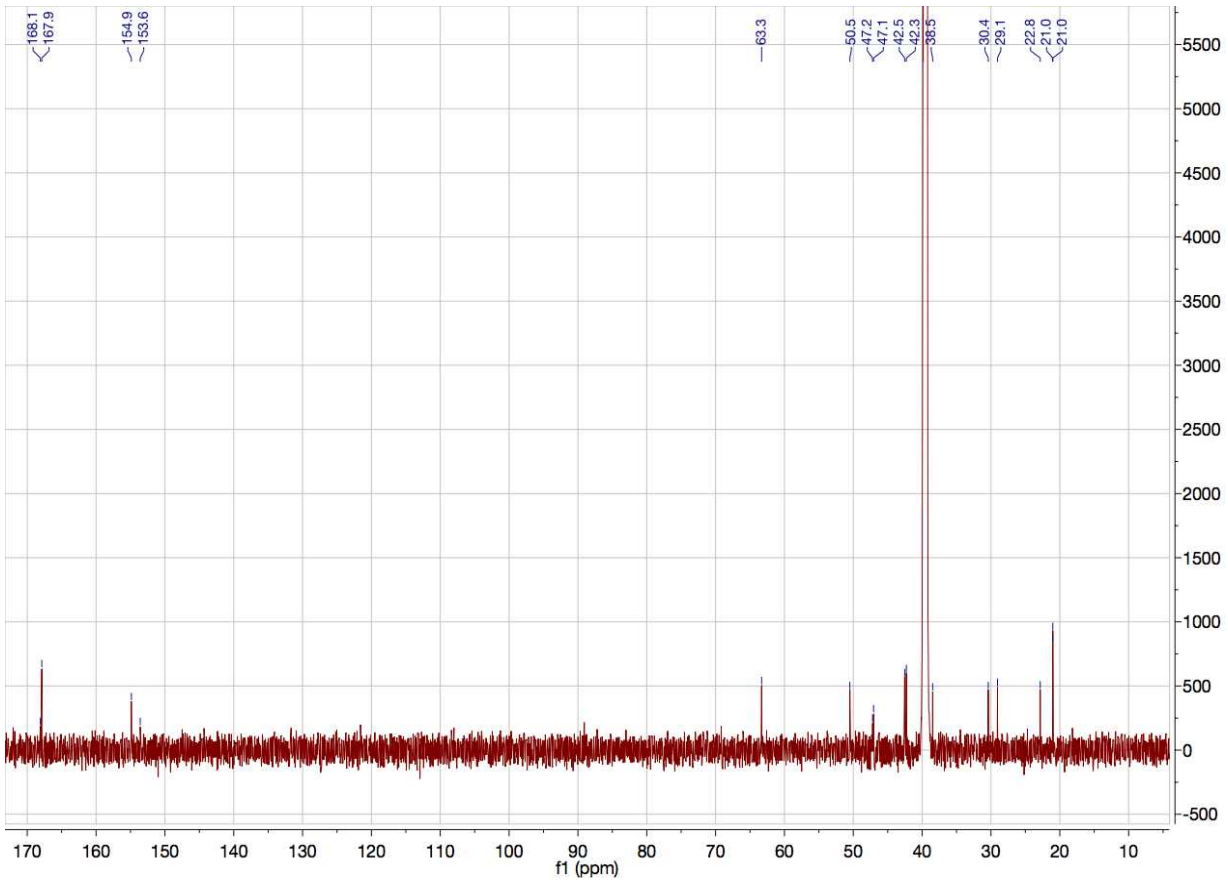


^{1}H, ^{15}N-HMBC spectrum (DMSO-d_6, 900 MHz) of **1.**

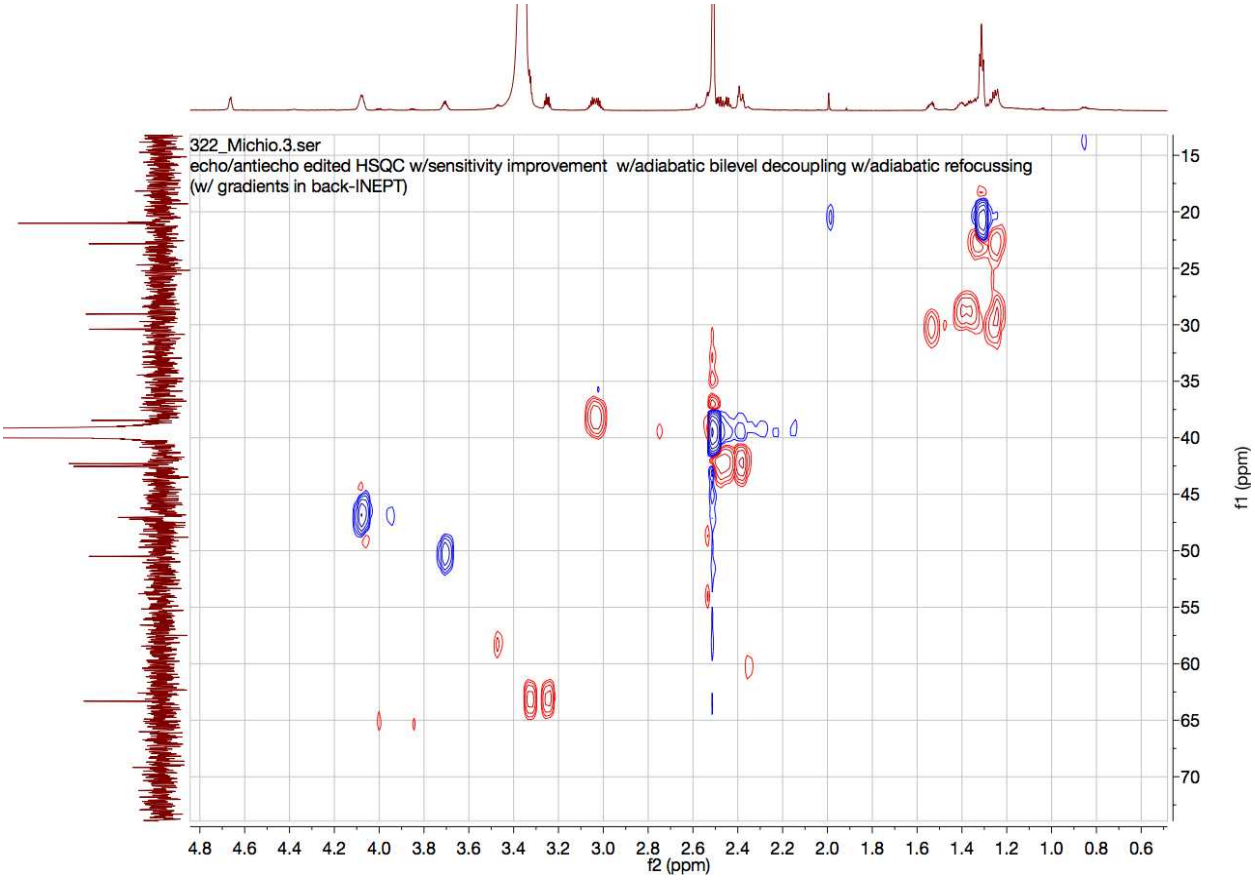
Figure S4. NMR spectra of compound **1**.



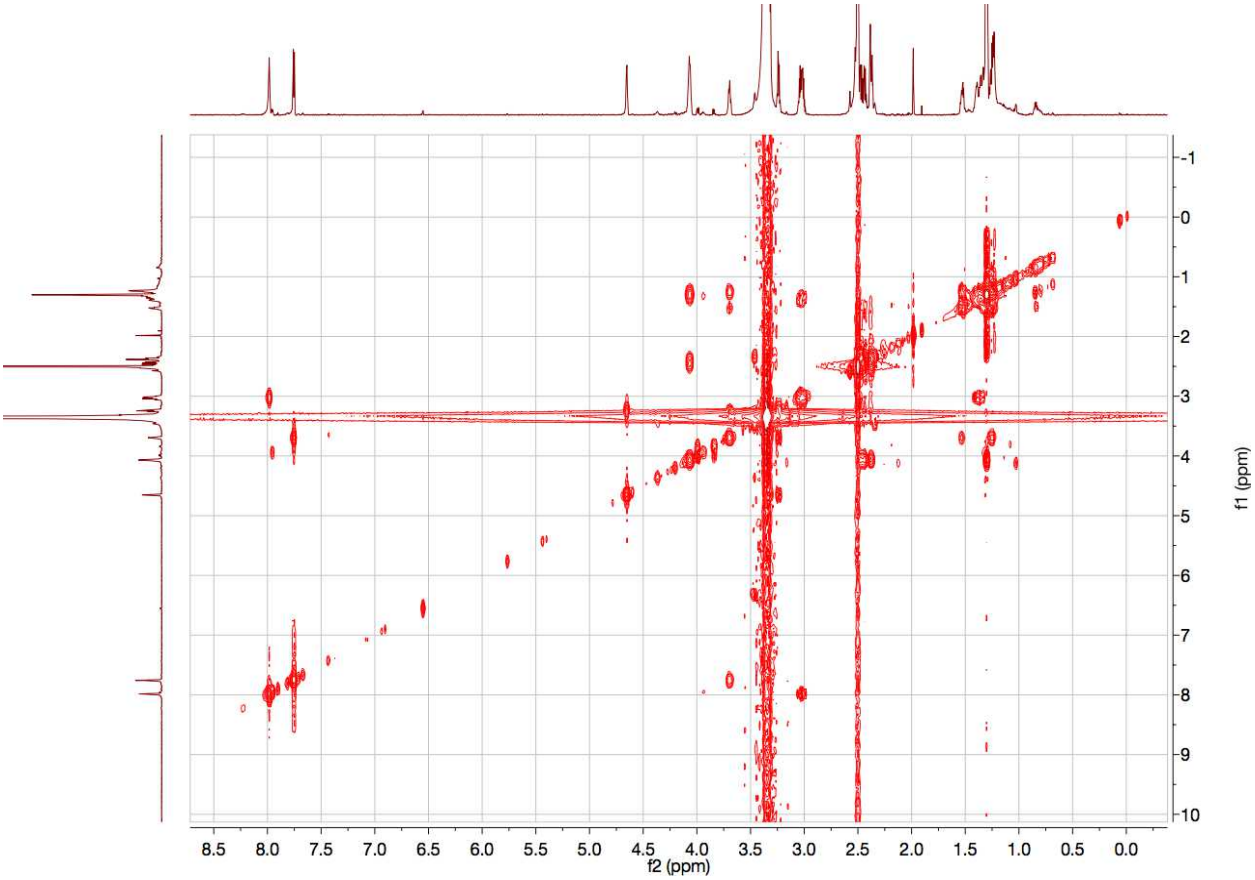
^{1}H NMR spectrum (DMSO-d_6, 900 MHz) of **2**.



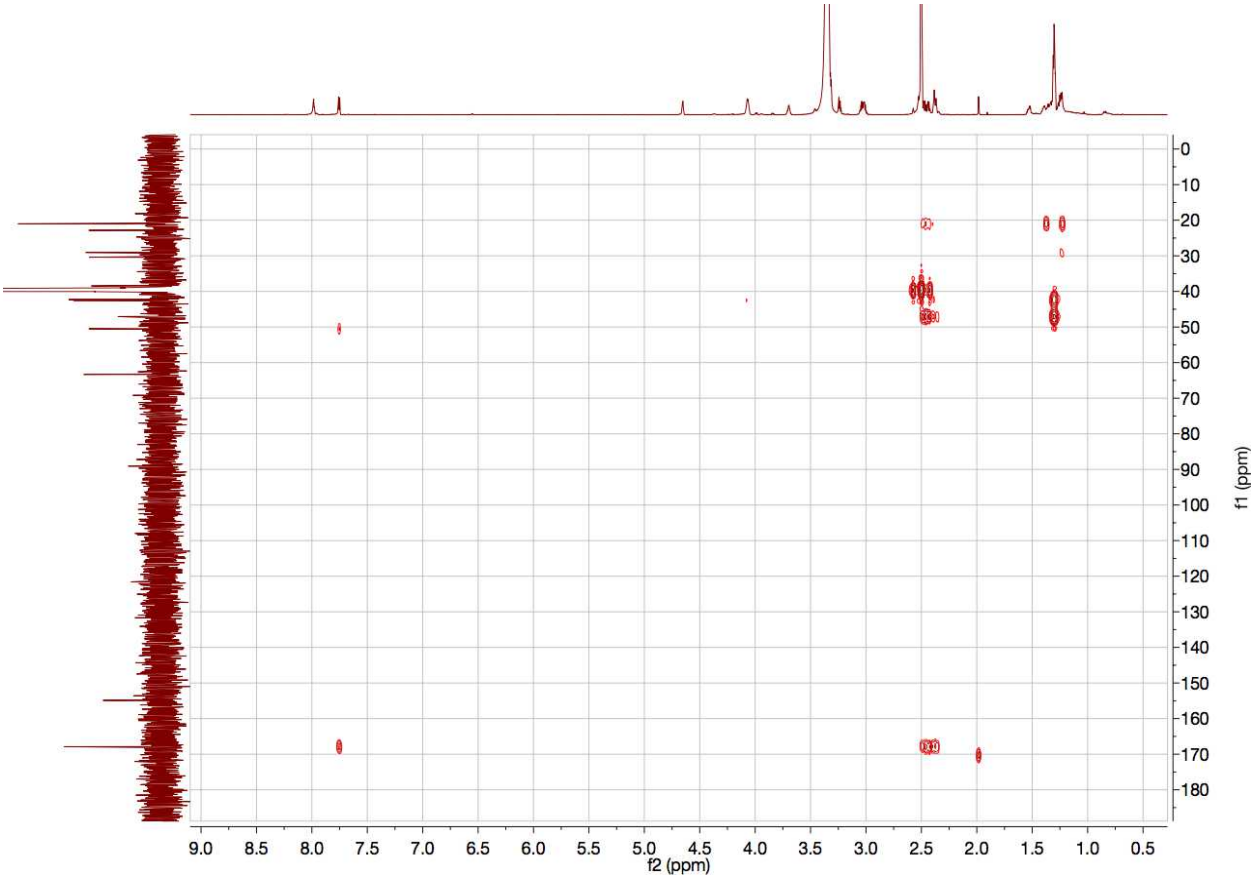
^{13}C NMR spectrum (DMSO-d_6, 225 MHz) of **2**.



HSQC spectrum (DMSO-d_6, 900 MHz) of **2**.



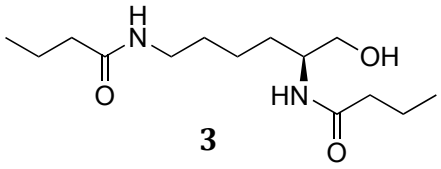
^{1}H, ^{1}H-COSY spectrum (DMSO-d_6, 900 MHz) of **2.**



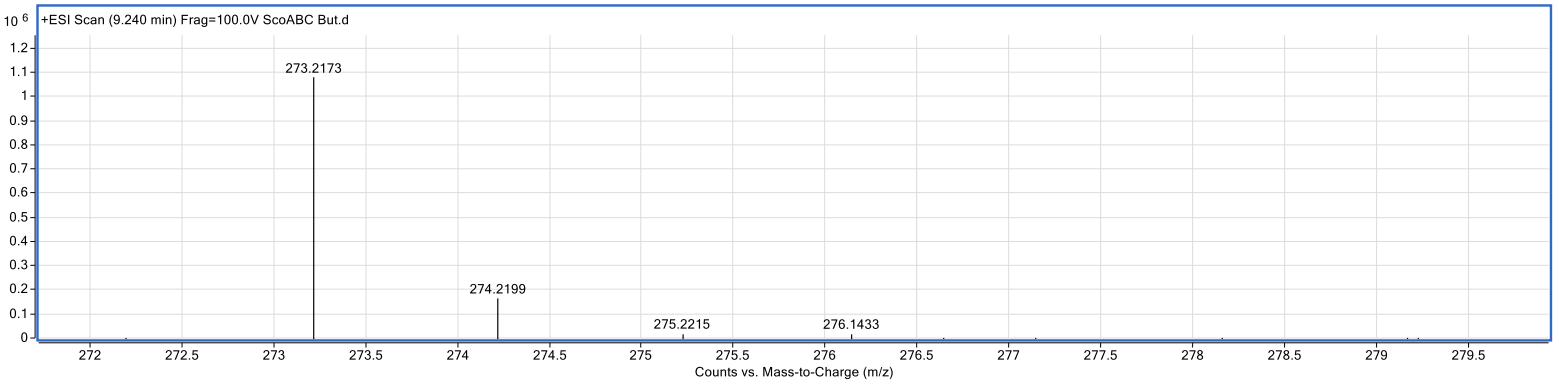
HMBC spectrum (DMSO-d_6, 900 MHz) of **2**.

Figure S5. NMR spectra of compound **2**.

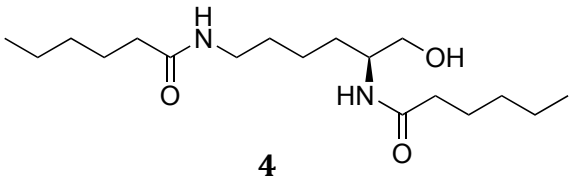
m/z $[M+H]^+$
Obs. 273.2173
Calc. 273.2173
Error: 0 ppm



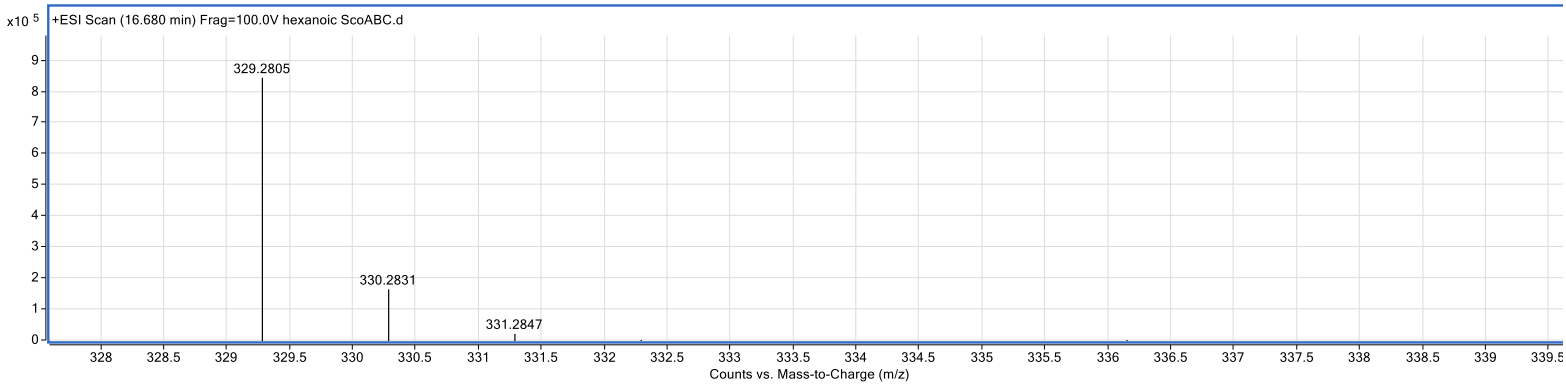
3



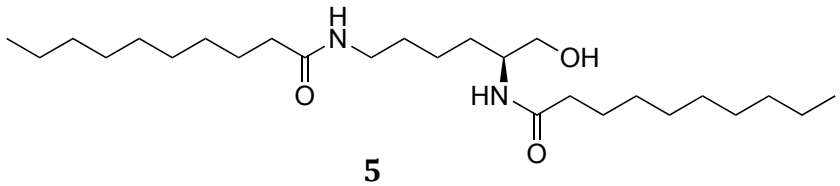
m/z $[M+H]^+$
Obs. 329.2805
Calc. 329.2799
Error: 1.8 ppm



4



m/z $[M+H]^+$
Obs. 441.4051
Calc. 441.4050
Error: 0.2 ppm



5

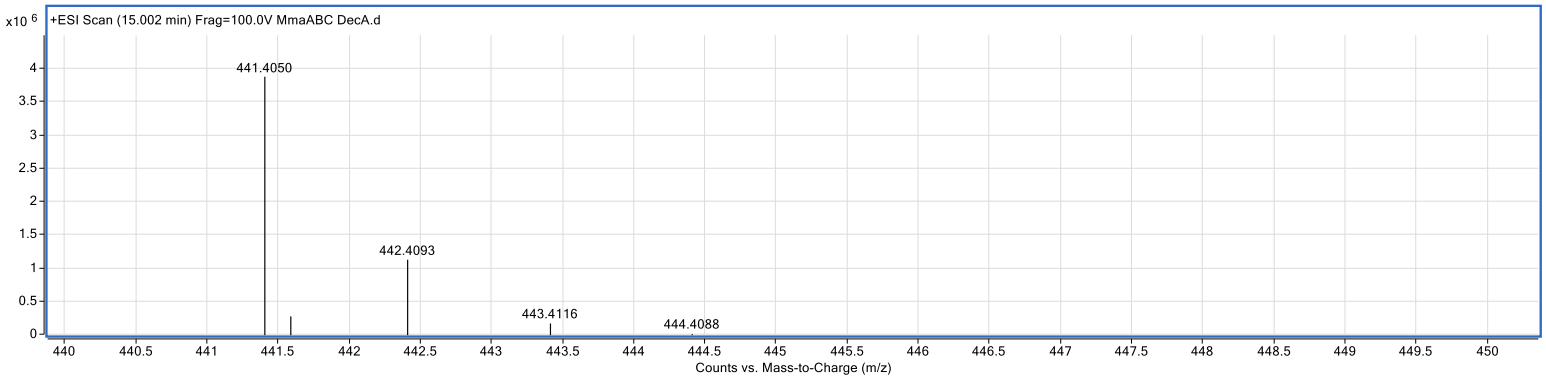
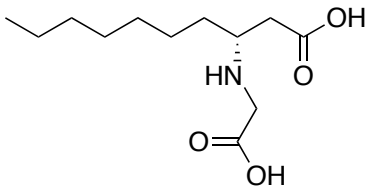
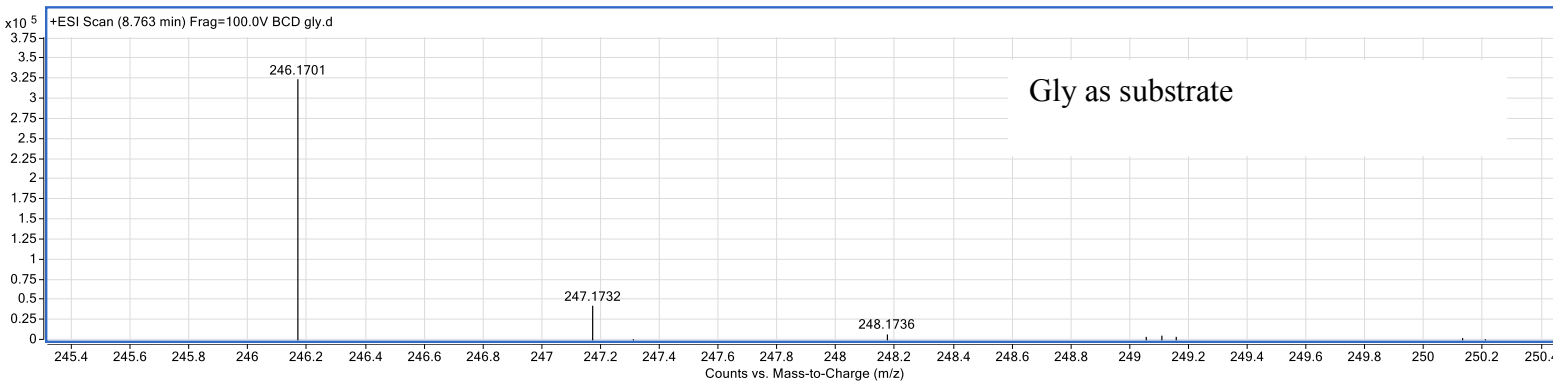
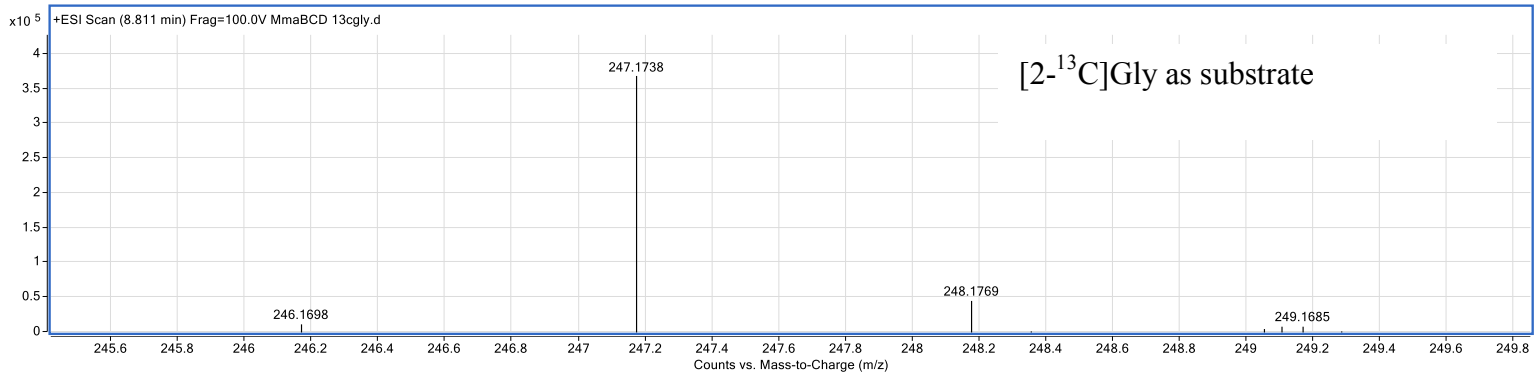


Figure S6. HRMS analysis of **3-5.**

m/z $[M+H]^+$
Obs. 246.1701
Calc. 246.1700
Error: 0.4 ppm







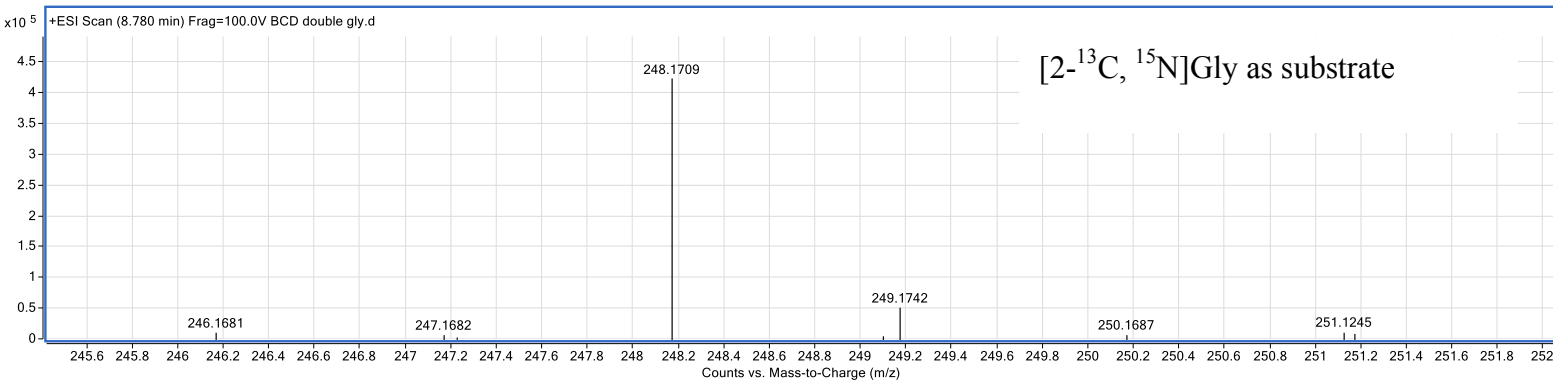
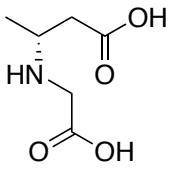
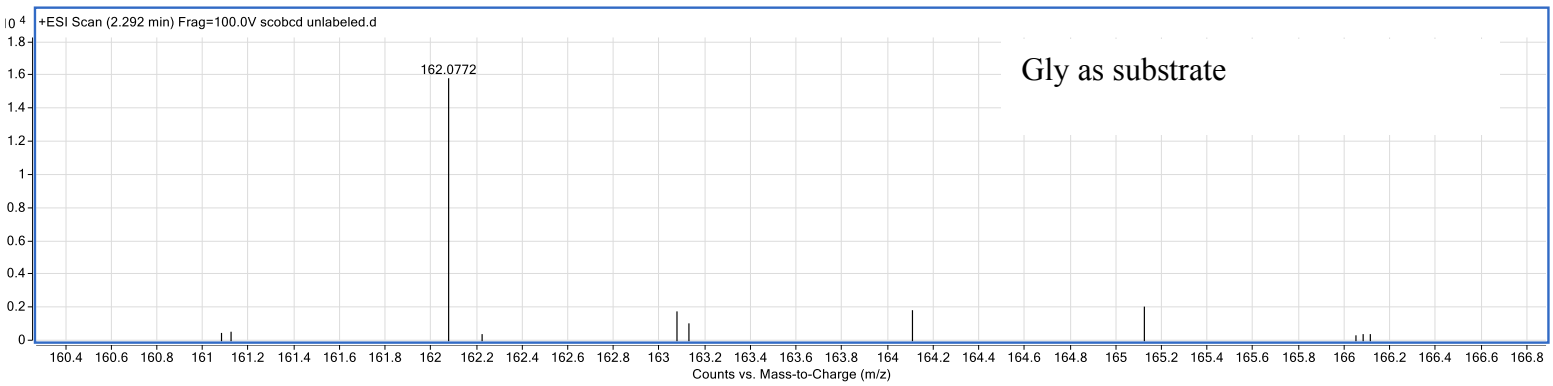


Figure S7. HRMS analysis of MmaBCD *in vitro* reaction products.

m/z $[M+H]^+$
Obs. 162.0772
Calc. 162.0761
Error: 6.7 ppm





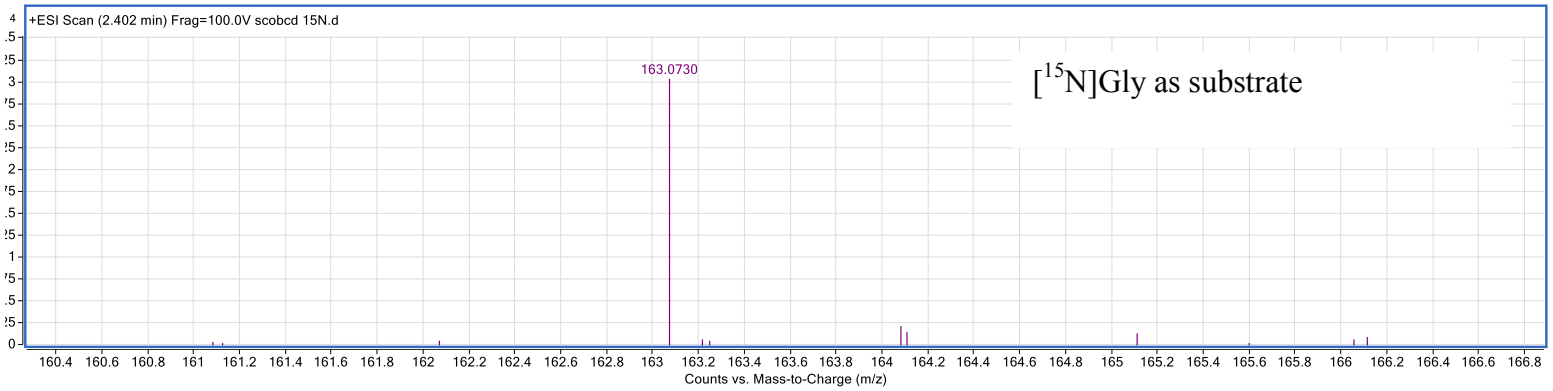
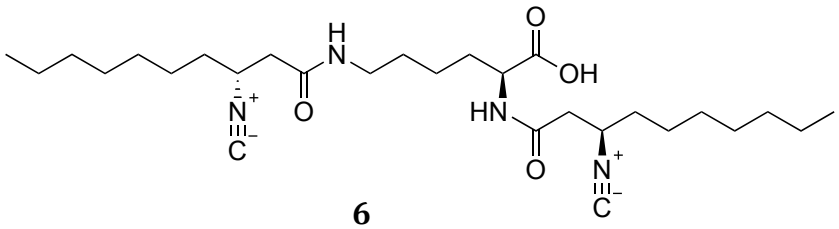
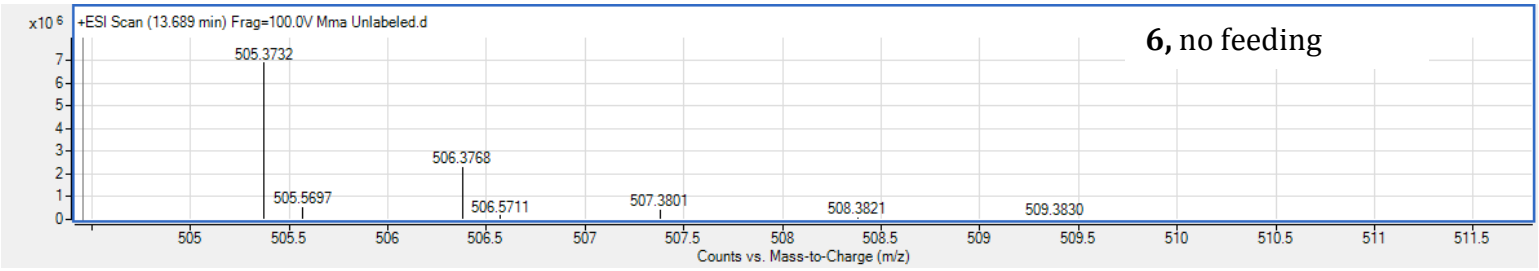


Figure S8. HRMS analysis of ScoBCD *in vitro* reaction products.

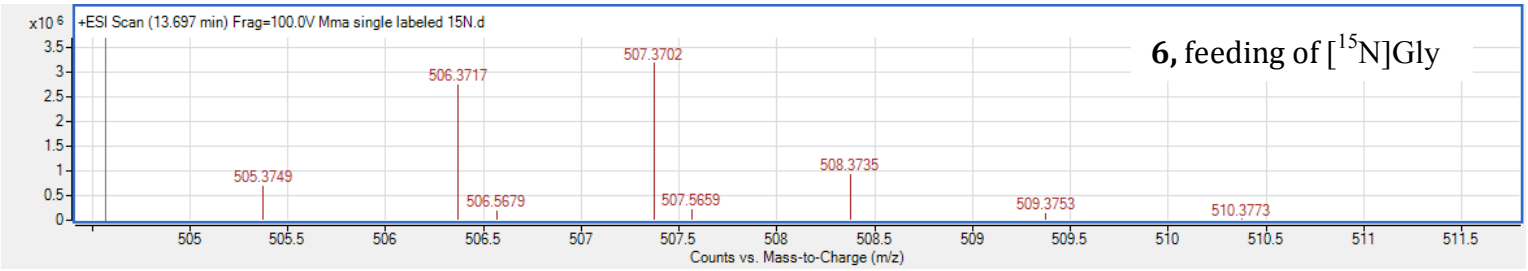
m/z $[M+H]^+$
Obs. 505.3748
Calc. 505.3748
Error: 0 ppm



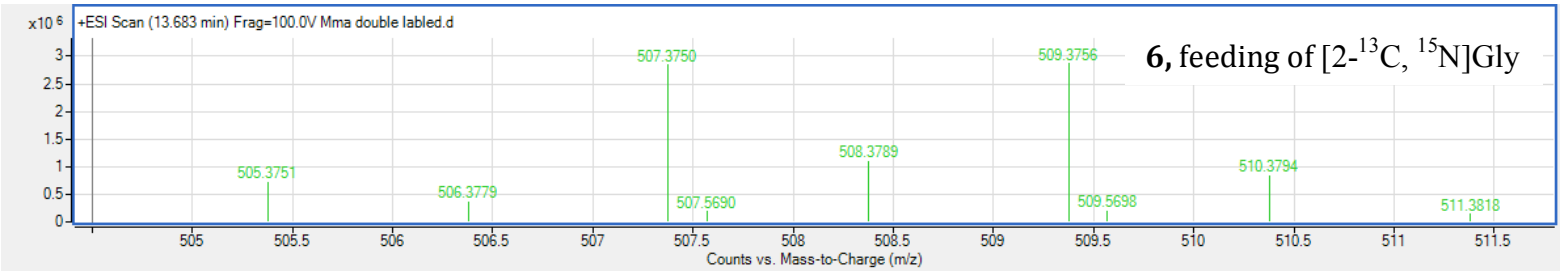
6



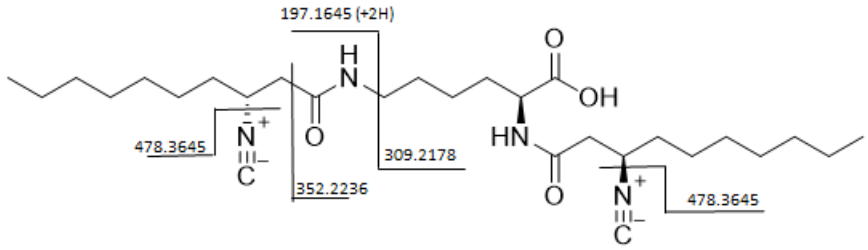
6, no feeding

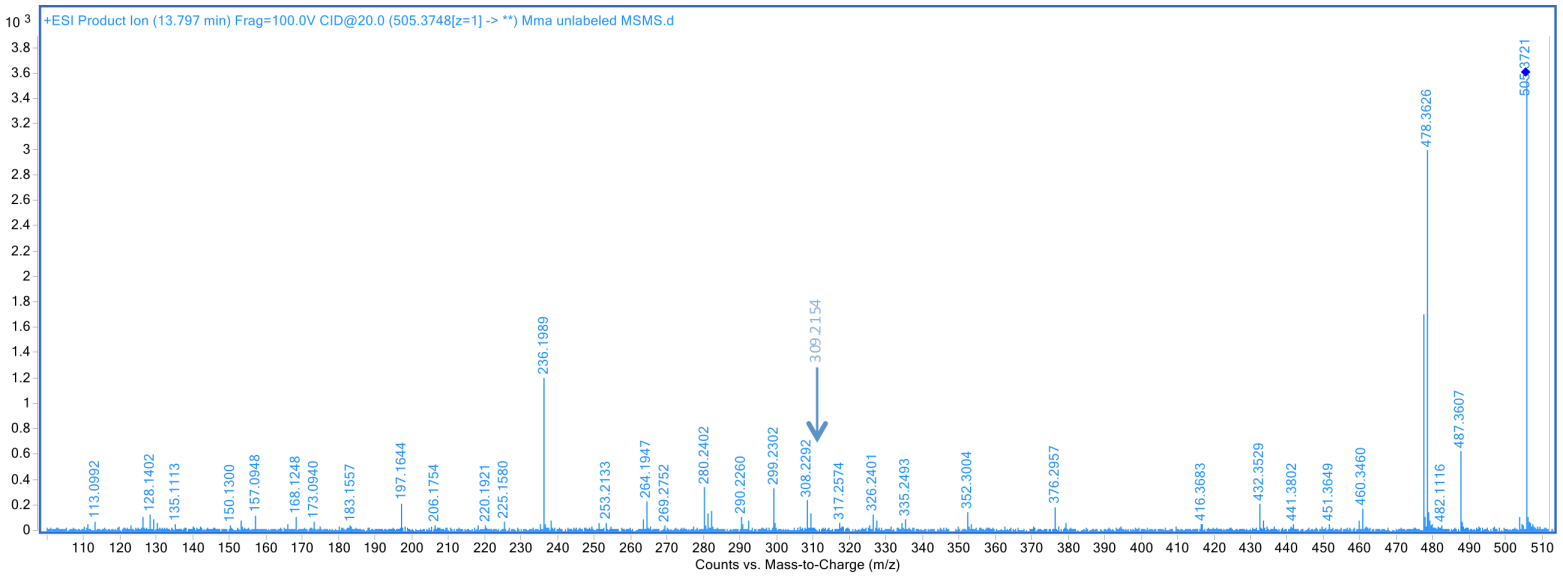


6, feeding of [^{15}N]Gly



6, feeding of [2-^{13}C, ^{15}N]Gly





m/z $[M+H]^+$
Obs. 561.4376
Calc. 561.4374
Error: 0.4 ppm

7

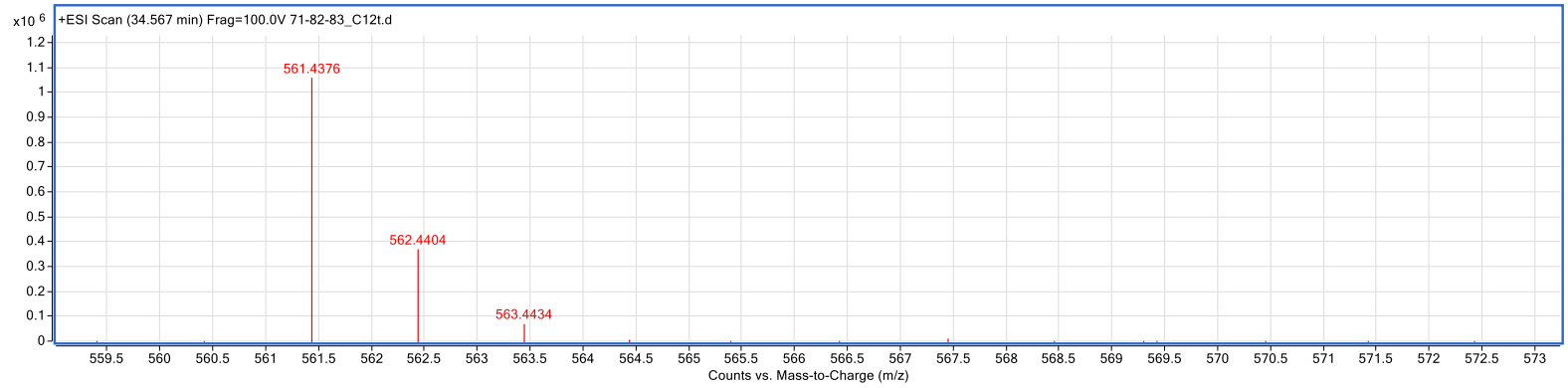
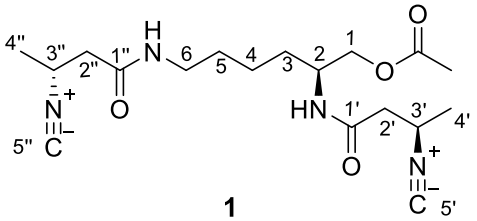


Figure S9. HRMS analysis of **6** and **7**.

SI Tables

Table S1. NMR data for compound **1** in DMSO-d6.

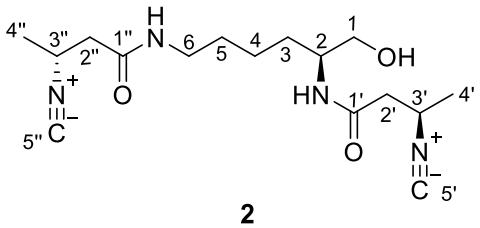


1

Position	δ_H [ppm]		mult. (*J* in Hz)	^{1}H-^{13}C HMBC	δ_C [ppm]
1	3.84	1H	dd (10.9, 6.5)	2,3,1-OAc(C)	65.4
	3.99	1H	dd (10.9, 4.8)		
2	3.94	1H	m	1′,3,4	47.3
3	1.47	1H	m	1,2,4,5	30.2
	1.34[a]	1H	m		
4	1.34[a]	1H	m	3,6	22.6
	1.26	1H	m		
5	1.39	2H	m	3,4,6	28.8
6	3.03	2H	m	1″,4,5	38.3
1′					168.0
2′	2.41[b]	1H	m	1’,3’,4’	42.4
	2.47[c]	1H	m		
3′	4.07[d]	1H	m	1′,2’,4′,5’	47.1
4′	1.31[e]	3H	m	1’,2’,3’	20.9
5′					155.0
1″					167.8
2″	2.37[b]	1H	m	1’’, 3’’, 4’’	42.3
	2.44[c]	1H	m		
3″	4.07[d]	1H	m	1″,2″,4″,5’’	47.0
4″	1.31[e]	3H	m	1″,2″,3″	21.0
5″					154.9
1-OAc					170.2
	1.99	3H	s	1, 1-OAc (C)	20.6
2-NH	7.93	1H	d (8.5)	1,2,3,1’,2’,3’’	
6-NH	7.97	1H	m	5,6,1’’	

[a], [b], [c], [d], [e], overlapped signals.

Table S2. NMR data for compound **2** in DMSO-d6.



2

Position	δ_H [ppm]		mult. (*J* in Hz)	^{1}H-^{13}C HMBC	δ_C [ppm]
1	3.24	1H	m		63.3
	3.32	1H	m		
2	3.70	1H	m		50.5
3	1.24[a]	1H	m		30.4
	1.53	1H	m		
4	1.24[a]	1H	m	3	22.8
	1.31[b]	1H	m		
5	1.35	1H	m		29.1
	1.40	1H	m		
6	3.03	2H	m	1″,4,5	38.5
1′					167.9
2′	2.44	1H	m	3’	42.5
	2.48	1H	m		
3′	4.07[c]	1H	m	2’, 4’	47.2
4′	1.30[b]	3H	m	2’, 3’	21.0
5′					154.9
1″					168.1
2″	2.37	1H	m	3’’	42.3
	2.38	1H	m		
3″	4.07[c]	1H	m	3’’	47.1
4″	1.30[b]	3H	m	3’’	21.0
5″					153.6
1-OH	4.65	1H	m		
2-NH	7.76	1H	d (8.5)	1’, 2	
6-NH	7.98	1H	m		

[a], [b], overlapped signals.

Table S3. Primers used in this study.

Primer	Sequence (5' -> 3')
Duet-mmar101-F	**aaagaattcgGTGACCGCGCCCGAAATCGG**
Duet-mmar101-R	**tataagcttCTAGGCCAGTGCGGGTCTTG**
pET24b-mmar100-F	**aaacataTGGCTGACCCGGTGCGCCA**
pET24b-mmar100-R	**aaactcgagCGTAGGACTTTCCAGTTCGG**
Duet-mmar99-F	**aaaggatccgATGTCCGACTTACCCGCTAC**
Duet-mmar99-R	**aaactgcagTCAGCCACCGGTTTGGACCT**
Duet-mmar98-F	**aaaggatccgcatATGAGCACCACCGATTTGAC**
Duet-mmar98-R	**tataagcttcaattgCTAGGGGATGTTCAGGGCCG**
Duet-mmar97-F	**aaaggatccgATGACGCTCAACGTGAAAGG**
Duet-mmar97-R	**tataagcttTCATGCCGGGTAGCCCGGCG**
MmaNRPS-ETDuet-F	**aaactgcagGTGACCGCGCCCGAAATCGG**
MmaNRPS-ETDuet-R	**aaaaagcttCTAGGCCAGTGCGGGTCTTG**
MmaAAL-RSFDuet-F	**aaagaattcgATGTCCGACTTACCCGCTAC**
MmaAAL-RSFDuet-R	**aaaaagcttTCAGCCACCGGTTTGGACCT**
MmaACP-RSFDuet-F	**aaaaaaacatatgCTGACCCGGTGCGCCA**
MmaACP-RSFDuet-R	**aaactcgagCGTAGGACTTTCCAGTTCGG**
MmaOXY-CDFDuet-F	**aaacatatgATGACGCTCAACGTGAAAGG**
MmaOXY-CDFDuet-R	**aaactcgagTCATGCCGGGTAGCCCGGCG**
MmaTE3-CDFDuet-F	**aaactgcagATGAGCACCACCGATTTGAC**
MmaTE3-CDFDuet-R	**aaaaagcttCTAGGGGATGTTCAGGGCCG**
MmaTe3-ACYCDuet-F	**aaactgcagATGAGCACCACCGATTTGAC**
MmaTe3-ACYCDuet-R	**aaaaagcttCTAGGGGATGTTCAGGGCCG**
AAL-RSFDuet-F	**aaagaattcgATGGACCGGCTCCACCACCC**
AAL-RSFDuet-R	**aaaaagcttTCAGTTGACCTTGCGTGCGG**
ACP2-RSFDuet-F	**aaaaaacatatgCTGCTCCCCTCACGCT**
ACP2-RSFDuet-R	**aaactcgagTCATGCGGTGACATGGCCCG**
Oxy-CDFDuet-F	**aaacatatgATGCAGATCGACGAACAGCC**
Oxy-CDFDuet-R	**aaactcgagTCATGCCGCCTGGATCCCGT**
TE3-CDFDuet-F	**aaactgcagATGACGGACGAAGCCCTGCT**
TE3-CDFDuet-R	**aaaaagcttTCAGGGGACGTTGACGAAGG**
NRPS-ETDuet-F	**aaactgcagATGTCACCGCATGACGACGC**
NRPS-ETDuet-R	**aaaaagcttCTACTTGGCGGGCATTGCCG**
TE3-ACYCDuet-F	**aaactgcagATGACGGACGAAGCCCTGCT**
TE3-ACYCDuet-R	**aaaaagcttTCAGGGGACGTTGACGAAGG**
Duet-sco101-F	**aaagaattcgATGTCACCGCATGACGACGC**
Duet-sco101-R	**tataagcttCTACTTGGCGGGCATTGCCG**
pET24b-sco100-F	**aaacataATGCCTGCTCCCCTCACGCT**
pET24b-sco100-R	**aaactcgagTCATGCGGTGACATGGCCCG**

Duet-sco99-F	**aaaggatccgATGGACCGGCTCCACCACCC**
Duet-sco99-R	**aaactgcagTCAGTTGACCTTGCGTGCGG**
Duet-sco98-F	**aaaggatccgcatATGACGGACGAAGCCCTGCT**
Duet-sco98-R	**tataagcttcaattgTCAGGGGACGTTGACGAAGG**
Duet-sco97-F	**aaaggatccgATGCAGATCGACGAACAGCC**
Duet-sco97-R	**tataagcttTCATGCCGCCTGGATCCCGT**
pMSG360 Hyg-F	**ATCTGGATCCACGAAGCTTC**
pMSG360 Hyg-R	**GGGGATCCTCTAGAGTCCTG**
pMSG360 vector-F	**GGGTACCTGAGAGCCTTCAA**
pMSG360 vector-R	**ATCCTGCAGGAATTCCTCGA**
MmaE KO-F	**ggatttcggacaggactctagaggatccccGAGGCCGTCCCGTGCTTTGC**
MmaE KO-R	**gctgactgggttgaaggctctcaggtacccACGCTCAACGTGAAAGGCGA**
MmaA KO-F	**accgtacgtctcgaggaattcctgcaggatCTAGGCCAGTGCGGGTCTTG**
MmaA KO-F	**gggccaccatgaagcttcgtggatccagatCGCGGTCCCTGATCAACTGC**