Supporting Information for: The molecular features of non-peptidic nucleophilic substrates and acceptor proteins determine the efficiency of sortagging.

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Contents:

Cmpd	Proteins												Software				
	EGF-Srt	TNFa-Srt	TNFa-GS-Srt	CAHII-SRT-6H	CAHII-GS-SRT-6H	CAHII-PAS-SRT-6H	EGF-Srt	TNFa-Srt	TNFa-GS-Srt	CAHII-SRT-6H	CAHII-GS-SRT-6H	CAHII-PAS-SRT-6H	Marvin	ACD/Labs	Schroedinger	MolGpK	Mean
	0.5/1 mM cmpd concentration, product yield, %						0.5/1mM cmpd concentration, hydrolytic side-product content, %						pKa values				
1	100/100	17/21	95/97	0/0	90/91	90/95	0/0	0/0	1/1	0/0	0/0	0/0	8.58	8.02	7.95	7.90	8.11
2	96/100	14/17	86/93	0/0	89/92	79/89	4/0	0/0	2/2	0/0	0/0	3/0	9.28	9.10	8.33	9.60	9.08
3	96/100	16/19	84/91	0/0	90/91	76/88	4/0	0/0	2/2	0/0	0/0	3/0	8.91	9.00	8.30	9.40	8.90
4	96/100	13/16	83/91	0/0	87/92	79/87	4/0	0/0	2/2	0/0	0/0	0/0	9.29	9.45	8.35	9.60	9.17
5	95/94	10/12	74/56	0/0	90/92	82/92	5/6	0/0	4/2	0/0	3/0	2/0	8.19	9.97	8.18	8.10	8.61
6	95/100	15/17	82/91	0/0	88/91	75/86	5/0	0/0	2/2	0/0	0/0	3/0	8.90	9.07	8.30	9.50	8.94
7	96/94	11/15	76/87	0/0	90/93	78/87	4/6	0/0	4/4	0/0	0/0	6/0	8.37	10.27	8.39	8.70	8.93
8	86/97	11/14	80/90	0/0	93/93	72/84	9/3	0/0	3/2	0/0	0/0	6/3	9.16	9.03	9.51	9.20	9.23
9	95/100	12/15	77/87	0/0	88/95	69/90	5/0	0/0	3/3	0/0	0/0	7/0	9.26	9.17	8.34	9.50	9.07
10	93/100	7/9	65/81	0/0	85/87	58/74	7/0	0/0	4/4	0/0	0/0	7/4	9.29	9.19	8.25	8.70	8.86
11	73/83	9/12	54/72	0/0	86/92	60/53	8/10	0/0	6/4	0/0	4/0	11/9	9.30	8.74	9.39	8.50	8.98
12	84/87	6/7	49/63	0/0	80/82	46/58	16/13	1/0	8/7	0/0	12/7	17/14	8.37	10.27	8.39	8.70	8.93
13	53/60	0/2	10/14	0/0	67/ 74	17/ 3	25/27	0/0	8/8	0/0	22/15	21/16	9.30	9.70	9.80	9.80	9.65
14	45/54	2/3	20/28	0/0	36/16	16/27	33/27	1/1	13/11	0/0	46/0	29/25	10.21	10.54	10.10	10.90	10.44
15	0/0	0/0	0/0	0/0	0/0	0/0	58/54	2/2	21/26	0/0	73/76	48/52	9.53	9.90	9.76	9.60	9.70
16	NA/61	NA/0	NA/28	NA/0	NA/20	NA/21	NA/25	NA/0	NA/22	NA/0	NA/23	NA/25	9.16	9.03	9.47	9.50	9.29
17	NA/100	NA/8	NA/60	NA/0	NA/53	NA/44	NA/0	NA/0	NA/7	NA/0	NA/14	NA/7	9.30	8.74	9.39	9.20	9.16

Table S1. Percentage yields of the conjugate products for all combinations of the nucleophiles and acceptor proteins for the sortagging reactions done at 0.5 mM and 1 mM nucleophile concentrations (values separated by slash). Percentage content of the hydrolytic side-products for the same reactions. For compounds **16** and **17** (DOTA derivatives) reactions at 0.5 mM nucleophile were not performed. The pKa values for the protonated nucleophilic amino groups of all substrates calculated by four computational tools, as well as their arithmetic means.

	Conjugate Yield, % at 0.5 mM nucleophile		Conjugate Yield, % at 1 mM nucleophile	
Acceptor Protein	**TNFα-GS-Srt**	**CAHII-GS-SRT-6His**	**TNFα-GS-Srt**	**CAHII-GS-SRT-6His**
Biotin Ethylenediamine, HCl, no TFA	**70**	**28**	**83**	**26**
Biotin Ethylenediamine, HCl, + TFA	**72**	**26**	**84**	**32**

Table S2. Effect of trifluoroacetate on sortagging reactions. Reactions with the 2 acceptor proteins were done in the standard conditions with or without addition of sodium trifluoroacetate in the equimolar concentration to the nucleophilic substrate.

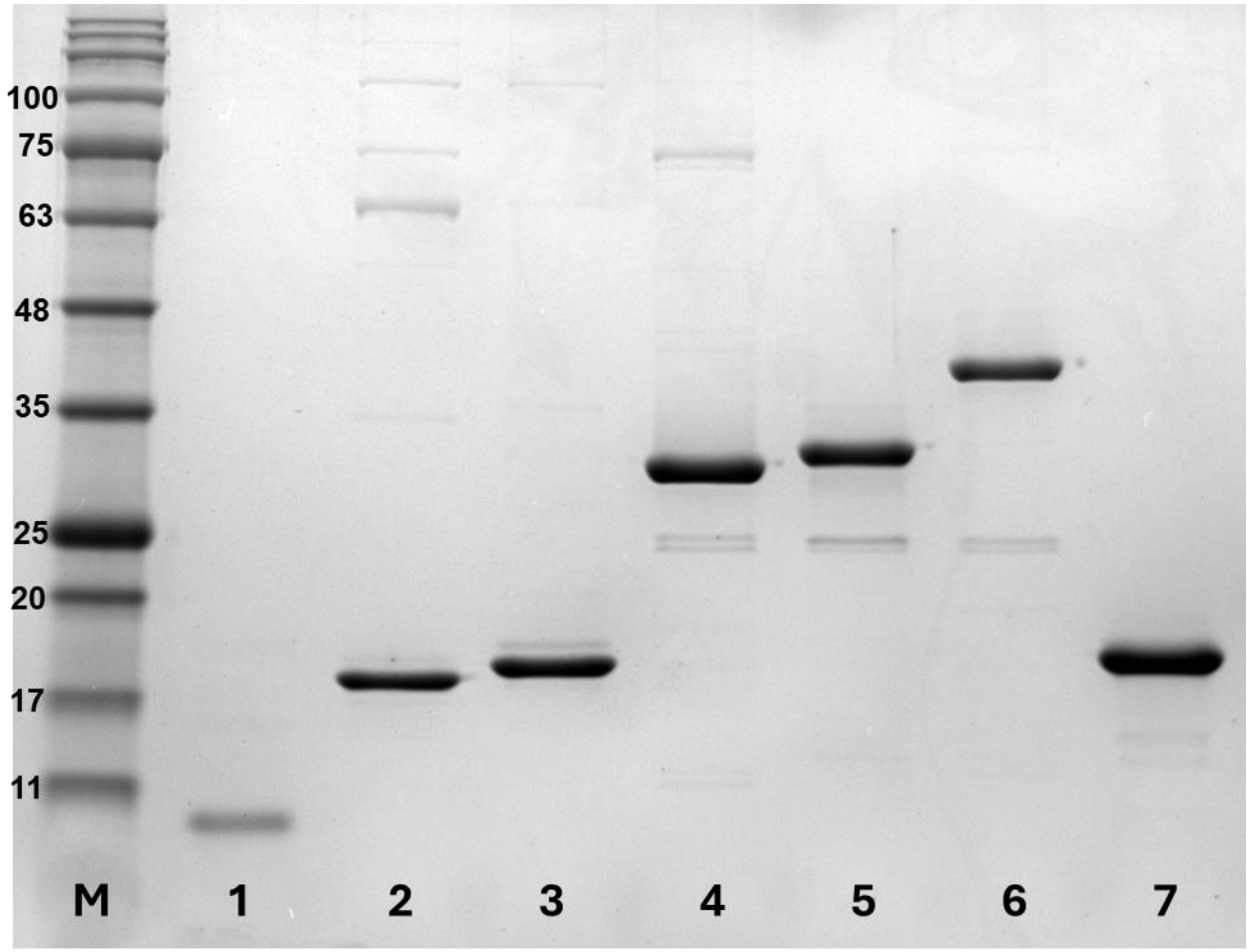


Fig. S1. Gradient SDS-PAAG (7-22%) electrophoresis of the recombinant proteins used in this work, Coomassie stain.
M – molecular weight markers in kD, 1 – EGF-SRT, 2 – TNFα-SRT, 3 – TNFα -GS-SRT, 4 – CAHII-SRT-6H, 5 – CAHII-GS-SRT-6H, 6 – CAHII-PAS-SRT-6H, 7 - SrtA7M.

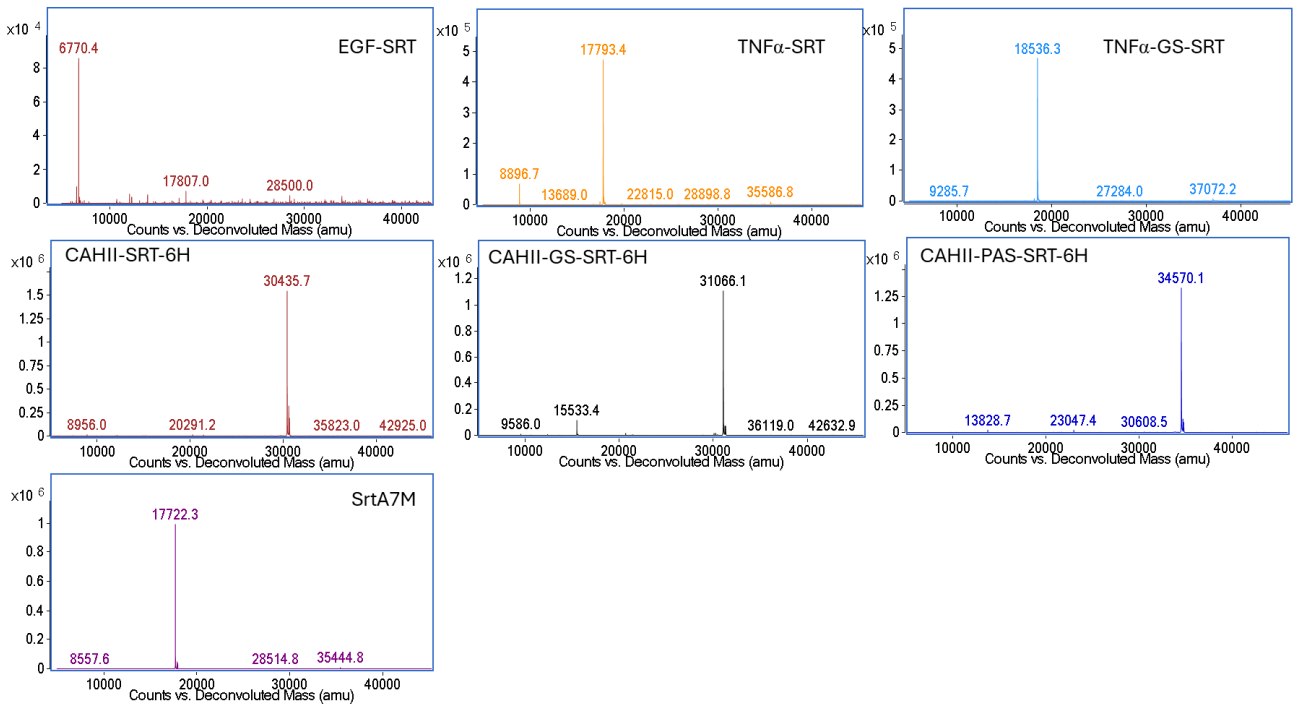


Fig. S2. QTOF LC-MS spectra of the recombinant proteins used in the study.

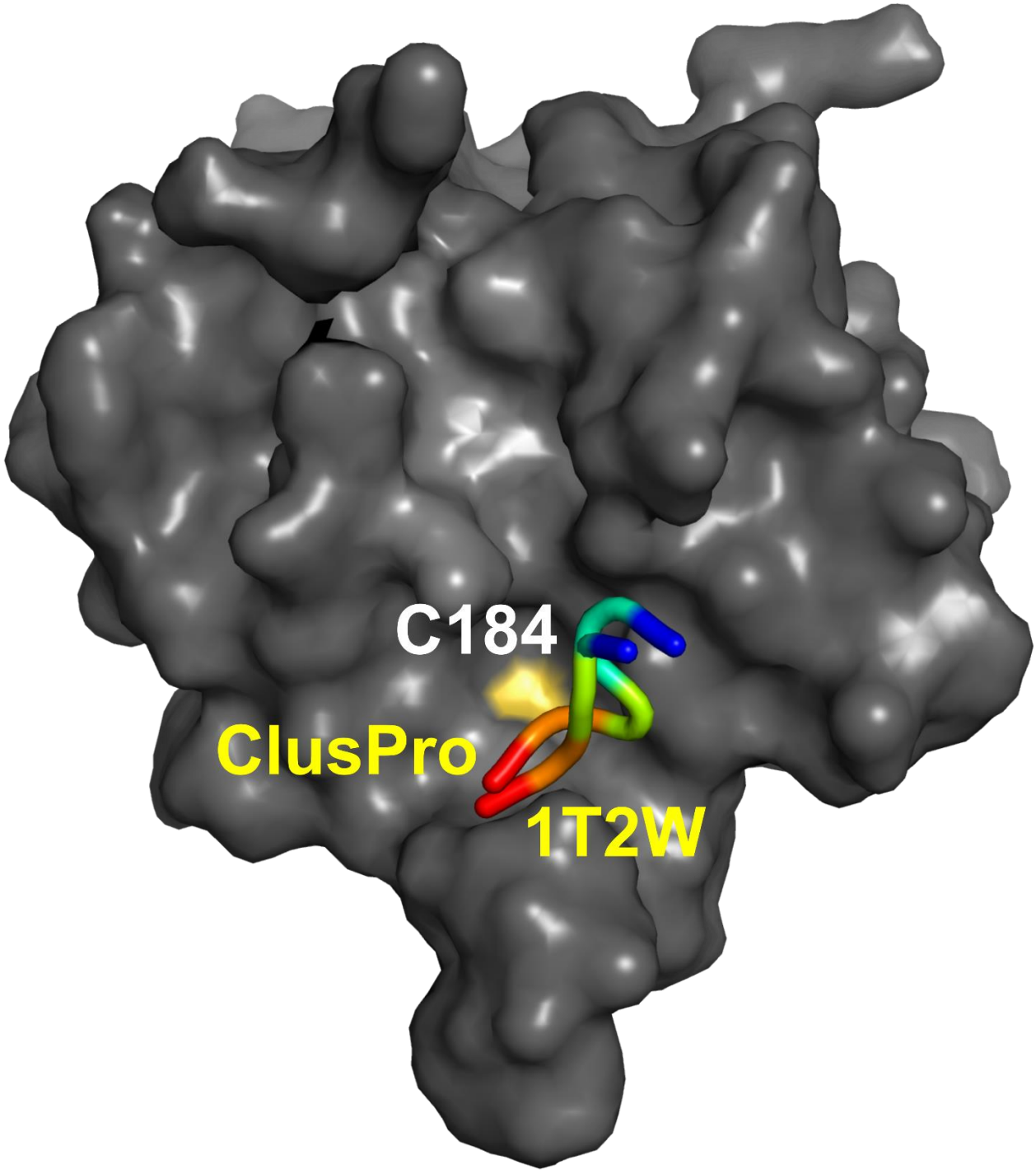
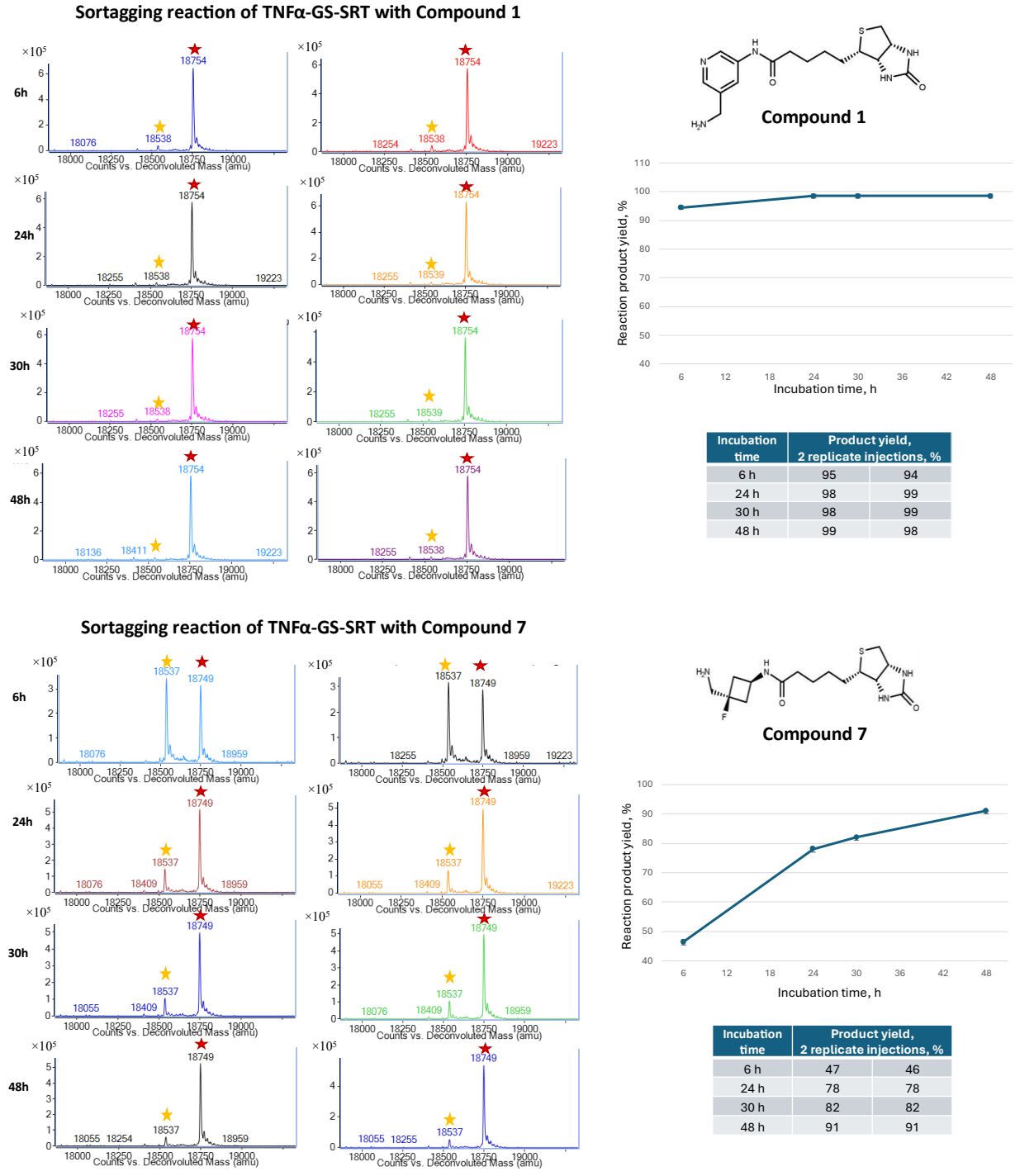


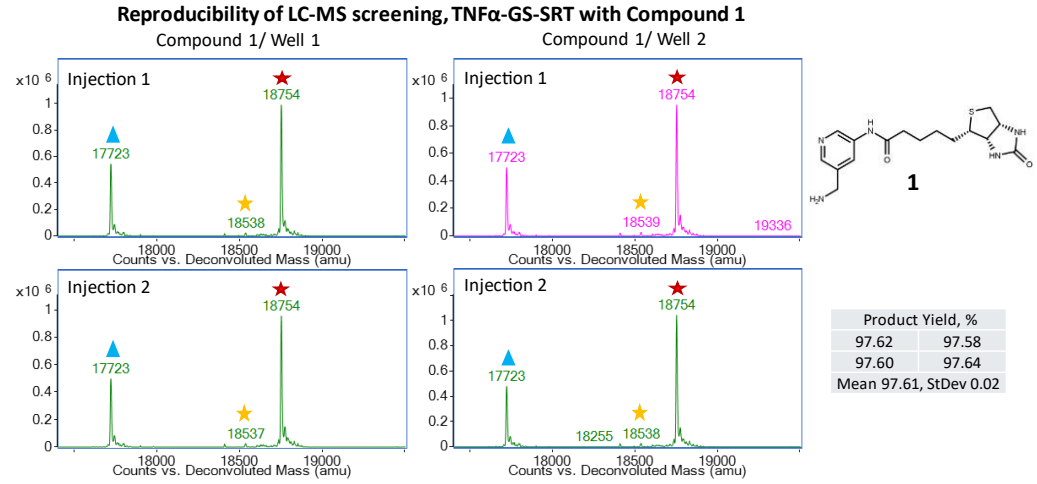
Fig. S3. PyMOL visualization of the superposition of the docked LPETG motifs in PDB entry 1T2W (Crystal Structure of Sortase A in Complex with a LPETG peptide, DOI: https://doi.org/10.2210/pdb1T2W/pdb,) with the LPETG-sequence of ClusPro protein-protein docking model between Sortase A and the I-TASSER model of human Carbonic Anhydrase II (Sortase-hCAHII-2GS-SRT-6H). Sulfur atom in the catalytic Cys184 of the active site is highlighted in yellow.



Incubation time	Product yield, 2 replicate injections, %	
6 h	95	94
24 h	98	99
30 h	98	99
48 h	99	98

Incubation time	Product yield, 2 replicate injections, %	
6 h	47	46
24 h	78	78
30 h	82	82
48 h	91	91

Fig. S4. Sortagging time course experiments for TNFα-GS-SRT - Compound **1** and TNFα-GS-SRT - Compound **7** protein acceptor-nucleophile pairs. Peaks of the initial protein and the conjugate product on mass-spectra are marked by yellow and red stars, correspondingly. Hydrolytic side-product of sortagging was not detectable in these experiments. Calculated product yields in two LC-MS injections for each time point (rounded to the nearest integer) are shown in the tables and their standard deviation values are shown on the graphs. The experiments were done as described in the Materials and Methods section, at 10:1 molar ratio of nucleophile to protein (1 mM:100 μM) and consecutive sampling at 4 time points.



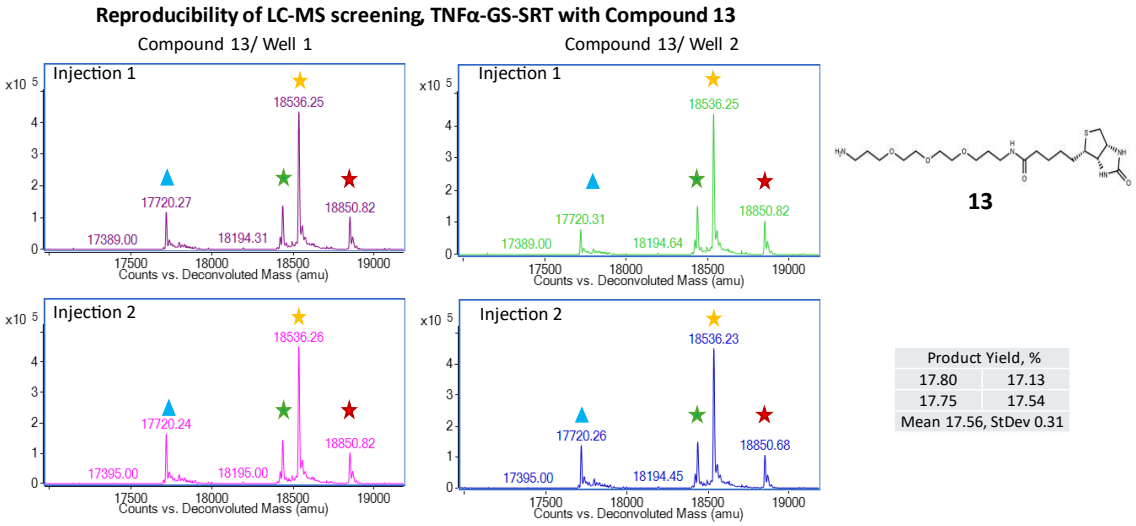
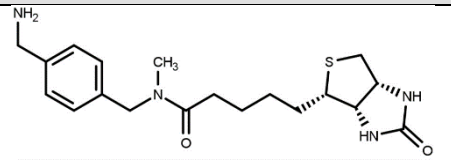
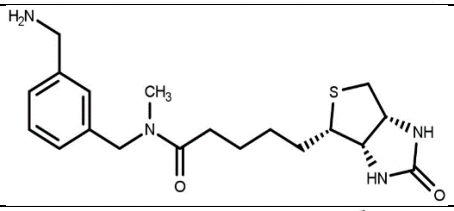
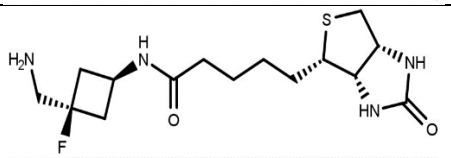
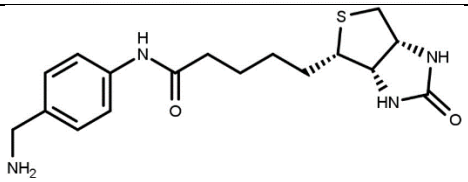
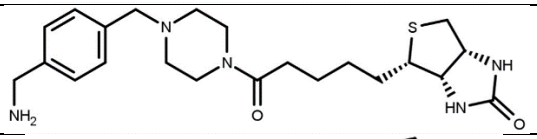
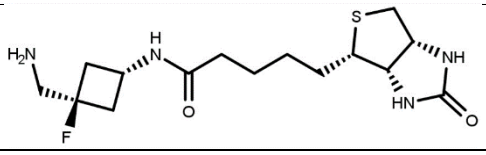


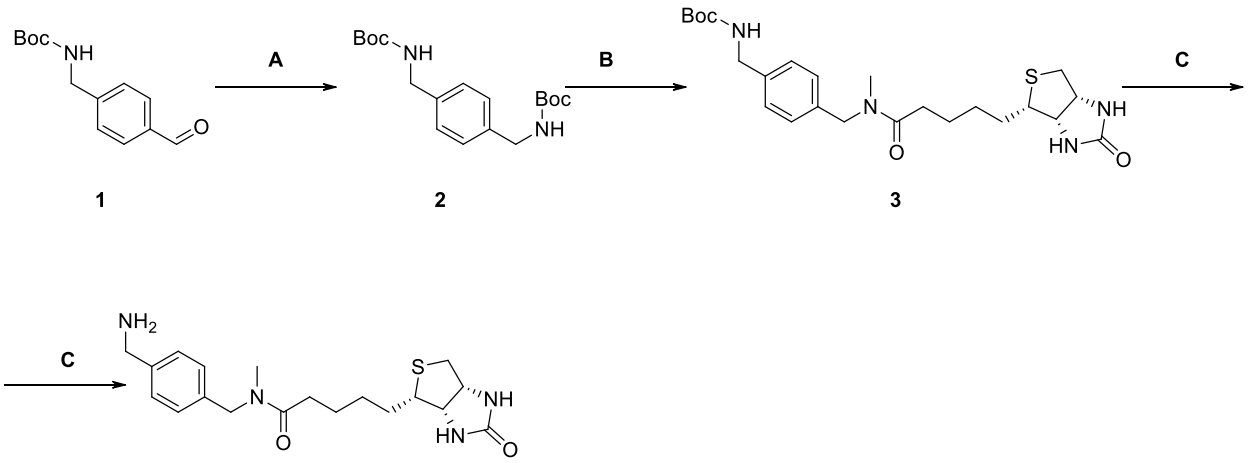
Fig. S5. Reproducibility checks of sortagging experiments analysis for TNFα-GS-SRT - Compound **1** and TNFα-GS-SRT - Compound **13** acceptor-nucleophile pairs. Peaks of the initial protein, the conjugate product and hydrolytic side-product on mass-spectra are marked by yellow, red and green stars, correspondingly. Peaks of SrtA7M are marked by blue triangles. Calculated product yields in two separate reactions with two consecutive LC-MS system injections for each reaction are shown in the tables. The experiments were done as described in the Materials and Methods section, at 10:1 molar ratio of nucleophile to protein (1 mM:100 µM) and 24 h incubation.

List of compounds

No.	Structure	Purity, %	Comments
compound 2		95	HCl salt
compound 6		95	HCl salt
compound 7		90	HCl salt
compound 9		91	
compound 10		91	HCl salt
compound 12		90	HCl salt

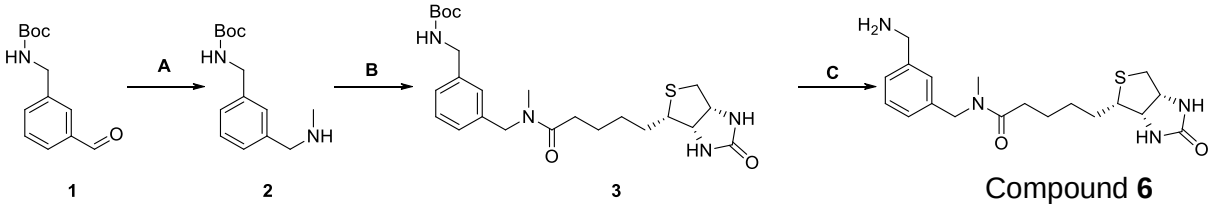
Synthesis Synthesis was carried out following the scheme given below:

Scheme 1



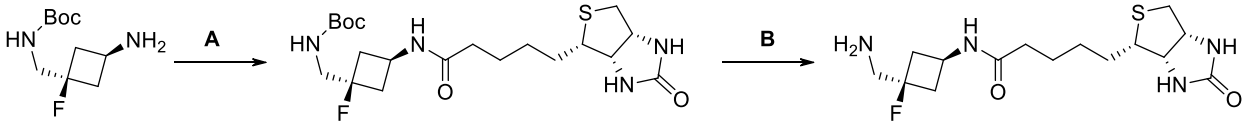
Compound **2**

Scheme 2



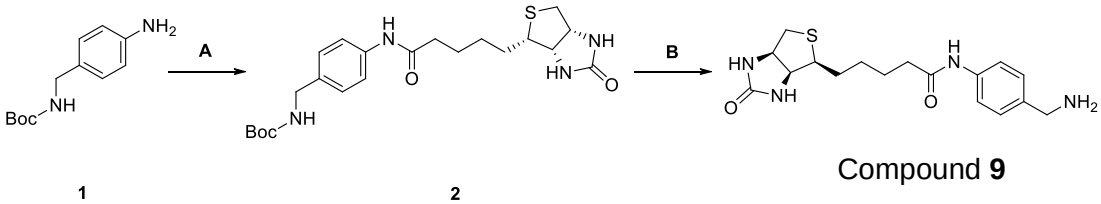
Compound **6**

Scheme 3



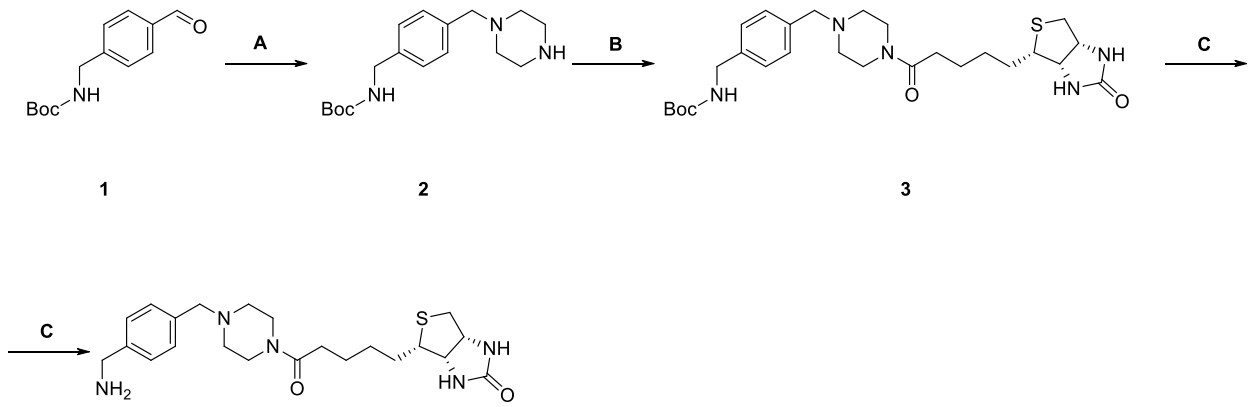
Compound **7**

Scheme 4



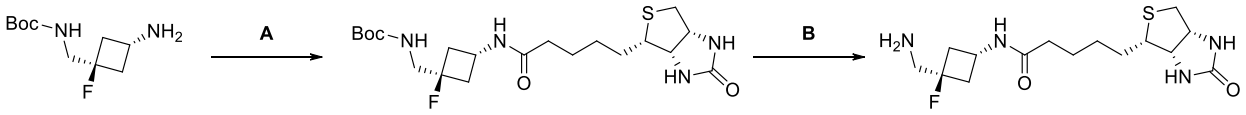
Compound **9**

Scheme 5



Compound **10**

Scheme 6



Compound **12**

Scheme 1 (compound **2)**

Step A

Aldehyde **1**(0.4 g, 1.7 mmol, 1 eq.) was dissolved in $MeNH_2$ (20% in MeOH, 20 ml) and the resulting mixture was stirred at room temperature overnight. Then $NaBH_4$ (0.07 g, 1.7 mmol, 1 eq.) was added portion wise and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with K_2CO_3 (50 mL), extracted with EtOAc (3*40 mL), washed with brine (3*20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude amine **2**(0.29 g, 93% purity, 1.16 mmol, 63% yield) obtained as yellow oil was used without further purification.

Step B

EDC (0.22 g, 1.74 mmol, 1.5 eq.) was added to a mixture of biotin (0.28 g, 1.16 mmol, 1 eq.), DIPEA (0.42 g, 3.48 mmol, 0.56 ml, 3 eq.) and HOBt (0.39 g, 2.9 mmol, 2.5 eq.) in DMF and the resulting mixture was stirred at room temperature for 30 min. Amine **2**(0.29 g, 1.16 mmol, 1 eq.) was then added slowly and reaction mixture was stirred at room temperature further 12 h. The reaction mixture was diluted with EtOAc (120 mL), washed with $NaHSO_4$ aq. (25 mL), $NaHCO_3$ aq. (25 mL), brine (3*20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude Boc-amine **3** (0.43 g, 63% purity, 0.9 mmol, 49% yield) obtained as brown oil was used without further purification.

Step C

Boc-amine **3** (0.43 g, 0.9 mmol) was treated with 3M HCl (dioxane solution, 10 ml) and resulting mixture was stirred at room temperature overnight. After consumption of the starting material (LCMS control) the resulting mixture was concentrated under reduced pressure to dryness. The residue obtained was subjected for prep HPLC purification (0-0-25-100% 0-2-7-7.1min; 30ml/min water+HCl-

MeCN (loading pump 4ml/min water); target mass 377 column PFP 5uM 19*100mm(R)) to afford compound 2 (0.058 g, 95% purity, 0.14 mmol, 15% yield) as light brown oil.

Scheme 2 (compound **6)**

Step A
Aldehyde **1**(0.4 g, 1.7 mmol, 1 eq.) was dissolved in $MeNH_2$ (20% in MeOH, 20 ml) and the resulting mixture was stirred at room temperature overnight. Then $NaBH_4$ (0.07 g, 1.7 mmol. 1 eq.) was added portion wise and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with K_2CO_3 (50 mL) and extracted with EtOAc (3*40 mL), washed with brine (20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude amine **2**(0.38 g, 82% purity, 1.52 mmol, 73% yield) obtained as yellow oil was used without further purification.

Step B
EDC (0.29 g, 2.28 mmol, 1.5 eq.) was added to a mixture of biotin (0.56 g, 1.52 mmol, 1 eq.), DIPEA (0.56 g, 4.56 mmol, 0.73 ml, 3 eq.) and HOBt (0.51 g, 3.8 mmol, 2.5 eq.) in DMF and the resulting mixture was stirred at room temperature for 30 min. Amine **2**(0.38 g, 1.52 mmol, 1 eq.) was then added slowly and reaction mixture was stirred at room temperature further 12 h. The reaction mixture was diluted with EtOAc (120 mL), washed with $NaHSO_4$ aq. (25 mL), $NaHCO_3$ aq. (25 mL), brine (3*20 mL) and dried over Na_2SO_4. The crude Boc-amine **3** (0.61 g, 37% purity, 1.28 mmol, 31% yield) obtained as yellow oil was used without further purification.

Step C
Boc-amine **3** (0.61 g, 1.28 mmol) was treated with 3M HCl (dioxane solution, 10 ml) and resulting mixture was stirred at room temperature overnight. After consumption of the starting material (LCMS control) the resulting mixture was concentrated under reduced pressure to dryness. The residue obtained was subjected for prep HPLC purification (0-0-25-100% 0-2-7-7.1min; 30ml/min water+HCl-MeCN (loading pump 4ml/min water); target mass 377 column Chromatorex C18 5uM 19*100mm(R)) to afford compound 6 (0.087 g, 95% purity, 0.21 mmol, 16% yield) as brown solid.

Scheme 3 (compound 7**)**

Step A
EDC (0.22 g,1.71 mmol, 1.5 eq.) was added to a mixture of biotin (0.27 g, 1.14 mmol, 1 eq.), DIPEA (0.42 g, 3.42 mmol, 0.55 ml, 3 eq.) and HOBt (0.38 g, 2.85 mmol, 2.5 eq.) in DMF and the resulting mixture was stirred at room temperature for 30 min. Amine **1**(0.25 g, 1.14 mmol, 1 eq.) was then added slowly and reaction mixture was stirred at room temperature further 12 h. The reaction mixture was diluted with EtOAc (120 mL), washed with $NaHSO_4$ aq. (25 mL), $NaHCO_3$ aq. (25 mL), brine (3*20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude Boc-amine **2** (0.5 g, 15% purity, 1.12 mmol, 14% yield) obtained as yellow oil was used without further purification.

Step B
Boc-amine **2** (0.5 g, 1.12 mmol) was treated with 3M HCl (dioxane solution, 10 ml) and resulting mixture was stirred at room temperature overnight. After consumption of the starting material (LCMS control) the resulting mixture was concentrated under reduced pressure to dryness. The residue obtained was subjected for prep HPLC purification (0-0-25-100% 0-2-7-7.1min; 30ml/min water+HCl-MeCN (loading pump 4ml/min water); target mass 345 column Chromatorex C18 5uM 19*100mm(R)) to afford compound 7 (0.051 g, 90% purity, 0.13 mmol, 10% yield) as pale-yellow oil.

Scheme 4 (compound **9)**

Step A
HATU (1 g, 2.7 mmol, 1.5 eq.) was added portion wise to a mixture of biotin (0.43 g, 1.8 mmol, 1 eq.) and DIPEA (0.66 g, 5.4 mmol, 0.87 ml, 3 eq.) in DMF and the resulting mixture was stirred at

room temperature for 30 min. Amine **1**(0.4 g, 1.8 mmol, 1 eq.) was then added slowly and reaction mixture was stirred at room temperature further 12 h. The reaction mixture was diluted with EtOAc (120 mL), washed with $NaHSO_4$ aq. (25 mL), $NaHCO_3$ aq. (25 mL), brine (3*20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude Boc-amine **2** (0.53 g, 28% purity, 1.18 mmol, 18% yield) obtained as yellow oil was used without further purification.

Step B
Boc-amine **2** (0.53 g, 1.18 mmol) was treated with 3M HCl (dioxane solution, 10 ml) and resulting mixture was stirred at room temperature overnight. After consumption of the starting material (LCMS control) the resulting mixture was concentrated under reduced pressure to dryness. The residue obtained was subjected for prep HPLC purification (3-10-40-100% 0-2-7-7.1 30ml/min water-MeOH (loading pump 4ml/min water); target mass 349; column SunFireC18 19*100mm (L)) to afford compound 9 (0.096 g, 91% purity, 0.27 mmol, 21% yield) as brown solid.

Scheme 5 (compound **10)**

Step A
Piperazine (1.02 g, 11.91 mmol, 7 eq.) was added to mixture of aldehyde **1**(0.4 g, 1.7 mmol, 1 eq.) in methanol (15 ml) and the resulting mixture was stirred at room temperature overnight. Then $NaBH_4$ (0.07 g, 1.7 mmol, 1 eq.) was added portion wise and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with K_2CO_3 (50 mL) and extracted with EtOAc (3*40 mL), washed with brine (20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude amine **2**(0.19 g, 94% purity, 0.62 mmol, 34% yield) obtained as colorless oil was used without further purification.

Step B
EDC (0.12 g, 0.93 mmol, 1.5 eq.) was added to a mixture of biotin (0.15 g, 0.62 mmol, 1 eq.), DIPEA (0.22 g, 1.86 mmol, 0.3 ml, 3 eq.) and HOBt (0.2 g, 1.55 mmol, 2.5 eq.) in DMF and the resulting mixture was stirred at room temperature for 30 min. Amine **2**(0.19 g, 0.62 mmol, 1 eq.) was then added slowly and reaction mixture was stirred at room temperature further 12 h. The reaction mixture was diluted with EtOAc (120 mL), washed with $NaHSO_4$ aq. (25 mL), $NaHCO_3$ aq. (25 mL), brine (3*20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude Boc-amine **3** (0.22 g, 50% purity, 0.44 mmol, 33% yield) obtained as brown oil was used without further purification.

Step C
Boc-amine **3** (0.22 g, 0.44 mmol) was treated with 3M HCl (dioxane solution, 10 ml) and resulting mixture was stirred at room temperature overnight. After consumption of the starting material (LCMS control) the resulting mixture was concentrated under reduced pressure to dryness. The residue obtained was subjected for prep HPLC purification (0-0-40-100% 0-2-7-7.1 min; 30ml/min water-MeOH+HCl (loading pump 4 ml/min water); target mass 432 column Chromatorex C18 5uM 19*100 uM) to afford compound 10 (0.036 g, 91% purity, 0.07 mmol, 16% yield) as brown oil.

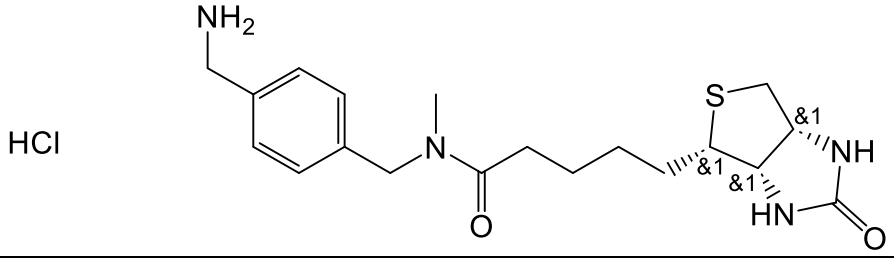
Scheme 6 (compound **12)**

Step A
EDC (0.22 g,1.71 mmol, 1.5 eq.) was added to a mixture of biotin (0.27 g, 1.14 mmol, 1 eq.), DIPEA (0.42 g, 3.42 mmol, 0.55 ml, 3 eq.) and HOBt (0.38 g, 2.85 mmol, 2.5 eq.) in DMF and the resulting mixture was stirred at room temperature for 30 min. Amine **1**(0.25 g, 1.14 mmol, 1 eq.) was then added slowly and reaction mixture was stirred at room temperature further 12 h. The reaction mixture was diluted with EtOAc (120 mL), washed with $NaHSO_4$ aq. (25 mL), $NaHCO_3$ aq. (25 mL), brine (3*20 mL) and dried over Na_2SO_4. The combined organic extract was concentrated under reduced pressure to dryness. The crude Boc-amine **2** (0.32 g, 8% purity, 0.72 mmol, 5% yield) obtained as brown oil was used without further purification.

Step B

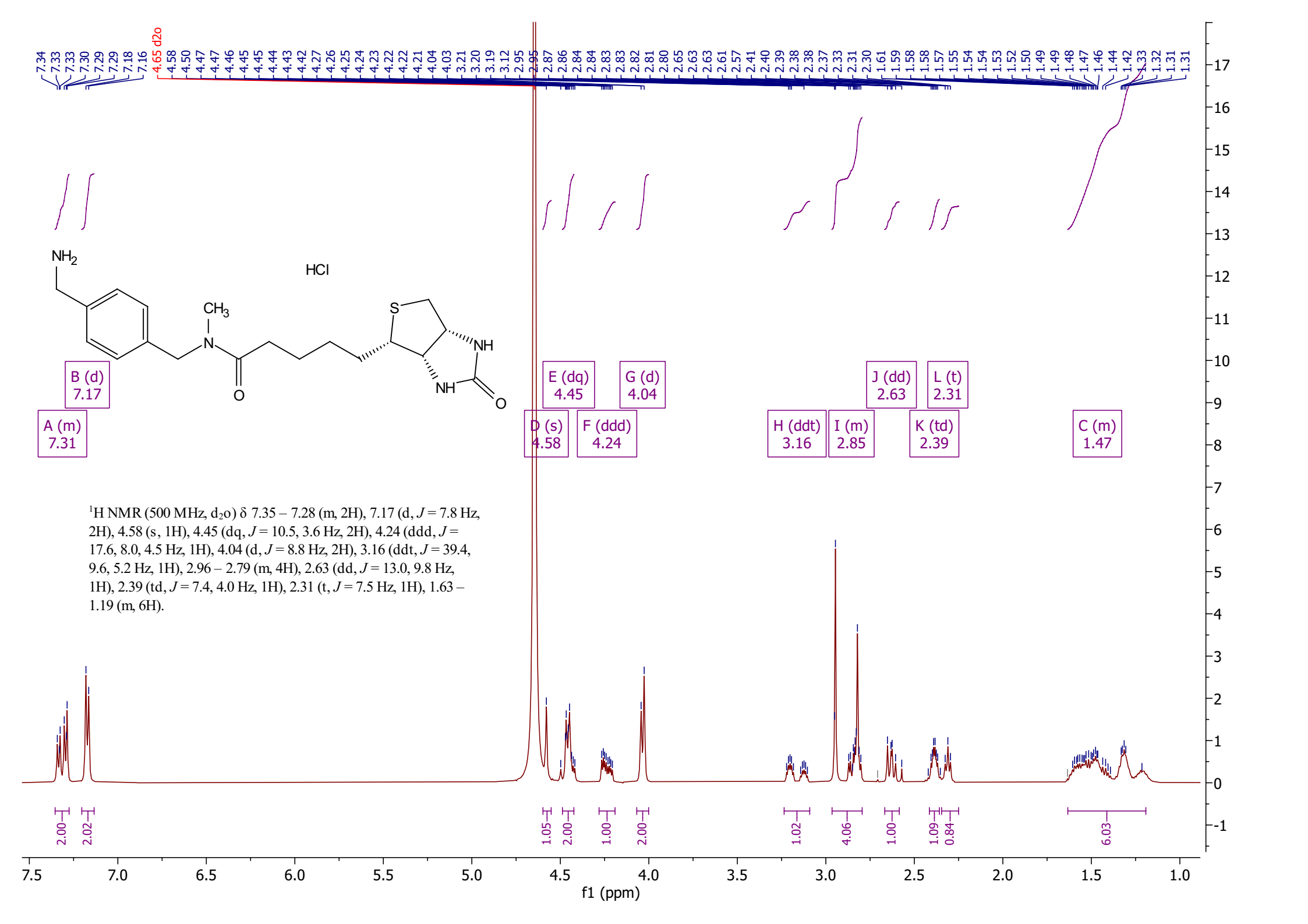
Boc-amine **2** (0.32 g, 0.72 mmol) was treated with 3M HCl (dioxane solution, 10 ml) and resulting mixture was stirred at room temperature overnight. After consumption of the starting material (LCMS control) the resulting mixture was concentrated under reduced pressure to dryness. The residue obtained was subjected for prep HPLC purification (0-0-25-100% 0-2-7-7.1min; 30ml/min water+HCl-MeCN (loading pump 4ml/min water); target mass 345 column Chromatorex C18 5uM 19*100mm(R)) to afford compound 12 (0.061 g, 90% purity, 0.16 mmol, 20% yield) as pale-yellow solid.

Compound 2

Mol Structure	
Mol Formula	$C_{19}H_{29}ClN_4O_2S$
Compound Name	Z8797397995
Mol. Wt.	412.98
Date Synthesized	December 05, 2023

Analytical Tests

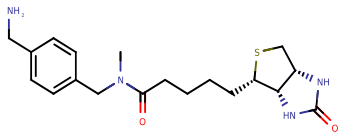
Analytical Tests	Results
LCMS	$[M-H]^-$: 375.0
LCMS (>95%)	Rt= 0.618 min, 100% (215nm)
1H-NMR	Consistent with structure



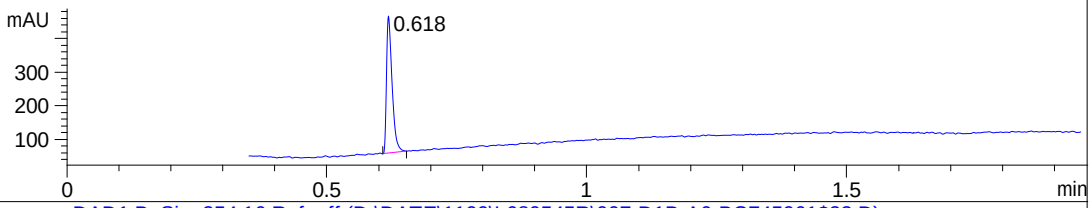
^{1}H NMR (500 MHz, d_2o) δ 7.35 – 7.28 (m, 2H), 7.17 (d, J = 7.8 Hz, 2H), 4.58 (s, 1H), 4.45 (dq, J = 10.5, 3.6 Hz, 2H), 4.24 (ddd, J = 17.6, 8.0, 4.5 Hz, 1H), 4.04 (d, J = 8.8 Hz, 2H), 3.16 (ddt, J = 39.4, 9.6, 5.2 Hz, 1H), 2.96 – 2.79 (m, 4H), 2.63 (dd, J = 13.0, 9.8 Hz, 1H), 2.39 (td, J = 7.4, 4.0 Hz, 1H), 2.31 (t, J = 7.5 Hz, 1H), 1.63 – 1.19 (m, 6H).

MaxPeak: 100.00%
Ret_Time: 0.618 min

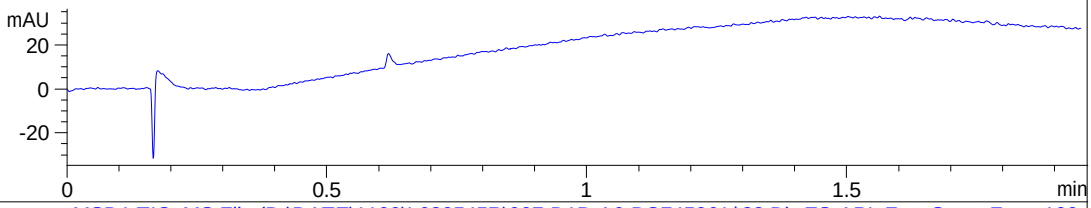
BC745961$22



DAD1 A, Sig=215,16 Ref=off (D:\DATE\1109\L680545R\007-D1B-A6-BC745961$22.D)



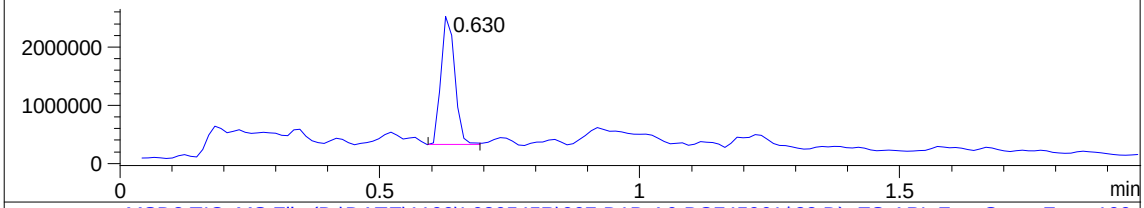
DAD1 B, Sig=254,16 Ref=off (D:\DATE\1109\L680545R\007-D1B-A6-BC745961$22.D)



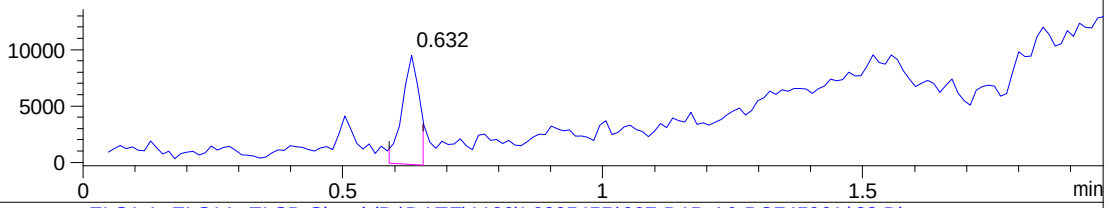
Mol Wt 412.98
Exact Mass 376.23

#	Time	Area%
1	0.618	100.00

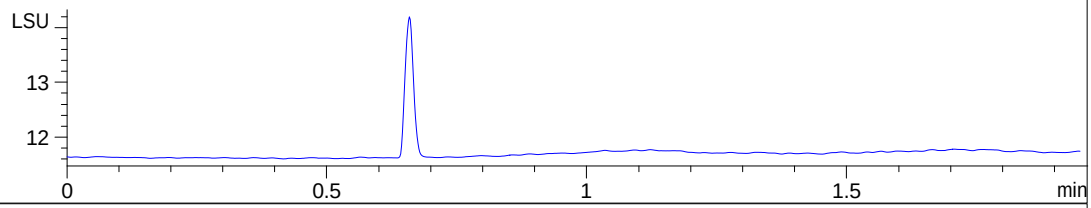
MSD1 TIC, MS File (D:\DATE\1109\L680545R\007-D1B-A6-BC745961$22.D) ES-API, Fast Scan, Frag: 100,



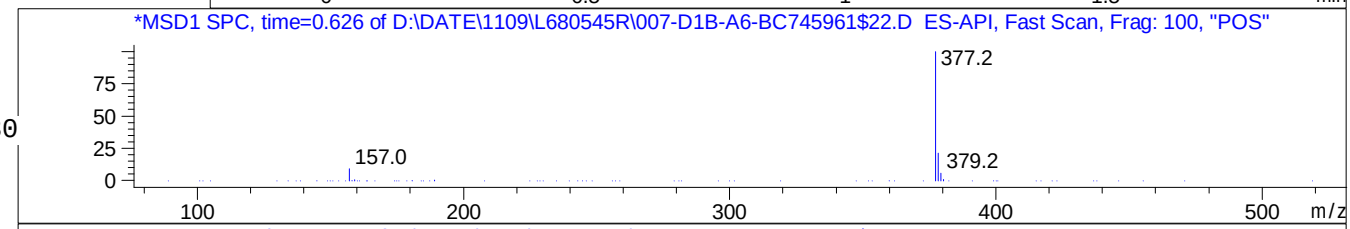
MSD2 TIC, MS File (D:\DATE\1109\L680545R\007-D1B-A6-BC745961$22.D) ES-API, Fast Scan, Frag: 100,



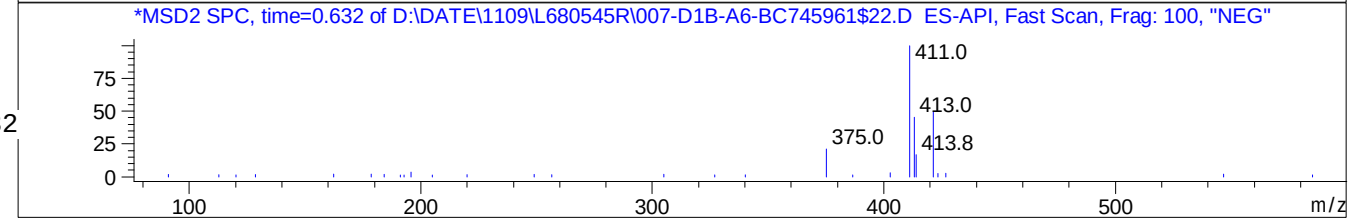
ELS1 A, ELS1A, ELSD Signal (D:\DATE\1109\L680545R\007-D1B-A6-BC745961$22.D)



RT 0.630

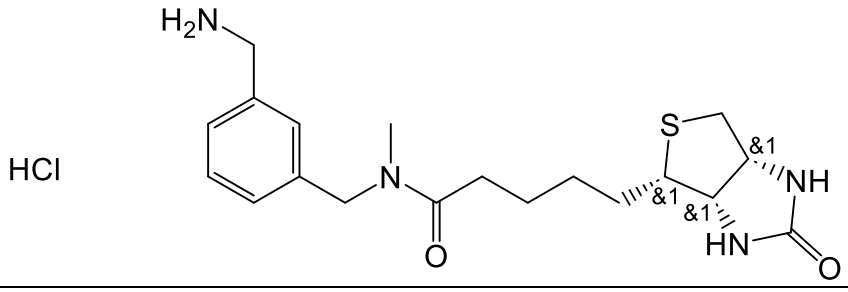


RT 0.632



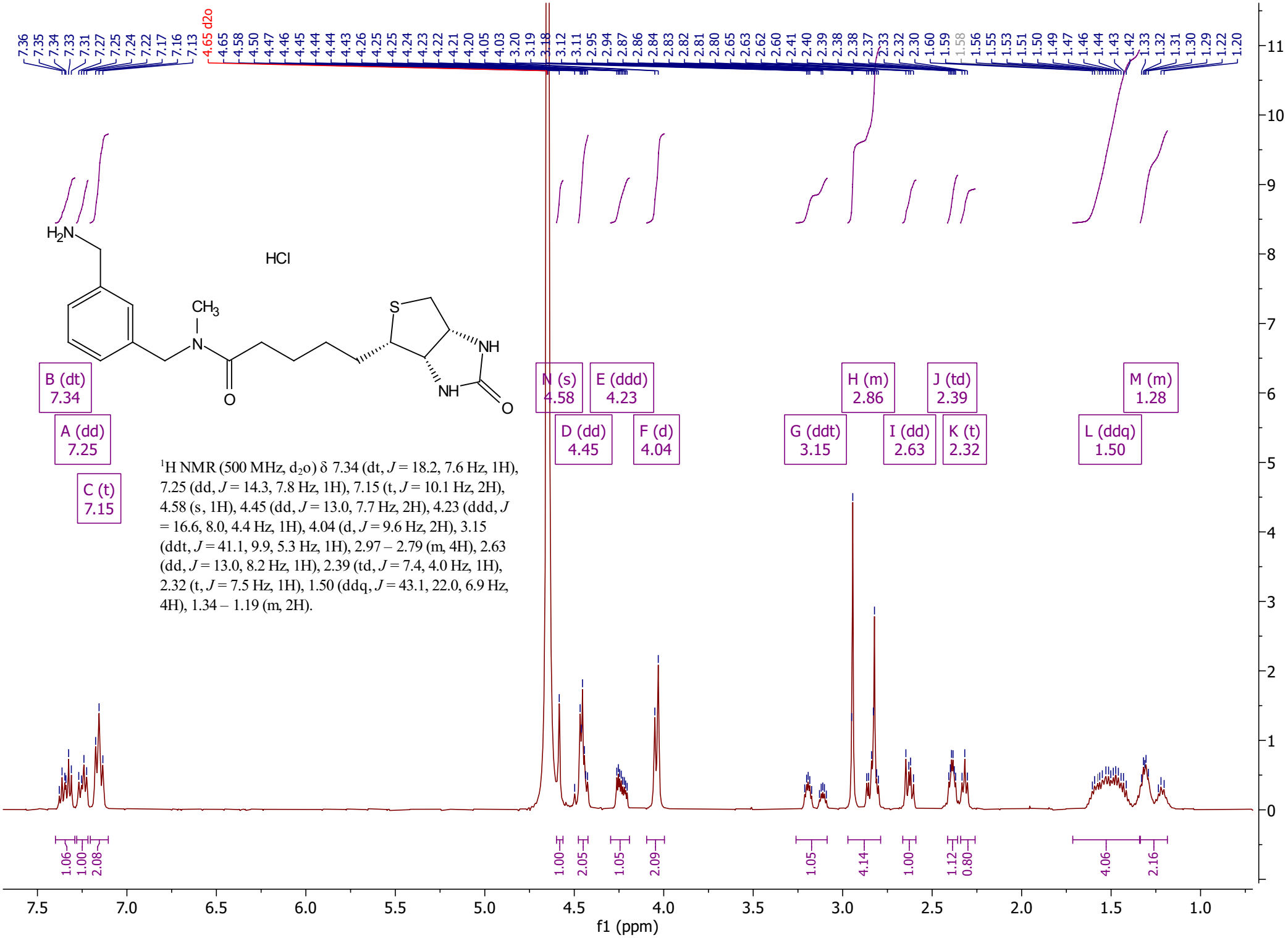
Inj.Date 11/9/2023 CH 21 Acq. Method C:\Users\ -> ->

Compound 6

Mol Structure	
Mol Formula	$C_{19}H_{29}ClN_4O_2S$
Compound Name	Z8797397992
Mol. Wt.	412.98
Date Synthesized	November 14, 2023

Analytical Tests

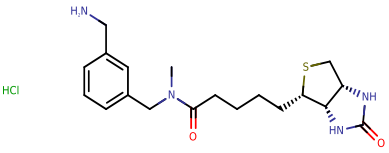
Analytical Tests	Results
LCMS	$[M-H]^-$: 375.0
LCMS (>95%)	Rt= 0.624 min, 100% (215nm)
1H-NMR	Consistent with structure



^{1}H NMR (500 MHz, d_2o) δ 7.34 (dt, *J* = 18.2, 7.6 Hz, 1H), 7.25 (dd, *J* = 14.3, 7.8 Hz, 1H), 7.15 (t, *J* = 10.1 Hz, 2H), 4.58 (s, 1H), 4.45 (dd, *J* = 13.0, 7.7 Hz, 2H), 4.23 (ddd, *J* = 16.6, 8.0, 4.4 Hz, 1H), 4.04 (d, *J* = 9.6 Hz, 2H), 3.15 (ddt, *J* = 41.1, 9.9, 5.3 Hz, 1H), 2.97 – 2.79 (m, 4H), 2.63 (dd, *J* = 13.0, 8.2 Hz, 1H), 2.39 (td, *J* = 7.4, 4.0 Hz, 1H), 2.32 (t, *J* = 7.5 Hz, 1H), 1.50 (ddq, *J* = 43.1, 22.0, 6.9 Hz, 4H), 1.34 – 1.19 (m, 2H).

MaxPeak: 100.00%
Ret_Time: 0.624 min

BC754326$2



Mol Wt 412.98

Exact Mass 376.23

#	Time	Area%
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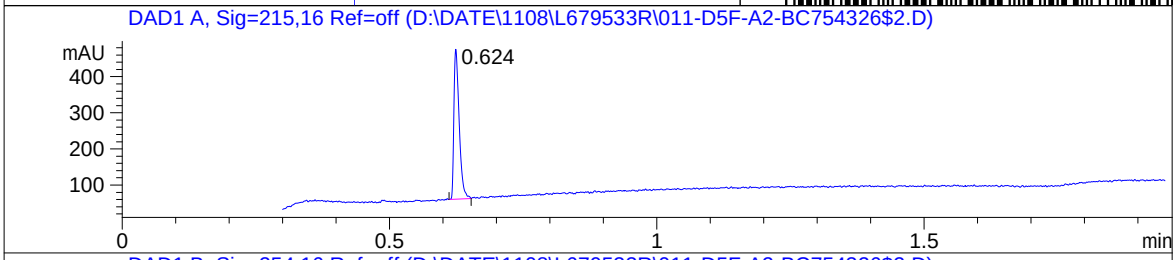
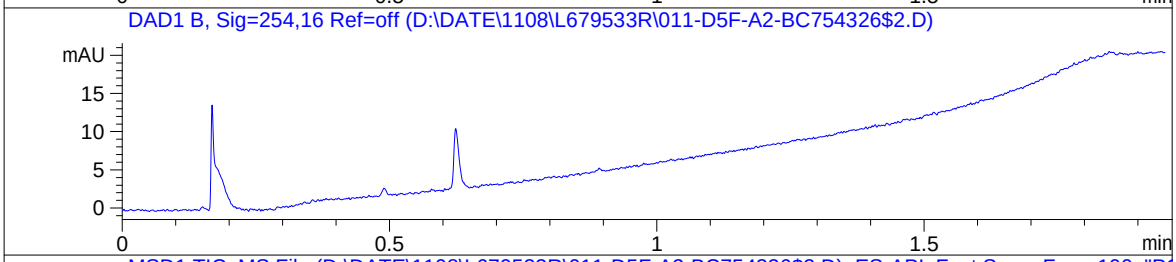
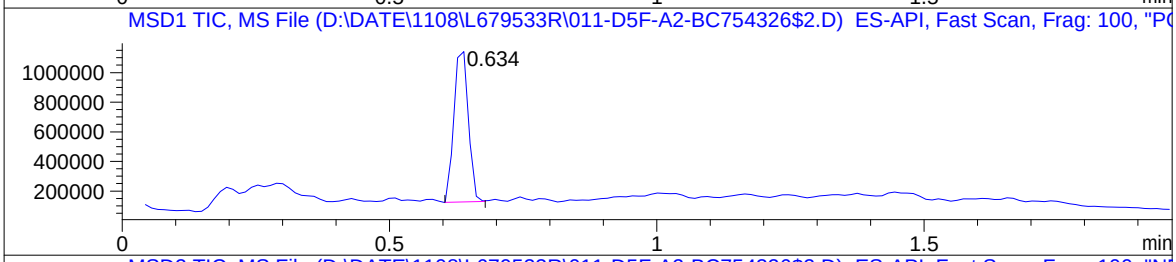
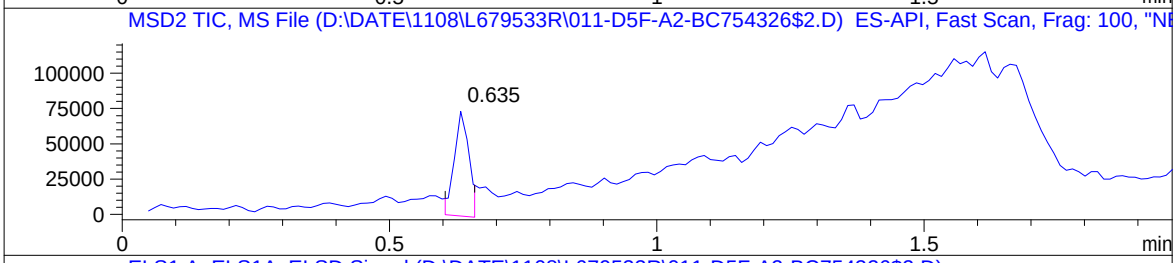
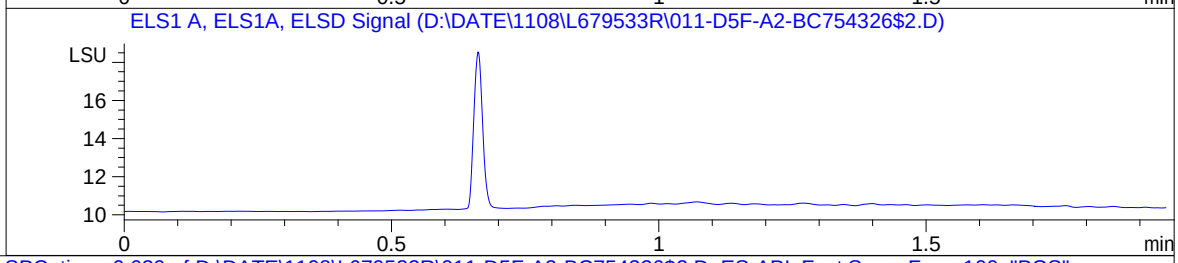


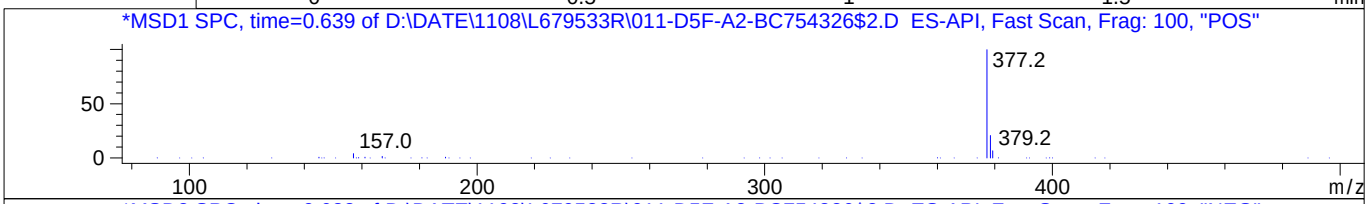




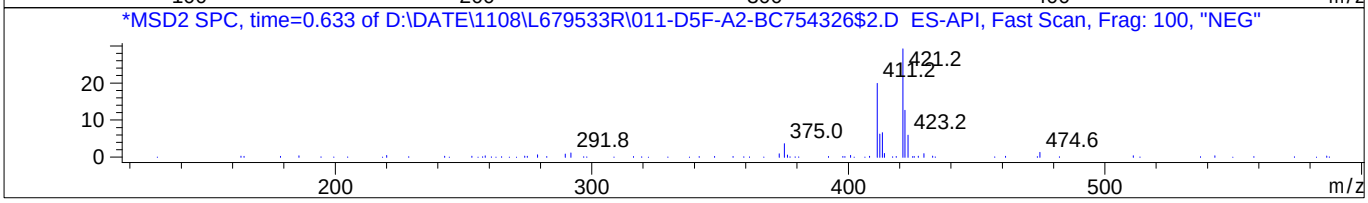




RT 0.634

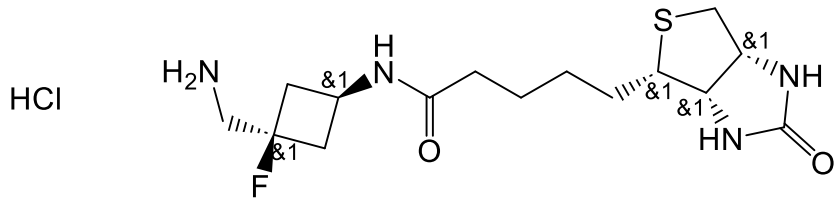


RT 0.635



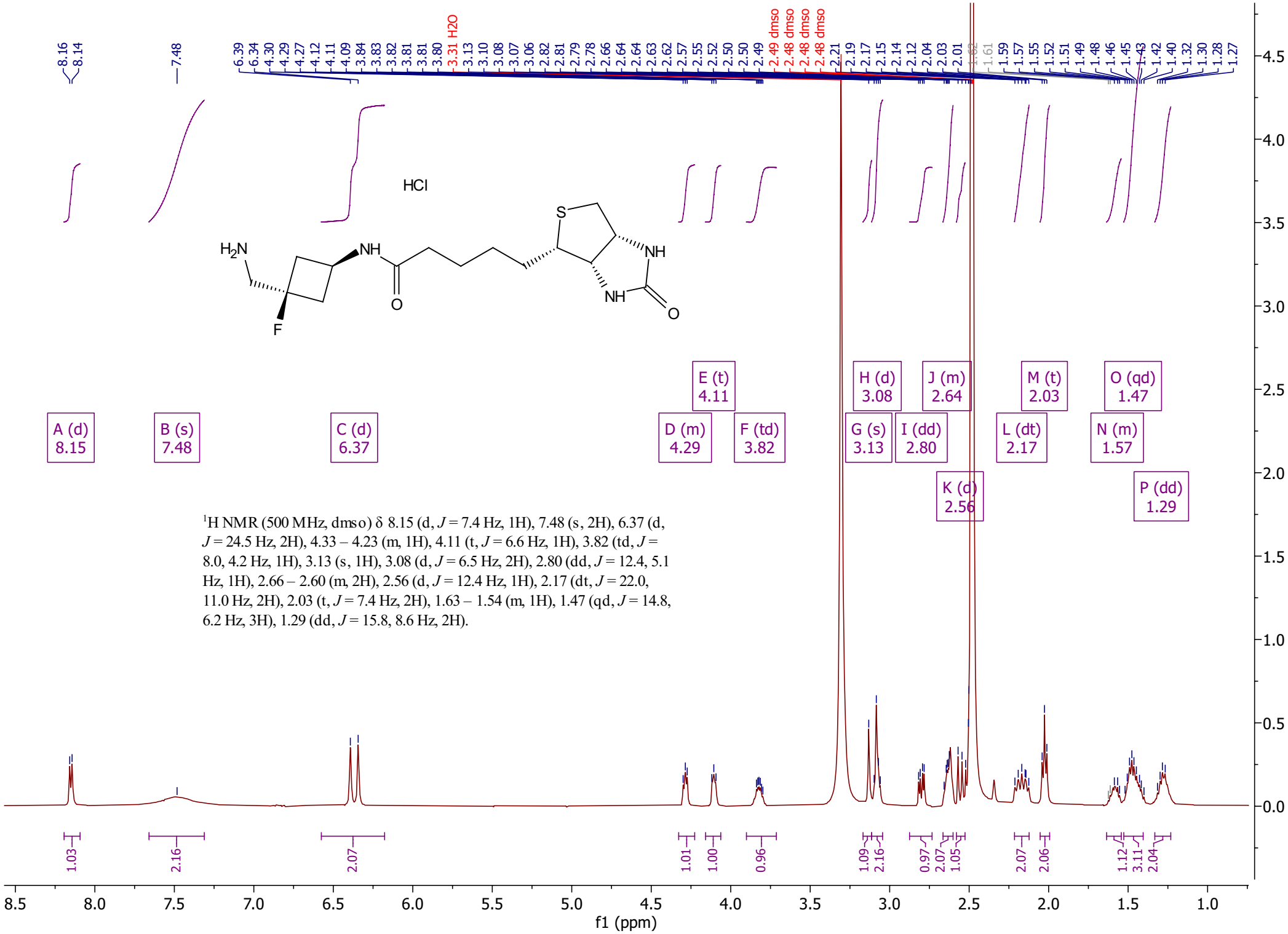
Inj.Date 11/8/2023 E Acq. Method C:\Users\ -> ->

Compound 7

Mol Structure	
Mol Formula	$C_{15}H_{26}ClFN_4O_2S$
Compound Name	Z3699935772
Mol. Wt.	380.91
Date Synthesized	November 09, 2023

Analytical Tests

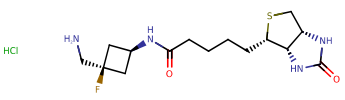
Analytical Tests	Results
LCMS	[M+H]+: 345.0
LCMS (>95%)	Rt= 0.685 min, 96.37% (215nm)
1H-NMR	Consistent with structure



^{1}H NMR (500 MHz, dmso) δ 8.15 (d, *J* = 7.4 Hz, 1H), 7.48 (s, 2H), 6.37 (d, *J* = 24.5 Hz, 2H), 4.33 – 4.23 (m, 1H), 4.11 (t, *J* = 6.6 Hz, 1H), 3.82 (td, *J* = 8.0, 4.2 Hz, 1H), 3.13 (s, 1H), 3.08 (d, *J* = 6.5 Hz, 2H), 2.80 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.66 – 2.60 (m, 2H), 2.56 (d, *J* = 12.4 Hz, 1H), 2.17 (dt, *J* = 22.0, 11.0 Hz, 2H), 2.03 (t, *J* = 7.4 Hz, 2H), 1.63 – 1.54 (m, 1H), 1.47 (qd, *J* = 14.8, 6.2 Hz, 3H), 1.29 (dd, *J* = 15.8, 8.6 Hz, 2H).

MaxPeak: 96.37%
Ret_Time: 0.685 min

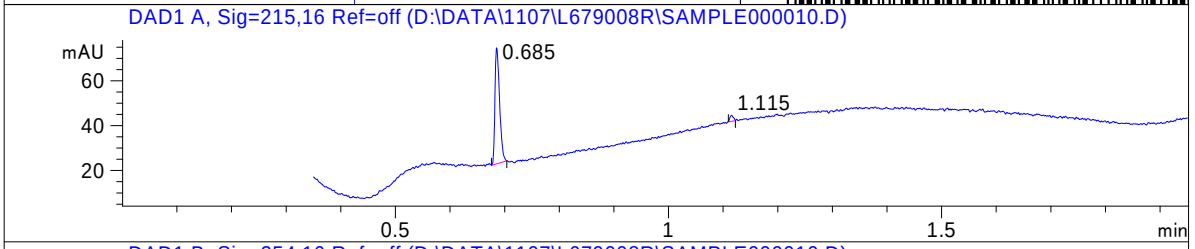
BC746832$2

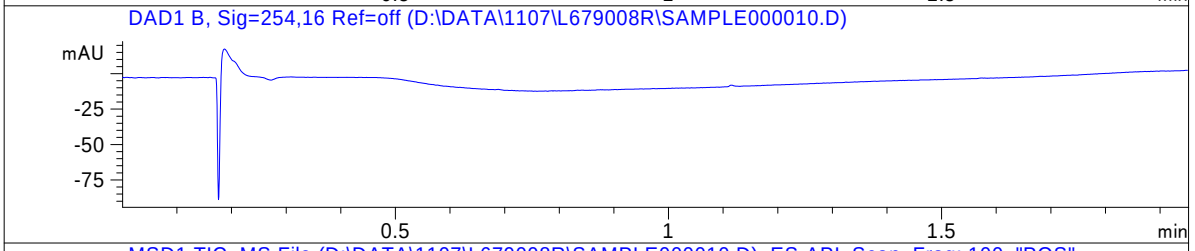


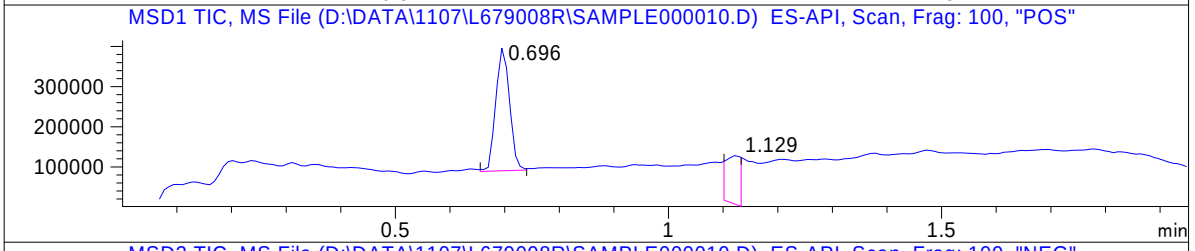
Mol Wt 380.91

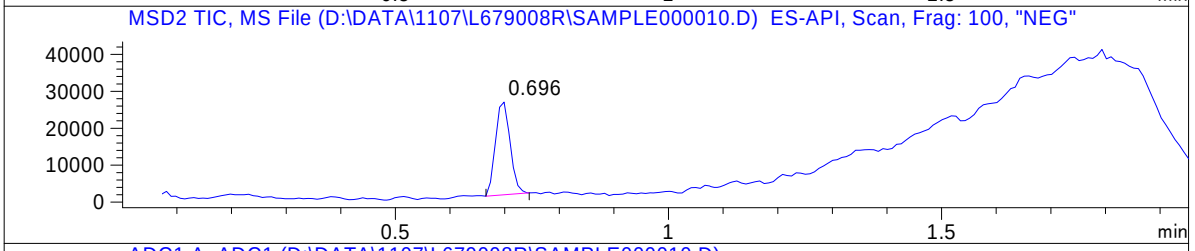
Exact Mass 344.2

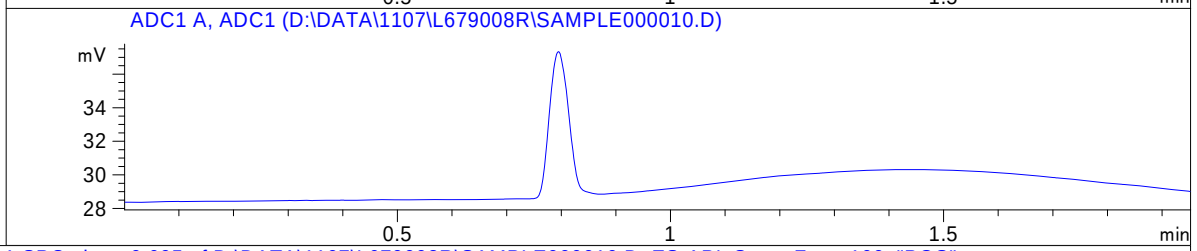
#	Time	Area%
1	0.685	96.37
2	1.115	3.63



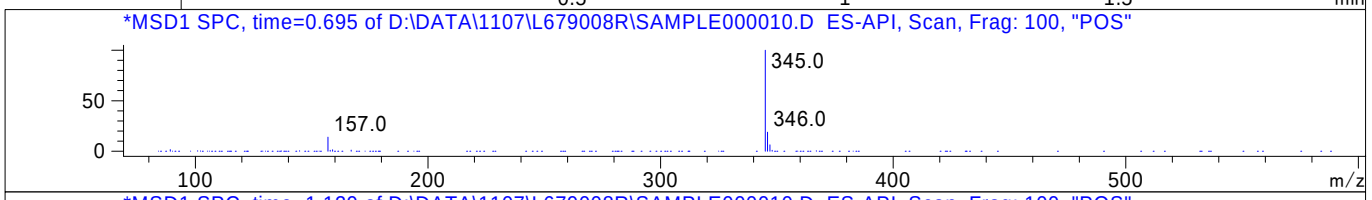




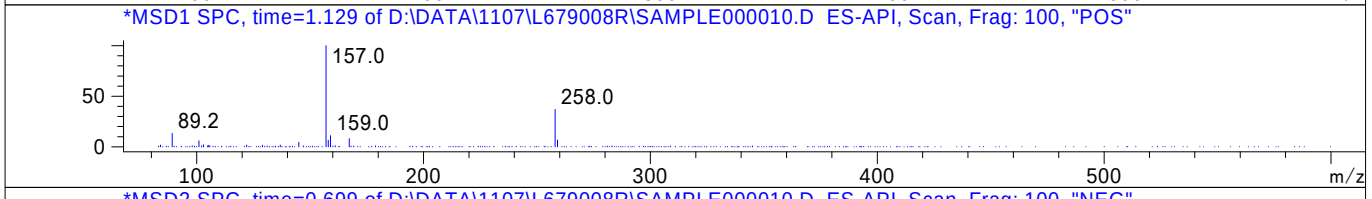




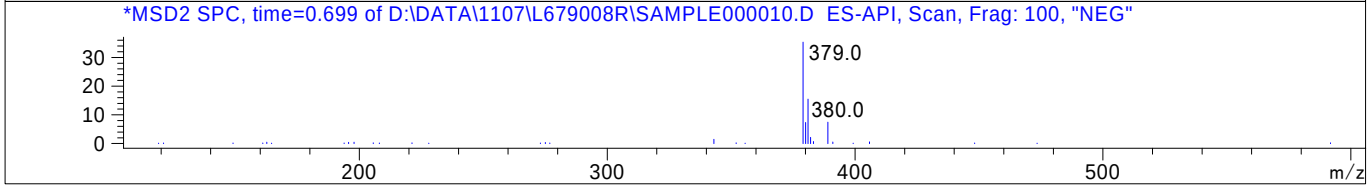
RT 0.696



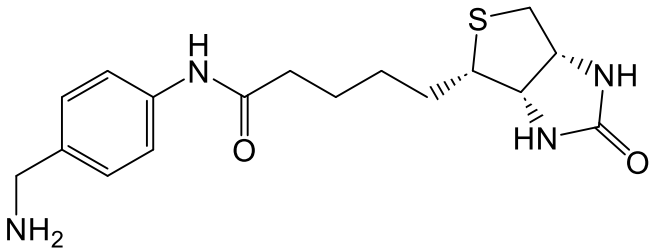
RT 1.129



RT 0.696

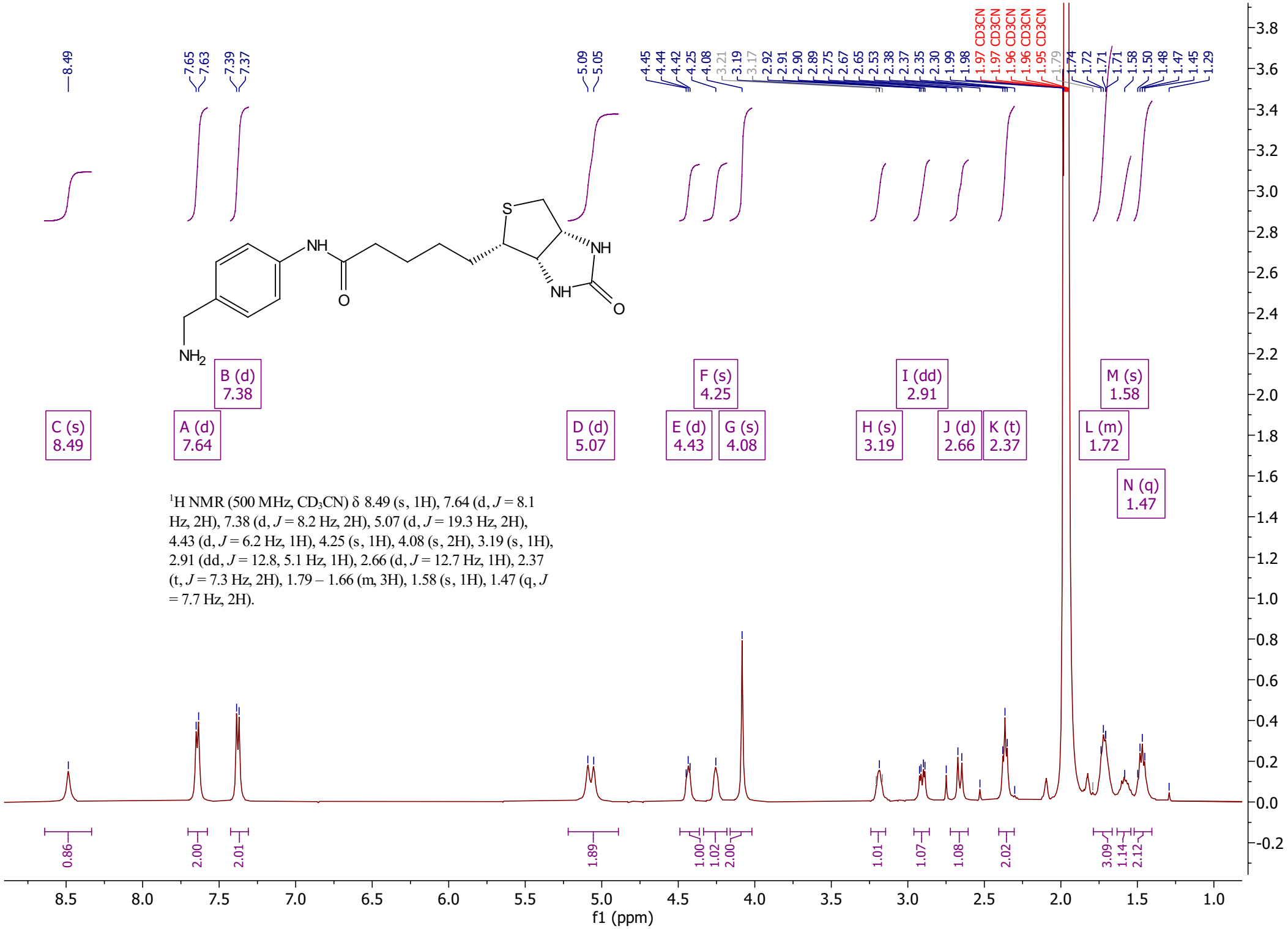


Compound 9

Mol Structure	
Mol Formula	$C_{17}H_{24}N_4O_2S$
Compound Name	Z2451166862
Mol. Wt.	348.16
Date Synthesized	October 20, 2023

Analytical Tests

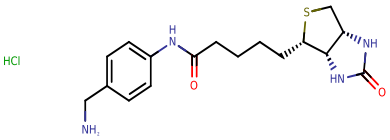
Analytical Tests	Results
LCMS	$[M-H]^-$: 347.2
LCMS (>95%)	Rt= 0.687 min, 97.98% (215nm)
1H-NMR	Consistent with structure

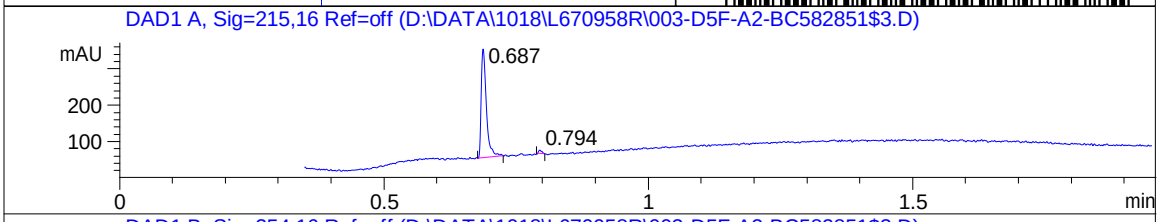


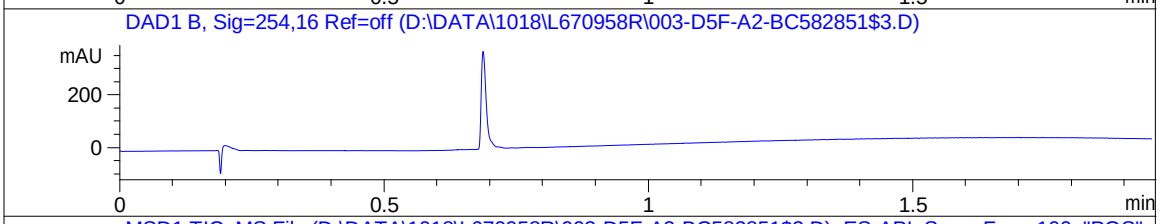
^{1}H NMR (500 MHz, CD_3CN) δ 8.49 (s, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 5.07 (d, J = 19.3 Hz, 2H), 4.43 (d, J = 6.2 Hz, 1H), 4.25 (s, 1H), 4.08 (s, 2H), 3.19 (s, 1H), 2.91 (dd, J = 12.8, 5.1 Hz, 1H), 2.66 (d, J = 12.7 Hz, 1H), 2.37 (t, J = 7.3 Hz, 2H), 1.79 – 1.66 (m, 3H), 1.58 (s, 1H), 1.47 (q, J = 7.7 Hz, 2H).

MaxPeak: 97.98%
Ret_Time: 0.687 min

BC582851$3



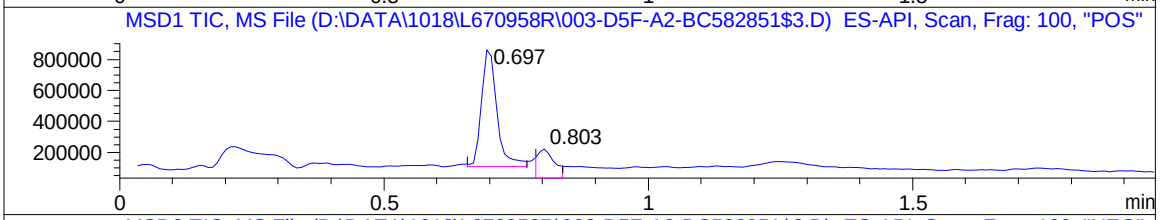


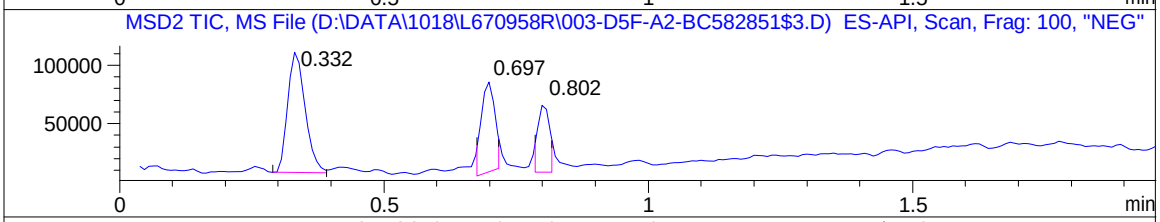


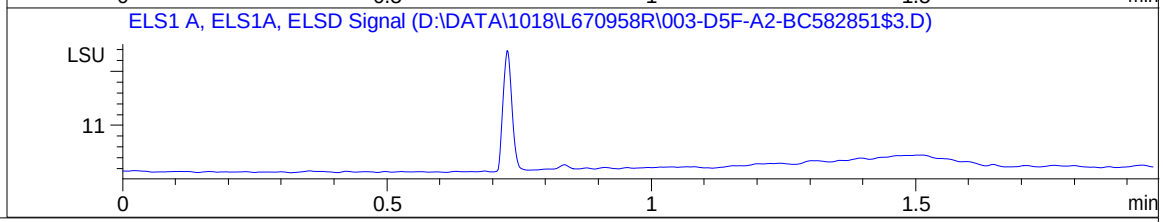
Mol Wt 384.92

Exact Mass 348.19

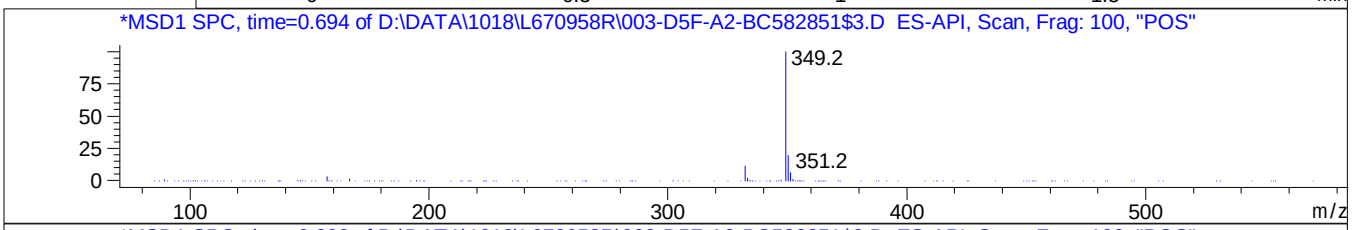
#	Time	Area%
1	0.687	97.98
2	0.794	2.02



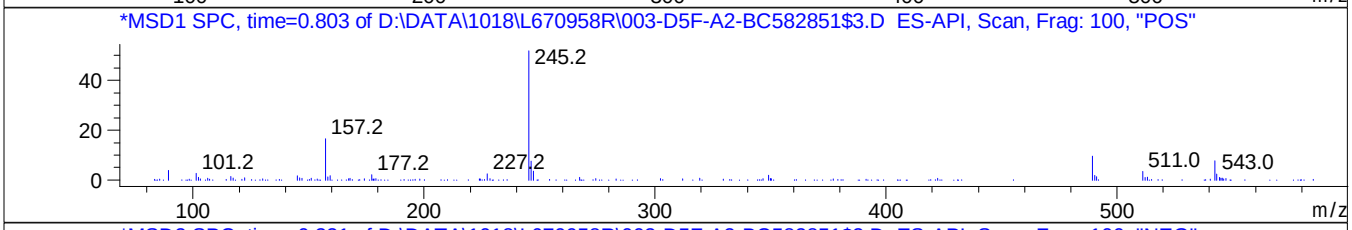




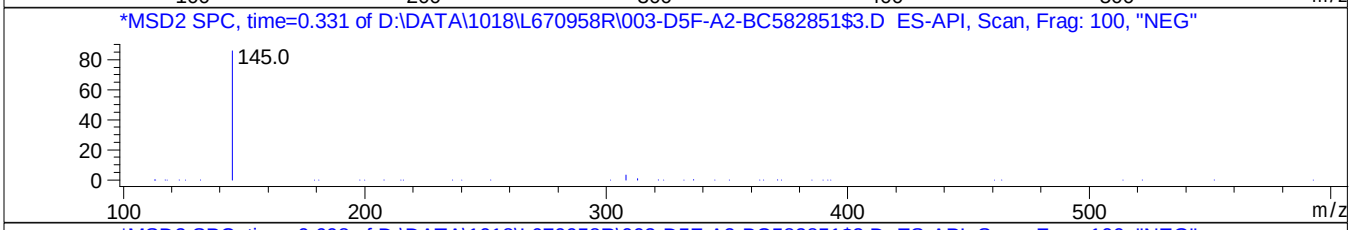
RT 0.697



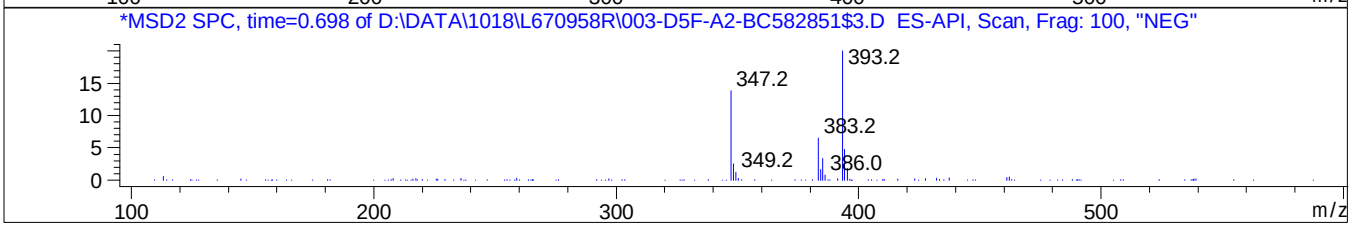
RT 0.803



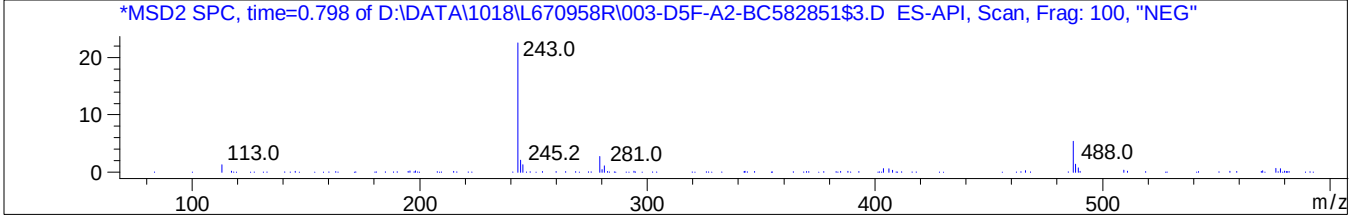
RT 0.332



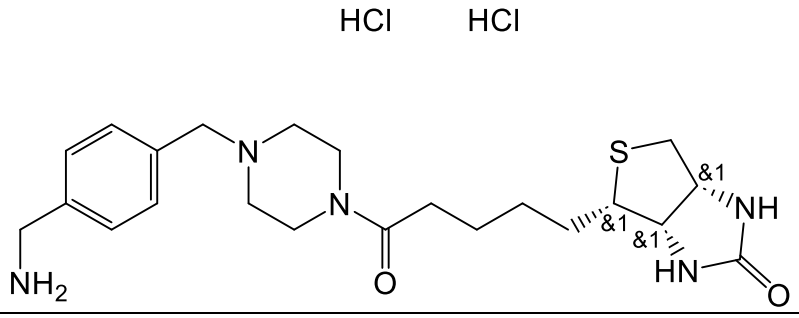
RT 0.697



RT 0.802

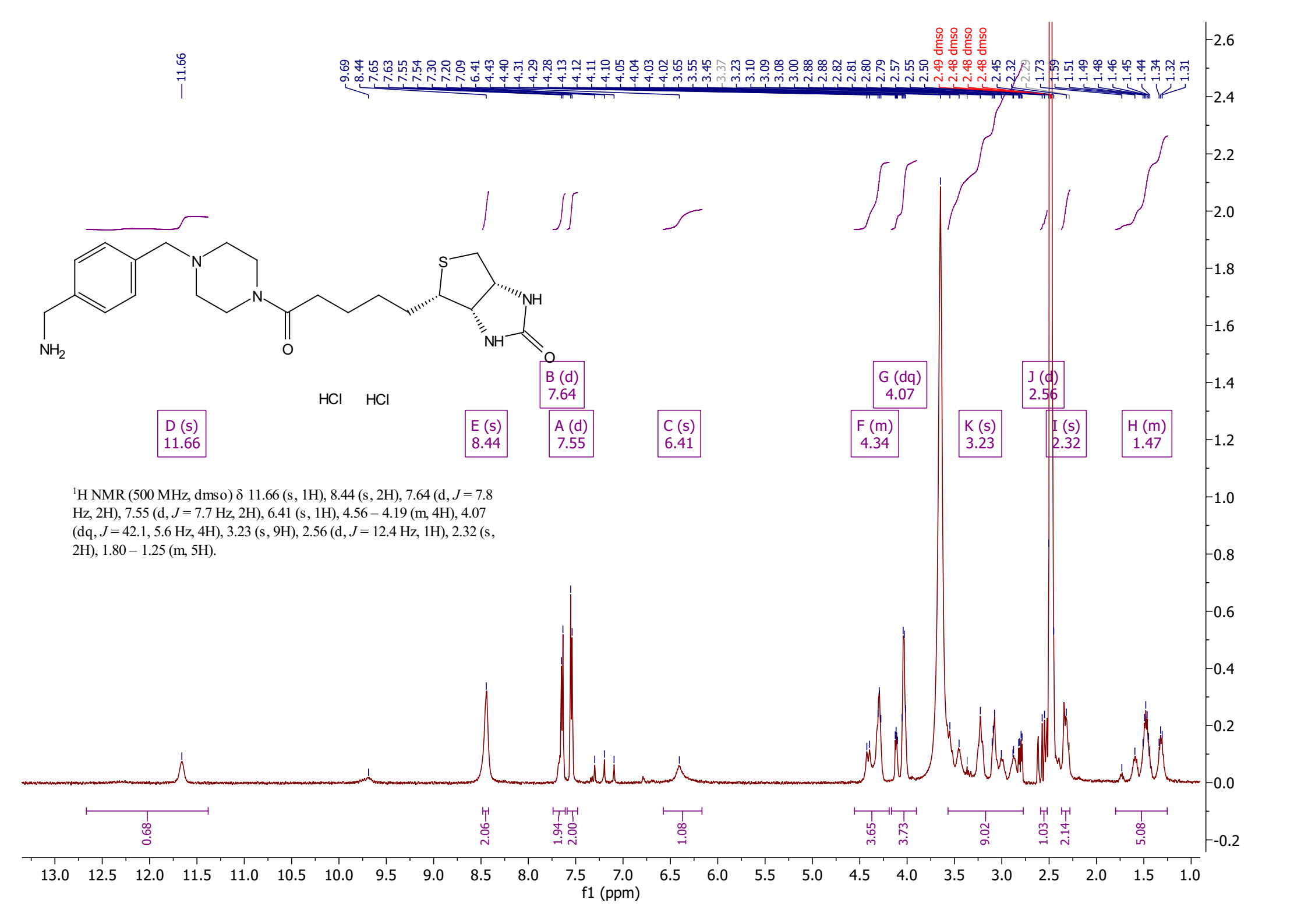

*MSD2 SPC, time=0.798 of D:\DATA\1018\L670958R\003-D5F-A2-BC582851$3.D ES-API, Scan, Frag: 100, "NEG"
20
10
0
243.0
113.0
245.2
281.0
488.0
100
200
300
400
500
m/z

Compound 10

Mol Structure	
Mol Formula	$C_{22}H_{37}Cl_2N_5O_2S$
Compound Name	Z8797397983
Mol. Wt.	431.24
Date Synthesized	November 21, 2023

Analytical Tests

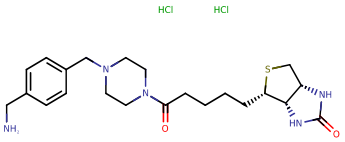
Analytical Tests	Results
LCMS	$[M-H]^-$: 429.2
LCMS (>95%)	Rt= 0.330 min, 100% (215nm)
1H-NMR	Consistent with structure



^{1}H NMR (500 MHz, dmso) δ 11.66 (s, 1H), 8.44 (s, 2H), 7.64 (d, J = 7.8 Hz, 2H), 7.55 (d, J = 7.7 Hz, 2H), 6.41 (s, 1H), 4.56 – 4.19 (m, 4H), 4.07 (dq, J = 42.1, 5.6 Hz, 4H), 3.23 (s, 9H), 2.56 (d, J = 12.4 Hz, 1H), 2.32 (s, 2H), 1.80 – 1.25 (m, 5H).

MaxPeak: 100.00%
Ret_Time: 0.330 min

BC746829$11



Mol Wt 504.52

Exact Mass 431.28

#	Time	Area%
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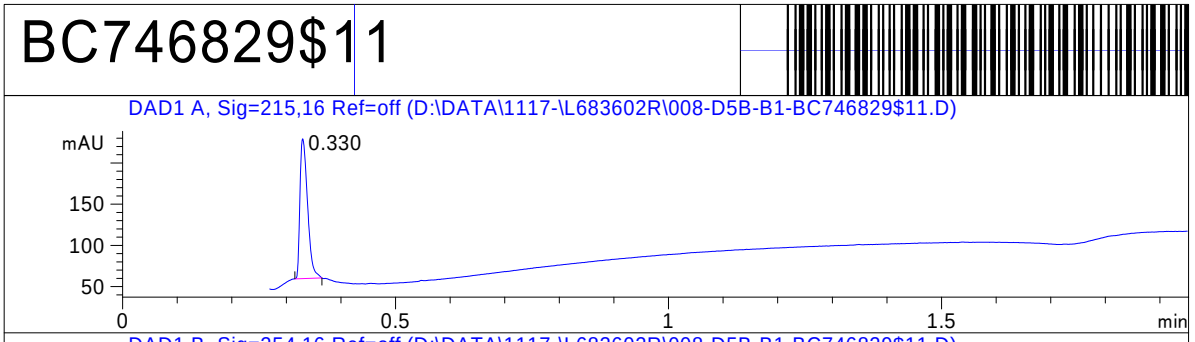
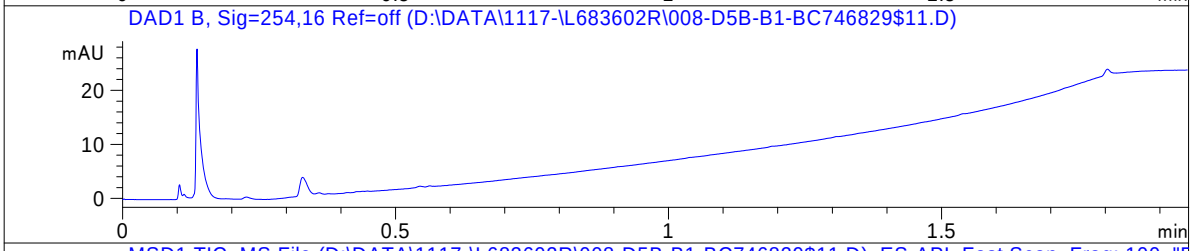
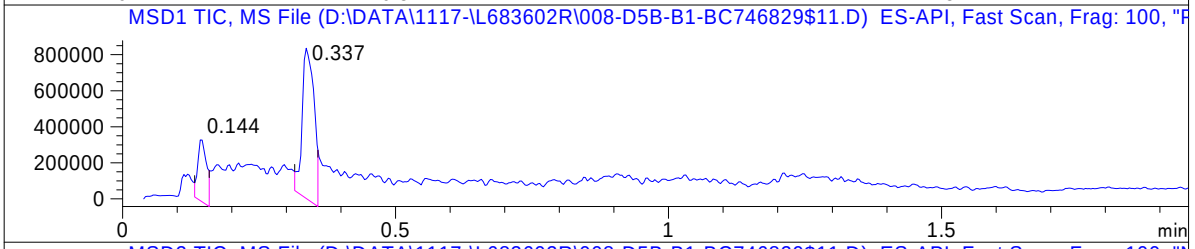
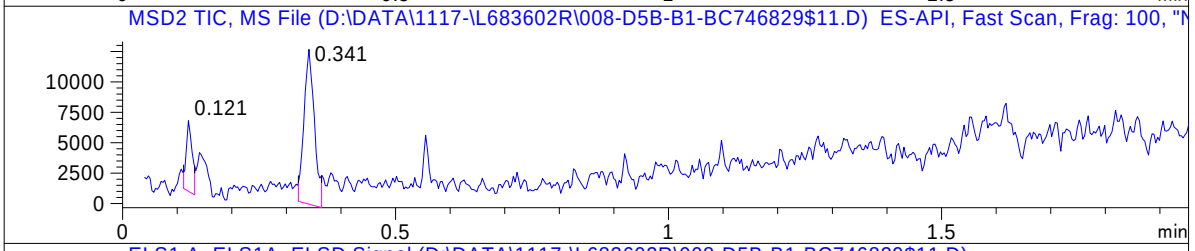
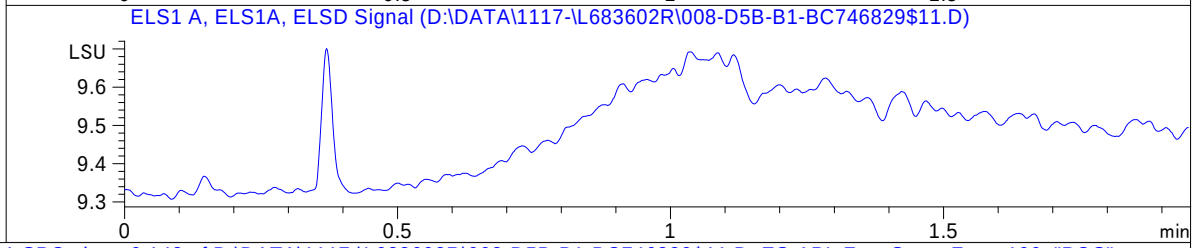


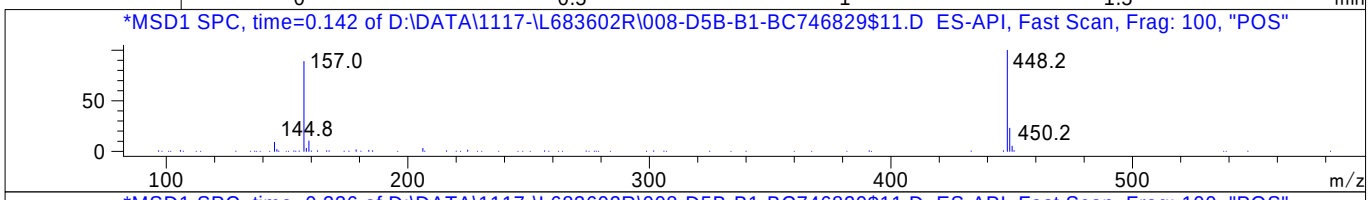




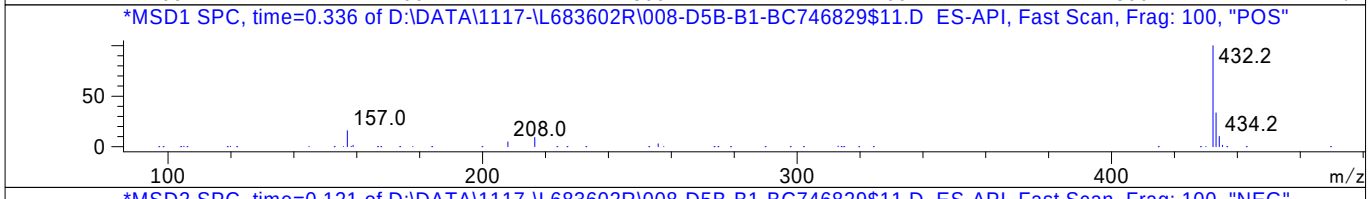




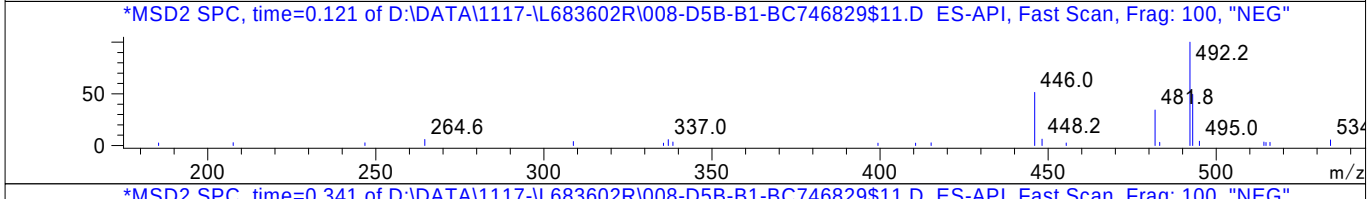
RT 0.144



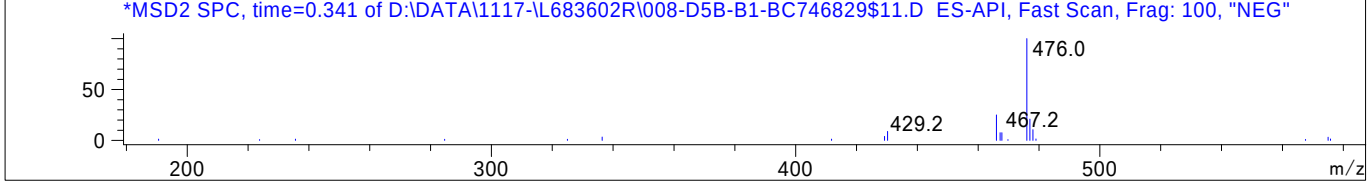
RT 0.337



RT 0.121

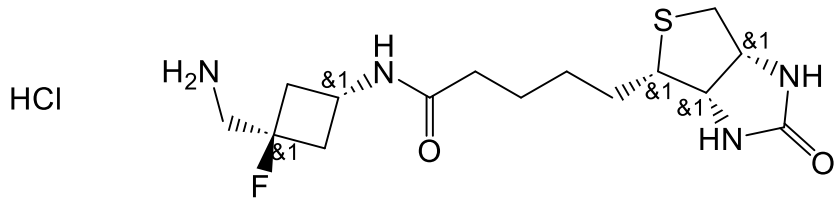


RT 0.341



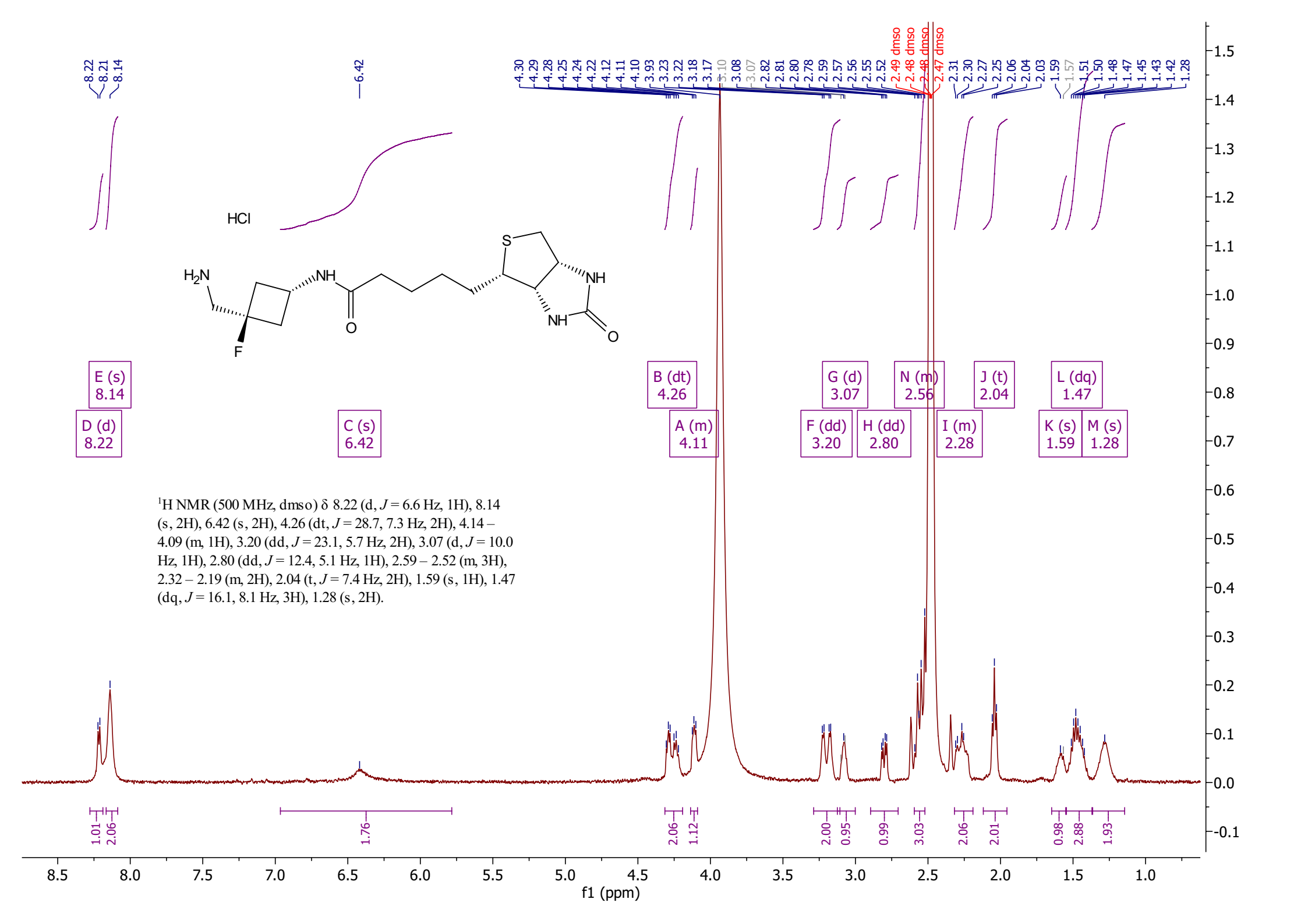
Inj.Date 11/17/2023 H Acq. Method C:\Users\ -> ->

Compound 12

Mol Structure	
Mol Formula	$C_{15}H_{26}ClFN_4O_2S$
Compound Name	Z3699944567
Mol. Wt.	380.91
Date Synthesized	November 09, 2023

Analytical Tests

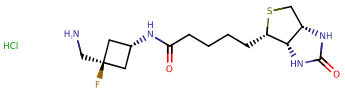
Analytical Tests	Results
LCMS	[M+H]+: 345.0
LCMS (>95%)	Rt= 0.682 min, 97.52% (215nm)
1H-NMR	Consistent with structure



^{1}H NMR (500 MHz, dmso) δ 8.22 (d, J = 6.6 Hz, 1H), 8.14 (s, 2H), 6.42 (s, 2H), 4.26 (dt, J = 28.7, 7.3 Hz, 2H), 4.14 – 4.09 (m, 1H), 3.20 (dd, J = 23.1, 5.7 Hz, 2H), 3.07 (d, J = 10.0 Hz, 1H), 2.80 (dd, J = 12.4, 5.1 Hz, 1H), 2.59 – 2.52 (m, 3H), 2.32 – 2.19 (m, 2H), 2.04 (t, J = 7.4 Hz, 2H), 1.59 (s, 1H), 1.47 (dq, J = 16.1, 8.1 Hz, 3H), 1.28 (s, 2H).

MaxPeak: 97.52%
Ret_Time: 0.682 min

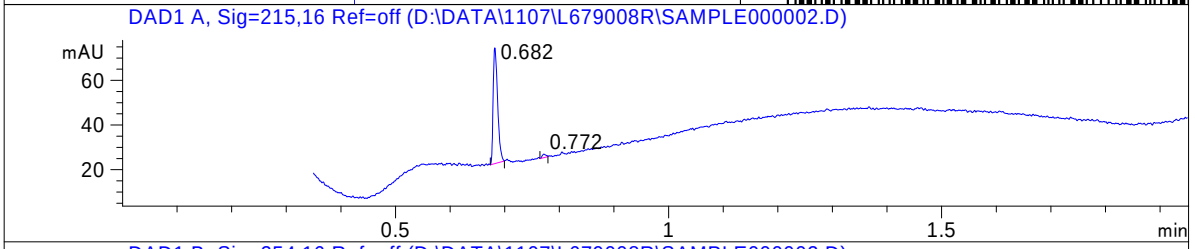
BC746830$3

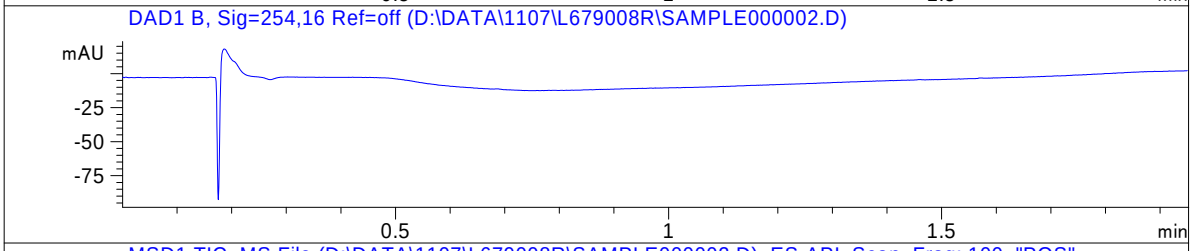


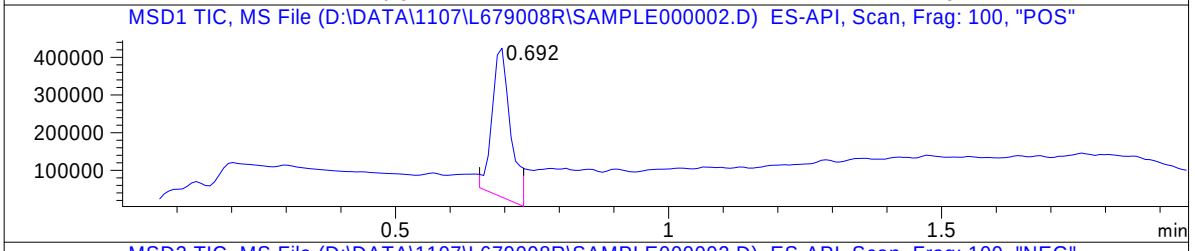
Mol Wt **380.91**

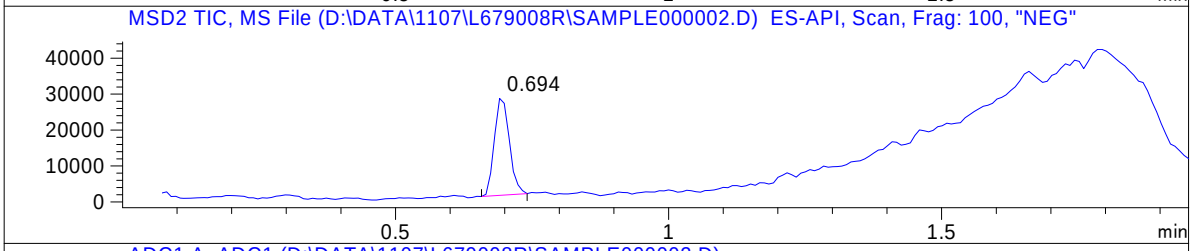
Exact Mass **344.2**

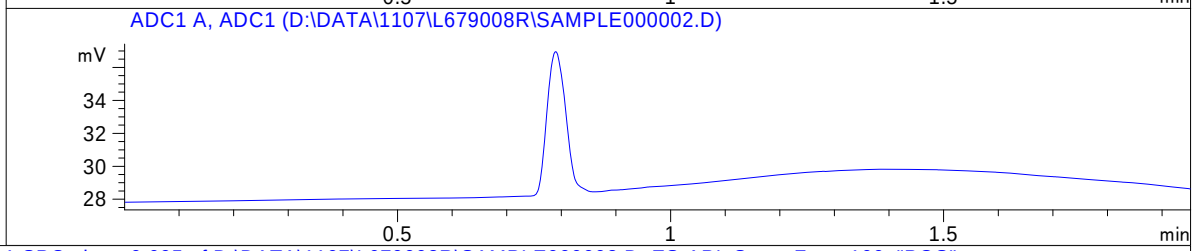
#	Time	Area%
1	0.682	97.52
2	0.772	2.48



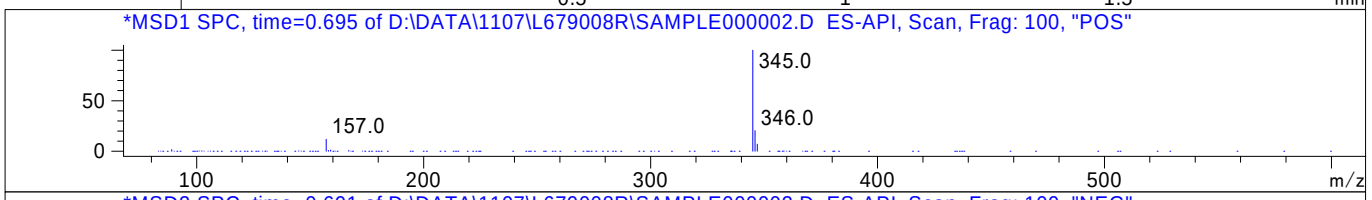








RT 0.692



RT 0.694