

Electronic Supplementary Information

Short protecting-group-free synthesis of 5-acetylsulfanyl-histidines in water:

Novel precursors of 5-sulfanyl-histidine and analogues

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Typical procedure S1: Optimisation of the synthesis of 5-acetylsulfanyl-histidine (11a) in aqueous solution

For the optimisation of reaction conditions, an aqueous solution of histidine hydrochloride hydrate (**6a** x HCl, 0.10-0.55M, 5-250 mmol, 1 equiv) and concentrated hydrochloric acid (0-1 equiv) was cooled in an ice-bath. Br₂ (1.3 equiv) was added rapidly dropwise, and thioacetic acid (**9**, 1-10 equiv) after 1 min. For entries 9 to 11, N-bromosuccinimide (NBS, 1.3 equiv) was added all at once as activating reagent instead of Br₂, and thioacetic acid (**9**, 4 equiv) after 2.5 min (instead of 1min). After stirring for 30min at 0°C, and rapid decantation of non-converted thioacetic acid, 200µL of the reaction mixture were diluted in 500µL D₂O, filtered, and the solution analysed by ¹H NMR (400MHz).

Characteristic imidazole H atoms of 5-acetylsulfanyl-histidine (**11a**) are detected at 8.85-8.95 ppm (H-2), non-converted histidine (**6a**) at 7.45 (H-5) and 8.87 ppm (H-2) and 2-thiohistidine (**8a**) at 6.9 ppm (H-5). The reactional yield was calculated by integration after normalization of the aromatic region between 6.6-9.5 ppm based on the assumption that all products formed are characterized by one aromatic H (either on position 2 or 5) besides non-converted histidine with 2 protons, see Fig. S2.

Figure S2. ¹H NMR (400MHz, H₂O/D₂O, 6.0-9.5 ppm) of the reaction mixture.

Reaction mixture corresponding to Table S3, entry 5.

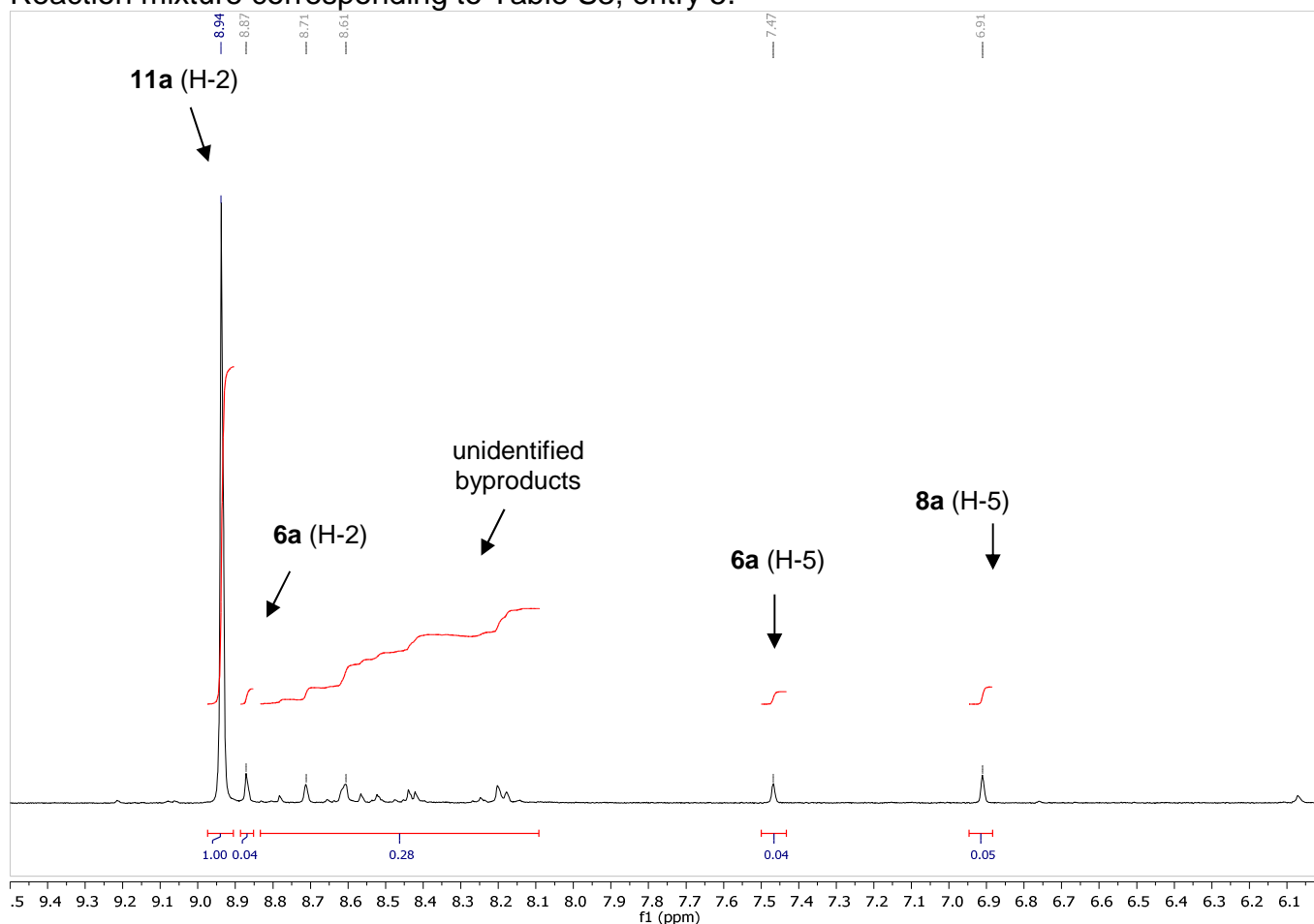
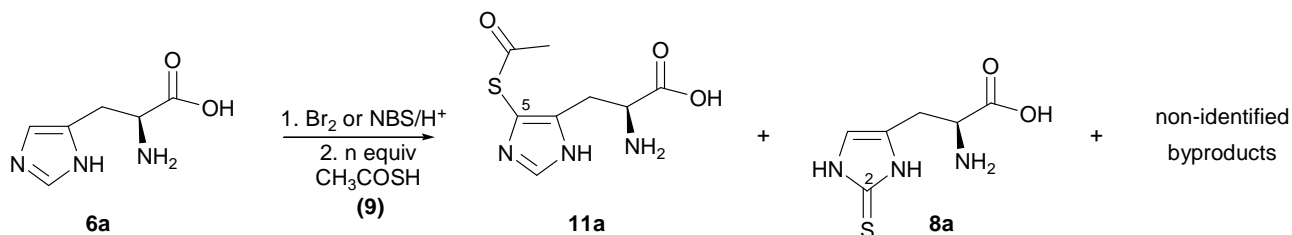


Table S3 Reaction parameters for the synthesis of 5-acetylsulfanyl-histidine (11a)

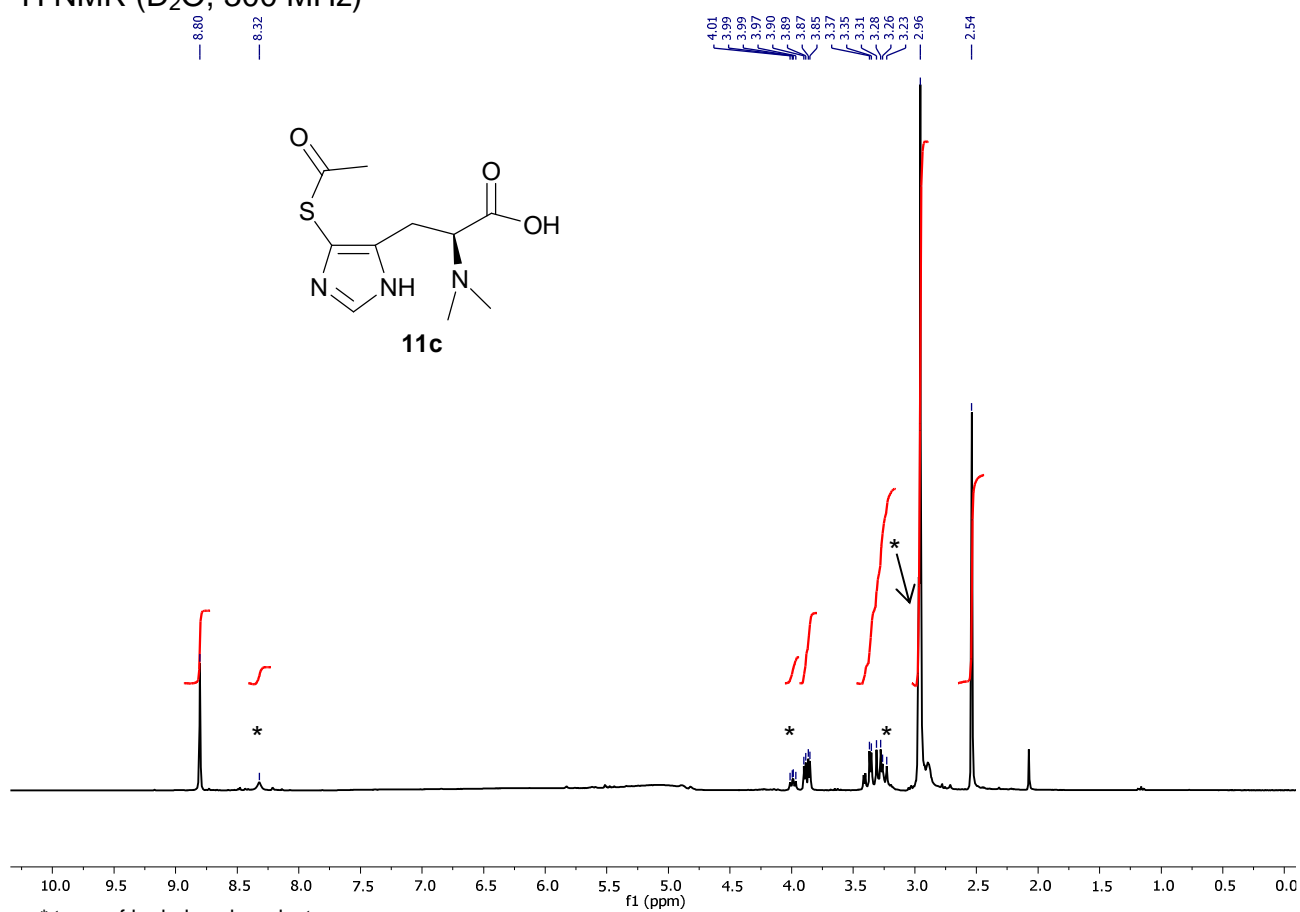
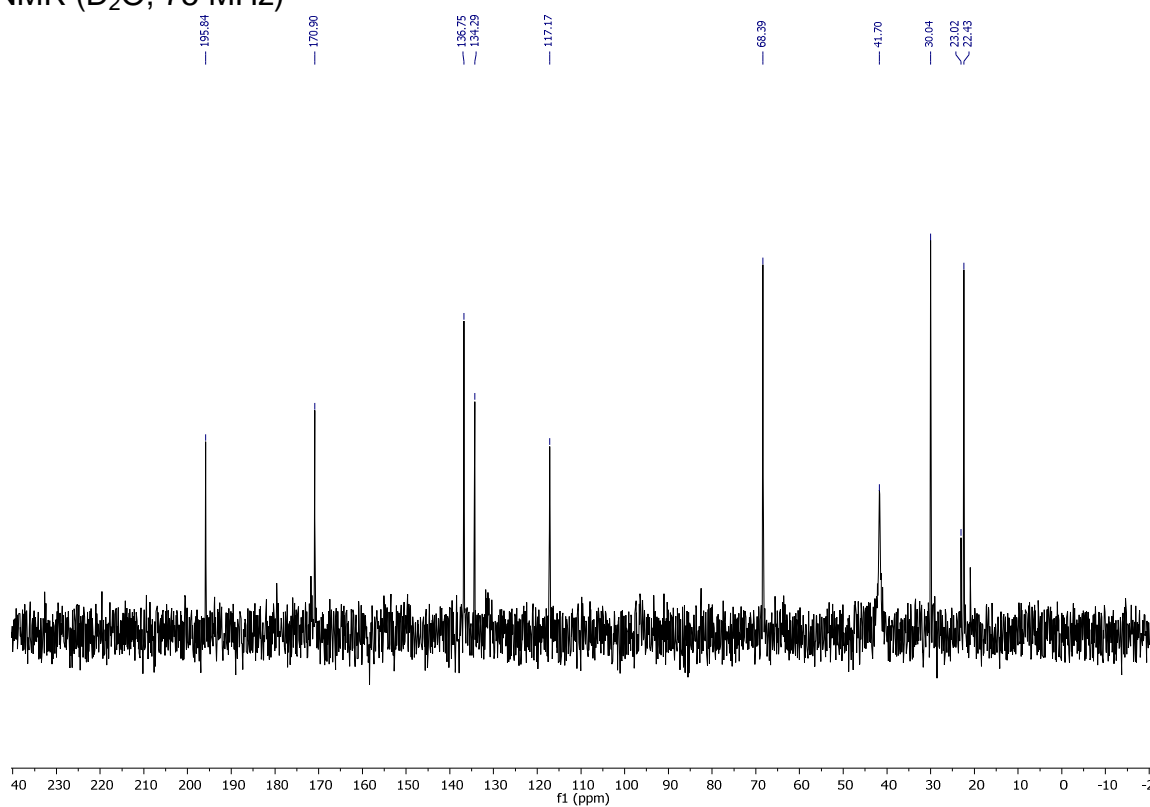
Entry	Scale (mmol)	HCl	Dilution	Reagent	Equiv CH ₃ COSH (9)	Yield (by ¹ H NMR of the reaction mixture)		
						11a	6a	8a
1	5	1	0.55 M	Br ₂	4	58%	4%	19%
2	5	1	0.25 M	Br ₂	4	64%	3%	8%
3	5	1	0.1 M	Br ₂	4	61%	0%	7%
4	50	1	0.45 M	Br ₂	4	65%	2%	11%
5	250	1	0.15 M	Br ₂	4	70%	3%	3%
6	5	1	0.25 M	Br ₂	10	76%	1%	6%
7	5	1	0.25 M	Br ₂	1	52%	5%	21%
8	5	2	0.25 M	Br ₂	4	63%	4%	14%
9	20	1	0.15 M	NBS	4	61%	8%	4%
10	50	2	0.15 M	NBS	4	67%	1%	3%
11	100	2	0.15 M	NBS	4	68%	0%	4%

Applying the optimal conditions identified for sulfur-introduction using cysteine as reagent, using a slight excess of bromine (1.3 equiv), followed immediately by the addition of an excess of thioacetic acid (entry 1), 5-acetylsulfanyl-histidine **11a** (58%), was formed besides a minor amount of 2-thiohistidine (**8a**) and residual histidine.

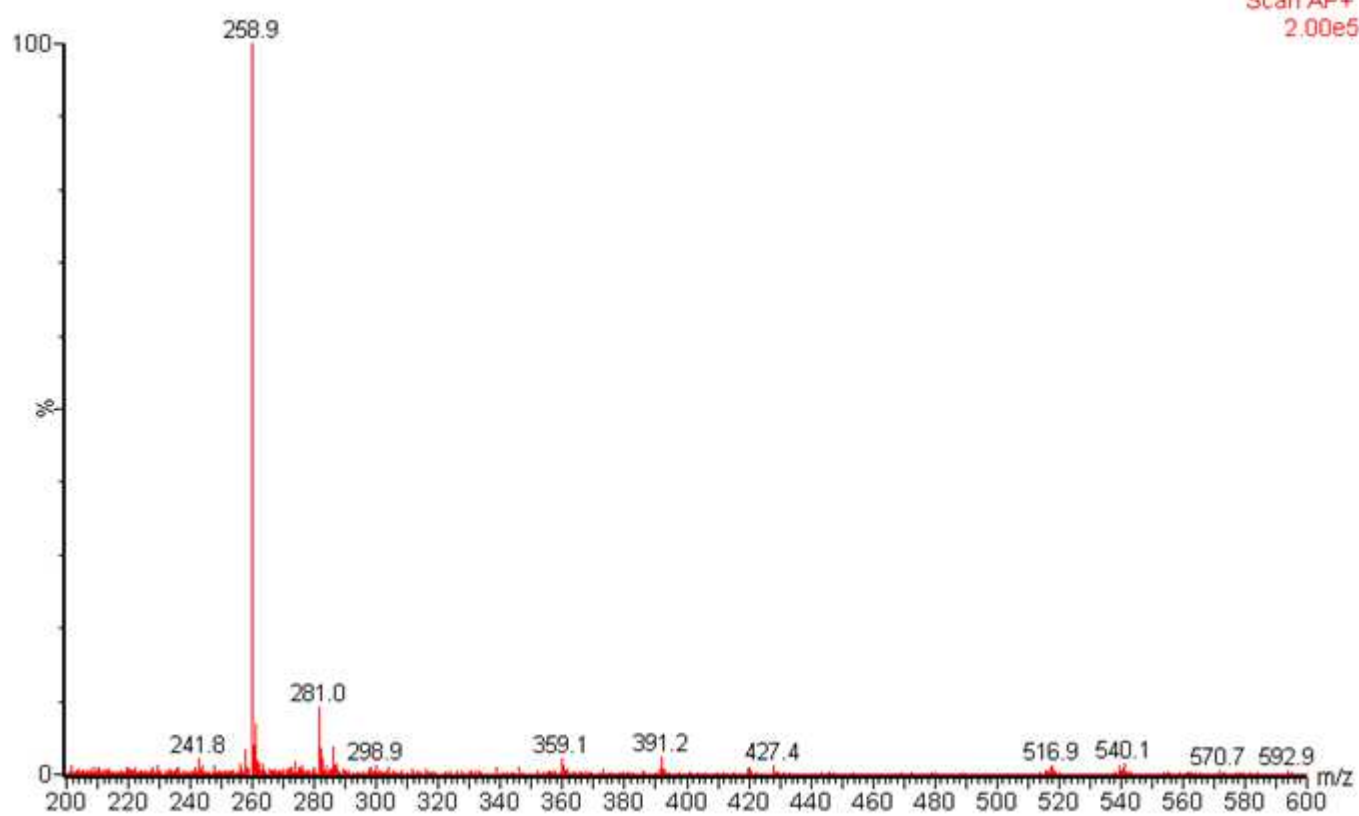
Variation of different reaction parameters showed that under stronger dilution (entry 2 and 3), and also on a higher scale (entry 4 and 5) a quite similar product profile is observed, with a reactional yield of 61-70% of the new product. Interestingly, the relative percentage of 2-thiohistidine **8a** seems to be slightly influenced by dilution, and the best results were obtained in the concentration range for **6a** from 0.15-0.25 M. Using ten instead of four equivalents of thioacetic acid **9**, the yield was only slightly improved (entry 6), and even with only one equivalent of **9**, **11a** was still obtained in 52% yield (entry 7), but then again a higher percentage of the 2-thiohistidine derivative was observed.

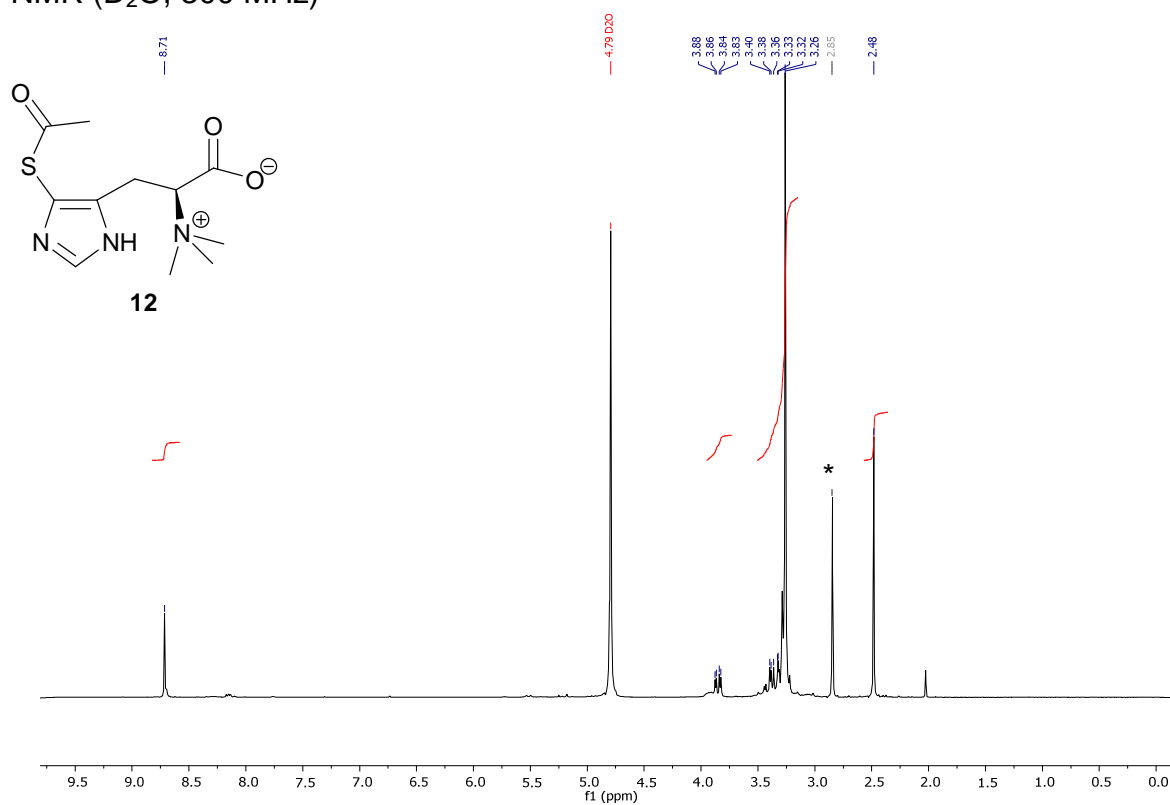
A higher percentage of 2-substituted product **8a** is also observed conducting the reaction in the presence of 2 equivalents of hydrochloric acid instead of 1 (entry 8), but the 5-substituted product **11a** is still obtained in good yield.

The same preference towards 5-substitution by thioacetic acid is observed with N-bromosuccinimide (NBS) instead of bromine to activate the histidine, with the best results in the presence of 2 equivalents of acid and a slightly longer activation time before addition of thioacetic acid (entry 9-11).

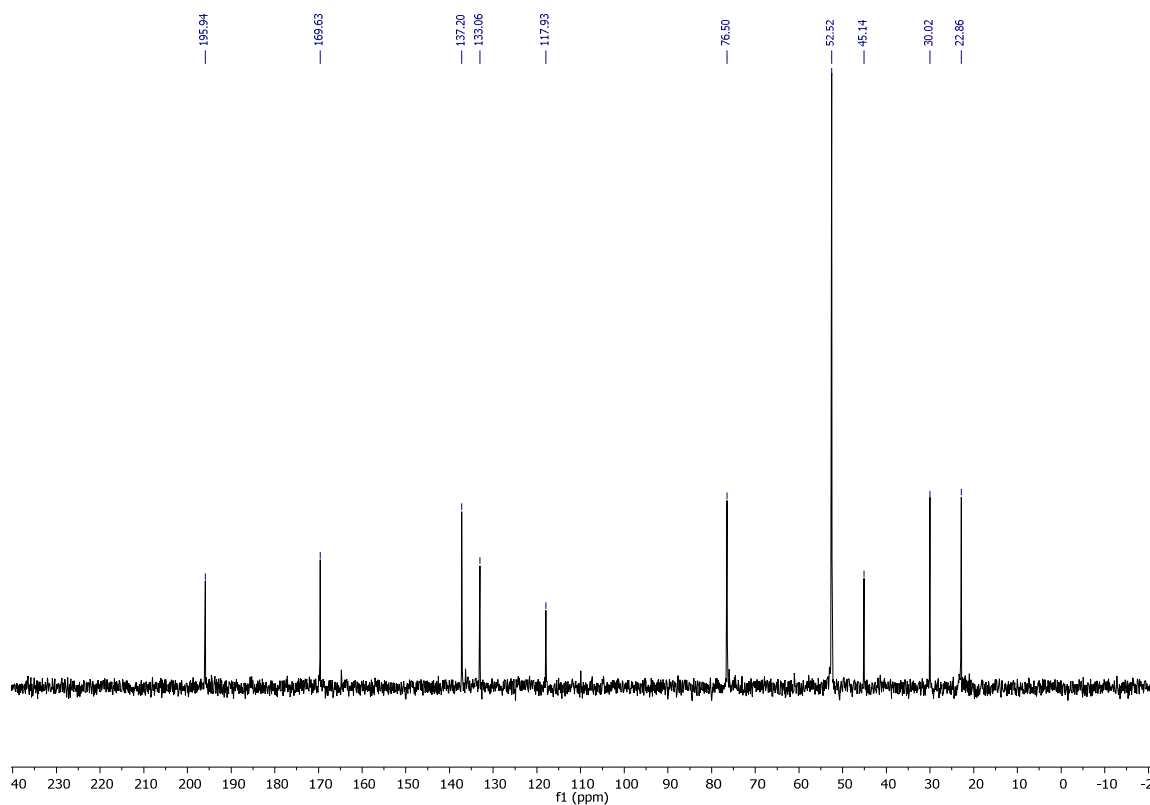
L-5-Acetylsulfanyl- α ,N,N-dimethyl-histidine (**11c**). ^1H NMR (D_2O , 300 MHz) ^{13}C NMR (D_2O , 75 MHz)

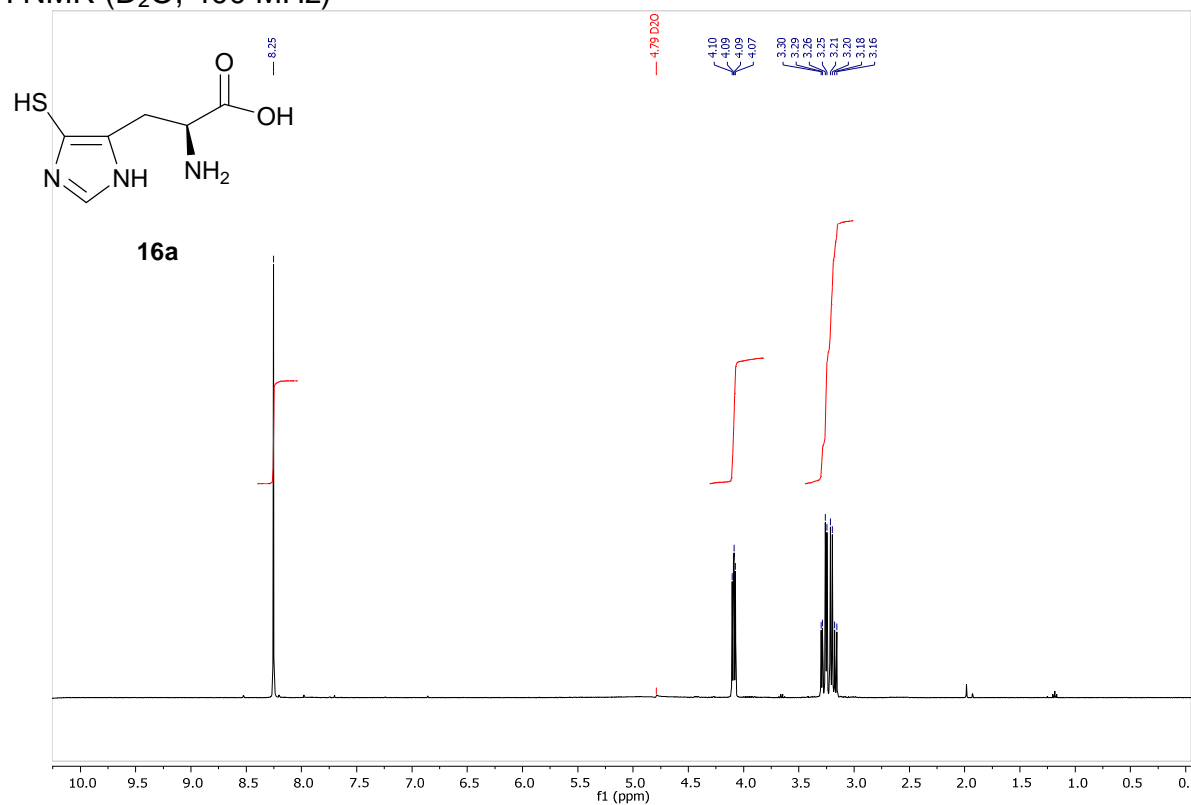
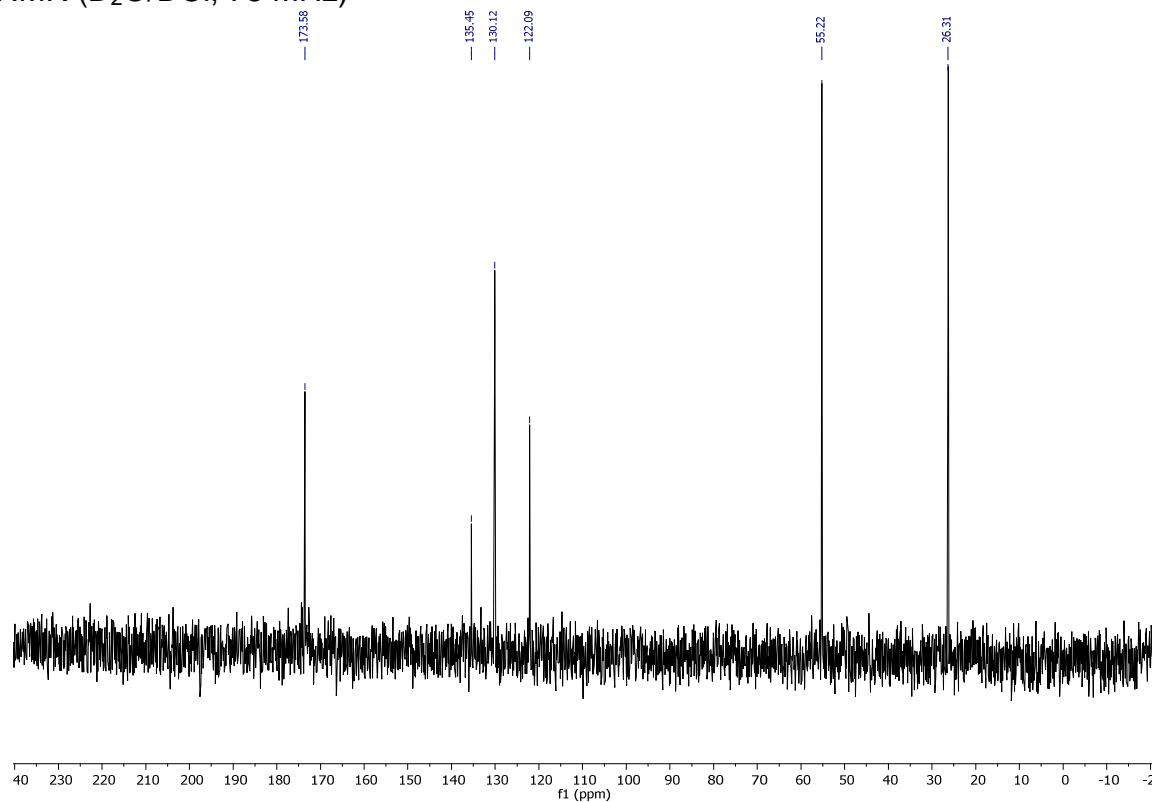
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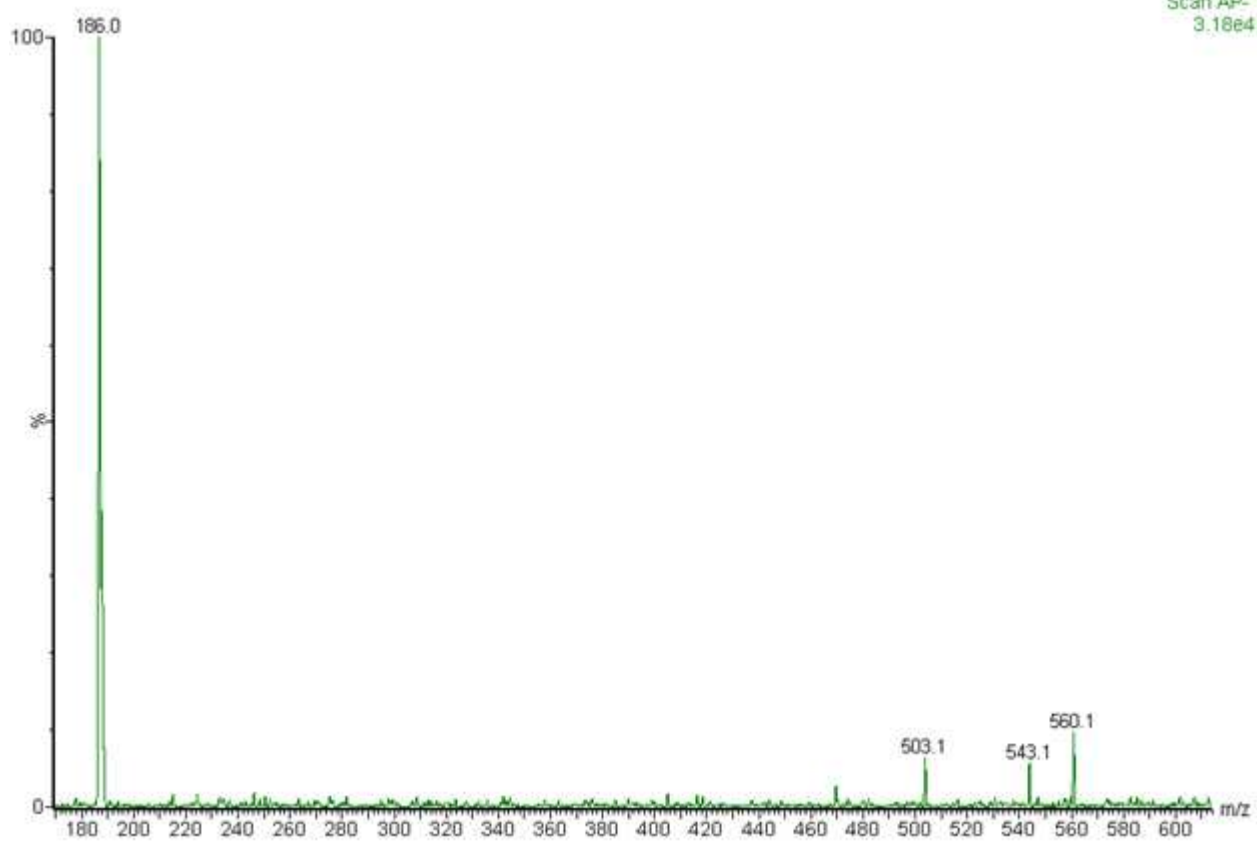
L-5-Acetylsulfanyl- α ,N,N,N-trimethyl-histidine (**12**). ^1H NMR (D_2O , 300 MHz)

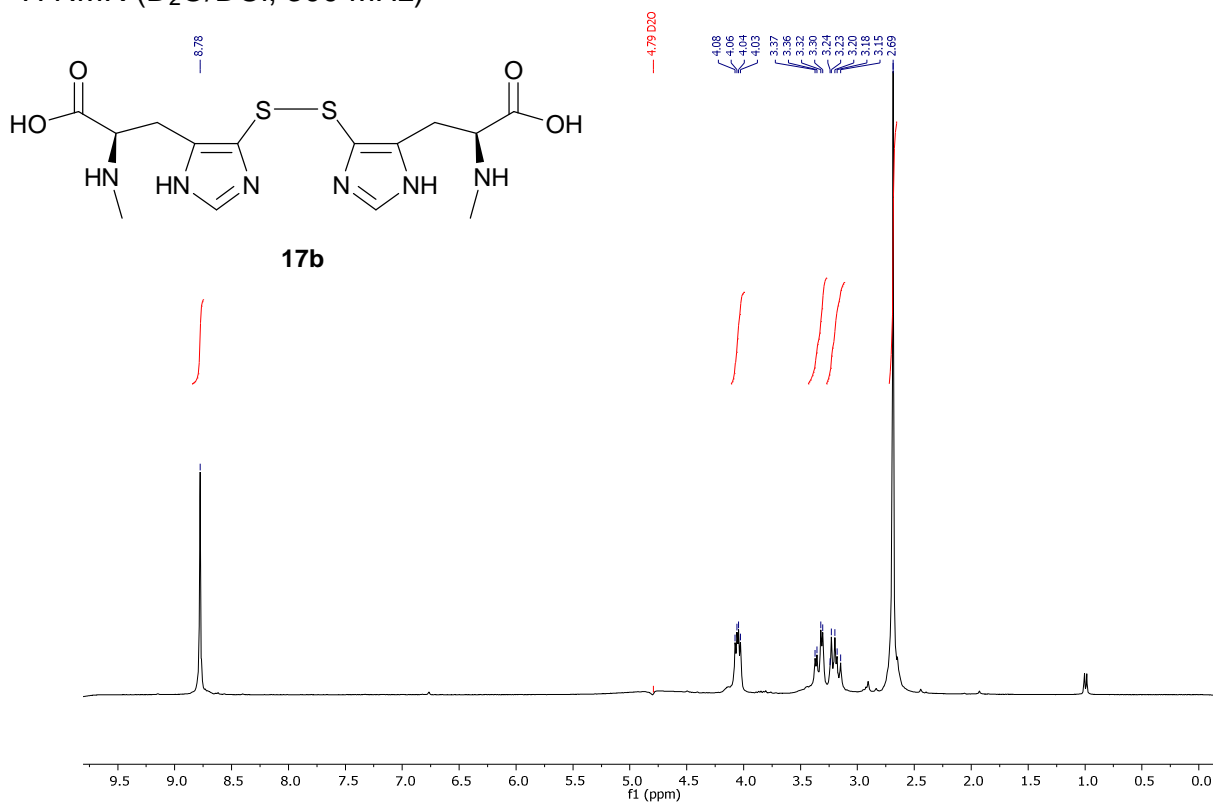
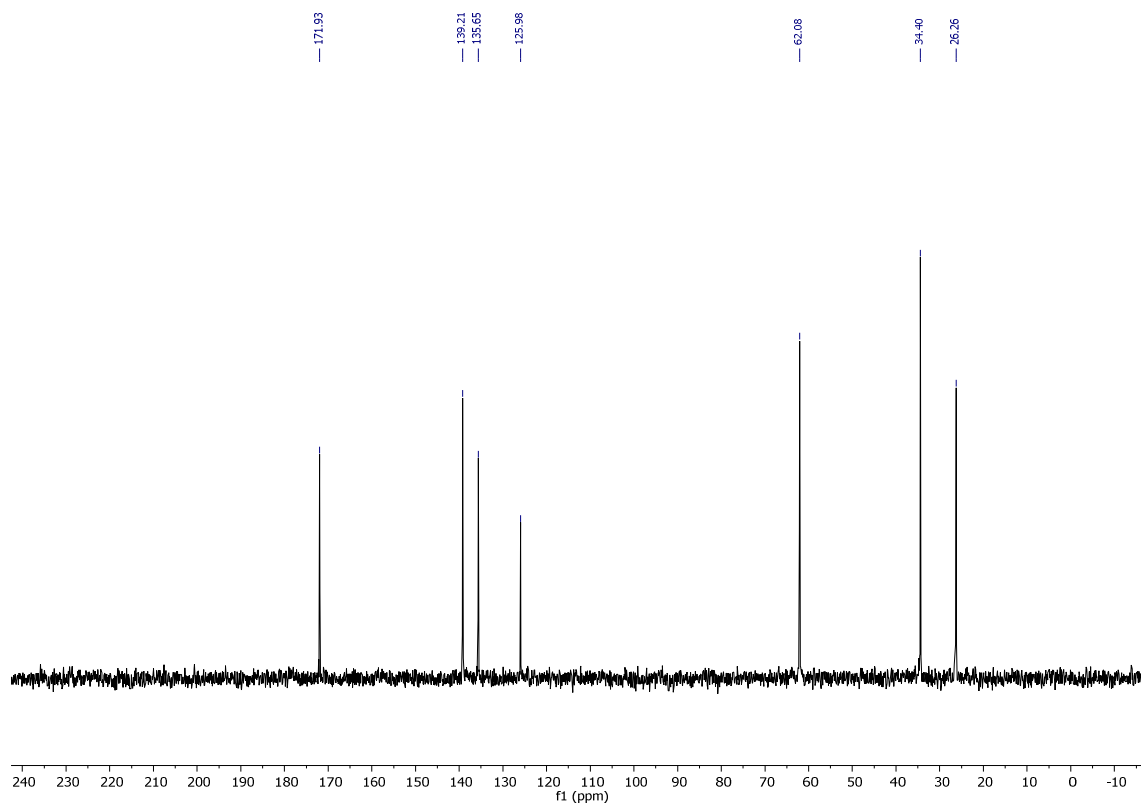
* trace of residual succinimide

 ^{13}C NMR (D_2O , 75 MHz)

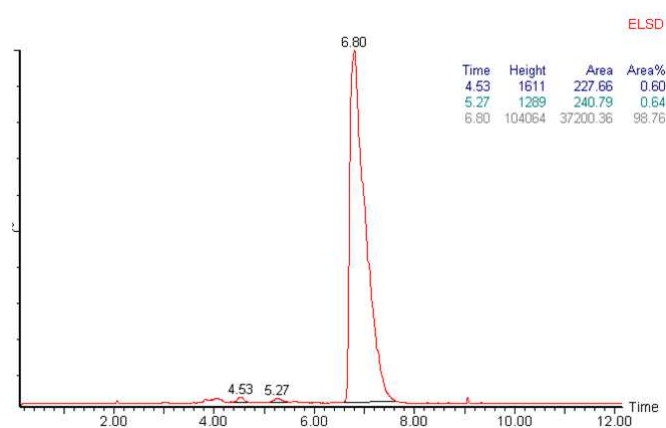
L-5-sulfanyl-histidine (16a) ^1H NMR (D_2O , 400 MHz) ^{13}C NMR ($\text{D}_2\text{O}/\text{DCI}$, 75 MHz)

APCI - MS

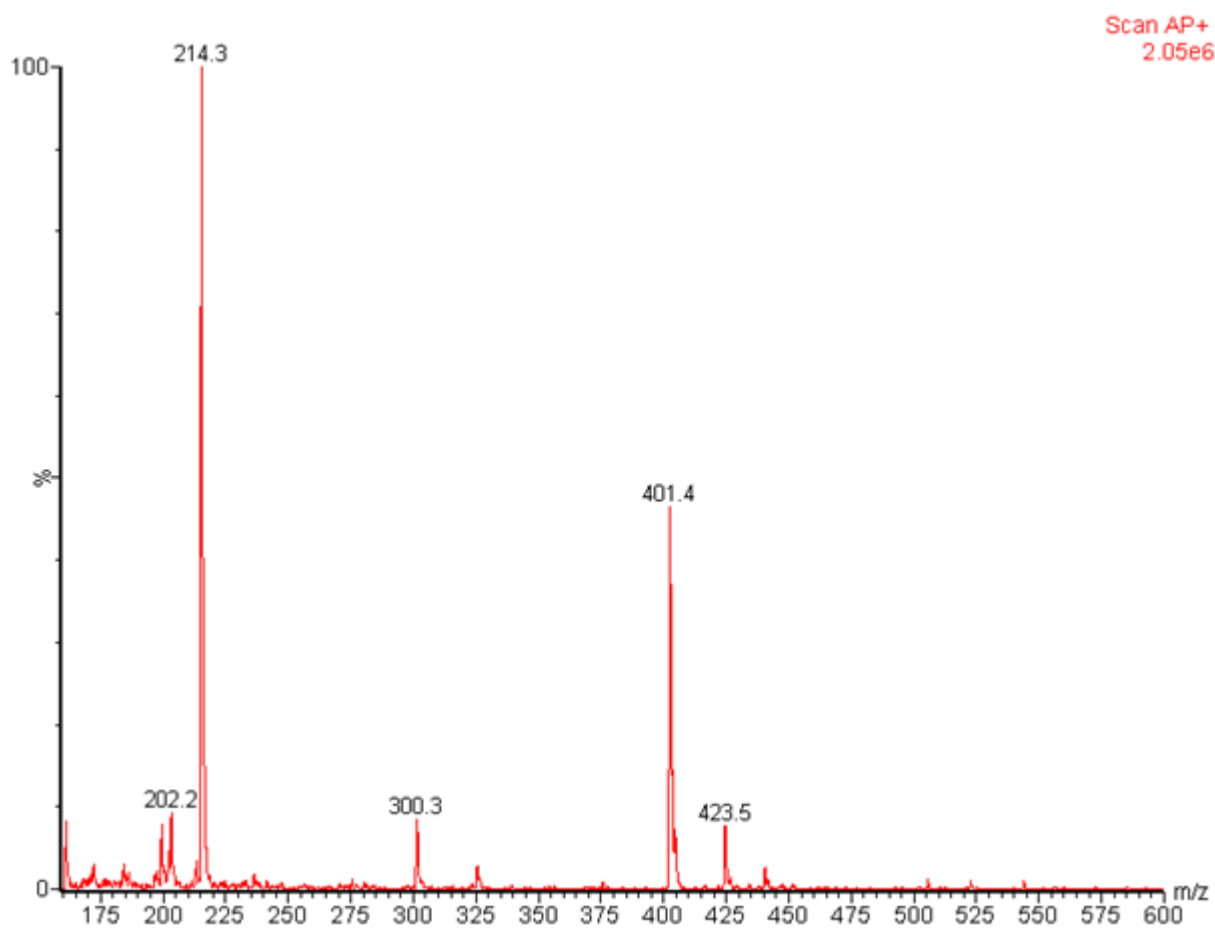
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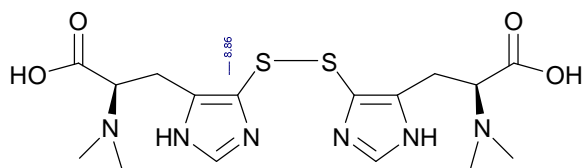
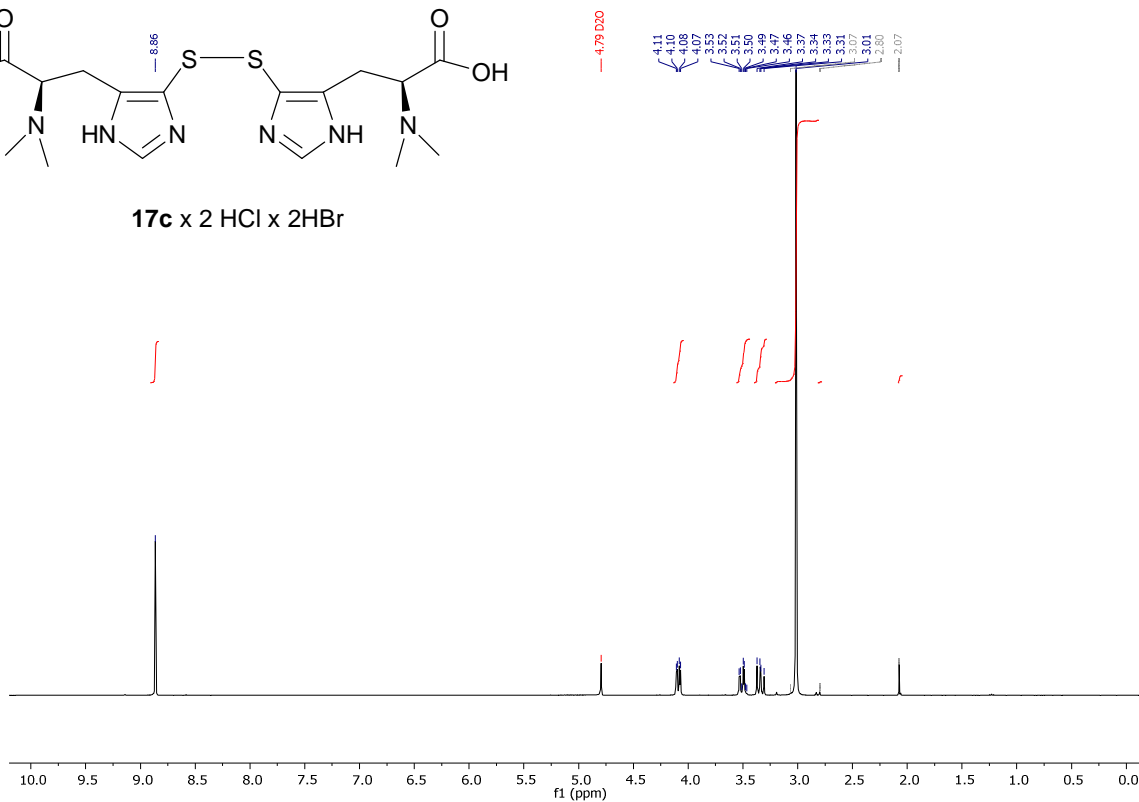
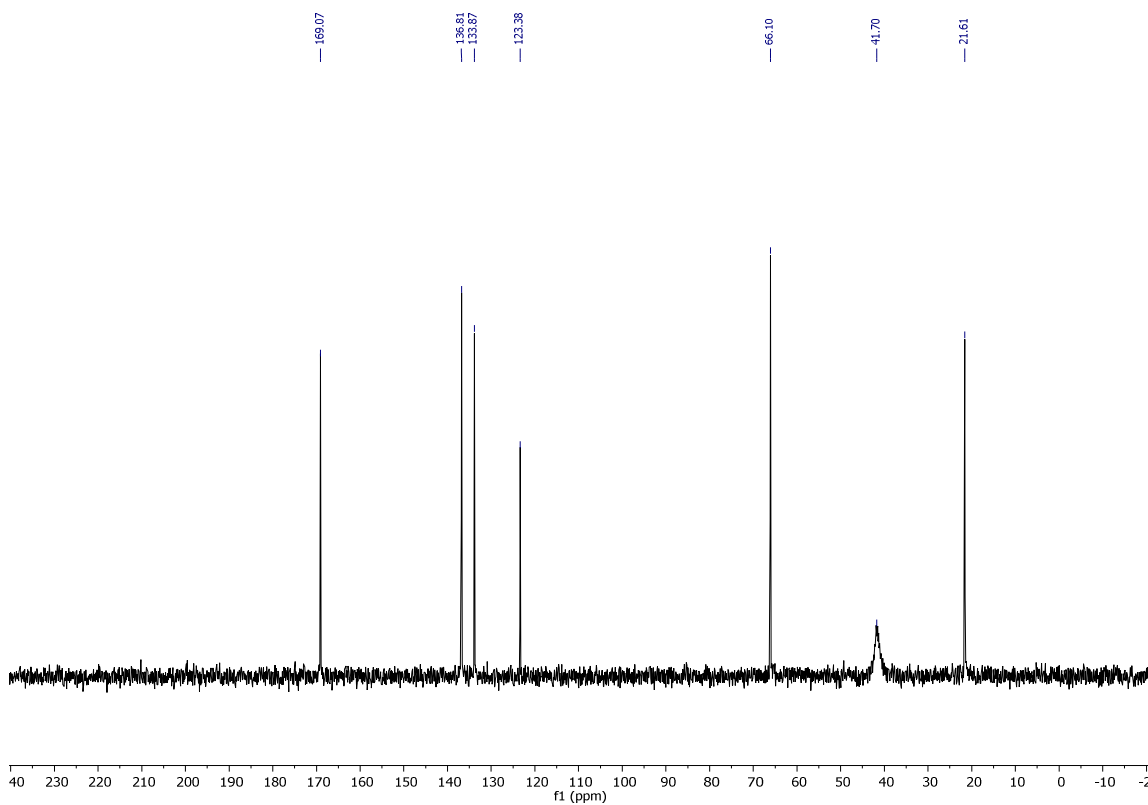
Disulfide 17b ^1H NMR ($\text{D}_2\text{O}/\text{DCI}$, 300 MHz) ^{13}C NMR ($\text{D}_2\text{O}/\text{DCI}$, 75 MHz)

LC (column B, see Experimental Part)

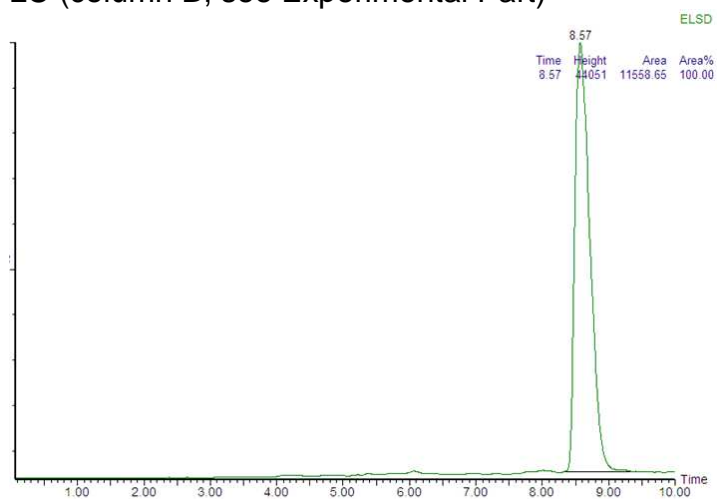


APCI - MS

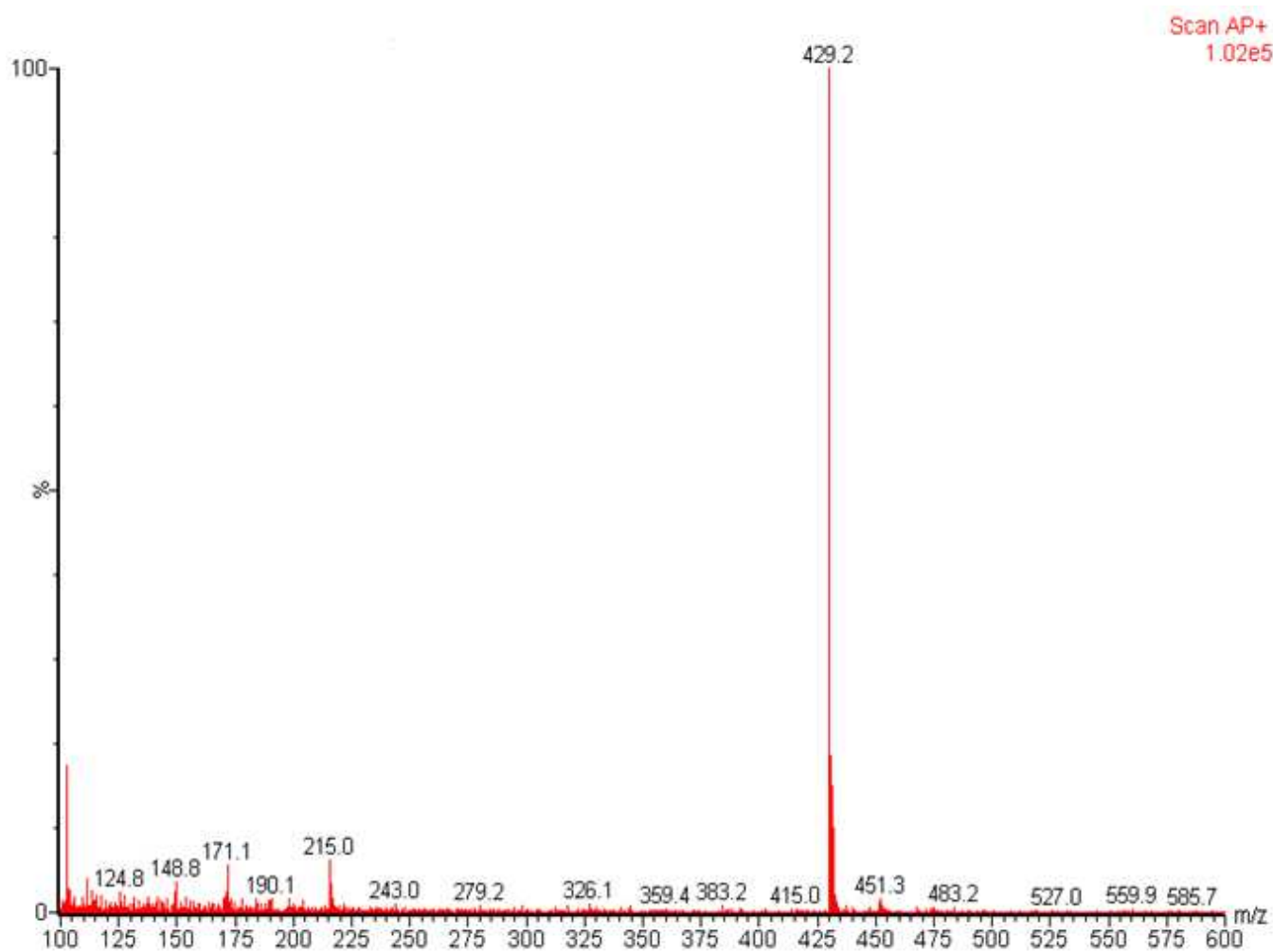


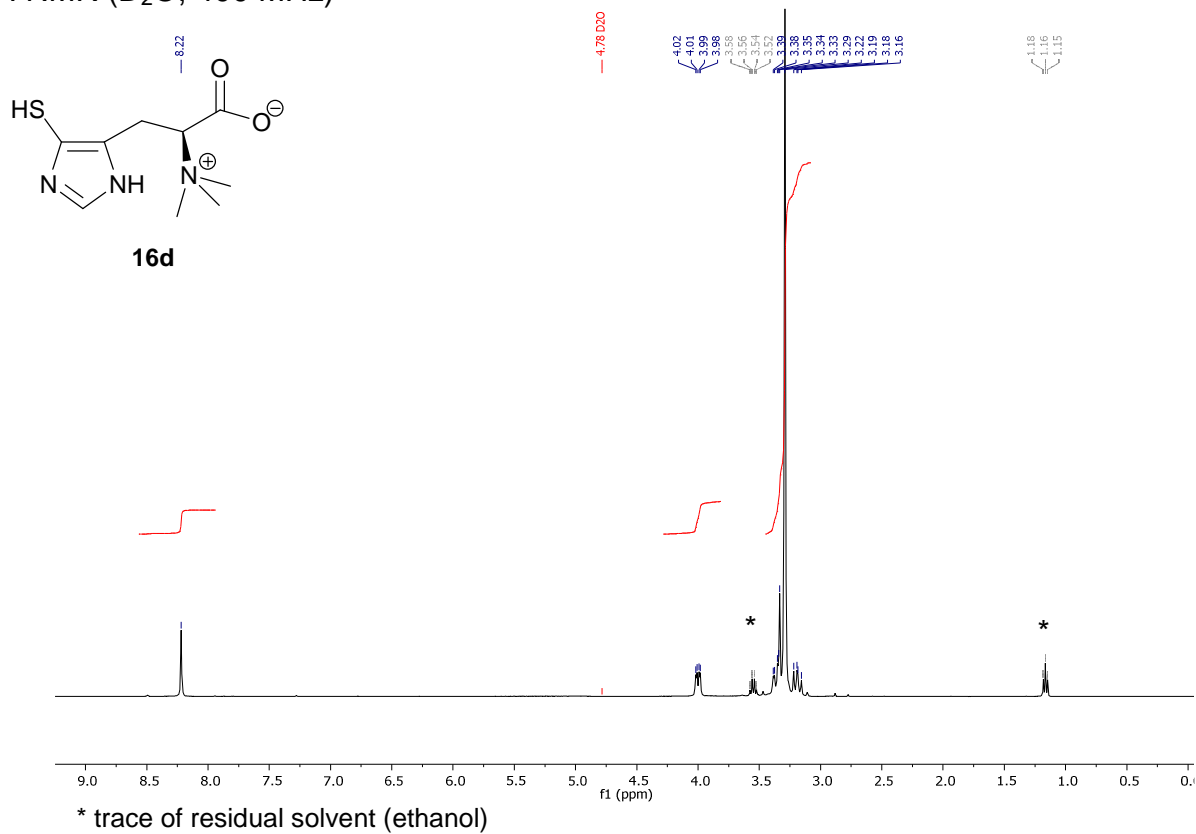
Disulfide 17c ^1H NMR (D_2O , 400 MHz)**17c x 2 HCl x 2 HBr** ^{13}C NMR (D_2O , 75 MHz)

LC (column B, see Experimental Part)



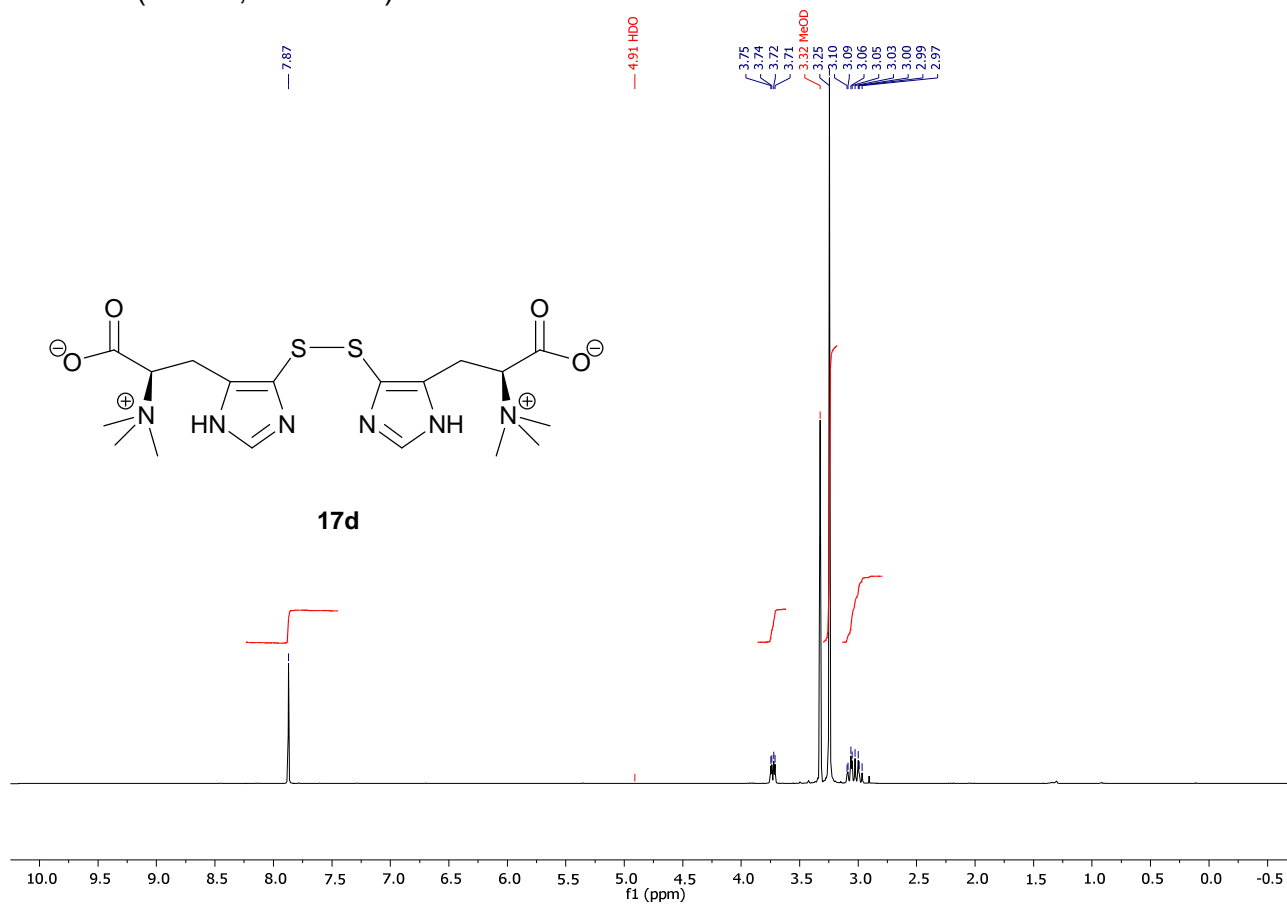
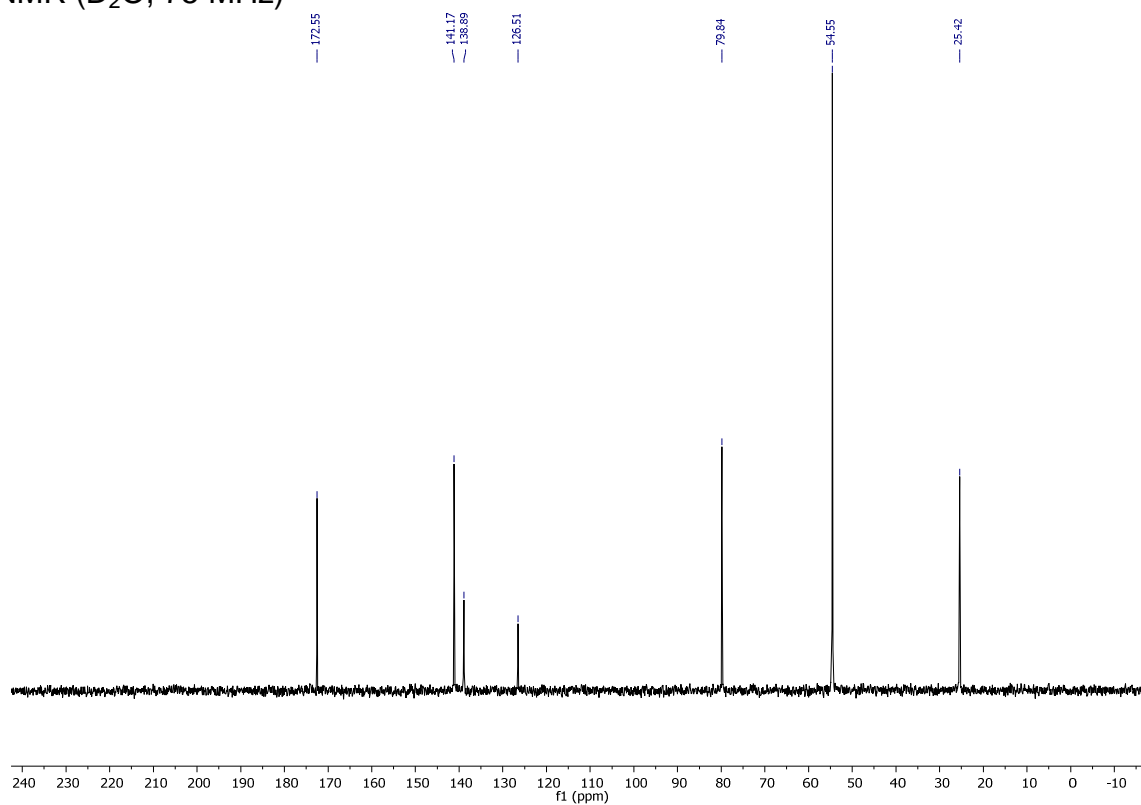
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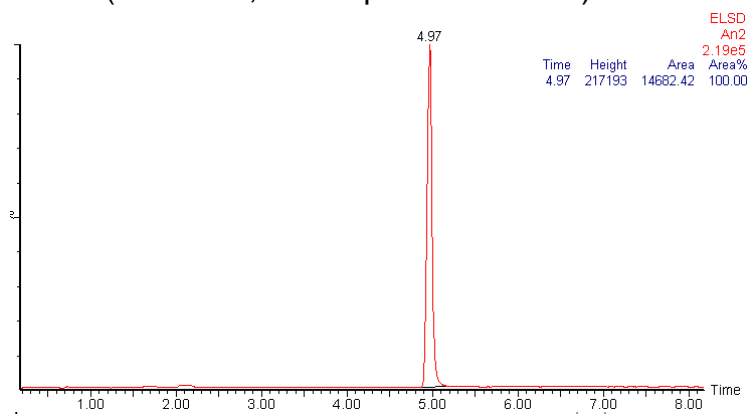
L-5-sulfanyl- α -N,N,N-trimethyl-histidine (16d) ^1H NMR (D_2O , 400 MHz)

APCI - MS:

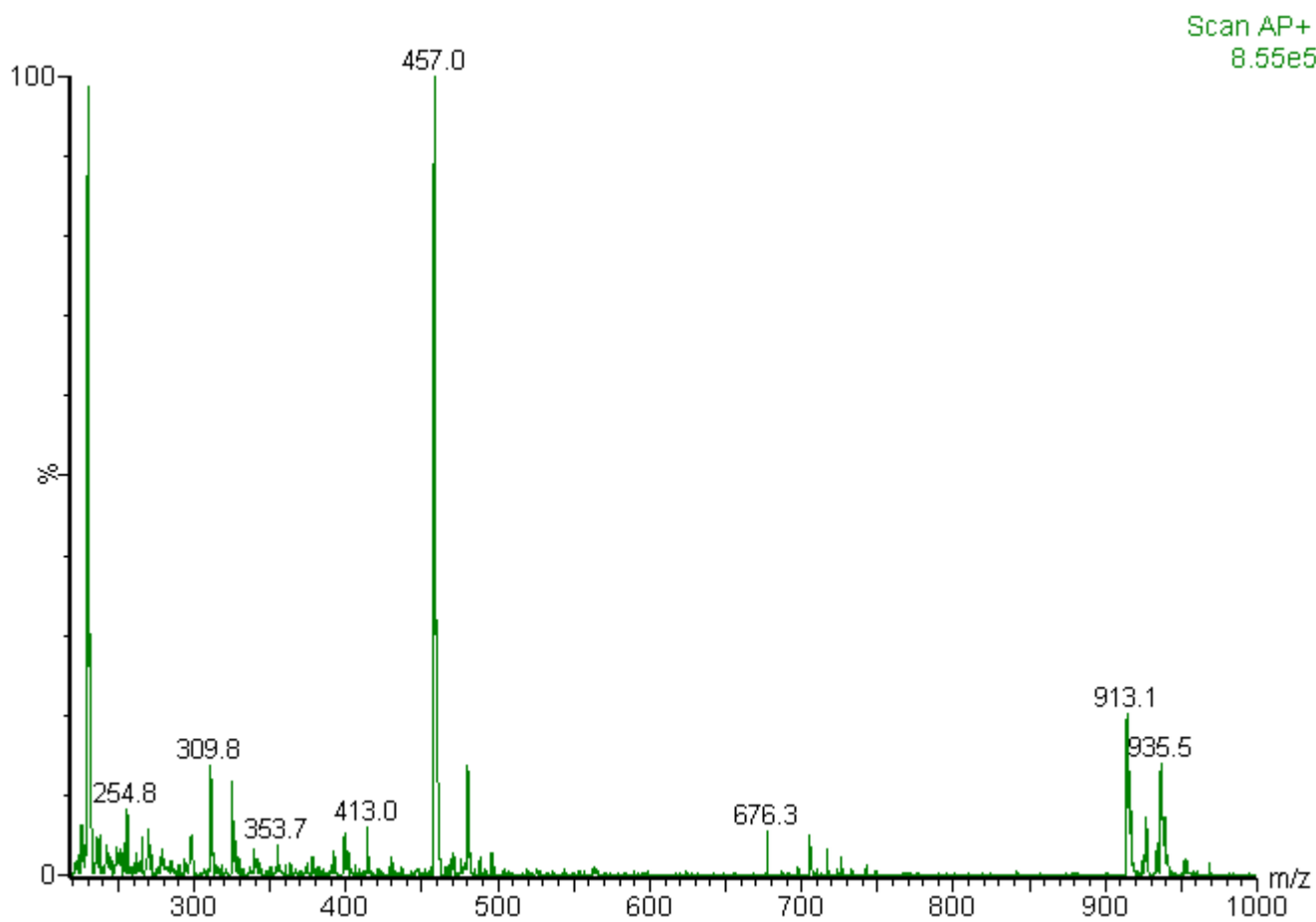


Disulfide 17d. ^1H NMR (MeOD, 400 MHz) ^{13}C NMR (D₂O, 75 MHz)

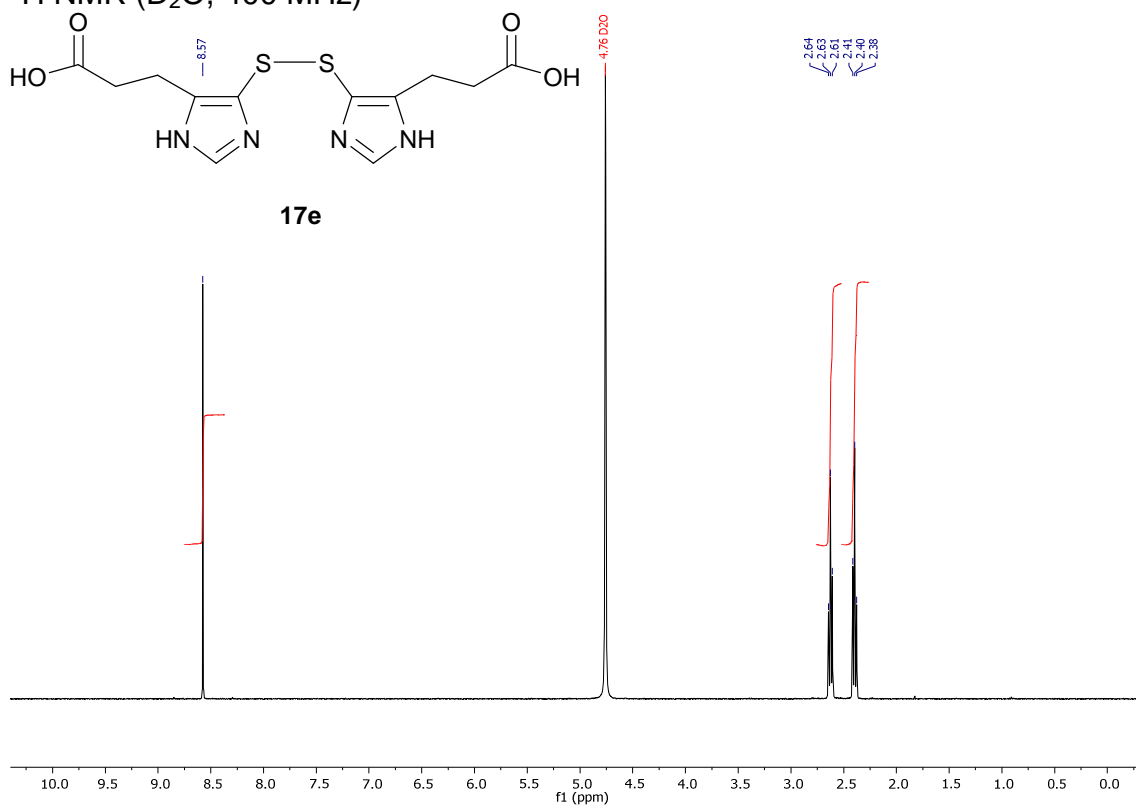
HPLC (column A, see Experimental Part)



APCI - MS:



Disulfide 17e

 ^1H NMR (D_2O , 400 MHz) ^{13}C NMR (D_2O , 75 MHz)