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Supporting information

Biosynthetic diversification of non-ribosomal peptides through activity-based protein profiling of adenylation domains

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Supplementary Discussion

Compound **5** contained D-Phe and L-Ser(Allyl), suggesting that the epimerization (E) domain of GrsA did not convert L-Ser(Allyl) to its D-configured form. The downstream C domain does not adhere to the inherent stereochemical preference toward a D-configured aminoacyl thioester.[1] The distinct substrate structure could impact the binding to the E and/or C domains. In fact, the GrsA-W239S/GrsB1 diketopiperazine (DKP) formation assay utilizing *O*-propargyl-L-Tyr and L-Pro yielded predominantly L,L-configured DKP.[2] The TE domain of GrsB catalyzes the dimerization of two assembled pentapeptides and subsequent cyclization, releasing GS (Fig. S2B).[3] Positions 1 and 5 are not amenable to changes in the stereogenic configuration for the elongation and/or cyclization reaction (Fig. S2).[3] Potential mechanisms for the biosynthesis of compound **5** have been postulated (Fig. S3). The first peptapeptide containing D-Phe, is composed of the synthetases and is transferred to the active-site Ser residue of the TE domain of GrsB. The second pentapeptide, containing L-Ser(Allyl), was presented by Ppant of the T domain adjacent to the TE domain of GrsB. The amino group of L-Ser(Allyl) of the second peptide loaded onto the T domain attacks the first pentapeptide on the TE domain, leading to a decapeptide bound to the active-site Ser residue of the TE domain. Subsequently, the TE domain catalyzes cyclization, resulting in the formation of compound **5**.

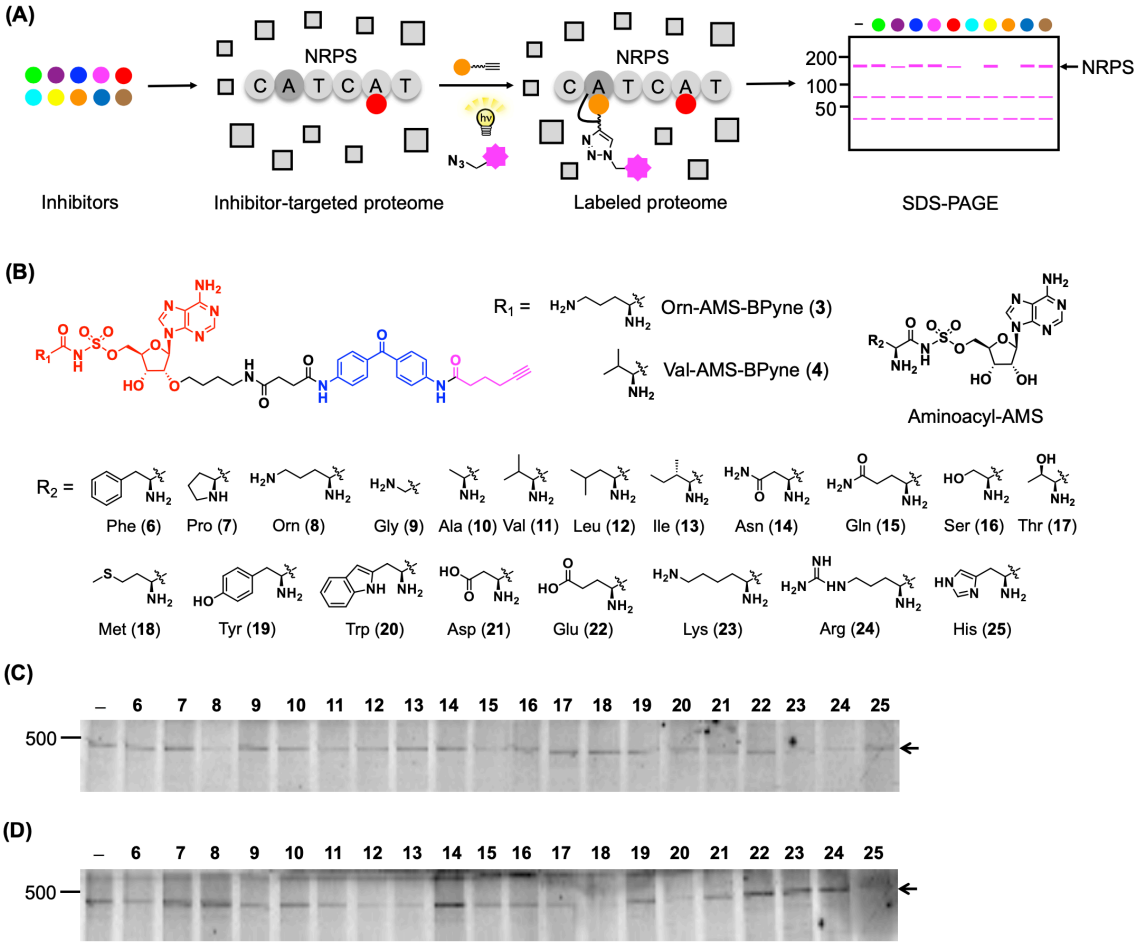


Figure S1. Functional profiling of adenylation domains in non-ribosomal peptide synthetases by competitive activity-based protein profiling (ABPP).[4] (A) Proteomic assays for the A-domains in NRPSs through competitive ABPP. In a general competitive ABPP experiment, bacterial proteomes treated with a competitor (or vehicle control) are reacted with an ABPP probe. Labeled proteomes are then irradiated, treated with a TAMRA-peg$_3$-azide under copper(I)-catalyzed azide-alkyne cycloaddition conditions, and resolved by SDS-PAGE. NRPS modules are composed of thiolation (T), adenylation (A), and condensation (C) domains. The rectangles represent nonspecific proteins. (B) Chemical structures of ABPP probes **3** and **4** targeting the Orn- and Val-activating domains of NRPS, respectively, and competitors **6**–**25**. (C) Competitive ABPP of **6**–**25** toward the Orn-activating domain of endogenous GrsB in the *A. migulanus* DSM 5759 proteome with probe **3**. (D) Competitive ABPP of **6**–**25** toward the Val-activating domain of endogenous GrsB in the *A. migulanus* DSM 5759 proteome with probe **4**.

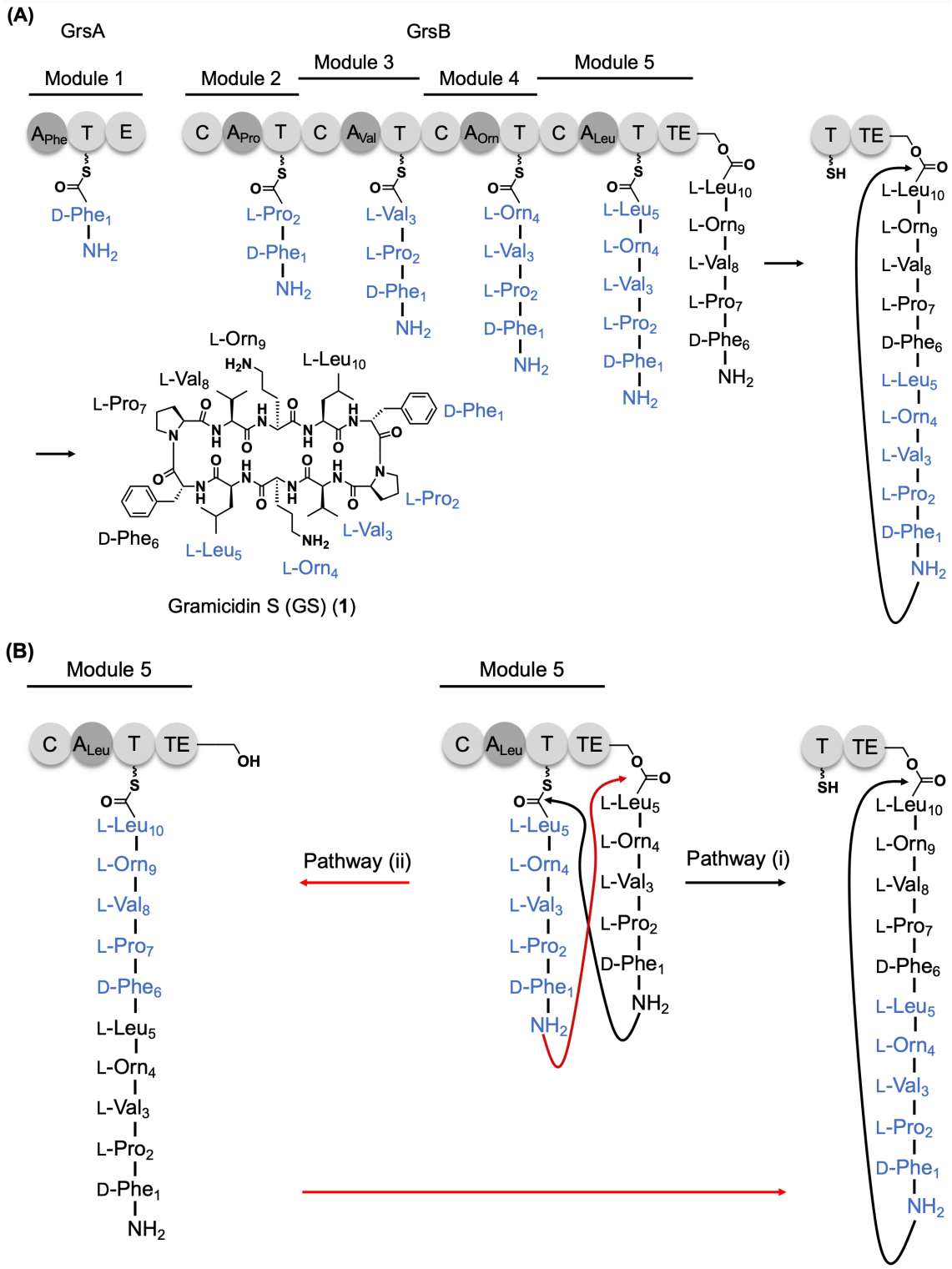


Figure S2. (A) Non-ribosomal peptide synthesis of the antibiotic gramicidin S (**1**). GrsA is a single NRPS module containing the L-Phe-activating A-domain and responsible for the incorporation of D-Phe into the peptide. GrsB consists of four NRPS modules containing the L-Pro, L-Val, L-Orn, and L-Leu-activating A-domains. (B) Possible mechanism of the dimerization

catalyzed by module 5 of GrsB.[3] In pathway (i), the pentapeptide bound to the active-site Ser residue of the TE domain attacks the thioester of pentapeptide-S-T domain, leading to a decapeptide bound to the active-site Ser residue of the TE domain. In pathway (ii), the amino group of pentapeptide-S-T domain attacks the ester of the pentapeptide bound to the active-site Ser residue of the TE domain. The decapeptide bound to the T domain is transferred to the active-site Ser residue of the TE domain. According to the literature, module 5 of GrsB utilizes the pathway (ii).[3] Modules comprise thiolation (T), adenylation (A), epimerization (E), condensation (C), and thioesterase (TE) domains. The TE domain catalyzes the dimerization of assembled pentapeptides and cyclization to produce gramicidin S.

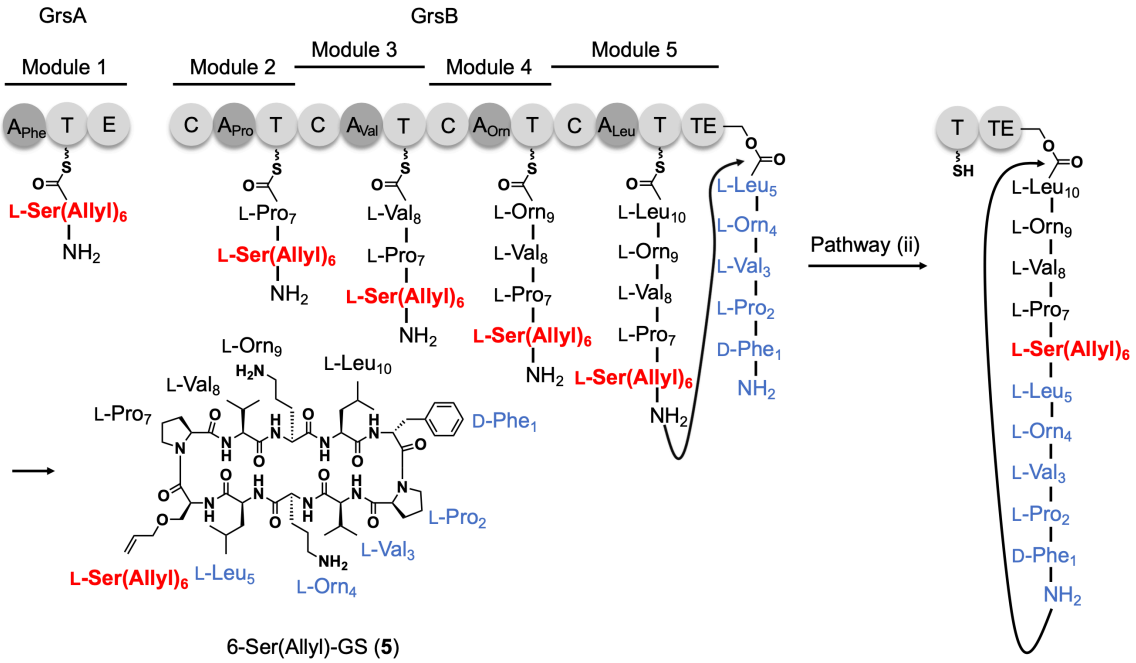


Figure S3. Possible mechanism of the biosynthesis of 6-Ser(Allyl)-GS (**5**). The first pentapeptide containing D-Phe, is composed of the synthetases and is transferred to the active-site Ser residue of the TE domain of GrsB. The second pentapeptide, containing L-Ser(Allyl), is presented by Ppant of the T domain adjacent to the TE domain of GrsB. Utilizing the pathway (ii) (Fig. S1B), the TE domain catalyzes the dimerization of assembled pentapeptides and cyclization to produce gramicidin S. Modules comprise thiolation (T), adenylation (A), epimerization (E), condensation (C), and thioesterase (TE) domains.

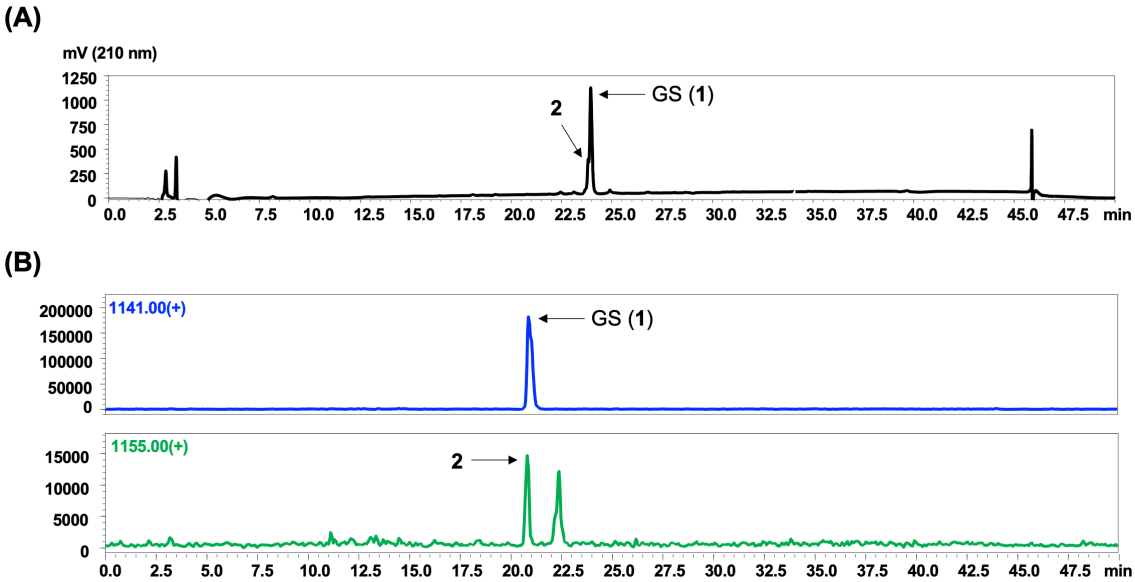


Figure S4. HPLC data (A) and LC-MS data (B) of the extracts of *A. migulanus* ATCC 9999 cultivated in a chemically defined media F3/6.

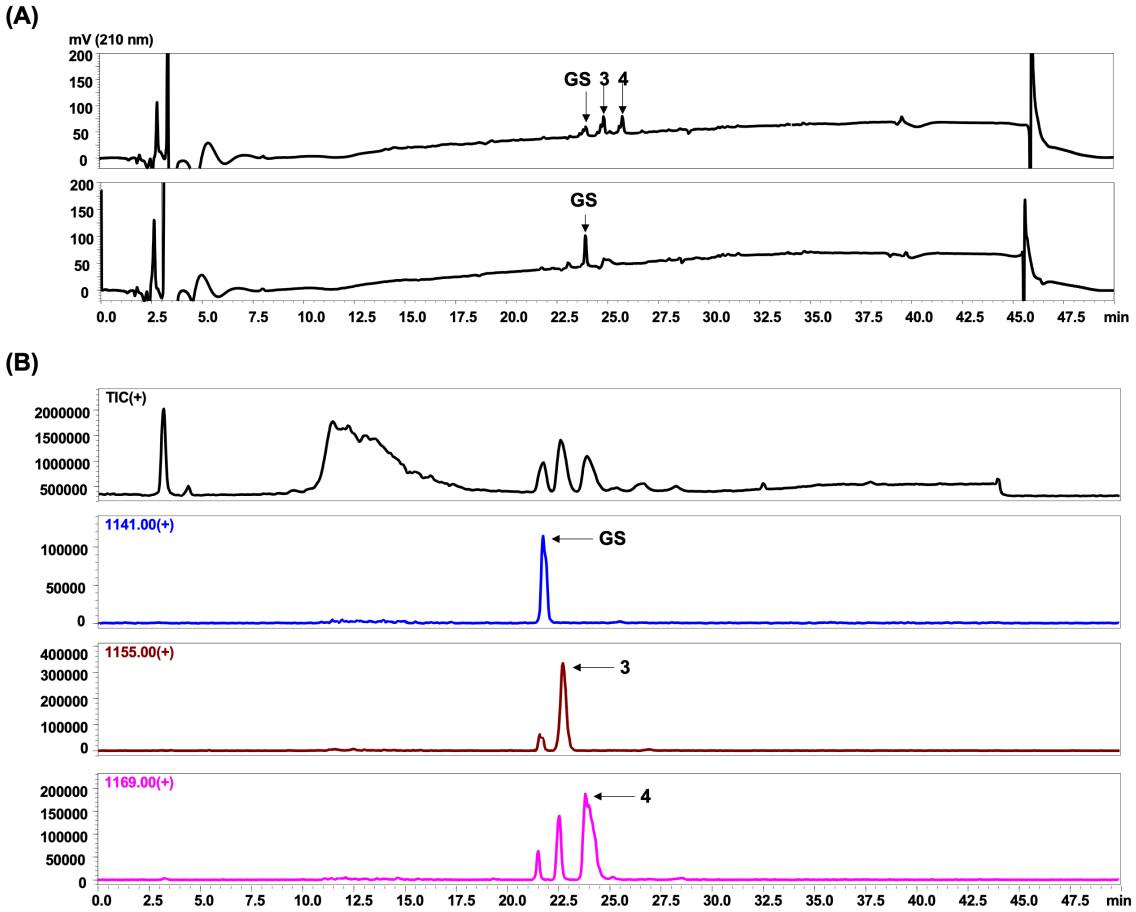


Figure S5. (A) HPLC data of the extracts of *A. migulanus* ATCC 9999 cultivated in the presence of Ile (0.5% w/v) (upper) and without the addition of L-Ile (bottom). (B) LC-MS data of the extracts of *A. migulanus* ATCC 9999 cultivated in the presence of Ile (0.5% w/v).

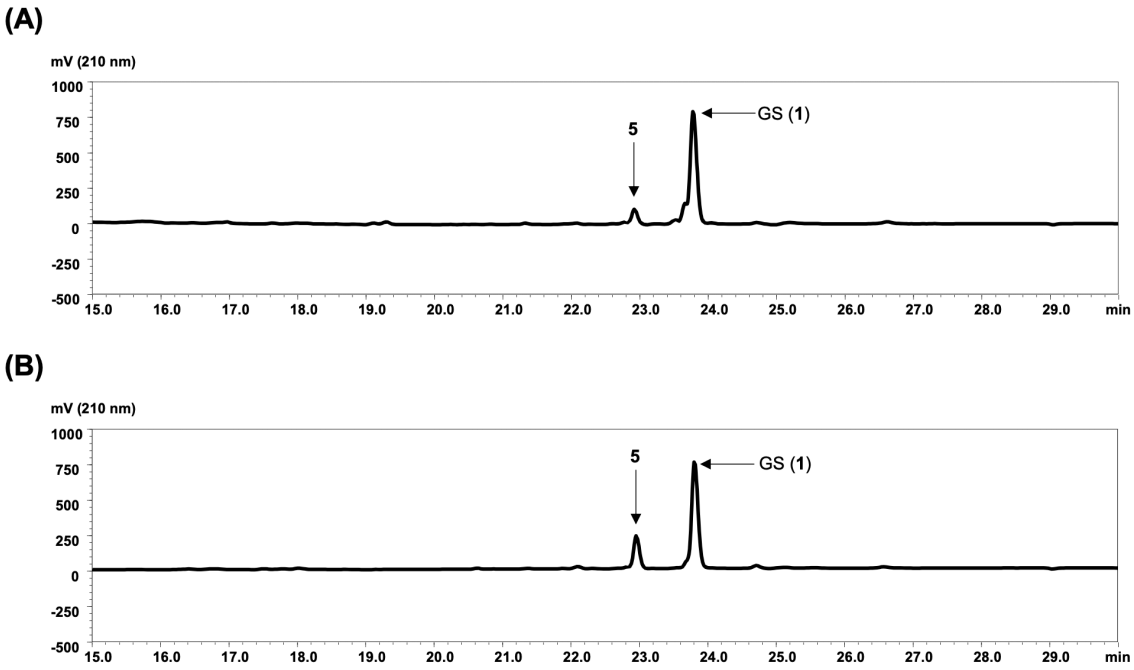


Figure S6. HPLC data of the extracts of *A. migulanus* ATCC 9999 cultivated in the presence of Ser(Allyl) (0.5% w/v) (A) and Ser(Allyl) (1% w/v) (B). We observed that Ser(Allyl) impaired the growth of A. migulanus ATCC 9999 at concentrations of 2% (w/v) and above.

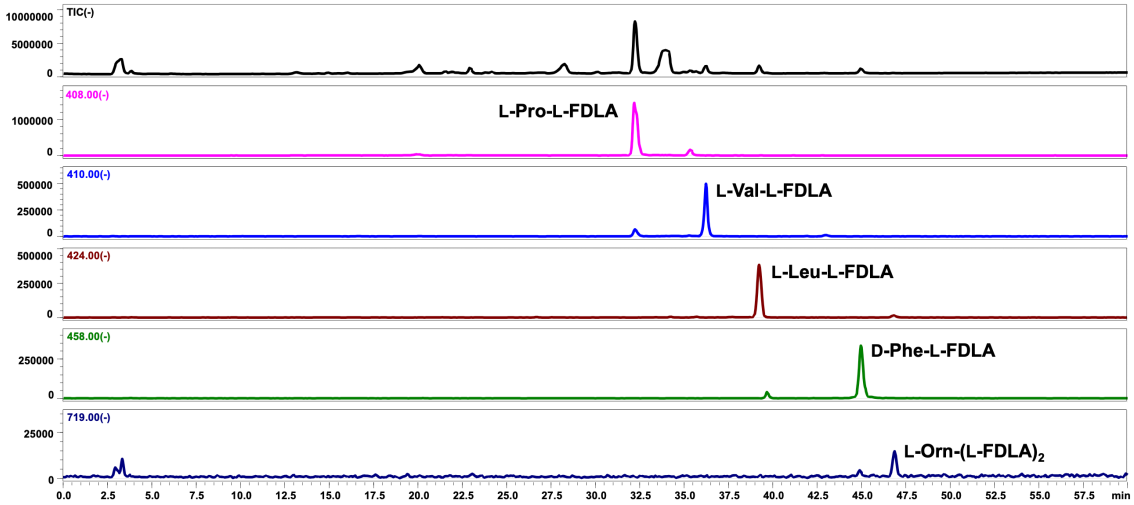


Figure S7. Advanced Marfey's analysis of GS (**1**). Mass chromatograms (negative ion mode) of L-FDLA derivatives of the hydrolyzed GS (**1**).

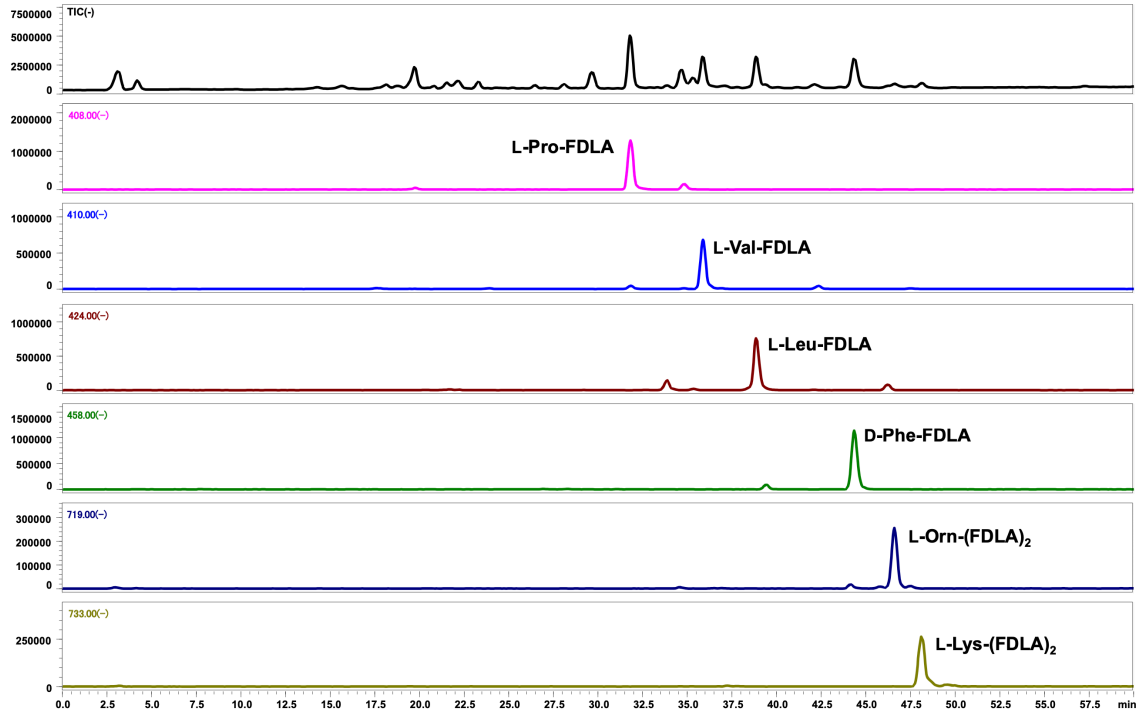


Figure S8. Advanced Marfey's analysis of 4-Lys-GS (**2**). Mass chromatograms (negative ion mode) of L-FDLA derivatives of the hydrolyzed 4-Lys-GS (**2**).

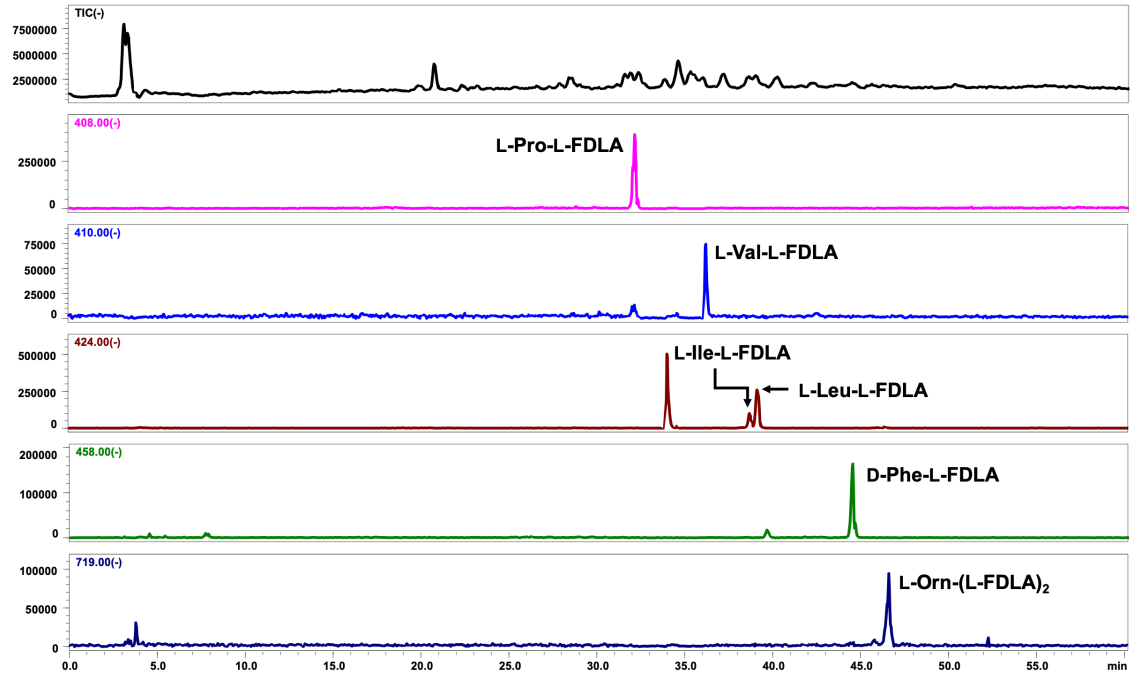


Figure S9. Advanced Marfey's analysis of 8-Ile-GS (**3**). Mass chromatograms (negative ion mode) of L-FDLA derivatives of the hydrolyzed 8-Ile-GS (**3**).

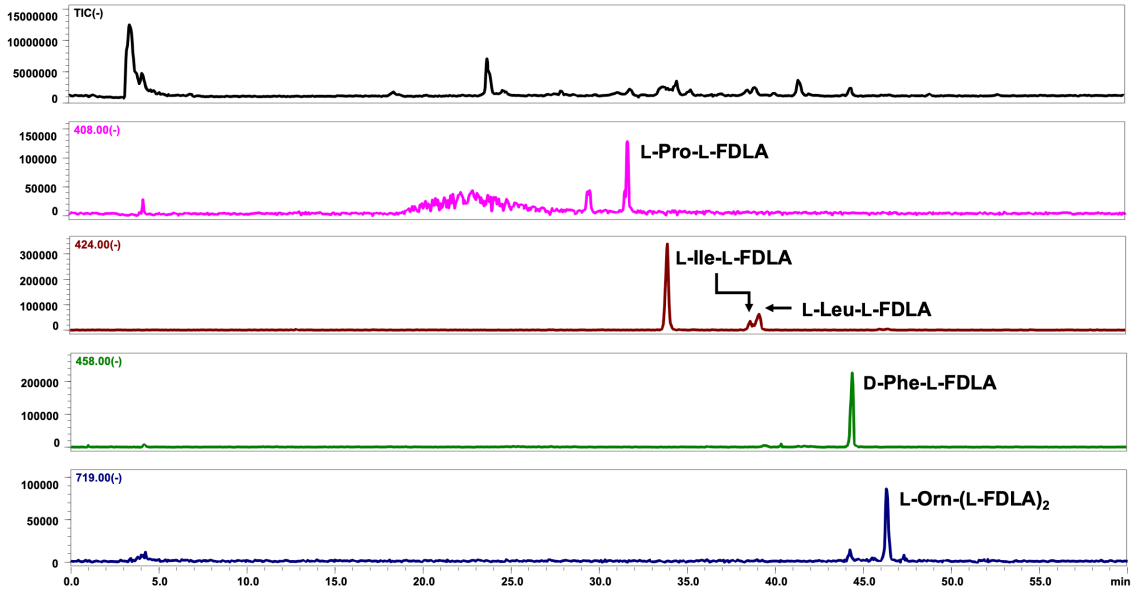


Figure S10. Advanced Marfey's analysis of 3,8-Ile-GS (**4**). Mass chromatograms (negative ion mode) of L-FDLA derivatives of the hydrolyzed 3,8-Ile-GS (**4**).

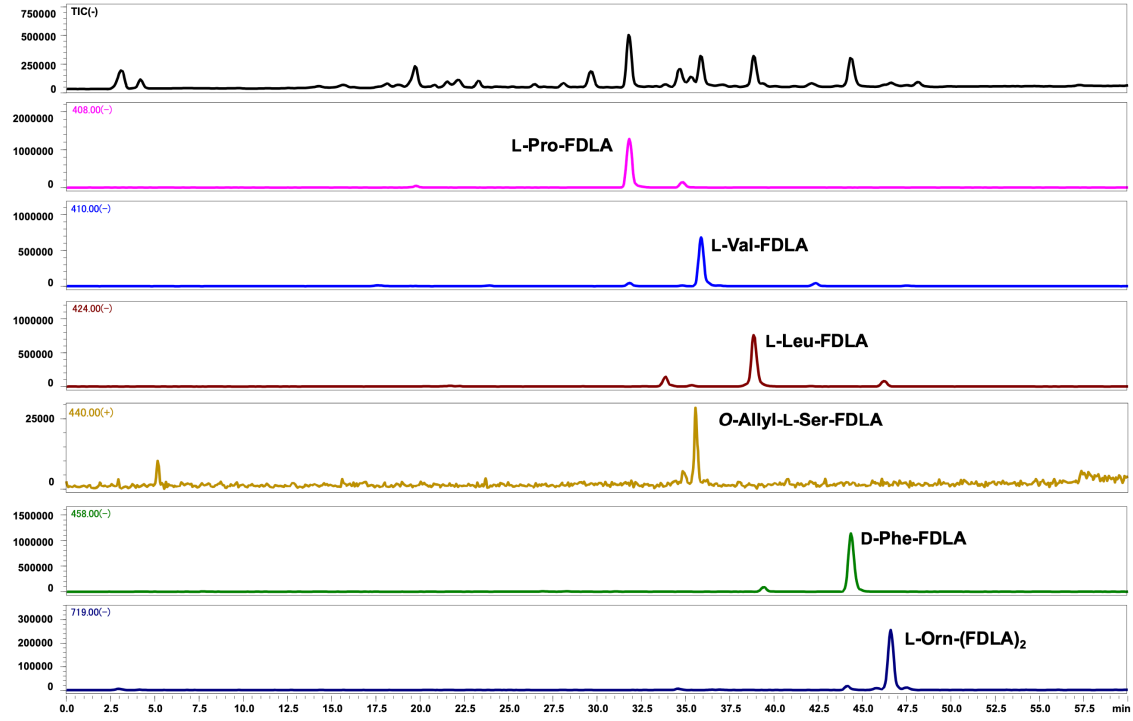


Figure S11. Advanced Marfey's analysis of 6-Ser(Allyl)-GS (**5**). Mass chromatograms of L-FDLA derivatives of the hydrolyzed 6-Ser(Allyl)-GS (**5**).

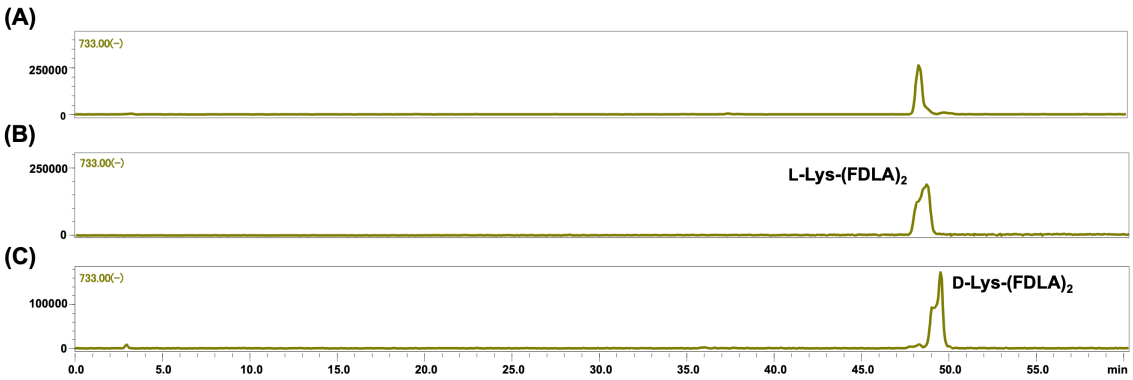


Figure S12. LC-MS analysis of L-Lys Marfey's derivative. (A) Mass chromatograms (negative ion mode) of L-FDLA derivatives of the hydrolyzed 4-Lys-GS (**2**). (B) Authentic sample of L-Lys-L-FDLA. (C) Authentic sample of D-Lys-L-FDLA.

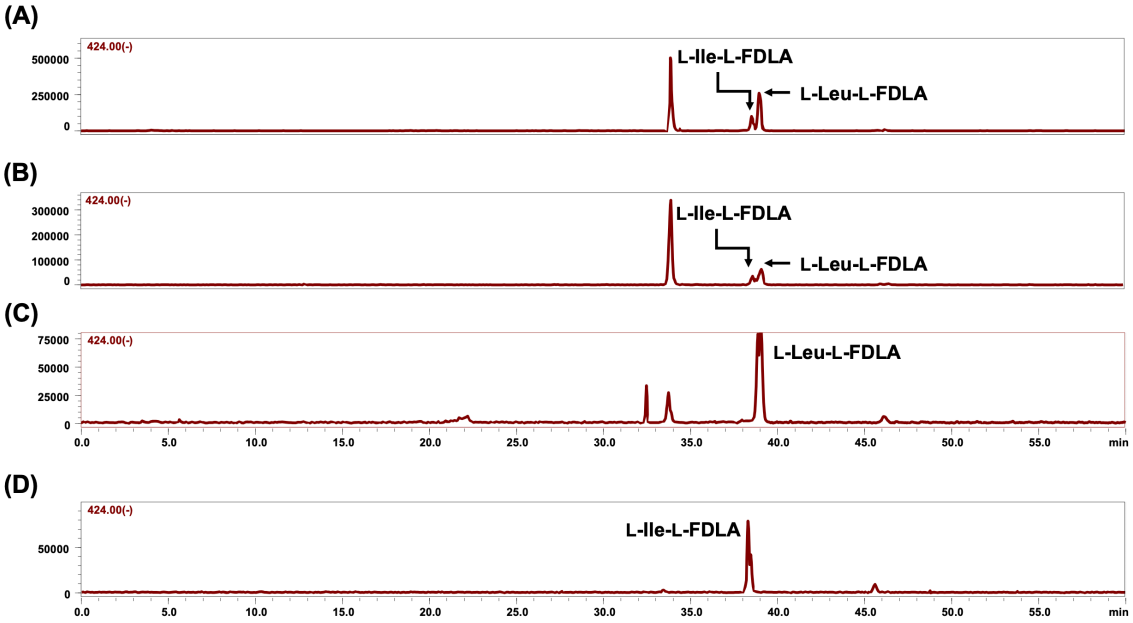


Figure S13. LC-MS analysis of L-Leu and L-Ile Marfey's derivatives. Mass chromatograms (negative ion mode) of L-FDLA derivatives of the hydrolyzed 8-Ile-GS (**3**) (A) and the hydrolyzed 3,8-Ile-GS (**4**) (B). (C) Authentic sample of L-Leu-L-FDLA. (D) Authentic sample of L-Ile-L-FDLA.

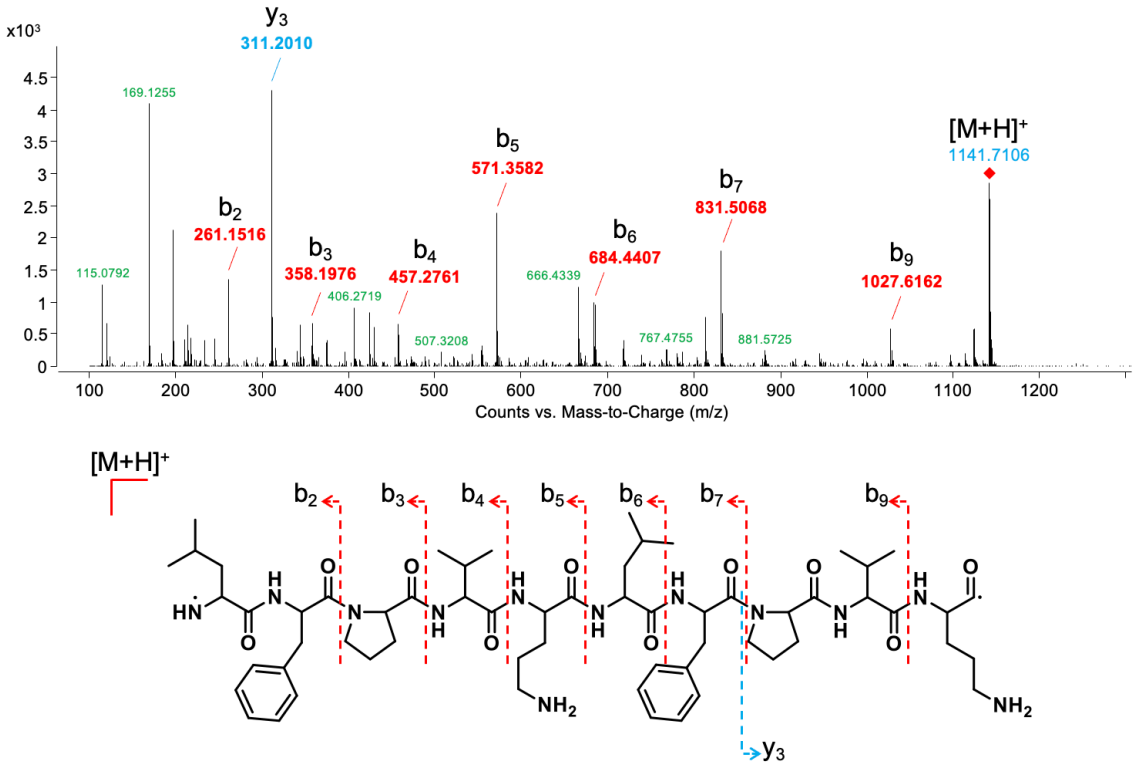


Figure S14. MS/MS analysis of GS (**1**). CID 55 eV.

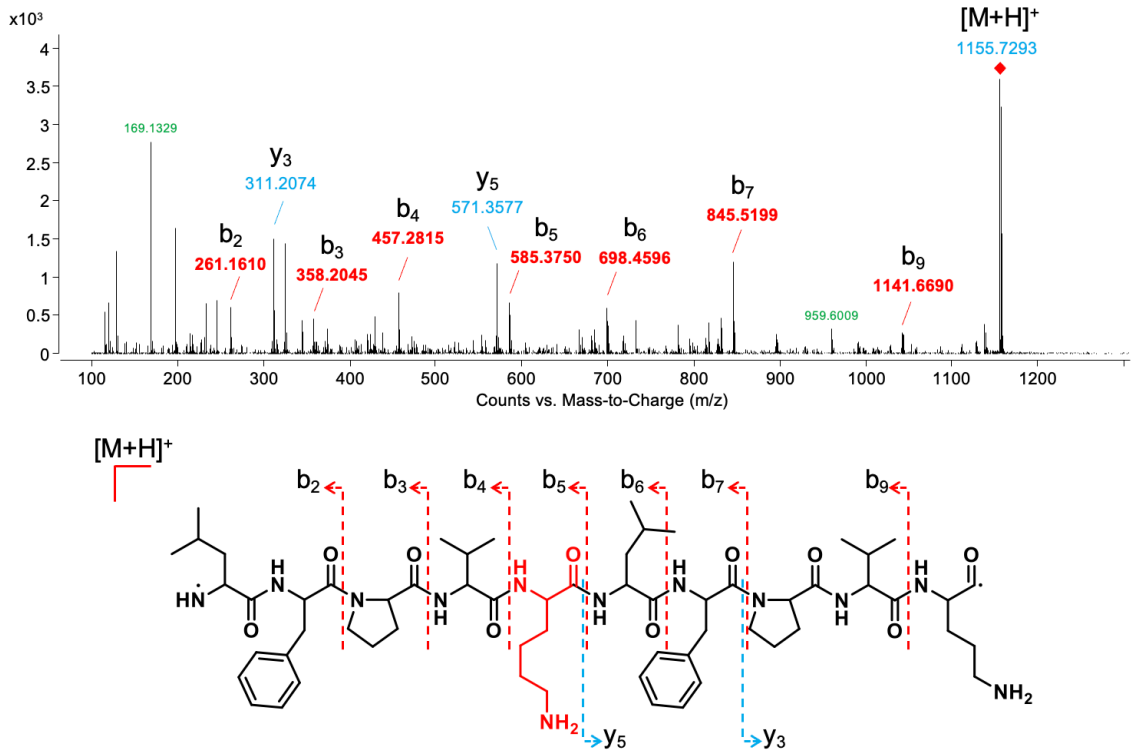


Figure S15. MS/MS analysis of 4-Lys-GS (**2**). CID 55 eV.

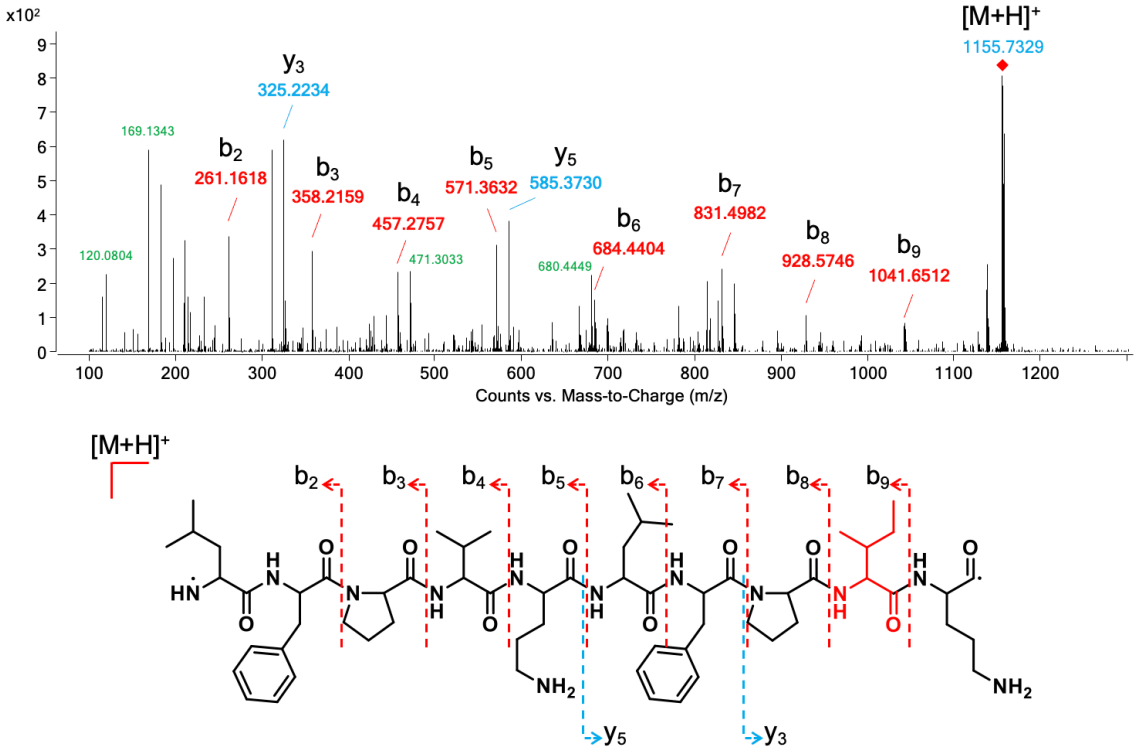


Figure S16. MS/MS analysis of 8-Ile-GS (**3**). CID 50 eV.

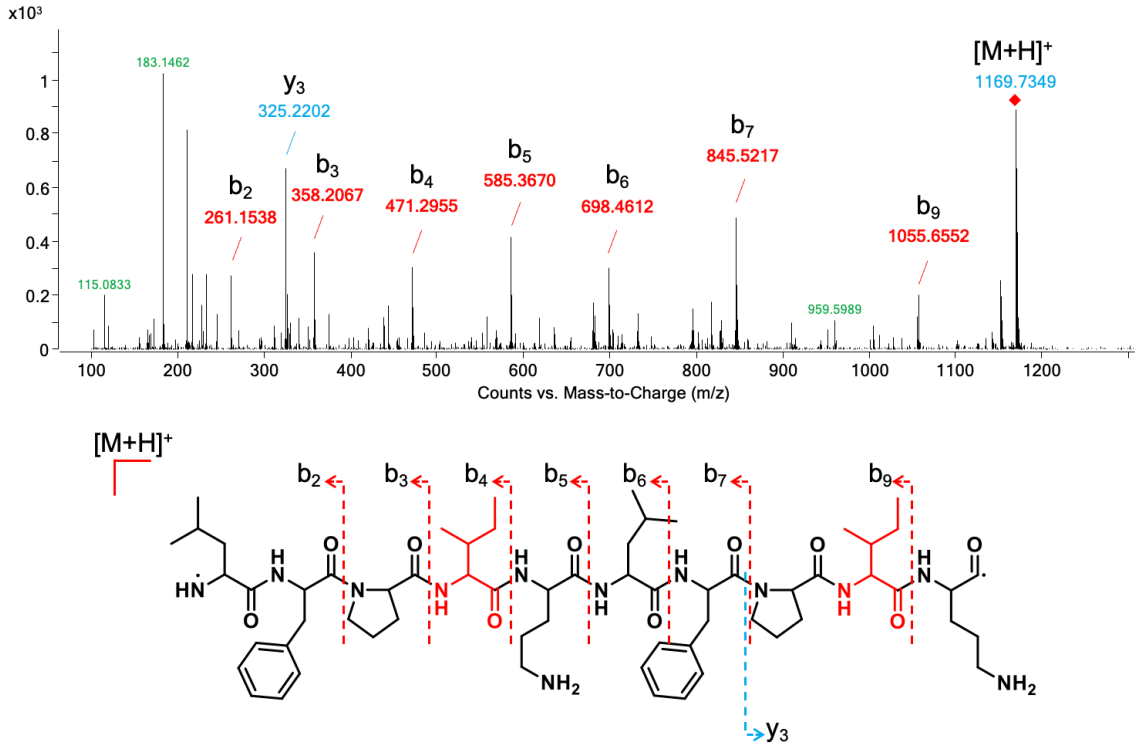


Figure S17. MS/MS analysis of 3,8-Ile-GS (**4**). CID 55 eV.

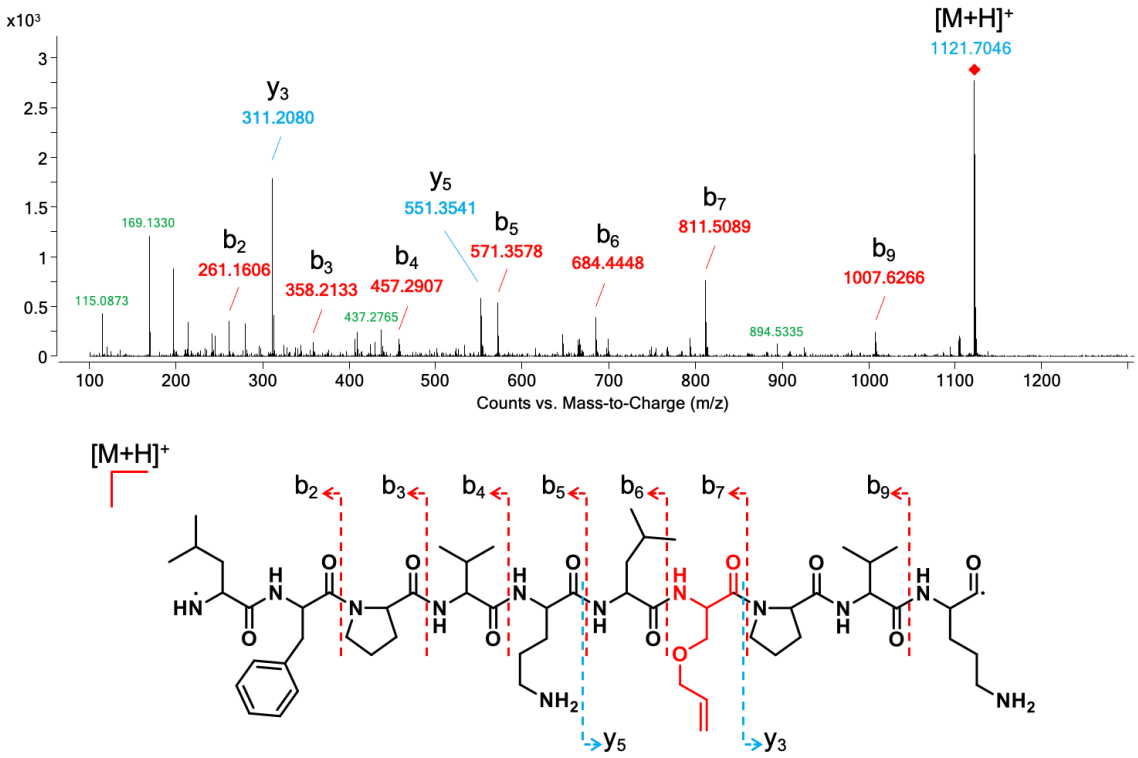


Figure S18. MS/MS analysis of 6-Ser(Allyl)-GS (**5**). CID 55 eV.

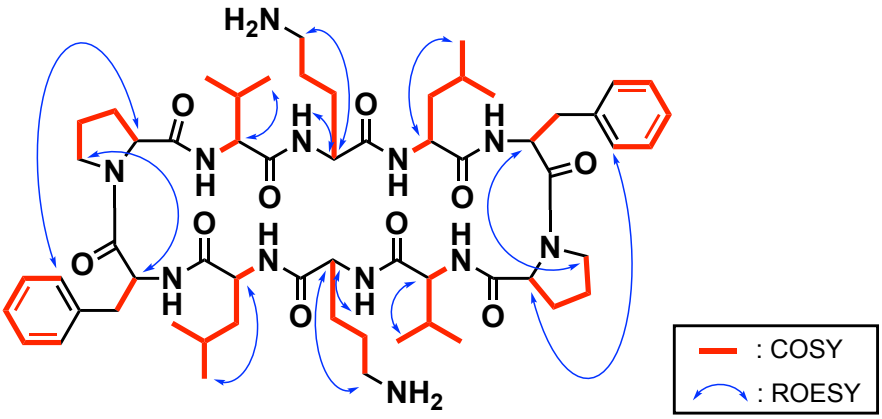


Figure S19. COSY and ROESY correlations for GS (**1**).

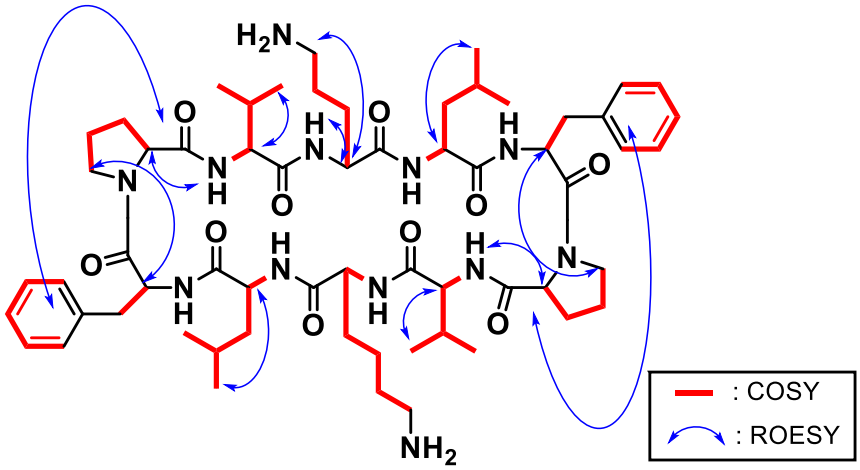


Figure S20. COSY and ROESY correlations for 4-Lys-GS (**2**).

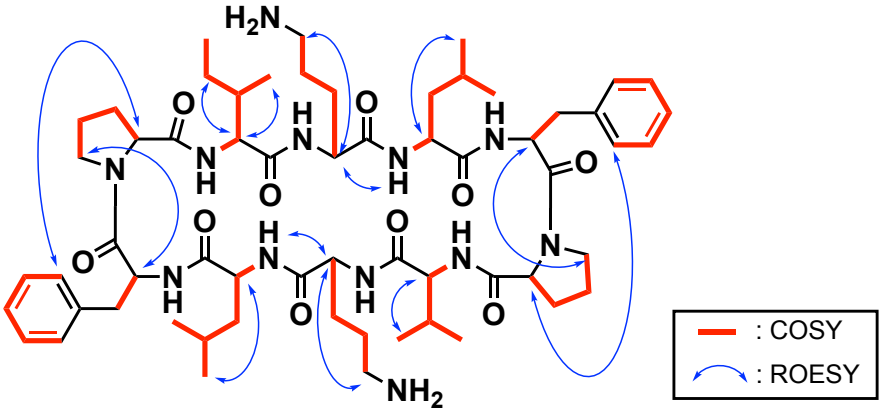


Figure S21. COSY and ROESY correlations for 8-Ile-GS (**3**).

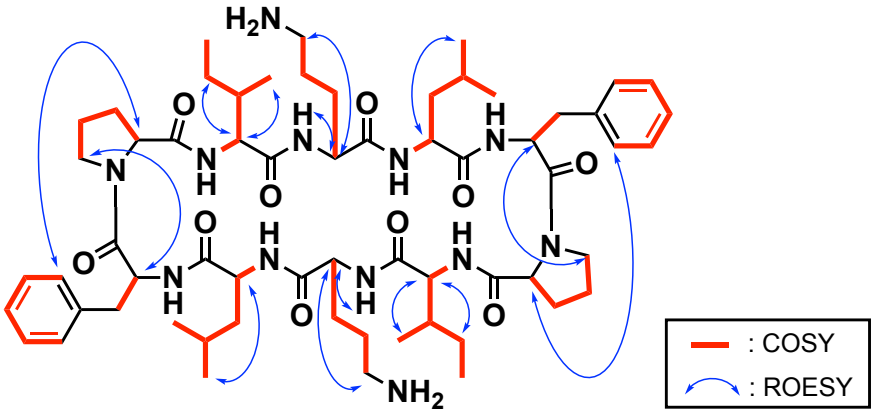


Figure S22. COSY and ROESY correlations for 3,8-Ile-GS (**4**).

Table S1. ^{1}H NMR data (800 MHz) for compounds **1-5** in CD_3OD.

position		GS (**1**)	4-Lys-GS (**2**)				8-Ile-GS (**3**)			3,8-Ile- GS (**4**)			6-Ser(Allyl)-GS (**5**)		
Leu	H_α	4.66 (td, *J* = 9.8, 6.7)	4.62/4.65 (*J* =9.2, 7.2)				4.65/4.66 (td, *J* =9.4, 7.4)			4.66 (td, *J* =9.2, 7.4)			4.61–4.66 (m)		
	H_βa	1.40 (ddd, *J* = 13.6, 9.8, 6.7)	1.28–2.05 (m)				1.40 (br dt like, *J* = *ca.* 13.0, 7.4)			1.39 (td, *J* =7.4, 6.7)			1.25–1.77 (m)		
	H_βb	1.48–1.55 (m)	1.28–2.05 (m)				1.46–1.64 (m)			1.47–1.63 (m)			1.25–1.77 (m)		
	N*H*	8.72 (d, *J* = 9.8)	8.74/8.77 (d, *J* = 9.2)				8.72/8.74 (each 1H, d, *J* = 9.4)			8.73 (br d, *J* = ca. 9.2)			8.70/8.72 (*J* = 9.3)		
Orn	H_α	4.97 (td, *J* = 9.2, 5.2)	4.93 (td, *J* = 9.2, 5.1)	Lys	H_α	4.97 (td, *J* = 8.9, 6.3)	4.97/4.99 (td, *J* = 9.9, 5.4)			5.02 (td, *J* = 9.9, 5.3)			4.94/4.95 (dd, *J* = 10.4, 5.1)		
	H_βa	1.67–1.80 (m)	1.28–2.05 (m)		H_βa	1.28–2.05 (m)	1.67–1.80 (m)			1.47–1.63 (m)			1.91–2.09 (m)		
	H_βb	2.05 (m)	1.28–2.05 (m)		H_βb	1.28–2.05 (m)	1.98–2.15 (m)			2.02–2.07 (m)			1.91–2.09 (m)		
	$H_\gamma$ab	1.48–1.55 (m)	1.28–2.05 (m)		$H_\gamma$ab	1.28–2.05 (m)	1.67–1.80 (m)			1.74–1.79 (m)			1.25–1.77 (m)		
	H_δa	2.86 (ddd, *J* = 12.0, 10.0, 6.7)	2.86–2.90 (m)		$H_\delta$ab	1.28–2.05 (m)	2.86–2.90 (m)			2.85–2.89 (m)			2.79/2.84 (td, *J* = 9.7, 6.7)		
	H_δb	3.03 (ddd, *J* = 12.0, 6.8, 6.2)	2.99–3.09 (m)		$H_\varepsilon$ab	2.99–3.09 (m)	3.02–3.06 (m)			3.02–3.06 (m)			2.96/3.01 (td, *J* = 9.7, 6.7)		
	N*H*	8.65 (d, *J* = 9.2)	8.49 (br d like, *J* = *ca.* 9.2)		N*H*	8.56 (br d like, *J* = *ca.* 8.9)	8.64/8.67 (br d, *J* = *ca.* 9.9			8.66 (br d, *J* = ca. 9.9)					
Val	H_α	4.13 (t, *J* = 11.0)	4.12/4.20 (t, *J* = 8.8)				4.14 (t, *J* = 8.8)	Ile	H_α 4.20 (t, *J* = 9.0)	H_α 4.19 (t, *J* = 9.0)	Ile	H_α 4.19 (t, *J* = 9.0)	4.12/4.15 (t, *J* = 8.8, 6.6)		
	N*H*	7.69 (d, *J* = 11.0)	7.64/7.66 (d, *J* = 8.8)				7.70 (br d, *J* = *ca.* 8.8)		H_β 1.98–2.15 (m) H_γa 1.19–1.33 (m) H_γb 1.67–1.80 (m) N*H* 7.69 (br d, *J* = *ca.* 9.0)	H_β 2.10–2.16 (m) H_γa 1.21–1.25 (m) H_γb 1.47–1.63 (m) N*H* 7.72 (d, *J* = 9.0)			7.67/7.74 (br d like, *J* = ca. 8.8)		
Pro	H_α	4.34 (dd, *J* = 7.7, 1.9)	4.34/4.36 (dd, *J* = 7.3, 1.0)				4.33 (br t like *J* =*ca.* 7.0)			4.33 (dd, *J* = 8.0, 2.1)			4.32 (br-d like, *J* = *ca.* 6.6)/4.32 (br-t like, *J* = *ca.* 5.5)		
	H_βa	1.67–1.80 (m)	1.28–2.05 (m)				1.67–1.80 (m)			1.65–1.71 (m)			1.25–1.77 (m)		
	H_βb	2.00 (td, *J* = 5.2, 1.9)	1.28–2.05 (m)				1.98–2.15 (m)			1.98–2.01 (m)			1.91–2.09 (m)		
	H_γa	1.67–1.80 (m)	1.28–2.05 (m)				1.46–1.64 (m)			1.47–1.63 (m)			1.25–1.77 (m)		
	H_γb	1.67–1.80 (m)	1.28–2.05 (m)				1.67–1.80 (m)			1.65–1.71 (m)			1.95–2.09 (m)		
	H_δa	2.48 (br ddd like, *J* = ca. 9.4, 7.7)	2.46/2.48 (br q like, *J* = *ca.* 8.5)				2.48 (br q like, *J* = *ca.* 9.2)			2.48 (br q like, *J* = ca. 9.6)			2.46/3.78 (q, *J* = 9.3)		
	H_δb	3.73 (td, *J* = 9.4, 2.1)	3.69–3.74 (br m)				3.72/3.73 (br dt like, *J* = *ca.* 8.1, 2.5)			3.72 (br ddd like, *J* = *ca.* 9.6, 8.4, 3.0)			3.72/4.08 (td, *J* = 9.3, 2.6)		
D-Phe	H_α	4.50 (dd, *J* = 11.5, 5.1)	4.47–4.50 (br m)				4.50 (dd, *J* = 11.3, 5.0)			4.50 (dd, *J* = 11.6, 5.0)			4.48 (dd, *J* = 12.0, 5.0)	Ser(Allyl)	H_α 4.61–4.66 (m)
	H_βa	2.94 (t, *J* = 11.5)	2.93/2.94(t, *J* = 11.2)				2.93 (t, *J* = 11.0)			2.93 (t, *J* = 11.6)			2.92 (t, *J* = 12.0)		H_βa 3.67 (1H, t, *J* = 9.1)
	H_βb	3.09 (dd, *J* = 11.5, 5.1)	2.99–3.09 (m)				3.09 (dd, *J* = 12.5, 4.9)			3.08 (dd, *J* = 11.6, 5.0)			3.07 (dd, *J* = 12.0, 5.0)		H_βb 3.70 (1H, dd, *J* = 9.1, 6.2)

Table S2. ^{13}C NMR data (200 MHz) for compounds **1-5** in CD_3OD.

position		GS (**1**)	4-Lys-GS (**2**)		8-Ile-GS (**3**)		3,8-Ile- GS (**4**)	6-Ser(Allyl)-GS (**5**)	
Leu	C_α	51.5	51.6		51.5/51.6		51.5	51.41/51.43	
	C_β	42.1	42.0/42.1		42.0/42.1		42.1	41.99/42.02	
Orn	C_α	52.5	52.6	C_α 53.6	52.4/52.5		52.4	52.5	
	C_β	30.8	30.6	C_β 30.8	30.7/30.8		30.8	30.8	
	C_γ	24.6	24.6	Lys C_γ 23.7	24.6		24.6	24.5/24.6	
	C_δ	40.4	40.4	C_δ 28.5	40.4		40.4	40.4	
				Cε 40.9					
Val	C_α	60.5	60.3/60.5		60.5	Ile C_α 59.0 C_β 37.8 C_γ 26.3	Ile C_α 59.0 C_β 37.7 C_γ 26.4	60.5/60.6	
Pro	C_α	62.1	61.9/62.1		62.1		62.1	62.1/62.4	
	C_β	30.7	30.7		30.7/30.8		30.7	30.7	
	C_γ	24.5	24.5		24.5		24.5	24.5/24.9	
	C_δ	48.0	47.8/48.0		48.0/48.1		48.1	48.0/48.7	
D-Phe	C_α	55.9	55.8/55.9		55.9/56.0		56.0	55.9	Ser(Allyl) C_α 53.9
	C_β	37.3	37.3/37.4		37.3		37.3	37.3	C_β 69.3

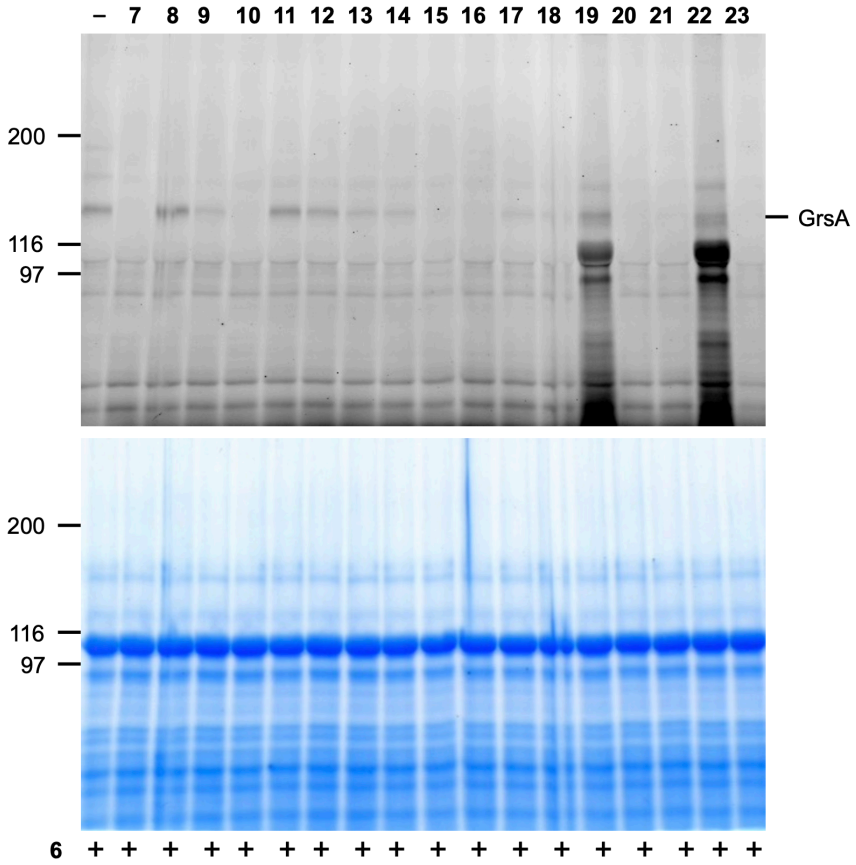


Figure S23. Full images of SDS-PAGE gels from Figure 3A. Competitive ABPP of **7**–**23** toward the A -domain of GrsA. Assessment of the inhibition potency (100 μM) in the *A. migulanus* ATCC 9999 proteome (2 mg/mL) with Phe-AMS-BPyne (**6**) (1 μM). The top image depicts the fluorescence observed with λ_{ex} = 532 nm and λ_{em} = 580 nm and the bottom denotes total protein content by staining with Coomassie (Colloidal Coomassie Blue Stain).

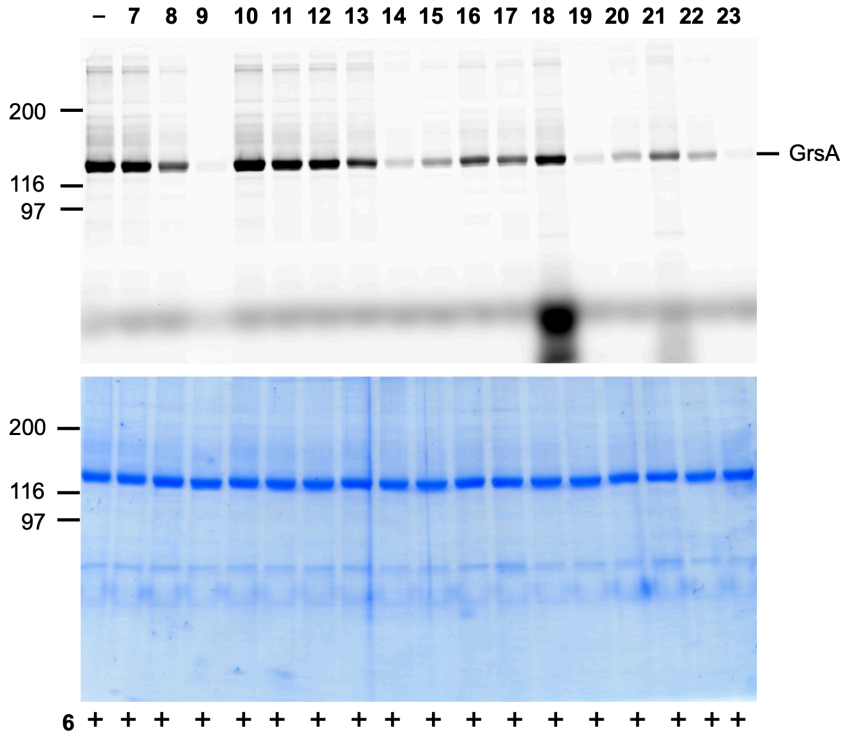


Figure S24. Full images of SDS-PAGE gels from Figure 3B. Competitive ABPP of **7**–**23** toward the A -domain of GrsA. Assessment of the inhibition potency (100 μM) in GrsA (1 μM) with Phe-AMS-BPyne (**6**) (1 μM). The top image depicts the fluorescence observed with λ_{ex} = 532 nm and λ_{em} = 580 nm and the bottom denotes total protein content by staining with Coomassie (Colloidal Coomassie Blue Stain).

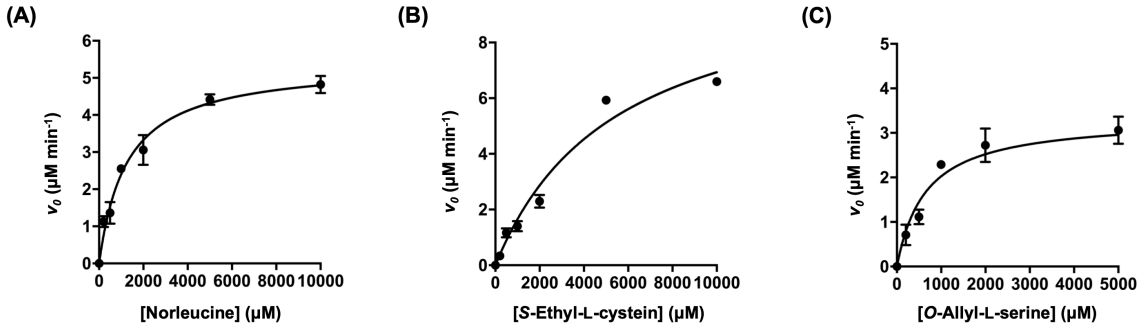


Figure S25. Steady-state kinetics of GrsA. (A) Each reaction contained 1 µM GrsA, 20 mM Tris (pH 8.0), 2.5 mM ATP, 1 mM $MgCl_2$, 1 mM TCEP, 150 mM hydroxylamine (pH 7.0), 0.1 U purine nucleoside phosphorylase, 0.04 U inorganic pyrophosphatase, 0.2 mM MesG, and 0–10000 µM norleucine. (B) The reactions contained 1 µM GrsA and 0–10000 µM *S*-ethyl-L-cystein. (C) The reactions contained 1 µM GrsA and 0–5000 µM *O*-allyl-L-serine. Velocities were fit to the Michaelis-Menten equation.

Table S3. Catalytic parameters of the adenylation reaction catalyzed by GrsA.[a]

Substrate	k_{cat} [min^{-1}]	K_m [µM]	k_{cat}/K_m [min^{-1} mM^{-1}]
Norleucine	5.4 ± 0.2	1249 ± 174	4.3
S-Ethyl-L-cystein	10 ± 1.3	5491 ± 1320	1.8
O-Allyl-L-serine	3.3 ± 0.2	650 ± 151	5.1
L-Phenylalanine[b]	500 ± 12	25 ± 2	20161

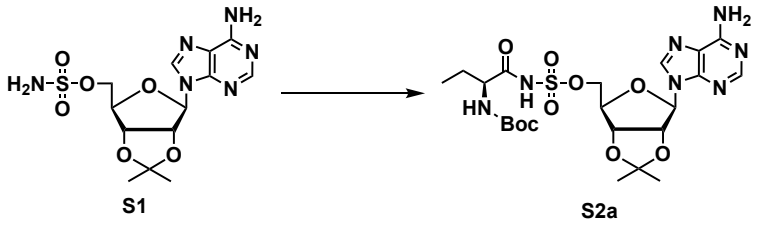
[a]Catalytic parameters were determined using a coupled hydroxamate-MesG continuous spectrophotometric assay.[5] Errors are given as the standard deviation of triplicate independent experiments. [b]Values obtained from Ref. 4. As expected, the A-domain of GrsA catalyzed the adenylation reactions of Nle (k_{cat}/K_m = 4.3 mM^{-1} min^{-1}), Cys(Et) (k_{cat}/K_m = 2.0 mM^{-1} min^{-1}), and Ser(Allyl) (k_{cat}/K_m = 5.1 mM^{-1} min^{-1}).

Chemical Synthetic Procedures

General Synthetic Methods. All commercial reagents were used as provided unless otherwise indicated. **S1** was prepared according to published literature procedures.[6] All reactions were carried out under an atmosphere of nitrogen in dry solvents with oven-dried glassware and constant magnetic stirring unless otherwise noted. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL NMR spectrometers and standardized to the NMR solvent signal as reported by Gottlieb.[7] TLC analysis was performed using Silica Gel 60 F254 plates (Merck) and visualization was accomplished with ultraviolet light (λ = 254 nm) and/or the appropriate stain [phosphomolybdic acid, iodine, ninhydrin, and potassium permanganate]. Silica gel chromatography was carried out with SiliaFlash F60 230-400 mesh (Silicycle), according to the method of Still.[8] Mass spectral data were obtained using a LCMS-IT-TOF mass spectrometer (Shimadzu).

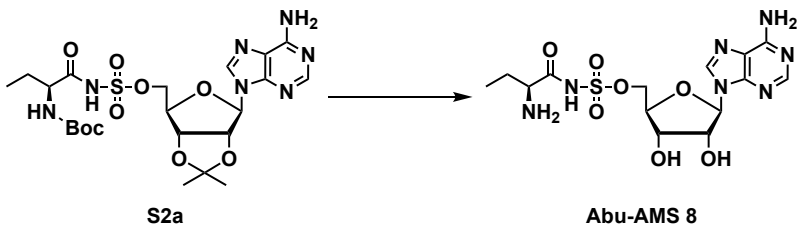
Chemical Synthesis of Abu-AMS 8

Compound S2a



1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (415 mg, 2.16 mmol) and *N*-hydroxysuccinimide (170 mg, 1.48 mmol) were added to a solution of Boc-Abu-OH (200 mg, 0.98 mmol) in CH_2Cl_2 (10 mL). After 4 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Abu-OSu (160 mg, 0.53 mmol) and cesium carbonate (347 mg, 1.07 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (137 mg, 0.36 mmol) in DMF (10 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (95:5:1 to 90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2a** as a colorless oil (83 mg, 41%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.47 (s, 1H), 8.21 (s, 1H), 6.23 (d, *J* = 3.5 Hz, 1H), 5.35 (dd, *J* = 5.8, 3.5 Hz, 1H), 5.12−5.07 (m, 1H), 4.55−4.52 (m, 1H), 4.23 (d, *J* = 3.5 Hz, 2H), 3.92 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.16 (q, *J* = 7.5 Hz, 14H, Et_3N-*CH*$_2$), 1.85−1.75 (m, 1H), 1.69−1.54 (m, 4H), 1.43 (s, 9H), 1.38 (s, 3H), 1.28 (t, *J* = 7.5 Hz, 21H, Et_3N-*CH*$_3$), 0.90 (ddd, *J* = 6.9, 6.9, 2.3 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 180.8, 157.7, 157.3, 154.0, 150.5, 141.4, 120.1, 115.2, 91.8, 85.7, 85.6, 83.3, 80.0, 69.7, 59.2, 47.5, 28.8, 27.5, 27.3, 26.1, 10.3, 9.21. HRMS (ESI−): $[M-H]^-$ calcd for $C_{22}H_{32}N_7O_9S$, 570.1982; found, 570.1988.

Abu-AMS 8

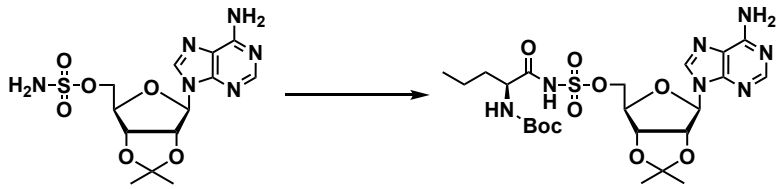


Compound **S2a** (50 mg, 0.090 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at

room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (89:11:1 to 83:17:1 to 60:40:1 $CHCl_3$/MeOH/Et_3N) to afford compound Abu-AMS **8** as a colorless oil (14 mg, 35%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.08 (d, *J* = 5.8 Hz, 1H), 4.63 (dd, *J* = 5.8, 5.8 Hz, 1H), 4.46–4.23 (m, 4H), 3.50 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.10 (q, *J* = 7.5 Hz, 6H, Et_3N-*CH*$_2$), 1.98–1.82 (m, 2H), 1.26 (t, *J* = 7.5 Hz, 9H, Et_3N-*CH*$_3$), 1.01 (q, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 174.5, 157.3, 153.9, 150.7, 141.2, 120.1, 89.5, 84.3, 76.2, 72.0, 69.1, 57.3, 47.6, 25.4, 9.82, 9.42. HRMS (ESI−): $[M-H]^-$ calcd for $C_{14}H_{20}N_7O_7S$, 430.1145; found, 430.1141.

Chemical Synthesis of Nva-AMS 9

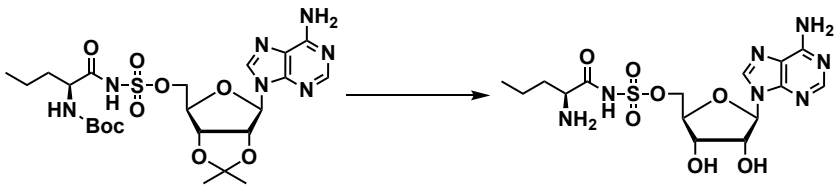
Compound S2b



1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (389 mg, 2.03 mmol) and *N*-hydroxysuccinimide (159 mg, 1.38 mmol) were added to a solution of Boc-Nva-OH (200 mg, 0.92 mmol) in CH_2Cl_2 (10 mL). After 2 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Nva-OSu (142 mg, 0.45 mmol) and cesium carbonate (293 mg, 0.90 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (116 mg, 0.30 mmol) in DMF (10 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (95:5:1 to 90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2b** as a colorless oil (86 mg, 49%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.45 (s, 1H), 8.22 (s, 1H), 6.23 (d, *J* = 3.4 Hz, 1H), 5.36 (dd, *J* = 5.8, 3.4 Hz, 1H), 5.11 (dd, *J* = 5.8, 3.8 Hz, 1H), 4.53 (dd, *J* = 5.8, 3.8 Hz, 1H), 4.29−4.17 (m, 2H), 3.97 (dd, *J* = 8.3, 4.8 Hz, 1H), 3.23 (q, *J* = 7.5 Hz, 5H, Et_3N-*CH*$_2$), 1.79−1.46 (m, 1H), 1.58−1.46 (m, 1H), 1.61 (s, 3H), 1.40 (s, 9H), 1.38 (s, 3H), 1.45−1.33 (m, 2H, overlapping with Boc-CH_3 and acetonide-CH_3), 1.28 (t, *J* = 7.5 Hz, 7.5H, Et_3N-*CH*$_3$), 0.89 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 181.6, 157.8, 157.3, 154.0, 150.5, 141.5, 120.1, 115.3, 91.8, 85.7, 85.6, 83.3, 80.1, 69.8, 58.0, 47.8, 36.6, 28.8, 27.5, 25.6, 20.2, 14.2,

9.27. HRMS (ESI−): $[M-H]^-$ calcd for $C_{23}H_{34}N_7O_9S$, 584.2139; found, 584.2138.

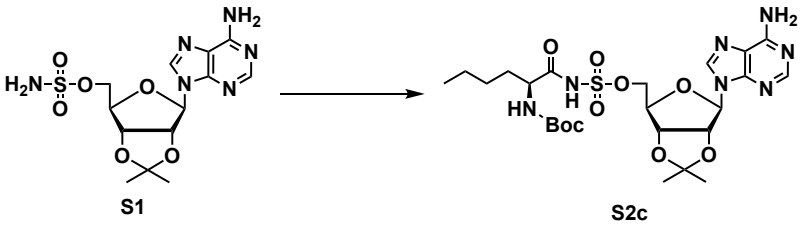
Nva-AMS 9



Compound **S2b** (51 mg, 0.090 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (89:11:1 to 83:17:1 to 75:25:1 $CHCl_3$/MeOH/Et_3N) to afford compound Nva-AMS **9** as a colorless oil (15 mg, 38%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.19 (s, 1H), 6.08 (d, J = 5.2 Hz, 1H), 4.63 (dd, J = 5.2, 5.2 Hz, 1H), 4.45–4.22 (m, 4H), 3.71–3.57 (m, 1H), 3.10 (q, J = 7.5 Hz, 4H, Et_3N-*CH*$_2$), 1.88–1.70 (m, 2H), 1.49–1.37 (m, 2H), 1.26 (t, J = 7.5 Hz, 6H, Et_3N-*CH*$_3$), 0.93 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 176.4, 157.2, 153.9, 150.7, 141.2, 120.1, 89.5, 84.2, 76.2, 72.0, 69.1, 56.9, 47.6, 35.0, 19.4, 14.1, 9.43. HRMS (ESI−): $[M-H]^-$ calcd for $C_{15}H_{22}N_7O_7S$, 444.1301; found, 444.1321.

Chemical Synthesis of Nle-AMS 10

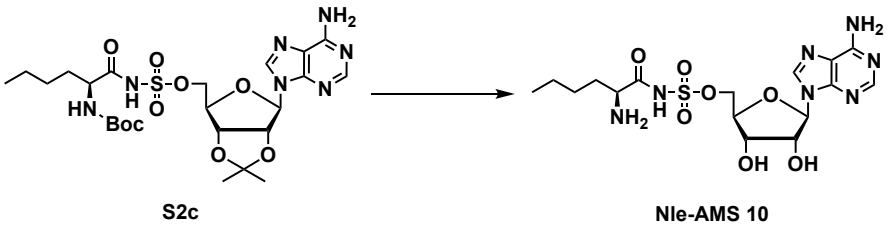
Compound S2c



1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (422 mg, 1.93 mmol) and *N*-hydroxysuccinimide (152 mg, 1.32 mmol) were added to a solution of Boc-Nle-OH (203 mg, 0.88 mmol) in CH_2Cl_2 (10 mL). After 14 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Nle-OSu (135 mg, 0.41 mmol) and cesium carbonate (267 mg, 0.82 mmol) were added to a solution of 5′-

O-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (106 mg, 0.27 mmol) in DMF (10 mL). The solution was stirred at room temperature for 15 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (95:5:1 to 90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2c** as a colorless oil (91 mg, 57%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.47 (s, 1H), 8.21 (s, 1H), 6.23 (d, *J* = 3.5 Hz, 1H), 5.34 (dd, *J* = 5.8, 3.5 Hz, 1H), 5.09 (d, *J* = 5.8 Hz, 1H), 4.54 (dd, *J* = 6.0, 3.7 Hz, 1H), 4.23 (d, *J* = 3.4 Hz, 2H), 4.00−3.94 (m, 1H), 3.18 (q, *J* = 7.5 Hz, 4H, Et_3N-*CH*$_2$), 1.85−1.66 (m, 1H), 1.61 (s, 3H), 1.64−1.52 (m, 1H, overlapping with acetonide-CH_3), 1.41 (s, 9H), 1.38 (s, 3H), 1.28 (t, *J* = 7.5 Hz, 6H, Et_3N-*CH*$_3$), 1.46−1.24 (m, 4H, overlapping with Boc-CH_3, acetonide-CH_3, and Et_3N-*CH*$_3$), 0.86 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 181.3, 157.7, 157.3, 154.0, 150.5, 141.4, 120.1, 115.2, 91.8, 85.7, 85.6, 83.3, 80.0, 69.7, 58.1, 47.7, 34.2, 29.0, 28.8, 27.5, 25.6, 23.6, 14.4, 9.19. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{36}N_7O_9S$, 598.2295; found, 598.2308.

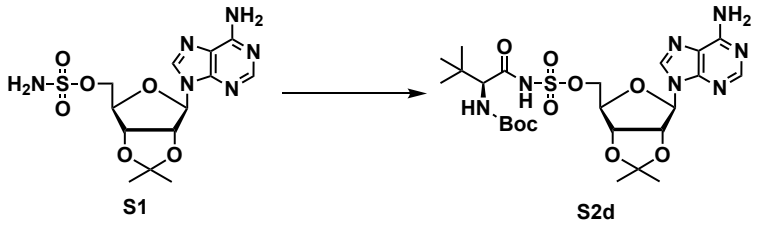
Nle-AMS 10



Compound **S2c** (45 mg, 0.080 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 4 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (83:17:1 to 75:25:1 $CHCl_3$/MeOH/Et_3N) to afford Nle-AMS **10** as a colorless oil (17 mg, 46%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.52 (s, 1H), 8.19 (s, 1H), 6.08 (d, *J* = 5.1 Hz, 1H), 4.63 (d, *J* = 5.1 Hz, 1H), 4.46–4.21 (m, 4H), 3.66–3.58 (m, 1H), 3.07 (q, *J* = 7.5 Hz, 4H, Et_3N-*CH*$_2$), 1.89–1.71 (m, 2H), 1.45–1.28 (m, 4H), 1.25 (t, *J* = 7.5 Hz, 6H, Et_3N-*CH*$_3$), 0.86 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 174.8, 157.3, 153.9, 150.7, 141.3, 120.1, 89.4, 84.2, 76.2, 71.9, 69.1, 57.1, 47.6, 32.2, 28.1, 23.5, 14.1, 9.51. HRMS (ESI−): $[M-H]^-$ calcd for $C_{16}H_{24}N_7O_7S$, 458.1458; found, 458.1401.

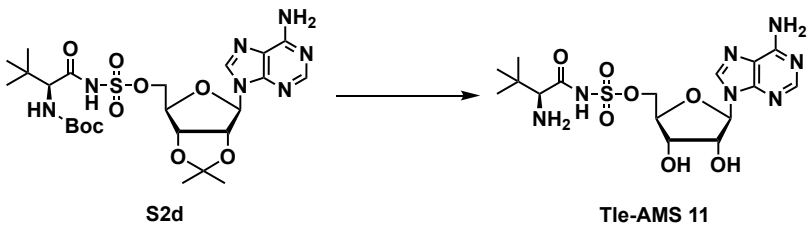
Chemical Synthesis of Tle-AMS 11

Compound S2d



1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (364 mg, 1.90 mmol) and *N*-hydroxysuccinimide (150 mg, 1.30 mmol) were added to a solution of Boc-Tle-OH (200 mg, 0.86 mmol) in CH_2Cl_2 (10 mL). After 3 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Tle-OSu (110 mg, 0.33 mmol) and cesium carbonate (218 mg, 0.67 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (85 mg, 0.22 mmol) in DMF (10 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2d** as a colorless oil (44 mg, 34%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.42 (s, 1H), 8.21 (s, 1H), 6.23 (d, *J* = 2.9 Hz, 1H), 5.37 (dd, *J* = 5.7, 2.9 Hz, 1H), 5.13 (dd, *J* = 6.3, 2.9 Hz, 1H), 4.53 (dd, *J* = 6.3, 4.0 Hz, 1H), 4.28 (dd, *J* = 10.9, 4.0 Hz, 1H), 4.23 (dd, *J* = 10.9, 4.0 Hz, 1H), 3.23 (q, *J* = 7.5 Hz, 3.5H, Et_3N-*CH*$_2$), 1.61 (s, 3H), 1.40 (s, 9H), 1.38 (s, 3H), 1.29 (t, *J* = 7.5 Hz, 5.3H, Et_3N-*CH*$_3$), 0.95 (s, 9H). ^{13}C NMR (125 MHz, CD_3OD) δ 180.2, 157.7, 157.3 154.0, 150.4, 141.5,120.2, 115.3, 91.8, 85.70, 85.67, 83.2, 80.2, 69.9, 66.2, 47.8, 35.6, 28.8, 27.5, 27.0, 25.6, 9.26. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{36}N_7O_9S$, 598.2295; found, 598.2290.

Tle-AMS 11

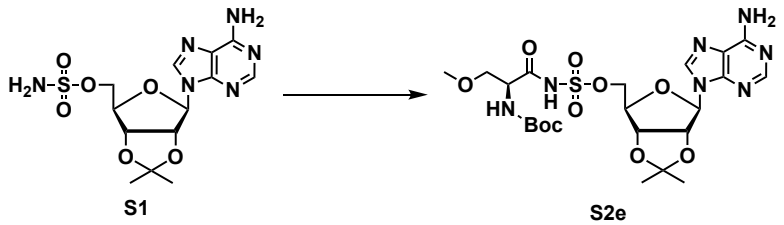


Compound **S2d** (58 mg, 0.10 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at

room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (89:11:1 to 83:17:1 $CHCl_3$/MeOH/Et_3N) to afford compound Tle-AMS **11** as a colorless oil (21 mg, 45%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.53 (s, 1H), 8.19 (s, 1H), 6.08 (d, J = 5.2 Hz, 1H), 4.63 (dd, J = 5.2, 5.2 Hz, 1H), 4.44–4.38 (m, 2H), 4.36–4.28 (m, 2H), 3.37–3.34 (m, 1H), 3.09 (q, J = 7.5 Hz, 3H, Et_3N-*CH*$_2$), 1.25 (t, J = 7.5 Hz, 4.5H, Et_3N-*CH*$_3$), 1.08 (s, 9H). ^{13}C NMR (500 MHz, CD_3OD) δ 175.6, 157.2, 153.9, 150.6, 141.2, 120.1, 89.6, 84.2, 76.1, 71.9, 68.8, 65.8, 47.6, 34.3, 27.2, 9.46. HRMS (ESI−): $[M-H]^-$ calcd for $C_{16}H_{24}N_7O_7S$, 458.1458; found, 458.1499.

Chemical Synthesis of Ser(Me)-AMS 12

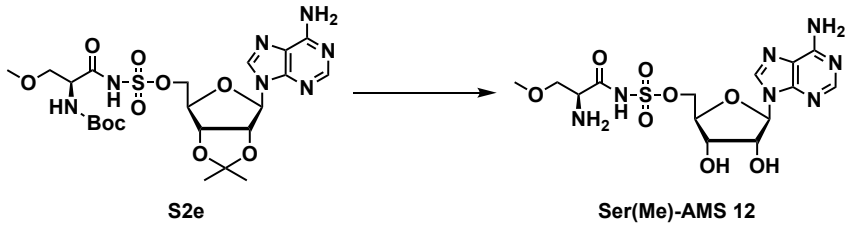
Compound S2e



To a solution of Boc-Ser(Me)-OMe (200 mg, 0.86 mmol) in methanol (10 mL) was added 1 M aq LiOH (1.3 mL) at room temperature. Stirring was continued at room temperature for 5 h. The flask was then placed on a rotary evaporator and the methanol and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford Boc-Ser(Me)-OH as a colorless oil (198 mg, quant.). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (268 mg, 1.4 mmol) and *N*-hydroxysuccinimide (203 mg, 1.4 mmol) were added to a solution of Boc-Ser(Me)-OH (198 mg, 0.90 mmol) in DMF (10 mL). After 5 h, the mixture was diluted with EtOAc, washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Ser(Me)-OSu (126 mg, 0.40 mmol) and cesium carbonate (389 mg, 1.2 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (231 mg, 0.60 mmol) in DMF (4 mL). The solution was stirred at room temperature for 12 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (87:13:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2e** as a white solid (90 mg, 38%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.40 (d, J = 10.9 Hz, 1H), 8.21 (s, 1H), 6.23 (d, J = 2.9 Hz,

1H), 5.37 (dd, *J* = 6.3, 2.8 Hz, 1H), 5.11 (ddd, *J* = 6.3, ,2.8, 2.8 Hz, 1H), 4.53 (dd, *J* = 6.3, 4.0 Hz, 1H), 4.32−4.19 (m, 2H), 4.19−4.14 (m, 1H), 3.74−3.68 (m, 1H), 3.62−3.55 (m, 1H), 3.28 (s, 3H), 2.78 (q, *J* = 7.5 Hz, 1H, Et_3N-*CH*$_2$), 1.59 (s, 3H), 1.40 (s, 9H), 1.37 (s, 3H), 1.12 (t, *J* = 7.5 Hz, 1.5H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 179.2, 157.7, 157.3, 154.0, 150.4, 141.5, 120.2, 115.3, 91.8, 85.6, 83.2, 83.1, 80.4, 74.5, 69.9, 59.3, 58.2, 47.2, 28.7, 27. 5, 25.6, 10.4. HRMS (ESI−): $[M-H]^-$ calcd for $C_{22}H_{32}N_7O_{10}S$, 586.1931; found, 586.1930.

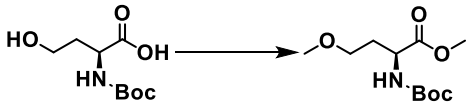
Ser(Me)-AMS 12



Compound **S2e** (60 mg, 0.10 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 2 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (80:20:1 to 67:33:1 $CHCl_3$/MeOH/Et_3N) to afford Ser(Me)-AMS **12** as a colorless solid (23 mg, 51%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.49 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 5.2 Hz, 1H), 4.64 (dd, *J* = 8.6, 5.2 Hz, 1H), 4.41−4.26 (m, 4H), 3.86 (dd, *J* = 6.3, 3.4 Hz, 1H), 3.83−3.68 (m, 2H), 3.35 (s, 3H), 3.19 (q, *J* = 7.5 Hz, 8H, Et_3N-*CH*$_2$), 1.29 (t, *J* = 7.5 Hz, 12H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.5, 157.2, 153.9, 150.7, 141.2, 120.1, 89.4, 84.3, 76.2, 72.2, 69.2, 68.6, 59.4, 56.9, 47.7, 9.24. HRMS (ESI−): $[M-H]^-$ calcd for $C_{14}H_{20}N_7O_8S$, 446.1094; found, 446.1073.

Chemical Synthesis of homoSer(Me)-AMS 13

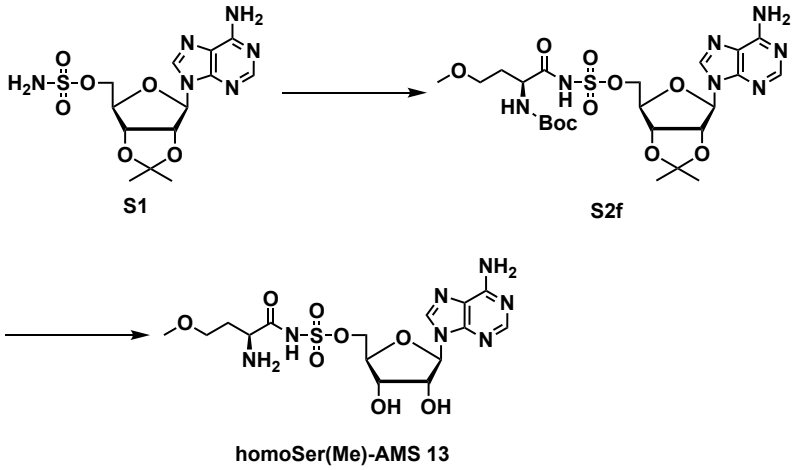
Boc-homoSer(Me)-OMe



To a solution of Boc-homoSer-OH (600 mg, 2.8 mmol) was dissolved in DMF (5 mL) was added NaH (60%, dispersion in paraffin liquid) (136 mg, 6.8 mmol) with stirring at 0 °C for 30 min. MeI (126 μL, 2.0 mmol) was added with stirring at room temperature for 4 h. The mixture was

diluted with EtOAc, washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (75:25 hexane/EtOAc) to afford Boc-homoSer(Me)-OMe as a colorless oil (601 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (d, J = 7.4 Hz, 1H), 4.25 (dd, J = 12.3, 4.2 Hz, 1H), 3.60 (s, 3H), 3.37−3.26 (m, 2H), 3.17 (s, 3H), 1.99−1.89 (m, 1H), 1.88−1.76 (m, 1H), 1.30 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 155.5, 79.9, 69.1, 58.9, 52.4, 51.8, 32.1, 28.4. HRMS (ESI+): $[M+H]^+$ calcd for $C_{11}H_{22}NO_5$, 248.1498; found, 248.1500.

homoSer(Me)-AMS 13

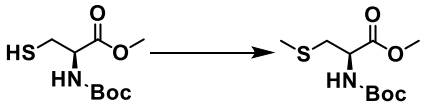


To a solution of Boc-homoSer(Me)-OMe (371 mg, 1.5 mmol) in methanol (10 mL) was added 1 M aq LiOH (2.3 mL) at room temperature. Stirring was continued at room temperature for 12 h. The flask was then placed on a rotary evaporator and the methanol and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford Boc-homoSer(Me)-OH (350 mg, quant.). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (863 mg, 4.5 mmol) and *N*-hydroxysuccinimide (675 mg, 4.5 mmol) were added to a solution of Boc-homoSer(Me)-OH (350 mg, 1,5 mmol) in DMF (10 mL). After 15 h, the mixture was diluted with EtOAc, washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-homoSer(Me)-OSu (190 mg, 0.58 mmol) and cesium carbonate (381 mg, 1.2 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (150 mg, 0.39 mmol) in DMF (3 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The crude mixture was dissolved in a 4:1 (v/v)

mixture of TFA and H_2O at room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (80:20:1 $CHCl_3$/MeOH/Et_3N) to afford homoSer(Me)-AMS **13** as a colorless oil (89 mg, 49%, over two steps). ^{1}H NMR (500 MHz, CD_3OD) δ 8.52 (s, 1H), 8.20 (s, 1H), 6.09 (d, J = 5.1 Hz, 1H), 4.63 (dd, J = 5.1, 5.1 Hz, 1H), 4.42−4.26 (m, 4H), 3.67 (dd, J = 7.8, 3.8 Hz, 1H), 3.57 (q, J = 7.4 Hz, 2H), 3.29 (s, 3H), 3.19 (q, J = 7.5 Hz, 3.5H), 2.26−2.14 (m, 1H), 2.13−1.98 (m, 1H), 1.30 (t, J = 7.5 Hz, 5.3H). ^{13}C NMR (125 MHz, CD_3OD) δ 175.4, 157.3, 153.9, 150.7, 141.2, 120.1, 89.5, 84.2, 76.2, 71.9, 71.3, 69.0, 59.2, 55.5, 47.7, 31.7, 9.21. HRMS (ESI−): $[M-H]^-$ calcd for $C_{15}H_{22}N_7O_8S$, 460.1251; found, 460.1244.

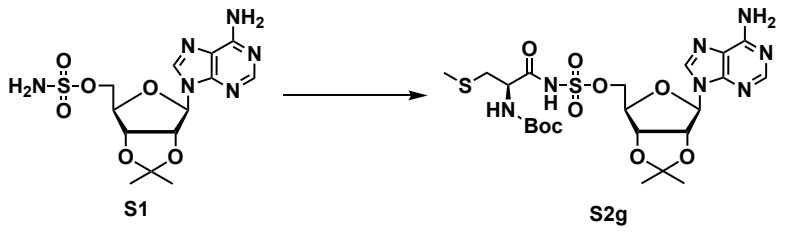
Chemical Synthesis of Cys(Me)-AMS 14

Boc-Cys(Me)-OMe



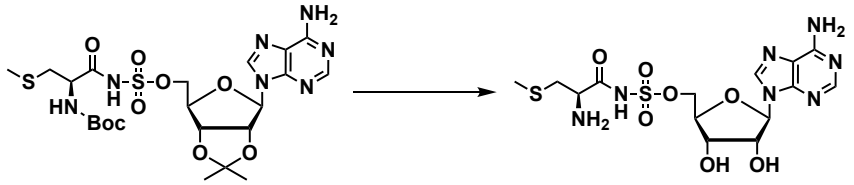
Boc-Cys-OMe (175 μL, 0.85 mmol) was dissolved in DMF (10 mL) containing methyl iodide (106 μL, 1.7 mmol) and then K_2CO_3 (231 mg, 1.7 mmol) was added. The mixture was stirred at 0 °C for 4 h. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (90:10 hexane/EtOAc) to afford Boc-Cys(Me)-OMe as a colorless oil (196 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (d, J = 7.5 Hz, 1H), 4.53 (dd, J = 13.2, 5.2 Hz, 1H), 3.76 (s, 3H), 2.93 (ddd, J = 19.5, 13.2, 4.6 Hz, 2H), 2.11 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 155.3, 80.3, 53.1, 52.7, 36.8, 28.4, 16.4. HRMS (ESI+): $[M+H]^+$ calcd for $C_{10}H_{20}NO_4S$, 250.1113; found, 5250.1101.

Compound S2g



To a solution of Boc-Cys(Me)-OMe (105 mg, 0.45 mmol) in THF (10 mL) and H_2O (10 mL) was added lithium hydroxide (53 mg, 2.2 mmol) at room temperature. Stirring was continued at room temperature for 3 h. The flask was then placed on a rotary evaporator and the THF and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (173 mg, 0.90 mmol) and *N*-hydroxysuccinimide (78 mg, 0.68 mmol) were added to a solution of Boc-Cys(Me)-OH (106 mg, 0.45 mmol) in CH_2Cl_2 (10 mL). After 12 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Cys(Me)-OSu (150 mg, 0.45 mmol) and cesium carbonate (293 mg, 0.90 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (116 mg, 0.30 mmol) in DMF (10 mL). The solution was stirred at room temperature for 12 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2g** as a yellow oil (107 mg, 59%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 6.23 (d, J = 2.9 Hz, 1H), 5.37 (dd, J = 5.8, 2.9 Hz, 1H), 5.12 (dd, J = 5.8, 3.5 Hz, 1H), 4.55 (dd, J = 5.8, 3.5 Hz, 1H), 4.26 (d, J = 3.5 Hz, 2H), 4.23−4.14 (m, 1H), 2.96 (q, J = 7.5 Hz, 11H, Et_3N-*CH*$_2$), 2.85−2.65 (m, 2H), 2.08 (s, 3H), 1.61 (s, 3H), 1.42 (s, 9H), 1.39 (s, 3H), 1.20 (t, J = 7.5 Hz, 16.5H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 157.5, 157.3, 154.0, 150.5, 141.4, 120.1, 115.2, 91.9, 85.7, 85.6, 83.3, 80.3, 69.8, 57.5, 47.5, 38.5, 28.8, 27.5, 25.6, 16.1, 9.93. (The ^{13}C signal of the sulfamoyloxy-linked carbonyl, around 180 ppm, was not observed.) HRMS (ESI−): $[M-H]^-$ calcd for $C_{22}H_{32}N_7O_9S_2$, 602.1703; found, 602.1714.

Cys(Me)-AMS 14



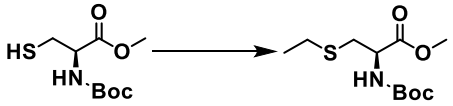
S2g **Cys(Me)-AMS 14**

Compound **S2g** (75 mg, 0.12 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (87:13:1 to 83:17:1 $CHCl_3$/MeOH/Et_3N) to afford Cys(Me)-AMS **14** as a colorless solid (21 mg, 37%). ^{1}H

NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.09 (d, J = 4.6 Hz, 1H), 4.64 (dd, J = 4.6, 4.6 Hz, 1H), 4.42−4.26 (m, 4H), 3.78−3.73 (m, 1H), 3.12 (q, J = 7.5 Hz, 6H, Et_3N-CH_2), 2.14 (s, 3H), 1.27 (t, J = 7.5 Hz, 7.5H, Et_3N-CH_3). ^{13}C NMR (125 MHz, CD_3OD) δ 175.9, 157.3, 153.9, 150.7, 141.2, 120.1, 89.5, 84.3, 76.1, 72.0, 69.1, 55.9, 47.6, 37.5, 15.5, 9.39. HRMS (ESI−): $[M-H]^-$ calcd for $C_{14}H_{20}N_7O_7S_2N$, 462.0866; found, 462.0858.

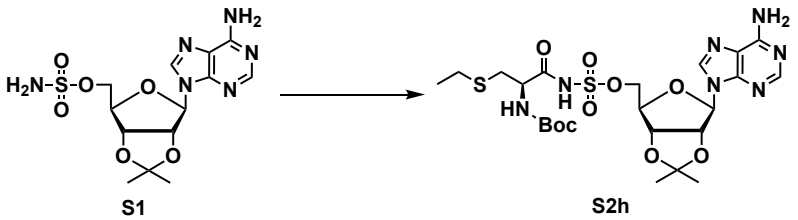
Chemical Synthesis of Cys(Et)-AMS 15

Boc-Cys(Et)-OMe



Boc-Cys(Et)-OMe (175 μL, 0.85 mmol) was dissolved in DMF (10 mL) containing ethyl iodide (136 μL, 1.7 mmol) and then K_2CO_3 (231 mg, 1.7 mmol) was added. The mixture was stirred at room temperature for 6 h. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (89:11 hexane/EtOAc) to afford Boc-Cys(Et)-OMe as a colorless oil (151 mg, 65%). ^1H NMR (500 MHz, $CDCl_3$) δ 5.35 (d, J = 7.5 Hz, 1H), 4.53 (dd, J = 13.2, 5.2 Hz, 1H), 3.76 (s, 3H), 2.97 (ddd, J = 17.8, 13.2, 4.6 Hz, 2H), 2.55 (q, J = 7.5 Hz, 2H), 1.45 (s, 9H), 1.44 (s, 9H), 1.24 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 155.3, 80.3, 53.1, 52.7, 36.8, 28.4, 16.4. HRMS (ESI+): $[M+Na]^+$ calcd for $C_{11}H_{21}NNaO_4S$, 286.1089; found, 286.1067.

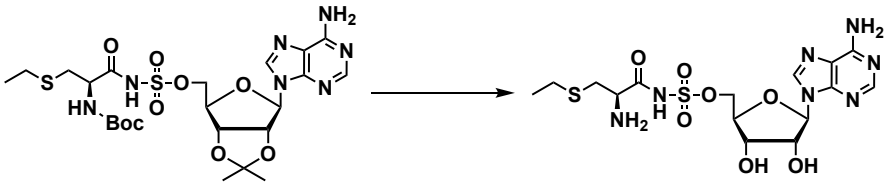
Compound S2h



To a solution of Boc-Cys(Et)-OMe (78 mg, 0.30 mmol) in THF (10 mL) and H_2O (10 mL) was added lithium hydroxide (36 mg, 1.5 mmol) at room temperature. Stirring was continued at room temperature for 4 h. The flask was then placed on a rotary evaporator and the THF and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The

aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (115 mg, 0.60 mmol) and *N*-hydroxysuccinimide (52 mg, 0.45 mmol) were added to a solution of Boc-Cys(Et)-OH (75 mg, 0.30 mmol) in CH_2Cl_2 (10 mL). After 4 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Cys(Et)-OSu (150 mg, 0.30 mmol) and cesium carbonate (195 mg, 0.90 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (77 mg, 0.20 mmol) in DMF (10 mL). The solution was stirred at room temperature for 10 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2h** as a white solid (90 mg, 73%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.42 (s, 1H), 8.22 (s, 1H), 6.23 (d, *J* = 2.8 Hz, 1H), 5.38 (dd, *J* = 5.7, 2.8 Hz, 1H), 5.12 (dd, *J* = 5.7, 2.3 Hz, 1H), 4.54 (dd, *J* = 5.7, 3.5 Hz, 1H), 4.33−4.23 (m, 2H), 4.22−4.08 (m, 1H), 3.15 (q, *J* = 7.5 Hz, 3.5H, Et_3N-*CH*$_2$), 3.04−2.92 (m, 1H), 2.88−2.77 (m, 1H), 2.54 (q, *J* = 7.5 Hz, 2H), 1.60 (s, 3H), 1.41 (s, 9H), 1.38 (s, 3H), 1.27 (t, *J* = 7.5 Hz, 5.3H, Et_3N-*CH*$_3$), 1.18 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 179.5, 157.5, 157.3, 154.0, 150.4, 141.5, 120.2, 115.3, 91.8, 85.6, 83.2, 80.4, 69.9, 58.3, 57.9, 47.7, 35.6, 28.8, 27.5, 27.1, 25.6, 15.1, 9.33. HRMS (ESI−): $[M-H]^-$ calcd for $C_{23}H_{34}N_7O_9S_2$, 616.1859; found, 616.1833.

Cys(Et)-AMS 15

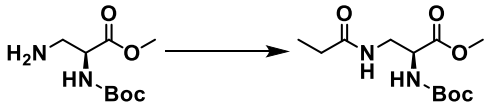


Compound **S2h** (47 mg, 0.080 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 4 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (87:13:1 to 83:17:1 $CHCl_3$/MeOH/Et_3N) to afford Cys(Et)-AMS **15** as a colorless solid (17 mg, 48%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 5.1 Hz, 1H), 4.64 (dd, *J* = 5.1, 5.1 Hz, 1H), 4.45−4.26 (m, 4H), 3.83−3.73 (m, 1H), 3.15 (q, *J* = 7.5 Hz, 7H, Et_3N-*CH*$_2$), 2.96−2.87 (m, 1H), 2.68−2.52 (m, 3H), 1.28 (t, *J* = 7.5 Hz, 10.5H), 1.22 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ157.3, 153.9, 150.7, 141.2, 120.1, 89.5, 84.3, 76.1, 72.0, 69.1, 56.2,

47.7, 34.4, 26.7, 14.9, 9.28. (The ^{13}C signal of the sulfamoyloxy-linked carbonyl, around 175 ppm, was not observed.) HRMS (ESI−): $[M-H]^-$ calcd for $C_{15}H_{22}N_7O_7S_2$, 476.1022; found, 476.1001.

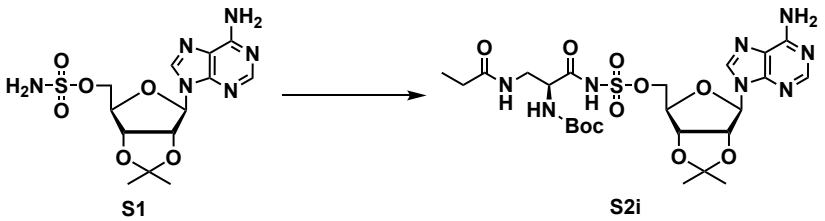
Chemical Synthesis of 3-propanoylamino-Ala-AMS 16

Methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-propionamidopropanoate



A solution of Boc-Dap-OMe (97 mg, 0.45 mmol), propionyl chloride (46 μL, 0.53 mmol), and *N*,*N*-diisopropylethylamine (170 μL, 0.98 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature. After 15 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (67:33 hexane/EtOAc) to afford methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-propionamidopropanoate as a colorless oil (66 mg, 53%). ^1H NMR (500 MHz, CD_3OD) δ 4.27 (dd, J = 6.0, 6.0 Hz, 1H), 3.72 (s, 3H), 3.56 (dd, J = 13.8, 5.2 Hz, 1H), 3.47 (dd, J = 13.8, 6.9 Hz, 1H), 2.19 (q, J = 7.7 Hz, 2H), 1.46 (s, 9H), 1.11 (t, J = 7.7 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 177.7, 172.8, 157.8, 80.8, 55.1, 52.8, 41.5, 30.0, 28.7, 10.4. HRMS (ESI+): $[M+H]^+$ calcd for $C_{12}H_{23}N_2O_5$, 275.1607; found, 275.1607.

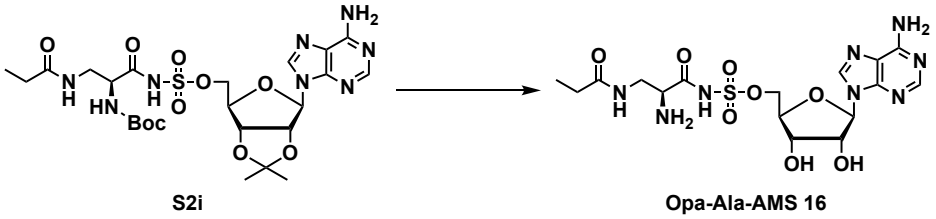
Compound S2i



To a solution of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-propionamidopropanoate (66 mg, 0.24 mmol) in 1:1 THF/H_2O (20 mL) was added LiOH (29 mg, 1.2 mmol) at room temperature. Stirring was continued at room temperature for 2 h. The flask was then placed on a rotary evaporator and the THF and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over

Na_2SO_4, and evaporated to dryness to afford (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-propionamidopropanoic acid as a white solid (52 mg, 84%). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (101 mg, 0.53 mmol) and *N*-hydroxysuccinimide (41 mg, 0.36 mmol) were added to a solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-propionamidopropanoic acid (52 mg, 0.20 mmol) in CH_2Cl_2 (10 mL). After 15 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-3-(propanoylamino)-Ala-OSu (65 mg, 0.17 mmol) and cesium carbonate (156 mg, 0.48 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (62 mg, 0.16 mmol) in DMF (10 mL). The solution was stirred at room temperature for 24 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (95:5:1 to 90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2i** as a colorless oil (57 mg, 59%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.47 (s, 1H), 8.22 (s, 1H), 6.24 (d, *J* = 3.4 Hz, 1H), 5.34 (dd, *J* = 5.7, 2.8 Hz, 1H), 5.15−5.07 (m, 1H), 4.55 (ddd, *J* = 3.4, 3.4, 3.4, Hz, 1H), 4.30−4.18 (m, 2H), 4.11 (dd, *J* = 6.9, 6.9 Hz, 1H), 3.54 (dd, *J* = 13.2, 4.6 Hz, 1H), 3.45 (dd, *J* = 13.2, 7.5 Hz, 1H), 3.10 (q, *J* = 7.5 Hz, 6H, Et_3N-*CH*$_2$), 2.18 (q, *J* = 8.0 Hz, 2H), 1.61 (s, 3H), 1.40 (s, 9H), 1.38 (s, 3H), 1.25 (t, *J* = 7.5 Hz, 9H, Et_3N-*CH*$_3$), 1.09 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 178.4, 177.4, 157.7, 157.3, 154.0, 150.5, 141.4, 120.1, 115.2, 91.8, 85.8, 85.6, 83.2, 80.3, 69.8, 58.0, 47.6, 43.0, 30.2, 28.7, 27.5, 25.6, 10.4, 9.43. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{35}N_8O_{10}S$, 627.2197; found, 627.2189.

3-[(1-Oxopropyl)amino]-Ala-AMS 16

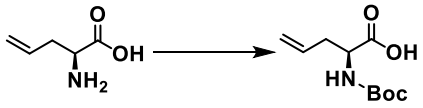


Compound **S2i** (30 mg, 0.050 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 4 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (83:17:1 to 75:25:1 $CHCl_3$/MeOH/Et_3N) to afford Opa-Ala-AMS **16** as a colorless oil (14 mg, 56%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.53 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 4.6 Hz, 1H), 4.68−4.55 (m, 1H), 4.45−4.25 (m, 4H), 3.76−3.65 (m, 2H), 3.61−3.50 (m, 1H), 3.11 (q, *J* = 7.5 Hz, 9H, Et_3N-*CH*$_2$), 2.21 (q, *J* = 7.5 Hz, 2H), 1.27 (t, *J* = 7.5 Hz, 13.5H, Et_3N-*CH*$_3$), 1.08 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 178.8, 157.3, 153.9, 150.7, 141.2, 120.1, 89.6, 84.1, 76.2, 71.8, 68.9, 57.8,

47.4, 41.8, 29.8, 9.95, 9.25. (The ^{13}C signal of the carbonyl was not observed.) HRMS (ESI−): $[M-H]^-$ calcd for $C_{16}H_{23}N_8O_8S$, 487.1360; found, 487.1325.

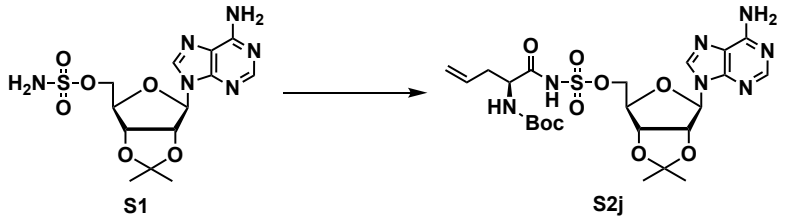
Chemical Synthesis of AllylGly-AMS 17

Boc-allylGly-OH



To a stirred solution of allylGly-OH (1 g, 8.67 mmol) in a mixed solvent of H_2O (9 mL), 1 M aq NaOH (9 mL), and 1,4-dioxane (18 mL) was added Boc_2O (2.2 mL, 9.56 mmol), and it was stirred for 14 h at room temperature. The reaction mixture was cooled to 0 °C and quenched with 1 M aq HCl, and aqueous layer was extracted with EtOAc. The organic layers were washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo to afford compound Boc-allylGly-OH as a colorless oil (1.9 g, quant). ^1H NMR (500 MHz, CD_3OD) δ 5.83−5.72 (m, 1H), 5.16−5.06 (m, 2H), 4.16 (dd, J = 8.2, 5.0 Hz, 1H), 2.60−2.51 (m, 1H), 2.46−2.38 (m, 1H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, CD_3OD) δ 175.3, 157.8, 134.6, 118.5, 80.5, 54.6, 37.1, 28.7. HRMS (ESI+): $[M+Na]^+$ calcd for $C_{10}H_{17}NNaO_4$, 238.1055; found, 238.1010.

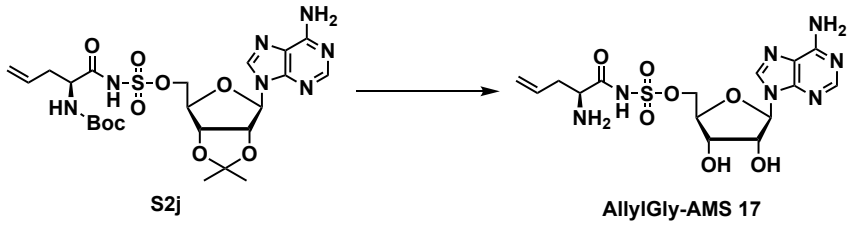
Compound S2j



1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (415 mg, 2.16 mmol) and *N*-hydroxysuccinimide (170 mg, 1.47 mmol) were added to a solution of Boc-allylGly-OH (212 mg, 0.98 mmol) in CH_2Cl_2 (10 mL). After 14 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-allylGly-OSu (129 mg, 0.41 mmol) and cesium carbonate (269 mg, 0.82 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (106 mg, 0.27 mmol) in DMF (10 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was filtered

through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (95:5:1 to 90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2j** as a colorless oil (75 mg, 48%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.42 (s, 1H), 8.21 (s, 1H), 6.23 (d, *J* = 2.9 Hz, 1H), 5.82−5.70 (m, 1H), 5.37 (dd, *J* = 5.7, 2.9 Hz, 1H), 5.11 (dd, *J* = 6.3, 2.3 Hz, 1H), 5.08 (dd, *J* = 17.2, 1.8 Hz, 1H), 5.00 (d, *J* = 10.3 Hz, 1H), 4.53 (ddd, *J* = 4.0, 4.0, 4.0 Hz, 1H), 4.30−4.19 (m, 2H), 4.03 (ddd, *J* = 5.1, 5.1, 5.1 Hz, 1H), 3.17 (q, *J* = 7.4 Hz, 2H, Et_3N-*CH*$_2$), 2.58−2.47 (m, 1H), 2.39−2.28 (m, 1H), 1.60 (s, 3H), 1.40 (s, 9H), 1.38 (s, 3H), 1.28 (t, *J* = 7.4 Hz, 3H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 180.6, 157.6, 157.3, 154.0, 150.4, 141.5, 135.4, 120.2, 118.0, 115.3, 91.8, 85.7, 85.6, 83.2, 80.2, 69.9, 57.8, 48.7, 38.6, 28.8, 27.5, 25.6, 9.26. HRMS (ESI+): $[M+H]^+$ calcd for $C_{23}H_{34}N_7O_9S$, 584.2139; found, 584.2068.

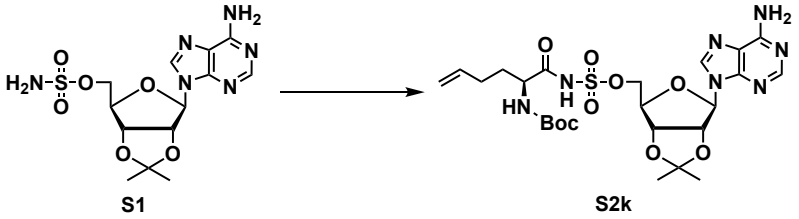
AllylGly-AMS 17



Compound **S2j** (30 mg, 0.050 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (83:17:1 to 60:40:1 $CHCl_3$/MeOH/Et_3N) to afford allylGly-AMS **17** as a colorless oil (11 mg, 51%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 5.1 Hz, 1H), 5.85−5.74 (m, 1H), 5.22 (d, *J* = 16.0 Hz, 1H), 5.15 (d, *J* = 10.3 Hz, 1H), 4.63 (dd, *J* = 5.1, 5.1 Hz, 1H), 4.42−4.28 (m, 4H), 3.71 (dd, *J* = 7.5, 4.6 Hz, 1H), 3.15 (q, *J* = 7.5 Hz, 3H, Et_3N-*CH*$_2$), 2.77−2.65 (m, 1H), 2.61−2.52 (m, 1H), 1.28 (t, *J* = 7.5 Hz, 4.5H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 175.4, 157.3, 153.9, 150.7, 141.2, 133.0, 120.6, 120.1, 89.4, 84.2, 76.1, 72.0, 69.1, 56.3, 47.7, 37.2, 9.29. HRMS (ESI−): $[M-H]^-$ calcd for $C_{15}H_{20}N_7O_7S$, 442.1145; found, 442.1179.

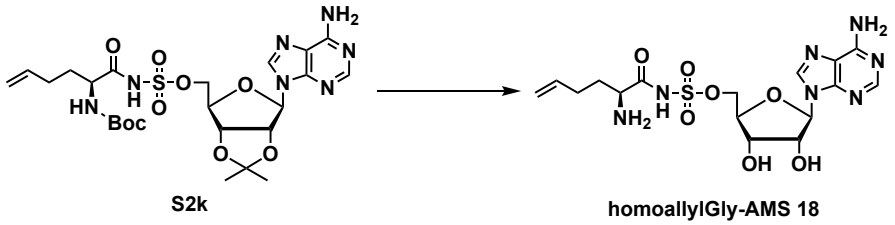
Chemical Synthesis of HomoallylGly-AMS 18

Compound S2k



To a solution of Boc-homoallylGly-OMe (112 mg, 0.46 mmol) in THF (10 mL) and H_2O (10 mL) was added lithium hydroxide (55 mg, 2.3 mmol) at room temperature. Stirring was continued at room temperature for 4 h. The flask was then placed on a rotary evaporator and the THF and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (176 mg, 0.92 mmol) and *N*-hydroxysuccinimide (79 mg, 0.69 mmol) were added to a solution of Boc-homoallylGly-OH (105 mg, 0.46 mmol) in CH_2Cl_2 (10 mL). After 4 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-homoallylGly-OSu (150 mg, 0.46 mmol) and cesium carbonate (303 mg, 0.93 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (118 mg, 0.31 mmol) in DMF (10 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (87:13:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2k** as a colorless oil (140 mg, 76%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 6.24 (d, *J* = 3.4 Hz, 1H), 5.89−5.73 (m, 1H), 5.36 (dd, *J* = 6.3, 3.4 Hz,, 1H), 5.15−5.08 (m, 1H), 5.07−4.81 (m, 2H), 4.53 (ddd, *J* = 4.0, 4.0, 4.0 Hz, 1H), 4.28−4.18 (m, 2H), 4.02−3.94 (m, 1H), 3.17 (q, *J* = 6.9 Hz, 12H, Et_3N-*CH*$_2$), 2.16−2.03 (m, 2H), 1.91−1.77 (m, 2H), 1.74−1.61 (m, 2H), 1.60 (s, 3H), 1.40 (s, 9H), 1.38 (s, 3H), 1.27 (t, *J* = 6.9 Hz, 18H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 180.8, 157.6, 157.3, 154.0, 150.4, 141.4, 139.4, 139.1, 120.1, 115.2, 91.7, 85.6, 85.6, 83.2, 80.0, 69.7, 57.6, 47.6, 33.9, 31.0, 28.8, 27.5, 25.6, 9.16. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{34}N_7O_9S$, 596.2139; found, 596.2138.

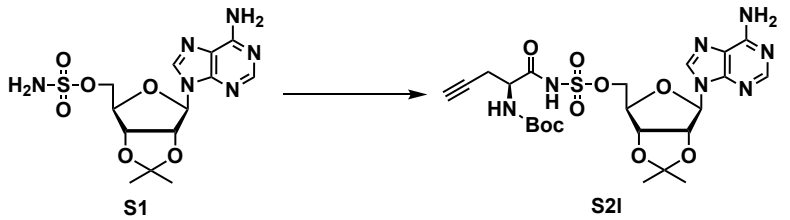
homoallylGly-AMS 18



Compound **S2k** (72 mg, 0.12 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 5 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (83:17:1 to 80:20:1 $CHCl_3$/MeOH/Et_3N) to afford homoallylGly-AMS **18** as a colorless oil (16 mg, 30%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.52 (s, 1H), 8.19 (s, 1H), 6.08 (d, *J* = 5.2 Hz, 1H), 5.88−5.74 (m, 1H), 5.13−4.94 (m, 2H), 4.63 (dd, *J* = 5.2, 5.2 Hz, 1H), 4.43−4.26 (m, 4H), 3.63 (dd, *J* = 5.8, 5.8 Hz, 1H), 3.09 (q, *J* = 7.5 Hz, 5H, Et_3N-*CH*$_2$), 2.22−2.14 (m, 2H), 2.02−1.93 (m, 1H), 1.89−1.80 (m, 1H), 1.25 (t, *J* = 7.5 Hz, 7.5H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 174.6, 157.3, 153.9, 150.7, 141.2, 138.1, 120.1, 116.1, 89.5, 84.2, 76.1, 71.9, 69.1, 56.7, 47.6, 31.9, 30.5, 9.47. HRMS (ESI−): $[M-H]^-$ calcd for $C_{16}H_{22}N_7O_7S$, 456.1301; found, 456.1339.

Chemical Synthesis of Pra-AMS 19

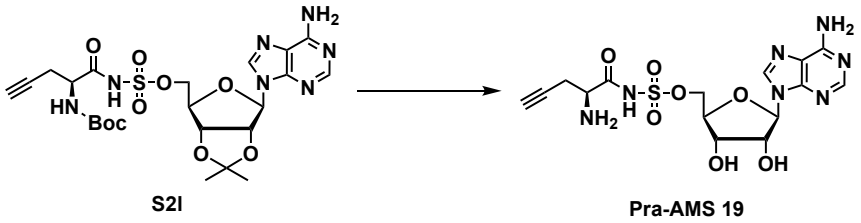
Compound S2l



1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (402 mg, 2.10 mmol) and *N*-hydroxysuccinimide (241 mg, 2.10 mmol) were added to a solution of Boc-Pra-OH (300 mg, 1.40 mmol) in DMF (10 mL). After 6 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Pra-OSu (104 mg, 0.33 mmol) and cesium carbonate (476 mg, 1.46 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (282 mg, 0.73 mmol) in DMF (5 mL). The solution was stirred at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite.

The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (88:12:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2l** as a colorless solid (90 mg, 47%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.22 (s, 1H), 7.90 (s, 1H), 6.23 (d, *J* = 2.8 Hz, 1H), 5.37 (dd, *J* = 6.3, 3.5 Hz, 1H), 5.11 (dd, *J* = 6.3, 1.7 Hz, 1H), 4.54 (ddd, *J* = 3.4, 3.4, 3.4 Hz, 1H), 4.25 (d, *J* = 4.0 Hz, 2H), 4.12 (dd, *J* = 5.7, 5.7 Hz, 1H), 3.13 (q, *J* = 7.5 Hz, 7H, Et_3N-*CH*$_2$), 2.77−2.55 (m, 2H), 2.26 (s, 1H), 1.60 (s, 3H), 1.42 (s, 9H), 1.38 (s, 3H), 1.25 (t, *J* = 7.5 Hz, 10H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 178.2, 157.29, 157.25, 154.0, 150.4, 141.4, 120.1, 115.2, 91.8, 85.6, 85.5, 83.3, 80.3, 79.5, 71.9, 69.8, 56.4, 47.7, 28.7, 27.5, 25.6, 24.1, 9.30. HRMS (ESI−): $[M-H]^-$ calcd for $C_{23}H_{30}N_7O_9S$, 580.1826; found, 580.1799.

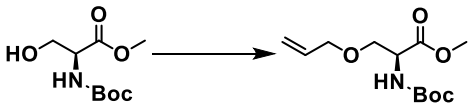
Pra-AMS 19



Compound **S2l** (44 mg, 0.076 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 2 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (80:20:1 $CHCl_3$/MeOH/Et_3N) to afford Pra-AMS **19** as a colorless solid (20 mg, 59%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.52 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 5.1 Hz, 1H), 4.64 (dd, *J* = 5.1, 5.1 Hz, 1H), 4.42−4.27 (m, 4H), 3.78 (dd, *J* = 5.7, 5.7 Hz, 1H), 3.18 (q, *J* = 7.5 Hz, 6H, Et_3N-*CH*$_2$), 2.95−2.71 (m, 2H), 2.52 (dd, *J* = 2.5, 2.5 Hz, 1H), 1.29 (t, *J* = 7.5 Hz, 9H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 175.0, 157.2, 153.9, 150.7, 141.2, 120.1, 89.4, 84.3, 78.9, 76.2, 74.1, 72.1, 69.2, 55.5, 47.7, 23.7, 9.23. HRMS (ESI−): $[M-H]^-$ calcd for $C_{15}H_{18}N_7O_7S$, 440.0988; found, 440.0949.

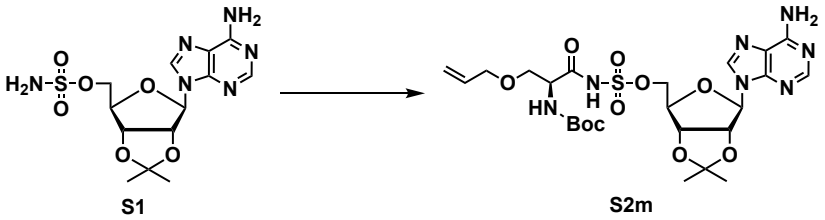
Chemical Synthesis of Ser(Allyl)-AMS 20

Boc-Ser(Allyl)-OMe



A three-necked flask was charged with Boc-Ser-OMe (480 mg, 2.19 mmol) and $Pd(PPh_3)_4$ (81 mg, 0.07 mmol) under N_2. Allylmethyl carbonate (371 μL, 3.30 mmol) in THF (40 mL) was syringed over 10 min. The reaction mixture was heated to 60 °C for 12 h. The solvent was evaporated in vacuo, and the residue was diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (67:33 hexane/EtOAc) to afford Boc-Ser(Allyl)-OMe as a colorless oil (498 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88−5.75 (m, 1H), 5.37 (d, J = 8.6 Hz, 1H), 5.26−5.11 (m, 2H), 4.46−4.36 (m, 1H), 4.02−3.90 (m, 2H), 3.83 (dd, J = 9.2, 2.6 Hz, 1H), 3.74 (s, 3H), 3.63 (dd, J = 9.2, 3.2 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 155.6, 134.2, 117.6, 80.1, 72.3, 70.0, 54.1, 52.6, 28.4. HRMS (ESI+): $[M+Na]^+$ calcd for $C_{12}H_{21}NNaO_5$, 282.1317; found, 282.1287.

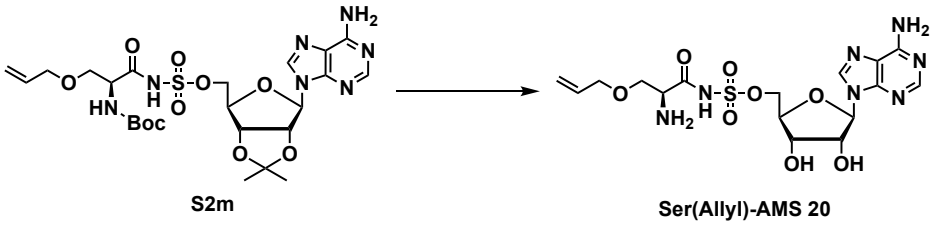
Compound S2m



To a solution of Boc-Ser(Allyl)-OMe (314 mg, 1.21 mmol) in methanol (10 mL) was added 1 M aq LiOH (1.8 mL) at room temperature. Stirring was continued at room temperature for 3 h. The flask was then placed on a rotary evaporator and the methanol and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford Boc-Ser(Allyl)-OH as a white solid (216 mg, 73%). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (230 mg, 1.2 mmol) and *N*-hydroxysuccinimide (180 mg, 1.2 mmol) were added to a solution of Boc-Ser(Allyl)-OH (98 mg, 0.40 mmol) in DMF (5 mL). After 12 h, the mixture was diluted with EtOAc, washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Ser(Allyl)-OSu (53 mg, 0.16 mmol) and cesium carbonate (100 mg, 0.31 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (40 mg, 0.10 mmol) in DMF (1 mL). The solution was stirred at room temperature for 12 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (84:16:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2m** as a colorless solid (30 mg, 48%). ^{1}H NMR (500

MHz, CD_3OD) δ 8.45 (s, 1H), 8.22 (s, 1H), 6.23 (d, J = 2.9 Hz, 1H), 5.88−5.78 (m, 1H), 5.39−5.32 (m, 1H), 5.26−5.18 (m, 1H), 5.14−5.05 (m, 2H), 4.56−4.50 (m, 1H), 4.26−4.19 (m, 1H), 4.18−4.13 (m, 1H), 3.97−3.91 (m, 2H), 3.80−3.72 (m, 1H), 3.70−4.62 (m, 1H), 3.09 (q, J = 7.4 Hz, 4H, Et_3N-CH_2), 1.61 (s, 3H), 1.42 (s, 9H), 1.39 (s, 3H), 1.24 (t, J = 7.4 Hz, 6H, Et_3N-CH_3). ^{13}C NMR (125 MHz, CD_3OD) δ 178.6, 157.6, 157.3, 154.0, 150.5, 141.4, 136.0, 120.1, 117.3, 115.2, 91.9, 85.7, 85.6, 83.3, 80.3, 73.1, 72.2, 69.8, 60.1, 58.3, 47.6, 28.8, 27.5, 25.6, 9.51. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{34}N_7O_{10}S$, 612.2088; found, 612.2103.

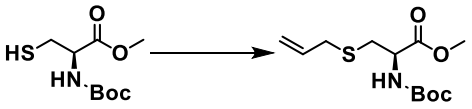
Ser(Allyl)-AMS 20



Compound **S2m** (29 mg, 0.047 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 2 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (75:25:1 $CHCl_3$/MeOH/Et_3N) to afford Ser(Allyl)-AMS **20** as a colorless oil (18 mg, 82%). ^1H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.08 (d, J = 5.2 Hz, 1H), 5.95−5.80 (m, 1H), 5.26 (dd, J = 17.0, 1.5 Hz, 1H), 5.13 (d, J = 10.5 Hz, 1H), 4.63 (dd, J = 9.9, 5.2 Hz, 1H), 4.43−4.24 (m, 4H), 4.01 (d, J = 5.8 Hz, 1H), 3.88−3.77 (m, 2H), 3.15 (q, J = 7.5 Hz, 6H, Et_3N-CH_2), 1.28 (t, J = 7.5 Hz, 9H, Et_3N-CH_3). ^{13}C NMR (125 MHz, CD_3OD) δ157.3, 153.9, 150.8, 141.2, 135.5, 120.1, 118.0, 89.3, 84.3, 76.2, 73.3, 72.1, 69.8, 69.2, 57.1, 47.7, 9.33. (The ^{13}C signal of the sulfamoyloxy-linked carbonyl, around 175 ppm, was not observed.) HRMS (ESI−): $[M-H]^-$ calcd for $C_{16}H_{22}N_7O_8S$, 472.1251; found, 472.1233.

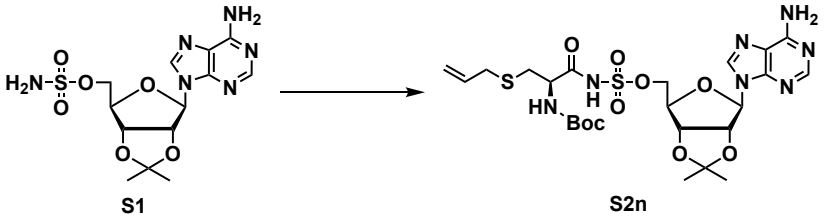
Chemical Synthesis of Cys(Allyl)-AMS 21

Boc-Cys(Allyl)-OMe



Boc-Cys-OMe (175 μL, 0.85 mmol) was dissolved in DMF (10 mL) containing allyl bromide (147 μL, 1.7 mmol) and then K_2CO_3 (231 mg, 1.7 mmol) was added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (67:33 hexane/EtOAc) to afford Boc-Cys(Allyl)-OMe as a colorless oil (131 mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.74−5.64 (m, 1H), 5.27 (d, J = 7.5 Hz, 1H), 5.11−5.03 (m, 2H), 4.47 (dd, J = 12.6, 5.5 Hz, 1H), 3.71 (s, 3H), 3.13−3.02 (m, 2H), 2.88 (dd, J = 13.8, 4.9 Hz, 1H), 2.80 (dd, J = 13.8, 5.5 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 155.3, 133.8, 118.0, 80.3, 53.3, 52.7, 35.3, 33.0, 28.4. HRMS (ESI+): $[M+H]^+$ calcd for $C_{12}H_{22}NO_4S$, 276.1270; found, 276.1286.

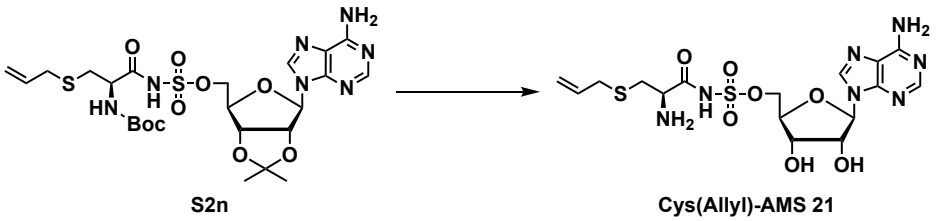
Compound S2n



To a solution of Boc-Cys(Allyl)-OMe (71 mg, 0.26 mmol) in THF (5 mL) and H_2O (5 mL) was added lithium hydroxide (31 mg, 1.3 mmol) at room temperature. Stirring was continued at room temperature for 4 h. The flask was then placed on a rotary evaporator and the THF and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (100 mg, 0.52 mmol) and *N*-hydroxysuccinimide (45 mg, 0.39 mmol) were added to a solution of Boc-Cys(Allyl)-OH (68 mg, 0.26 mmol) in CH_2Cl_2 (10 mL). After 4 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Cys(Allyl)-OSu (93 mg, 0.26 mmol) and cesium carbonate (215 mg, 0.66 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (84 mg, 0.22 mmol) in DMF (10 mL). The solution was stirred at room temperature for 10 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2n** as a colorless oil (38 mg, 27%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.47 (s, 1H), 8.22 (s, 1H), 6.23 (d, J = 2.9 Hz, 1H), 5.82−5.66 (m, 1H), 5.37 (dd, J = 5.7, 2.9 Hz, 1H), 5.17−5.08 (m, 2H), 5.03 (d, J = 10.3 Hz, 1H), 4.55 (ddd, J = 4.0, 4.0, 4.0 Hz, 1H), 4.25 (d, J = 3.4 Hz, 2H), 4.21−4.14 (m, 1H),

3.16 (q, J = 6.9 Hz, 10H, Et_3N-CH_2), 2.91 (dd, J = 13.7, 4.6 Hz, 1H), 2.77 (dd, J = 13.7, 6.9 Hz, 1H), 1.61 (s, 3H), 1.42 (s, 9H), 1.39 (s, 3H), 1.27 (t, J = 6.9 Hz, 12H, Et_3N-CH_3). ^{13}C NMR (125 MHz, CD_3OD) δ 178.7, 164.8, 157.3, 154.0, 150.5, 141.4, 135.7, 120.1, 117.6, 115.2, 91.9, 85.7, 85.6, 83.3, 80.3, 69.8, 57.6, 47.7, 35.9, 34.9, 28.7, 27.5, 25.6, 9.25. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{34}N_7O_9S_2$, 628.1859; found, 628.1882.

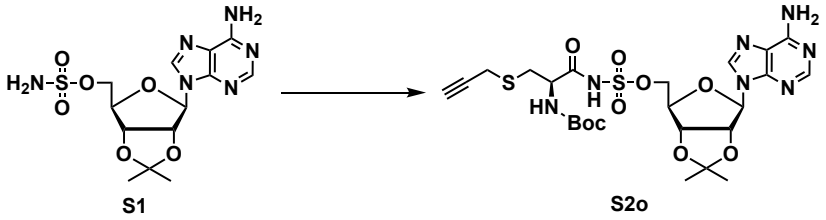
Cys(Allyl)-AMS 21



Compound **S2n** (38 mg, 0.060 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (84:17:1 $CHCl_3$/MeOH/Et_3N) to afford Cys(Allyl)-AMS **21** as a yellow solid (18 mg, 61%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.50 (s, 1H), 8.20 (s, 1H), 6.08 (d, J = 5.2 Hz, 1H), 5.20 (d, J = 17.2 Hz, 1H), 5.08 (d, J = 10.3 Hz, 1H), 4.64 (dd, J = 5.2, 5.2 Hz, 1H), 4.45−4.27 (m, 4H), 3.80−3.72 (m, 1H), 3.15 (q, J = 7.5 Hz, 16H), 3.09−2.96 (m, 1H), 2.86 (dd, J = 14.3, 7.4 Hz, 1H), 1.28 (t, J = 7.5 Hz, 24H). ^{13}C NMR (125 MHz, CD_3OD) δ 179.9, 157.3, 153.9, 150.7, 141.2, 135.1, 120.1, 118.4, 89.5, 84.3, 76.1, 72.0, 69.1, 56.3, 47.6, 35.4, 23.9, 9.28. HRMS (ESI−): $[M-Na]^-$ calcd for $C_{16}H_{22}N_7O_7S_2$, 488.1022; found, 488.1020.

Chemical Synthesis of Cys(Pra)-AMS 22

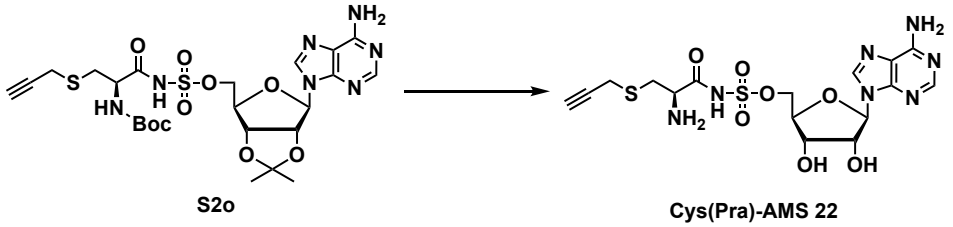
Compound S2o



To a solution of Boc-Cys(Pra)-OMe (127 mg, 0.46 mmol) in THF (10 mL) and H_2O (10 mL) was

added lithium hydroxide (60 mg, 2.5 mmol) at room temperature. Stirring was continued at room temperature for 3 h. The flask was then placed on a rotary evaporator and the THF and H_2O were removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (176 mg, 0.92 mmol) and *N*-hydroxysuccinimide (79 mg, 0.69 mmol) were added to a solution of Boc-Cys(Pra)-OH (119 mg, 0.46 mmol) in CH_2Cl_2 (10 mL). After 12 h, the mixture was washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Cys(Pra)-OSu (150 mg, 0.46 mmol) and cesium carbonate (254 mg, 0.78 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (100 mg, 0.26 mmol) in DMF (10 mL). The solution was stirred at room temperature for 10 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (90:10:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2o** as a yellow oil (118 mg, 72%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 6.23 (d, *J* = 3.4 Hz, 1H), 5.37 (dd, *J* = 5.7, 2.8 Hz, 1H), 5.15−5.09 (m, 1H), 4.55 (ddd, *J* = 4.0, 4.0, 4.0 Hz, 1H), 4.31−4.10 (m, 3H), 3.20−3.12 (m, 1H), 3.08 (q, *J* = 7.5 Hz, 12H, Et_3N-*CH*$_2$), 2.95 (dd, *J* = 13.8, 6.9 Hz, 1H), 2.66 (s, 1H), 1.61 (s, 3H), 1.42 (s, 9H), 1.39 (s, 3H), 1.25 (t, *J* = 7.5 Hz, 18H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 178.6, 157.4, 157.3, 154.0, 150.5, 141.4, 120.1, 115.2, 91.9, 85.7, 85.6, 83.3, 81.0, 80.3, 72.4, 69.8, 57.5, 47.6, 35.9, 28.8, 27.5, 25.6, 20.3, 9.54. HRMS (ESI−): $[M-H]^-$ calcd for $C_{24}H_{32}N_7O_9S_2$, 626.1703; found, 626.1686.

Cys(Pra)-AMS 22

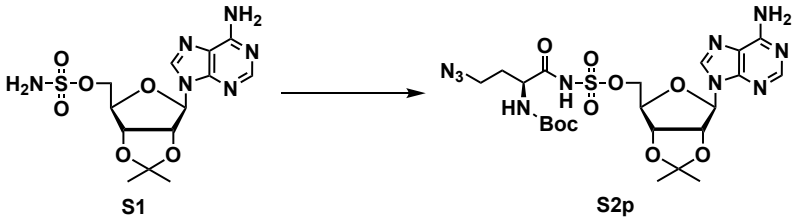


Compound **S2o** (69 mg, 0.11 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 3 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (87:13:1 to 86:14:1 to 80:20:1 $CHCl_3$/MeOH/Et_3N) to afford Cys(Pra)-AMS **22** as a colorless solid (17 mg, 31%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 5.2 Hz, 1H), 4.64 (dd, *J* = 5.2, 5.2 Hz, 1H), 4.47−4.23 (m,4H), 3.83−3.73 (m, 1H), 3.40−3.36 (m, 2H), 3.12 (q, *J* =

7.5 Hz, 9H), 3.07−2.97 (m, 2H), 2.62 (s, 1H), 1.27 (t, J = 7.5 Hz, 13.5H). ^{13}C NMR (125 MHz, CD_3OD) δ 180.0, 157.3, 153.9, 150.6, 141.2, 120.1, 89.4, 84.3, 80.8, 76.1, 72.9, 72.0, 69.2, 56.3, 47.6, 35.6, 20.1, 9.38. HRMS (ESI−): $[M-Na]^-$ calcd for $C_{16}H_{20}N_7O_7S_2$, 486.0866; found, 486.0835.

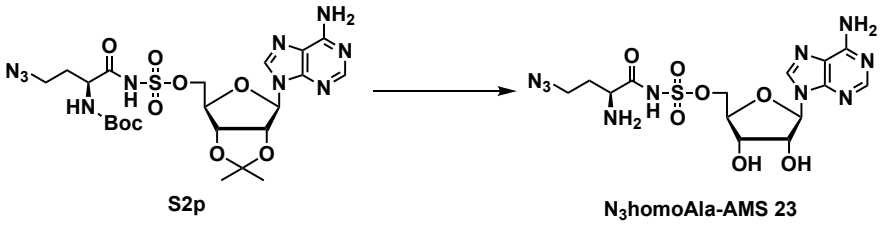
Chemical Synthesis of Dab(N_3)-AMS 23

Compound S2p



1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (328 mg, 1.7 mmol) and *N*-hydroxysuccinimide (257 mg, 1.7 mmol) were added to a solution of Boc-Dab(N_3)-OH (139 mg, 0.57 mmol) in DMF (5 mL). After 16 h, the mixture was diluted with EtOAc, washed with 5% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Boc-Dab(N_3)-OSu (133 mg, 0.39 mmol) and cesium carbonate (254 mg, 0.78 mmol) were added to a solution of 5′-*O*-sulfamoyl-2′,3′-isopropylideneadenosine **S1** (100 mg, 0.26 mmol) in DMF (2 mL). The solution was stirred at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (95:5:1 $CHCl_3$/MeOH/Et_3N) to afford compound **S2p** as a colorless solid (121 mg, 76%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.44 (s, 1H), 8.22 (s, 1H), 6.24 (d, J = 2.8 Hz, 1H), 5.36 (dd, J = 5.8, 2.8 Hz, 1H), 5.15−5.08 (m, 1H), 4.54 (ddd, J = 3.4, 3.4, 3.4 Hz, 1H), 4.30−4.18 (m, 2H), 4.10−3.96 (m, 1H), 3.45−3.32 (m, 2H), 3.17 (q, J = 7.5 Hz, 4H, Et_3N-*CH*$_2$), 2.10−1.97 (m, 1H), 1.88−1.72 (m, 1H), 1.61 (s, 3H), 1.41 (s, 9H), 1.38 (s, 3H), 1.28 (t, J = 7.5 Hz, 7H, Et_3N-*CH*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 180.1, 157.7, 157.3, 154.0, 150.4, 141.4, 120.1, 115.3, 91.7, 85.7, 83.2, 80.2, 69.7, 63.4, 55.9, 55.8, 47.4, 33.5, 28.8, 27.5, 25.6, 9.97. HRMS (ESI−): $[M-H]^-$ calcd for $C_{22}H_{31}N_{10}O_9S$, 611.1996; found, 611.1992.

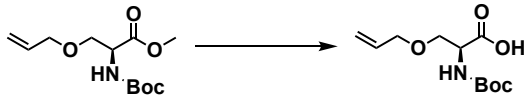
N_3homoAla-AMS 23



Compound **S2p** (36 mg, 0.059 mmol) was dissolved in a 4:1 (v/v) mixture of TFA and H_2O at room temperature. After 2 h, the flask was placed on the rotary evaporator and the TFA and H_2O were removed at reduced pressure. The residue was purified by flash chromatography (80:20:1 $CHCl_3$/MeOH/Et_3N) to afford N_3homoAla-AMS **23** as a colorless solid (16 mg, 59%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.20 (s, 1H), 6.09 (d, *J* = 5.1 Hz, 1H), 4.62 (dd, *J* = 5.1, 5.1 Hz, 1H), 4.46−4.23 (m, 4H), 3.71 (dd, *J* = 6.9, 6.9 Hz, 1H), 3.61−3.47 (m, 2H), 3.18 (q, *J* = 7.5 Hz, 3H), 2.23−2.11 (m, 1H), 2.05−1.93 (m, 1H), 1.29 (t, *J* = 7.5 Hz, 4.5H). ^{13}C NMR (125 MHz, CD_3OD) δ 180.1, 157.3, 153.9, 150.7, 141.2, 120.1, 89.5, 84.2, 76.1, 71.9, 69.1, 55.2, 32.8, 24.1, 9.39. HRMS (ESI−): [M−H]− calcd for $C_{14}H_{19}N_{10}O_7S$, 471.1159; found, 471.1172.

Chemical Synthesis of *O*-allyl-L-Ser

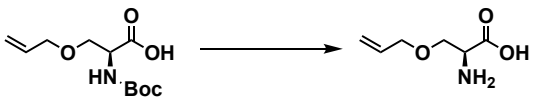
Boc-Ser(Allyl)-OH



To a solution of Boc-Ser(Allyl)-OMe (5.4 g, 19.7 mmol) in a 1:1 (v/v) mixture of THF and H_2O was added LiOH (2.4 g, 98.4 mmol) at room temperature. Stirring was continued at room temperature for 16 h. The flask was then placed on a rotary evaporator and the THF was removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford Boc-Ser(Allyl)-OH as a white solid (4.9 g, quant.). ^{1}H NMR (800 MHz, CD_3OD) δ 5.94−5.83 (m, 1H), 5.28 (dddd, *J* = 17.3, 1.6, 1.6, 1.6 Hz, 1H), 5.16 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.30 (dd, *J* = 4.1, 4.1 Hz, 1H), 4.06−3.94 (m, 2H), 3.79 (dd, *J* = 9.8, 4.9 Hz, 1H), 3.68 (dd, *J* = 9.8, 3.7 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (200 MHz, CD_3OD) δ 173.9, 157.9, 135.7, 117.4, 80.7, 73.1, 70.9, 55.3, 28.7.

HRMS (ESI+): $[M+Na]^+$ calcd for $C_{11}H_{19}NNaO_5$, 268.1161; found, 268.1146.

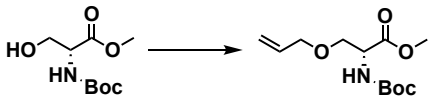
_O_-Allyl-L-Ser



Boc-Ser(Allyl)-OH (4.8 g, 20.0 mmol) was dissolved in a 4N-HCl/dioxane at room temperature. After 6 h, the flask was placed on the rotary evaporator to afford *O*-allyl-L-Ser as a white solid (3.2 g, quant.). ^{1}H NMR (800 MHz, CD_3OD) δ 5.98−5.86 (m, 1H), 5.32 (dddd, *J* = 17.4, 1.6, 1.6, 1.6 Hz, 1H), 5.22 (dd, *J* = 10.5, 1.6 Hz, 1H), 4.17 (dd, *J* = 4.7, 3.3 Hz, 1H), 4.13−4.01 (m, 2H), 3.91 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.83 (d, *J* = 10.5, 3.2 Hz, 1H). ^{13}C NMR (200 MHz, CD_3OD) δ 173.5, 135.2, 118.3, 73.4, 68.1, 54.4. HRMS (ESI+): $[M+Na]^+$ calcd for $C_6H_{11}NNaO_3$, 168.0637; found, 168.0627.

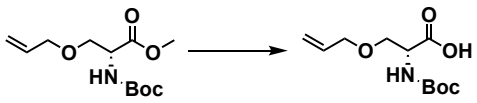
Chemical Synthesis of _O_-allyl-D-Ser

Boc-D-Ser(Allyl)-OMe



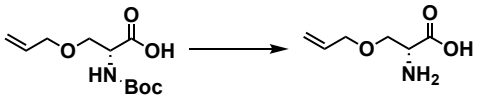
A three-necked flask was charged with Boc-D-Ser-OMe (1 g, 4.56 mmol) and $Pd(PPh_3)_4$ (1.1 g mg, 0.91 mmol) under N_2. Allylmethyl carbonate (610 μL, 5.47 mmol) in THF (50 mL) was syringed over 10 min. The reaction mixture was heated to 60 °C for 22 h. The solvent was evaporated in vacuo, and the residue was diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (6:1 hexane/EtOAc) to afford Boc-D-Ser(Allyl)-OMe as a yellow oil (235 mg, 20%). ^{1}H and ^{13}C spectra of Boc-D-Ser(Allyl)-OMe agreed well with those of the corresponding antipode Boc-Ser(Allyl)-OMe. HRMS (ESI+): $[M+Na]^+$ calcd for $C_{12}H_{21}NNaO_5$, 282.1317; found, 282.1312.

Boc-D-Ser(Allyl)-OH

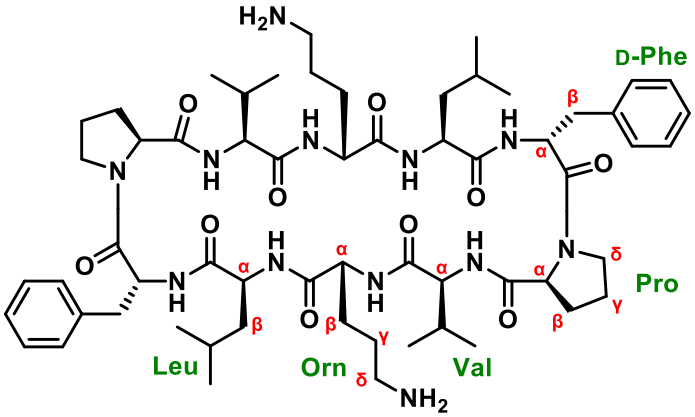


To a solution of Boc-D-Ser(Allyl)-OMe (190 mg, 0.73 mmol) in a 1:1 (v/v) mixture of THF and H_2O was added LiOH (153 mg, 3.65 mmol) at room temperature. Stirring was continued at room temperature for 24 h. The flask was then placed on a rotary evaporator and the THF was removed at reduced pressure. The residue was diluted with H_2O and washed with EtOAc. The aqueous layer was acidified with citric acid monohydrate and back extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford Boc-D-Ser(Allyl)-OH as a white solid (169 mg, 94%). ^{1}H and ^{13}C spectra of Boc-D-Ser(Allyl)-OH agreed well with those of the corresponding antipode Boc-Ser(Allyl)-OH. HRMS (ESI+): $[M+Na]^+$ calcd for $C_{11}H_{19}NNaO_5$, 268.1161; found, 268.1157.

***O*-Allyl-D-Ser**



Boc-D-Ser(Allyl)-OH (160 mg, 0.65 mmol) was dissolved in a 4N-HCl/dioxane at room temperature. After 6 h, the flask was placed on the rotary evaporator to afford *O*-Allyl-D-Ser as a white solid (97 mg, quant.). ^{1}H and ^{13}C spectra of *O*-Allyl-D-Ser agreed well with those of the corresponding antipode *O*-Allyl-L-Ser. HRMS (ESI+): $[M+H]^+$ calcd for $C_6H_{11}NO_3$, 146.0817; found, 146.0812.



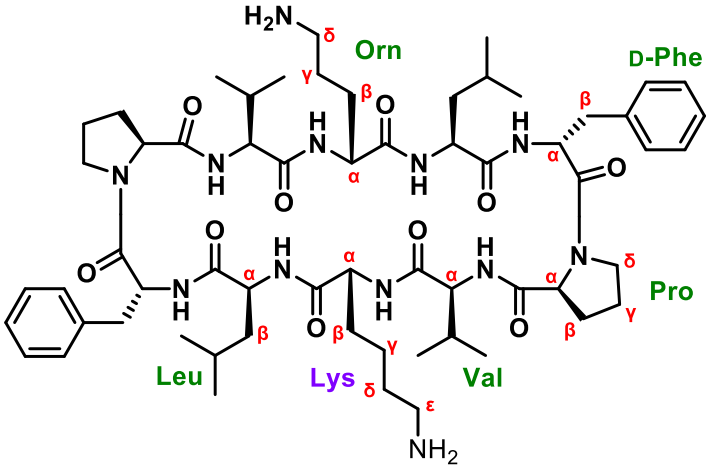
GS (**1**)

GS (**1**)

^{1}H NMR (800 MHz, CD_3OD) δ 0.88/0.89 [each 3H, d, *J* = 6.7, CH(C*H*$_3$)$_2$, Leu], 0.89/0.95 [each 3H, d, *J* = 6.7, CH(C*H*$_3$)$_2$, Val], 1.40 (1H, ddd, *J* = 13.6, 9.8, 6.7 H$_\beta$a, Leu), 1.48–1.55 [{4H, m, C*H*(CH$_3$)$_2$ and H$_\beta$b, Leu}, {H$_\gamma$ab, Orn}], 1.67–1.80 [4H, m, (H$_\beta$a, Orn), (H$_\beta$a and H$_\gamma$ab, Pro)], 2.00 (1H, td, *J* = 5.2, 1.9, H$_\beta$b, Pro), 2.05 (1H, m, H$_\beta$b, Orn), 2.26 [1H, dsep, *J* = 11.0, 6.7, C*H*(CH$_3$)$_2$, Val], 2.48 (1H, br ddd like, *J* = *ca*. 9.4, 7.7, H$_\delta$a, Pro), 2.86 (1H, ddd, *J* = 12.0, 10.0, 6.7, H$_\delta$a, Orn), 2.94 (1H, t, *J* = 11.5, H$_\beta$a, D-Phe), 3.03 (1H, ddd, *J* = 12.0, 6.8, 6.2, H$_\delta$b, Orn), 3.09 (1H, dd, *J* = 11.5, 5.1, H$_\beta$b, D-Phe), 3.73 (1H, td, *J* = 9.4, 2.1, H$_\delta$b, Pro), 4.13 (1H, t, *J* = 11.0, H$_\alpha$, Val), 4.34 (1H, dd, *J* = 7.7, 1.9,H$_\alpha$, Pro), 4.50 (1H, dd, *J* = 11.5, 5.1, H$_\alpha$, D-Phe), 4.66 (1H, td, *J* = 9.8, 6.7, H$_\alpha$, Leu), 4.97 (1H, td, *J* = 9.2, 5.2, H$_\alpha$, Orn), 7.24-7.32 (5H, m, arom. D-Phe), 7.69 (1H, d, *J* = 11.0, N*H*, Val), 8.65 (1H, d, *J* = 9.2, N*H*, Orn), 8.72 (1H, d, *J* = 9.8, N*H*, Leu).

^{13}C NMR (200 MHz, CD_3OD) δ 19.6/19.7 [CH(*C*H$_3$)$_2$, Val], 23.1/23.3 [CH(*C*H$_3$)$_2$, Leu], 24.5 (C$_\gamma$, Pro), 24.6(C$_\gamma$, Orn), 25.7 [*C*H(CH$_3$)$_2$, , Leu], 30.7 (C$_\beta$, Pro), 30.8 (C$_\beta$, Orn), 32.1 [*C*H(CH$_3$)$_2$, Val], 37.3 (C$_\beta$, D-Phe), 40.4 (C$_\delta$, Orn), 42.1 (C$_\beta$, Leu),48.0 (C$_\delta$, Pro), 51.5 (C$_\alpha$, Leu), 52.5 (C$_\alpha$, Orn), 55.9 (C$_\alpha$, D-Phe), 60.5 (C$_\alpha$, Val), 62.1 (C$_\alpha$, Pro), 128.6/129.8(2C)/130.5(2C) (d, arom, D-Phe), 137.0 (s, arom, D-Phe), 172.5/172.8/173.5/ 173.6/173.7 (N*C*O).

HRMS (ESI+): [M+H]$^+$ calcd for $C_{60}H_{93}N_{12}O_{10}$, 1141.7138; found, 1141.7106.



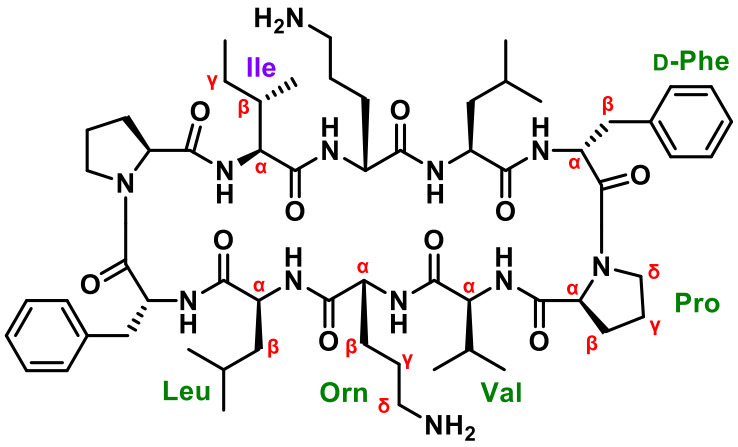
4-Lys-GS (**2**)

4-Lys-GS (**2**)

^{1}H-NMR (800 MHz, CD_3OD) δ 0.87/0.88/0.94/0.95 [each 3H, d, *J* = 6.7, CH(C*H*$_3$)$_2$, Leu], 0.88–0.89 [12H, m, CH(C*H*$_3$)$_2$, Val], 1.28–2.05 [24H, m, { H$_\beta$ab and C*H*(CH$_3$)$_2$, Leu}, {H$_\beta$ab, H$_\gamma$ab, Orn}, {H$_\beta$ab, H$_\gamma$ab and H$_\delta$ab, Lys}, {H$_\beta$ab and H$_\gamma$ab, Pro}], 2.24/2.25 [each 1H, dsep, *J* = 8.8, 6.7, C*H*(CH$_3$)$_2$, Val], 2.46/2.48 (each 1H, br q like, *J* = *ca*. 8.5, H$_\delta$a, Pro), 2.85–2.88 (1H, br m, H$_\delta$a, Orn), 2.93/2.94 (each 1H, t, *J* = 11.2, H$_\beta$a, D-Phe), 2.99–3.09 [5H, m, (H$_\delta$b, Orn), (H$_\varepsilon$ab, Lys), (H$_\beta$b, D-Phe)], 3.69–3.74 (2H, br m, H$_\delta$b, Pro), 4.12/4.20 (each 1H, t, *J* = 8.8, H$_\alpha$, Val), 4.34/4.36 (each 1H, dd, *J* = 7.3, 1.0, H$_\alpha$, Pro), 4.47–4.50 (2H, br m, H$_\alpha$, D-Phe), 4.62/4.65 (each 1H, td, *J* =9.2, 7.2, H$_\alpha$, Leu), 4.93 (1H, td, *J* = 9.2, 5.1, H$_\alpha$, Orn), 4.97 (1H, td, *J* = 8.9, 6.3, H$_\alpha$, Lys), 7.23/7.24 (each 2H, d, *J* = 6.5, arom., D-Phe), 7.270/7.274 (each 1H, t, *J* = 6.5, arom., D-Phe), 7.308/7.309 (each 2H, t, *J* = 6.5, arom., D-Phe), 7.64/7.66 (each 1H, d, *J* = 8.8, N*H*, Val), 8.49 (1H, br d like, *J* = *ca*. 9.2, N*H*, Orn), 8.56 (1H, br d like, *J* = *ca*. 8.9, N*H*, Lys), 8.74/8.77 (each 1H, d, *J* = 9.2, N*H*, Leu[1]).

^{13}C-NMR (200 MHz, CD_3OD) δ 19.3/19.5/19.7/19.8 [CH(*C*H$_3$)$_2$, Val], 23.0/23.1/23.2/23.3 [CH(*C*H$_3$)$_2$,Leu], 23.7 (C$_\gamma$, Lys), 24.5 (2C, C$_\gamma$, Pro), 24.6 (C$_\gamma$, Orn), 25.7/25.8 [*C*H(CH$_3$)$_2$, Leu], 28.5 (C$_\delta$, Lys), 30.6 (C$_\beta$, Orn), 30.7 (2C, C$_\beta$, Pro), 30.8 (C$_\beta$, Lys), 32.1/32.2 [C*H*(CH$_3$)$_2$, Val], 37.3/37.4 (C$_\beta$, D-Phe), 40.4 (C$_\delta$, Orn), 40.9 (C$_\varepsilon$, Lys), 42.0/42.1 (C$_\beta$, Leu), 47.8/48.0 (C$_\delta$, Pro), 51.6 (2C, C$_\alpha$, Leu), 52.6 (C$_\alpha$, Orn), 53.6 (C$_\alpha$, Lys), 55.8/55.9 (C$_\alpha$, D-Phe), 60.3/60.5 (C$_\alpha$, Val), 61.9/62.1 (C$_\alpha$, Pro), 128.6(2C)/ 129.8(4C)/130.5(4C) (d, arom, D-Phe), 136.9/137.0 (s, arom, D-Phe), 172.5/172.6/ 172.7/173.0/173.4/173.5/173.6 (2C)/173.7/173.9 (N*C*O).

HRMS (ESI+): [M+H]$^+$ calcd for $C_{61}H_{95}N_{12}O_{10}$, 1155.7294; found, 1156.5050.



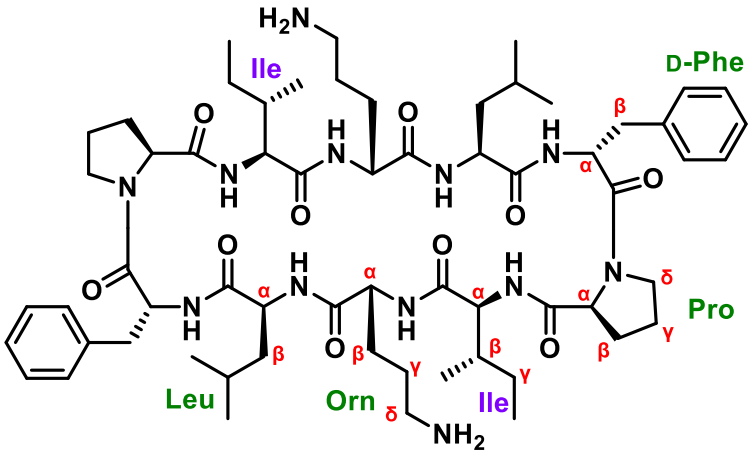
8-Ile-GS (**3**)

8-Ile-GS (**3**)

^{1}H-NMR (800 MHz, CD_3OD) δ 0.83-0.90 [18H, m, { $CH(CH_3)_2$, Leu}, {$CHCH_3$ and CH_2CH_3, Ile}], 0.90/0.96 [each 3H, d, *J* = 6.8, $CH(CH_3)_2$, Val], 1.19-1.33 (1H, m, H_γa, Ile), 1.40 (2H, br dt like, *J* = *ca*. 13.0, 7.4, H_βa, Leu), 1.46-1.64 [6H, m, {H_βb and $CH(CH_3)_2$, Leu}, {H_γa, Pro}], 1.67-1.80 [11H, m, (H_βa and $H_\gamma$ab, Orn), (H_γb, Ile), (H_βa and H_γb, Pro)], 1.98-2.15 [5H, m, { H_βb, Orn}, {H_β, Ile}, {H_βb, Pro}], 2.24-2.28 [2H, m, $CH(CH_3)_2$, Val], 2.48 (2H, br q like, *J* = *ca*. 9.2, H_δa, Pro), 2.86-2.90 (2H, m, H_δa, Orn), 2.93 (2H, t, *J* = 11.0, H_βa, D-Phe), 3.02-3.06 (2H, m, H_δb, Orn), 3.09 (2H, dd, *J* = 12.5, 4.9, H_βb, D-Phe), 3.72/3.73 (each 1H, br de like, *J* = *ca*. 8.1, 2.5, H_δb, Pro), 4.14 (1H, t, *J* = 8.8, H_α, Val), 4.20 (1H, t, *J* = 9.0, H_α, Ile), 4.33 (2H, br t like, *J* = *ca*. 7.0, H_α, Pro), 4.50 (2H, dd, *J* = 11.3, 5.0, H_α, D-Phe), 4.65/4.66 (each 1H, td, *J* = 9.4, 7.4, H_α, Leu), 4.97/4.99 (each 1H, td, *J* = 9.9, 5.4, H_α, Orn), 7.24-7.32 (10H, m, arom. D-Phe), 7.69 (1H, br d, *J* = *ca*. 9.0, N*H*, Ile), 7.70 (1H, br d, *J* = *ca*. 8.8, N*H*, Val), 8.64/8.67 (each 1H, br d, *J* = *ca*. 9.9, N*H*, Orn), 8.72/8.74 (each 1H, d, *J* = 9.4, N*H*, Leu).

^{13}C-NMR (200 MHz, CD_3OD) δ 10.9 (CH_2CH_3, Ile), 15.9 ($CHCH_3$, Ile), 19.5/19.7 [$CH(CH_3)_2$, Val], 23.08/23.15/23.19/23.3 [$CH(CH_3)_2$, Leu], 24.5 (2C, C_γ, Pro), 24.6 (2C, C_γ, Orn), 25.7/25.8 [$CH(CH_3)_2$, Leu], 26.3 (C_γ, Ile), 30.7(3C)/30.8 (C_β, Orn and Pro), 32.0 [$CH(CH_3)_2$, Val], 37.3 (2C, C_β, D-Phe), 37.8 (C_β, Ile), 40.4 (2C, C_δ, Orn), 42.0/42.1 (C_β, Leu), 48.0/48.1 (H_δ, Pro), 51.5/51.6 (C_α, Leu), 52.4/52.5 (C_α, Orn), 55.9/56.0 (C_α, D-Phe), 59.0 (C_α, Ile), 60.5 (C_α, Val), 62.1(2C, C_α, Pro), 128.6(2C)/ 129.8(4C)/130.5(4C) (d, arom., D-Phe), 137.0(2C, s, arom., D-Phe), 173.5(2C)/ 173.6(2C)/173.7/173.8/176.4(2C) /176.4(2C) (N*C*O).

HRMS (ESI+): $[M+H]^+$ calcd for $C_{61}H_{95}N_{12}O_{10}$, 1155.7294; found, 1155.7329.



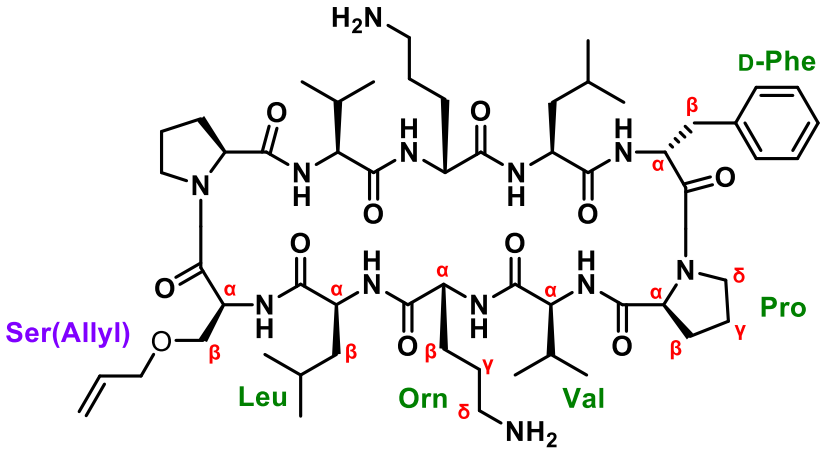
3,8-Ile-GS (**4**)

3,8-Ile-GS (**4**)

^{1}H-NMR (800 MHz, CD_3OD) δ 0.84–0.86 [6H, m CH_2CH_3 and $CHCH_3$, Ile], 0.89/0.90 [each 3H, d, *J* = 6.5, $CH(CH_3)_2$, Leu], 1.21–1.25 [1H, m, Hγa, Ile], 1.39 (1H, td, *J* = 7.4, 6.7, Hβa, Leu), 1.47–1.63 [5H, m, {$CH(CH_3)_2$ and Hβb, Leu}, {Hβa, Orn}, {Hγb, Ile}, {Hγa, Pro}], 1.65–1.71 [2H, m, (Hβa and Hγb, Pro)], 1.74–1.79 (2H, m, Hγab, Orn), 1.98-2.01 (1H, m, Hβb, Pro), 2.02–2.07 (1H, m, Hβb, Orn), 2.10–2.16 (1H, m, Hβ, Ile), 2.48 (1H, br q like, *J* = *ca*. 9.6, Hδa, Pro), 2.85–2.89 (1H, m, Hδa, Orn), 2.93 (1H, t, *J* = 11.6, Hβa, D-Phe), 3.02–3.06 (1H, m, Hδb, Orn), 3.08 (1H, dd, *J* = 11.6, 5.0, Hβb, D-Phe), 3.72 (1H, br ddd like, *J* = *ca*. 9.6, 8.4, 3.0, Hδb, Pro), 4.19 (1H, t, *J* = 9.0, Hα, Ile), 4.33 (1H, dd, *J* = 8.0, 2.1, Hα, Pro), 4.50 (1H, dd, *J* = 11.6, 5.0, Hα, D-Phe), 4.66 (1H, td, *J* = 9.2, 7.4, Hα, Leu), 5.02 (1H, td, *J* = 9.9, 5.3, Hα, Orn), 7.24–7.32 (5H, m, arom., D-Phe), 7.72 (1H, d, *J* = 9.0, N*H*, Ile), 8.66 (1H, br d , *J* = *ca*. 9.9, Orn, N*H*), 8.73 (1H, br d-like, *J* = *ca*. 9.2, N*H*, Leu).

^{13}C-NMR (200 MHz, CD_3OD) δ 10.9 (CH_2CH_3, Ile), 15.9 ($CHCH_3$, Ile), 23.1/23.2 [$CH(CH_3)_2$, , Leu], 24.5 (C_γ, Pro), 24.6 (H_γ, Orn), 25.7 [$CH(CH_3)_2$, , Leu], 26.4 (C_γ, Ile), 30.7 (C_β, Pro), 30.8 (H_β, Orn), 37.3 (C_β, D-Phe), 37.7 (C_β, Ile), 40.4 (H_δ, Orn), 42.1 (C_β, Leu), 48.1 (C_δ, Pro), 51.5 (C_α, Leu), 52.4 (C_α, Orn), 56.0 (C_α, D-Phe), 59.0 (C_α, Ile), 62.1 (C_α, Pro), 128.6/129.8(2C)/130.5(2C)(d, arom., D-Phe), 137.0 (s, arom. D-Phe), 173.1/173.5/173.6/173.7 /176.4 (NCO).

HRMS (ESI+): $[M+H]^+$ calcd for $C_{62}H_{97}N_{12}O_{10}$, 1169.7451; found, 1169.7349.



6-Ser(Allyl)-GS (**5**)

6-Ser(Allyl)-GS (**5**)

^{1}H-NMR (800 MHz, CD_3OD) δ 0.86/0.90/0.93/0.10 [each 3H, d, *J* = 6.7, CH(CH_3)$_2$, Leu], 0.867–0.90 [12H, m, CH(CH_3)$_2$, Val], 1.25–1.77 [12H, m, {$H_\beta$ab and C*H*(CH_3)$_2$, Leu},{$H_\gamma$ab Orn}, {H_βa and H_γa, Pro}],1.91–2.09 [10H, m, {$H_\beta$ab Orn},{H_βb and H_γb, Pro}], 2.24/2.30 [each 1H, dsep, *J* = 8.8, 6.7, C*H*(CH_3)$_2$, Val], 2.46/3.78 (each 1H, q, *J* = 9.3, H_δa, Pro), 2.79/2.84/2.96/3.01 (each 1H, td, *J* = 9.7, 6.7, $H_\delta$ab, Orn), 2.92 (1H, t, *J* = 12.0, H_βa, D-Phe), 3.07 (1H, dd, *J* = 12.0, 5.0, H_βb, D-Phe), 3.67 [1H, t, *J* = 9.1, H_βa, Ser(Allyl)], 3.70 [1H, dd, *J* = 9.1, 6.2, H_βb, Ser(Allyl)], 3.72/4.08 (each 1H, td, *J* = 9.3, 2.6, H_δb, Pro), 3.98/4.02 [each 1H, br ddt like, *J*= *ca*. 12.9, 5.4, 1.6, C*H*$_2$CH=CH$_2$, Ser(Allyl)], 4.12/4.15 (each 1H, t, *J* = 8.8, 6.6, H_α, Val), 4.32 (1H, br d like, *J* = *ca*. 6.6, H_α, Pro), 4.32 (1H, br t like, *J* = *ca*. 5.5, Hα, Pro), 4.48 (1H, dd, *J* = 12.0, 5.0, H_α, D-Phe), 4.61–4.66 [3H, m, H_α, Leu and H_α, Ser(Allyl)], 4.94/4.95 (each 1H, dd, *J* = 10.4, 5.1, H_α, Orn), 5.17 [1H, br dd like, *J* = *ca*. 10.4, 1.6, CH$_2$CH=C*H*$_2$, Ser(Allyl)], 5.24 [1H, br qd like, *J* = *ca*.17.3, 1.6, CH$_2$CH=C*H*$_2$, Ser(Allyl)], 5.85–5.90 [1H, m, CH$_2$C*H*=CH$_2$, Ser(Allyl)], 7.23 (2H, d, *J* = 7.5, arom., D-Phe), 7.26 (1H, t, *J* = 7.5, arom., D-Phe), 7.30 (2H, t, *J* = 7.5, arom., D-Phe), 7.67/7.74 (each 1H, br d like, *J* = *ca*. 8.8, N*H*, Val), 8.70/8.72 (each 1H, d, *J* = 9.3, N*H*, Leu).

^{13}C-NMR (200 MHz, CD_3OD) δ 19.5/19.6/19.70/19.72 [CH(*C*H$_3$)$_2$, Val], 23.0/23.1/23.27/23.31 [CH(*C*H$_3$)$_2$, Leu], 24.5 (2C, C_γ, Orn and Pro), 24.6 (C_γ, Orn), 24.9 (C_γ,Pro), 25.7 [2C, *C*H(CH_3)$_2$, Leu], 30.7 (2C, C_β, Pro), 30.8 (2C, C_β, Orn), 32.0/32.1 [C*H*(CH_3)$_2$, Val], 37.3 (C_β, D-Phe), 40.4 (2C, C_δ, Orn), 41.99/42.02 (C_β, Leu), 48.0/48.7 (C_δ, Pro), 51.41/51.43 (C_α, Leu), 52.5 (2C, C_α, Orn), 53.9 [C_α, Ser(Allyl)], 55.9 (C_α, D-Phe), 60.5/60.6 (C_α, Val), 62.1/62.4 (C_α, Pro), 69.3 [C_β, Ser(Allyl)], 73.4 [*C*H$_2$CH=CH$_2$, Ser(Allyl)], 117.6 [CH$_2$CH=*C*H$_2$, Ser(Allyl)], 128.6/129.8(2C) /130.5(2C) (d, arom, D-Phe), 135.5 [CH$_2$*C*H=CH$_2$, Ser(Allyl)], 137.0 (s, arom, D-Phe); 172.4 /172.75/172.80/172.85/173.5/173.64 (2C)/173.66/173.7/173.9 (N*C*O).

HRMS (ESI+): $[M+H]^+$ calcd for $C_{57}H_{93}N_{12}O_{11}$, 1121.7087; found, 1122.4440.

Chemical Biology Procedures

Protein Expression and Materials: Recombinant *holo*-GrsA was expressed and purified as previously described.[9,10]

Bacterial strains: *Aneurinibacillus migulanus* ATCC 9999 was obtained from the American Type Culture Collection (ATCC).

Preparation of cellular lysates for proteomic labeling experiments: *A. migulanus* ATCC 9999 was maintained on nutrient agar. Single colonies were used to inoculate YPG medium (50 g/L yeast extract, 50 g/L bacto peptone, and 5 g/L glucose) and cultures were shaken for 24 h at 37 °C. The seed culture (2 mL) was transferred to YPG media (250 mL) and the resulting mixture was incubated at 37 °C. Growth was routinely monitored at A_{660} on a U-2910 spectrophotometer (Hitachi). The cells (OD_{600} = 10) were harvested by centrifugation and stored in the freezer until used. The frozen cell pellets were resuspended in Tris pH 8.0 (20 mM), $MgCl_2$ (1 mM), and TCEP (1 mM). Because of the lability of the synthetase during mechanical cell disruption processes,[11] a gentle treatment of cells with lysozyme (0.2 mg/mL) was used to release intracellular protein. The cell suspension was incubated at 0 °C for 30 min. The mixture was then incubated at 30°C for 30 min. The solution was centrifuged for 5 min at 15,000 rpm and the pellets were discarded. The total protein concentration was quantitated by the method of Bradford.[12]

Competitive ABPP of the A-domain of recombinant GrsA in a purified system: Recombinant GrsA (1 μM) were treated with Phe-AMS-BPyne **6** (1 μM from a 100 μM stock in DMSO) in 20 mM Tris (pH 8.0), 1 mM $MgCl_2$, 1 mM TCEP, and 0.0025% NP-40. Inhibition studies were performed by pre-incubation of GrsA (1 μM) with inhibitors **7**–**23** (100 μM from a 10 mM stock in DMSO) for 10 min at room temperature, respectively. In all experiments total DMSO concentration was kept at 2.2%. After 10 min at room temperature, these samples were irradiated at 365 nm for 30 min on ice. To initiate the click reaction, rhodamine (Rh)-azide, TCEP, TBTA ligand, and $CuSO_4$ were added to provide final concentrations of 100 μM, 1 mM, 100 μM, and 1 mM, respectively. After 1 h at room temperature, 5× SDS-loading buffer (strong reducing) was added and the samples were heated at 95 °C for 5 min. Samples were separated by 1D SDS-PAGE and fluorescent gel visualization was performed using a Typhoon 9410 Gel and Blot Imager (GE Healthcare).

Competitive ABPP of the A-domain of endogenous GrsA in a complex system: *A. miglanus* ATCC 9999 proteome (2.0 mg/mL) was individually treated with inhibitors **7**–**23** (100 μM from a 10 mM stock in DMSO) in 20 mM Tris (pH 8.0), 1 mM $MgCl_2$, 1 mM TCEP, 0.2 mg/mL

lysozyme. These samples were incubated for 10 min at room temperature and subsequently treated with Phe-AMS-BPyne **6** (1 μM from a 100 μM stock in DMSO). In all experiments, the total DMSO concentration was kept at 2.2%. After 10 min at room temperature, these samples were irradiated at 365 nm for 30 min on ice and reacted with TAMRA-azide for 1 h at room temperature. Reactions were treated with 5× SDS-loading buffer (strong reducing) and subjected to 1D SDS-PAGE. Fluorescent gel visualization was performed using a Typhoon 9410 Gel and Blot Imager (GE Healthcare).

Hydroxamate-MesG assay

Standard assay conditions: Reactions contained 1 μM GrsA in 20 mM Tris (pH 8.0), 2.5 mM ATP, 1 mM $MgCl_2$, 1 mM TCEP, 150 mM hydroxylamine (pH 7.0), 0.1 U purine nucleoside phosphorylase (Sigma–Aldrich, N8264), 0.04 U inorganic pyrophosphatase (Sigma–Aldrich, I1643), 0.2 mM MesG (Berry & Associates), and varying concentrations of substrates. The reactions (100 μL) were run in 96-well half-area plates (Corning, 3881) and the cleavage of MesG was monitored at A_{355} on an EnVision Multilabel Reader (PerkinElmer). Working stocks of hydroxylamine were prepared fresh by combining 500 μL of 4 M hydroxylamine, 250 μL of water and 250 μL of 7 M NaOH on ice.

Determination of kinetic parameters: Steady-state kinetic parameters of the substrates were determined for each enzyme using standard assay conditions as described above. The substrate concentrations are listed here: Nle (200–10000 μM), Cys(Et) (200–10000 μM), and Ser(Allyl) (200–10000 μM). Initial velocities were fit to the Michaelis-Menten equation using Prism 7 (GraphPad Software).

Production of gramicidin S and L-Lys-containing gramicidin S: The preparation of spores was performed as previously described.[13] The inoculum was prepared by introducing 100 μL of spore suspension into 50 mL of chemically defined medium F3/6, and cultures were incubated for 48 h at 37 °C. The defined F3/6 contained the following components per liter of Milli-Q water: 10 g of D-fructose, 6.5 g of K_2HPO_4, 1.7 g of KH_2PO_4, 203 mg of $MgCl_2{\cdot}6H_2O$, 103 mg of $CaCl_2{\cdot}2H_2O$, 10 mg of $MnCl_2{\cdot}4H_2O$, 0.27 mg of $FeCl_3{\cdot}6H_2O$, 1.5 g of L-Pro, 1.3 g of L-His, 1.3 g of L-Gln, 0.5 g of L-Met, 0.3 g of L-Arg, and 1.0 g of L-Phe.[14] The phosphates were autoclaved separately as a 10-fold concentrated solution. The Mg, Ca, and Mn salts were combined and stored in a 1000-fold concentrate. The Fe was also stored as a 1000-fold concentrate.

Supplementation experiments: The inoculum was prepared by introducing 100 μL of spore suspension into 50 mL of chemically defined medium F3/6, supplemented with 0.25 g of Ile, 0.25 g of Nle, 0.25 g of Cys(Et), or 0.25 g of *O*-allyl-L-Ser and cultures were taken for 48 h at 37 °C.

For concentration dependence of *O*-allyl-L-Ser supplementation, PDB experiments were performed using *O*-allyl-L-Ser (0.25–2 g).

Extraction and isolation: The cells were centrifuged (7500 ×*g*, 15 min, 4 °C). The pellet was suspended in the pre-extraction solution (150 mM NaCl, 20 mM HCl) and incubated at 80 °C for 15 min. The suspensions were diluted 1:1 with ethanol, and gramicidin S and its derivatives were extracted by stirring at room temperature for 1 h. Cell debris was removed by centrifugation. The supernatant was concentrated *in vacuo* to obtain the crude extract. The extract was separated by semipreparative HPLC [Cosmosil AR-II (Nacalai Tesque, Japan), ϕ10 × 250 mm, MeCN/H_2O = 60:40, 0.1% TFA, 3 mL/min, 210 nm to give GS (**1**) (t_R = 12.5 min), 4-Lys-GS (**2**) (t_R = 11.9 min) and 6-Ser(Allyl)-GS (**5**) (t_R = 9.8 min); Wakopak Navi C30-5 (Fujifilm, Japan), ϕ20 × 250 mm, MeCN/H_2O = 65:35, 0.1% TFA, 9 mL/min, 210 nm to give 8-Ile-GS (**3**) (t_R = 12.3 min) and 3,8-Ile-GS (**4**) (t_R = 16.0 min)].

HPLC and LC-MS analysis of the crude extract: The extract was analyzed by HPLC [Cosmosil AR-II (Nacalai Tesque, Japan), ϕ4.6 × 250 mm, MeCN/H_2O containing 0.1% TFA (10% MeCN 0 min, 10−100% MeCN 30 min, 100% MeCN 40 min, 10% MeCN 50 min), 1 mL/min, 210 nm] and LC-MS [Cosmosil AR-II (Nacalai Tesque, Japan), ϕ3.0 × 150 mm, MeCN/H_2O containing 0.1% FA (10% MeCN 0 min, 10−100% MeCN 30 min, 100% MeCN 40 min, 10% MeCN 50 min), 0.2 mL/min, 210 nm].

Hydrolysis of GS (1), 4-Lys-GS (2), 8-Ile-GS (3), 3,8-Ile-GS (4), and 6-Ser(Allyl)-GS (5) and advanced Marfey's method: GS (**1**), 4-Lys-GS (**2**), 8-Ile-GS (**3**), 3,8-Ile-GS (**4**), and 6-Ser(Allyl)-GS (**5**) (1 mg) were hydrolyzed in 6 M HCl for 24 h at 100 °C and then dried *in vacuo*. The resulting hydrolysate was dissolved in 1 M $NaHCO_3$ (20 μL). To the hydrolysate was added L-FDLA (1% w/v in acetone, 100 μL), and the mixtures were stirred for 1 h at 70 °C. The solution was cooled to room temperature, neutralized with 1 M HCl (20 μL), evaporated, and then dissolved in MeCN (100 μL). The derivatives were analyzed by LC−MS; LC separation was conducted by a reverse-phase column (Cosmosil AR-II (Nacalai Tesque, Japan), ϕ3.0 × 150 mm) with a gradient elution system of MeCN/H_2O containing 0.1 % FA (20% MeCN 5 min, 20−80% MeCN 60 min, 80−100% MeCN 70 min, 100% MeCN 80 min, 20% MeCN 90 min, 0.2 mL/min, 340 nm). L-Lys, D-Lys, L-Leu, D-Leu, L-Ile, D-Ile, *O*-allyl-L-Ser, and *O*-allyl-D-Ser (1 mg) were dissolved in 1 M $NaHCO_3$ (20 μL). To the solution was added L-FDLA (1% w/v in acetone, 100 μL), and the mixtures were stirred for 1 h at 70 °C. The solution was cooled to room temperature, neutralized with 1 M HCl (20 μL), evaporated, and then dissolved in MeCN (100 μL). The materials were used as standards without further purification.

MS/MS analysis of GS (1), 4-Lys-GS (2), 8-Ile-GS (3), 3,8-Ile-GS (4), and 6-Ser(Allyl)-GS (5): Dried samples were dissolved in DMSO and placed in 96 well plate (Agilent Co.) sealed with a Silicone Sealing Film (Agilent Co.). Compounds (0.1 μg each for analogs) were analyzed by using Ultra Performance Liquid Chromatography (UPLC, Agilent 1290 Infinity LC system, Agilent Co.) connected to Electrospray Ionization/Quadrupole Time of Flight/Mass Spectrometry (ESI/QTOF/MS, Agilent 6550A iFunnel Q-TOF syetem, Agilent Co.). The UPLC-ESI/QTOF/MS System was controlled using the MassHunter software (Agilent Co.).

LC settings: The UPLC system was equipped with a UPLC column (ACQUITY BEH C_{18}, 2.1×100 mm, Waters) with a pre-column (ACQUITY BEH C_{18} VanGuard Pre-column 2.1×5 mm, Waters). Acetonitrile (LC-MS grade, Fujifilm Wako) and H_2O (Milli-Q, Merck Millipore) containing 0.1% formic acid (LC-MS grade, Fujifilm Wako) were used as mobile phases. First concentration of acetonitrile was kept at 5% for 0.5 min, and then increased to 95% by linear gradient until 8.5 min, and then kept at 95% for 11.5 min. Column temperatures were kept at 40 ℃ and the flow rate was 0.3 mL/min.

QTOF/MS settings: Mass spectra were acquired in the positive ion mode over a mass range of 100–1700 *m/z*. Purine (*m/z* 121.050873; Agilent) and hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazene (*m/z* 922.009798; Agilent) were used as a lock-mass internal calibrants during data acquisition. The ion source was the Dual Agilent Jet Stream (AJS) ESI. The following instrument parameters were used for the MS and targeted MS/MS data acquisition: gas temperature of 200°C, drying gas flow of 14 L/min, nebulizer gas (nitrogen) pressure of 35 psig, sheath gas temperature of 350 °C, sheath gas flow of 11 L/min, funnel exit DC of 50 eV, funnel RF HP of 130 eV, and funnel RF LP of 60 eV. The following scan source parameters were used for the MS or targeted MS-MS data acquisition: capillary voltage of 3500 V (positive), nozzle voltage of 1000 V, fragmentor voltage of 175 V, skimmer voltage of 65 V, and octopole RF peak voltage of 750 V. Minutes 1.5–10.5 were recorded using MS or targeted MS/MS data acquisition. For MS or targeted MS/MS data acquisition, the scan rate setting was 1.0 spectra/sec. The selected ion(s) per MS1 scan were subjected to collision-induced dissociation (CID) according to the following parameters: isolation width (amu): 4, acquisition time, 200 ms/spec; collision energy, 50, 55, and 60 per scans; and scanning rt width: 0.5 min. GS (**1**): 1141.7132 (*z*=1), rt 6.65 min. 4-Lys-GS (**2**): 1155.7289 (*z*=1), rt 6.63 min. 8-Ile-GS (**3**): 1155.7289 (*z*=1), rt 6.80 min. 3,8-Ile-GS (**4**): 1169.7445 (*z*=1), rt 6.99 min. 6-Ser(Allyl)-GS (**5**): 1121.7081 (*z*=1), rt 6.46 min.

Determination of minimum inhibitory concentration (MIC): *Bacillus subtilis* ATCC 6051 was cultured overnight at 37°C in cation-adjusted Mueller Hinton Broth II (CA-MHB-II) and adjusted

to obtain turbidity comparable to 0.5 McFarland standards before MIC determination. The antibiotic activity of GS (**1**), 4-Lys-GS (**2**), 8-Ile-GS (**3**), 3,8-Ile-GS (**4**), and 6-Ser(Allyl)-GS (**5**) was assayed for antibiotic activity by using the broth microdilution method in a 96-well microtiter plate.[15] The MIC was determined at concentrations ranging from 0.0625–64 μg/mL. The test compound was added to sterile CA-MHB-II in a microtiter plate before the bacterial suspension was prepared as described above Inoculated and uninoculated wells of compound-free broth were also included (the former controls the adequacy of the broth to support the growth of the organism, whereas the latter is a sterility check). The 96-well plates were incubated for 24 h at 37°C. The assay was performed in duplicate. Resazurin was used as an indicator to support visualization by using a method similar to that reported by Sarker *et al*.[16] A sample of sterile aqueous resazurin solution (10 μL, 0.7% w/v) was added to the wells. After further incubation for 24 h at 37 °C, the plates were then evaluated.

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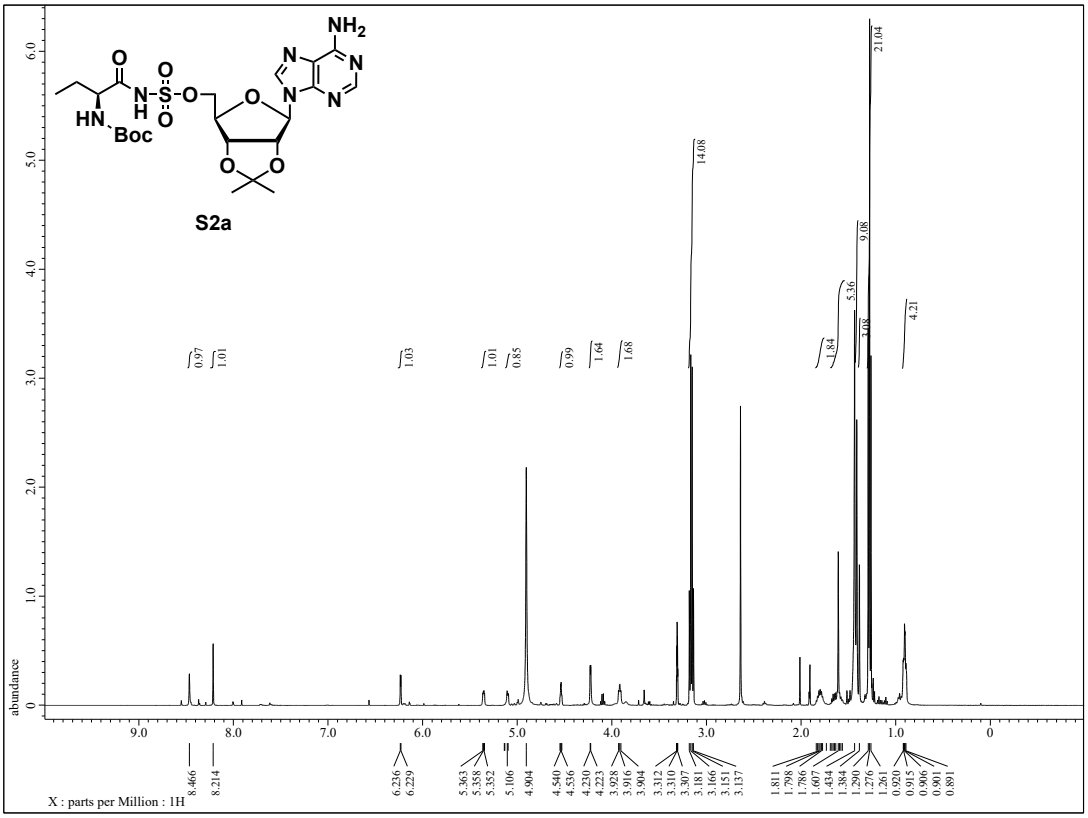
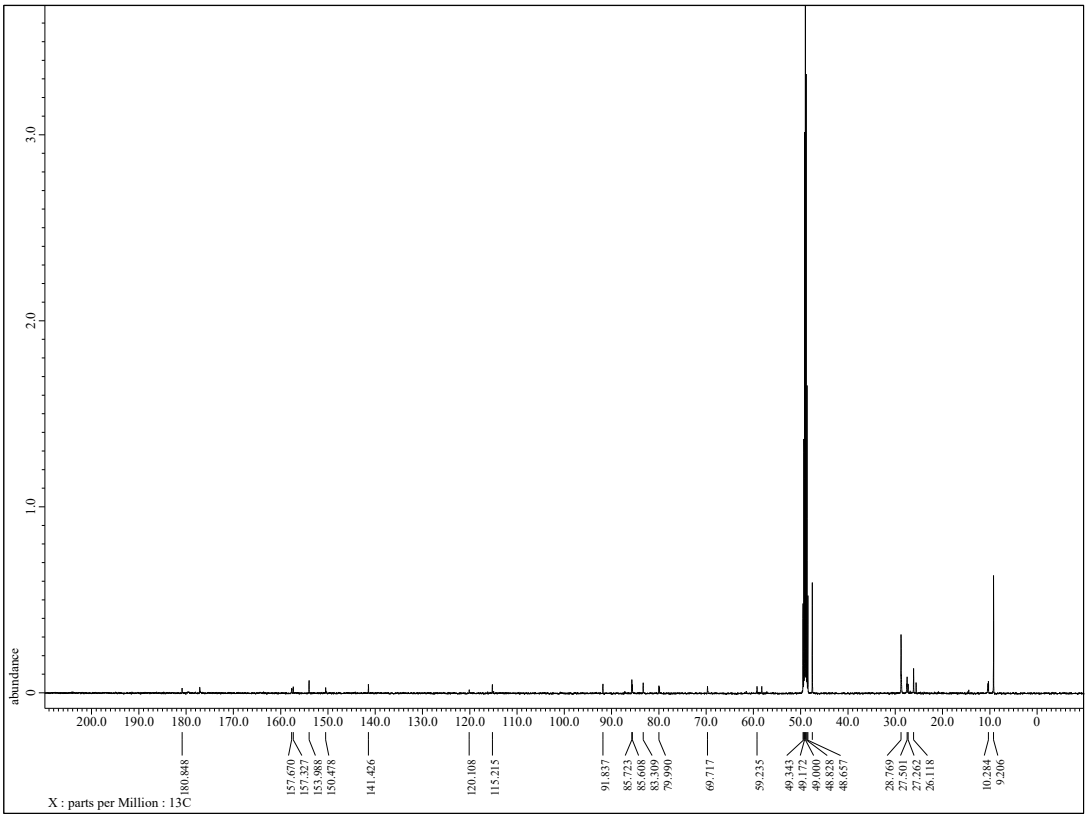
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2a** in CD_3OD





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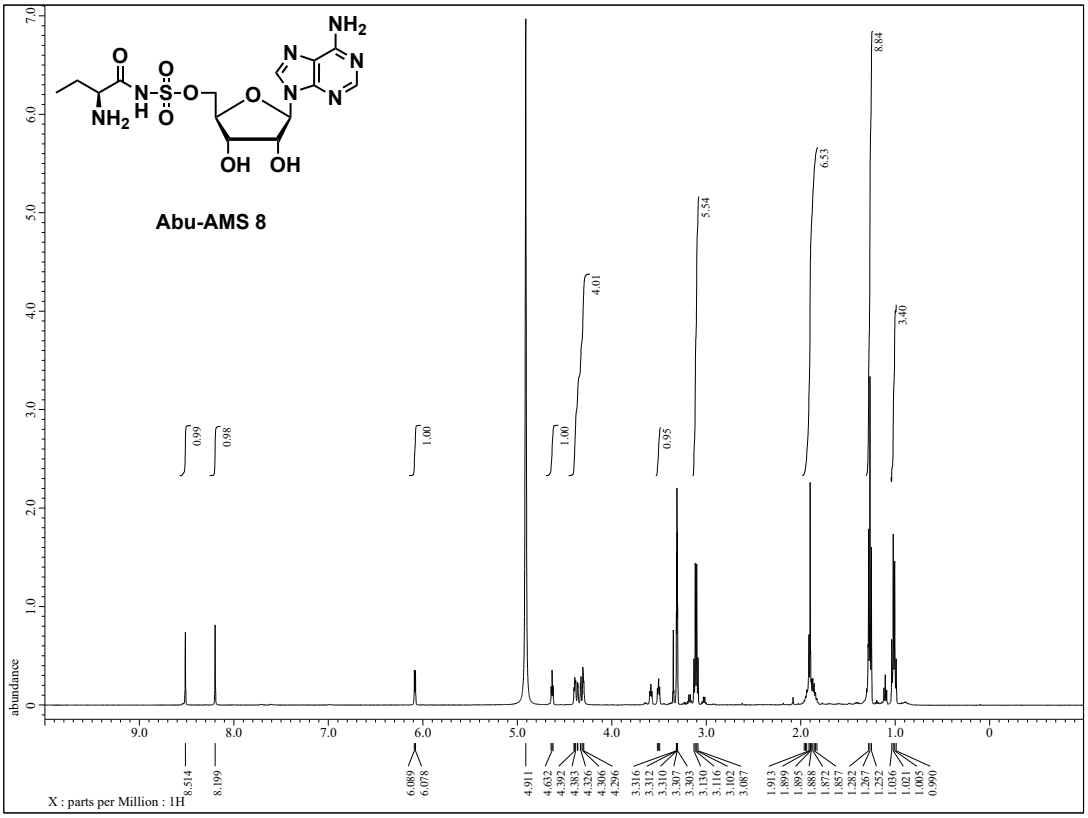

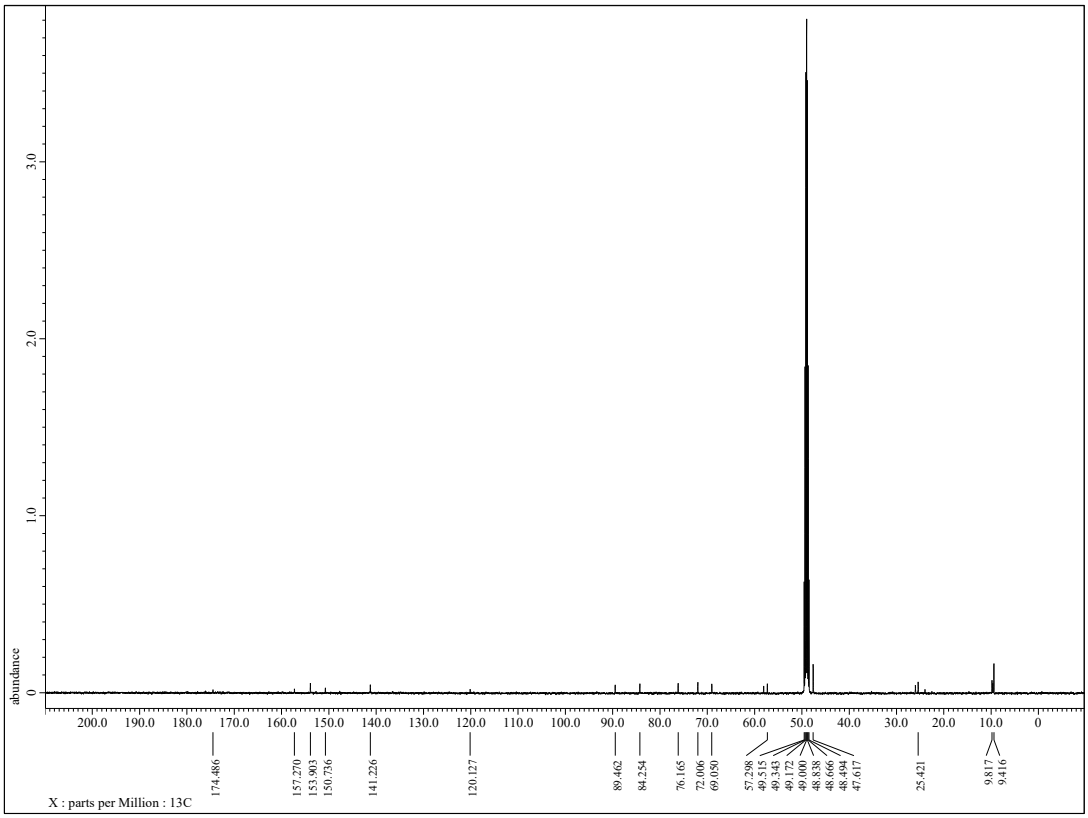

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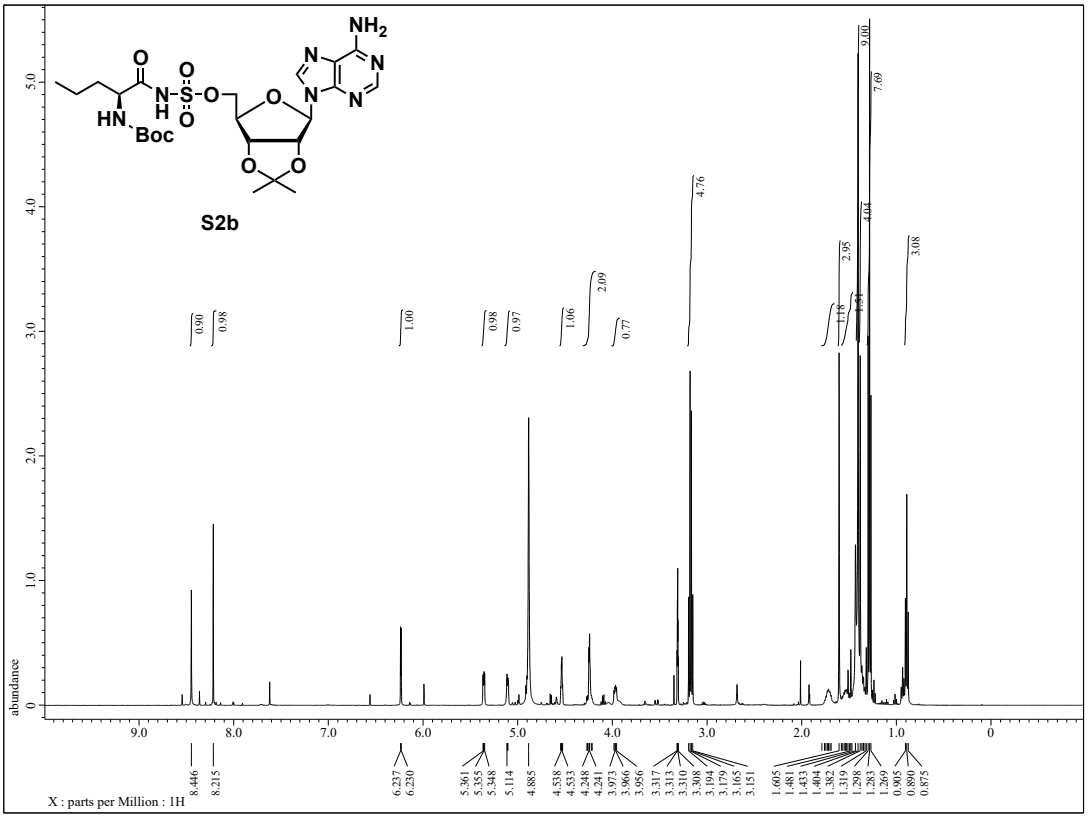

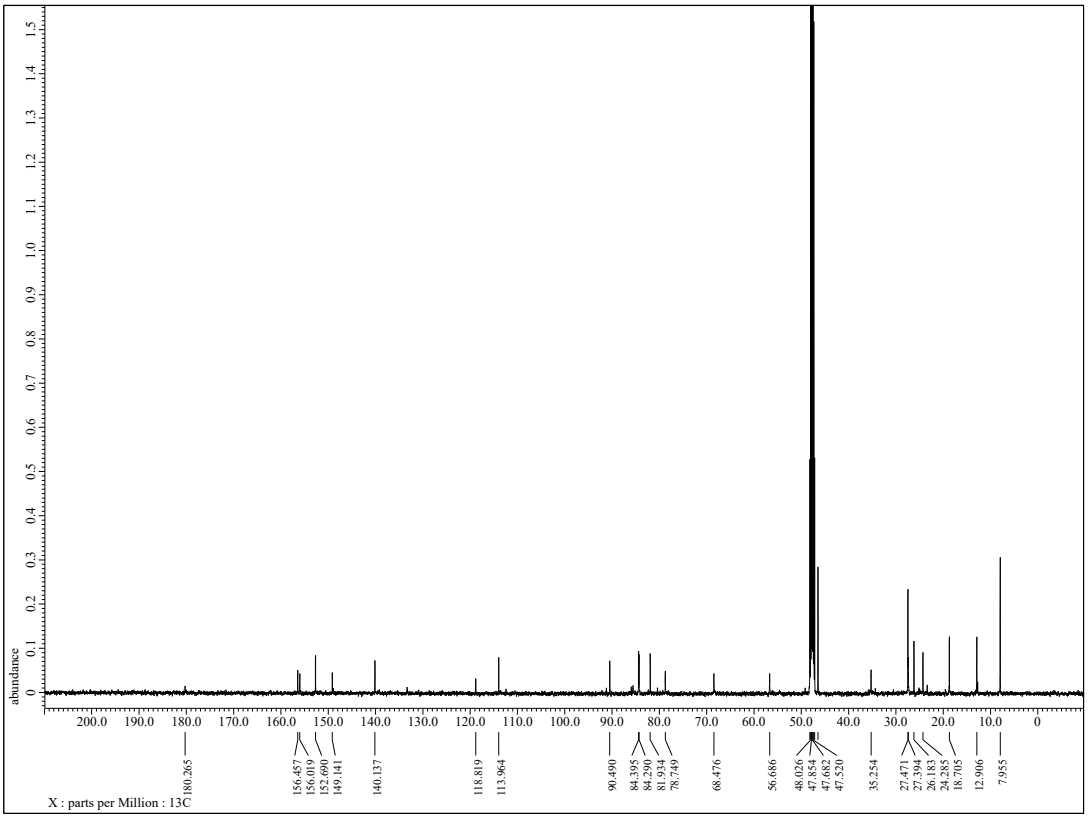

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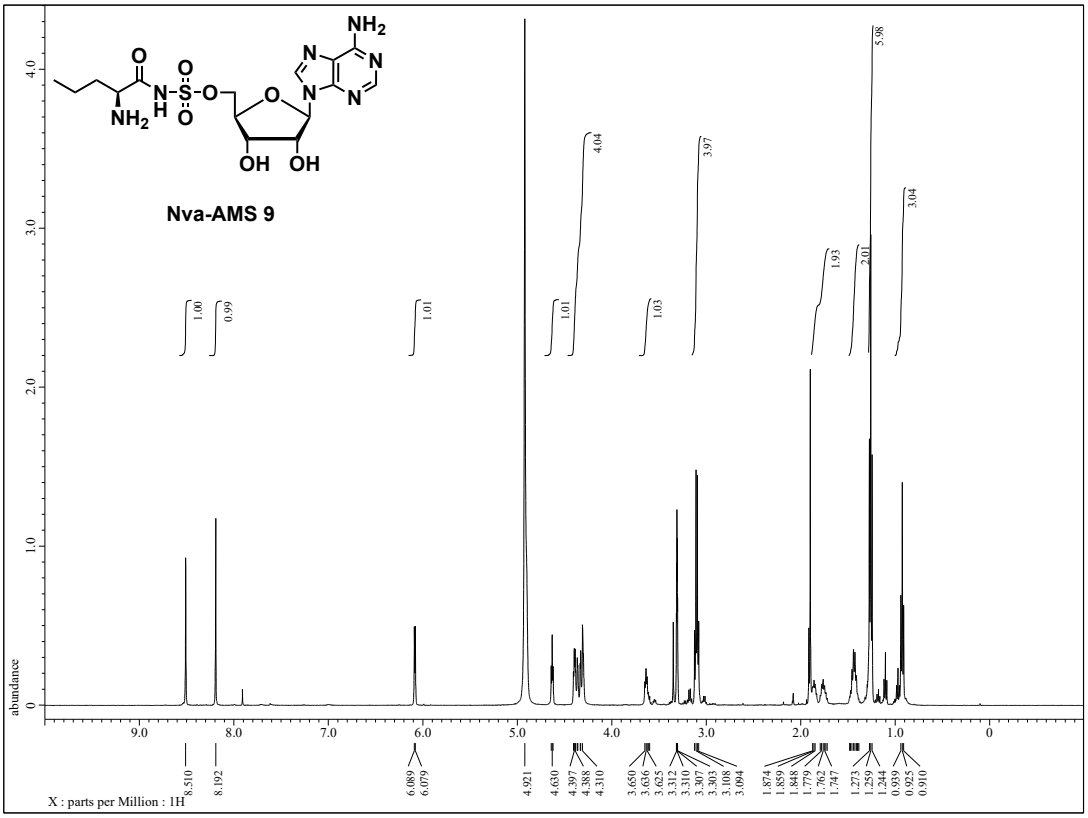

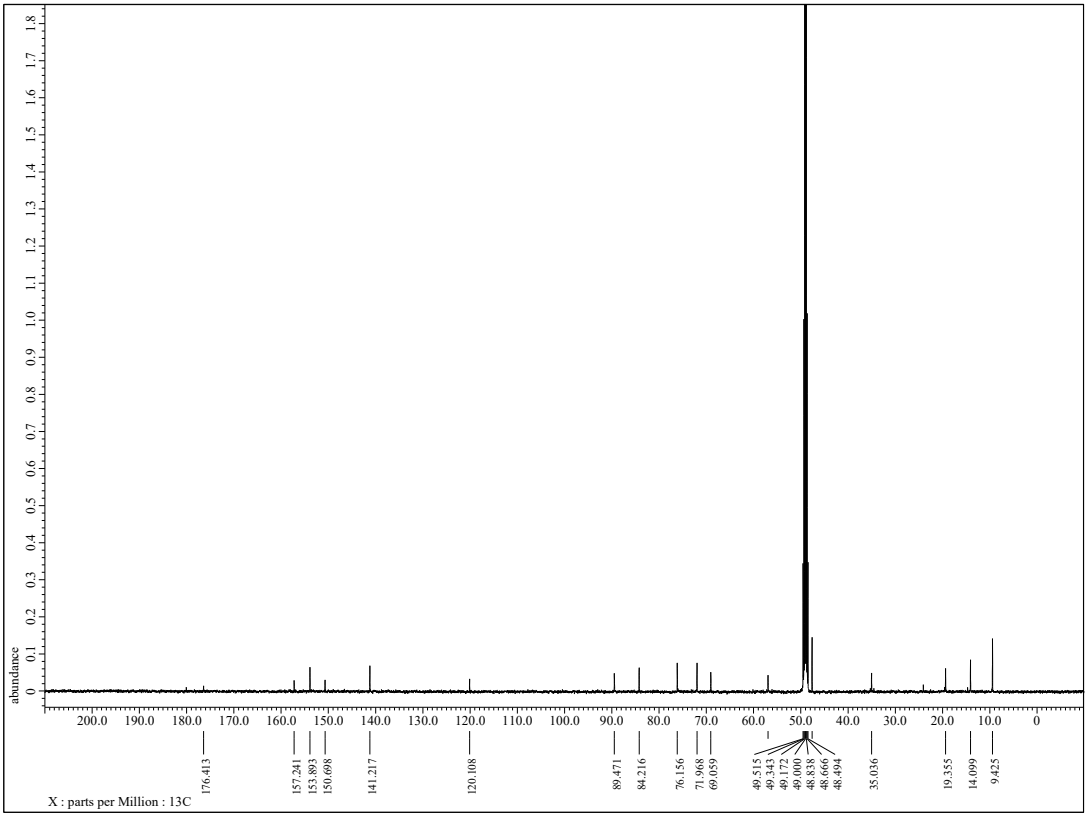

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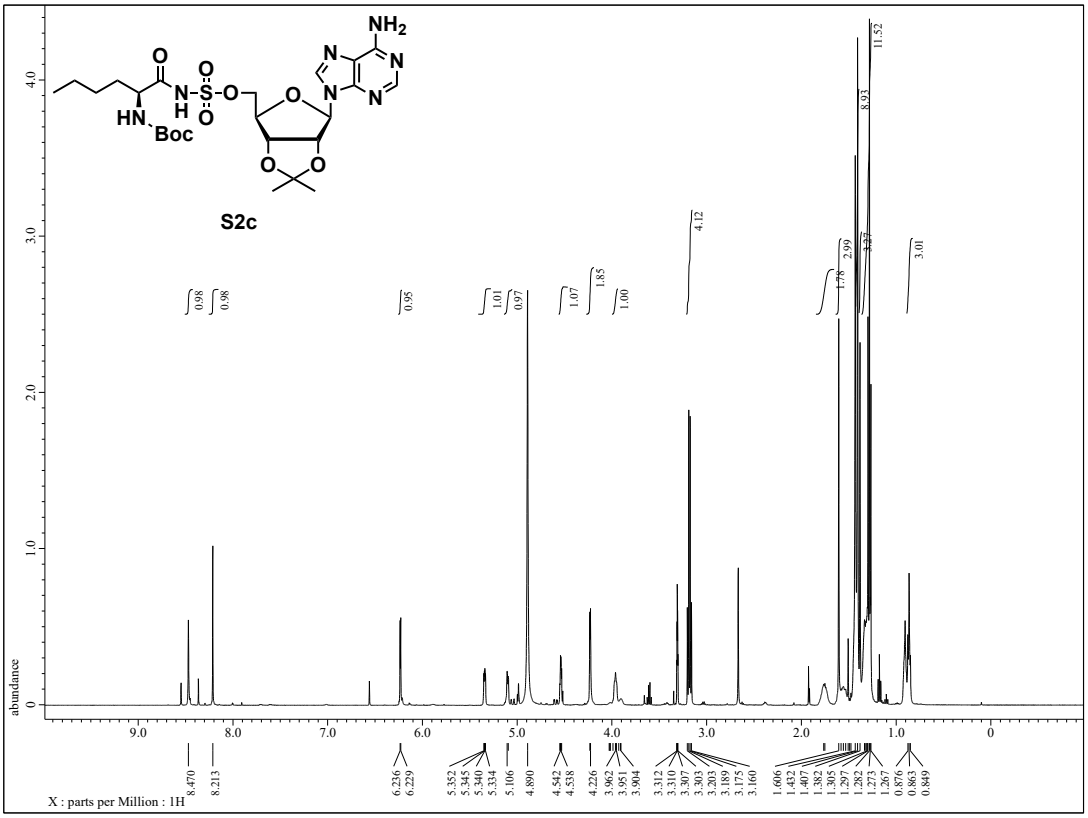

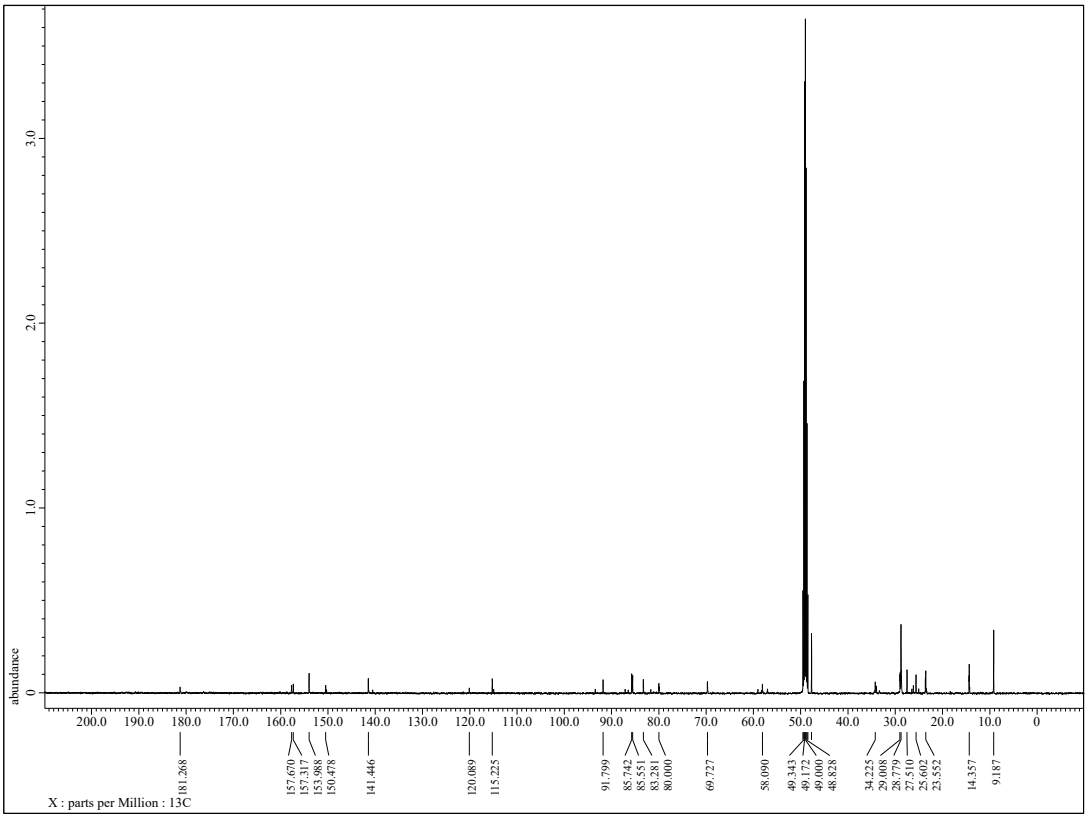

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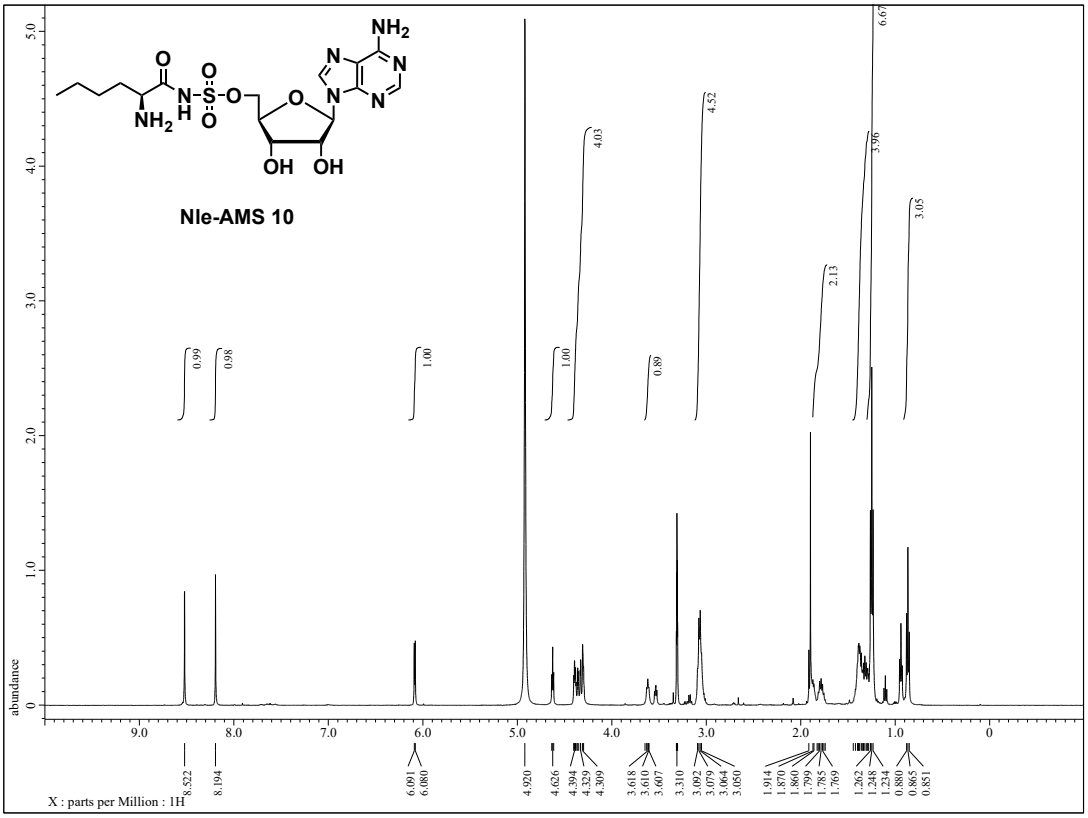

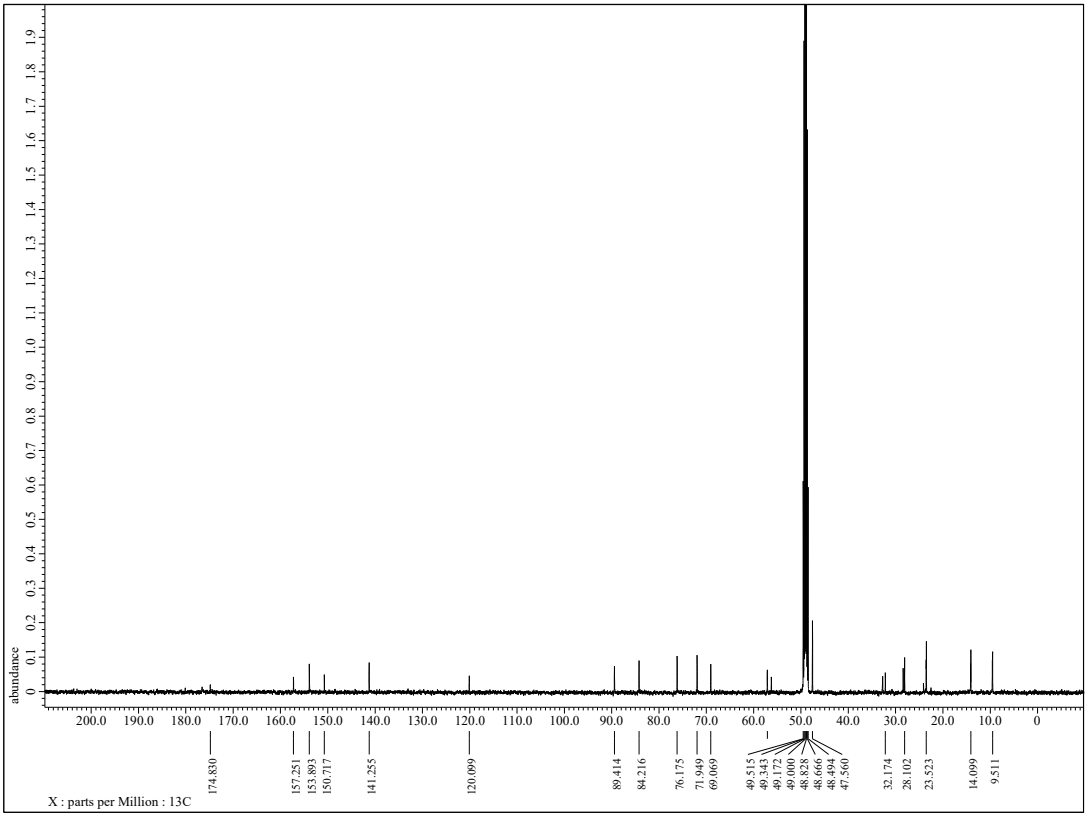

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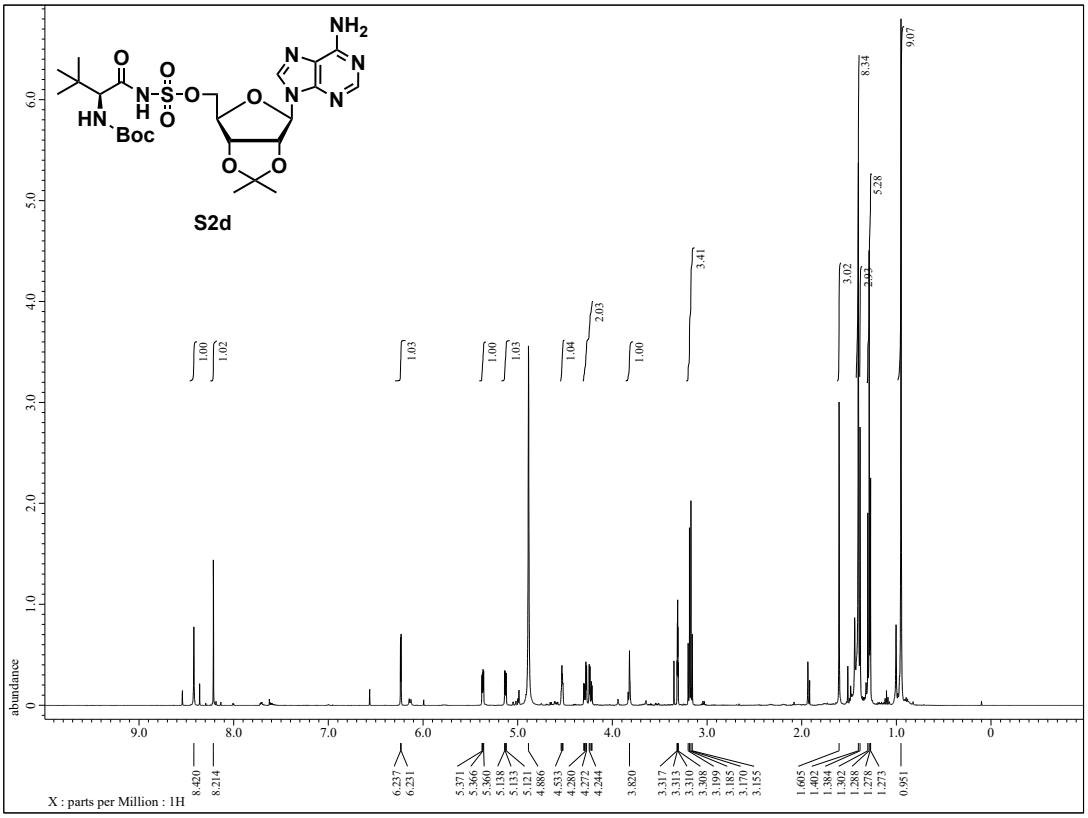

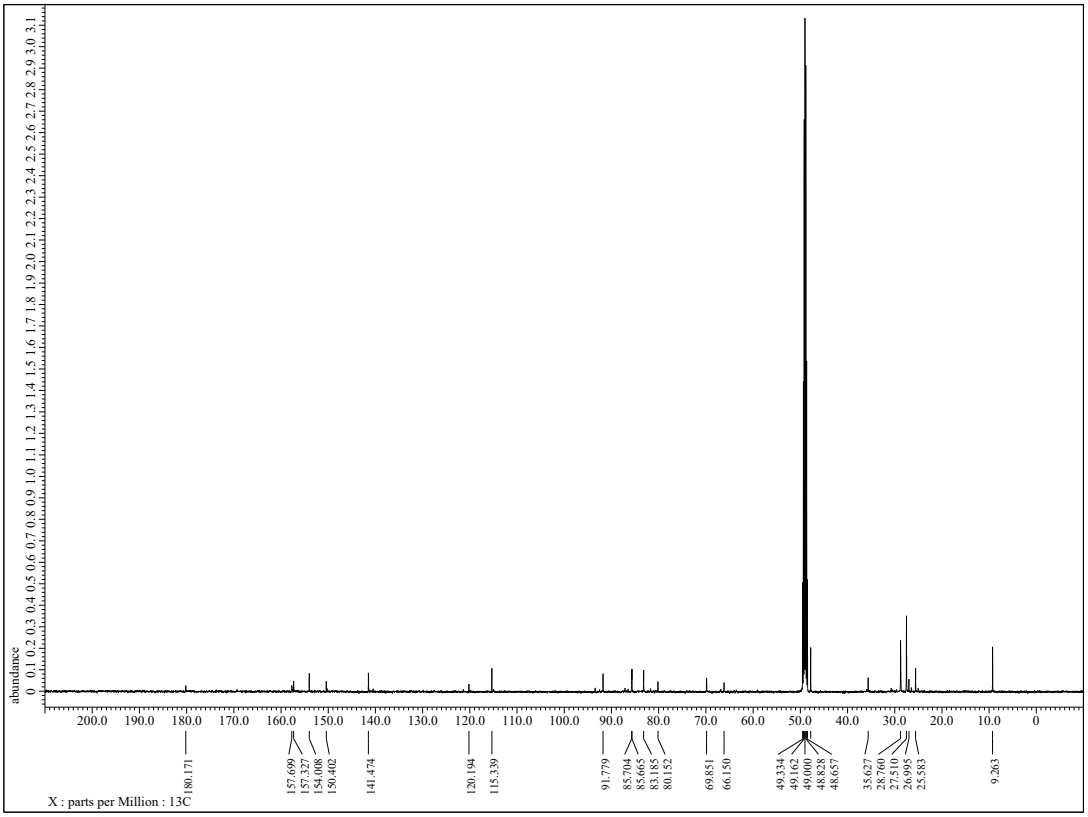

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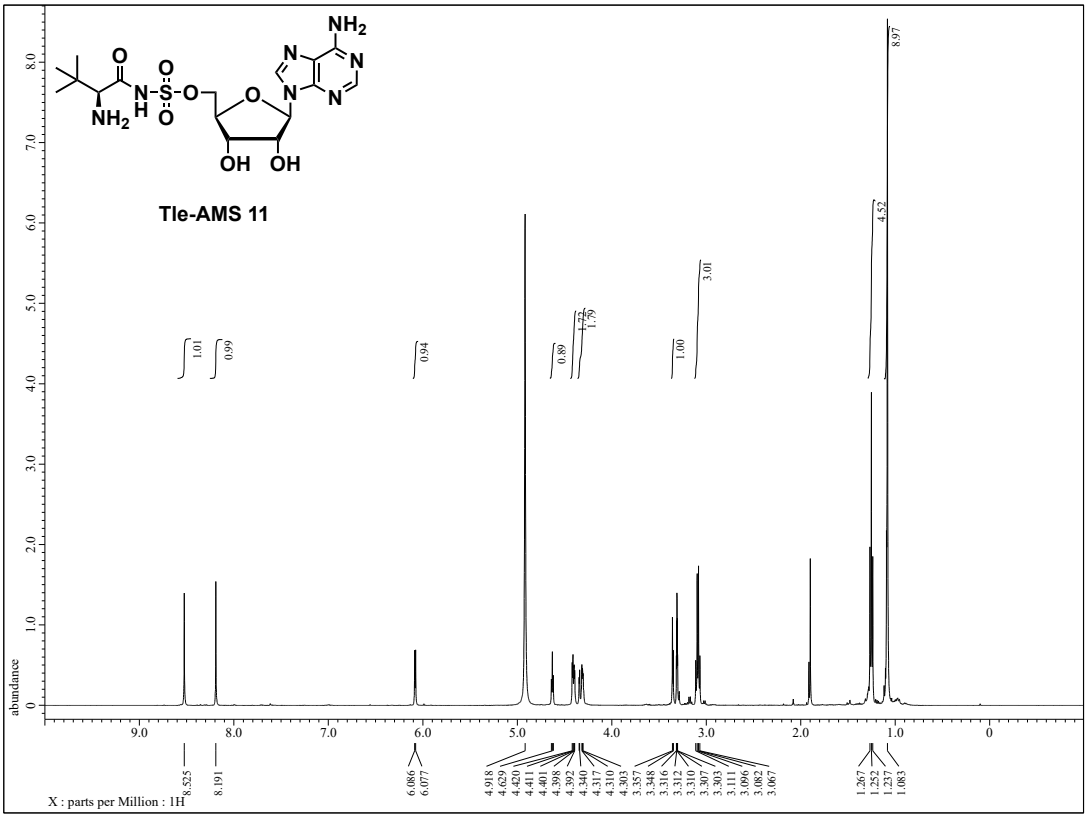

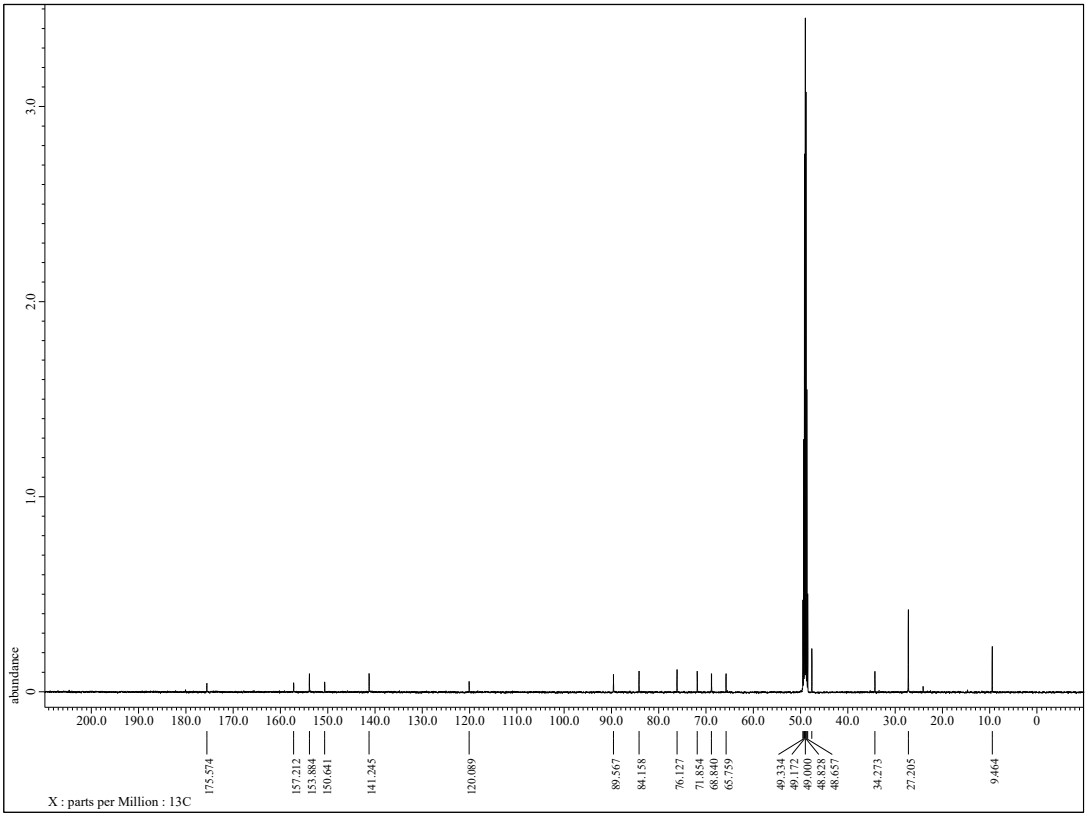

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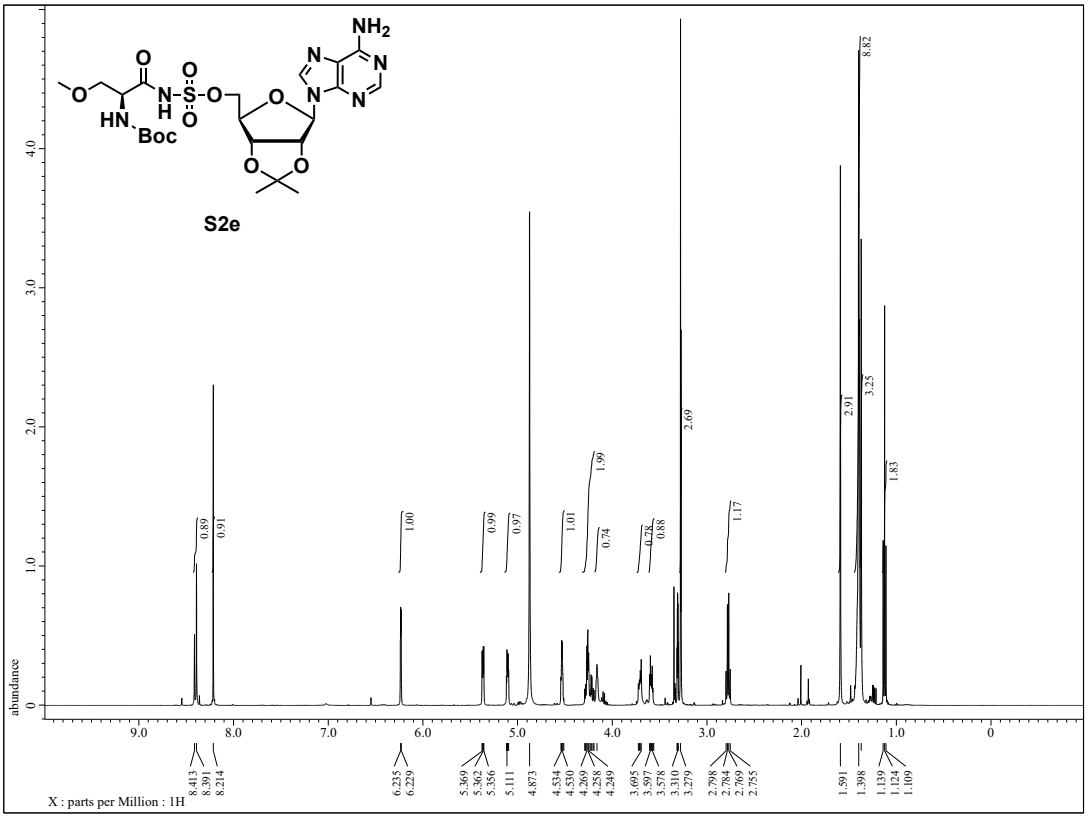
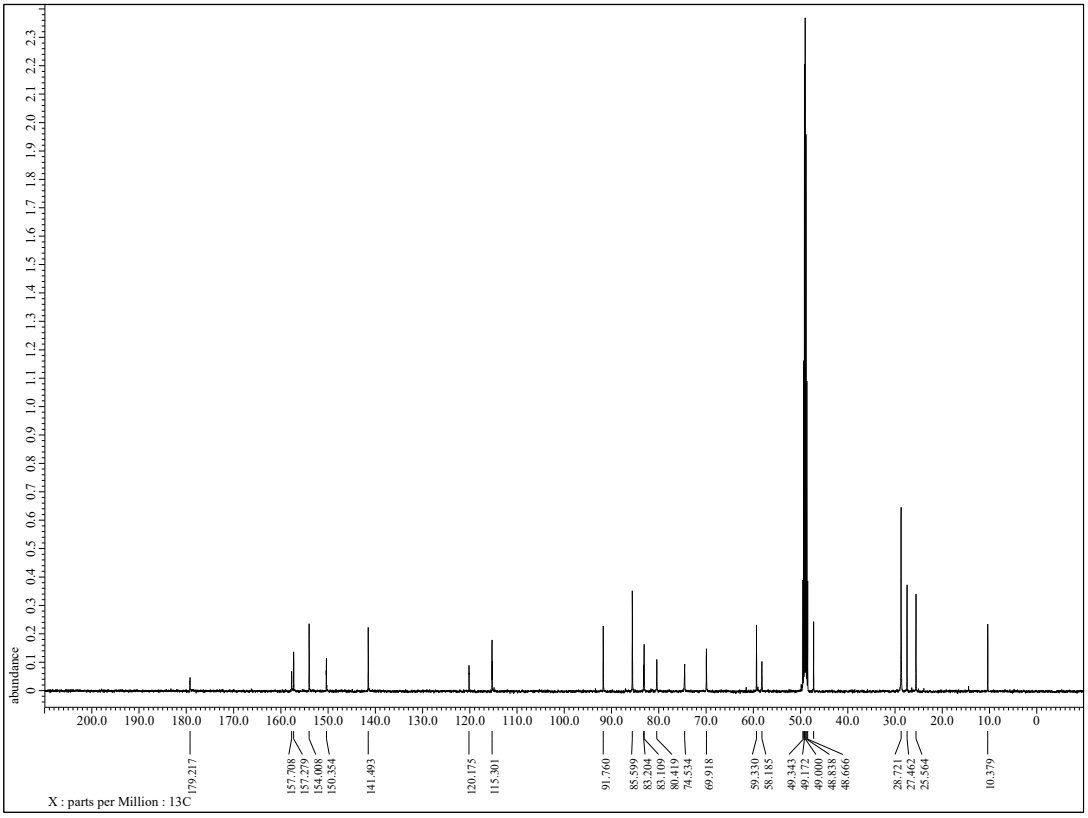
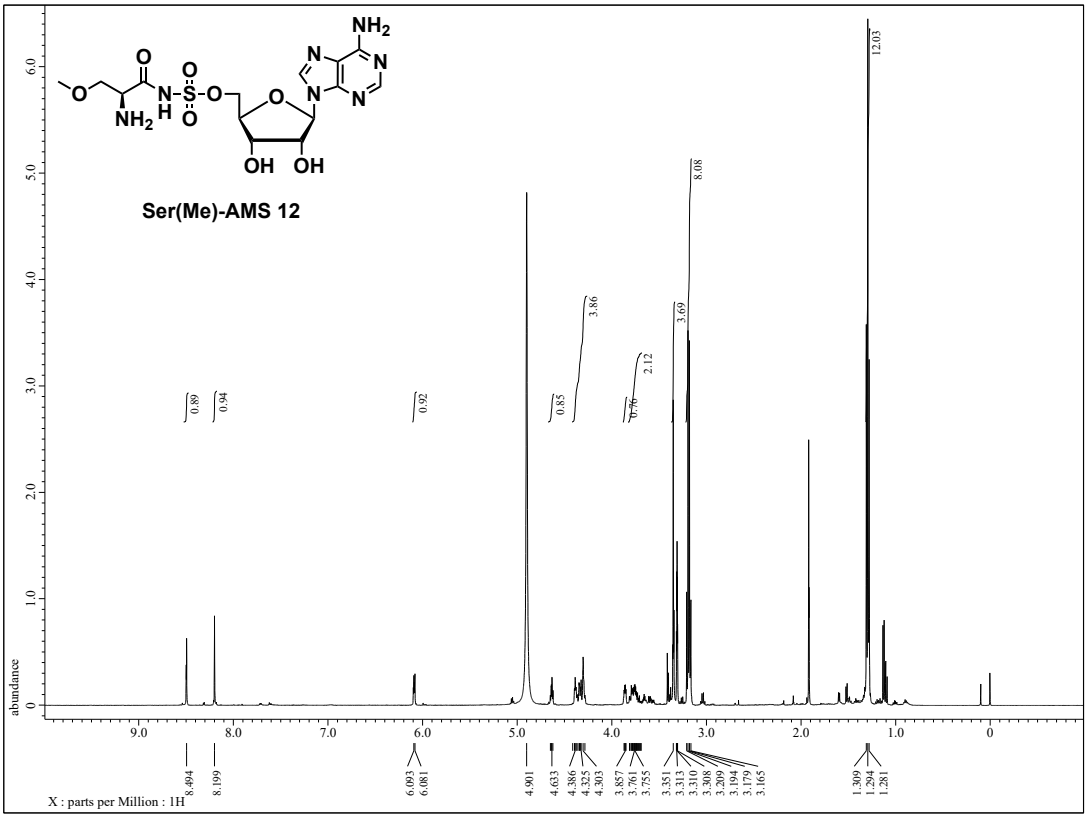
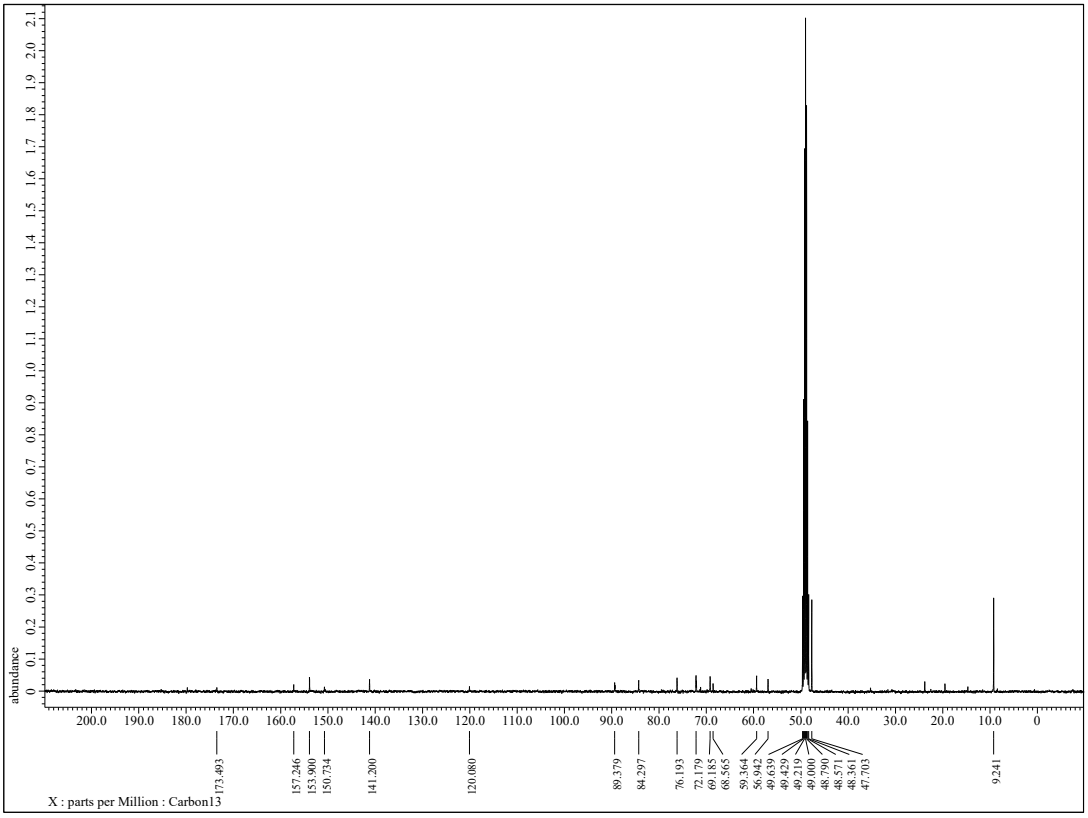




^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Ser(Me)-AMS 12** in CD_3OD





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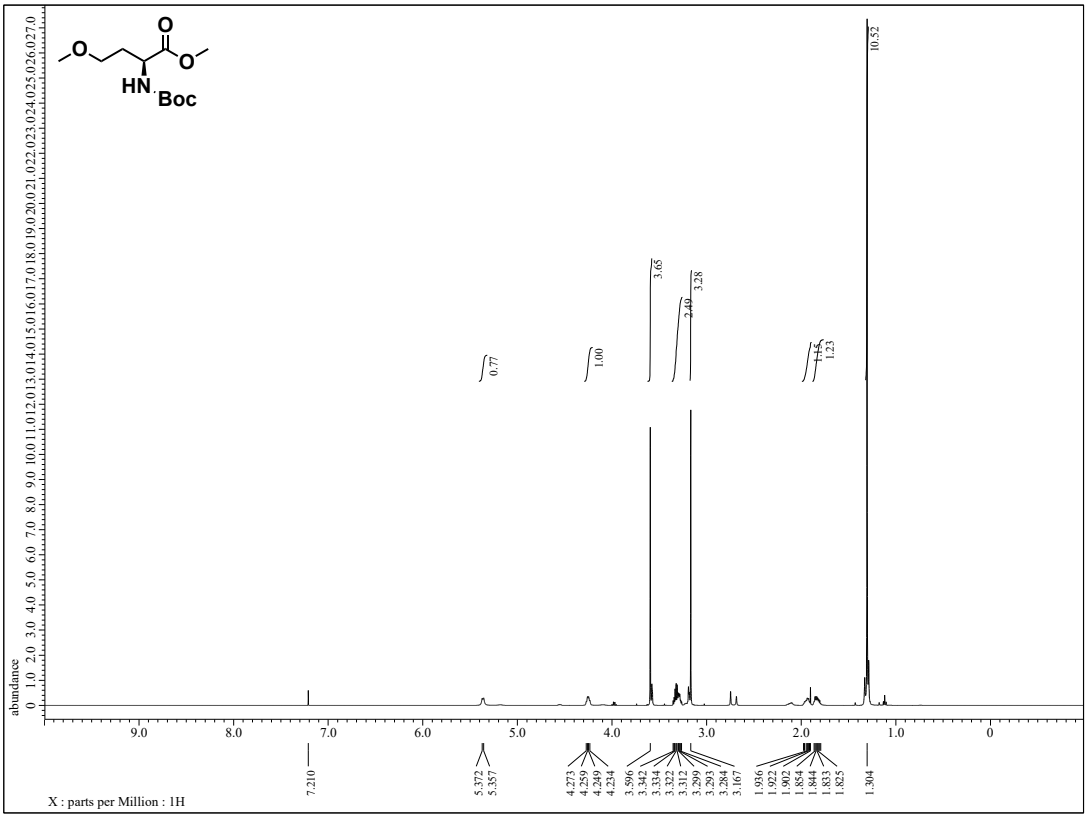
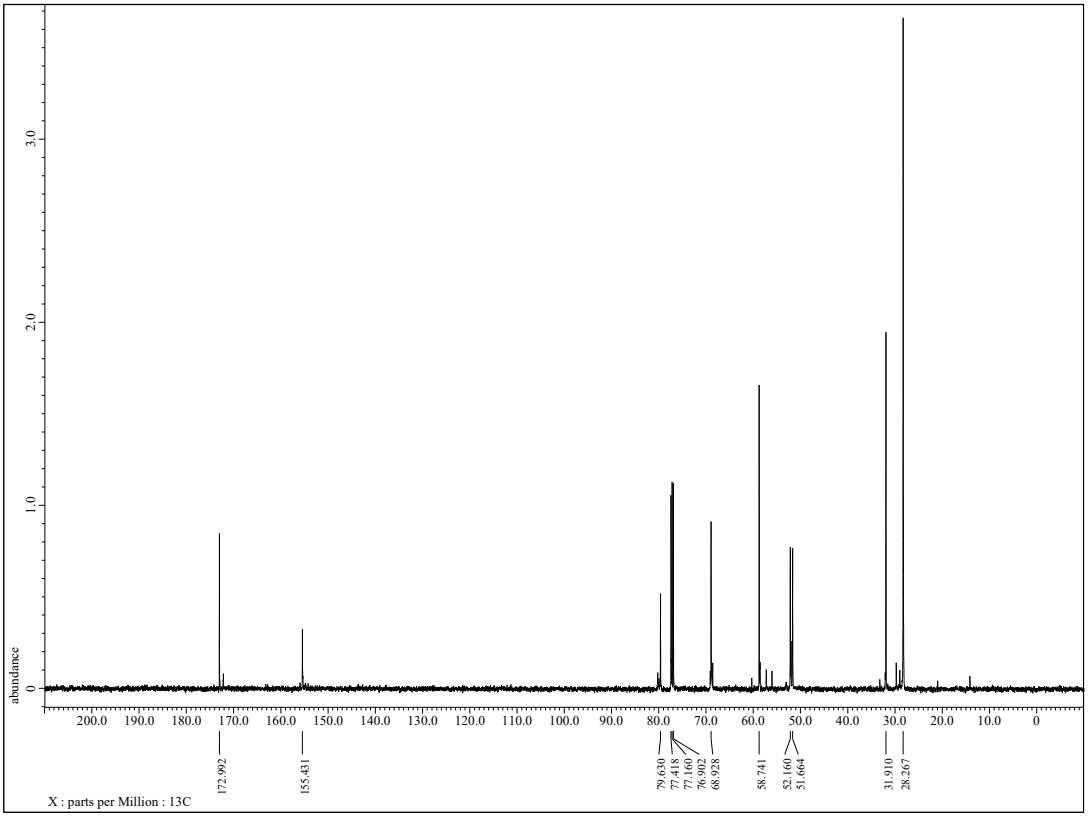
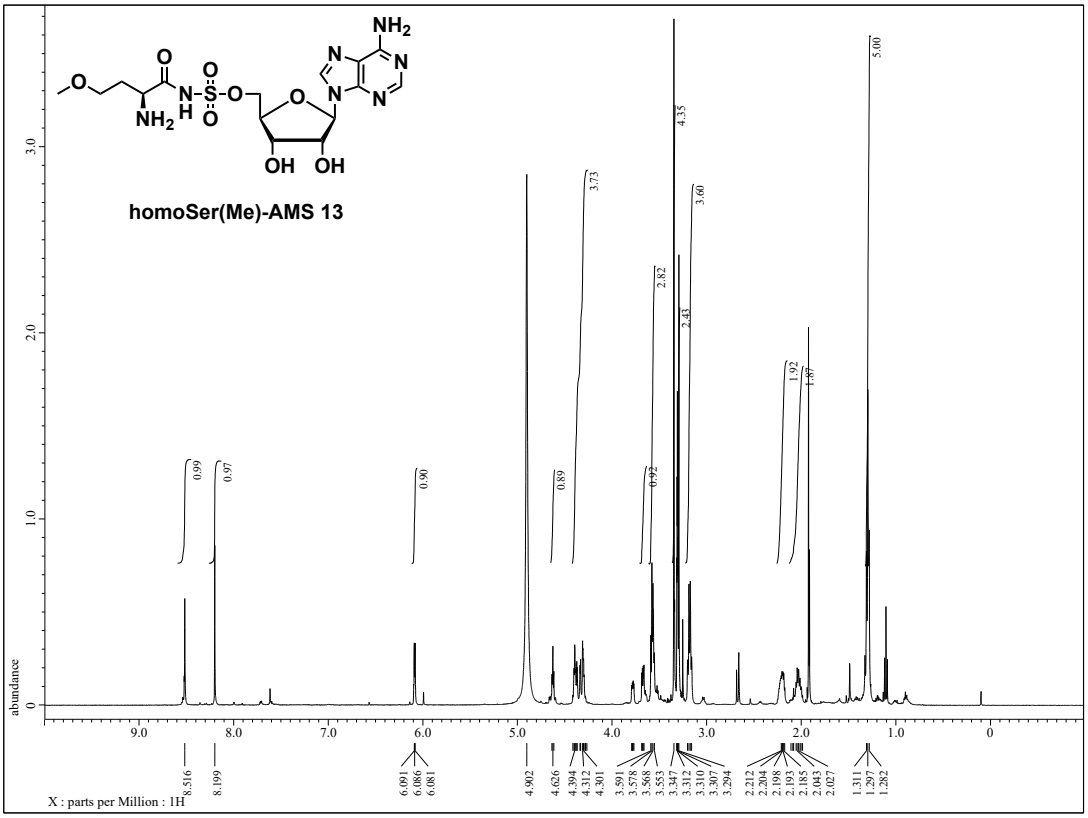
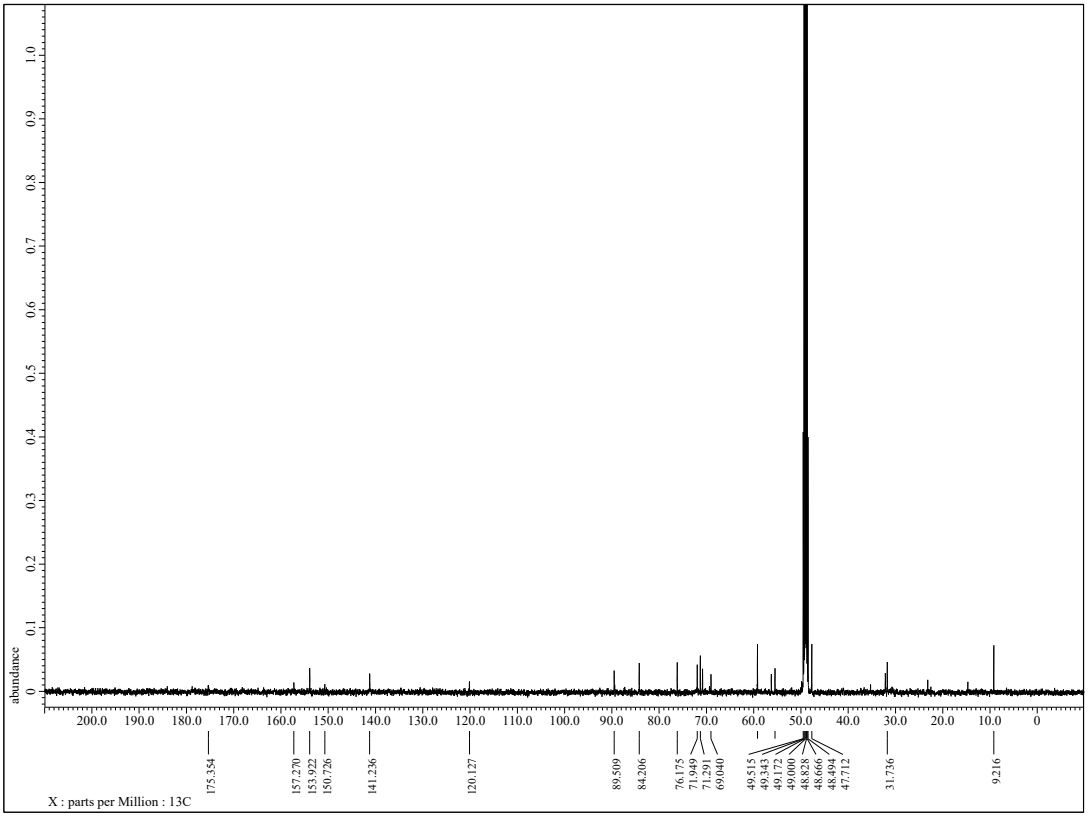


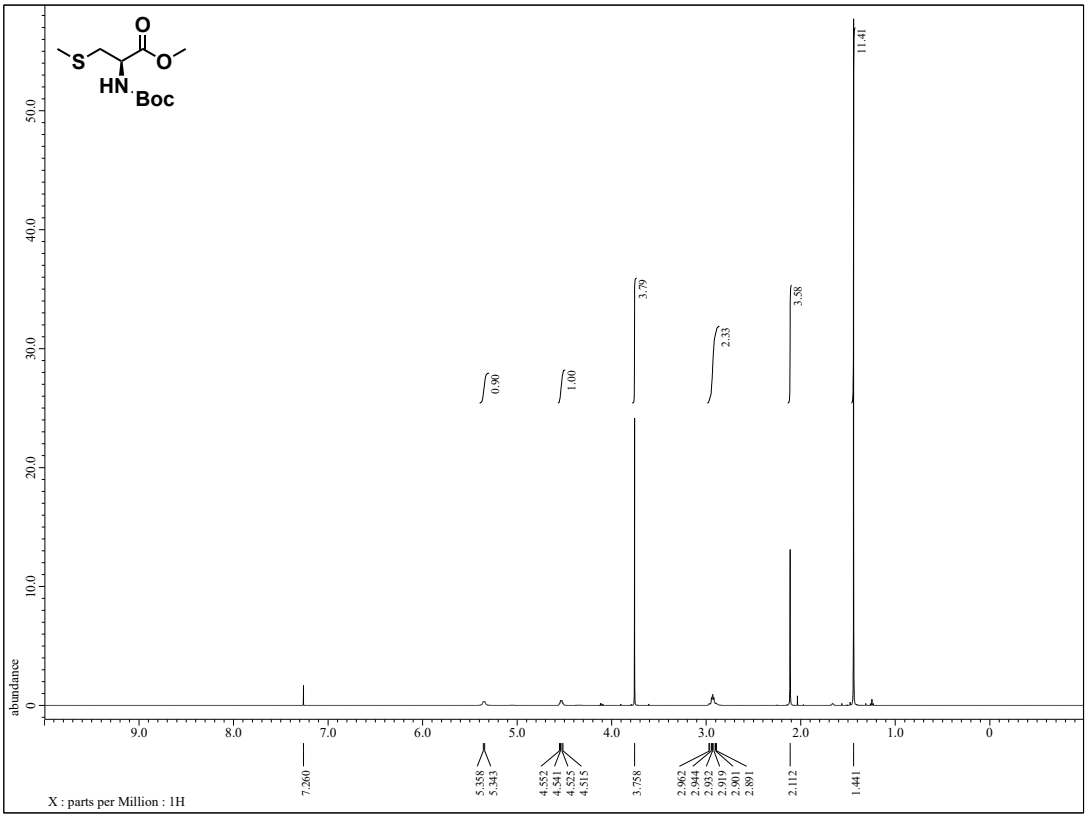


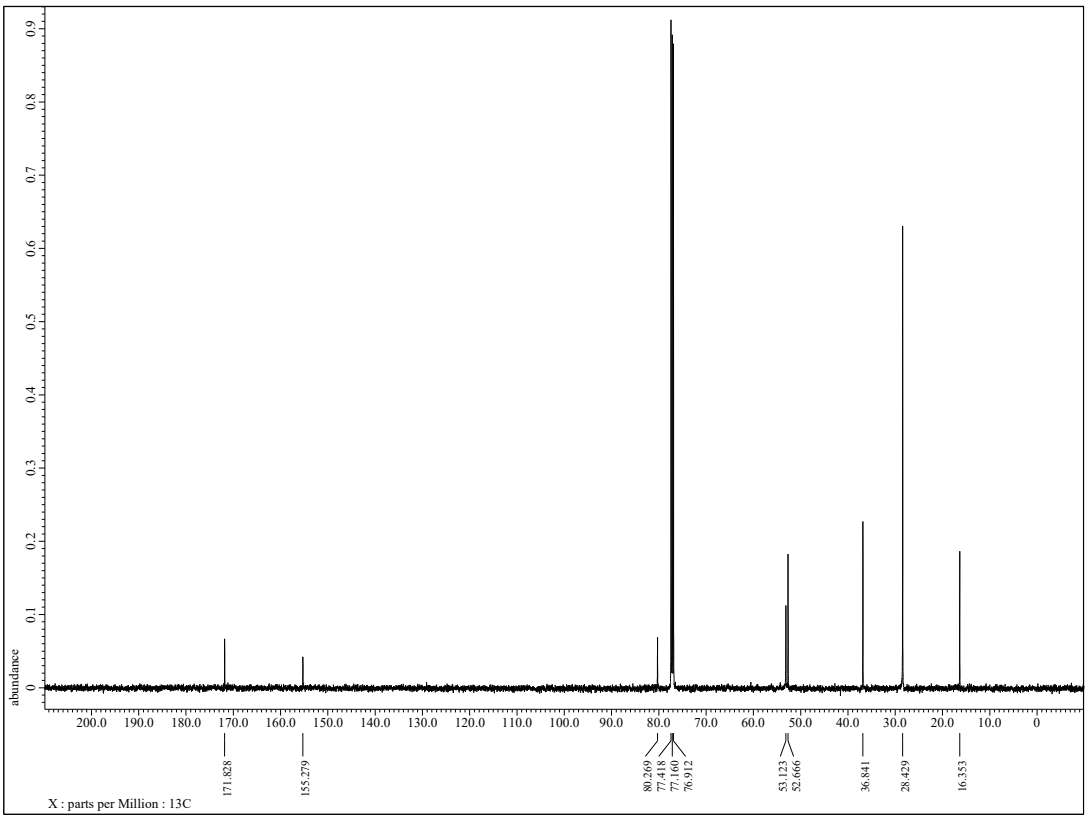
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **homoSer(Me)-AMS 13** in CD_3OD



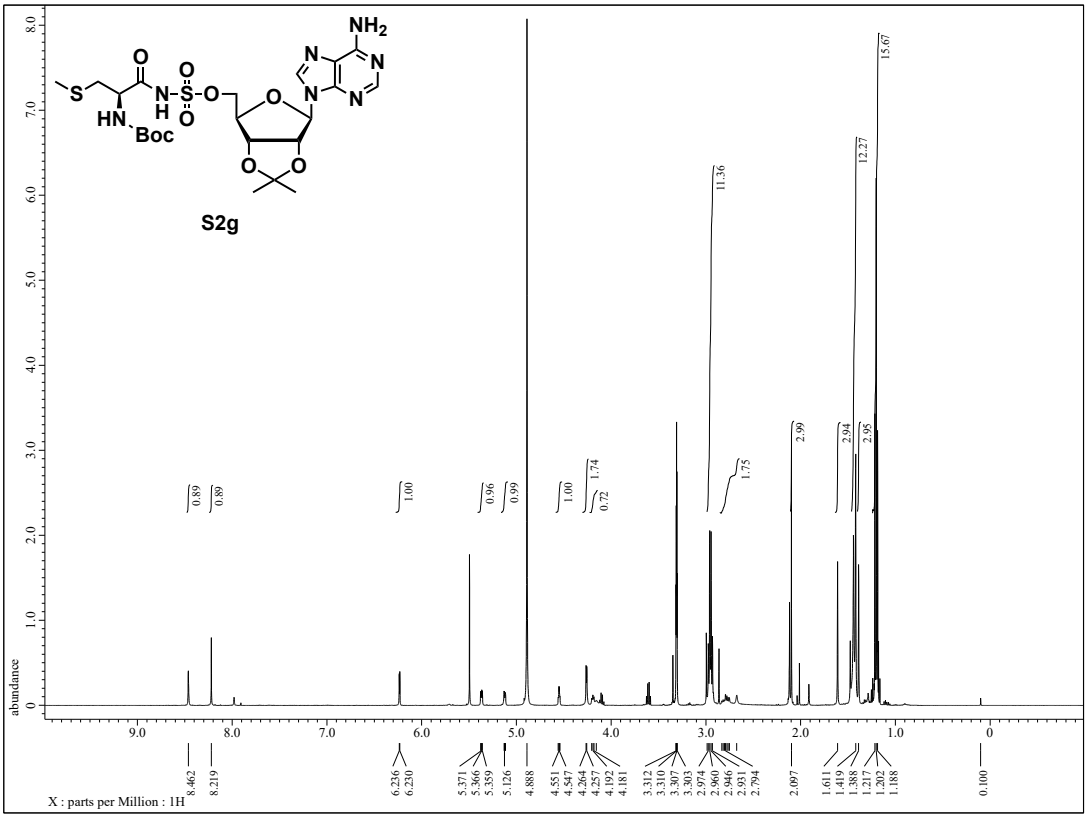


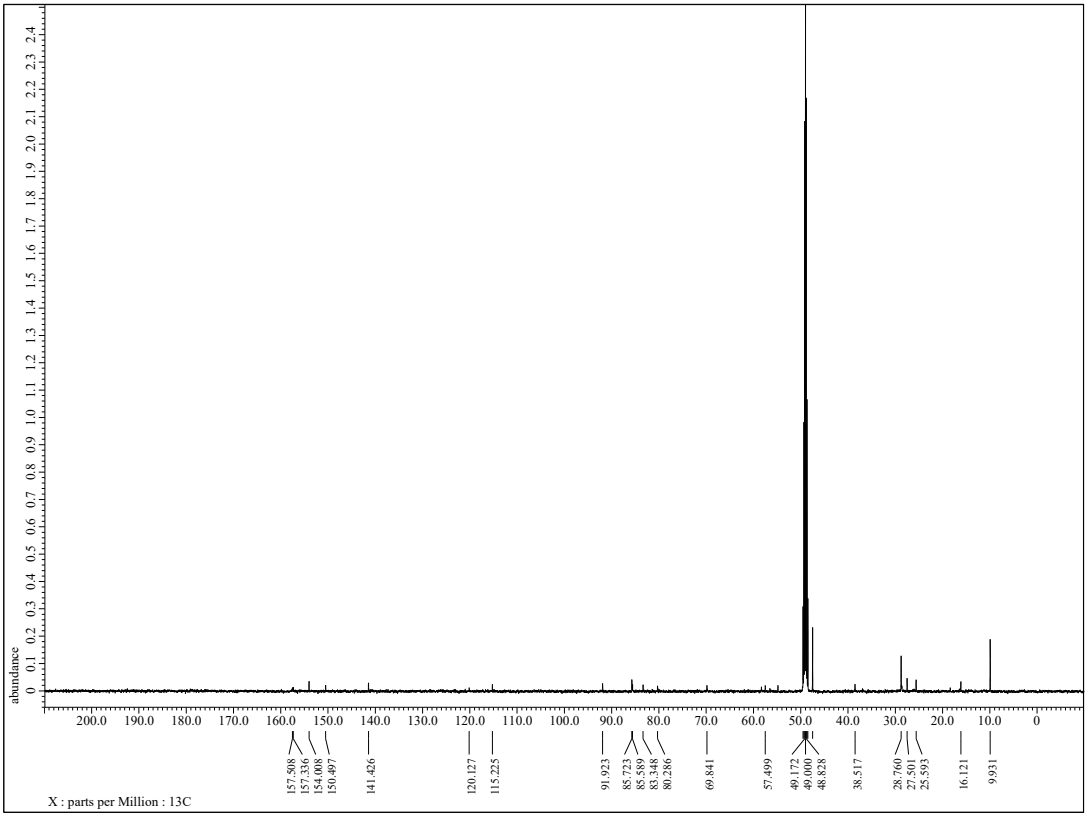
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Boc-Cys-OMe** in $CDCl_3$



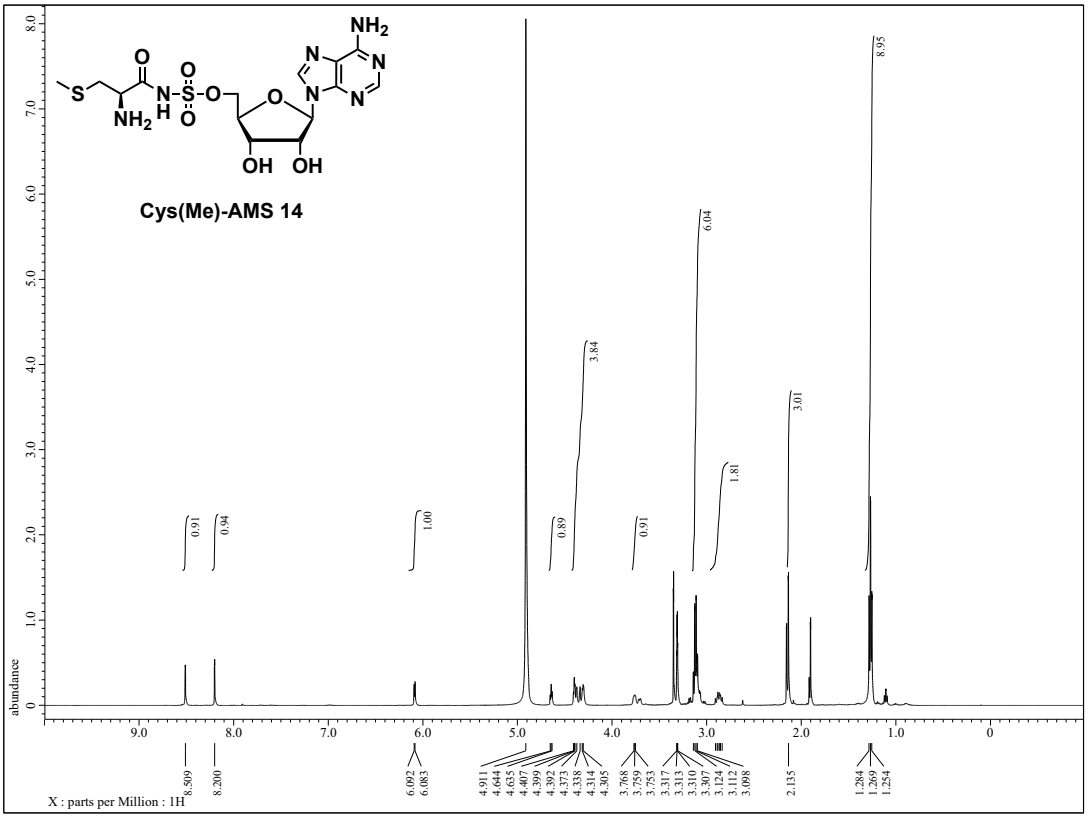


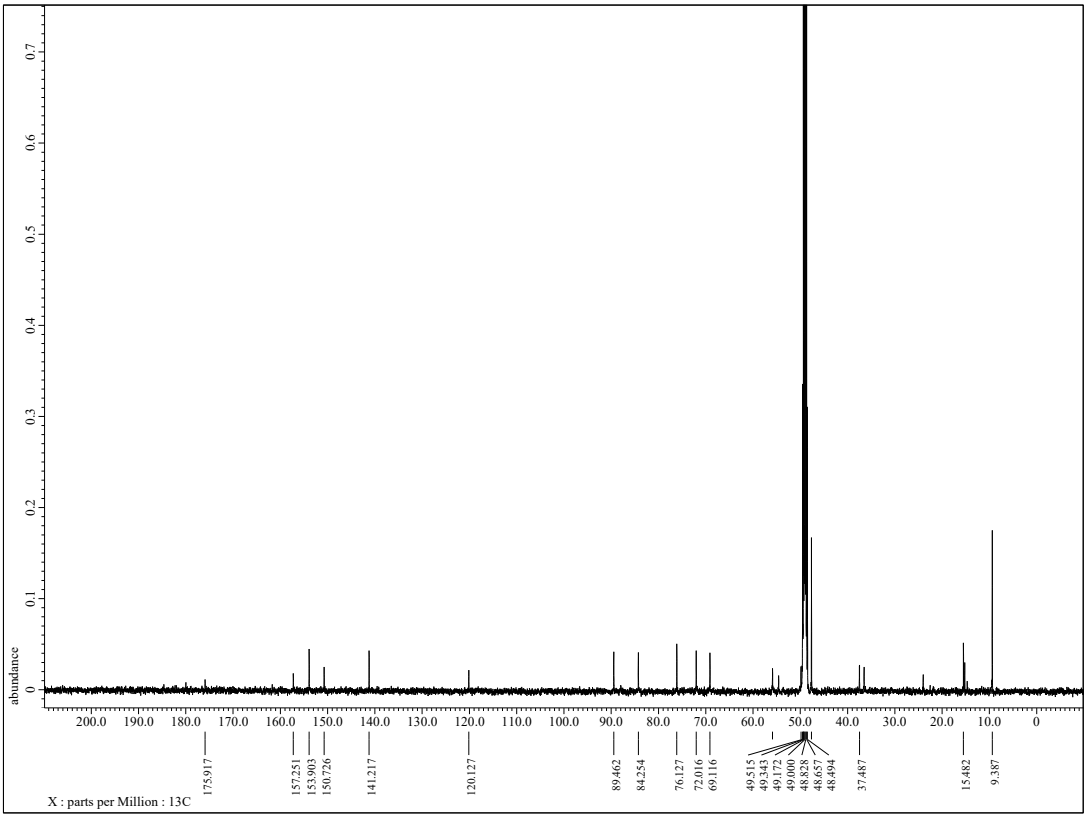
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2g** in CD_3OD



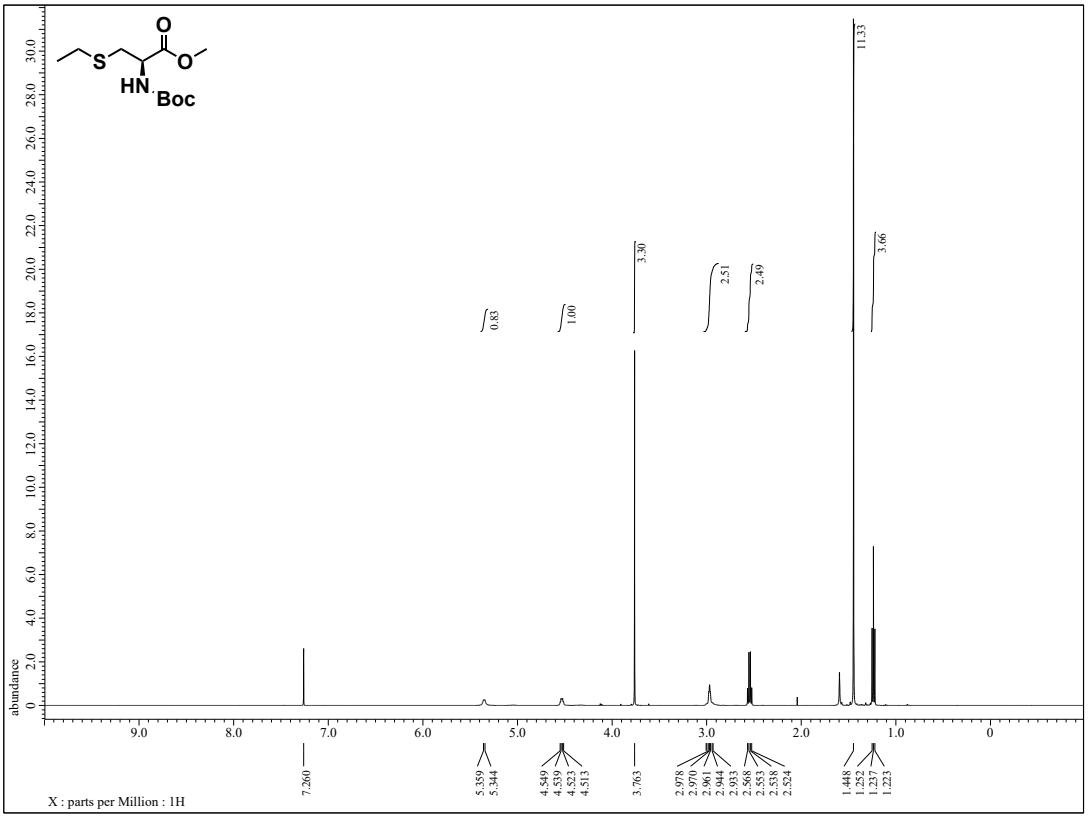


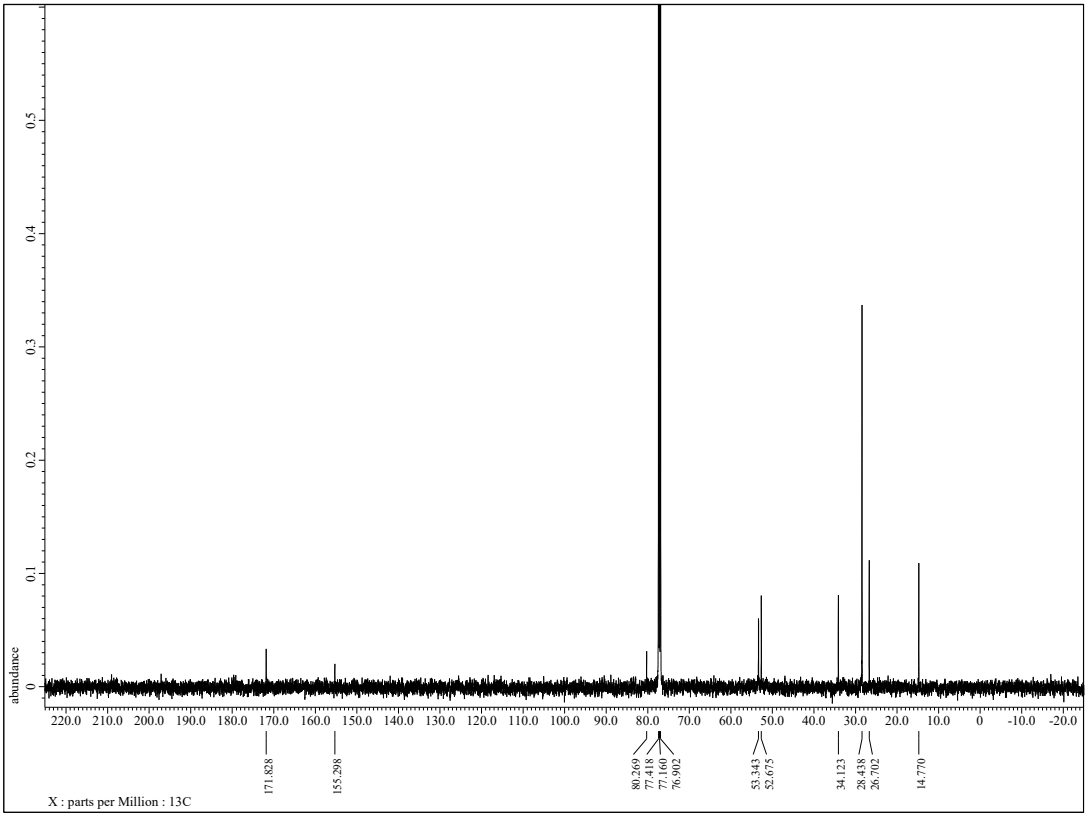
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Cys(Me)-AMS 14** in CD_3OD



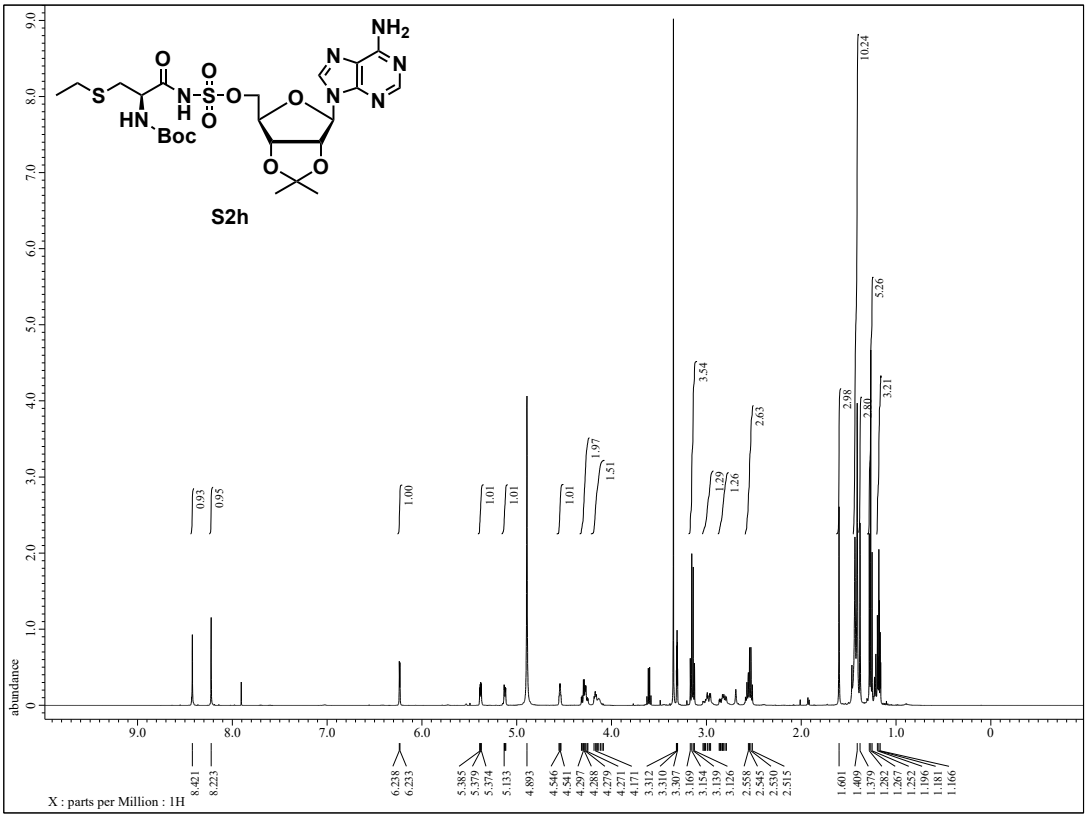


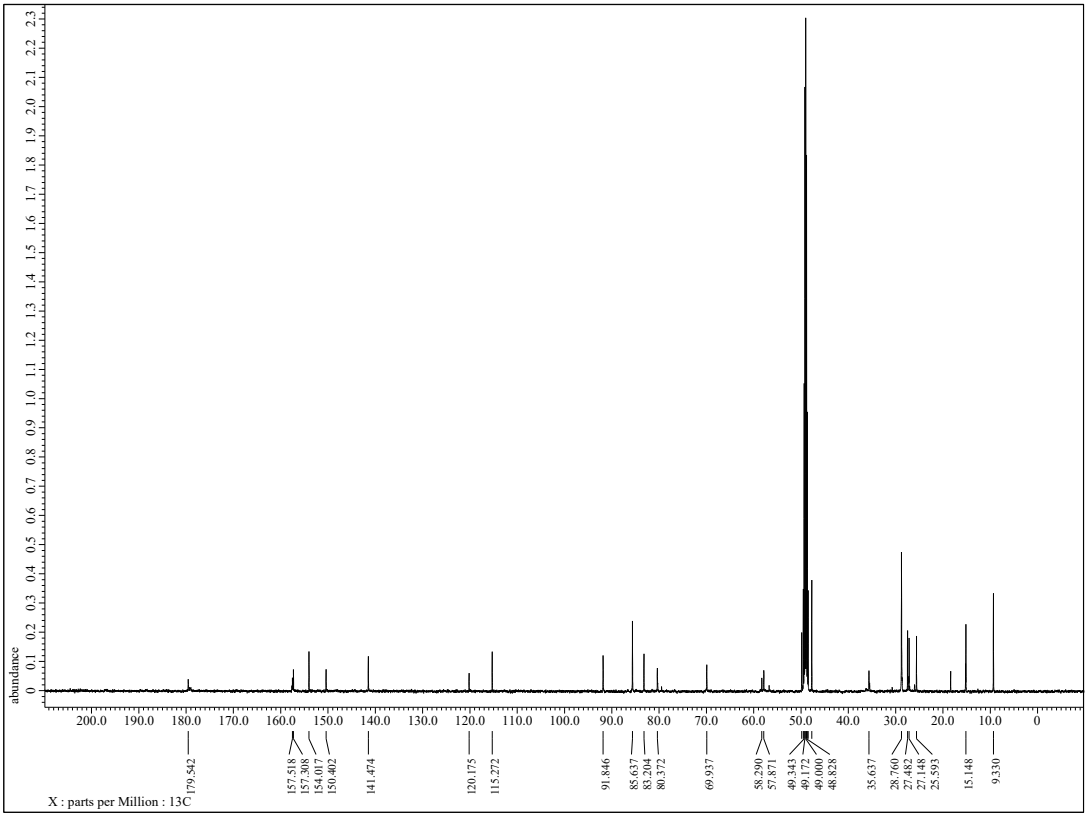
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Boc-Cys(Et)-OMe** in $CDCl_3$



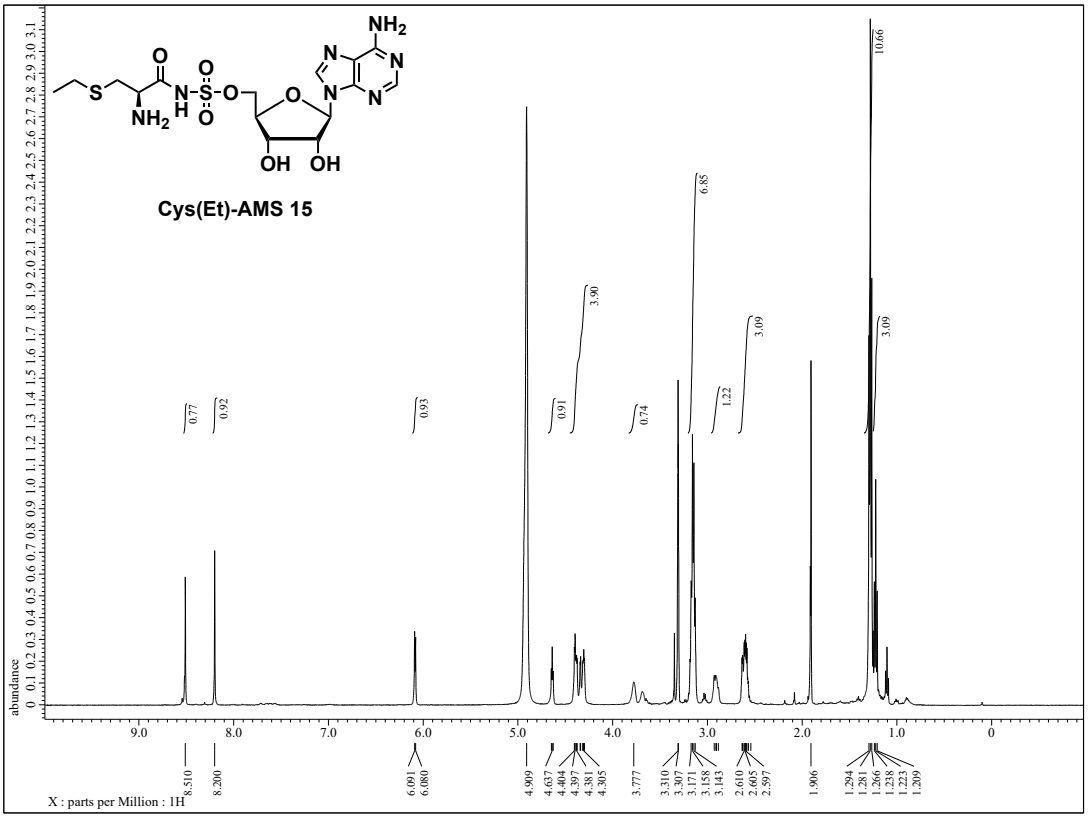


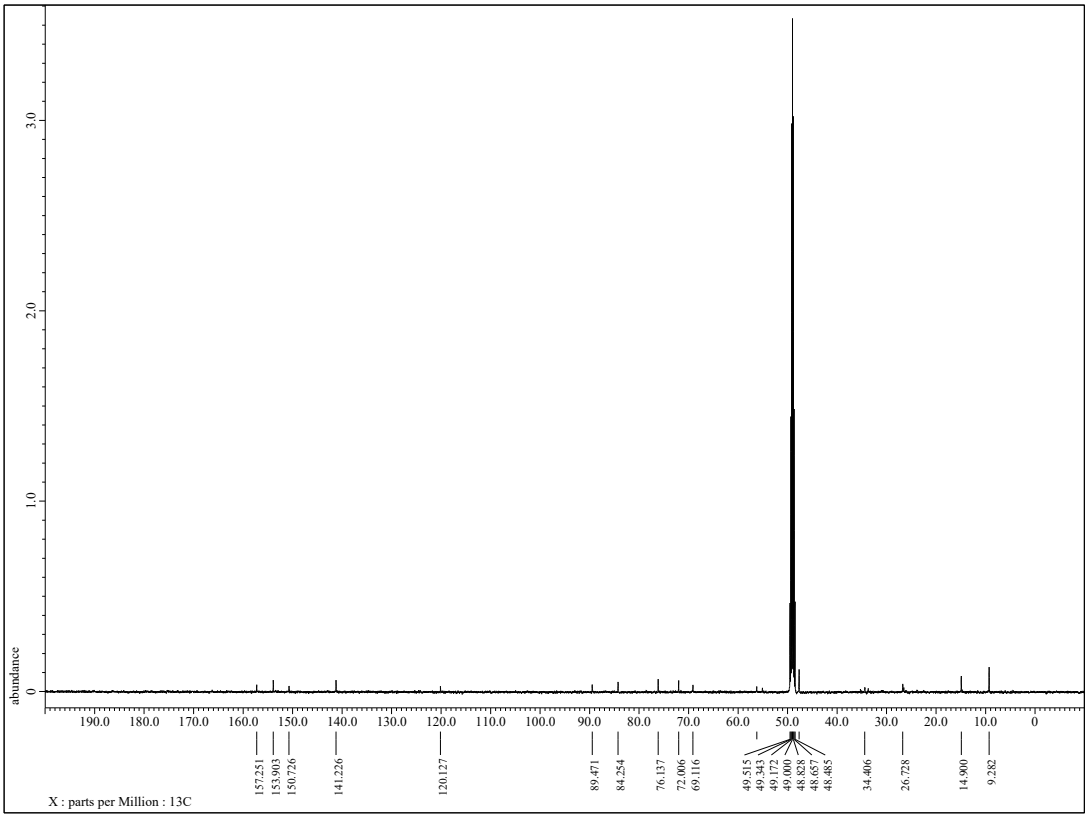
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2h** in CD_3OD



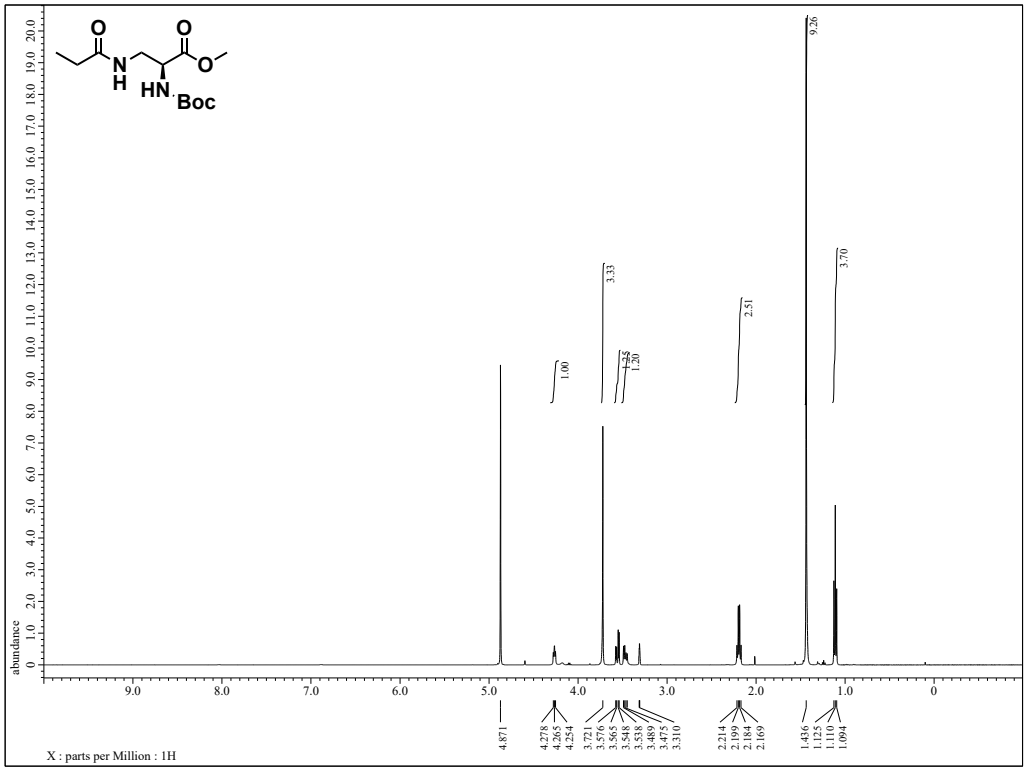


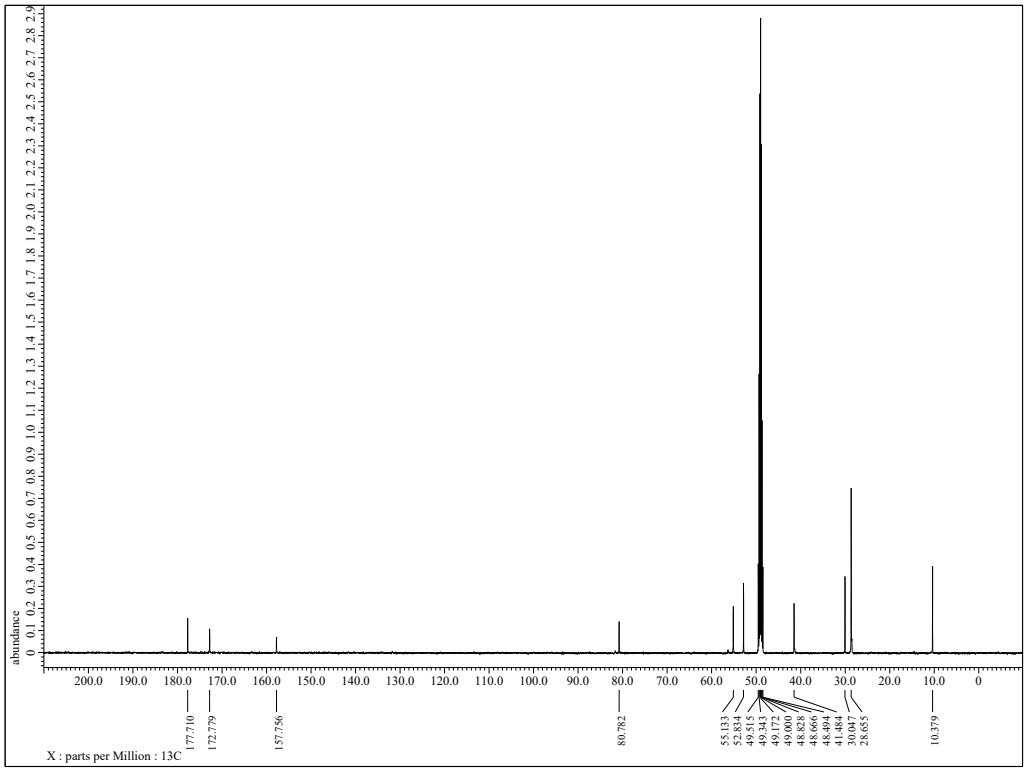
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Cys(Et)-AMS 15** in CD_3OD



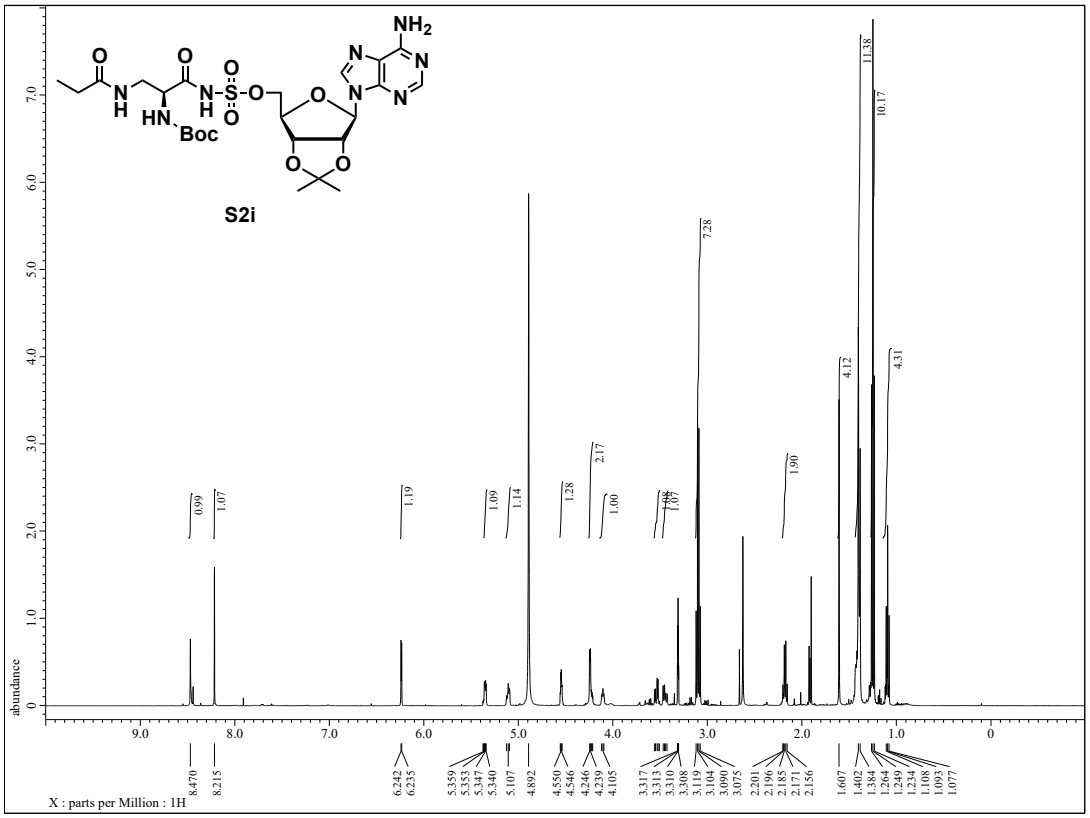


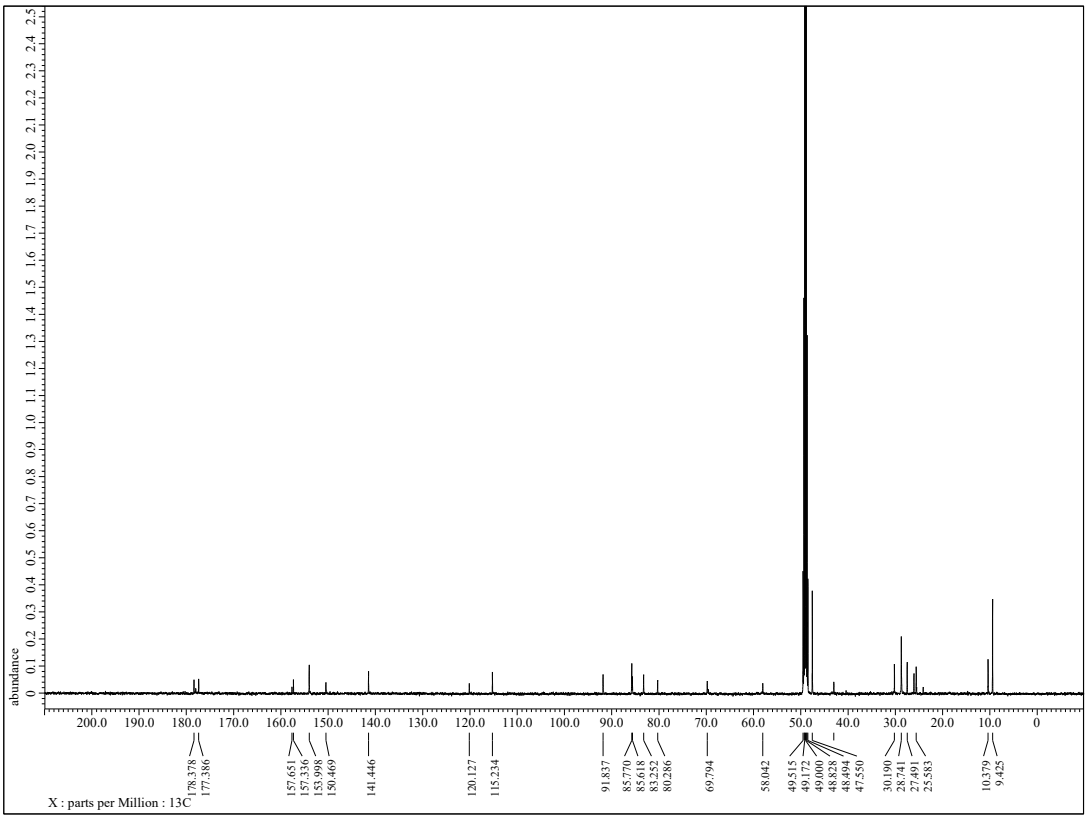
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-propionamidopropanoate** in CD_3OD



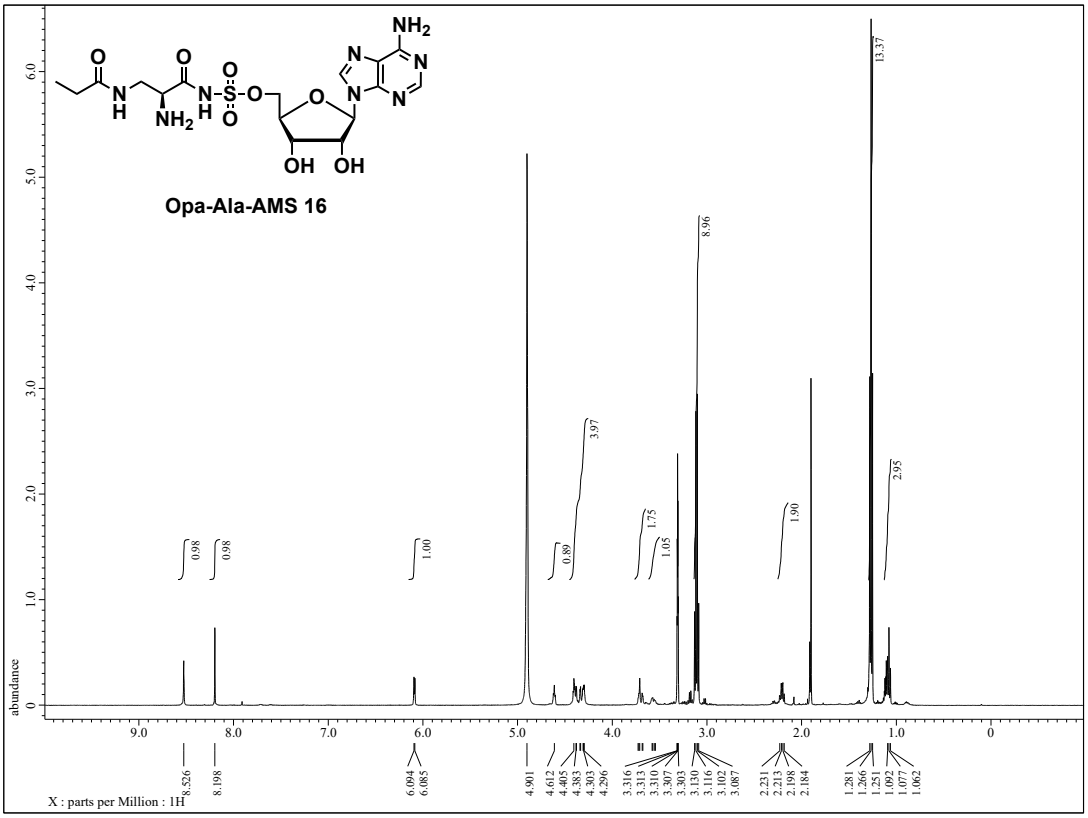


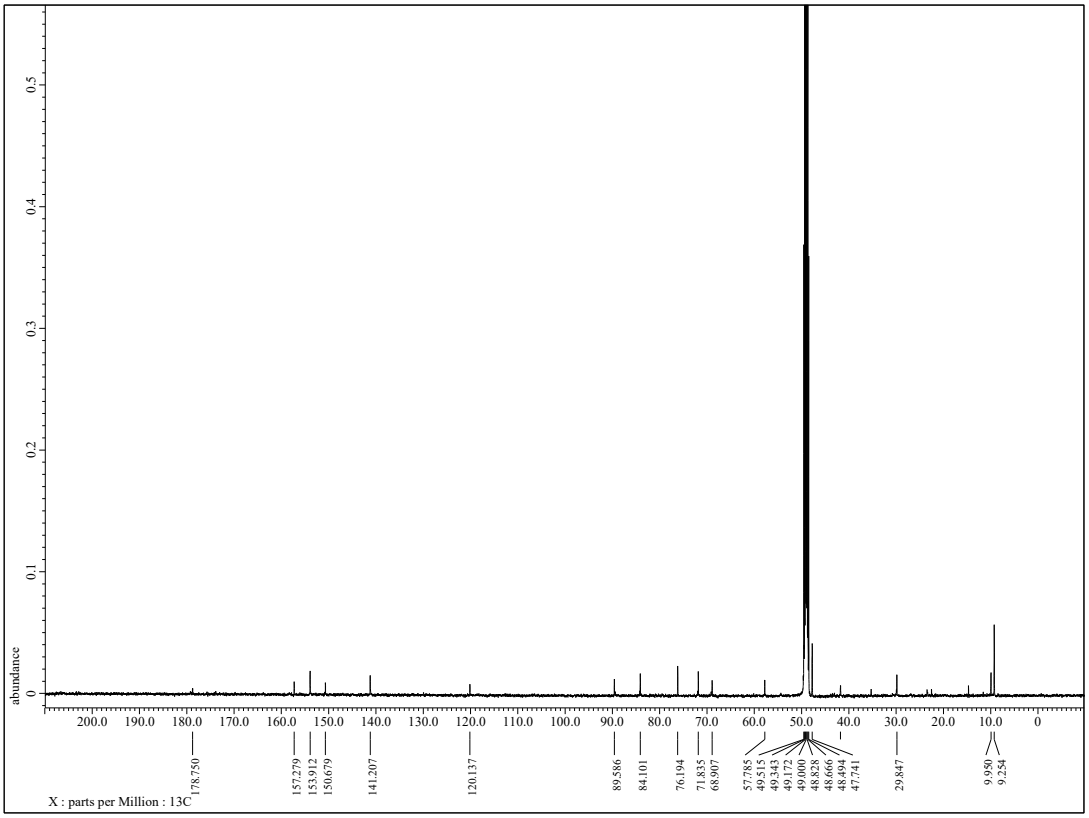
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2i** in CD_3OD



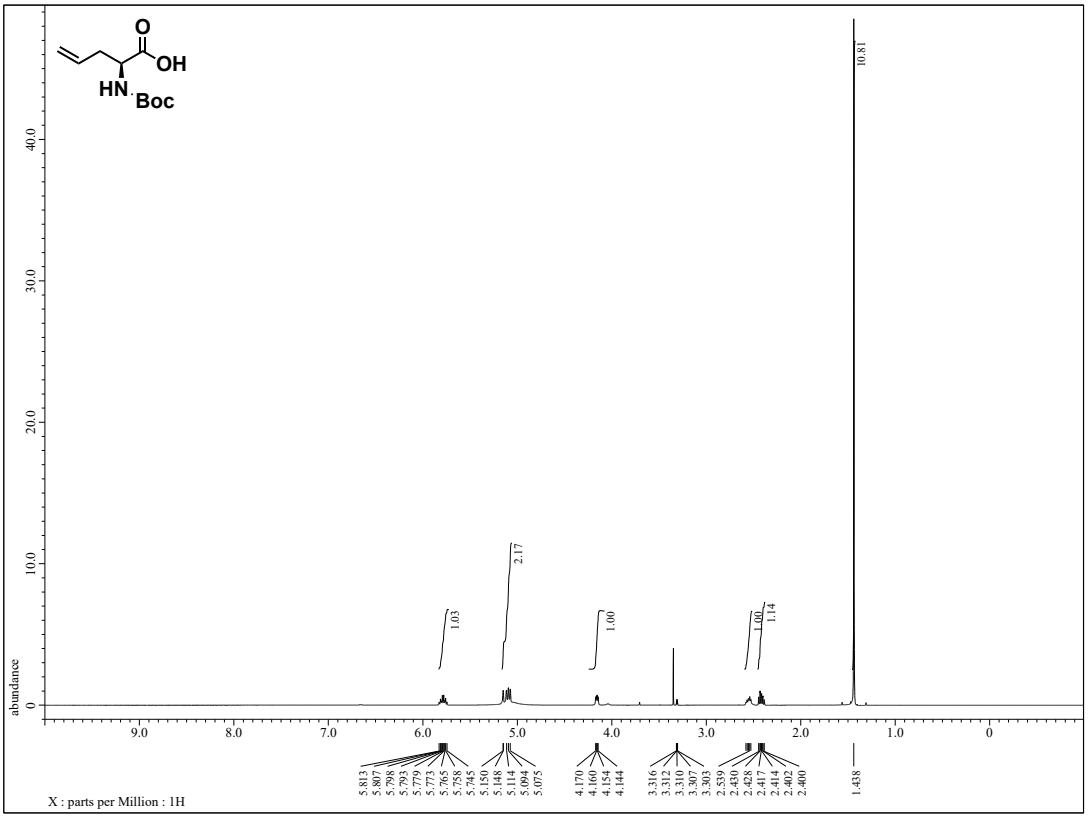


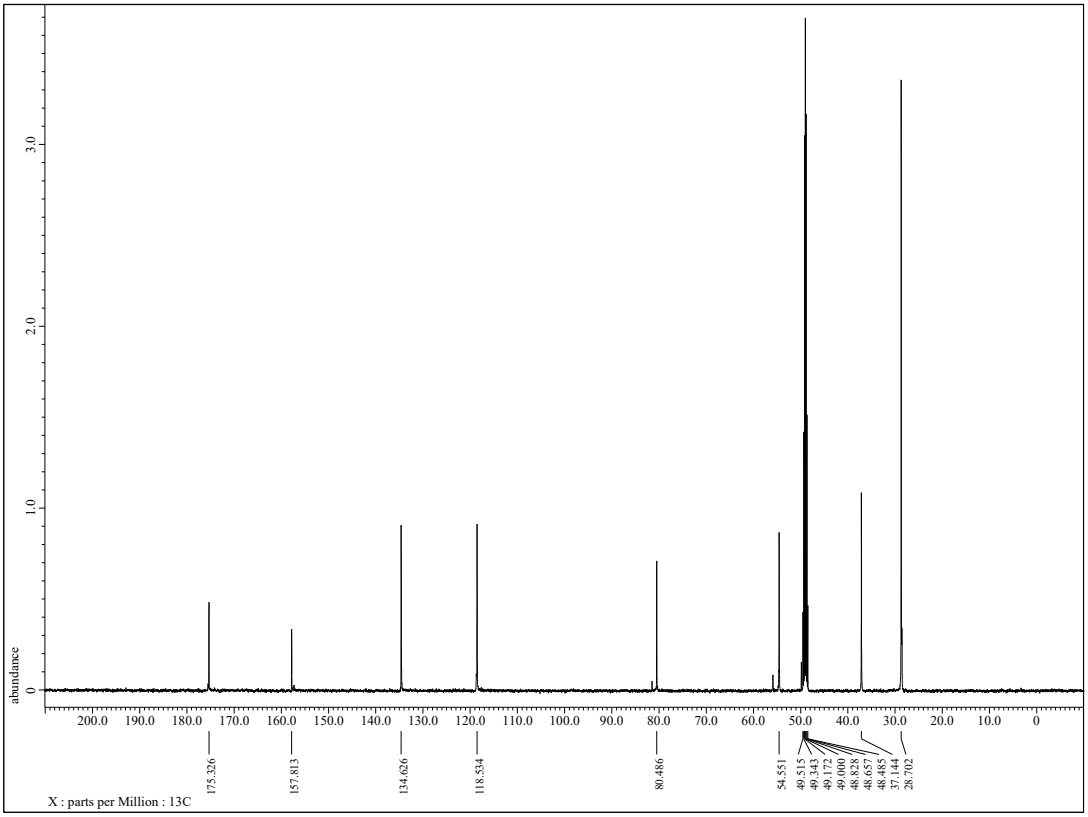
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Opa-Ala-AMS 16** in CD_3OD



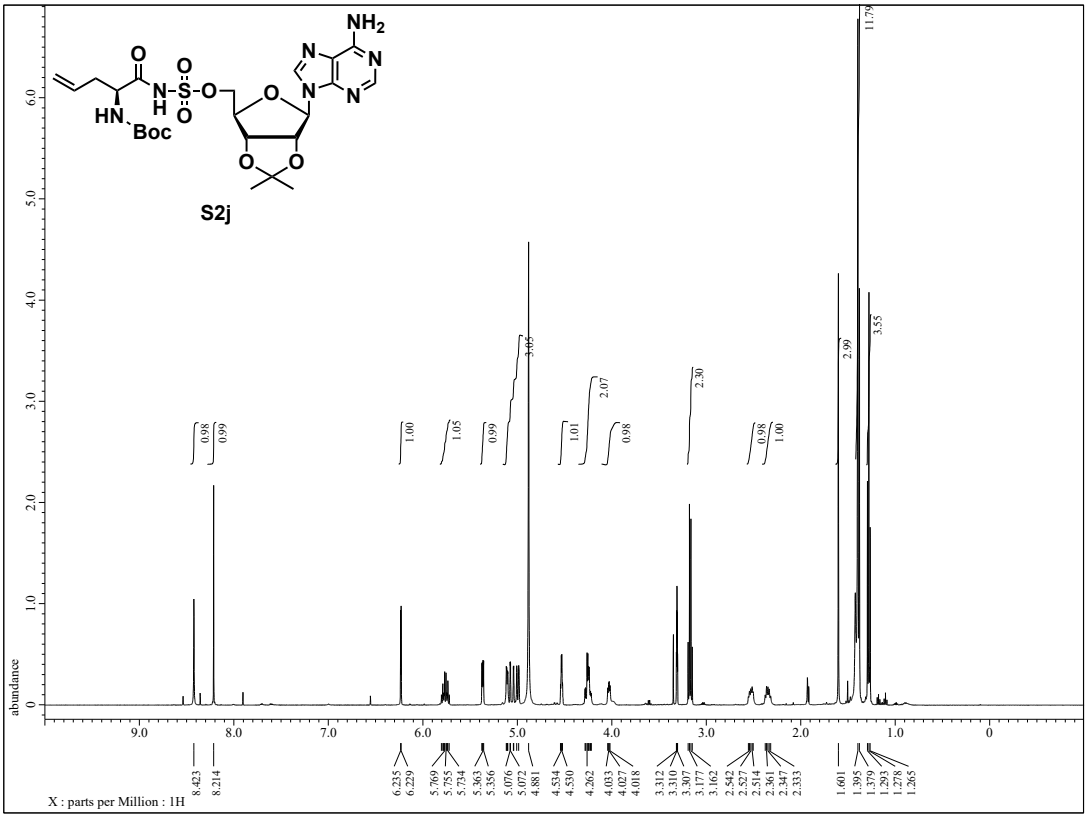


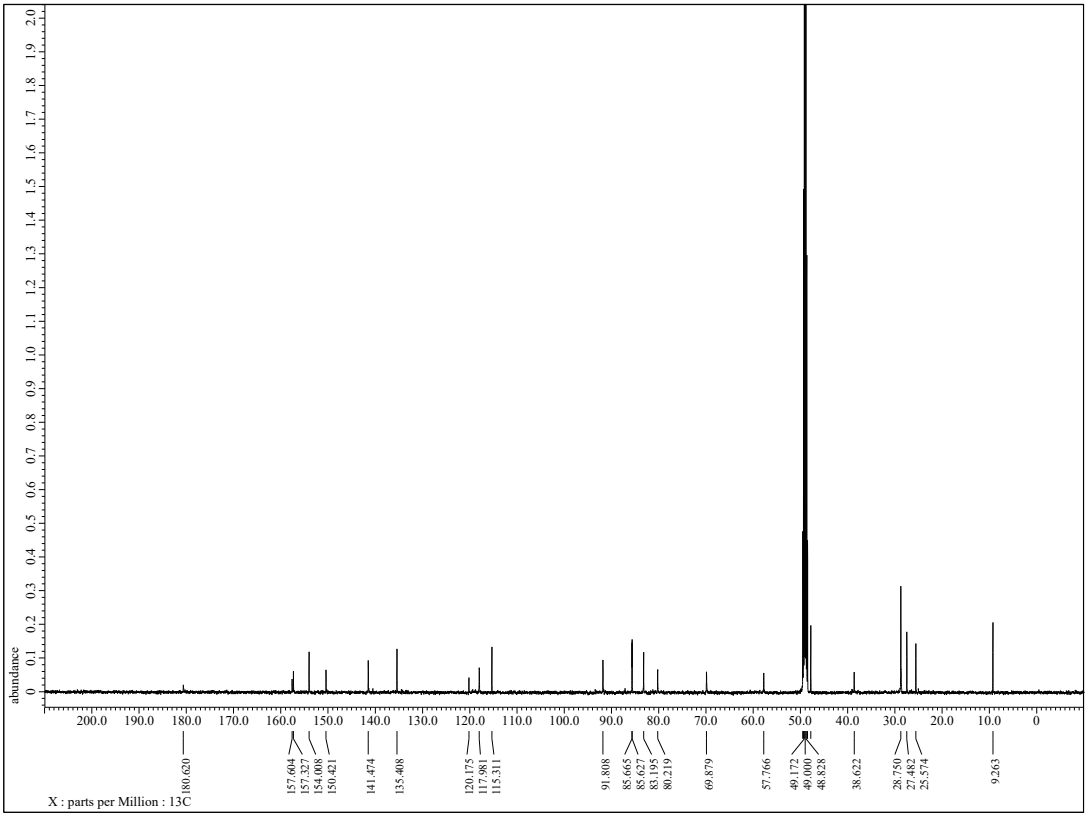
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Boc-allylGly-OH** in CD_3OD



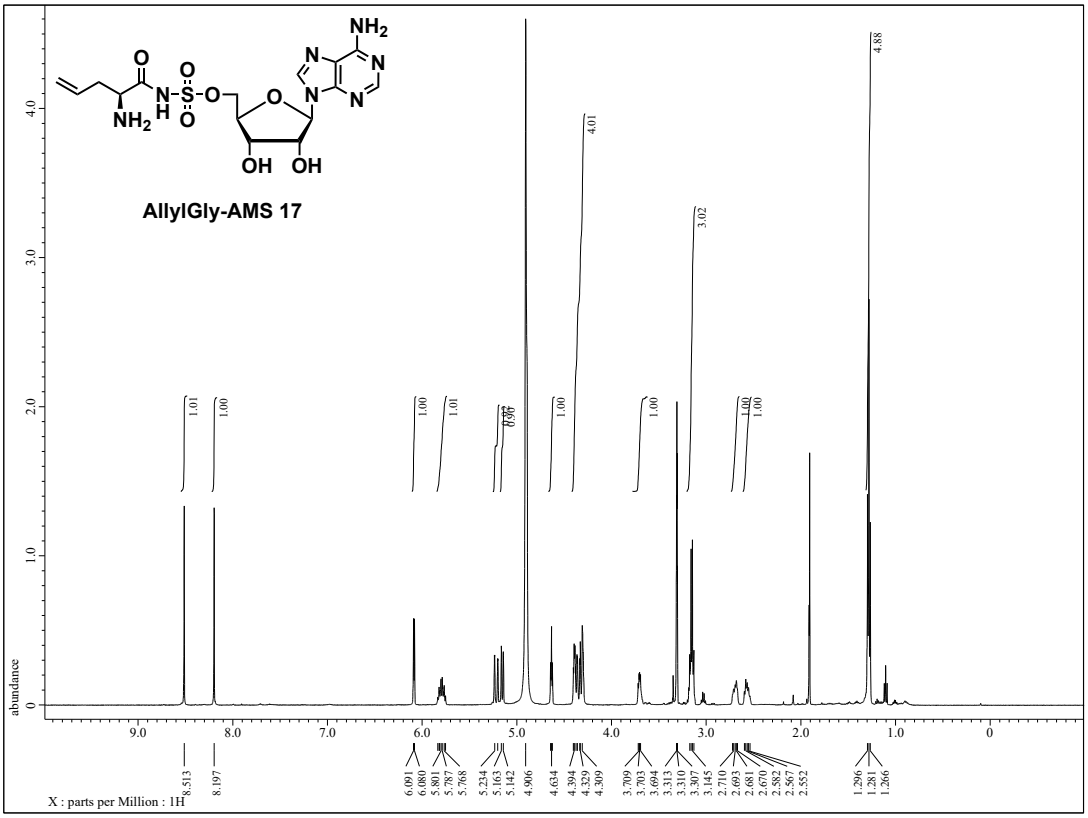


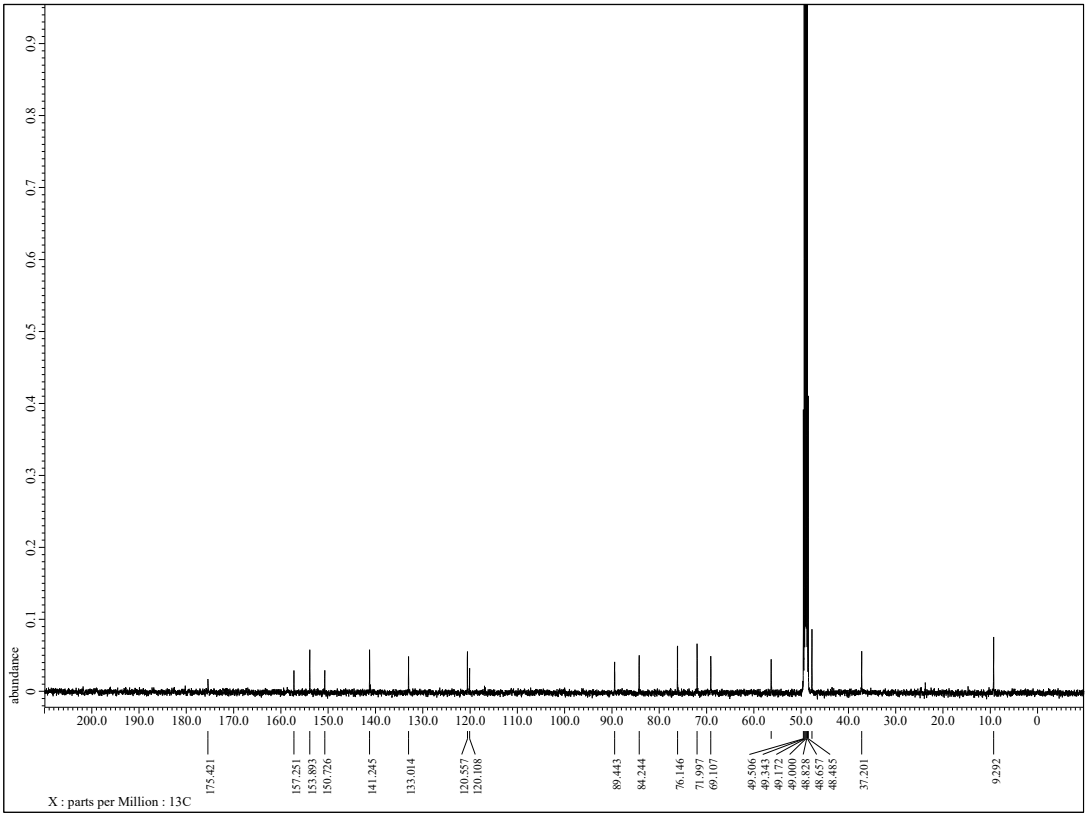
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2j** in CD_3OD



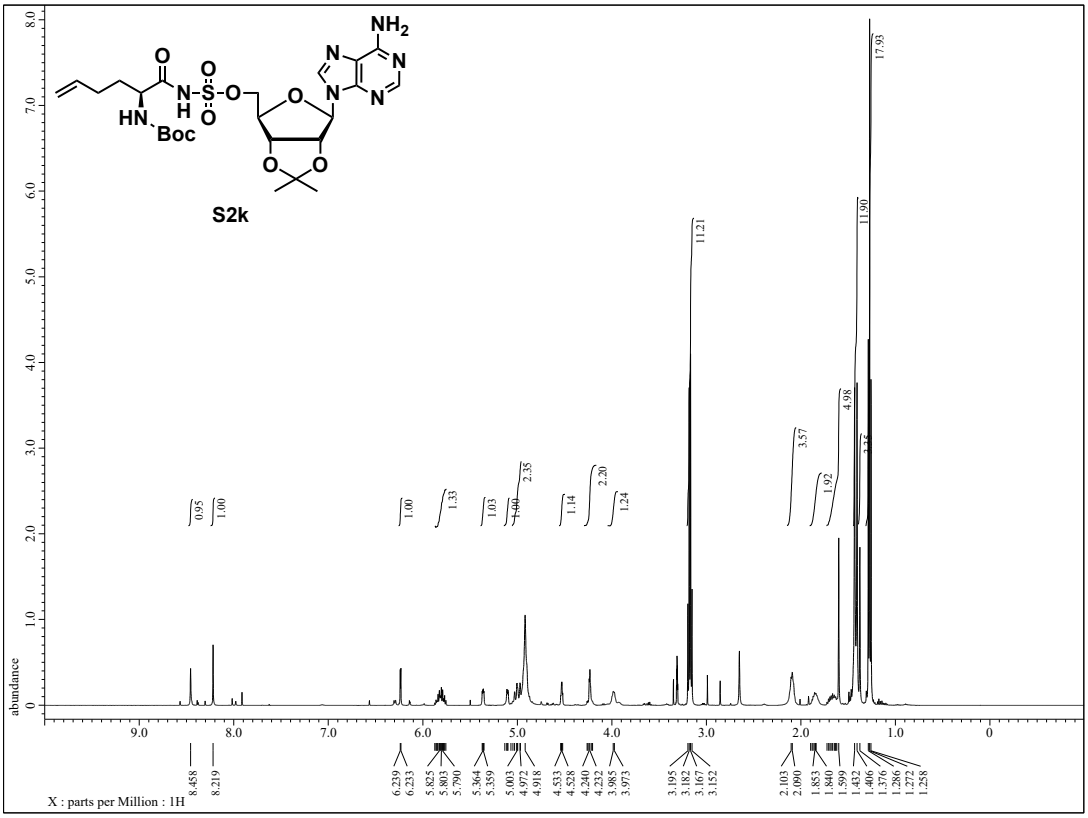


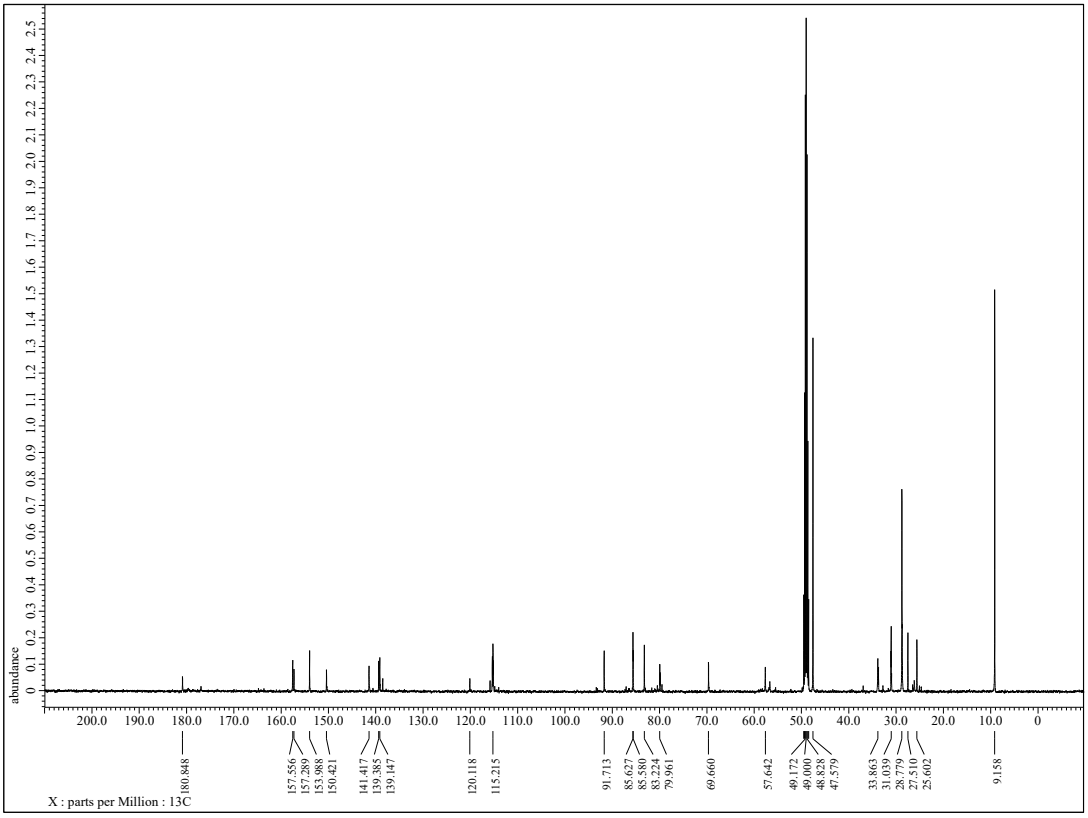
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **AllylGly-AMS 17** in CD_3OD



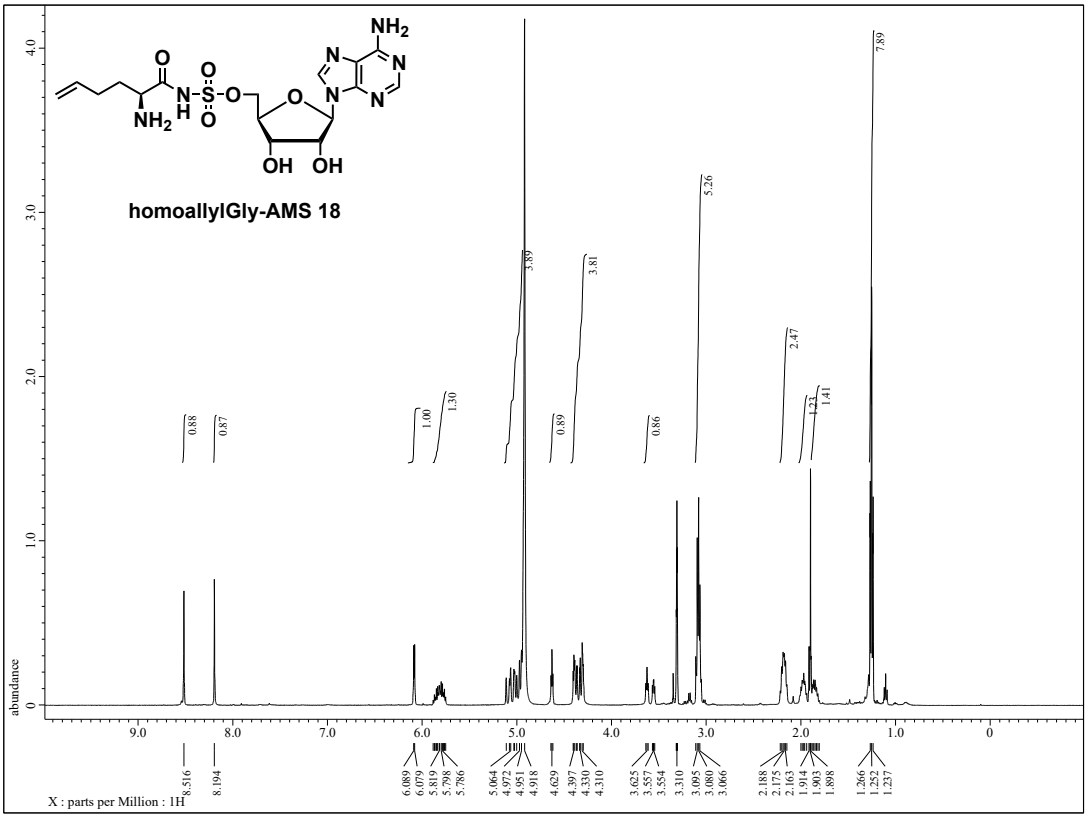


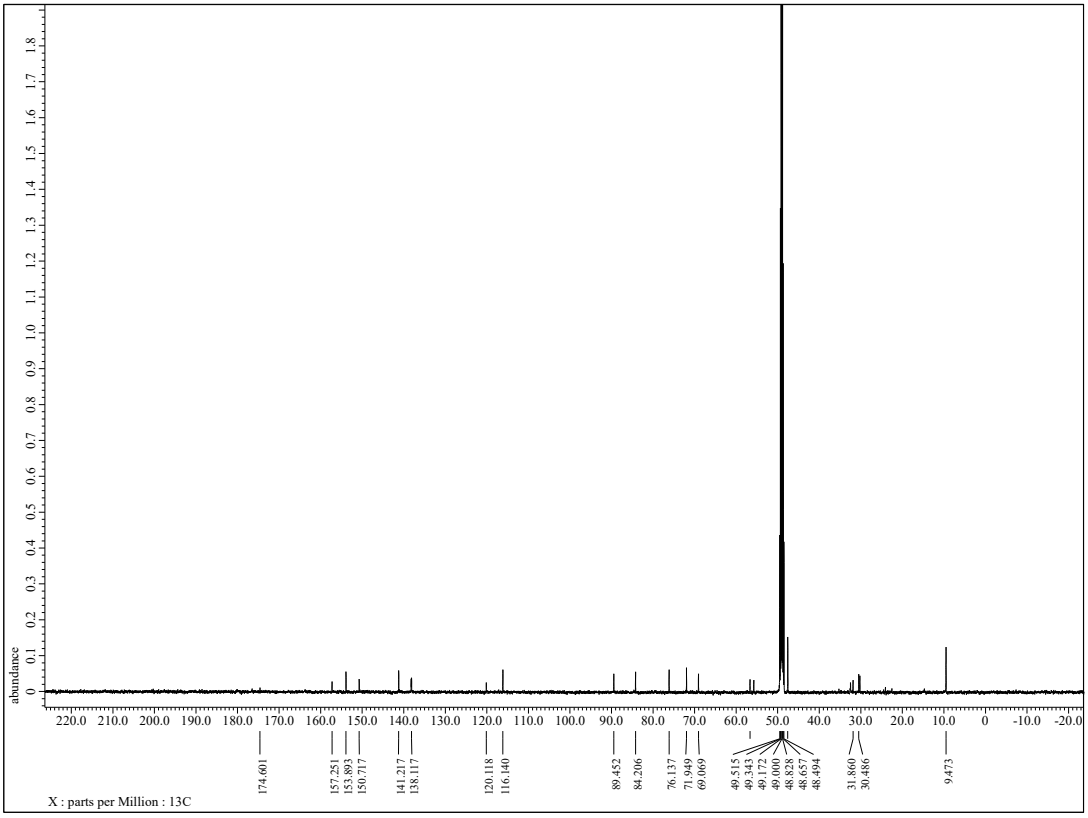
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2k** in CD_3OD



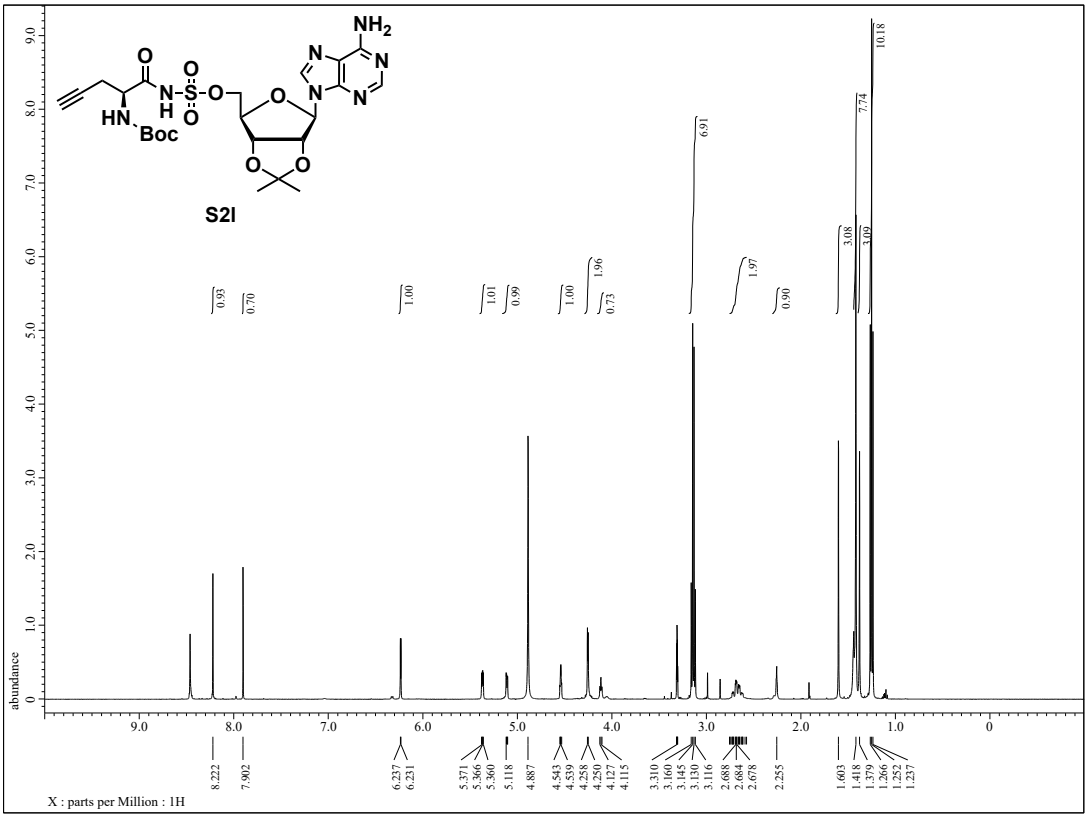


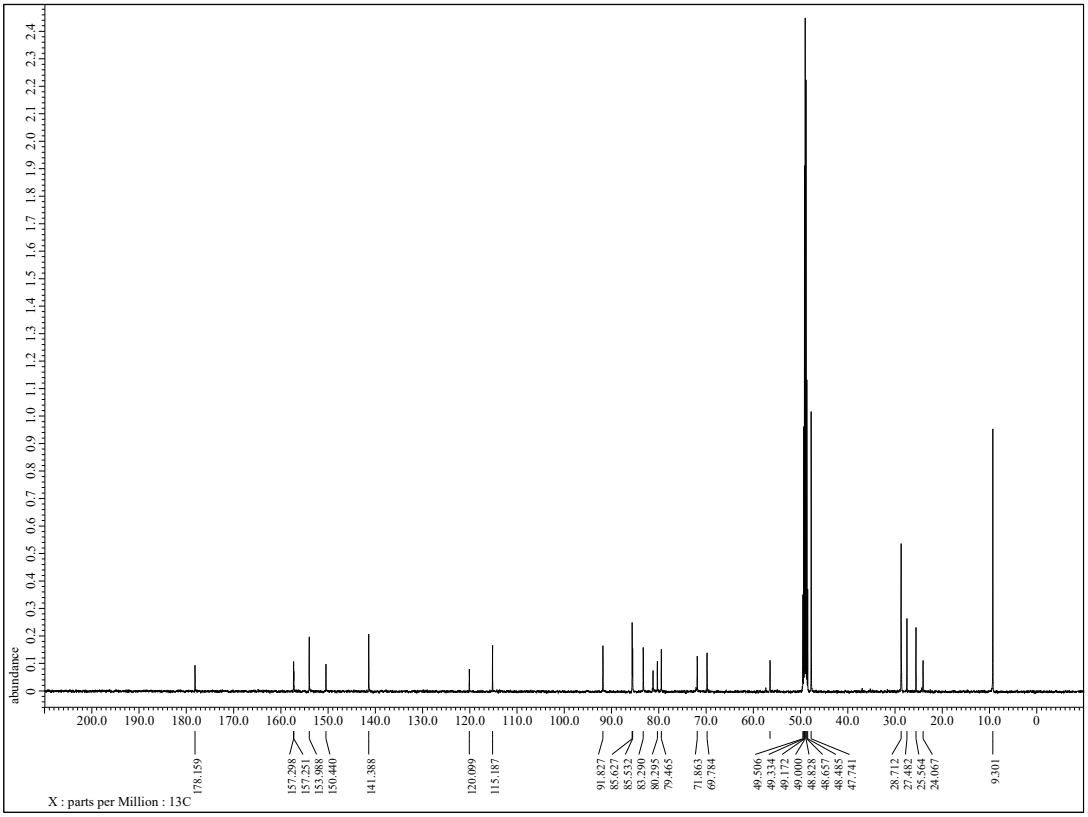
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **homoallylGly-AMS 18** in CD_3OD



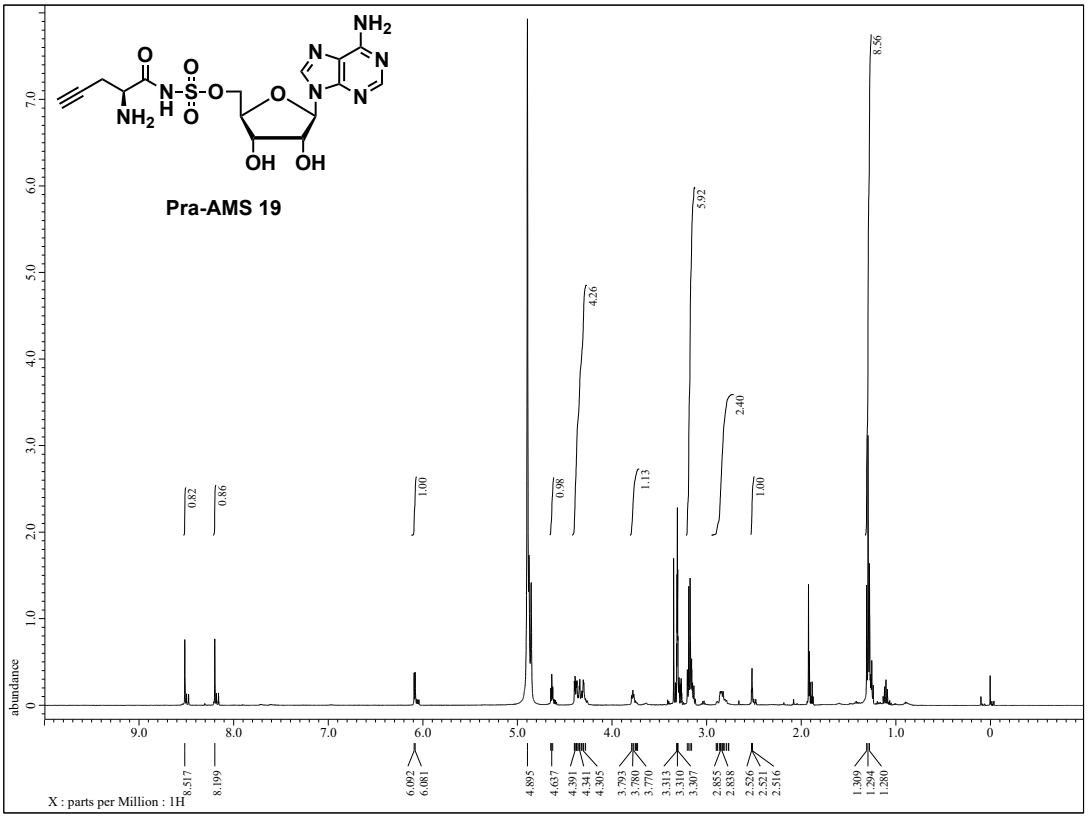


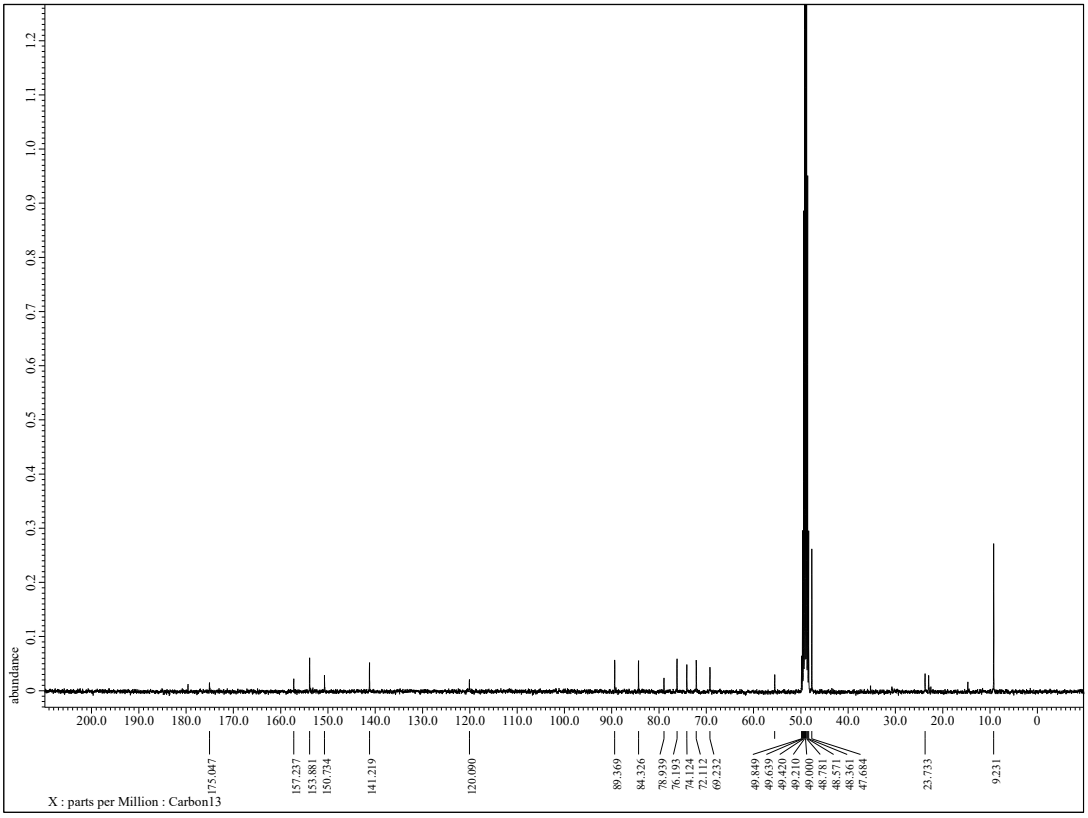
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2l** in CD_3OD



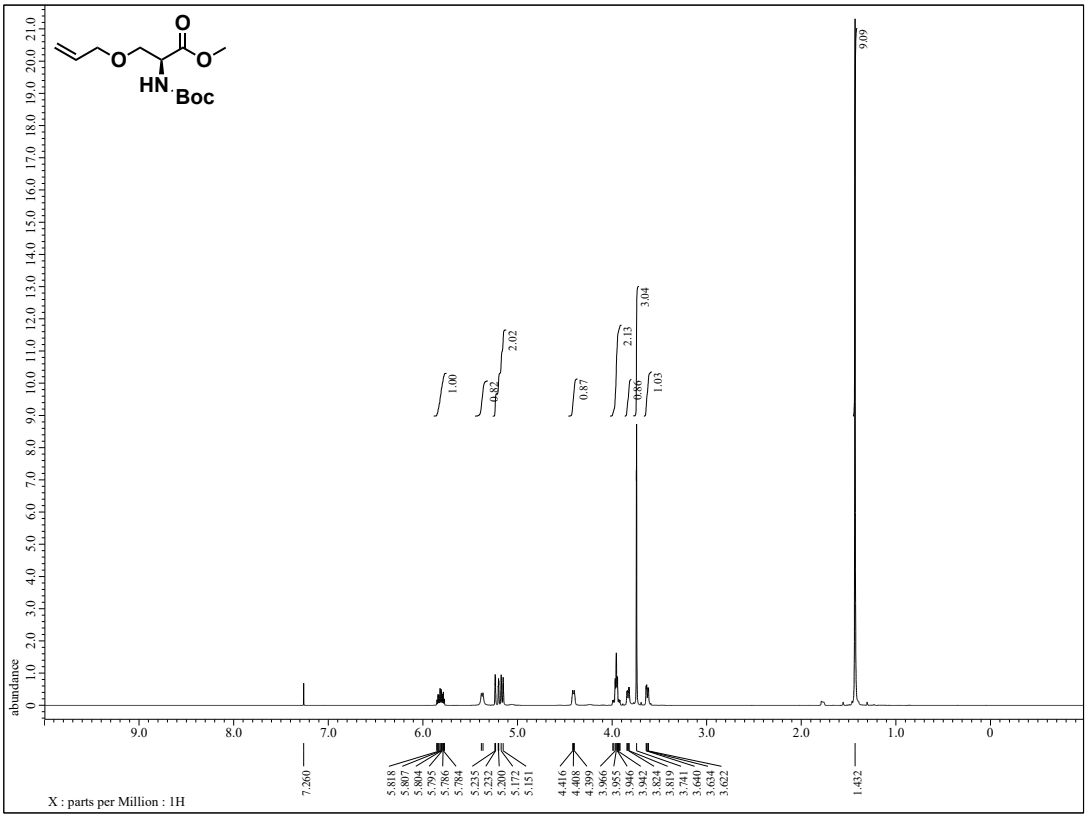


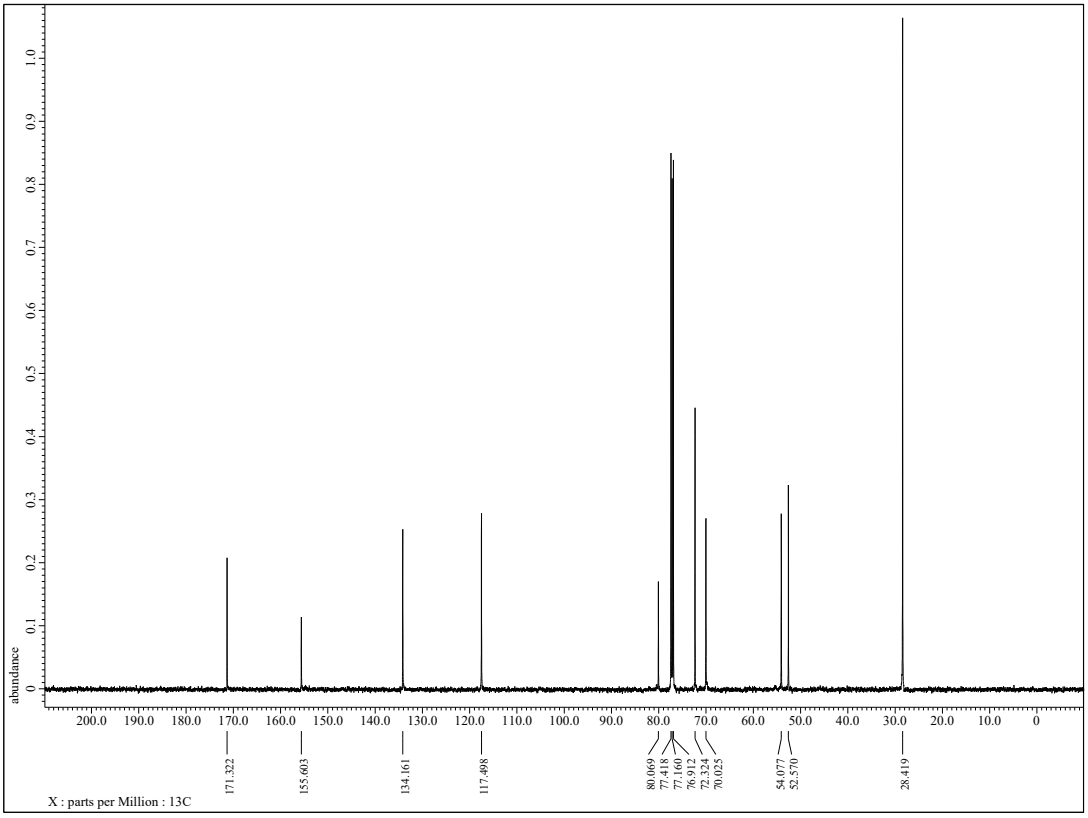
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Pra-AMS 19** in CD_3OD



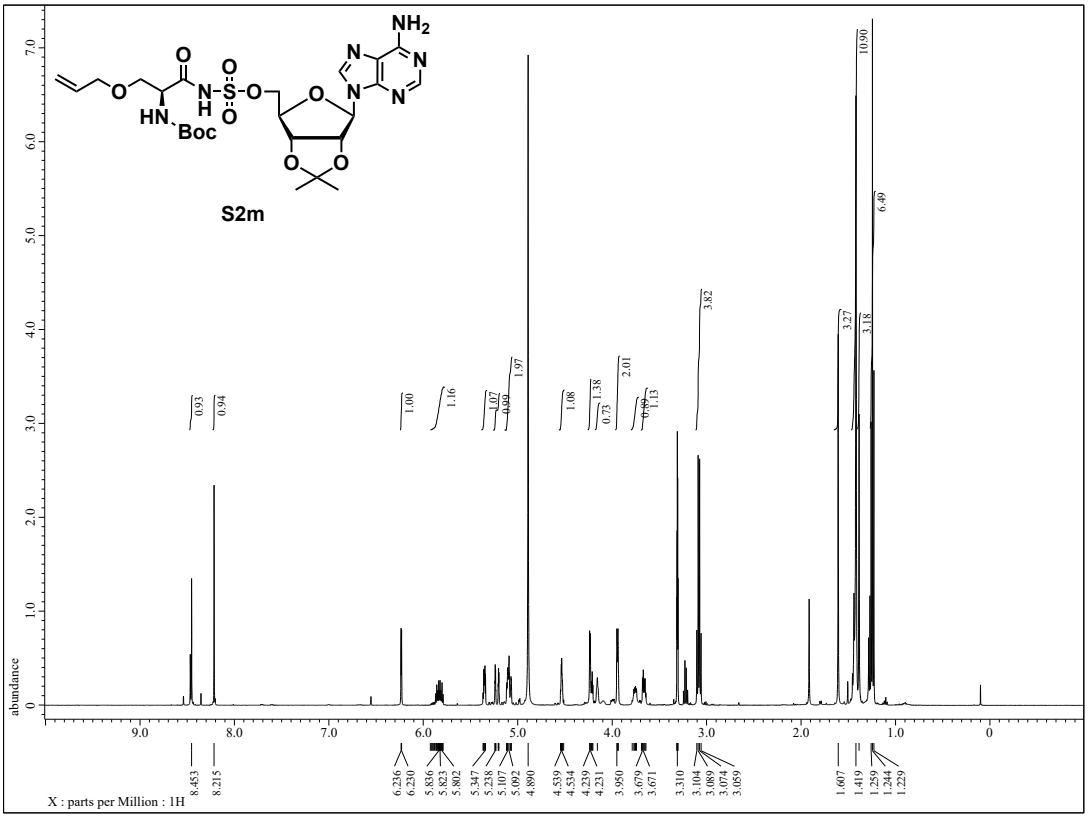


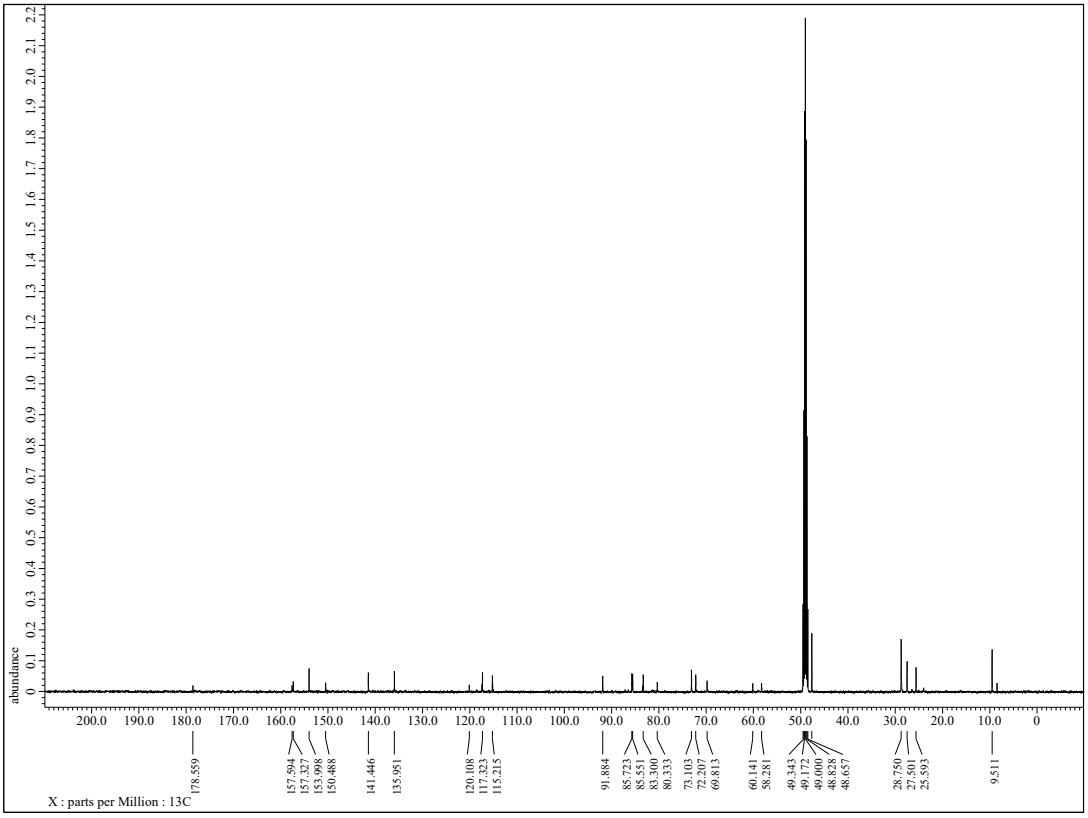
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Boc-Ser(Allyl)-OMe** in $CDCl_3$



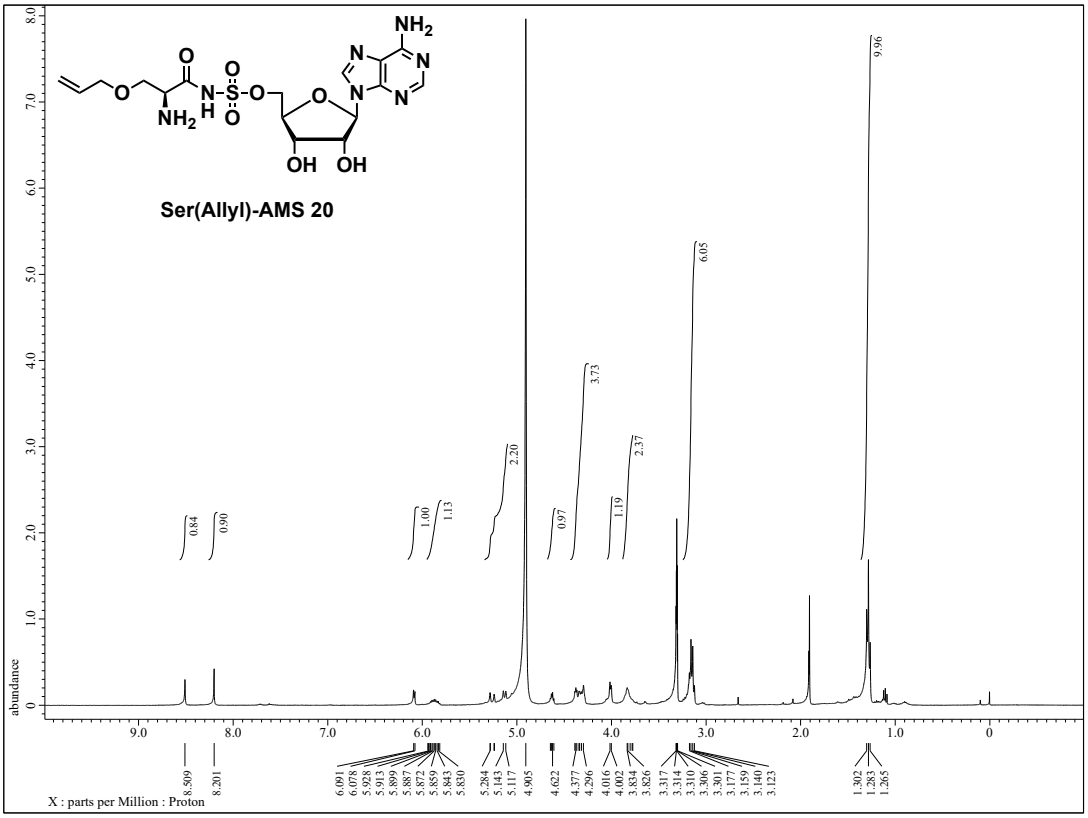


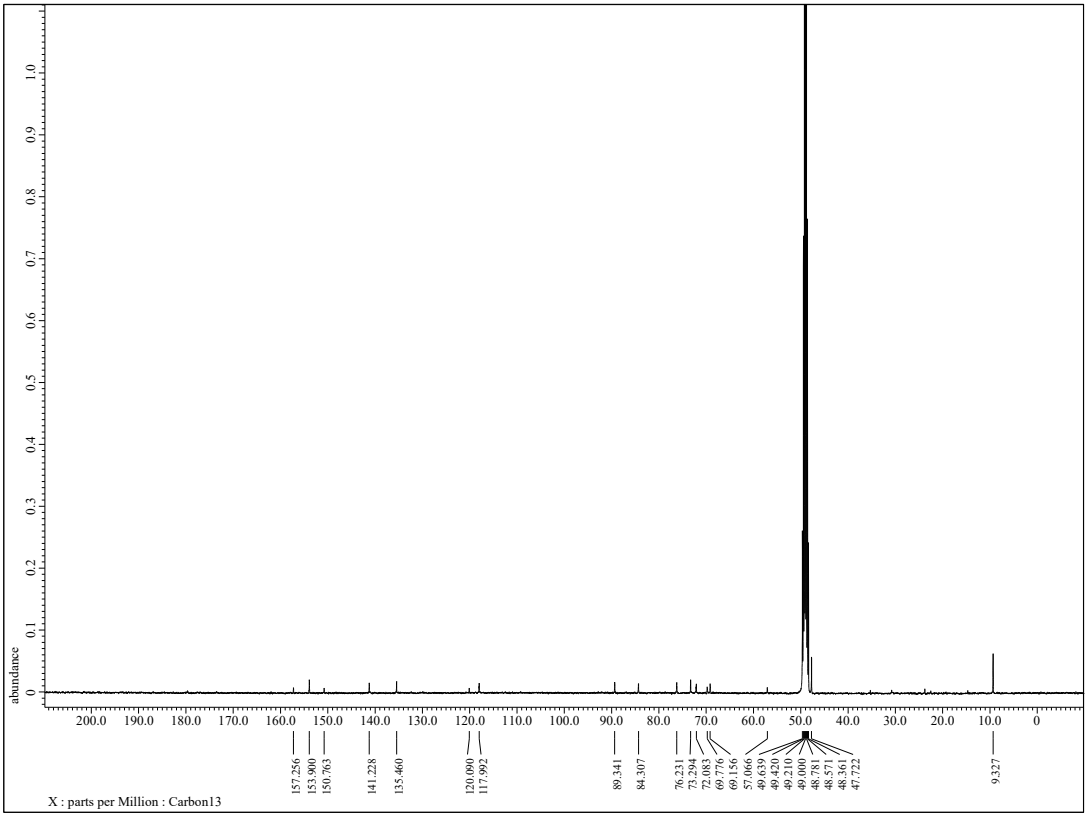
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2m** in CD_3OD



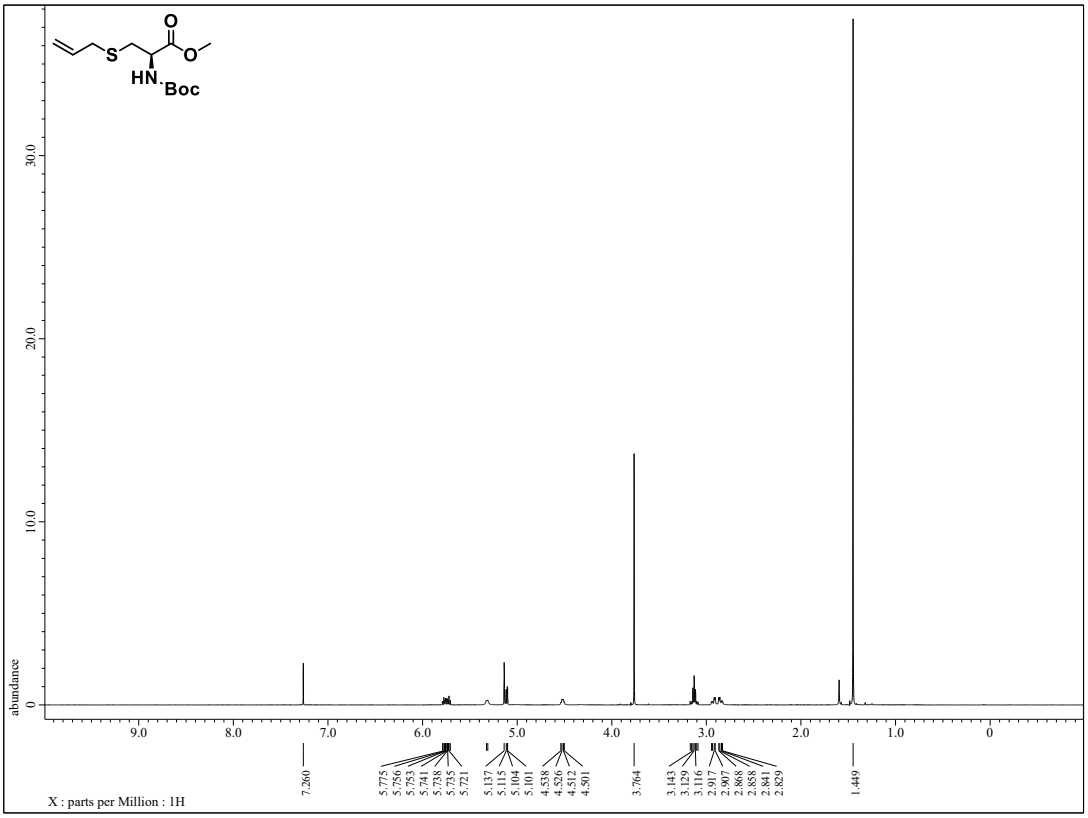


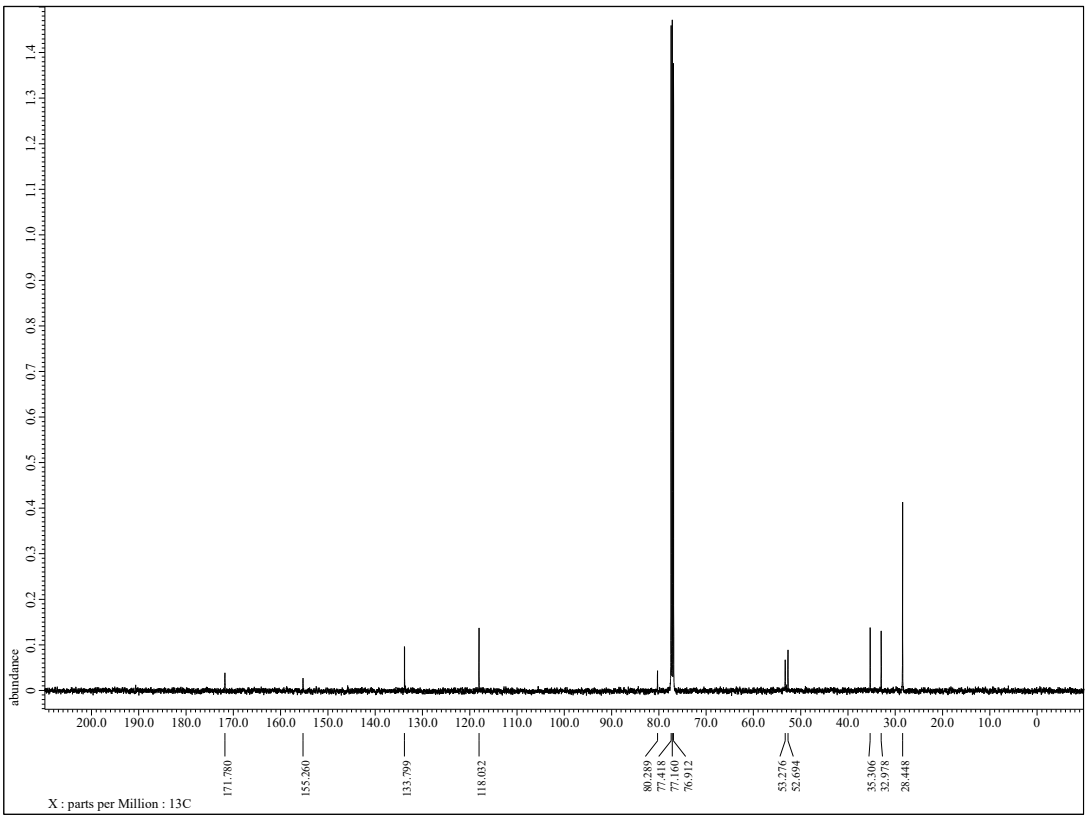
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Ser(Allyl)-AMS 20** in CD_3OD



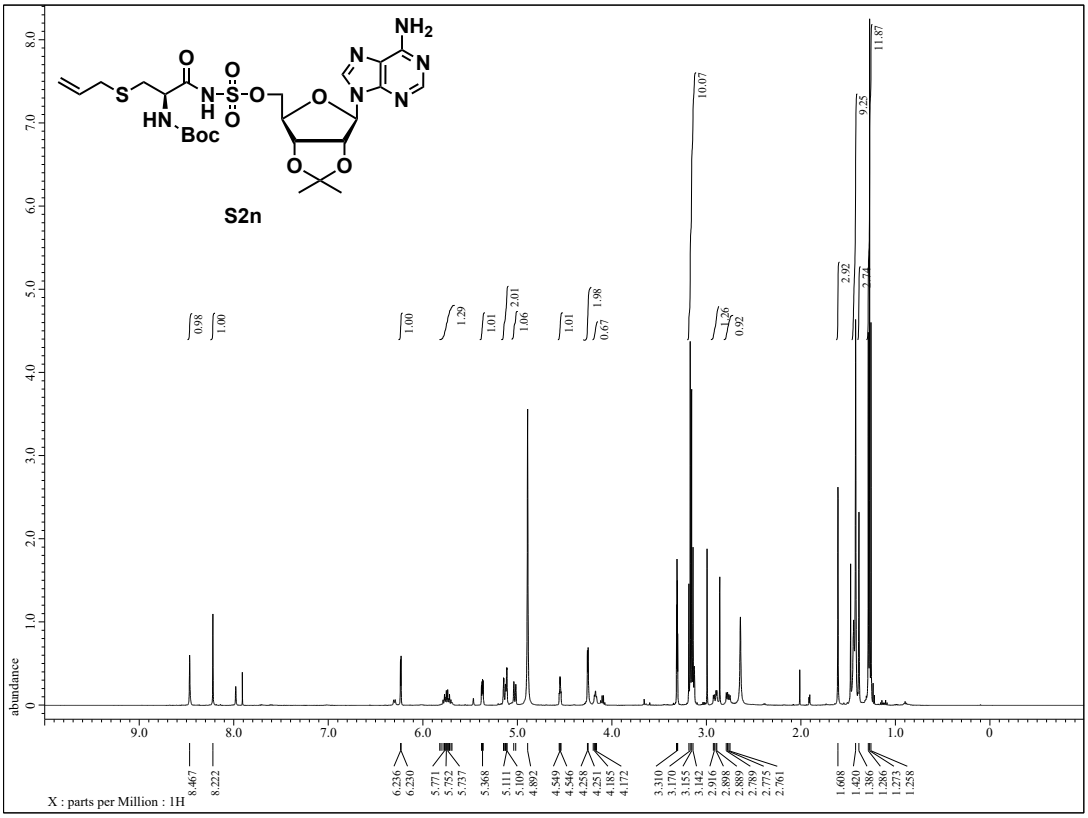


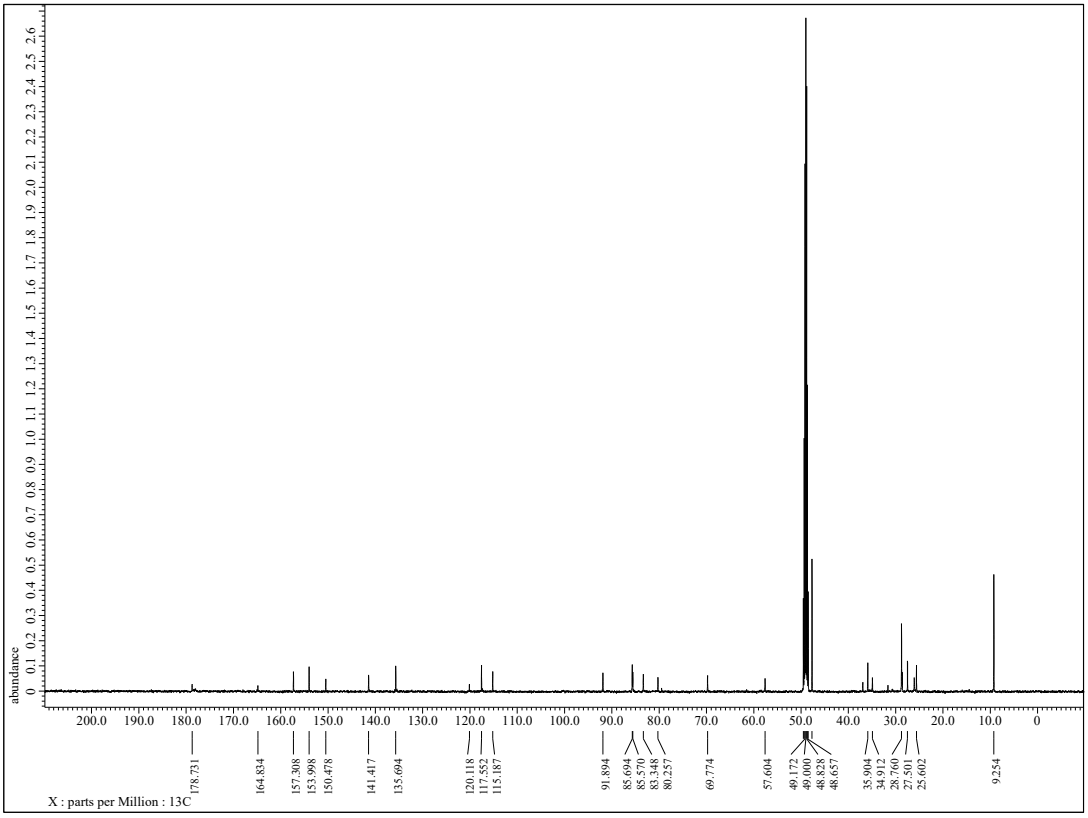
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Boc-Cys(Allyl)-OMe** in $CDCl_3$



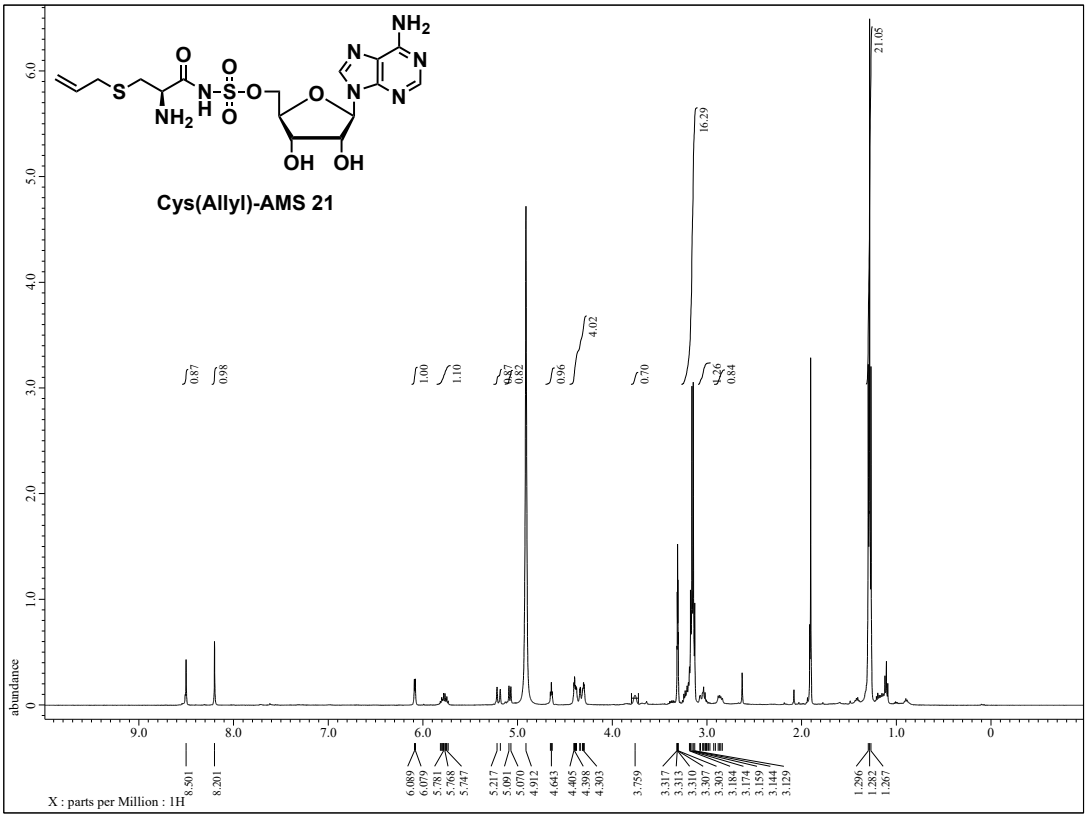


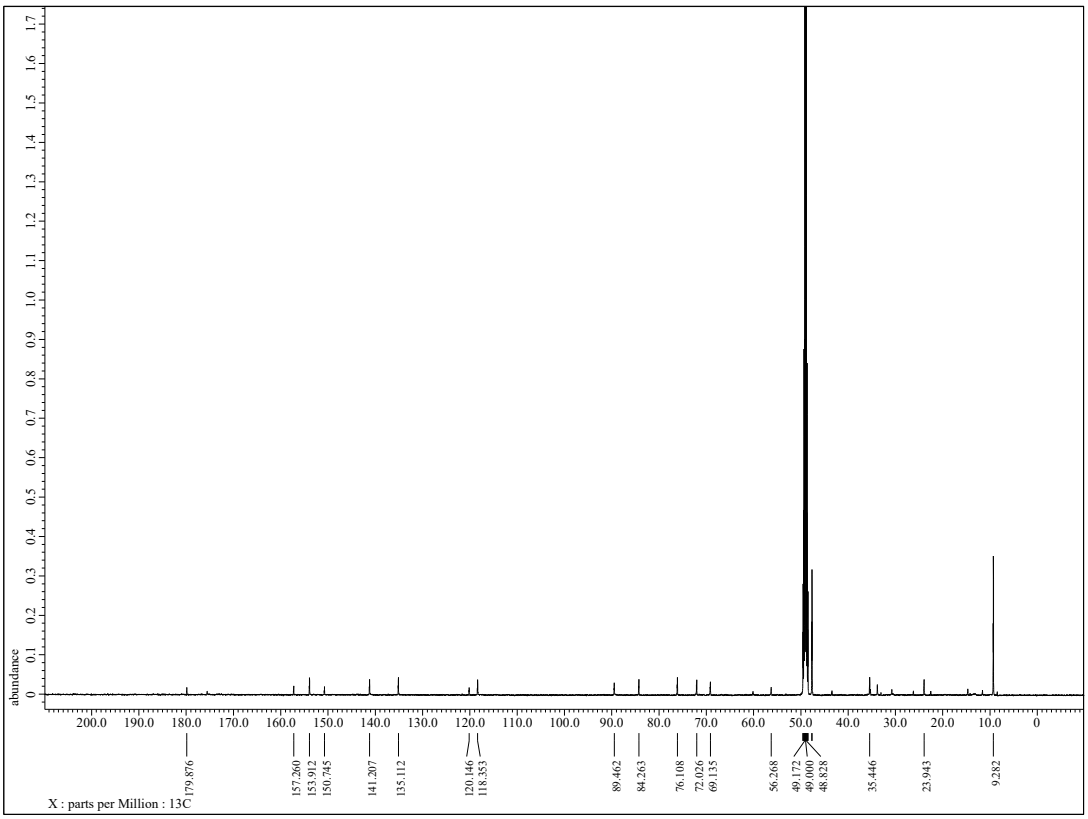
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2n** in CD_3OD



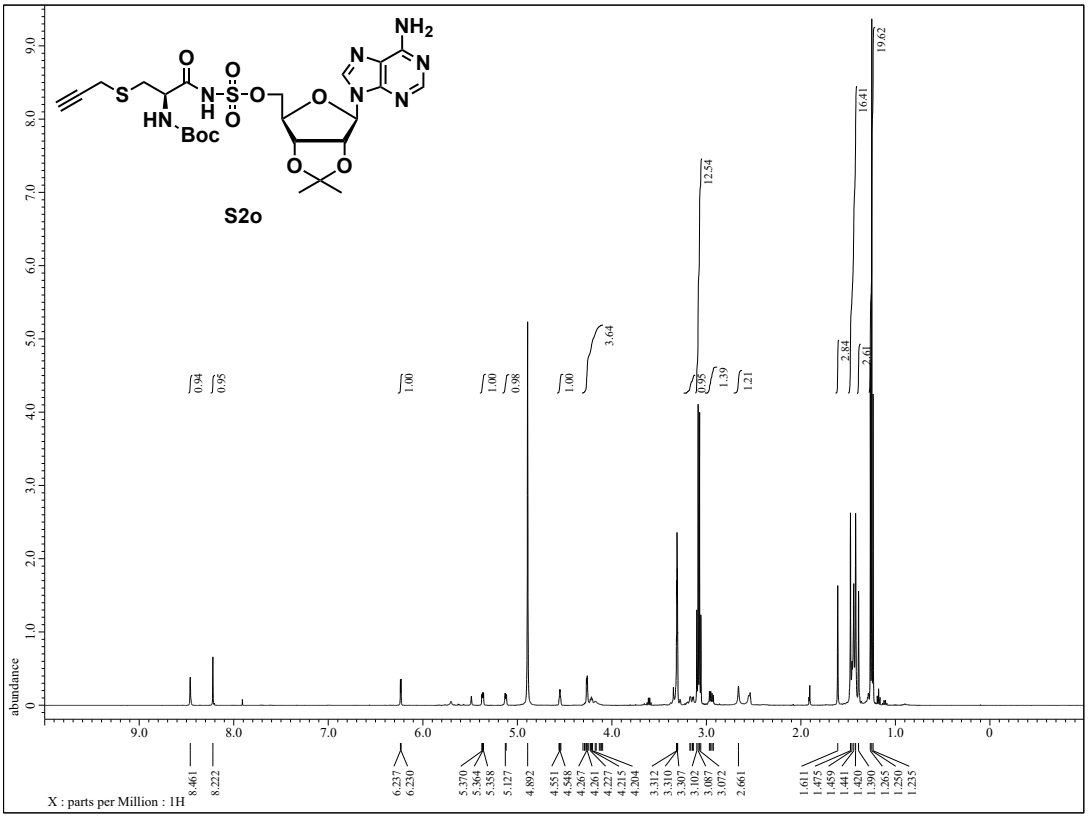


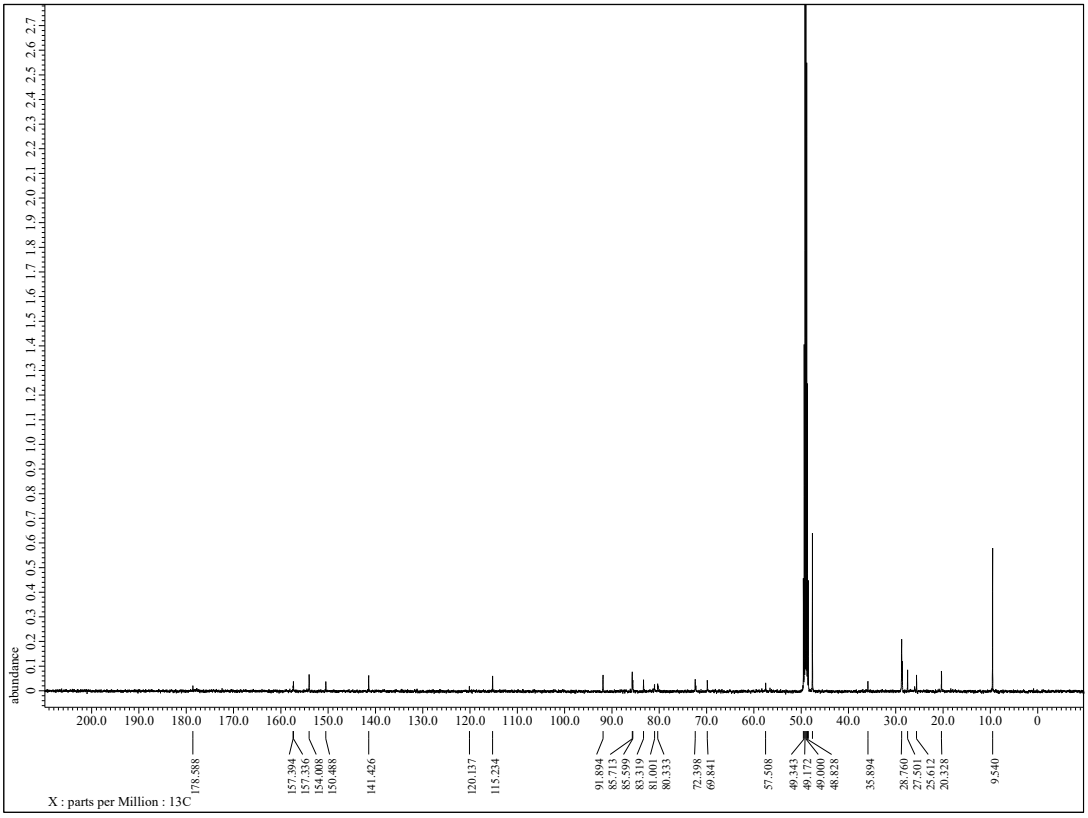
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Cys(Allyl)-AMS 21** in CD_3OD



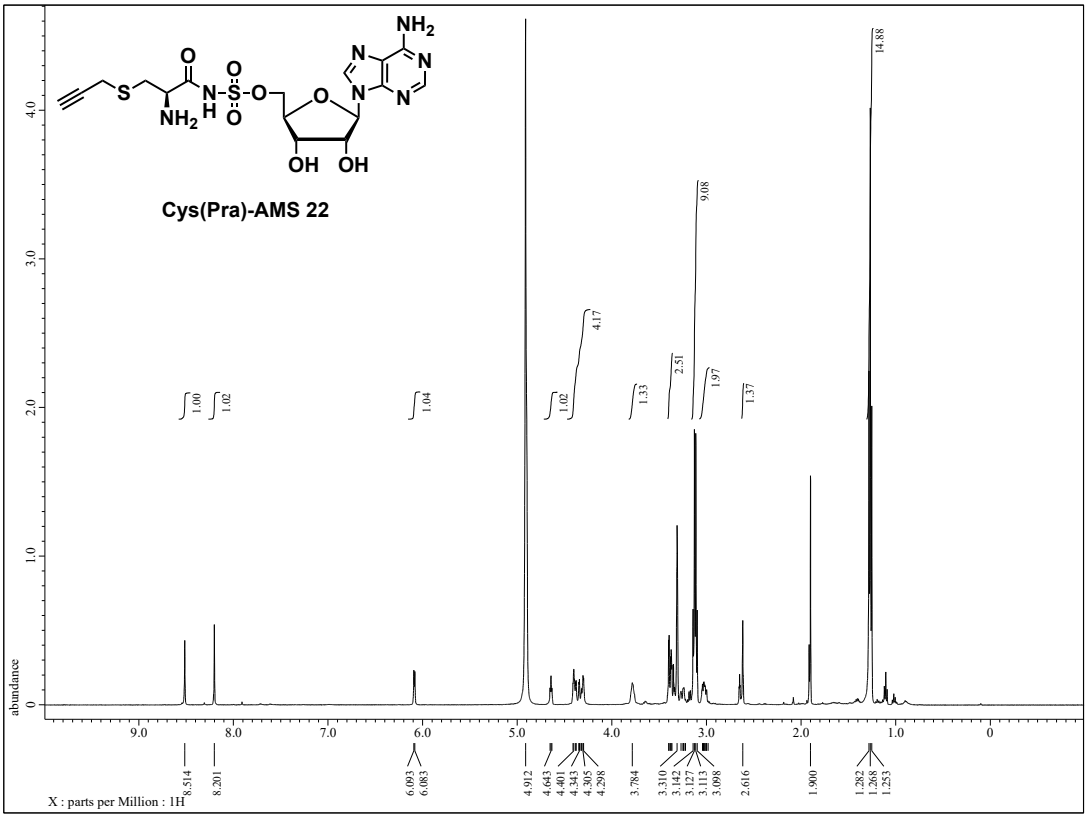


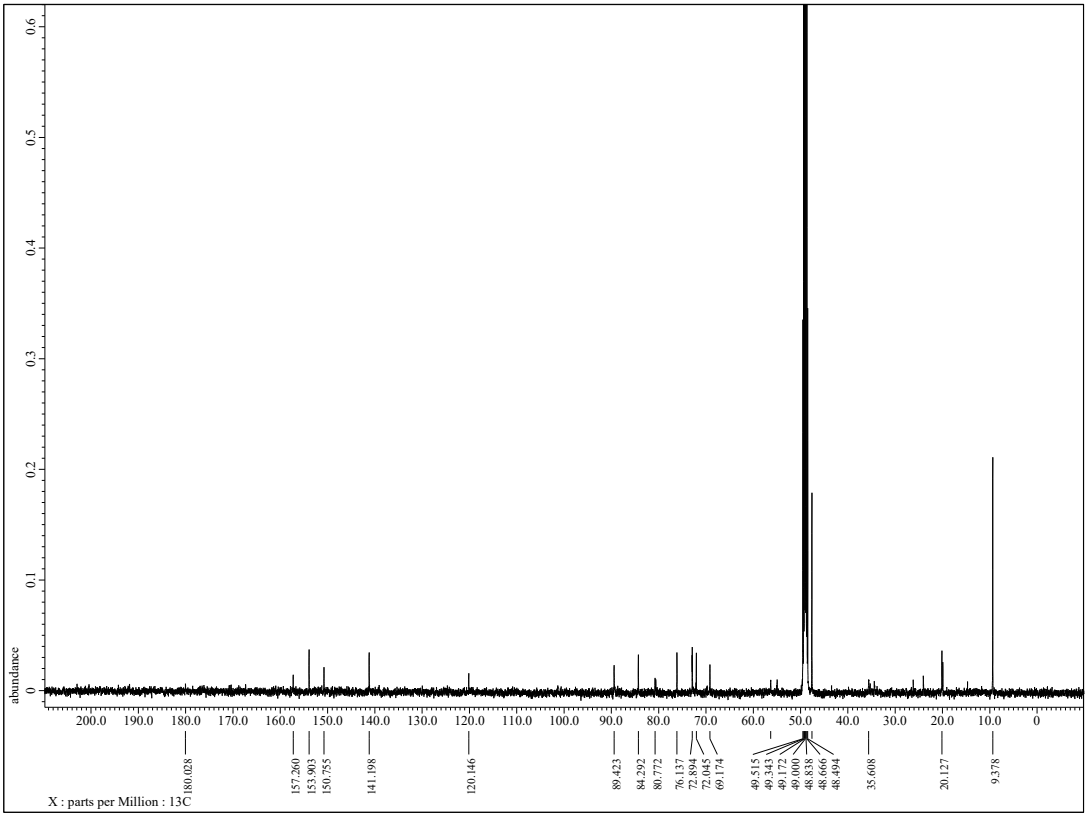
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2o** in CD_3OD



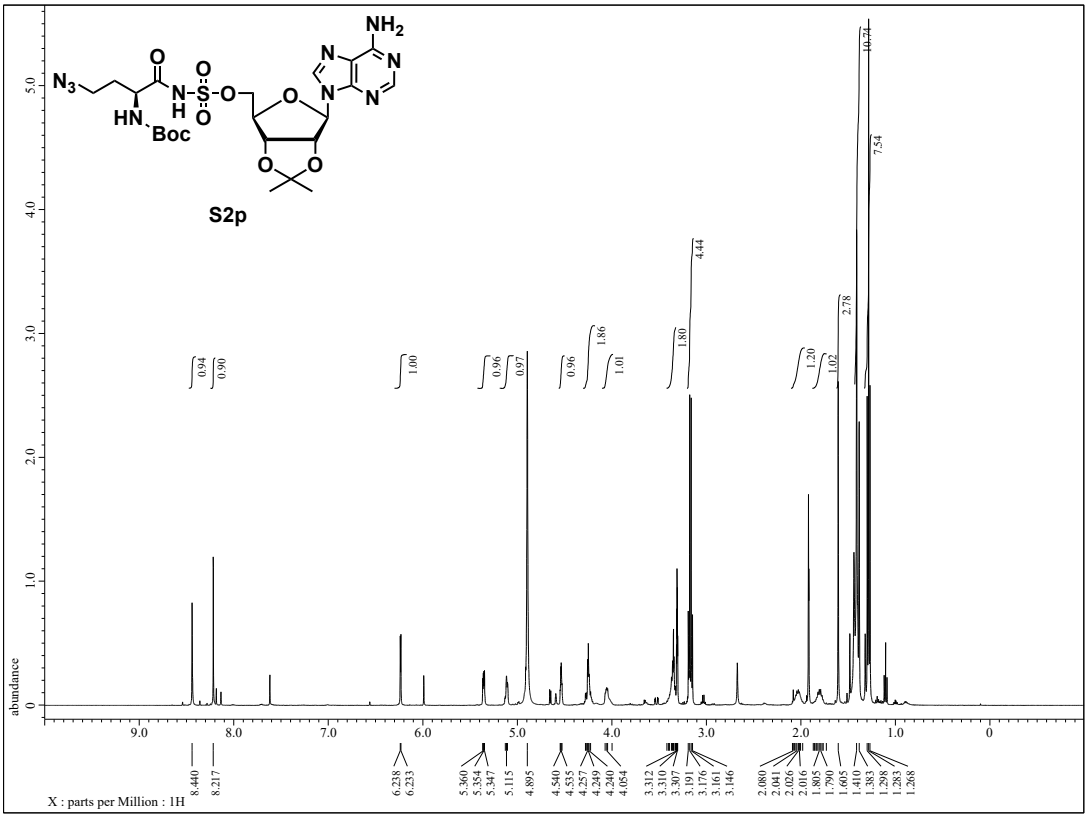


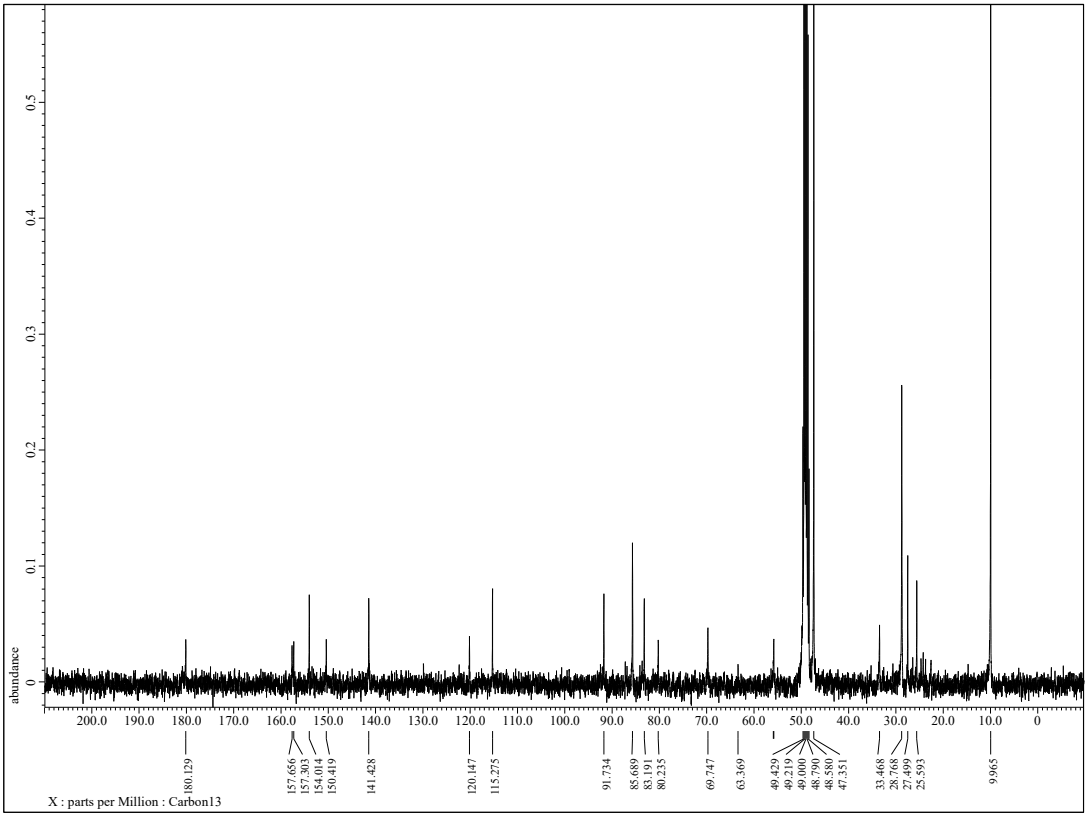
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **Cys(Pra)-AMS 22** in CD_3OD



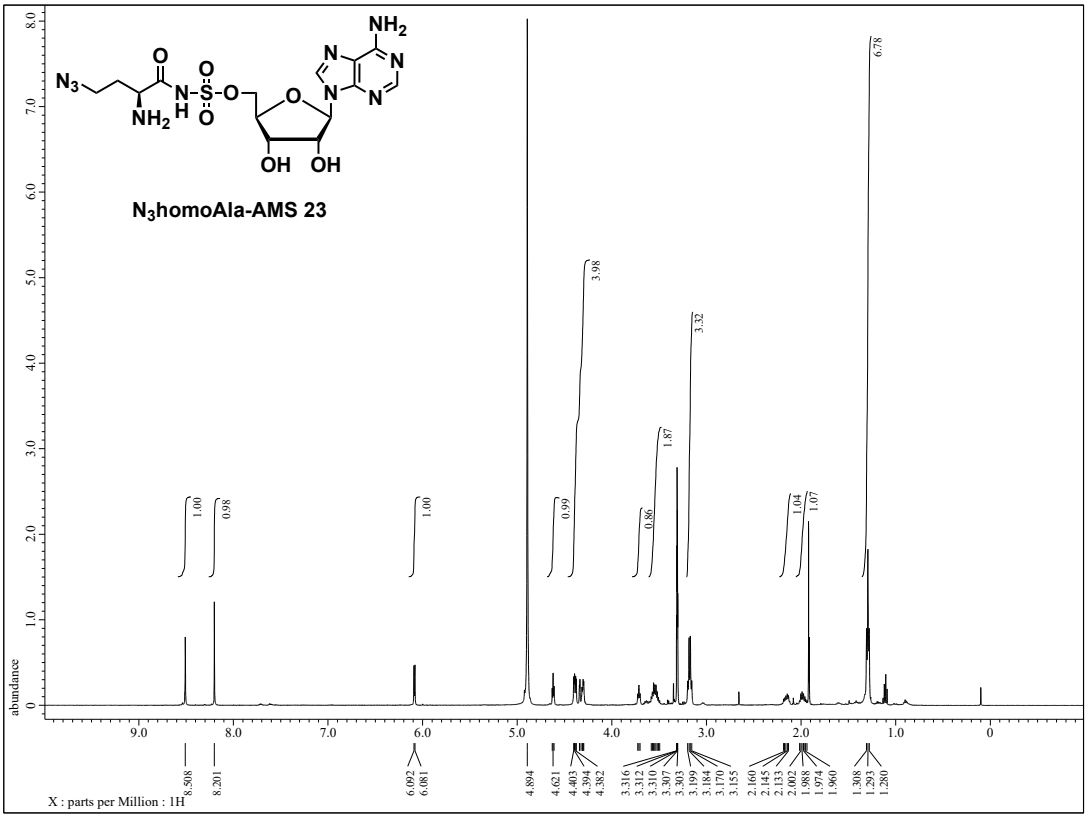


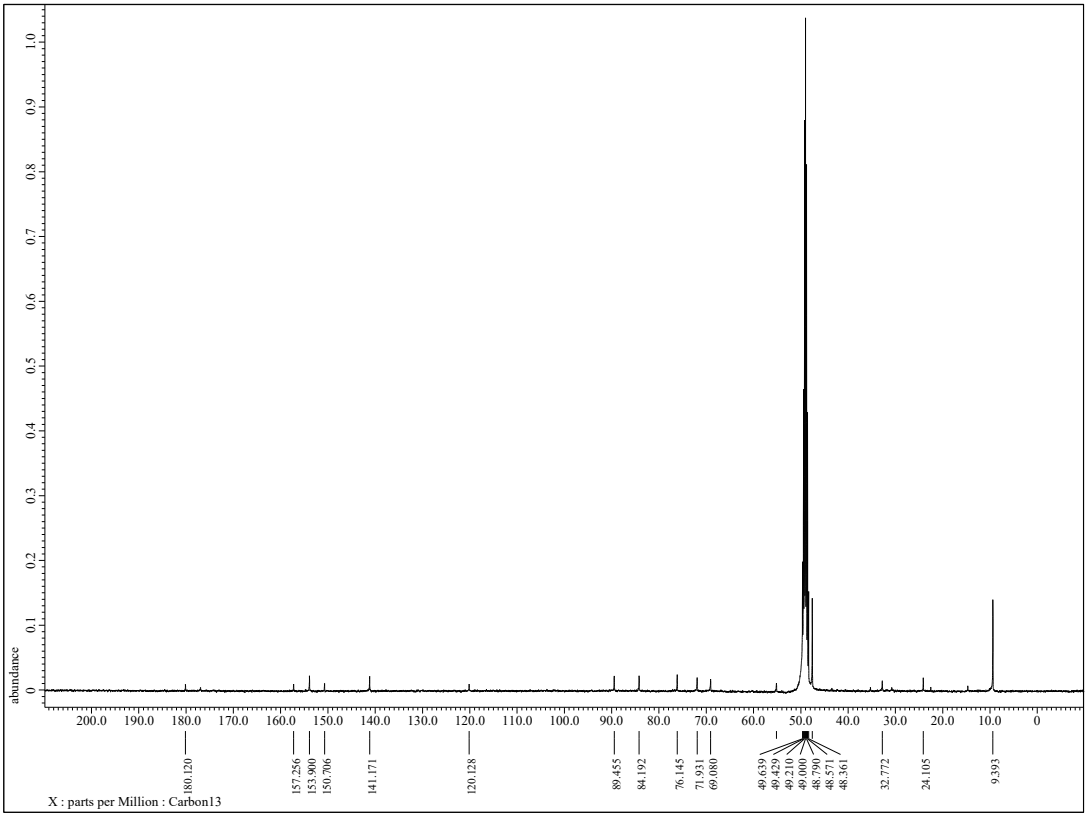
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **S2p** in CD_3OD



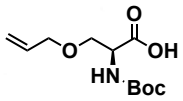


^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **N_3homoAla-AMS 23** in CD_3OD



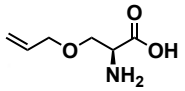


^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of **Boc-Ser(Allyl)-OH** in CD_3OD

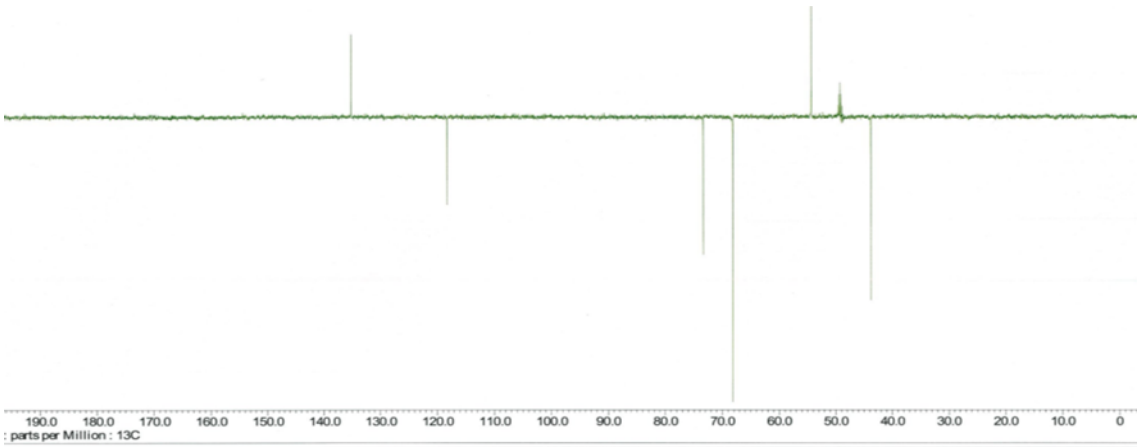


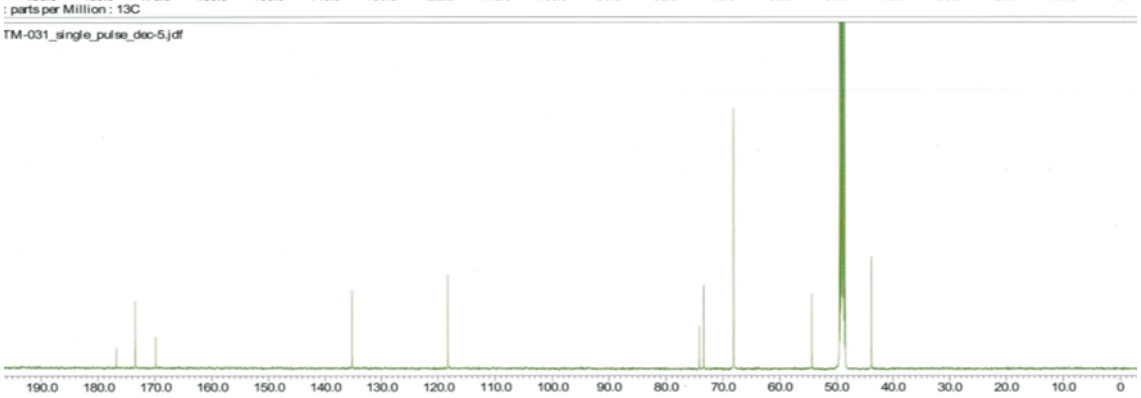
Boc-Ser(Allyl)-OH

^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of ***O*-Allyl-L-Ser** in CD_3OD

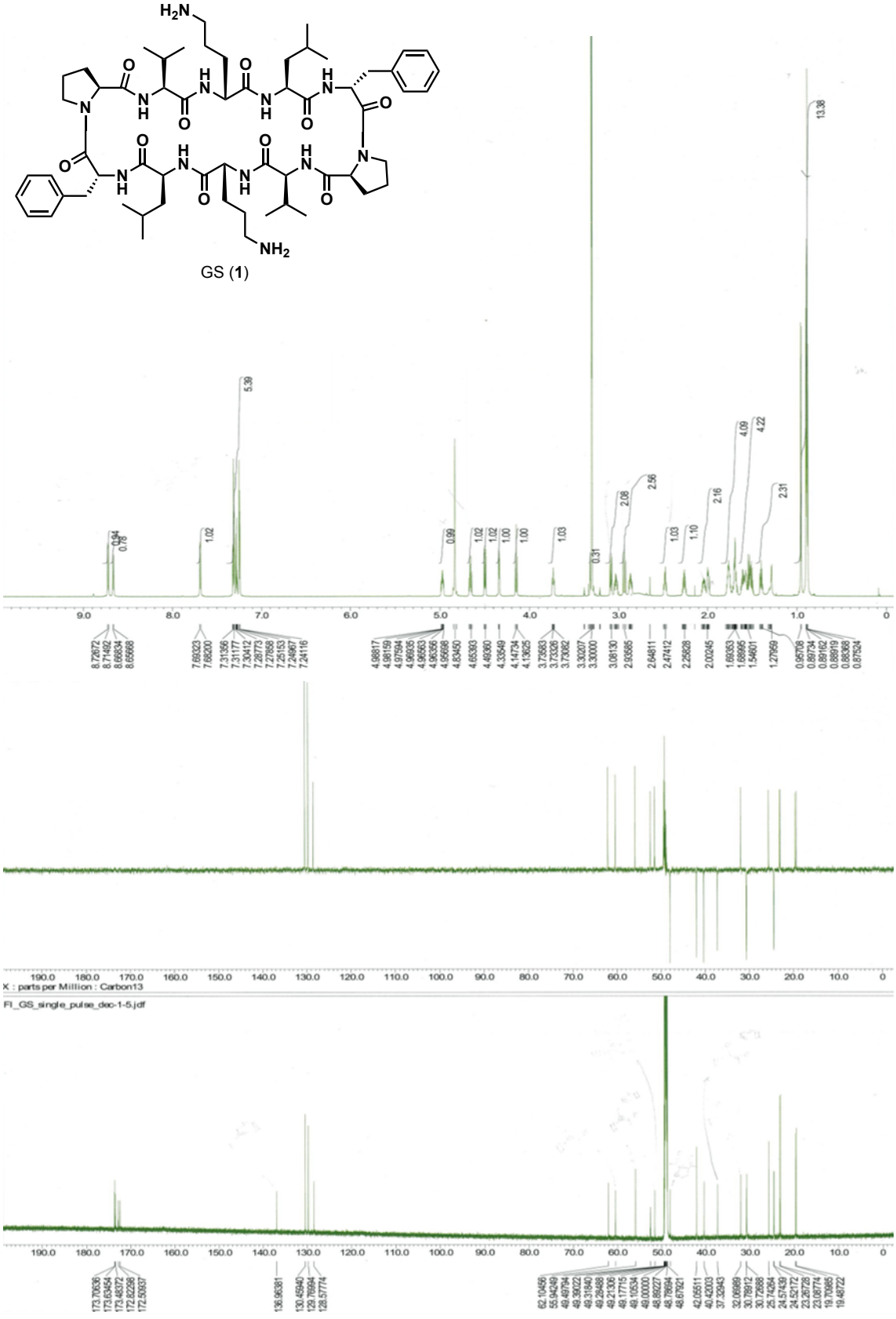


***O*-Allyl-L-Ser**

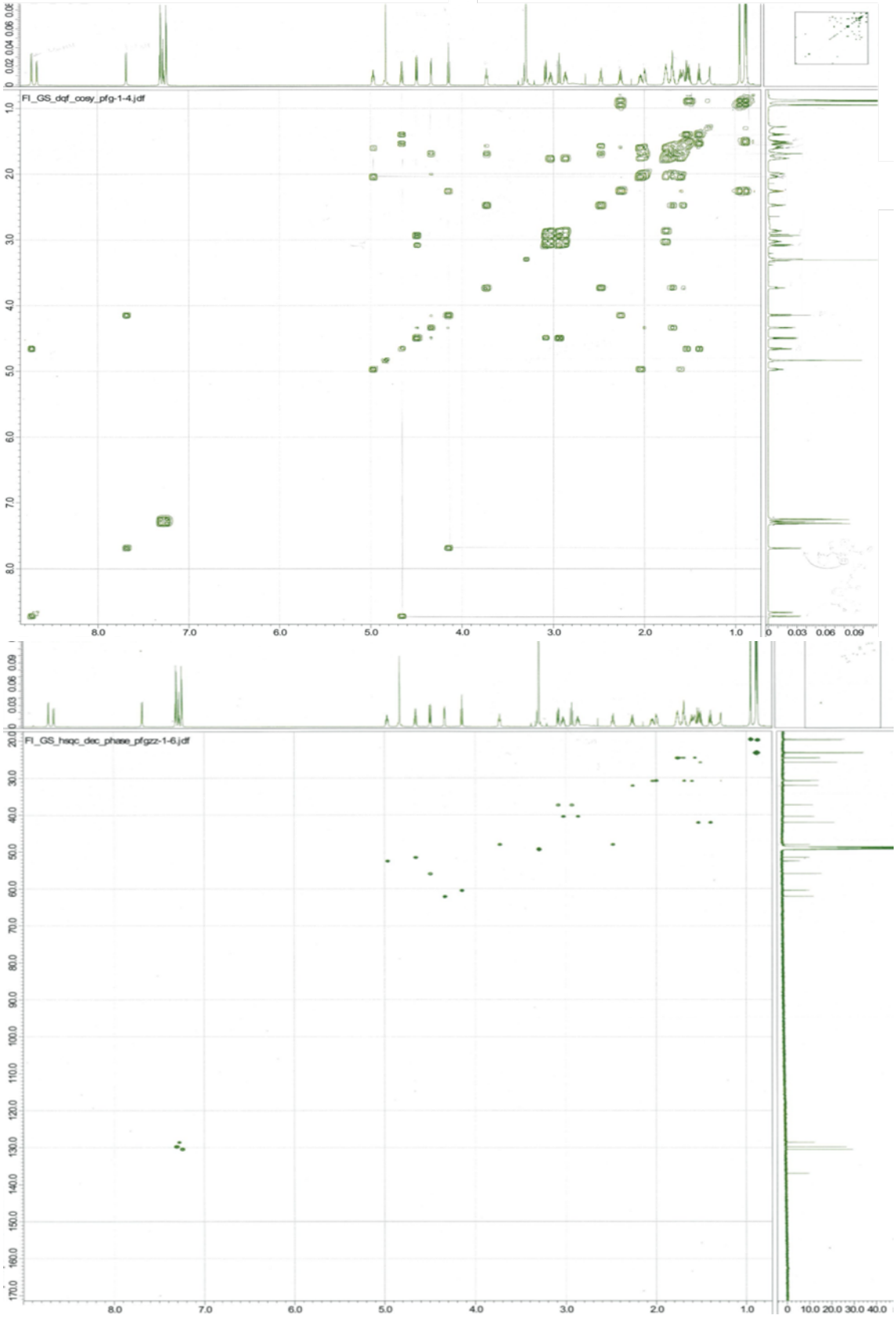




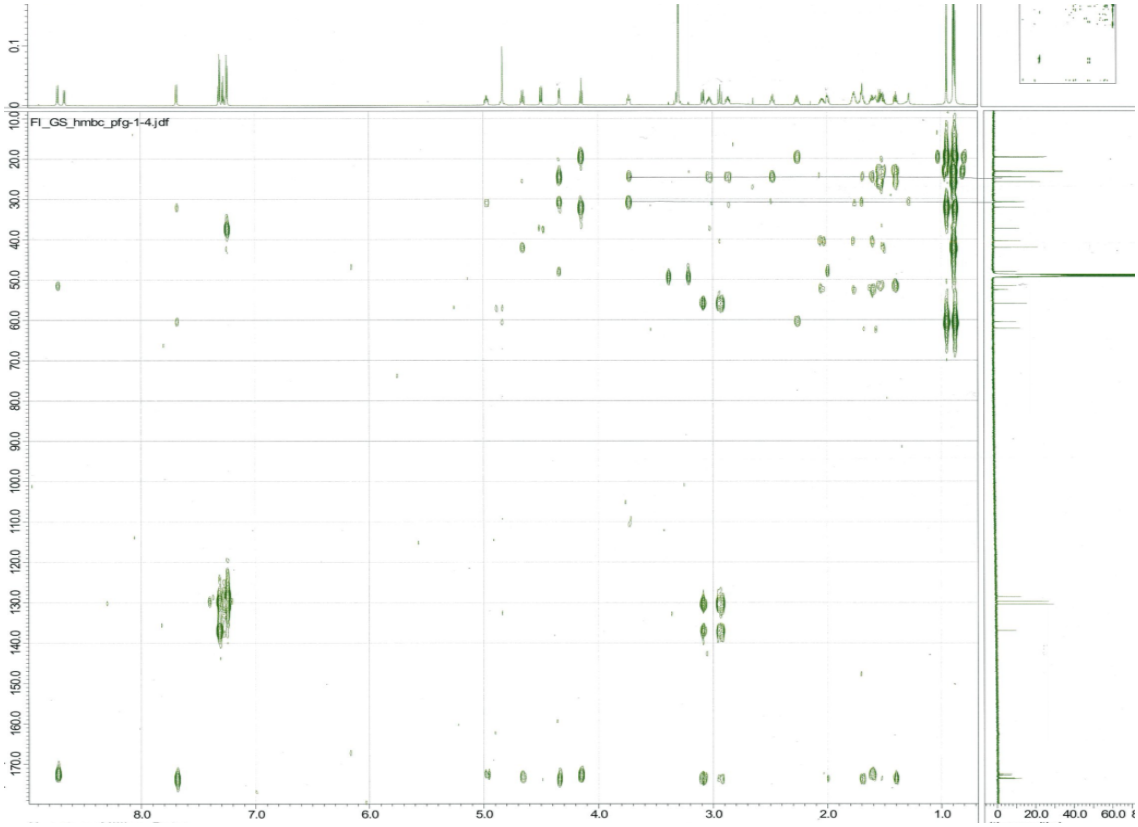
^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of GS (**1**) in CD_3OD

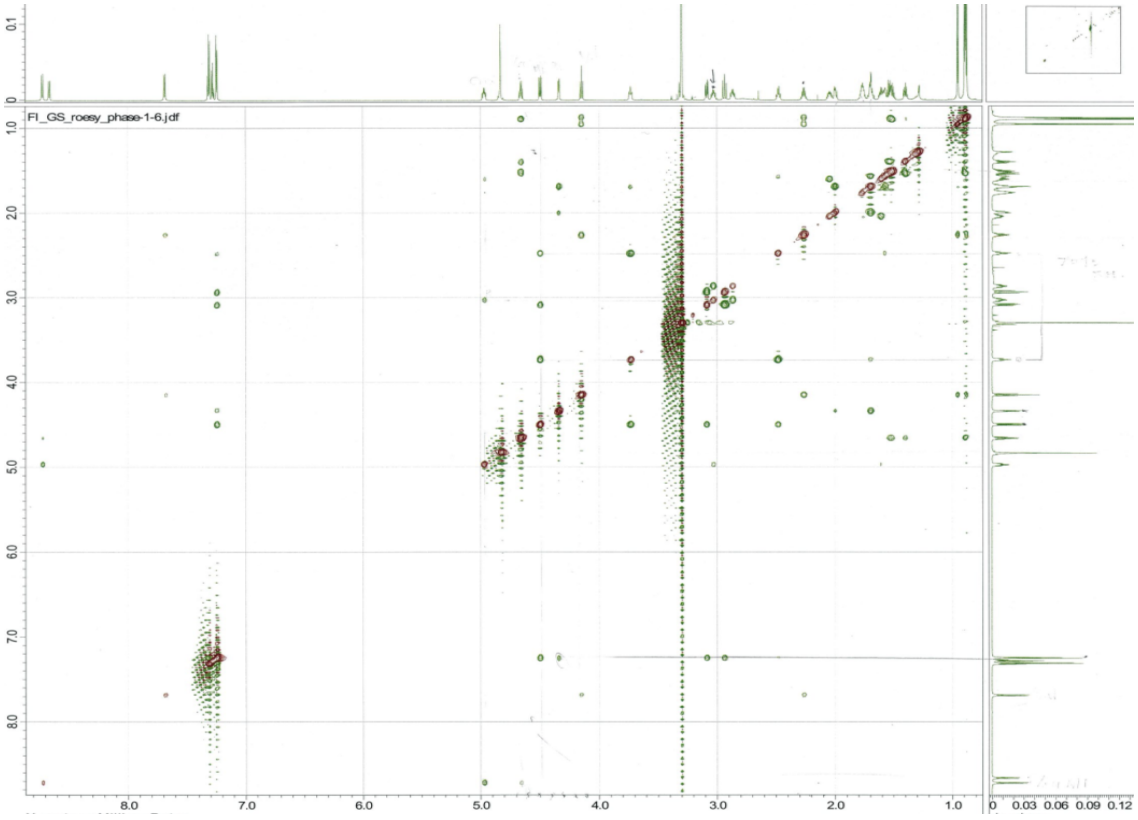


COSY and HSQC spectra (800 MHz) of GS (**1**) in CD_3OD

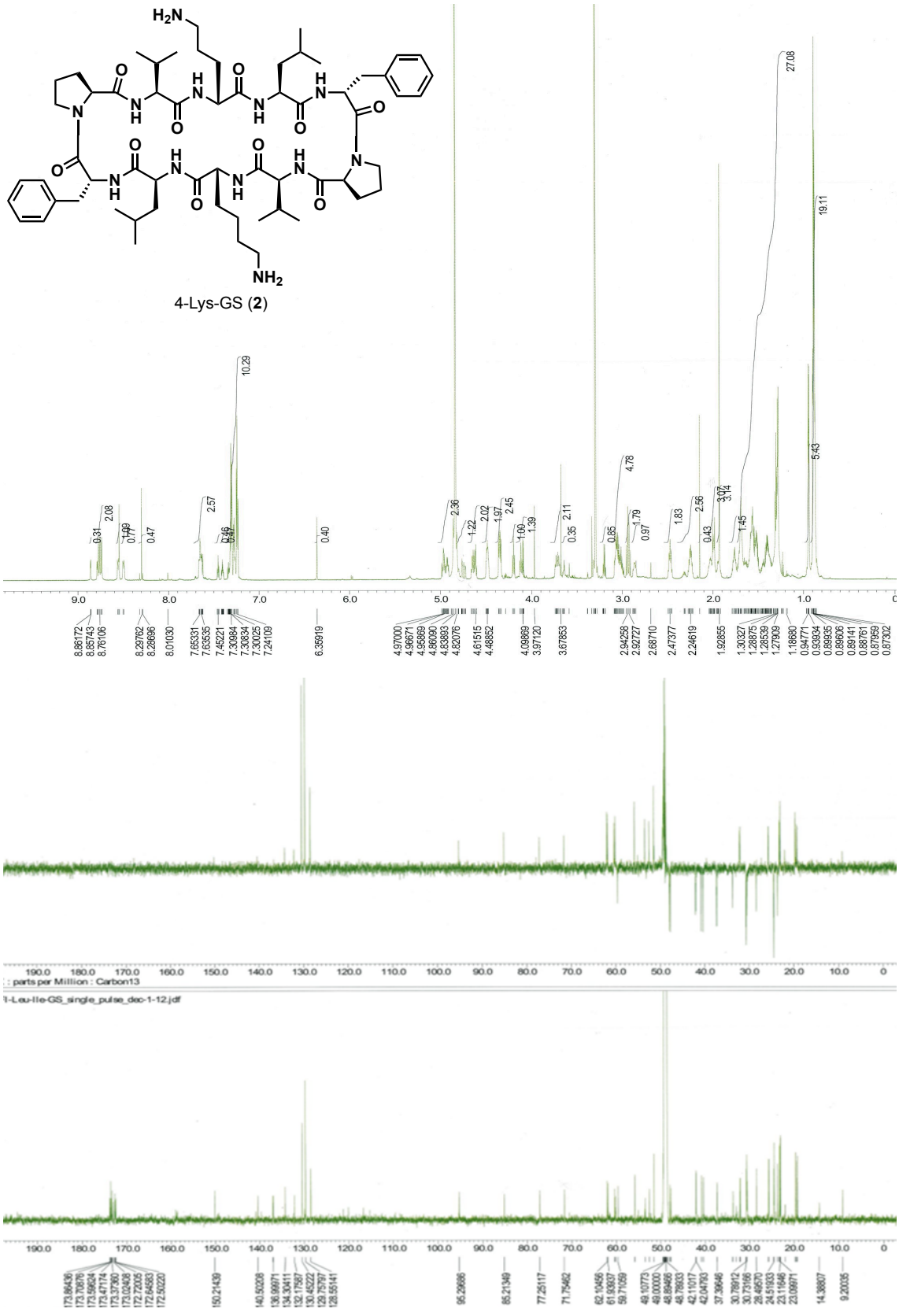


HMBC and ROESY spectra (800 MHz) of GS (**1**) in CD_3OD

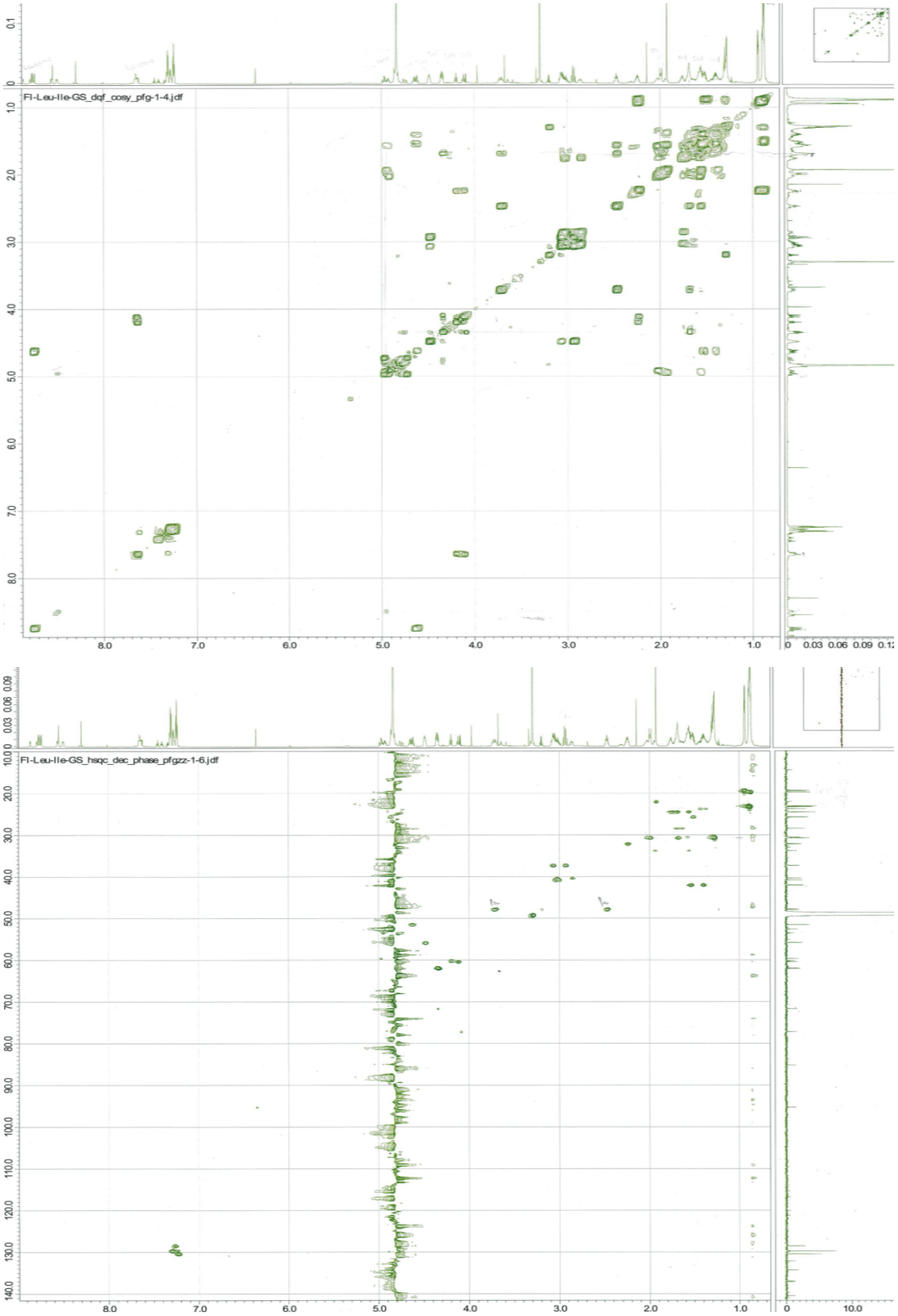




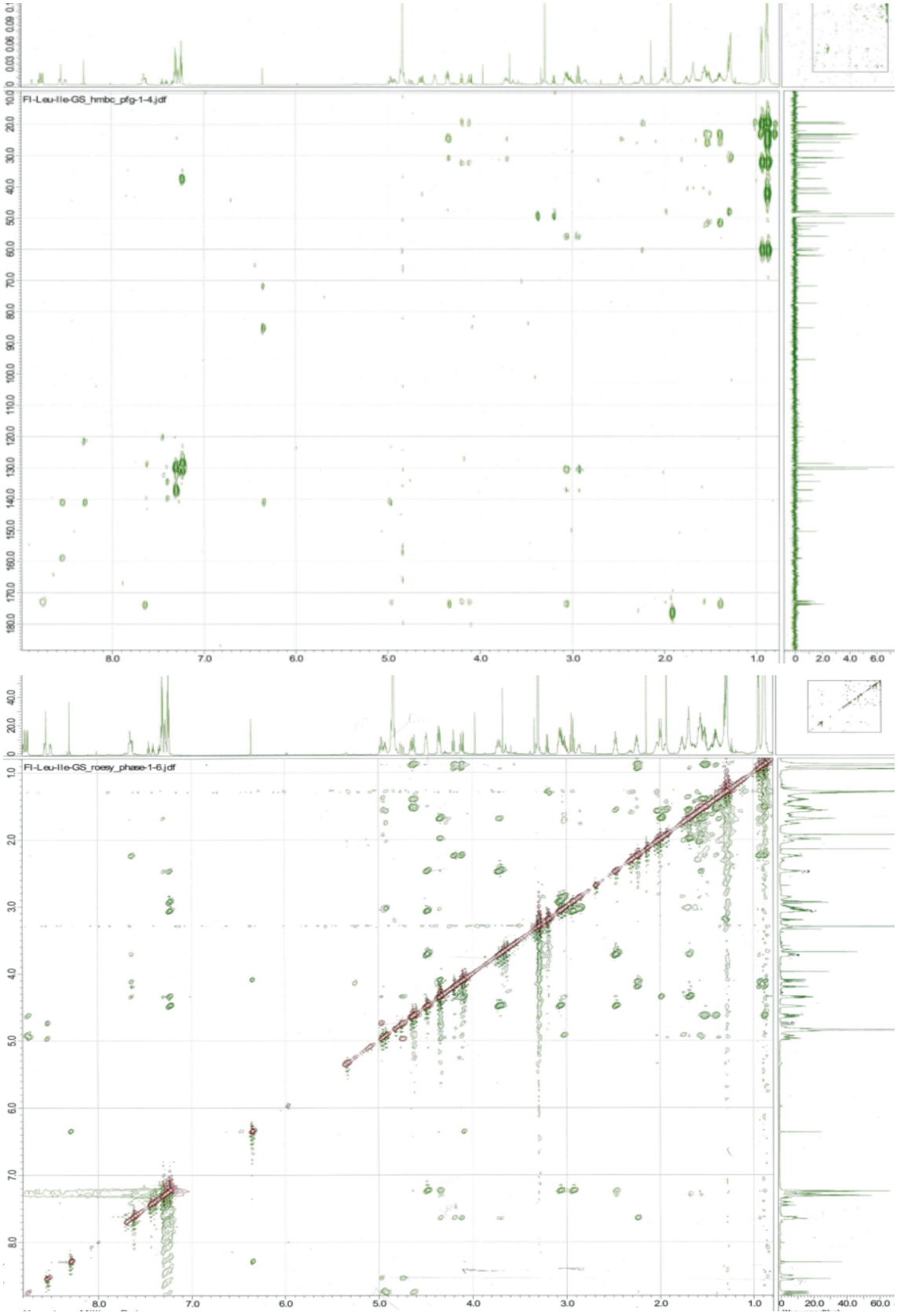
^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of 4-Lys-GS (**2**) in CD_3OD



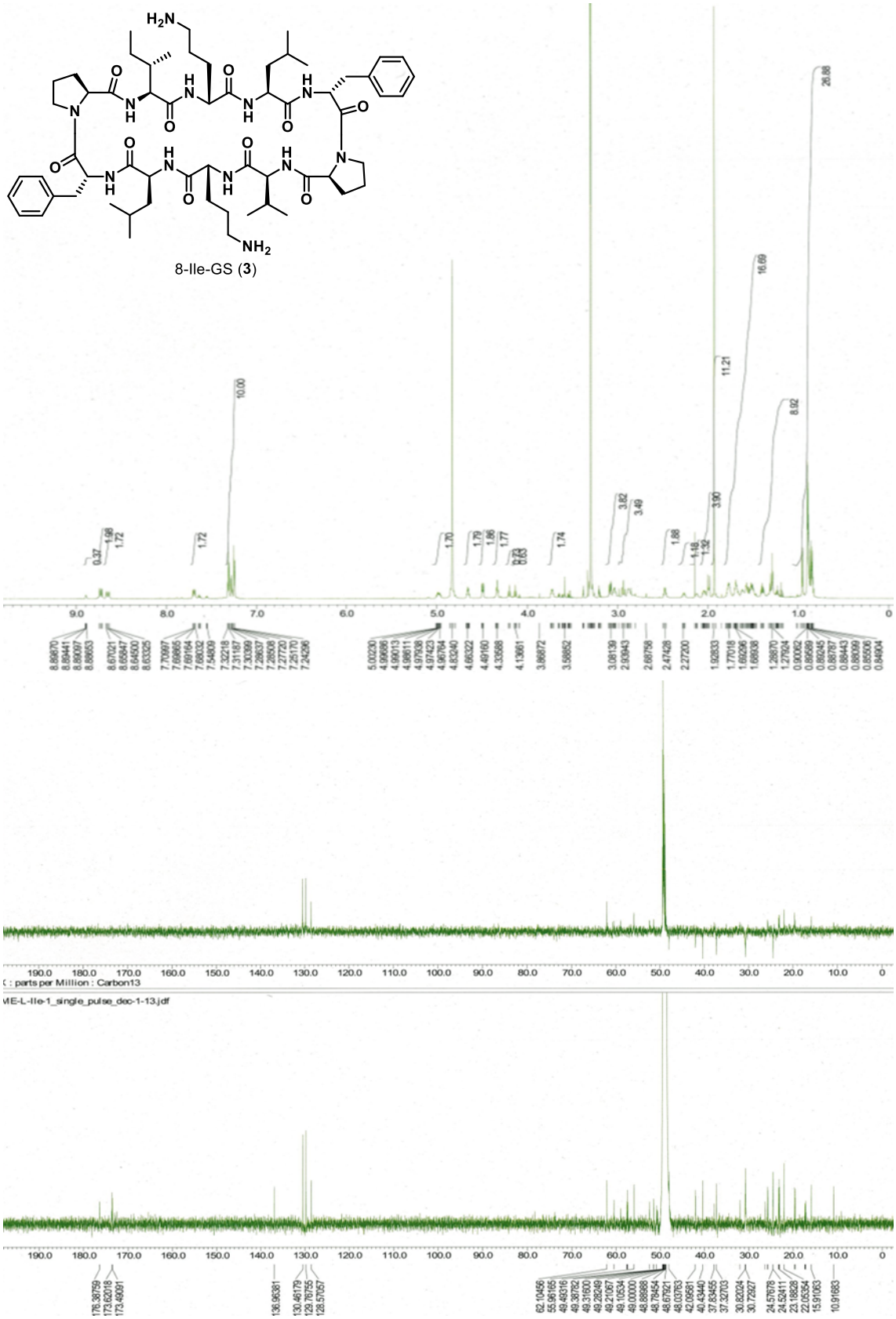
COSY and HSQC spectra (800 MHz) of 4-Lys-GS (**2**) in CD_3OD



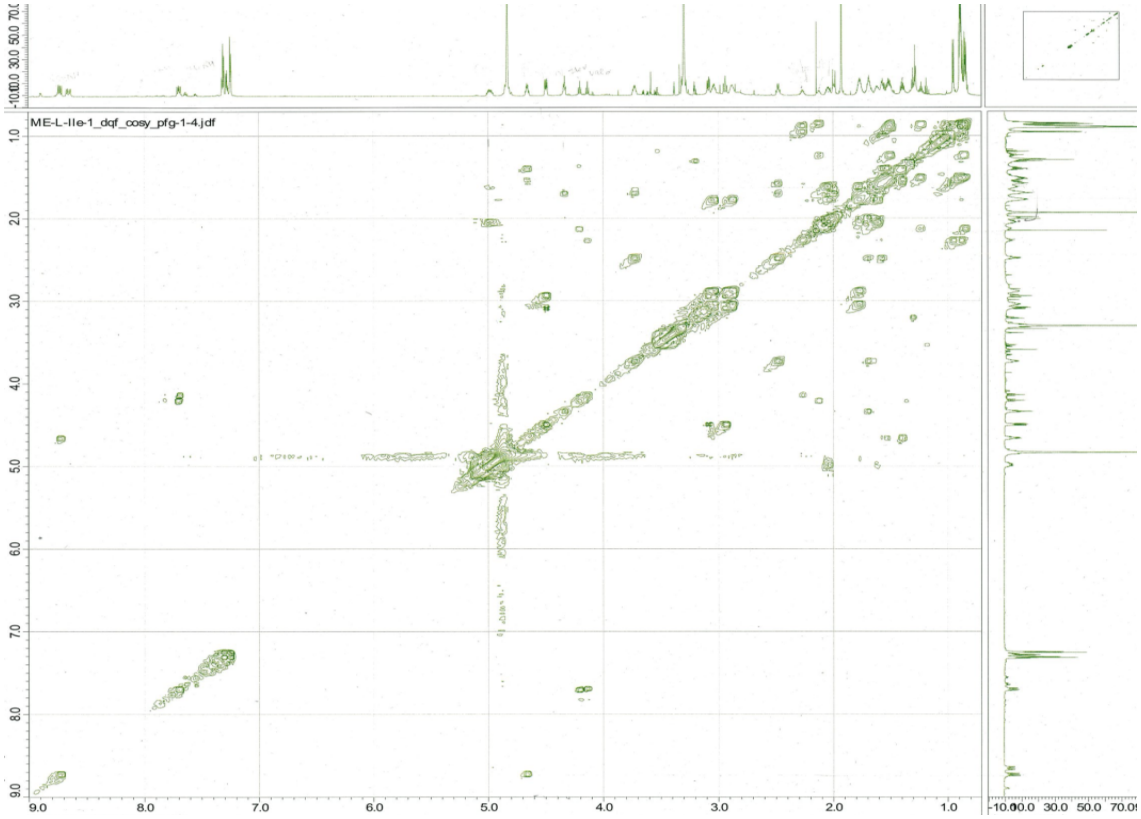
HMBC and ROESY spectra (800 MHz) of 4-Lys-GS (**2**) in CD_3OD

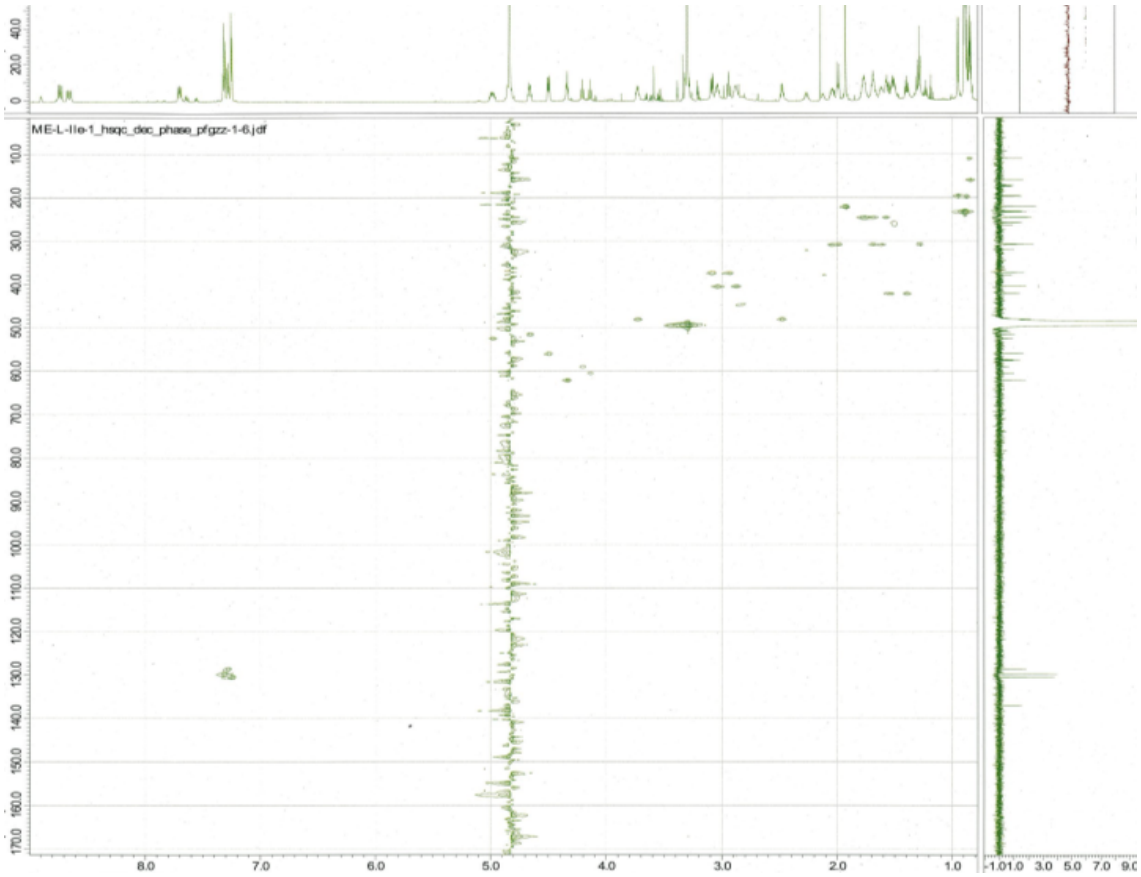


^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of 8-Ile-GS (**3**) in CD_3OD

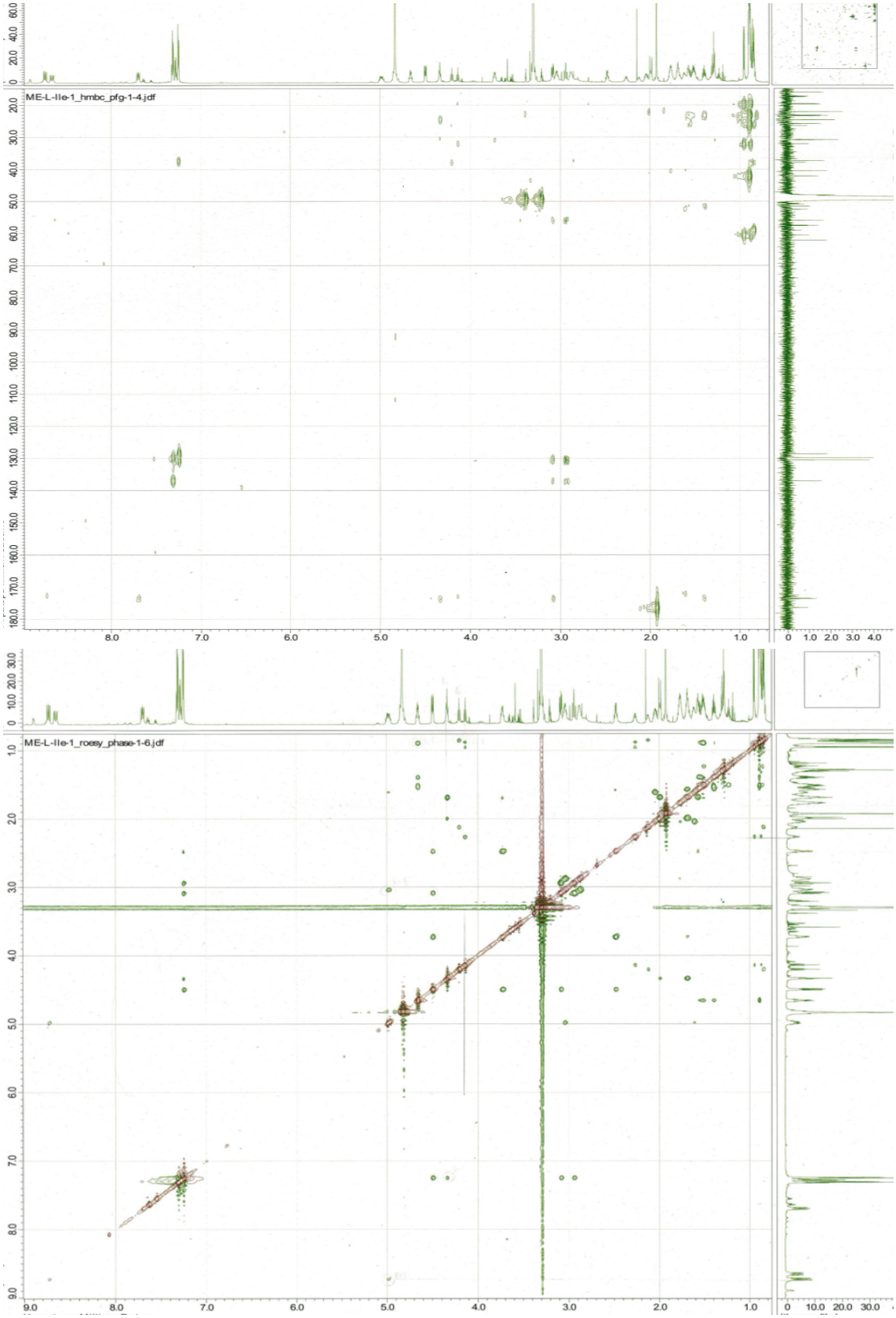


COSY and HSQC spectra (800 MHz) of 8-Ile-GS (**3**) in CD_3OD

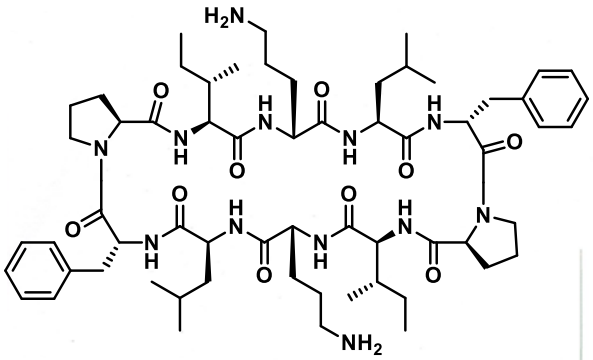




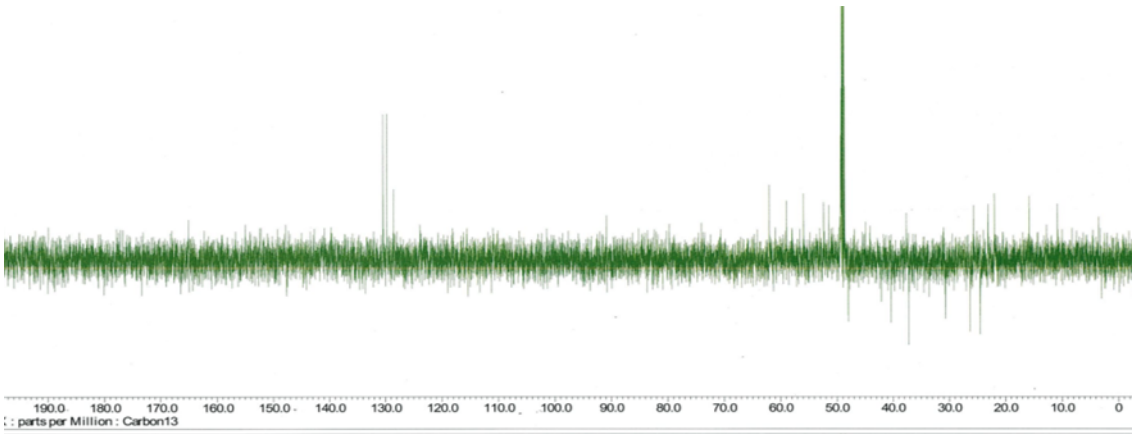
HMBC and ROESY spectrum (800 MHz) of 8-Ile-GS (**3**) in CD_3OD

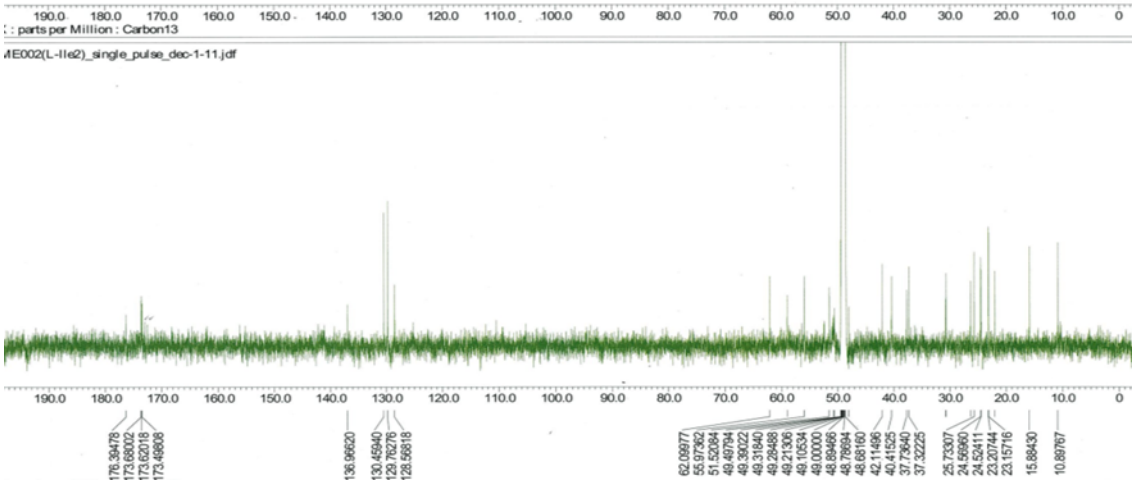


^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of 3,8-Ile-GS (**4**) in CD_3OD

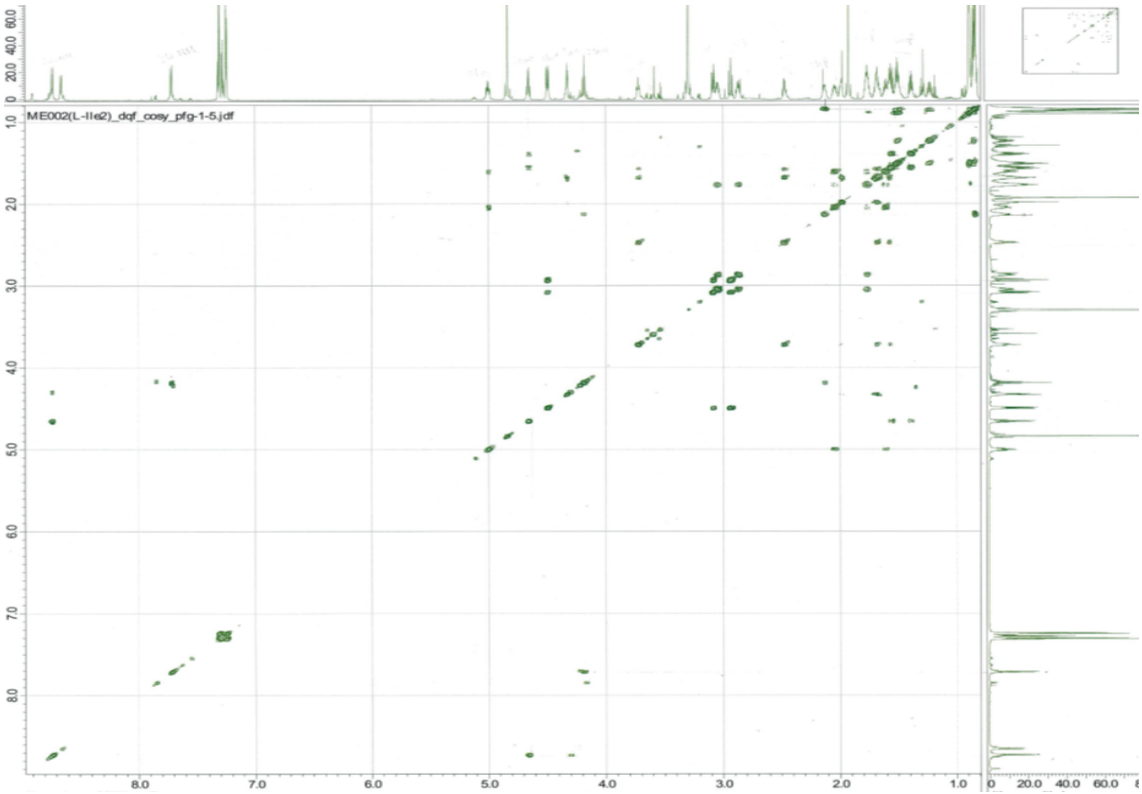


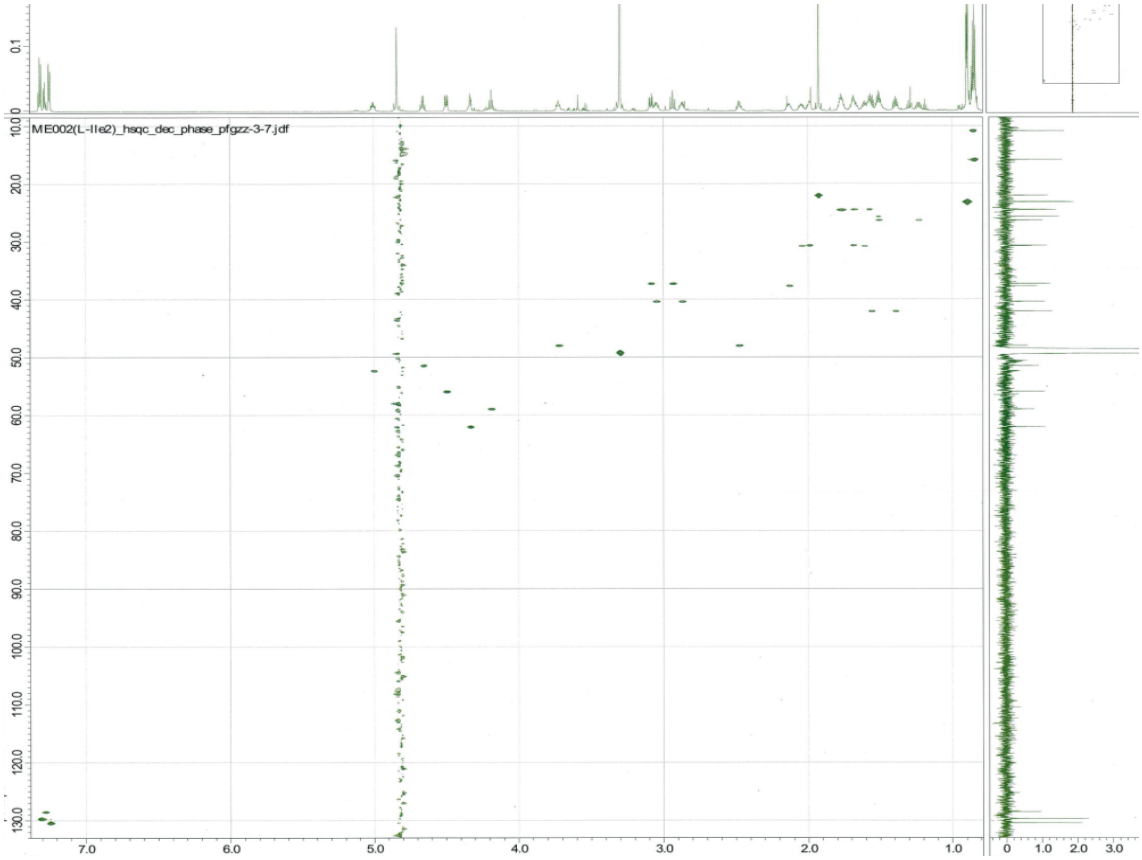
3,8-Ile-GS (**4**)





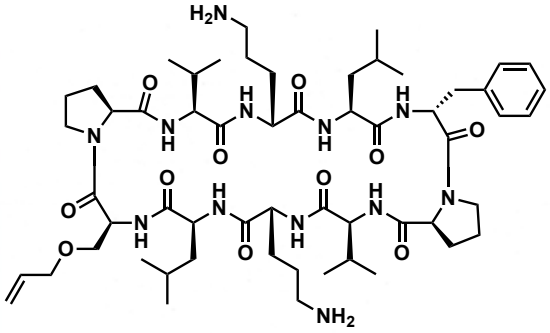
COSY and HSQC spectra (800 MHz) of 3,8-Ile-GS (**4**) in CD_3OD



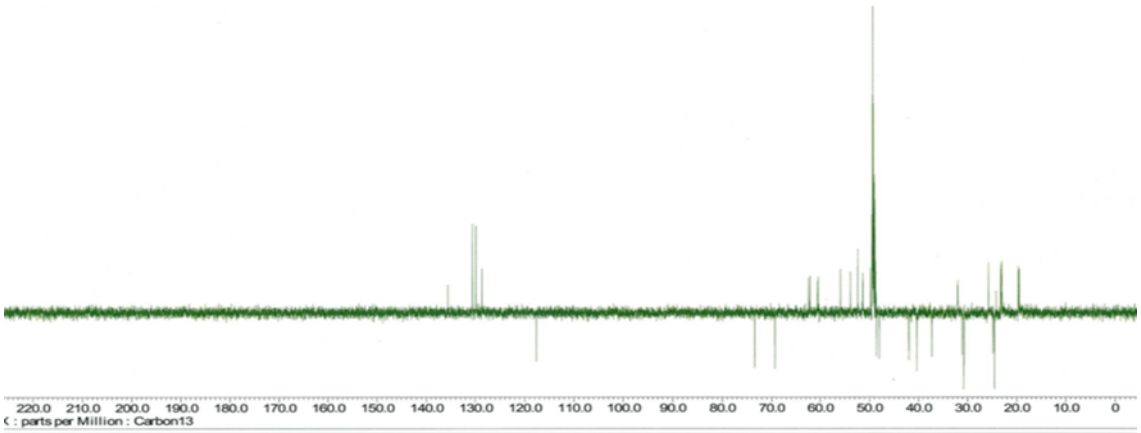


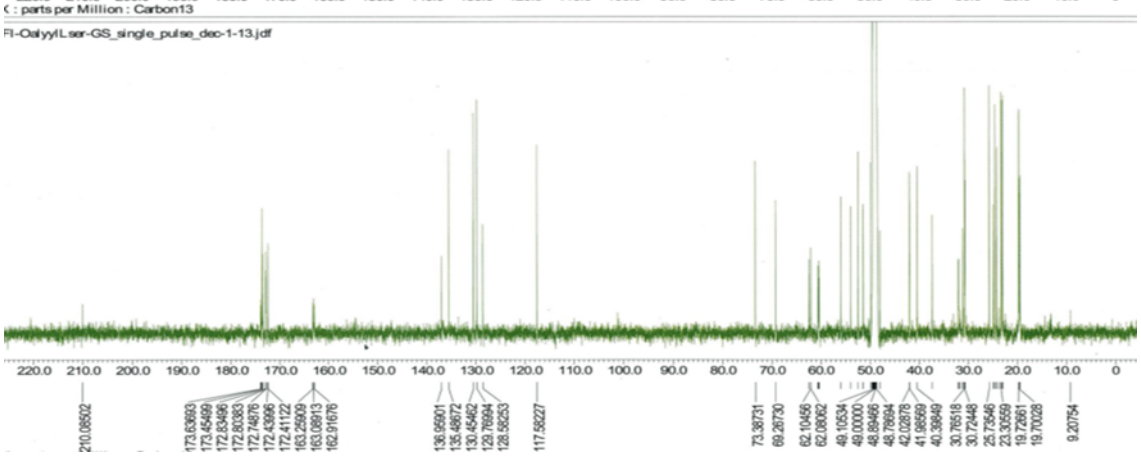
HMBC and ROESYspectra (800 MHz) of 3,8-Ile-GS (**4**) in CD_3OD

^{1}H-NMR (800 MHz) and ^{13}C-NMR (200 MHz) spectra of 6-Ser(Allyl)-GS (**5**) in CD_3OD

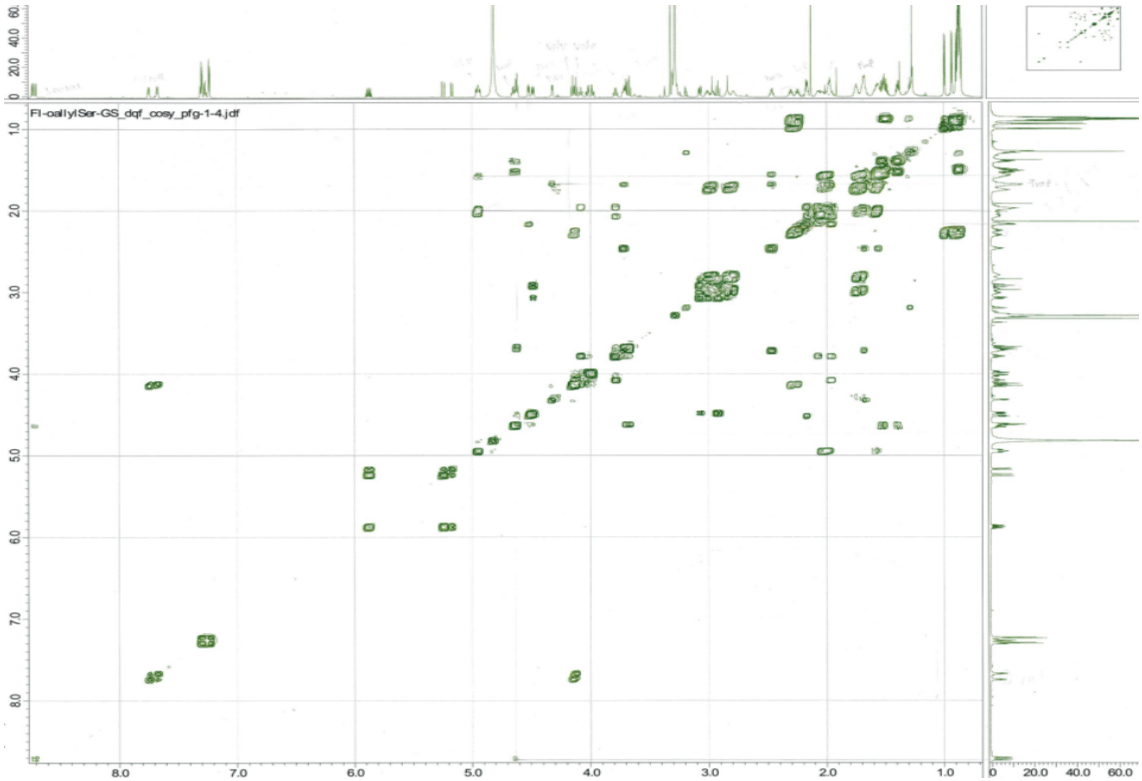


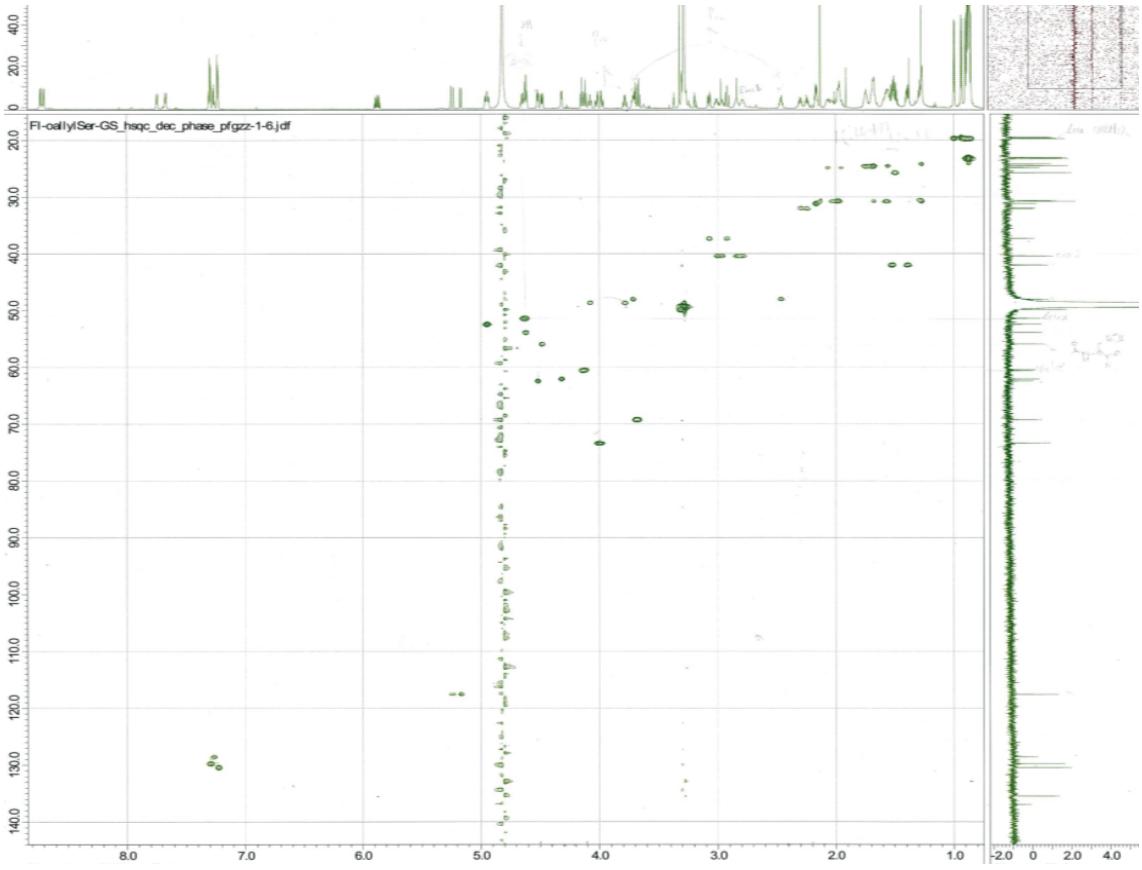
6-Ser(Allyl)-GS (**5**)





COSY and HSQC spectra (800 MHz) of 6-Ser(Allyl)-GS (**5**) in CD_3OD





HMBC and ROESY spectra (800 MHz) of 6-Ser(Allyl)-GS (**5**) in CD_3OD