

Supporting Information

Natalenamides A-C, Cyclic Tripeptides from the Termite-associated *Actinomadura* sp. RB99

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Figure S1. HR-ESIMS data of **1**

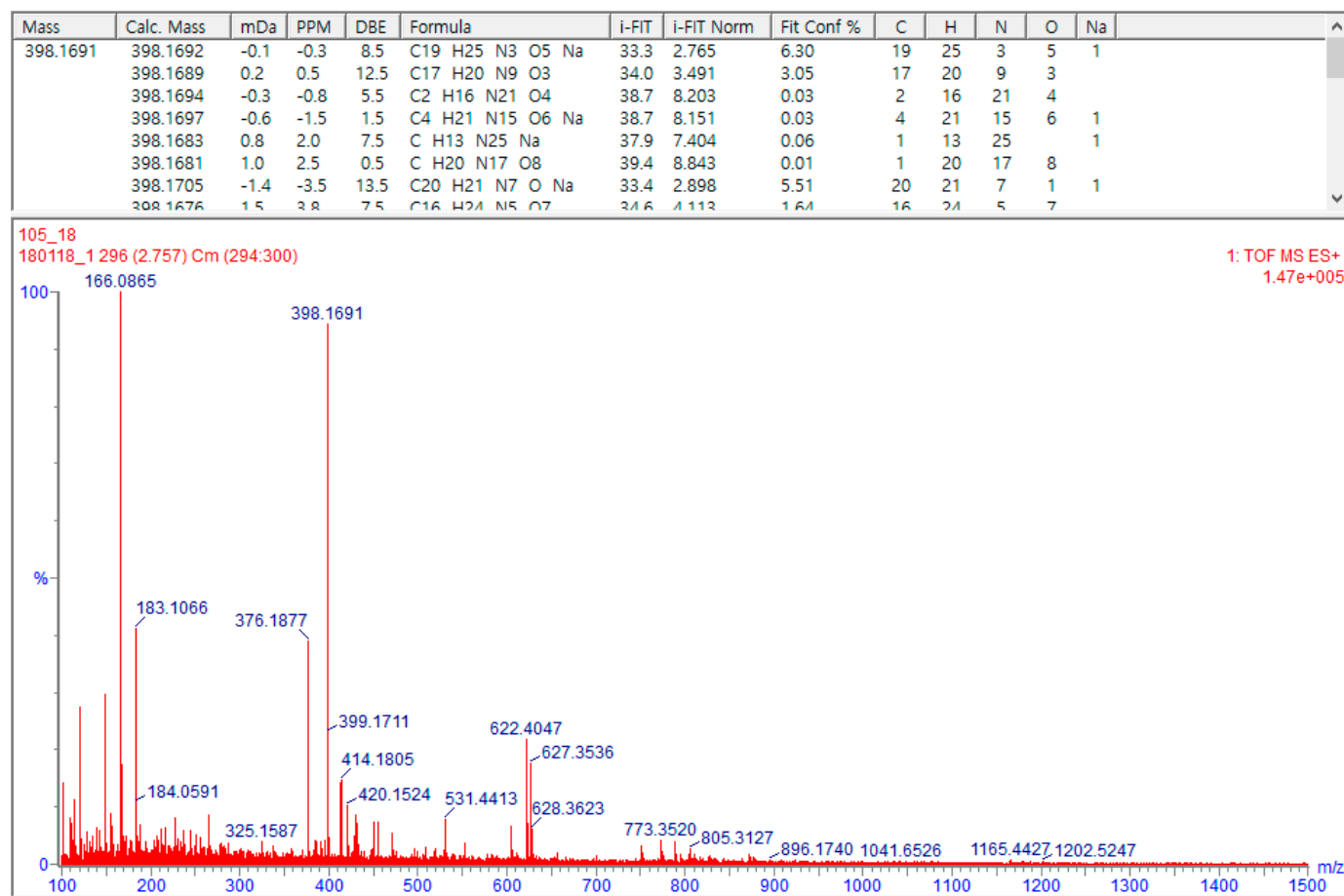


Figure S2. ^1H NMR spectrum of **1** (CD_3OD , 800 MHz)

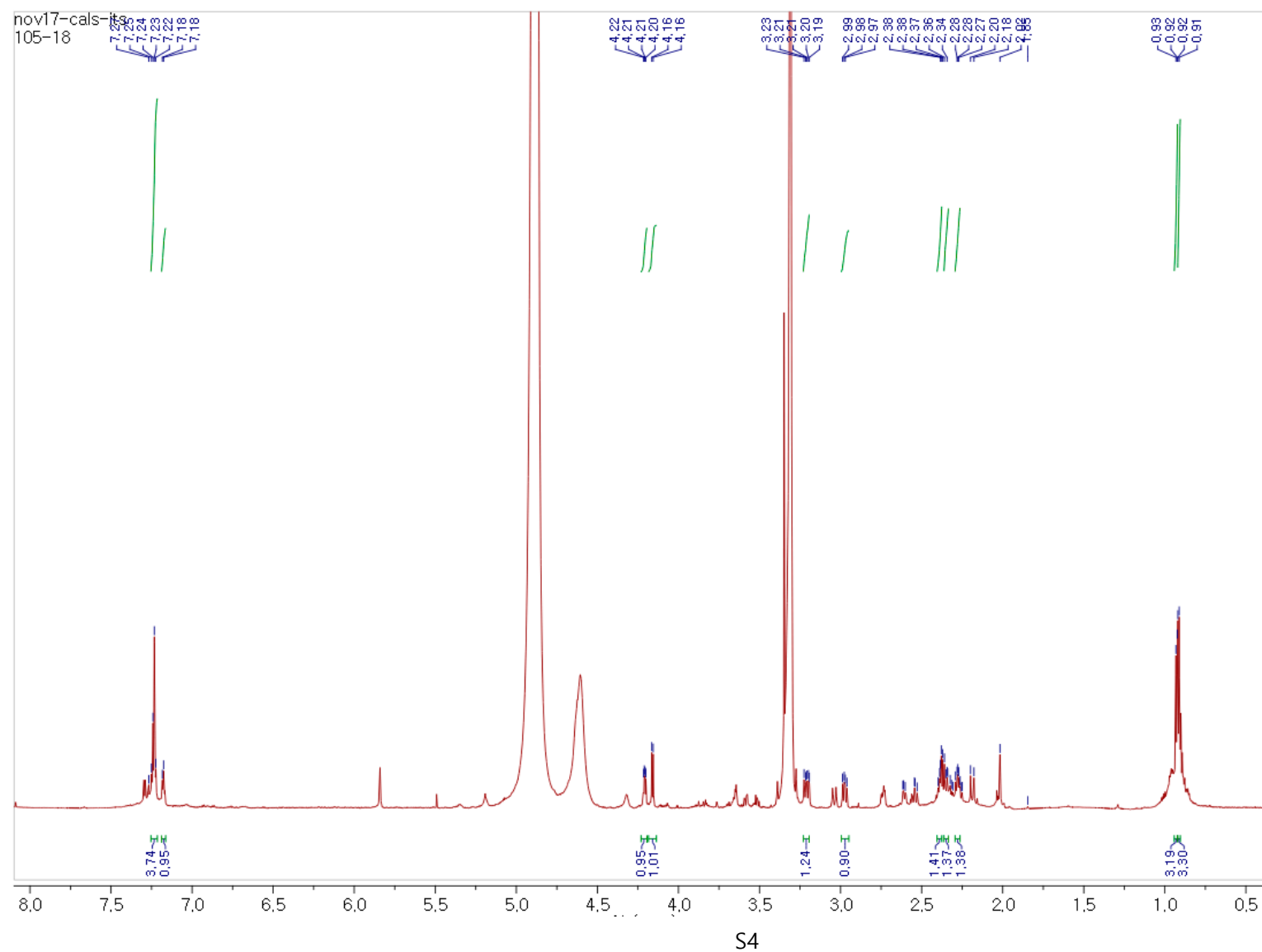


Figure S3. ^1H - ^1H COSY spectrum of **1** (CD_3OD)

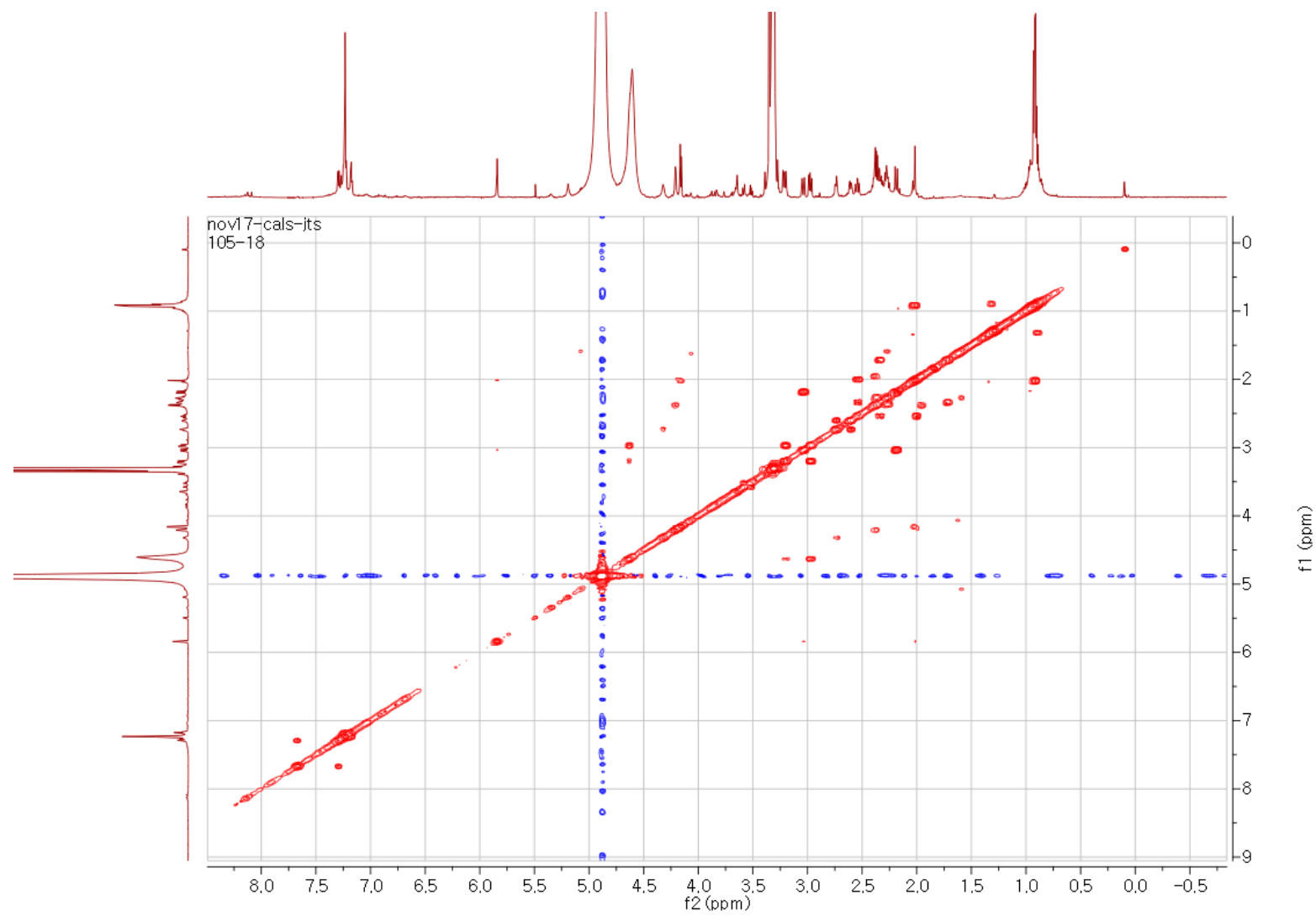


Figure S4. HSQC spectrum of **1** (CD₃OD)

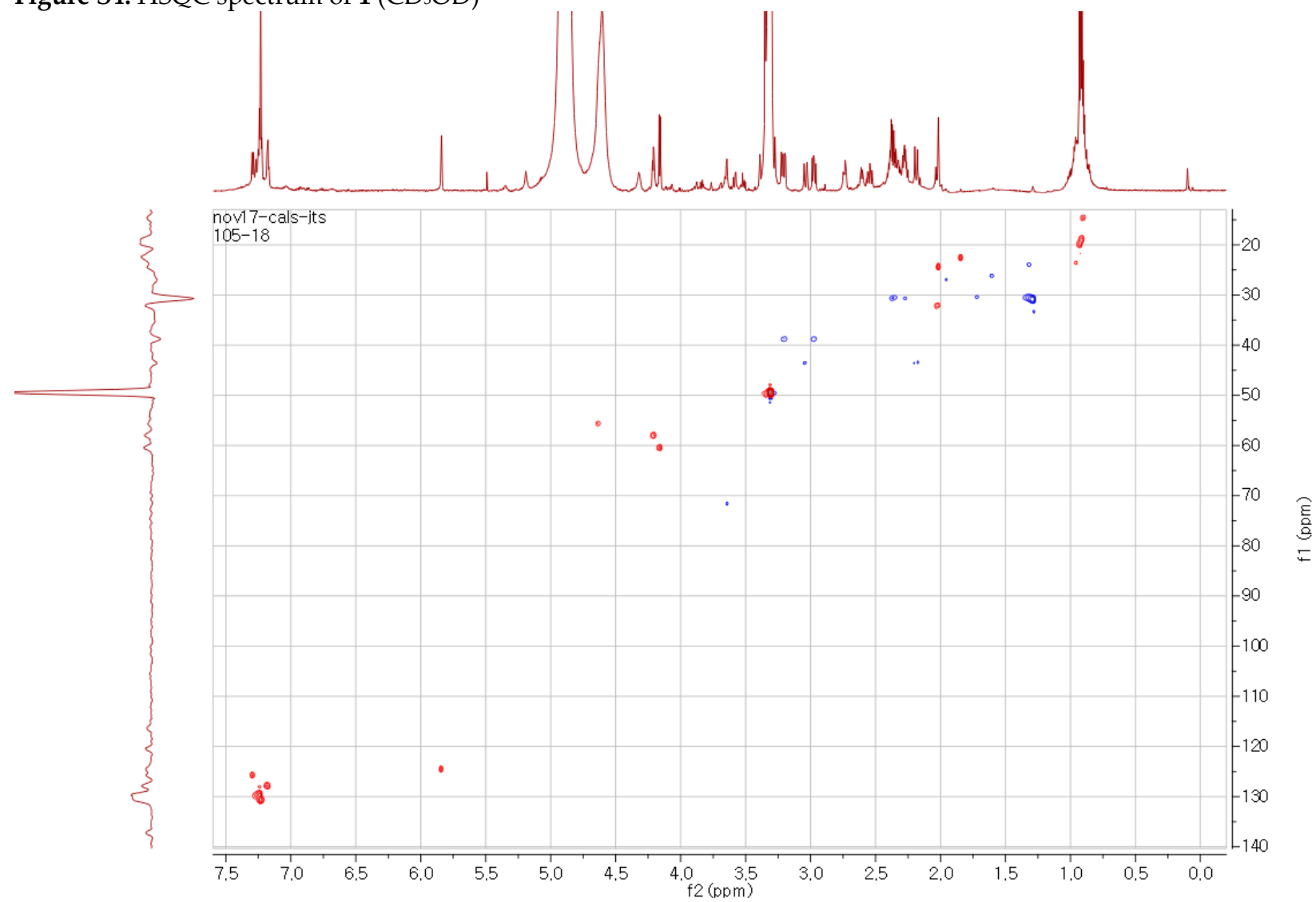


Figure S5. HMBC spectrum of **1** (CD₃OD)

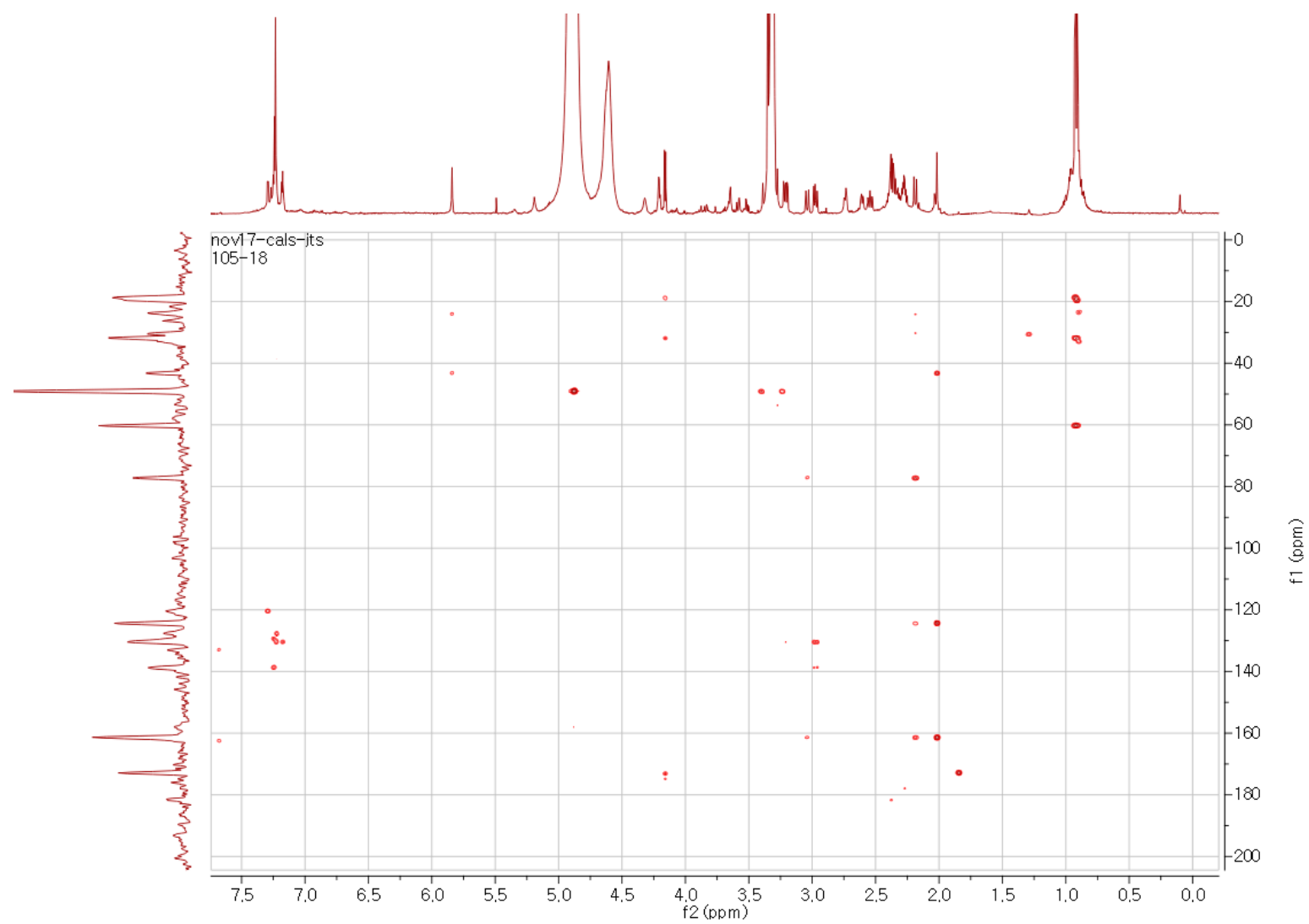


Figure S6. HR-ESIMS data of **2**

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na
390.2032	390.2029	0.3	0.8	8.5	C20 H28 N3 O5	558.7	0.596	55.10	20	28	3	5	
	390.2005	2.7	6.9	5.5	C18 H29 N3 O5 Na	559.3	1.152	31.61	18	29	3	5	1
	390.2018	1.4	3.6	10.5	C19 H25 N7 O Na	561.2	3.110	4.46	19	25	7	1	1
	390.2045	-1.3	-3.3	9.5	C23 H29 N O3 Na	561.4	3.255	3.86	23	29	1	3	1
	390.2042	-1.0	-2.6	13.5	C21 H24 N7 O	562.0	3.881	2.06	21	24	7	1	
	390.2002	3.0	7.7	9.5	C16 H24 N9 O3	562.1	4.025	1.79	16	24	9	3	
	390.1989	4.3	11.0	4.5	C15 H28 N5 O7	563.0	4.911	0.74	15	28	5	7	
	390.2060	-2.7	-0.5	12.5	C25 H28 N O3	563.0	5.781	0.31	25	28	1	3	

105_20

180118_2 344 (3.205)

1: TOF MS ES+
5.72e+005

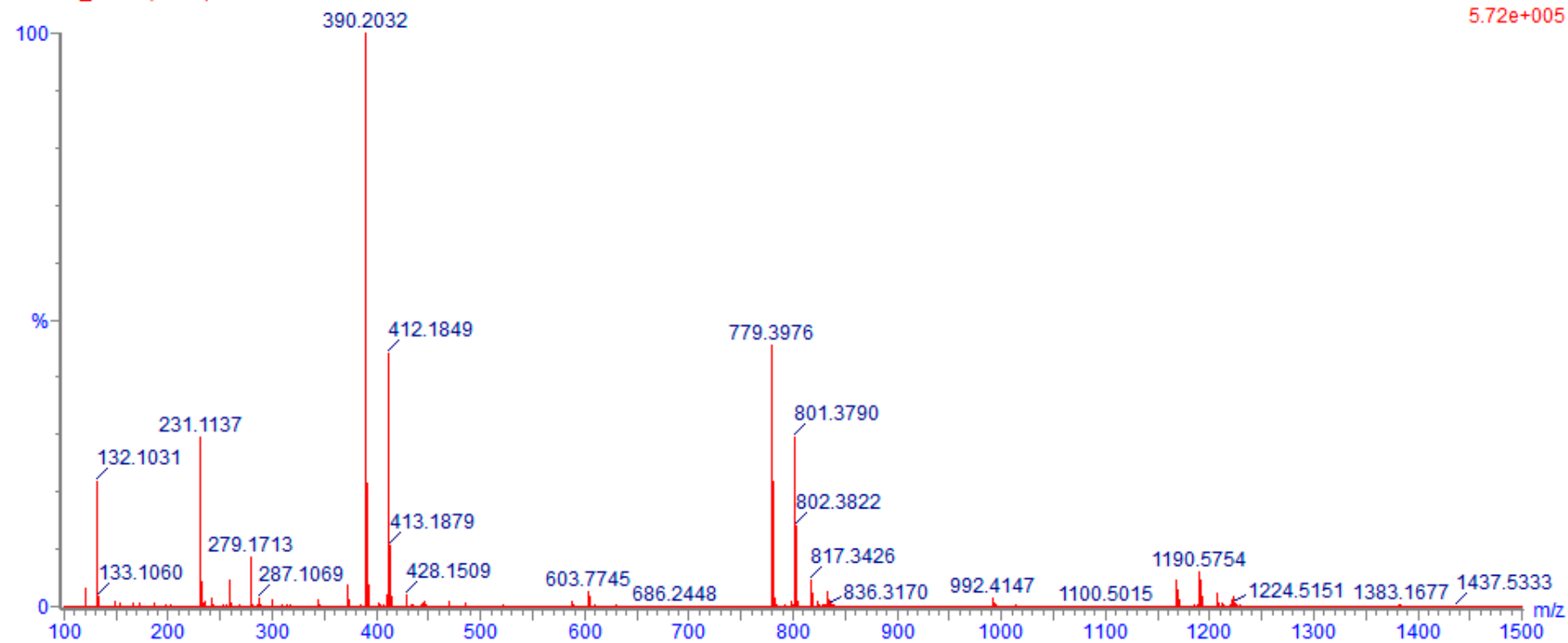


Figure S7. ^1H NMR spectrum of **2** (CD_3OD , 800 MHz)

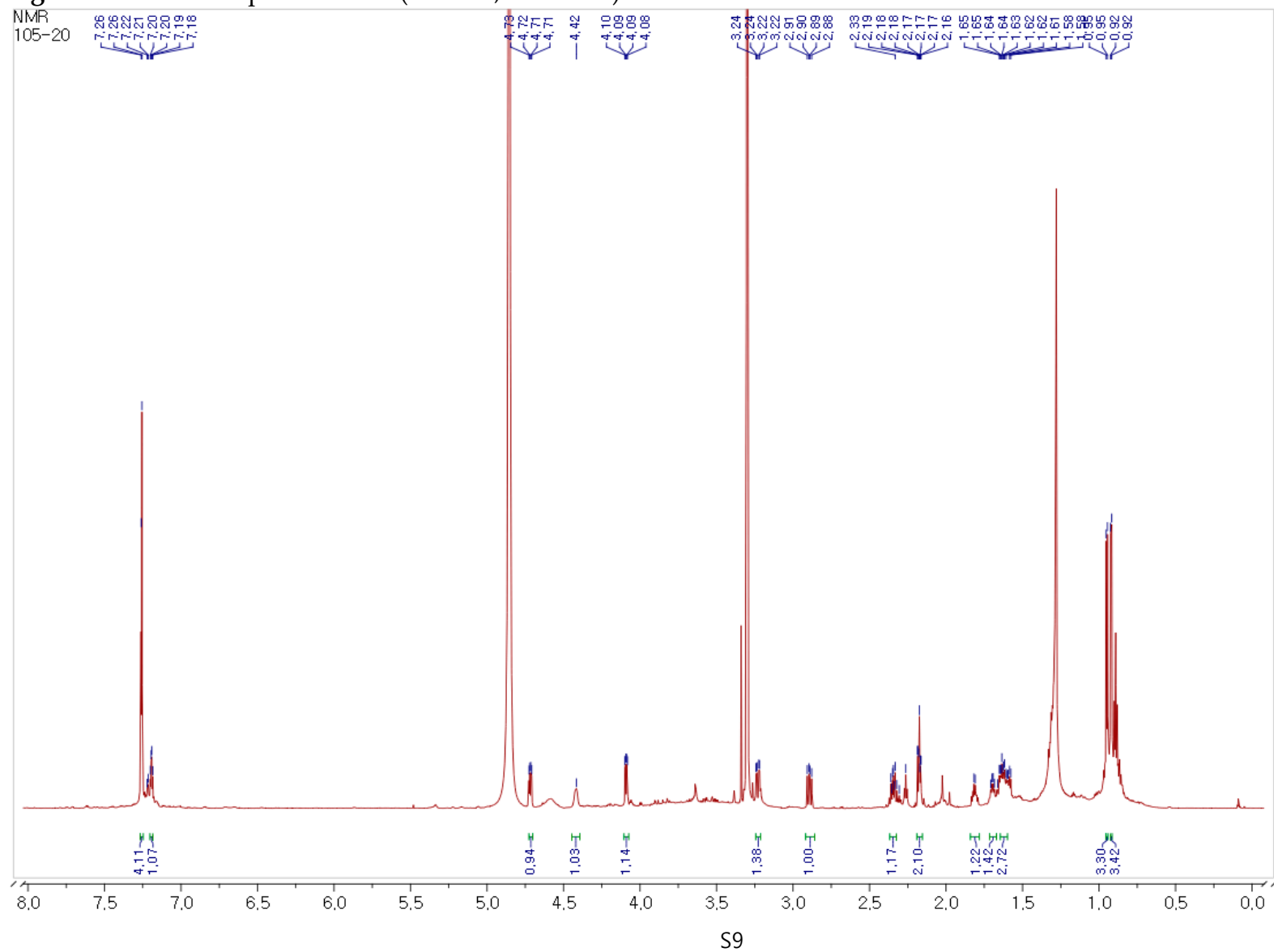


Figure S8. ^1H - ^1H COSY spectrum of **2** (CD_3OD)

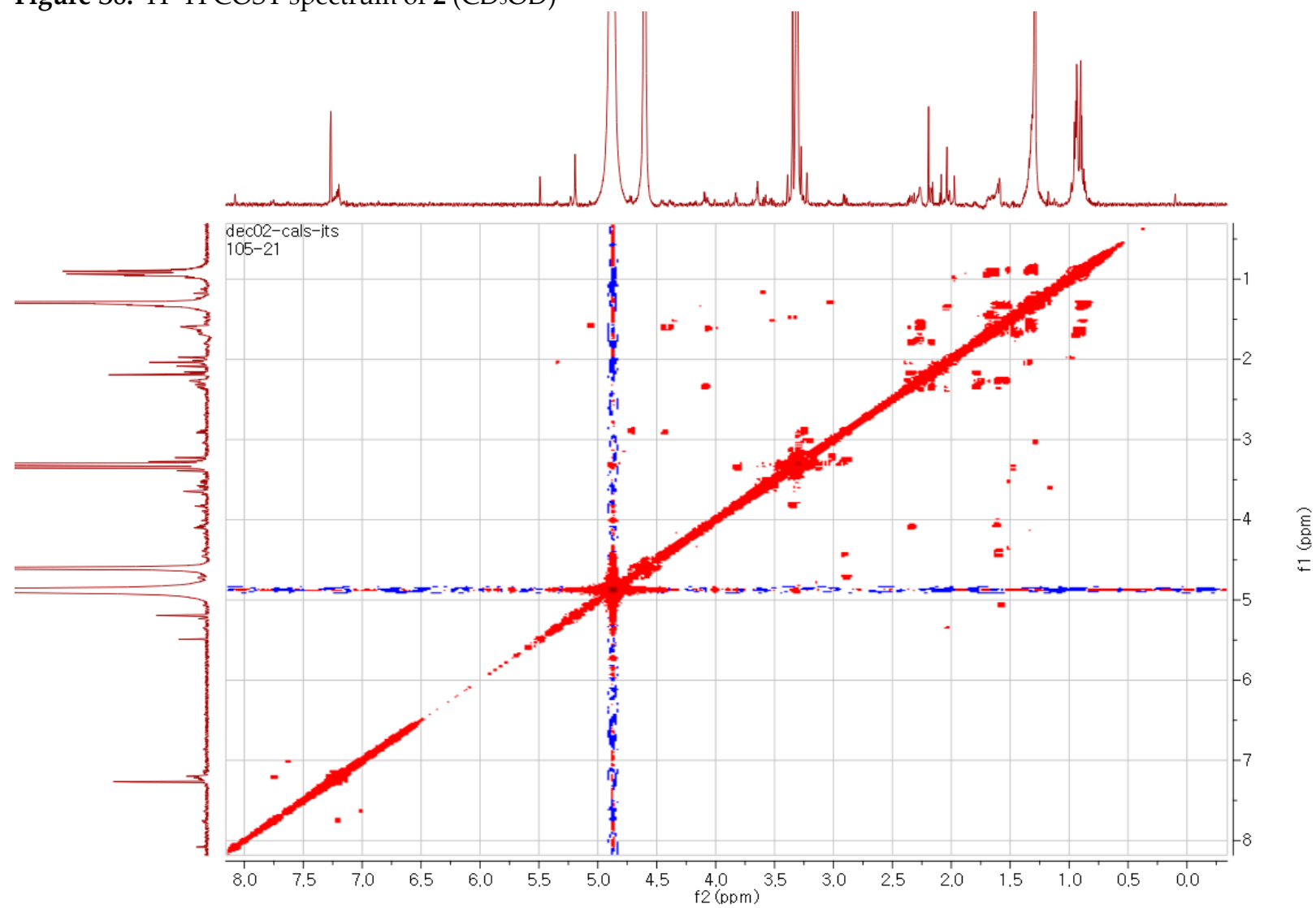


Figure S9. HSQC spectrum of **2** (CD₃OD)

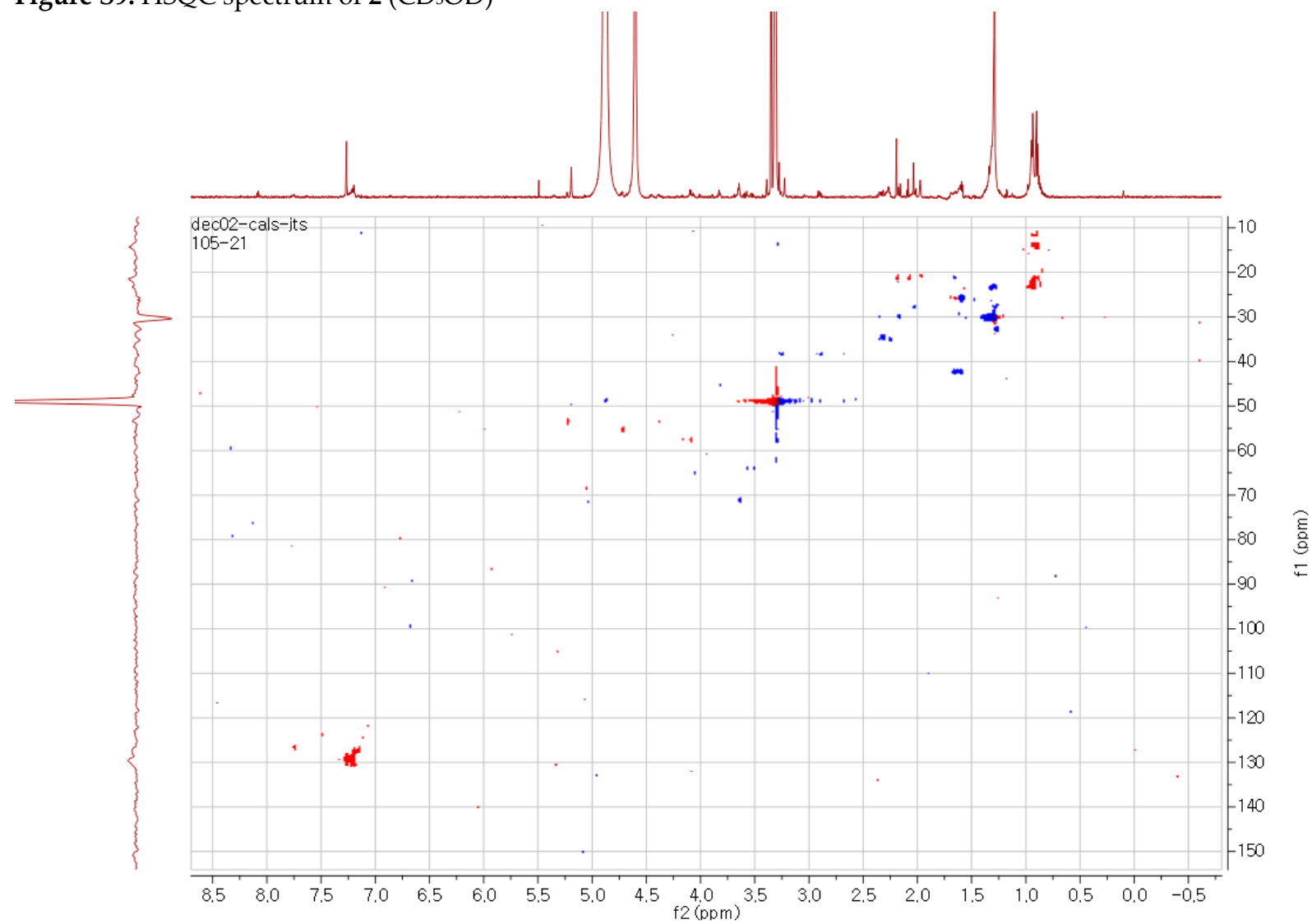


Figure S10. HMBC spectrum of **2** (CD₃OD)

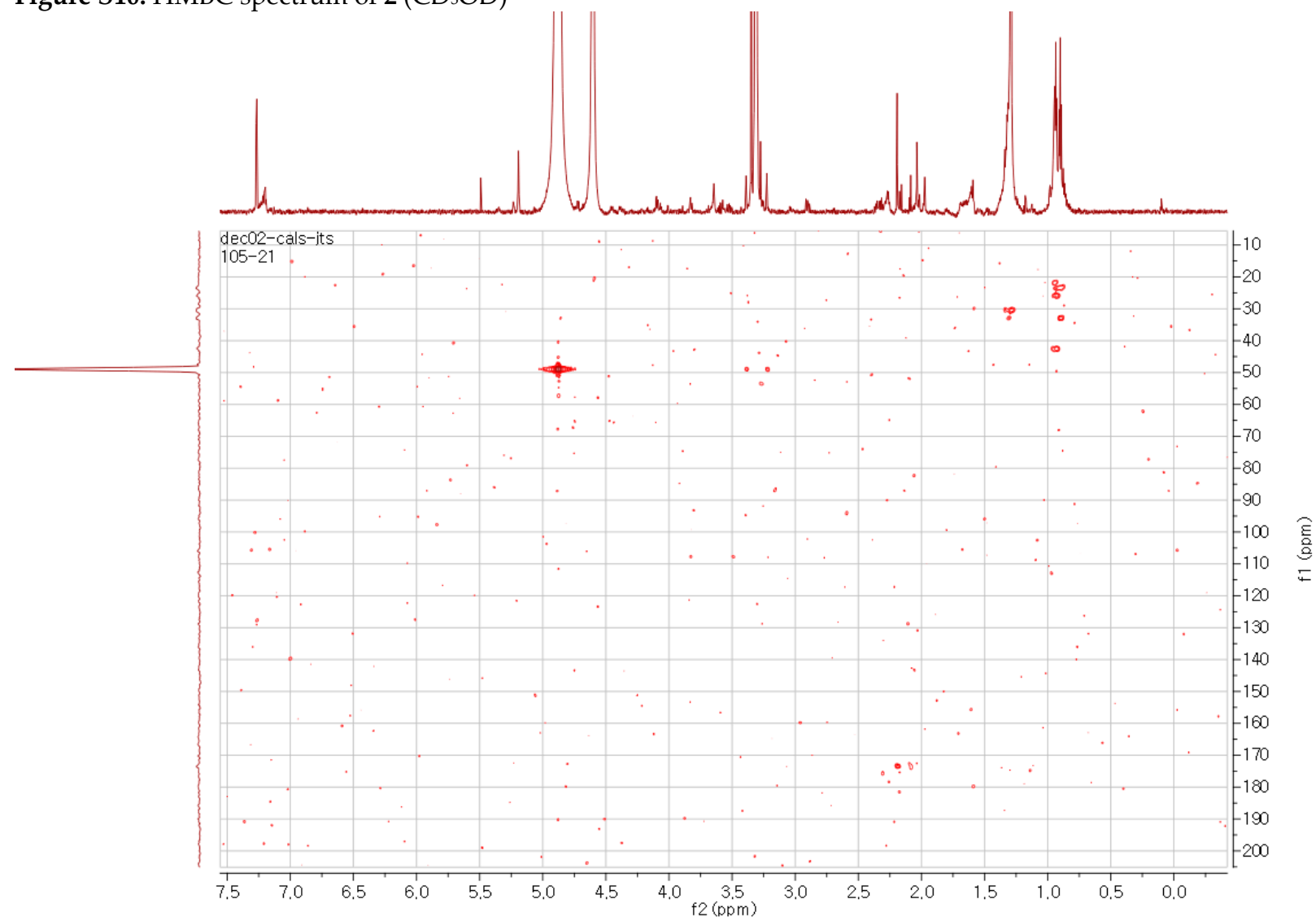


Figure S11. HR-ESIMS data of **3**

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na	
446.1694	446.1705	-1.1	-2.5	17.5	C24 H21 N7 O Na	223.7	0.369	69.12	24	21	7	1	1	
	446.1689	0.5	1.1	16.5	C21 H20 N9 O3	225.2	1.846	15.79	21	20	9	3		
	446.1692	0.2	0.4	12.5	C23 H25 N3 O5 Na	225.4	2.086	12.42	23	25	3	5	1	
	446.1716	-2.2	-4.9	15.5	C25 H24 N3 O5	227.6	4.269	1.40	25	24	3	5		
	446.1676	1.8	4.0	11.5	C20 H24 N5 O7	228.5	5.150	0.58	20	24	5	7		
	446.1729	-3.5	-7.8	20.5	C26 H20 N7 O	229.1	5.704	0.33	26	20	7	1		
	446.1665	2.9	6.5	13.5	C19 H21 N9 O3 Na	229.5	6.162	0.21	19	21	9	3	1	
	446.1732	-3.8	-8.5	16.5	C28 H25 N3 O3 Na	230.7	7.350	0.06	28	25	1	3	1	

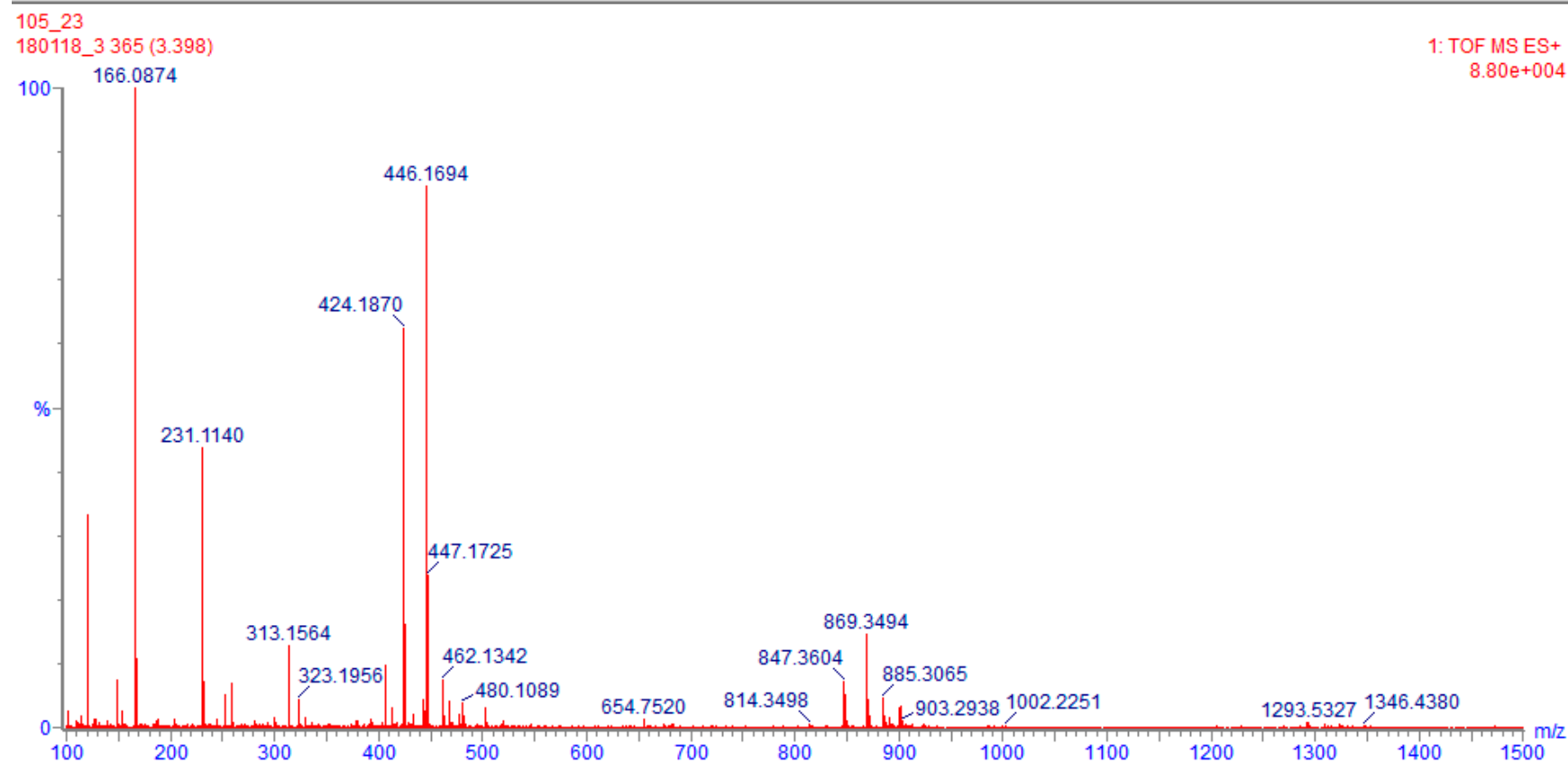


Figure S12. ^1H NMR spectrum of **3** (CD_3OD , 800 MHz)

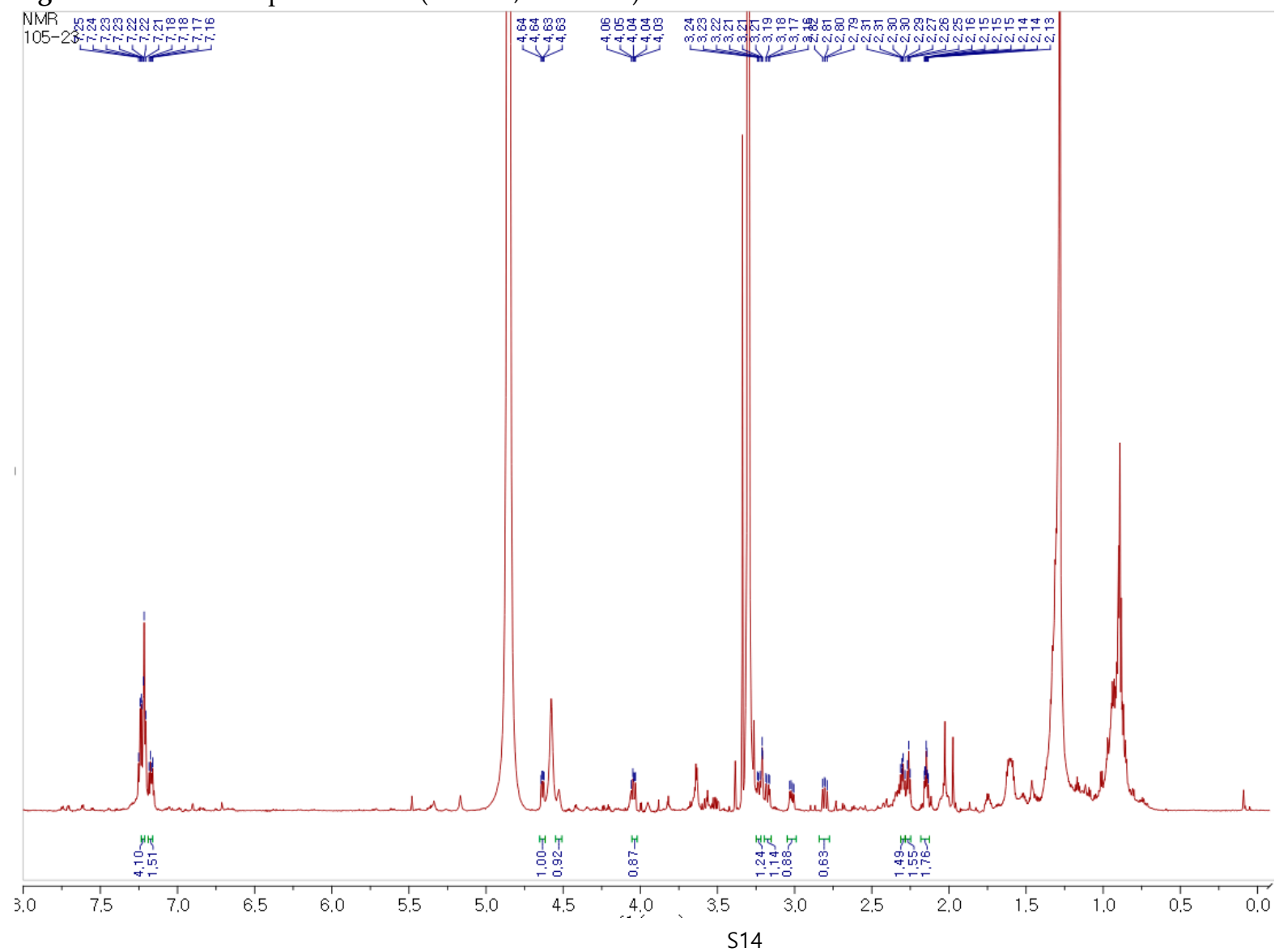


Figure S13. ^1H - ^1H COSY spectrum of **3** (CD_3OD)

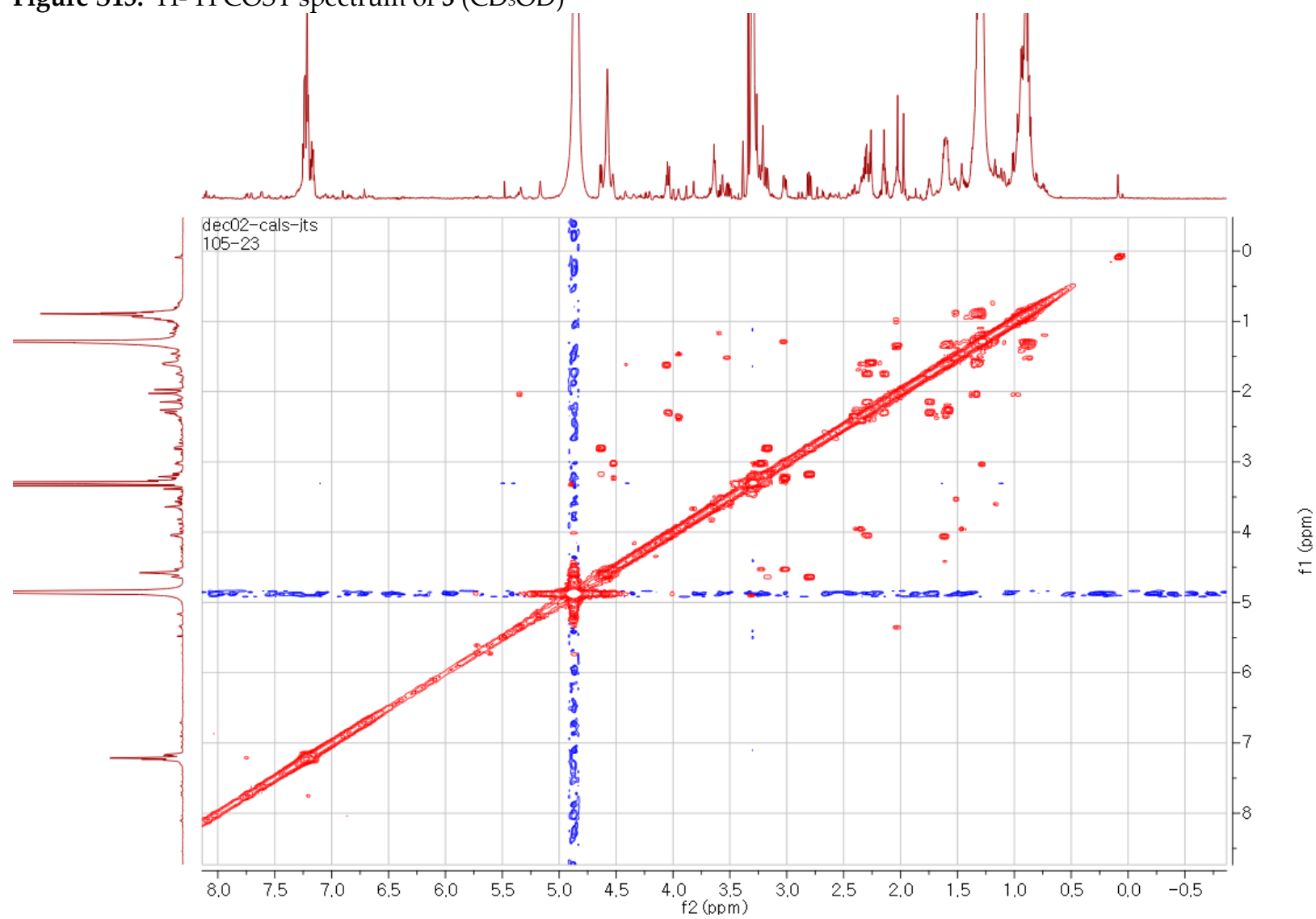


Figure S14. HSQC spectrum of **3** (CD₃OD)

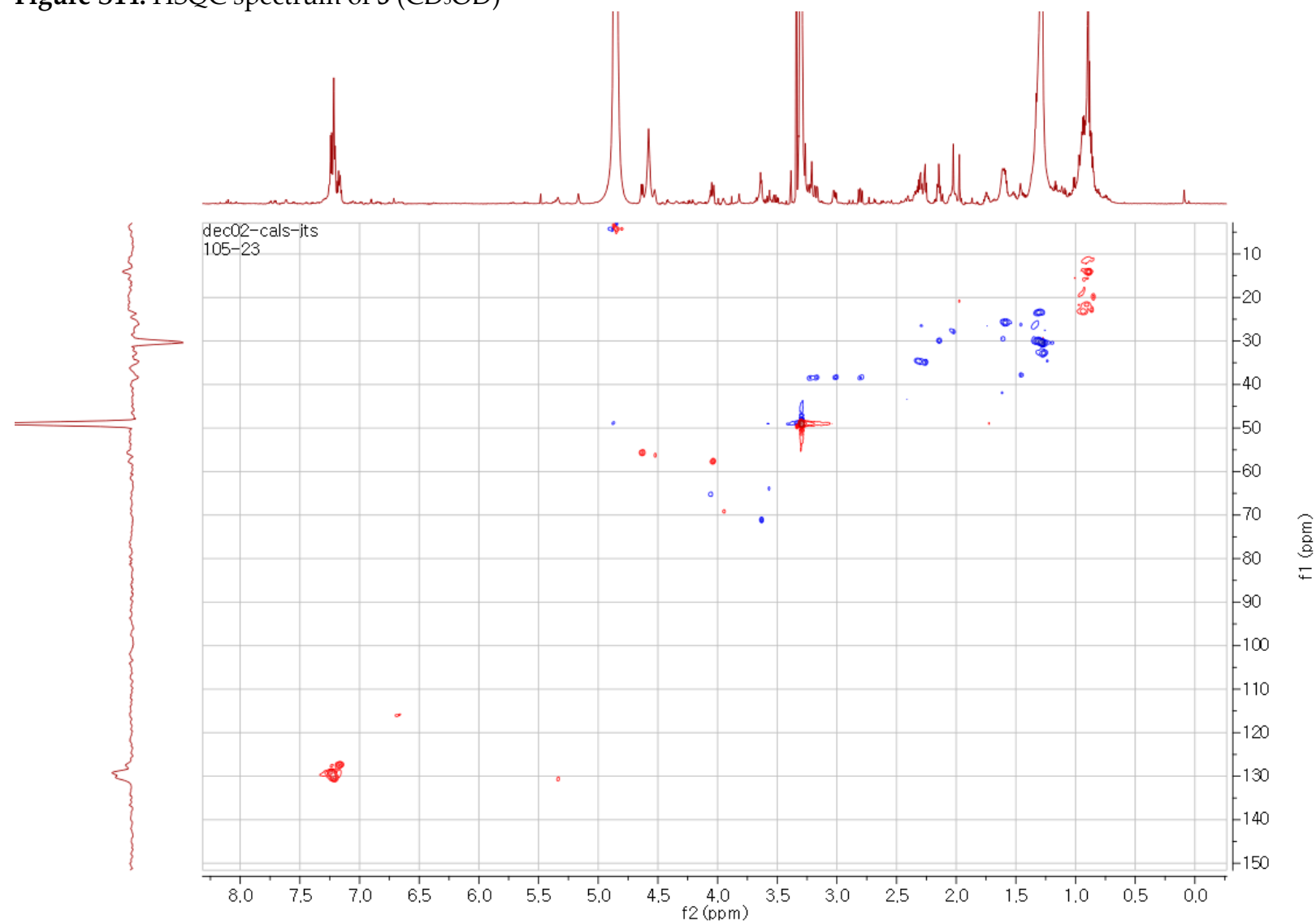


Figure S15. HMBC spectrum of **3** (CD₃OD)

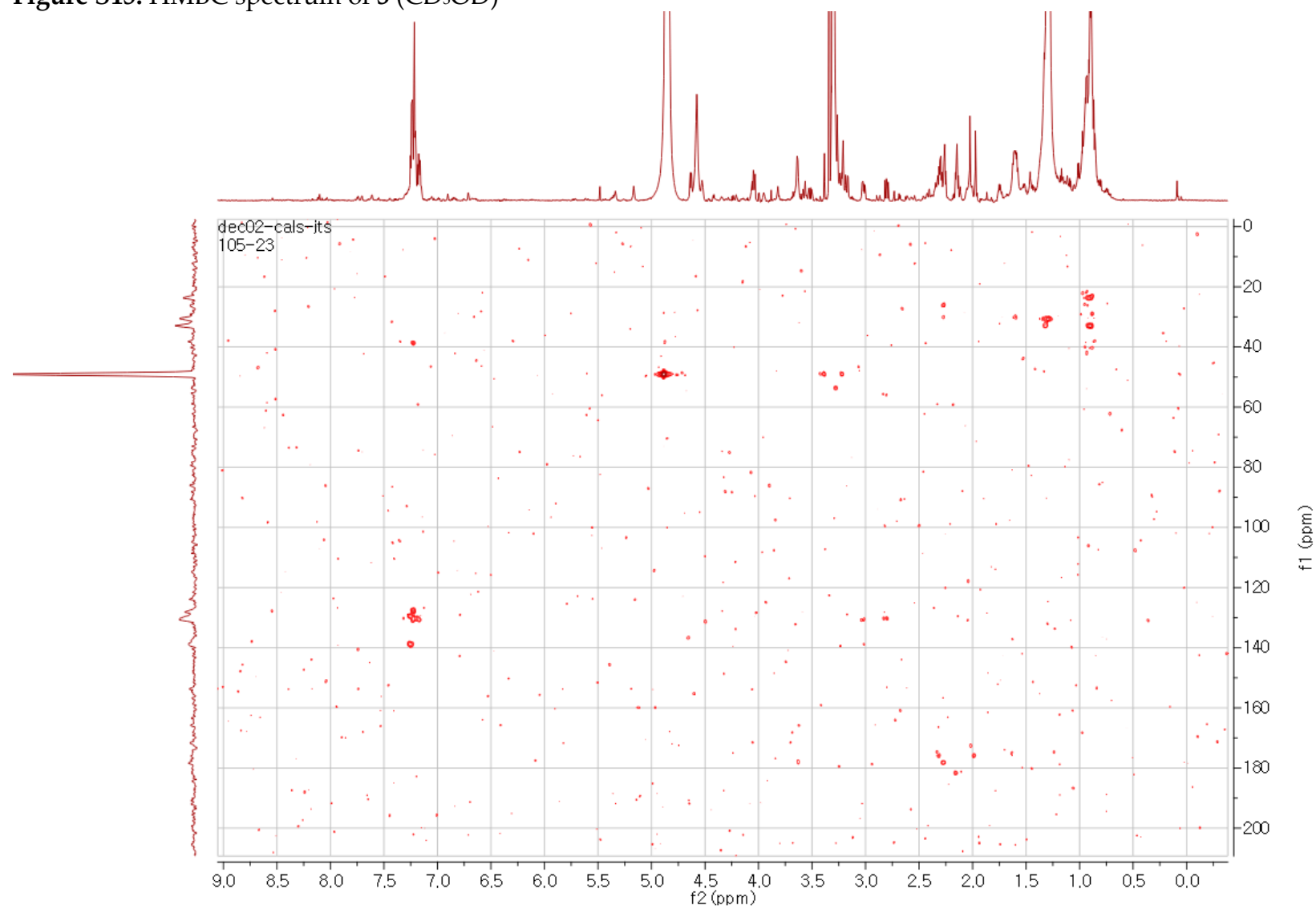
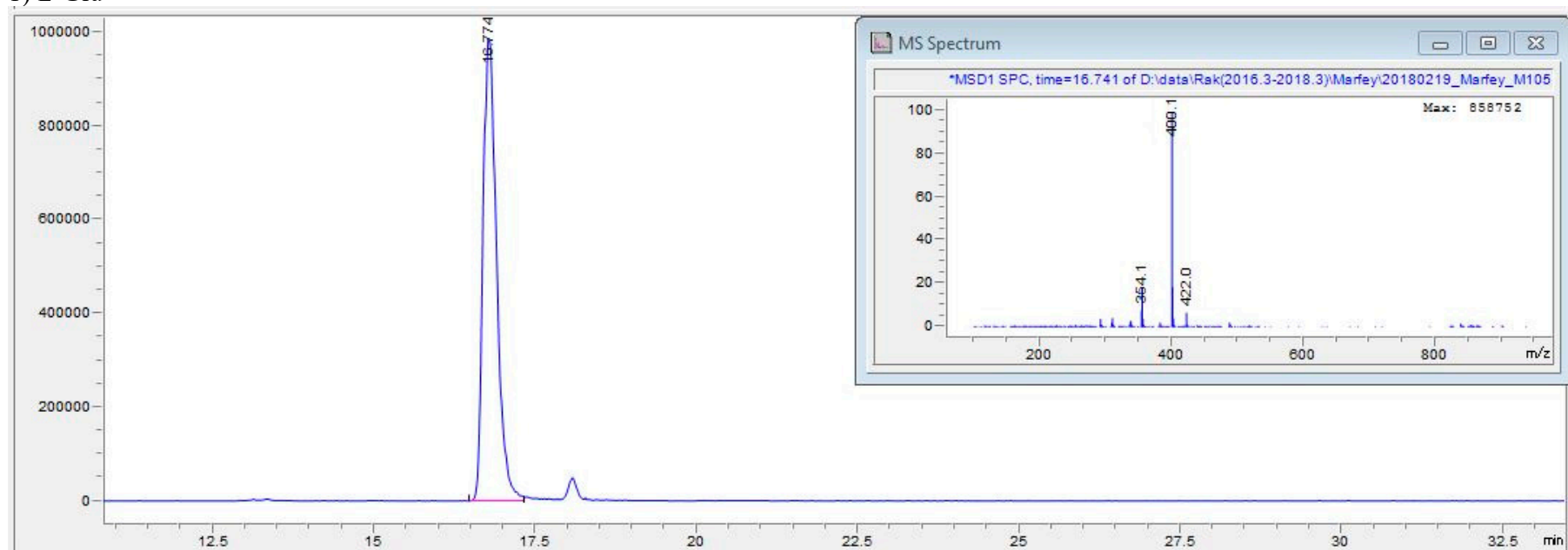
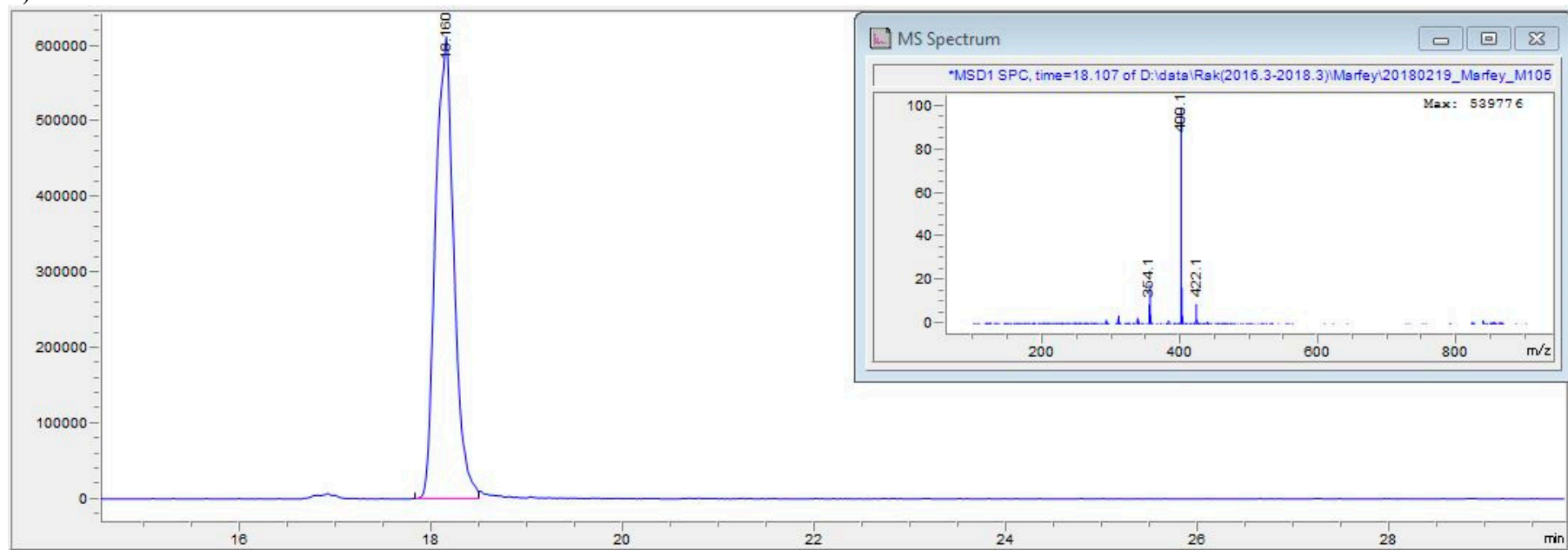


Figure S16. Retention times of the L-FDAA derivatized amino acids of standards

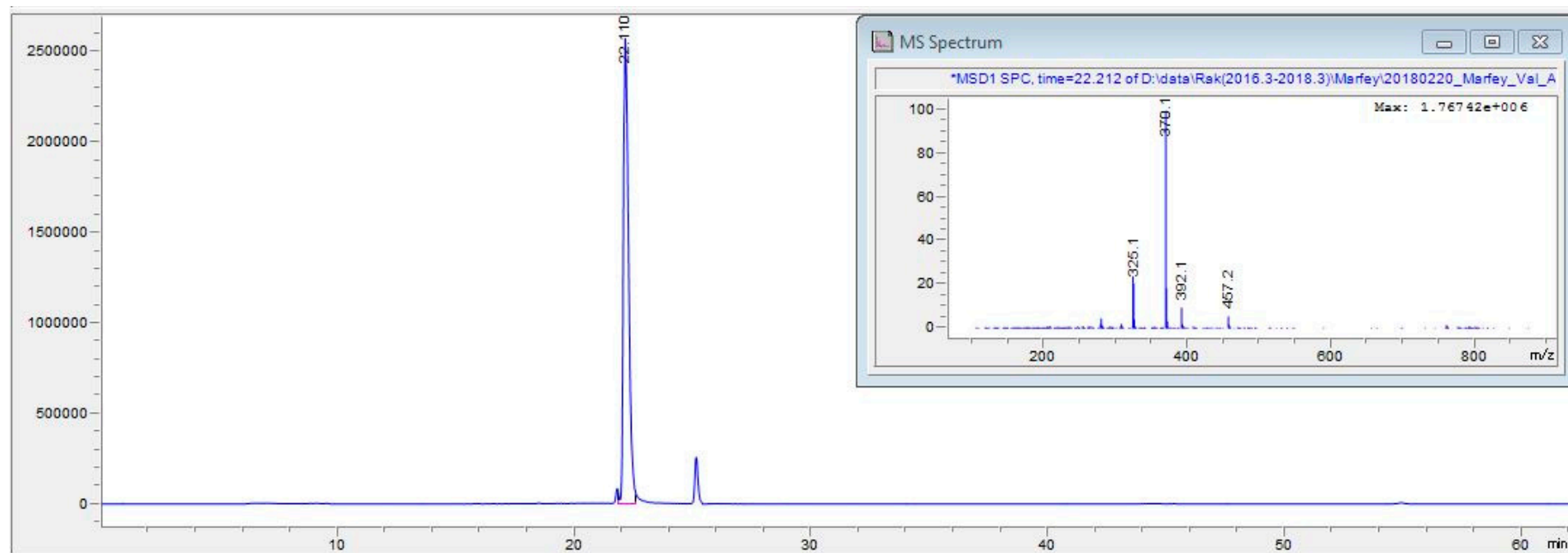
1) L-Glu



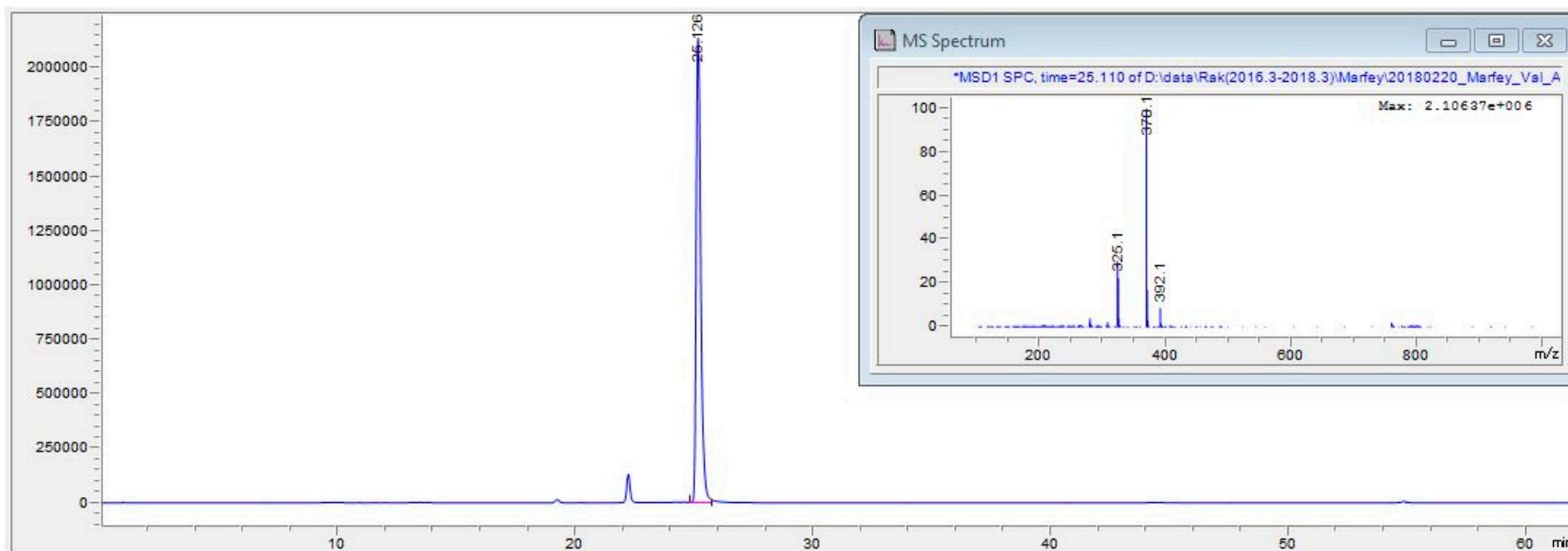
2) D-Glu



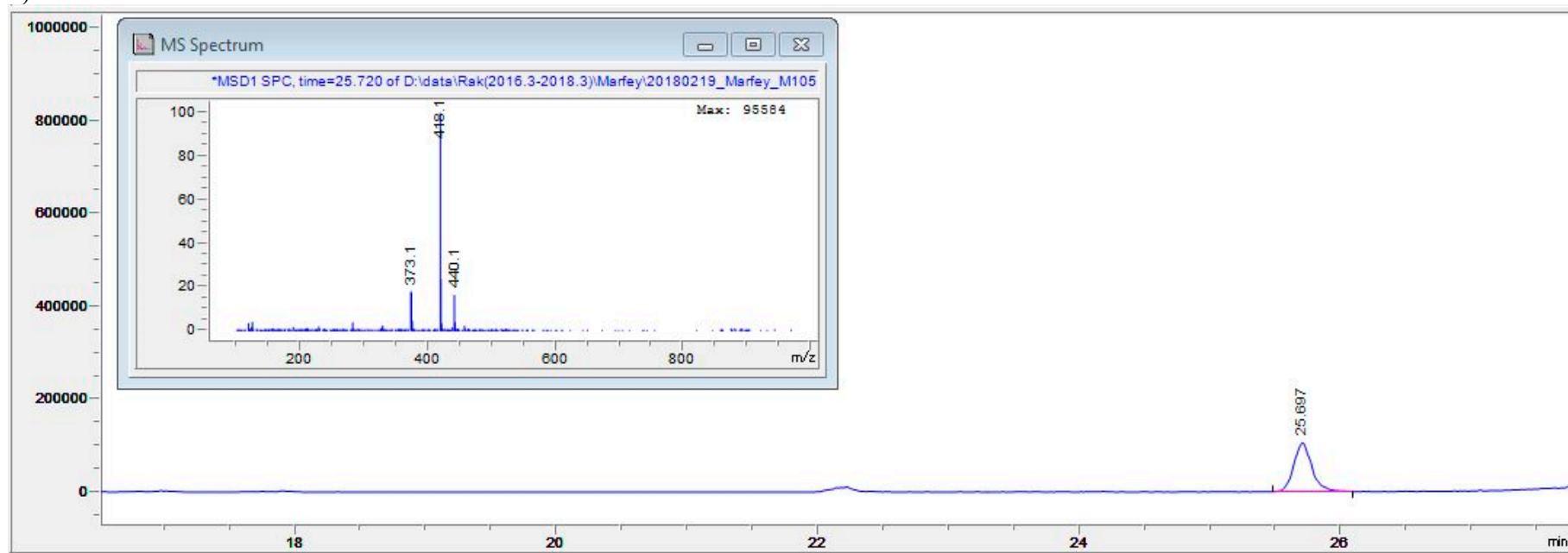
3) L-Val



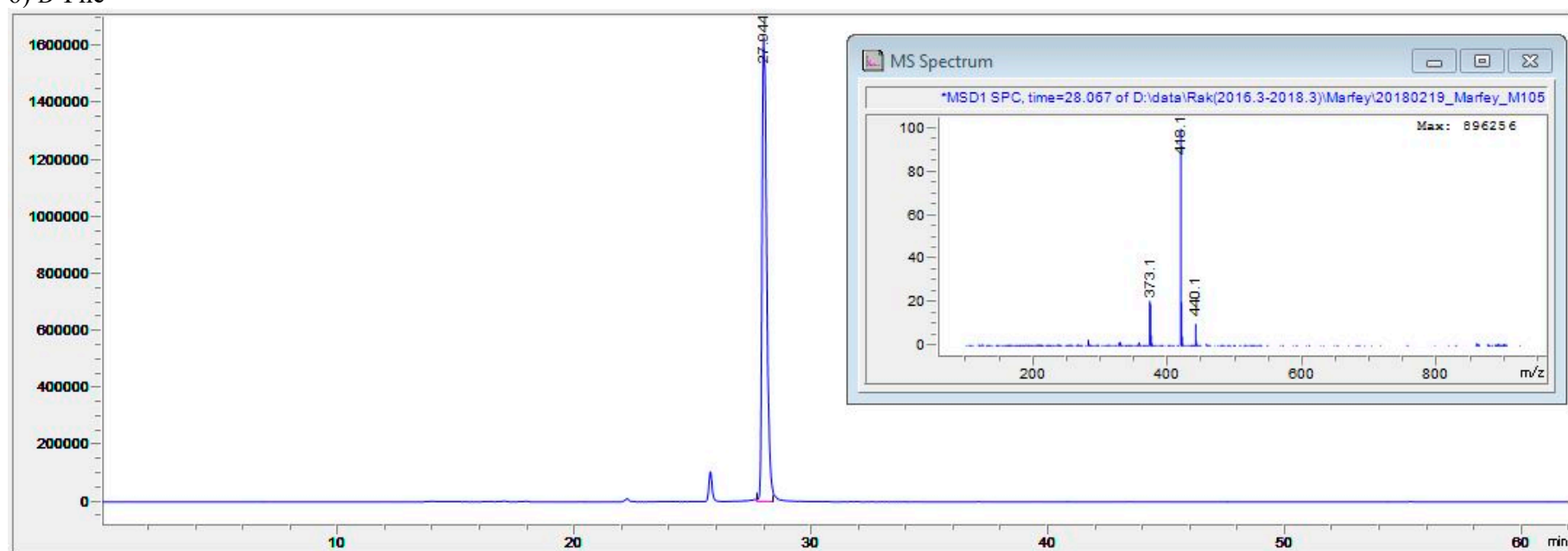
4) D-Val



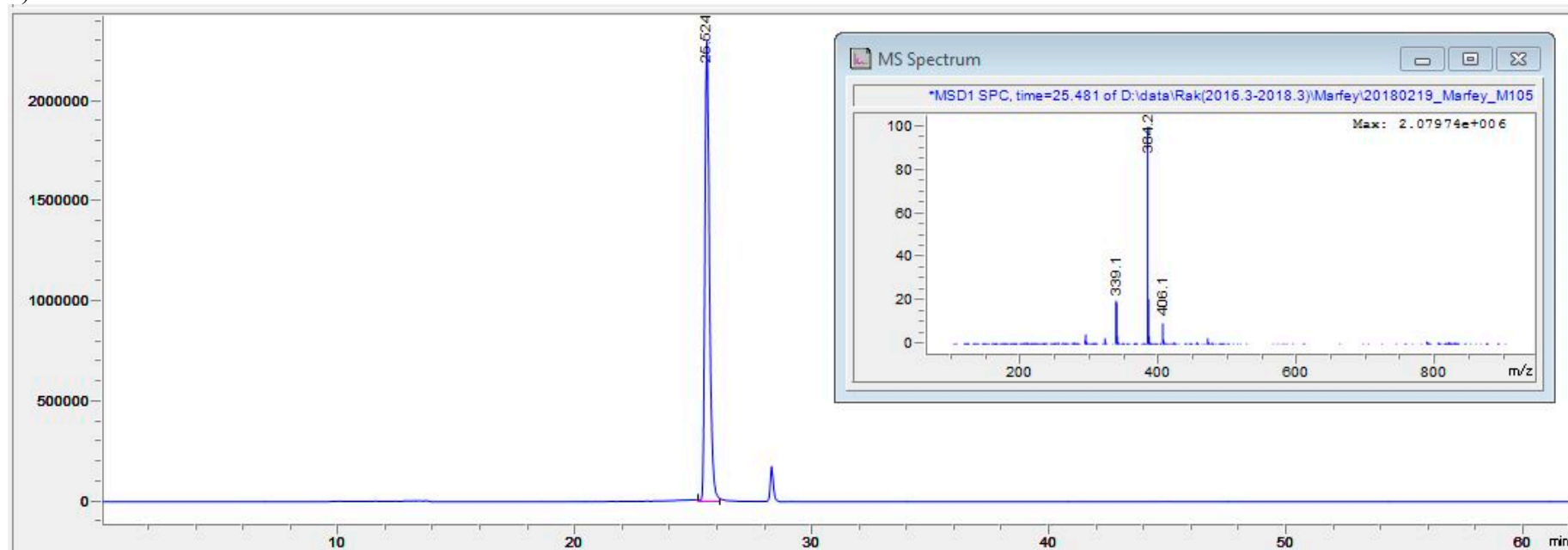
5) L-Phe



6) D-Phe



7) L-Leu



8) D-Leu

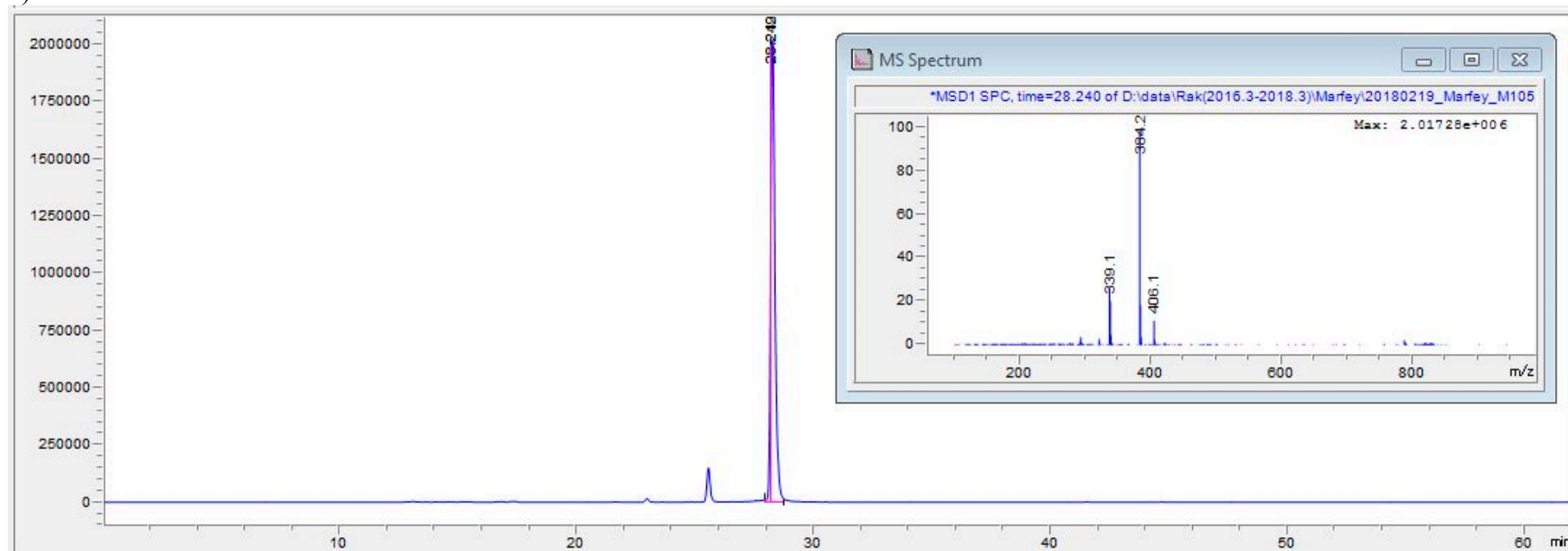
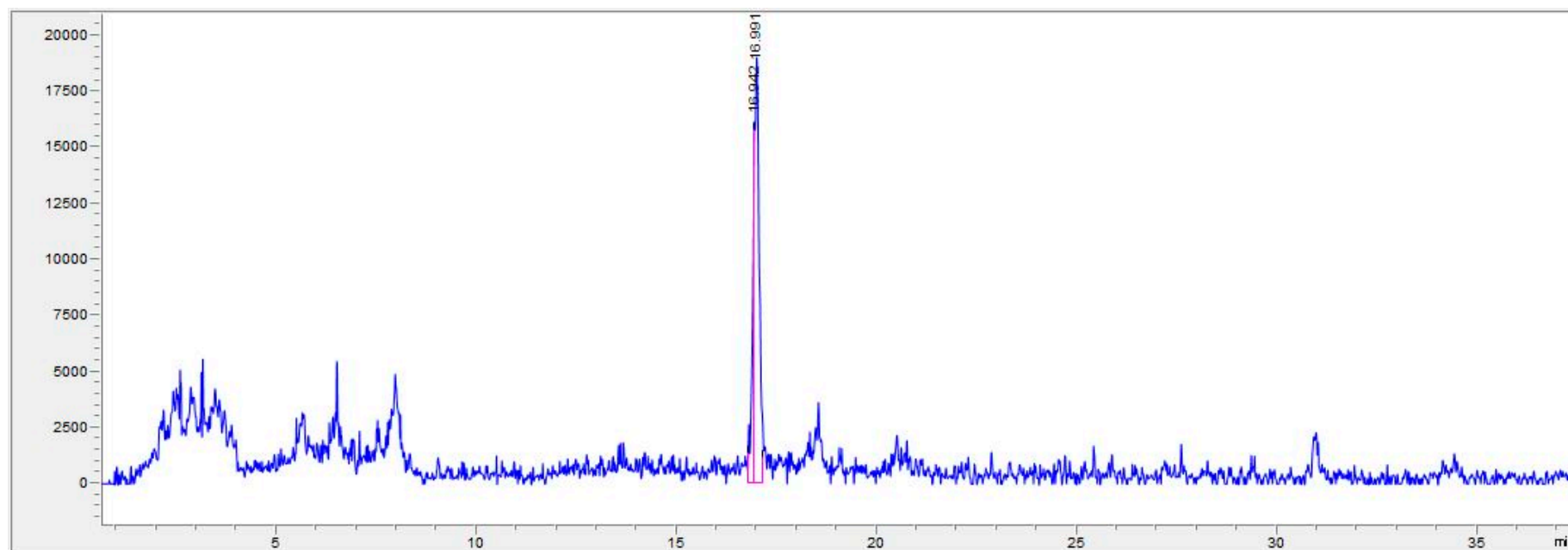
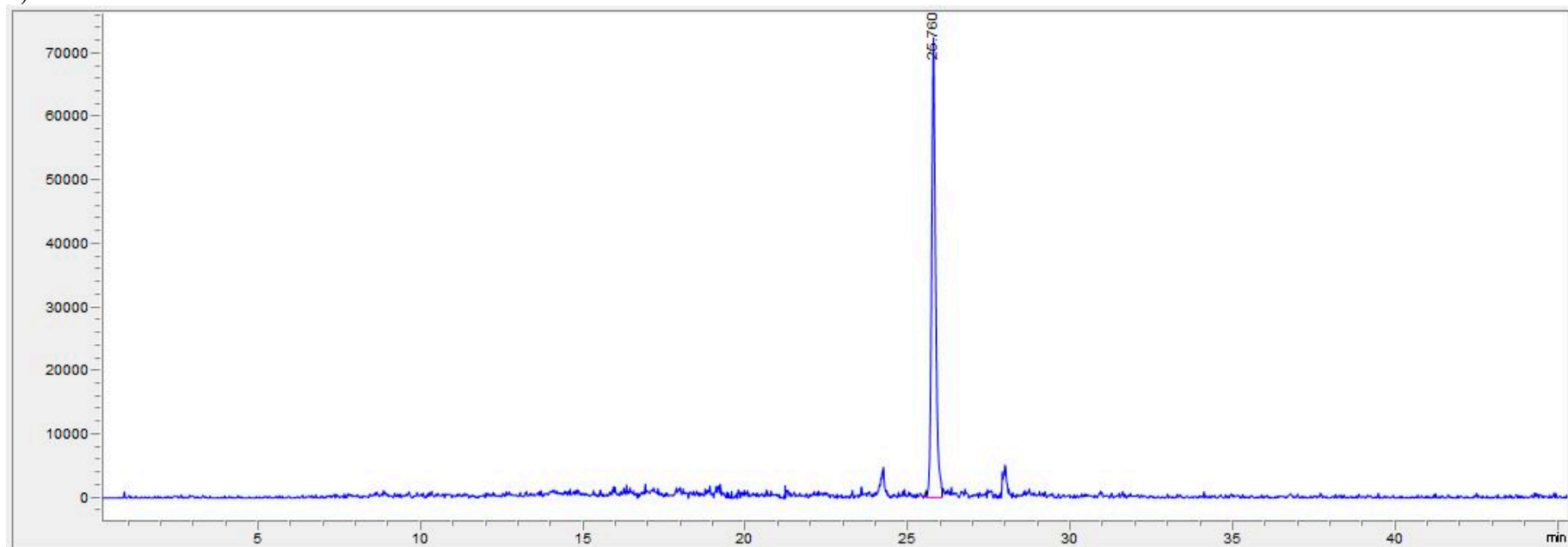


Figure S17. Retention times of the L-FDAA derivatized amino acids from compound **1**

1) L-Glu



2) L-Phe



3) L-Val

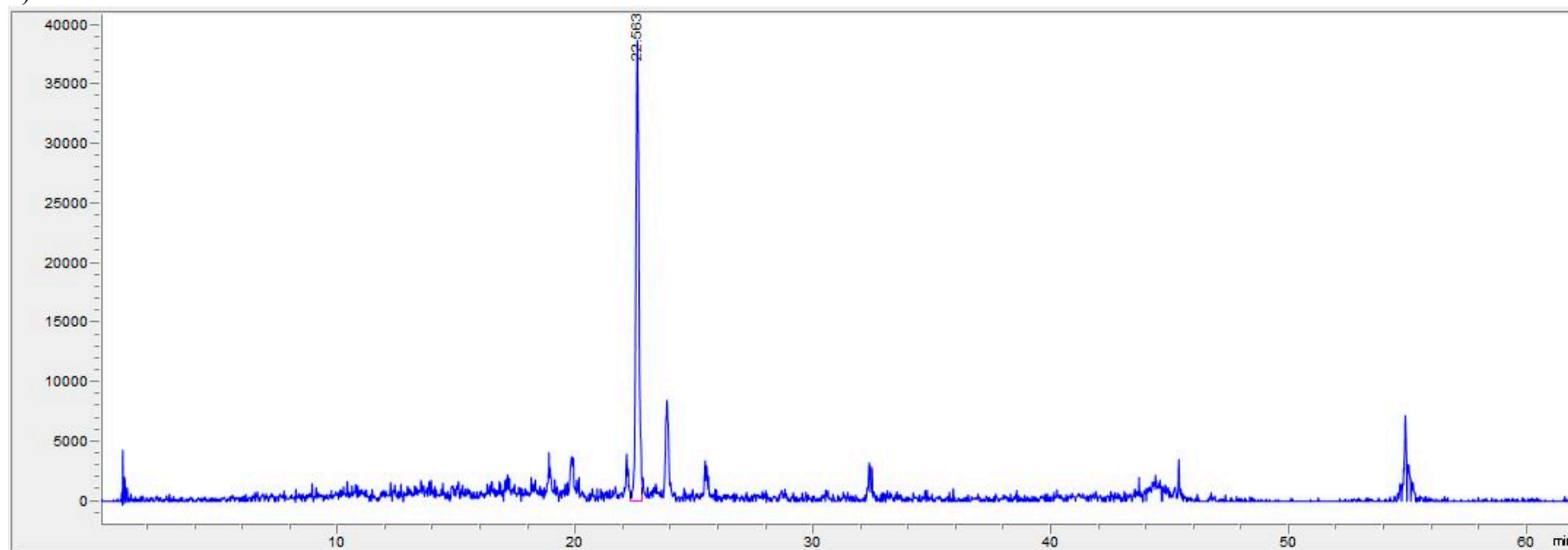


Figure S18. Retention times of the L-FDAA derivatized L-Leu from compound 2

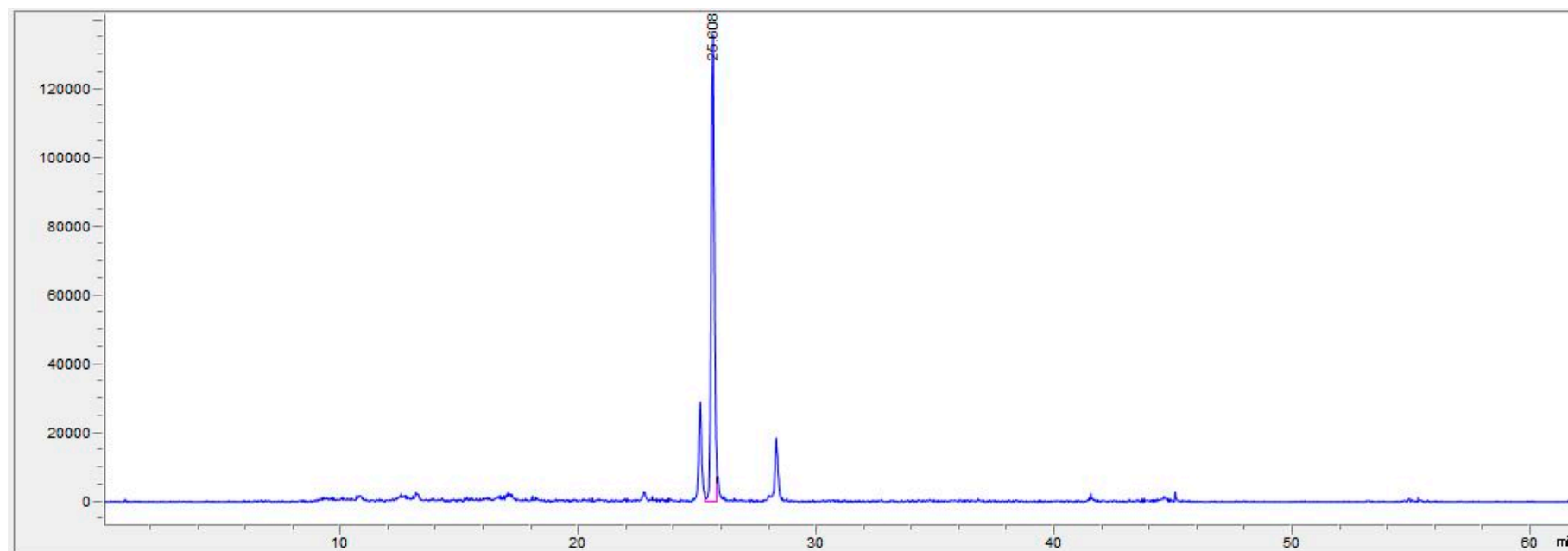


Figure S19. Stimulation of production of compounds 1-3

Actinomadura sp. RB99 was grown in 500 mL ISP2 broth supplemented with varying NaCl concentrations (1-3%) for 10 days at 30 °C. Culture supernatant was extracted with activated HP20 resin using the following steps: 1. Wash step using 1 L dH₂O, 2. elution with 20% MeOH (500 mL), 50% MeOH (500 mL), 100% MeOH (500 mL) and 100% Acetone (500 mL). Organic solvents were evaporated under reduced pressure and resuspended in 20% MeOH and purified using an activated SPE C18 column using the following step gradient (20% MeOH, 50% MeOH, 80% MeOH, 100% MeOH). SPE fractions were analysed using HRMS and HPLC-UV and main metabolite signals dereplicated using an in-house MS/UV data base.

