

GenBank Accession: SPY04274.1; Region 191-450

*Nm*NAT nucleotide sequence

ATGTCCCCCAGCCGTTTCAATCAAACGGGCCCCAAAATCGGCCTGAGCGTACGCCTTGCAGAAACC
 CAAGCTGAAATCGAAGCCGCCCAAAGATTGCGCTATCAGGTTTTTGCCCAAGAGTTGGGGGCGGAA
 ATCGAAAGCGATGACGGCCGTGATGTTCGATCCTTATGATGAGCATTGCCACCACCTGCTTGCCTTCG
 ACGATGCAACCGGCCGAAGTTATCGGCTGCTACCGCCTGATTACCGAAGAAACCGCGAAAAAAGTCG
 GCGGCTGGTACAGCGAGCATGAATTCGACCATGAGCCTTTGAAAGACATTCTGCCGCAAACCGTCG
 AACTCGGTGCGCCTGTACCCACCCGGACTACCGCAACGGCGGCTTGGTCATGCTGTTGTGGACCG
 GTTTGGTCAAATTCATGAAAGACGAAAACCTGCGCTTTATGATTGGTTGCGGCAGTATCGAAATGCG
 CGACGGCGGCAACGATGCGGGCGGGCCTGTATCATGCTTTGAAAGACAAATACCTCGCTCCGGAACA
 ATGGCGCGTCAAACCGCTCAACCCGCTCAAATGGGACAGCATCACGCCGTCTGAAAATCCGCCTGT
 ACCCGCACTGATCAAAGGCTATCTCAAAGCAGGCGCATGGTTCTGCGGCGAGCCTTGCCTCGATGA
 AGCATTCAACTGCGCGGATGTGCTGATCATGATGGACATCAGCCACCTTTCGGACCGCTACTTGCAG
 CGTTTTGCCCTAAAACCGATTCATAA

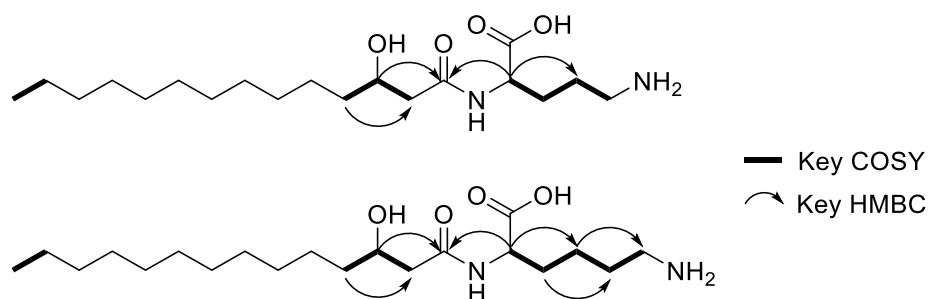


Figure S1. Key COSY and HMBC NMR correlations for **1** and **2**.

***N*-3-hydroxymyristoyl-L-ornithine (MH+ 359) (MeOD, 600 MHz)**

	NH	α H	β H	γ H	δ H	
<i>N</i> -3-hydroxymyristoyl		2.35	3.95	1.48		CH2 1.23-1.37 Terminal CH3 0.88
Orn		4.45	1.74 1.99	1.49	2.94	

	CO	α C	β C	γ C	δ C	
<i>N</i> -3-hydroxymyristoyl	172.92	43.16	68.38	36.87		Terminal CH3 12.93
Orn	173.14	51.20	28.37	22.29	38.76	

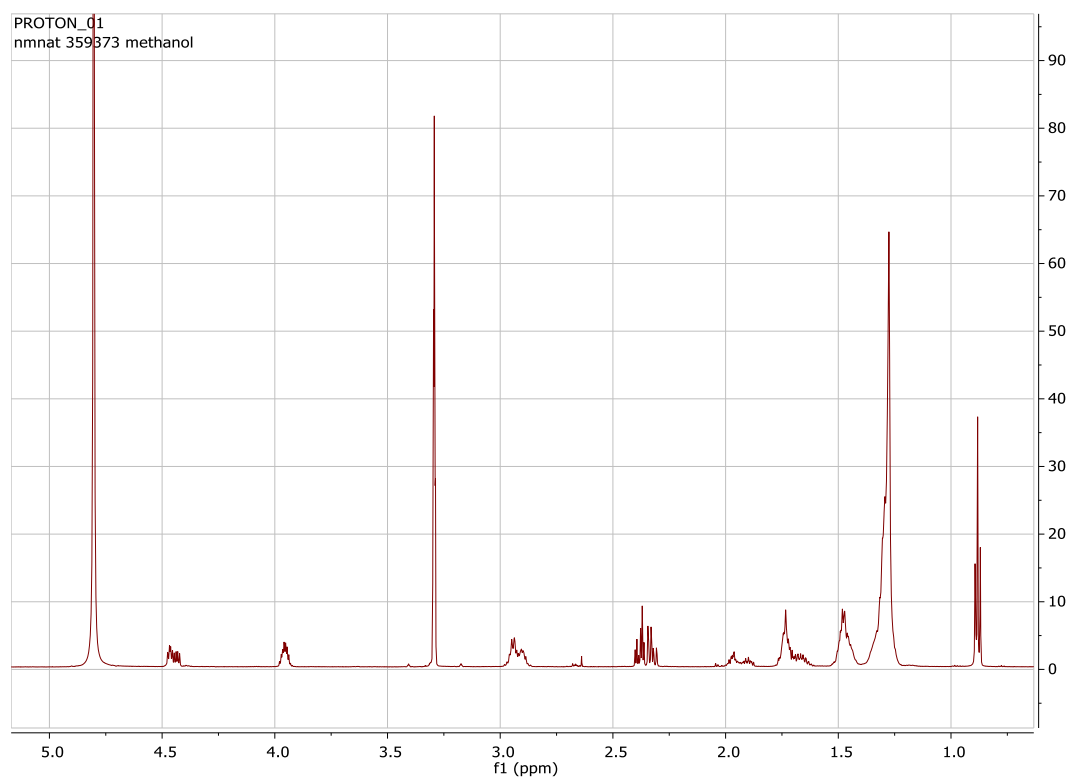
***N*-3-hydroxymyristoyl-L-lysine (MH+ 373) (MeOD, 600 MHz)**

	NH	α H	β H	γ H	δ H	
<i>N</i> -3-hydroxymyristoyl		2.35	3.95	1.48		CH2 1.23-1.37

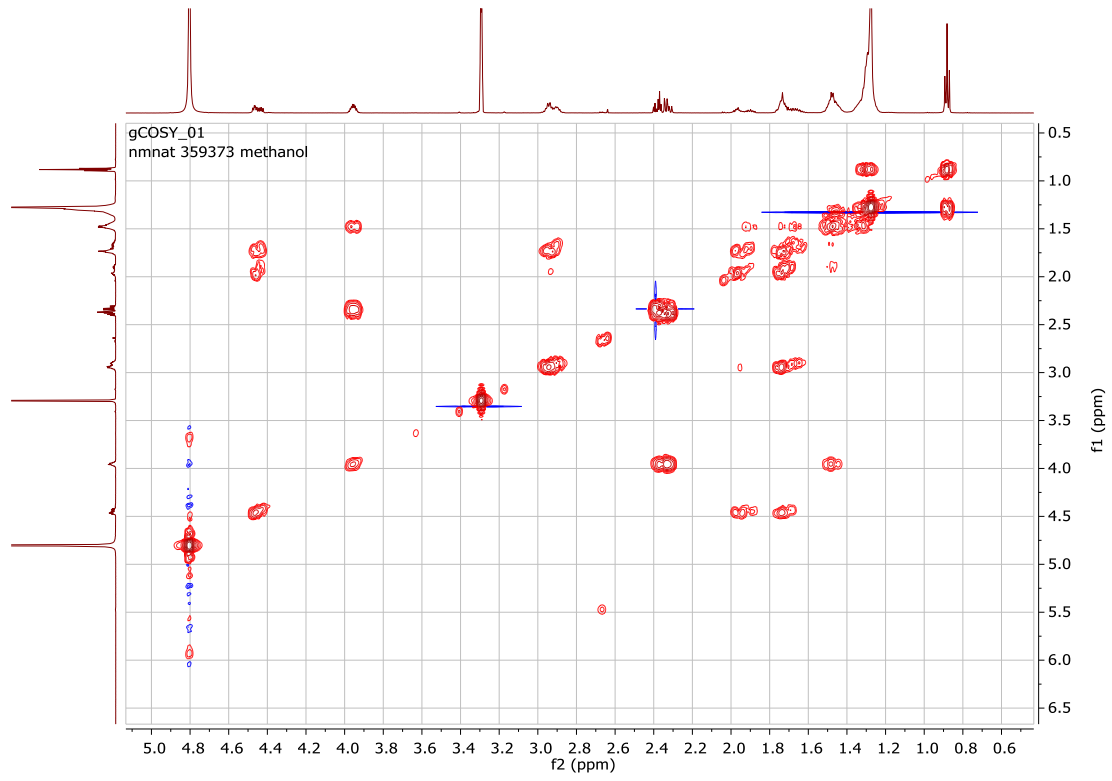
Lys		4.42	1.92 1.70	1.49	1.66	Terminal CH3 0.88 εH 2.90
<i>N</i> -3-hydroxymyristoyl	CO	172.92	αC 43.16	βC 68.38	γC 36.87	δC
Lys		173.66	51.47	30.76	22.29	26.52 Terminal CH3 12.93 εC 39.09

Table S1. Chemical shifts of natural (bacterially-produced) **1** and **2** as a mixture identified by 1D- and 2D-NMR (gCOSY, gHSQC, gHMBC)

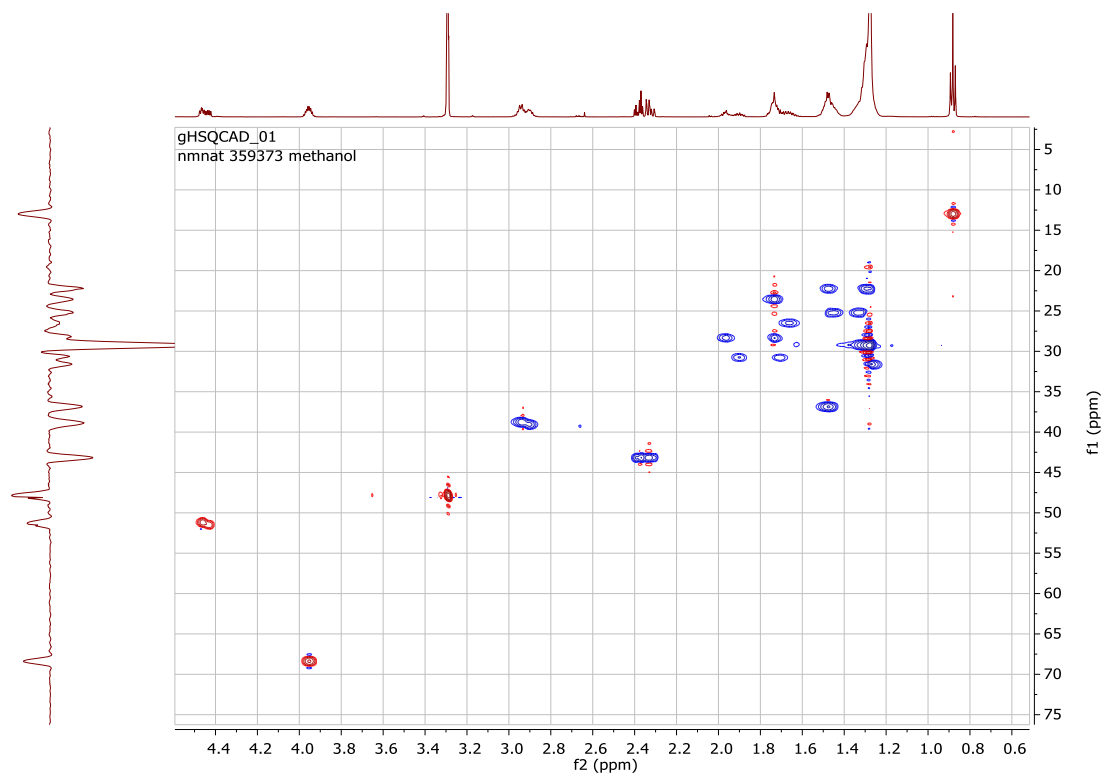
A



B



C



D

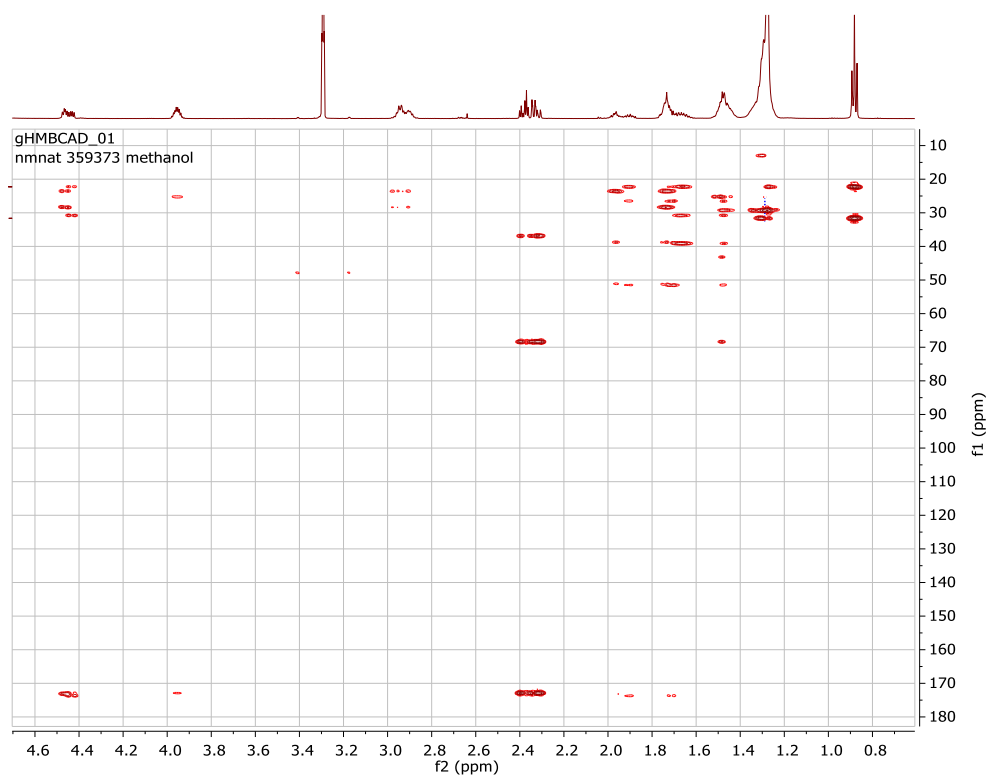


Figure S2. NMR spectra of natural **1** and **2** as a mixture (MeOD, 600 MHz)

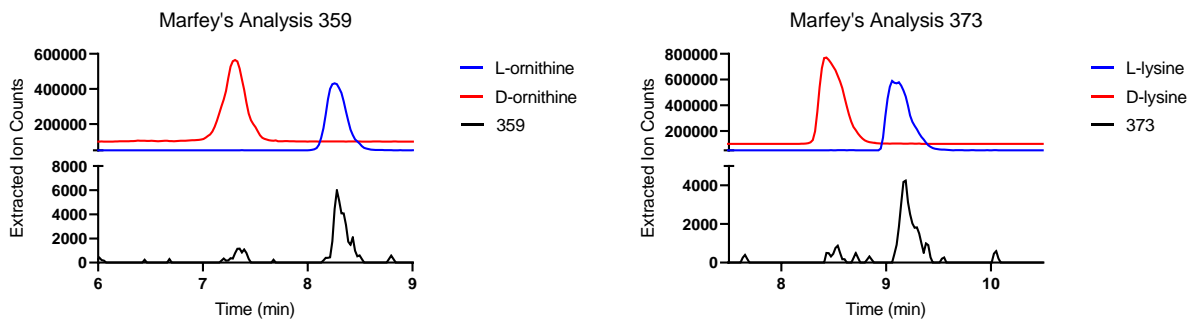


Figure S3. Marfey's analysis of **1** and **2**. Extracted ion chromatograms (m/z 385.1466, m/z 399.1623) of the Marfey's product of L-ornithine, D-ornithine, L-lysine, D-lysine, hydrolyzed **1**, hydrolyzed **2** are shown, both (**1** and **2**) of which align in retention time with that of the *S*- configuration of their respective amino acid head groups.

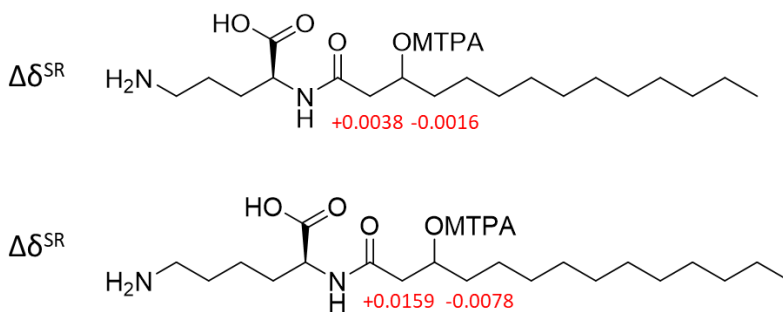


Figure S4. The $\Delta\delta_{S-R}$ in ppm for the MTPA esters of compounds **1** and **2**.

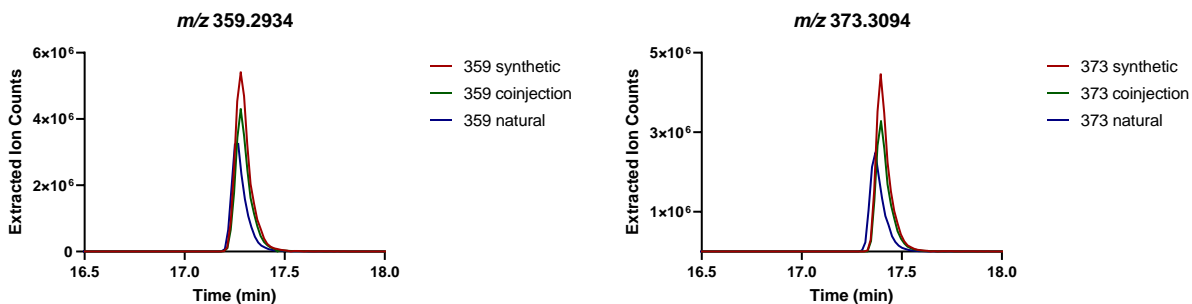


Figure S5. Extracted ion chromatogram of crude *NmNAT* extract, synthetic, and co-injection of crude and synthetic **1** and **2**.

***N*-3-hydroxymyristoyl-L-ornithine (MH+ 359) (DMSO, 600 MHz)**

	NH	α H	β H	γ H	δ H	
<i>N</i> -3-hydroxymyristoyl		2.16	3.72	1.30, 1.26		CH2 1.20-1.32 Terminal CH3 0.82
Orn	7.84	4.02	1.58, 1.72	1.55	2.74	

	CO	α C	β C	γ C	δ C	
<i>N</i> -3-hydroxymyristoyl	171.13	44.10	67.95	37.34		Terminal CH3 14.40
Orn	173.92	52.51	29.27	24.26	38.99	

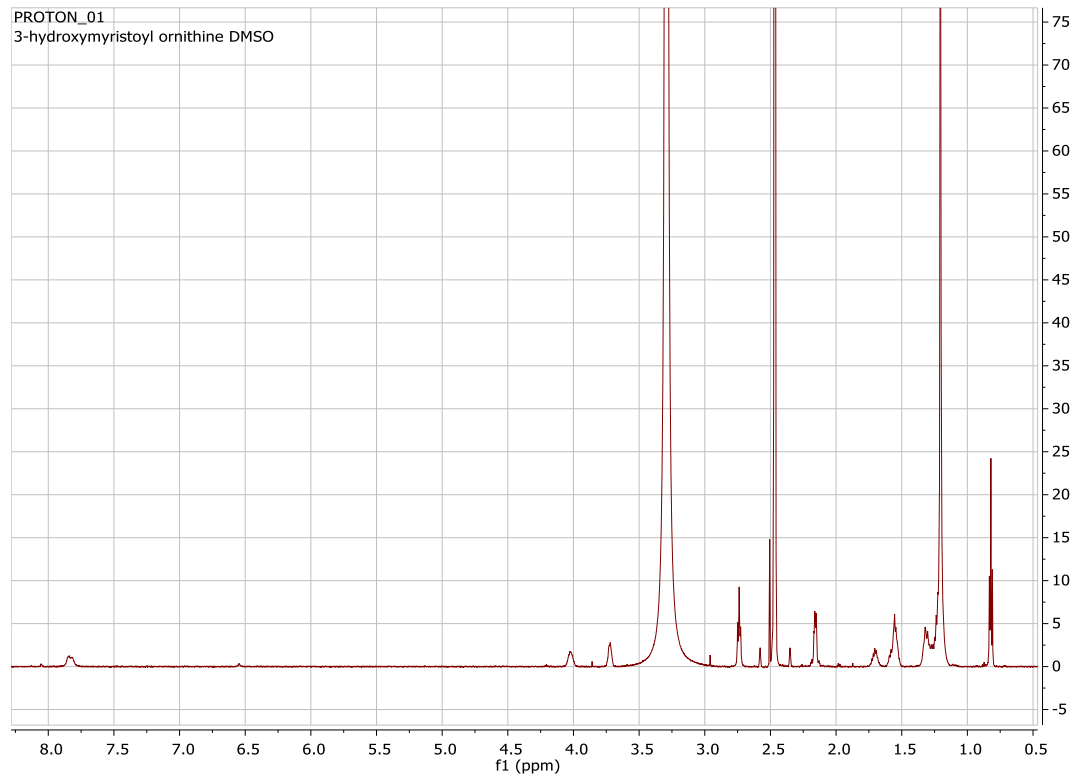
***N*-3-hydroxymyristoyl-L-lysine (MH+ 373) (DMSO, 600 MHz)**

	NH	α H	β H	γ H	δ H	
<i>N</i> -3-hydroxymyristoyl		2.17	3.73			CH2 1.20-1.32 Terminal CH3 0.82
Lys	7.95	4.11	1.66, 1.52	1.30	1.49	ϵ H 2.72

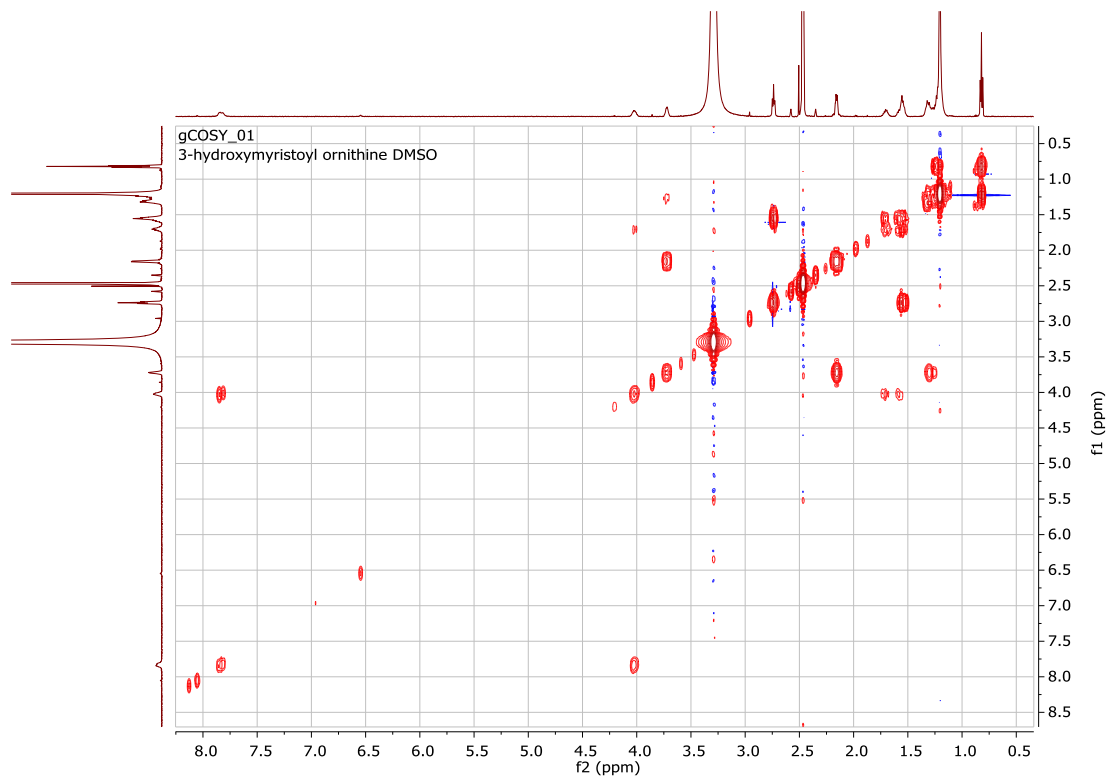
	CO	α C	β C	γ C	δ C	
<i>N</i> -3-hydroxymyristoyl	171.33	43.98		36.87		Terminal CH3 14.45
Lys	174.20	52.05	31.13	22.66	26.91	ϵ C 39.11

Table S2. Chemical shifts of synthetic **1** and **2** identified by 1D- and 2D- NMR (gCOSY, gHSQC, gHMBC)

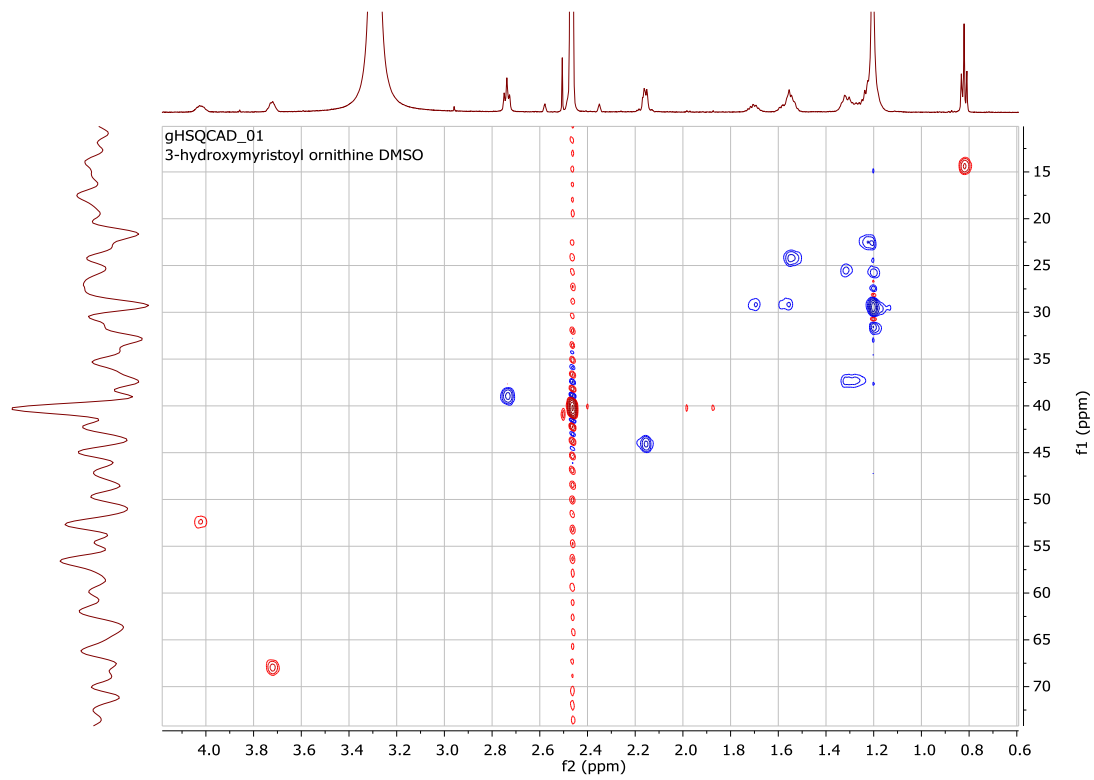
A



B



C



D

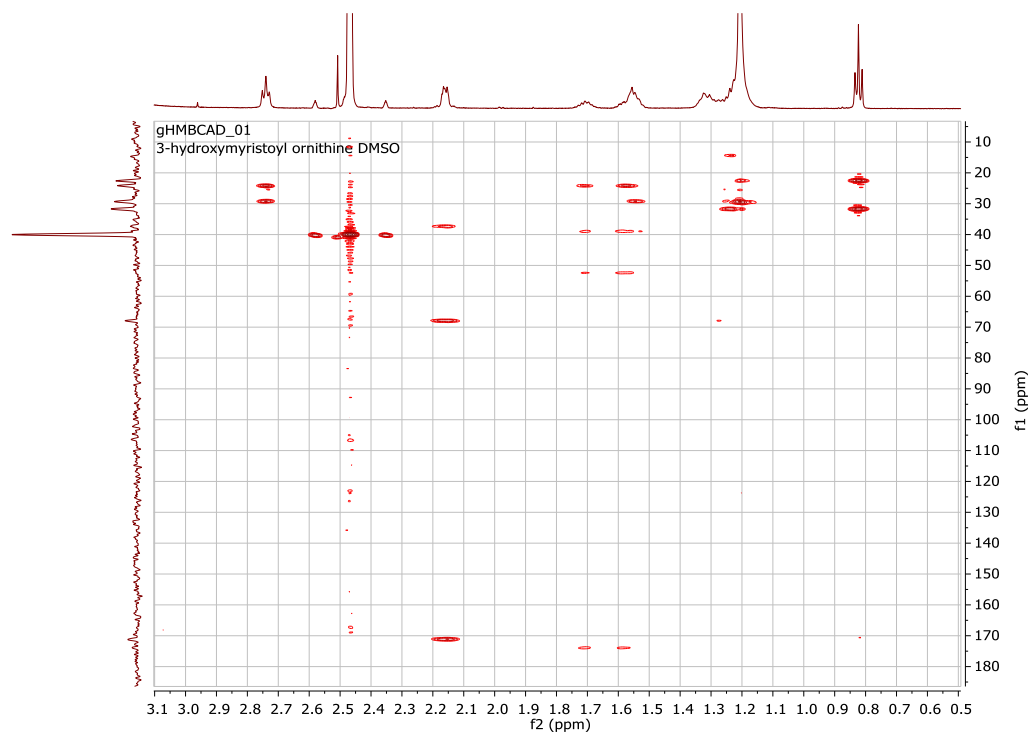
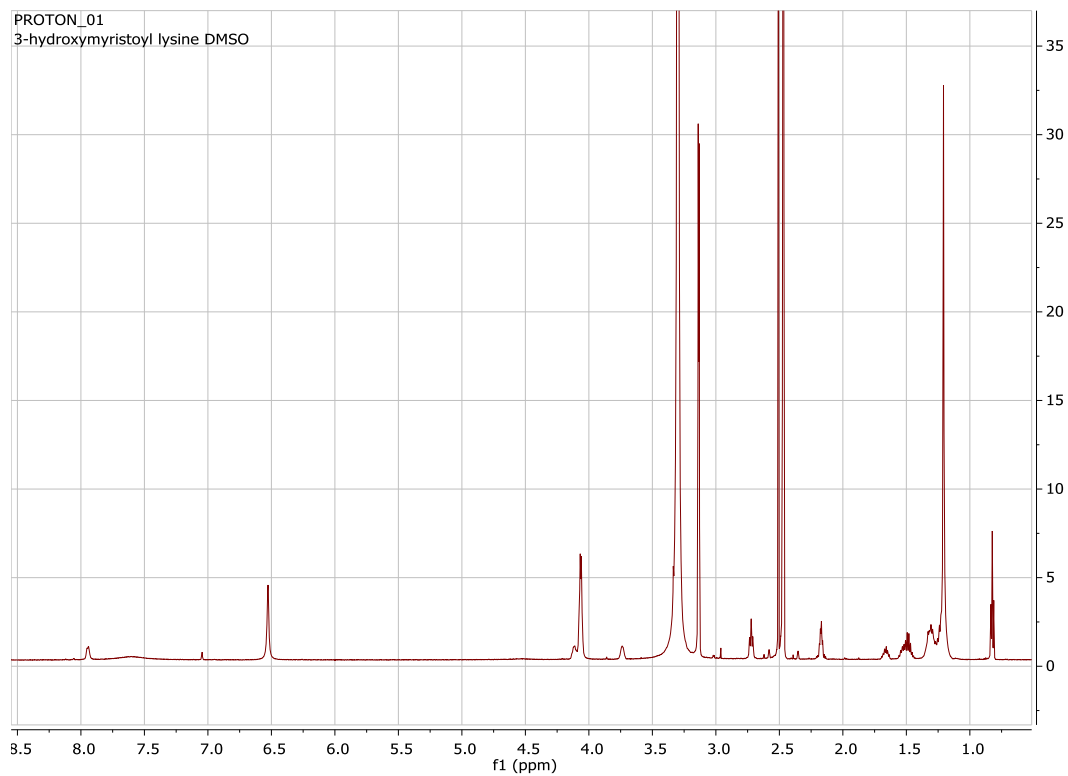
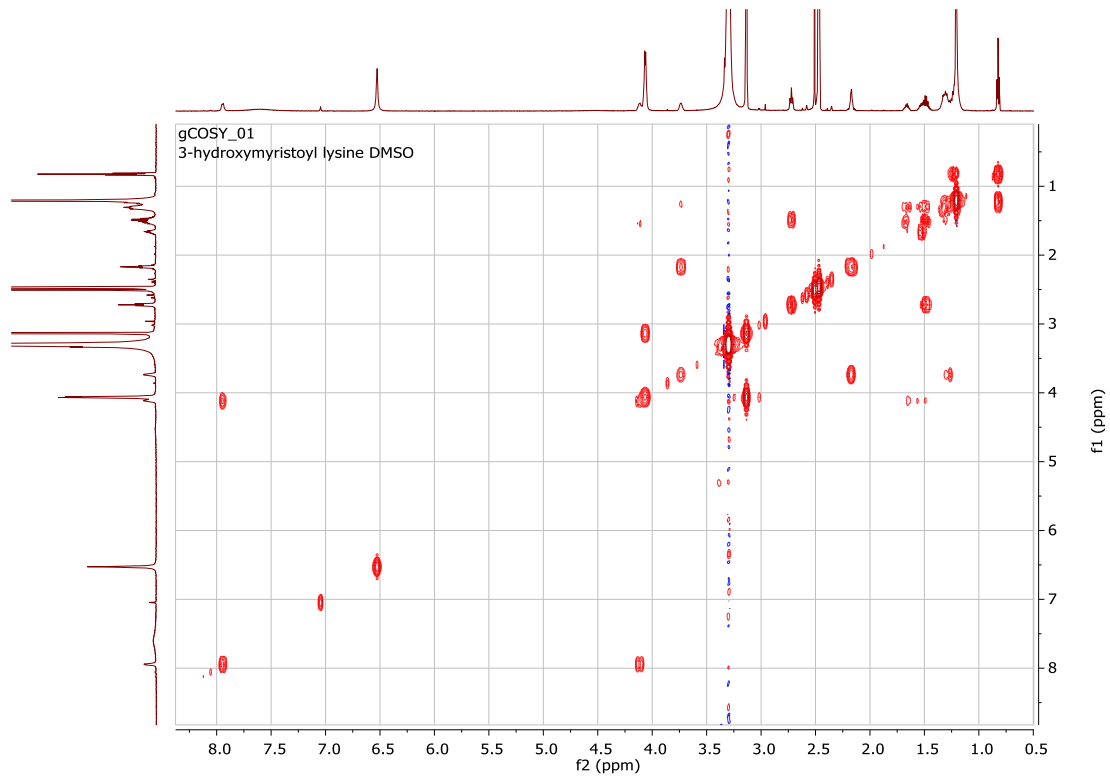


Figure S6. NMR spectra of synthetic compound **1** (DMSO, 600 MHz)

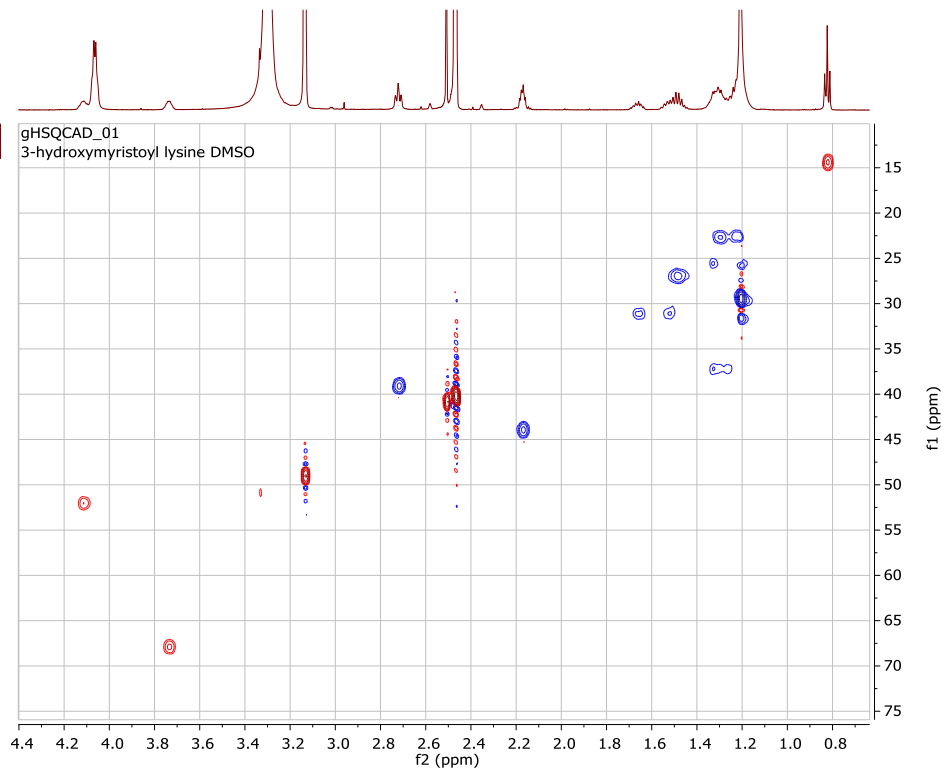
A



B



C



D

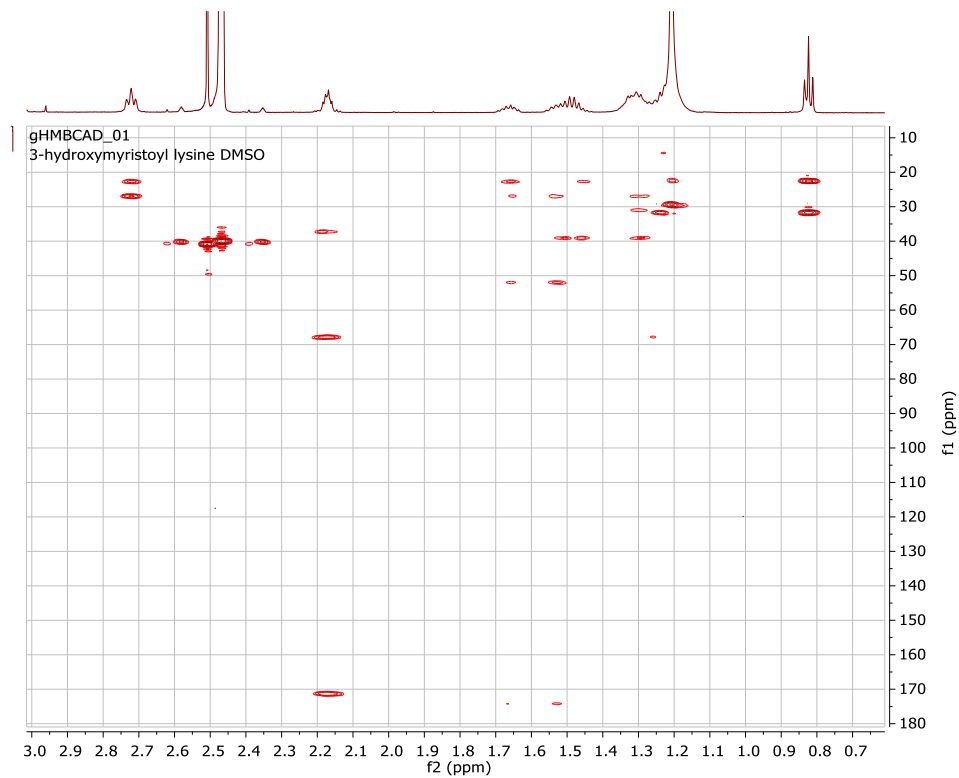
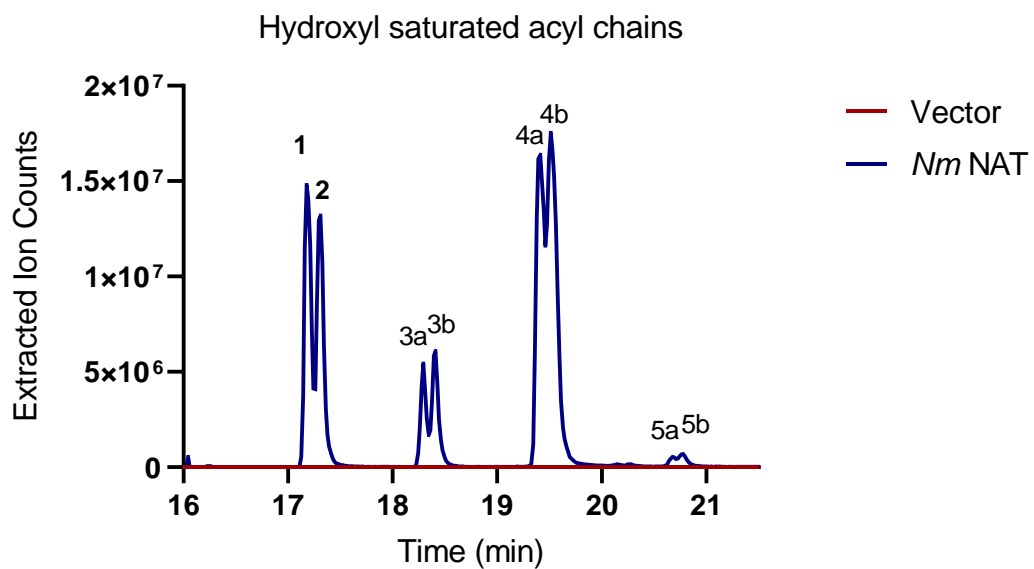
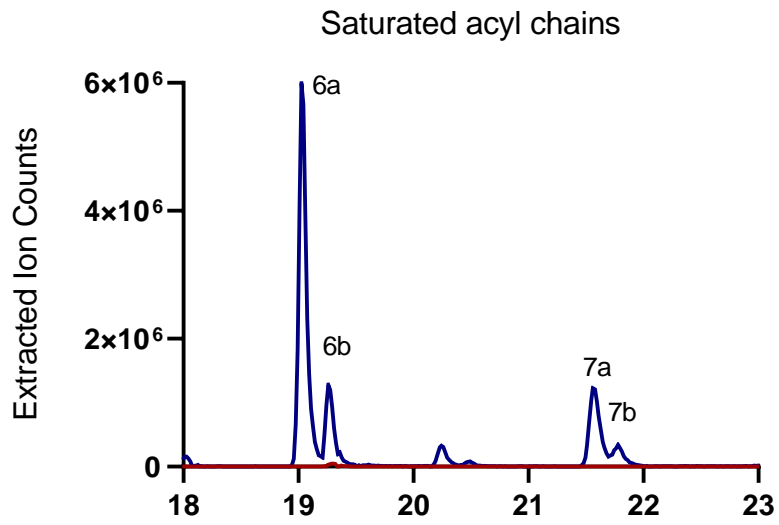


Figure S7. NMR spectra of synthetic compound **2** (DMSO, 600 MHz)

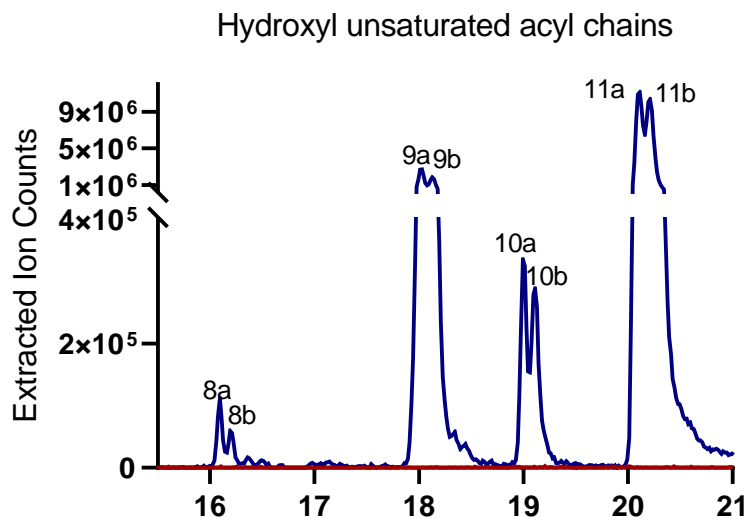
A



B



C



D

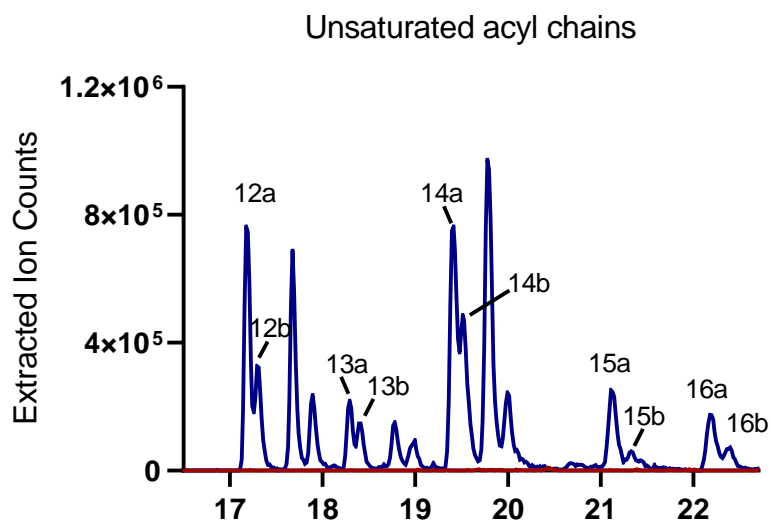


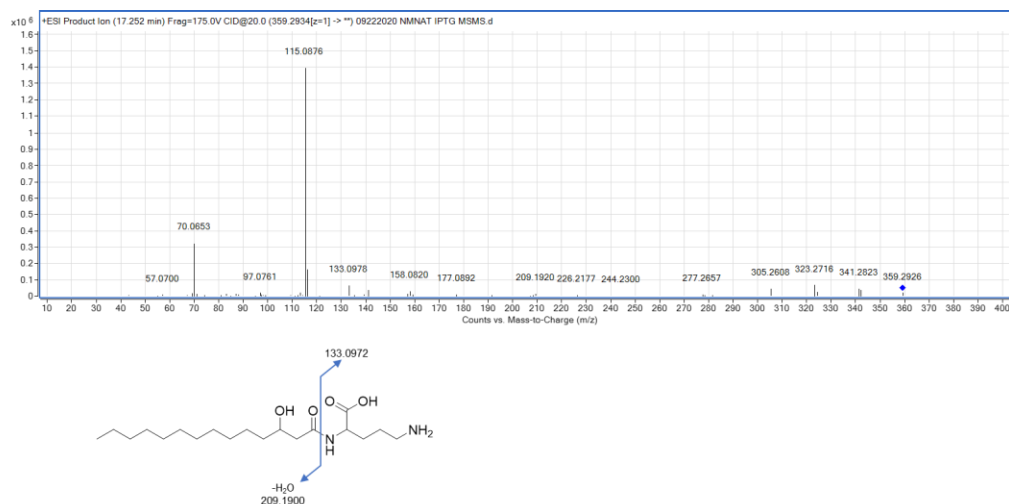
Figure S8. Extracted ion chromatograms of all identified *N*-acyl ornithines and lysines from *NmNAT* expression in *E. coli*

Head Group	Acyl Chain	Compound	Retention Time (min)	Theoretical <i>m/z</i>	Observed <i>m/z</i>	Ppm error
Orn	OH-C14:0	1	17.177	359.2904	359.2934	8.349792
	OH-C15:0	3a	18.294	373.3061	373.30857	6.616554
	OH-C16:0	4a	19.411	387.3217	387.3246	7.487316
	OH-C17:0	5a	20.678	401.3374	401.33806	1.644502
Lys	OH-C14:0	2	17.31	373.3061	373.3086	6.696917
	OH-C15:0	3b	18.41	387.3217	387.3234	4.389116
	OH-C16:0	4b	19.511	401.3374	401.33929	4.709255
	OH-C17:0	5b	20.778	415.353	415.3536	1.444554
Orn	C14	6a	19.027	343.2955	343.29833	8.243627
	C16	7a	21.561	371.3268	371.3279	2.96235
Lys	C14	6b	19.261	357.3112	357.3132	5.597362
	C16	7b	21.778	385.3425	*385.34231	-0.519019

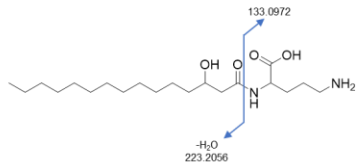
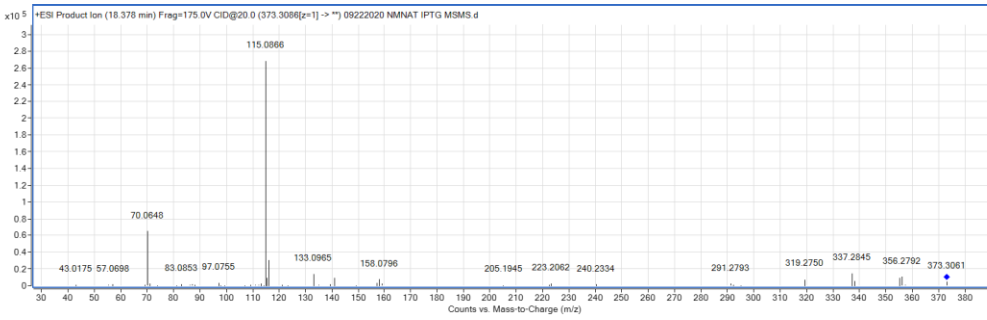
Orn	OH-C14:1	8a	16.093	357.2748	357.2746	-0.559793
	OH-C16:1	9a	18.027	385.3061	385.3071	2.595339
	OH-C17:1	10a	19.011	399.3217	399.3218	0.250425
	OH-C18:1	11a	20.111	413.3374	413.3387	3.14513
Lys	OH-C14:1	8b	16.193	371.2904	*371.2915	2.96241
	OH-C16:1	9b	18.127	399.3217	399.3224	1.752973
	OH-C17:1	10b	19.111	413.3374	*413.3376	0.483866
	OH-C18:1	11b	20.211	427.353	427.3548	4.211975
Orn	C14:1	12a	17.177	341.2799	341.28111	3.545477
	C15:1	13a	18.294	355.2955	355.2959	1.125823
	C16:1	14a	19.411	369.3112	369.3113	0.270774
	C17:1	15a	21.111	383.3268	383.3278	2.60874
	C18:1	16a	22.195	397.3425	397.3426	0.251672
Lys	C14:1	12b	17.293	355.2955	355.2962	1.970191
	C15:1	13b	18.41	369.3112	*369.31151	0.8394
	C16:1	14b	19.527	383.3268	383.3275	1.826118
	C17:1	15b	21.311	397.3425	*397.3427	0.503344
	C18:1	16b	22.395	411.3581	*411.3589	1.944778

Table S3. HRMS of all identified *N*-acyl ornithines and lysines from expression of *NmNAT* in *E. coli*. Ions marked with * are detected but have inconclusive tandem MS spectra

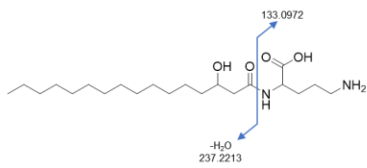
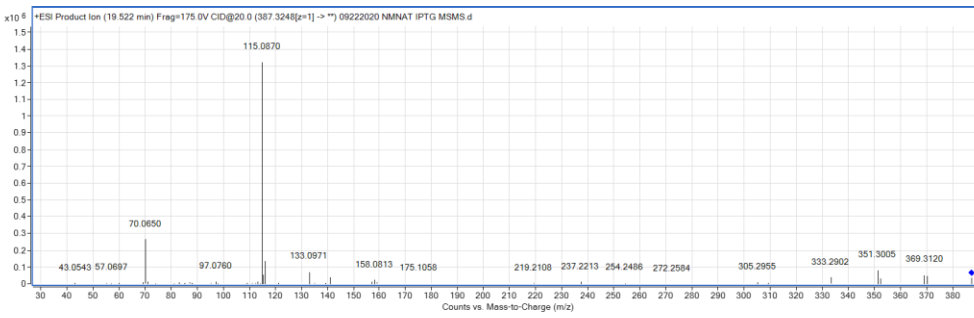
OH-C14:0 Ornithine



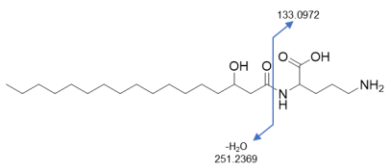
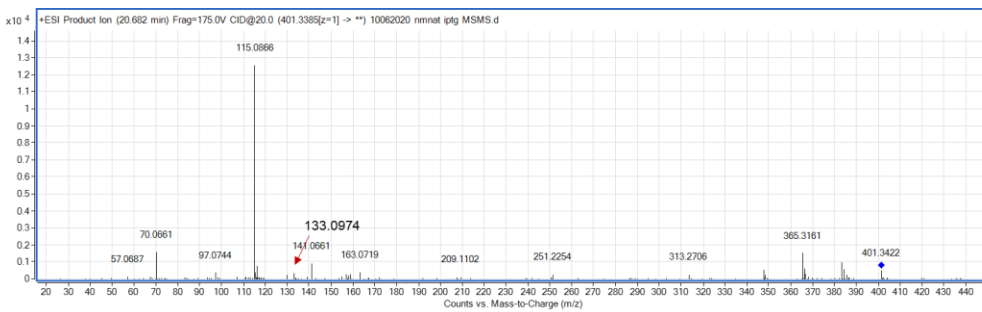
OH-C15:0 Ornithine



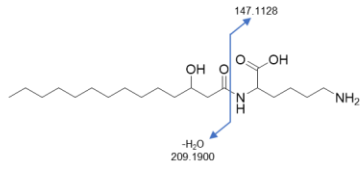
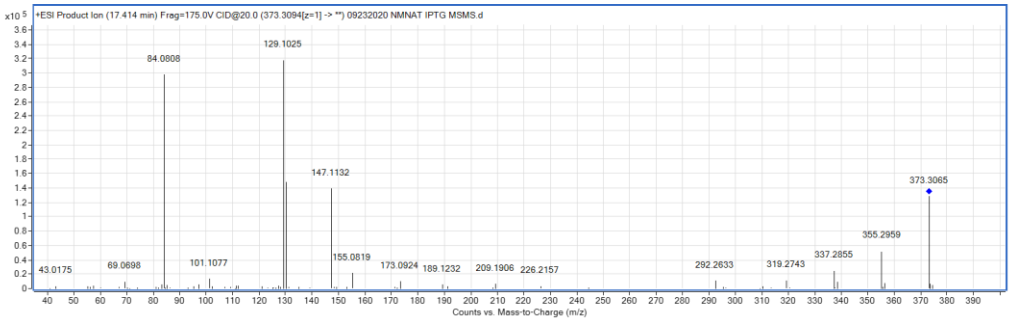
OH-C16:0 Ornithine



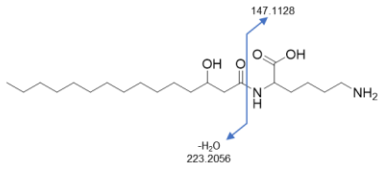
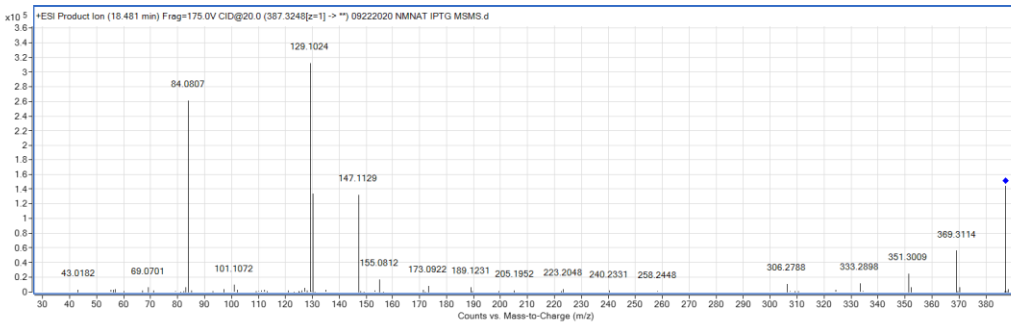
OH-C17:0 Ornithine



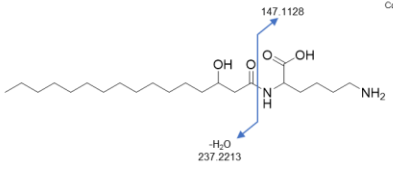
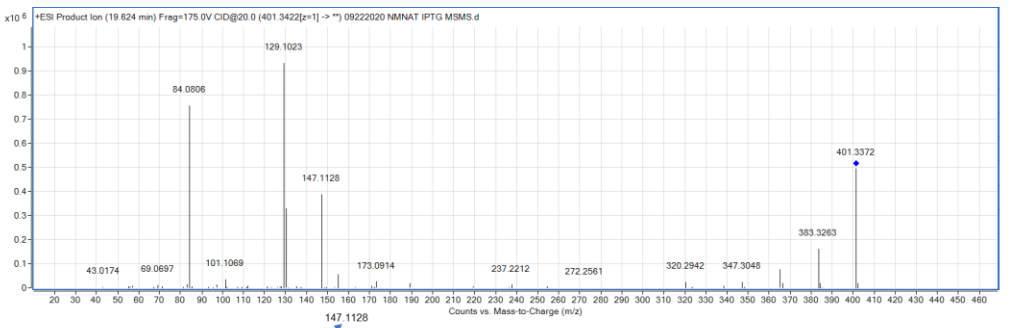
OH-C14:0 Lysine



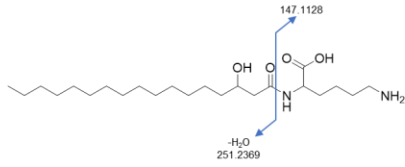
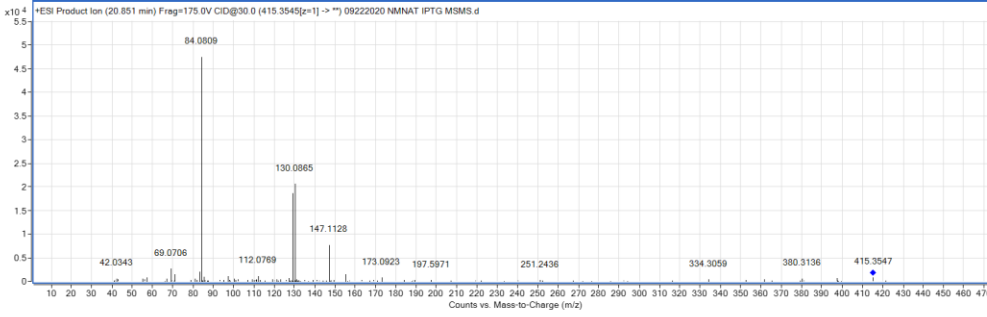
OH-C15:0 Lysine



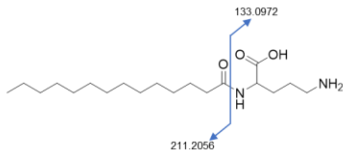
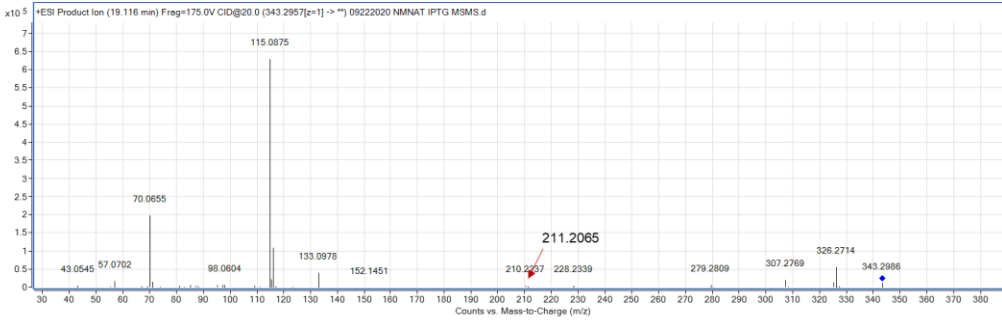
OH-C16:0 Lysine



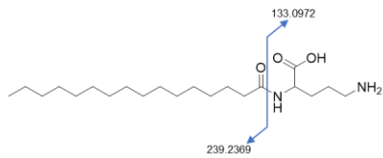
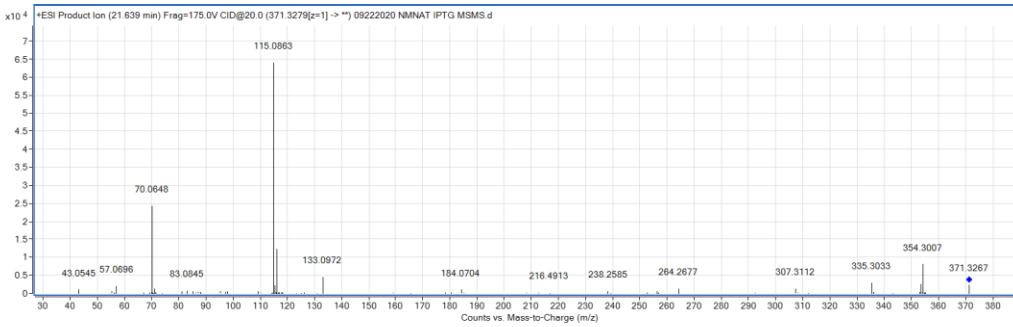
OH-C17:0 Lysine



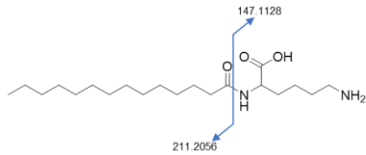
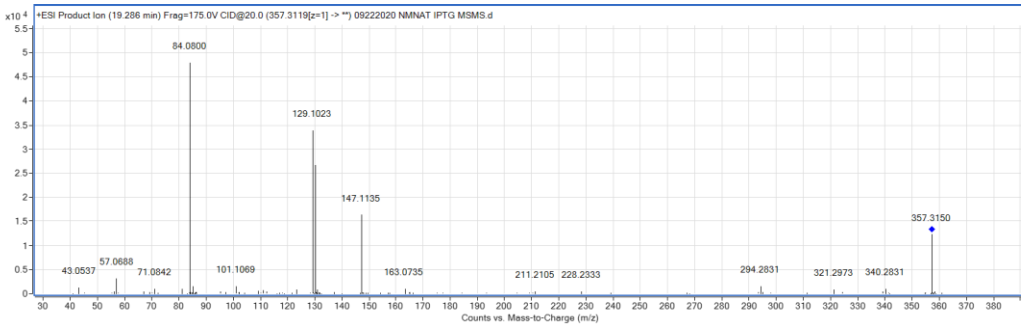
C14:0 Ornithine



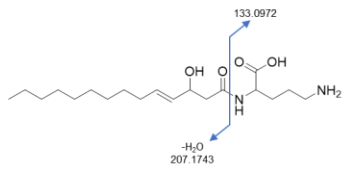
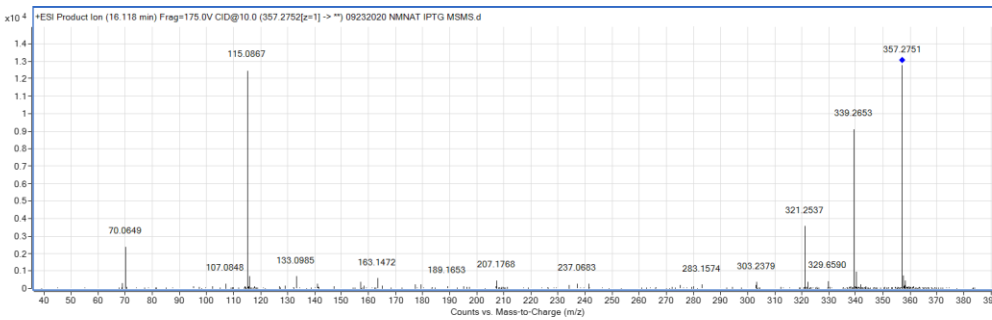
C16:0 Ornithine



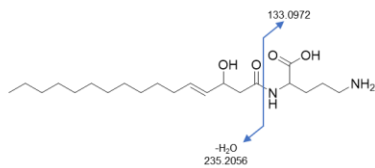
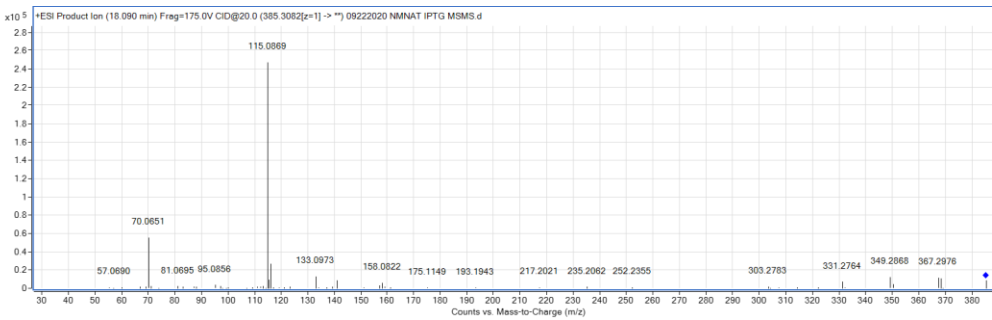
C14:0 Lysine



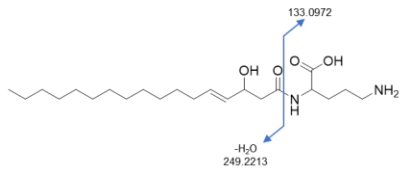
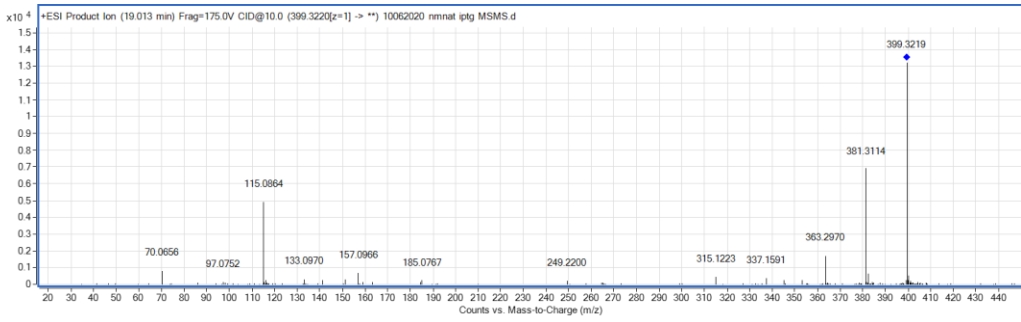
OH-C14:1 Ornithine



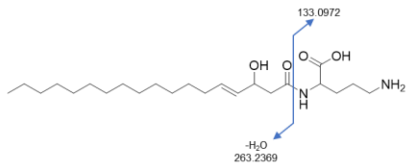
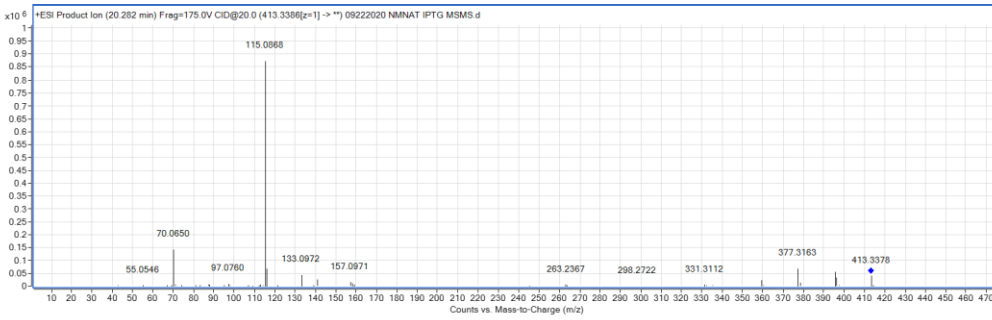
OH-C16:1 Ornithine



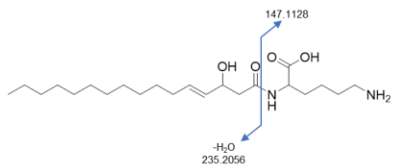
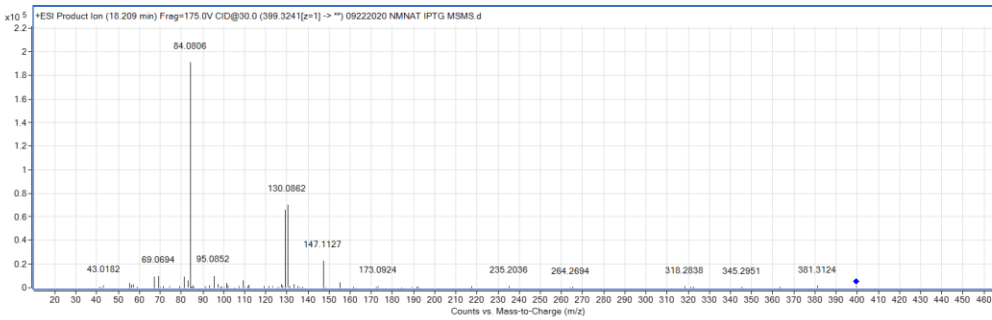
OH-C17:1 Ornithine



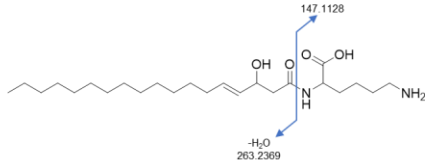
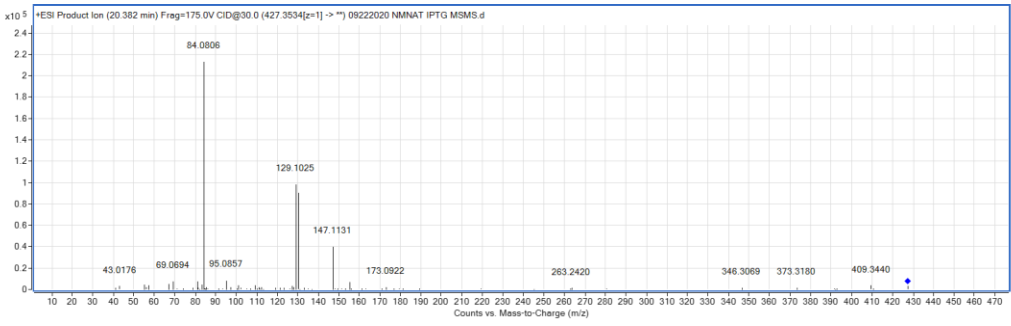
OH-C18:1 Ornithine



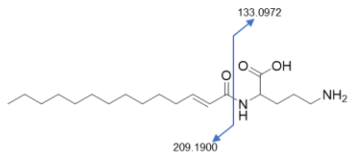
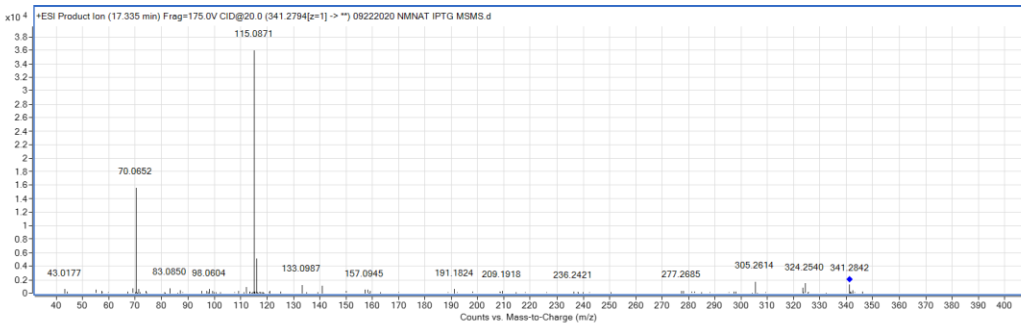
OH-C16:1 Lysine



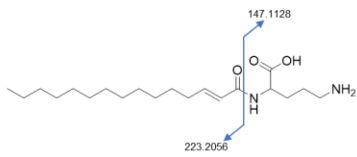
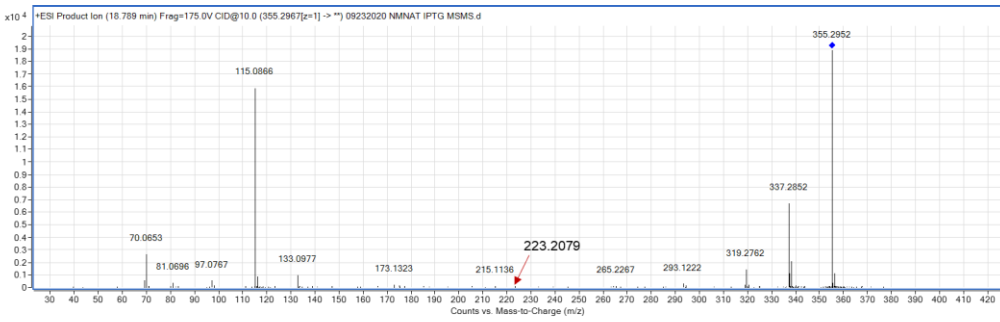
OH-C18:1 Lysine



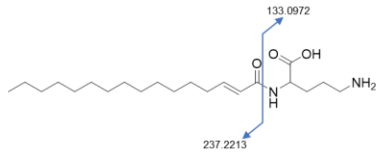
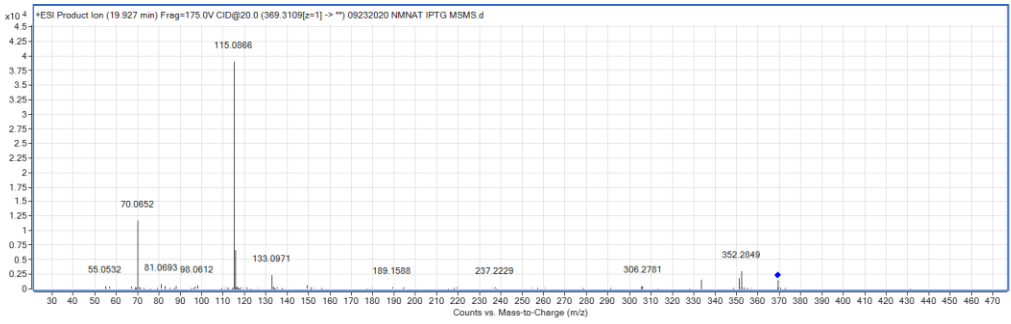
C14:1 Ornithine



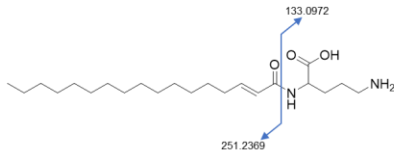
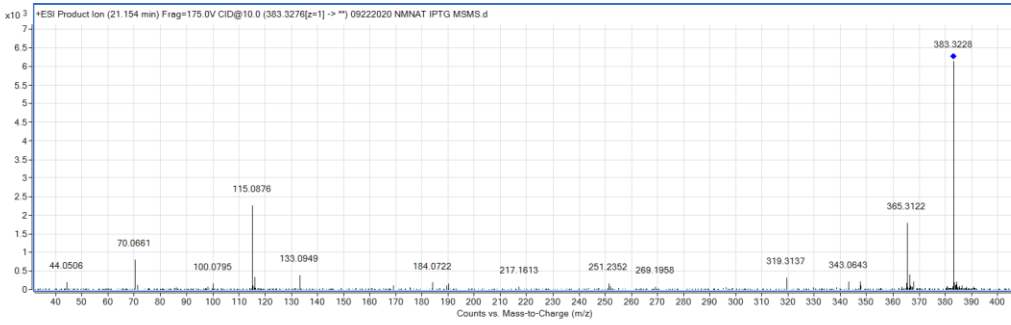
C15:1 Ornithine



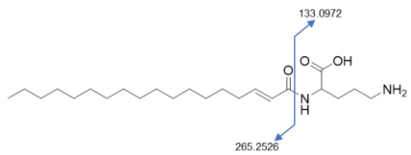
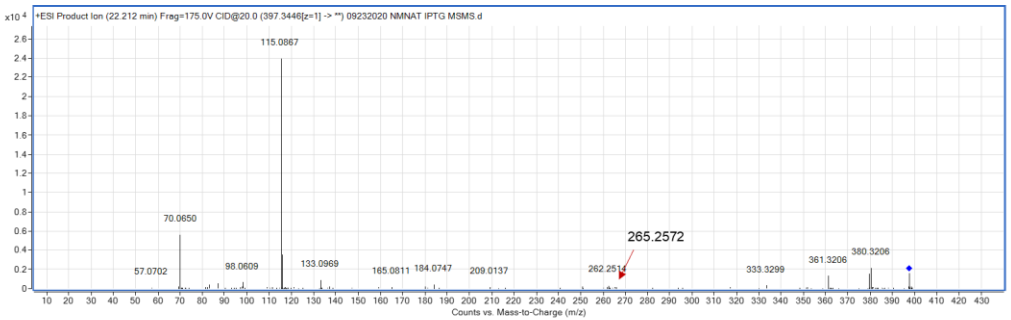
C16:1 Ornithine



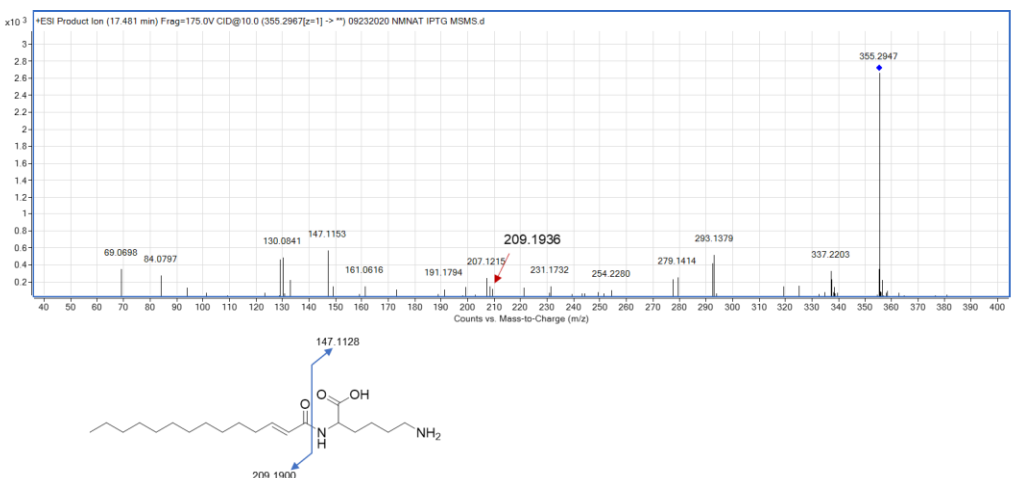
C17:1 Ornithine



C18:1 Ornithine



C14:1 Lysine



C16:1 Lysine

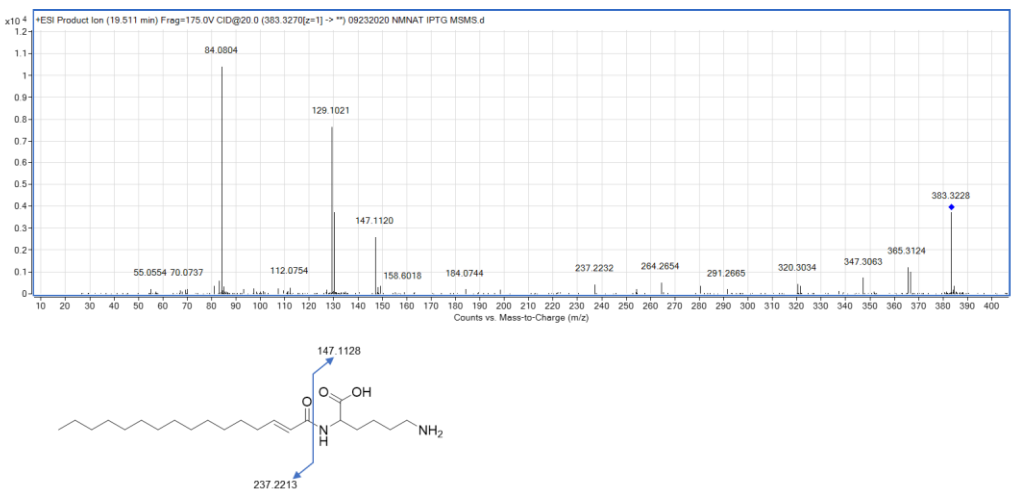


Figure S9. Tandem MS of 24 of the 30 *N*-acyl amides detected from *NmNAT* expression. Corresponding structures show deduced structures, where regiochemistry of hydroxyl groups and unsaturation, as well as stereochemistry of points of unsaturation are assumed. The straight acyl chain is also a simplification, as they may occur as branched chain fatty acids.

Structural elucidation of 3 and 4

Compound	Molecular Formula	Theoretical <i>m/z</i>	Observed <i>m/z</i>	Ppm error
3	C ₁₆ H ₃₁ NO ₃	286.2377	286.2381	1.39744
4	C ₁₉ H ₂₉ NO ₃	320.222	320.2229	2.81055

Table S4. Theoretical and observed HRMS values of compounds 3 and 4

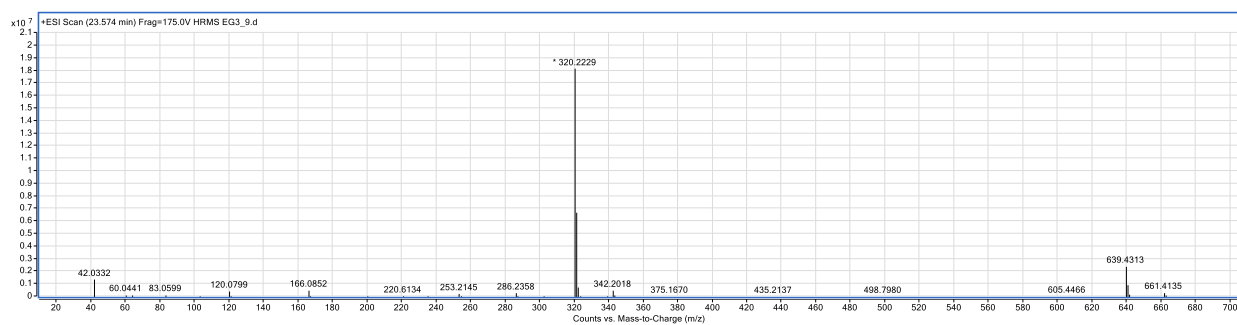
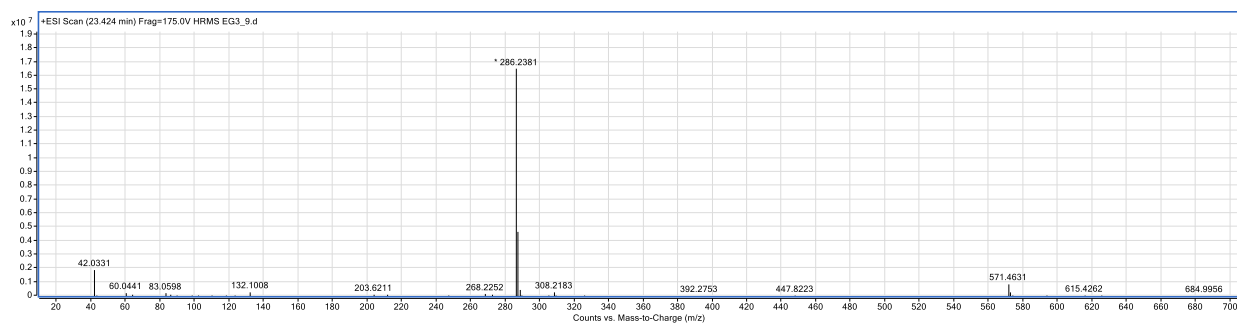


Figure S10. HRMS of compounds **3** and **4**

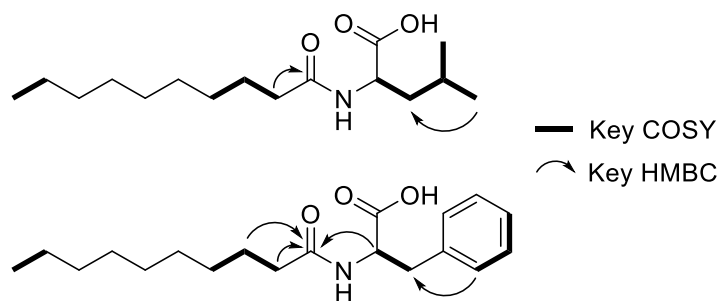


Figure S11. Key COSY and HMBC correlations of natural **3** and **4** identified from a mixture

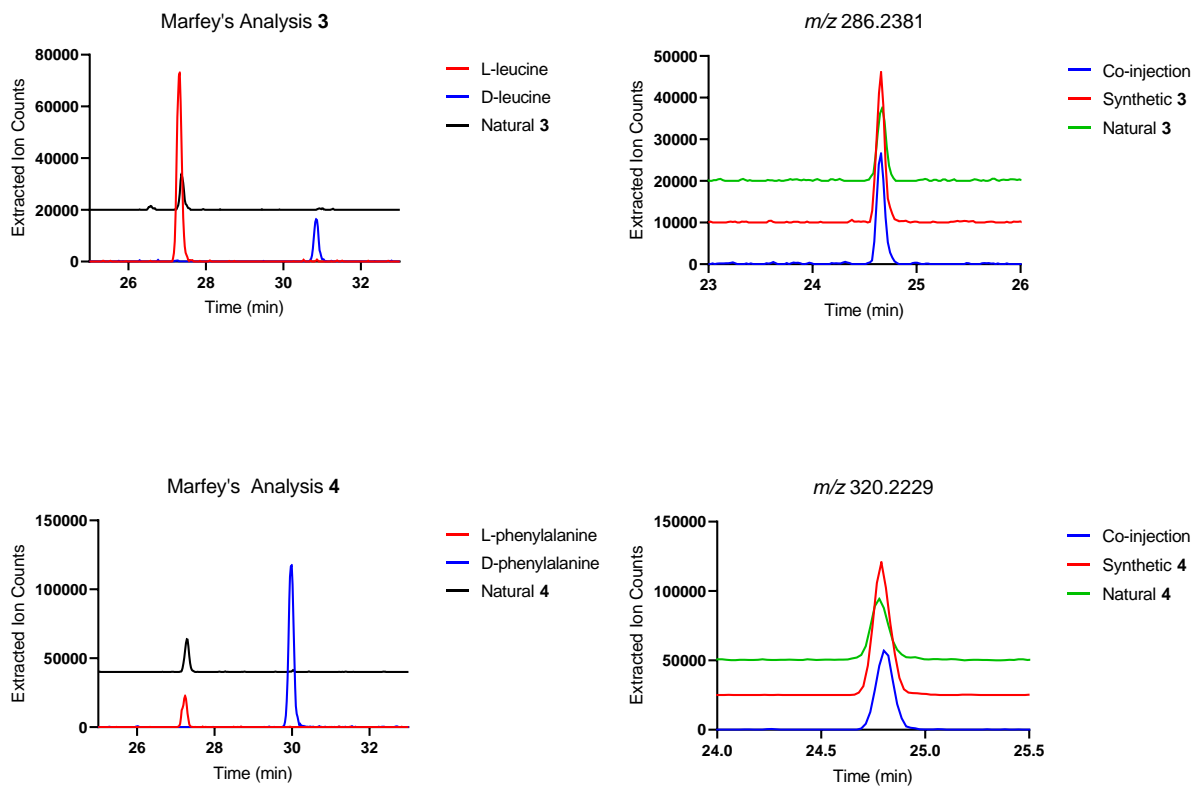


Figure S12. Marfey's analysis for stereochemical elucidation of compounds **3** and **4** (left). Co-injection with synthetic **3** and **4** for validation of structure (right).

***N*-decanoyl-L-leucine (MH+ 286) (CDCl₃, 400 MHz)**

	NH	α H	β H	γ H	δ H
<i>N</i> -decanoyl		2.26	1.64		CH ₂ 1.25-1.35 Terminal CH ₃ 0.89
Leu	8.05	4.62	1.74 1.60	1.73	0.97

	CO	α C	β C	γ C	δ C
<i>N</i> -decanoyl	174.17	36.50	25.61		CH ₂ 21.86, 29.19-31.86 Terminal CH ₃ 14.07
Leu	176.39	50.93	41.16	24.91	22.65 22.83

Table S5. Chemical shifts of synthetic **3** as identified by 1D- (¹H and ¹³C) and 2D- (gCOSY) NMR

N-decanoyl-L-phenylalanine (MH+ 320) (DMSO, 400 MHz)

	NH	α H	β H		
N-decanoyl		2.03	1.39	CH2 1.08-1.32	
Phe	8.07	4.43	2.84	Terminal CH3 0.87	12.65
			3.06	Aromatic	COOH
				7.17-7.29	

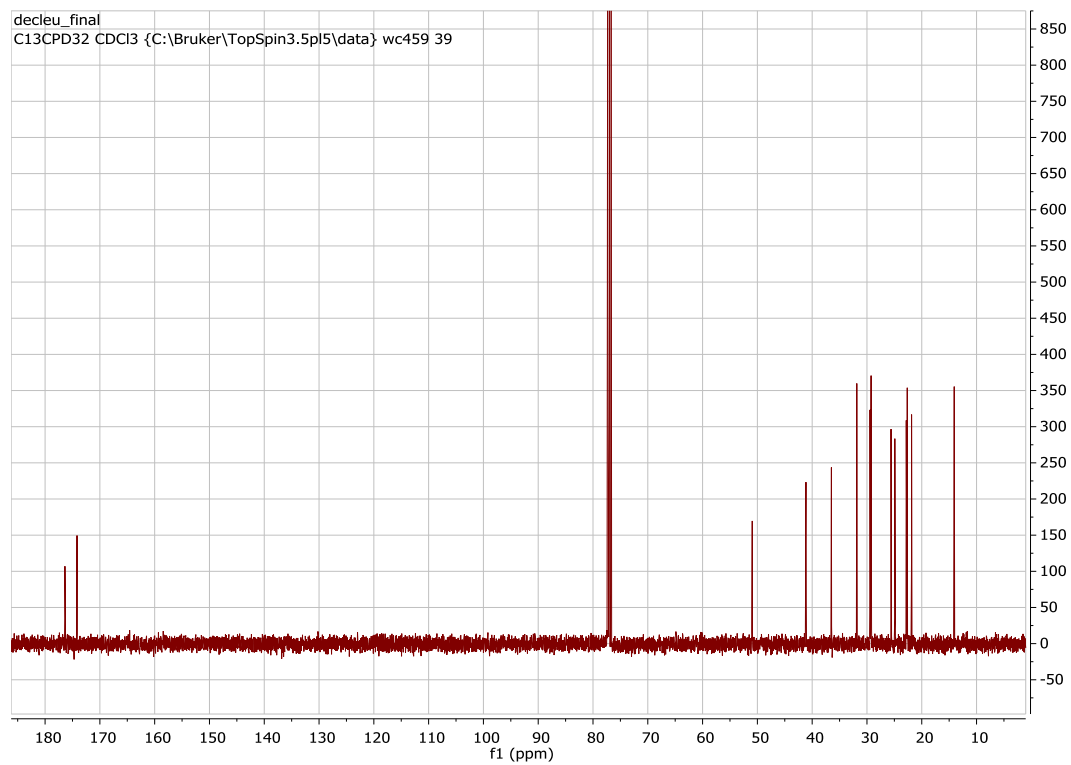
	CO	α C	β C	
N-decanoyl	172.57	35.53	25.63	CH2 22.55-31.69
Phe	173.69	53.77	37.24	Terminal CH3 14.39
				Aromatic
				126.77-138.26

Table S6. Chemical shifts of synthetic **3** as identified by 1D- (^1H and ^{13}C) and 2D- (gCOSY) NMR

A



B



C

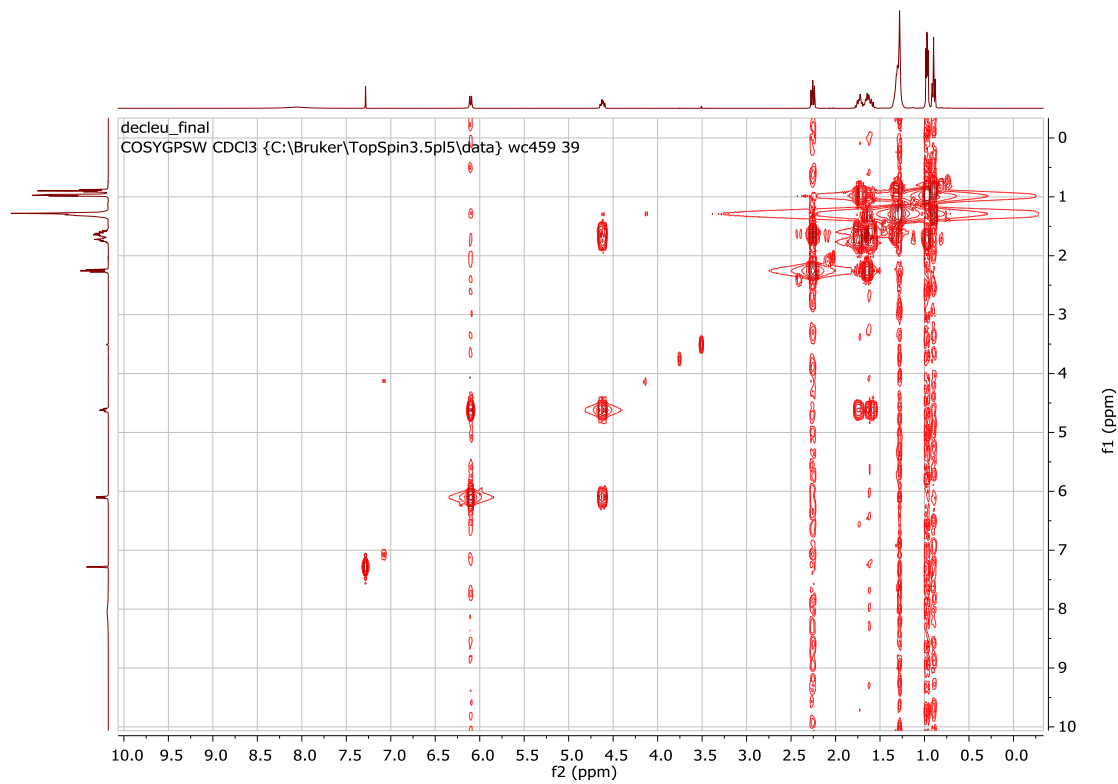
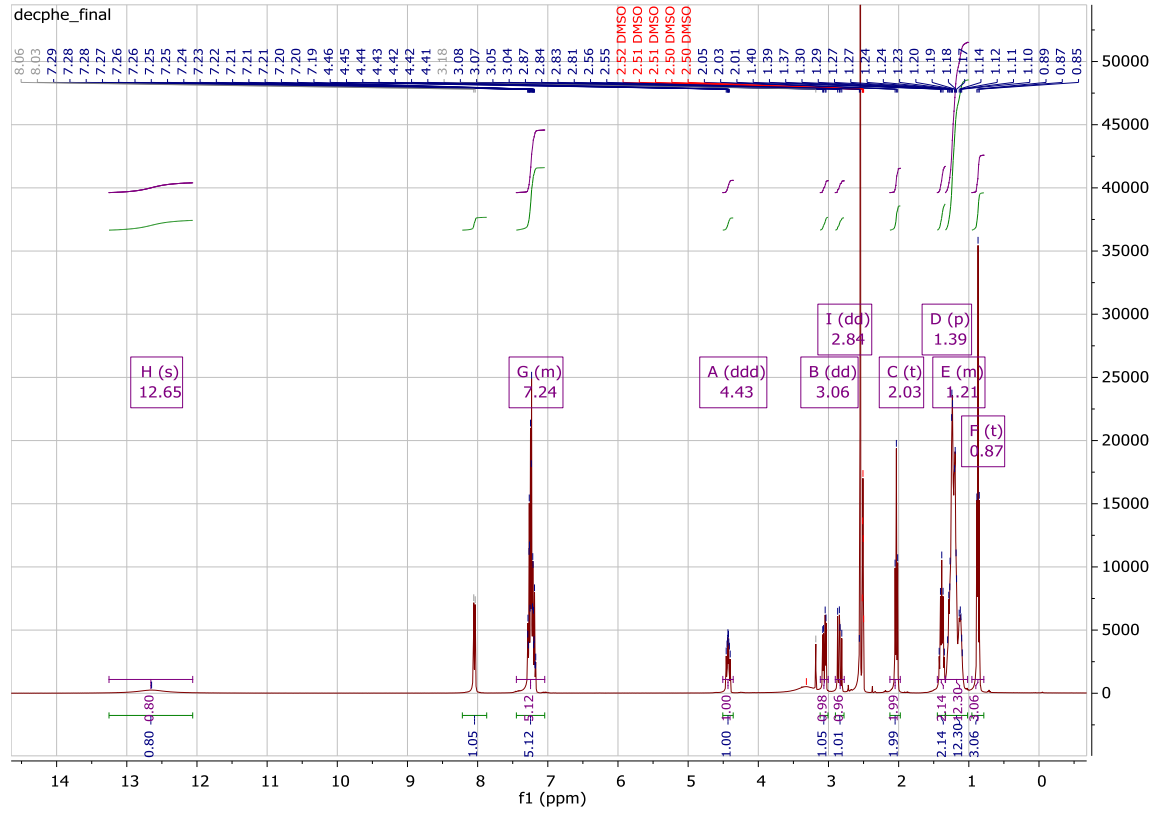
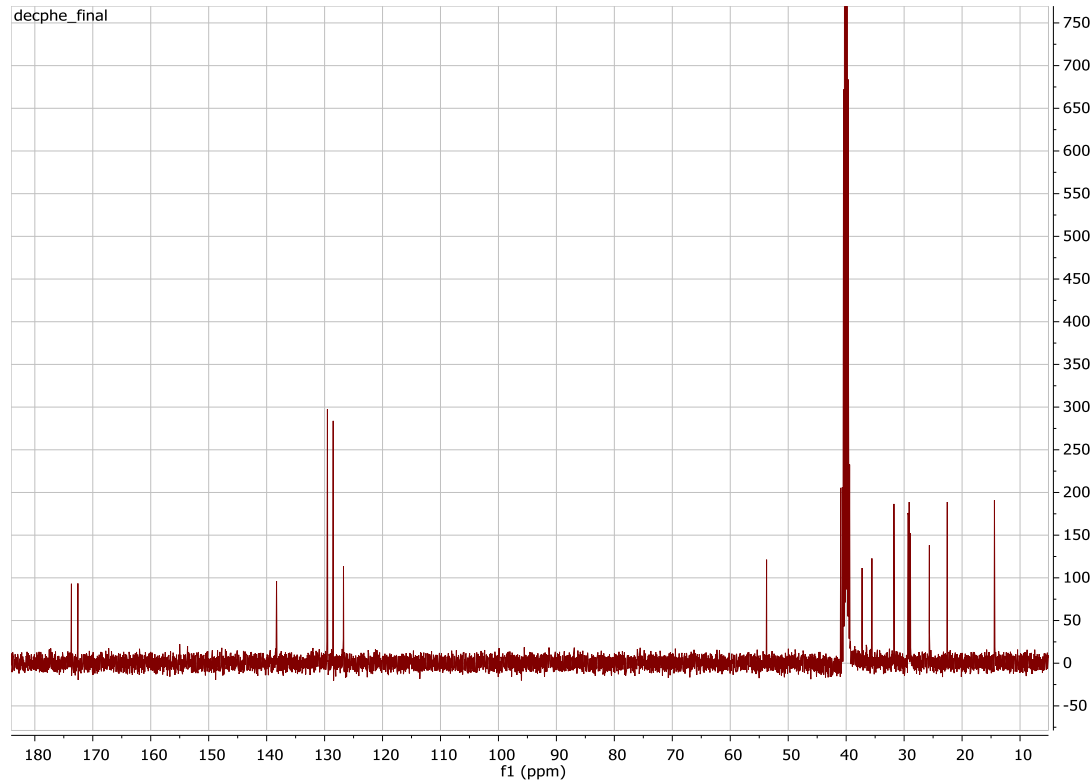


Figure S13. NMR spectra of synthetic compound **3** (CDCl₃, 400 MHz)

A



B



C

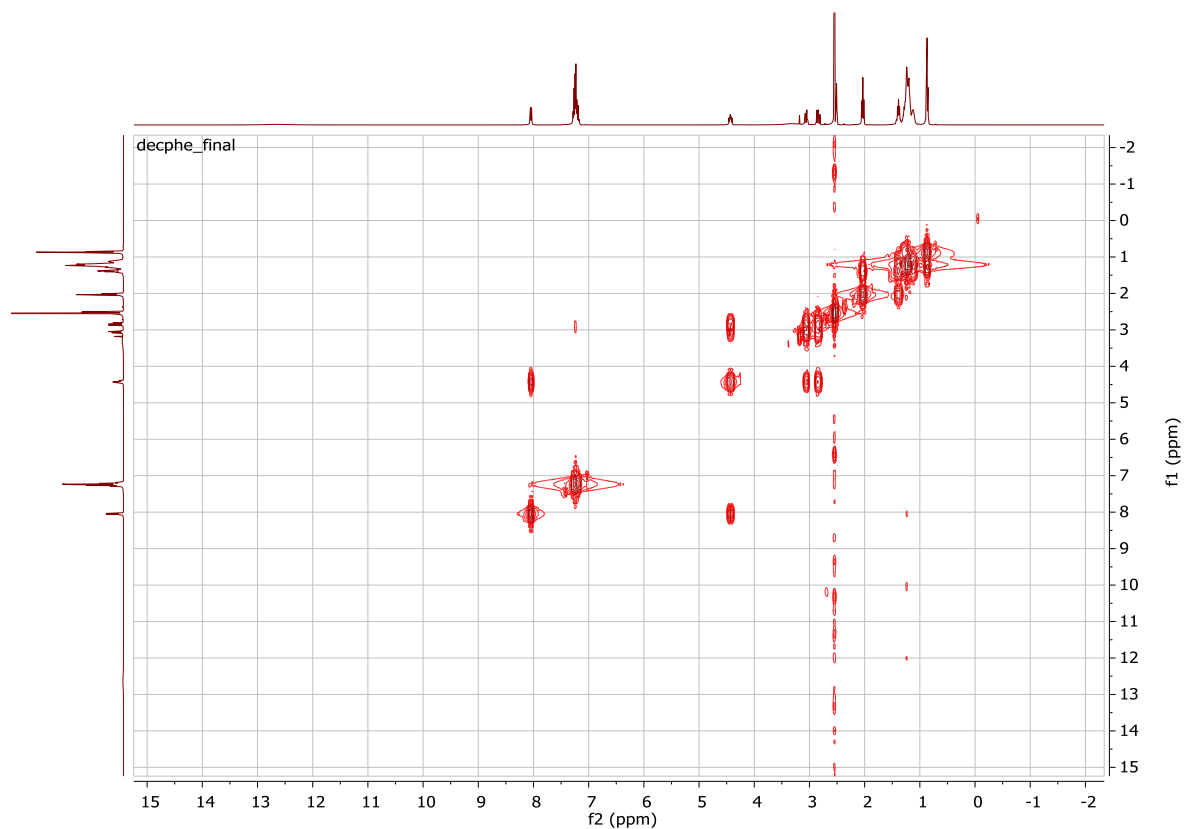


Figure S14. NMR spectra of synthetic compound 4 (DMSO, 600 MHz)

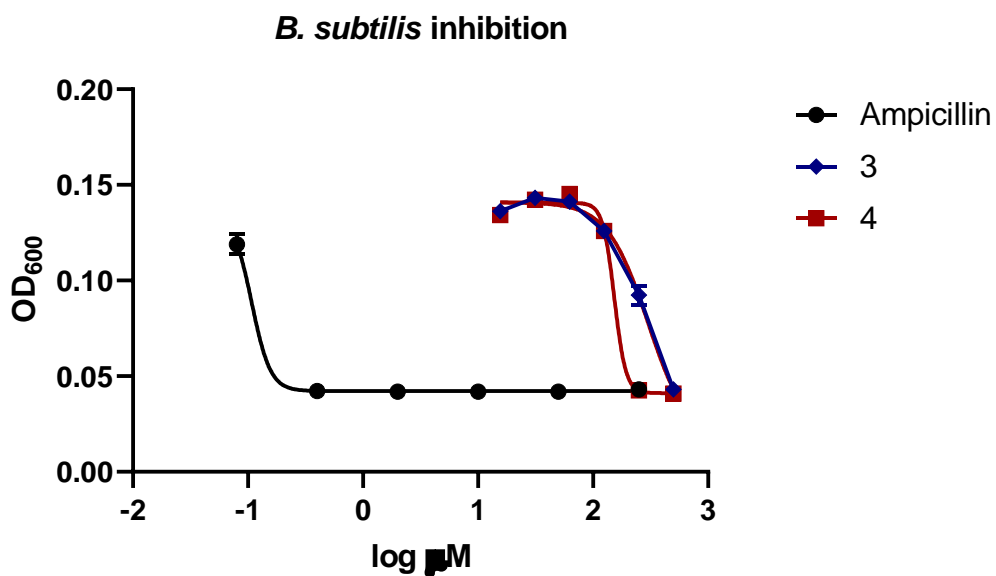


Figure S15. Antibacterial assay of compounds 3 and 4 against *Bacillus subtilis*. IC₅₀ was calculated as 301.2 μM and 153 μM for 3 and 4, respectively, through a variable slope (four parameters) fitting on GraphPad Prism.

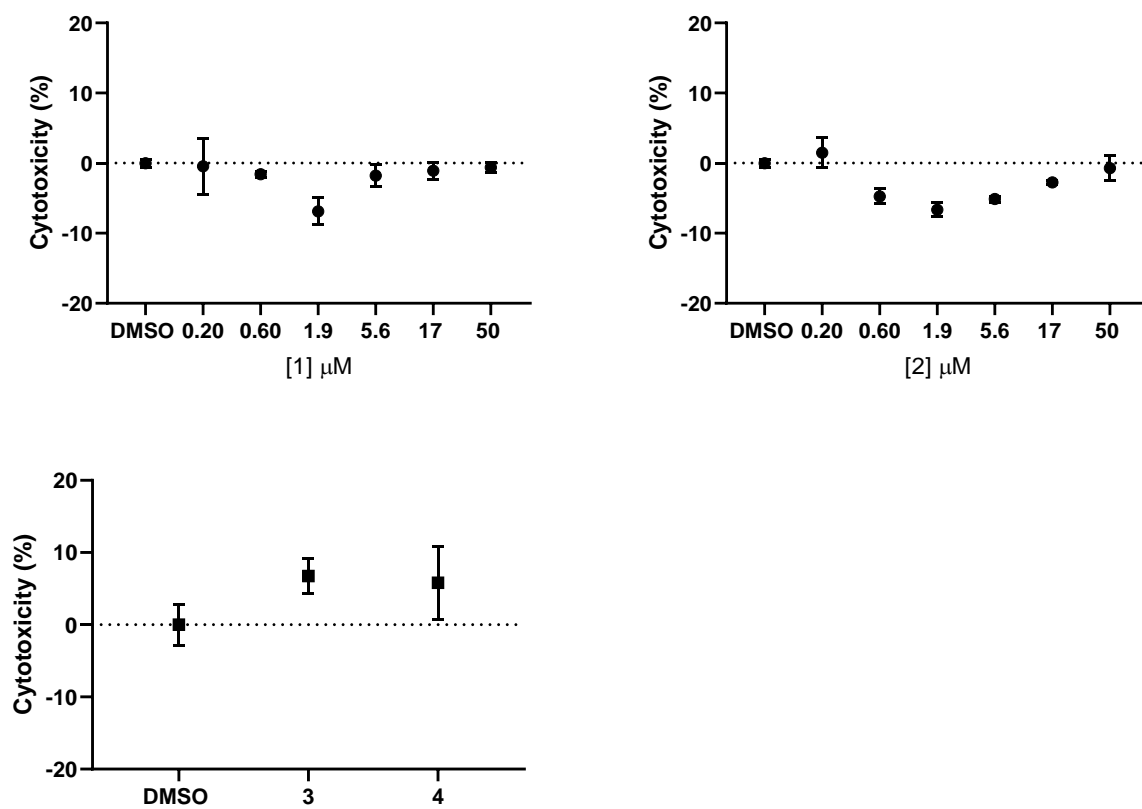


Figure S16. Cytotoxicity (LDH readout) measurements of compounds **1** and **2** at varying concentrations, and of compounds **3** and **4** at fixed concentration of 30 μM .