

New Cytotoxic Secondary Metabolites from Marine Bryozoan *Cryptosula pallasiana*

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Supplementary Information

S1. HR-ESI-MS (positive) spectrum of compound **1**

S2. ESI-MS (positive) spectrum of compound **1**

S3. ¹H-NMR (CDCl₃, 500 MHz) spectrum of compound **1**

S4. ¹³C-NMR (CDCl₃, 125 MHz) spectrum of compound **1**

S5. DEPT135 spectrum of compound **1**

S6. ¹H-¹H COSY spectrum of compound **1**

S7. HSQC spectrum of compound **1**

S8. HMBC spectrum of compound **1**

S9. NOESY spectrum of compound **1**

S10. HR-ESI-MS (positive) spectrum of compound **6**

S11. EI-MS spectrum of methyl tetradecanoate obtained from methanolysis of ceramide **6**

S12. ¹H-NMR (CDCl₃, 500 MHz) spectrum of compound **6**

S13. ¹³C-NMR (CDCl₃, 125 MHz) spectrum of compound **6**

S14. HR-ESI-MS (positive) spectrum of compound **7**

S15. EI-MS spectrum of (*R*)-methyl 2-hydroxytetradecanoate obtained from methanolysis of ceramide

7

S16. ¹H-NMR (CDCl₃, 500 MHz) spectrum of compound **7**

S17. ^{13}C -NMR (CDCl_3 , 125 MHz) spectrum of compound **7**

S18. ^1H -NMR (CDCl_3 , 500 MHz) data of compound **2**

S19. ^1H -NMR (CDCl_3 , 500 MHz) data of compound **3**

S20. ^1H -NMR (CDCl_3 , 500 MHz) data of compound **4**

S21. ^{13}C -NMR (CDCl_3 , 125 MHz) data of compounds **2–4**

S22. ^1H -NMR (CD_3OD , 500 MHz) and ^{13}C NMR (CD_3OD , 125 MHz) data of compound **5**

S23. ^1H -NMR (CDCl_3 , 500MHz), ^{13}C -NMR (CDCl_3 , 125 MHz) and ESI-MS data of compound **8**

S24. ^1H -NMR (CDCl_3 , 500MHz), ^{13}C -NMR (CDCl_3 , 125 MHz) and ESI-MS data of compound **9**

S1. HR-ESI-MS (positive) spectrum of compound 1

Elemental Composition Report

Multiple Mass Analysis: 2 mass(es) processed

Tolerance = 30.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: None

Monoisotopic Mass, Even Electron Ions

16 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 5-34 H: 10-55 O: 1-2 Na: 1-1

SIPI

XR-73 M.W=414

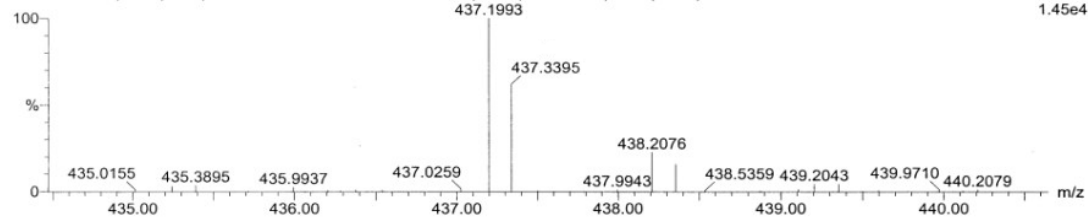
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26-Mar-2011, 15:53:57

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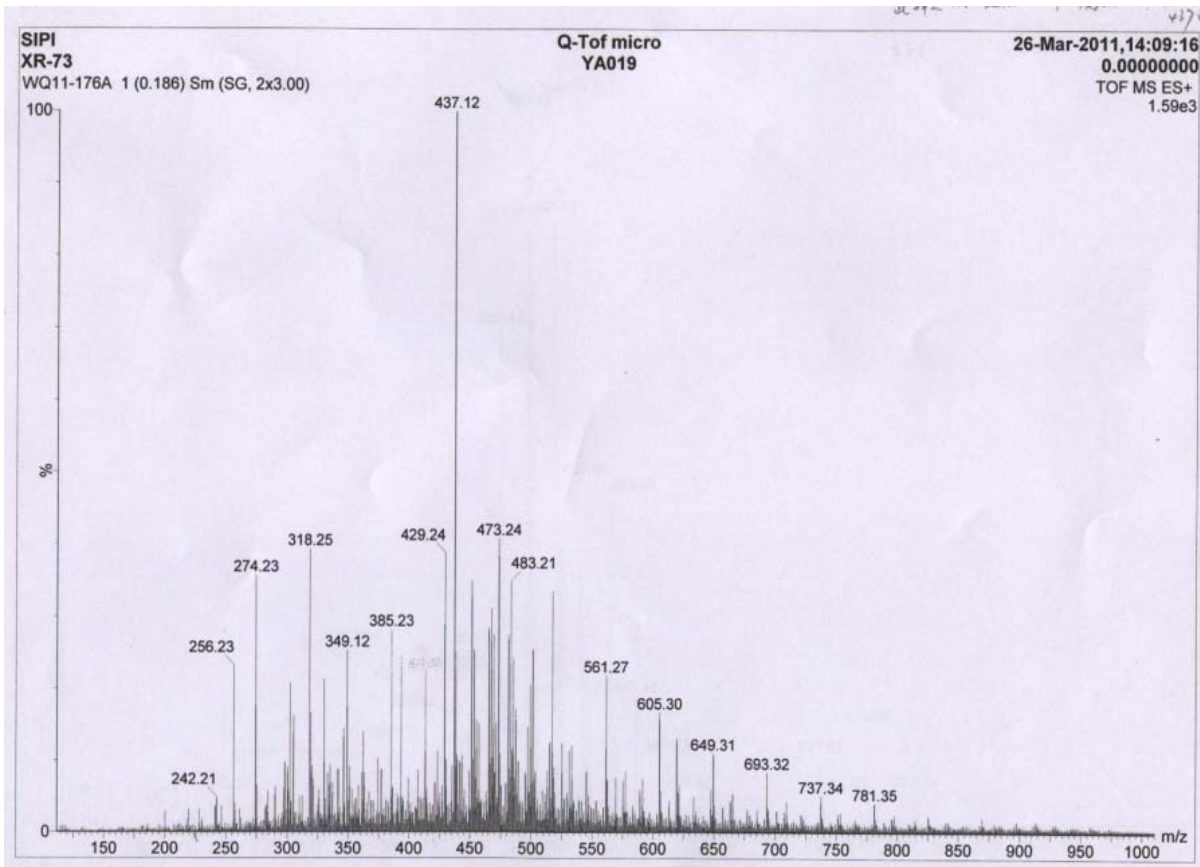
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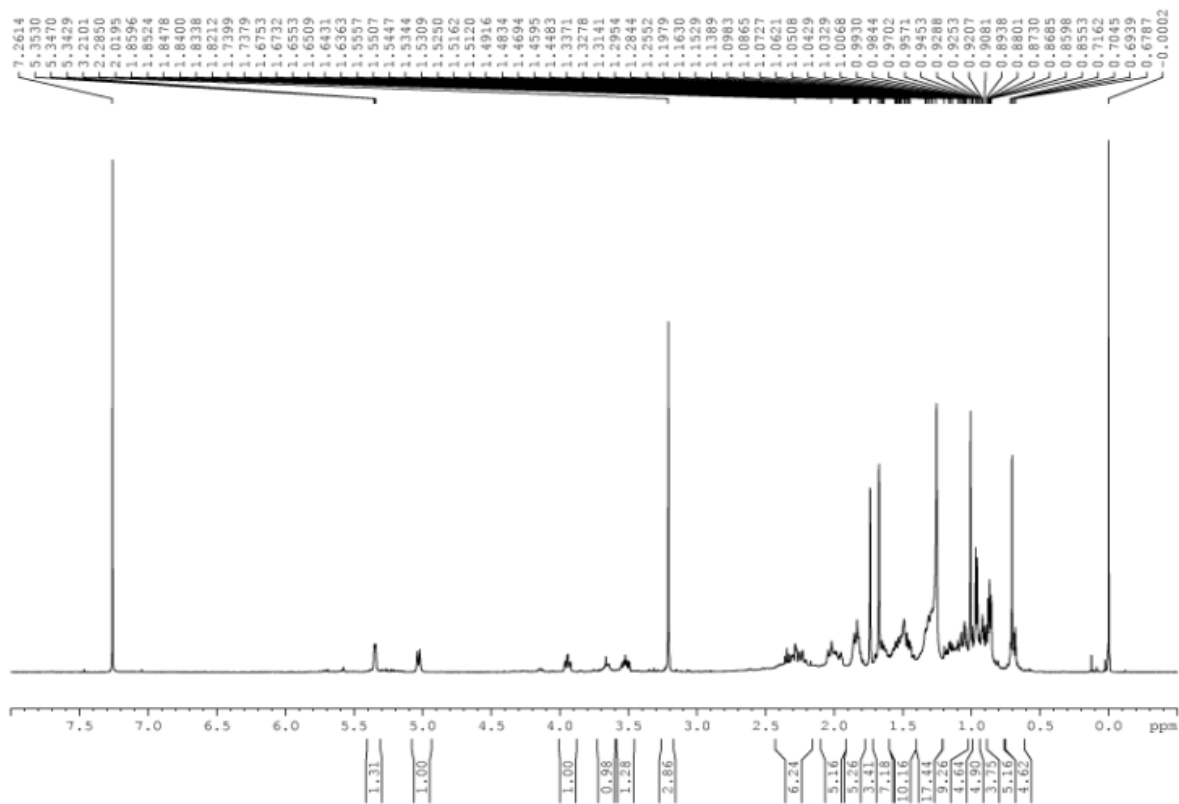
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437.1993	100.00	437.1881	11.5	26.3	18.5	378.3	C31 H26 O Na
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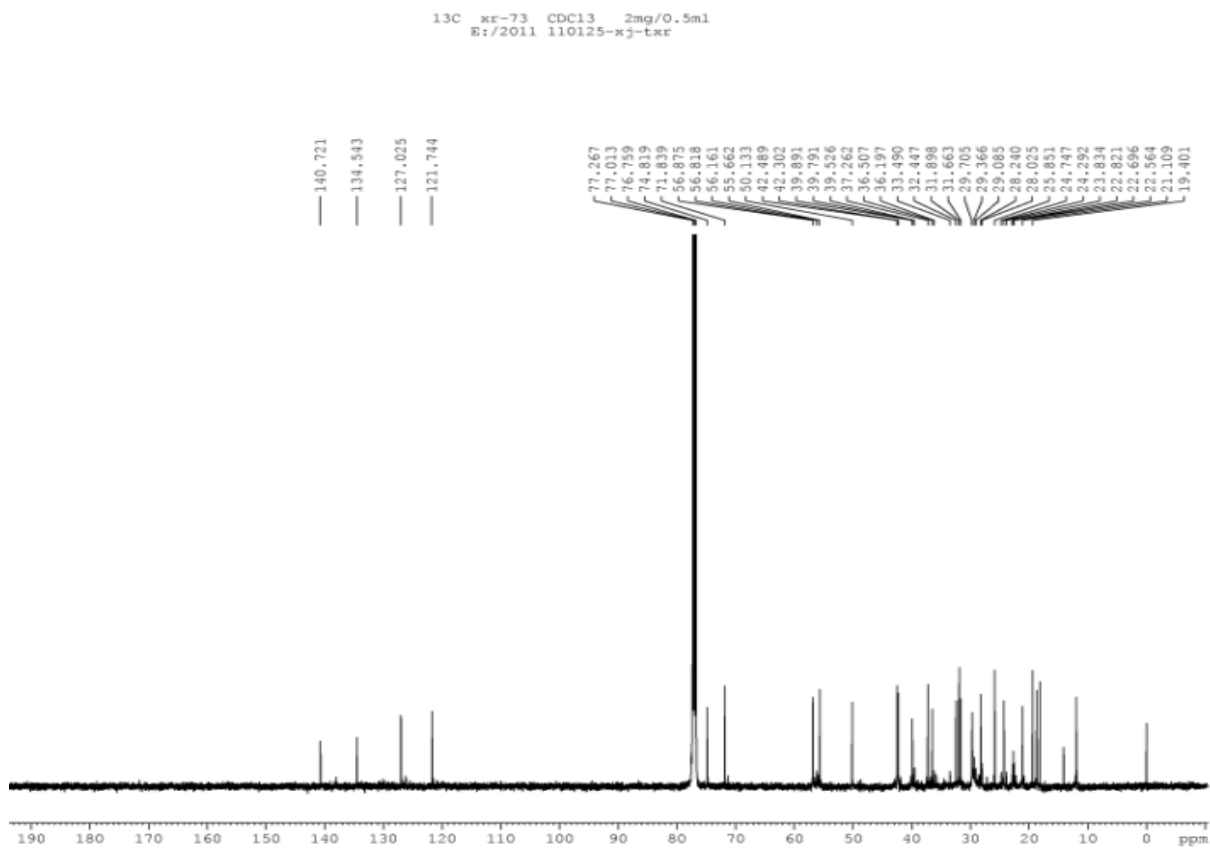
S2. ESI-MS (positive) spectrum of compound 1



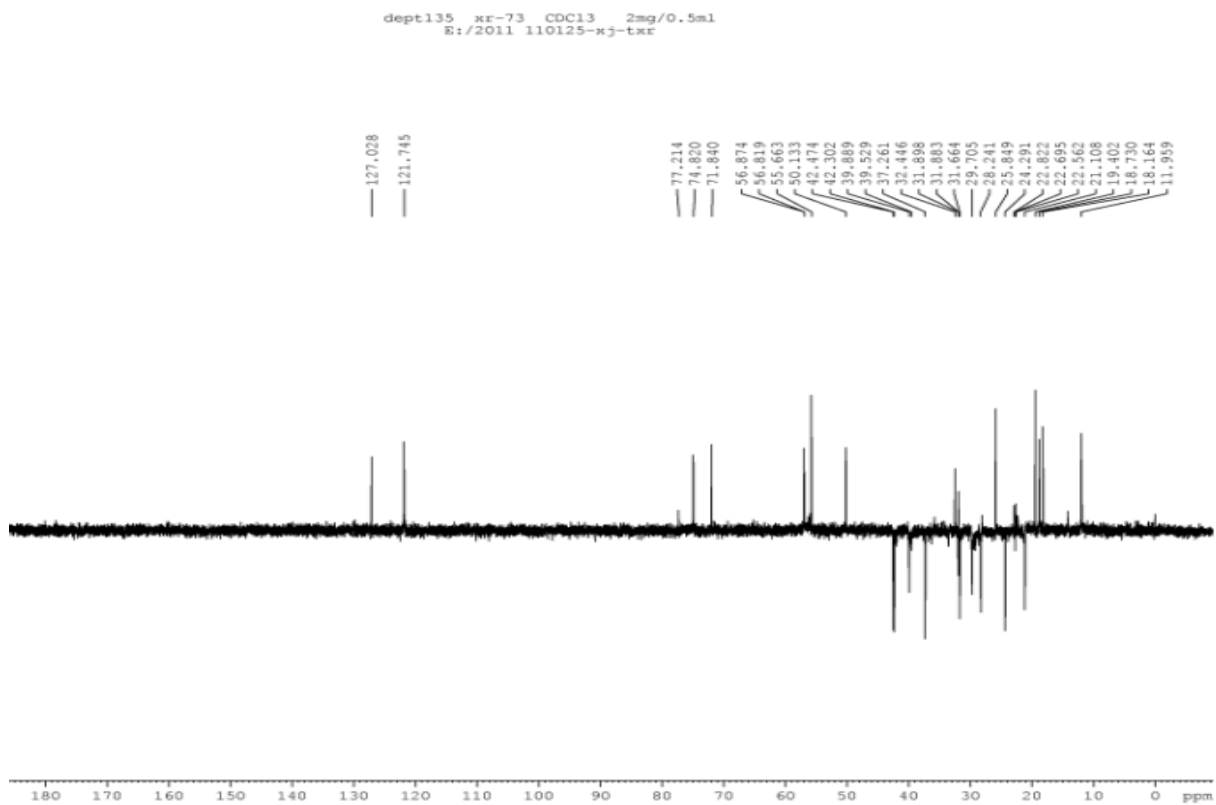
S3. ¹H-NMR (CDCl₃, 500 MHz) spectrum of compound 1



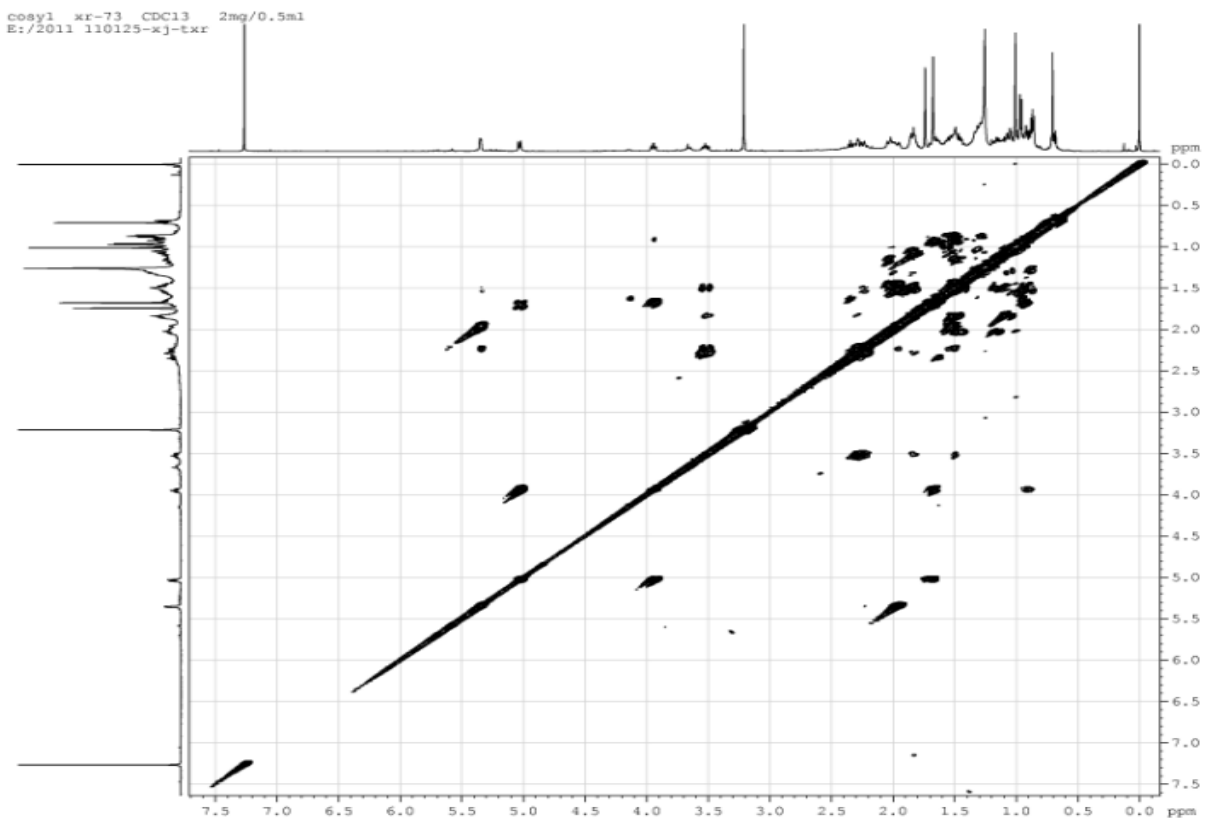
S4. ¹³C-NMR (CDCl₃, 125 MHz) spectrum of compound 1



S5. DEPT135 spectrum of compound 1

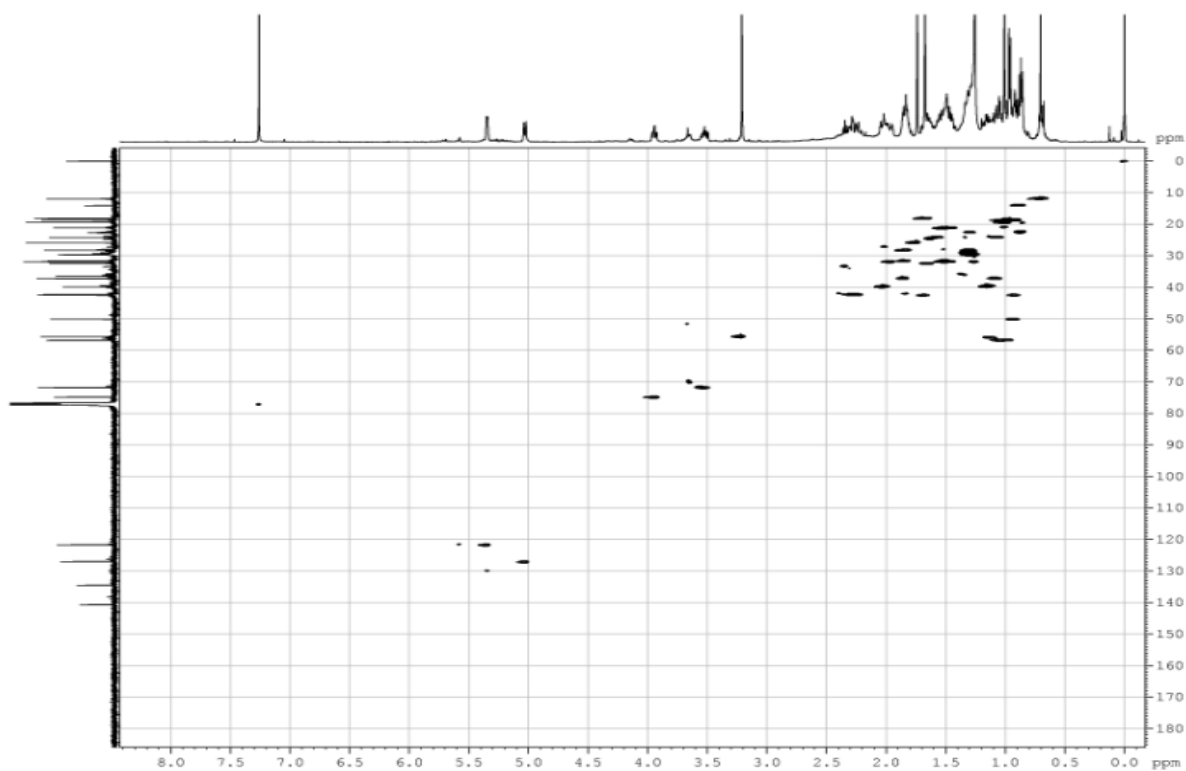


S6. ¹H-¹H COSY spectrum of compound 1



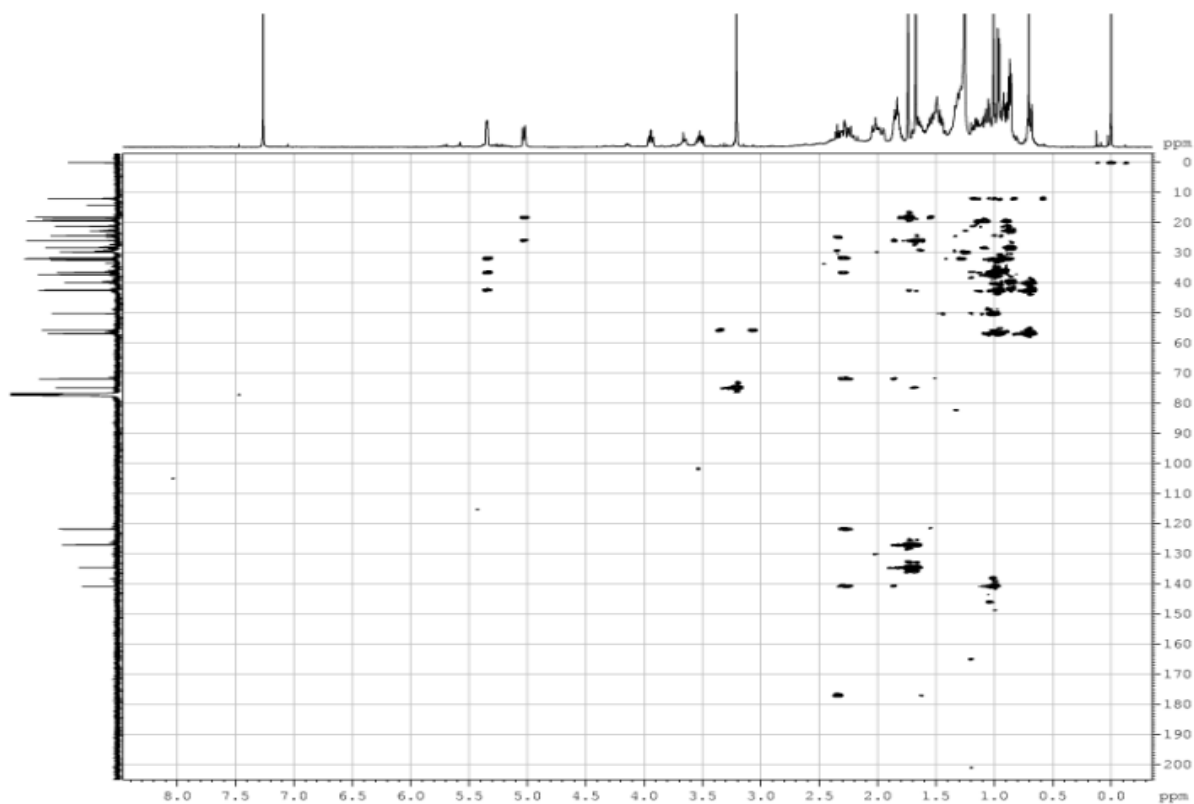
S7. HSQC spectrum of compound 1

HSQC xr-73 CDC13 2mg/0.5ml
E:/2011 110125-xj-txr



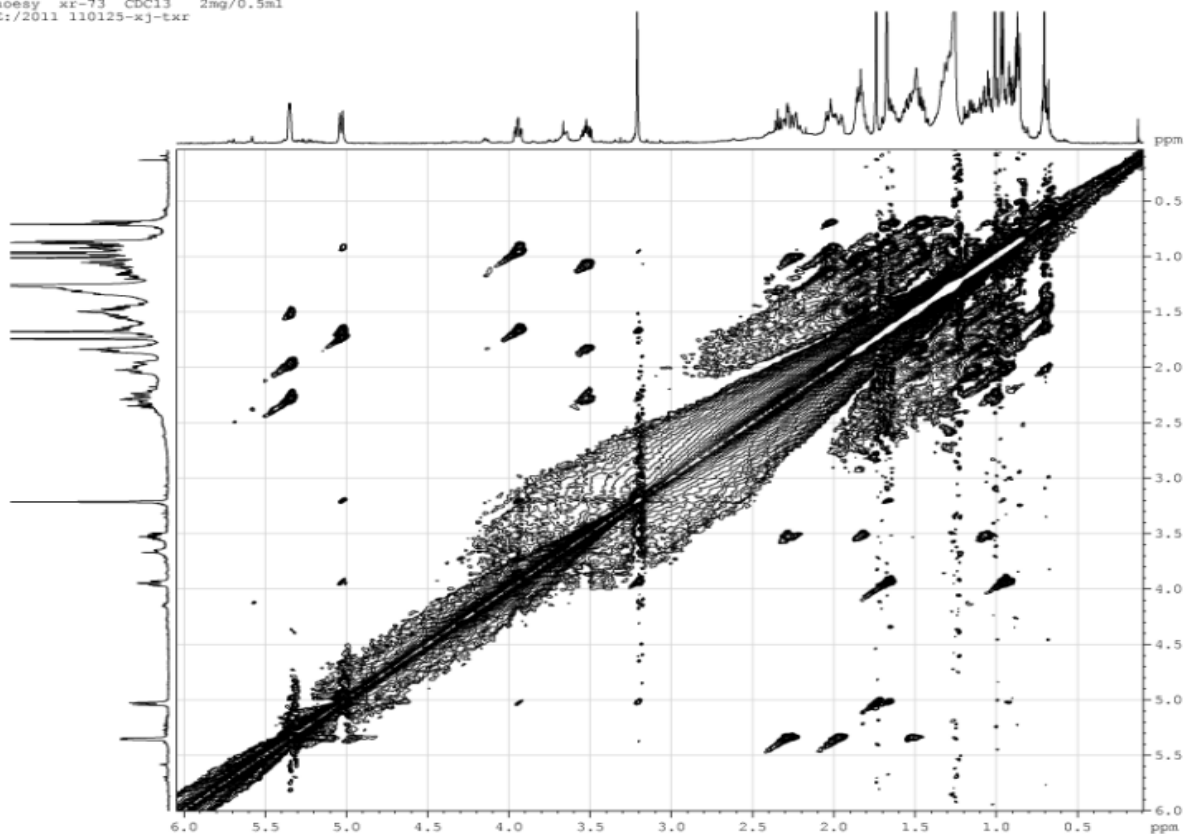
S8. HMBC spectrum of compound 1

hmhc xr-73 CDC13 2mg/0.5ml
E:/2011 110125-xj-txr



S9. NOESY spectrum of compound 1

noesy xr-73 CDCl3 2mg/0.5ml
E:/2011 110125-xj-txr



S10. HR-ESI-MS (positive) spectrum of compound 6

Elemental Composition Report

Page 1

Multiple Mass Analysis: 2 mass(es) processed

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: None

Monoisotopic Mass, Even Electron Ions

16 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C:10-35 H:5-57 N:1-1 O:1-6 Na:1-1

SIP1

XR-70 M.W=507

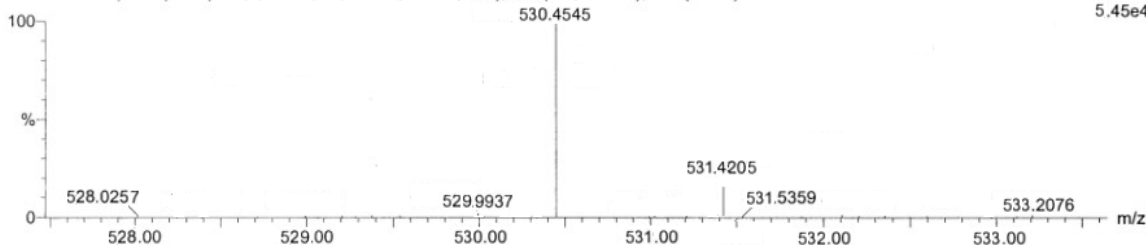
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26-Mar-2011, 15:57:53

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TOF MS ES+

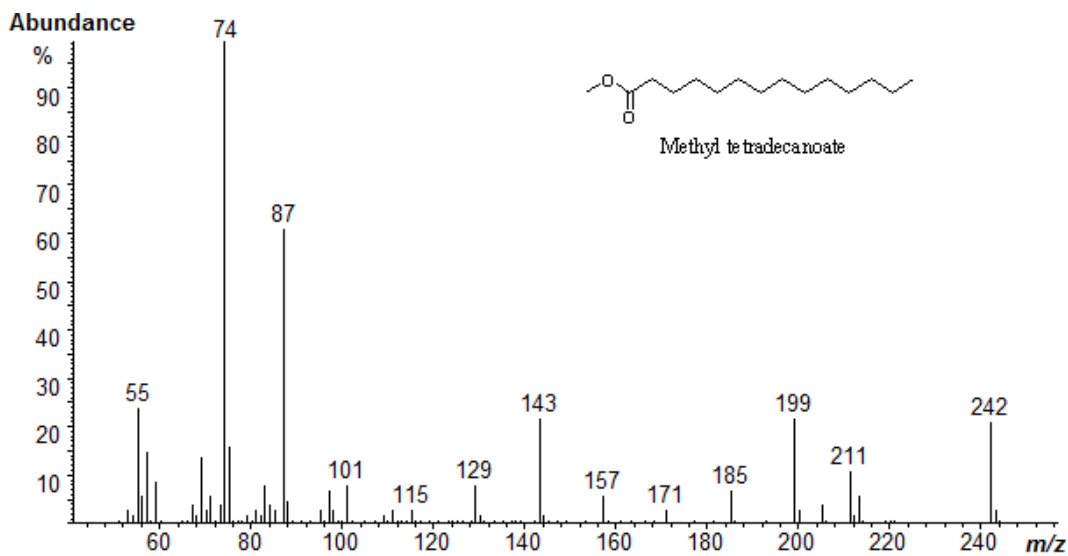
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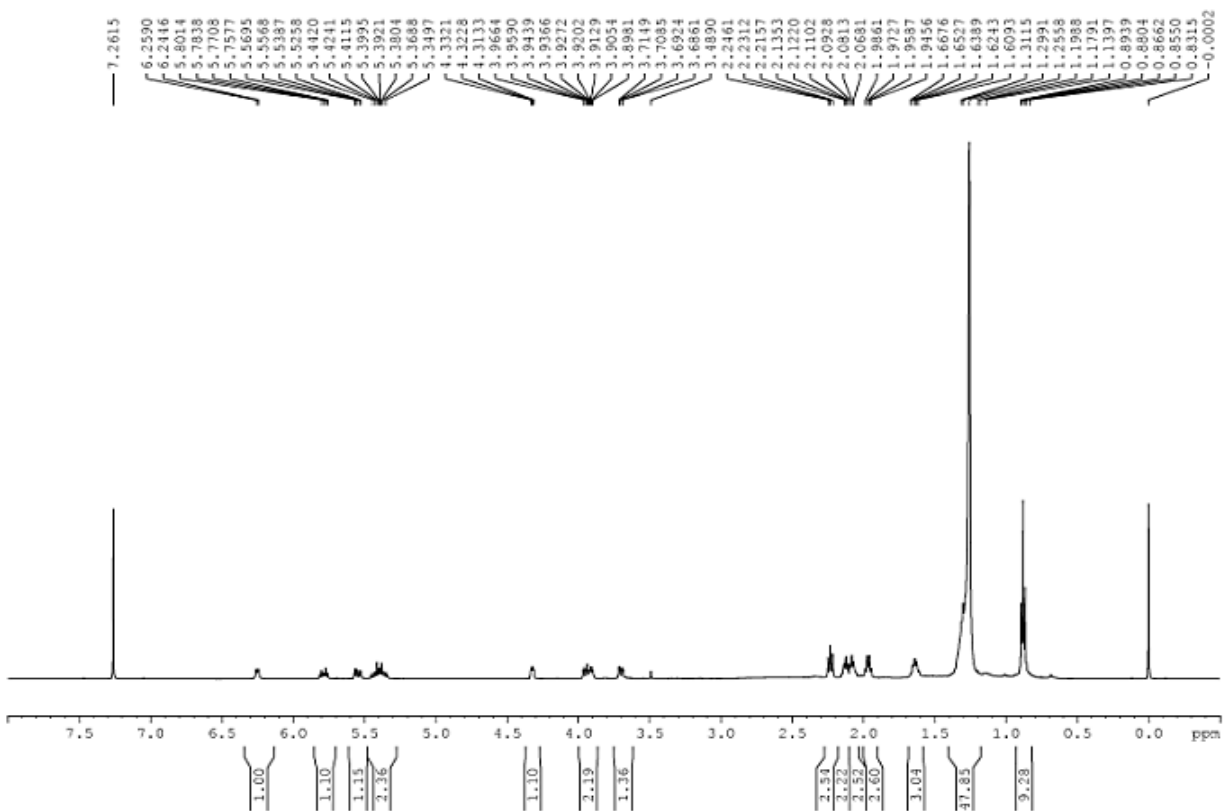
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S11. EI-MS spectrum of methyl tetradecanoate obtained from methanolysis of ceramide 6

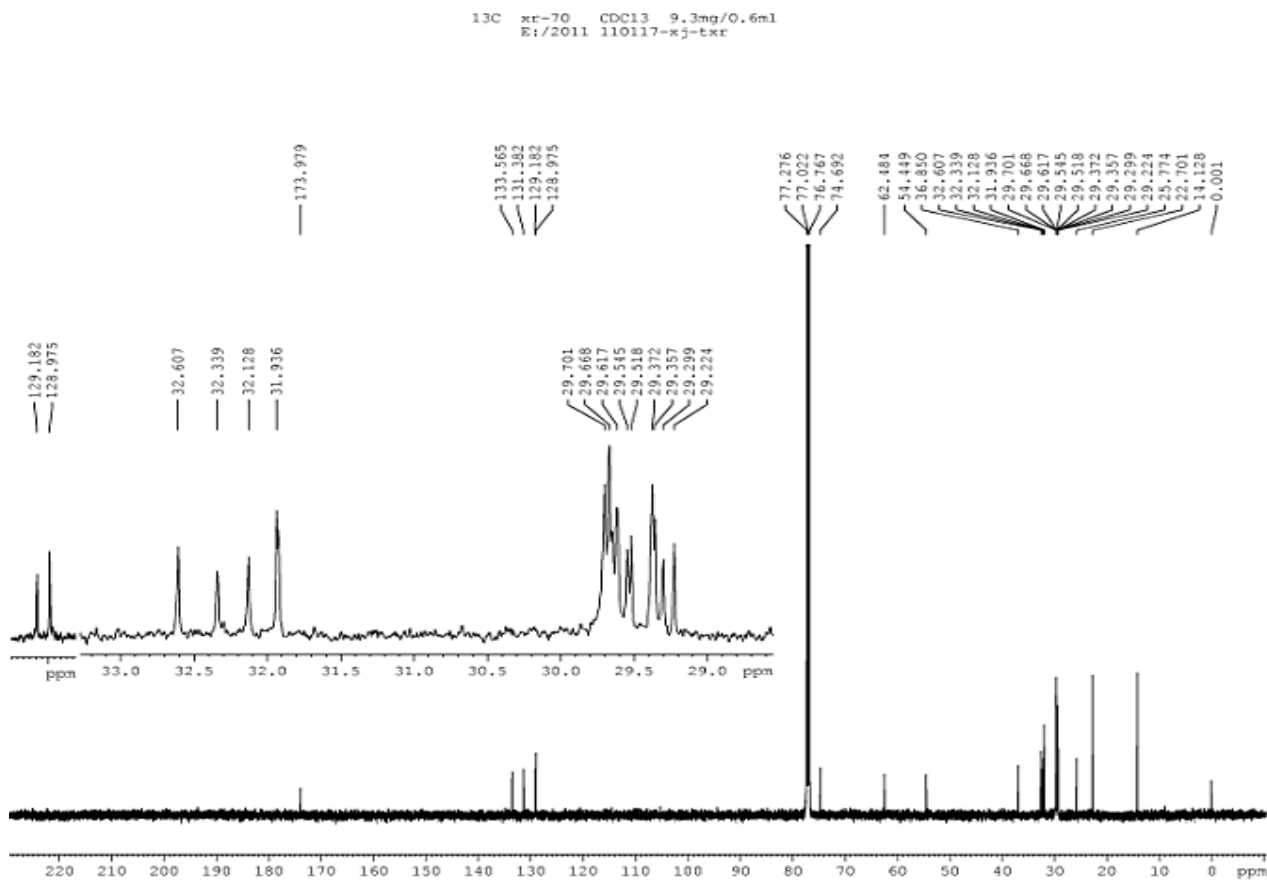


S12. ¹H-NMR (CDCl₃, 500 MHz) spectrum of compound 6

¹H_xr-70 CDCl₃ 9.3mg/0.6ml
E:/2011 110117-xj-txr



S13. ¹³C-NMR (CDCl₃, 125 MHz) spectrum of compound 6



S14. HR-ESI-MS (positive) spectrum of compound 7

Elemental Composition Report

Multiple Mass Analysis: 2 mass(es) processed

Tolerance = 1 0.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions

16 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C:10-35 H:5-57 N:1-1 O:1-6 Na:1-1

SIPI

XR-71 M.W= 523

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Q-ToF micro

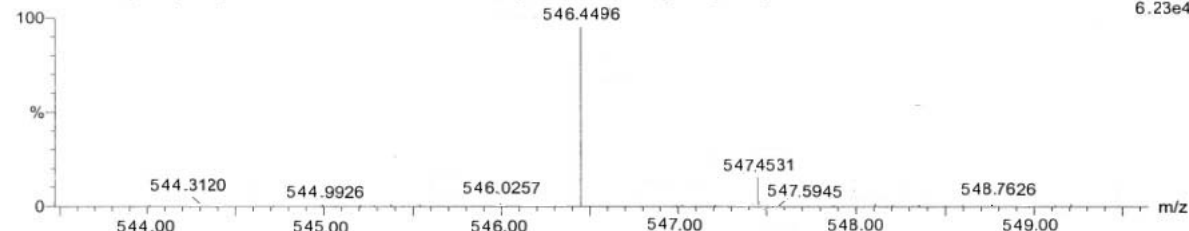
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26-Mar-2011,16:25:53

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TOF MS ES+

6.23e4

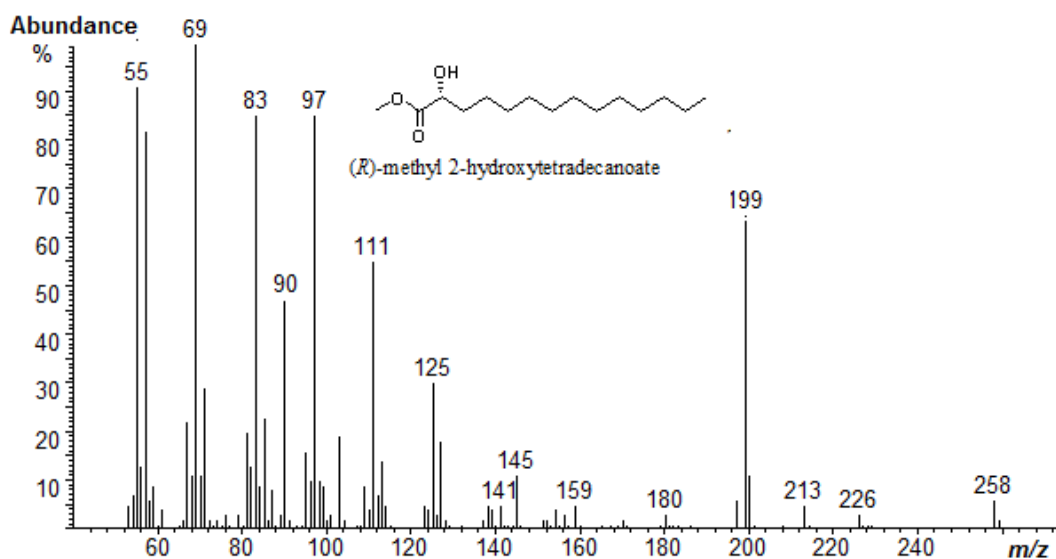
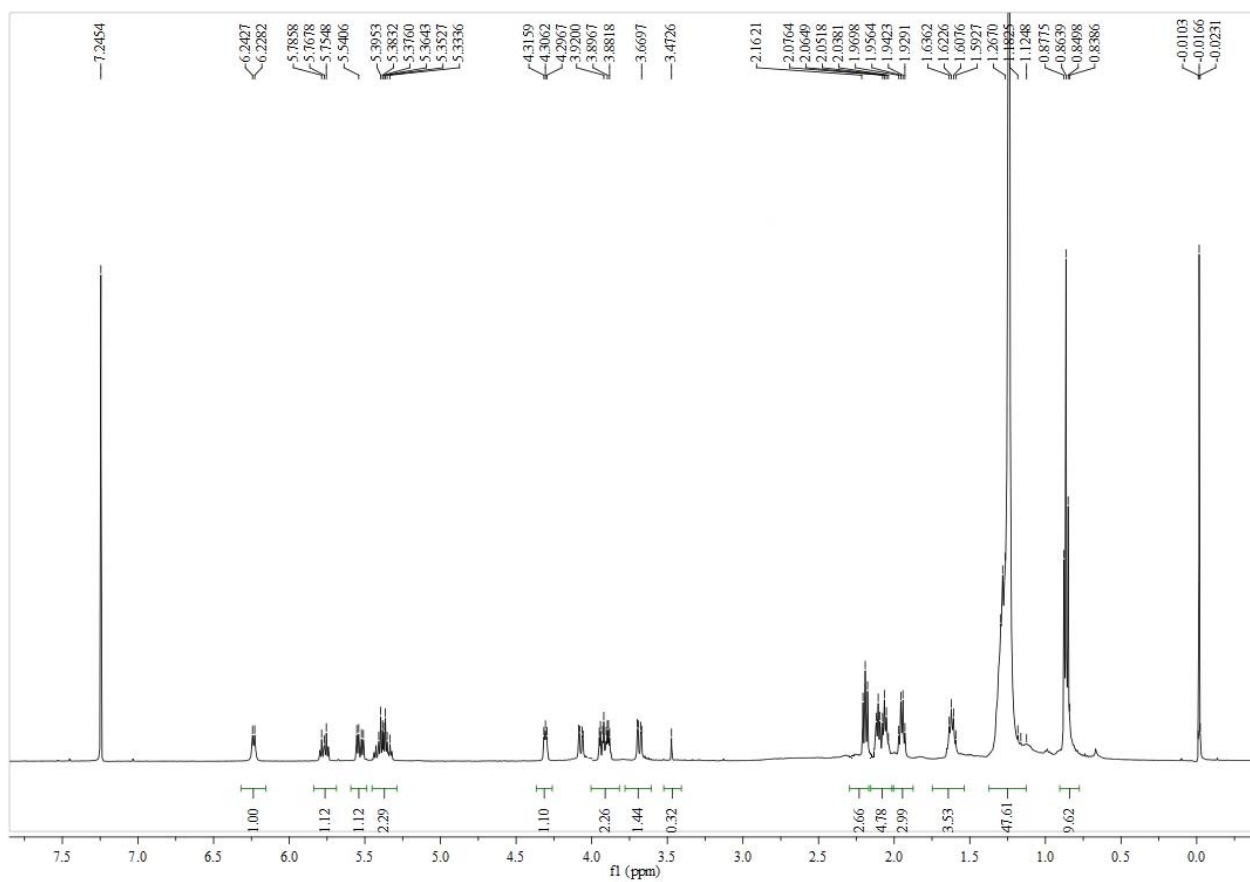


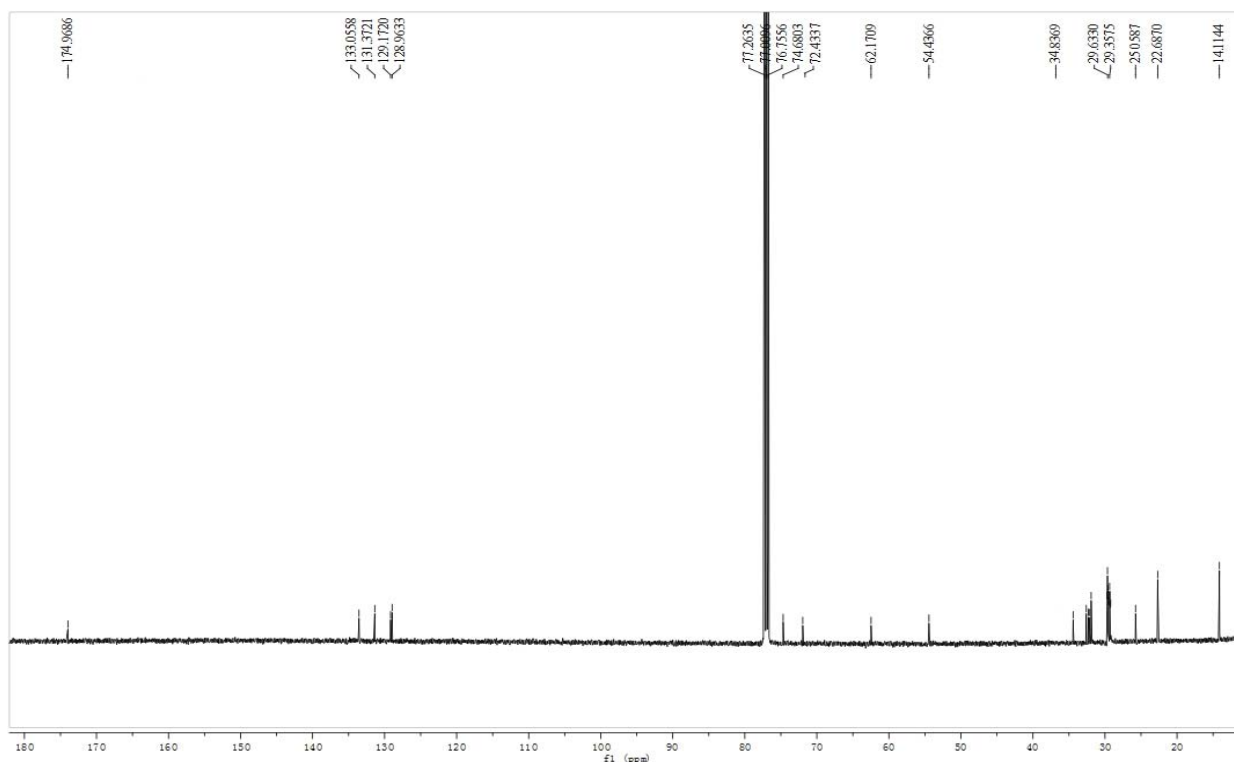
Minimum: 45.00
Maximum: 100.00

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
546.4496	100.00	546.4498	-0.2	4.5	16.8	30.5	C32 H61 N O4 Na

S15. EI-MS spectrum of (R)-Methyl 2-hydroxytetradecanoate obtained from methanolysis of ceramide

7

**S16.** $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) spectrum of compound 7

S17. ^{13}C -NMR (CDCl_3 , 125 MHz) spectrum of compound **7****S18.** ^1H -NMR (CDCl_3 , 500MHz) data of compound **2**

^1H -NMR (500MHz, CDCl_3) δ : 5.35 (1H, *m*, H-6), 3.52 (1H, *m*, H-3), 1.00 (3H, *s*, H₃-19), 0.91 (3H, *d*, $J = 6.5$ Hz, H₃-21), 0.87 (3H, *d*, $J = 6.5$ Hz, H₃-26), 0.86 (3H, *d*, $J = 6.5$ Hz, H₃-27), 0.68 (3H, *s*, H₃-18).

S19. ^1H -NMR (CDCl_3 , 500MHz) data of compound **3**

^1H NMR (500 MHz, CDCl_3) δ : 5.35 (1H, *t*, $J = 2.6$ Hz, H-7), 5.28 (1H, *dd*, $J = 15.3, 6.7$ Hz, H-23), 5.20 (1H, *dd*, $J = 15.5, 7.0$ Hz, H-22), 4.08 (1H, *m*, H-3), 3.63 (1H, *d*, $J = 5.1$ Hz, H-6), 1.18 (3H, *s*, H₃-19), 1.00 (3H, *d*, $J = 6.6$ Hz, H₃-21), 0.86 (6H, *d*, $J = 6.6$ Hz, H₃-26, H₃-27), 0.69 (3H, *s*, H-18).

S20. ^1H -NMR (CDCl_3 , 500MHz) data of compound **4**

^1H -NMR (500 MHz, CDCl_3) δ : 5.35 (1H, *m*, H-7), 5.21 (1H, *dd*, $J = 15.2, 7.5$ Hz, H-23), 5.16 (1H, *dd*, $J = 15.1, 7.4$ Hz, H-22), 4.07 (1H, *m*, H-3), 3.63 (1H, *d*, $J = 5.1$ Hz, H-6), 1.09 (3H, *s*, H₃-19), 1.02 (3H, *d*, $J = 6.7$ Hz, H₃-21), 0.92 (3H, *d*, $J = 6.8$ Hz, H₃-28), 0.84 (3H, *d*, $J = 6.4$ Hz, H₃-26), 0.82 (3H, *d*, $J = 6.5$ Hz, H₃-27), 0.60 (3H, *s*, H₃-18).

S21. ^{13}C -NMR (CDCl_3 , 125 MHz) data of compounds **2 – 4** †

NO.	2	3	4
	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)
1	37.3 <i>t</i>	33.0 <i>t</i>	33.0 <i>t</i>
2	31.7 <i>t</i>	30.8 <i>t</i>	30.9 <i>t</i>
3	71.8 <i>d</i>	67.8 <i>d</i>	67.8 <i>d</i>
4	42.3 <i>t</i>	39.5 <i>t</i>	39.2 <i>t</i>
5	140.8 <i>s</i>	76.1 <i>s</i>	76.1 <i>s</i>
6	121.7 <i>d</i>	73.7 <i>d</i>	73.7 <i>d</i>
7	31.9 <i>t</i>	117.5 <i>d</i>	117.5 <i>d</i>
8	31.9 <i>d</i>	144.1 <i>s</i>	144.0 <i>s</i>
9	50.1 <i>d</i>	43.5 <i>d</i>	43.5 <i>d</i>
10	36.5 <i>s</i>	37.1 <i>s</i>	37.2 <i>s</i>
11	21.1 <i>t</i>	22.1 <i>t</i>	22.1 <i>t</i>
12	39.8 <i>t</i>	39.8 <i>t</i>	39.5 <i>t</i>
13	42.3 <i>s</i>	43.8 <i>s</i>	43.8 <i>s</i>
14	56.8 <i>d</i>	54.7 <i>d</i>	54.8 <i>d</i>
15	24.3 <i>t</i>	22.9 <i>t</i>	22.9 <i>t</i>
16	28.2 <i>t</i>	28.6 <i>t</i>	28.2 <i>t</i>
17	56.2 <i>d</i>	56.0 <i>d</i>	55.9 <i>d</i>
18	11.9 <i>q</i>	12.3 <i>q</i>	12.3 <i>q</i>
19	19.4 <i>q</i>	18.8 <i>q</i>	18.8 <i>q</i>
20	35.8 <i>d</i>	40.2 <i>d</i>	40.5 <i>d</i>
21	18.7 <i>q</i>	20.8 <i>q</i>	21.1 <i>q</i>
22	36.2 <i>t</i>	138.1 <i>d</i>	135.6 <i>d</i>
23	23.8 <i>t</i>	126.3 <i>d</i>	132.3 <i>d</i>
24	39.5 <i>t</i>	42.0 <i>t</i>	43.1 <i>d</i>
25	28.3 <i>d</i>	28.5 <i>d</i>	33.2 <i>d</i>
26	22.8 <i>q</i>	22.2 <i>q</i>	19.7 <i>q</i>
27	22.6 <i>q</i>	22.3 <i>q</i>	20.2 <i>q</i>
28			18.0 <i>q</i>

† Assignments aided by DEPT experiments and literatures.

S22. $^1\text{H-NMR}$ (CD_3OD , 500 MHz) and $^{13}\text{C-NMR}$ (CD_3OD , 125 MHz) data of compound **5**

$^1\text{H-NMR}$ (CD_3OD , 500 MHz) δ : 5.74 (1H, *s*, H-7), 4.21 (1H, *tt*, $J = 7.0, 3.7$ Hz, H-3), 2.41 (1H, *dt*, $J = 13.9, 2.5$ Hz, H-4b), 1.98 (1H, *dt*, $J = 14.4, 2.5$ Hz, H-2b), 1.75 (3H, *s*, H₃-11), 1.72 (1H, *d*, $J =$

4.1 Hz, H-4a), 1.51 (1H, *dd*, $J = 14.4, 5.0$ Hz, H-2a), 1.46 (3H, *s*, H₃-10), 1.26 (3H, *s*, H₃-9); ¹³C-NMR (CD₃OD, 125 MHz) δ : 185.7 (*s*, C-6), 174.4 (*s*, C-8), 113.3 (*d*, C-7), 89.0 (*s*, C-5), 67.2 (*d*, C-3), 48.0 (*t*, C-2), 46.4 (*t*, C-4), 37.2 (*s*, C-1), 31.0 (*q*, C-9), 27.4 (*q*, C-11), 27.0 (*q*, C-10).

S23. ¹H-NMR (CDCl₃, 500MHz), ¹³C-NMR (CDCl₃, 125 MHz) and ESI-MS data of compound **8**

¹H-NMR (500 MHz, CDCl₃) δ : 6.25 (1H, *d*, $J = 7.2$ Hz, NH), 5.78 (1H, *dt*, $J = 15.0, 6.6$ Hz, H-5), 5.53 (1H, *dd*, $J = 15.3, 6.5$ Hz, H-4), 4.32 (1H, *br t*, $J = 4.4$ Hz, H-3), 3.95 (1H, *dd*, $J = 11.2, 3.7$ Hz, H-2), 3.90 (1H, *m*, H-1b), 3.70 (1H, *dd*, $J = 11.2, 3.2$ Hz, H-1a), 2.23 (2H, *t*, $J = 7.5$ Hz, H-2'), 2.05 (4H, *q*, $J = 7.1$, H-6), 1.63 (2H, *m*, H-3'), 1.26-1.37 (42H, *m*, H-7 – H-17, H-4' – H-13'), 0.88 (6H, *t*, $J = 7.0$ Hz, H-18, H-14'); ¹³C-NMR (125 MHz, CDCl₃) δ : 174.0 (C-1'), 134.3 (C-5), 128.8 (C-4), 74.7 (C-3), 62.5 (C-1), 54.5 (C-2), 36.9 (C-2'), 32.3 (C-6), 31.9 (C-12', C-7, C-16), 29.1-29.7 (C-8 – C-15, C-4' – C-11'), 25.8 (C-3'), 22.7 (C-17, C-13'), 14.1 (C-18, C-14'); ESI-MS (+) m/z : 532 [M + Na]⁺, 437, 330, 302, 274, 264, 256, 219; ESI-MS (–) m/z : 554, [M + COOH][–], 544 [M + Cl][–], 508 [M – H][–], 447, 394, 339, 325, 311, 283, 265, 255, 227, 143, 126.

S24. ¹H-NMR (CDCl₃, 500MHz), ¹³C-NMR (CDCl₃, 125 MHz) and ESI-MS data of compound **9**

¹H-NMR (500 MHz, CDCl₃) δ : 6.25 (1H, *d*, $J = 7.5$ Hz, NH), 5.77 (1H, *dt*, $J = 15.2, 6.65$ Hz, H-5), 5.54 (1H, *dd*, $J = 15.4, 6.4$ Hz, H-4), 5.42 (1H, *dt*, $J = 15.2, 6.3$ Hz, H-9), 5.37 (1H, *dt*, $J = 15.2, 6.4$ Hz, H-8), 4.32 (1H, *br s*, H-3), 3.94 (1H, *dd*, $J = 11.3, 3.7$ Hz, H-2), 3.91 (1H, *m*, H-1b), 3.71 (1H, *dd*, $J = 11.3, 3.5$ Hz, H-1a), 2.23 (2H, *t*, $J = 7.5$ Hz, H-2'), 2.12 (2H, *m*, H-7), 2.07 (2H, *m*, H-10), 1.96 (2H, *q*-like, $J = 6.7$ Hz, H-6), 1.64 (2H, *m*, H-3'), 1.26 (38H, *m*, H-11 – H-17, H-4' – H-15'), 0.88 (6H, *t*, $J = 6.8$ Hz, H-18, H-16'); ¹³C-NMR (125 MHz, CDCl₃) δ : 174.0 (C-1'), 133.6 (C-5), 131.4 (C-8), 129.2 (C-4), 129.0 (C-9), 74.7 (C-3), 62.5 (C-1), 54.5 (C-2), 36.9 (C-2'), 32.6 (C-10), 32.3 (C-6), 32.1 (C-7), 31.9 (C-14', C-16), 29.2-29.7 (C-11 – C-15, C-4' – C-13'), 25.8 (C-3'), 22.7 (C-17, C-15'), 14.1 (C-18, C-16'); ESI-MS (+) m/z : 558 [M + Na]⁺, 437, 330, 274, 262, 256; ESI-MS (–) m/z : 570 [M + Cl][–], 534 [M – H][–], 325, 311, 283, 255, 155, 113.