

Bathyptilones: Terpenoids from an Antarctic sea pen, *Anthoptilum grandiflorum* (Verrill, 1879).

Santana A.L. Thomas,[†] Anthony Sanchez,[‡] Younghoon Kee,[‡] Nerida G. Wilson,[§] Bill J. Baker^{†,*}

Email: bjbaker@usf.edu; Phone: (813) 974-0274; Fax: (813) 905-9933

[†]Department of Chemistry, University of South Florida, Tampa, FL 33620, United States

[‡]Department of Cellular, Microbiology and Molecular Biology, University of South Florida, Tampa, FL 33620, United States

[§]Western Australia Museum, Perth, Western Australia, Australia

Figure S1. ¹ H NMR spectrum for bathyptilone A (1) (500 MHz, CDCl ₃)	3
Figure S2. ¹³ C NMR spectrum for bathyptilone A (1) (125 MHz, CDCl ₃)	3
Figure S3. ¹ H- ¹ H COSY NMR spectrum for bathyptilone A (1) (500 MHz, CDCl ₃)	4
Figure S5. ¹ H- ¹³ C HSQC NMR spectrum for bathyptilone A (1) (800 MHz, CDCl ₃)	4
Figure S4. ¹ H- ¹³ C HMBC NMR spectrum for bathyptilone A (1) (500 MHz, CDCl ₃)	5
Figure S6. ¹ H- ¹ H NOESY NMR spectrum for bathyptilone A (1) (500 MHz, CDCl ₃)	5
Figure S7. HRESIMS spectrum for bathyptilone A (1)	6
Figure S8. ¹ H NMR spectrum for bathyptilone B (2) (400 MHz, CDCl ₃)	6
Figure S9. ¹³ C NMR spectrum for bathyptilone B (2) (125 MHz, CDCl ₃)	6
Figure S10. ¹ H- ¹ H COSY NMR spectrum for bathyptilone B (2) (500 MHz, CDCl ₃)	7
Figure S11. ¹ H- ¹³ C HSQC NMR spectrum for bathyptilone B (2) (500 MHz, CDCl ₃)	7
Figure S12. ¹ H- ¹³ C HMBC NMR spectrum for bathyptilone B (2) (500 MHz, CDCl ₃)	8
Figure S13. ¹ H- ¹ H ROESY NMR spectrum for bathyptilone B (2) (500 MHz, CDCl ₃)	8
Figure S14. HRESIMS spectrum for bathyptilone B (2)	9
Figure S15. ¹ H NMR spectrum for bathyptilone C (3) (800 MHz, CDCl ₃)	9
Figure S16. ¹³ C NMR spectrum for bathyptilone C (3) (200 MHz, CDCl ₃)	10
Figure S17. Zoomed-in region of Figure S16 from 0-75 ppm	10
Figure S18. Zoomed-in region of Figure S16 from 78-205 ppm	11

Figure S19. ^1H - ^1H COSY NMR spectrum for bathyptilone C (3) (500 MHz, CDCl_3)	11
Figure S20. ^1H - ^{13}C HSQC NMR spectrum for bathyptilone C (3) (800 MHz, CDCl_3)	12
Figure S21. ^1H - ^{13}C HMBC NMR spectrum for bathyptilone C (3) (500 MHz, CDCl_3)	12
Figure S22. ^1H - ^1H NOESY NMR spectrum for bathyptilone C (3) (800 MHz, CDCl_3).....	13
Figure S23. HRESIMS spectrum for bathyptilone C (3)	13
Figure S24. ^1H NMR spectrum for enbepeanone A (4) (500 MHz, CDCl_3).....	14
Figure S25. Zoomed-in region of Figure S24 from 0.9-1.53 ppm.....	14
Figure S26. Zoomed-in region of Figure S24 from 1.59-6.7 ppm.....	15
Figure S27. ^{13}C NMR spectrum for enbepeanone A (4) (125 MHz, CDCl_3)	15
Figure S28. Zoomed in region of Figure S27 from 0-50 ppm.	16
Figure S29. Zoomed in region of Figure S27 from 78-200 ppm	16
Figure S30. ^1H - ^1H COSY NMR spectrum for enbepeanone A (4) (500 MHz, CDCl_3)	17
Figure S31. ^1H - ^{13}C HSQC NMR spectrum for enbepeanone A (4) (500 MHz, CDCl_3)	17
Figure S32. ^1H - ^{13}C HMBC NMR spectrum for enbepeanone A (4) (500 MHz, CDCl_3)	18
Figure S33. ^1H - ^1H NOESY NMR spectrum for enbepeanone A (4) (500 MHz, CDCl_3).....	18
Figure S34. HRESIMS spectrum for enbepeanone A (4)	19
Figure S35. Maximum Likelihood tree topology comparing our <i>Anthoptilum</i> msh1 sequences with those available on Genbank.....	20
Table S1. Crystal data and structure refinement for bathyptilone A (1).....	21
Table S2. Crystal data and structure refinement for bathyptilone B (2).....	21
Table S3. Crystal data and structure refinement for bathyptilone C (3).....	22
Table S4. Crystal data and structure refinement for enbepeanone A (4).....	22
Table S5. Bijvoet-Pair analysis and Bayesian statistics for (1) and (4).....	23

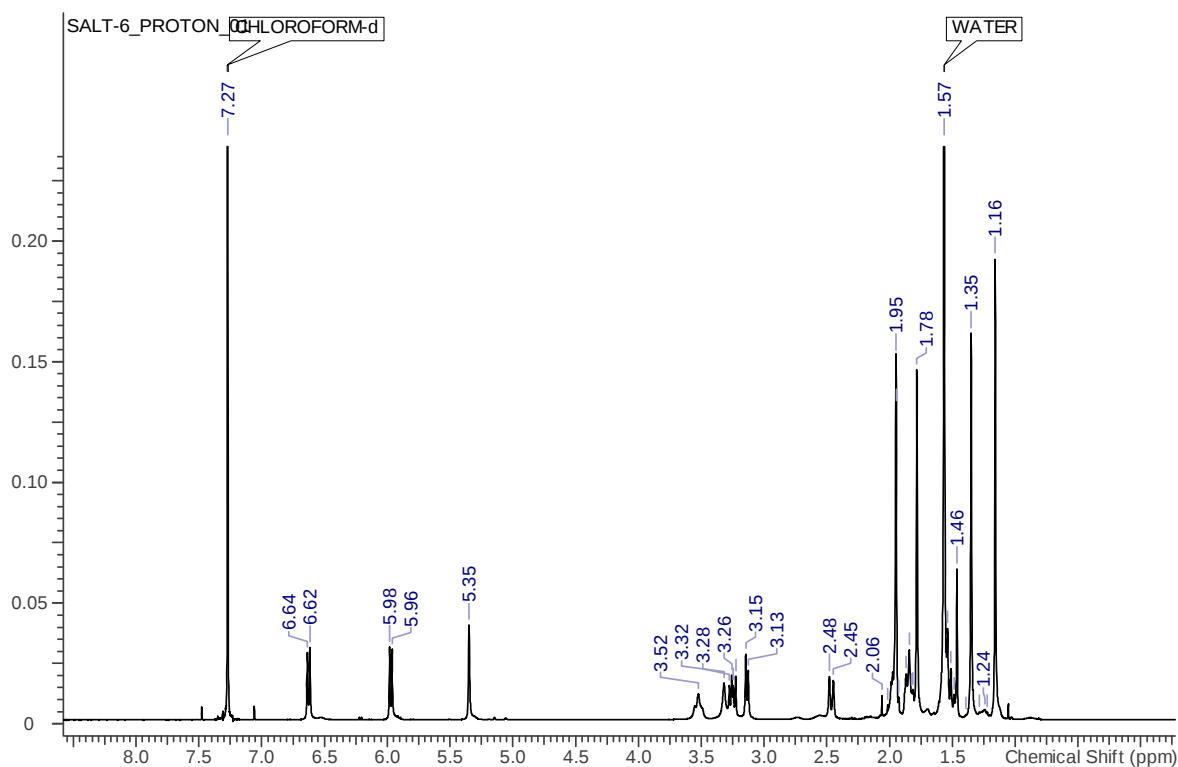


Figure S1. ^1H NMR spectrum for bathyptilone A (**1**) (500 MHz, CDCl_3).

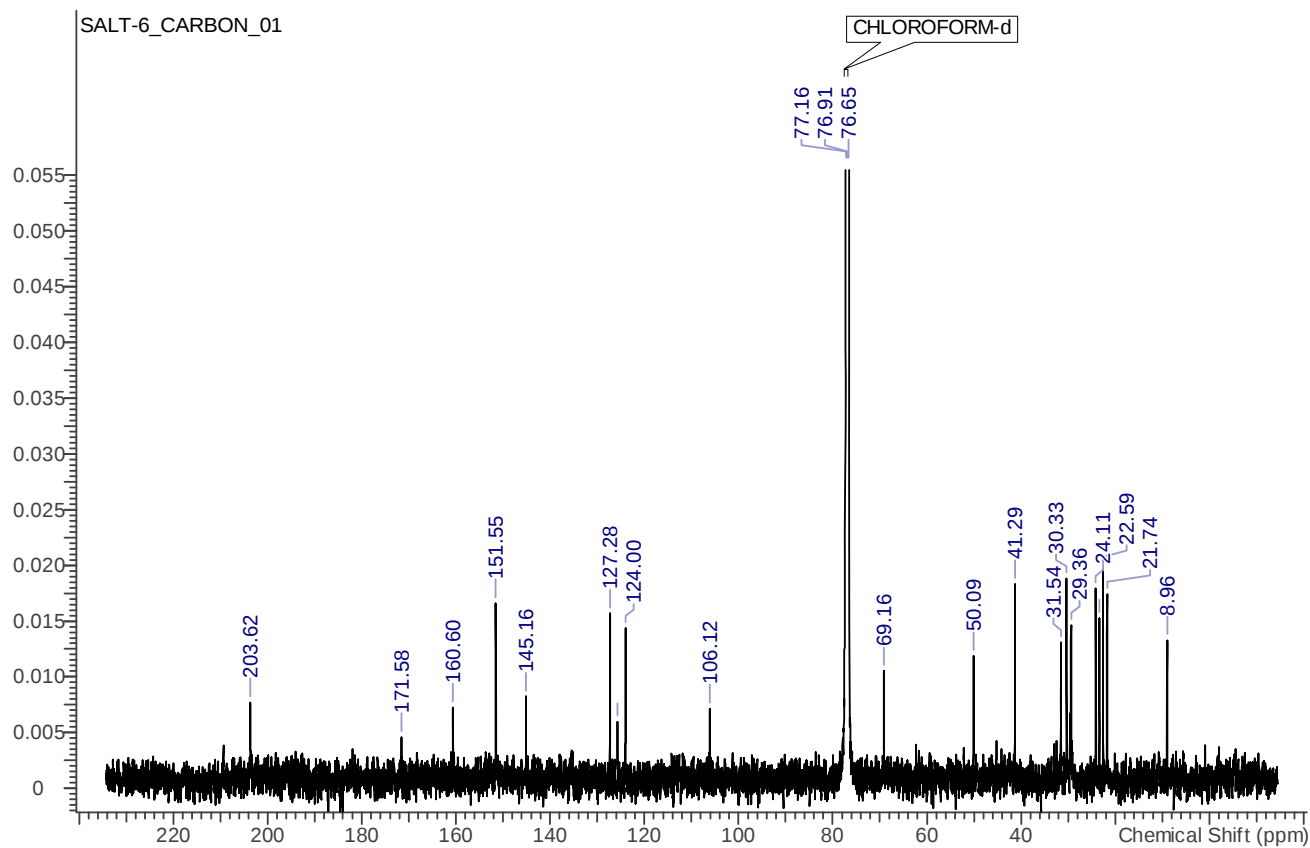


Figure S2. ^{13}C NMR spectrum for bathyptilone A (**1**) (125 MHz, CDCl_3).

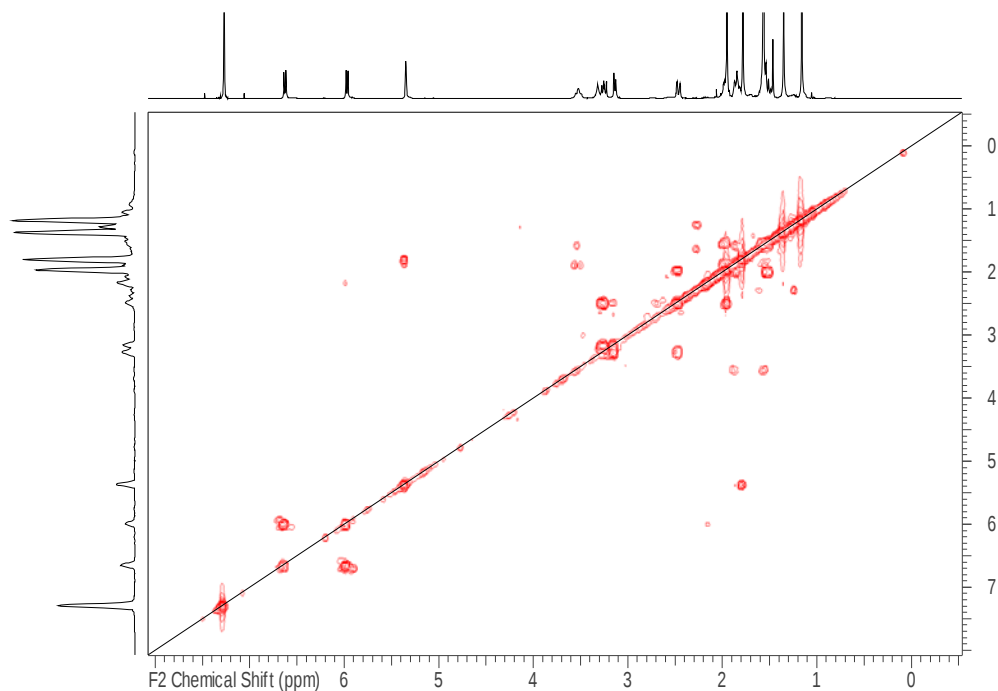


Figure S3. ^1H - ^1H COSY NMR spectrum for bathyptilone A (**1**) (500 MHz, CDCl_3).

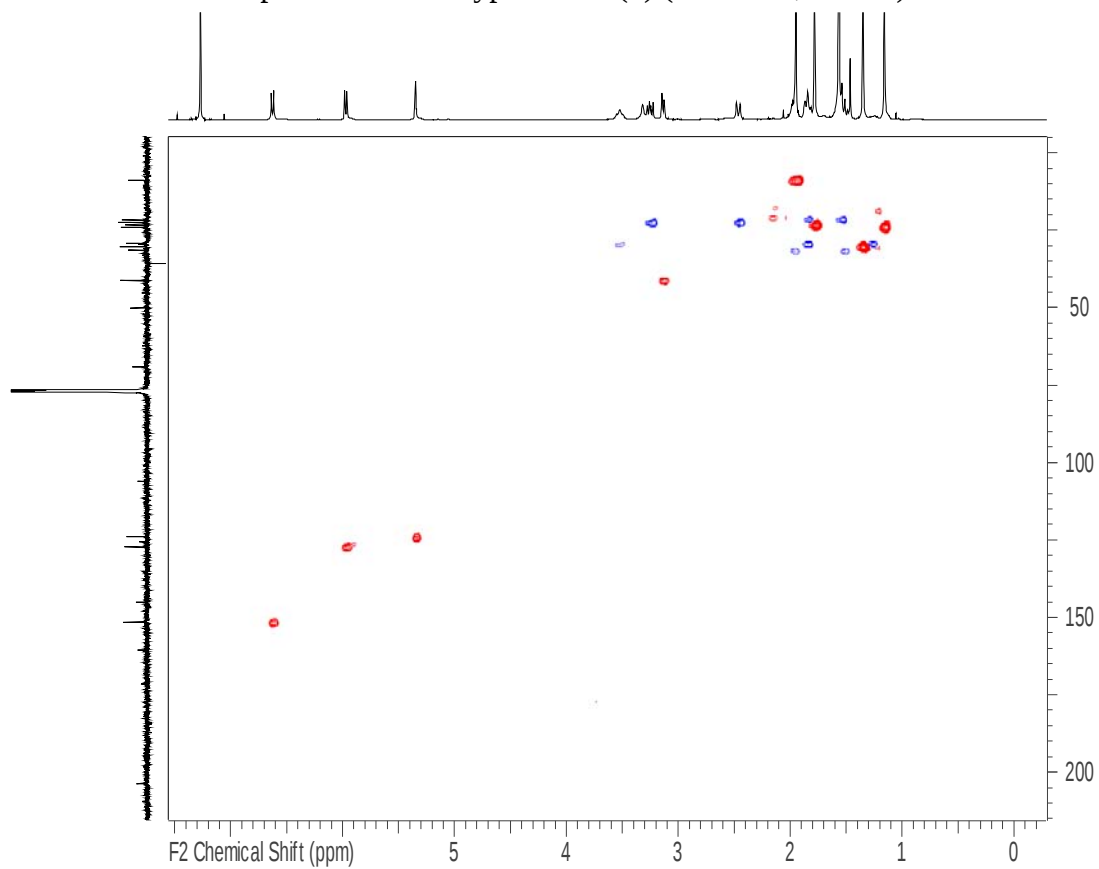


Figure S4. ^1H - ^{13}C HSQC NMR spectrum for bathyptilone A (**1**) (500 MHz, CDCl_3).

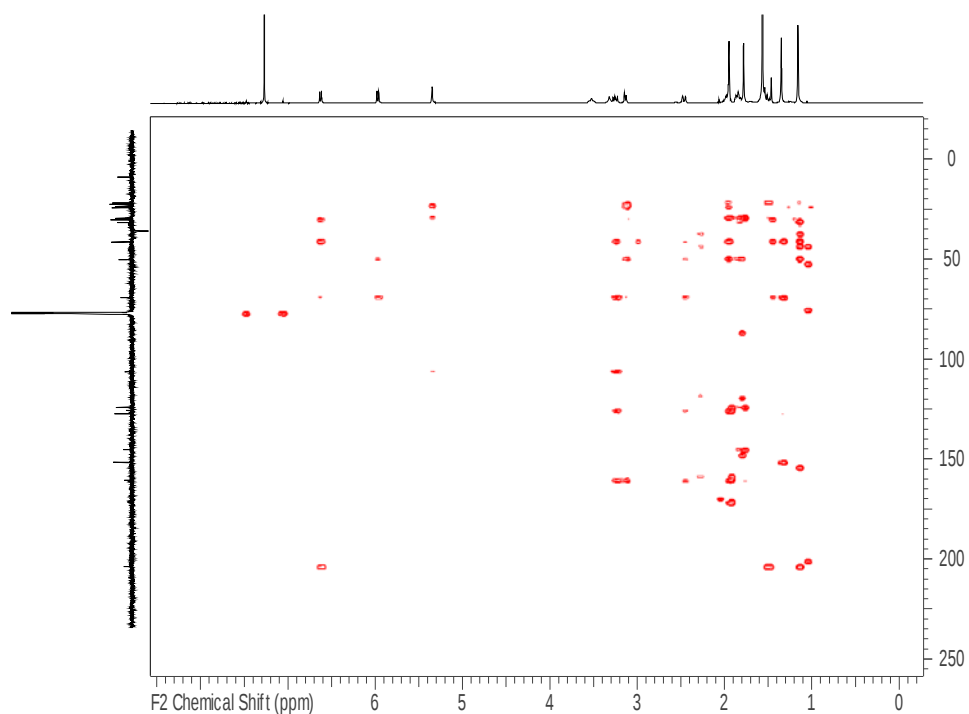


Figure S5. ^1H - ^{13}C HMBC NMR spectrum for bathyptilone A (**1**) (500 MHz, CDCl_3).

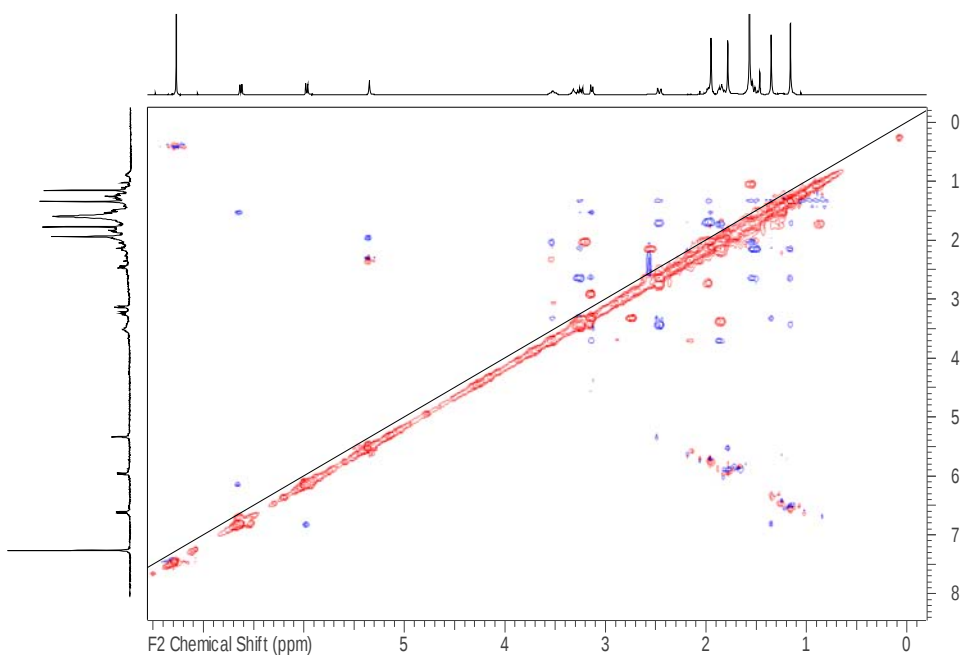


Figure S6. ^1H - ^1H ROESY NMR spectrum for bathyptilone A (**1**) (500 MHz, CDCl_3).

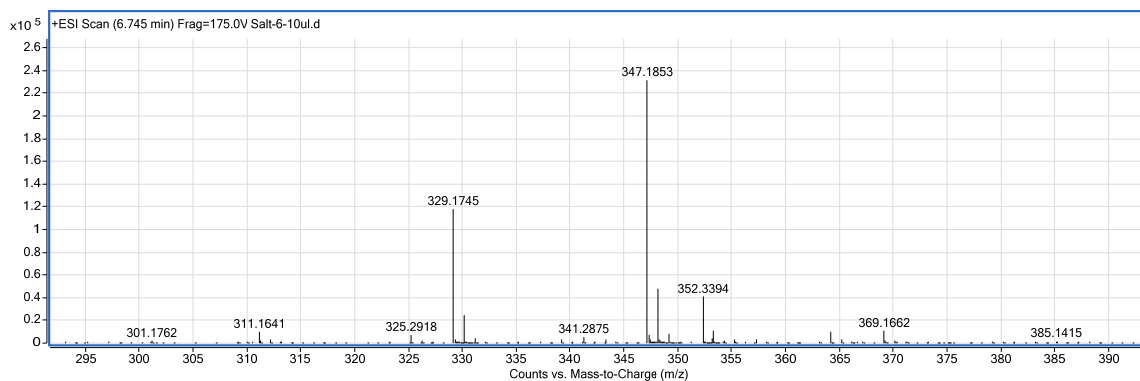


Figure S7. High resolution ESI-MS spectrum for bathyptilone A (1).

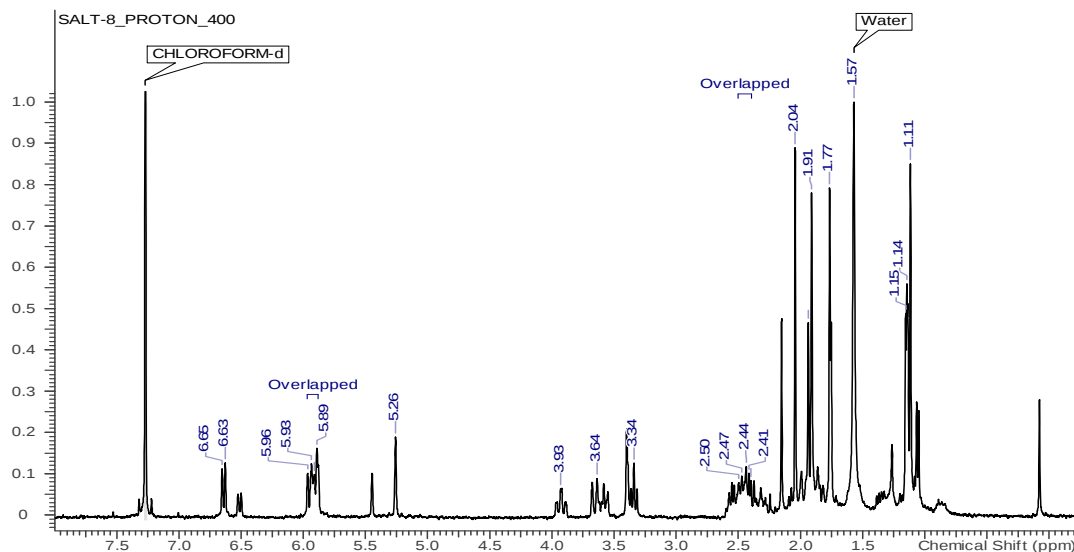


Figure S8. ^1H NMR spectrum for bathyptilone B (2) (400 MHz, CDCl_3). Minor peaks from inseparable derivative not marked.

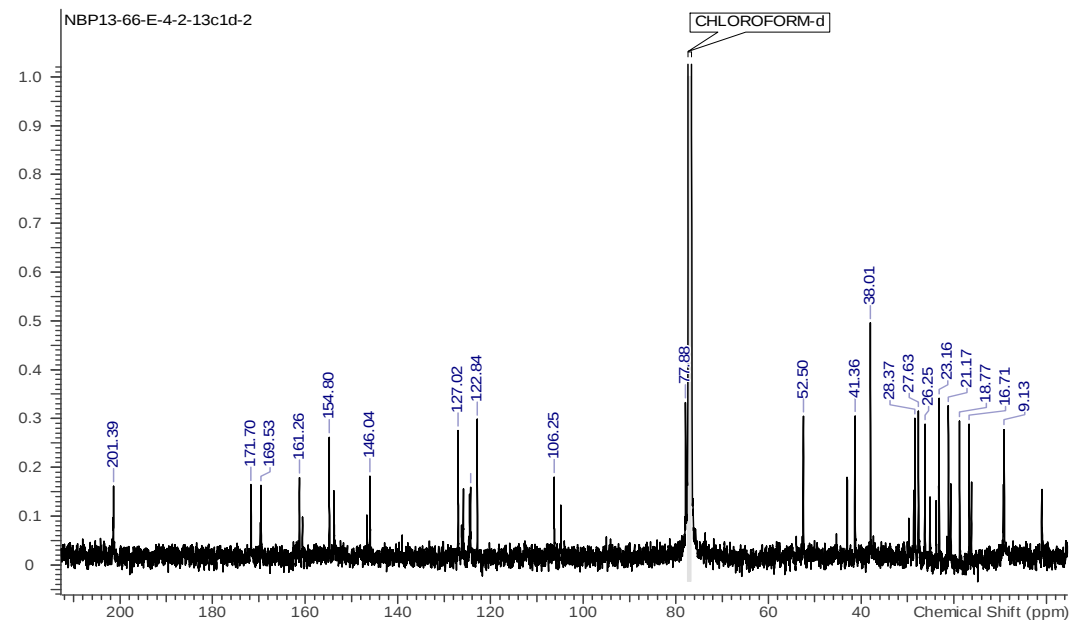


Figure S9. ^{13}C NMR spectrum for bathyptilone B (2) (125 MHz, CDCl_3). Minor peaks from inseparable derivative not marked.

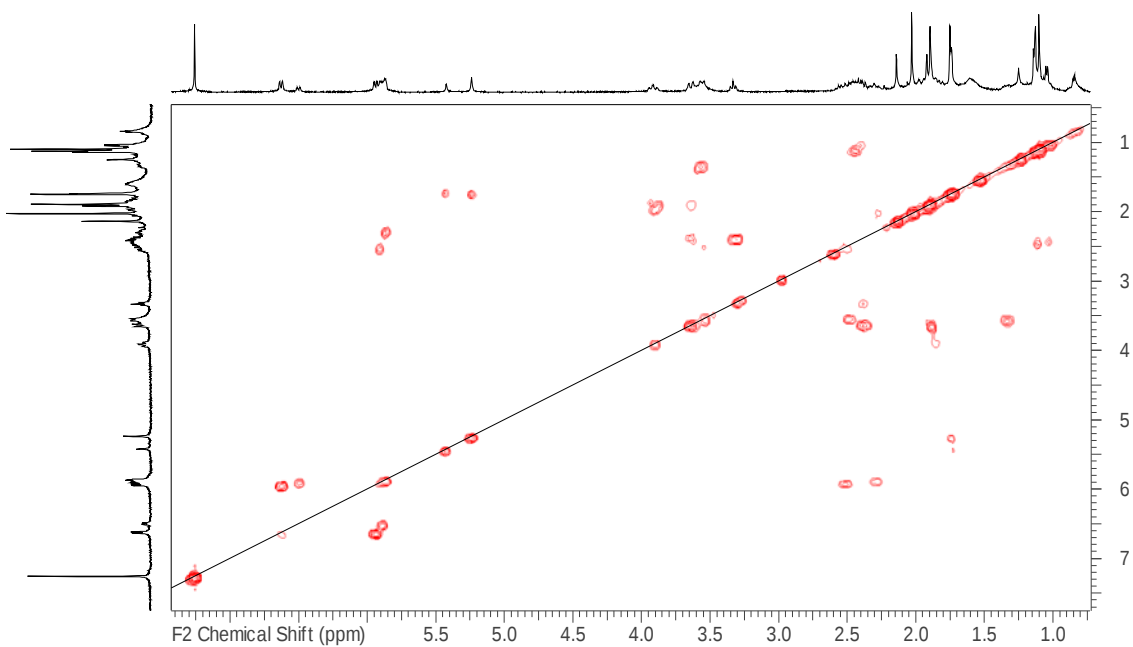


Figure S10. ^1H - ^1H COSY NMR spectrum for bathyptilone B (**2**) (500 MHz, CDCl_3).

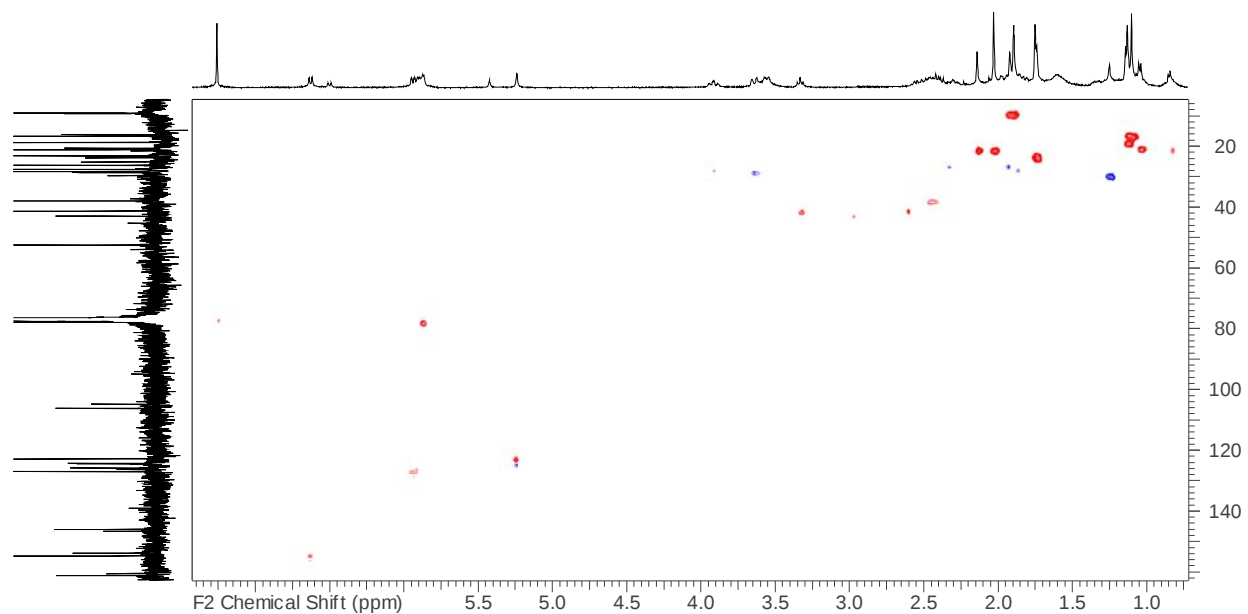


Figure S11. ^1H - ^{13}C HSQC NMR spectrum for bathyptilone B (**2**) (500 MHz, CDCl_3).

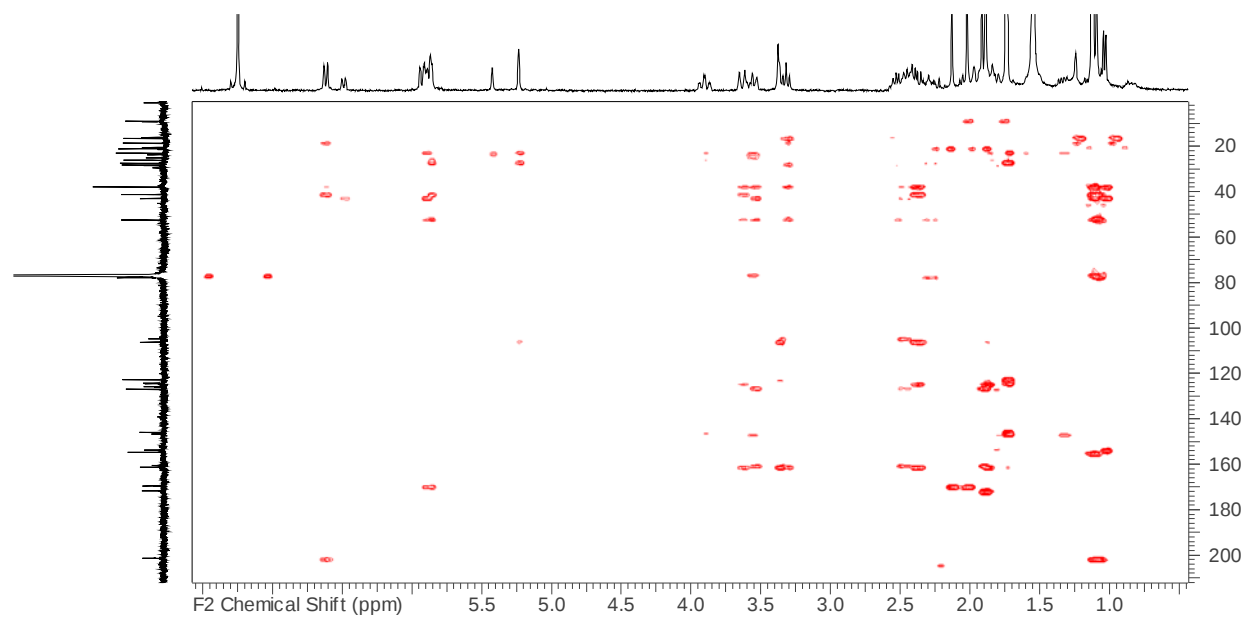


Figure S12. ^1H - ^{13}C HMBC NMR spectrum for bathyptilone B (2) (500 MHz, CDCl_3).

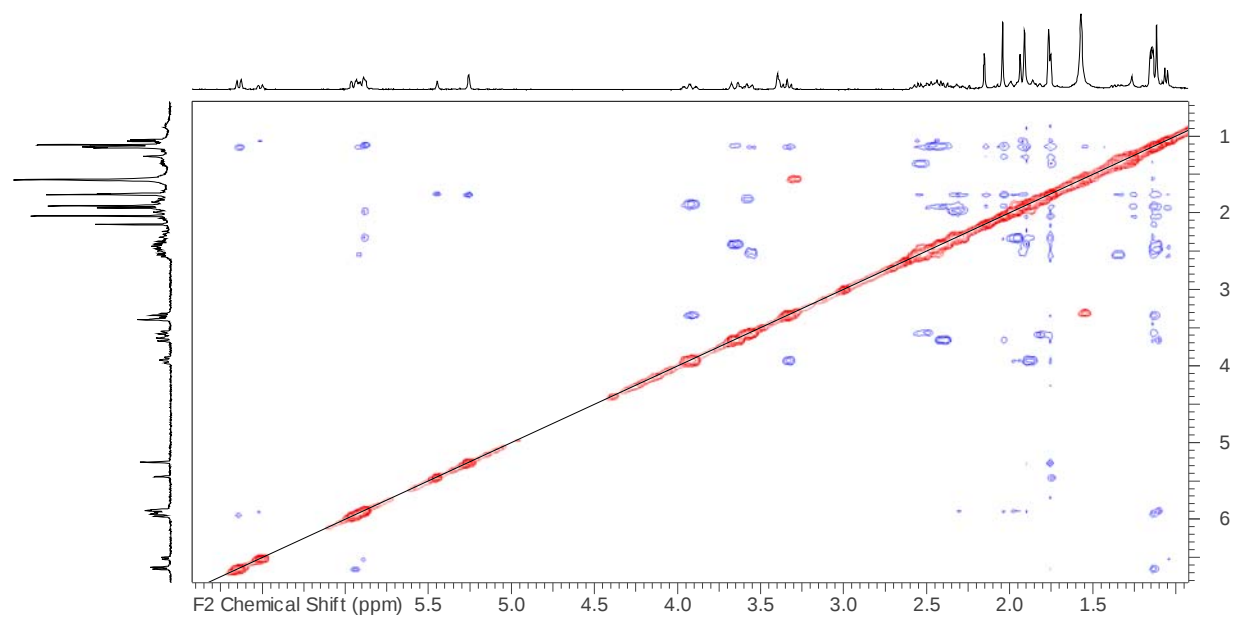


Figure S13. ^1H - ^1H ROESY NMR spectrum for bathyptilone B (2) (500 MHz, CDCl_3).

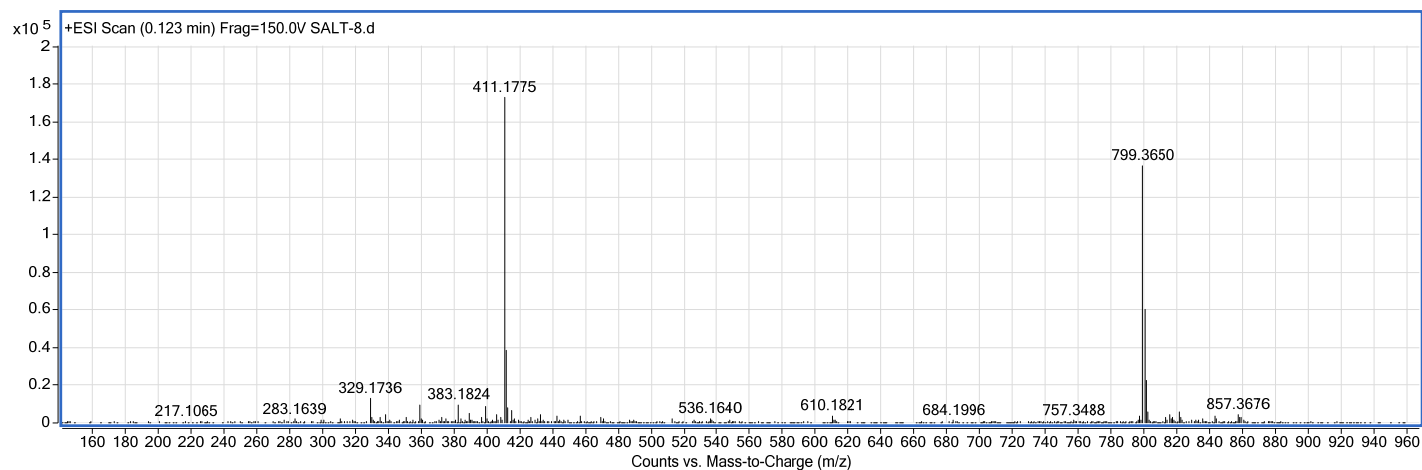


Figure S14. High resolution ESI-MS spectrum for bathyptilone B (**2**).

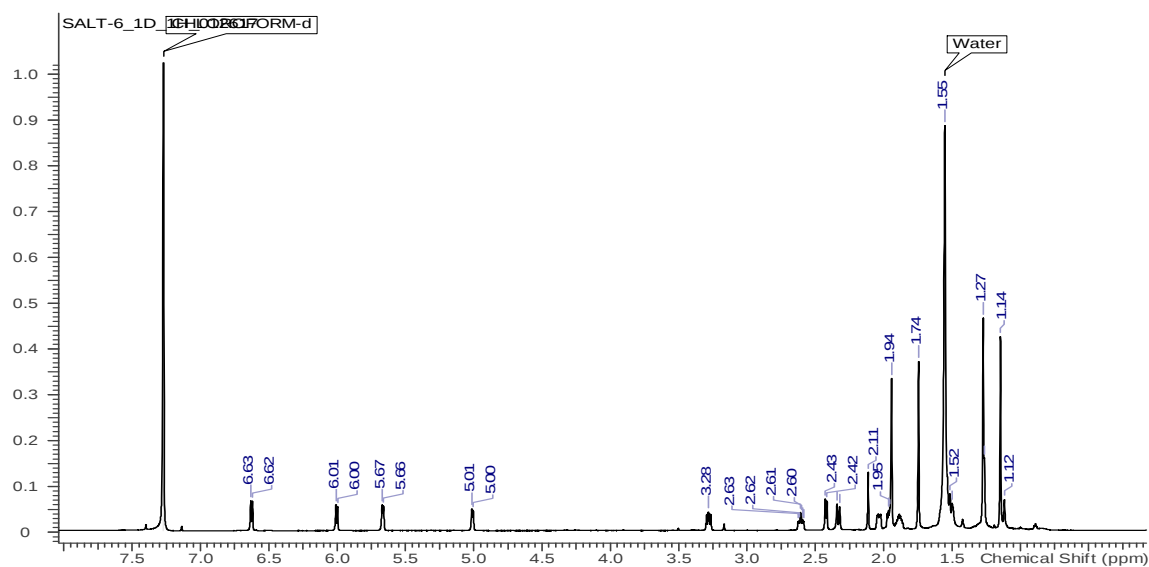


Figure S15. ¹H NMR spectrum for bathyptilone C (**3**) (800 MHz, CDCl₃).

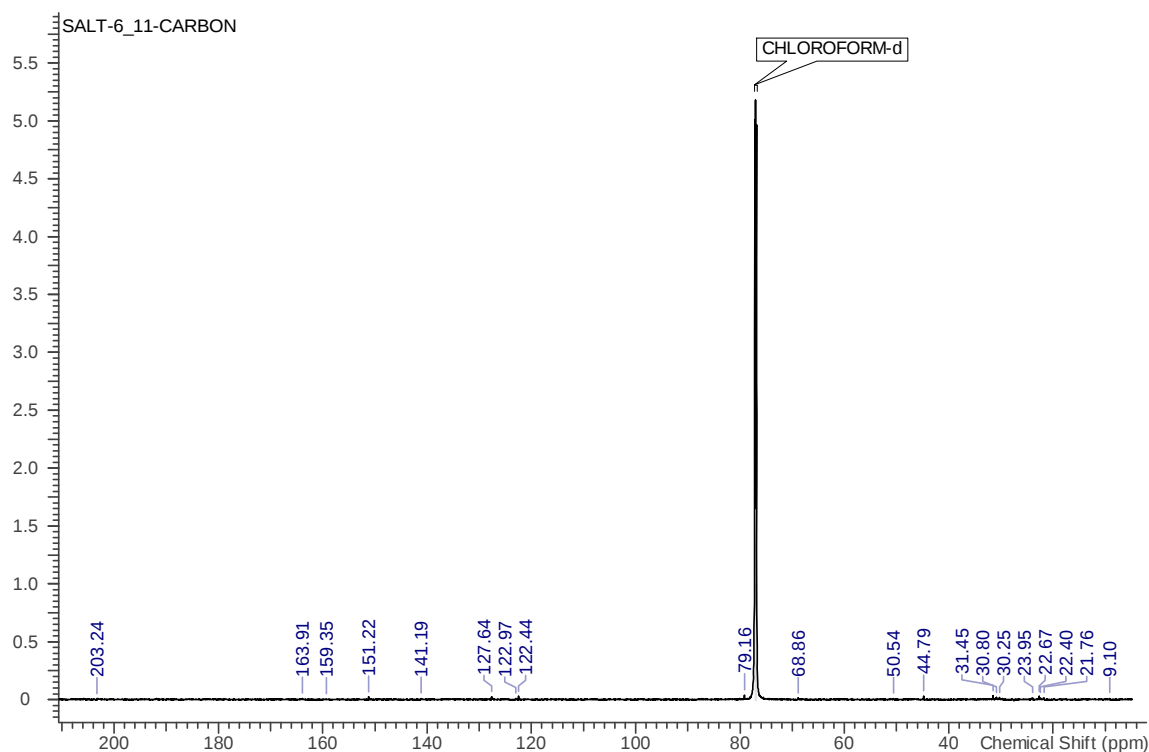


Figure S16. ^{13}C NMR spectrum for bathyptilone C (3) (200 MHz, CDCl_3).

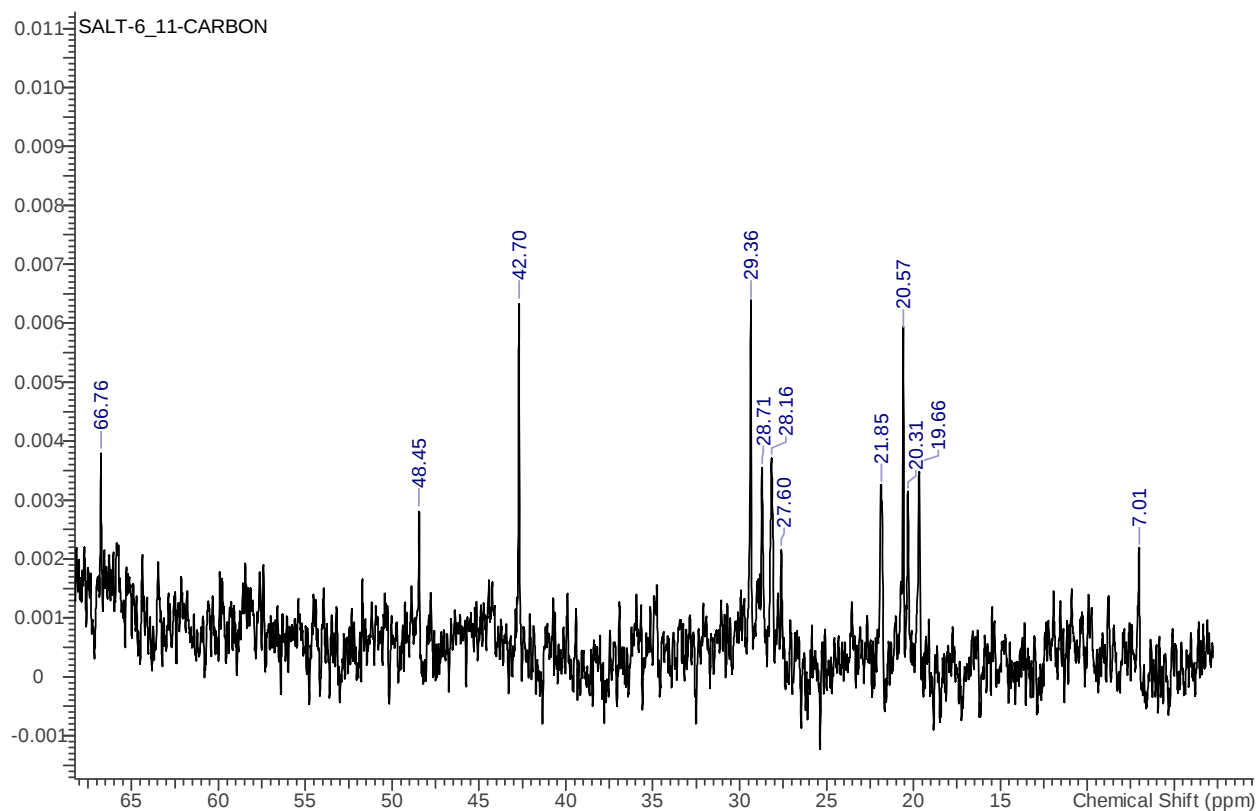


Figure S17. Zoomed-in region of Figure S16 from 0-75 ppm.

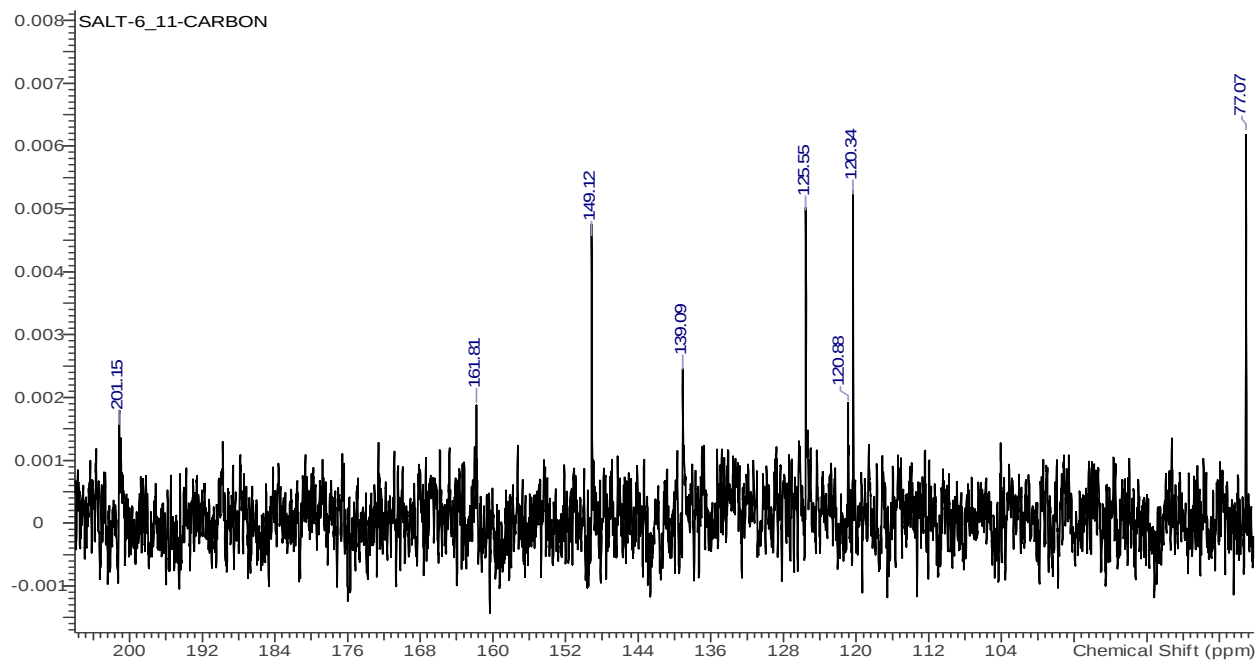


Figure S18. Zoomed-in region of Figure S16 from 76-205 ppm.

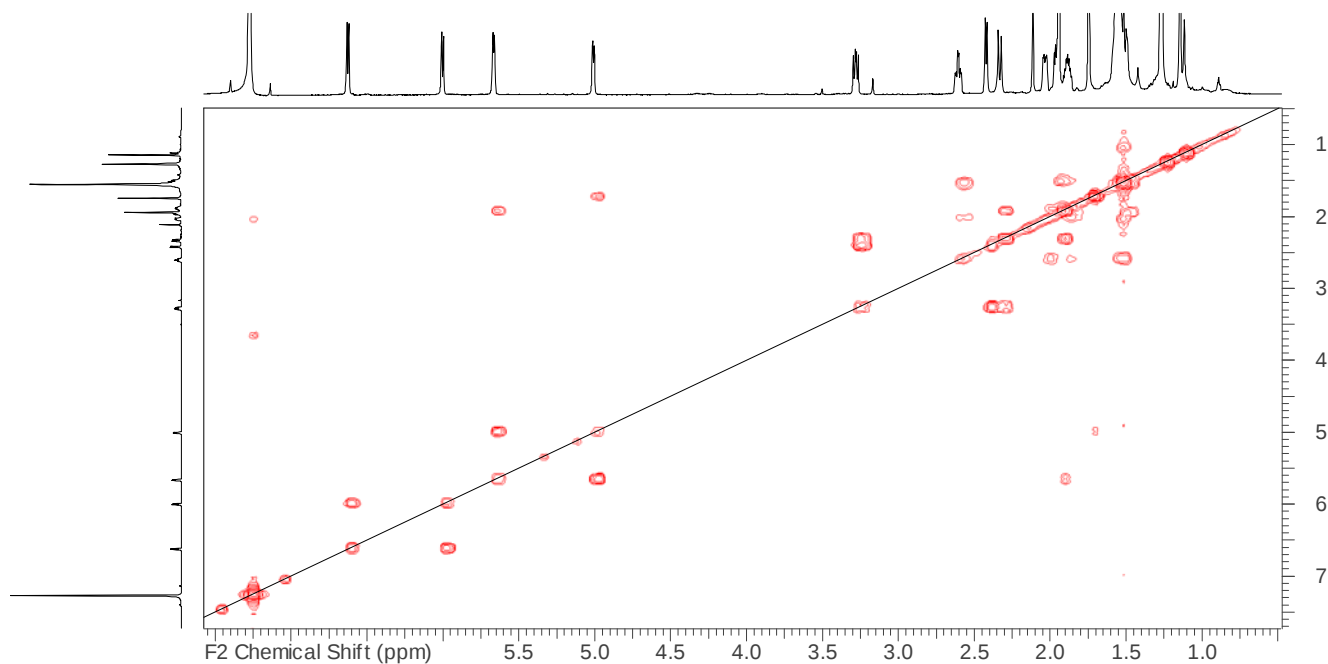


Figure S19. ^1H - ^1H COSY NMR spectrum for bathyptilone C (**3**) (500 MHz, CDCl_3).

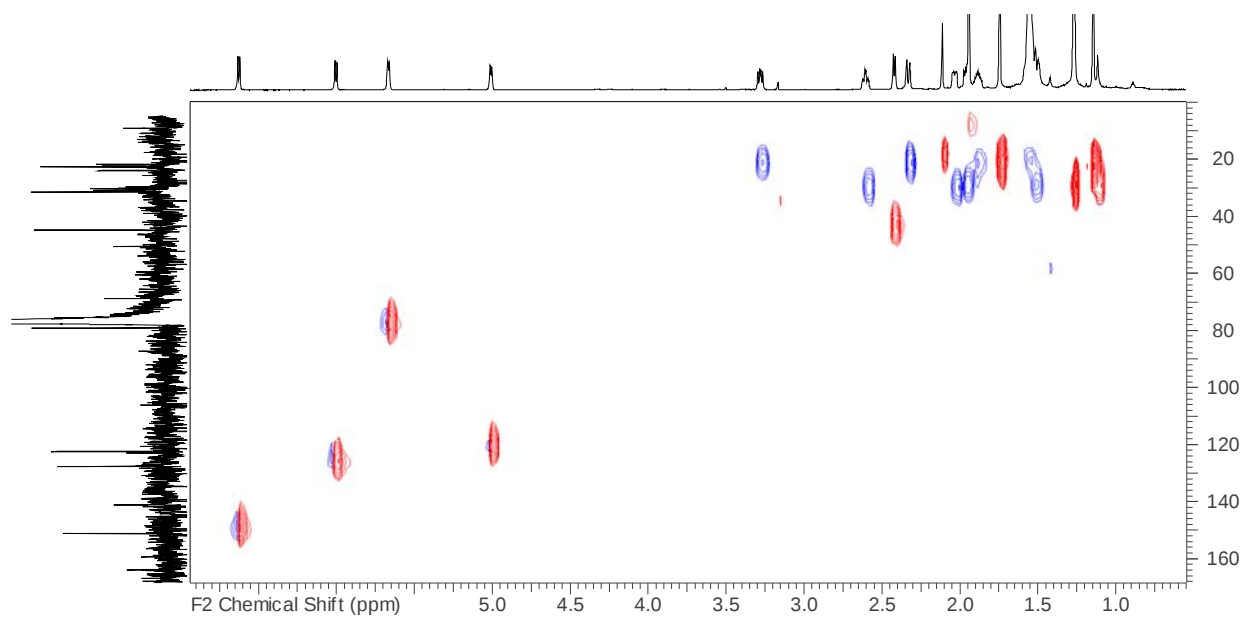


Figure S20. ^1H - ^{13}C HSQC NMR spectrum for bathyptilone C (**3**) (800 MHz, CDCl_3).

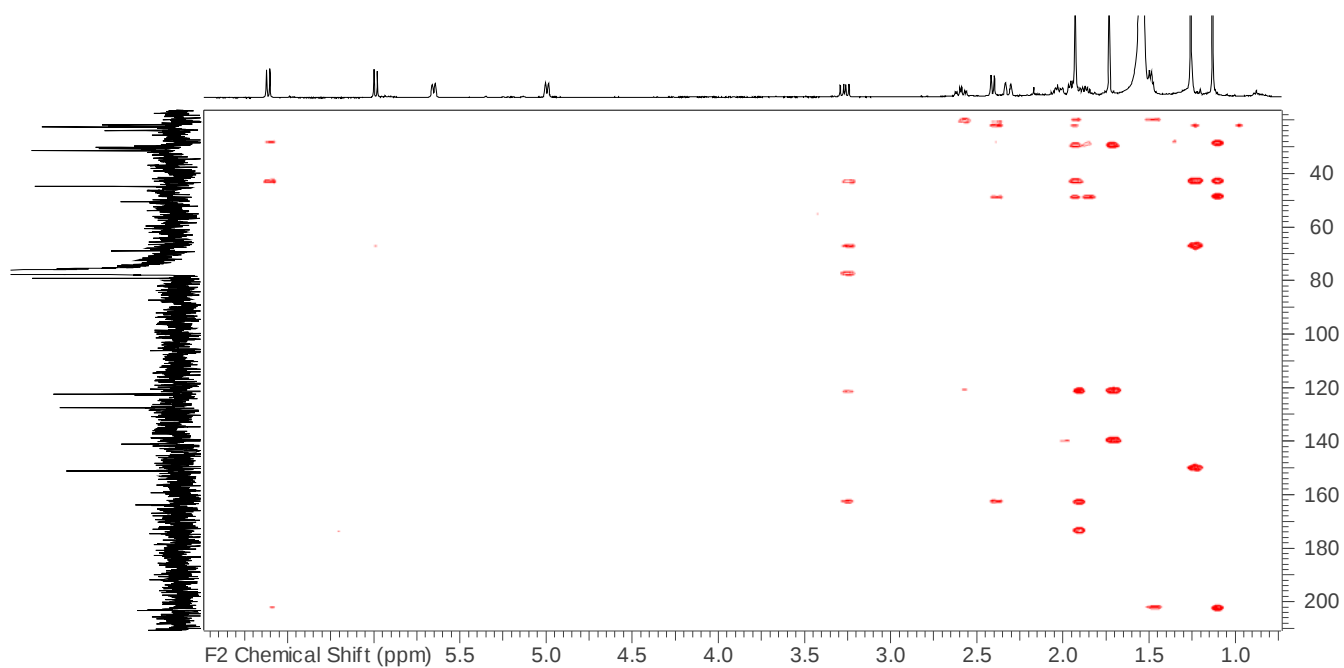


Figure S21. ^1H - ^{13}C HMBC NMR spectrum for bathyptilone C (**3**) (500 MHz, CDCl_3).

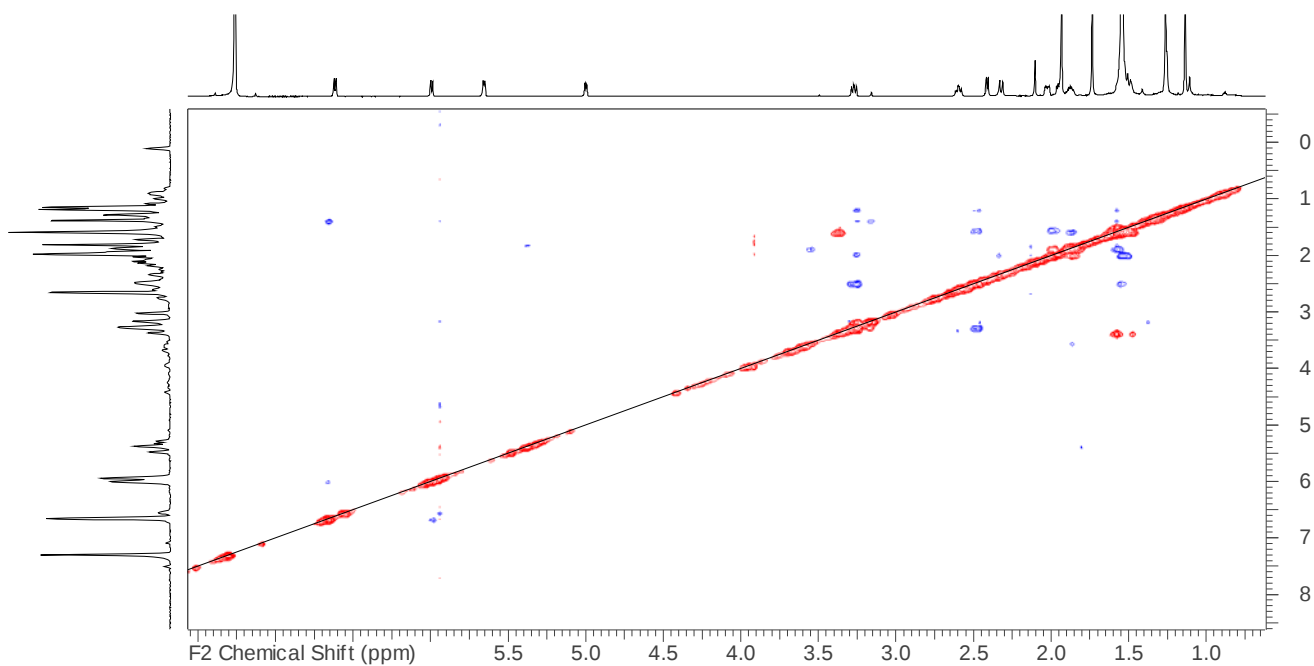


Figure S22. ^1H - ^1H NOESY NMR spectrum for bathyptilone C (**3**) (800 MHz, CDCl_3).

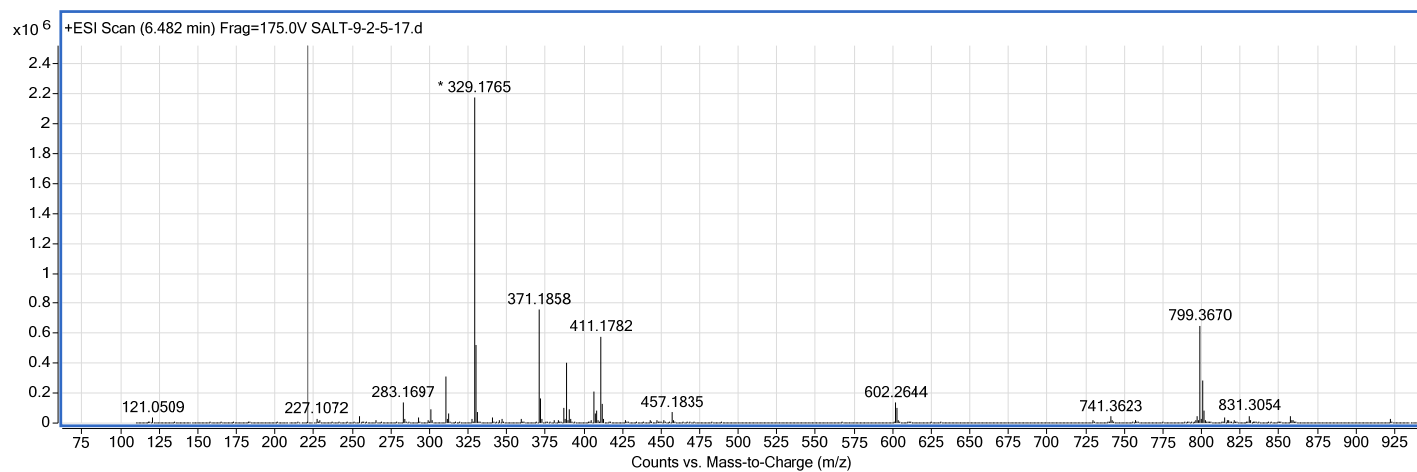


Figure S23. High resolution ESI-MS spectrum for bathyptilone C (**3**).

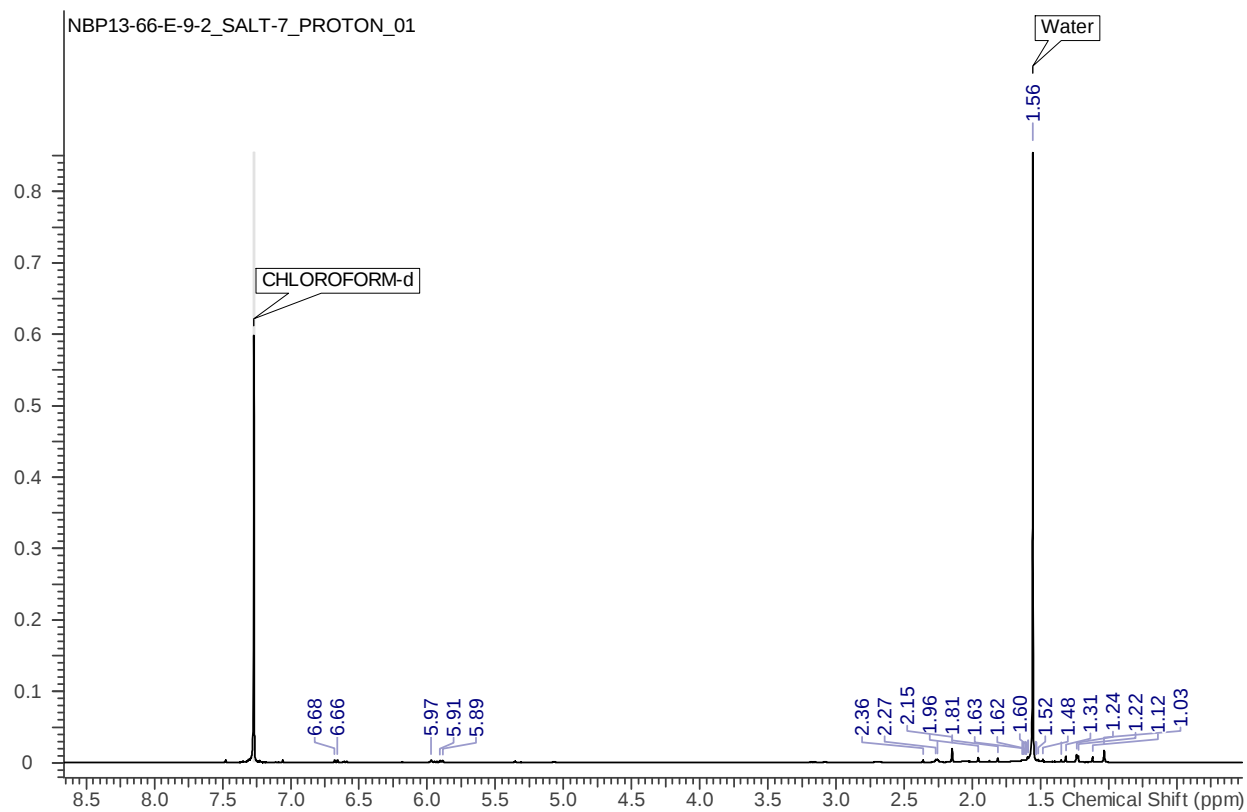


Figure S24. ^1H NMR spectrum for enbepeanone A (**4**) (500 MHz, CDCl_3).

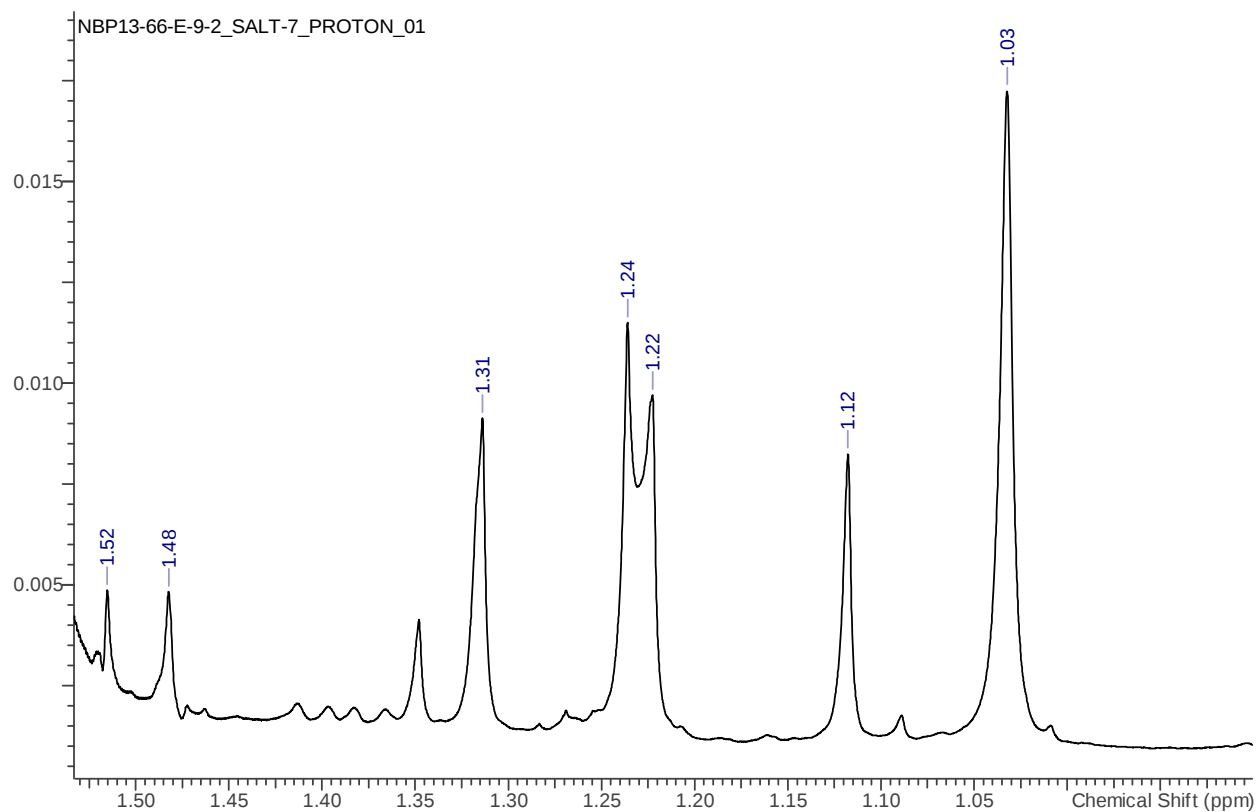


Figure S25. Zoomed-in region of Figure S24 from 0.9-1.53 ppm.

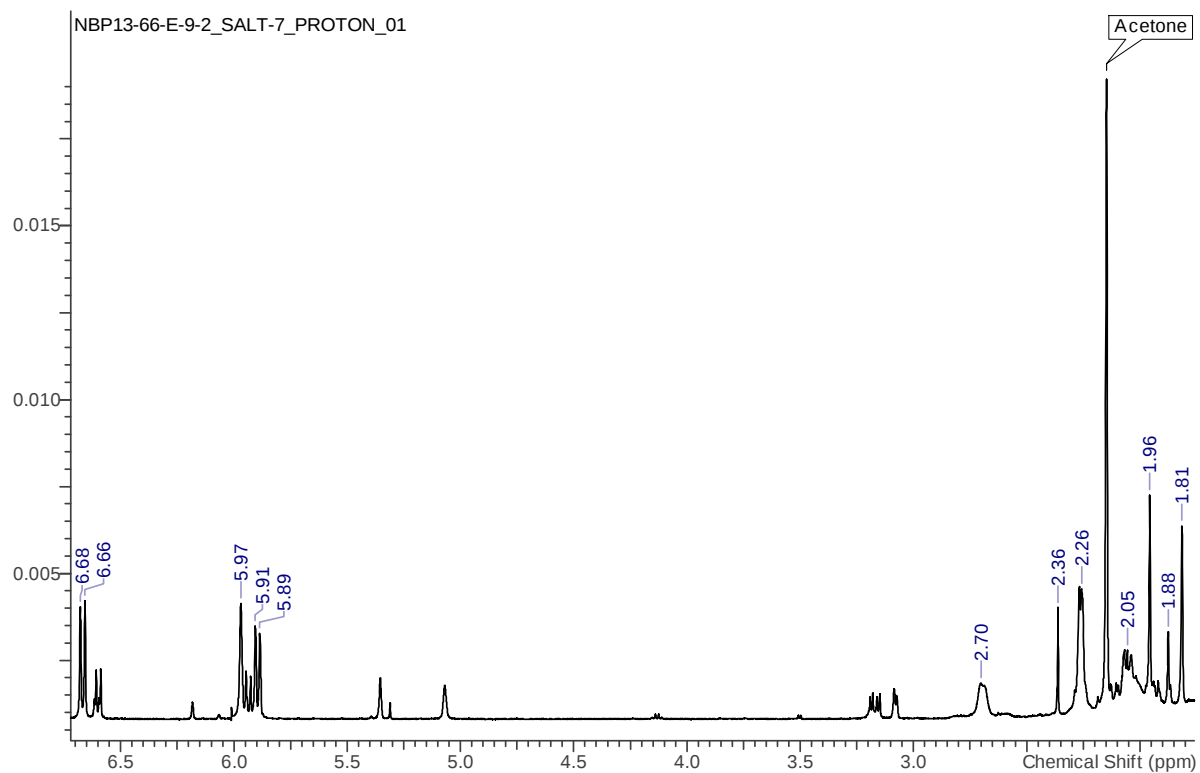


Figure S26. Zoomed-in region of Figure S24 from 1.59-6.7 ppm.

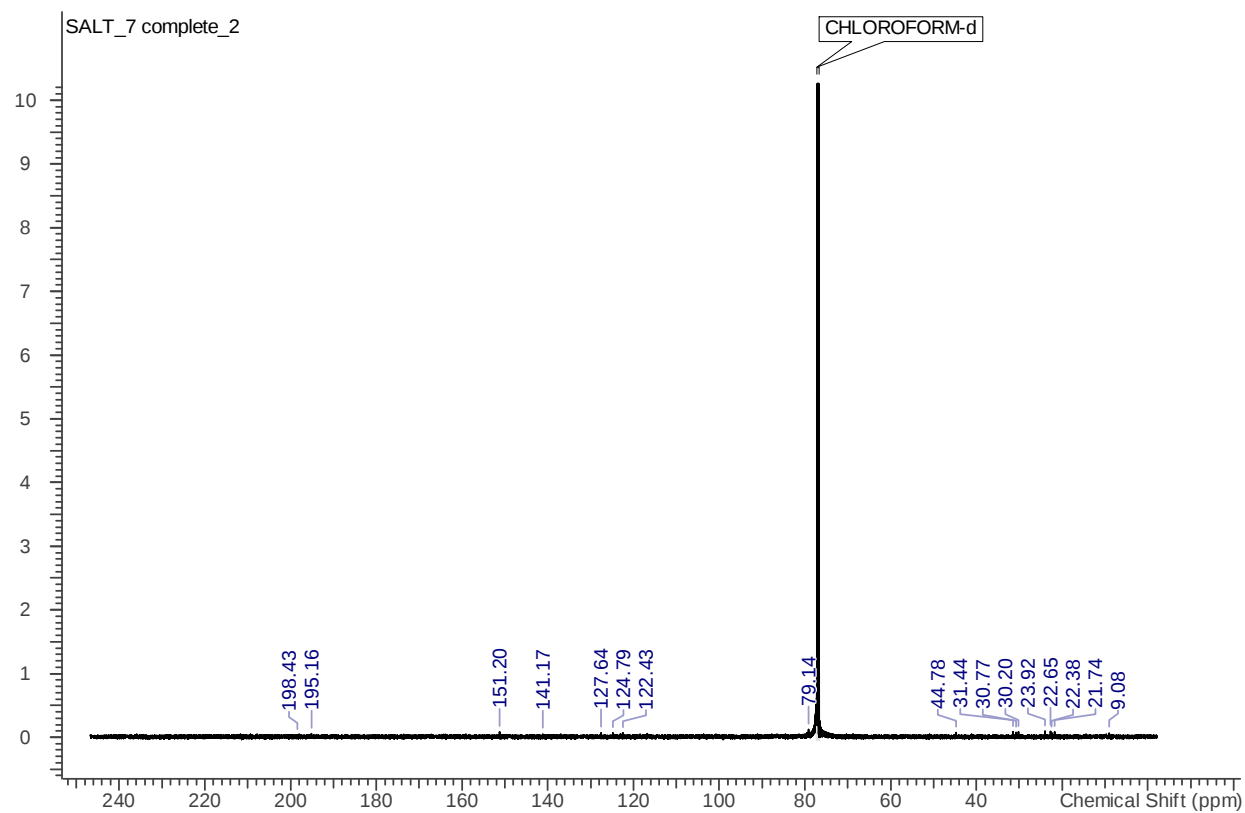


Figure S27. ^{13}C NMR spectrum for enbepeanone A (4) (200 MHz, CDCl_3).

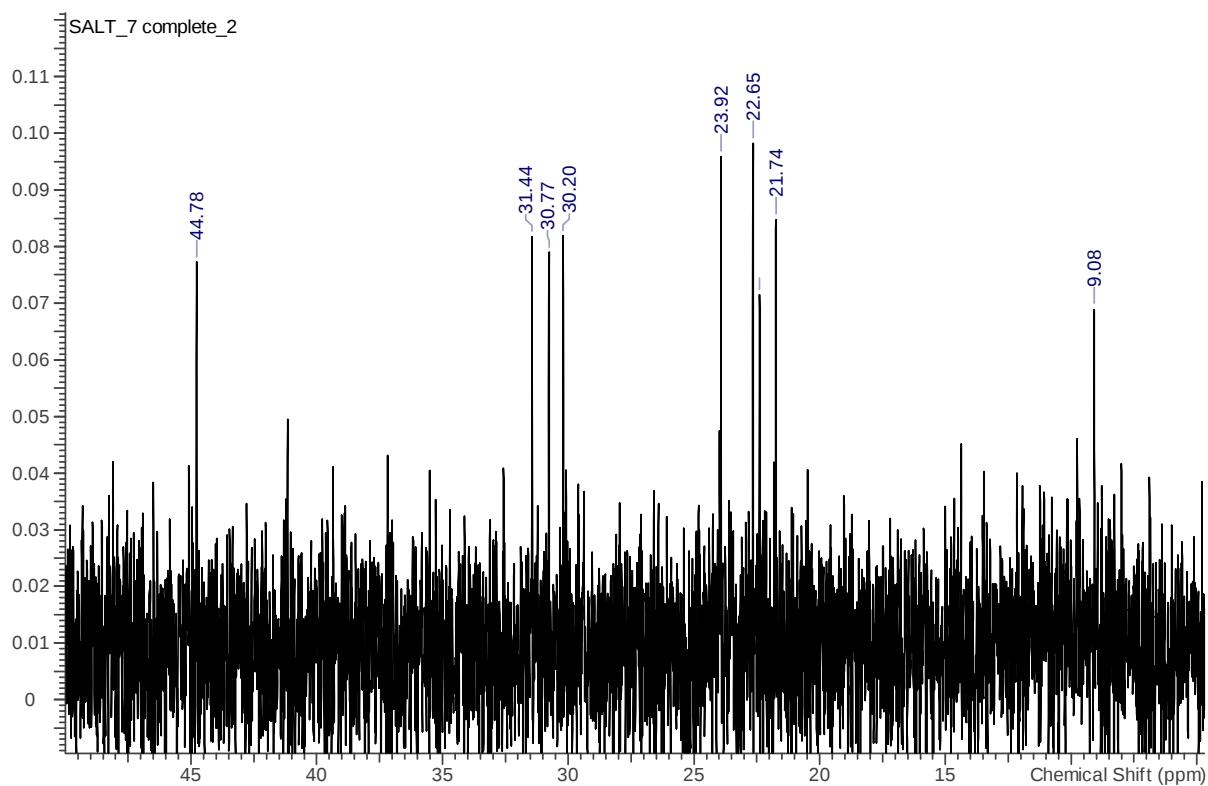


Figure S28. Zoomed in region of Figure S27 from 0-50 ppm.

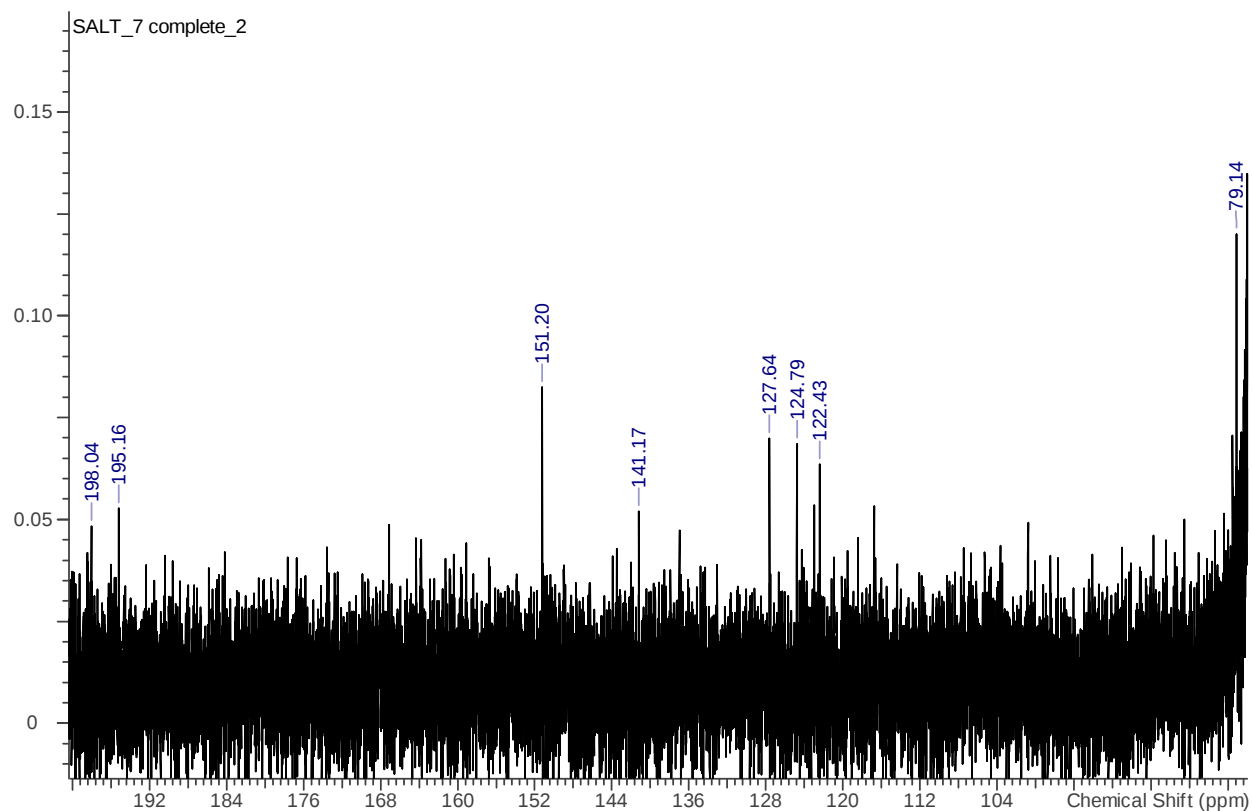


Figure S29. Zoomed in region of Figure S27 from 78-200 ppm.

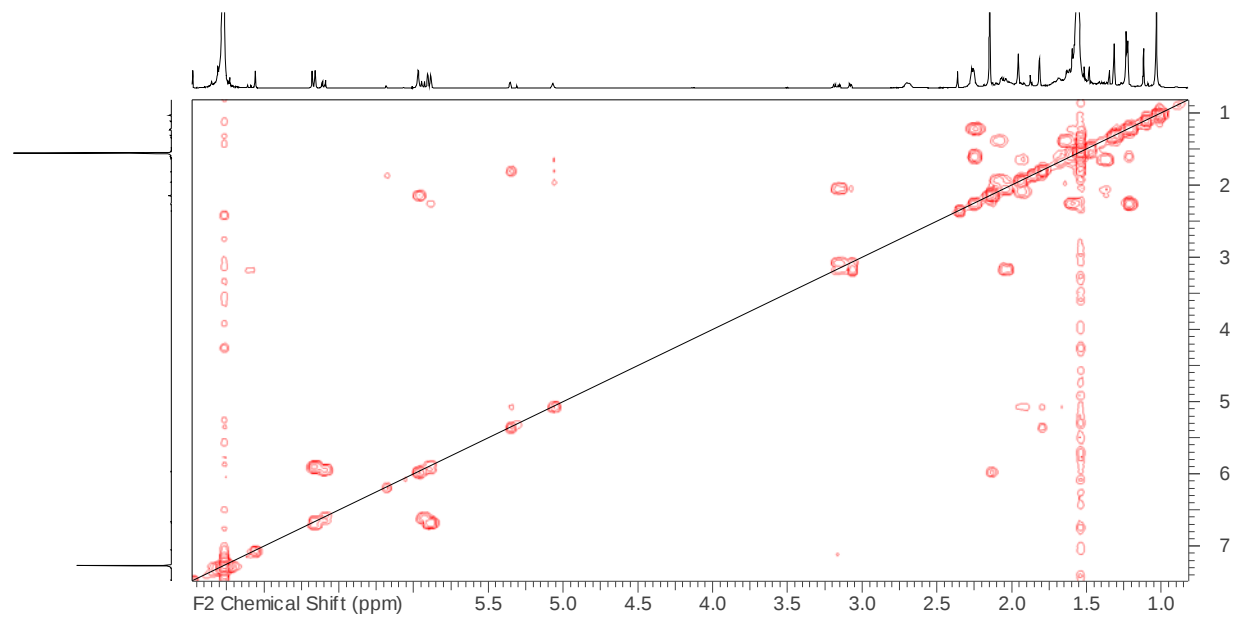


Figure S30. ^1H - ^1H COSY NMR spectrum for enbepeanone A (**4**) (500 MHz, CDCl_3).

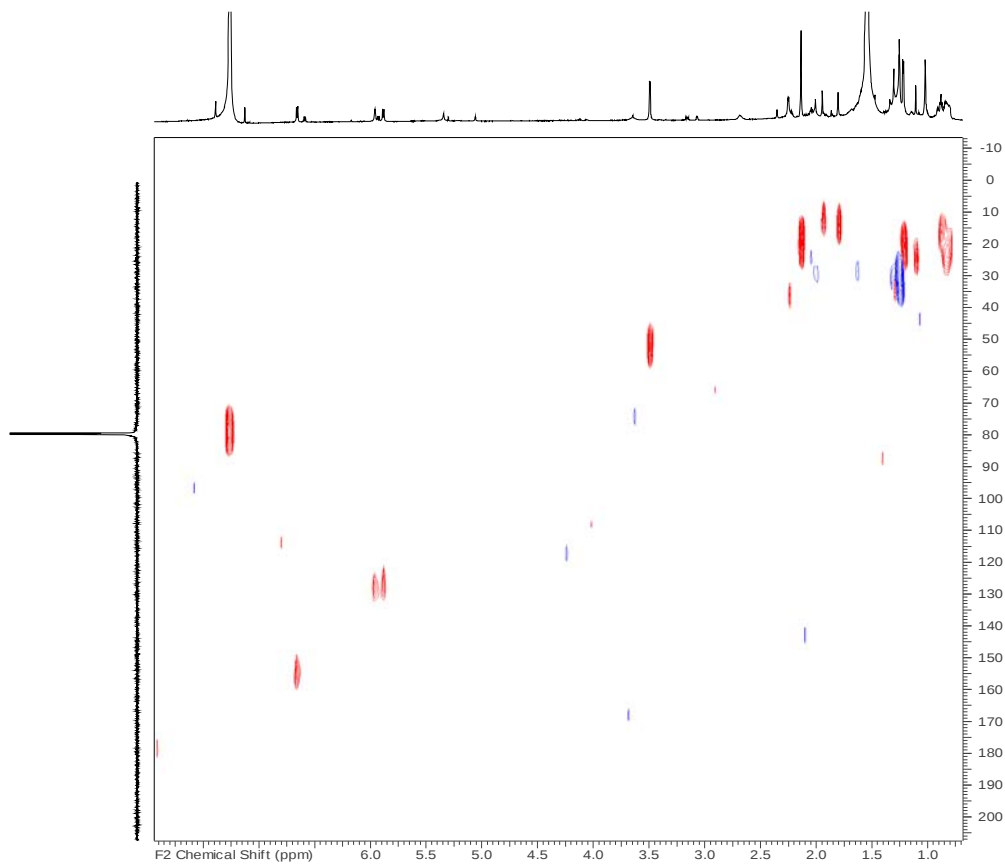


Figure S31. ^1H - ^{13}C HSQC NMR spectrum for enbepeanone A (**4**) (800 MHz, CDCl_3).

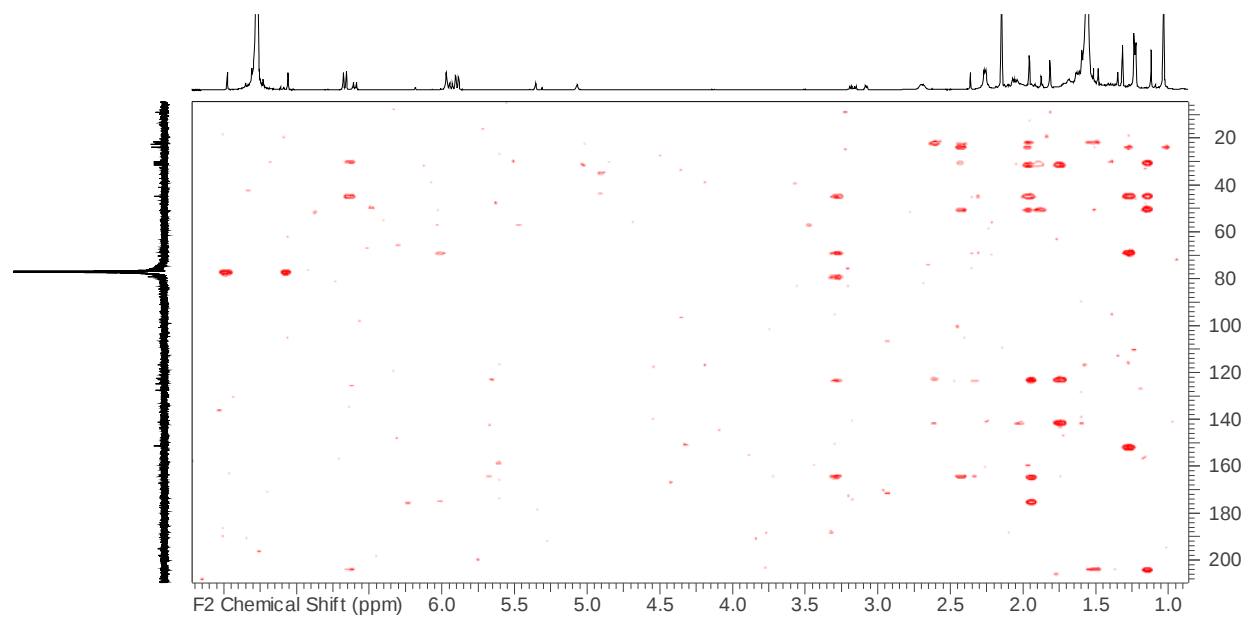


Figure S32. ^1H - ^{13}C HMBC NMR spectrum for enbepeanone A (**4**) (500 MHz, CDCl_3).

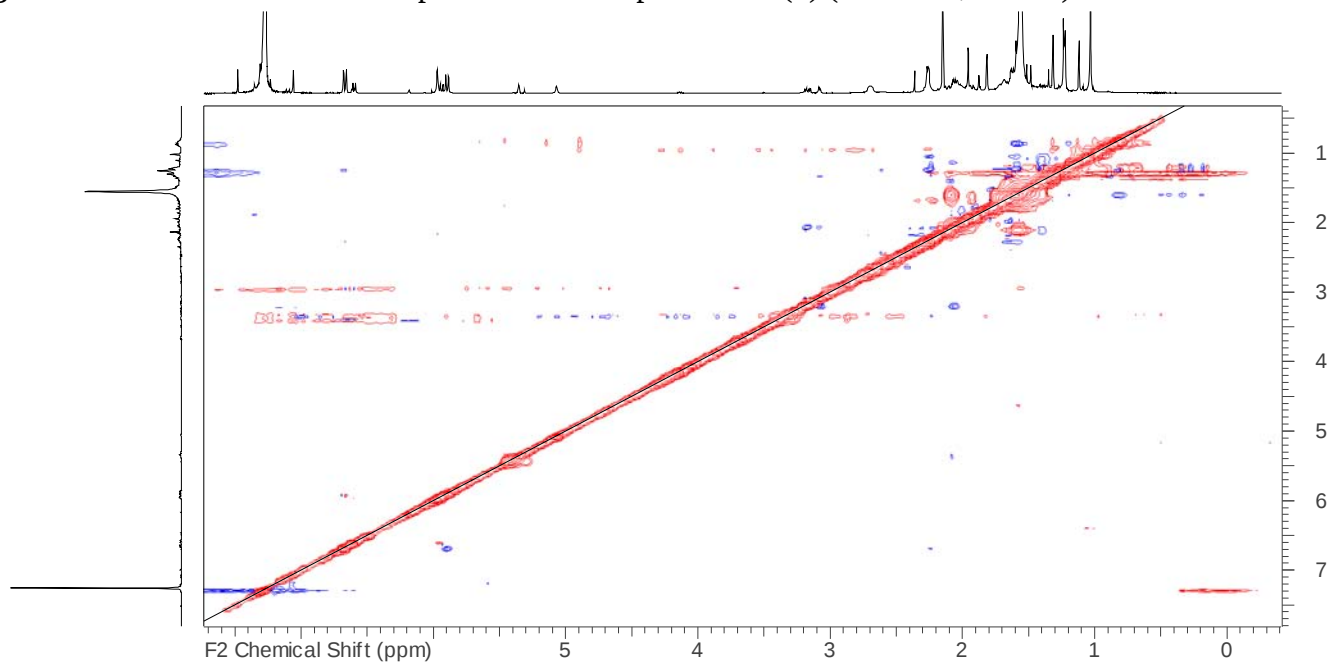


Figure S33. ^1H - ^1H NOESY NMR spectrum for enbepeanone A (**4**) (500 MHz, CDCl_3).

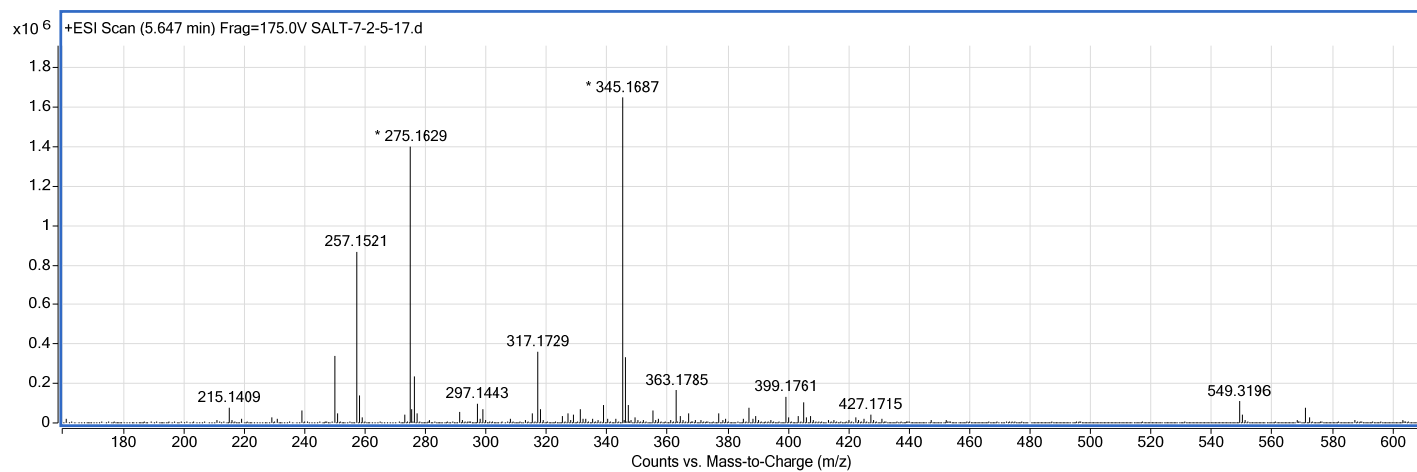


Figure S34. High resolution ESI-MS spectrum for enbepeanone A (4).



Figure S35. Maximum Likelihood tree topology comparing our *Anthoptilum msh1* sequences with those available on Genbank

Table S1. Crystal data and structure refinement for bathyptilone A (1).

Identification code	NBP13_66_E
Empirical formula	C ₂₀ H ₂₆ O ₅
Formula weight	346.41
Temperature/K	100.01
Crystal system	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
a/Å	8.7926(2)
b/Å	12.2611(2)
c/Å	16.4222(3)
α/°	90
β/°	90
γ/°	90
Volume/Å ³	1770.43(6)
Z	4
ρ _{calc} /cm ³	1.300
μ/mm ⁻¹	0.753
F(000)	744.0
Crystal size/mm ³	0.093 × 0.038 × 0.034
Radiation	CuKα (λ = 1.54178)
2θ range for data collection/°	9 to 132.984
Index ranges	-10 ≤ h ≤ 10, -14 ≤ k ≤ 14, -19 ≤ l ≤ 19
Reflections collected	23978
Independent reflections	3123 [R _{int} = 0.0453, R _{sigma} = 0.0213]
Data/restraints/parameters	3123/0/238
Goodness-of-fit on F ²	1.088
Final R indexes [I > 2σ (I)]	R ₁ = 0.0288, wR ₂ = 0.0674
Final R indexes [all data]	R ₁ = 0.0312, wR ₂ = 0.0688
Largest diff. peak/hole / e Å ⁻³	0.19/-0.18
Flack parameter	-0.04(6)

Table S2. Crystal data and structure refinement for bathyptilone B (2).

Identification code	NB_P13_66_E_4_2
Empirical formula	C ₂₂ H ₂₈ O ₆
Formula weight	388.44
Temperature/K	100.02
Crystal system	Monoclinic
Space group	P2 ₁
a/Å	9.5139(4)
b/Å	10.3255(5)
c/Å	10.4615(4)
α/°	90
β/°	90.924(3)
γ/°	90
Volume/Å ³	1027.56(8)
Z	2
ρ _{calc} /cm ³	1.255
μ/mm ⁻¹	0.744
F(000)	416.0
Crystal size/mm ³	0.04 × 0.02 × 0.005
Radiation	CuKα (λ = 1.54178)
2θ range for data collection/°	8.452 to 154.192
Index ranges	-11 ≤ h ≤ 11, -13 ≤ k ≤ 13, -12 ≤ l ≤ 13
Reflections collected	8460
Independent reflections	3614 [R _{int} = 0.1118, R _{sigma} = 0.1052]
Data/restraints/parameters	3614/1/262
Goodness-of-fit on F ²	1.041
Final R indexes [I > 2σ (I)]	R ₁ = 0.0576, wR ₂ = 0.1076
Final R indexes [all data]	R ₁ = 0.0859, wR ₂ = 0.1184
Largest diff. peak/hole / e Å ⁻³	0.25/-0.24
Flack parameter	0.2(3)

Table S3. Crystal data and structure refinement for bathyptilone C (3).

Identification code	ST_NBP13_66_E_9_4_b
Empirical formula	C ₂₀ H ₂₆ O ₄
Formula weight	330.41
Temperature/K	99.99
Crystal system	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
a/Å	8.2529(2)
b/Å	10.8806(3)
c/Å	19.0878(5)
α/°	90
β/°	90
γ/°	90
Volume/Å ³	1714.02(8)
Z	4
ρ _{calc} /cm ³	1.280
μ/mm ⁻¹	0.707
F(000)	712.0
Crystal size/mm ³	0.467 × 0.058 × 0.055
Radiation	CuKα (λ = 1.54178)
2θ range for data collection/°	9.266 to 154.388
Index ranges	-10 ≤ h ≤ 9, -13 ≤ k ≤ 13, -23 ≤ l ≤ 23
Reflections collected	25817
Independent reflections	3597 [R _{int} = 0.0725, R _{sigma} = 0.0359]
Data/restraints/parameters	3597/0/225
Goodness-of-fit on F ²	1.071
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0356, wR ₂ = 0.0852
Final R indexes [all data]	R ₁ = 0.0398, wR ₂ = 0.0885
Largest diff. peak/hole / e Å ⁻³	0.27/-0.18
Flack parameter	0.03(9)

Table S4. Crystal data and structure refinement for enbepeanone A (4).

Identification code	NB_P13_66_E_9_2_Salt_7
Empirical formula	C ₁₇ H ₂₂ O ₃
Formula weight	274.34
Temperature/K	100.0
Crystal system	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
a/Å	7.1483(2)
b/Å	7.7391(2)
c/Å	25.4330(7)
α/°	90
β/°	90
γ/°	90
Volume/Å ³	1406.99(7)
Z	4
ρ _{calc} /cm ³	1.295
μ/mm ⁻¹	0.698
F(000)	592.0
Crystal size/mm ³	0.2 × 0.06 × 0.02
Radiation	CuKα (λ = 1.54178)
2θ range for data collection/°	6.95 to 153.75
Index ranges	-8 ≤ h ≤ 9, -9 ≤ k ≤ 9, -31 ≤ l ≤ 31
Reflections collected	9932
Independent reflections	2884 [R _{int} = 0.0551, R _{sigma} = 0.0432]
Data/restraints/parameters	2884/0/188
Goodness-of-fit on F ²	1.105
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0374, wR ₂ = 0.0789
Final R indexes [all data]	R ₁ = 0.0455, wR ₂ = 0.0825
Largest diff. peak/hole / e Å ⁻³	0.19/-0.23
Flack parameter	-0.03(13)

Table S5. Bijvoet-Pair analysis and Bayesian statistics	
bathpytilone A (1)	Enbepeanone A (4)
Space Group P212121	Space Group P212121
Wavelength 1.54178	Wavelength 1.54178
Flack x -0.04(6)	Flack x -0.03(13)
Parsons z .. -0.03(7)	Parsons z .. 0.03(14)
Bijvoet Pairs 1319	Bijvoet Pairs 1162
Coverage ... 100	Coverage ... 94
DiffCalcMax. 40.69	DiffCalcMax. 23.94
Outlier Crit 81.38	Outlier Crit 47.88
Scatter Plot	Scatter Plot
Sigma Crit.. 0.25	Sigma Crit.. 0.25
Select Pairs 270	Select Pairs 44
Number Plus 176	Number Plus 32
Number Minus 94	Number Minus 12
Slope 1.139	Slope 0.860
Student-T Prob. Plot	Student-T Prob. Plot
Sample Size. 1309	Sample Size. 1152
Corr. Coeff. 0.999	Corr. Coeff. 0.999
Intercept .. 0.008	Intercept .. 0.003
Slope 0.856	Slope 0.851
Bayesian Statistics	Bayesian Statistics
Student_T Nu 100	Student_T Nu 100
Select Pairs 1319	Select Pairs 1162
Theta_Min .. 6.76	Theta_Min .. 8.62
Theta_Max .. 66.49	Theta_Max .. 76.45
P2(true).... 1.000	P2(true).... 1.000
P3(true).... 1.000	P3(true).... 1.000
P3(rac-twin) 0.9E-18	P3(rac-twin) 0.4E-03
P3(false) .. 0.1E-67	P3(false) .. 0.4E-14
G 1.0641	G 0.9433
G (su) 0.1164	G (su) 0.2388
Hooft y ... -0.03(6)	Hooft y ... 0.03(12)