

Supplementary Material

New Ansamycins from the Deep-Sea-Derived Bacterium *Ochrobactrum* sp. OUCMDZ-2164

Yaqin Fan ^{1,2,†}, **Cong Wang** ^{1,3,†}, **Liping Wang** ⁴, **Arthit Chairoungdua** ⁵, **Pawinee Piyachaturawat** ⁵, **Peng Fu** ^{1,2*} and **Weiming Zhu** ^{1,2,4*}

¹ Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China; fanyaqin.826@163.com (Y.F.); wangcong123206@163.com (C.W.)

² Laboratory for Marine Drugs and Bioproducts, Qingdao National Laboratory for Marine Science and Technology; Qingdao 266003, China

³ Guangxi Key Laboratory of Chemistry and Engineering of Forest Products, School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, Nanning 530006, China

⁴ State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550014, China; lipingw2006@163.com

⁵ Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand; arthit.chi@mahidol.ac.th (A.C.); pawinee.pia@mahidol.ac.th (P.P.)

* Correspondence: fupeng@ouc.edu.cn (P.F.); weimingzhu@ouc.edu.cn (W.Z.);
Tel./Fax: +86-532-8203-1268 (W.Z.)

† These authors contributed equally to this paper.

List of Supplementary Material

Figure S1. HRESIMS spectrum of trienomycin H (1).....	S3
Figure S2. ¹ H-NMR spectrum of trienomycin H (1) in DMSO- <i>d</i> ₆	S4
Figure S3. ¹³ C-NMR spectrum of trienomycin H (1) in DMSO- <i>d</i> ₆	S5
Figure S4. DEPT spectrum of trienomycin H (1) in DMSO- <i>d</i> ₆	S6
Figure S5. HSQC spectrum of trienomycin H (1) in DMSO- <i>d</i> ₆	S7
Figure S6. ¹ H- ¹ H COSY spectrum of trienomycin H (1) in DMSO- <i>d</i> ₆	S8
Figure S7. HMBC spectrum of trienomycin H (1) in DMSO- <i>d</i> ₆	S9
Figure S8. HRESIMS spectrum of trienomycin I (2)	S10
Figure S9. ¹ H-NMR spectrum of trienomycin I (2) in DMSO- <i>d</i> ₆	S11
Figure S10. ¹³ C-DEPTQ-NMR spectrum of trienomycin I (2) in DMSO- <i>d</i> ₆	S12
Figure S11. HSQC spectrum of trienomycin I (2) in DMSO- <i>d</i> ₆	S13
Figure S12. ¹ H- ¹ H COSY spectrum of trienomycin I (2) in DMSO- <i>d</i> ₆	S14
Figure S13. HMBC spectrum of trienomycin I (2) in DMSO- <i>d</i> ₆	S15
Figure S14. The determination of the Ala configuration of 1 by Marfey's Method	S16
Figure S15. HPLC profiles for the water-insoluble hydrolysate of 1 and 3	S17
Figure S16. ESIMS spectrum for the water-insoluble hydrolysate of 1	S18
Figure S17. ESIMS spectrum of trienomycinol (3)	S19
Figure S18. ¹ H NMR spectra of 3 and the water-insoluble hydrolysate of 1 and 2	S20
Figure S19. HPLC profiles for the water-insoluble hydrolysate of 2 and 3	S21
Figure S20. ESIMS spectrum for the water-insoluble hydrolysate of 2	S22
Figure S21. Phylogenetic tree mapping for the <i>Ochrobactrum</i> sp. OUCMDZ-2164	S23
The Physical Properties of trienomycinol (3)	S24

Figure S1. HRESIMS spectrum of trienomycin H (**1**)

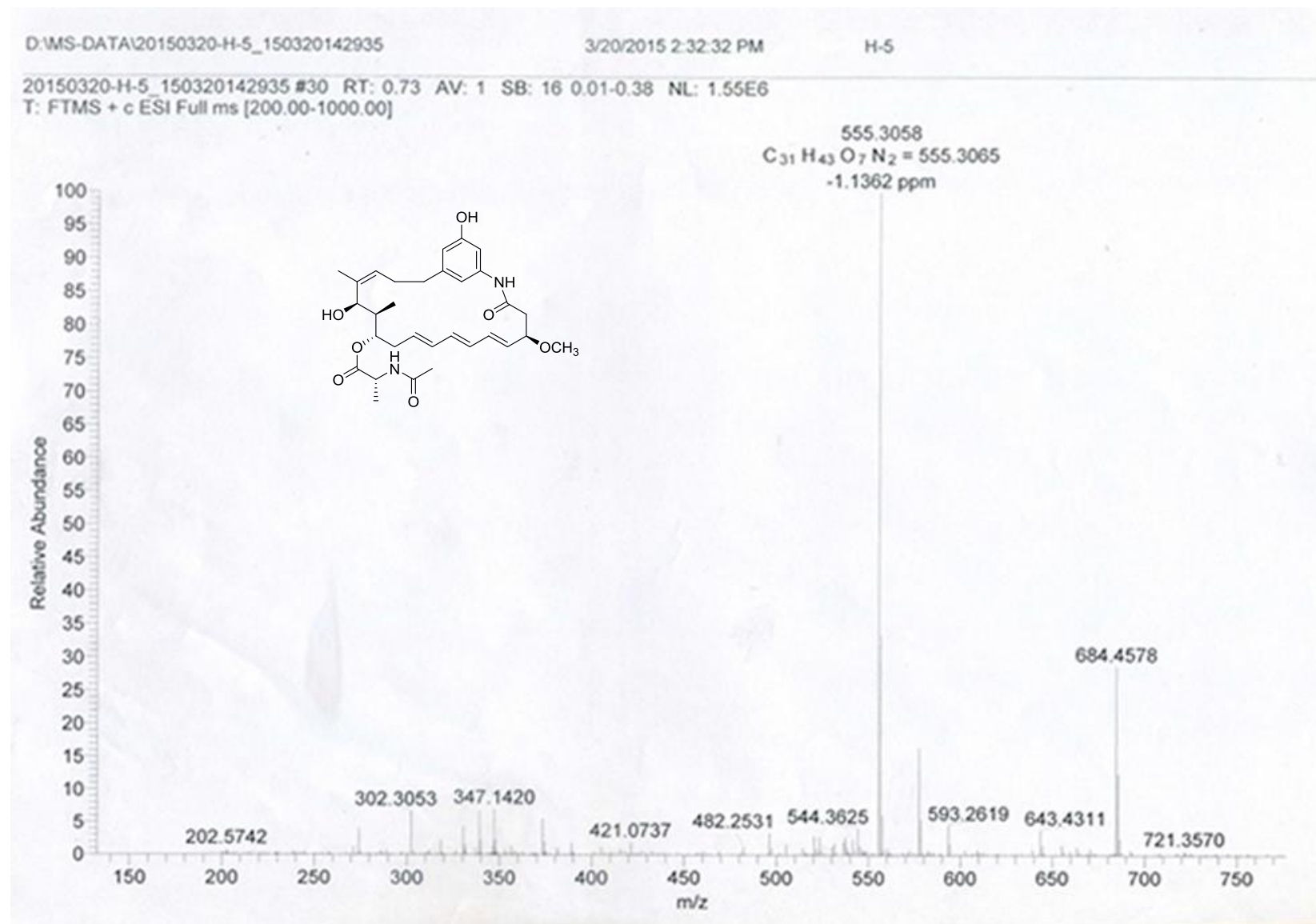


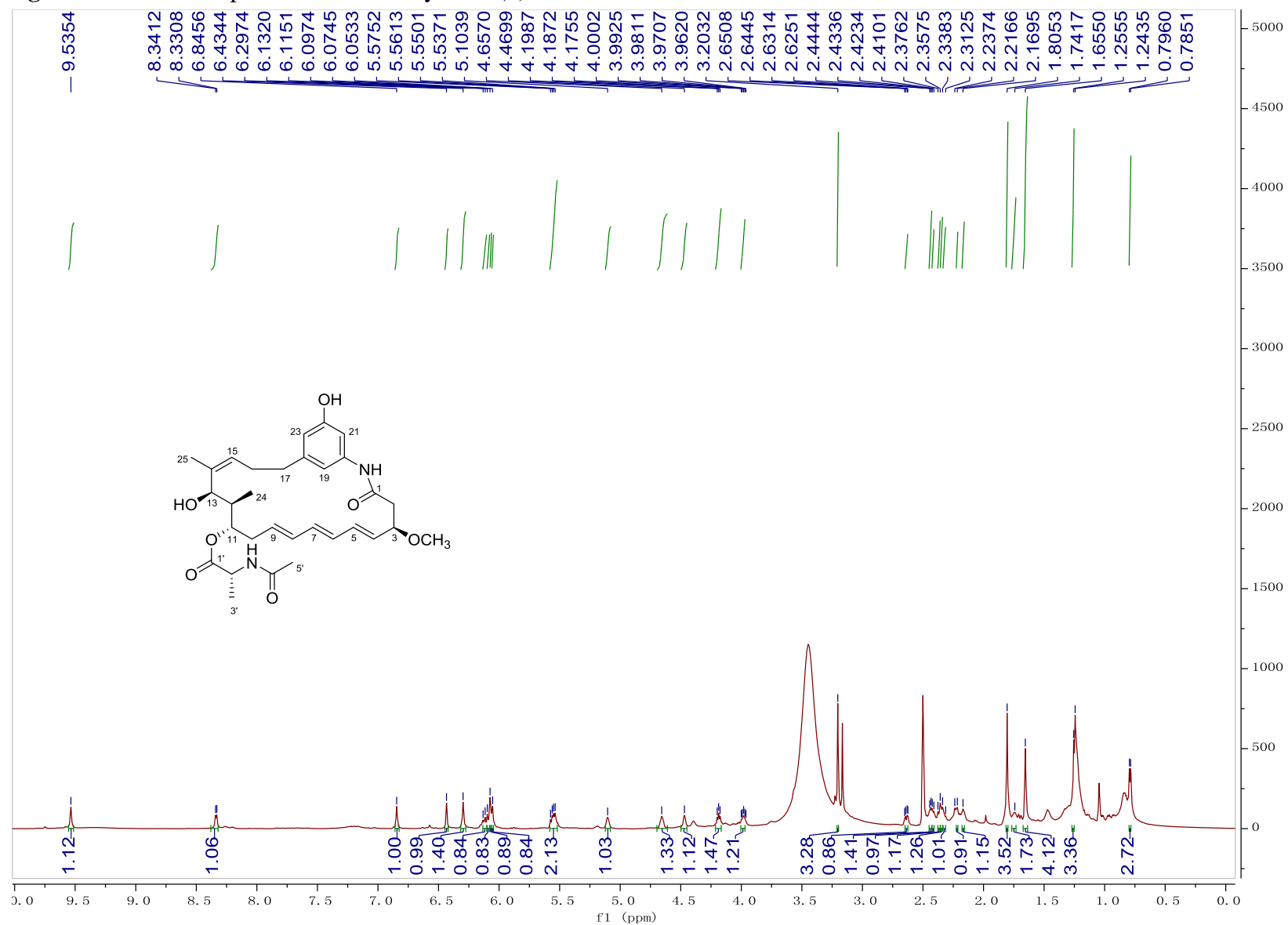
Figure S2. $^1\text{H-NMR}$ spectrum of trienomycin H (**1**) in $\text{DMSO-}d_6$ 

Figure S3. ^{13}C -NMR spectrum of trienomycin H (**1**) in $\text{DMSO-}d_6$

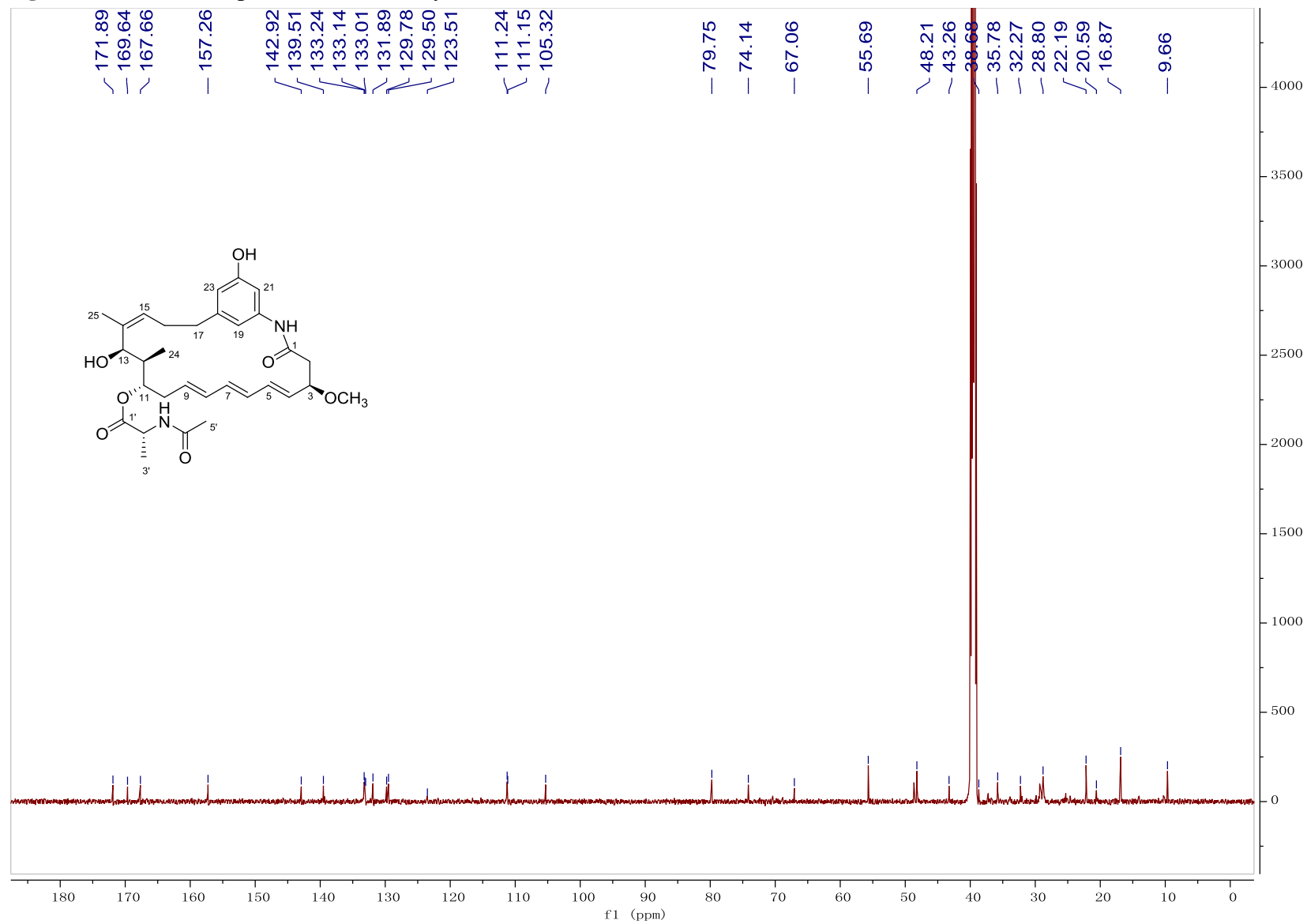


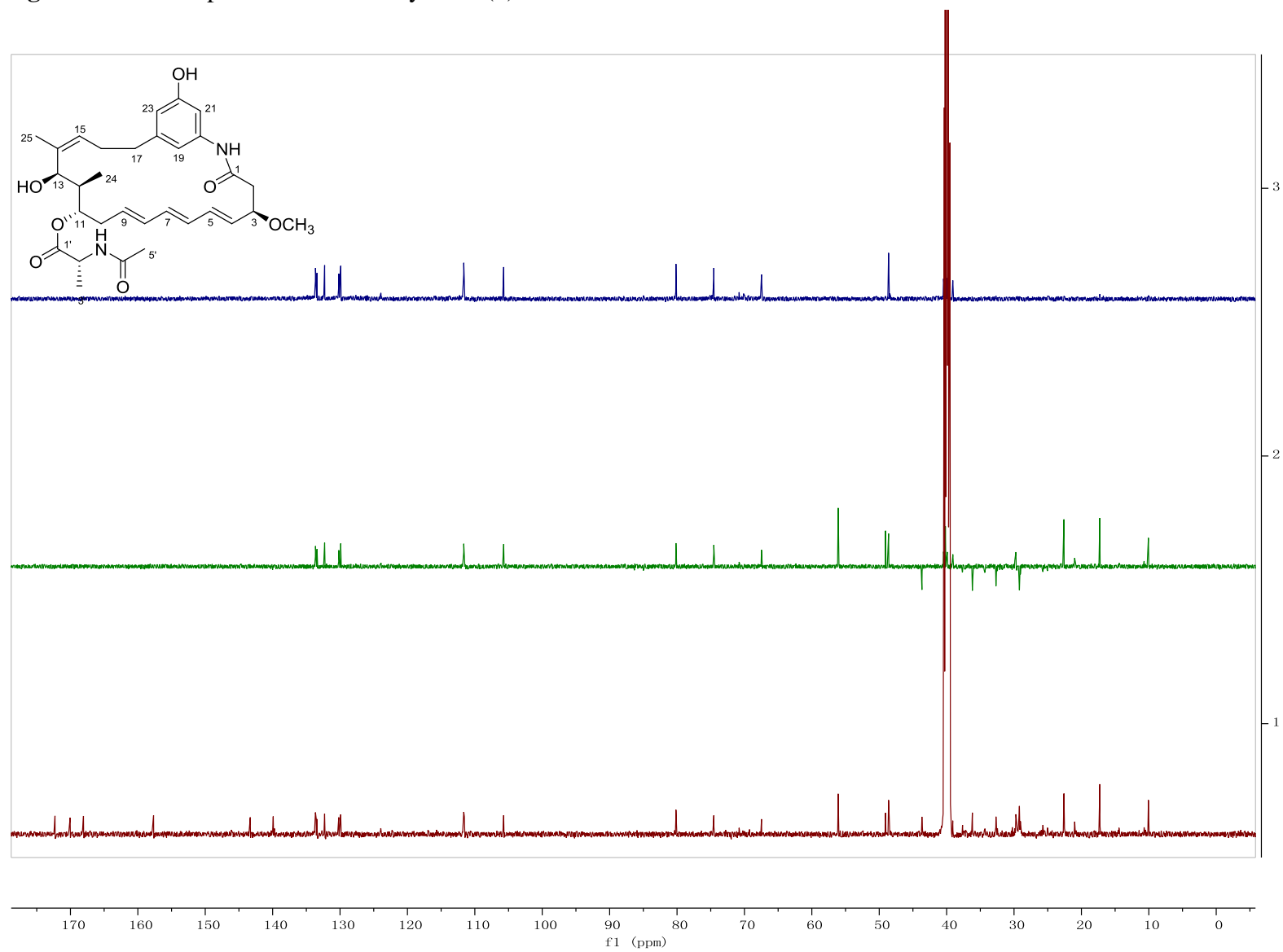
Figure S4. DEPT spectrum of trienomycin H (**1**) in DMSO-*d*₆

Figure S5. HSQC spectrum of trienomycin H (**1**) in DMSO-*d*₆

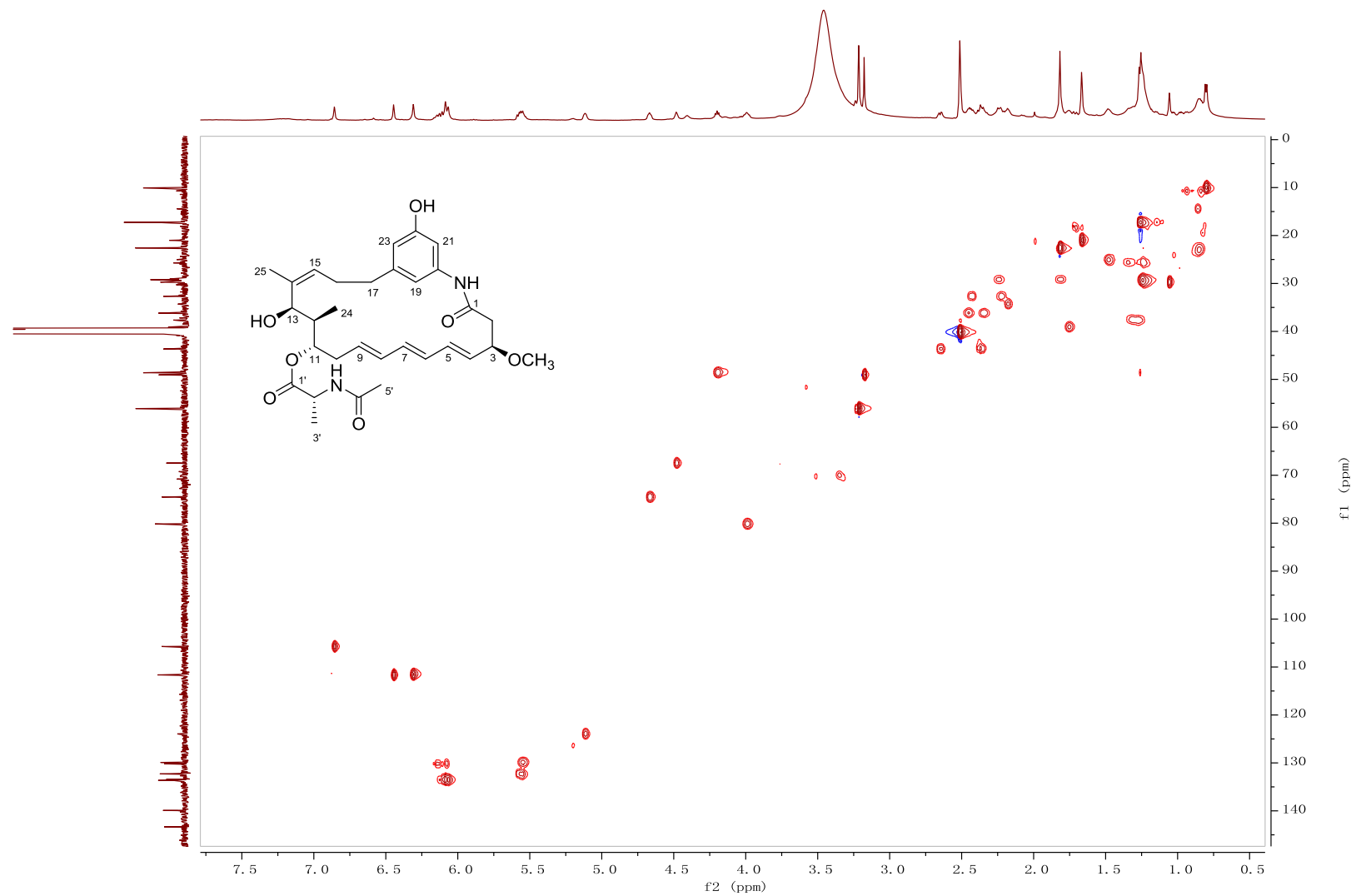


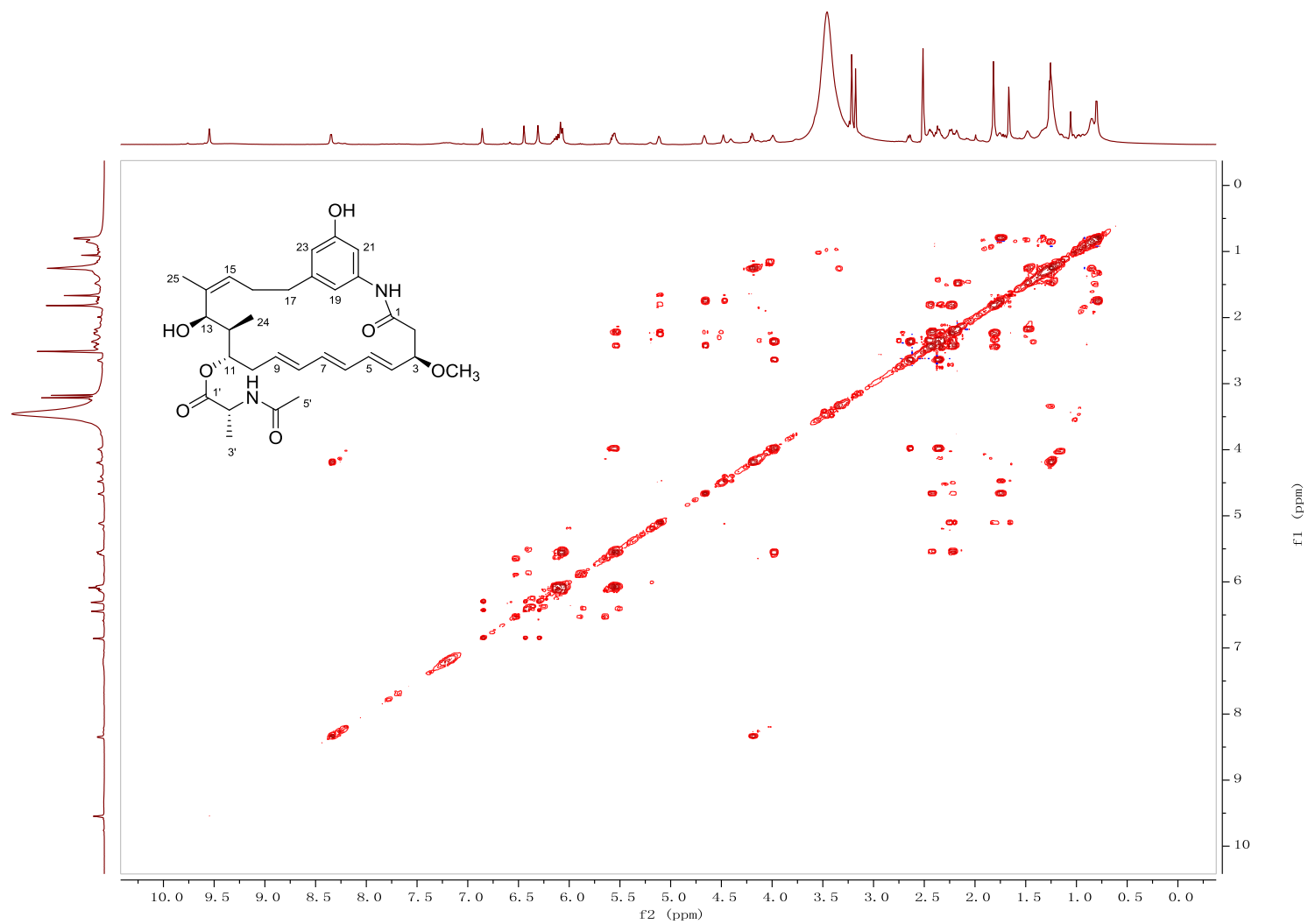
Figure S6. ^1H - ^1H COSY spectrum of trienomycin H (**1**) in $\text{DMSO-}d_6$ 

Figure S7. HMBC spectrum of trienomycin H (**1**) in DMSO-*d*₆

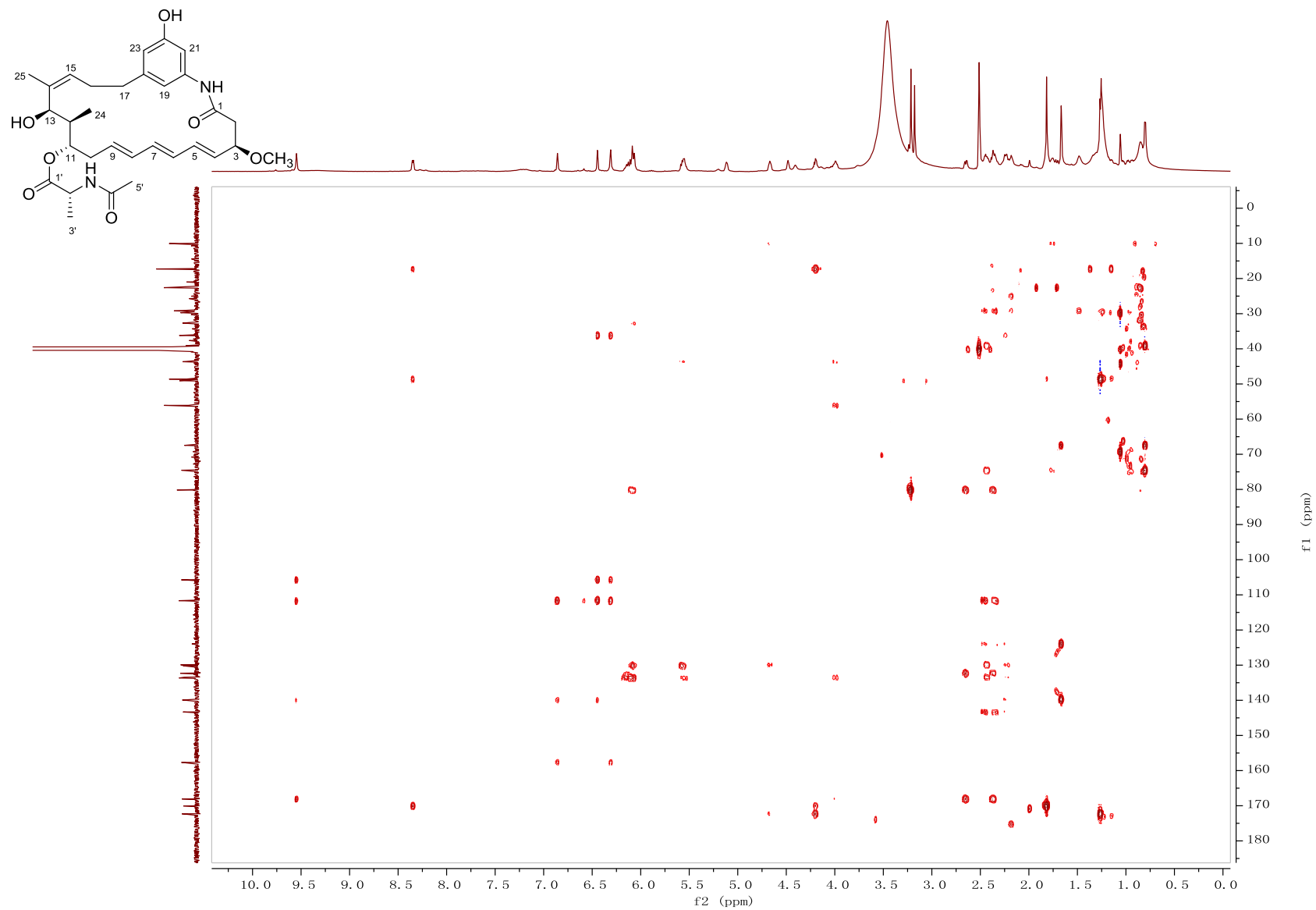


Figure S8. HRESIMS spectrum of trienomycin I (2)

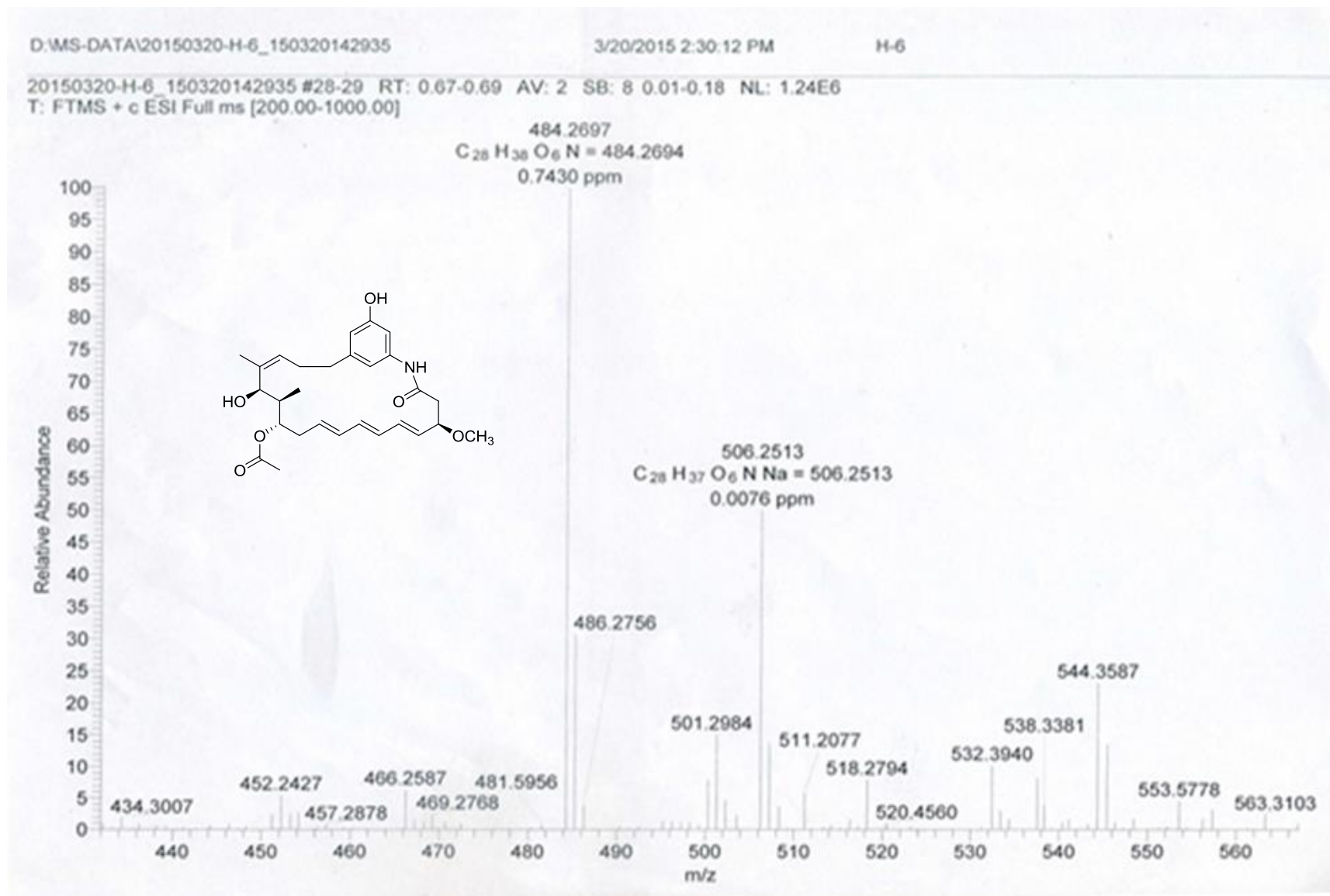


Figure S9. $^1\text{H-NMR}$ spectrum of trienomycin I (**2**) in $\text{DMSO-}d_6$

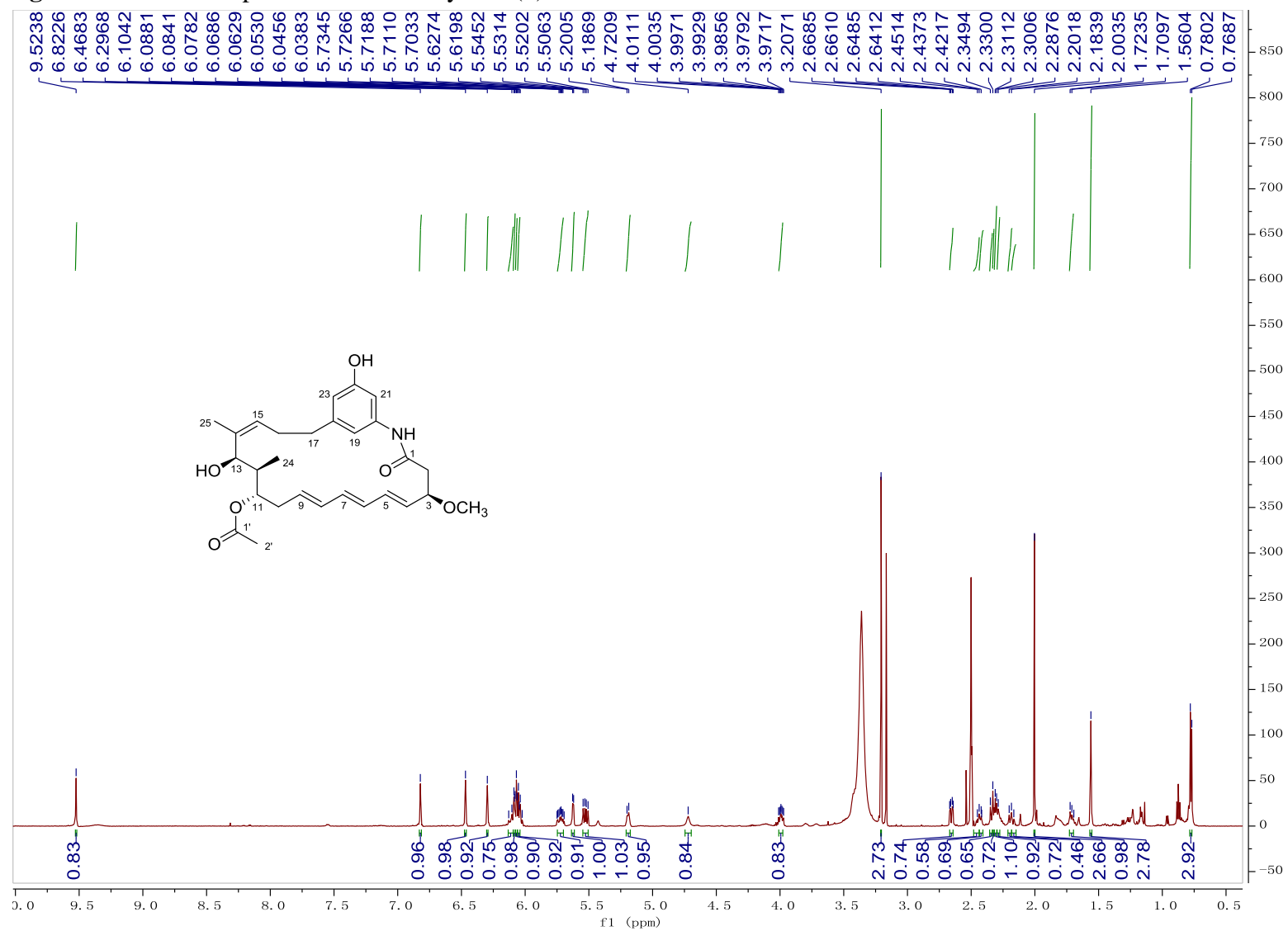


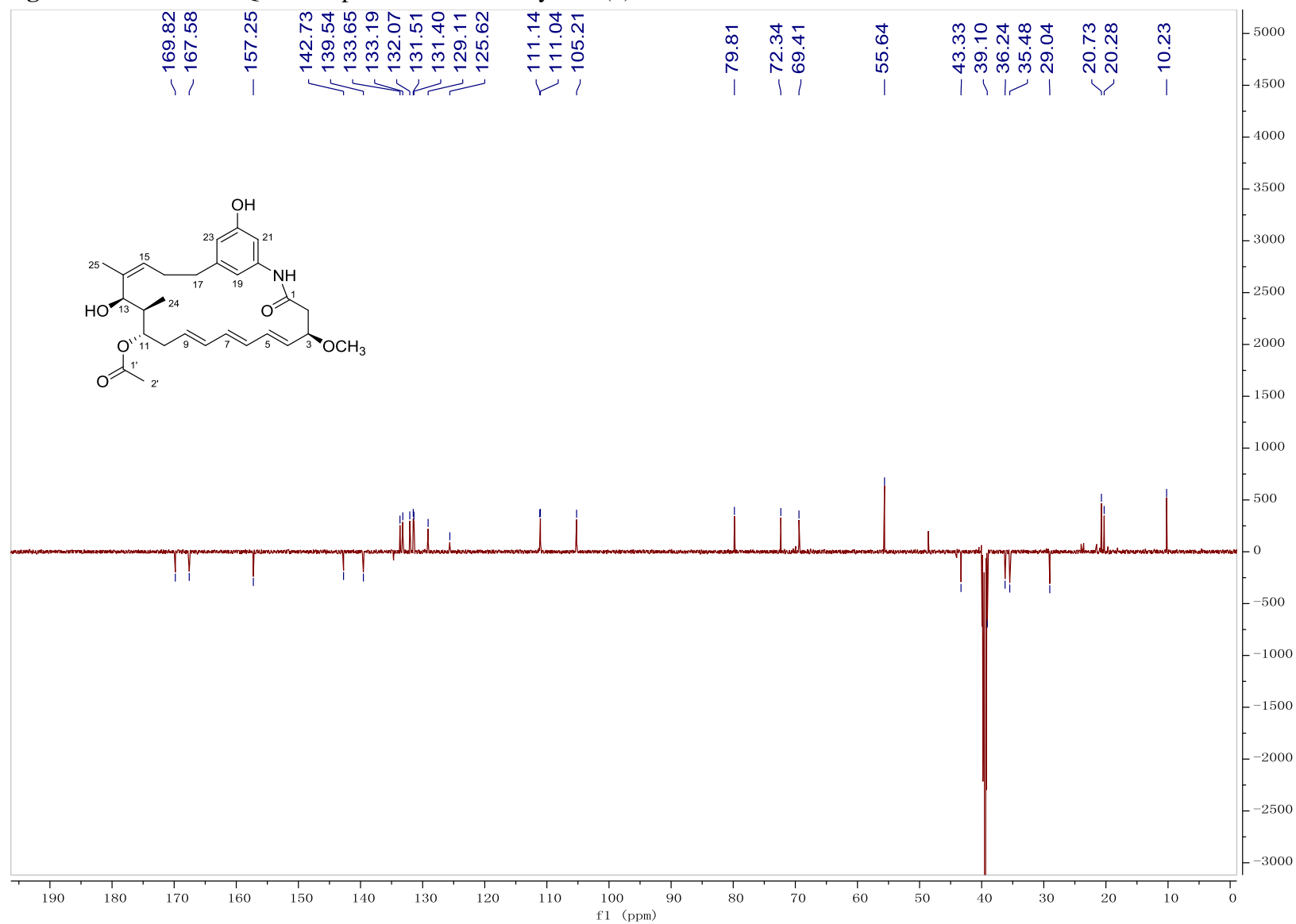
Figure S10. ^{13}C -DEPTQ-NMR spectrum of trienomycin I (**2**) in $\text{DMSO-}d_6$ 

Figure S11. HSQC spectrum of trienomycin I (**2**) in DMSO-*d*₆

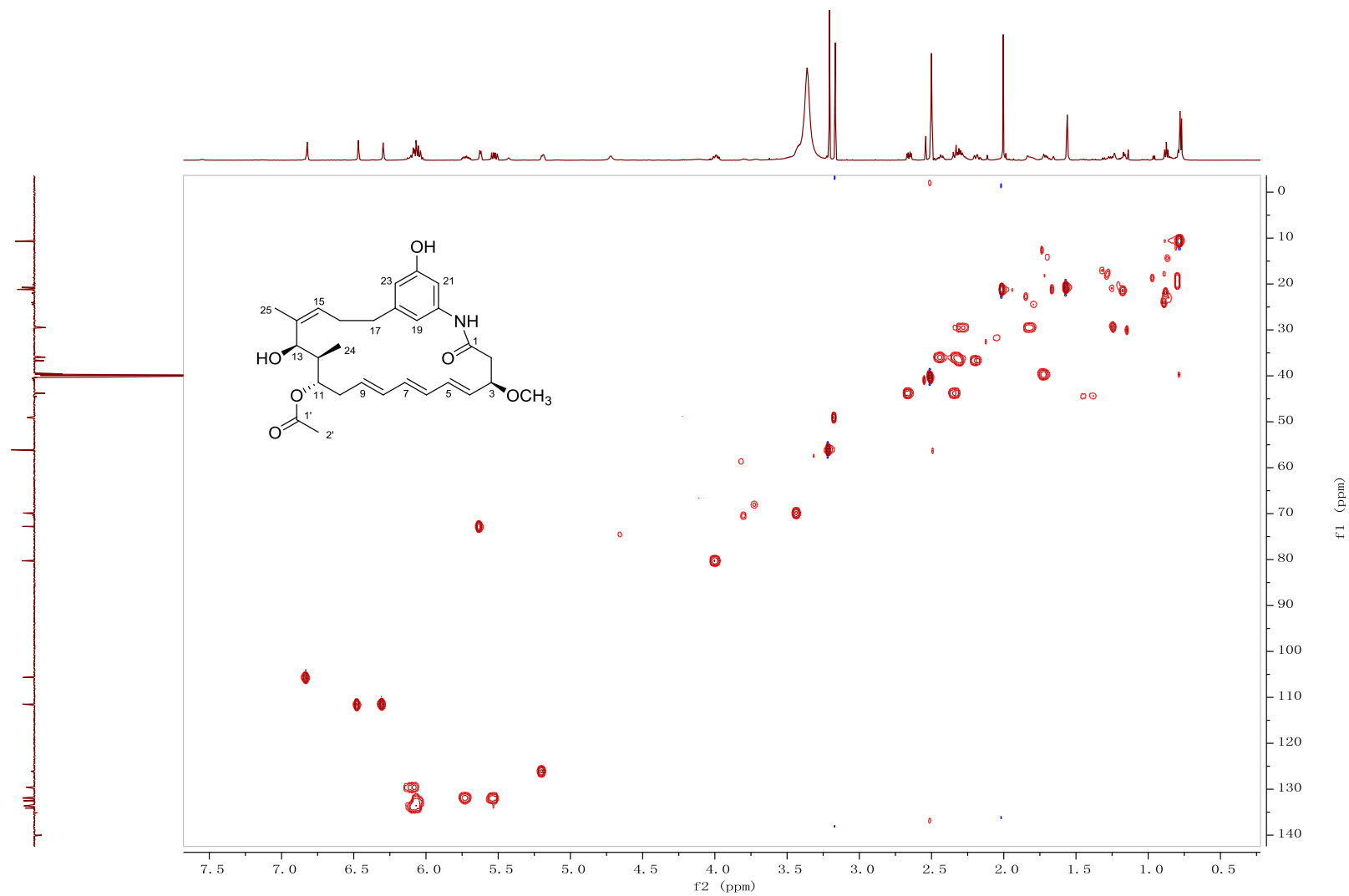


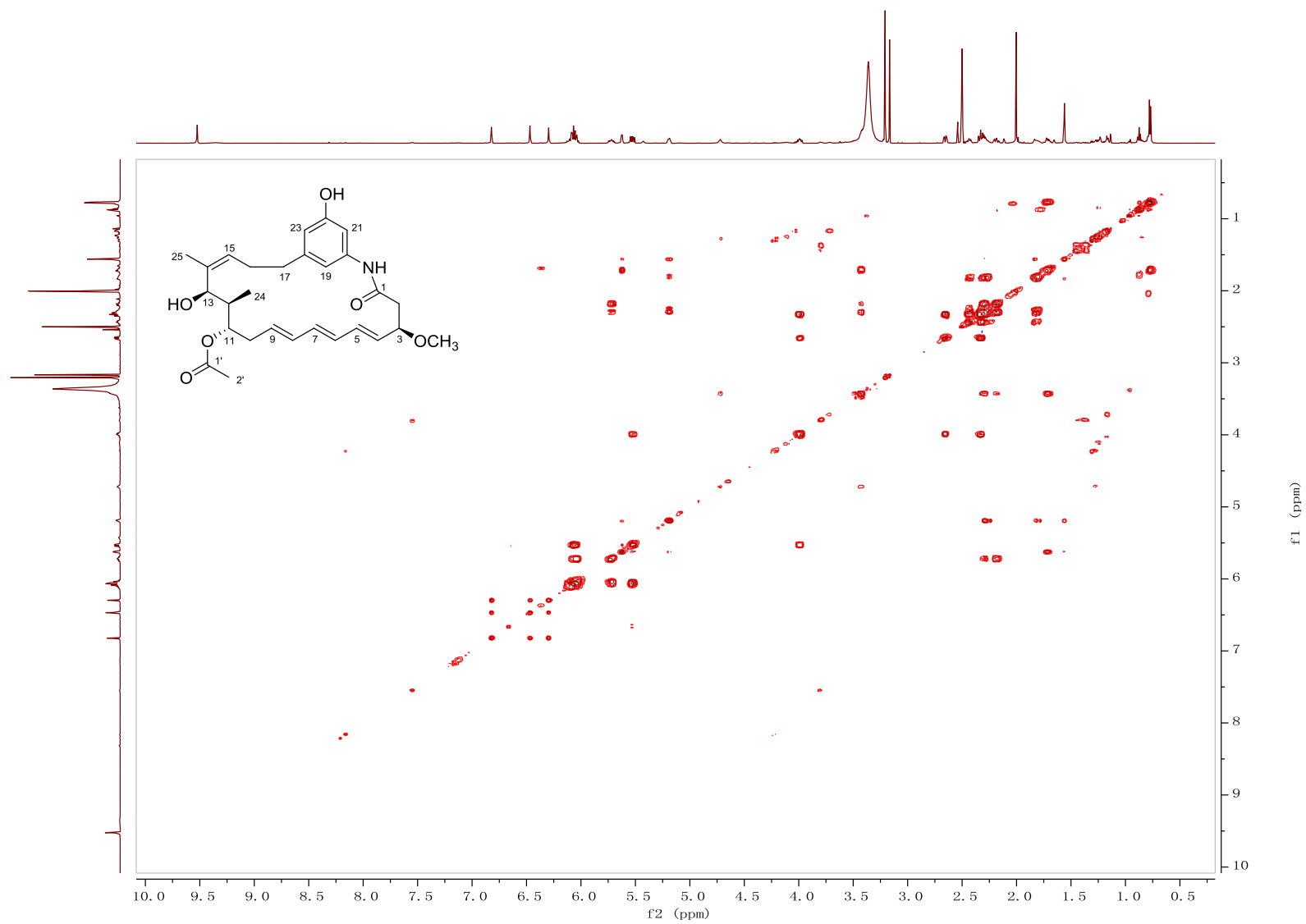
Figure S12. ^1H - ^1H COSY spectrum of trienomycin I (**2**) in $\text{DMSO-}d_6$ 

Figure S13. HMBC spectrum of trienomycin I (**2**) in DMSO-*d*₆

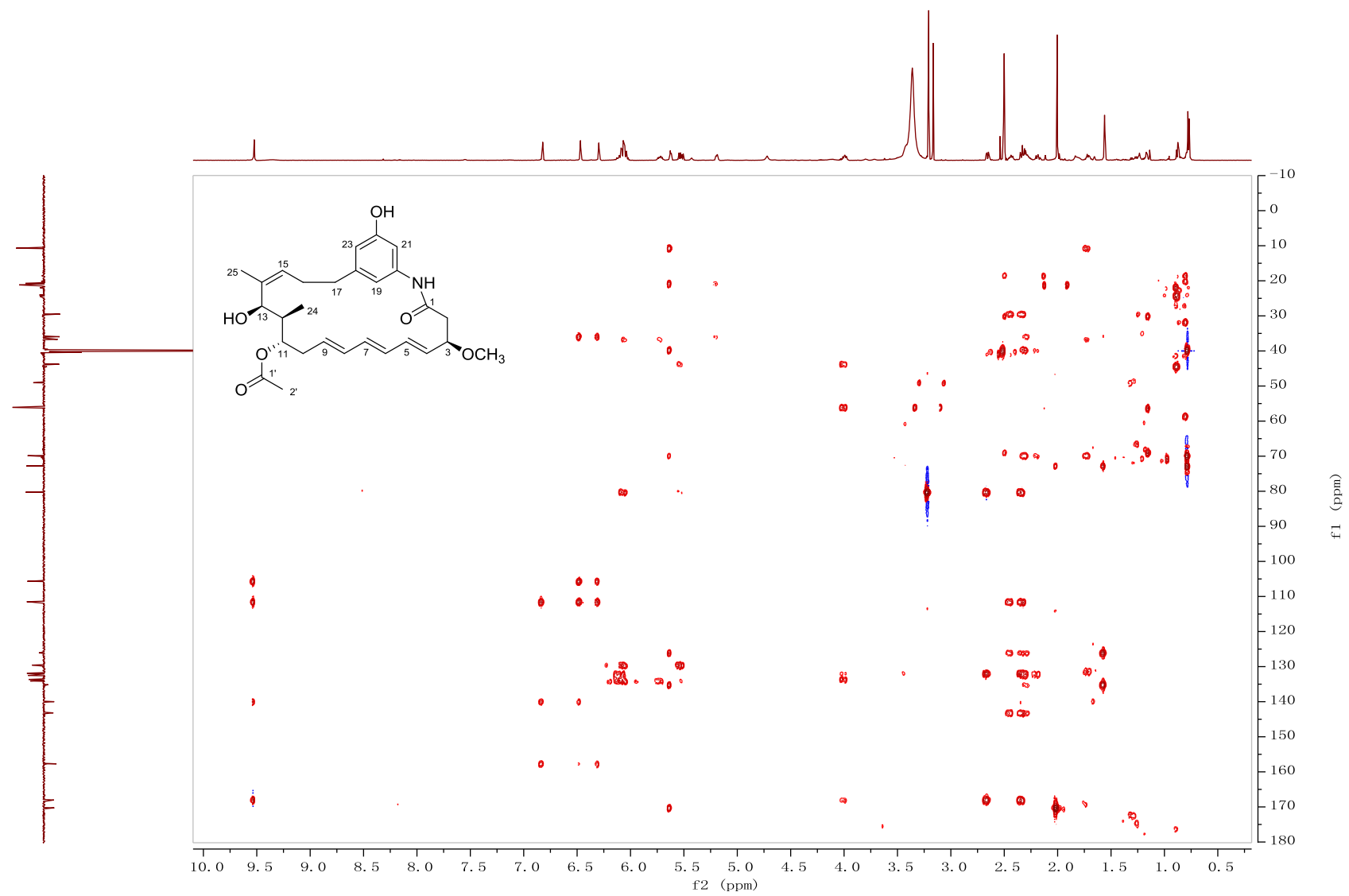


Figure S14. The determination of the Ala configuration of **1** by Marfey's Method (Solvents: A water + 0.2% TFA, B MeCN; linear gradient: 0 min, 25% B; 40 min, 60% B; 45 min, 100% B; temperature, 30 °C; flow rate, 1 mL/min; UV detection at λ 340 nm; FDAA, 14.2 min)

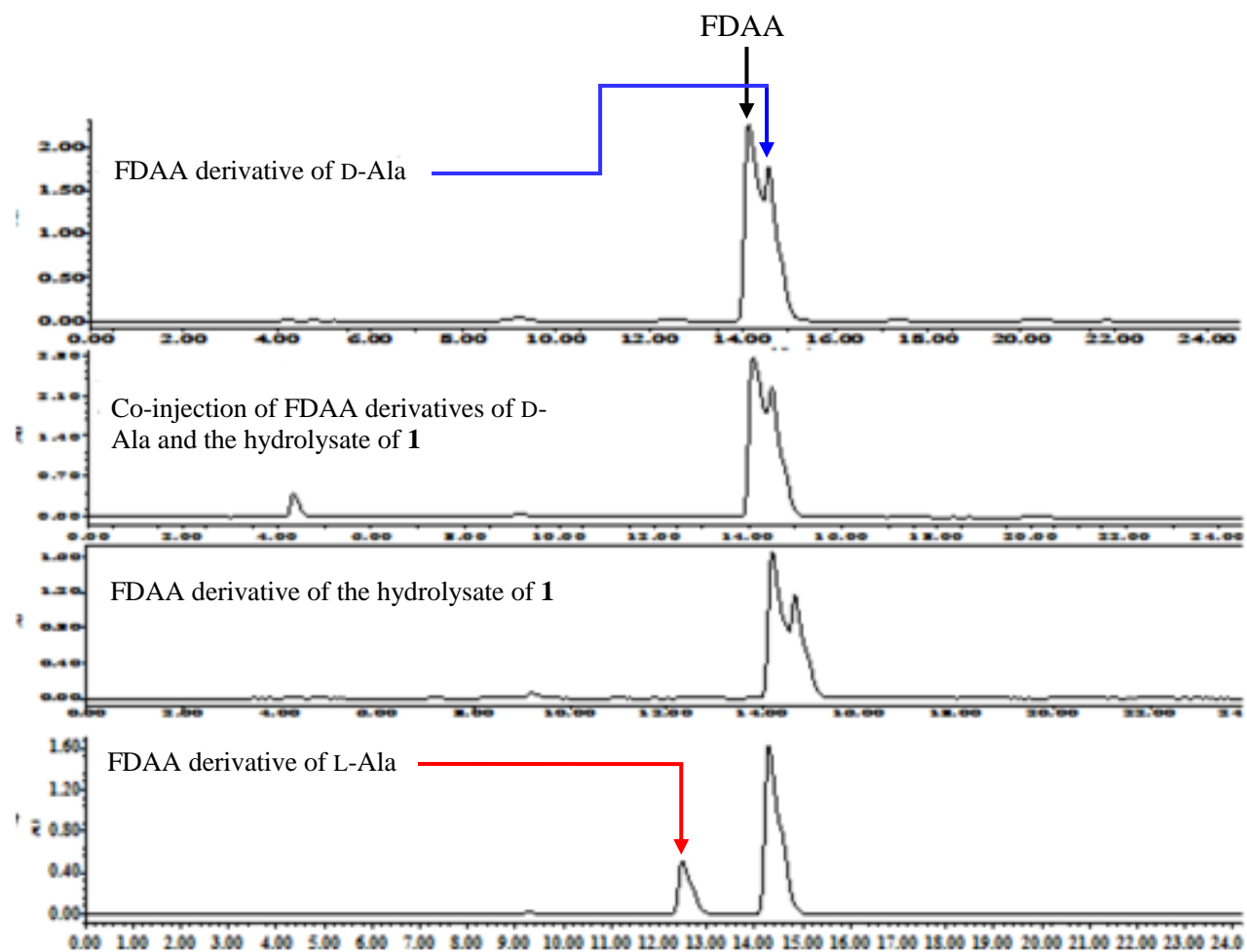


Figure S15. HPLC profiles for the water-insoluble hydrolysate of **1** and **3**

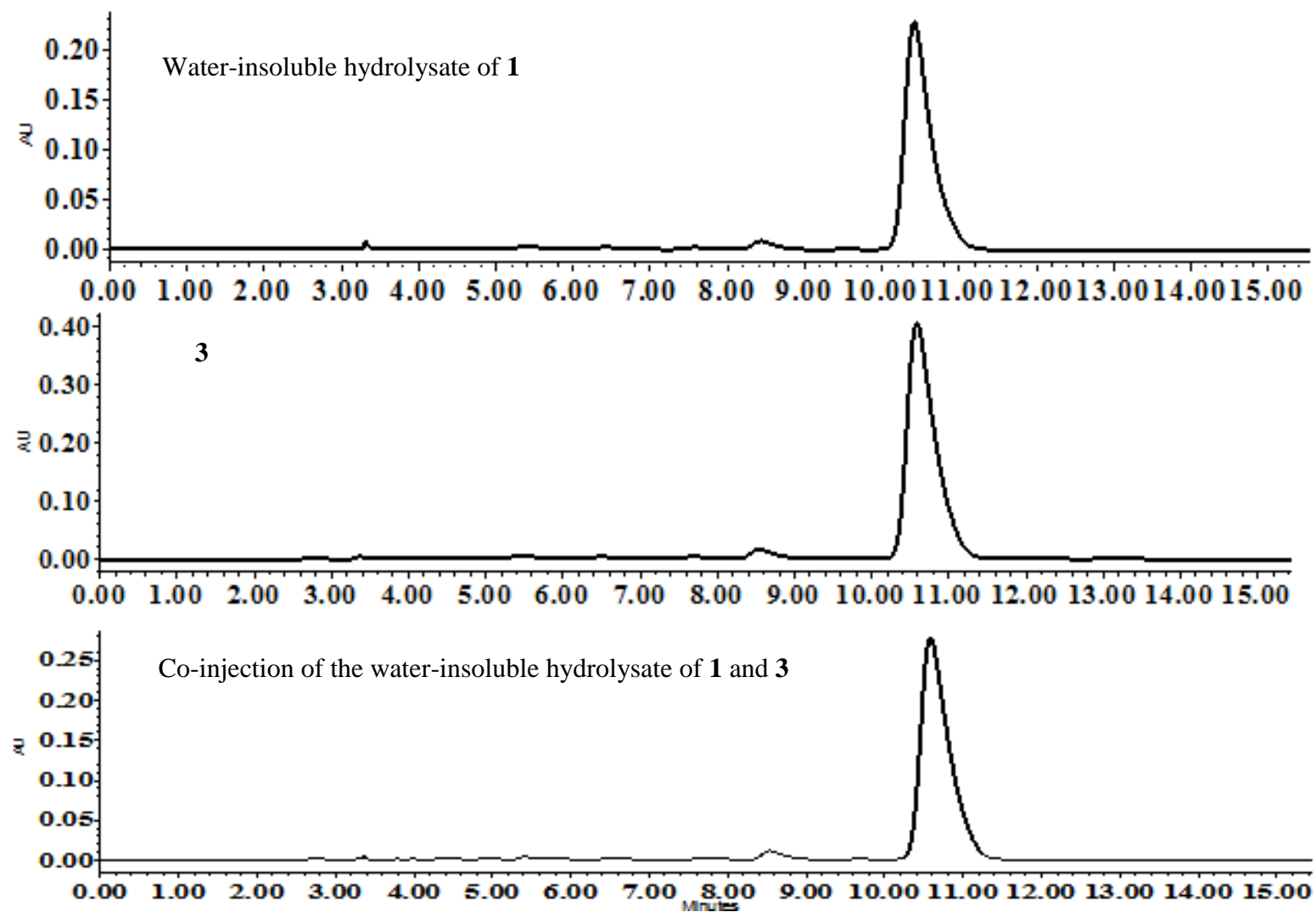


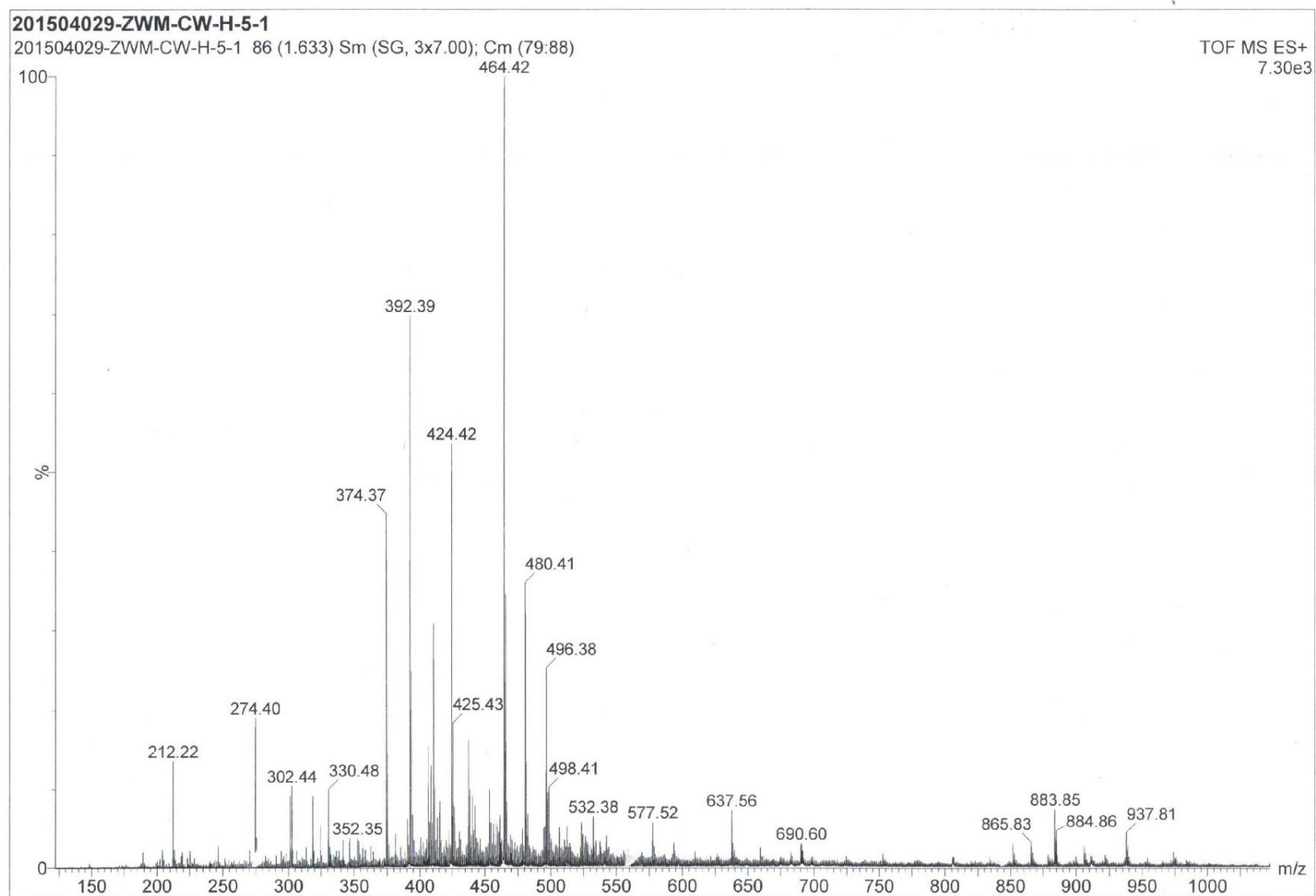
Figure S16. ESIMS spectrum for the water-insoluble hydrolysate of **1**

Figure S17. ESIMS spectrum for trienomycinol (**3**)

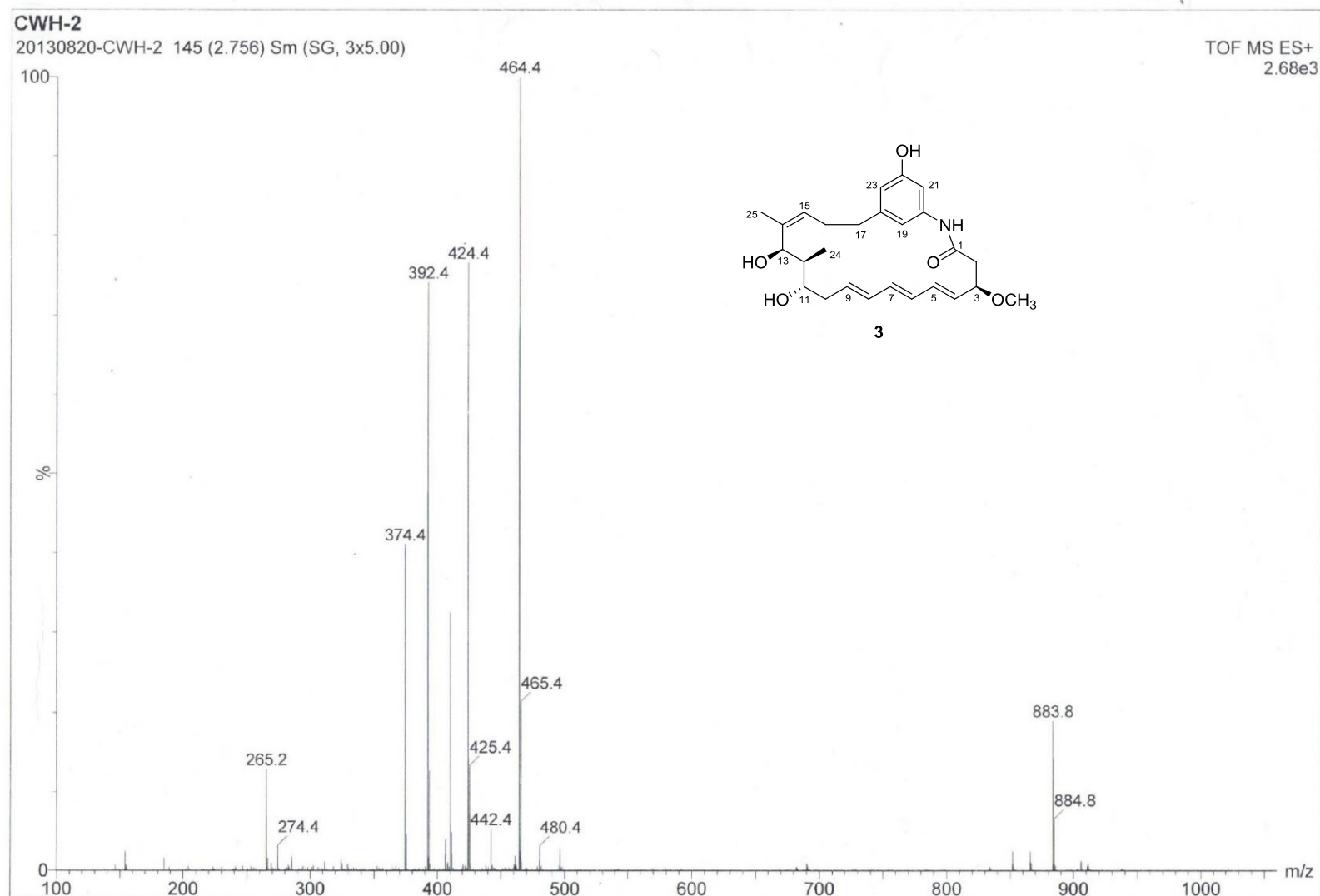


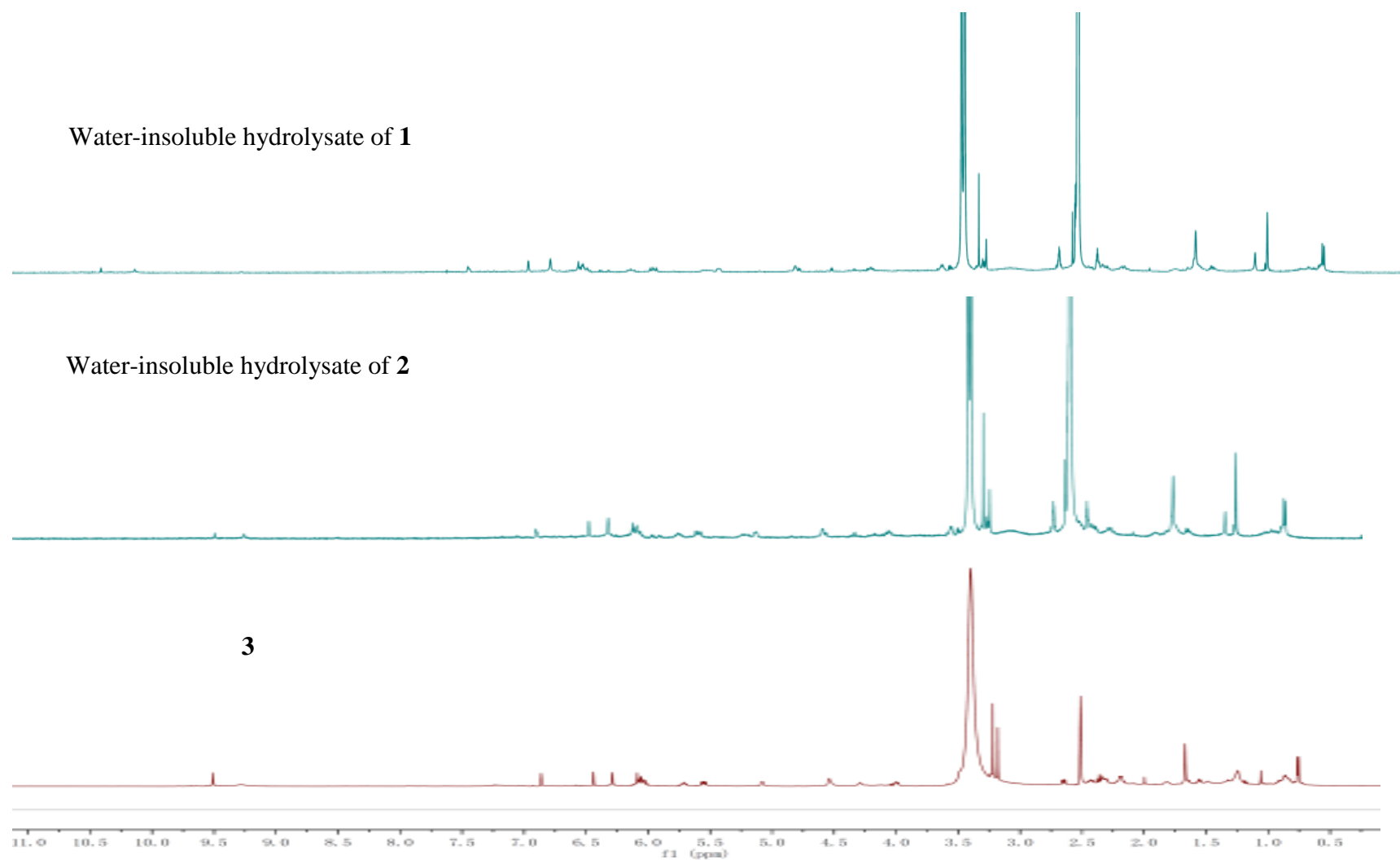
Figure S18. ^1H NMR spectra for **3** and the water-insoluble hydrolysate of **1** and **2**

Figure S19. HPLC profiles for the water-insoluble hydrolysate of **2** and **3**

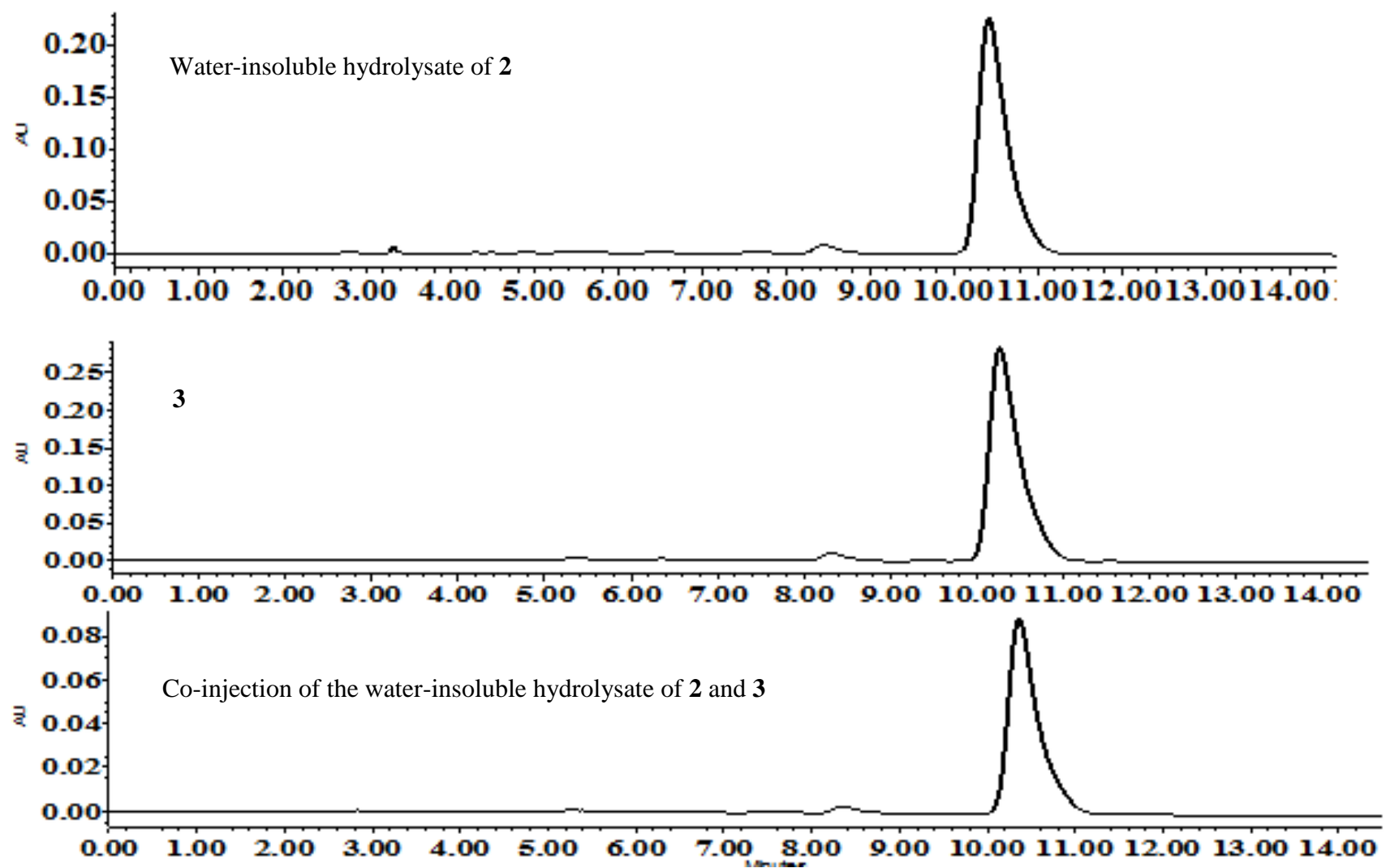


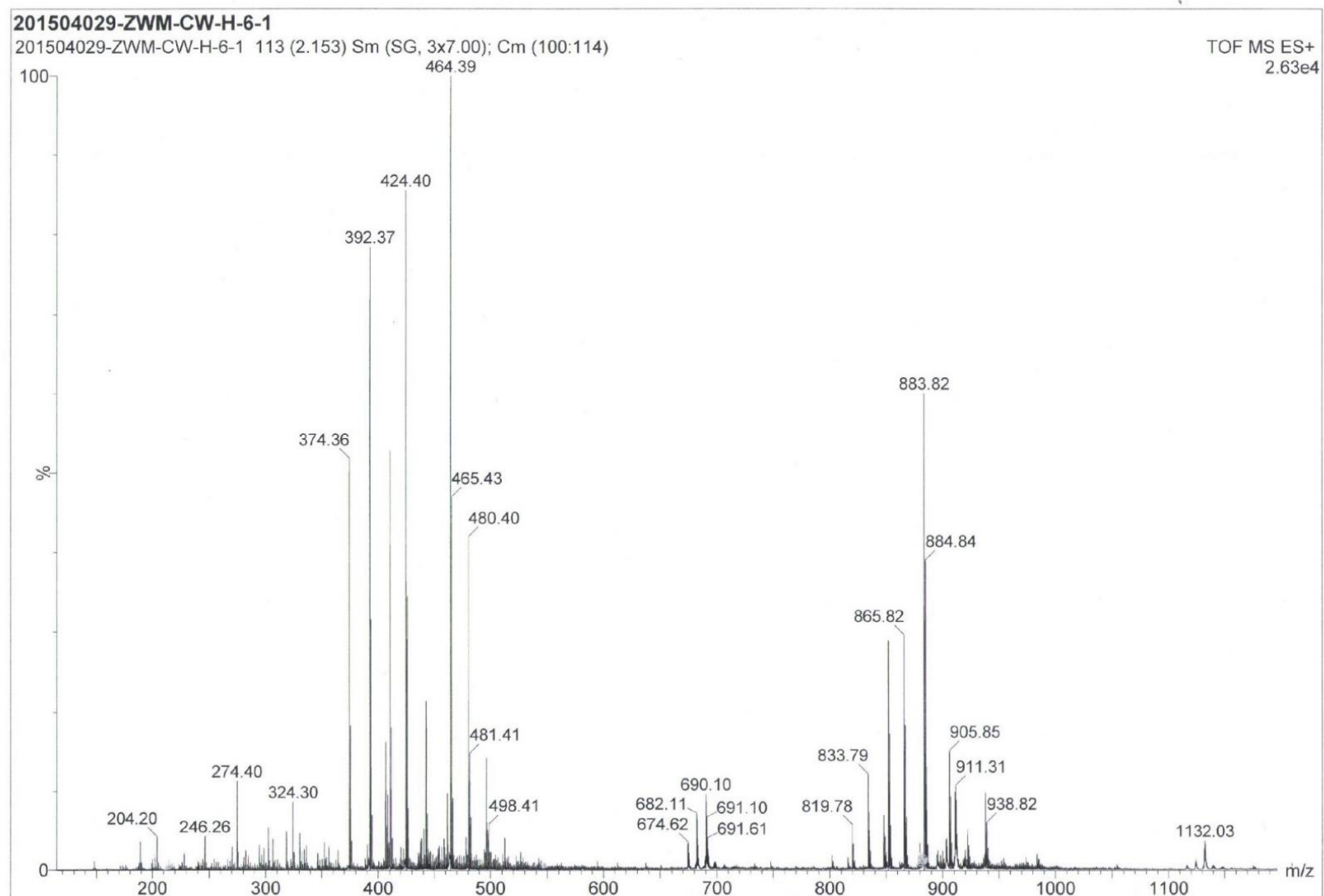
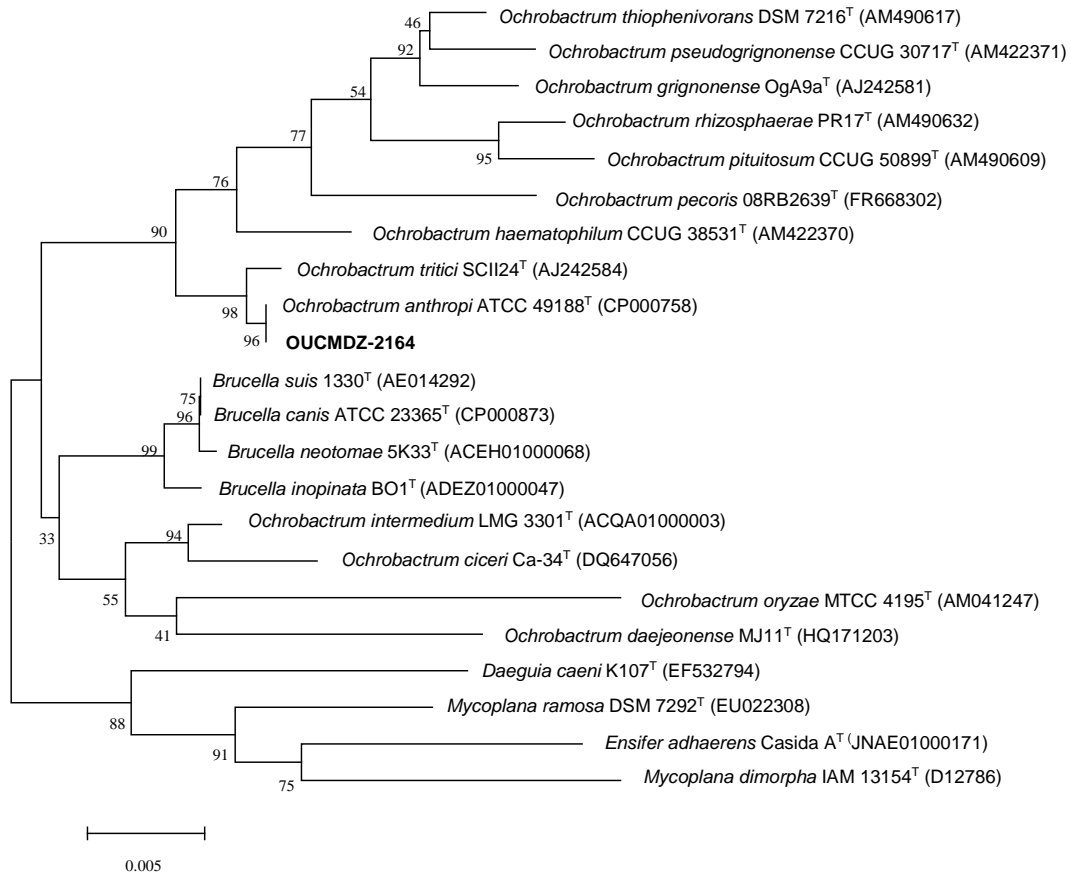
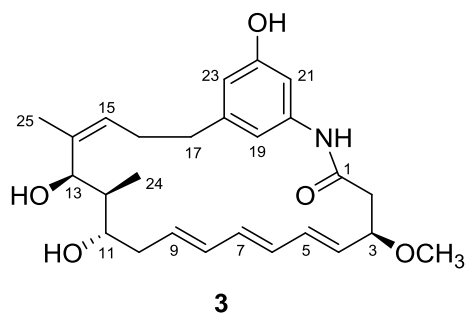
Figure S20. ESIMS spectrum for the water-insoluble hydrolysate of **2**

Figure S21. Phylogenetic tree mapping for the *Ochrobactrum* sp. OUCMDZ-2164



The Physical Properties of trienomycinol (3)



Trienomycinol (3): Yellow oil; $[\alpha]_{\text{D}}^{25} +62.8$ (c 0.1, MeOH); ECD (0.0011 M , MeOH) λ_{max} ($\Delta\epsilon$) 211 (-6.3), 266 (-2.8), 269 ($+13.0$) nm; ^1H NMR (DMSO- d_6 , 600 MHz) δ 9.51, (s, 1H, 20-NH), 6.86 (s, 1H, H-21), 6.44 (s, 1H, H-23), 6.29 (s, 1H, H-19), 6.09 (dd, $J = 15.3$ Hz, $J = 11.0$ Hz, 1H, H-6), 6.08 (dd, $J = 15.3$ Hz, $J = 10.3$ Hz, 1H, H-7), 6.07 (dd, $J = 15.0$ Hz, $J = 9.3$ Hz, 1H, H-5), 6.06 (dd, $J = 15.0$ Hz, $J = 11.5$ Hz, 1H, H-8), 5.71 (m, 1H, H-9), 5.56 (dd, $J = 15.0$ Hz, $J = 8.3$ Hz, 1H, H-4), 5.08 (m, 1H, H-15), 4.54 (brs, 1H, H-13), 4.00 (m, 1H, H-3), 3.48 (m, 1H, H-11), 3.18 (s, 3H, CH₃O-3), 2.65 (m, 1H, H_a-2), 2.42 (overlap, 1H, H_a-10), 2.37 (m, 1H, H_b-2), 2.33 (overlap, 1H, H_b-10), 2.19 (overlap, 1H, H_a-17), 2.18 (overlap, 1H, H_a-16), 2.00 (overlap, 1H, H_b-17), 1.82 (overlap, 1H, H_b-16), 1.67 (overlap, 1H, H-12), 1.67 (overlap, 3H, H₃-25), 0.75 (d, $J = 6.6$ Hz, 3H, H₃-24); ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 168.1 (C, C-1), 157.6 (C, C-22), 143.5 (C, C-18), 140.0 (C, C-14), 140.0 (C, C-20), 134.1 (CH, C-6), 133.5 (CH, C-5), 132.5 (CH, C-9), 132.1 (CH, C-8), 131.8 (CH, C-4), 129.4 (CH, C-7), 124.0 (CH₂, C-15), 111.5 (CH, C-19), 111.5 (CH, C-23), 105.7 (CH, C-21), 80.1 (CH, C-3), 70.7 (CH, C-11), 68.2 (CH, C-13), 56.1 (CH₃, CH₃O-3), 43.7 (CH₂, C-2), 41.5 (CH, C-12), 36.8 (CH₂, C-10) 36.2 (CH₂, C-17), 29.3 (CH₂, C-16), 20.9 (CH₃, C-25), 10.5 (CH₃, C-24). ESIMS m/z 464.4 $[\text{M} + \text{Na}]^+$.