Supporting Information

Identification of (4*S*/*R*)-4,5-dihydro-hydroxygeldanamycins as shunt products in geldanamycin biosynthesis

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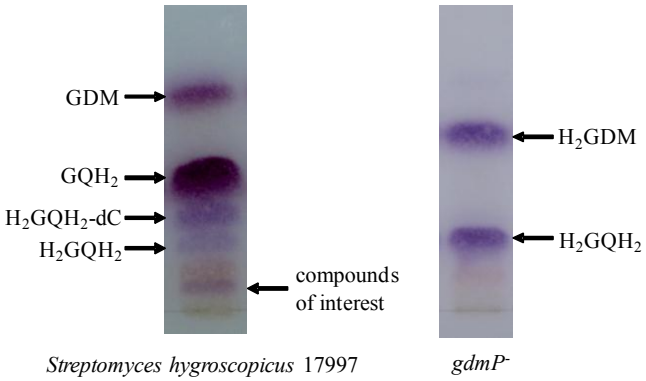
Supporting Information Available

Index

Figure S1. TLC comparison of secondary metabolites of *Streptomyces hygroscopicus* 17997 and *gdmP*$^-$: EtOAc extracts were chromatographed on normal phase TLC (EtOAc: C_6H_{14}: CH_2Cl_2: MeOH = 9: 6: 6: 1.5), and then sprayed with 2.0 mol/L NaOH for color reaction.* A unique blue band with a small R_f value from *Streptomyces hygroscopicus* 17997 was later proved to contain the putative C-4/5 hydroxylated intermediate(s) of GDM.

* Liu, A. M.; Wu, L. Z.; Wang, Y. G.; Zhang, H. T.; He, W. Q.; Li, Y. H.; Zhang, K. *Chin. J. Antibiot*. **2008**, *33*, 403-406.



GDM, geldanamycin;

GQH_2, hydroquinone geldanamycin;

H_2GQH_2-dC, hydroquinone 4,5-dihydro-7-descarbamoyl-7-hydroxygeldanamycin;

H_2GQH_2, hydroquinone 4,5-dihydrogeldanamycin;

H_2GDM, 4,5-dihydrogeldanamycin.

Figure S2. ESIMS spectrum of **1**

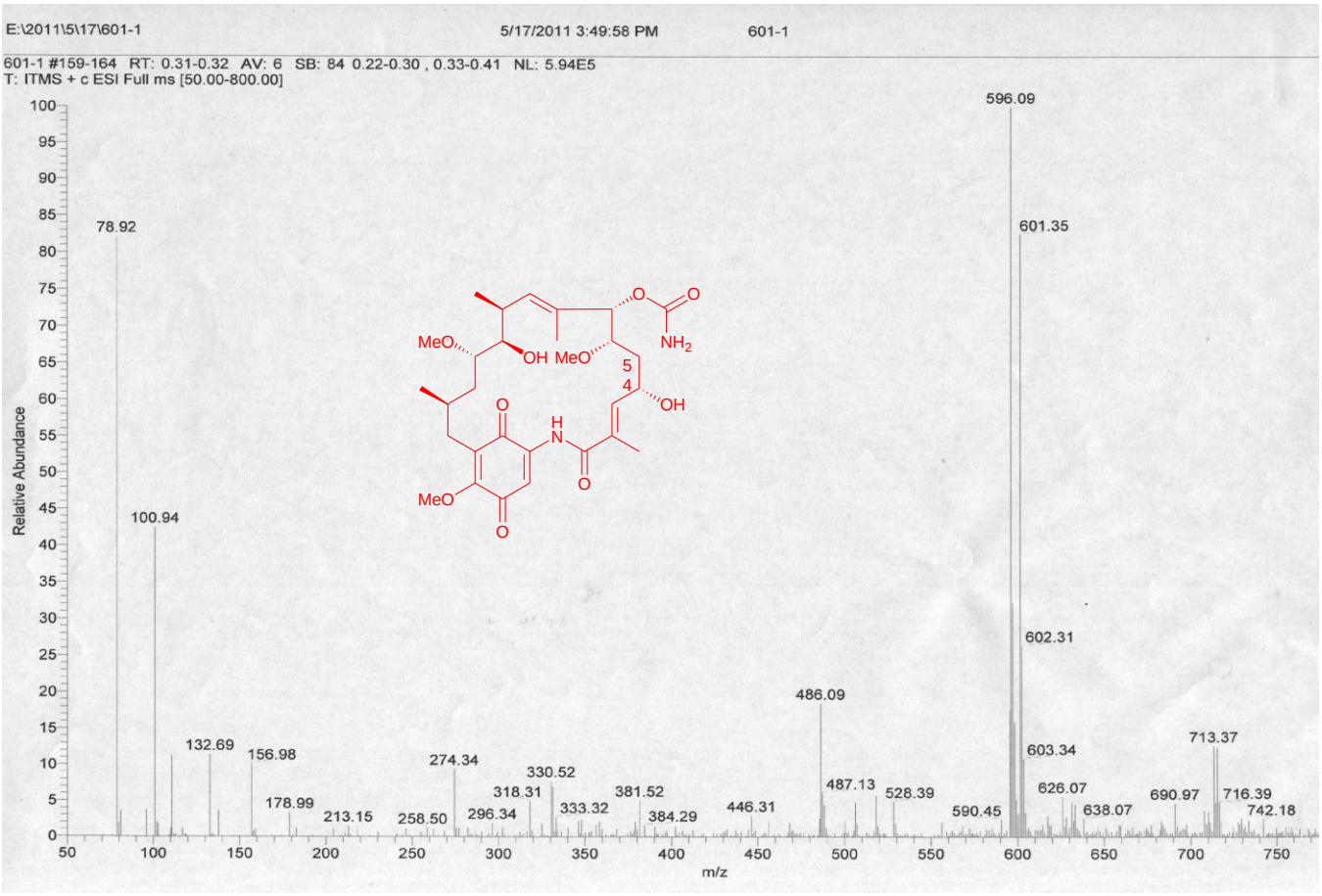


Figure S3. MS^2 spectrum of **1**

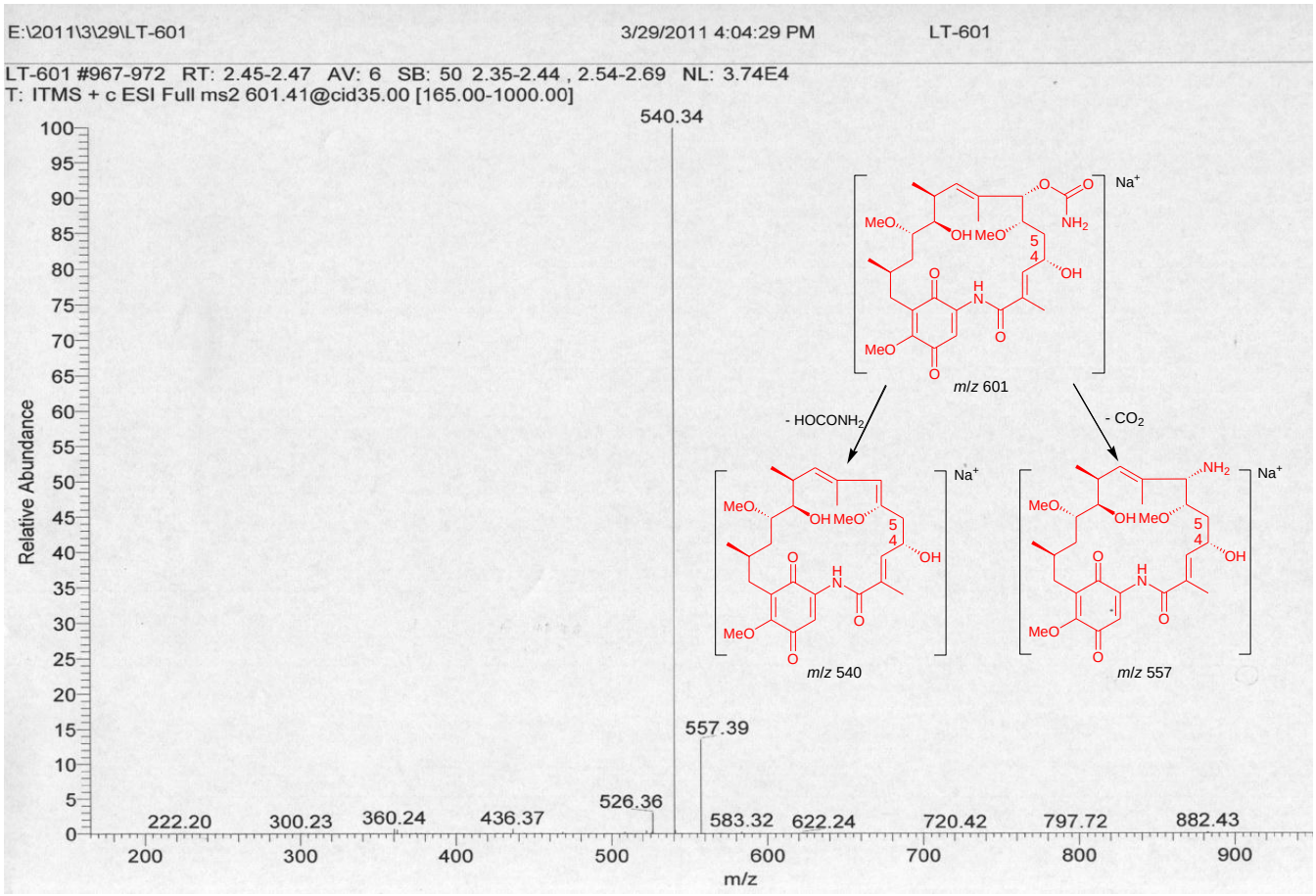
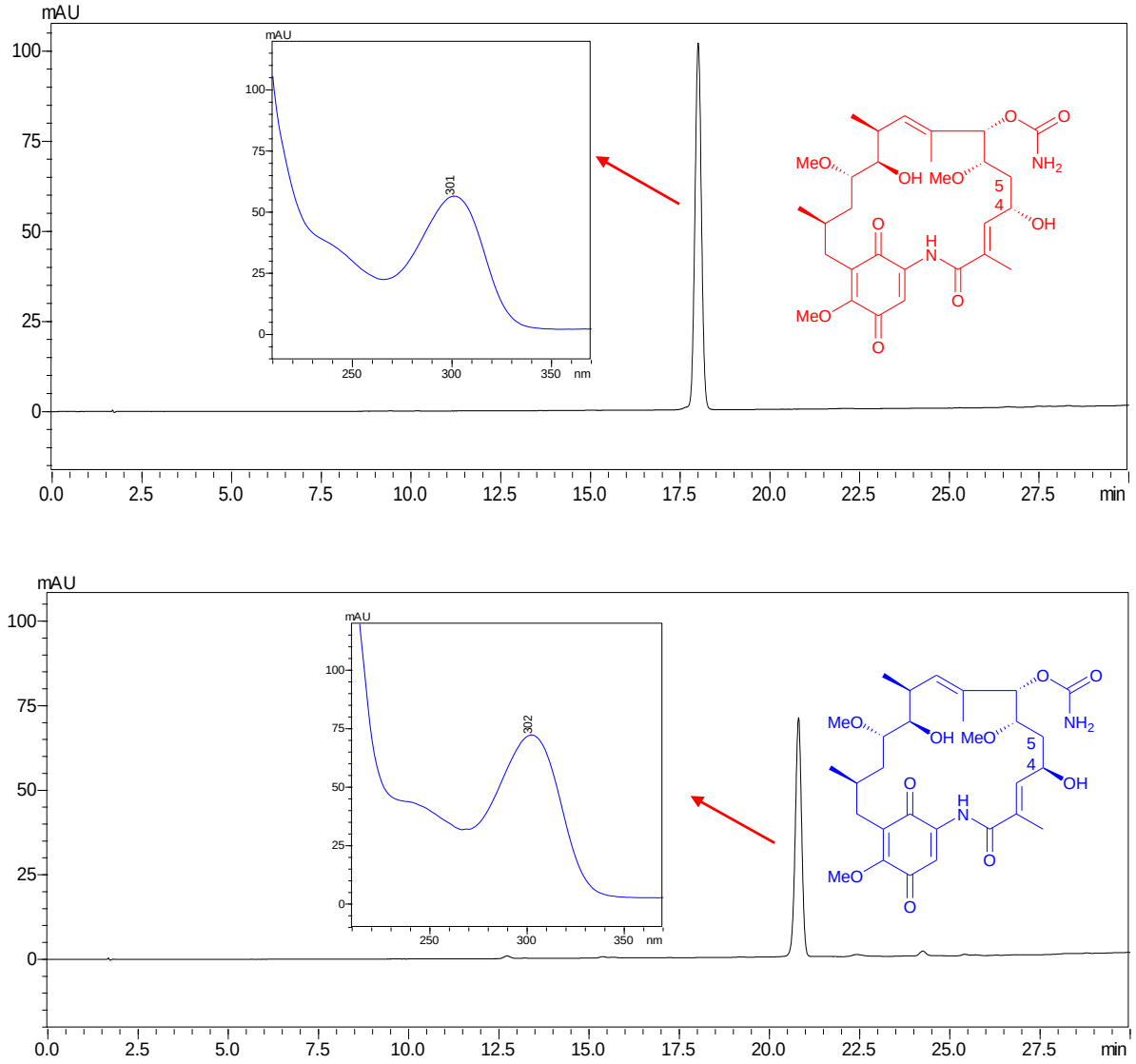


Figure S4. HPLC analysis of **1** and **2**: The two compounds had different retention time (18.1 min and 20.8 min, respectively), but nearly identical UV absorption profiles. Compared to GDM, their loss of characteristic absorption peak at 254 nm indicated some alteration(s) in the chromophore of –NH-CO-C(CH_3)-CH=CH-CH=CH-. HPLC parameters: Dikma Diamonsil C18 column (4.6 × 150 mm, 5 μm), mobile phase MeOH-H_2O, 40-100% in 30 min, 1.0 ml/min, wavelength 301 nm.



(Upper: **1**; Lower: **2**, with UV absorption profile as inset)

Figure S5. HRESIMS spectrum of **1**

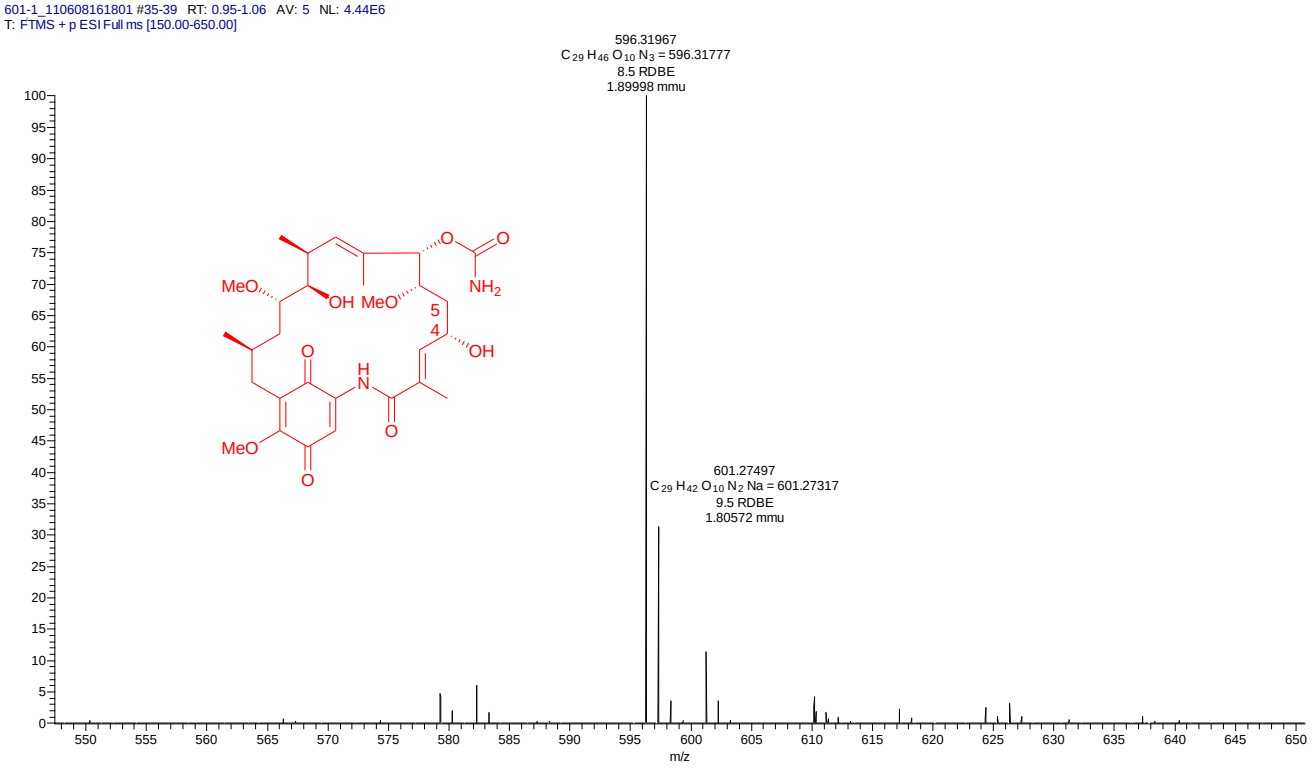


Figure S6. IR spectrum of **1**

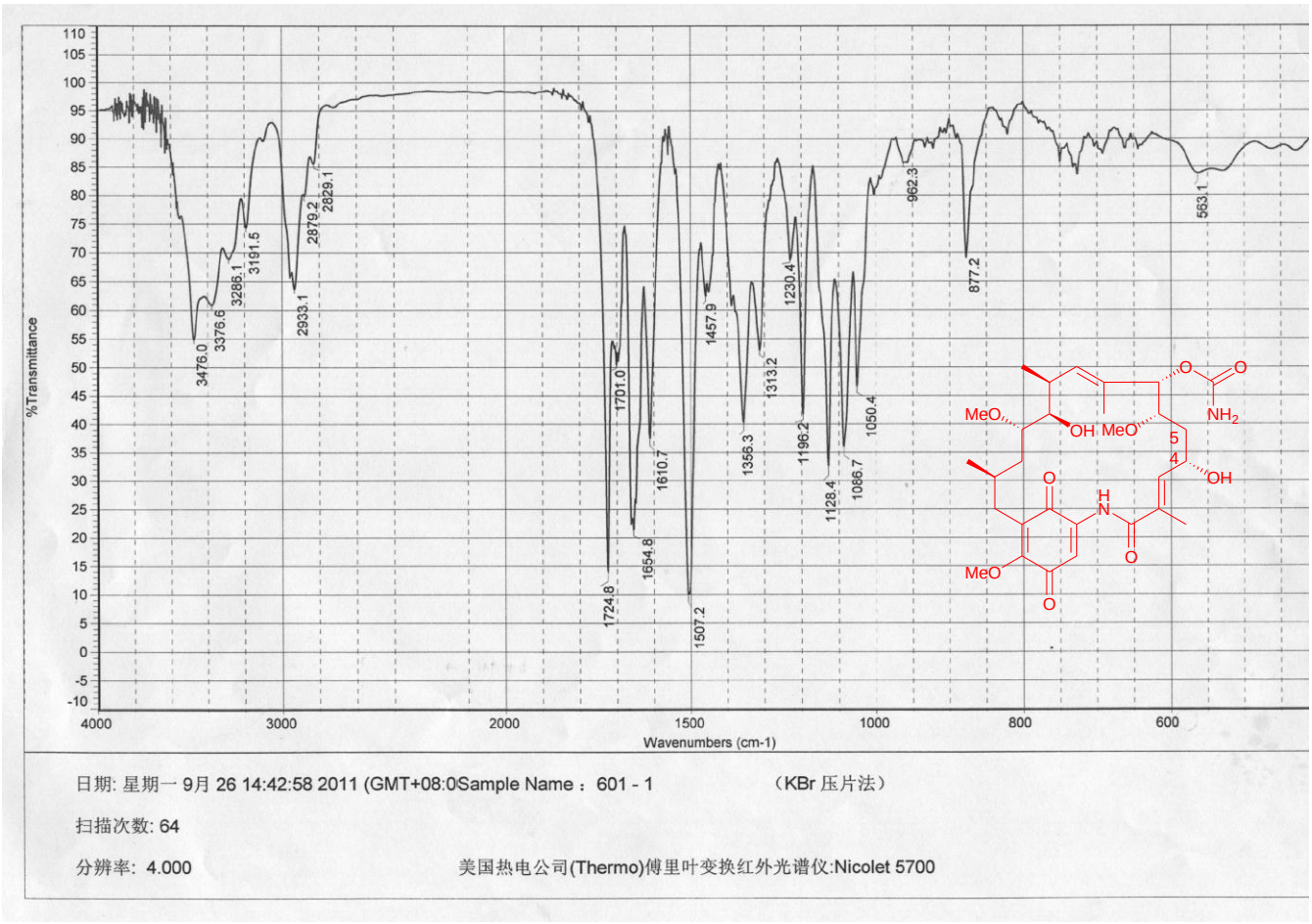


Figure S7. ^{1}H NMR spectrum (600 MHz) of **1** in $CDCl_3$-d_1

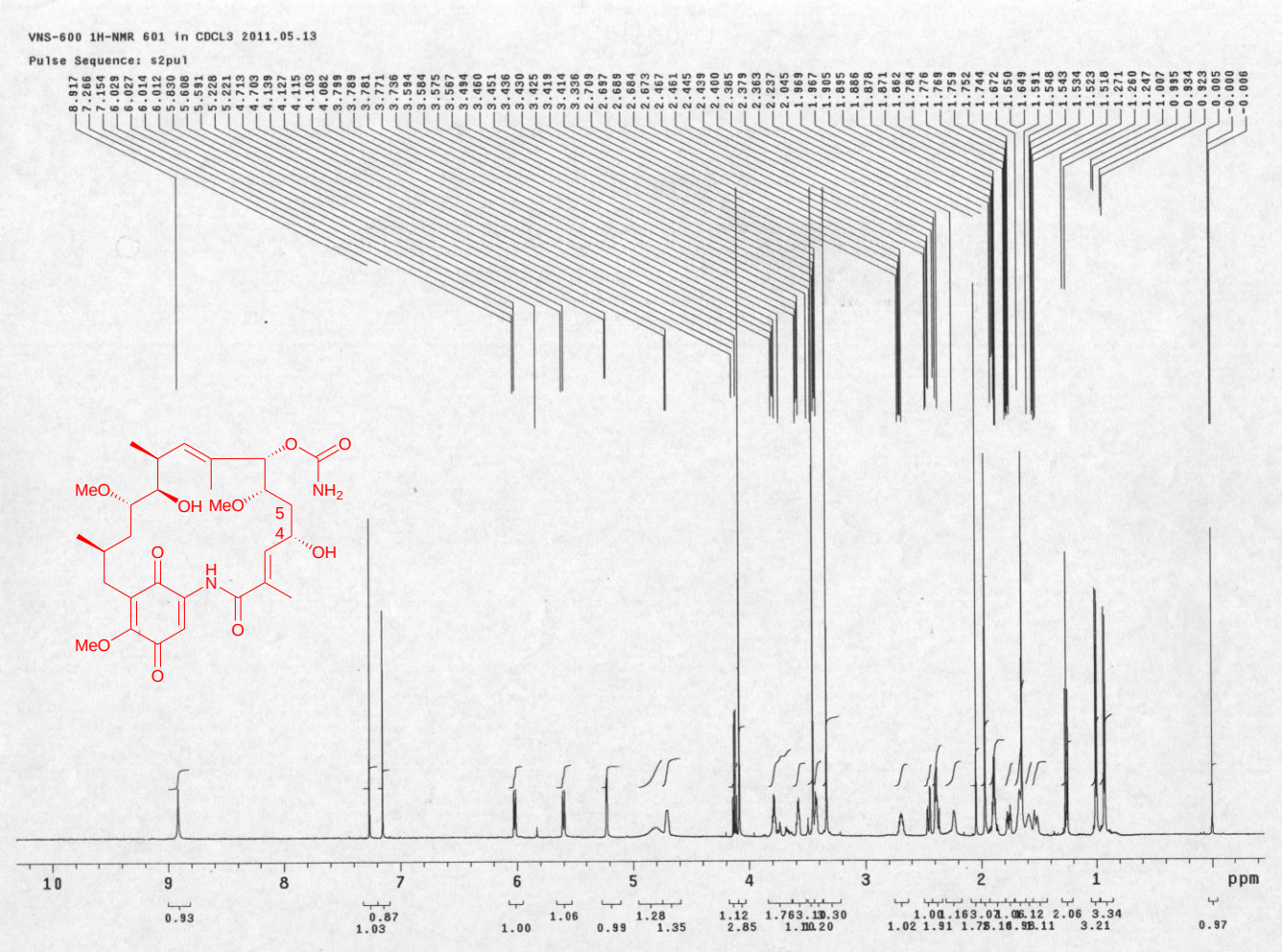


Figure S8. ^{13}C NMR spectrum (150 MHz) of **1** in $CDCl_3$-d_1

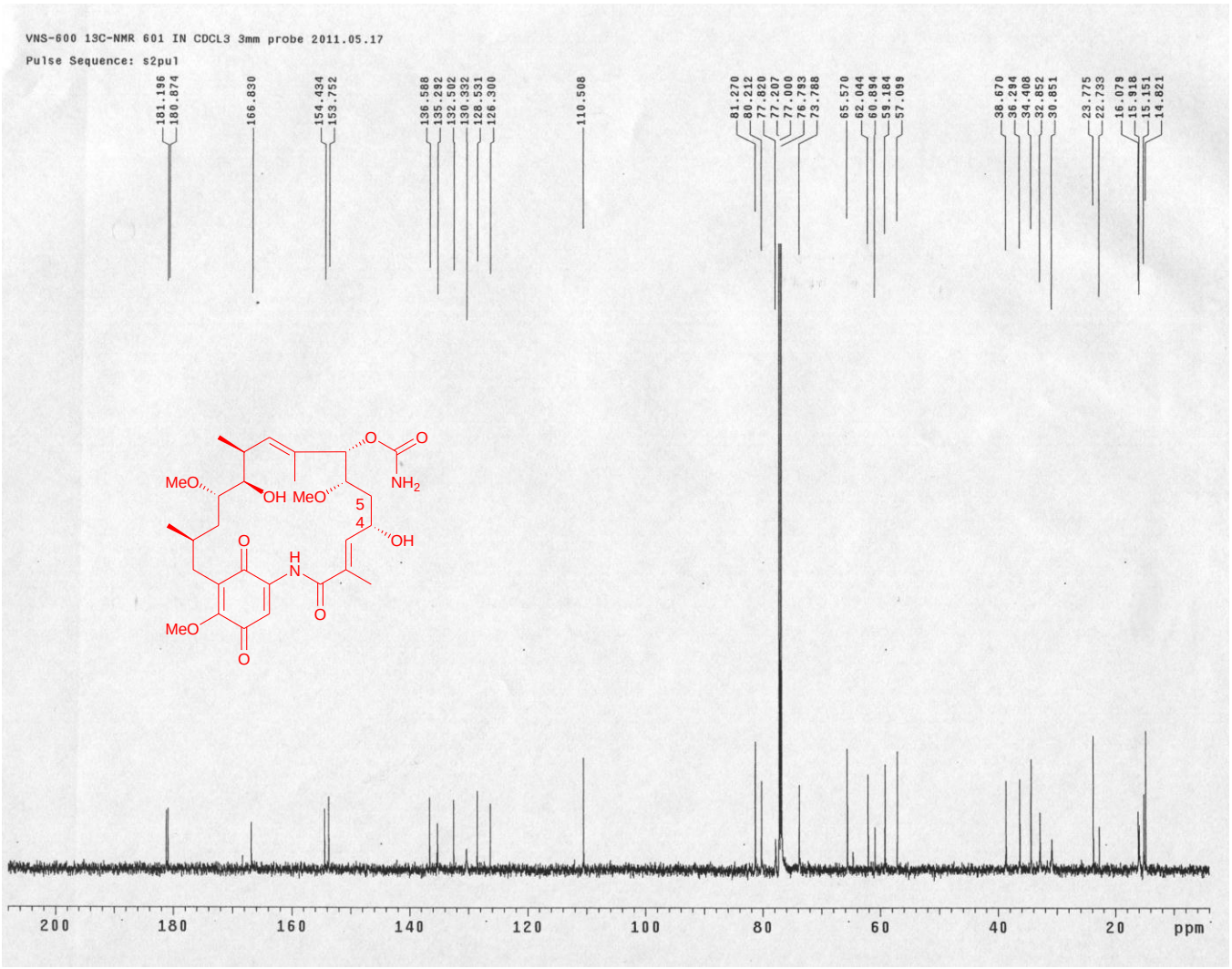


Figure S9. DEPT spectrum (150 MHz) of **1** in $CDCl_3$-d_1

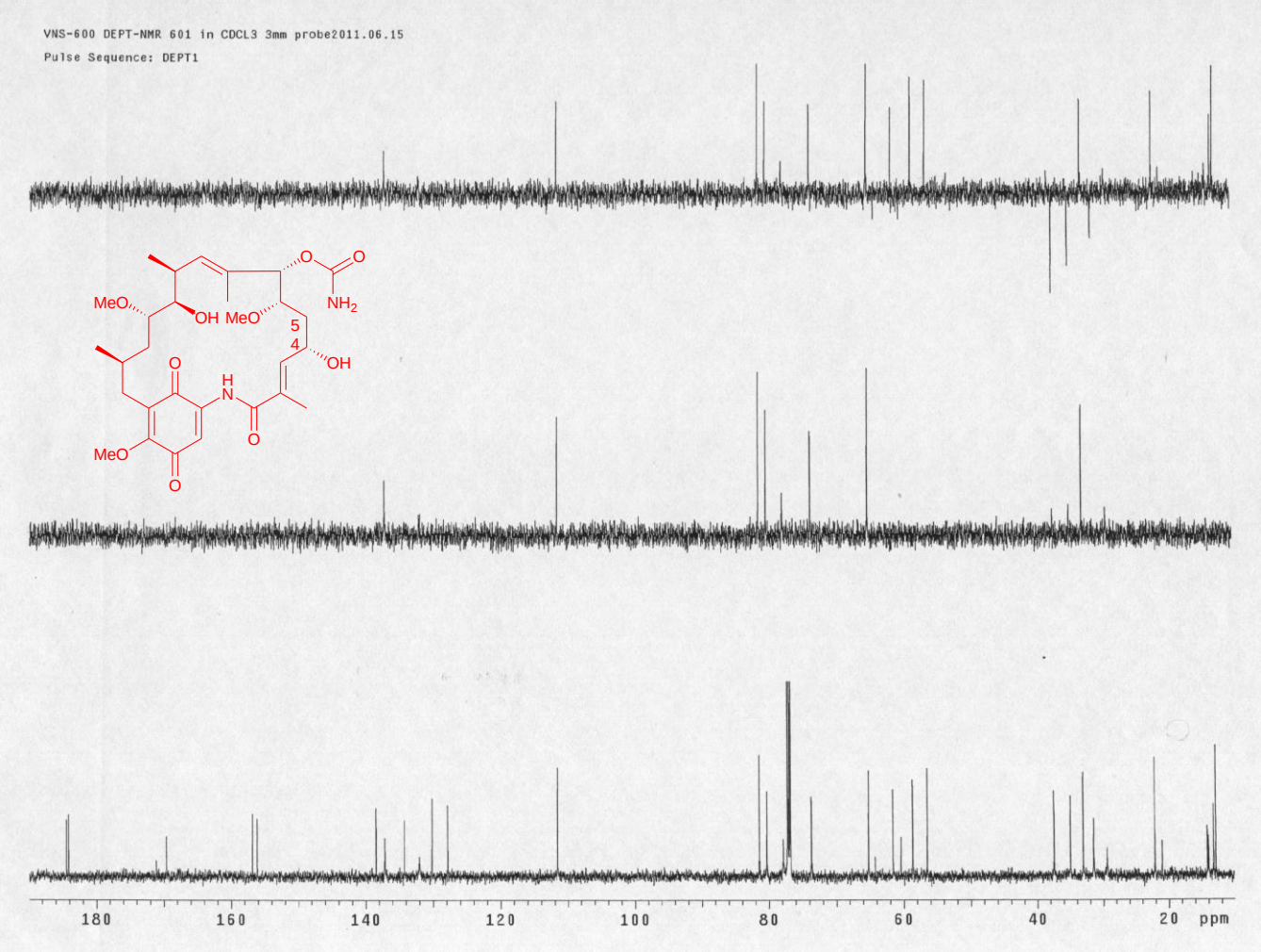


Figure S10. COSY spectrum (600 MHz) of **1** in $CDCl_3$-d_1

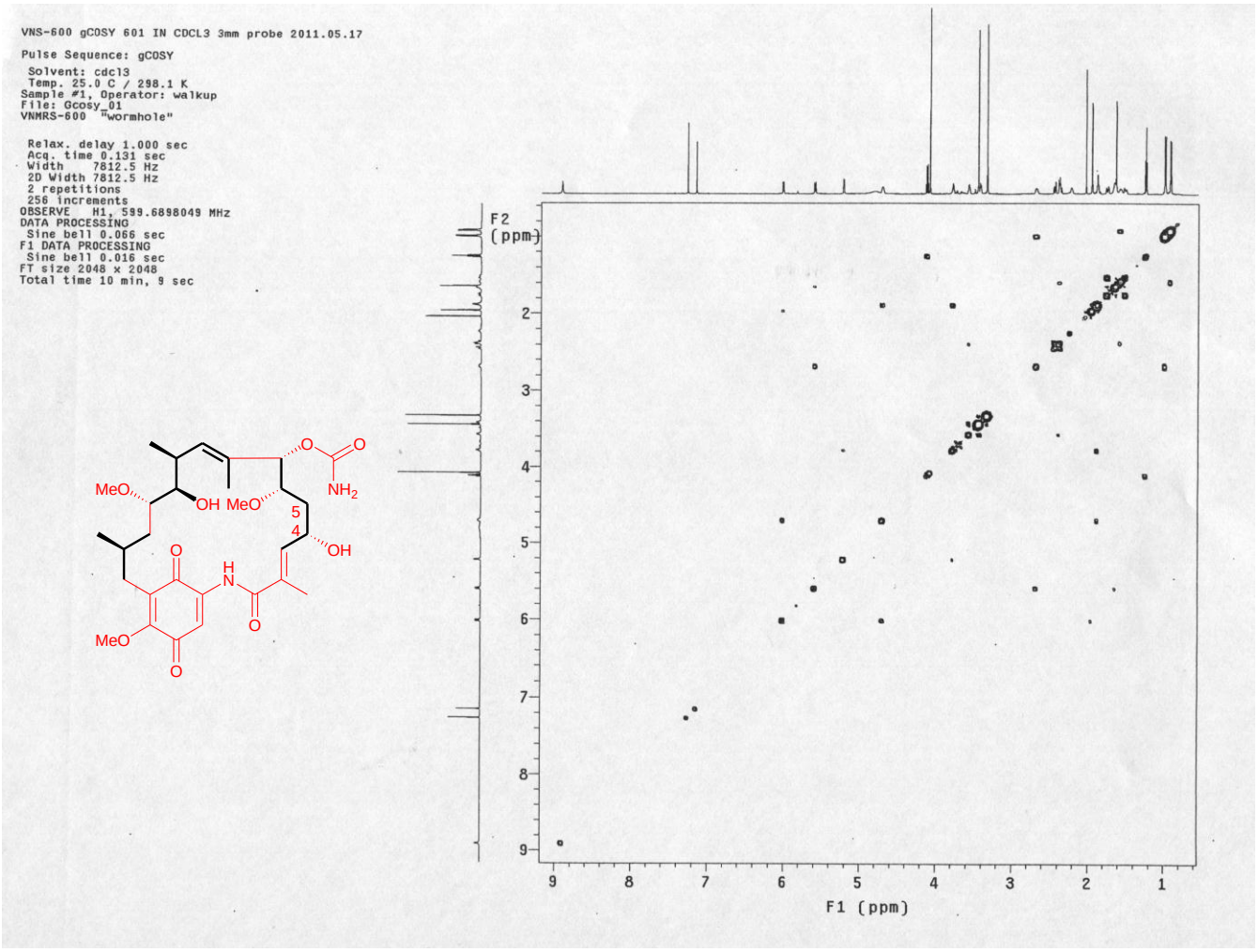


Figure S11. HSQC spectrum (600 MHz) of **1** in $CDCl_3$-d_1

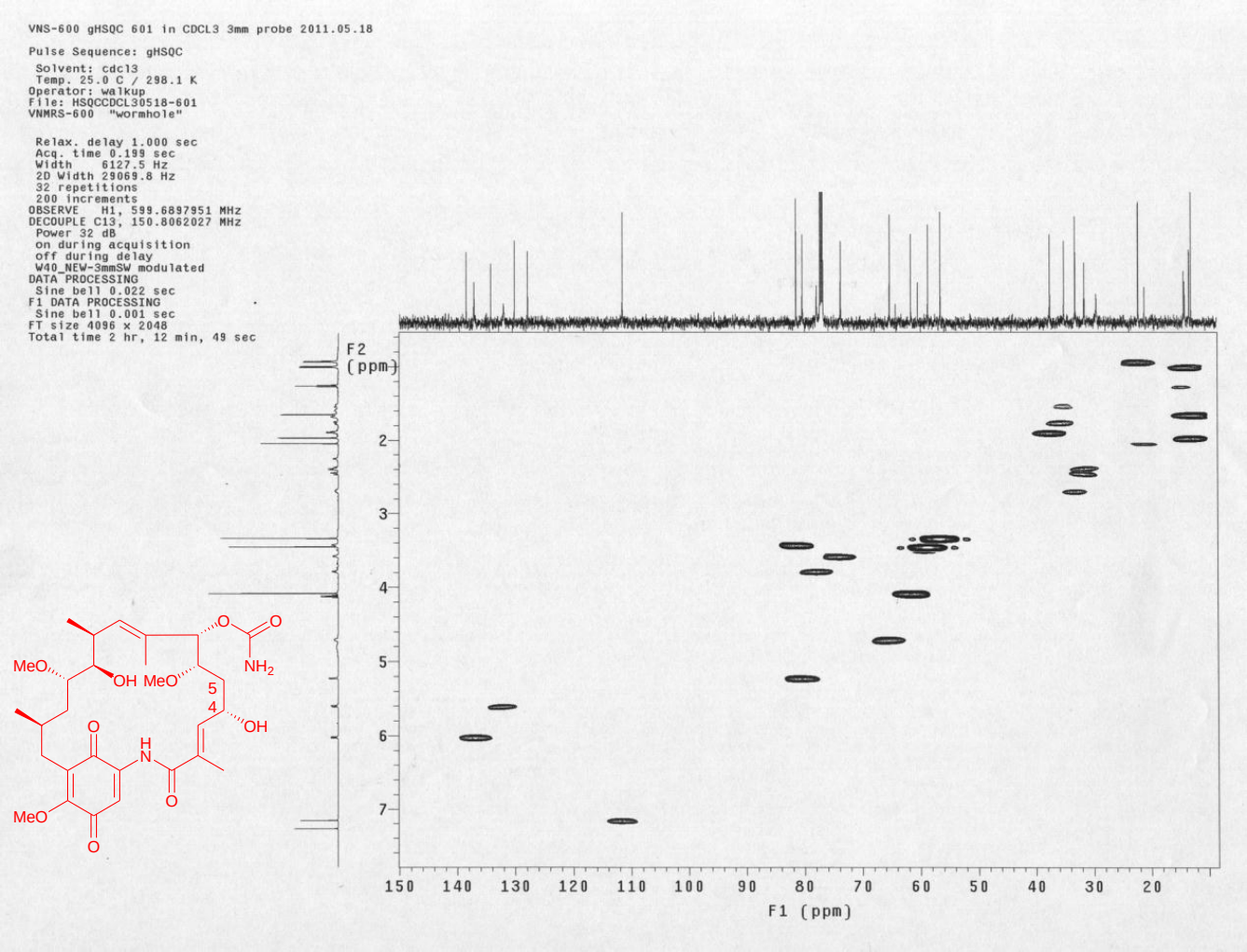


Figure S12. HMBC spectrum (600 MHz) of **1** in $CDCl_3$-d_1

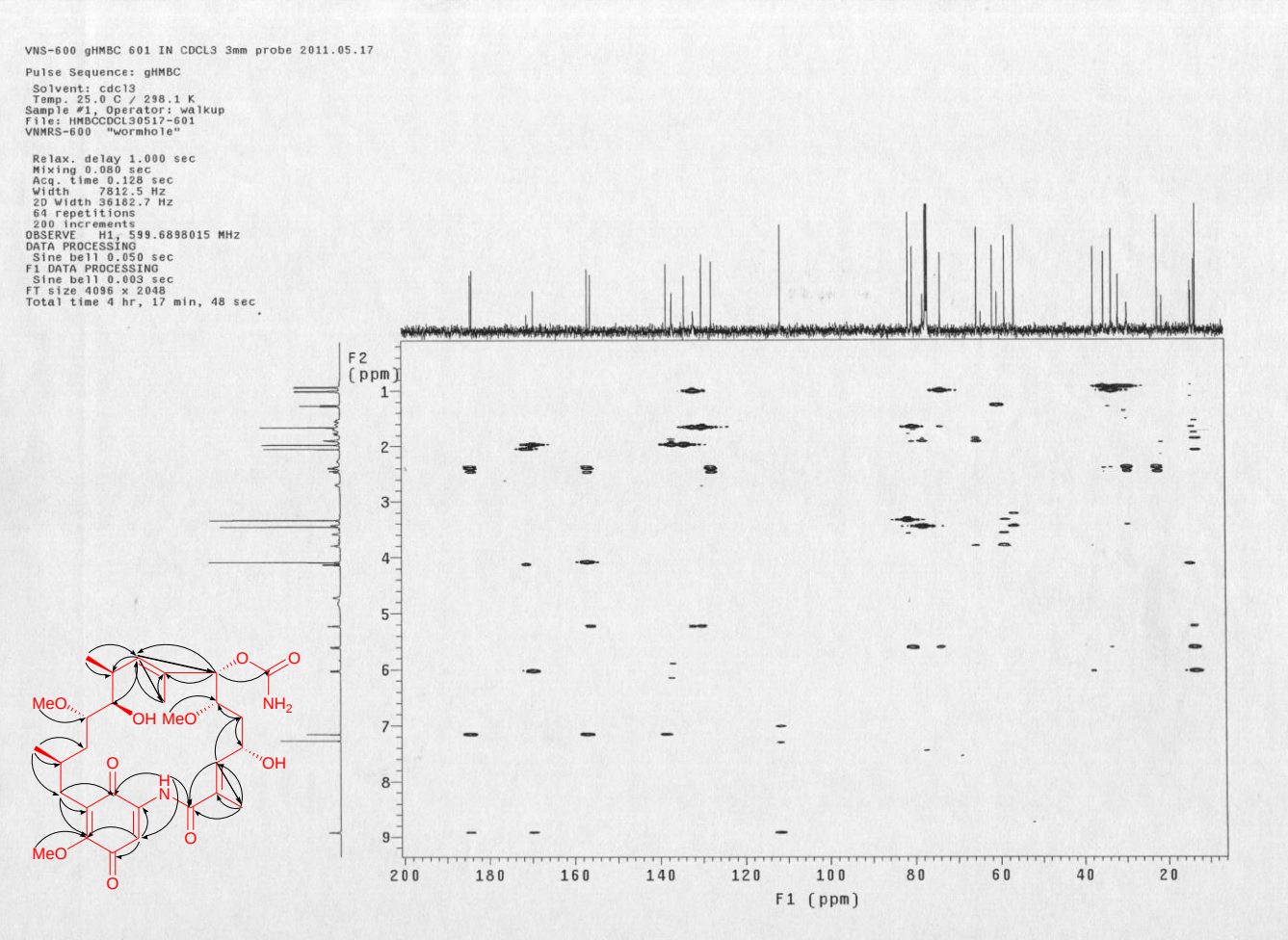


Figure S13. ESIMS spectrum of a sample containing **2**

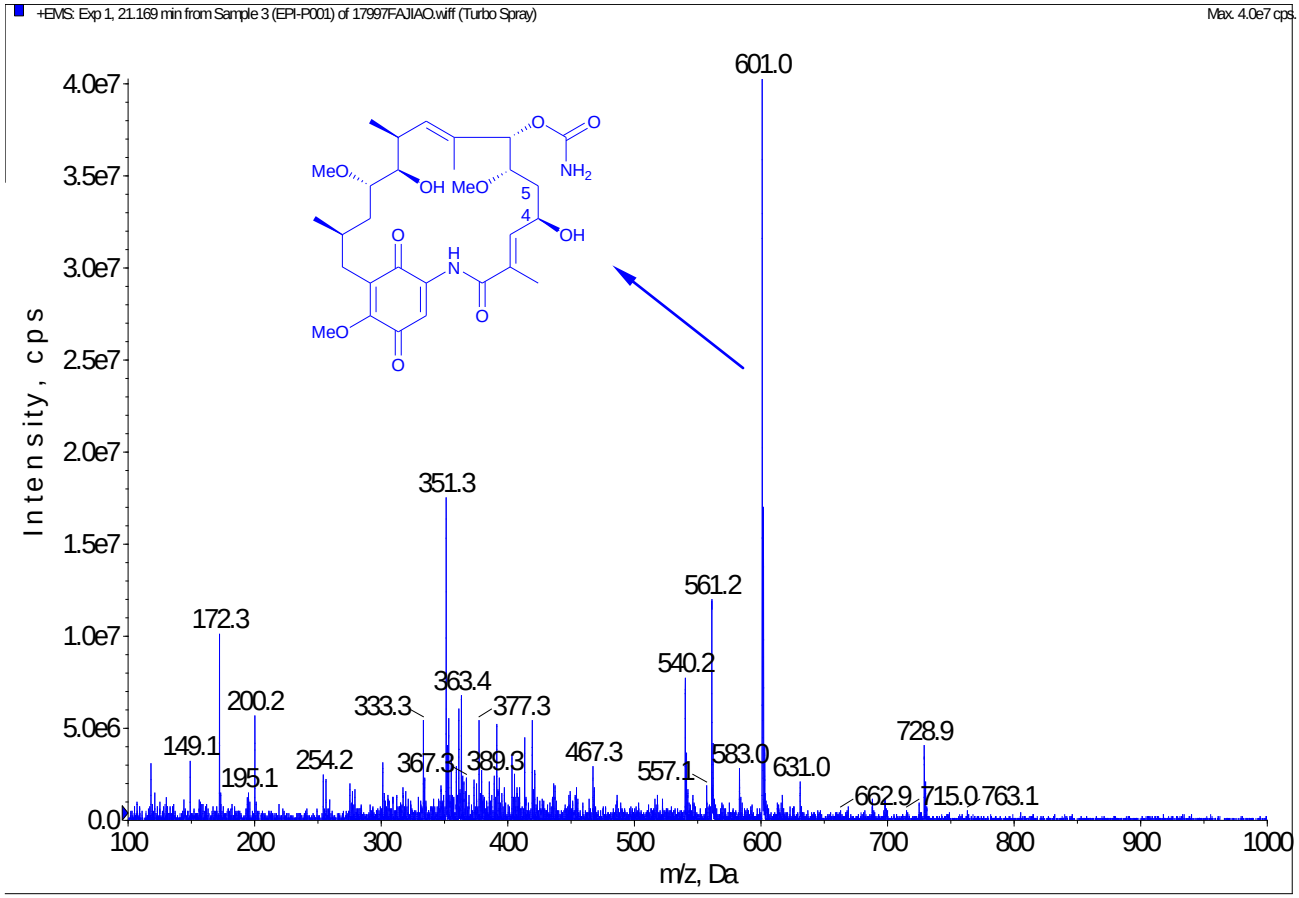


Figure S14. MS^2 spectrum of **2**

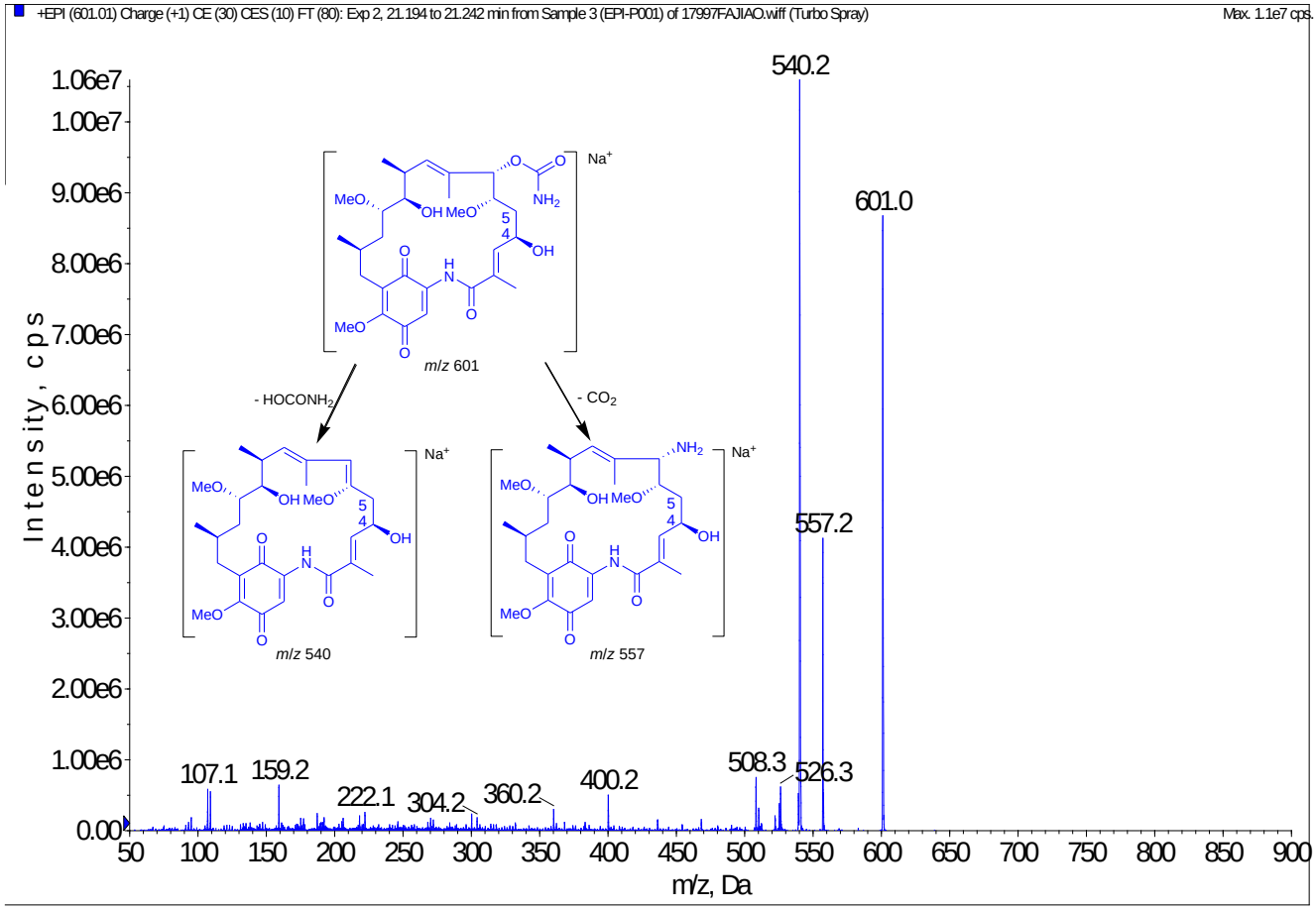


Figure S15. HRESIMS spectrum of **2**

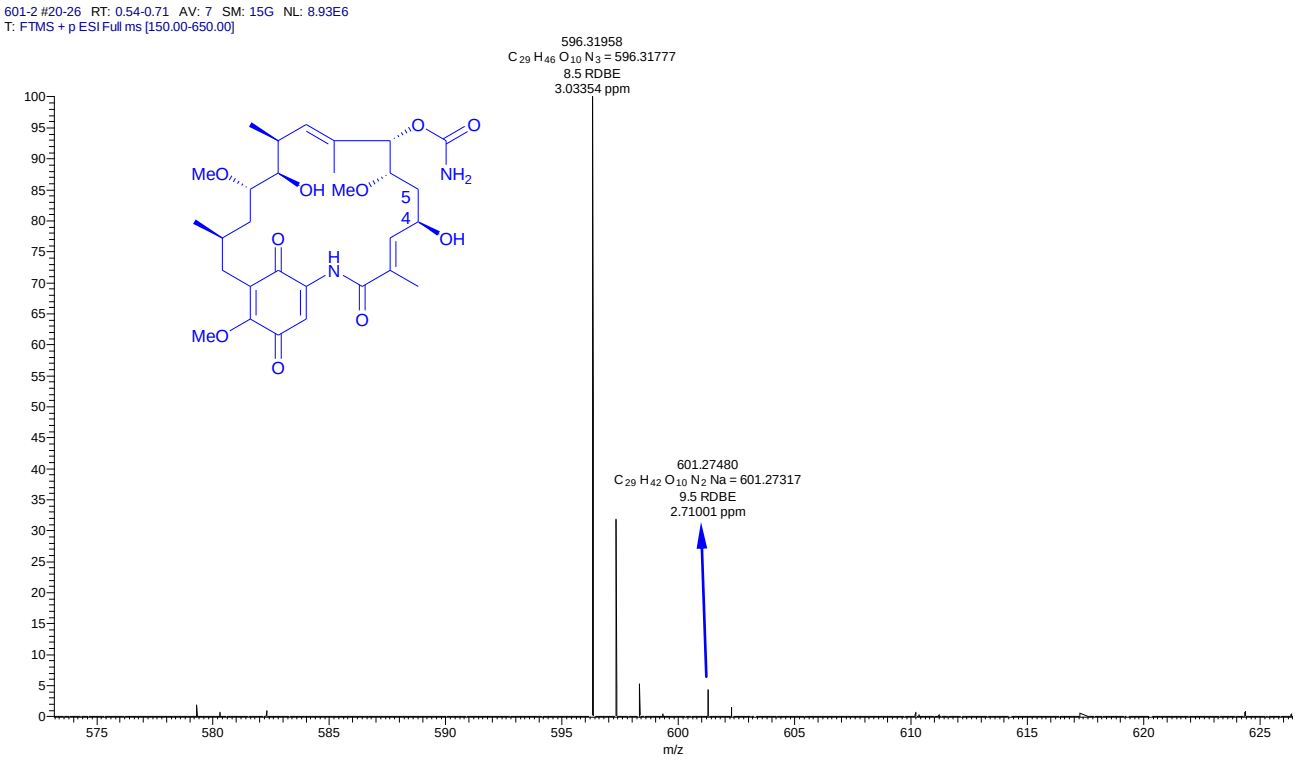


Figure S16. IR spectrum of **2**

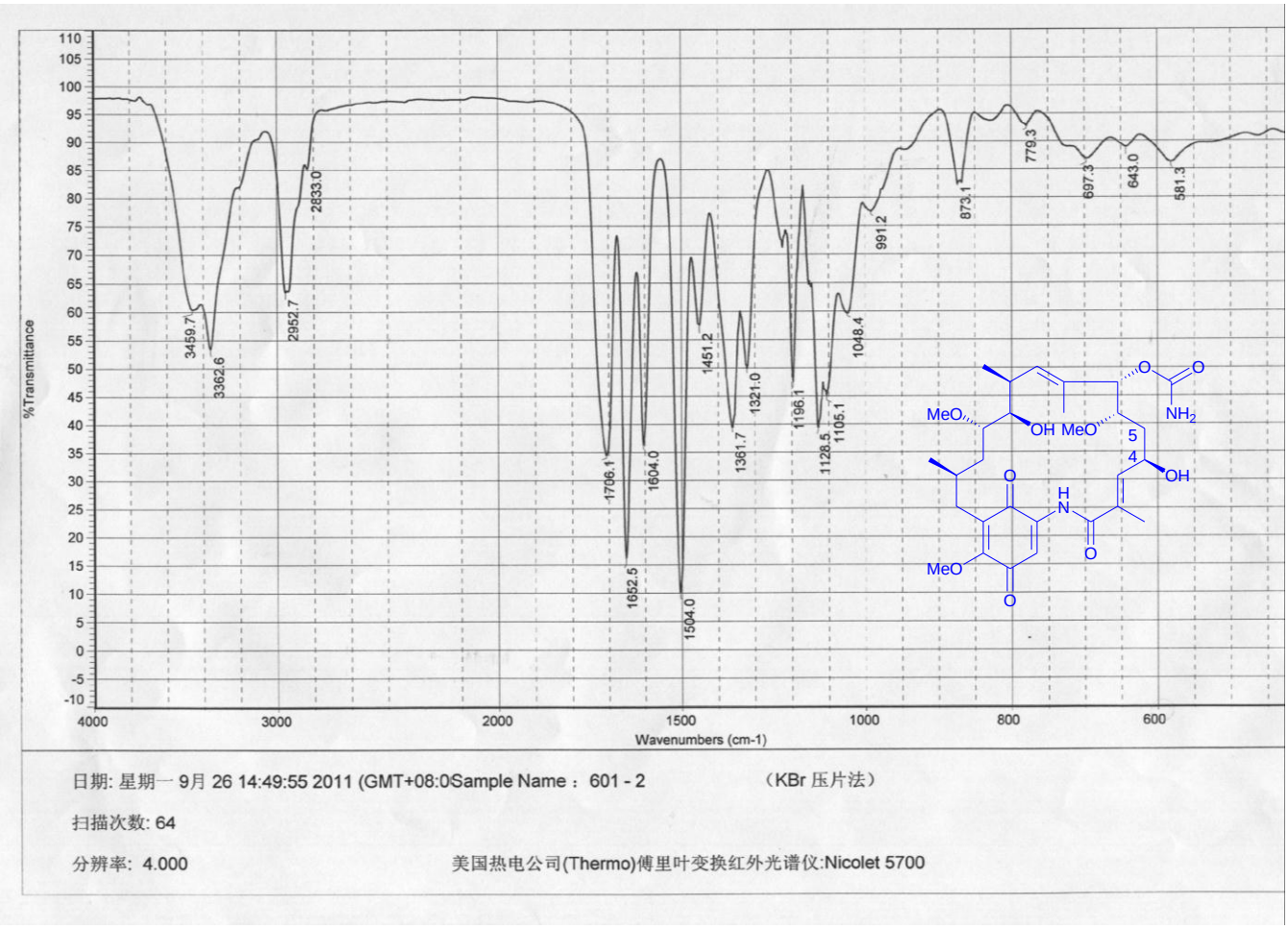


Figure S17. ^{1}H NMR spectrum (600 MHz) of **2** in $CDCl_3$-d_1

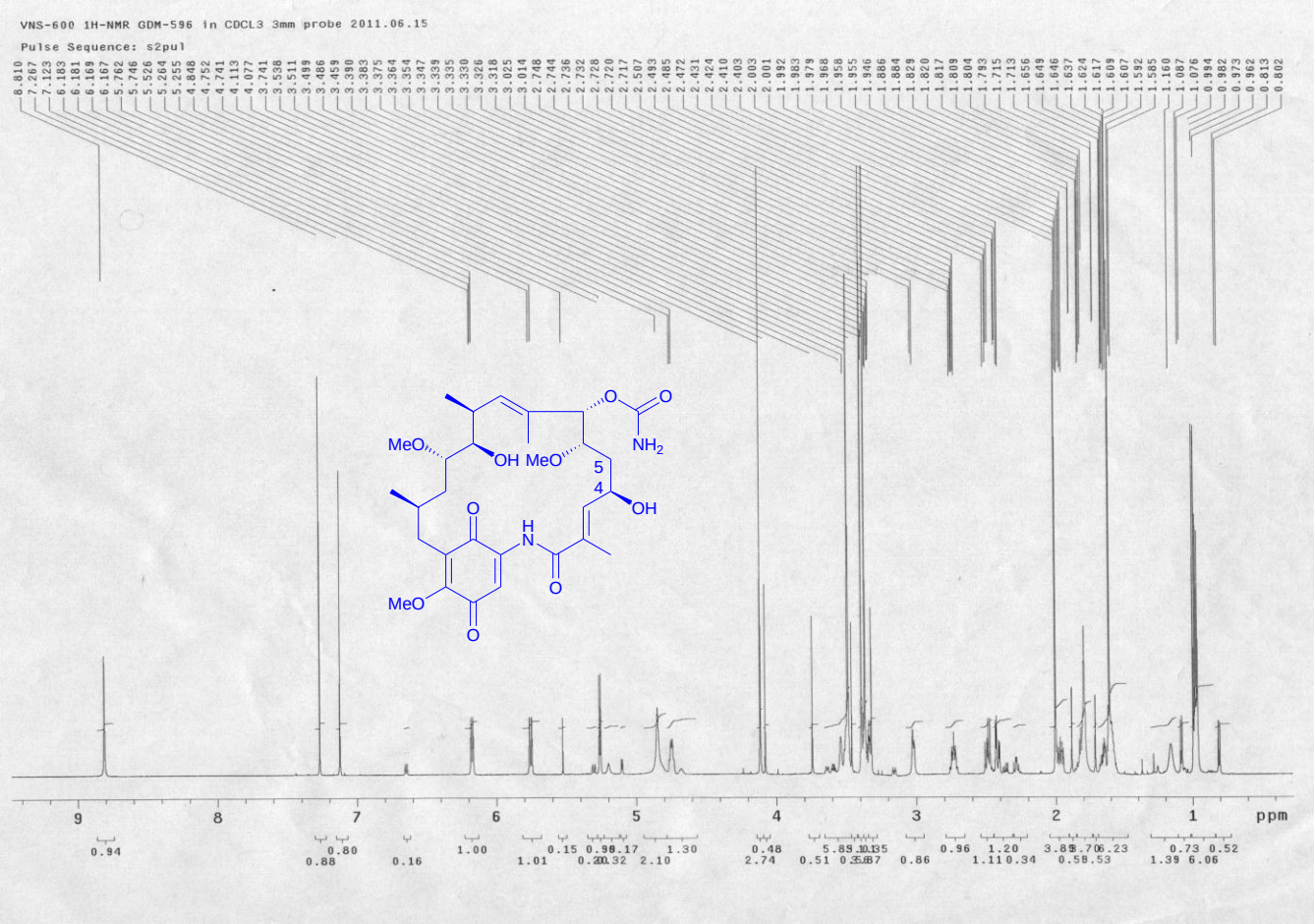


Figure S18. ^{13}C NMR spectrum (150 MHz) of **2** in $CDCl_3$-d_1

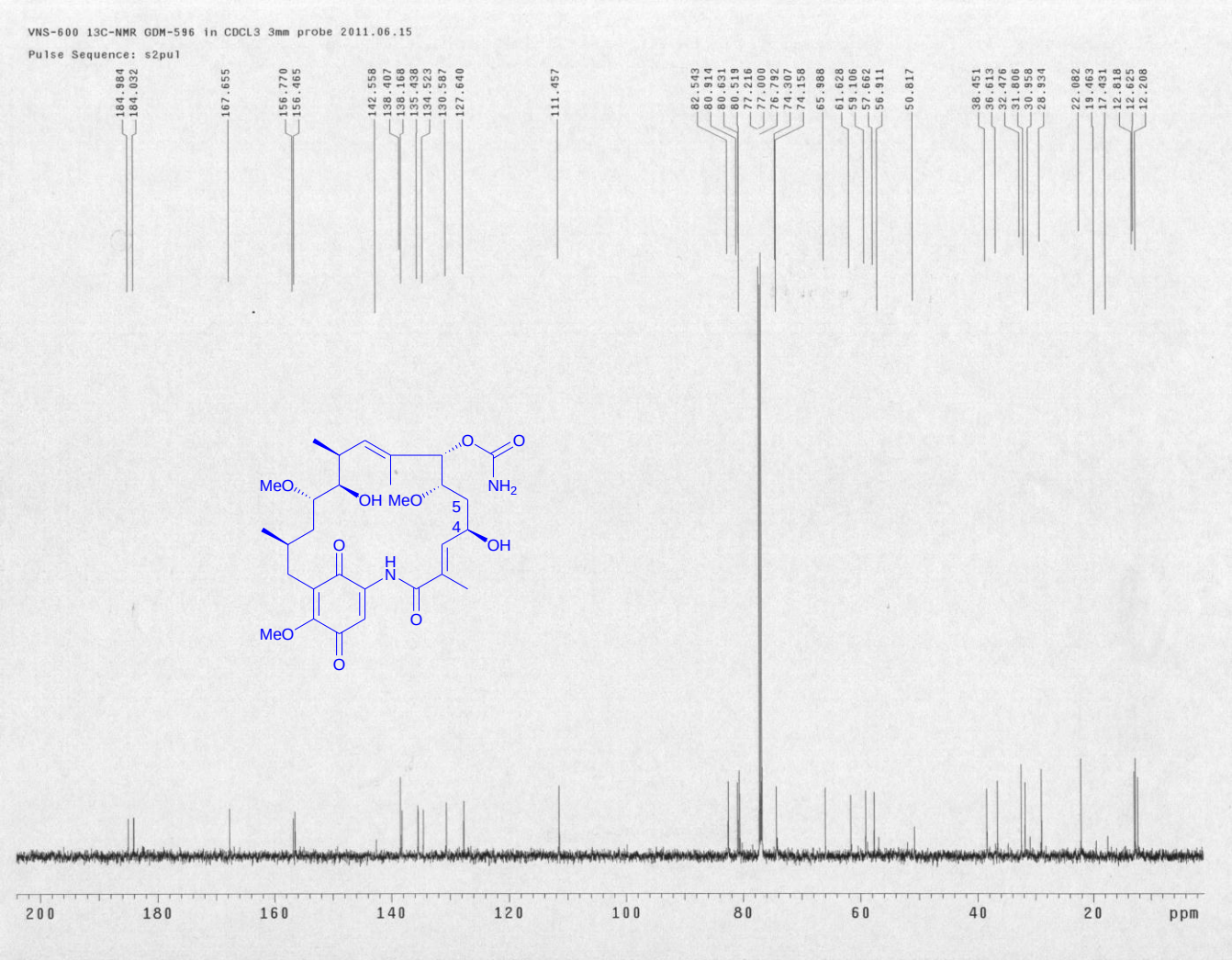


Figure S19. DEPT spectrum (150 MHz) of **2** in $CDCl_3$-d_1

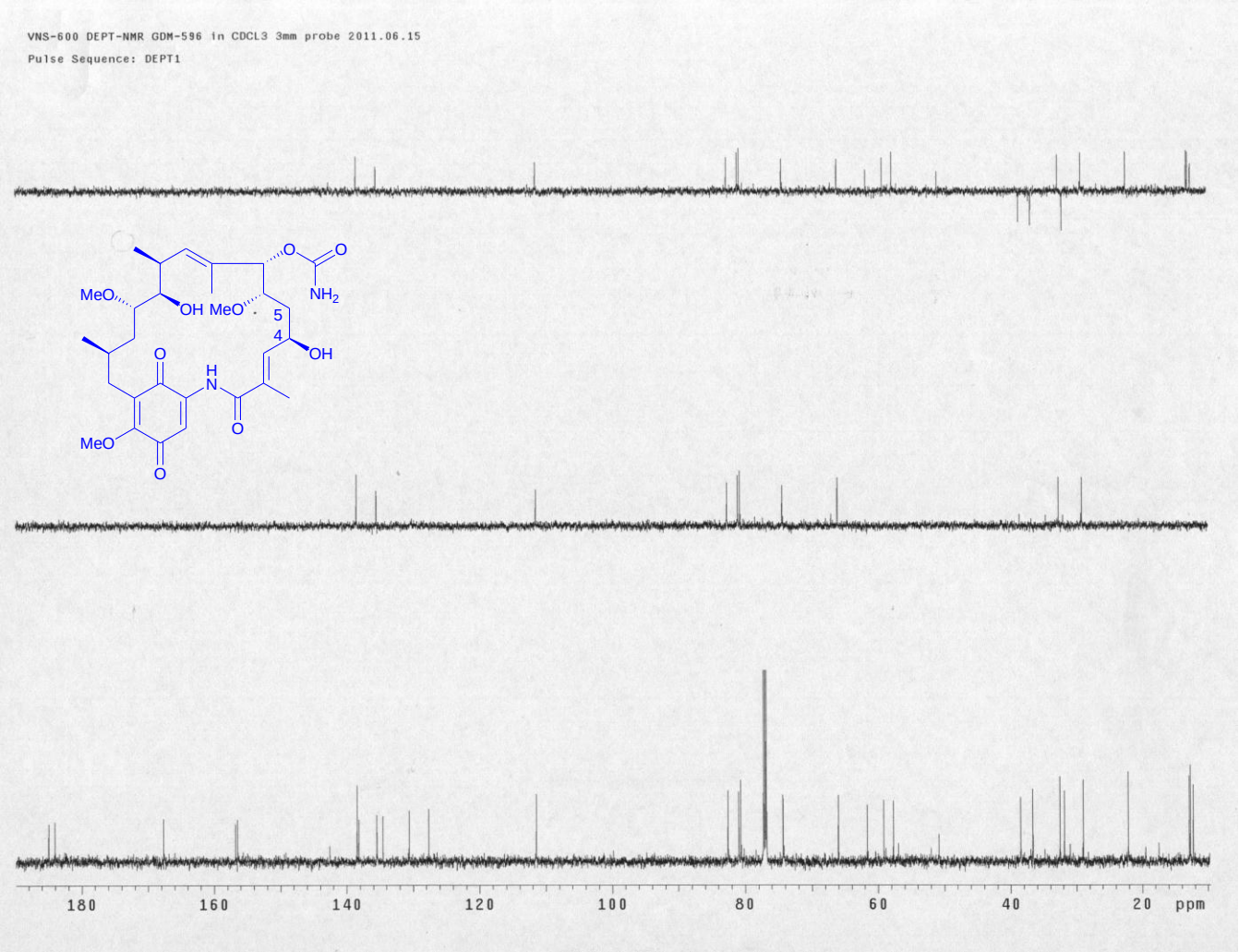


Figure S20. COSY spectrum (600 MHz) of **2** in $CDCl_3$-d_1

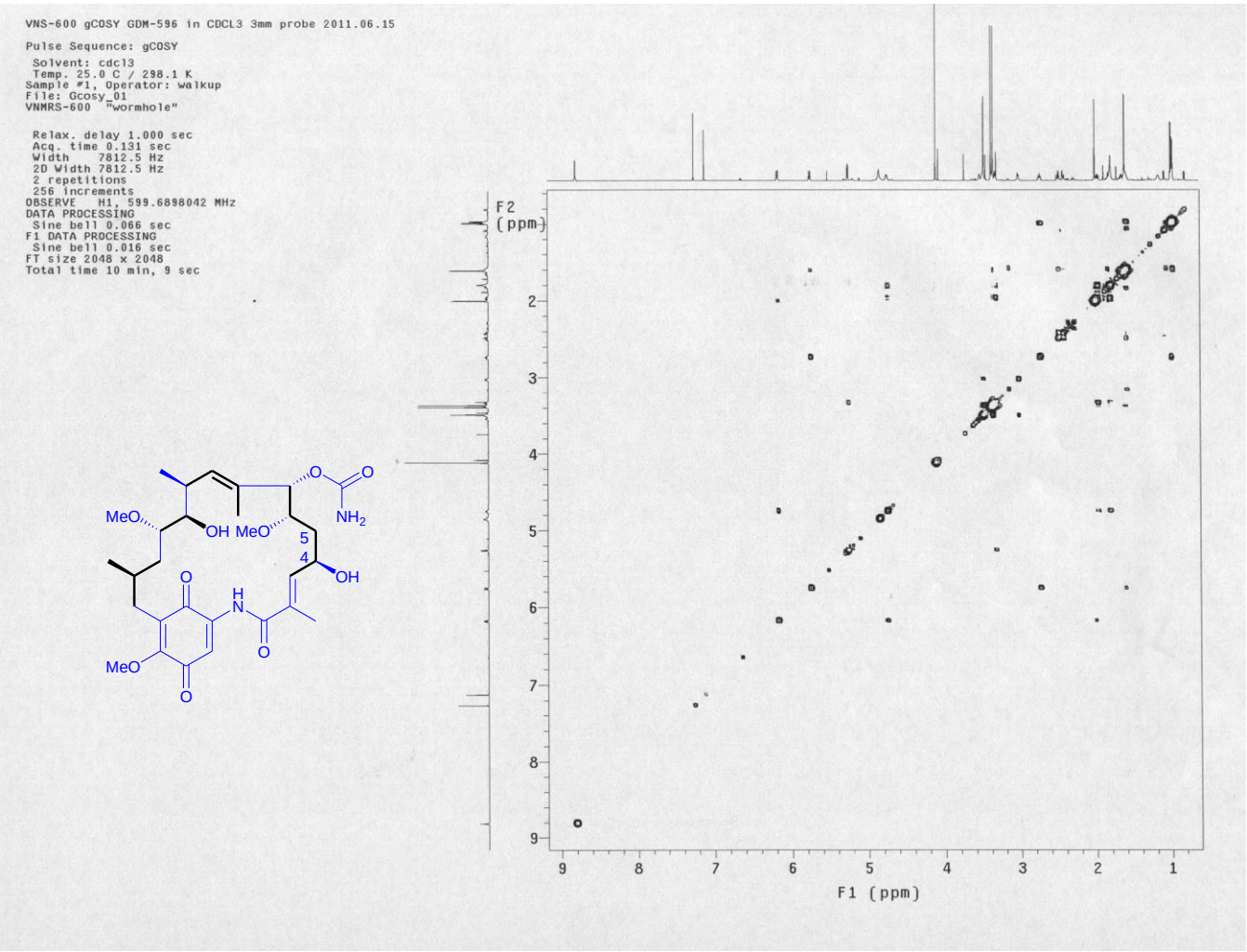


Figure S21. HSQC spectrum (600 MHz) of **2** in $CDCl_3$-d_1

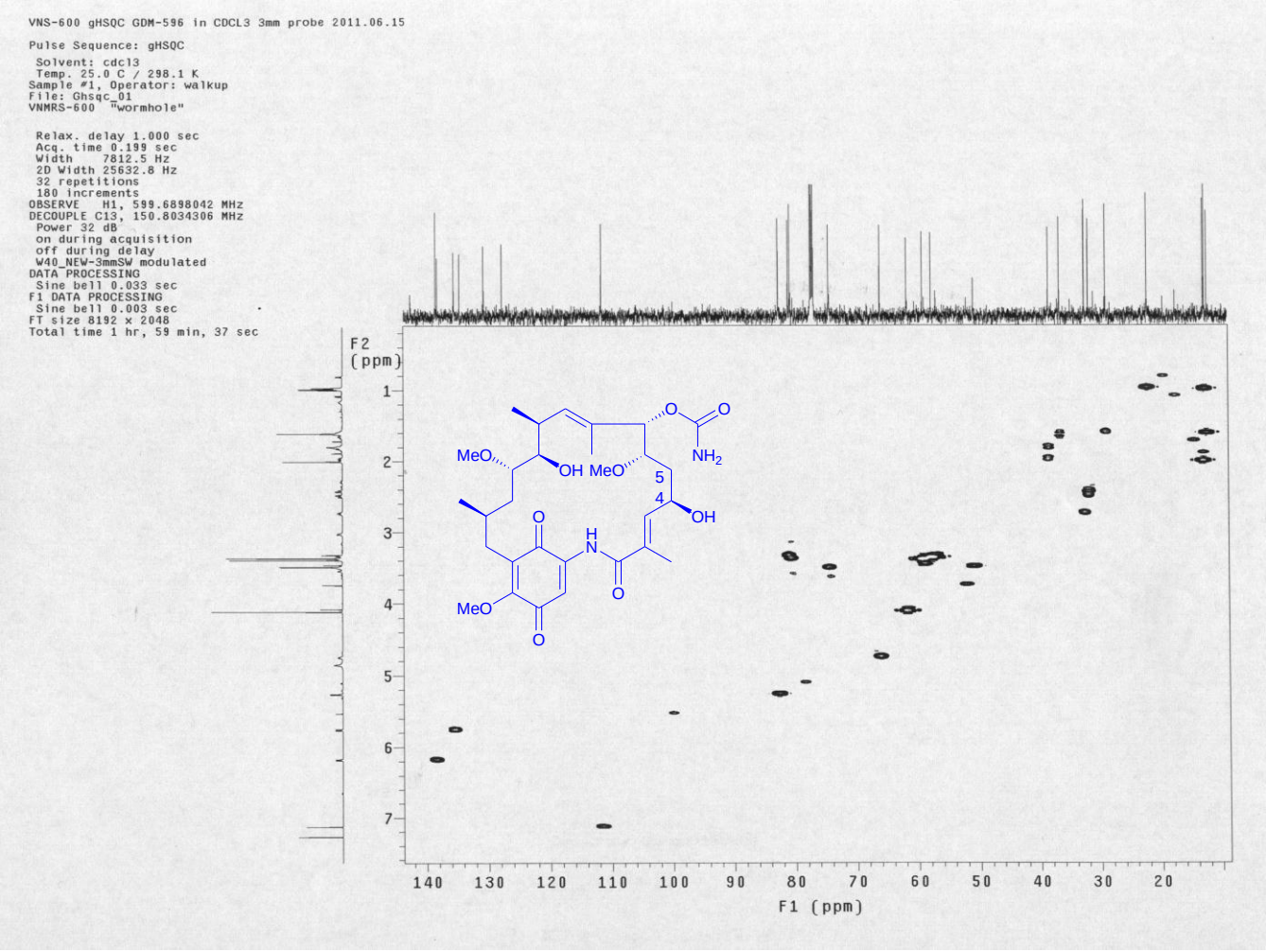


Figure S22. HMBC spectrum (600 MHz) of **2** in $CDCl_3$-d_1

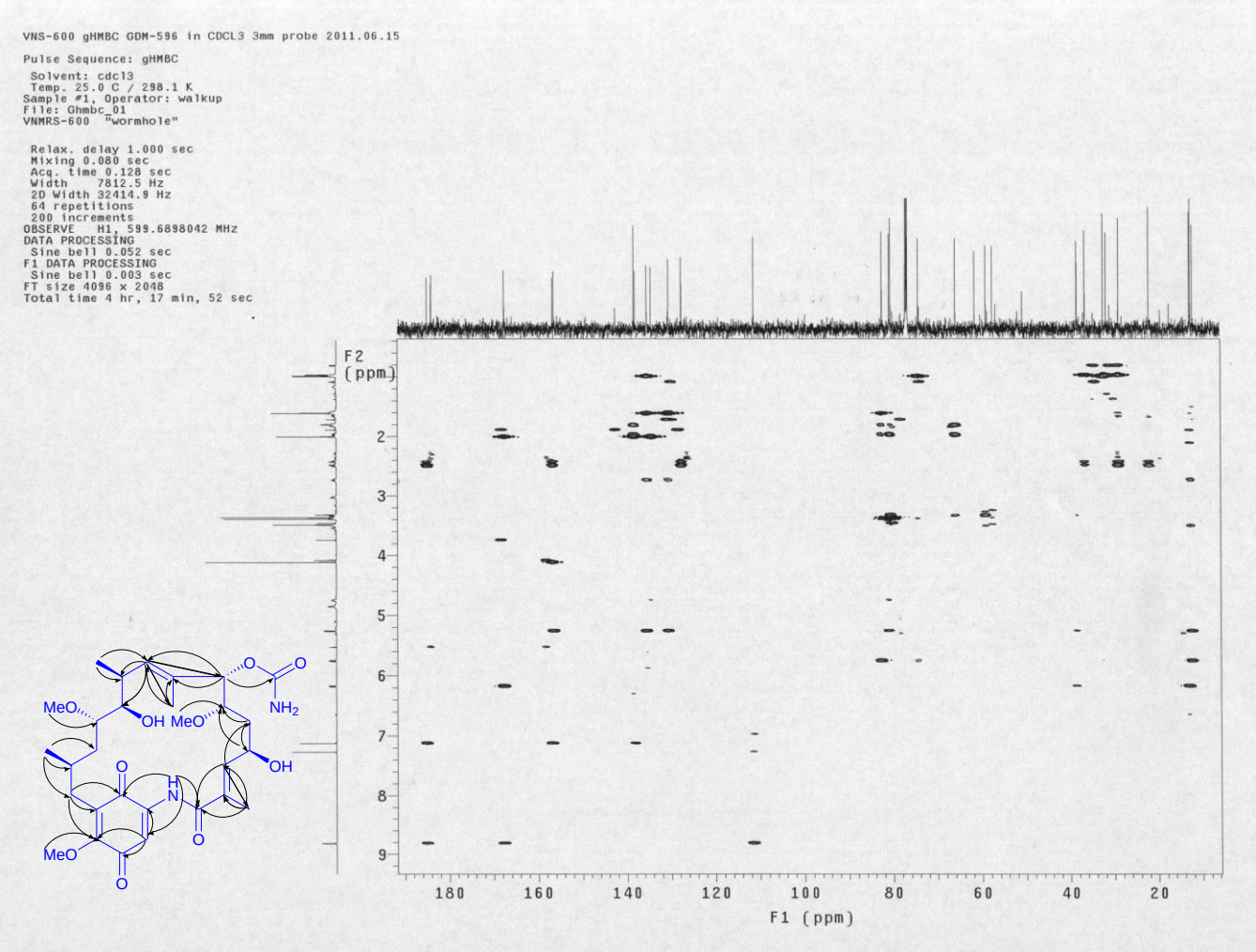


Figure S23. ^{1}H NMR spectrum (600 MHz) of **1** in DMSO-d_6 (70 °C)

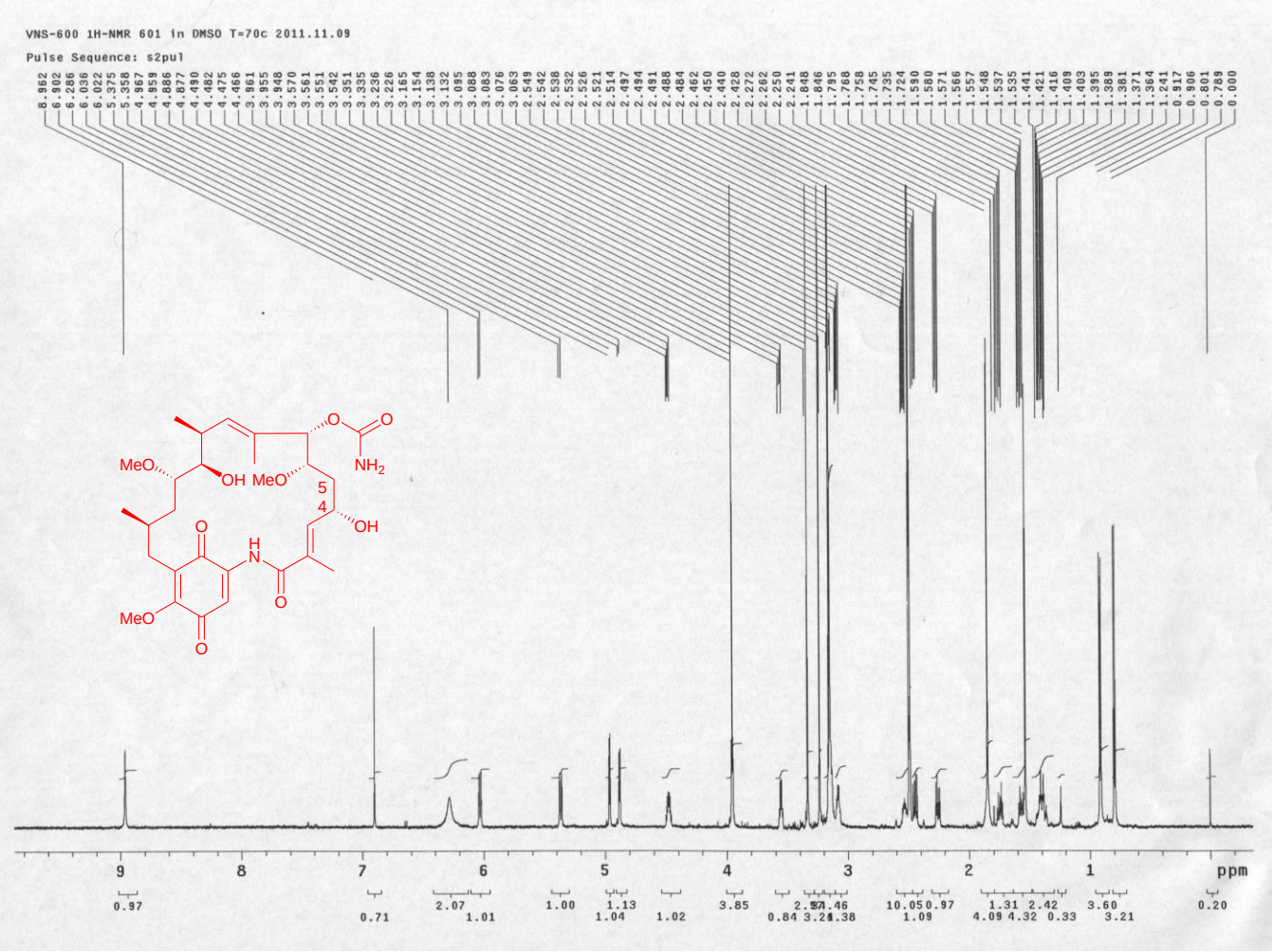


Figure S24. MS spectrum of **1*R***

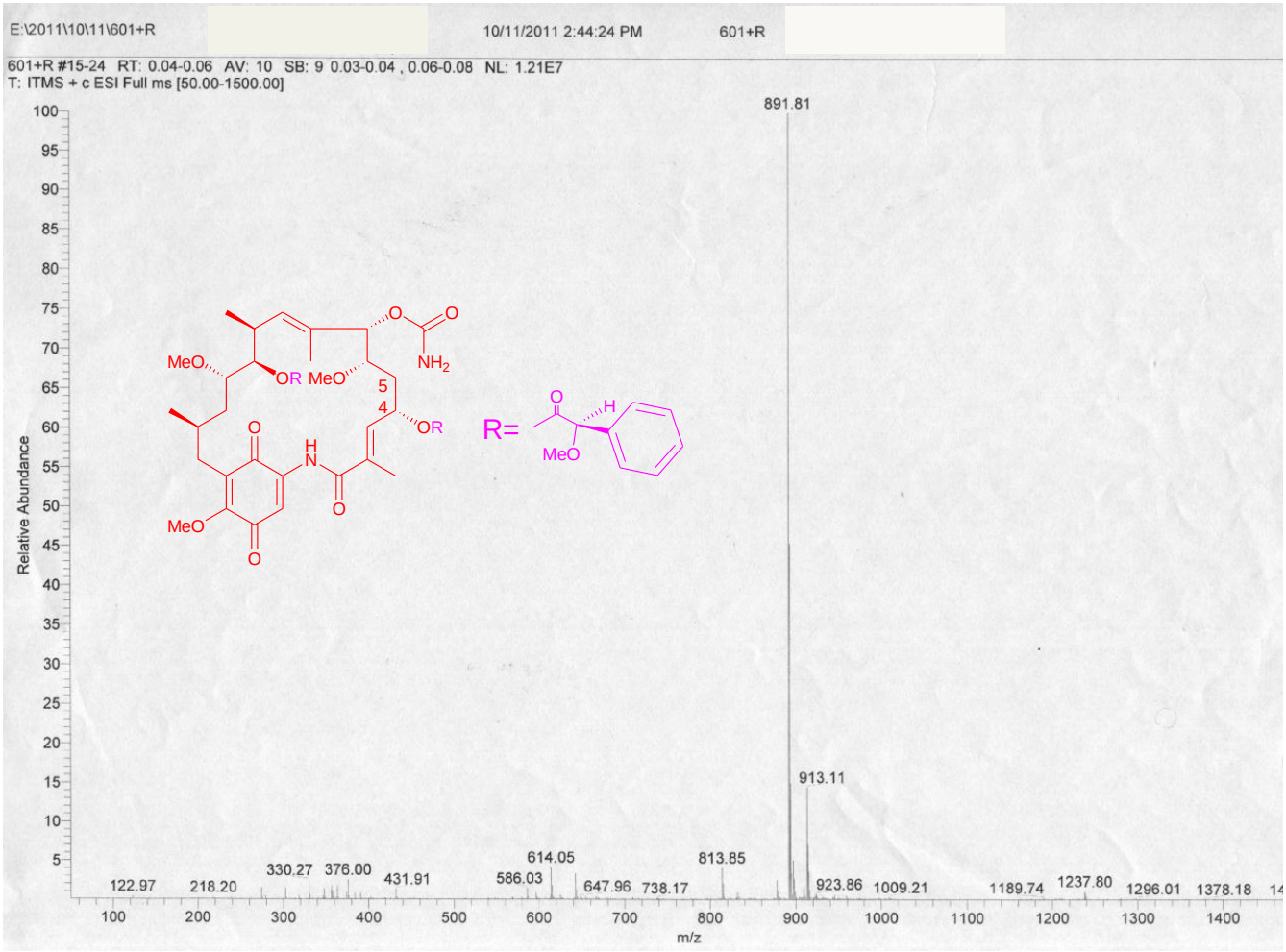


Figure S25. ^{1}H NMR spectrum (600 MHz) of **1*R*** in DMSO-d_6 (70 °C)

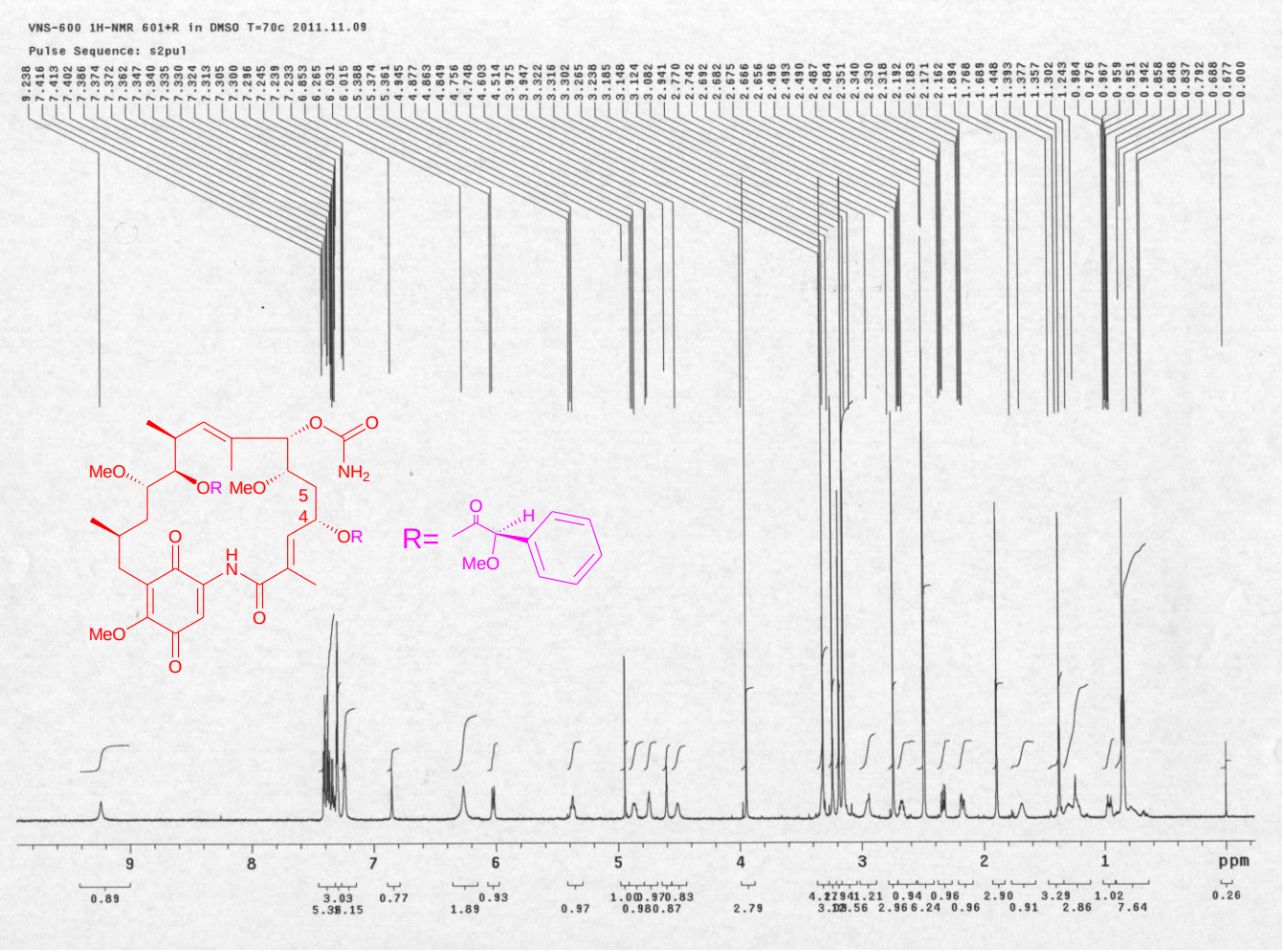


Figure S26. MS spectrum of **1*S***

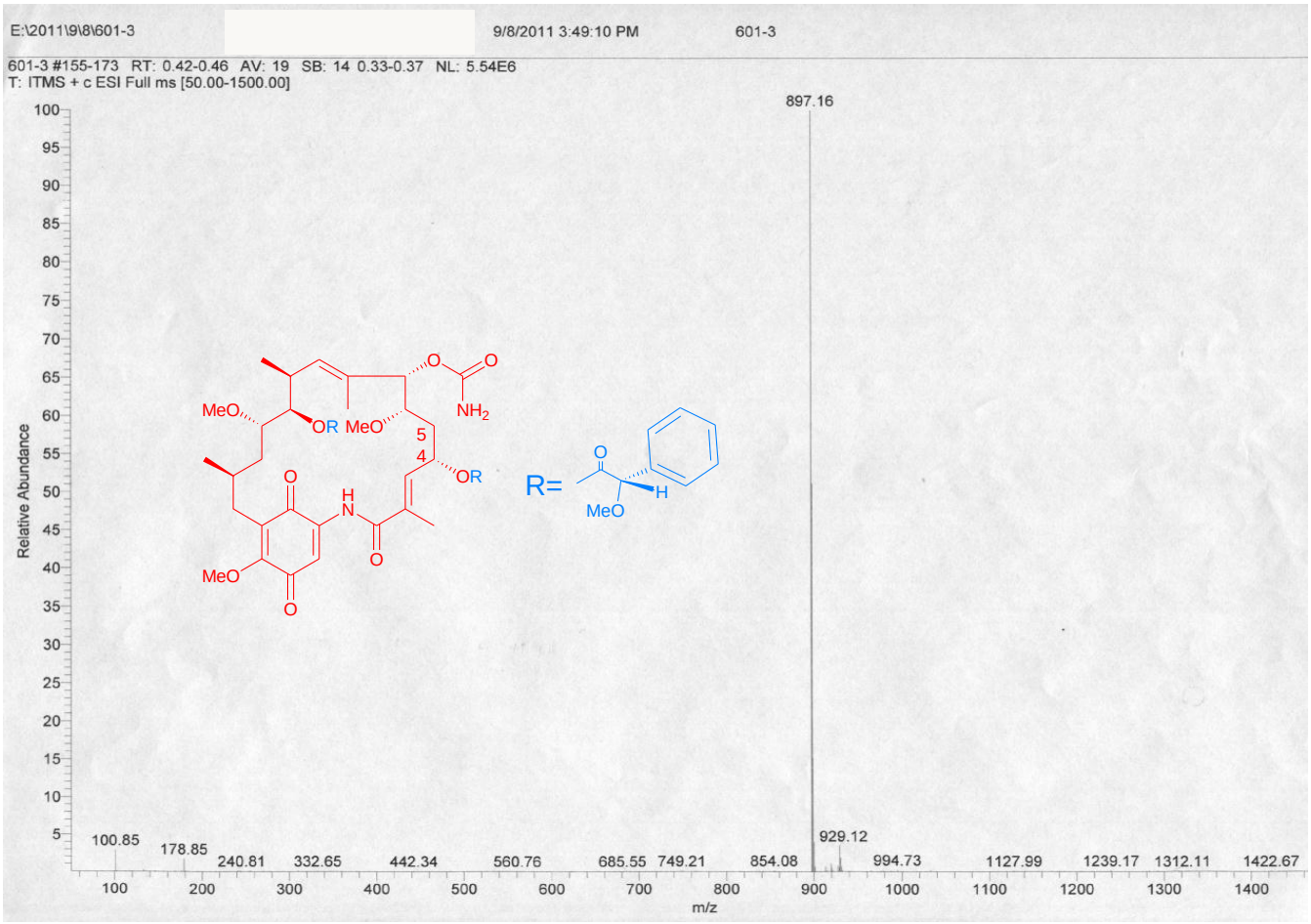


Figure S27. ^{1}H NMR spectrum (600 MHz) of **1*S*** in DMSO-d_6 (70 °C)

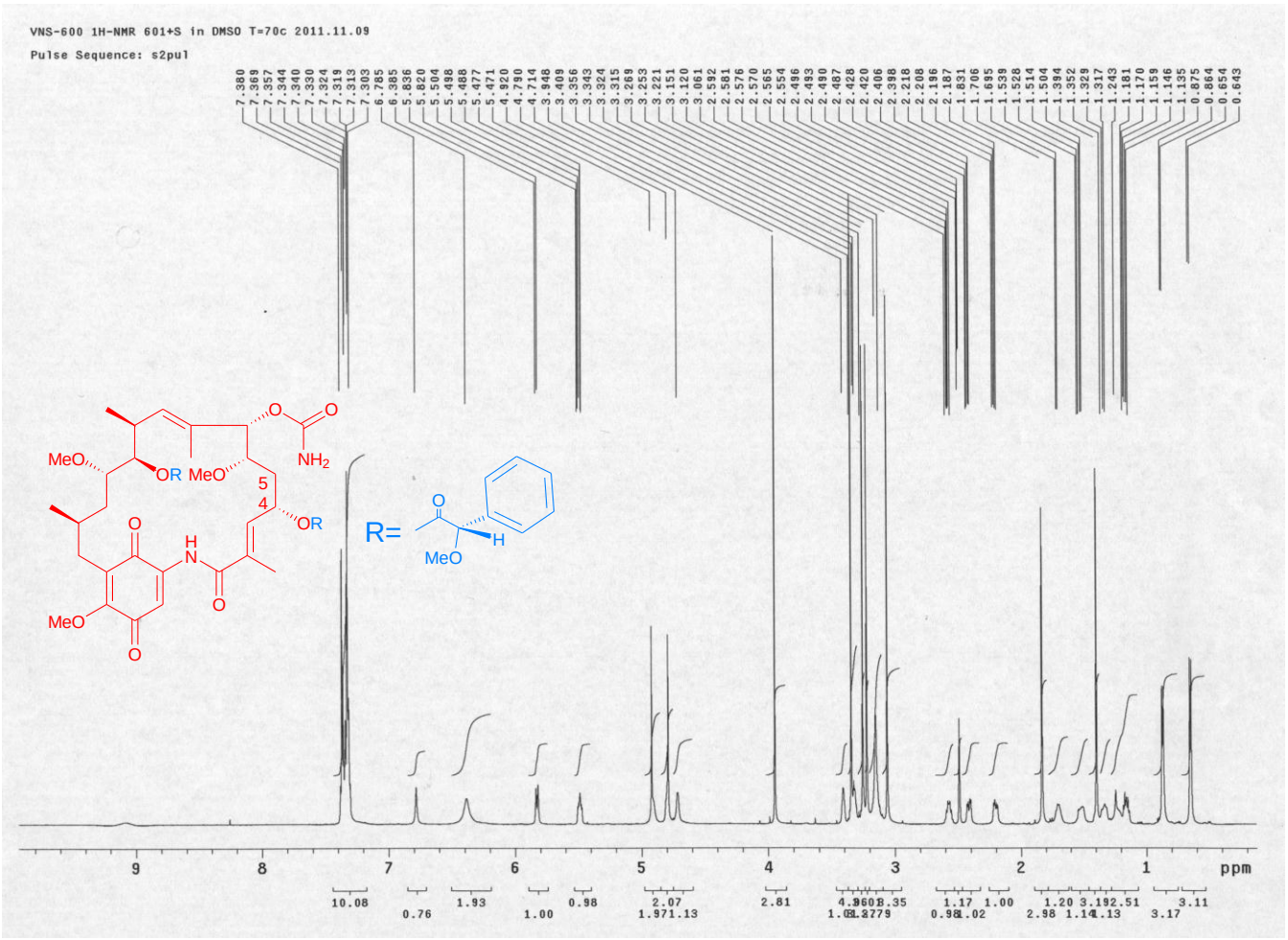


Figure S28. The proposed scheme of C-4,5 oxidation by GdmP in GDM biosynthesis

GDM is the major (normal) product, while **1** and **2** are minor (shunt) products, of C-4,5 oxidation. The oxidation is carried out with hydroquinone form of substrate and product *in vivo*.

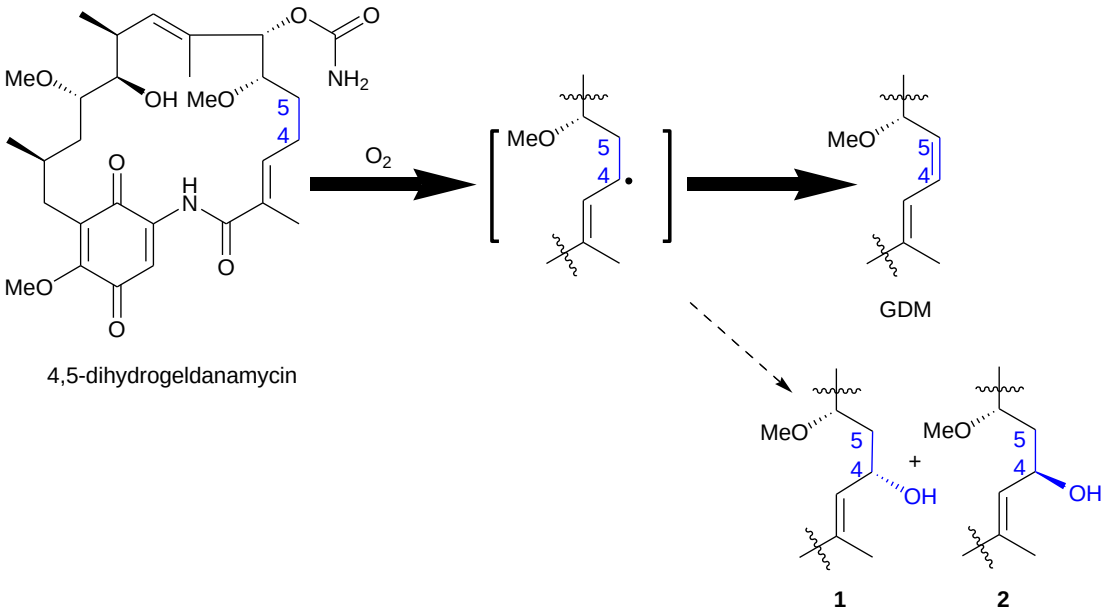
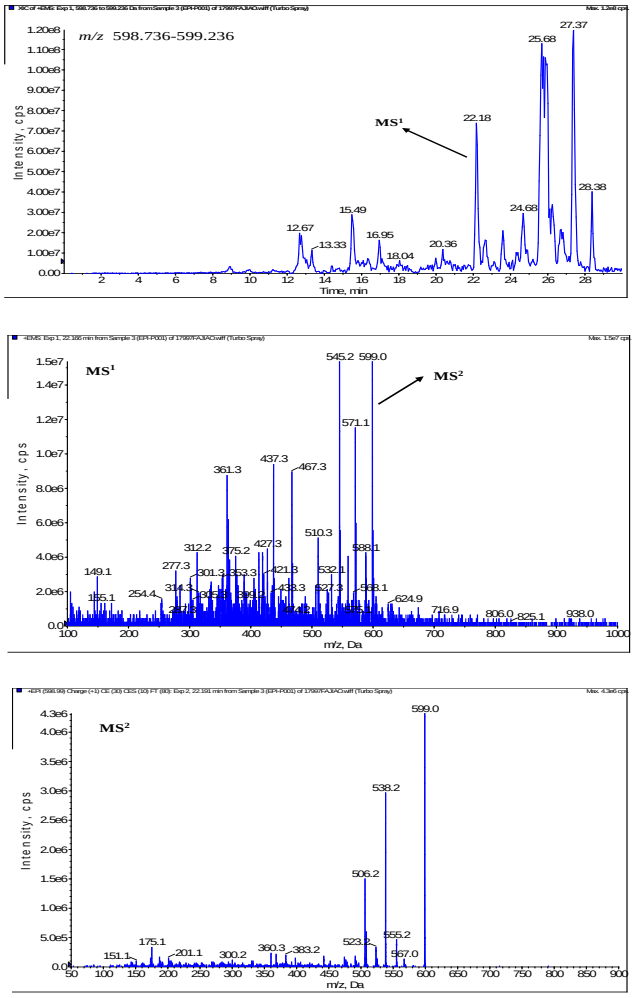
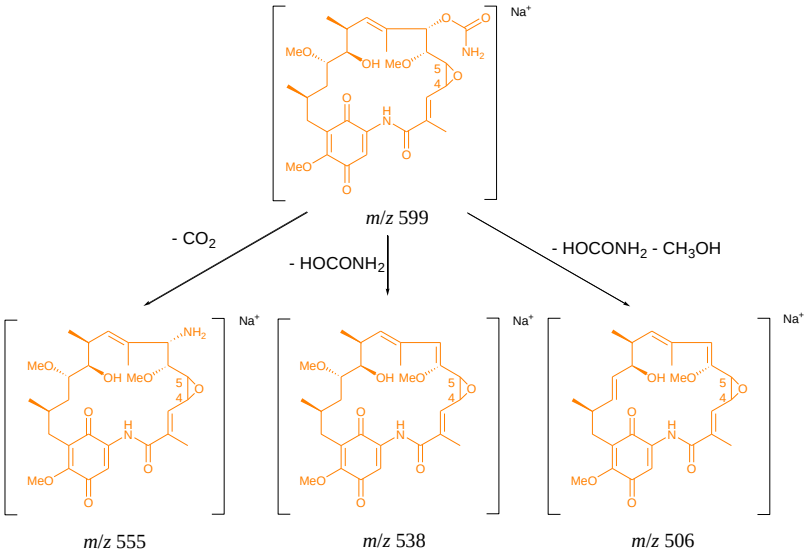


Figure S29. LCMS analysis of putative 4,5-epoxygeldanamycin from *Streptomyces hygroscopicus* 17997

An expected *m/z* 599 $[M + Na]^+$ (22.18 min; GDM at 24.68 min), in accordance with *m/z* of 4,5-epoxygeldanamycin ($C_{29}H_{40}N_2O_{10}Na$, 599), displayed a typical GDM analogue's MS^2 fragment pattern (555 $[M + Na - CO_2]^+$, 538 $[M + Na - HOCONH_2]^+$, and 506 $[M + Na - HOCONH_2 - HOCH_3]^+$). Besides, a key fragment ion *m/z* 175, which is one oxygen atom (16 u) more than *m/z* 159 [C_{2-10}, $^+C(CH_3)$=CH-CH=CH-C≡C-$C(CH_3)$=CH-CH_2CH_3, a fragment containing C-4,5 of GDM in MS^2 , see: Lang, W.; Caldwell, G. W.; Li, J.; et al. *Drug. Metab. Dispos*. 2007, *35*, 21-29.], made us speculate that this *m/z* 599 was most probably 4,5-epoxygeldanamycin.





Upper: selected ion (*m/z* 599) chromatogram. Middle: MS^1 of eluent at 22.18min. Lower: MS^2 of *m/z* 599.

Figure S30. HPLC analysis of the two red compounds degraded from **1** and **2**

The two red compounds had the same retention time (15.1 min) and UV absorption profile. Compared to **1** and **2**, the two red compounds showed maximal absorption wavelength at 307nm and no absorption peak at 254nm. HPLC parameters: Dikma Diamonsil RP-C18 column (4.6×150 mm, 5 μm), MeOH-H_2O, 40-100% in 30 min, 1.0 ml/min, 301 nm (Upper: red compound degraded from **1**; Lower: red compound degraded from **2**).