

# Tetrocarcins N and O, glycosidic spirotetronates from a marine-derived *Micromonospora* sp. identified by PCR-based screening

Yi Tan,<sup>a</sup> Yuanyuan Hu,<sup>a</sup> Qiang Wang,<sup>a</sup> Hongxia Zhou,<sup>a</sup> Yiguang Wang<sup>a</sup> and Maoluo Gan<sup>a,b\*</sup>

<sup>a</sup> Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

<sup>b</sup> State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Correspondence e-mail: ganml@hotmail.com

## Supporting Information

## Contents

<b>Figure S1.</b> The amino acid sequence alignment ( <b>a</b> ) and the phylogenetic tree ( <b>b</b> ) of cloned dTDP-Glucose-4,6-dehydratase fragments with their known analogues.....	S4
<b>Figure S2.</b> The (+)-HRESIMS spectrum of compound <b>1</b> .....	S5
<b>Figure S3.</b> The IR spectrum of compound <b>1</b> .....	S6
<b>Figure S4.</b> The $^1\text{H}$ NMR spectrum of compound <b>1</b> .....	S7
<b>Figure S5.</b> The $^{13}\text{C}$ NMR spectrum of compound <b>1</b> .....	S8
<b>Figure S6.</b> The DEPT spectrum of compound <b>1</b> .....	S9
<b>Figure S7.</b> The $^1\text{H}$ - $^1\text{H}$ COSY spectrum of compound <b>1</b> .....	S10
<b>Figure S8.</b> The HSQC spectrum of compound <b>1</b> .....	S11
<b>Figure S9.</b> The HMBC spectrum of compound <b>1</b> .....	S12
<b>Figure S10.</b> The ROESY spectrum of compound <b>1</b> .....	S13
<b>Figure S11.</b> The (+)-HRESIMS spectrum of compound <b>2</b> .....	S14
<b>Figure S12.</b> The IR spectrum of compound <b>2</b> .....	S15
<b>Figure S13.</b> The $^1\text{H}$ NMR spectrum of compound <b>2</b> .....	S16
<b>Figure S14.</b> The $^{13}\text{C}$ NMR spectrum of compound <b>2</b> .....	S17
<b>Figure S15.</b> The DEPT spectrum of compound <b>2</b> .....	S18
<b>Figure S16.</b> The $^1\text{H}$ - $^1\text{H}$ COSY spectrum of compound <b>2</b> .....	S19
<b>Figure S17.</b> The TOCSY spectrum of compound <b>2</b> .....	S20
<b>Figure S18.</b> The HSQC spectrum of compound <b>2</b> .....	S21
<b>Figure S19.</b> The HMBC spectrum of compound <b>2</b> .....	S22
<b>Figure S20.</b> The ROESY spectrum of compound <b>2</b> .....	S23
<b>Figure S21.</b> The CD spectra of compounds <b>1–3</b> .....	S24

ChaH1 -MRILITGGAGFIGSHFVRSLLDDRYAGWEGARVTVDKLTYAGNRASLP--ASHPRLTF 60  
 Ch1C2 -MRYFVTGGAGFIGGHHYVRALLAGAYPGTAGCEVTVDKLTYAAHLDNLP--LDHDRLT 60  
 ChmAII -MRVLVTGAAGFIGSHFVRQLLSGSYPELAGAHVSLDKLTYAGNTENLAEVAGHPRHTF 60  
 ChryE TTLLLVTGGAGFIGSHYVRTLLSPEGP--GDISVTVDALTYAGNPANLDPVRAHPGFTF 60  
 LanH MKKILITGGAGFIGSHYVRTLLNDGYEDWKGAHVTVDKLTYAGNRDNL--EAHPRLTF 60  
 PdmY -MRILVTGGAGFIGSHYVRSLAGRYPAVADALTVVDKLTYAGNTDNL--IGDPLTF 60  
 RhoH -MNIPVTGAAGFIGSHFVRTLLSGGYPGHEDDRVTVDKLTYAGTLNNLP--PRHPLTF 60  
 StrE ATRLLVTGGAGFIGSHYVRTLLGPDGP--PDAVVTVDALSYAGNLANLDPVRDHPPVRF 60  
 TcaB2 SKRVVVTGGAGFIGSHYVRQLIGGAYPAFADASVVVDKLTYAGNLDNLAAVSGDERFEL 60  
 UrdH -MNILVTGAAGFIGSHFVRSLADTYSGWEGARVTALDKLTYAGNRNNLP--PSNPRLEF 60  
 dTGD\_5-297a -----VVVLDKLTYAGNLDNLAAVSGDERFEL 60  
 dTGD\_5-297b -----YVGfedchVTVDKLTYASDRNNLP--AHHPLTF 60

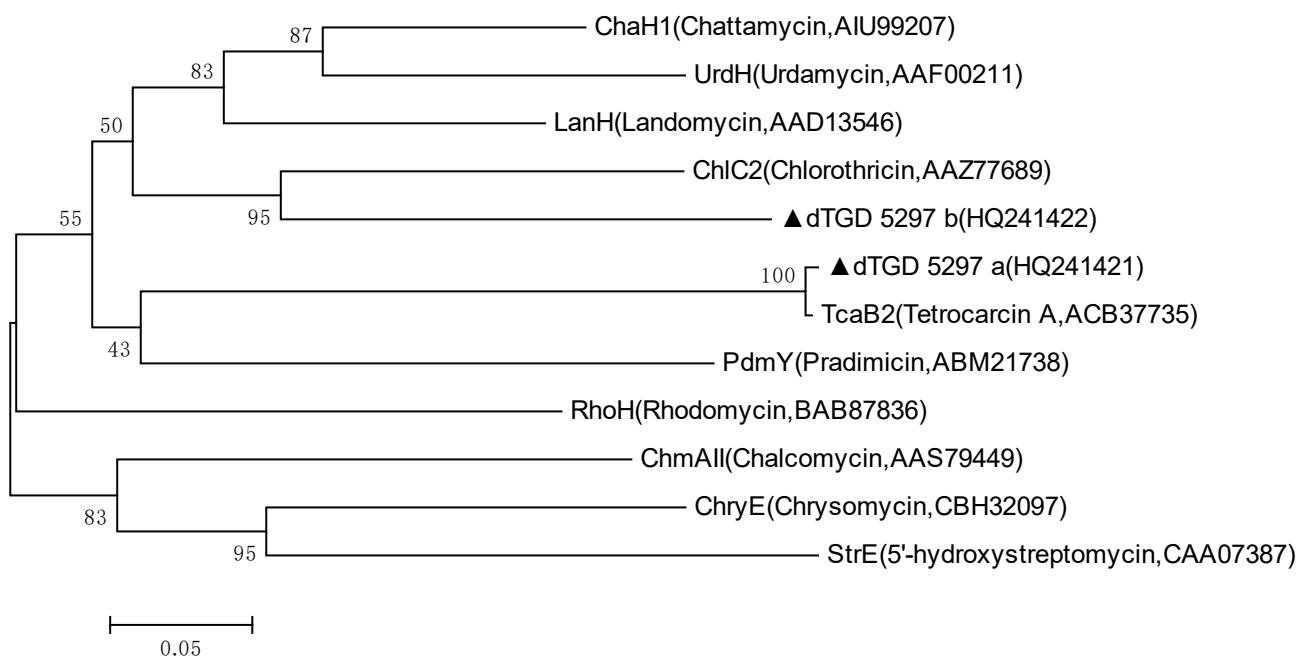
\* : \* : \*\*. . \* . :

ChaH1 VQGDVCD S ALLREVLP GH DAVV HFA ESH VDRS ITGAAEFFRTNV L GT Q ALL D A V LATGV 120  
 Ch1C2 VRGDICDAGL LADL P GHD AVV HFA ESH VDRS LADGT P FVT NV L GT Q ILL D AC ARTGV 120  
 ChmAII LHGDICDPPTVAQALRGTDLVHFA ESH VDRS ITDSA AFVTTNV L GT Q ILL D A AL RAGT 120  
 ChryE VRGDICDAPL VRS LARRADQIVHFA ESH VDRS ITDPA AFT RTN VM GT Q ILL D A AL RAGT 120  
 LanH VQGDICDFELL L ELL PGH DAVV HFA ESH VDRS LESAEEF VHTN VTGT Q RLL D A V LATRV 120  
 PdmY VKGDICDVPL RELL PGH DAVV HFA ESH VDRS LVSAGE FAVTN VL GT Q SLL D C C V LAGI 120  
 RhoH VHGDICDTPLLGKV FPGHEA VV HFA ESH VDRS VAGAEAF VRTN VL GT Q ALLEA AL RHGT 120  
 StrE VHGDICDADL V RV MAG-QDQV VH LAA ESH VDRS LLL DAA AF VRTN NAGGT Q TLL D A AL RHGV 120  
 TcaB2 VVGDICD A Q LLA GLL PGH DAVV V HFA ESH VDRS IVGAADF MHTN V Q GT YTVL Q A CL D AGV 120  
 UrdH VRGD CDR ALL RELL PGH HAVV HFA ESH VDRS LSEGAGE FFR TN VL GT Q TLL D A V L D SGV 120  
 dTGD\_5-297a VVGDICD A Q LLA GLL PGH DAVV V HFA ESH VDRS IVGAADF MHTN V Q GT YTVL Q A CL D AGV 120  
 dTGD\_5-297b VRGDICD RPL LADL VPGH DALV HFA ESH VDRS I DD PAP FF ETN VM SH HLL A CARA AV 120  
 : \*\*\*:\*\*\* : . . : \*\*:\*\*\*\*\*: . \* \*. \*: :\* .

ChaH1 ERVHVSTDEVYGSIEEGAWTEEWPLLNPNTPYAASKAGSDLVARSYWRTHGV DVS VTRCS 180  
 Ch1C2 PRFHVSTDEVYGS LRGHSWTEDTLLEPNSPY AASKAAS DLMARAY WRTSDV AVS I TRCS 180  
 ChmAII SRFHVSTDEVYGS I PEGSSTEADPLDPN SPY AASKA S D LLA LF HRT HGLD VR VTRCS 180  
 ChryE RTFVHISTDEVYGSIDEGSWPEDHPLRPSSPYAASKA S D LLA SHHH HGLD VR VTRCS 180  
 LanH KRVHVSTDEVYGSIDEGSWTEEWPLAPNSPYSASKAAS D LLA RSYWRTHGLD LS I TRCS 180  
 PdmY GRVHVSTDEVHGSIDTGSWDEA APVDRSPSPY AASKAAS DHFALAY HH THRLP VMI TRCS 180  
 RhoH GV FVQVSTDETYGSIAEGSWTEDEPLLPNSPSPY AASKA S ADL I ARSYWRTHGLD VR VTRCA 180  
 StrE APFVQVSTDEVYGSLETGSWTEDEA V RPNSPSPY AASKA S G D L L A L A M H V S H G L D V R I T R C S 180  
 TcaB2 GRV VQVSTDEVYGSIAETGSW PESDPLEPNSPSPY SASKAC GDL I ARSYFRTYGLPVCLTRCS 180  
 UrdH ERVHVSTDEVYGSIEQGSWTEDWPLQPNSPSPY AASKA C S D L V A R A Y C A P T E V D L S I TRCS 180  
 dTGD\_5-297a GRV VQVSTDEVYGSIAETGSW PESDPLEPNSPSPY SASKAC GDL I ARSYFRTYGLPVCLTRCS 180  
 dTGD\_5-297b GRVHVSTDEVYGSIDGSWTERCVLEPNSPSPY AASKAAS DCV R SYWRTYD DV S I TRCA 180  
 . \* : \*\*\*\*. : \*\*: \*: \* : \* : \*\*:\*\*\*\* . \* .. : . : : :\*\*\*:

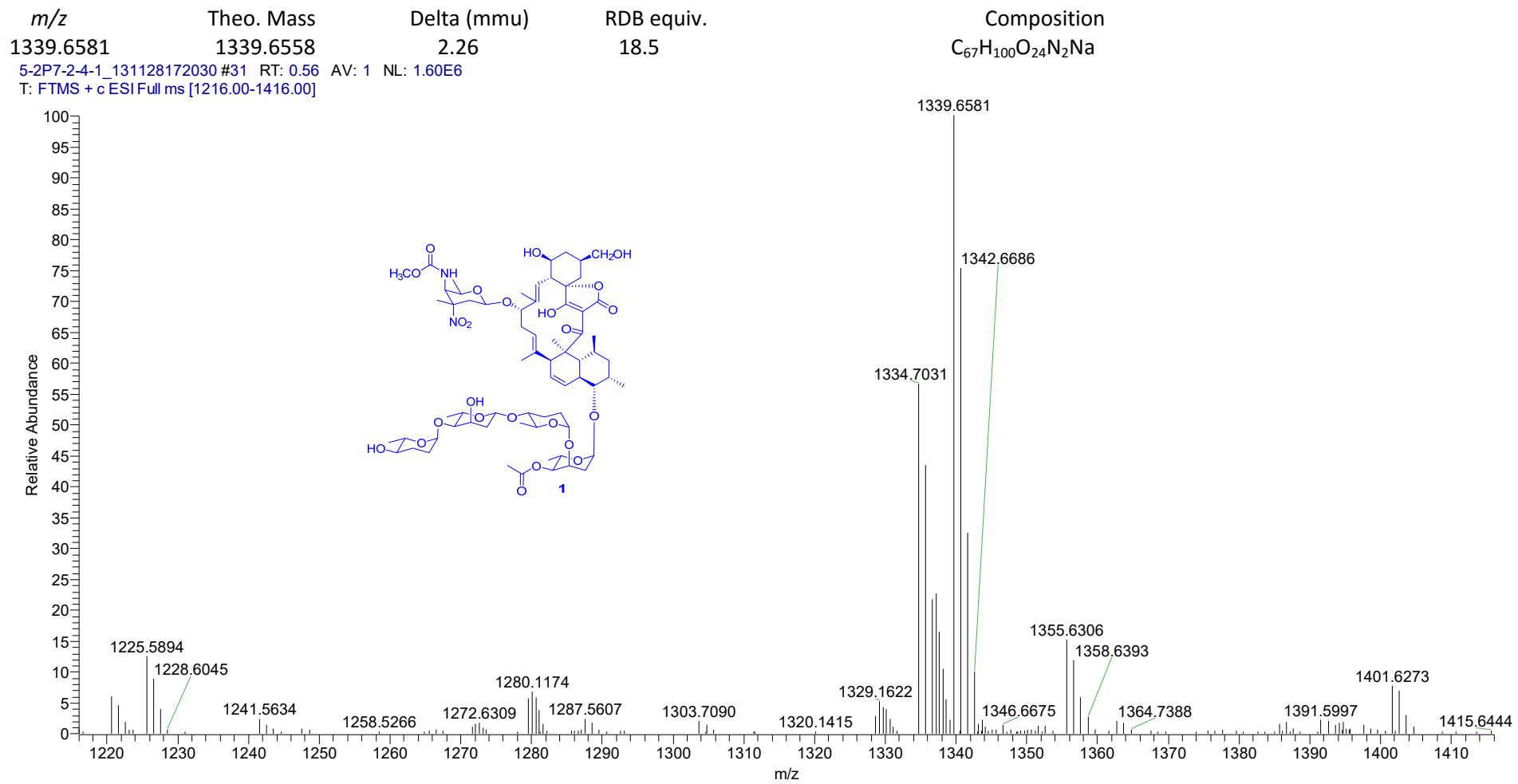
ChaH1 NNYGPYQHPEKLIPLFITNLLEG R GPV PLYGDGRNVREW M H V D D C R A L H L V L N N G R A G E V 240  
 Ch1C2 NNYGTHQFIEKVVP LF VTNLL D G L D V PLYGDGH H V REWL H V D E H C R A L H L V L T E G R A G E I 240  
 ChmAII NNYGPHQHPEKVVPLFVT HLL E G L R L PLYGDGLHRRDWLH V D D H C R G I A M V A A R G R A G E V 240  
 ChryE NNYGHHHFPEKAIPLFVTRLLDGRKV PLYGDGRNV RDWLH I D D H V R A V E L V R T S G R P G E V 240  
 LanH NNYGPYQHPEKLIPLFVTNLL EGEQV PLYGDGGNIREWLH V D D H C R A I D L V L N K G R A G E I 240  
 PdmY NNYGPYQFPEKIVPLFVTNLLA G R P V P L F G D G L H R R Q W L H V D H C D A V Q R V L E D G A P G E V 240  
 RhoH NNYGPQHPEKLVPLFVT RLLD G Q P V P L YGDGSNL R EWLH V D D H C R A V R V L D E G R P G E I 240  
 StrE NNYGPYQFPEKLVPRFVTLL EGR KV PLYGDGLH V RDWLH V D D H V R G I E A V R A R G R A G R V 240  
 TcaB2 NNYGPQHYPEK I I PL FITNLLEG R GPV PLYGDGH N V RDWLH V D D H C R A I Q L V V E G G R P G E V 240  
 UrdH NNYGPQHPEKVI P RF VTNLL EGR QV PLYGDGRNVREW L H V D E H C R G I H L V L N K G Q A G E I 240  
 dTGD\_5-297a NNY----- 240  
 dTGD\_5-297b NNY----- 240  
 \*\*\*

(a)



(b)

**Figure S1.** The amino acid sequence alignment (a) and the phylogenetic tree (b) of cloned dTDP-Glucose-4,6-dehydratase fragments with their known analogues. dTGD 5-297a (NCBI accession no. HQ241421) and dTGD 5-297b (accession no. HQ241422) are the cloned dTDP-glucose-4,6-dehydratase gene fragments identified from *Micromonospora* sp. 5-297; ChaH1 (accession no. AIU99207) for chattamycin biosynthesis from *Streptomyces chattanoogensis*; UrdH (accession no. AAF00211) for urdamycin A biosynthesis *S. fradiae*; LanH (accession no. AAD13546) for landomycin biosynthesis from *S. cyanogenus*; ChIC2 (accession no. AAZ77689) for chlorothricin biosynthesis from *S. antibioticus*; PdmY (accession no. ABM21738) for pradimicin biosynthesis from *Actinomadura hibisca*; TcaB2 (accession no. ACB37735) for tetrocacin A biosynthesis from *Micromonospora chalcea*; RhoH (accession no. BAB87836) for rhodomycin biosynthesis from *S. violaceus*; ChmAll (accession no. AAS79449) for chalcomycin biosynthesis from *S. bikiniensis*; ChryE (accession no. CBH32097) for chrysomycin biosynthesis from *S. albaduncus*; StrE (accession no. CAA07387) for 5'-hydroxystreptomycin biosynthesis from *S. glaucescens*.



**Figure S2.** The (+)-HRESIMS spectrum of compound 1

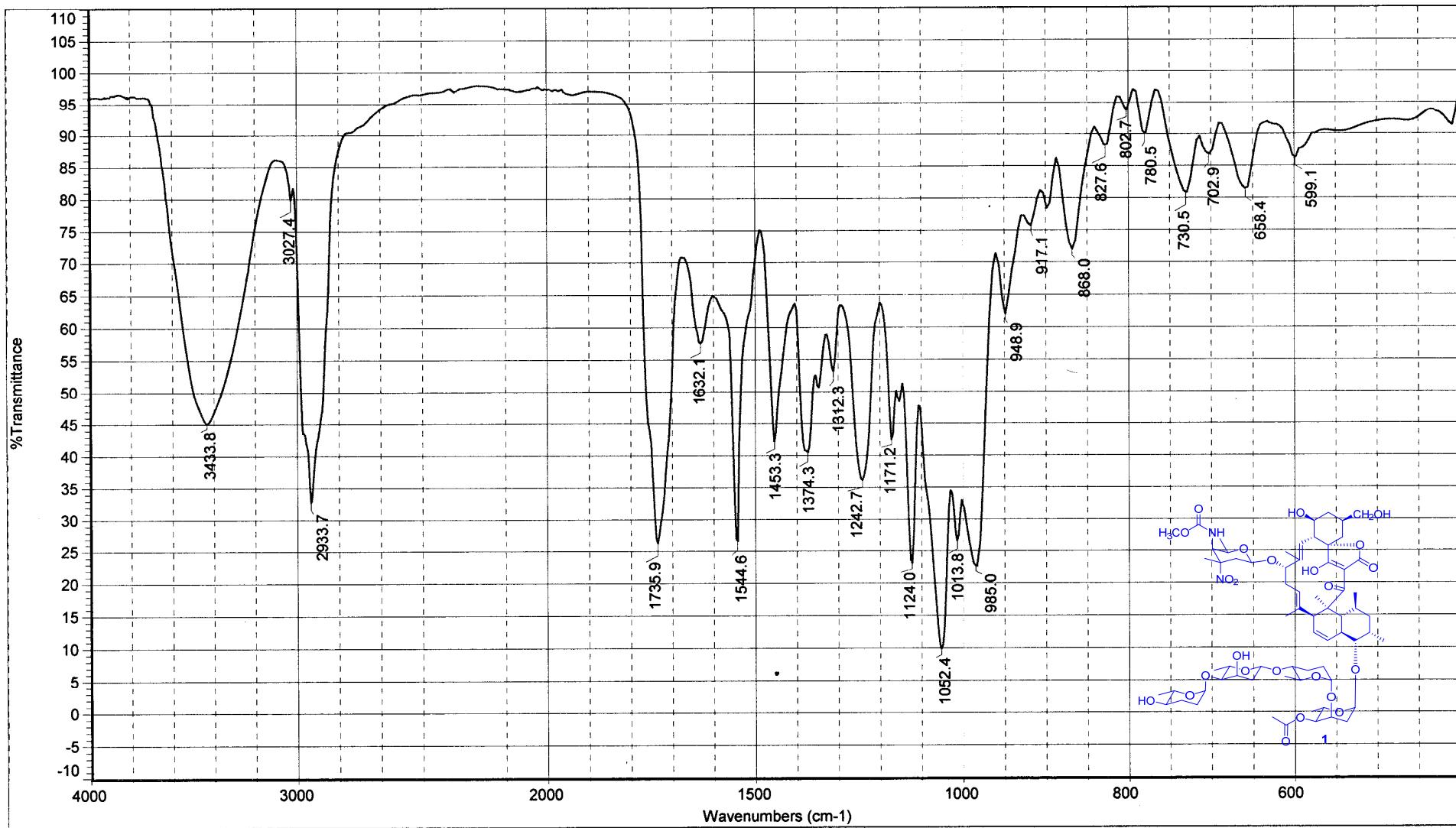


Figure S3. The IR spectrum of compound 1

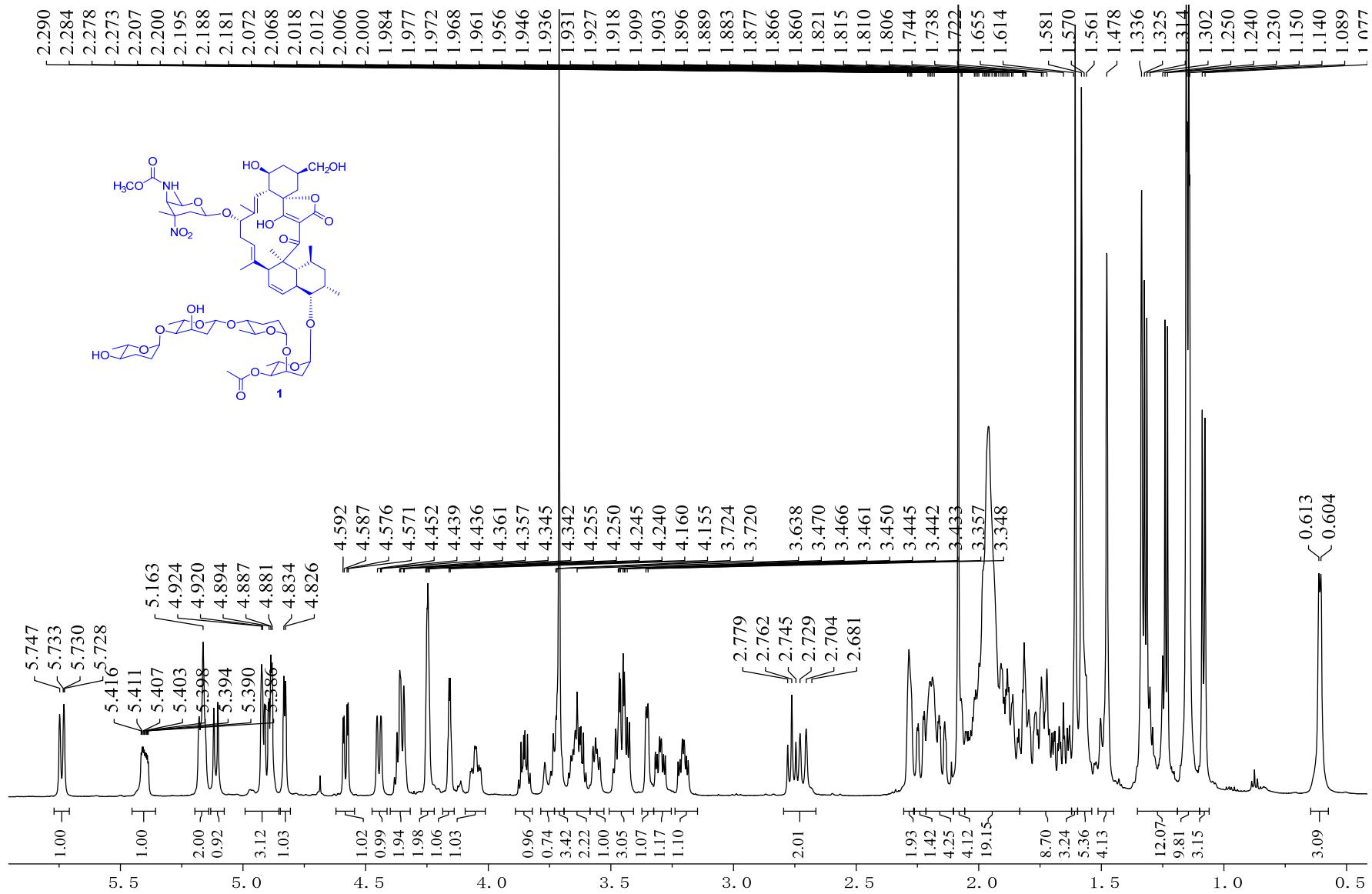
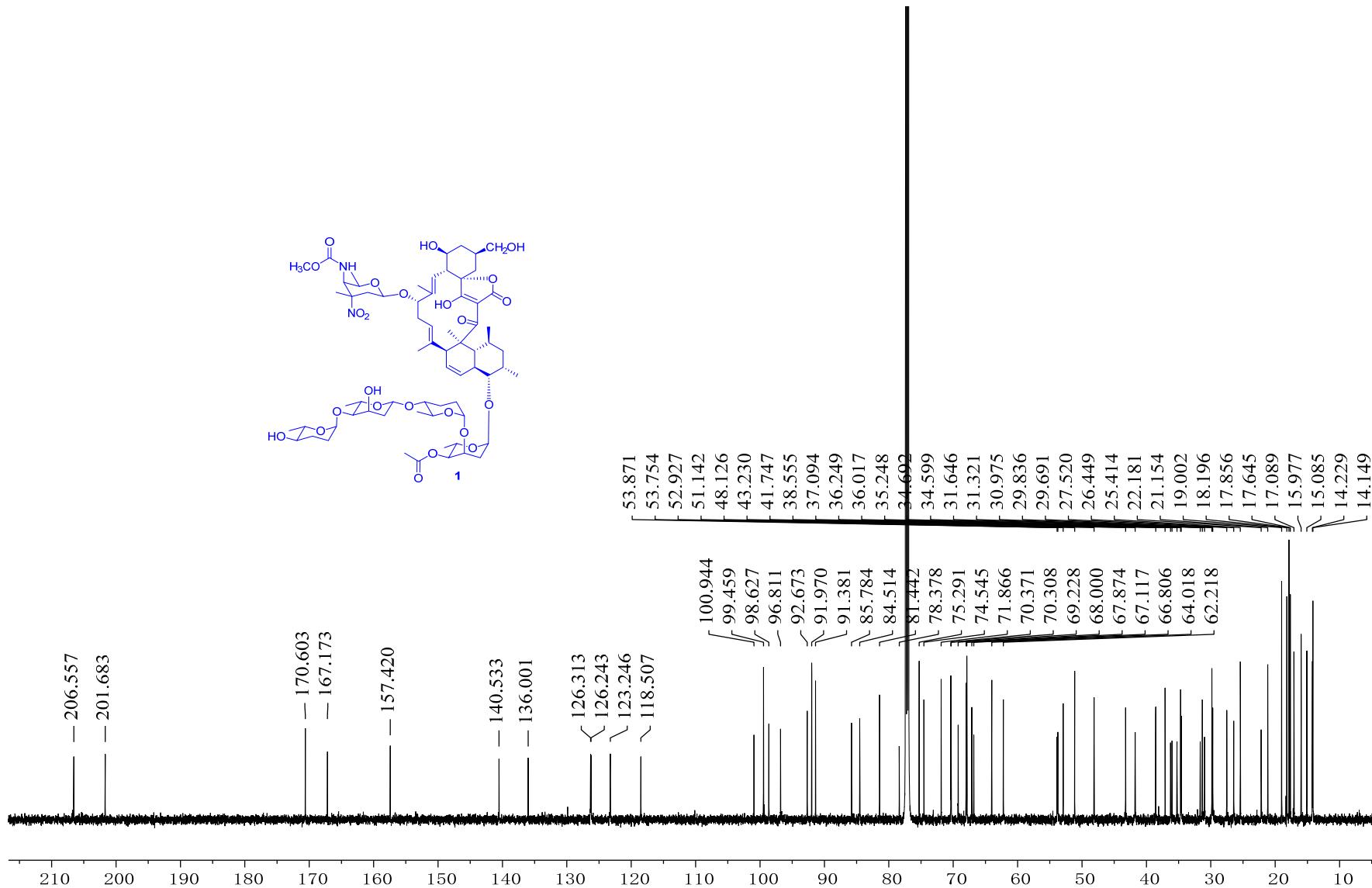
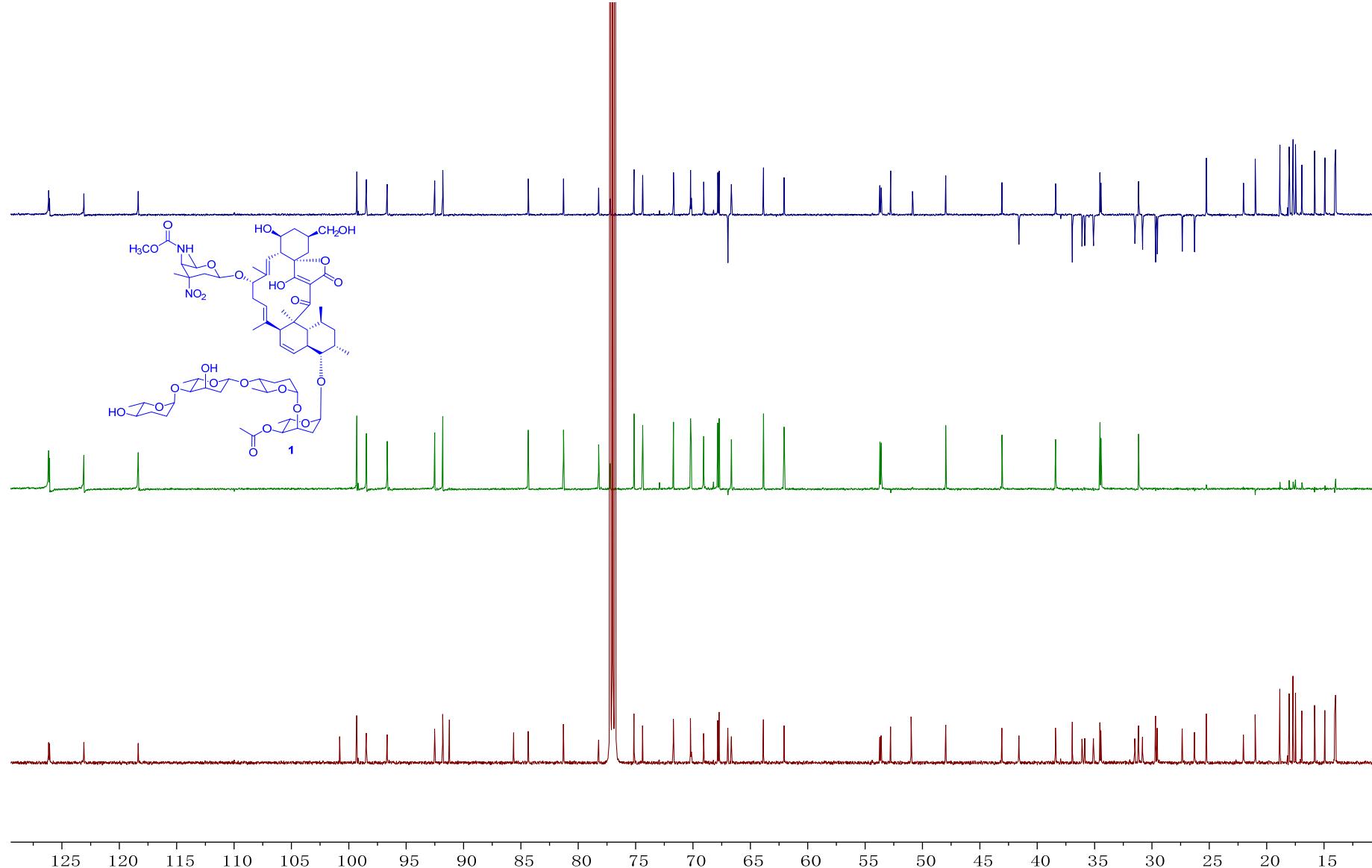


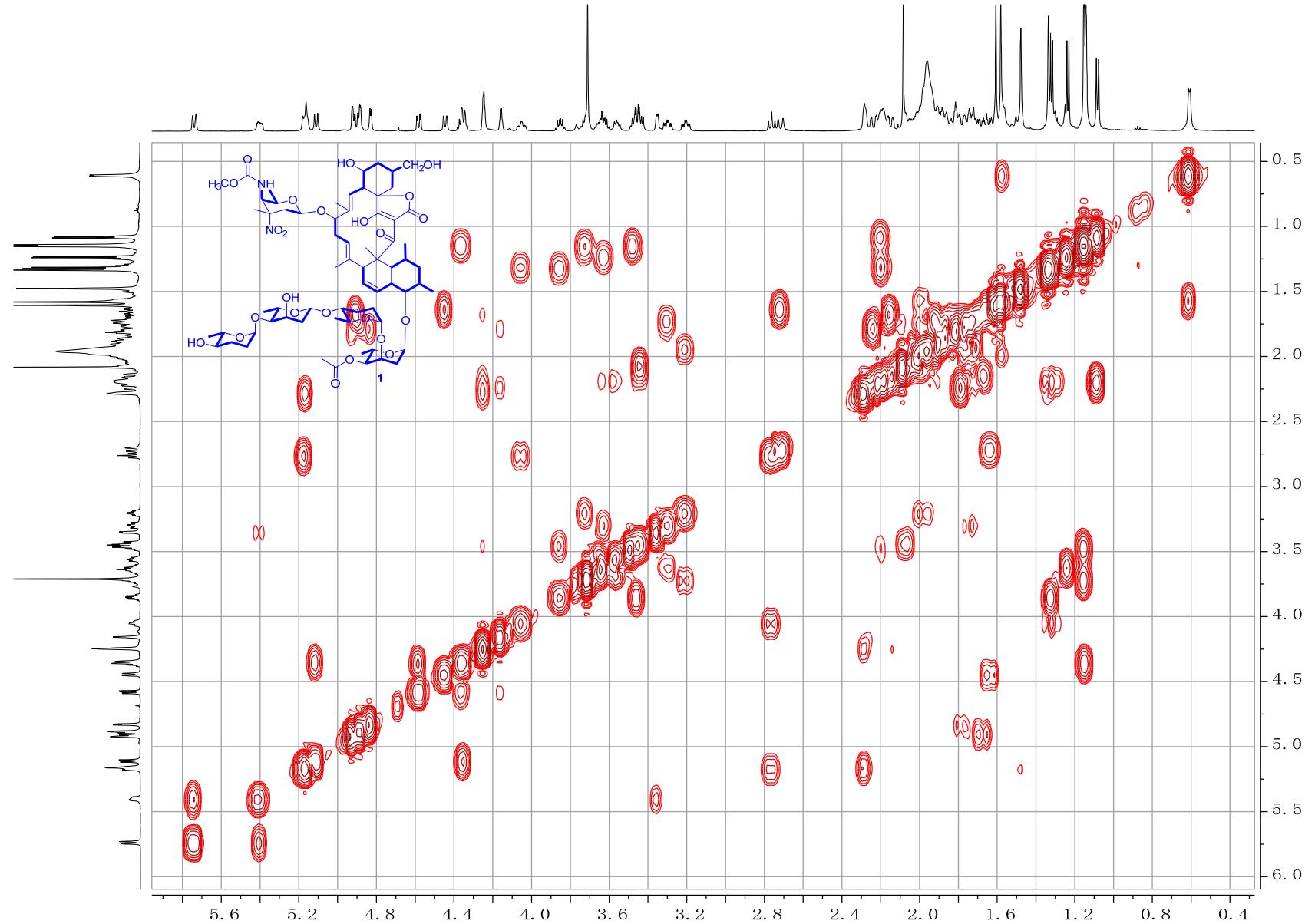
Figure S4. The  $^1\text{H}$  NMR spectrum of compound **1**



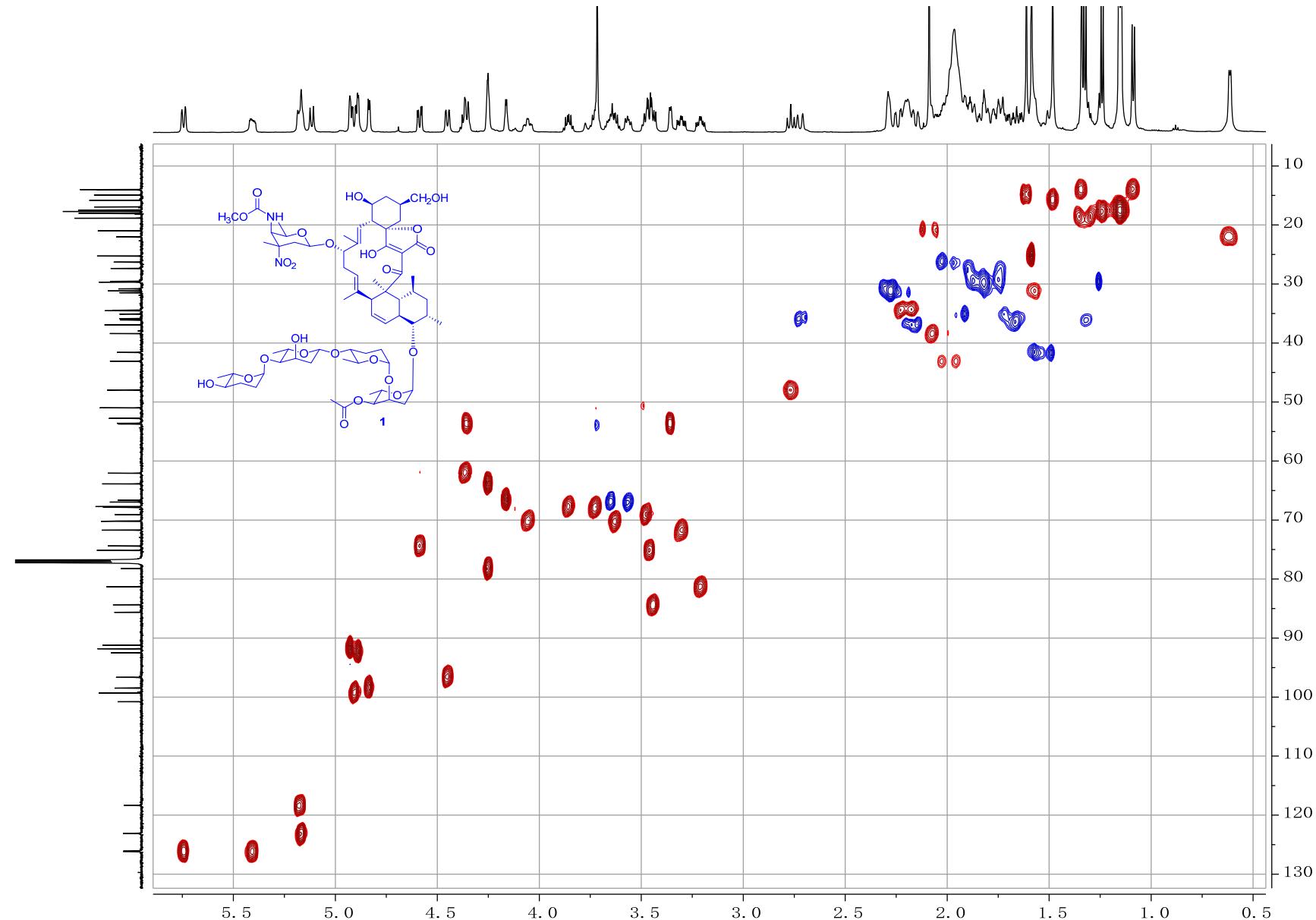
**Figure S5.** The  $^{13}\text{C}$  NMR spectrum of compound **1**



**Figure S6.** The DEPT spectrum of compound **1**



**Figure S7.** The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 1



**Figure S8.** The HSQC spectrum of compound **1**

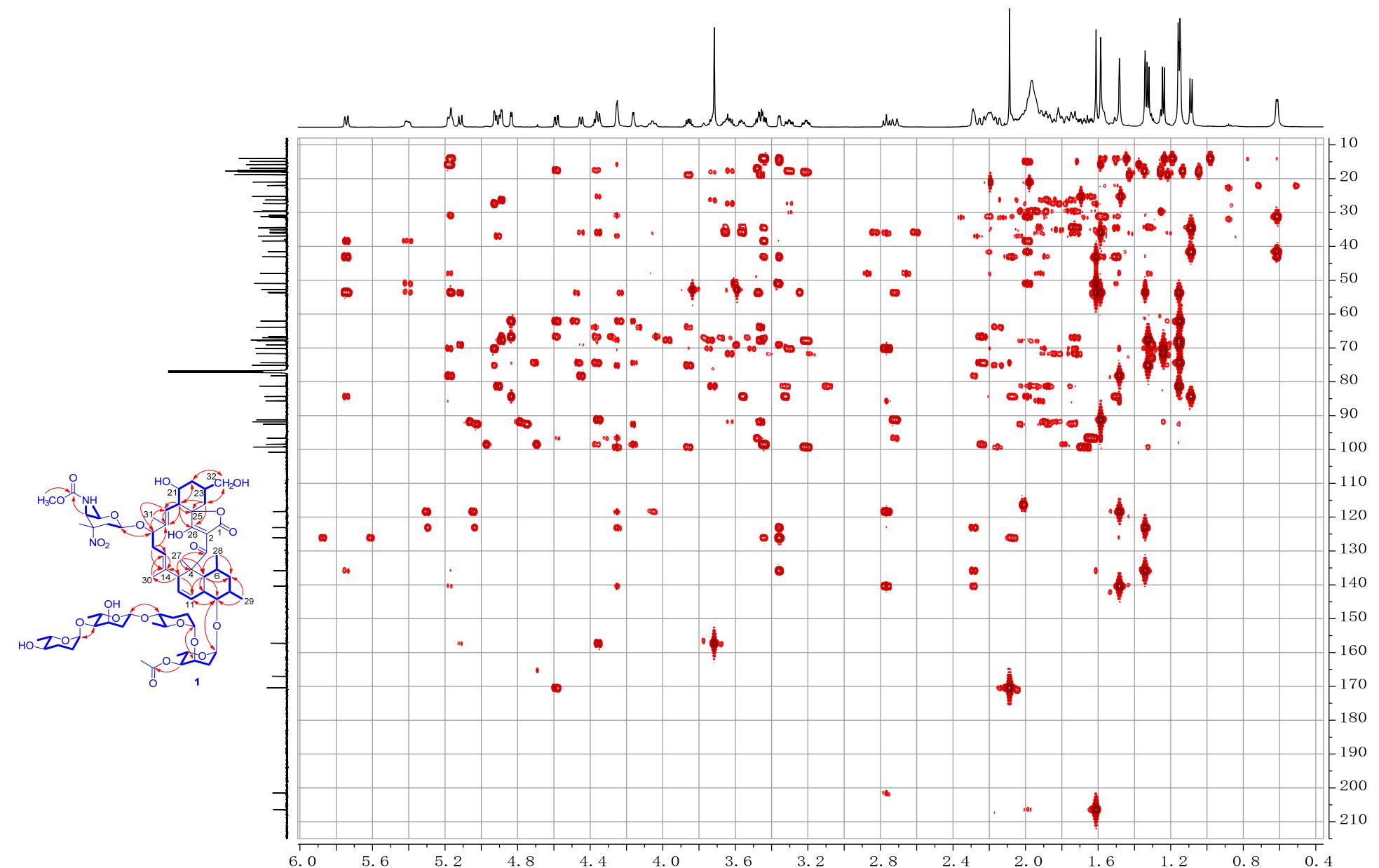


Figure S9. The HMBC spectrum of compound 1

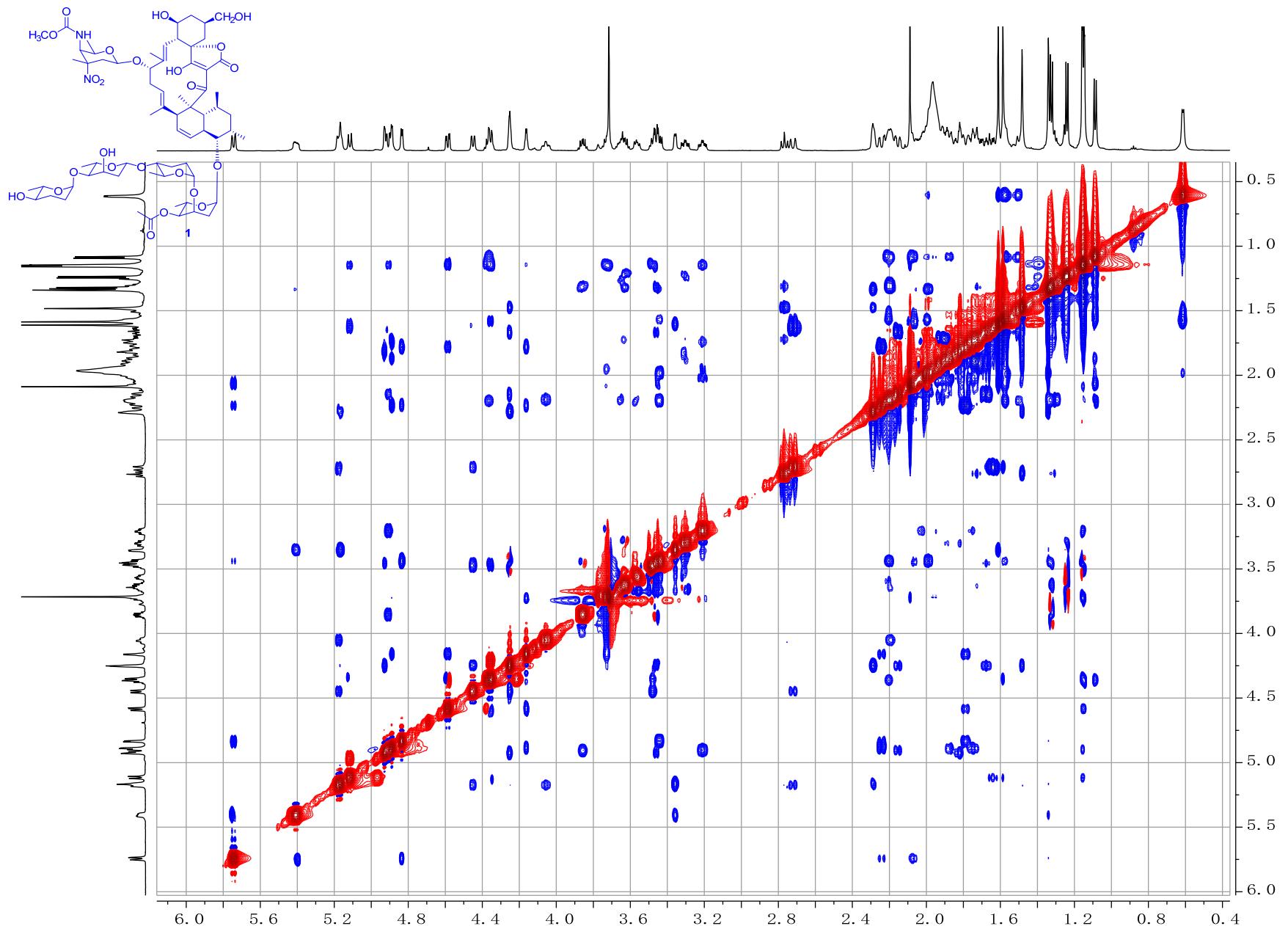
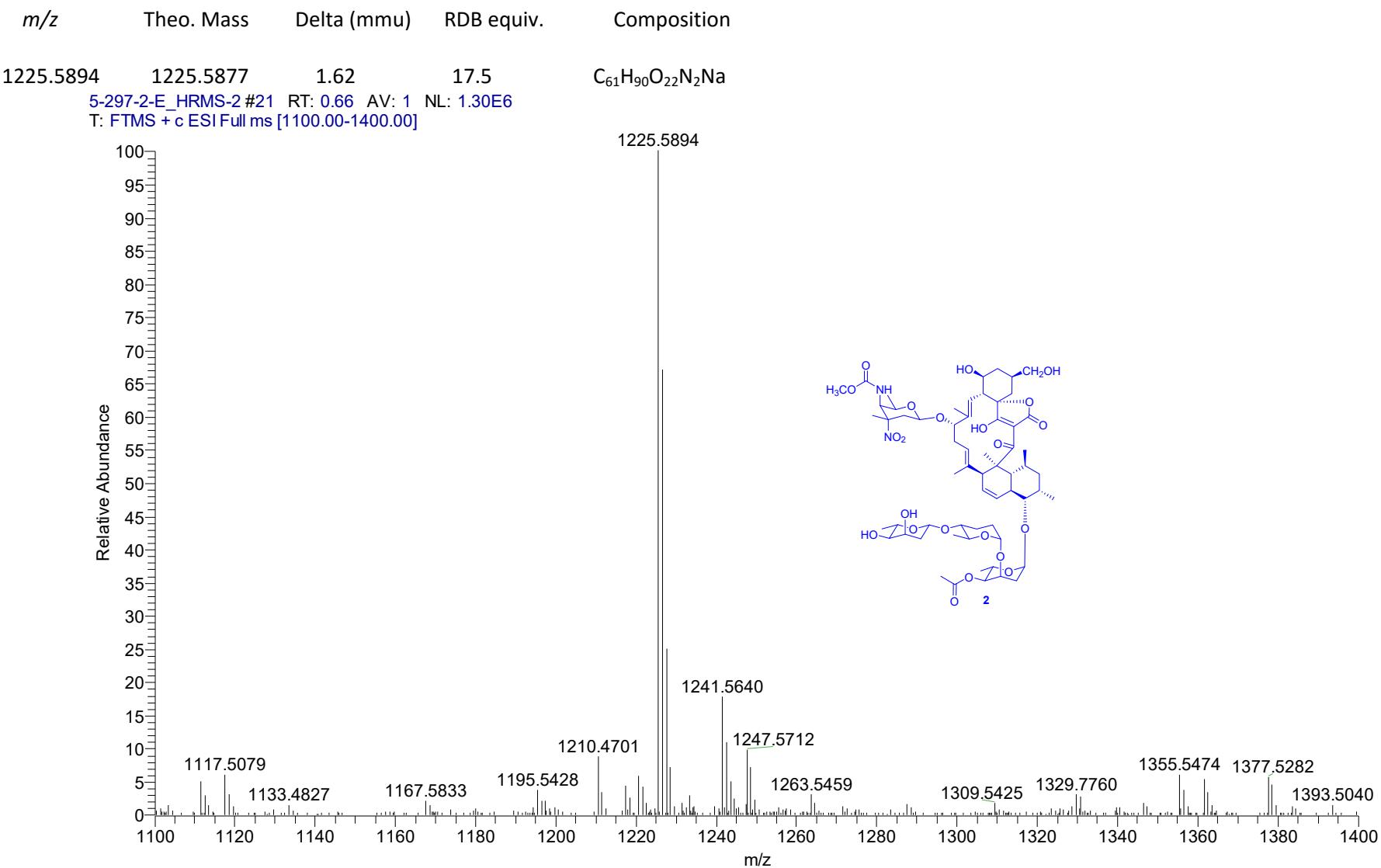
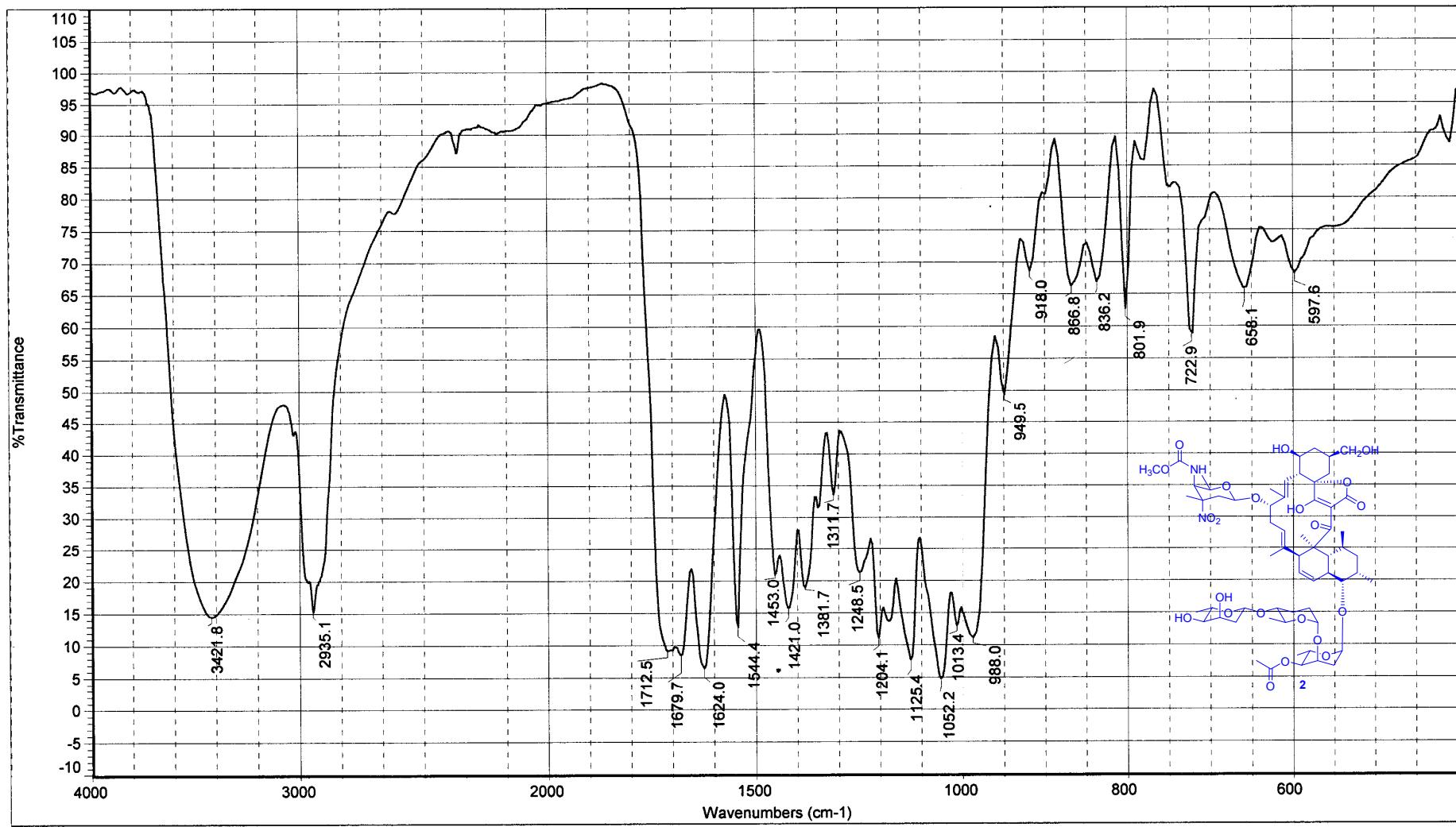


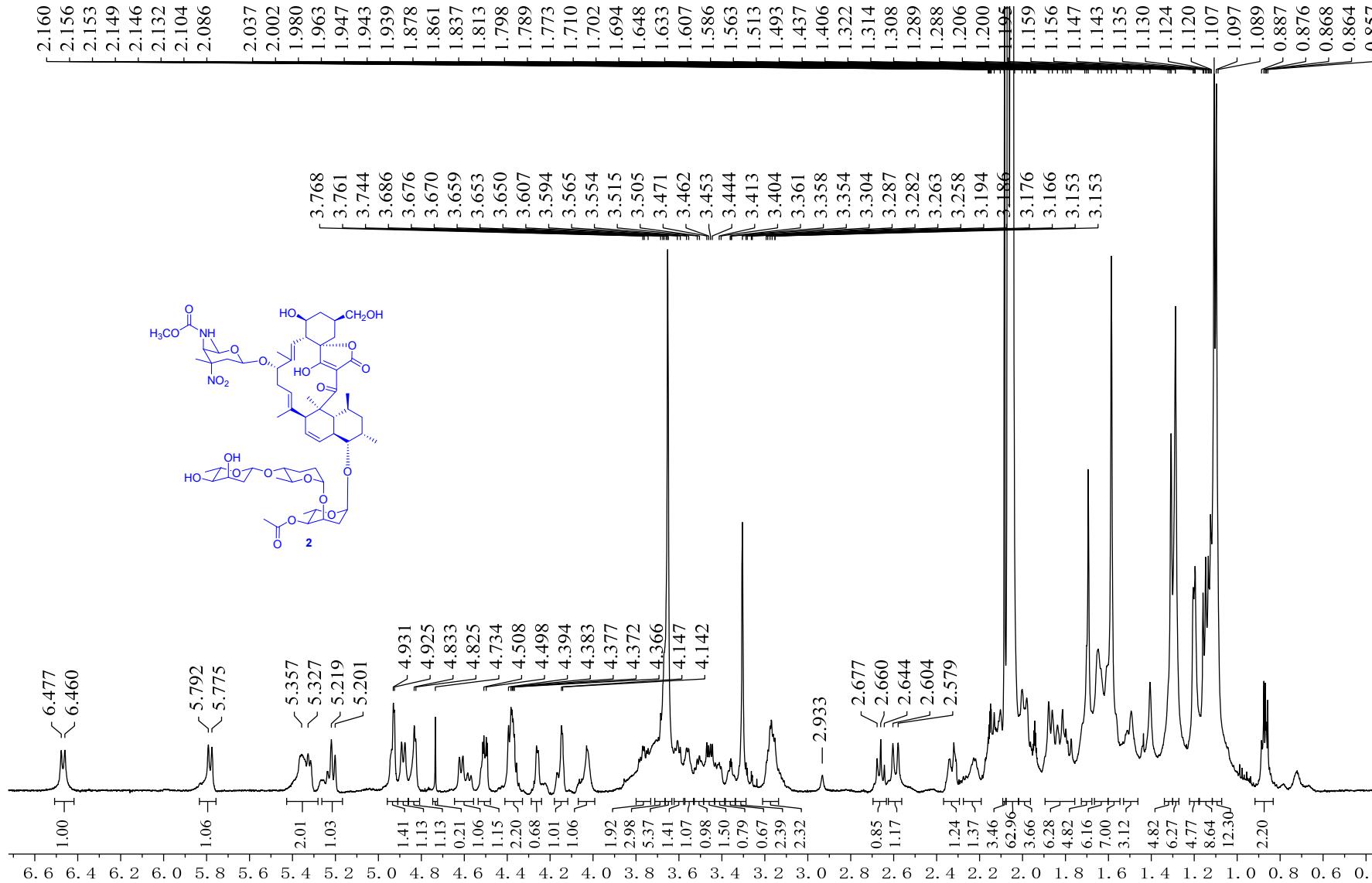
Figure S10. The ROESY spectrum of compound 1

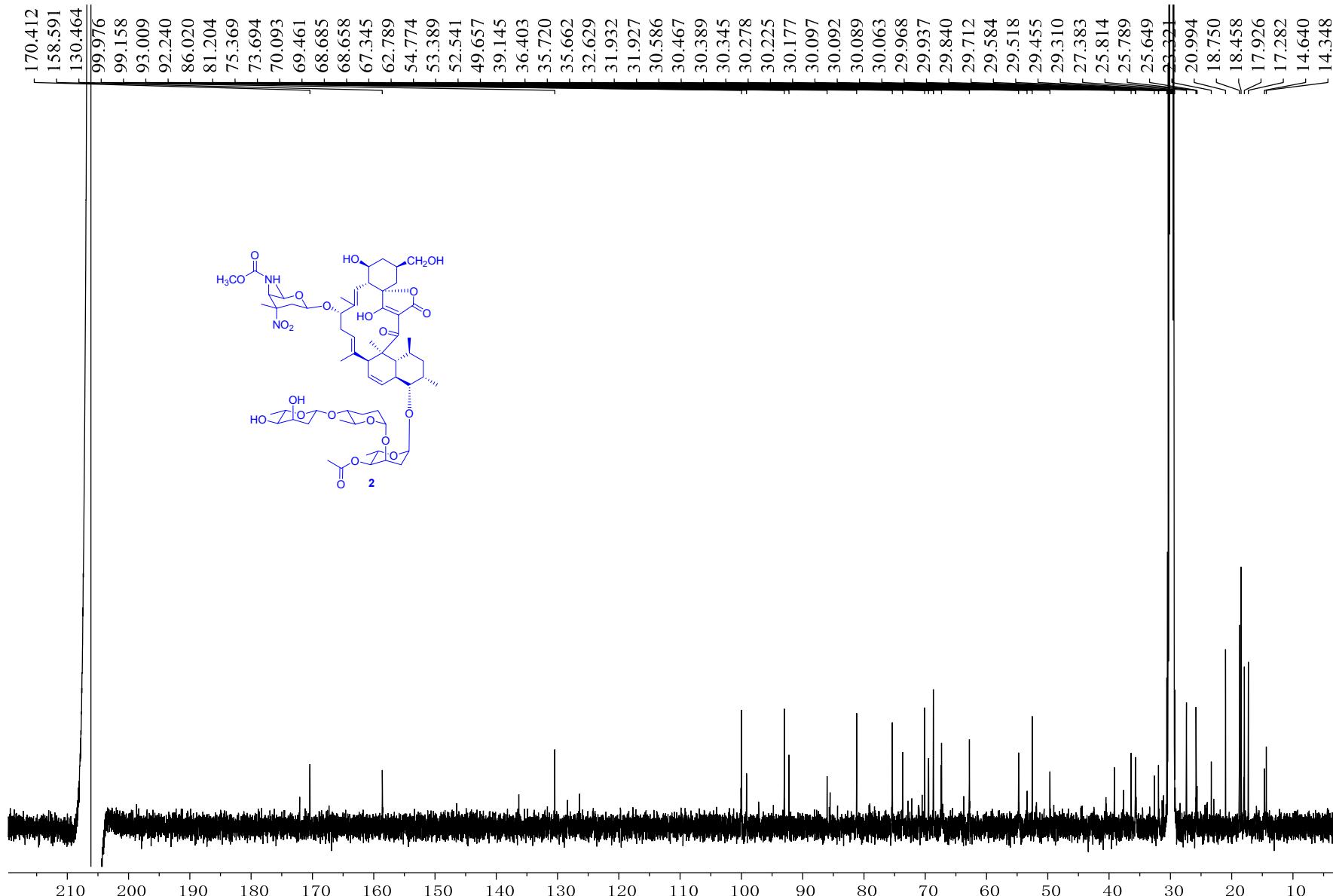


**Figure S11.** The (+)-HRESIMS spectrum of compound 2

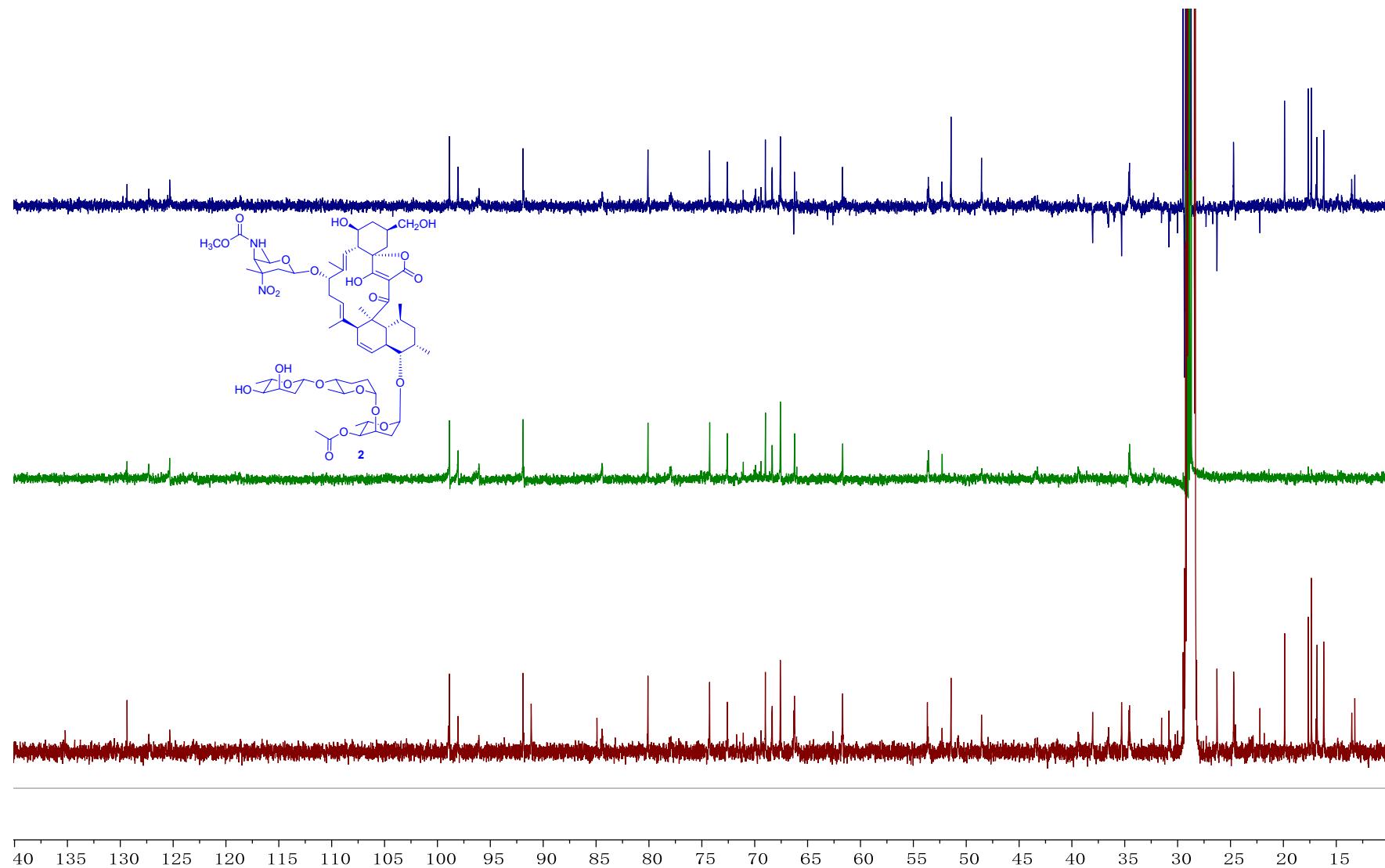


**Figure S12.** The IR spectrum of compound **2**

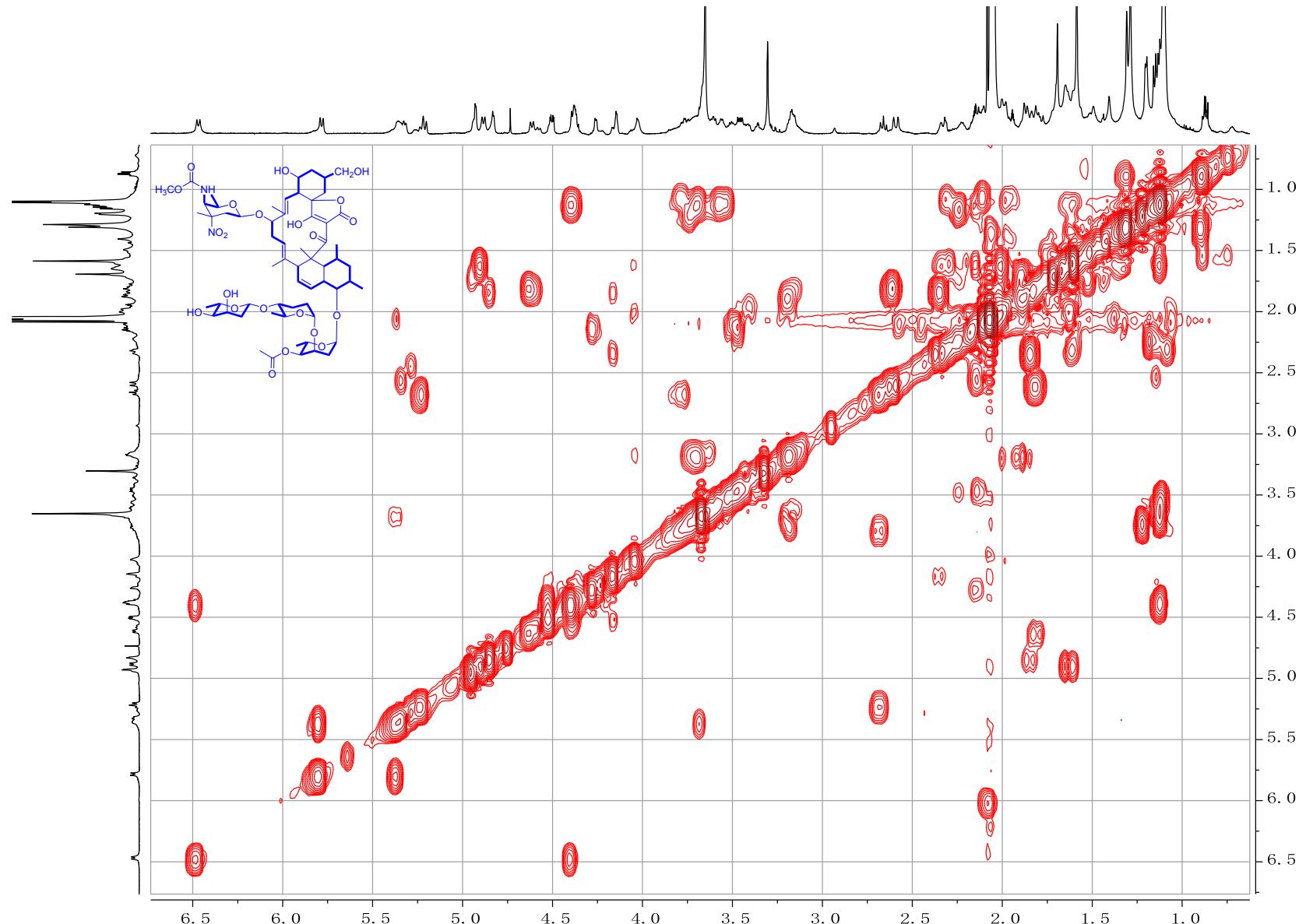




**Figure S14.** The  $^{13}\text{C}$  NMR spectrum of compound 2



**Figure S15.** The DEPT spectrum of compound **2**



**Figure S16.** The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 2

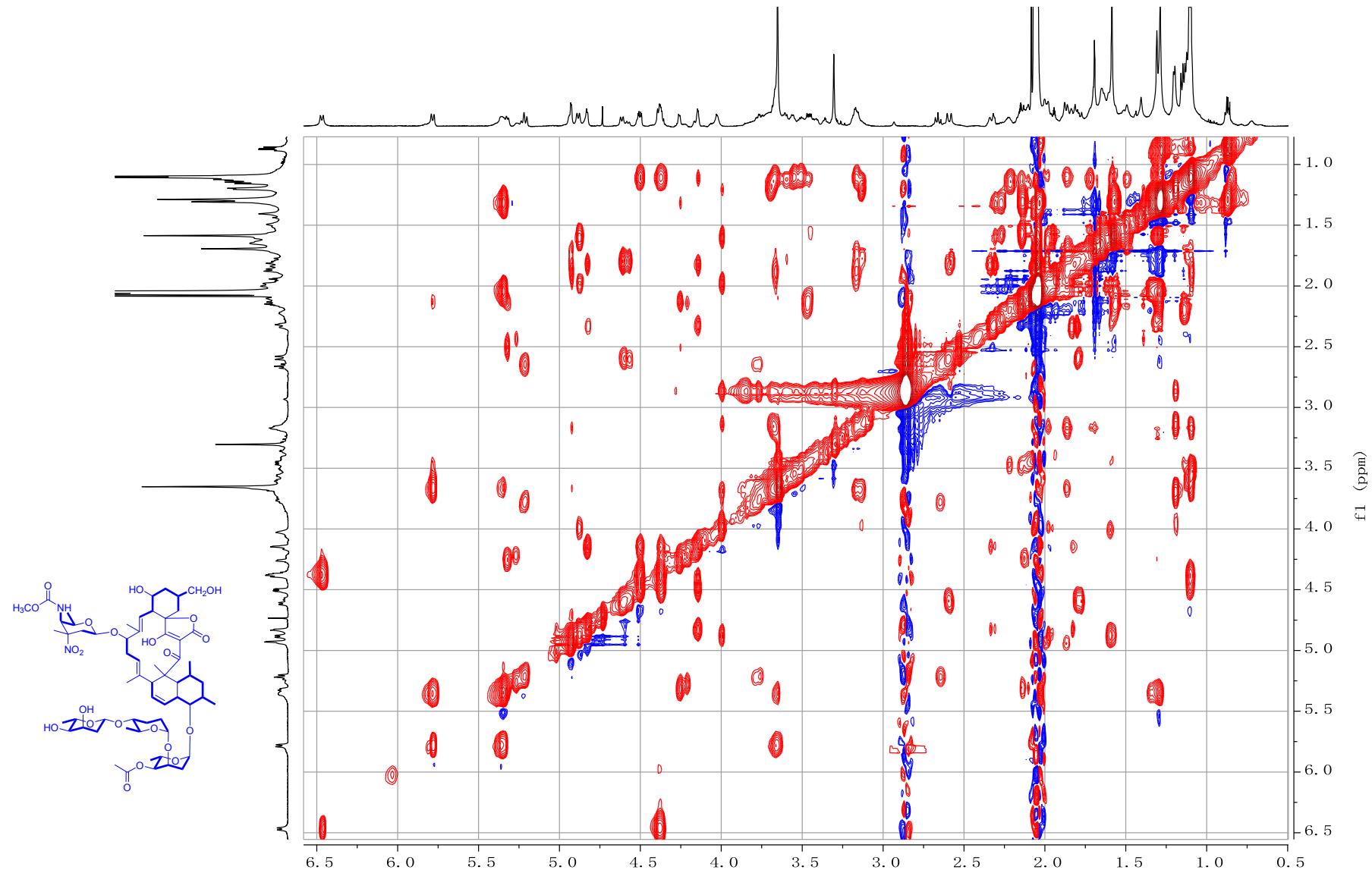
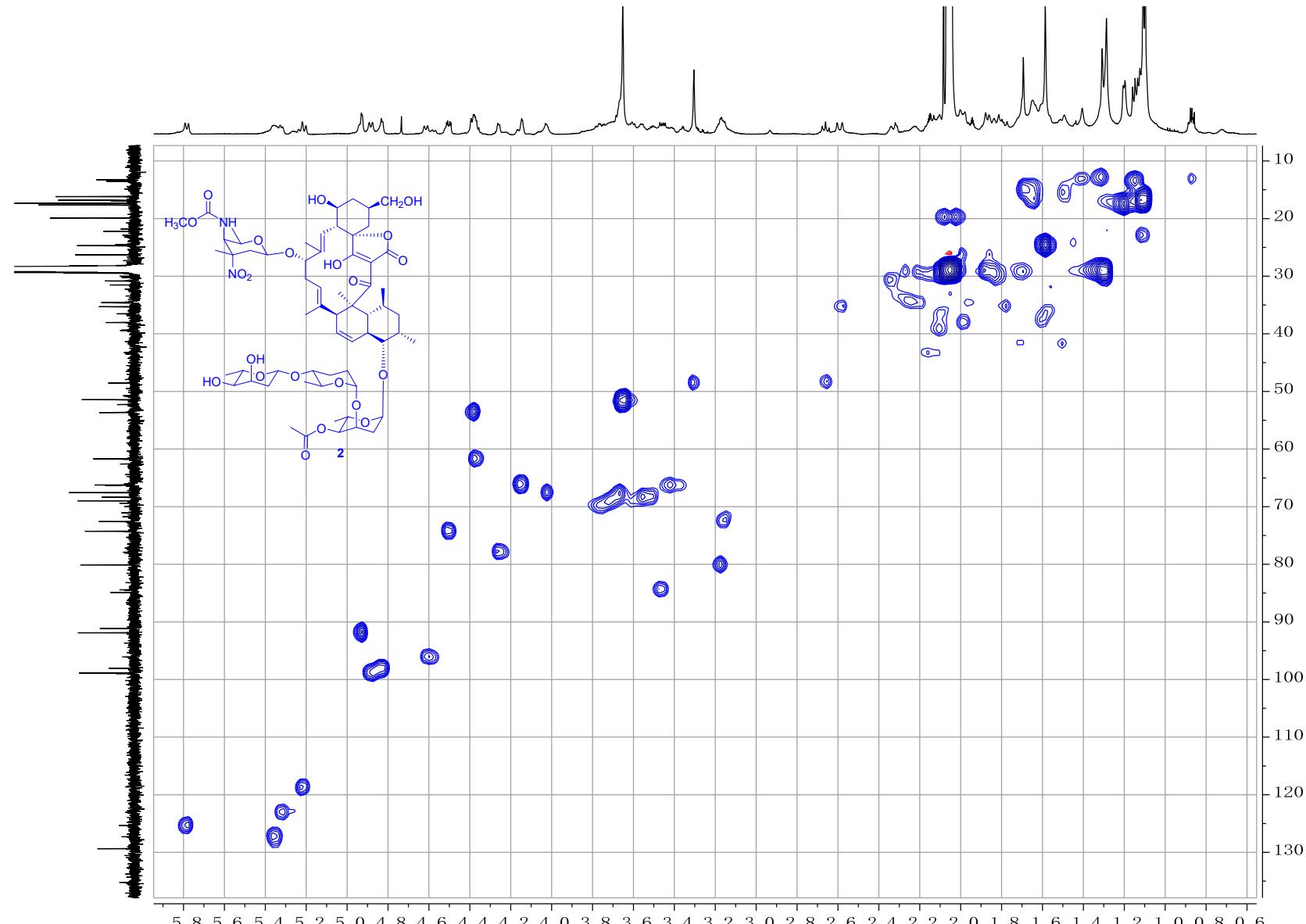


Figure S17. The TOCSY spectrum of compound 2



**Figure S18.** The HSQC spectrum of compound 2

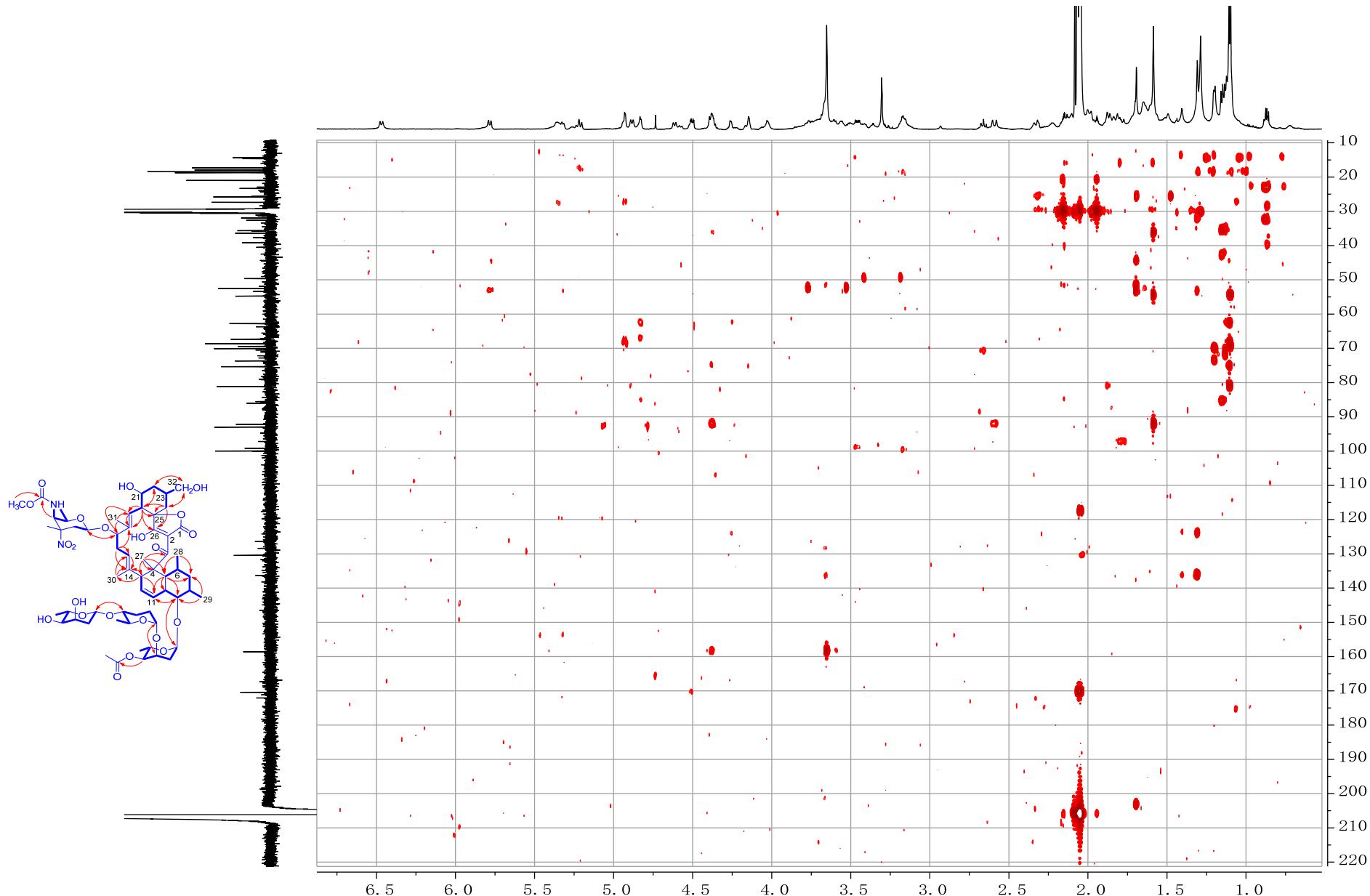
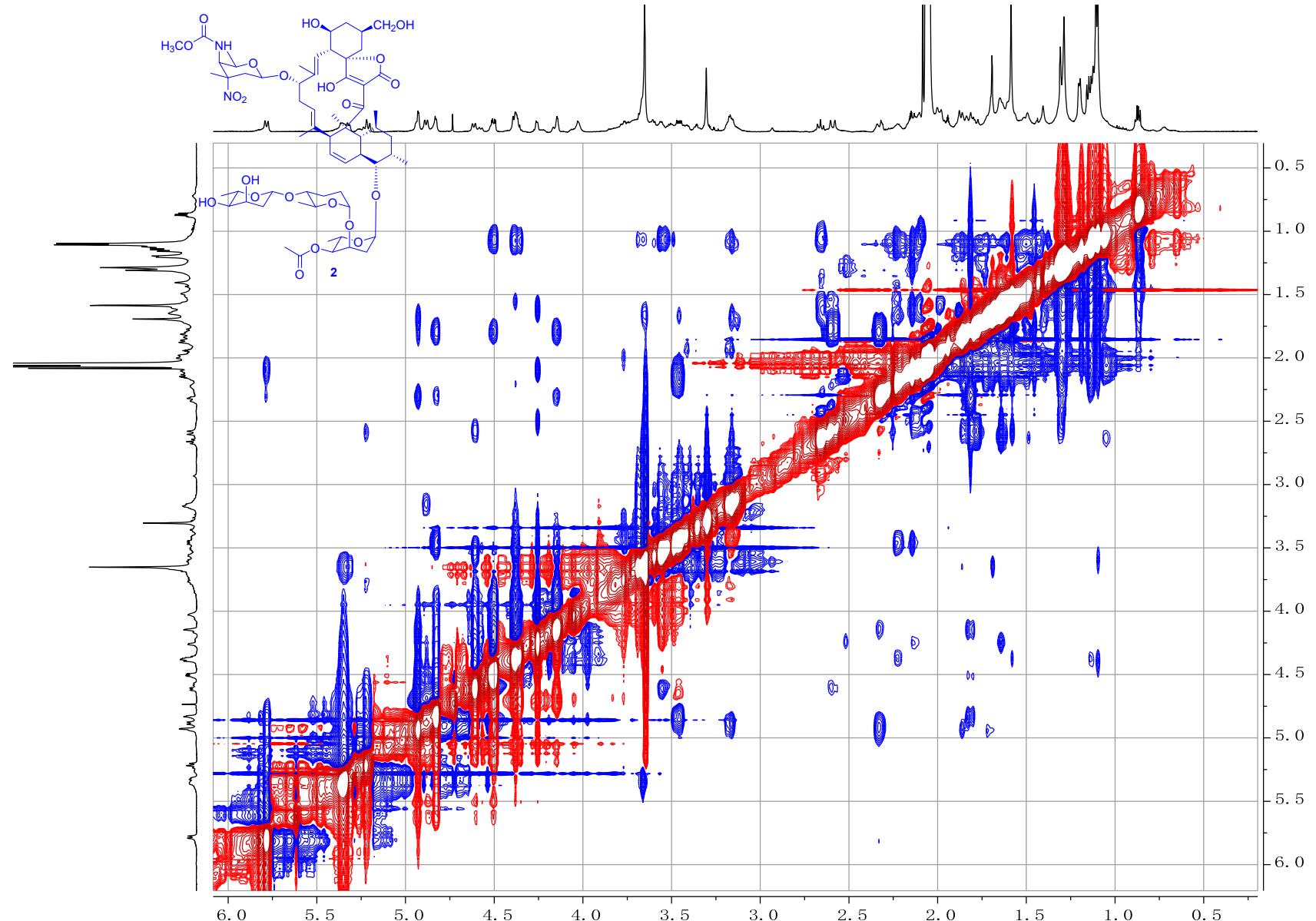
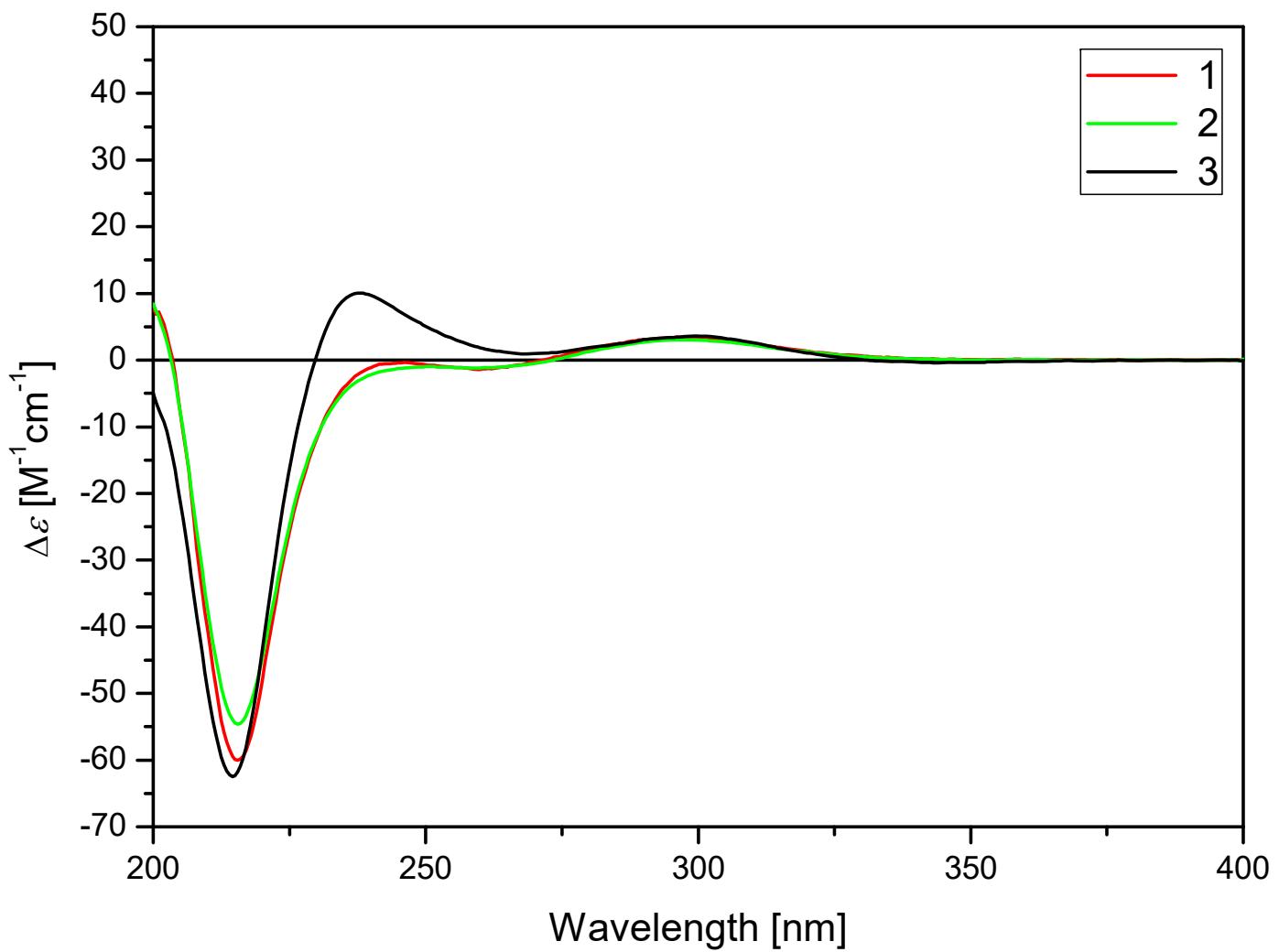


Figure S19. The HMBC spectrum of compound 2



**Figure S20.** The ROESY spectrum of compound 2



**Figure S21.** The CD spectra of compounds **1–3**