Supporting Information

Biosynthesis of antitumor warkmycins reveals spatiotemporal order of the tailoring steps involving multiple glycosylation, hydroxylation, dual *O*-acetylation and carbamoylation

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General Methods and Materials

General experimental procedures.

Strains and plasmids used in this study are listed in **Table S5**. All 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) spectra were acquired using a Bruker Ascend 700 spectrometer (Bruker BioSpin GmbH) in $CDCl_3$ with TMS as internal standard (Sigma-Aldrich Inc.) A MaXis 4G UHR59 TOFMS spectrometer was used to measure HRESIMS spectra. Silica gel (100−200 mesh and 200−300 mesh; Yantai Jiangyou Silica Gel Development) was used for column chromatography. Analytic HPLC was performed by Agilent 1260 HPLC system equipped with a G1311C isocratic pump and an Agilent G1315D diode array detector (DAD), using a reversed-phase column Basic C18 120A (Agilent, 4.6×250 mm, 5 μm). Semipreparative HPLC was performed by HITACHI primaide system equipped with a 1110 isocratic pump and a 1430 DAD detector, using ODS-A column (YMC, 10 mm×250 mm, 5 μm). All primers were synthesized by Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China)

The media and solution formulations used in this article

LB medium: tryptone 10 g/L, NaCl 10 g/L, yeast extract 5 g/L, pH 7.0.

TB medium: containing 180 mL solution A: tryptone 12 g/L, glycerol 4 mL/L, yeast extract 24 g/L, and 20 mL solution B: 0.17 M KH_2PO_4, 0.72 M K_2HPO_4, pH 7.0.

MS agar plates: mannitol 20 g/L, soybean powder 20 g/L, agar 20 g/L, pH 7.2−7.4.

Modified-P3 medium: oat powder 20 g/L, glucose 20 g/L, $FeSO_4 \cdot 7H_2O$ 0.1 g/L, $ZnSO_4 \cdot 7H_2O$ 0.1 g/L, $MnCl_2 \cdot 4H_2O$ 0.1 g/L, 1 mL/L microelements (0.08% $ZnCl_2$, 0.4 % $FeCl_3 \cdot 6H_2O$, 0.02% $CuCl_2 \cdot 6H_2O$, 0.01% $MnCl_2 \cdot 4H_2O$, 0.02%$NaB_4O_7 \cdot 10H_2O$) and 0.2% $CaCO_3$, pH 7.2−7.4.

Storage buffer: 10% glycerol, Tris-HCl (50 mM), NaCl (100 mM), pH 8.0.

Binding buffer: Tris-HCl (50 mM), NaCl (500 mM), imidazole (10 mM) and glycerol 100 mL/L, pH 8.0.

Wash buffer: Tris-HCl (50 mM), NaCl (500 mM) and imidazole (500 mM), pH 8.0.

Bis-Tris buffer: Bis-Tris (50 mM), NaCl (500 mM), pH 7.0.

HPLC analysis.

The metabolites of *in vivo* gene disruption mutant strains and *in vitro* enzyme reaction products analyzed using a reversed-phase column Basic C18 120A (Agilent, 4.6×250 mm, 5 um) with DAD detector using the solvent system (phase A, 15% CH_3CN+0.1% HAc; phase B, 85% CH_3CN+0.1% HAc): 0−20 min 0%−80% phase B; 20−21.5 min 80%−100% phase B; 21.5−27 min 100% phase B; 27−27.1 min 100%−0% phase B; 27.1−30 min 0% phase B at a flow rate of 1 mL/min.

Bioinformatic Analysis

The antiSMASH V7.1.1 software was used to predict the secondary metabolite biosynthesis gene clusters.[1] The TMHMM -2.0 (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) was used to analysis the transmembrane domain of the acetyltransferase War21.

Construction of Gene Mutant Strains.

Common gene inactivation methods: four cosmids: 8-9A, 1-4G, 9-4F, 5-12E, were selected for gene disruption. Functional genes of the *war* cluster from *S. pratensis* SCSIO LCY05 were inactivated individually or together by λ-RED recombination technology,[2] according to REDIRECT protocols and previously published methods of our group.[3, 4]

The in-frame gene deletion methods: the 39 bp genes' homologous arms with 6 bp *Spe*I digest sites located on each end of fragment were elongated from *oriT/aac(3)IV* fragment by PCR. The in-frame deletion of core synthetic genes of Δ*BGC 24* (encoded for anthracimycins) was achieved by digestion with *Spe*I and ligation with T4 ligase to leave 6 bp scar. Then the correct cosmid was introduced into BW/pIJ790. The apramycin resistance gene cassette *oriT/aac(3)IV* introduced from BW/pIJ773 was used to partially replace the *neo* gene of cosmid. The constructed mutant cosmid was transferred into *E. coli* ET12567/pUZ8002 and then into SCSIO LCY05 by conjugation using a method similar to those employed for common gene inactivation as described before. The double exchange mutant strains were then screened using drug resistance screening (apramycin sensitivity) and then verified by PCR.[3] The in-frame deletion of core synthetic genes of Δ*BGC 22* and Δ*BGC 2* was achieved using the same methods as those above.

Cloning, overexpression, and purification of War1 and War9

The War1-encoding gene was PCR amplified from the cosmid 1-4G using primers pET28a-*war1*-F, pET28a-*war1*-R and cloned into the pET28a vector (linearized with *Nde*I/*Eco*RI sites). The reconstruction plasmid pET28a-*war1* was transformed into *E. coli* BL21(DE3) for overexpression of the *war1* gene. The culture and purification methods of War1 were performed as described previously.[5]

The War9-encoding gene was PCR amplified from the cosmid 5-12E using primers pET28a-*war9*-F, pET28a-*war9*-R. The recombinant plasmid pET28a-*war9* was constructed and cultured as described above for War1. The difference is that War9 was induced with addition of $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$(0.5 mM) and 5-aminolevulinic acid (5-ALA, 1 mM) in addition to IPTG (100 μM). The purification methods for War9 were similar those used for War1 production.

Preparation of microsomal fraction of War21.

The War21-encoding gene was PCR amplified from the cosmid 5-12E using primers pGEX-4T1-*war21*-F, pGEX-4T1-*war21*-R and cloned into the pGEX-4T1 vector (linearized with *Bam*H I/*Xho* I sites). The reconstruction plasmid pGEX-4T1-*war21* was transformed into *E. coli* BL21(DE3) for overexpression of the *war21* gene. The preparation of microsomal fraction of War21 was according to previously published methods.[5]

***In vitro* enzymatic reaction of War1 War9 and War21.**

Purified War1 (10 μmol/L) was incubated with ATP (200 μmol/L), $MgCl_2$ (2 mmol/L), carbamyl phosphate disodium salt (1 mmol/L) and substrate (50 μmol/L) in a total volume of 100 μL at 30 ℃ with shaking at 300 rpm for 1 h in Bis-Tris Buffer.

Purified War9 (10 μmol/L) was incubated with NADPH (2 mmol/L), Fdx (10 μmol/L), Fdr (10 μmol/L) and substrate (50 μmol/L) in HEPES buffer (50 mmol/L, pH 7.2) at 30 ℃ with shaking at 300 rpm for 1 h in a total volume of 100 μL.

A total of 100 μL reaction mixture, containing 97.5 μL microsomal fraction of the War21, 2 mmol/L acetyl-CoA and 50 μmol/L substrate was incubated at 30 °C with shaking at 300 rpm for 1 h. The microsomal fraction of empty vector pGEX-4T-1 in *E. coli* BL21(DE3) served as a negative control for these experiments. 100 μL methanol was then added to each reaction and centrifuged to remove the precipitated protein. The supernatant was then used for HPLC analysis.

Antitumor activity assays

Compounds **1**-**16**, **18** and **20** were examined for cell growth inhibitory activity against 12 different human cell lines according to our previously published methods.[6] All experiments were performed three times with cisplatin and adriamycin as positive controls. GraphPad software version 8.0 was used to test the IC_{50} values.

Small-scale fermentation and analyses of WT and mutant strains.

The WT and mutant strains were inoculated to modified-MS agar plates and incubated at 28 °C for 5-7 days. The mycelium was transferred into 250 mL flasks containing 50 mL of modified- adding 2% XAD16N resins, and then incubated on

rotary shakers (28 °C, 200 rpm) for 9 days. The fermentation products were then centrifuged at 3900 rpm for 10 min, the resin and the cell mixture were extracted with ethanol. Extracts were then concentrated under reduced pressure, and then concentrated to 1 mL with methanol, and 50 μL of which were analyzed using HPLC as aforementioned.

Fermentation and isolation.

S. pratensis SCSIO LCY05Δ*A* was inoculated onto MS agar plates and incubated at 28 °C for 5–7 days. The mycelium was then transferred into 250 mL flasks containing 50 mL of modified-P3 medium (2% oat powder, 2% glucose, 0.01% $FeSO_4 \cdot 7H_2O$, 0.01% $ZnSO_4 \cdot 7H_2O$, 0.01% $MnCl_2 \cdot 4H_2O$, 0.01% microelements (0.08% $ZnCl_2$, 0.4 % $FeCl_3 \cdot 6H_2O$, 0.02% $CuCl_2 \cdot 6H_2O$, 0.01% $MnCl_2 \cdot 4H_2O$, 0.02%$NaB_4O_7 \cdot 10H_2O$) and 0.2% $CaCO_3$, pH 7.2−7.4) and then incubated on rotary shakers (28 °C, 200 rpm) for 36 h. After 36 h of growth, each of the seed flasks was respectively transferred to two 1 L flasks that contained 200 mL of modified-P3 medium adding 2% XAD16N resins, and then incubated on rotary shakers (28 °C, 200 rpm) for 9 days.

About 25 L of fermentation culture were filtered through a sieve to separate the XAD16N resins from the fermentation broth. Fermentation broth was then centrifuged at 3900 rpm for 10 min to separate the supernatant and mycelia. The XAD16N resins were extracted using EtOH and the mycelia were extracted using acetone by ultra-sonication for three times until no major compounds could be detected by HPLC. The extracts were combined and 36 g crude material was obtained by vacuum concentration. The crude material was then subjected to a silica gel CC using gradient elution with $CHCl_3$ and MeOH mixtures (100:0, 98:2, 96:4, 94:6, 92:8, 90:10, 80:20, 70:30, 60:40, 50:50) to give ten fractions (Fr. 1−Fr. 10). Fr. 4 was then subjected to a silica gel CC using gradient elution with $CHCl_3$ and MeOH mixtures (100:0, 98:2, 96:4, 94:6, 92:8, 90:10) to give six sub-fractions (Fr. 4.1−Fr. 4.6).

The components of Fr. 4.4 were separated by semi-preparative HPLC (MeCN−H_2O, 32:68) to yield compound **7** (45.1 mg, t_R=17 min), compound **1** (25.6 mg, t_R=24 min) and compound **5** (13.7 mg, t_R=27 min). Fr. 4.5 was separated by semi-preparative HPLC (MeCN−H_2O, 28:72) to yield compound **2** (86.9 mg, t_R = 26 min), compound **6** (3.9 mg, t_R = 35 min). The components of Fr. 4.6 were separated by semi-preparative HPLC (MeCN−H_2O, 33:67) to yield compound **3** (11.3 mg, t_R = 18 min), compound **8** (8.8 mg, t_R = 21 min), and compound **4** (10.6 mg, t_R = 25 min).

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war7* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5–7 days. Fermentation and extraction of LCY05/Δ*ASC*/Δ*war7* was performed in the same method as in the LCY05Δ*A* strain. The crude material was subjected to a silica gel CC using gradient elution with $CHCl_3$ and MeOH mixtures (100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50) to give seven fractions (Fr. 1−Fr. 7). Fr. 2 was subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) to give six fractions (Fr. 2.1−Fr. 2.6). Fr. 2.3, Fr. 2.4 and Fr. 2.5 were separated by semi-preparative HPLC (MeCN−H_2O, 18:82) to yield compound **10** (12.1 mg, t_R = 18 min).

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war8* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5-7 days. Fermentation and extraction of LCY05/Δ*ASC*/Δ*war8* was performed in the same method as in the LCY05Δ*A* strain. The crude material was subjected to a silica gel CC using gradient elution with $CHCl_3$ and MeOH mixtures (100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50) to give seven fractions (Fr. 1−Fr. 7). Fr. 3 was then subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) to give six fractions (Fr. 3.1−Fr. 3.6). Fr. 3.3 and Fr. 3.4 were separated by semi-preparative HPLC (MeCN−H_2O, 15:85) to yield compound **9** (1.8 mg, t_R = 16 min). The components of Fr. 3.5 were separated by semi-preparative HPLC (MeCN−H_2O, 20:80) to yield compound **13** (2.6 mg, t_R = 13 min) and compound **14** (5.1 mg, t_R = 24 min).

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war9* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5-7 days. Fermentation and extraction of LCY05/ΔASC/Δ*war9* was performed in the same way as had been applied to the LCY05Δ*A* strain. The crude material was then subjected to reversed phase liquid chromatography (RPLC) using gradient elution with MeCN and H_2O mixtures (100:0, 2:8, 4:6, 6:4, 7:3, 8:2, 0:100) to give seven fractions (Fr. 1−Fr. 7). Fr. 3 was subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) affording six fractions (Fr. 3.1−Fr. 3.6). The components of Fr. 3.3 were separated by semi-preparative HPLC (MeCN−H_2O, 30:70) to yield compound **3** (8.3 mg, t_R = 15 min) and compound **18** (21 mg, t_R = 18 min). Fr. 3.4 was separated by semi-preparative HPLC (MeOH−H_2O, 20:80) to yield compound **19** (2.3 mg, t_R = 23 min) and compound **20** (7.5 mg, t_R = 28 min)

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war10* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5–7 days. Fermentation and extraction of LCY05/Δ*ASC*/Δ*war10* was performed in the same fashion as had been applied to the LCY05Δ*A* strain. The crude material was subjected to silica gel CC using gradient elution with $CHCl_3$ and MeOH mixtures (100:0, 95:5, 90:10, 80:20, 70:30, 60:40) to give six fractions (Fr. 1−Fr. 6). Fr. 3 was subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) to give six sub-fractions (Fr. 3.1−Fr. 3.6). The components of Fr. 3.4 were separated by semi-preparative HPLC (MeCN−H_2O, 24:76) to yield compound **11** (10.3 mg, t_R = 15 min).

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war11* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5–7 days. Fermentation and extraction of LCY05/Δ*ASC*/Δ*war11* was performed in the same way as had been applied to the LCY05Δ*A* strain. The crude material was subjected to a silica gel CC using gradient elution with $CHCl_3$ and MeOH mixtures (100:0, 98:2, 96:4, 94:6, 92:8, 90:10, 80:20, 70:30, 60:40) to give nine fractions (Fr. 1−Fr. 9). Fr. 6 was subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) to give five sub-fractions (Fr. 6.1−Fr. 6.5). Fr. 6.3 and Fr. 6.4 were then separated by semi-preparative HPLC (MeCN−H_2O, 23:77) to yield compound **12** (10.3 mg, t_R = 18 min).

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war21* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5–7 days. Fermentation and extraction of LCY05/Δ*ASC*/Δ*war21* was performed using methods previously applied to the LCY05Δ*A* strain. The crude material was subjected to reversed phase liquid chromatography (RPLC) using gradient elution with MeCN and H_2O mixtures (100:0, 2:8, 4:6, 6:4, 7:3, 8:2, 0:100) to give seven fractions (Fr. 1−Fr. 7). Fr. 4 was subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) to give 12 sub-fractions (Fr. 4.1−Fr. 4.12). Fr. 4.4 −Fr.4.8 were separated by semi-preparative HPLC (MeCN−H_2O, 28.5:71.5) to yield compound **15** (8.5 mg, t_R = 16 min) and **16** (13.2 mg, t_R = 22 min).

S. pratensis SCSIO LCY05/Δ*ASC*/Δ*war23* was inoculated onto modified-MS agar plates containing 0.1% Apr and 0.1% Tmp and incubated at 28 °C for 5–7 days. Fermentation and extraction of LCY05/Δ*ASC*/Δ*war21* was performed using methods previously applied to the LCY05Δ*A* strain. The crude material was subjected to reversed phase liquid chromatography (RPLC) using gradient elution with MeCN and H_2O mixtures (100:0, 2:8, 4:6, 6:4, 7:3, 8:2, 0:100) to give seven fractions (Fr. 1−Fr. 7). Fr. 5 was subjected to a gel filtration chromatography using gradient elution with $CHCl_3$ and MeOH mixtures (1:1) to give 12 sub-fractions (Fr. 5.1−Fr. 5.12). Fr. 5.4 −Fr.5.6 were separated by semi-preparative HPLC (MeCN−H_2O, 30:70) to yield compound **17** (15.2 mg, t_R = 22 min).

Glycohydrolysis and derivatization of compounds

Accurately weigh compound **10** (1.0 mg) into a reaction vial, add 1 mL of 2 N HCl solution, and hydrolyze at 100 °C in a water bath for 2 h. After completion, evaporate the solvent under reduced pressure. Extract the residue with ethyl acetate (3 × 2 mL). Combine the aqueous phases and concentrate to dryness under reduced pressure. Redissolve the resulting residue in 1 mL of anhydrous pyridine, then sequentially add methyl cysteinate hydrochloride (1.0 mg) and react at 60 °C for 1 h. Subsequently, add *O*-tolyl isothiocyanate (15 μL) and continue the reaction at 60 °C for an additional hour. Concentrate the final reaction mixture under reduced pressure and prepare the sample for HPLC analysis. The same experimental procedure was applied to avermectin for both hydrolysis and derivatization reactions.

NMR Calculations

The conformational search was carried out using Spartan'14 (Wavenfunction, Irvine, CA, USA) using the Monte Carlo algorithm and Merck molecular force field (MMFF) with standard parameters and convergence criteria. Those conformers accounting for over 99% population were optimized with DFT calculations at B3LYP/6-31G(d) level using Gaussian 09 program. Then, frequency analysis of all optimized conformations was undertaken at the same level of theory to ensure they were no imaginary frequencies. NMR shielding constants were calculated with the GIAO method at mPW1PW91-6-311+G(d,p) level with IEFPCM solvent model in methanol solvent. The shielding constants obtained were converted into chemical shifts by referencing to TMS at 0 ppm (Δcal = σTMS – σcal), where the σTMS was the shielding constant of TMS calculated at the same level.

Characterization of new compounds.

Warkmycin C (**3**): yellow powder; $[\alpha]^{20}_{D}$ +14.07 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.63), 278(3.90), 217(4.56) nm; IR (film) ν_{max} 3319, 2947, 2833, 2537, 1655, 1449, 1410, 1111, 1018, 667 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S8**; HRESIMS *m/z* 1034.4219 $[M + H]^+$ (calcd for $C_{50}H_{68}NO_{22}$, 1034.4227).

Warkmycin D (**4**): yellow powder; $[\alpha]^{20}_{D}$ +6.67 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.57), 278(3.89), 217(4.54) nm; IR (film) ν_{max} 3329, 2943, 2833, 2361, 1653, 1454, 1109, 1020, 671 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S8**; HRESIMS *m/z* 1009.4399 $[M + NH_4]^+$ (calcd for $C_{48}H_{69}N_2O_{21}$, 1009.4387).

Warkmycin E (**5**): yellow powder; $[\alpha]^{20}_{D}$ +5.06 (*c* 0.10, MeOH); λ_{max} (log ε) 428(2.82), 279(3.25), 205(4.07) nm; IR (film) ν_{max} 3337, 2945, 2833, 2362, 1653, 1418, 1113, 1020, 667 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S8**; HRESIMS *m/z* 991.4157 $[M + H]^+$ (calcd for $C_{49}H_{67}O_{21}$, 991.4169).

Warkmycin F (**6**): yellow powder; $[\alpha]^{20}_{D}$ +6.29 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.16), 279(3.46), 205(4.29) nm; IR (film) ν_{max} 3327, 2941, 2831, 2343, 1653, 1452, 1416, 1111, 1020, 669 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S9**; HRESIMS *m/z* 1024.4393 $[M + NH_4]^+$ (calcd for $C_{49}H_{70}NO_{22}$, 1024.4384).

Warkmycin G (**7**): yellow powder; $[\alpha]^{20}_{D}$ +6.74 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.59), 279(3.76), 206(4.53) nm; IR (film) ν_{max} 3325, 2945, 2822, 2363, 1647, 1514, 1454, 1112, 1020, 671 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S9**; HRESIMS *m/z* 966.4337 $[M + NH_4]^+$ (calcd for $C_{47}H_{68}NO_{20}$, 966.4329).

Warkmycin H (**8**): yellow powder; $[\alpha]^{20}_{D}$ +14.08 (*c* 0.10, MeOH); λ_{max} (log ε) 429(3.66), 278(3.91), 218(4.61) nm; IR (film) ν_{max} 3314, 2941, 2832, 2360, 1647, 1454, 1109, 1020, 673 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S9**; HRESIMS *m/z* 982.4297 $[M + NH_4]^+$ (calcd for $C_{47}H_{68}NO_{21}$, 982.4278). the molecular formula of **8** was established as $C_{48}H_{64}O_{21}$ by HRESIMS.

Warkmycin I (**9**): yellow powder; $[\alpha]^{20}_{D}$ +4.27 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.22), 278(3.43), 217(4.05) nm; IR (film) ν_{max} 3345, 2926, 2361, 1663, 1454, 1290, 1260, 1108, 1020, 679 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S10**; HRESIMS *m/z* 373.0636 $[M - H]^-$ (calcd for $C_{19}H_{17}O_6$, 373.0929).

Warkmycin J (**10**): yellow powder; $[\alpha]^{20}_{D}$ +13.23 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.17), 278(3.41), 217(4.02) nm; IR (film) ν_{max} 3352, 2938, 2833, 2317, 1643, 1456, 1258, 1198, 1022, 664 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S11**; HRESIMS *m/z* 519.1861$[M + H]^+$ (calcd for $C_{26}H_{31}O_{11}$, 519.1861).

Warkmycin K (**11**): yellow powder; $[\alpha]^{20}_{D}$ +69.04 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.35), 278(3.67), 217(4.30) nm; IR (film) ν_{max} 3358, 2924, 2833, 1645, 1454, 1254, 1096, 1020, 671 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S11**; HRESIMS *m/z* 708.2892 $[M + NH_4]^+$ (calcd for $C_{34}H_{46}NO_{15}$, 708.2862).

Warkmycin L (**12**): yellow powder; $[\alpha]^{20}_{D}$ +29.95 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.89), 278(4.02), 217(4.58) nm; IR (film) ν_{max} 3350, 2934, 2833, 2357, 1636, 1418, 1099, 1020, 662 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S11**; HRESIMS *m/z* 833.3257 $[M - H]^-$ (calcd for $C_{41}H_{53}O_{18}$, 833.3237).

Warkmycin M (**13**): yellow powder; $[\alpha]^{20}_{D}$ +5.97 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.30), 278(3.17), 217(3.77) nm; IR (film) ν_{max} 3319, 2941, 2833, 2359, 1661, 1456, 1256, 1111, 1020, 671 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S10**; HRESIMS *m/z* 391.4399 $[M + H]^+$ (calcd for $C_{19}H_{19}O_9$, 391.4387)

Warkmycin N (**14**): yellow powder; $[\alpha]^{20}_{D}$ +2.07 (*c* 0.10, MeOH); λ_{max} (log ε) 428(2.92), 278(3.05), 217(3.51) nm; IR (film) ν_{max} 3364, 2938, 2361, 1697, 1655, 1454, 1287, 1020, 764, 671 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S10**; HRESIMS *m/z* 391.4399 $[M + H]^+$ (calcd for $C_{19}H_{19}O_9$, 391.4387).

Warkmycin O (**15**): yellow powder; $[\alpha]^{20}_{D}$ +20.23 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.22), 278(4.29, 217(4.45) nm; IR (film) ν_{max} 3360, 2928, 2833, 2322, 1645, 1454, 1260, 1098, 1020, 671 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S12**; HRESIMS *m/z* 923.3911 $[M + H]^+$ (calcd for $C_{45}H_{63}O_{20}$, 923.3907).

Warkmycin P (**16**): yellow powder; $[\alpha]^{20}_{D}$ +19.38 (*c* 0.10, MeOH); λ_{max} (log ε) 428(3.55), 278(4.69), 217(4.66) nm; IR (film) ν_{max} 3312, 2943, 2833, 2302, 1645, 1454, 1260, 1109, 1020, 667 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S12**; HRESIMS *m/z* 966.3931 $[M + H]^+$ (calcd for $C_{46}H_{64}NO_{21}$, 966.3965).

Warkmycin Q (**18**): yellow powder; $[\alpha]^{20}_{D}$ +30.0 (*c* 0.10, MeOH); λ_{max} (log ε) 424(3.33), 275(3.62), 219(4.27) nm; IR (film) ν_{max} 3340, 2938, 2833, 1719, 1636, 1396, 1254, 1020, 675 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S13**; HRESIMS *m/z* 1009.4415 $[M + H]^+$ (calcd for $C_{48}H_{69}N_2O_{21}$, 1009.4387).

Warkmycin R (**19**): yellow powder; $[\alpha]^{20}_{D}$ +16.31 (*c* 0.10, MeOH); λ_{max} (log ε) 427(3.48), 278(3.73), 218(4.41) nm; IR (film) ν_{max} 3336, 2924, 2833, 2367, 1638, 1418, 1098, 1020, 667 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S13**; HRESIMS *m/z* 905.3817 $[M - H]^-$ (calcd for $C_{45}H_{61}O_{19}$, 905.3813).

Warkmycin S (**20**): yellow powder; $[\alpha]^{20}_{D}$ +14.96 (*c* 0.10, MeOH); λ_{max} (log ε) 429(3.05), 279(3.35), 219(3.92) nm; IR (film) ν_{max} 3332, 2943, 2833, 2302, 1645, 1454, 1260, 1109, 1020, 667 cm^{-1}; ^{1}H and ^{13}C NMR data, **Table S13**; HRESIMS *m/z* 993.4464 $[M + NH_4]^+$ (calcd for $C_{48}H_{69}N_2O_{20}$, 993.4464)

Table S1. AntiSMASH-predicted BGCs embedded in *Streptomyces pratensis* SCSIO LCY05.

BGC	From	To	Type	Most similar known cluster	Similarity
Cluster 1	86,525	139,397	NRPS, T1PKS	balhimycin	8%
Cluster 2	193,399	265,976	NRPS-like, lanthipeptide-class-iv, transAT-PKS	cycloheximide	94%
Cluster 3	291,498	397,604	NRPS, T3PKS	cyclofaulknamycin	25%
Cluster 4	430,666	441,166	melanin	melanin	100%
Cluster 5	441,935	453,308	RiPP-like	streptamidine	58%
Cluster 6	483,021	536,390	NRPS, T1PKS	dutomycin	4%
Cluster 7	555,102	600,933	T1PKS	C-1027	16%
Cluster 8	702,617	713,414	RiPP-like	tetronasin	3%
Cluster 9	715,768	765,322	T1PKS, NRPS	SGR PTMs	100%
Cluster 10	806,152	857,206	NRPS, NRPS-like	nucleocidin	47%
Cluster 11	887,657	914,232	terpene	hopene	69%
Cluster 12	1,280,599	1,301,987	terpene	formicamycins A-M	16%
Cluster 13	1,499,889	1,511,274	RiPP-like		
Cluster 14	1,573,964	1,653,393	T2PKS, oligosaccharide, NRPS	warkmycin CS1/ warkmycin CS2	97%
Cluster 15	1,729,662	1,742,514	hydrogen-cyanide	aborycin	21%
Cluster 16	1,849,033	1,881,754	NI-siderophore	kinamycin	19%
Cluster 17	2,288,577	2,309,584	terpene		
Cluster 18	2,689,132	2,711,762	lanthipeptide-class-iii	AmfS	100%
Cluster 19	2,753,144	2,763,566	melanin	melanin	100%
Cluster 20	2,779,885	2,808,770	thiopeptide, LAP	bombyxamycin A/B	11%
Cluster 21	2,811,410	2,866,680	T1PKS, NRPS-like	enduracidin	8%
Cluster 22	3,799,816	3,938,124	ectoine, butyrolactone, arylpolyene, NRPS, T1PKS	skyllamycins	97%
Cluster 23	4,711,250	4,721,660	ectoine	ectoine	75%
Cluster 24	4,967,455	5,054,748	transAT-PKS	anthracimycin	73%
Cluster 25	5,601,391	5,631,169	NI-siderophore	desferrioxamin B	100%
Cluster 26	5,697,002	5,728,692	lanthipeptide-class-iii, lanthipeptide-class-ii	cyclothiazomycin	9%
Cluster 27	6,110,394	6,179,619	lanthipeptide-class-ii, RRE-containing, NRPS	streptozotocin	19%
Cluster 28	6,821,224	6,831,622	ectoine	ectoine	100%
Cluster 29	7,267,787	7,288,863	terpene	steffimycin D	19%
Cluster 30	7,597,301	7,641,554	NRPS	collismycin A	14%
Cluster 31	7,668,605	7,698,792	thiopeptide, LAP	kanamycin	8%
Cluster 32	7,914,456	7,955,574	T3PKS	naringenin	100%
Cluster 33	7,967,895	8,018,316	NRPS	retimycin A	13%
Cluster 34	8,061,900	8,157,778	NRP-metallophore, NRPS	griseobactin	100%
Cluster 35	8,221,867	8,244,080	terpene	geosmin	100%

Cluster 36	8,275,491	8,286,435	butyrolactone	coelimycin P1	8%
Cluster 37	8,324,487	8,414,937	NRPS-like, NRPS	leupeptins	62%

Table S2. The deduced functions of ORFs from *war* gene cluster in SCSIO LCY05.

Gene	Size(aa)	Proposed function	ID/SI	Origin	Protein homolog
orf(-2)	324	sugar ABC transporter permease	99/99	*Streptomyces* sp. CS057	WP_087764562.1
orf(-1)	294	carbohydrate ABC transporter permease	99/99	*Streptomyces* sp. CS057	WP_087764563.1
war1	595	carbamoyltransferase	99/99	*Streptomyces anulatus*	WP_164222014.1
war2	409	methyltransferase domain-containing protein	99/99	*Streptomyces* sp. CS057	WP_087764565.1
war3	258	class I SAM-dependent methyltransferase	100/99	*Streptomyces*	WP_047179338.1
war4	342	NAD-dependent epimerase/dehydratase	99/99	*Streptomyces* sp. MNU77	WP_079190940.1
war5	309	glucose-1-phosphate thymidylyltransferase RfbA	100/100	*Streptomyces*	WP_063780464.1
war6	211	dTDP-4-dehydrorhamnose 3,5-epimerase	99/99	*Streptomyces* sp. CS057	WP_087764567.1
war7	385	C-glycosyltransferase_SaqGT5	100/100	*Streptomyces* sp. CS057	OWA25227.1
war8	377	C-glycosyltransferase_SaqGT5	100/48	*Micromonospora* sp. Tu 6368	ACP19370.1
war9	395	cytochrome P450	100/100	*Streptomyces*	WP_047179334.1
war10	401	glycosyltransferase	100/100	*Streptomyces* sp. CS057	OWA25230.1
war11	414	glycosyltransferase	100/100	*Micromonospora s*p. Tu 6368	ACP19375.1
war12	193	TetR/AcrR family transcriptional regulator	99/99	*Streptomyces*	WP_047179331.1
war13	267	hypothetical protein	99/99	*Streptomyces anulatus*	WP_164222001.1
war14	355	Gfo/Idh/MocA family oxidoreductase	98/99	*Streptomyces* sp. CS057	WP_087764573.1
war15	469	NDP-hexose 2,3-dehydratase family protein	100/99	*Streptomyces anulatus*	WP_164221997.1
war16	251	NAD-dependent epimerase/dehydratase	99/99	*Streptomyces anulatus*	WP_164221995.1
war17	436	lipopolysaccharide biosynthesis protein RfbH	99/99	*Streptomyces*	WP_047179328.1
war18	332	dTDP-glucose 4,6-dehydratase	100/100	*Streptomyces*	WP_047179327.1
war19	349	aldo/keto reductase	100/100	*Streptomyces anulatus*	WP_164221993.1
war20	194	NAD(P)H-dependent oxidoreductase	100/100	*Streptomyces*	WP_047179326.1
war21	397	acyltransferase	100/100	*Streptomyces*	WP_047179325.1

war22	423	MFS transporter	99/99	*Streptomyces anulatus*	WP_164341436.1
war23	253	SDR family oxidoreductase	99/99	*Streptomyces*	WP_047179324.1
war24	503	FAD-dependent monooxygenase	99/99	*Streptomyces* sp. MNU77	WP_047181057.1
war25	315	cyclase	99/99	*Streptomyces*	WP_047179323.1
war26	260	ketoacyl reductase	99/99	*Streptomyces* sp. CS057	OWA25242.1
war27	87	acyl carrier protein	99/99	*Streptomyces*	WP_047179321.1
war28	409	ketosynthase chain-length factor	99/99	*Streptomyces* sp. CS057	OWA25244.1
war29	434	*β*-ACP synthase	99/99	*Streptomyces* sp. CS057	OWA25245.1
war30	109	Polyketide synthesis cyclase	100/72	*Streptomyces*	CAG14964.1
war31	489	FAD-dependent monooxygenase	99/99	*Streptomyces*	WP_087764577.1
war32	266	hypothetical protein	98/92	*Streptomyces* sp. MNU77	WP_143657716.1
war33	207	response regulator transcription factor	100/92	*Streptomyces*	WP_047181055.1
war34	200	NAD(P)H-dependent oxidoreductase	100/100	*Streptomyces*	WP_047179316.1
orf(+1)	530	MFS transporter	100/100	*Streptomyces* sp. CS057	WP_087764579.1

Table S3. Comparison of ORFs in the *war* gene cluster with the literature.

Gene	Size (aa)	Proposed function	Homologues genes in other angucyclines BGCs (identity/similarity% in amino acid sequence)	
			Landomycin *(lan)*	**Saquayamycin *(saq)***
war1	595	carbamoyltransferase		
war2	409	methyltransferase domain-containing protein		
war3	258	class I SAM-dependent methyltransferase		
war4	342	NAD-dependent epimerase/dehydratase	*lanZ3* (38/49)	*saqZ3* (34/48)
war5	309	glucose-1-phosphate thymidylyltransferase RfbA		
war6	211	dTDP-4-dehydrorhamnose 3,5-epimerase	*lanZ1* (38/53)	*saqZ1* (37/55)
war7	385	glycosyltransferase	*lanGT2* (50/69)	*saqGT5* (55/72)
war8	377	glycosyltransferase	*lanGT2* (45/62)	*saqGT5* (49/66)
war9	395	cytochrome P450		
war10	401	glycosyltransferase	*lanGT1* (48/64)	*saqGT3* (52/68)
war11	414	glycosyltransferase	*lanGT4* (30/44)	*saqGT6* (52/67)
war12	193	TetR/AcrR family transcriptional regulator	*lanK* (49/63)	*saqK* (40/55)
war13	267	hypothetical protein		
war14	355	oxidoreductase	*lanT* (56/66)	*saqT* (54/64)
war15	469	NDP-hexose 2,3-dehydratase	*lanS* (68/78)	*saqS* (66/75)
war16	251	NAD-dependent epimerase/dehydratase	*lanR* (64/76)	*saqR* (66/78)
war17	436	lipopolysaccharide biosynthesis protein RfbH	*lanQ* (84/92)	*saqQ* (85/92)
war18	332	dTDP-glucose 4,6-dehydratase	*lanH* (68/78)	*saqH* (67/79)
war19	349	aldo/keto reductase		
war20	194	NAD(P)H-dependent oxidoreductase	*lanO* (64/73)	*saqO* (59/73)
war21	397	acyltransferase		
war22	423	MFS transporter		*saqJ* (43/59)
war23	253	SDR family oxidoreductase	*lanV* (68/81)	*saqN* (64/80)
war24	503	FAD-dependent monooxygenase	*lanE* (48/60)	*saqE* (48/59)
			lanM (54/66)	*saqM* (53/63)
war25	315	cyclase	*lanL* (68/76)	*saqL* (61/74)
war26	260	ketoacyl reductase	*lanD* (78/85)	*saqD* (75/83)
war27	87	acyl carrier protein	*lanC* (54/73)	*saqC* (54/75)
war28	409	ketosynthase chain-length factor	*lanB* (66/80)	*saqB* (68/79)
war29	434	*β*-ACP synthase	*lanA* (73/80)	*saqA (*74/81*)*
war30	109	cyclase	*lanF* (78/86)	*saqF (*66/74)
war31	489	FAD-dependent monooxygenase	*lanE* (66/76)	*saqE* (71/79)
war32	266	hypothetical protein		*saqP* (39/52)
war33	207	response regulator transcription factor		
war34	200	NAD(P)H-dependent oxidoreductase	*lanO* (58/72)	*saqO* (60/74)
war35	530	MFS transporter	*lanJ* (48/65)	*saqJ1* (48/66)

Table S4. Analysis of gene annotation related to deoxysugars biosynthesis of warkmycin.

Gene	proposed function	Homologues genes in other BGCs and the glycogroup species they encode (identity/similarity% in amino acid sequence)				
		Avermectin (*ave*)	Oleandomycin (*ole*)	Urdamycin (*udr*)	Mithramycin (*mtm*)	
		L-oleandrose	L-oleandrose	D-olivose	D-olivomycose D-olivose	D-amicetose
war2	3-*C* methyltransferase				*mtmC* (31/46)	
war3	3-*O* methyltransferase	*aveBVII* (58/68)				
war4	NDP-4-keto-6-deoxyhexose_reductase	*aveBIV* (31/45)	*oleU* (28/38)	*urdZ3* (34/45)		
war5	glucose-1-phosphate_thymidyltransferase	*aveBIII* (62/77)	*oleS* (32/50)	*urdG* (32/52)	*mtmD* (37/57)	*oleS* (32/50)
war6	dTDP-4-dehydrorhamnose 3,5-epimerase	*aveBV* (50/62)	*oleL* (43/57)	*urdZ1* (38/53)		
war14	NDP-D-glucose_3-ketoreductase		*oleW* (45/60)	*urdT* (43/53)	*mtmU* (46/58)	*oleW* (45/60)
war15	NDP-hexose 2,3-dehydratase	*aveBVI* (58/68)	*oleV* (47/62)	*urdS* (67/77)	*mtmV* (52/64)	*oleV* (47/62)
war16	NDP-hexose_4-ketoreductase			*urdR* (64/75)		*urdR* (64/75)
war17	NDP-hexose_3,4-dehydratase			*urdQ* (84/91)		*urdQ* (84/91)
war18	dTDP-glucose 4,6-dehydratase	*aveBII* (58/69)	*oleE* (59/70)	*urdH* (68/80)	*mtmE* (61/70)	*oleE* (59/70)
war19	dTDP-4-keto-6-deoxyhexose_2,3-reductase	*aveBVIII* (32/43)			*mtmW* (31/48)	

Table S5. Primers used in this study.

Primers	Sequence (5'-3')
For gene disruption	
*war*1-Del-F	CTGGAACCAGCCGACGACCTTCTTGGCGGACAGCAGGTCATTCCGGGGATCCGTCGACC
*war*1-Del-R	GGCCGCAGCGCACTGGTGGAGGACTTCACCCGGTCCACCTGTAGGCTGGAGCTGCTTC
*war*2-Del-F	CTGTCGCTCATGTACGGCGAGGGGTACGGATACCGCTCCATTCCGGGGATCCGTCGACC
*war*2-Del-R	GAACTGGAGGATGACGTTGCCCTTGGTGGAGGCCCCGTATGTAGGCTGGAGCTGCTTC
*war*7-Del-F	GTGTTCATGGCCGCCAACCACGAGGTAATGCCTGCGATCATTCCGGGGATCCGTCGACC

*war*7-Del-R	GTGGTGGACGAGCAGGTCGCAGGTGGGCGCGACCACGTCTGTAGGCTGGAGCTGCTTC
*war*8-Del-F	CTCAAGGCGACCCGGCGCTCCGGCGAACTTGAGCACGTCATTCCGGGGATCCGTCGACC
*war*8-Del-R	CCTGTCCTCGGTCGCCTCGTCGGGCAGCAGGCTGATCCCTGTAGGCTGGAGCTGCTTC
*war*9-Del-F	TCCTCCGATCTGAGCGTCCTCGCACCCGAGGGCAAGCGCATTCCGGGGATCCGTCGACC
*war*9-Del-R	CATGCAGAAGTGCGAGCCTCGGCCGAACGCCAGGTGGGCTGTAGGCTGGAGCTGCTTC
*war*10-Del-F	GTCGCGTTCGGCGAACCGTTCGACACCGAACAGCTGGTGATTCCGGGGATCCGTCGACC
*war*10-Del-R	GTACGAGGGGTCGTCCAGCAGCAGGGACAGGGACGCGCGTGTAGGCTGGAGCTGCTTC
*war*11-Del-F	ATGCCGGTACCGCTGCTCGAAGCGATGGACATGACCGTCATTCCGGGGATCCGTCGACC
*war*11-Del-R	CCGGTCGGCGATCACTTCCTGGTCGGATCCGGCATGGATTGTAGGCTGGAGCTGCTTC
*war*13-Del-F	TACCTGCTGGGCGGCAGCCACCACTTCGAGGCGGACCGCATTCCGGGGATCCGTCGACC
*war*13-Del-R	CGGGTCGAGGAGGGTGAAGTCGCCGGTGAGGCGGCGTACTGTAGGCTGGAGCTGCTTC
*war*19-Del-F	TTCATCGACACCGCGAACGTCTACGGCGGGGGACGCTCGATTCCGGGGATCCGTCGACC
*war*19-Del-R	GAACTTGCCGTGGCGCATCAGCTCCCGGGGGAAGCCGAGTGTAGGCTGGAGCTGCTTC
*war*20-Del-F	ACCGAACGCTTCGGTCACACCGTCGCCGCCTGGTTCGCCATTCCGGGGATCCGTCGACC
*war*20-Del-R	CTGGTCCAGCAGGACCTTGGCGGCGGCTCCGCAGCTCTCTGTAGGCTGGAGCTGCTTC
*war*21-Del-F	GTCGCAGCCGACTACTACCGCTGGTTCGGCAACGCCGGCATTCCGGGGATCCGTCGACC
*war*21-Del-R	CATGACGGGGCGTTCGACGATCGCGTACAGGGCCCACGCTGTAGGCTGGAGCTGCTTC
*war*23-Del-F	GAGGAAGCCGCCCTGAGCACGGTCGCCGACATCGAGGCGATTCCGGGGATCCGTCGACC
*war*23-Del-R	GTCGGAGGCGAGGAACGCCACCACGTCACCGACGTCCGCTGTAGGCTGGAGCTGCTTC
*war*31-Del-F	CACTTCGGGGGCCGGCCGGTCGACTTCGGGGTACTCGAAATTCCGGGGATCCGTCGACC
*war*31-Del-R	GTGCAGCAGTTCGGTGGTGCTGGTCTTGCCGTCGGCCCGTGTAGGCTGGAGCTGCTTC
*war*34-Del-F	CACGCCGACTTCGACGTGGAGGTCATCGACGCGGCGCAGATTCCGGGGATCCGTCGACC
*war*34-Del-R	ACGCAGCGTCGTCCCCCACCACGCGAGCTGATCCAGCATTGTAGGCTGGAGCTGCTTC
*war*26-29-Del-F	GCTCGTGAGGTTGGTGGCGATGACGTCGTCCCACAGCTCactagtATTCCGGGGATCCGTCGACC

For confirmation

*war*1-VF	ATCTTCTCGGTGTACTCCGGG
*war*1-VR	CGACGAGAAGTACGCGGAC
*war*2-VF	GCGCTGACCGGGGTCTTC
*war*2-VR	TAGATCCAGGGCAGCACCAG
*war*7-VF	ATGCGCGTACTCTTCGTCAC
*war*7-VR	TCCGACAGCACCTGACGG

*war*8-VF	TGAGGCTGCTGTTCGTCACG
*war*8-VR	TTCACGCCAGGGAGGTCAC
*war*9-VF	AGGACCAGTTCTGGATGCGC
*war*9-VR	GCTGTTCCACACCCATCACG
*war*10-VF	CCGATCCGAGCCATCTGC
*war*10-VR	TTCCAGTTCGGGGACGACC
*war*11-VF	ATGCGCGTTCTGTTCACGG
*war*11-VR	GACTCCATCTCGTCCCTCAGG
*war*19-VF	ATGAAGTACGACCTCCTCGGC
*war*19-VR	TCACAGGAGGGTGGCGAC
*war*20-VF	GACTCGCATGTCGCAGCAG
*war*20-VR	GCTCACGCCGGTCACTC
*war*21-VF	AGACTTCCCTCGCTCACCGG
*war*21-VR	AGTCCTTGTCGGGTGTGTGG
*war*23-VF	ACGGCCCATCGCAAGAAC
*war*23-VR	TCAGCCGAGGAGTGTGCC
*war*24-VF	TCGTGCTGGAACGACGCAC
*war*24-VR	GACGAGGACTCCGCGACC
*war*31-VF	CTGTCATTGTCGTGGGCGC
*war*31-VR	AGATCCAGGCCACGTATCCG
*war*34-VF	GCGCCTGGTCGTTCTCATC
*war*34-VR	GGTGGAGTCCTACACTCCCG
*war*26-29-VF	CATGCACAGCACGTTGATCG
*war*26-29-VR	TCAGAAGTTGCCCAGGCC
For plasmids construction	
pET28a-War1-F	TGGTGCCGCGCGGCAGCCATATGATCGTTCTTGGATACAACGGC
pET28a-War1-R	TGTCGACGGAGCTCGAATTCTCAGTTCTTGGAGACCACGAAG
pET28a-War9-F	CGCCATATGATGACCGCGACCGGCACG
pET28a-War9-R	CCCAAGCTTTCAGGTCAGCAGGTGCAGCTG
pGEX-4T1-War21-F	ATCTGGTTCCGCGTGGATCCATGGCGCCATCGAAC

pGEX-4T1-War21-R TCACGATGCGGCCGCTCGAGTCAGTGGGCGGCGG

Table S6. Cytotoxic activity of warkmycin congeners against various human cancer cell lines (IC_{50}, μM) [a]

Compounds	Human cell line/ IC_{50} (μM) of compound											
	HUVEC	MCF-7	Hela	MDA-MB-231	HGC-27	HCT-116	HepG2	A549	Panc-1	5-8F	A375	U87MG
Adriamycin	0.21	0.61	0.21	0.47	0.29	0.27	0.69	0.28	0.53	0.48	0.20	0.38
Cisplatin	1.59	4.50	1.17	9.74	7.30	4.25	3.63	1.12	2.42	1.51	1.34	2.40
1	1.48	0.48	1.46	1.38	1.74	0.55	1.57	2.36	2.23	2.39	2.45	4.93
2	1.71	0.81	2.64	3.69	3.35	0.93	3.28	1.99	2.67	3.85	3.72	9.20
3	3.23	1.36	3.69	3.51	4.91	1.86	4.71	3.43	4.27	4.29	4.95	17.86
4	3.84	1.76	5.84	4.72	8.28	2.27	7.26	3.73	6.88	4.97	8.00	23.47
5	3.14	0.99	3.16	3.13	4.45	1.00	5.10	2.08	3.50	4.09	5.27	15.95
6	2.83	1.28	3.20	4.02	2.75	0.84	3.19	2.94	3.91	5.19	4.81	8.75
7	1.87	0.58	2.75	3.28	3.87	1.08	2.96	1.68	2.53	5.20	3.27	7.28
8	2.73	1.30	2.99	2.37	4.63	1.51	4.18	2.21	1.11	4.95	5.58	10.80
9	3.24	0.91	2.97	4.22	4.02	1.44	2.73	5.36	3.21	3.50	5.80	9.32
10	3.14	1.60	3.68	6.04	6.71	1.88	6.94	5.48	3.36	7.61	6.53	11.36
11	14.88	16.34	33.85	>50	32.27	12.28	36.30	35.15	25.49	>50	>50	>50
12	23.05	18.27	>50	>50	28.05	16.59	32.15	30.25	36.44	>50	>50	>50
13	7.85	4.49	8.93	12.05	18.89	6.55	6.58	13.09	24.30	6.14	14.70	30.26
14	8.75	5.06	12.86	11.01	16.12	7.10	8.80	12.26	12.47	6.76	16.60	31.70
15	15.20	18.02	27.24	22.89	23.57	23.53	27.22	33.36	13.20	37.25	33.25	>50
16	7.13	6.01	14.64	15.86	11.26	6.31	16.31	14.16	8.25	19.24	13.17	24.83
18	0.92	0.34	1.03	0.97	0.86	0.46	1.45	1.00	1.18	1.49	0.90	2.25
20	1.58	0.49	1.30	1.73	1.48	0.84	2.46	2.31	1.88	1.80	2.36	4.40

[a] HUVEC, Human umbilical vein epithelial cells; MCF7, breast cancer cell line, HeLa, cervical cell line; MDA-MB-231, triple negative breast cancer cell line; HGC-27, gastric cancer line; HCT-116, colorectal cancer cell lines; HEPG2, hepatocellular cancer line; A549, lung cancer cell line; Panc-1, pancreatic cancer cell line; 5-8F, nasopharynx cancer cell line; A375, melanoma cell line and U87MG, spongioblastoma. Cisplatin and adriamycin were used as positive control agents, and all the experiments were carried out in triplicate.

Table S7. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin (**1**) and 4-*O*-deacetyl warkmycin (**2**) (δ in ppm, J in Hz, $CDCl_3$)

position	warkmycin (**1**)		4-*O*-deacetylation warkmycin (**2**)	
	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
1	79.4, CH	4.33, d (5.8)	80.5, CH	4.31, d (5.8)
2	124.1, CH	5.98, d (5.8)	121.2, CH	5.83, d (5. 8)
3	134.0, C		137.9, C	
4	68.2, CH	5.33, d	73.8, CH	5.81, s
4a	74.1, C		73.7, C	
5	74.2, CH	5.81, d (6.3)	74, CH	5.83, d (6.7)
6	68.2, CH	5.01, m	68.3, CH	5.04, d (6.7)
6a	140.1, C		140.3, C	
7	188.4, C		188.5, C	
7a	114.0, C		114.1, C	
8	157.8, C		157.9, C	
9	139.3, C		139.4, C	
10	133.1, CH	7.87, d (7.9)	133.0, CH	7.9, d (7.8)
11	119.8, CH	7.64, d (7.9)	119.7, CH	7.65, d (7.8)
11a	130.1, C		130.1, C	
12	187.1, C		187.3, C	
12a	145.9, C		146.4, C	
12b	77.3, C		79.6, C	
Me-3	21.0, CH	1.78, s	21.6, CH_3	1.95, s
COMe-4	171.2, C			
COMe-4	20.9, CH_3	2.3, s		
COMe-5	171.0, C		170.7, C	
COMe-5	20.9, CH_3	2.16, s	20.9, CH_3	2.23, s
OH-8		12.44		12.50
Sugar A(oleandrose)				
1'	99.3, CH	4.70, d (4.4)	99.3, CH	4.71, d (4.2)
2'	30.0, CH_2	1.41, overlap	30.3, CH_2	1.42, m
		1.93, m		1.98, m
3'	76.2, CH	3.41, m	76.2, CH	3.41, m
4'	65.7, CH	3.58, m	65.9, CH	3.59, m
5'	61.9, CH	4.33, m	61.9, CH	4.38, m
6'	16.4, CH_3	1.22, d (6.7)	16.4, CH_3	1.26, d (6.6)
OMe-3'	57.3, CH_3	3.3, s	57.3, CH_3	3.3, s
$CONH_2$-4'	155.9, C		155.9, C	
Sugar B(olivose)				
1''	71.2, CH	4.85, d (11.4)	71.2, CH	4.88, d (11.2)
2''	37.8, CH_2	1.49, m	37.8, CH_2	1.53, m
		2.45, m		2.49, m
3''	83.4, CH	3.70, m	83.4, CH	3.73, m

4''	75.5, CH	3.18, m	75.5, CH	3.21, m
5''	76.4, CH	3.47, m	76.4, CH	3.51, m
6''	18.3, CH	1.43, d (6.1)	18.4, CH_3	1.46, d (6.2)
Sugar C				
(olivomycose)				
1'''	99.6, CH	4.64, dd (1.9, 10.1)	99.7, CH	4.67, dd (2.0, 10.2)
2'''	44.3, CH	1.75, m	44.3, CH_2	1.78, m
		2.01, m		2.04, m
3'''	69.8, C		69.8, C	
4'''	89.7, CH	3.13, d (9.6)	89.7, CH	3.17, d (9.6)
5'''	70.7, CH	3.51, m	70.8, CH	3.54, m
6'''	18.1, CH_3	1.32, d (6.1)	18.2, CH_3	1.35, d (6.0)
Me-3'''	22.3, CH_3	1.26, s	22.4, CH_3	1.28, s
Sugar D(amicetose)				
1''''	103.2, CH	4.46, dd (2.0, 9.6)	103.3, CH	4.5, d (2.2, 9.6)
2''''	30.2, CH_2	1.70, m	30.1, CH_2	1.74, m
		1.92, m		1.98, m
3''''	27.8, CH_2	1.51, m	27.9, CH	1.53, m
		2.22, m		2.25, m
4''''	72.8, CH	4.36, m	72.9, CH	4.38, m
5''''	74.0, CH	3.55, m	70.3, CH	3.67, m
6''''	17.7, CH_3	1.25, d (6.1)	17.8, CH_3	1.28, d (6.6)

Table S8. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin C-E (**3**-**5**) (δ in ppm, J in Hz, $CDCl_3$)

position	warkmycin C (**3**)		warkmycin D (**4**)		warkmycin E (**5**)	
	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
1	79.2, CH	4.31, d (5.7)	79.4, CH	4.31, d (4.5)	80.4, CH	4.24, d (5.4)
2	124.3, CH	5.93, d (5.7)	124.1, CH	5.96, d (4.5)	121.4, CH	5.74, dd (5.4)
3	133.3, C		134.0, C		136.8, C	
4	67.9, CH	5.58, s	68.2, CH	5.33, d (4.0)	68.8, CH	5.60, s
4a	74.0, C		74.1, C		72.0, C	
5	68.0, CH	5.67, dd (8.5, 8.7)	74.2, CH	5.81, d (6.6)	67.4, CH	5.62, dd (8.8, 9.6)
			68.2, CH	5.00, d (6.6)		
6	27.5, CH_2	2.80, dd (8.5, 19.6)	27.8, CH_2	2.79, dd (8.8, 19.7)	27.7, CH_2	2.79, dd (8.8, 19.7)
		3.19, dd (8.7, 19.6)		3.22, dd (9.6, 19.7)		3.22, dd (9.6, 19.7)
6a	140.7, C		140.2, C		140.9, C	
7	188.8, C		188.4, C		188.8, C	
7a	113.5, C		114.0, C		113.6, C	
8	157.8, C		157.8, C		157.8, C	
9	138.7, C		139.3, C		138.7, C	
10	133.1, CH	7.87, d (7.4)	133.1, CH	7.87, d (7.8)	133.2, CH	7.87, d (7.7)
11	119.9, CH	7.64, d (7.4)	119.8, CH	7.63, d (7.8)	119.8, CH	7.62, d (7.7)
11a	130.4, C		130.2, C		130.4, C	
12	186.5, C		187.1, C		186.5, C	
12a	145.6, C		145.8, C		146.1, C	
12b	77.5, C		77.2, C		79.8, C	
Me-3	21.1, CH_3	1.77, s	21.0, CH_3	1.76, s	21.6, CH	1.92, s
COMe-4	171.0, C		171.2, C		170.0, C	
COMe-4	21.0, CH	2.24, s	20.9, CH_3	2.15, s	21.0, CH	2.14, s
COMe-5	170.4, C		171.1, C			
COMe-5	21.0, CH_3	2.18, s	20.9, CH_3	2.29, s		
OH-8		12.39		12.43		12.41
Sugar A						
(oleandrose)						
1'	99.0, CH	4.61, d (4.9)	99.3, CH	4.68, d (4.3)	99.0, CH	4.60, d (4.8)
2'	28.5, CH_2	1.69, m	28.6, CH_2	1.26, m	30.6, CH_2	1.49, m
		1.95, m		1.68, m		1.94, m
3'	75.9, CH	3.26, m	76.3, CH	3.37, m	75.9, CH	3.27, m
4'	70.7, CH	3.50, m	65.7, CH	3.56, m	68.2, CH	3.45, m
5'	62.6, CH	4.31, m	62.0, CH	4.32, m	62.6, CH	4.31, m
6'	16.3, CH_3	1.15, d (6.2)	16.4, CH_3	1.20, d (6.8)	16.2, CH	1.17, d (6.7)
OMe-3'	56.9, CH_3	3.23, s	57.3, CH_3	3.27, s	56.9, CH	3.23, s
$CONH_2$-4'	155.9, C				156.1, C	
Sugar B						
(olivose)						
1''	71.2, CH	4.83, d (11.4)	71.2, CH	4.84, d (11.3)	71.3, CH	4.83, d (11.4)

2''	37.8, CH_2	1.52, m	37.8, CH_2	1.51, m	37.8, CH_2	1.50, m
		2.49, m		2.49, m		2.49, m
3''	83.6, CH	3.69, m	83.3, CH	3.7, m	83.6, CH	3.71, m
4''	75.5, CH	3.19, m	75.5, CH	3.18, m	75.5, CH	3.18, m
5''	76.4, CH	3.47, m	76.4, CH	3.47, m	76.4, CH	3.47, m
6''	18.3, CH_3	1.43, d (6.1)	18.4, CH_3	1.42, d (6.1)	18.1, CH	1.26, d (6.4)
Sugar C						
(olivomycose)						
1'''	99.7, CH	4.62, dd (2.2, 10.5)	99.6, CH	4.62, m	99.7, CH	4.63, dd (2.0, 10.1)
2'''	44.3, CH_2	1.74, m	44.3, CH_2	1.76, m	44.3, CH	1.75, m
		2.00, m		2.00, m		2.00, m
3'''	69.9, C		69.8, C		69.7, C	
4'''	89.7, CH	3.14, d (9.6)	89.4, CH	3.12, m	89.7, CH	3.14, d (9.6)
5'''	68.4, CH	3.47, m	70.8, CH	3.49, m	70.7, CH	3.49, m
6'''	17.7, CH_3	1.25, d (6.0)	18.2, CH_3	1.31, d (6.8)	18.3, CH	1.31, d (6.1)
Me-3'''	22.3, CH_3	1.24, s	22.4, CH_3	1.25, s	22.3, CH	1.24, s
Sugar D						
(amicetose)						
1''''	103.2, CH	4.47, d (9.6)	103.2, CH	4.44, d (9.5)	103.2, CH	4.47, d (9.6)
2''''	30.2, CH_2	1.94, m	30.2, CH_2	1.68, m	31.1, CH	1.64, m
		1.69, m		1.94, m		2.08, m
3''''	27.8, CH_2	1.50, m	27.8, CH_2	1.50, m	28.5, CH	1.28, m
		2.24, m		2.24, m		1.69, m
4''''	72.8, CH	4.37, m	72.8, CH	4.36, m	72.8, CH	4.36, m
5''''	74.0, CH_2	3.54, m	74, CH	3.55, m	74.0, CH	3.53, m
6''''	18.1, CH_3	1.32, d (6.0)	17.8, CH_3	1.3, d (6.8)	17.7, CH_3	1.43, d (6.1)

Table S9. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin F-H (**6-8**) (δ in ppm, J in Hz, $CDCl_3$)

position	warkmycin F (**6**)		warkmycin G (**7**)		warkmycin H (**8**)	
	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
1	79.4, CH	4.31, d (4.5)	79.4, CH	4.31, d (4.5)	80.5, CH	4.31, d (5.8)
2	124.1, CH	5.96, d (4.5)	124.1, CH	5.96, d (4.5)	121.2, CH	5.84, d (5.8)
3	134.0, C		134.0, C		137.9, C	
4	68.2, CH	5.33, s	68.2, CH	5.33, s	70.3, CH	3.57, s
4a	74.1, C		74.1, C		73.7, C	
5	74.2, CH	5.81, d (6.6)	74.2, CH	5.81, d (6.6)	73.9, CH	5.82, d (6.8)
6	68.2, CH	5.00, d (6.6)	68.2, CH	5.00, d (6.6)	68.4, CH	5.04, d (6.8)
6a	140.2, C		140.2, C		140.3, C	
7	188.4, C		188.4, C		188.5, C	
7a	114.0, C		114.0, C		114.1, C	
8	157.8, C		157.8, C		157.9, C	
9	139.3, C		139.3, C		139.4, C	
10	133.1, CH	7.87, d (7.8)	133.1, CH	7.87, d (7.8)	133.0, CH	7.90, d (7.8)
11	119.8, CH	7.63, d (7.8)	119.8, CH	7.63, d (7.8)	119.7, CH	7.65, d (7.8)
11a	130.2, C		130.2, C		130.1, C	
12	187.1, C		187.1, C		187.3, C	
12a	145.8, C		145.8, C		146.3, C	
12b	77.2, C		77.2, C		79.6, C	
Me-3	21.0, CH_3	1.76, s	21.0, CH_3	1.76, s	21.6, CH	1.95, s
COMe-4	171.2, C		171.2, C			
COMe-4	20.9, CH_3	2.15, s	20.9, CH_3	2.15, s		
COMe-5	171.1, C		171.1, C		170.7, C	
COMe-5	20.9, CH_3	2.29, s	20.9, CH_3	2.29, s	20.9, CH	2.24, s
OH-8		12.43		12.43		12.52
Sugar A (oleandrose)						
1'	99.3, CH	4.68, d (4.3)	99.3, CH	4.68, d (4.3)	99.3, CH	4.71, d (4.5)
2'	30.6, CH_2	1.62, m 1.92, m	30.6, CH_2	1.67, m 1.94, m	30.6, CH	1.68, m 1.95, m
3'	76.3, CH	3.37, m	76.3, CH	3.37, m	76.2, CH	3.42, m
4'	65.7, CH	3.56, m	65.7, CH	3.56, m	65.7, CH	3.59, m
5'	62.0, CH	4.32, m	62.0, CH	4.32, m	61.9, CH	4.39, m
6'	16.4, CH_3	1.20, d (6.8)	16.4, CH_3	1.20, d (6.8)	16.4, CH	1.26, d (6.7)
OMe-3'	57.3, CH_3	3.27, s	57.3, CH_3	3.27, s	57.3, CH_3	3.30, s
Sugar B (olivose)						
1''	71.2, CH	4.84, d (11.3)	71.2, CH	4.84, d (11.3)	71.0, CH	4.89, d (11.5)
2''	37.8, CH	1.48, m 2.45, m	37.8, CH	1.48, m 2.45, m	37.9, CH	1.53, m 2.49, m

3''	83.3, CH	3.7, m	83.3, CH	3.7, m	83.5, CH	3.73, m
4''	75.5, CH	3.18, m	75.5, CH	3.18, m	75.5, CH	3.22, m
5''	76.4, CH	3.47, m	76.4, CH	3.47, m	65.9, CH	3.59, m
6''	18.4, CH_3	1.42, d (6.1)	18.4, CH_3	1.42, d (6.1)	18.2, CH	1.35, d (6.1)
Sugar C						
(olivomycose)						
1'''	99.6, CH	4.62, m	99.6, CH	4.62, m	99.7, CH	4.66, dd (2.0, 10.1)
2'''	44.3, CH_2	1.76, m 2.00, m	44.3, CH_2	1.76, m 2.00, m	44.4, CH	1.78, m 2.04, m
3'''	69.8, C		69.8, C		69.7, C	
4'''	89.4, CH	3.12, m	89.4, CH	3.12, m	89.5, CH	3.17, d (9.6)
5'''	70.8, CH	3.49, m	70.8, CH	3.49, m	70.8, CH	3.34, m
6'''	18.2, CH_3	1.31, d (6.8)	18.2, CH_3	1.31, d (6.8)	18.4, CH	1.33, d (6.1)
Me-3'''	22.4, CH_3	1.25, s	22.4, CH_3	1.25, s	22.4, CH_3	1.29, s
Sugar D						
(amicetose)						
1''''	103.2, CH	4.44, d (9.5)	103.2, CH	4.44, d (9.5)	103.2, CH	4.48, dd (2.1, 9.6)
2''''	31.8, CH_2	1.48, m 2.07, m	31.2, CH_2	1.50, m 2.10, m	31.1, CH	1.53, m 2.14, m
3''''	30.0, CH_2	1.38, m 1.92, m	30.0, CH_2	1.40, m 1.90, m	30.1, CH	1.43, m 1.95, m
4''''	74, CH	3.55, m	74, CH	3.55, m	76.3, CH	3.41, m
5''''	76.3, CH	3.49, m	76.4, CH	3.49, m	76.4, CH	3.51, m
6''''	17.8, CH_3	1.3, d (6.8)	17.8, CH_3	1.3, d (6.8)	17.8, CH_3	1.46, d (6.2)

Table S10. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin I (**9**), warkmycin M (**13**) and warkmycin N (**14**). (δ in ppm, J in Hz, CD_3OD)

position	warkmycin I (**9**)		warkmycin M (**13**)		warkmycin N (**14**)	
	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)
1	72.0, CH	4.46, d (5.6)	74.3, CH	4.79, d (7.6)	68.0, CH	3.90, d (6.5)
2	120.0, CH	5.47, d (5.6)	120.7, CH	5.43, d (7.6)	120.4, CH	5.88, d (6.5)
3	134.7, C		134.1, C		135, C	
4	34.5, CH_2	2.64, d (17.2) 2.25, d (17.2)	35.4, CH_2	2.77, d (17.3) 2.10, d (17.3)	37.4, CH_2	2.72, d (15.1) 2.60, d (15.1)
4a	74.9, C		75.1, C		77.0, C	
5	71.1, CH	4.18, d (7.1)	76.4, CH	3.98, d (7.5)	64.3, CH	4.25, d (10.6)
6	70.1, CH	4.86, overlap (7.1)	64.8, CH	3.75, d (7.5)	68.6, CH	3.53, d (10.6)
6a	143.0, C		65.3, C		72.2, C	
7	190.0, C		197.0, C		200.0, C	
7a	114.9, C		114.0, C		115.2, C	
8	161.1, C		160.8, C		162.1, C	
9	123.8, CH	7.27, t (8.5)	123.8, CH	7.31, d (8.4)	124.8, CH	7.40, d (8.4)
10	136.1, CH	7.67, t (7.3)	136.5, CH	7.70, dd (8.4,7.5)	142.5, CH	7.76, dd (8.4,7.5)
11	118.5, CH	7.62, t (7.5)	119.2, CH	7.63, d (7.5)	118.2, CH	7.60, d (7.5)
11a	132.8, C		132.7, C		136.5, C	
12	186.2, C		189.5, C		192.6, C	
12a	145.1, C		66.8, C		72.6, C	
12b	77.7, C		73.4, C		72.0, C	
Me-3	22.0, CH_3	1.76, s	21.8, CH_3	1.74, s	23.2, CH_3	1.90, s

Table S11. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin J-L (**10-12**). (δ in ppm, J in Hz, $CDCl_3$)

position	warkmycin J (**10**)		warkmycin K (**11**)		warkmycin L (**12**)	
	δ_{C}, type	δ_{H}, mult. (J in Hz)	δ_{C}, type	δ_{H}, mult. (J in Hz)	δ_{C}, type	δ_{H}, mult. (J in Hz)
1	81.2, CH	4.23, d (7.6)	80.8, CH	4.22, d (4.5)	80.8, CH	4.25, d (4.5)
2	119.0, CH	5.61, d (7.6)	119.0, CH	5.64, d (4.5)	119.3, CH	5.68, d (4.5)
3	136.4, C		136.1, C		136.4, C	
4	35.6, CH_2	2.83, d (17.4) 2.27, d (17.4)	36.1, CH	2.09, d (17.8) 2.29, d (17.8)	36.2, CH_2	2.12, d (18.4) 2.30, d (18.4)
4a	75.1, C		74.2, C		74.2, C	
5	73.6, CH	4.18, d (6.2)	74.3, CH	5.86, d (6.7)	74.5, CH	5.87, d (6.7)
6	71.3, CH	4.86, d (6.2)	68.2, CH	4.96, d (6.7)	68.2, CH	4.99, d (6.7)
6a	141.2, C		140.1, C		140.7, C	
7	188.8, C		188.4, C		188.8, C	
7a	114.8, C		114.0, C		114.1, C	
8	161.6, C		157.8, C		157.8, C	
9	125.5, CH	7.33, d (9.5)	139.3, C		139.2, C	
10	136.4, CH	7.64, dd (10.6,9.5)	132.9, CH	7.85, d (7.8)	133.0, CH	7.89, d (7.8)
11	119.5, CH	7.63, d (10.6)	119.8, CH	7.63, d (7.8)	119.7, CH	7.65, d (7.8)
11a	131.7, C		130.1, C		130.1, C	
12	187.5, C		187.1, C		187.4, C	
12a	145.9, C		145.9, C		146.2, C	
12b	77.4, C		77.6, C		77.6, C	
Me-3	23.3, CH_3	1.82, s	23.3, CH_3	1.72, s	23.4, CH	1.75, s
COMe-5			170.7, C		170.8, C	
COMe-5			20.9, CH_3	2.24, s	21.0, CH_3	2.27, s
Sugar A (oleandrose)						
1'	99.3, CH	4.59, d (4.5)	99.1, CH	4.65, d (4.4)	99.3, CH	4.67, d (4.5)
2'	29.5, CH_2	2.04, m 1.68, m	30.2, CH_2	1.32, m 1.86, m	30.4, CH_2	1.89, m 1.39, m
3'	76.6, CH	3.34, m	76.2, CH	3.36, m	76.3, CH	3.39, m
4'	66.4, CH	3.50, m	65.9, CH	3.56, m	65.9, CH	3.59, m
5'	62.0, CH	4.22, m	62.0, CH	4.37, m	61.9, CH	4.40, m
6'	16.3, CH_3	1.21, d (6.7)	16.4, CH_3	1.25, d (6.7)	16.4, CH	1.24, d (6.7)
OMe-3'	57.7, CH_3	3.30, s	57.3, CH_3	3.26, s	57.3, CH	3.30, s
Sugar B (olivose)						
1''			71.3, CH	4.90, m	71.2, CH	4.87, d (11.5)

2''	39.4, CH_2	1.43, m 2.49, m	37.8, CH_2	1.49, m 2.49, m
3''	72.9, CH	3.85, m	83.4, CH	3.73, m
4''	77.8, CH	3.18, m	75.6, CH	3.19, m
5''	76.0, CH	3.50, m	76.6, CH	3.51, m
6''	18.1, CH_3	1.41, d (6.2)	18.3, CH	1.39, d (6.2)
Sugar C (olivomycose)				
1'''			99.5, CH	4.69, m
2'''			45.3, CH_2	1.78, m 2.02, m
3'''			71.8, C	
4'''			78.9, CH	3.29, m
5'''			71.4, CH	3.50, m
6'''			18.4, CH_3	1.45, d (6.0)
Me-3'''			20.4, CH_3	1.33, s

Table S12. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin O-P (**15-16**) (δ in ppm, J in Hz, [a] CD_3OD, [b] $CDCl_3$)

position	warkmycin O (**15**)[a]		warkmycin P (**16**)[b]	
	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)
1	81.7, CH	4.43, d (4.6)	80.7, CH	4.26, d (4.7)
2	123.1, CH	5.72, d (4.6)	121.4, CH	5.76, d (4.7)
3	138.7, C		137.9, C	
4	70.5, CH	4.18, s	69.9, CH	4.19, s
4a	76.1, C		74.6, C	
5	74.3, CH	4.11, d (6.5)	73.8, CH	4.11, d (5.9)
6	71.9, CH	4.81, d (6.5)	71.2, CH	4.83, d (5.9)
6a	146.6, C		140.5, C	
7	190.8, C		188.9, C	
7a	115.5, C		114.1, C	
8	158.5, C		157.9, C	
9	139.3, C		139.2, C	
10	134.3, CH	7.86, d (7.9)	133.0, CH	7.87, d (7.9)
11	120.0, CH	7.62, d (7.9)	119.7, CH	7.62, d (7.9)
11a	132.3, C		130.2, C	
12	187.3, C		187.3, C	
12a	144.6, C		146.3, C	
12b	79.6, C		79.1, C	
Me-3	21.8, CH_3	1.95, s	21.6, CH_3	2.01, s
Sugar A (oleandrose)				
1'	100.9, CH	4.58, d (4.3)	99.6, CH	4.63, d (4.3)
2'	31.8, CH_2	1.50, m 1.98, m	29.6, CH_2	1.40, m 1.86, m
3'	78.4, CH	3.27, m	76.4, CH	3.37, m
4'	67.3, CH	3.43, m	66.2, CH	3.50, m
5'	63.6, CH	4.10, m	62.1, CH	4.19, m
6'	16.9, CH_3	1.17, d (6.8)	16.3, CH_3	1.21, d (6.5)
OMe-3'	57.7, CH_3	3.30, s	57.7, CH_3	3.36, s
$CONH_2$-4'			156.2, C	
Sugar B(olivose)				
1''	72.4, CH	4.90, m	71.2, CH	4.85, m
2''	38.5, CH	1.40, m 2.49, m	37.9, CH	1.49, m 2.47, m
3''	82.2, CH	3.83, m	83.4, CH	3.72, m
4''	76.8, CH	3.11, m	75.5, CH	3.19, m
5''	77.7, CH	3.38, m	76.4, CH	3.37, m
6''	18.3, CH_3	1.30, d (6.7)	18.4, CH_3	1.44, d (6.4)

Sugar C				
(olivomycose)				
1'''	99.2, CH	4.77, m	99.6, CH	4.63, m
2'''	45.6, CH_2	1.67, m 1.92, m	44.3, CH_2	1.83, m 2.03, m
3'''	71.5, C		69.7, C	
4'''	90.5, CH	3.17, m	89.7, CH	3.17, m
5'''	71.8, CH	3.53, m	70.7, CH	3.53, m
6'''	18.4, CH_3	1.28, d (6.6)	18.2, CH_3	1.32, d (6.6)
Me-3'''	22.6, CH_3	1.23, s	22.4, CH_3	0.90, s
Sugar D				
(amicetose)				
1''''	104.5, CH	4.55, m	103.2, CH	4.49, m
2''''	31.9, CH_2	1.50, m 2.00, m	30.3, CH_2	1.52, m 1.96, m
3''''	30.8, CH_2	1.31, m 1.86, m	27.8, CH_2	1.51, m 2.20, m
4''''	72.0, CH	4.81, m	72.9, CH	4.37, m
5''''	77.6, CH	3.47, m	74.0, CH	3.57, m
6''''	18.7, CH_3	1.26, d (6.7)	17.7, CH_3	1.26, d (6.7)

Table S13. Summary of ^{1}H (700 MHz) and ^{13}C NMR (176 MHz) Data for warkmycin Q (**18**), warkmycin R (**19**) and warkmycin S (**20**). (δ in ppm, J in Hz, CD_3OD)

position	warkmycin Q (**18**)		warkmycin R (**19**)		warkmycin S (**20**)	
	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)	$\delta_{C'}$ type	$\delta_{H'}$ mult. (J in Hz)
1	80.6, CH	4.33, d (4.6)	81.6, CH	4.30, d (4.6)	81.6, CH	4.27, d (4.6)
2	119.4, CH	5.60, d (4.6)	119.9, CH	5.57, d (4.6)	119.9, CH	5.55, d (4.6)
3	135.1, C		136.3, C		134.7, C	
4	34.8, CH_2	1.95, d (17.3) 2.34, d (17.3)	34.8, CH_2	2.61, d (17.3) 2.27, d (17.3)	35.0, CH	2.20, d (17.3) 2.37, d (17.3)
4a	74.2, C		75.5, C		72.7, C	
5	74.8, CH	5.82, d (6.8)	75.8, CH	5.82, d (6.8)	69.0, CH	5.62, t (6.8，8.9)
6	68.1, CH	4.88, d (6.8)	70.8, CH	4.76, d (6.8)	27.2, CH	3.13, dd (18.2, 8.9) 2.64, dd (18.2, 6.8)
6a	143.7, C		144.0, C		144.0, C	
7	188.9, C		189.9, C		190.1, C	
7a	117.1, C		114.6, C		114.4, C	
8	158.5, C		157.6, C		157.8, C	
9	137.8, C		138.2, C		138.2, C	
10	132.8, CH	7.84, d (7.6)	132.8, CH	7.84, d (7.6)	133.2, CH	7.84, d (7.8)
11	118.7, CH	7.62, d (7.6)	119.0, CH	7.62, d (7.6)	119.8, CH	7.63, d (7.8)
11a	131.0, C		131.6, C		131.9, C	
12	185.8, C		186.7, C		186.3, C	
12a	144.5, C		145.6, C		145.3, C	
12b	77.0, C		77.5, C		78.0, C	
Me-3	21.2, CH_3	1.67, s	22.6, CH_3	1.78, s	21.6, CH_3	21.2, s
COMe-5	170.8, C				171.8, C	
COMe-5	19.5, CH_3	2.20, s			20.2, CH	2.13, s
Sugar A						
(oleandrose)						
1'	99.0, CH	4.59, d (4.3)	99.7, CH	4.58, d (4.3)	99.9, CH	4.50, d (4.3)
2'	29.2, CH_2	1.29, m 1.87, m	30.8, CH_2	1.81, m 1.83, m	28.4, CH_2	1.64, m 2.13, m
3'	76.8, CH	3.28, m	76.7, CH	3.28, m	76.8, CH	3.18, m
4'	65.7, CH	3.49, m	66.4, CH	3.43, m	68.2, CH	3.34, m
5'	62.0, CH	4.27, m	62.5, CH	4.13, m	63.2, CH	4.21, m
6'	16.8, CH_3	1.17, d (6.2)	15.8, CH_3	1.15, d (6.3)	16.0, CH_3	1.12, d (6.3)
OMe-3'	56.0, CH_3	3.22, s	56.7, CH_3	3.30, s	56.3, CH_3	3.20, s
$CONH_2$-4'	157.7, C				157.7, C	
Sugar B						
(olivose)						
1''	71.0, CH	4.84, m	70.9, CH	4.90, m	72.6, CH	4.86, m
2''	37.1, CH	1.39, m 2.46, m	37.6, CH	1.41, m 2.49, m	37.8, CH	1.42, m 2.47, m

3''	80.9, CH	3.83, m	81.3, CH	3.83, m	81.4, CH	3.83, m
5''	75.4, CH	3.10, m	75.4, CH	3.10, m	76.1, CH	3.11, m
4''	76.2, CH	3.45, m	76.7, CH	3.45, m	76.9, CH	3.45, m
6''	18.6, CH_3	1.38, d (6.1)	17.8, CH_3	1.36, d (6.6)	17.7, CH_3	1.37, d (6.7)
Sugar C (olivomycose)						
1'''	97.8, CH	4.77, m	98.3, CH	4.76, m	98.4, CH	4.76, m
2'''	44.3, CH_2	1.67, m 1.95, m	44.6, CH_2	1.67, m 1.94, m	44.9, CH_2	1.67, m 1.94, m
3'''	70.1, C		70.5, C		70.8, C	
4'''	89.1, CH	3.17, m	89.4, CH	3.16, m	89.7, CH	3.16, m
5'''	70.4, CH	3.53, m	70.3, CH	3.53, m	71.7, CH	3.54, m
6'''	18.4, CH_3	1.30, d (6.1)	17.5, CH_3	1.30, d (6.5)	18.0, CH_3	1.37, d (6.6)
Me-3'''	22.7, CH_3	1.23, s	21.6, CH_3	1.23, s	21.9, CH_3	1.26, s
Sugar D (amicetose)						
1''''	103.0, CH	4.61, m	103.6, CH	4.55, m	103.7, CH	4.60, m
2''''	29.8, CH_2	1.63, m 1.95, m	30.9, CH_2	1.50, m 1.93, m	30.5, CH_2	1.58, m 1.97, m
3''''	27.4, CH_2	1.57, m 2.12, m	29.5, CH_2	1.58, m 2.01, m	28.1, CH_2	1.21, m 2.13, m
4''''	72.4, CH	4.22, m	73.1, CH	4.16, m	73.0, CH	4.24, m
5''''	73.8, CH	3.61, m	76.7, CH	3.45, m	74.5, CH	3.62, m
6''''	18.1, CH_3	1.21, d (6.6)	17.3, CH_3	1.24, d (6.6)	17.4, CH_3	1.21, d (6.7)

Table S14. Conformational analysis of the B3LYP/6-31G(d) optimized conformers of (6a*R*,12a*S*)-**13** and (6a*S*,12a*R*)-**14**

Conformers	E (Hartree) [a]	Population [b]
(6a*R*,12a*S*)-**13-a**	-1411.8954169	97.69%
(6a*R*,12a*S*)-**13-b**	-1411.8902096	0.39%
(6a*R*,12a*S*)-**13-c**	-1411.8917056	1.92%
(6a*S*,12a*R*)-**14-a**	-1411.8954169	26.18%
(6a*S*,12a*R*)-**14-b**	-1411.8902096	1.69%
(6a*S*,12a*R*)-**14-c**	-1411.8917056	72.12%

[a] Electronic energy obtained at B3LYP/6-31G(d) level of theory; [b] The Boltzmann distribution of each conformer. (T=298.15 K)

Table S15. Experimental chemical shifts of **d1** and **d2**, calculated ^{13}C-NMR chemical shifts of (6a*R*,12a*S*)-**13** and (6a*S*,12a*R*)-**14**, and related parameters.

No.					Dd(**13-14**)	Dd(**d1-d2**)	Dd(**d2-d1**)	|X| [a]	|Y| [b]	$MAE_{\|X\|}$ [c]	$MAE_{\|Y\|}$ [d]
	Cal. **13**	Cal. **14**	Exp. **d1**	Exp. **d2**							
1	74.3	68.1	74.6	73	6.2	1.6	-1.6	4.6	7.8		
2	120.7	120.4	131.2	129.1	0.3	2.1	-2.1	1.8	2.4		
3	134.1	135	138.5	140.9	-0.9	-2.4	2.4	1.5	3.3		
4	35.3	37.4	42.3	41.7	-2.1	0.6	-0.6	2.7	1.5		
4a	75.1	77	81	78.3	-1.9	2.7	-2.7	4.6	0.8		
5	76.6	64.3	68.7	72	12.3	-3.3	3.3	15.6	9		
6	64.8	68.5	67.4	63.4	-3.7	4	-4	7.7	0.3		
6a	65.3	72.2	70.3	72.6	-6.9	-2.3	2.3	4.6	9.2		
7	197	200	208.5	207	-3	1.5	-1.5	4.5	1.5		
7a	114	115.2	118.7	122	-1.2	-3.3	3.3	2.1	4.5	4.17	4.01
8	160.8	162.1	171.1	165.7	-1.3	5.4	-5.4	6.7	4.1		
9	123.8	124.8	132.4	130.6	-1	1.8	-1.8	2.8	0.8		
10	136.5	142.5	147.1	147.2	-6	-0.1	0.1	5.9	6.1		
11	119.2	118.2	127.1	127	1	0.1	-0.1	0.9	1.1		
11a	132.7	136.5	145.5	145.4	-3.8	0.1	-0.1	3.9	3.7		
12	189.5	192.6	196.8	202.3	-3.1	-5.5	5.5	2.4	8.6		
12a	66.8	72.6	76.5	76.2	-5.8	0.3	-0.3	6.1	5.5		
12b	73.4	71.9	80	78	1.5	2	-2	0.5	3.5		
3-Me	21.8	23.2	25.2	26.2	-1.4	-1	1	0.4	2.4		

[a] $X=\Delta\Delta\delta_{Calcd\_(D1-D2)}-\Delta\delta_{Exptl\_(d1-d2)}$; [b] $Y=\Delta\Delta\delta_{Calcd\_(D1-D2)}-\Delta\delta_{Exptl\_(d2-d1)}$; [cd] the mean absolute error (MAE) defined as $\Sigma n\ |\Delta cal - \Delta exp|/n$, and the value $MAE_{|X|} > MAE_{|Y|}$, which means that the calculated ^{13}C NMR data for compound **13** is more compatible with experimental data of **d2** (compound **14** is more compatible with experimental data of **d1**).

Scheme S1. The protein sequence of War1.

MIVLGYNGFSRVAELFGRLYGHTPESVDRHNLIGHDAAACLFVDGRLVAAVEEERLNRQKKTSAFPALAIRWC
LDQAGLDFDDVDRFAFGWNHDEKYADAVVTGVAGSPEPAEARLRTLTTFGELYLGAIGRSALVEDFTRSTGYA
LPDAKLVTVPHHHAHLACGRLFSGAADAAFLVNDGTAERHSAIMGEVRNGKVEVFDRFTIDAGHSIAQLFGAIT
RYLGFVPNNDEYKVMGLAGFGVAPEKNPLLGEVVTLEEGGRYSLRLAHDRRGPRAYDGLLDELFGGDDATRH
TFDHRVRLACAAQQMIETVTAHQLRALAGATDLRDLIFEGGLALNCVNNTKLLEETPFERMEVSFGASDPGVSI
GAAAHVAFEQAEPVTAEMAPYTGPAYGADEIRKVLRRHEPALVWEEIPTAEVARRTTDLLSAKKVVGWFQGR
TEFGPRALGNRSILADPSHADMKDVINNRVKHREPFRPFAPIVLERDAAKVFELGKKDRSPYMTFVFPVRPEYTE
KIAAATHVDATSRIQTVTEEGNPLLAELLTEFTARTGVPCLVNTSFNVAGEPIVCSPEDAVACFLGTDIDHLVIGD
FVVSKN

Scheme S2. The protein sequence of War9.

MTATGTPWTFHQDQFWMRGEEPPGRVVHDEAKGLWNIYGWDESLQALGDPETFSSDLSVLAPEGKRQIFPGN
LTTMDPPDHTKLRKIVSGVFTRGMVAALEPRIKELTLELLAGTEPGSSFDLVEELAHPLPVIVIAELLGVPSSDRH
LFREWVSKLLANNQSFSTGEDTPELRAQRALTFEQIENLSGYLREHVESRRVTPRDDLLGRLVEAEVDGQRLST
AEVVNFAFVLLVAGHITTTMLLGNTILCLDAHPQAMKTVREDRERIPSAIEESLRLFSPLASLRRVTTRATRIGDA
EIAAMQVVMVWTAVASRDTRQFGDPHTFDIDRRANAHLAFGRGSHFCMGAPLARLEGRLALDILFDRYPGLRR
DPDRDPVFMPGANVMGVEQLHLLT

Scheme S3. The protein sequence of War21.

MAPSNATSRLPSLTGMRFLAALMVFSVHGAAAGVFKDQGVAADYYRWFGNAGAVGVSFFFMLSGFVLTWSV
RPADTVRGFWRRRLLKIFPNHLVTFVVAIVLLTVTSTAVAFPETLANLFLVHAWVPDSGYVETANTVSWSLSVE
LLFYLSFPLLIKGVSRIAPAVLWYAAGGIVLVIMLMPLIAQTLLPDTPGFMFMQISWTQIWFVYVFPVARLLEFVL
GMLLARIVLSGRWIGLGVLPPALLTVVAYVGAVQIDHNPLYNYVAITVVPLALLIPAAAAADARGRESLLSKRP
MVWLGEISFAFYCVHYLILIYGHRLFGSDPNIFGKPSGPAWSTPGGLLFLAAALVVSVLAAWALYAIVERPVMR
RWGRPPGPRHTEQAAVRPERAAEAPAAH

Scheme S4. The ^{1}H NMR and ^{13}C NMR spectrum of rabelomycin (**17**).

The HRESI-MS data of compound **17** showed $[M-H]^{+}$=337.0716, the molecular formula of **3** was established as $C_{19}H_{14}O_6$, ^{1}H NMR ($CDCl_3$, 700 MHz,) δ12.28 (s, 1H), 11.64 (s, 1H), 7.69 (dd, *J* = 7.8, 7.4 Hz, 1H), 7.64 (dd, *J* = 7.4 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 6.99 (s, 1H), 3.49 (s, 1H), 3.08 (s, 2H), 3.01 (d, *J* = 15.1 Hz, 1H), 2.97 (d, *J* = 15.1 Hz, 1H), 1.49 (s, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ196.1, 192.5, 183.2, 163.1, 162.0, 150.7, 138.0, 137.8, 135.4, 128.9, 124.0, 122.0, 120.0, 116.8, 115.0, 72.1, 53.8, 44.2, 30.0. The structure of compound **17** was elucidated by comparing the data with previous literature, they were identified as rabelomycin (**17**).

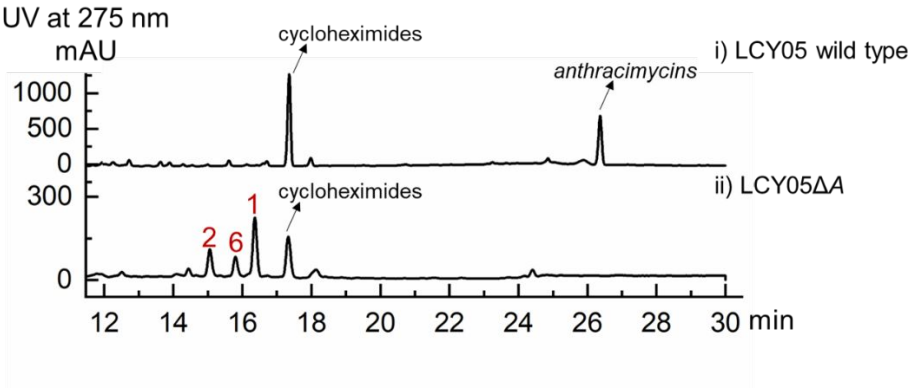


Figure S1. HPLC analyses of SCSIO LCY05 wild-type strain and SCSIO LCY05Δ*A* mutant strain.

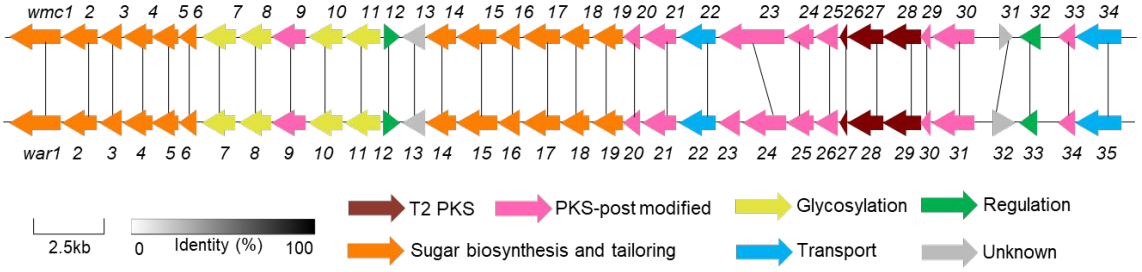


Figure S2. Genetic organization and comparison of the warkmycin biosynthetic gene cluster from *Streptomyces* sp. CS057 (*wmc*, upper) and the *S. pratensis* SCSIO LCY05 strain reported in this study (*war*, lower) using clinker.

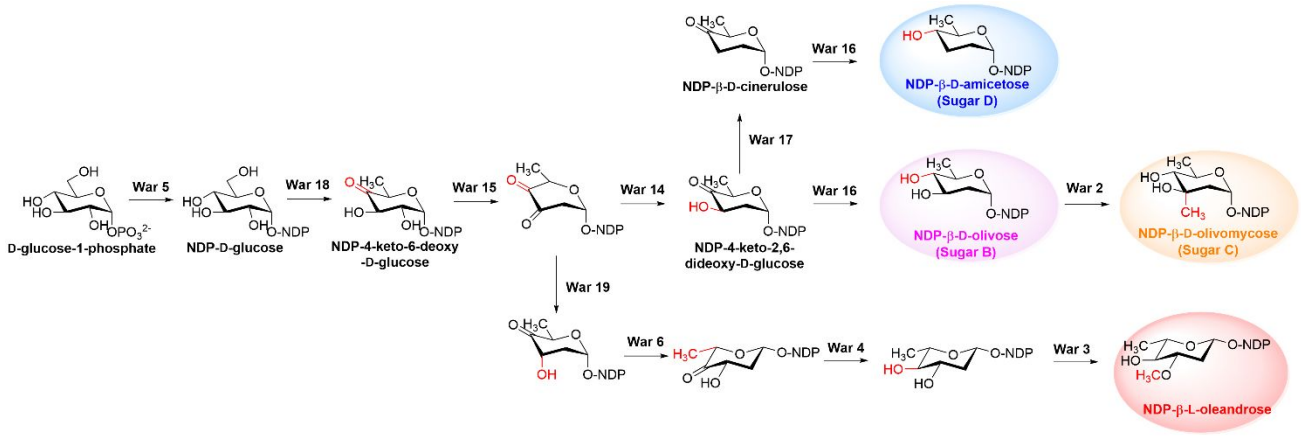


Figure S3. Proposed biosynthetic pathway to sugars A−D.

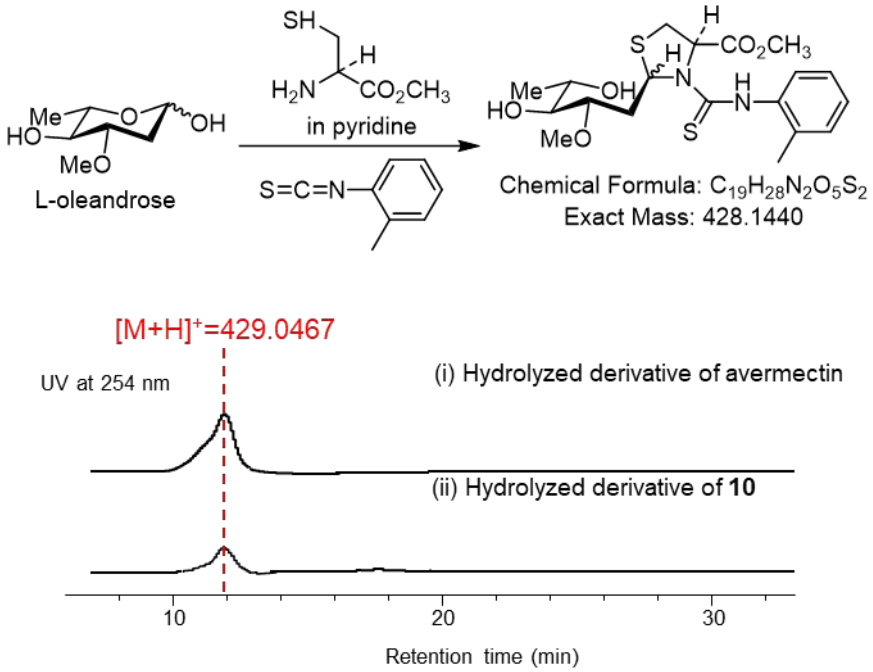


Figure S4. HPLC comparison of sugar hydrolysis and derivatization products of avermectin and compound **10**[7-8]

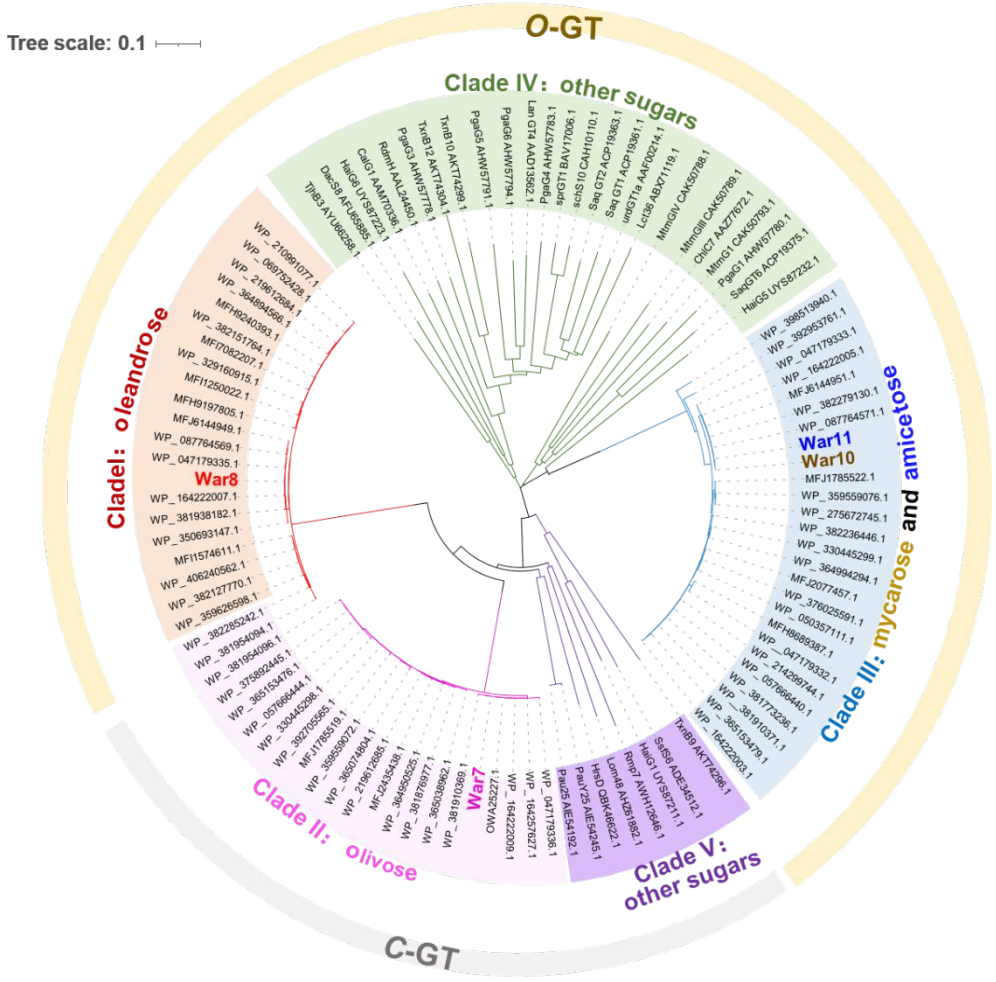


Figure S5. Phylogenetic analysis of glycosyltransferase.

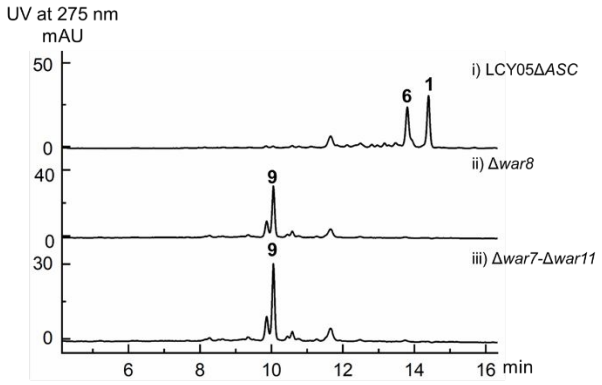


Figure S6. HPLC analysis of metabolites from LCY05Δ*ASC*/Δ*war7*−*war11*

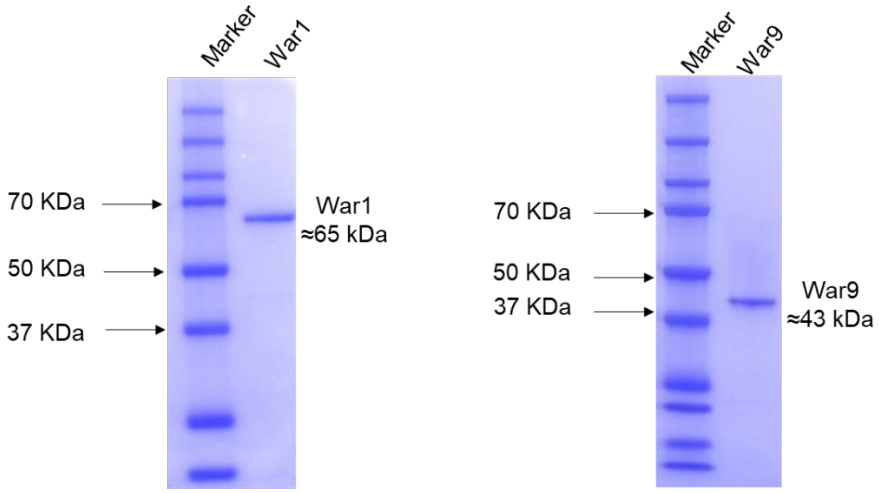


Figure S7. SDS-PAGE analysis of purified War1 and War9 enzymes.

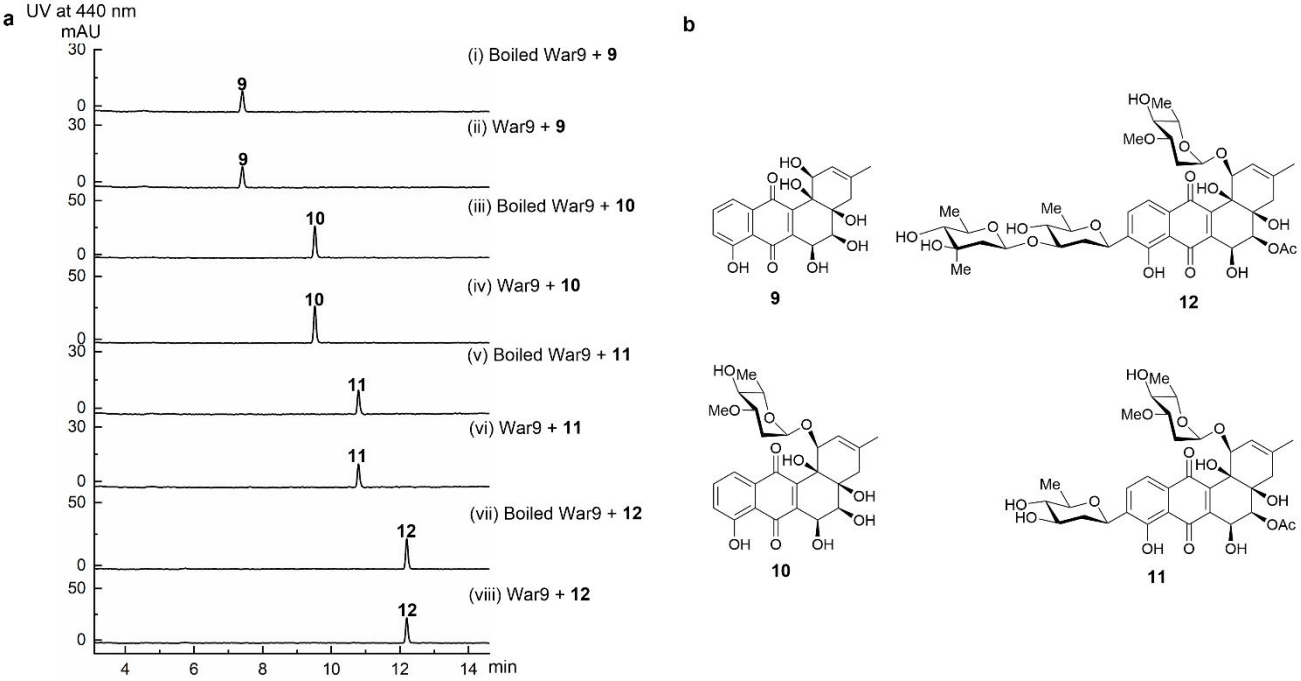


Figure S8. *In vitro* analysis of the cytochrome P450 War9. (**a**) HPLC analysis of War9-catalyzed reactions; (**b**) The structures of substrates acted upon by War9.

TMHMM result

```
# War21 Length: 397
# War21 Number of predicted TMHs:  10
# War21 Exp number of AAs in TMHs: 222.73796
# War21 Exp number, first 60 AAs:  35.98005
# War21 Total prob of N-in:        0.99656
# War21 POSSIBLE N-term signal sequence
War21        TMHMM2.0  inside      1   12
War21        TMHMM2.0  TMhelix    13   35
War21        TMHMM2.0  outside    36   44
War21        TMHMM2.0  TMhelix    45   67
War21        TMHMM2.0  inside     68   87
War21        TMHMM2.0  TMhelix    88  110
War21        TMHMM2.0  outside   111  136
War21        TMHMM2.0  TMhelix   137  159
War21        TMHMM2.0  inside    160  165
War21        TMHMM2.0  TMhelix   166  188
War21        TMHMM2.0  outside   189  202
War21        TMHMM2.0  TMhelix   203  225
War21        TMHMM2.0  inside    226  233
War21        TMHMM2.0  TMhelix   234  256
War21        TMHMM2.0  outside   257  259
War21        TMHMM2.0  TMhelix   260  282
War21        TMHMM2.0  inside    283  294
War21        TMHMM2.0  TMhelix   295  317
War21        TMHMM2.0  outside   318  340
War21        TMHMM2.0  TMhelix   341  363
War21        TMHMM2.0  inside    364  397
```

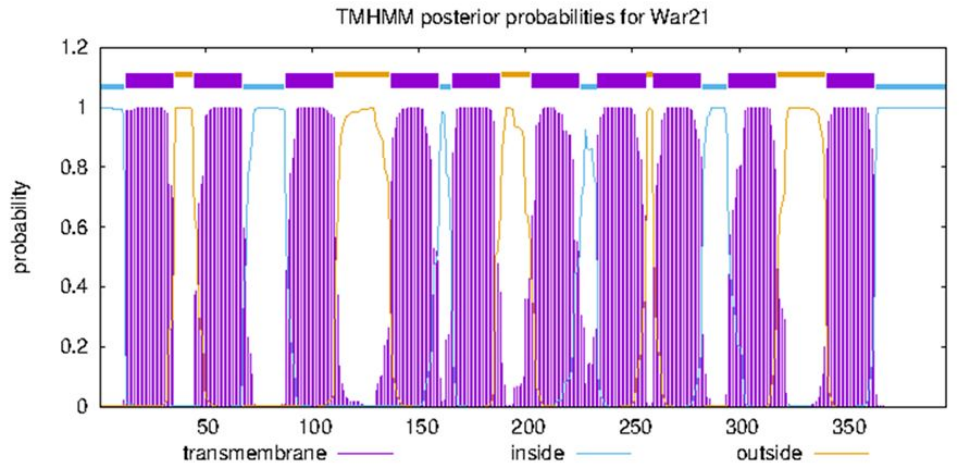


Figure S9. The transmembrane regions of War21 predicted by TMHMM Server v. 2.0.

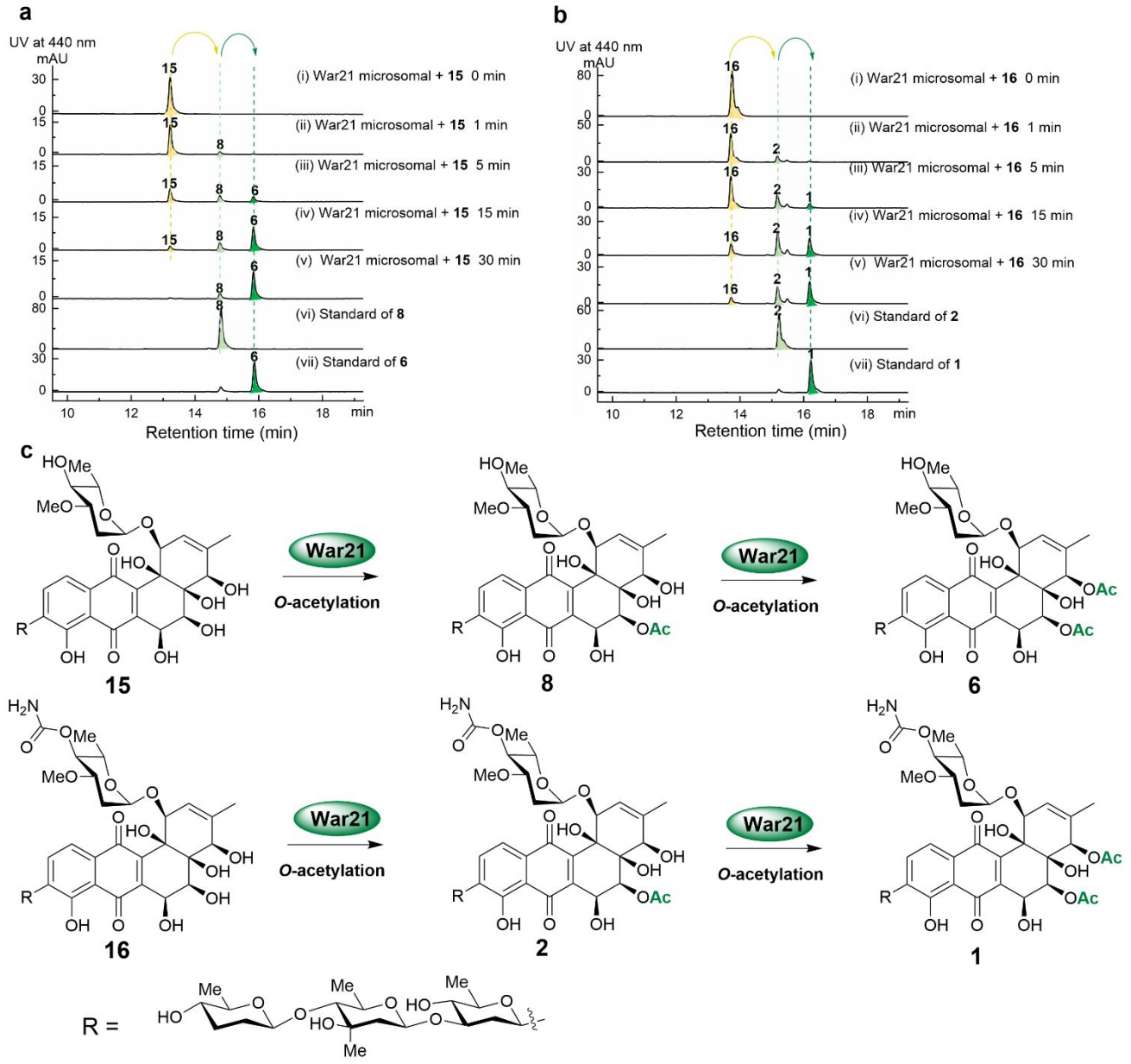


Figure S10. *In vitro* analysis of the acetyltransferase War21 activity. (**a**) HPLC traces of time-course using **15** as the substrate; (**b**) HPLC traces of time-course using **16** as the substrate; (**c**) The verified substrates and reactions catalyzed by War21.

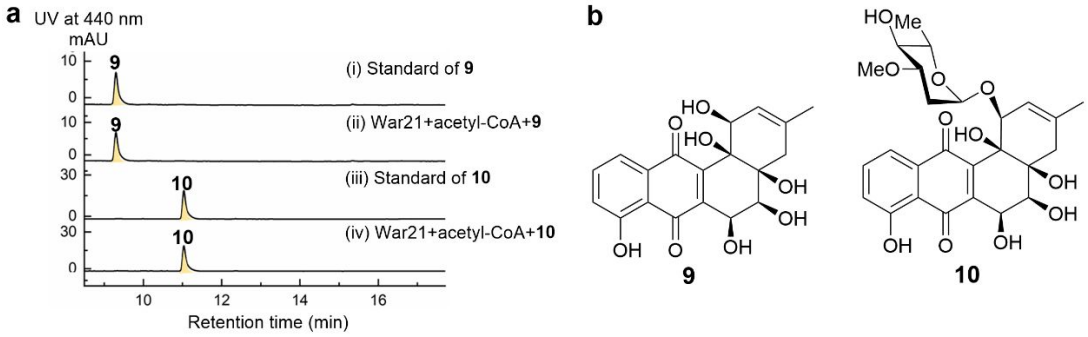


Figure S11. *In vitro* analysis of the acetyltransferase War21 activity using **9** and **10** as the substrates. (**a**) HPLC traces of using **9** and **10** as the substrate; (**b**) The substrates structure of **9** and **10**.

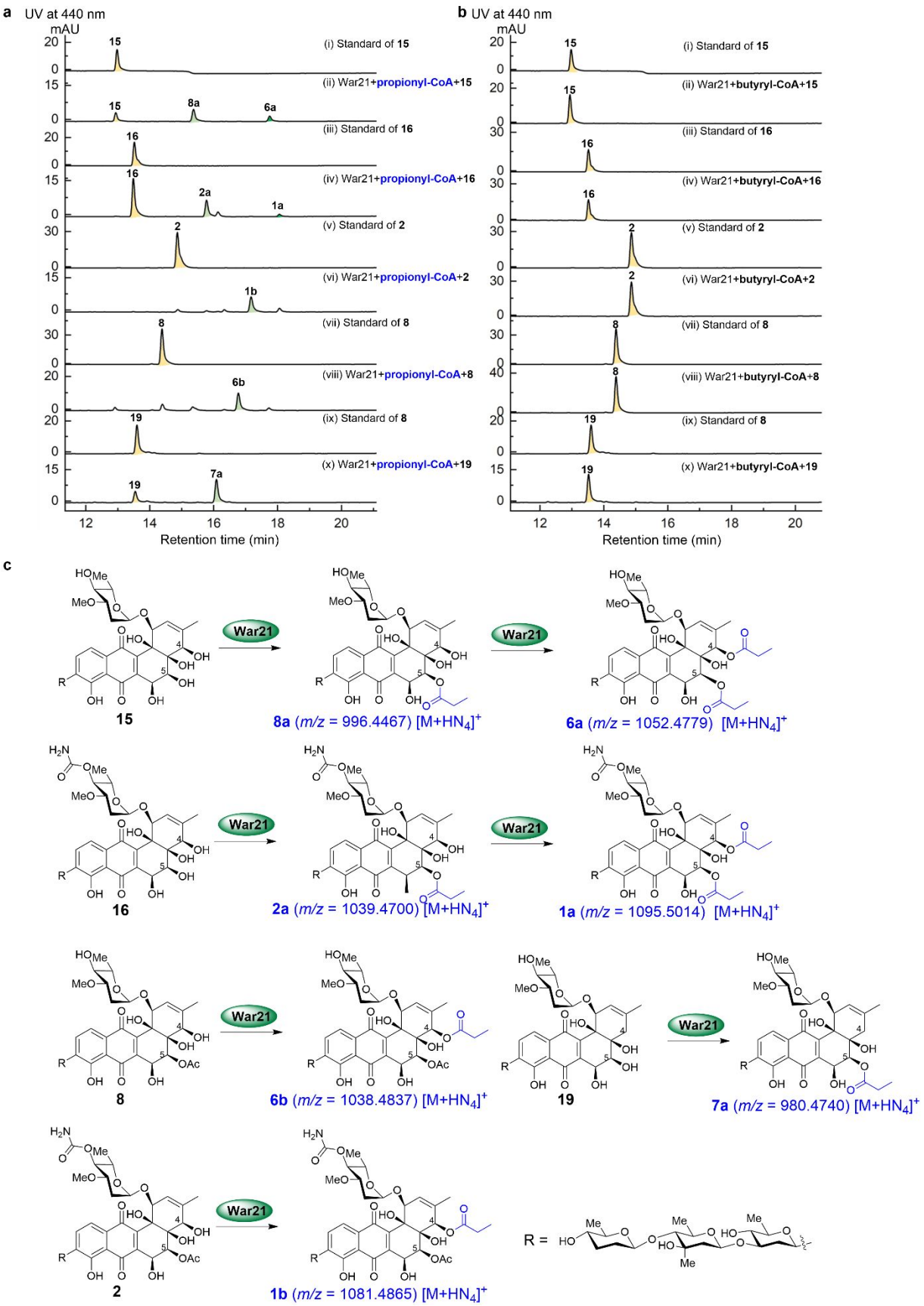


Figure S12. *In vitro* analysis of the acetyltransferase War21 activity use propionyl-CoA and butyryl-CoA were used as donors. (**a**) HPLC traces of using propionyl-CoA as donor; (**b**) HPLC traces of using butyryl-CoA as donor; (**c**) The verified substrates and reactions catalyzed by War21 using propionyl-CoA as donor.

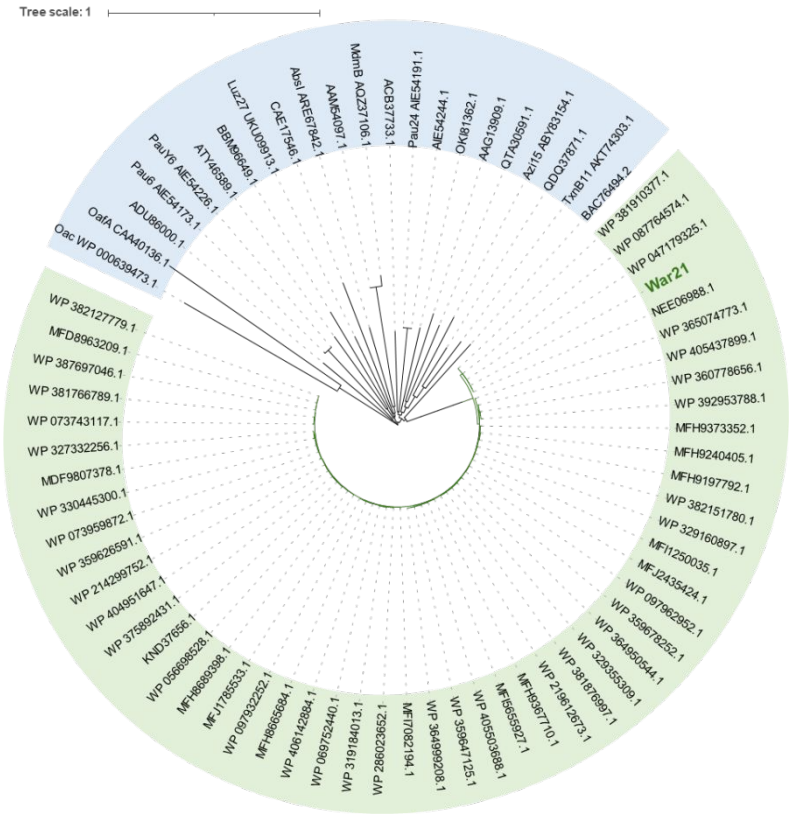


Figure S13. Phylogenetic analysis of acetyltransferase.

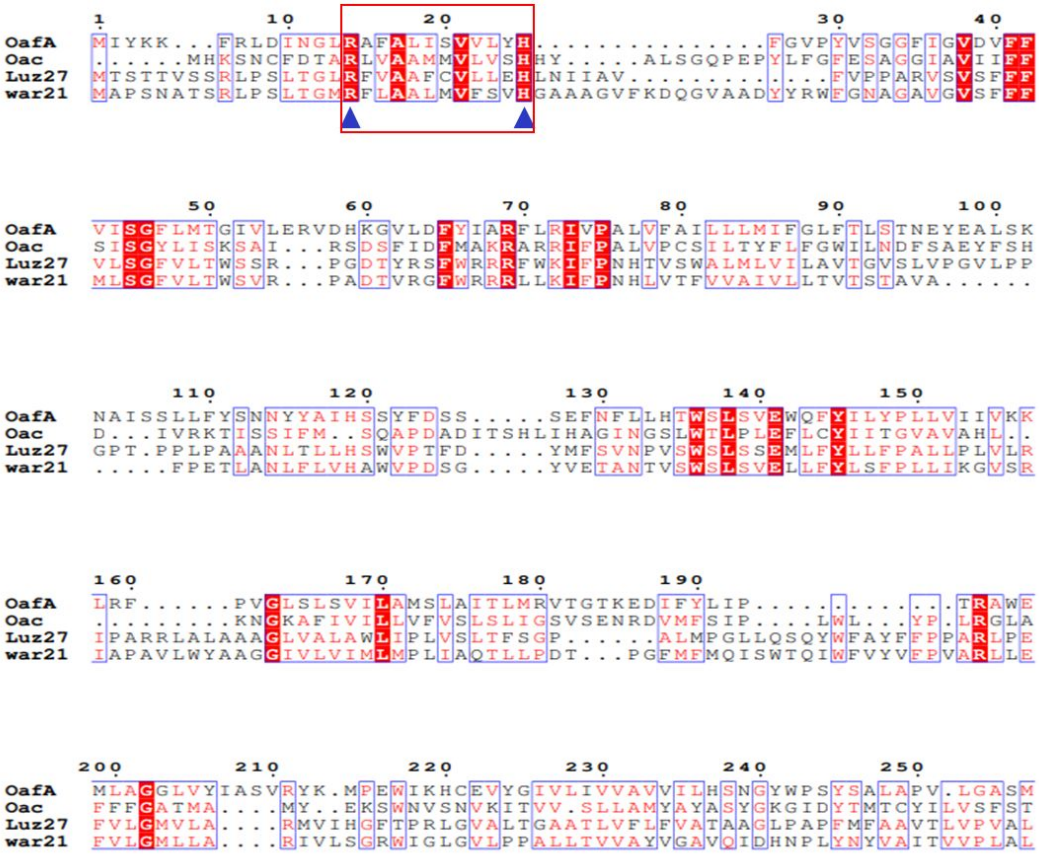


Figure S14. Sequence alignment of War21 and other acetyltransferases Luz27 (accession UKU09913.1), OafA (accession CAA40136) and Oac (accession WP_000639473). The two key residues are indicated by blue triangles.

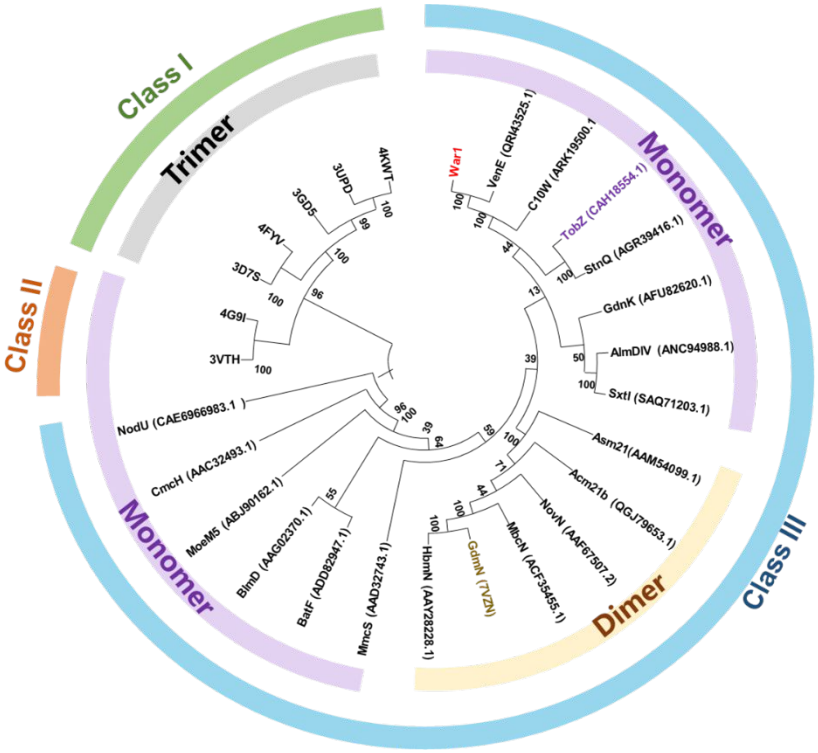


Figure S15. Phylogenetic analysis and aggregation states of CTases.

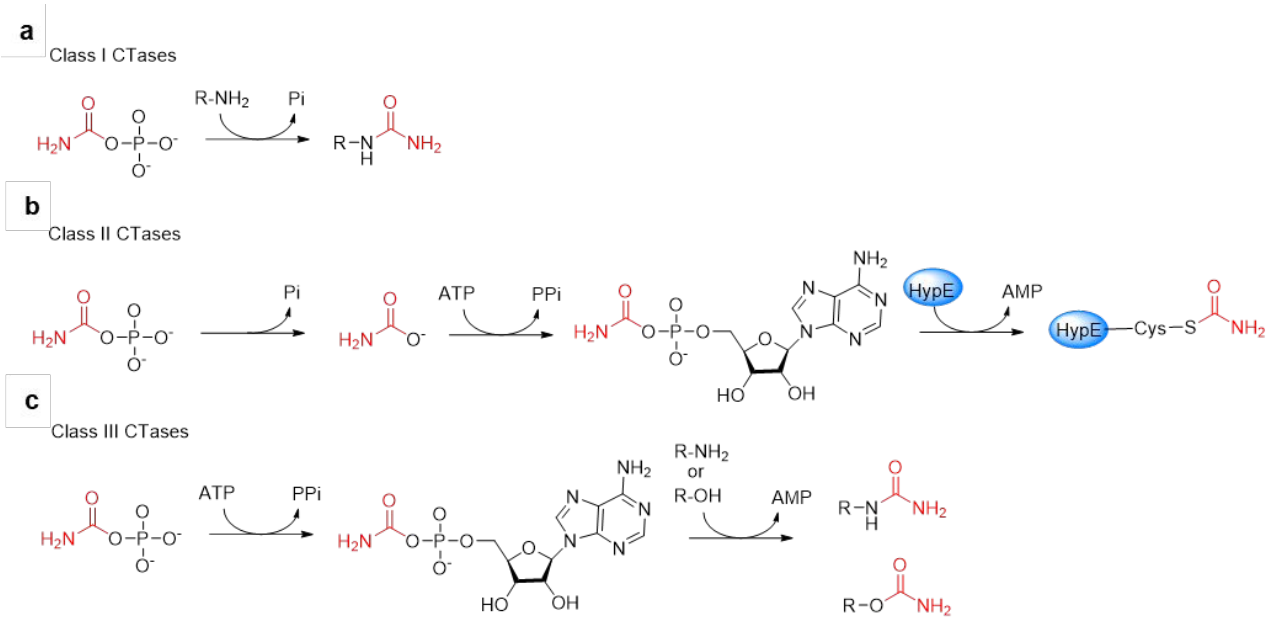


Figure S16. Schemes for carbamoylation catalyzed by different classes of CTases.

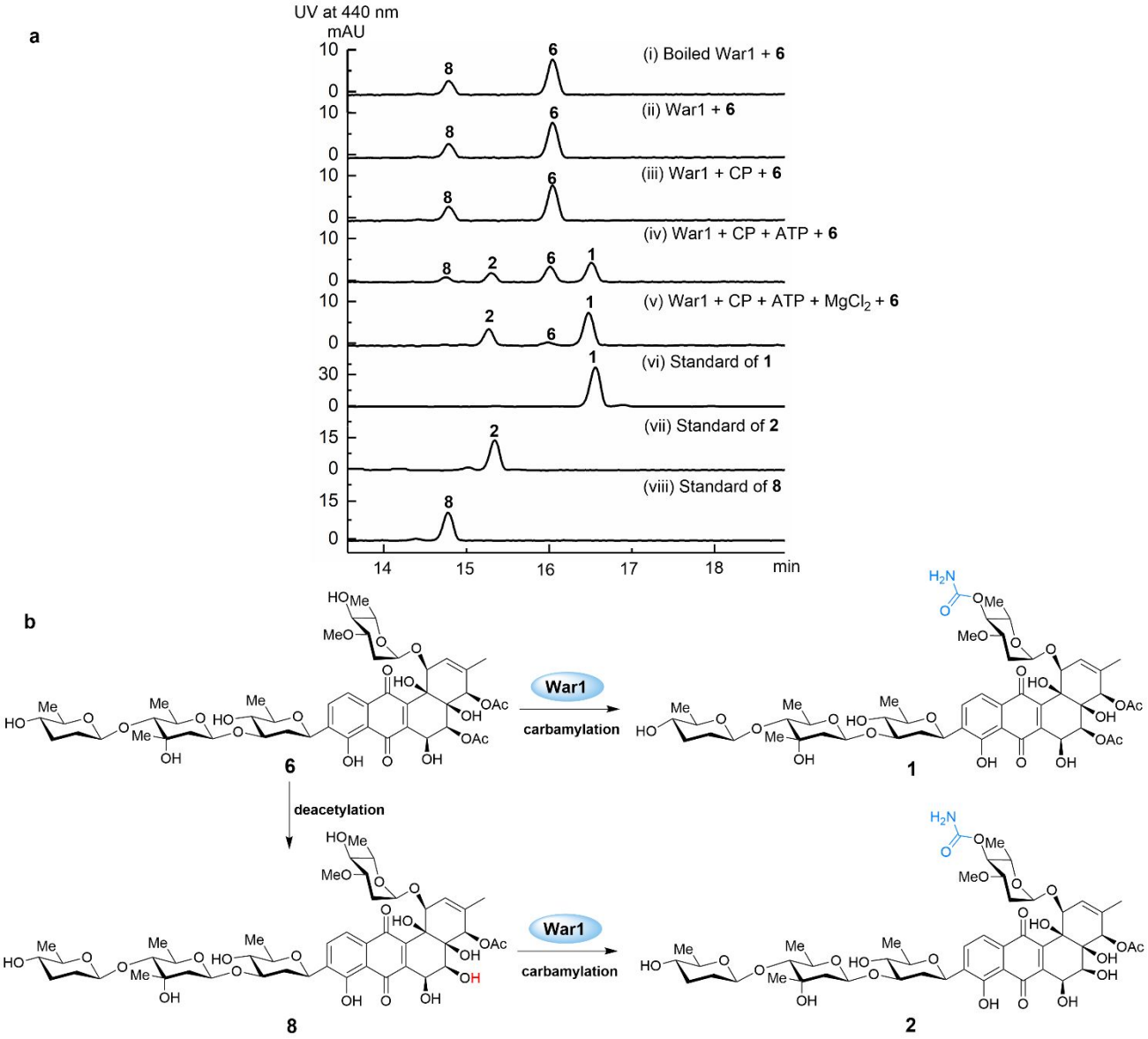


Figure S17. *In vitro* analysis of the carbamoyltransferase War1. (**a**) HPLC traces of the War1-catalyzed reactions; (**b**) Verified substrates and reactions catalyzed by War1.

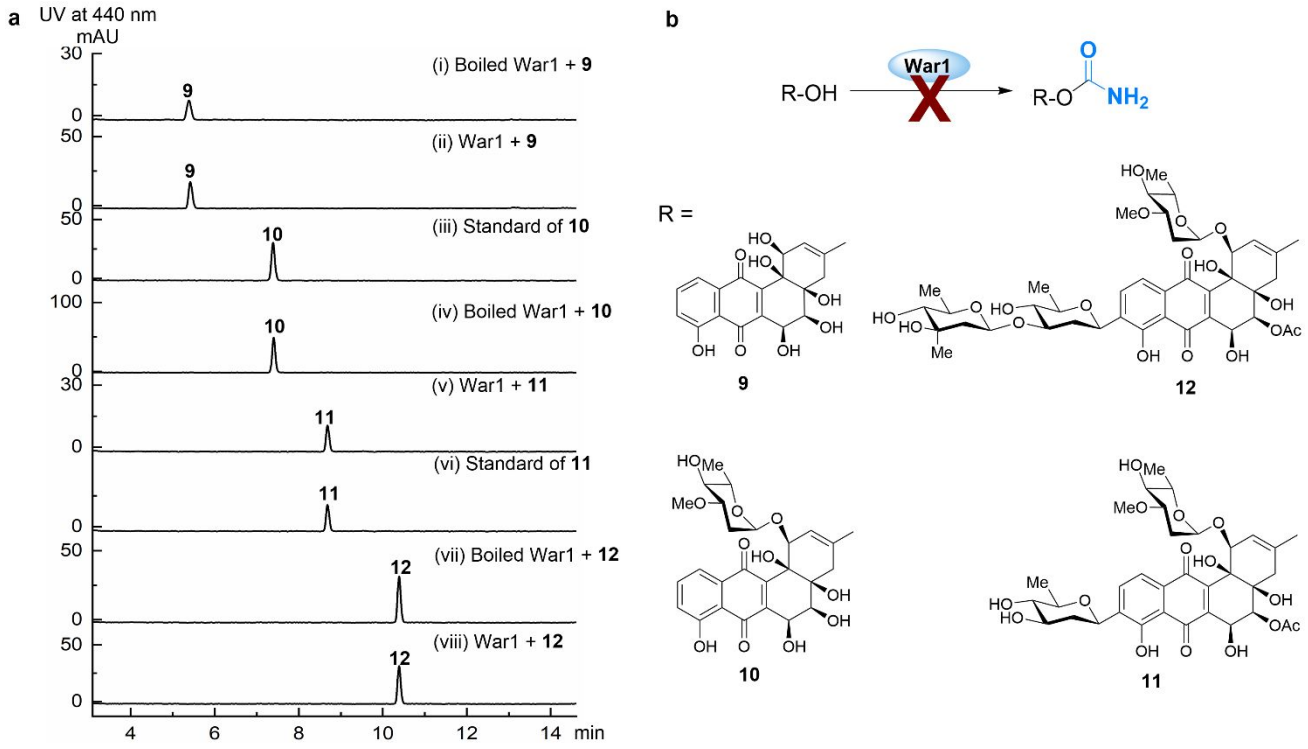


Figure S18. *In vitro* analysis of the carbamoyltransferase War1 using non-tetrasaccharide substrates. (**a**) HPLC traces of the War1-catalyzed reactions; (**b**) The structures of substrates acted upon by War1.

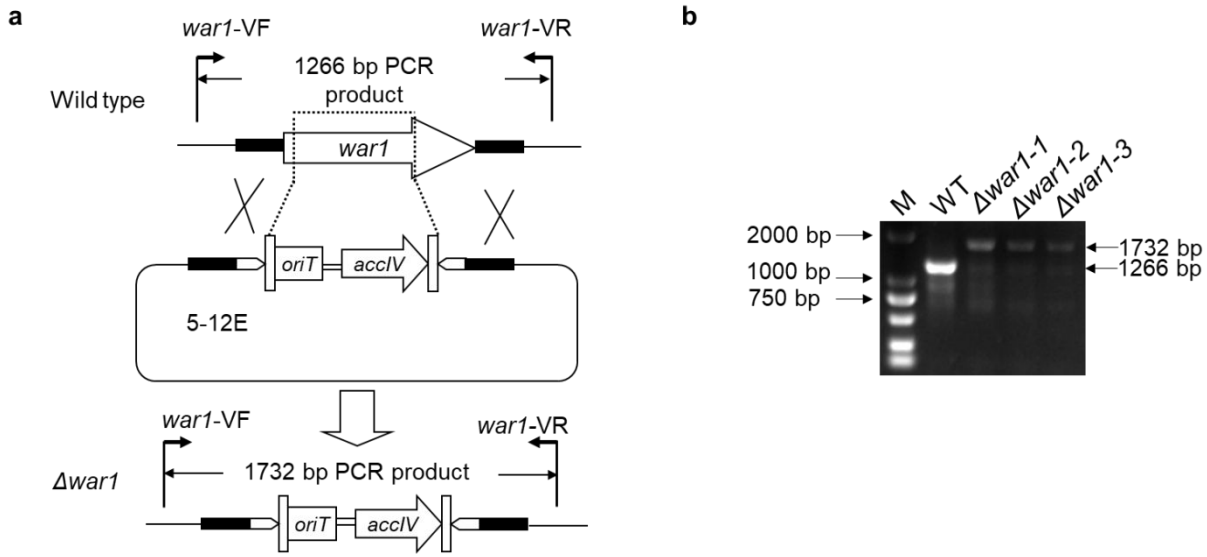


Figure S19. Construction and gel electrophoretic analyses of mutant *war1*. (**a**) Construction of mutant strain *war1* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war1* mutant by PCR DNA templates.

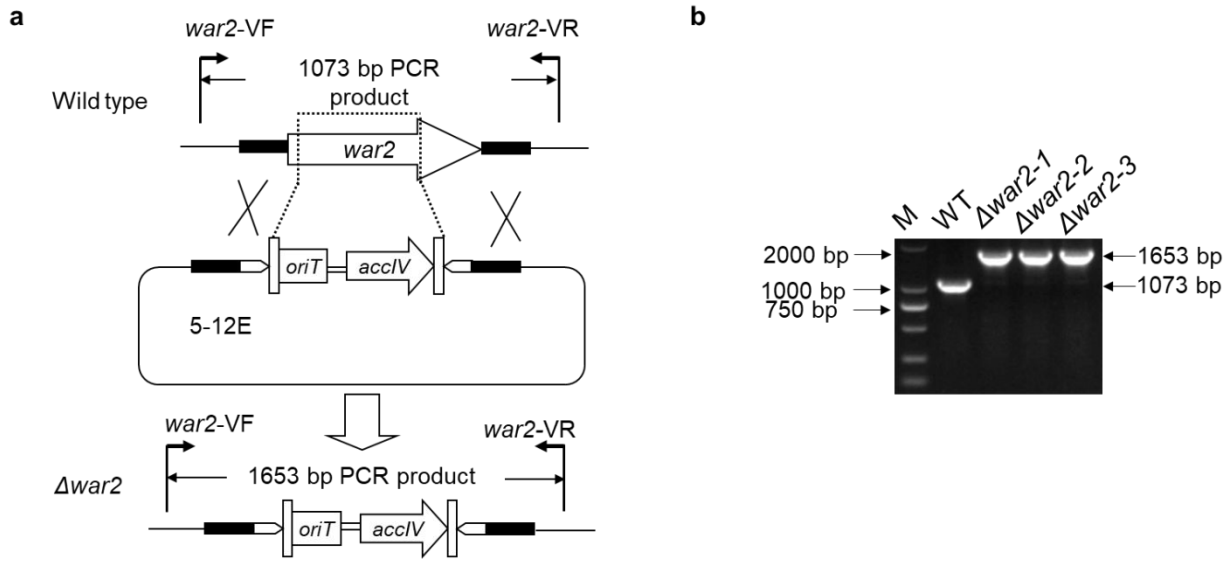


Figure S20. Construction and gel electrophoretic analyses of mutant *war2*. (**a**) Construction of mutant strain *war2* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war2* mutant by PCR DNA templates.

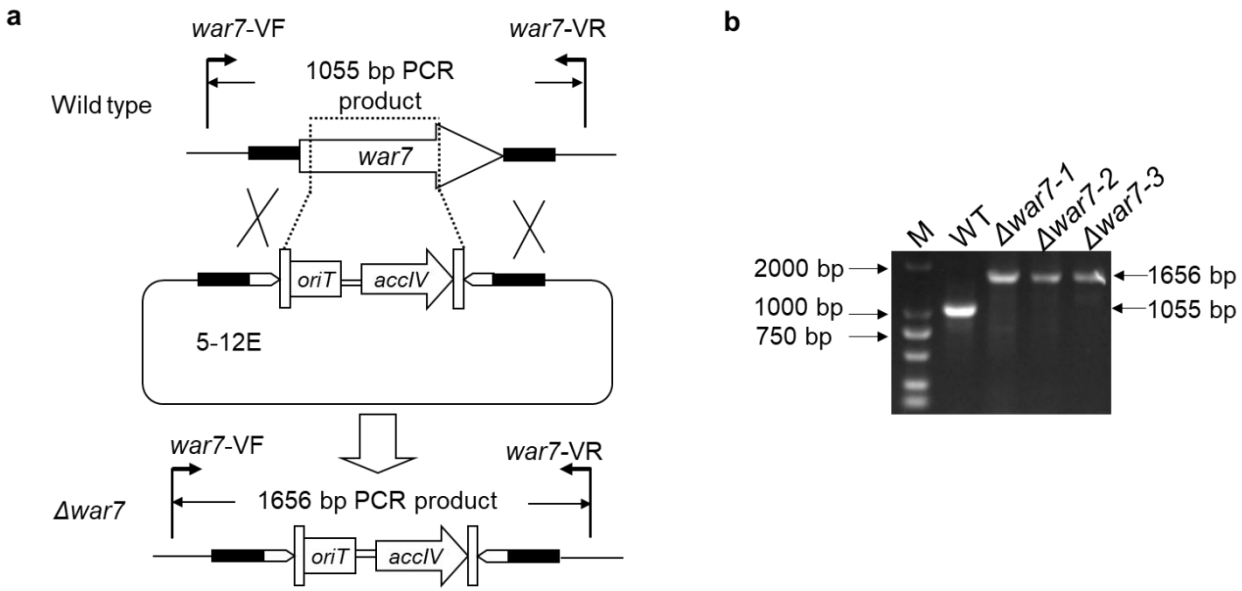


Figure S21. Construction and gel electrophoretic analyses of mutant *war7*. (**a**) Construction of mutant strain *war7* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war7* mutant by PCR DNA templates.

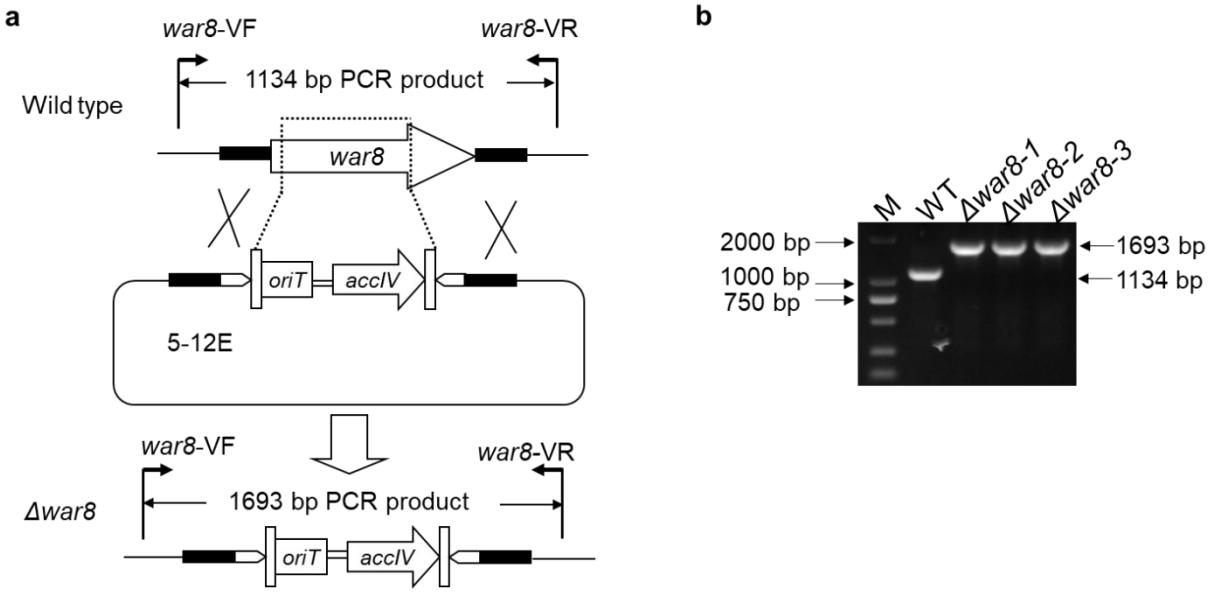


Figure S22. Construction and gel electrophoretic analyses of mutant *war8*. (**a**) Construction of mutant strain *war8* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war8* mutant by PCR DNA templates.

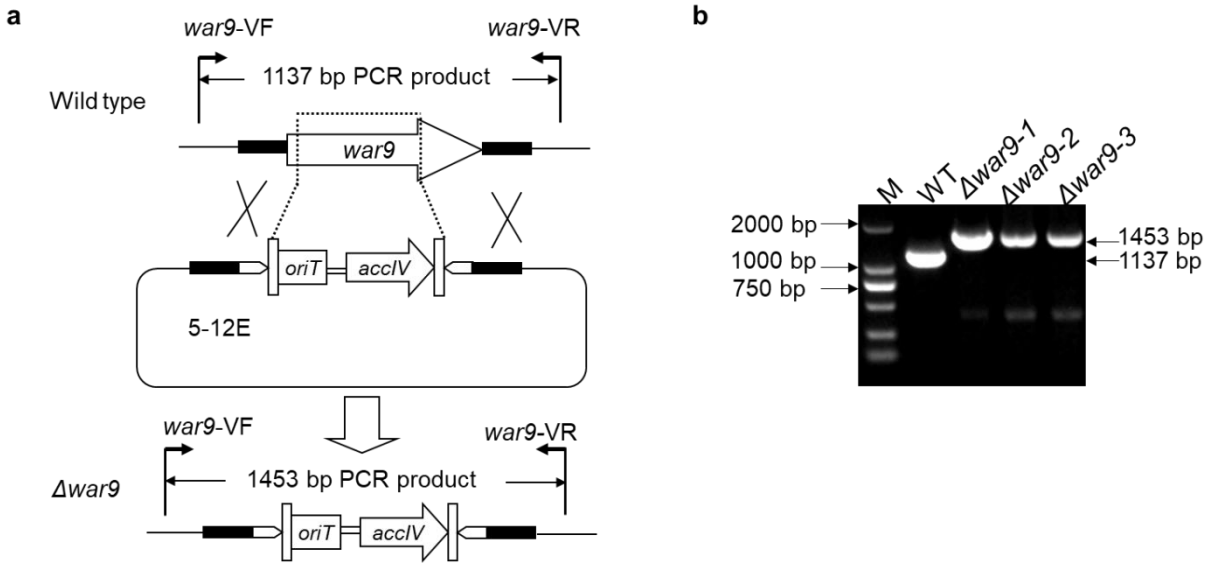


Figure S23. Construction and gel electrophoretic analyses of mutant *war9*. (**a**) Construction of mutant strain *war9* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war9* mutant by PCR DNA templates.

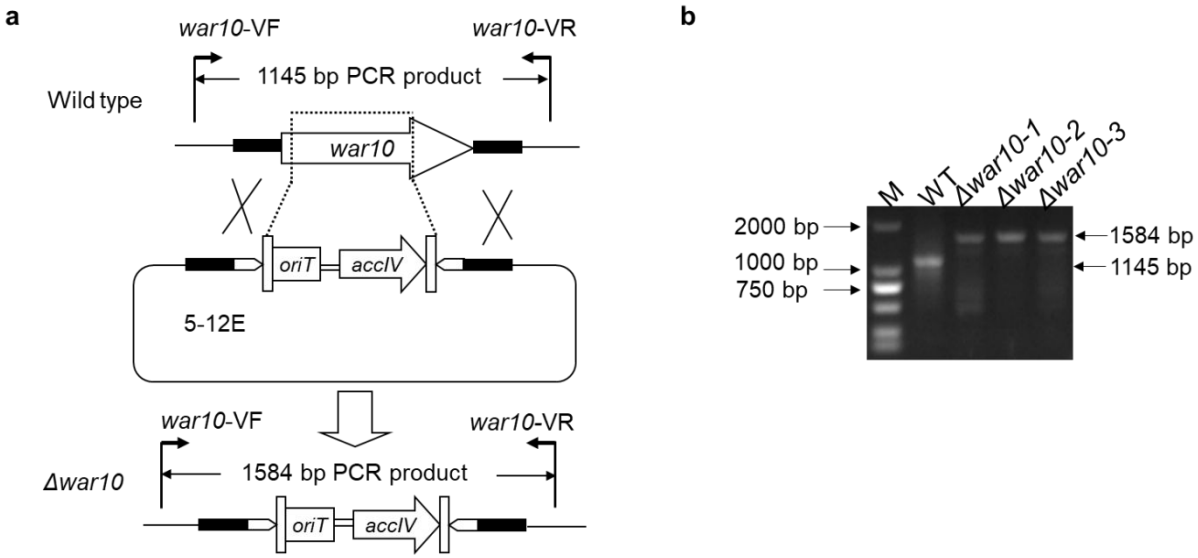


Figure S24. Construction and gel electrophoretic analyses of mutant *war10*. (**a**) Construction of mutant strain *war9* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war9* mutant by PCR DNA templates.

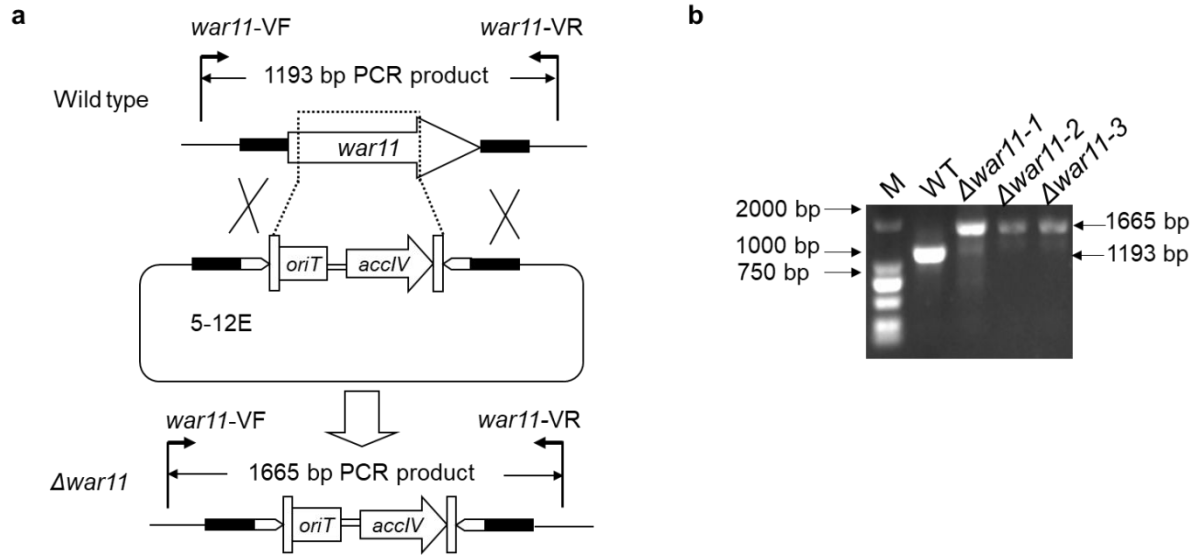


Figure S25. Construction and gel electrophoretic analyses of mutant *war11*. (**a**) Construction of mutant strain *war11* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war11* mutant by PCR DNA templates.

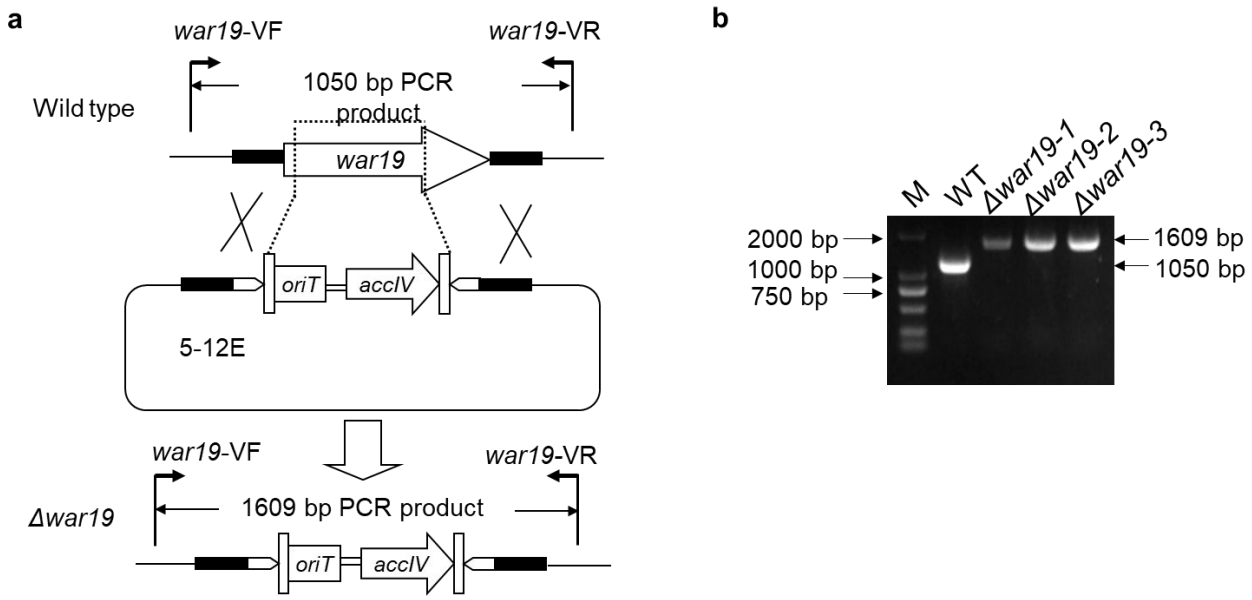


Figure S26. Construction and gel electrophoretic analyses of mutant *war19*. (**a**) Construction of mutant strain *war19* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war19* mutant by PCR DNA templates.

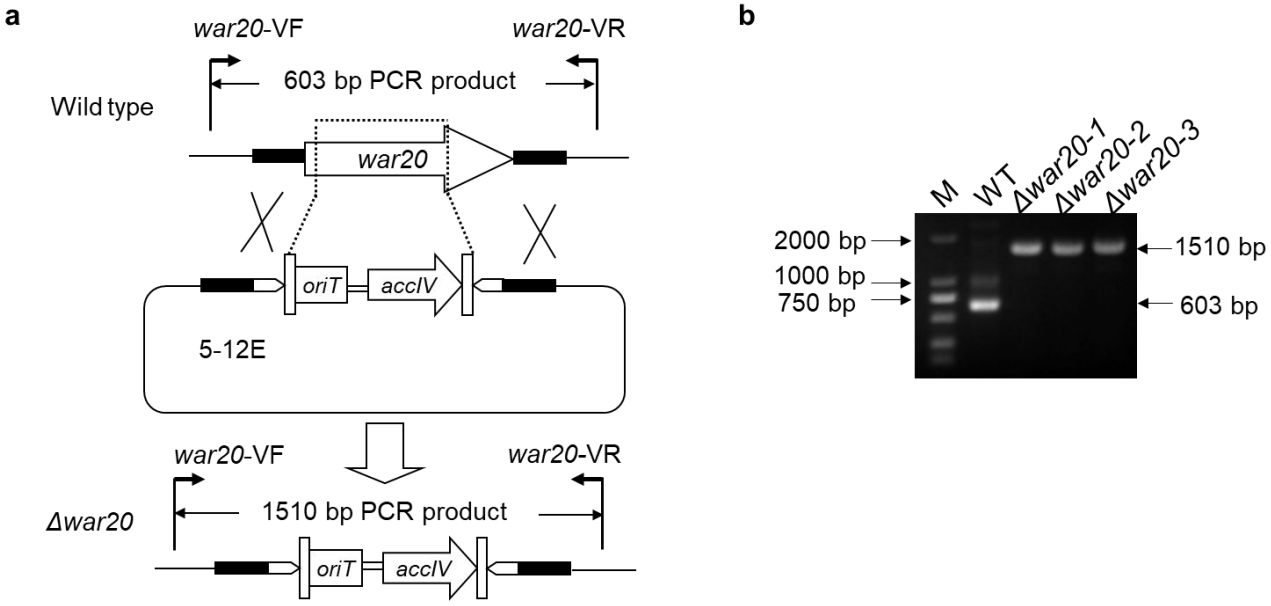


Figure S27. Construction and gel electrophoretic analyses of mutant *war20*. (**a**) Construction of mutant strain *war20* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war20* mutant by PCR DNA templates.

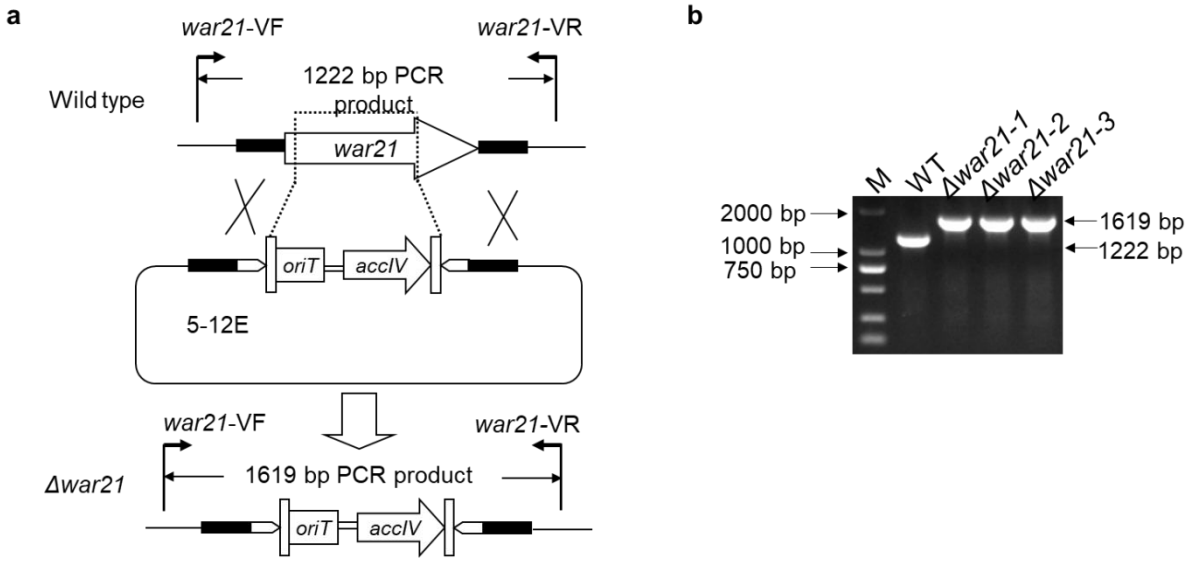


Figure S28. Construction and gel electrophoretic analyses of mutant *war21*. (**a**) Construction of mutant strain *war*21 and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war21* mutant by PCR DNA templates.

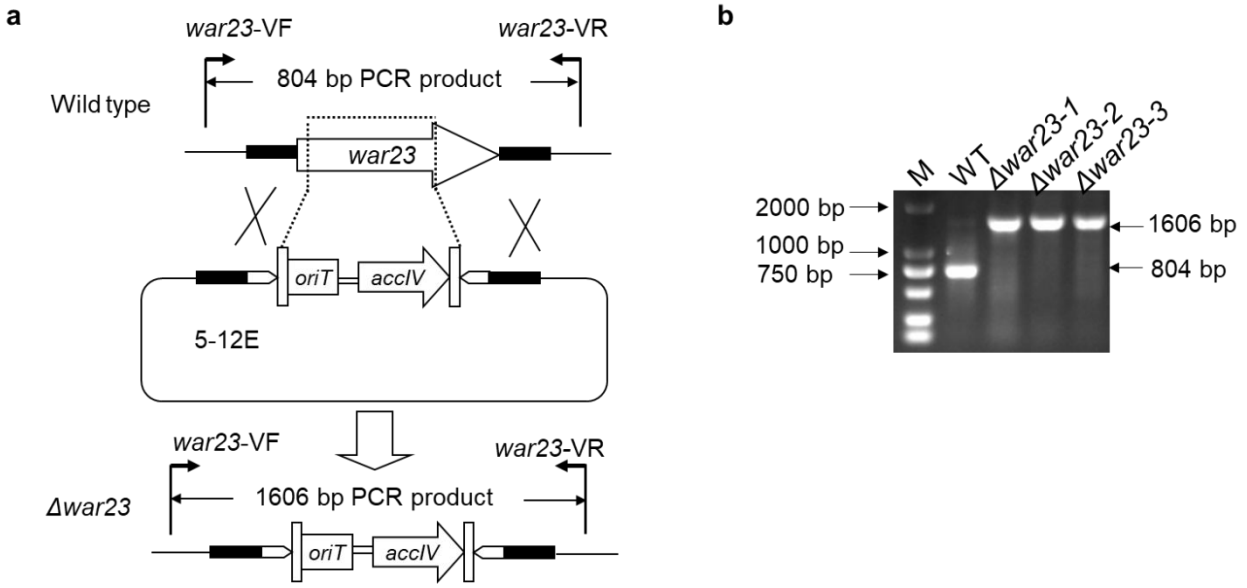


Figure S29. Construction and gel electrophoretic analyses of mutant *war23*. (**a**) Construction of mutant strain *war23* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war23* mutant by PCR DNA templates.

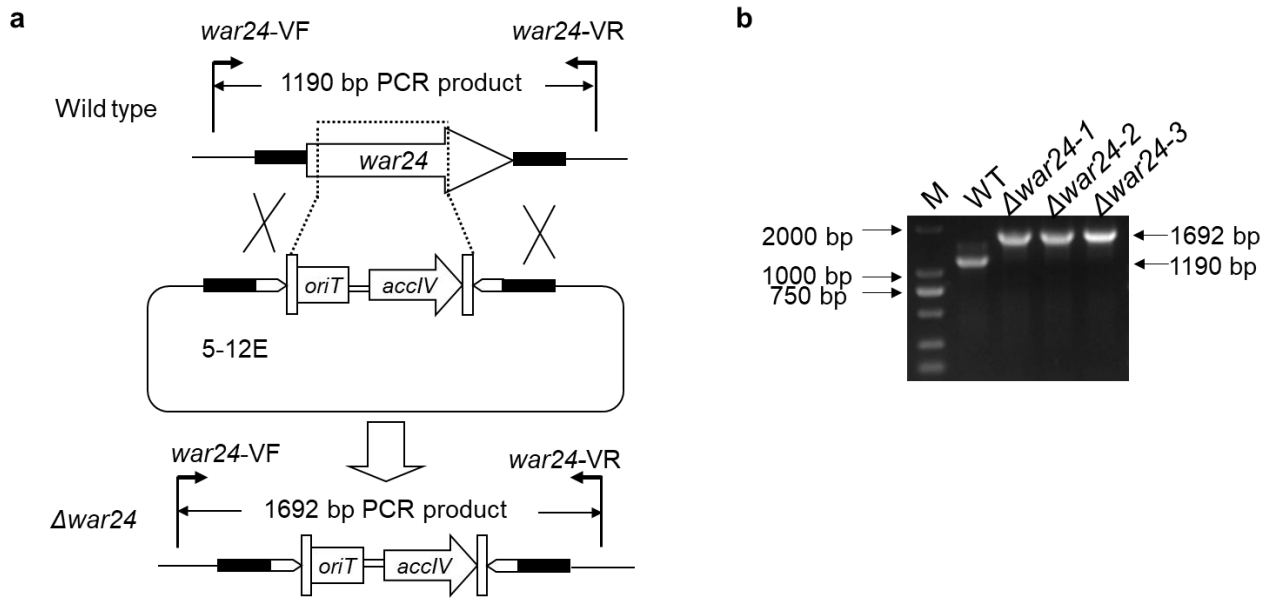


Figure S30. Construction and gel electrophoretic analyses of mutant *war24*. (**a**) Construction of mutant strain *war24* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war24* mutant by PCR DNA templates.

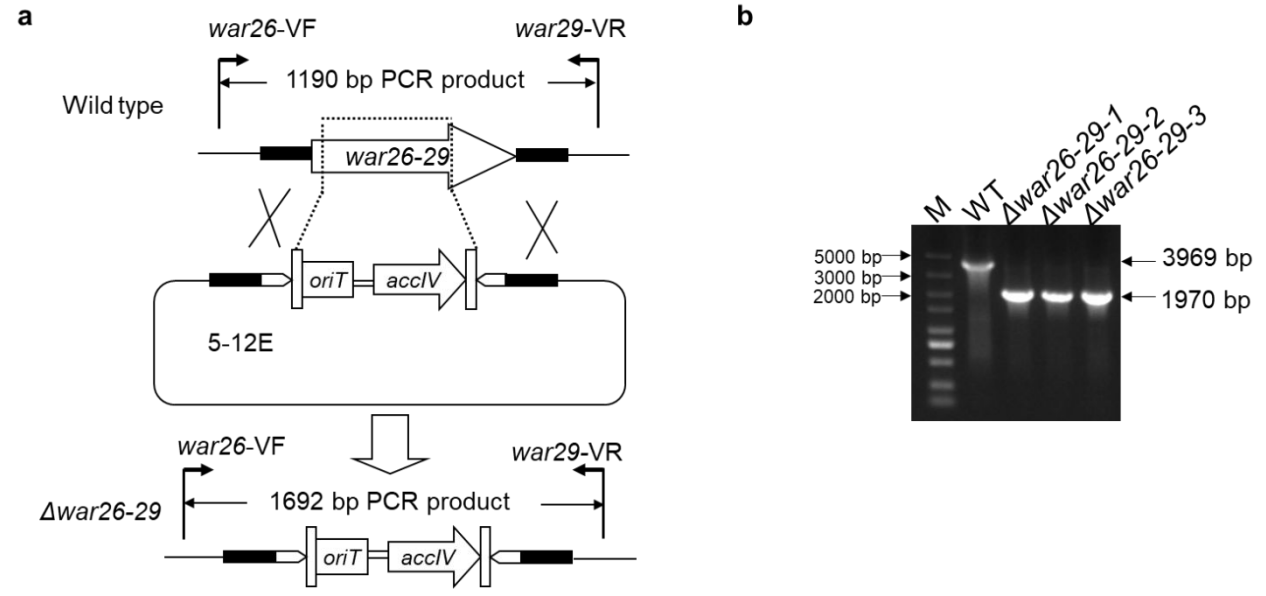


Figure S31. Construction and gel electrophoretic analyses of mutant *war26−29*. (**a**) Construction of mutant strain *war26-29* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war26-29* mutant by PCR DNA templates.

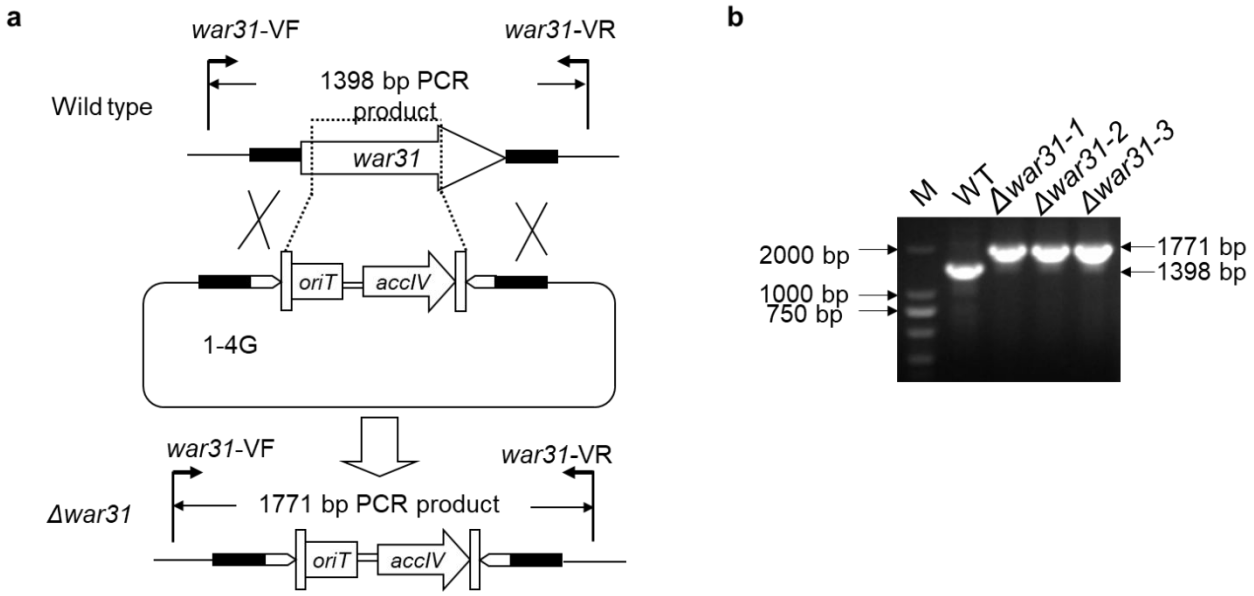


Figure S32. Construction and gel electrophoretic analyses of mutant *war31*. (**a**) Construction of mutant strain *war31* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war31* mutant by PCR DNA templates.

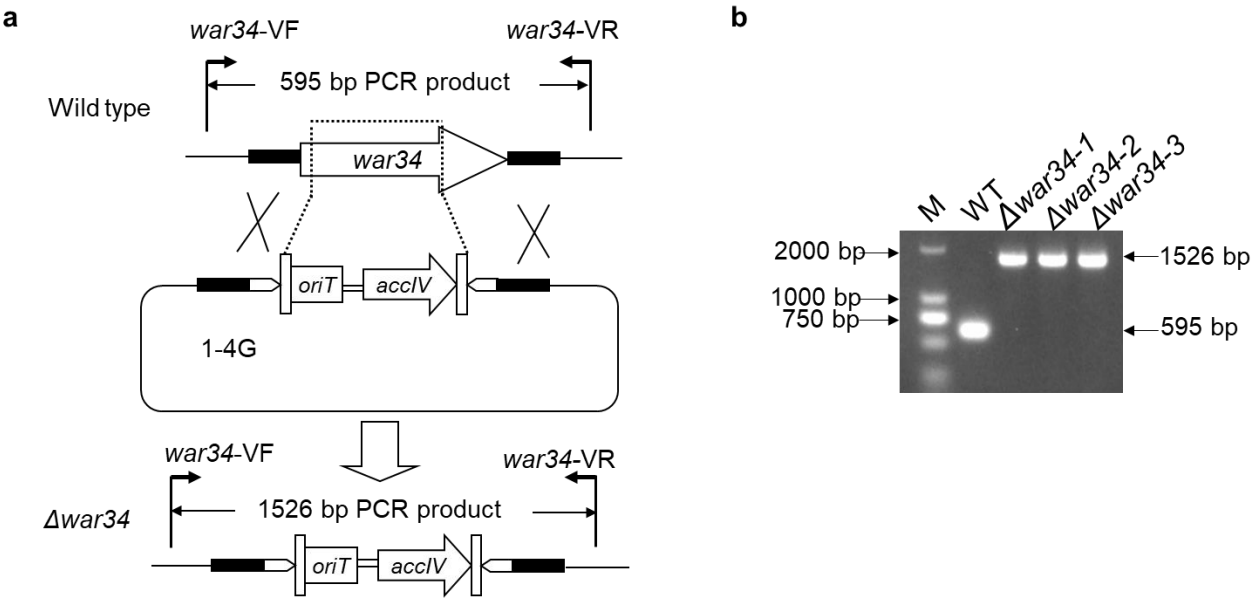


Figure S33. Construction and gel electrophoretic analyses of mutant *war34*. (**a**) Construction of mutant strain *war34* and predicted PCR fragment size from wild-type and mutant; (**b**) Verification of the *war34* mutant by PCR DNA templates.

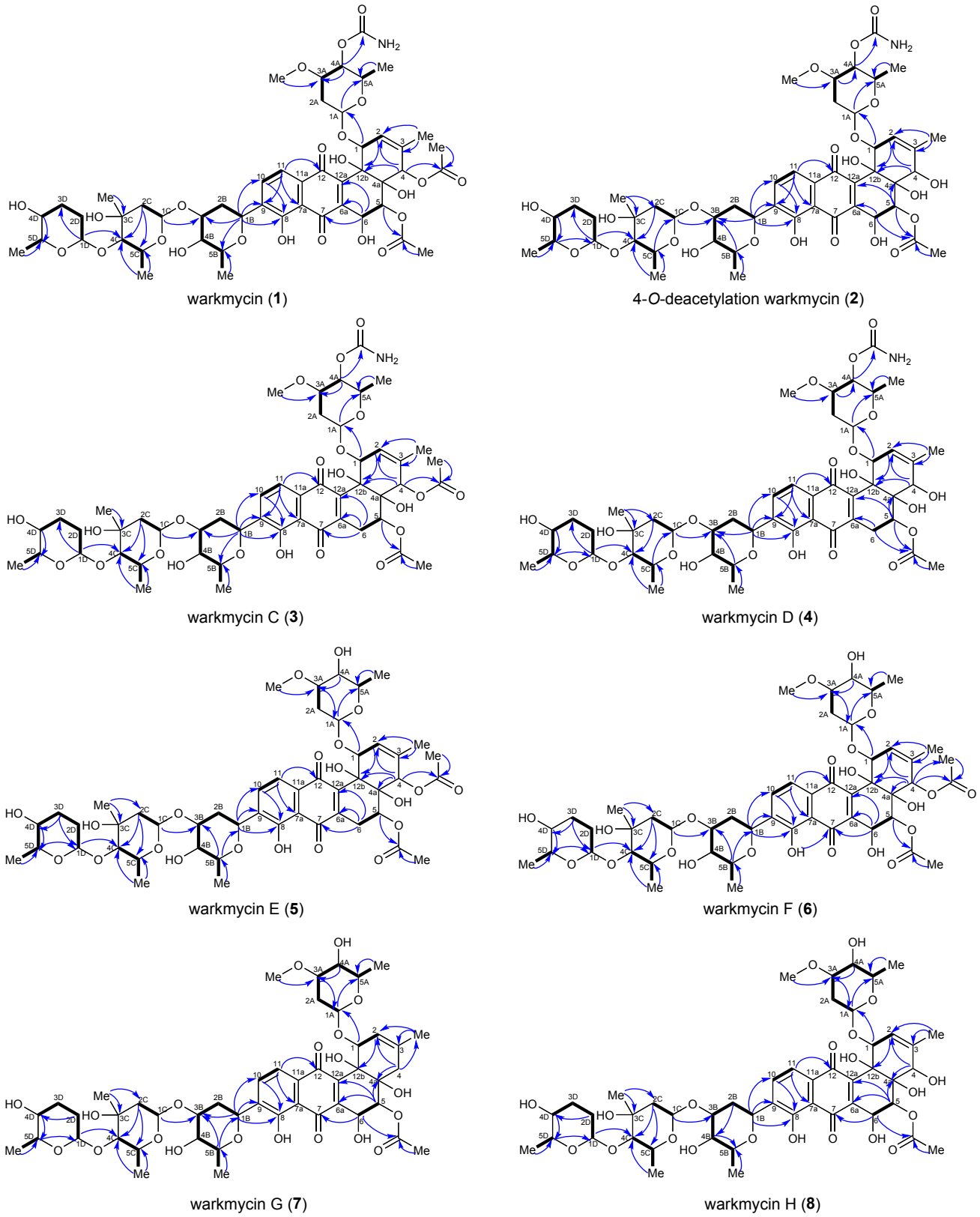


Figure S34. The Selected HMBC and ^{1}H-^{1}H COSY of warkmycins (**1-8**)

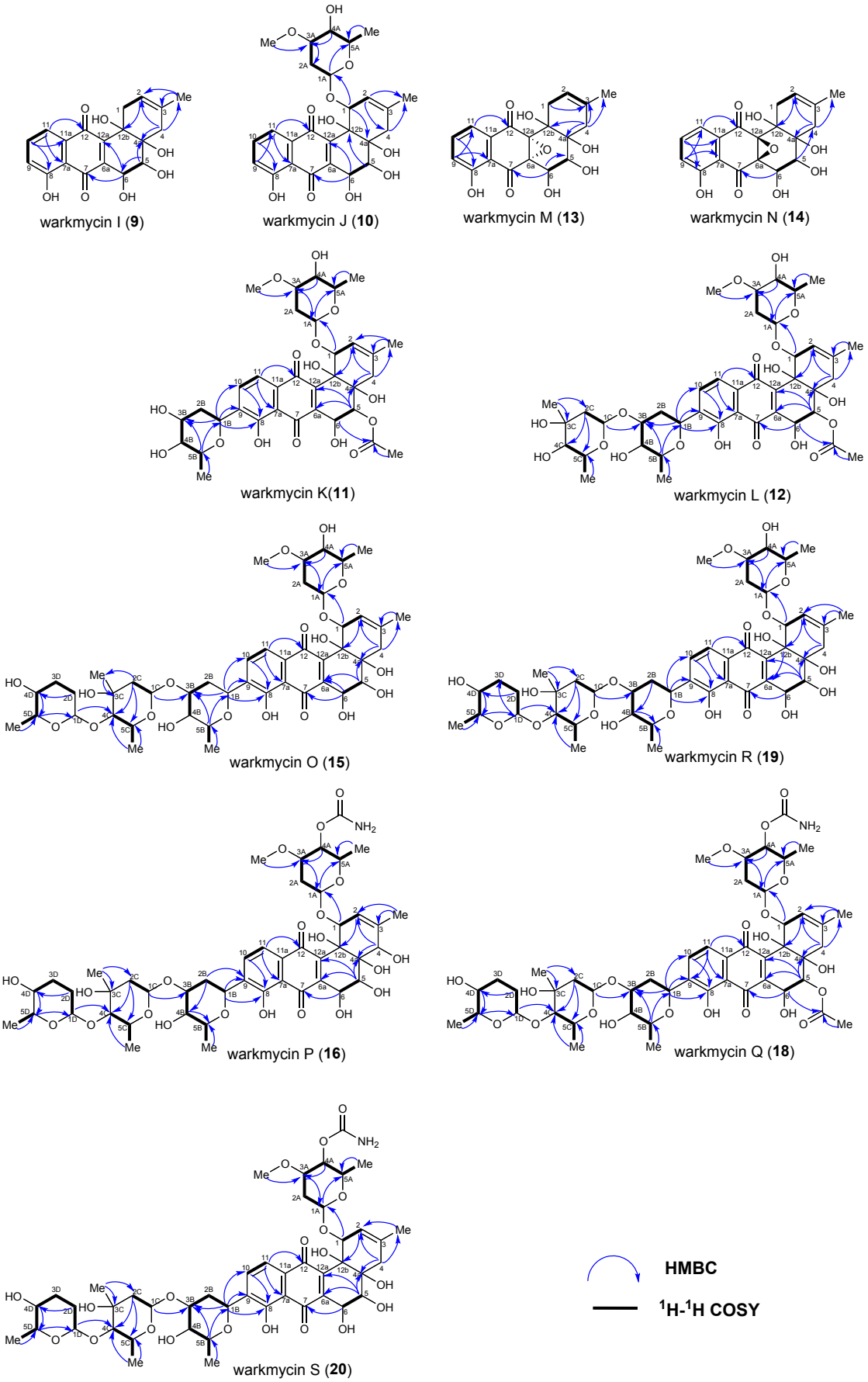


Figure S35. The Selected HMBC and ^{1}H-^{1}H COSY of warkmycins (**9-16, 18-20**)

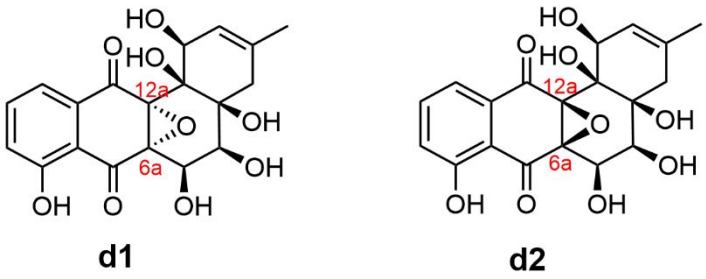


Figure S36. The structures of (6a*R*,12a*S*)-**13** and (6a*S*,12a*R*)-**14**

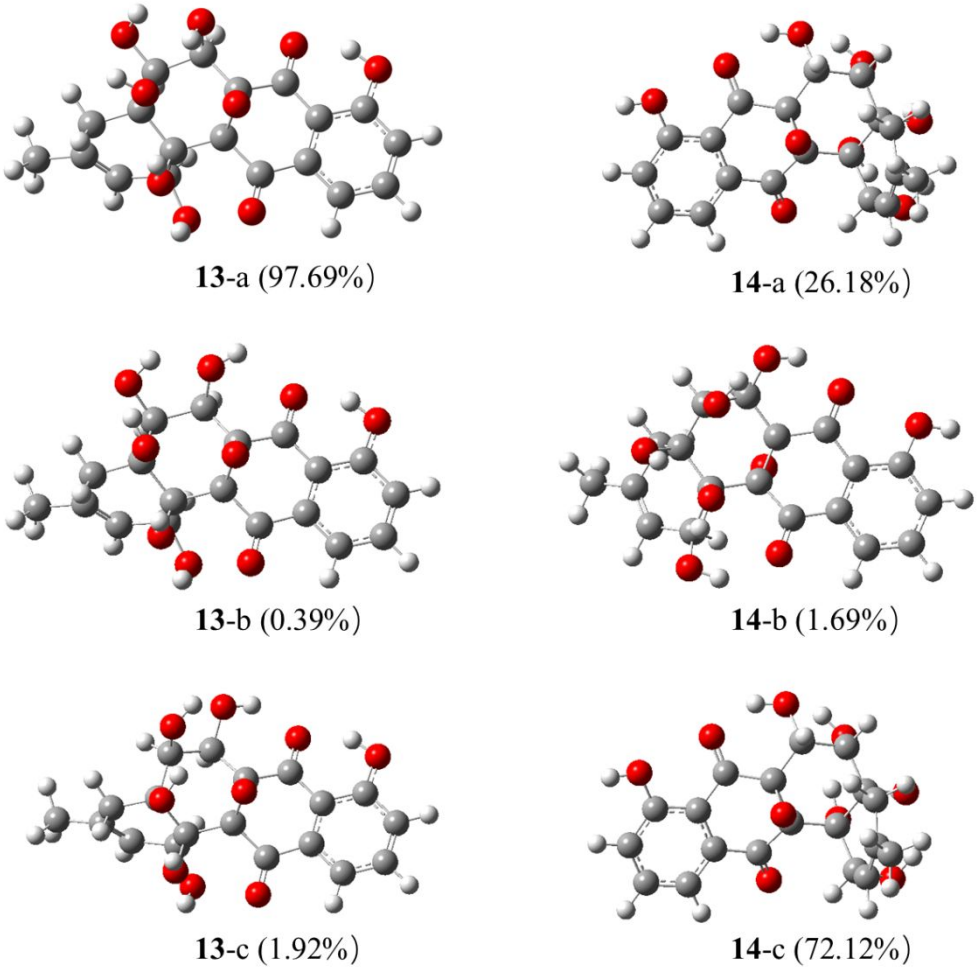


Figure S37. Optimized geometries of dominant conformers of (6a*R*,12a*S*)-**13-a** and (6a*S*,12a*R*)-**14-c**, respectively, at the B3LYP/6-31G(d) level. The Boltzmann distribution of each conformer was showed.

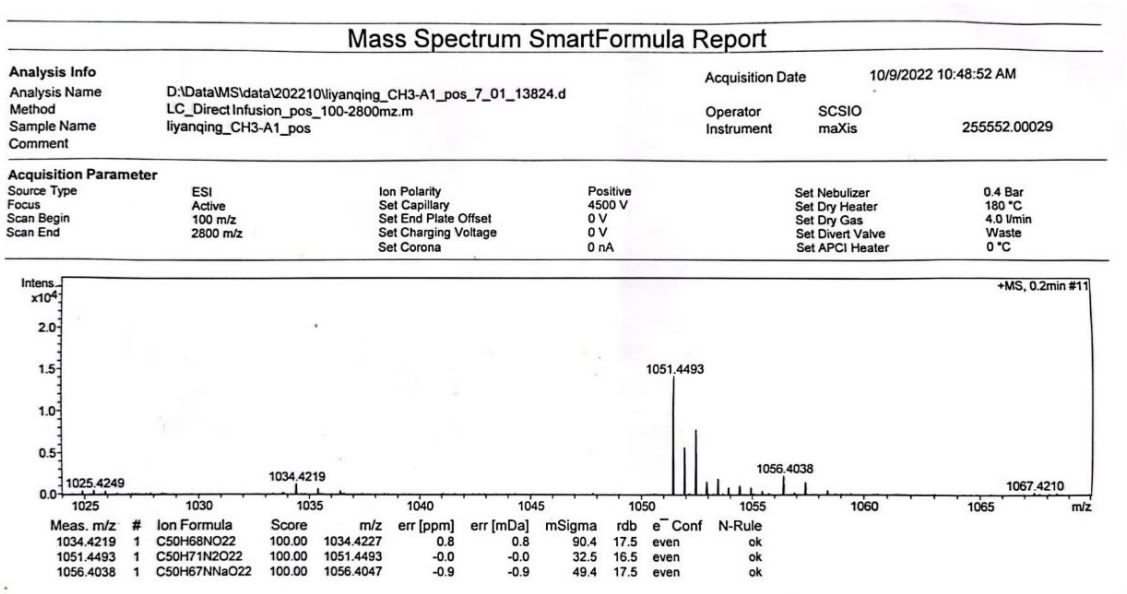


Figure S38. HRESIMS spectrum of compound **1**

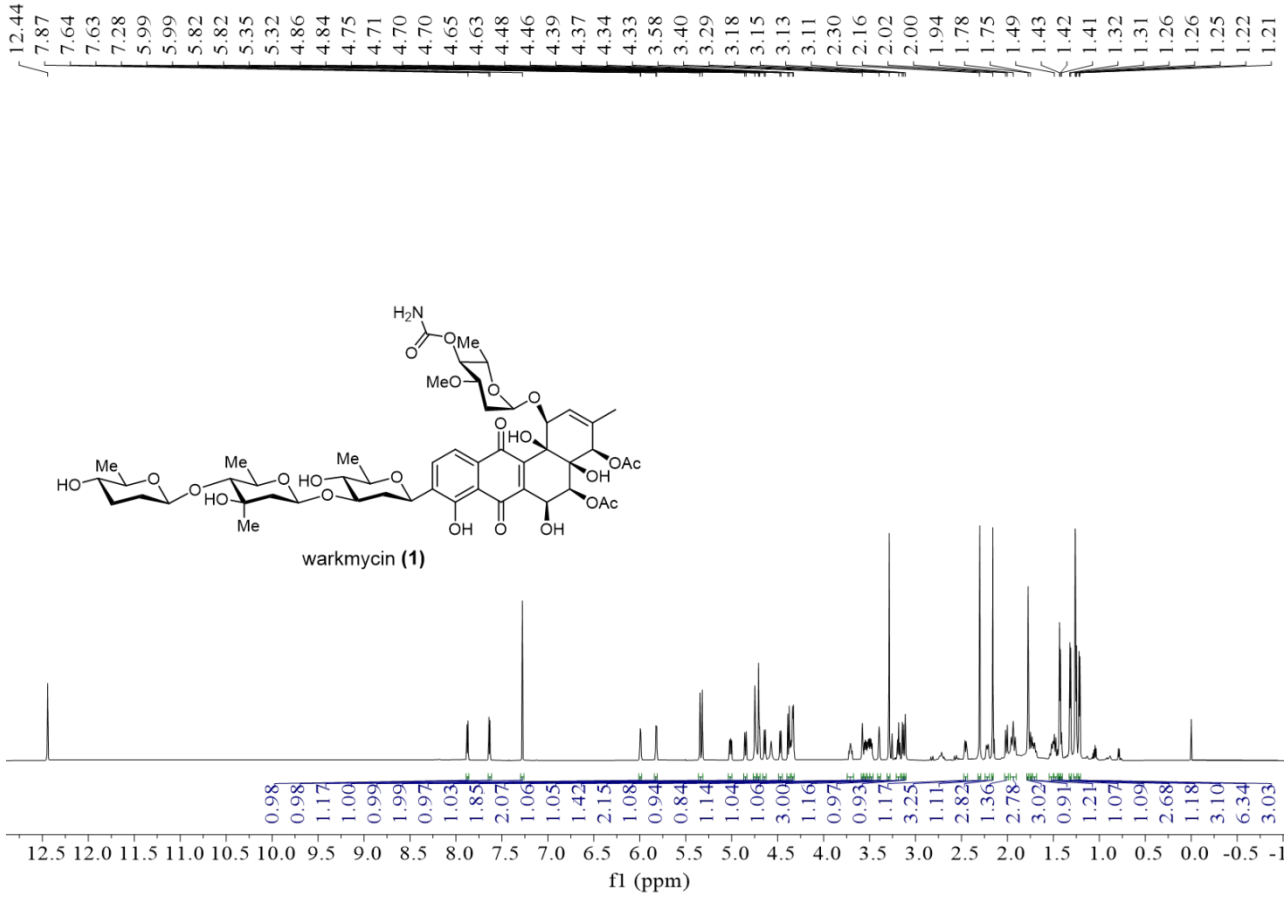


Figure S39. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **1**

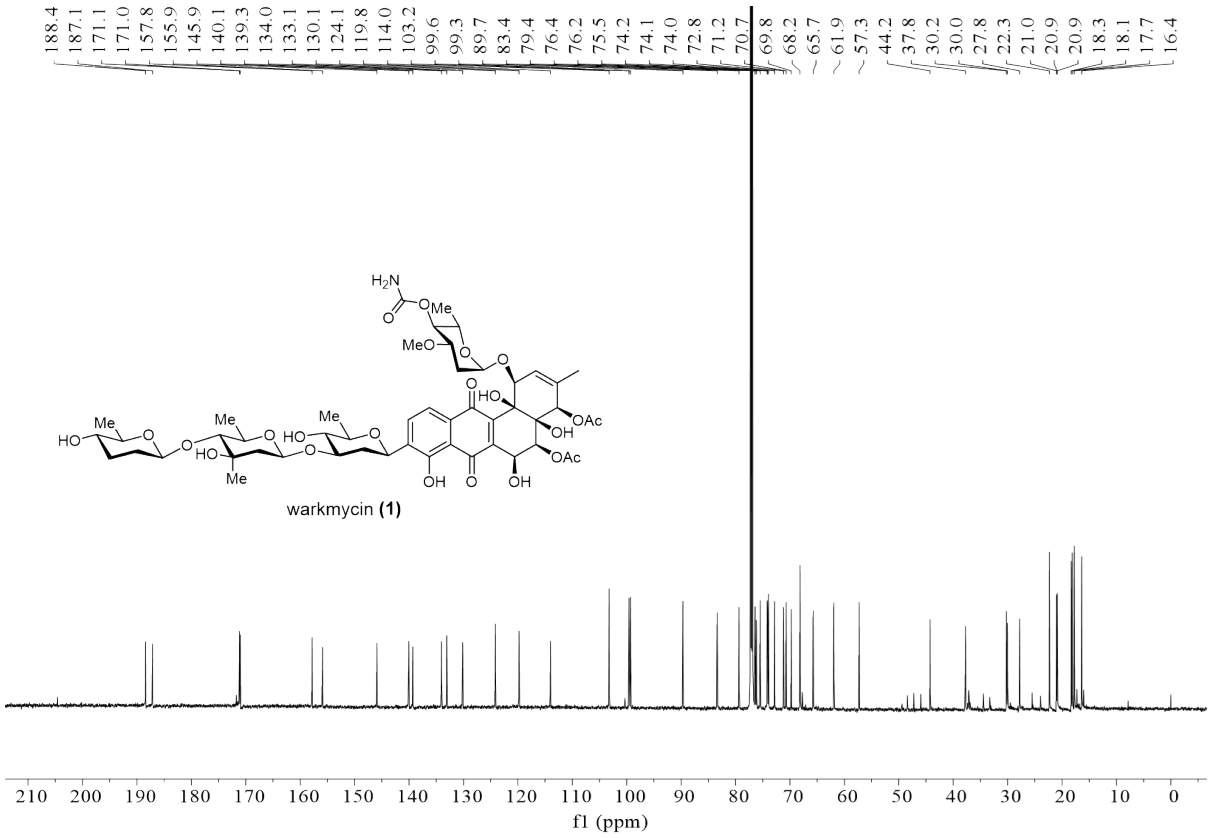


Figure S40. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **1**

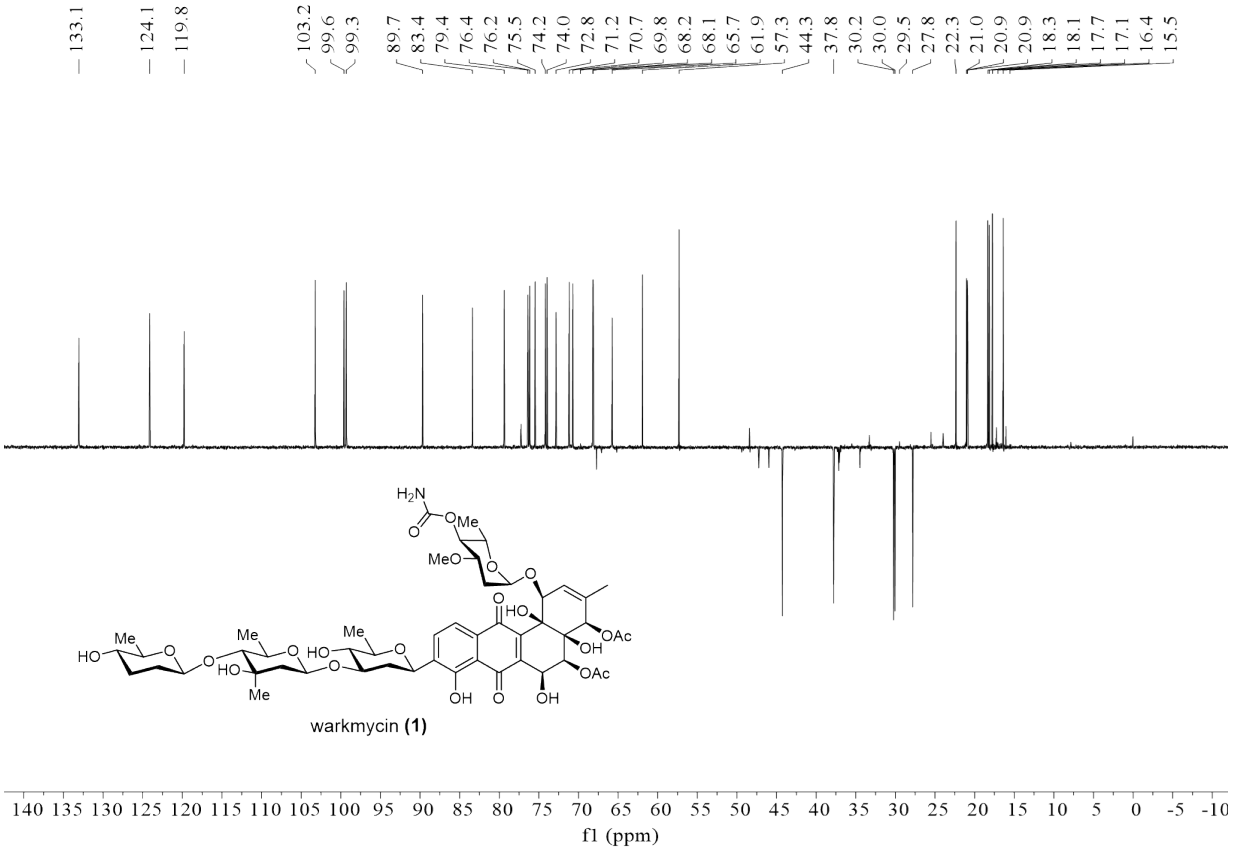


Figure S41. DEPT 135 spectrum of compound **1**

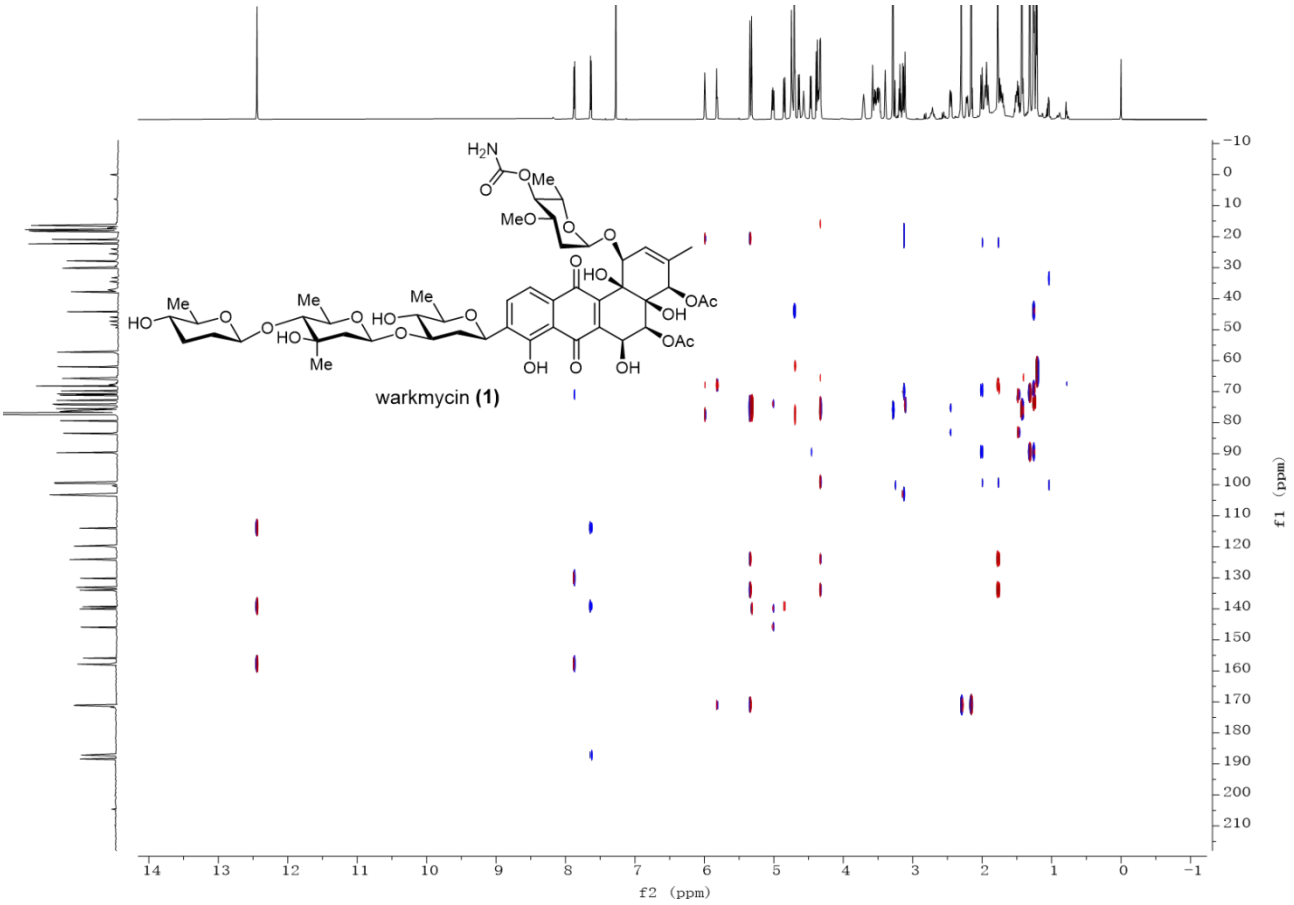


Figure S42. HSQC spectrum of compound **1**

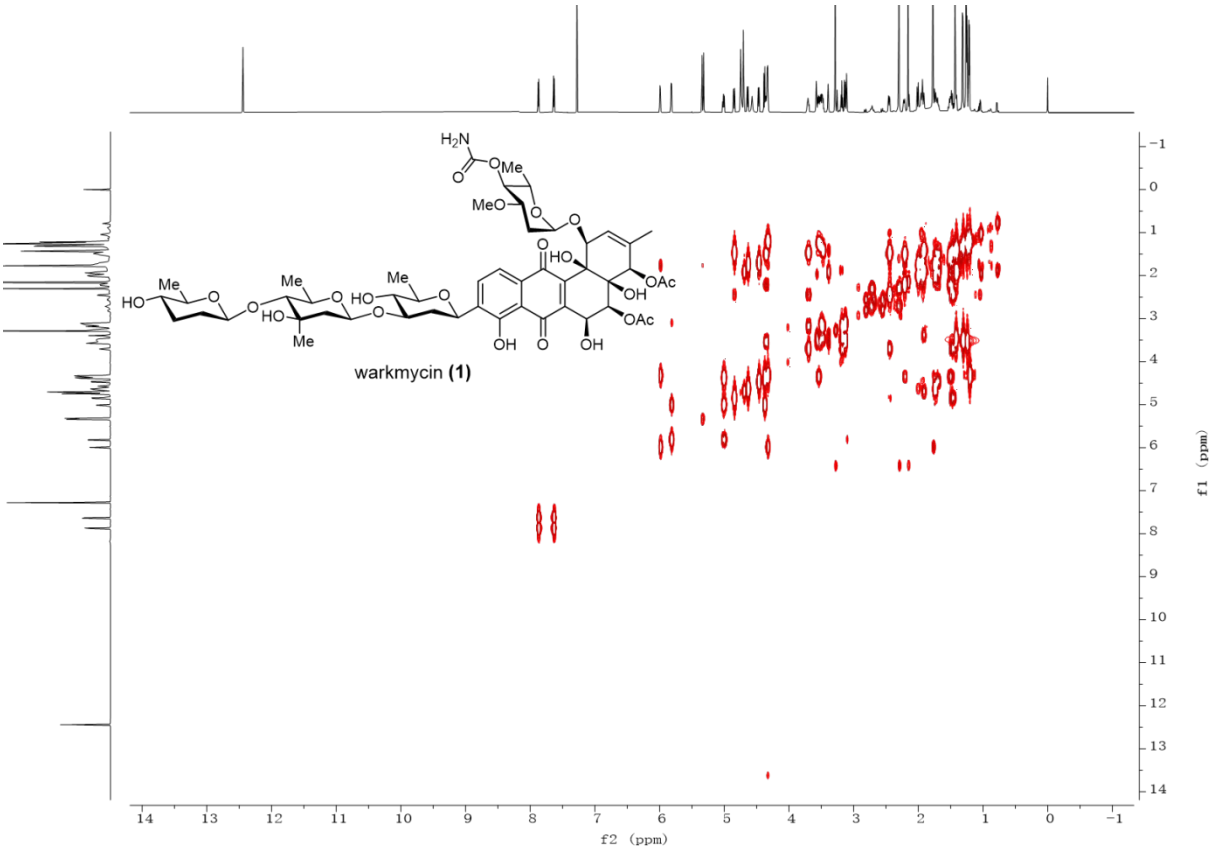


Figure S43. HMBC spectrum of compound **1**

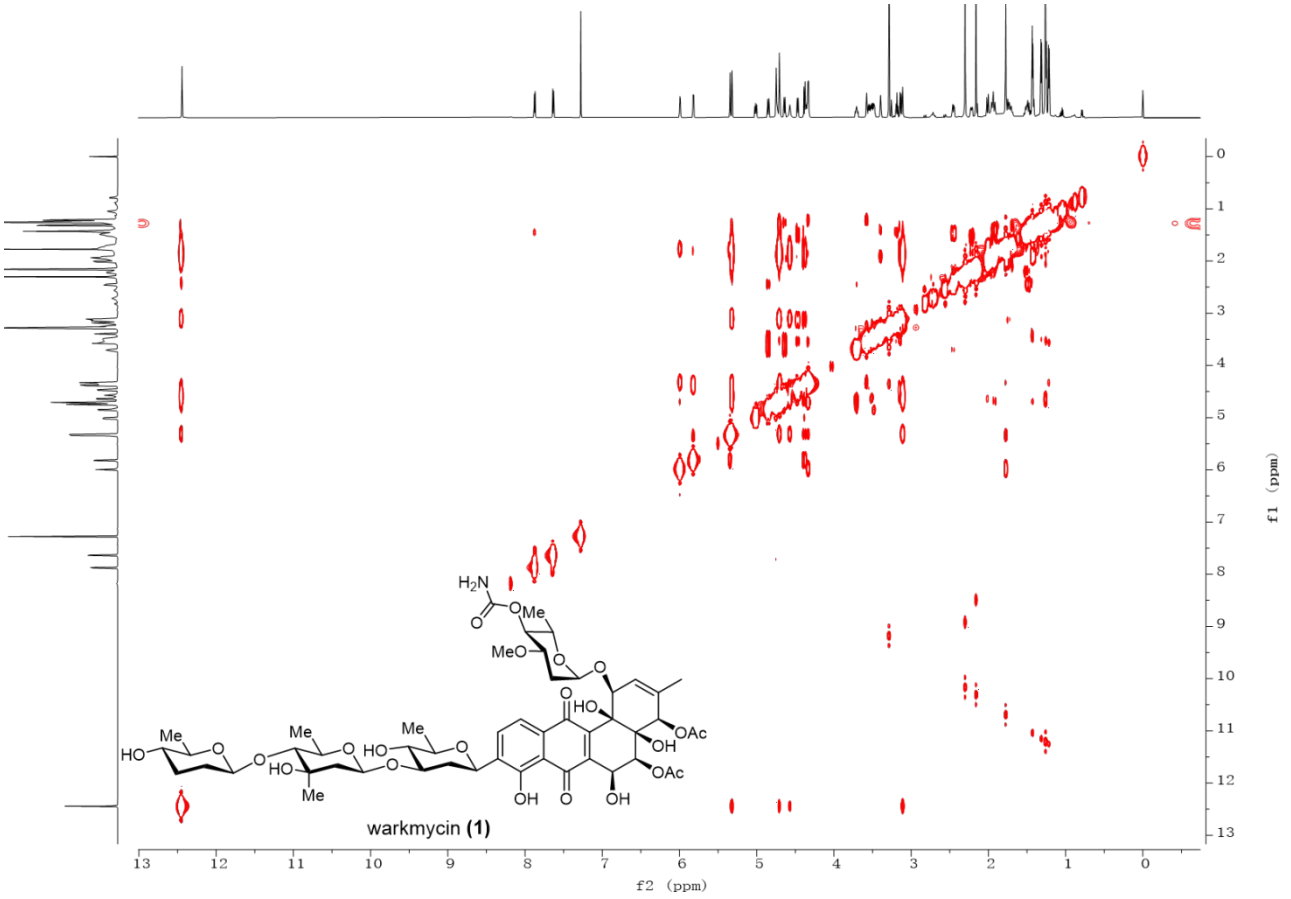


Figure S44. ^{1}H-^{1}H COSY spectrum of compound **1**

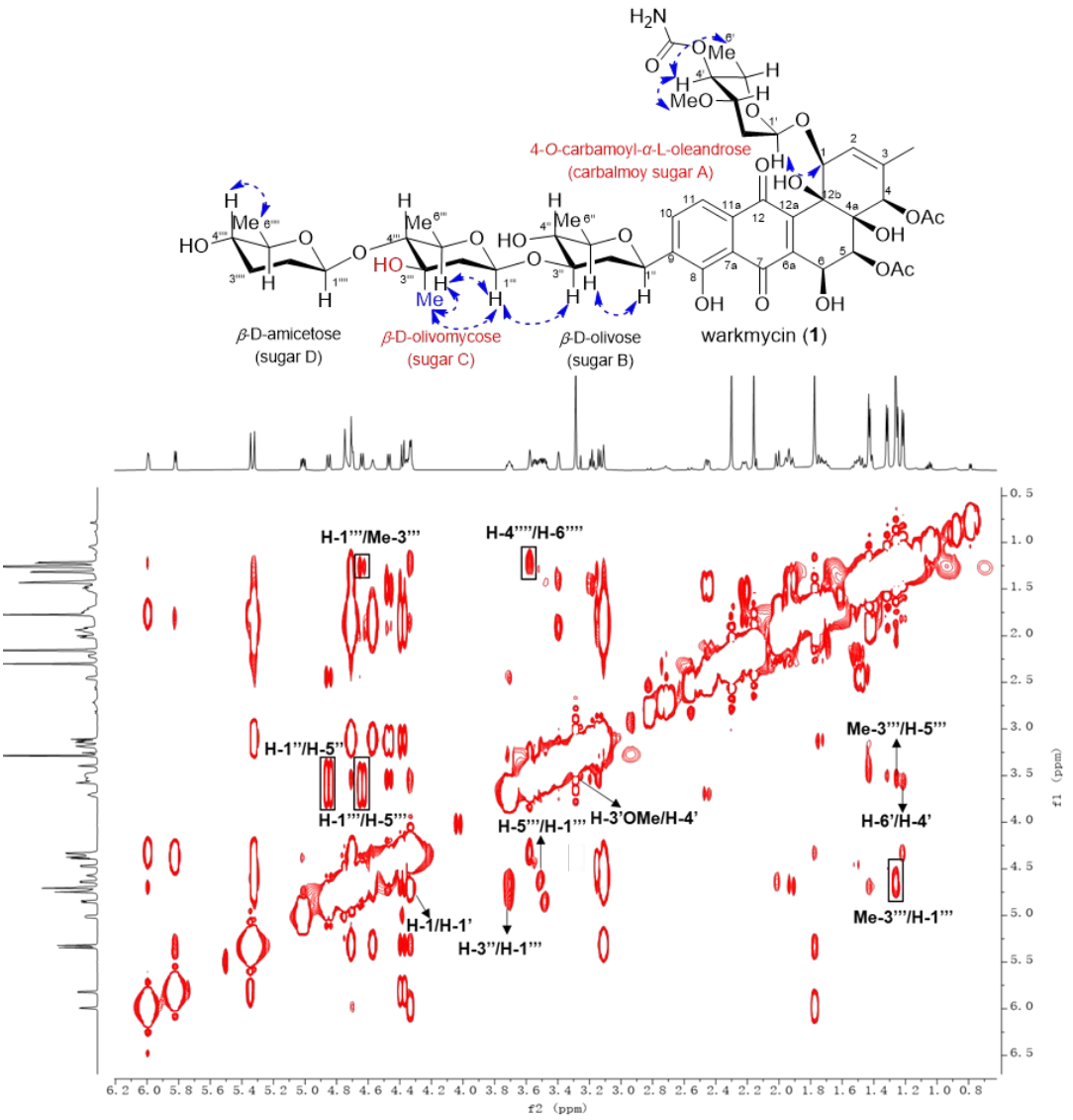


Figure S45. NOESY spectrum of compound **1**

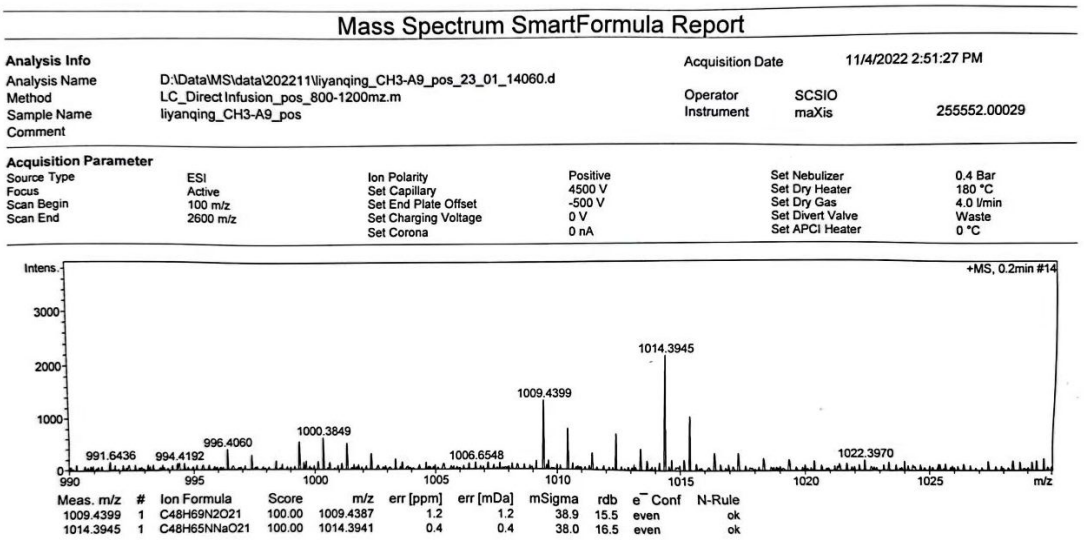


Figure S46. HRESIMS spectrum of compound **2**

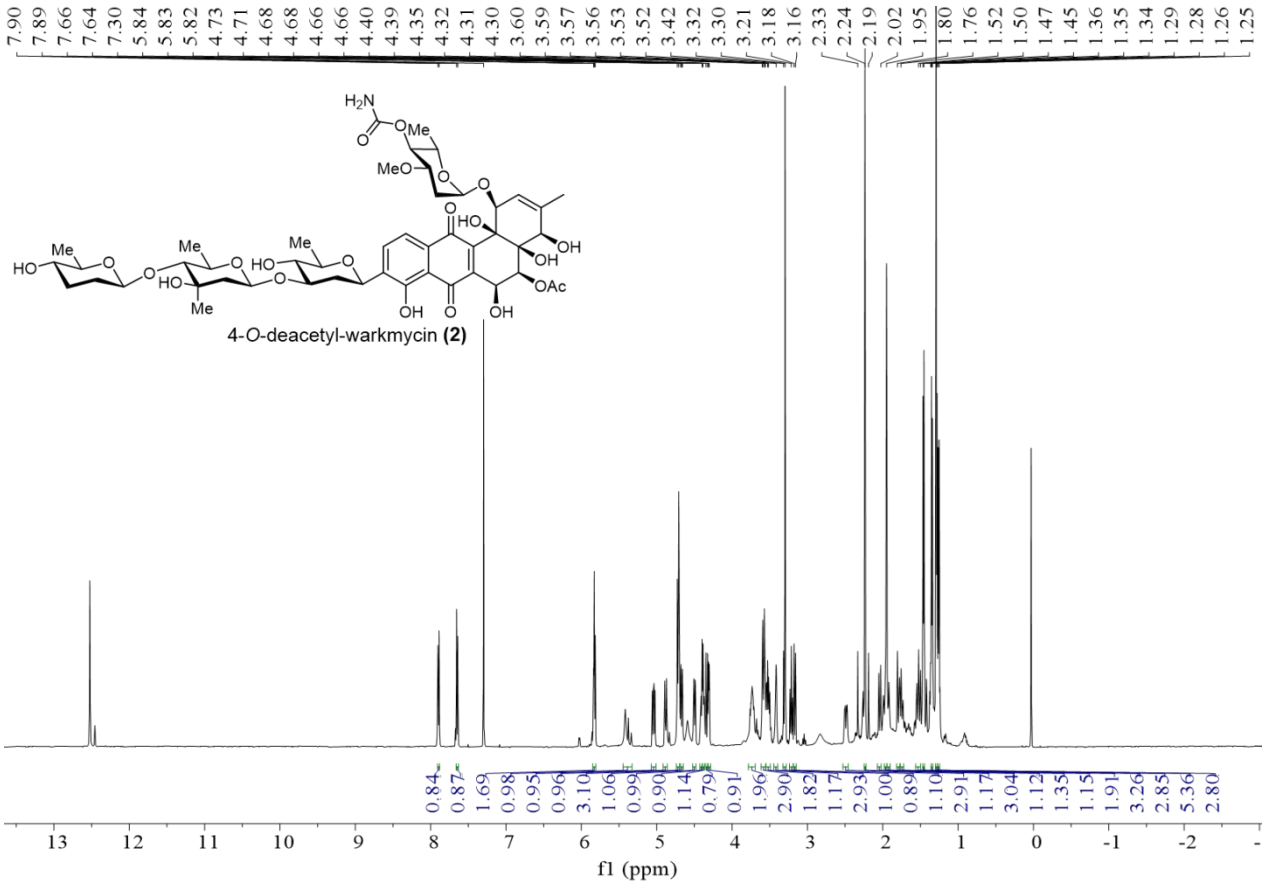


Figure S47. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **2**

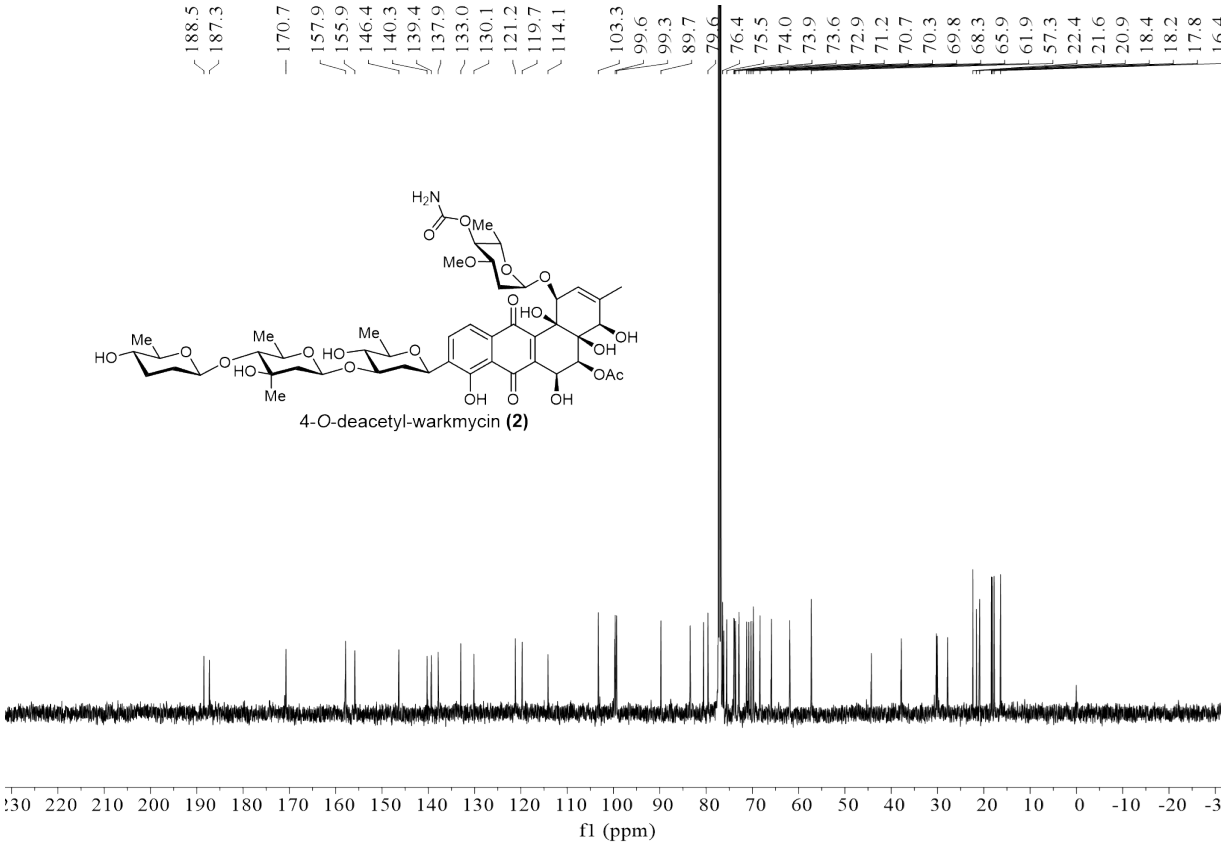


Figure S48. ^{13}C NMR (176 MHz, Chloroform-*d*) spectrum of compound **2**

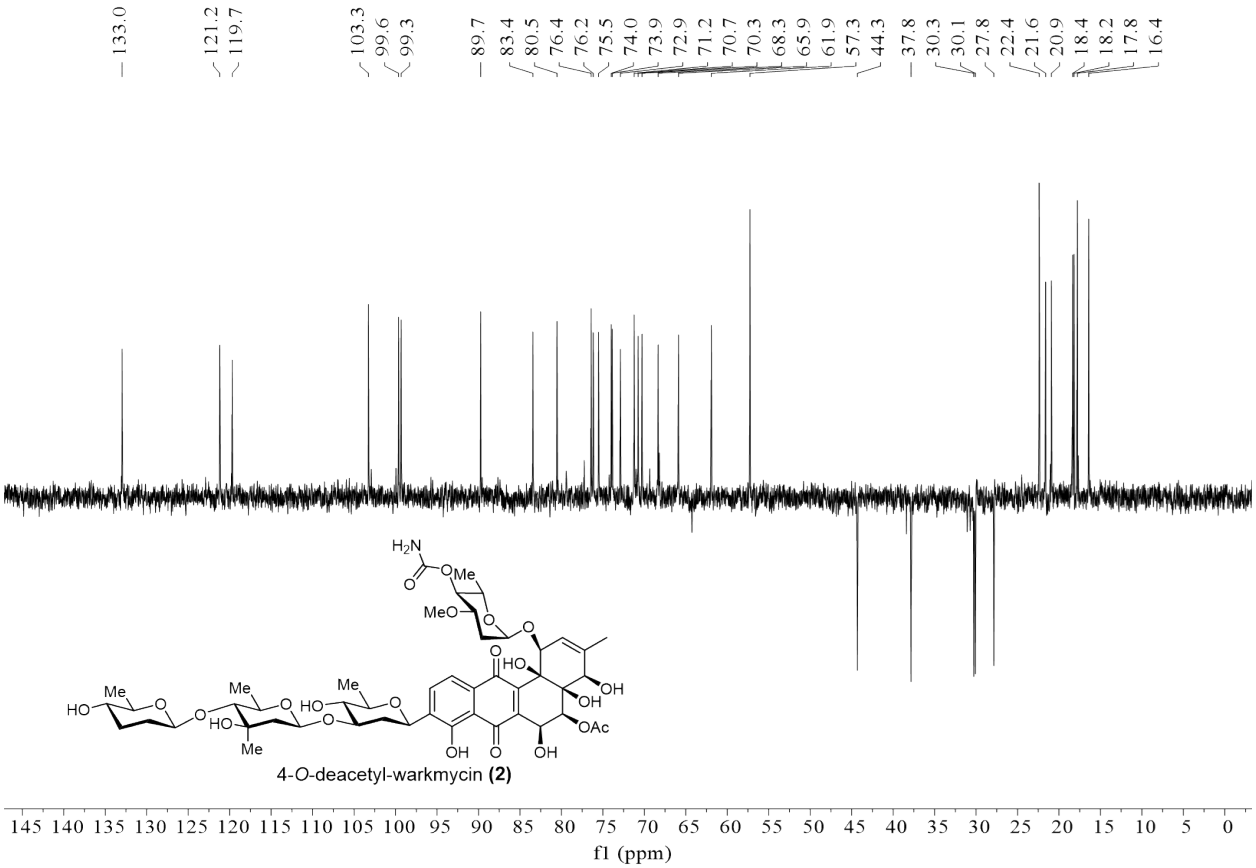


Figure S49. DEPT 135 spectrum of compound **2**

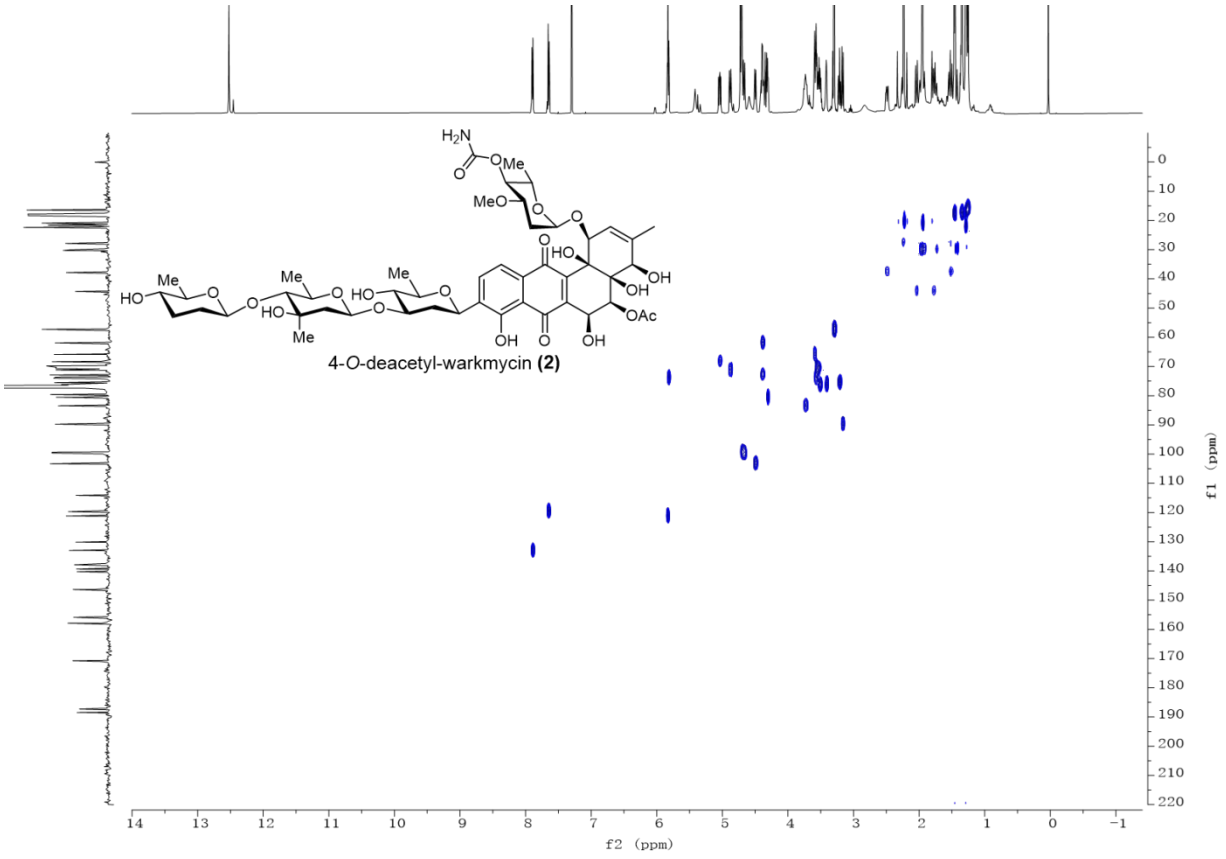


Figure S50. HSQC spectrum of compound **2**

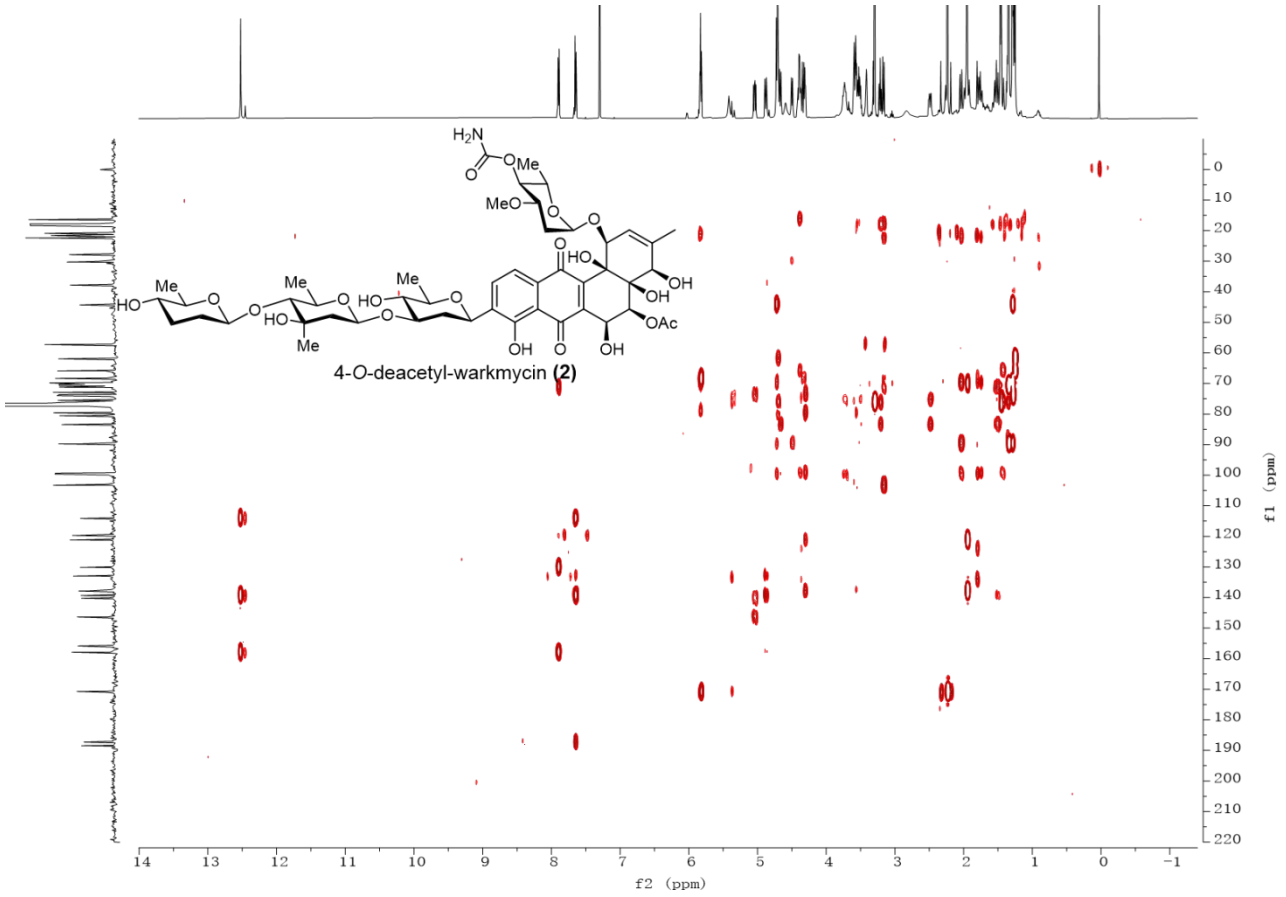


Figure S51. HMBC spectrum of compound **2**

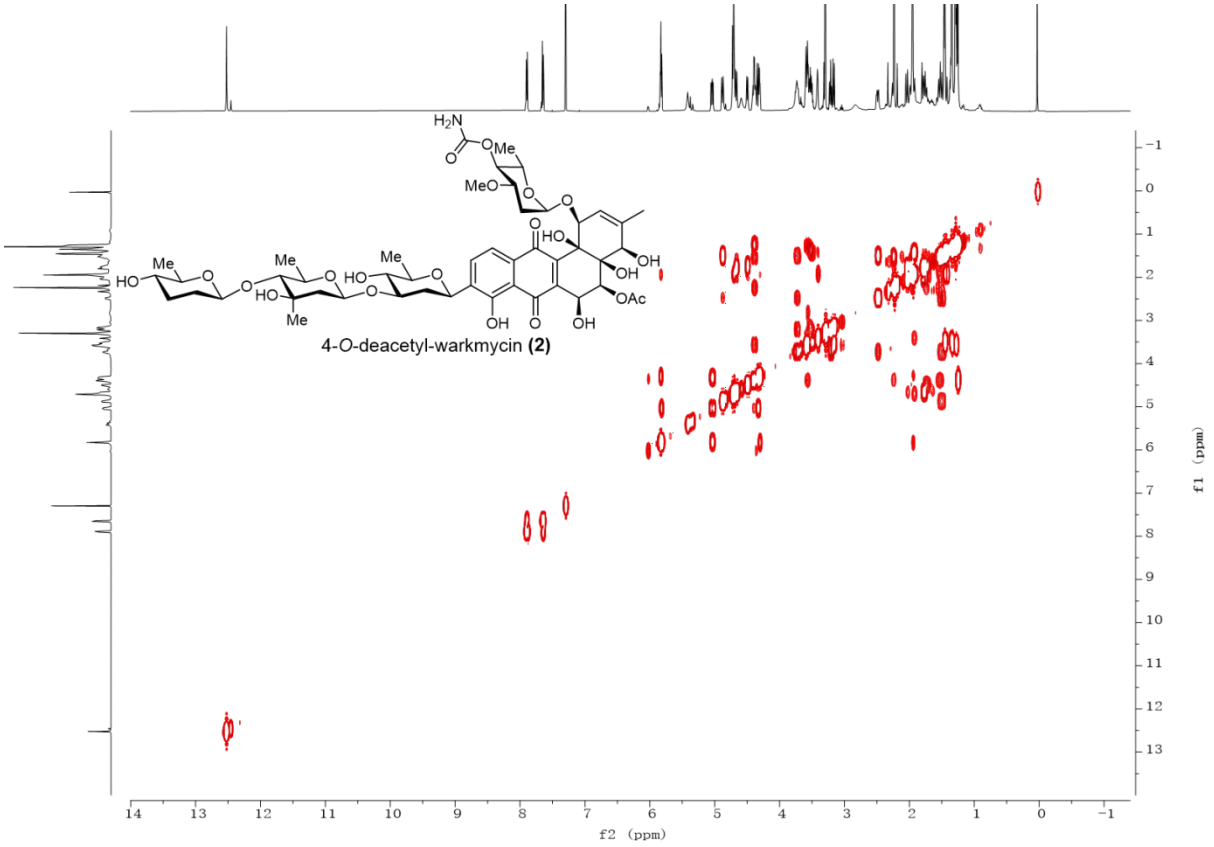


Figure S52. ^{1}H-^{1}H COSY spectrum of compound **2**

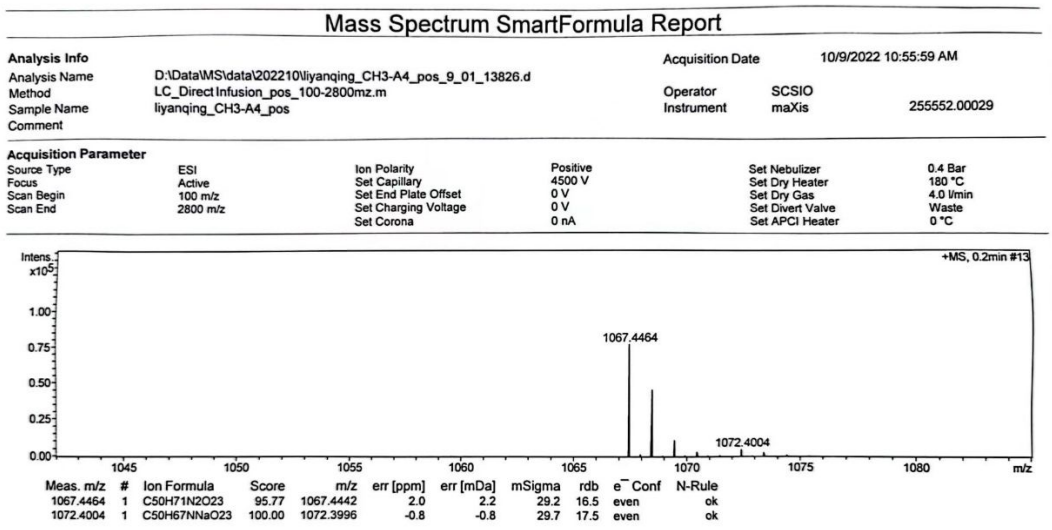


Figure S53. HRESIMS spectrum of compound **3**

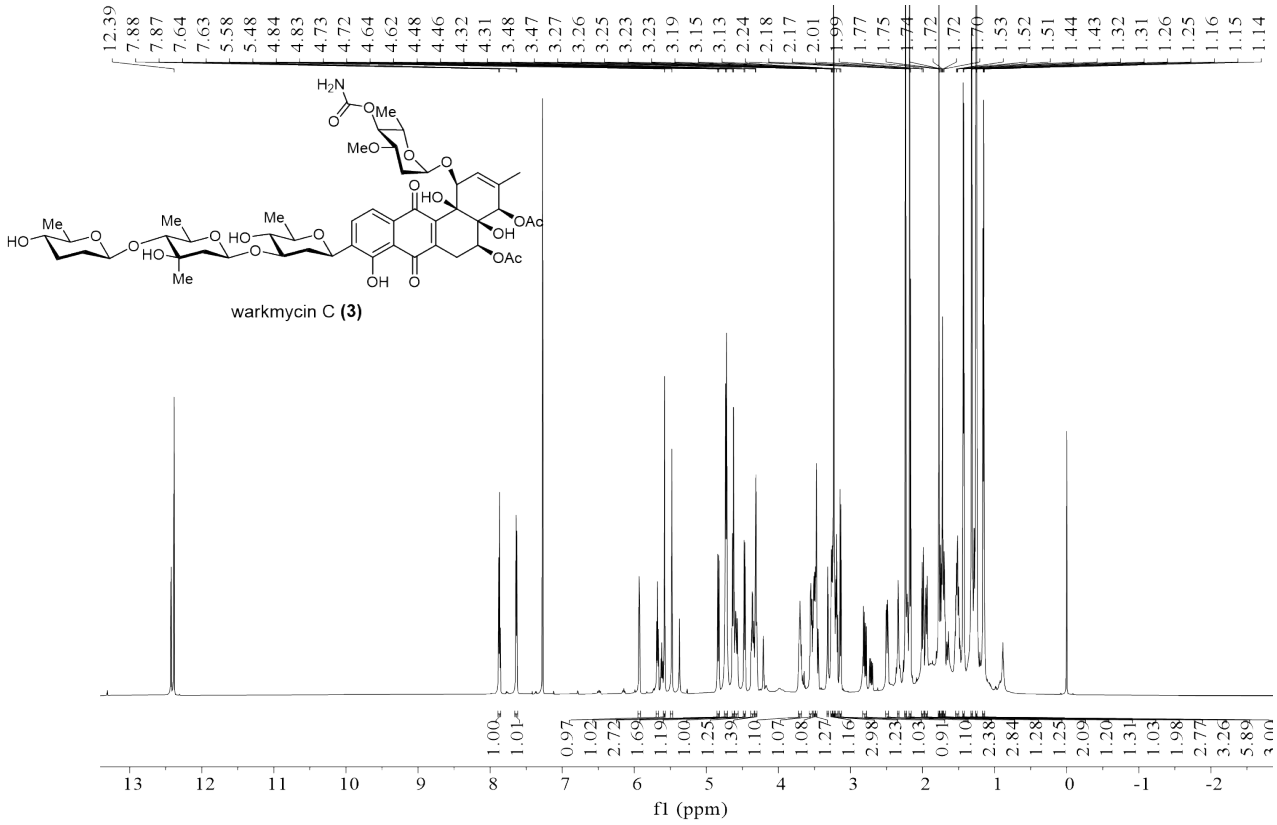


Figure S54. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **3**

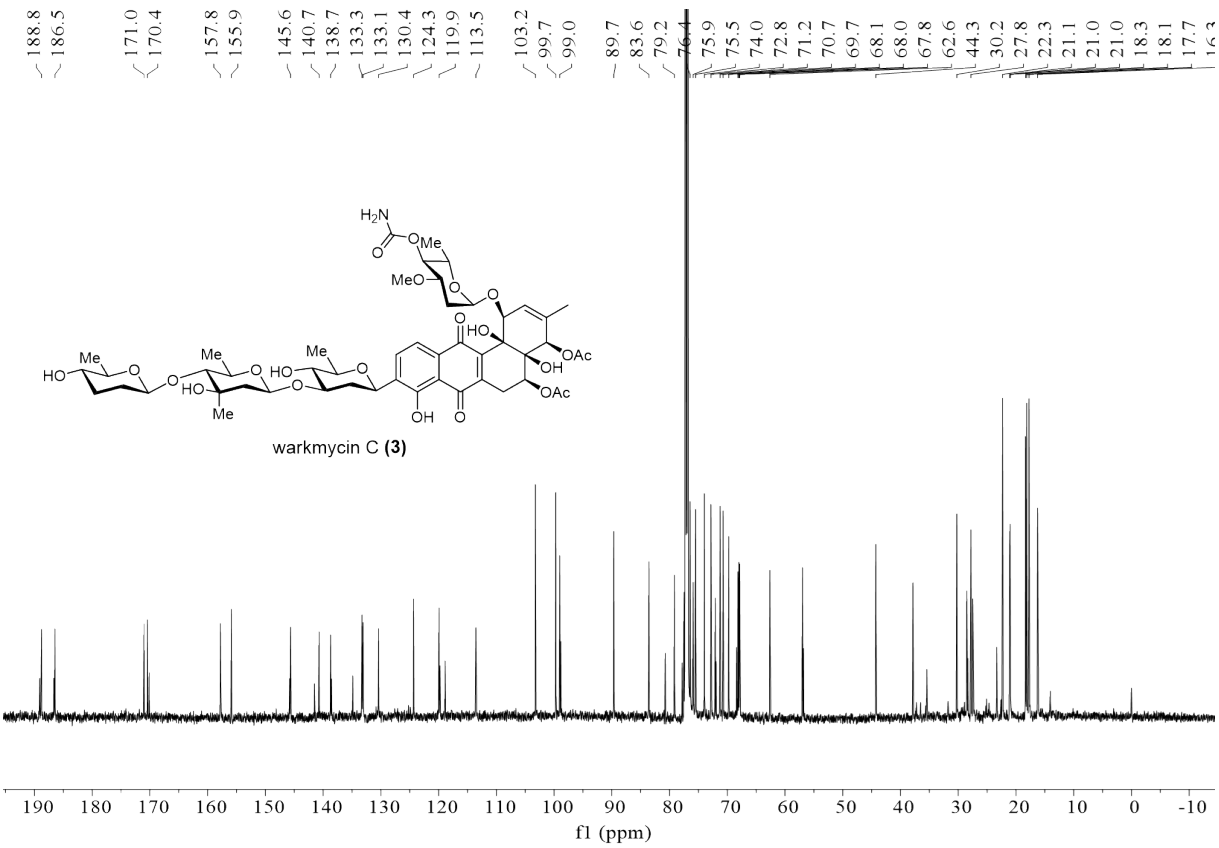


Figure S55. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **3**

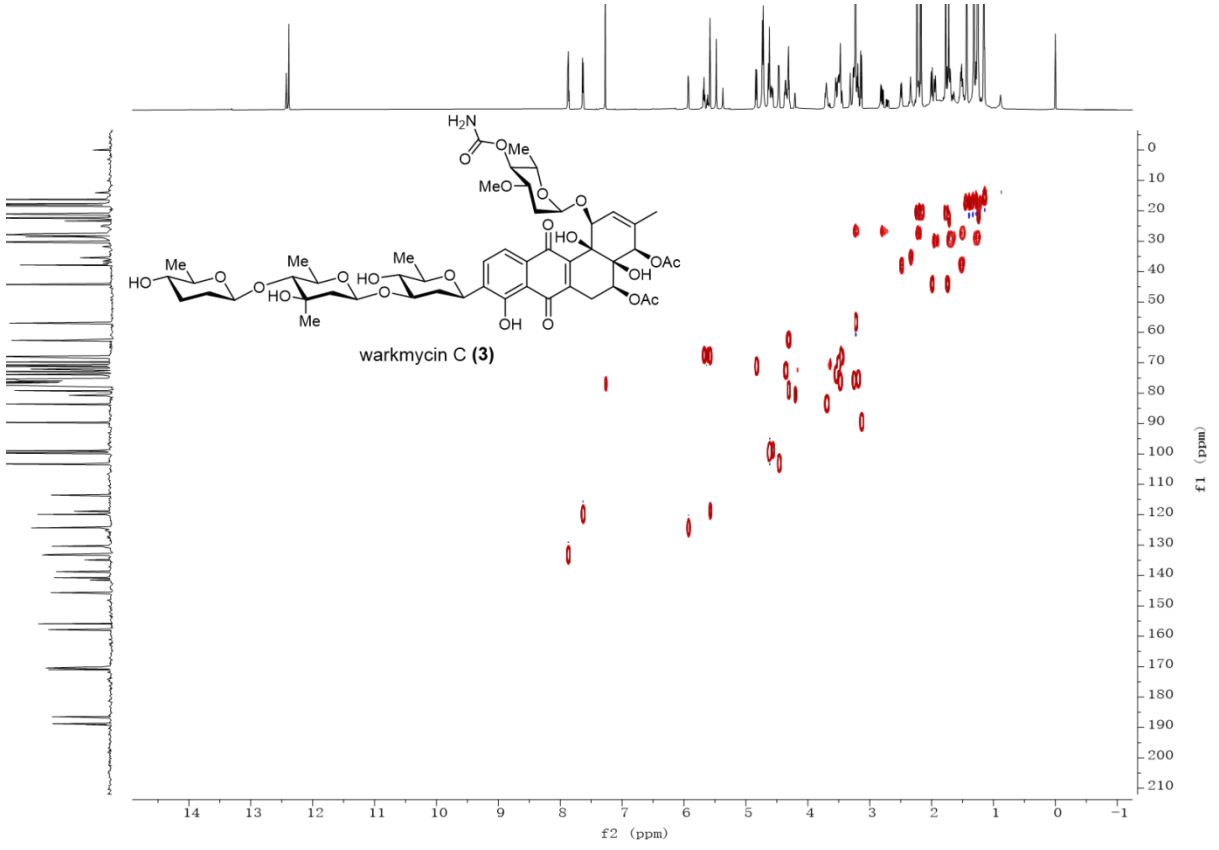


Figure S56. HSQC spectrum of compound **3**

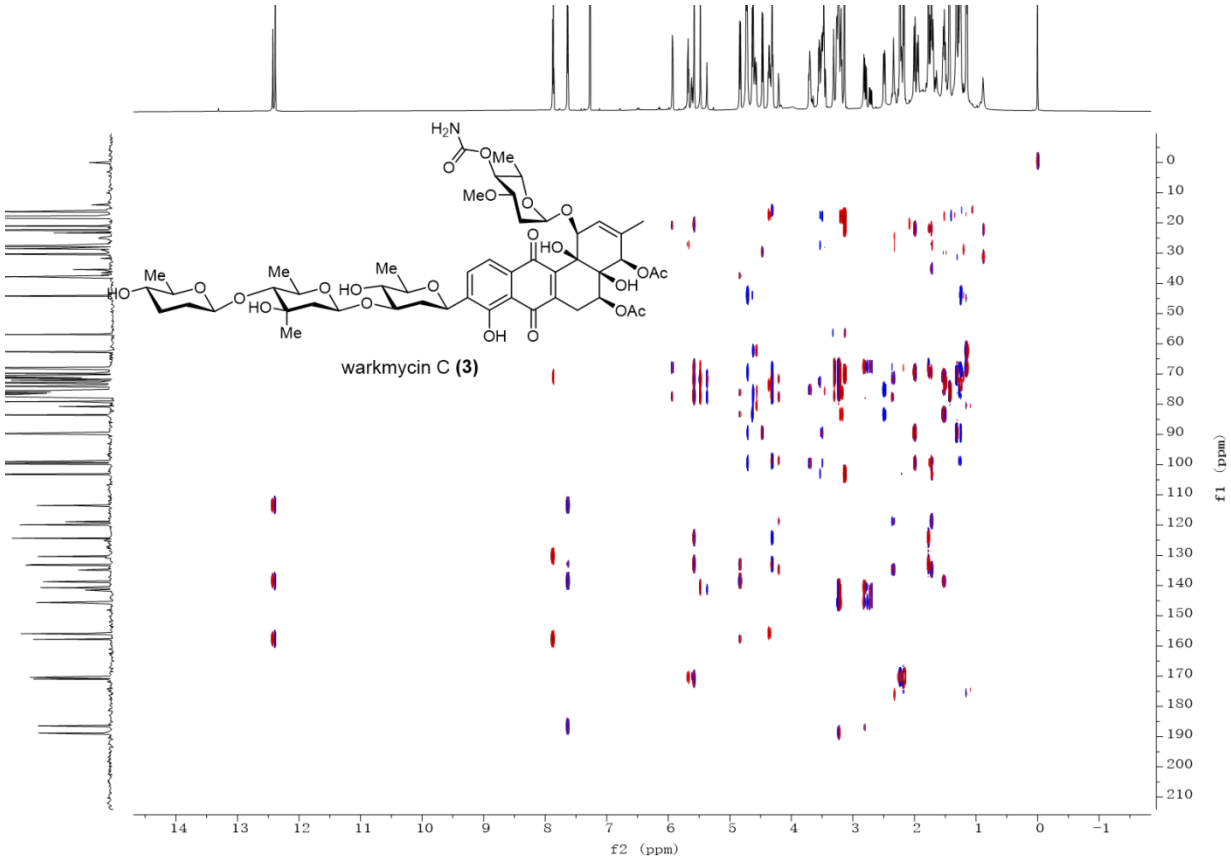


Figure S57. HMBC spectrum of compound **3**

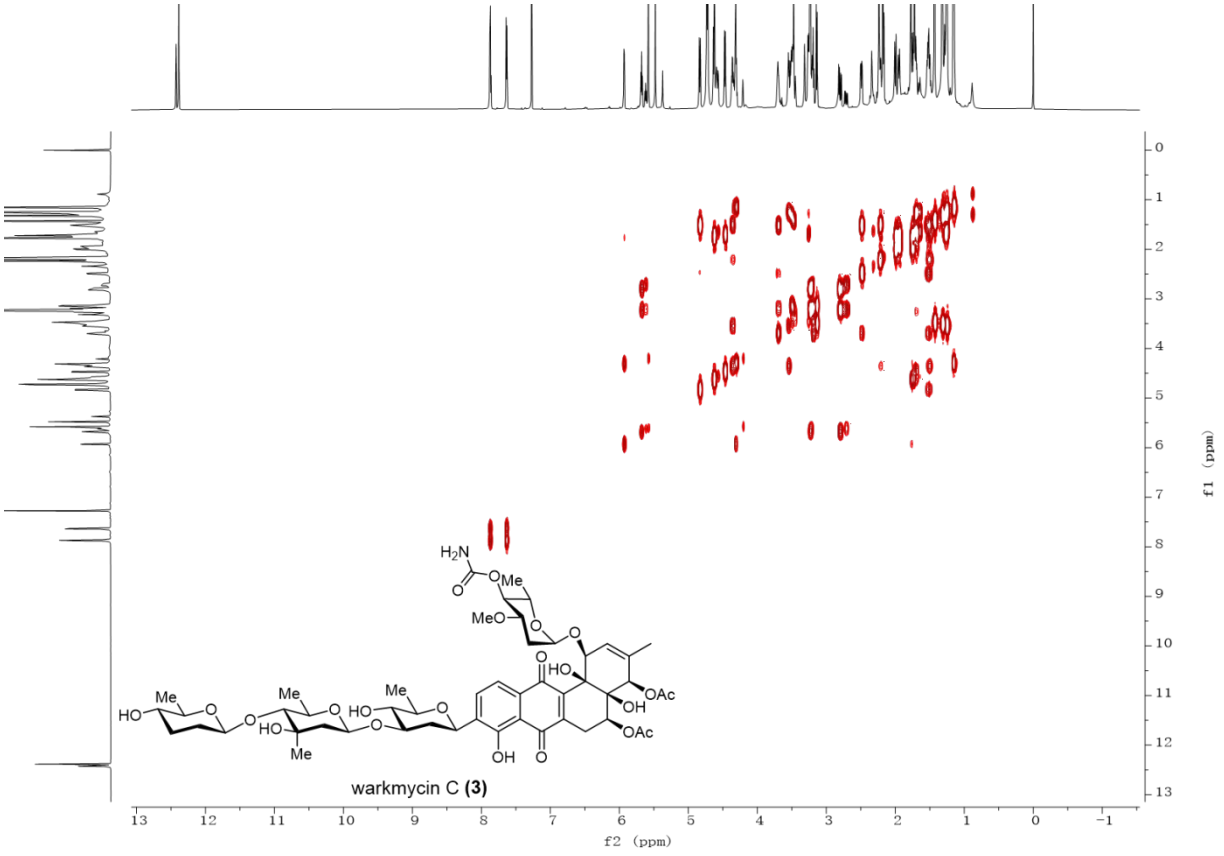


Figure S58. ^{1}H-^{1}H COSY spectrum of compound **3**

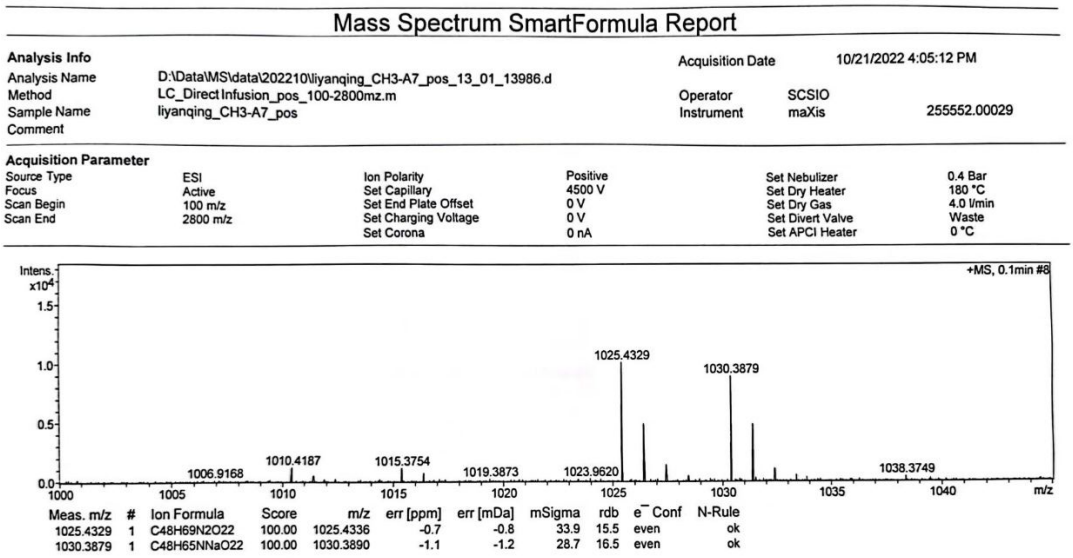


Figure S59. HRESIMS spectrum of compound **4**

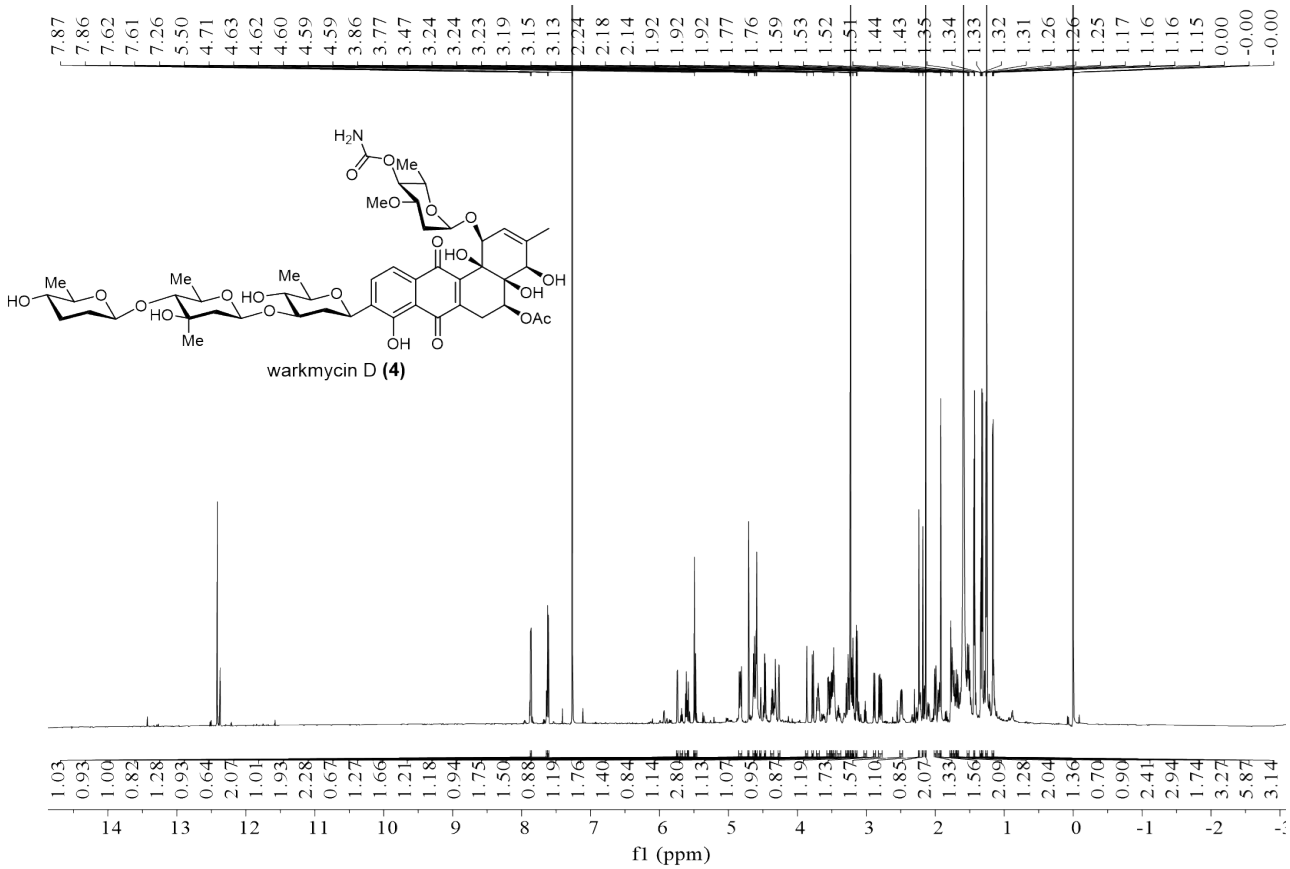


Figure S60. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **4**

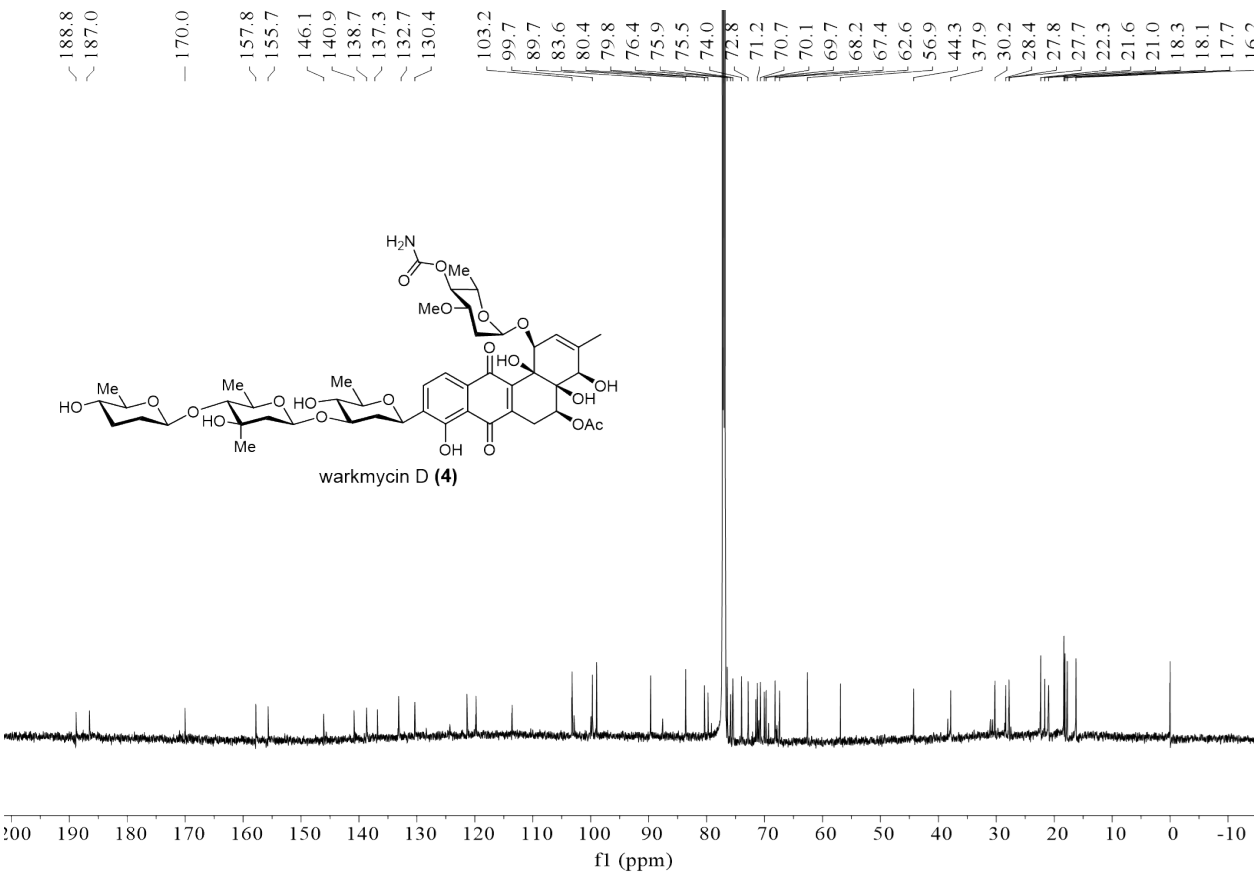


Figure S61. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **4**

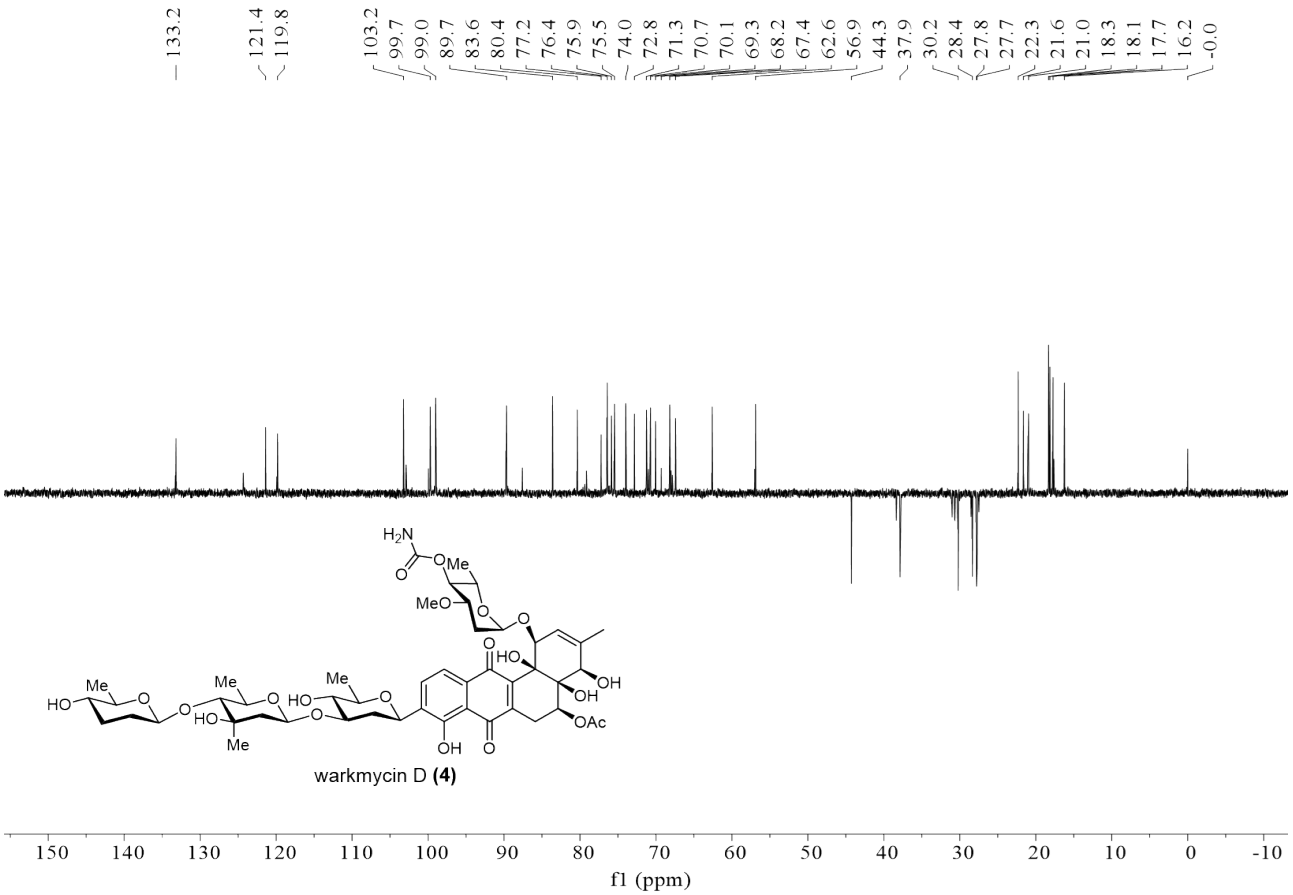


Figure S62. DEPT 135 spectrum of compound **4**

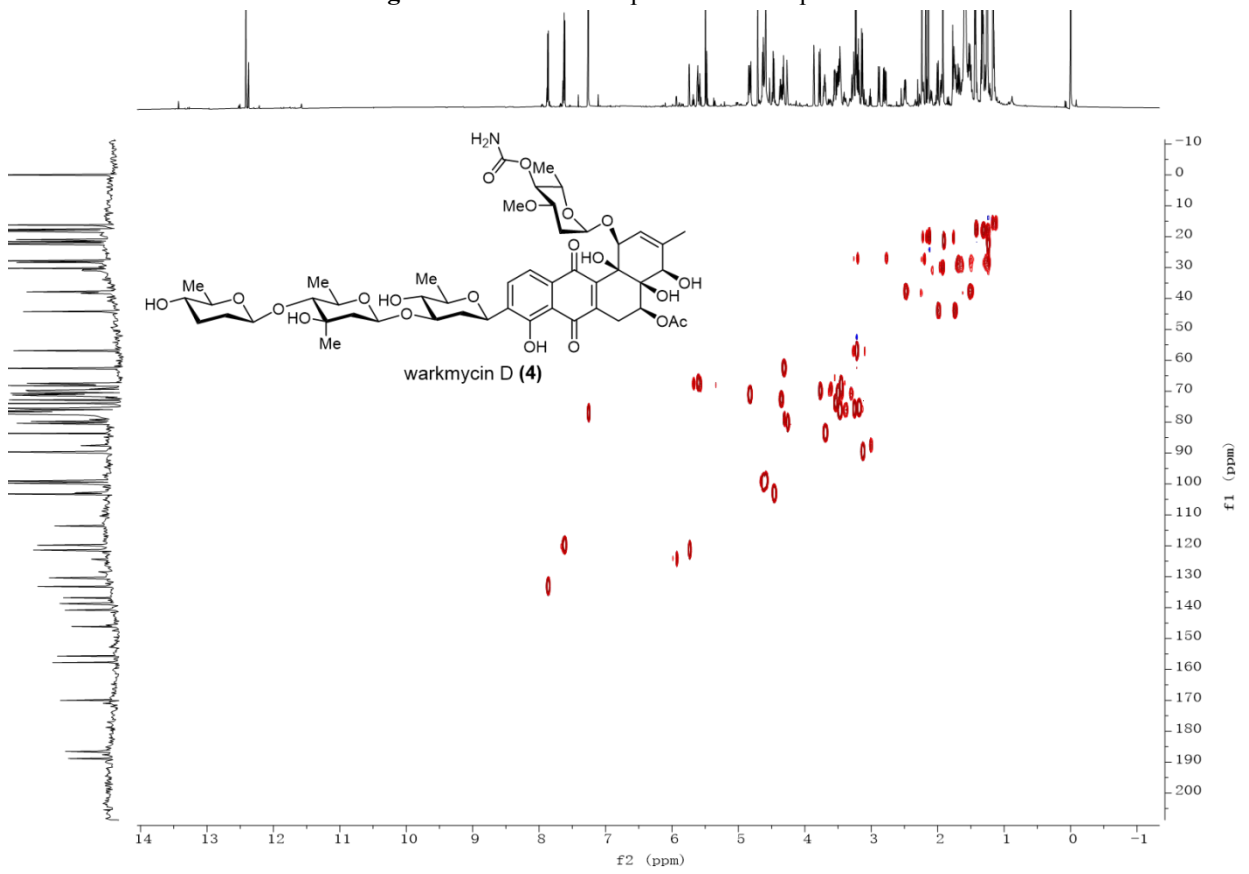


Figure S63. HSQC spectrum of compound **4**

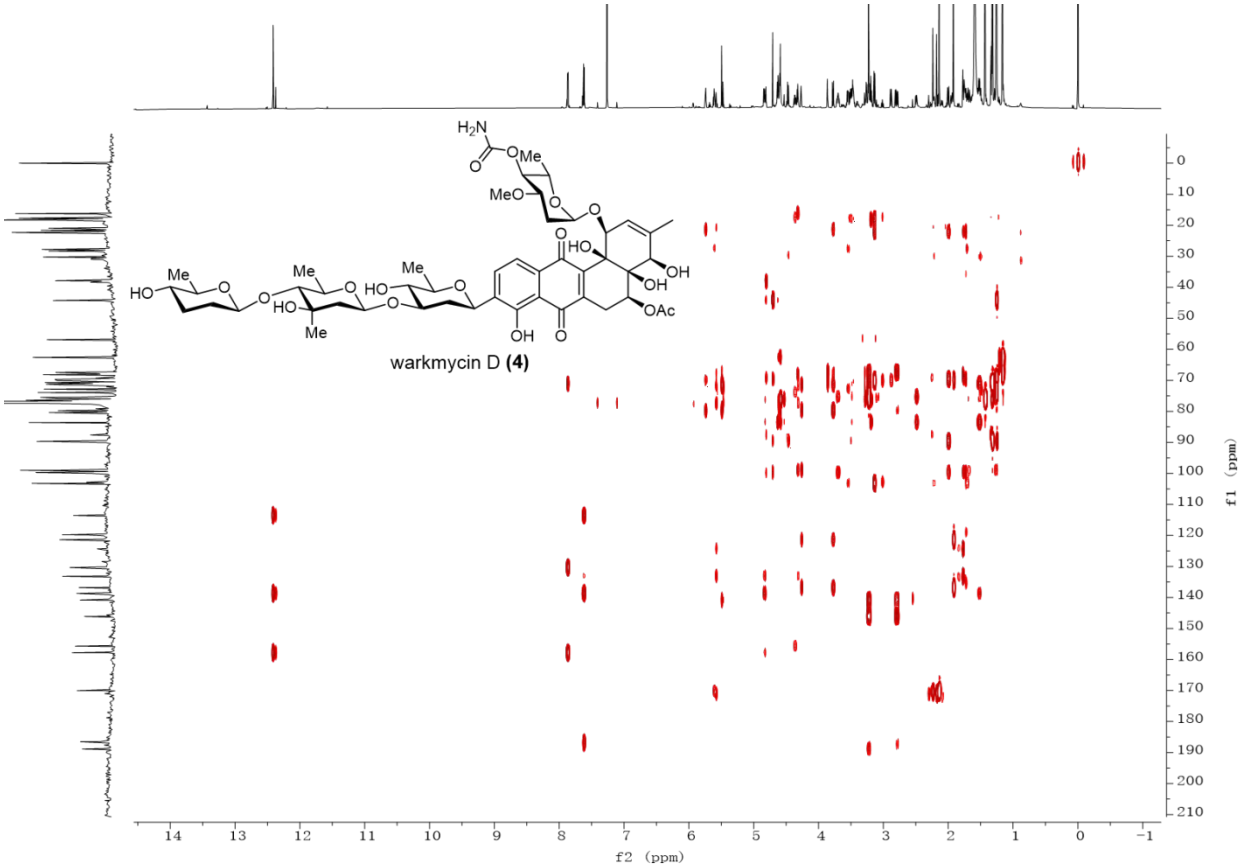


Figure S64. HMBC spectrum of compound **4**

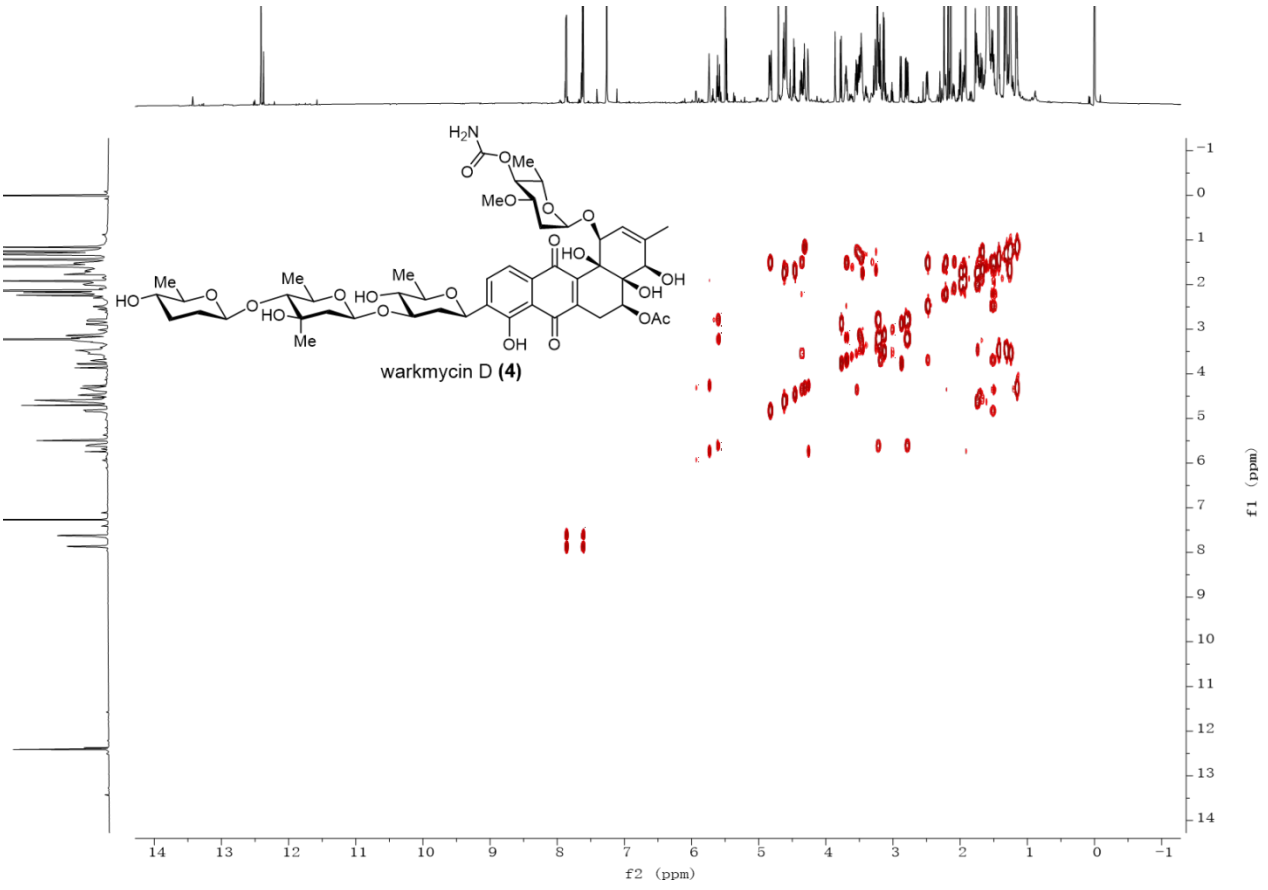


Figure S65. ^{1}H-^{1}H COSY spectrum of compound **4**

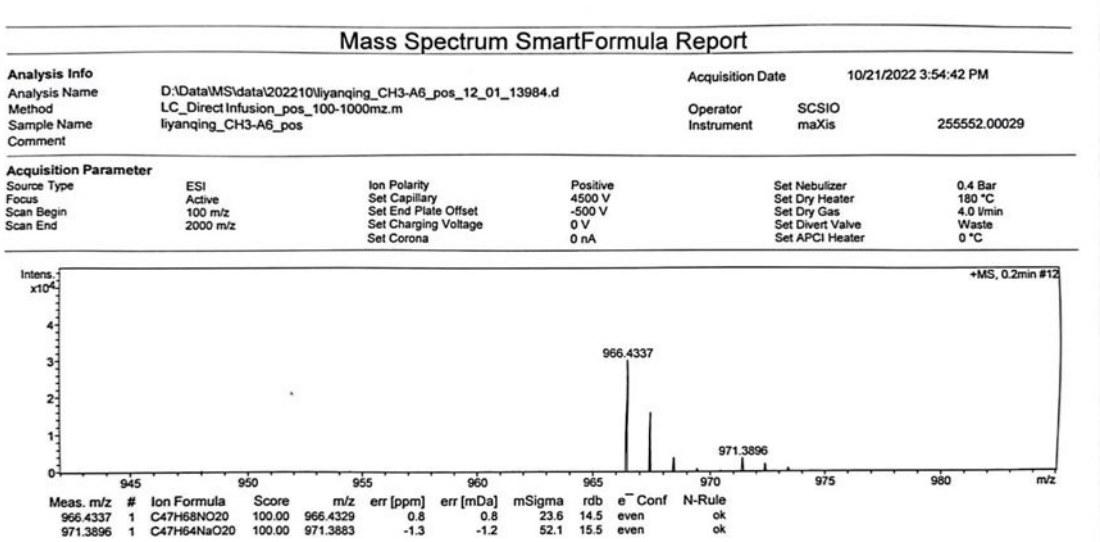


Figure S66. HRESIMS spectrum of compound **5**

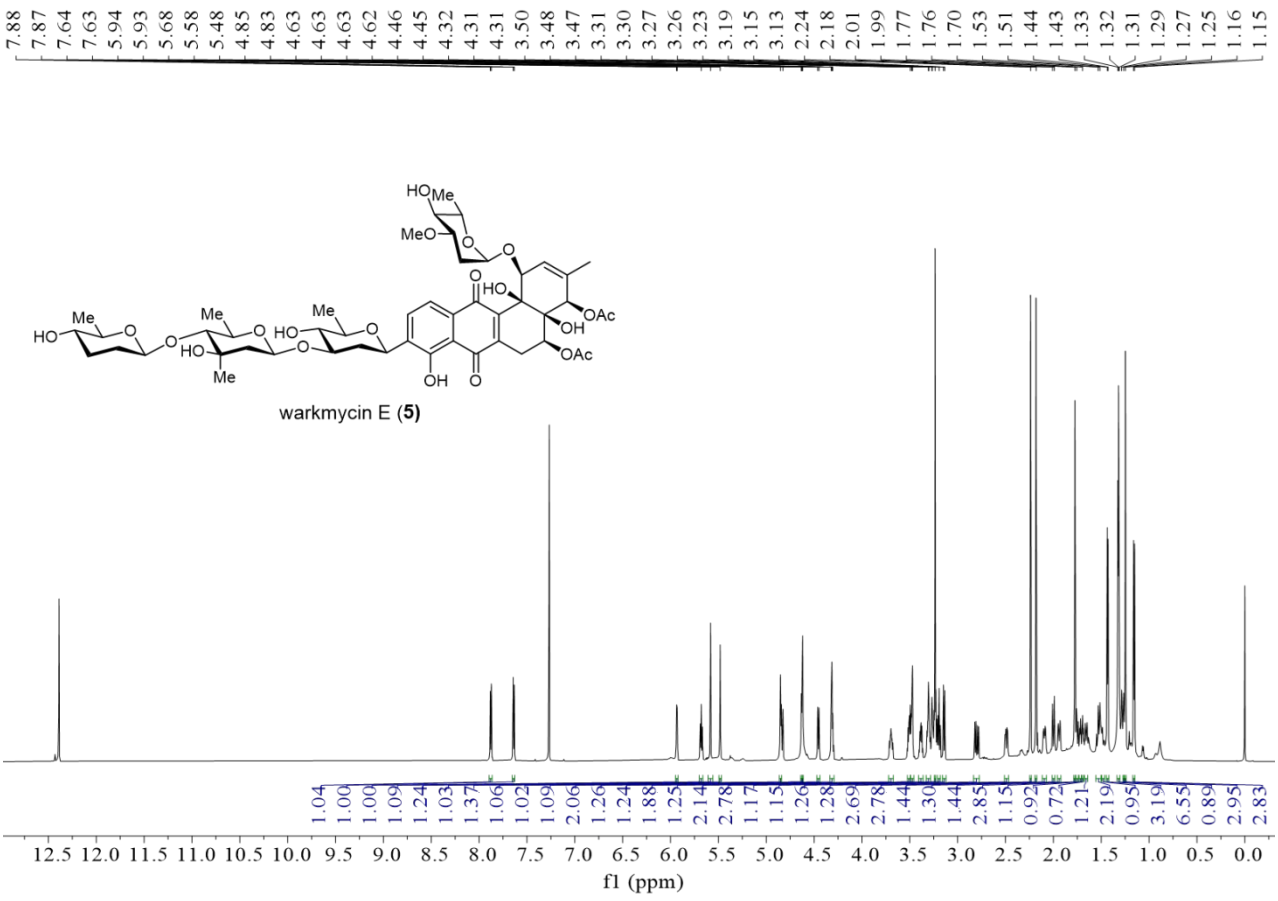


Figure S67. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **5**

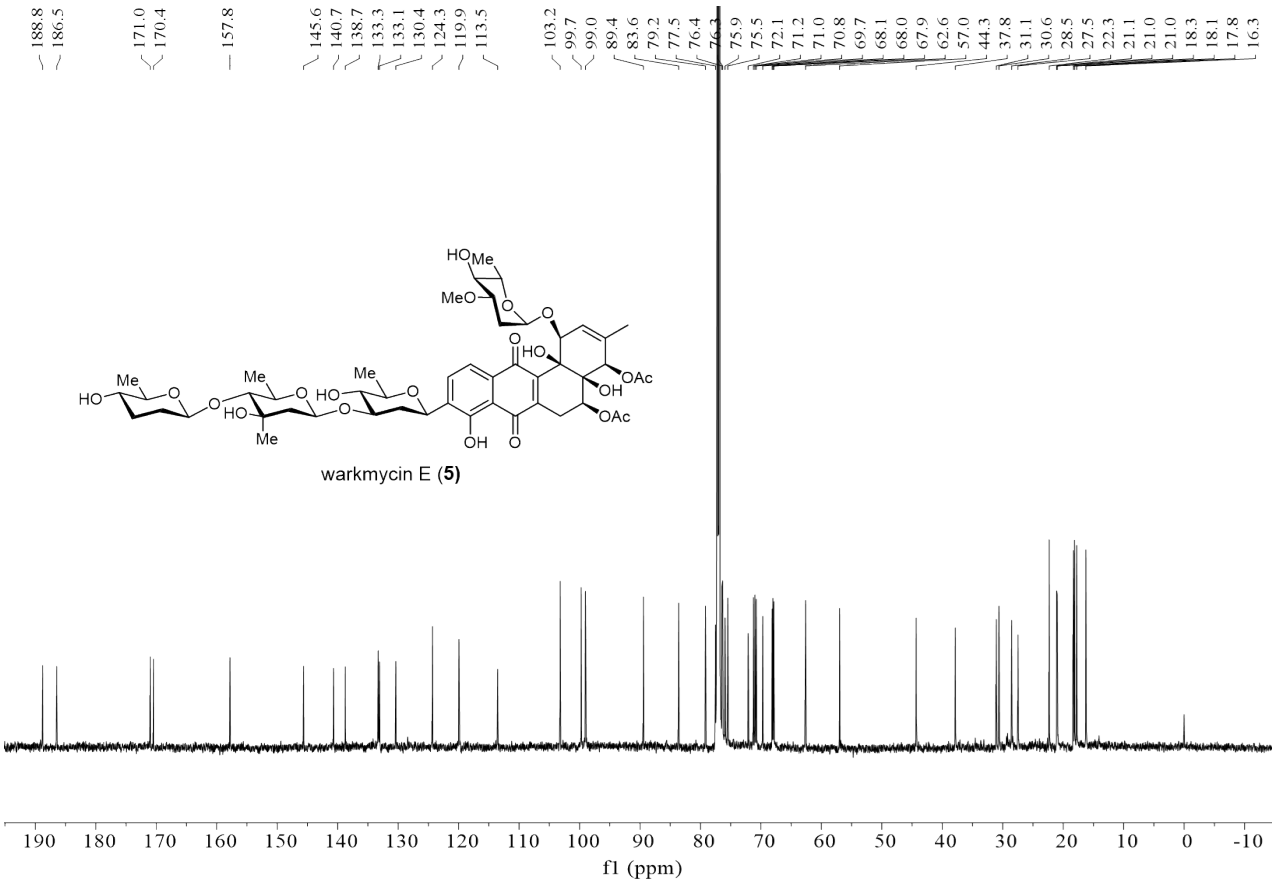


Figure S68. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **5**

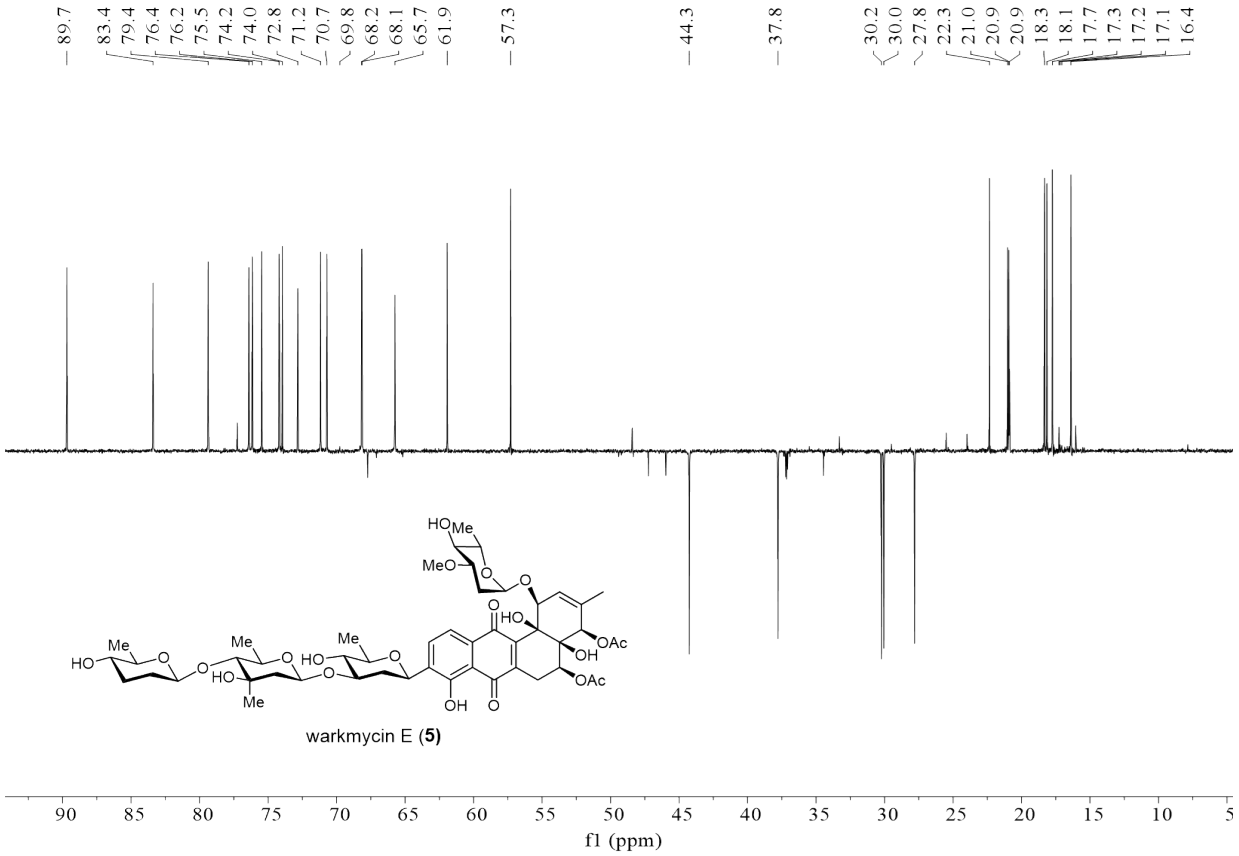


Figure S69. DEPT135 spectrum of compound **5**

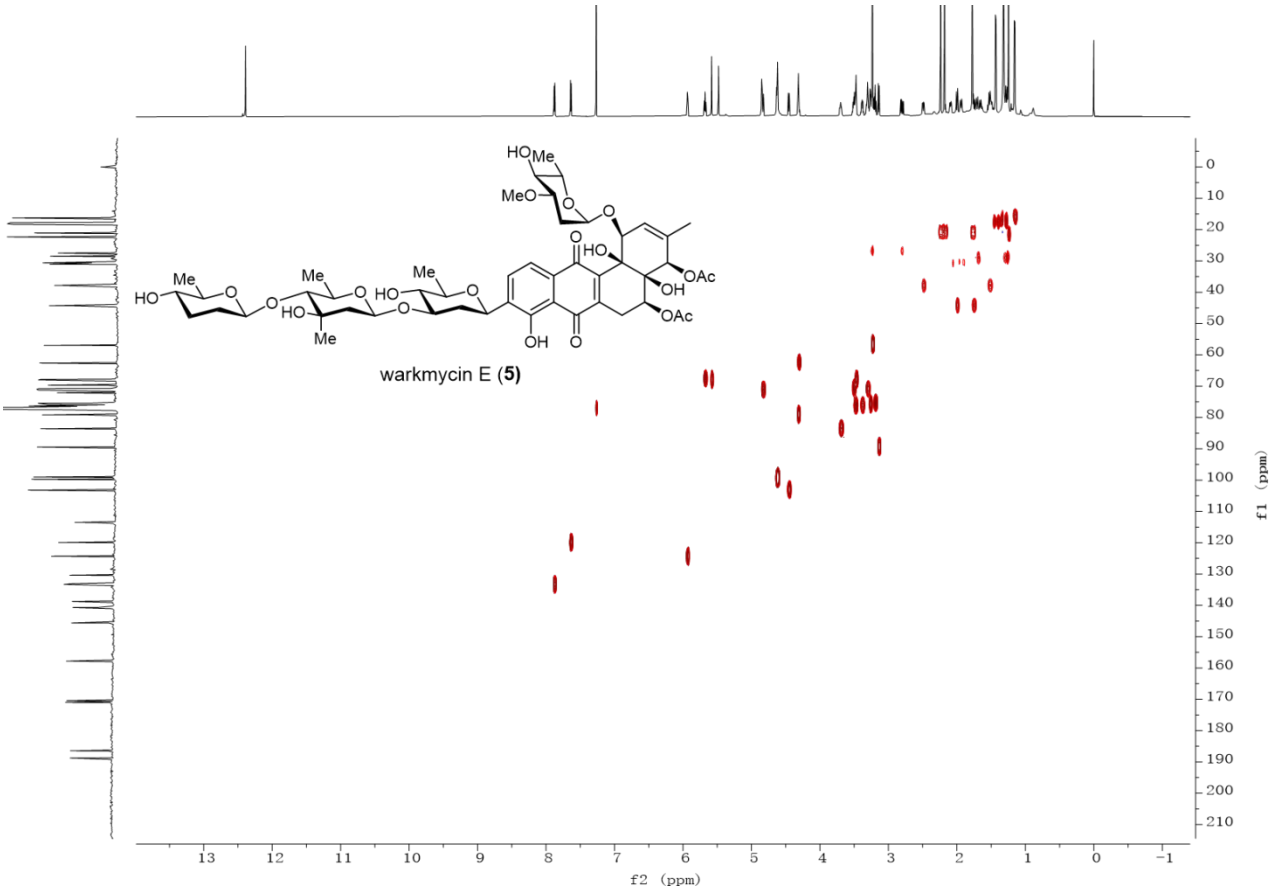


Figure S70. HSQC spectrum of compound **5**

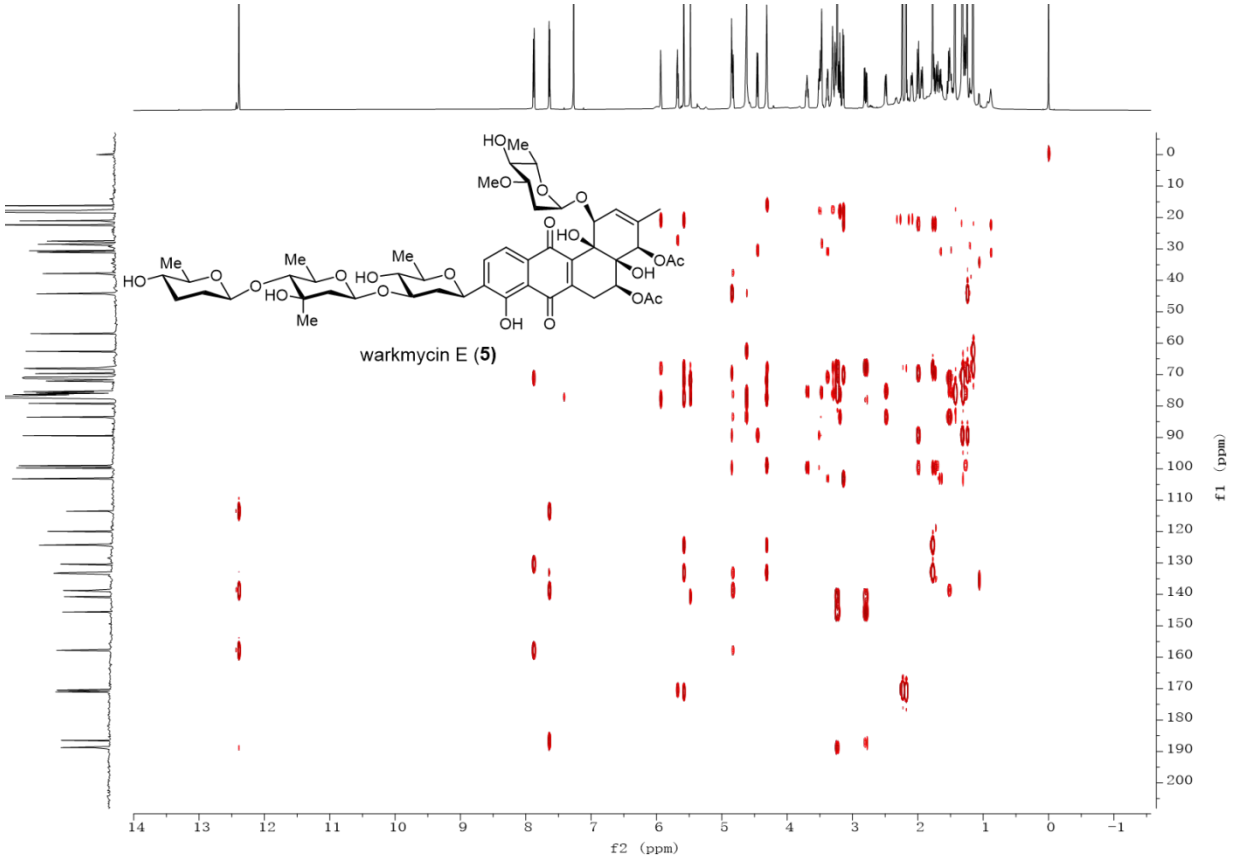


Figure S71. HMBC spectrum of compound **5**

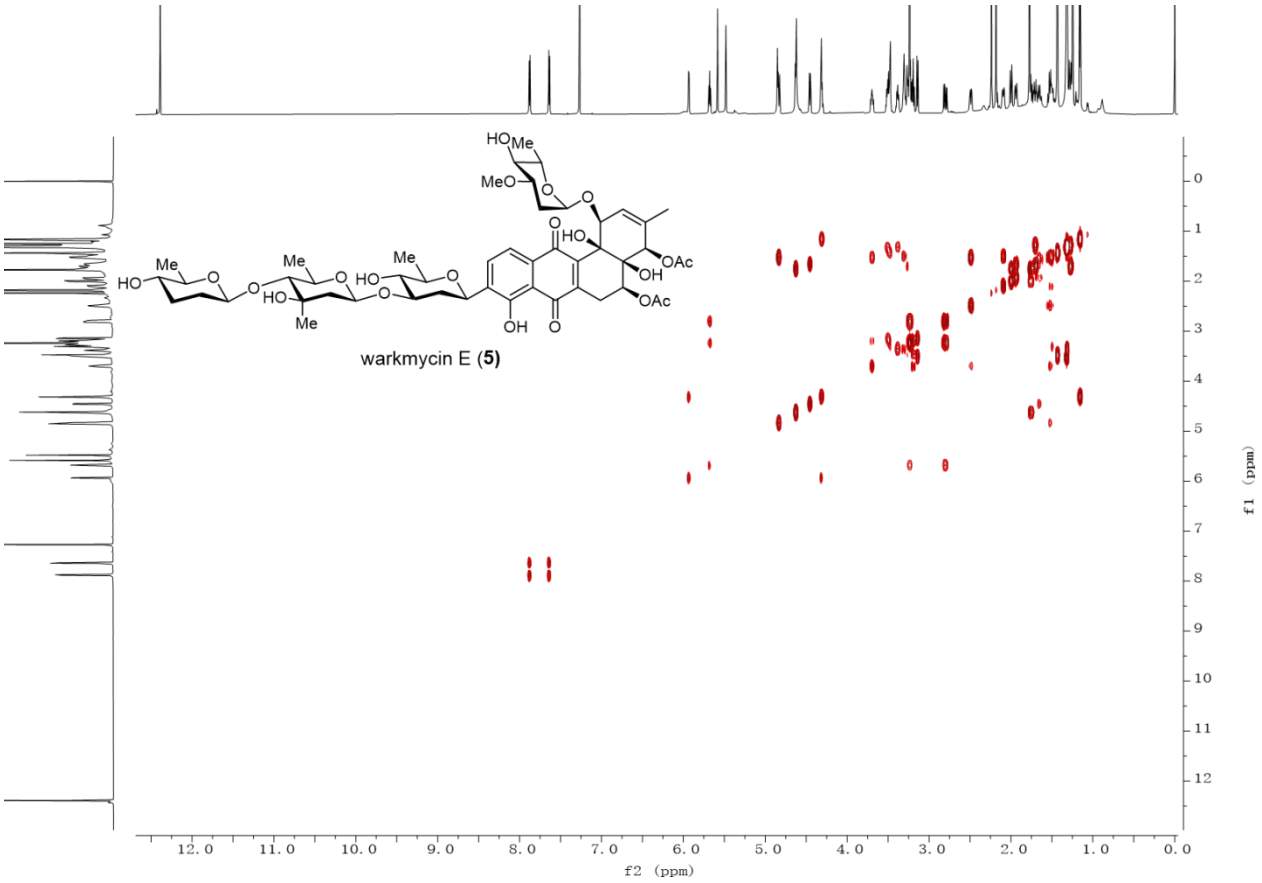


Figure S72. ^{1}H-^{1}H COSY spectrum of compound **5**

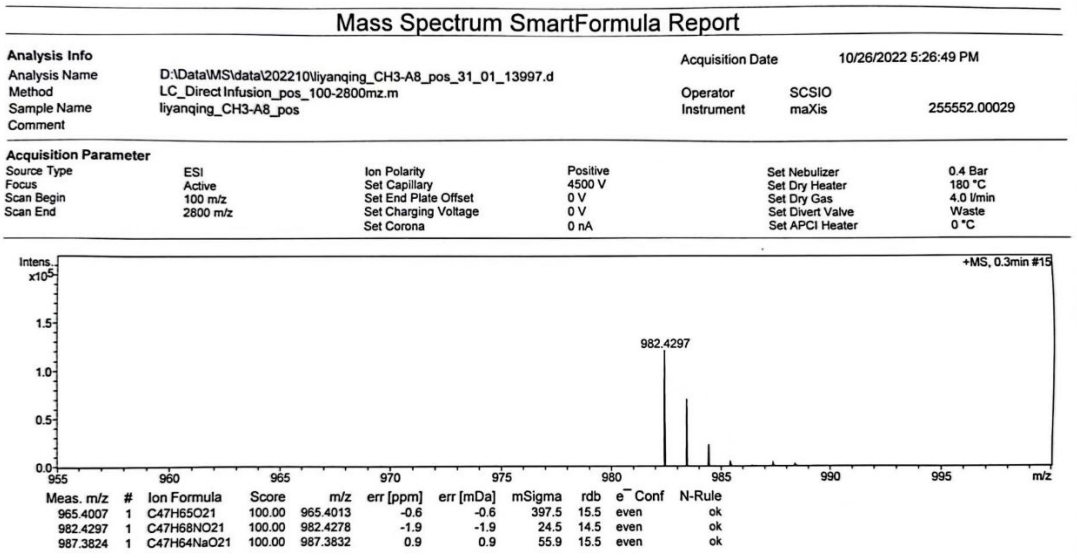


Figure S73. HRESIMS spectrum of compound **6**

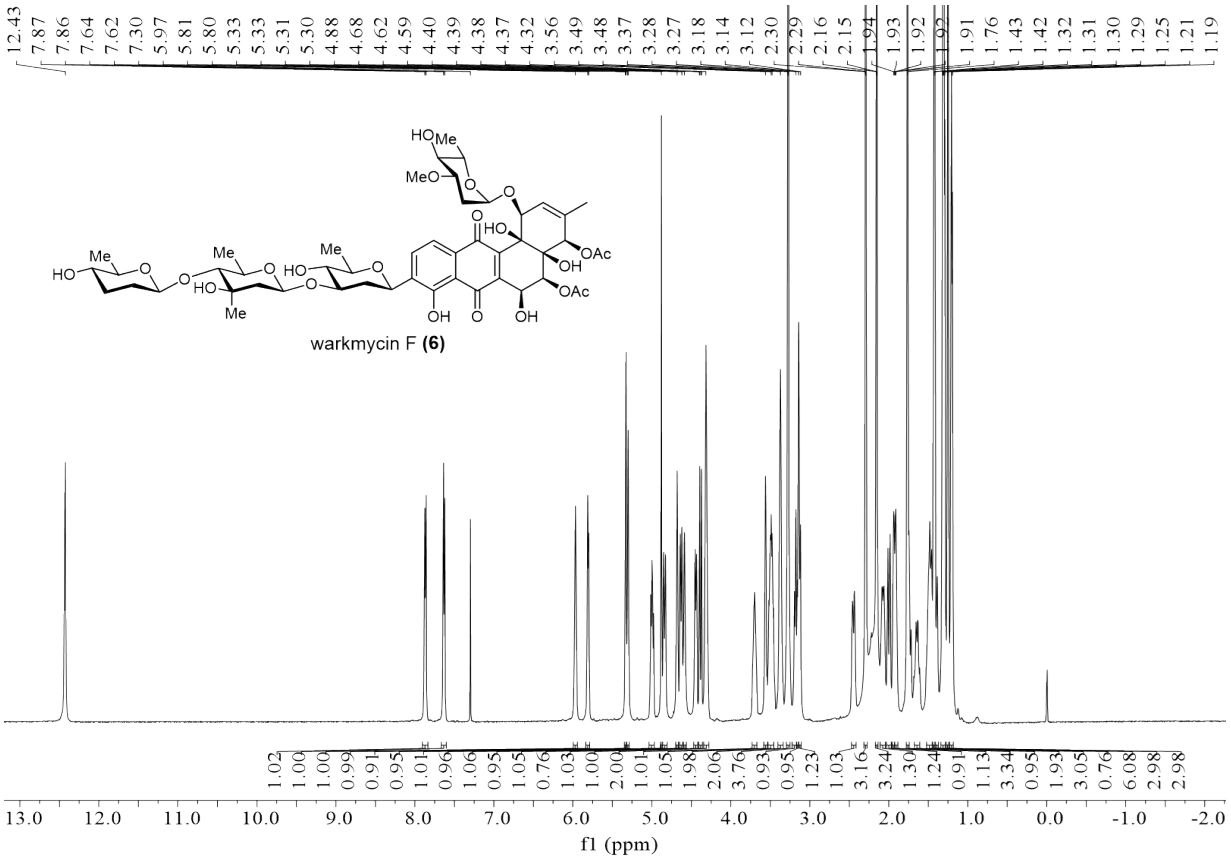


Figure S74. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **6**

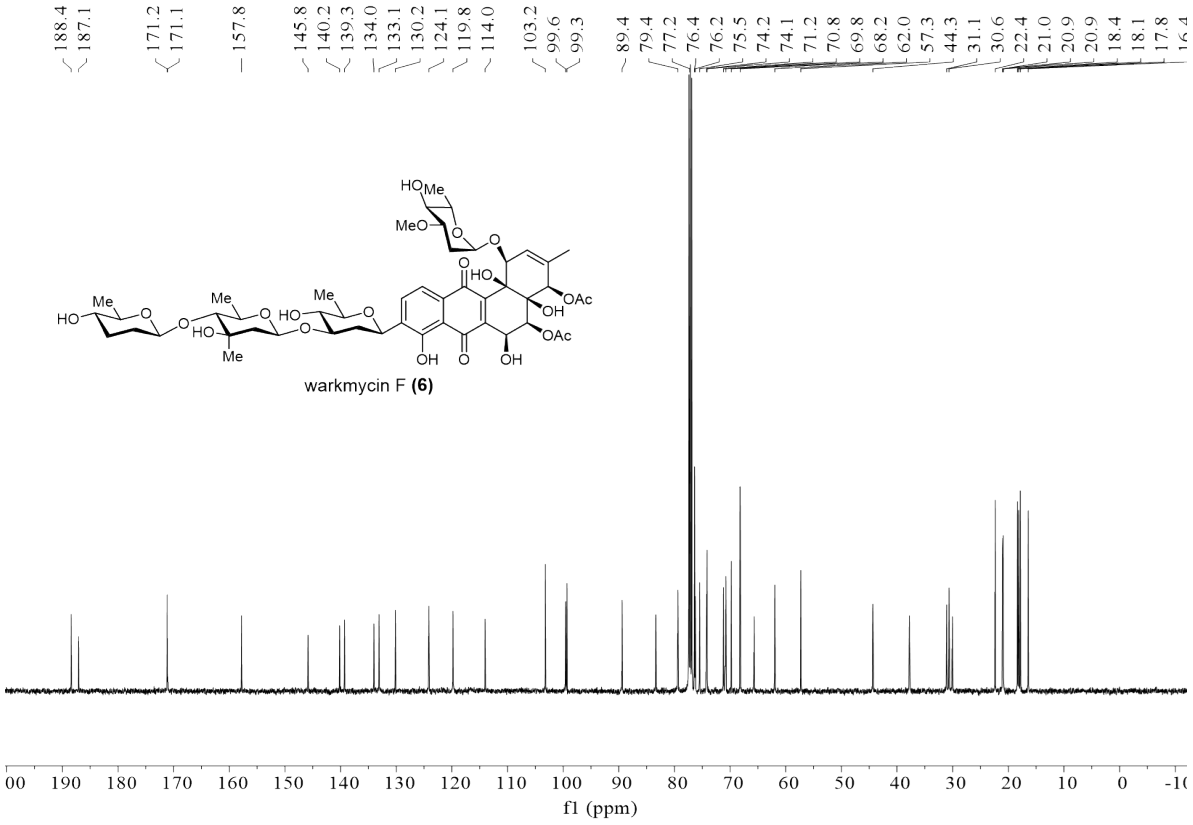


Figure S75. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **6**

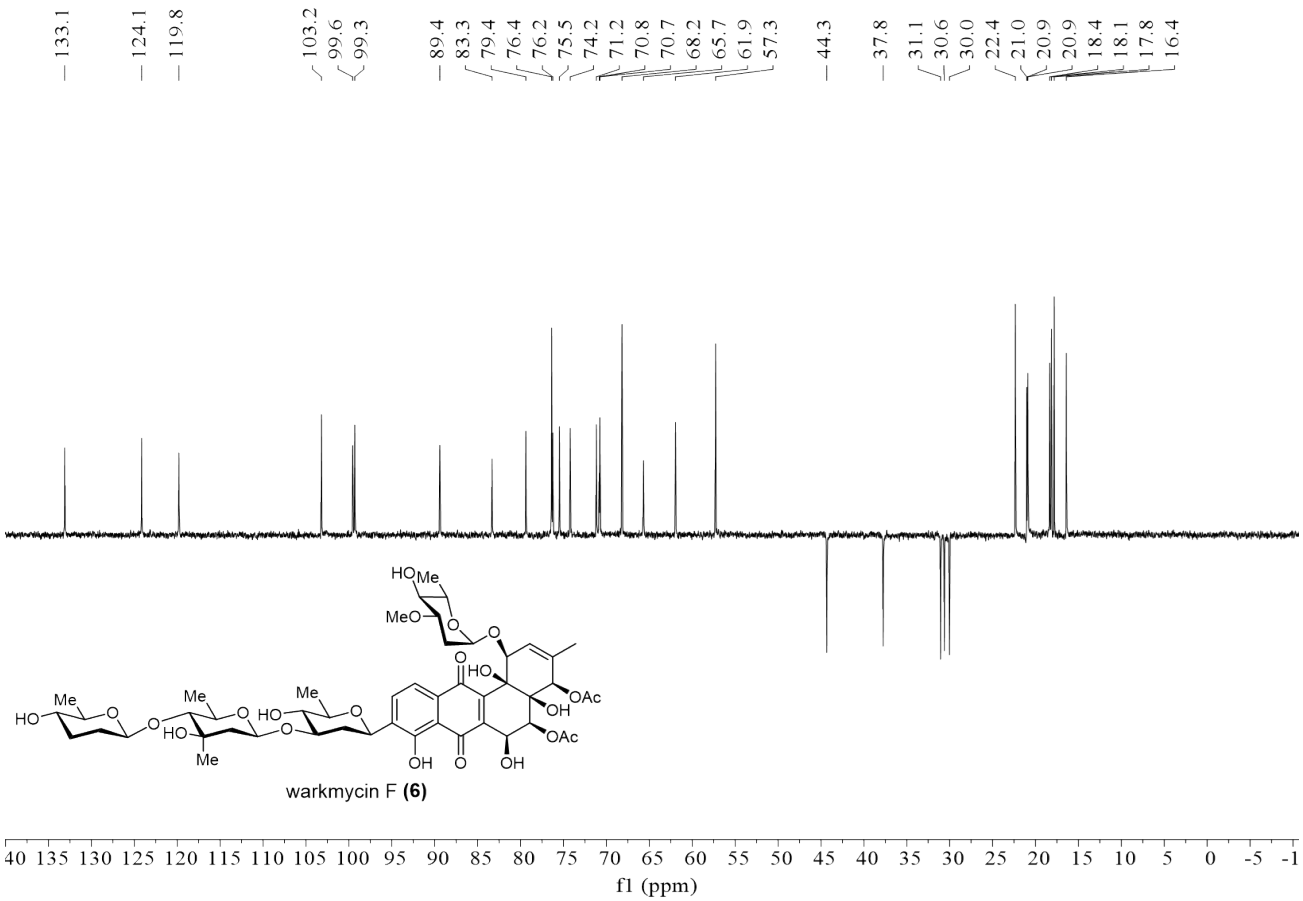


Figure S76. DEPT 135 spectrum of compound **6**

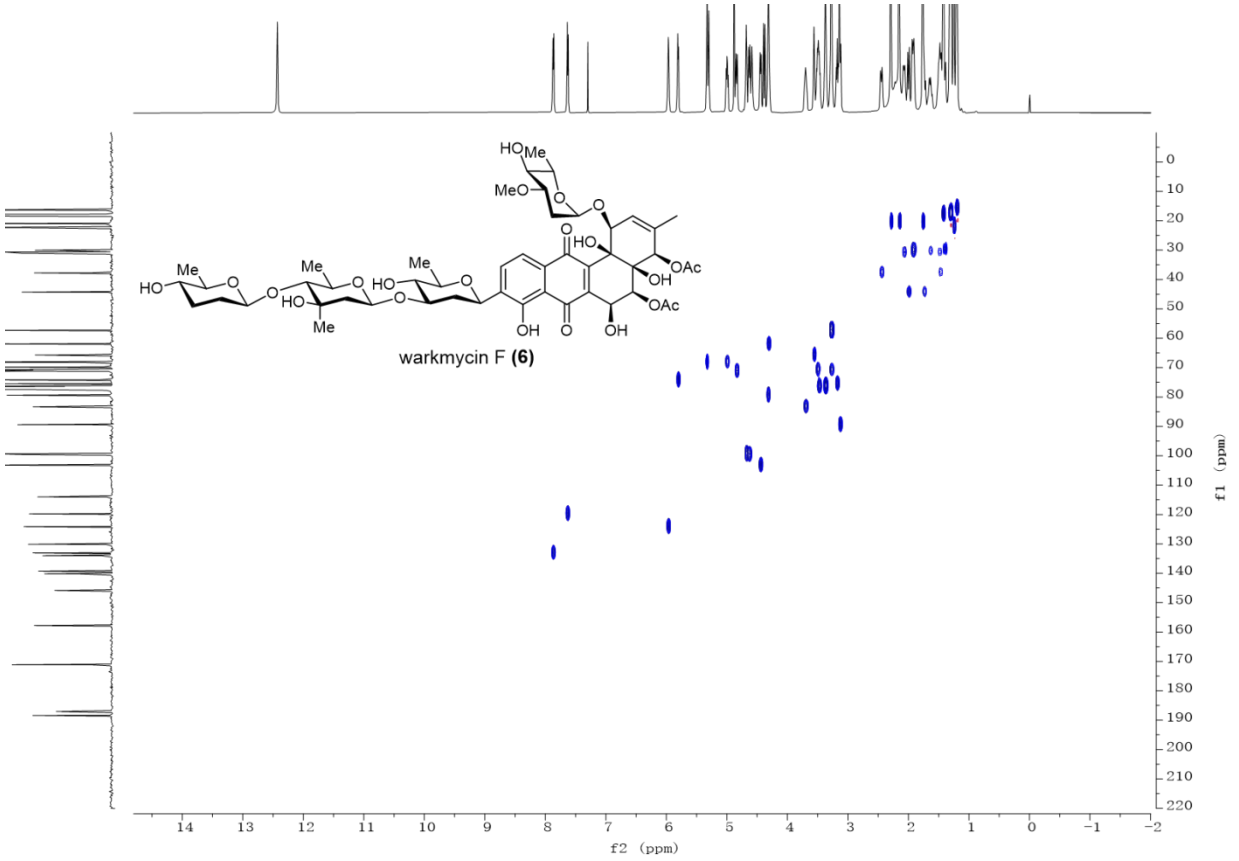


Figure S77. HSQC spectrum of compound **6**

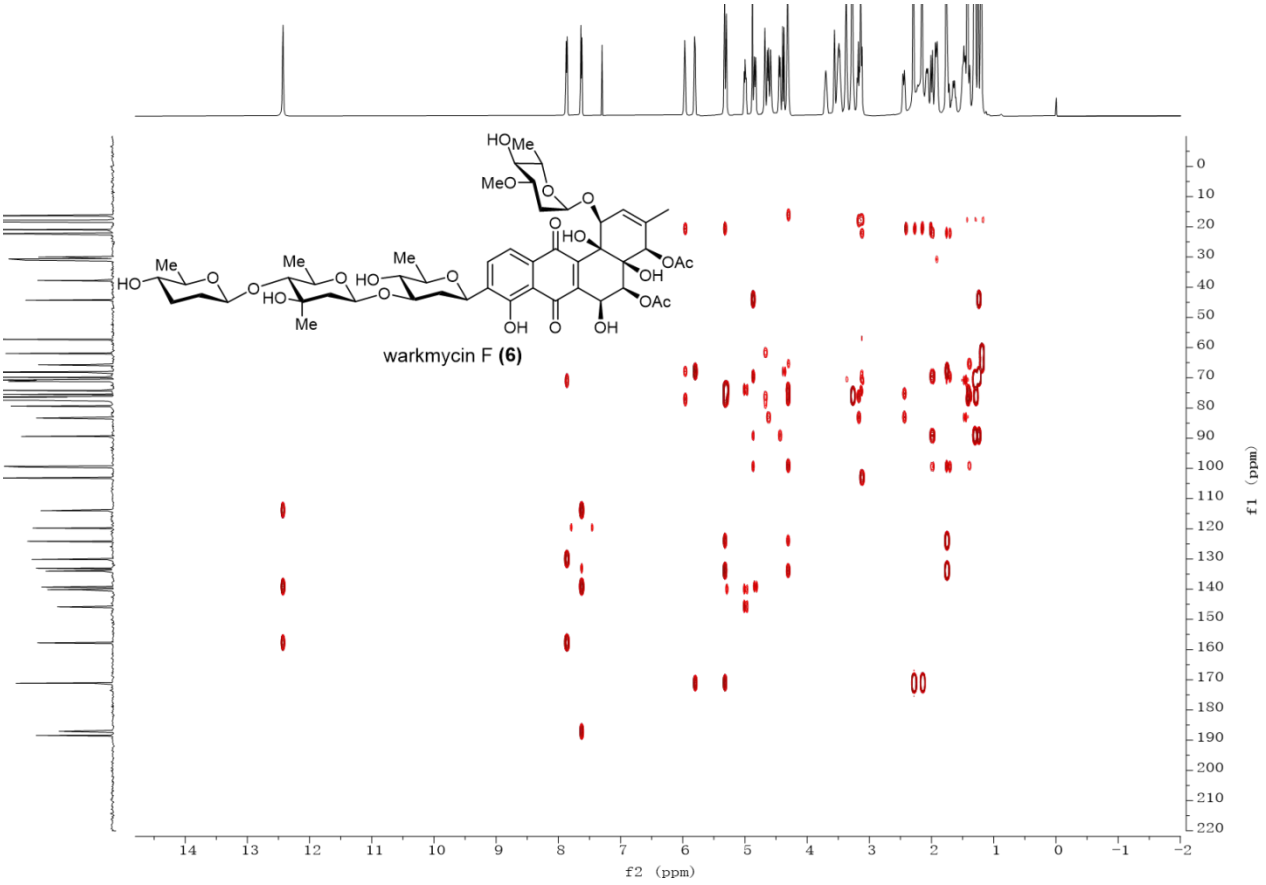


Figure S78. HMBC spectrum of compound **6**

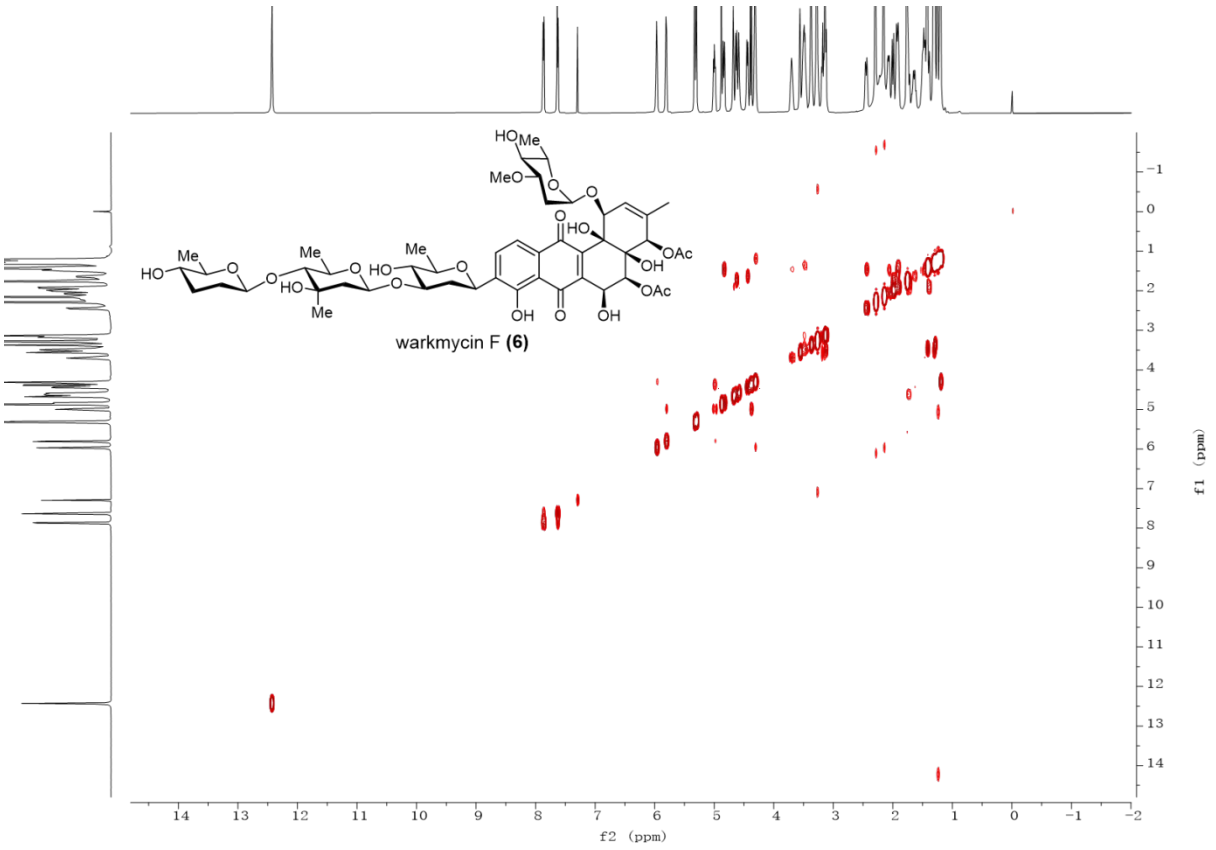


Figure S79. ^{1}H-^{1}H COSY spectrum of compound **6**

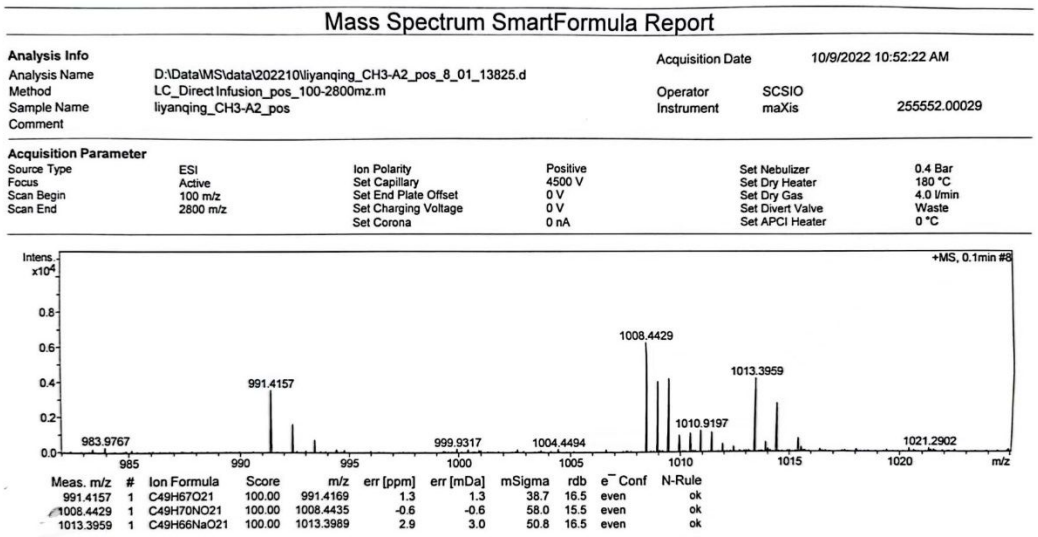


Figure S80. HRESIMS spectrum of compound **7**

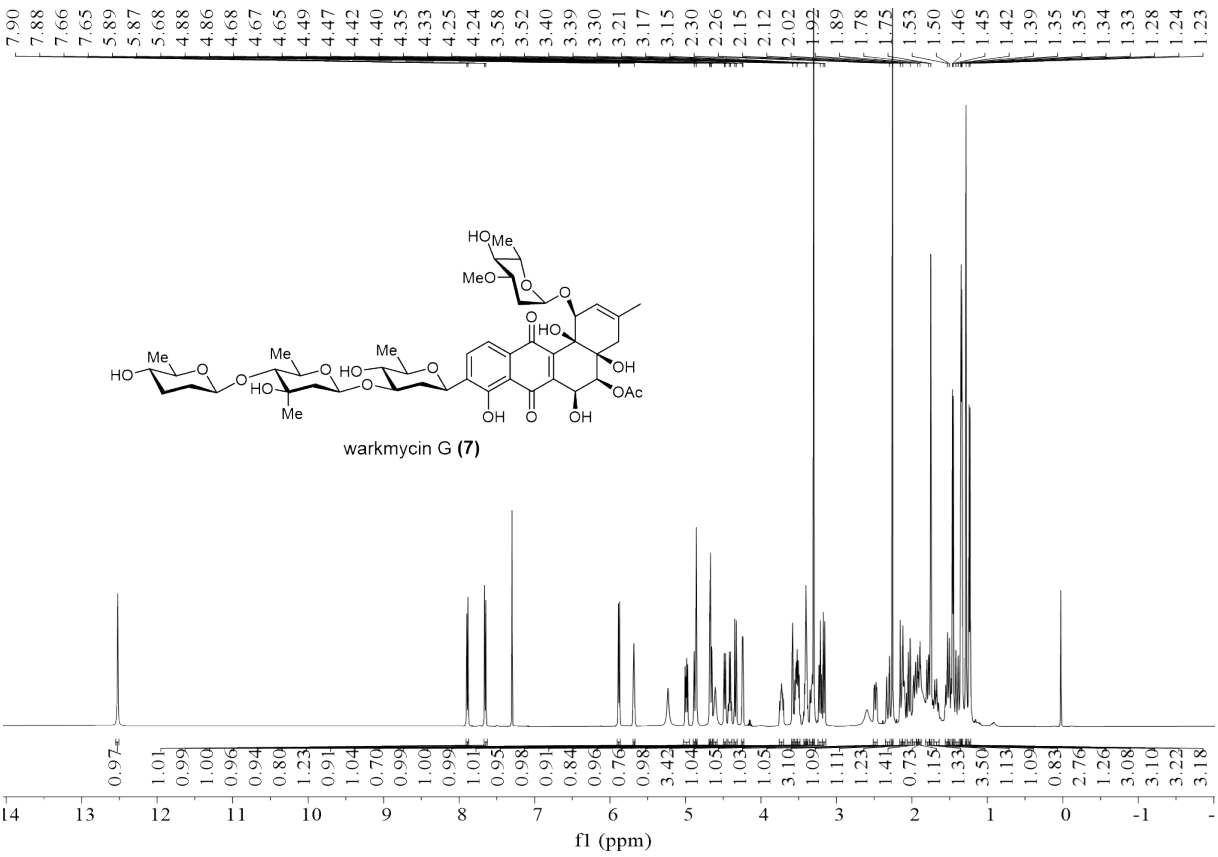


Figure S81. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **7**

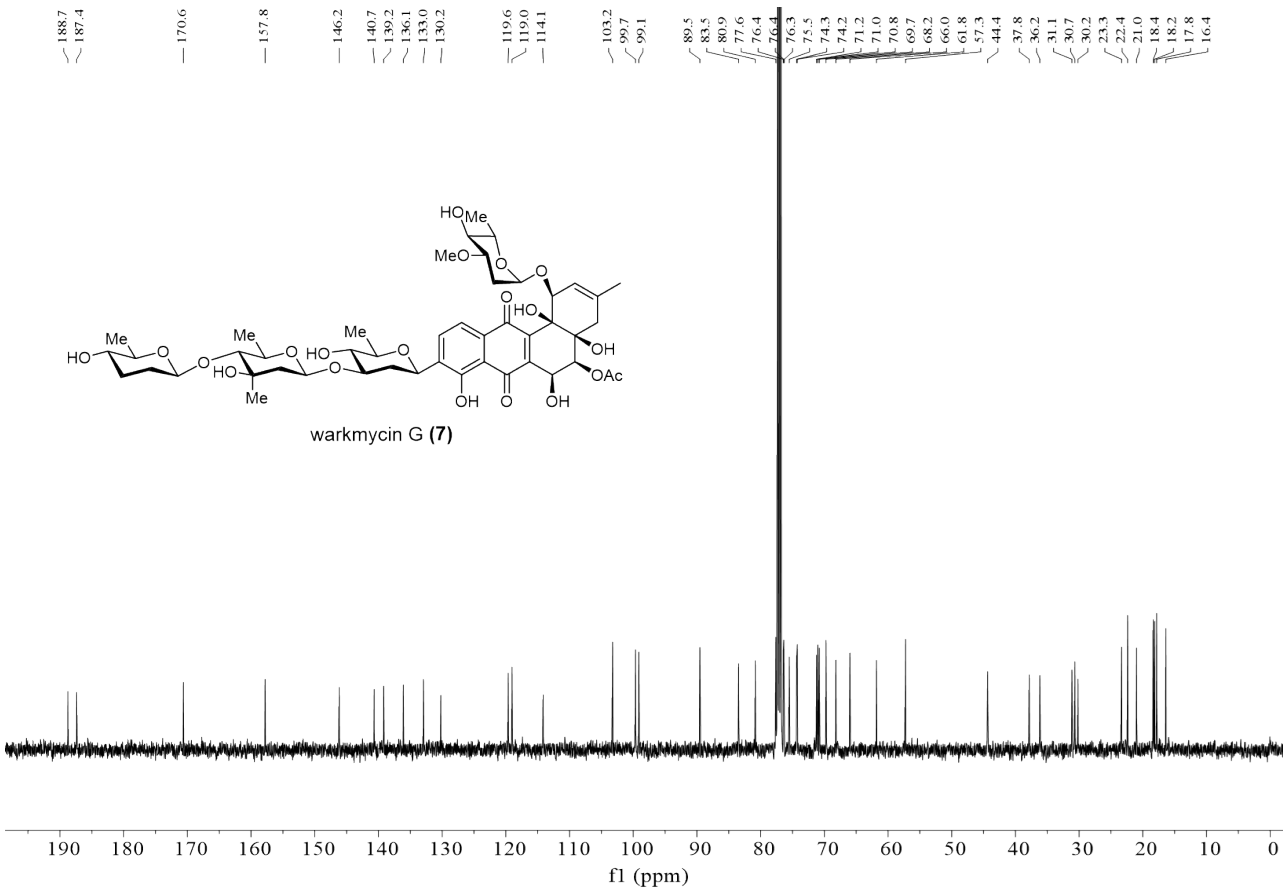


Figure S82. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **7**

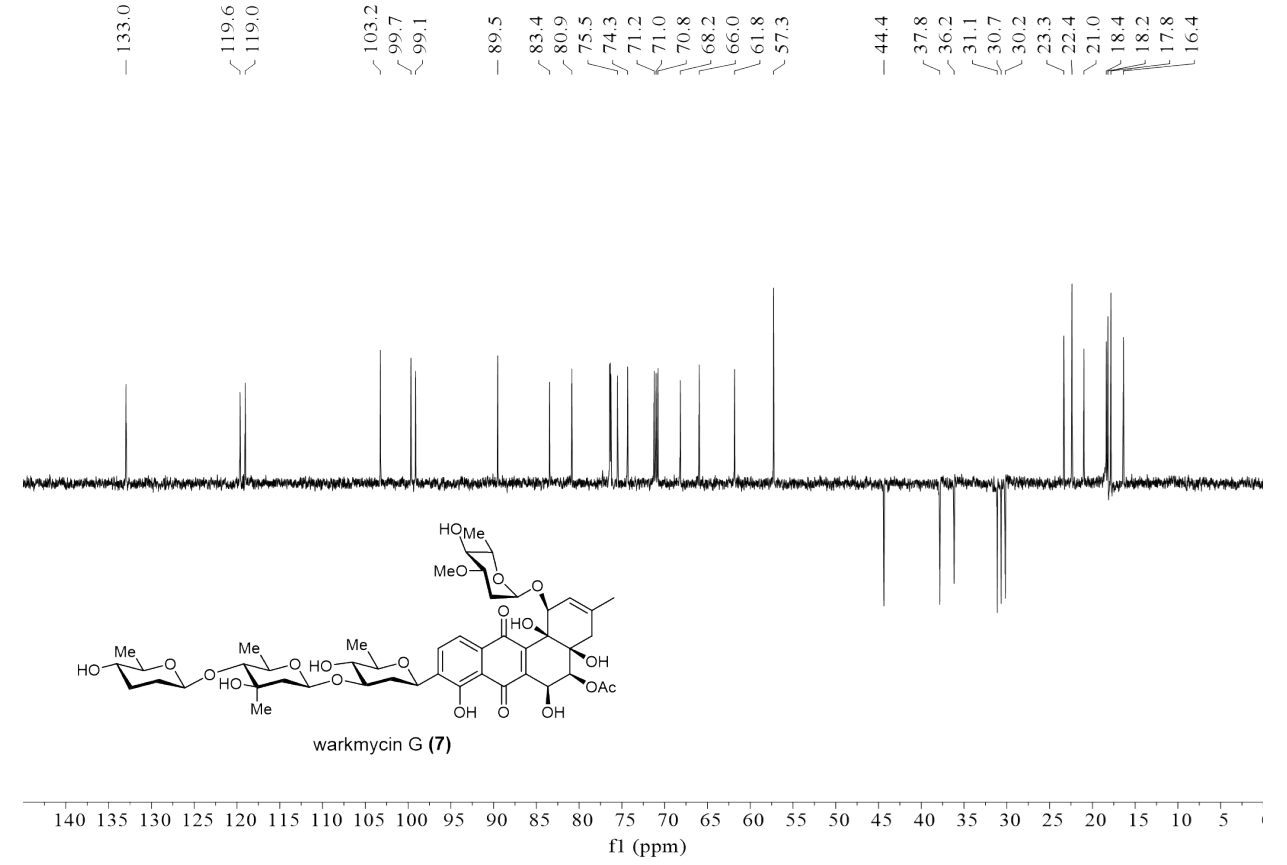


Figure S83. DEPT 135 spectrum of compound **7**

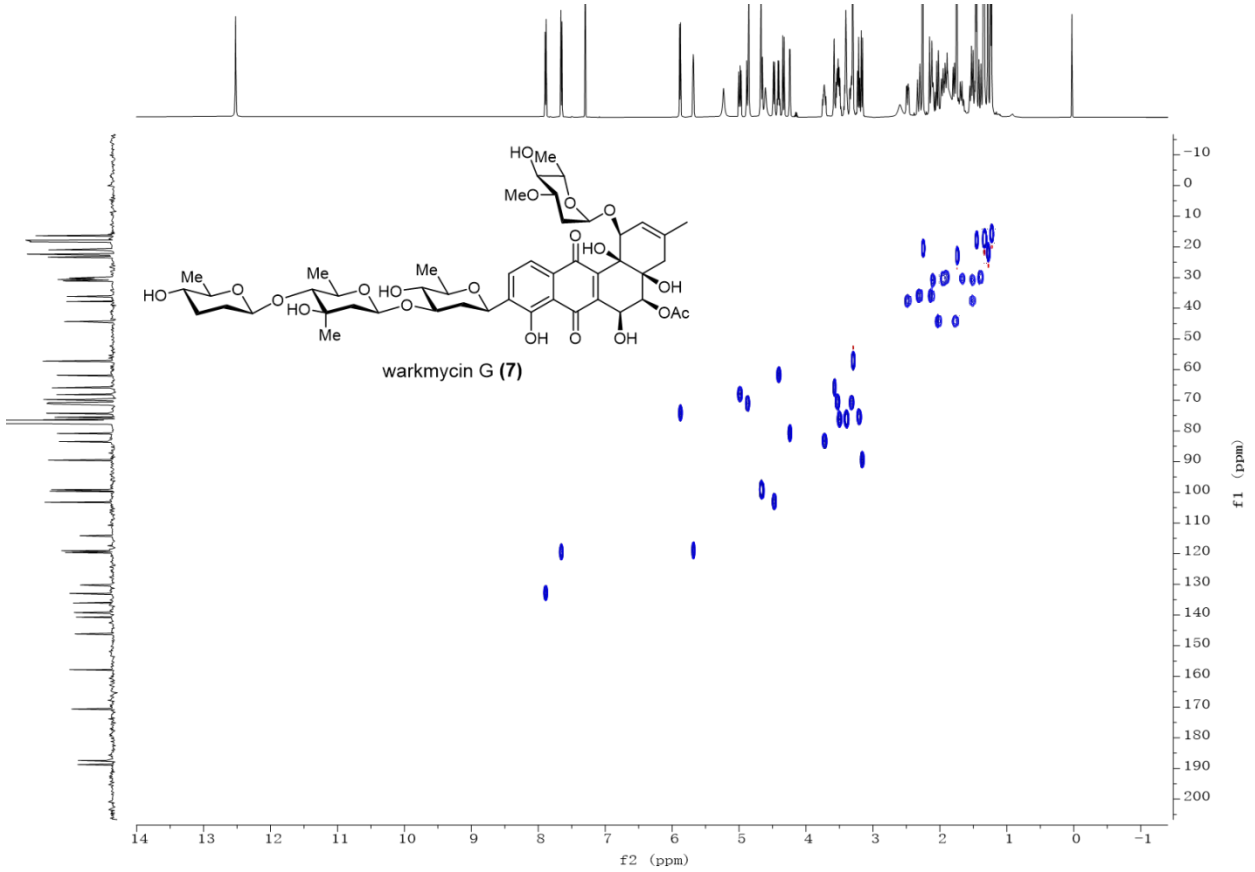


Figure S84. HSQC spectrum of compound **7**

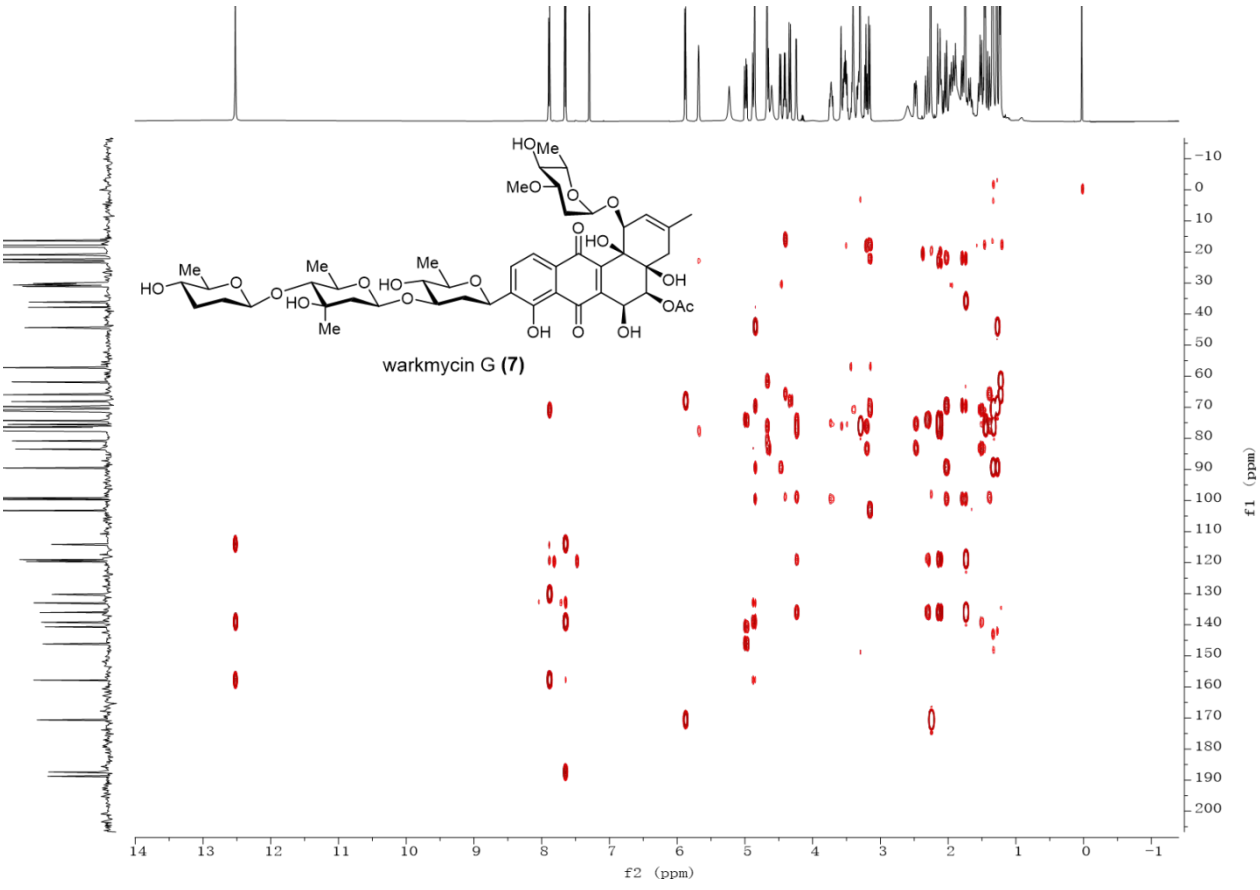


Figure S85. HMBC spectrum of compound **7**

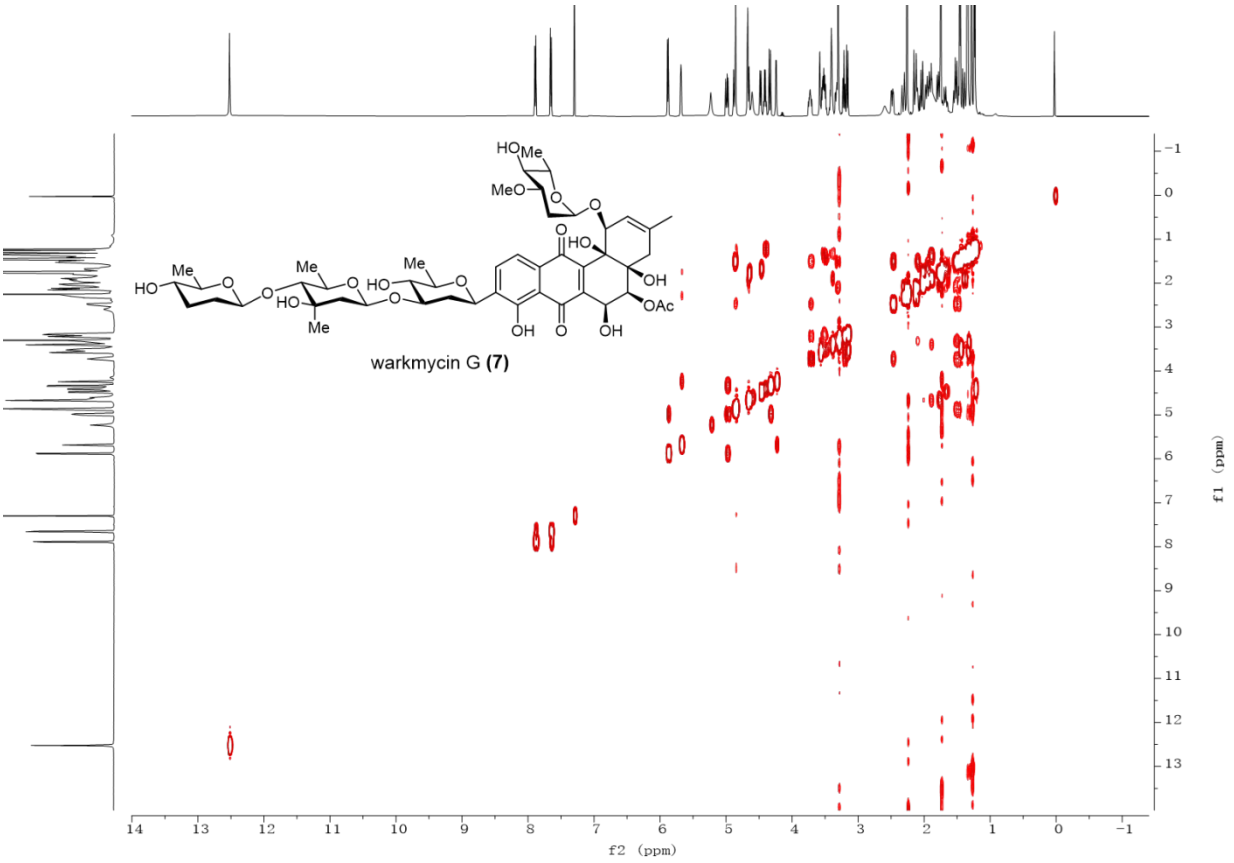


Figure S86. ^{1}H-^{1}H COSY spectrum of compound **7**

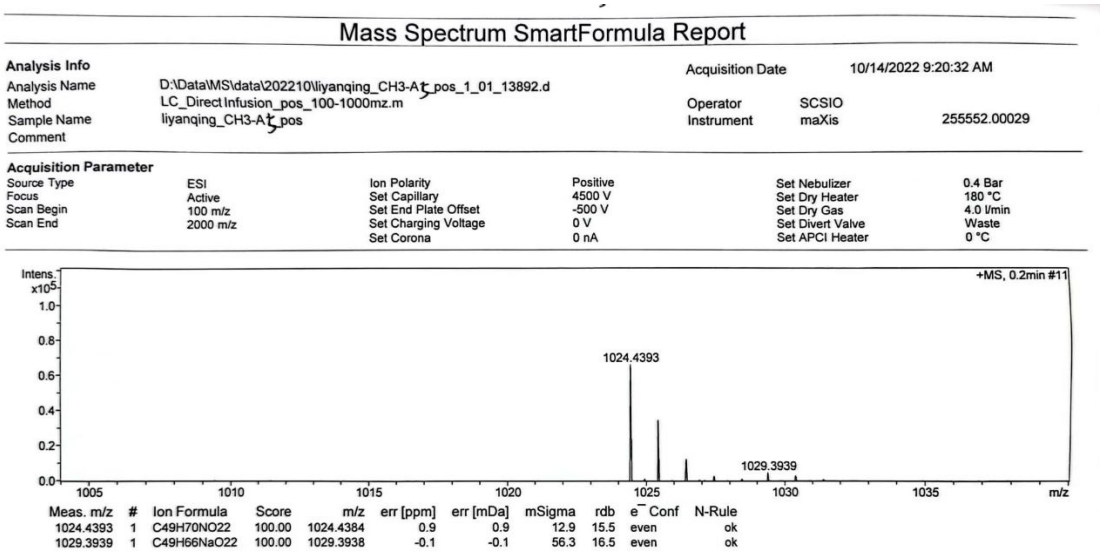


Figure S87. HRESIMS spectrum of compound **8**

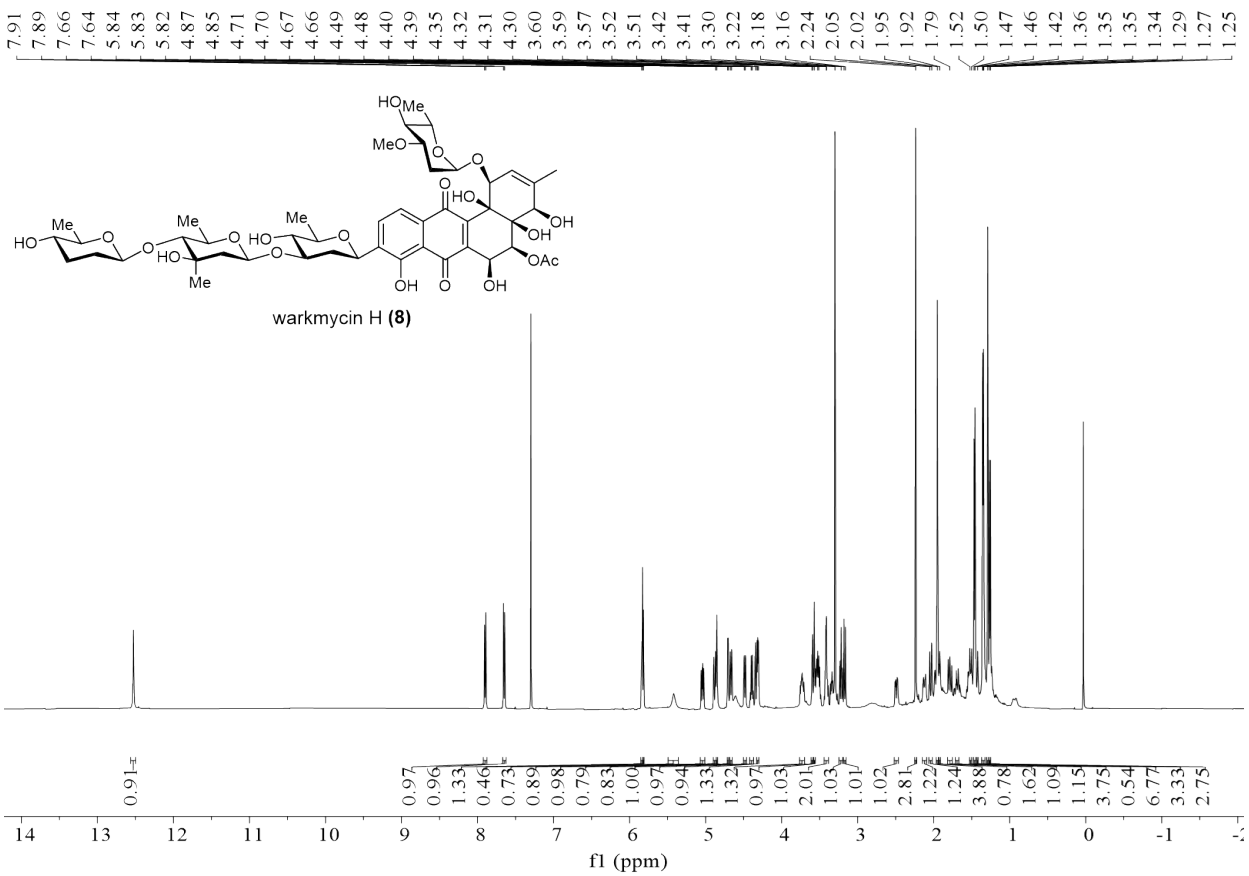


Figure S88. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **8**

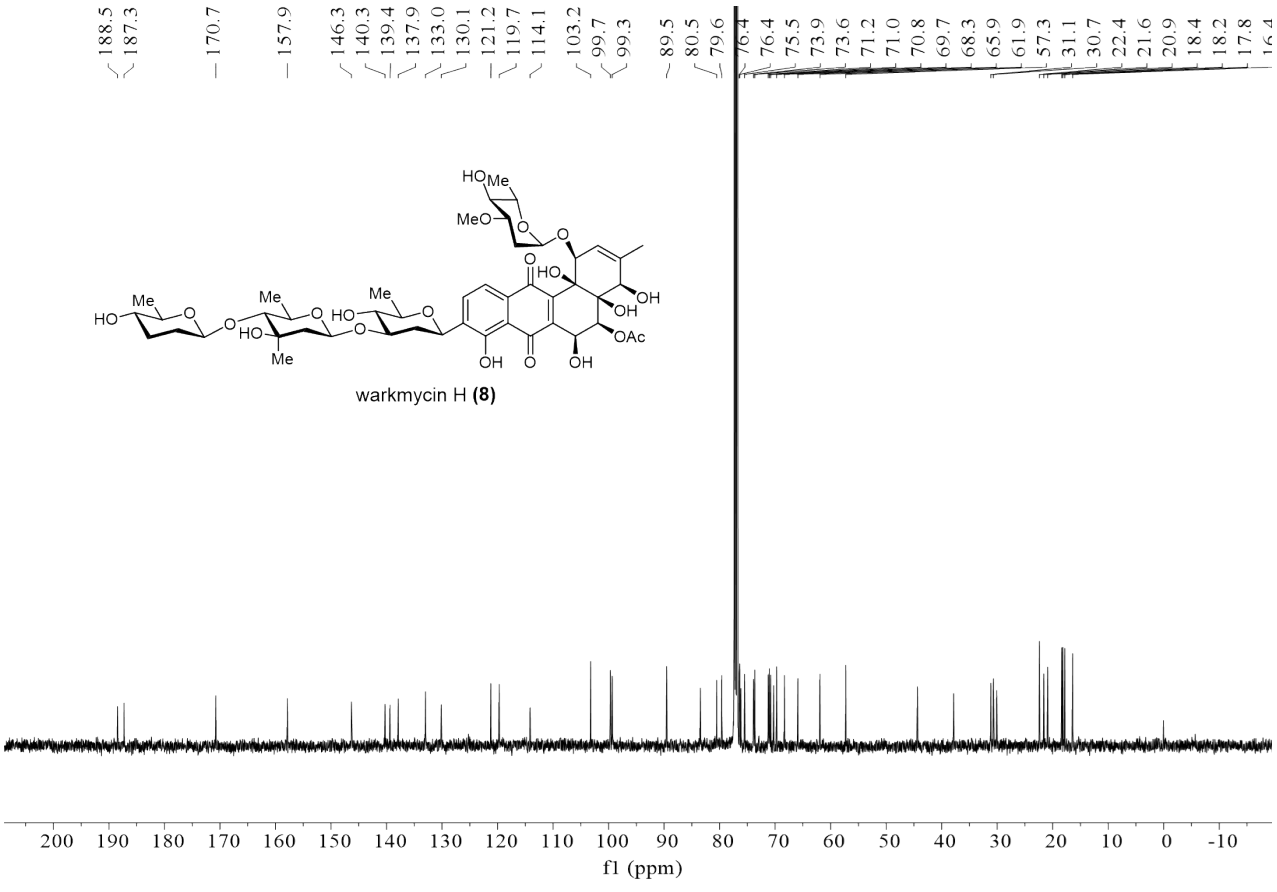


Figure S89. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **8**

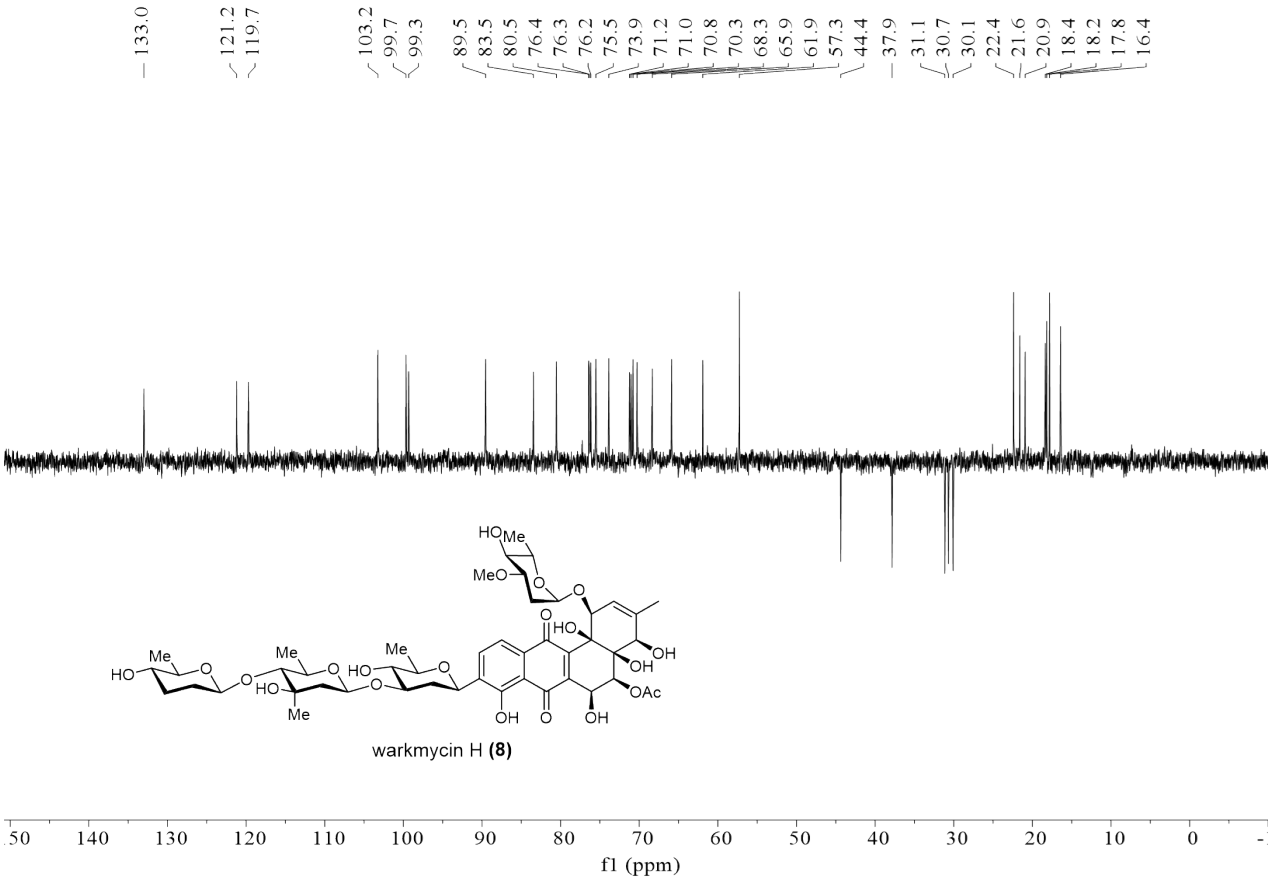


Figure S90. DEPT 135 spectrum of compound **8**

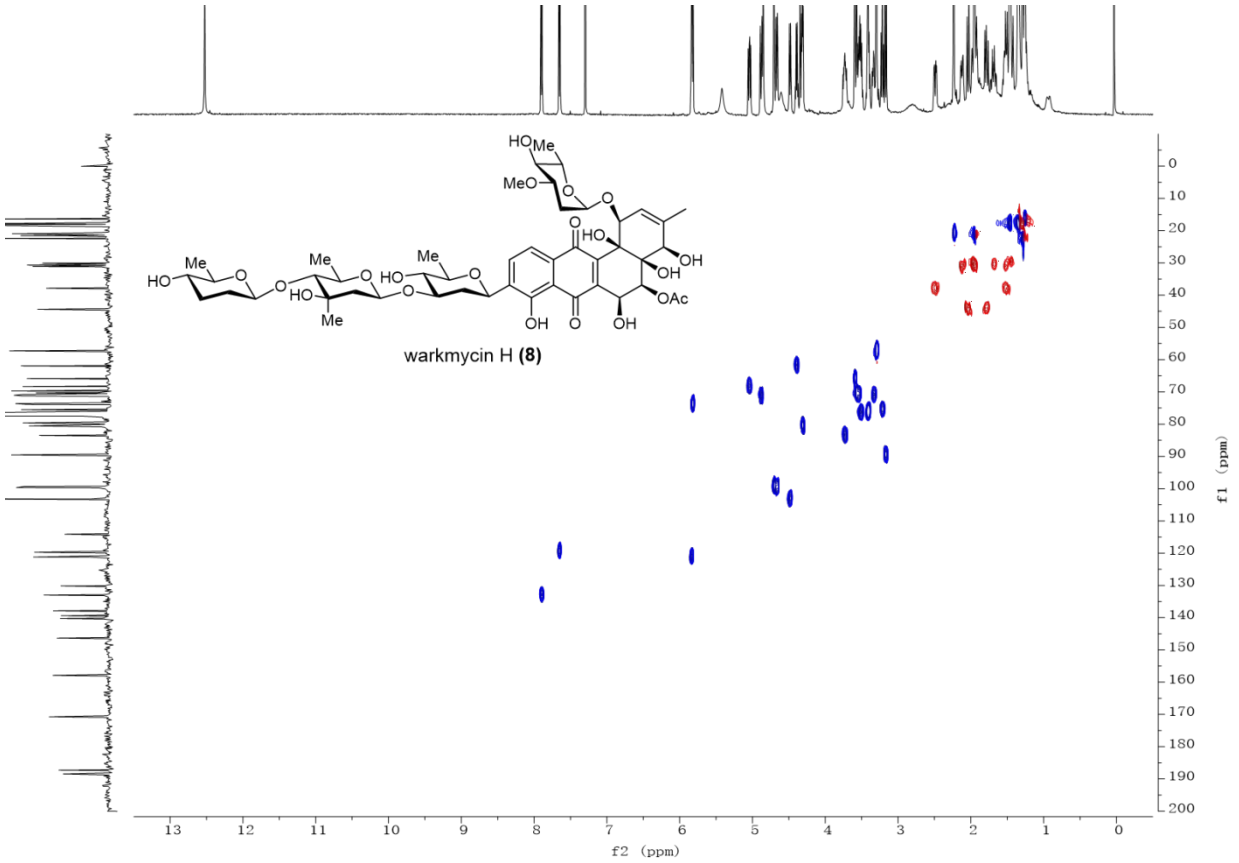


Figure S91. HSQC spectrum of compound **8**

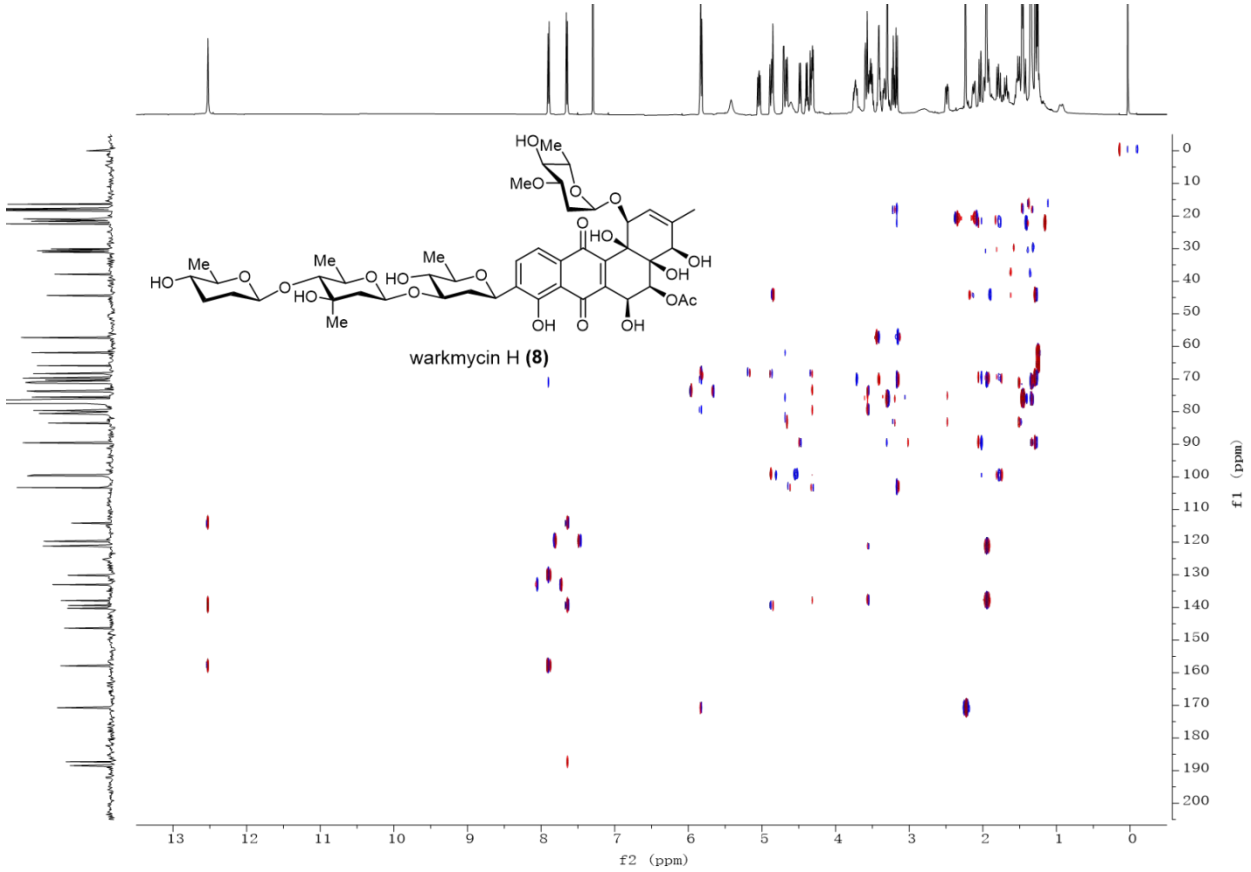


Figure S92. HMBC spectrum of compound **8**

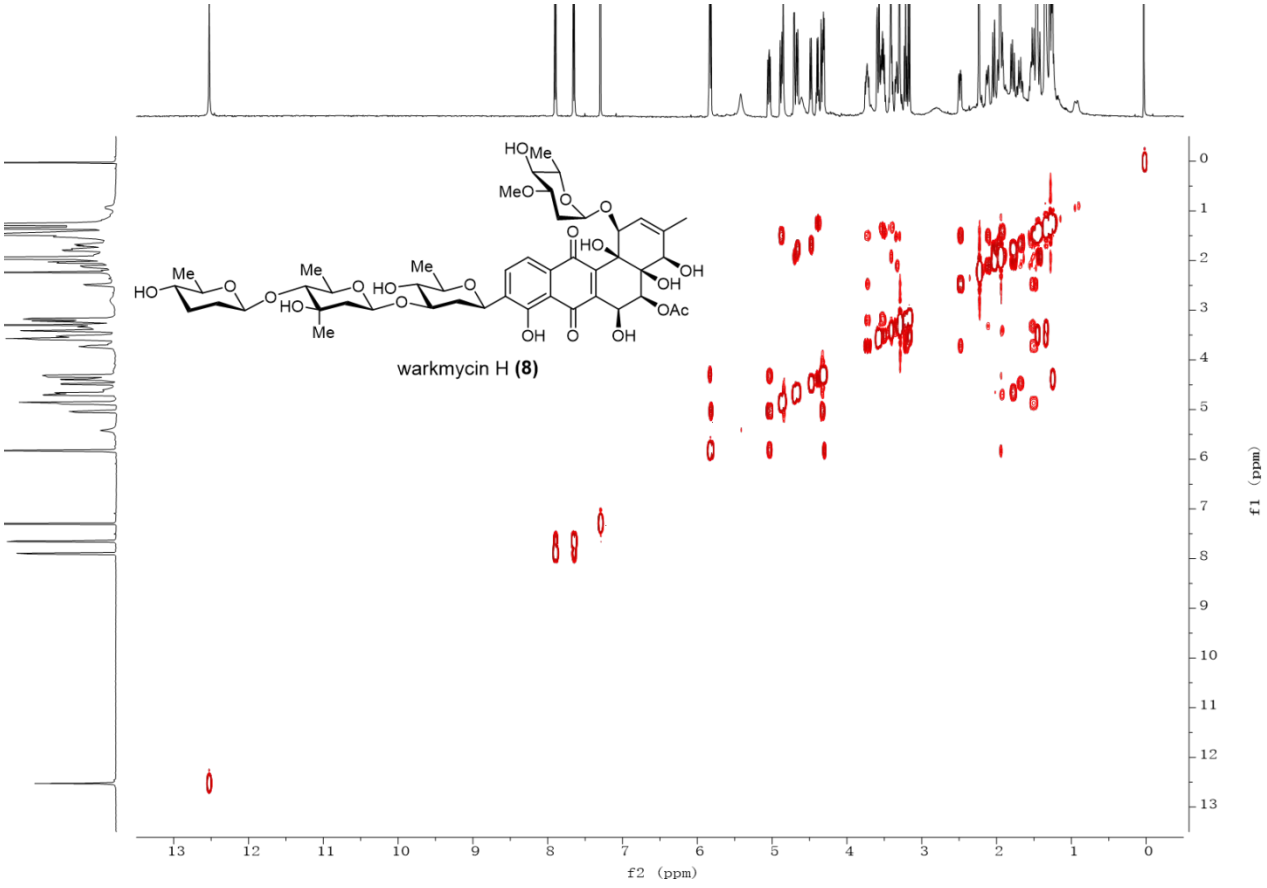


Figure S93. ^{1}H-^{1}H COSY spectrum of compound **8**

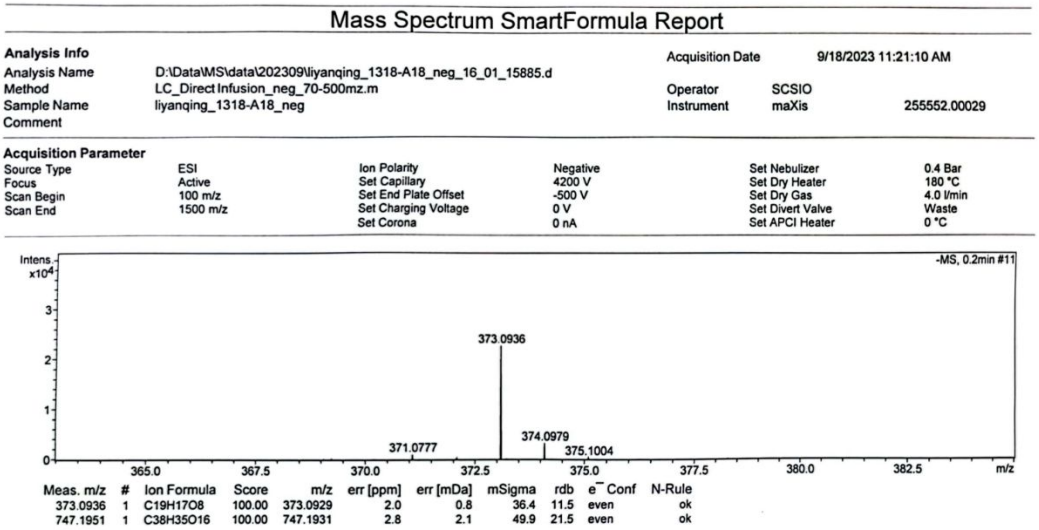


Figure S94. HRESIMS spectrum of compound **9**

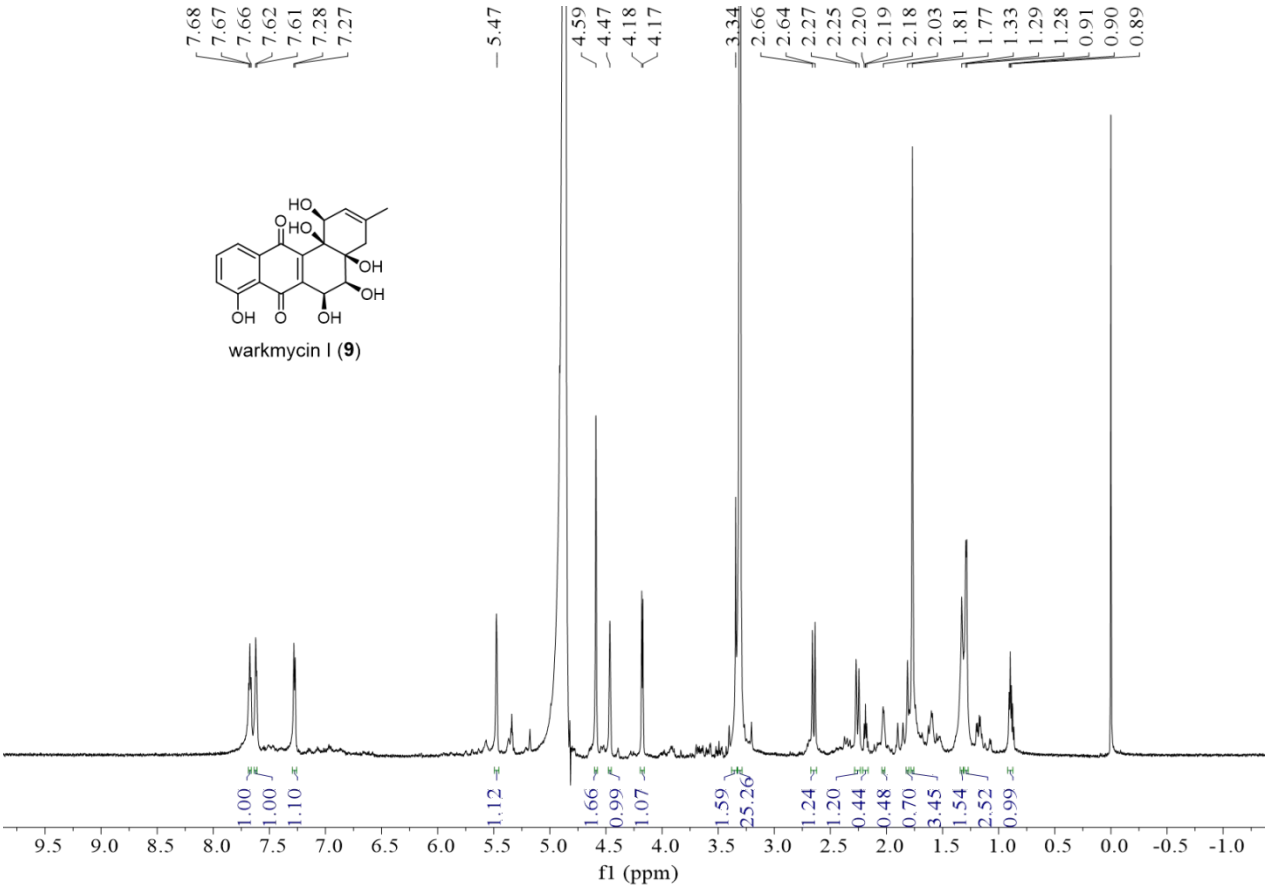


Figure S95. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **9**

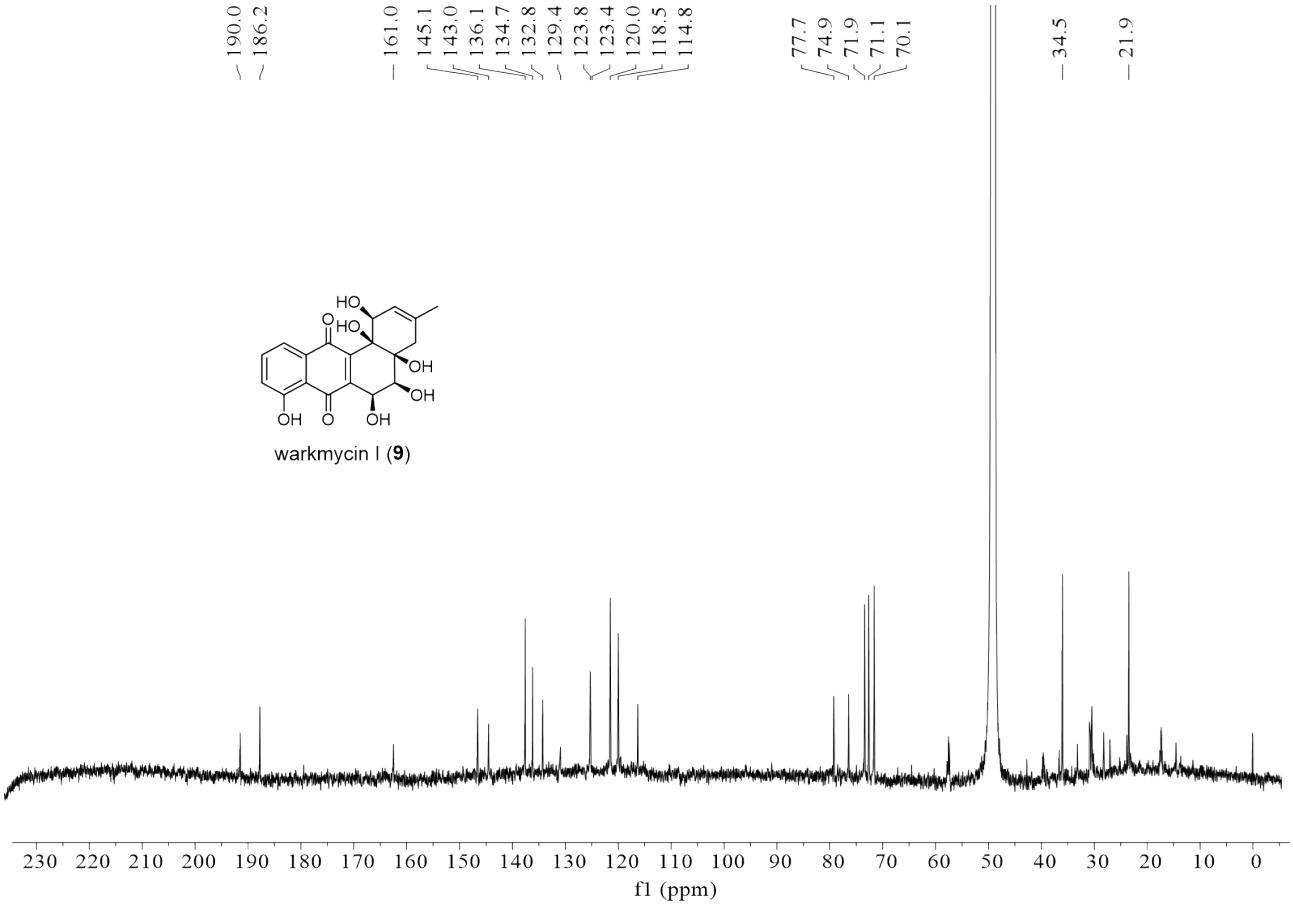


Figure S96. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **9**

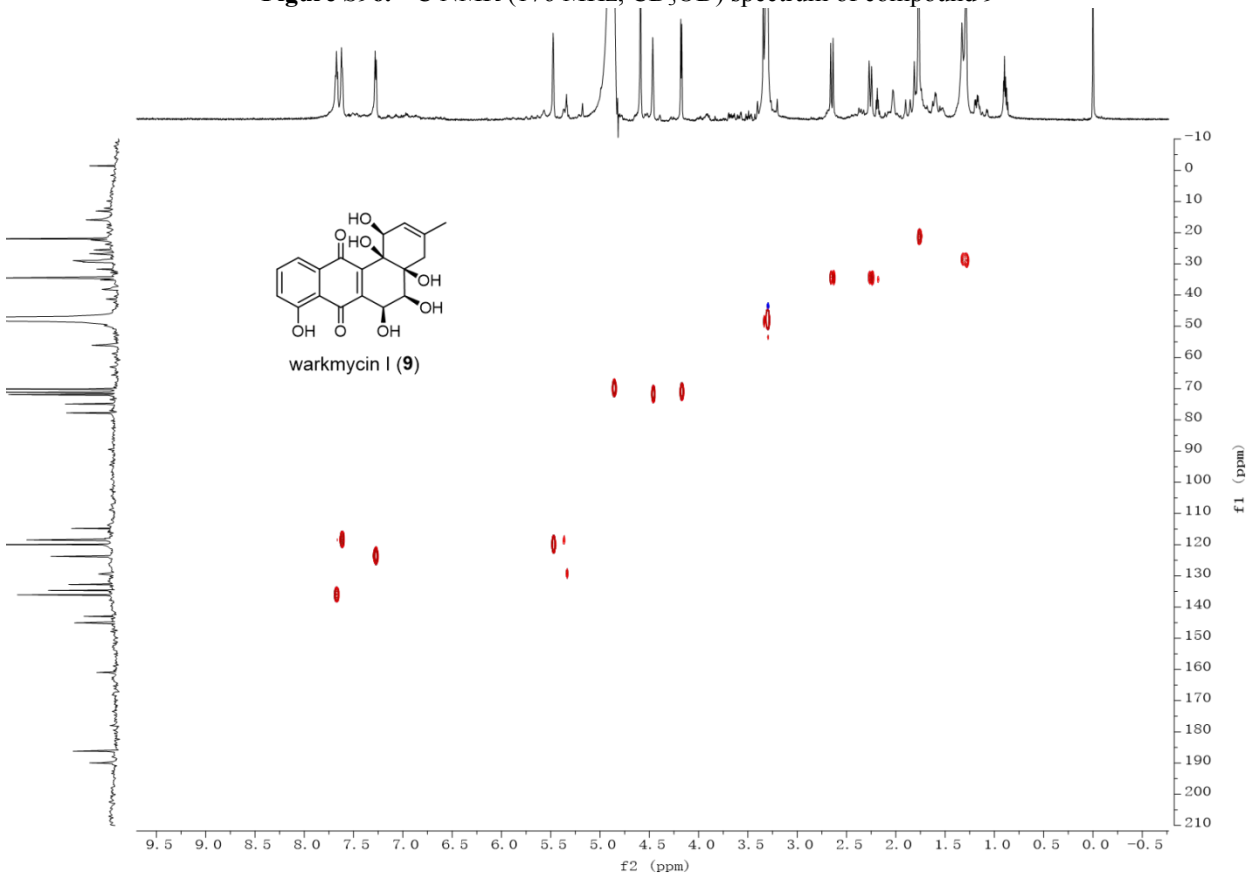


Figure S97. HSQC spectrum of compound **9**

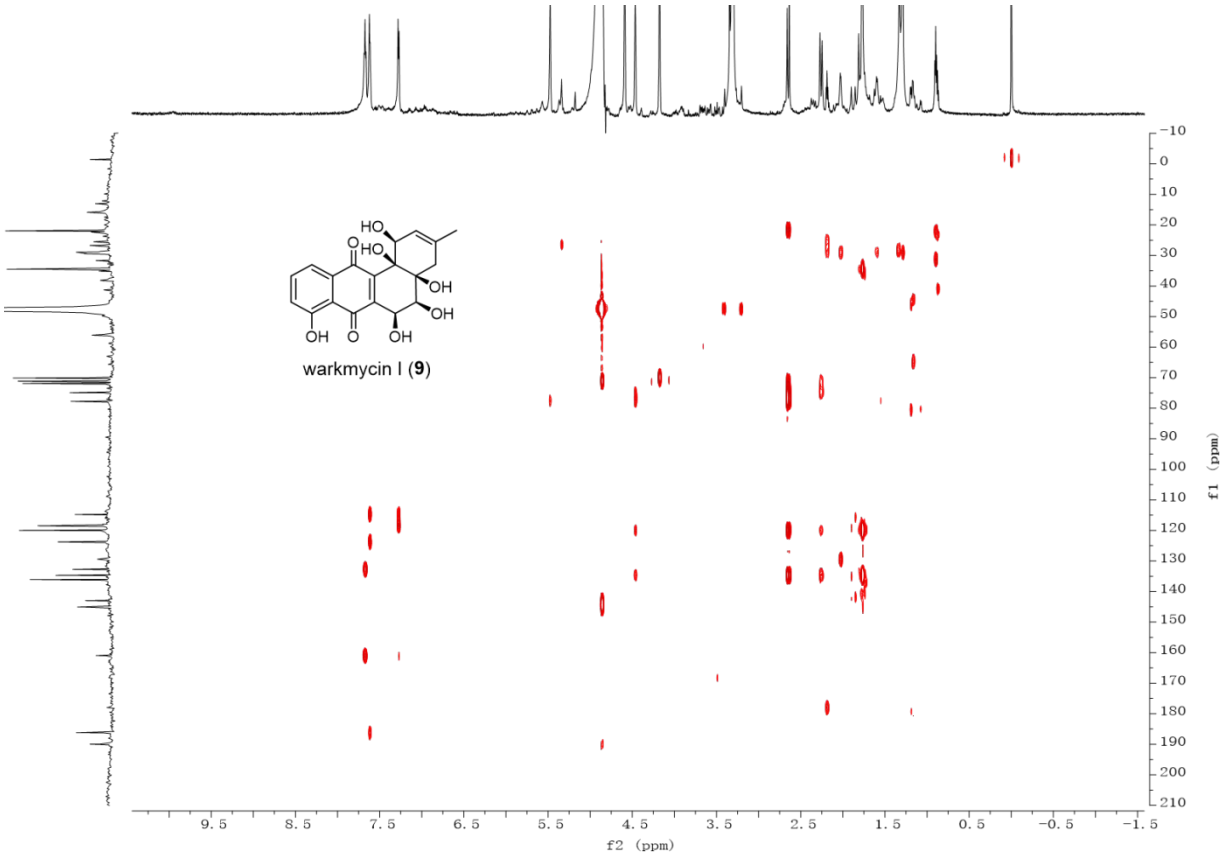


Figure S98. HMBC spectrum of compound **9**

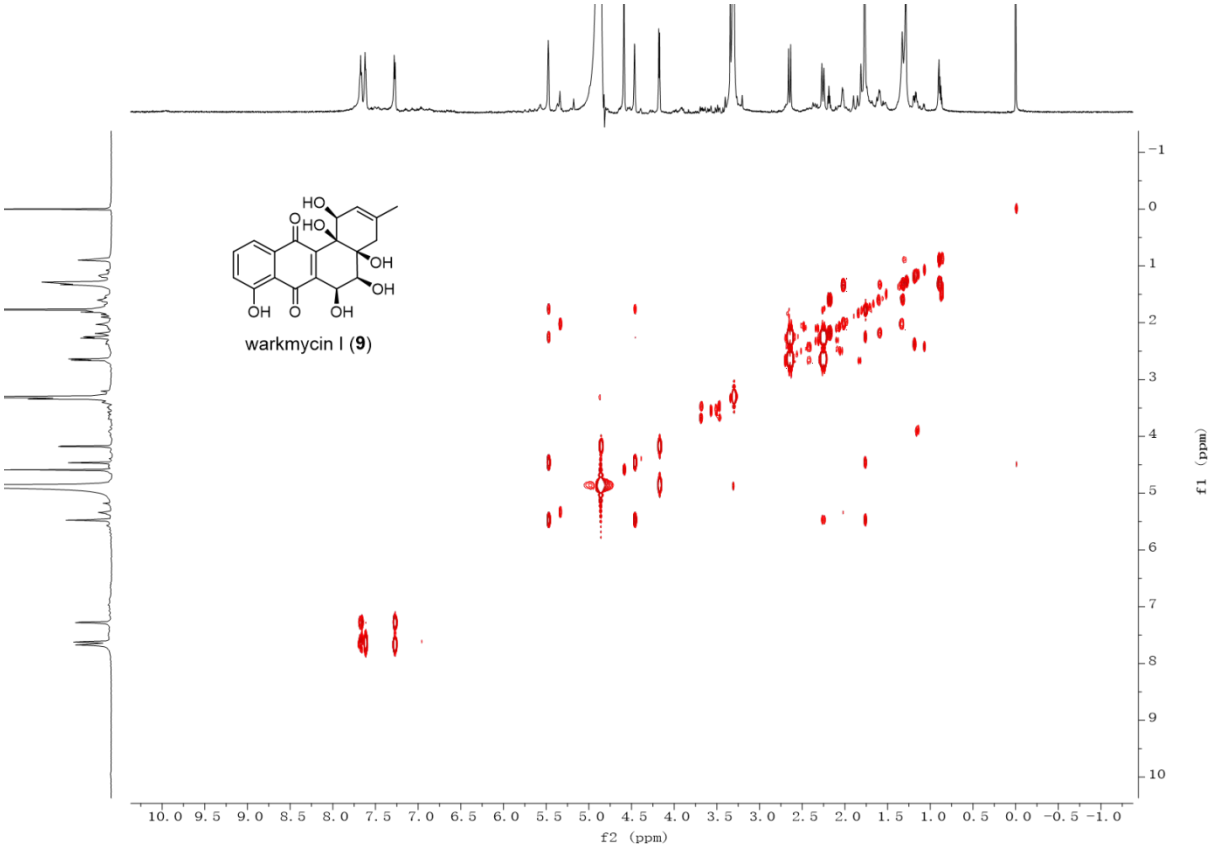


Figure S99. ^{1}H-^{1}H COSY spectrum of compound **9**

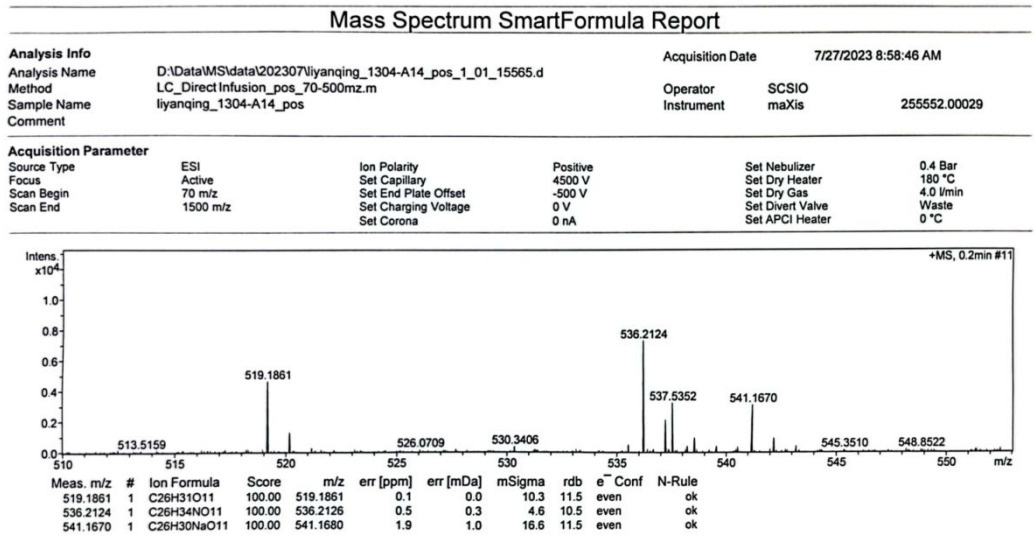


Figure S100. HRESIMS spectrum of compound **10**

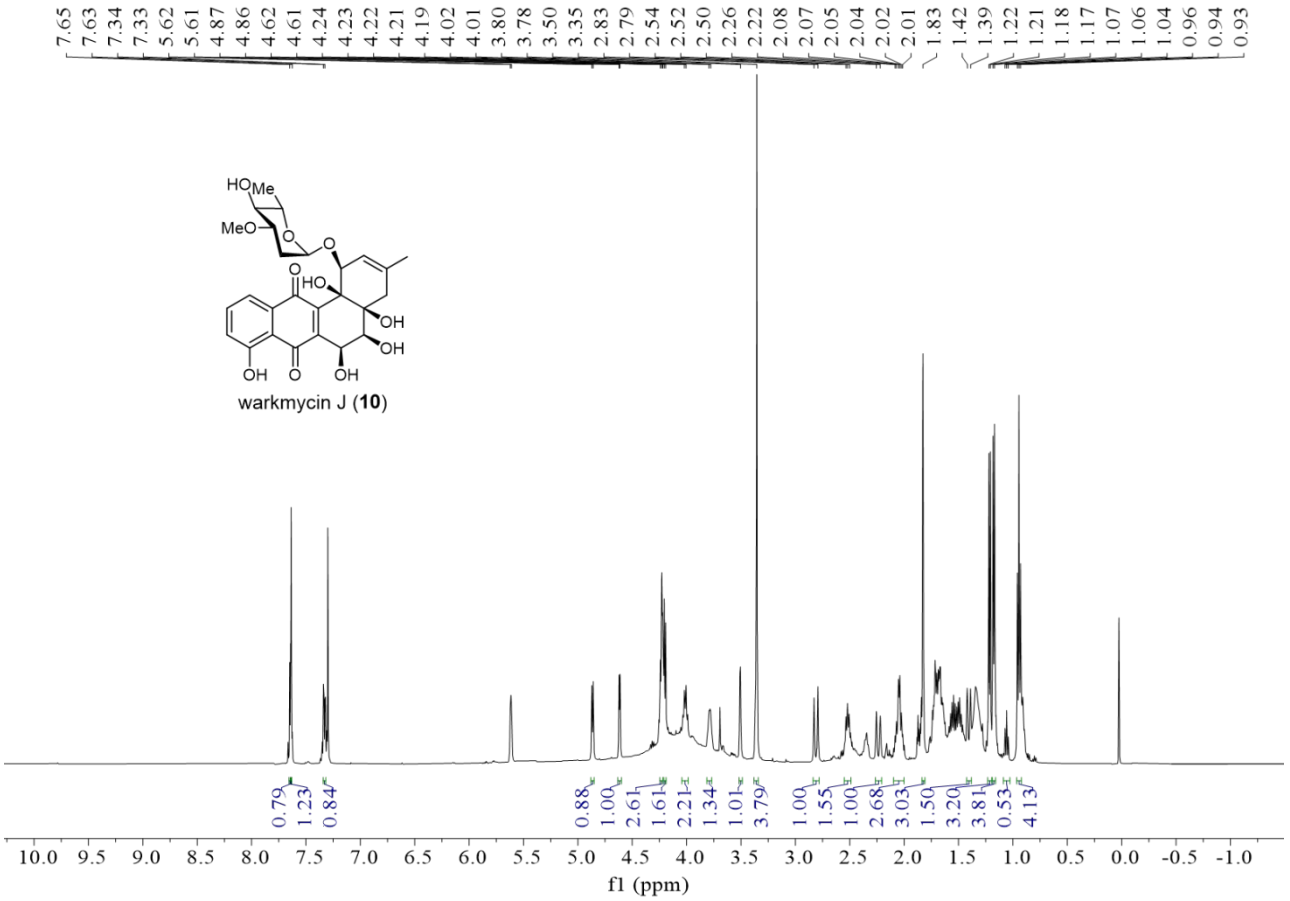


Figure S101. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **10**

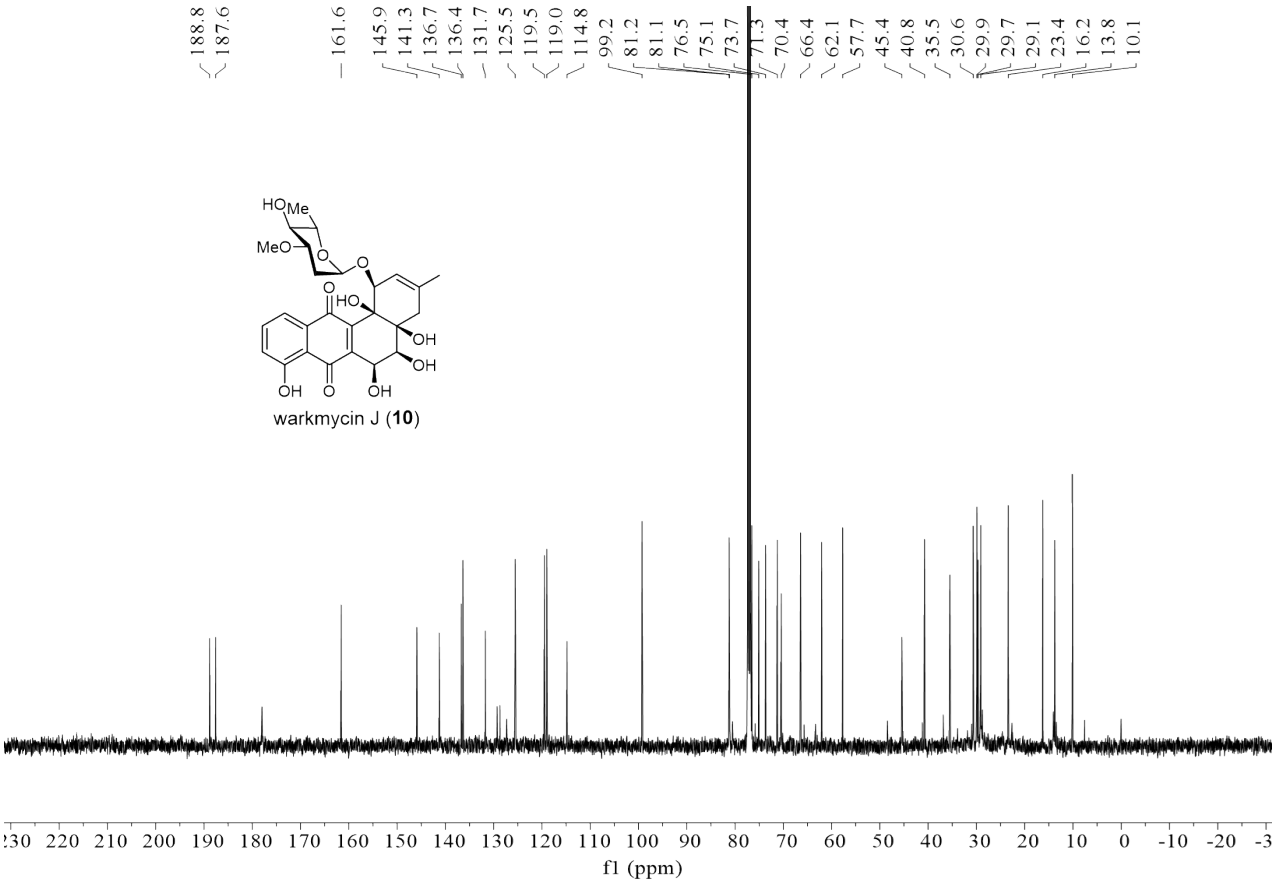


Figure S102. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **10**

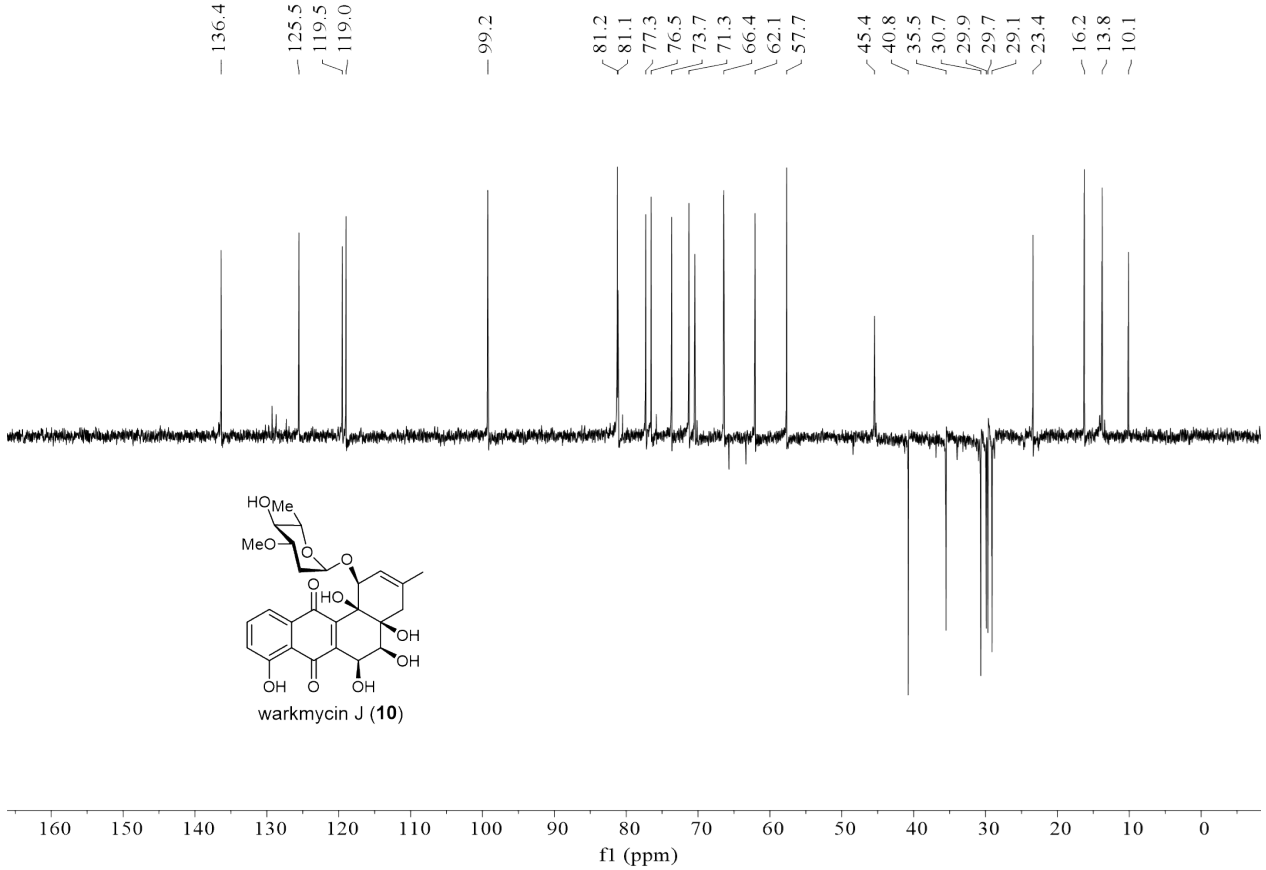


Figure S103. DEPT 135 spectrum of compound **10**

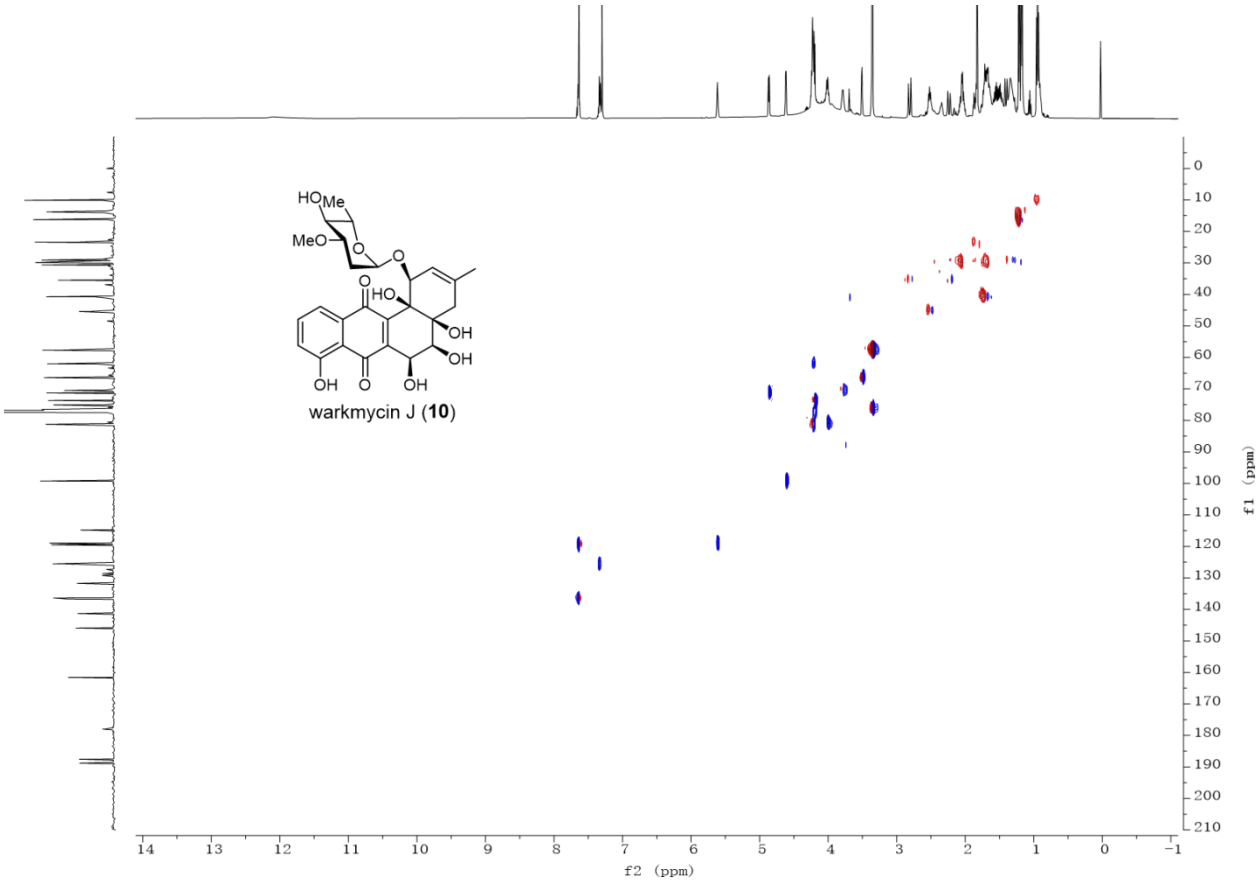


Figure S104. HSQC spectrum of compound **10**

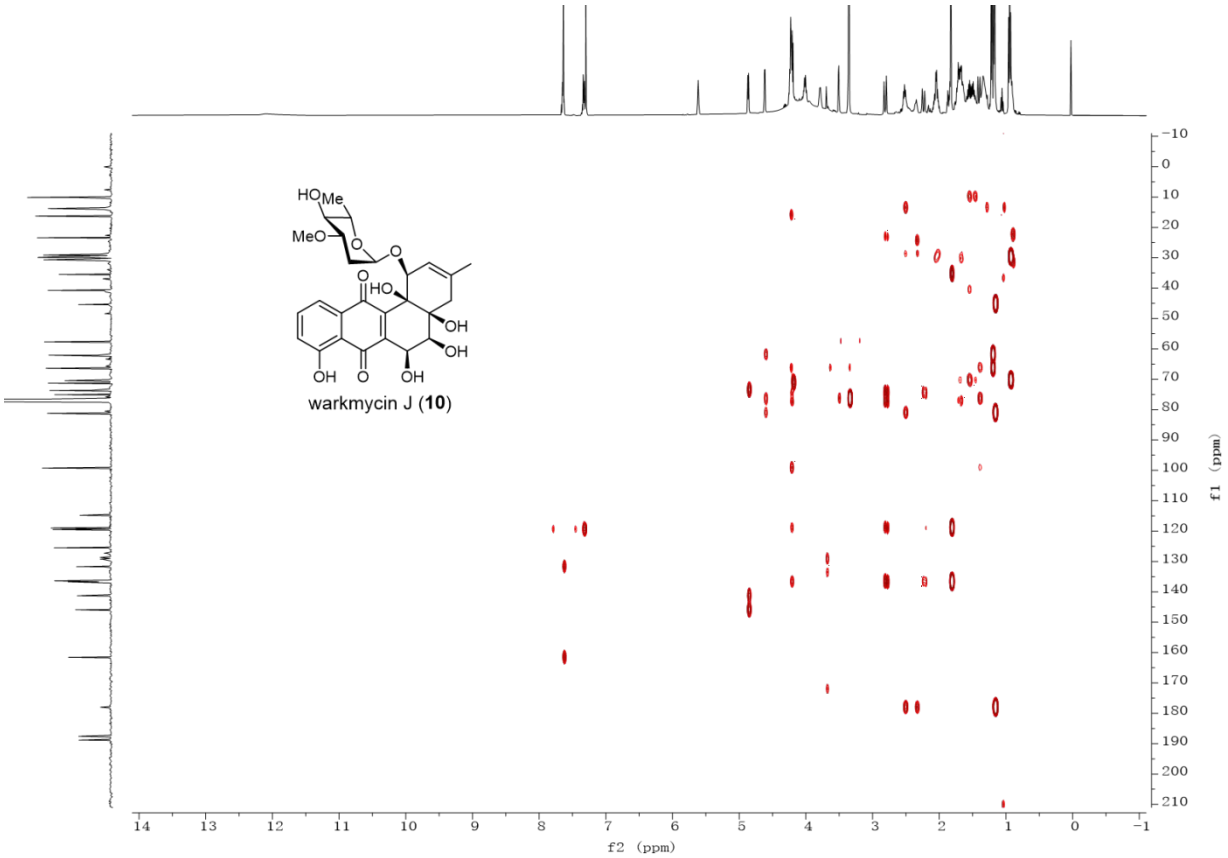


Figure S105. HMBC spectrum of compound **10**

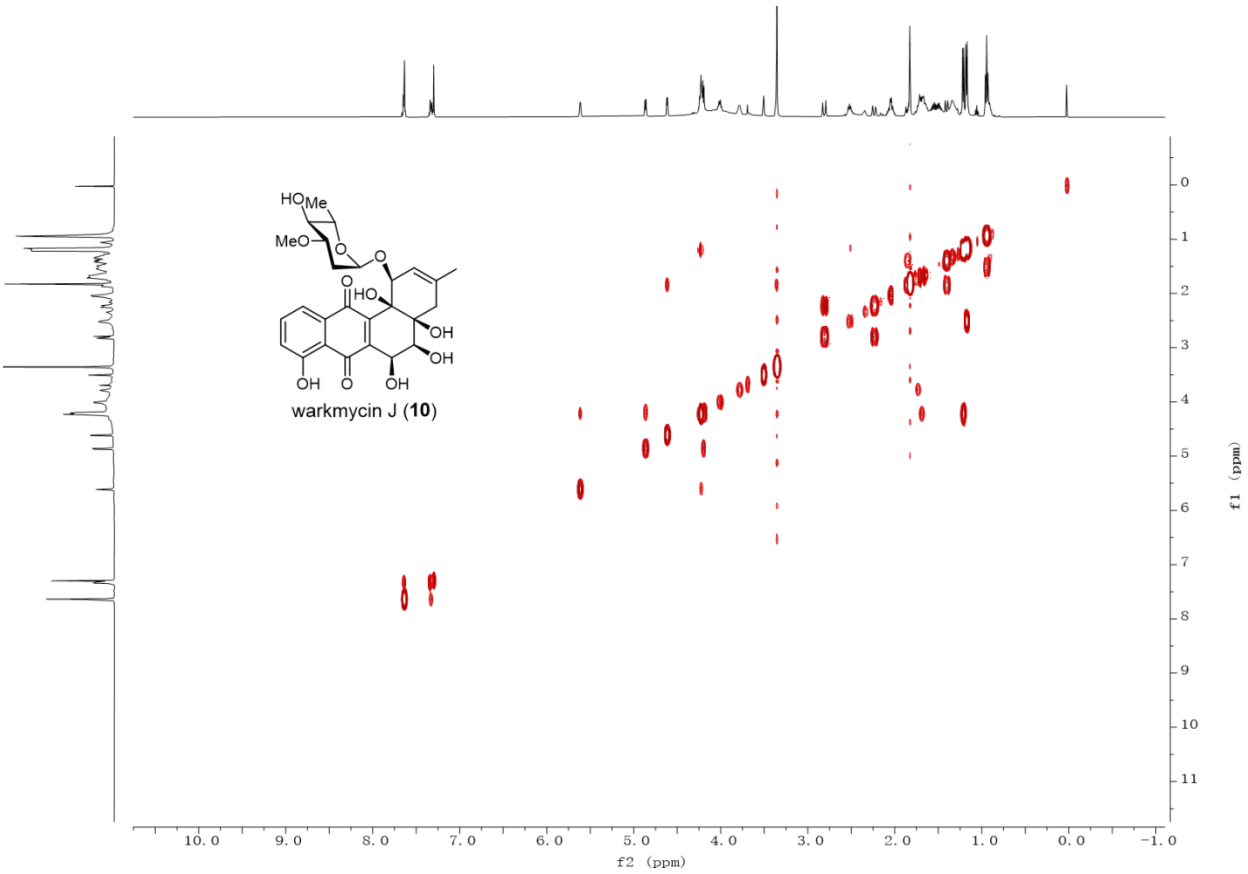


Figure S106. ^{1}H-^{1}H COSY spectrum of compound **10**

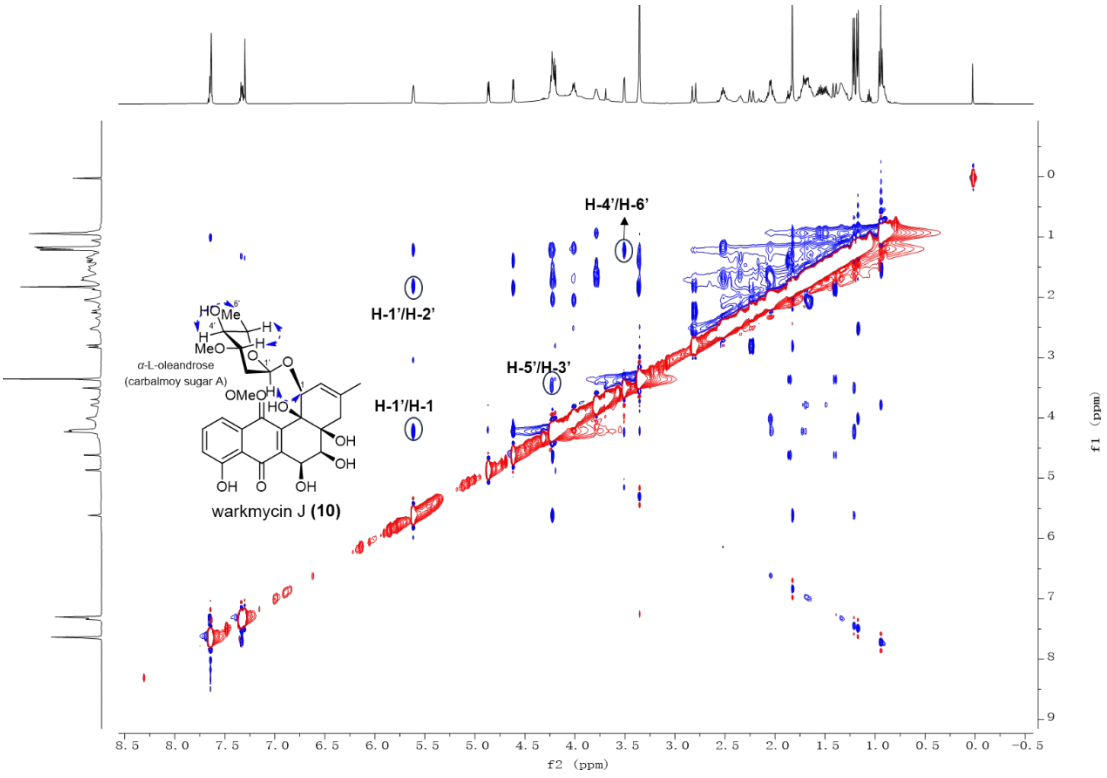


Figure S107. NOESY spectrum of compound **10**

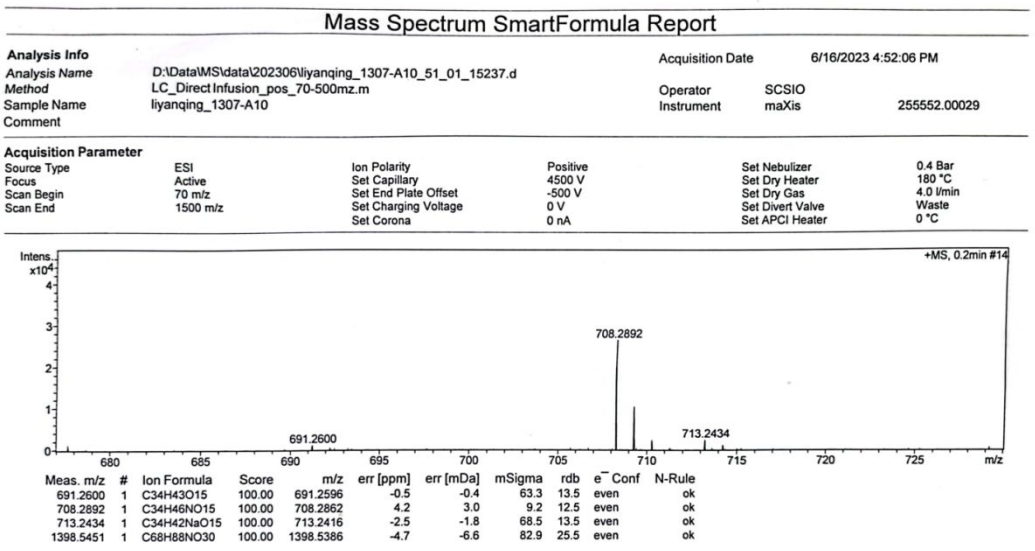


Figure S105. HRESIMS spectrum of compound **11**

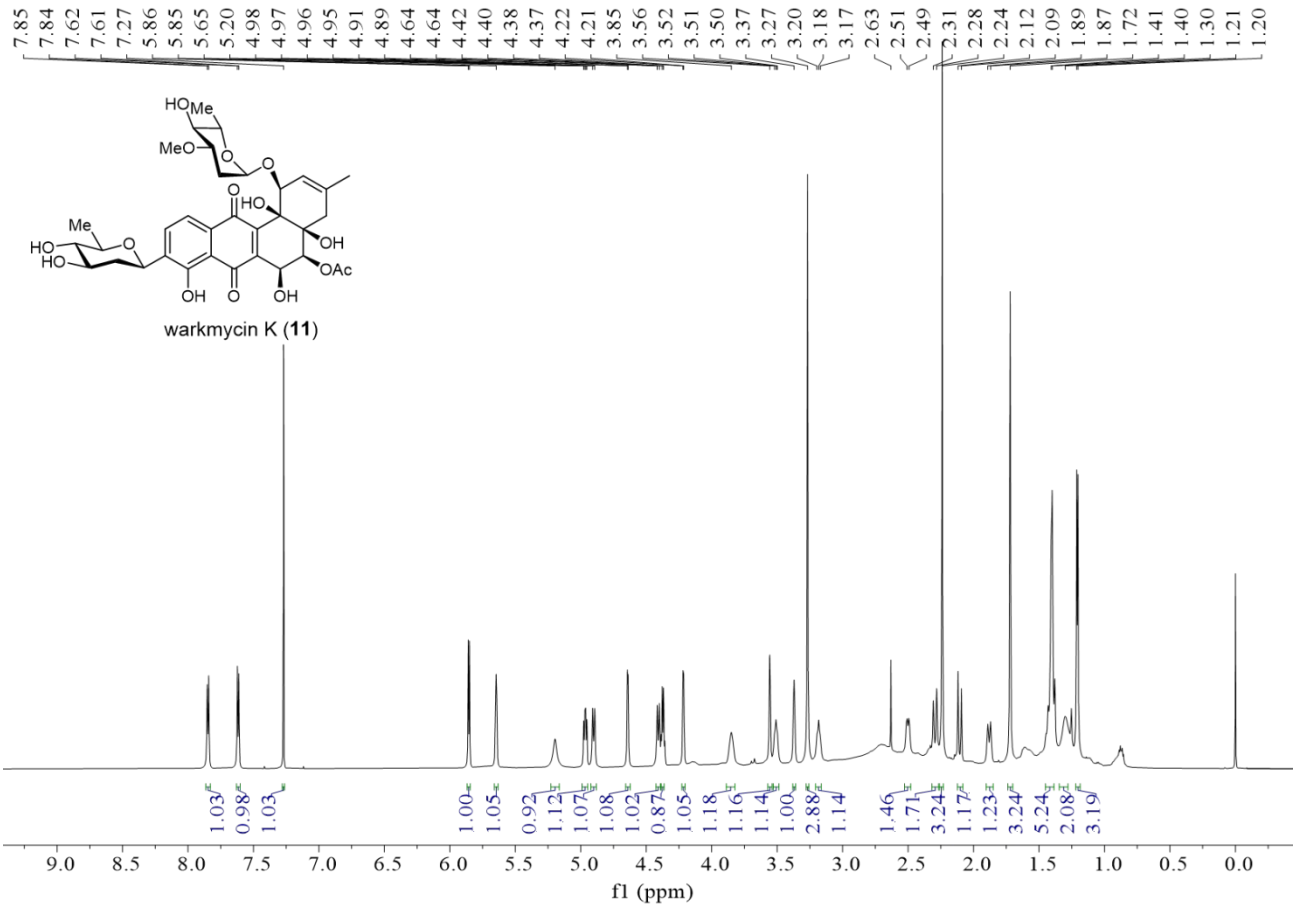


Figure S106. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **11**

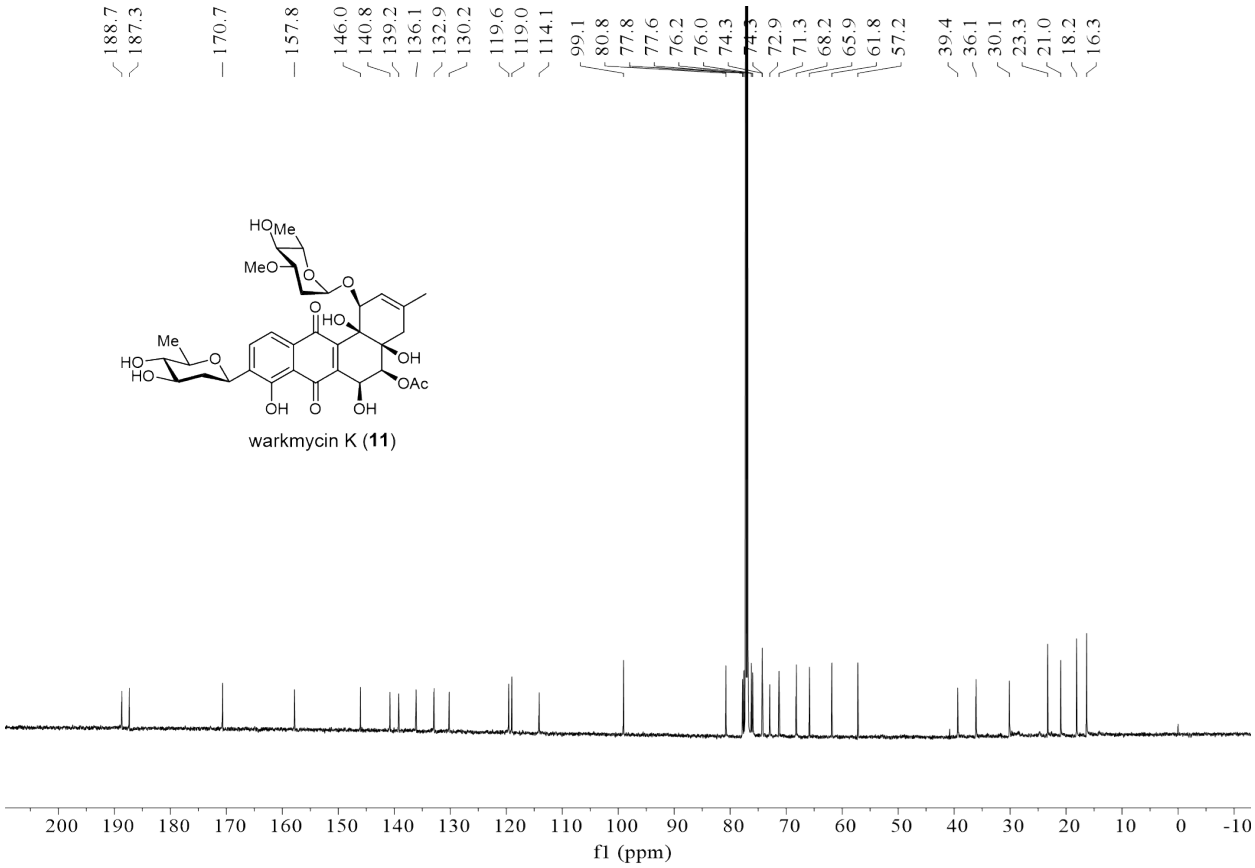


Figure S107. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **11**

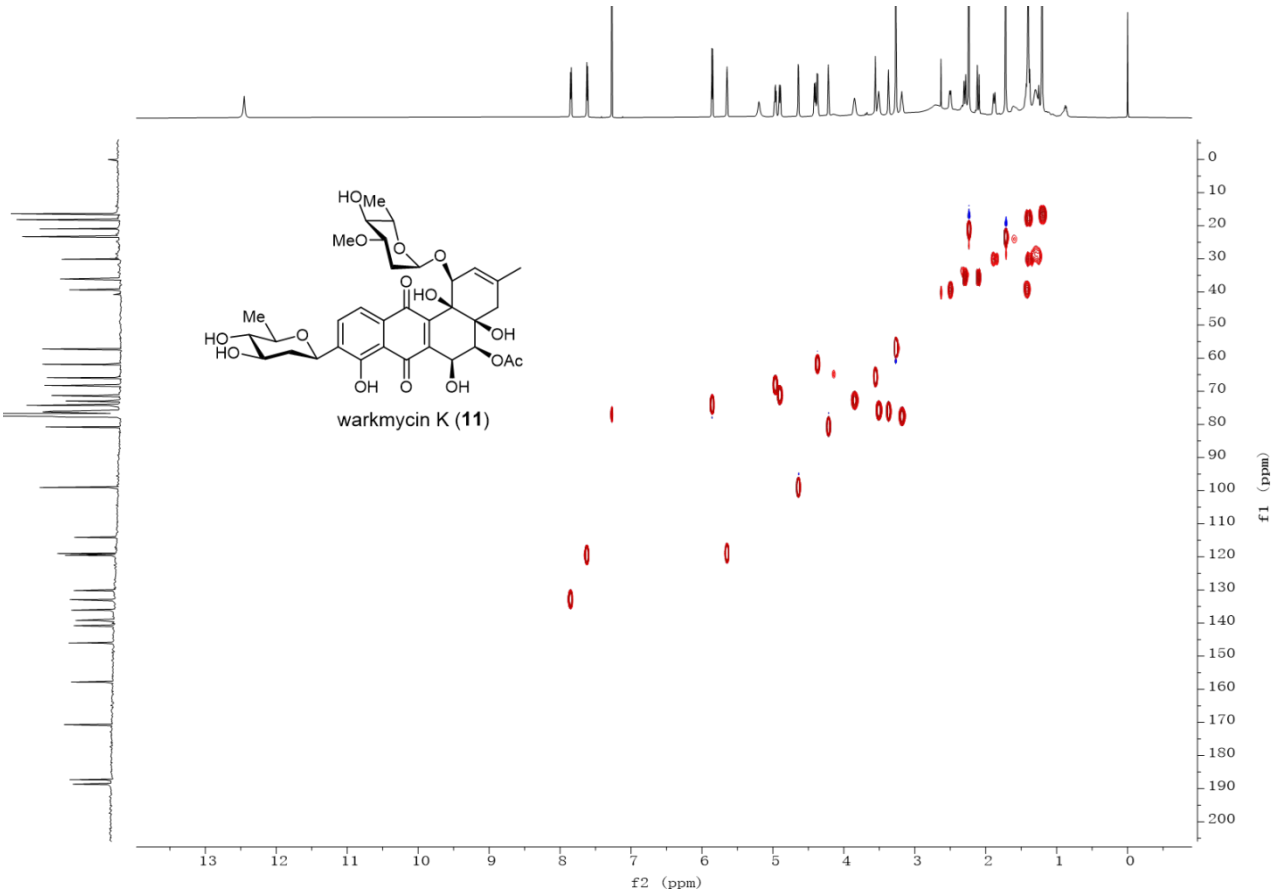


Figure S108. HSQC spectrum of compound **11**

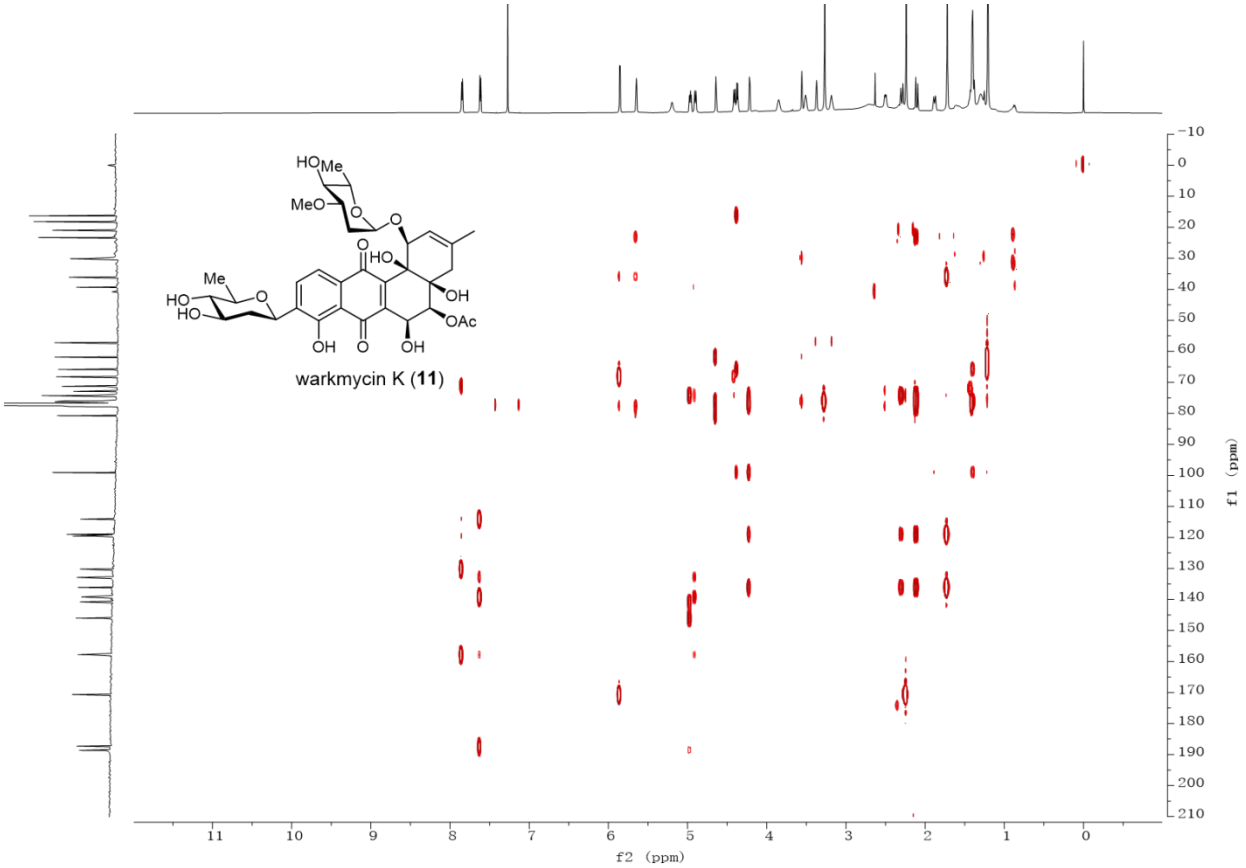


Figure S109. HMBC spectrum of compound **11**

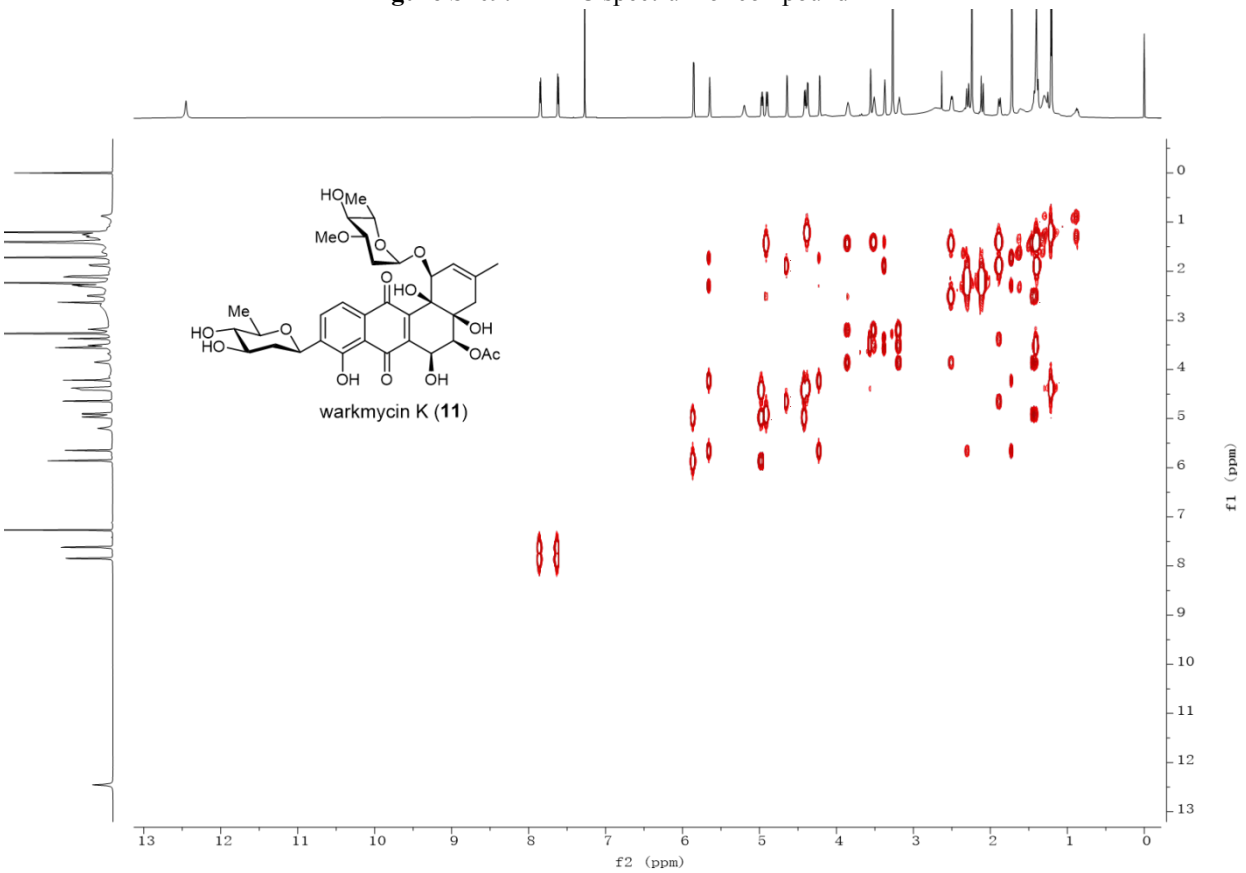


Figure S110. ^{1}H-^{1}H COSY spectrum of compound **11**

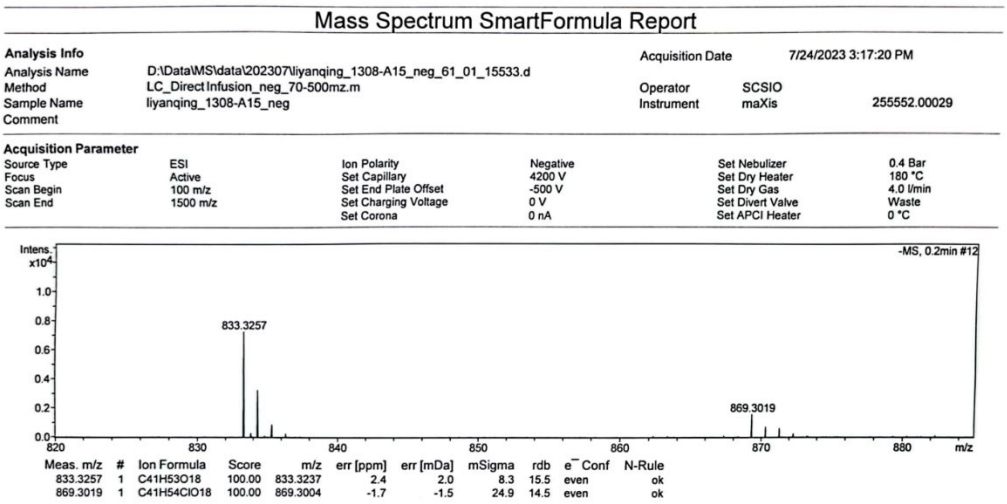


Figure S111. HRESIMS spectrum of compound **12**

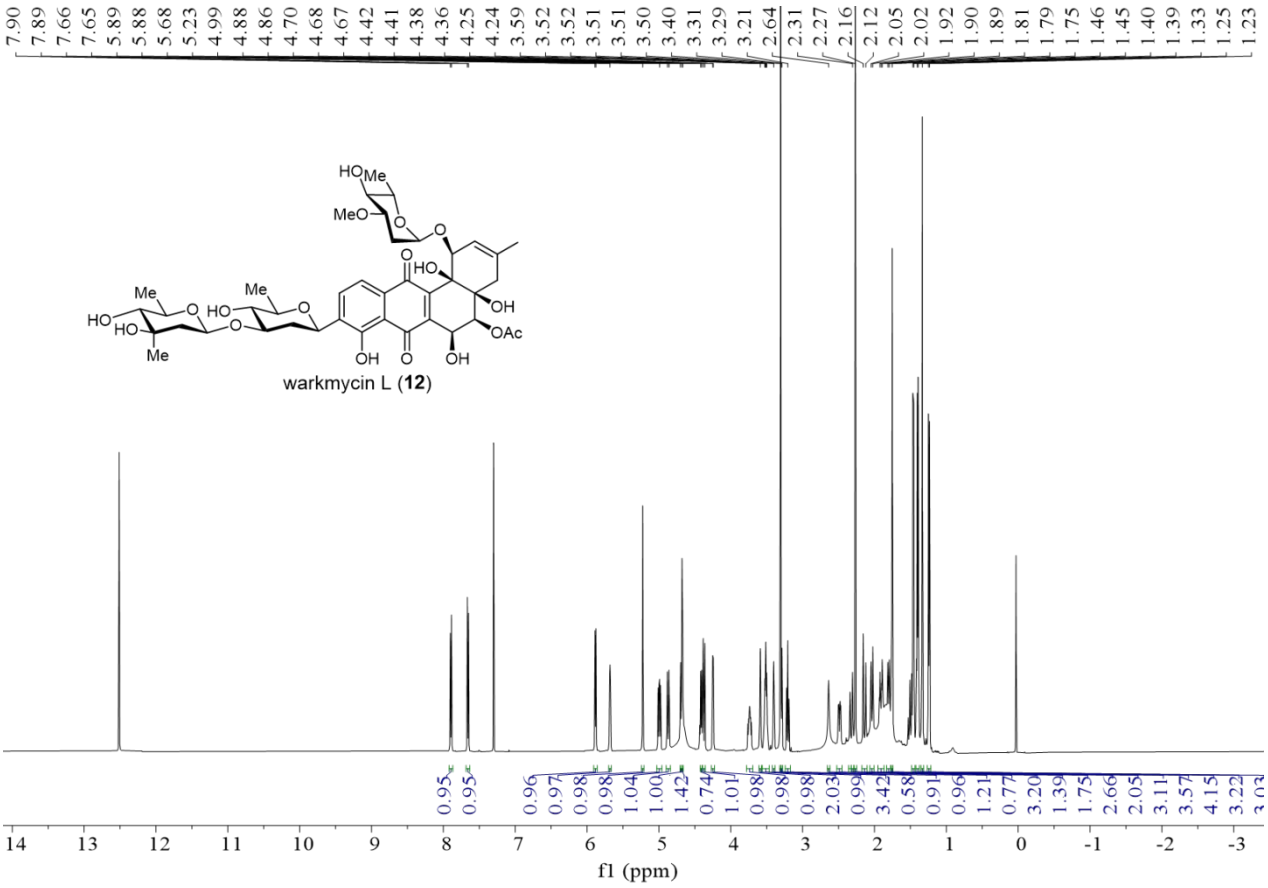


Figure S112. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **12**

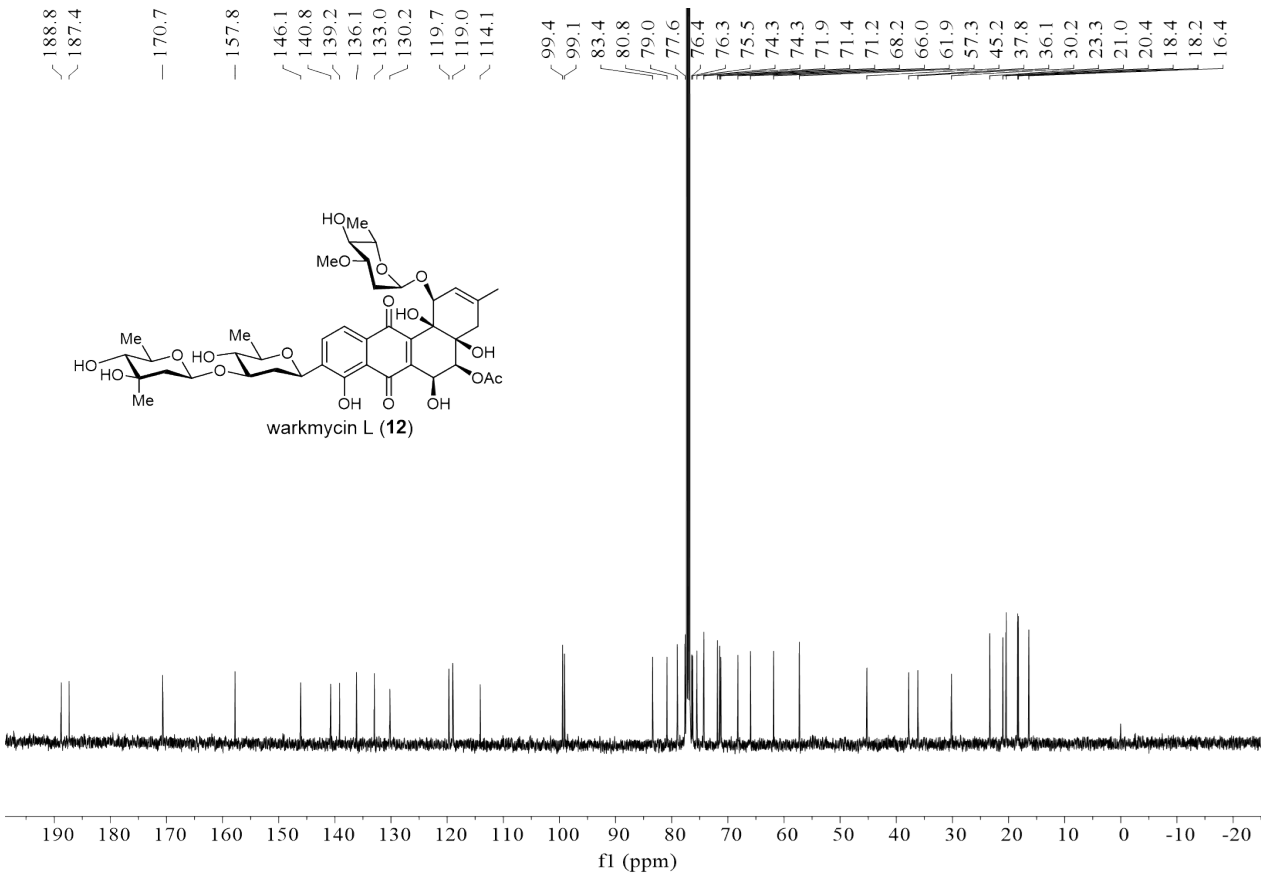


Figure S113. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **12**

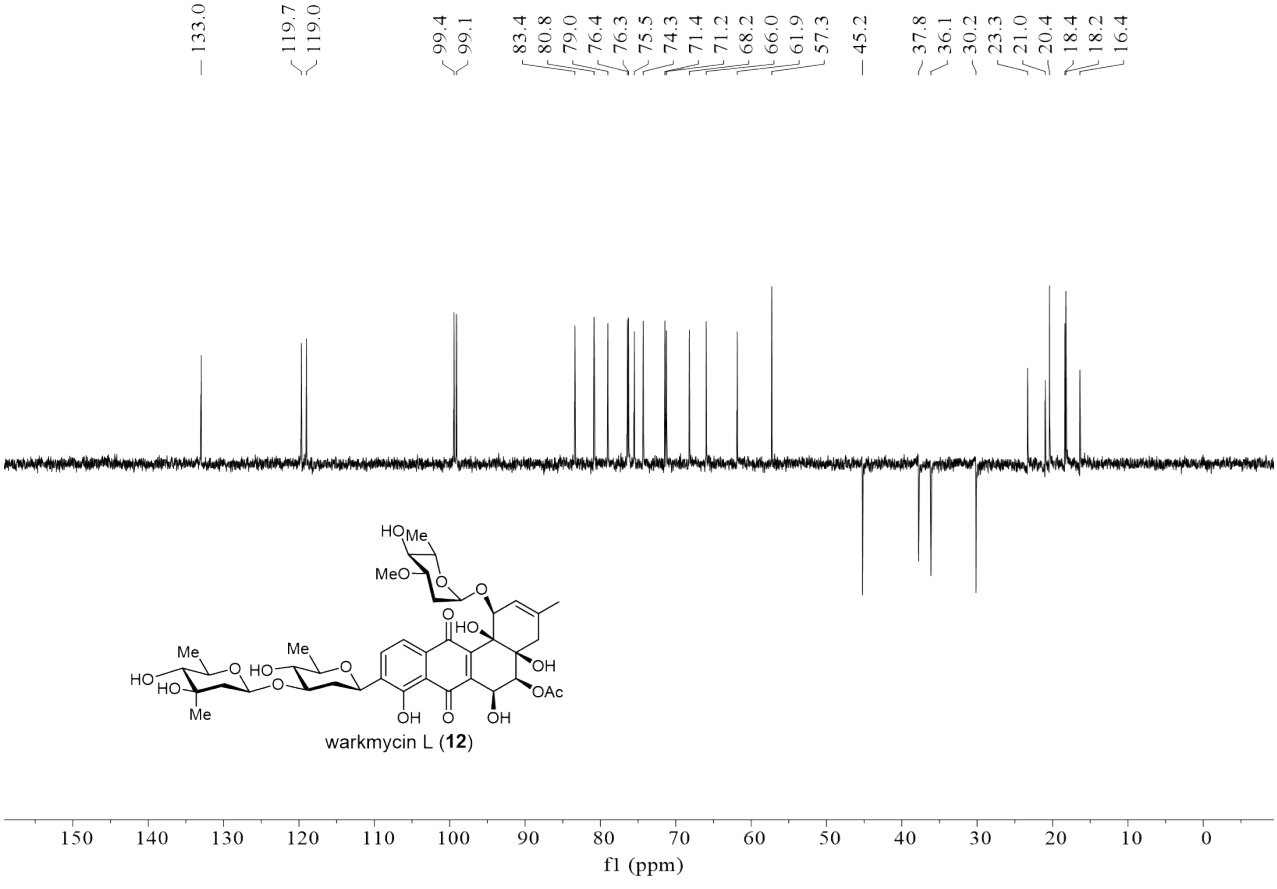


Figure S114. DEPT 135 spectrum of compound **12**

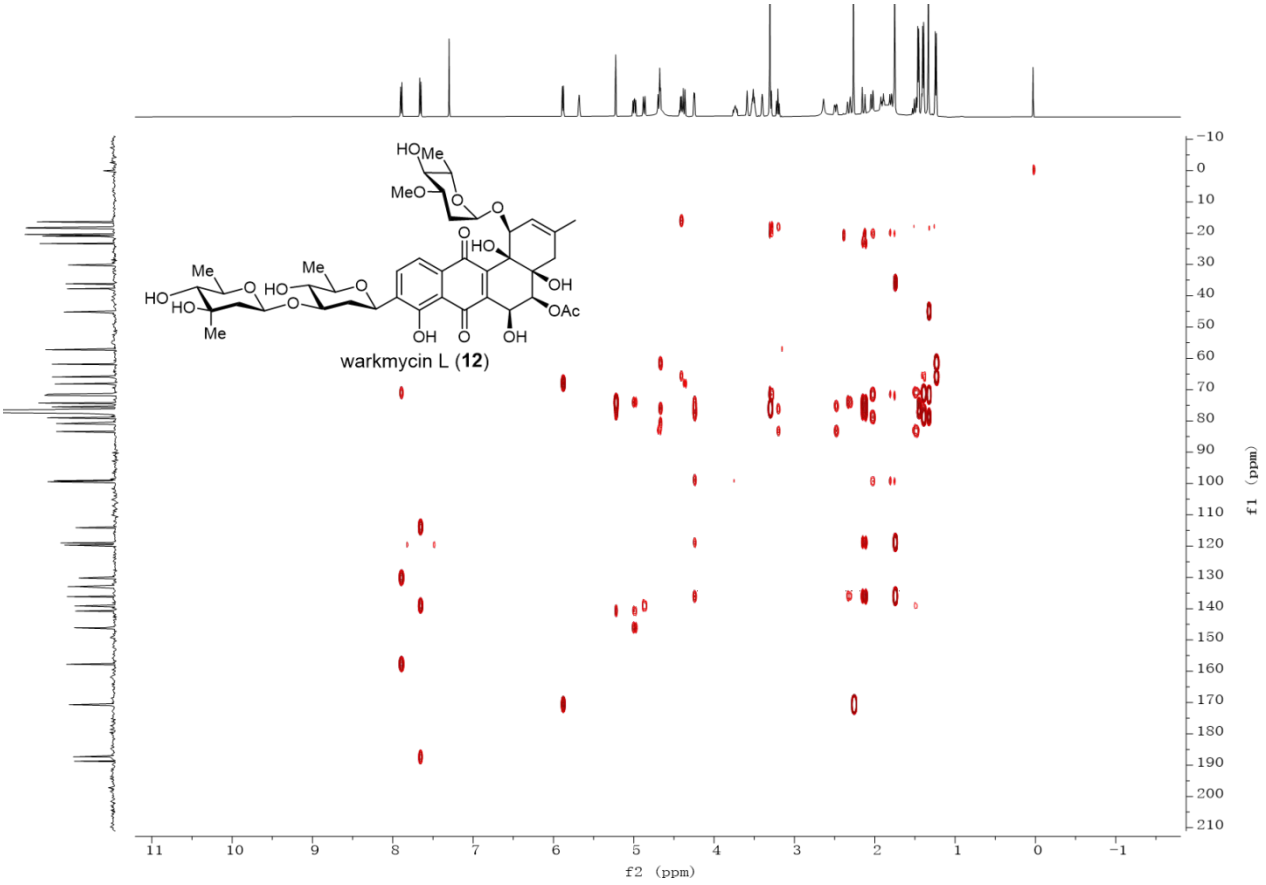


Figure S115. HSQC spectrum of compound **12**

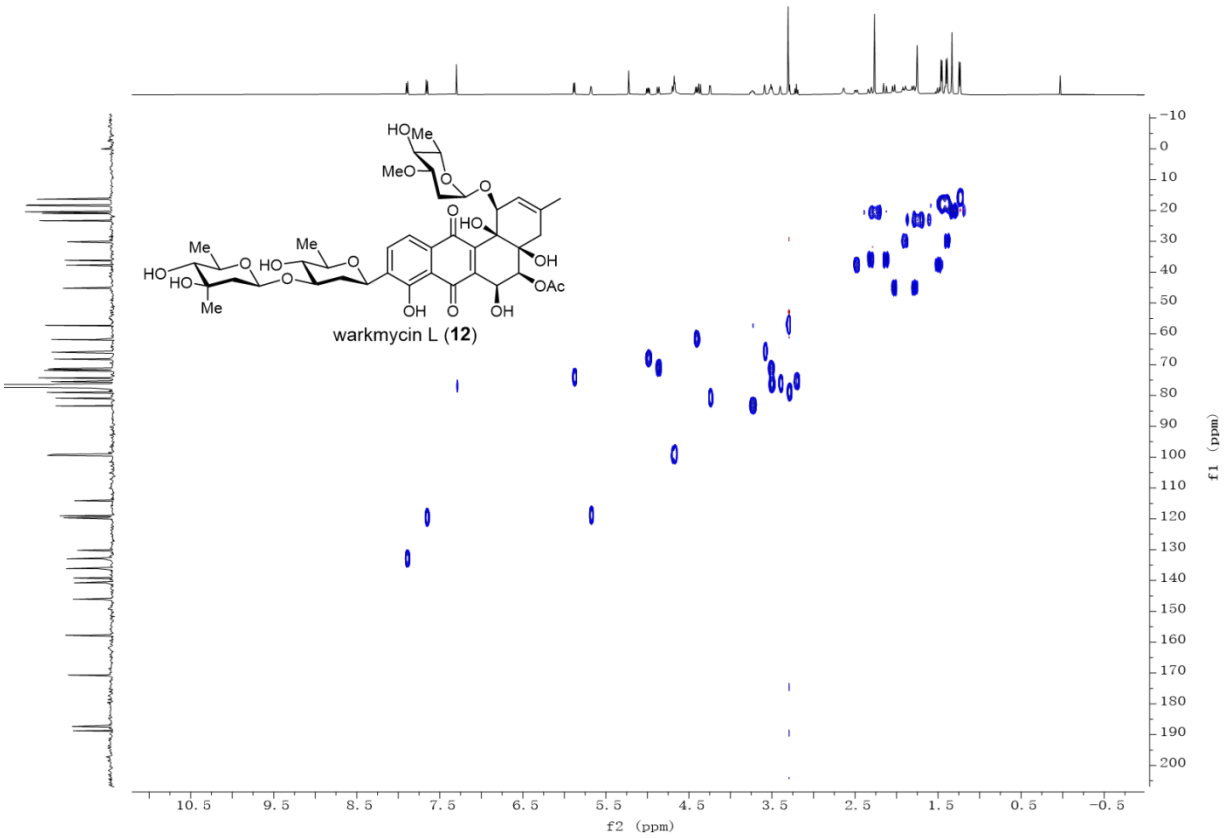


Figure S116. HMBC spectrum of compound **12**

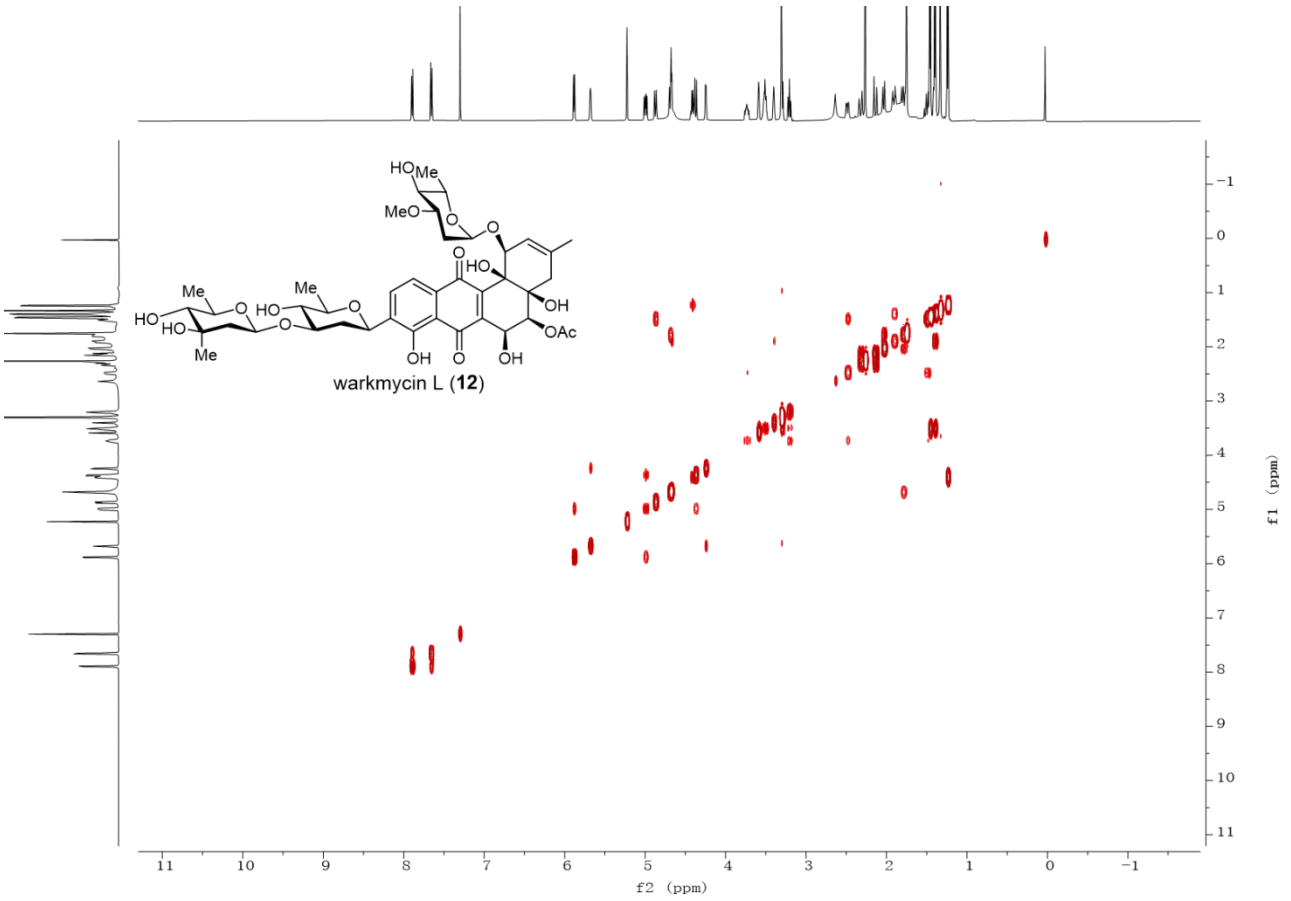


Figure S117. ^{1}H-^{1}H COSY spectrum of compound **12**

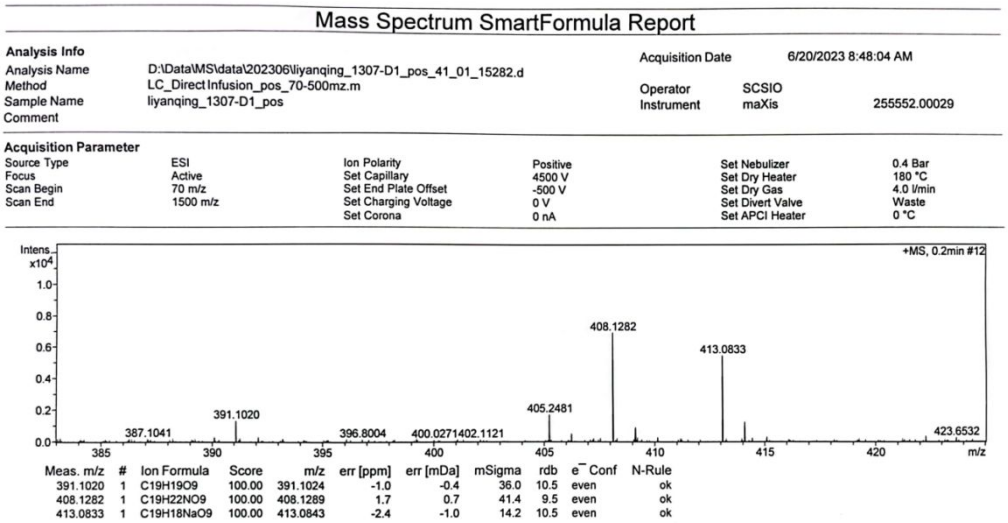


Figure S118. HRESIMS spectrum of compound **13**

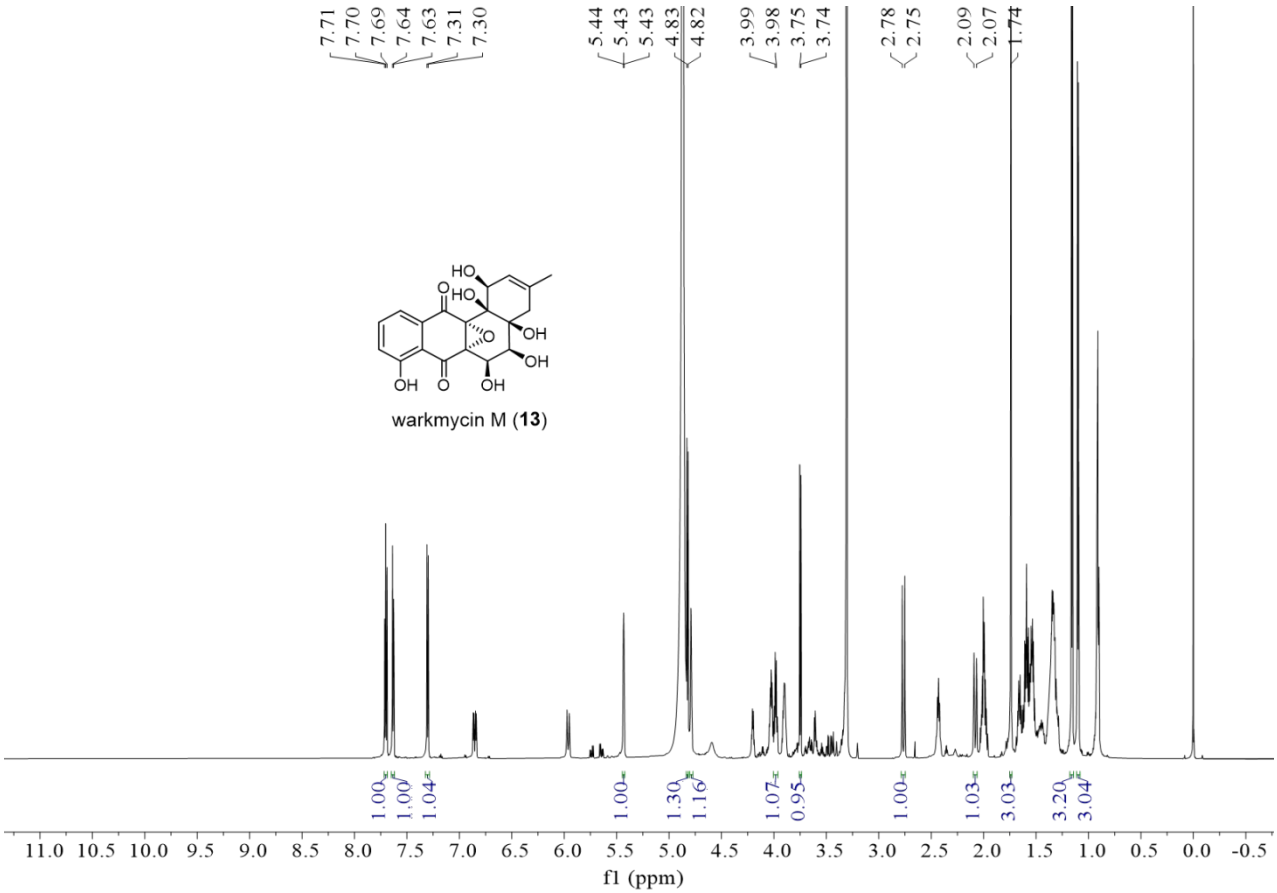


Figure S119. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **13**

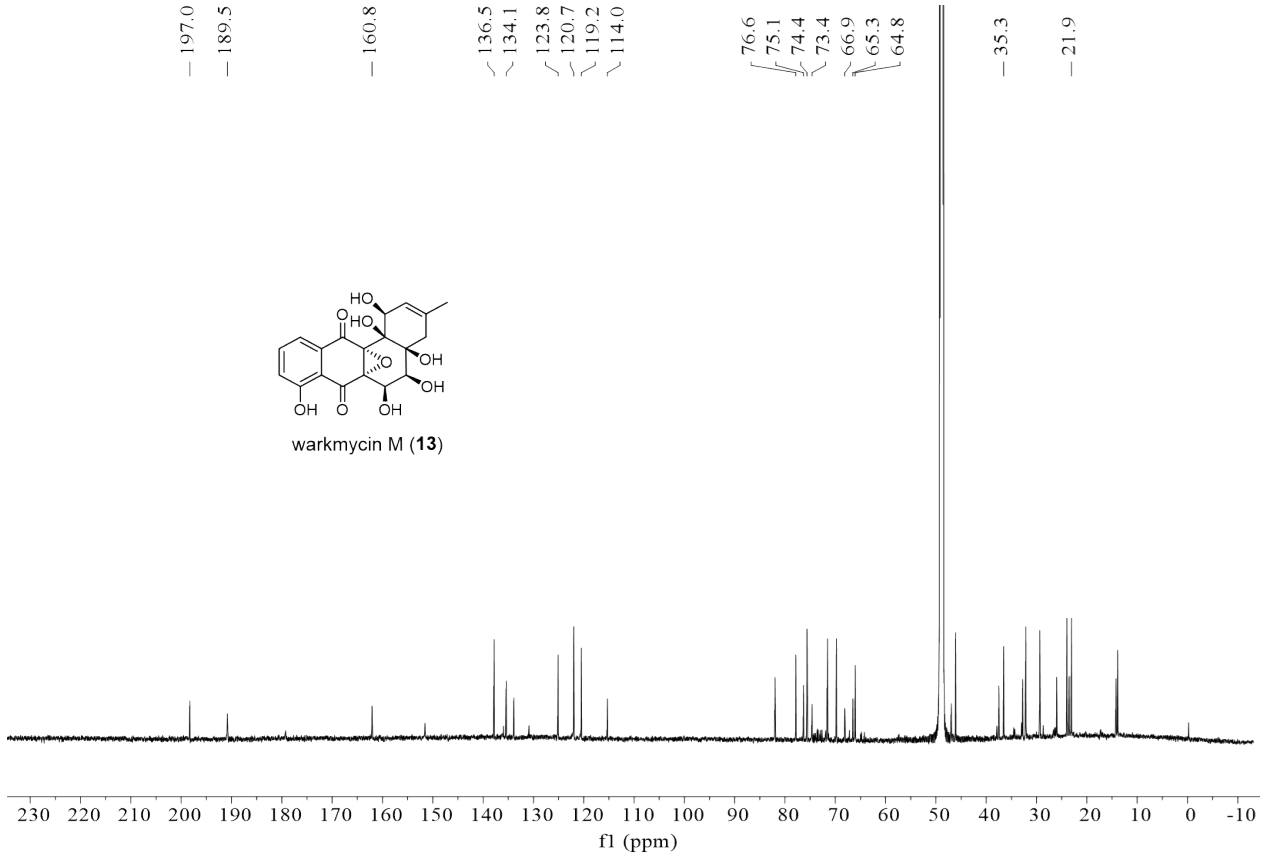


Figure S120. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **13**

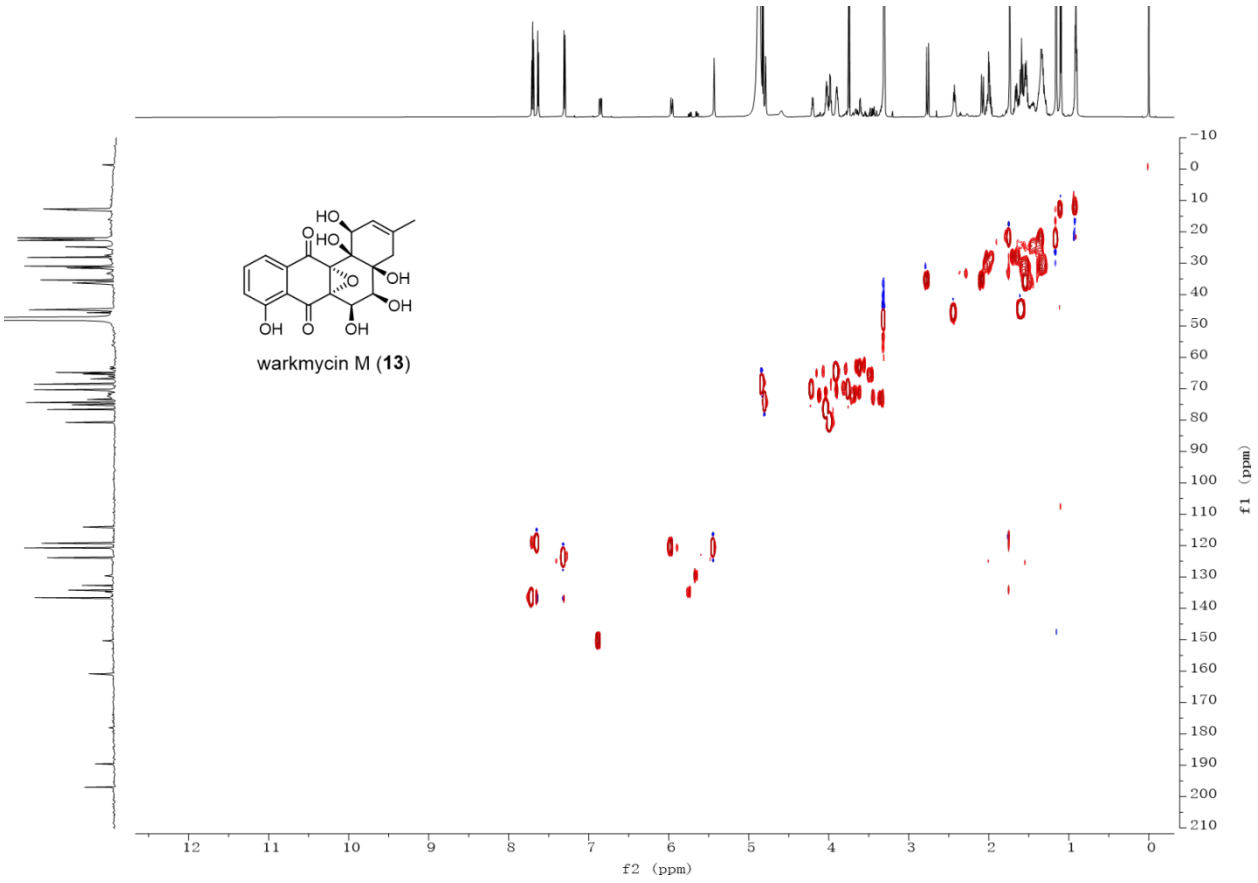


Figure S121. HSQC spectrum of compound **13**

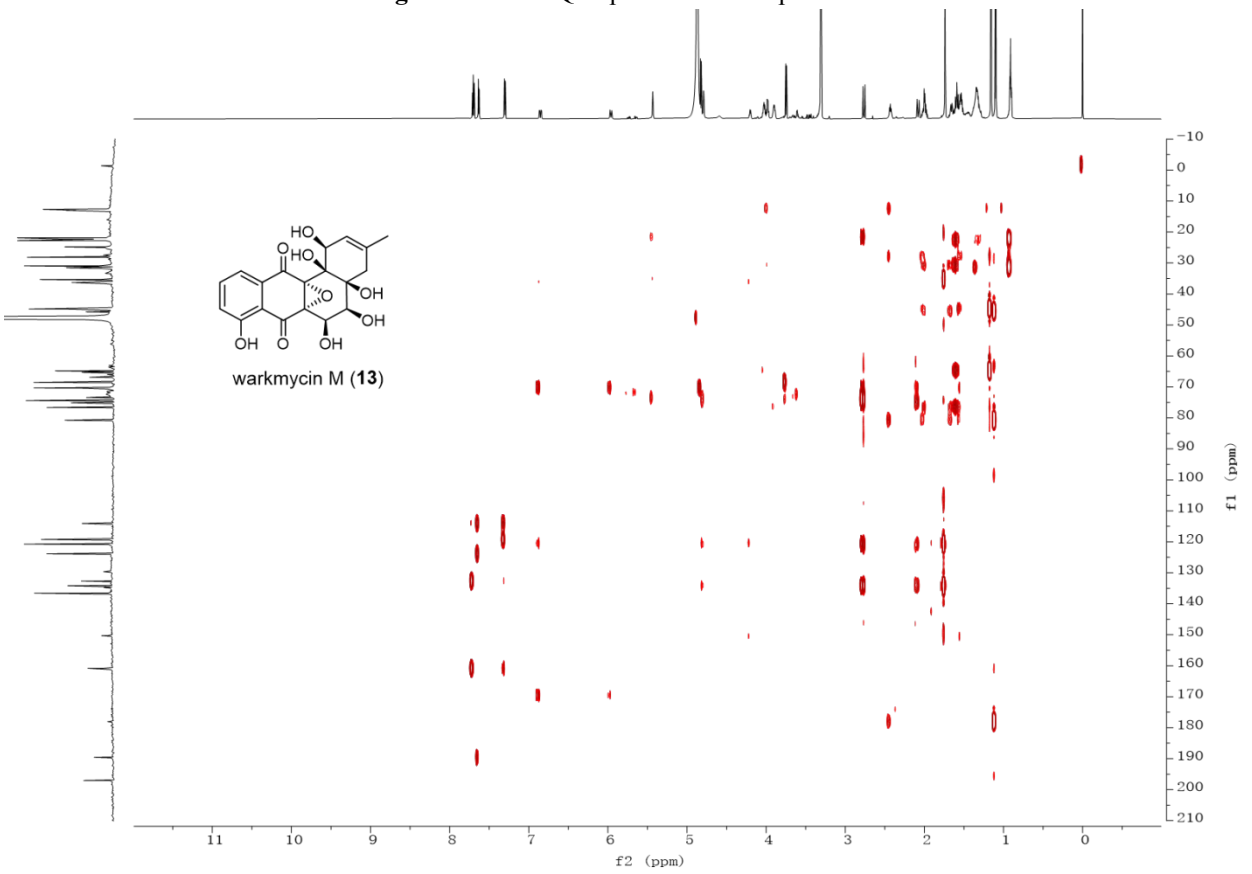


Figure S122. HMBC spectrum of compound **13**

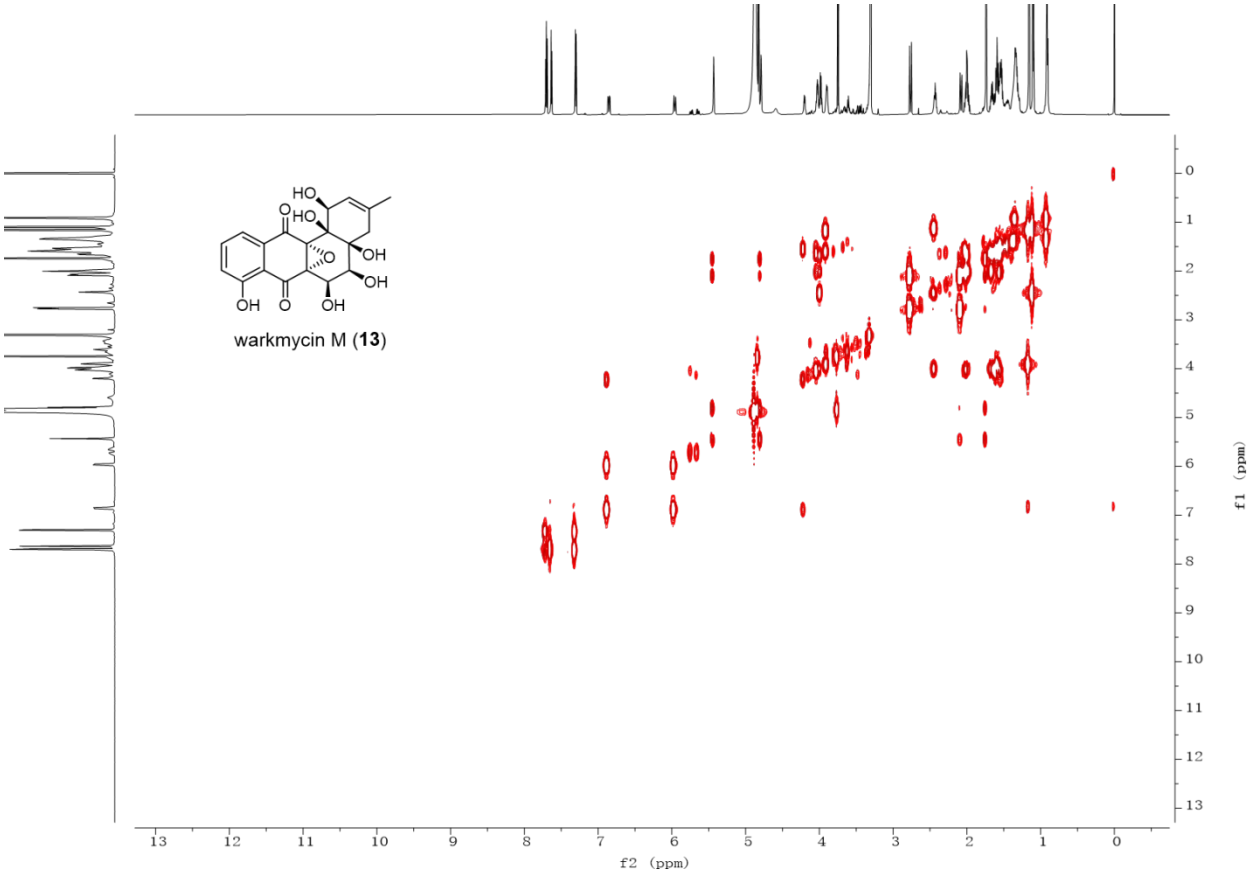


Figure S123. ^{1}H-^{1}H COSY spectrum of compound **13**

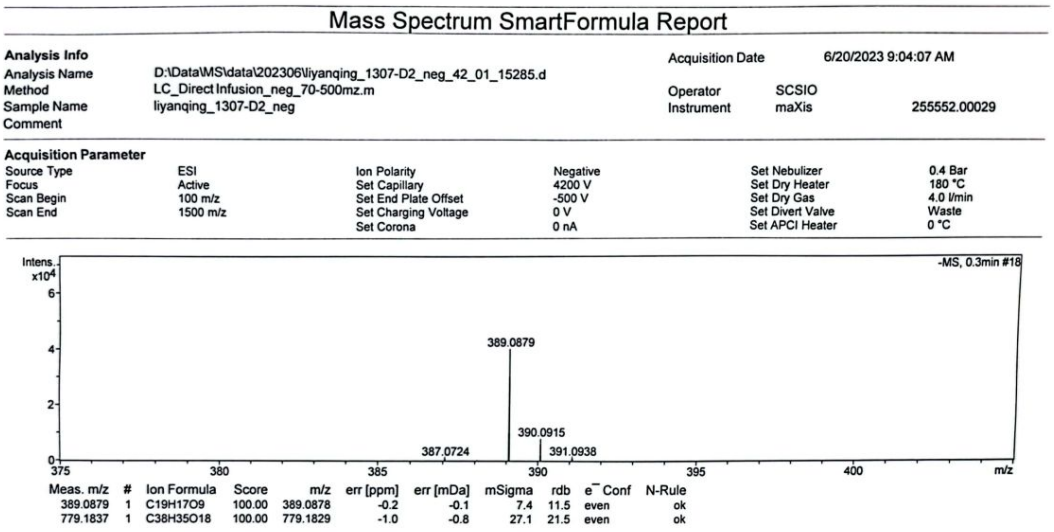


Figure S124. HRESIMS spectrum of compound **14**

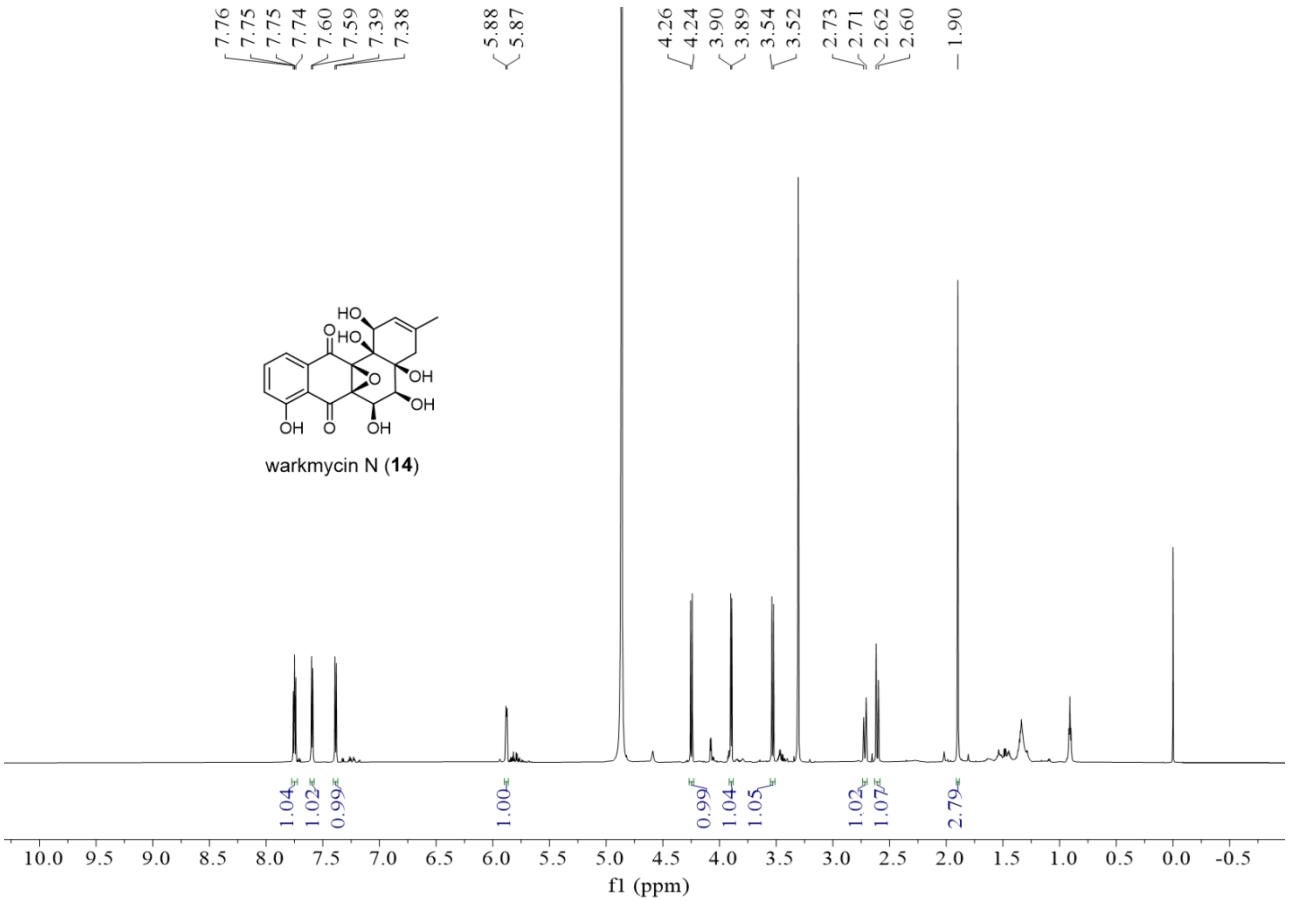


Figure S125. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **14**

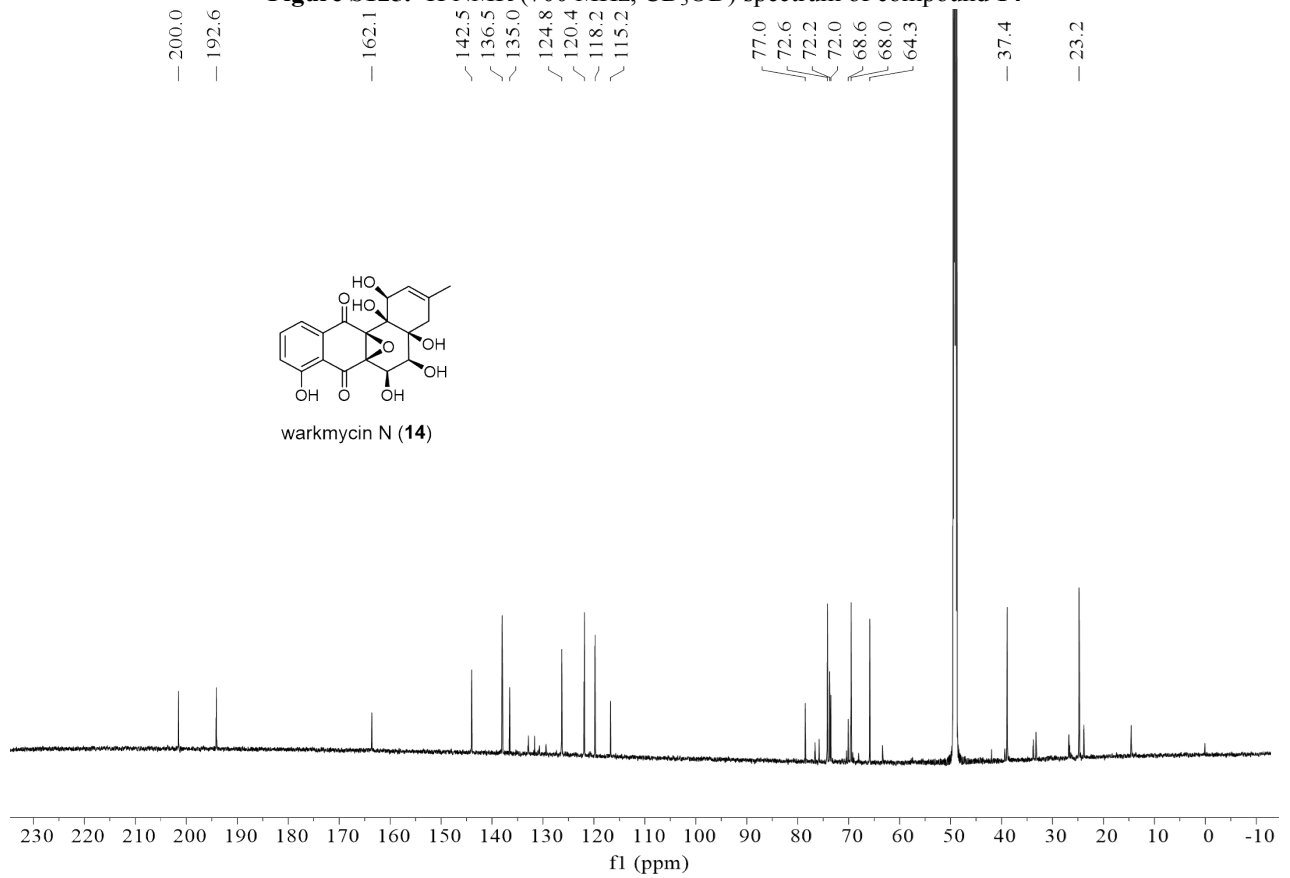


Figure S126. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **14**

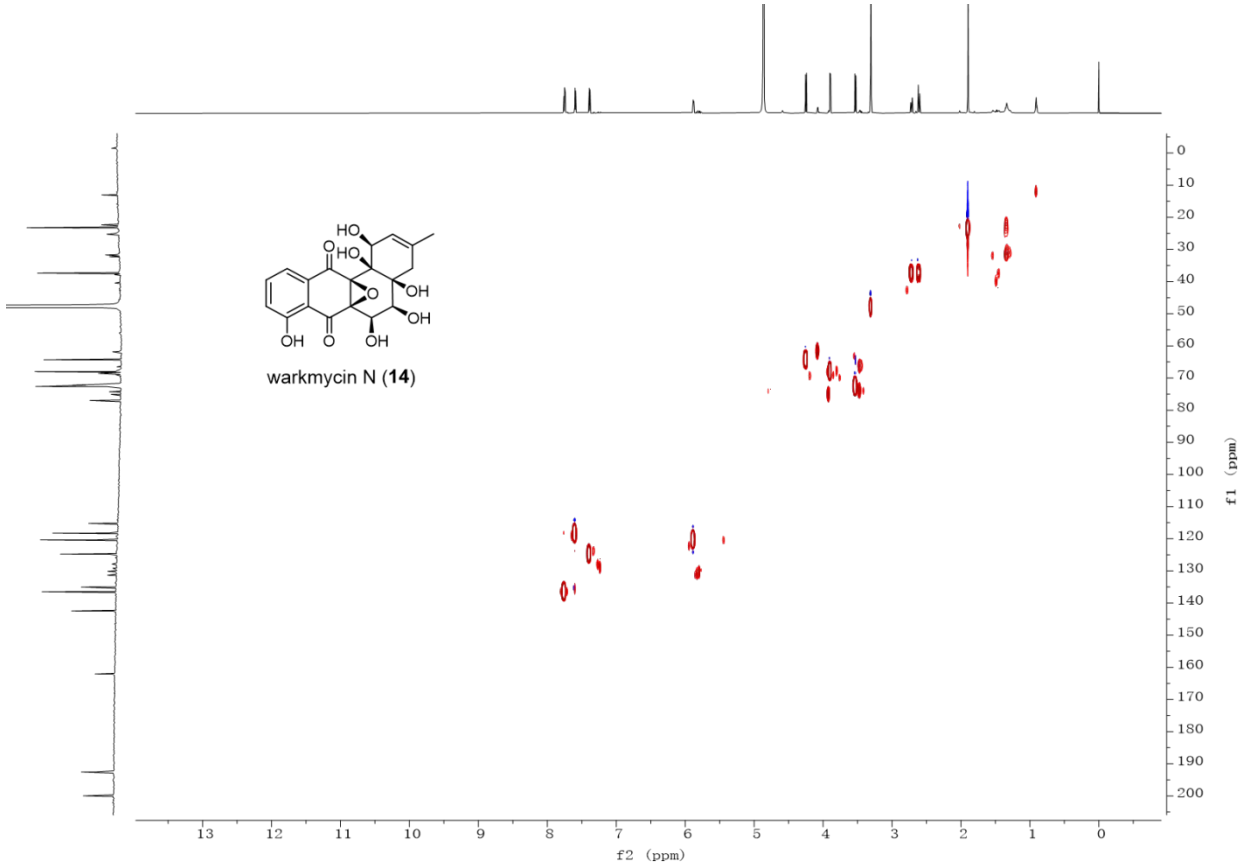


Figure S127. HSQC spectrum of compound **14**

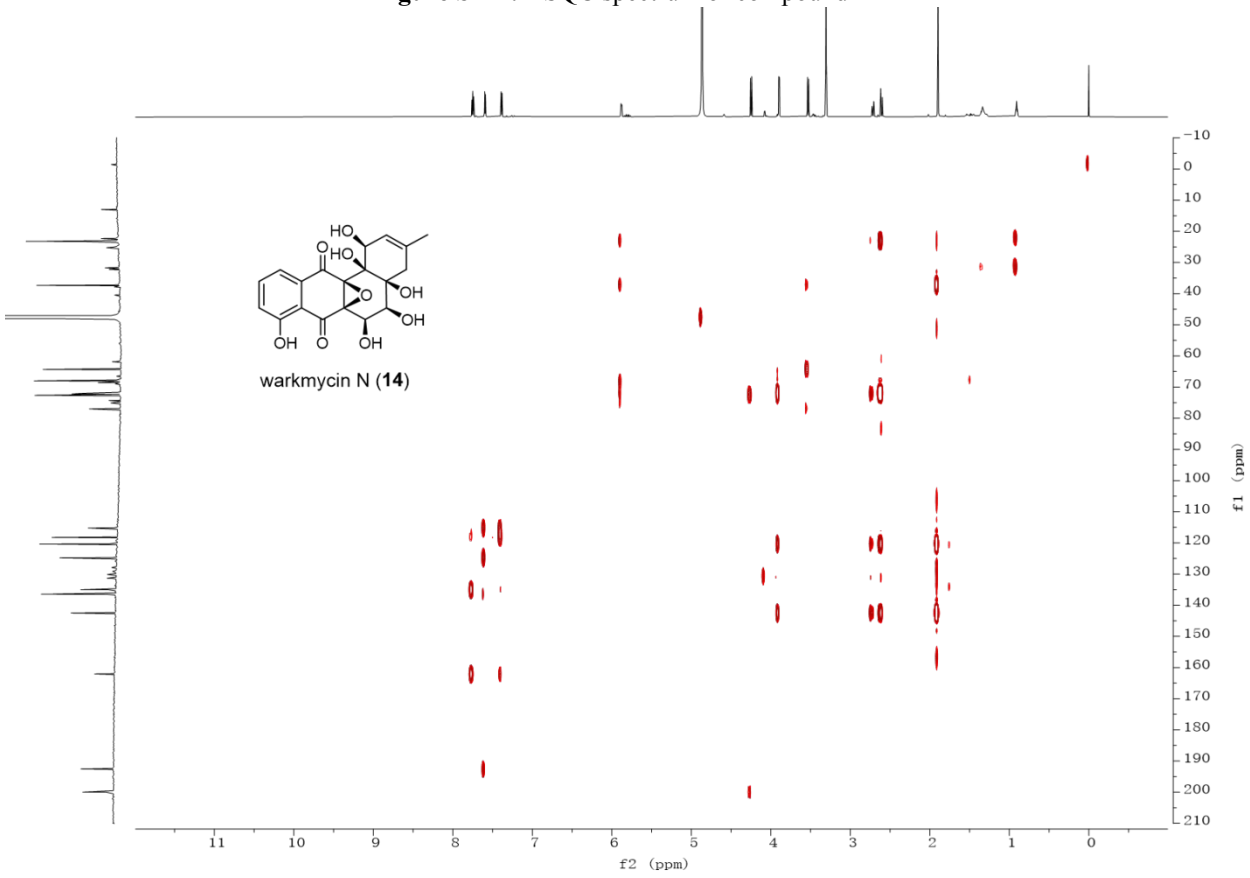


Figure S128. HMBC spectrum of compound **14**

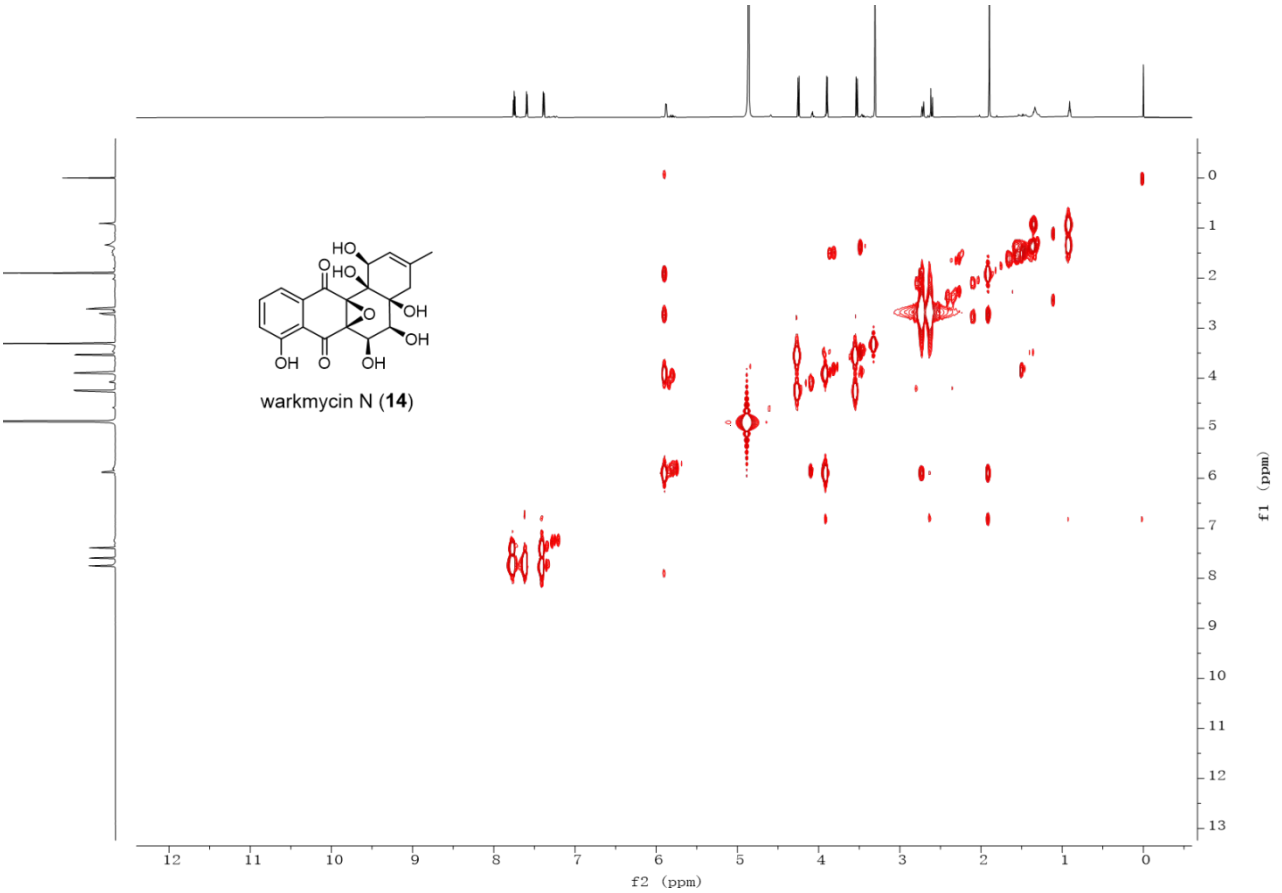


Figure S129. ^{1}H-^{1}H COSY spectrum of compound **14**

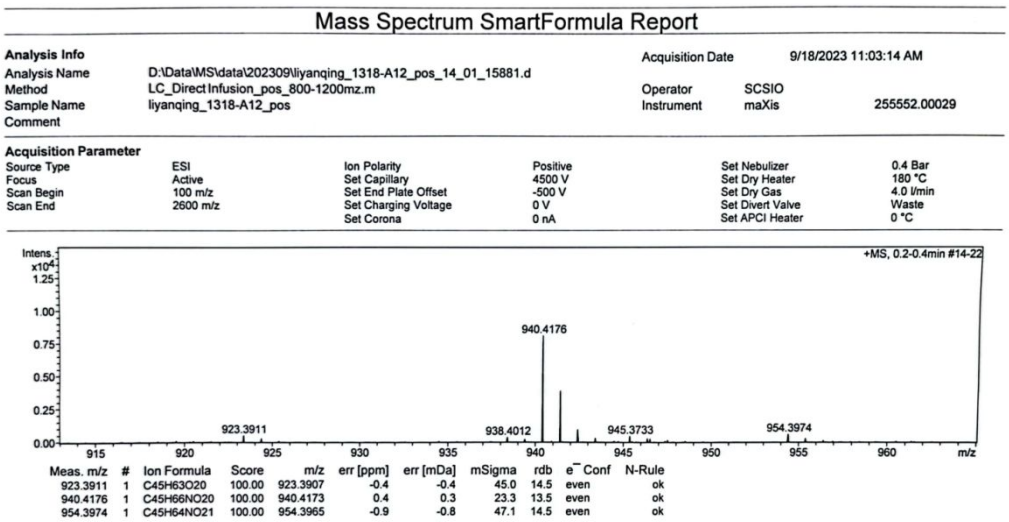


Figure S130. HRESIMS spectrum of compound **15**

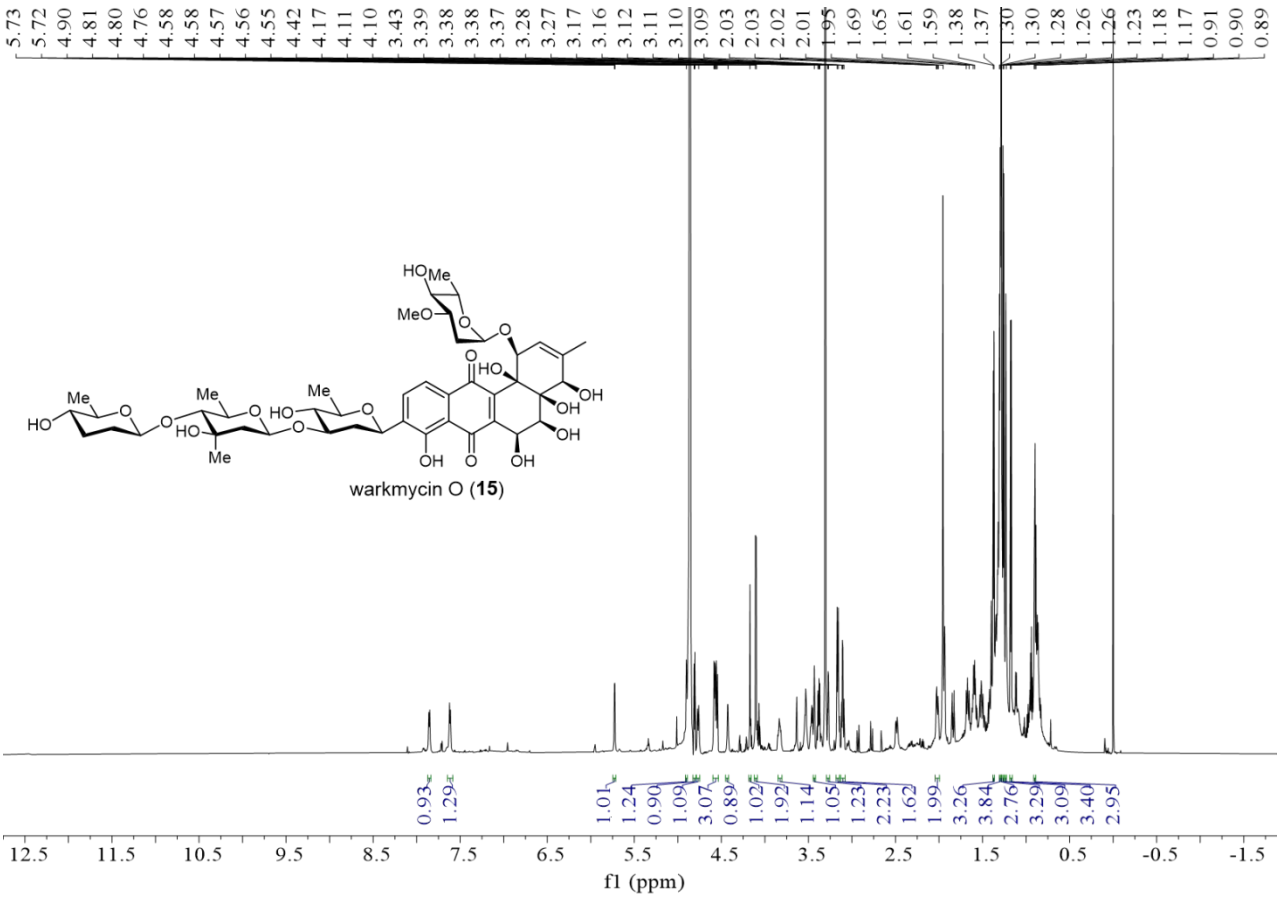


Figure S131. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **15**

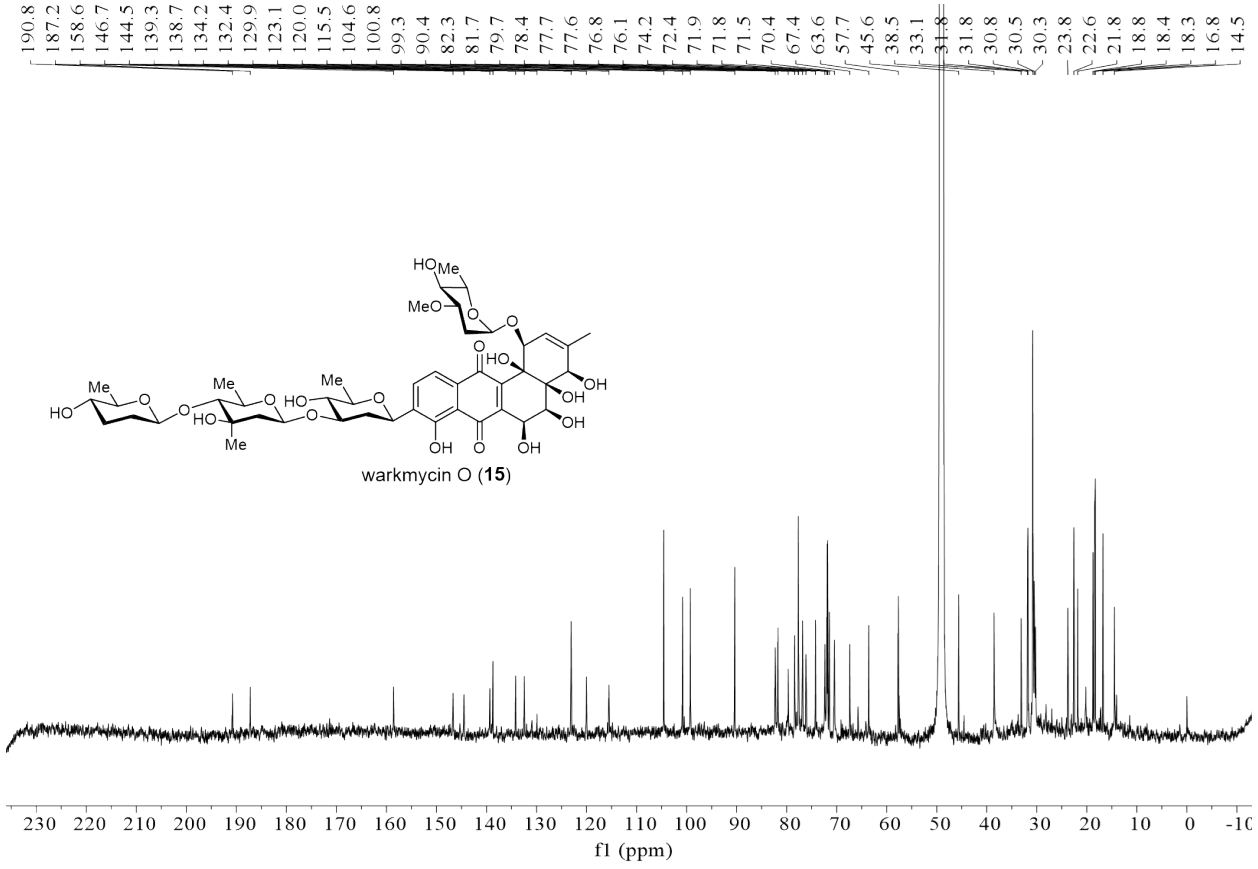


Figure S132. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **15**

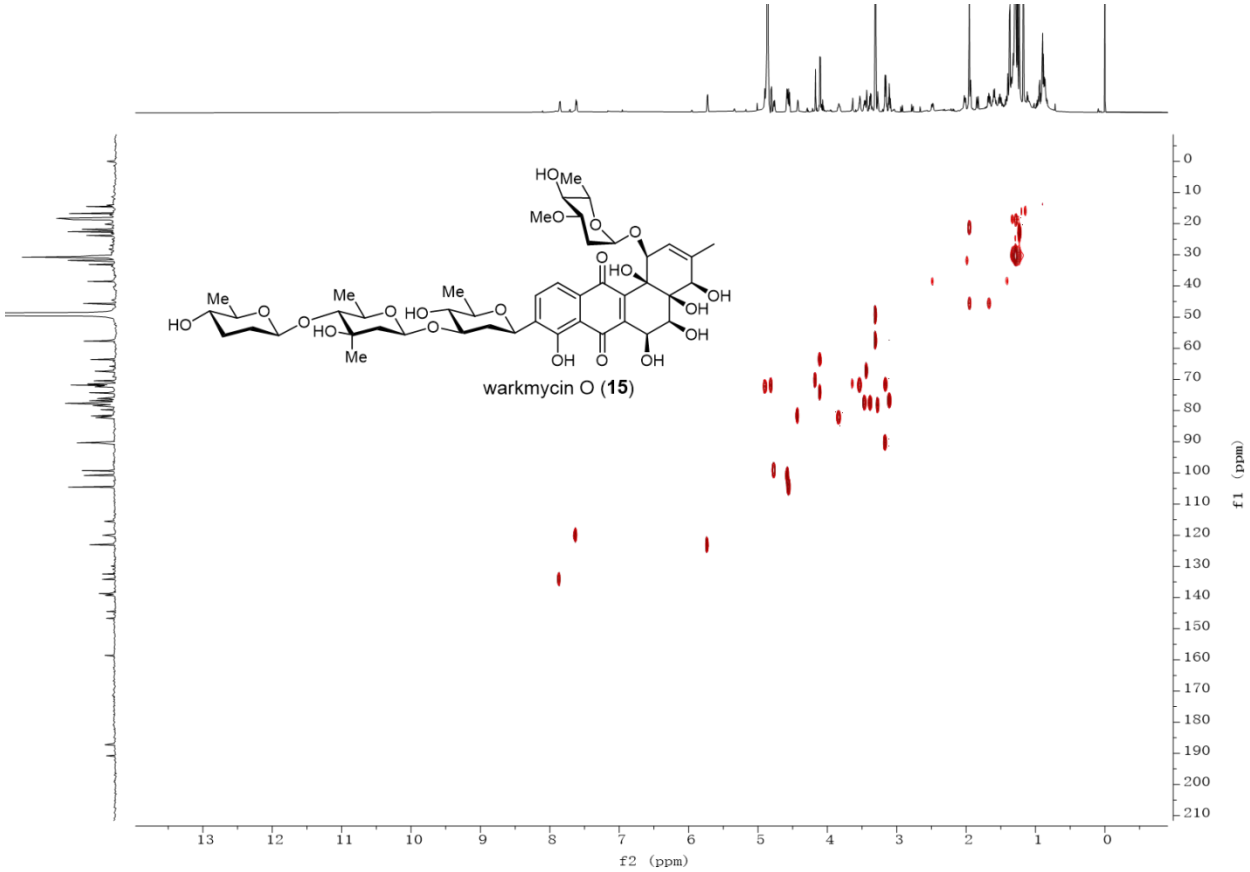


Figure S133. HSQC spectrum of compound **15**

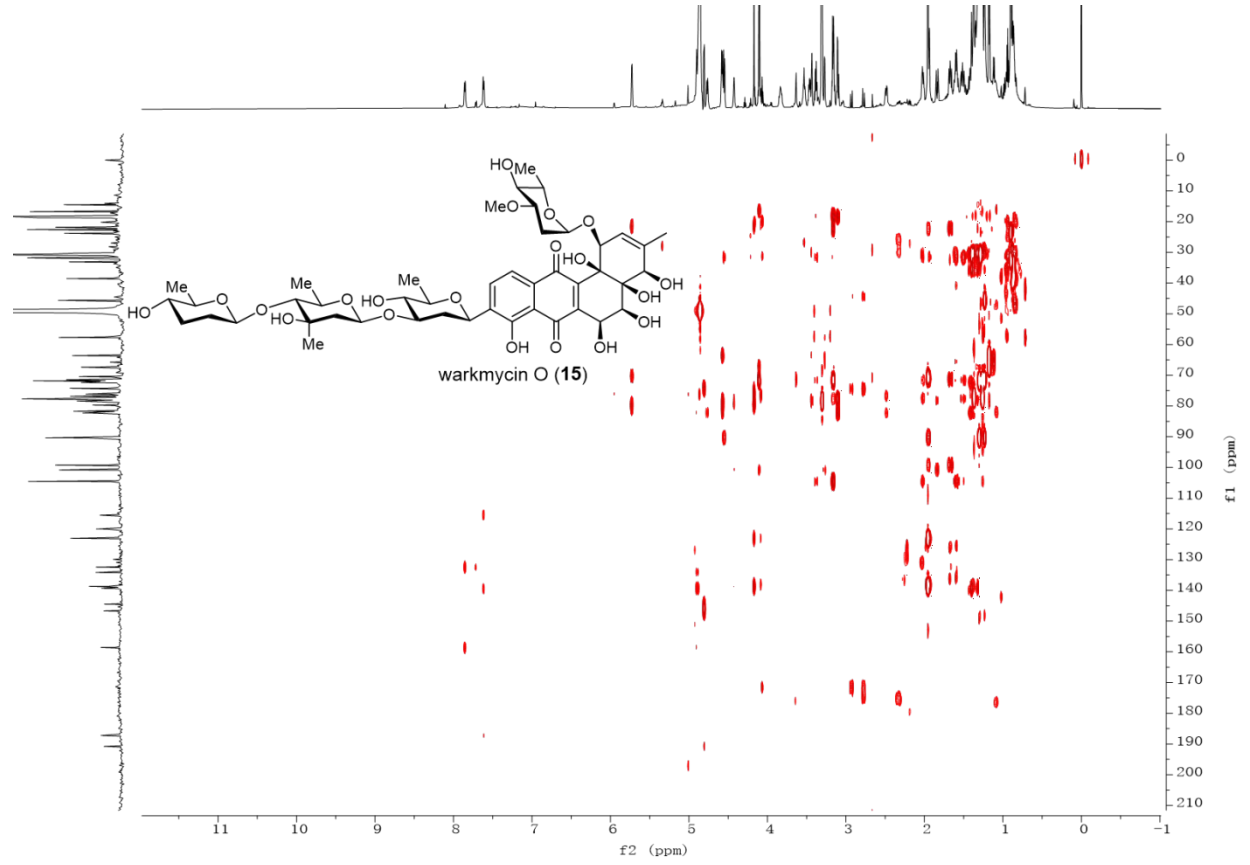


Figure S134. HMBC spectrum of compound **15**

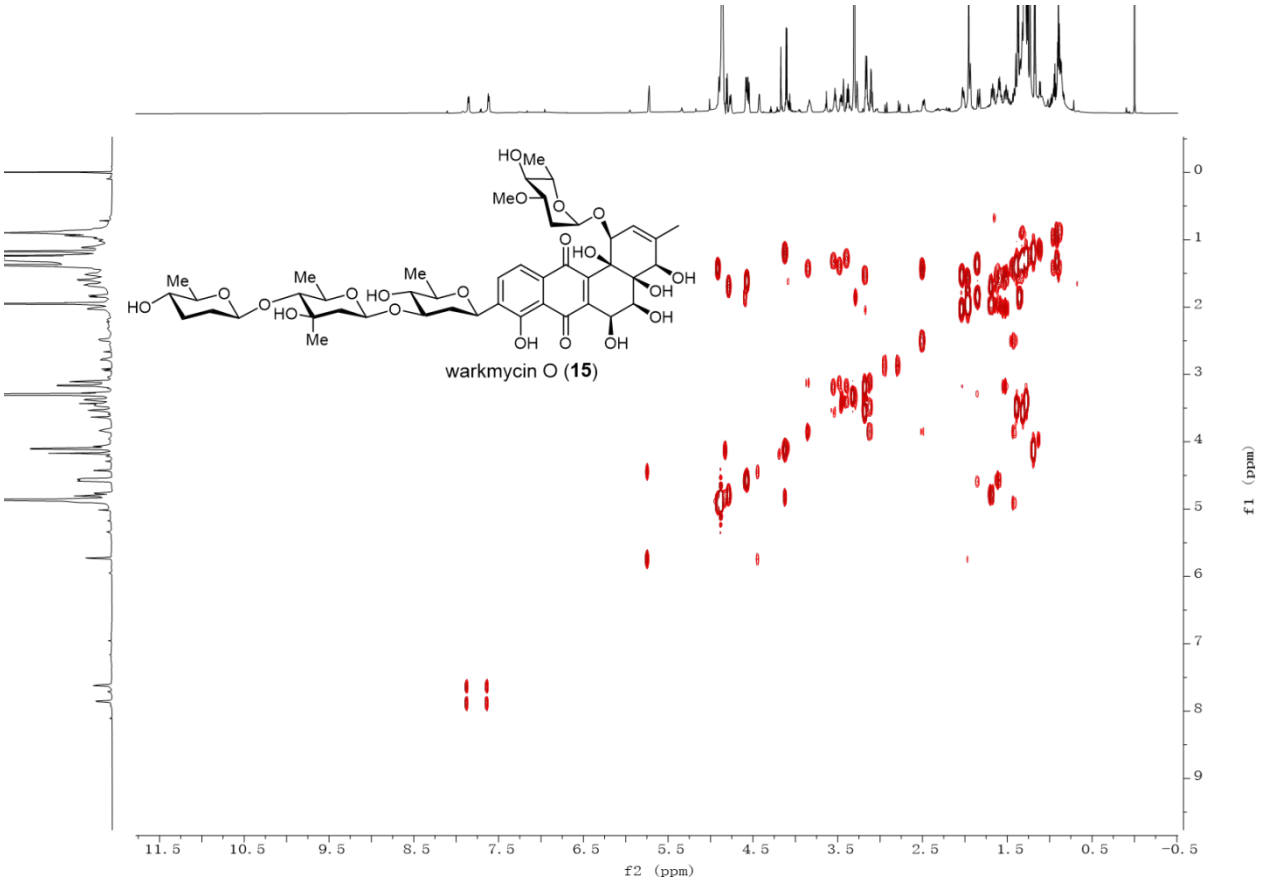


Figure S135. ^{1}H-^{1}H COSY spectrum of compound **15**

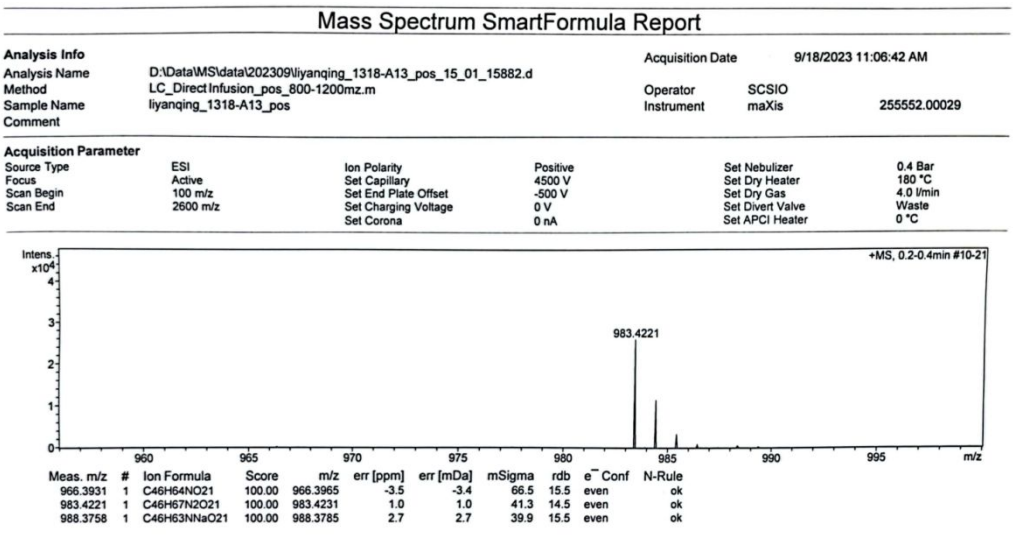


Figure S136. HRESIMS spectrum of compound **16**

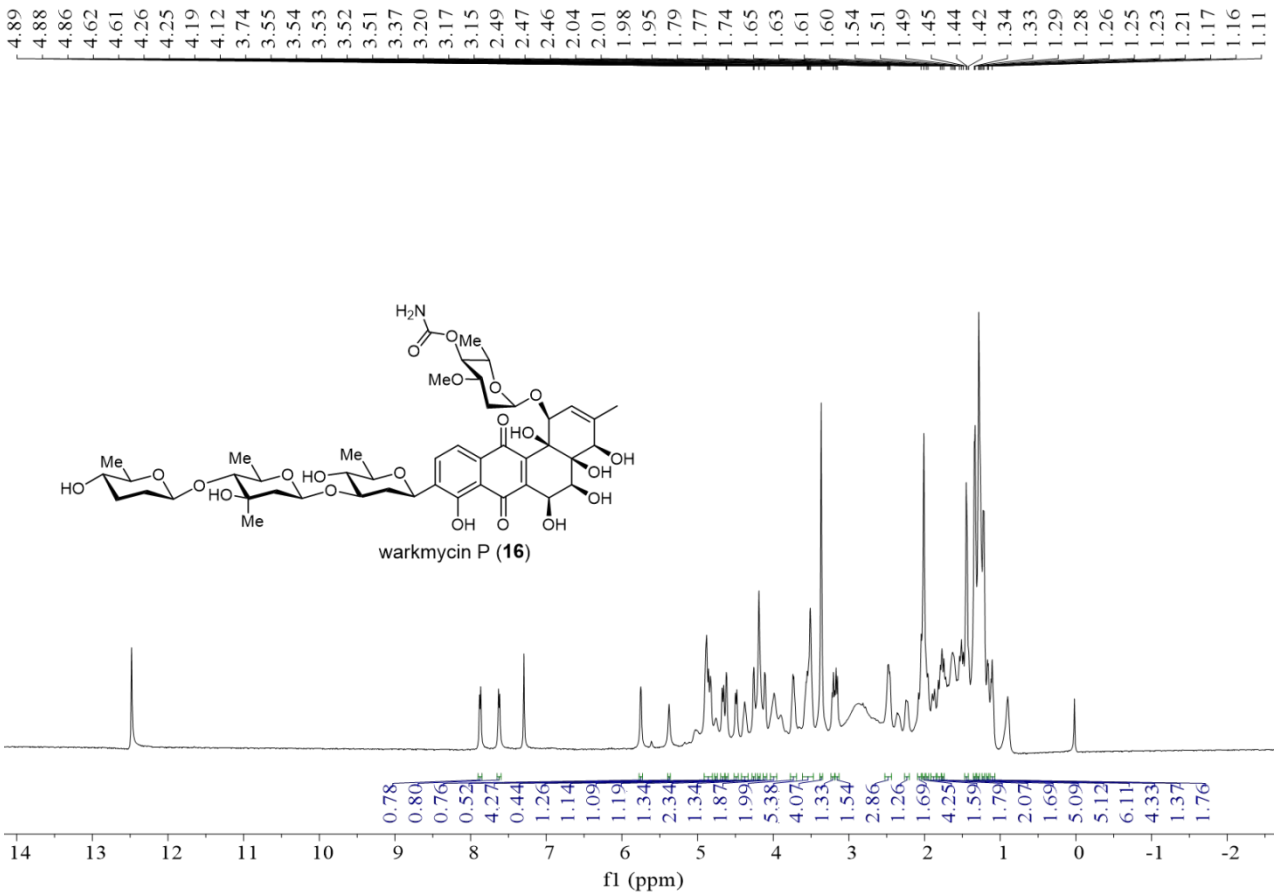


Figure S137. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **16**

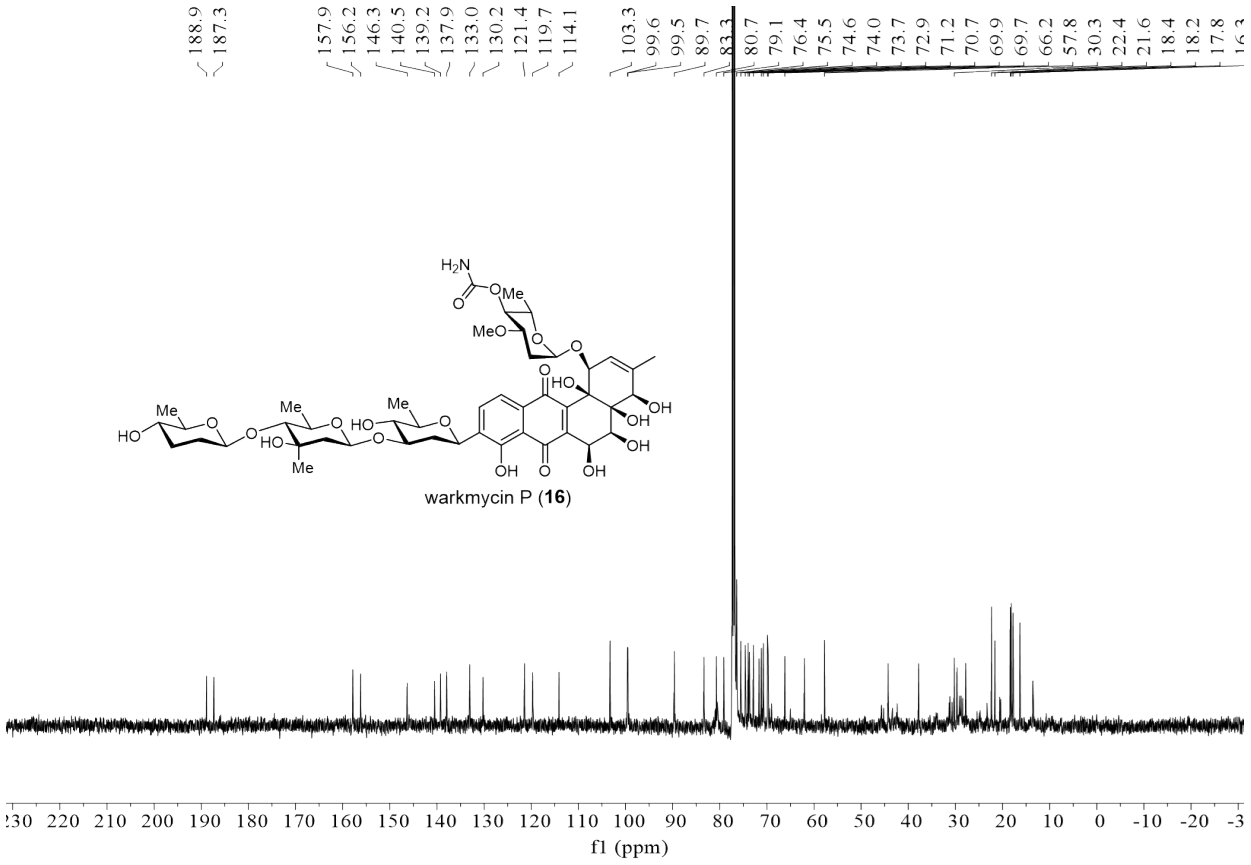


Figure S138. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **16**

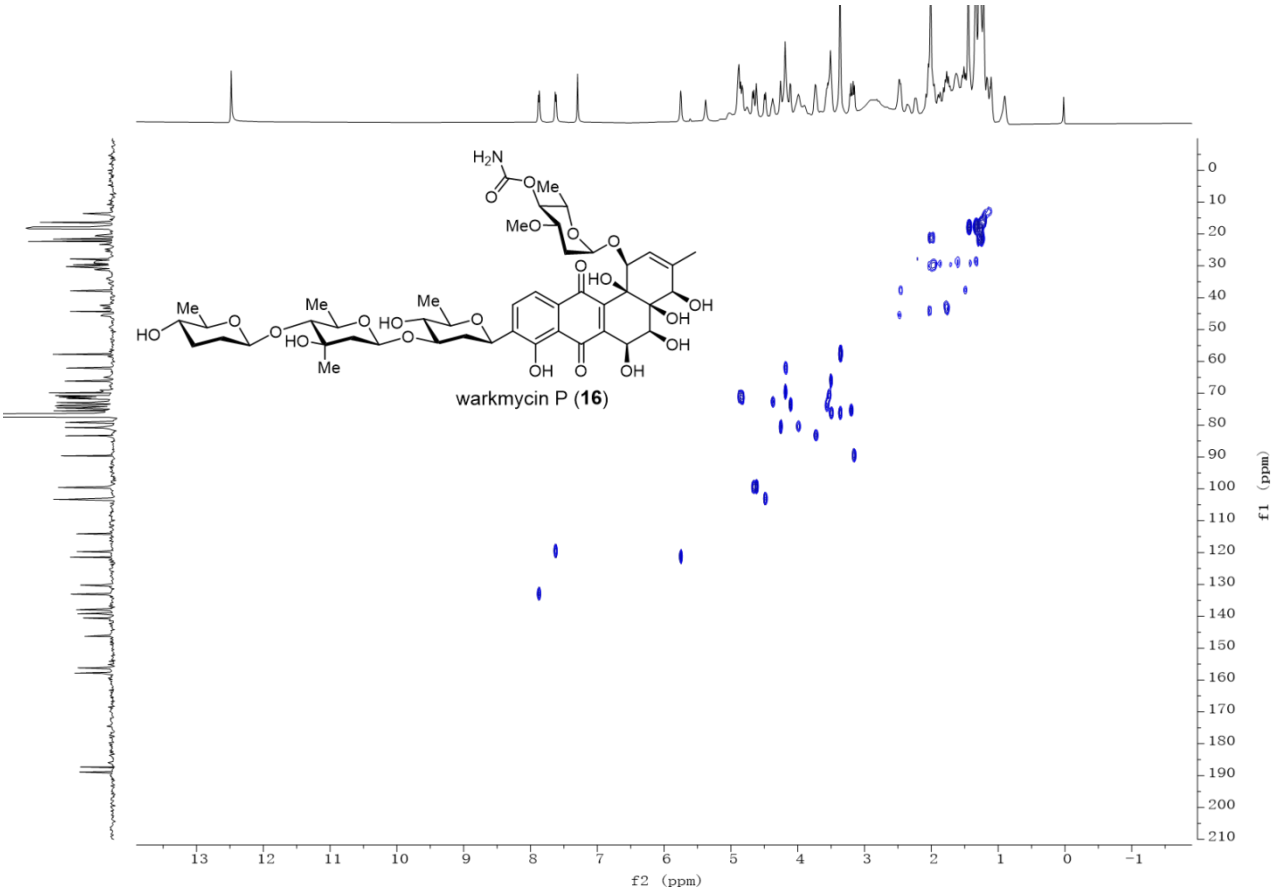


Figure S139. HSQC spectrum of compound **16**

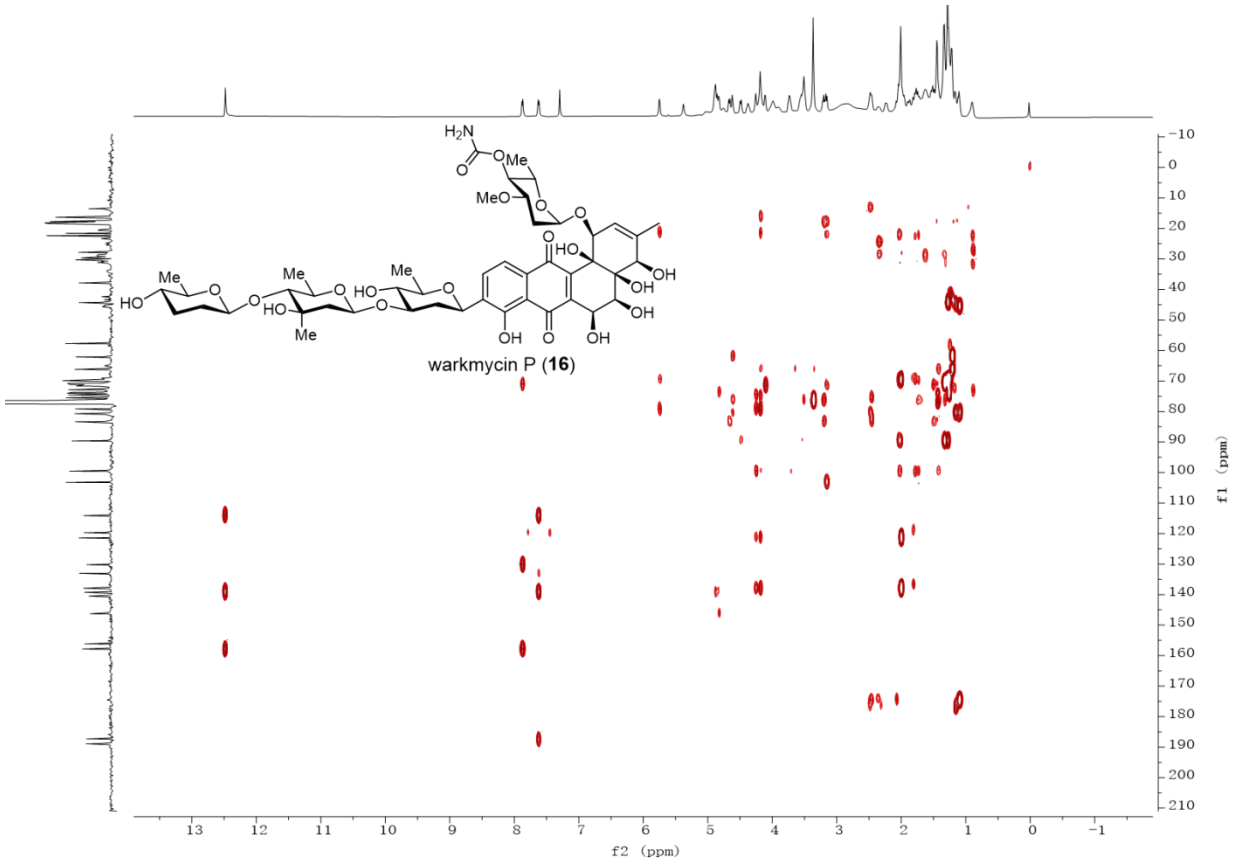


Figure S140. HMBC spectrum of compound **16**

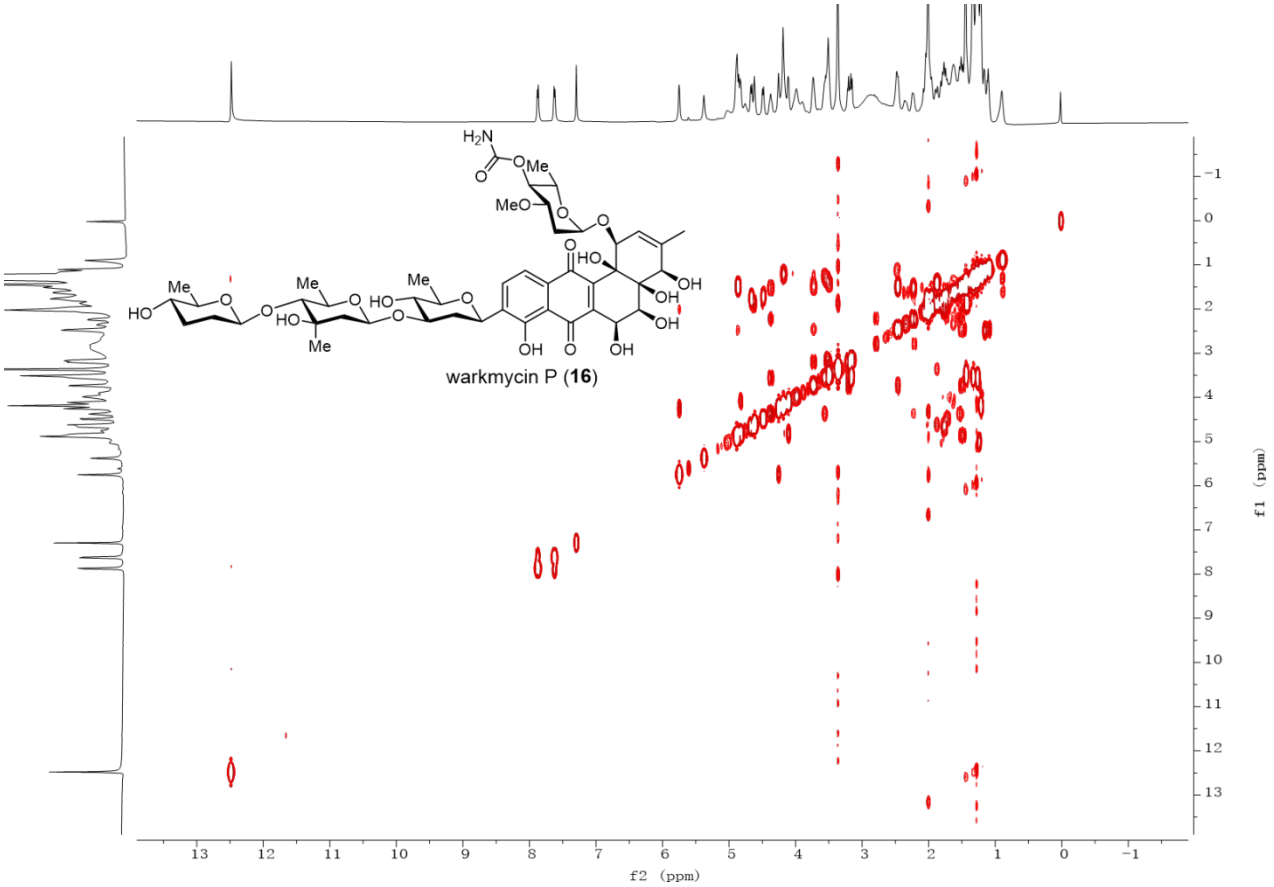


Figure S141. ^{1}H-^{1}H COSY spectrum of compound **16**

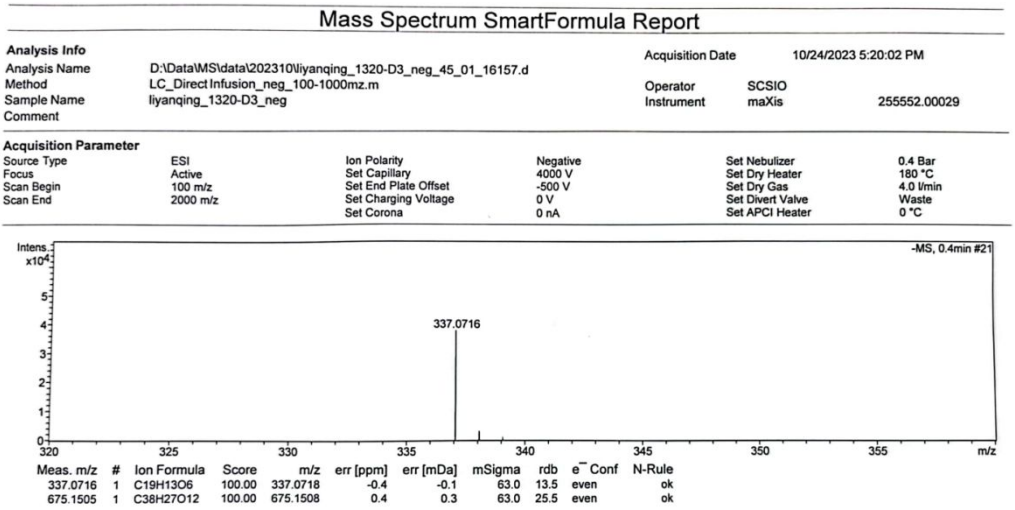


Figure S142. HRESIMS spectrum of compound **17**

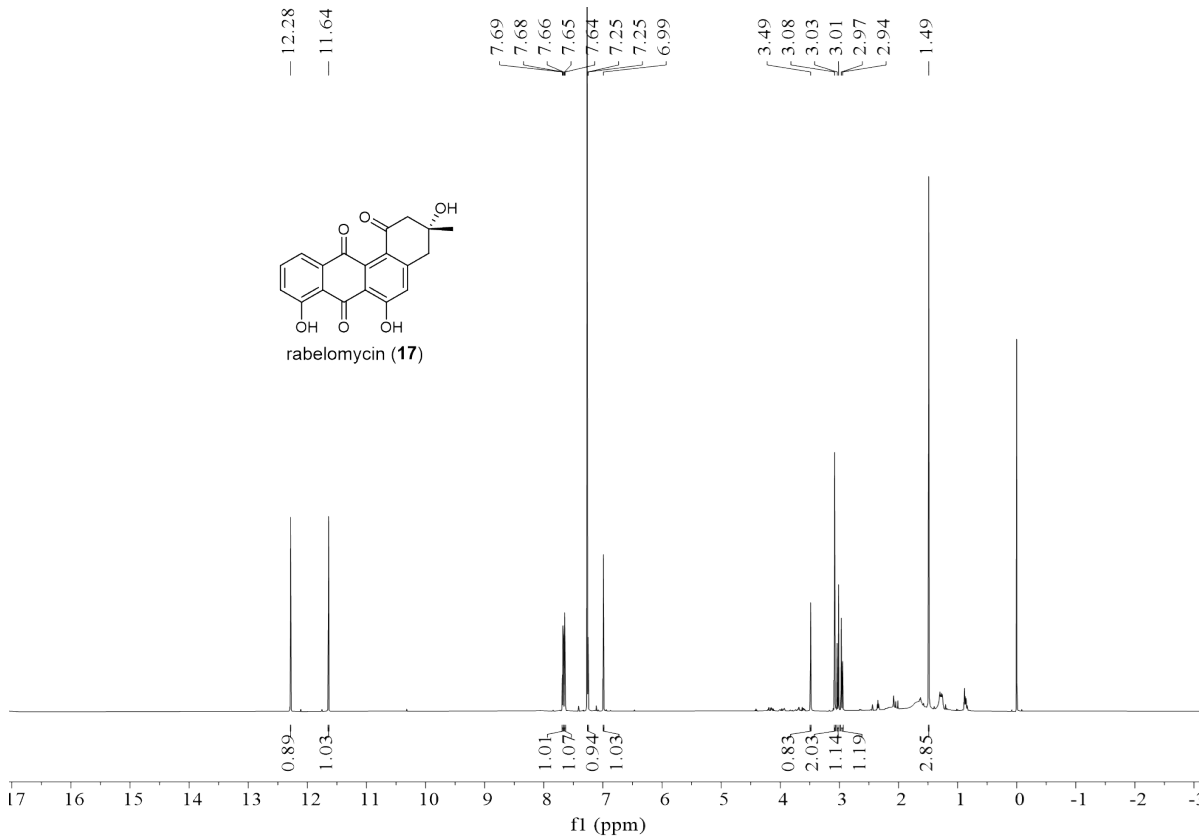


Figure S143. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **17**

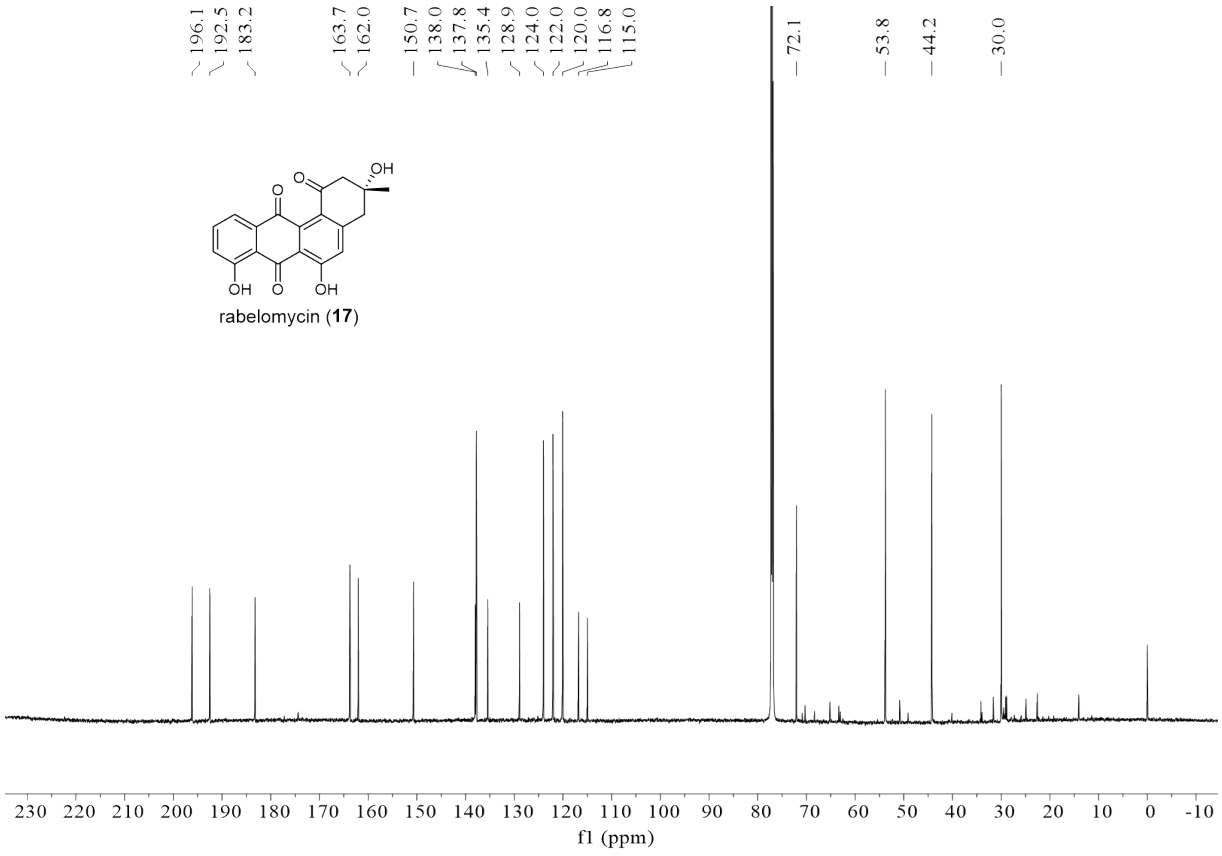


Figure S144. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **17**

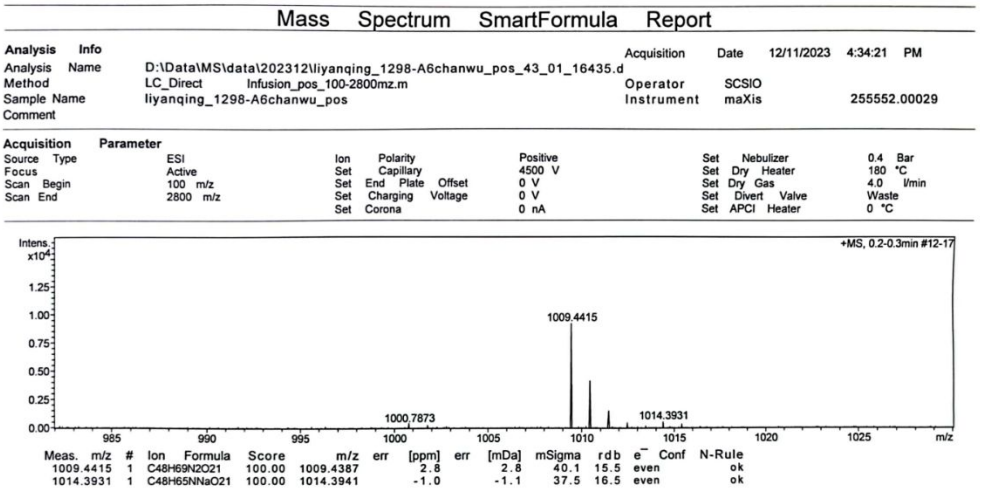


Figure S145. HRESIMS spectrum of compound **18**

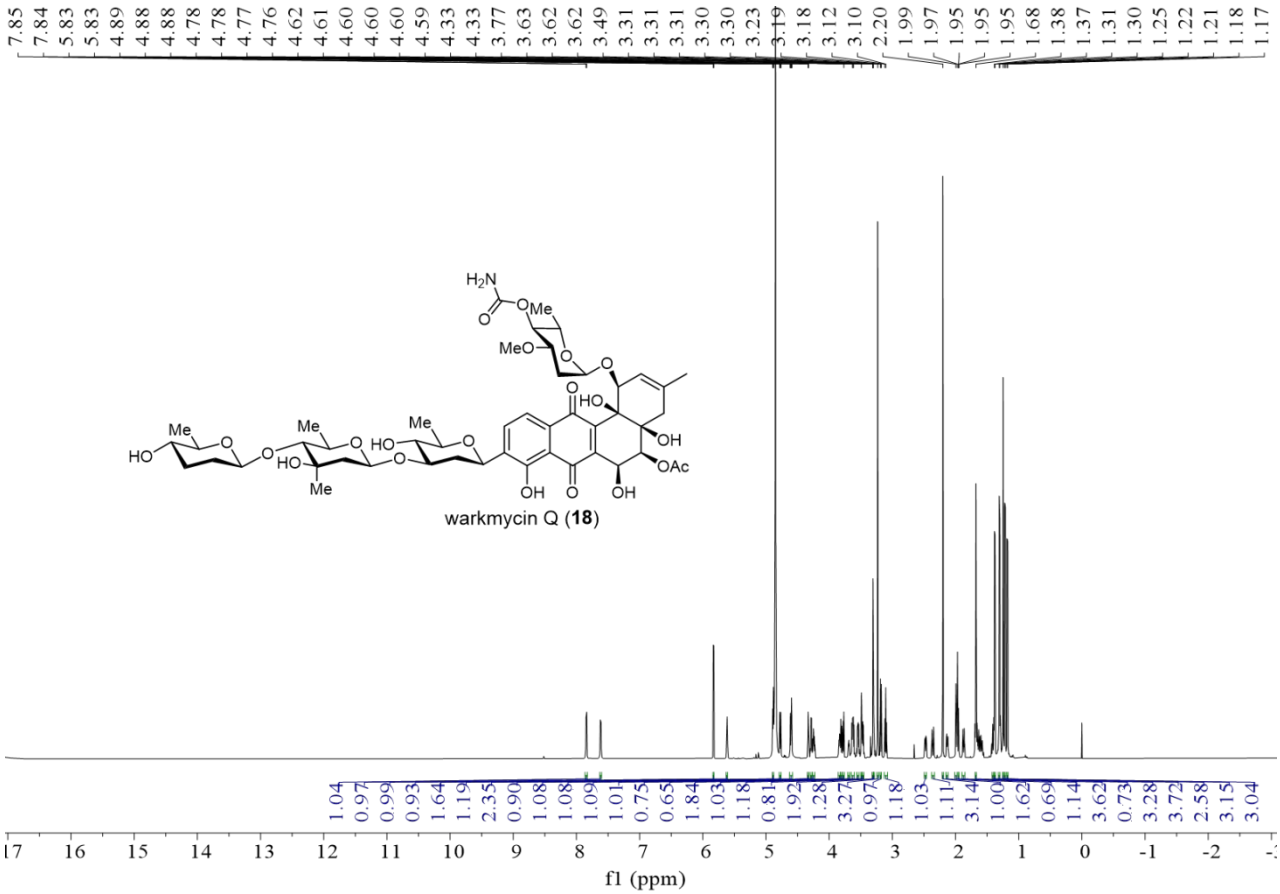


Figure S146. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **18**

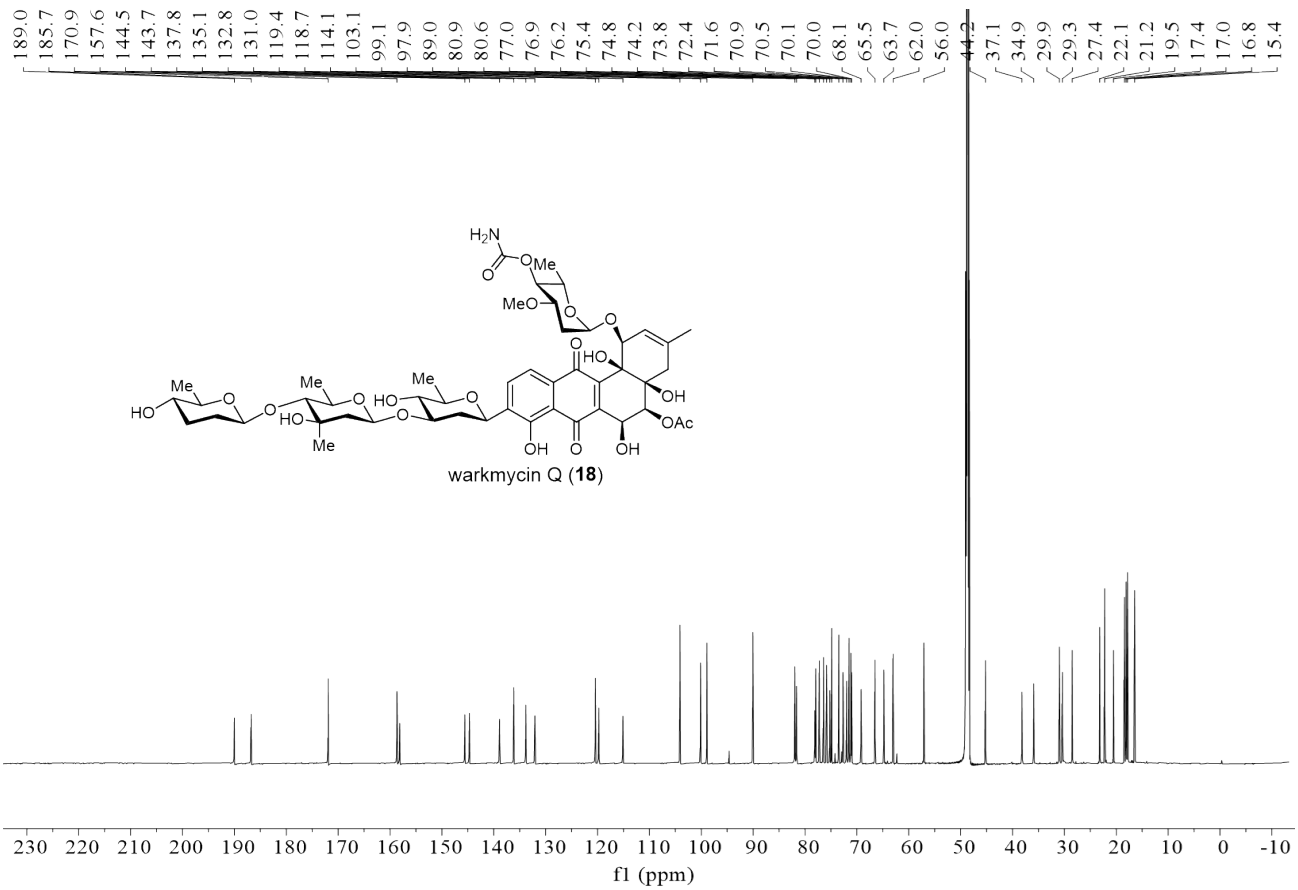


Figure S147. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **18**

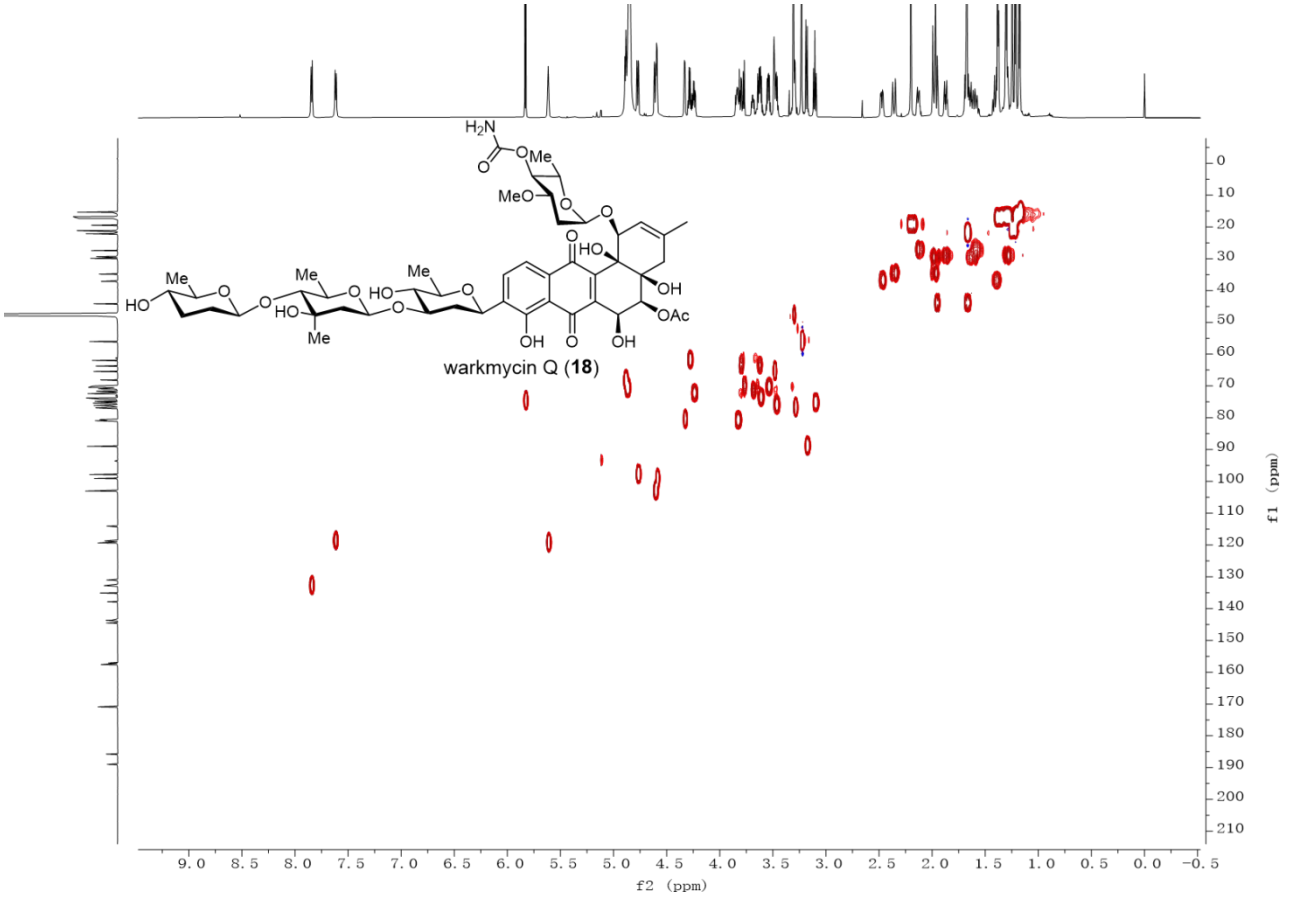


Figure S148. HSQC spectrum of compound **18**

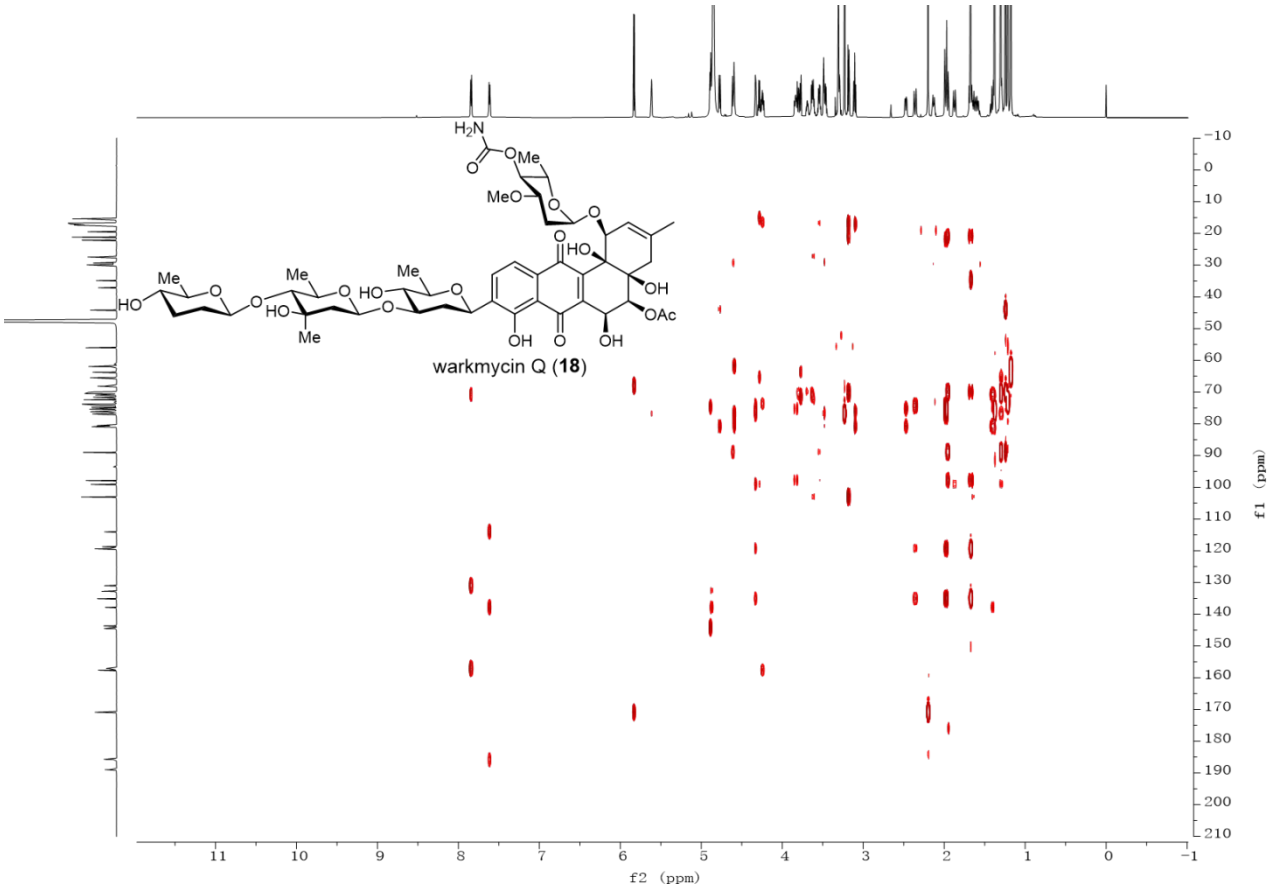


Figure S149. HMBC spectrum of compound **18**

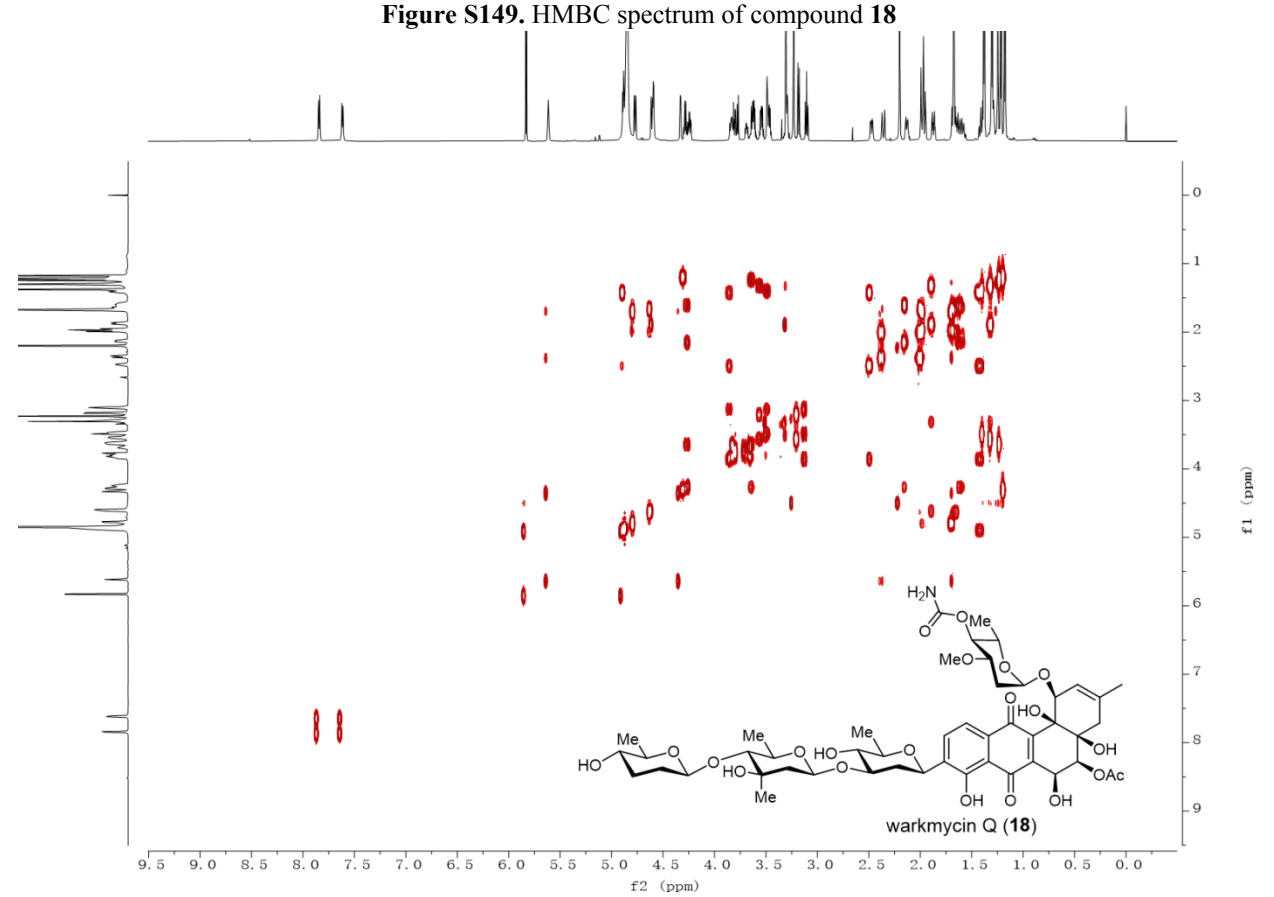
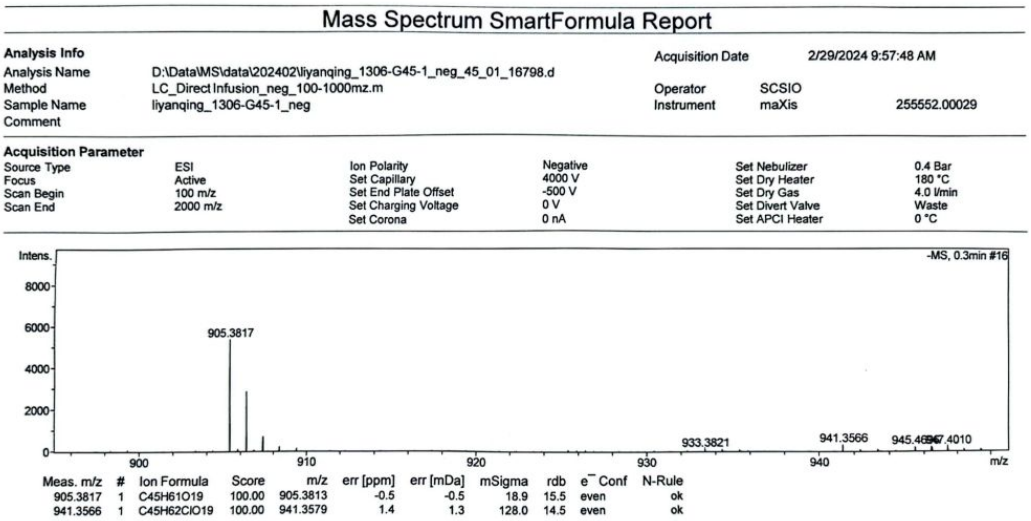


Figure S150. ^{1}H-^{1}H COSY spectrum of compound **18**



Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\MS\data\202402\liyanqing_1306-G45-1_neg_45_01_16798.d	Acquisition Date	2/29/2024 9:57:48 AM
Method	LC_Direct Infusion_neg_100-1000mz.m	Operator	SCSIO
Sample Name	liyanqing_1306-G45-1_neg	Instrument	maXis 255552.00029
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e⁻ Conf	N-Rule
905.3817	1	C45H61O19	100.00	905.3813	-0.5	-0.5	18.9	15.5	even	ok
941.3566	1	C45H62ClO19	100.00	941.3579	1.4	1.3	128.0	14.5	even	ok

Figure S151. HRESIMS spectrum of compound **19**

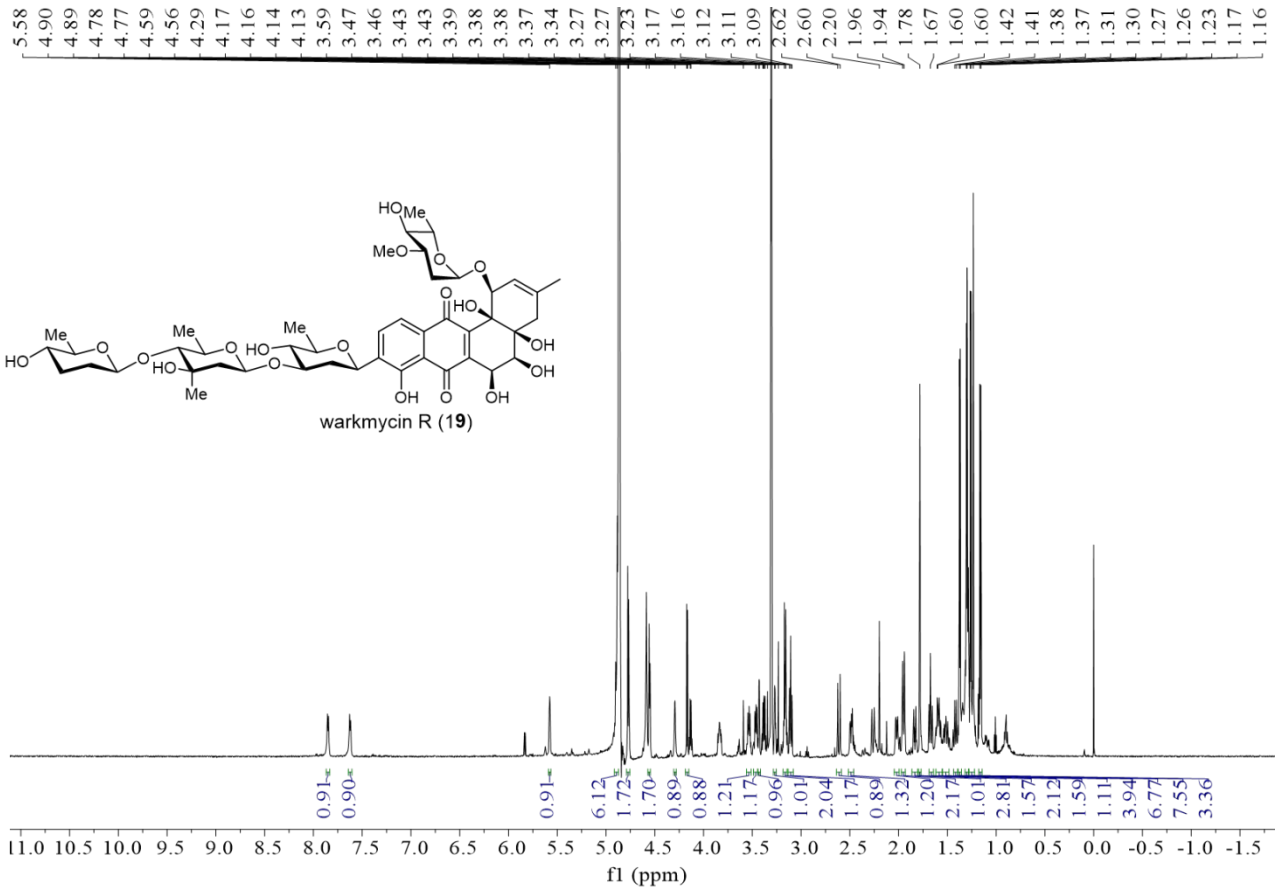


Figure S152. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **19**

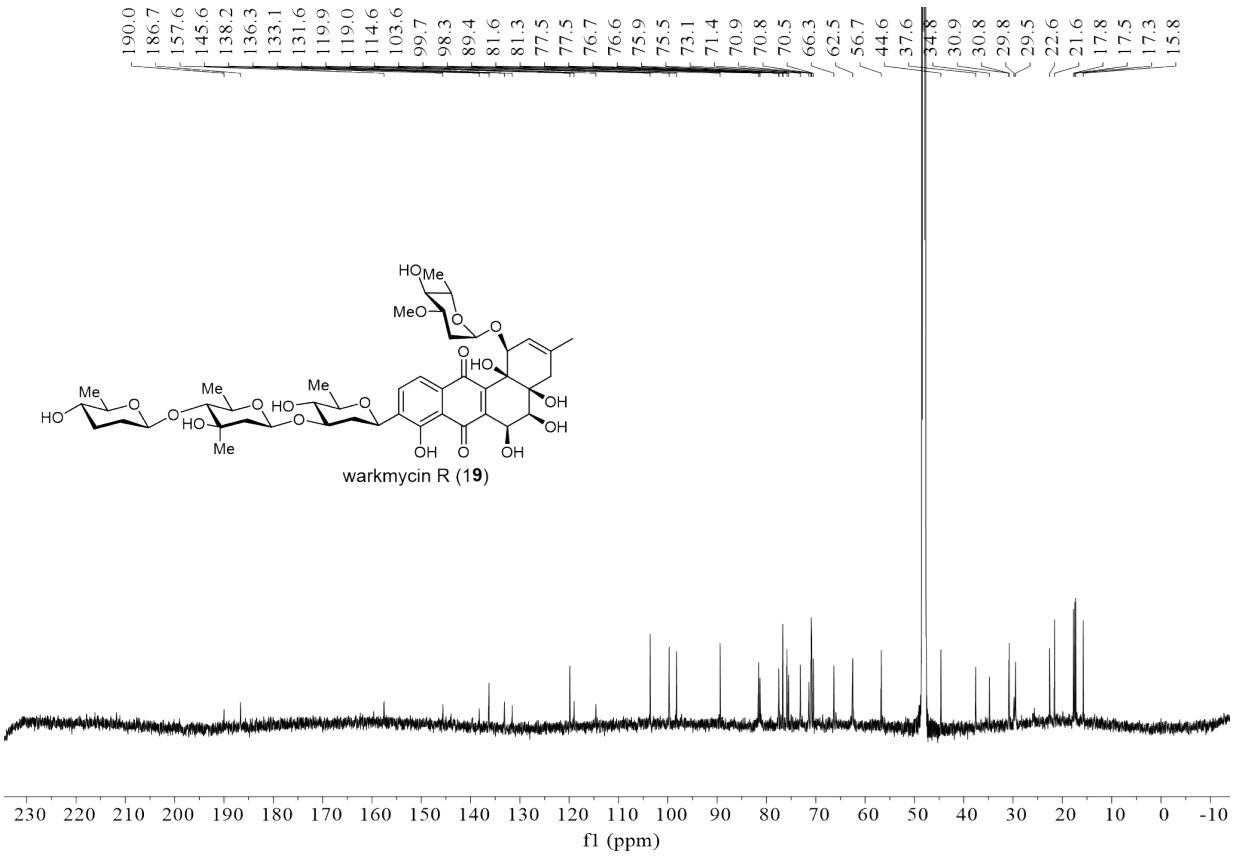


Figure S153. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **19**

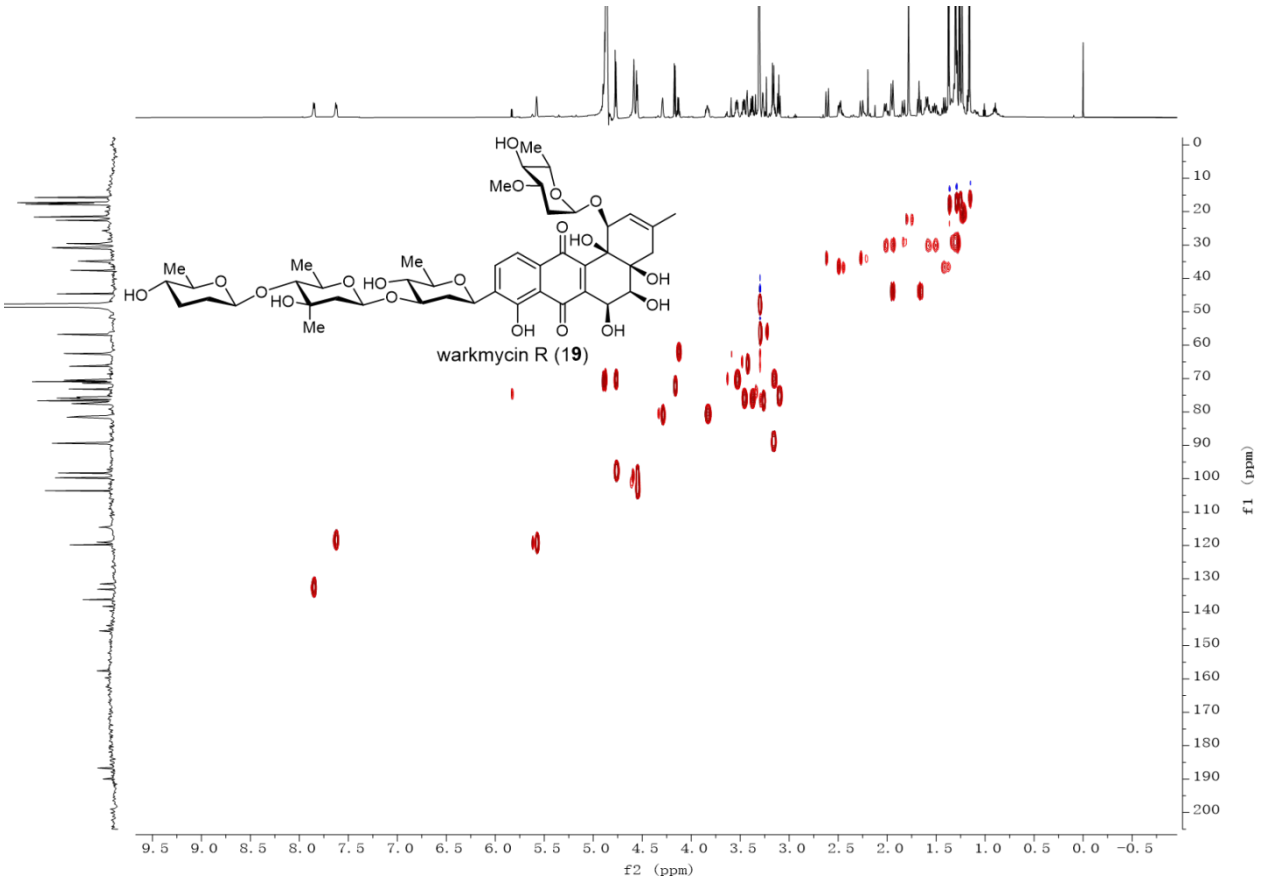


Figure S154. HSQC spectrum of compound **19**

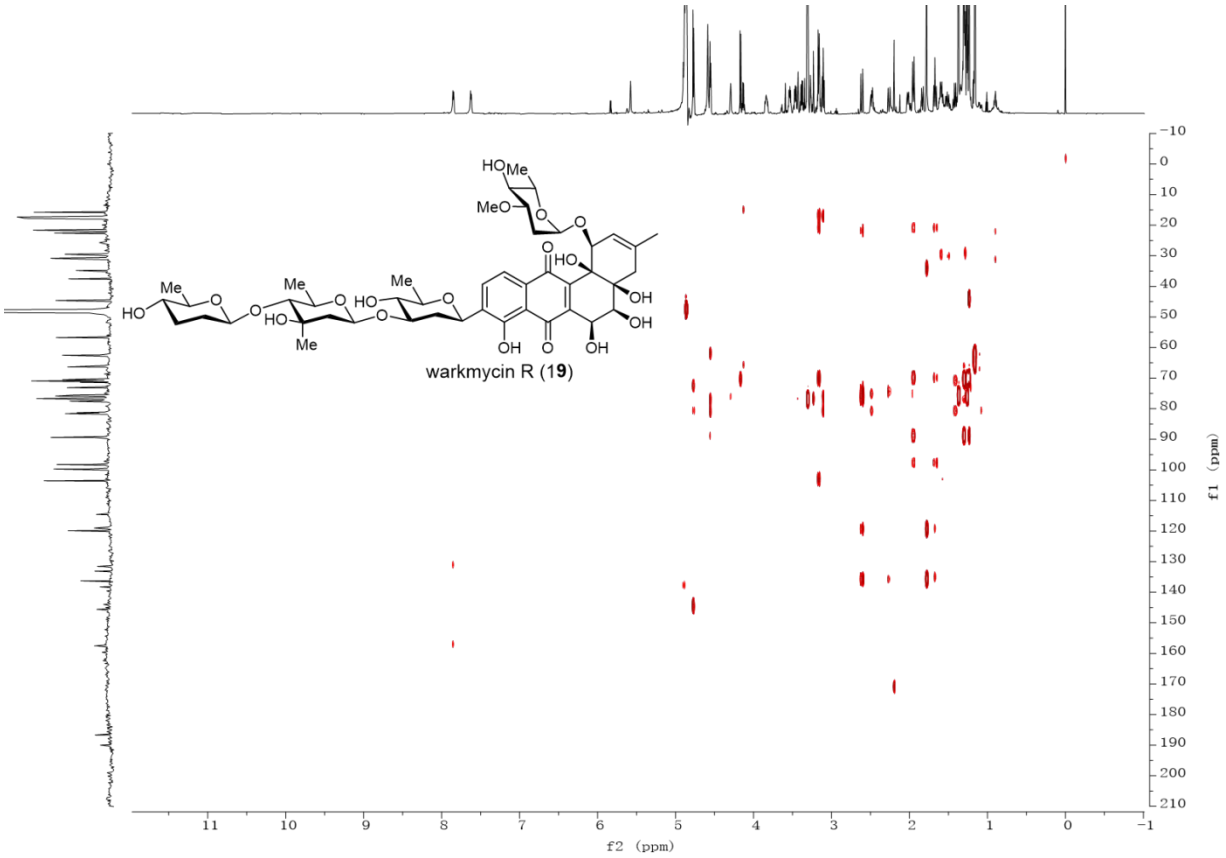


Figure S155. HMBC spectrum of compound **19**

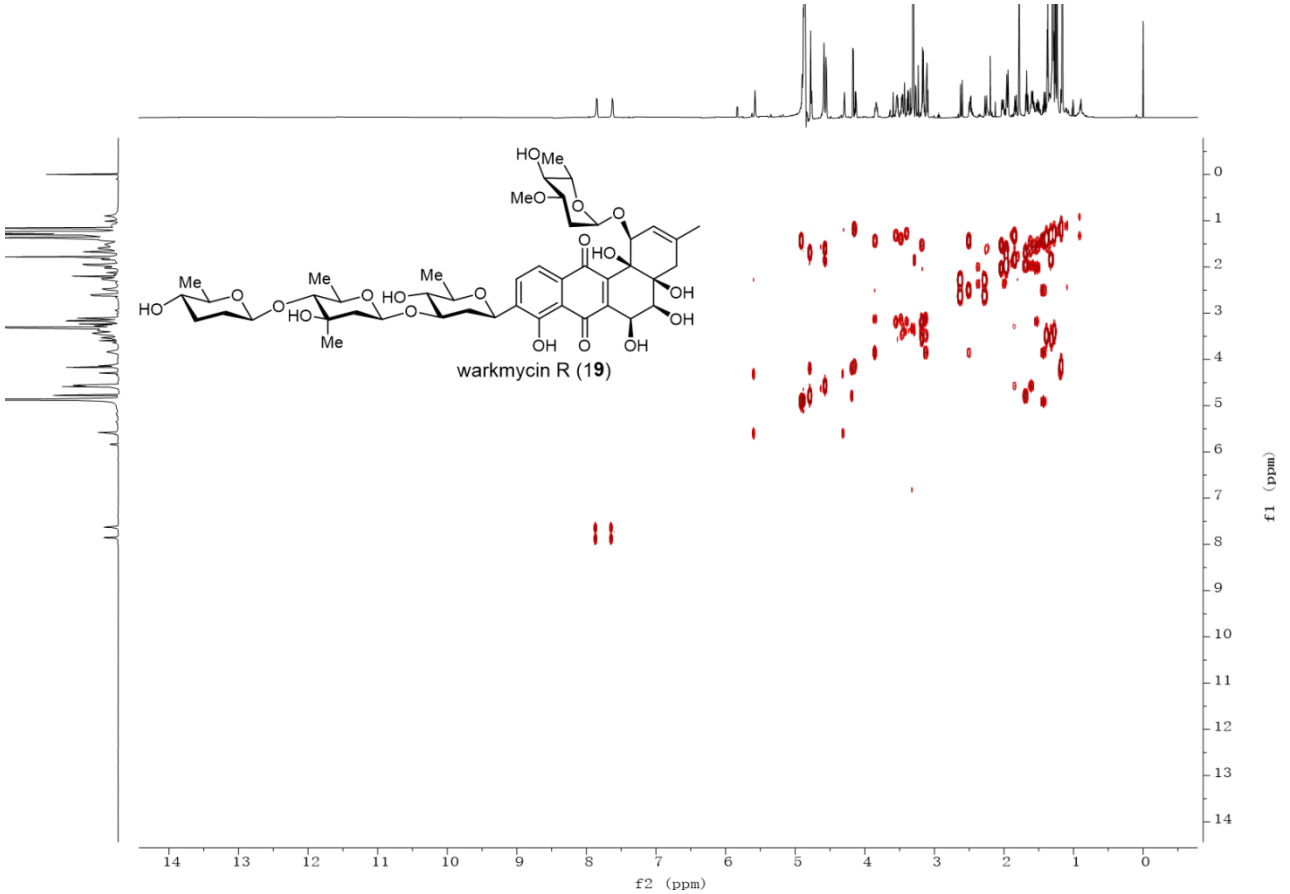


Figure S156. ^{1}H-^{1}H COSY spectrum of compound **19**

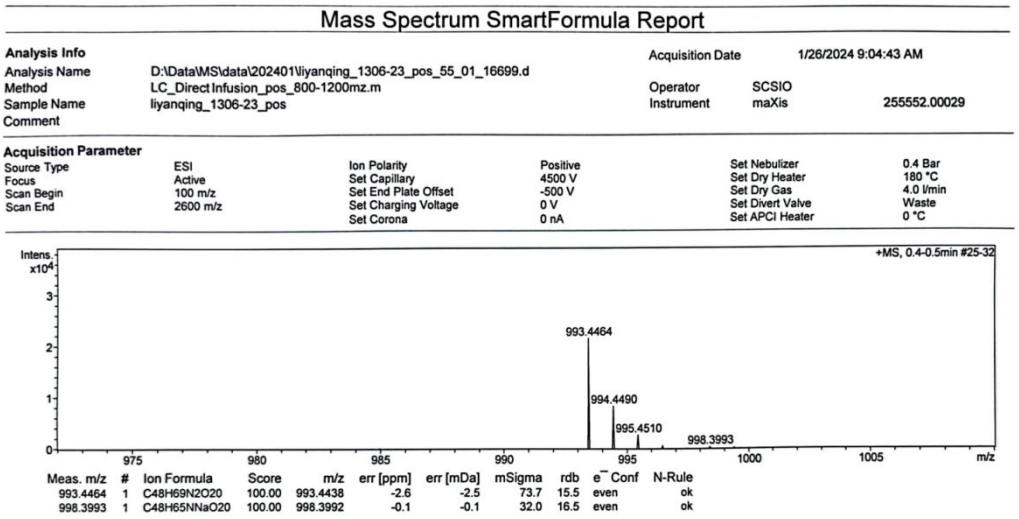


Figure S157. HRESIMS spectrum of compound **20**

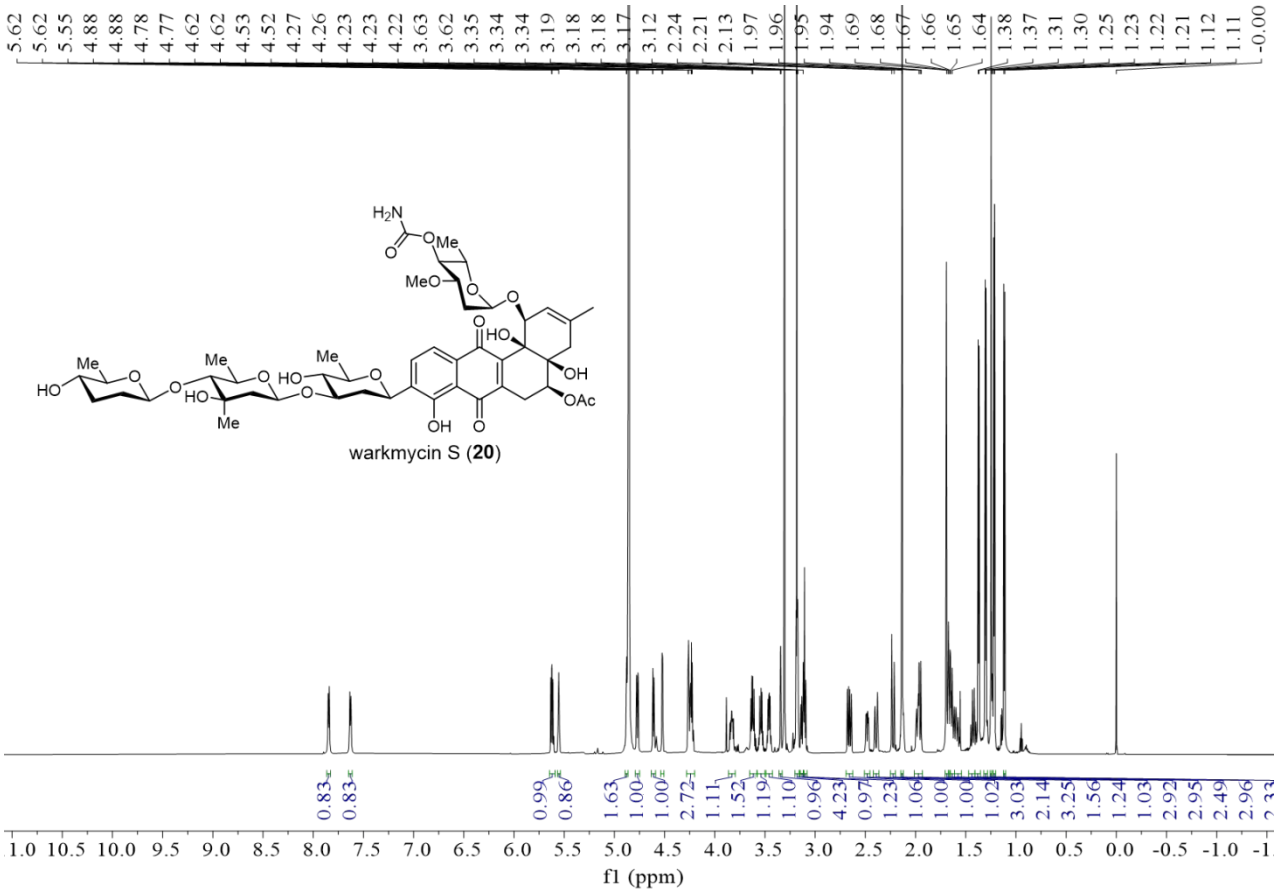


Figure S158. ^{1}H NMR (700 MHz, CD_3OD) spectrum of compound **20**

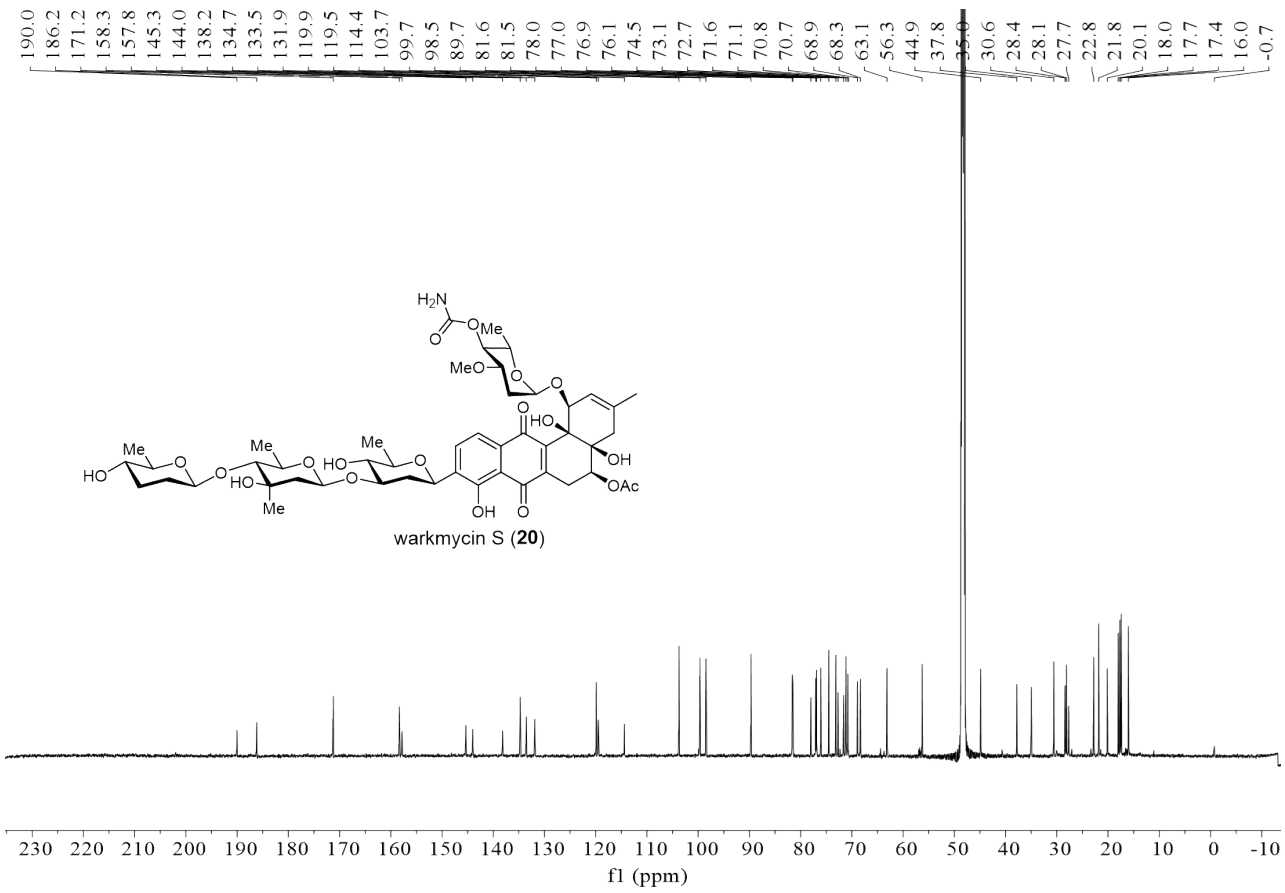


Figure S159. ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **20**

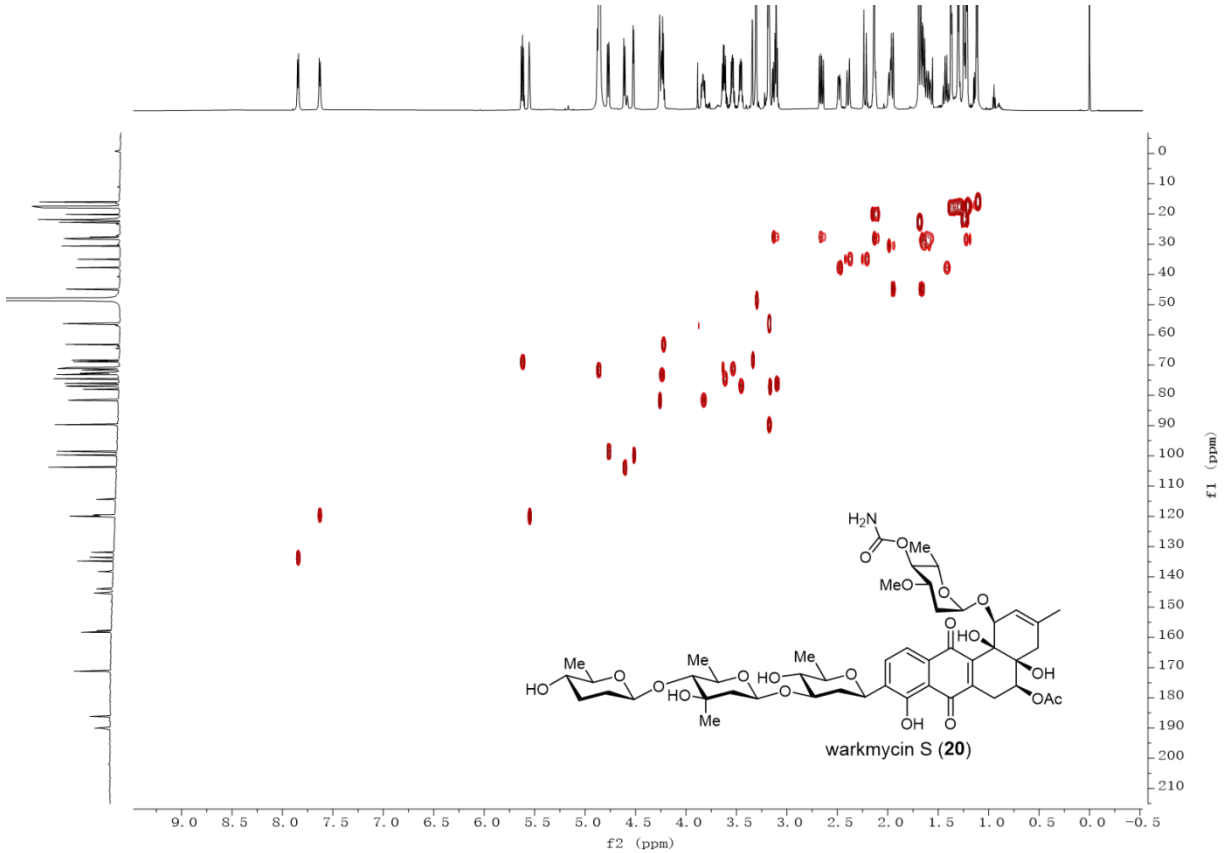


Figure S160. HSQC spectrum of compound **20**

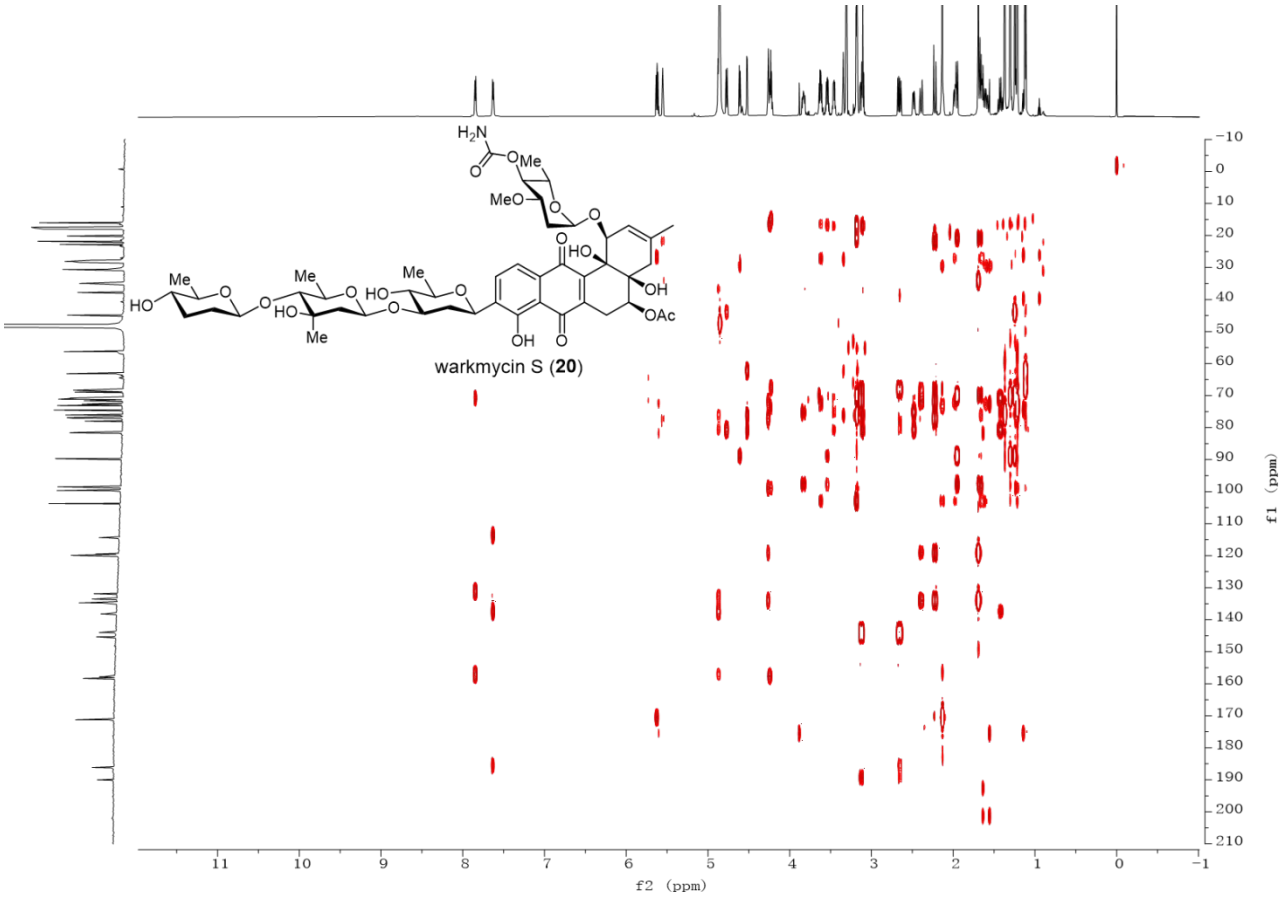


Figure S161. HMBC spectrum of compound **20**

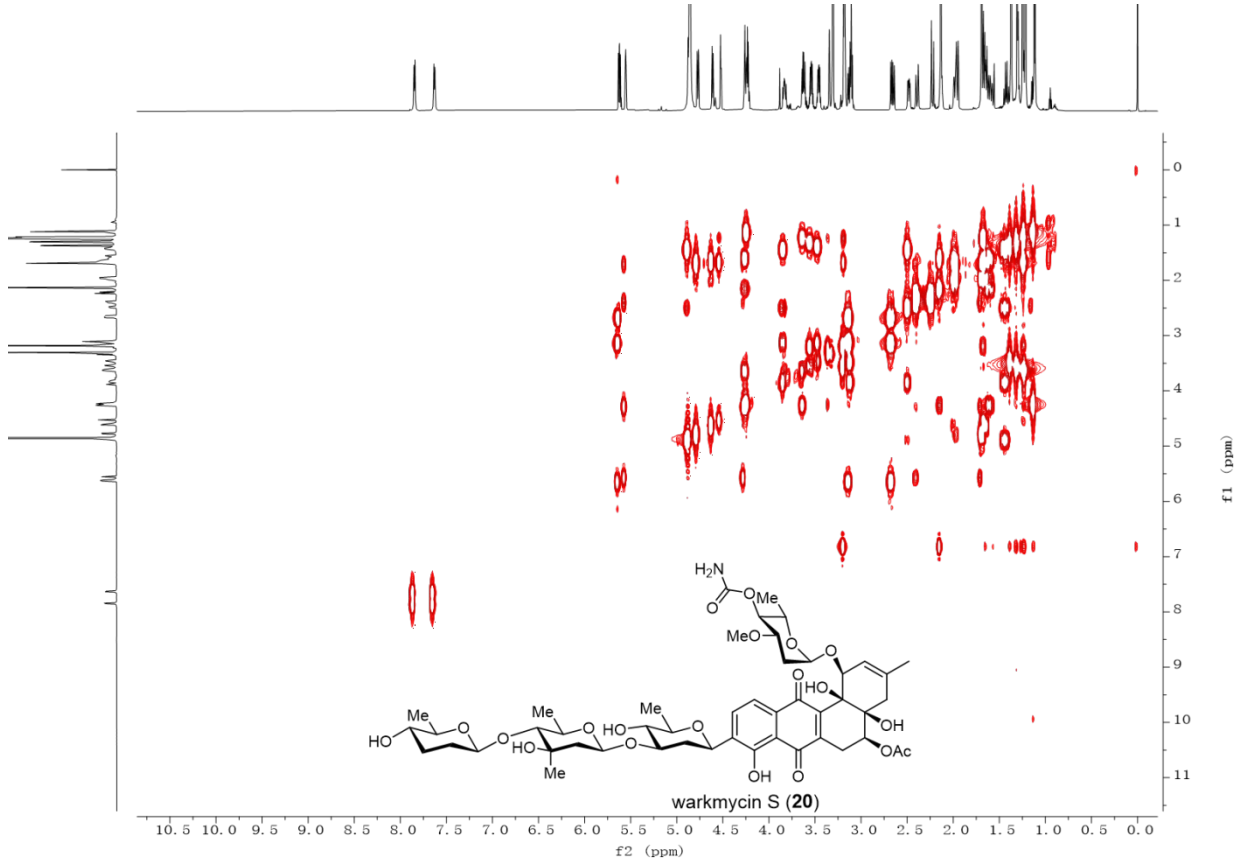


Figure S162. ^{1}H-^{1}H COSY spectrum of compound **20**

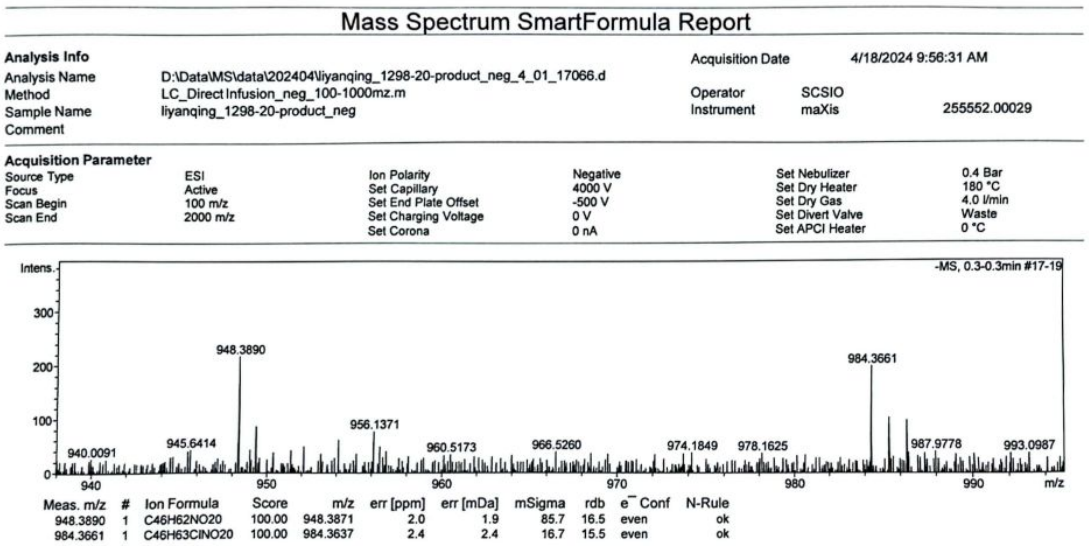


Figure S163. HRESIMS spectrum of compound **21**

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