

Supporting Information

Genome mining of albocandins A-E from *Streptomyces* sp. YINM00030

Zhou-Tian-Le Zhang^{a, 1}, Zhen Ren^{b, 1}, Xiaoyu Su^a, Tian-Peng Xie^a, Mengzhuo Yi^a, Hao Zhou^{a, *}, Min Yin^{a, *} and Zhong-Tao Ding^{a, c, *}

^a School of Chemical Science and Technology, School of Medicine, Yunnan University, University Town East Outer Ring South Road, Kunming, Yunnan 650500, China

^b School of Agriculture and Life Sciences, Kunming University, 2 Pu Xin Road, Kunming, Yunnan 650214, China

^c College of Traditional Chinese Medicine, Yunnan University of Chinese Medicine, 1076 Yu Hua Road Kunming, Yunnan 650500, China

*Corresponding authors. E-mail address: yinmin@ynu.edu.cn (M. Yin), haozhou@ynu.edu.cn (H. Zhou) and ztding@ynu.edu.cn (Z. Ding); mailing address: Yunnan University, University Town East Outer Ring South Road, Kunming, Yunnan 650500, China

¹ These authors contributed equally to this study

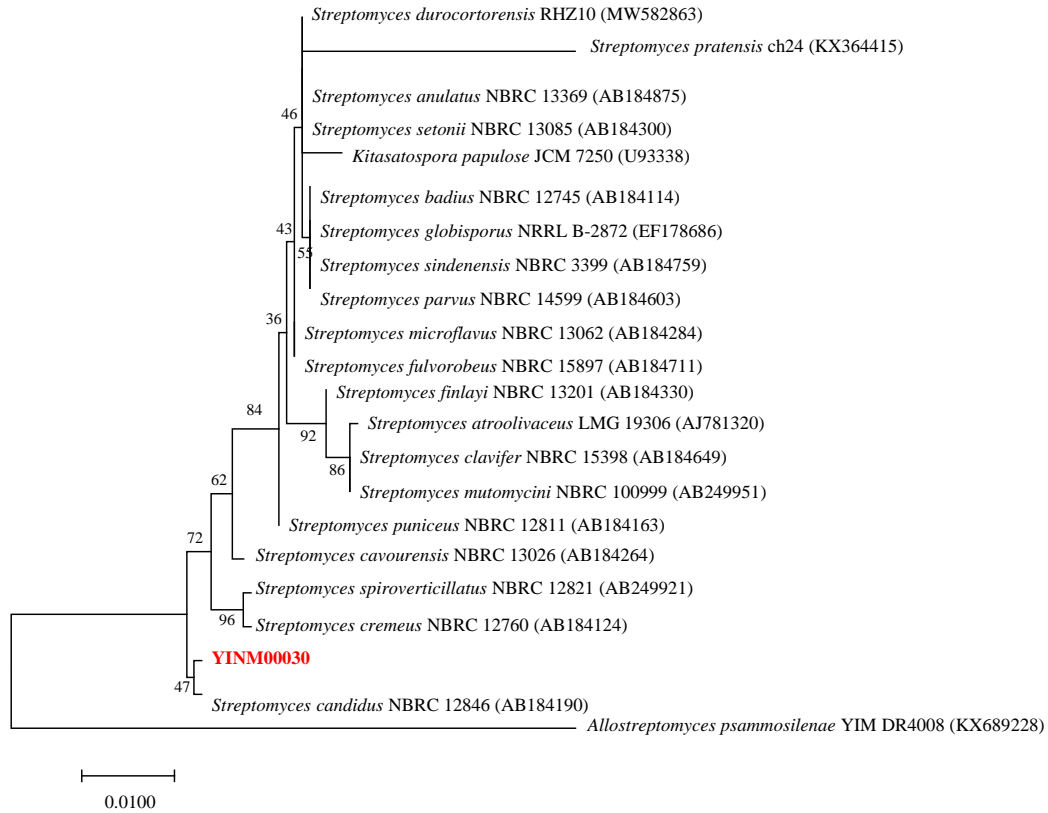


Fig. S1 Maximum-likelihood tree showing the relationships between strain YINM00030 and related members of the genus *Streptomyces*. Bootstrap values (>50%) based on 1000 replicates are shown at the branch nodes. *Allostreptomyces psammosileneae* YIM DR4008 was used as the outgroup. Bar, 1% sequence divergence.

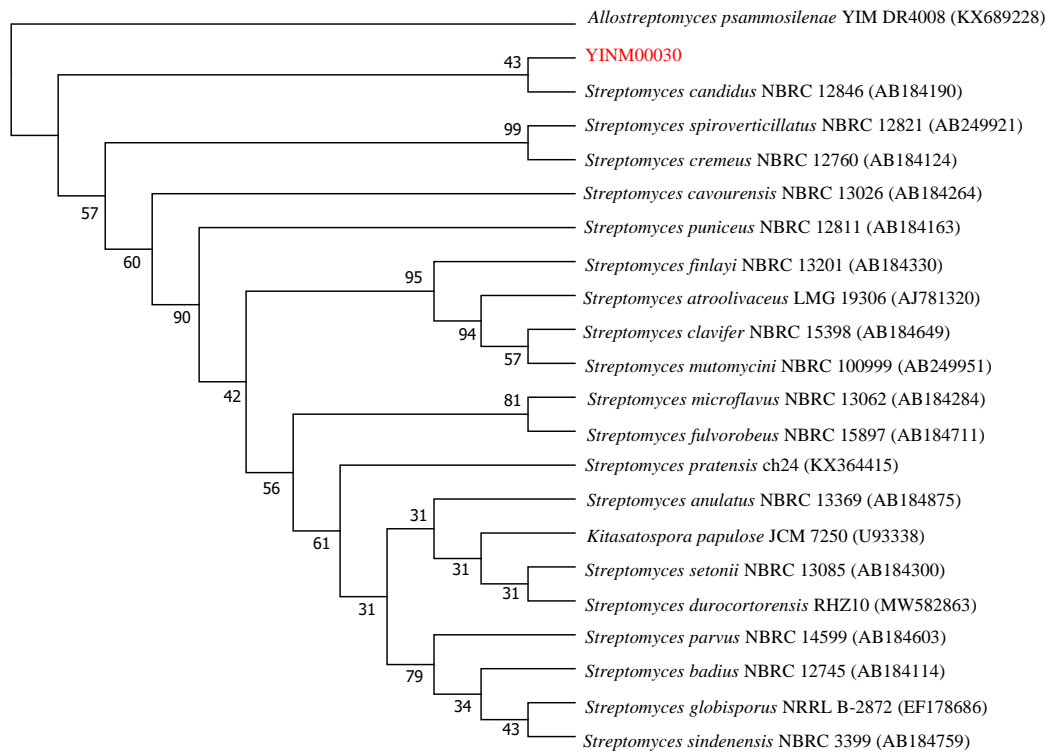


Fig. S2 Maximum-parsimony tree showing the relationships between strain YINM00030 and related members of the genus *Streptomyces*. Bootstrap values (>50%) based on 1000 replicates are shown at the branch nodes. *Allostreptomyces psammosilena* YIM DR4008 was used as the outgroup.

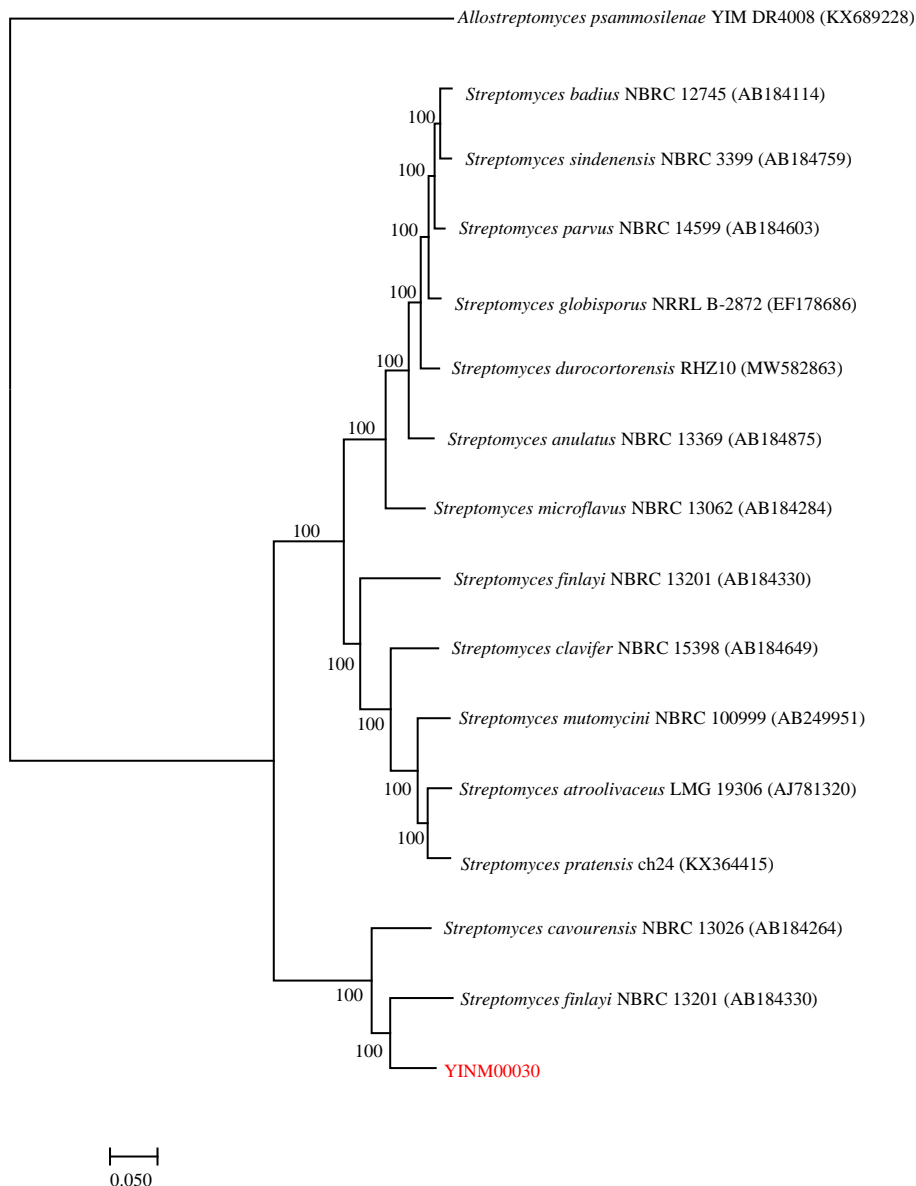


Fig. S3 The RAxML neighbor-joining phylogenomic tree of strain YINM00030 and its closest relatives from the genus *Streptomyces* based on marker genes. Bootstrap values (>70%) based on 100 resamplings are given at the nodes. *Allostreptomyces psammosileneae* YIM DR4008 was used as outgroup. Bar, 0.05 substitutions per nucleotide position.

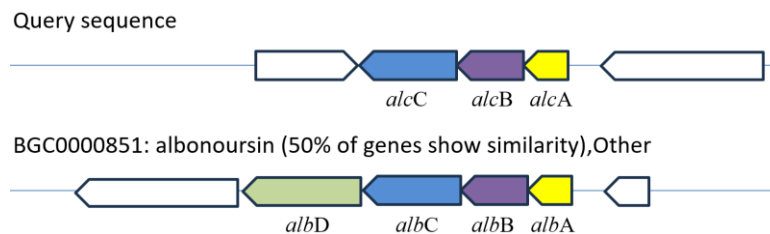


Fig. S4 The similarity of albocandin biosynthetic gene cluster in the YINM00030 with the albonourin biosynthetic gene cluster.

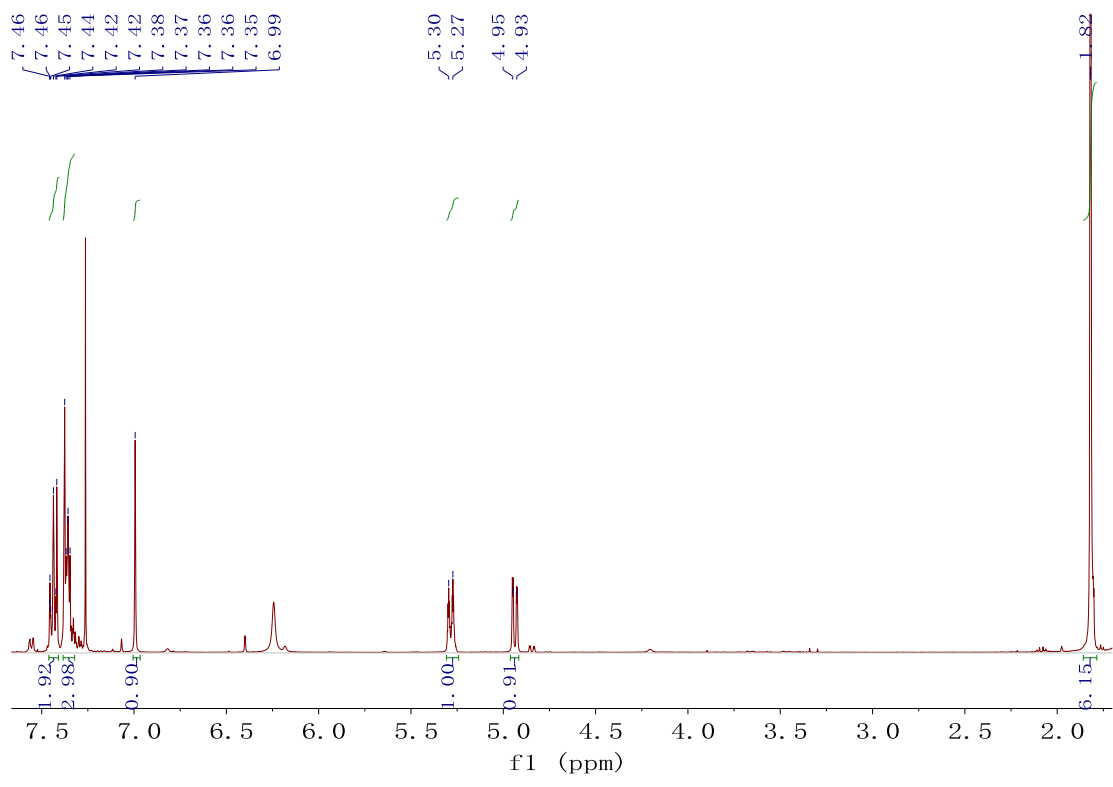


Fig. S5 ^1H NMR spectrum of albocandin A (**1**, in CDCl_3 , 400 MHz)

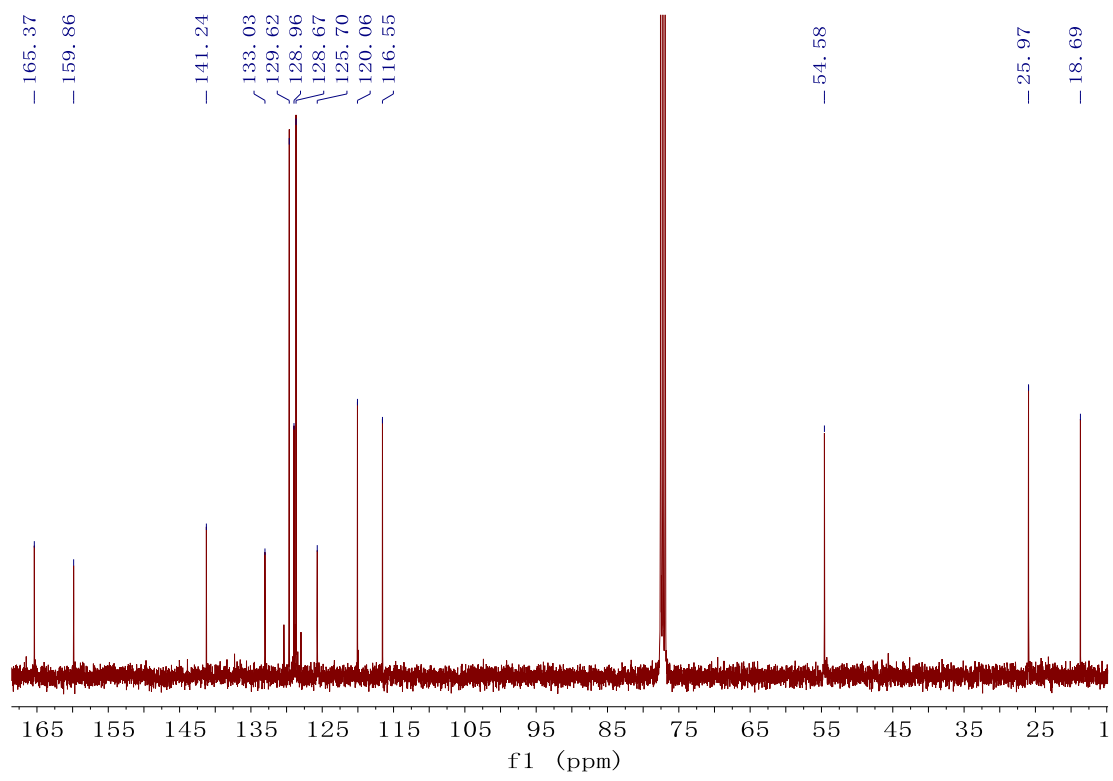


Fig. S6 ^{13}C NMR spectrum of albocandin A (**1**, in CDCl_3 , 100 MHz).

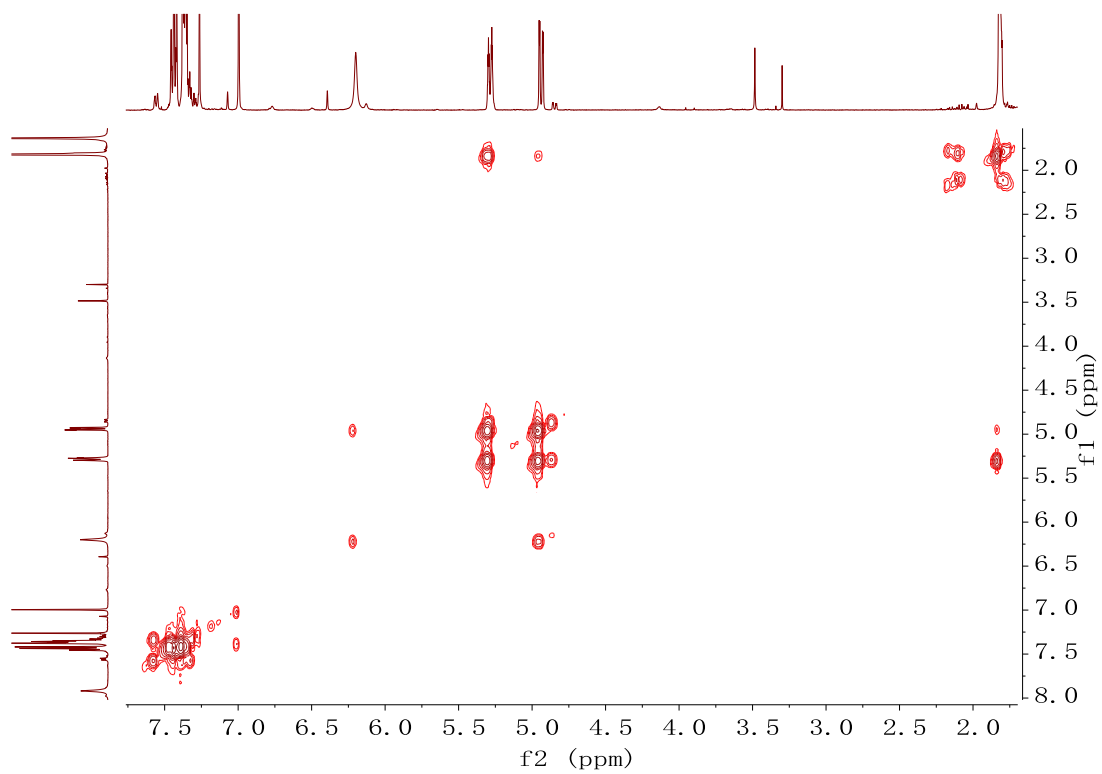


Fig. S7 COSY spectrum of albocandin A (**1**, in CDCl₃, 400 MHz).

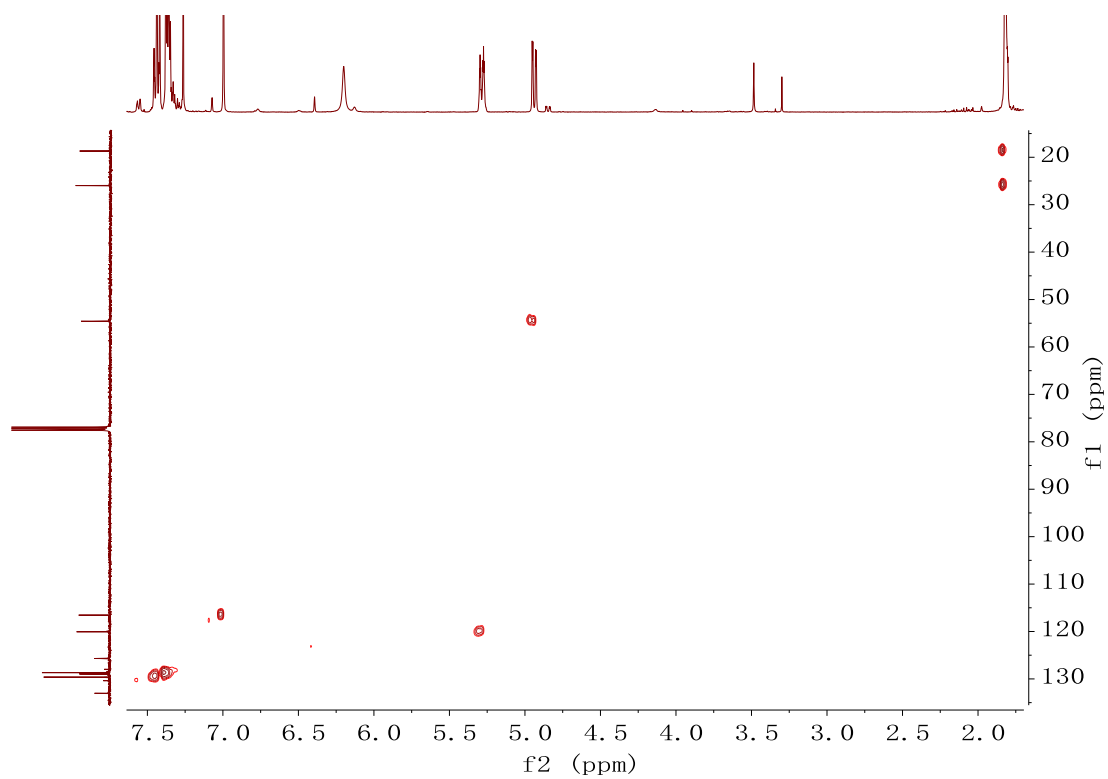


Fig. S8 HSQC spectrum of albocandin A (**1**, in CDCl₃, 400 MHz).

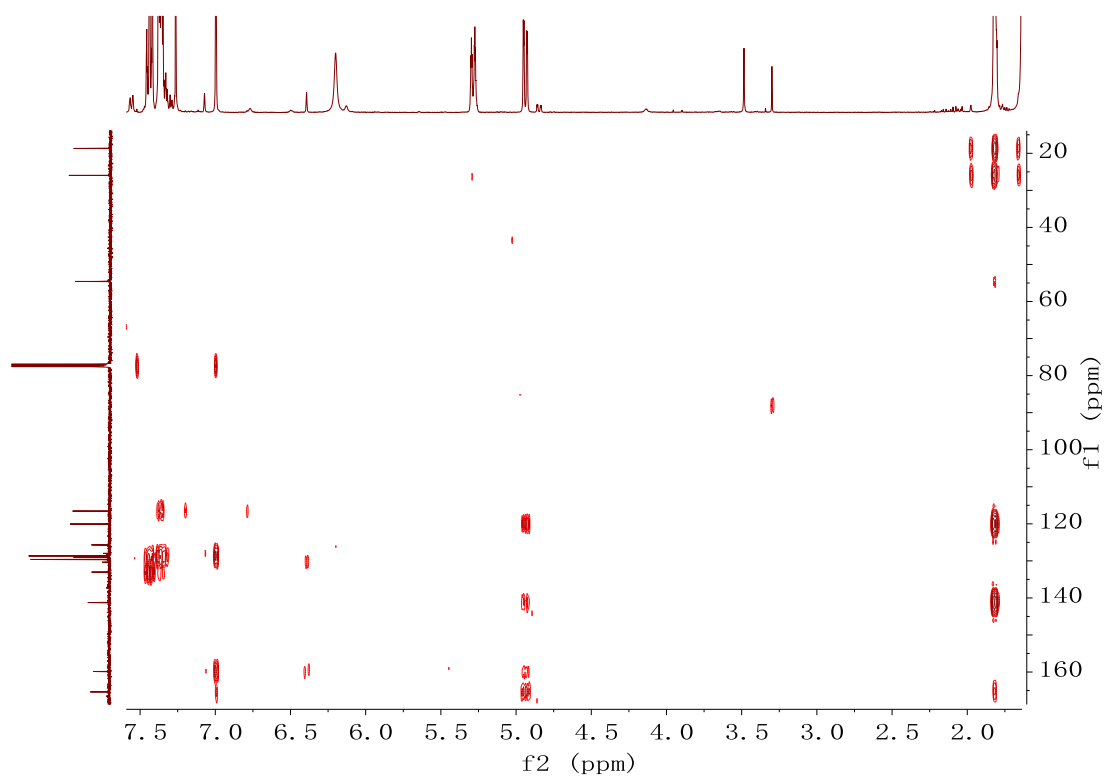


Fig. S9 HMBC spectrum of albocandin A (**1**, in CDCl_3 , 400 MHz).

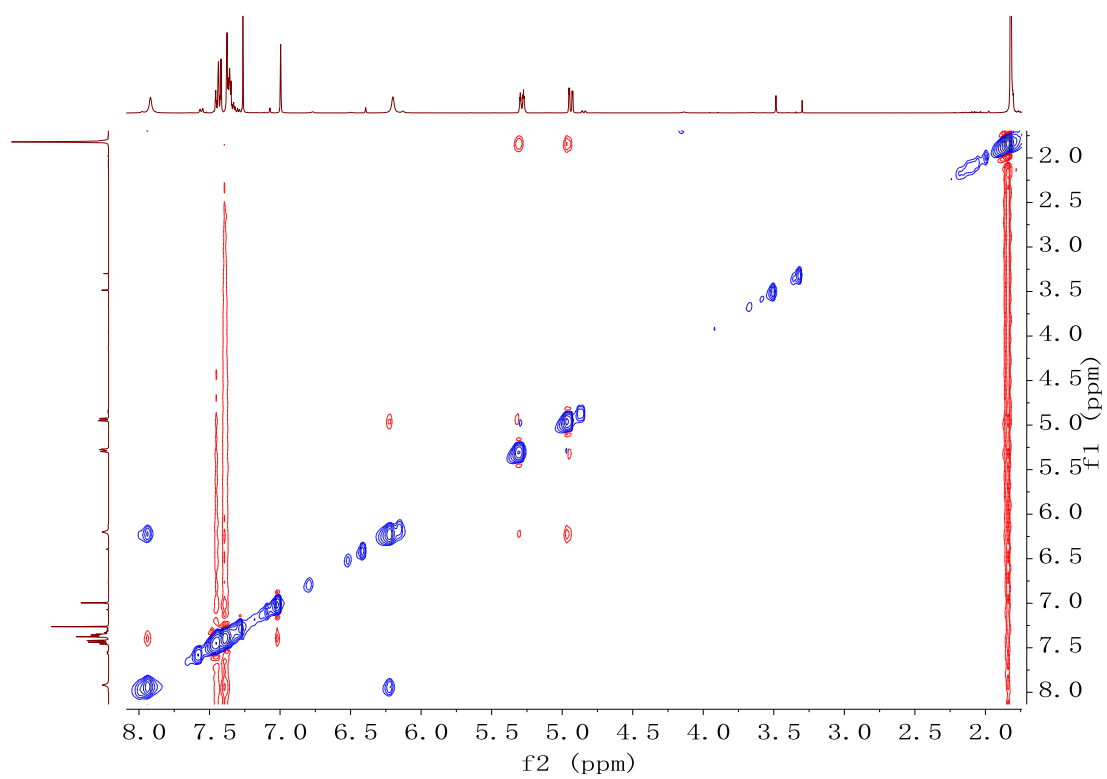


Fig. S10 NOESY spectrum of albocandin A (**1**, in CDCl_3 , 400 MHz).

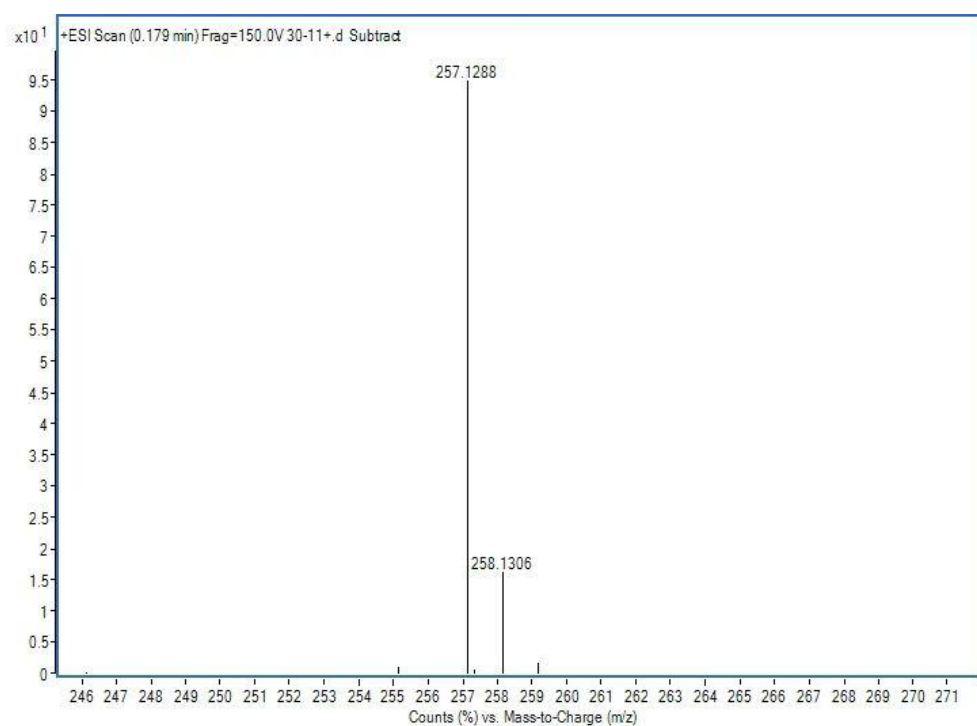


Fig. S11 (+) HRESIMS data of albocandin A (**1**).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650

Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2024/4/3

Method Name : Specific Rotation @25C

Set Temperature : 25.0°C

Time Delay : 10

Delay between measurement : 1 Sec

| N | Avg. | Std.Dev. | %RSD | Min | Max |
|---|-------|----------|------|-------|-------|
| 5 | 5.140 | 0.313 | 6.08 | 5.000 | 5.700 |

| S.No | Sample ID | Time | Result | Scale | QR *Arc | WL.G.nm | Lg.mm | Conc.g/100mL | Temp |
|------|-----------|----------|--------|-------|---------|---------|-------|--------------|--------|
| 1 | 30-11 | 15:12:58 | 5.000 | SR | 0.0050 | 589 | 100 | 0.100 | 25.0°C |
| 2 | 30-11 | 15:13:04 | 5.000 | SR | 0.0050 | 589 | 100 | 0.100 | 25.0°C |
| 3 | 30-11 | 15:13:11 | 5.000 | SR | 0.0050 | 589 | 100 | 0.100 | 25.0°C |
| 4 | 30-11 | 15:13:18 | 5.000 | SR | 0.0050 | 589 | 100 | 0.100 | 25.0°C |
| 5 | 30-11 | 15:13:25 | 5.700 | SR | 0.0057 | 589 | 100 | 0.100 | 25.0°C |

Fig. S12 The rotation value of albocandin A (**1**, 0.1 g/100 mL in MeOH).

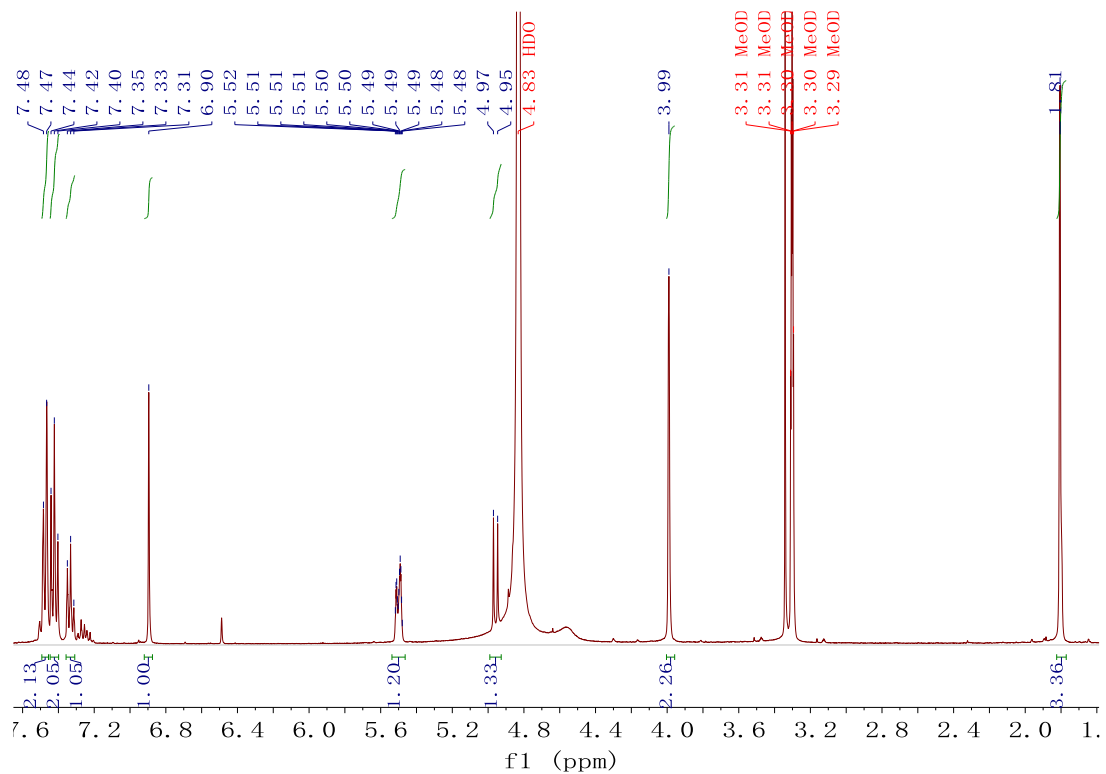


Fig. S13 ¹H NMR spectrum of albocandin B (**2**, in MeOD, 400 MHz).

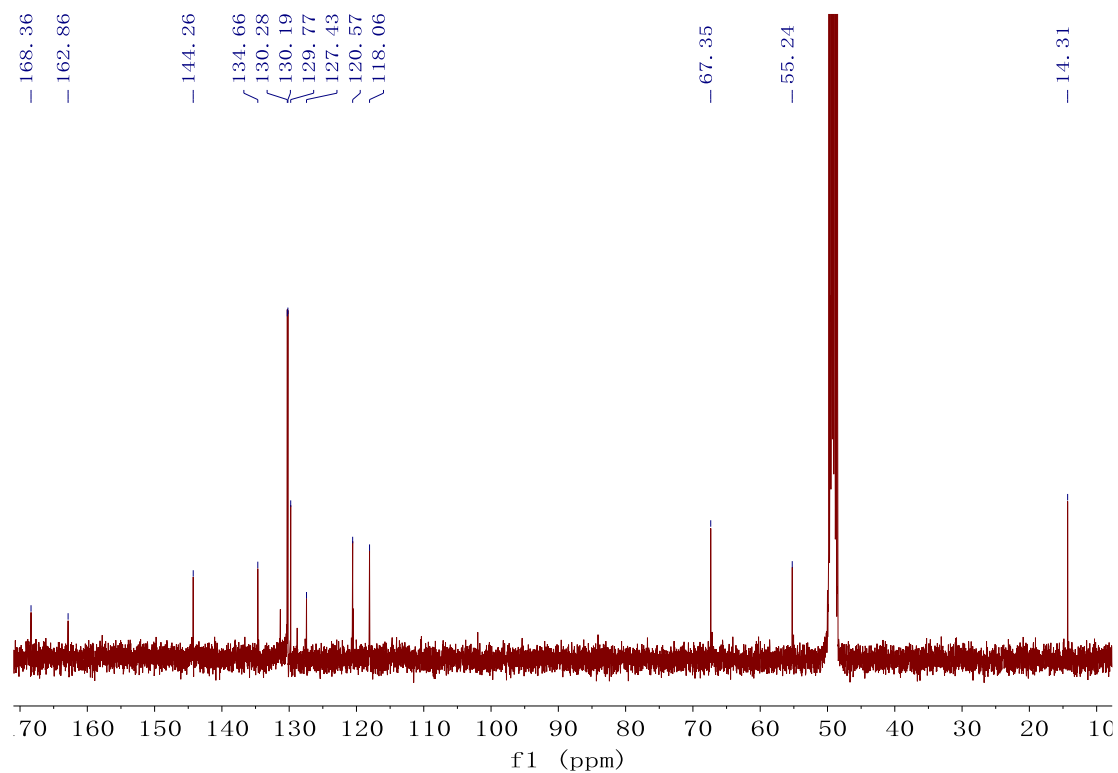


Fig. S14 ¹³C NMR spectrum of albocandin B (**2**, in MeOD, 100 MHz).

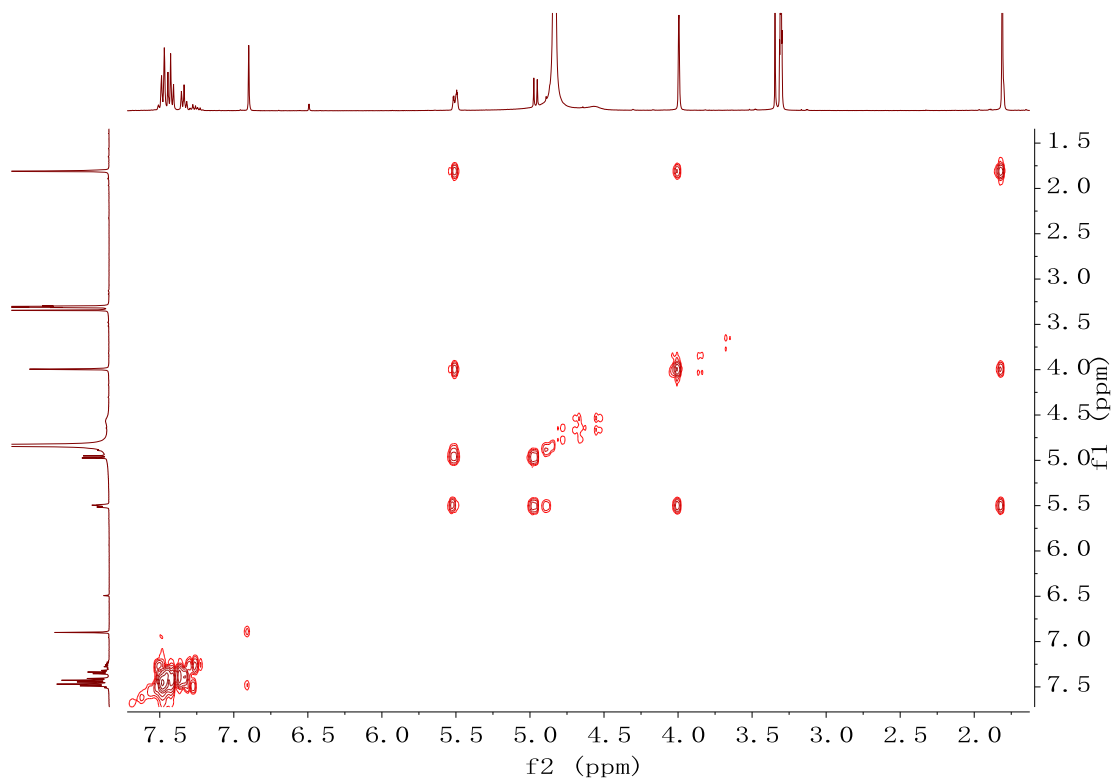


Fig. S15 COSY spectrum of albocandin B (**2**, in MeOD, 400 MHz).

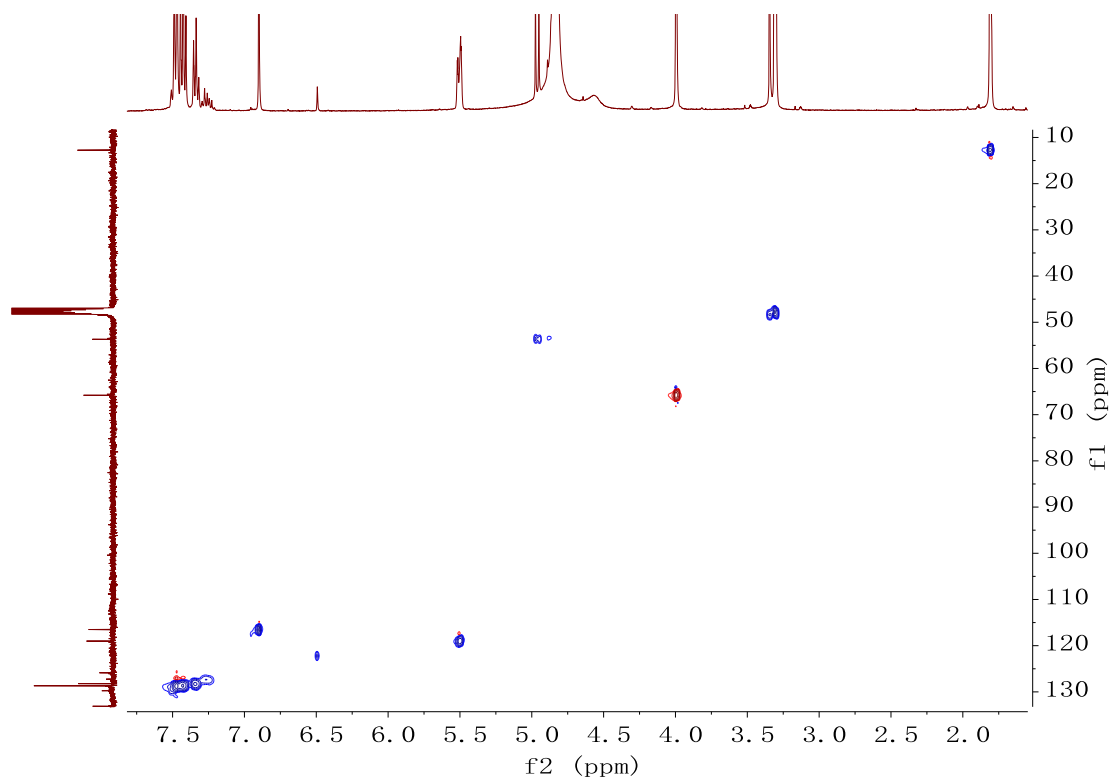


Fig. S16 HSQC spectrum of albocandin B (**2**, in MeOD, 400 MHz).

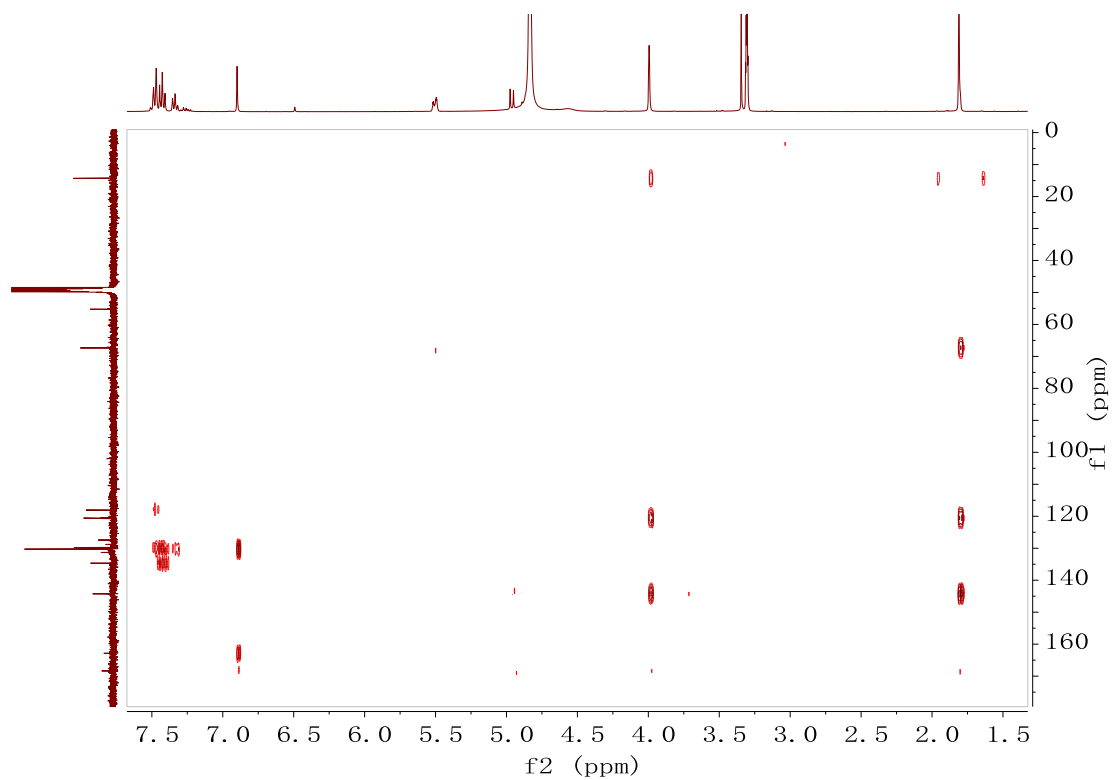


Fig. S17 HMBC spectrum of albocandin B (**2**, in MeOD, 400 MHz).

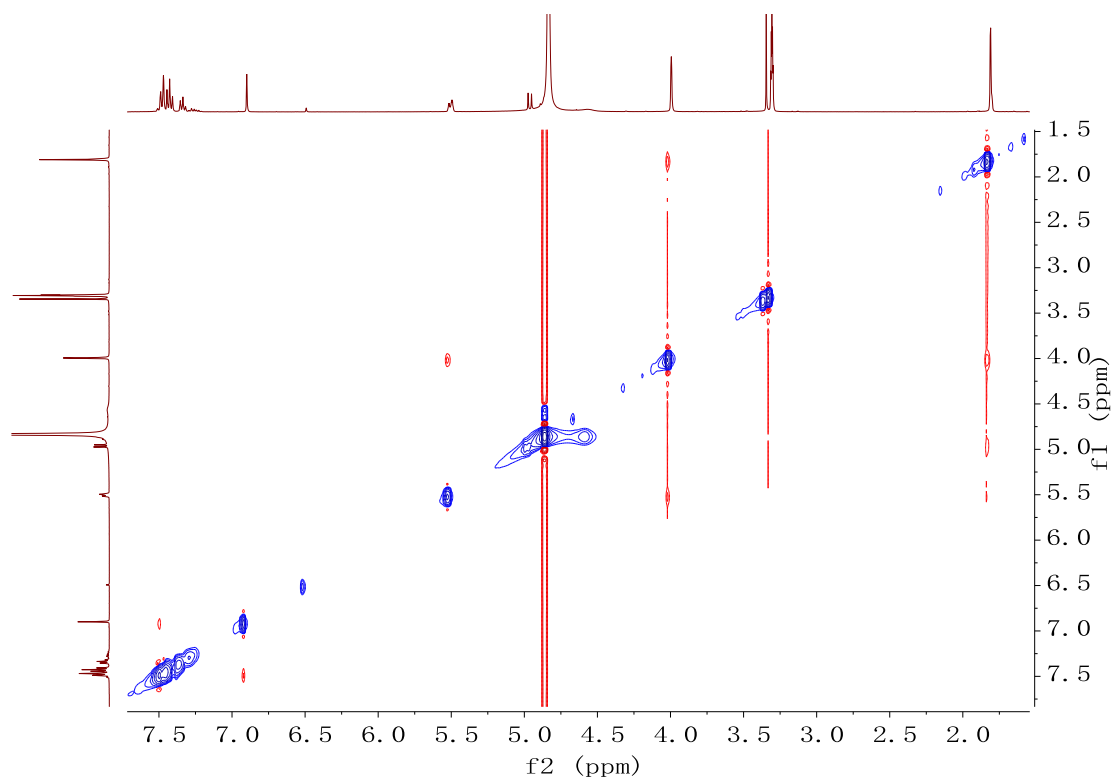


Fig. S18 NOESY spectrum of albocandin B (**2**, in MeOD, 400 MHz).

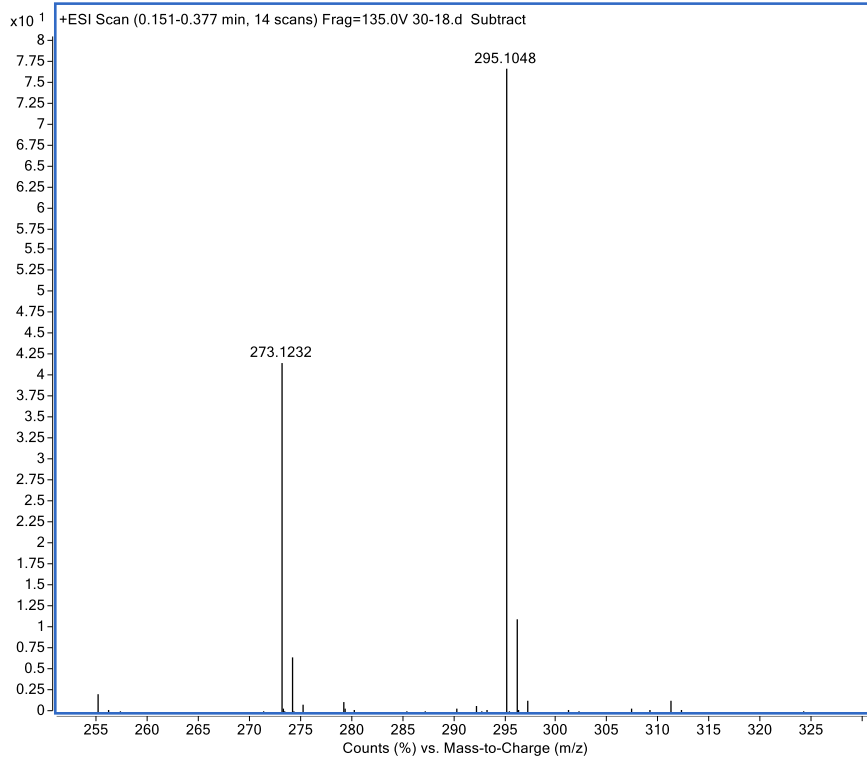


Fig. S19 (+) HRESIMS data of albocandin B (2).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650
 Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2024/4/3

Method Name : Specific Rotation @25C

Set Temperature : 25.0°C

Time Delay : 10

Delay between measurement : 1 Sec

| N | Avg. | Std.Dev. | %RSD | Min | Max |
|---|--------|----------|--------|--------|--------|
| 5 | -1.680 | 0.610 | -36.30 | -2.000 | -0.600 |

| S.No | Sample ID | Time | Result | Scale | OR*Arc | WLG.nm | Lg.mm | Conc.g/100mL | Temp |
|------|-----------|----------|--------|-------|---------|--------|-------|--------------|--------|
| 1 | 30-18 | 15:22:59 | -0.600 | SR | -0.0003 | 589 | 100 | 0.050 | 25.0°C |
| 2 | 30-18 | 15:23:06 | -2.000 | SR | -0.0010 | 589 | 100 | 0.050 | 25.0°C |
| 3 | 30-18 | 15:23:13 | -2.000 | SR | -0.0010 | 589 | 100 | 0.050 | 25.0°C |
| 4 | 30-18 | 15:23:19 | -2.000 | SR | -0.0010 | 589 | 100 | 0.050 | 25.0°C |
| 5 | 30-18 | 15:23:26 | -1.800 | SR | -0.0009 | 589 | 100 | 0.050 | 25.0°C |

Fig. S20 The rotation value of albocandin B (2, 0.1 g/100 mL in MeOH).

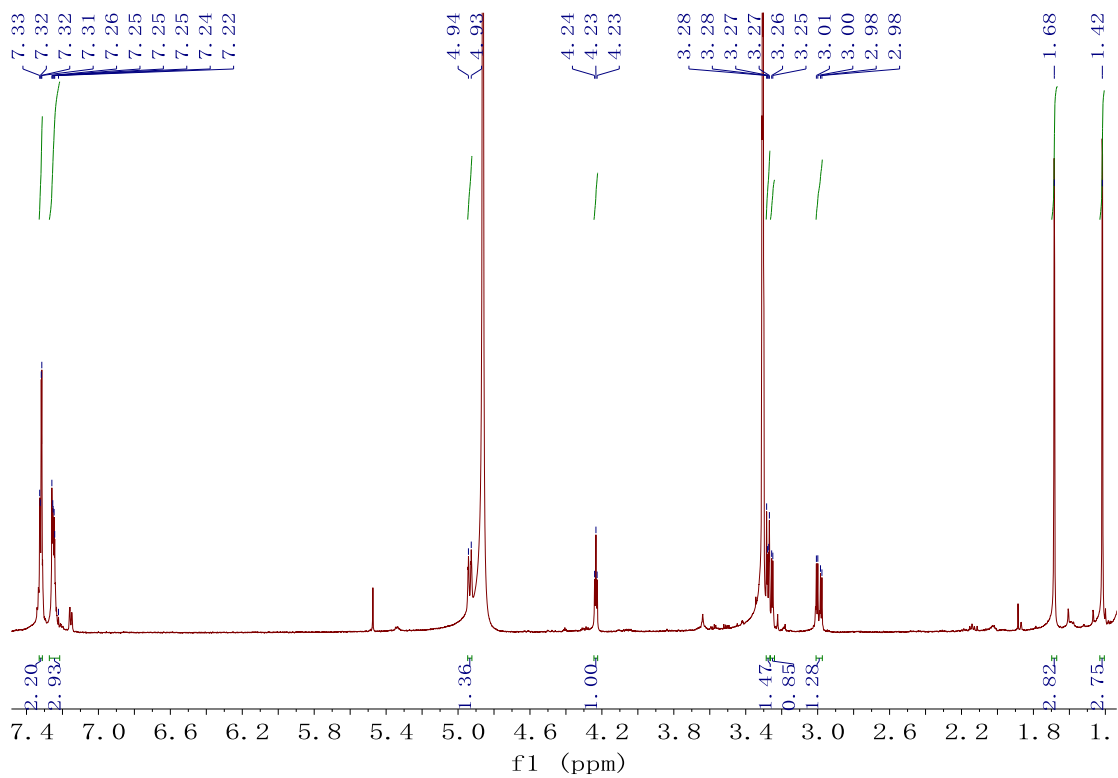


Fig. S21 ^1H NMR spectrum of albocandin C (**3**, in MeOD, 600 MHz).

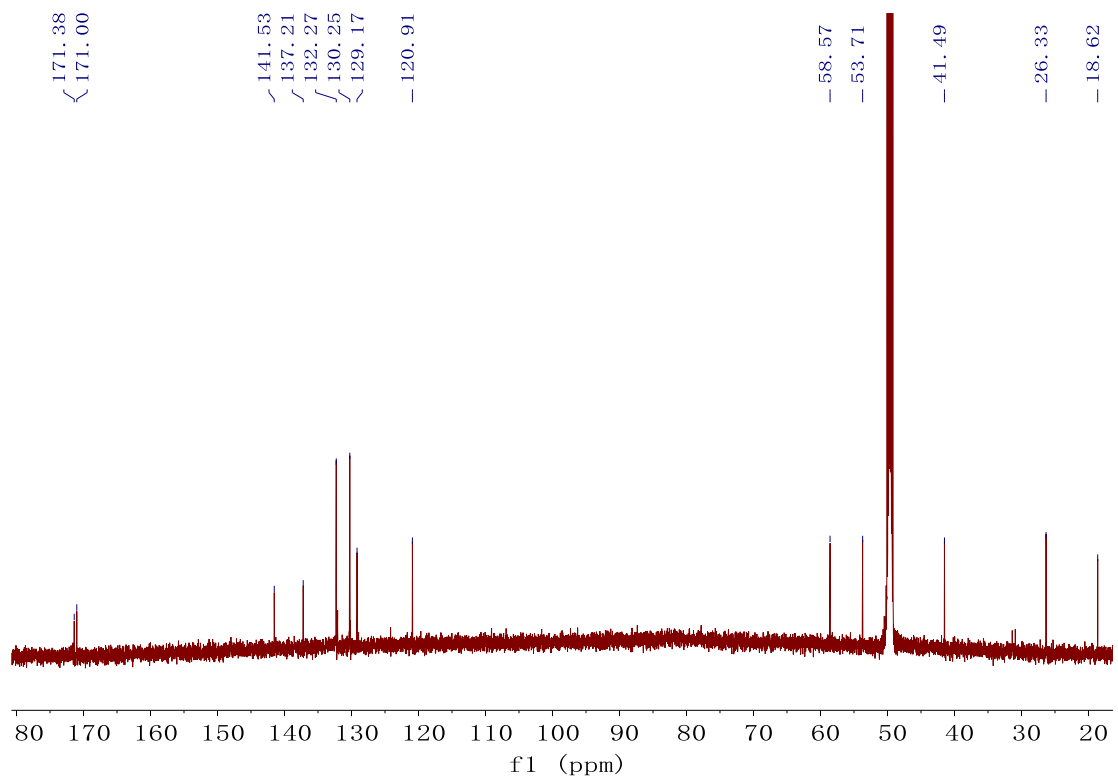


Fig. S22 ^{13}C NMR spectrum of albocandin C (**3**, in MeOD, 150 MHz).

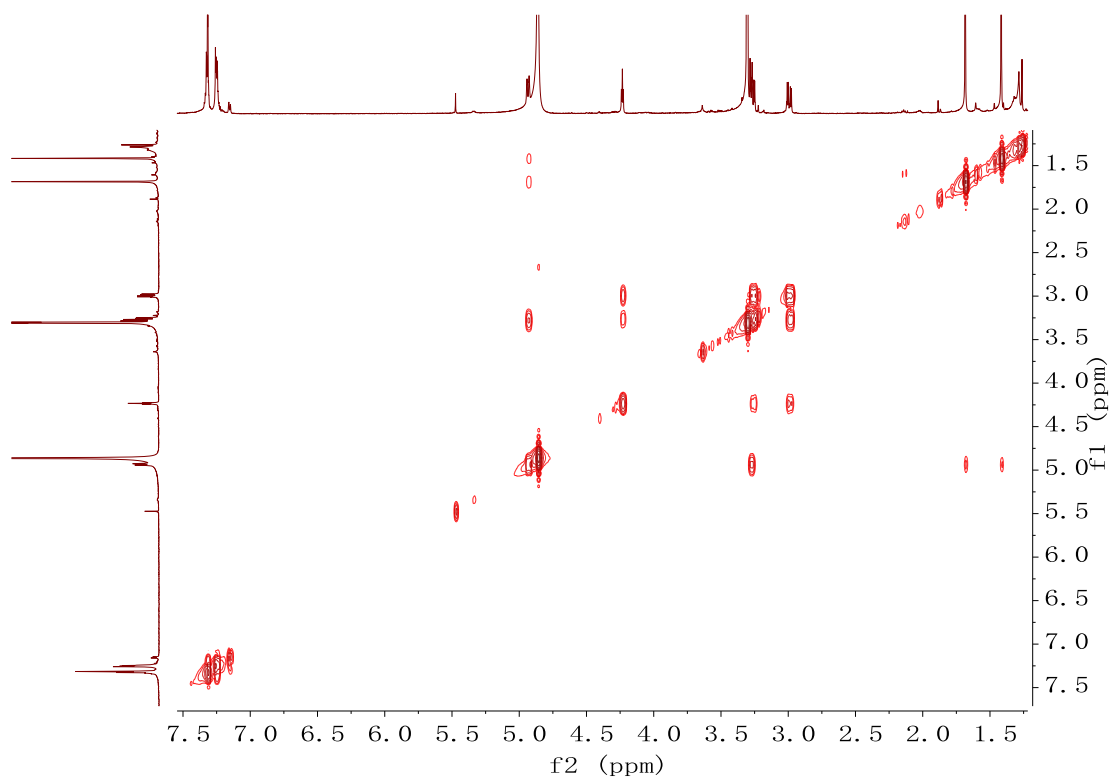


Fig. S23 COSY spectrum of albocandin C (**3**, in MeOD, 600 MHz).

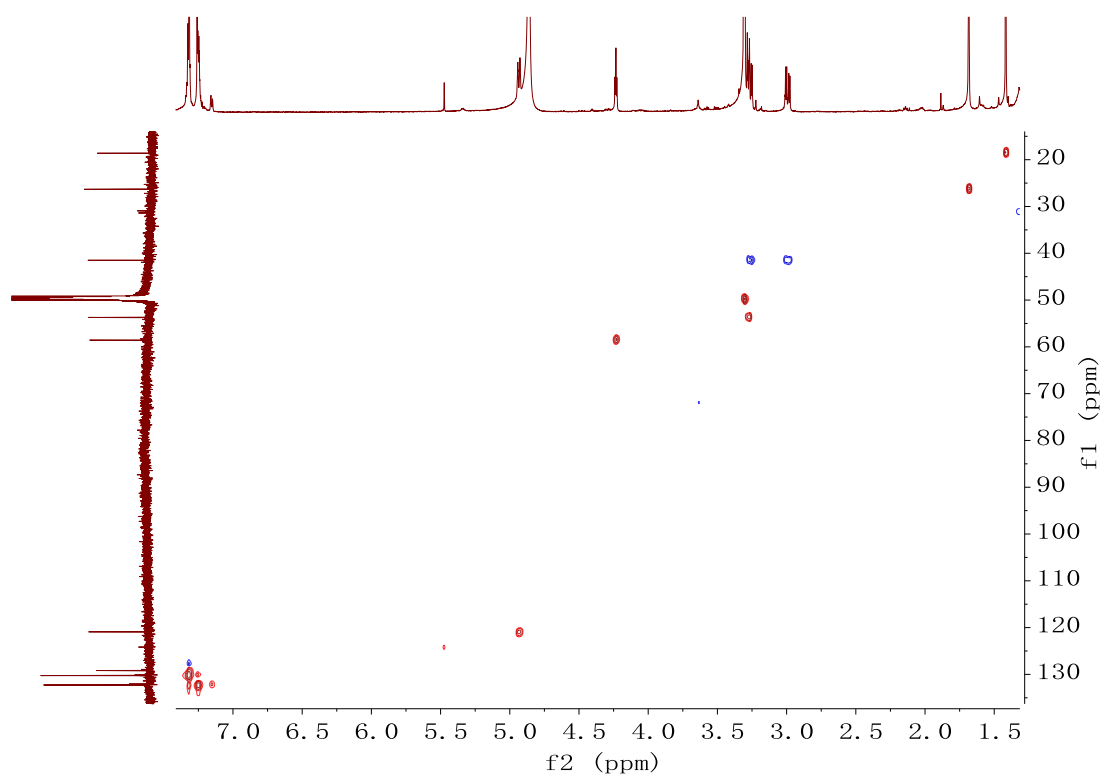


Fig. S24 HSQC spectrum of albocandin C (**3**, in MeOD, 600 MHz).

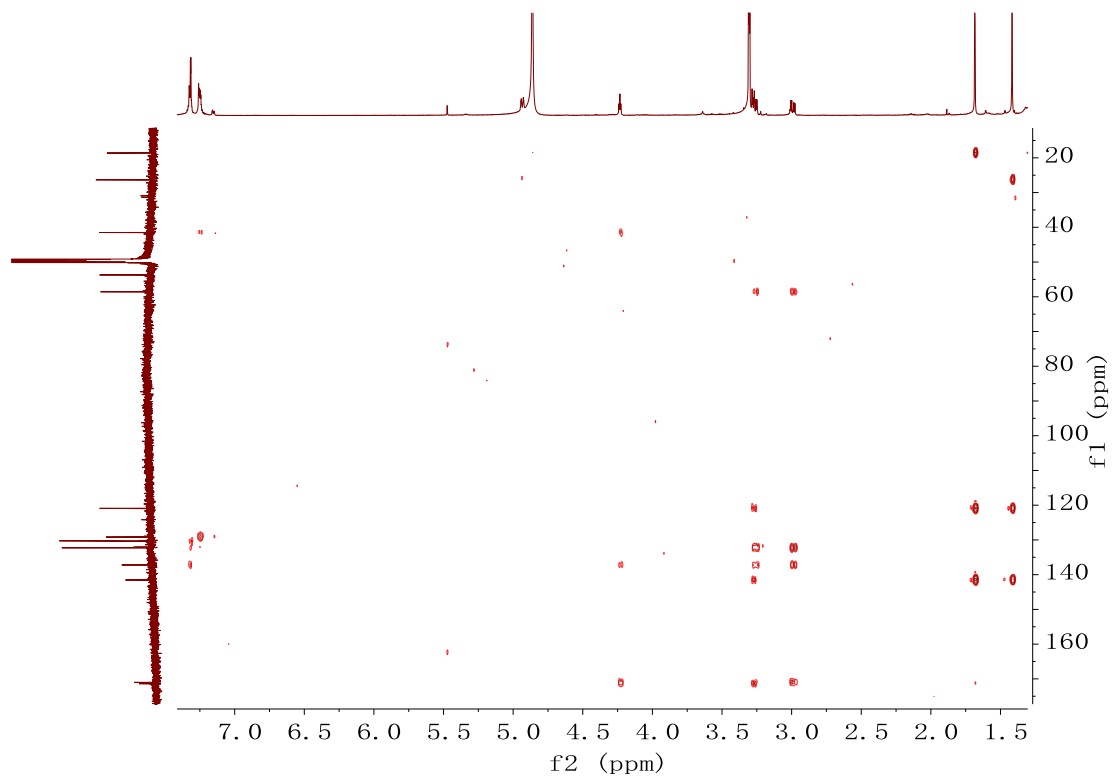


Fig. S25 HMBC spectrum of albocandin C (**3**, in MeOD, 600 MHz).

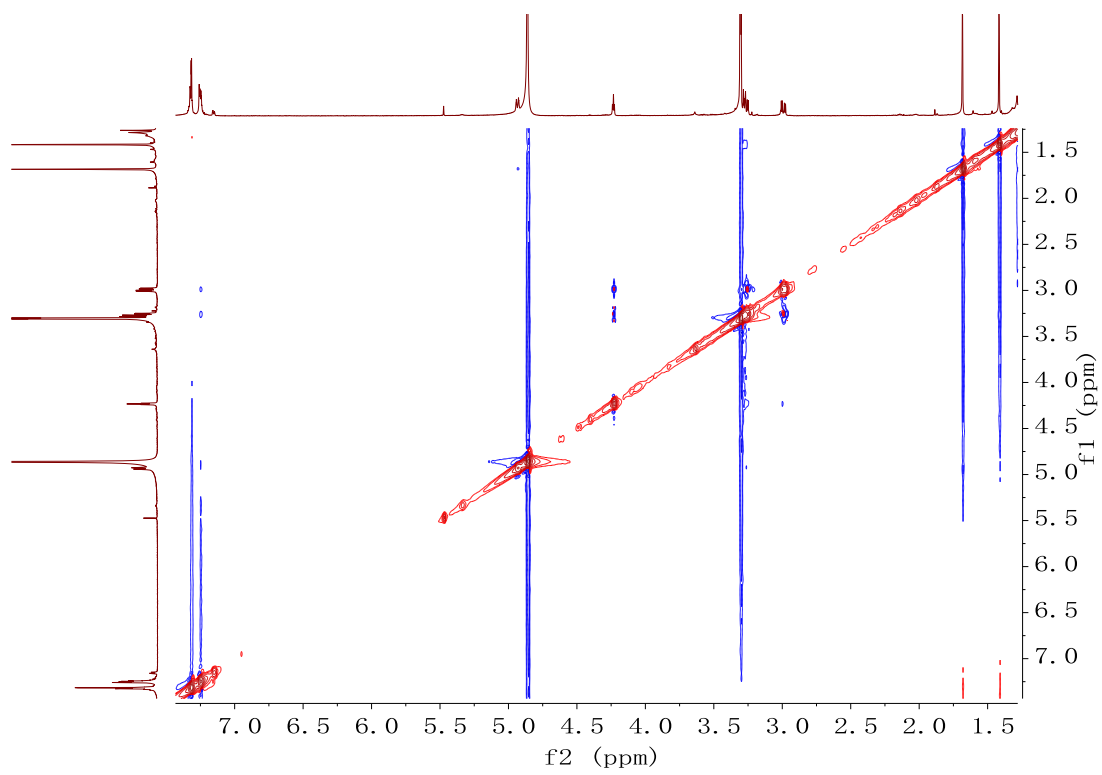


Fig. S26 NOESY spectrum of albocandin C (**3**, in MeOD, 600 MHz).

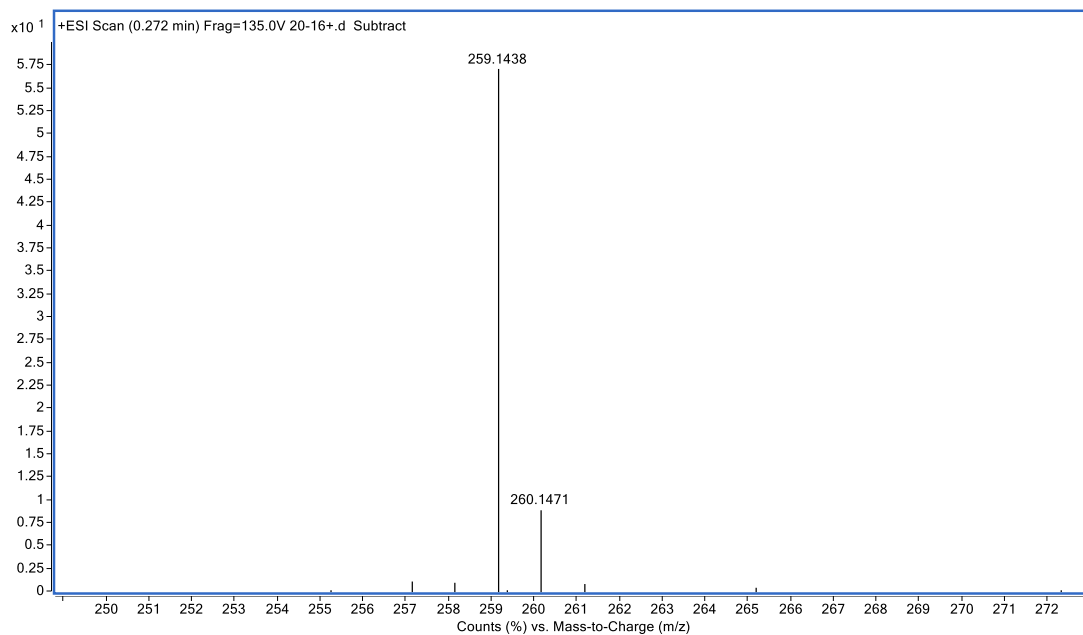


Fig. S27 (+) HRESIMS data of albocandin C (**3**).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650

Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2024/4/3

Method Name : Specific Rotation @25C

Set Temperature : 25.0°C

Time Delay : 10

Delay between measurement : 1 Sec

| N | Avg. | Std.Dev. | %RSD | Min | Max |
|---|-------|----------|-------|-------|-------|
| 5 | 1.640 | 0.422 | 25.73 | 1.300 | 2.100 |

| S.No | Sample ID | Time | Result | Scale | OR*Arc | WLG.nm | Lg.mm | Conc.g/100mL | Temp |
|------|-----------|----------|--------|-------|--------|--------|-------|--------------|--------|
| 1 | 30-16-1 | 15:29:47 | 1.300 | SR | 0.0013 | 589 | 100 | 0.100 | 24.9°C |
| 2 | 30-16-1 | 15:29:54 | 2.100 | SR | 0.0021 | 589 | 100 | 0.100 | 24.9°C |
| 3 | 30-16-1 | 15:30:01 | 1.400 | SR | 0.0014 | 589 | 100 | 0.100 | 25.0°C |
| 4 | 30-16-1 | 15:30:08 | 2.100 | SR | 0.0021 | 589 | 100 | 0.100 | 25.0°C |
| 5 | 30-16-1 | 15:30:15 | 1.300 | SR | 0.0013 | 589 | 100 | 0.100 | 25.0°C |

Fig. S28 The rotation value of albocandin C (**3**, 0.1 g/100 mL in MeOH).

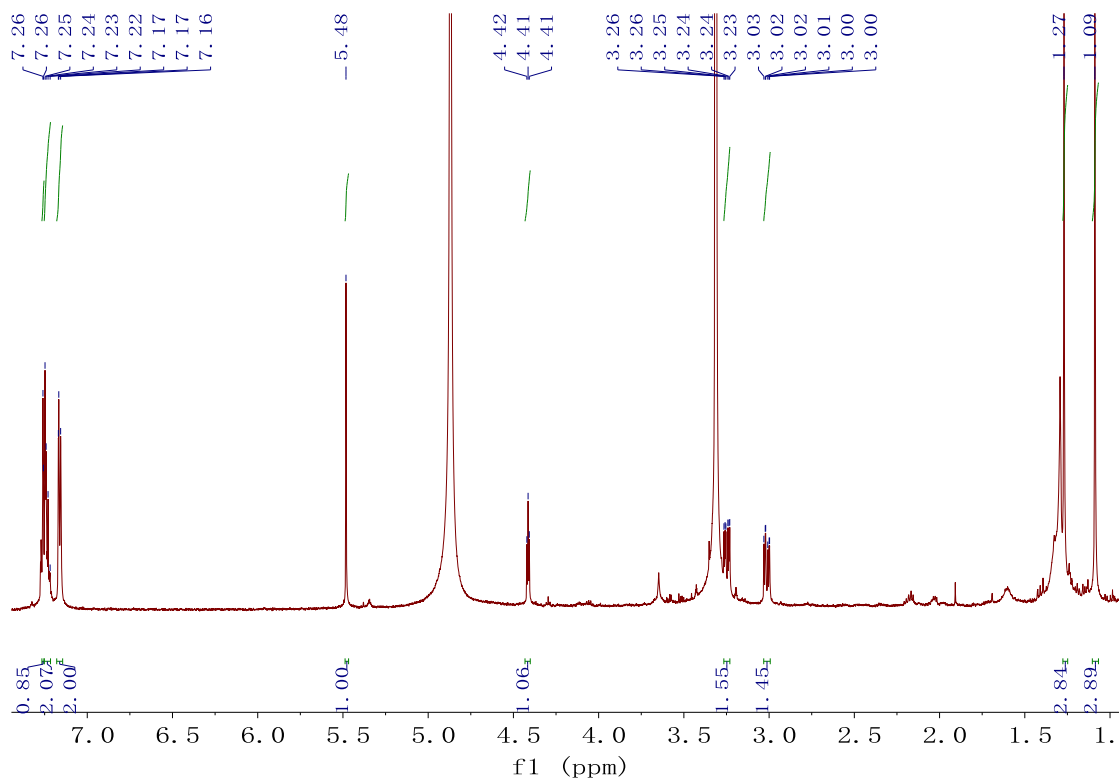


Fig. S29 ^1H NMR spectrum of albocandin D (**4**, in MeOD, 600 MHz).

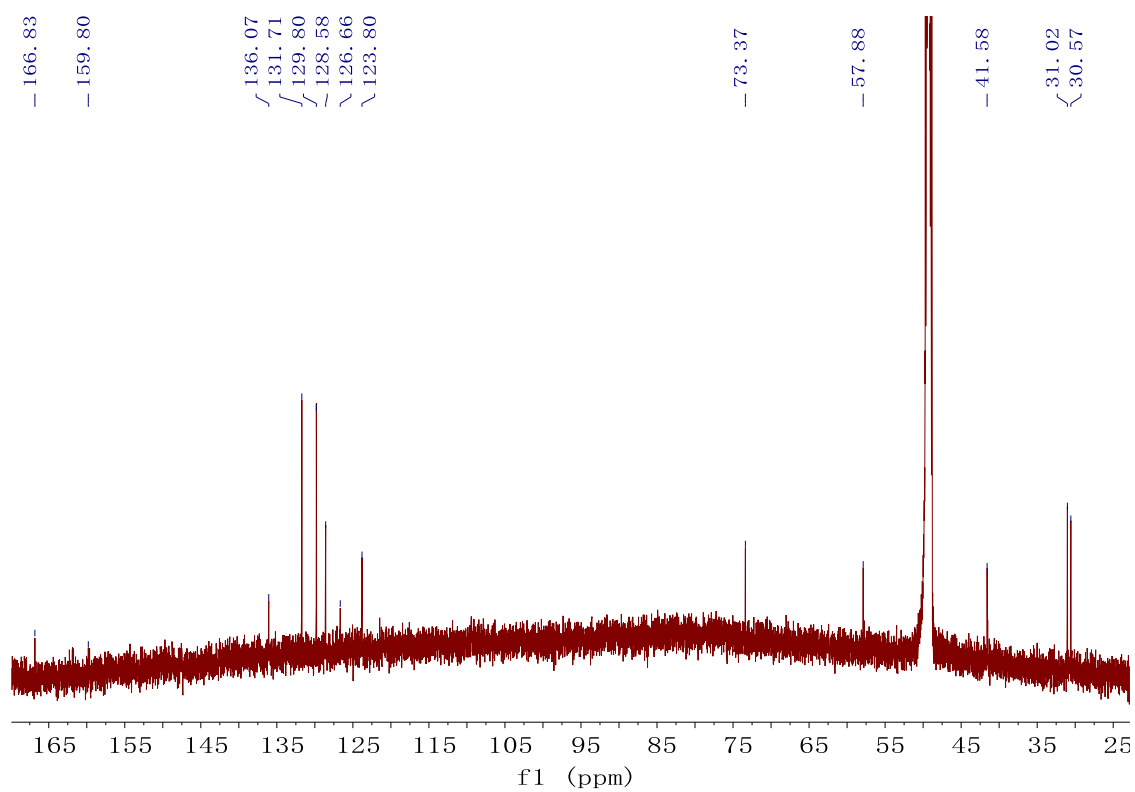


Fig. S30 ^{13}C NMR spectrum of albocandin D (**4**, in MeOD, 150 MHz).

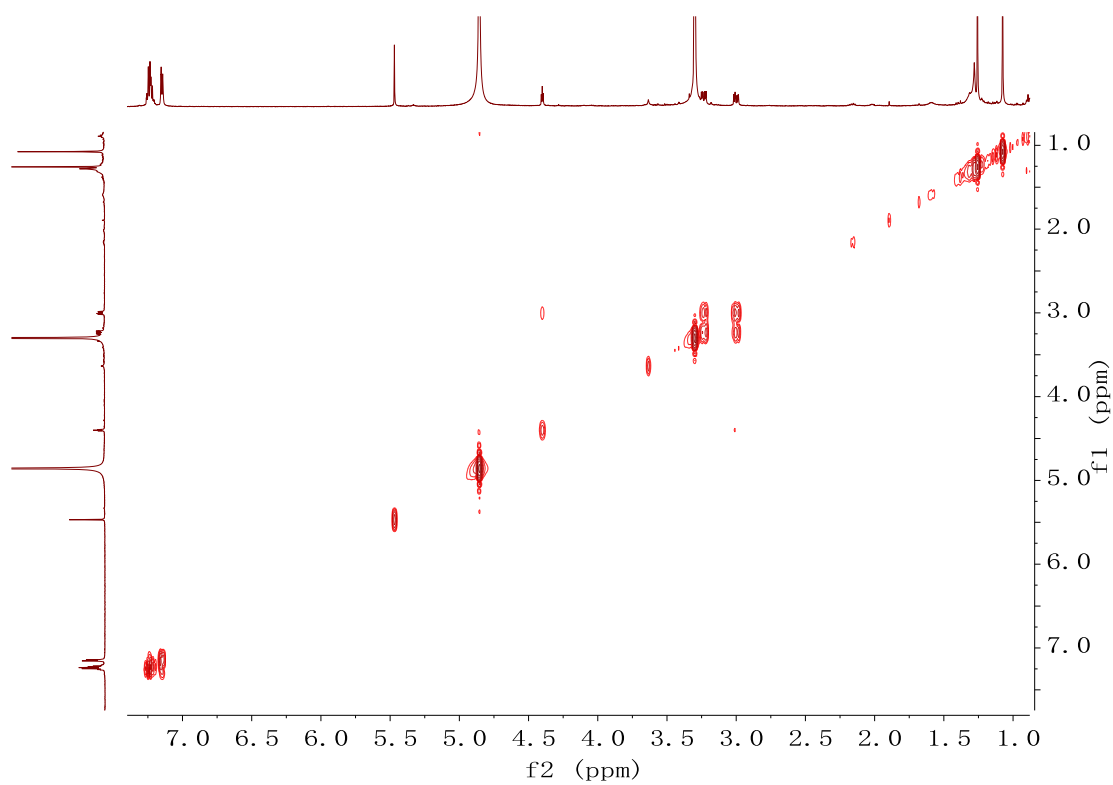


Fig. S31 COSY spectrum of albocandin D (**4**, in MeOD, 600 MHz).

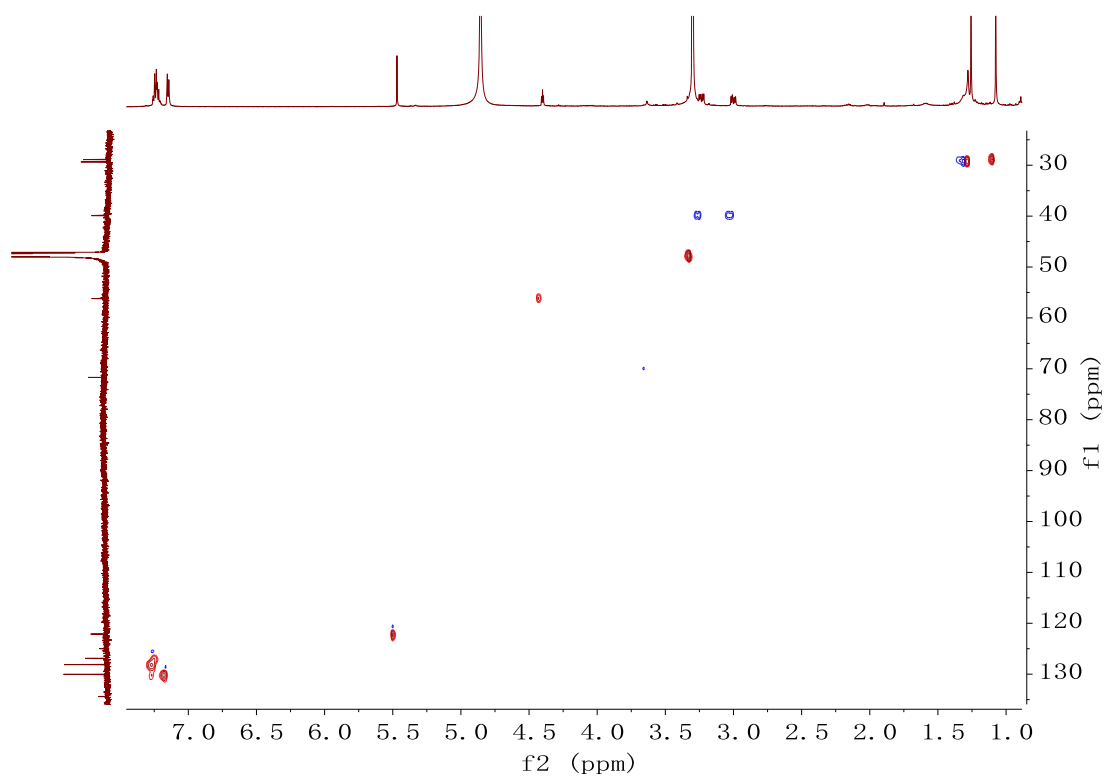


Fig. S32 HSQC spectrum of albocandin D (**4**, in MeOD, 600 MHz).

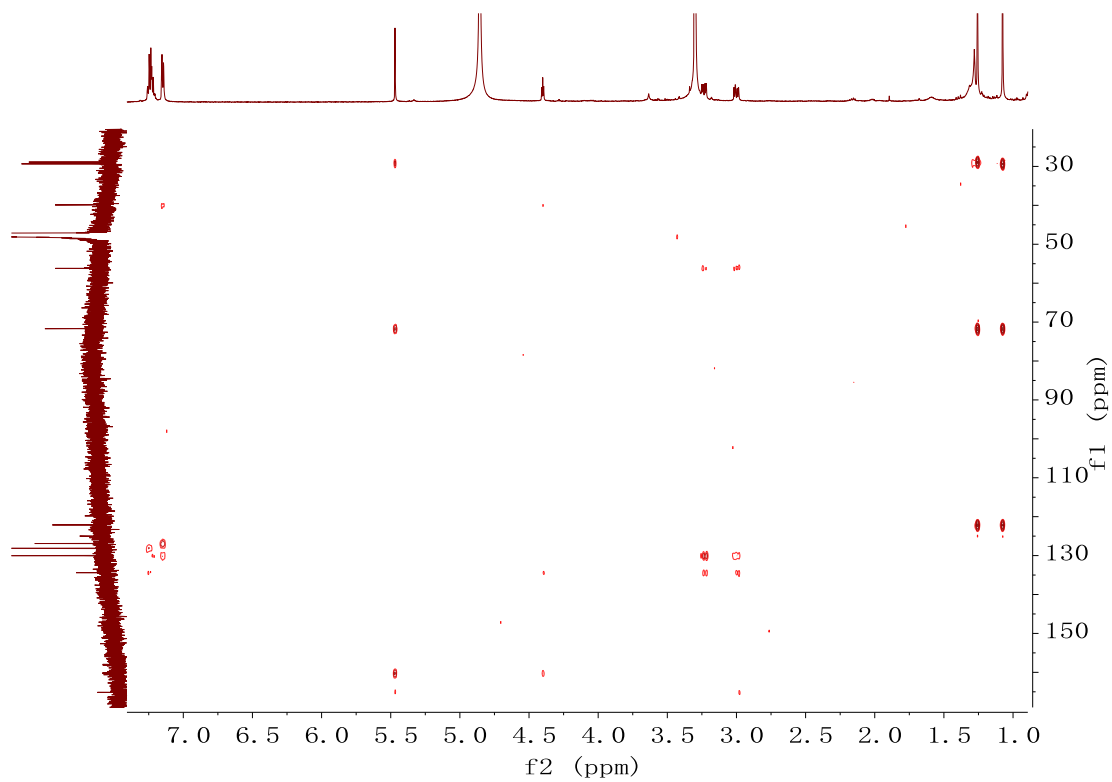


Fig. S33 HMBC spectrum of albocandin D (**4**, in MeOD, 600 MHz).

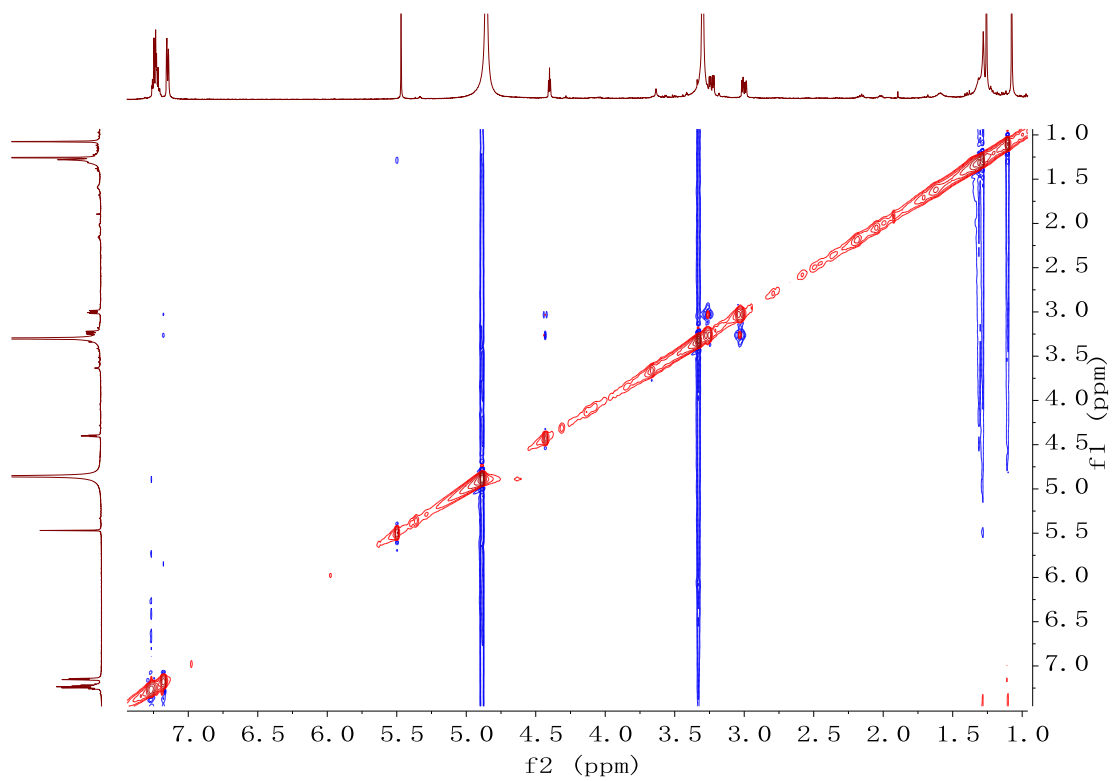


Fig. S34 NOESY spectrum of albocandin D (**4**, in MeOD, 600 MHz).

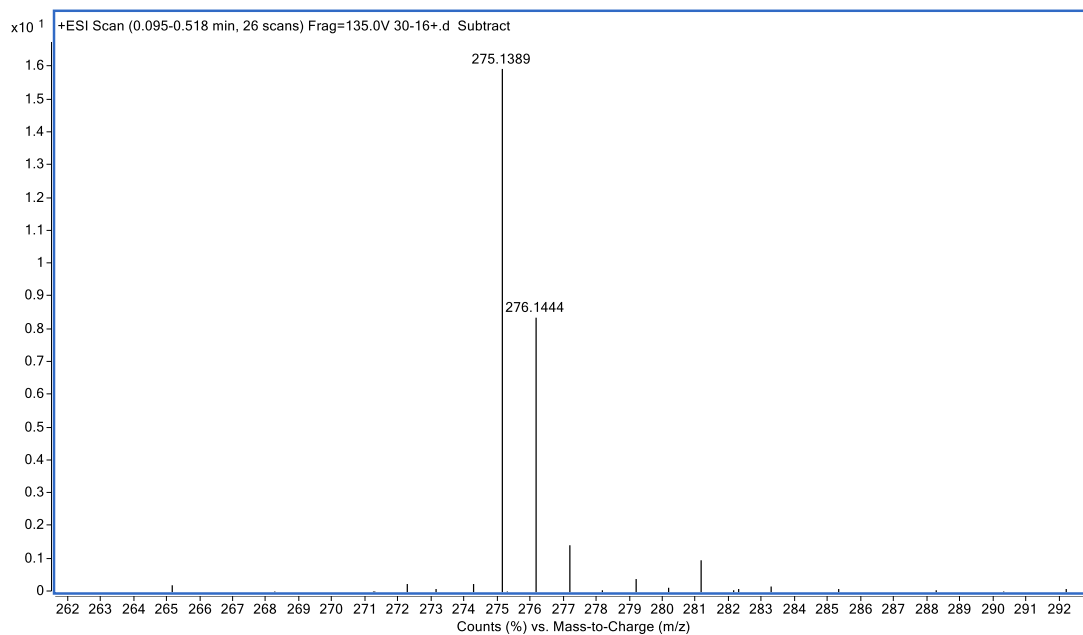


Fig. S35 (+) HRESIMS data of albocandin D (4).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83850

Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2024/4/3

Method Name : Specific Rotation @25C

Set Temperature : 25.0°C

Time Delay : 10

Delay between measurement : 1 Sec

| N | Avg. | Std.Dev. | %RSD | Min | Max |
|---|---------|----------|-------|---------|---------|
| 5 | -11.040 | 0.723 | -6.54 | -12.200 | -10.500 |

| S.No | Sample ID | Time | Result | Scale | OR.*Arc | WLG.nm | Lg.mm | Conc.g/100mL | Temp |
|------|-----------|----------|---------|-------|---------|--------|-------|--------------|--------|
| 1 | 30-16-2 | 15:32:54 | -12.200 | SR | -0.0122 | 589 | 100 | 0.100 | 25.0°C |
| 2 | 30-16-2 | 15:33:01 | -10.800 | SR | -0.0106 | 589 | 100 | 0.100 | 25.0°C |
| 3 | 30-16-2 | 15:33:08 | -10.800 | SR | -0.0106 | 589 | 100 | 0.100 | 24.9°C |
| 4 | 30-16-2 | 15:33:15 | -10.500 | SR | -0.0105 | 589 | 100 | 0.100 | 24.9°C |
| 5 | 30-16-2 | 15:33:22 | -11.300 | SR | -0.0113 | 589 | 100 | 0.100 | 24.9°C |

Fig. S36 The rotation value of albocandin D (4, 0.1 g/100 mL in MeOH).

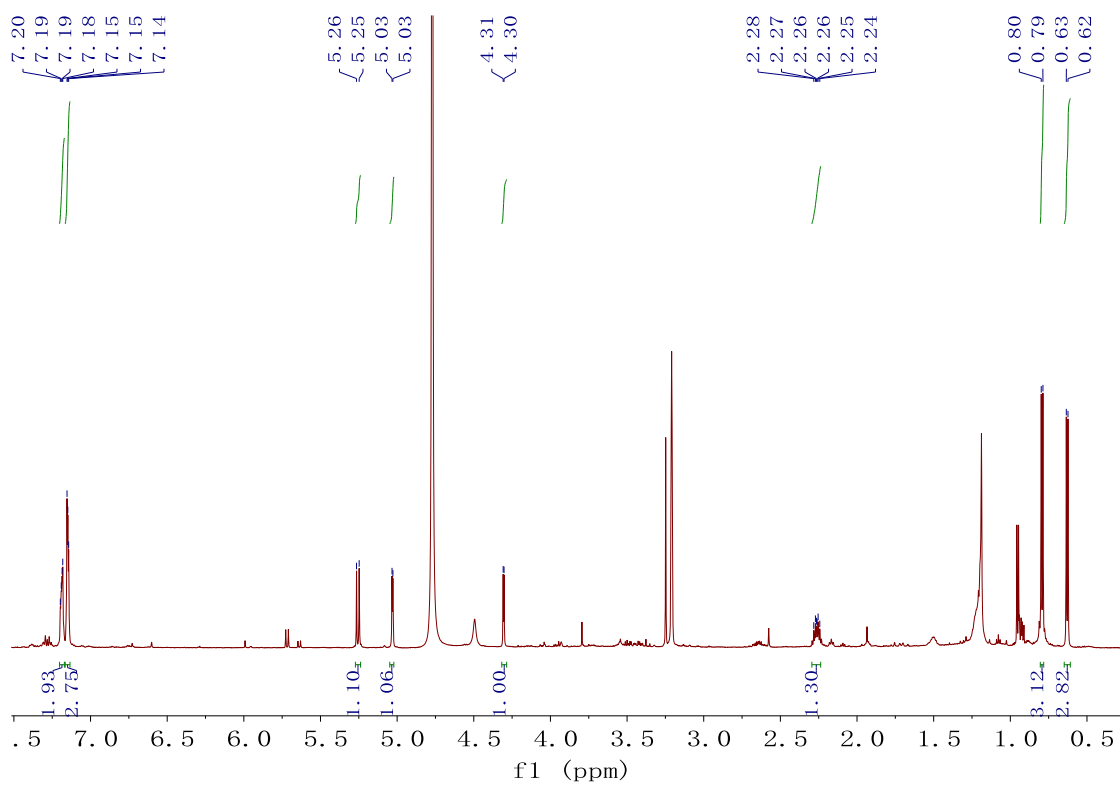


Fig. S37 ^1H NMR spectrum of albocandin E (**5**, in MeOD, 600 MHz).

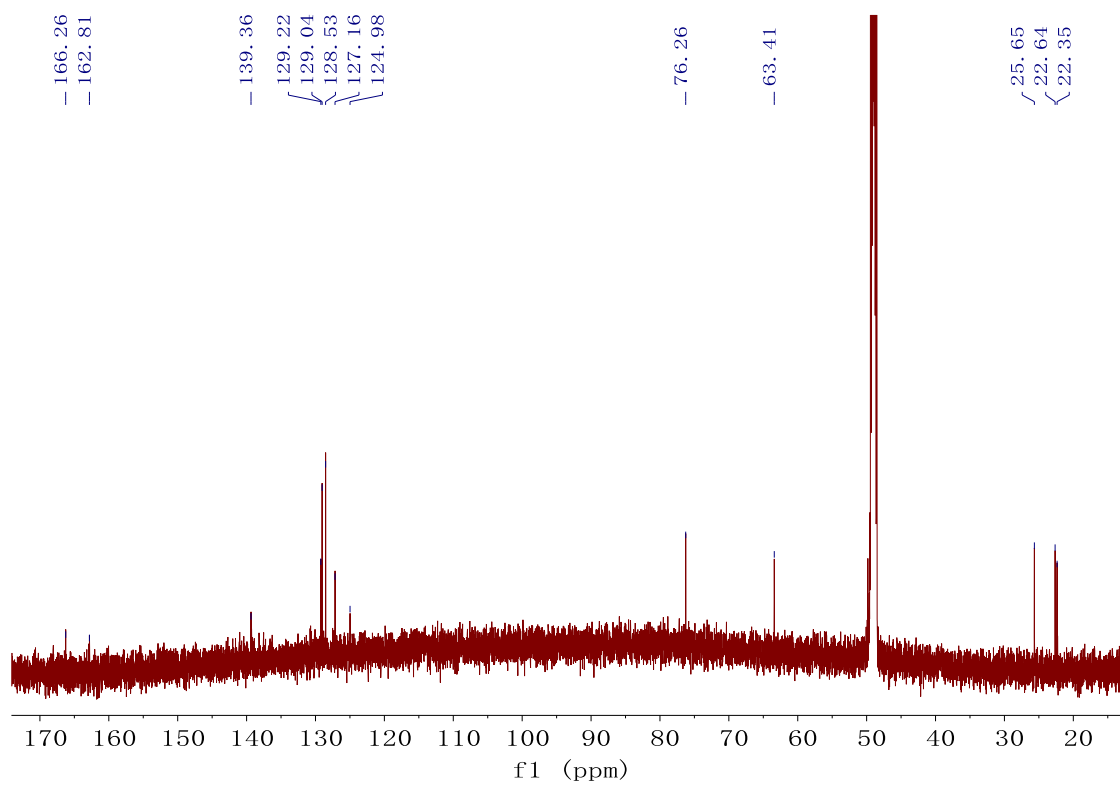


Fig. S38 ^{13}C NMR spectrum of albocandin E (**5**, in MeOD, 150 MHz).

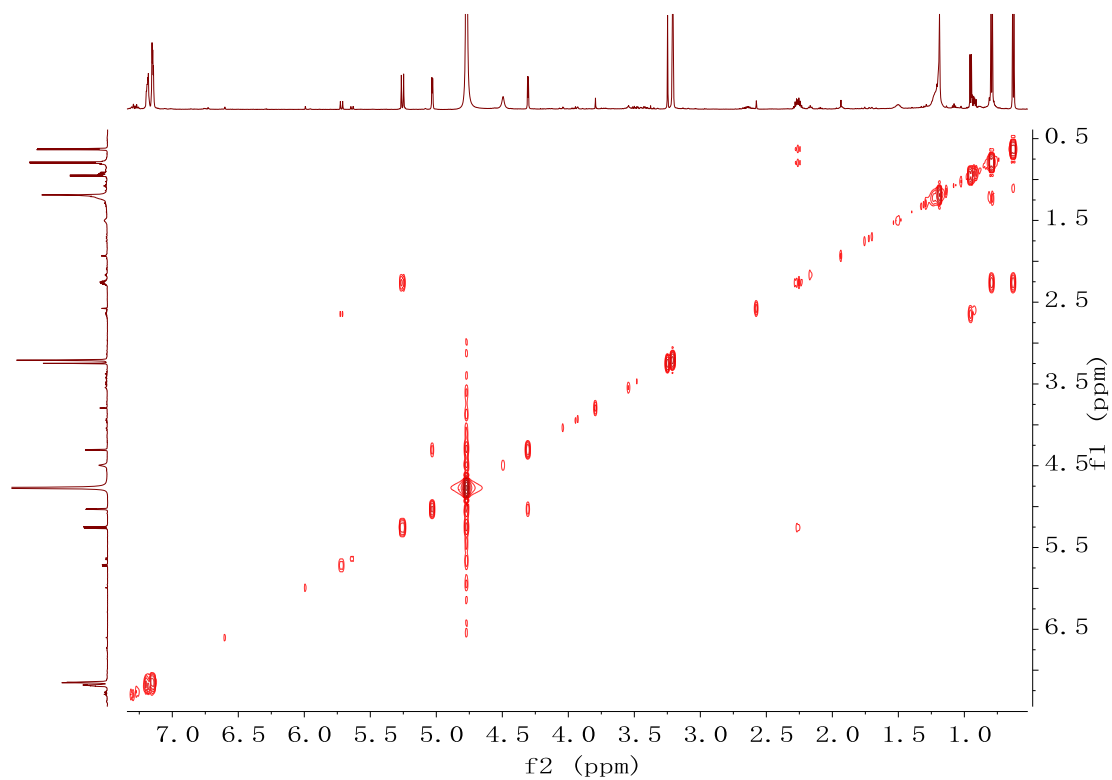


Fig. S39 COSY spectrum of albocandin E (**5**, in MeOD, 600 MHz).

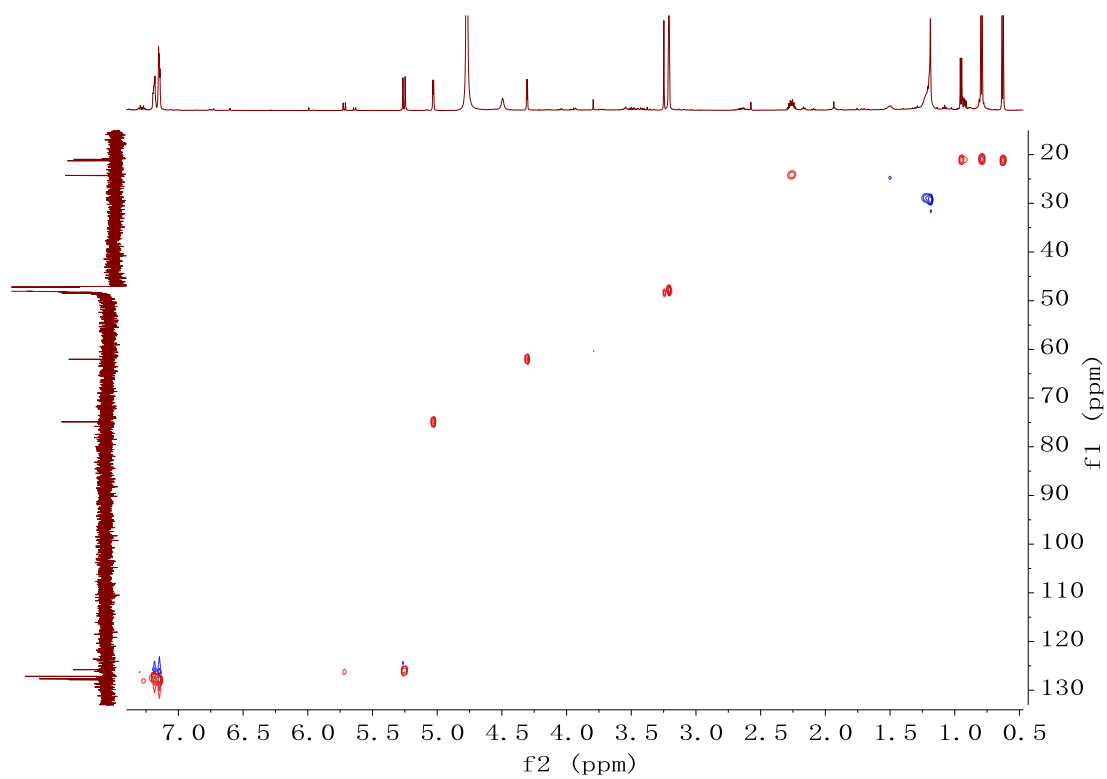


Fig. S40 HSQC spectrum of albocandin E (**5**, in MeOD, 600 MHz).

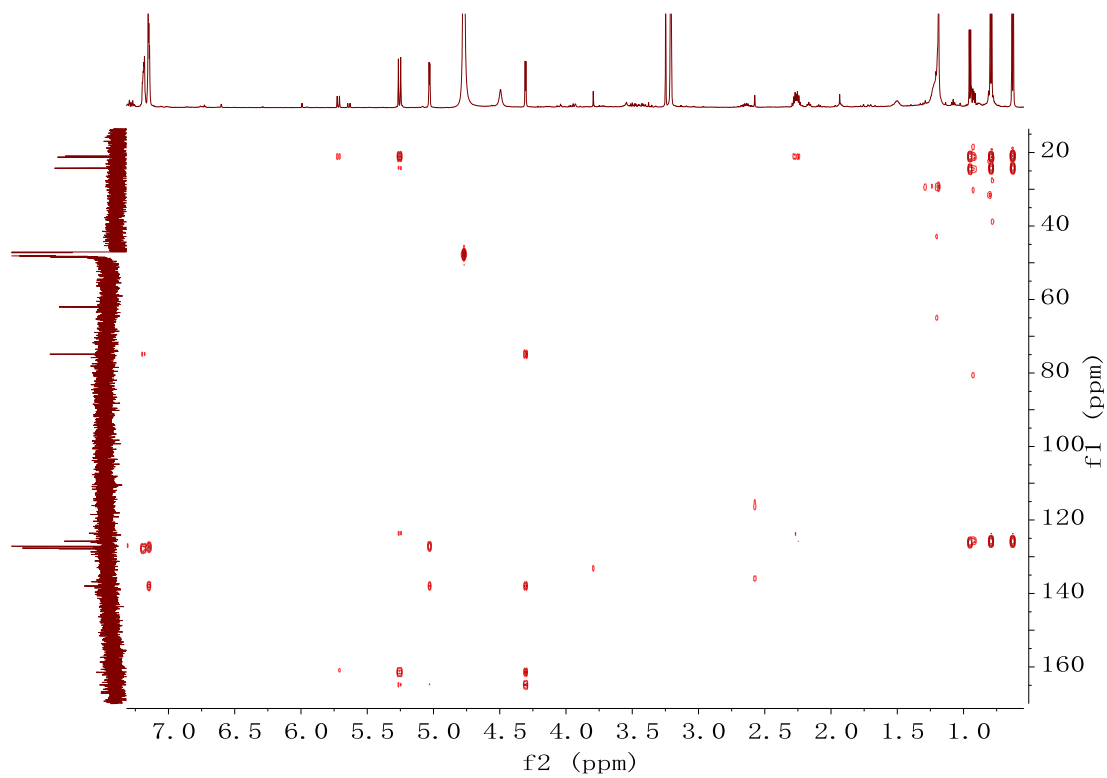


Fig. S41 HMBC spectrum of albocandin E (**5**, in MeOD, 600 MHz).

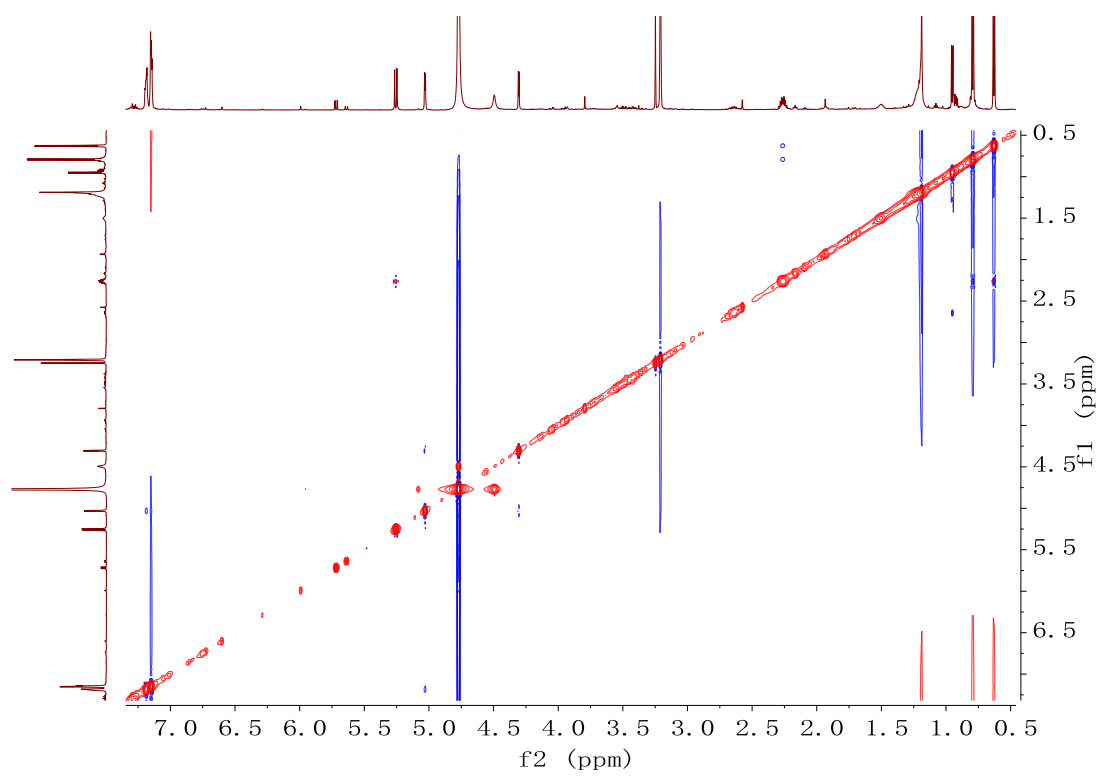


Fig. S42 NOESY spectrum of albocandin E (**5**, in MeOD, 600 MHz).

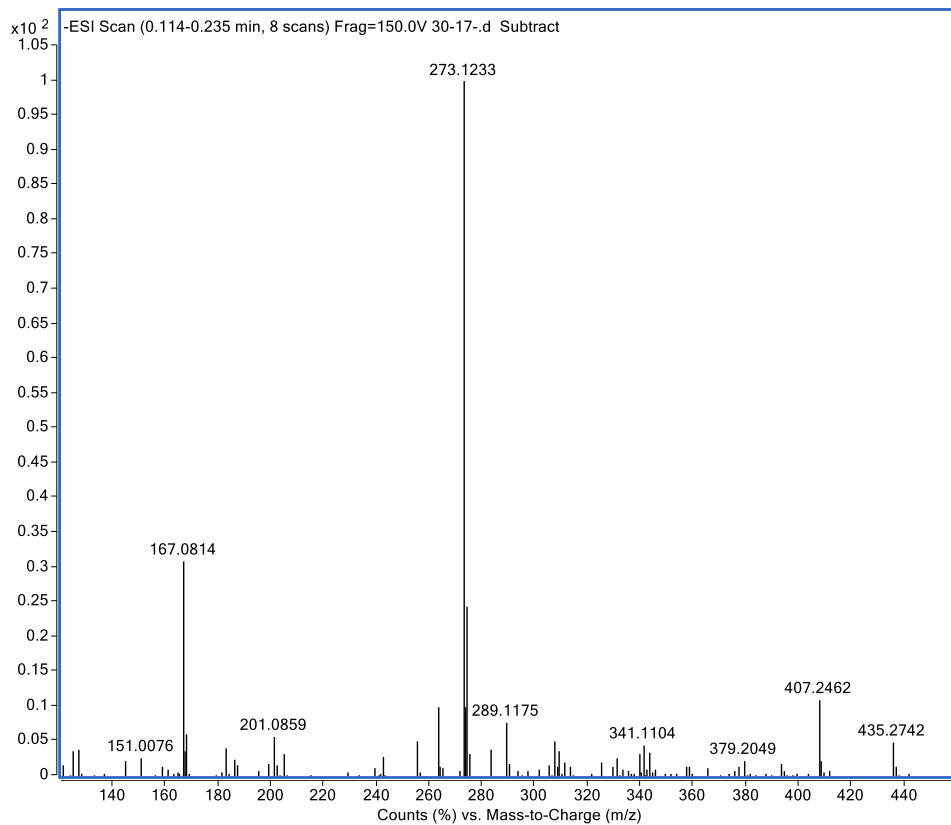


Fig. S43 (-) HRESIMS data of albocandin E (**5**).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650

Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2024/4/3

Method Name : Specific Rotation @25C

Set Temperature : 25.0°C

Time Delay : 10

Delay between measurement : 1 Sec

| N | Avg. | Std.Dev. | %RSD | Min | Max |
|---|--------|----------|--------|--------|--------|
| 5 | -5.400 | 0.800 | -14.81 | -6.200 | -4.600 |

| S.No | Sample ID | Time | Result | Scale | OR °Arc | WL.G.nm | Lg.mm | Conc.g/100mL | Temp |
|------|-----------|----------|--------|-------|---------|---------|-------|--------------|--------|
| 1 | 30-17 | 15:18:17 | -4.600 | SR | -0.0046 | 589 | 100 | 0.100 | 25.0°C |
| 2 | 30-17 | 15:18:23 | -6.200 | SR | -0.0062 | 589 | 100 | 0.100 | 25.0°C |
| 3 | 30-17 | 15:18:30 | -6.200 | SR | -0.0062 | 589 | 100 | 0.100 | 25.1°C |
| 4 | 30-17 | 15:18:37 | -4.600 | SR | -0.0046 | 589 | 100 | 0.100 | 25.1°C |
| 5 | 30-17 | 15:18:44 | -5.400 | SR | -0.0054 | 589 | 100 | 0.100 | 25.1°C |

Fig. S44 The rotation value of albocandin E (**5**, 0.1 g/100 mL in MeOH).

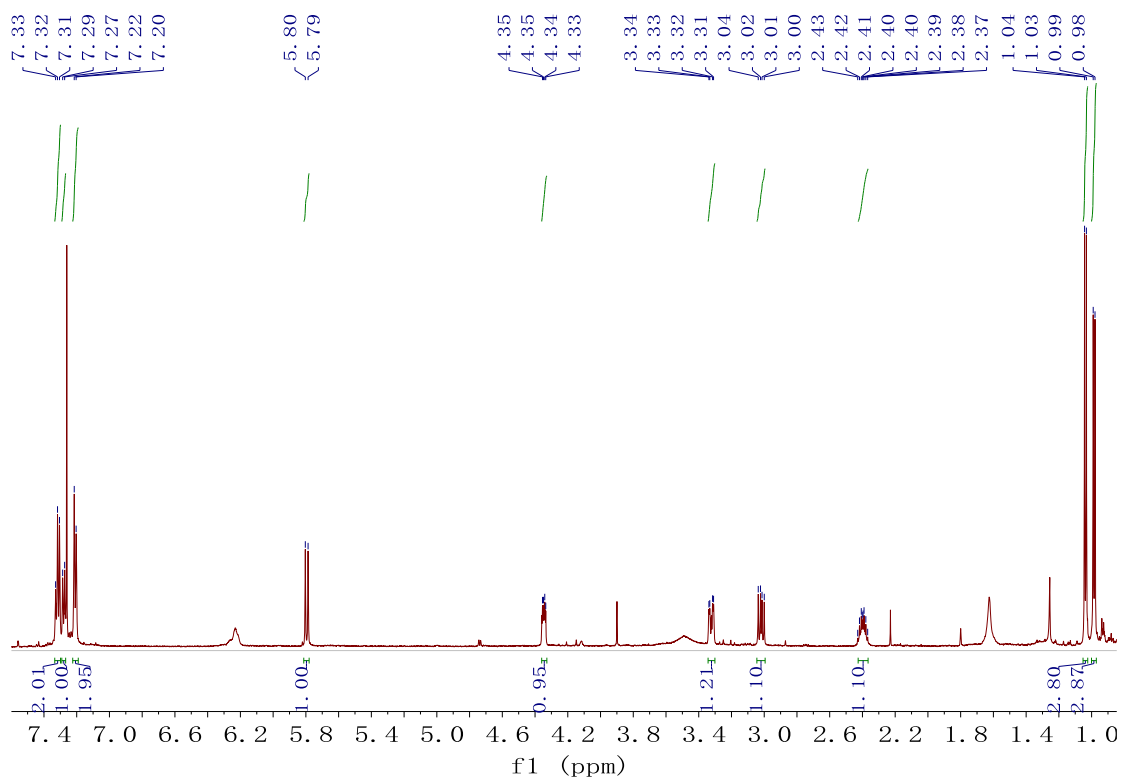


Fig. S45 ^1H NMR spectrum of (Z)-3-benzyl-6-isobutylidene DKP (**6**, in CDCl_3 , 600 MHz).

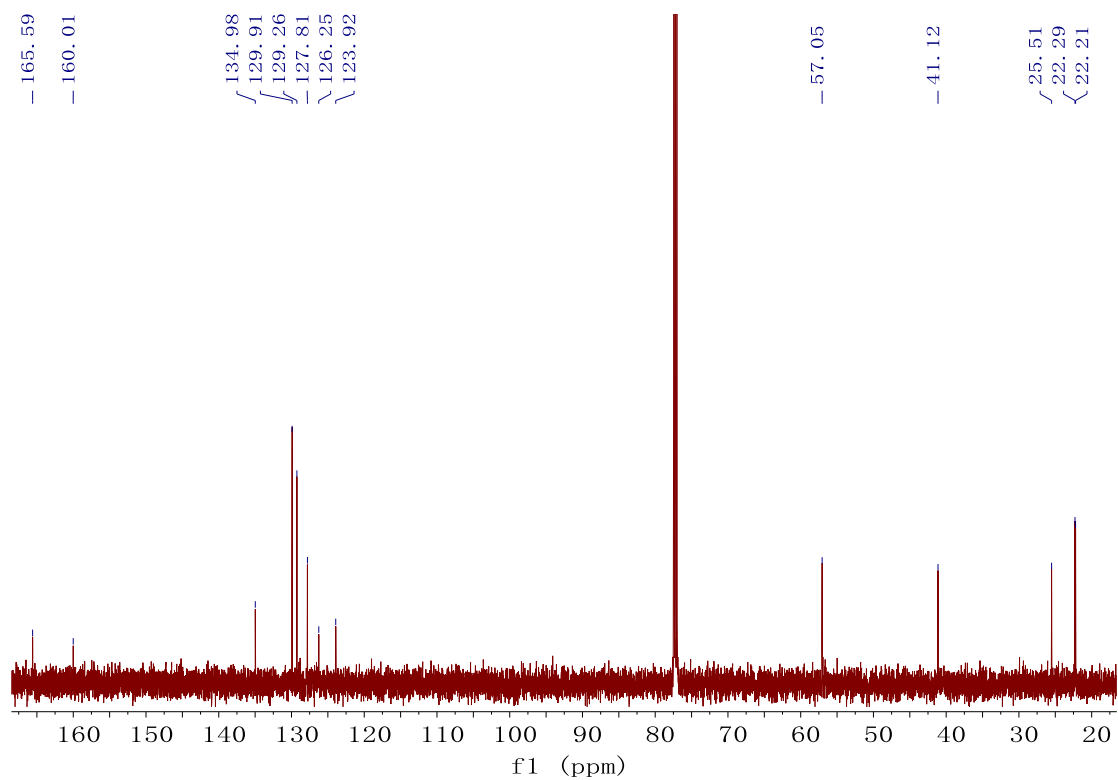


Fig. S46 ^{13}C NMR (150 MHz) spectrum of (Z)-3-benzyl-6-isobutylidene DKP (**6**, in CDCl_3 , 150 MHz).

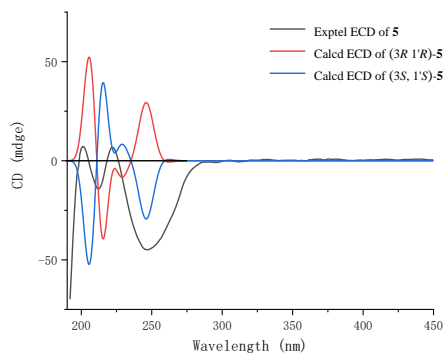


Fig. S47 Experimental and calculated ECD spectra of (3*R*, 1'*R*)-**5** and (3*S*, 1'*S*)-**5** (in MeOH).

Table S1 Annotation of the main functional gene in the *alc* cluster

| identifer | size ^a | Protein homolog and origin | ID/SM (%) | Origin (protein ID) | Origin (strain) |
|--------------|-------------------|---------------------------------------|-----------|---------------------|--------------------------------|
| <i>orf-1</i> | 255 | DJ-1/PfpI family protein | 82/100 | WP_214921736.1 | <i>Streptomyces</i> sp. ISL-98 |
| <i>alc C</i> | 248 | albonoursin biosynthesis protein AlbC | 55/94 | AAN07909.1 | <i>Streptomyces noursei</i> |
| <i>alc B</i> | 102 | albonoursin biosynthesis protein AlbB | 67/100 | WP_205366763.1 | <i>Streptomyces noursei</i> |
| <i>alc A</i> | 189 | albonoursin biosynthesis protein AlbA | 64/93 | AAN07907.1 | <i>Streptomyces noursei</i> |
| <i>orf+1</i> | 467 | glutamine synthetase | 83/100 | WP_280697006.1 | Kitasatospora sp. GP82 |

^aSize in units of amino acids (aa).