Electronic Supplementary Information (ESI)

# Naphthylisoindolinone Alkaloids: The First Ring-Contracted Naphthylisoquinolines, from the Tropical Liana *Ancistrocladus abbreviatus*, with Cytotoxic Activity

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‖ Deceased on January 14, 2014.

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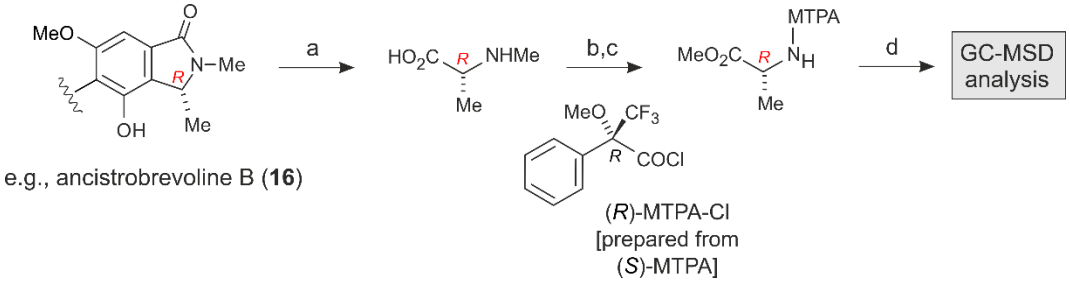
## 5. Ancistrobrevoline D (17)

## 1. General Procedure for Oxidative Degradation of Naphthylisoindolinone Alkaloids on an Analytical Scale




**Scheme:** Oxidative degradation of ancistrobrevoline B (**16**) to give readily analyzable *N*-methyl-D-alanine: (a) $RuCl_3$, $NaIO_4$; (b) $SOCl_2$, MeOH; (c) (*R*)-MTPA-Cl (Mosher's chloride); (d) stereoanalysis of the Mosher derivative of the methyl ester of *N*-methyl-D-alanine by GC-MSD.

The naphthylisoindolinone alkaloids ancistrobrevolines A-D (**14**-**17**) were subjected to a ruthenium(VIII)-mediated periodate degradation following a miniaturized procedure (see Scheme) as described earlier (G. Bringmann, R. God and M. Schäffer, *Phytochemistry*, 1996, **43**, 1393-1403). with subsequent derivatization of the resulting amino acid *N*-methylalanine with $CH_3OH$/HCl and (*R*)-α-trifluoromethylphenyl-acetylchloride [(*R*)-MTPA-Cl, prepared from (*S*)-MTPA]. The absolute configurations of **14**-**17** at the stereogenic center at C-1 were assigned by gas chromatography (GC) on a dimethylpolysiloxane-coated capillary column coupled to a mass-selective detector (MSD) and comparison with the corresponding derivatives of the authentic amino acids *N*-methyl-D-alanine and *N*-methyl-L-alanine.

Reactions were performed in 2.5 mL Wheaton screw-cap vials. The pure ancistrobrevolines (ca. 0.5 mg each) and 0.1 mg of $RuCl_3 \cdot H_2O$ (as catalyst) were added with stirring to a two-phase mixture consisting of MeCN (100 μL), $CCl_4$ (100 μL), and 0.1 M sodium phosphate buffer (pH 6.0) (200 μL) at room temperature. Over a period of 60 min, 26 mg of $NaIO_4$ were added in several portions and the mixture was allowed to stir at room temperature for another 1.5 h. For extraction of the resulting *N*-methylalanine, $H_2O$ (700 μL) were added and after a short period of additional stirring (10 min), the aqueous phase was separated, washed (× 2) with 300 μL portions of $CHCl_3$ and then lyophilized. The residue was extracted with dry MeOH (1.5 mL) followed by separation of the insoluble inorganic salts by centrifugation, providing a methanolic solution of the resulting amino acids, which was submitted to esterification. For this purpose, freshly

distilled $SOCl_2$ (70 μL) was added dropwise at 0°C with vigorously stirring. The mixture was then allowed to stand at room temperature for 12 h and another portion of $SOCl_2$ (70 μL) was added in the same manner. After 6 h standing at room temperature and evaporation of the solvent, the residue was suspended in dry $CH_2Cl_2$ (500 μL), with subsequent addition of 0.2 M (*R*)-MTPA-Cl in $CH_2Cl_2$ (100 μL) and up to 20 μL of $NEt_3$ until the mixture became alkaline. After stirring for 30 min, 1 μL of the resulting mixture was used directly for GC-MSD analysis on a non-polar fused silica capillary column (HP Ultra 2, 25 m ×0.32 mm × 0.52 μm). Helium was used as carrier gas with a column head pressure of 40 kPa. For chromatographic separation, an on-column injector maintained at 210 °C was used, with the column temperature program 100°C–160°C (30°C/min), then 160°C–190°C (1°C/min) and finally increased to 270°C (40°C/min).

Comparison with the respective derivatives of authentic amino acids of known configuration (from a test racemate routinely used for the analysis of usual naphthylisoquinoline alkaloids consisting of *N*-methylalanine, 3-aminobutyric acid, and *N*-methyl-3-aminobutyric acid) finally provided direct information about the absolute configuration at the chiral center at C1 of ancistrobrevoline A (**14**) (see Figure S11), ancistrobrevoline B (**15**) (see Figure S22), ancistrobrevoline C (**16**) (see Figure S33), and ancistrobrevoline D (**17**) (see Figure S44).

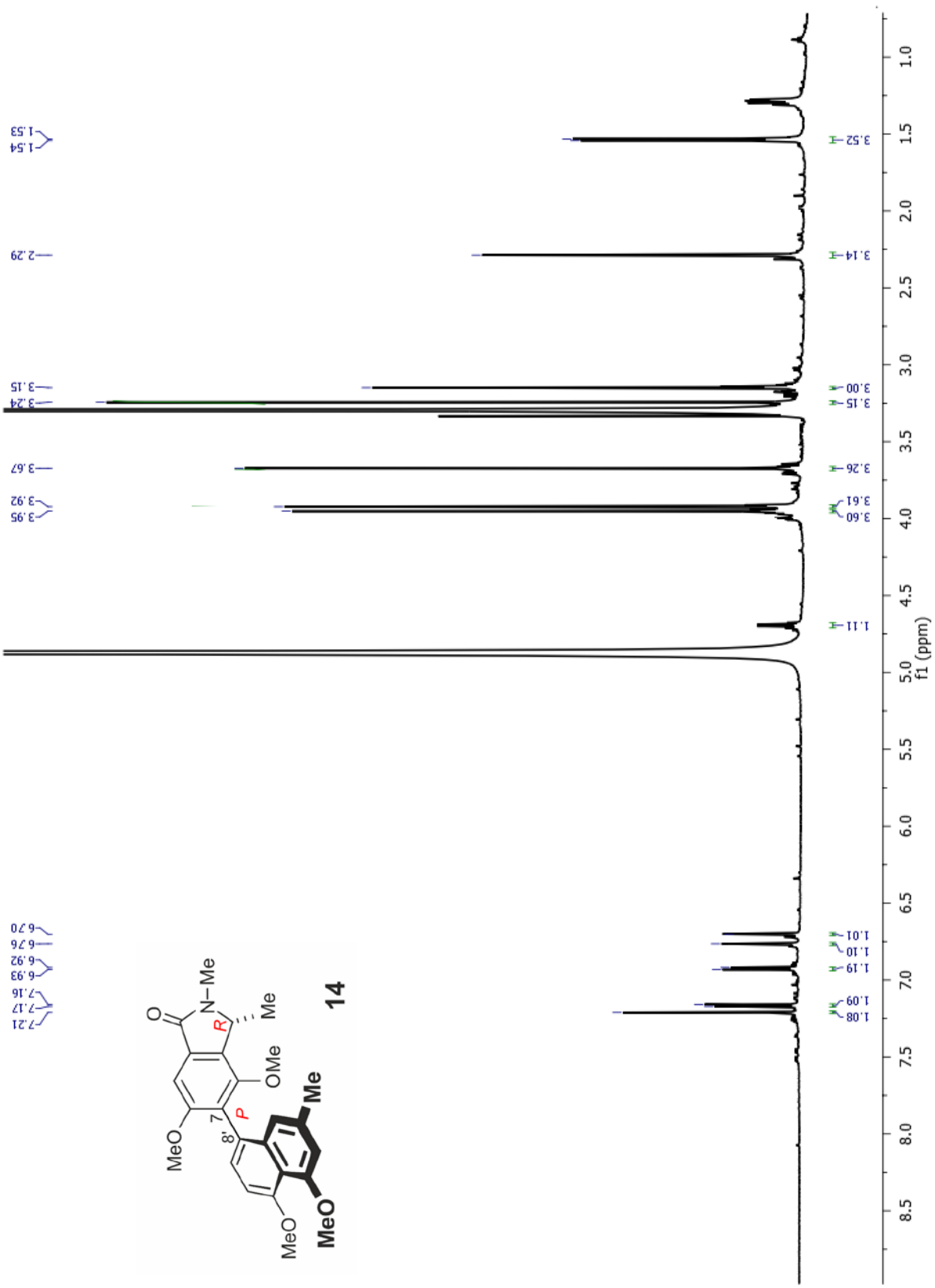



**Figure S1:** $^{1}$H NMR spectrum of ancistrobrevoline A (**14**) in methanol-$d_4$.

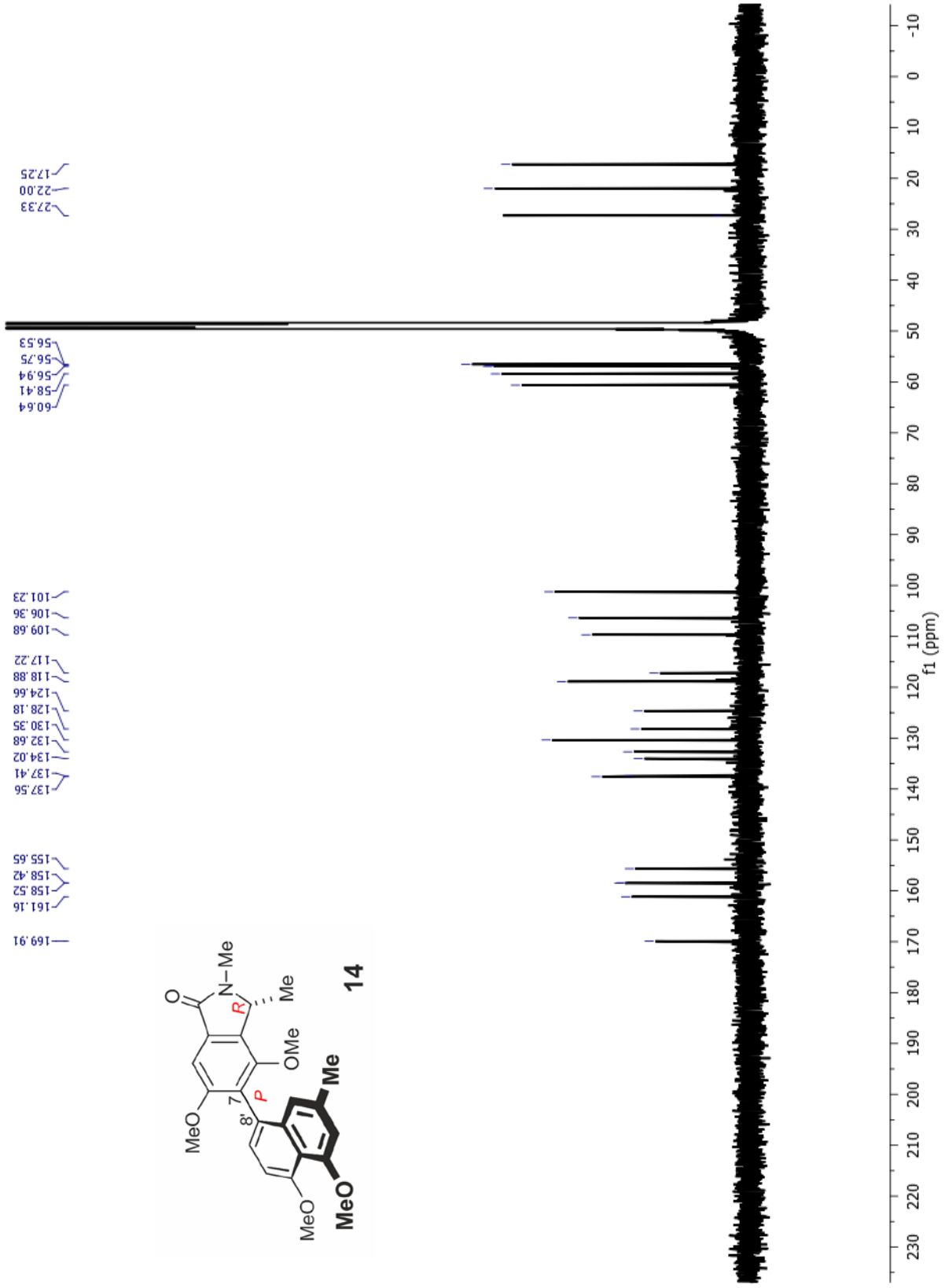



**Figure S2:** $^{13}$C NMR spectrum of ancistrobrevoline A (**14**) in methanol-$d_4$.

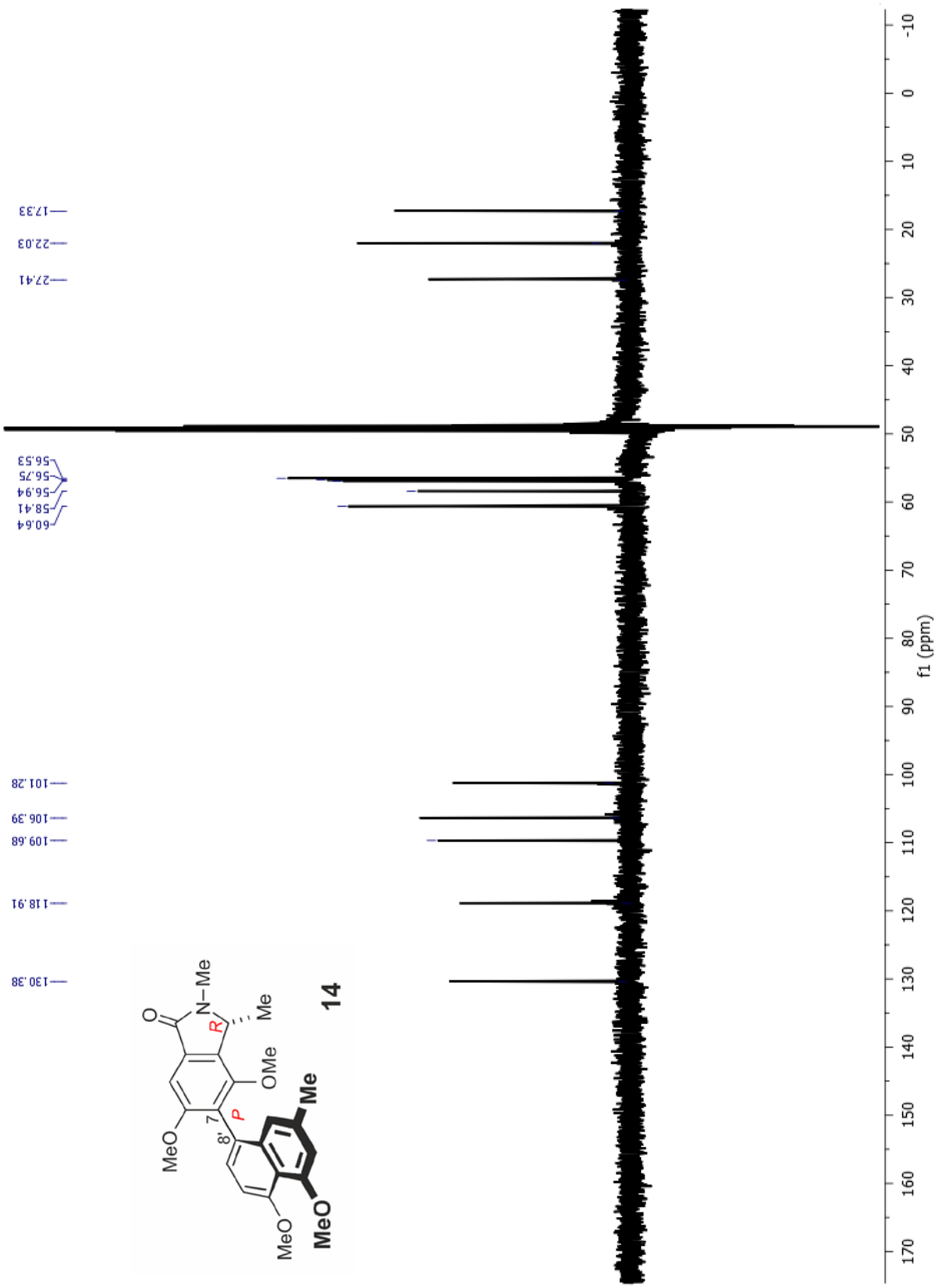



**Figure S3:** $^{13}$C DEPT 135 NMR spectrum ancistrobrevoline A (**14**) in methanol-$d_4$.

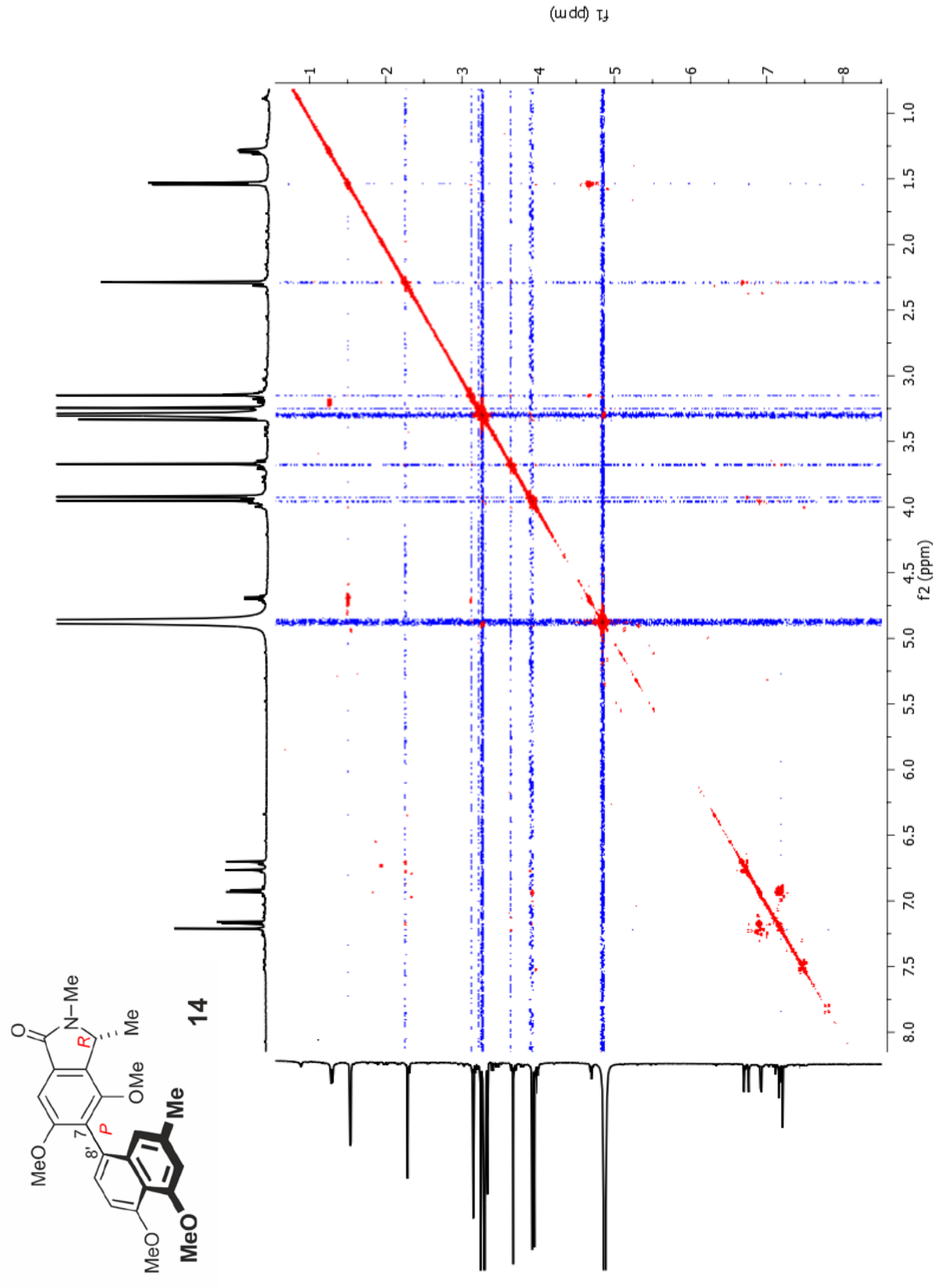



**Figure S4:** $^{1}$H-$^{1}$H COSY spectrum of ancistrobrevoline A (**14**) in methanol-$d_4$.

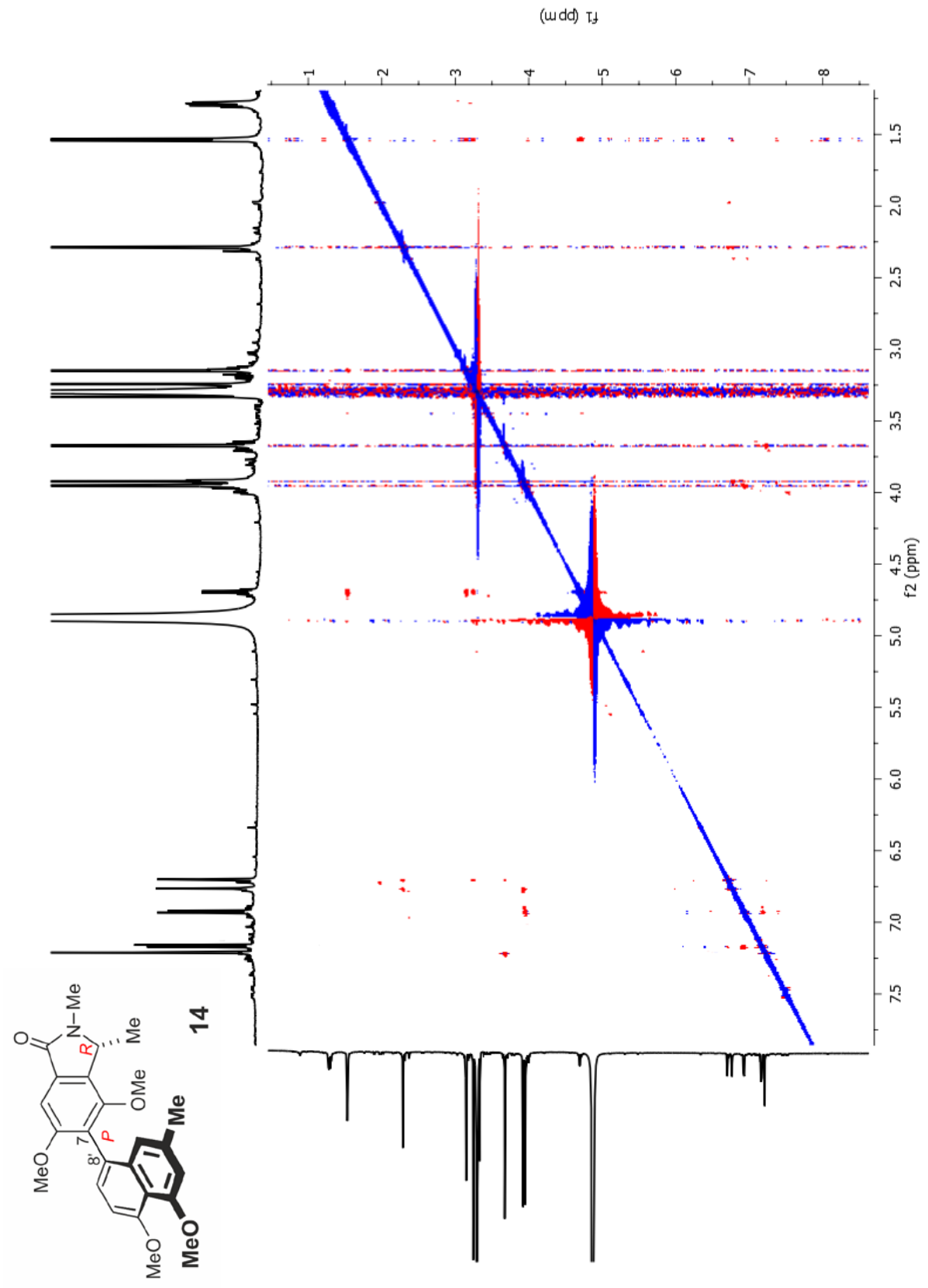



**Figure S5:** $^{1}$H-$^{1}$H NOESY spectrum of ancistrobrevoline A (**14**) in methanol-$d_4$.

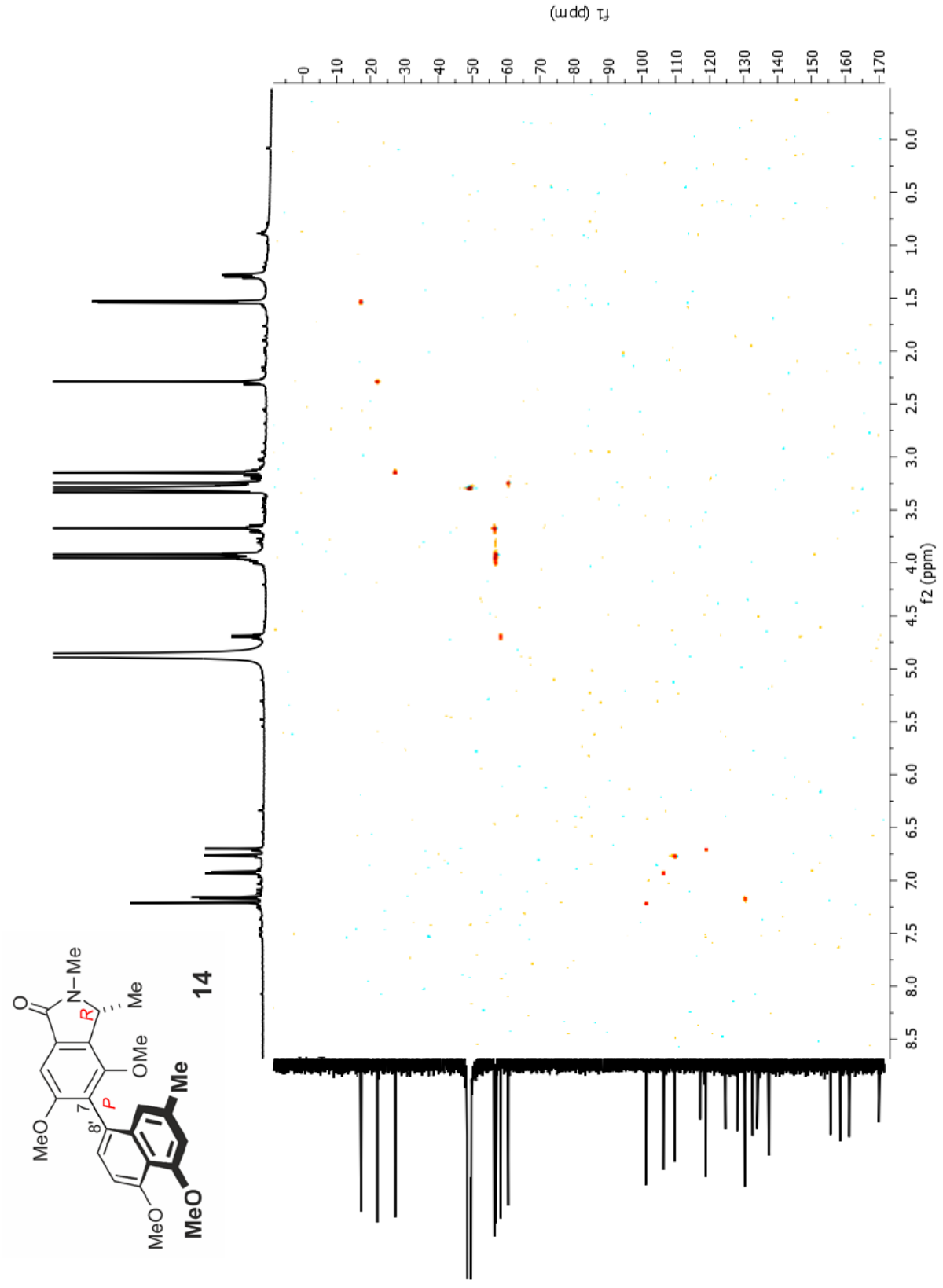



**Figure S6:** $^{1}$H-$^{13}$C HSQC spectrum of ancistrobrevoline (**14**) in methanol-$d_4$.

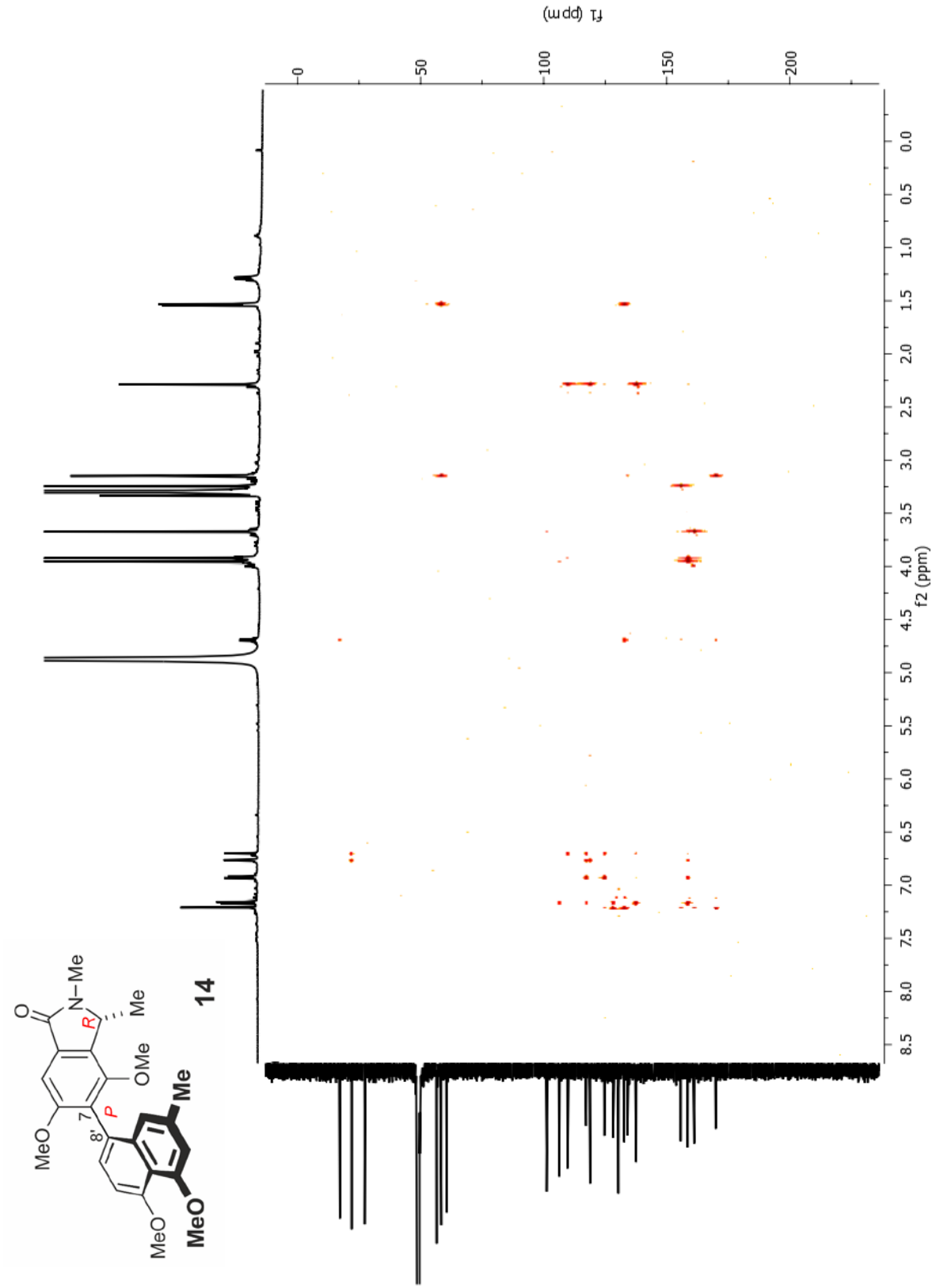



**Figure S7:** $^{1}$H-$^{13}$C HMBC spectrum of ancistrobrevoline (**14**) in methanol-$d_4$.

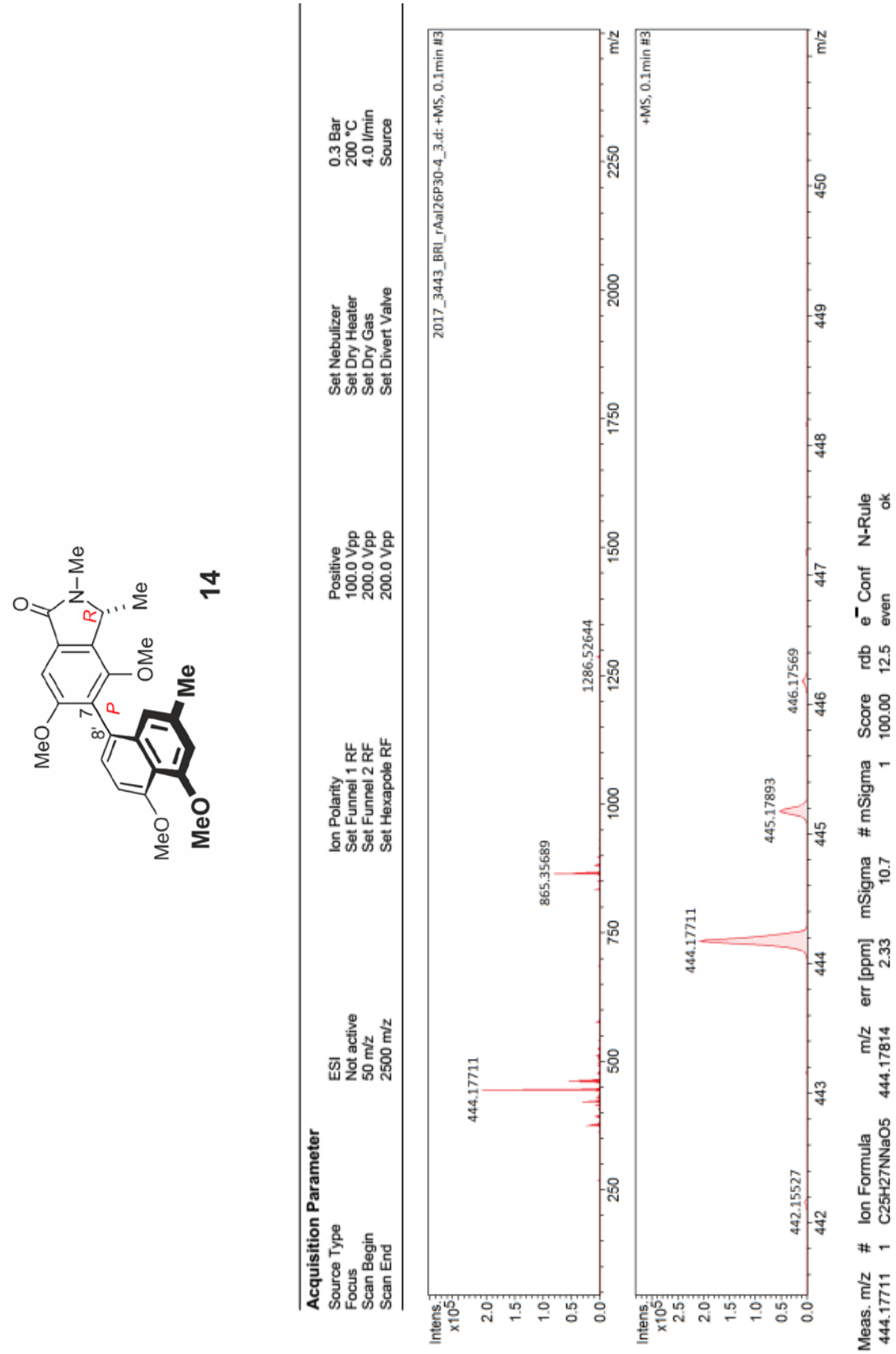
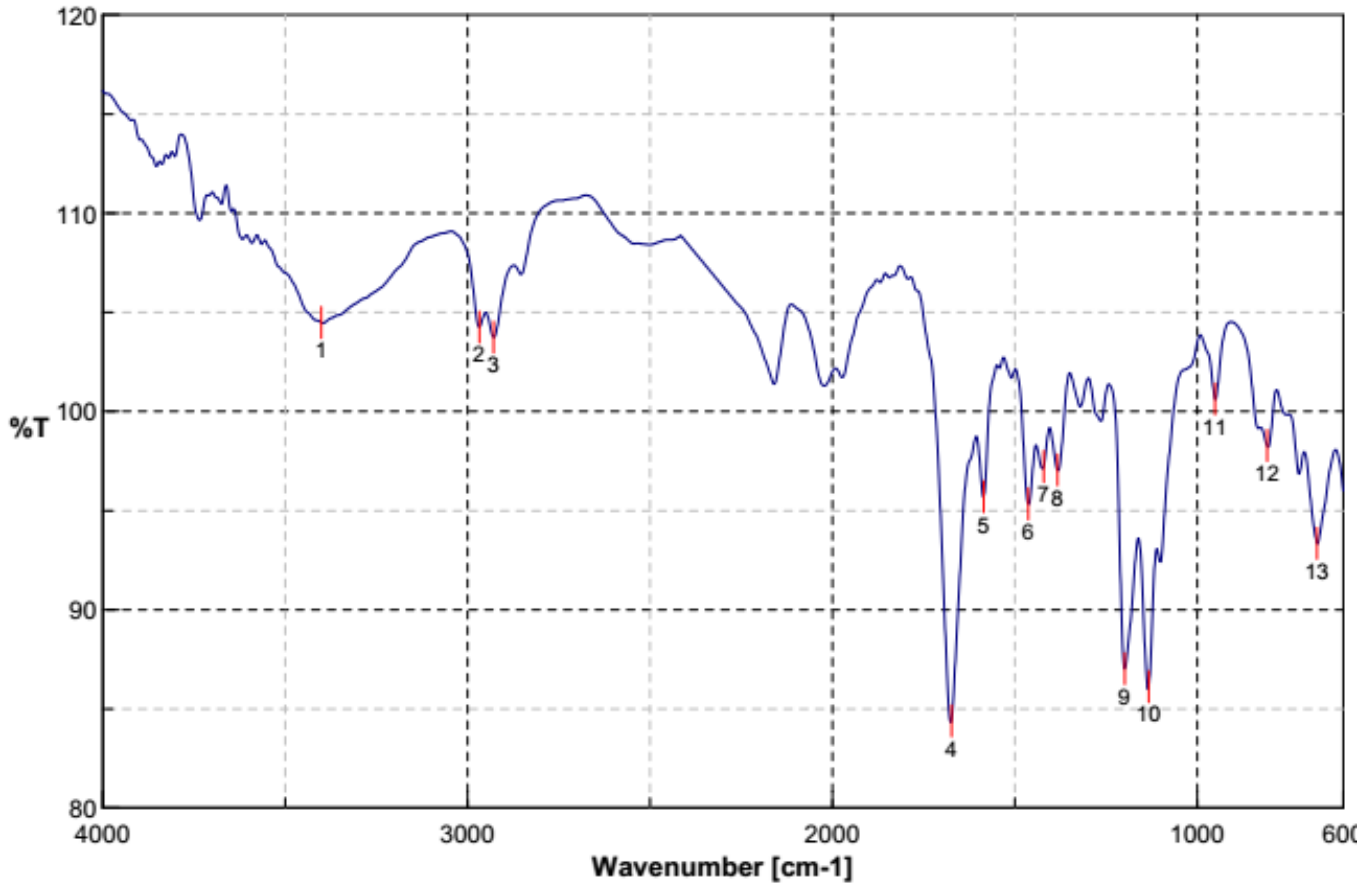



**Figure S8:** HR-ESI-MS spectrum of ancistrobrevoline A (**14**).

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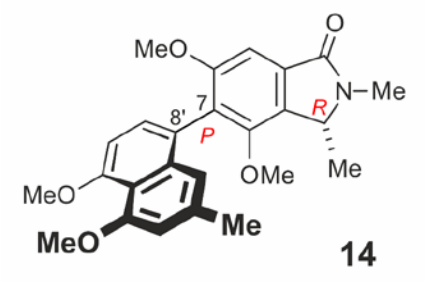


14

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| 4 | 1673.91 | 84.4025 | 5 | 1585.2 | 95.7079 | 6 | 1463.71 | 95.3412 |
| 7 | 1421.28 | 97.2293 | 8 | 1383.68 | 97.0615 | 9 | 1198.54 | 87.0345 |
| 10 | 1132.97 | 86.1198 | 11 | 951.698 | 100.63 | 12 | 808.992 | 98.2803 |
| 13 | 672.071 | 93.3576 | | | | | | |

**Figure S9:** IR spectrum of ancistrobrevoline A (**14**).

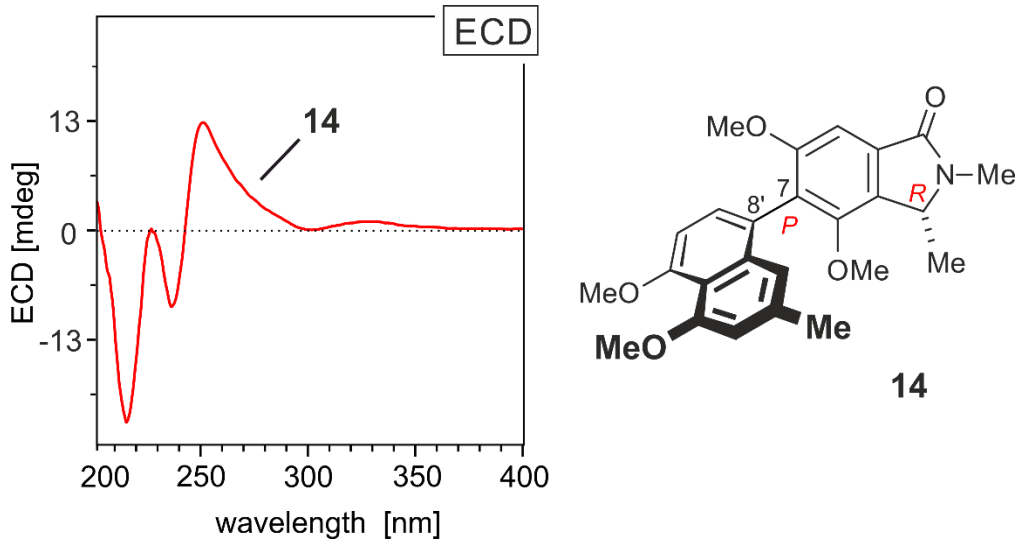



**Figure S10a:** ECD spectrum of ancistrobrevoline A (**14**).

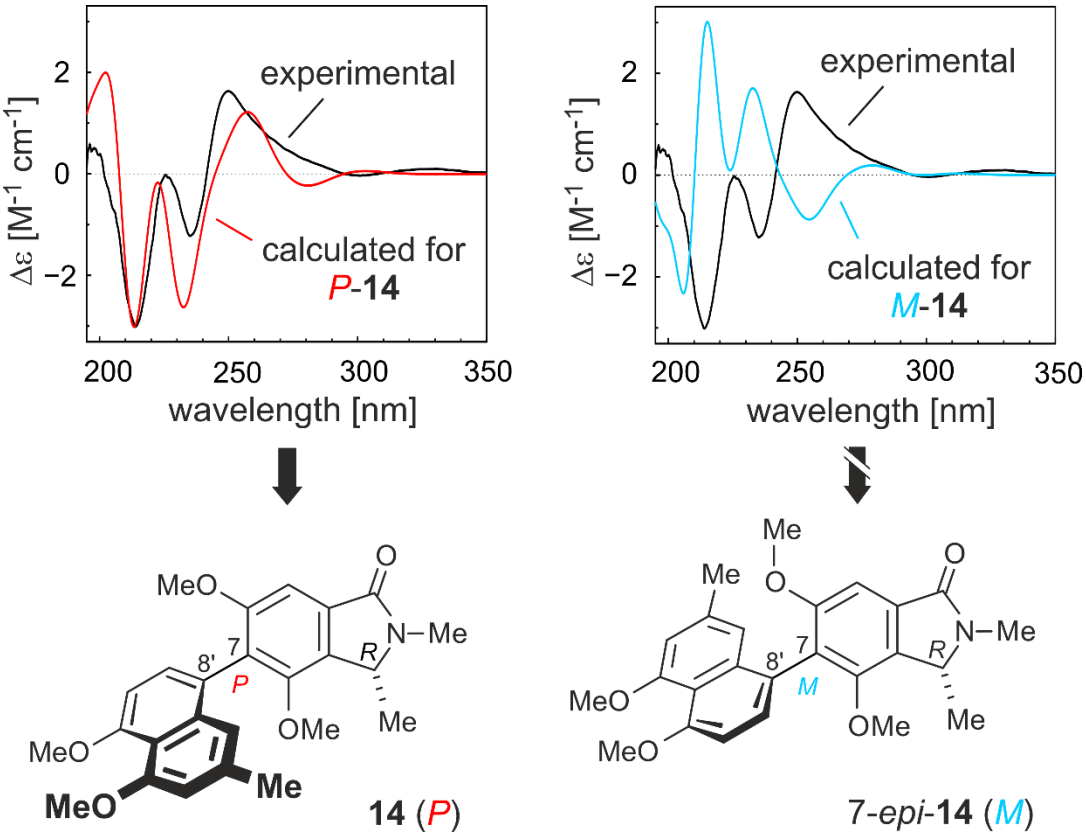
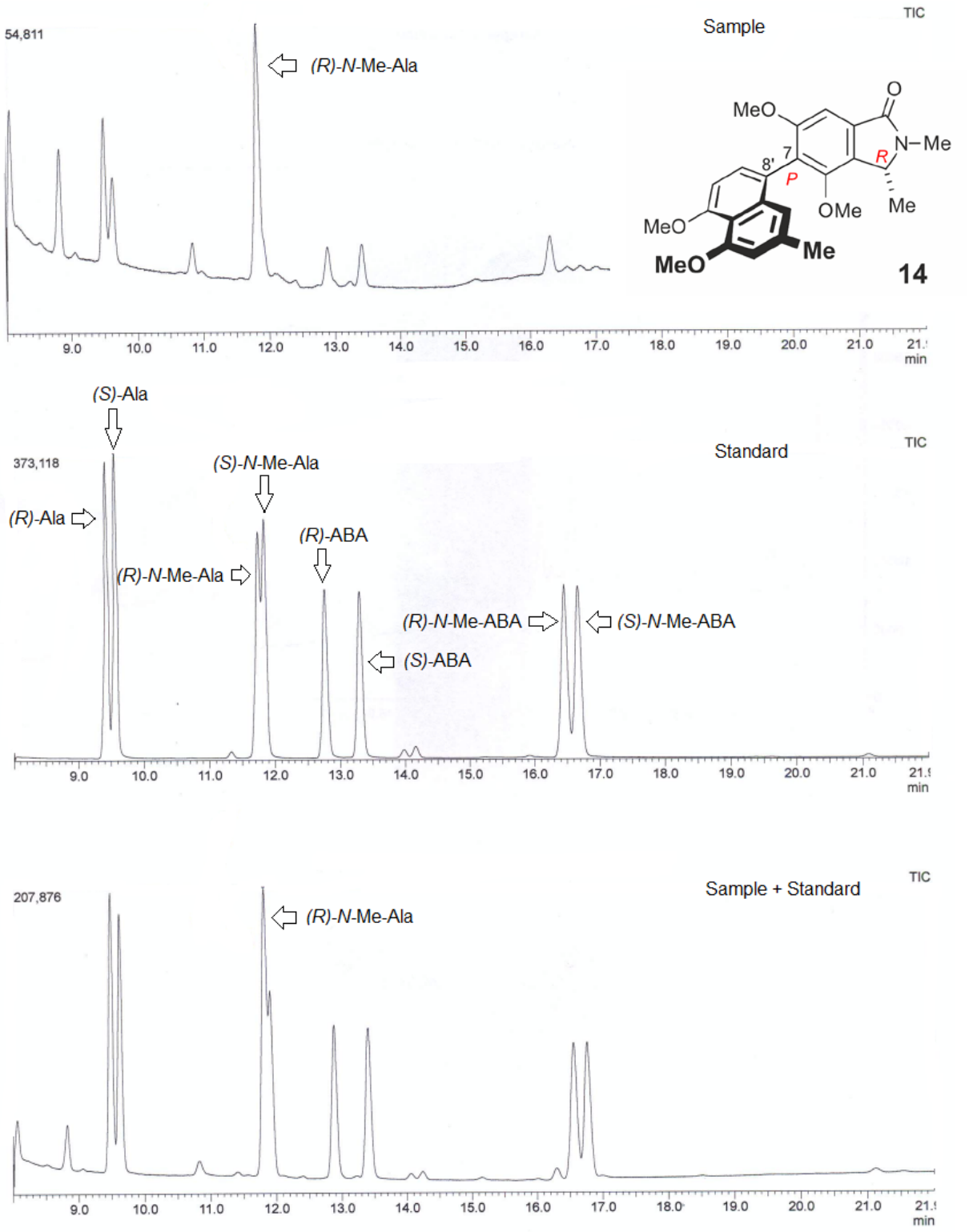



**Figure S10b:** Assignment of the absolute axial configuration of ancistrobrevoline A (**14**), by comparison of its experimental ECD spectrum with the spectra calculated for *P*-**14** and *M*-**14** by using TDωB97XD3/def2-TZVP//B3LYP-D3/def2-TZVP.




Ala = Alanine *N*-Me-Ala = *N*-Methylalanine

ABA = 3-Aminobutyric acid *N*-Me-ABA = *N*-Methyl-3-aminobutyric acid

**Figure S11:** Oxidative degradation of ancistrobrevoline A (**14**).

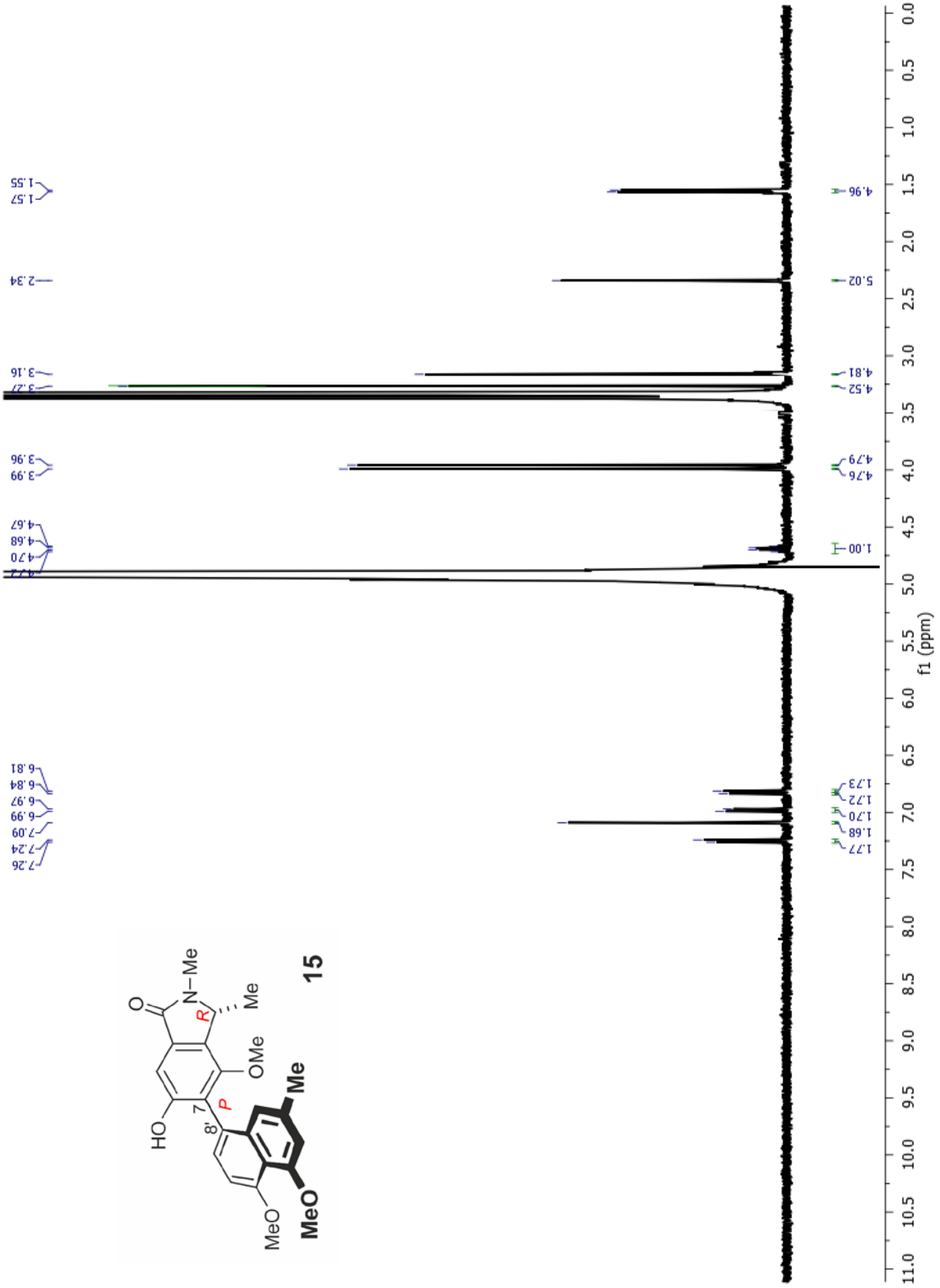



**Figure S12:** $^{1}$H NMR spectrum of ancistrobrevoline B (**15**) in methanol-$d_4$.

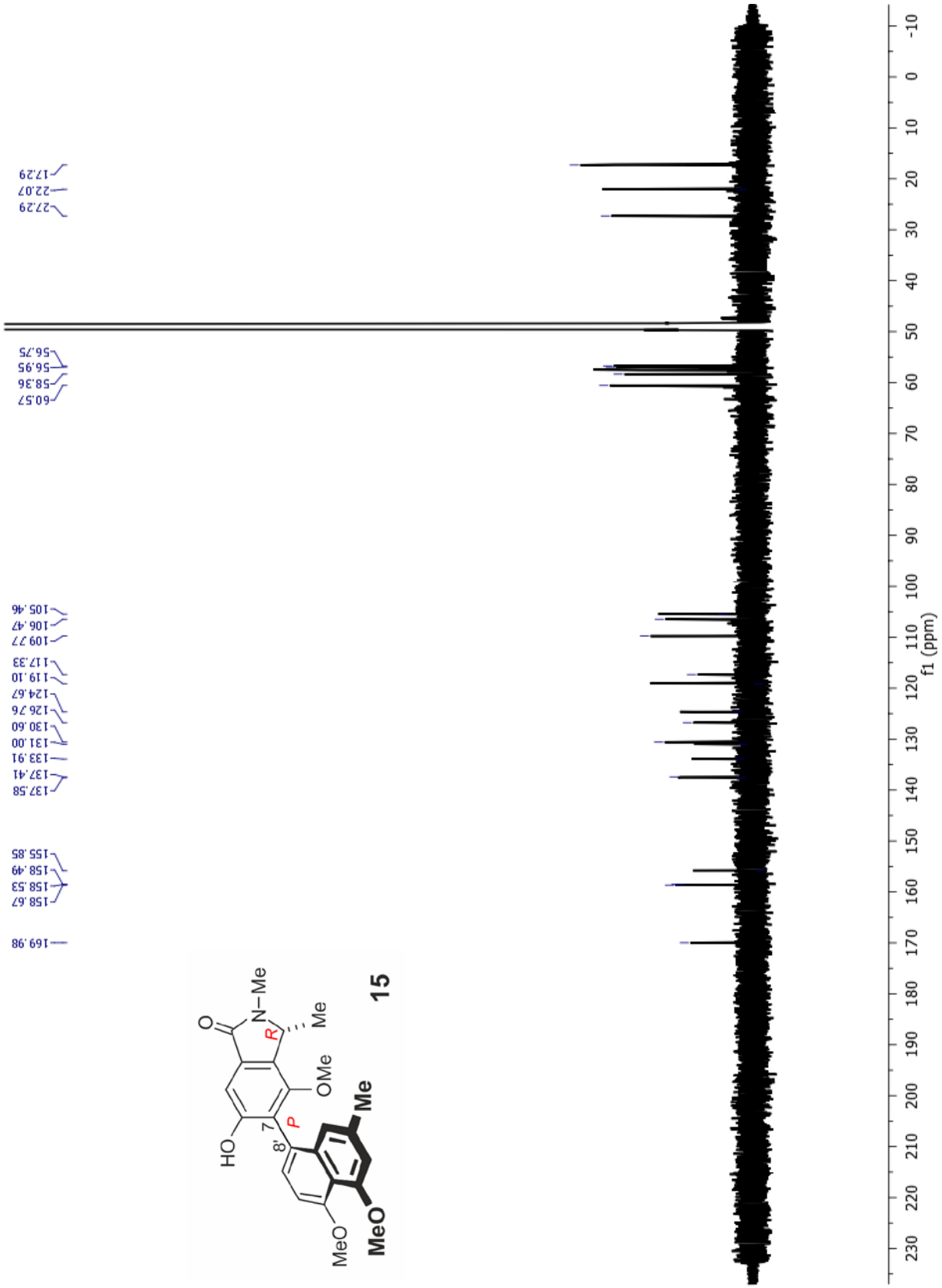



**Figure S13:** $^{13}$C NMR spectrum of ancistrobrevoline B (**15**) in methanol-$d_4$.

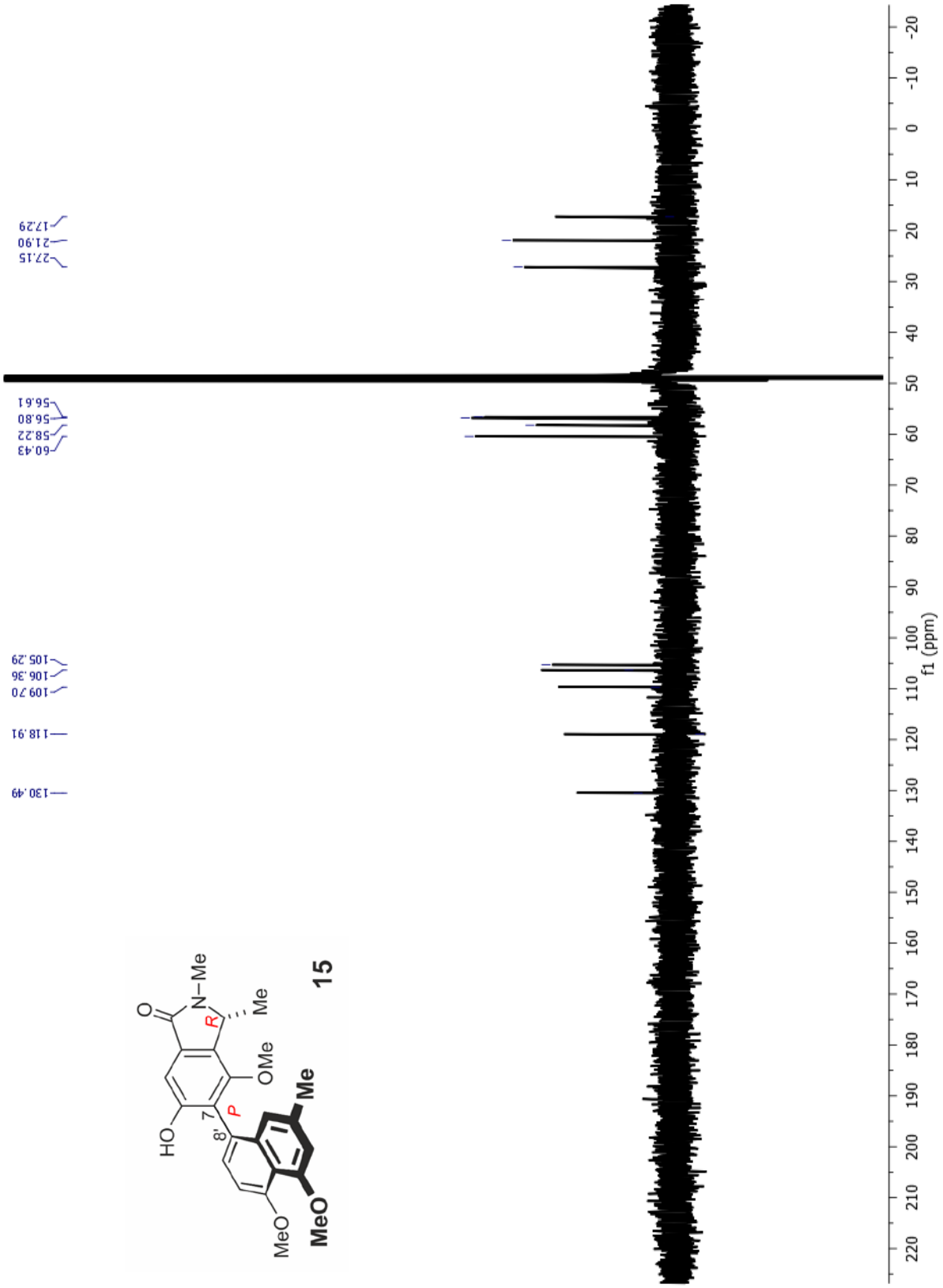



**Figure S14:** $^{13}$C DEPT 135 NMR spectrum ancistrobrevoline B (**15**) in methanol-$d_4$.

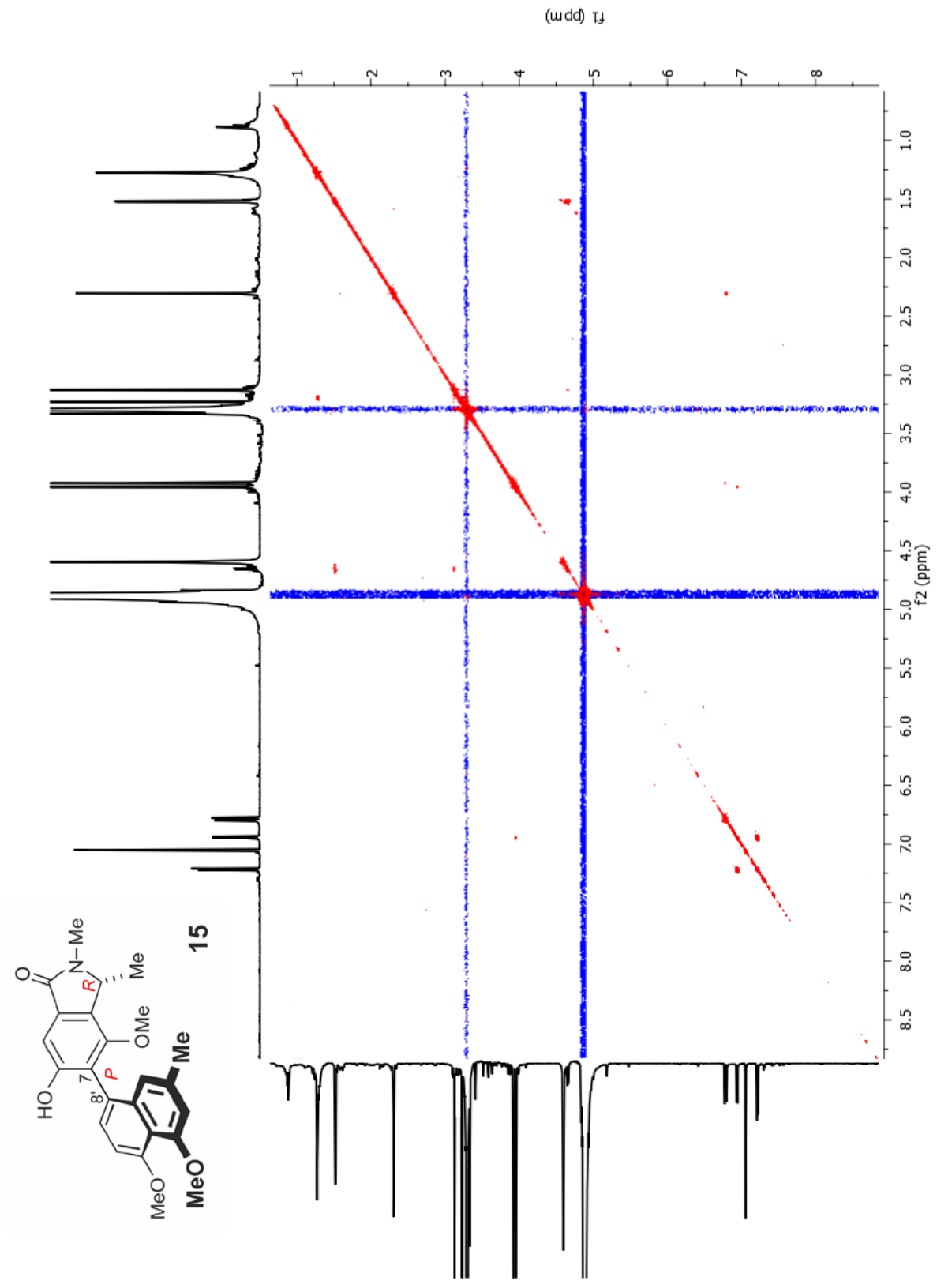



**Figure S15:** $^{1}$H-$^{1}$H COSY spectrum of ancistrobrevoline B (**15**) in methanol-$d_4$.

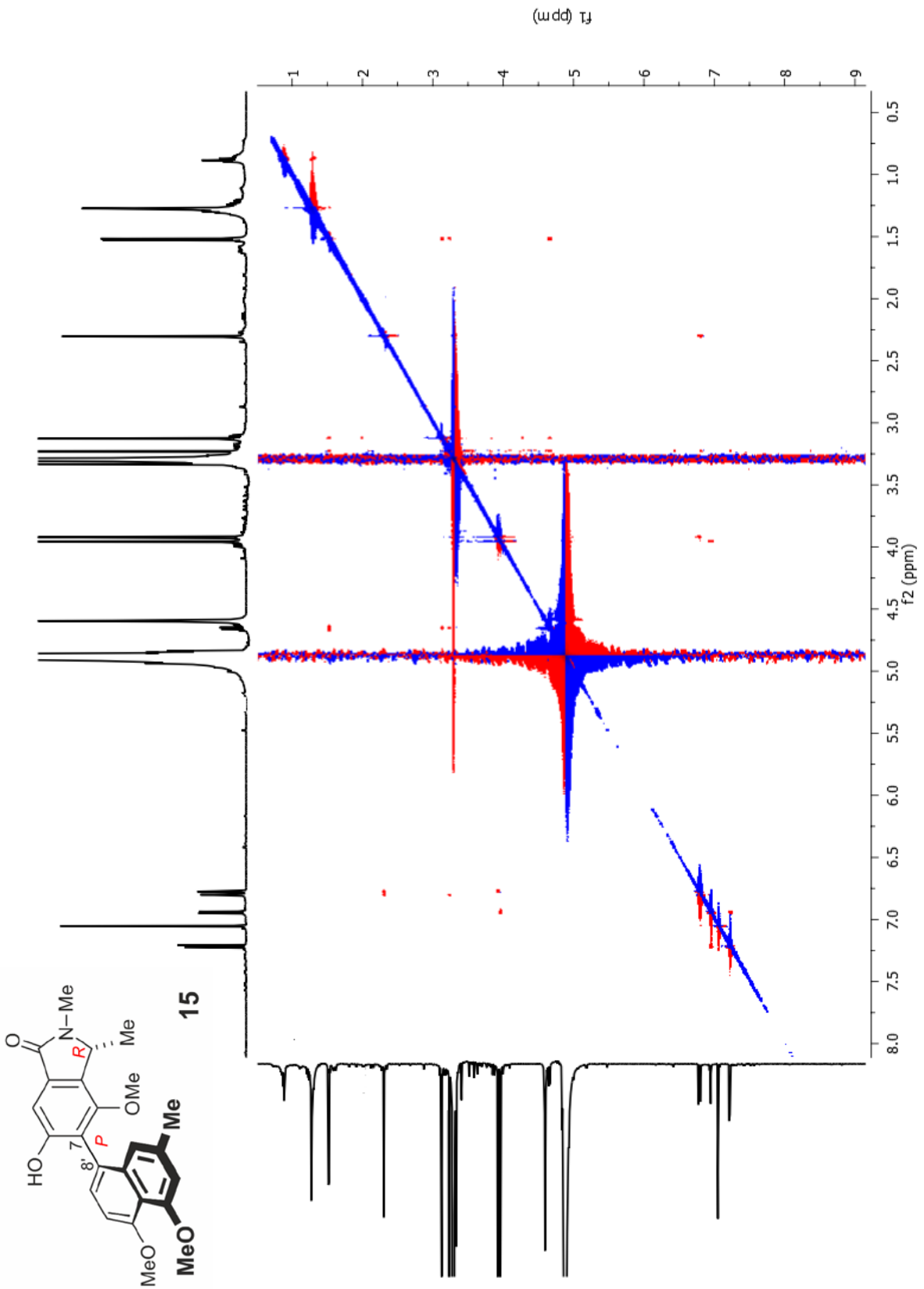



**Figure S16:** $^{1}$H-$^{1}$H NOESY spectrum of ancistrobrevoline B (**15**) in methanol-$d_4$.

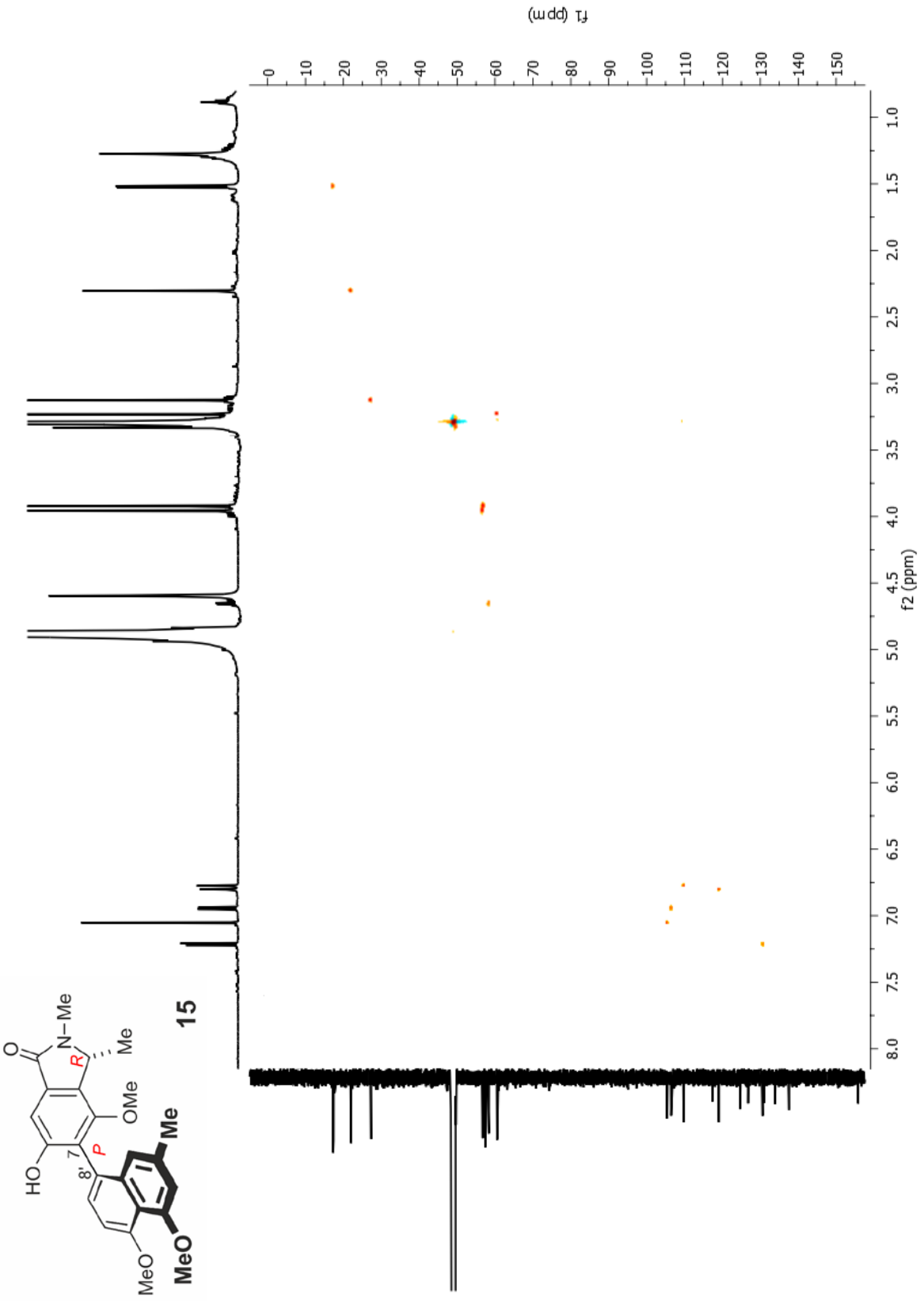



**Figure S17:** $^{1}$H-$^{13}$C HSQC spectrum of ancistrobrevoline B (**15**) in methanol-$d_4$.

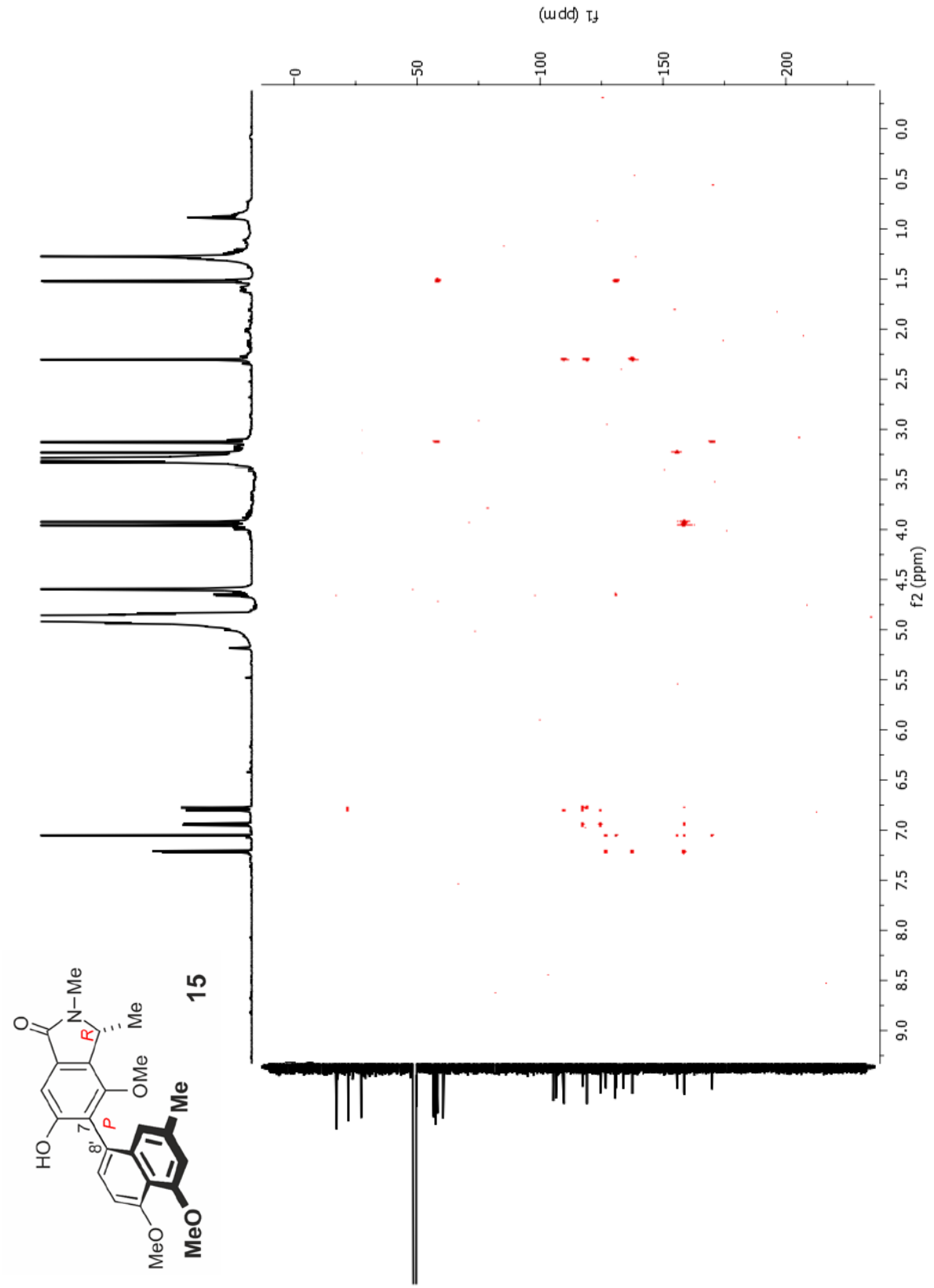



**Figure S18:** $^{1}$H-$^{13}$C HMBC spectrum of ancistrobrevoline B (**15**) in methanol-$d_4$.

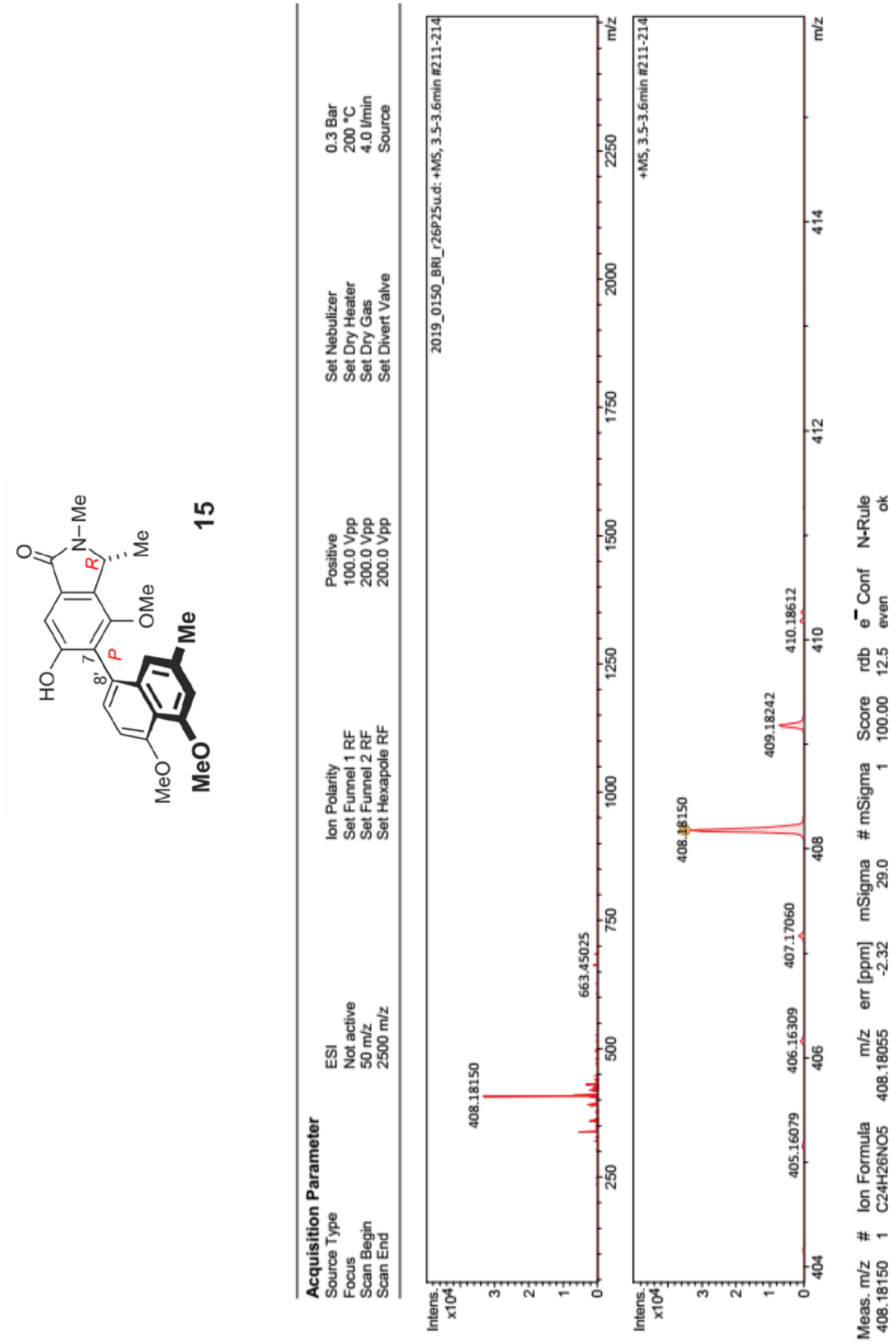
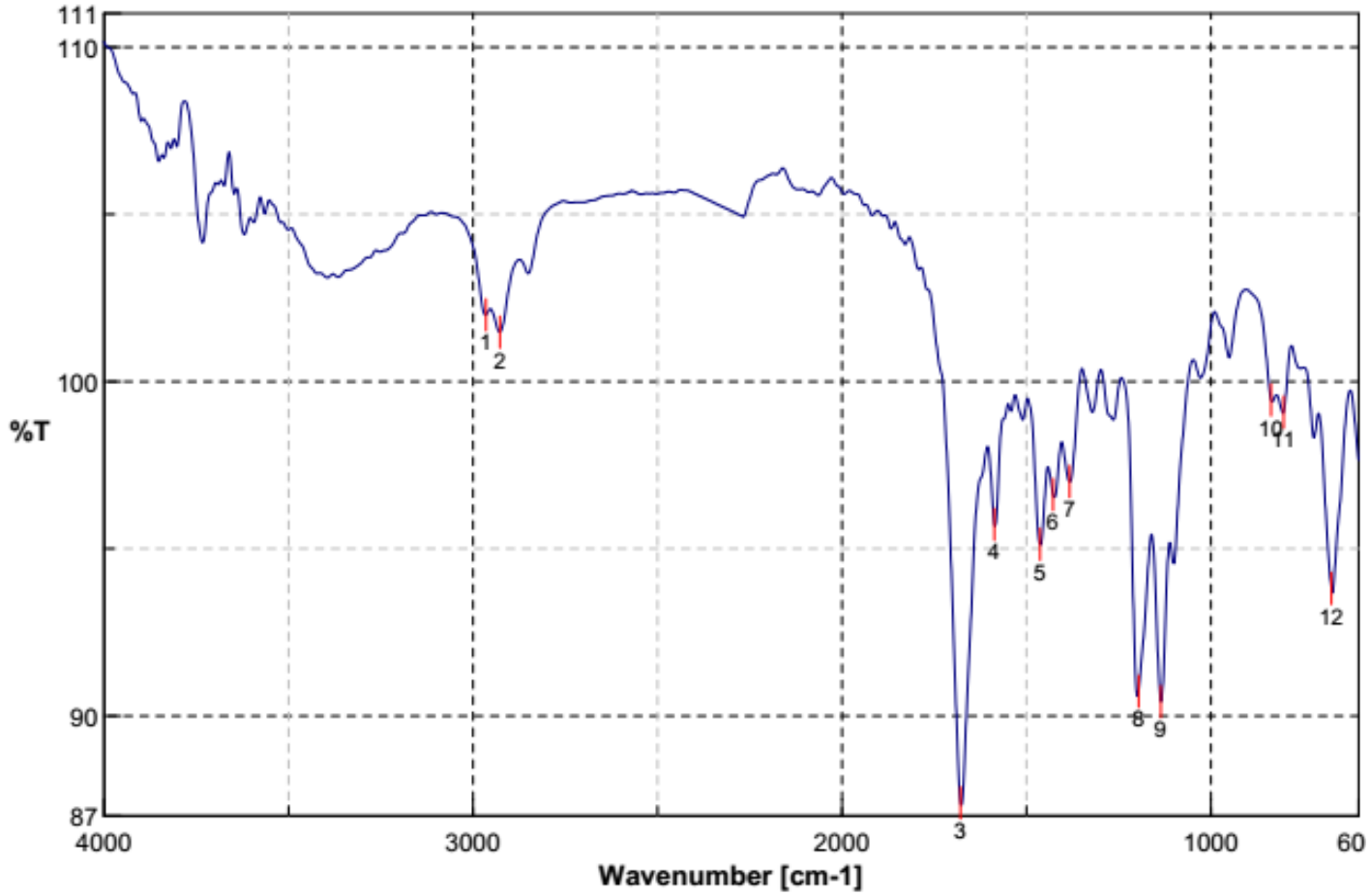



**Figure S19:** HR-ESI-MS spectrum of ancistrobrevoline B (**15**).

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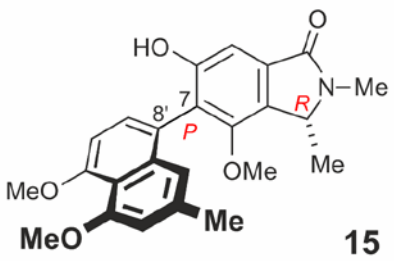


**15**

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| 4 | 1587.13 | 95.7282 | 5 | 1463.71 | 95.13 | 6 | 1428.03 | 96.6252 |
| 7 | 1383.68 | 97.0048 | 8 | 1196.61 | 90.7378 | 9 | 1135.87 | 90.4213 |
| 10 | 836.955 | 99.4485 | 11 | 803.206 | 99.0842 | 12 | 673.999 | 93.8082 |

**Figure S20:** IR spectrum of ancistrobrevoline B (**15**).

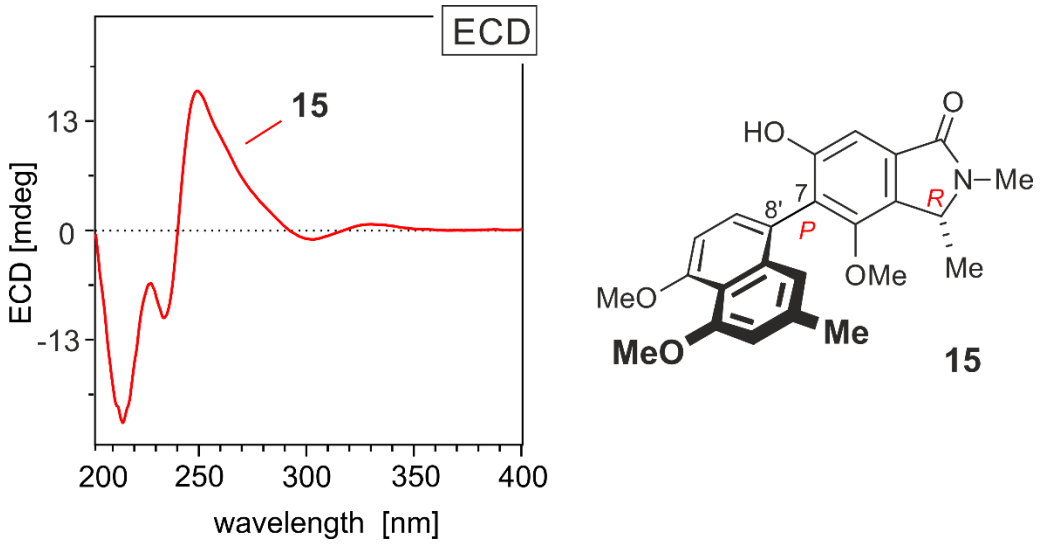



**Figure S21a:** ECD spectrum of ancistrobrevoline B (**15**).

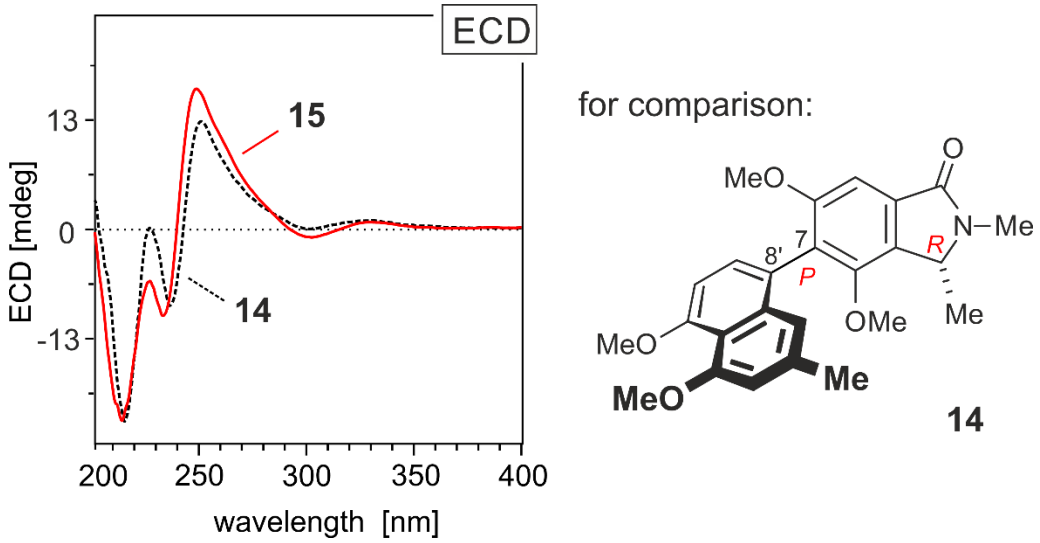
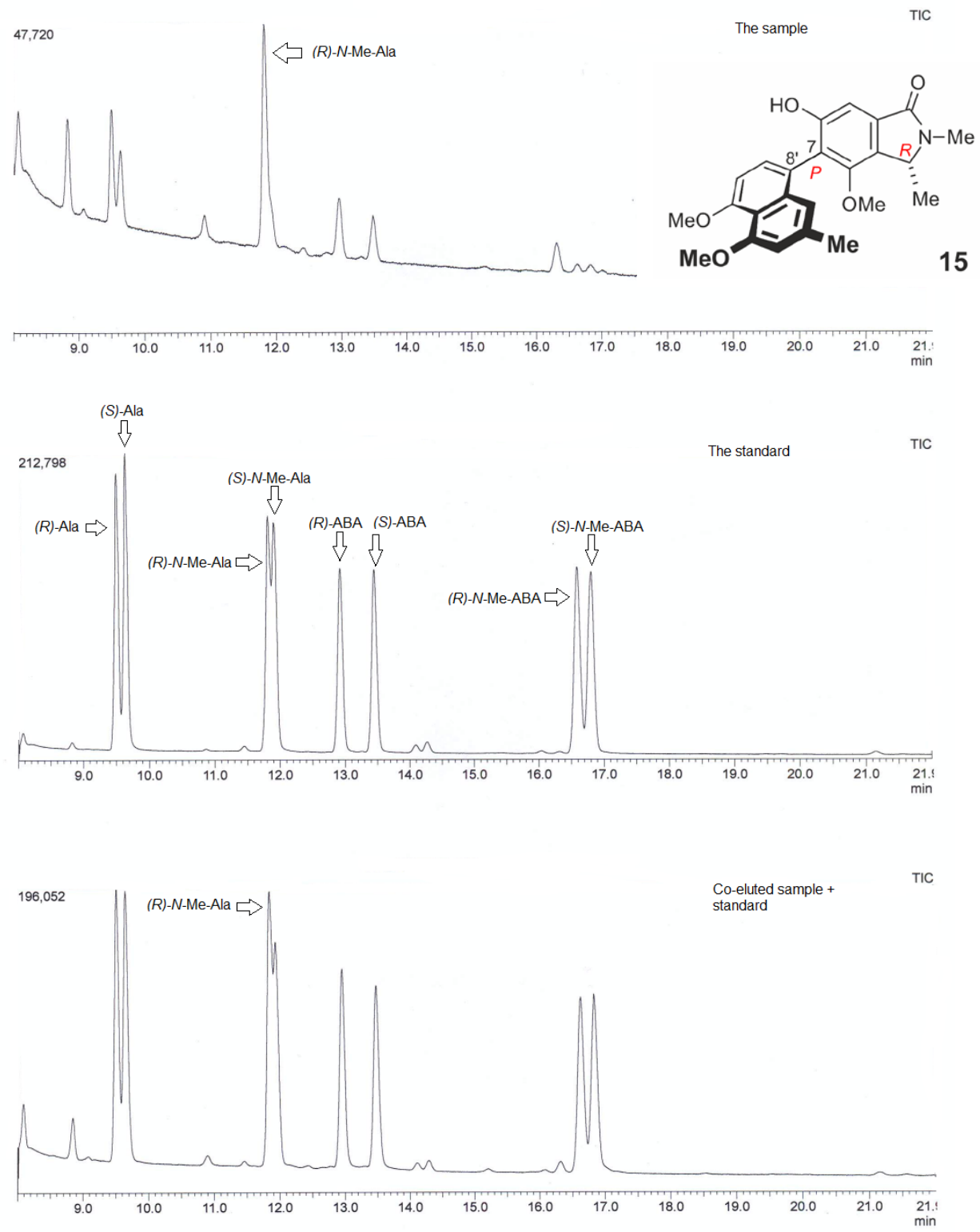



**Figure S21b:** Assignment of the absolute configuration of ancistrobrevoline B (**15**) by comparison of its ECD spectrum with that of ancistrobrevoline A (**14**).




Ala = Alanine *N*-Me-Ala = *N*-Methylalanine

ABA = 3-Aminobutyric acid *N*-Me-ABA = *N*-Methyl-3-aminobutyric acid

**Figure S22:** Oxidative degradation of ancistrobrevoline B (**15**).

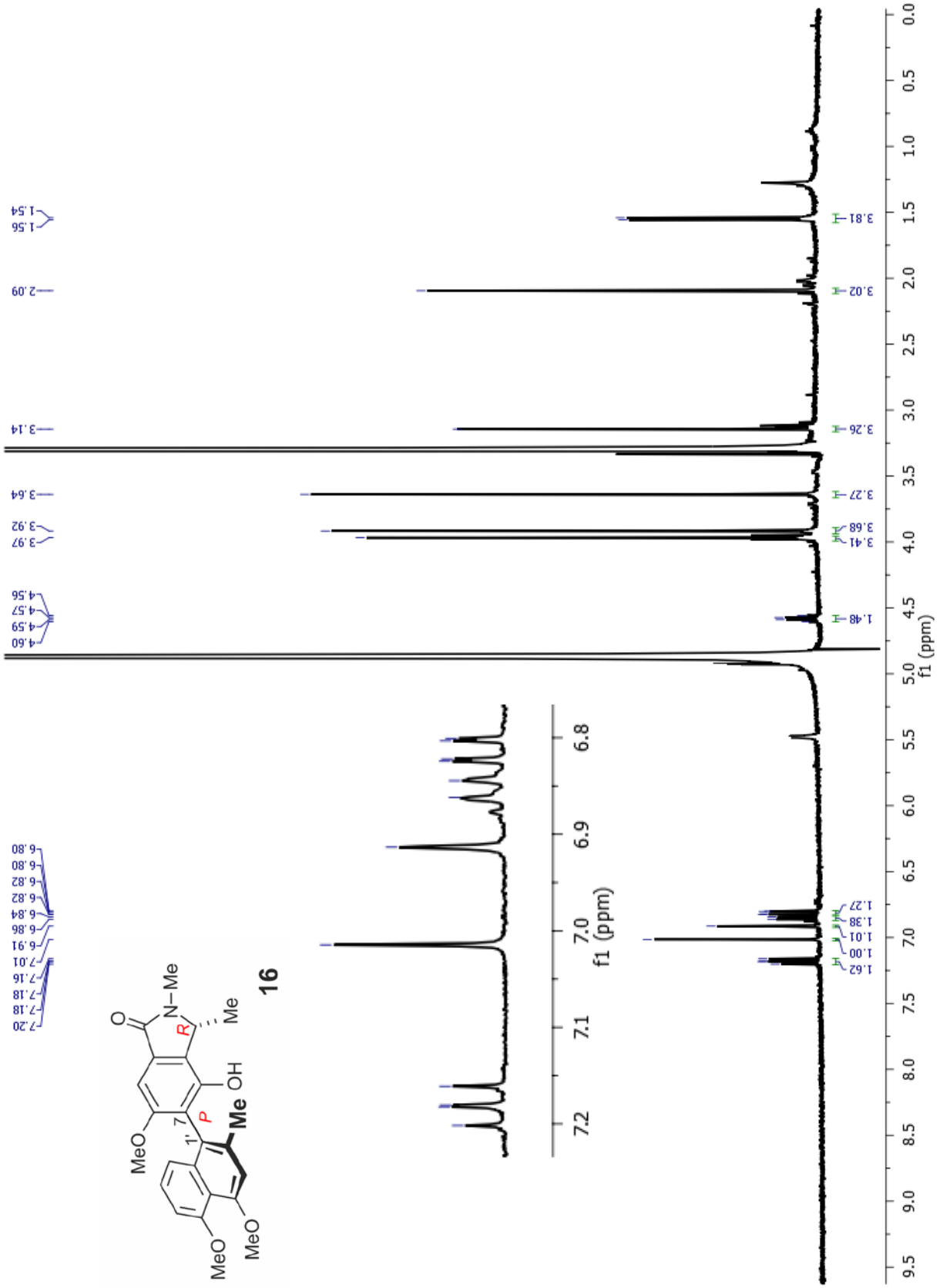



**Figure S23:** $^{1}$H NMR spectrum of ancistrobrevoline C (**16**) in methanol-$d_4$.

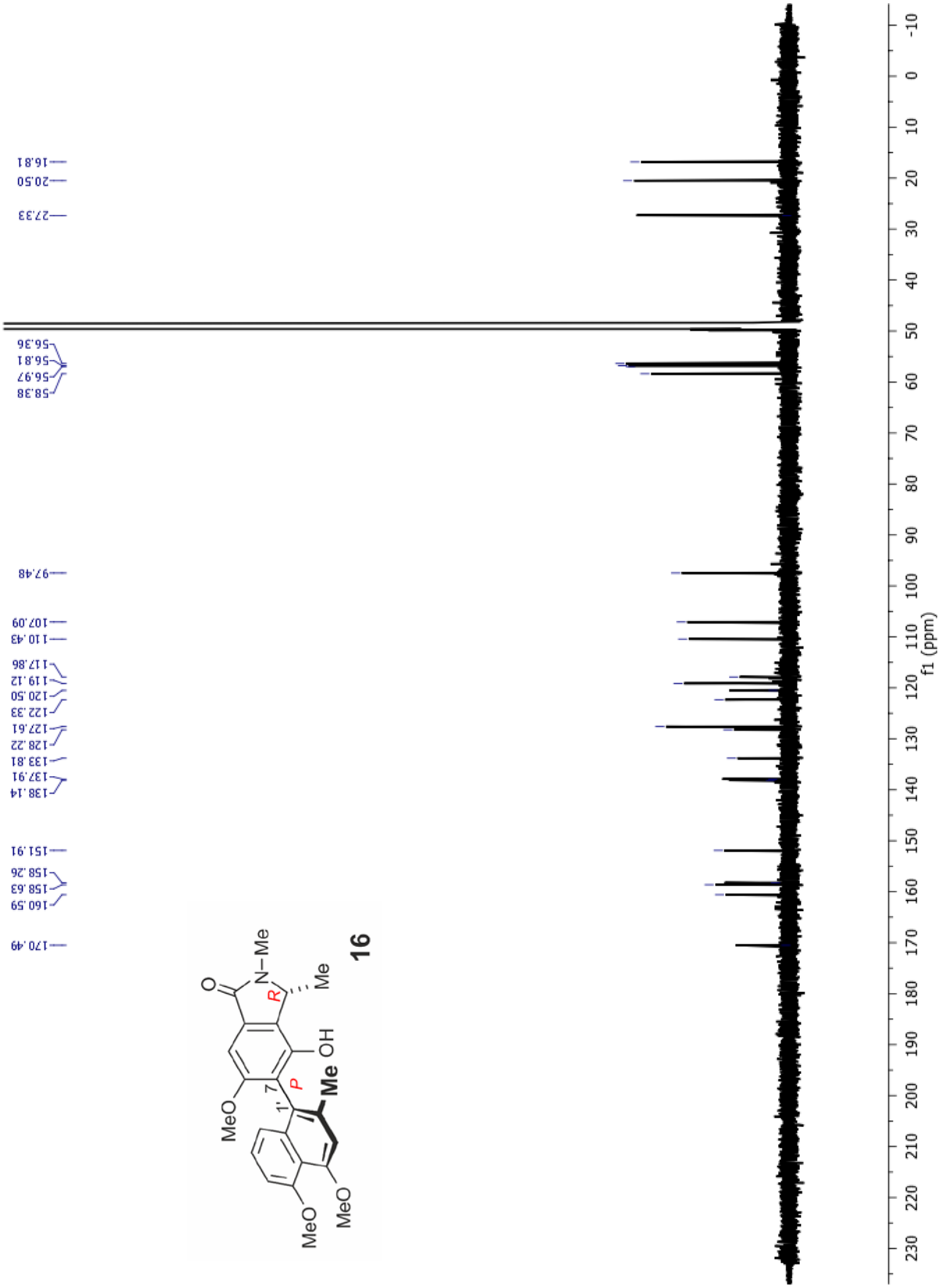



**Figure S24:** $^{13}$C NMR spectrum of ancistrobrevoline C (**16**) in methanol-$d_4$.

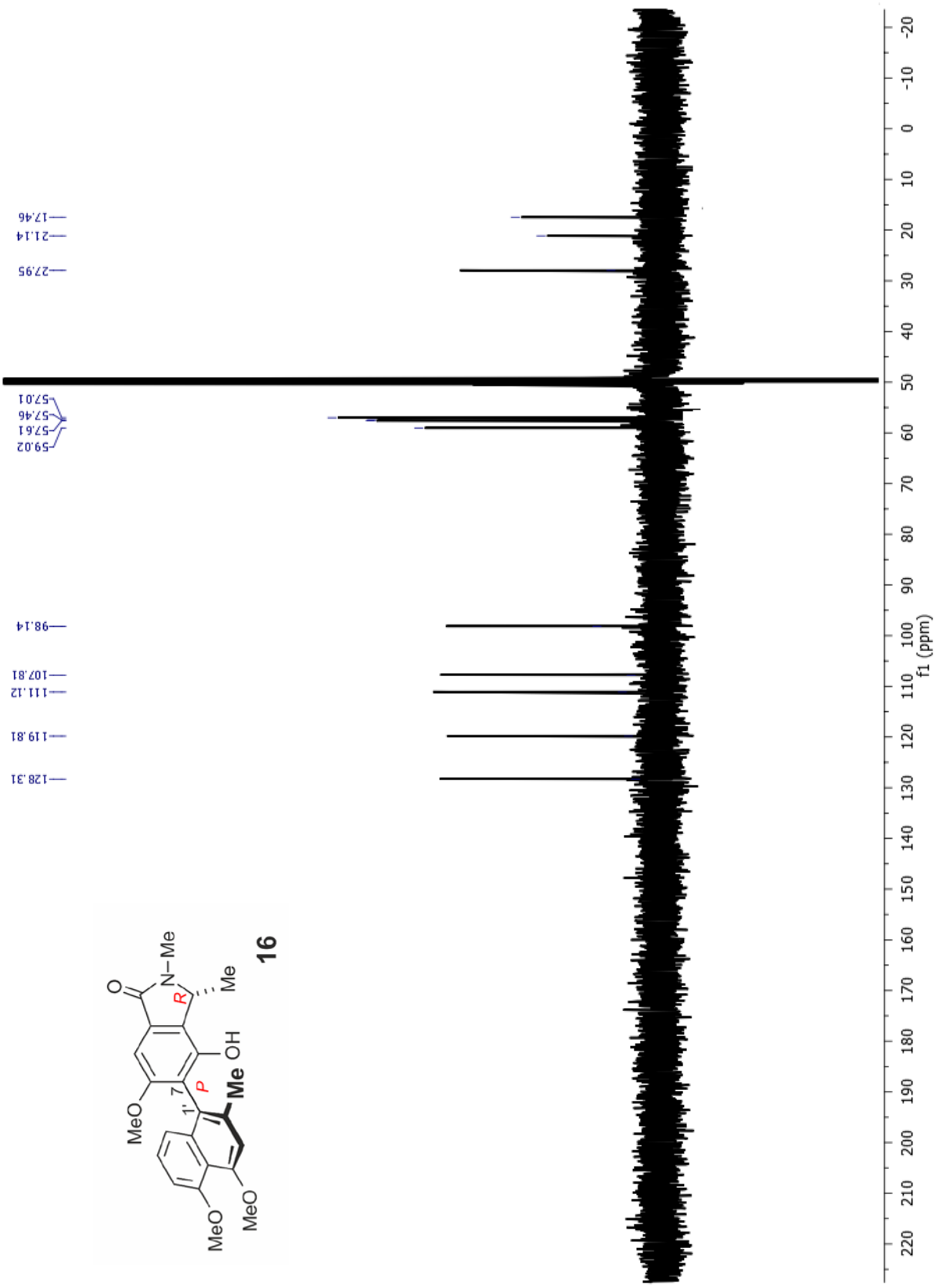



**Figure S25:** $^{13}$C DEPT 135 NMR spectrum ancistrobrevoline C (**16**) in methanol-$d_4$.

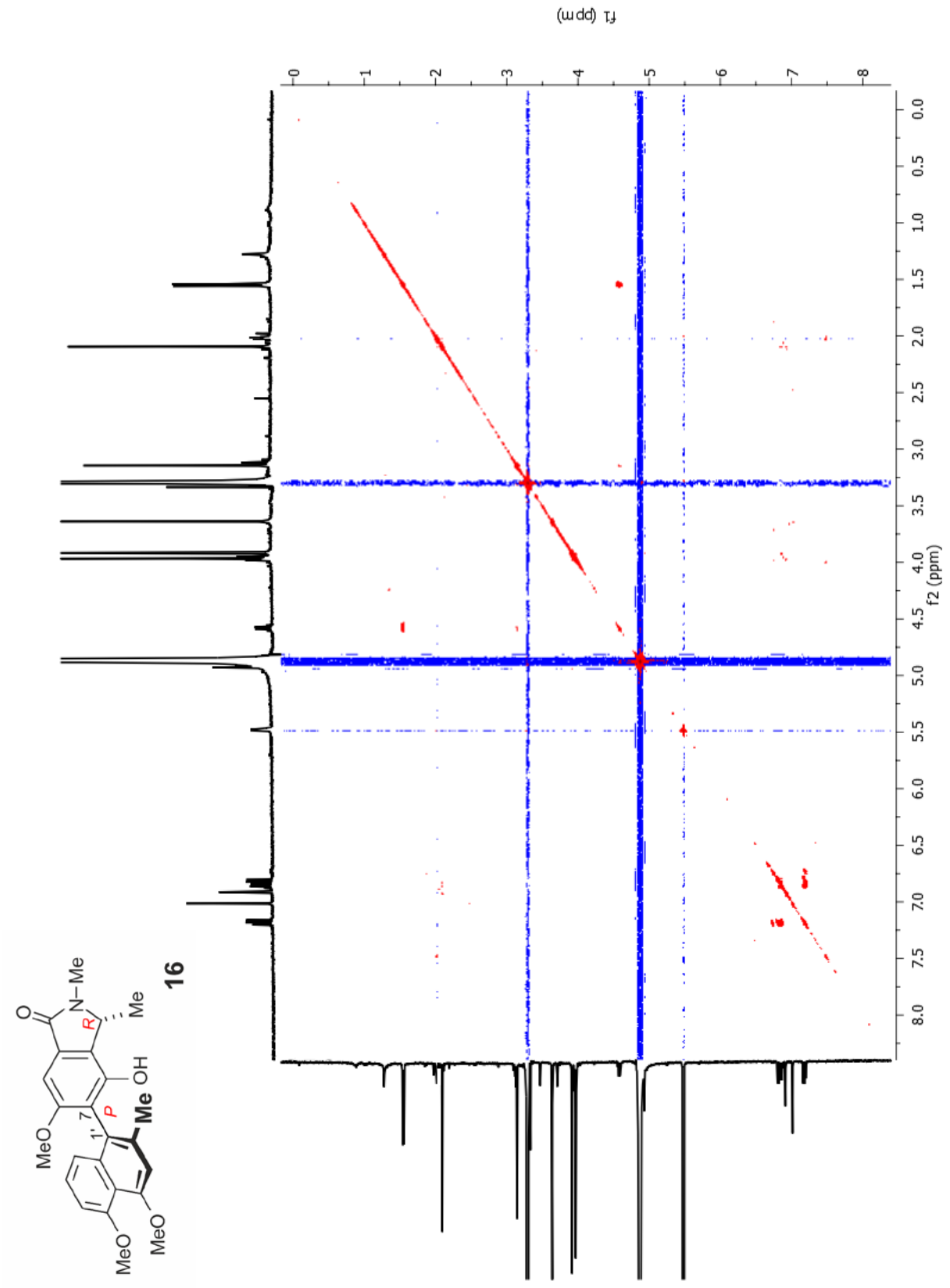



**Figure S26:** $^{1}$H-$^{1}$H COSY spectrum of ancistrobrevoline C (**16**) in methanol-$d_4$.

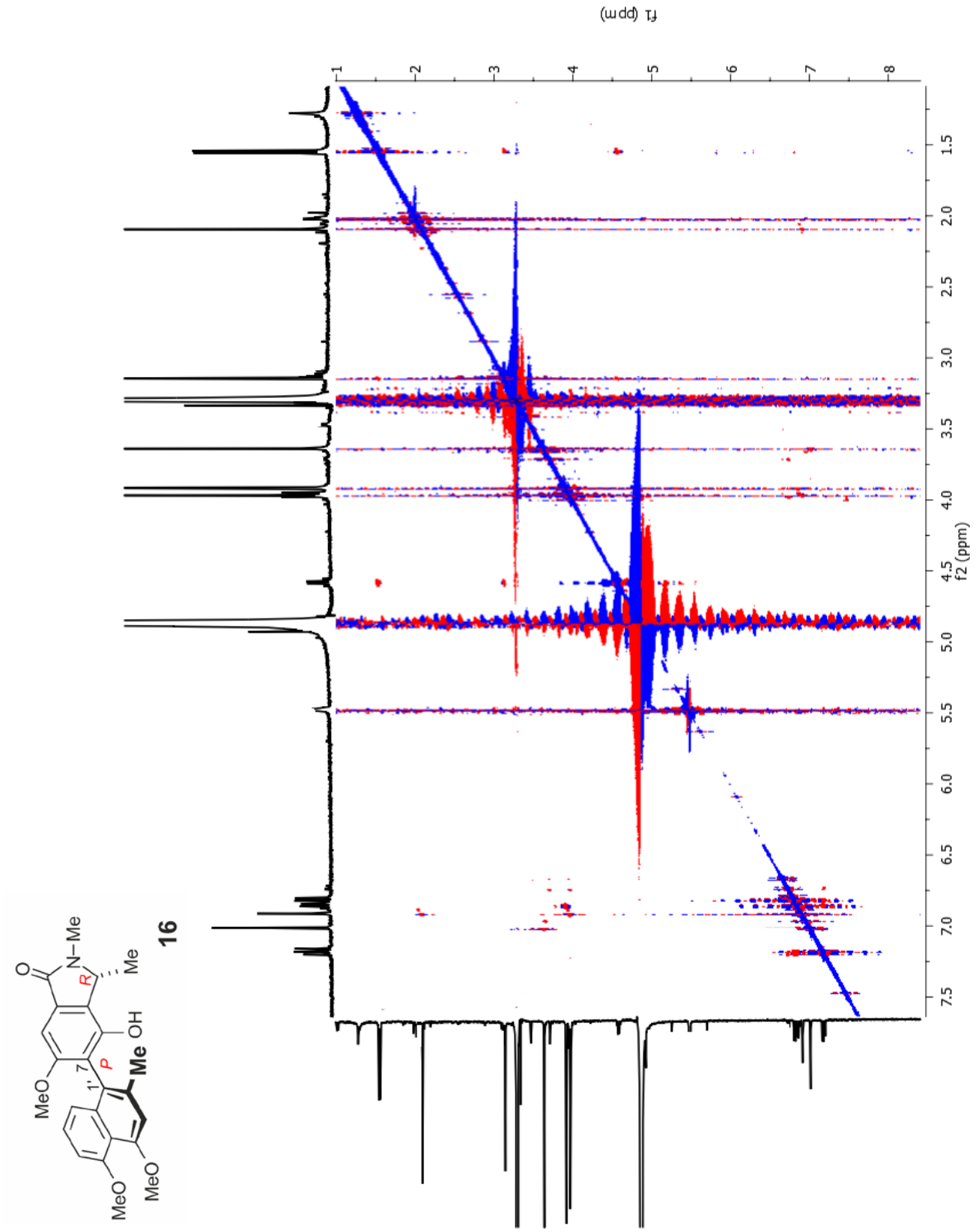



**Figure S27:** $^{1}$H-$^{1}$H NOESY spectrum of ancistrobrevoline C (**16**) in methanol-$d_4$.

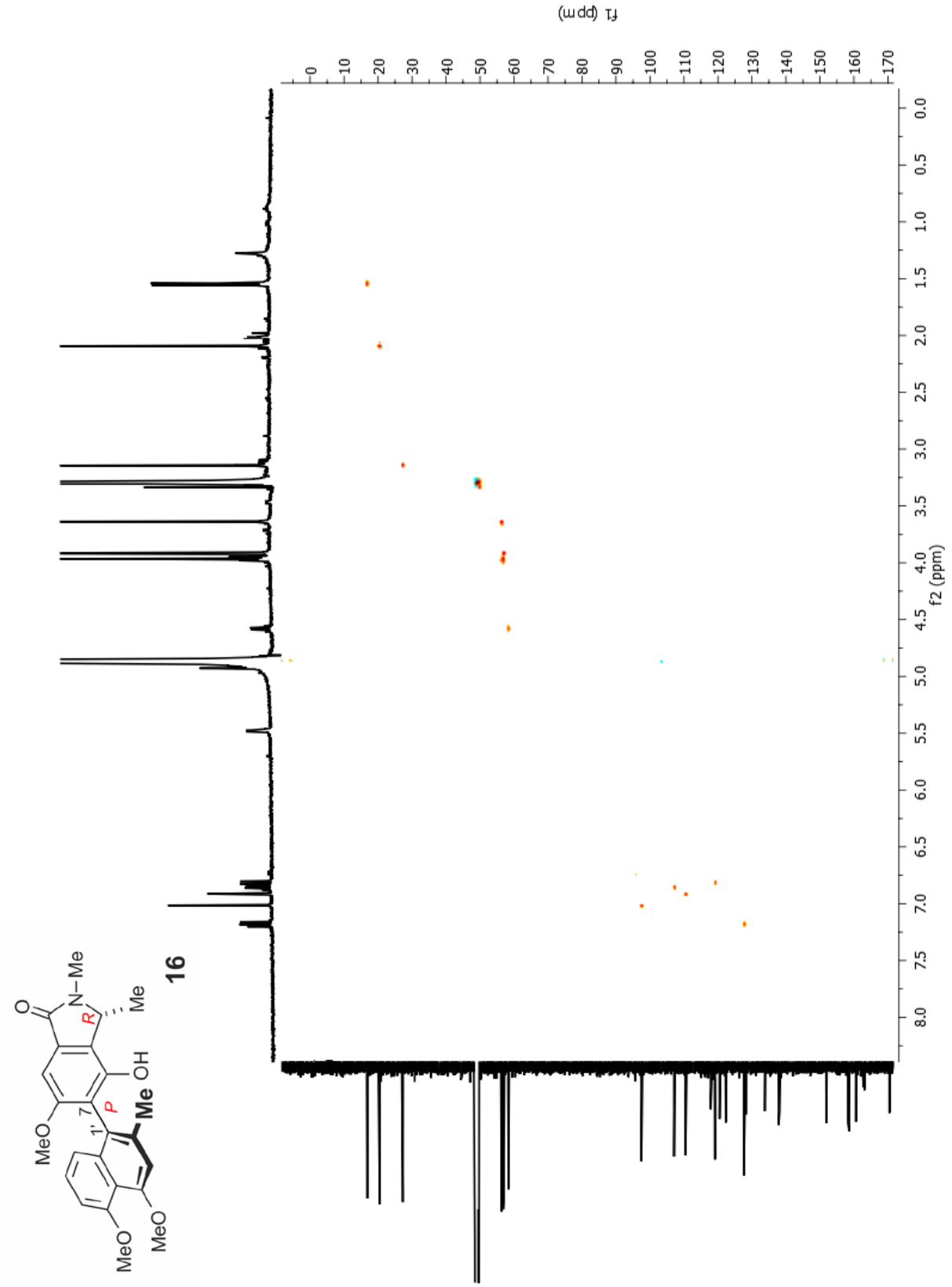



**Figure S28:** $^{1}H$-$^{13}C$ HSQC spectrum of ancistrobrevoline C (**16**) in methanol-$d_4$.

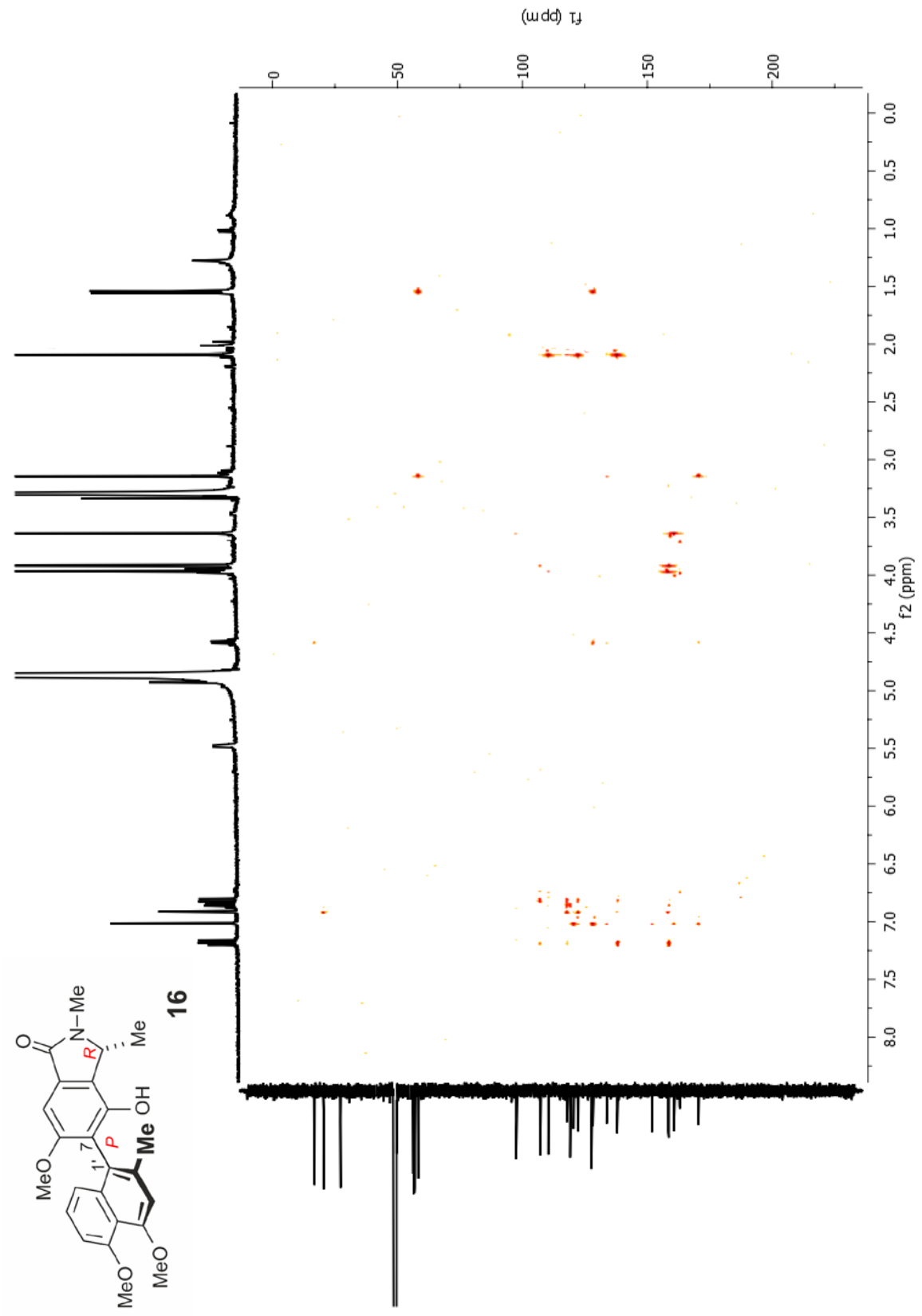



**Figure S29:** $^{1}$H-$^{13}$C HMBC spectrum of ancistrobrevoline C (**16**) in methanol-$d_4$.

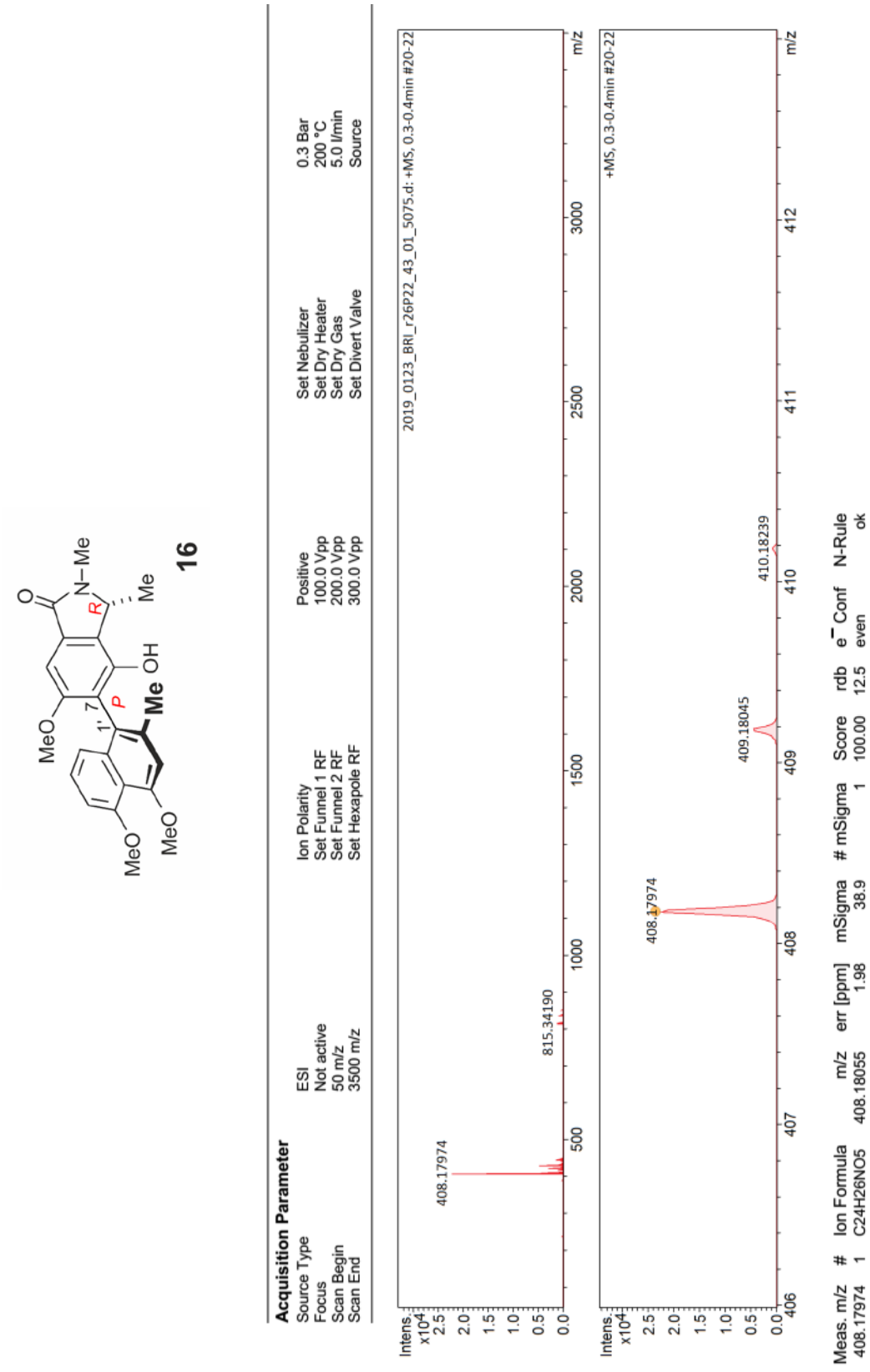
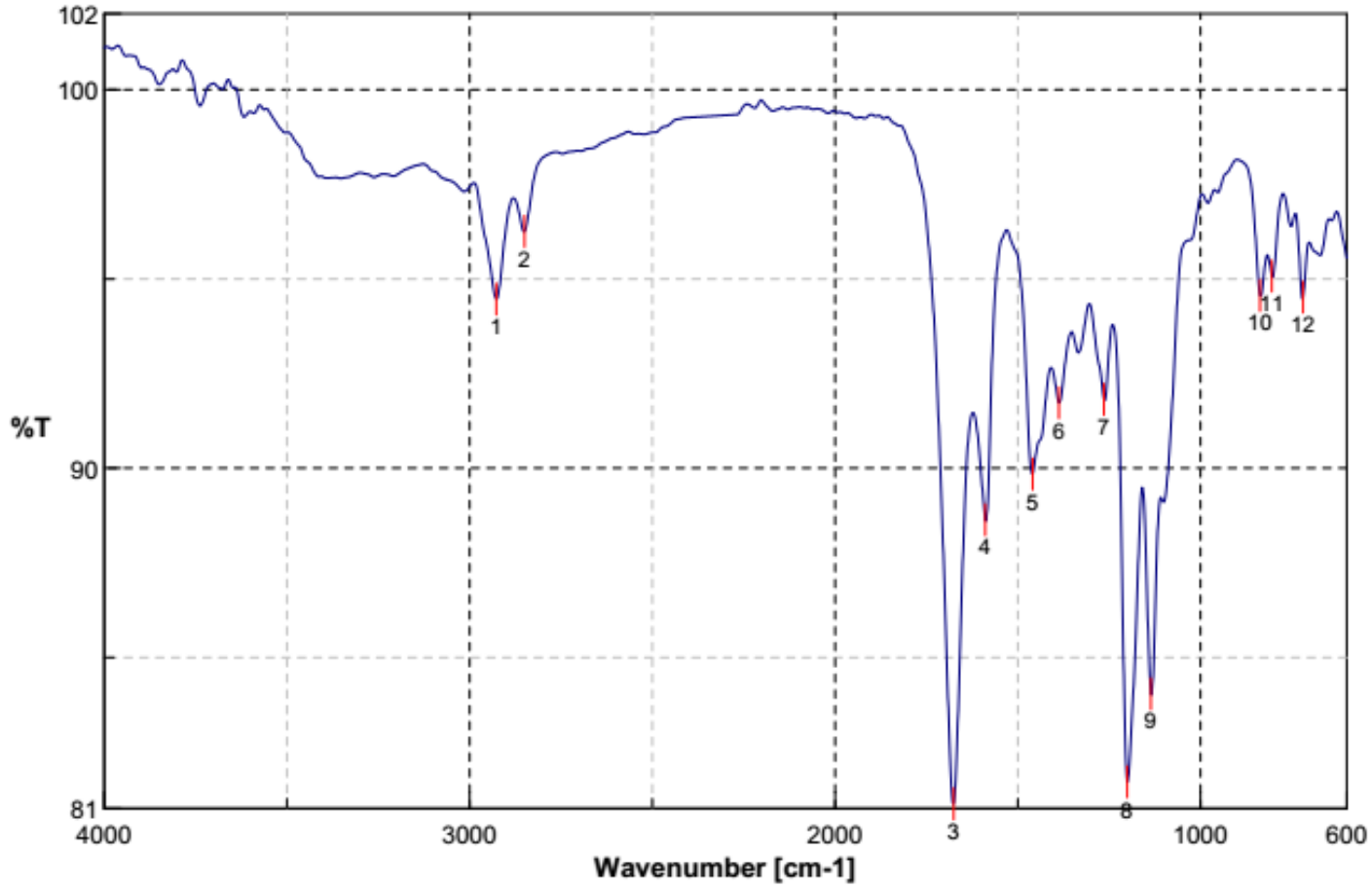



**Figure S30:** HR-ESI-MS spectrum of ancistrobrevoline C (**16**).

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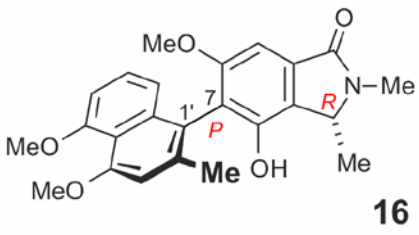
| Sample Name | |
| Comment | |




**16**

| No. | cm-1 | %T | No. | cm-1 | %T | No. | cm-1 | %T |
|---|---|---|---|---|---|---|---|---|
| 1 | 2927.41 | 94.4691 | 2 | 2850.27 | 96.2528 | 3 | 1675.84 | 81.1278 |
| 4 | 1589.06 | 88.6392 | 5 | 1459.85 | 89.846 | 6 | 1387.53 | 91.7289 |
| 7 | 1264.11 | 91.819 | 8 | 1200.47 | 81.7163 | 9 | 1135.87 | 84.0448 |
| 10 | 836.955 | 94.5601 | 11 | 805.135 | 95.0775 | 12 | 719.318 | 94.5178 |

**Figure S31:** IR spectrum of ancistrobrevoline C (**16**).

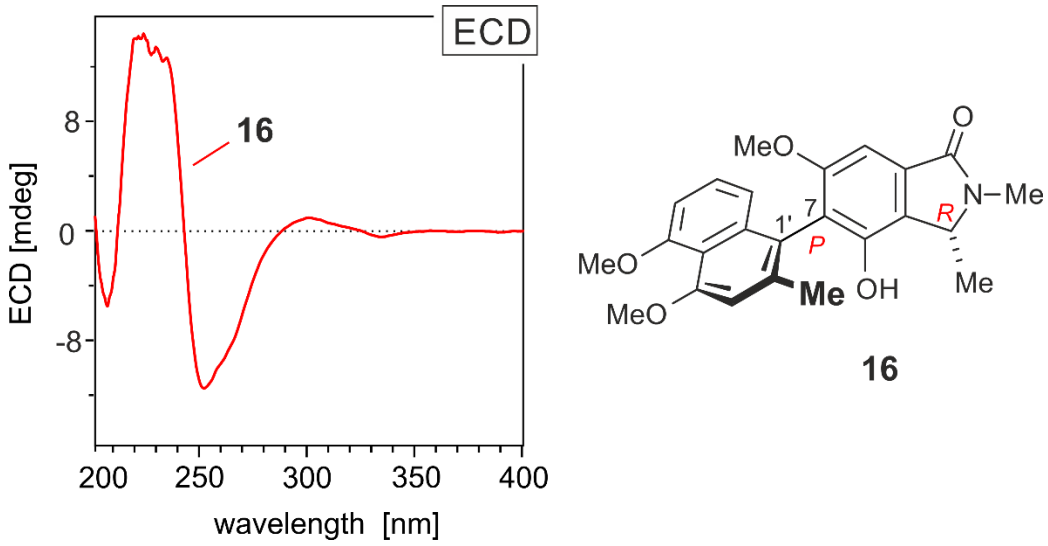



**Figure S32a:** ECD spectrum of ancistrobrevoline C (**16**).

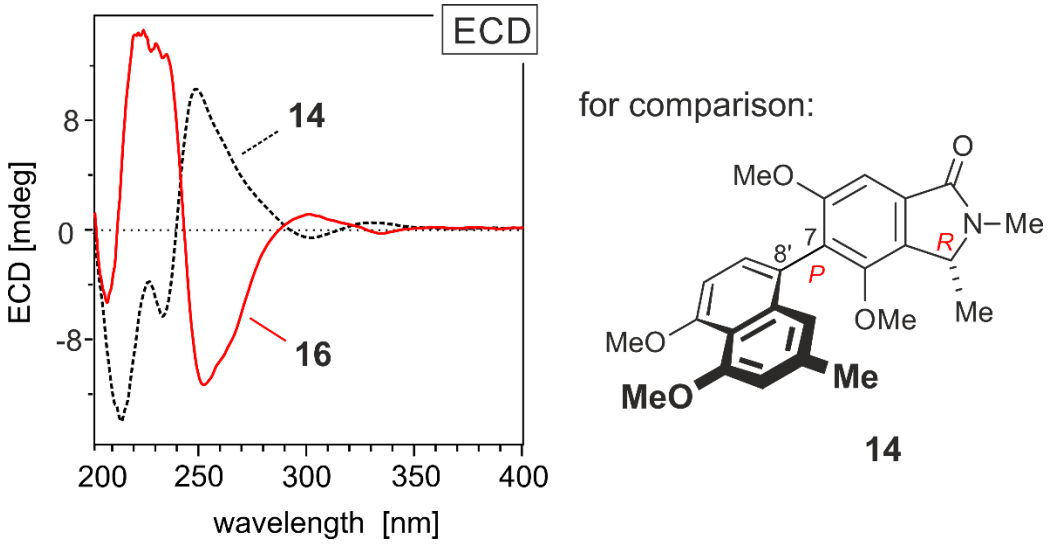
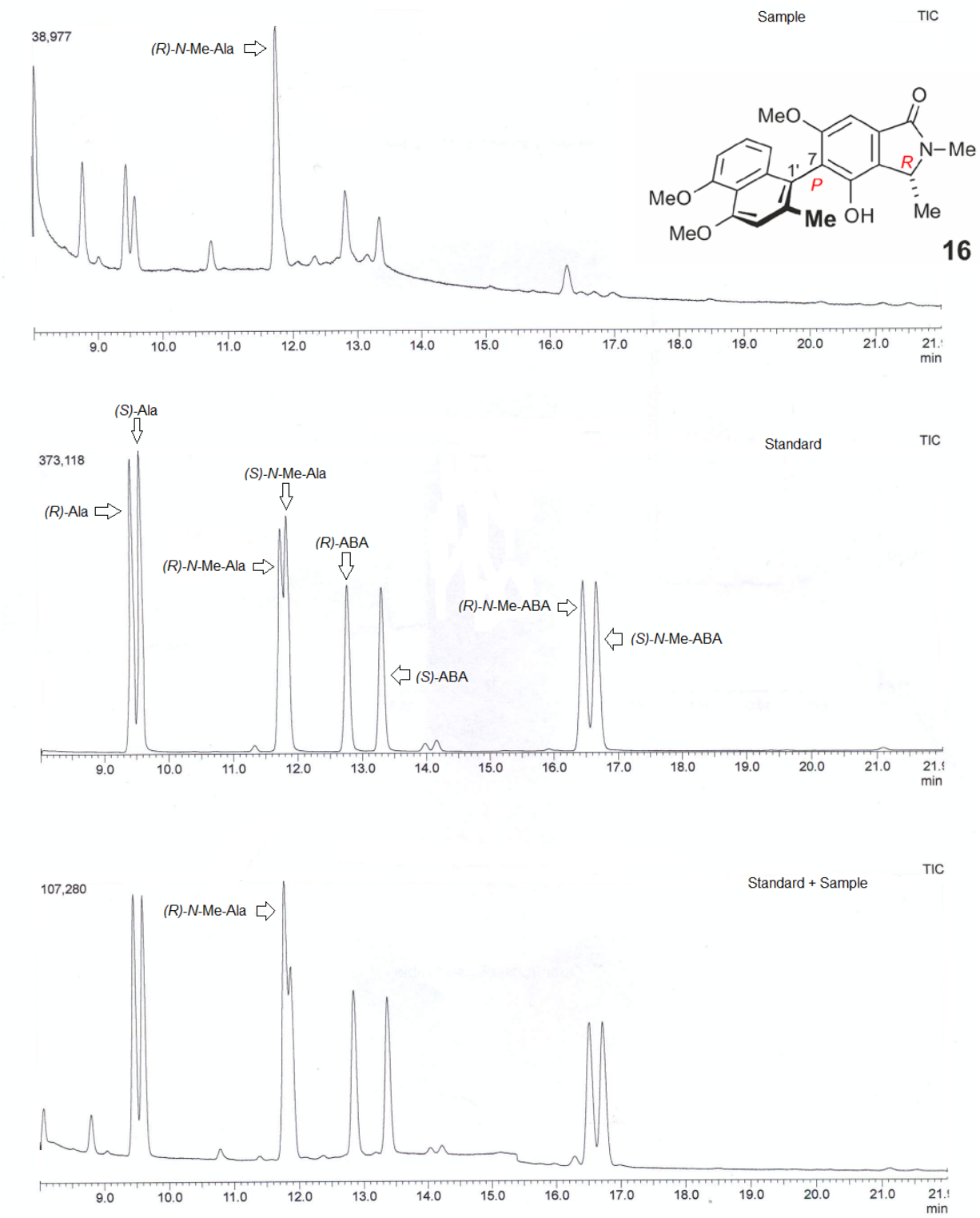



**Figure S32b:** Assignment of the absolute configuration of ancistrobrevoline C (**16**) by comparison of its ECD spectrum with that of ancistrobrevoline A (**14**).




Ala = Alanine *N*-Me-Ala = *N*-Methylalanine

ABA = 3-Aminobutyric acid *N*-Me-ABA = *N*-Methyl-3-aminobutyric acid

**Figure S33:** Oxidative degradation of ancistrobrevoline C (**16**).

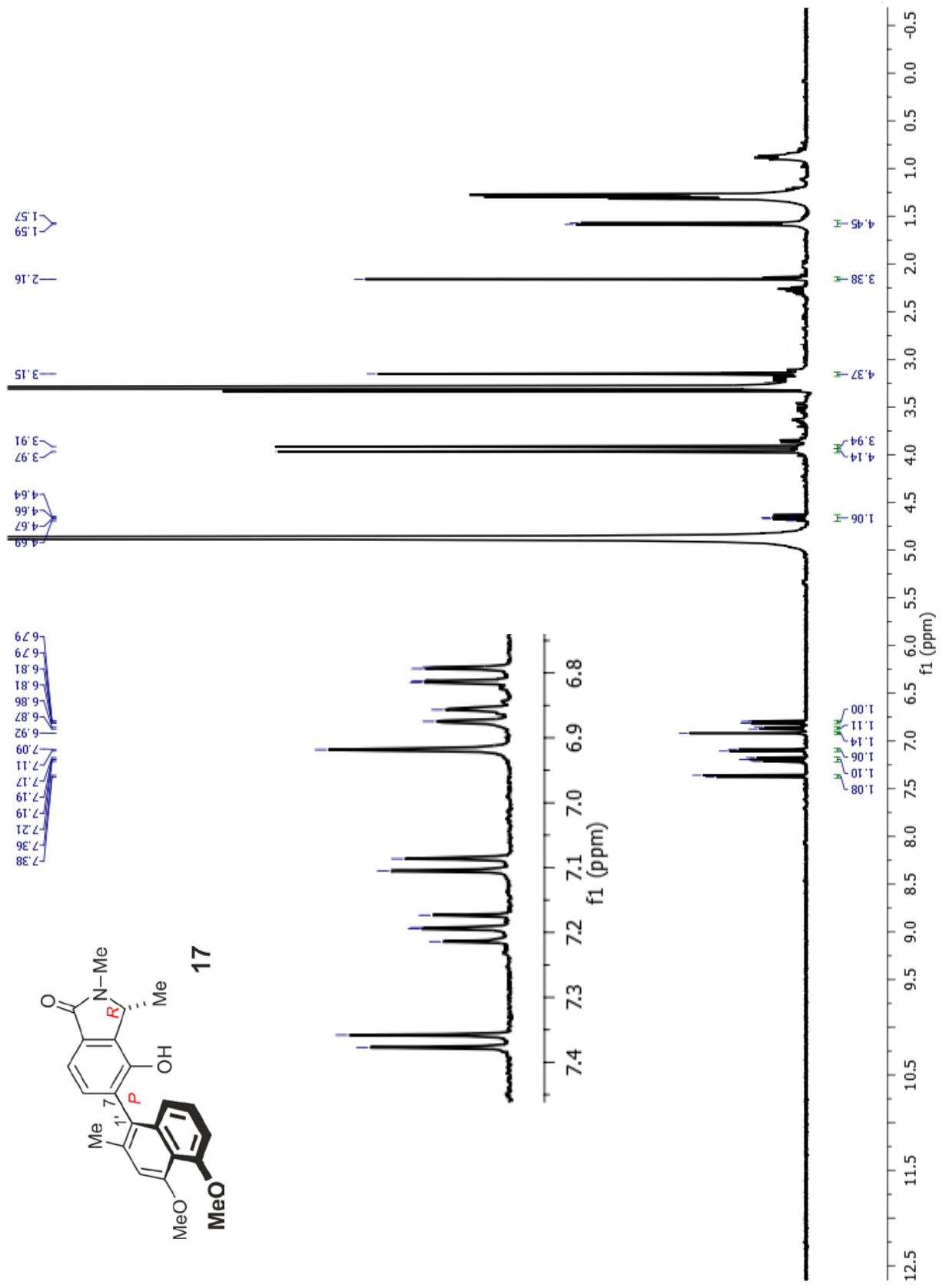



**Figure S34:** $^{1}$H NMR spectrum of ancistrobrevoline D (**17**) in methanol-$d_4$.

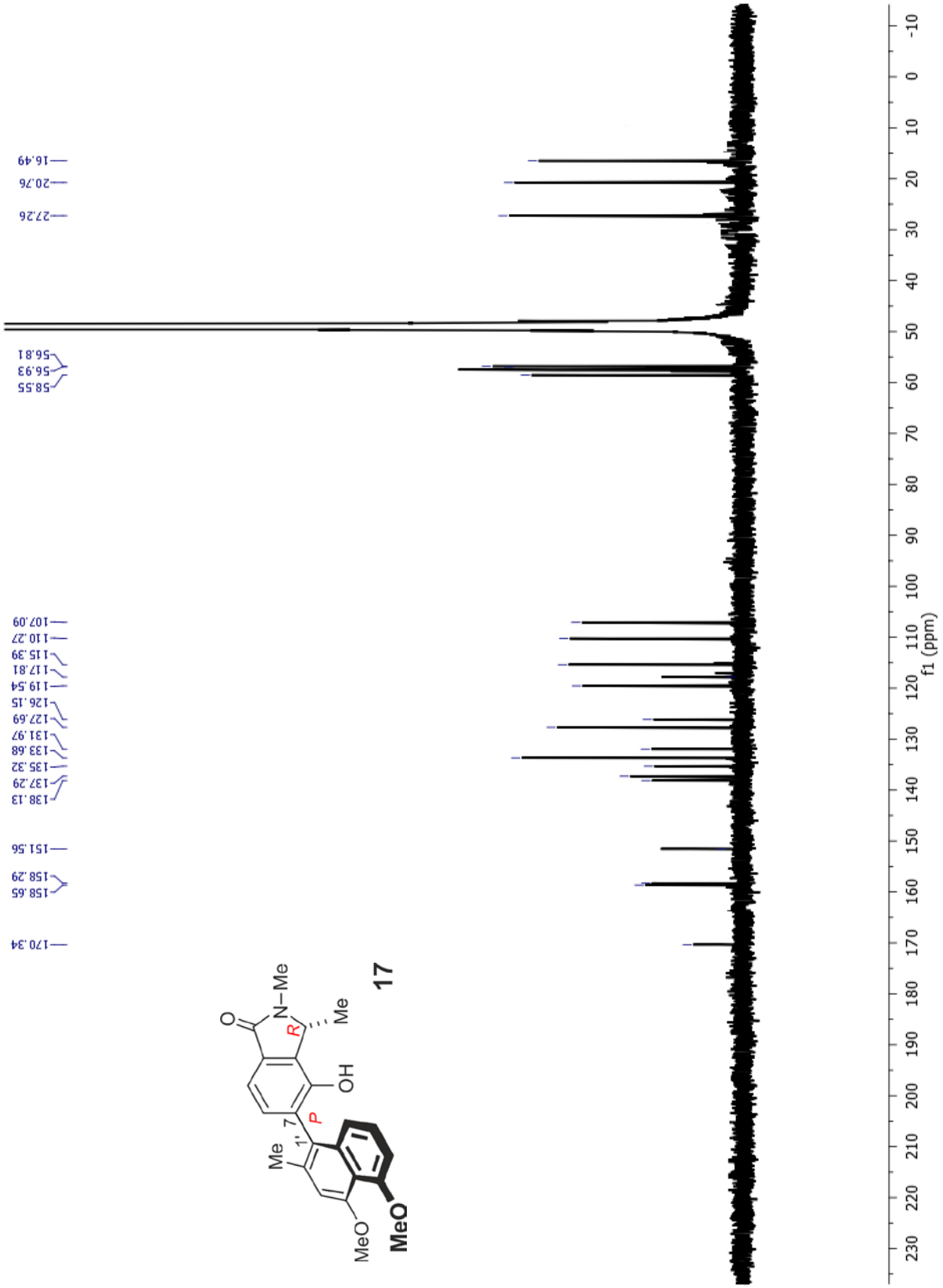



**Figure S35:** $^{13}$C NMR spectrum of ancistrobrevoline D (**17**) in methanol-$d_4$.

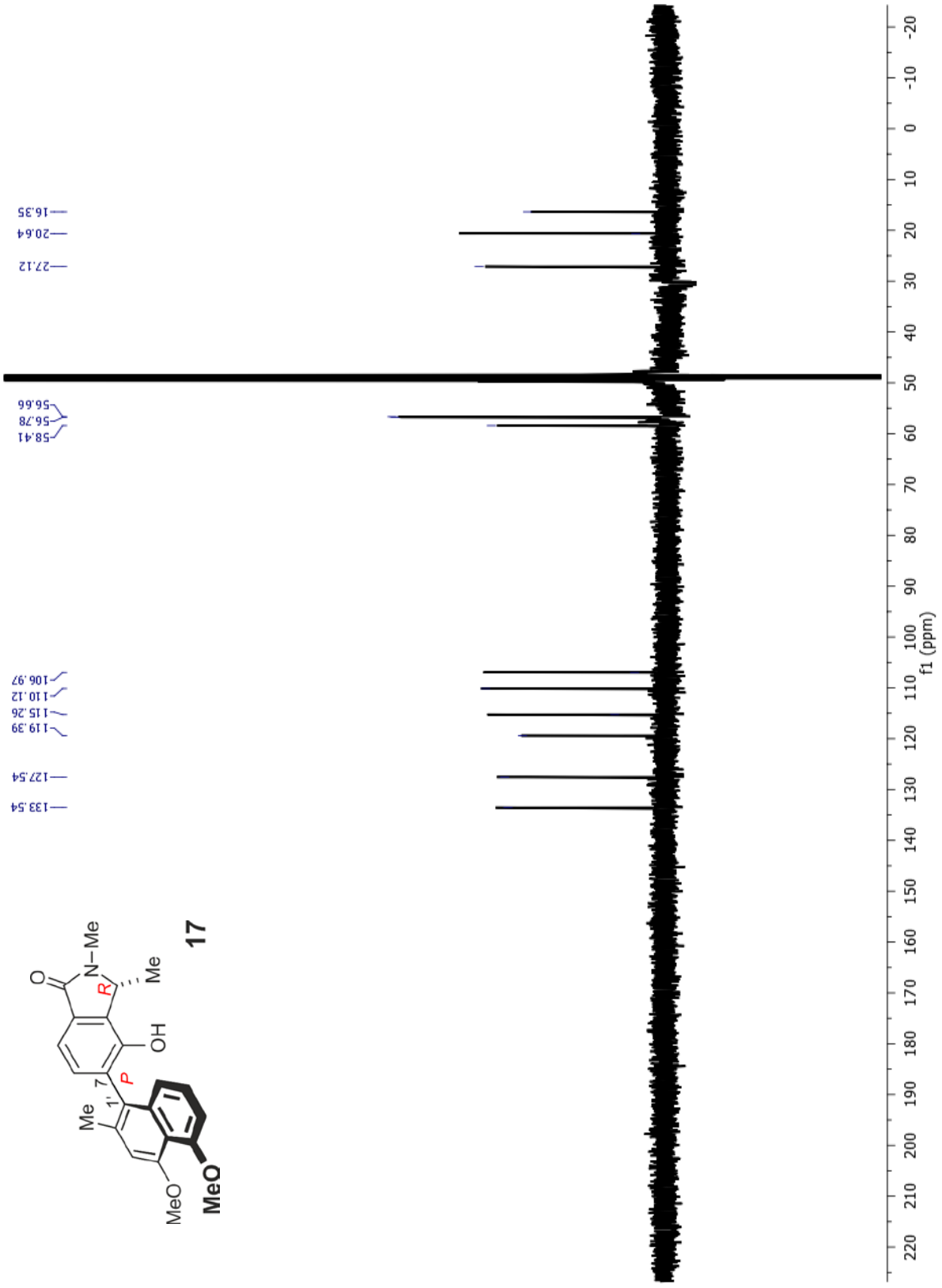



**Figure S36:** $^{13}$C DEPT 135 NMR spectrum ancistrobrevoline D (**17**) in methanol-$d_4$.

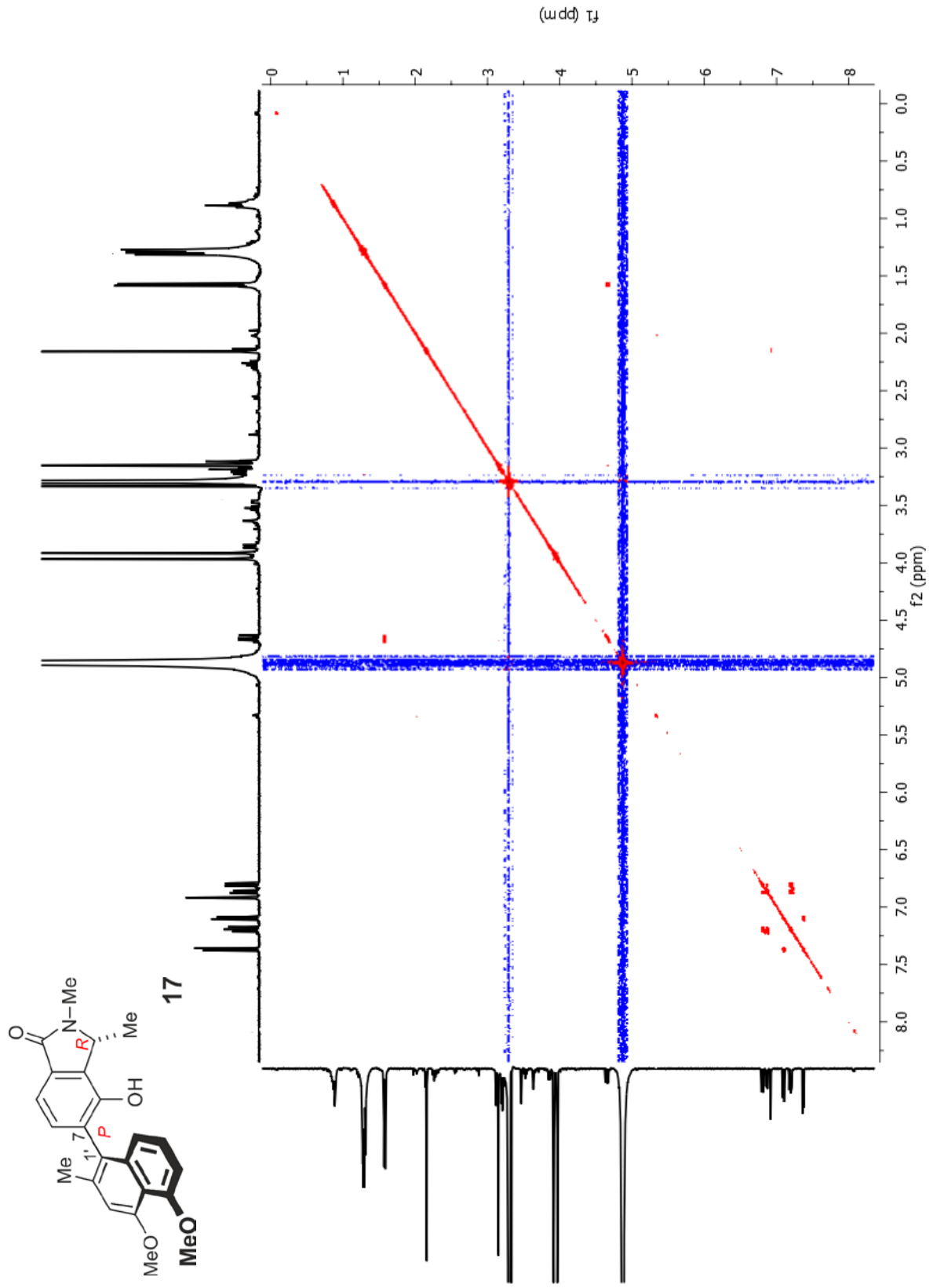



**Figure S37:** $^{1}$H-$^{1}$H COSY spectrum of ancistrobrevoline D (**17**) in methanol-$d_4$.

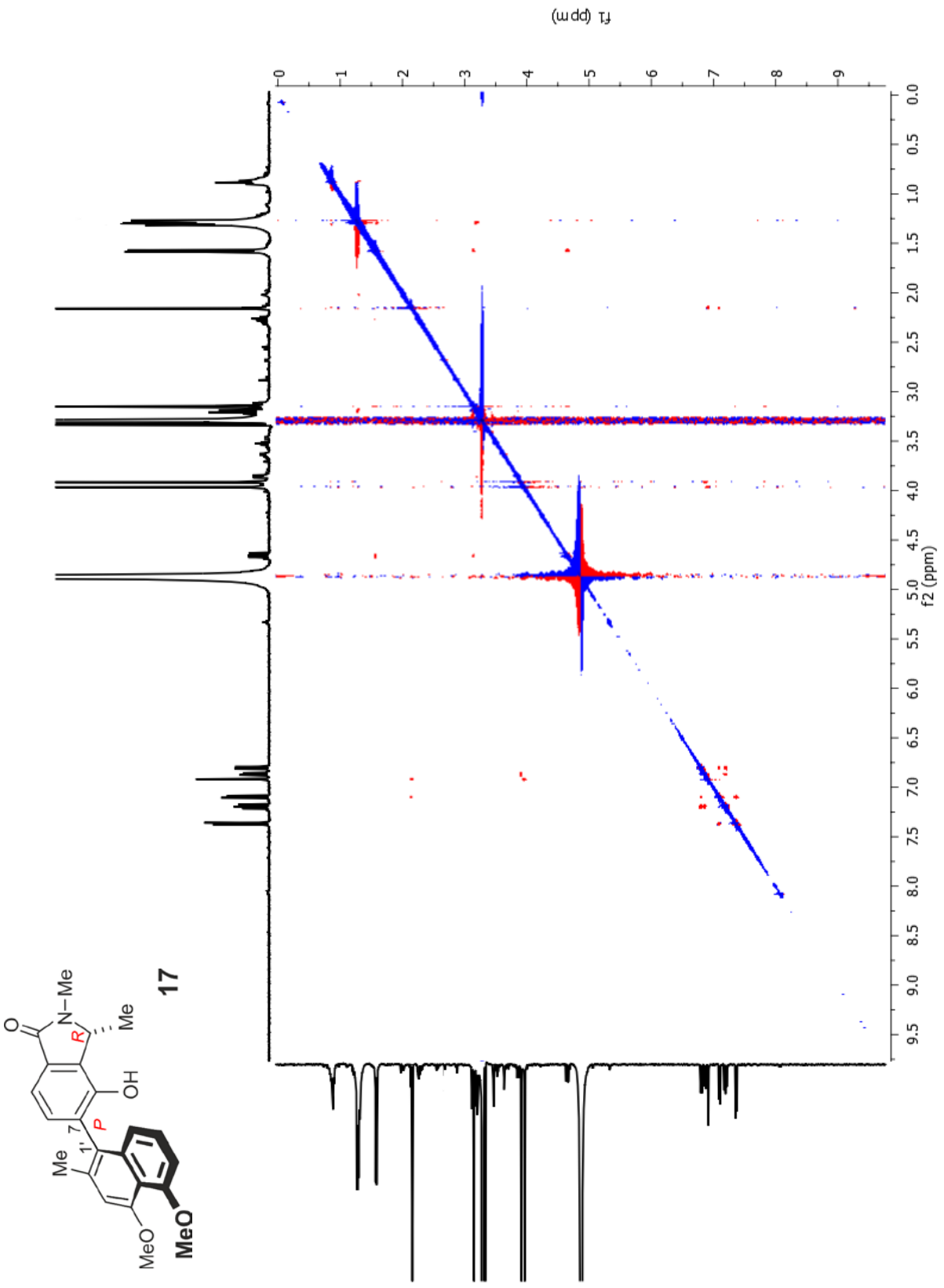



**Figure S38:** $^{1}$H-$^{1}$H NOESY spectrum of ancistrobrevoline D (**17**) in methanol-$d_4$.

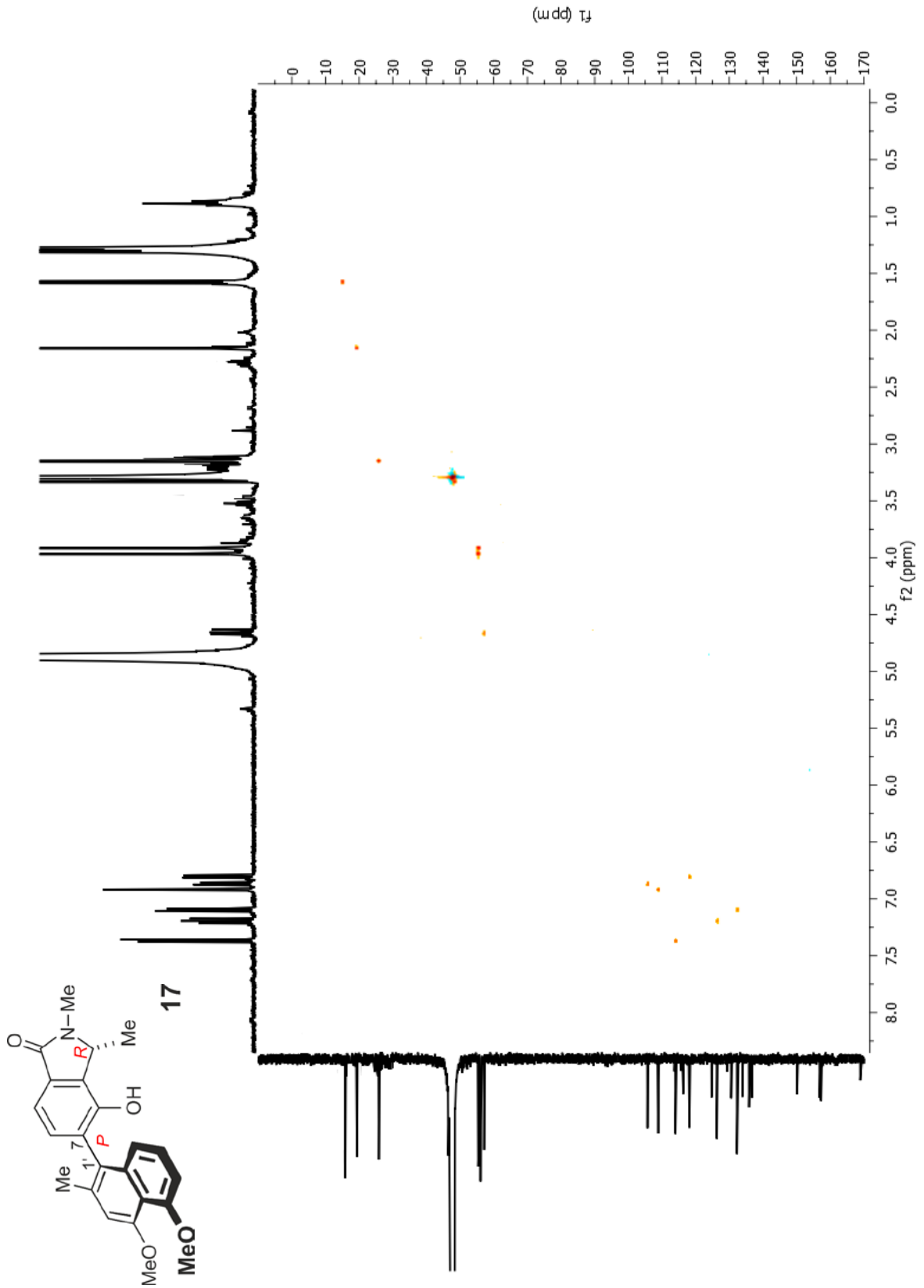



**Figure S39:** $^{1}$H-$^{13}$C HSQC spectrum of ancistrobrevoline D (**17**) in methanol-$d_4$.

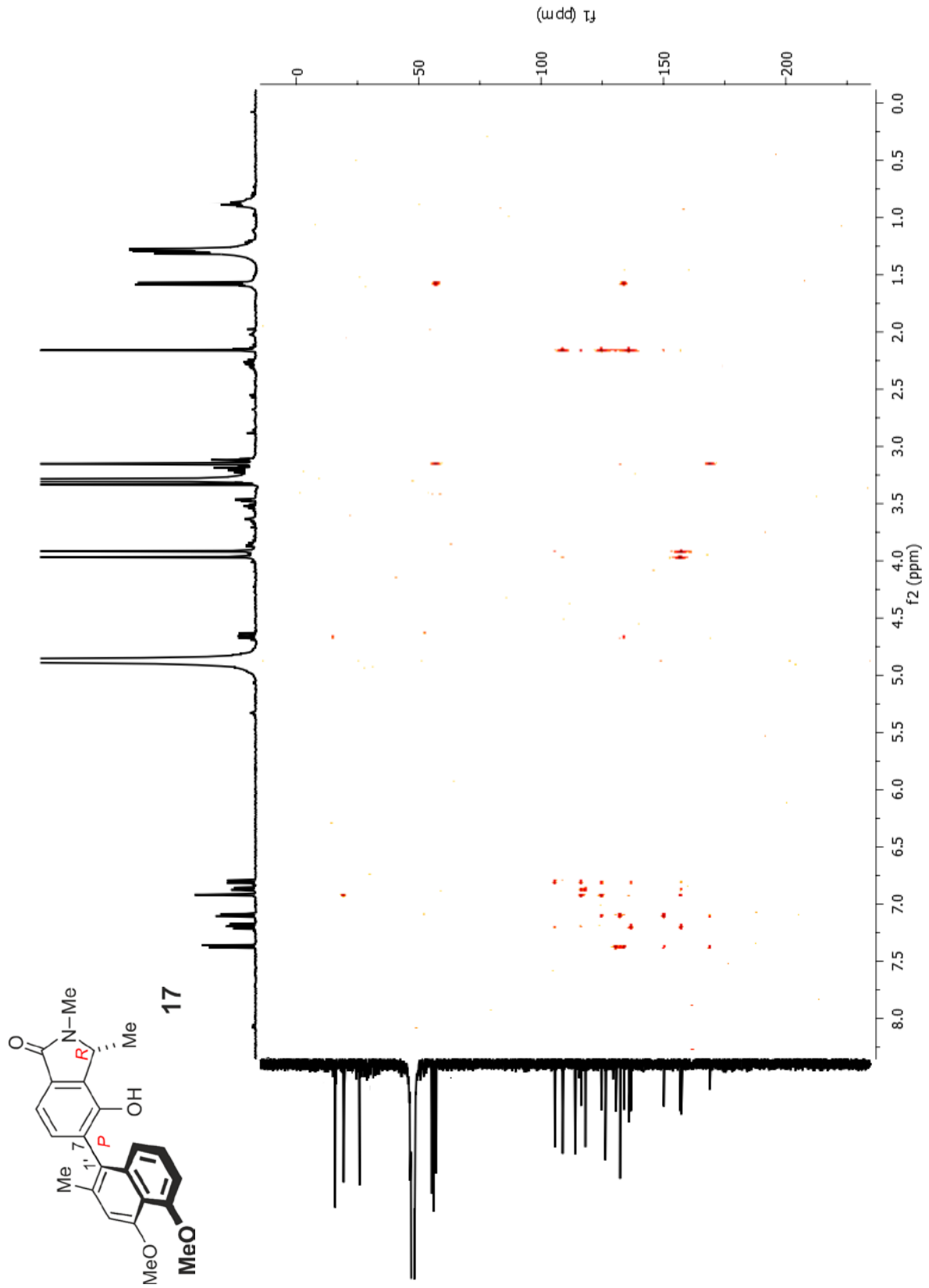
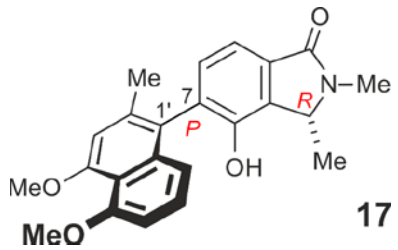


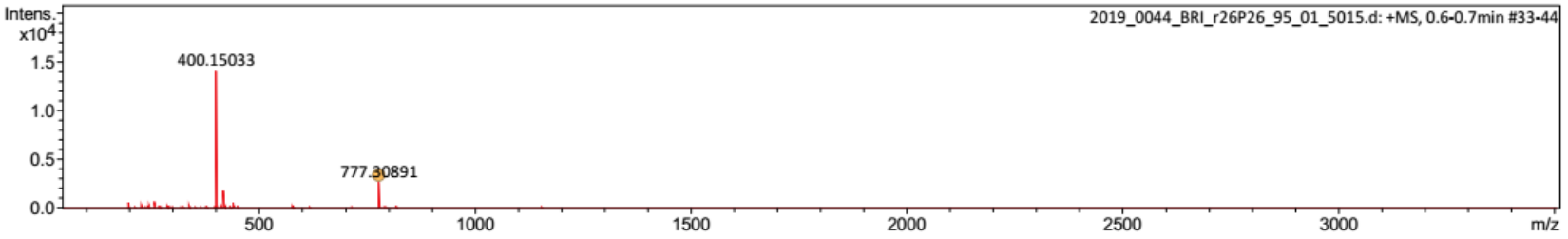

**Figure S40:** $^{1}$H-$^{13}$C HMBC spectrum of ancistrobrevoline D (**17**) in methanol-*d*$_4$.

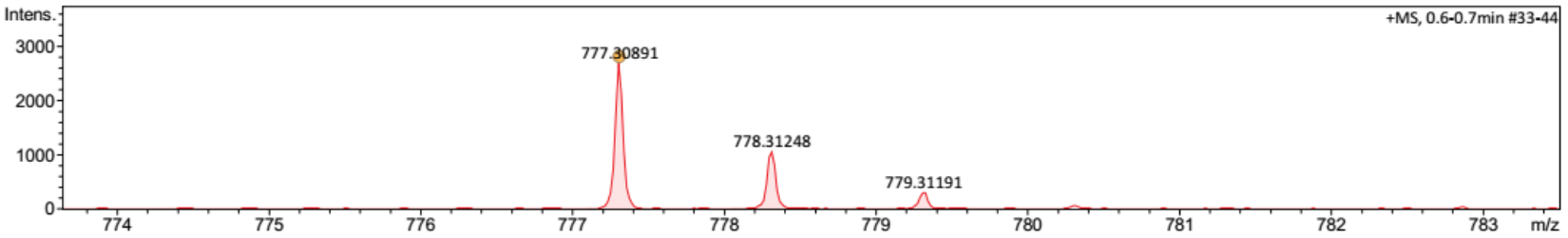
**Figure S41:** HR-ESI-MS spectrum of ancistrobrevoline D (**17**).



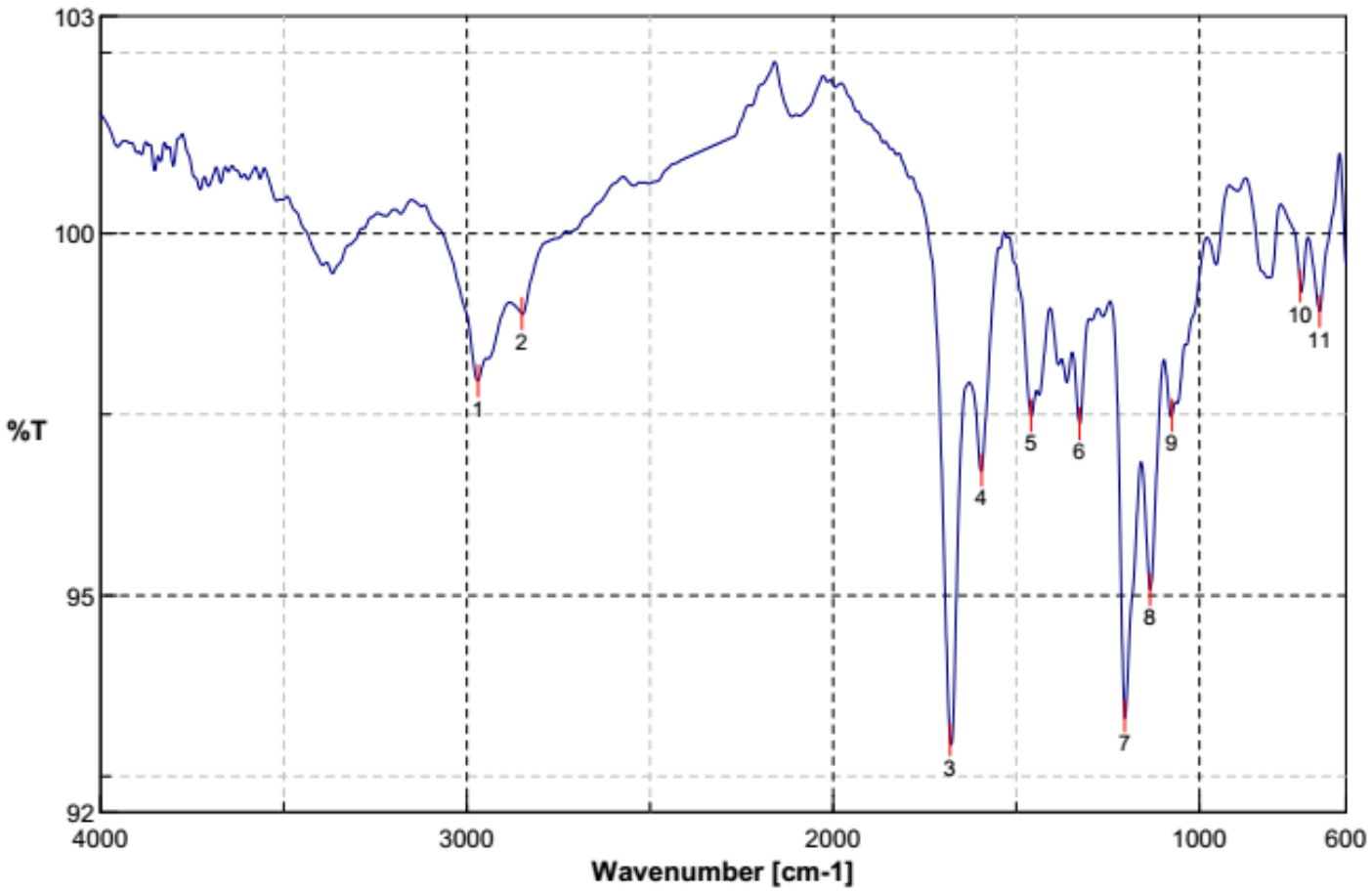

**17**

| **Acquisition Parameter** | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.3 Bar |
| Focus | Not active | Set Funnel 1 RF | 100.0 Vpp | Set Dry Heater | 200 °C |
| Scan Begin | 50 m/z | Set Funnel 2 RF | 200.0 Vpp | Set Dry Gas | 5.0 l/min |
| Scan End | 3500 m/z | Set Hexapole RF | 300.0 Vpp | Set Divert Valve | Source |

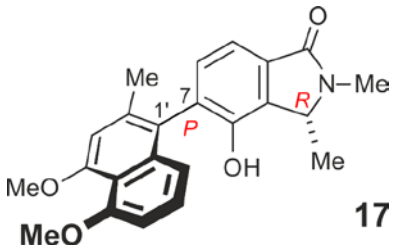



| Meas. m/z | # | Ion Formula | m/z | err [ppm] | mSigma | # mSigma | Score | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 400.15033 | 1 | C23H23NNaO4 | 400.15193 | 3.99 | 48.9 | 1 | 100.00 | 12.5 | even | ok |




| | |
|---|---|
| Accumulation | 8 |
| Resolution | 4 cm-1 |
| Zero Filling | ON |
| Apodization | Cosine |
| Gain | Auto (4) |
| Scanning Speed | Auto (2 mm/sec) |
| Date/Time | 28.02.2019 9:22 |
| Update | 28.02.2019 10:57 |
| Operator | Student |
| File Name | Memory#1 |
| Sample Name | |
| Comment | |




**17**

| No. | cm-1 | %T | No. | cm-1 | %T | No. | cm-1 | %T |
|---|---|---|---|---|---|---|---|---|
| 1 | 2969.84 | 97.9683 | 2 | 2850.27 | 98.8938 | 3 | 1680.66 | 93.0048 |
| 4 | 1594.84 | 96.73 | 5 | 1459.85 | 97.4823 | 6 | 1327.75 | 97.3716 |
| 7 | 1204.33 | 93.3327 | 8 | 1134.9 | 95.0794 | 9 | 1075.12 | 97.4866 |
| 10 | 725.104 | 99.2795 | 11 | 672.071 | 98.9257 | | | |

**Figure S42:** IR spectrum of ancistrobrevoline D (**17**).

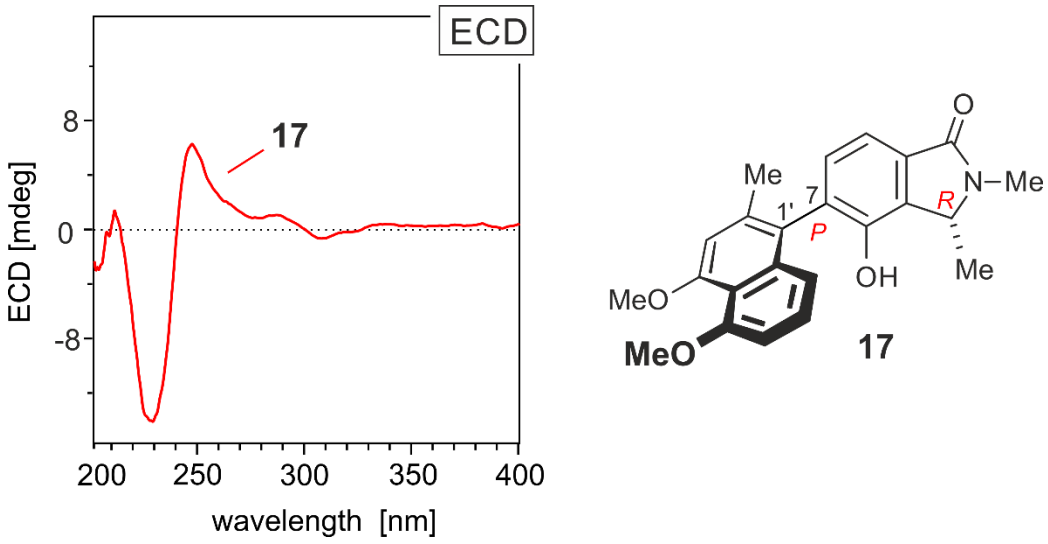



**Figure S43a:** ECD spectrum of ancistrobrevoline D (**17**).

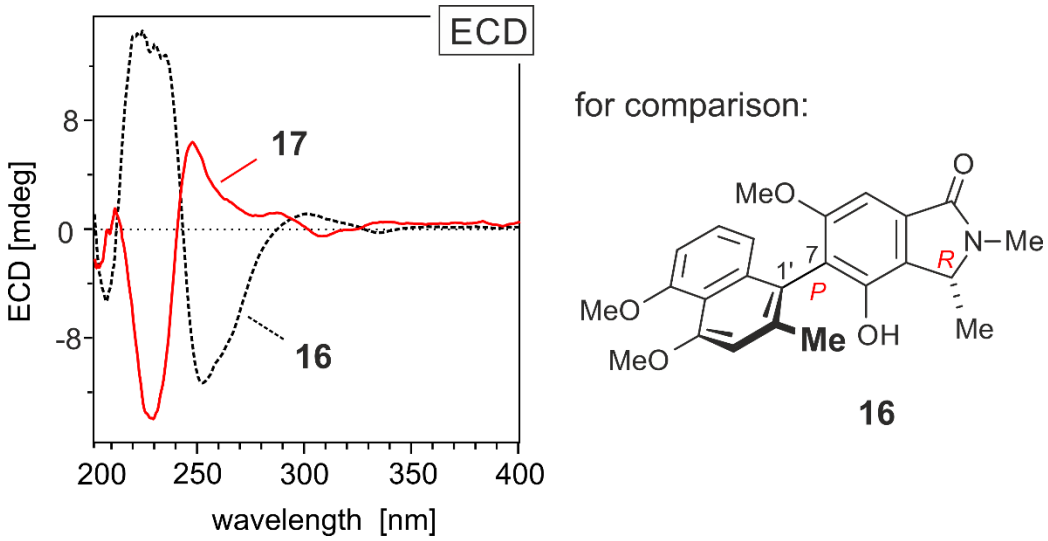
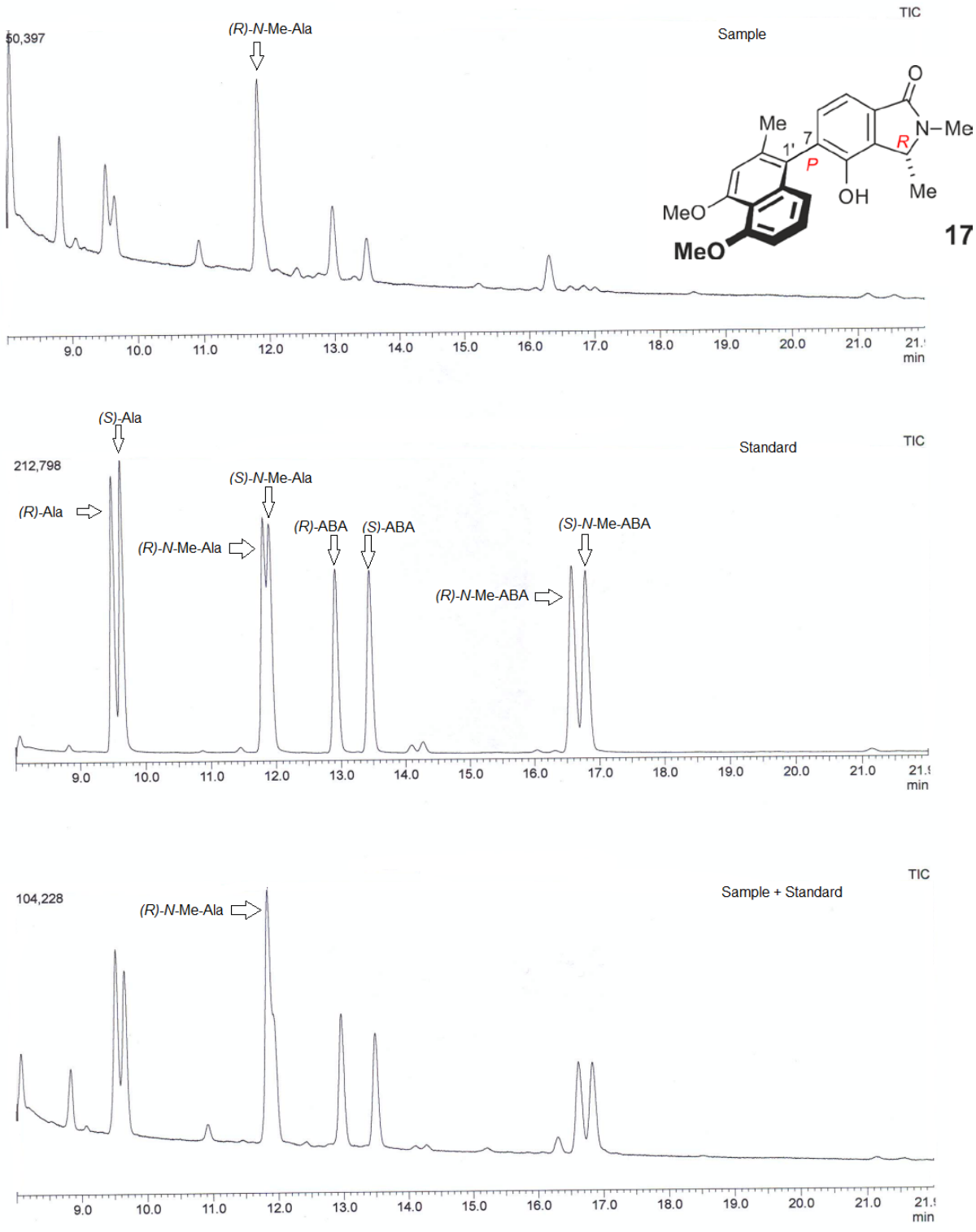



**Figure S43b:** Assignment of the absolute configuration of ancistrobrevoline D (**17**) by comparison of its ECD spectrum with that of ancistrobrevoline C (**16**).




| | |
|---|---|
| Ala = Alanine | *N*-Me-Ala = *N*-Methylalanine |
| ABA = 3-Aminobutyric acid | *N*-Me-ABA = *N*-Methyl-3-aminobutyric acid |

**Figure S44:** Oxidative degradation of ancistrobrevoline D (**17**).