Supporting Information

Semi-Synthesis of *S*-Desoxybrevetoxin-B2 and Brevetoxin-B2, and Assessment of their Acute Toxicities

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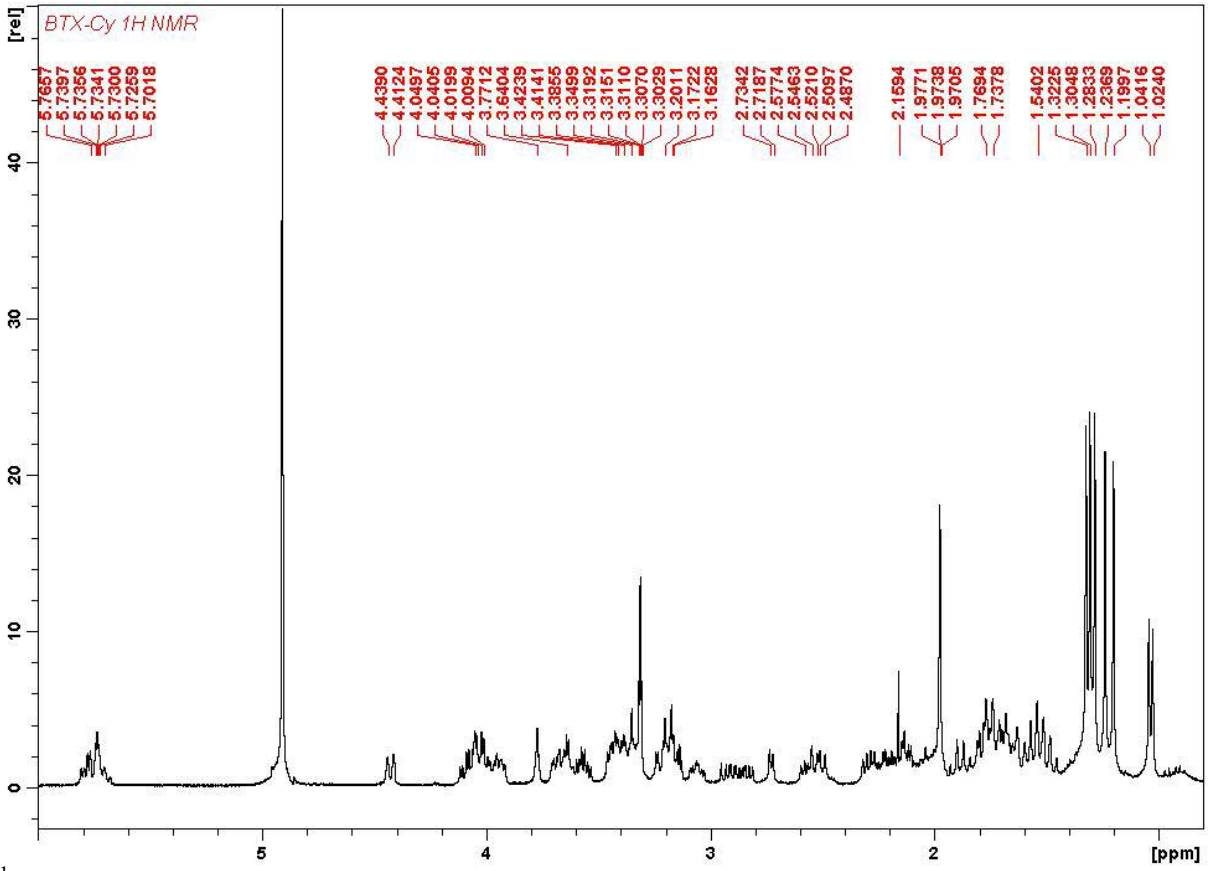
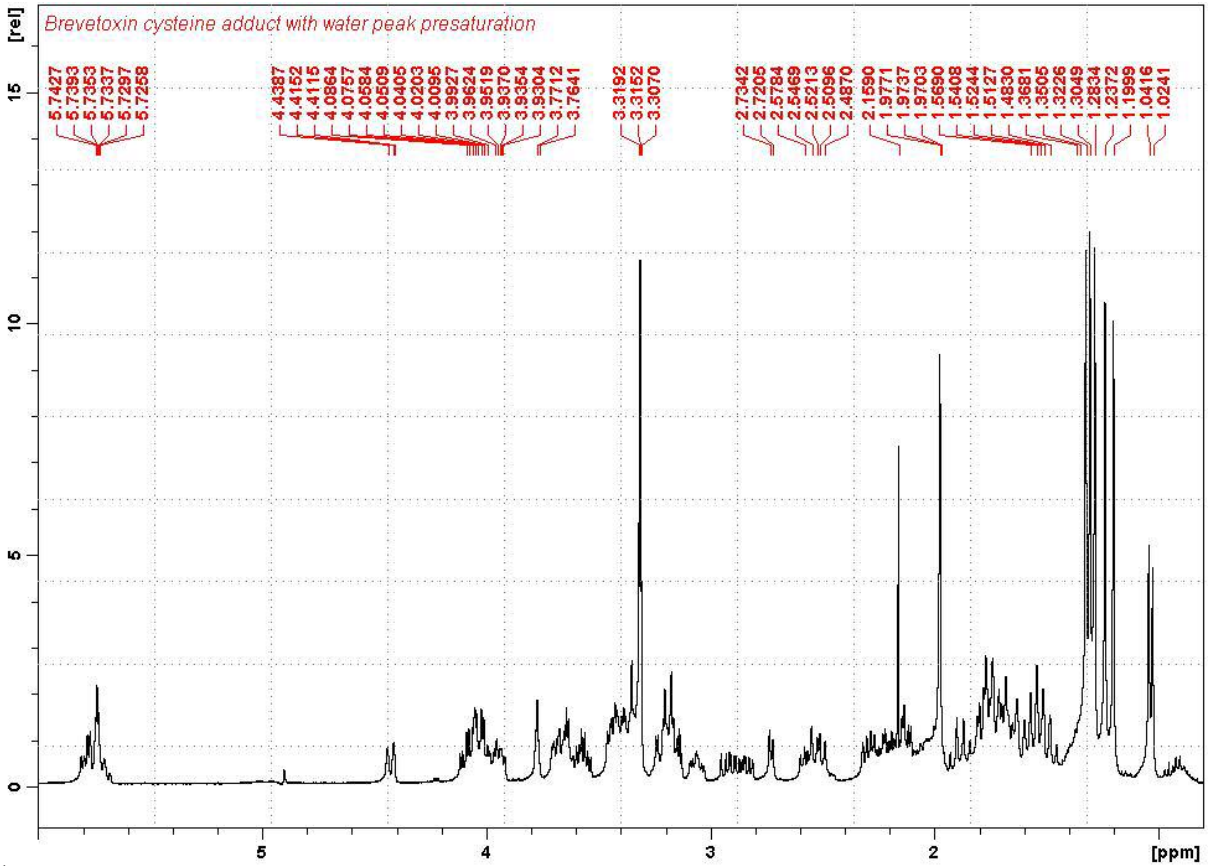
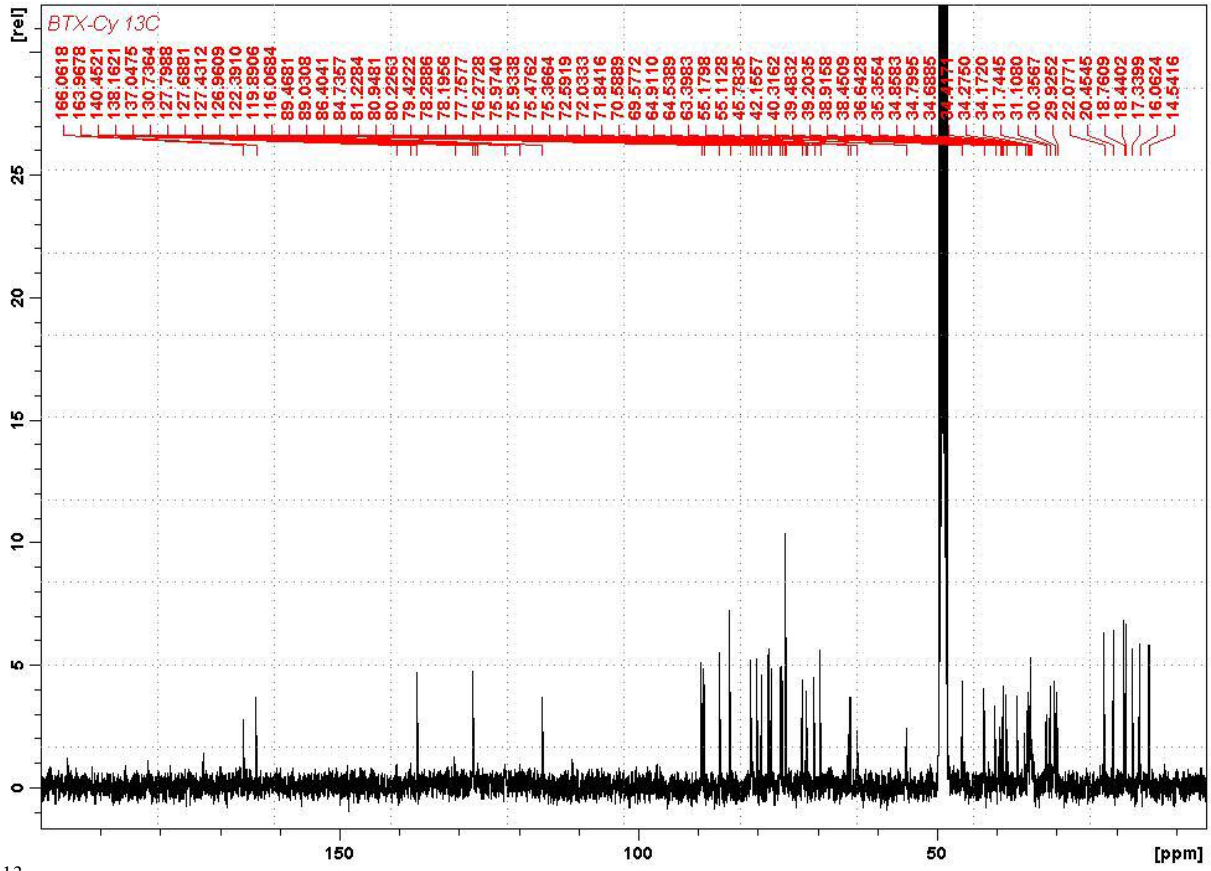
Supporting Information Available: ^{1}H NMR (with and without solvent suppression), ^{13}C, DEPT135, COSY (with expansion), TOCSY, HMBC, HSQC and ROESY NMR spectra of **4**; MS/MS spectra of **4** (positive and negative modes); ^{1}H NMR (with solvent suppression), ^{13}C, DEPT135, COSY (with expansion), TOCSY, HMBC expansion, and HSQC NMR spectra of **5**; MS/MS spectrum of **5** (positive mode); LC-MS analyses of trial reactions of **1** with L-cysteine, 2-hydroxythiepan, and 2-[(5-fluoresceinyl)aminocarbonyl]ethyl mercaptan; dose rates and time-to-death for **4**, **5**, and **6** administered i.p. to mice.



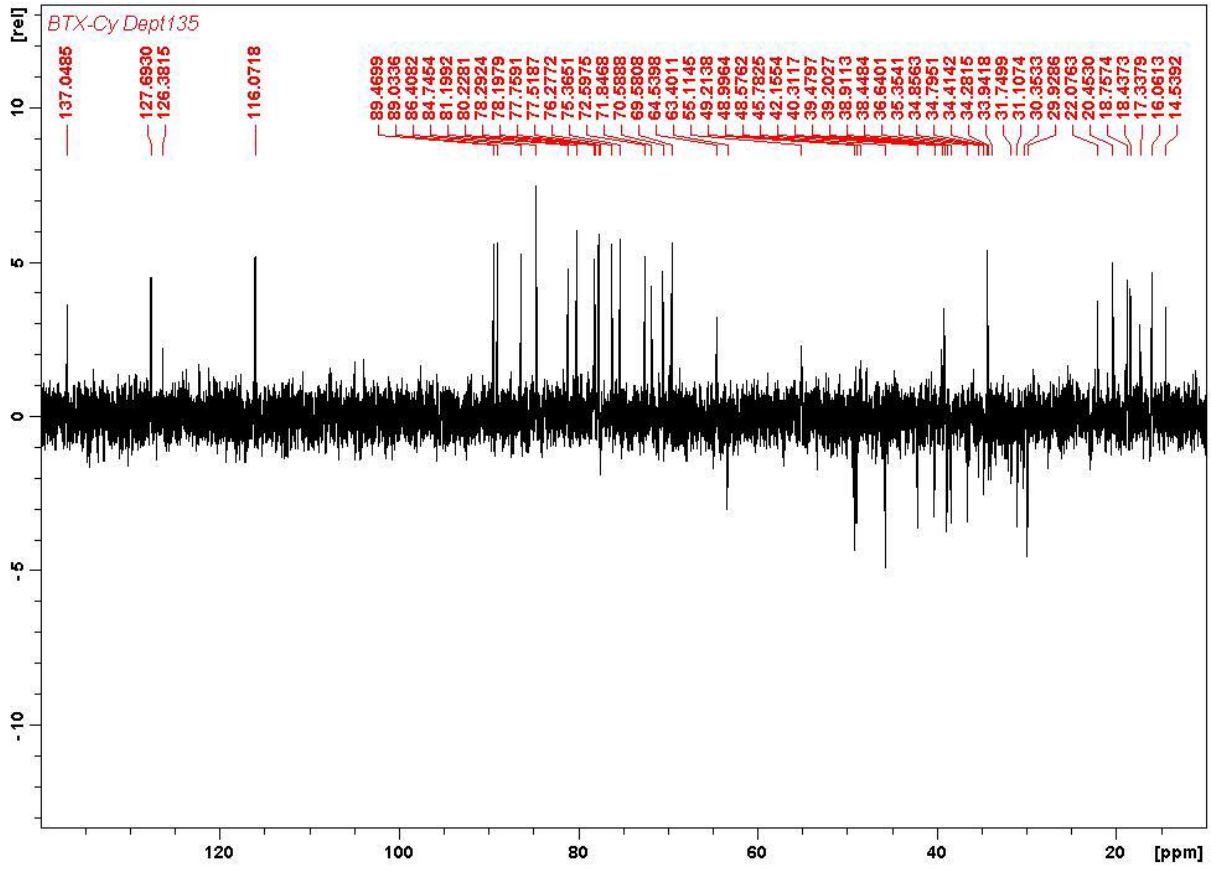
^{1}H NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



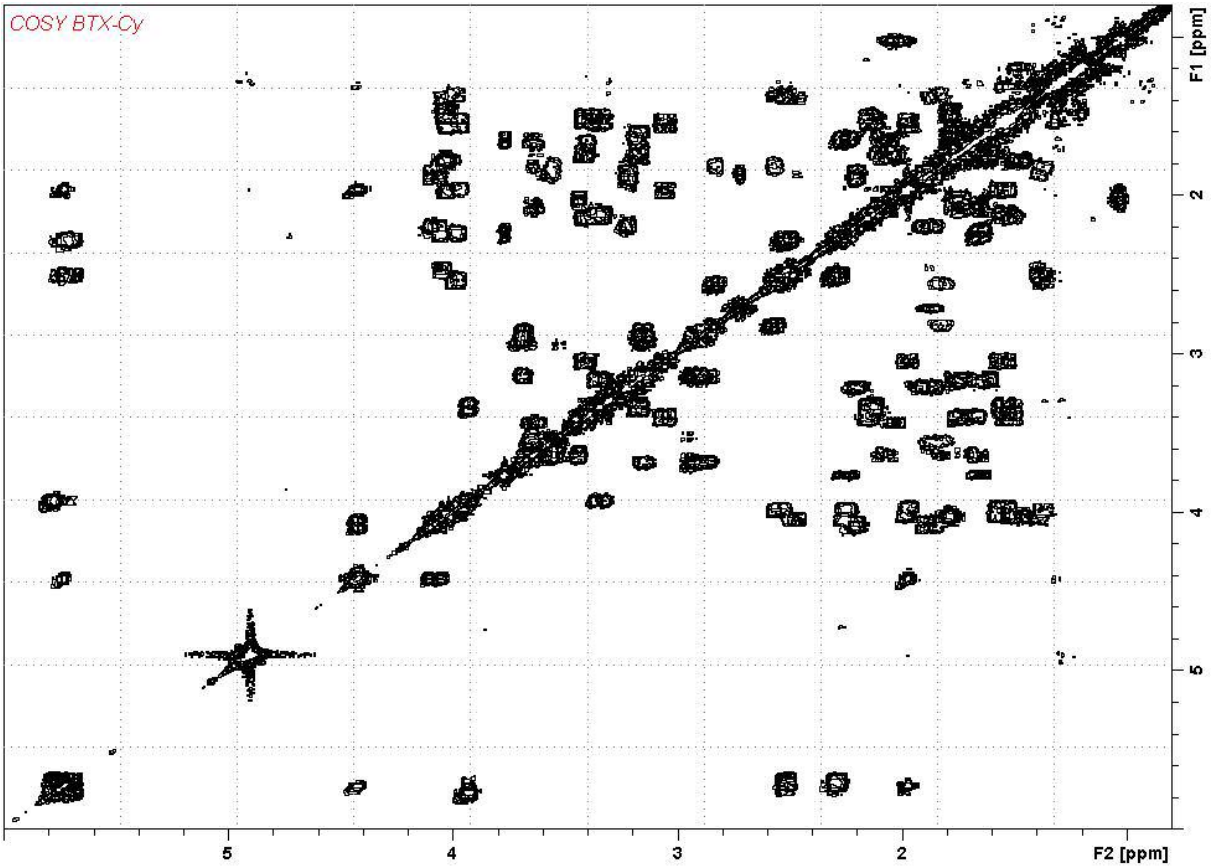
^{1}H NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD with presaturation of water peak.



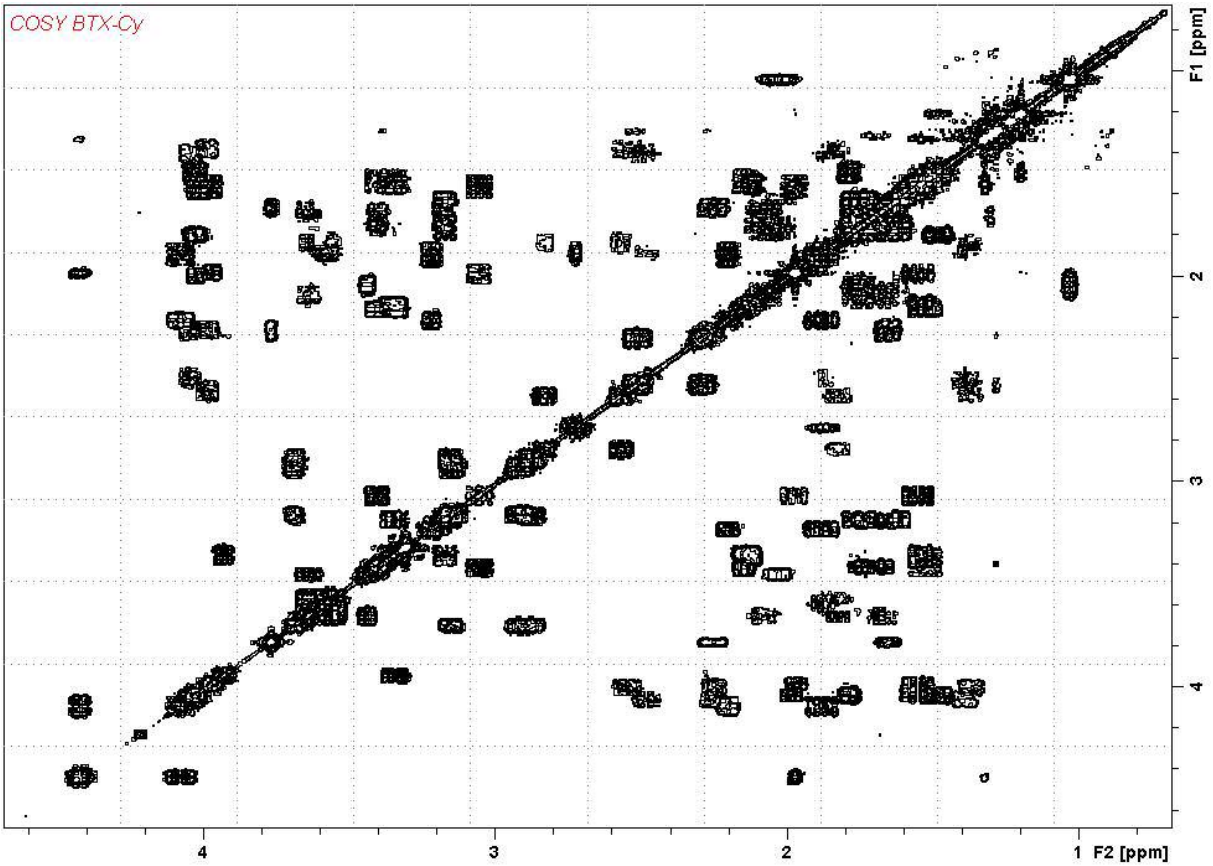
^{13}C NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



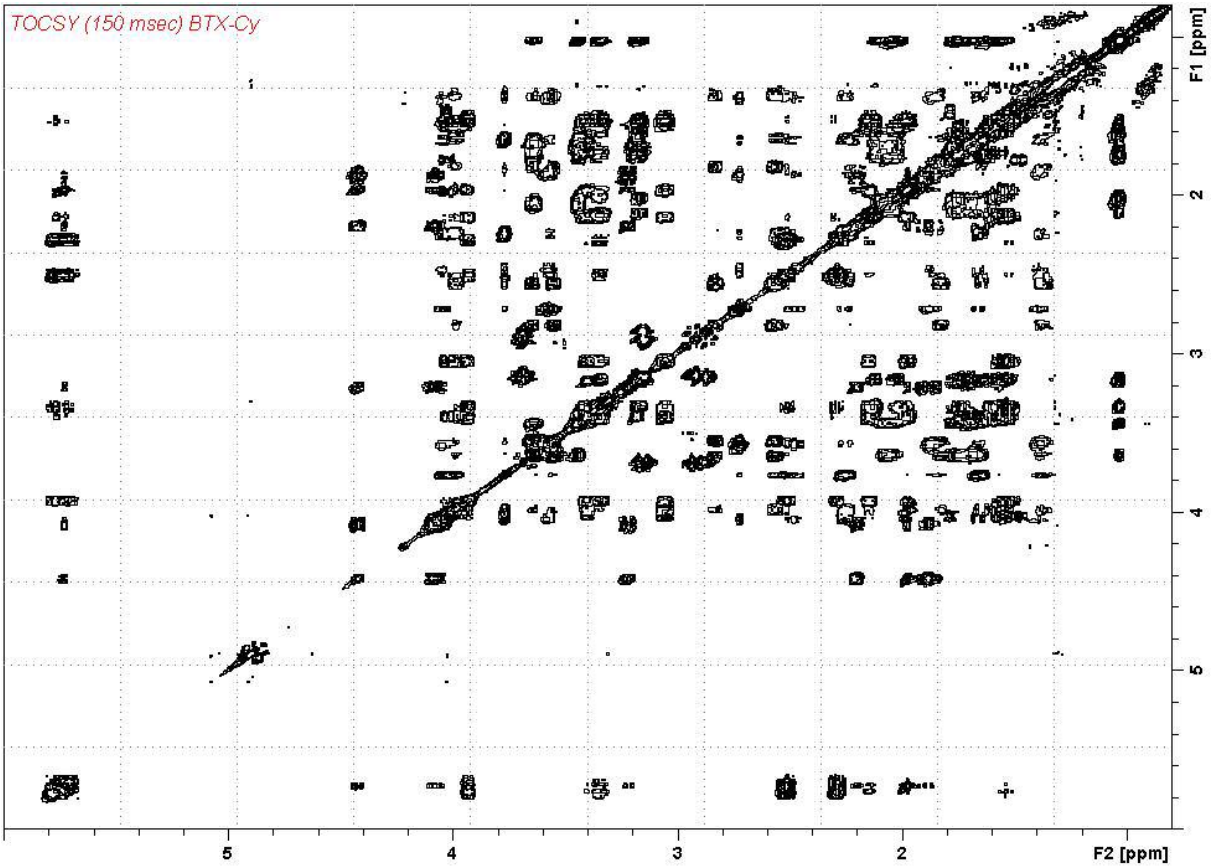
DEPT NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



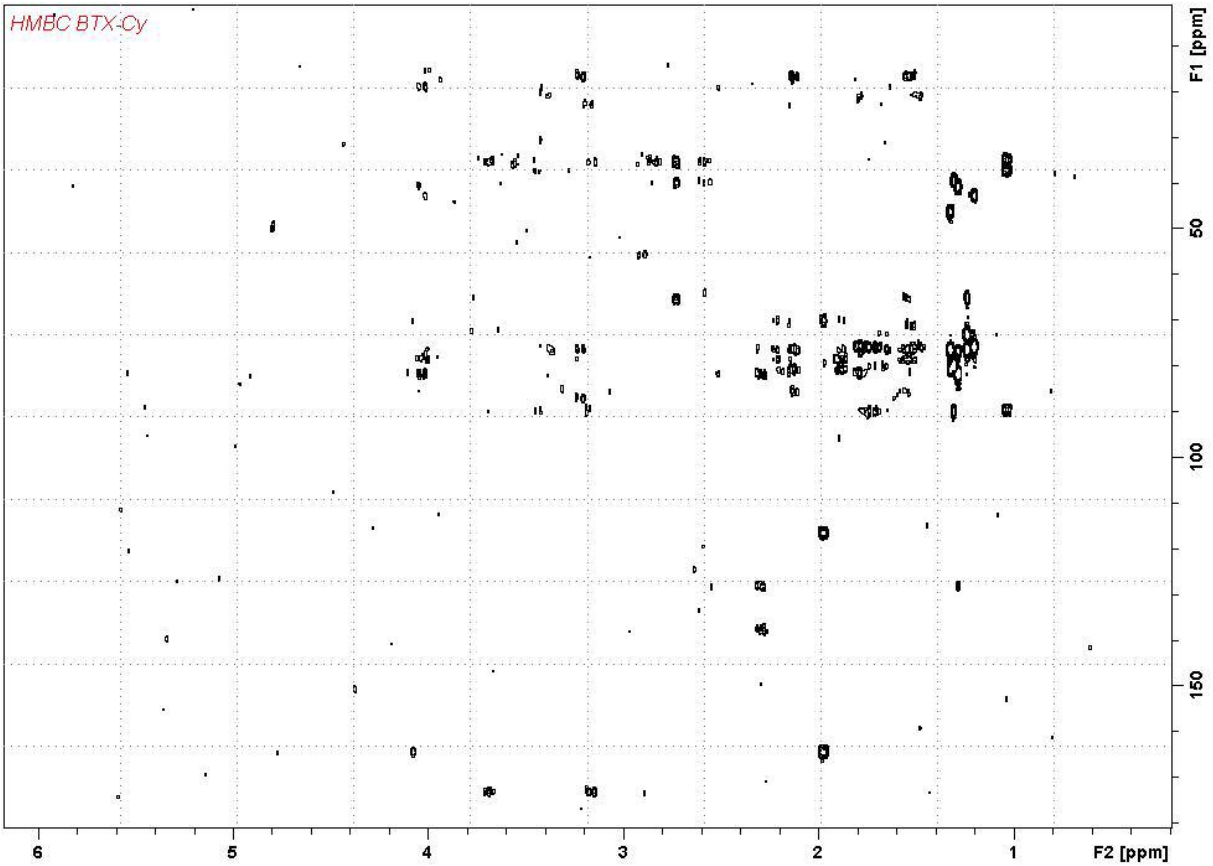
COSY NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



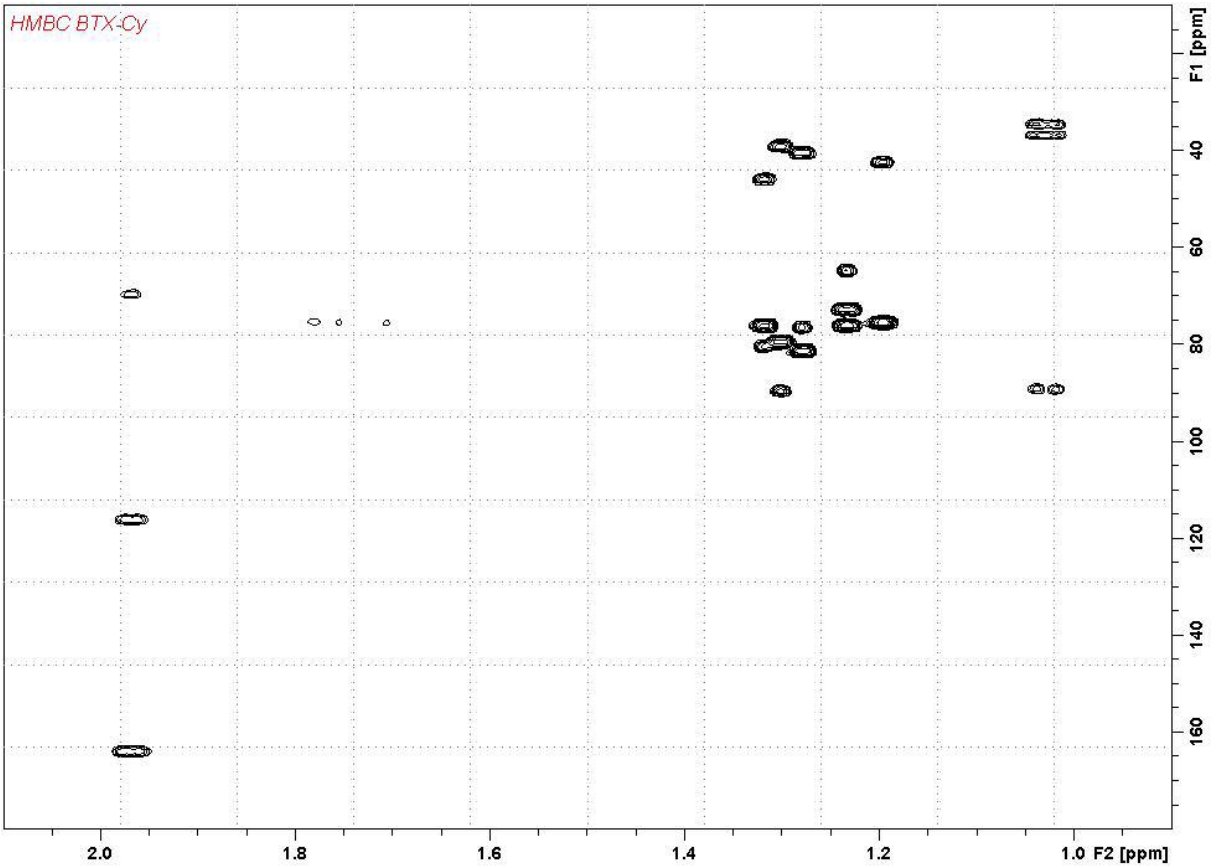
Expansion of the COSY NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



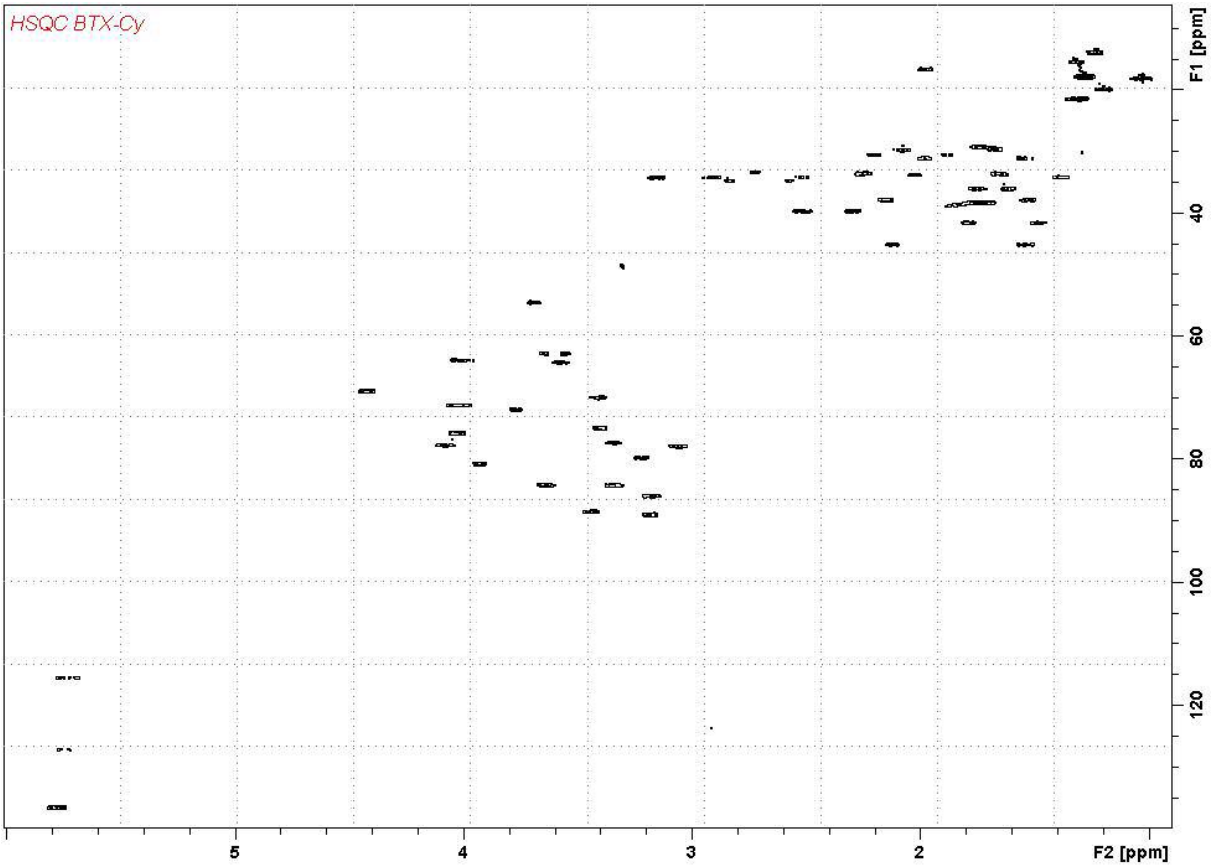
TOCSY NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



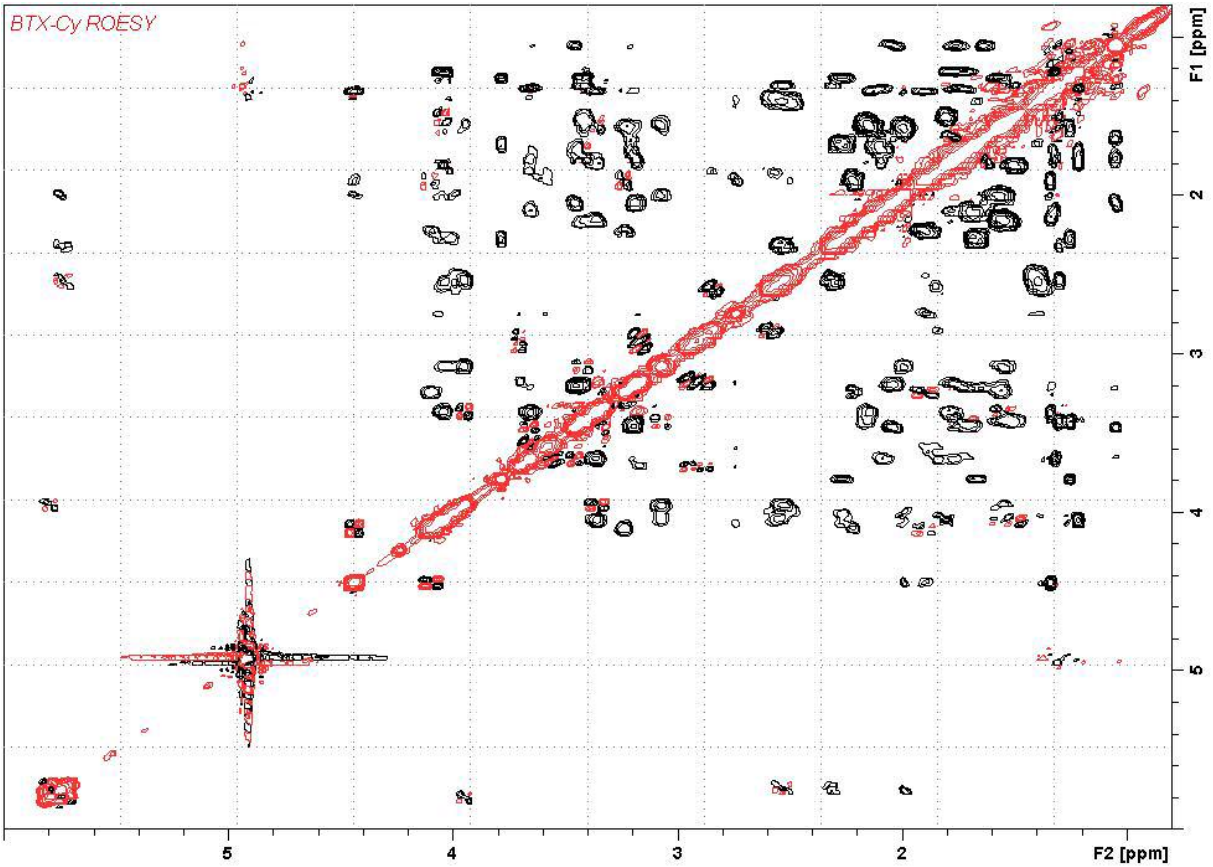
HMBC NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



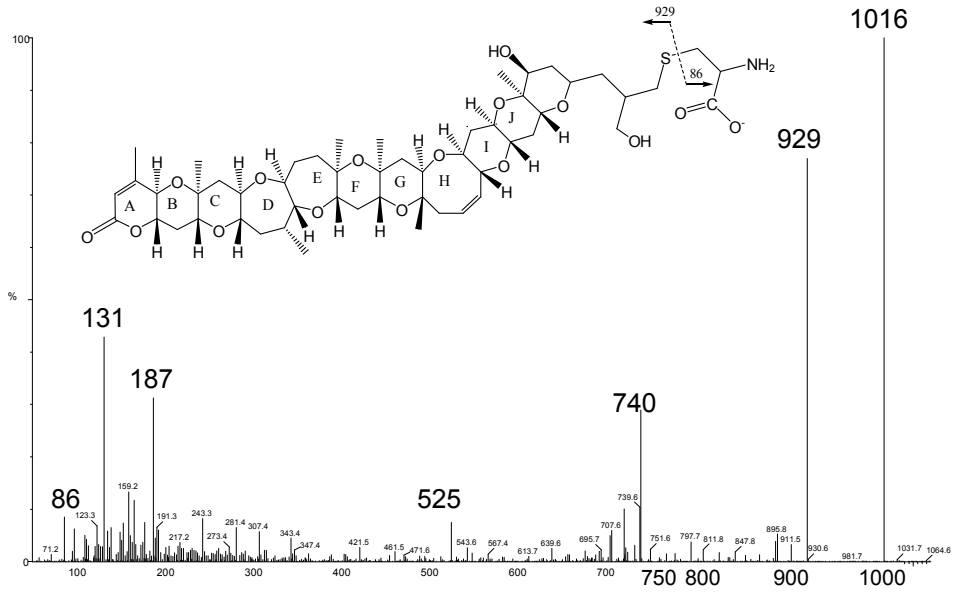
Expansion of the HMBC NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.



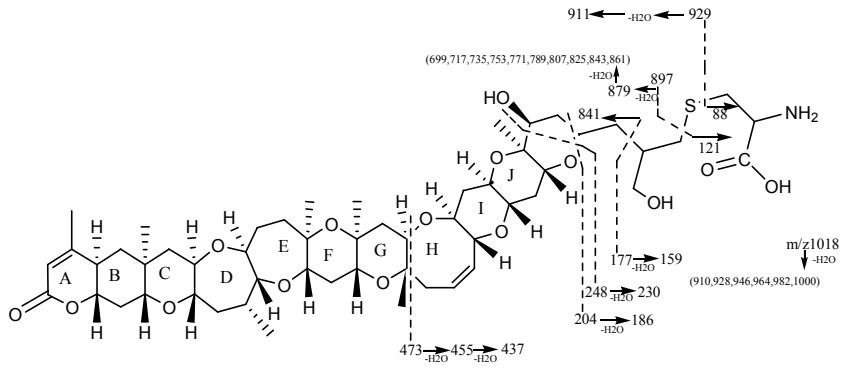
HSQC NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.

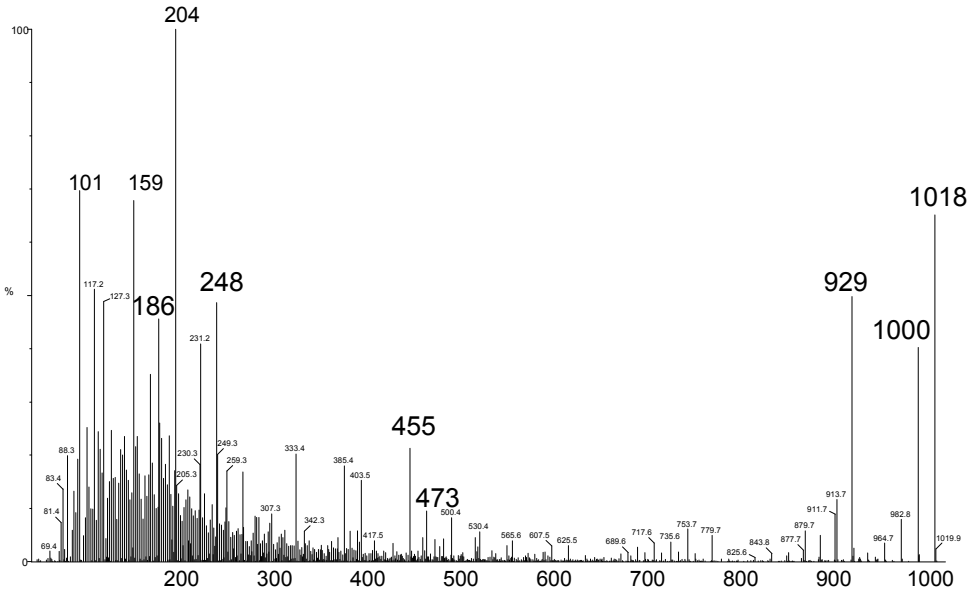


ROESY NMR spectrum of *S*-desoxybrevetoxin-B2 (**4**) in CD_3OD.

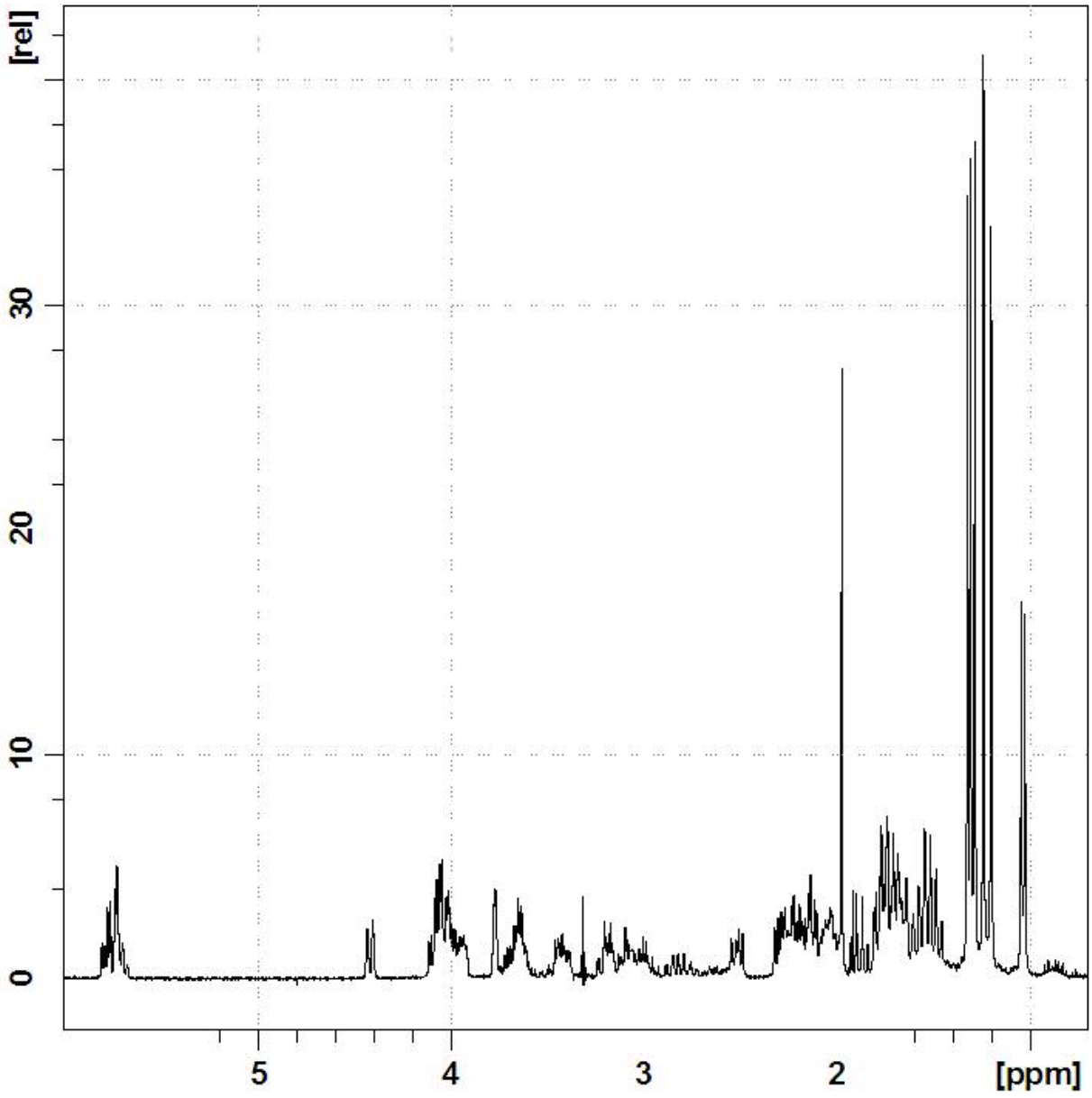


Negative ion MS/MS spectrum of *S*-desoxybrevetoxin-B2 (**4**) from LC-MS analysis

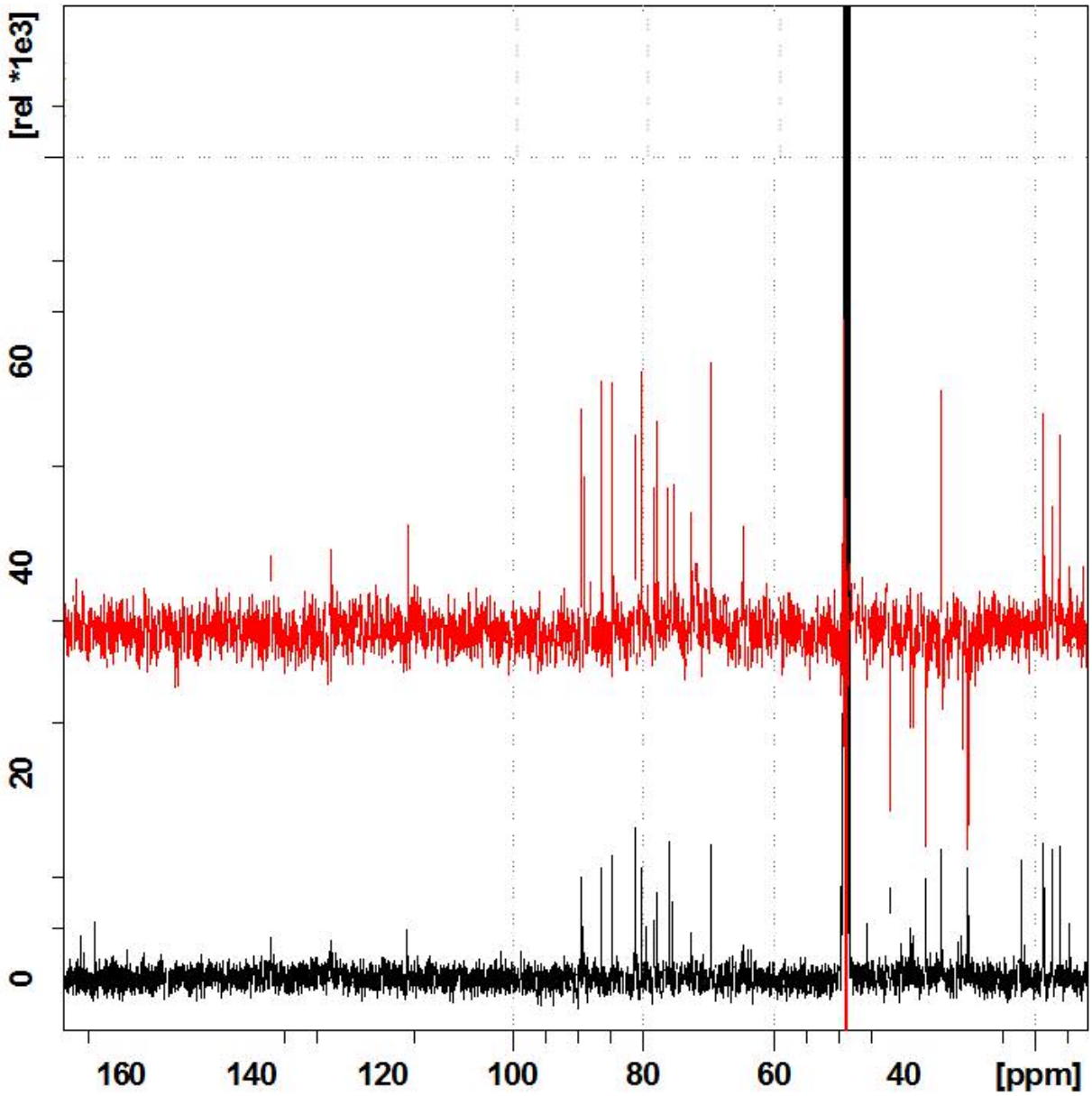




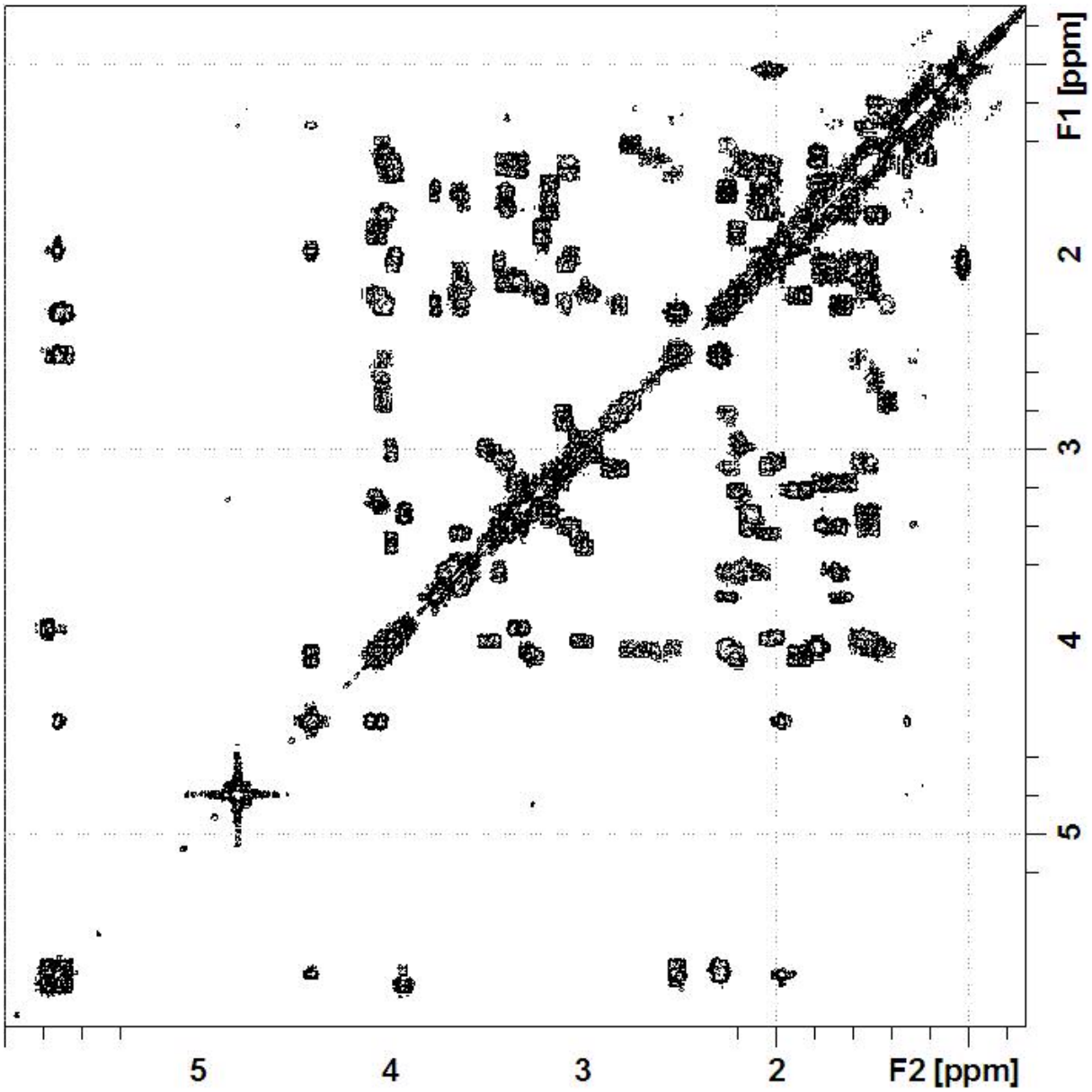
Positive ion MS/MS spectrum of *S*-desoxybrevetoxin-B2 (**4**) from LC-MS analysis



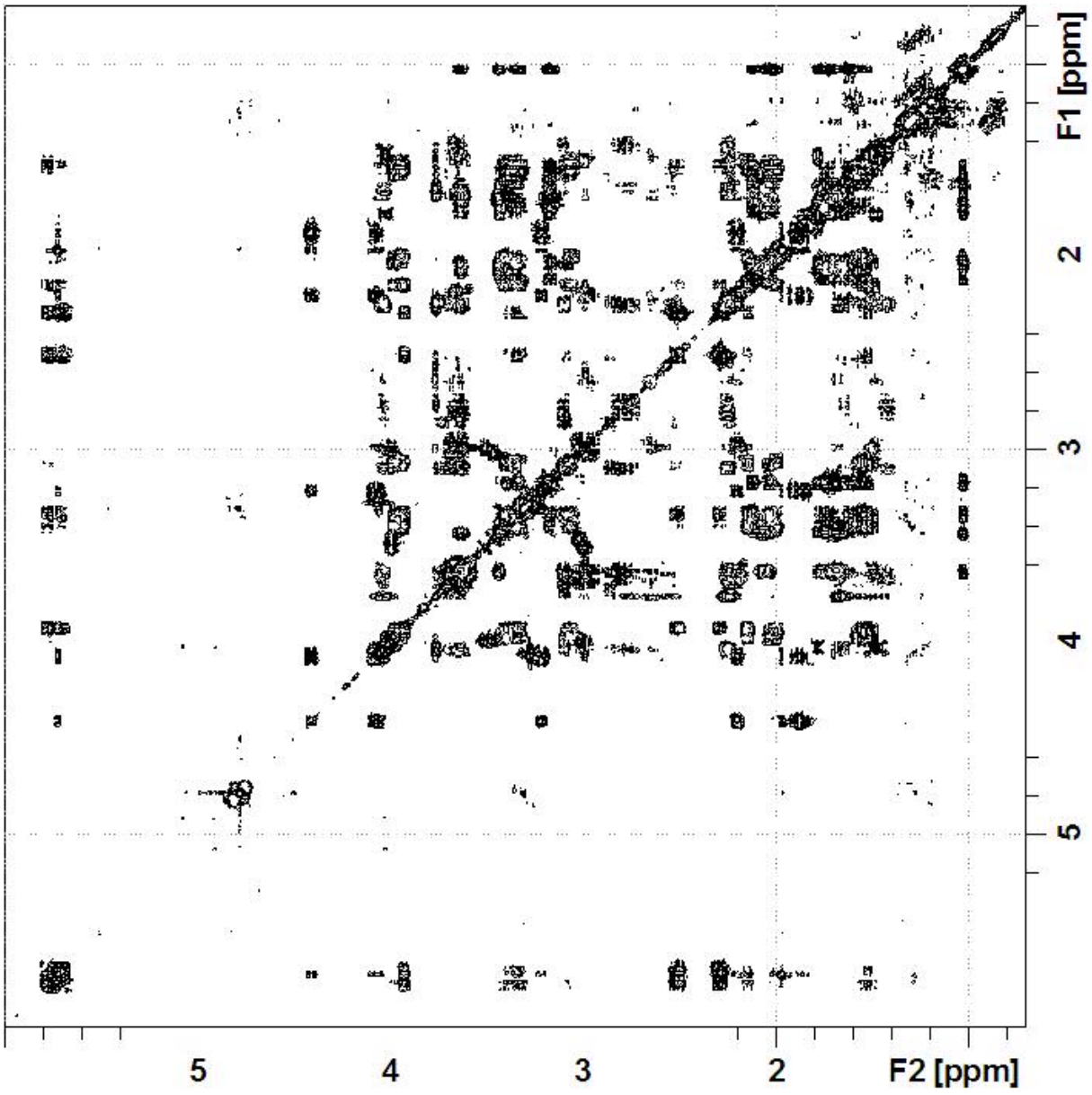
^{1}H NMR spectrum of brevetoxin-B2 (**5**) in CD_3OD with presaturation of water peak.



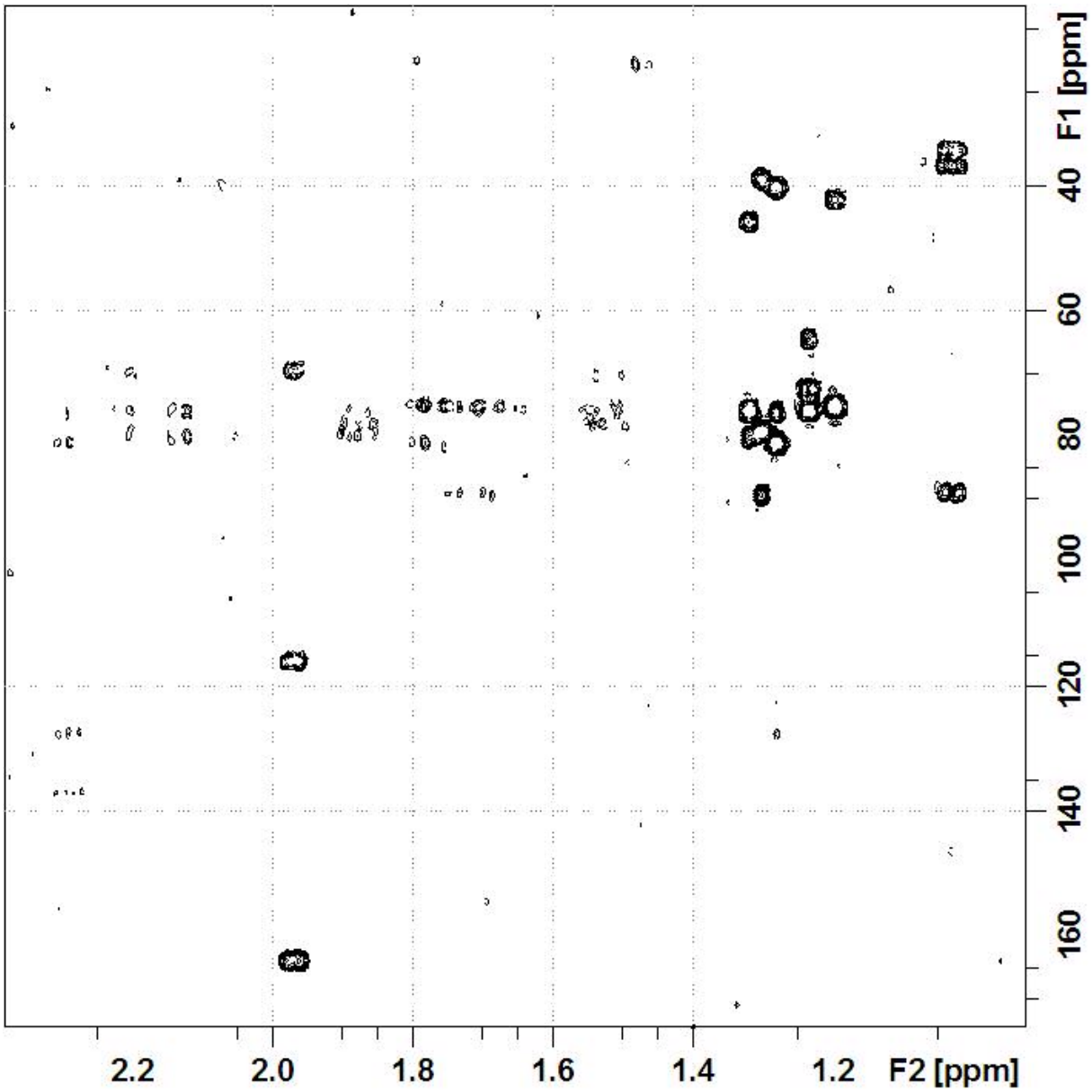
^{13}C (bottom, in black) and DEPT135 (top, in red) NMR spectra of brevetoxin-B2 (**5**) in CD_3OD.



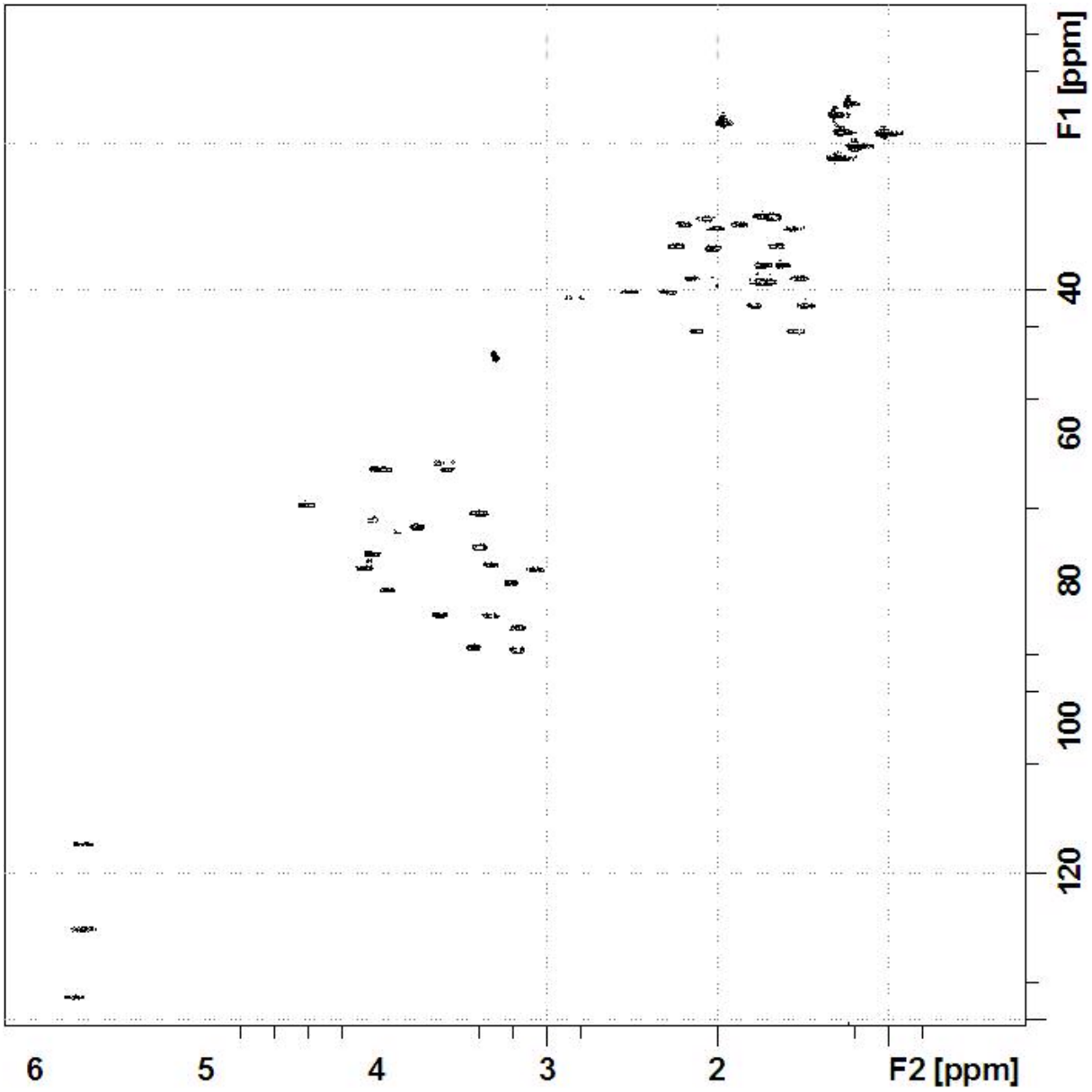
COSY NMR spectrum of brevetoxin-B2 (**5**) in CD_3OD.



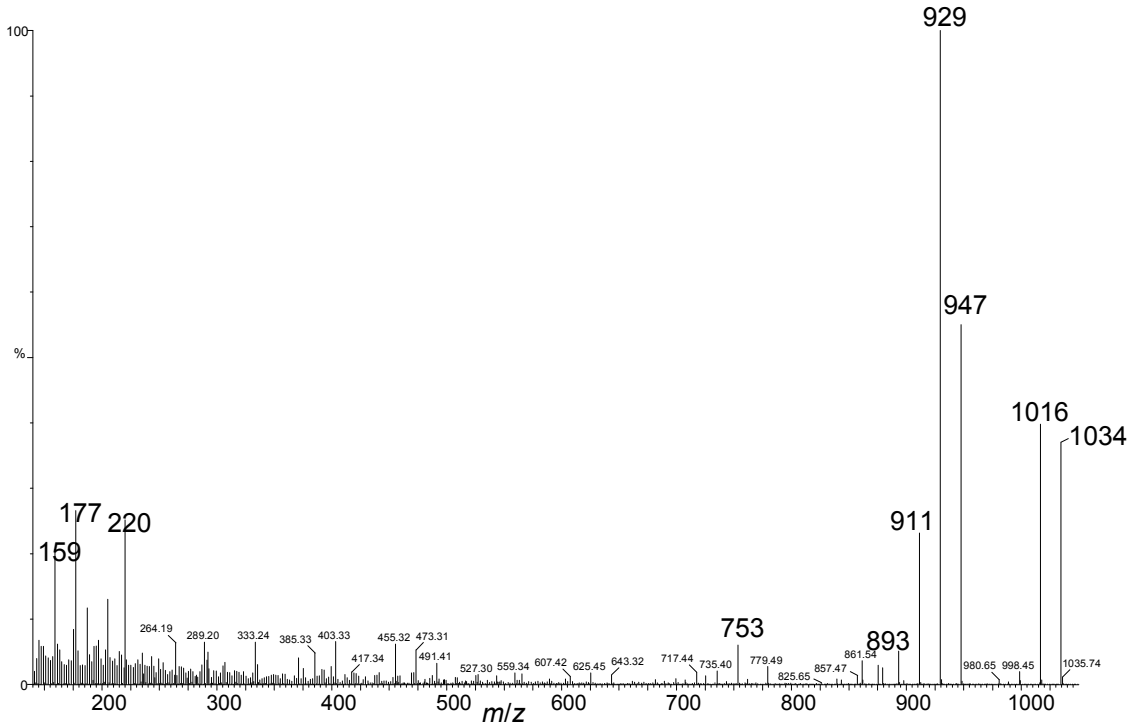
TOCSY NMR spectrum of brevetoxin-B2 (**5**) in CD_3OD.



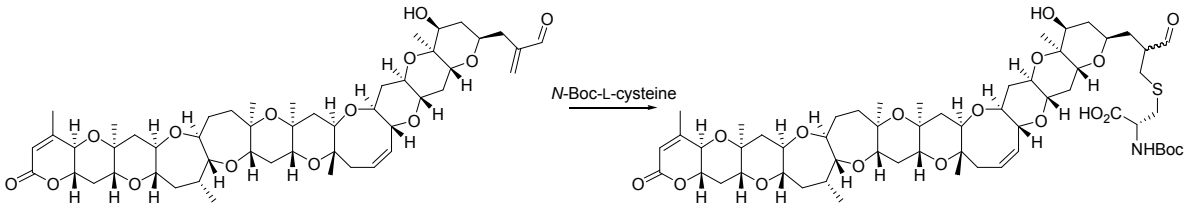
Expansion of HMBC NMR spectrum of brevetoxin-B2 (**5**) in CD_3OD, showing correlations exhibited by the methyl group protons.



HSQC NMR spectrum of brevetoxin-B2 (**5**) in CD_3OD, showing correlations exhibited by atoms in rings A–K. Correlations attributable to isomeric side-chain atoms were only visible when plotted into the noise level.

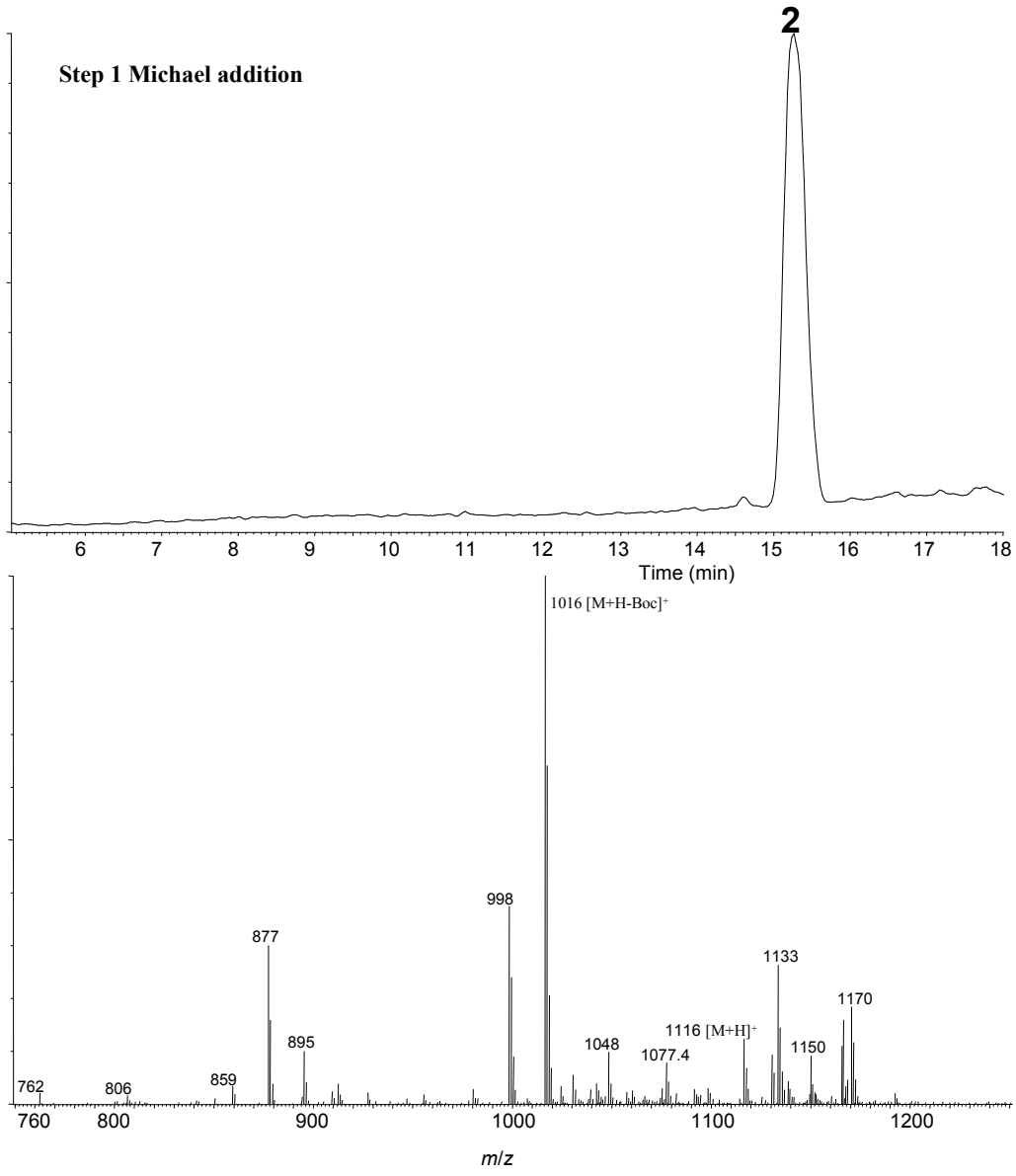


Positive ion MS/MS spectrum of purified semi-synthetic brevetoxin-B2 (**5**) obtained during LC-MS analysis.

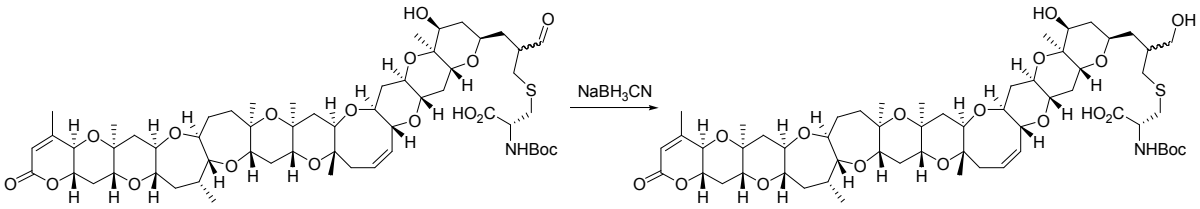


Brevetoxin-B (**1**)
$[M + H]^+$ *m/z* 895.5

N-Boc-*S*-desoxy-42-oxobrevetoxin-B2 (**2**)
$[M + H]^+$ *m/z* 1116.6

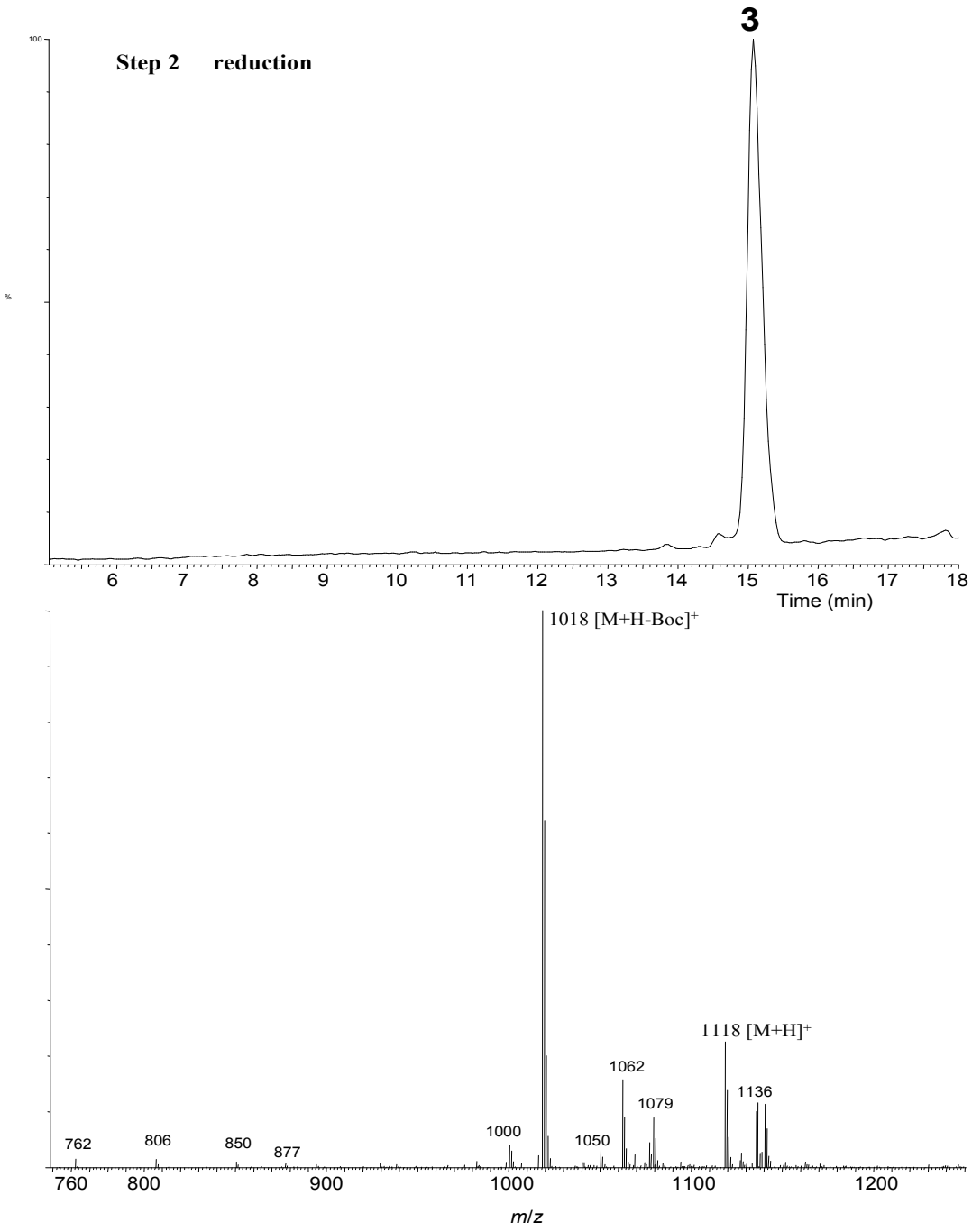


LC-MS analysis (method 1) of the crude reaction from the Michael addition of cysteine to **1**, and (bottom) mass spectrum from the peak for **2** extracted from the chromatogram. Note the facile in-source loss of the Boc group from $[M+H]^+$.

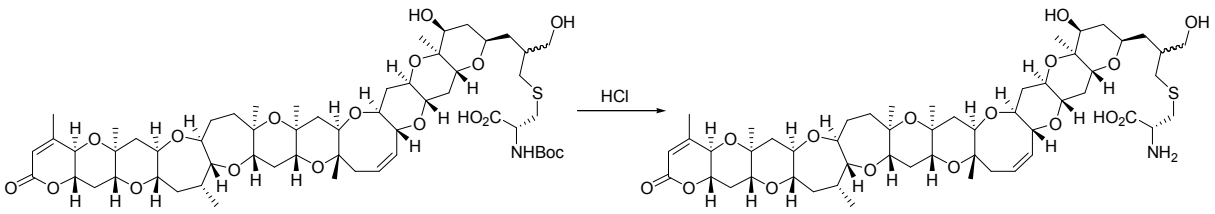


N-Boc-*S*-desoxy-42-oxobrevetoxin-B2 (**2**)
$[M + H]^+$ *m/z* 1116.6

N-Boc-*S*-desoxybrevetoxin-B2 (**3**)
$[M + H]^+$ *m/z* 1118.6



LC-MS analysis (method 1) of the crude reaction from the reduction of **2** with $NaBH_3CN$, and (bottom) mass spectrum from the peak for **3** extracted from the chromatogram. Note the ready loss of Boc group in-source.

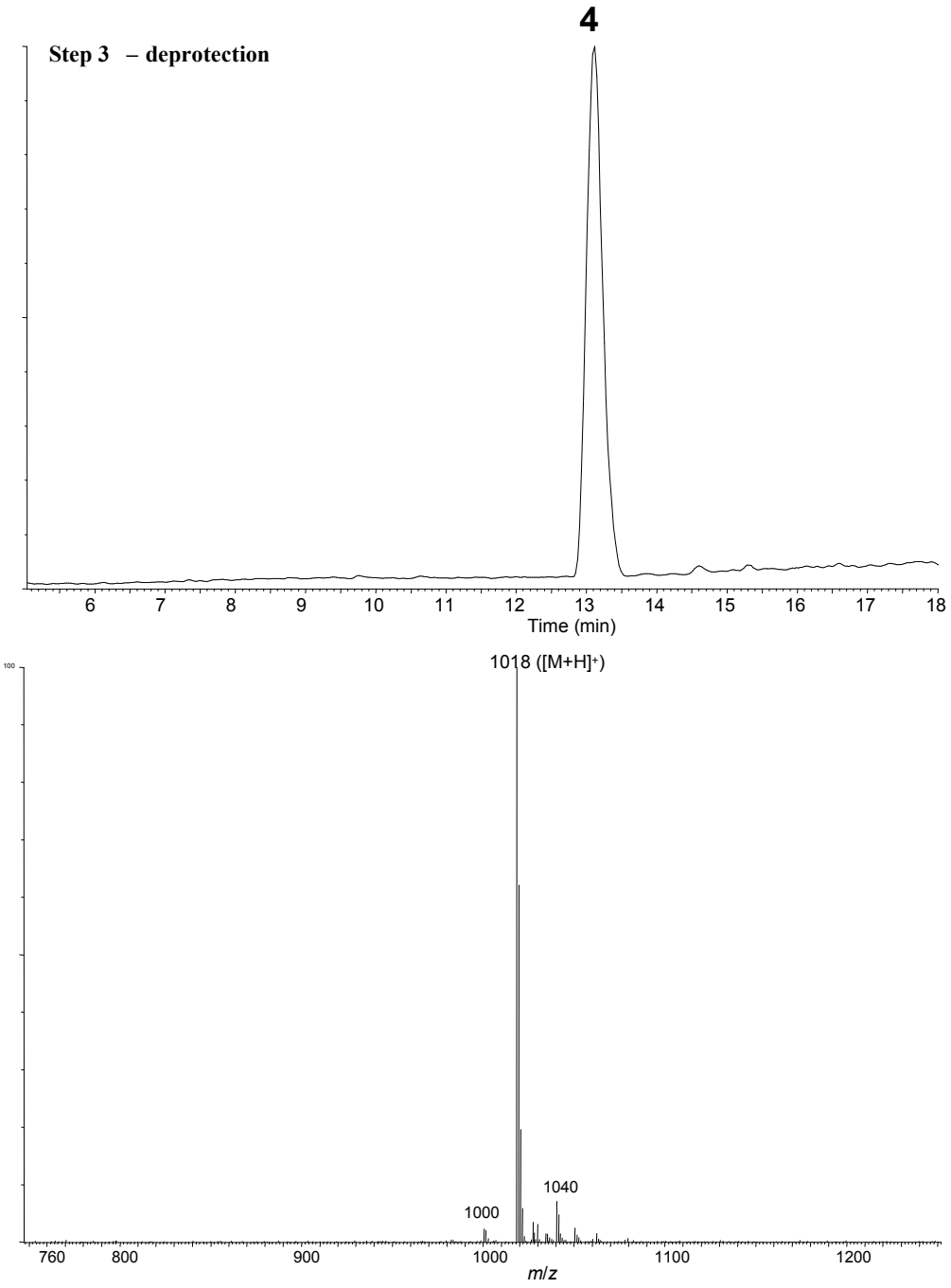


N-Boc-*S*-desoxybrevetoxin-B2 (**3**)

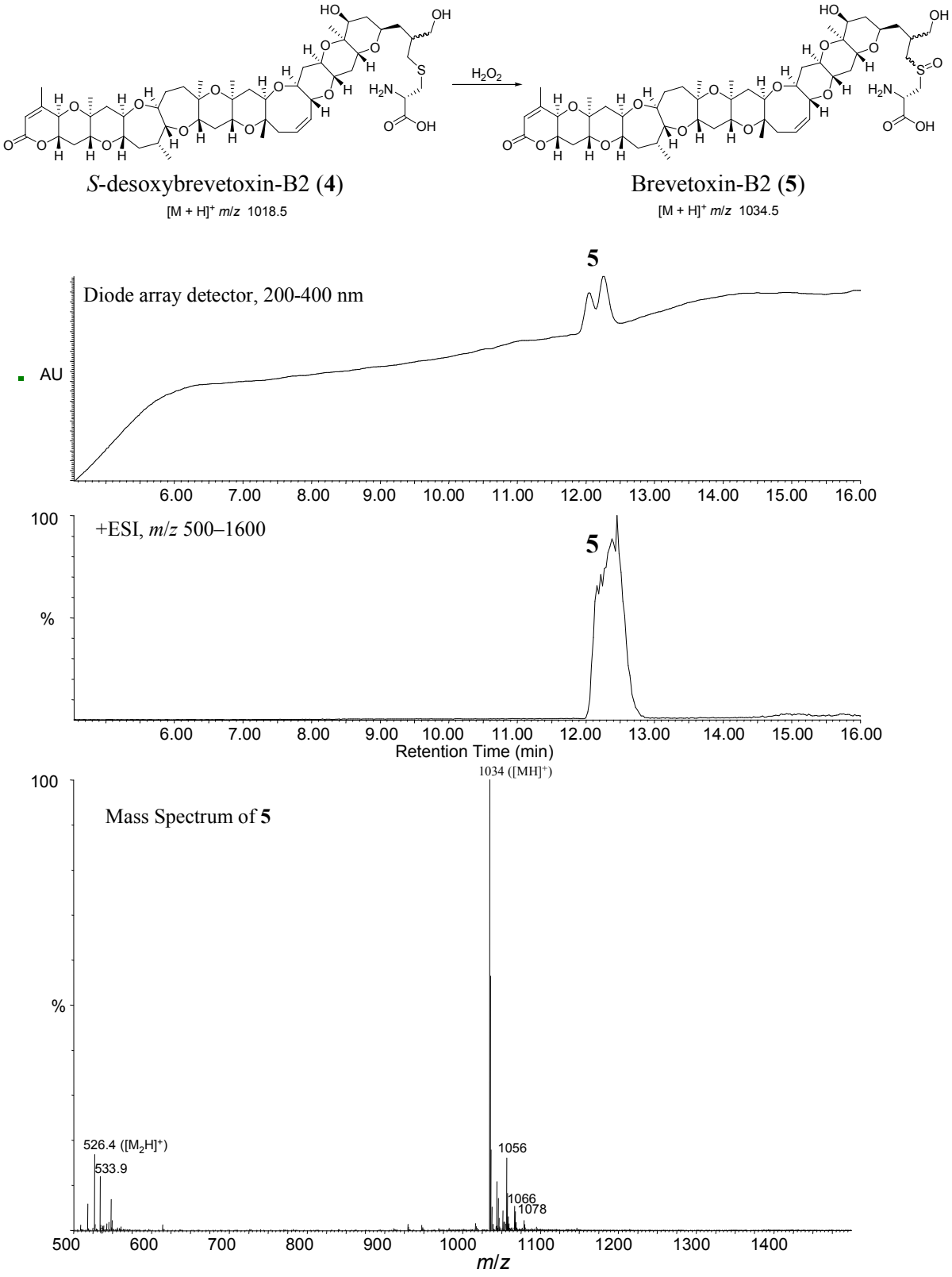
$[M + H]^+$ *m/z* 1118.6

S-desoxybrevetoxin-B2 (**4**)

$[M + H]^+$ *m/z* 1018.5

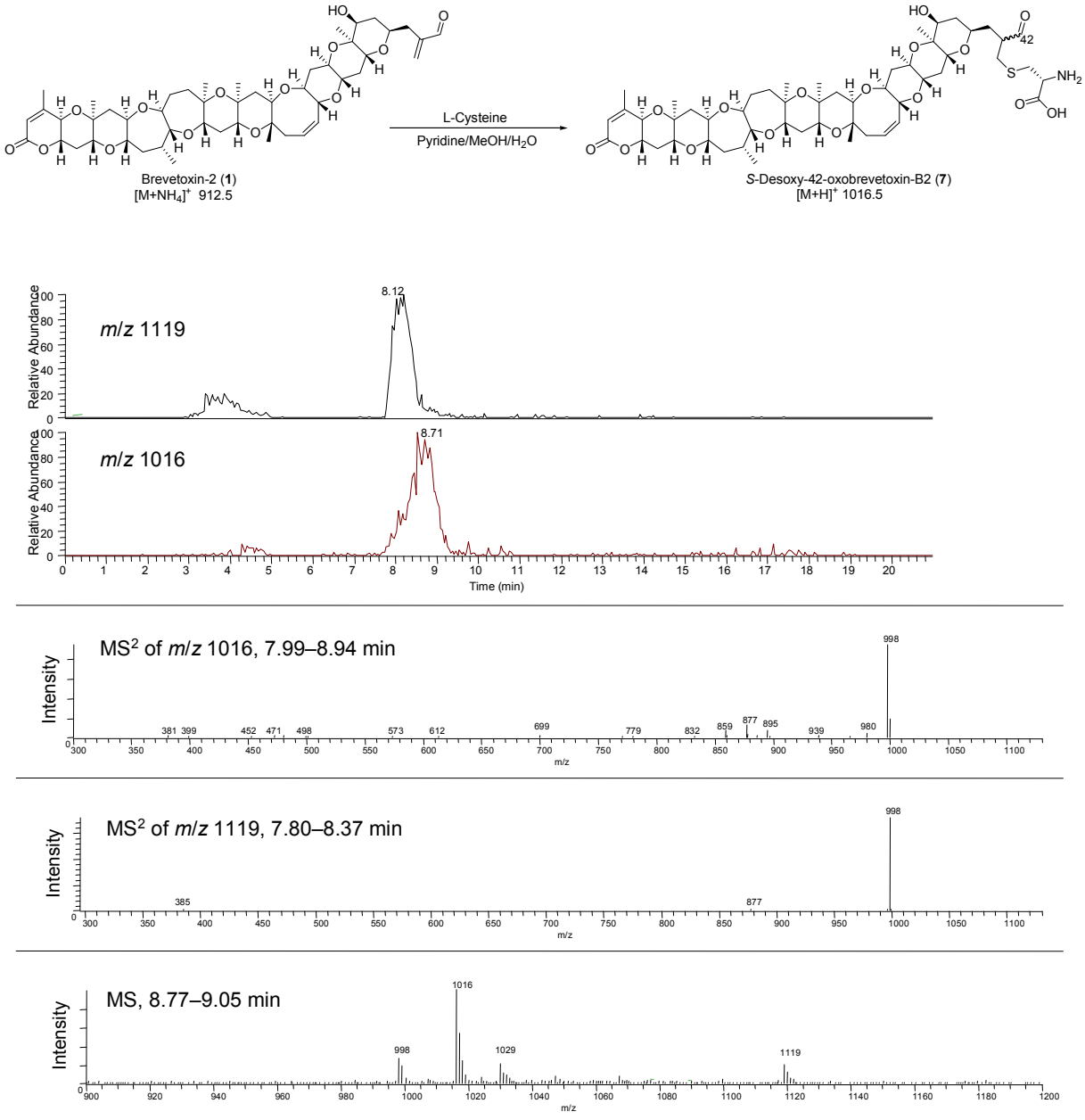


LC-DAD-MS analysis (method 1) of the crude reaction from the deprotection of **3** with HCl, and (bottom) mass spectrum from the peak for **4** extracted from the chromatogram.



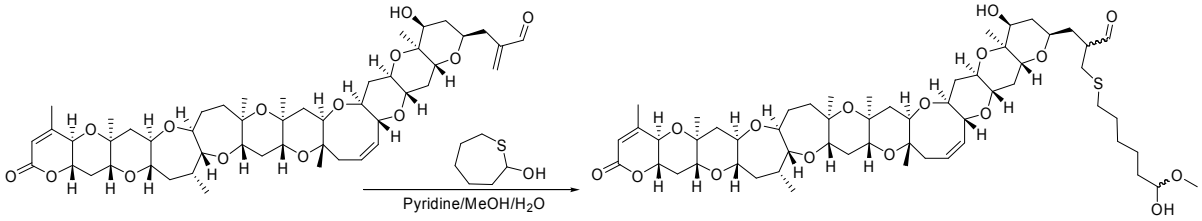
LC-DAD-MS analysis (method 1) of the crude reaction product from the oxidation of **4** with H_2O_2, and (bottom) mass spectrum from the peak for **5** extracted from the chromatogram.

LC-MS analysis of brevetoxin-2 (**1**) after reaction with excess cysteine



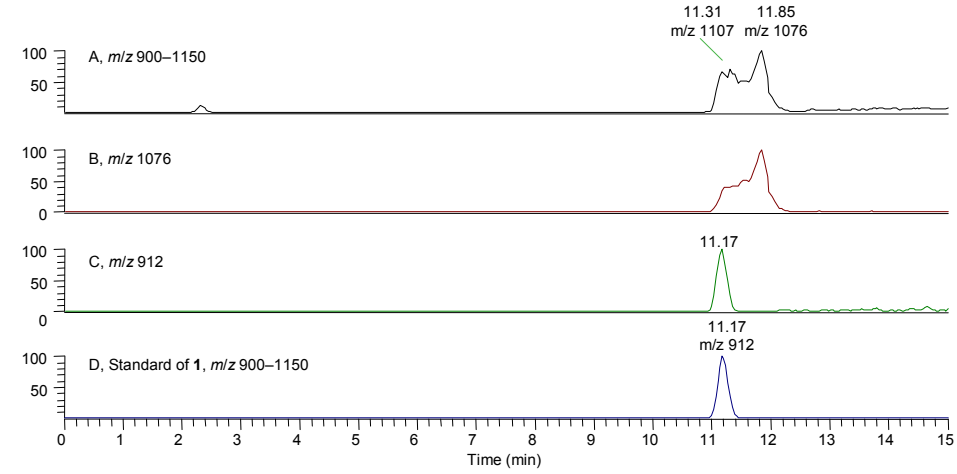
LC-MS analysis (method 2) of brevetoxin-2 (**1**) after reaction with excess cysteine. Note two products formed. The minor product's mass was consistent with the expected adduct (**7**, *m*/*z* 1016), but the major product corresponded to addition of a second cysteine with loss of water (*m*/*z* 1119). Below are shown MS^2 spectra of *m*/*z* 1016 (**7**) and 1119, respectively, followed by the mass spectrum of **7** (8.77–9.05 min) overlapping slightly with the dicysteinyl adduct (*m*/*z* 1119).

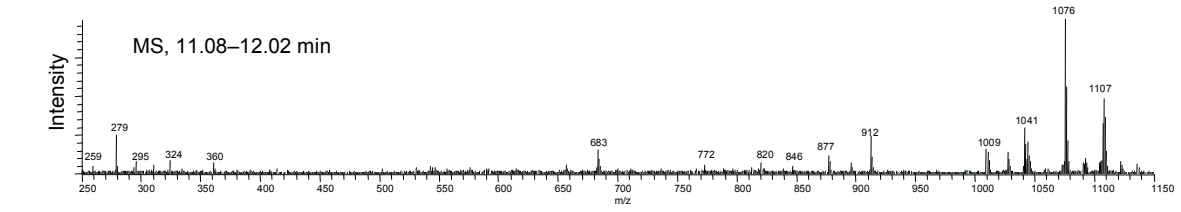
LC-MS analysis of brevetoxin-2 (**1**) after reaction with excess 2-hydroxythiepan

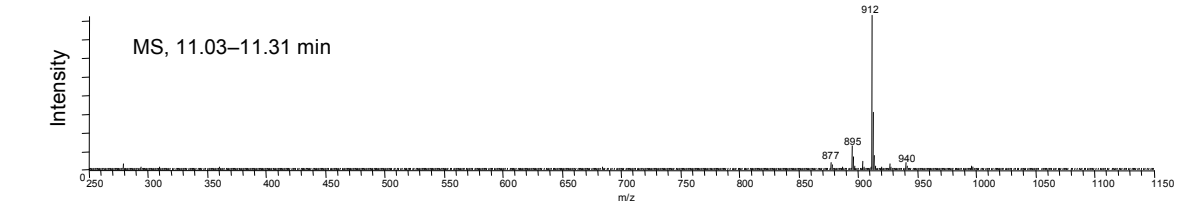


Brevetoxin-2 (**1**)
$[M+NH_4]^+$ 912.5

8
$[M+NH_4]^+$ 1076.6

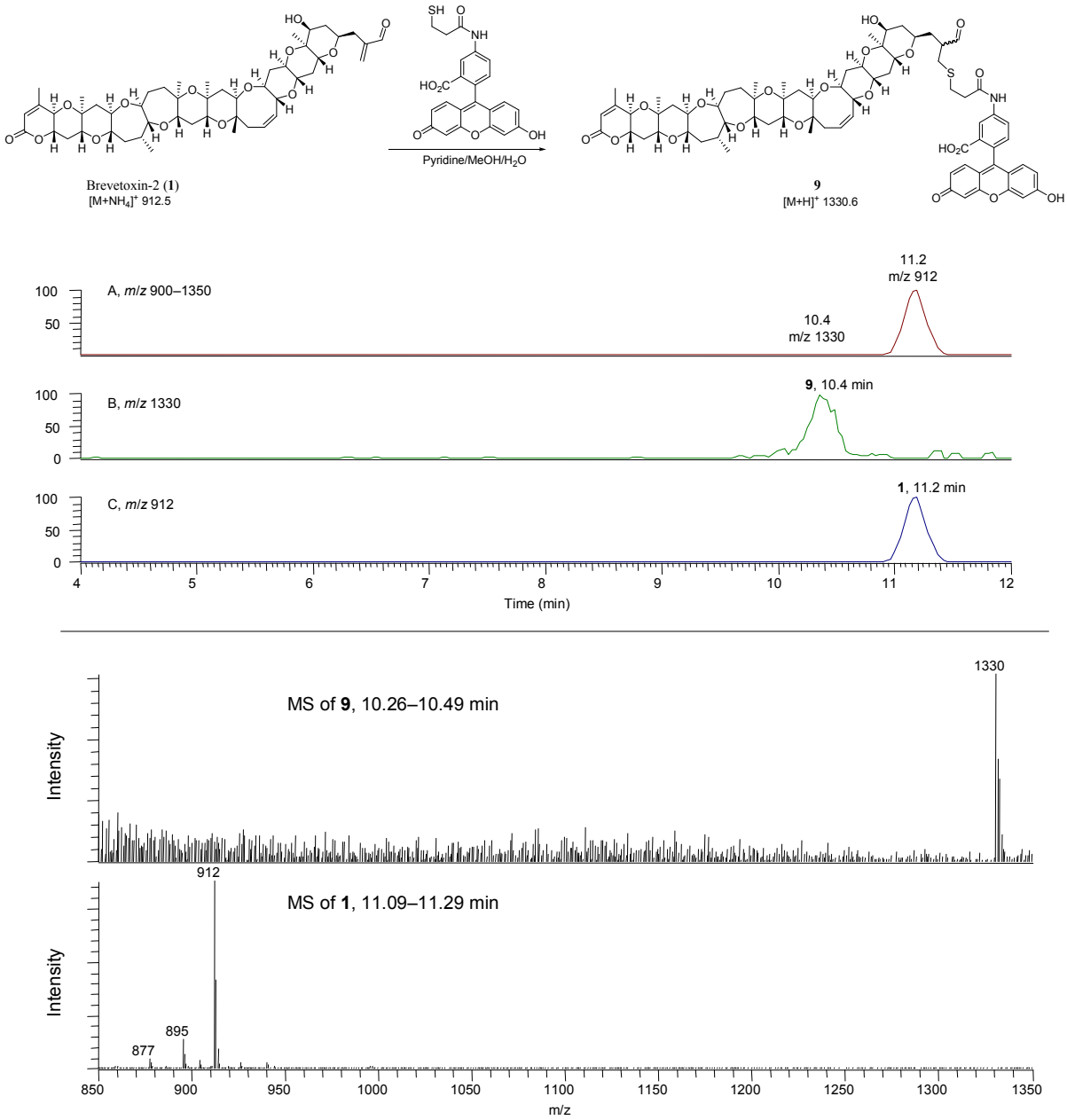






The top three chromatograms (A–C) are from LC-MS analysis (method 2) of brevetoxin-2 (**1**) after reaction with excess 2-hydroxythiepan, while the bottom chromatogram (D) is from a standard of **1**. The mass of the major product (**8**) (*m*/*z* 1076) was consistent with the ammonium ion adduct of the expected product in its hemiacetal form, while a small amount of unreacted **1** (*m*/*z* 912) remained. Below are shown mass spectra of the product peak (11.08–12.02 min) and of a standard of **1** (11.03–11.31 min) extracted from the chromatograms.

LC-MS analysis of brevetoxin-2 (**1**) after reaction with excess 2-[(5-fluoresceinyl)aminocarbonyl]ethyl mercaptan



The chromatograms (A–C) show LC-MS analysis (method 2) of brevetoxin-2 (**1**) after reaction with excess 2-[(5-fluoresceinyl)aminocarbonyl]ethyl mercaptan. Most of the brevetoxin did not react, but a small proportion was converted to a product with the appropriate mass for the $[M + H]^+$ ion (*m*/*z* 1330) of the expected fluoresceinyl adduct (**9**). The bottom sections show mass spectra of the product peak (10.22–12.52 min) and of a standard of **1**.

Summary of Doses, Deaths and Death Times for Dihydrobrevetoxin-B (**6**), *S*-Desoxybrevetoxin-B2 (**4**), andBrevetoxin-B2 (**5**) Administered i.p. to Mice

1. Dihydrobrevetoxin-B. LD_{50} 250 μg/kg

Dose (μg/kg)	Result	Time to death
199	Survived	-
250	Died	0.5 hr
199	Survived	-
250	Survived	-
310	Killed in extremis	24.0 hr
250	Killed in extremis	24.0 hr

2. *S*-Desoxybrevetoxin-B2. LD_{50} 211 μg/kg

Dose (μg/kg)	Result	Time to death
320	Died	0.6 hr
250	Died	0.4 hr
200	Survived	-
250	Died	6.5 hr
200	Survived	-
250	Died	1.0 hr

3. Brevetoxin-B2. LD_{50} 400 μg/kg

Dose (μg/kg)	Result	Time to death
320	Survived	-
400	Survived	-
500	Died	2.5 hr
400	Died	1.0 hr
320	Survived	-
400	Died	1.2 hr