

**Neo-actinomycins A and B, natural actinomycins bearing the
5*H*-oxazolo[4,5-*b*]phenoxazine chromophore, from the
marine-derived *Streptomyces* sp. IMB094**

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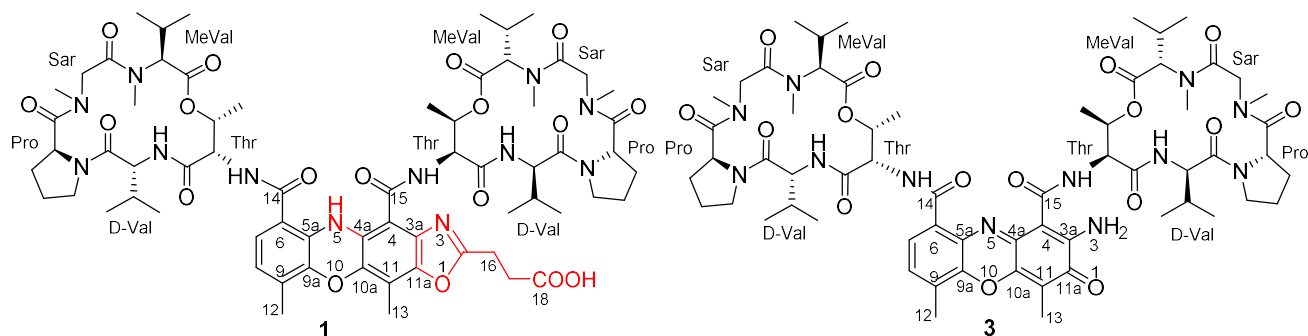


Table S1. ^{13}C NMR Spectroscopic Data for Neo-actinomycin (**1**) and Actinomycin D (**3**) in $\text{DMSO-}d_6^a$

no.	pentapeptidolactone (α -ring)			no.	pentapeptidolactone (β -ring)		
	1	3	$\Delta\delta$		1	3	$\Delta\delta$
Thr 1	169.2	168.2	1.0	Thr 1	169.4	168.3	1.1
2	53.9	54.4	-0.5	2	54.9	54.4	0.5
3	72.3	73.5	-1.2	3	71.9	73.5	-1.6
4	16.3	16.9	-0.6	4	16.7	16.9	-0.2
Val 1	170.6	172.7	-2.1	Val 1	170.7	172.7	-2.0
2	57.4	57.6	-0.2	2	57.4	57.7	-0.3
3	30.1	30.8	-0.7	3	30.2	30.8	-0.6
4	18.6	18.6	0.0	4	18.6	18.6	0.0
5	19.1	19.1	0.0	5	19.1	19.1	0.0
Pro 1	173.0	172.3	0.7	Pro 1	173.0	172.3	0.7
2	54.8	55.9	-1.1	2	54.8	55.9	-1.1
3	31.1	30.5	0.6	3	31.2	30.5	0.7
4	22.7	22.5	0.2	4	22.8	22.5	0.3
5	46.4	46.8	-0.4	5	46.4	46.8	-0.4
Sar 1	167.0	166.7	0.3	Sar 1	167.1	166.9	0.2
2	51.2	50.9	0.3	2	51.2	50.9	0.3
NMe	34.4	34.3	0.1	NMe	34.4	34.3	0.1
MeVal 1	168.1	168.1	0.0	MeVal 1	168.2	168.1	0.1
2	69.5	69.3	0.2	2	69.6	69.4	0.2
3	26.5	26.7	-0.2	3	26.5	26.7	-0.2
4	21.0	21.1	-0.1	4	21.1	21.1	0.0
5	18.8	18.6	0.2	5	18.8	18.6	0.2
NMe	38.6	38.5	0.1	NMe	38.8	38.5	0.3
chromophore							
2	165.8			10a	139.5	144.7	-5.2
3a	132.9	146.5	-13.6	11	111.8	111.9	-0.1
4	100.2	102.2	-2.0	11a	143.5	178.7	-35.2
4a	132.0	145.5	-13.5	12	14.8	14.6	0.2
5a	131.3	129.3	2.0	13	8.9	7.6	1.3
6	113.4	131.6	-18.2	14	166.6	166.1	0.5
7	122.0	125.1	-3.1	15	165.4	165.9	-0.5
8	122.0	129.6	-7.6	16	23.3		
9	127.5	127.0	0.5	17	30.3		
9a	140.7	140.0	0.7	18	173.1		

^a ^{13}C NMR data were recorded at 150 MHz. For comparison of NMR data of **1** and **3**, the atom numbers in **3** are labelled as the same as

1.

Table S2. ¹H NMR Spectroscopic Data for Neo-actinomycin (1) and Actinomycin D (3) in DMSO-*d*₆^a

pentapeptidolactone (α -ring)			pentapeptidolactone (β -ring)		
no.	1	3	no.	1	3
Thr 2	4.97, dd (9.0, 2.4)	4.50, dd (7.2, 2.4)	Thr 2	4.84, dd (8.4, 2.4)	4.50, dd (7.2, 2.4)
3	5.14, qd (6.0, 2.4)	5.07, m	3	5.24, qd (6.0, 2.4)	5.07, m
4	1.17, d (6.0)	1.18, d (6.6)	4	1.20, d (6.0)	1.18, d (6.6)
NH	7.25, d (9.0)		NH	9.66, d (8.4)	
Val 2	3.50, m	3.55, m	Val 2	3.49, m	3.55, m
3	1.88, m	1.96, m	3	1.88, m	1.96, m
4	0.92, d (6.6)	0.91, d (6.0)	4	0.94, d (6.6)	0.91, d (6.0)
5	0.69, d (7.2)	0.71, d (6.6)	5	0.70, (7.2)	0.71, d (6.6)
NH	8.42, d (6.0)		NH	8.29, d (5.4)	
Pro 2	6.24, dd (9.0, 3.0)	6.06, brd (10.2)	Pro 2	6.21, dd (9.0, 3.0)	6.05, brd (10.2)
3	2.10, m; 1.74, m	2.43, m; 1.73, m	3	2.10, m, 1.74, m	2.43, m; 1.73, m
4	1.91, m; 1.67, m	2.00, m; 1.96, m	4	1.91, m, 1.67, m	2.00, m; 1.96, m
5	3.49, m; 3.27, m	3.81, m; 3.50, m	5	3.50, m, 3.27, m	3.81, m; 3.50, m
Sar 2	4.80, d (18.0); 4.08, d (18.0)	4.56, d (18.0); 4.04, d (18.0)	Sar 2	4.78, d (18.0); 4.08, d (18.0)	4.56, d (18.0); 4.04, d (18.0)
NMe	2.75, s	2.72, s	NMe	2.75, s	2.72, s
MeVal 2	3.23, d (9.6)	3.13, d (9.6)	MeVal 2	3.23, d (9.6)	3.07, d (9.6)
3	2.54, m	2.46, m	3	2.54, m	2.46, m
4	0.97, d (6.6)	0.97, d (6.6)	4	0.98, d (6.6)	0.97, d (6.6)
5	0.79, d (6.6)	0.74, d (6.6)	5	0.80, d (6.6)	0.74, d (6.6)
NMe	3.19, s	2.94, s	NMe	3.19, s	2.92, s
chromophore					
5	11.74, s		13	2.27, s	2.50, s
7	7.11, d (8.4)	7.41, d (7.8)	16	3.17, m	
8	6.62, d (8.4)	7.41, d (7.8)	17	2.89, m	
12	2.13, s	2.14, s			

^a ¹H NMR data were recorded at 600 MHz. For comparison of NMR data of **1** and **3**, the atom numbers in **3** are labelled as the same as **1**.

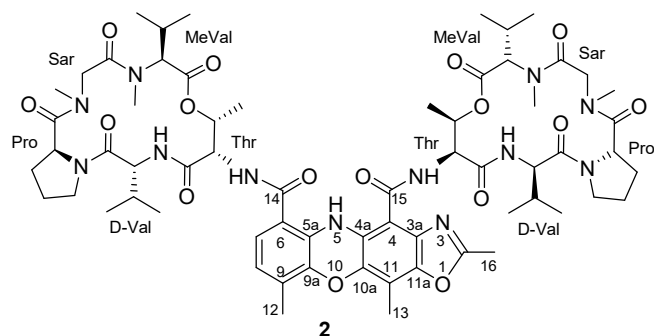


Table S3. ^{13}C NMR Spectroscopic Data for Neo-actinomycin B (2)^a

no.	pentapeptidolactone (α -ring)		no.	pentapeptidolactone (β -ring)	
	DMSO- <i>d</i> ₆	MeOH- <i>d</i> ₄		DMSO- <i>d</i> ₆	MeOH- <i>d</i> ₄
Thr 1	169.2, C	171.3, C	Thr 1	169.4, C	171.6, C
2	54.0, CH	55.8, CH	2	54.8, CH	56.2, CH
3	72.2, CH	74.6, CH	3	71.9, CH	74.0, CH
4	16.4, CH ₃	17.3, CH ₃	4	16.8, CH ₃	17.2, CH ₃
Val 1	170.6, C	173.8, C	Val 1	170.8, C	173.9, C
2	57.4, CH	59.6, CH	2	57.4, CH	59.5, CH
3	30.1, CH	32.0, CH	3	30.2, CH	32.0, CH
4	18.7, CH ₃	19.8, CH ₃	4	18.6, CH ₃	19.8, CH ₃
5	19.1, CH ₃	19.5, CH ₃	5	19.1, CH ₃	19.5, CH ₃
Pro 1	173.0, C	175.5, C	Pro 1	173.1, C	175.7, C
2	54.7, CH	57.6, CH	2	54.4, CH	57.4, CH
3	31.3, CH ₂	32.5, CH ₂	3	31.2, CH ₂	32.5, CH ₂
4	22.8, CH ₂	24.1, CH ₂	4	22.8, CH ₂	24.1, CH ₂
5	46.4, CH ₂	48.4, CH ₂	5	46.4, CH ₂	48.3, CH ₂
Sar 1	167.2, C	168.7, C	Sar 1	167.0, C	168.6, C
2	51.3, CH ₂	53.0, CH ₂	2	51.1, CH ₂	52.9, CH ₂
NMe	34.4, CH ₃	35.7, CH ₃	3	34.4, CH ₃	35.7, CH ₃
MeVal 1	168.2, C	169.7, C	MeVal 1	168.3, C	169.8, C
2	69.5, CH	72.1, CH	2	69.5, CH	72.4, CH
3	26.5, CH	28.2, CH	3	26.3, CH	27.9, CH
4	21.0, CH ₃	21.7, CH ₃	4	21.1, CH ₃	21.7, CH ₃
5	18.8, CH ₃	19.4, CH ₃	5	18.8, CH ₃	19.4, CH ₃
NMe	38.6, CH ₃	39.6, CH ₃	NMe	38.6, CH ₃	40.1, CH ₃
chromophore					
2	165.5, C	164.5, C	9a	140.7, C	142.8, C
3a	133.2, C	135.1, C	10a	139.4, C	141.5, C
4	100.1, C	101.7, C	11	111.7, C	113.5, C
4a	132.1, C	134.1, C	11a	143.7, C	145.6, C
5a	131.4, C	132.8, C	12	14.8, CH ₃	15.4, CH ₃
6	113.5, C	115.6, C	13	8.9, CH ₃	9.2, CH ₃
7	122.0, CH	122.3, CH	14	166.6, C	168.8, C
8	122.1, CH	123.4, CH	15	163.7, C	167.7, C
9	127.5, C	129.1, C	16	14.4, CH ₃	14.5, CH ₃

^a ^{13}C NMR data were recorded at 600 MHz.

Table S4. ¹H NMR Spectroscopic Data for Neo-actinomycin B (2)^a

pentapeptidolactone (α -ring)			pentapeptidolactone (β -ring)		
no.	DMSO- <i>d</i> ₆	MeOH- <i>d</i> ₄	no.	DMSO- <i>d</i> ₆	MeOH- <i>d</i> ₄
Thr 2	4.94, brd (9.6)	4.95, d (2.4)	Thr 2	4.94, brd (9.6)	5.01, d (2.4)
3	5.15, qd (6.6, 2.4)	5.33, d (6.0, 2.4)	3	5.23, qd (6.6, 2.4)	5.39, qd (6.0, 2.4)
4	1.17, d (6.6)	1.31, d (6.0)	4	1.18, d (6.6)	1.29, d (6.0)
NH	7.28, d (9.6)		NH	9.64, d (9.6)	
Val 2	3.51, m	3.70, d (10.2)	Val 2	3.51, m	3.66, d (10.2)
3	1.86, m	2.04, m	3	1.86, m	2.06, m
4	0.93, d (6.6)	1.08, d (6.6)	4	0.94, d (6.6)	1.08, d (6.6)
5	0.69, d (6.6)	0.86, d (6.0)	5	0.70, d (6.6)	0.85, d (6.6)
NH	8.36, d (6.0)		NH	8.31, d (6.0)	
Pro 2	6.25, dd (9.0, 3.6)	6.42, dd (9.0, 3.6)	Pro 2	6.21, dd (9.0, 3.6)	6.48, dd (9.0, 3.6)
3	2.09, m; 1.74, m	2.28, m; 1.94, m	3	2.09, m; 1.74, m	2.24, m; 1.90, m
4	1.90, m; 1.67, m	2.05, m; 1.83, m	4	1.90, m; 1.67, m	2.05, m; 1.83, m
5	3.48, m	3.61, m; 3.51, m	5	3.48, m	3.59, m; 3.49, m
Sar 2	4.82, d (18.0)	4.96, d (17.4)	Sar 2	4.78, d (18.0)	4.90, d (18.0)
	4.08, d (18.0)	4.12, d (17.4)		4.06, d (18.0)	4.09, d (18.0)
NMe	2.75, s	2.92, s	3	2.75, s	2.91, s
MeVal 2	3.23, d (9.6)	3.19, d (9.0)	MeVal 2	3.25, d (9.6)	3.21, d (9.0)
3	2.58, m	2.73, m	3	2.55, m	2.67, m
4	1.00, d (6.6)	1.04, d (6.0)	4	0.97, d (6.6)	1.06, d (6.0)
5	0.79, d (6.6)	0.88, d (6.0)	5	0.80, d (6.6)	0.89, d (6.0)
NMe	3.19, s	3.26, s	NMe	3.27, s	3.37, s
chromophore					
5	11.79, s		12	2.15, s	2.10, s
7	7.13, d (8.4)	6.96, d (8.4)	13	2.28, s	2.26, s
8	6.63, d (8.4)	6.51, d (8.4)	16	2.63, s	2.58, s

^a ¹H NMR data were recorded at 600 MHz.

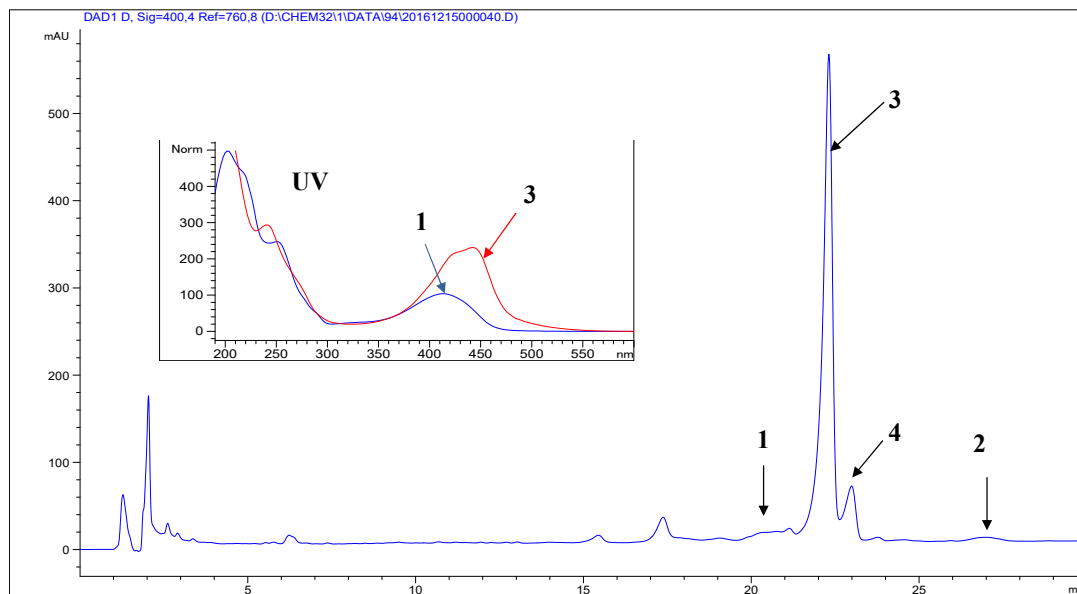


Figure S1. Typical HPLC chromatogram at 400 nm and UV spectra for crude extracts of strain IMB094 cultures under normal fermentation conditions.

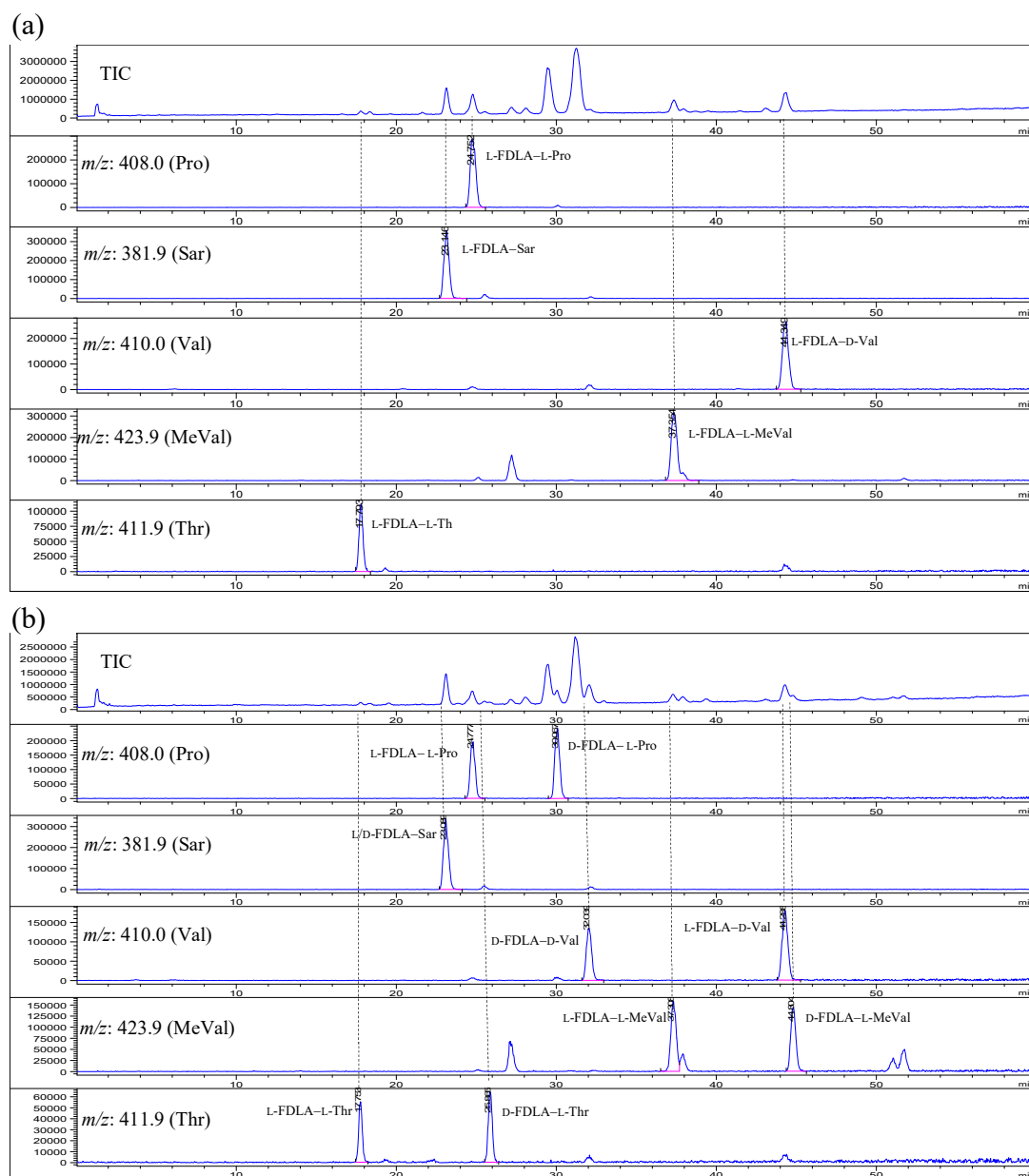


Figure S2. LC-MS chromatograms of (a) L-FDLA and (b) L/D-FDLA derivatives of the hydrolyzate of **1** recorded at negative ion mode

Table S2. LC-MS RetentionTimes (t_R , minute) of the Derivatized Amino Acids from the Hydrolyzate of **1**.

	m/z [M-H] ⁻	L-FDLA-derivate	L/D-FDLA-derivate
L-Pro	408.0	24.75	24.78
			30.07
Sar	381.9	23.15	23.09
D-Val	410.0	44.35	32.04
			44.29
L-MeVal	423.9	37.35	37.31
			44.80
L-Thr	411.9	17.79	17.75
			25.87

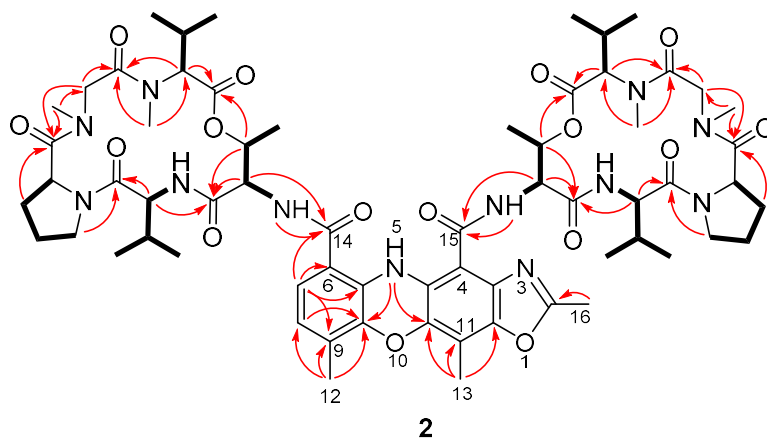


Figure S3. Key HMBC and ^1H - ^1H COSY correlations of **2**.

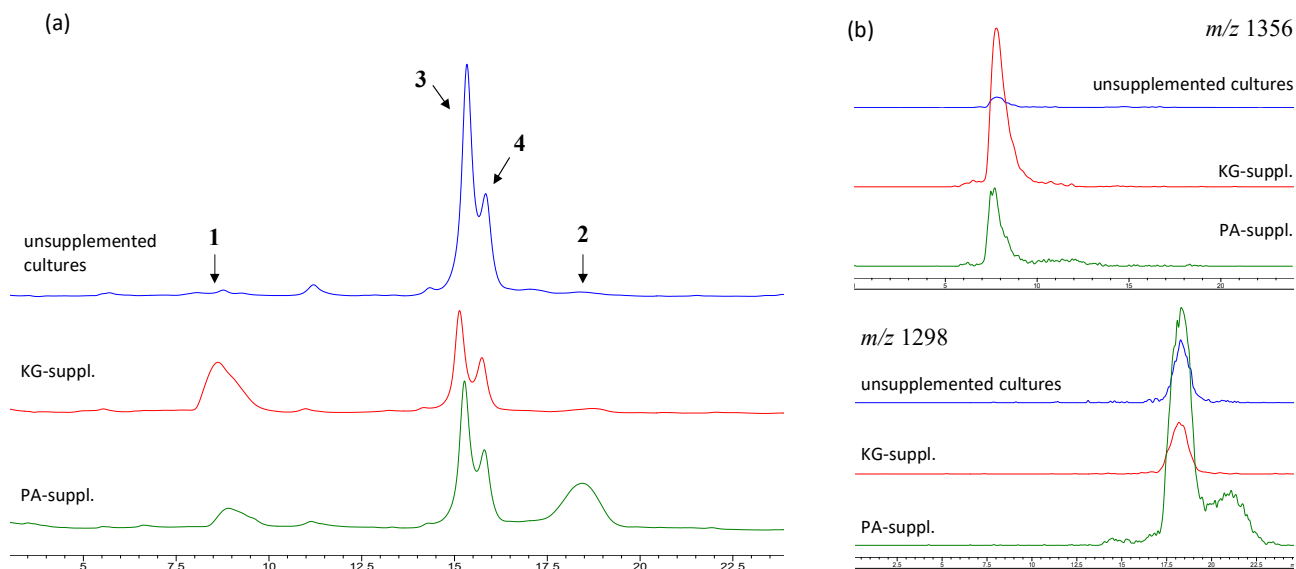


Figure S4. LC-MS analysis of neo-actinomycins in the cultures extracts of strain IMB094 in the feeding experiments (a) Chromatograms at 400 nm for unsupplemented cultures; cultures supplemented with 1mg/mL of α -ketoglutaric acid (KG) and cultures supplemented with 1mg/mL of pyruvic acid (PA); (b) Extracted ion chromatographs are shown for neo-actinomycins A (**1**, $[\text{M}+\text{NH}_4]^+ = 1356$) and B (**2**, $[\text{M}+\text{NH}_4]^+ = 1298$)

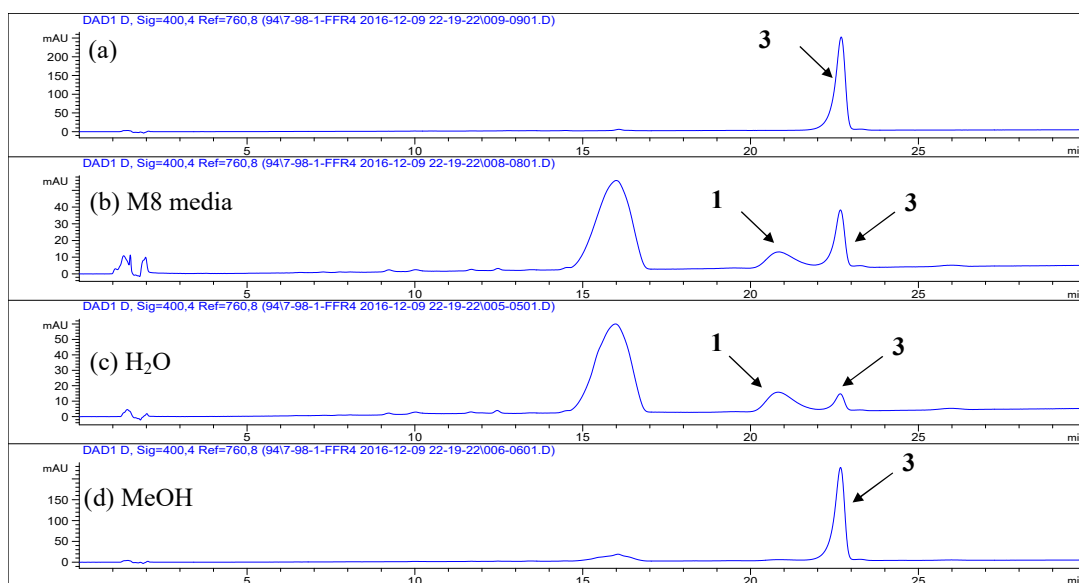


Figure S5. LC-MS traces at 400 nm for (a) actinomycin D (**3**) and conversion of **3** to **1** with α -ketoglutaric acid in the fermentation M8 media (b), H₂O (c), and MeOH (d) (LC-MS condition: Capcell MGII C-18 3 μ M, 3.0 mm \times 150 mm; mobile phase A: H₂O containing 0.1% formic acid; B: MeCN containing 0.1% formic acid; gradient conditions: 0–30 min linear gradient 35–80% B; flow rate: 0.5 mL/min)

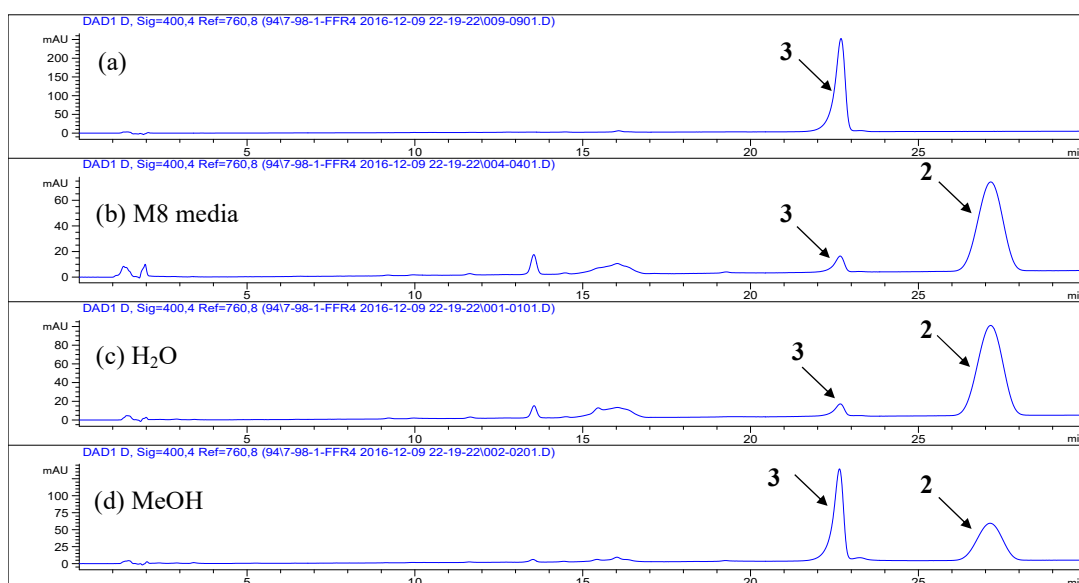


Figure S6. LC-MS traces at 400 nm for (a) actinomycin D (**3**) and conversion of **3** to **2** with pyruvic acid in the fermentation M8 media (b), H₂O (c), and MeOH (d) after 36 h. (LC-MS condition: Capcell MGII C-18 3 μ M, 3.0 mm \times 150 mm; mobile phase A: H₂O containing 0.1% formic acid; B: MeCN containing 0.1% formic acid; gradient conditions: 0–30 min linear gradient 35–80% B; flow rate: 0.5 mL/min)

Table S6. Conversion rates (%) of actinomycin D with α -ketoglutaric acid and pyruvic acid into neo-actinomycins A (**1**) and B (**2**) in MeOH and H₂O under different pH ^a

pH condition	1		2	
	MeOH	H ₂ O	MeOH	H ₂ O
pH 1.0	0%	14.9%	4.0%	36.6%
pH 2.0	0%	18.7%	5.1%	52.3%
pH 4.0	0%	0%	0.8%	3.1%

^a The pH 2.0 is the original pH value of the reaction mixtures (actinomycin D with α -ketoglutaric acid or pyruvic acid) without adjusting. The pH 1.0 and 4.0 was adjusted using 5 M HCl and 5 M ammonium hydroxide, respectively.

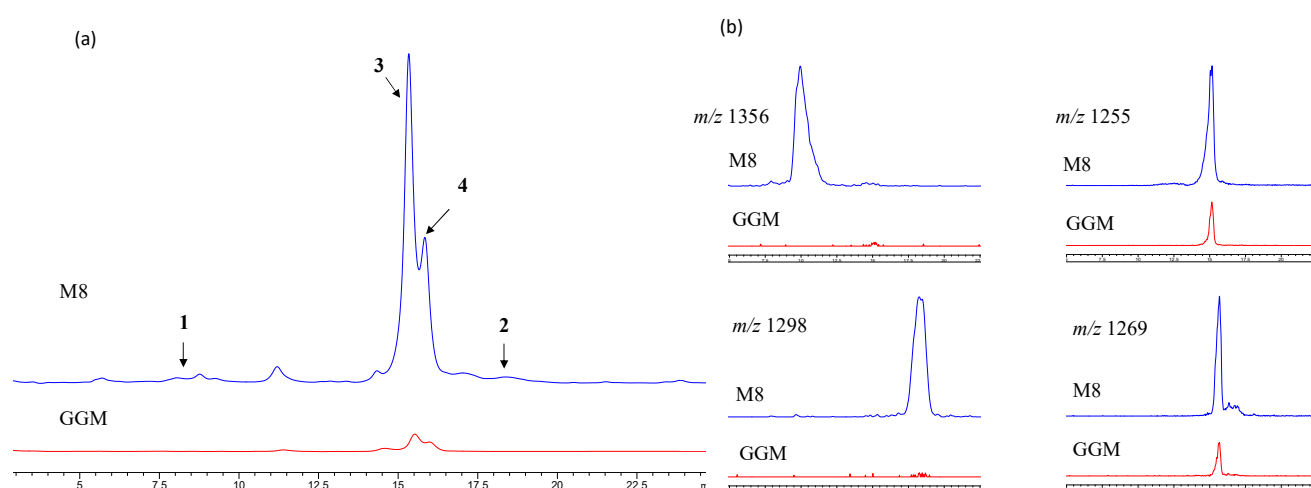


Figure S7. LC-MS analysis of actinomycin analogs in the cultures extracts of strain IMB094 grown in the M8 and GGM media. (a) Chromatograms at 400 nm; (b) Extracted ion chromatographs are shown for neo-actinomycins A (**1**, $[M+NH_4]^+ = 1356$), B (**2**, $[M+NH_4]^+ = 1298$), actinomycins D (**3**, $[M+H]^+ = 1255$) and X2 (**4**, $[M+H]^+ = 1269$).

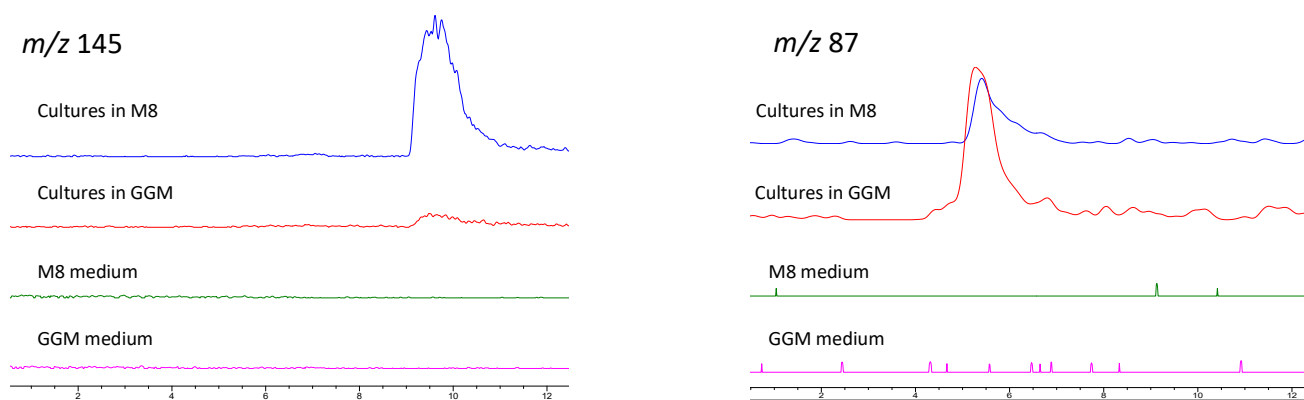


Figure S8. LC-MS analysis of α -ketoglutarate and pyruvate in the cultures extracts of strain IMB094 and in the media prior to cultivation. Extracted ion chromatographs are shown for α -ketoglutarate ($[M-H]^- = 145$) and pyruvate ($[M-H]^- = 87$).

Table S7. Concentrations of compounds **1–4**, α -ketoglutarate, and pyruvate in the fermentation broth cultured in M8 and GGM media ($\mu\text{g/mL}$)

Media	1	2	3	4	α -ketoglutarate	pyruvate
M8	12.2	3.7	39.9	23.3	39.4	7.7
GGM	0.0	0	2.8	1.6	3.9	21.4

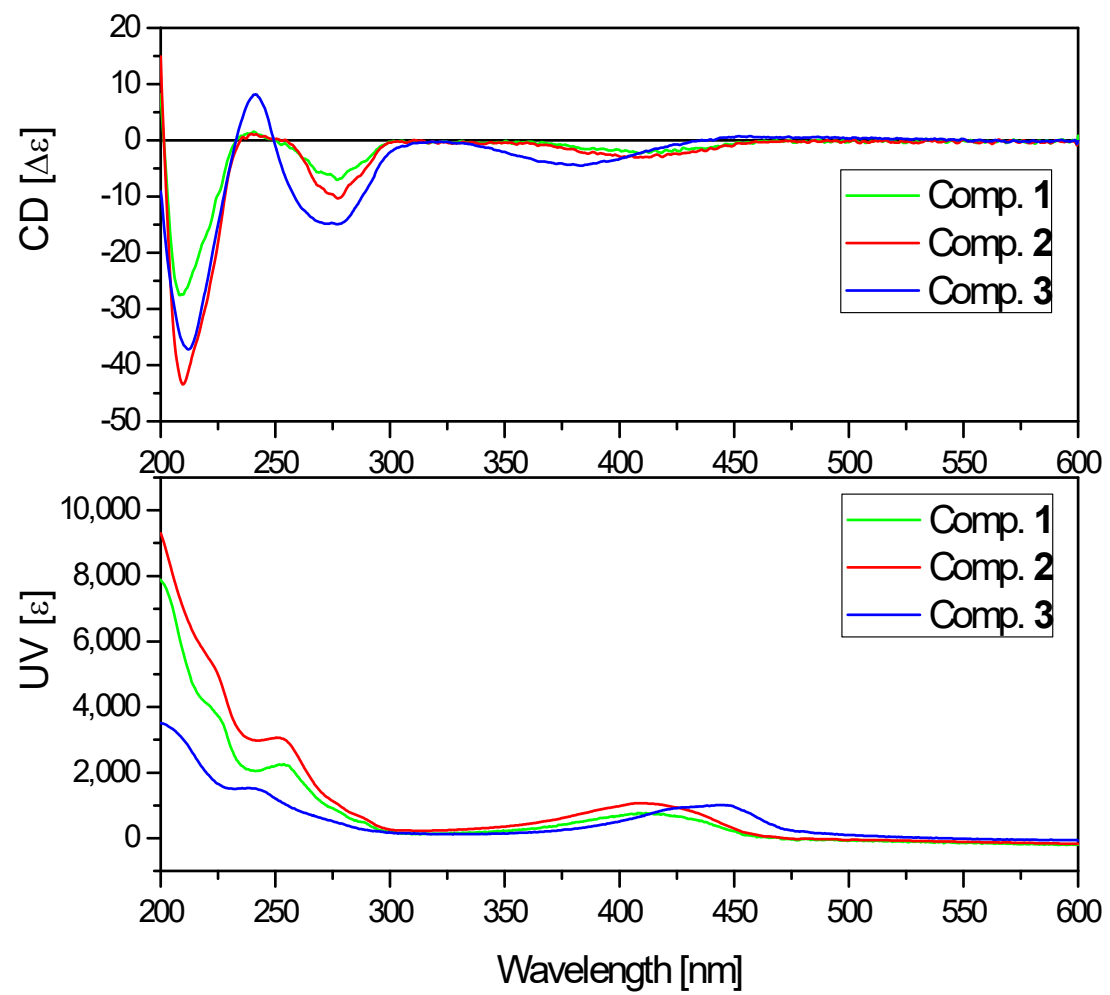


Figure S9. The CD and UV spectra of compounds 1–3 recorded in MeOH.

94-7 #58 RT: 0.85 AV: 1 NL: 1.46E7
T: FTMS + c ESI Full ms [150.00-2000.00]

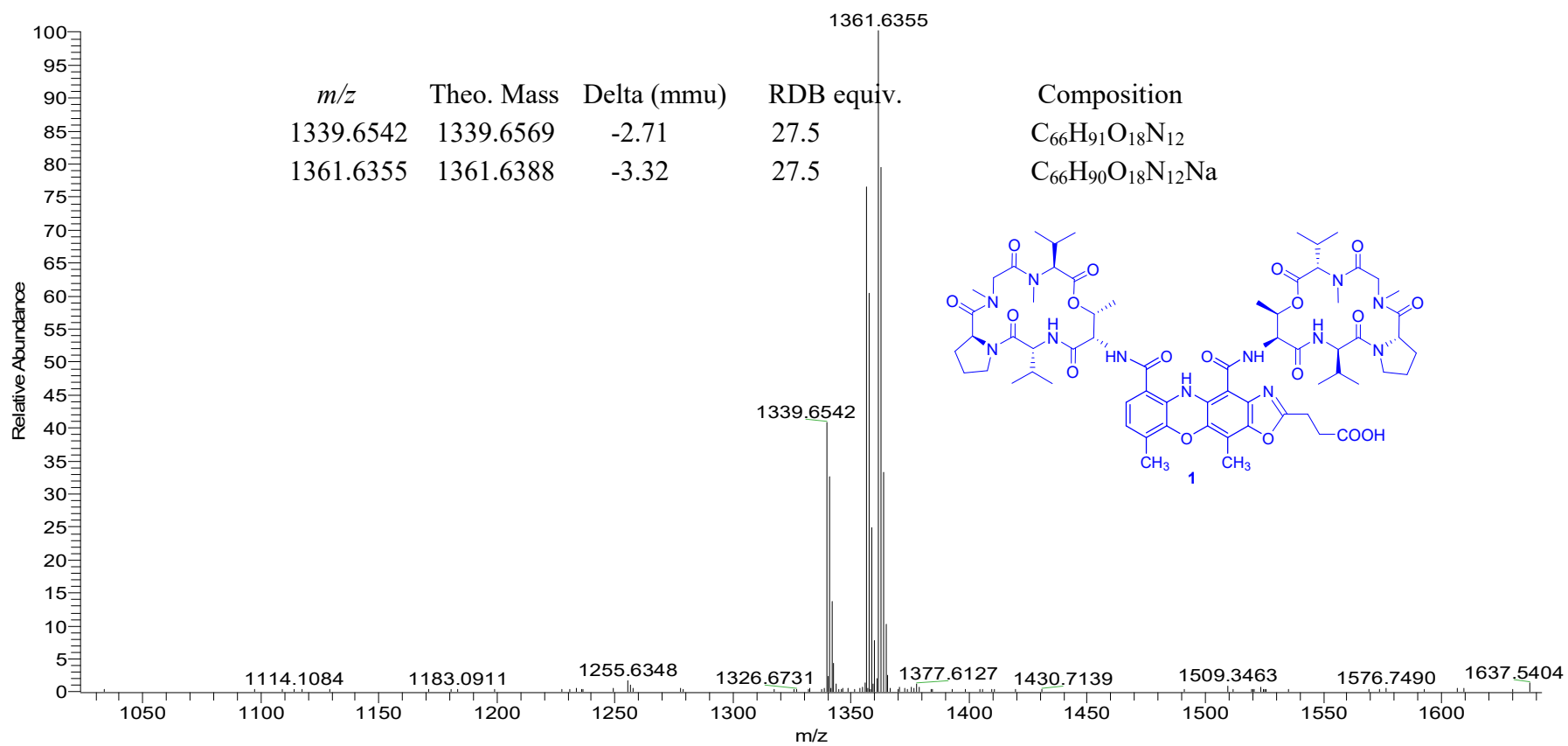


Figure S10. The (+)-HRESIMS spectrum of neo-actinomycin A (1).

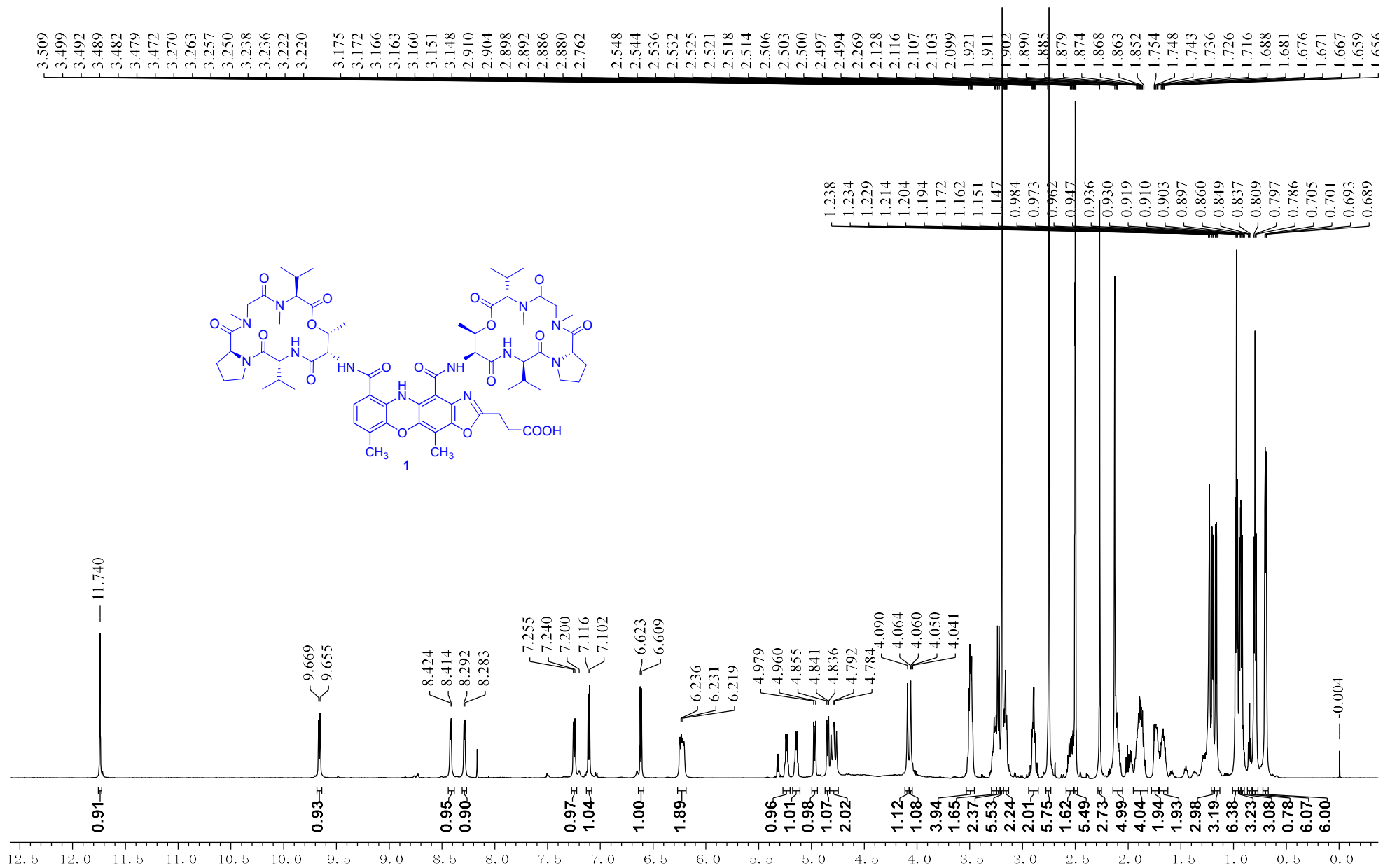


Figure S11. The ¹H NMR spectrum of neo-actinomycin A (1) in DMSO-d₆ (600 MHz).

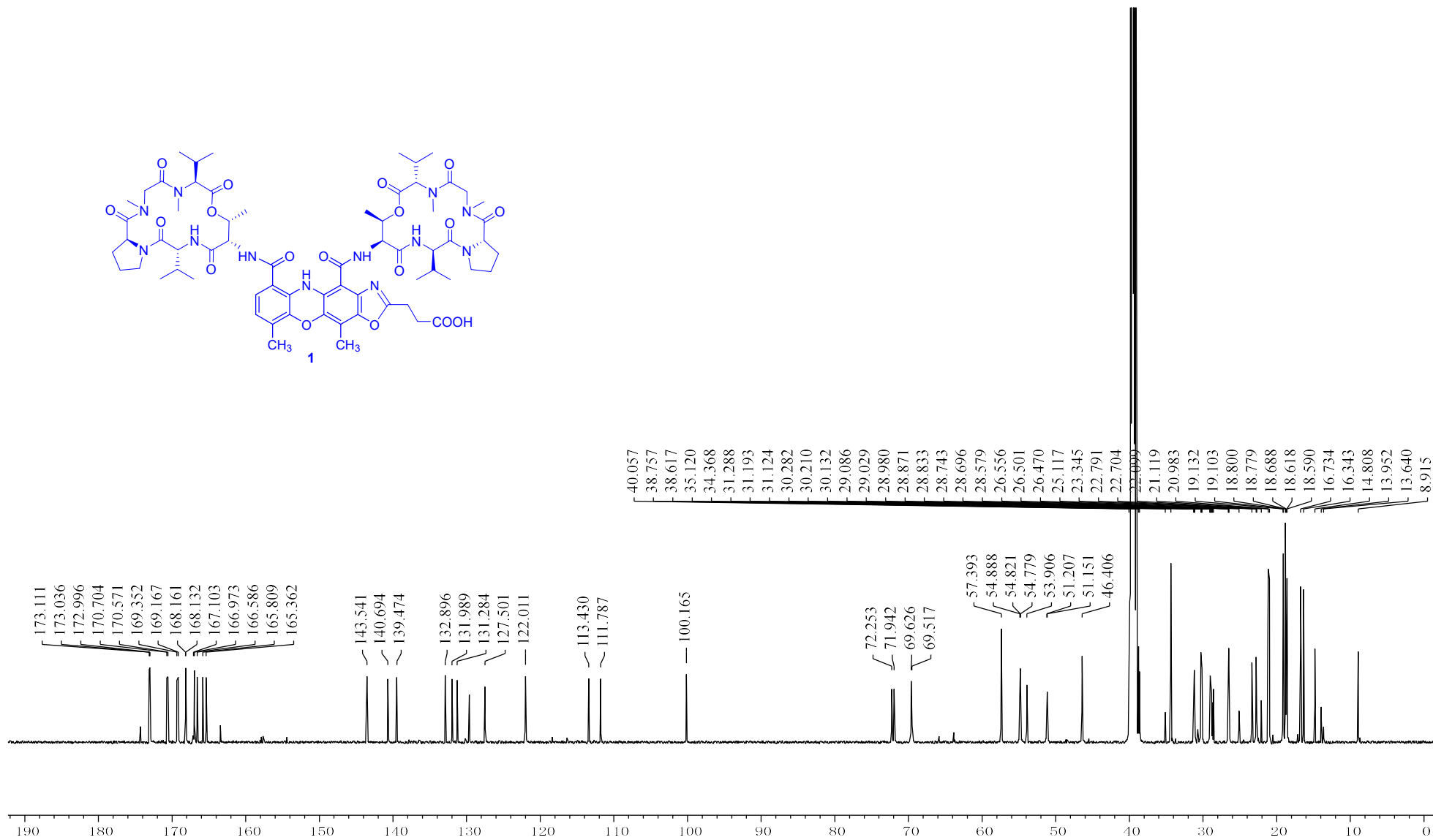


Figure S12. The ¹³C NMR spectrum of neo-actinomycin A (1) in DMSO-*d*₆ (150 MHz).

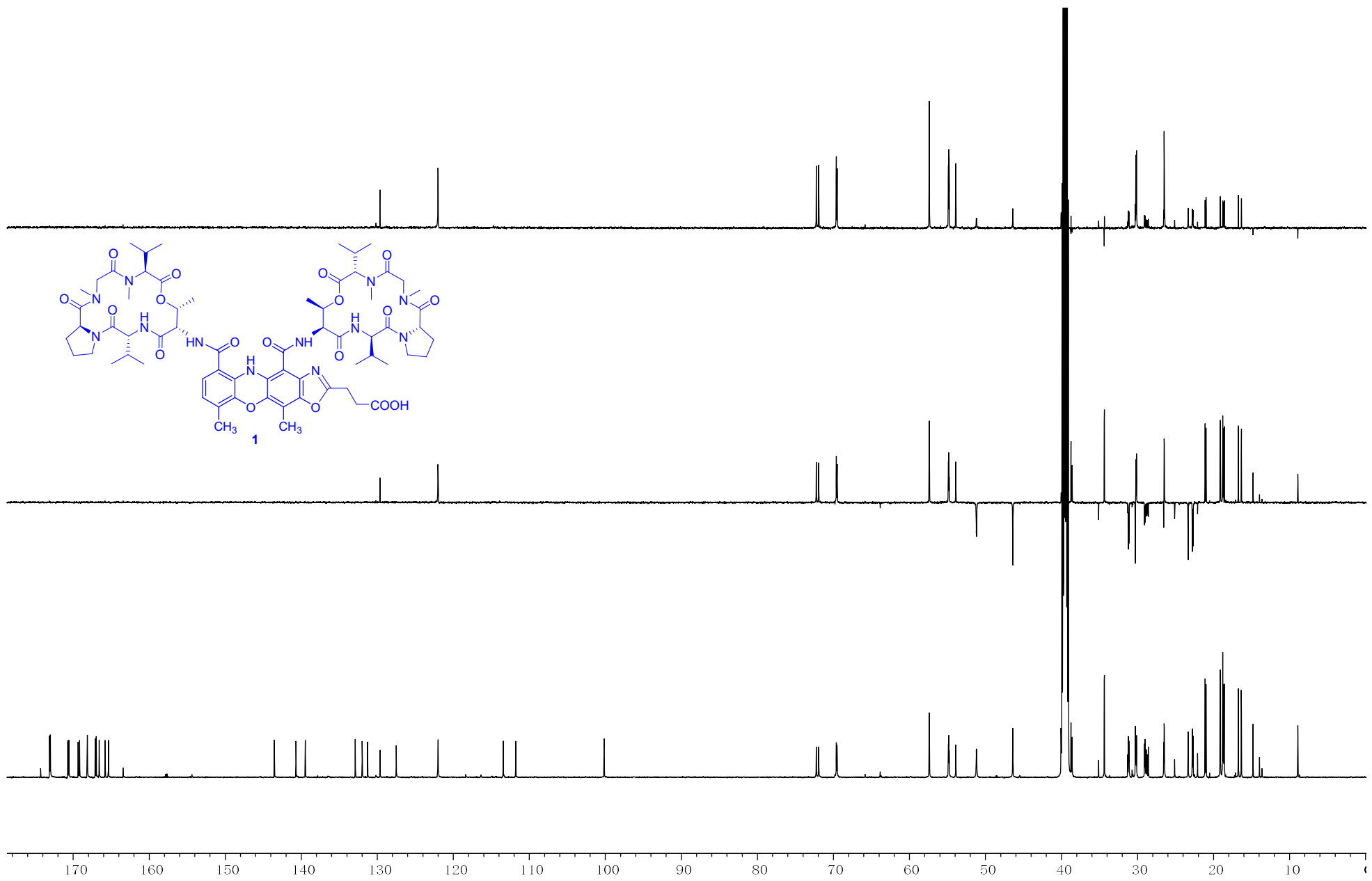


Figure S13. The DEPT spectrum of neo-actinomycin A (**1**) in DMSO-*d*₆ (150 MHz).

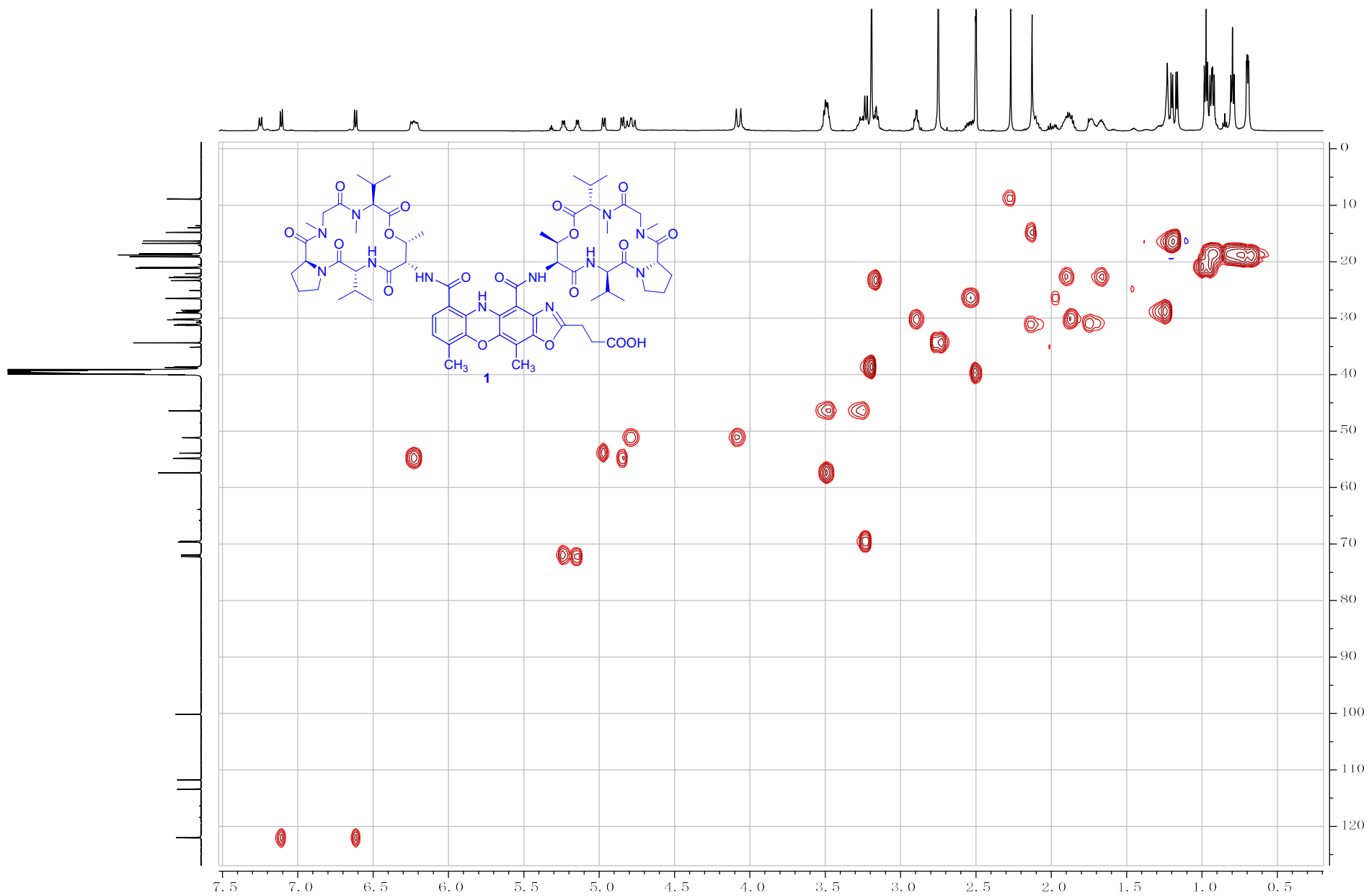


Figure S15. The HSQC spectrum of neo-actinomycin A (**1**) in DMSO-*d*₆ (600 MHz).

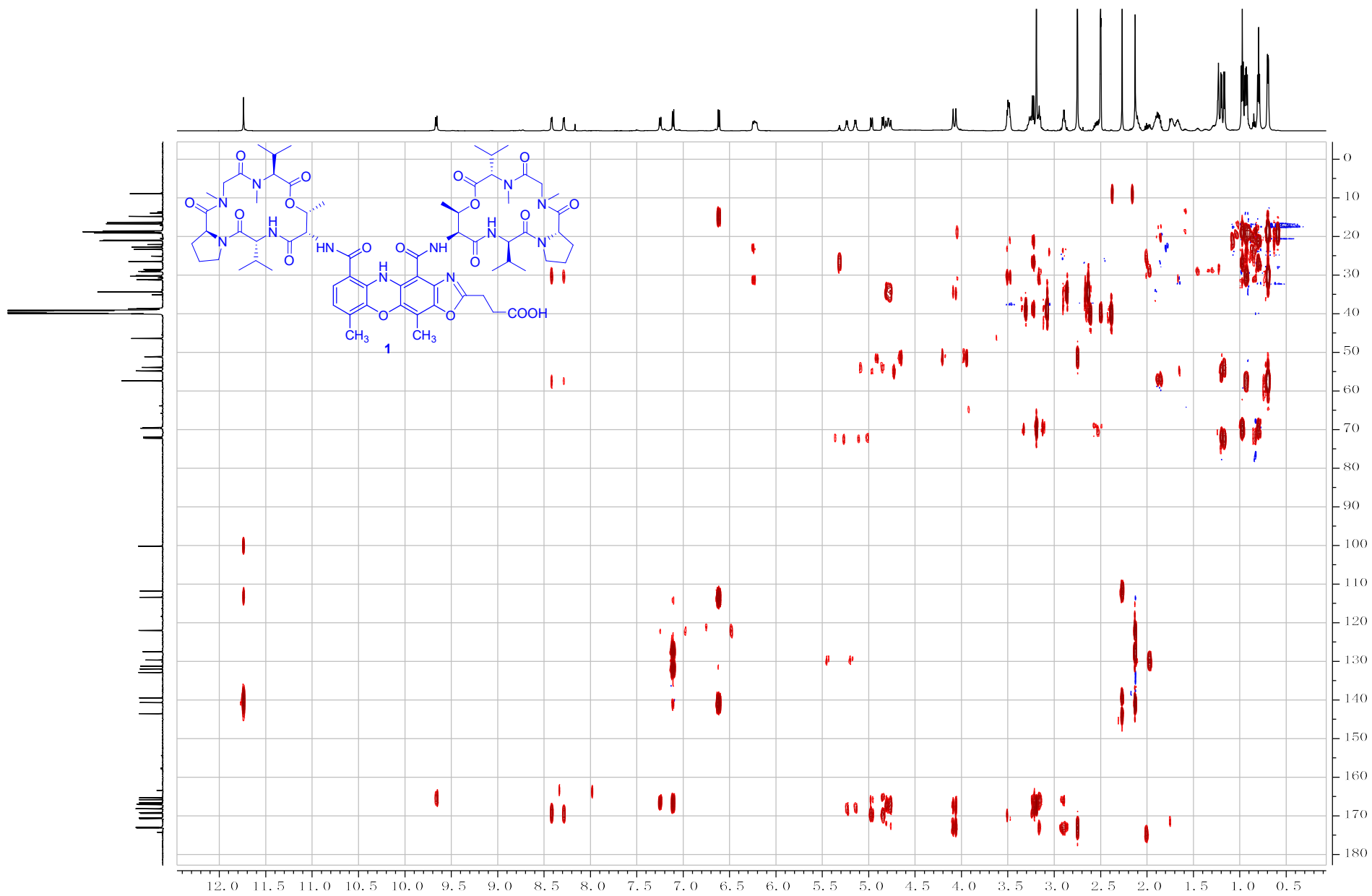


Figure S16. The HMBC spectrum of neo-actinomycin A (**1**) in DMSO- d_6 (600 MHz).

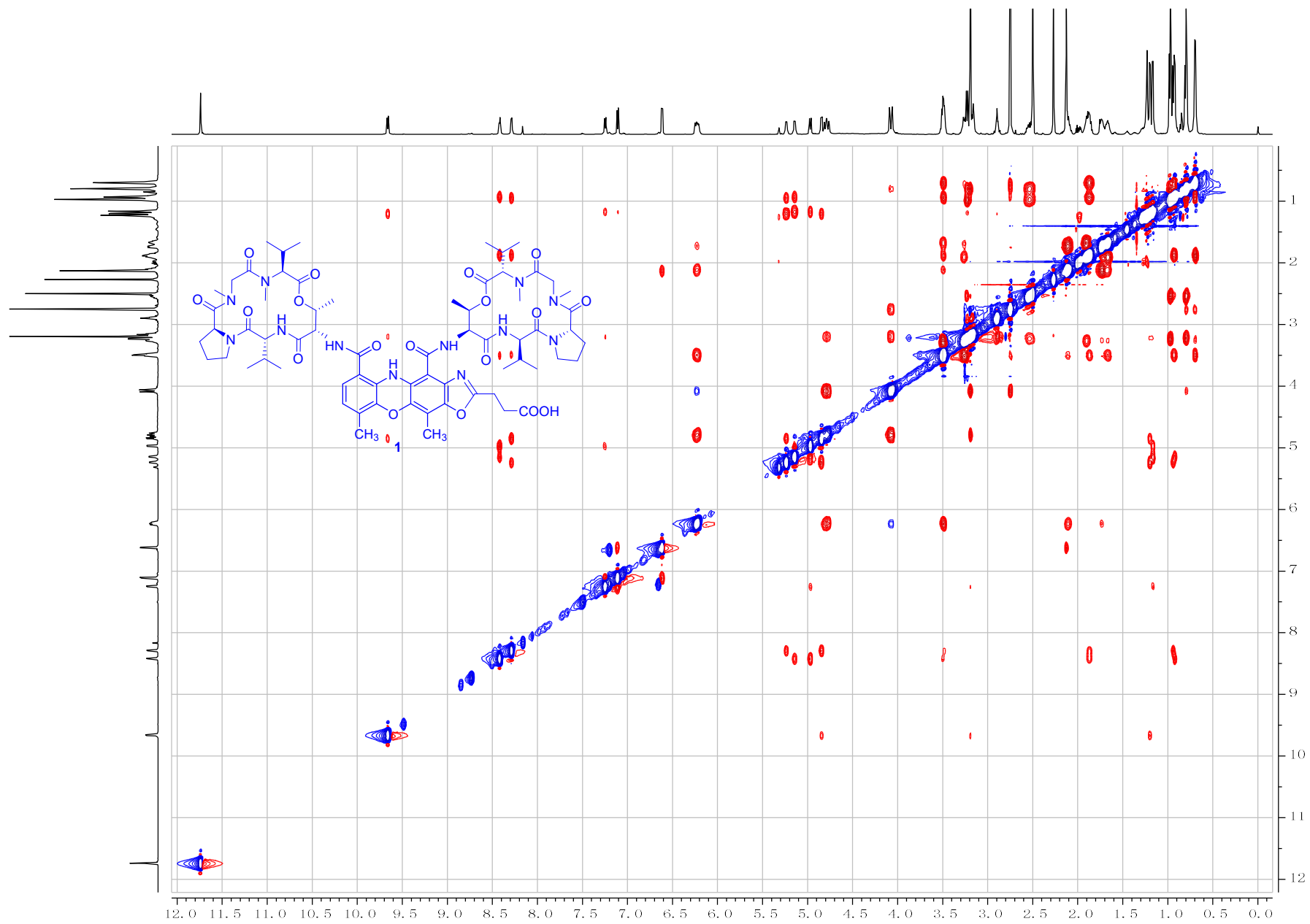


Figure S17. The ROESY spectrum of neo-actinomycin A (1) in DMSO- d_6 (600 MHz).

94-1 HRESIMS #4 RT: 0.08 AV: 1 NL: 5.56E6
T: FTMS + p ESI Full ms [100.00-2000.00]

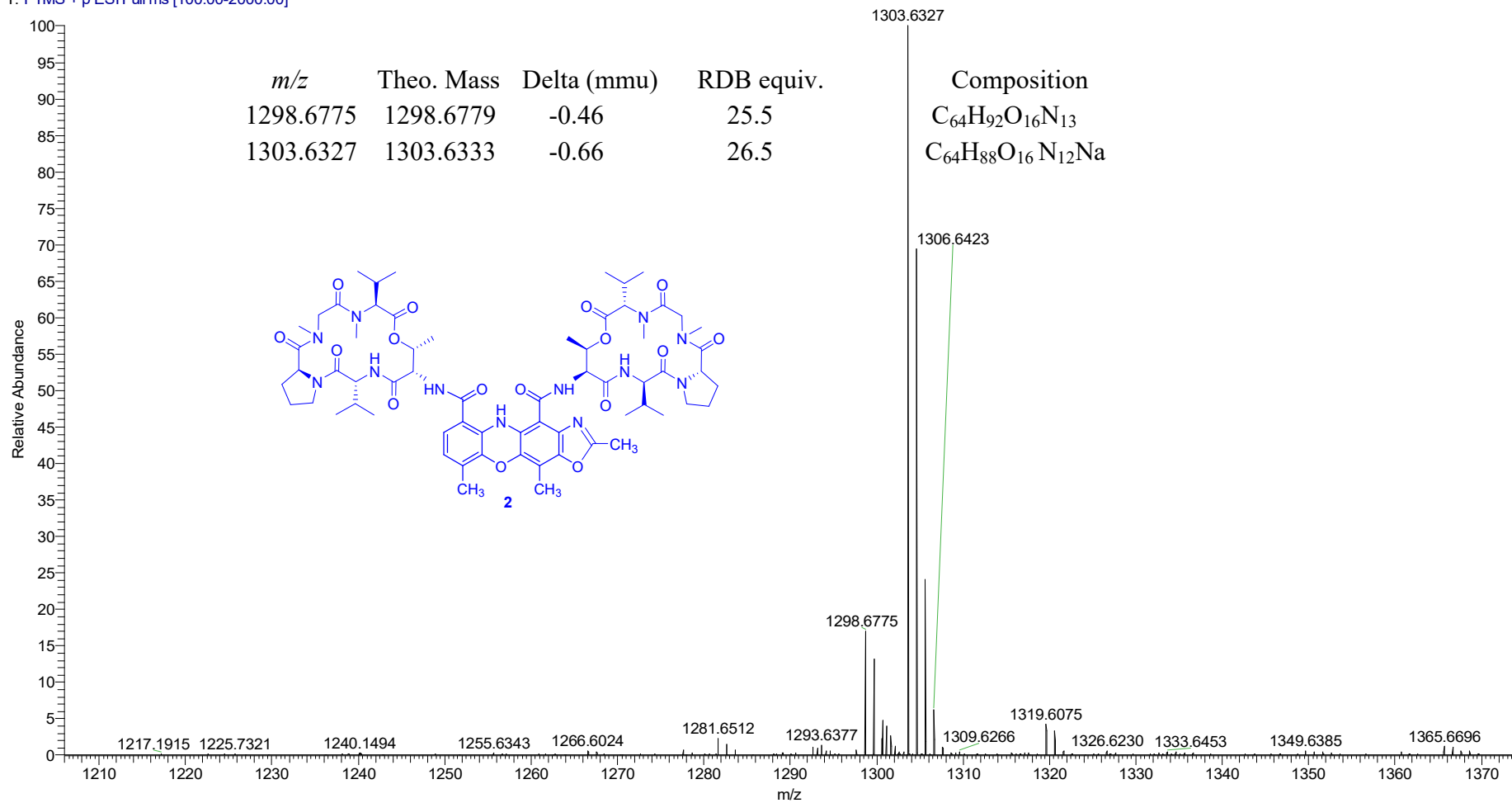


Figure S18. The (+)-HRESIMS spectrum of neo-actinomycin B (2).

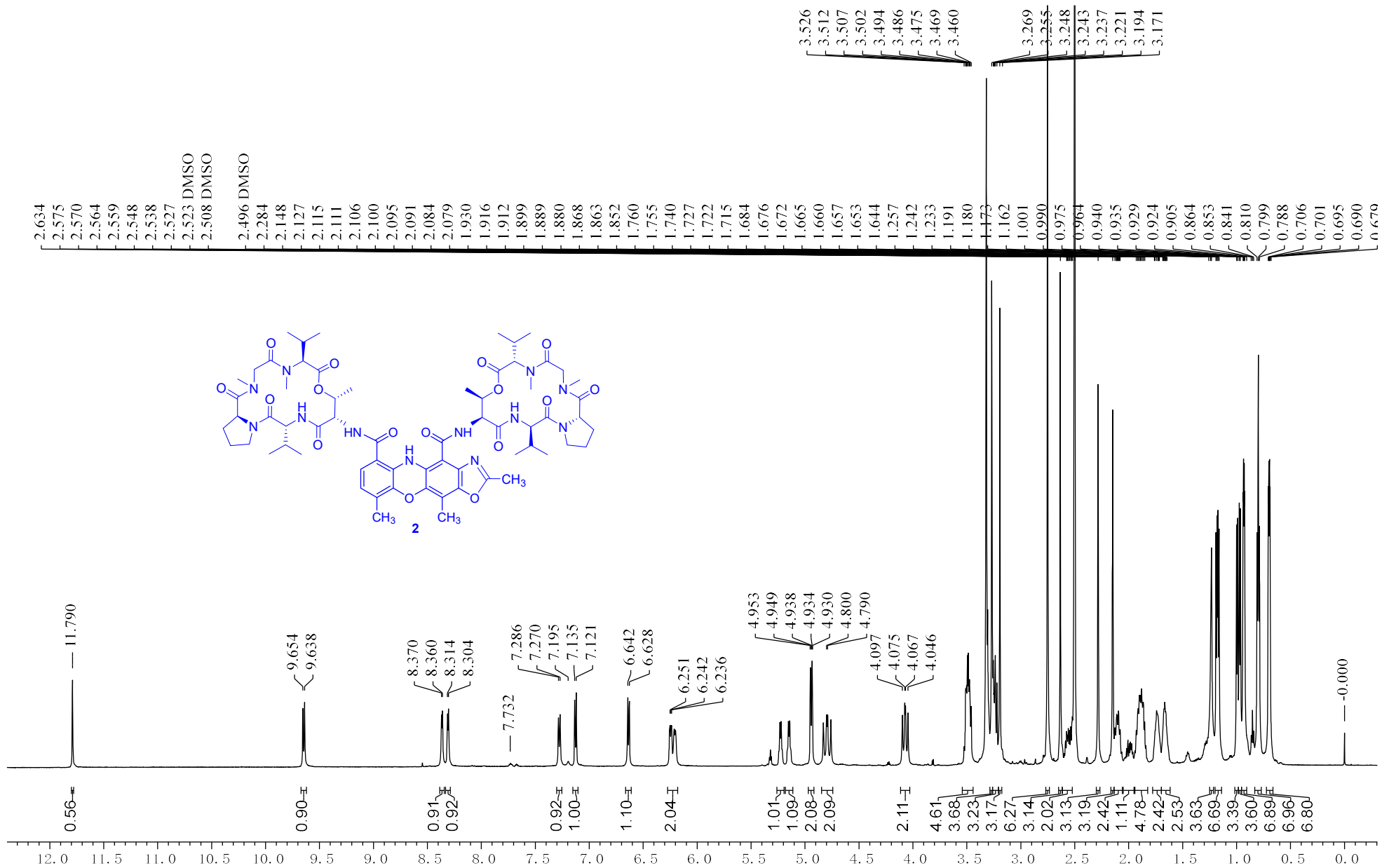


Figure S19. The ¹H NMR spectrum of neo-actinomycin B (2) in DMSO-*d*₆ (600 MHz).

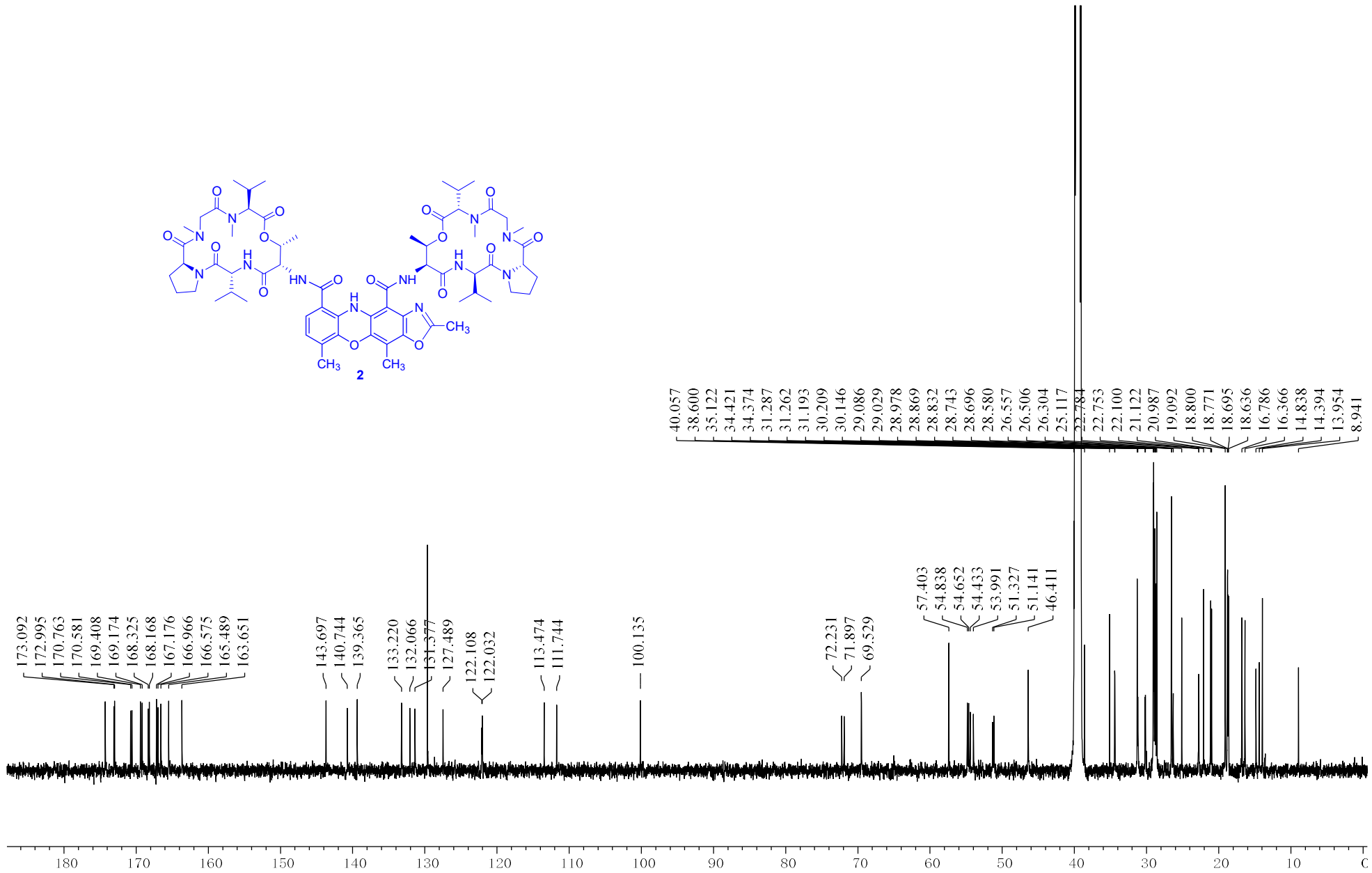


Figure S20. The ¹³C NMR spectrum of neo-actinomycin B (2) in DMSO-*d*₆ (150 MHz).

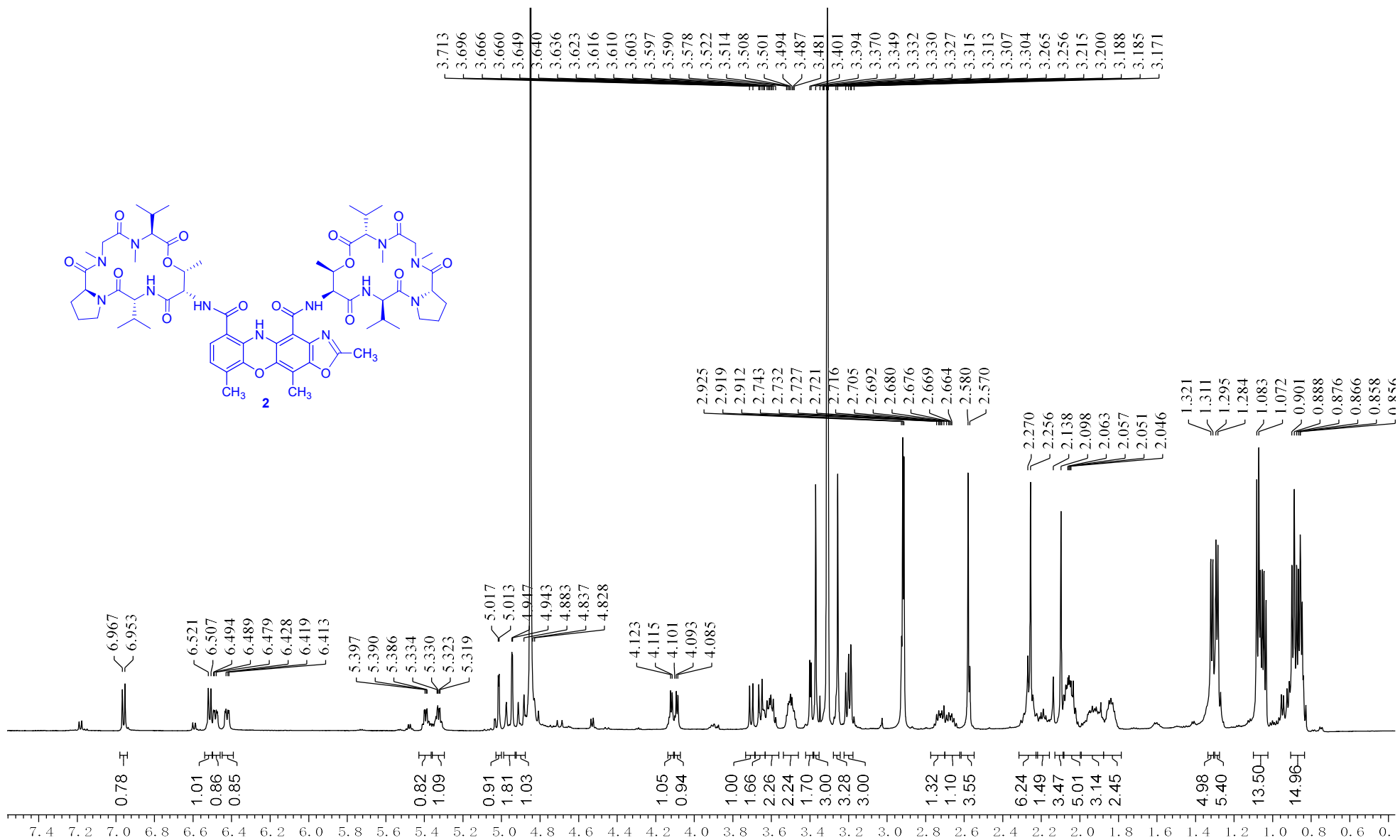


Figure S21. The ¹H NMR spectrum of neo-actinomycin B (2) in MeOH-d₄ (600 MHz).

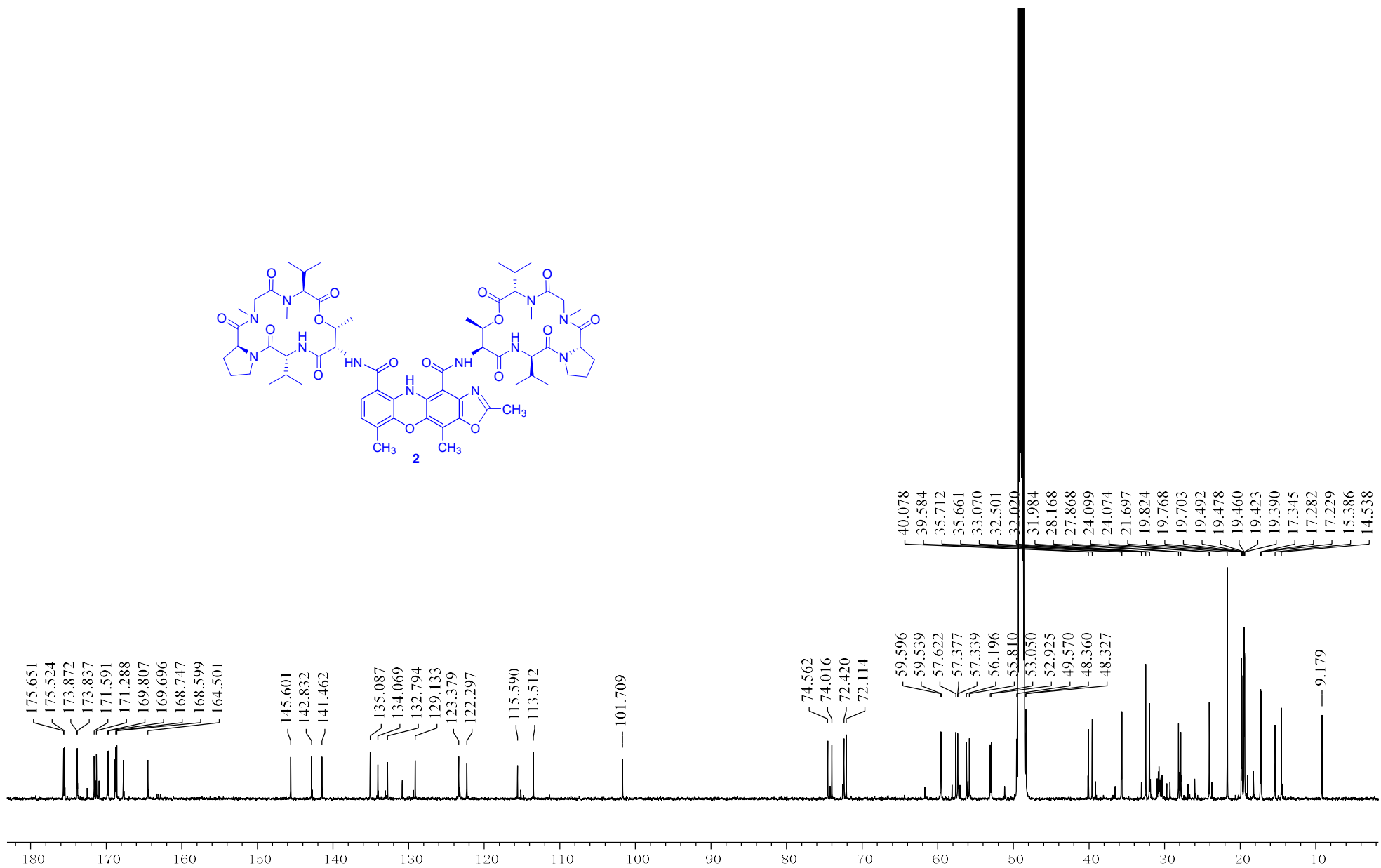


Figure S22. The ¹³C NMR spectrum of neo-actinomycin B (2) in MeOH-*d*₄ (150 MHz).

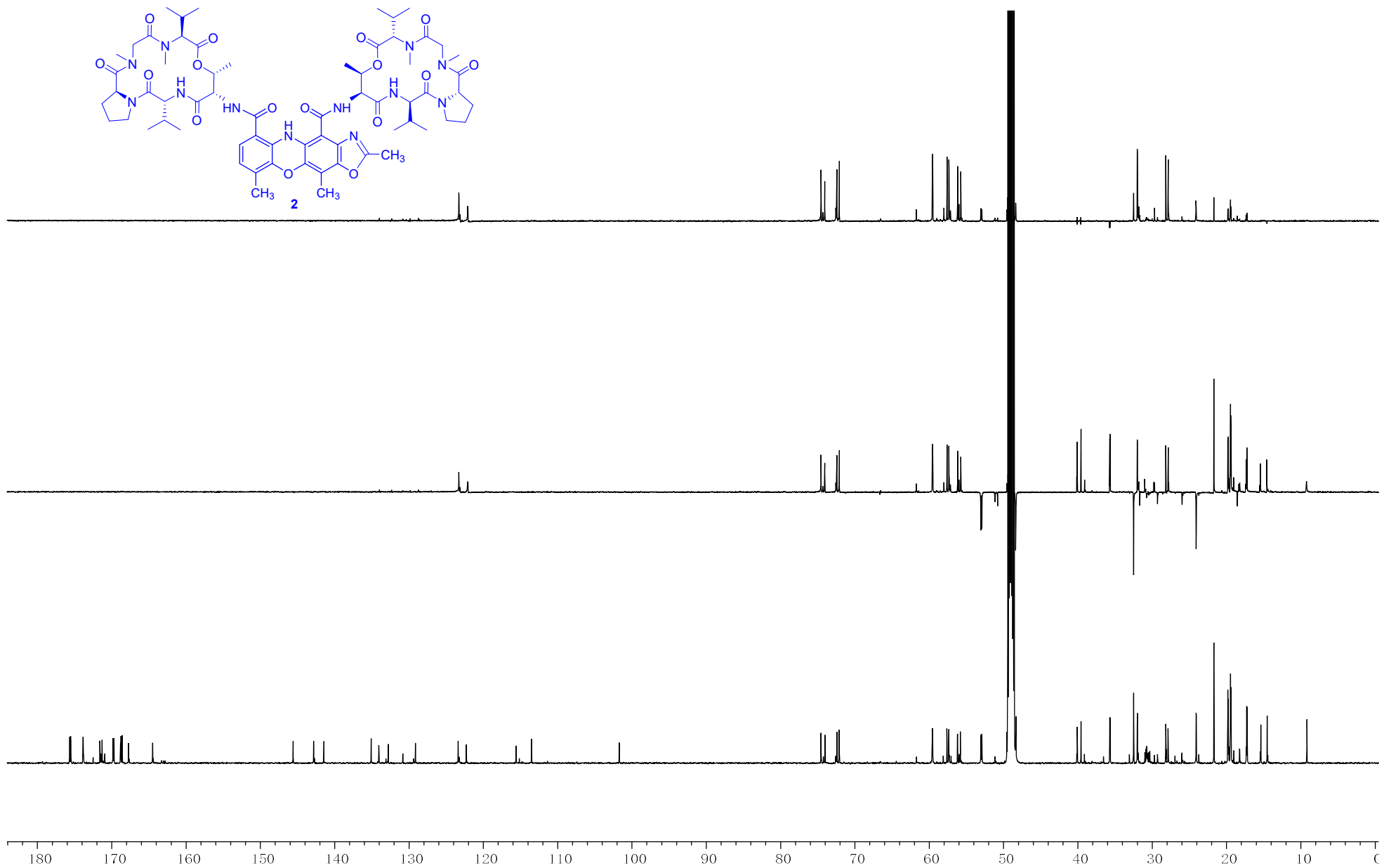


Figure S23. The DEPT spectrum of neo-actinomycin B (**2**) in MeOH-*d*₄ (150 MHz).

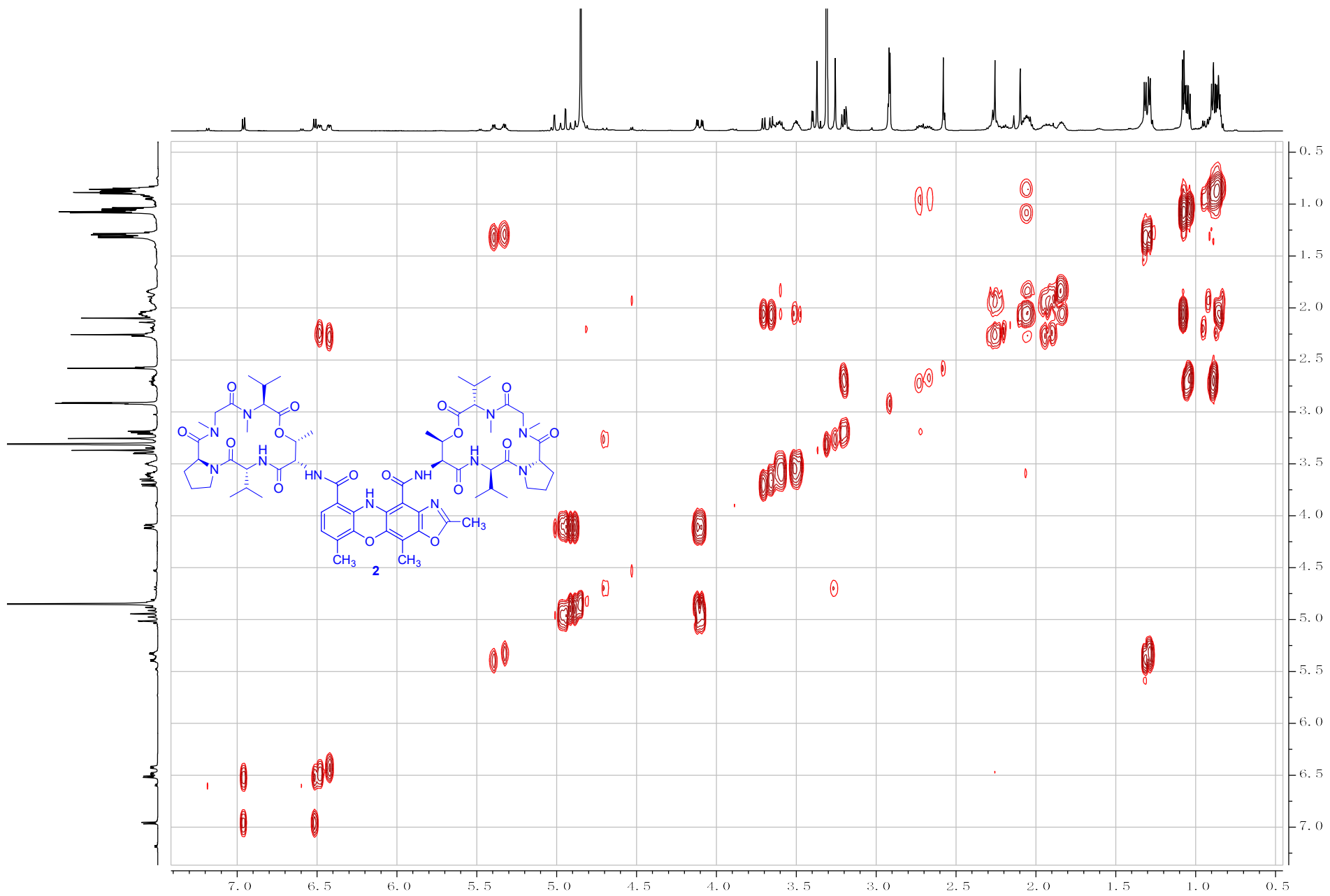


Figure S24. The COSY spectrum of neo-actinomycin B (**2**) in MeOH-*d*₄ (600 MHz).

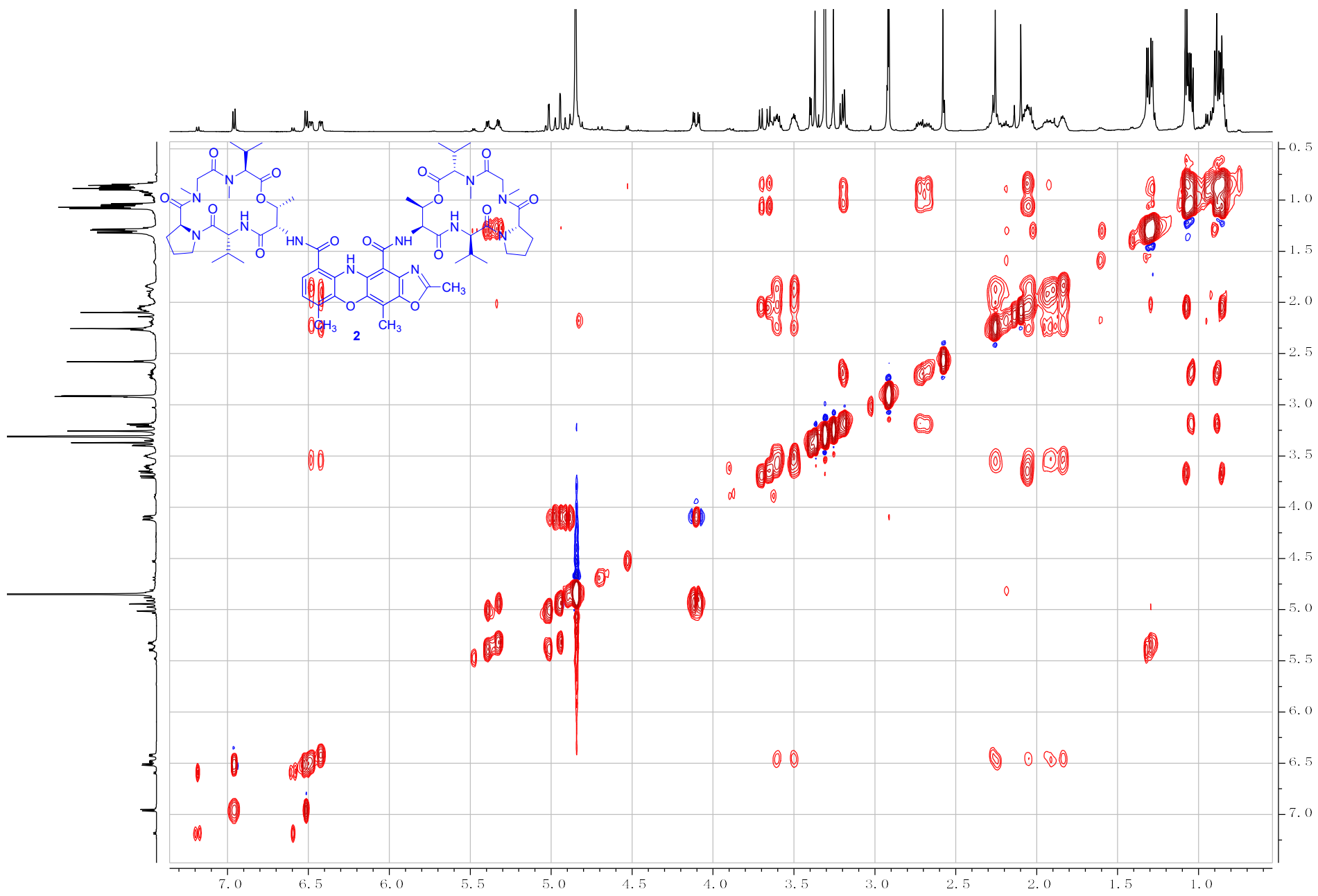


Figure S25. The TOCSY spectrum of neo-actinomycin B (2) in MeOH-d₄ (600 MHz).

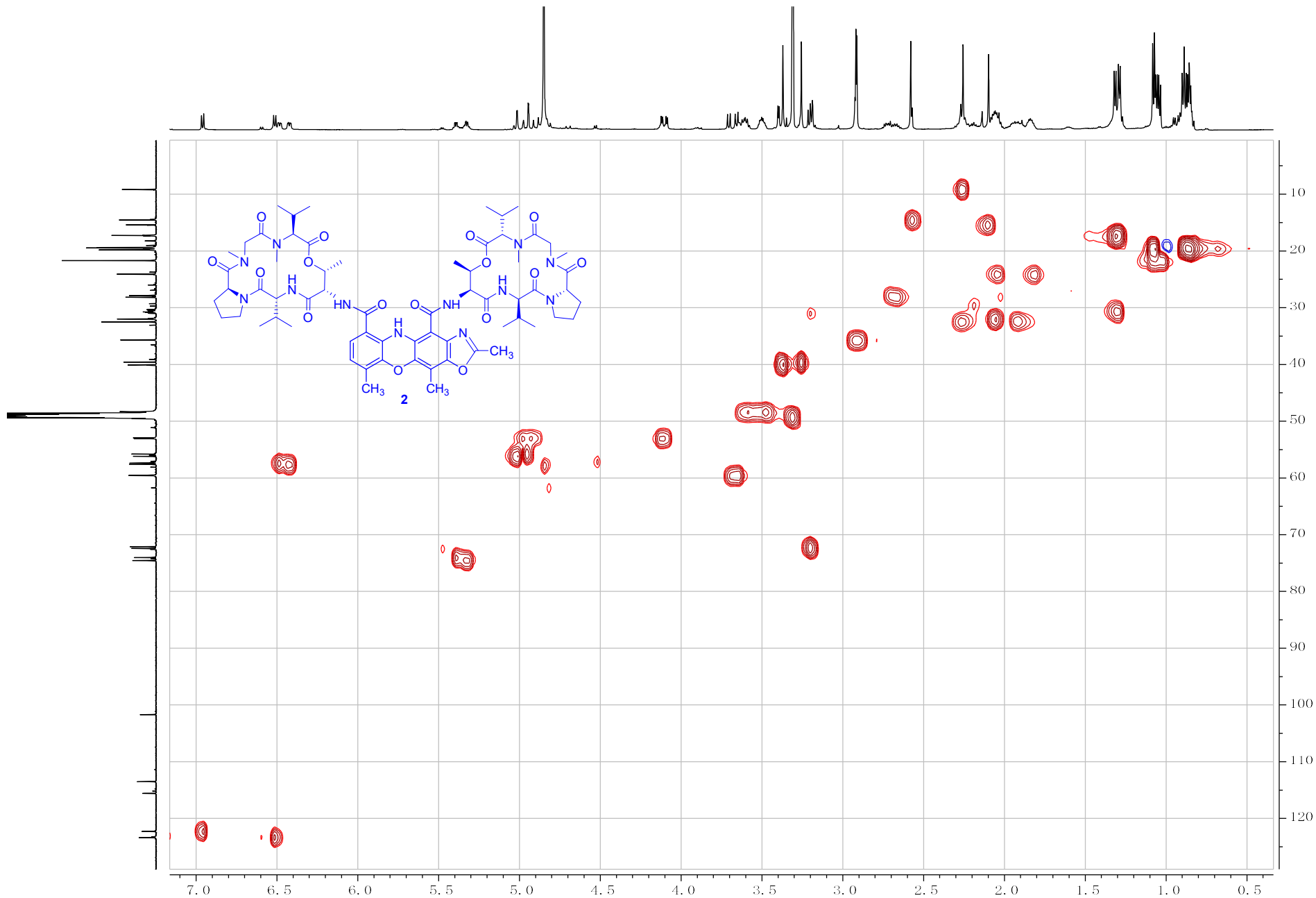


Figure S26. The HSQC spectrum of neo-actinomycin B (2) in MeOH-*d*₄ (600 MHz).

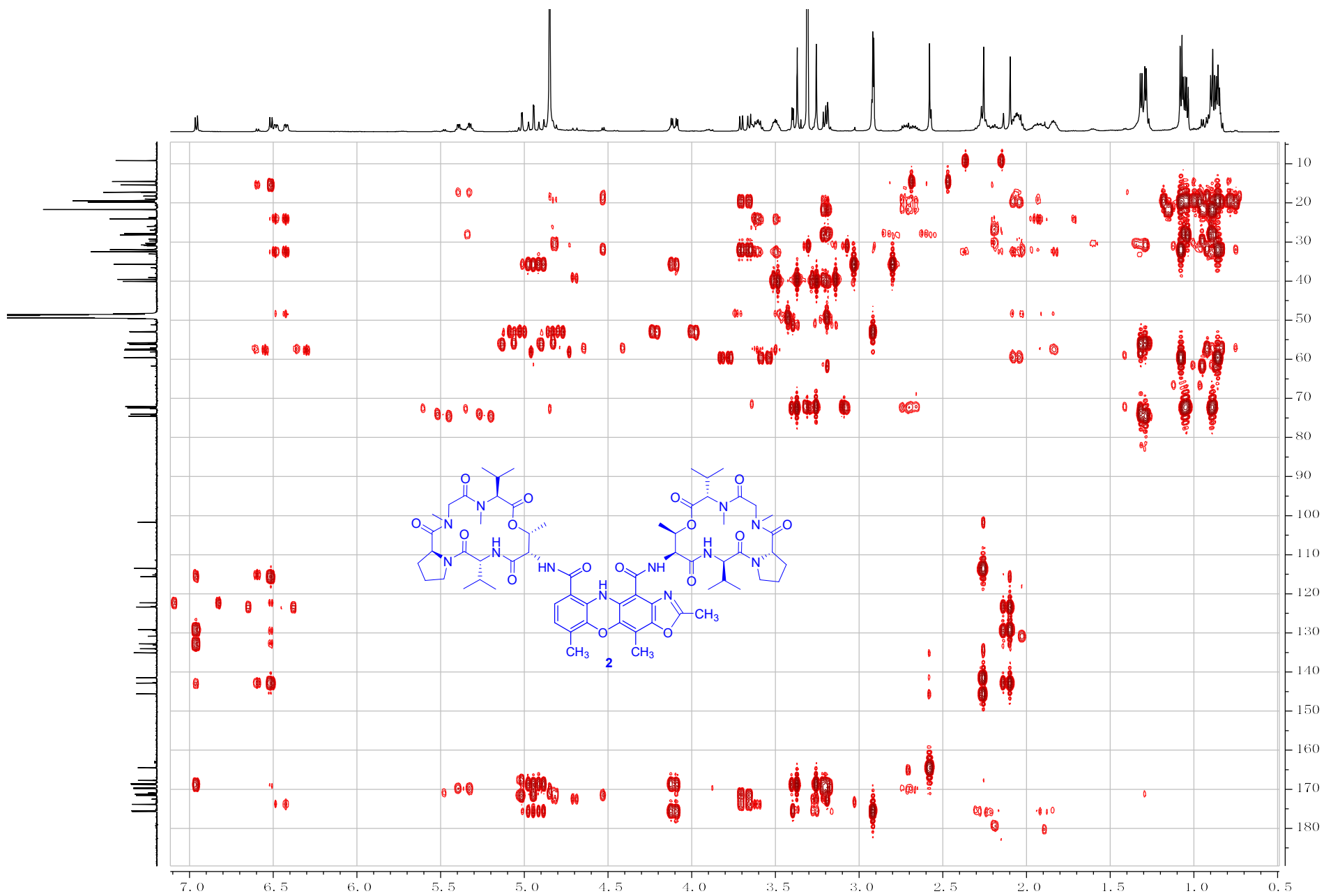


Figure S27. The HMBC spectrum of neo-actinomycin B (2) in MeOH-*d*₄ (600 MHz).

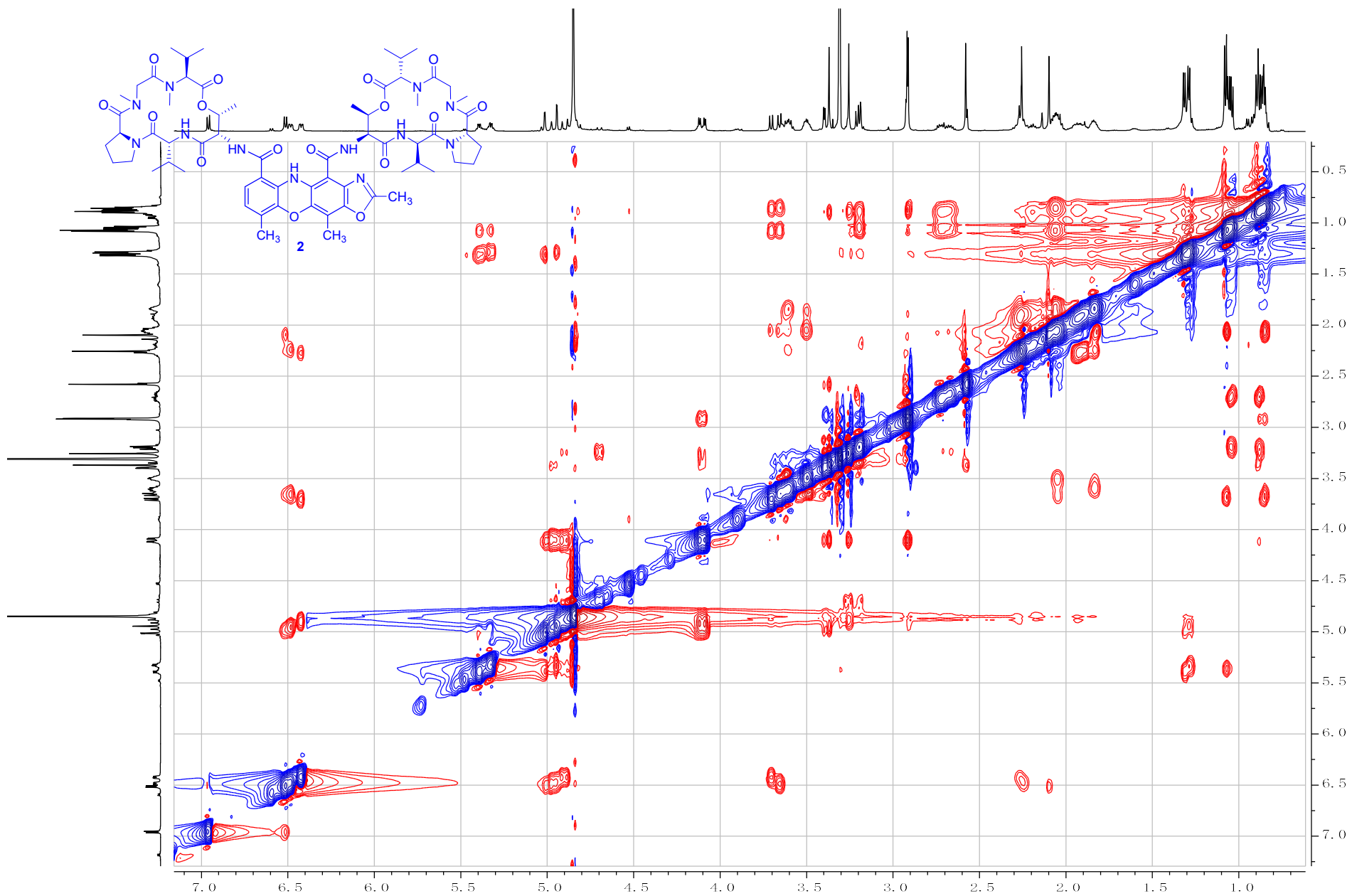


Figure S28. The ROESY spectrum of neo-actinomycin B (2) in MeOH-d₄ (600 MHz).