

Supporting Information

Cytotoxic Anthracycline Metabolites from a Recombinant *Streptomyces*

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Table of Contents

	Page
Experimentals	S2
Table S1.	S3
Figure S1–7.	S4–10
Figure S8–14.	S11–17
Figure S15–21.	S18–24
Figure S22–28.	S25–31
Figure S29–35.	S32–38
Figure S36–42.	S39–45
Figure S43–49.	S46–52
Figure S50–56.	S53–59
Figure S57–63.	S60–66
Figure S64–70.	S67–73
Figure S71–77.	S74–80
Figure S78–84.	S81–87

Cytotoxicity assay for compounds 1–15.

Human tumor cell lines MDA-MB-435, MDA-MB-231, NCI-H460, HCT-116, HepG2, and MCF10A cells were purchased from the ATCC. The cell growth inhibitory activity for compounds **1–15** were determined using the MTT method. All tumor cell lines were cultured at a density of 1×10^4 cells/well at 37°C in a humidified incubator (5% CO₂) for 24 h, followed by testing for 48 h in various concentrations of the compounds. Then 20 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) solution was added to each well and incubated for another 4 h. Culture supernatant was removed and replaced by 150 µL of dimethyl sulfoxide in each well. Cell growth inhibition was determined by measuring absorbance and calculated using the formula: cell viability (%) = (the average A₅₅₀ nm of the treated group/the average A₅₅₀ nm of the untreated group) × 100. Three independent repeated trials were conducted for each compound (n = 3). IC₅₀ values and 95% confidence interval (CI) were estimated using Excel's forecast and confidence functions (Microsoft Excel 2007).¹

References

(1) Ding, B.; Yuan, J.; Huang, X.; Wen, W.; Zhu, X.; Liu, Y.; Li, H.; Lu, Y.; He, L.; Tan, H.; She, Z. *Mar. Drugs* **2013**, *11*, 4961–4972.

Table S1. ^1H and ^{13}C NMR spectroscopic data for compound **13** recorded in CDCl_3 .

position	δ_{C}	δ_{H} mult. (J in Hz)	13		
			position	δ_{C}	δ_{H} mult. (J in Hz)
1	119.8	7.86, d (7.4)	13	32.5	1.44, dd (14.1, 7.2);
2	137.2	7.69, t (7.9)			1.85, m
3	124.9	7.30, d (8.3)	14	6.9	1.11, t (6.8)
4	162.8		15	171.6	
4-OH		12.16, s	16	52.6	3.71, s
4a	116.2		1'	101.7	5.44, d (2.7)
5	190.9		2'	23.7	1.54-2.15, m;
5a	111.3				2.06, m
6	157.1		3'	24.6	1.54-2.15, m
6-OH		12.86, s	4'	75.0	3.52, s
6a	136.3		5'	67.2	3.96, q (6.4)
7	70.8	5.26, d (2.1)	6'	17.3	1.11, d (6.8)
8	33.4	2.21, dd (14.9, 4.0);	1''	99.6	4.83, d (2.9)
		2.38, d (14.9)	2''	24.8	1.54-2.15, m
9	71.3		3''	26.1	1.54-2.15, m
10	52.3	4.28, s	4''	74.4	3.55, s
			5''	67.6	4.11, q (6.2)
10a	135.6		6''	17.3	1.22, d (6.4)
11	157.1		1'''	99.7	4.91, s
11-OH		13.52, s	2'''	24.6	1.54-2.15, m
11a	111.7		3'''	24.8	1.54-2.15, m
12	186.3		4'''	67.7	3.59, s
12a	133.6		5'''	66.9	4.06, q (6.5)
			6'''	17.3	1.17, d (6.6)

Figure S1. ^1H NMR (500 MHz) spectrum of compound **1** in CDCl_3 .

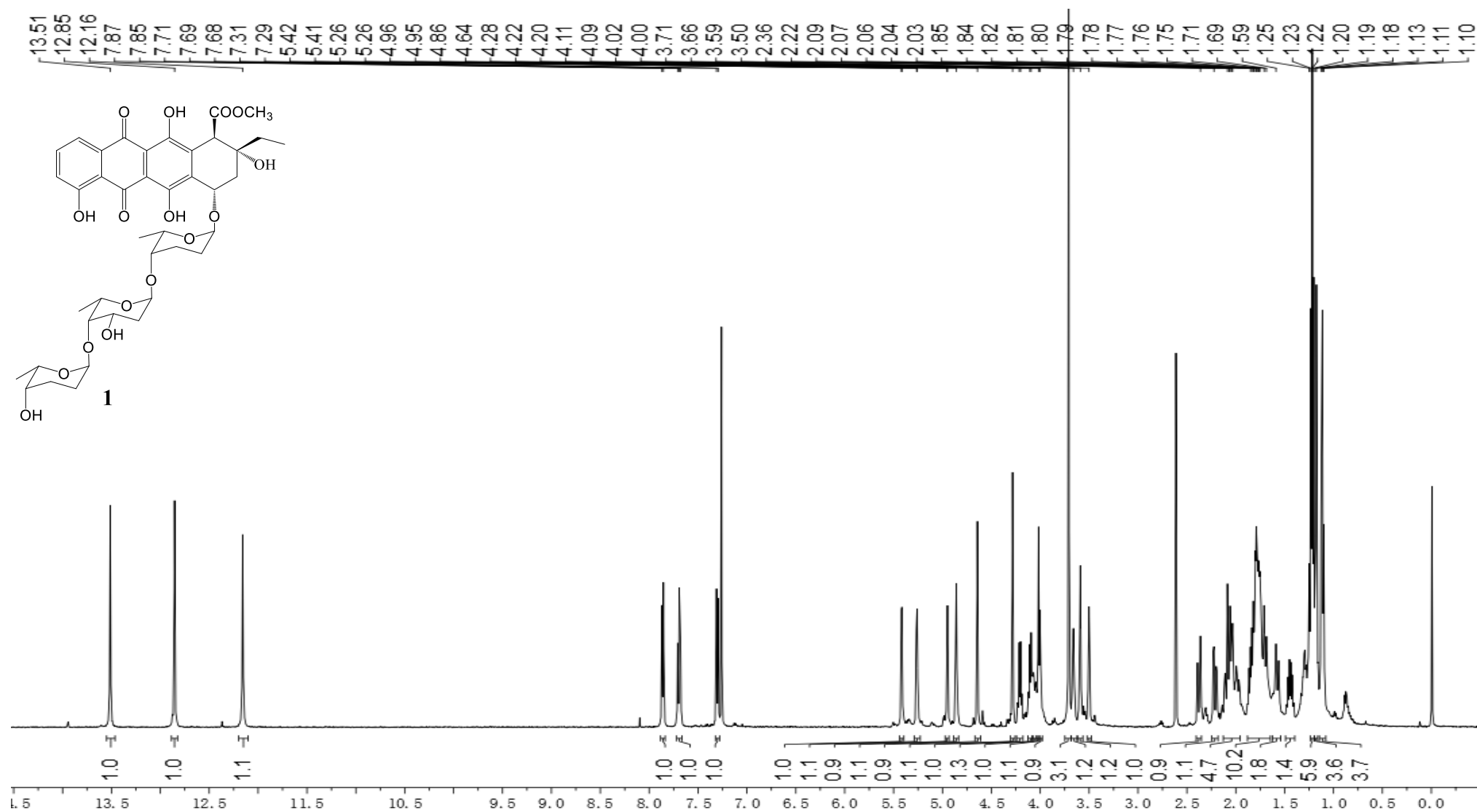


Figure S2. ^{13}C NMR (125 MHz) spectrum of compound **1** in CDCl_3 .

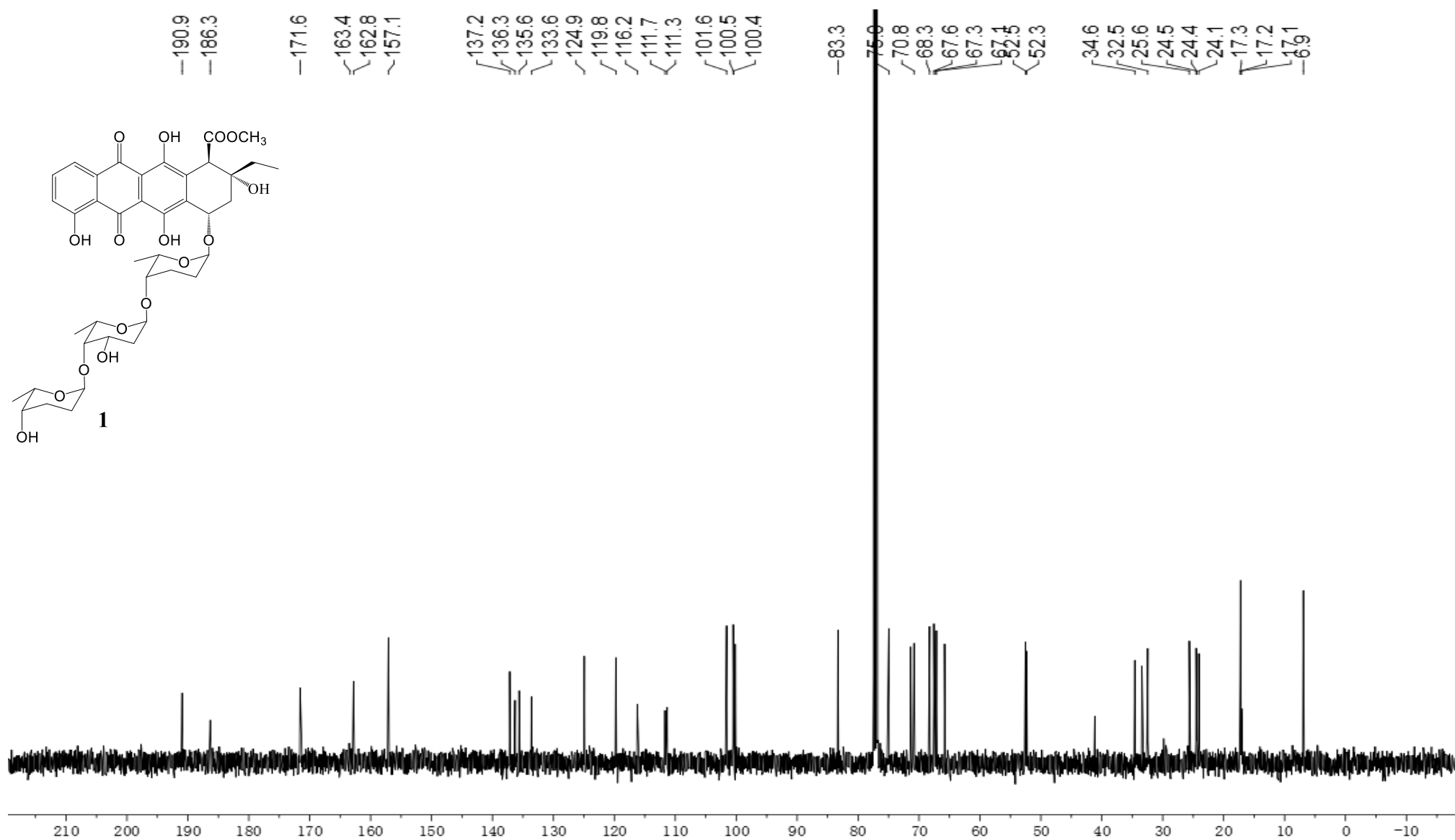


Figure S3. DEPT 135 spectrum of compound **1** in CDCl₃.

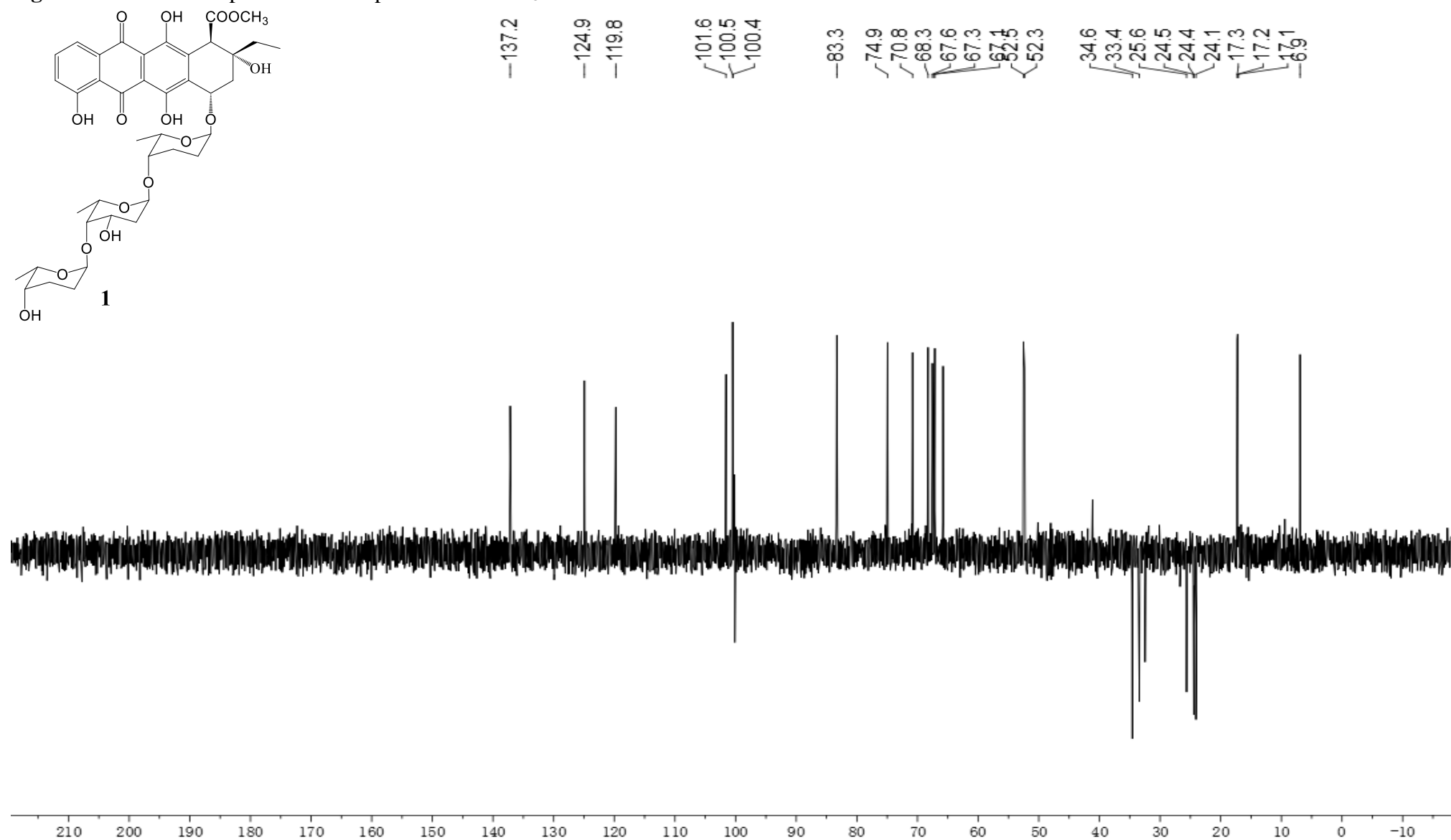


Figure S4. ^1H - ^1H COSY spectrum of compound **1** in CDCl_3 .

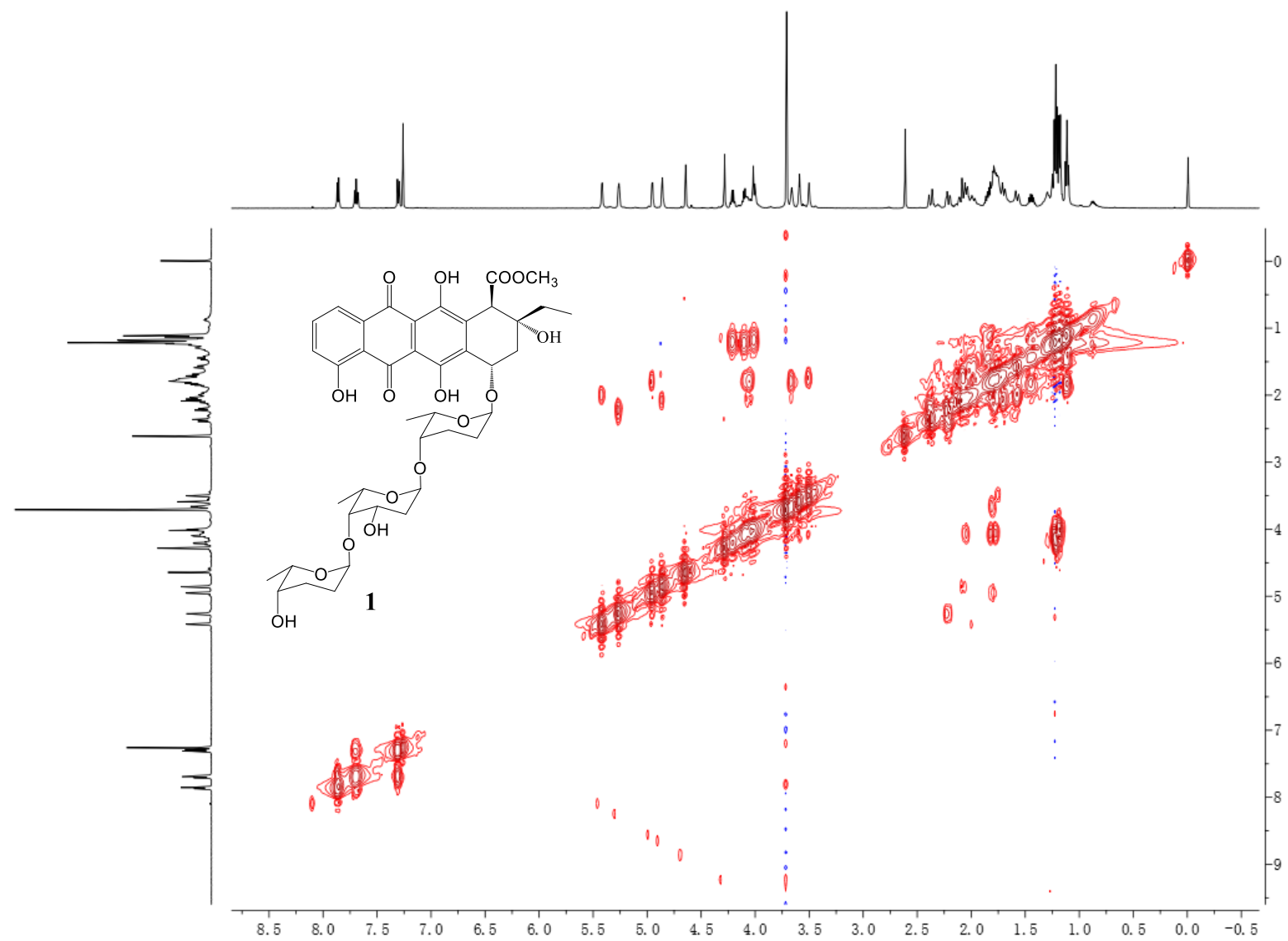


Figure S5. HSQC spectrum of compound **1** in CDCl₃.

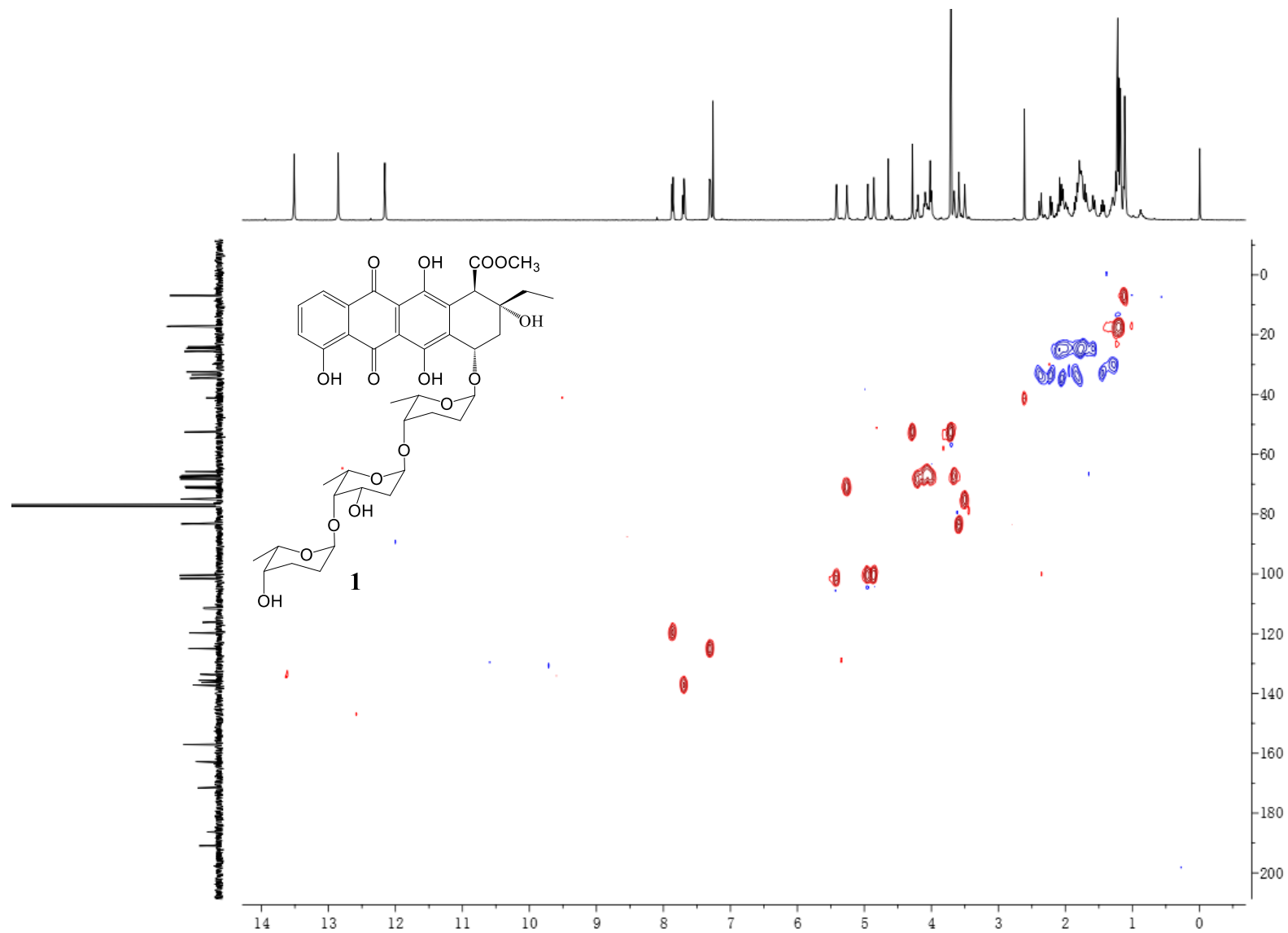


Figure S6. HMBC spectrum of compound **1** in CDCl₃.

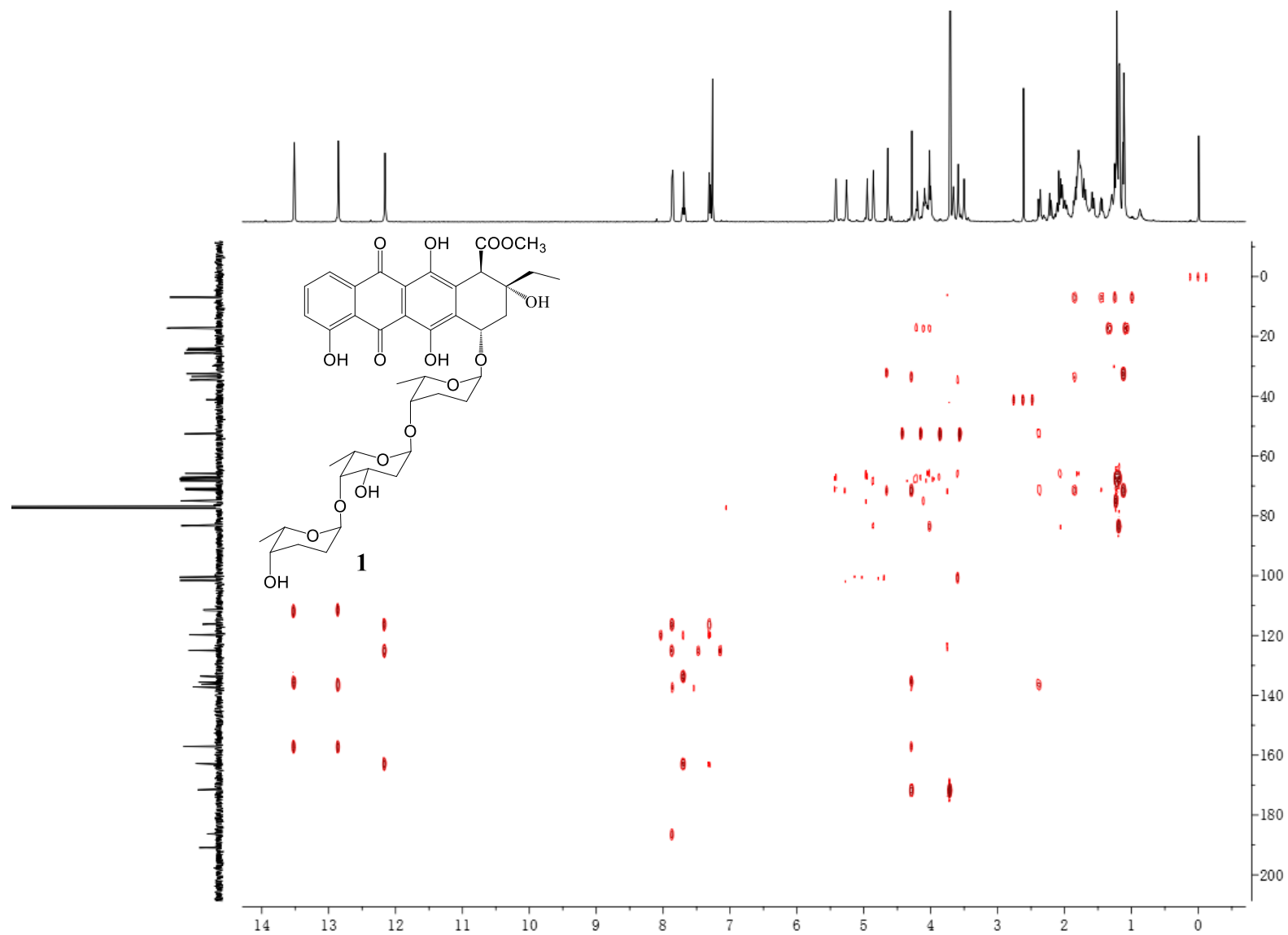


Figure S7. NOESY spectrum of compound **1** in CDCl₃.

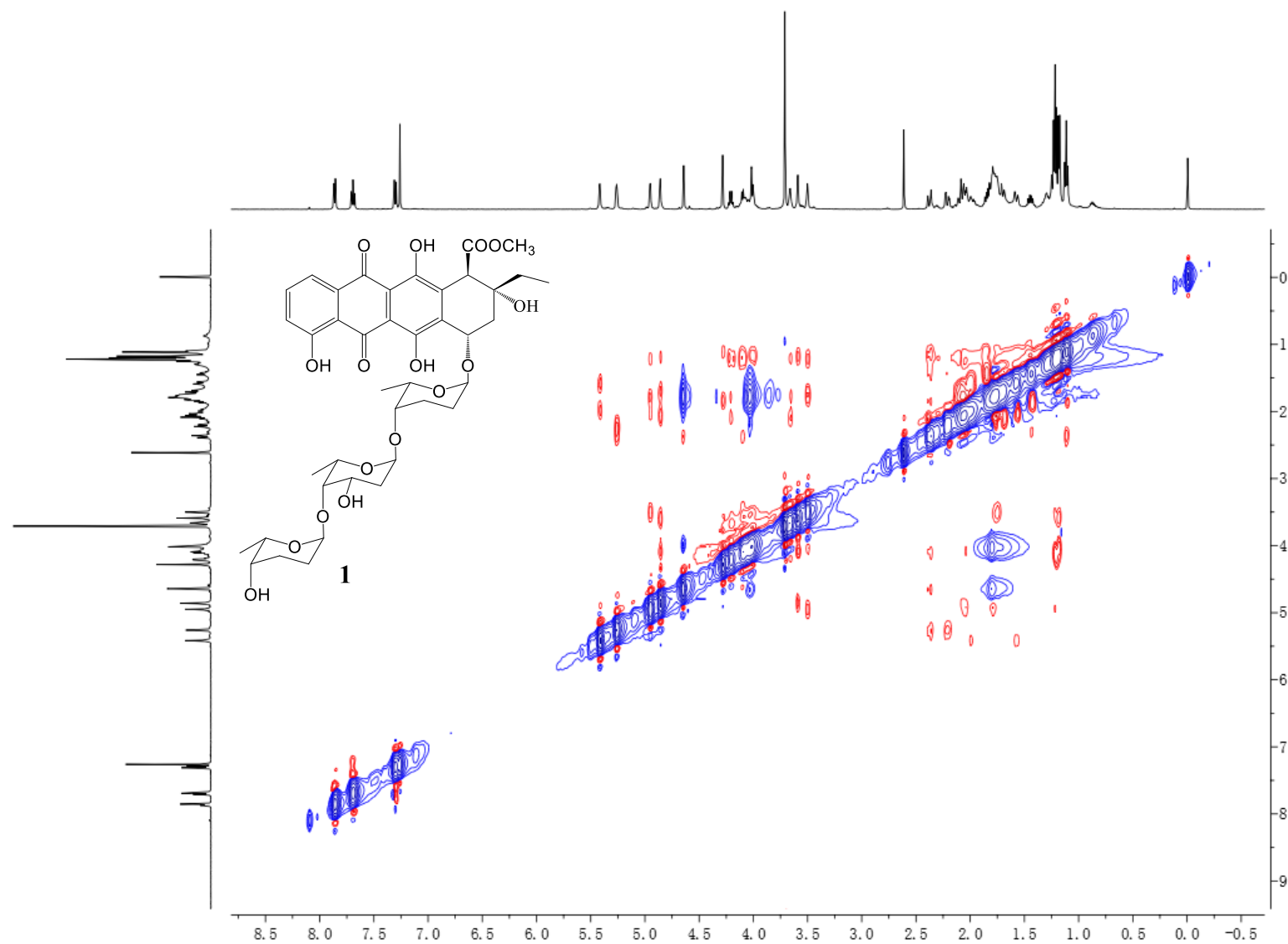


Figure S8. ^1H NMR (500 MHz) spectrum of compound **2** in CDCl_3 .

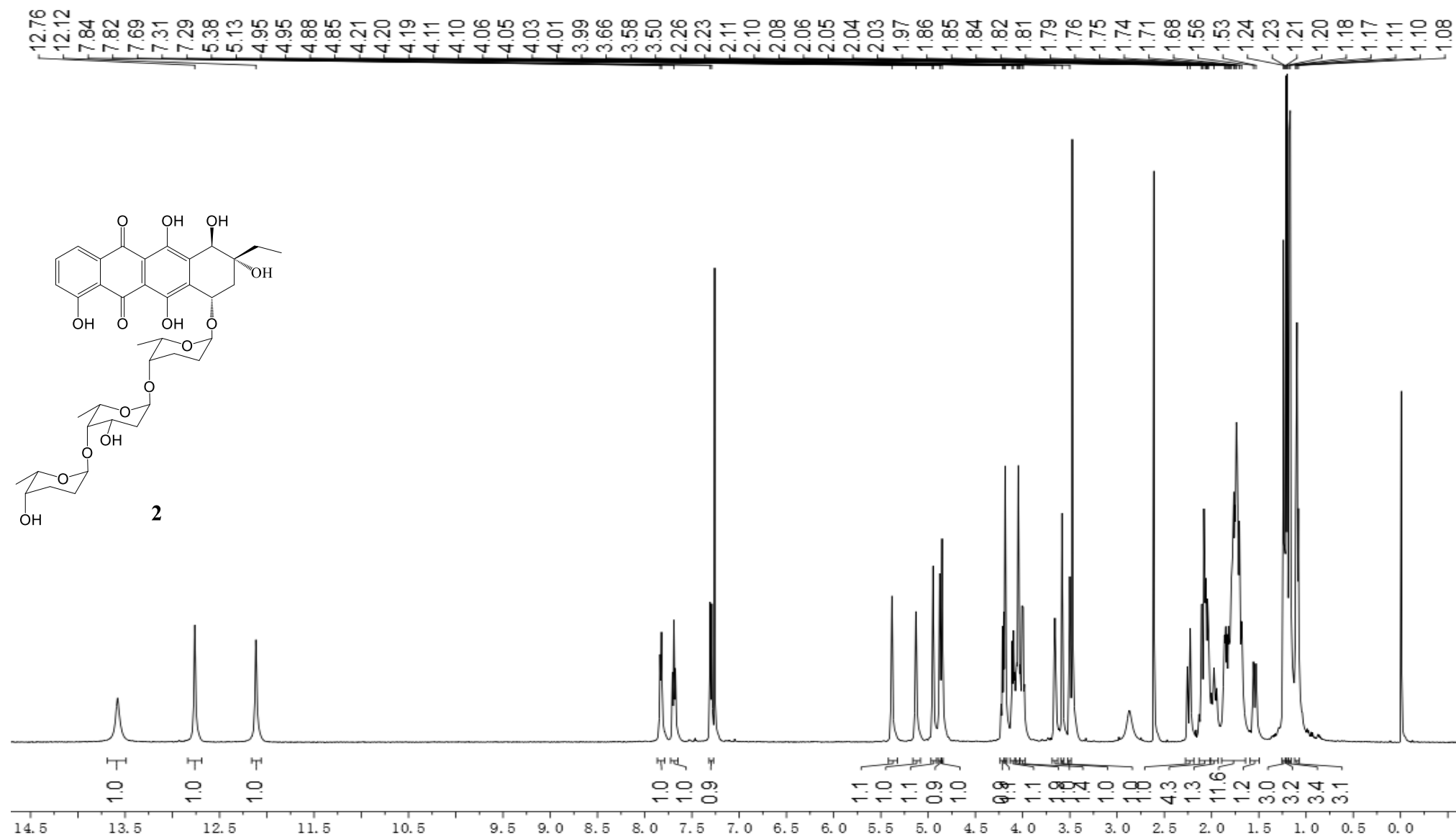


Figure S9. ^{13}C NMR (125 MHz) spectrum of compound **2** in CDCl_3 .

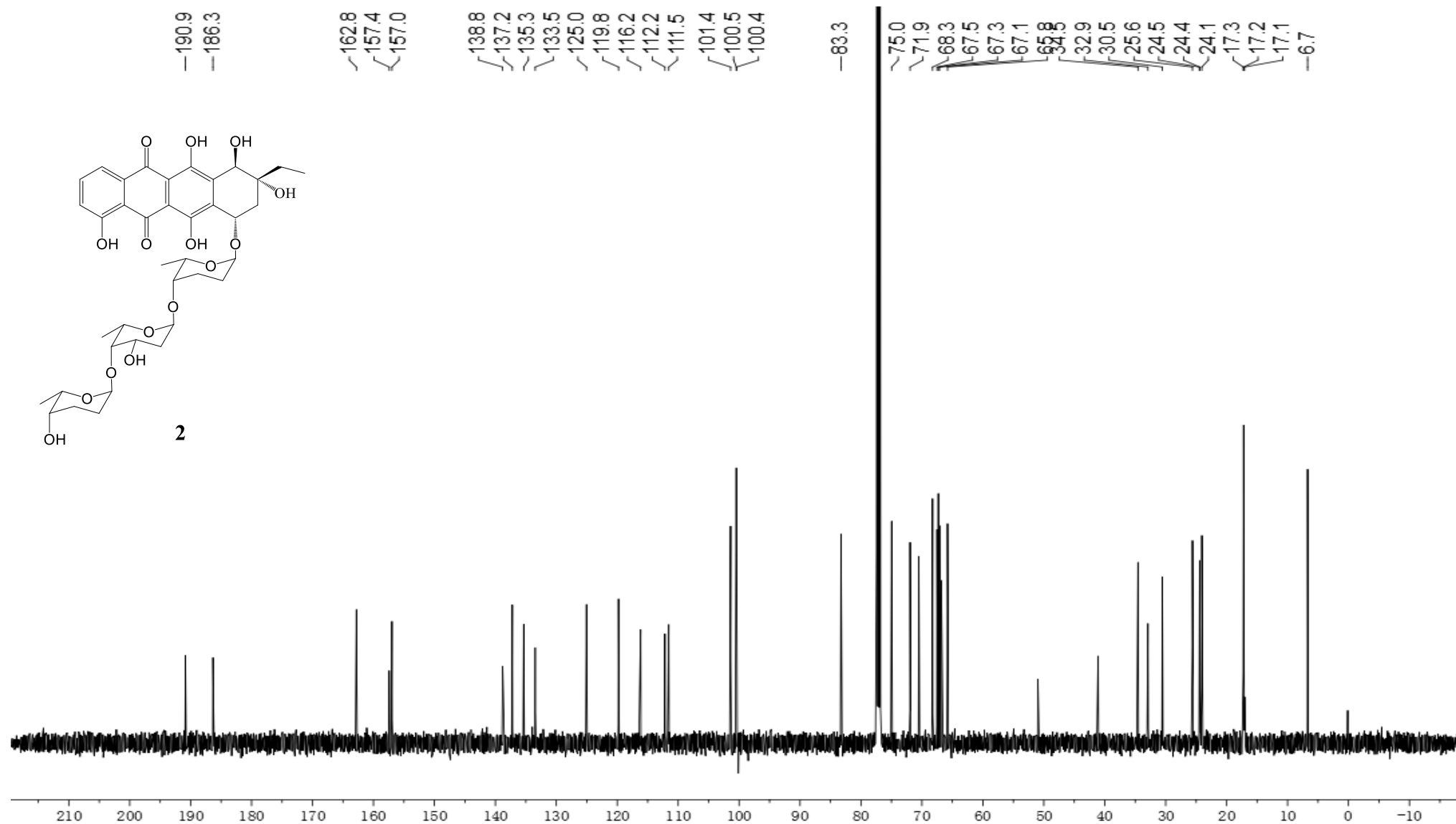


Figure S10. DEPT 135 spectrum of compound **2** in CDCl₃.

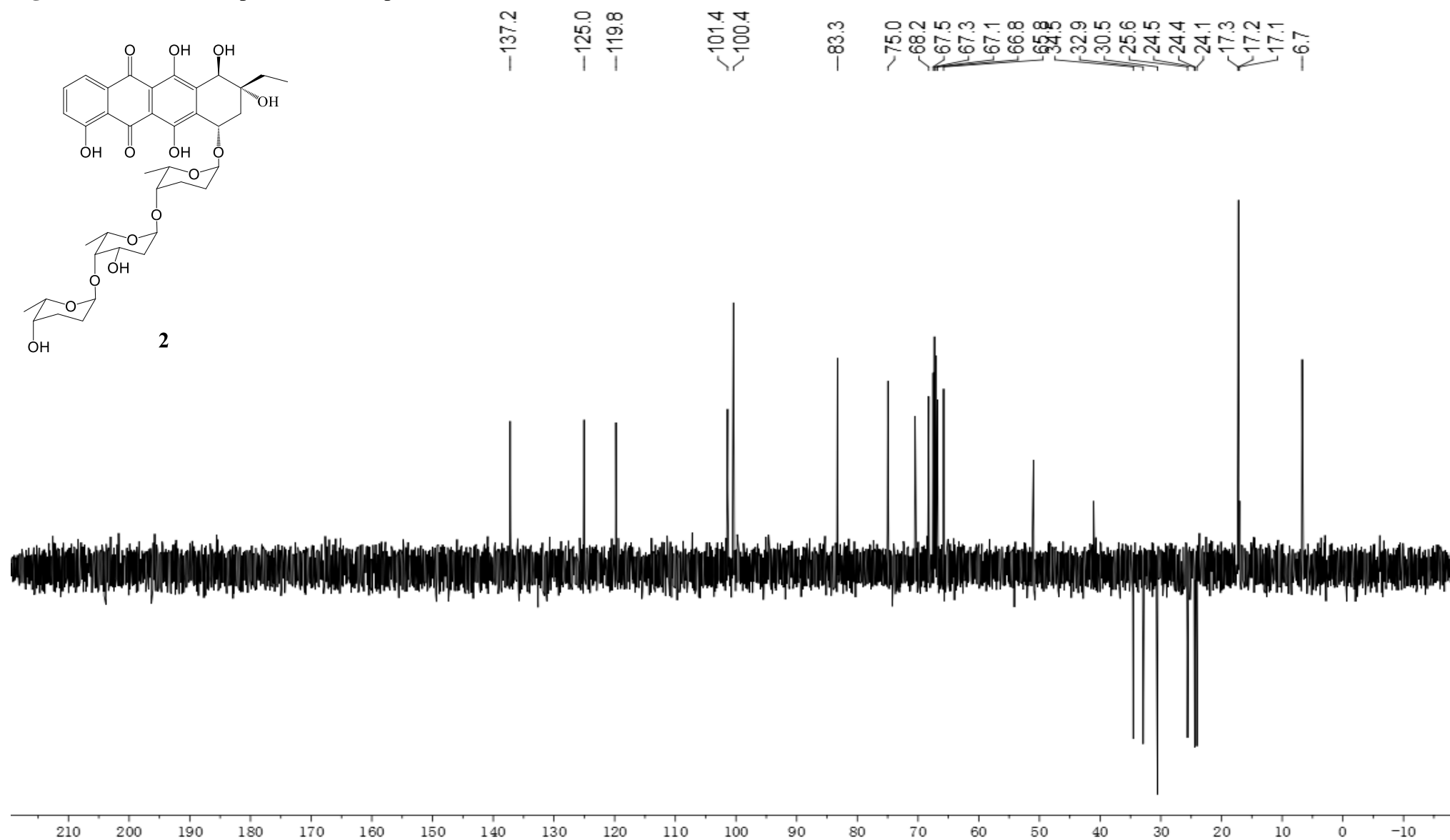


Figure S11. ^1H - ^1H COSY spectrum of compound **2** in CDCl_3 .

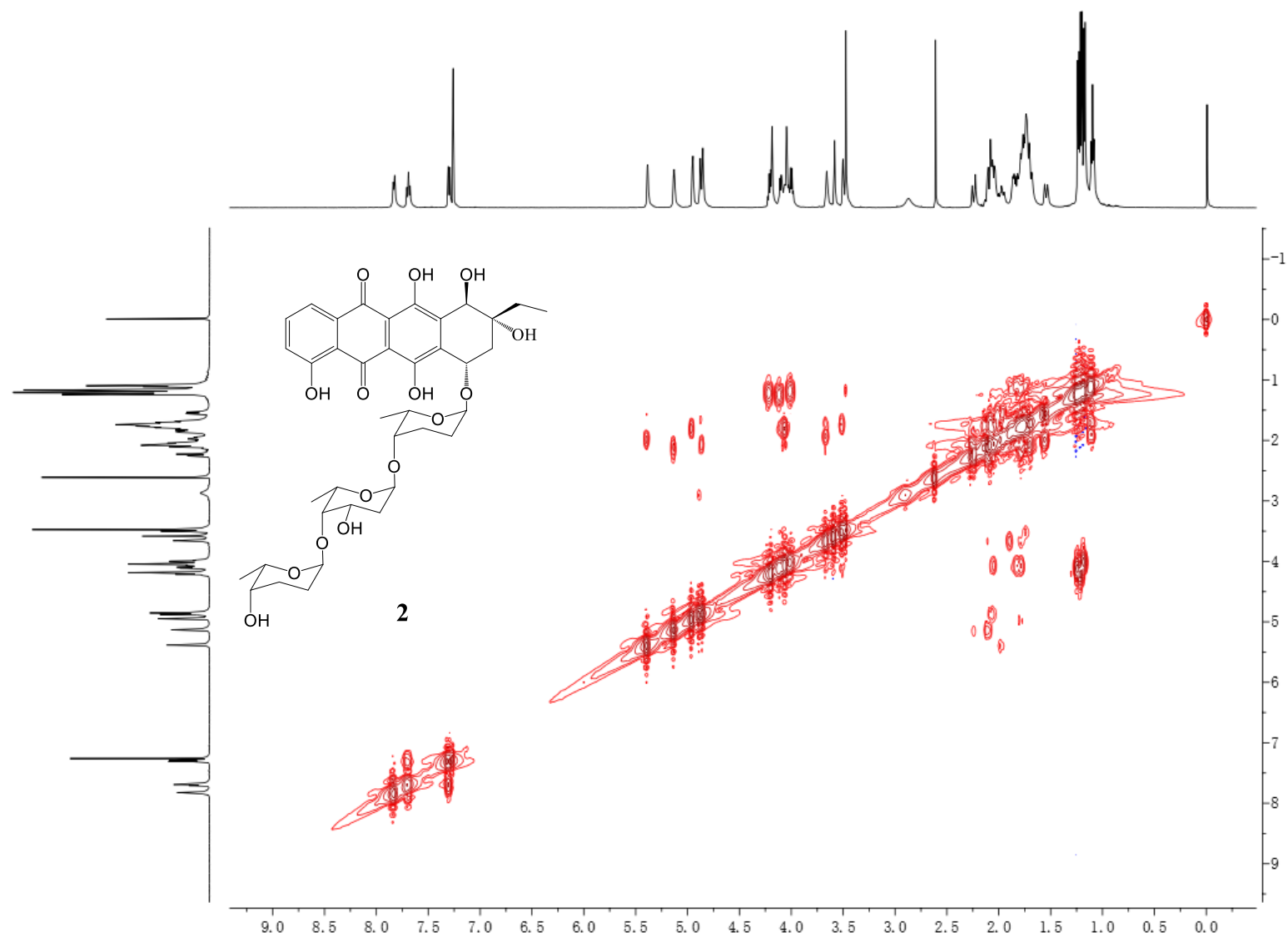


Figure S12. HSQC spectrum of compound **2** in CDCl₃.

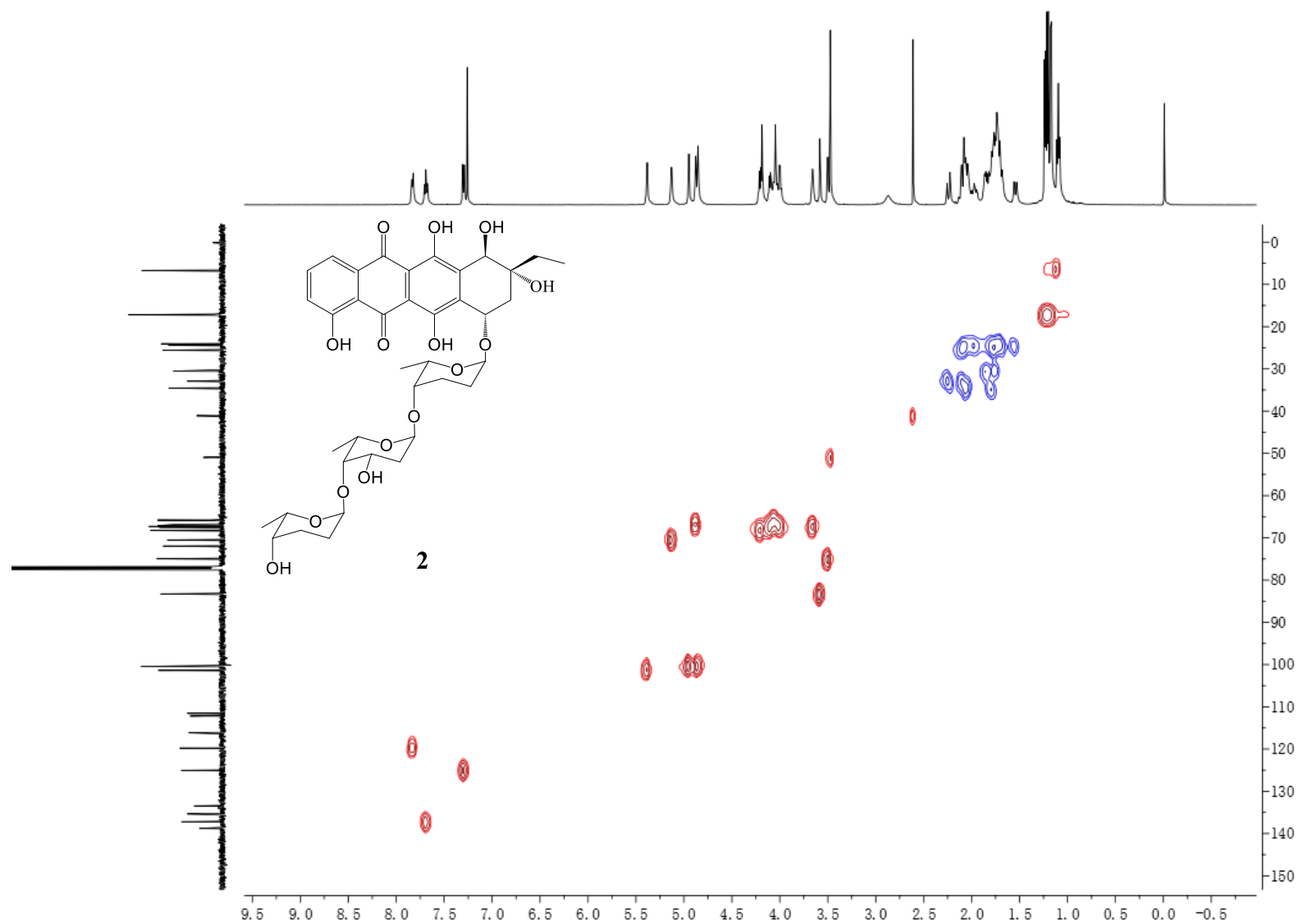


Figure S13. HMBC spectrum of compound **2** in CDCl₃.

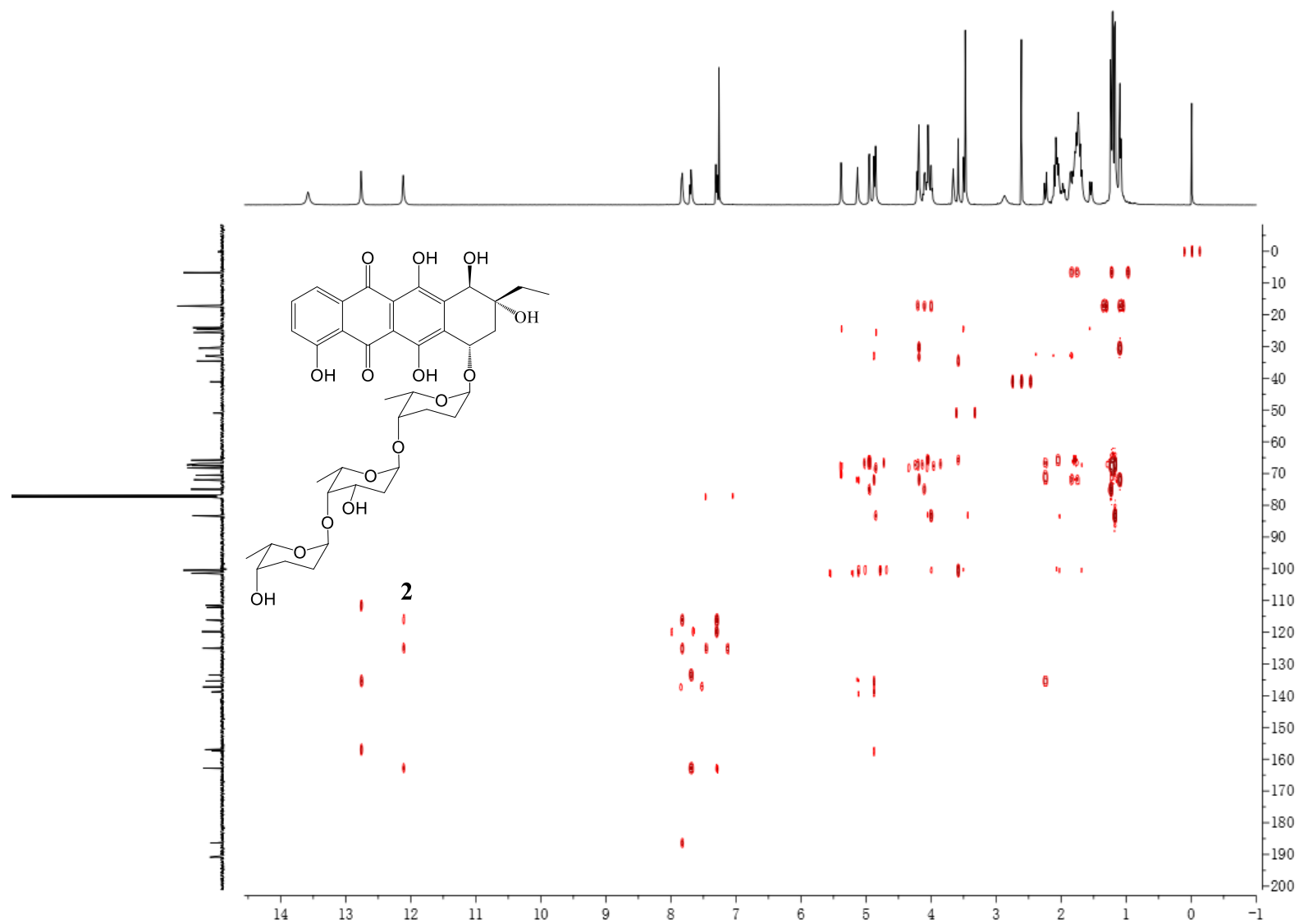


Figure S14. NOESY spectrum of compound **2** in CDCl₃.

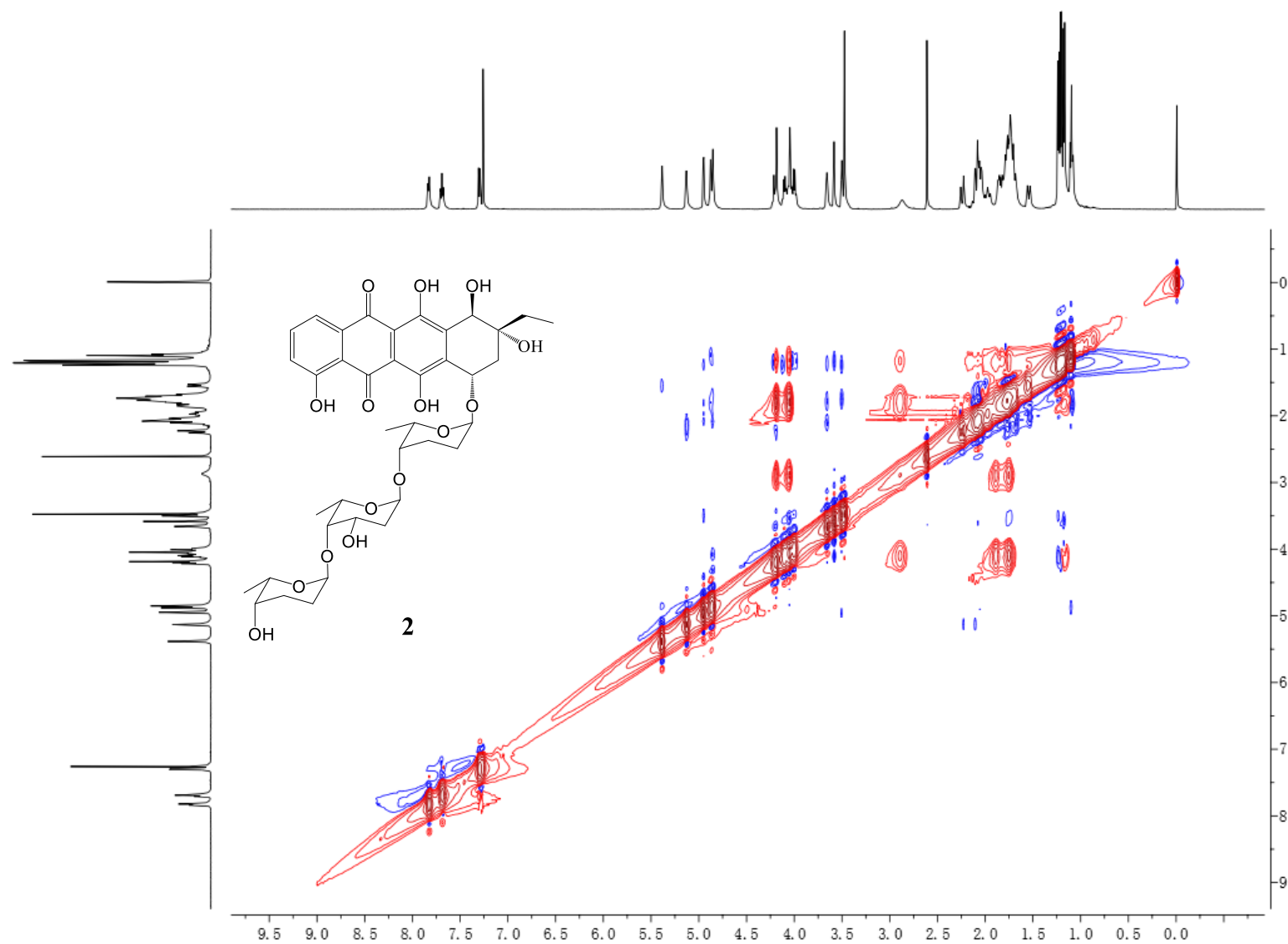


Figure S15. ^1H NMR (500 MHz) spectrum of compound **3** in CDCl_3 .

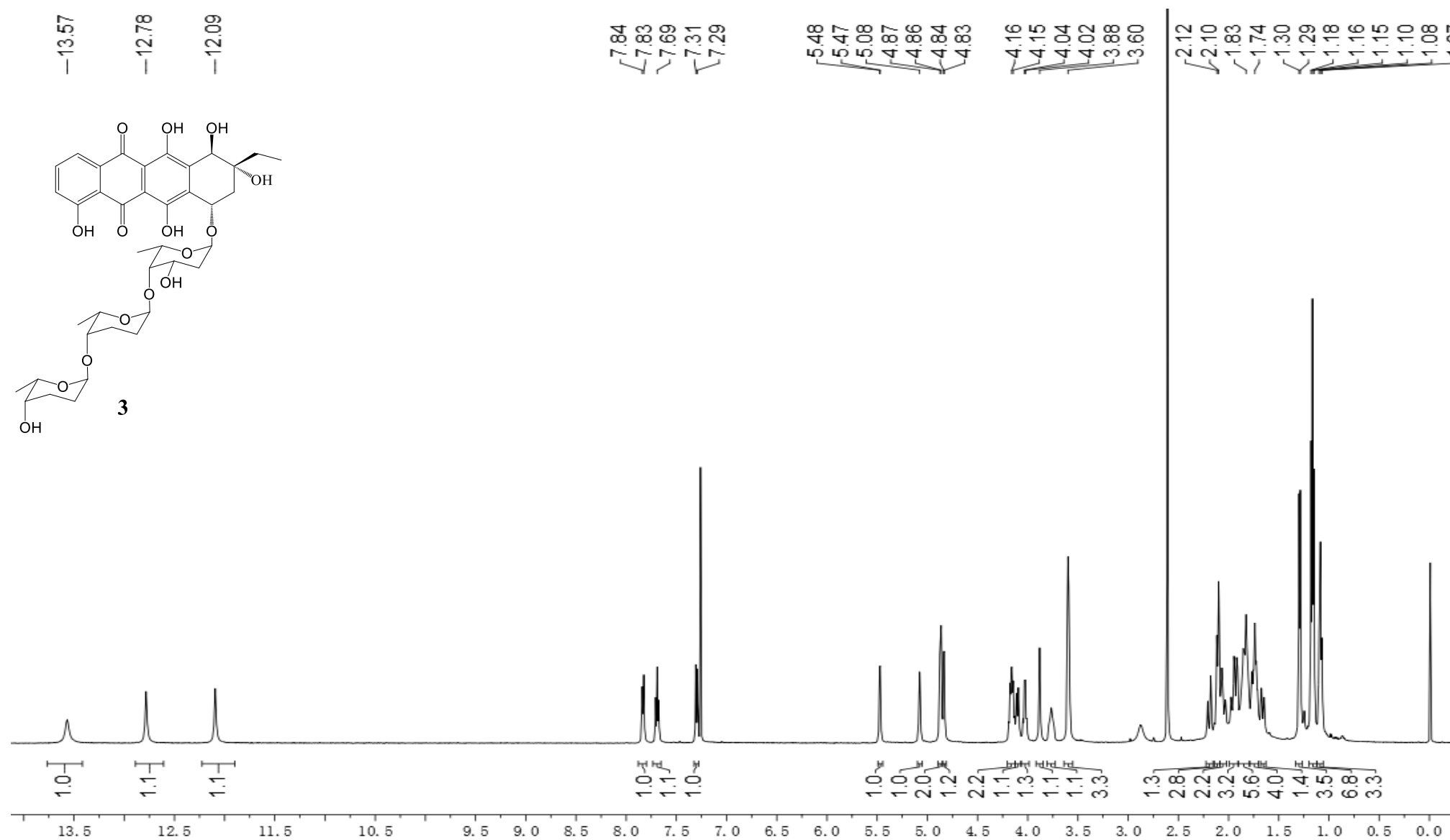


Figure S16. ^{13}C NMR (125 MHz) spectrum of compound **3** in CDCl_3 .

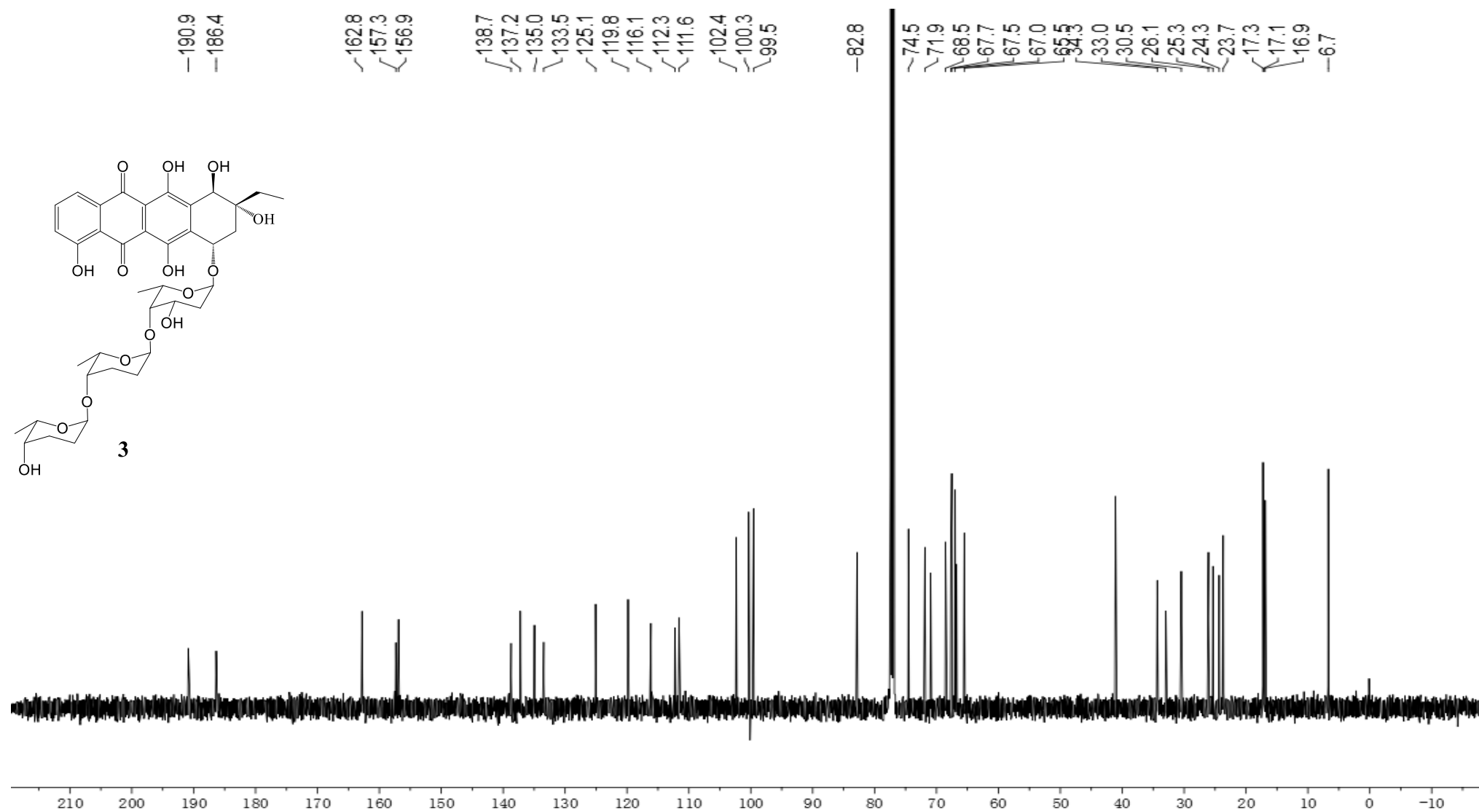


Figure S17. DEPT 135 spectrum of compound **3** in CDCl₃.

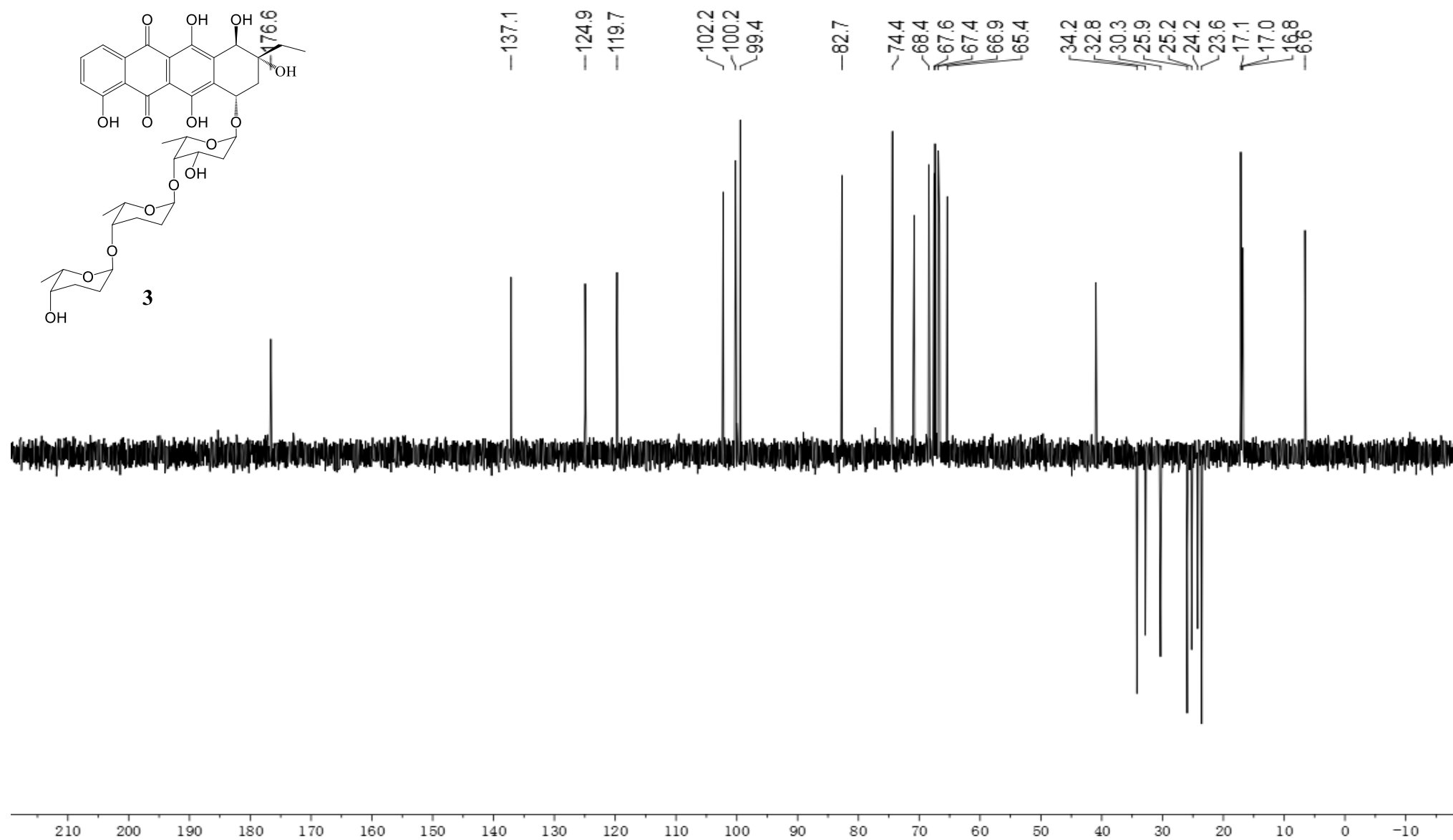


Figure S18. ^1H - ^1H COSY spectrum of compound **3** in CDCl_3 .

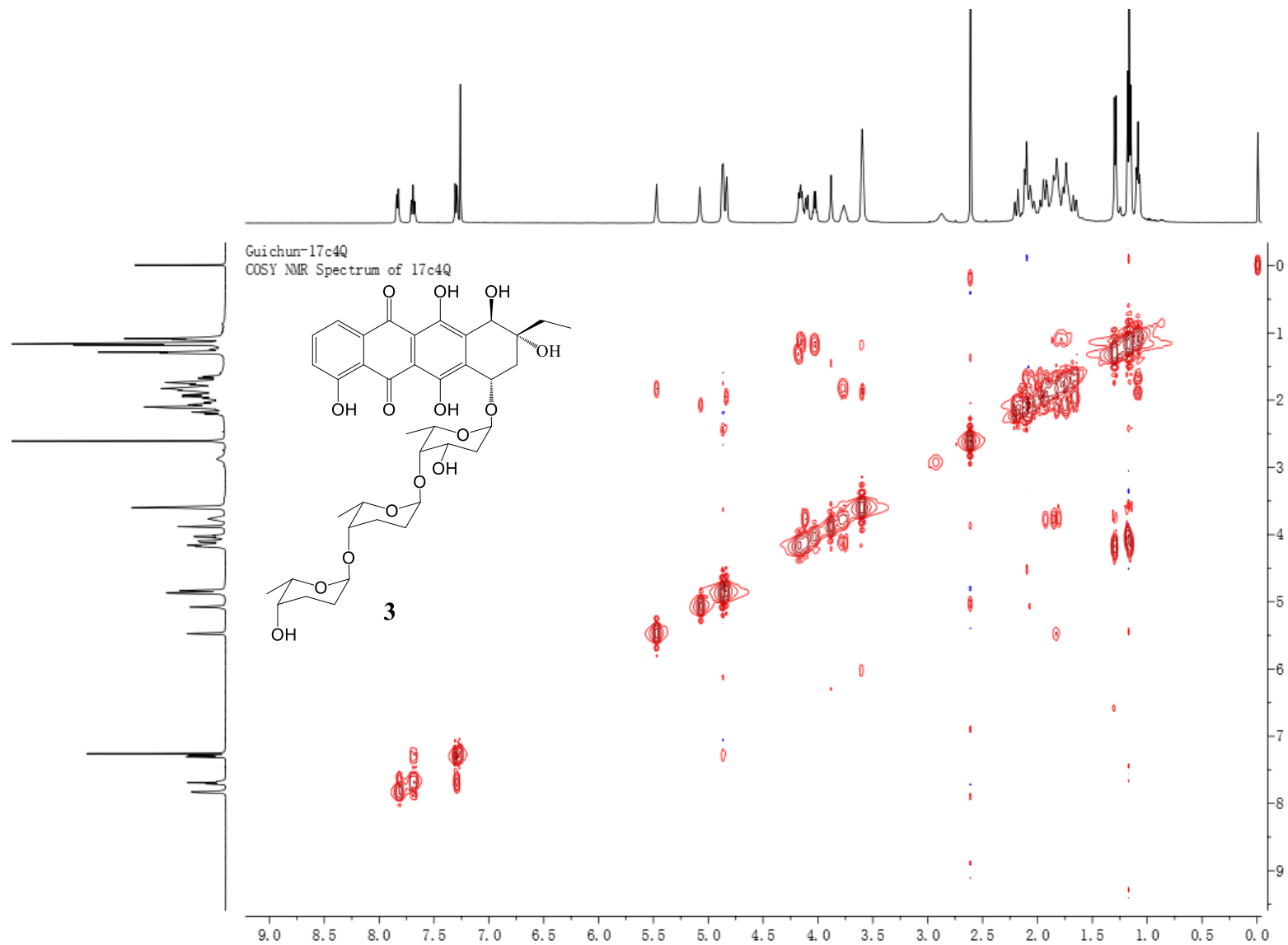


Figure S19. HSQC spectrum of compound **3** in CDCl₃.

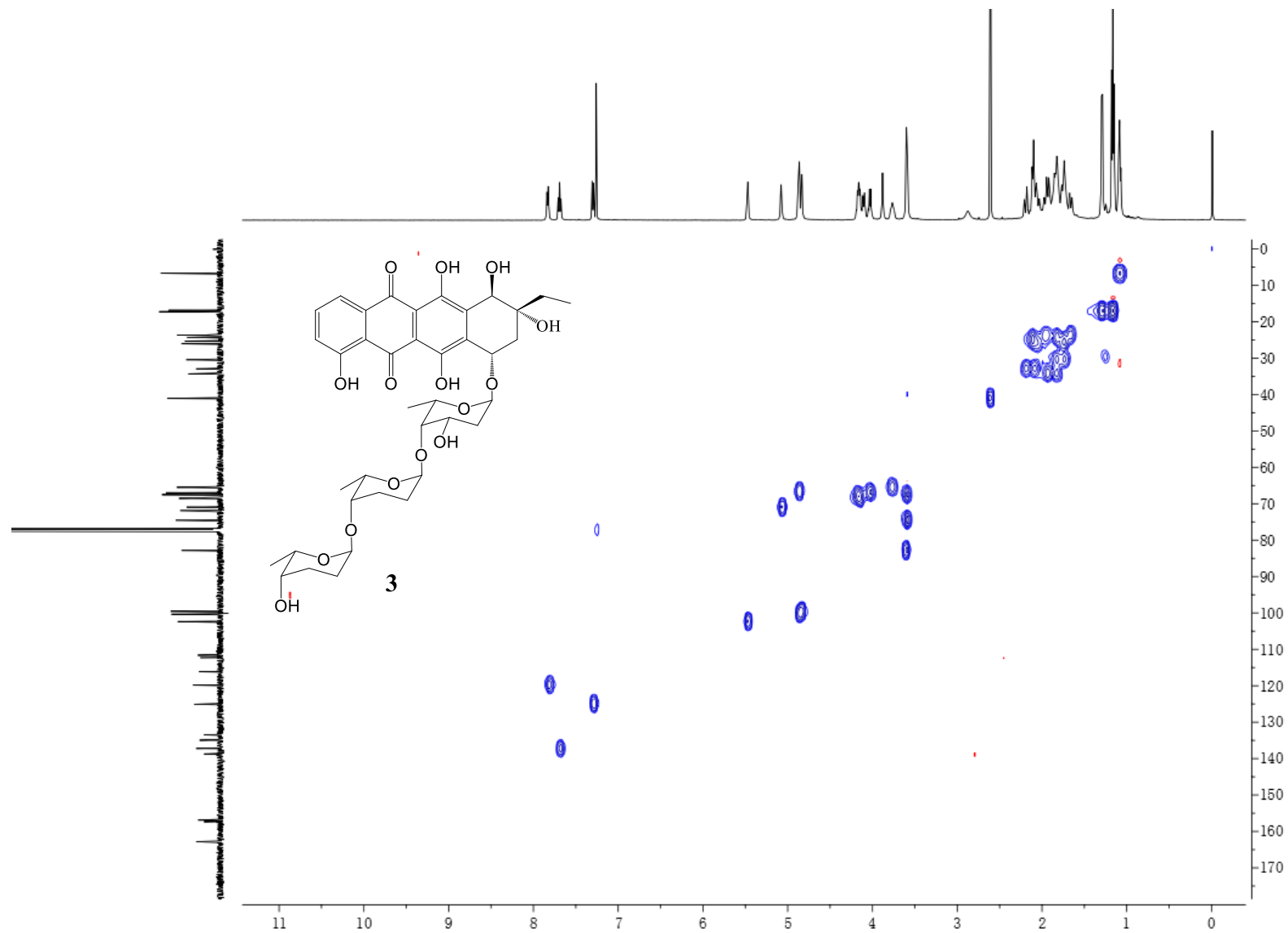


Figure S20. HMBC spectrum of compound **3** in CDCl₃.

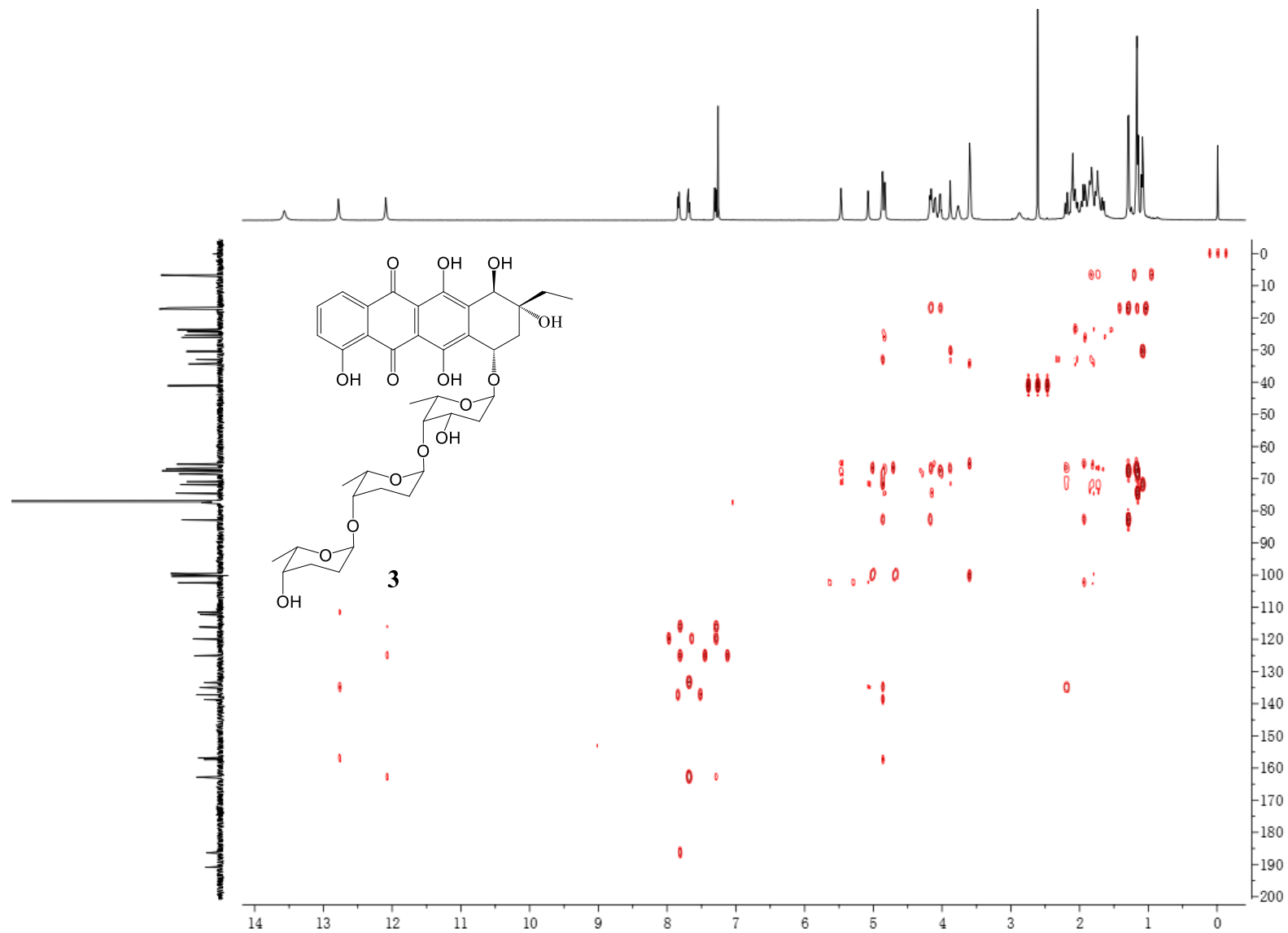


Figure S21. NOESY spectrum of compound **3** in CDCl₃.

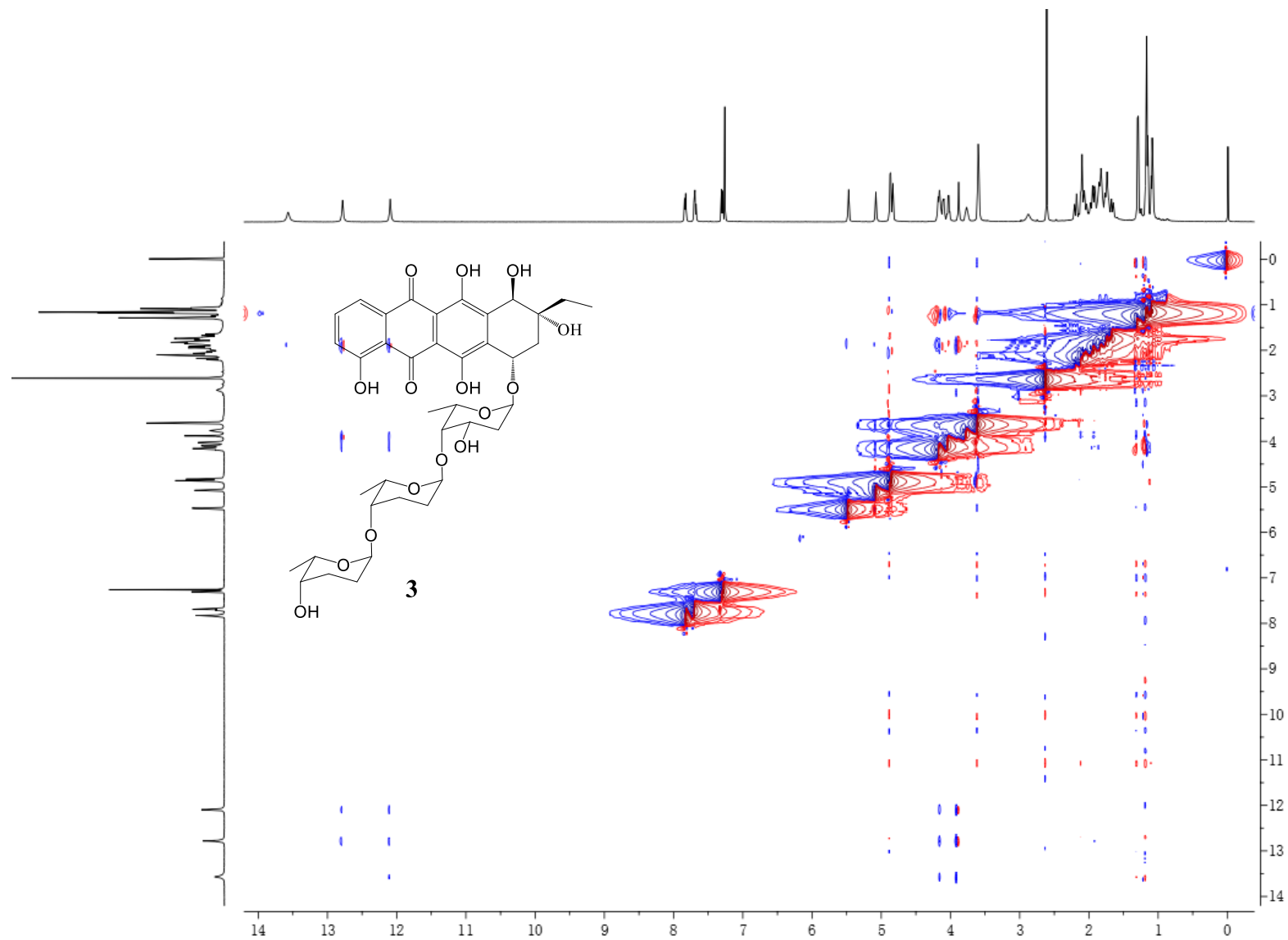


Figure S22. ^1H NMR (500 MHz) spectrum of compound **4** in CDCl_3 .

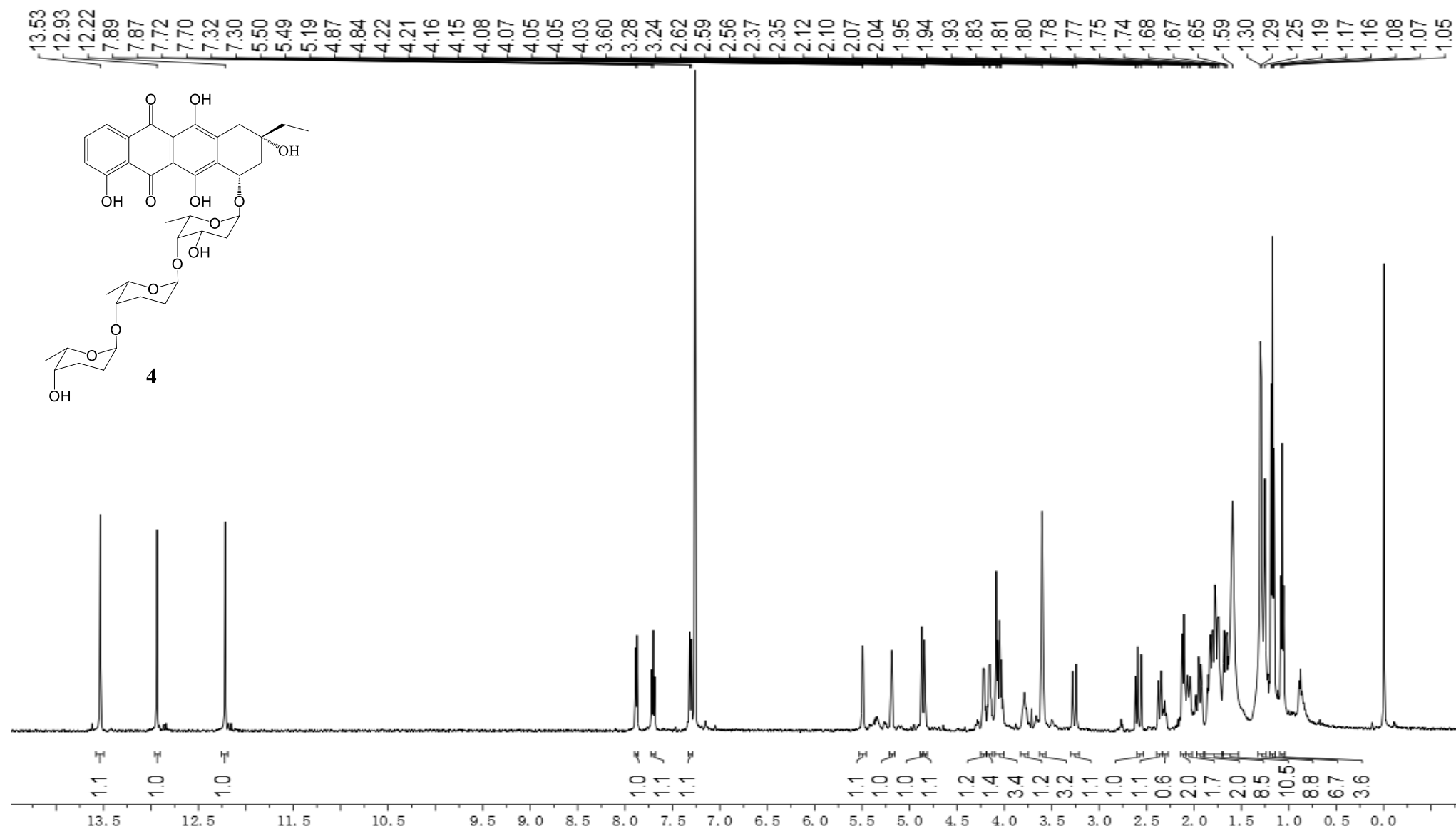


Figure S23. ^{13}C NMR (125 MHz) spectrum of compound **4** in CDCl_3 .

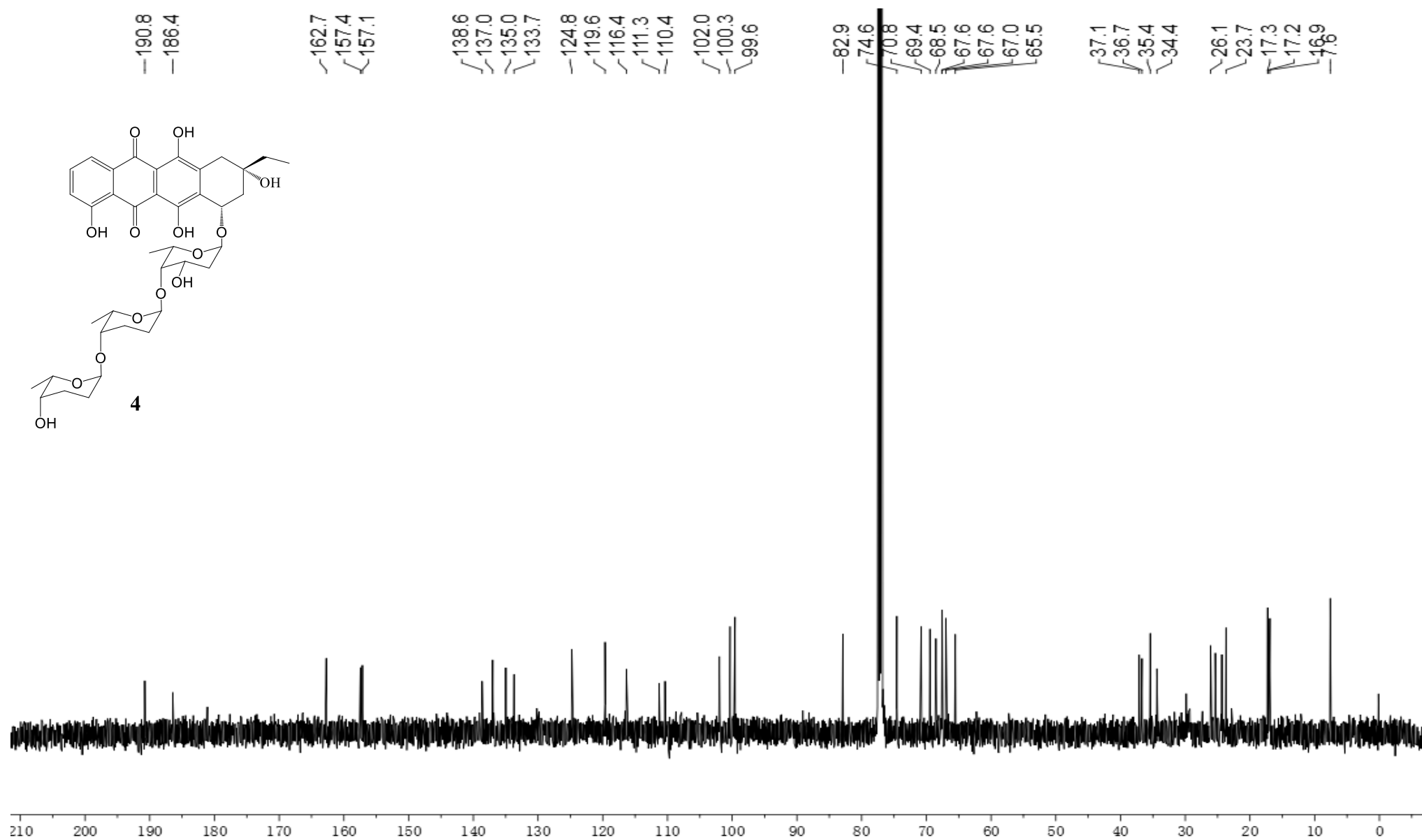


Figure S24. DEPT 135 spectrum of compound **4** in CDCl₃.

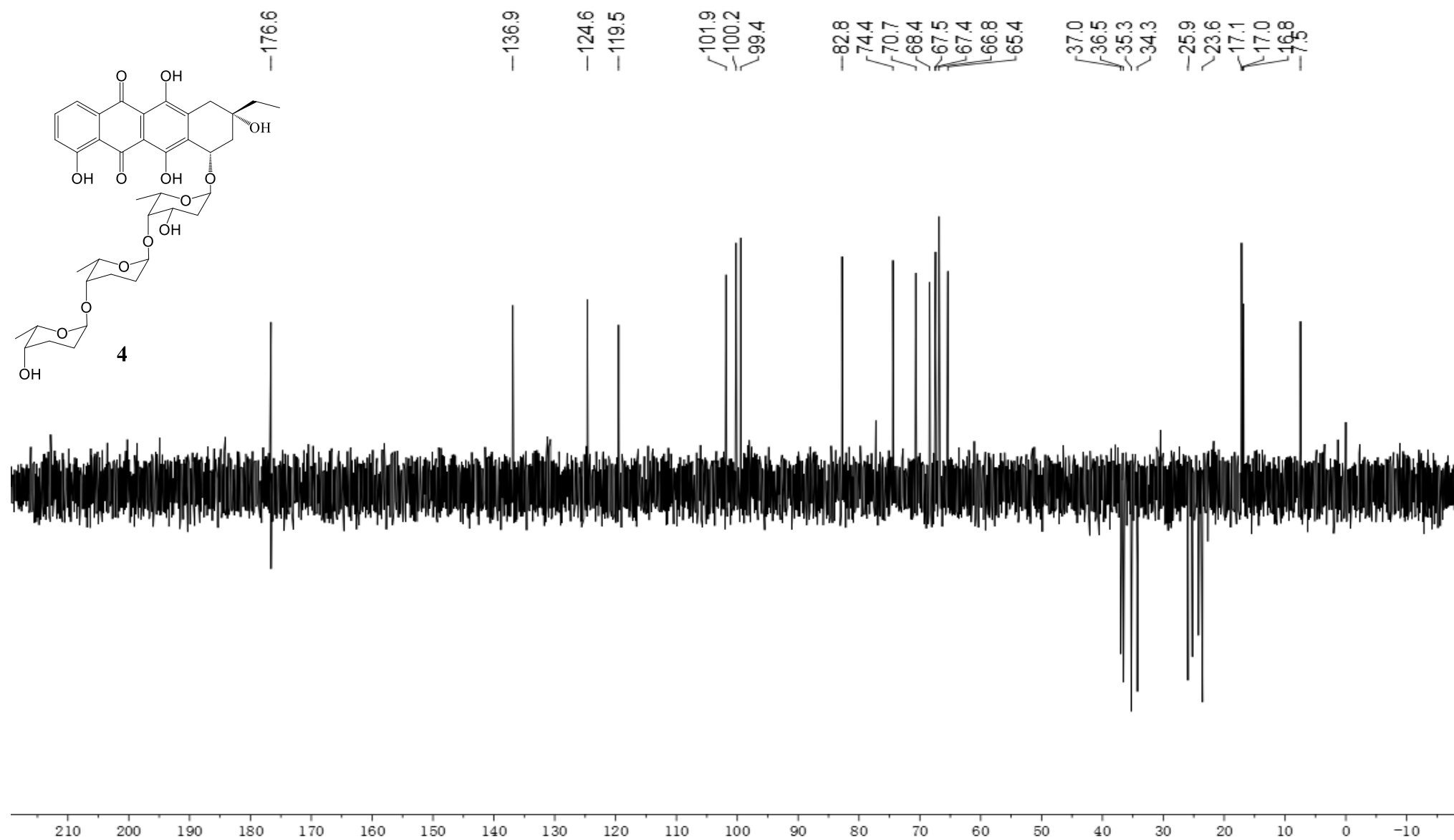


Figure S25. ^1H - ^1H COSY spectrum of compound **4** in CDCl_3 .

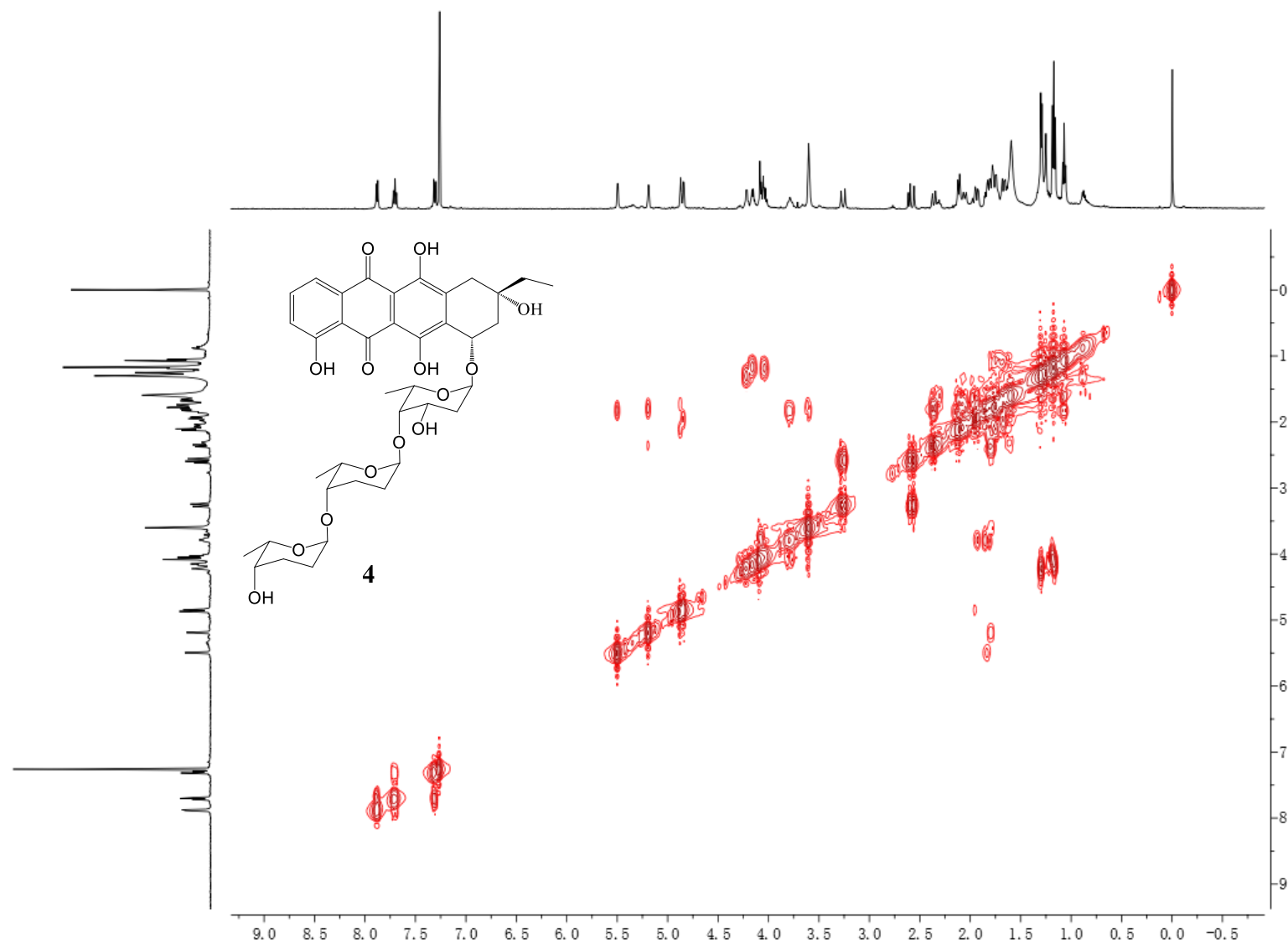


Figure S26. HSQC spectrum of compound **4** in CDCl₃.

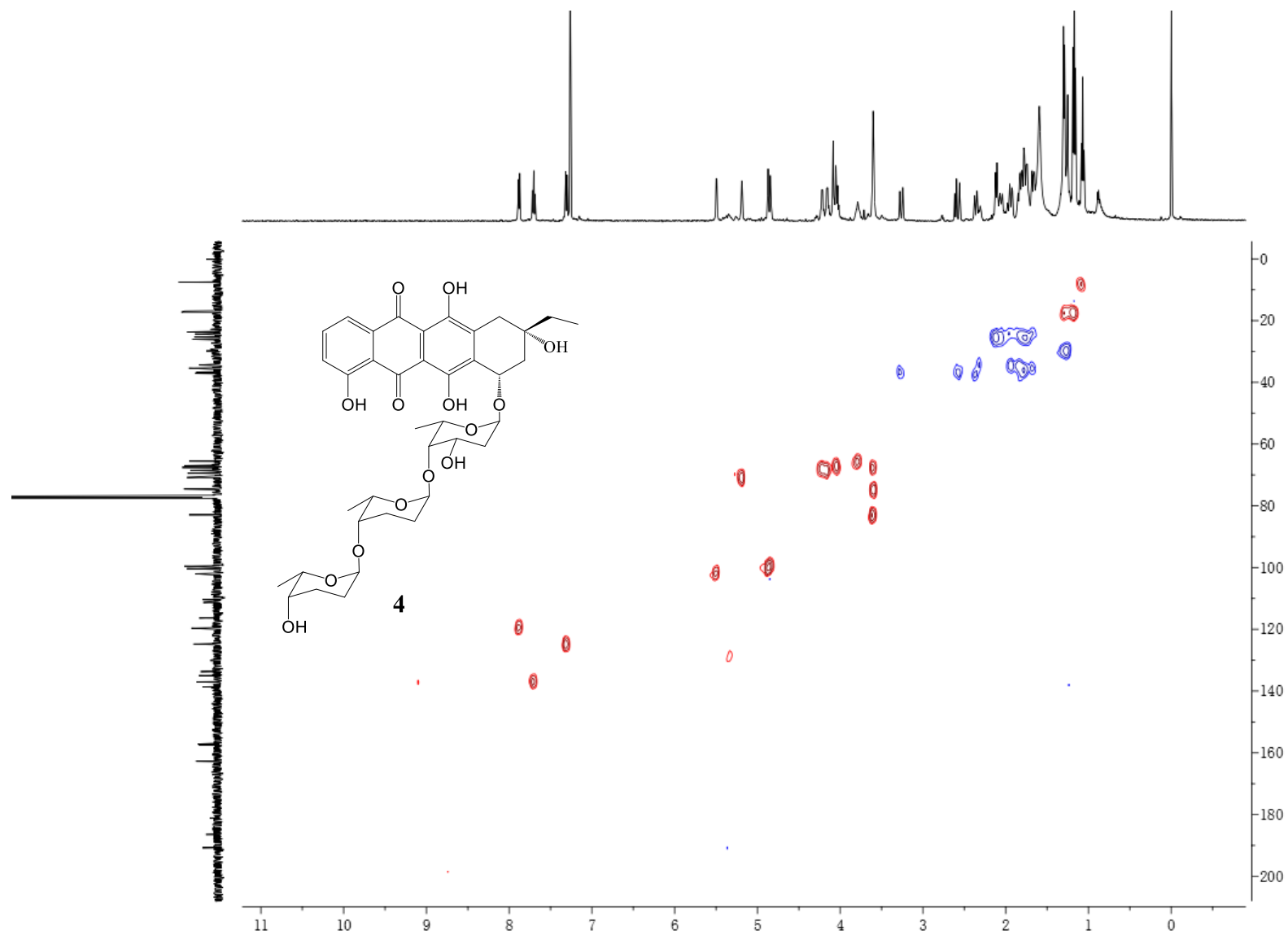


Figure S27. HMBC spectrum of compound **4** in CDCl₃.

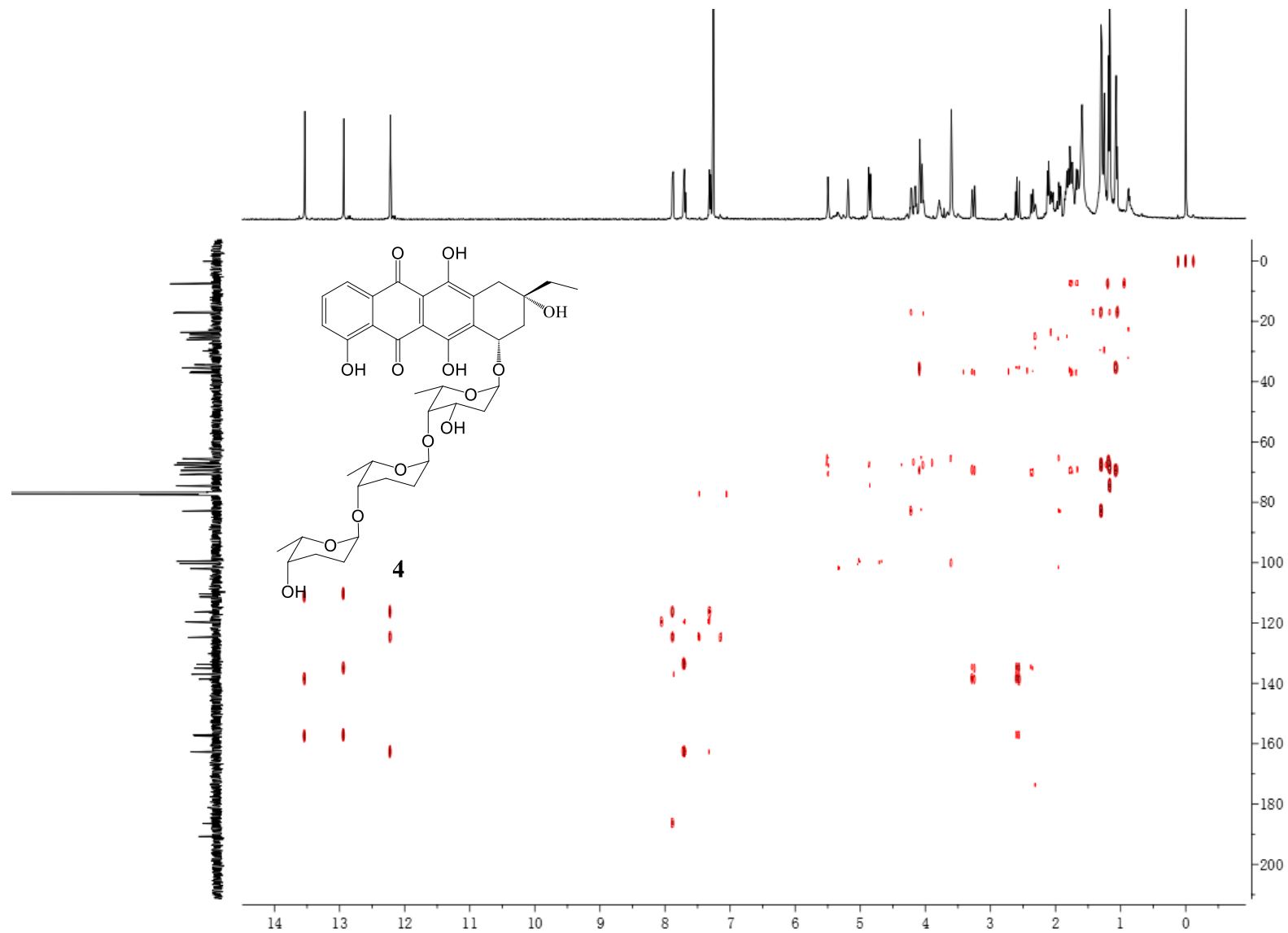


Figure S28. NOESY spectrum of compound **4** in CDCl₃.

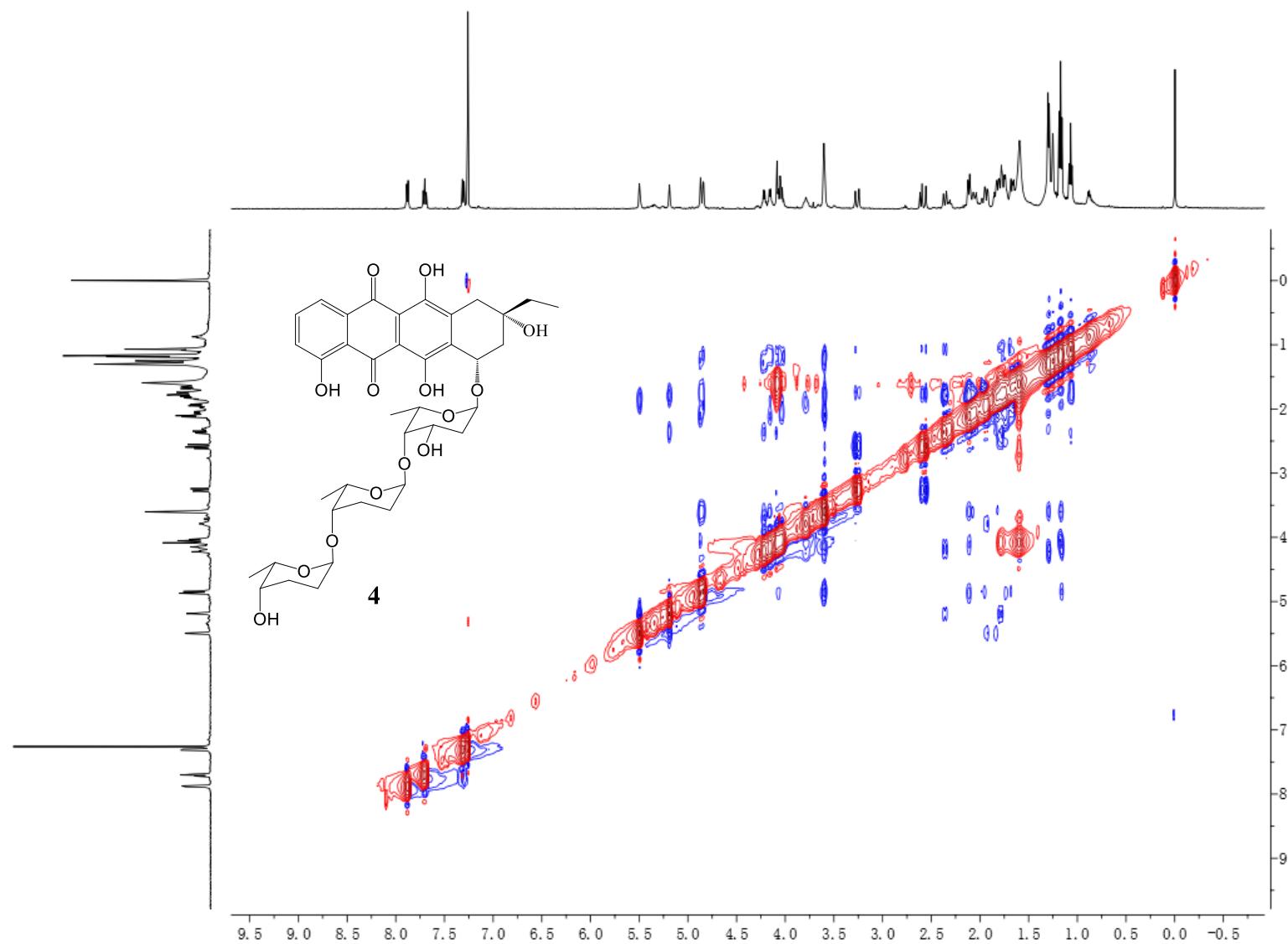


Figure S29. ^1H NMR (500 MHz) spectrum of compound **5** in CDCl_3 .

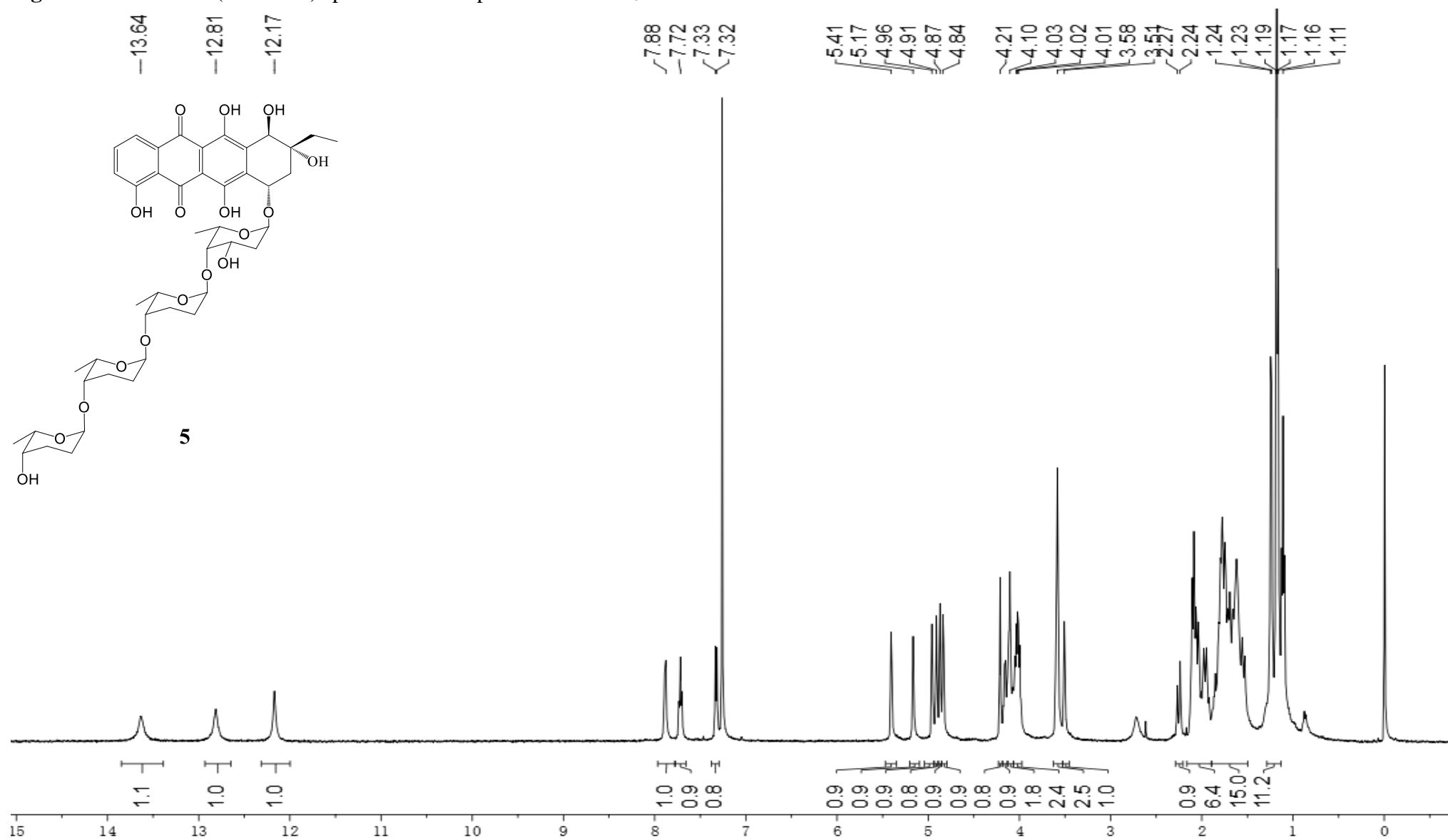


Figure S30. ^{13}C NMR (125 MHz) spectrum of compound **5** in CDCl_3 .

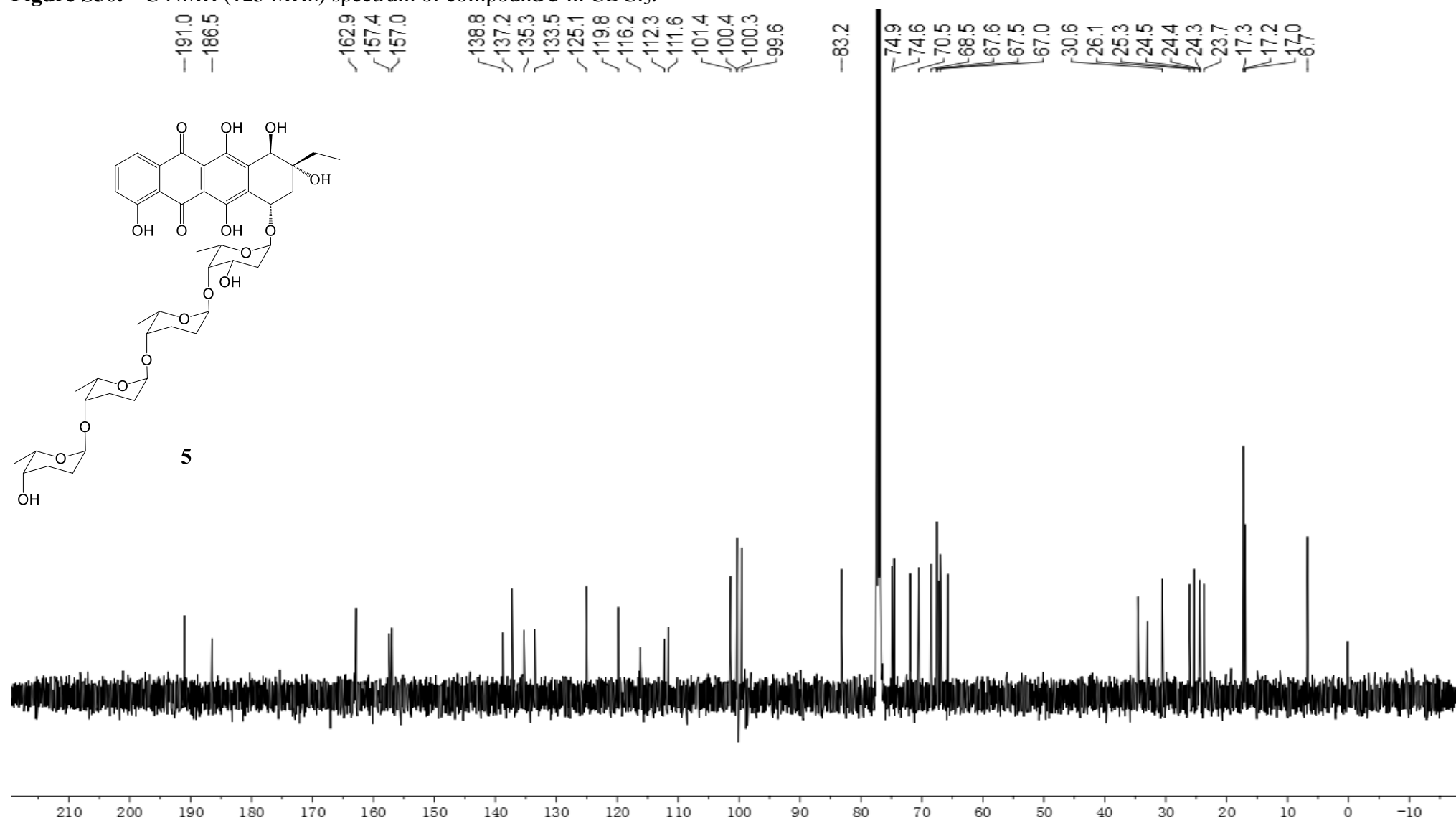


Figure S31. DEPT 135 spectrum of compound **5** in CDCl₃.

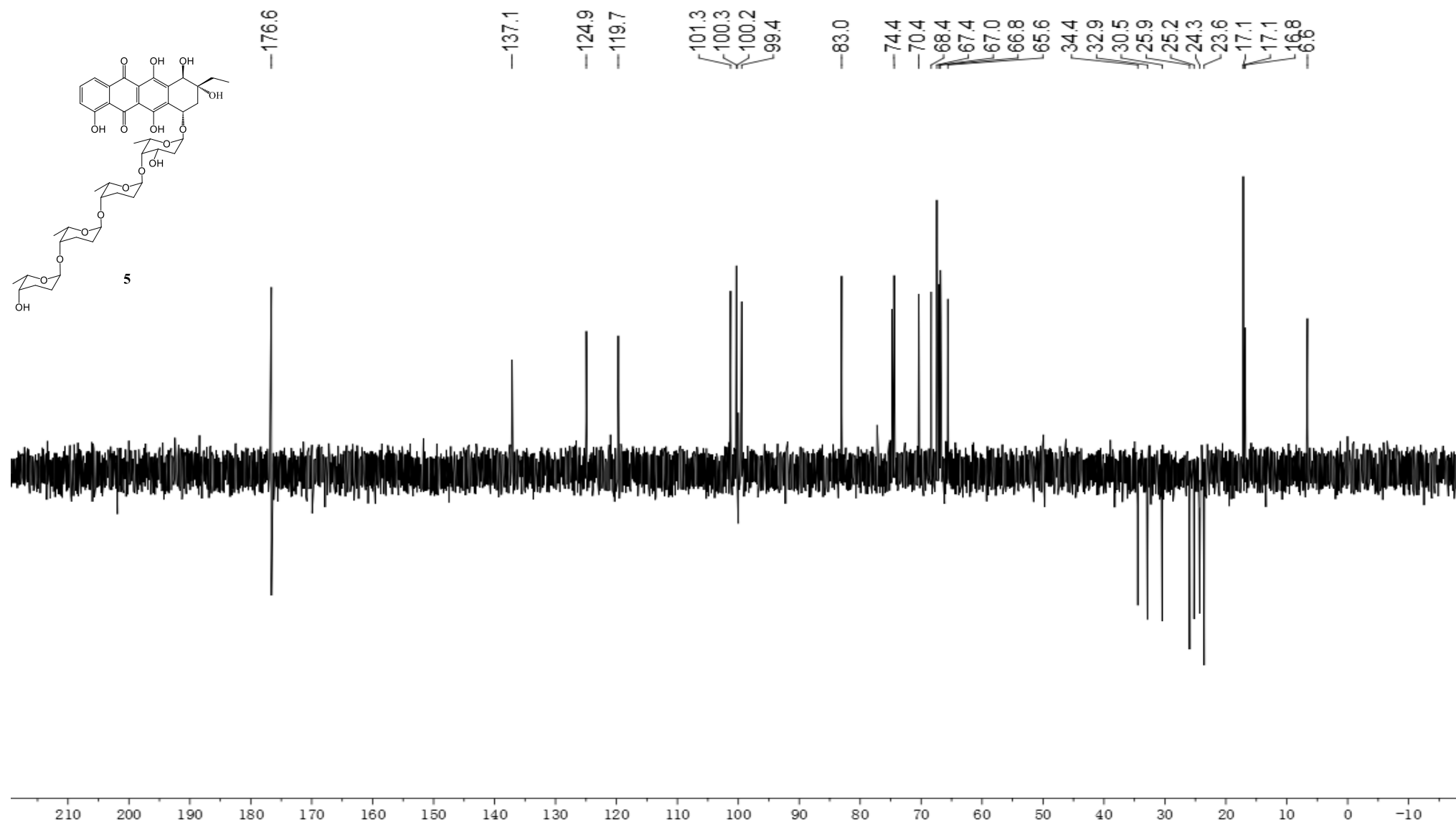


Figure S32. ^1H - ^1H COSY spectrum of compound **5** in CDCl_3 .

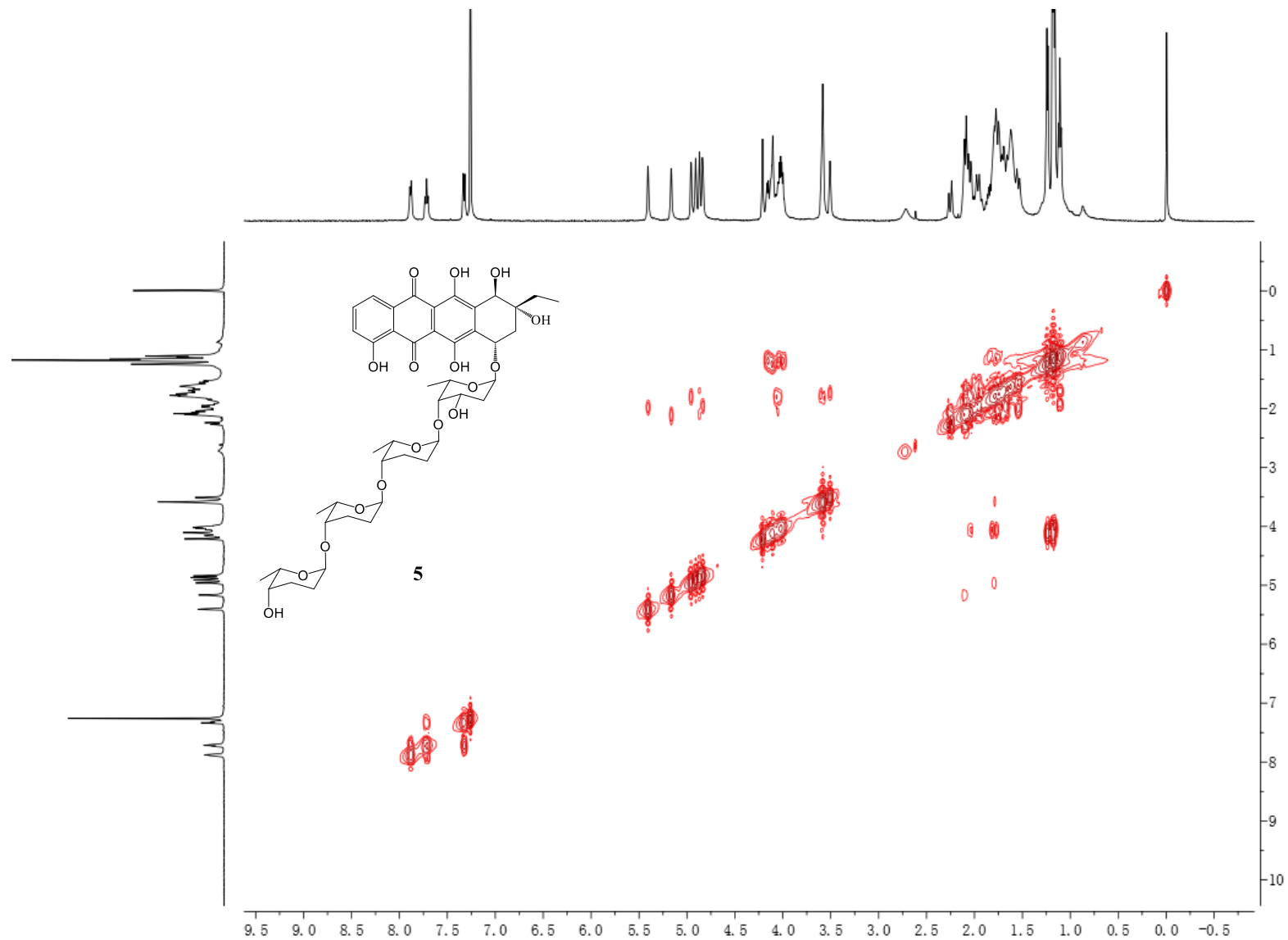


Figure S33. HSQC spectrum of compound **5** in CDCl₃.

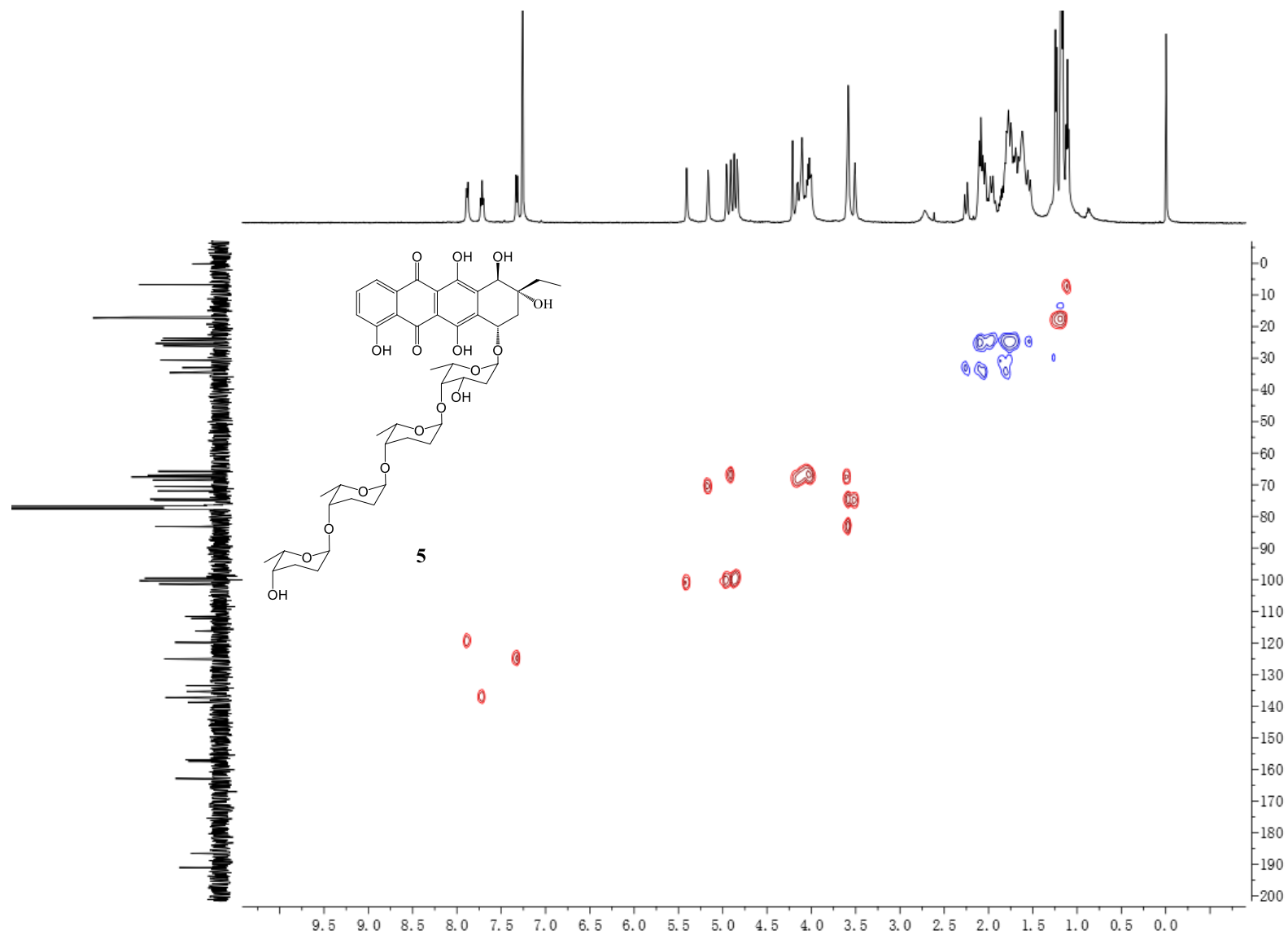


Figure S34. HMBC spectrum of compound **5** in CDCl₃.

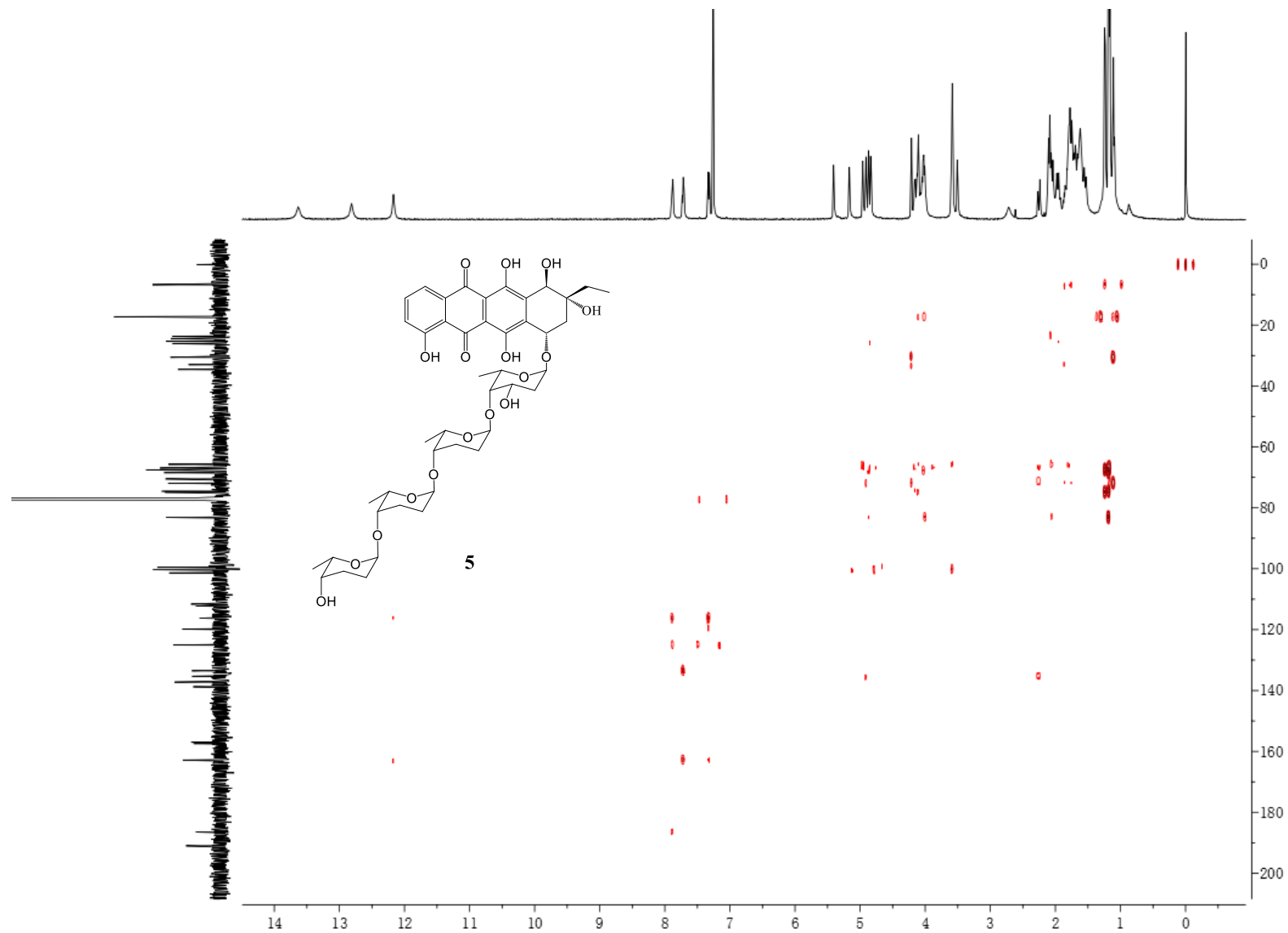
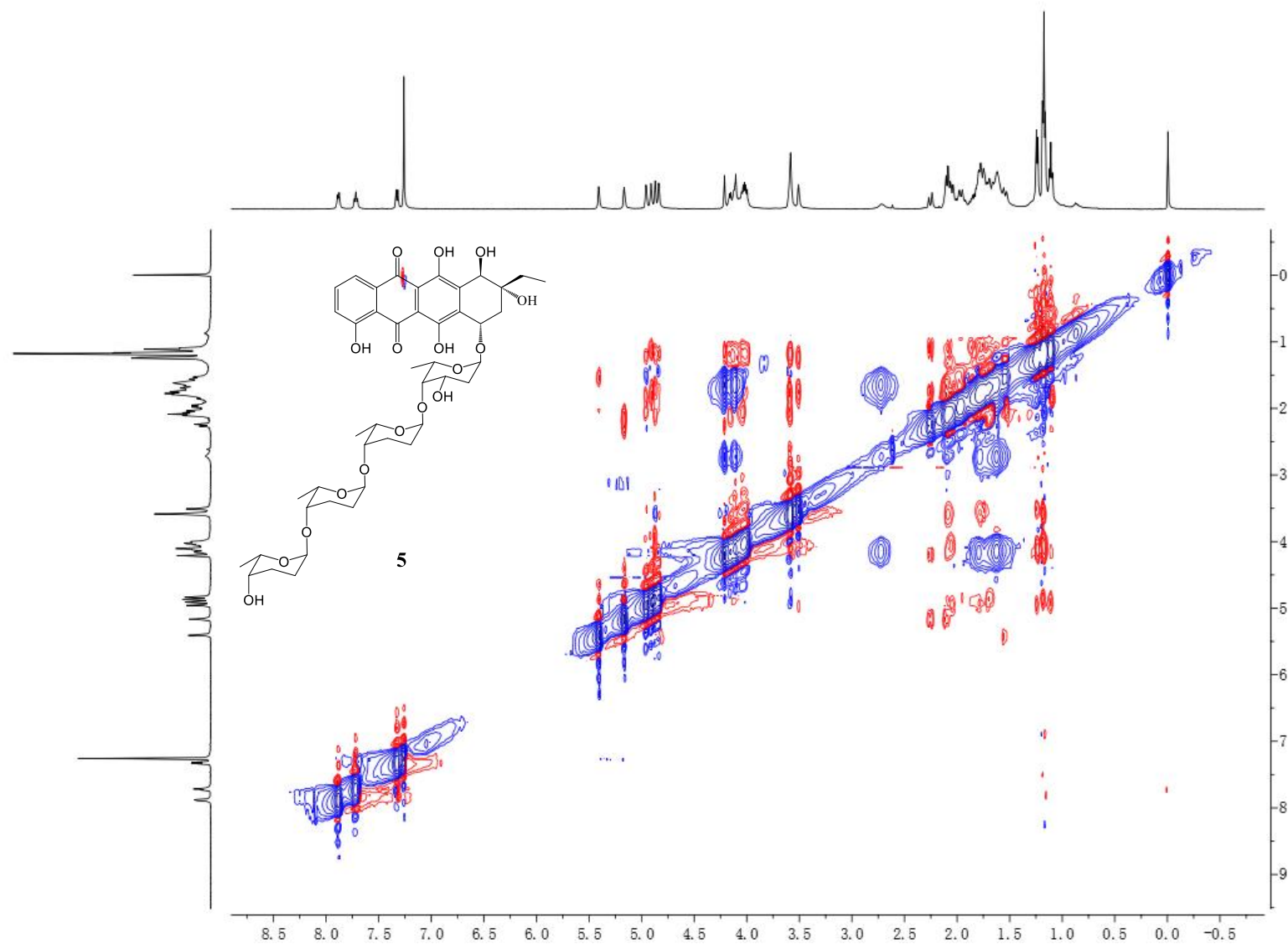


Figure S35. NOESY spectrum of compound **5** in CDCl₃.



[illegible]

Figure S37. ^{13}C NMR (125 MHz) spectrum of compound **6** in CDCl_3 .

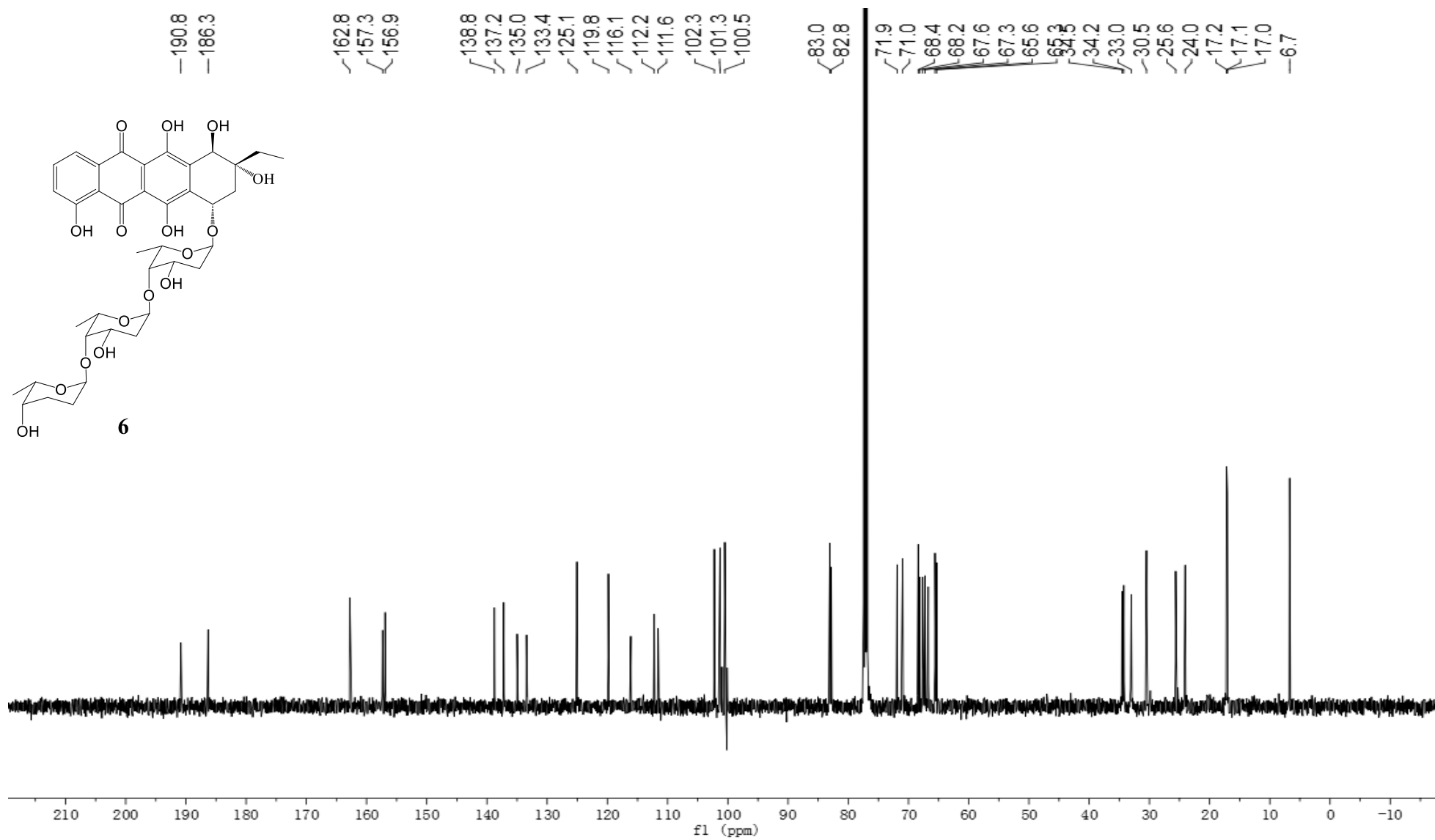


Figure S38. DEPT 135 spectrum of compound **6** in CDCl₃.

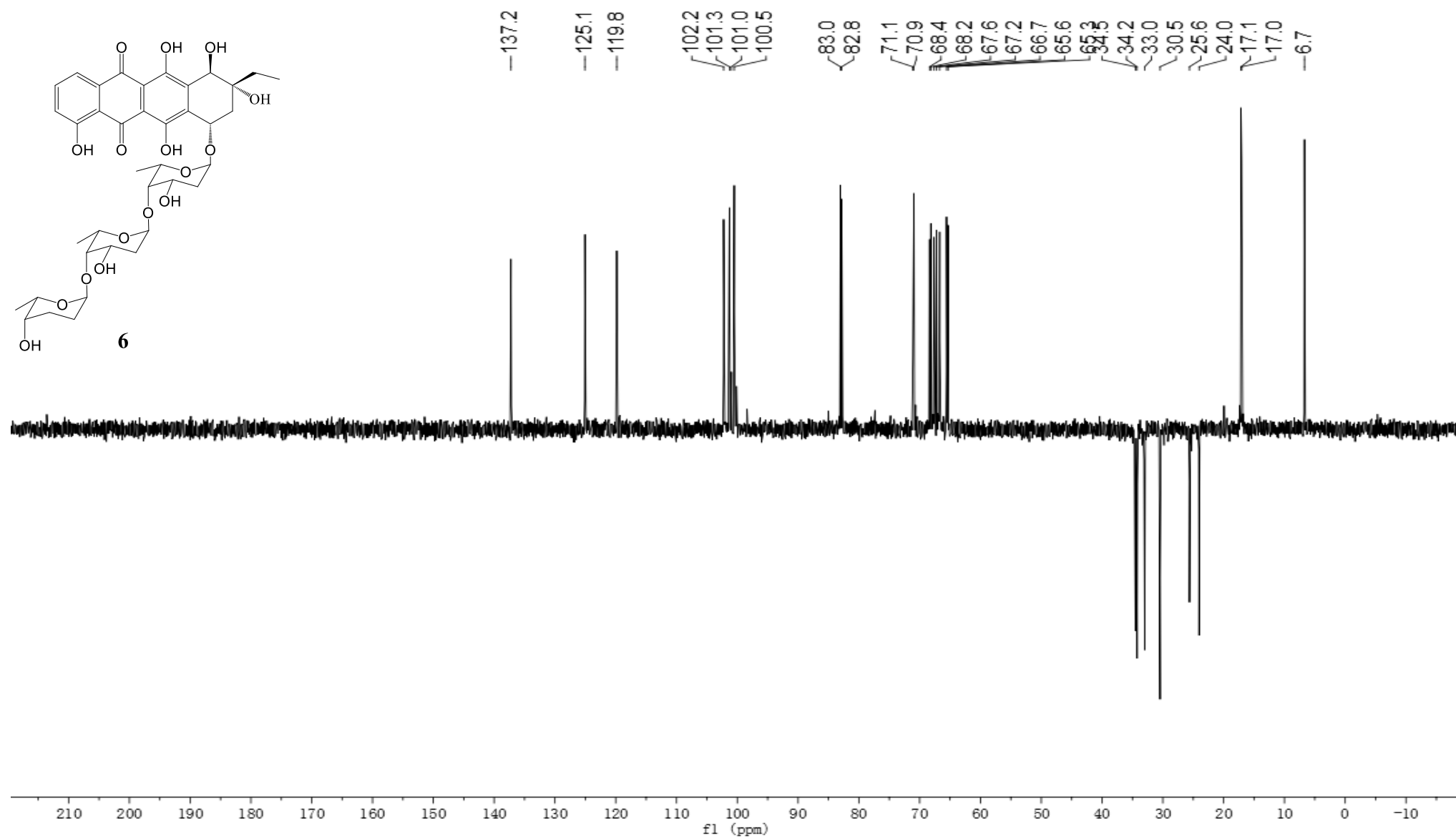


Figure S39. ^1H - ^1H COSY spectrum of compound **6** in CDCl_3 .

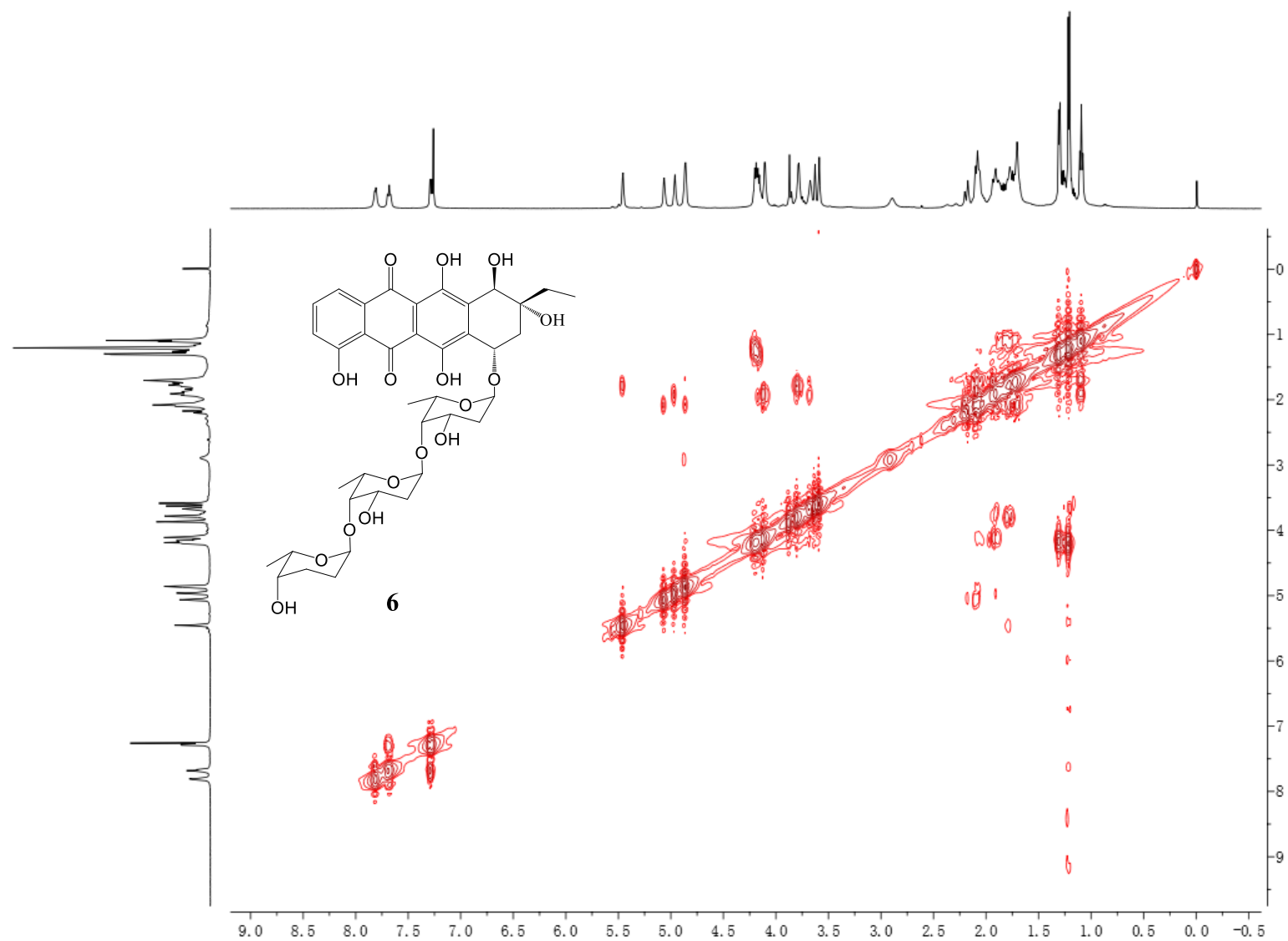


Figure S40. HSQC spectrum of compound **6** in CDCl₃.

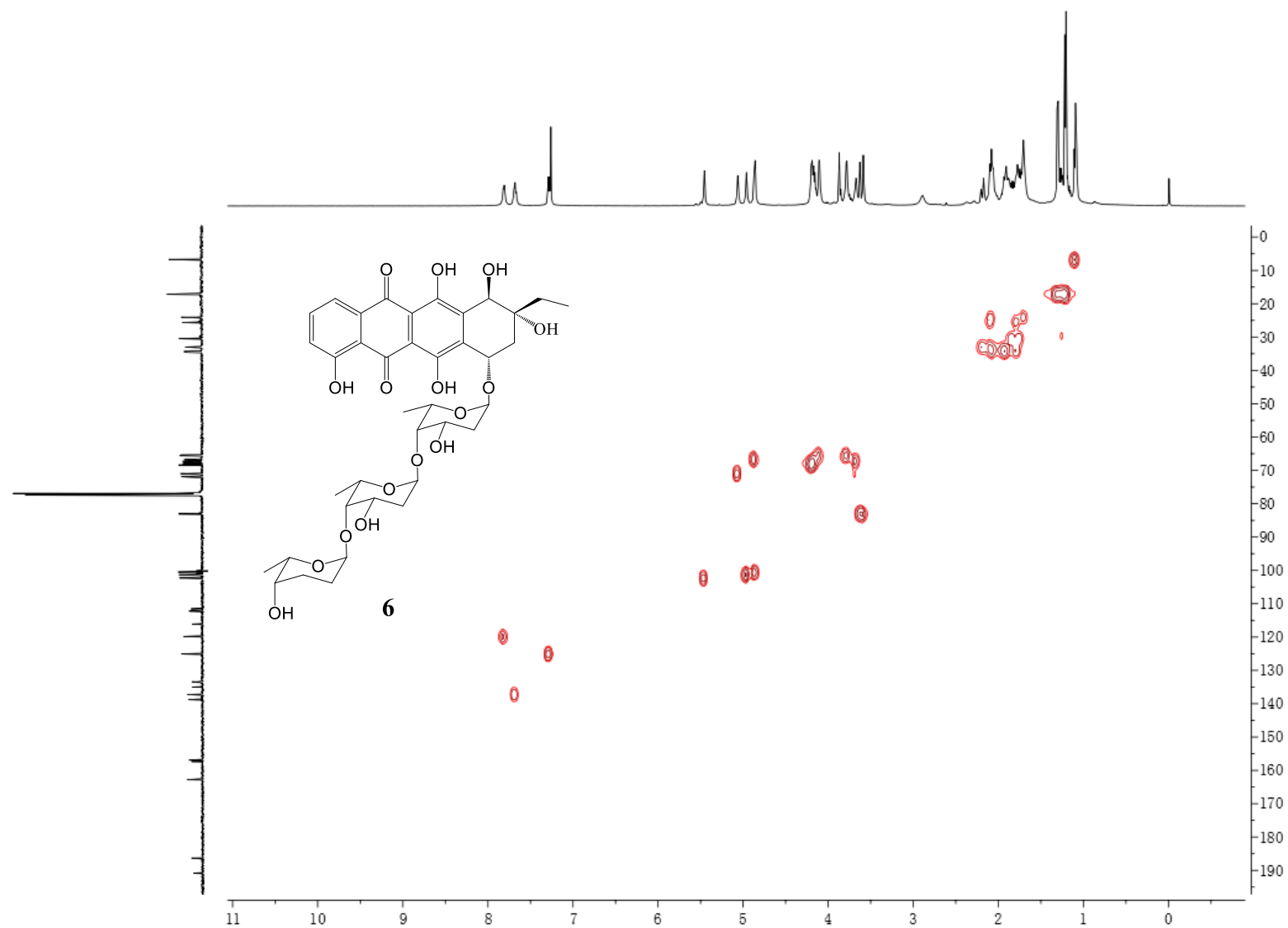


Figure S41. HMBC spectrum of compound **6** in CDCl₃.

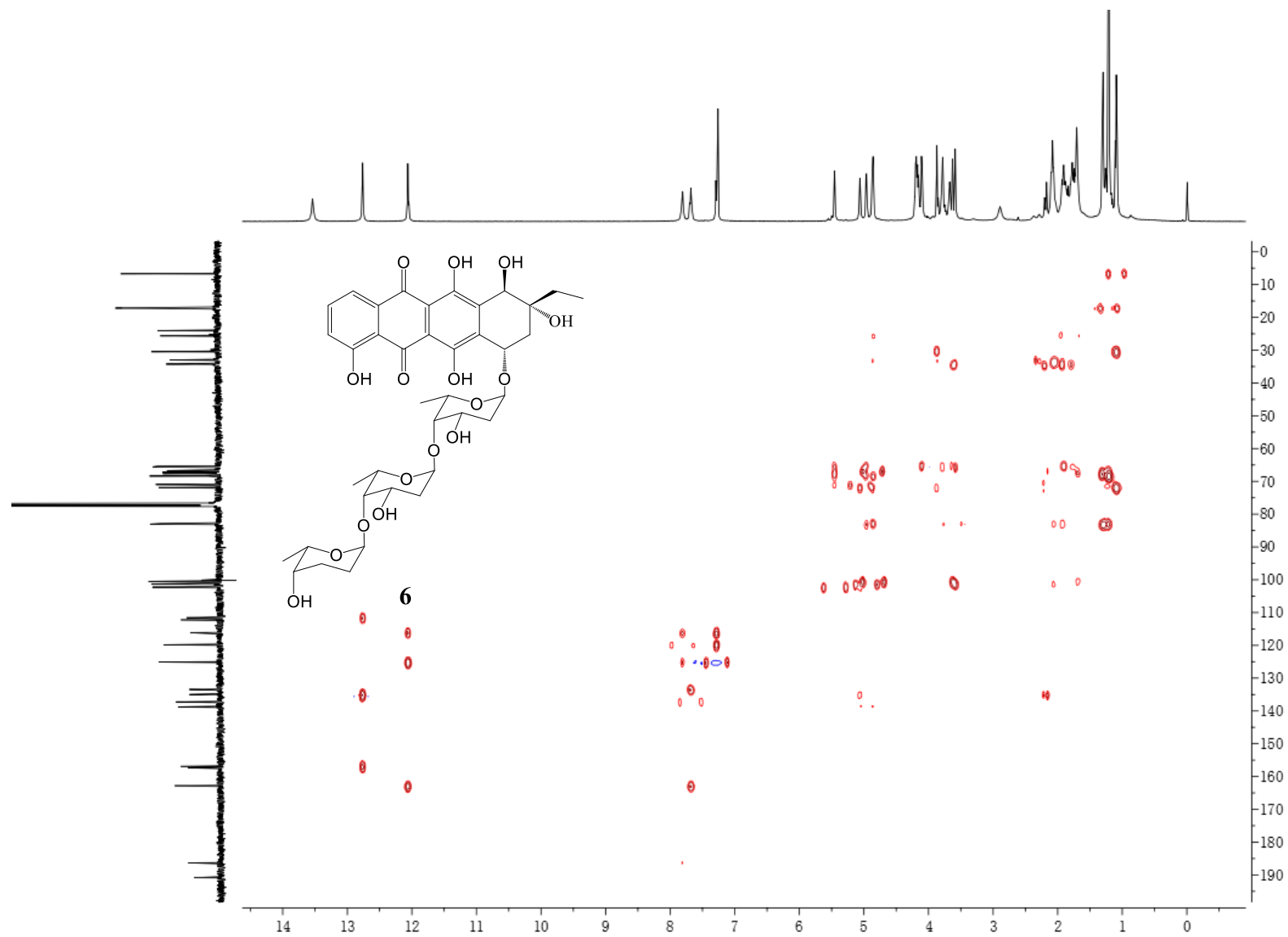


Figure S42. NOESY spectrum of compound **7** in CDCl₃.

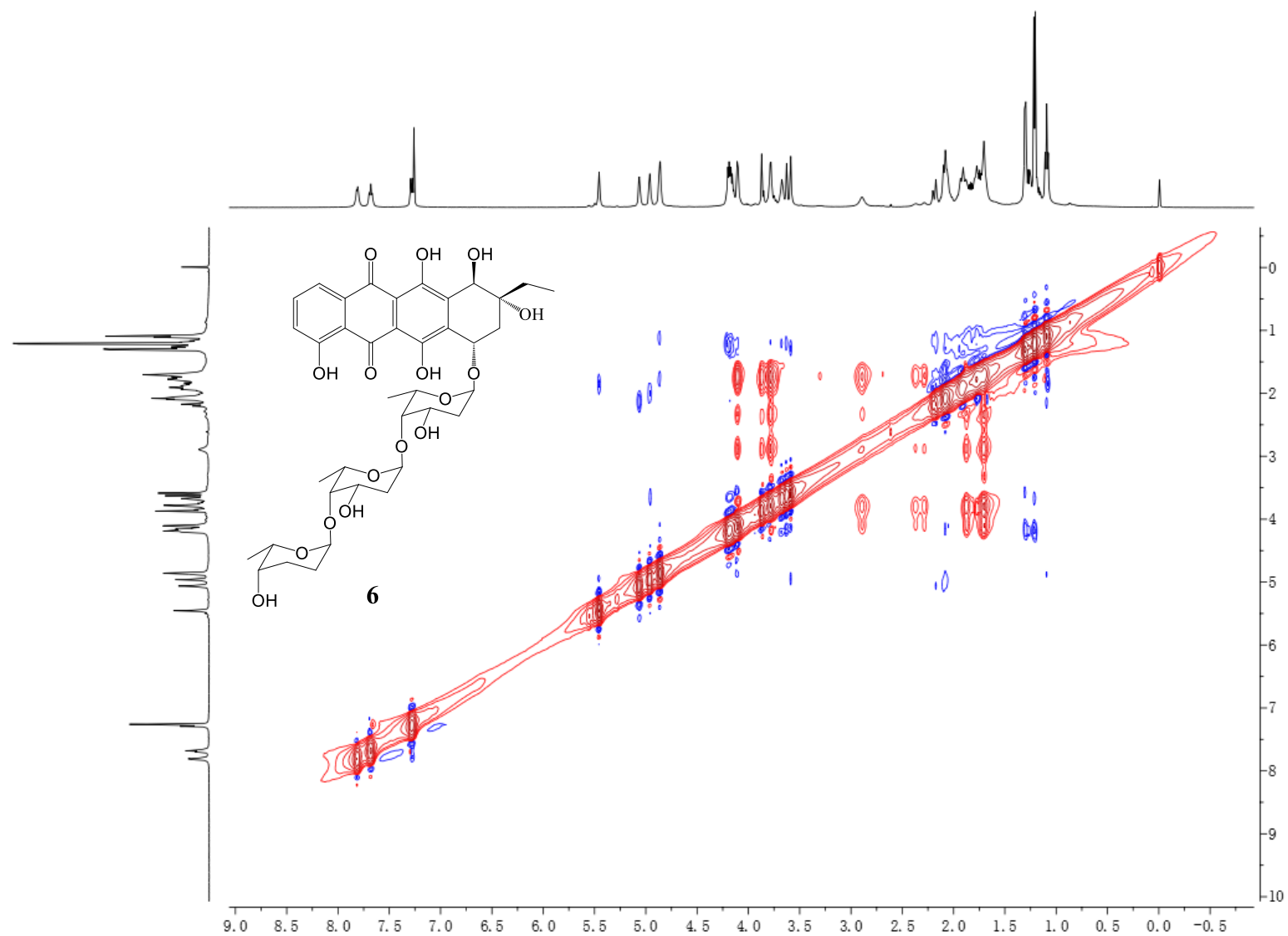


Figure S43. ^1H NMR (500 MHz) spectrum of compound **7** in CDCl_3 .

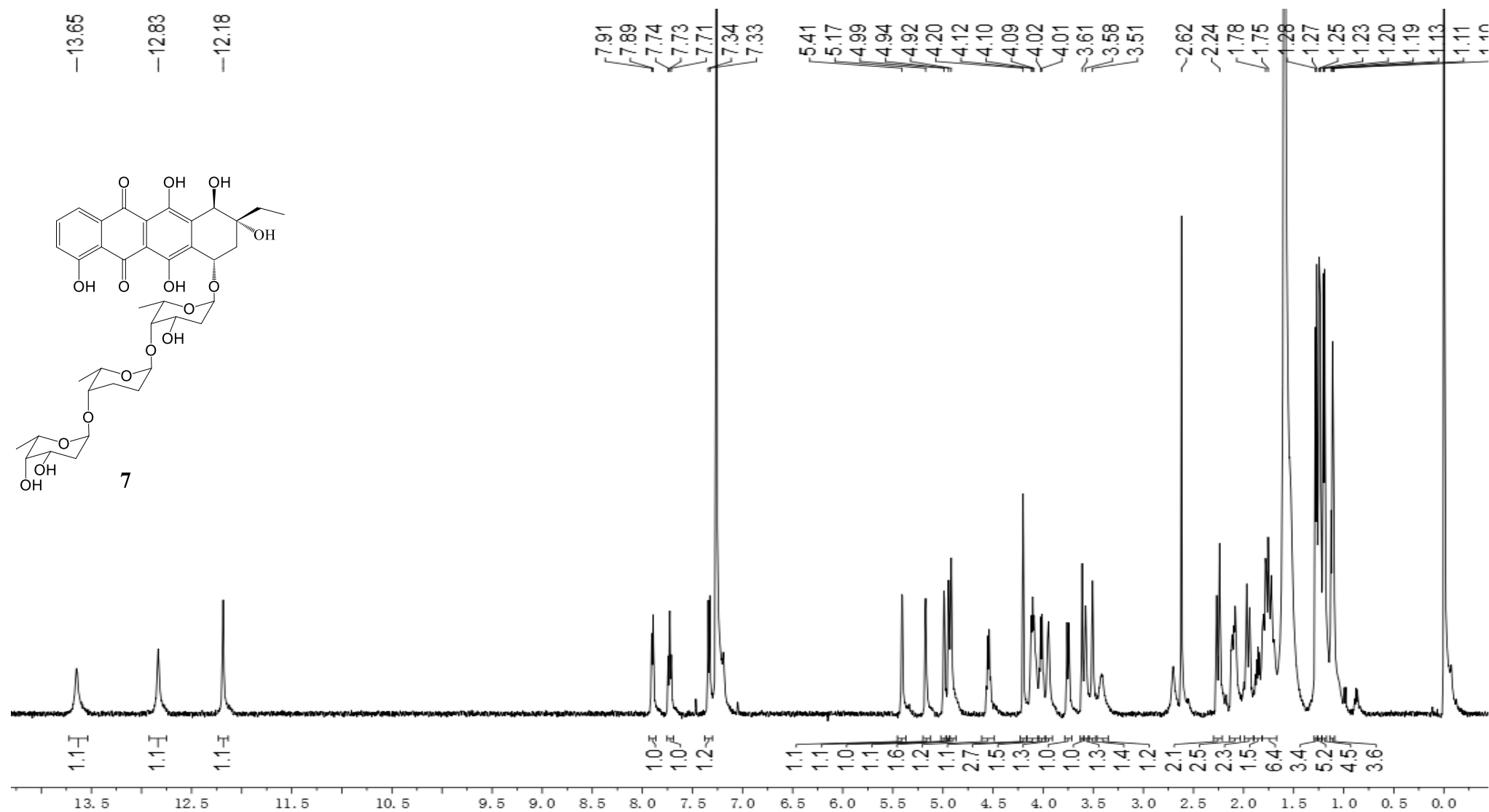


Figure S44. ^{13}C NMR (125 MHz) spectrum of compound **7** in CDCl_3 .

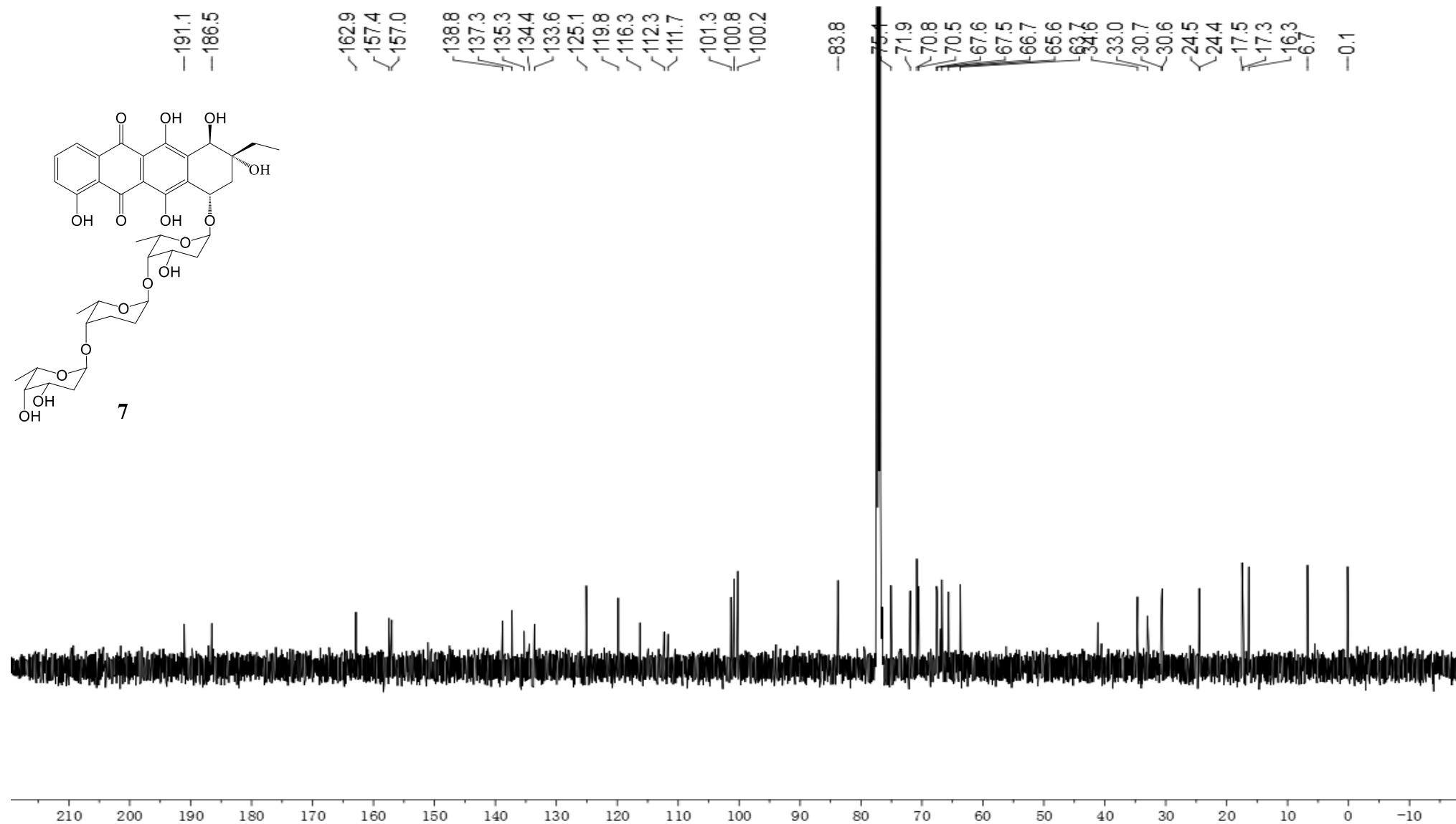


Figure S45. DEPT 135 spectrum of compound **7** in CDCl₃.

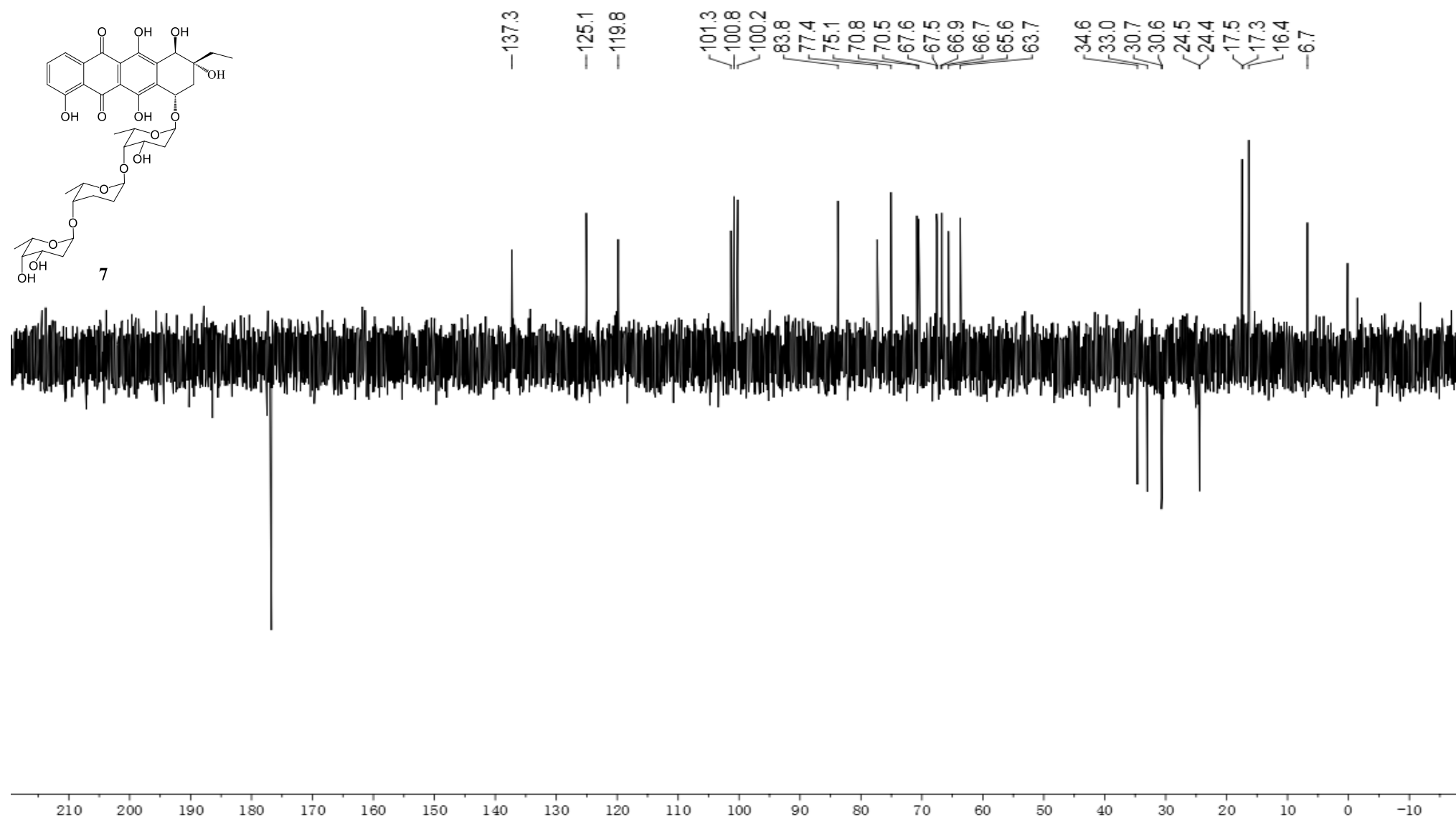


Figure S46. ^1H - ^1H COSY spectrum of compound **7** in CDCl_3 .

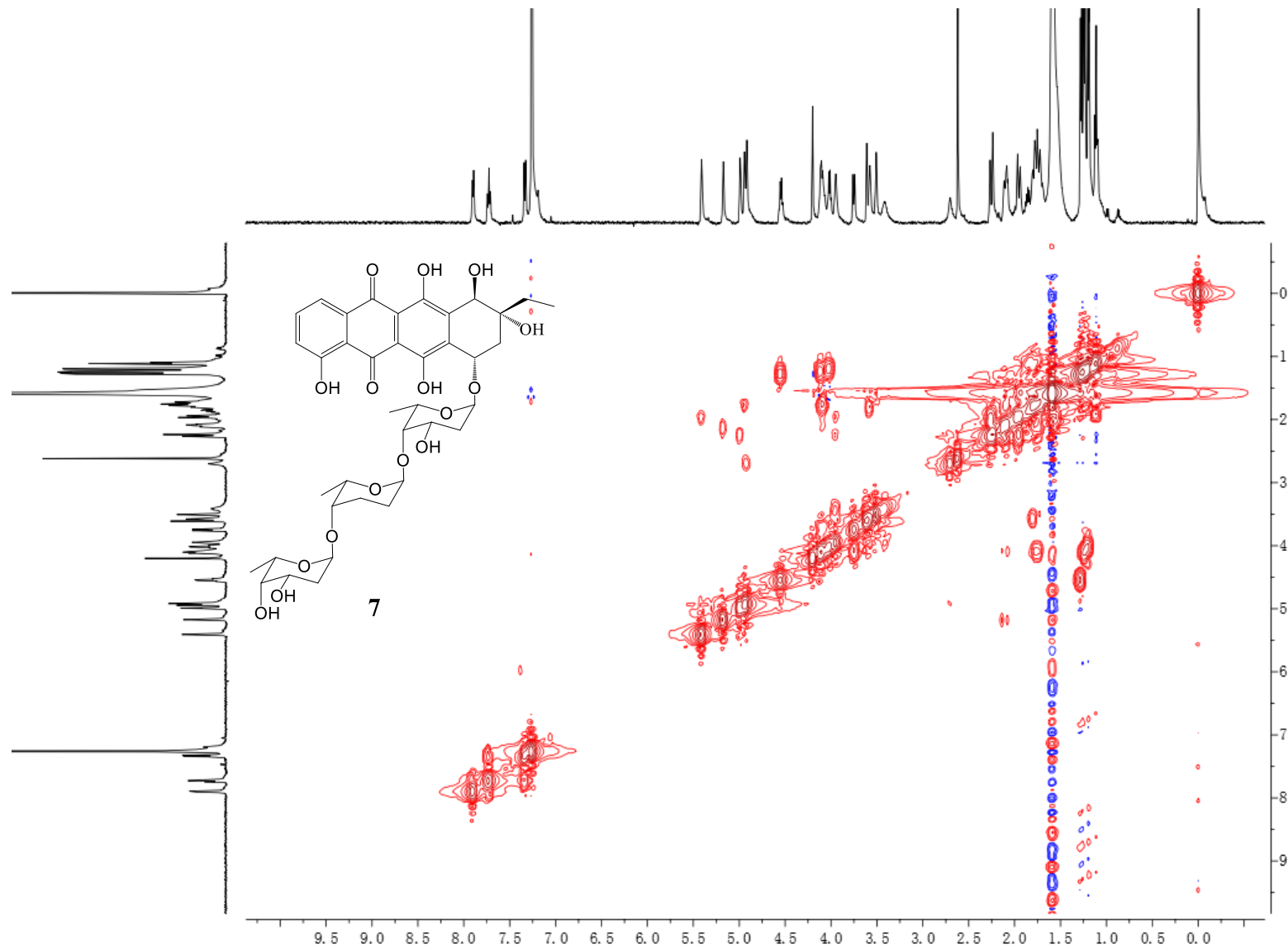


Figure S47. HSQC spectrum of compound **7** in CDCl₃.

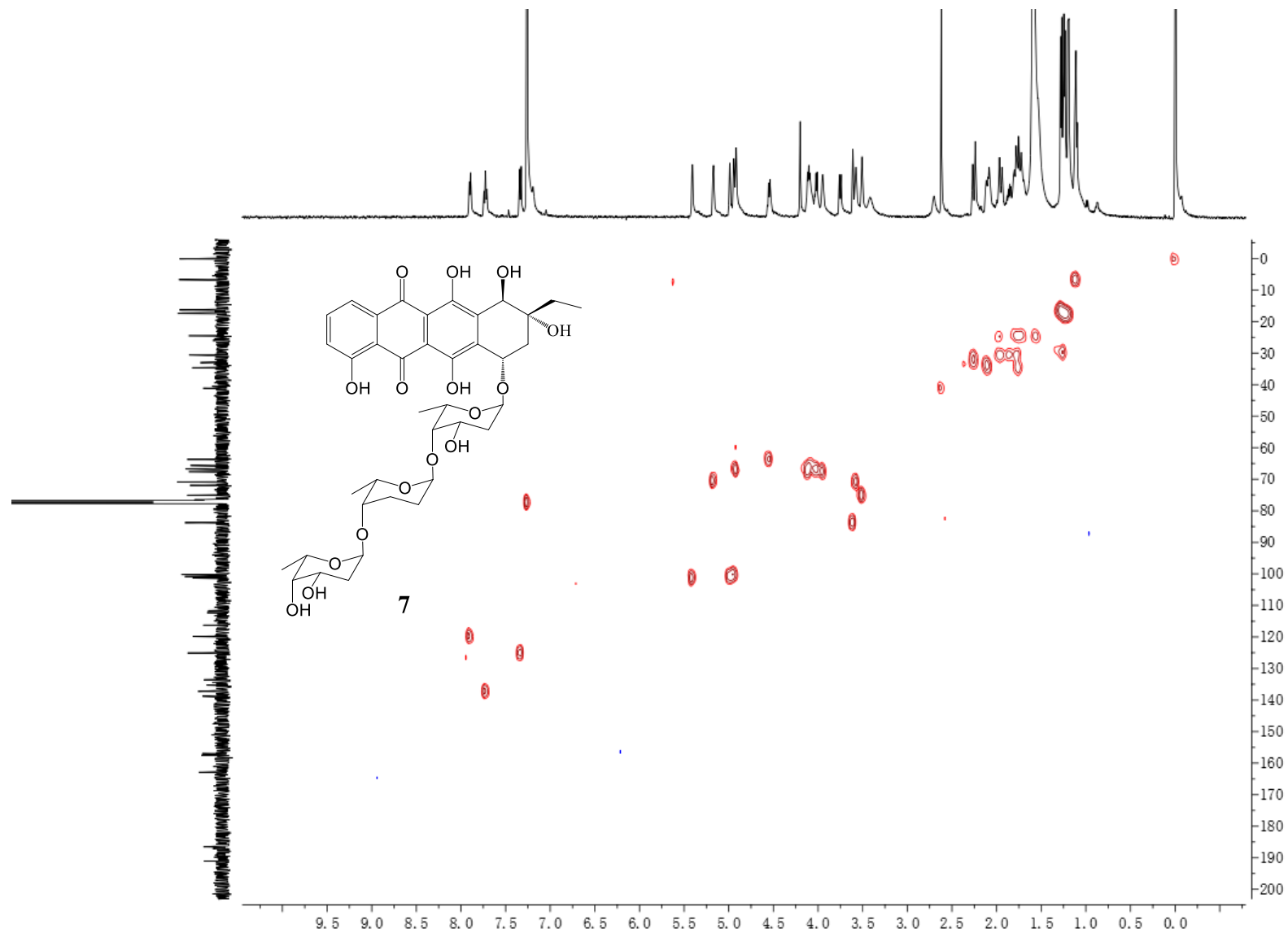


Figure S48. HMBC spectrum of compound **7** in CDCl₃.

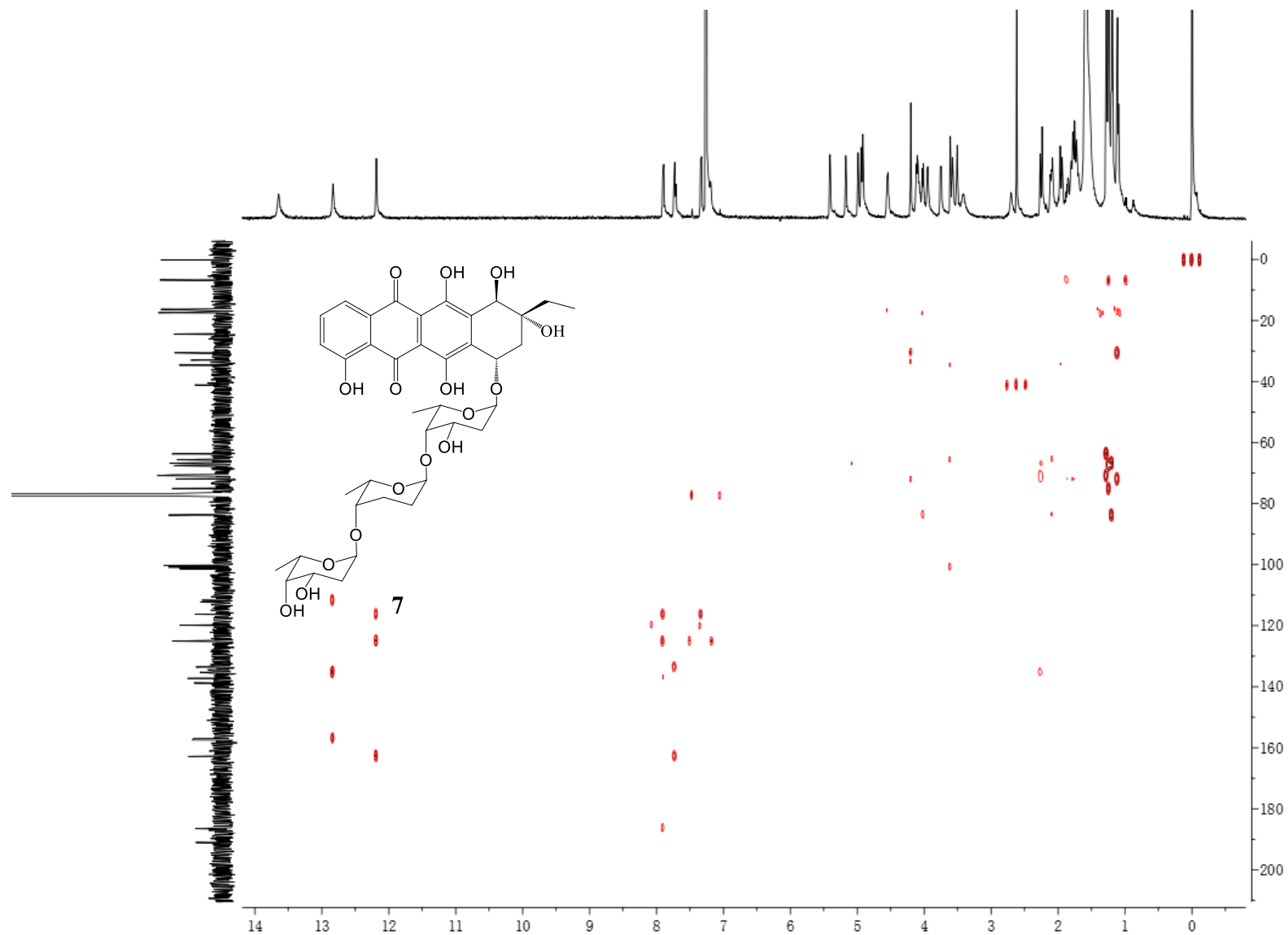


Figure S49. NOESY spectrum of compound **7** in CDCl₃.

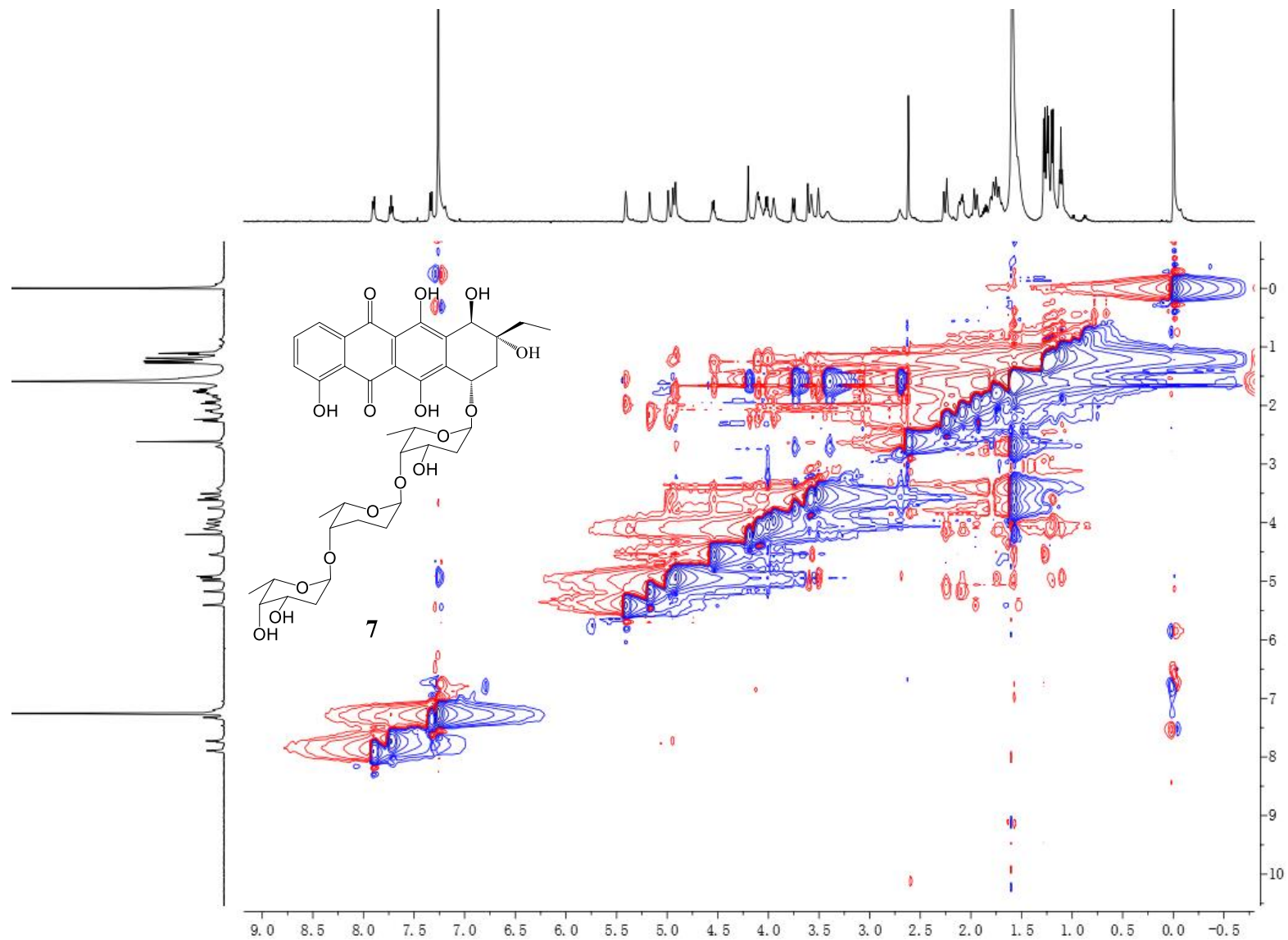


Figure S50. ^1H NMR (700 MHz) spectrum of compound **8** in CDCl_3 .

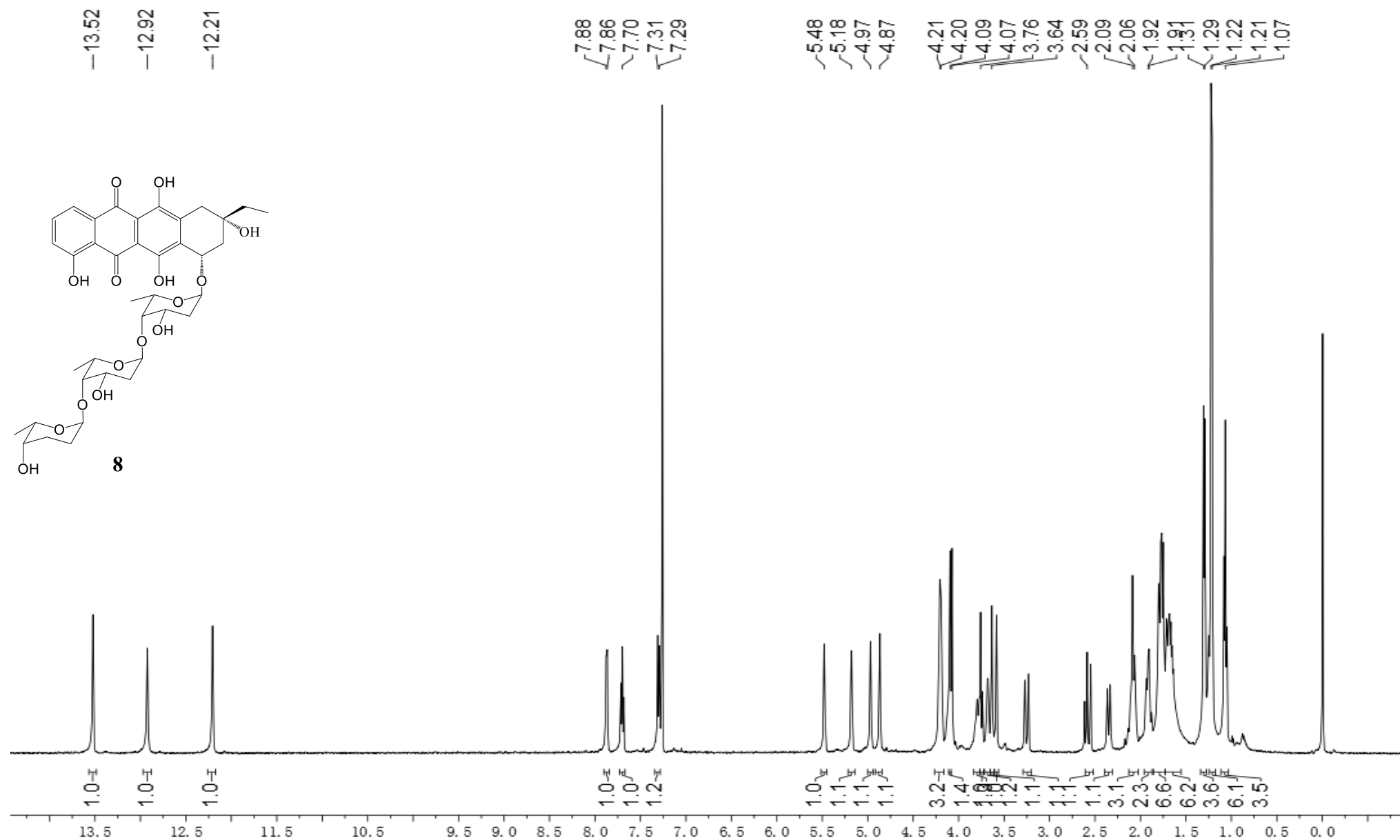


Figure S51. ^{13}C NMR (176 MHz) spectrum of compound **8** in CDCl_3 .

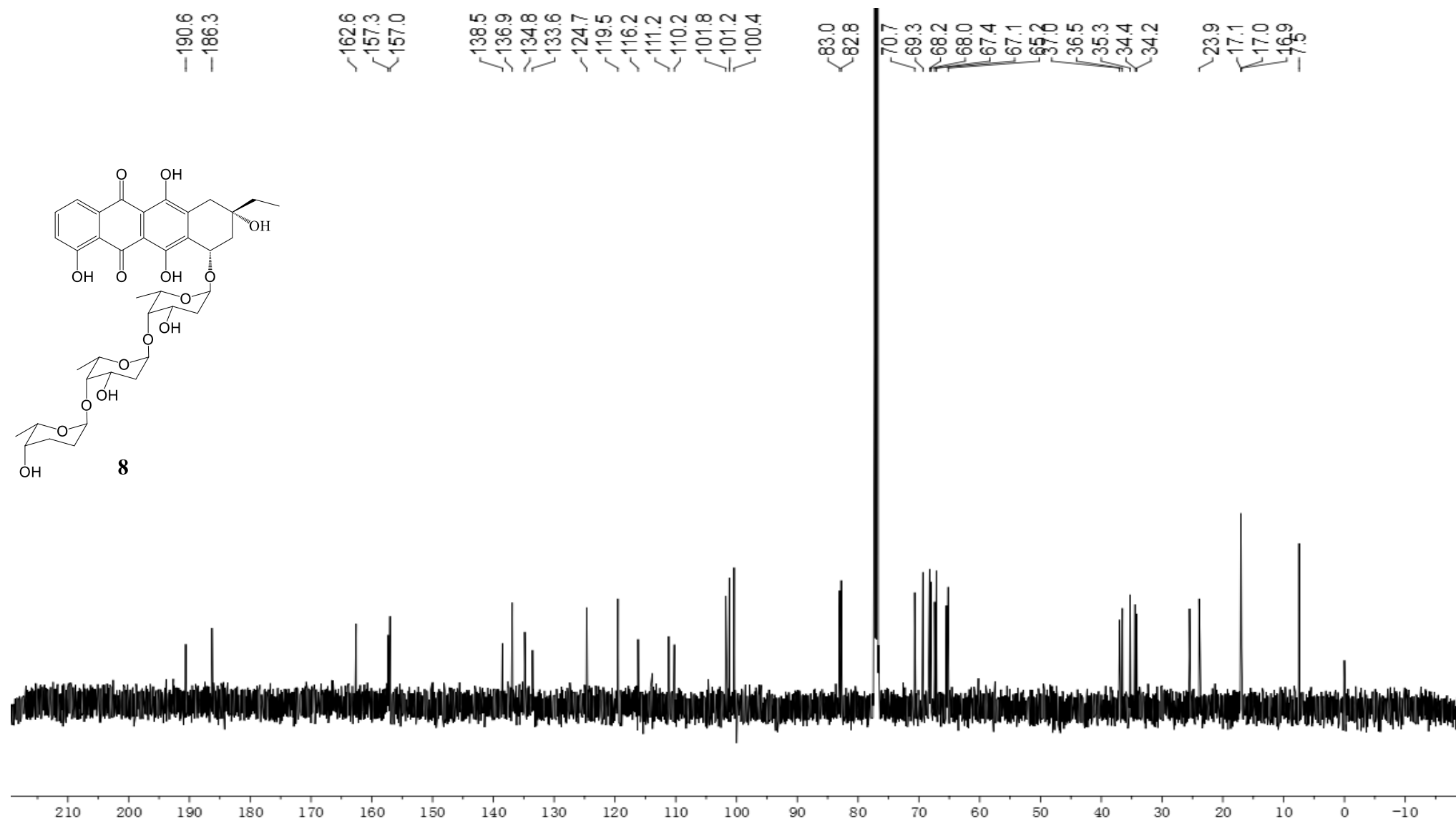


Figure S52. DEPT 135 spectrum of compound **8** in CDCl₃.

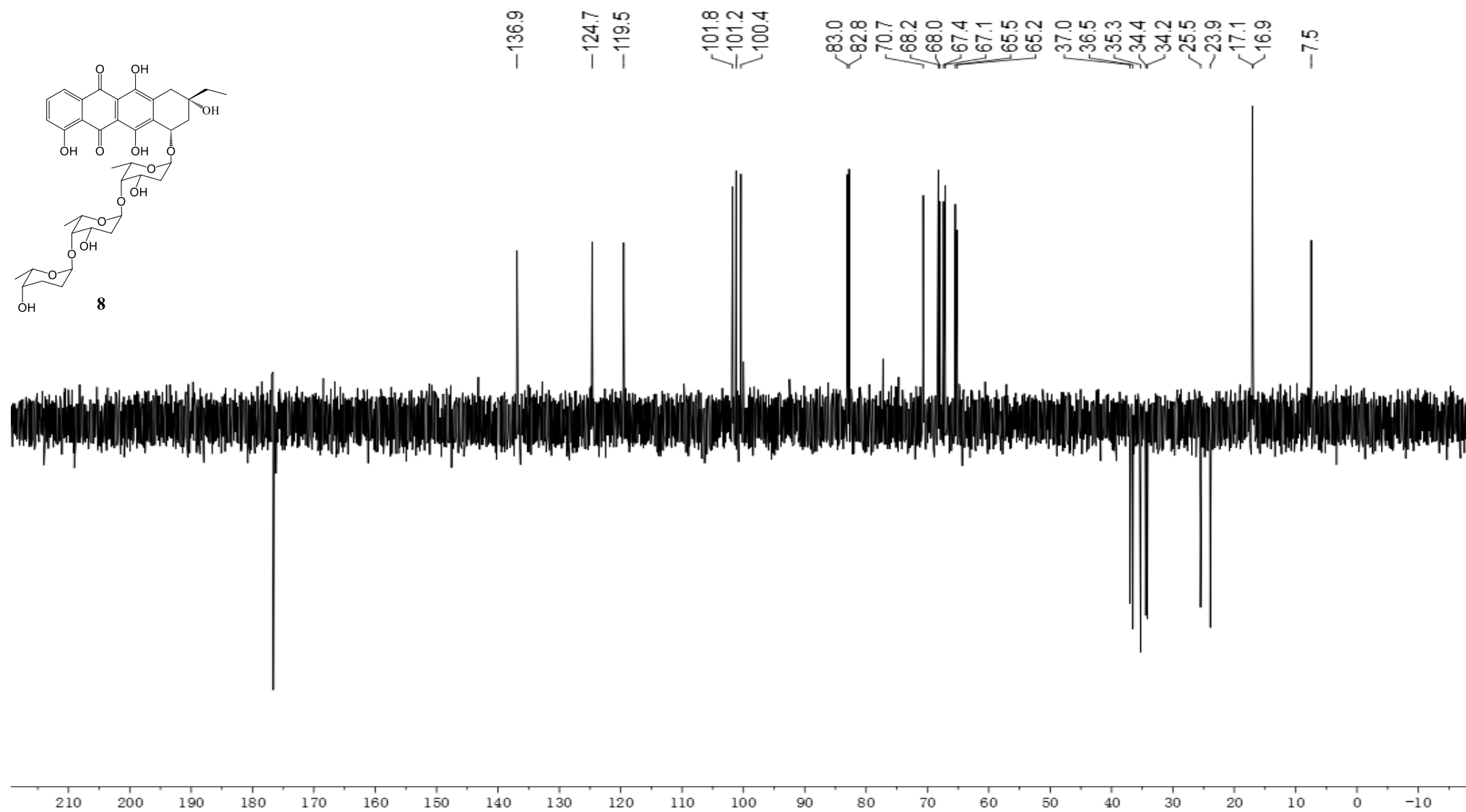


Figure S53. ^1H - ^1H COSY spectrum of compound **8** in CDCl_3 .

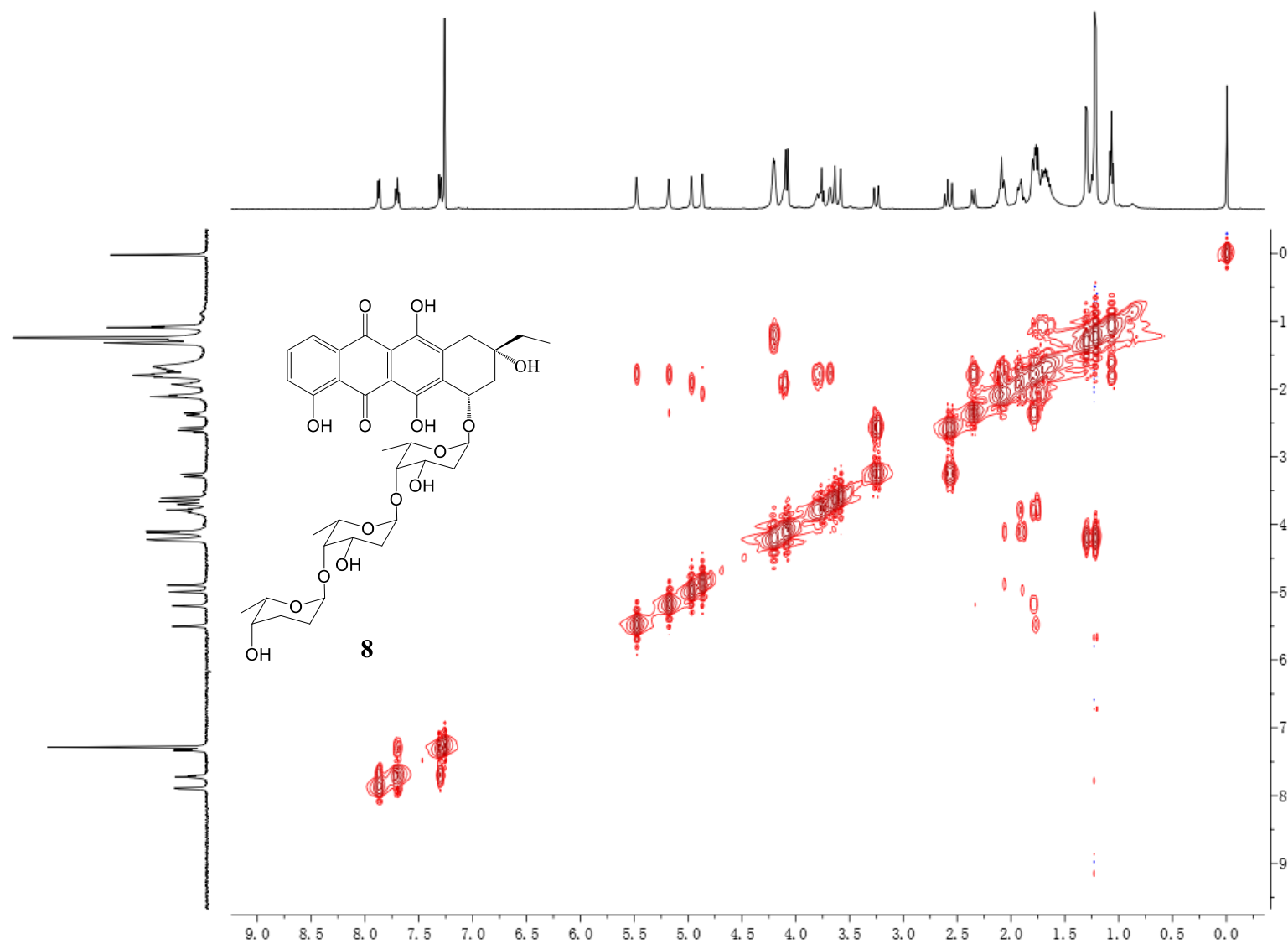


Figure S54. HSQC spectrum of compound **8** in CDCl₃.

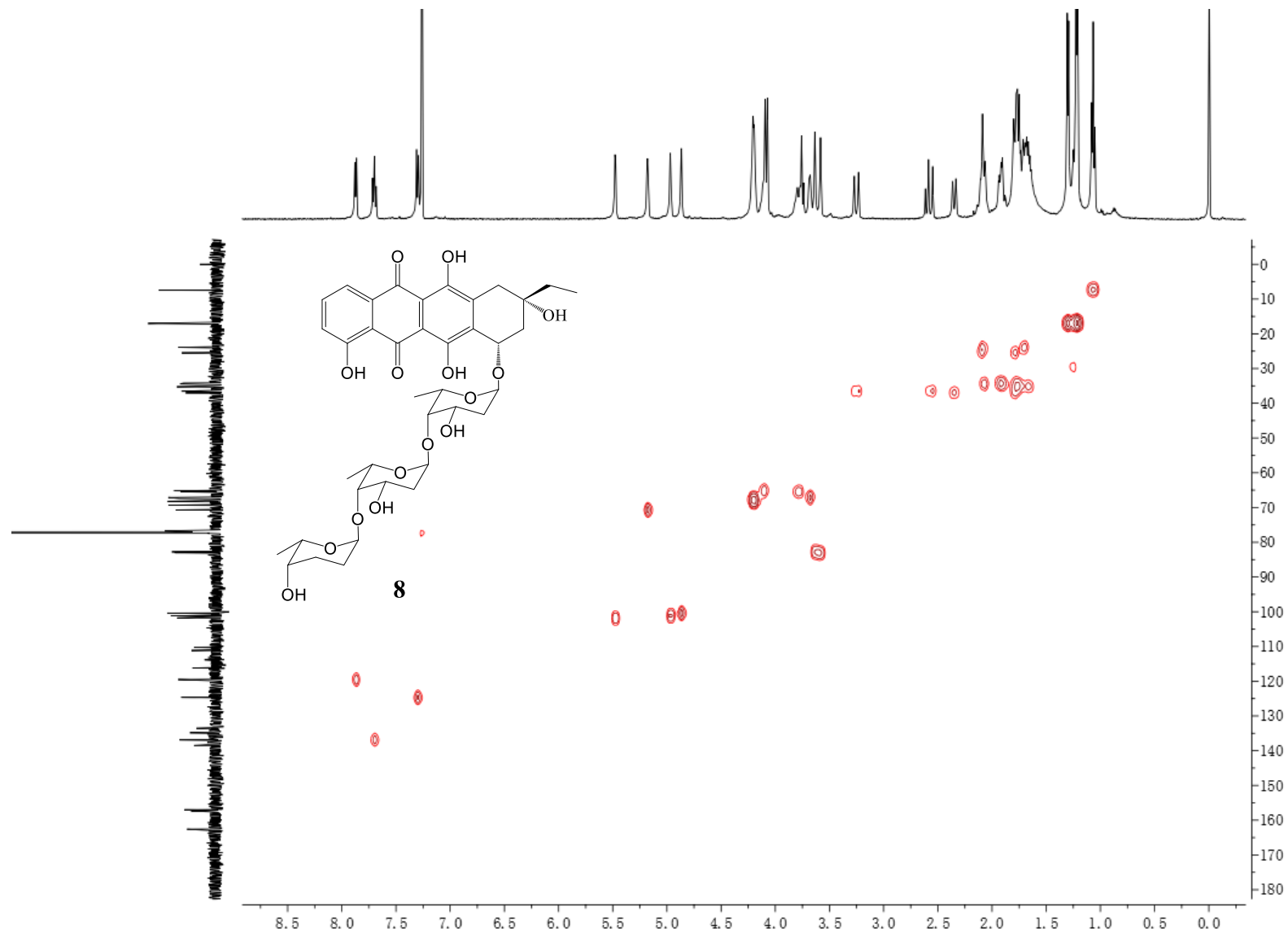


Figure S55. HMBC spectrum of compound **8** in CDCl₃.

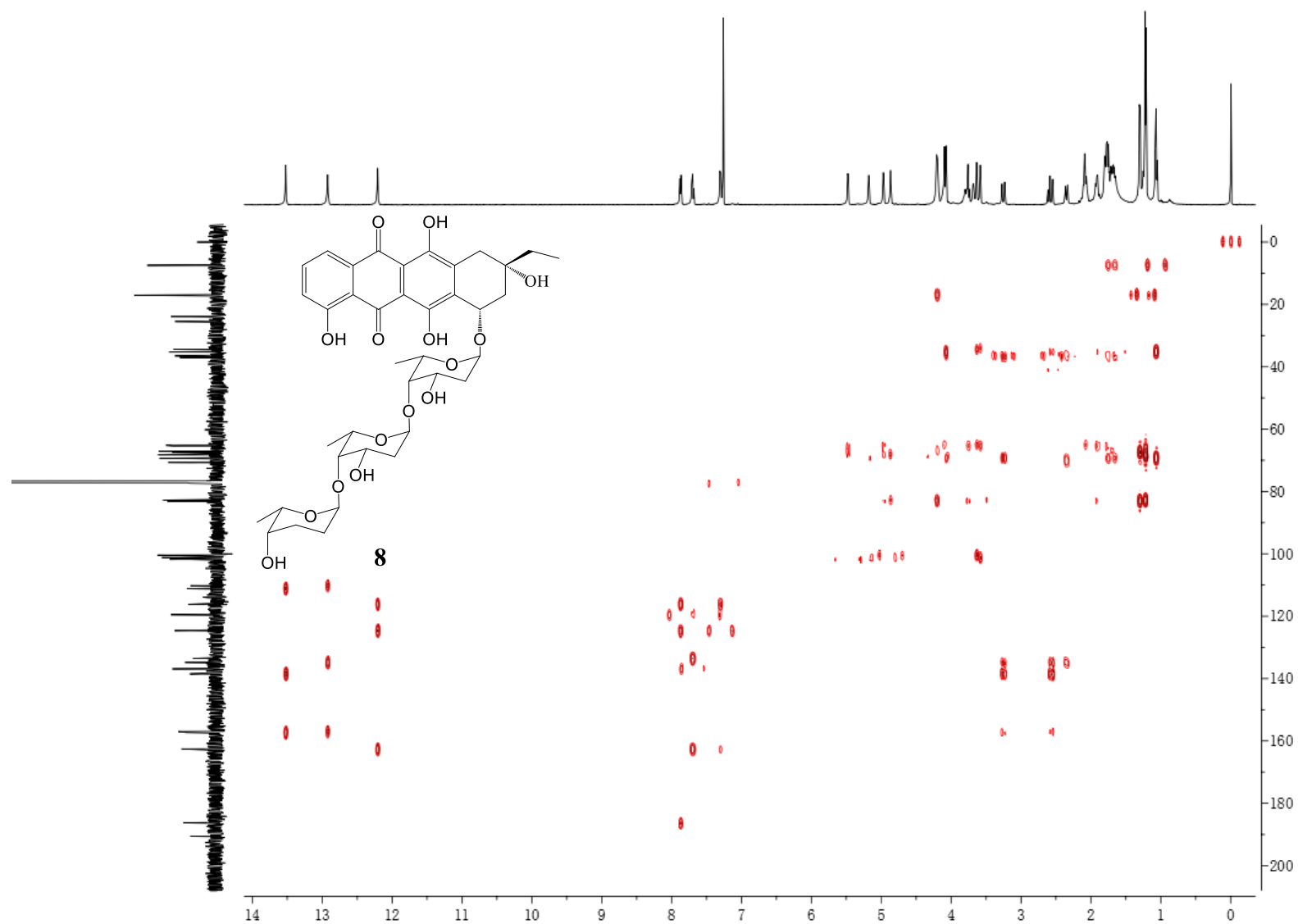


Figure S56. NOESY spectrum of compound **8** in CDCl₃.

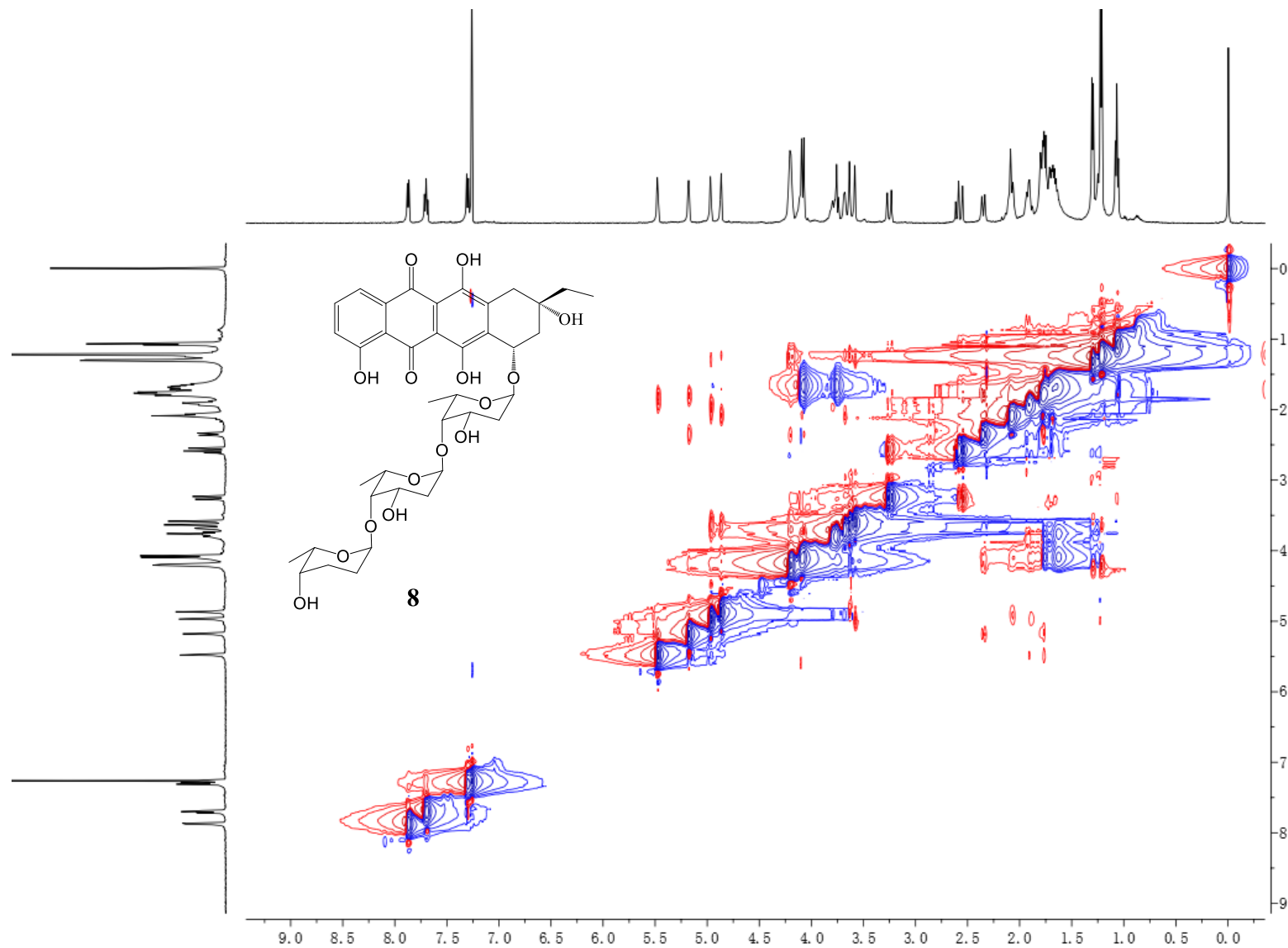


Figure S57. ^1H NMR (500 MHz) spectrum of compound **9** in CDCl_3 .

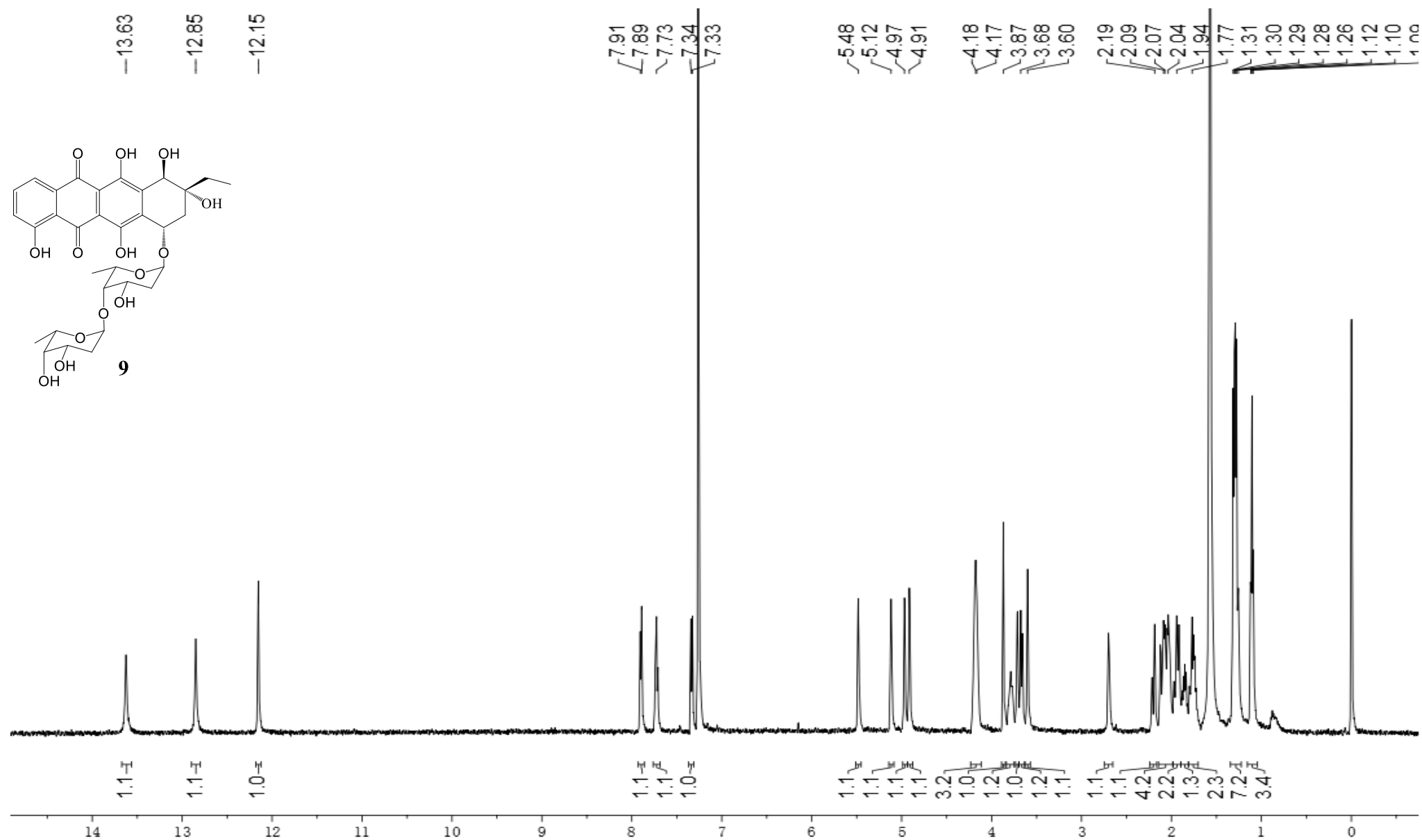


Figure S58. ^{13}C NMR (125 MHz) spectrum of compound **9** in CDCl_3 .

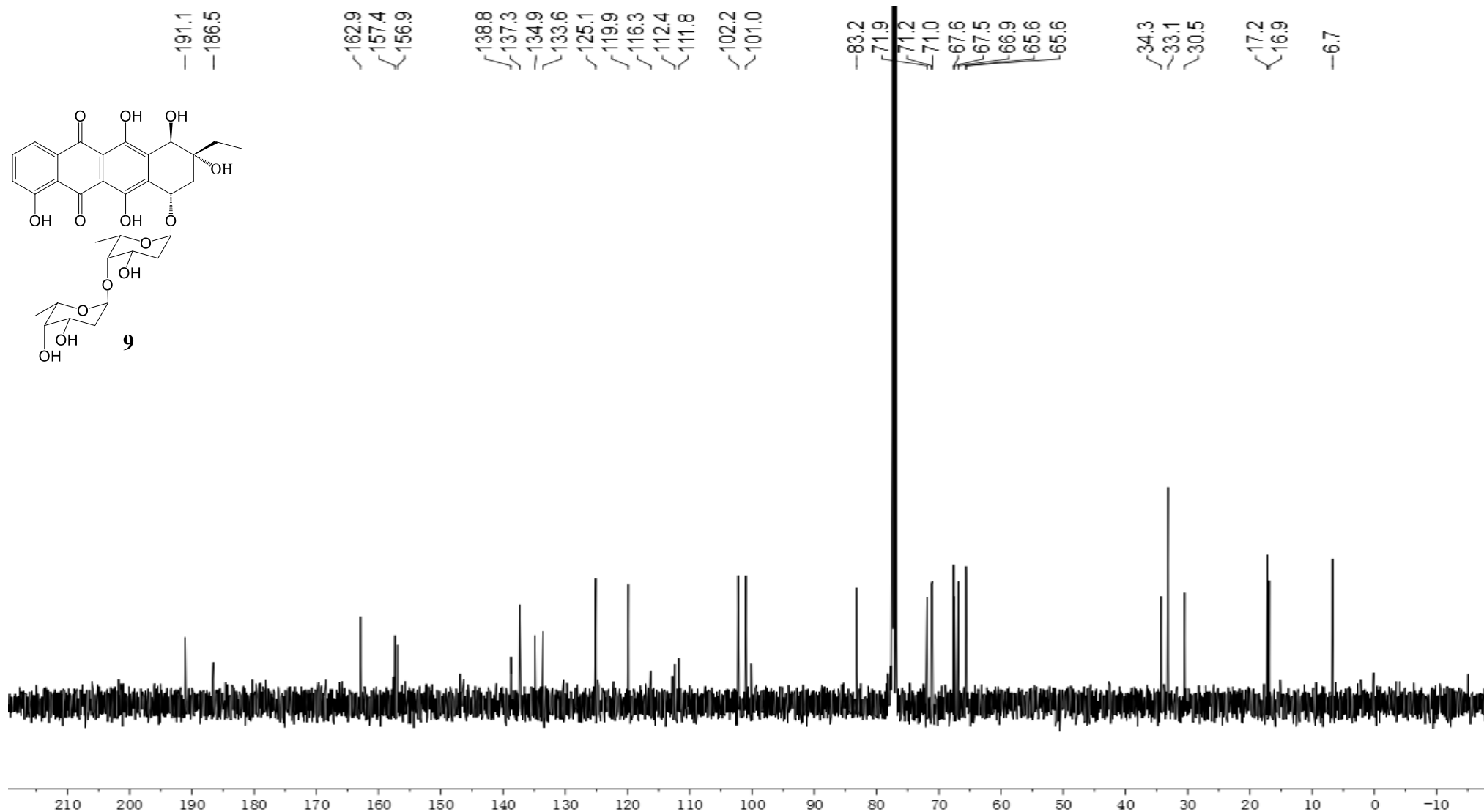


Figure S59. DEPT 135 spectrum of compound **9** in CDCl₃.

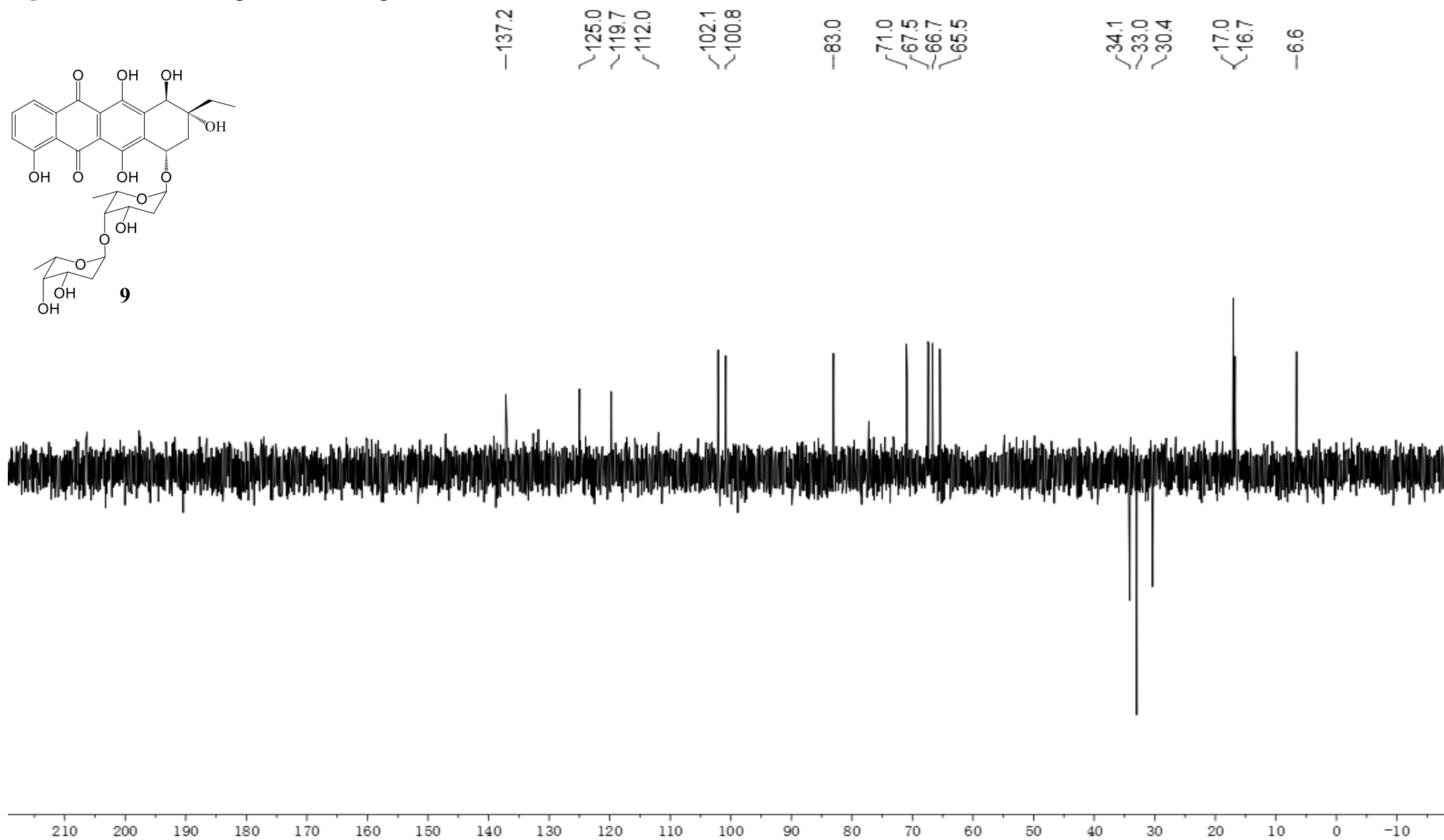


Figure S60. ^1H - ^1H COSY spectrum of compound **9** in CDCl_3 .

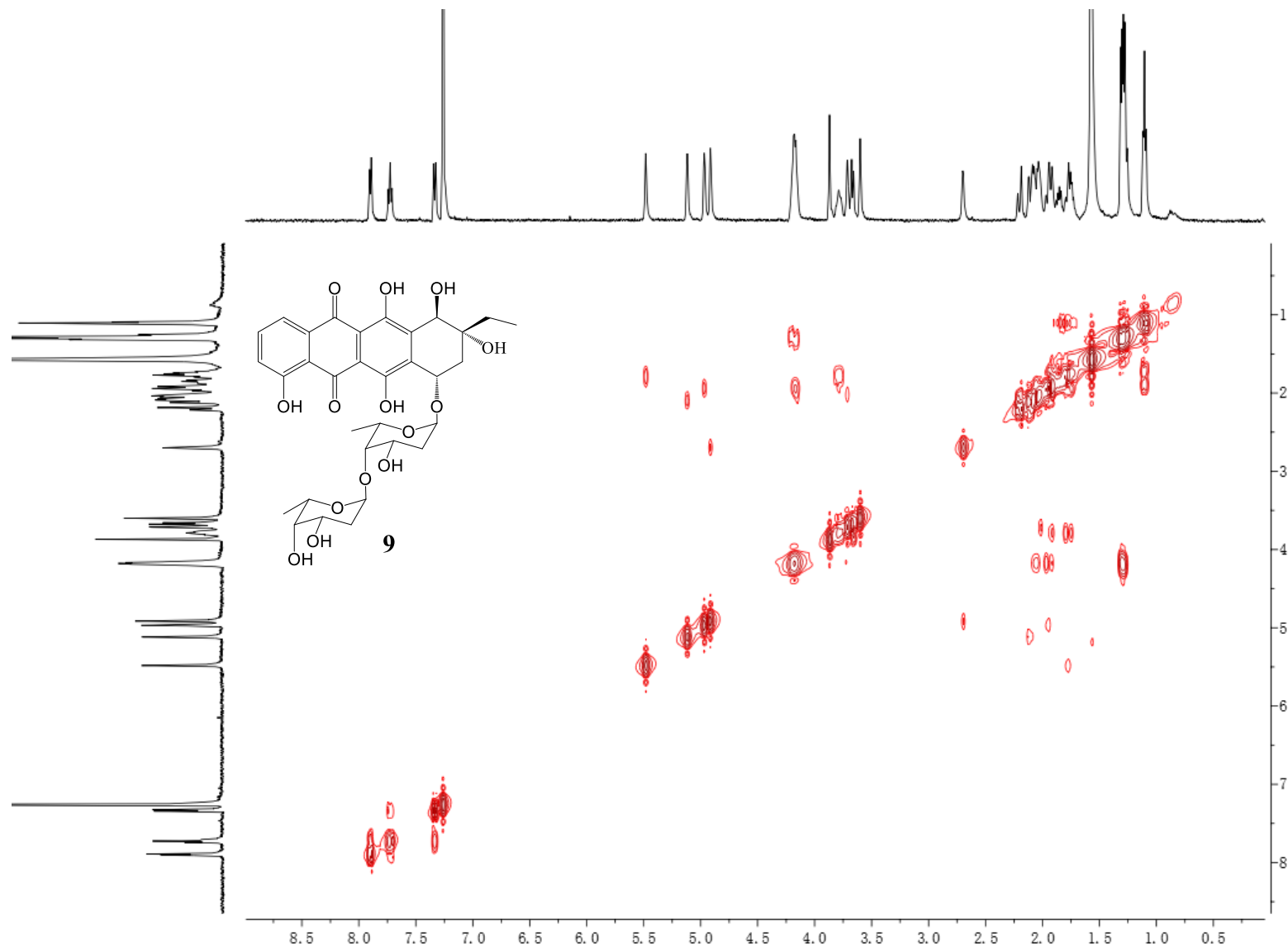


Figure S61. HSQC spectrum of compound **9** in CDCl₃.

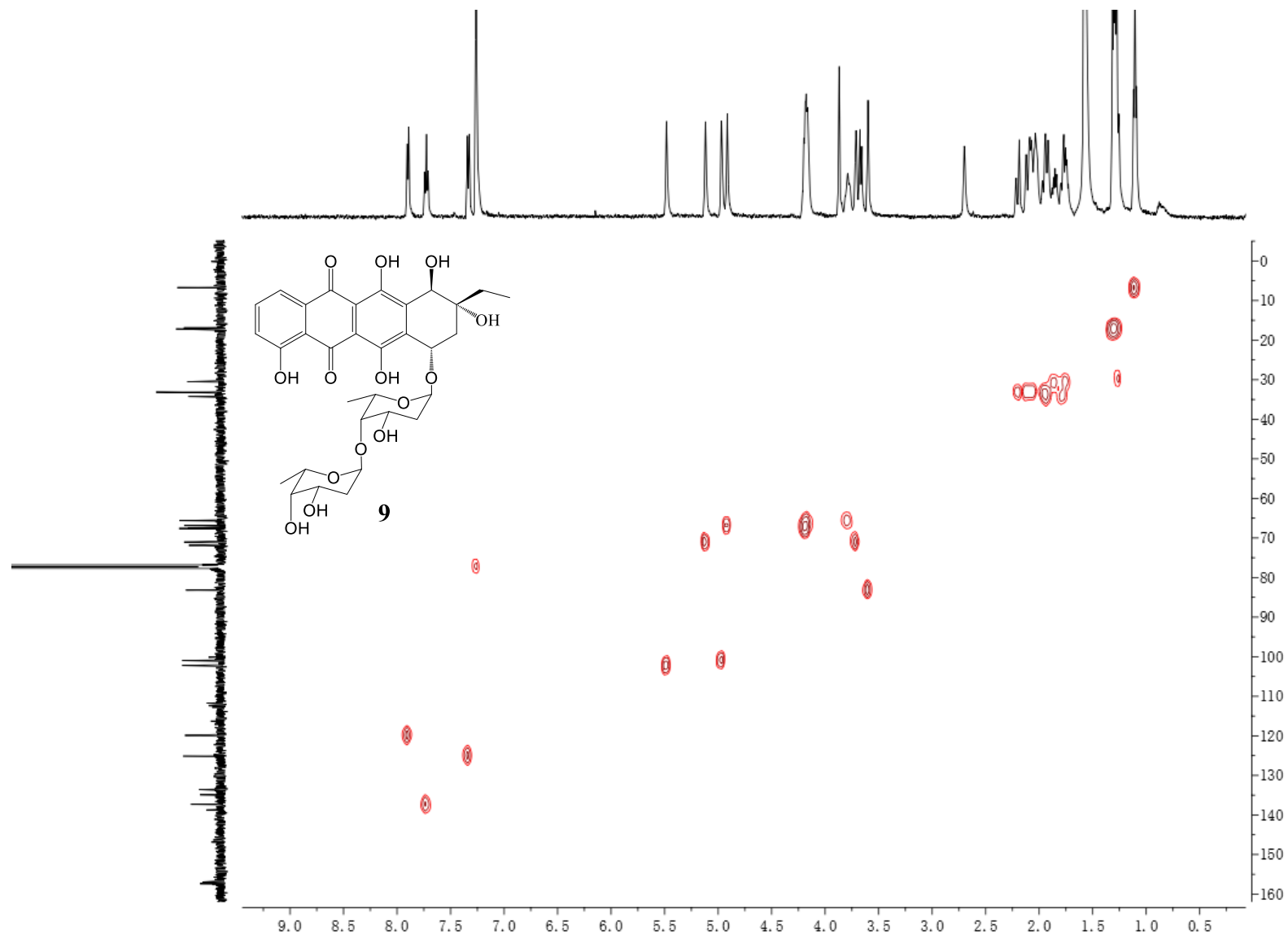


Figure S62. HMBC spectrum of compound **9** in CDCl₃.

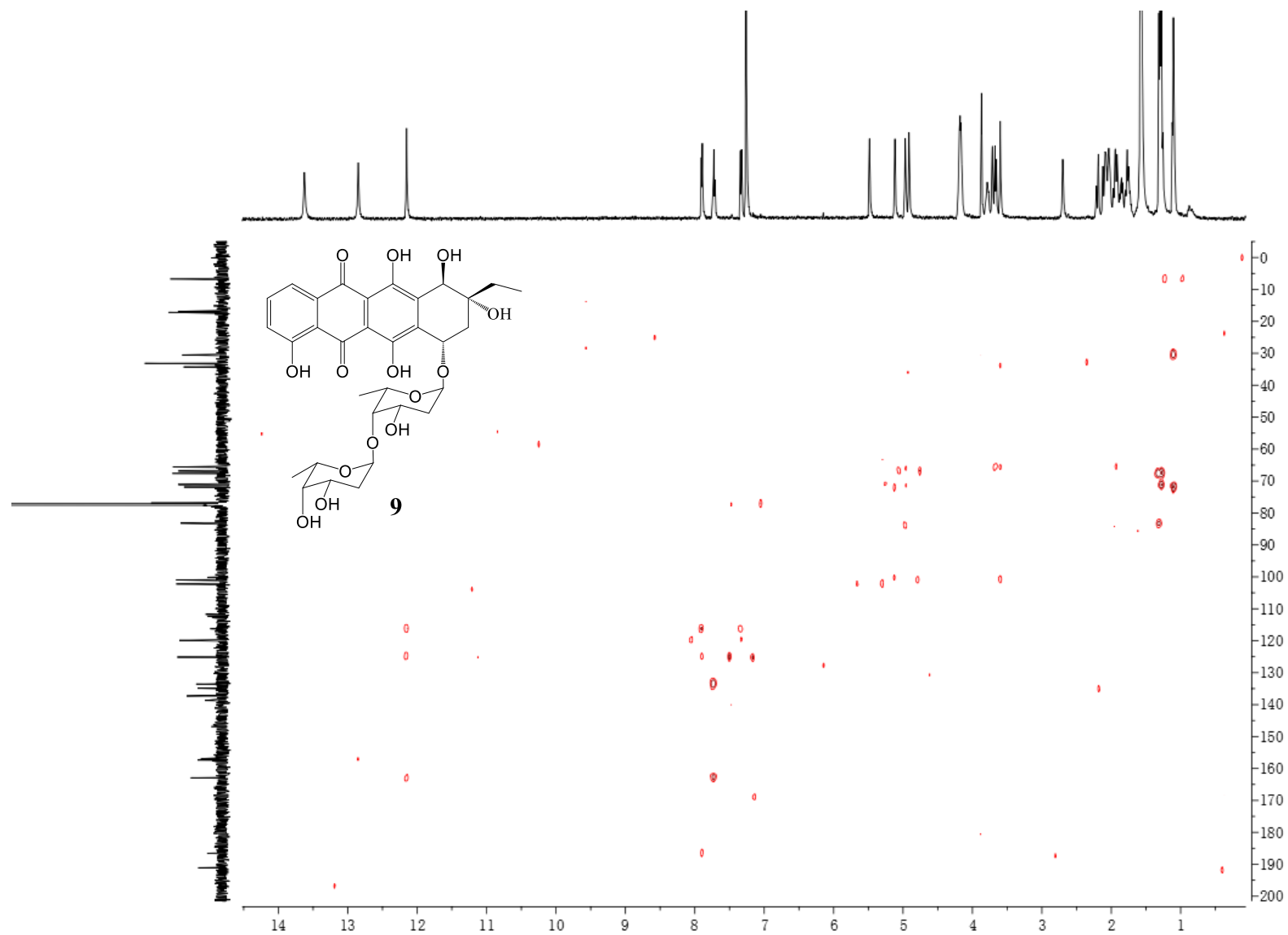


Figure S63. NOESY spectrum of compound **9** in CDCl₃.

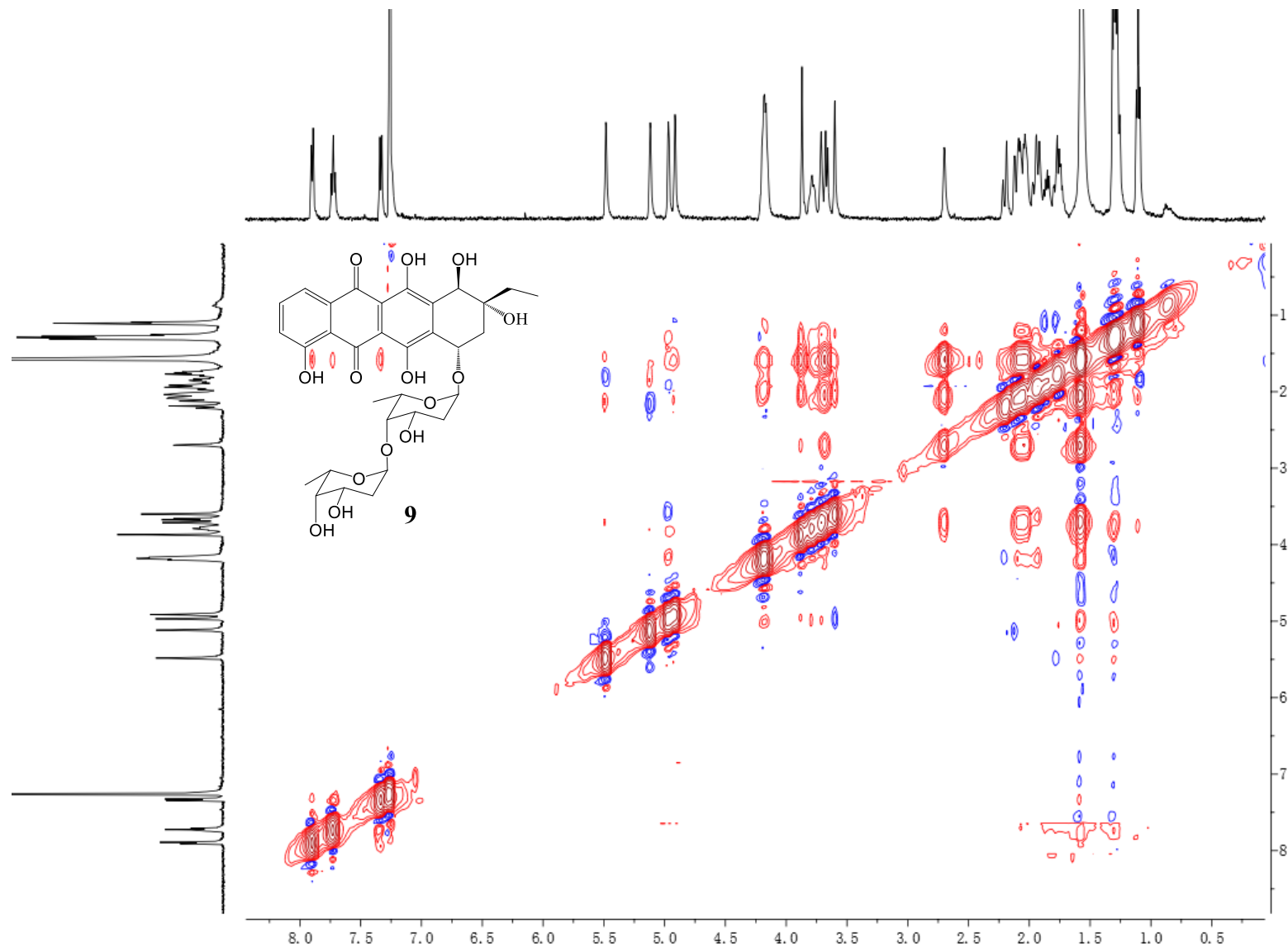


Figure S64. ^1H NMR (500 MHz) spectrum of compound **10** in $\text{CDCl}_3/\text{CD}_3\text{OD}$.

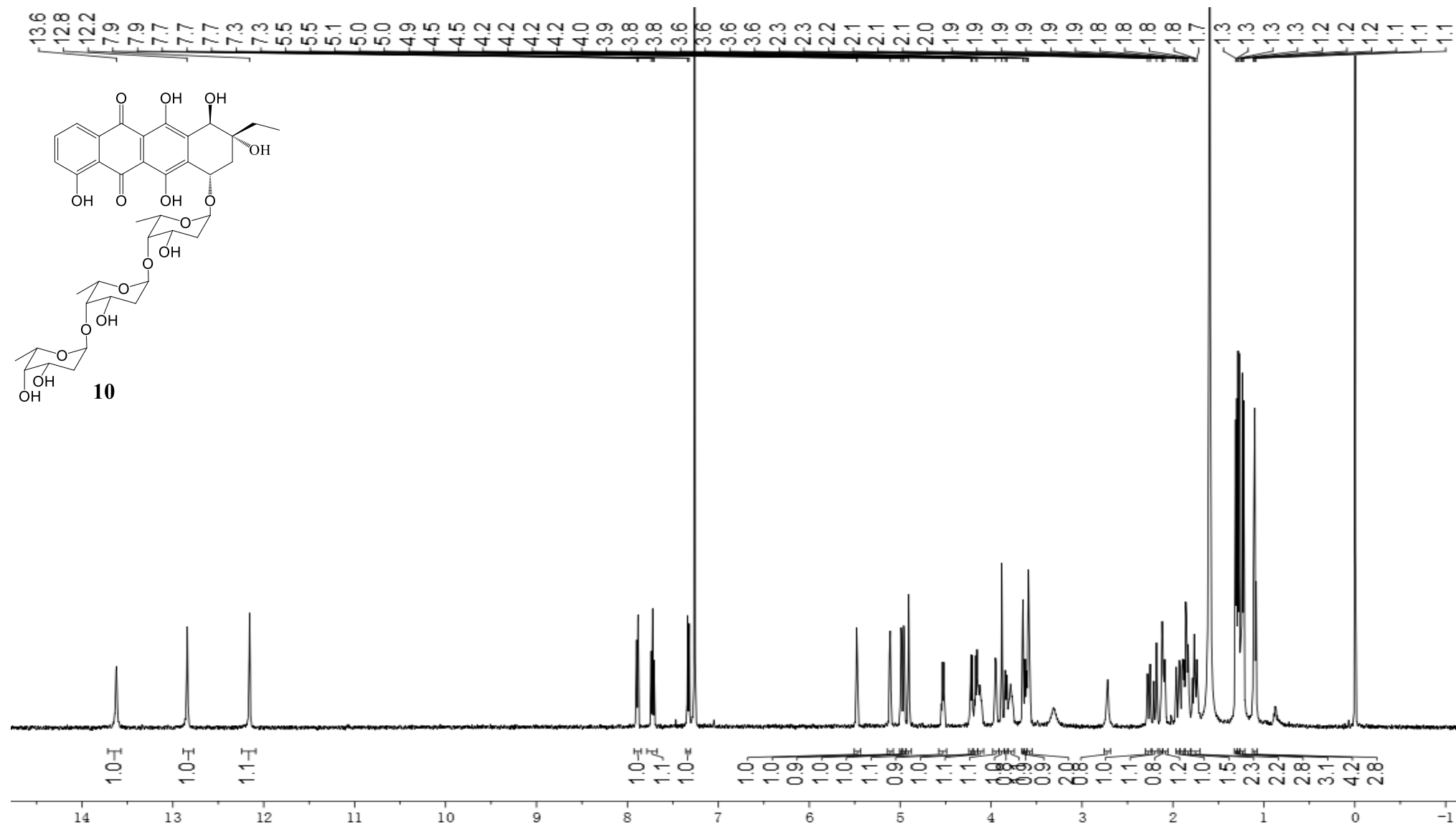


Figure S65. ^{13}C NMR (125 MHz) spectrum of compound **10** in $\text{CDCl}_3/\text{CD}_3\text{OD}$.

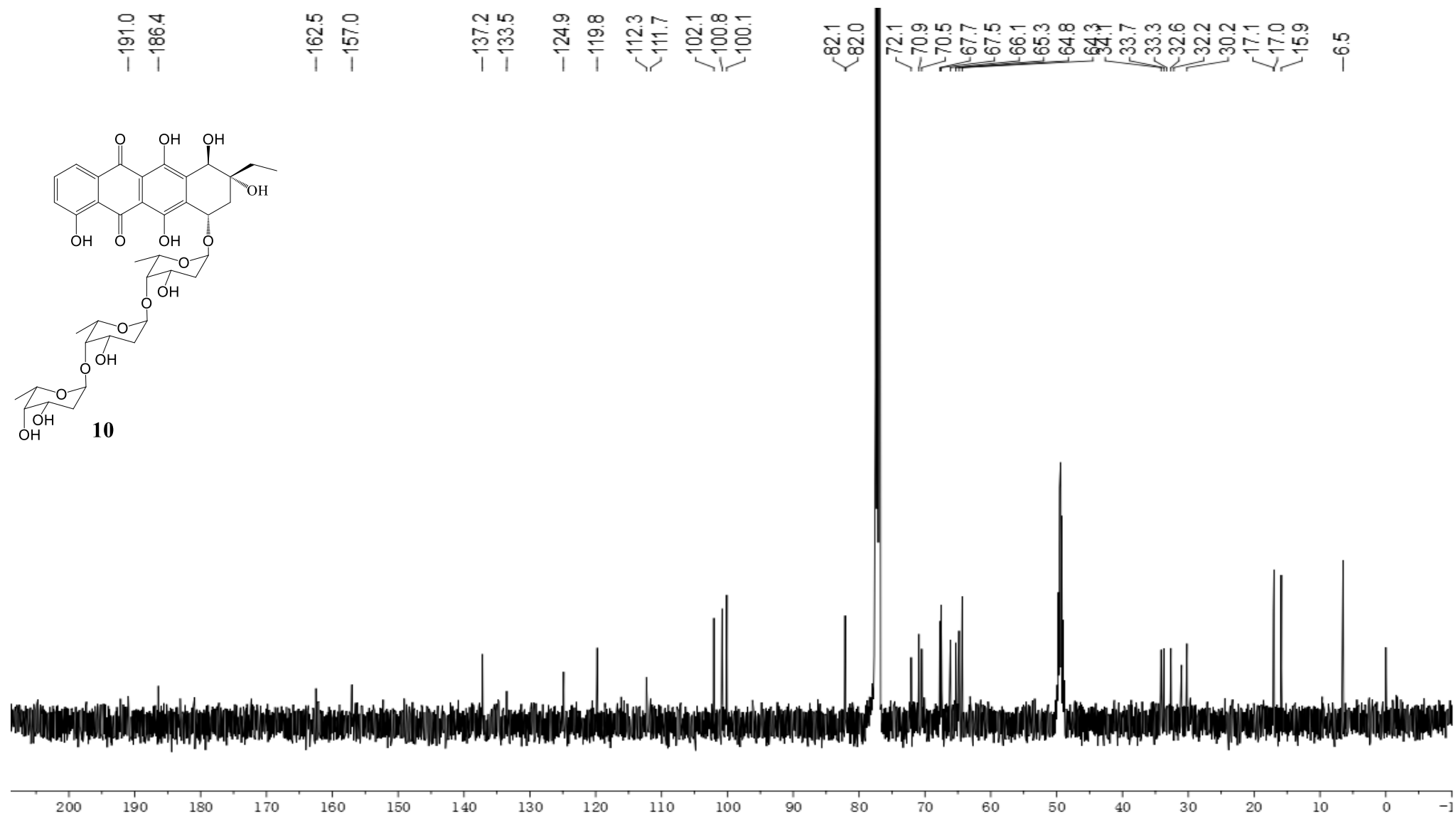


Figure S66. DEPT 135 spectrum of compound **10** in CDCl₃/CD₃OD.

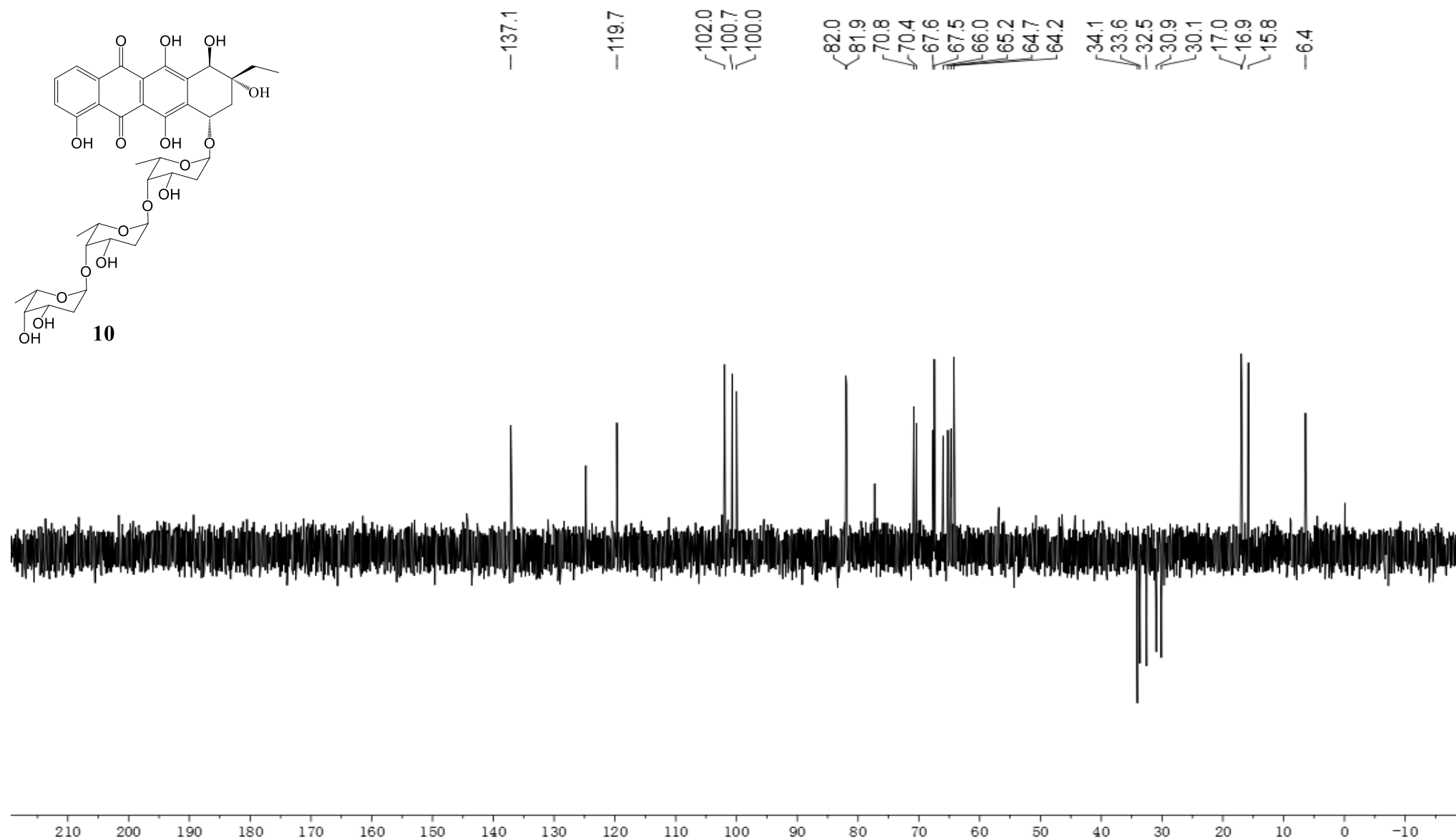


Figure S67. ^1H - ^1H COSY spectrum of compound **10** in $\text{CDCl}_3/\text{CD}_3\text{OD}$.

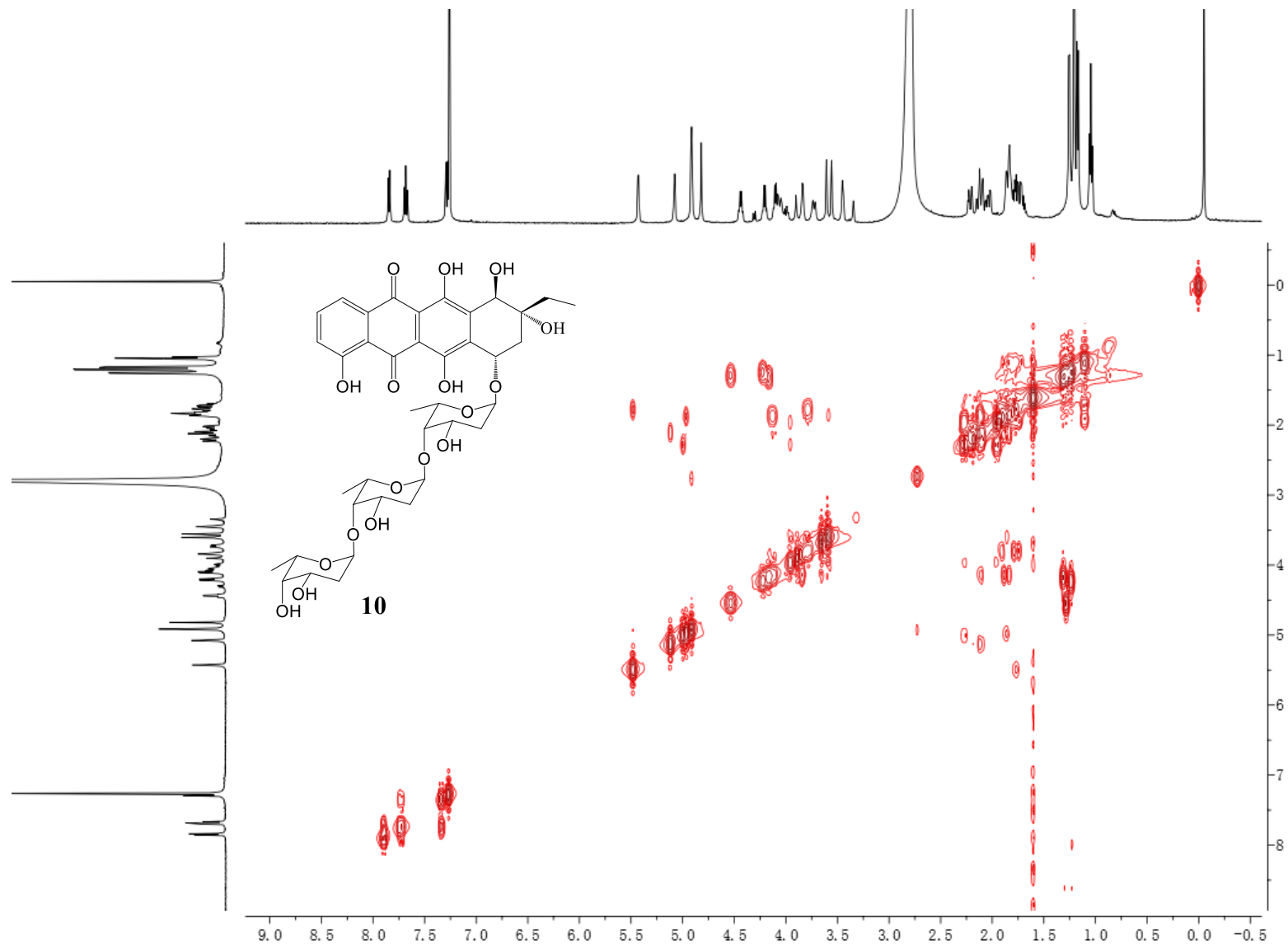


Figure S68. HSQC spectrum of compound **10** in CDCl₃/CD₃OD.

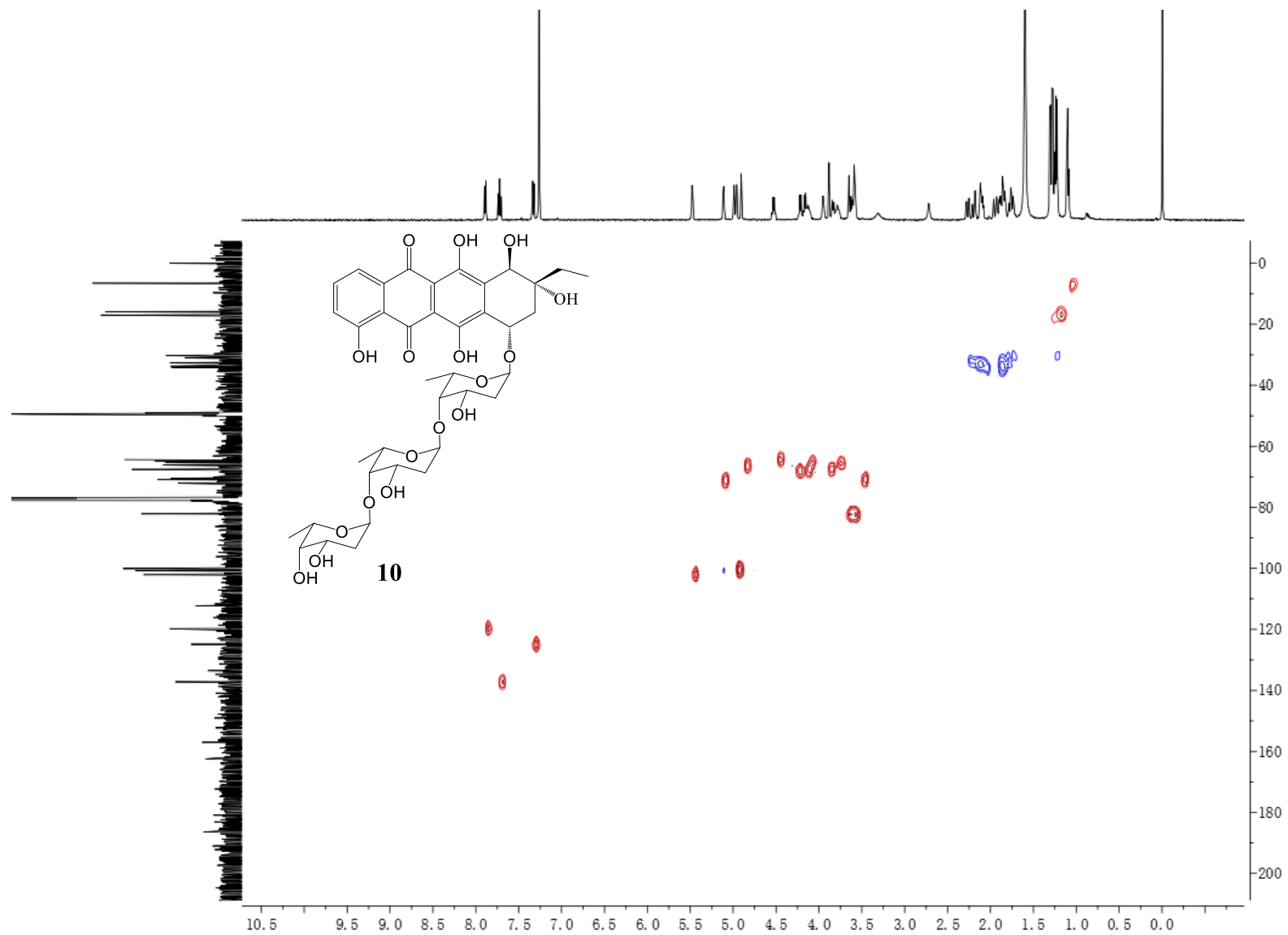


Figure S69. HMBC spectrum of compound **10** in CDCl₃/CD₃OD.

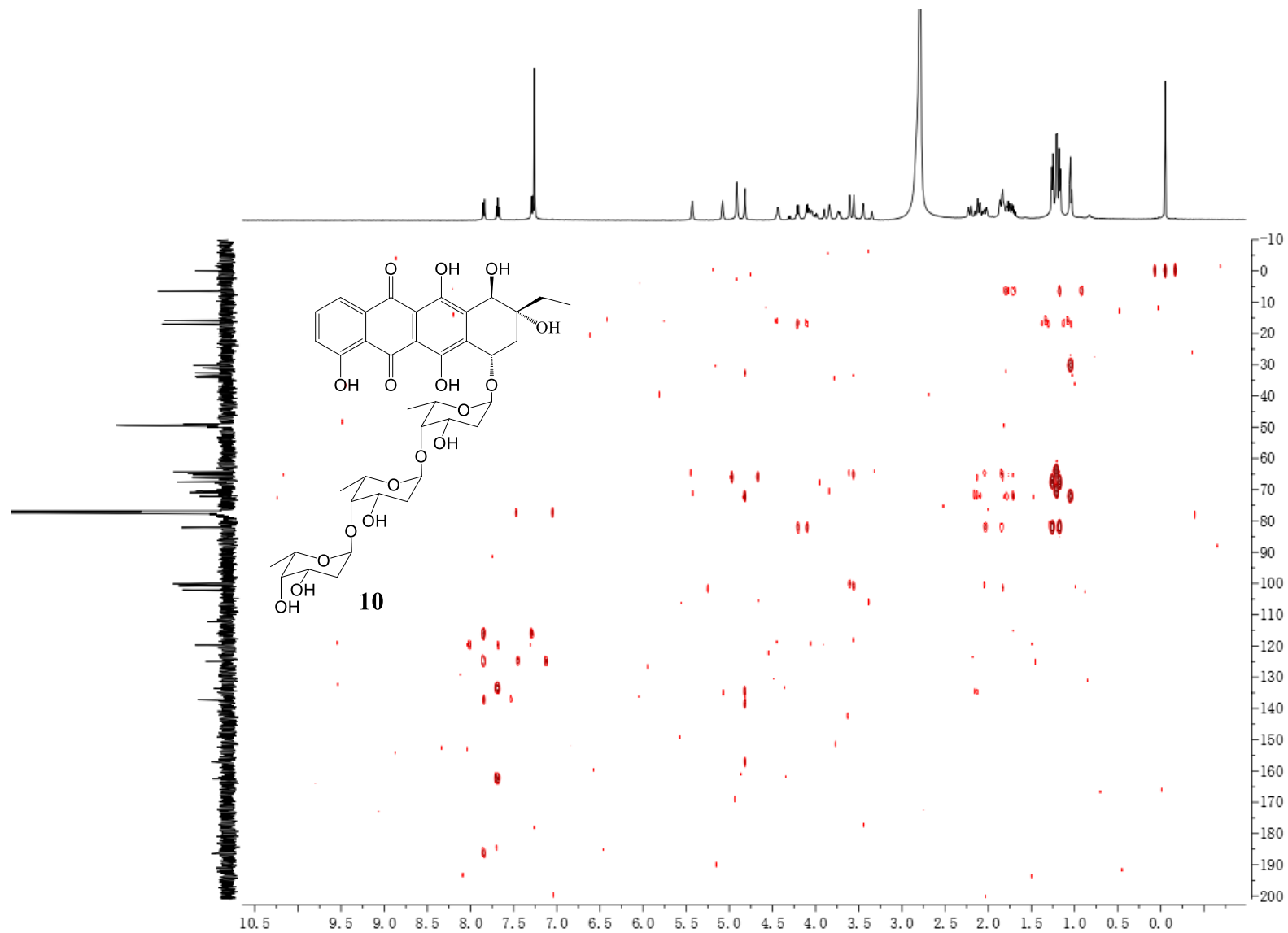


Figure S70. NOESY spectrum of compound **10** in CDCl₃/CD₃OD.

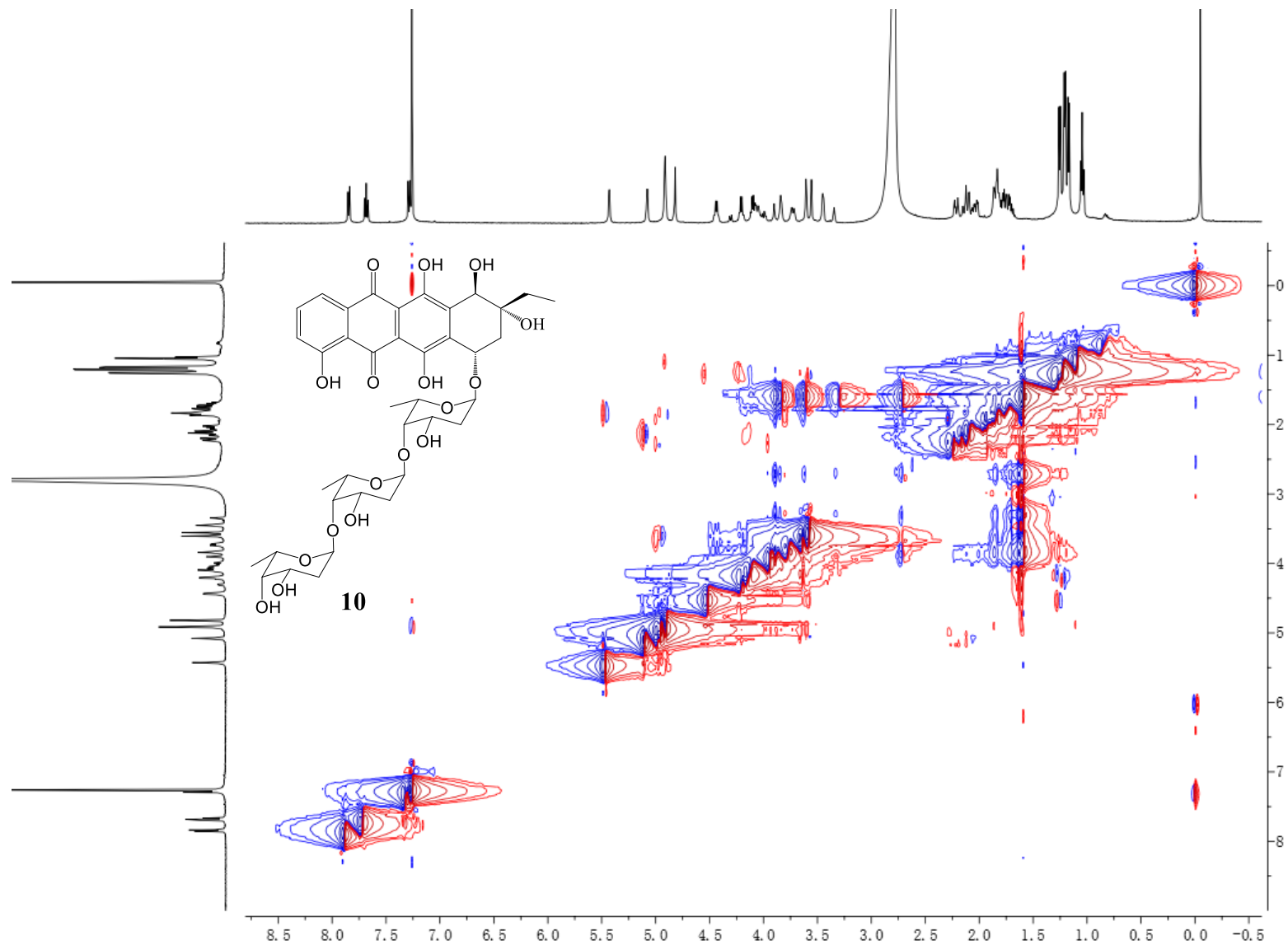


Figure S71. ^1H NMR (500 MHz) spectrum of compound **11** in CDCl_3 .

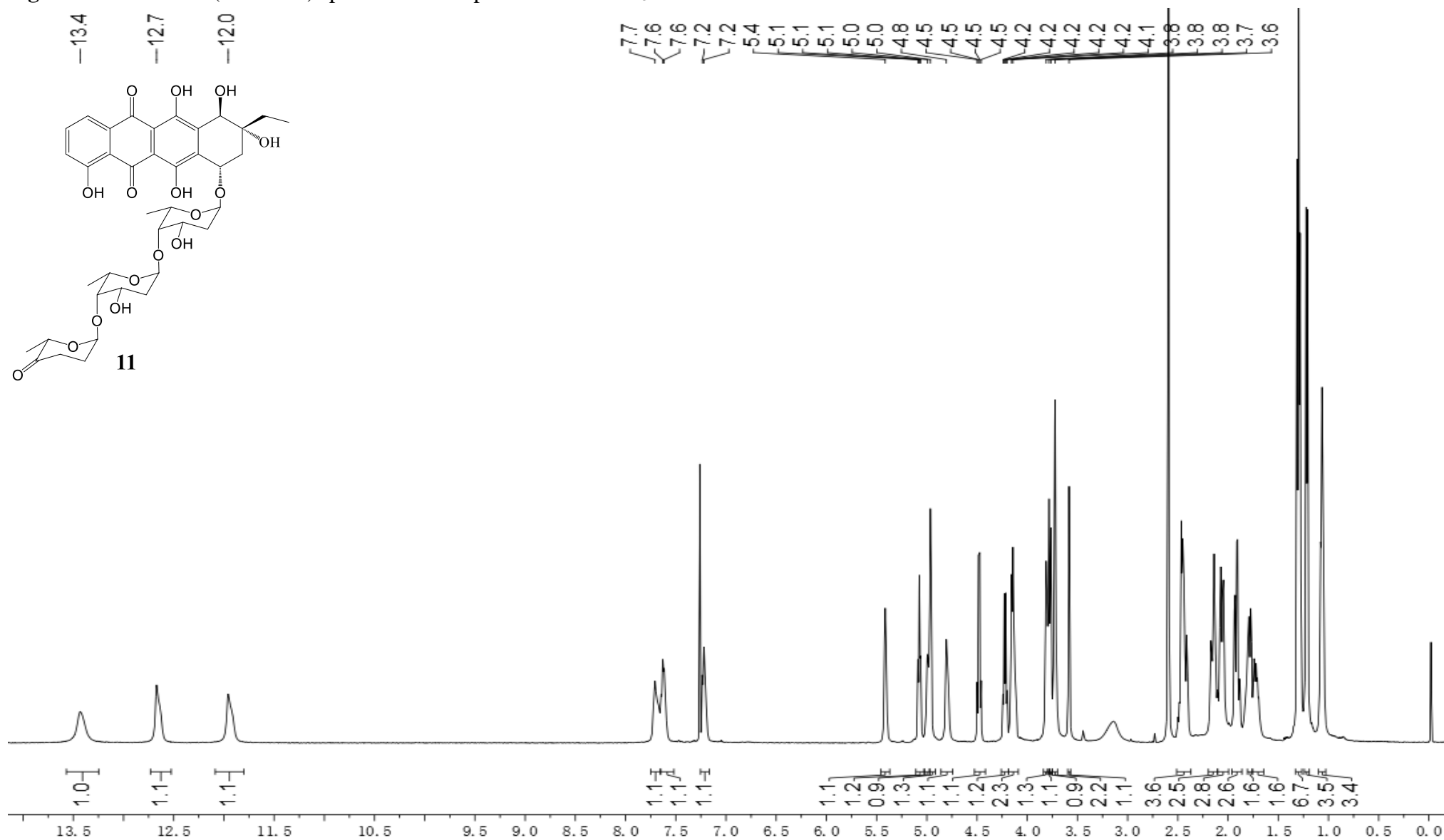


Figure S72. ^{13}C NMR (125 MHz) spectrum of compound **11** in CDCl_3 .

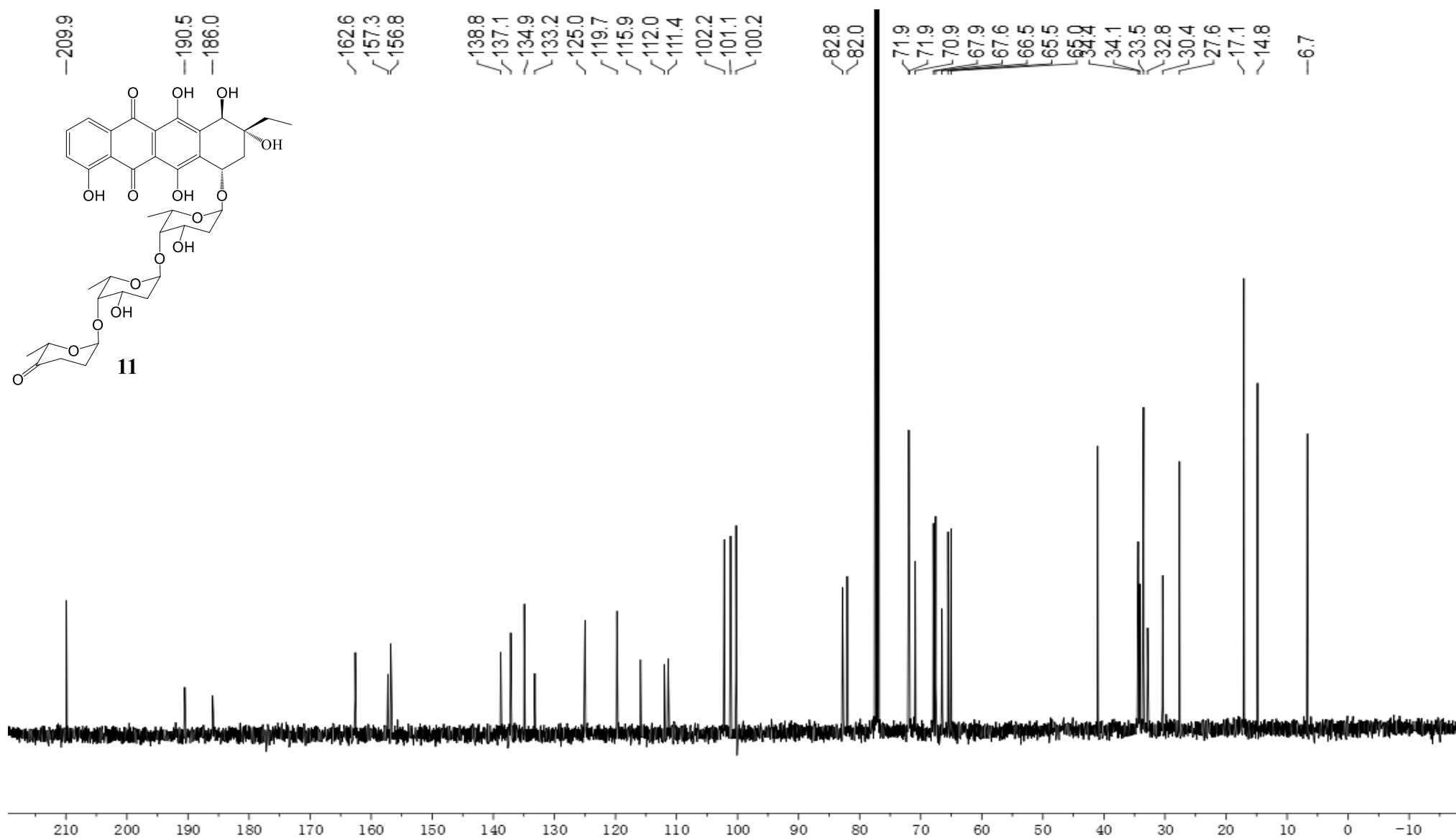


Figure S73. DEPT 135 spectrum of compound **11** in CDCl₃.

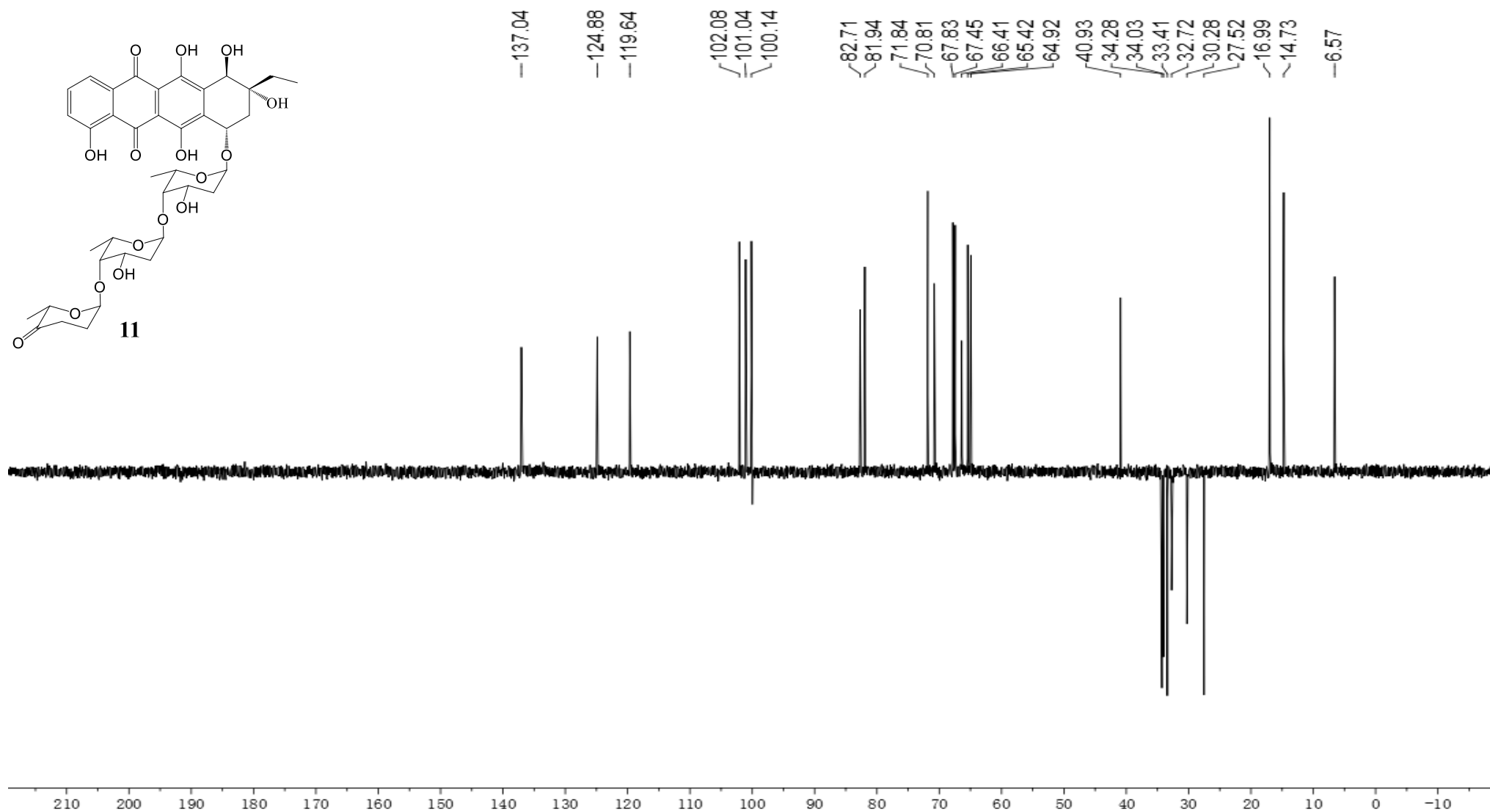


Figure S74. ^1H - ^1H COSY spectrum of compound **11** in CDCl_3 .

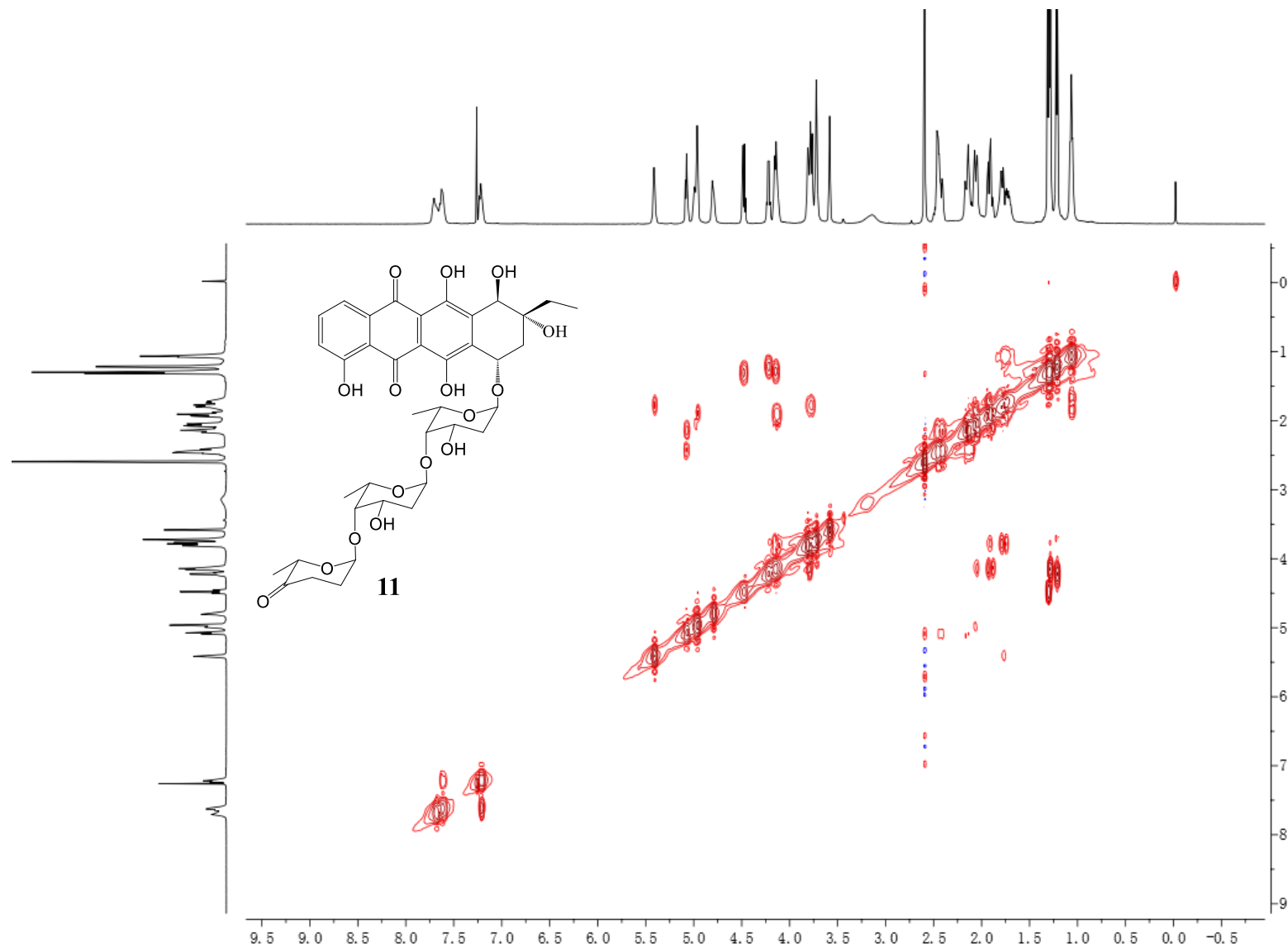


Figure S75. HSQC spectrum of compound **11** in CDCl₃.

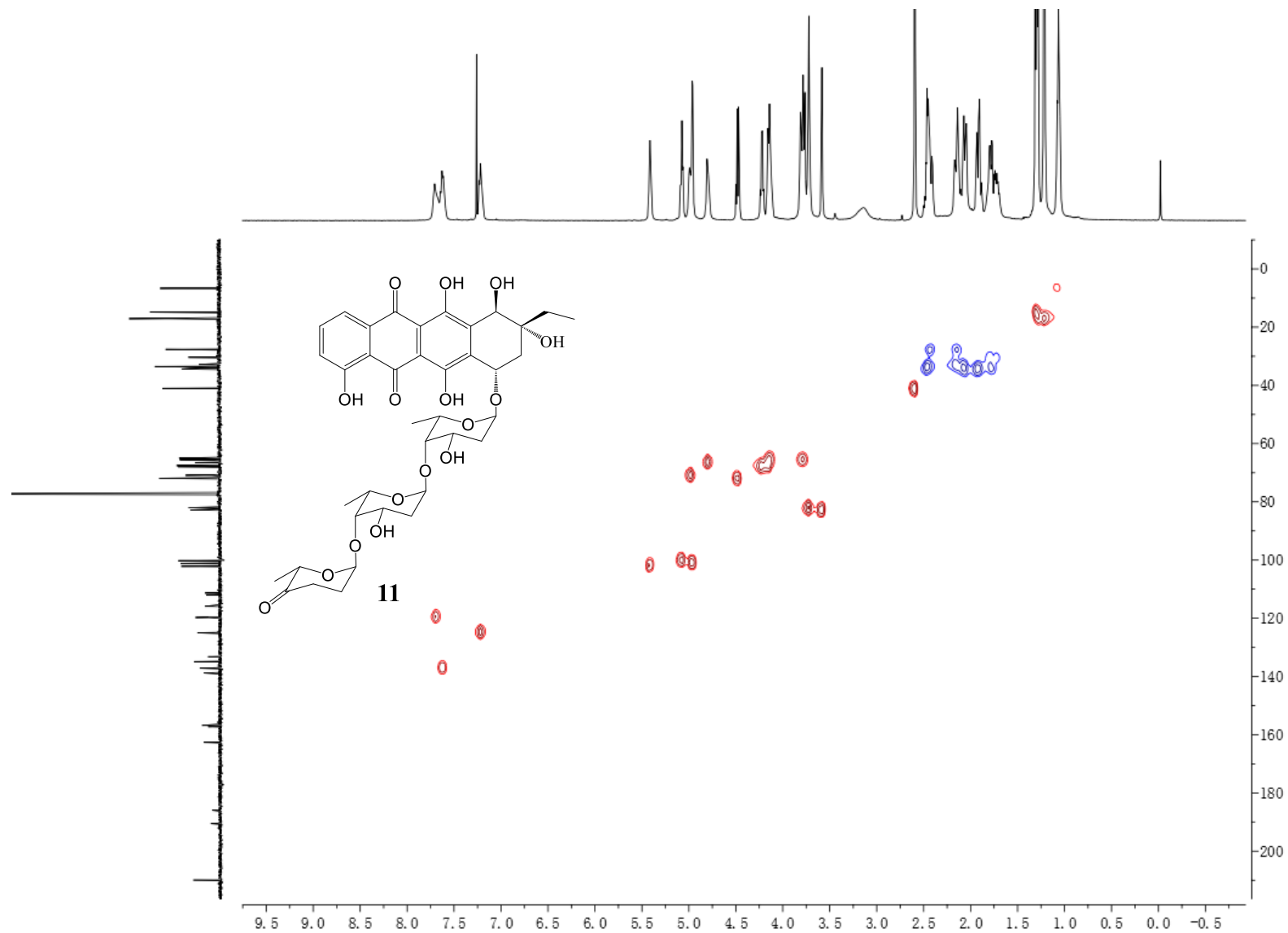
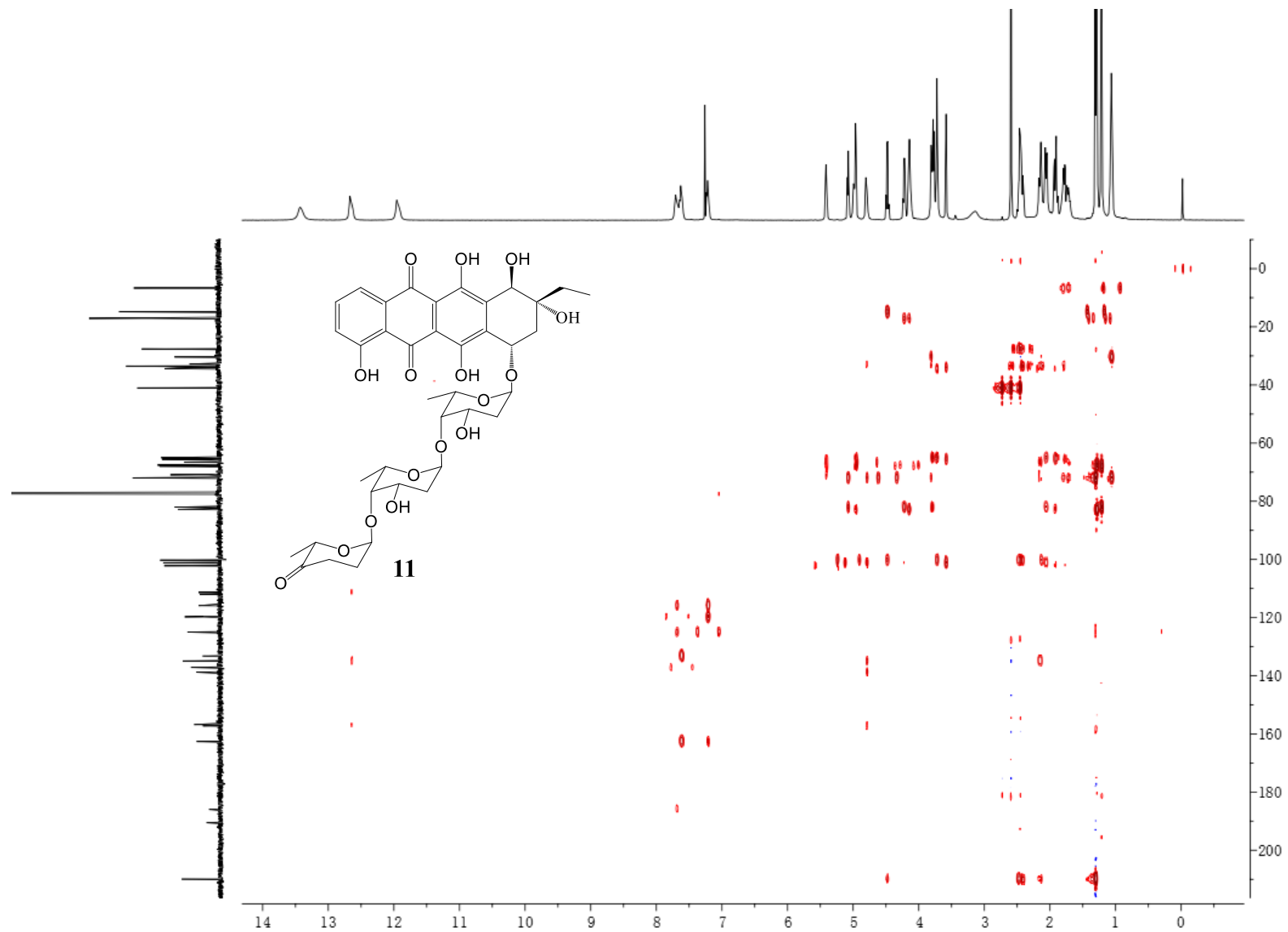


Figure S76. HMBC spectrum of compound **11** in CDCl₃.



The figure displays the chemical structure of compound **11** and its corresponding NMR spectra. The chemical structure is a complex molecule featuring a central benzene ring substituted with two hydroxyl groups and two carbonyl groups. It is linked via an ether bridge to a side chain containing multiple hydroxyl groups and a terminal hydroxyl group. The NMR spectra include a 1D ¹H NMR spectrum at the top, a 2D COSY spectrum in the middle, and a 1D ¹³C NMR spectrum at the bottom. The 1D ¹H NMR spectrum shows peaks in the aromatic region (6.5-7.5 ppm), a sugar region (3.5-5.5 ppm), and an aliphatic region (1.0-2.5 ppm). The 2D COSY spectrum shows correlations between protons in the sugar and aliphatic regions. The 1D ¹³C NMR spectrum shows peaks in the aromatic region (110-160 ppm), a sugar region (60-80 ppm), and an aliphatic region (1.0-2.5 ppm).

Figure S78. ^1H NMR (500 MHz) spectrum of compound **12** in CDCl_3 .

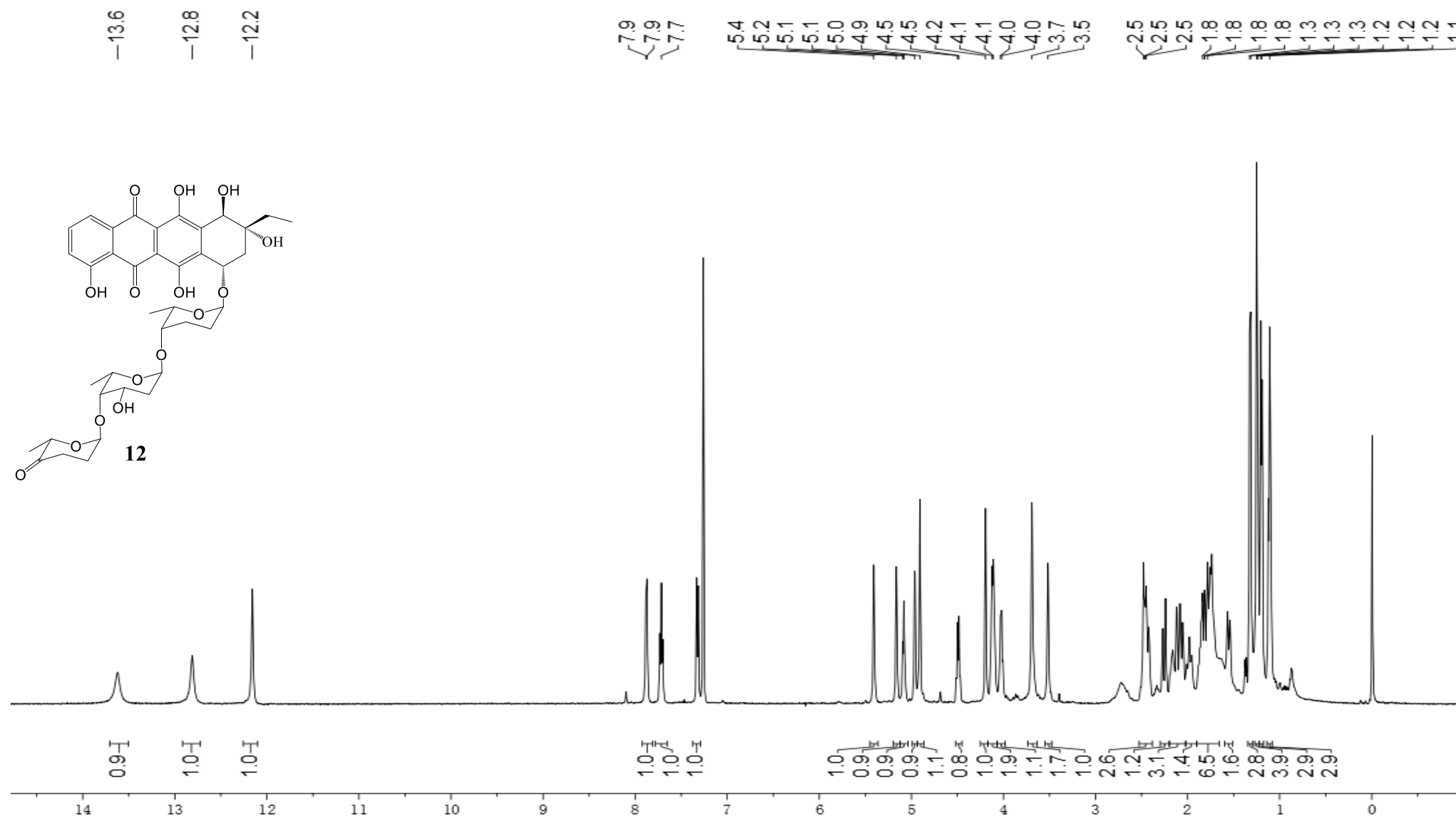


Figure S79. ^{13}C NMR (125 MHz) spectrum of compound **12** in CDCl_3 .

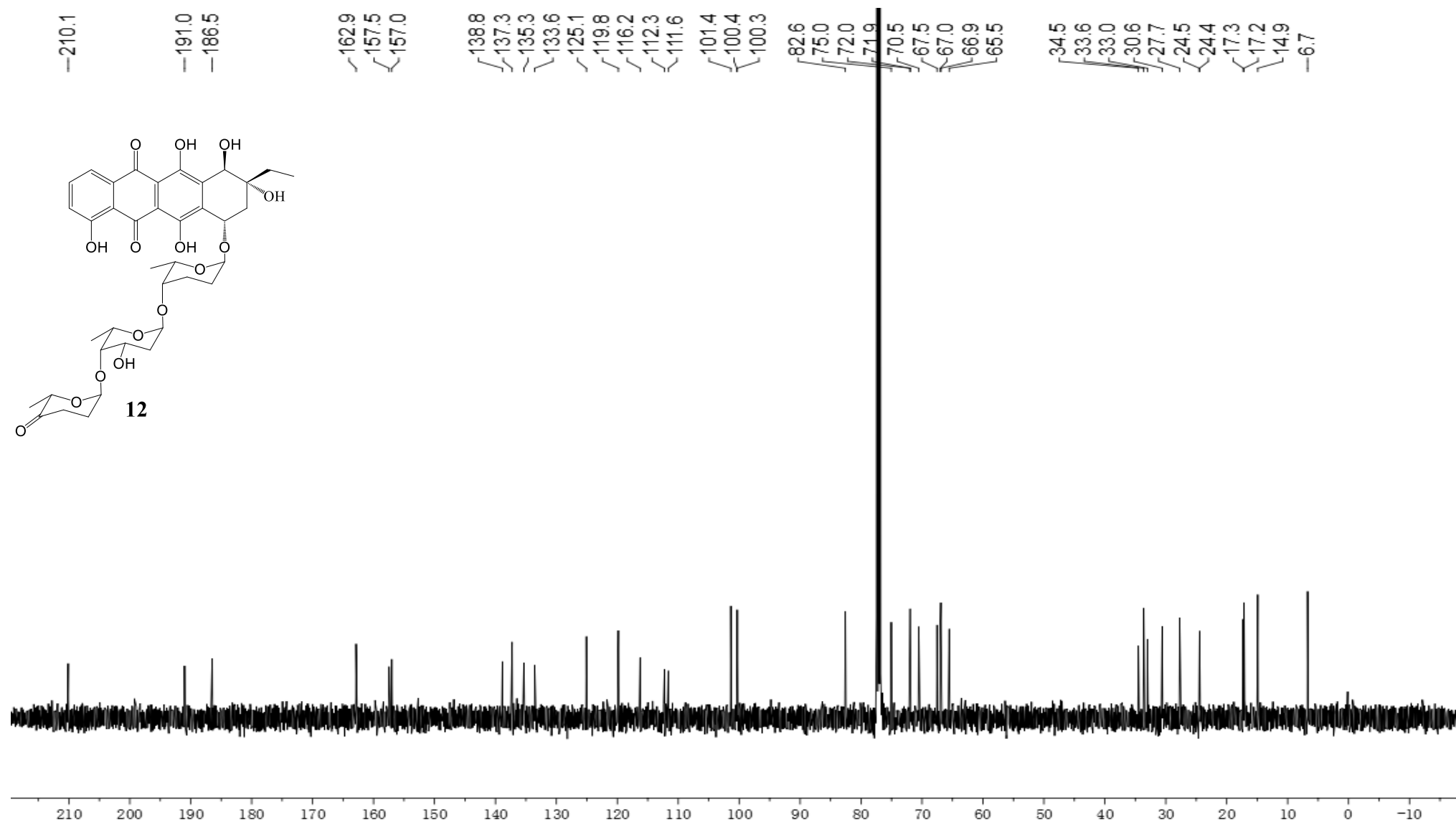


Figure S80. DEPT 135 spectrum of compound **12** in CDCl₃.

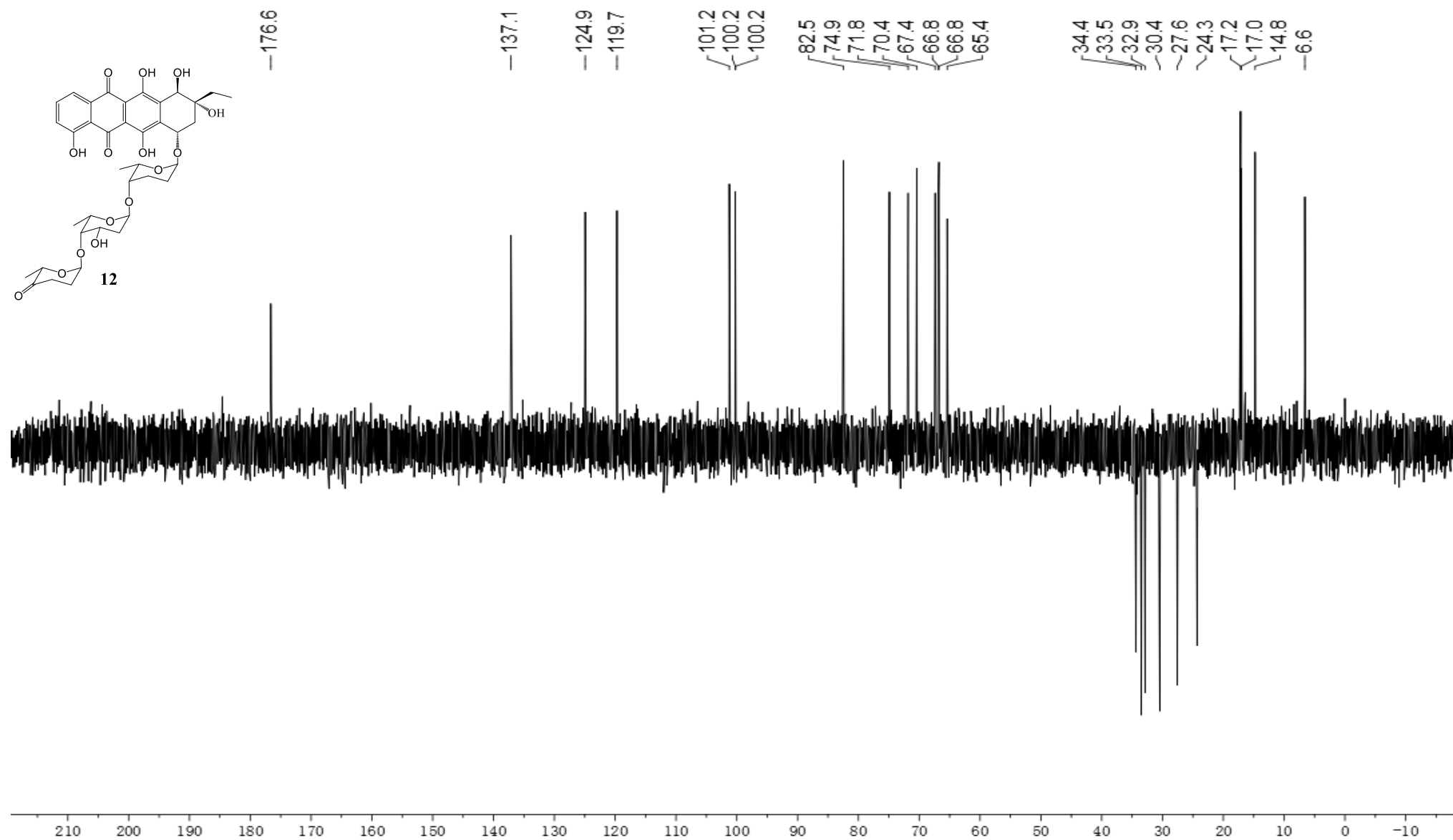


Figure S81. ^1H - ^1H COSY spectrum of compound **12** in CDCl_3 .

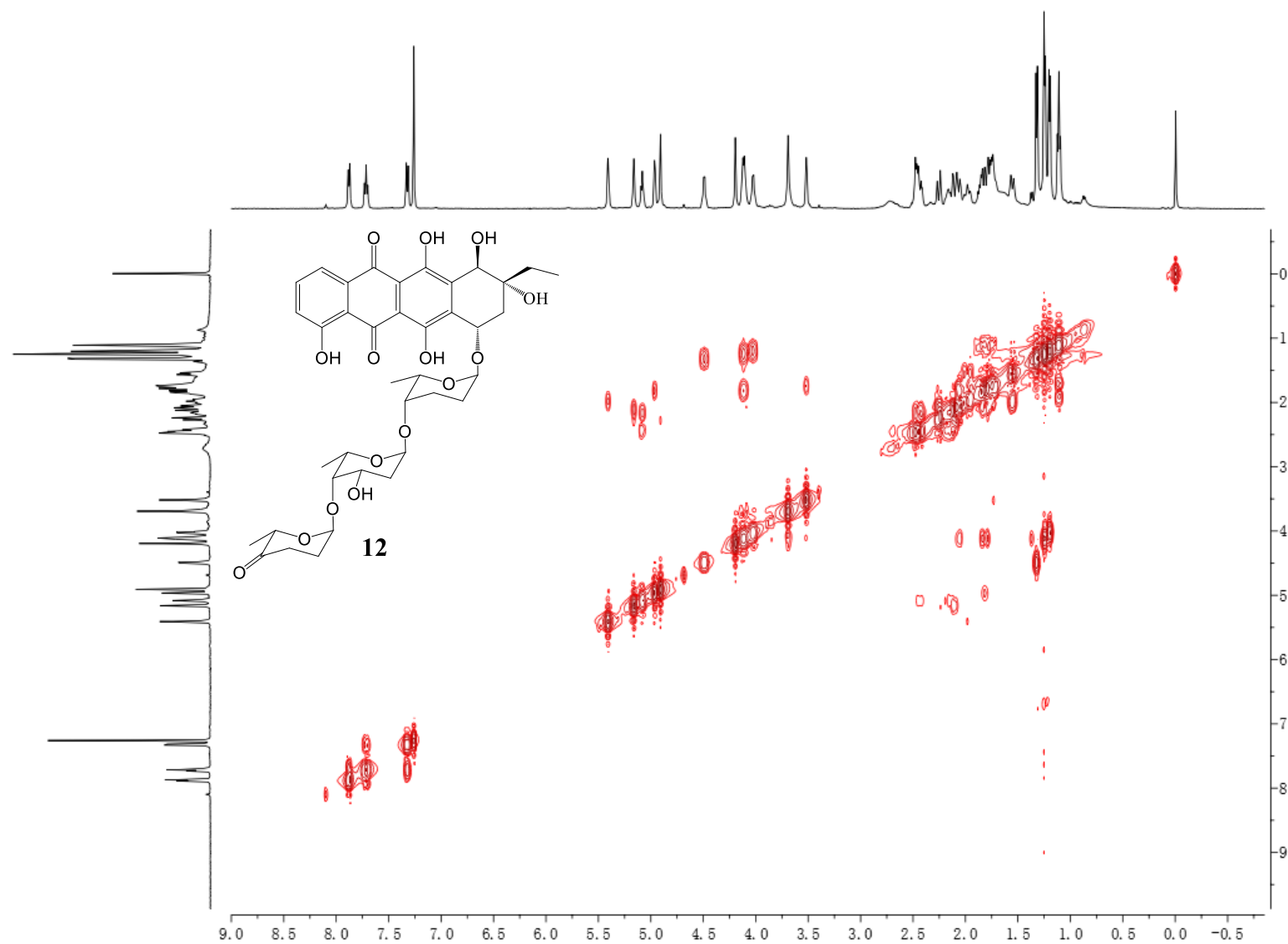


Figure S82. HSQC spectrum of compound **12** in CDCl₃.

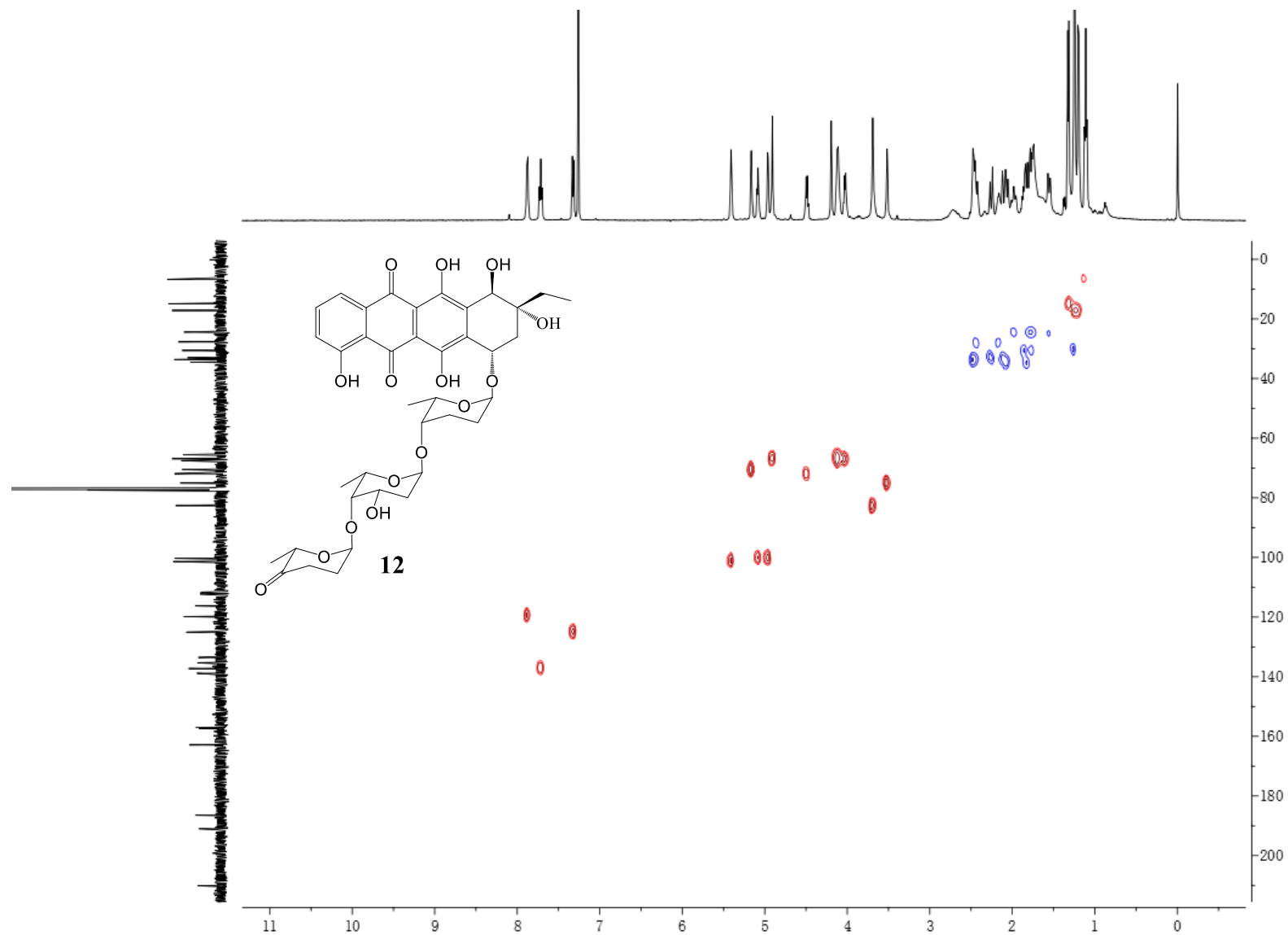


Figure S83. HMBC spectrum of compound **12** in CDCl₃.

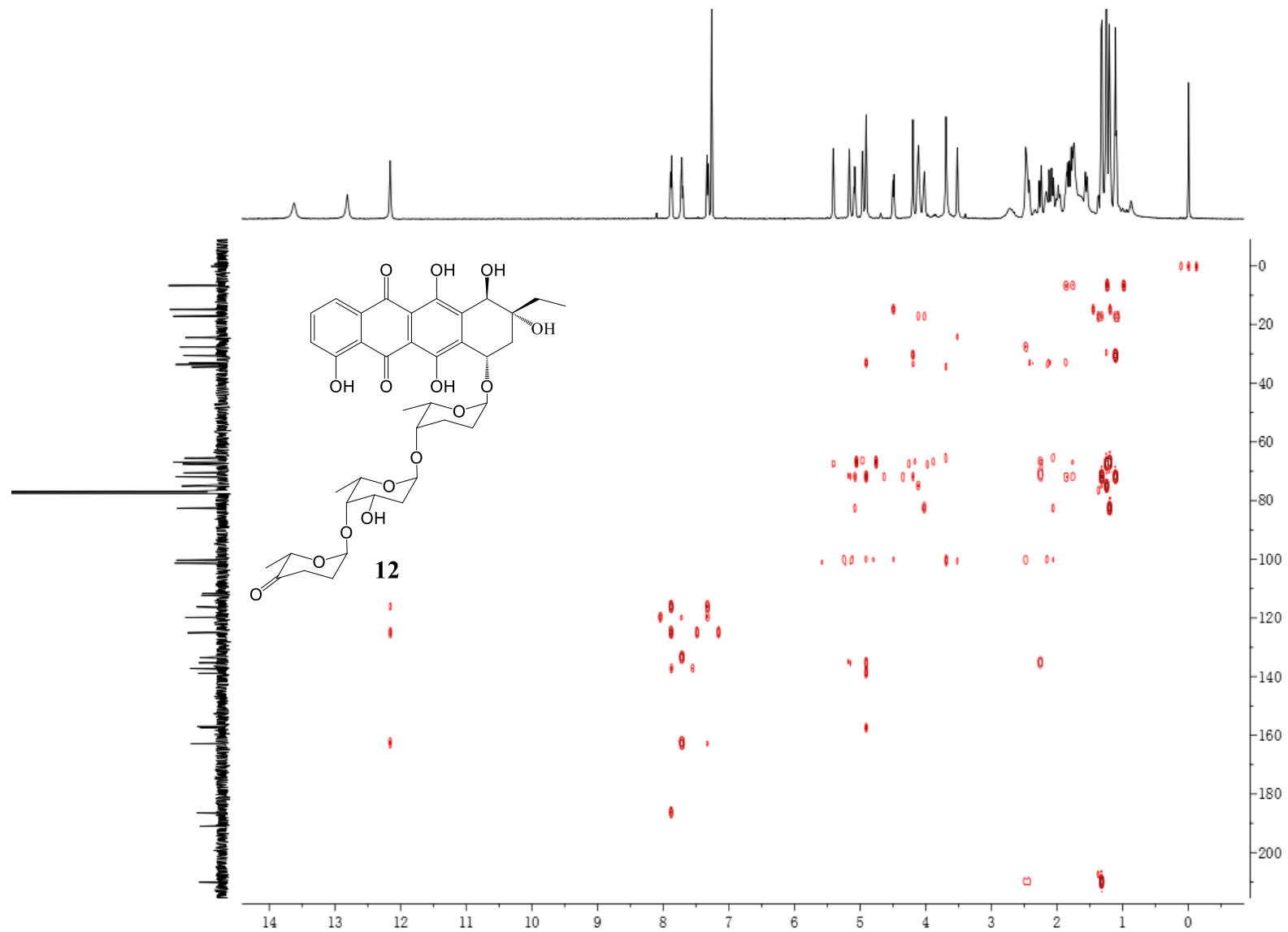


Figure S84. NOESY spectrum of compound **12** in CDCl₃.

