Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Syntheses of 7-dehydrocholesterol peroxides and their improved anticancer activity and selectivity over ergosterol peroxide

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Experimental section

Materials.

7-Dehydrocholesterol (7-DHC) was provided by Vidistone Chemical Company. Ergosterol, succinic anhydride, acetic anhydride, hematoporphyrin, eosin Y, methylene blue and *meso*-tetraphenylporphyrin (TPP) were purchased from Sigma-Aldrich. *n*-Hexane, methanol, benzene, CH_2Cl_2 and pyridine of analytical grade were purchased from SCRC (Sinopharm Chemical Reagent Co., Ltd) and used without further treatment.

Instruments and methods.

^1H NMR, ^{13}C NMR, HMBC and COSY spectra were recorded on a Bruker DMX-400 MHz and 100 MHz spectrophotometer. High-resolution ESI mass spectra (HR ESI-MS) were determined on a Brucker APEX IV (7.0T) FT_MS.

Syntheses of 7-dehydrocholesteryl-3-o-β-acetate (2) and 7-dehydrocholestryl-3-o-β-hemisuccinate (3).

7-dehydrocholesterol (1.00 g, 2.60 mmol) in 5 mL acetic anhydride (52 mmol) was stirred and refluxed for 0.5 h at 140℃. After removal of acetic anhydride in vacuo, 10 mL distilled water was added. The solid was filtered and washed with water and then purified on silica gel using *n*-hexane/ethyl acetate (15:1 in volume ratio) as eluent. The yield of 7-dehydrocholesteryl-3-o-β-acetate (**2**) was 81%. 7-dehydrocholestryl-3-o-β-hemisuccinate (**3**) was prepared in a similar way in a yield of 73%.

Cell culture.

Breast cancer cells SKOV-3, cervical carcinoma cells HeLa, lung cancer cells A549, prostatic carcinoma cells DU145 and human normal liver cells L-02 were provided by Cancer Institute, Chinese Academy of Medical Science. The cells were cultured in DMEM medium containing 10% FBS, 100U/mL penicillin/streptomycin at 37°C under a 5% CO_2 atmosphere, then plated at 2×10^5 per well in 96 well plates and incubated for 24 h in 150 μL DMEM medium in the same conditions.

Cell cytotoxicity assay.

The cytotoxicity of **1-3**, **1′-3′** and EEP was evaluated by MTT assay. The cells were plated at 2×10^5 per well in 96 well plates. After incubation for 24 h, the cells were treated with varied concentrations of the examined compounds for 48 h at 37°C. The culture medium was removed and 200 μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) solution was added and the cells were maintained at 37°C for 4 h. Then a mixed solution of CH_3OH/DMSO (1:1) was added and the absorbance at 595 nm was determined by a Multimode Plate Reader (EnSpire). The untreated cells served as the control and their viability was set as 100%.

Table S1. Reaction optimization for the synthesis of EEP, **2´** and **3´**.[a]

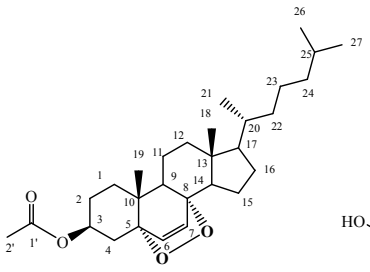
Entry	Substrate	Product	Catalyst (mol%)	solvent	t [b] (h)	Yield [c] (%)
1	ergosterol	EEP	TPP (0.1)	pyridine	1	58
2	ergosterol	EEP	TPP (0.1)	benzene	1	53
3	ergosterol	EEP	TPP (0.1)	CH_2Cl_2	3	44
4	ergosterol	EEP	TPP (0.1)	*n*-hexane/ methanol (3:1)	1	67
5	2	2´	TPP (0.1)	*n*-hexane/ methanol (3:1)	3	78
6	3	3´	TPP (0.1)	*n*-hexane/ methanol (3:1)	3	81

[a] Reaction conditions: 1.56 mmol substrate and 1.9 μM TPP in 20 mL of solvent was subjected to visible light irradiation (≥ 400 nm) at 0℃ under magnetic stirring and bubbling with oxygen; [b] by which all substrate was consumed; [c] isolated yield.

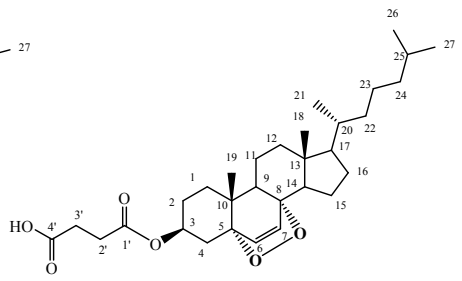
Table S2. ^{1}H NMR chemical shifts of **2, 3, 1´-3´**and EEP.

Position	^{1}H (δ, ppm) (in $CDCl_3$)					
	2	**3**	**EEP**	**1´**	**2´**	**3´**
H-3	4.70(m,1H)	4.73(m,1H)	3.92(m, 1H)	3.97(m, 1H)	4.98(m, 1H)	5.02(m, 1H)
H-6	5.56(dd, 1H, 2.4, 5.6Hz)	5.56(dd, 1H, 2.4, 4.8Hz)	6.47(d, 1H, 8.4 Hz)	6.51(d, 1H, 8.4 Hz)	6.51(d, 1H, 8.4 Hz)	6.51(d, 1H, 8.4 Hz)
H-7	5.38(dd, 1H, 2.4, 5.6Hz)	5.38(dd, 1H, 2.4, 4.8Hz)	6.21(d, 1H, 8.4 Hz)	6.24(d, 1H, 8.4 Hz)	6.23(d, 1H, 8.4 Hz)	6.22(d, 1H, 8.4 Hz)
H-18	0.61(s, 3H)	0.61(s, 3H)	0.81(s, 3H)	0.80(s, 3H)	0.79(s, 3H)	0.80(s, 3H)
H-19	0.93(s, 3H)	0.93(s, 3H)	0.88(s, 3H)	0.88(s, 3H)	0.89(s, 3H)	0.89(s, 3H)
H-20						
H-21	0.95(s, 3H)	0.95(s, 3H)	1.00(d, 3H, 6.4 Hz)	0.90(d, 3H, 6.8 Hz,)	0.91(s, 3H)	0.91(s, 3H)
H-22			5.14(dd, 1H, 15.2, 8.0 Hz,)			
H-23			5.22(dd, 1H, 15.2, 7.6 Hz,)			
H-26	0.86(d,3H, 1.6 Hz,)	0.85(d,3H, 1.2 Hz,)	0.81(d, 3H, 6.4 Hz,)	0.85(d,3H, 1.6 Hz,)	0.85(d,3H, 1.6 Hz,)	0.85(d,3H, 2.0 Hz,)
H-27	0.87(d,3H, 1.6 Hz,)	0.87(d,3H, 1.2 Hz,)		0.87(d, 3H, 1.6 Hz,)	0.87(d, 3H, 1.6 Hz)	0.87(d,3H, 2.0 Hz,)
H-28						
H-1´						
H-2´	2.04(s, 3H)	2.63(t, 2H, 4.8 Hz)			2.01(s, 3H)	2.59(t, 2H, 6.4 Hz)
H-3´		2.67(t, 2H, 4.8 Hz)				2.68(t, 2H, 6.4, Hz)
H-4´						

Atom numbering of **2´** and **3´** are as follows.



compound 2'



compound 3'

Table S3. ^{13}**C** NMR chemical shifts of **2, 3, 1´-3´**and EEP.

Position	**^{13}C (δ, ppm) (in $CDCl_3$)**					
	2	**3**	**EEP**	**1´**	**2´**	**3´**
C-1	28.1	28.1	34.8	28.0	26.4	26.3
C-2	36.3	36.2	30.1	30.2	33.4	33.2
C-3	72.9	73.5	66.4	66.4	69.6	70.2
C-4	38.1	38.0	37.0	37.1	37.1	37.1
C-5	138.7	138.5	82.2	82.3	81.8	81.8
C-6	120.4	120.4	130.7	130.8	131.0	131.1
C-7	116.4	116.4	135.6	135.6	135.2	135.1
C-8	141.7	141.6	79.4	79.5	79.5	79.5
C-9	46.2	46.2	51.2	51.3	51.3	51.2
C-10	37.3	37.2	37.0	36.1	36.1	36.1
C-11	23.2	23.1	23.4	23.5	23.5	23.5
C-12	39.7	39.6	39.4	39.6	39.6	39.6
C-13	43.1	43.1	44.6	44.9	44.9	44.9
C-14	54.6	54.6	51.8	51.8	51.7	51.7
C-15	21.2	21.2	20.9	20.7	20.7	20.7
C-16	28.3	28.2	28.6	28.3	28.3	28.3
C-17	56.1	56.1	56.3	56.6	56.6	56.6
C-18	11.9	11.9	12.9	12.7	12.8	12.7
C-19	16.3	16.3	18.2	18.2	18.1	18.1
C-20	39.4	39.3	39.7	39.6	39.6	39.6
C-21	19.0	19.0	19.7	18.7	18.7	18.7
C-22	36.3	36.3	132.4	34.9	34.5	34.4
C-23	24.0	24.0	135.2	23.9	23.9	23.9
C-24	36.8	36.7	42.8	35.3	35.3	35.3
C-25	28.2	28.1	33.1	28.1	28.1	28.1
C-26	22.6	22.6	20.0	22.6	22.6	22.6
C-27	22.9	22.9	20.7	22.9	22.9	22.8
C-28			17.6			
C-1´	170.5	171.584			170.0	171.2
C-2´	21.4	29.1			21.3	29.0
C-3´		29.4				29.3
C-4´		177.7				177.4

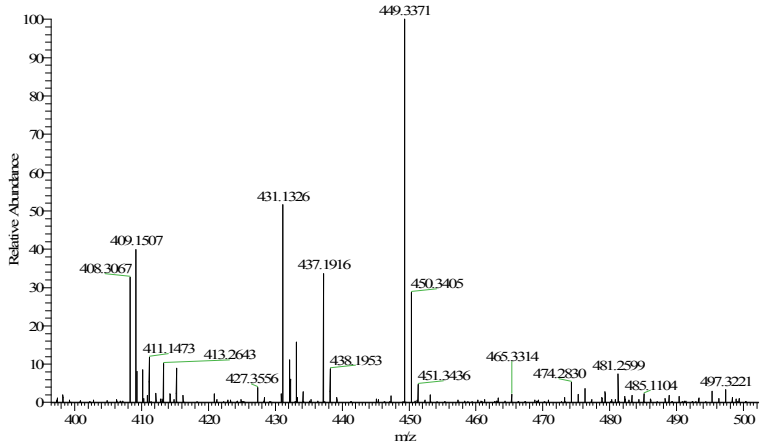


Figure S1. HR ESI-MS spectrum of **2**.

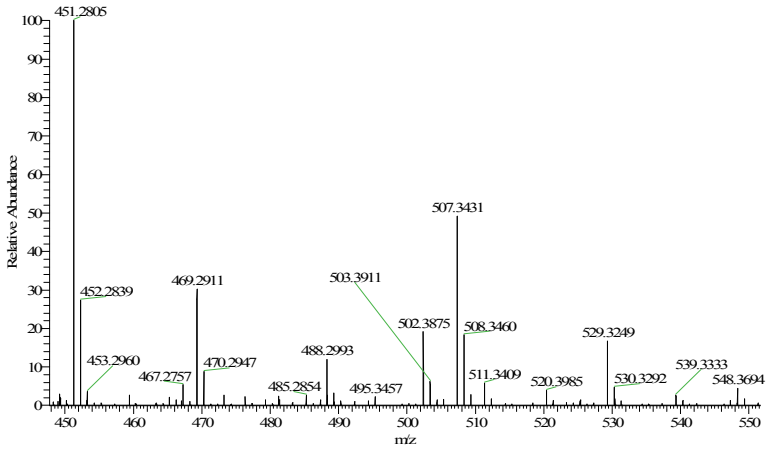


Figure S2. HR ESI-MS spectrum of **3**.

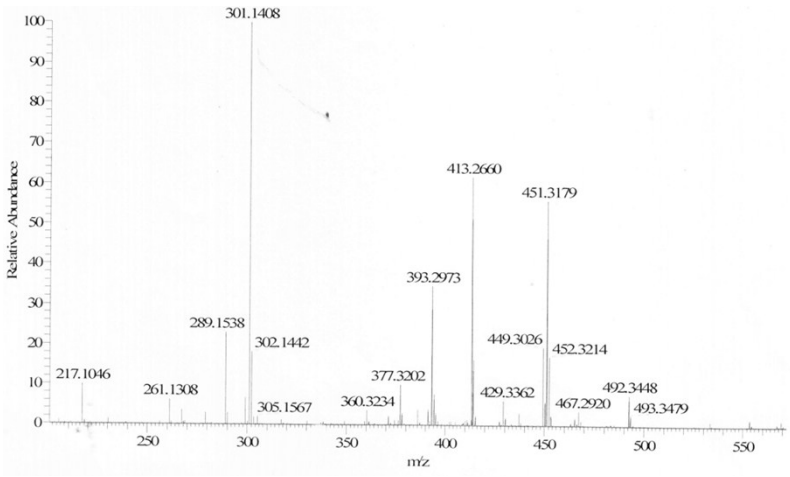


Figure S3. HR ESI-MS spectrum of EEP**.**

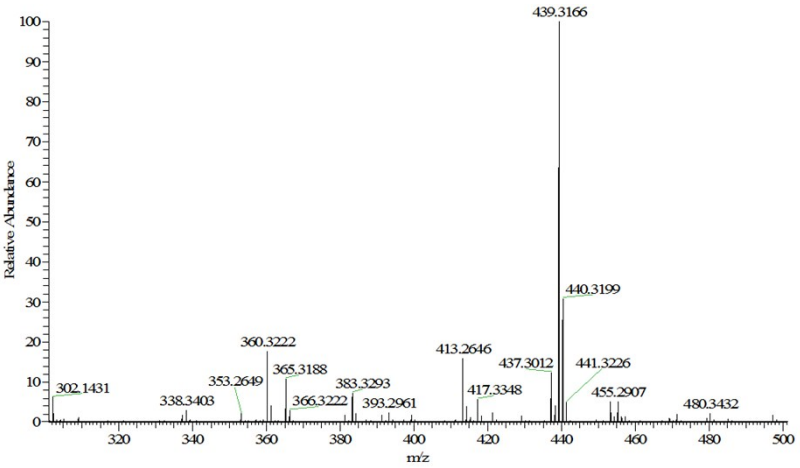


Figure S4. HR ESI-MS spectrum of CEP (**1´**).

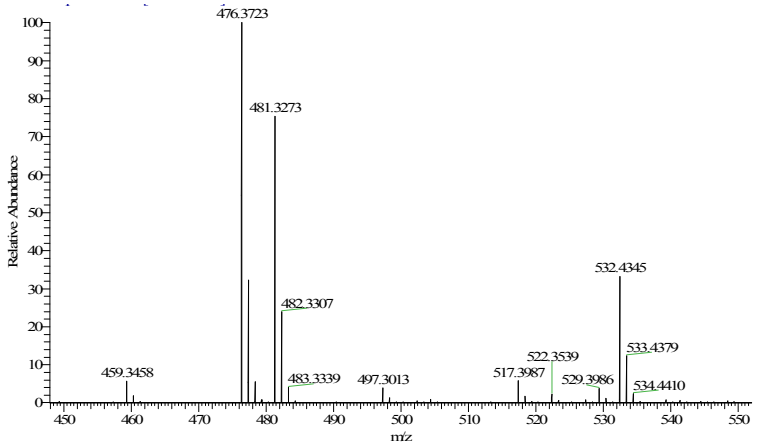


Figure S5. HR ESI-MS spectrum of **2´**.

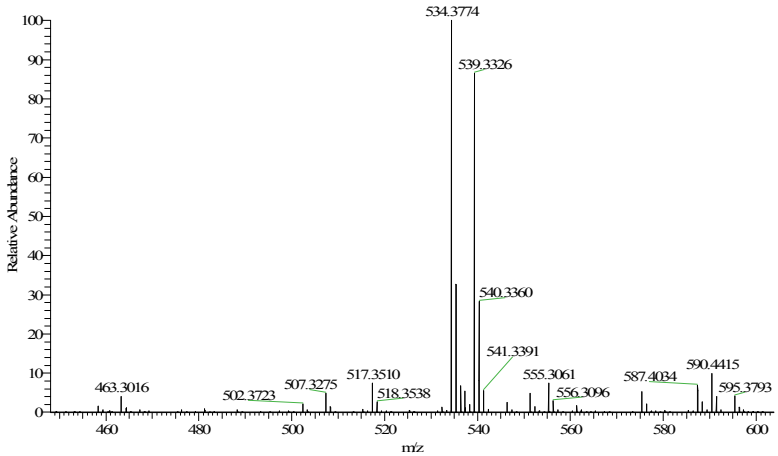


Figure S6. HR ESI-MS spectrum of **3´**.

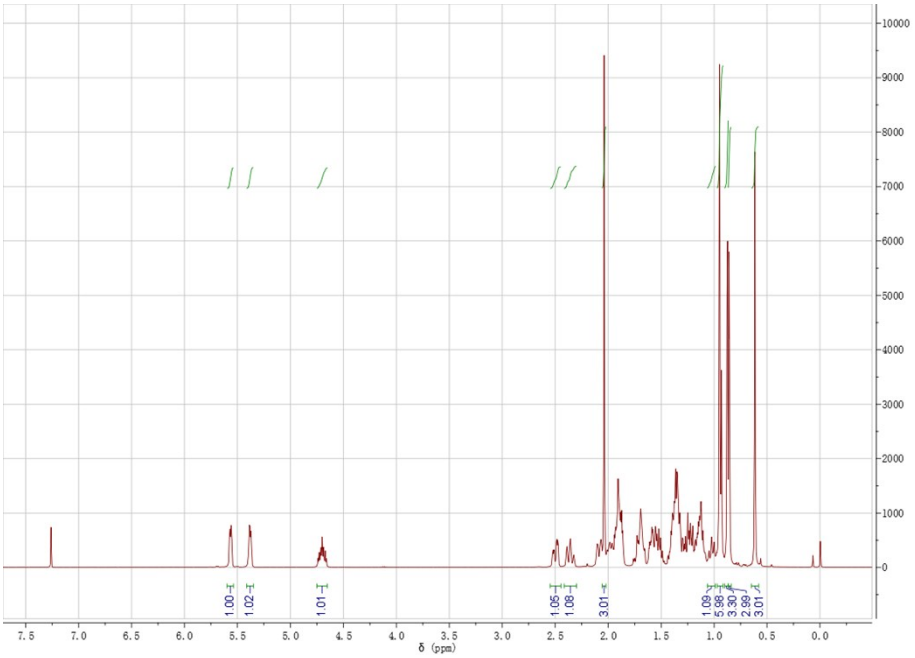


Figure S7. ^{1}H NMR spectrum of **2**.

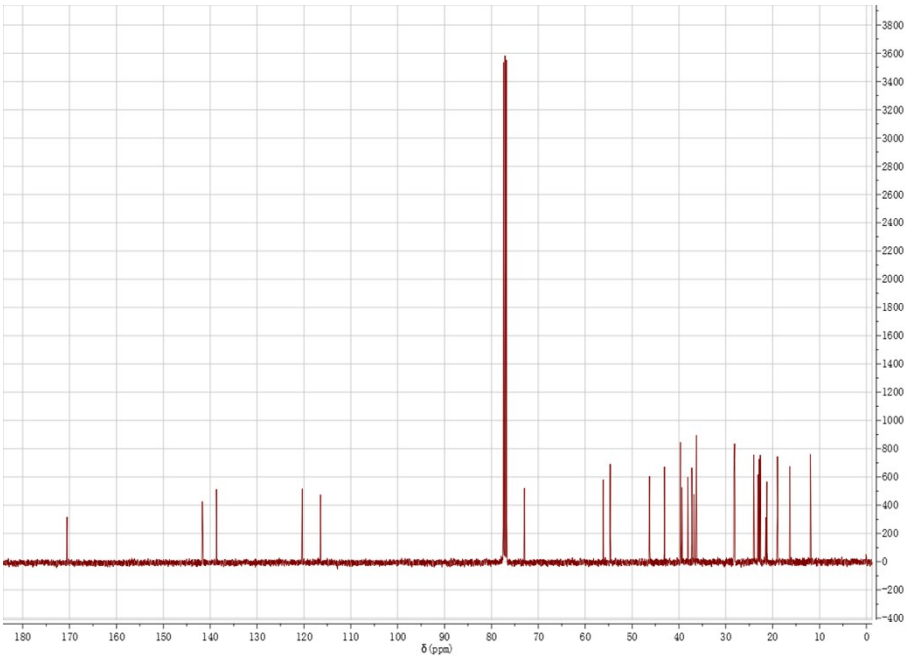


Figure S8. ^{13}C NMR spectrum of **2**.

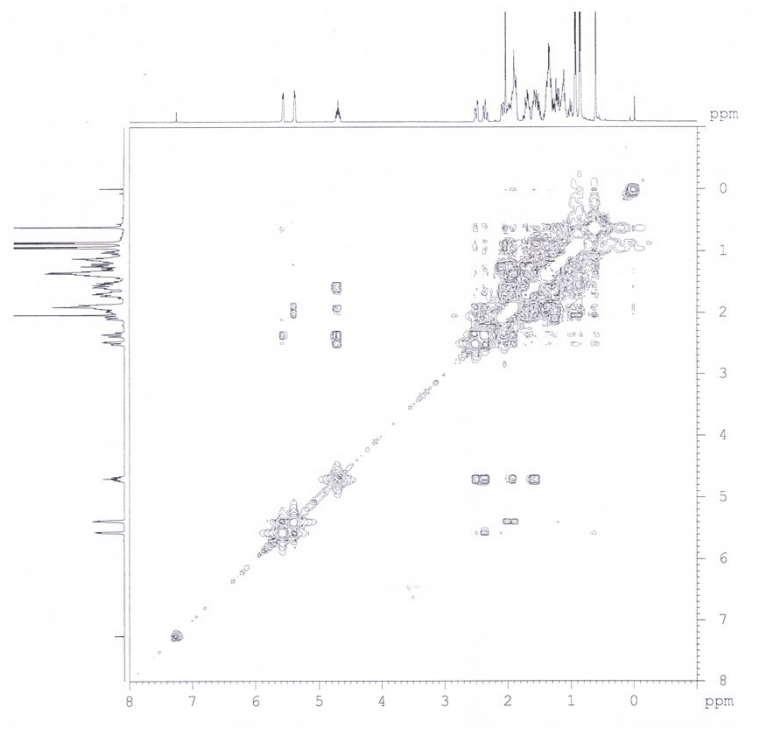


Figure S9. COSY spectrum of **2**.

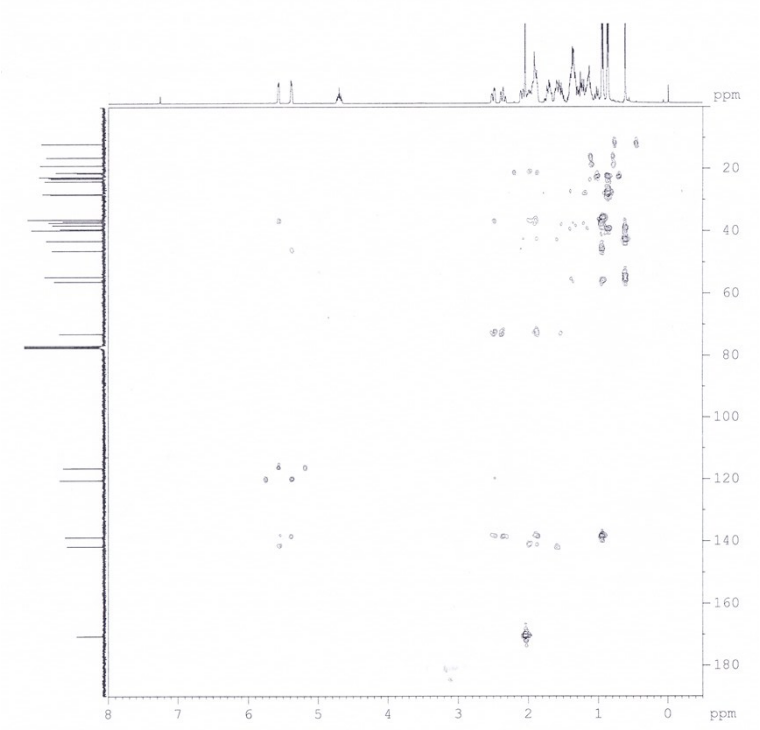


Figure S10. HMBC spectrum of **2**.

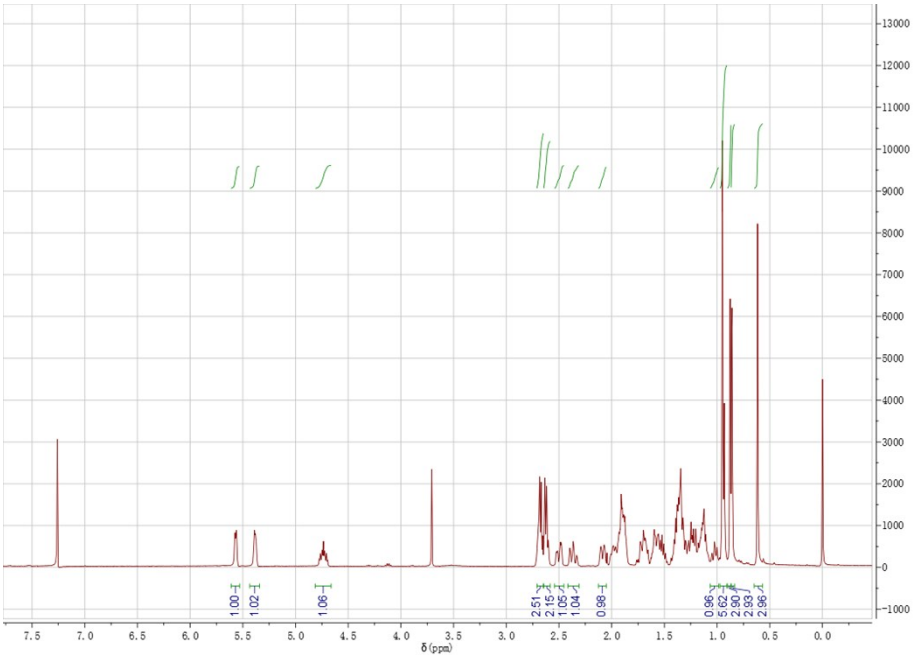


Figure S11. ^{1}H NMR spectrum of **3**.

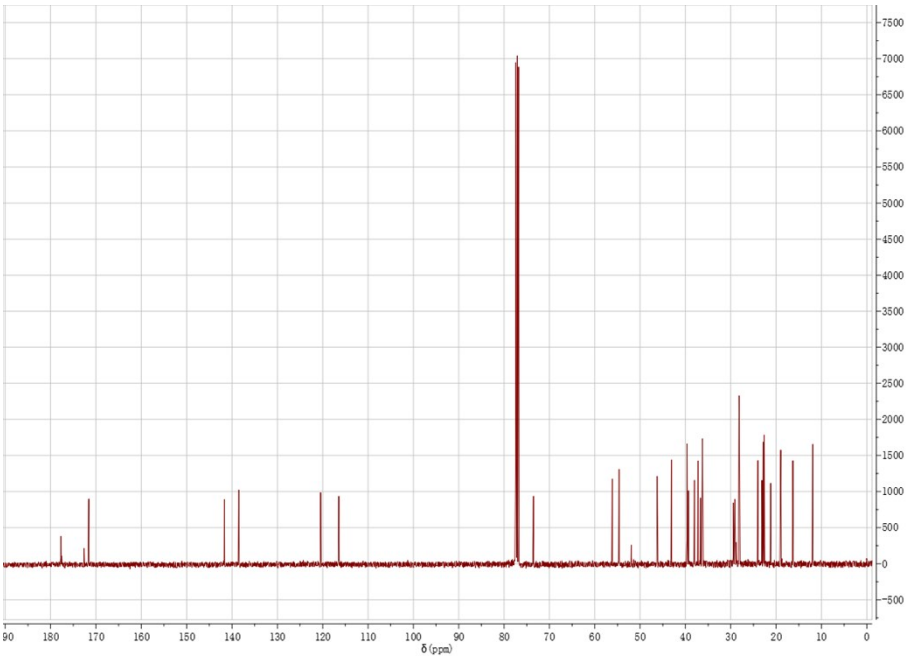


Figure S12. ^{13}C NMR spectrum of **3**.

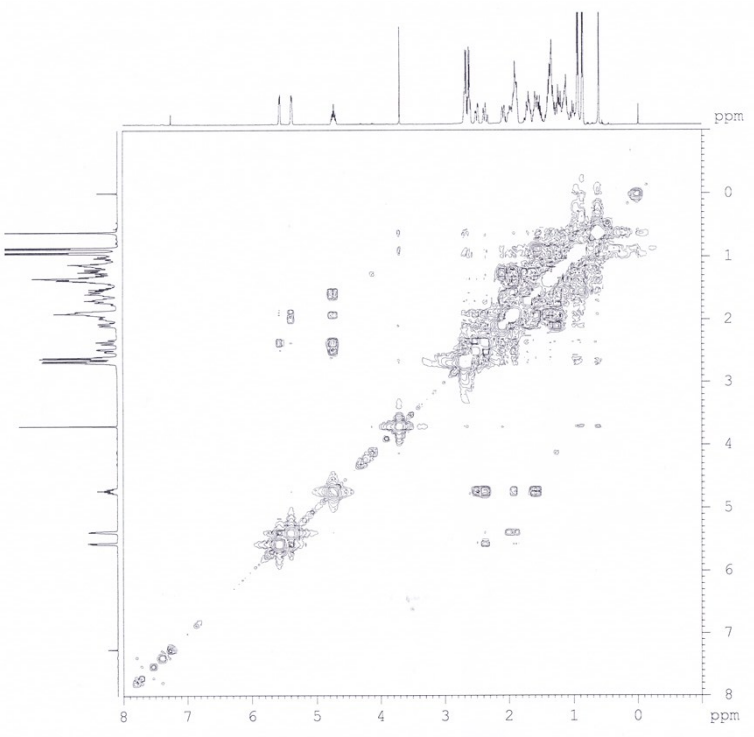


Figure S13. COSY spectrum of **3**.

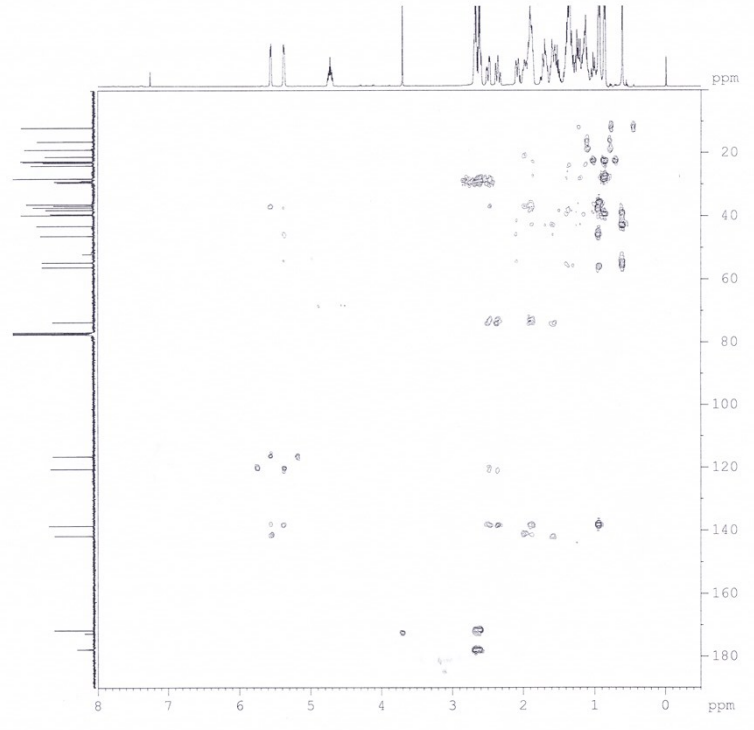


Figure S14. HMBC spectrum of **3**.

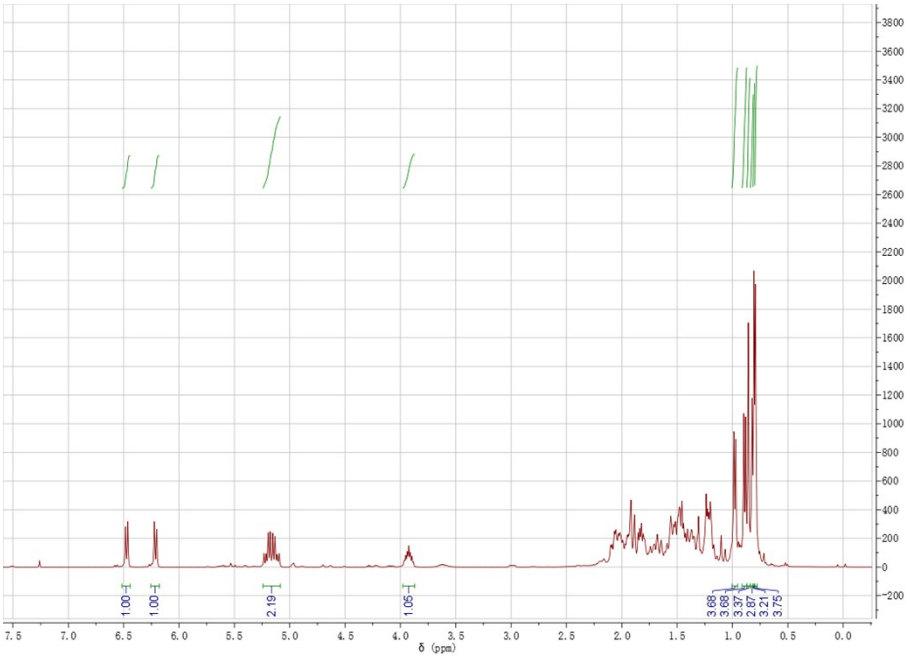


Figure S15. ^{1}H NMR spectrum of EEP.

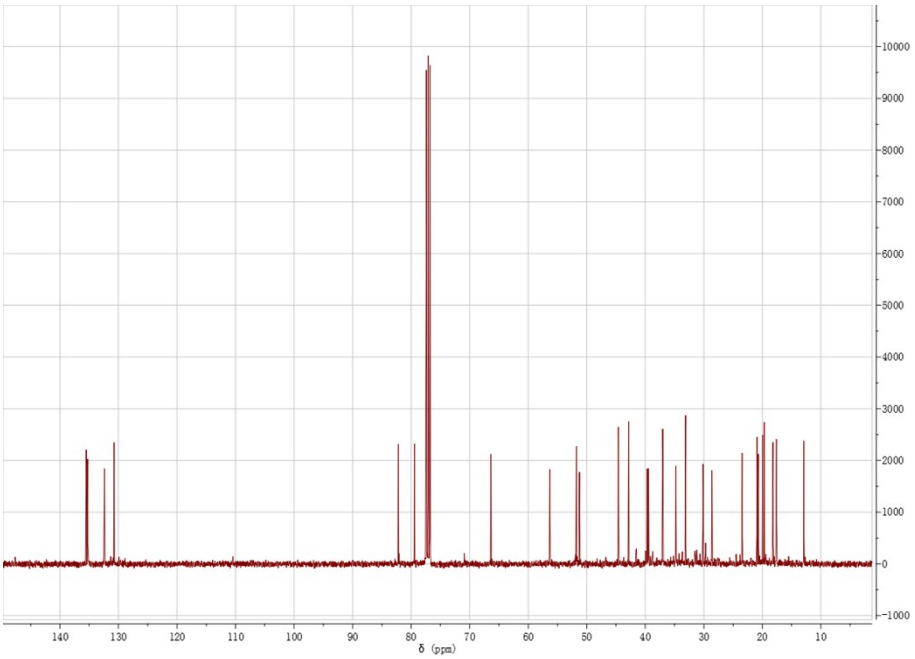


Figure S16. ^{13}C NMR spectrum of EEP.

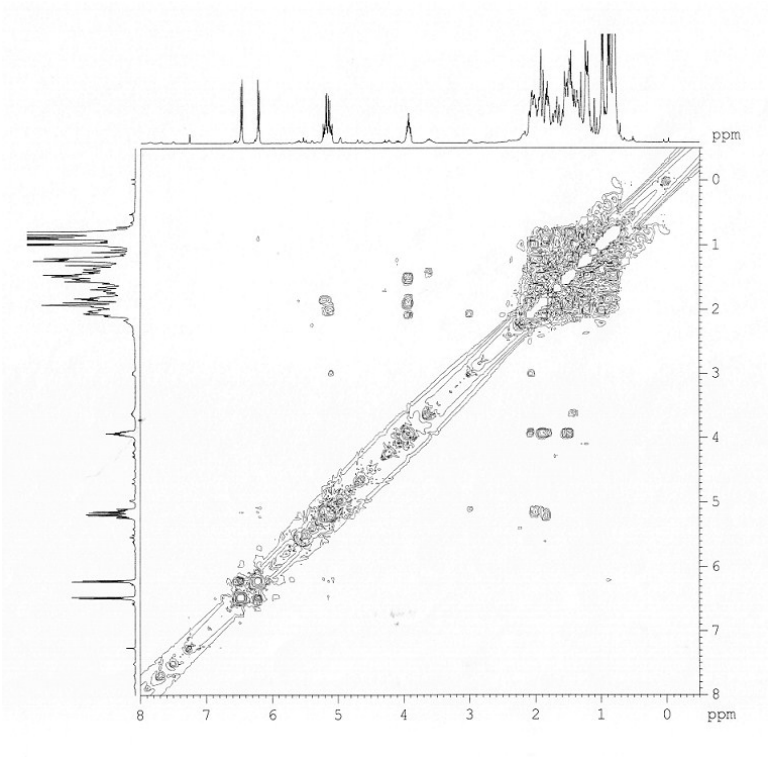


Figure S17. COSY spectrum of EEP.

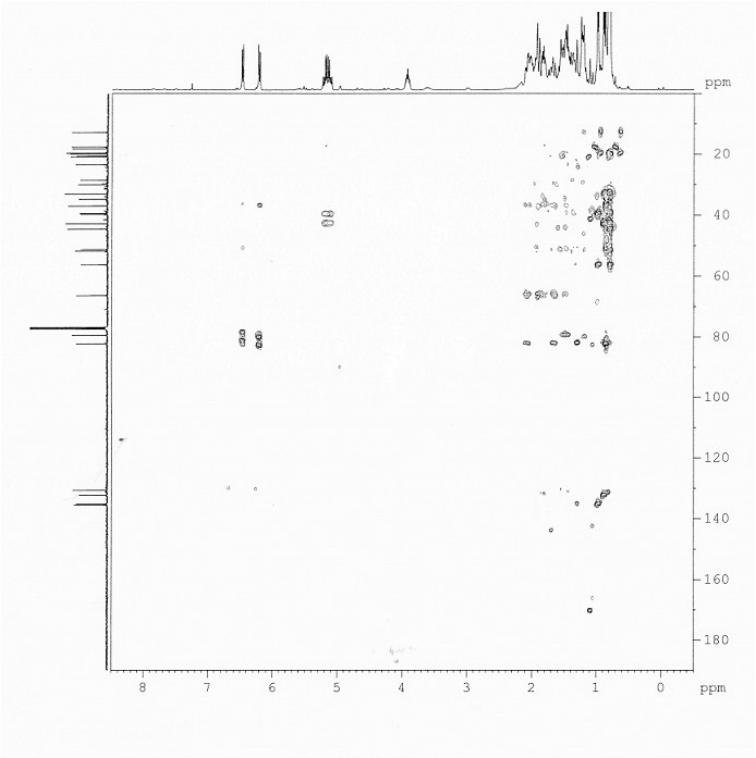


Figure S18. HMBC spectrum of EEP.

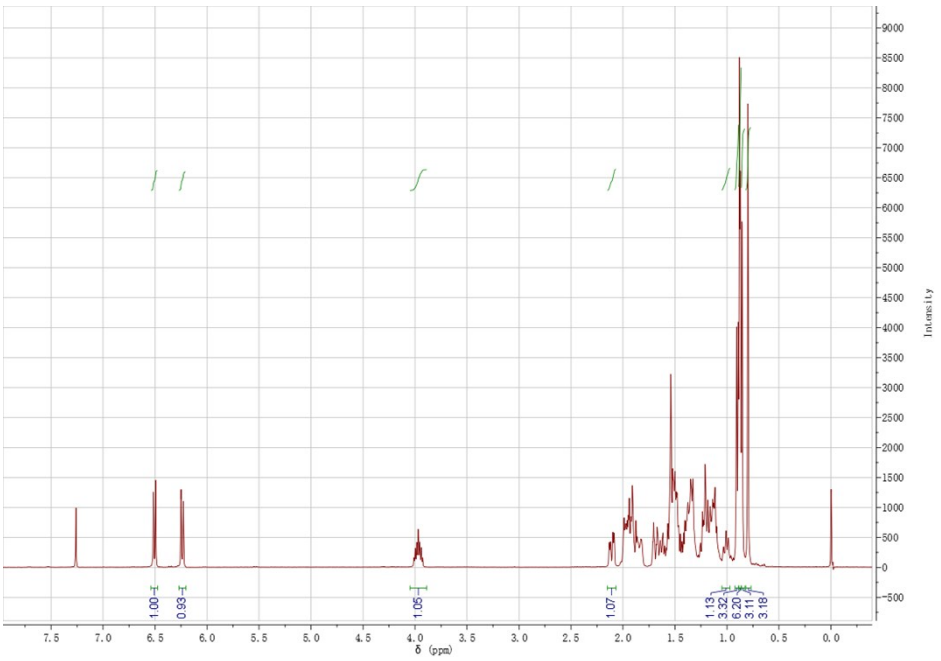


Figure S19. ^{1}H NMR spectrum of **1′**.

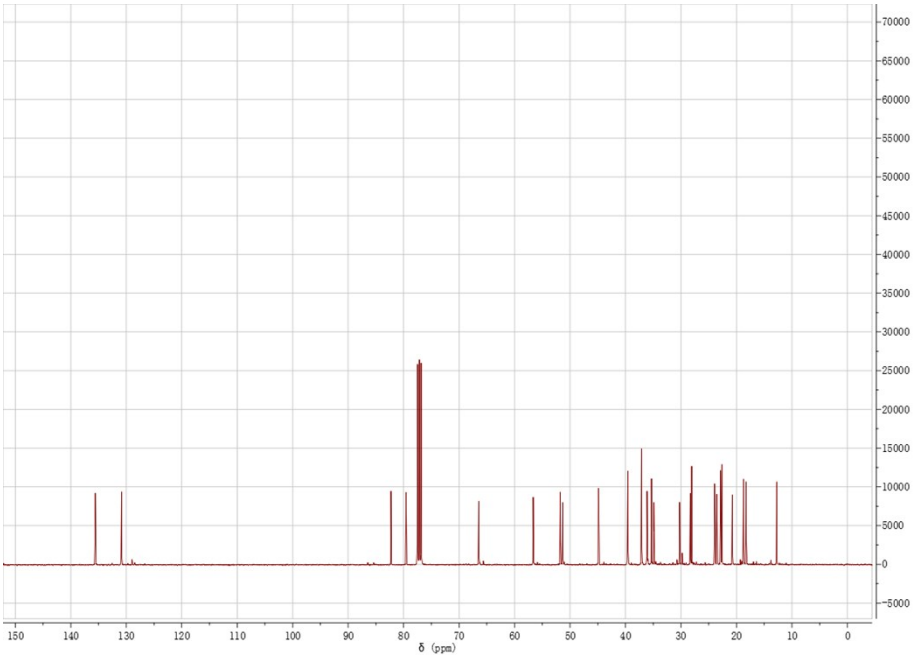


Figure S20. ^{13}C NMR spectrum of **1′**.

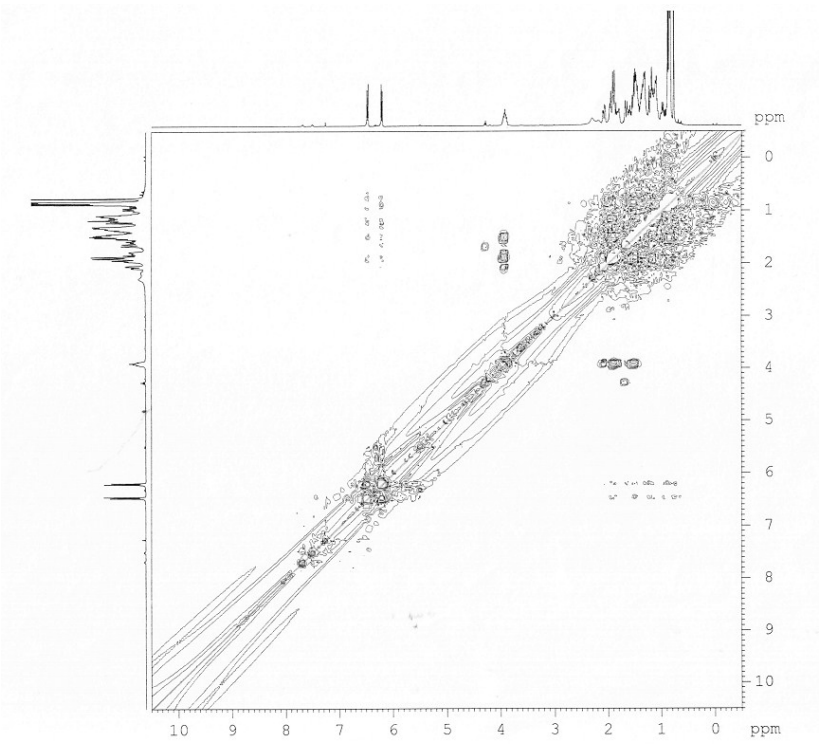


Figure S21. COSY spectrum of **1′.**

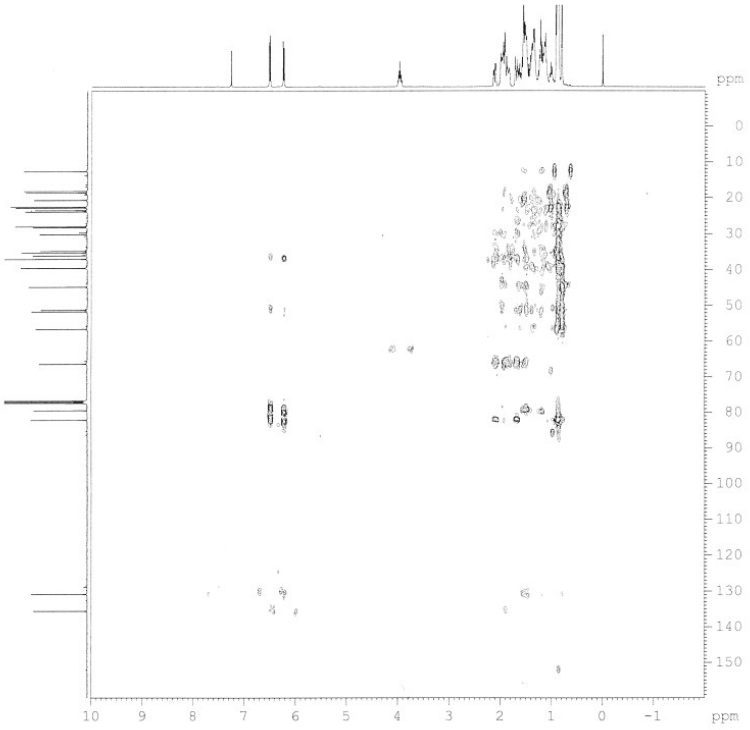


Figure S22. HMBC spectrum of **1′**.

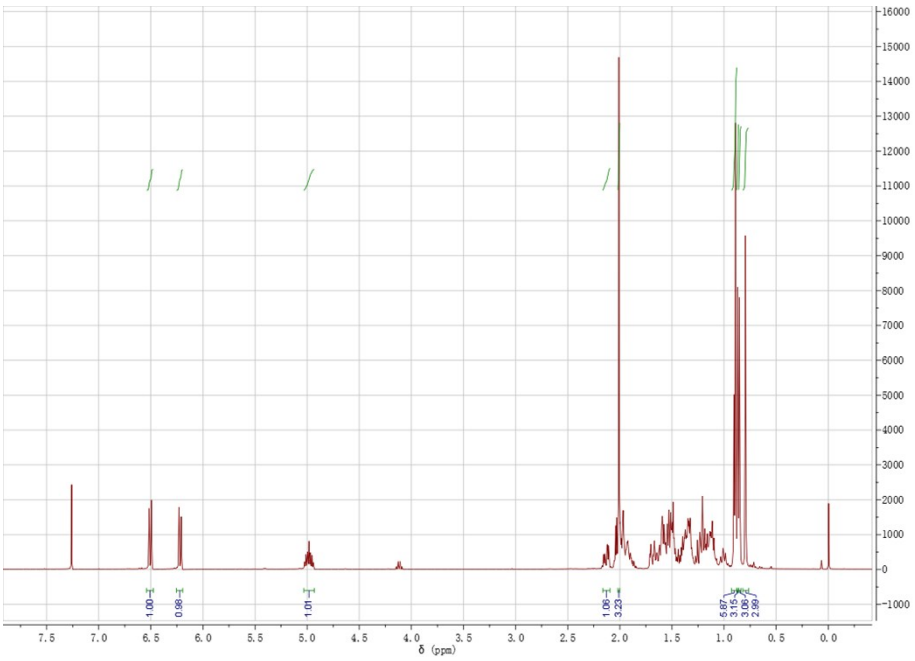


Figure S23. ^{1}H NMR spectrum of **2´**.

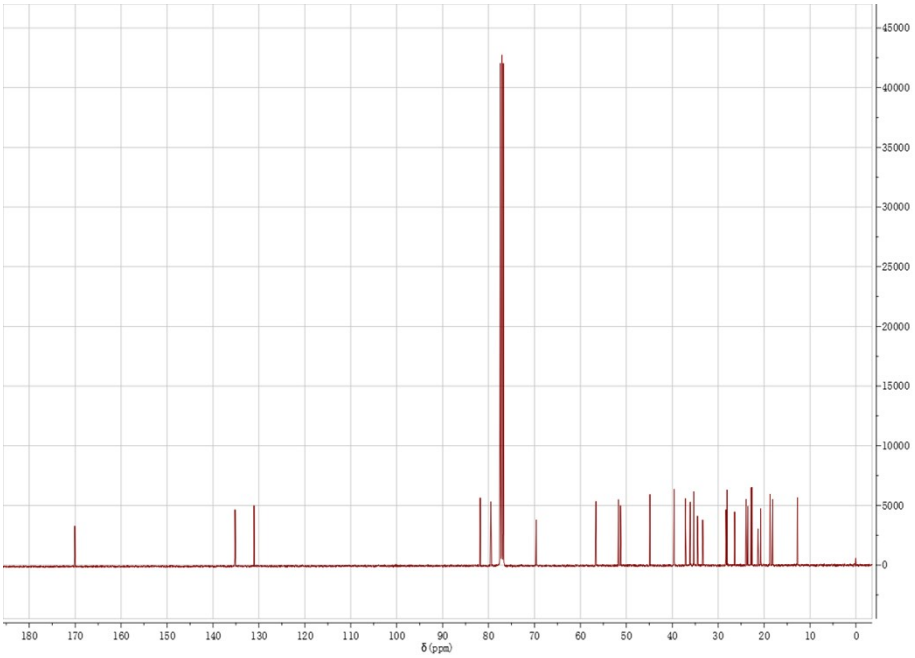


Figure S24. ^{13}C NMR spectrum of **2´**.

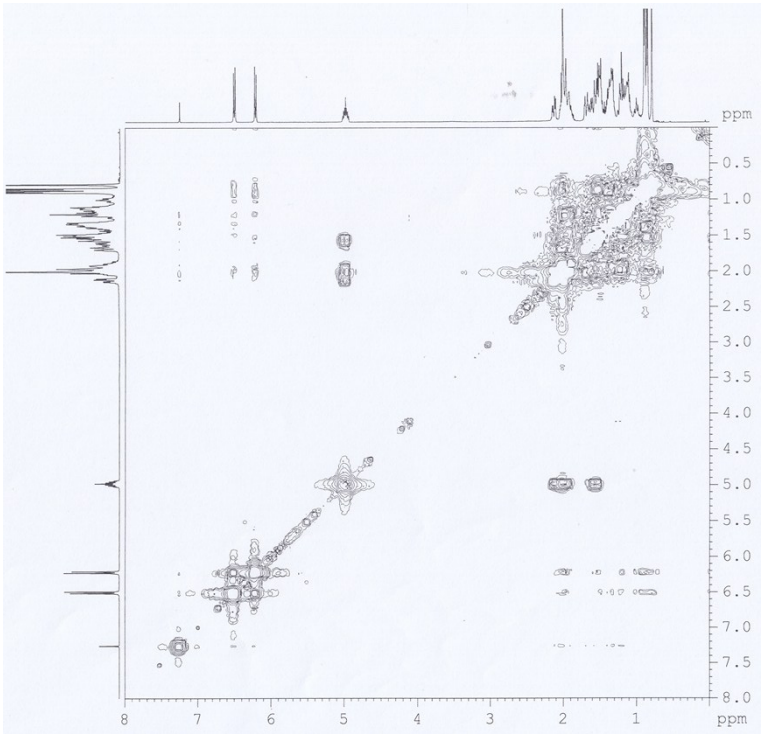


Figure S25. COSY spectrum of **2′.**

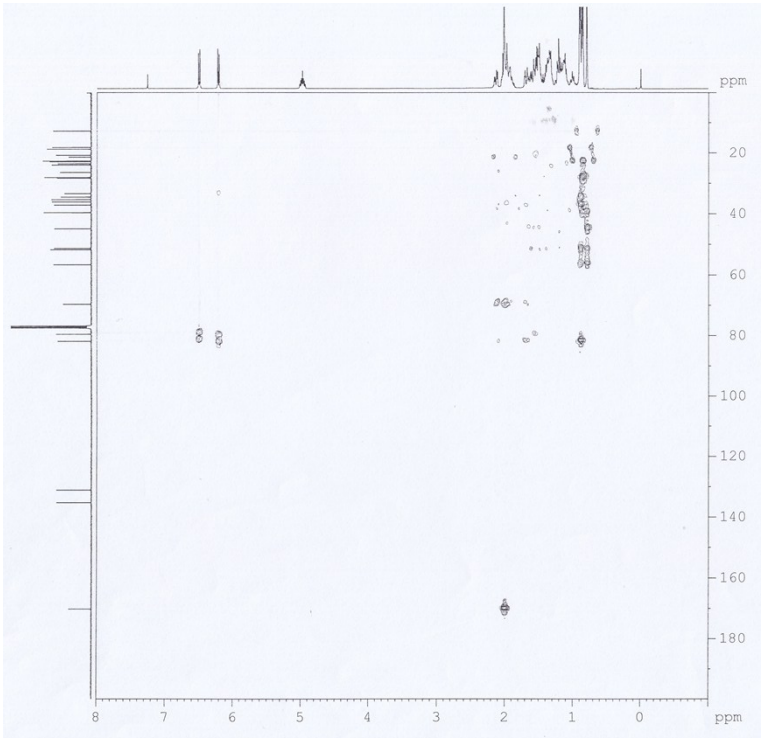


Figure S26. HMBC spectrum of **2′**.

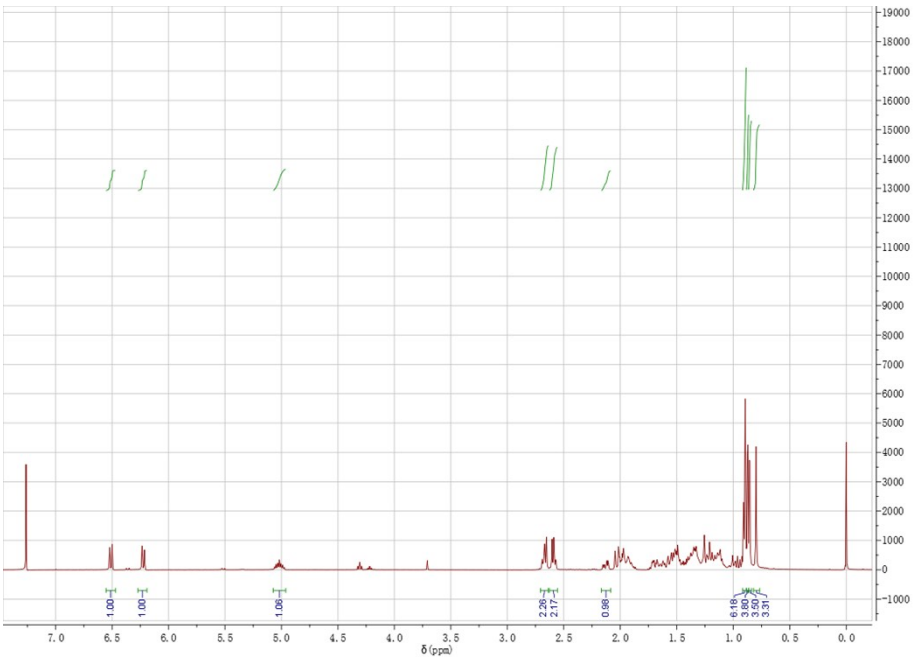


Figure S27. ^{1}H NMR spectrum of **3′**.

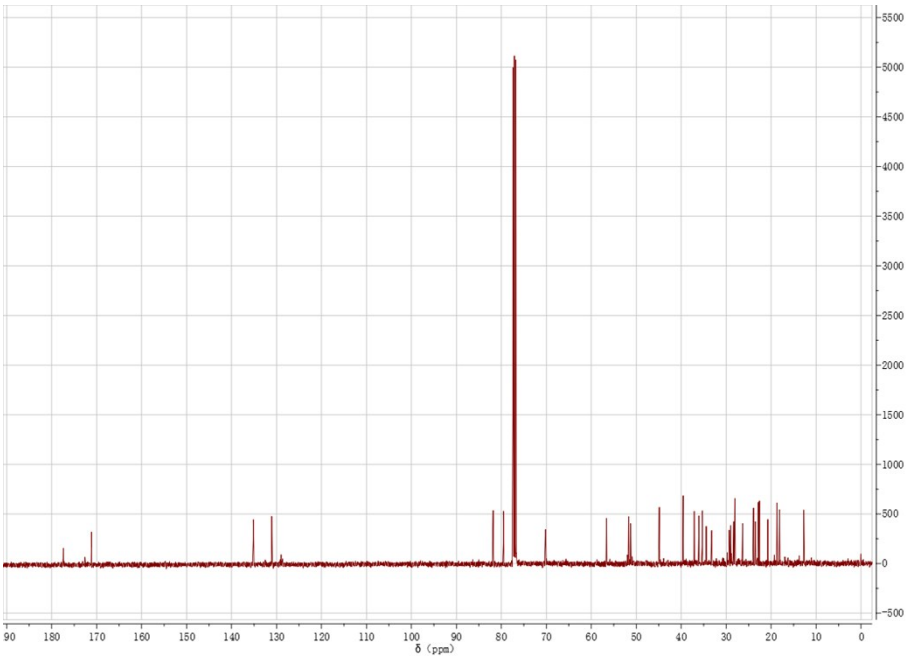


Figure S28. ^{13}C NMR spectrum of **3′**.

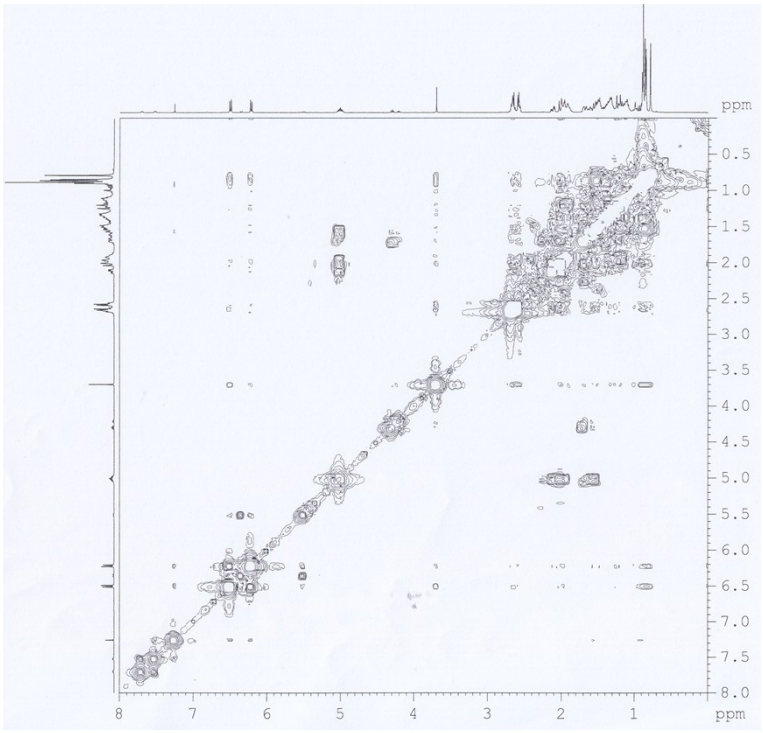


Figure S29. COSY spectrum of **3′.**

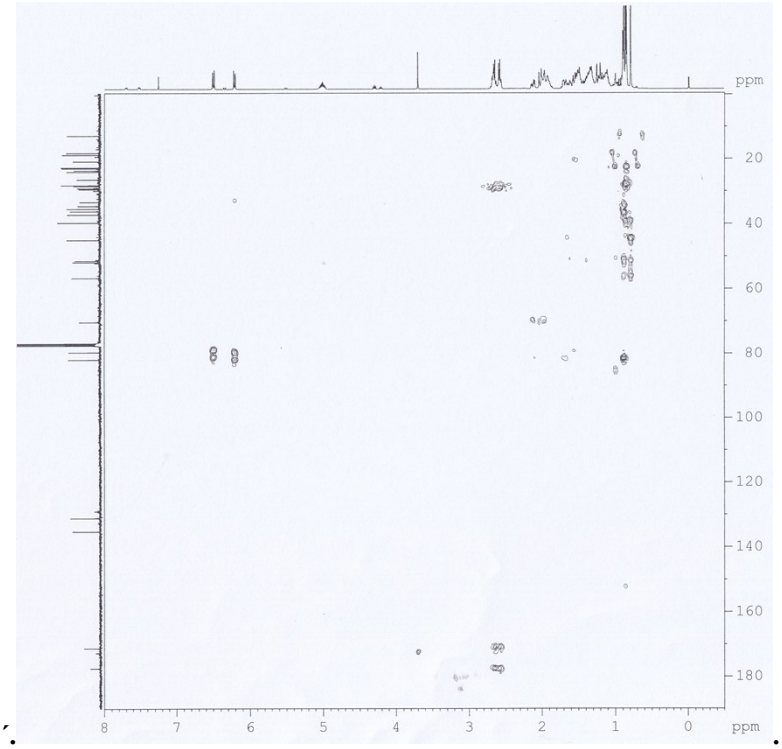


Figure S30. HMBC spectrum of **3′**.