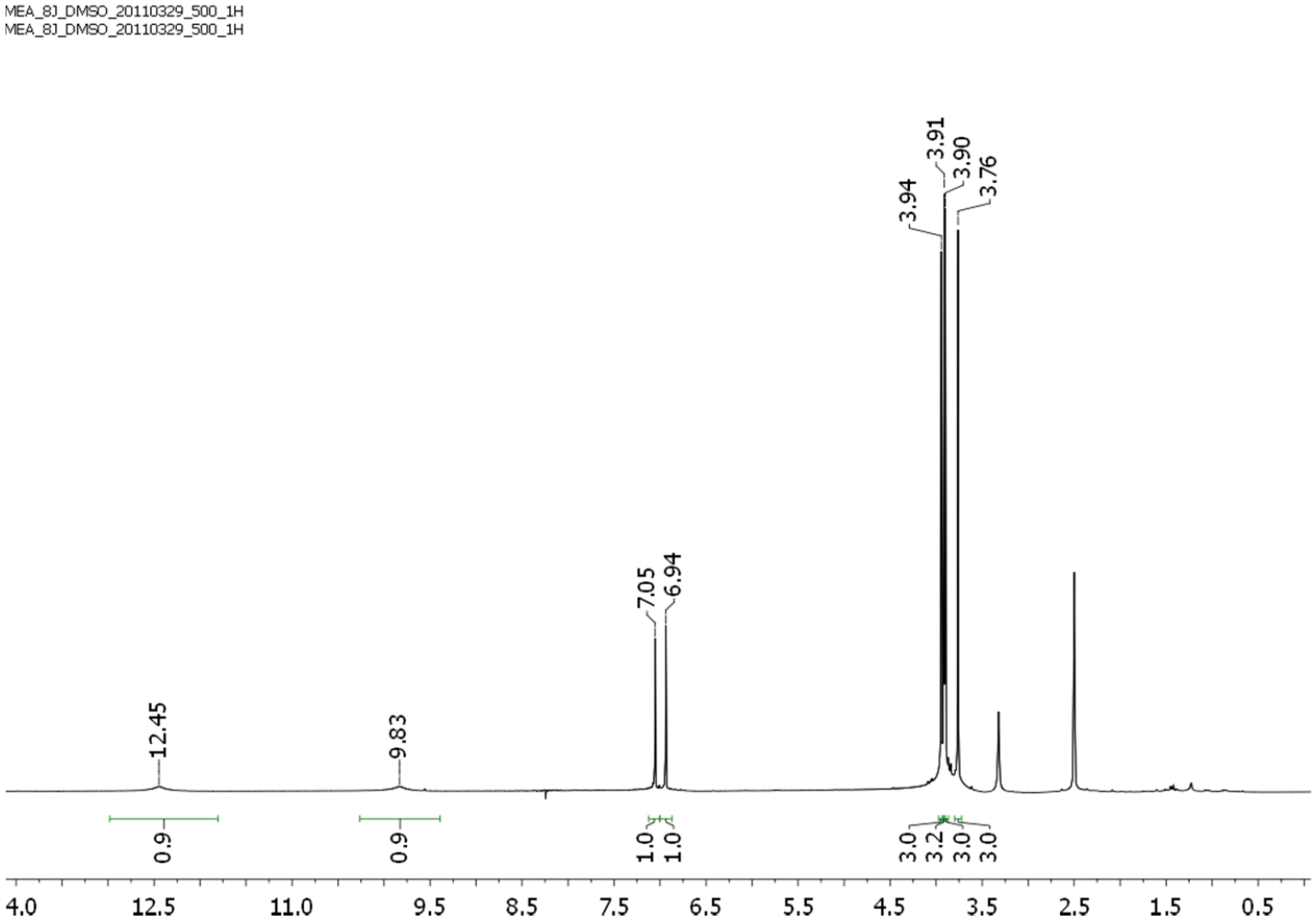
SUPPORTING INFORMATION

Busseihydroquinones A-D from the Roots of *Pentas bussei*

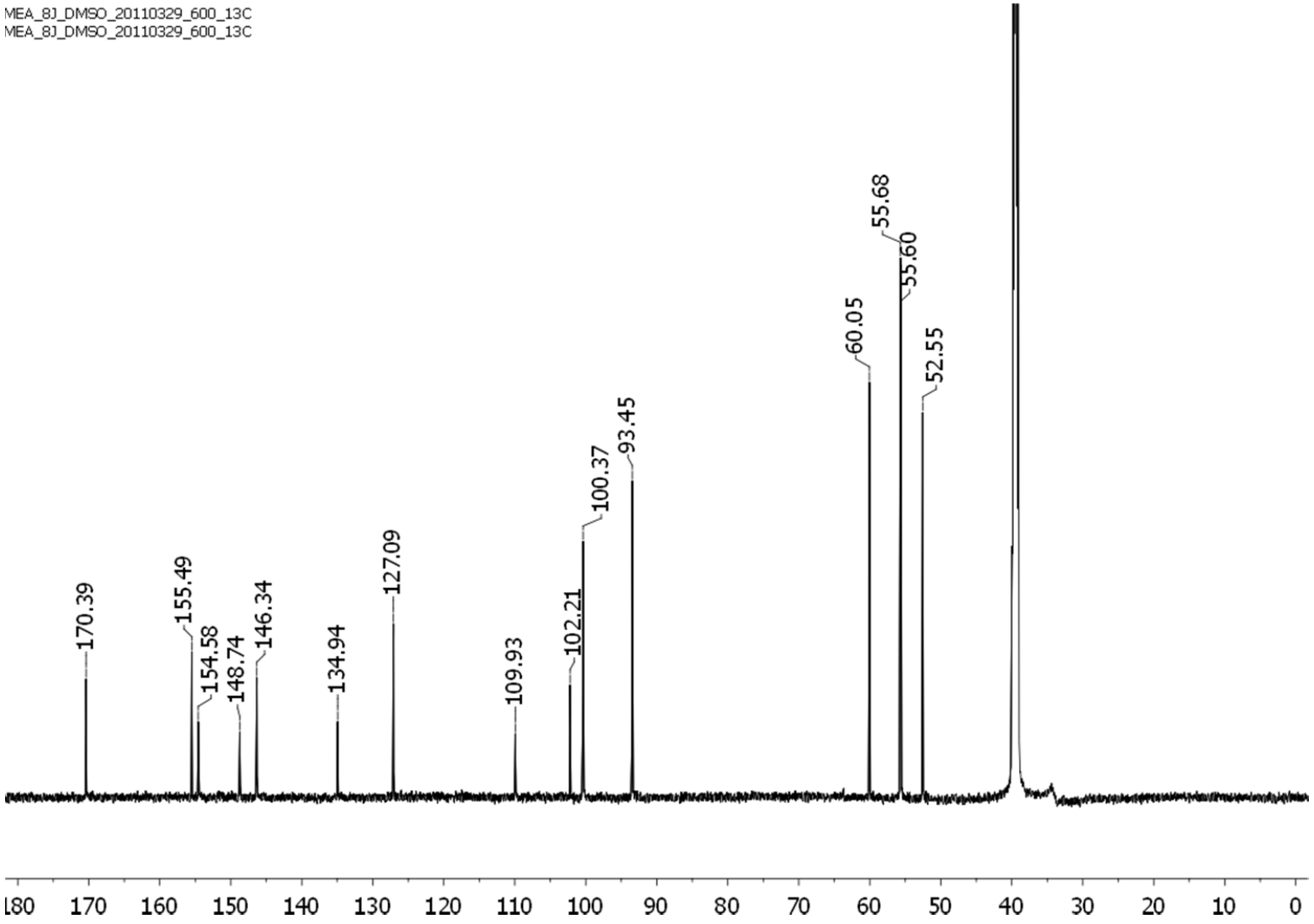
Milkyas Endale, Annabel Ekberg, Hoseah M. Akala, John Patrick Alao, Per Sunnerhagen, Abiy Yenesew,* Máté Erdelyi*

Table of contents

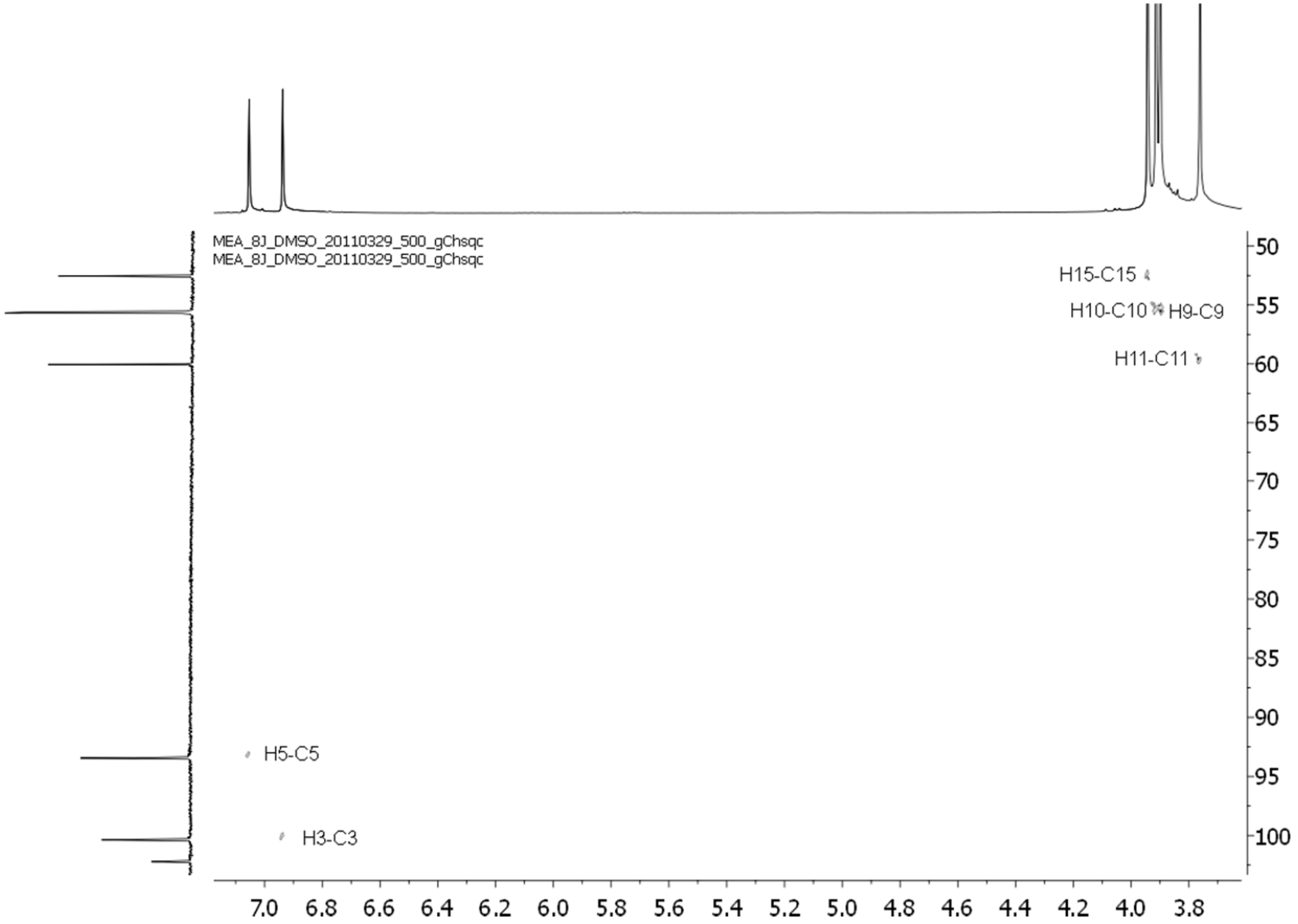
S2 NMR, MS and UV spectra and LC-MS/UV chromatogram of compound **1**.
S9 NMR, MS and UV spectra and LC-MS/UV chromatogram of compound **2**.
S17 NMR, MS and UV spectra and LC-MS/UV chromatogram of compound **3**.
S26 NMR, MS and UV spectra and LC-MS/UV chromatogram of compound **4**.
S34 NMR, MS and UV spectra and LC-MS/UV chromatogram of compound **5**.
S41 CD spectra.
S45 Cytotoxicity data.



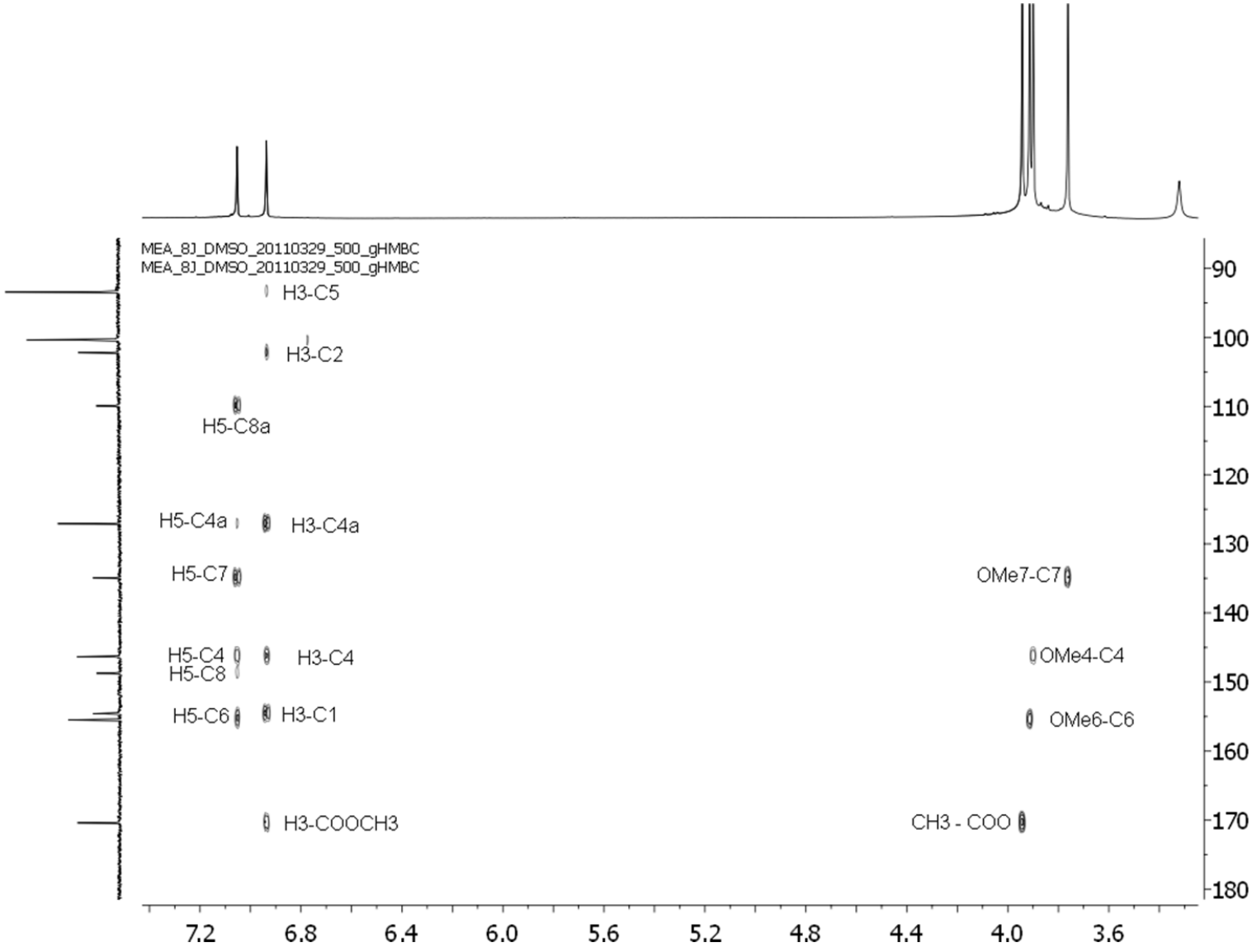
FigS1. ^{1}H NMR spectrum of compound **1** (DMSO-d_6, 500.30 MHz, 25˚C). Structure is shown in Fig 1, assignation is given in Table 1.



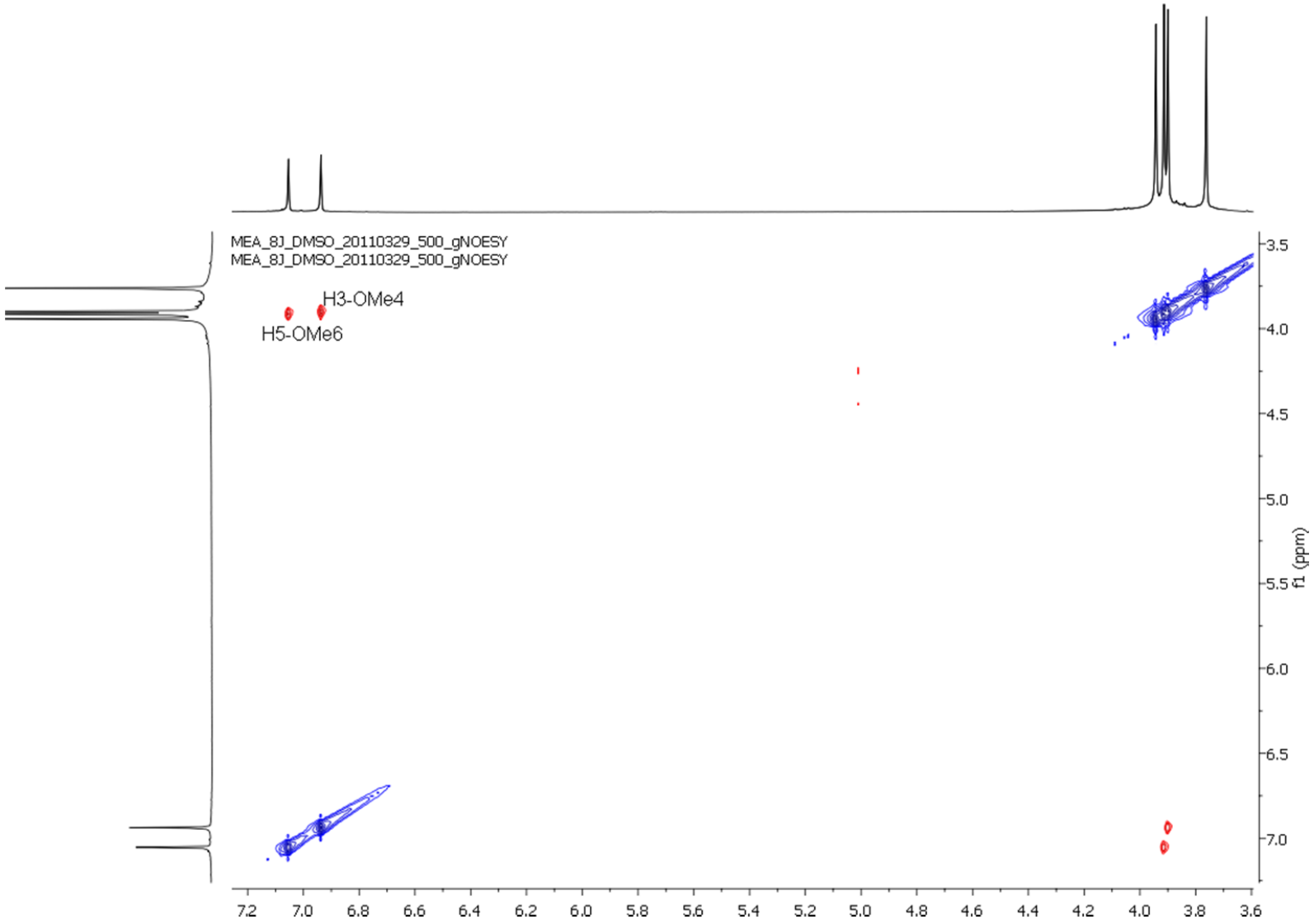
FigS2. ^{13}C NMR spectrum of compound **1** (DMSO-d_6, 150.84 MHz, 25˚C). Structure is shown in Fig 1, assignation is given in Table 1.



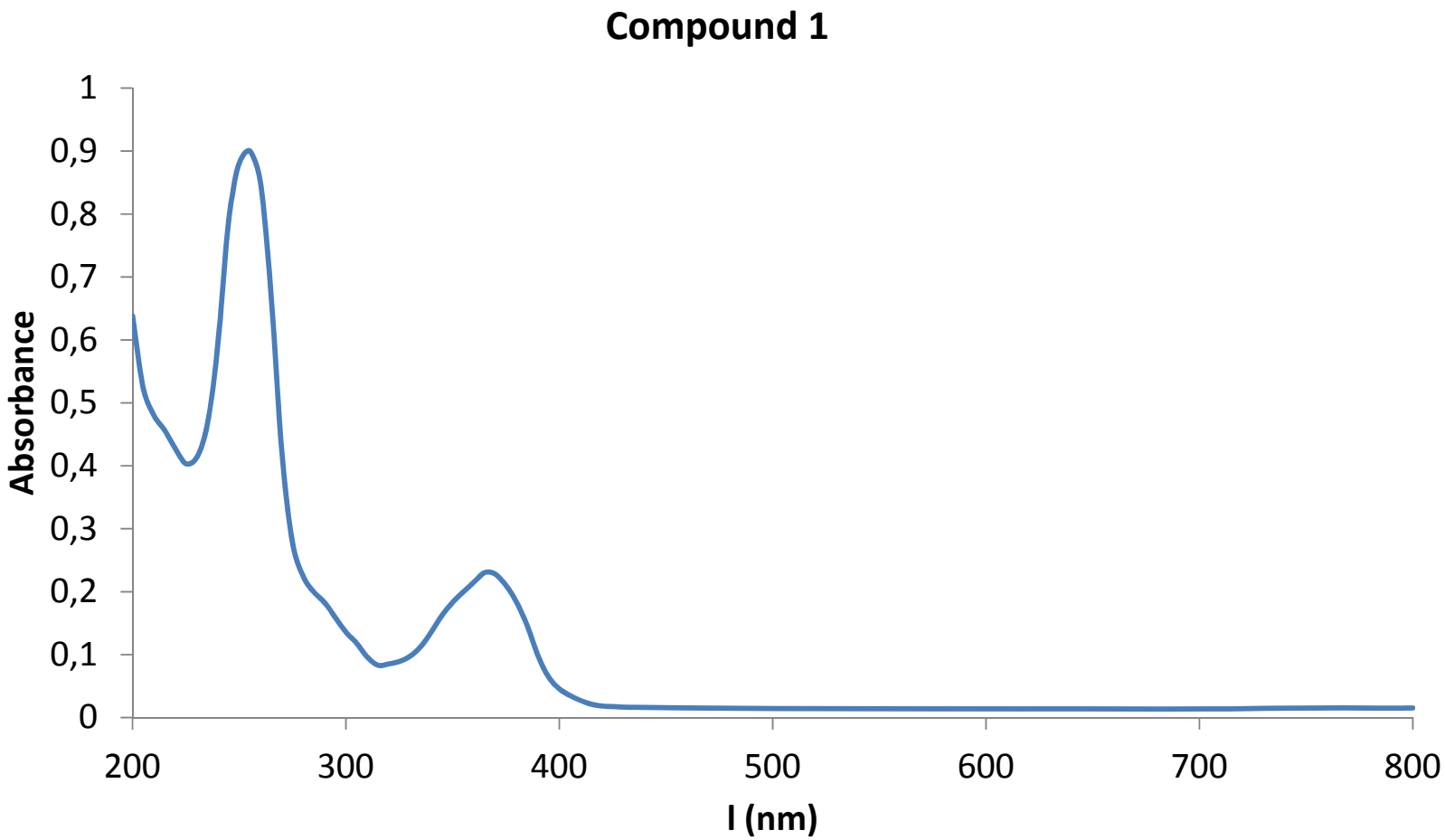
FigS3. $^{1}H,^{13}C$ HSQC NMR spectrum of compound **1** (DMSO-d_6, 500.30, 125.80 MHz, 25˚C); assignation is given in Table 1.



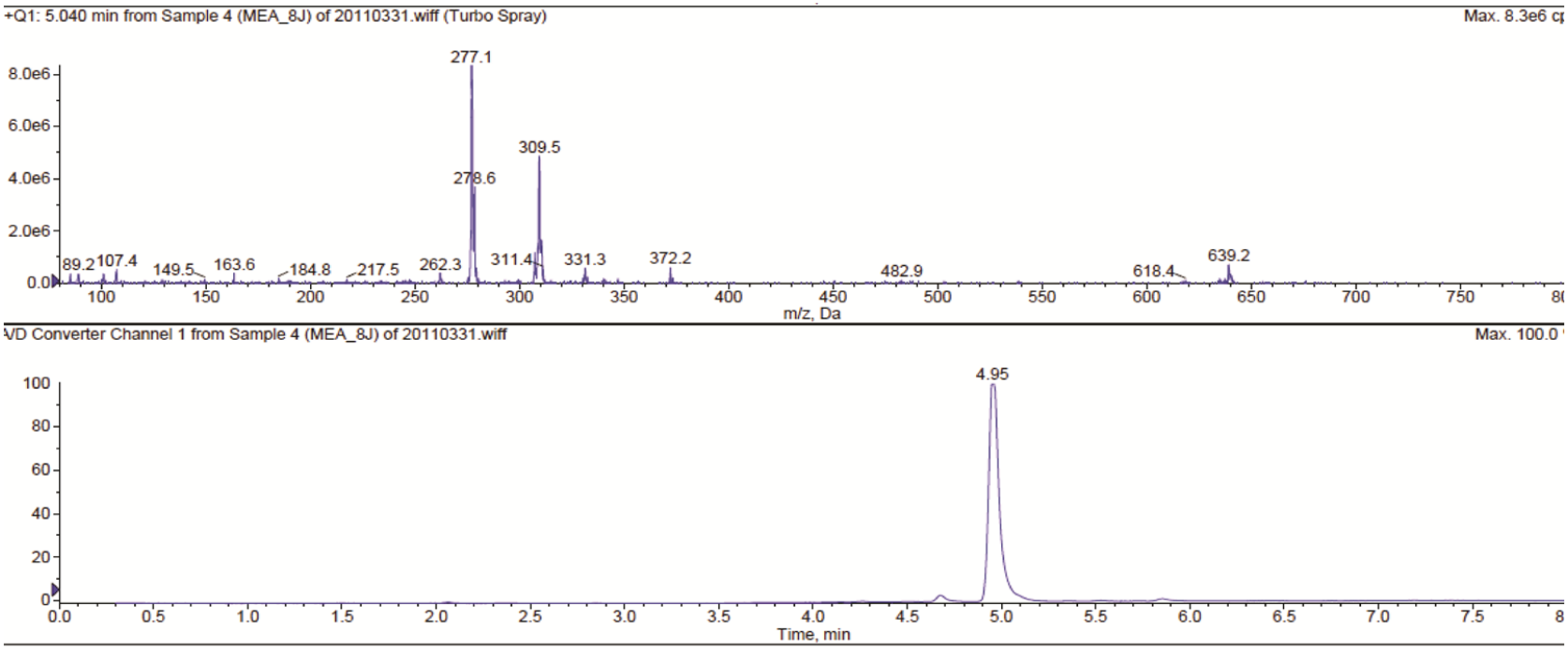
FigS4. $^{1}H,^{13}C$ HMBC NMR spectrum of compound **1** (DMSO-d_6, 500.30, 125.80 MHz, 25˚C); assignation is given in Table 1.



FigS5. NOESY spectrum of compound **1** (mix=700 ms, DMSO-d_6, 500.30, 125.80 MHz, 25˚C); assignation is given in Table 1.

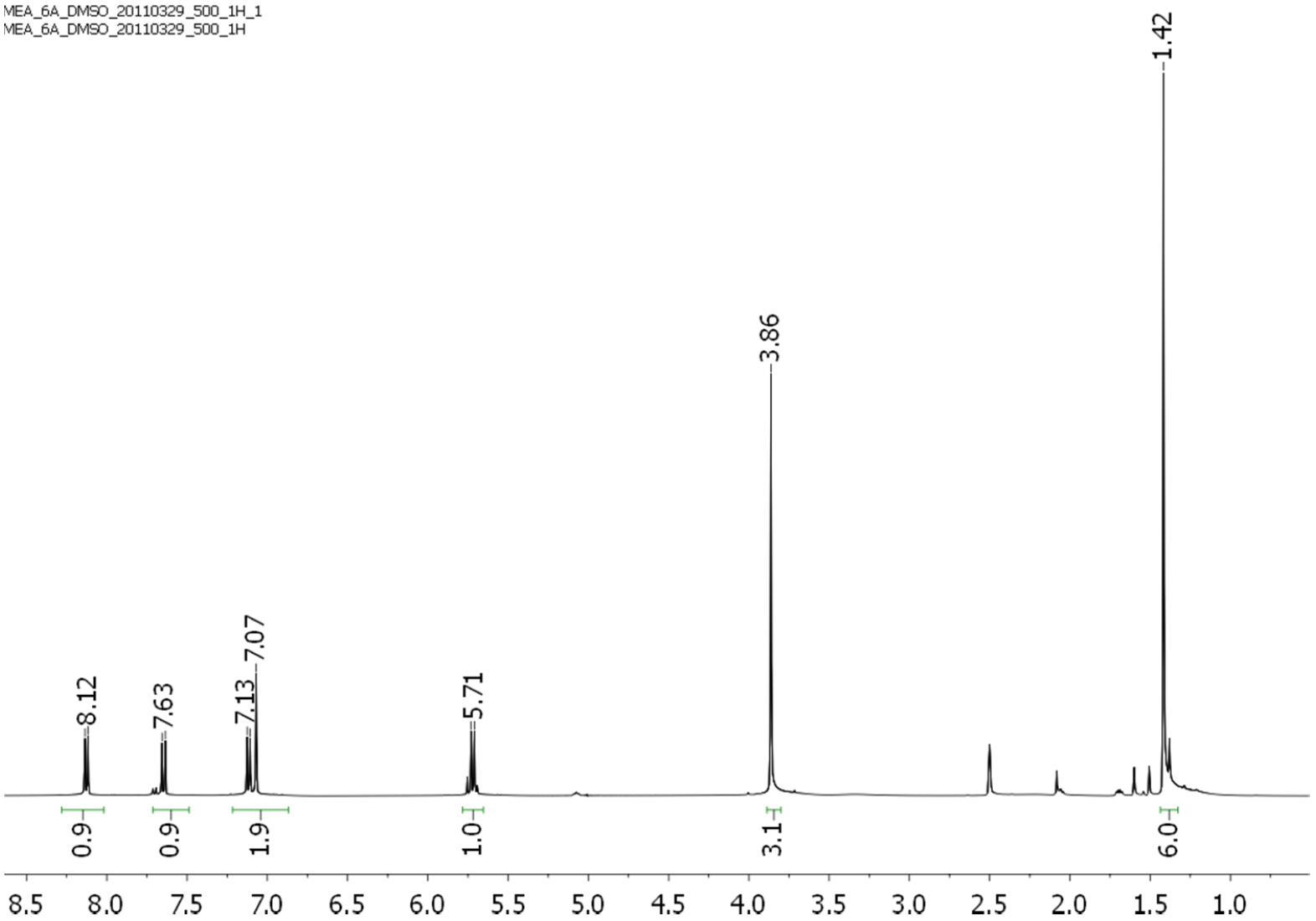


FigS6. The UV spectrum of compound **1** (Methanol, 25˚C). Absorption maxima were observed at 365 and 250 nm.

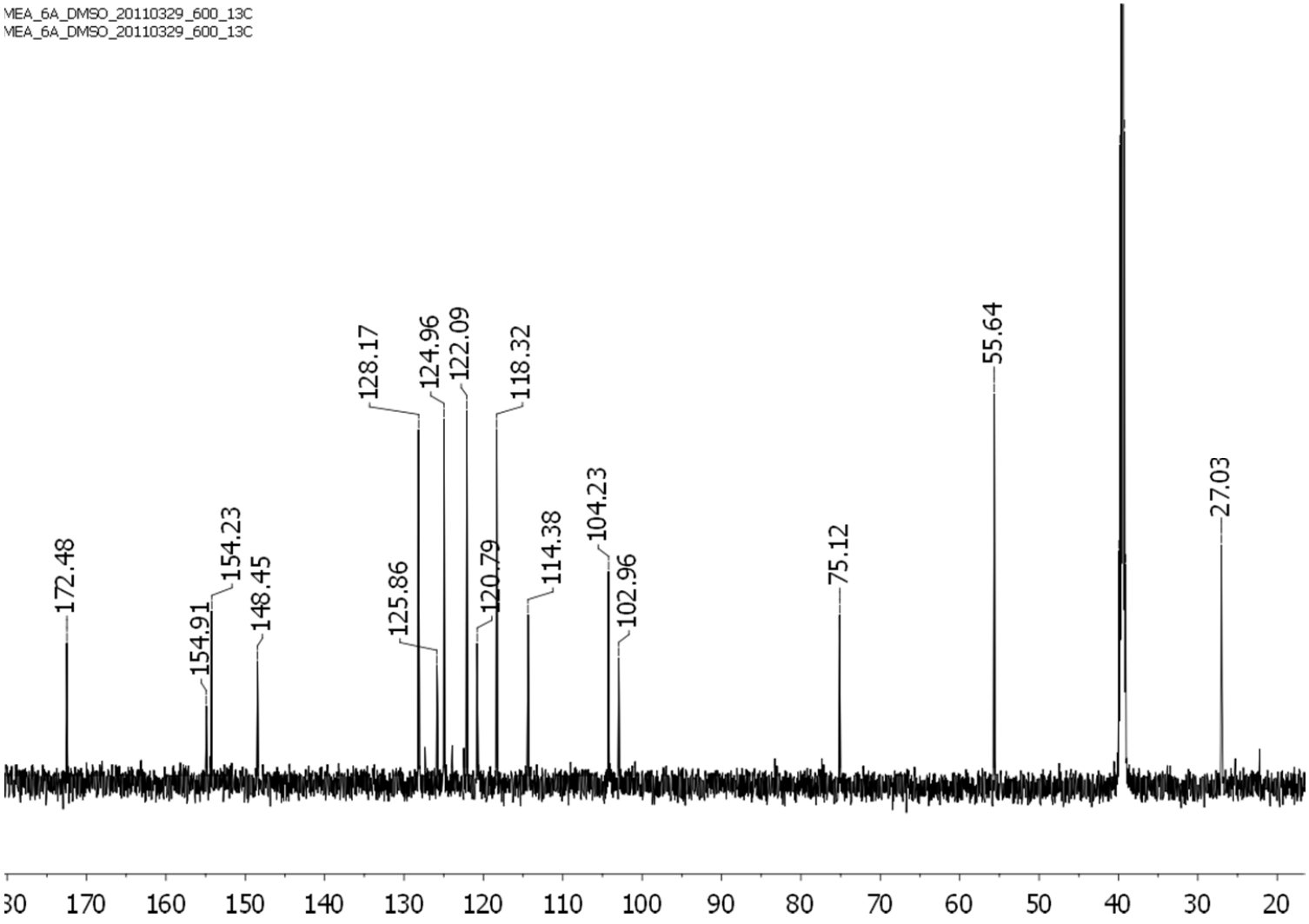


FigS7. The HPLC-UV/MS chromatogram of compound **1**.

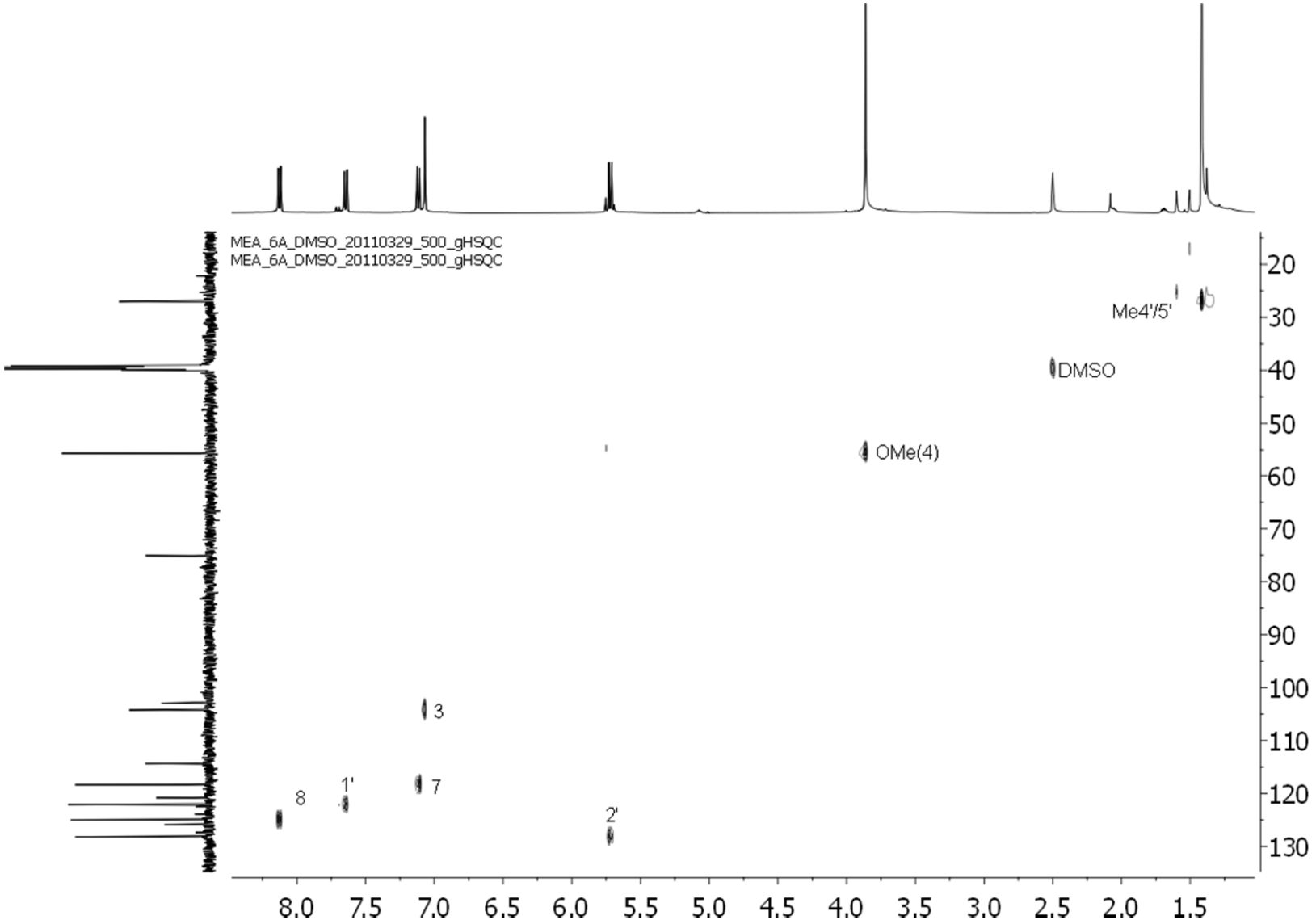
HRMS: m/z 309.2300 $[M+H]^+$, calculated for $C_{15}H_{16}O_7$ $+H^+$: 309.2379.



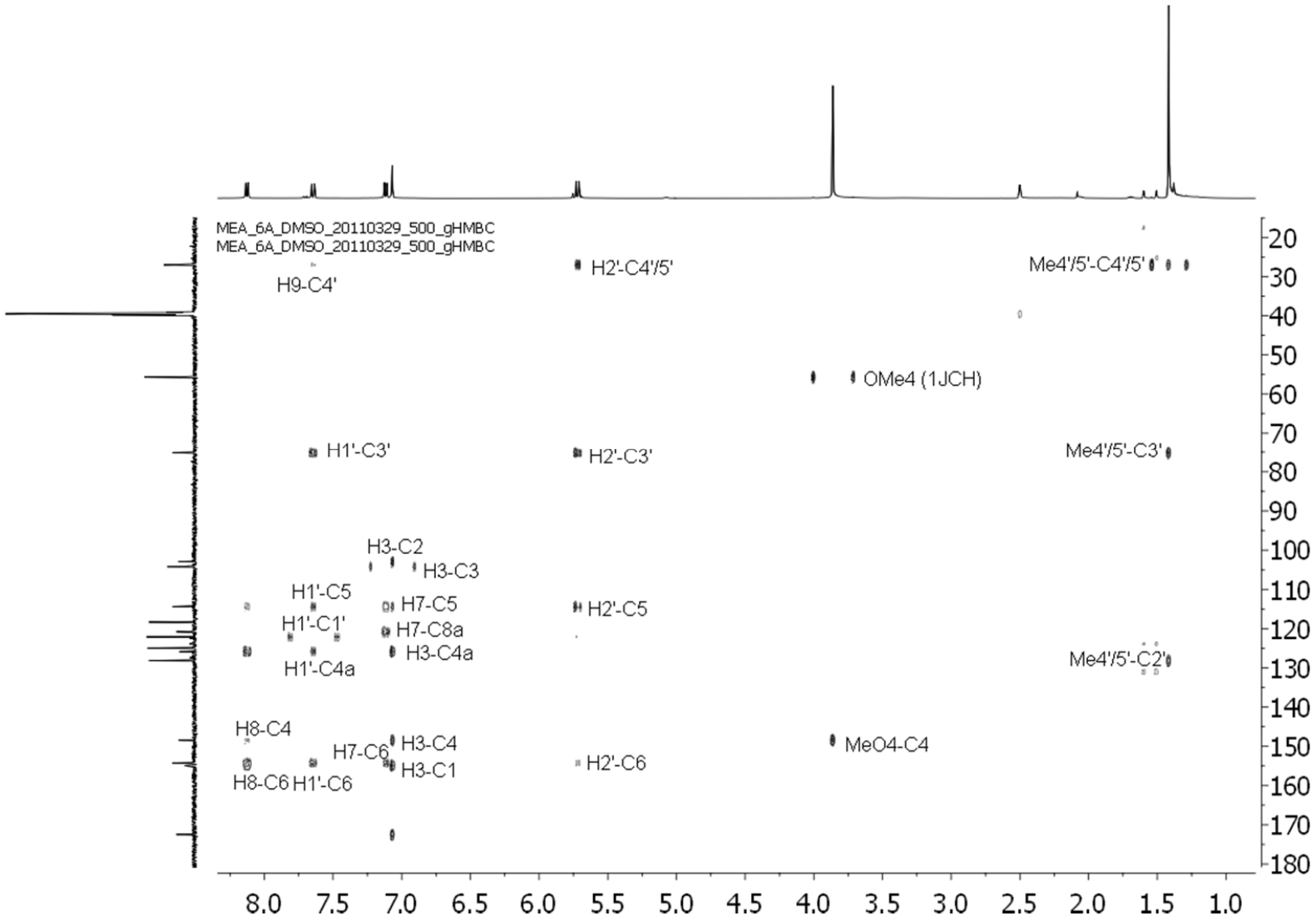
FigS8. ^{1}H NMR spectrum of compound **2** (DMSO-d_6, 500.30 MHz, 25˚C). Assignation is given in Table 1, structure is shown in figure 1.



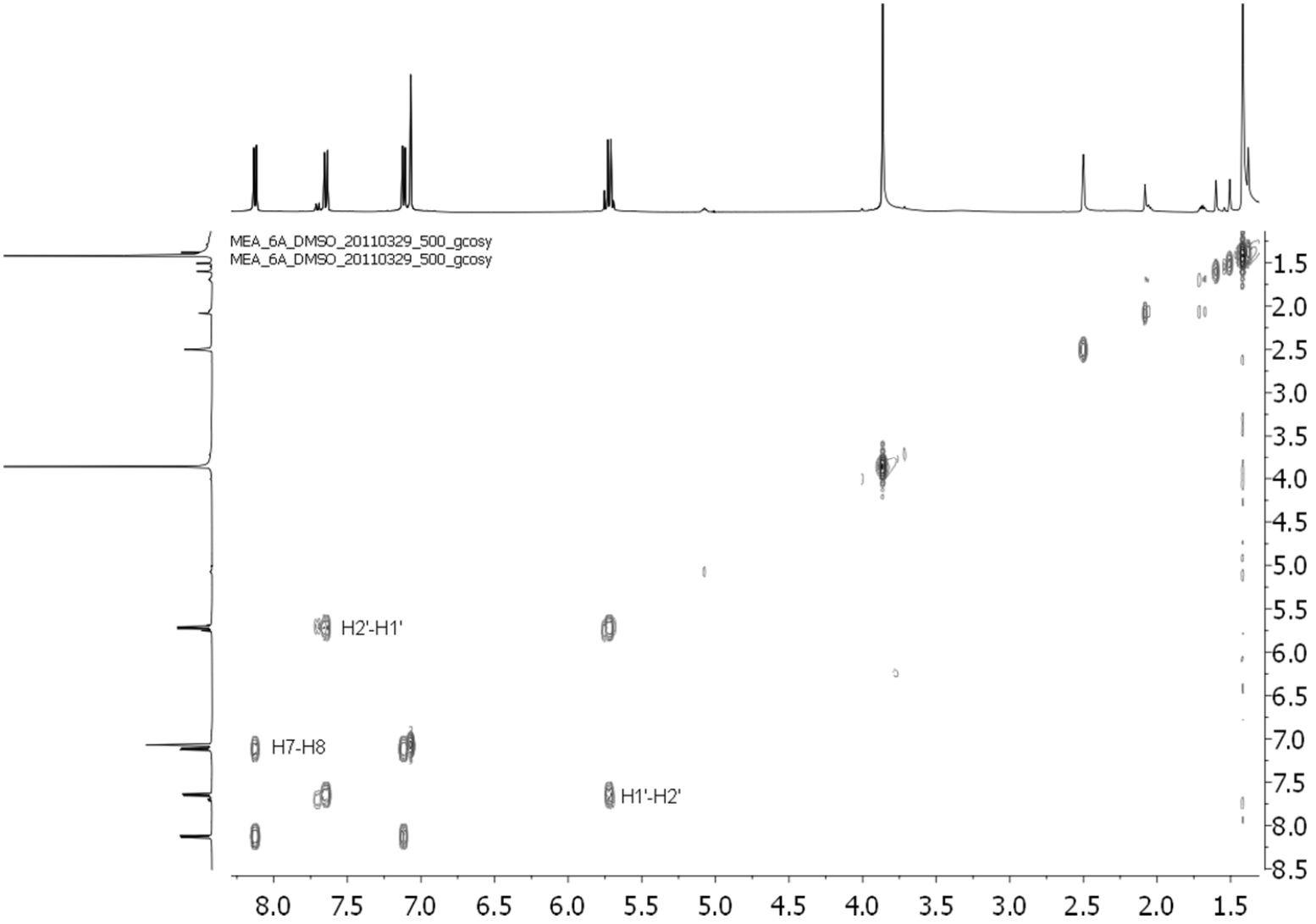
FigS9. ^{13}C NMR spectrum of compound **2** (DMSO-d_6, 150.84 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Figure 1.



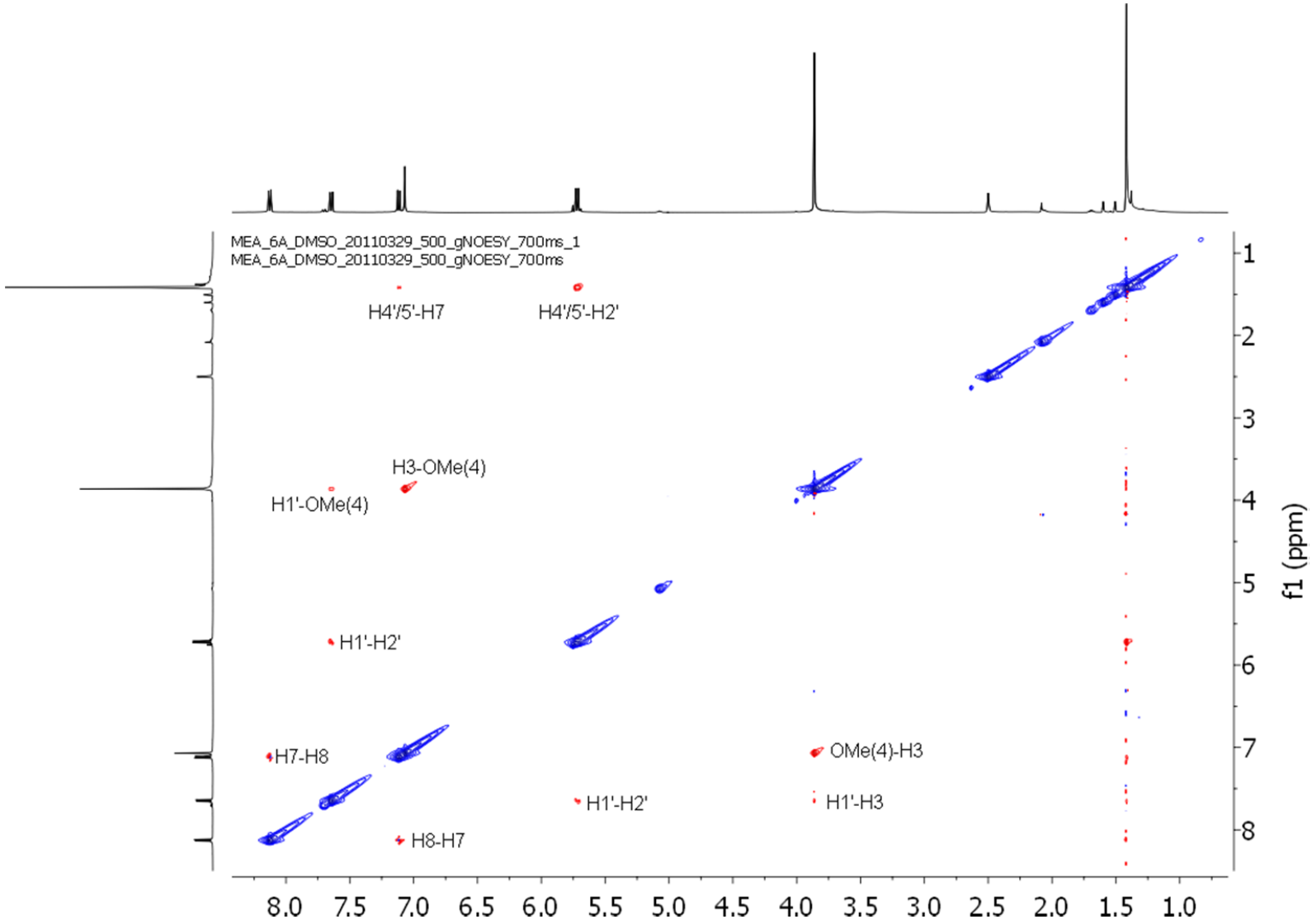
FigS10. $^{1}H,^{13}C$ HSQC NMR spectrum of compound **2** (DMSO-d_6, 500.30, 125.80 MHz, 25˚C); assignation is given in Table 1.



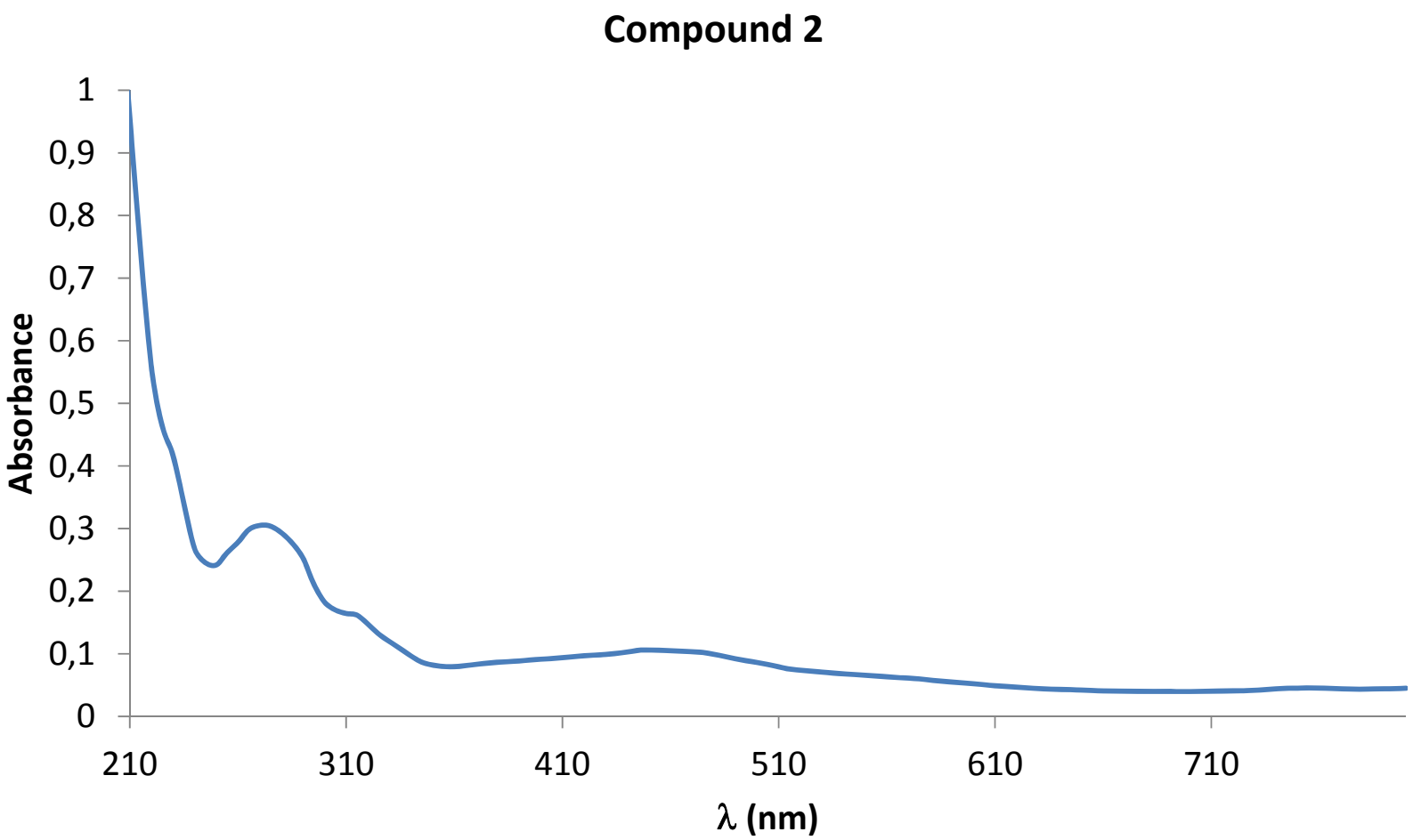
FigS11. $^{1}H,^{13}C$ HMBC spectrum of compound **2** (DMSO-d_6, 500.30, 125.80 MHz, 25˚C); assignation is given in Table 1.



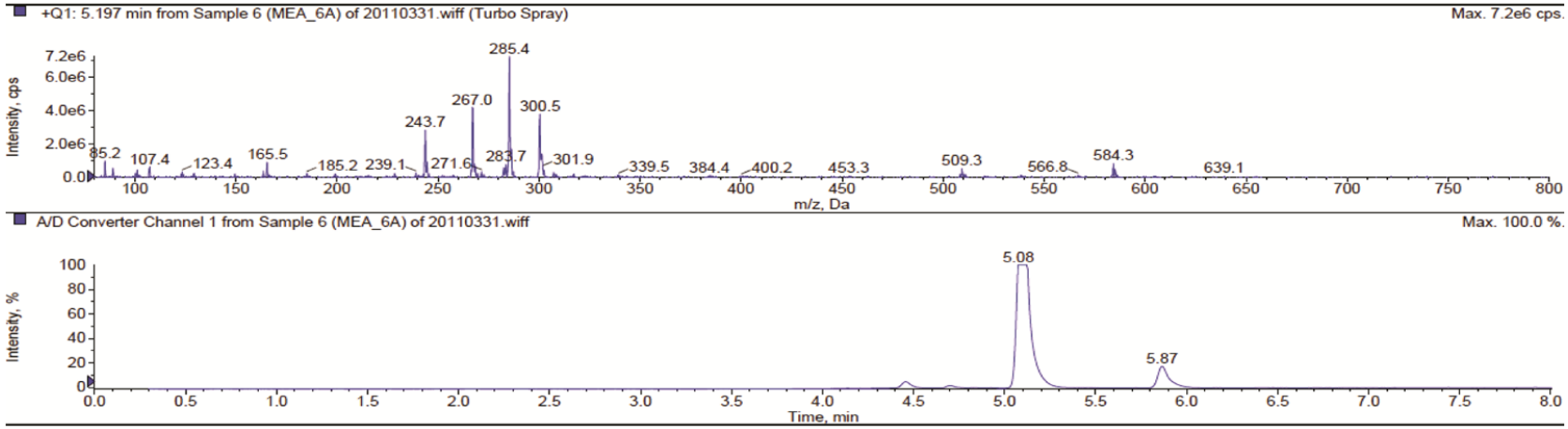
FigS12. COSY spectrum of compound **2** (DMSO-d_6, 500.30 MHz, 25˚C); assignation is given in Table 1.



FigS13. NOESY spectrum of compound **2** (DMSO-d_6, 500.30 MHz, mix=700 ms, 25˚C); assignation is given in Table 1.

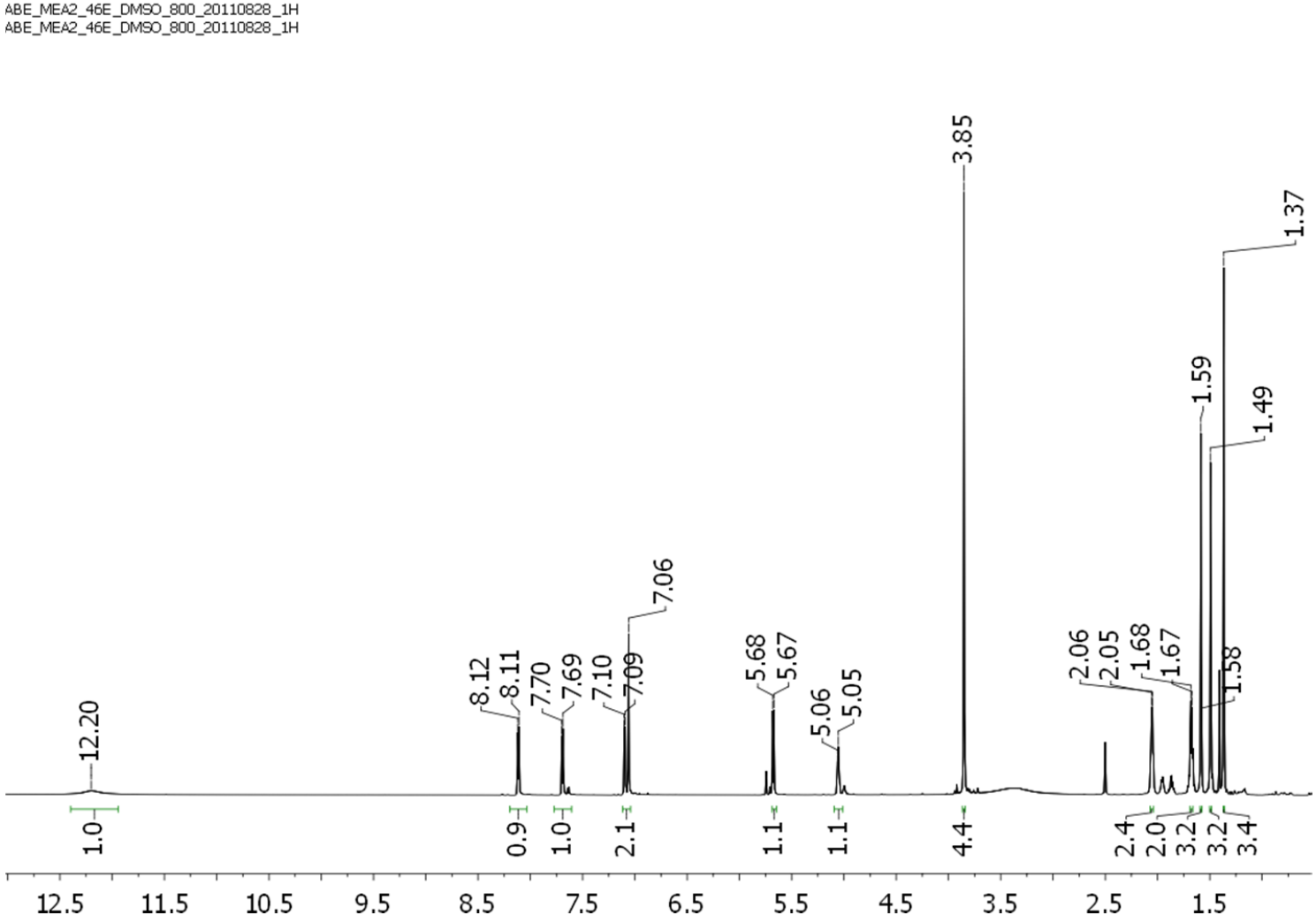


FigS14. UV spectrum of compound **2** (methanol, 25˚C); absorption maxima were observed at 275, 315 and 455 nm.

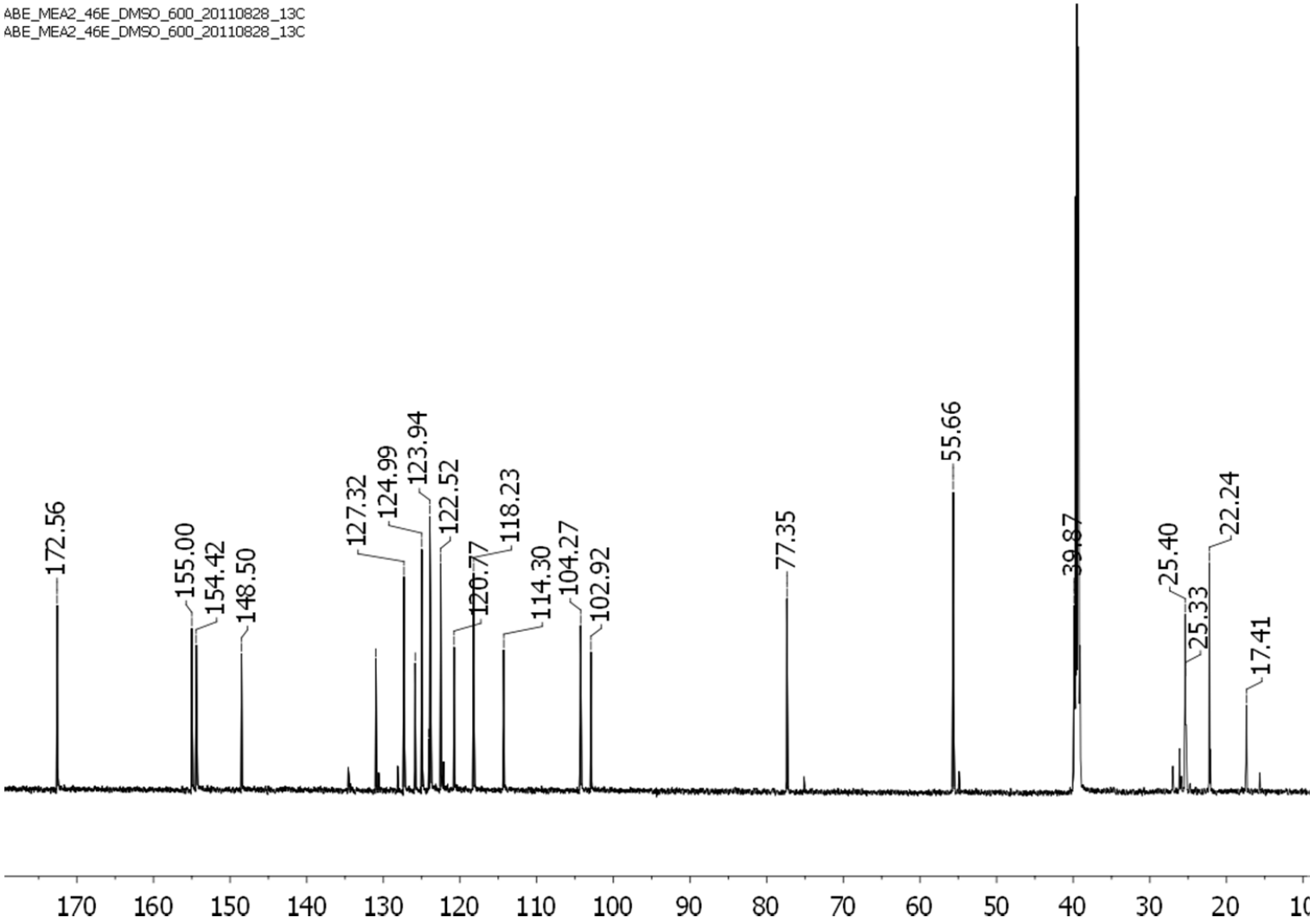


FigS15. The HPLC-UV/MS chromatogram of compound **2**.

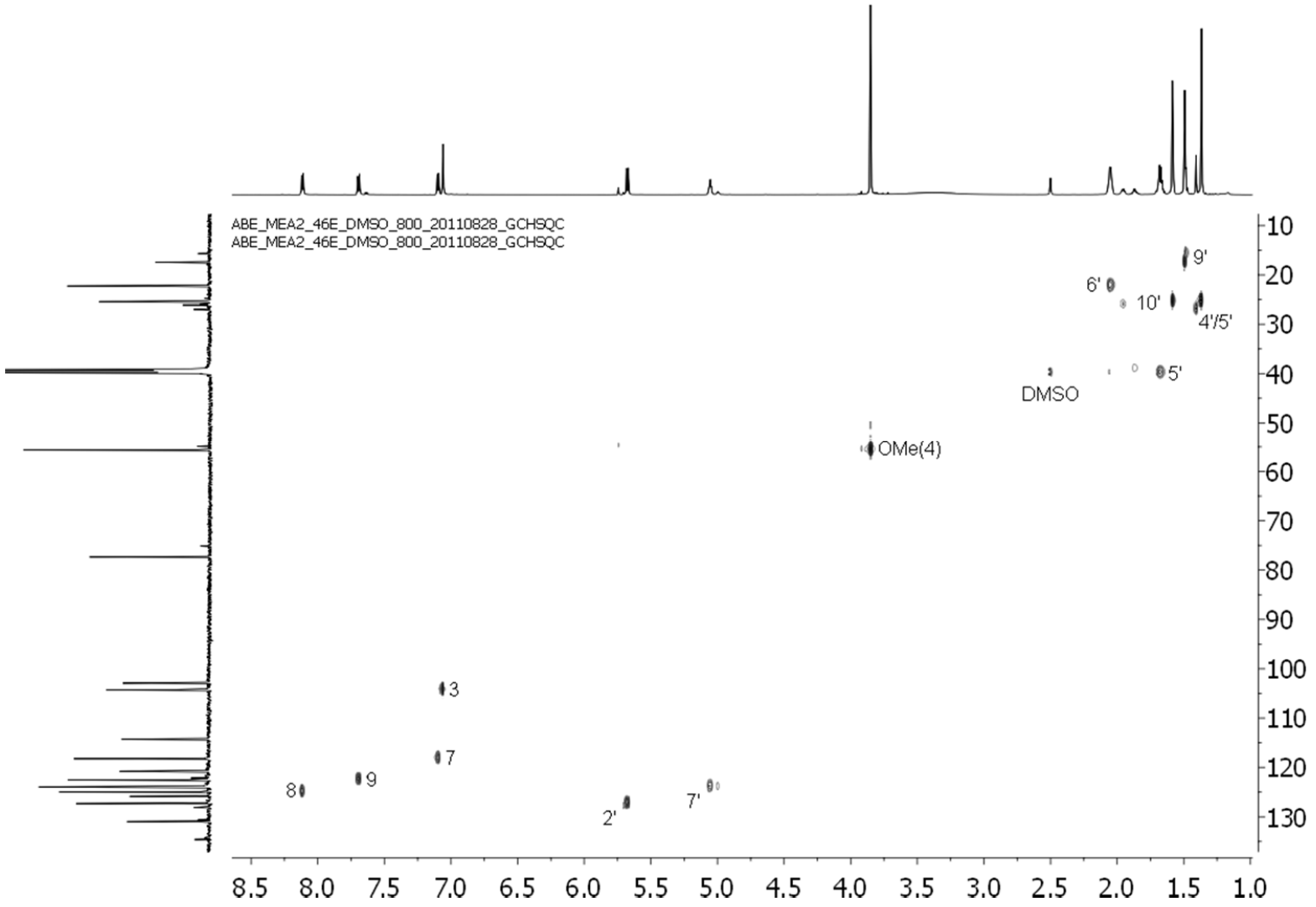
HRMS: m/z 299.0919 $[M-H]^-$, calculated for $C_{17}H_{15}O_5$ –H: 299.0953.



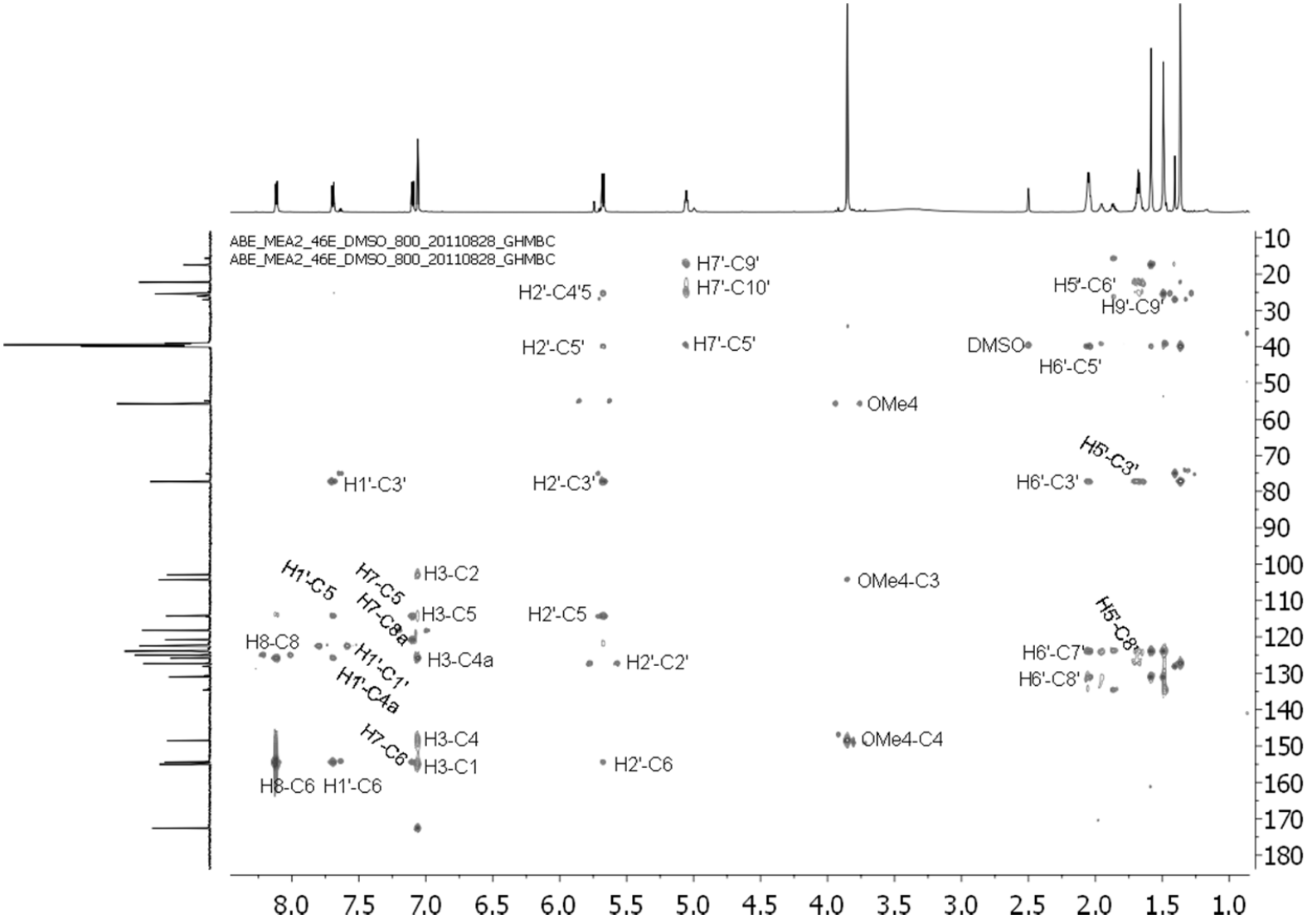
FigS16. ^{1}H NMR spectrum of compound **3** (DMSO-d_6, 800.11 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Fig. 1.



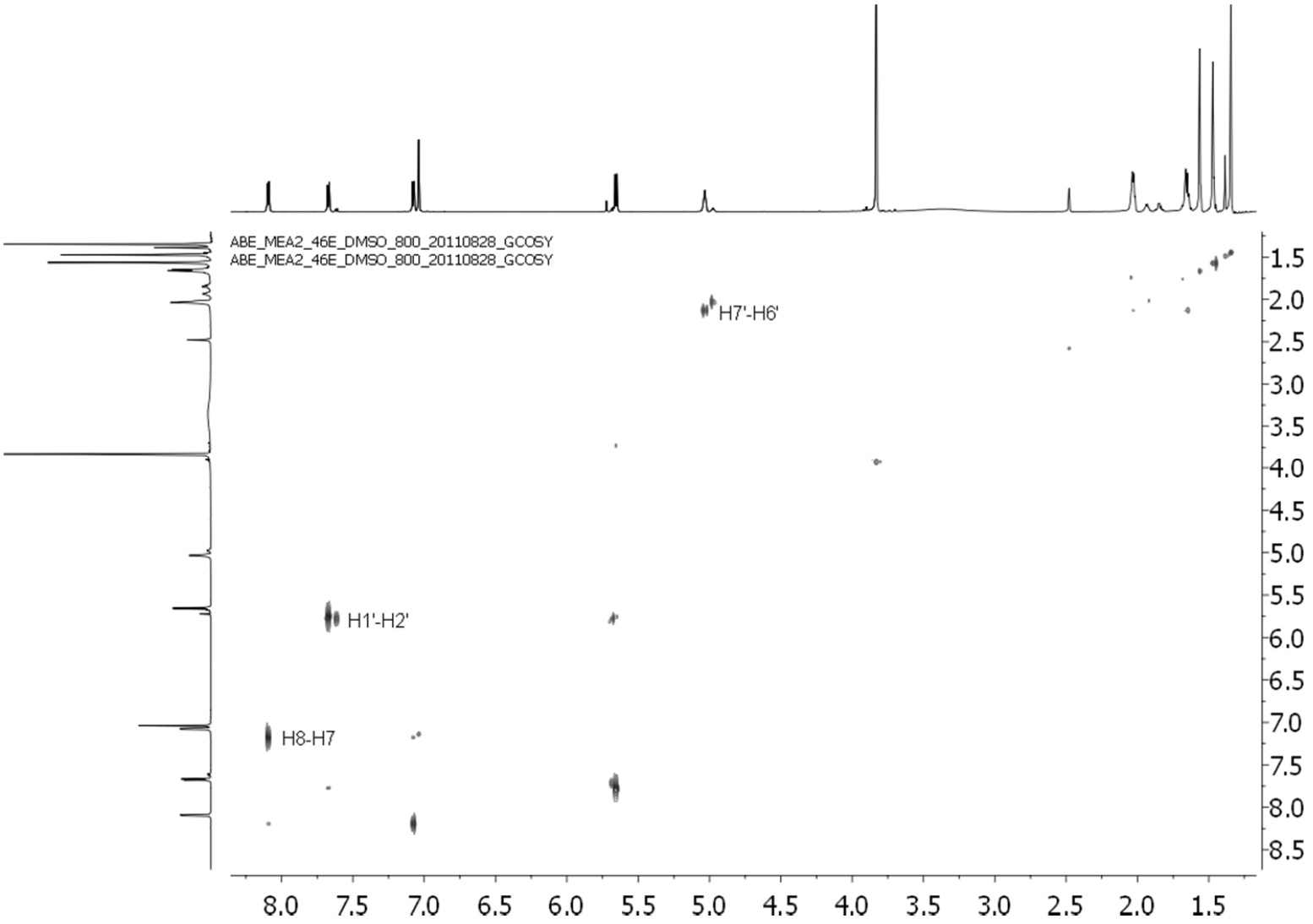
FigS17. ^{13}C NMR spectrum of compound **3** (DMSO-d_6, 150.30 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Fig. 1.



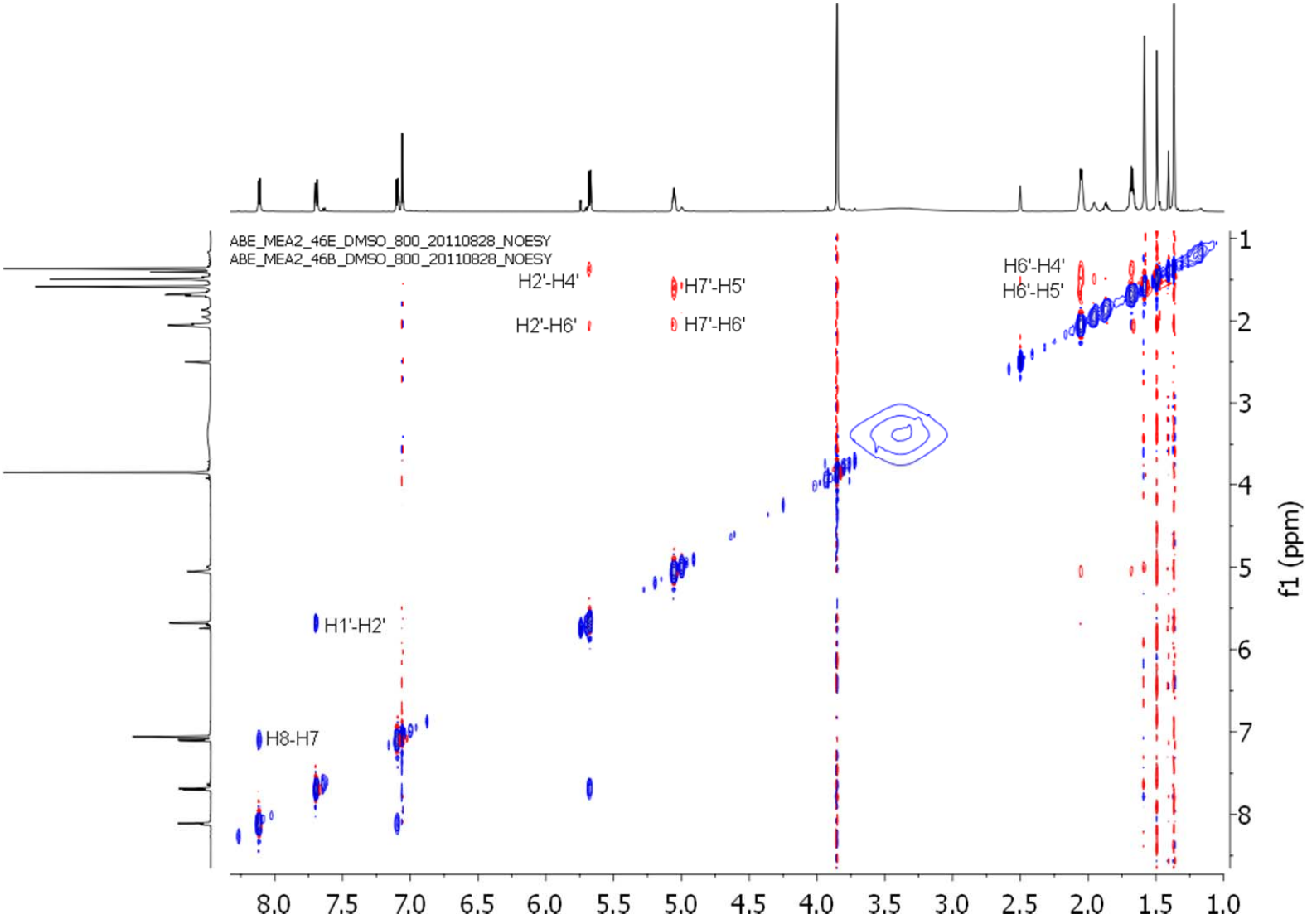
FigS18. $^{1}H,^{13}C$ HSQC NMR spectrum of compound **3** (DMSO-d_6, 800.30, 201.21 MHz, 25˚C); assignation is given in Table 1.



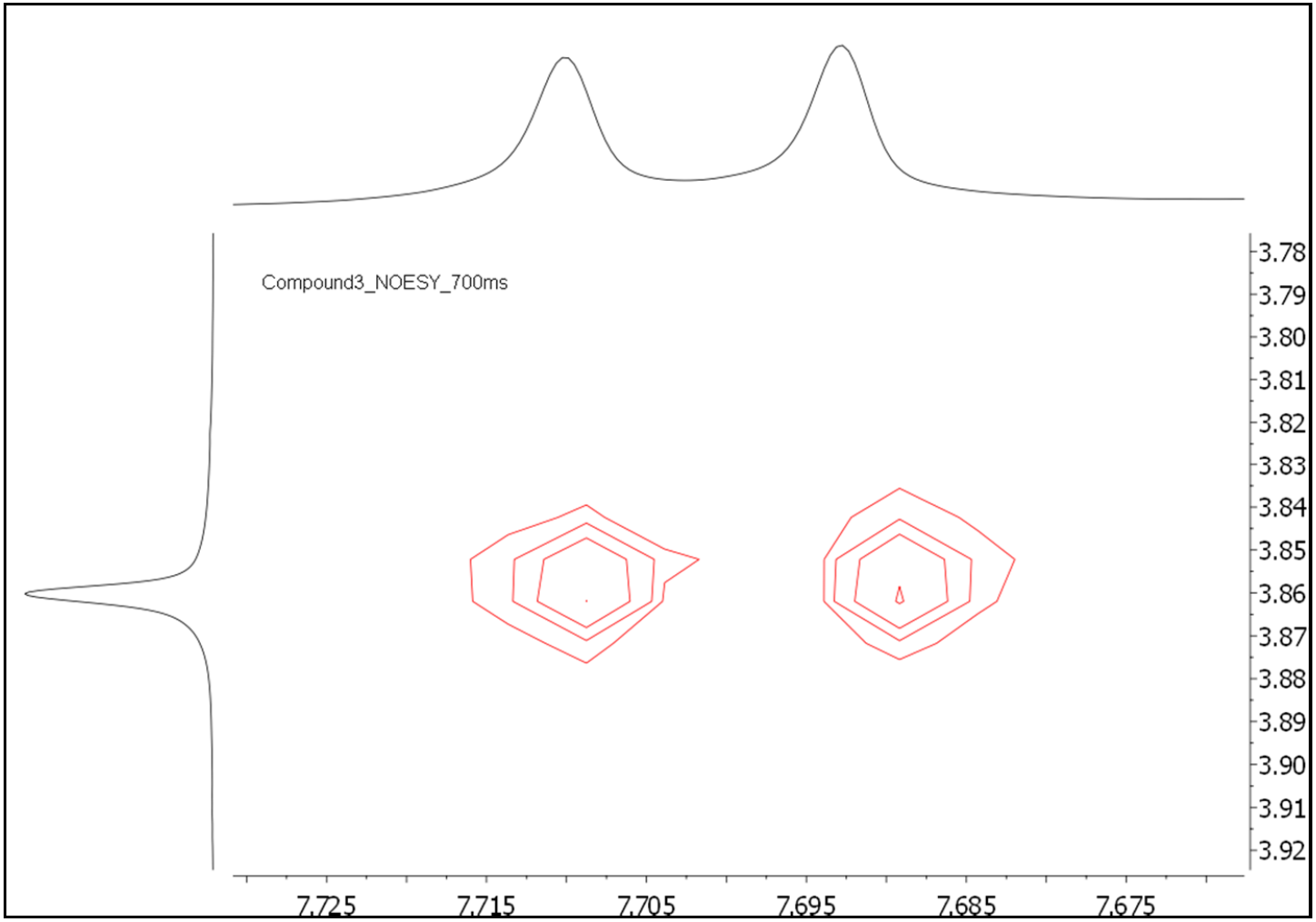
FigS19. $^{1}H,^{13}C$ HMBC NMR spectrum of compound **3** (DMSO-d_6, 800.30, 201.21 MHz, 25˚C); assignation is given in Table 1.



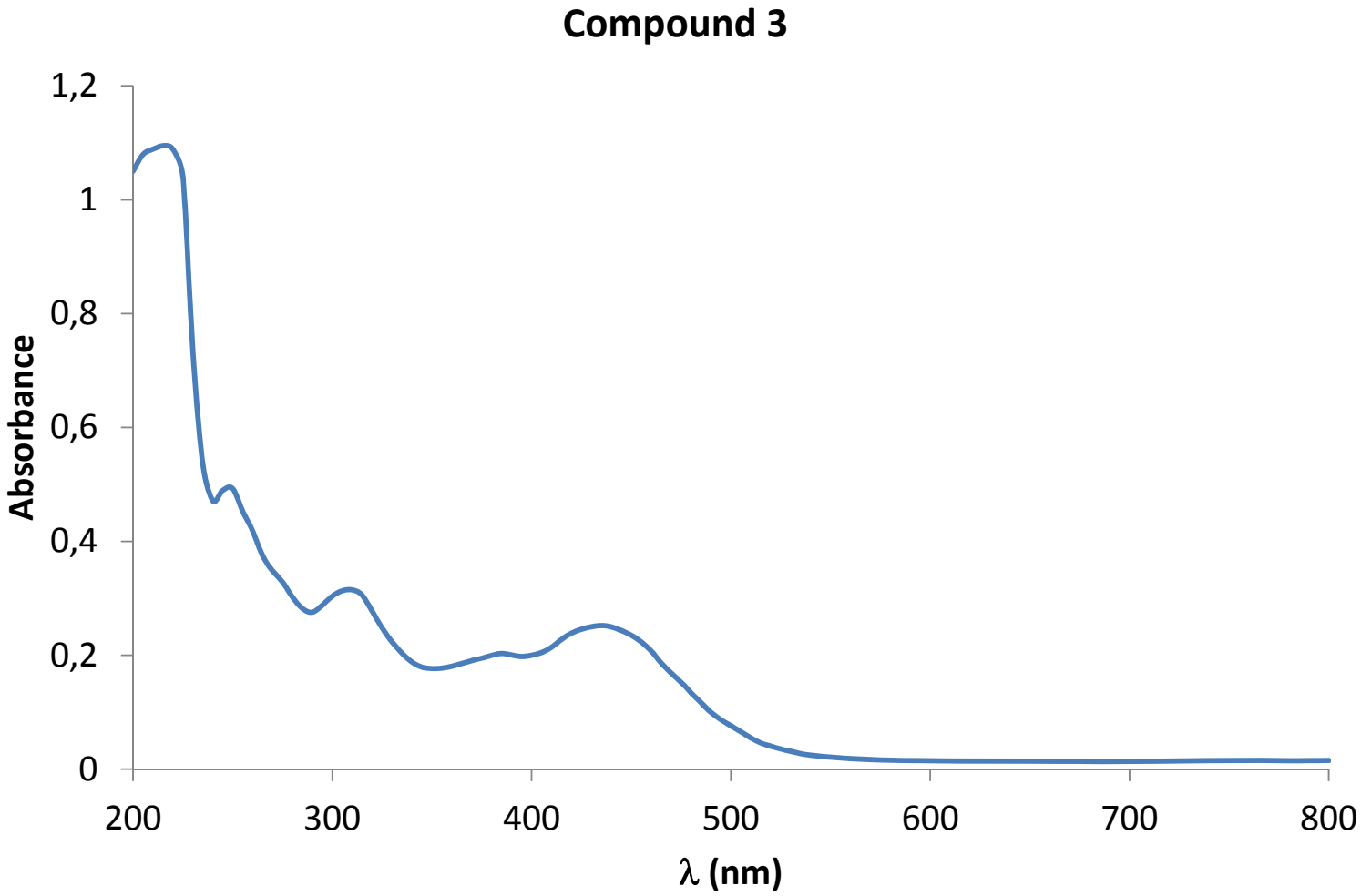
FigS20. COSY spectrum of compound **3** (DMSO-d_6, 800.30 MHz, 25˚C); assignation is given in Table 1.



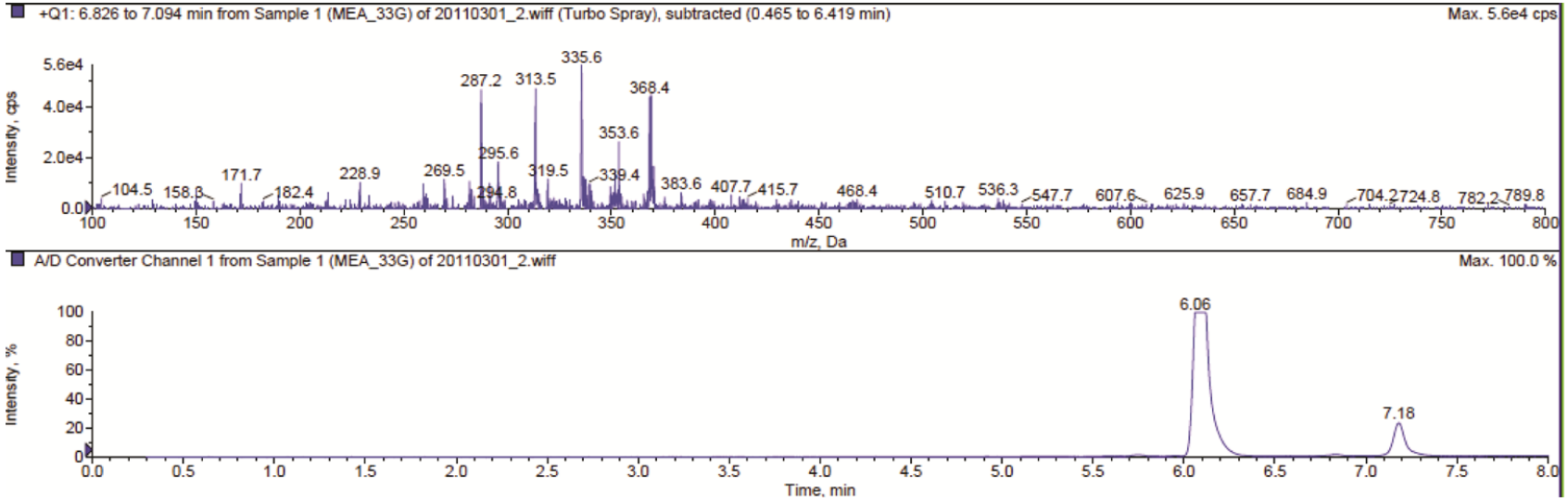
FigS21. NOESY spectrum of compound **3** (DMSO-d_6, 800.30 MHz, mix = 700 ms, 25˚C); assignation is given in Table 1.



FigS22. Decisive NOE cross peak between OCH_3-4 and H1' in the NOESY spectrum of compound **3** (DMSO-d_6, 800.30 MHz, mix = 700 ms, 25˚C); assignation is given in Table 1, structure is shown in Fig. 1.

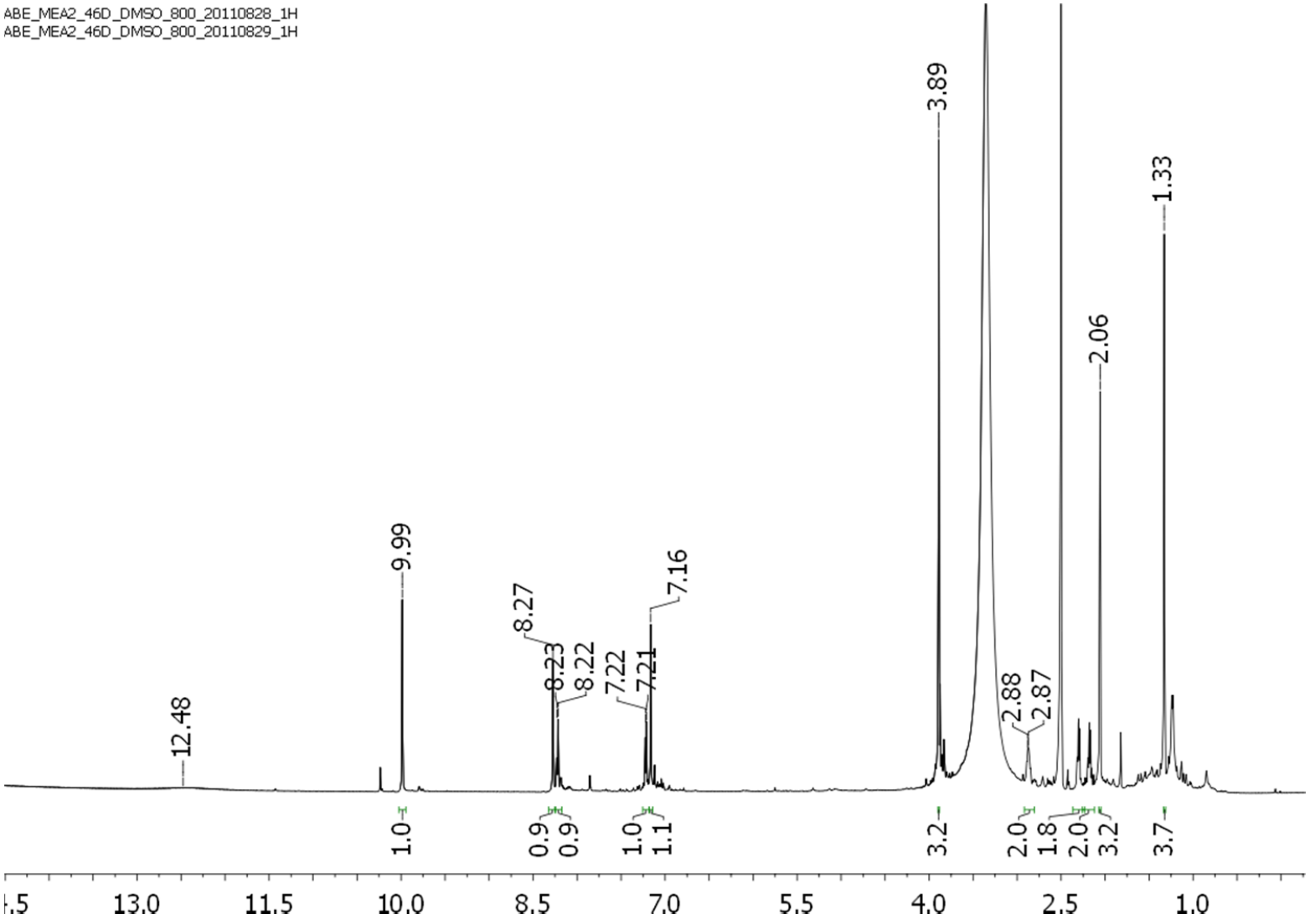


FigS23. UV spectrum of compound **3** (methanol, 25˚C); absorption maxima were observed at 210, 245, 300, and 430 nm.

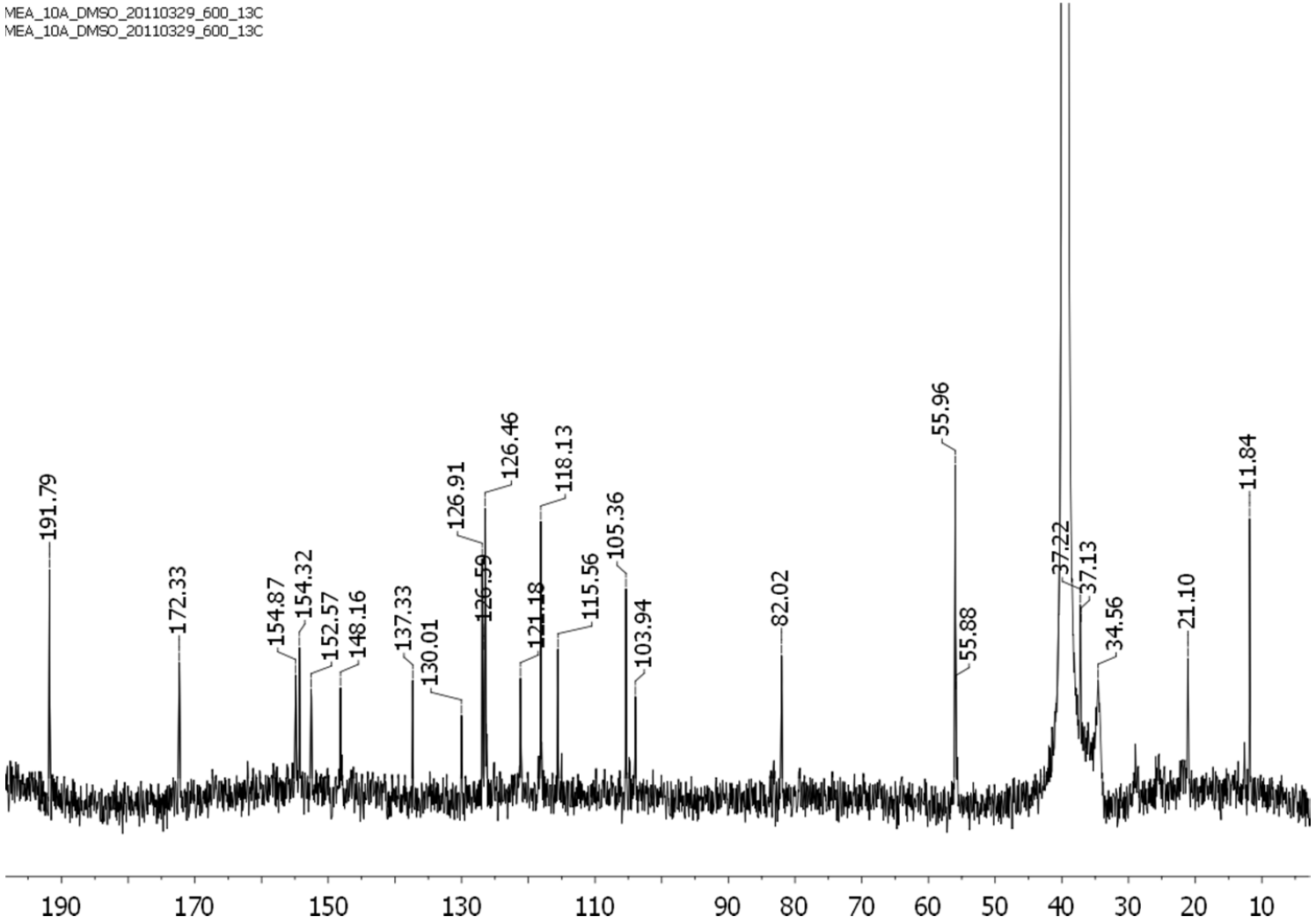


FigS24. The HPLC-UV/MS chromatogram of compound **3**.

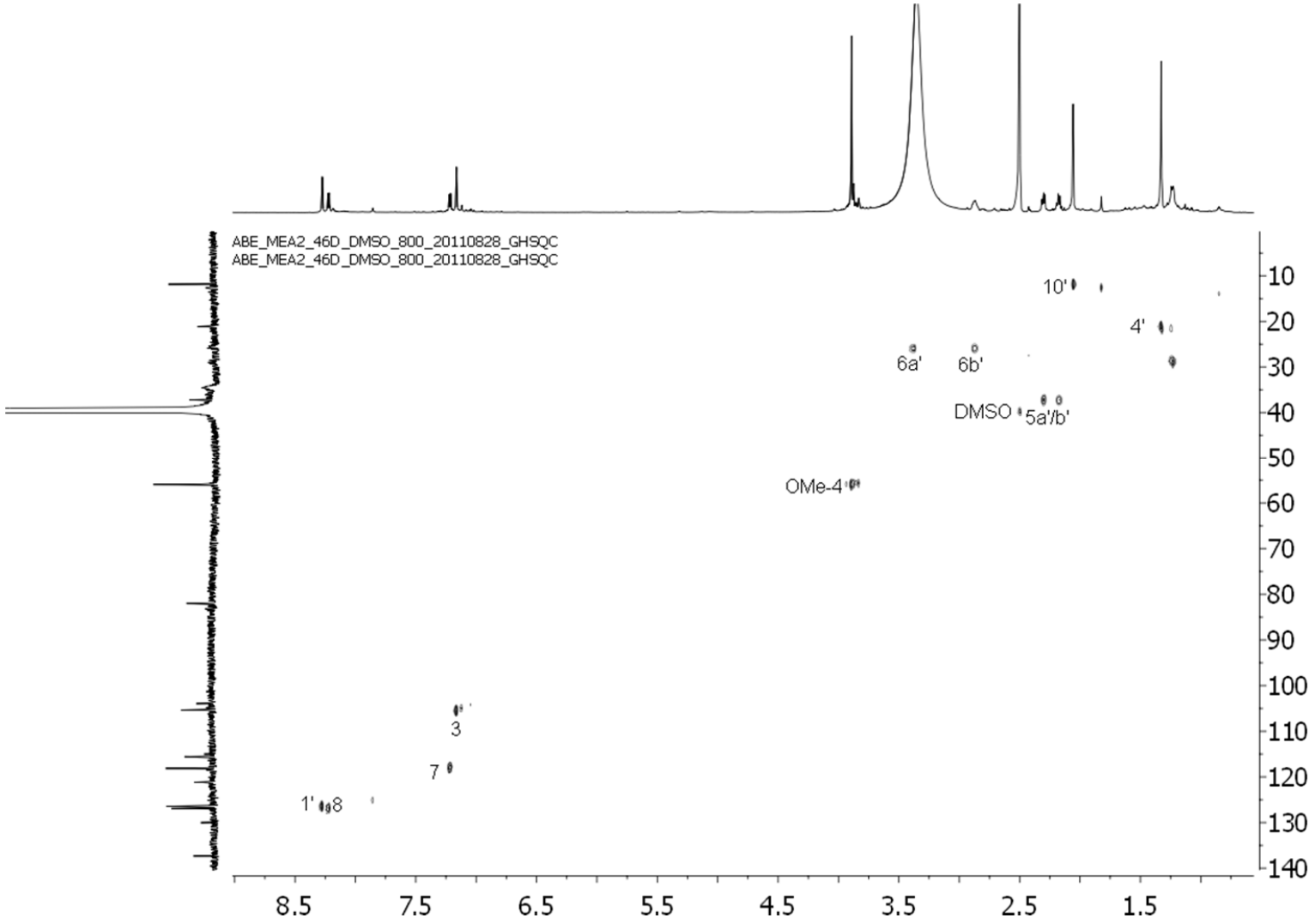
HRMS: m/z 386.1418 $[M-H]^-$, calculated for $C_{17}H_{15}O_5$ 386.1460.



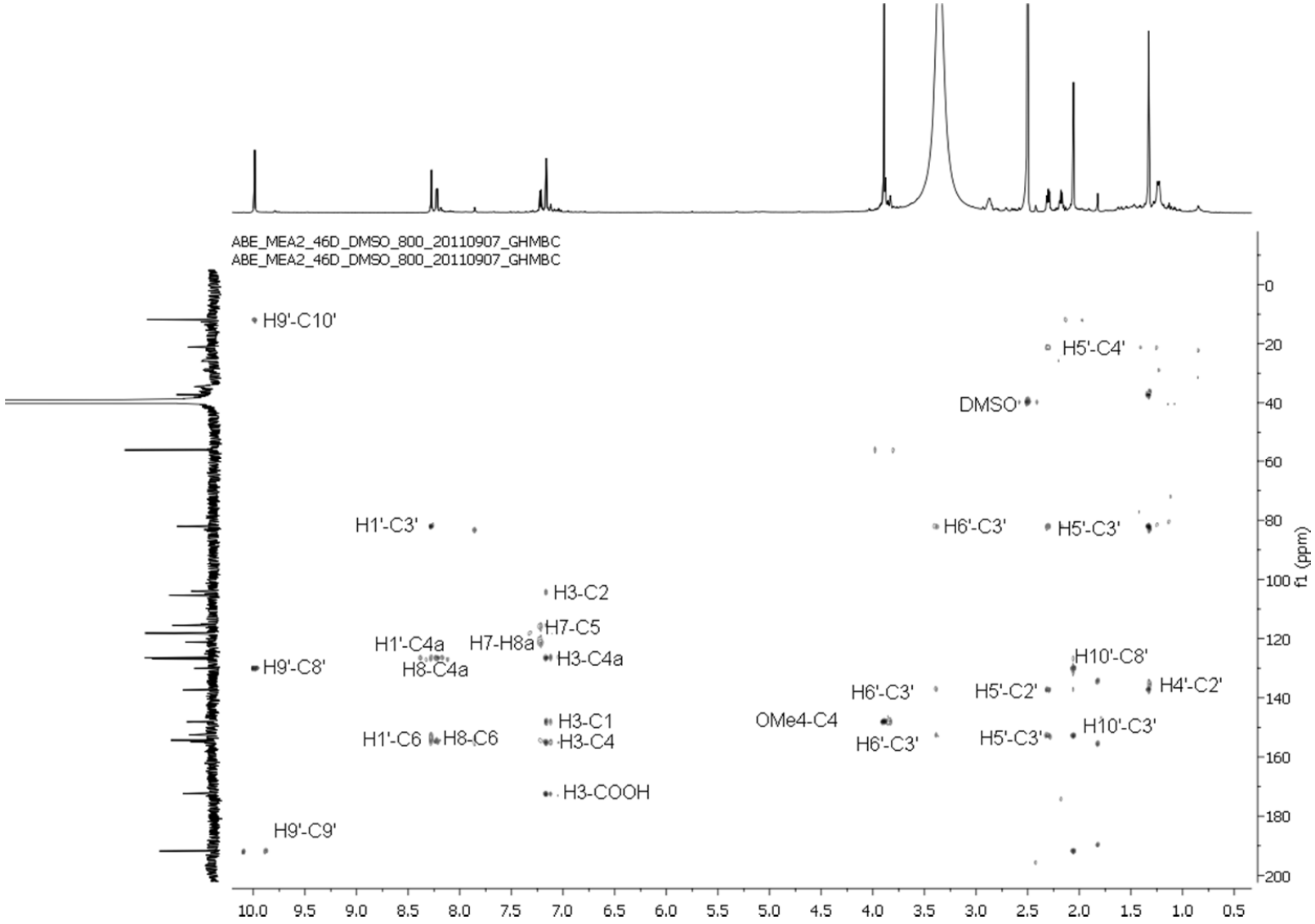
FigS25. ^{1}H NMR spectrum of compound **4** (DMSO-d_6, 800.11 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Fig.1.



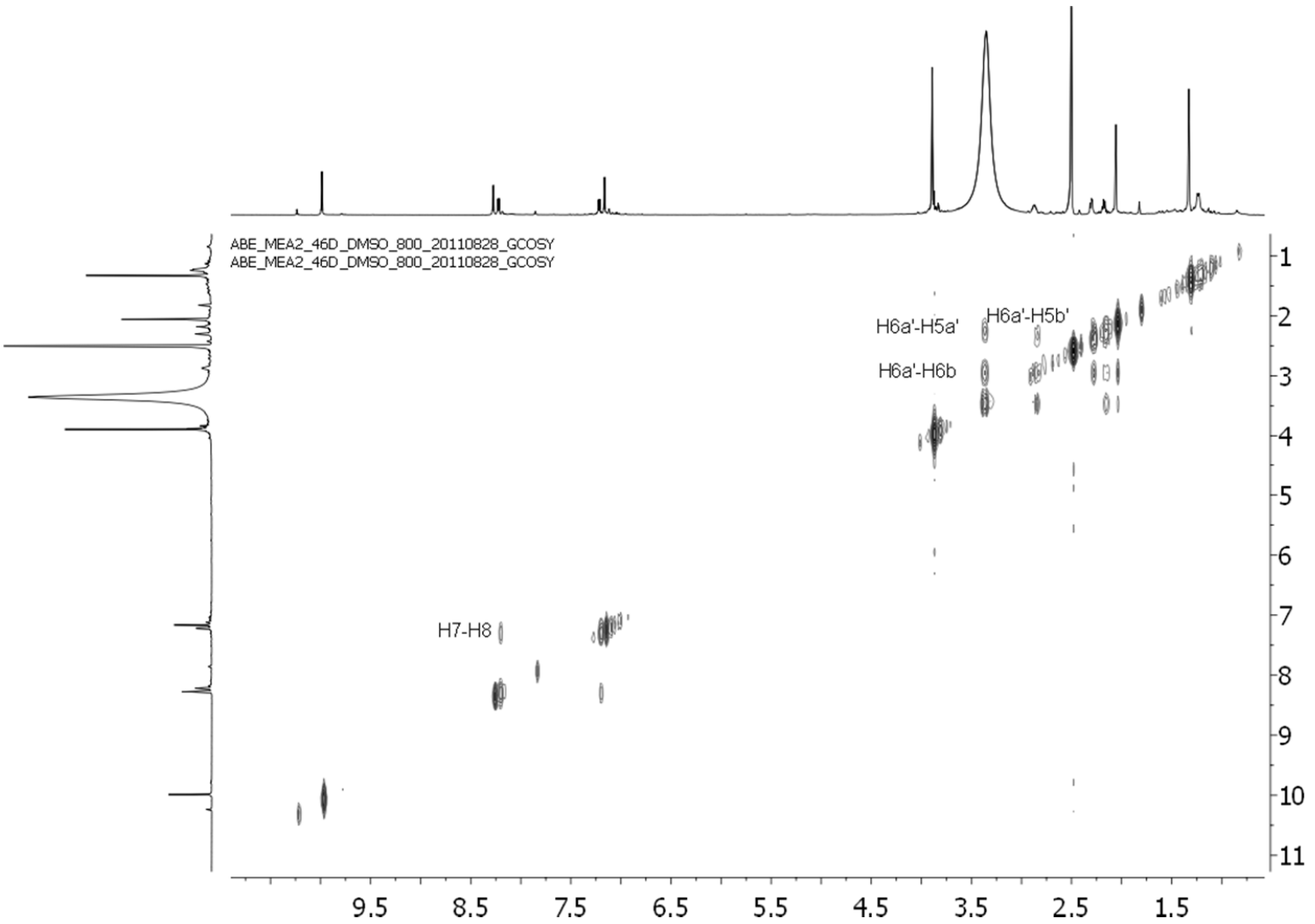
FigS26. ^{13}C NMR spectrum of compound **4** (DMSO-d_6, 150.30 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Fig.1.



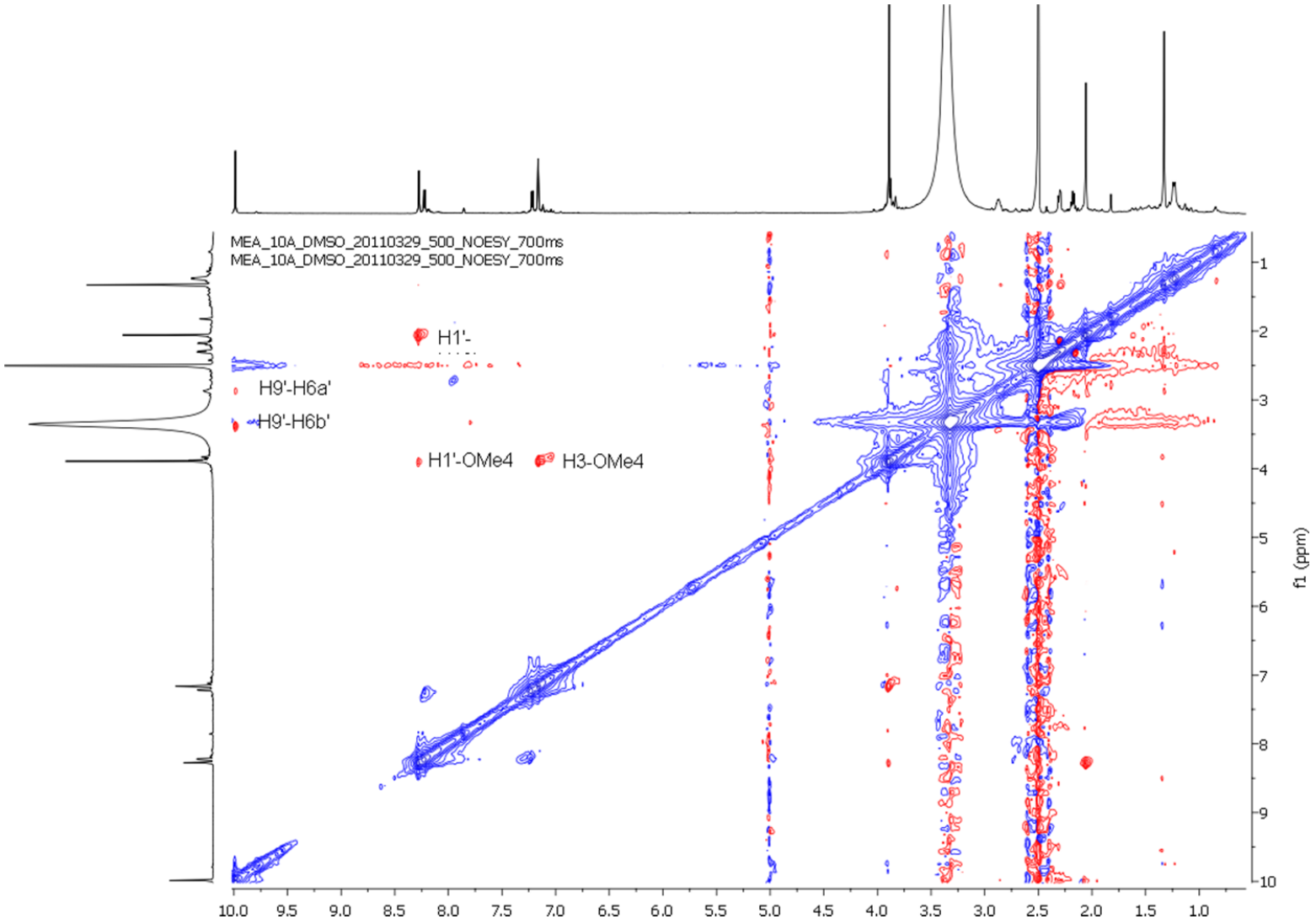
FigS27. $^{1}H,^{13}C$ HSQC NMR spectrum of compound **4** (DMSO-d_6, 800.30, 201.21 MHz, 25˚C); assignation is given in Table 1.



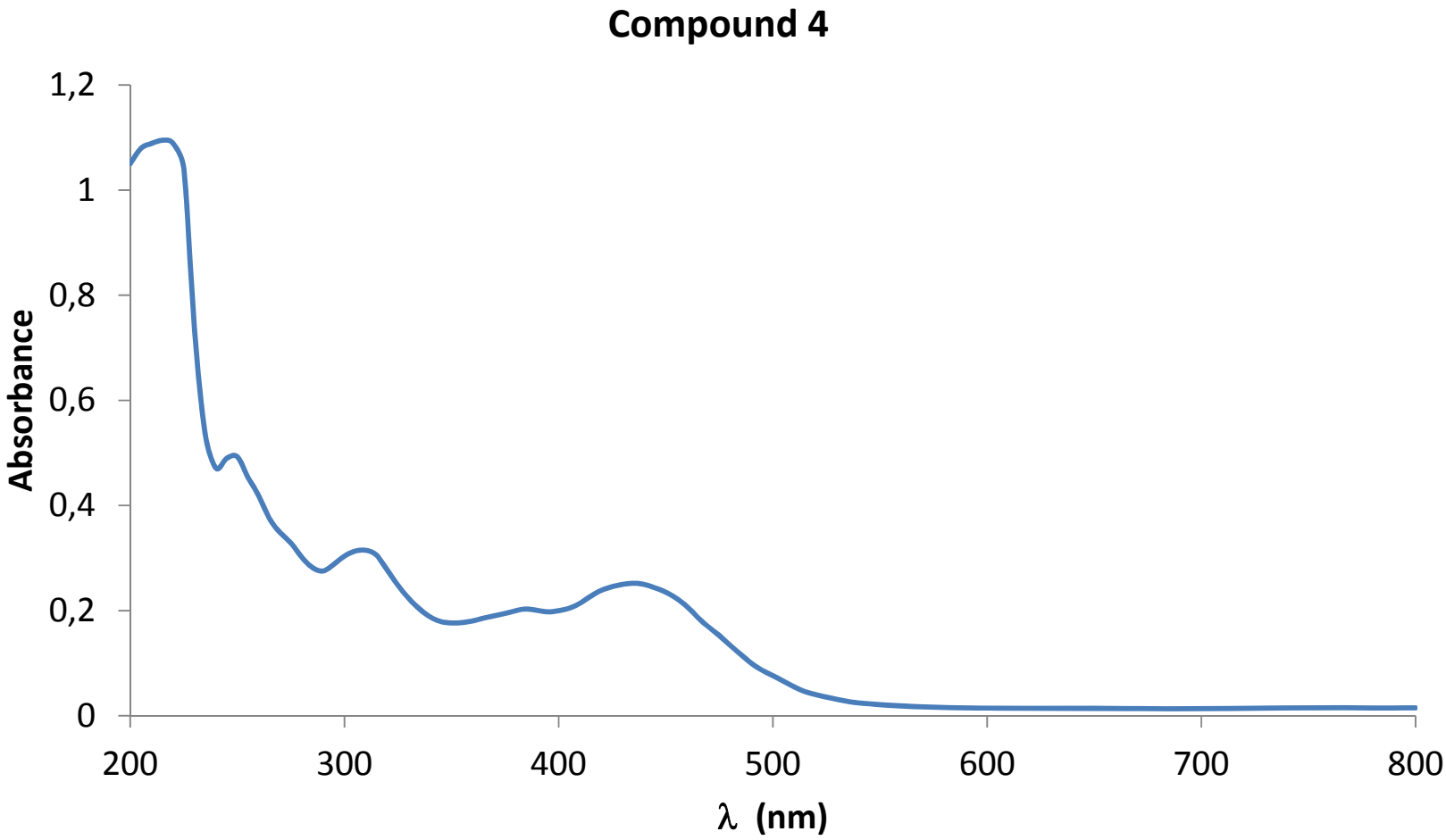
FigS28. $^{1}H,^{13}C$ HMBC spectrum of compound **4** (DMSO-d_6, 800.30, 201.21 MHz, 25˚C); assignation is given in Table 1.



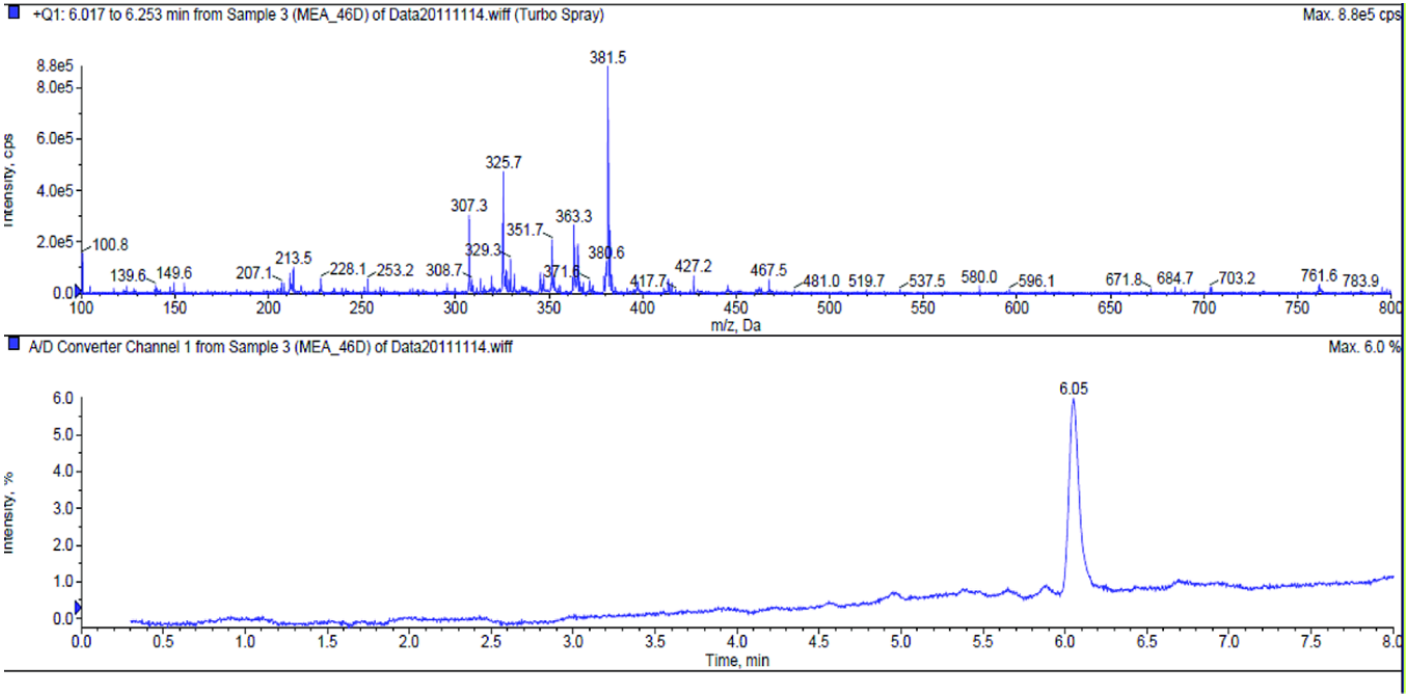
FigS29. COSY spectrum of compound **4** (DMSO-d_6, 800.30 MHz, 25˚C); assignation is given in Table 1.



FigS30. NOESY spectrum of compound **4** (DMSO-d_6, 800.30 MHz, mix = 700 ms, 25˚C); assignation is given in Table 1.

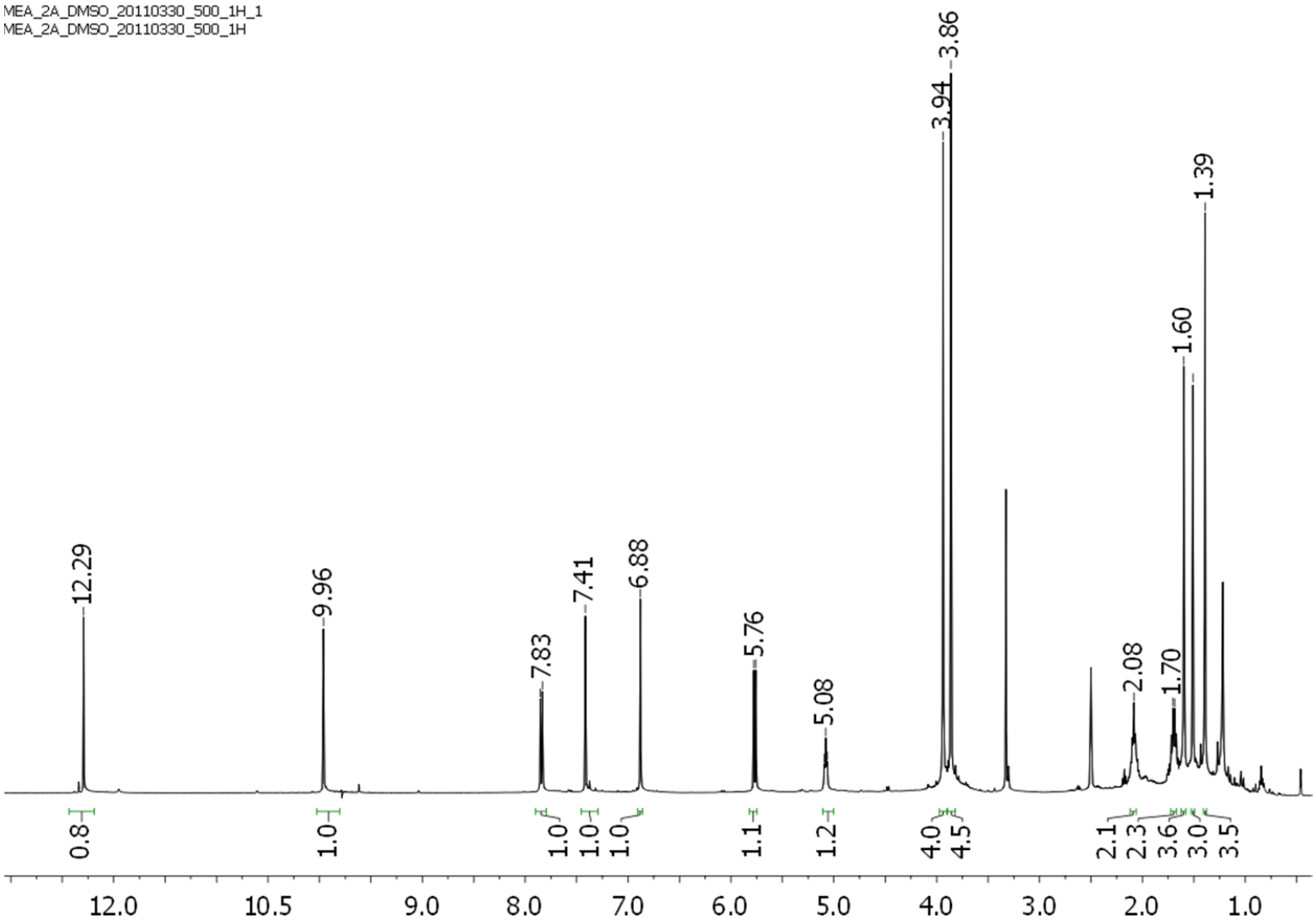


FigS31. UV spectrum of compound **4** (methanol, 25˚C); absorption maxima were observed at 215, 250, 310, 385, 430 nm.

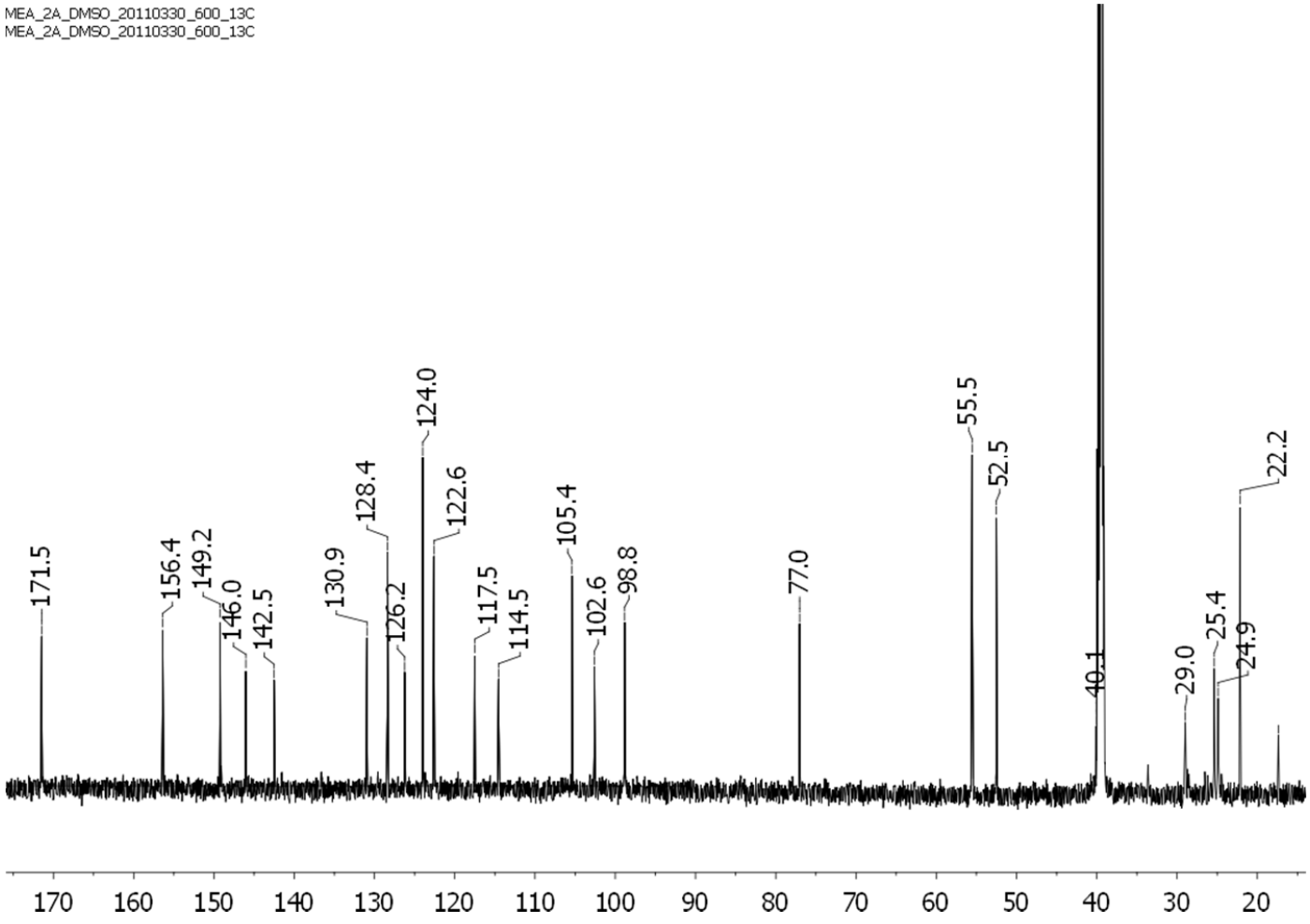


FigS32. The HPLC-UV/MS chromatogram of compound **4**.

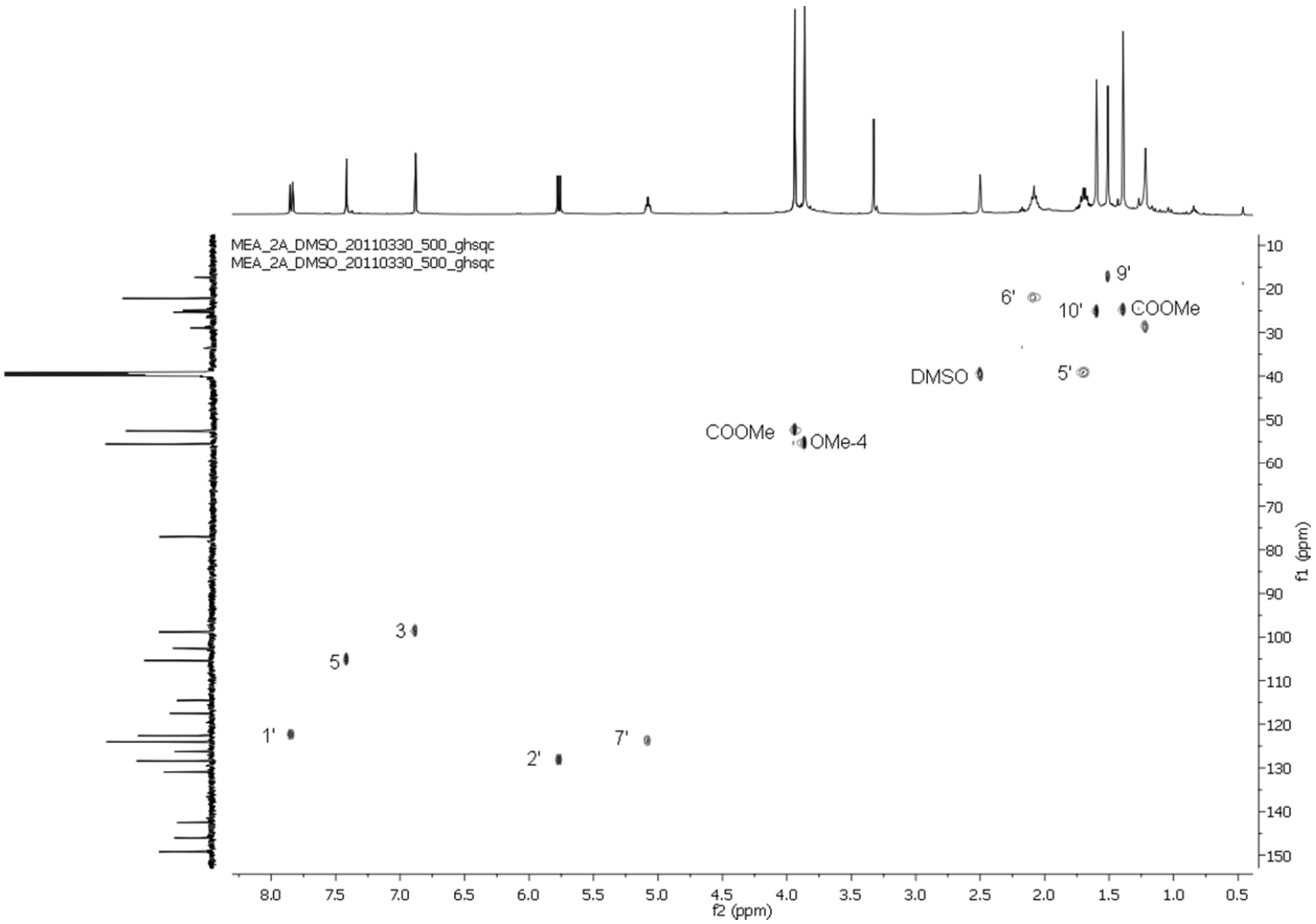
HRMS: m/z 379.1182 $[M-H]^-$, calculated for $C_{22}H_{20}O_6$ –H: 379.1176.



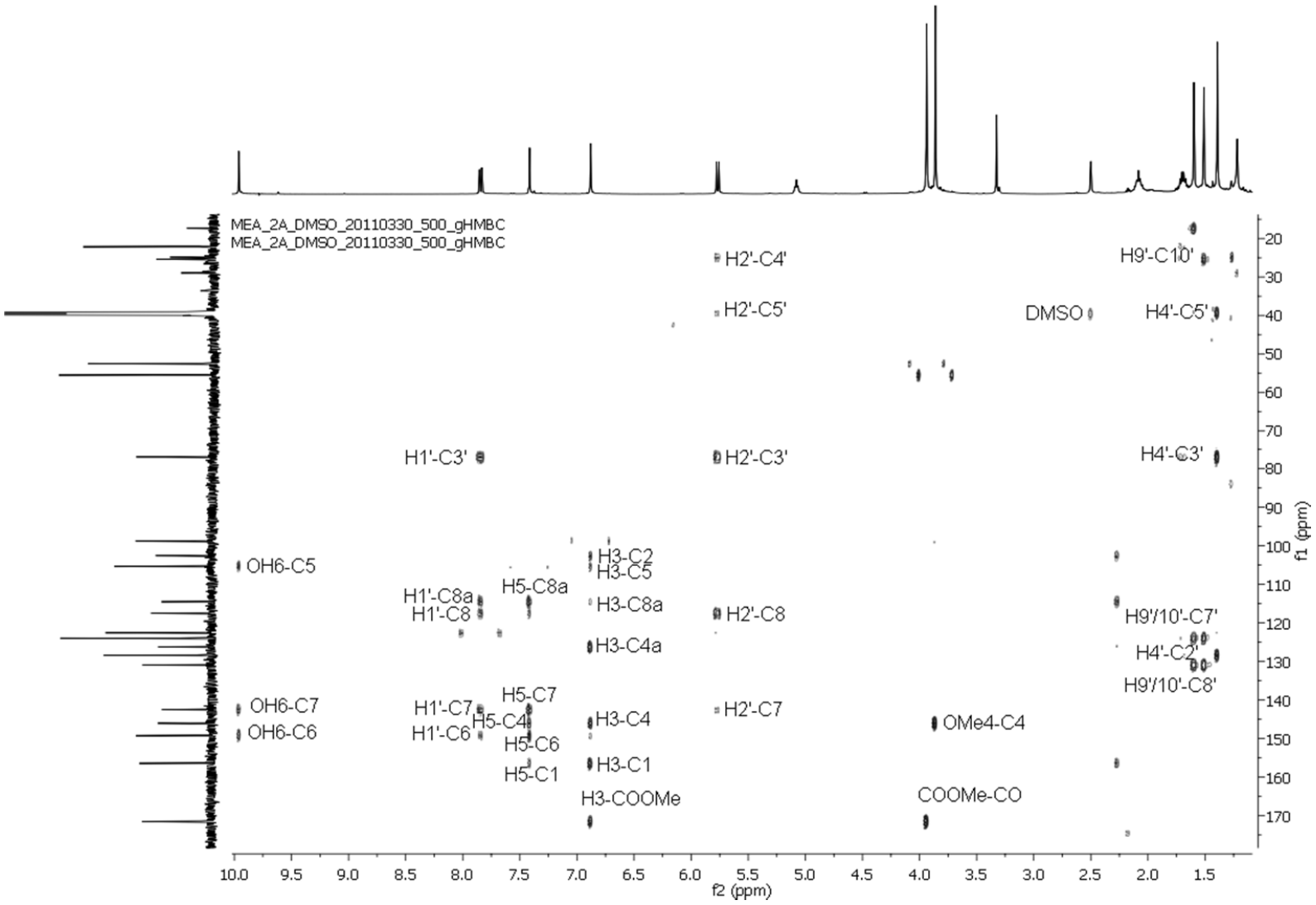
FigS33. [1]H NMR spectrum of compound **5** (DMSO-d_6, 500.30 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Fig. 1.



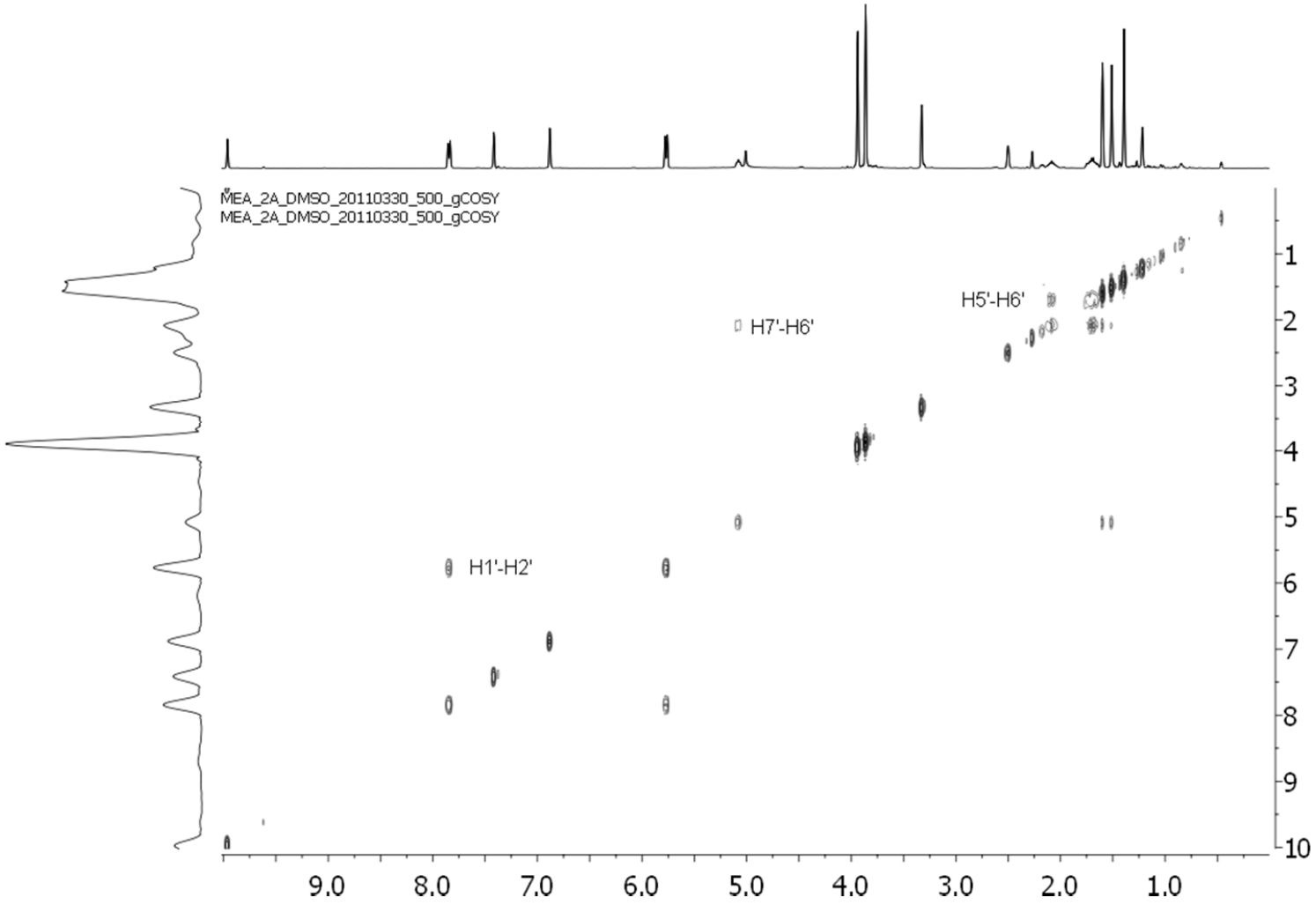
FigS34. ^{13}C NMR spectrum of compound **5** (DMSO-d_6, 150.30 MHz, 25˚C). Assignation is given in Table 1, structure is shown in Fig.1.



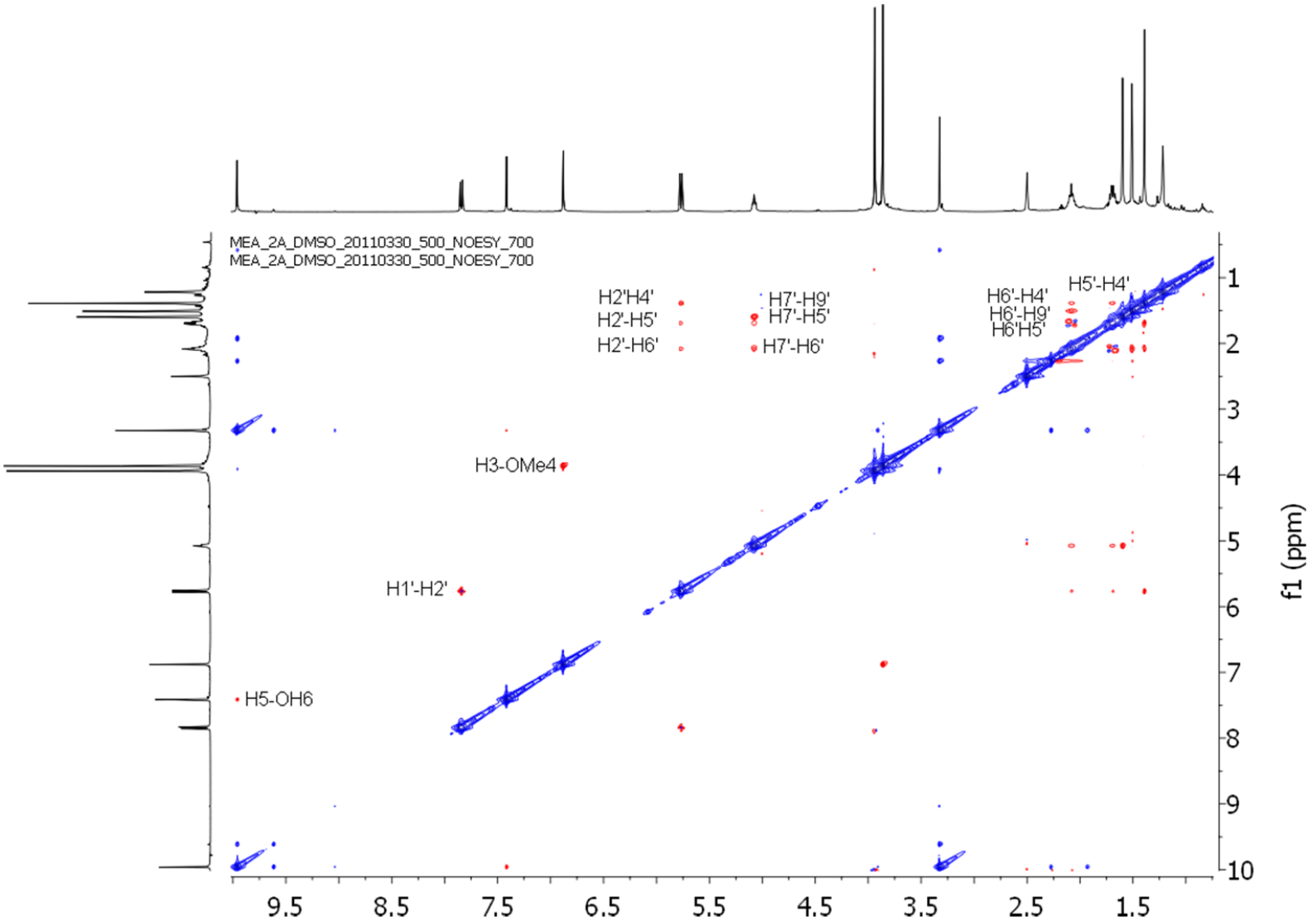
FigS35. HSQC spectrum of compound **5** (DMSO-d_6, 500.30 MHz, 150.30 MHz, 25˚C). Assignation is given in Table 1.



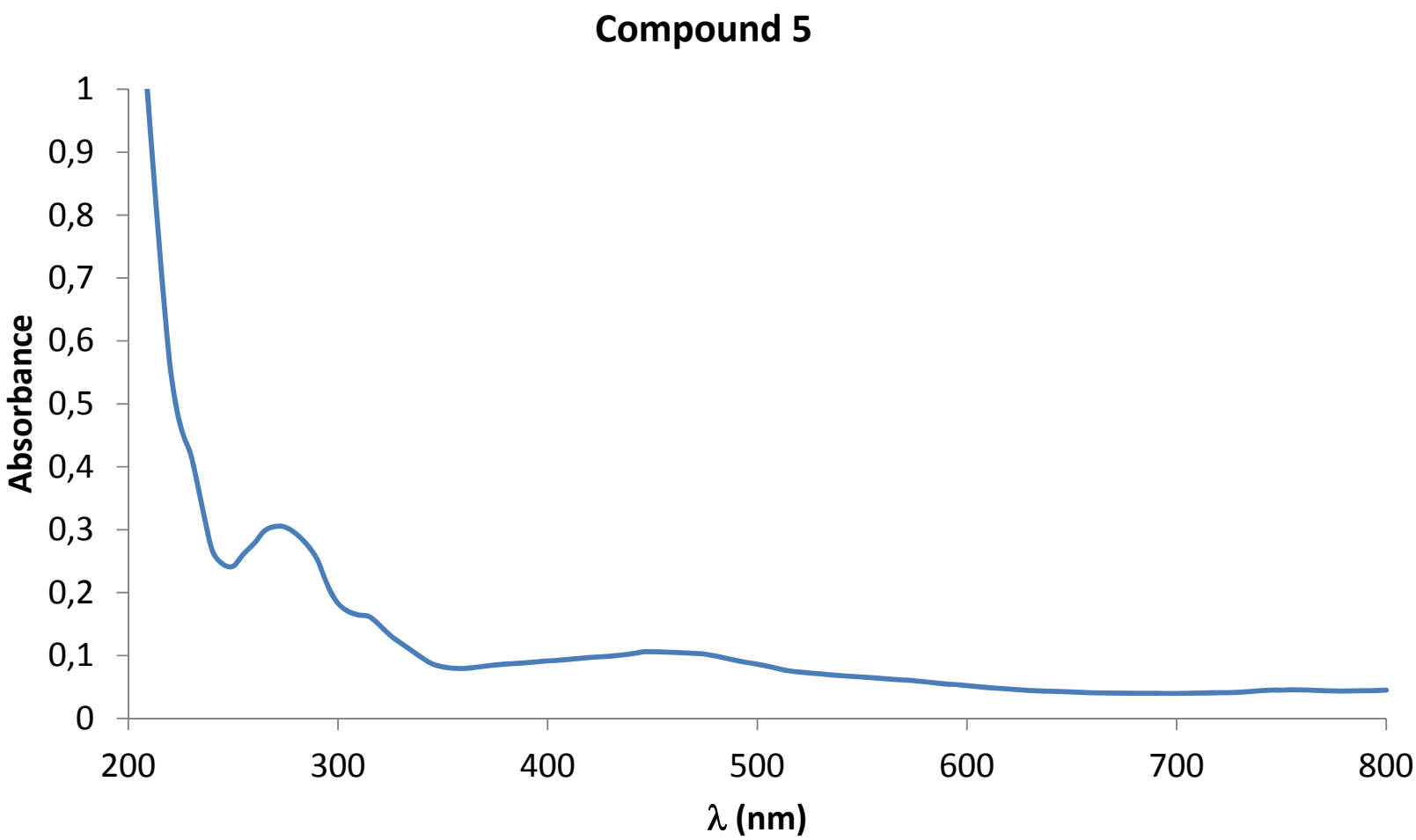
FigS36. HMBC spectrum of compound **5** (DMSO-d_6, 500.30 MHz, 150.30 MHz, 25˚C). Assignation is given in Table 1.



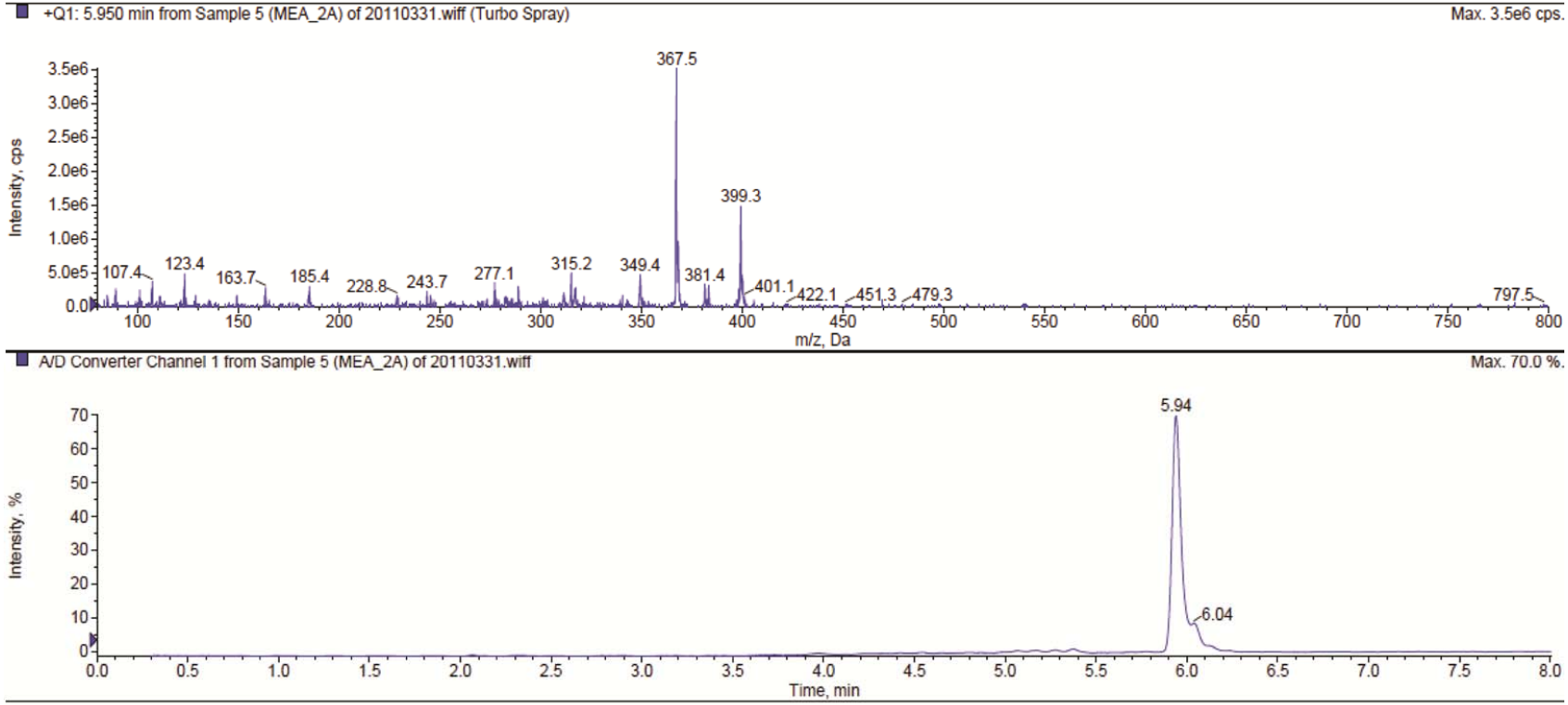
FigS37. COSY spectrum of compound **5** (DMSO-d_6, 500.30 MHz, 25˚C). Assignation is given in Table 1.



FigS38. NOESY spectrum of compound **5** (DMSO-d_6, 500.30 MHz, mix 700 ms, 25˚C). Assignation is given in Table 1

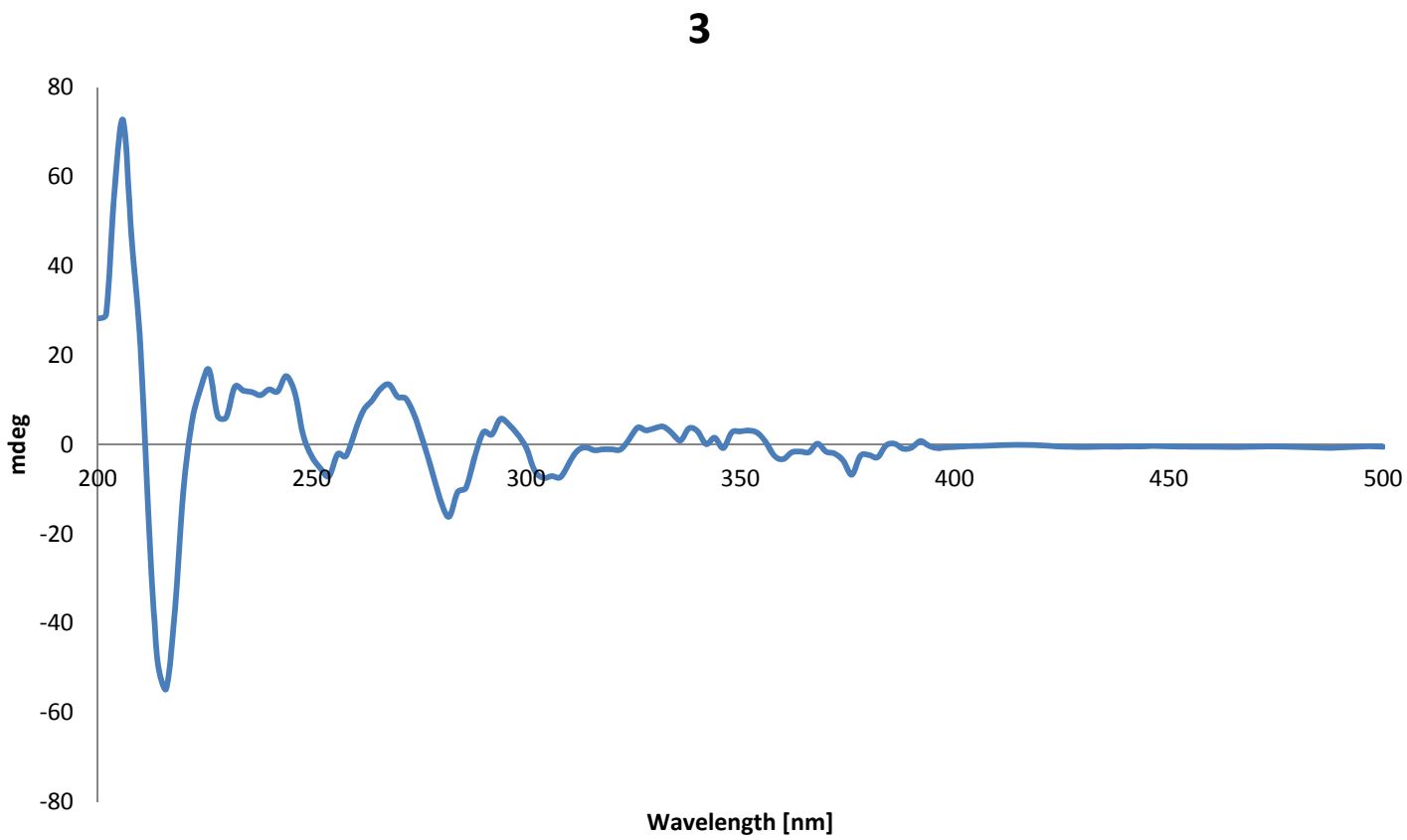


FigS39. UV spectrum of compound **5** (methanol, 25˚C); absorption maxima were observed at 270, 315, 450 nm.

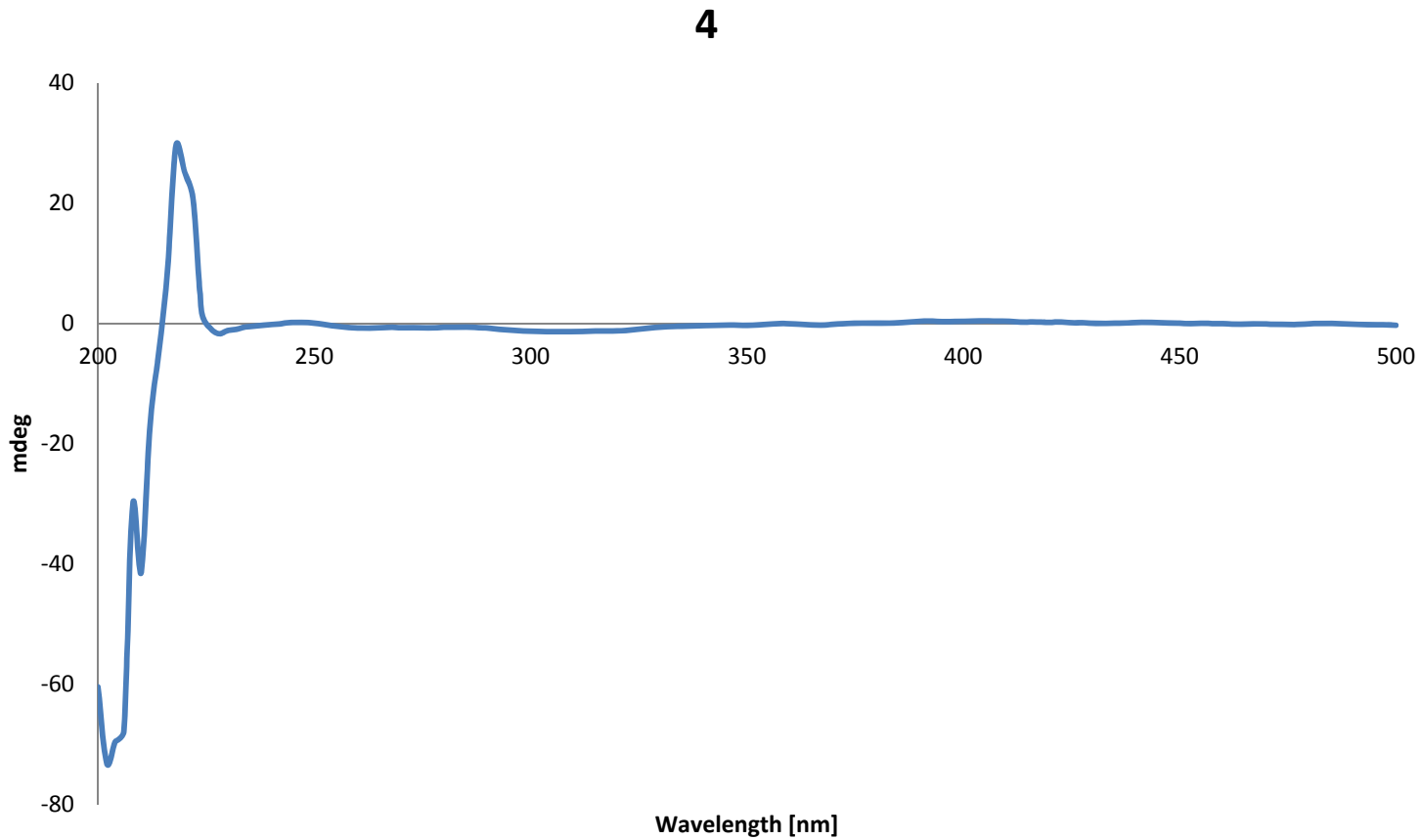


FigS40. The HPLC-UV/MS chromatogram of compound **5**.

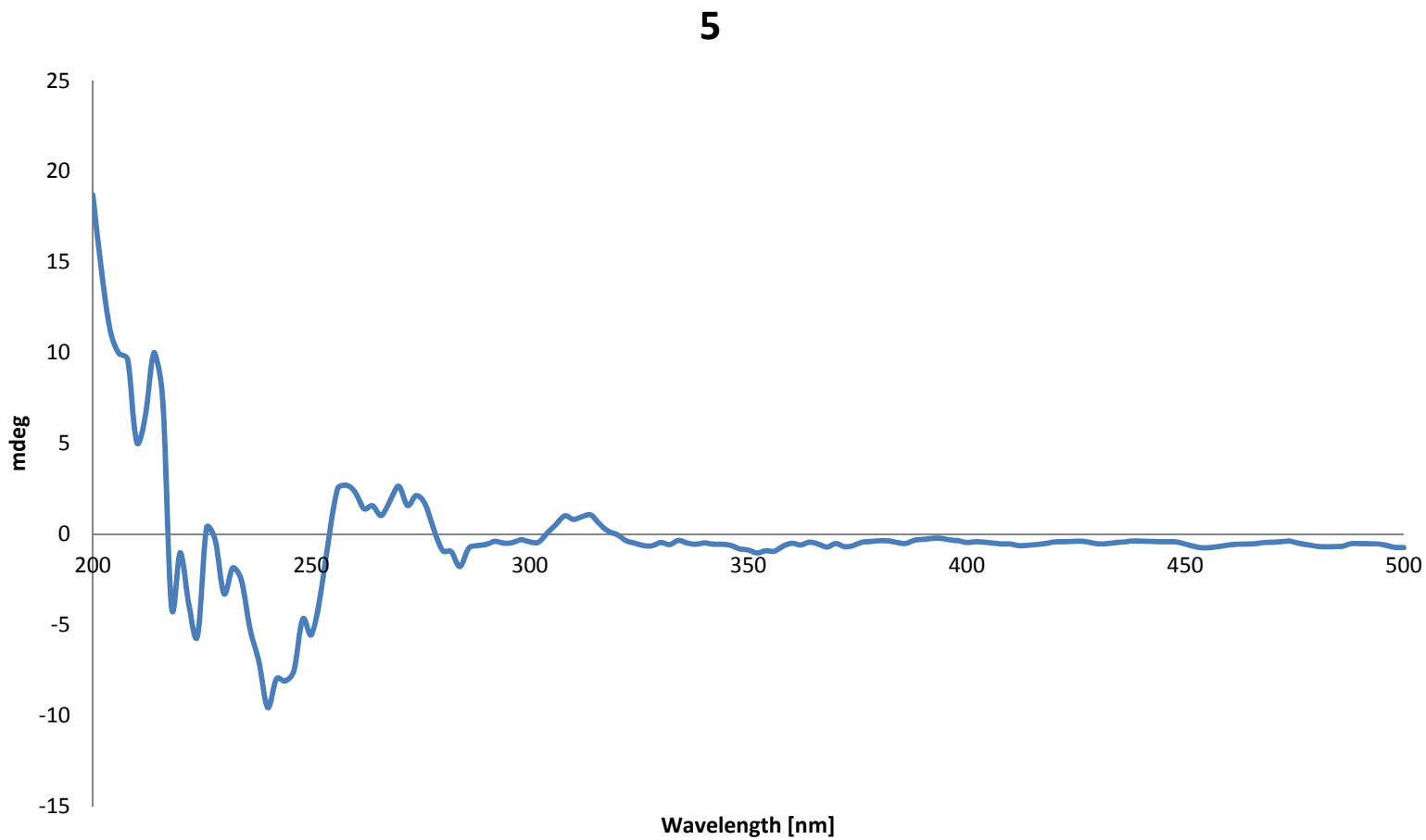
HRMS: m/z 399.2020 $[M+H]^+$, calculated for $C_{23}H_{27}O_6$ 399.1808.



FigS41. The CD spectrum of compound **3** in methanol. An increased high tension voltage on the 2-300 nm frequency interval indicates that the low frequency signals are artifacts arising from excessive absorbance.



FigS42. The CD spectrum of compound **4** in methanol. An increased high tension voltage on the 2-300 nm frequency interval indicates that the low frequency signals are artifacts arising from excessive absorbance.



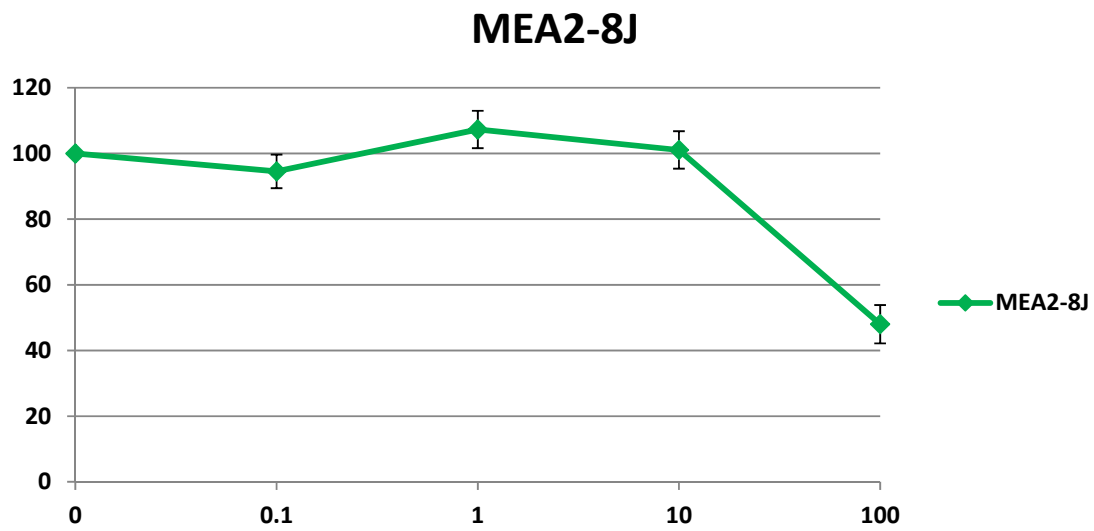
FigS43. The CD spectrum of compound **5** in methanol. An increased high tension voltage on the 2-300 nm frequency interval indicates that the low frequency signals are artifacts arising from excessive absorbance.

Cytotoxicity

Confidence intervals for the cytotoxicity data (LD_{50} 95%)

Compound	LD50 (µg/ml)	95% confidence interval (µg/ml)
Compound 1	93.7	49.4 – 177.9
Compound 2	112.1	49.9 – 251.8
Compound 3	> 100	Not determined
Compound 4	> 100	Not determined
Compound 5	22.8	13.8 – 37.7

Compound 1

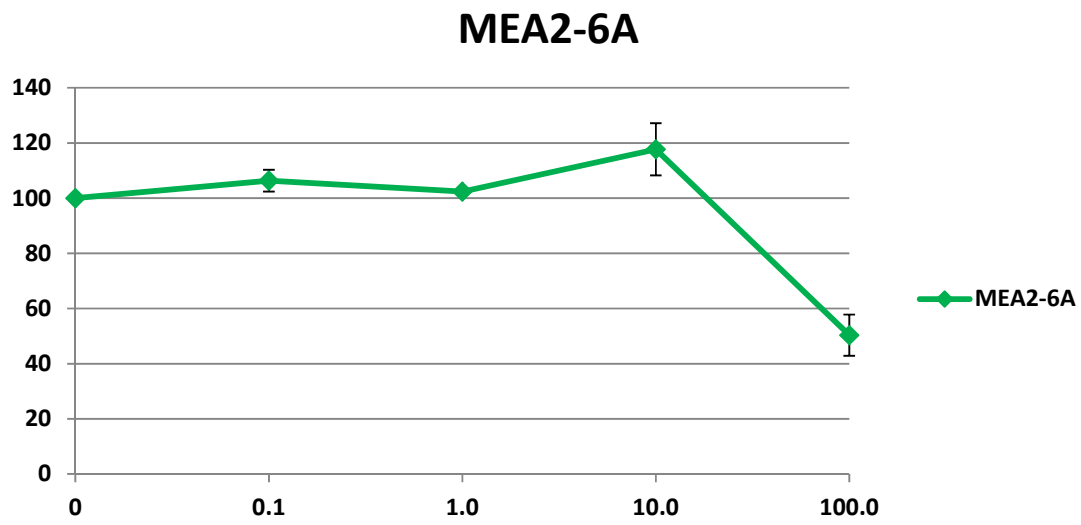


	(µg/ml)				
	0	**0.1**	**1**	**10**	**100**
	100	86.7	118.6	108.2	39.8
	100	104.1	103.1	105.3	59.3
	100	92.8	100.2	89.8	44.8
Mean	**100**	**94.5**	**107.3**	**101.1**	**48.0**
SE	**0**	**5.1**	**5.7**	**5.7**	**5.8**

Sigmoidal dose-response
LD50 93.7

LD50 95% Confidence Interval 49.37 to 177.9
Goodness of Fit R square 0.9429 Absolute Sum of Squares 0.1303

Compound 2



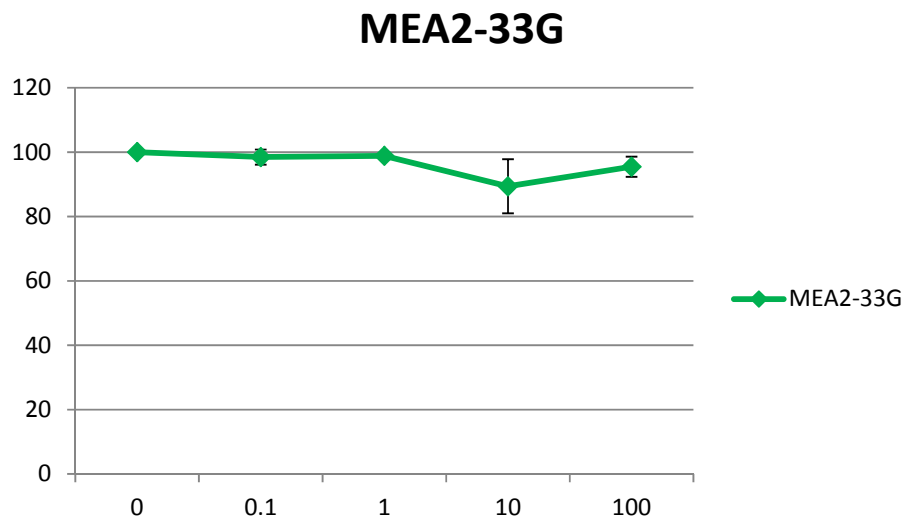
	(µg/ml)				
	0	**0.1**	**1**	**10**	**100**
	100	98.6	101.1	108.0	41.7
	100	109.0	102.4	108.4	44.1
	100	111.5	103.6	136.6	65.2
Mean	**100**	**106.3**	**102.4**	**117.7**	**50.3**
SE	**0**	**4.0**	**0.7**	**9.5**	**7,5**

Sigmoidal dose-response
LD50 112.1

LD50 95% Confidence Interval 49.87 to 251.8

Goodness of Fit	R square	0,9109	Absolute Sum of Squares	0.2398

Compound 3

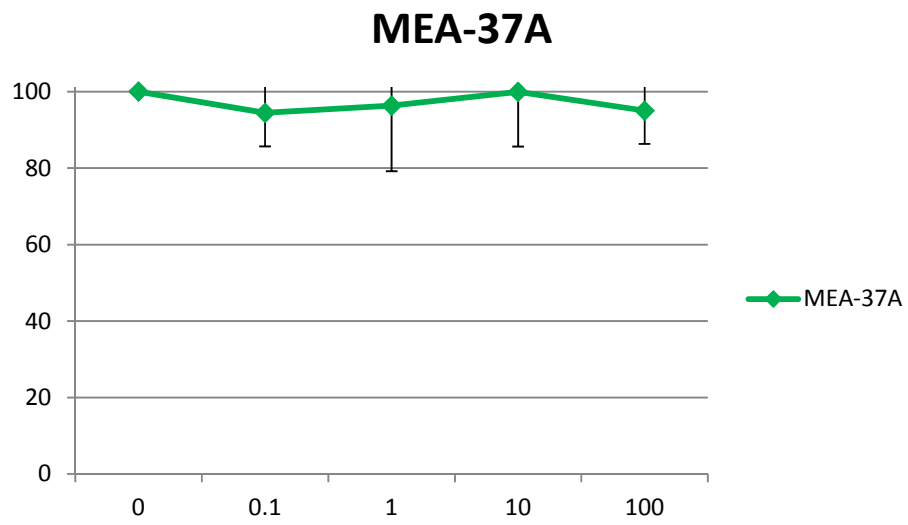


	0	0.1	1	10	100
	100	100.8	97.3	72.7	97.8
	100	100.8	99.4	95.5	89.3
	100	93.8	99.9	99.9	99.4
Mean	**100**	**98.5**	**98.9**	**89.4**	**95.5**
SE	**0**	**2.34**	**0.8**	**8.4**	**3.1**

Sigmoidal dose-response
Best-fit values:

LD50 Undetermined, >100

Compound 4

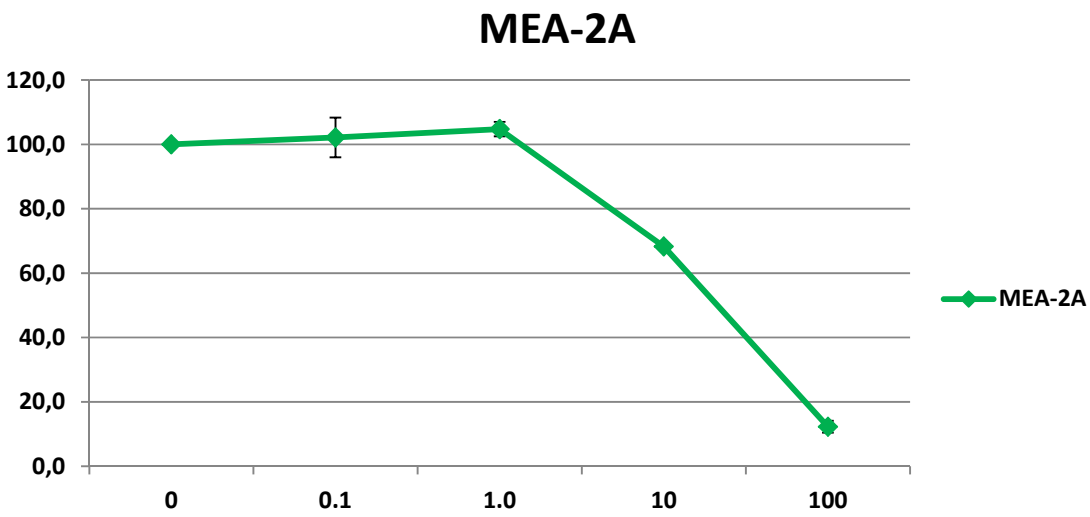


	(µg/ml)				
	0	**0.1**	**1**	**10**	**100**
	100	97.8	127.5	120.2	93.4
	100	107.7	93.0	107.2	110.8
	100	77.8	68.5	72.3	80.8
Mean	**100**	**94.4**	**96.3**	**99.9**	**95.0**
SE	**0**	**8.8**	**17.1**	**14.3**	**8.7**

Sigmoidal dose-response
Best-fit values:

LD50 Undetermined, >100

Compound 5



(µg/ml)

	0	**0.1**	**1**	**10**	**100**
	100	90.1	100.3	68.2	16.0
	100	105.8	106.4	66.9	10.5
	100	110.6	107.6	69.7	10.4
Mean	**100**	**102.1**	**104.8**	**68.3**	**12.3**
SE	**0**	**6.2**	**2.2**	**0.8**	**1.9**

Sigmoidal dose-response
LD50 22.8

LD50 95% Confidence Intervals **13.80 to 37.73**

Goodness of Fit R square 0.9771 Absolute Sum of Squares 0.04316