Electronic Supplementary Material (ESI) for RSC Advances.

Endophyte inspired chemical diversity from *beta*-caryophyllene

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Contents

General Procedures 2

Fermentatiuon Biotransformation of *β*-Caryophyllene 2

Calculation Procedures 4

Figure S1. HPLC profiles of *A. tubingensis* KJ-9 fermentation 4

Figure S2. Assignments of ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR Data 5

Figure S3. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 1 7

Figure S4. HSQC of 1 8

Figure S5. ^{1}H-^{1}H COSY 9

Figure S6. HMBC of 1 10

Figure S7. NOESY of 1 11

Figure S8. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 2 12

Figure S9. HSQC spectrum of 2 13

Figure S10. COSY spectrum of 2 14

Figure S11. HMBC spectrum of 2 15

Figure S12. NOESY spectrum of 2 16

Figure S13. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 3 17

Figure S14. HSQC of 3 18

Figure S15. COSY of 3 19

Figure S16. HMBC of 3 20

Figure S17. NOESY of 3 21

Figure S18. ^{1}H (125M Hz) and ^{13}C NMR of 4 22

Figure S19. HSQC of 4 23

Figure S20. ^{1}H-^{1}H COSY of 4 24

Figure S21. HMBC of 4 25

Figure S22. NOESY of 4 26

Figure S23. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 5 27

Figure S24. HSQC of 5 28

Figure S25. ^{1}H-^{1}H COSY of 5 29
Figure S26. HMBC of 5 30
Figure S27. NOESY of 5 31
Figure S28. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR spectra of 6 32
Figure S29. HSQC of 6 33
Figure S30. ^{1}H-^{1}H COSY of 6 34
Figure S31. HMBC of 6 35
Figure S32. NOESY of 6 36
Figure S33. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 7 37
Figure S34. HSQC of 6 38
Figure S35. ^{1}H-^{1}H COSY of 6 39
Figure S36. HMBC of 6 40
Figure S37. NOESY of 6 41

General Procedures

UV spectra were obtained using a Evolution 300 UV-vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). IR were recorded on a Bruker Tensor 27 spectrophotometer with KBr disks (Bruker Corp., German). Optical rotations were measured on a Autopol III automatic polarimeter (Rudolph Research Analytical, USA). CD spectrum were obtained on a Chirascan CD Spectrometer (Applied Photophysics Ltd., United Kingdom). ESI-MS was performed on a LTQ Fleet instrument (Thermo Fisher Scientific Inc., USA). HR ESIMS was performed on an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies Ltd., USA). 1D and 2D NMR spectra were recorded on a AVANCE III (500 MHz) instrument (Bruker Corp., German). Chemical shifts were reported using solvent residual as the internal standard. High performance liquid chromatography (HPLC) analysis and semi-preparation was performed on a Waters 1525 instrument (Waters Corp.). Column chromatography was performed on silica gel (90−150 μm; Qingdao Marine Chemical Inc., China), MCI gel (75−150 μm; Mitsubishi Chemical Corp., Japan), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex C_{18} gel (40−75 μm; Fuji Silysia Chemical LTD., JPN). GF_{254} plates (Qingdao Marine Chemical Inc., China) were used for thin-layer chromatography (TLC).

Fermentatiuon Biotransformation of β-Caryophyllene

The microorganism *Aspergillus tubingensis* KJ-9 was incubated in potato dextrose agar (PDA) at 25℃ for 3 days. One piece (approximately 7 mm^2) of mycelium and liquid state β-Caryophyllene with no solvent (333 μL) was fermented aseptically to a

500ml Erlenmeyer flask containing 150 mL of potato dextrose (PD) liquid medium. 47 flasks were shook at 130 rpm, 27±0.5 ^{O}C for 10 days. Another 3 parallel controls were conducted at the same time. Ethyl acetate (EtOAc) extraction of the fermentation and the controls were compared on HPLC with an Agilent TC-C18 column (250×4.6 mm, 10--100% methanol in 30 min, 1ml/min, detected at 254 and 210nm).

The fermentation broth was extracted by EtOAc to yield 12g of residue A. The residue was then chromatographed on a silica flash collumn (eluted with gradient Chloroform-Methanol) to give four fractions (Fr.1-Fr.4). Fr.2 (3.4g) was subjected on a Sephadex LH-20 (Methanol) and silica flash chromatography (eluted with gradient Petroleum ether-Acetone) to give three subfractions (Fr.21-Fr.23). Fr.21 (760mg) was purified on a semi-preparative HPLC C_{18} column (Thermo BDS Hypersil 250×10 mm, eluted with 64% Methanol) to afford compound **3** (14mg), compound **5** (24mg) and compound **7** (21mg). Fr.23 (380mg) was seperated on the semi-preparative HPLC C_{18} column (eluted with 55% Methanol) to yield compound **1** (16mg), compound **2** (2.8mg), compound **4** (24mg). Fr.3 (3.9g) was chromatographed with Sephadex LH-20 (methanol) and flash ODS column to give seven subfractions (Fr.31-Fr.37). Fr.36 (197mg) was separated on the semi-preparative HPLC C_{18} column (eluted with 63% methanol) to yield compound **6** (18mg).

Compound 1: white amorphous solid; $[\alpha]_D^{29}$ –129.0 (*c* 0.14, $CHCl_3$); UV (MeCN) $\lambda_{max}(\log\varepsilon)$ 248 nm (3.91); CD (MeCN) λ ($\Delta\varepsilon$ in $cm^{-1}M^{-1}$) 205 (–9.3), 224 (–3.1), 248 (–7.8), 329 (+1.1) nm; IR (KBr) ν_{max} 3402, 2956, 2873, 2801, 1653, 1602, 1459, 1380, 1283, 1205, 1048, 1031 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Figure S2; ESIMS *m/z* 235.10 $[M+H]^+$; HR ESIMS *m/z* 257.1508 $[M+Na]^+$(calcd. for $C_{15}H_{22}O_2$ $[M+Na]^+$ 257.1512).

Compound 2: white amorphous solid; $[\alpha]_D^{26}$ –115.6 (*c* 0.09, $CHCl_3$); UV (MeCN) $\lambda_{max}(\log\varepsilon)$ 244 nm (3.98); CD (MeCN) λ ($\Delta\varepsilon$ in $cm^{-1}M^{-1}$) 210 (+10.8), 246 (–7.4), 335 (+1.7) nm; IR (KBr) ν_{max} 3423, 2925, 2873, 1645, 1613, 1461, 1379, 1272, 1234, 1032 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Figure S2; ESIMS *m/z* 235.21 $[M+H]^+$; HR ESIMS *m/z* 257.1504 $[M+Na]^+$(calcd. for $C_{15}H_{22}O_2$ $[M+Na]^+$ 257.1512).

Compound 3: colorless oil; $[\alpha]_D^{26}$ –11.8 (*c* 0.12, $CHCl_3$); UV (MeCN) $\lambda_{max}(\log\varepsilon)$ 193 nm (3.85); CD (MeCN) λ ($\Delta\varepsilon$ in $cm^{-1}M^{-1}$) 207 (–4.7), 239 (+8.1) nm; IR (KBr) ν_{max} 3464, 2952, 2928, 1669, 1459, 1388, 1373, 1308, 1265, 1198, 1089, 1024 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Figure S2; ESIMS *m/z* 235.23 $[M+H]^+$; HR ESIMS *m/z* 257.1505 $[M+Na]^+$(calcd. for $C_{15}H_{22}O_2$ $[M+Na]^+$ 257.1512).

Compound 4: colorless oil; $[\alpha]_D^{29}$ –89.3 (*c* 0.08, $CHCl_3$); UV (MeCN) $\lambda_{max}(\log\varepsilon)$ 237 nm (4.13); CD (MeCN) λ ($\Delta\varepsilon$ in $cm^{-1}M^{-1}$) 201 (+8.4), 234 (–4.4) nm; IR (KBr) ν_{max} 3440, 2949, 2870, 2852, 1660, 1442, 1379, 1685, 1319, 1296, 1252, 1048 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Figure S2; ESIMS *m/z* 257.12 $[M+Na]^+$; HR ESIMS *m/z* 257.1506 $[M+Na]^+$(calcd. for $C_{15}H_{22}O_2$ $[M+Na]^+$ 257.1512).

Compound 5: colorless oil; $[\alpha]_D^{26}$ –93.1 (*c* 0.11, $CHCl_3$); UV (MeCN) $\lambda_{max}(\log\varepsilon)$ 193 nm (4.28); CD (MeCN) λ ($\Delta\varepsilon$ in $cm^{-1}M^{-1}$) 200 (+11.7), 226 (–3.0) nm; IR (KBr) ν_{max} 3437, 2952, 2931, 2874, 1726, 1665, 1631, 1437, 1378, 1316, 1250, 1134 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Figure S2; ESIMS *m/z* 263.20 $[M+H]^+$; HR ESIMS *m/z* 285.1452 $[M+Na]^+$(calcd. for

$C_{16}H_{22}O_3$ $[M+Na]^+$ 285.1461).

Compound 6: crystal; $[\alpha]_D^{21}$ +60.5 (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 3277, 2959, 2924, 2868, 1448, 1386, 1033, 993 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Figure S2; EIMS *m/z* 236 $[M]^+$; HR EIMS *m/z* 236.1775 $[M]^+$(calcd. for $C_{15}H_{24}O_2$ $[M]^+$ 236.1776).

Compound 7: colorless oil; $[\alpha]_D^{29}$ +46.1 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 3441, 2927, 2868, 1459, 1732, 1379, 1068, 1016 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Figure S2; EIMS *m/z* 250 $[M]^+$; HR EIMS *m/z* 250.1935 $[M]^+$(calcd. for $C_{16}H_{26}O_2$ $[M]^+$ 250.1933).

Calculation Procedures

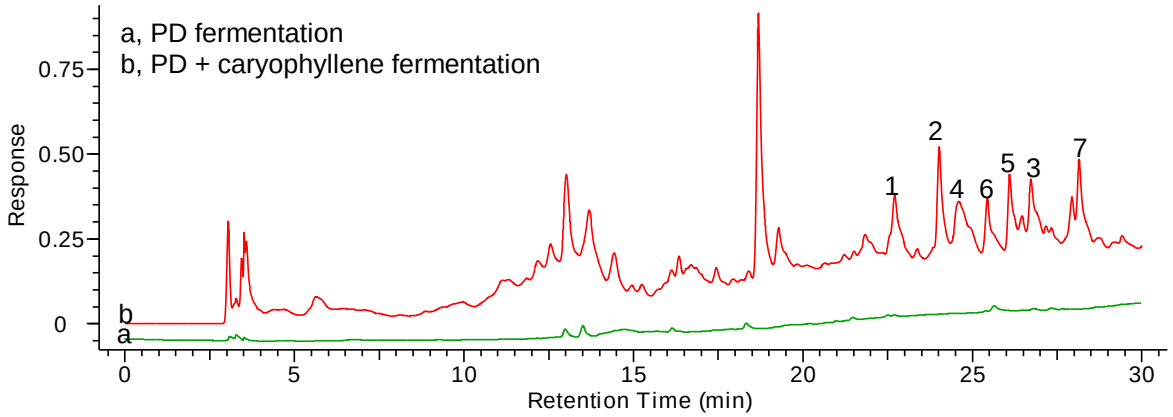
A preliminary conformational search was performed in Conflex6.7 using MMFF94s forcefield.[1] Conformers were saved and further optimized using the density functional theory (DFT) method and CPCM solvent model at B3LYP/6-311++G(d,p) level in Gaussian 09 software package.[2] Frequency was calculated at the same level of theory to check optimized results. The stable conformers with populations greater than 1% and without imaginary frequencies were submitted to ECD calculation by the TDDFT [B3LYP/aug-cc-pVDZ or cam-B3LYP/TZVP] method associated with CPCM solvent model in MeCN. The excitation energies (E), oscillator strength (f), rotatory strength in velocity form (R_{vel}), and rotatory strength in length form (R_{len}) of the lowest 32 excited states were calculated. ECD spectra of different conformers were summated in SpecDis 1.62 according to their Boltzmann-calculated distributions.[3] The half bandwidths and UV-shifts of CD summation were recorded as Table S1.

Table S1 Parameters in CD curves summation

Compd.	NC*	CD calculation method	half bandwidth (σ, eV)	UV-shifts (nm)
1	6	Cam-B3LYP/TZVP	0.30	+6
2	6	B3LYP/aug-cc-pVDZ	0.30	–11
3	1	Cam-B3LYP/TZVP	0.30	+5
5	2	B3LYP/aug-cc-pVDZ	0.29	–9

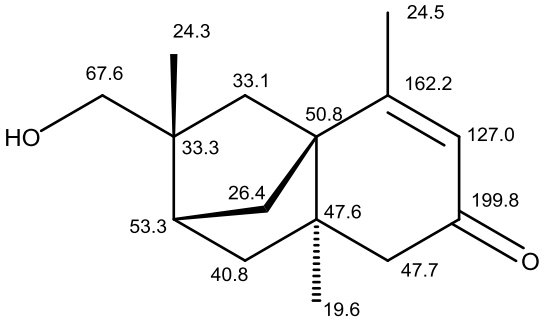
*NC, number of stable conformers

Figure S1. HPLC profiles of *A. tubingensis* KJ-9 fermentation



Chromatographi ccondition: Agilent TC-C18 column, gradient 10-100% MeOH in 30 min, detected by 230 nm

Figure S2. Assignments of ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR Data



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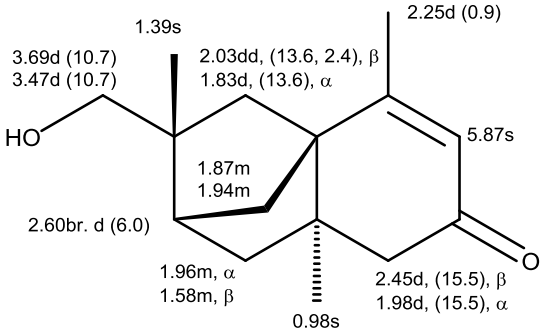

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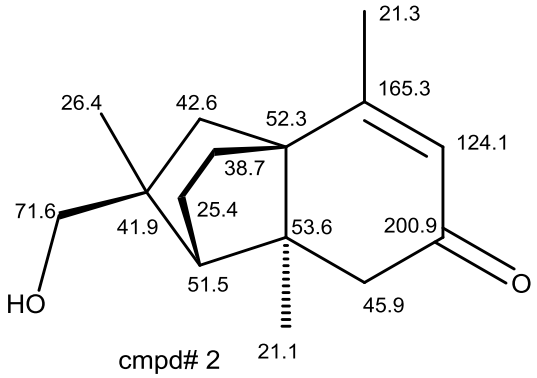

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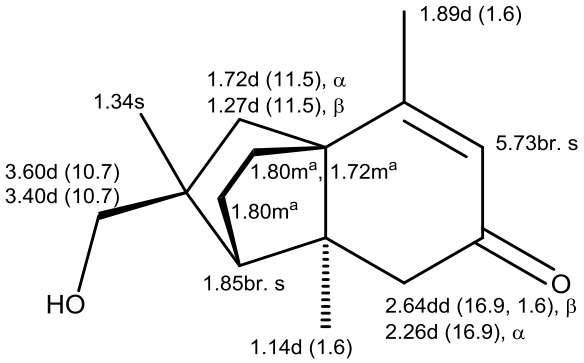

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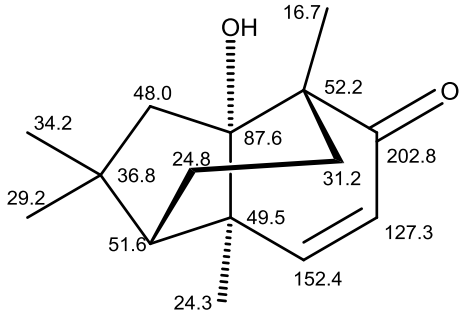

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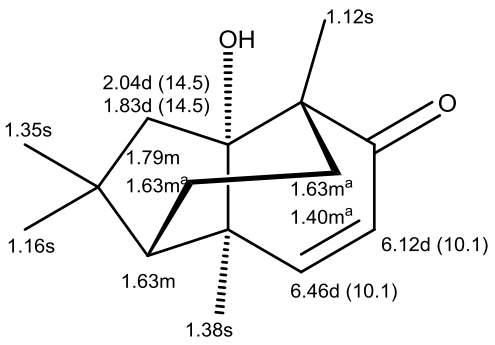

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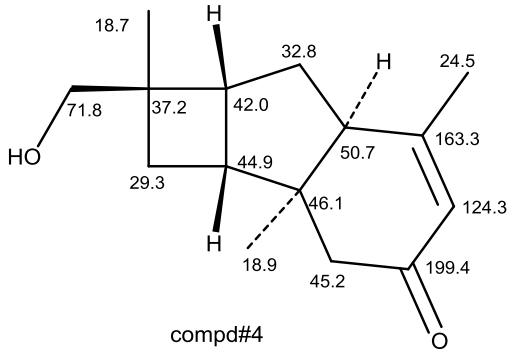

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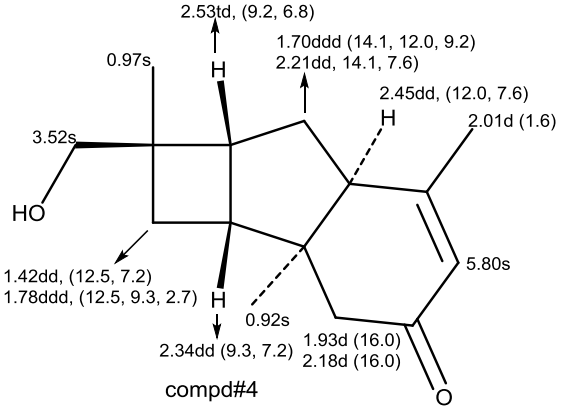

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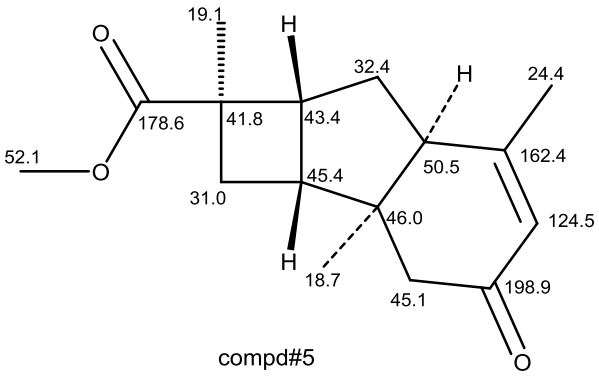

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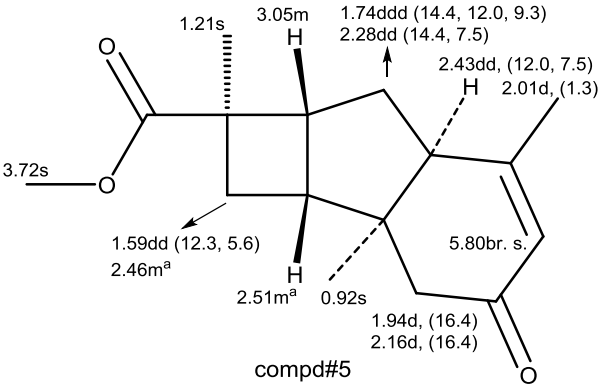

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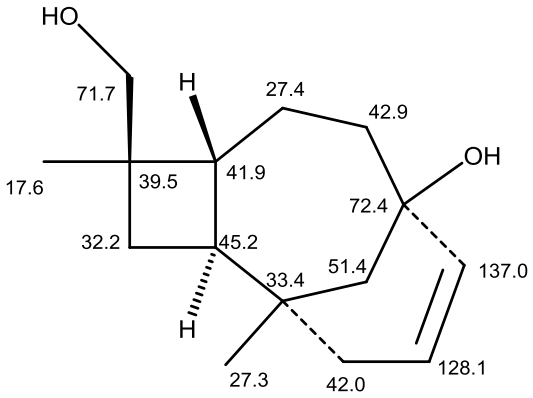

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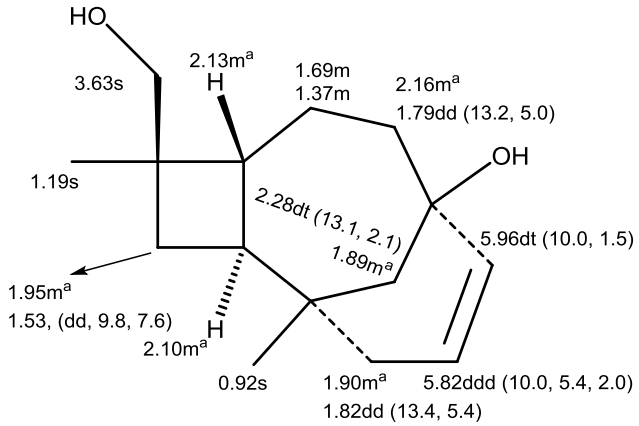

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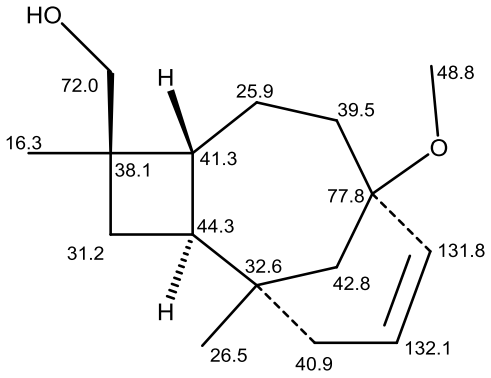

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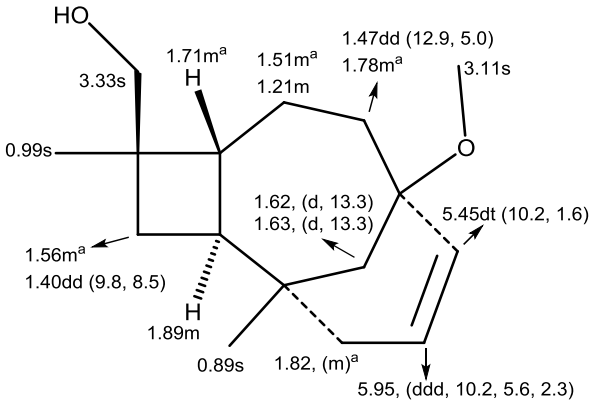

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δ in ppm, J in Hz; [a]overlapped

Figure S3. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 1

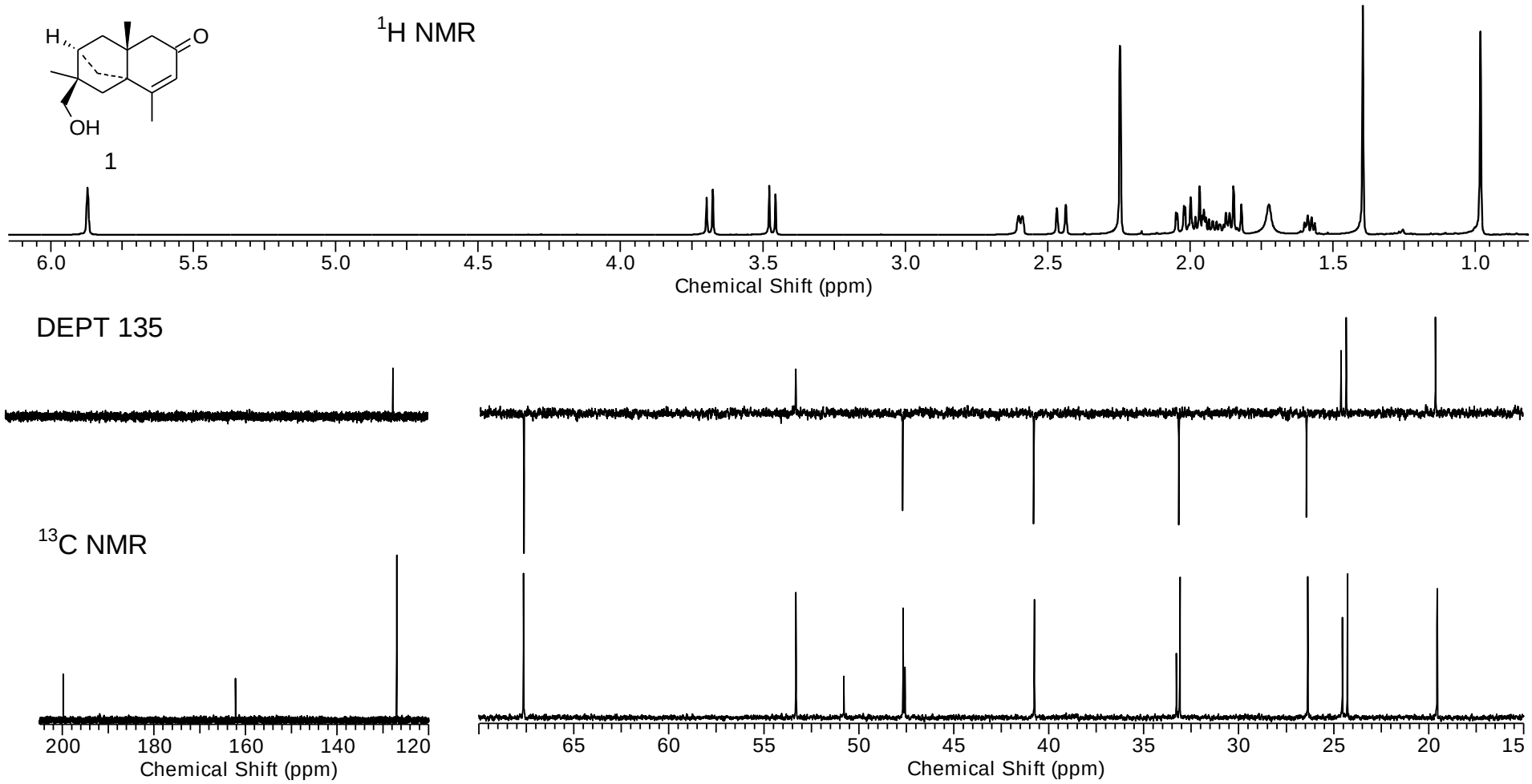


Figure S4. HSQC of 1

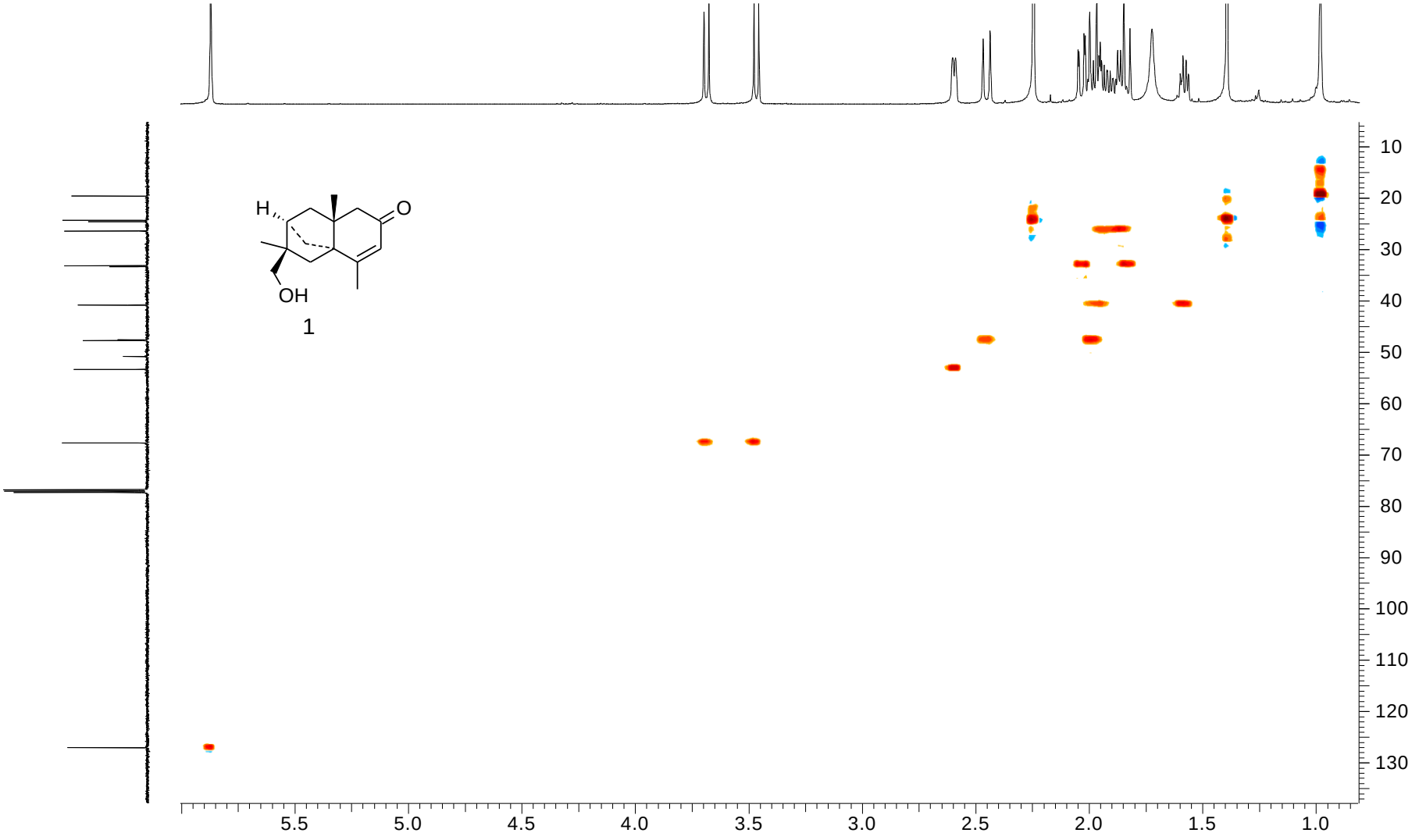


Figure S5. ^{1}H-^{1}H COSY

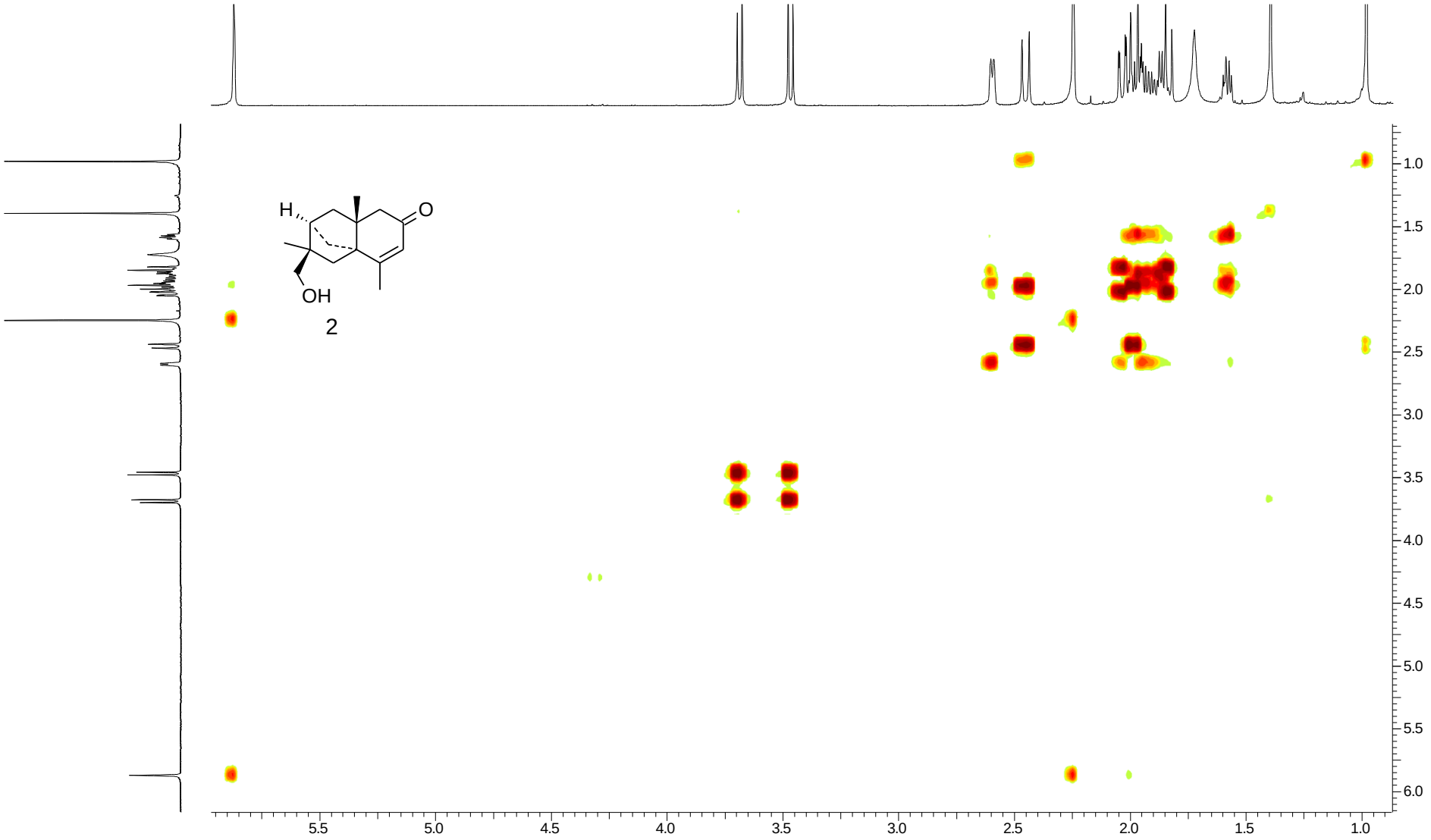


Figure S6. HMBC of 1

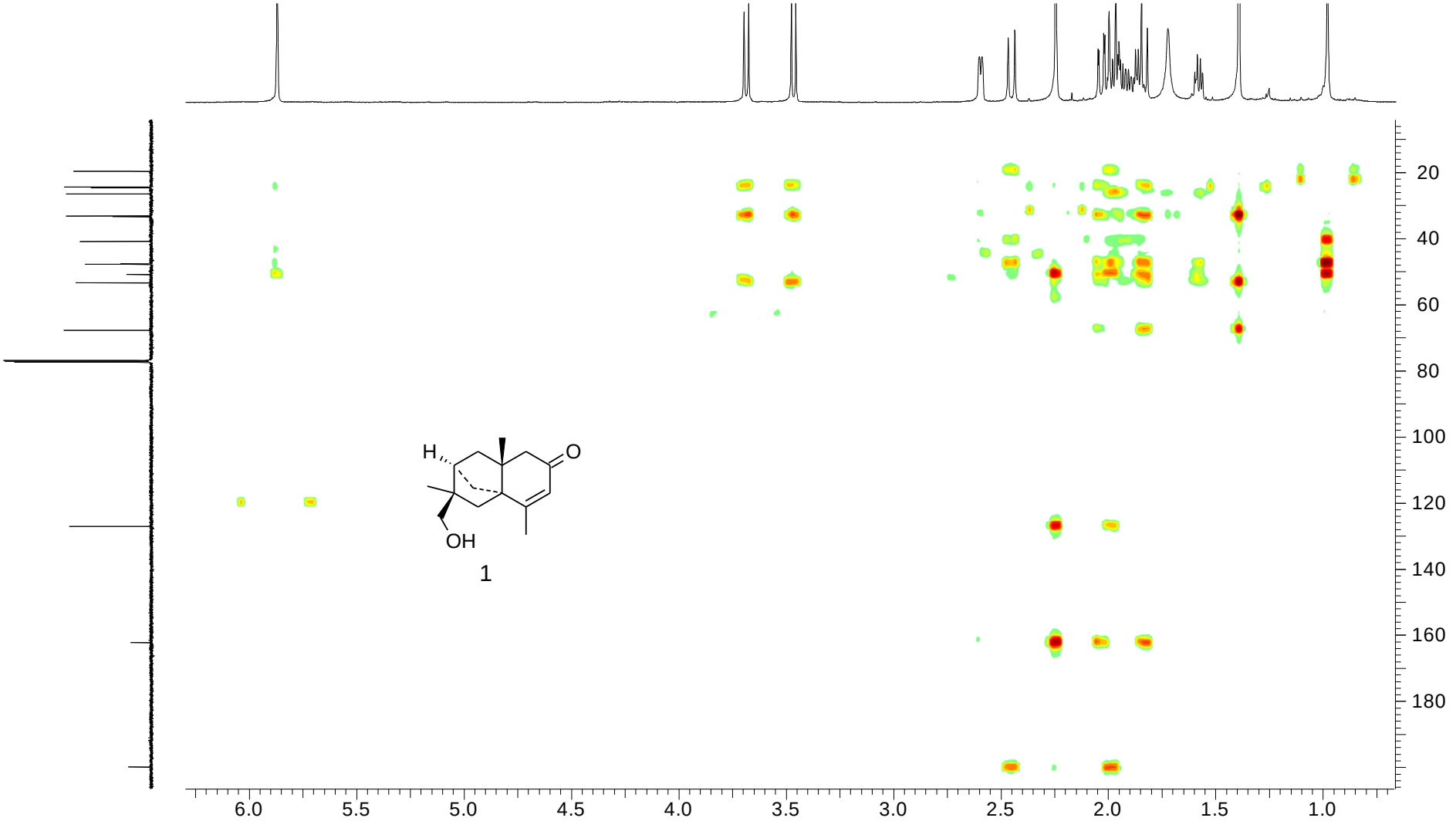


Figure S7. NOESY of 1

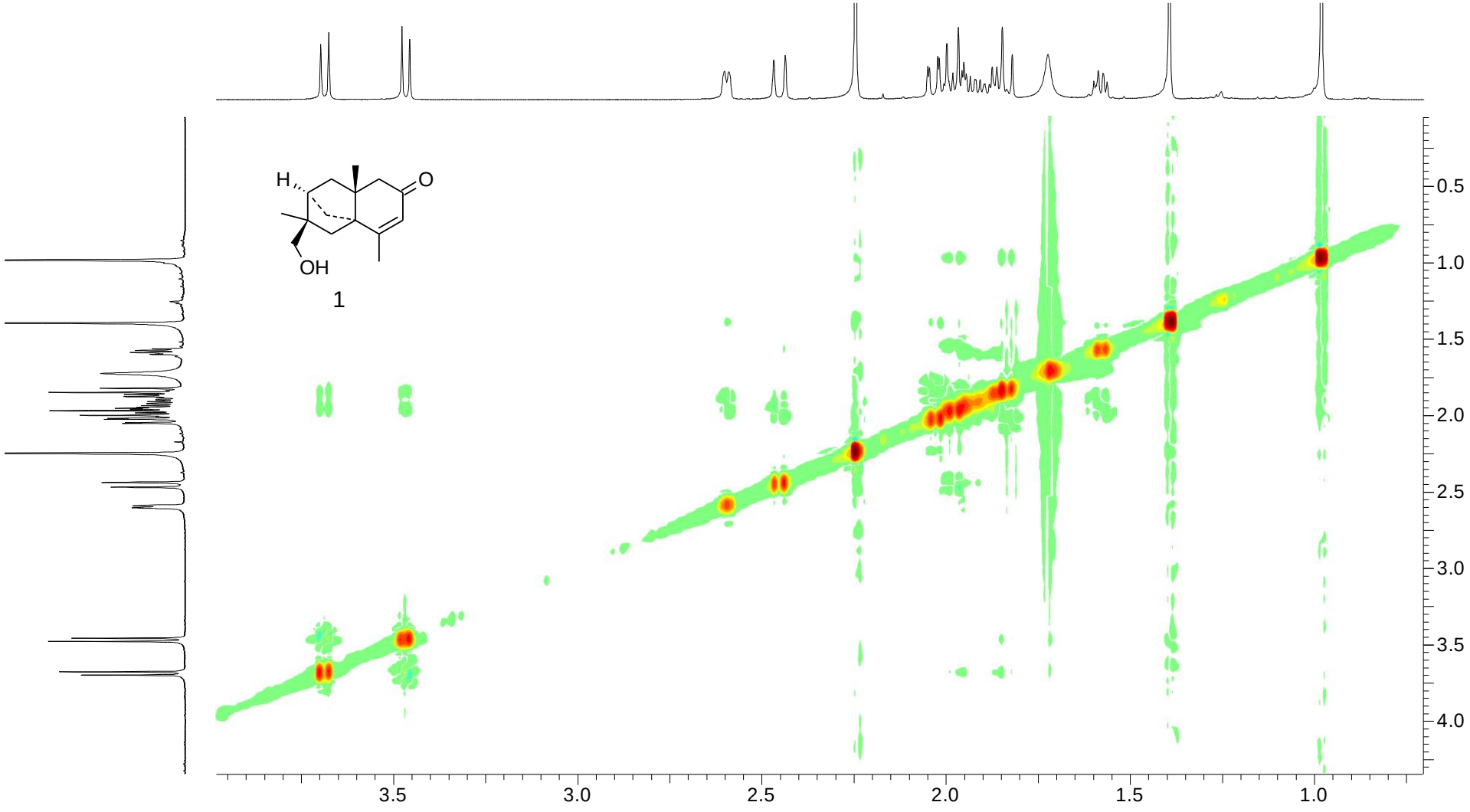


Figure S8. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 2

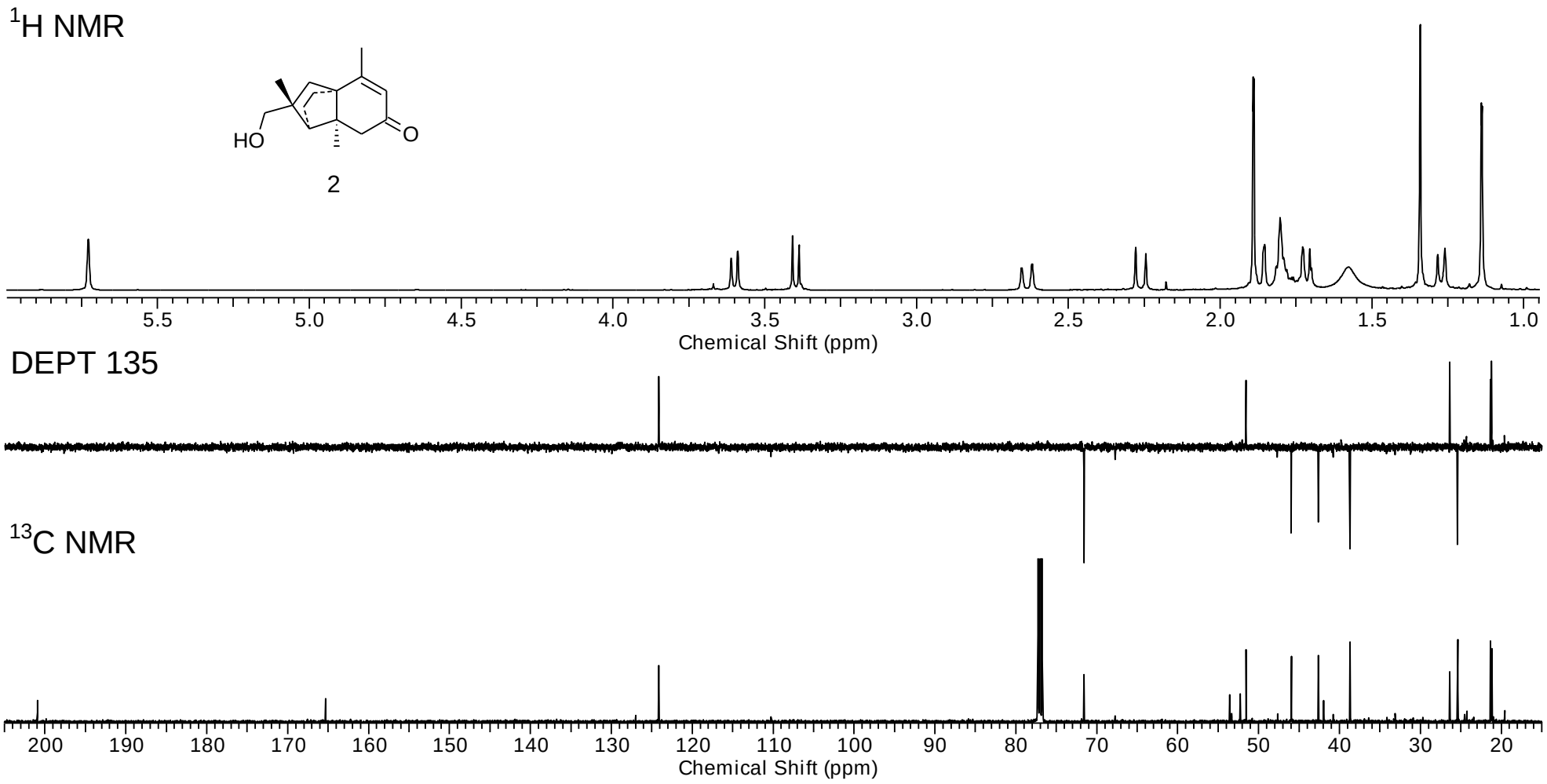


Figure S9. HSQC spectrum of 2

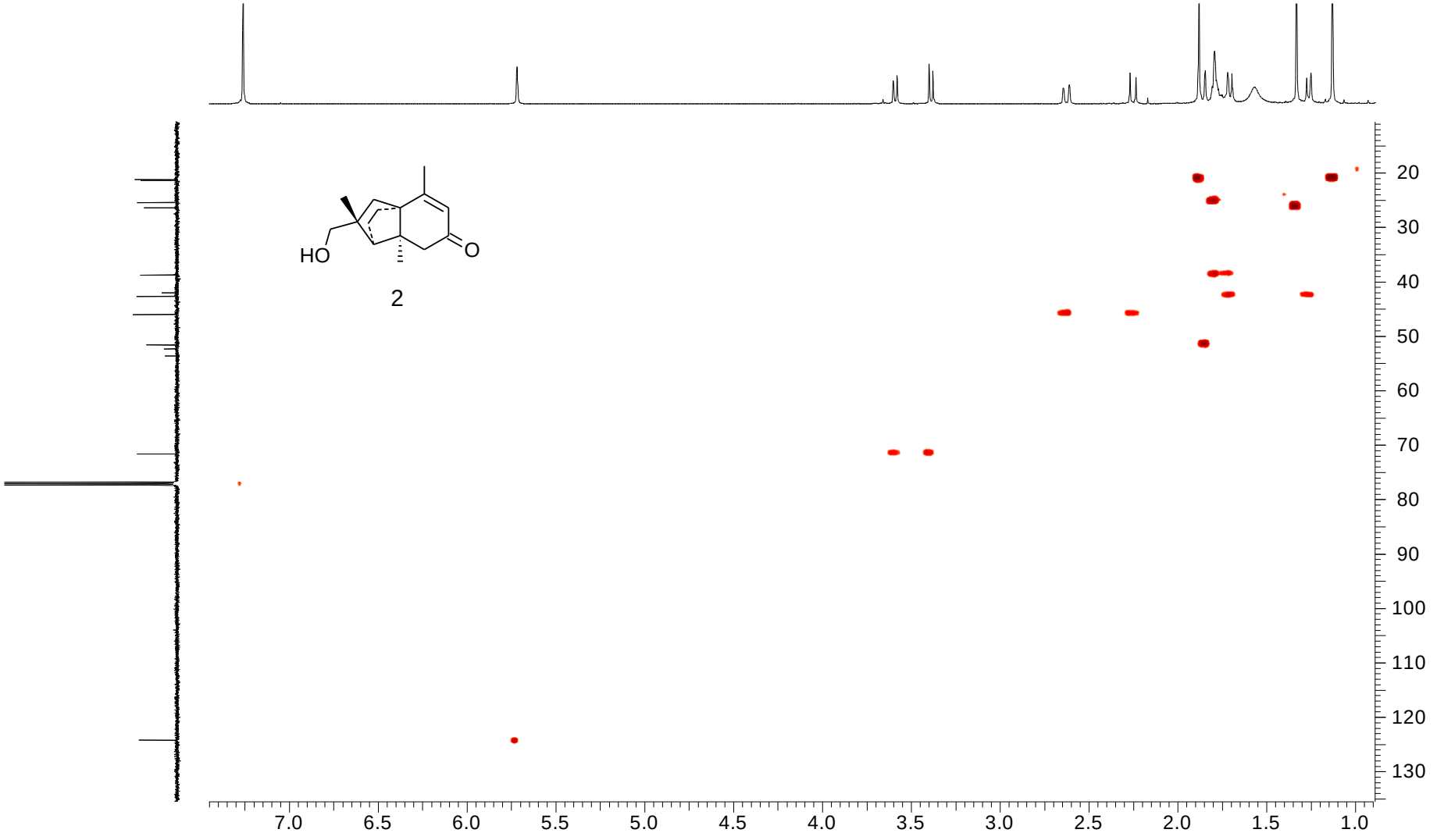


Figure S10. COSY spectrum of 2

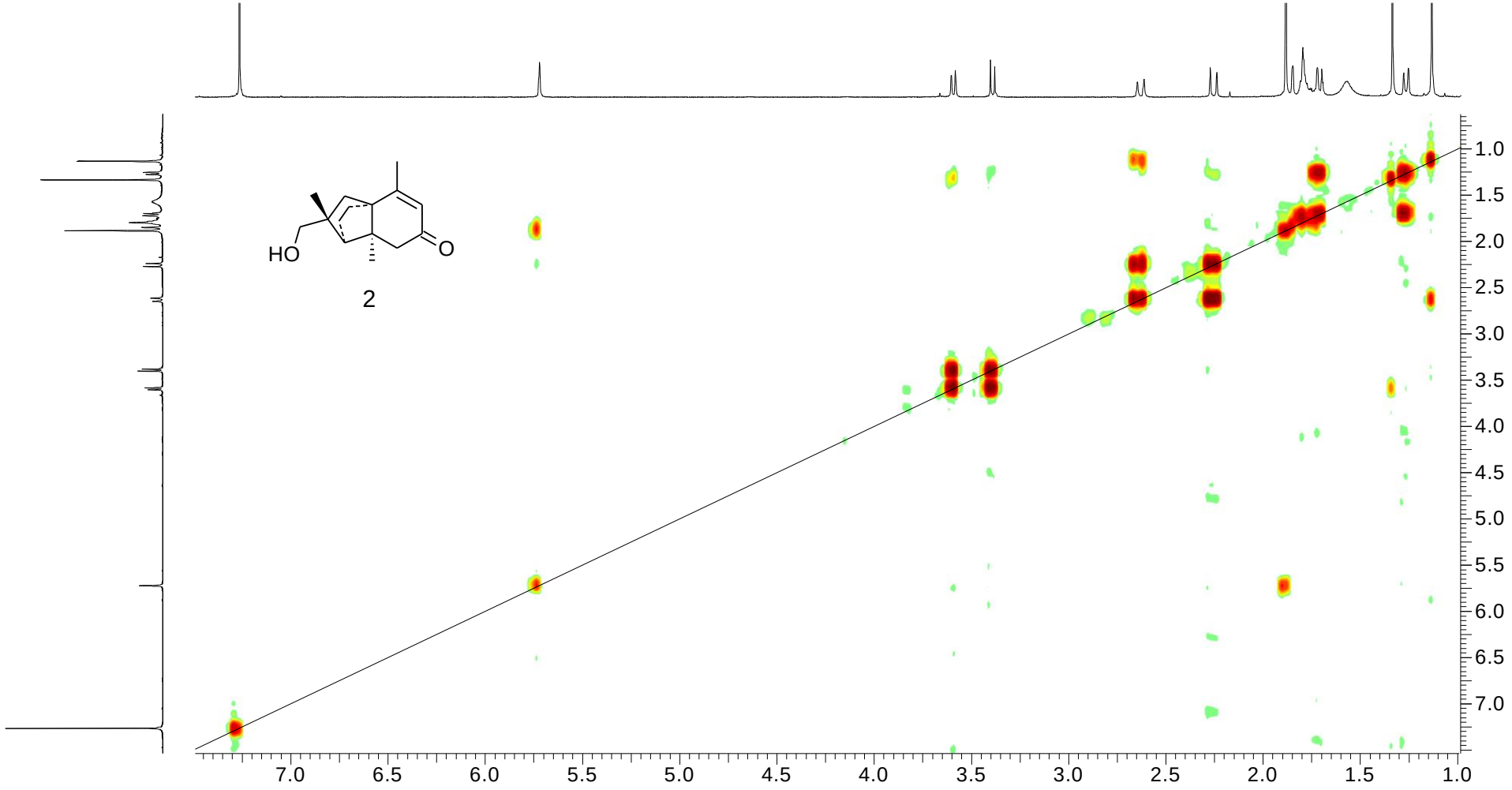


Figure S11. HMBC spectrum of 2

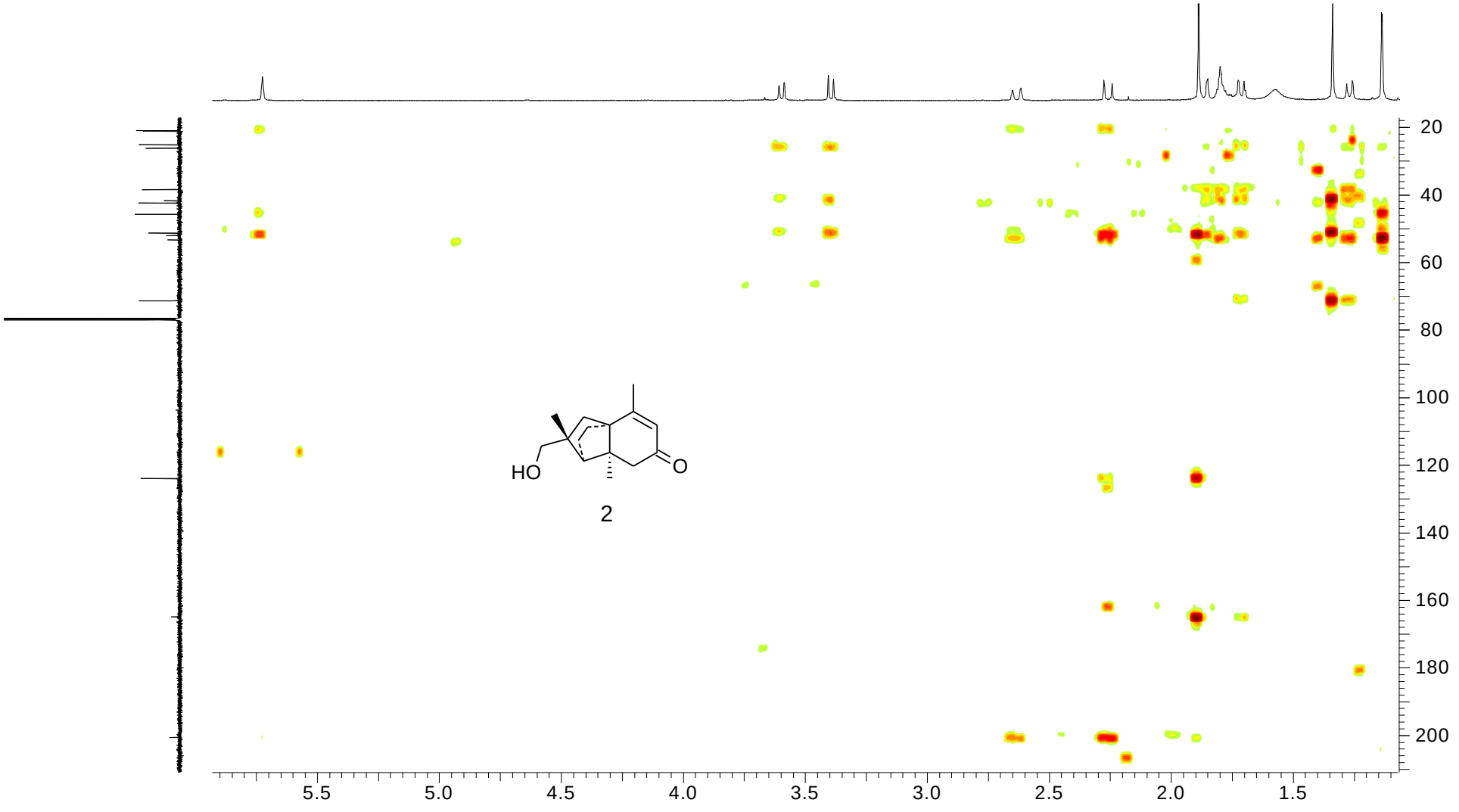


Figure S12. NOESY spectrum of 2

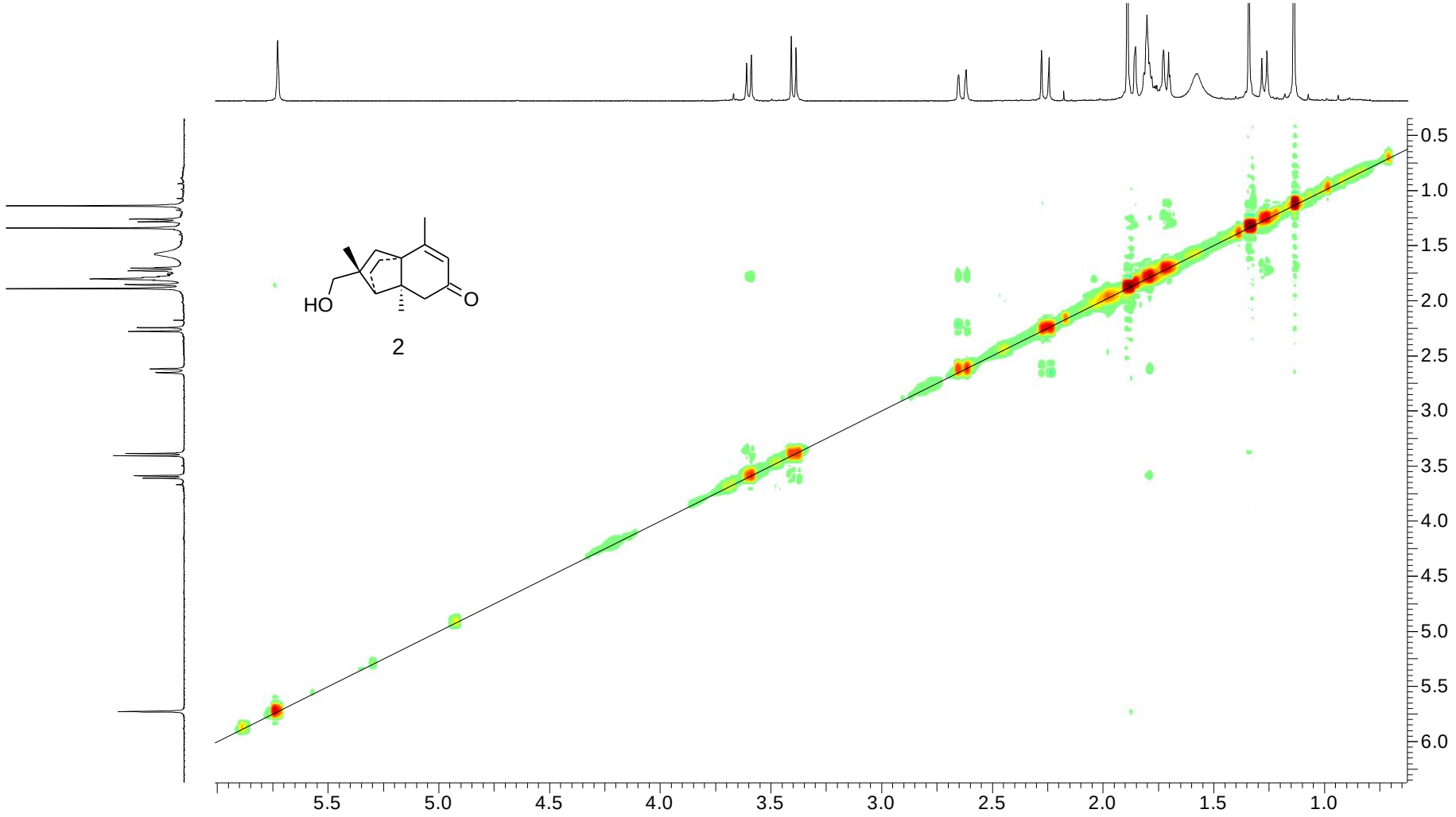


Figure S13. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 3

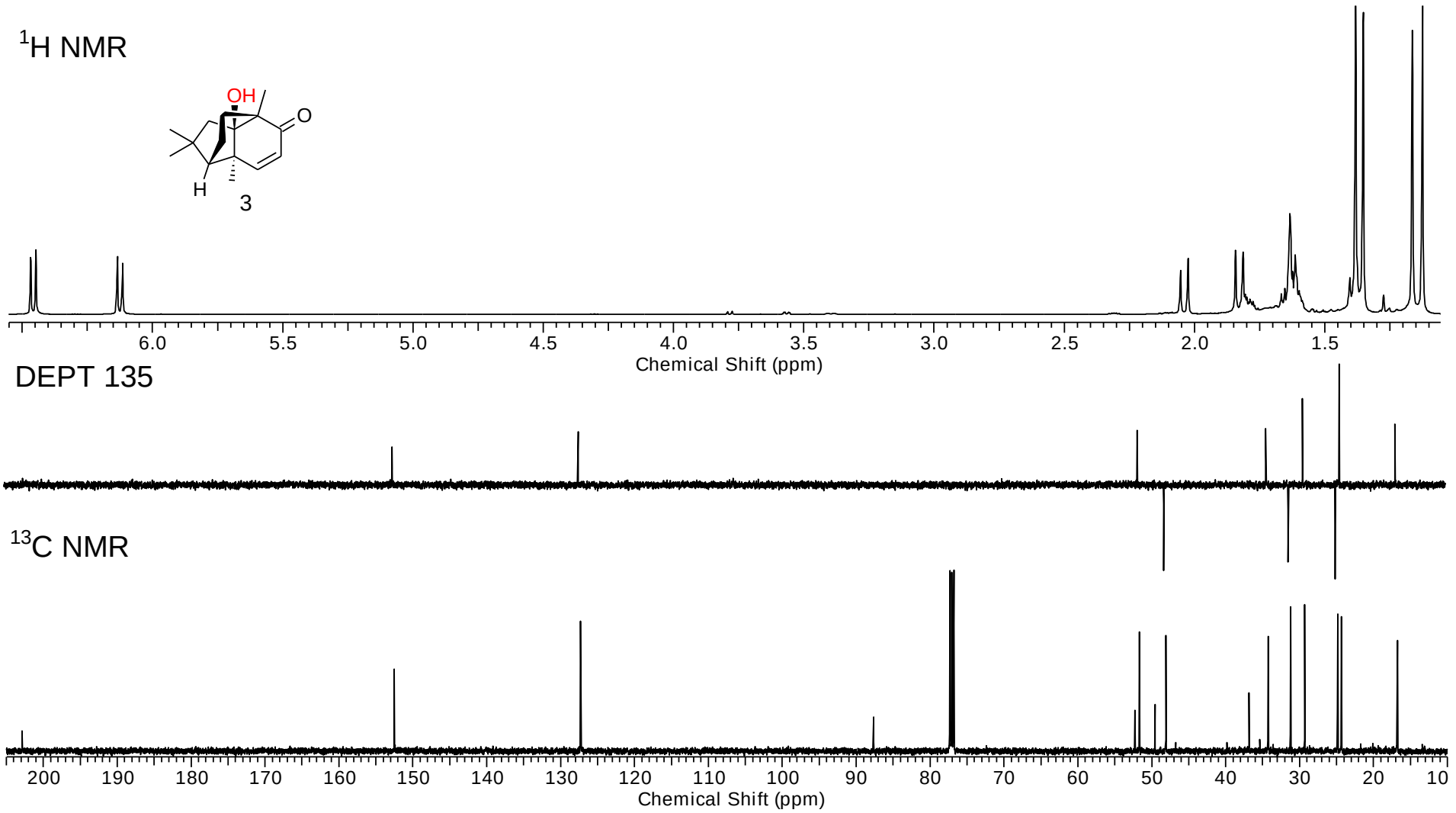


Figure S14. HSQC of 3

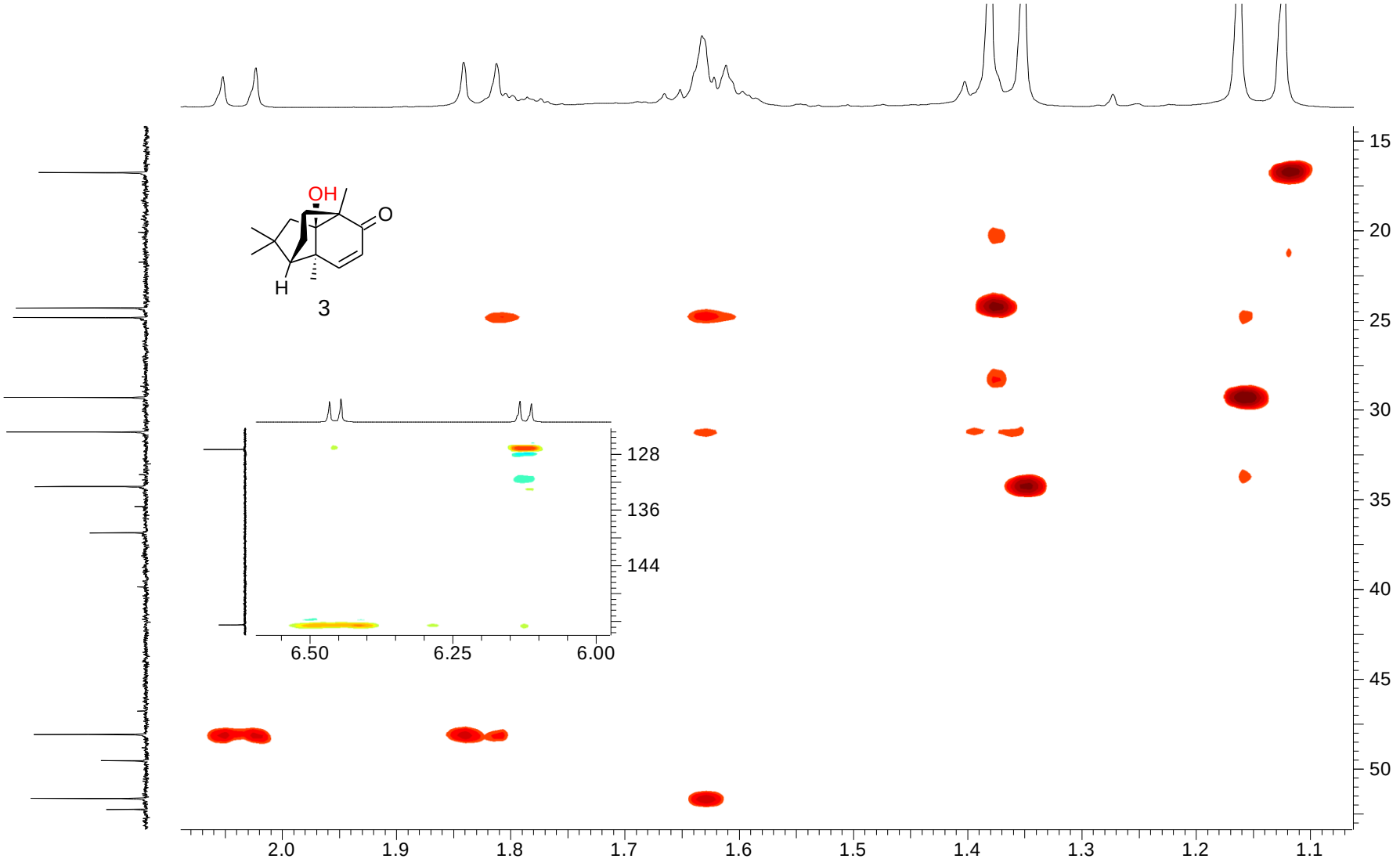


Figure S15. COSY of 3

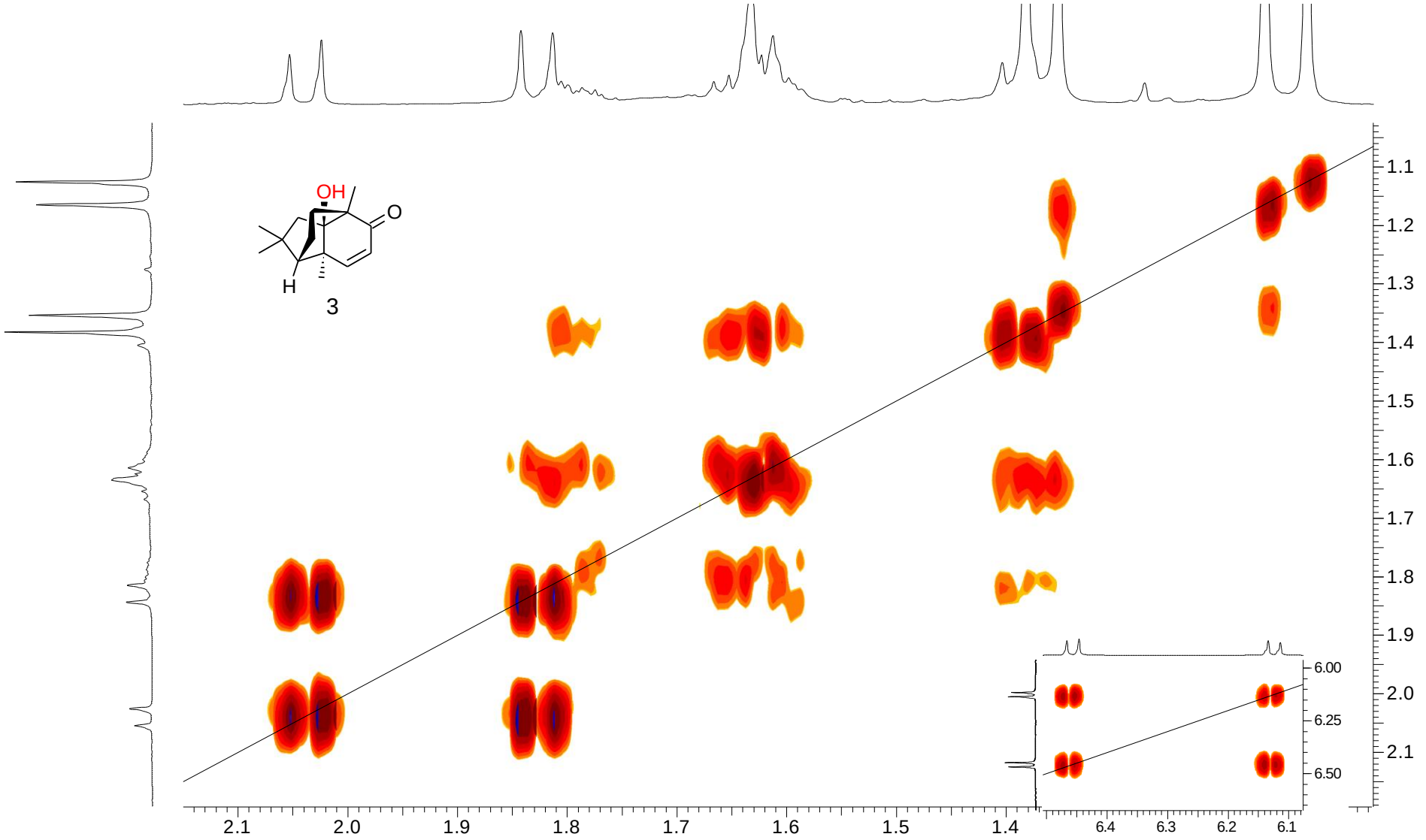
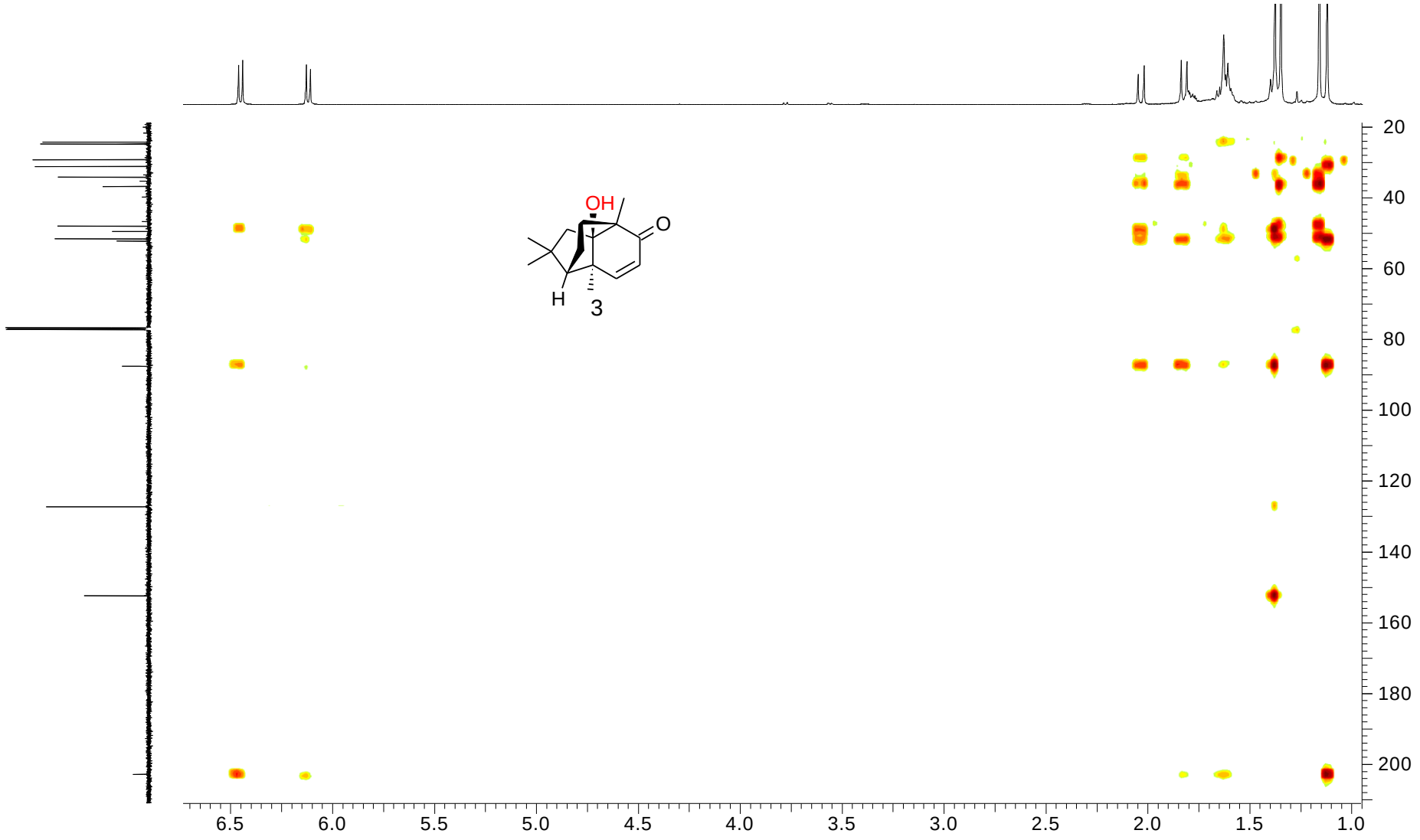


Figure S16. HMBC of 3



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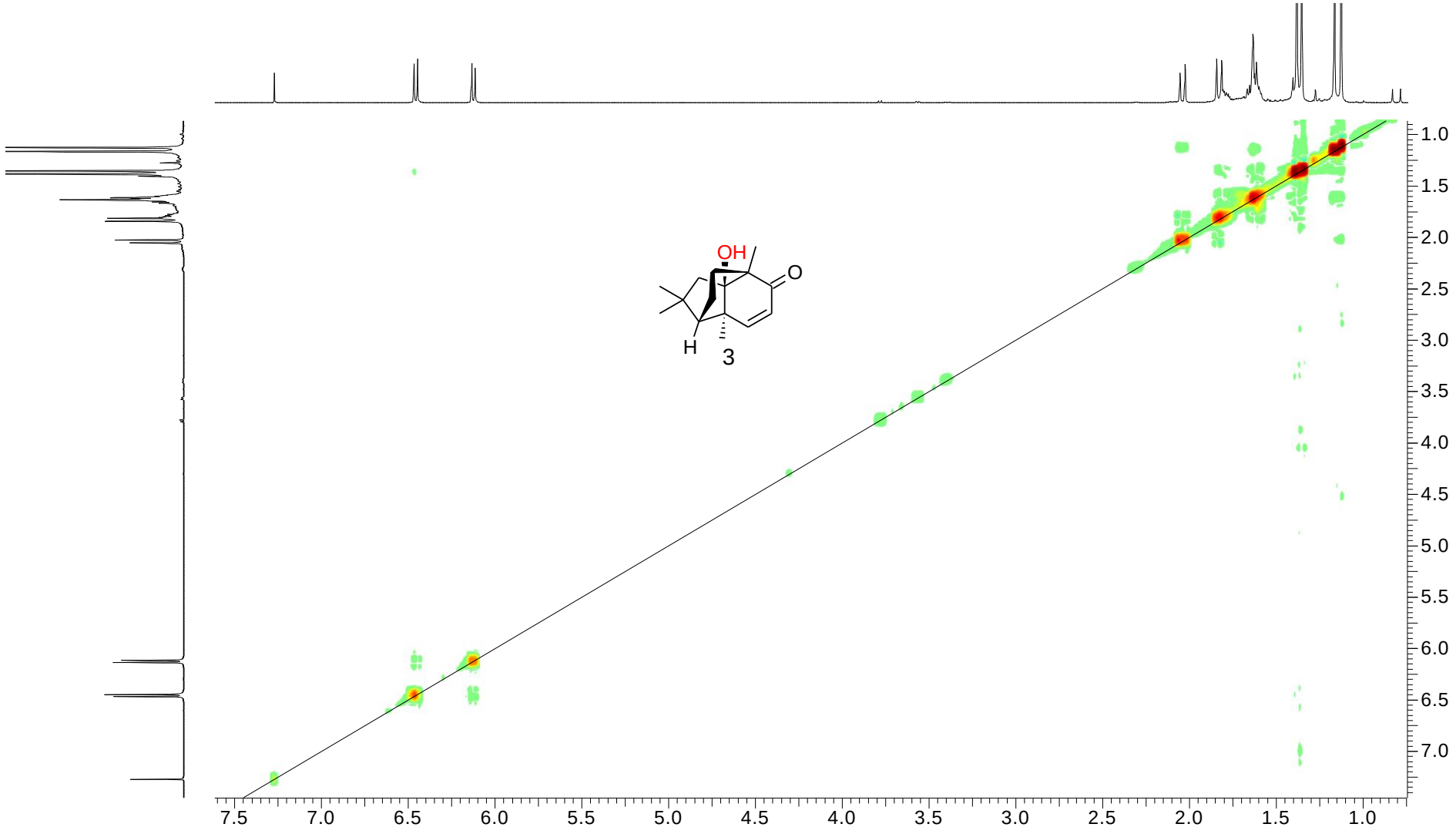

Figure S18. ^{1}H (125M Hz) and ^{13}C NMR of 4

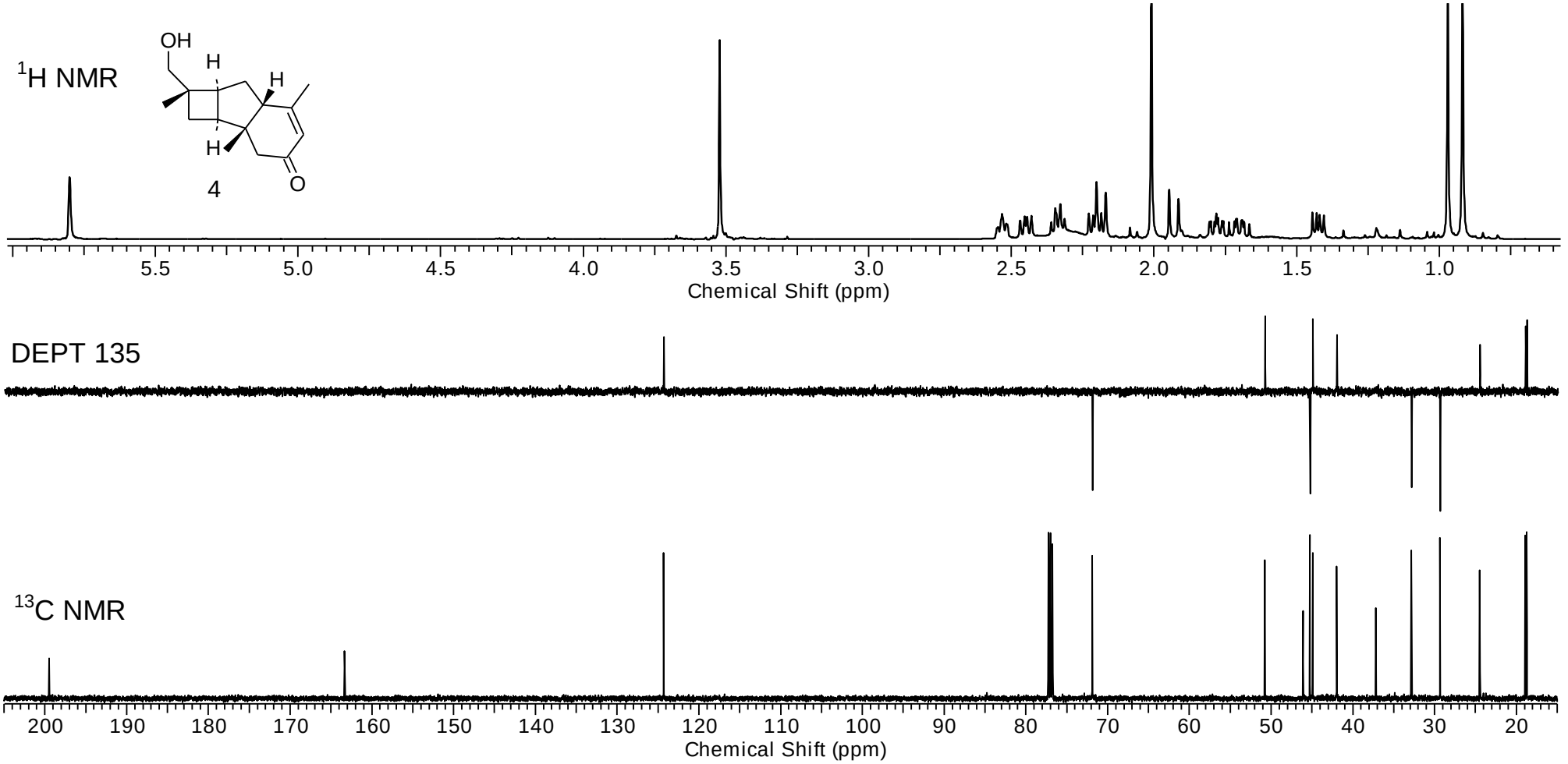

Figure S19. HSQC of 4

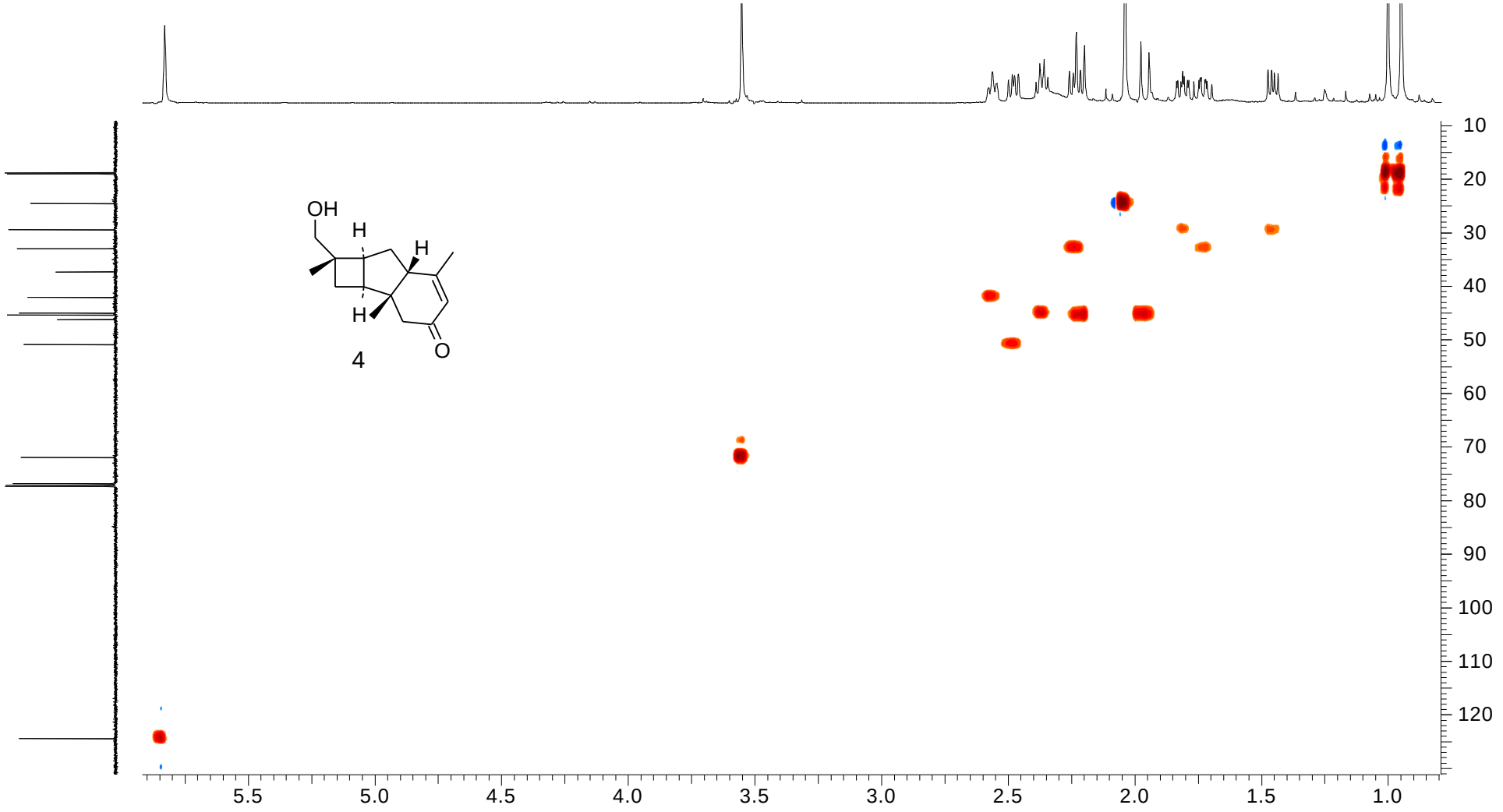

Figure S20. ^{1}H-^{1}H COSY of 4

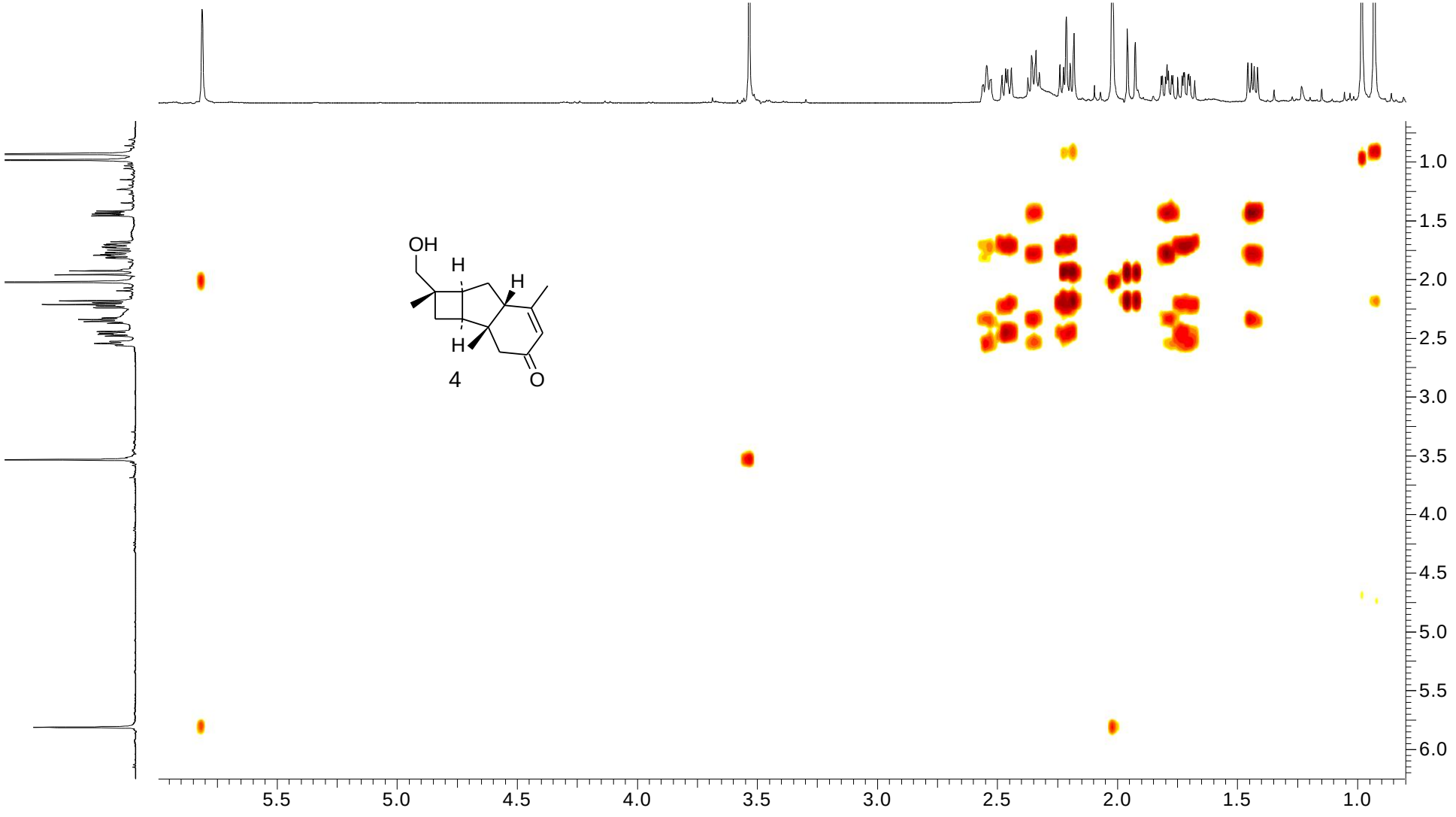

Figure S21. HMBC of 4

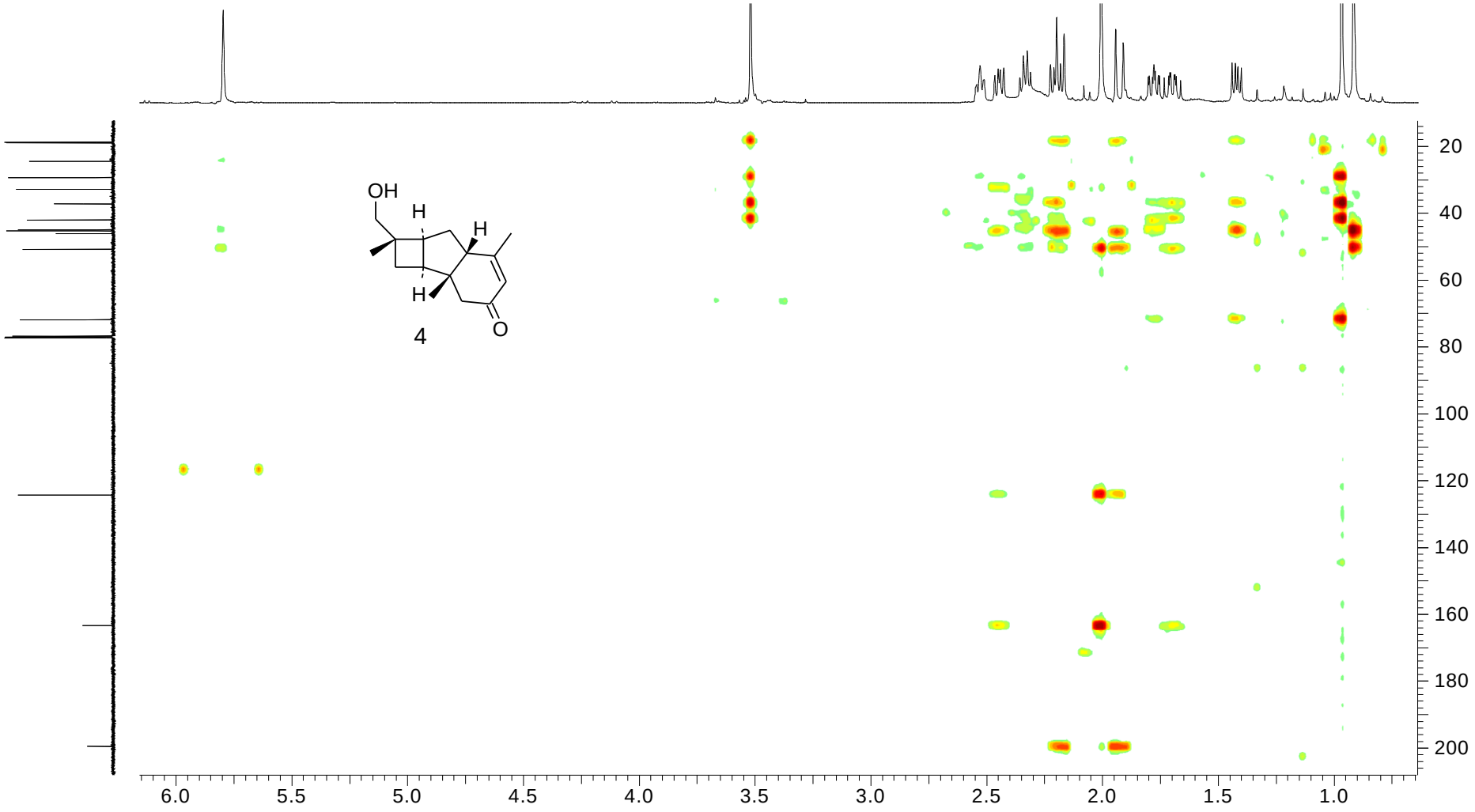

Figure S22. NOESY of 4

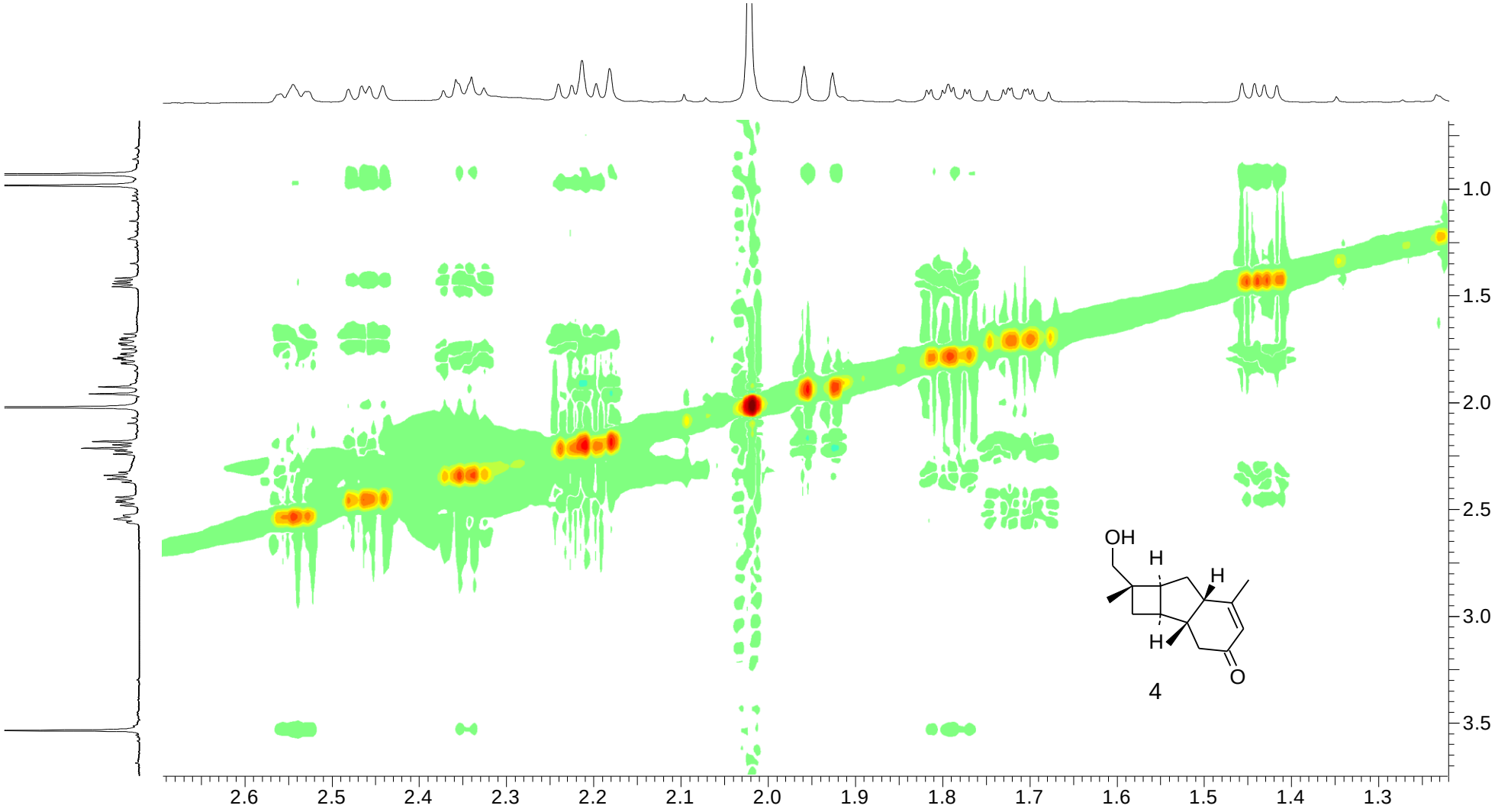

Figure S23. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 5

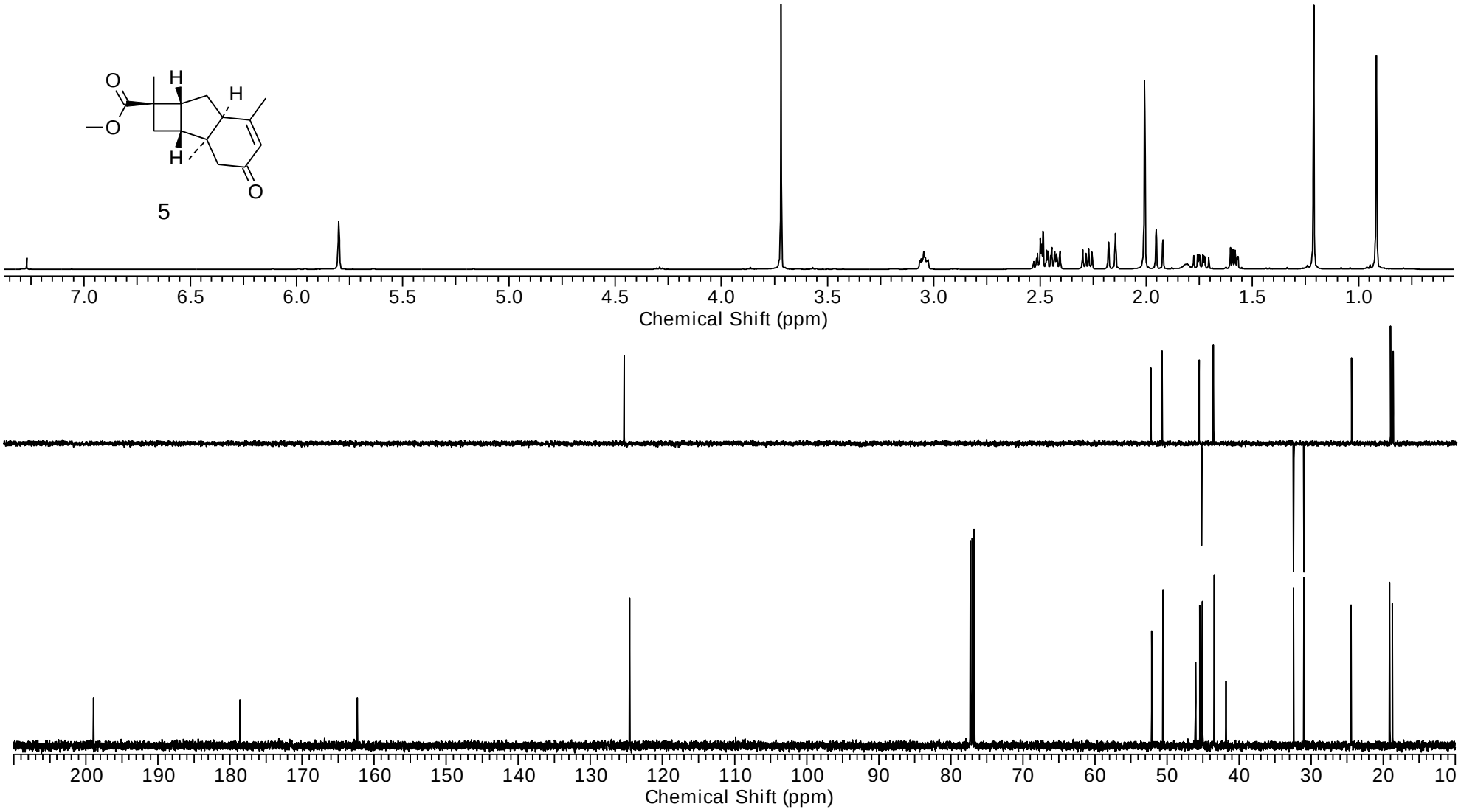

Figure S24. HSQC of 5

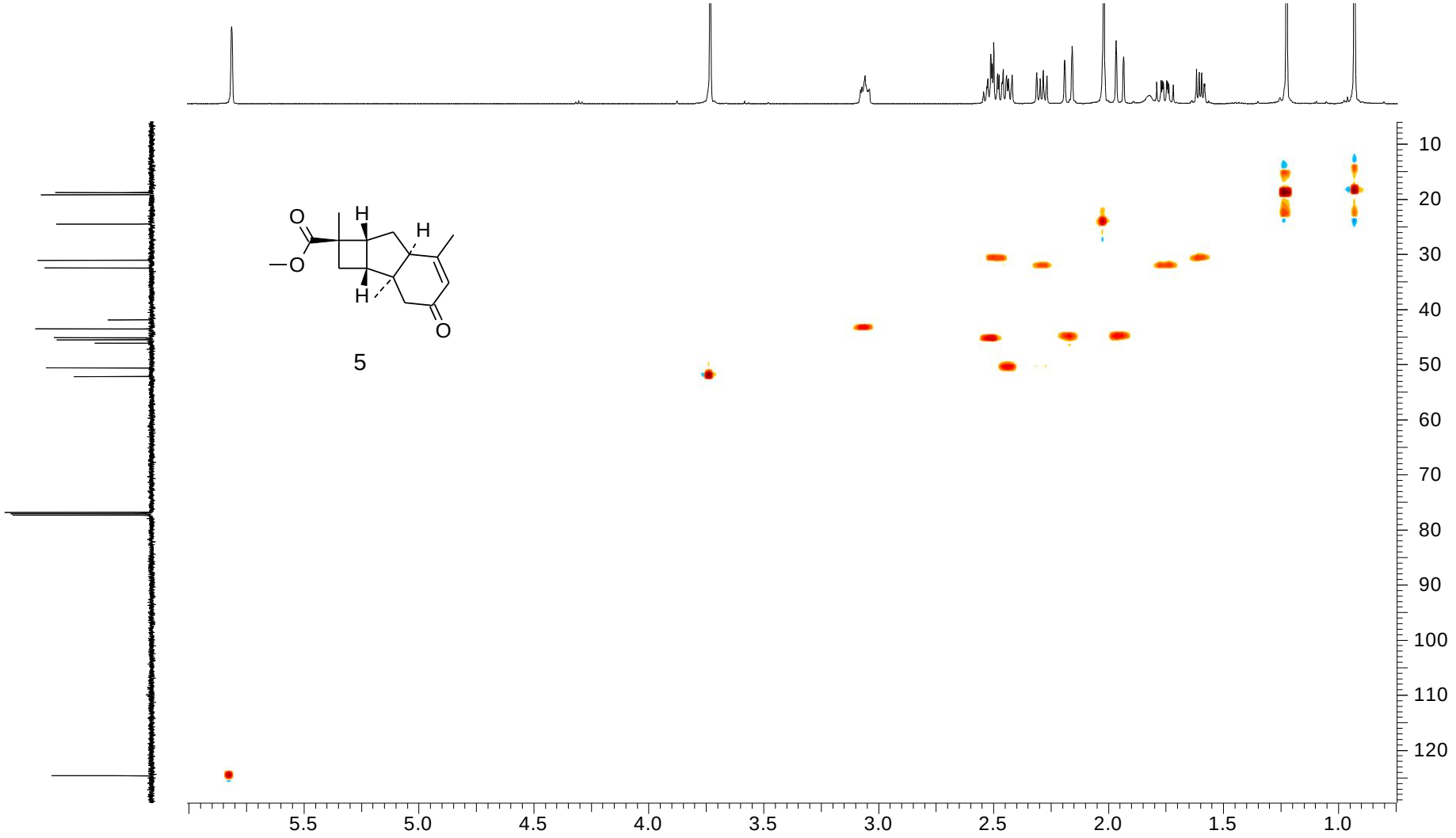

Figure S25. ^{1}H-^{1}H COSY of 5

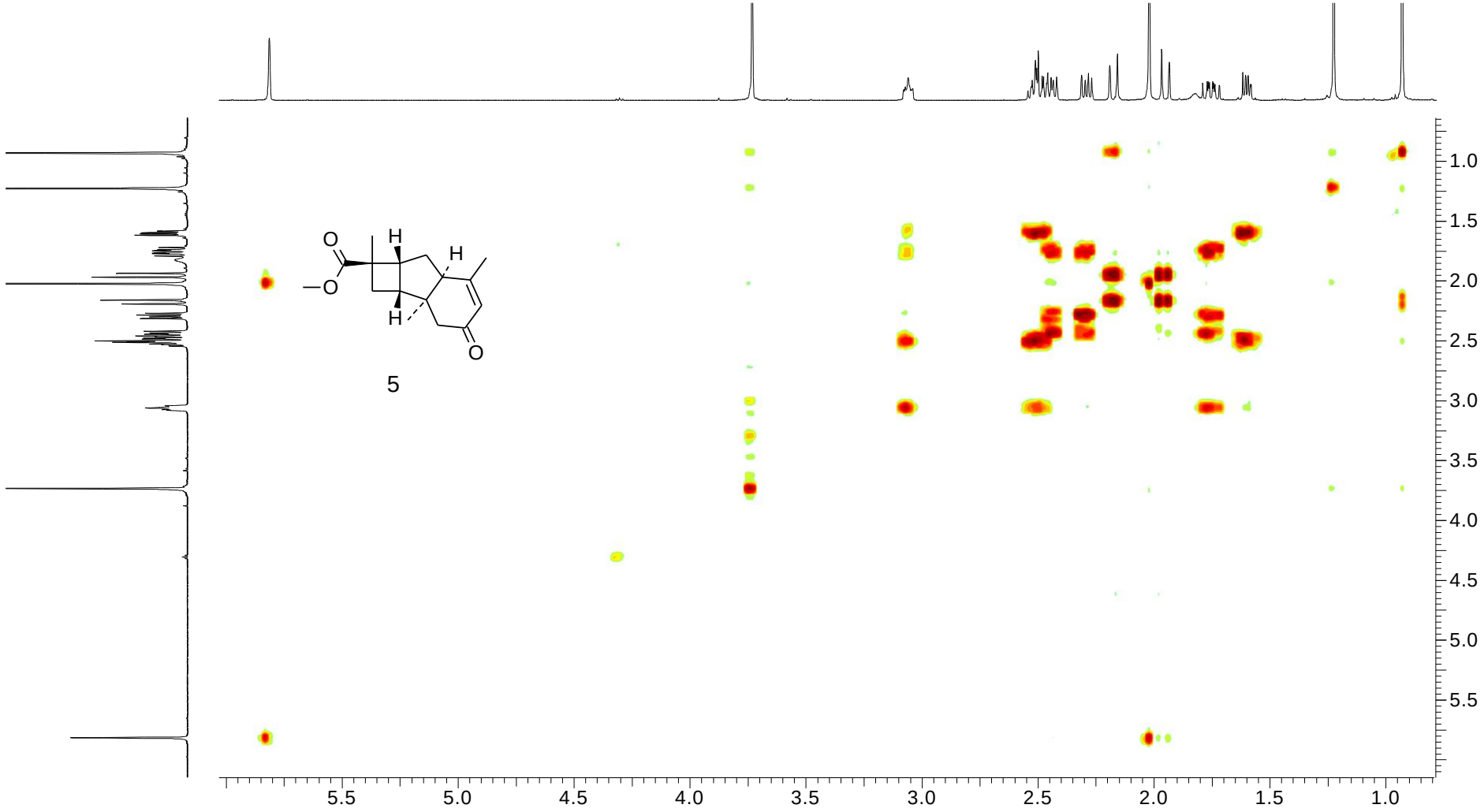

Figure S26. HMBC of 5

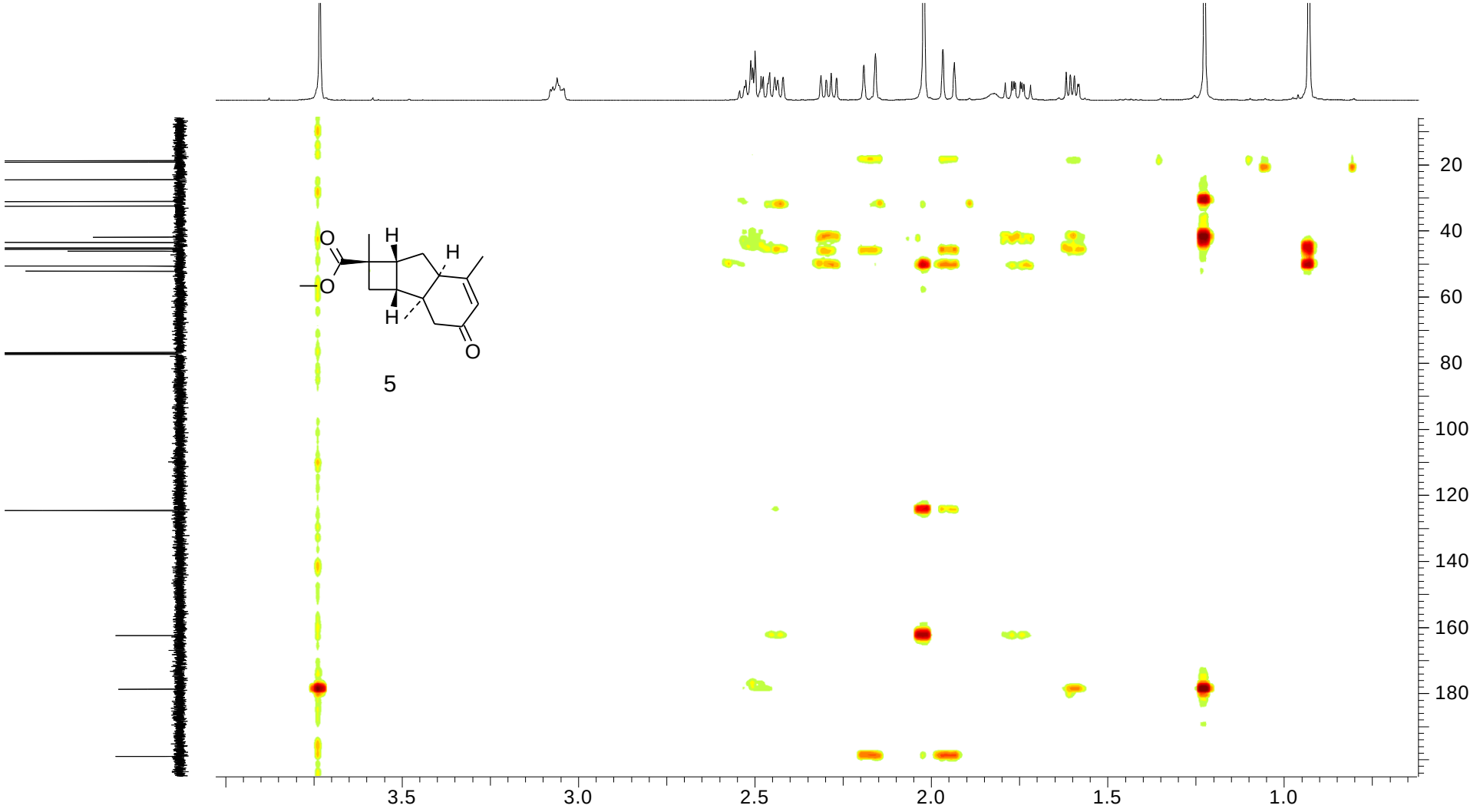

Figure S27. NOESY of 5

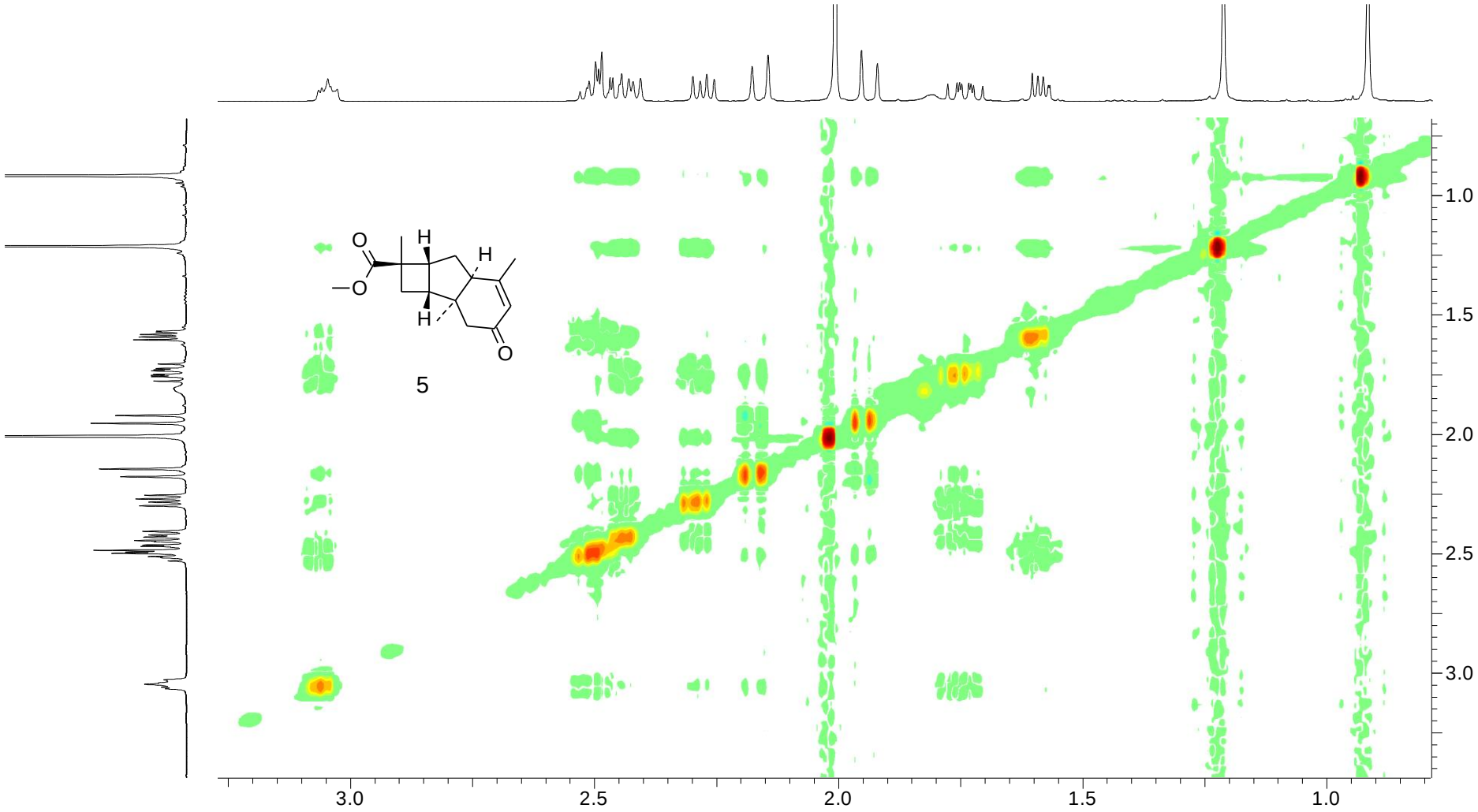

Figure S28. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR spectra of 6

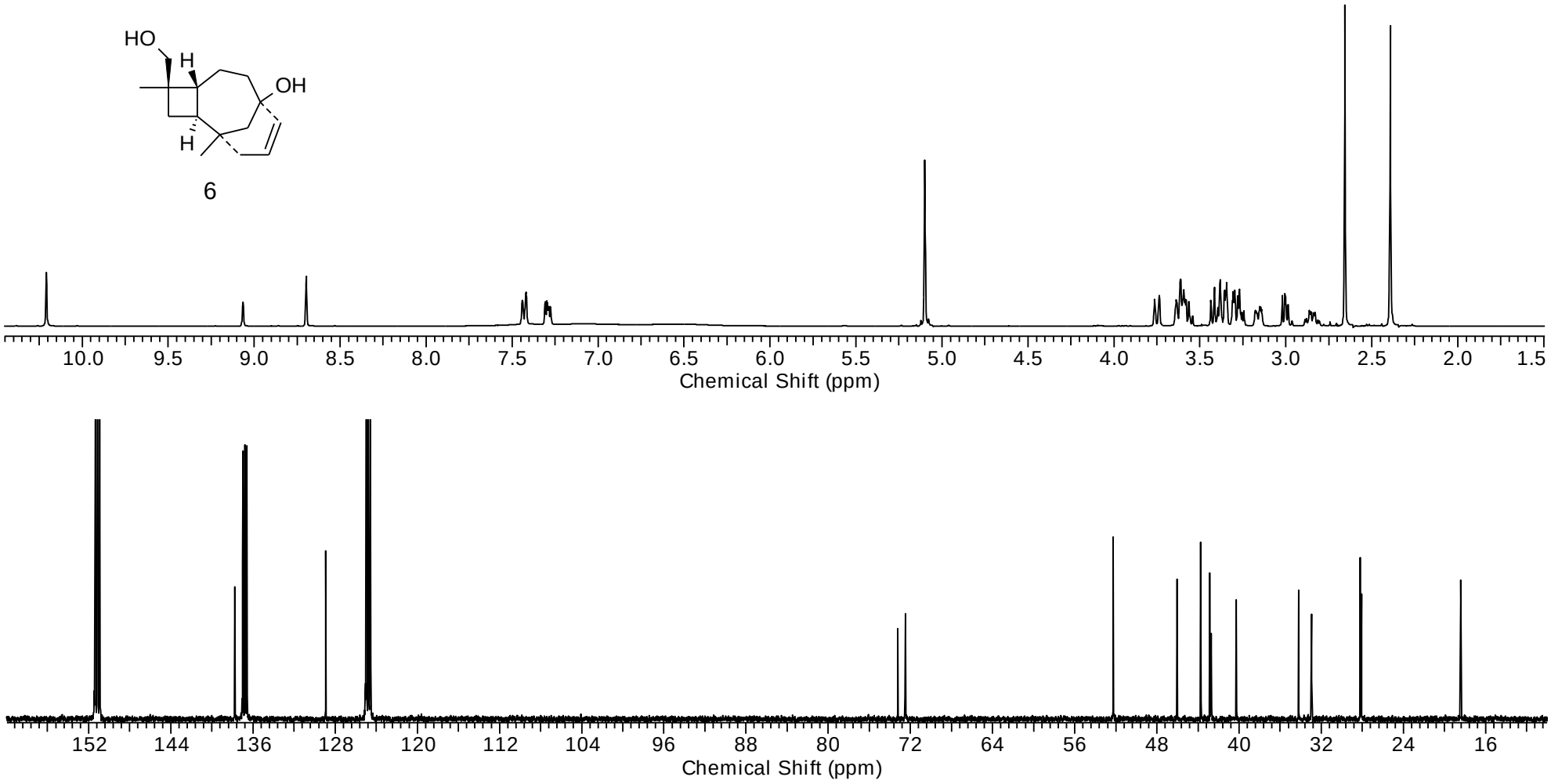

Figure S29. HSQC of 6

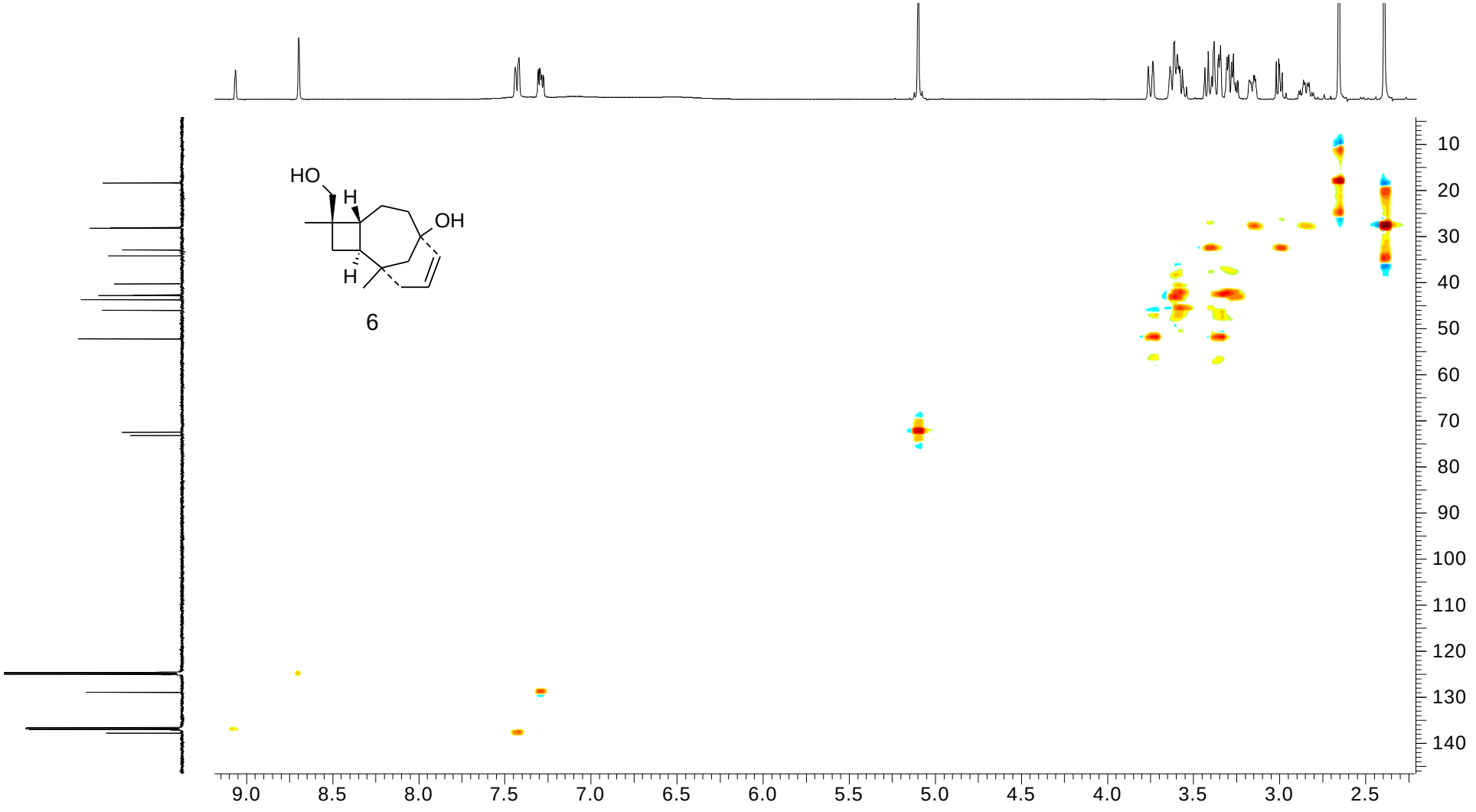

Figure S30. ^{1}H-^{1}H COSY of 6

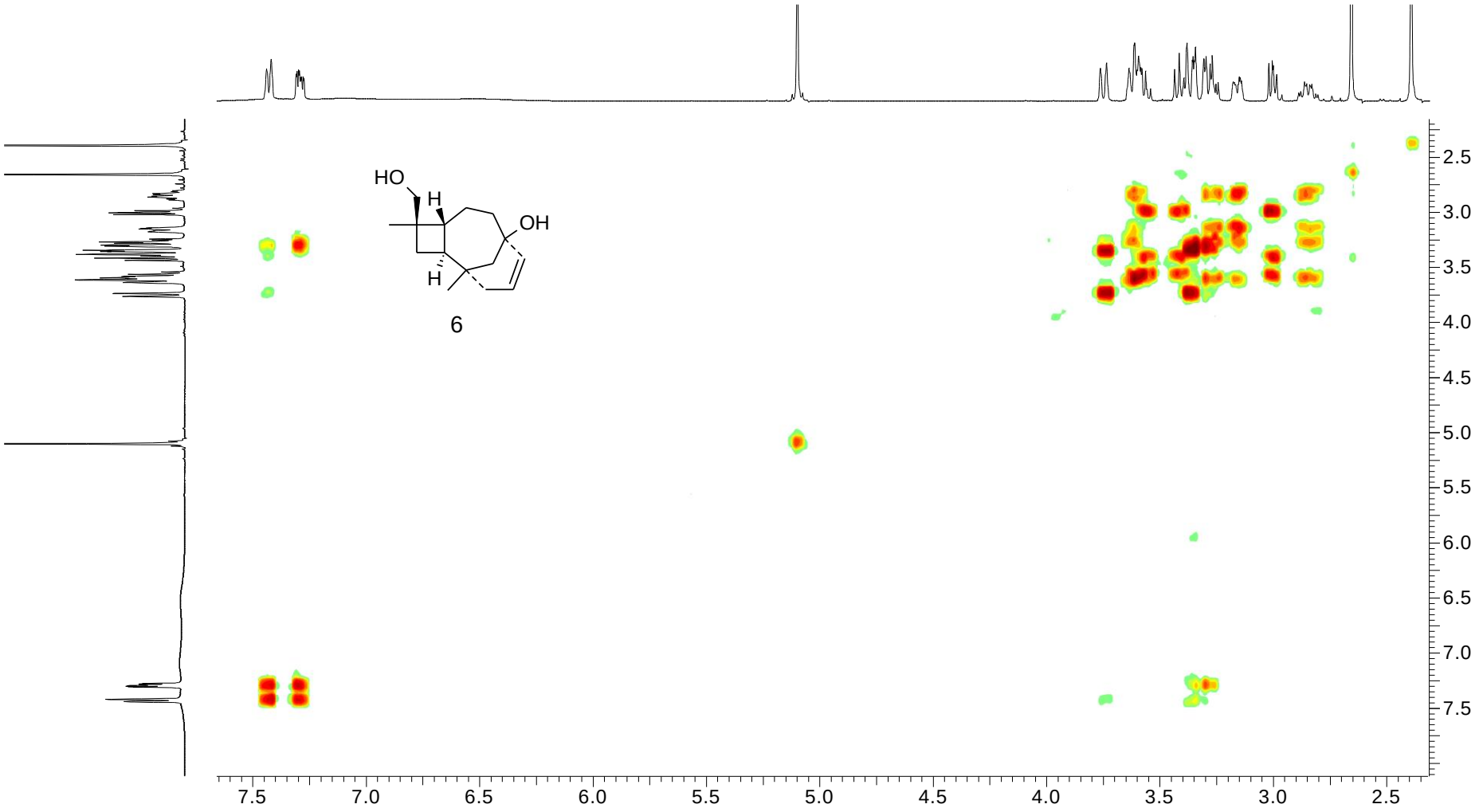

Figure S31. HMBC of 6

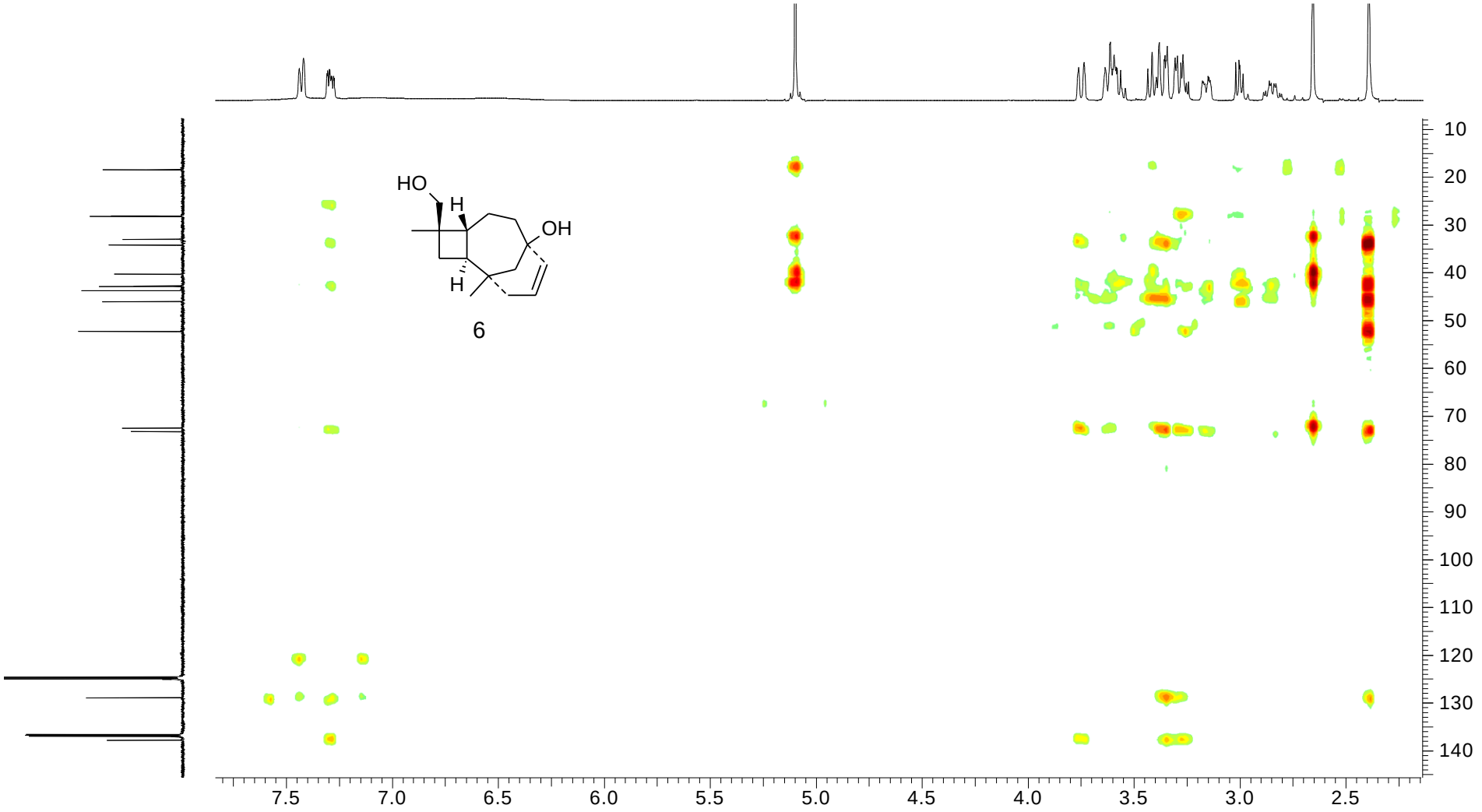

Figure S32. NOESY of 6

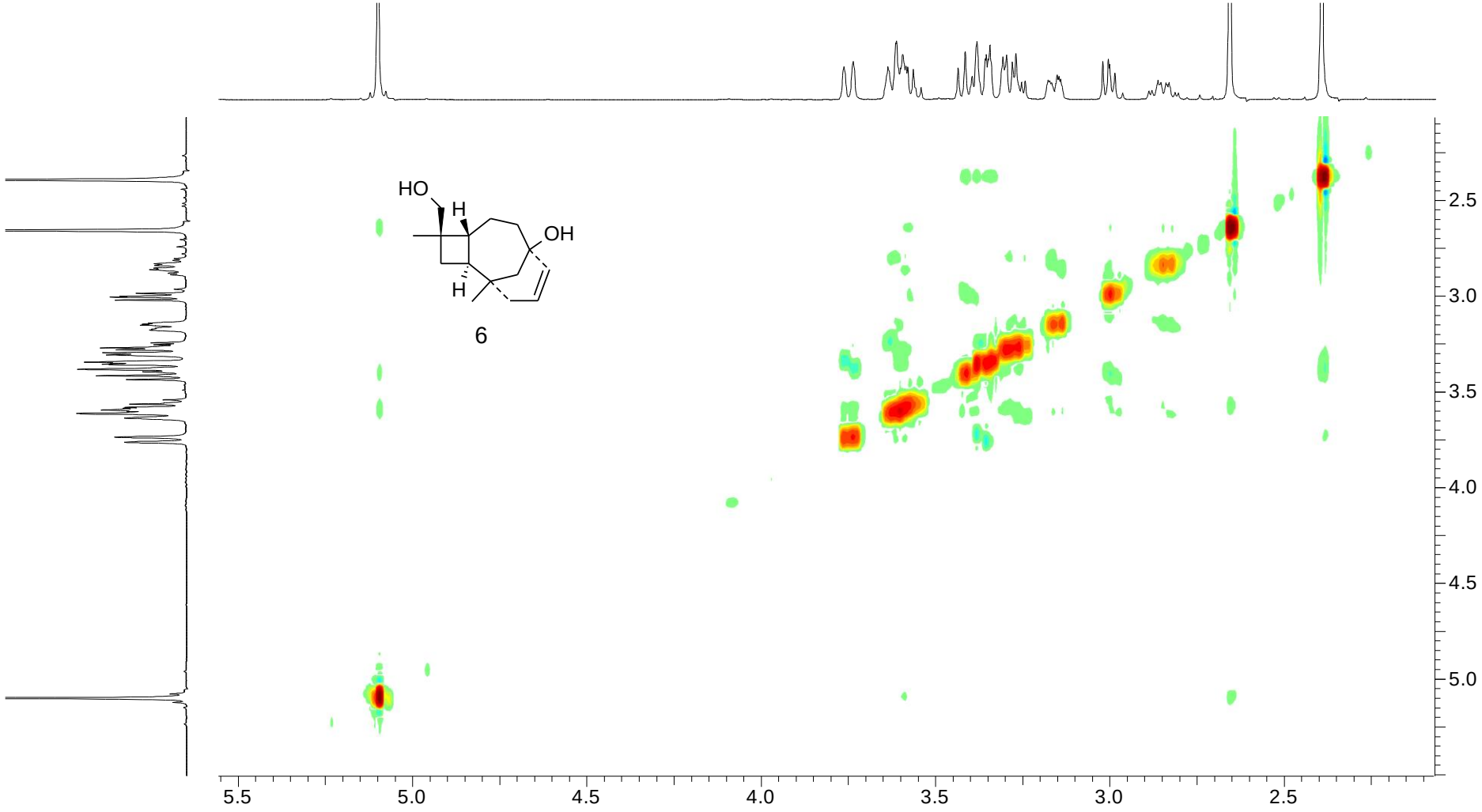

Figure S33. ^{1}H (500M Hz) and ^{13}C (125M Hz) NMR and DEPT spectra of 7

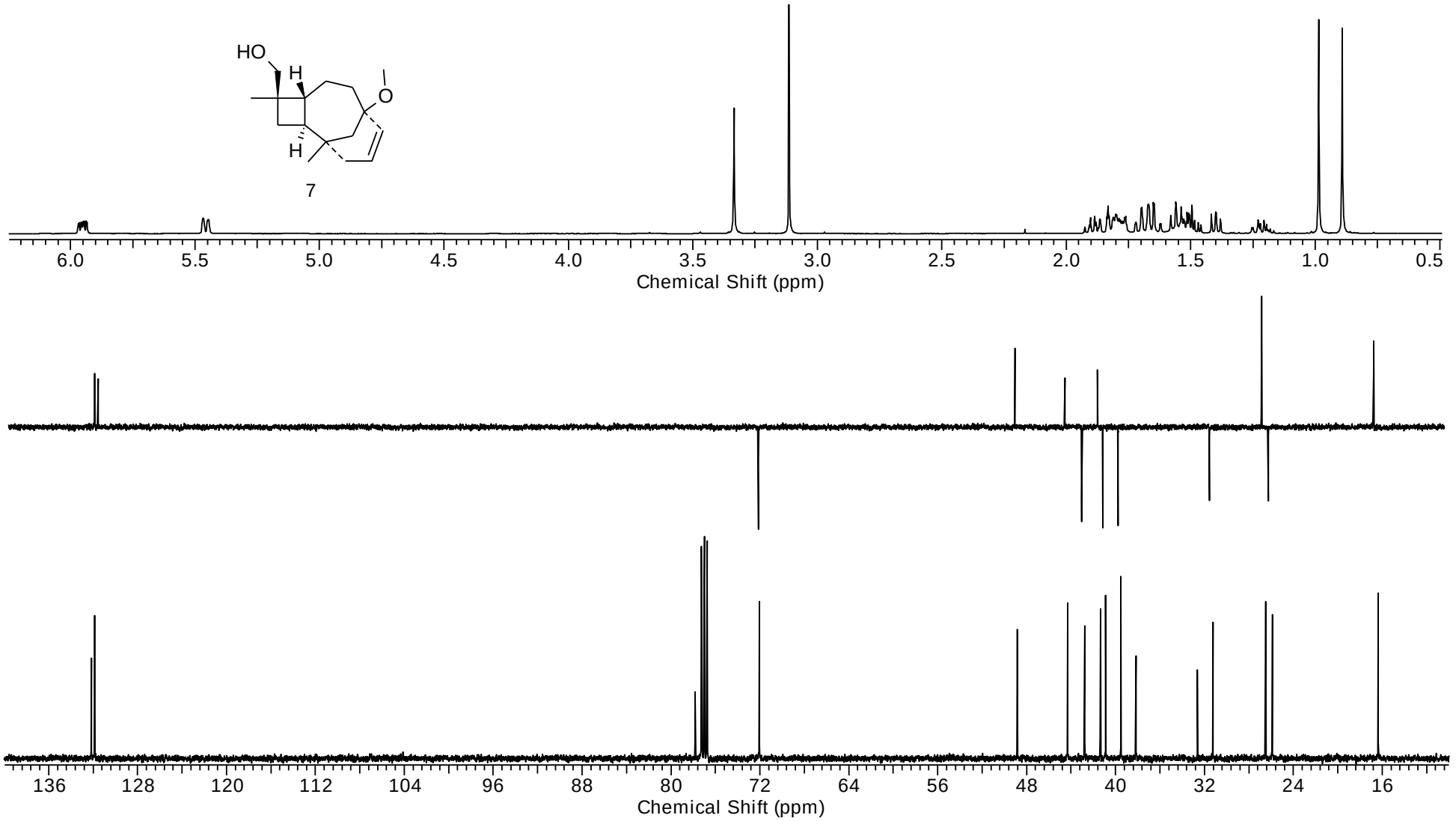

Figure S34. HSQC of 6

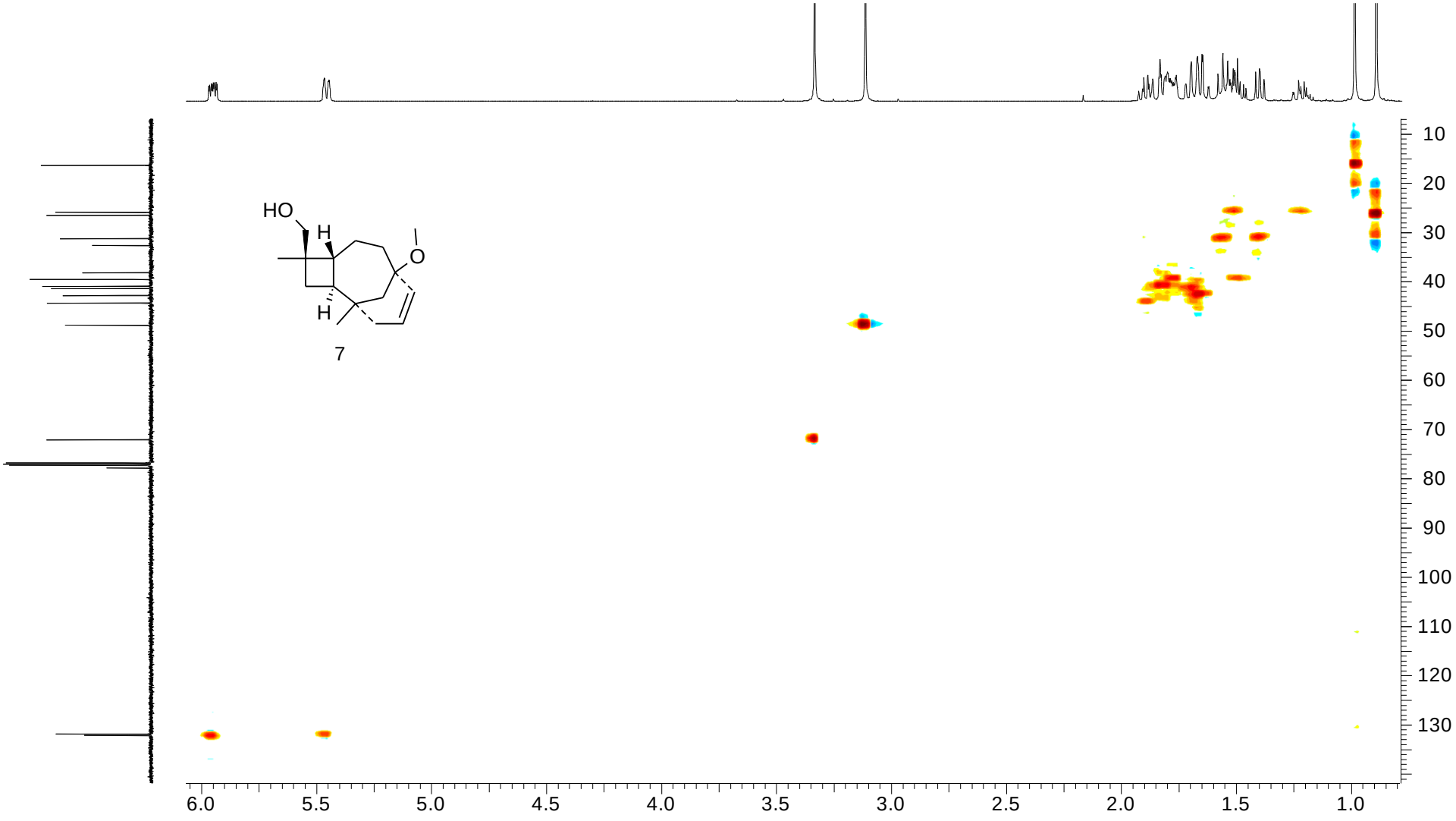

Figure S35. ^{1}H-^{1}H COSY of 6

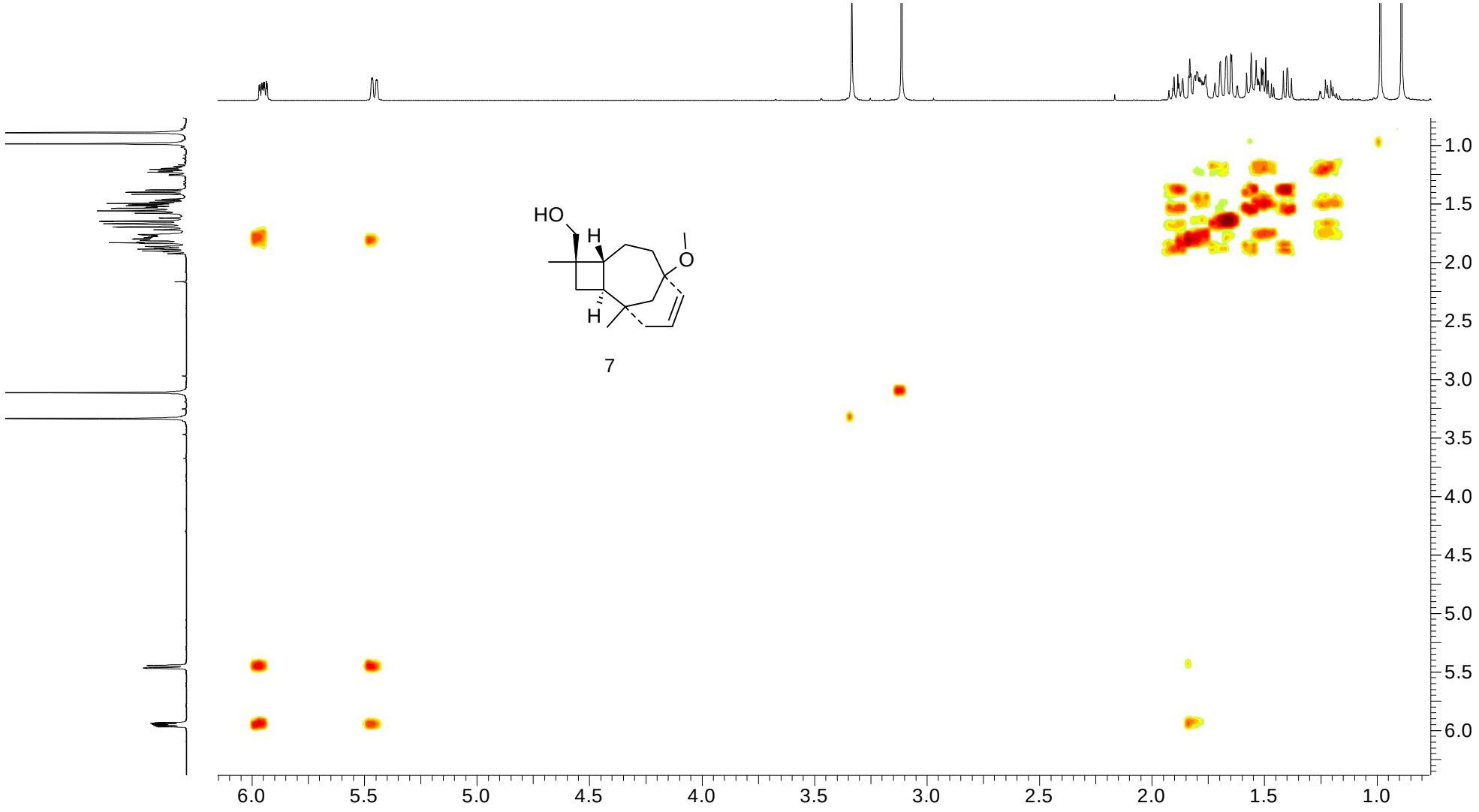

Figure S36. HMBC of 6

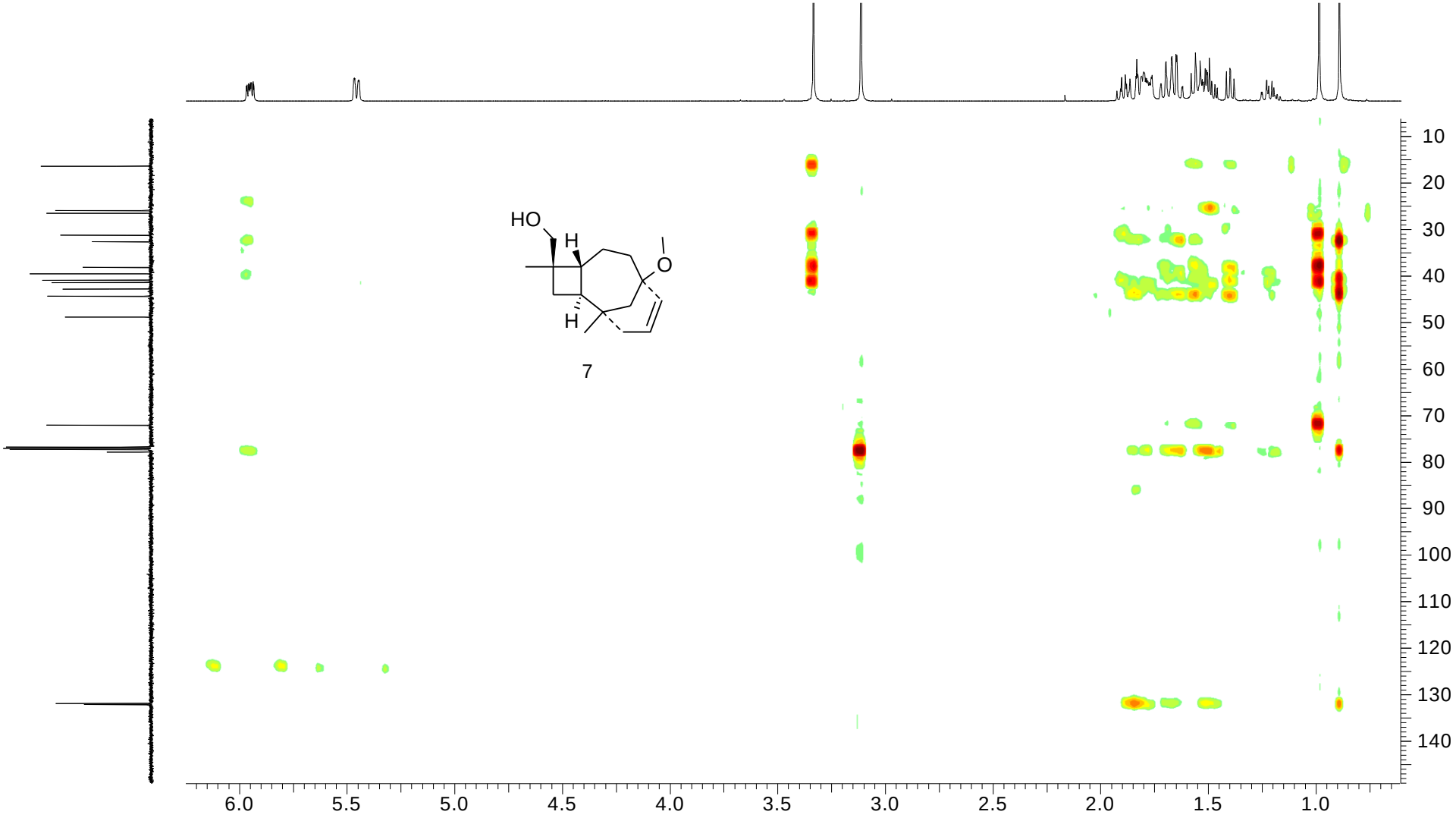

Figure S37. NOESY of 6