

Supplementary material

Table S1. Effects of the A02W extract (obtained in small scale and in large scale) on PAO1 *pqsA::gfp* and *lasB::gfp* reporter strain after 15h of growth. Azithromycin at 2 µg/mL was used as a positive control. DMSO 1.28 % was used as solvent control. Fluorescence was normalized to 100% of the reporter strain grown with DMSO 1.28%. All experiments were done in triplicate manner (technical and biological replicates).

	PqsA (% fluo)	SD	LasB (% fluo)	SD	CMI <i>P. aeruginosa</i> ATCC 27853
A02W 128 µg/mL (Small scale)	37	0.4	51	0.6	> 128 µg/mL
A02W 128 µg/mL (Up scale)	48	1.2	72	5.4	> 128 µg/mL
Azt 2 µg/mL (C+)	25	5.3	20	3.3	-

Table S2. Effects of the A02W fractions on PAO1 *pqsA::gfp* and *lasB::gfp* reporter strain after 15h of growth. Azithromycin at 2 µg/mL was used as a positive control, DMSO 1.28 % was used as solvent control. Fluorescence was normalized to 100% of the reporter strain grown with DMSO 1.28%. All experiments were done in triplicate manner (technical and biological replicates).

A02W Fractions (128 µg/mL)	PqsA (% fluo)	SD	LasB (% fluo)	SD	CMI <i>P. aeruginosa</i> ATCC 27853
F1	95	2.8	88	2.6	> 128
F2	93	0.7	78	1.5	> 128
F3	76	14.2	62	0.2	> 128
F4	30	4.2	36	6.8	> 128
F5	91	6.0	73	1.2	> 128
F9	86	4.3	75	2.2	> 128
F11	26	2.8	23	1.2	> 128
F12	101	3.8	74	1.4	> 128
F13	87	19.4	102	8.3	> 128
F14	100	6.0	84	6.4	> 128
F18	90	1.7	88	1.0	> 128
F19	89	1.3	72	1.1	> 128
Azt 2 µg/mL (C+)	25	5.3	20	3.3	> 128

Table S3. Annotation table of cluster C_1.

	RT (min)	m/z	Molecular formula	Molecule Name	Taxonomy							Classification			Inchi
		[M+H] ⁺			Kingdom	Phylum	Class	Order	Family	Genus	Species	Class	SubClass	Parent level 1	
Compound 2	0.42	215.0913	C10H14O5	(5S,6S)-6-((3'S,4'S,Z)-3',4'-dihydroxypent-1-en-1-yl)-5-hydroxy-5,6-dihydro-2H-pyran-2-one	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	Lasiodiplodia	Lasiodiplodia venezuelensis	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C10H14O5/c1-6(11)/12(2-4-9-8(13)-5-10(14)15-8h2-9,11-13h,1H3/b4-2-/67,77,8-9-/m0/s1
Compound 4	0.8	214.1072	C10H15NO4	(Z)-3-((2R,3R,6R)-3-hydroxy-6-((R)-1-hydroxyethyl)-3,6-dihydro-2H-pyran-2-yl)acrylamide	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	Lasiodiplodia	Lasiodiplodia venezuelensis	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C10H15NO4/c1-6(12)8-3-2-7(13)9(15-8)4-5-10(11)14h2-9,12-13h,1H3,(H2,11,14)h5-4-/6-7-8-9-/m0/s1
Node n1	0.26	197.0807	C10H12O4	Diplopyrone	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	Diplodia	Diplodia mutila	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C10H12O4/c1-6(11)7-2-3-8-8(13)7-4-5-10(12)14-9h2-9,11h,1H3
Node n2	0.98	239.0910	C12H14O5	Radicinol3-Epimer	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	Alternaria radicina	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C12H14O5/c1-3-4-7-5-8-9(12)15(17-7)11(14)10(13)6(2)16-8h3-6,10-11,13-14h,1-2h3/b4-3-
Node n3	0.71	260.1123 [M+NH3] ⁺ of 242.079	C11H14O6	Scytolide3S,10-Dihydro	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	-	-	-	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C11H14O6/c1-5-10(13)17-9-7(12)3-6(11)14(15-24-8)9(16-5h3,5,7-2,12h)4h2,1-2h3
Node n4	1.02	243.1222	C12H18O5	Antibiotic SEN 366F	Fungi	Ascomycota	-	-	-	-	-	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C12H18O5/c1-7-9(13)3-6-12(16)7-17-10-4-5-11(14)15-8(10)2h3,6-8,10-12,14h,4-5h2,1-2h3
Node n5	2.36	319.1537	C18H22O5	Solanapyrone B6(E)-Hydroxy, 1"-aldehyde	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	tenuissima SP-07	Pyrans	Pyranones and derivatives	Butenolide	InChI=1S/C18H22O5/c1-10-6-7-11-12(4-3-5-14)11(20)17(10)15-8-9-15(22-2)13(9-19)18(21)23-16h6-12,14,17,20h,3-5h2,1-2h3

Table S4. Effect of compound Y on PAO1 *pqsA::gfp* and *lasB::gfp* reporter strain after 15h of growth. Azithromycin at 2 µg/mL was used as a positive control. DMSO 1.28 % was used as solvent control. Fluorescence was normalized to 100% of the reporter strain grown with DMSO 1.28 %. All experiments were done in triplicate manner (technical and biological replicates).

	PqsA (% fluo)	SD	LasB (% fluo)	SD	CMI <i>P. aeruginosa</i> ATCC 27853
Compound Y 128 µg/mL	68	3.9	72	0.6	> 128 µg/mL
Azt 2 µg/mL (C+)	25	5.3	20	3.3	-

Table S5. Effect of compounds 2 and 4 on the expression on QS regulated genes: *lasI*, *lasR*, *rhlI*, *rhlR*, *MvfR*, expressed as relative expression. Cultures of PAO1 were grown in triplicate with the compound of interest at 128 µg/ml during 4 hours. DMSO 1.28 % was used as solvent control. Error bars indicate standard deviations.

	<i>lasI</i>	SD	<i>lasR</i>	SD	<i>rhlI</i>	SD	<i>rhlR</i>	SD	<i>pqsR</i>	SD
Compound 2 128 µg/mL	0.34	0.34	0.60	0.14	0.36	0.39	0.64	0.40	1.03	0.20
Compound 4 128 µg/mL	0.61	0.67	0.47	0.65	0.47	0.58	0.41	0.26	0.98	0.86
DMSO	1.05	0.36	1.08	0.49	1.05	0.34	1.03	0.27	1.06	0.40

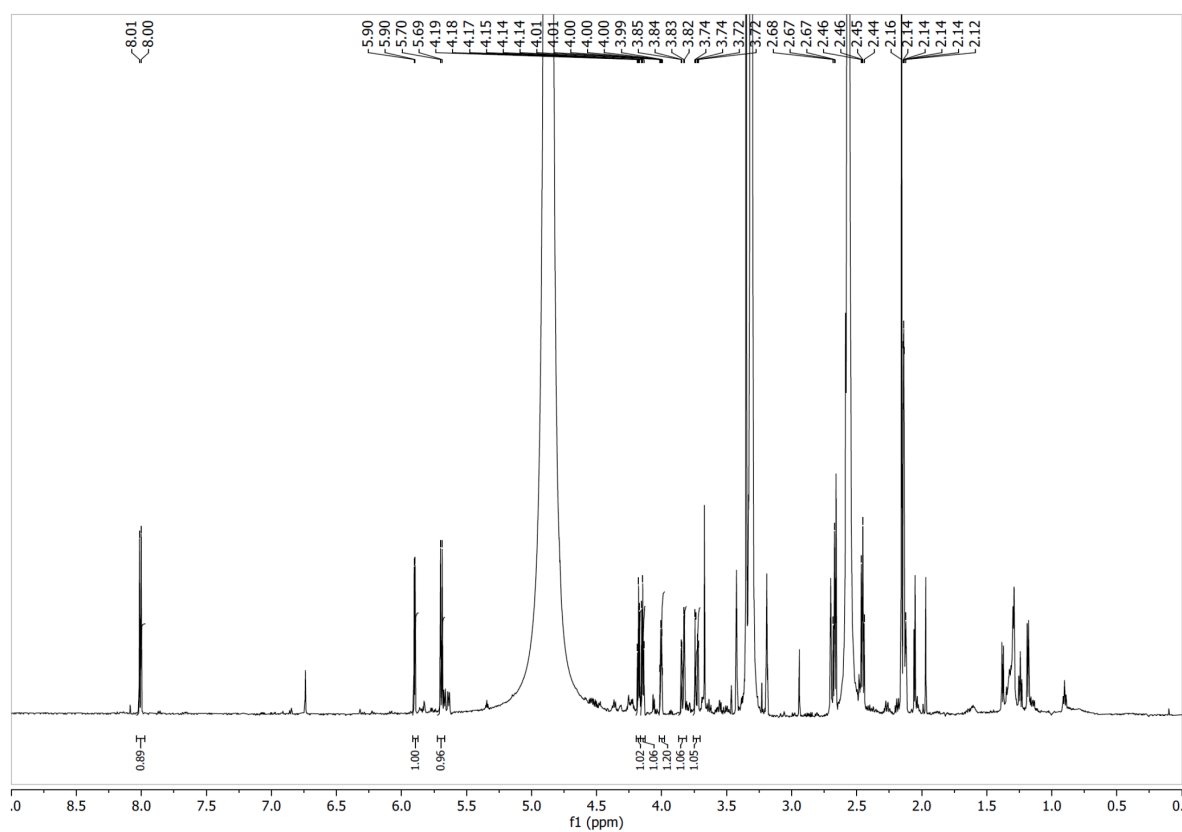


Figure S1. ¹H NMR spectrum of compound **1** in CD₃OD at 600 MHz

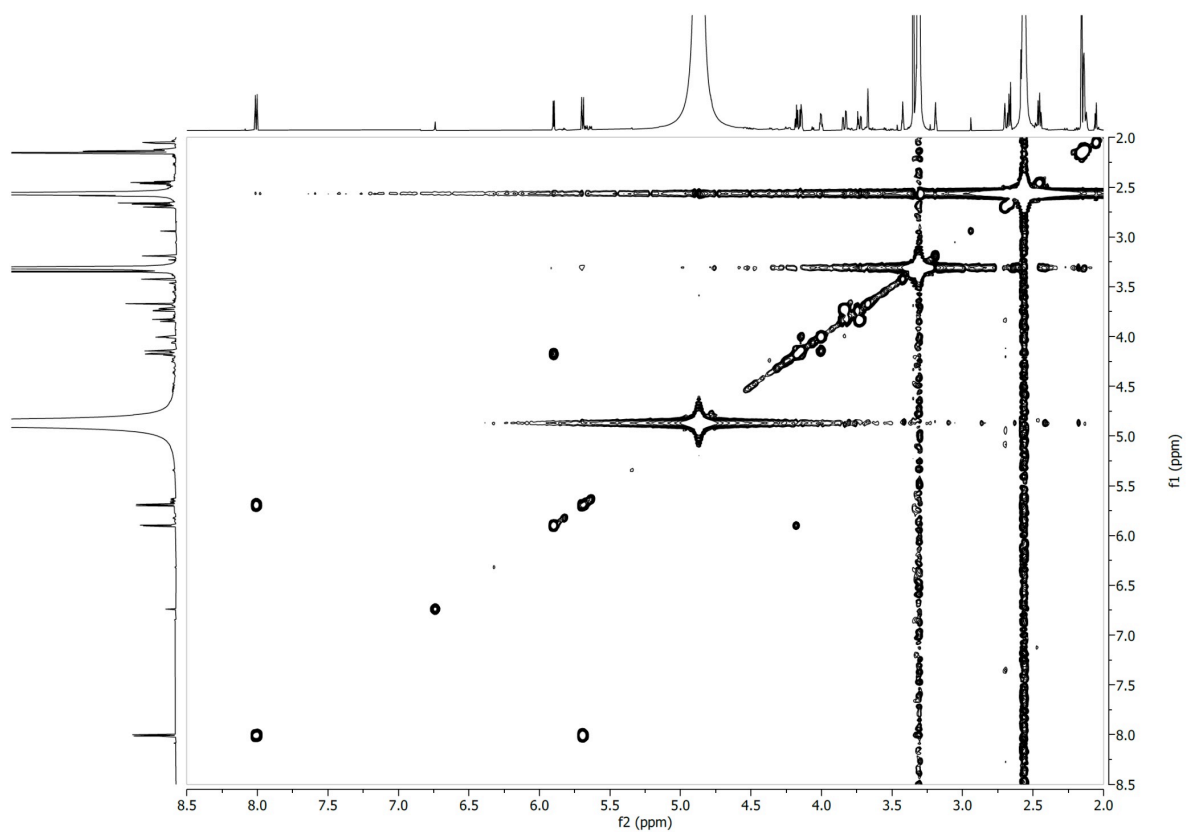


Figure S2. COSY NMR spectrum of compound **1** in CD₃OD

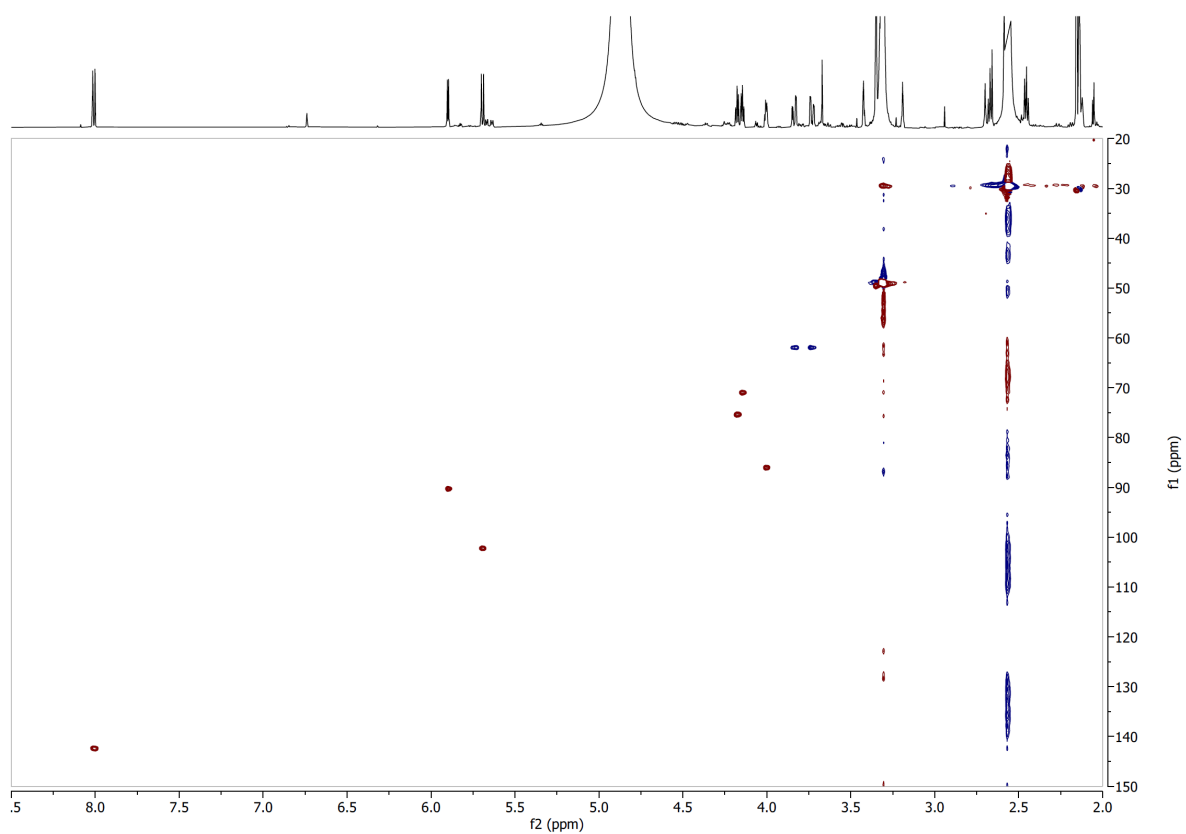


Figure S3. Edited-HSQC NMR spectrum of compound **1** in CD₃OD

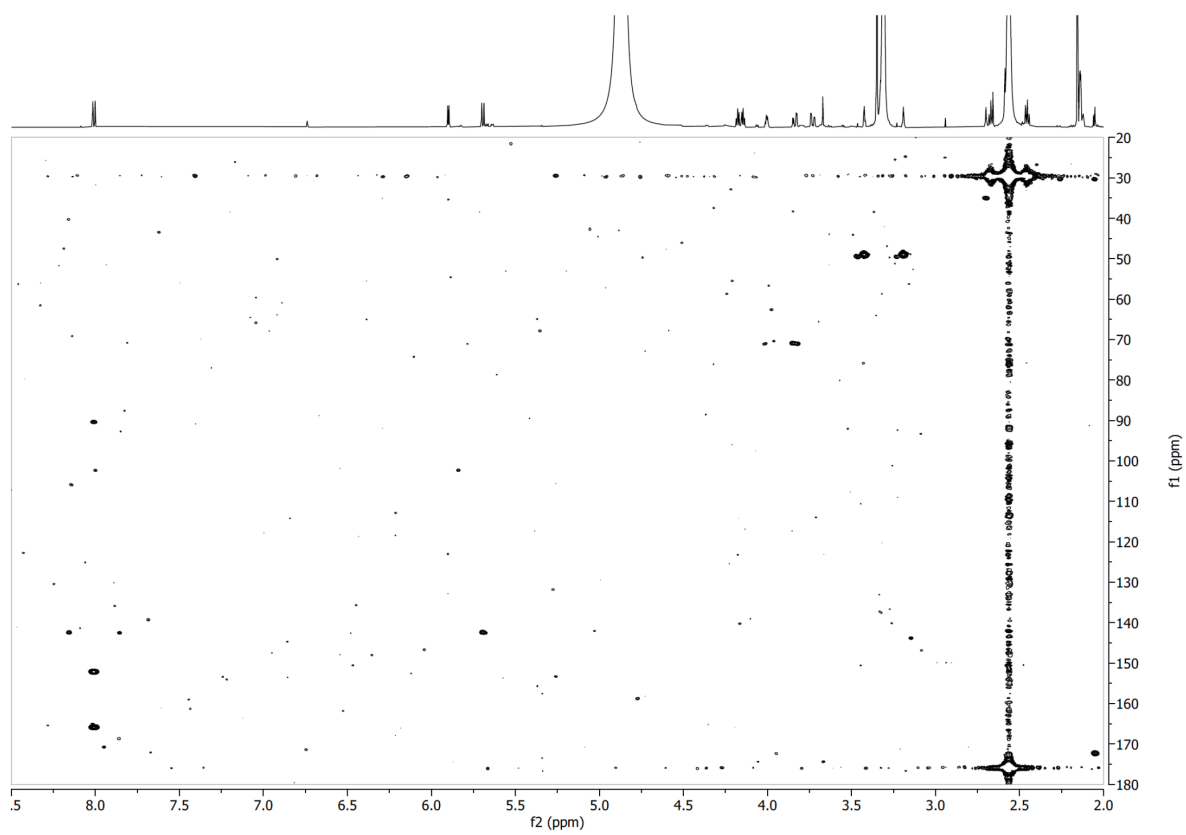


Figure S4. HMBC NMR spectrum of compound **1** in CD₃OD

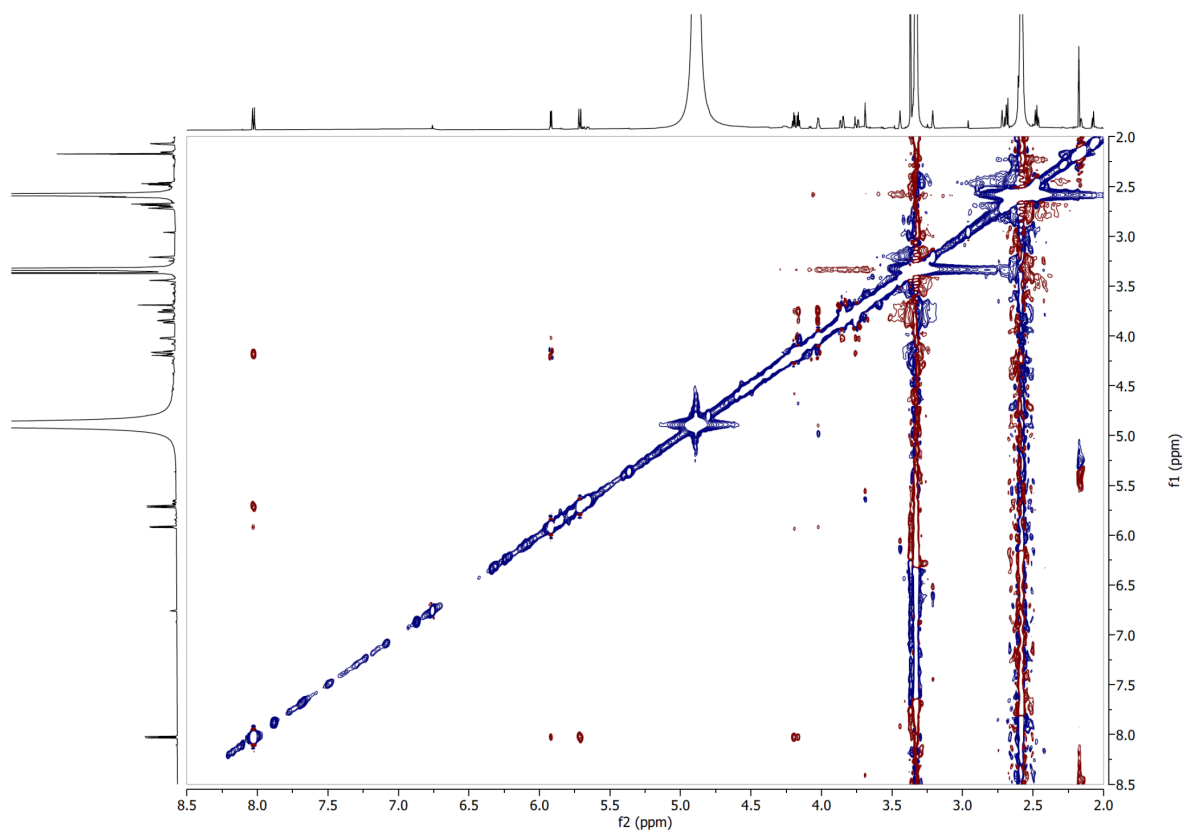


Figure S5. ROESY NMR spectrum of compound **1** in CD₃OD

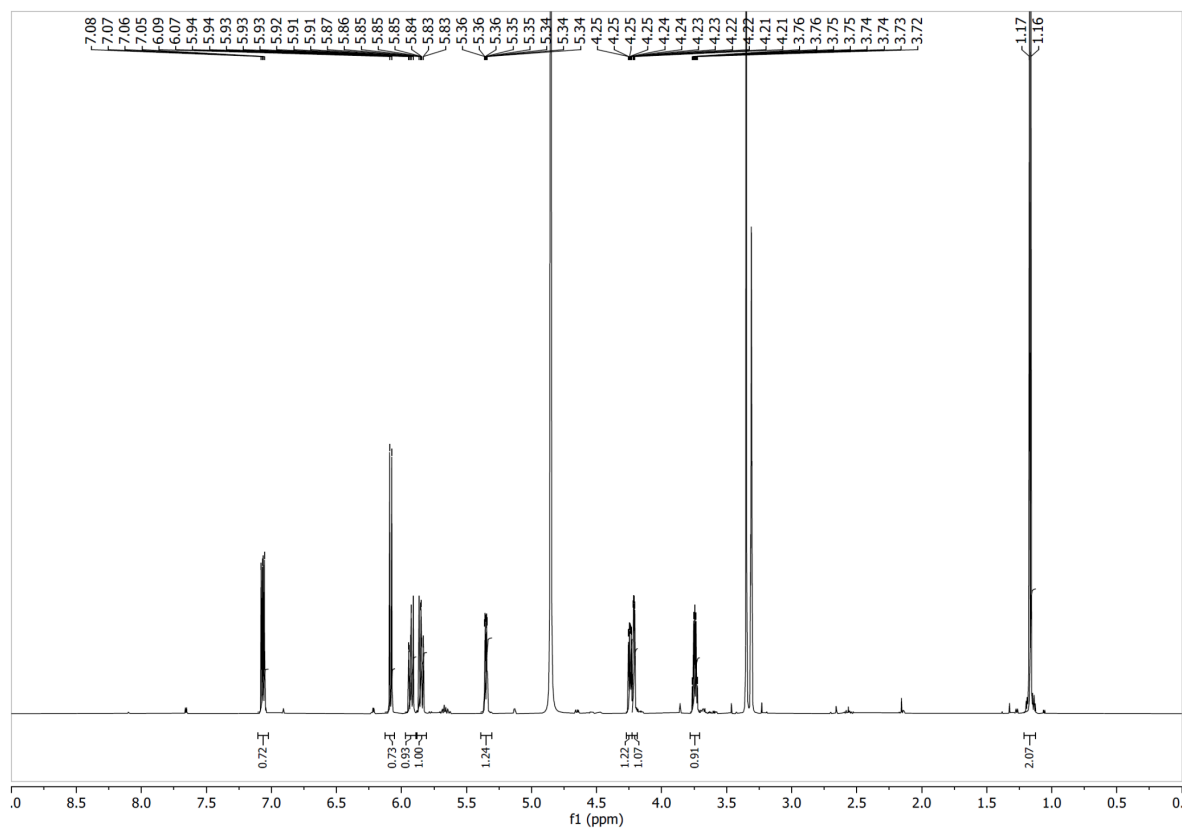


Figure S6. ¹H NMR spectrum of compound **2** in CD₃OD at 600 MHz

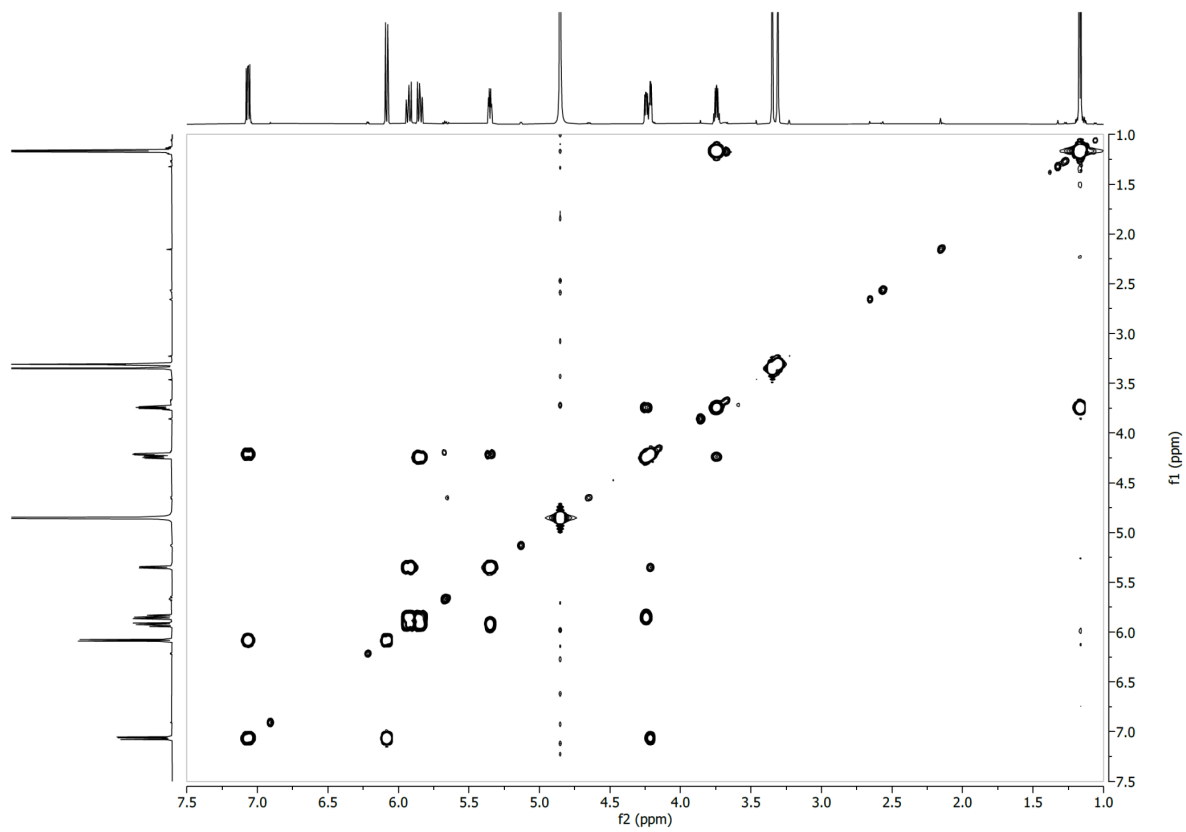


Figure S7. COSY NMR spectrum of compound **2** in CD₃OD

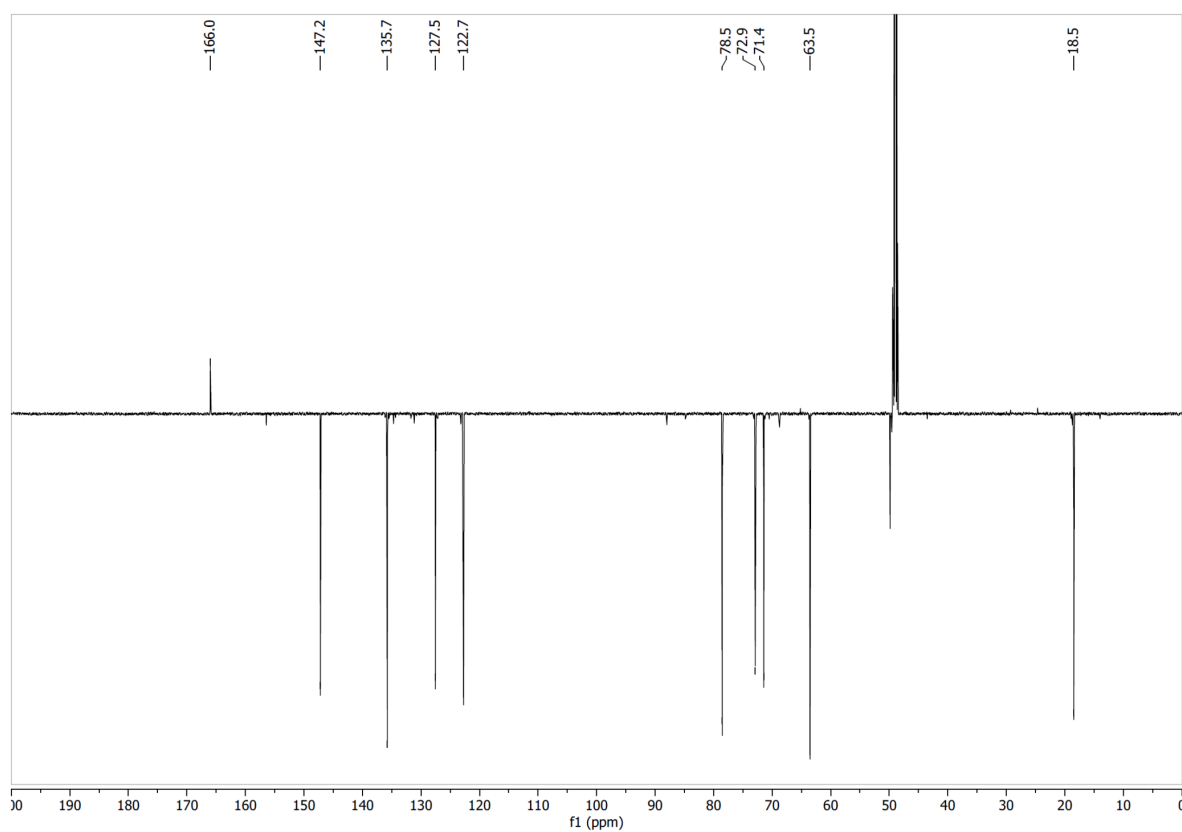


Figure S8. ^{13}C -DEPTQ NMR spectrum of compound **2** in CD_3OD at 151 MHz

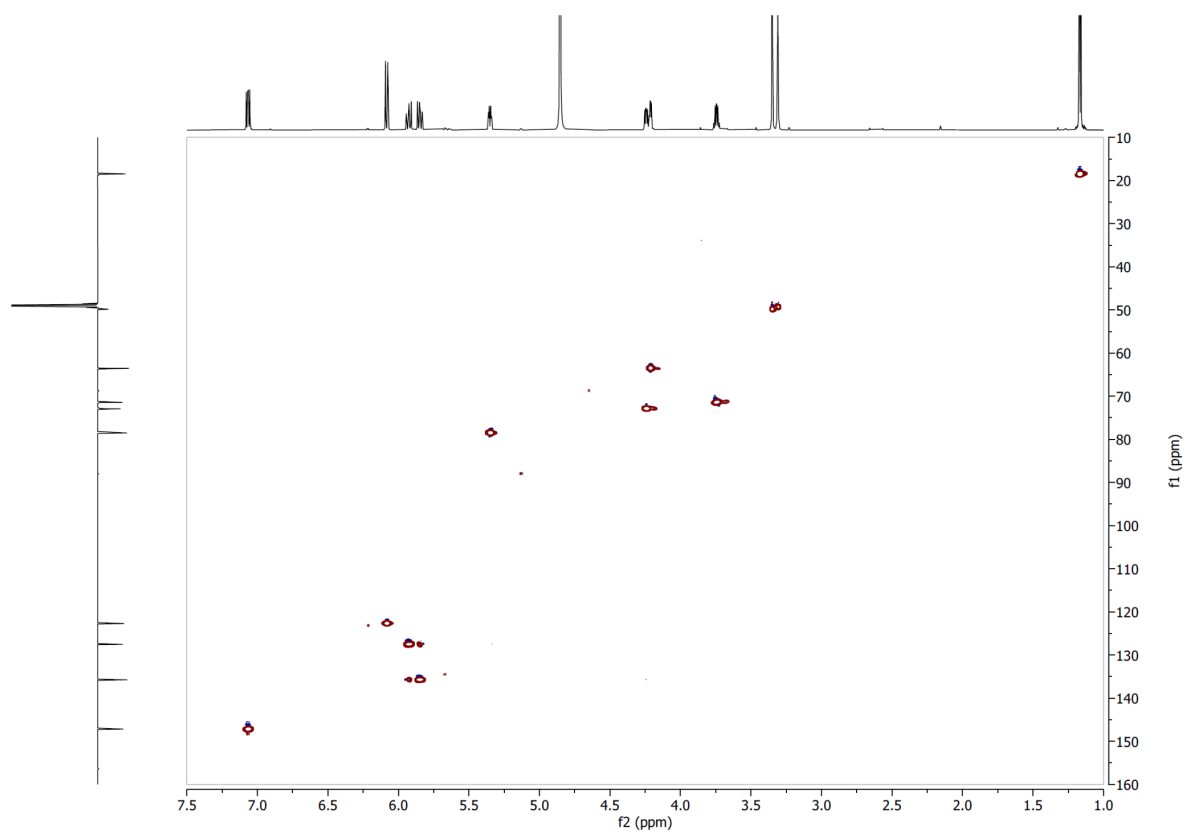


Figure S9. Edited-HSQC NMR spectrum of compound **2** in CD_3OD

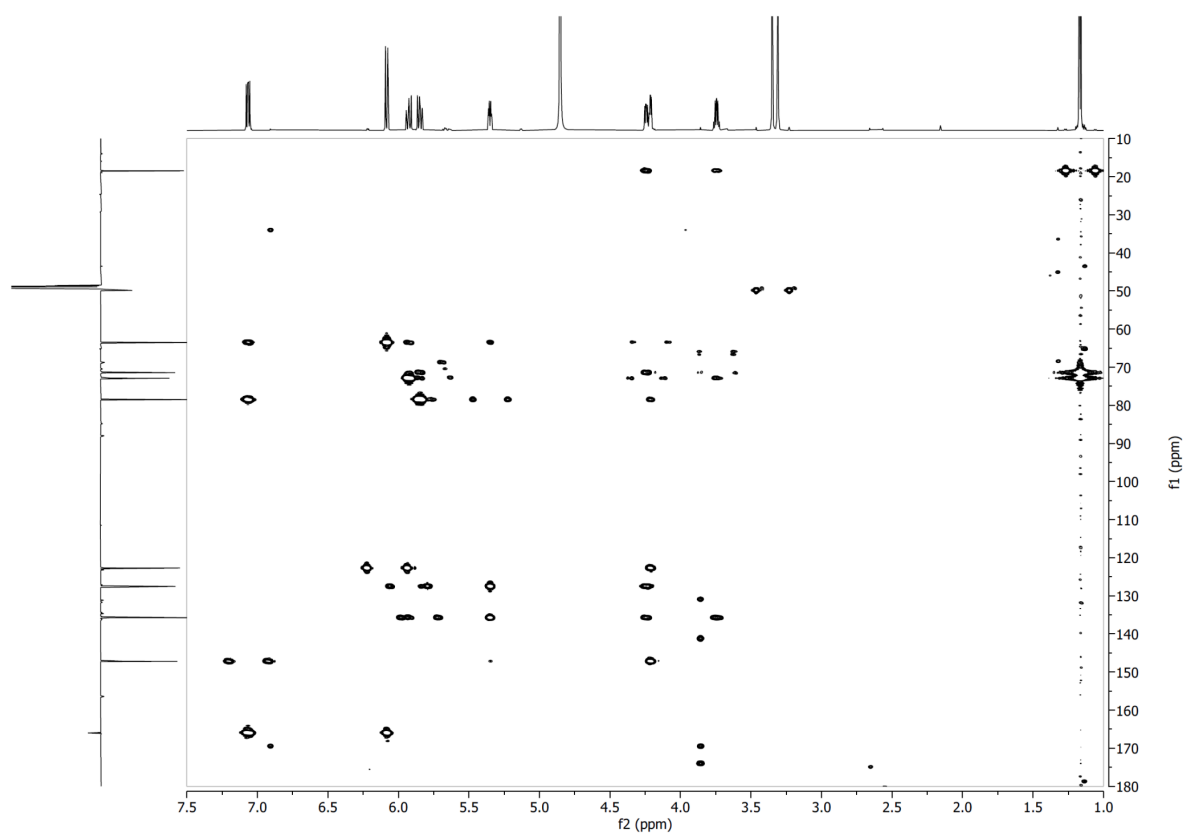


Figure S10. HMBC NMR spectrum of compound **2** in CD₃OD

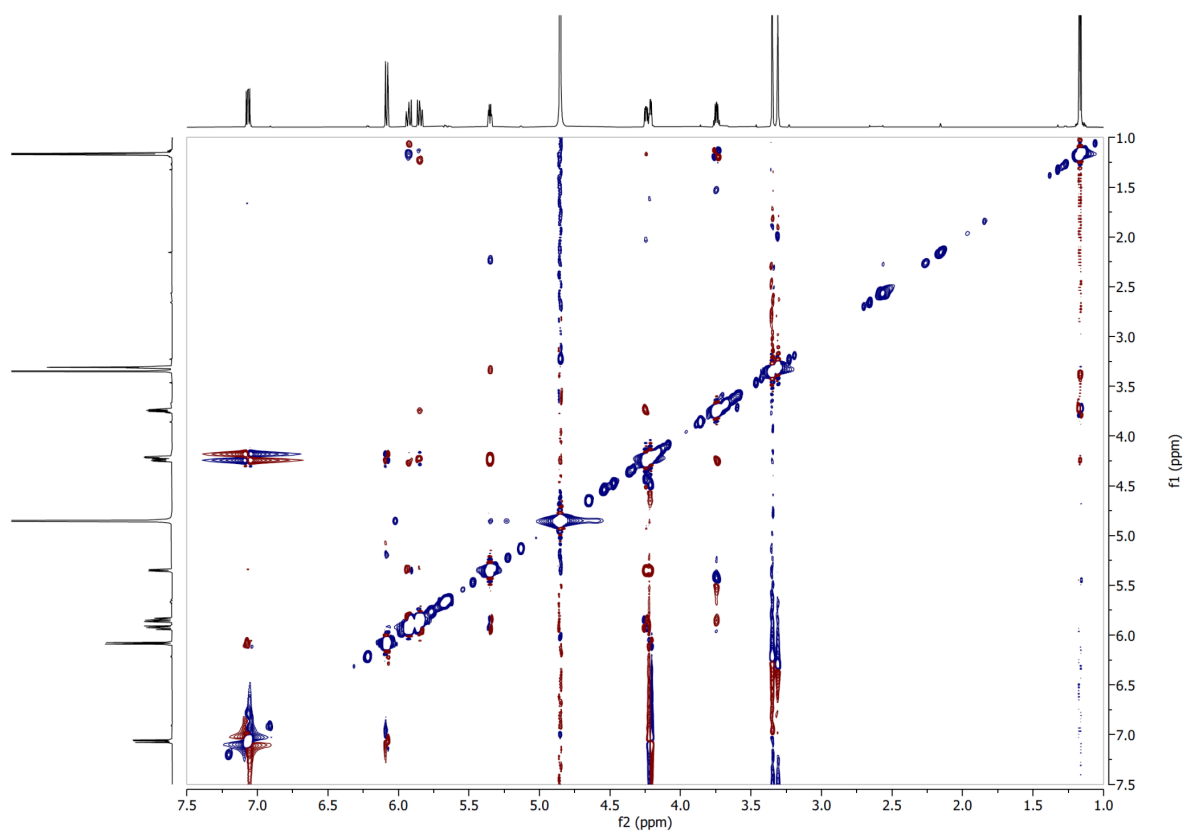


Figure S11. ROESY NMR spectrum of compound **2** in CD₃OD

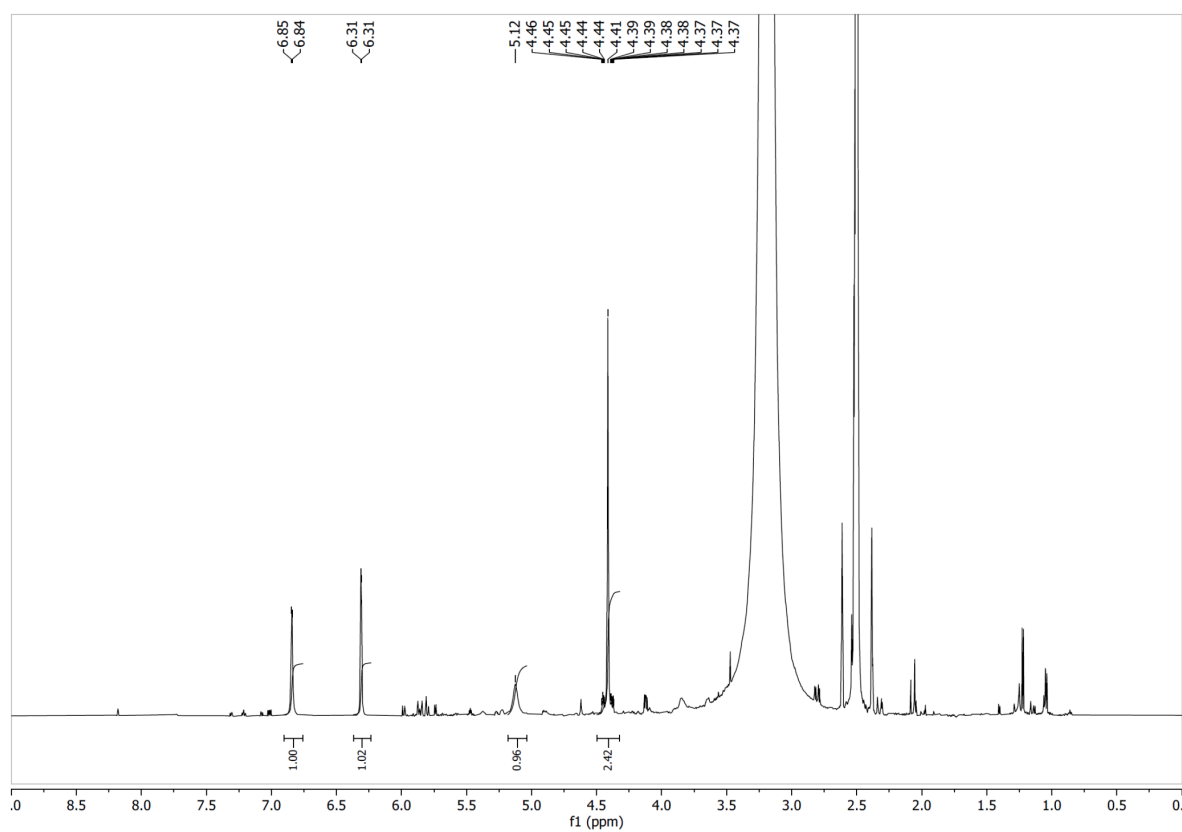


Figure S12. ¹H NMR spectrum of compound **3** in DMSO-*d*₆ at 600 MHz

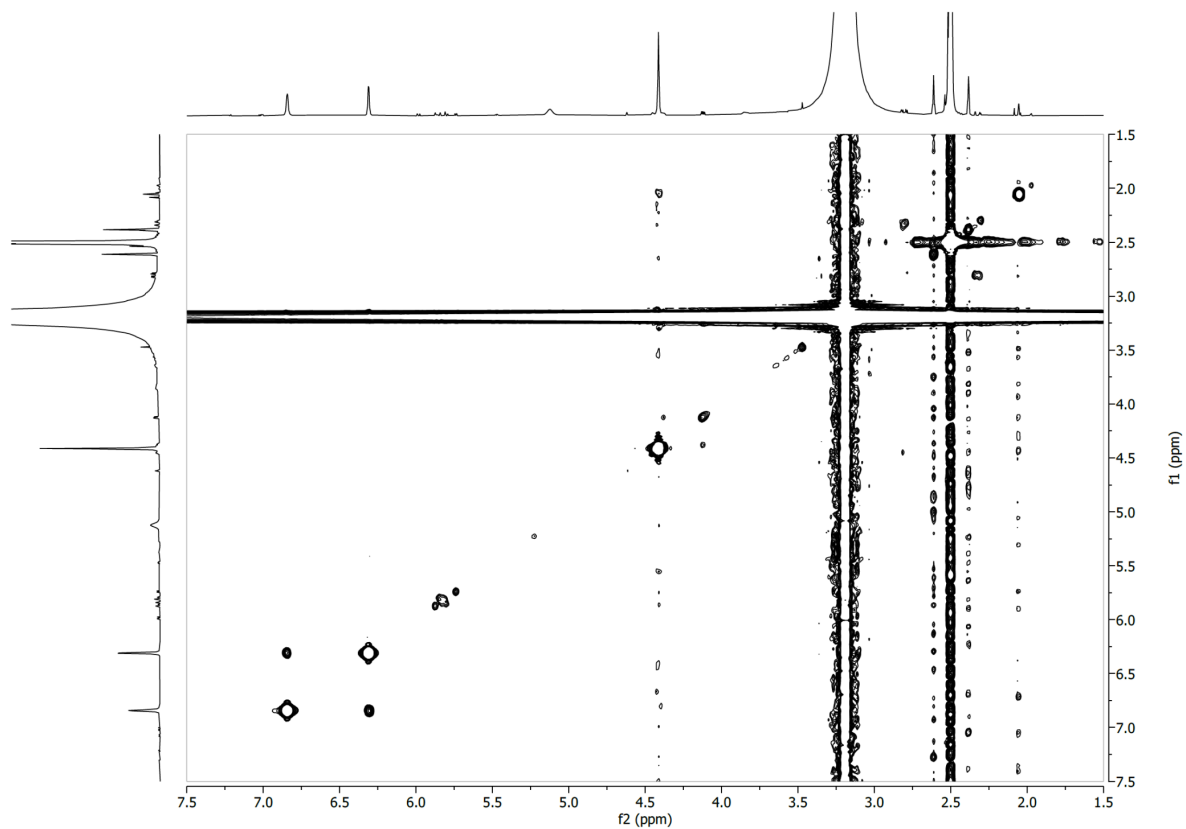


Figure S13. COSY NMR spectrum of compound **3** in DMSO-*d*₆

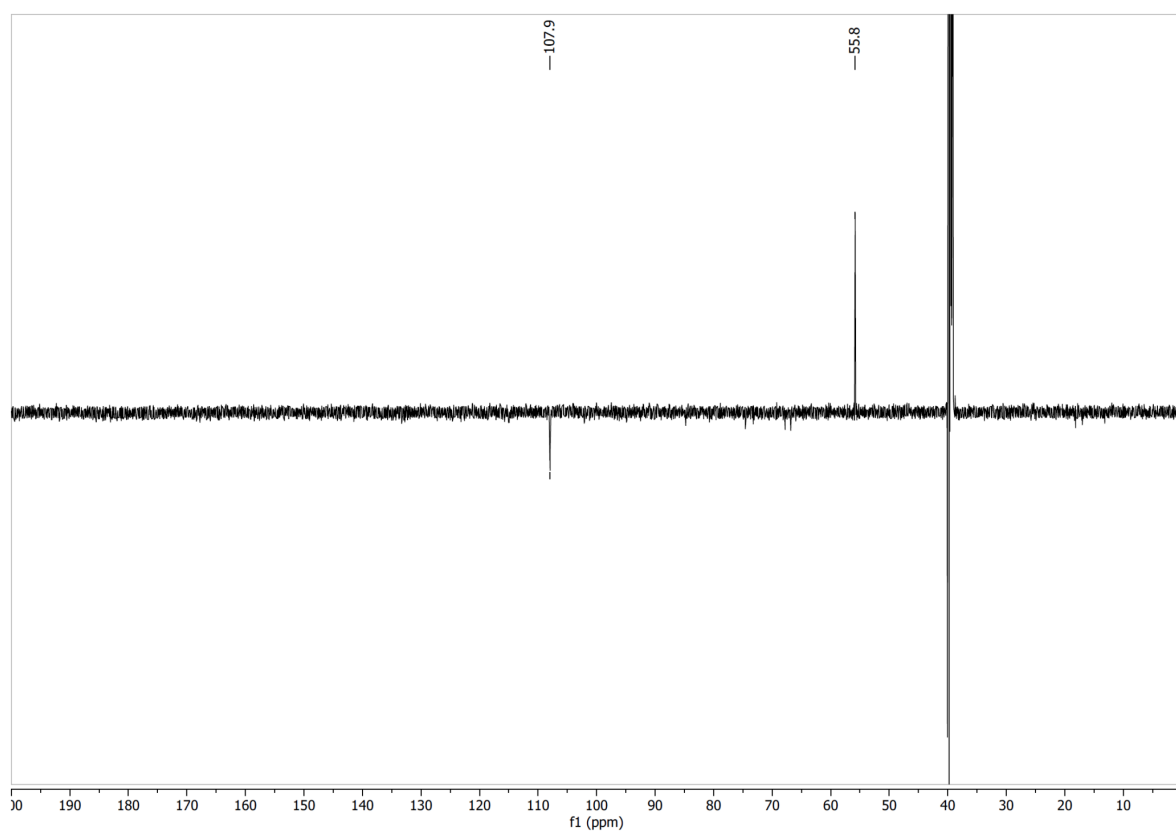


Figure S14. ^{13}C -DEPTQ NMR spectrum of compound **3** in $\text{DMSO}-d_6$

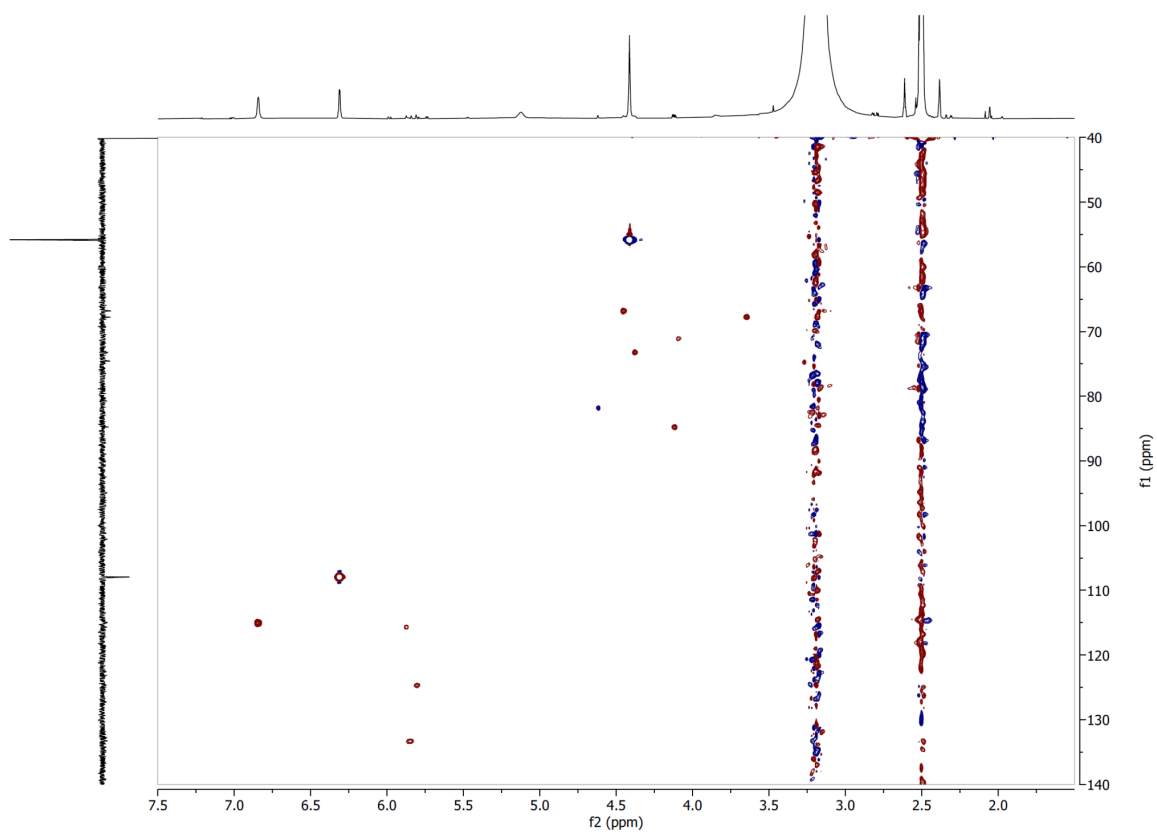


Figure S15. Edited-HSQC NMR spectrum of compound **3** in $\text{DMSO}-d_6$

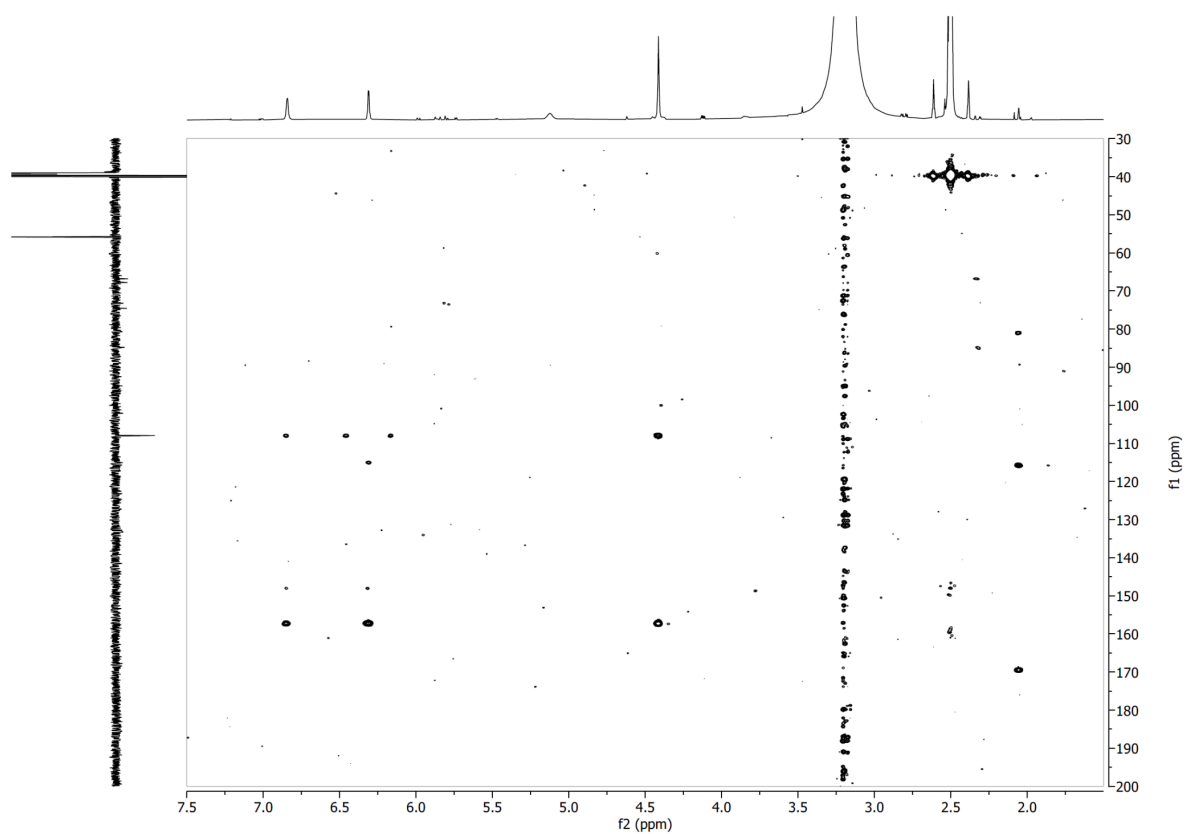


Figure S16. HMBC NMR spectrum of compound **3** in DMSO- d_6

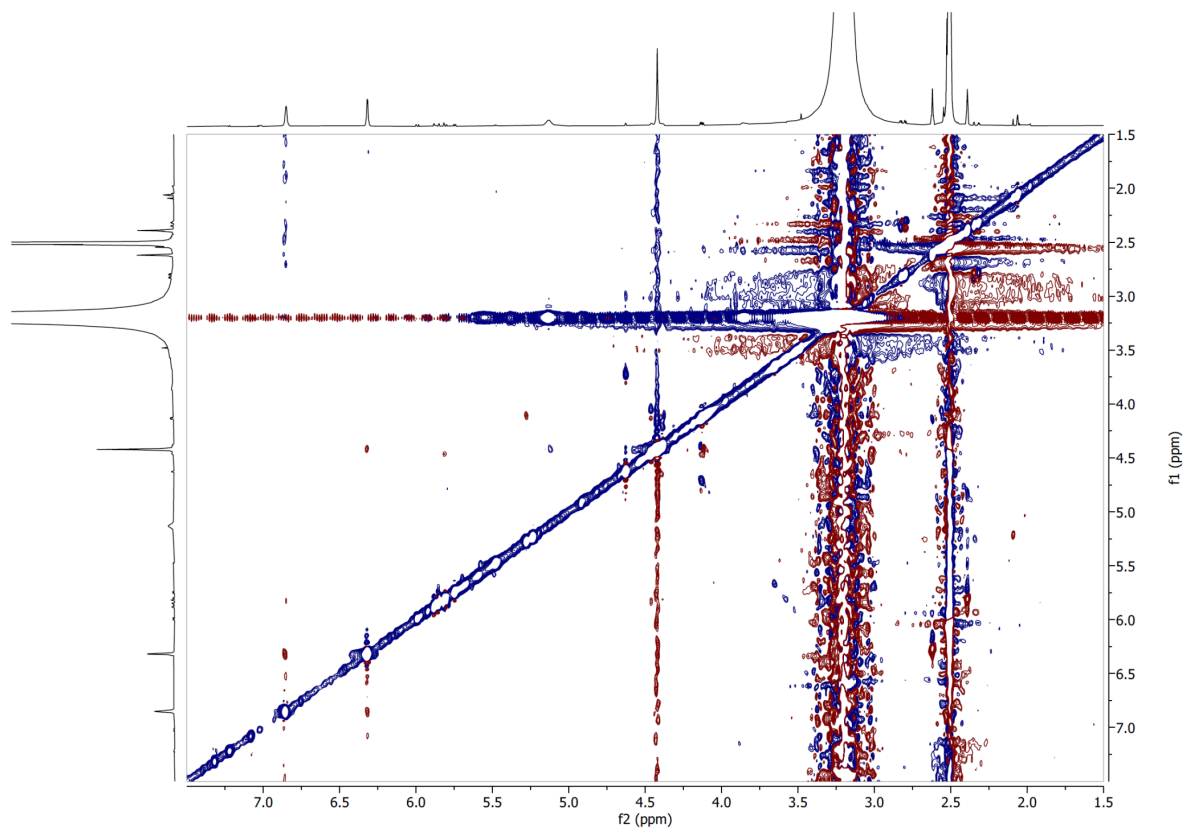


Figure S17. ROESY NMR spectrum of compound **3** in DMSO- d_6

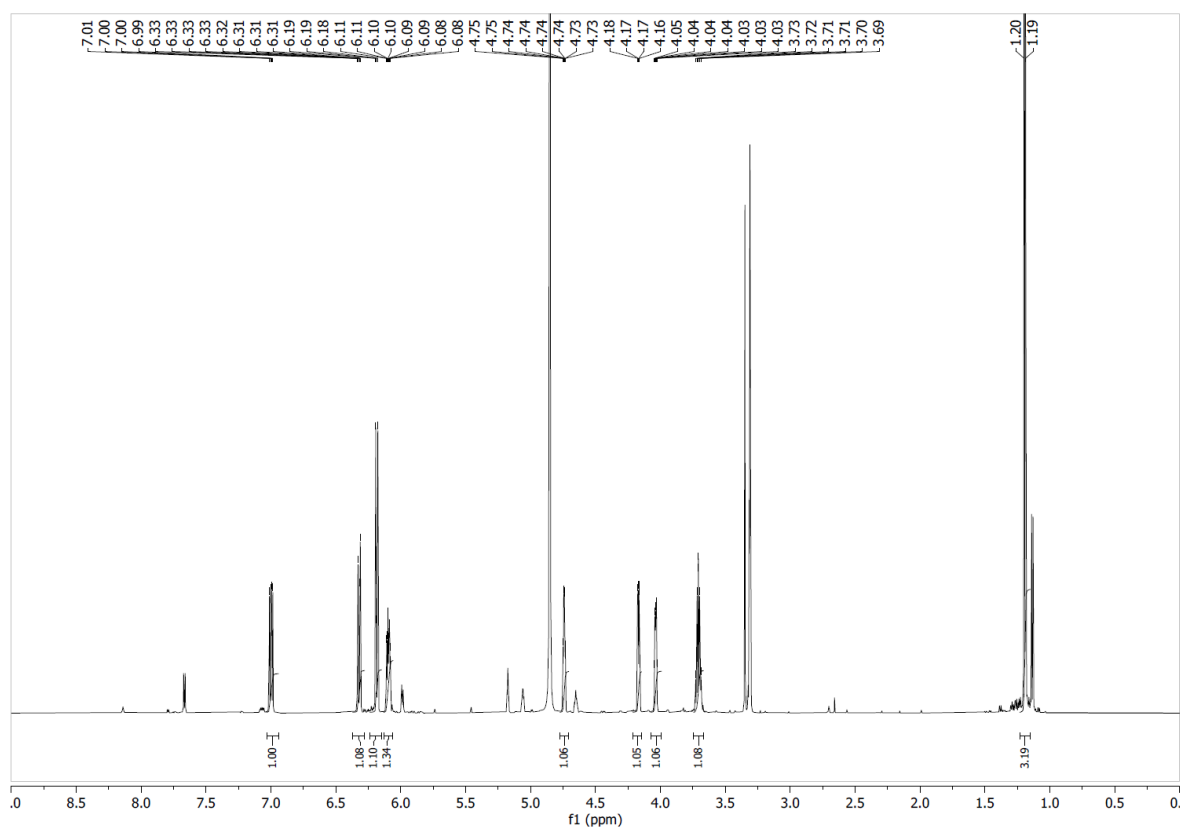


Figure S18. ¹H NMR spectrum of compound **4** in CD₃OD

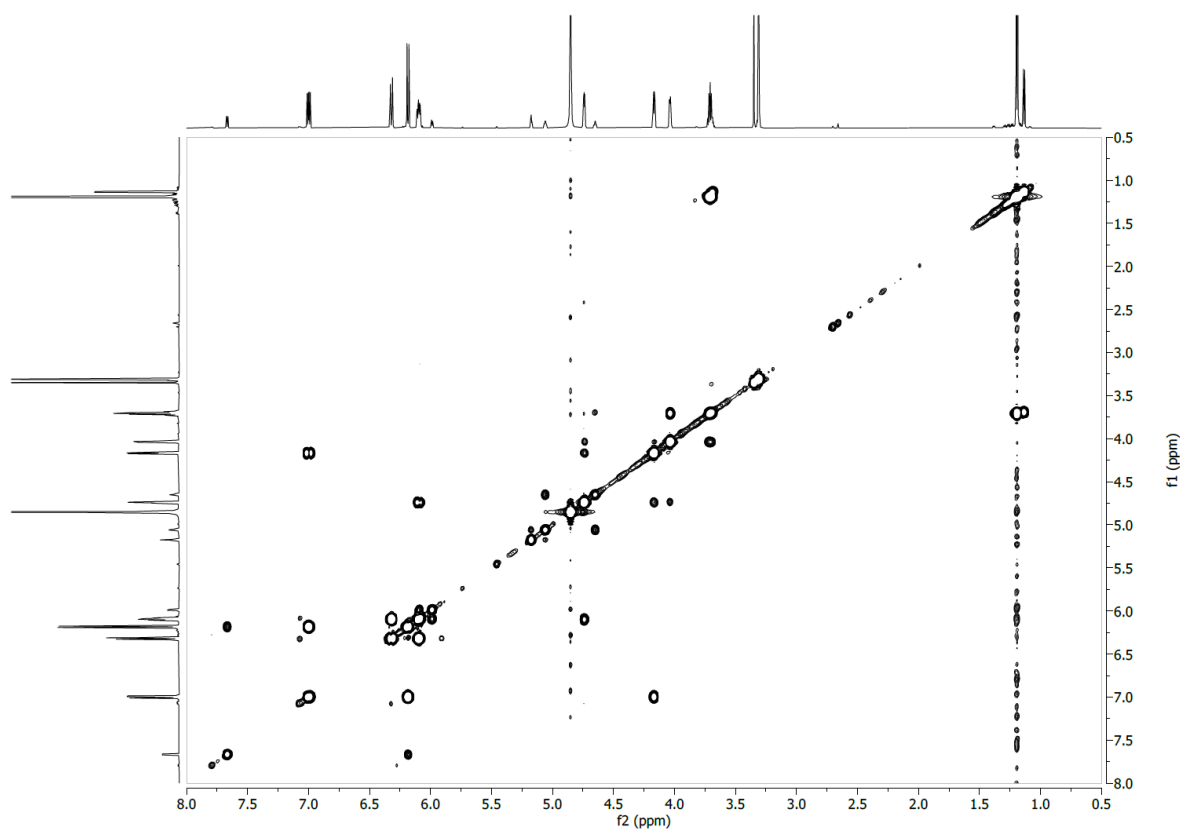


Figure S19. COSY NMR spectrum of compound **4** in CD₃OD

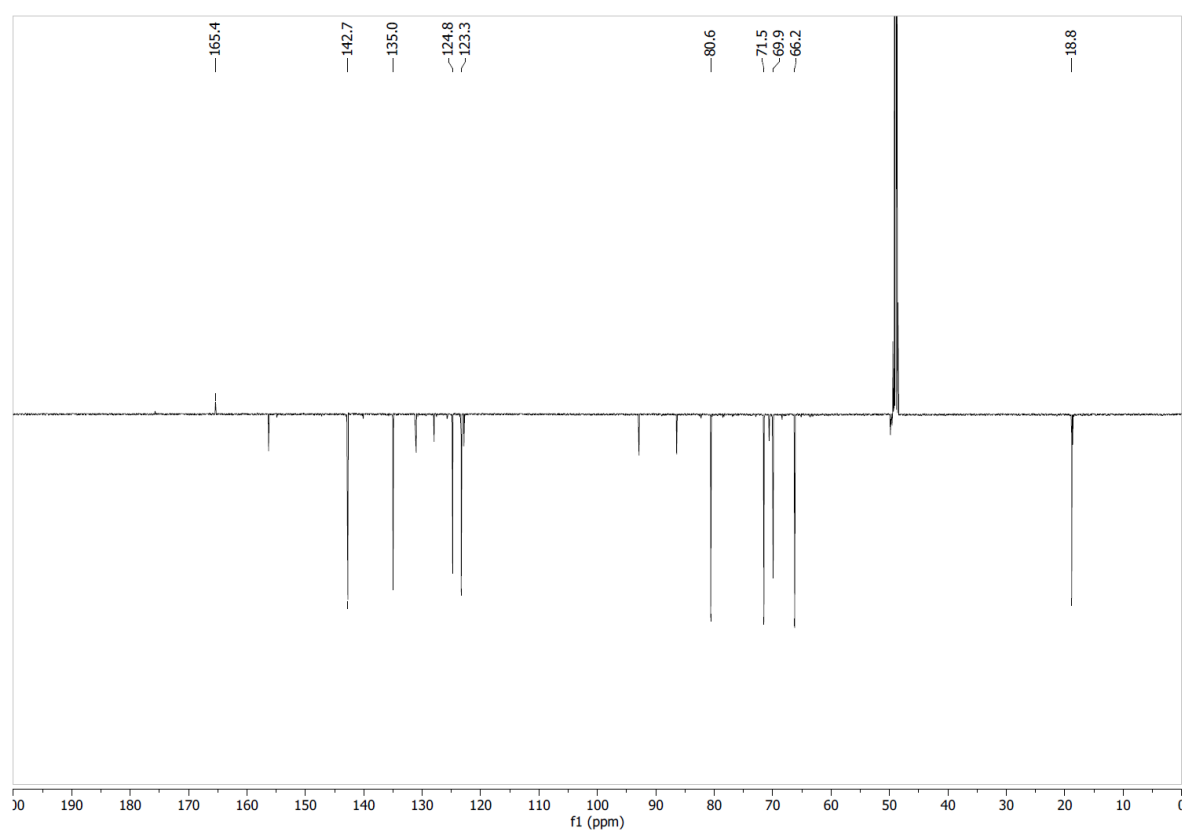


Figure S20. ¹³C-DEPTQ NMR spectrum of compound **4** in CD₃OD at 151 MHz

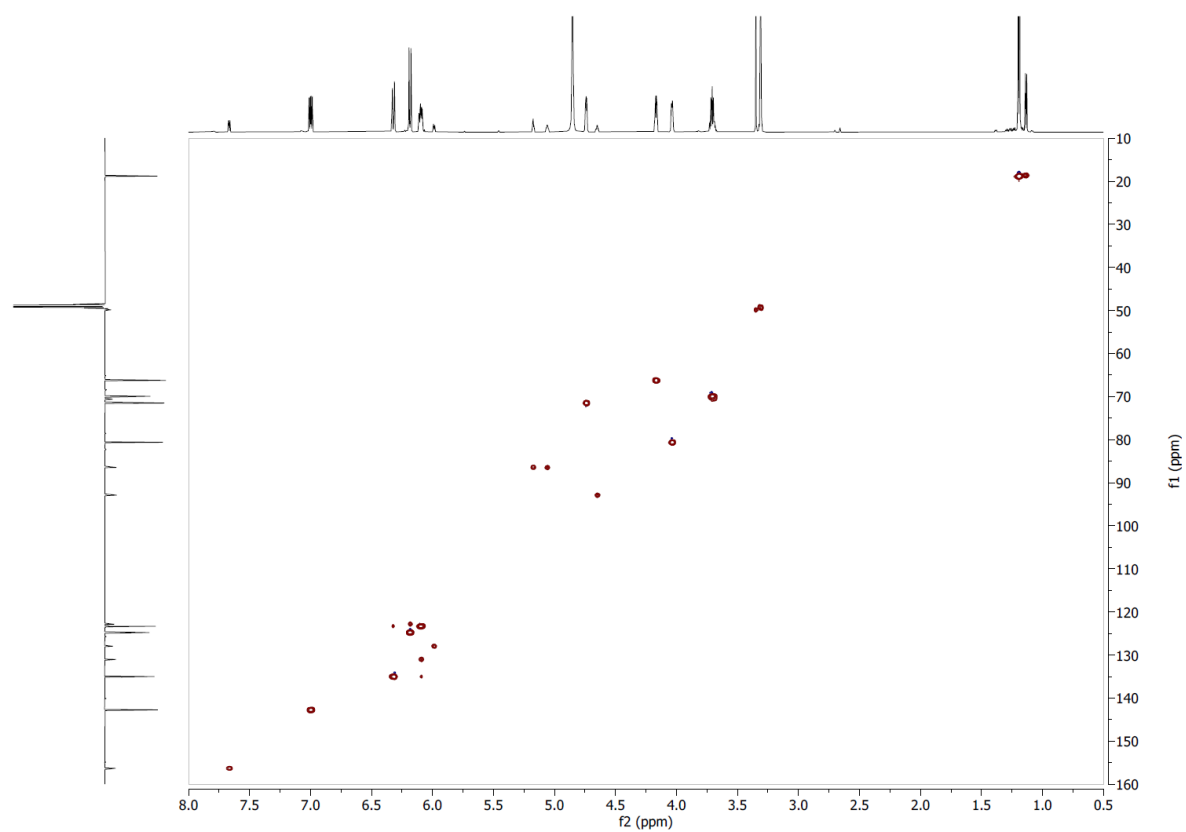


Figure S21. Edited-HSQC NMR spectrum of compound **4** in CD₃OD

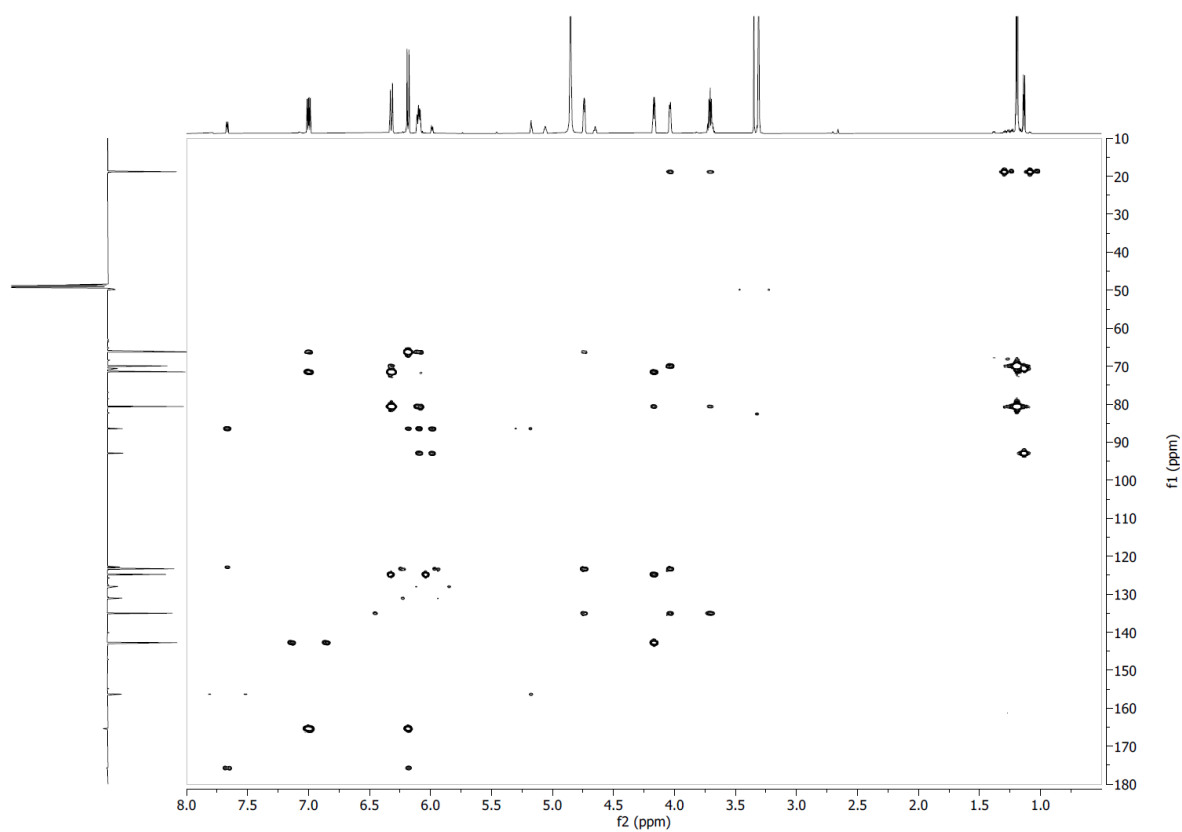


Figure S22. HMBC NMR spectrum of compound **4** in CD₃OD

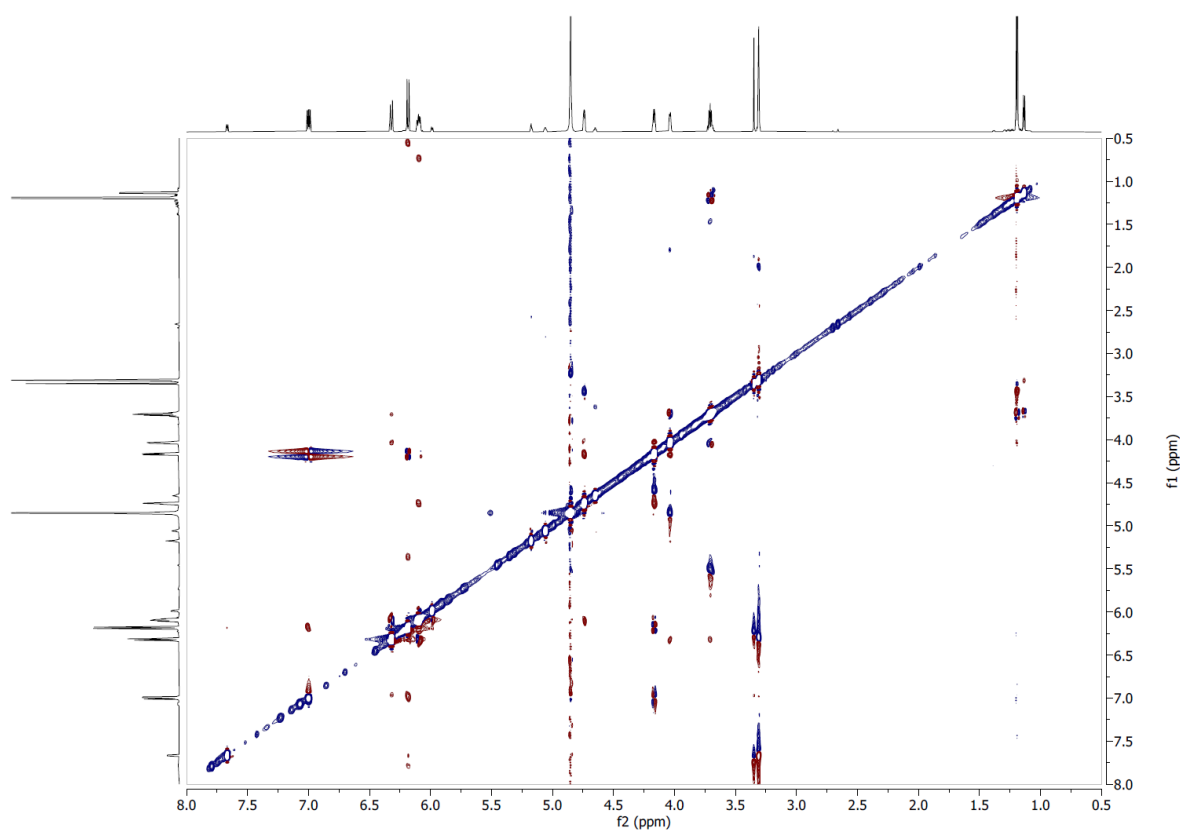


Figure S23. ROESY NMR spectrum of compound **4** in CD₃OD

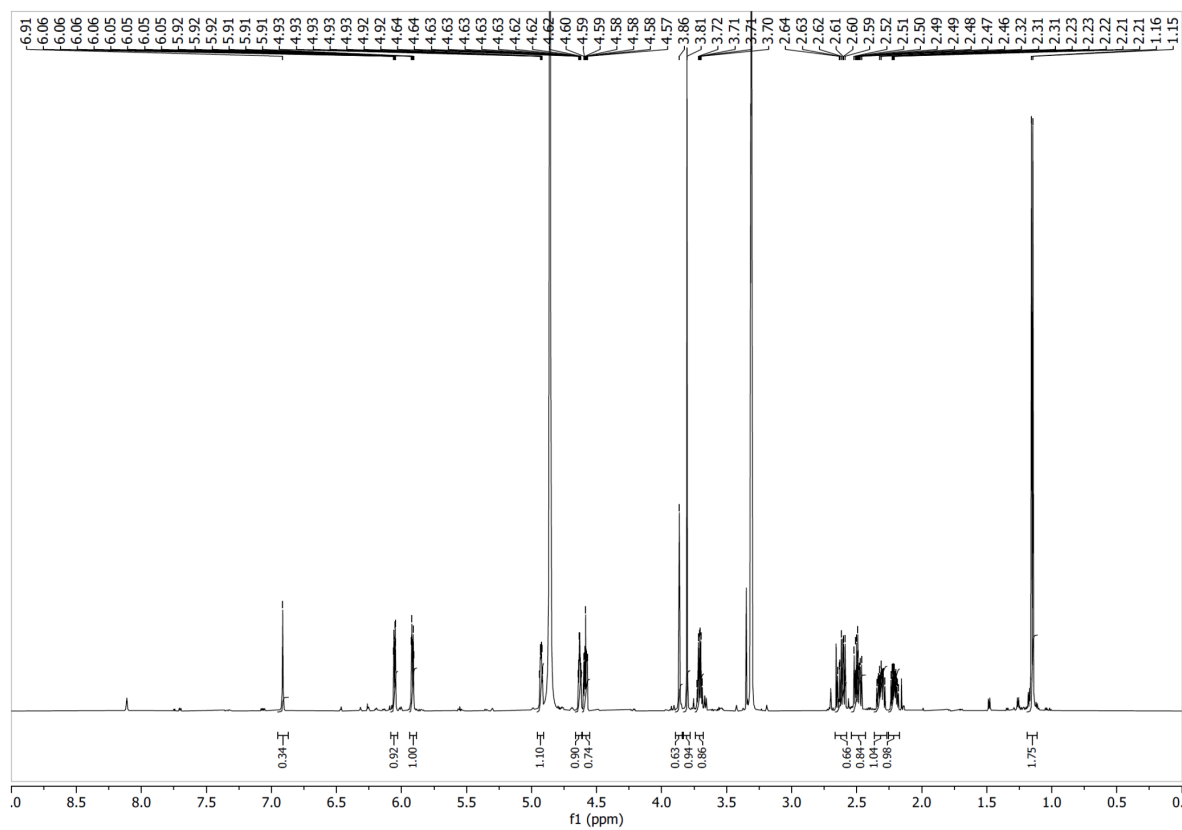


Figure S24. ¹H NMR spectrum of compound **5** in CD₃OD at 600 MHz

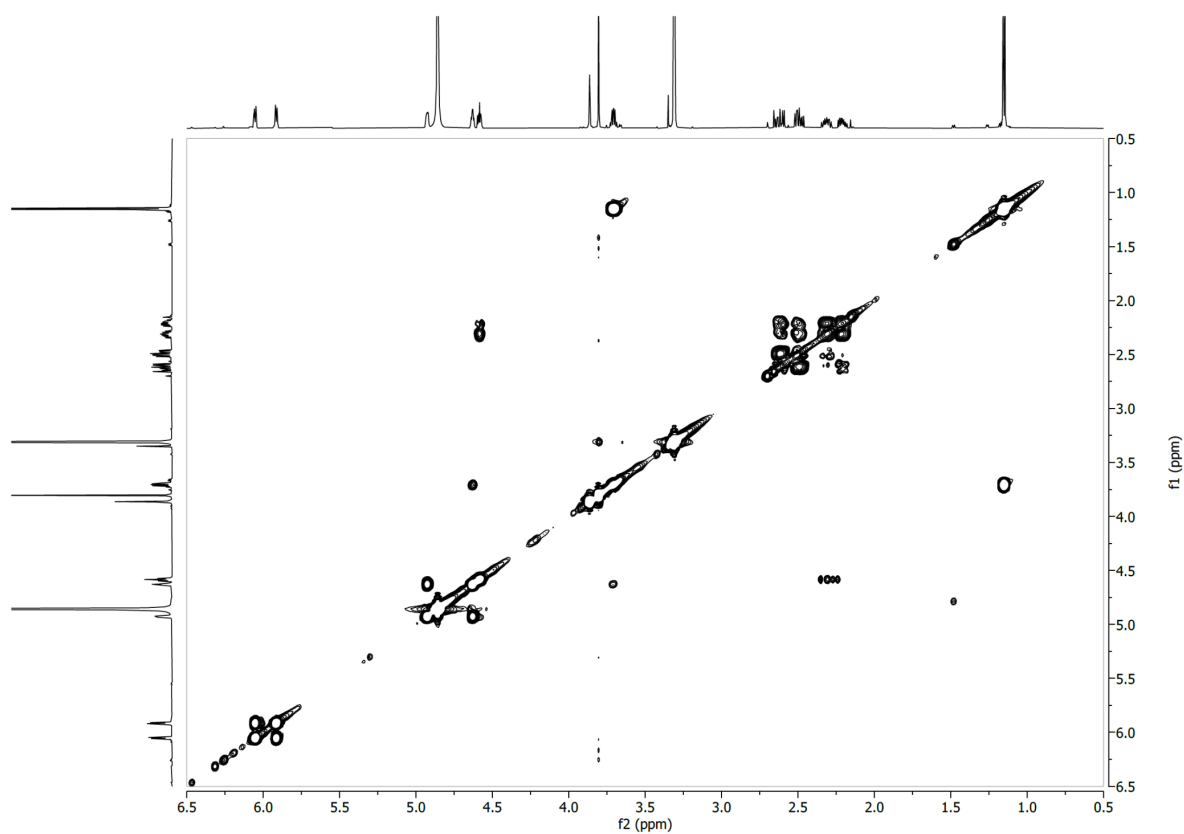


Figure S25. COSY NMR spectrum of compound **5** in CD₃OD

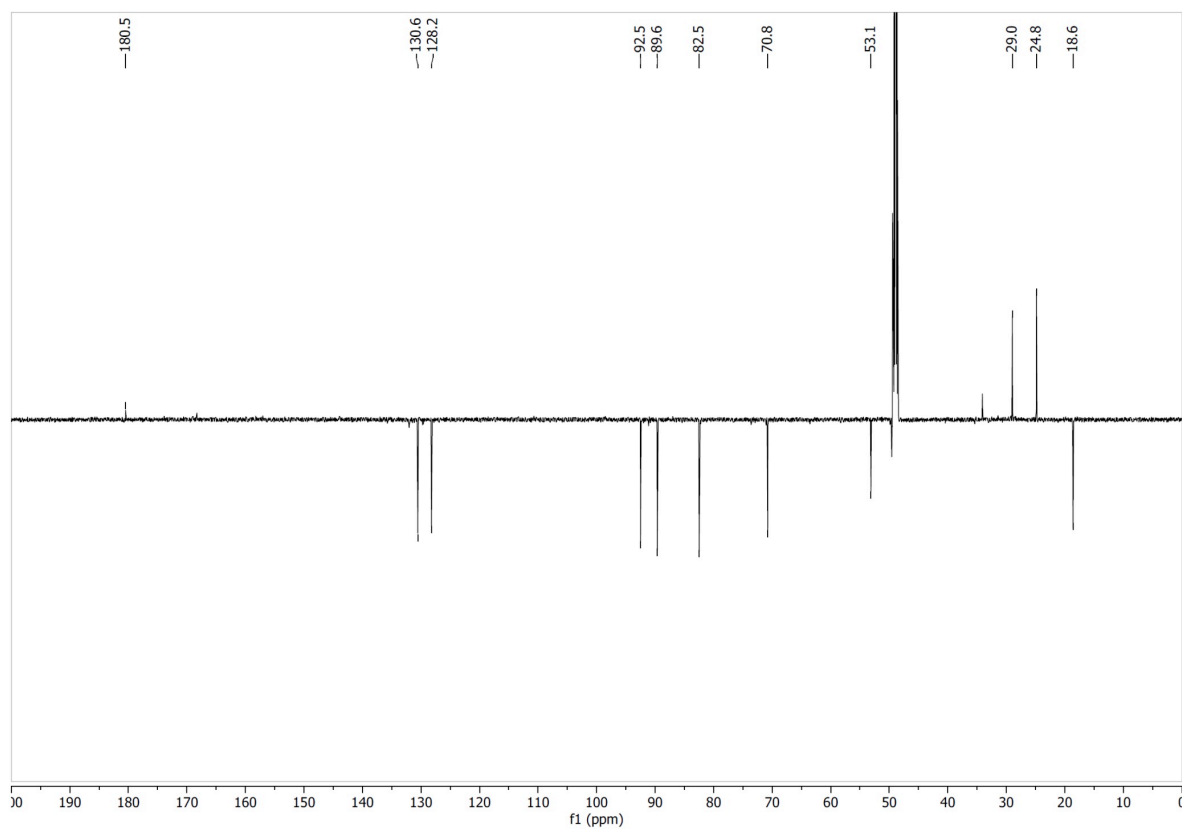


Figure S26. ¹³C-DEPTQ NMR spectrum of compound **5** in CD₃OD at 156 MHz

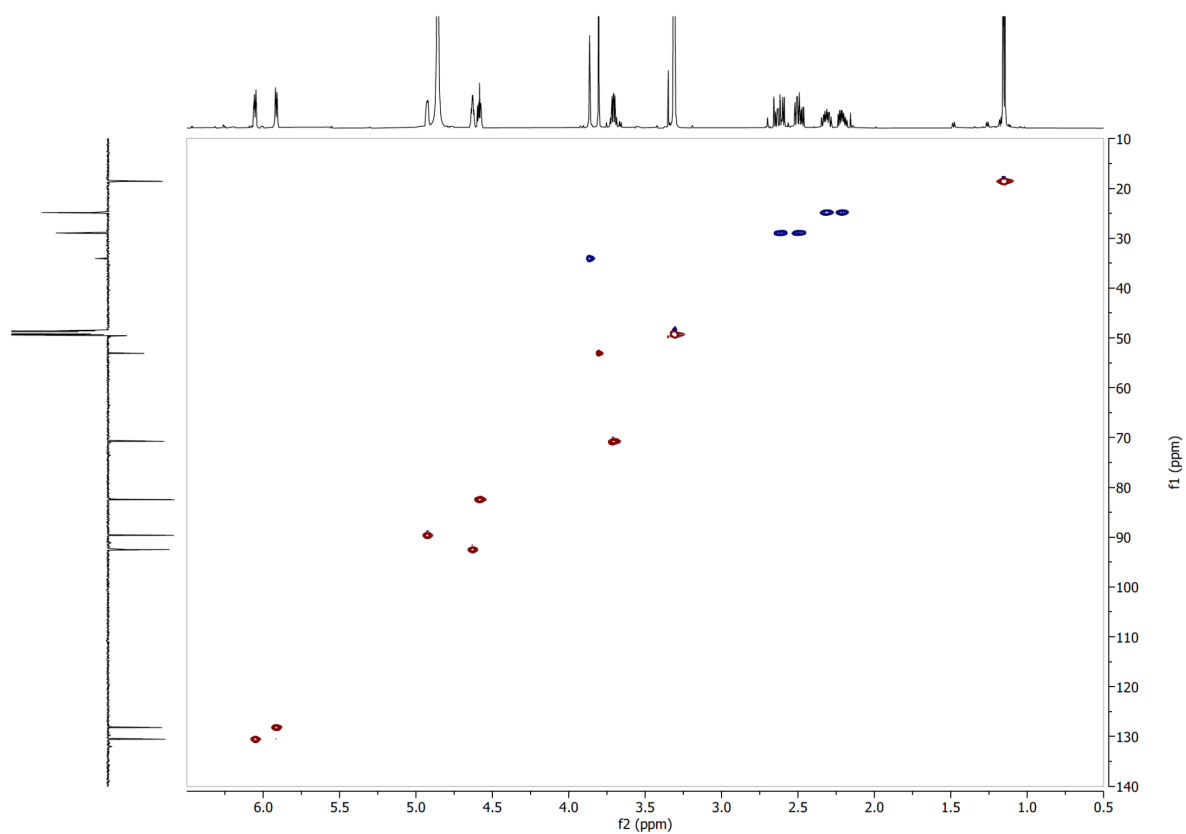


Figure S27. Edited-HSQC NMR spectrum of compound **5** in CD₃OD

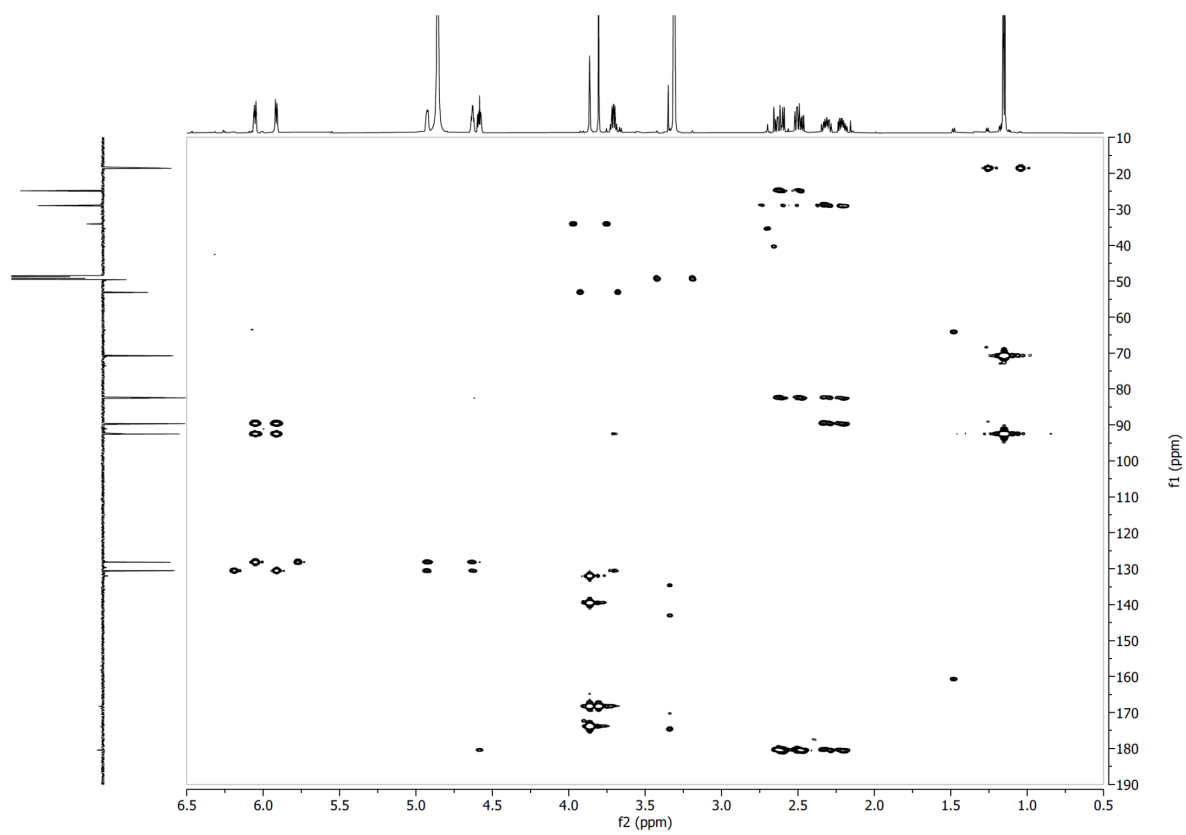


Figure S28. HMBC NMR spectrum of compound **5** in CD₃OD

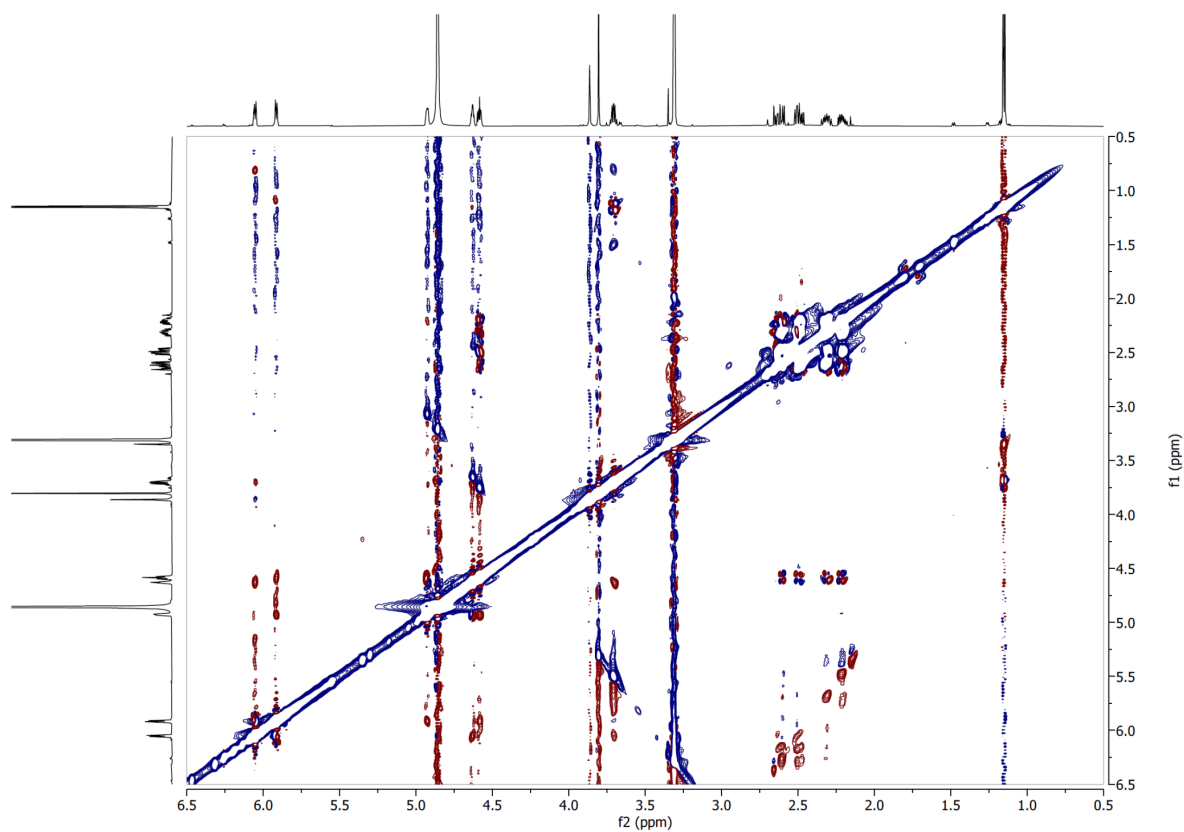


Figure S29. ROESY NMR spectrum of compound **5** in CD₃OD

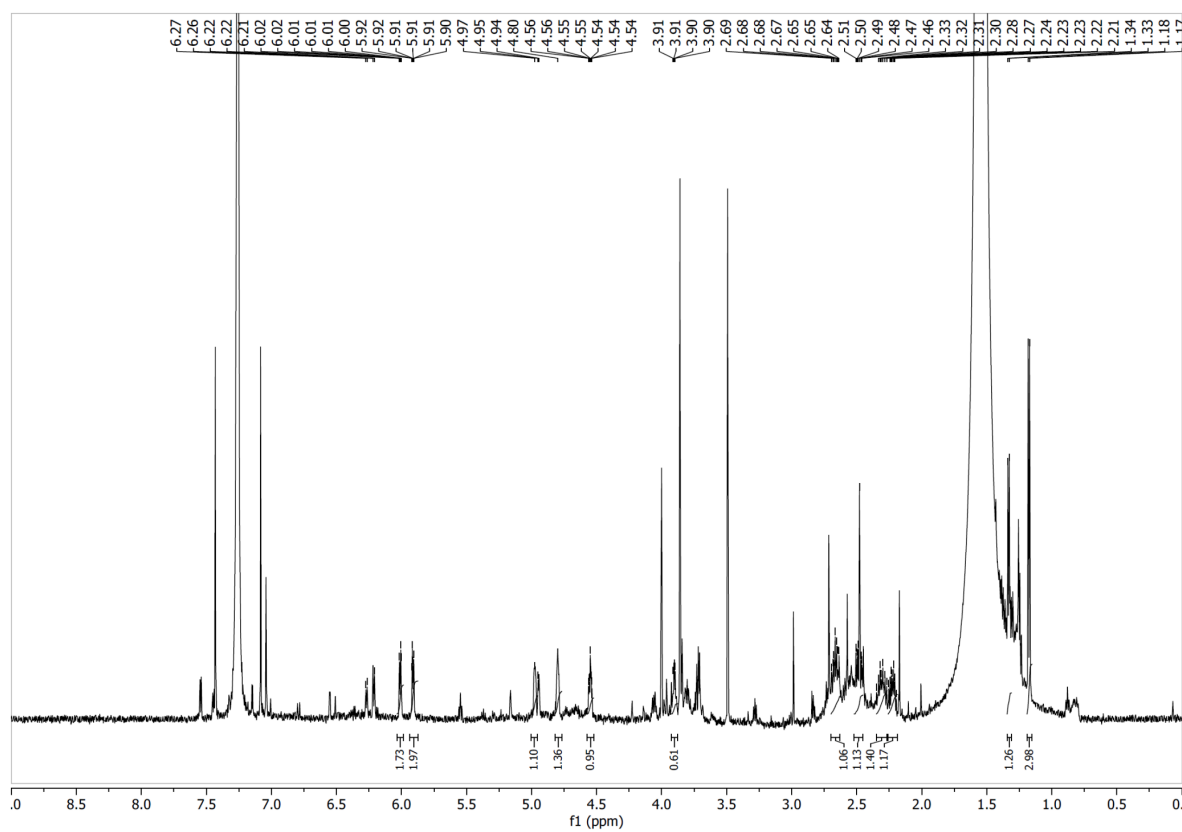


Figure S30. ^1H NMR spectrum of compound **5** in CDCl_3 at 600 MHz

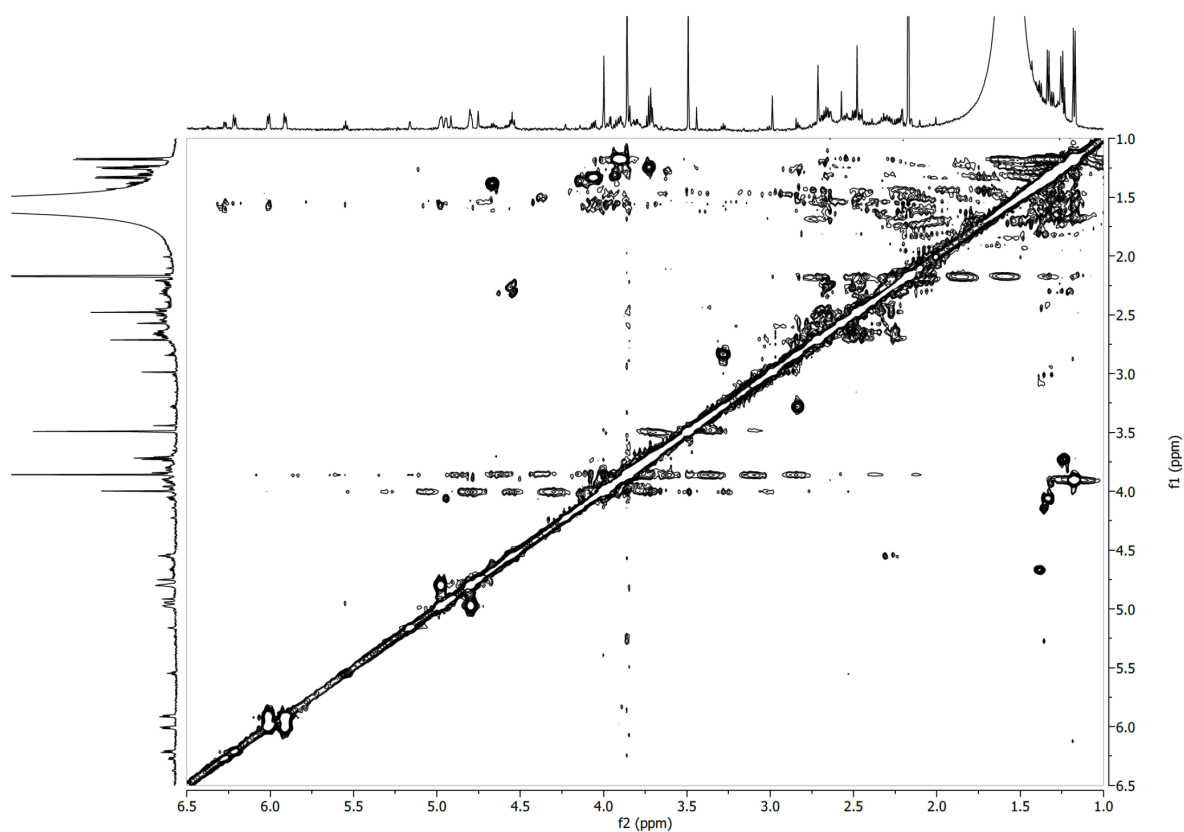


Figure S31. COSY NMR spectrum of compound **5** in CDCl_3

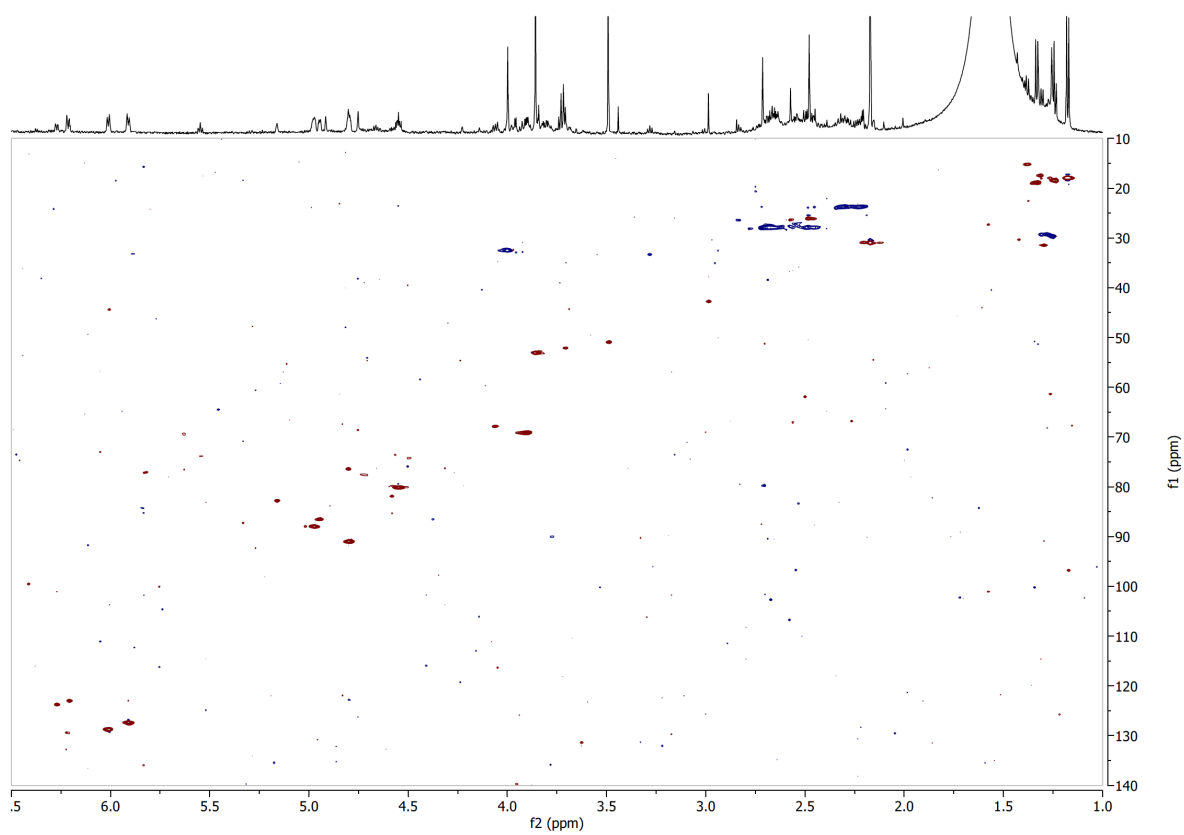


Figure S32. Edited-HSQC NMR spectrum of compound **5** in CDCl₃

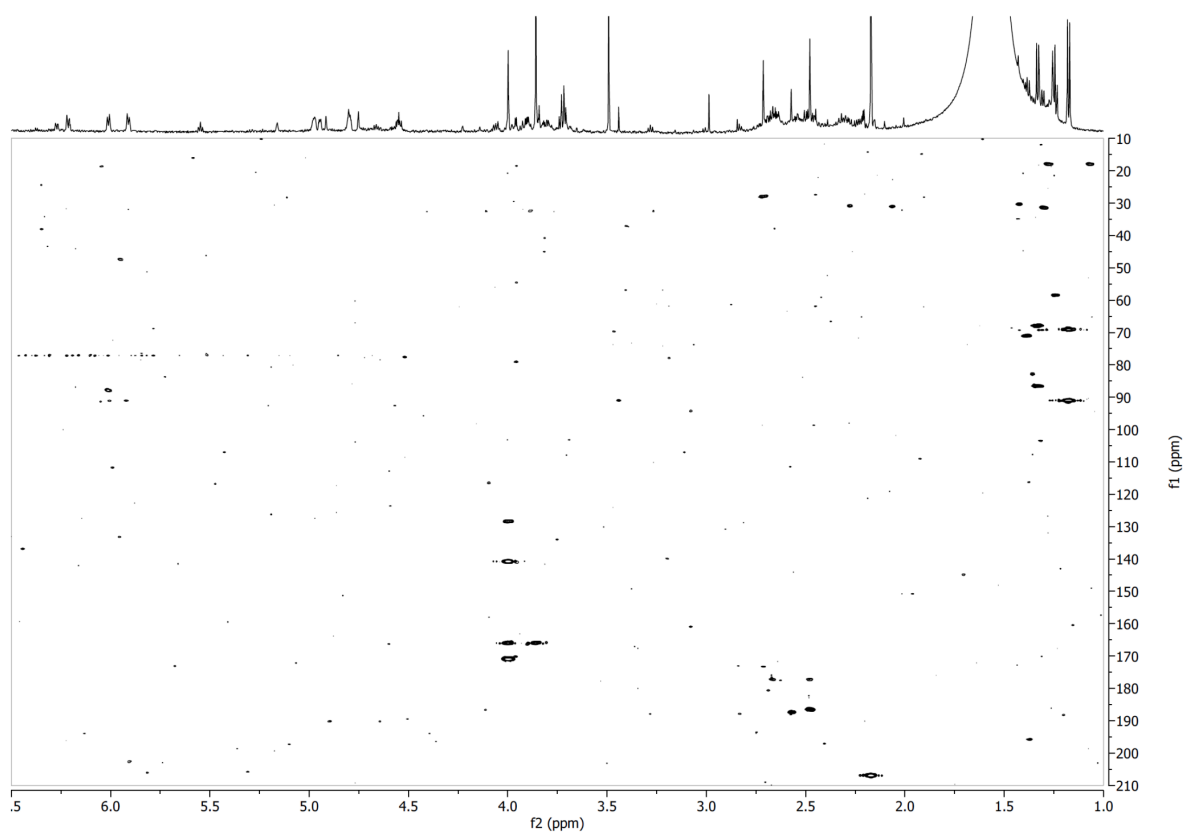


Figure S33. HMBC NMR spectrum of compound **5** in CDCl₃

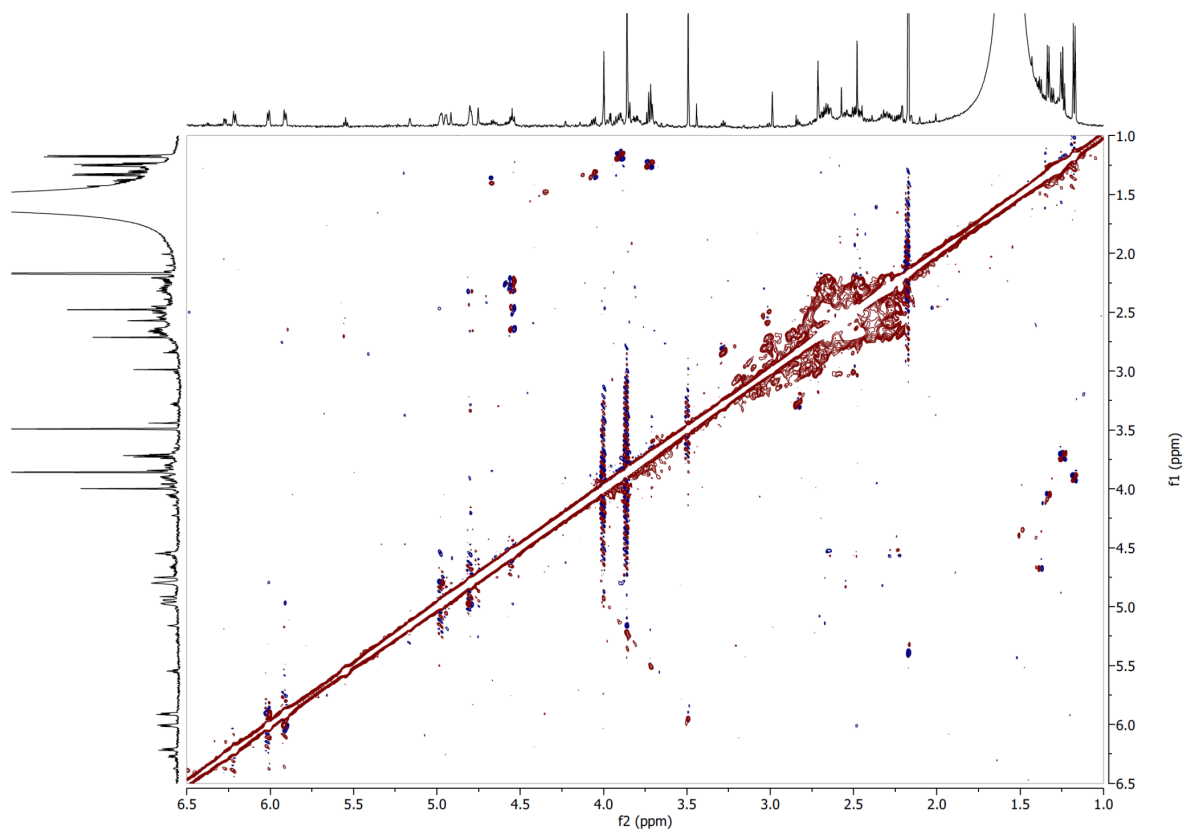


Figure S34. ROESY NMR spectrum of compound **5** in CDCl_3

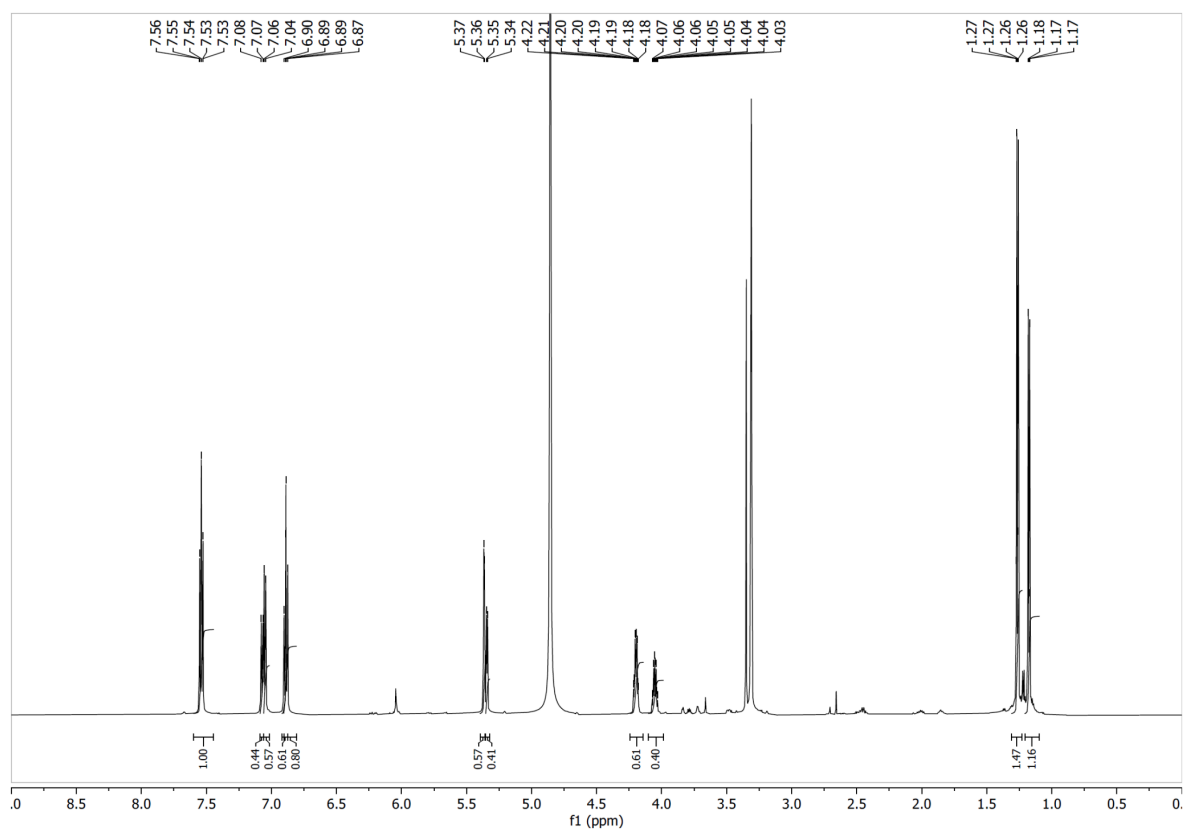


Figure S35. ^1H NMR spectrum of compound **6a** and **6b** in CD_3OD

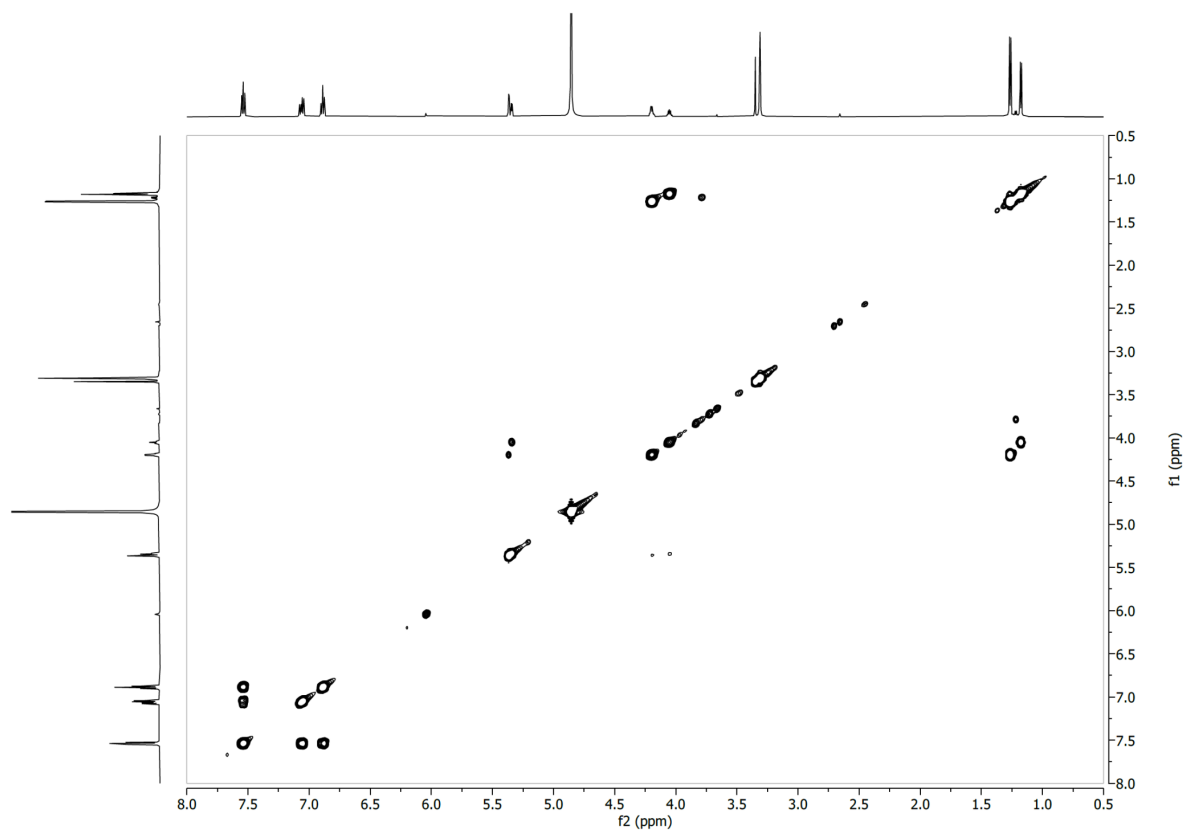


Figure S36. COSY NMR spectrum of compound **6a** and **6b** in CD₃OD

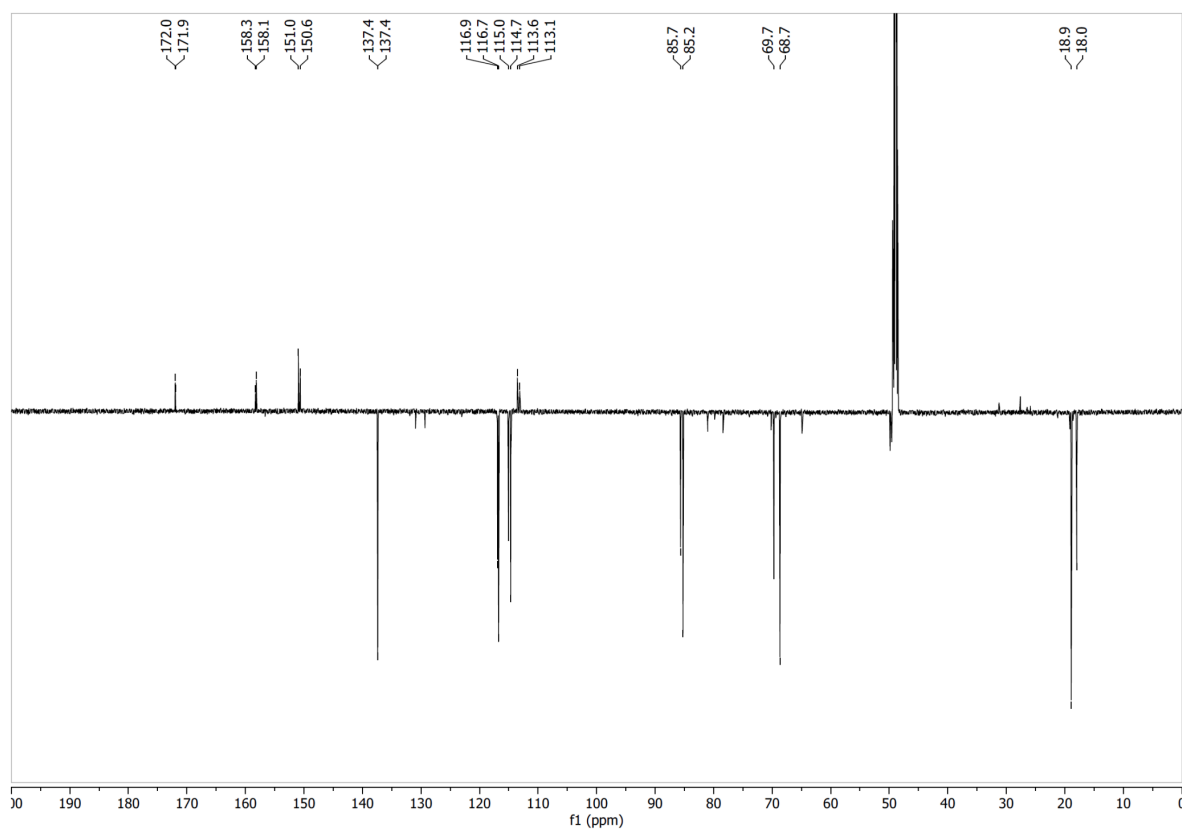


Figure S37. ¹³C-DEPTQ NMR spectrum of compound **6a** and **6b** in CD₃OD

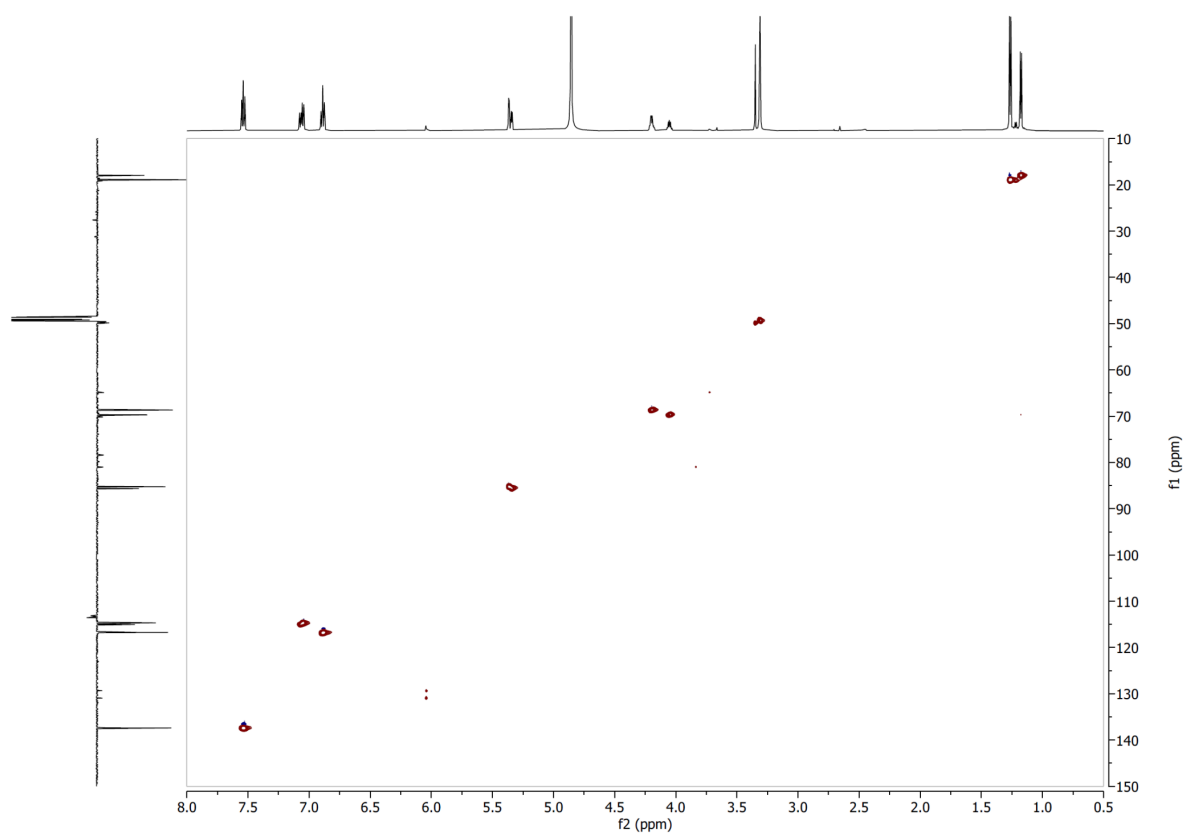


Figure S38. Edited-HSQC NMR spectrum of compound **6a** and **6b** in CD₃OD

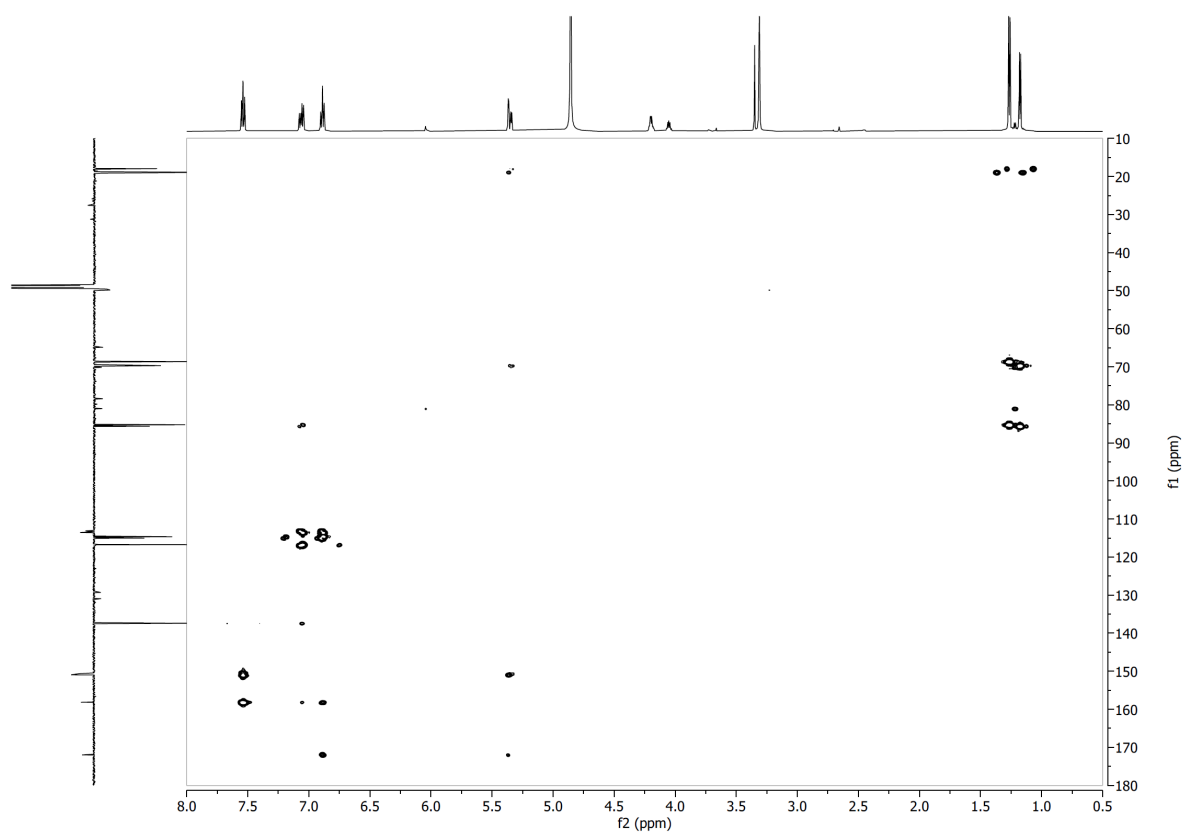


Figure S39. HMBC NMR spectrum of compound **6a** and **6b** in CD₃OD

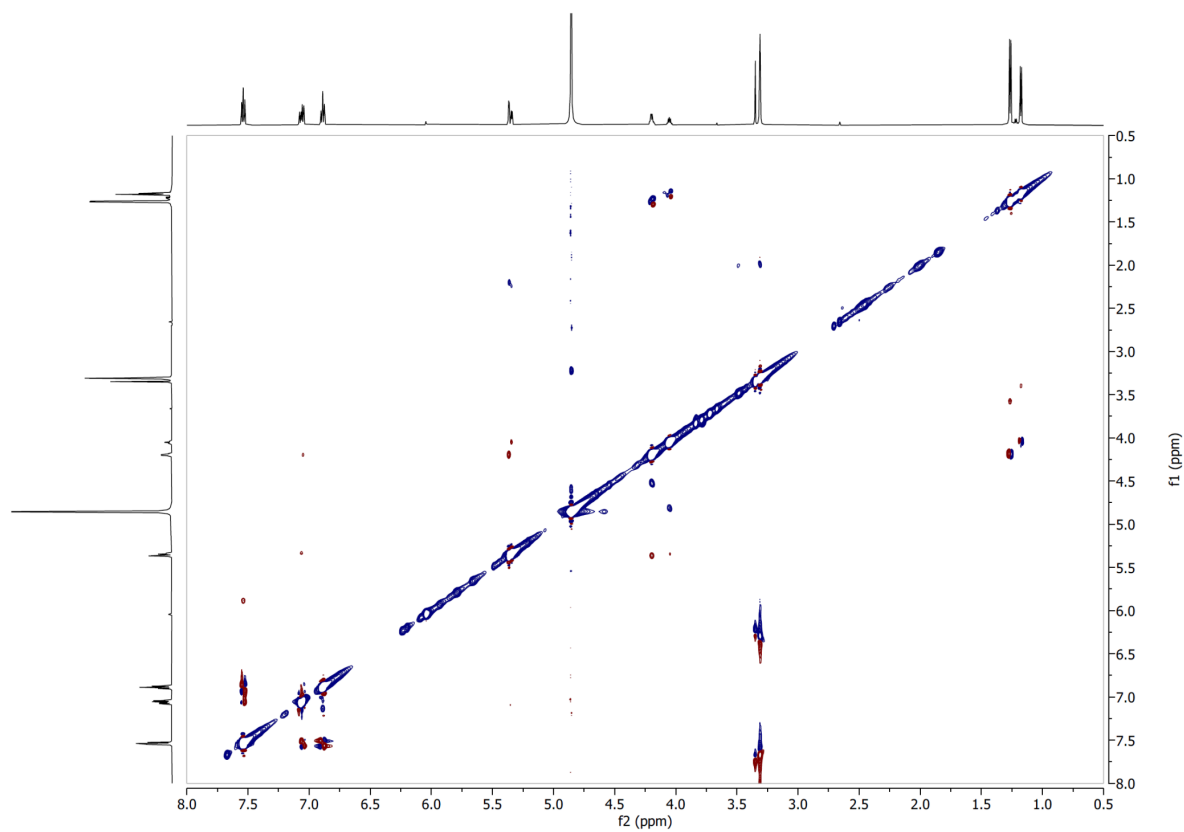


Figure S40. ROESY NMR spectrum of compound **6a** and **6b** in CD₃OD

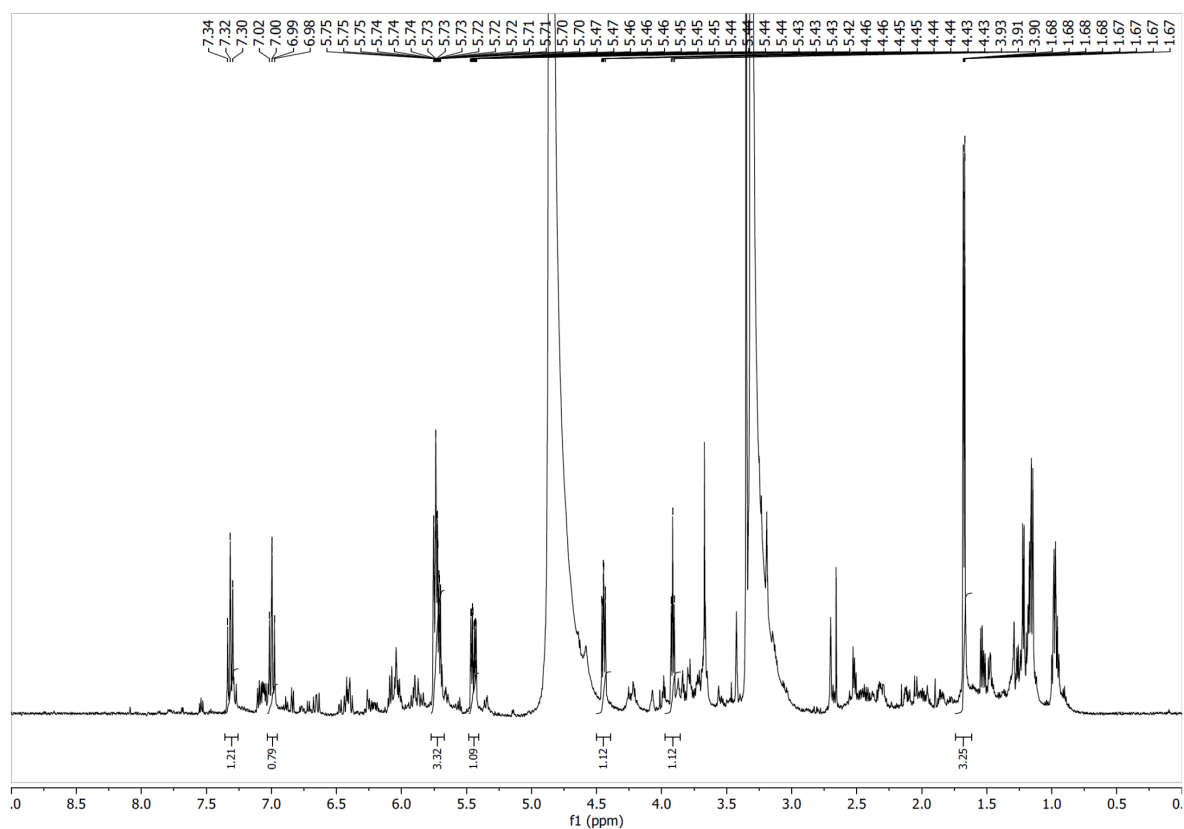


Figure S41. ¹H NMR spectrum of compound **7** in CD₃OD

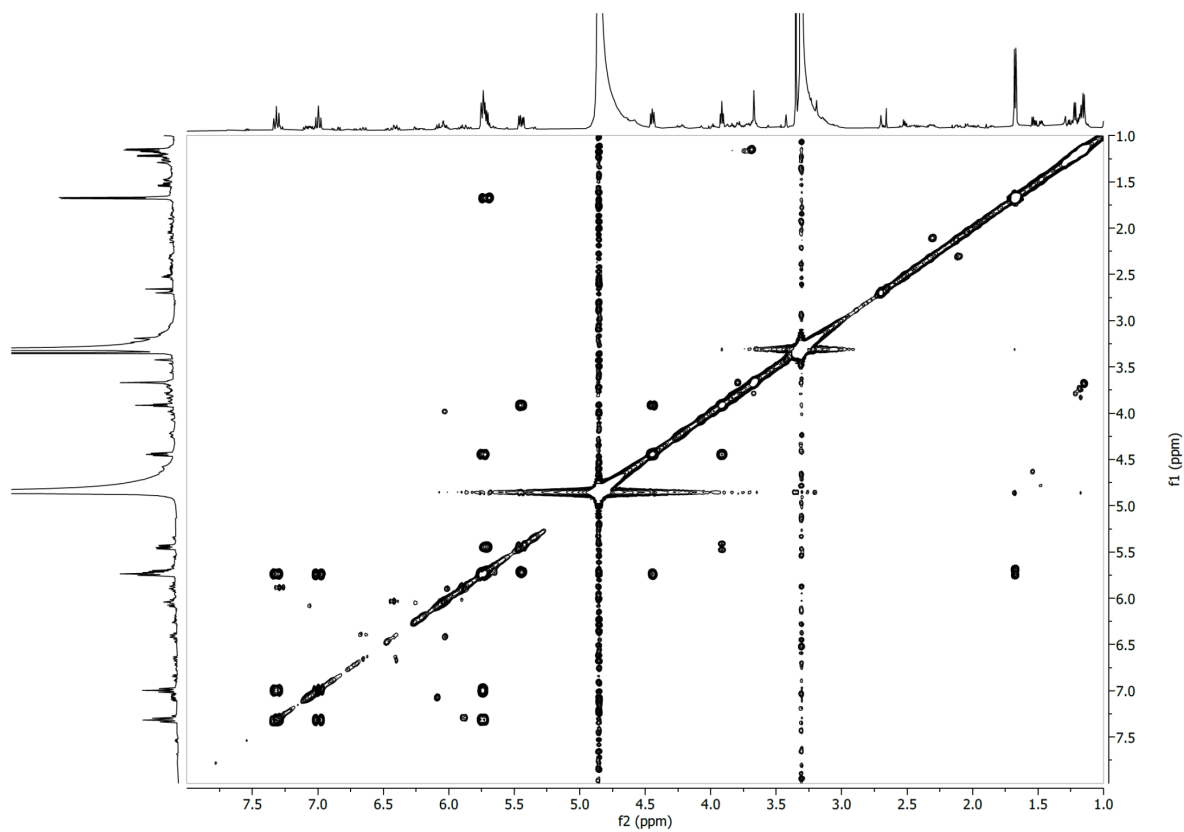


Figure S42. COSY NMR spectrum of compound **7** in CD₃OD

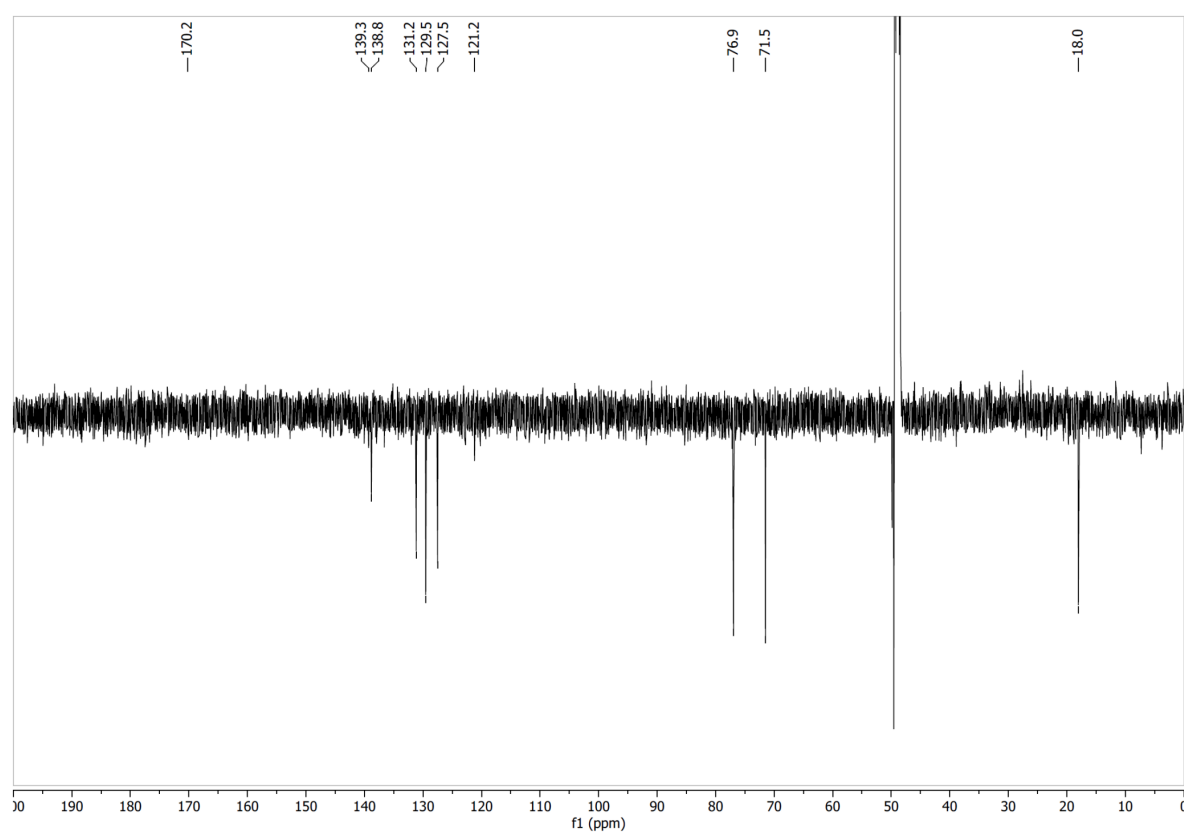


Figure S43. ¹³C-DEPTQ NMR spectrum of compound **7** in CD₃OD at 151 MHz

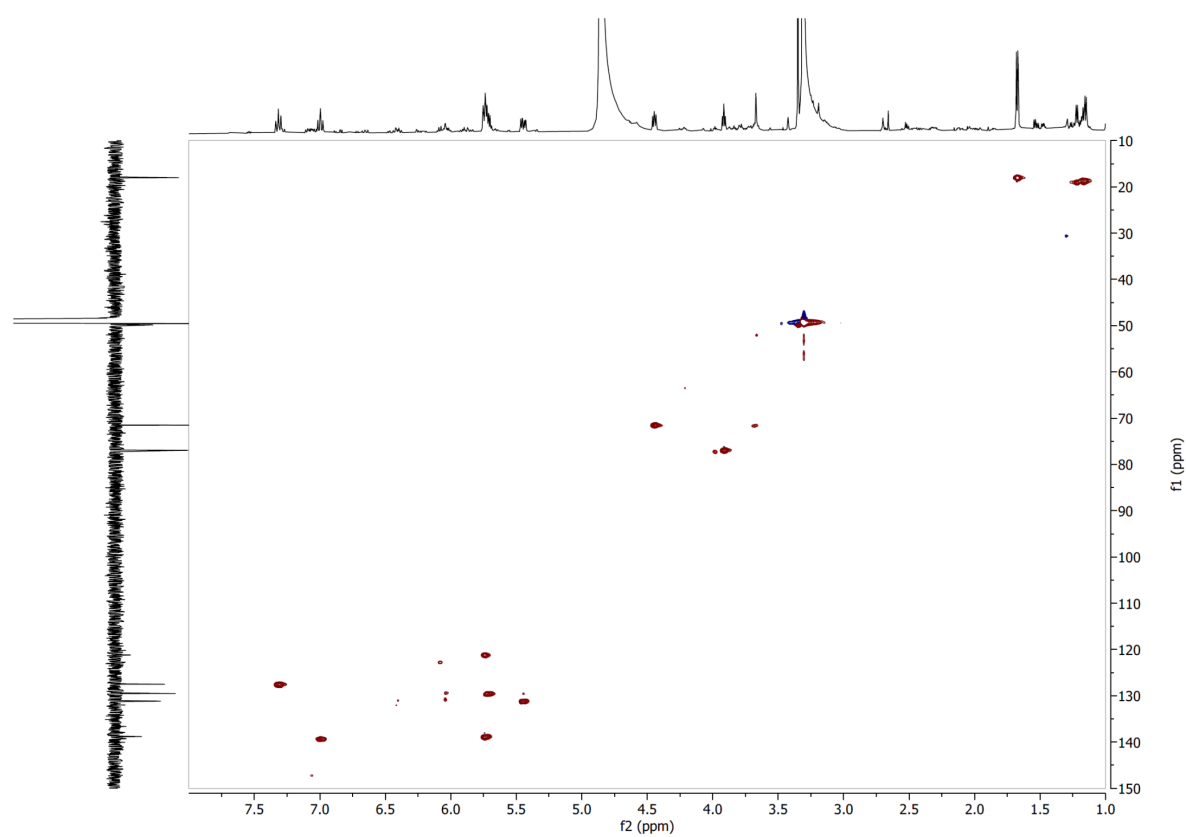


Figure S44. Edited-HSQC NMR spectrum of compound **7** in CD₃OD

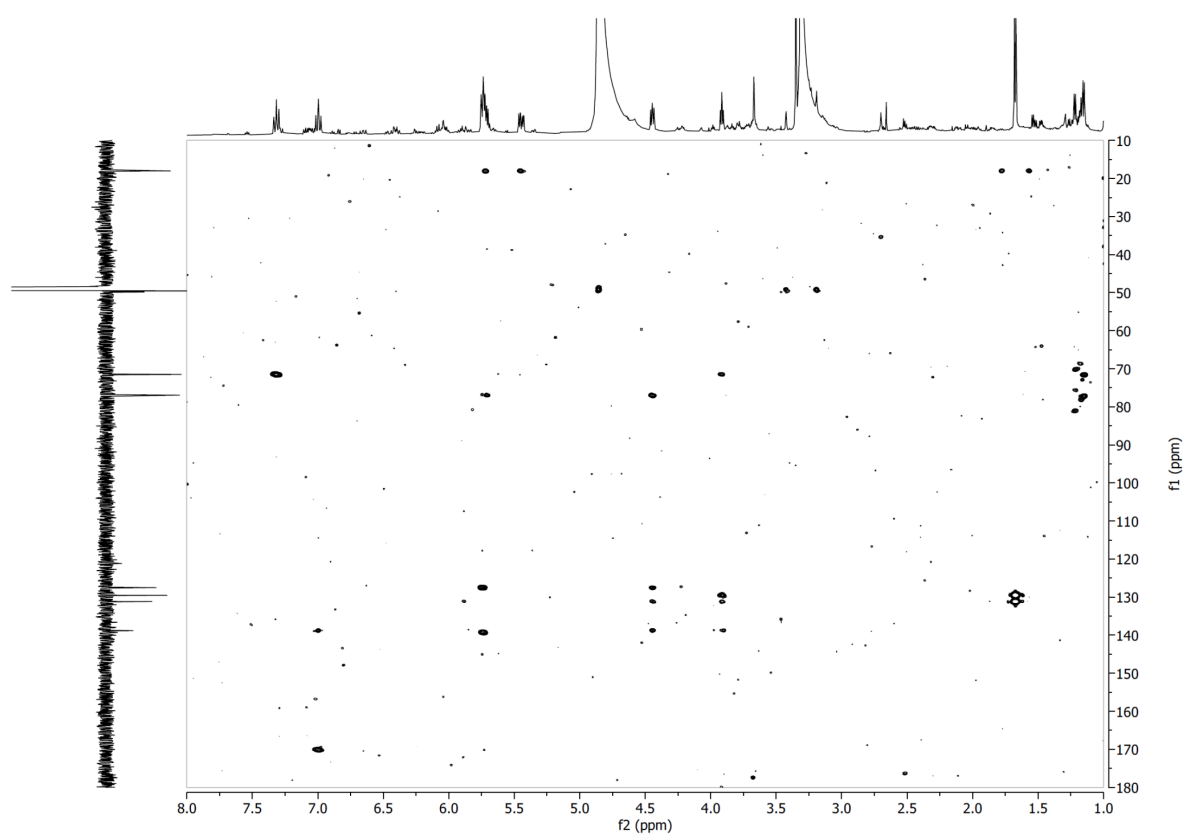


Figure S45. HMBC NMR spectrum of compound **7** in CD₃OD

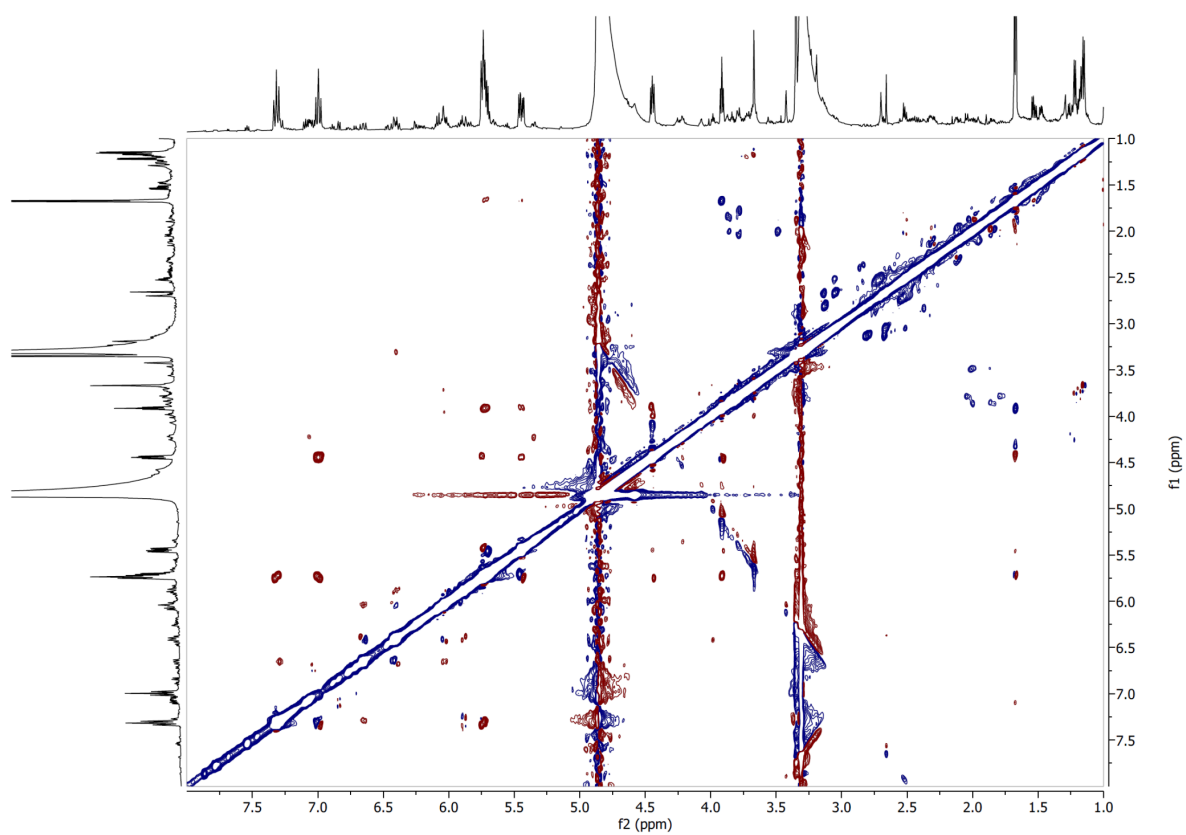


Figure S46. ROESY NMR spectrum of compound **7** in CD₃OD

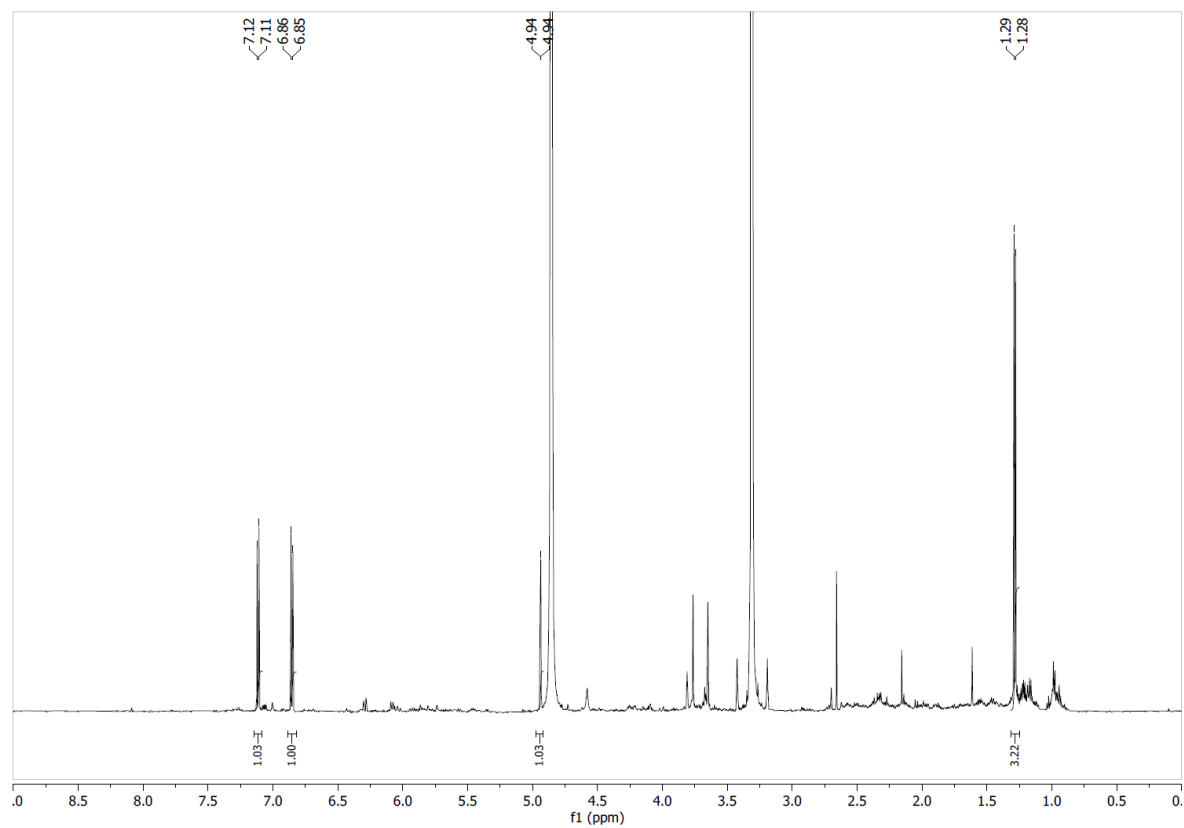


Figure S47. ¹H NMR spectrum of compound **8** in CD₃OD at 600 MHz

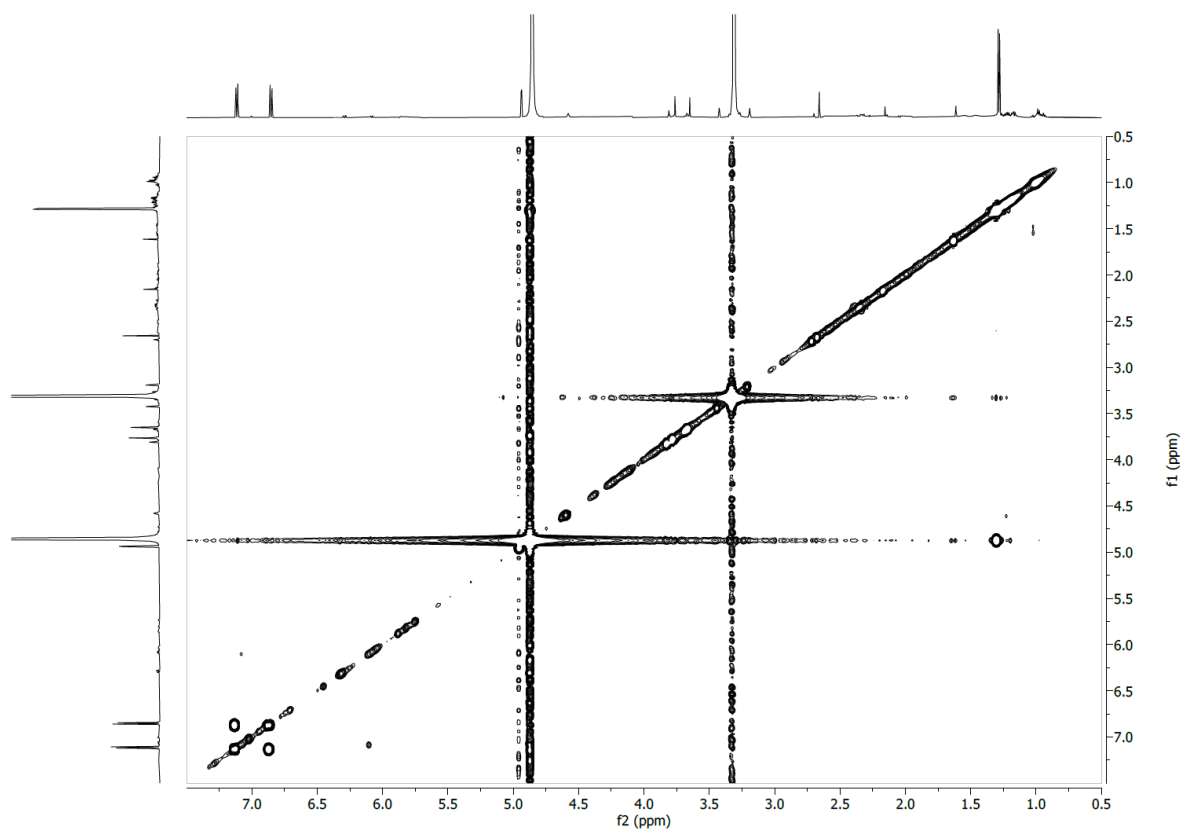


Figure S48. COSY NMR spectrum of compound **8** in CD₃OD

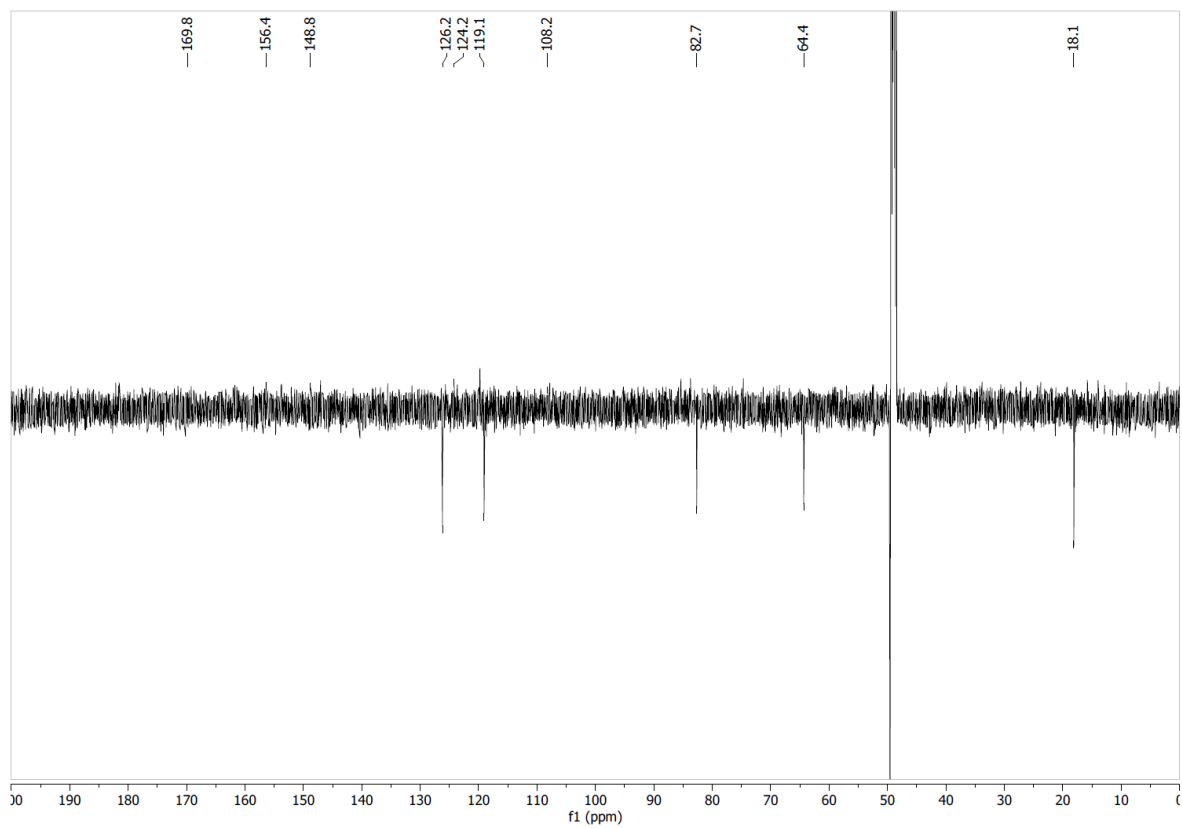


Figure S49. ¹³C-DEPTQ NMR spectrum of compound **8** in CD₃OD at 151 MHz

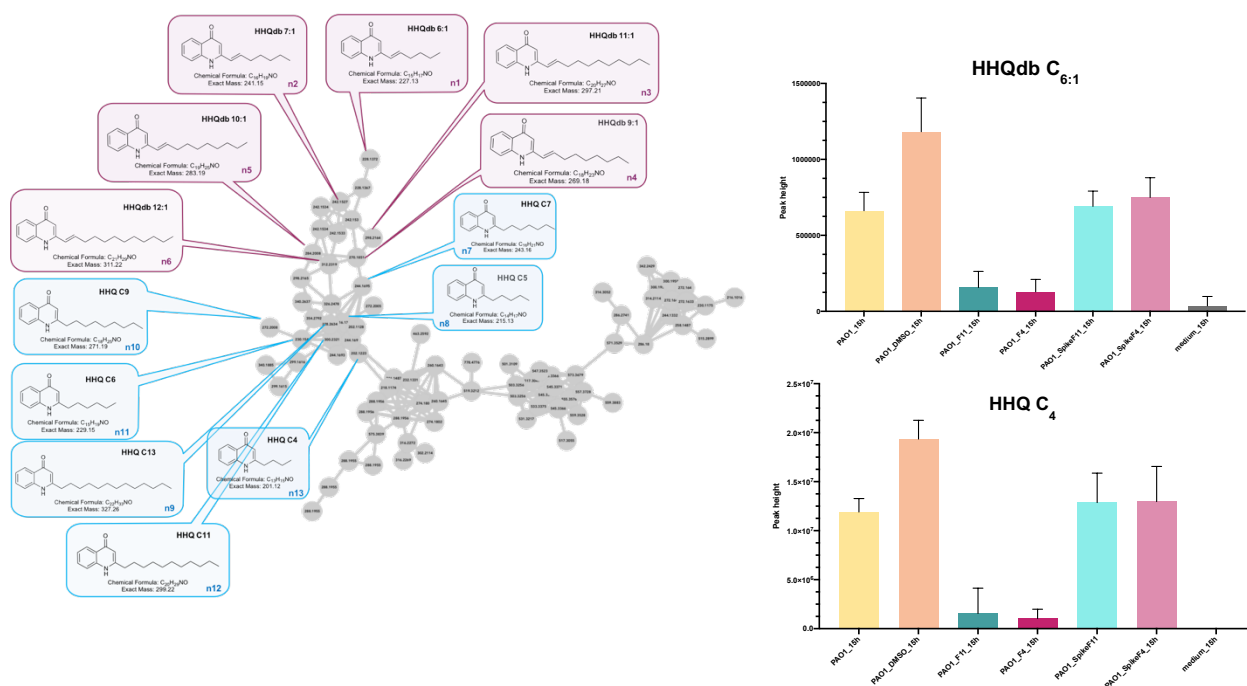


Figure S52. Cluster of alkyl quinolones with annotated HHQs and intensities under the different conditions of the features corresponding to the two HHQs that are significantly decreased with both **2** and **4**. HHQ: 4-heptyl-4(*1H*)-quinolone, db: double bond.

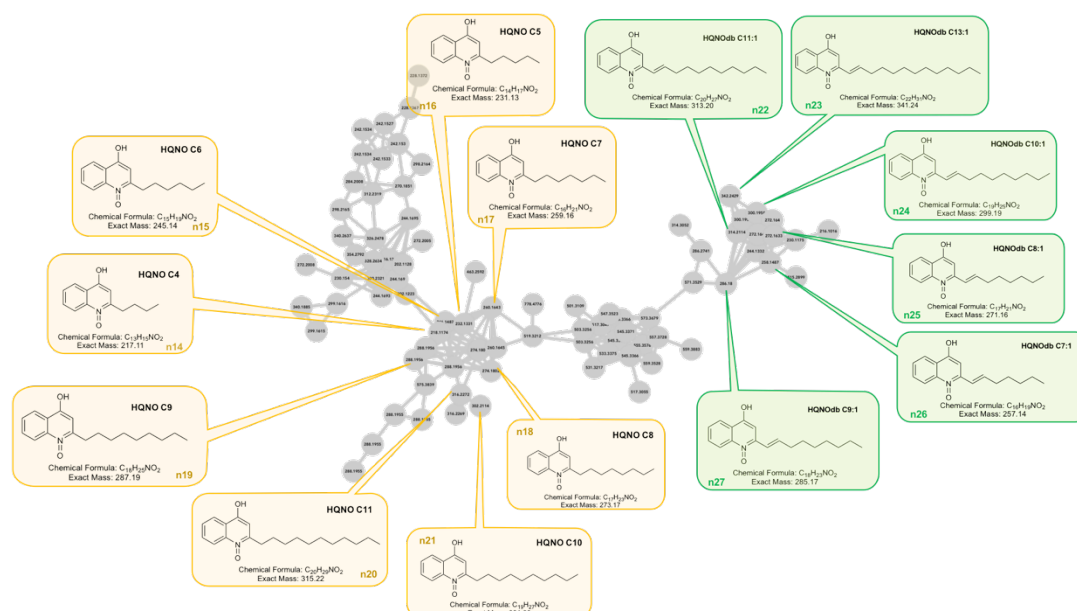


Figure S53. Cluster of alkyl quinolones with annotated HQNOs. HQNO: 2-heptyl-4-hydroxyquinoline-N-oxide.

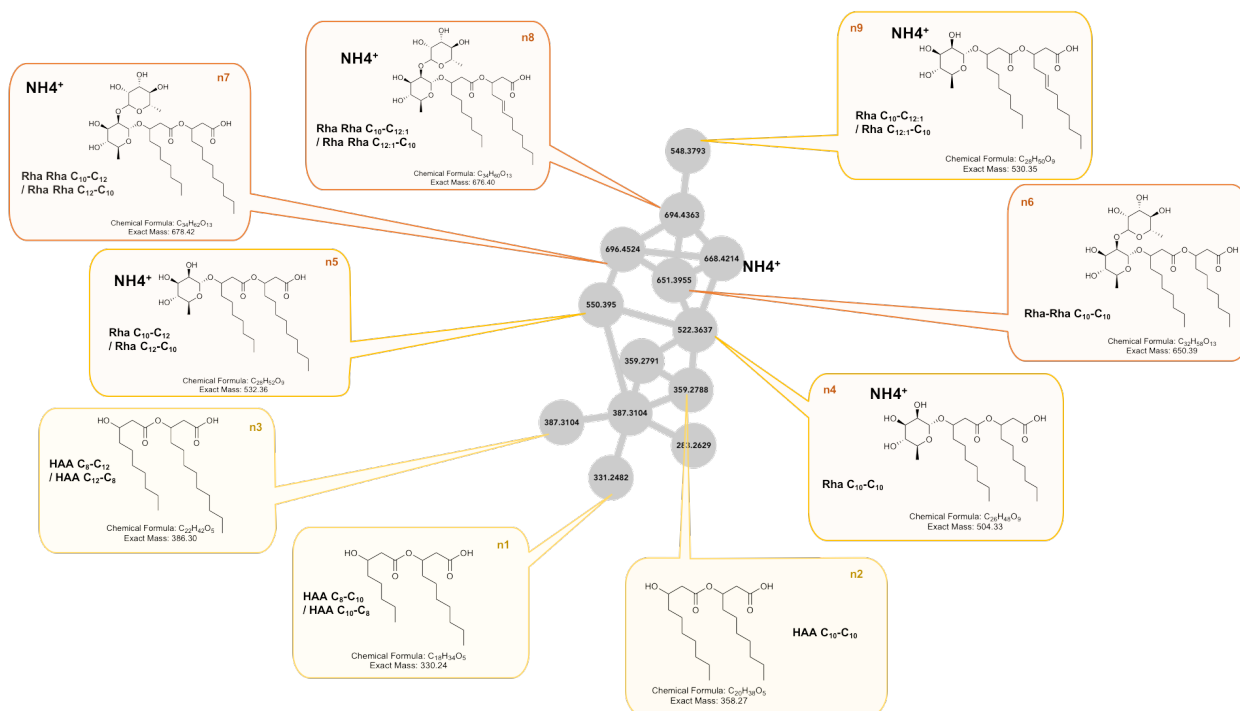


Figure S54. Cluster of rhamnolipids with annotated precursors without rhamnose HAA, mono-rhamnolipids Rha, and di-rhamnolipids Rha-Rha.
HAA: 3-(3-hydroxyalkanoyloxy)alkanoic acids
Rha: rhamnolipid

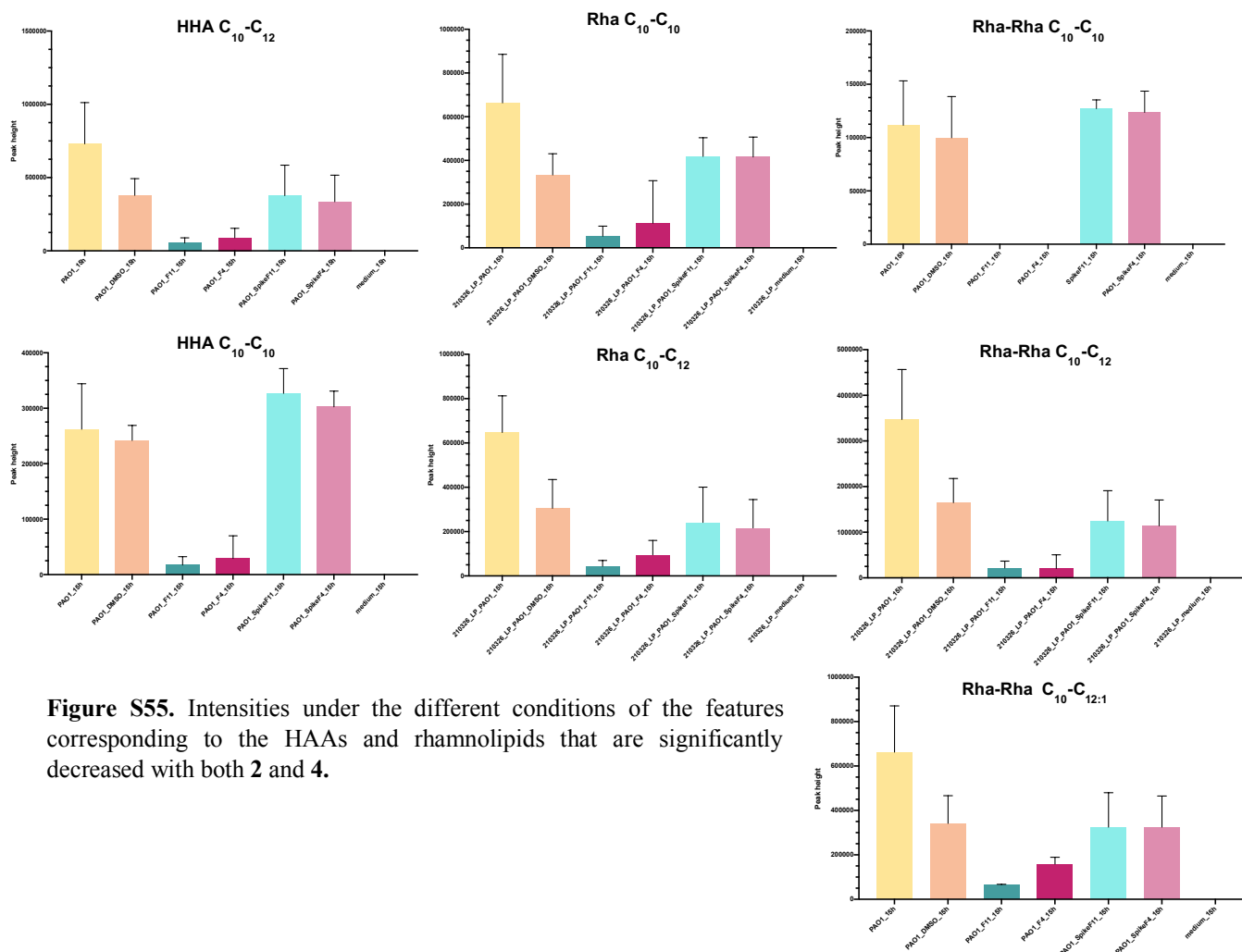


Figure S55. Intensities under the different conditions of the features corresponding to the HAAs and rhamnolipids that are significantly decreased with both 2 and 4.

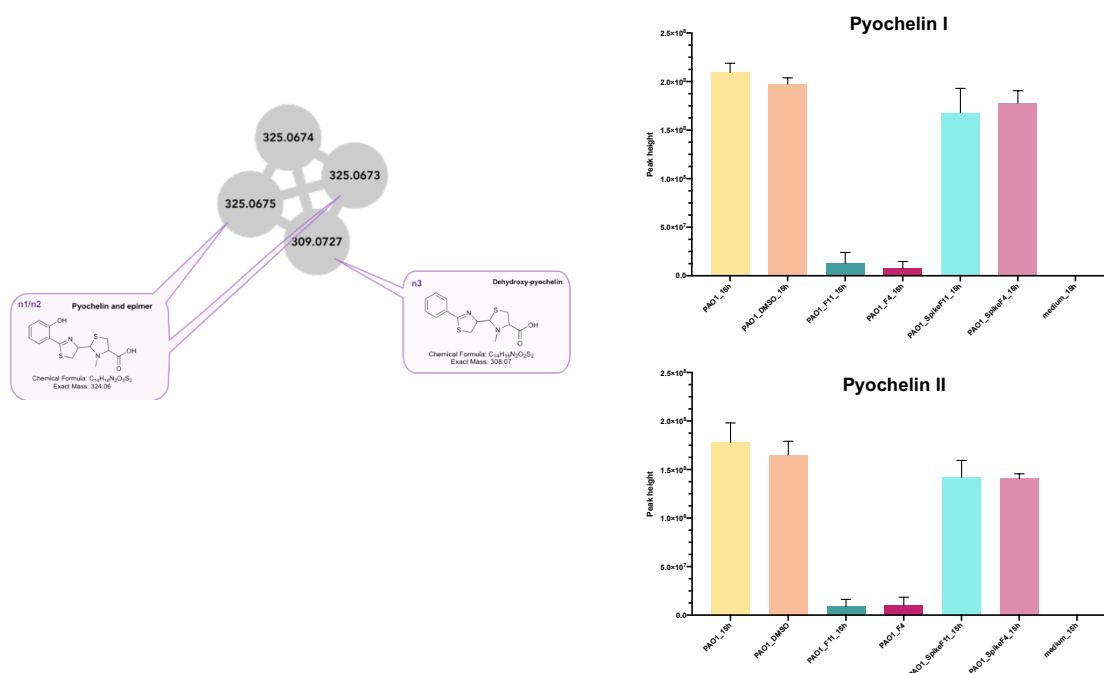


Figure S56. Cluster of pyochelin derivatives with annotated pyochelin I and epimer pyochelin II, dihydroxypyochelin and intensities under the different conditions of the features that are significantly decreased with both **2** and **4**.

