Calothrixamides A and B from the Cultured Cyanobacterium *Calothrix* sp. UIC 10520

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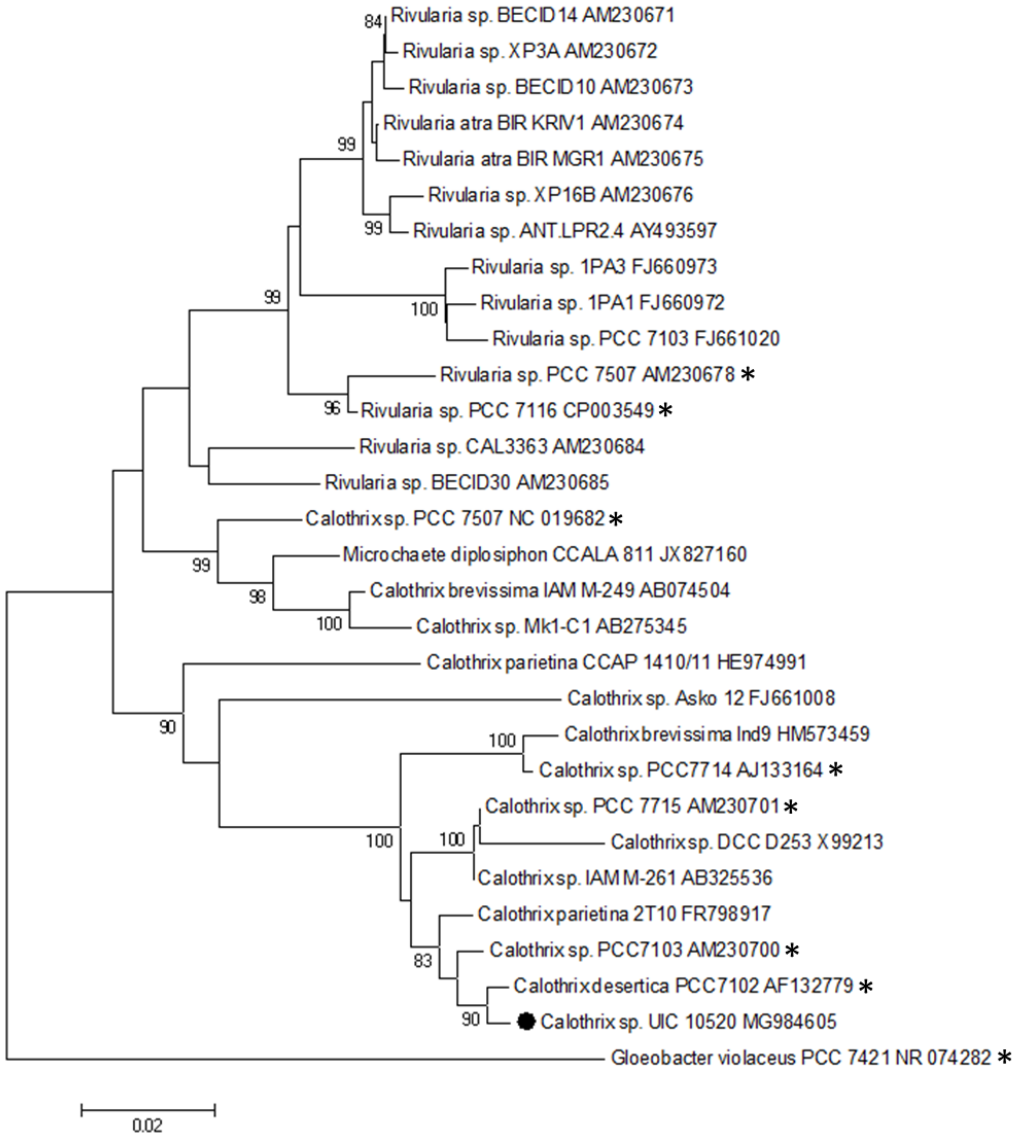
S1. Photomicrograph (40x, bright field) of *Calothrix* sp. UIC 10520

S2. Phylogenetic analysis of *Calothrix* sp. 10520

Phylogenetic analysis of *Calothrix* sp. UIC 10520 was performed using Mega 7.0.[1] The electropherogram obtained from Sanger sequencing was manually inspected and trimmed to 1176 bp (GenBank accession number MG984605.1). Microscopic analysis of the strain revealed a morphology similar to the morphologically distinct *Rivulariaceae* family. Partial 16S rRNA sequences of over 1000 bp of *Rivulariaceae* strains were obtained from GenBank and used to create a multiple sequence alignment with the 16S rRNA sequence of UIC 10520. ClustalW with default gap opening and extension penalties was used to perform the multiple sequence alignment. This multiple sequence alignment was used to create a phylogenetic tree. Evolutionary distances were computed using a Kimura-2 parameter method with a gamma distribution parameter of 0.47. The evolutionary history was inferred using the Neighbor-Joining tree building method and 1000 bootstrap replicates. Reference strains of the *Rivulariaceae* family from Bergey's Manual of Systematic Bacteriology[2] were included in the alignment and phylogenetic tree and used to determine the taxonomic identification of UIC 10520. UIC 10520 falls within the *Calothrix* clade and is genetically most similar to *Calothrix desertica*, a *Calothrix* sp. type strain.

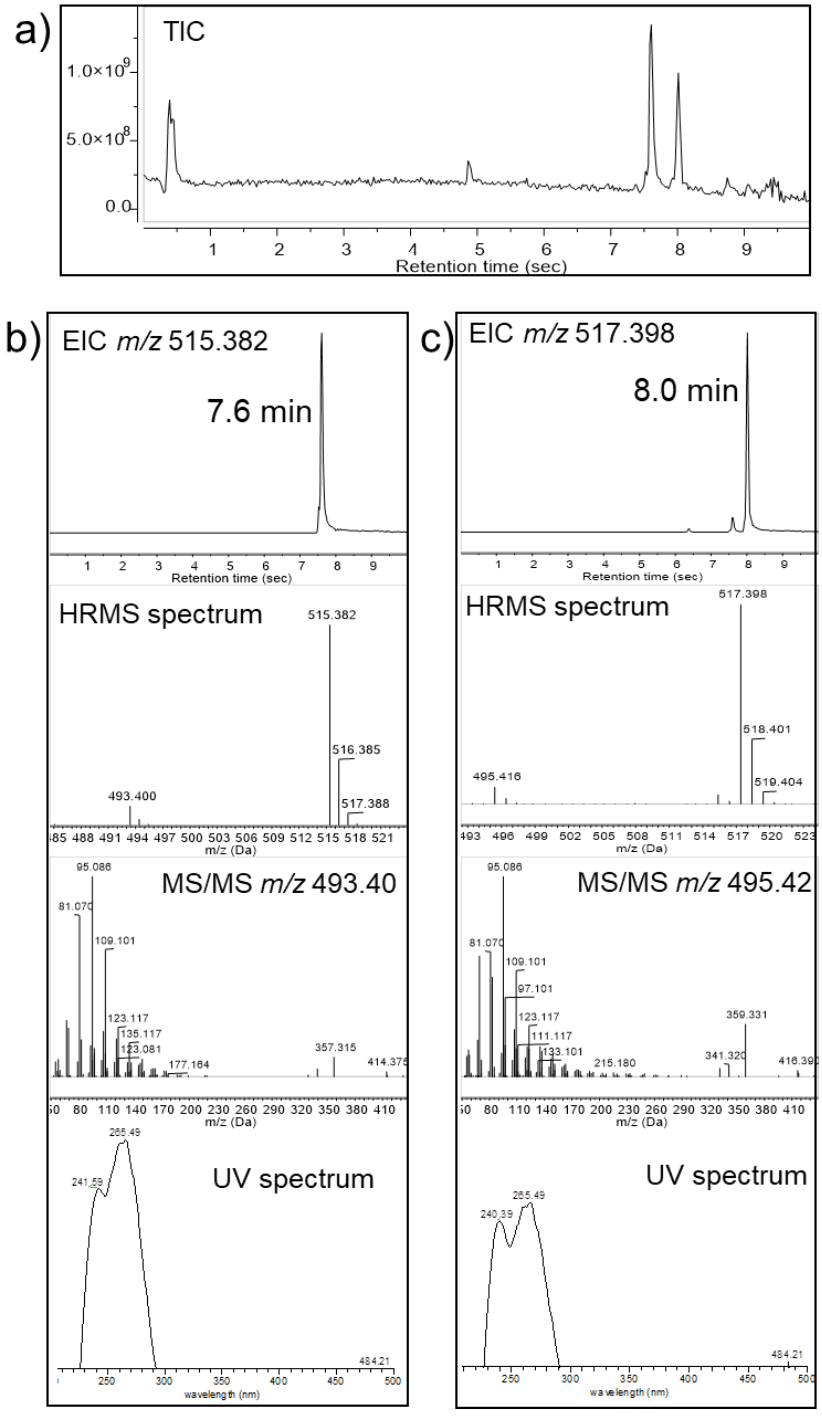


* Reference strains are indicated by an asterisk

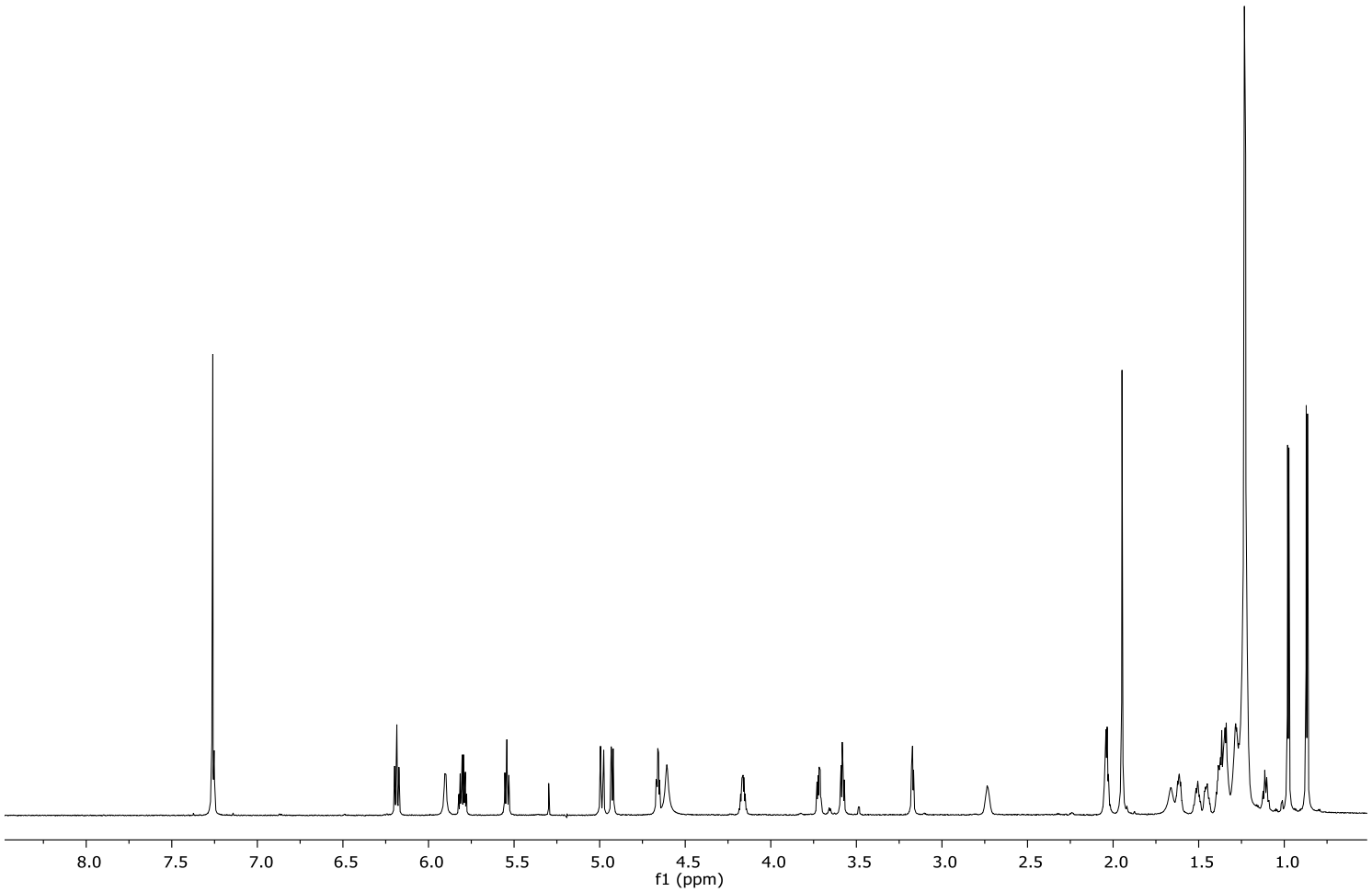
(1) Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33* (7), 1870–1874.

(2) Castenholz, R. W.; Wilmotte, A.; Herdman, M.; Rippka, R.; Waterbury, J. B.; Iteman, I.; Hoffmann, L. Phylum BX. Cyanobacteria. In *Bergey's Manual® of Systematic Bacteriology*; Springer New York: New York, NY, 2001; pp 473–599.

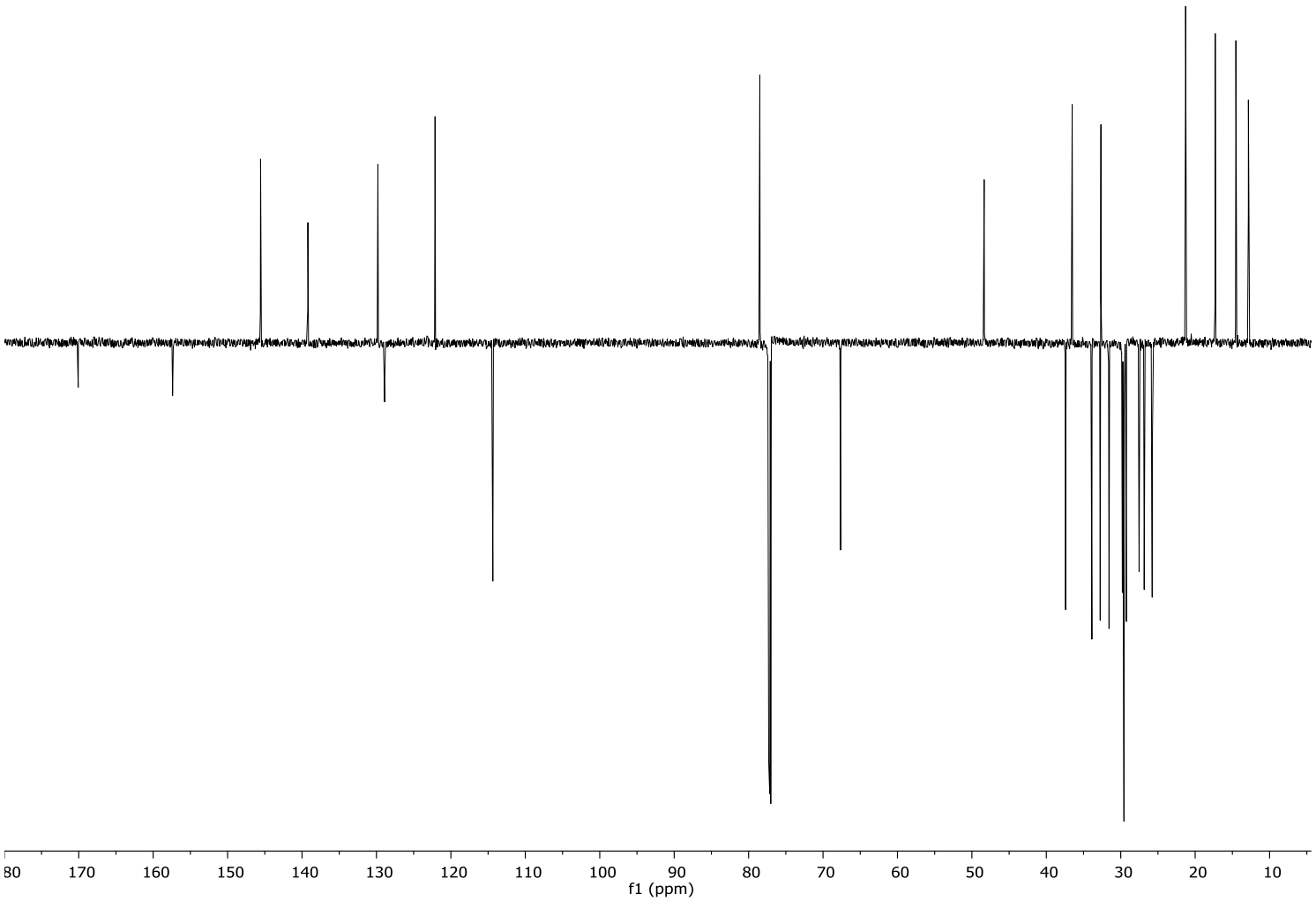
S3. Data acquired for *Calothrix* sp. UIC 10520 by droplet probe-UPLC-UV-HRMS-MS/MS. **a)** Total ion chromatogram (TIC); **b-c)** spectroscopic data used for the dereplication of calothrixamides A (**1**) and B (**2**), respectively



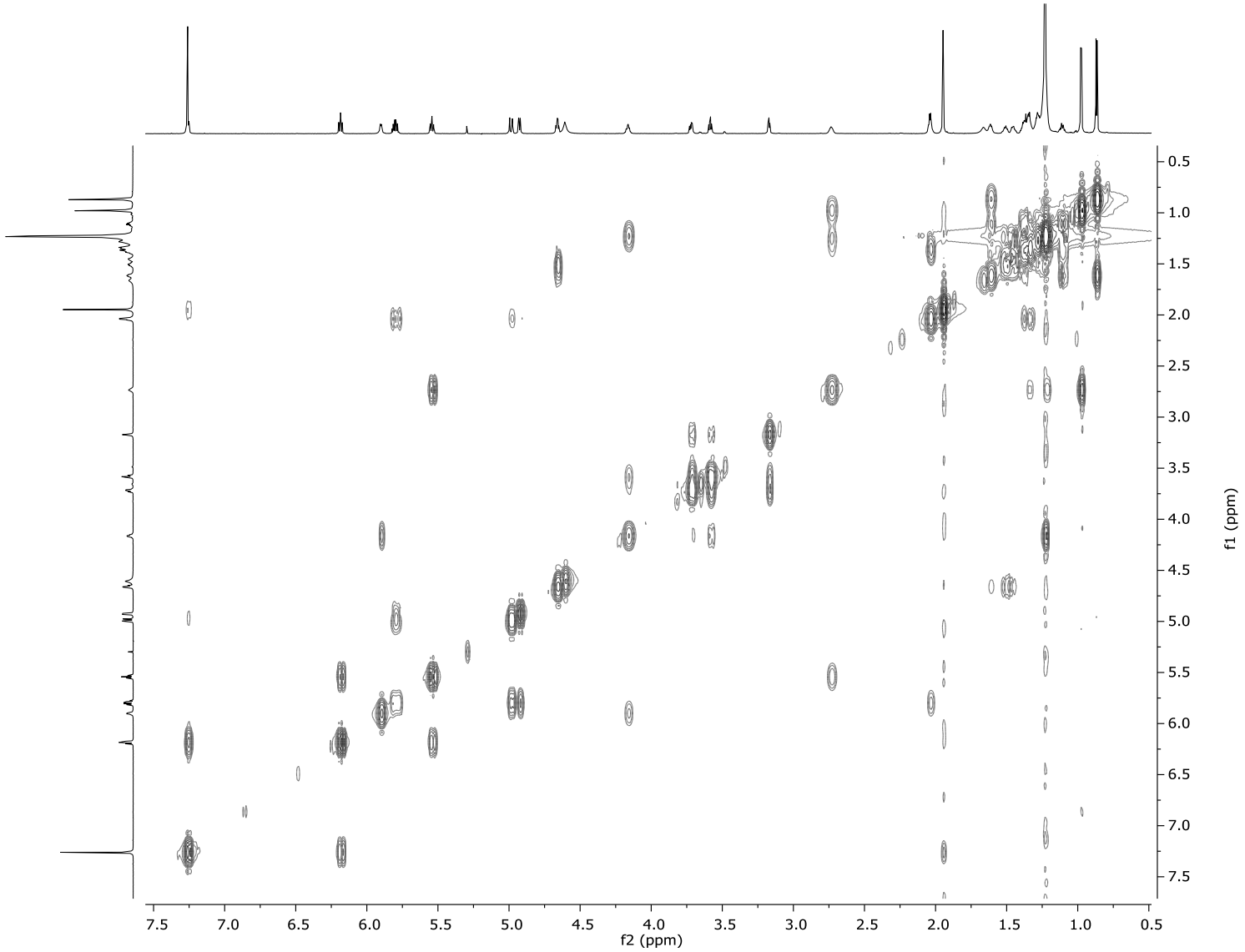
S4. ^{1}H NMR spectrum (900 MHz, $CDCl_3$) of **1**



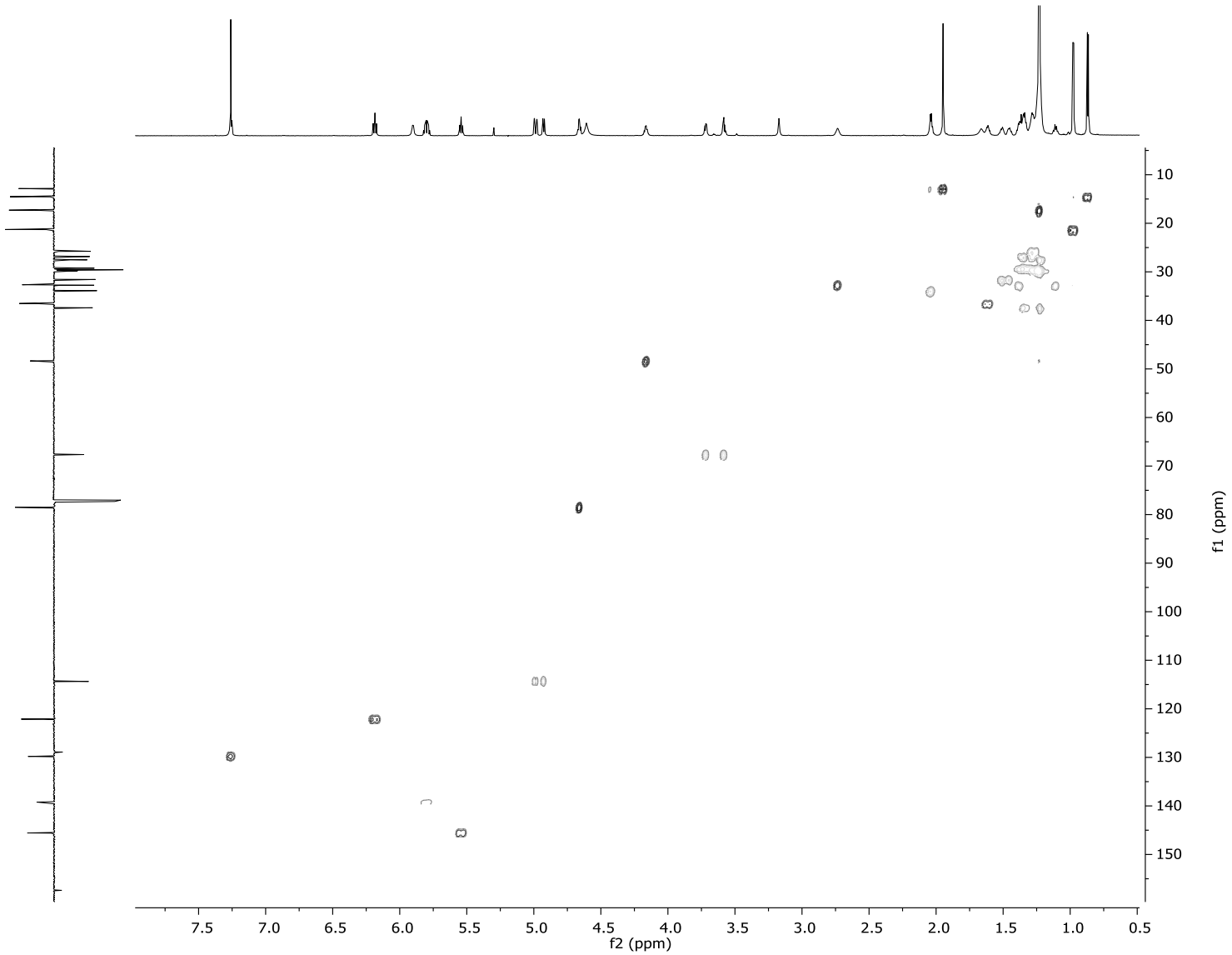
S5. DEPTQ spectrum (226 MHz, $CDCl_3$) of **1**



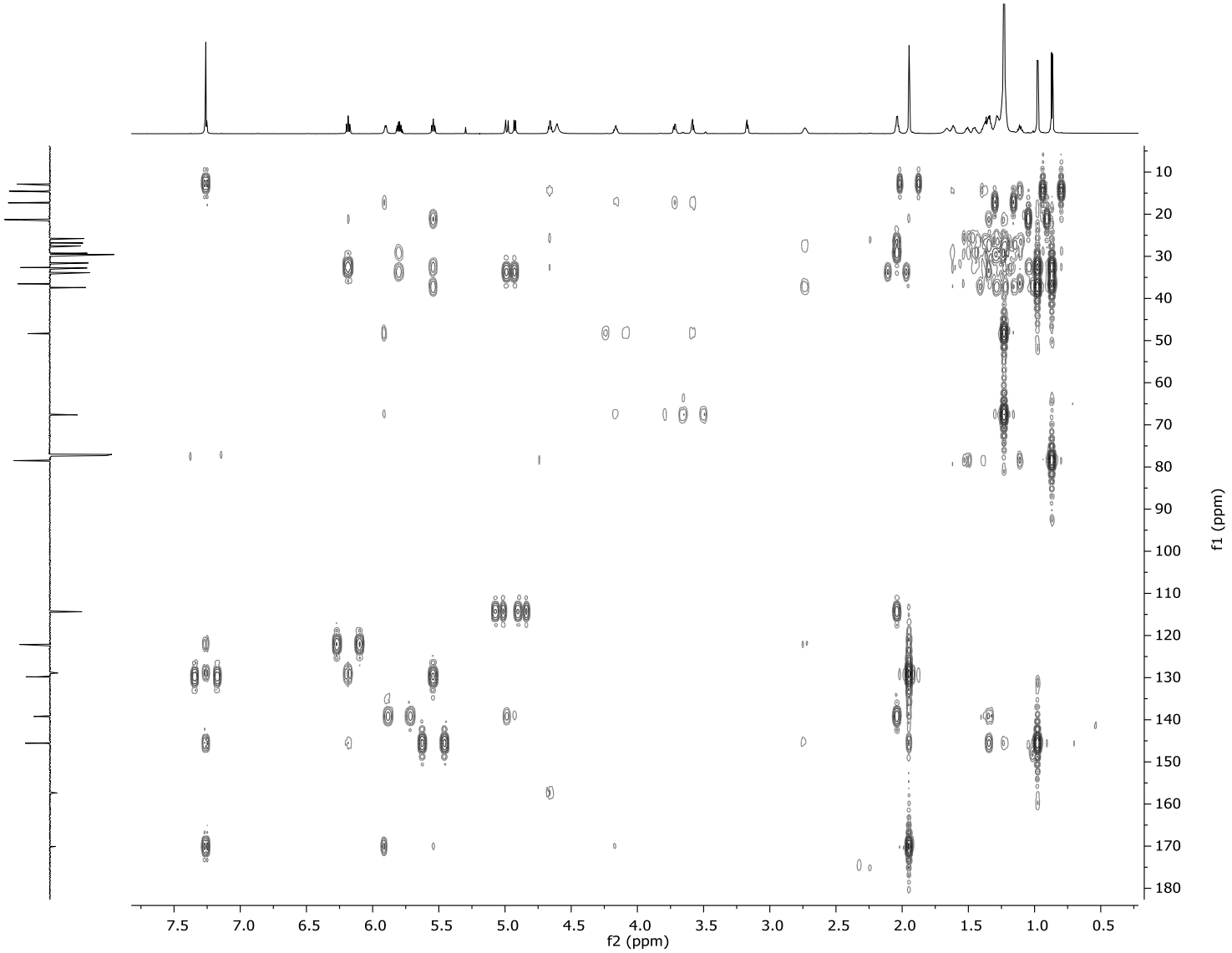
S6. COSY spectrum (900 MHz, $CDCl_3$) of **1**



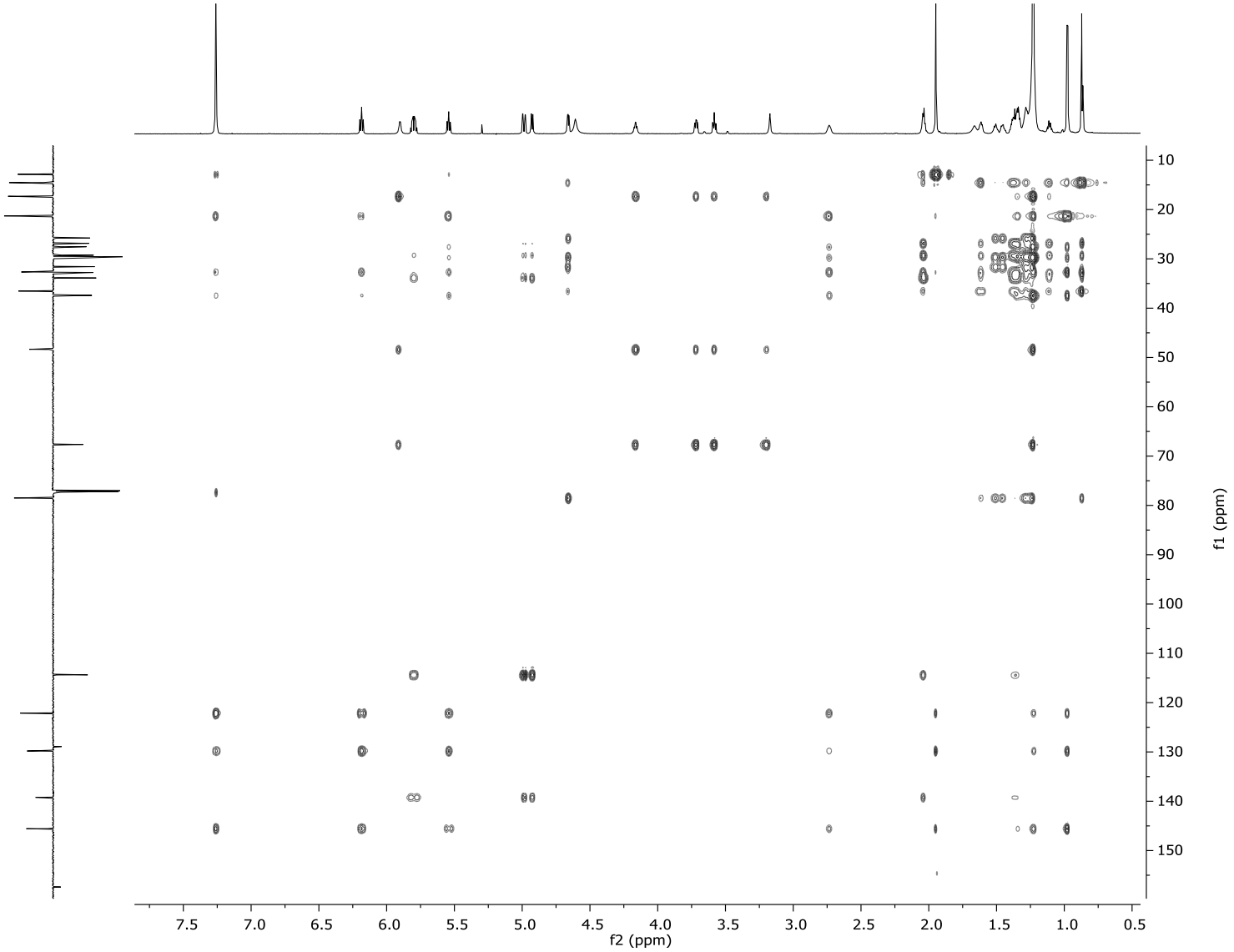
S7. HSQC spectrum (900 MHz, $CDCl_3$) of **1**



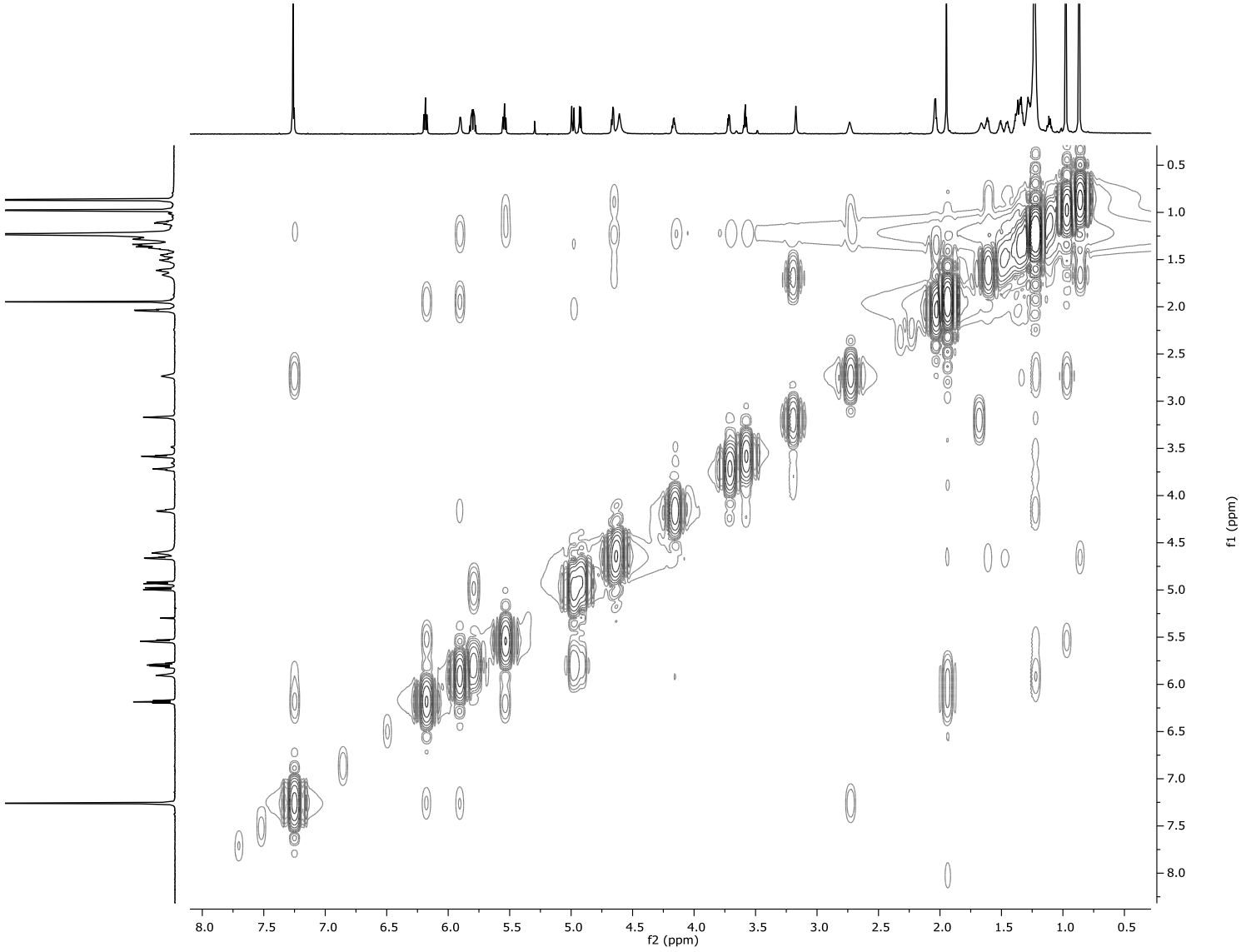
S8. HMBC spectrum (900 MHz, $CDCl_3$) of **1**



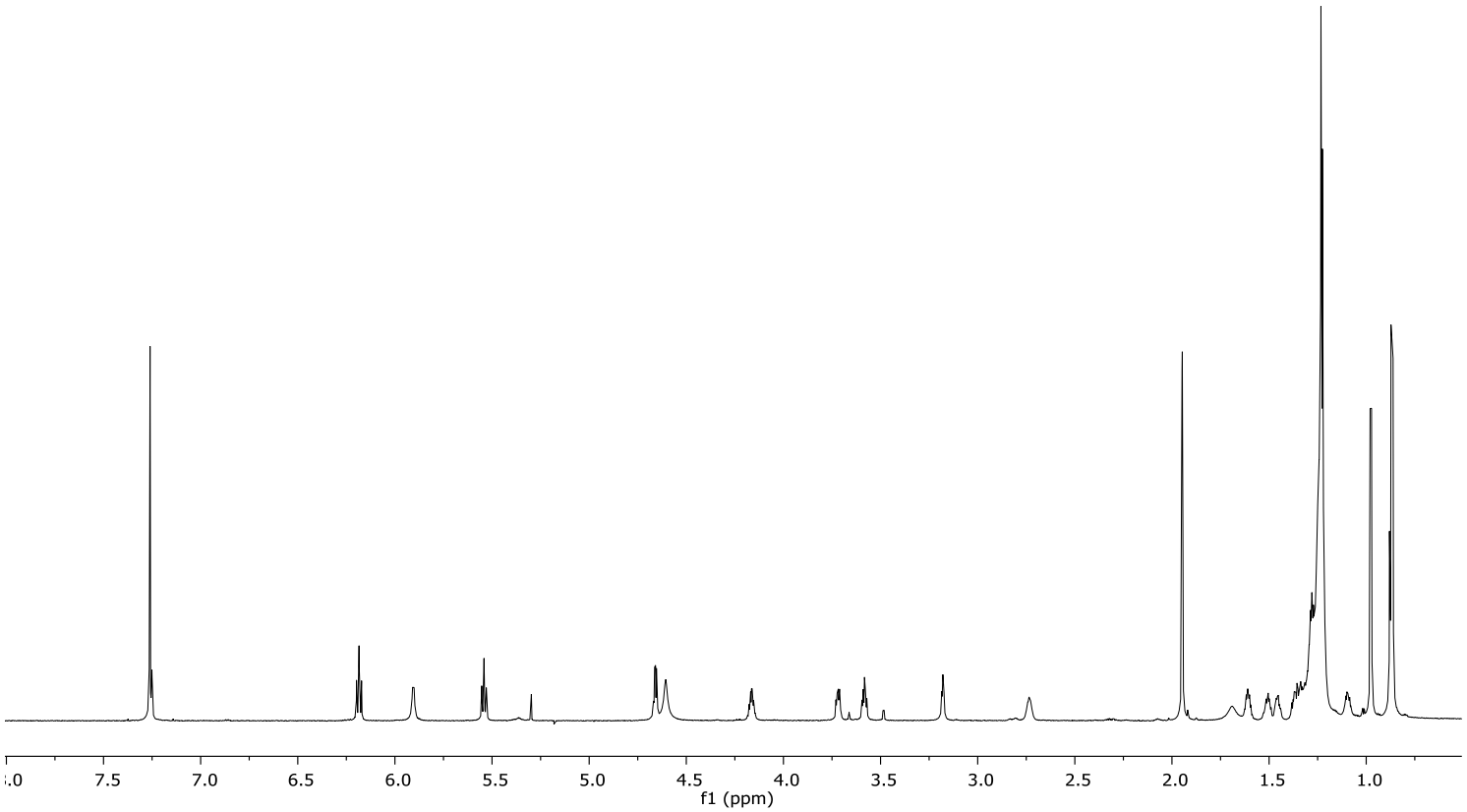
S9. ^{1}H-^{13}C HSQC-TOCSY spectrum (900 MHz, $CDCl_3$, 90 ms mixing time) of **1**



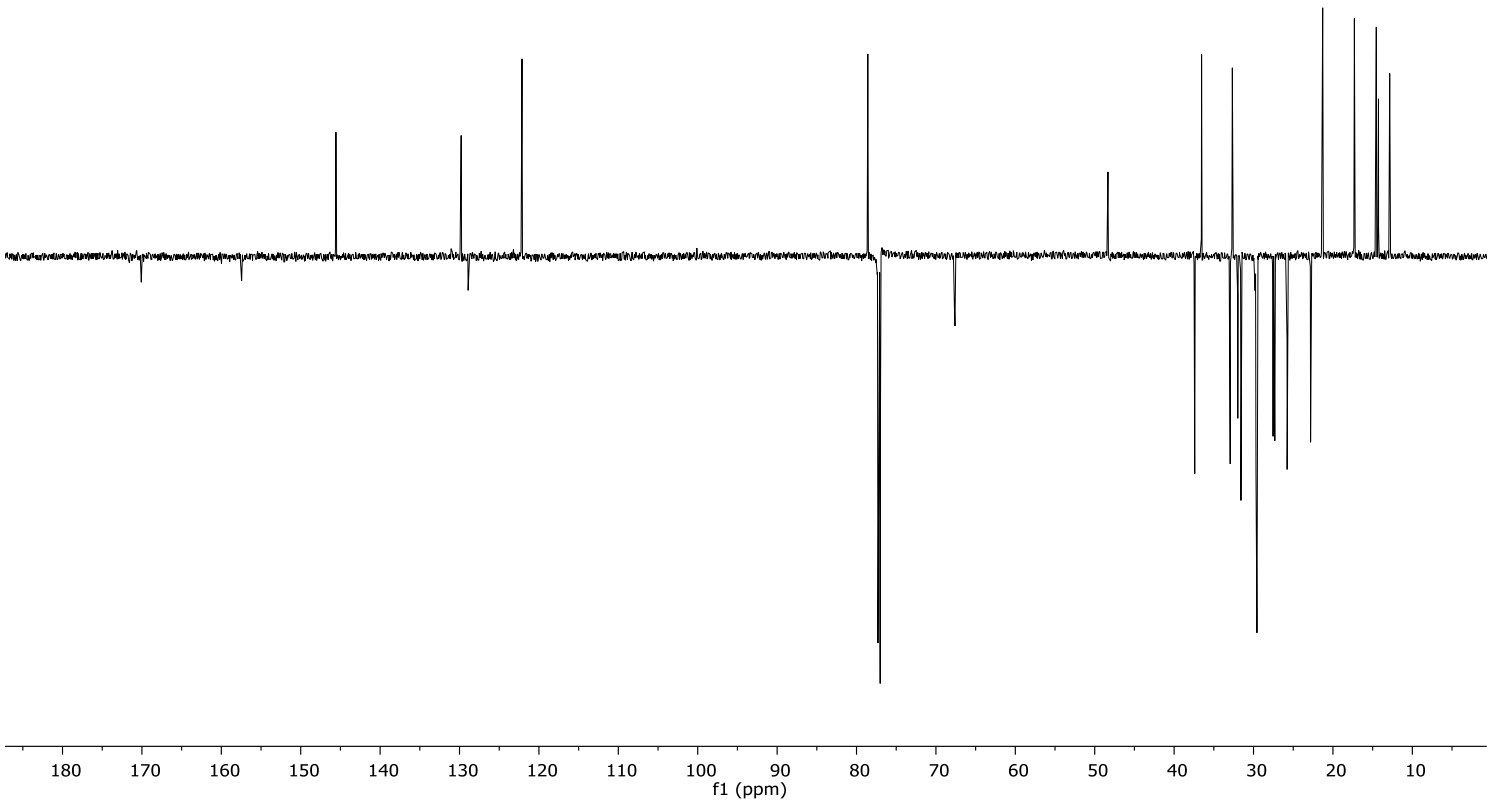
S10. NOESY spectrum (900 MHz, $CDCl_3$) of **1**



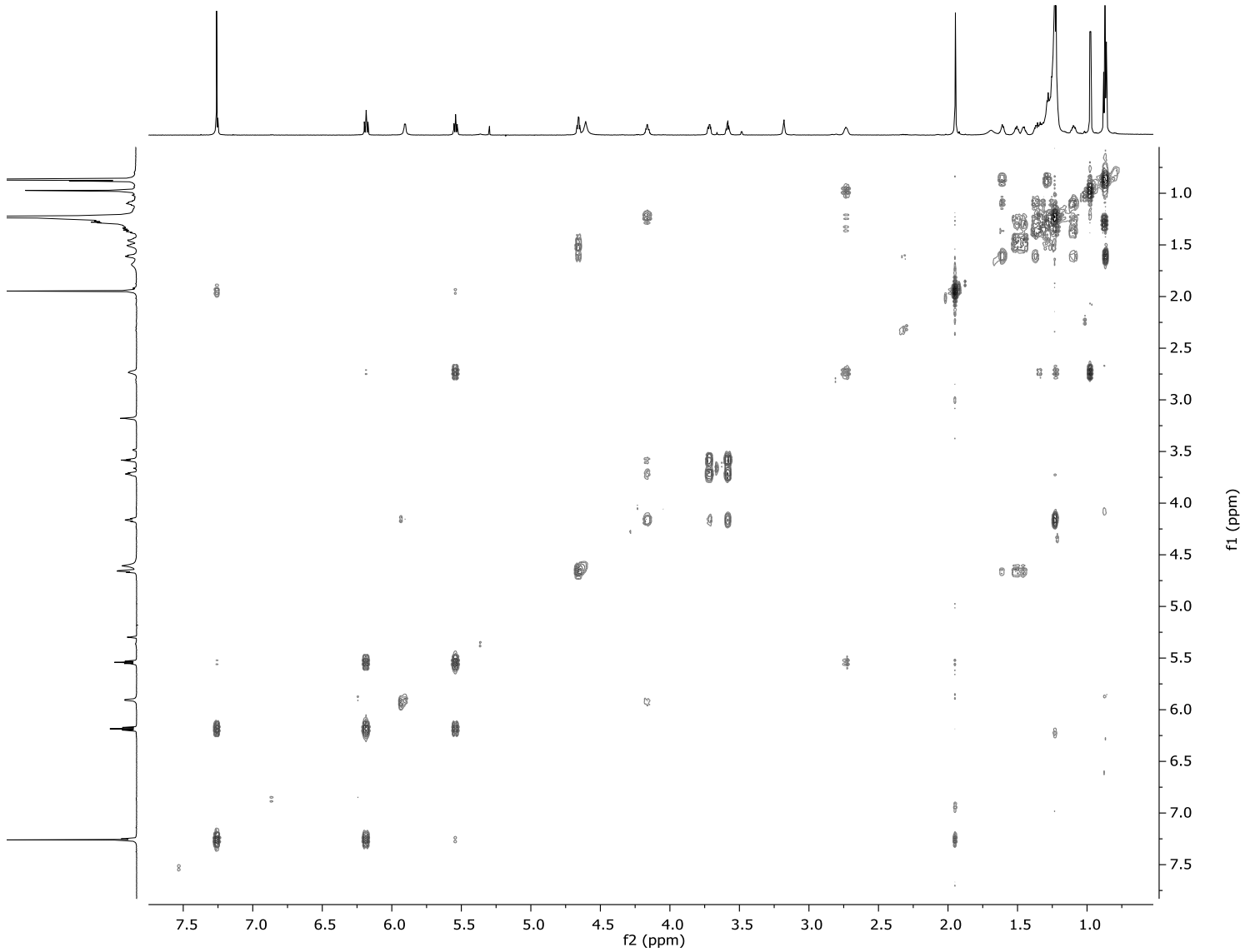
S11. ^{1}H NMR spectrum (900 MHz, $CDCl_3$) of **2**



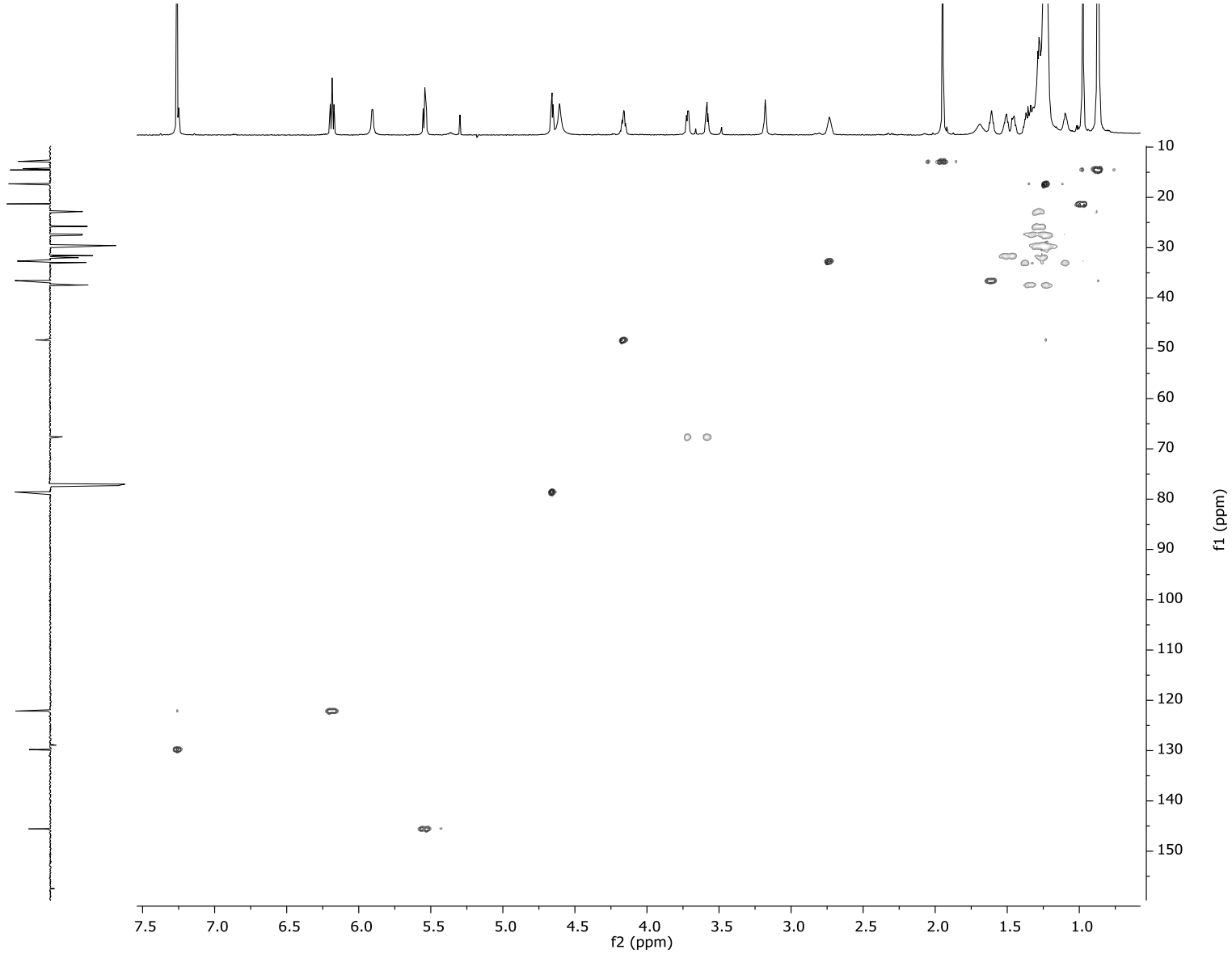
S12. DEPTQ spectrum (226 MHz, $CDCl_3$) of **2**



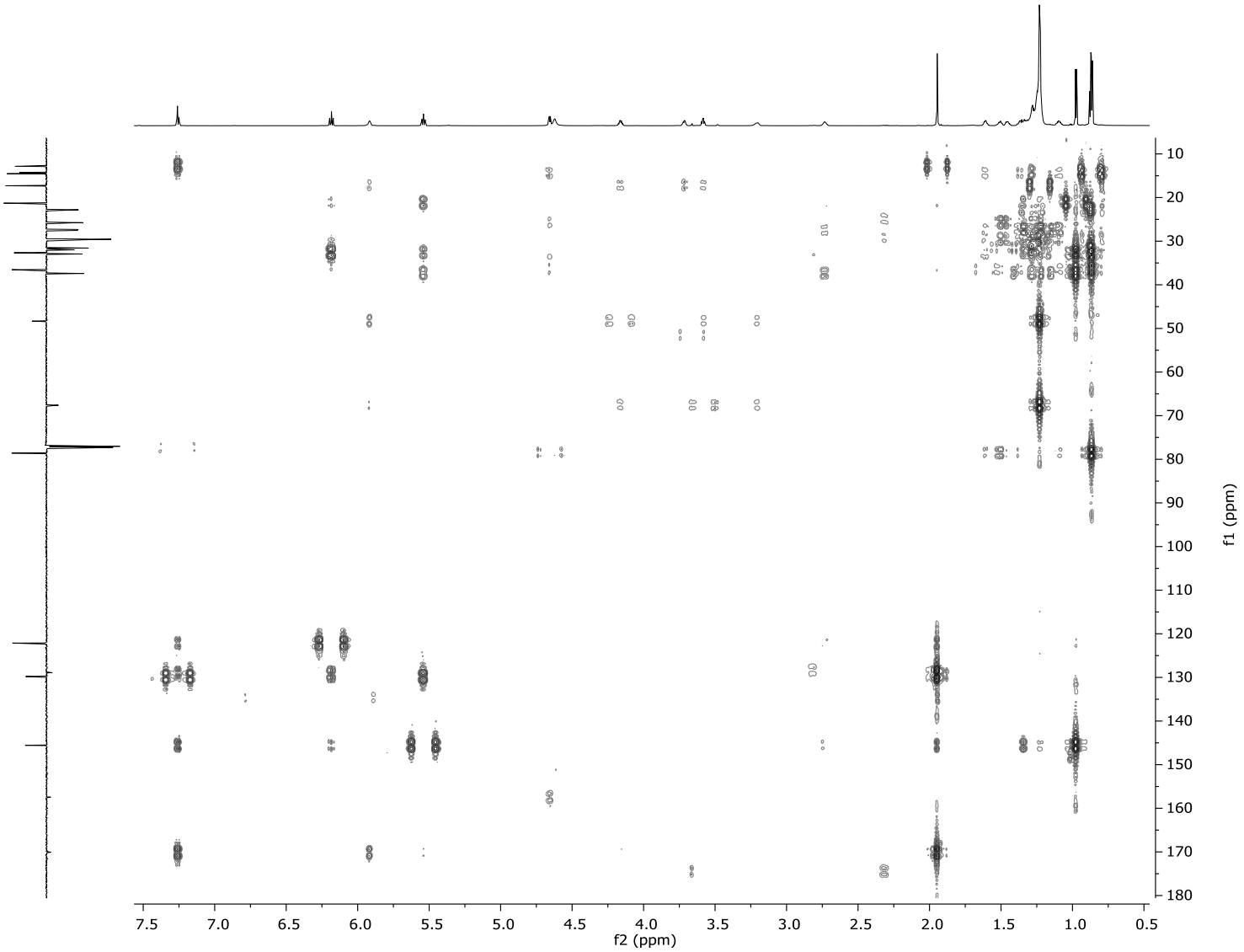
S13. COSY spectrum (900 MHz, $CDCl_3$) of **2**



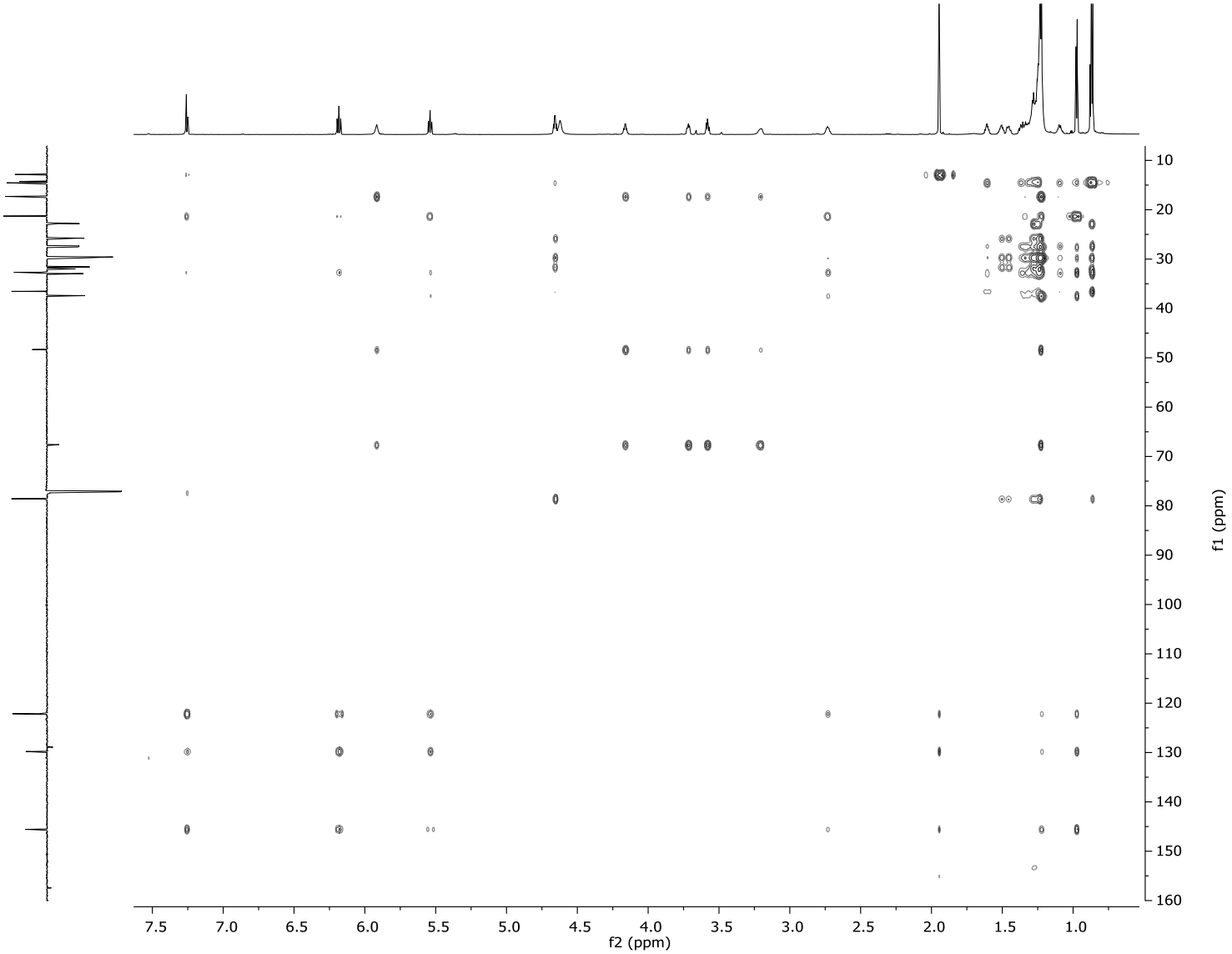
S14. HSQC spectrum (900 MHz, $CDCl_3$) of **2**



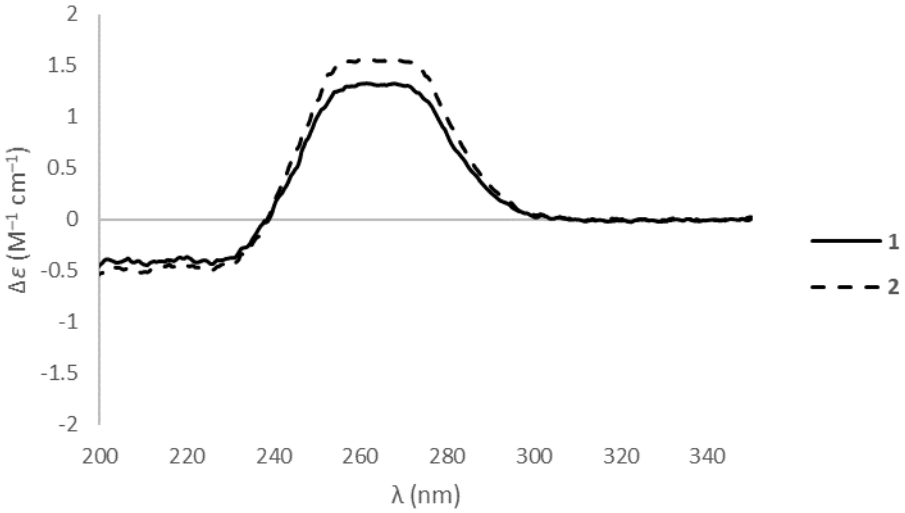
S15. HMBC spectrum (900 MHz, $CDCl_3$) of **2**



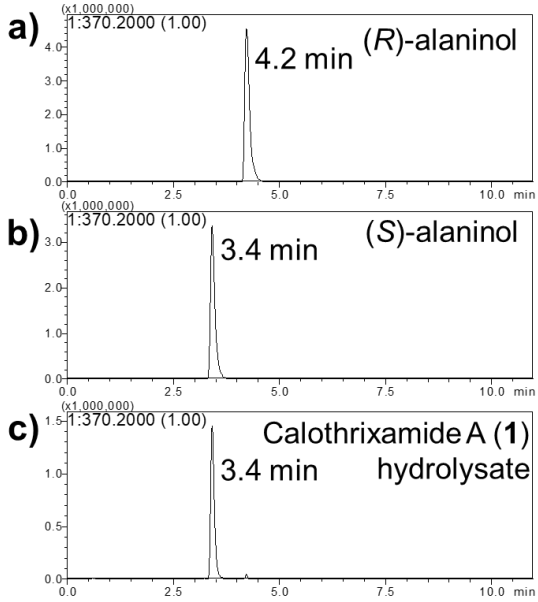
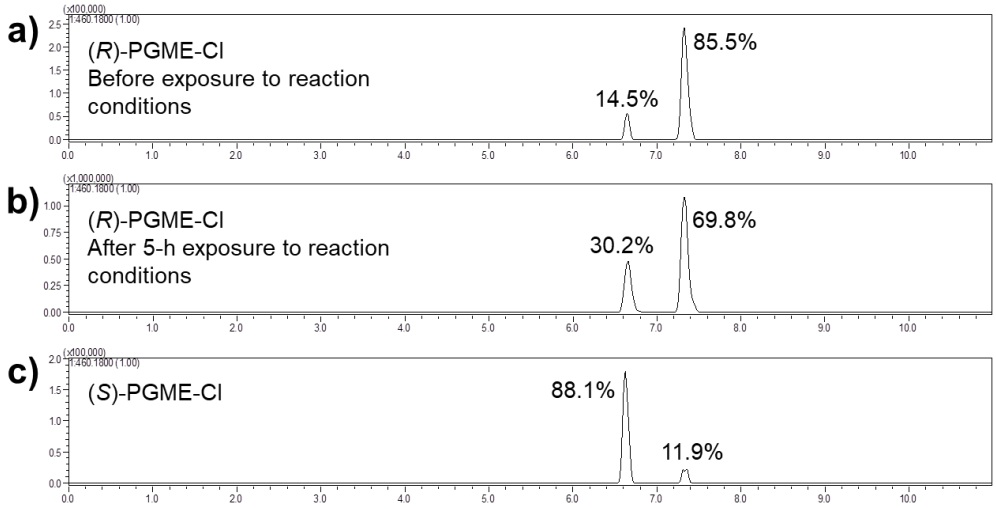
S16. ^{1}H-^{13}C HSQC-TOCSY spectrum (900 MHz, $CDCl_3$, 90 ms mixing time) of **2**



S17. ECD spectra of **1** and **2**



S18. Marfey's analysis of **1**. LC-MS analysis of the L-FDLA derivatives of **a)** *S*-alaninol, **b)**, *R*-alaninol, and **c)** hydrolysate of **1** (EIC *m/z* 370.17).



S19. Marfey's analysis of the (*R*)-PGME-Cl reagent before and after exposure to the derivatization conditions with no addition of substrate (see Experimental Section for details). LC-MS analysis of the L-FDLA derivatives of a) (*R*)-PGME-Cl before exposure, b) (*R*)-PGME-Cl after 5-h exposure, and c) non-exposed (*S*)-PGME-Cl for comparison (EIC m/z 460.18, % of total area).