SUPPLEMENTARY DATA

Immunomodulatory pinguisane-type sesquiterpenes from the liverwort *Porella cordaeana* (Porellaceae): The "new old" furanopinguisanol and its oxidation product exert mutually different effects on rat splenocytes

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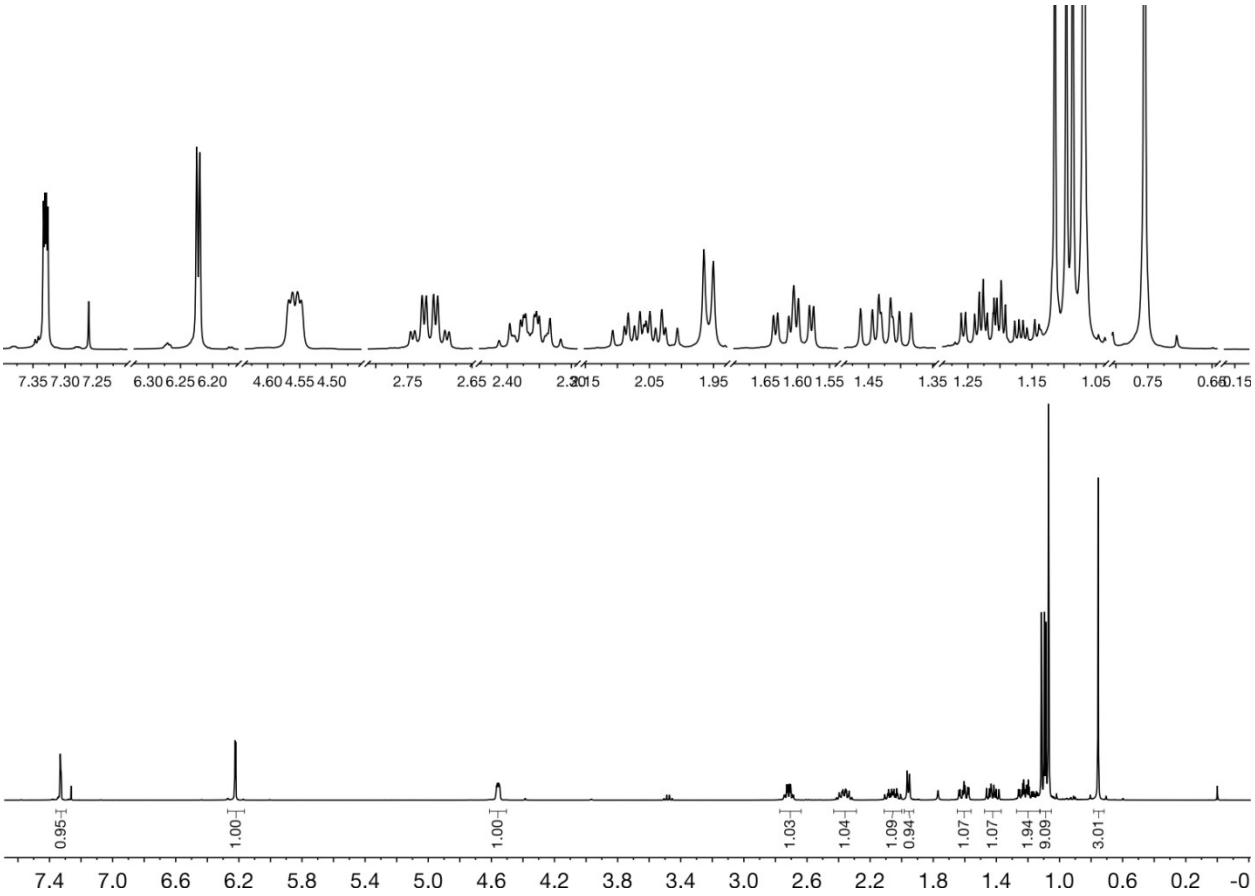


Fig. S1. ^{1}H NMR spectrum of α-furanopinguisanol (**1**) ($CDCl_3$).

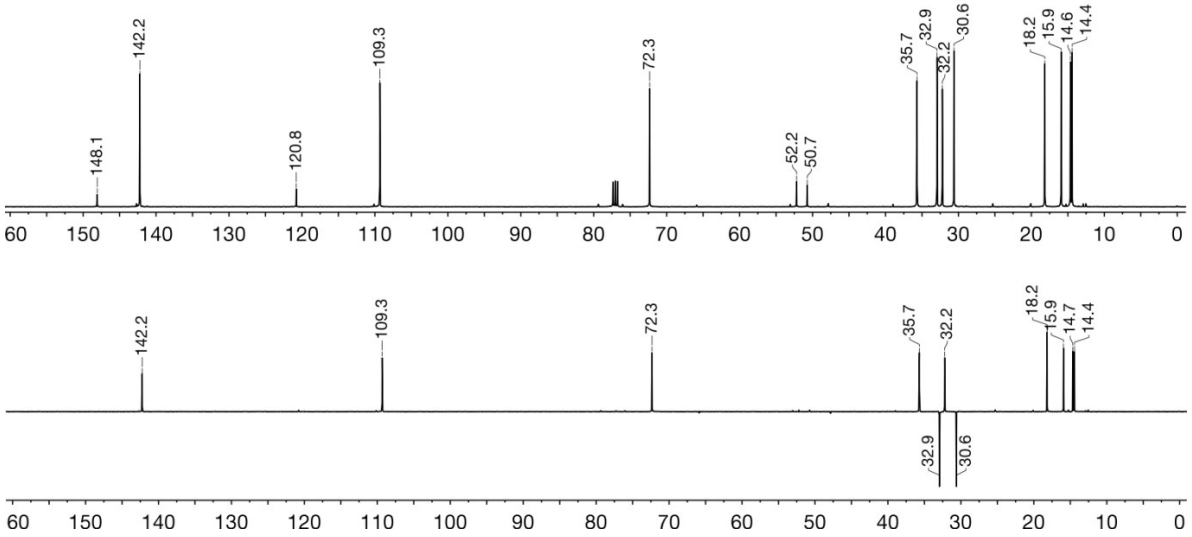


Fig. S2. ^{13}C NMR spectrum of **1** (above), DEPT-135 spectrum (below). CH_3 and CH signals are above, CH_2 signals are below the baseline ($CDCl_3$).

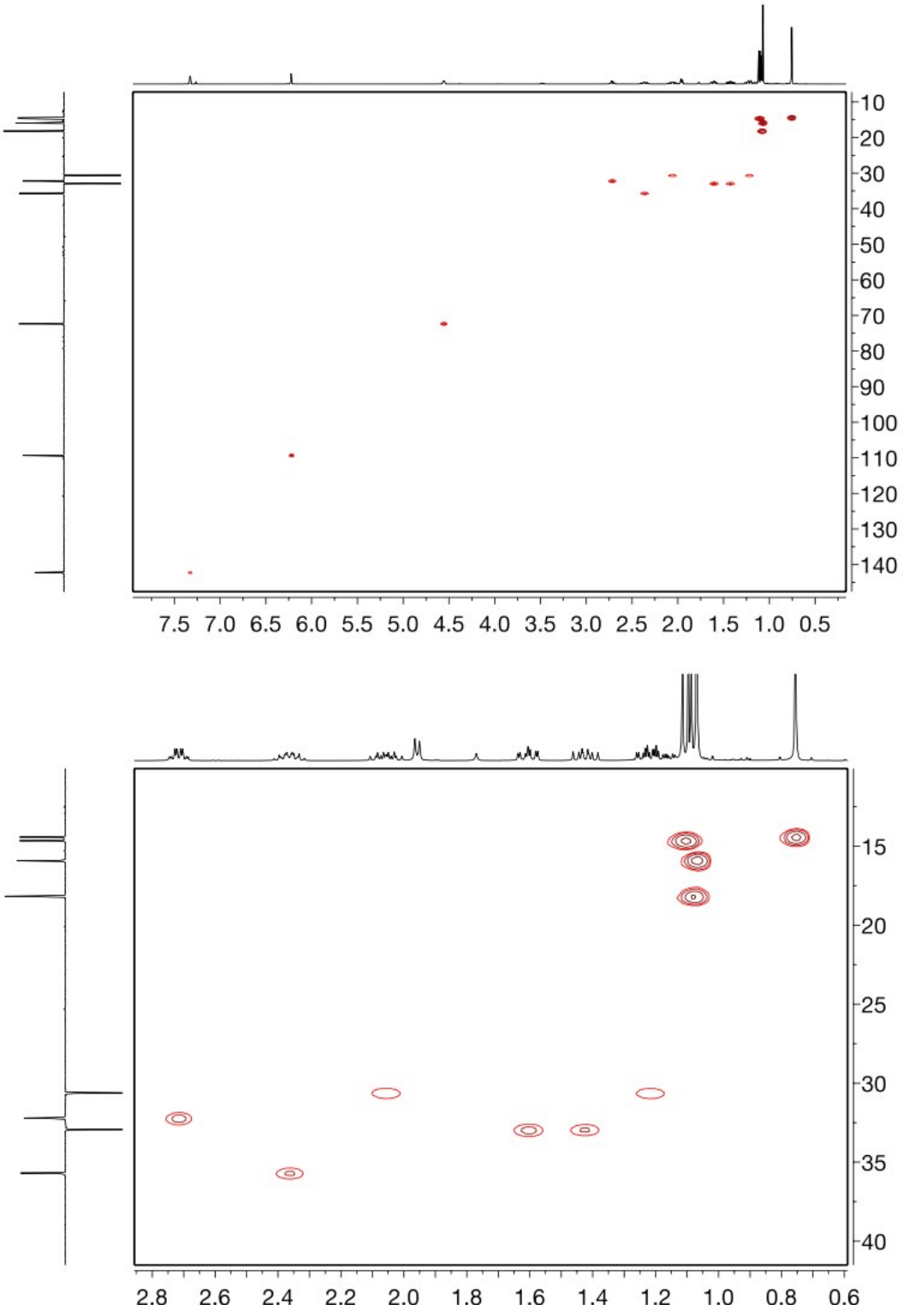


Fig. S3. HSQC spectrum of compound **1** and an expanded region of HSQC spectrum (0.5 – 3.0 ppm and 10 – 45 ppm) ($CDCl_3$).

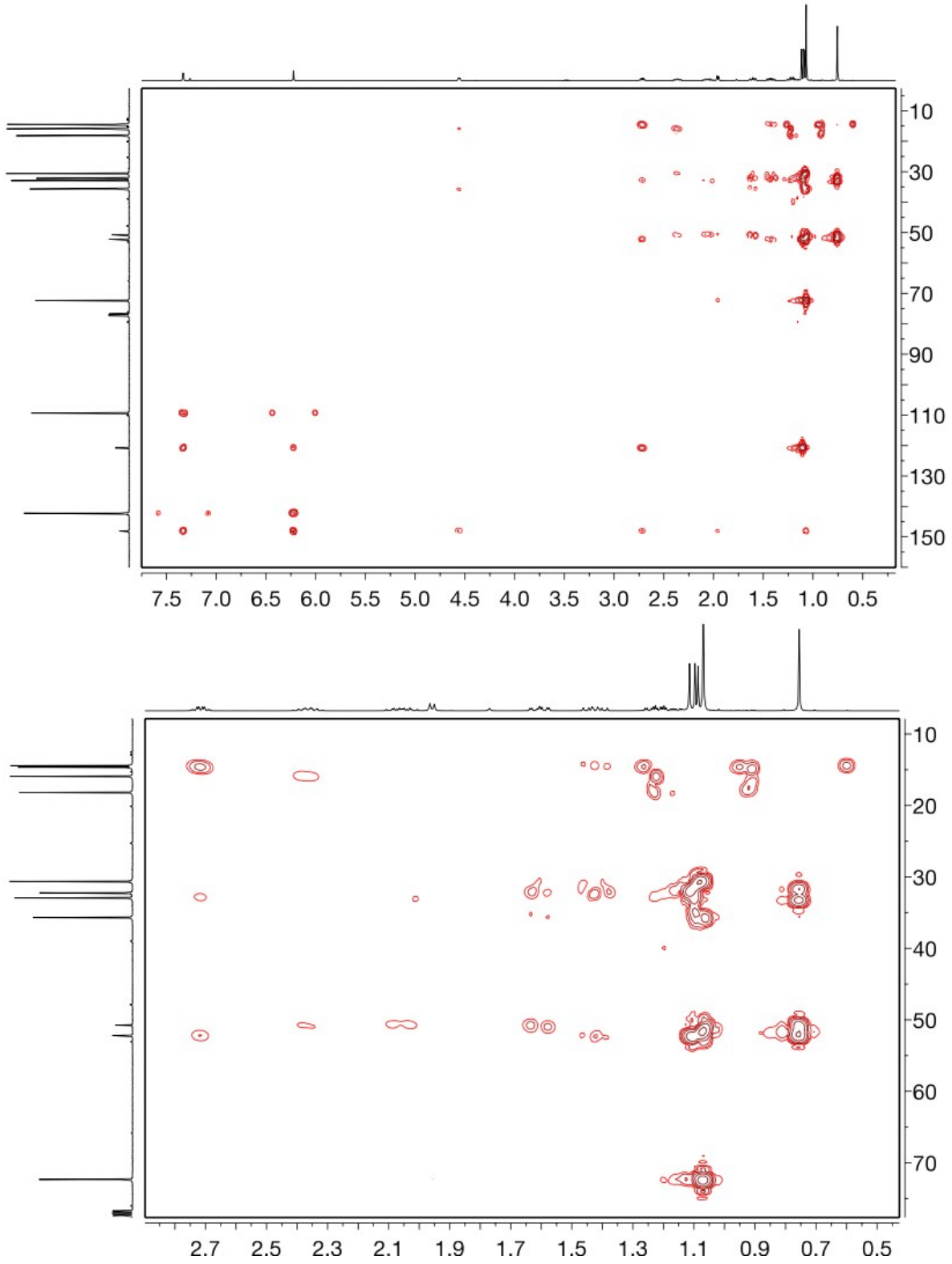


Fig. S4. HMBC spectrum of compound **1** and an expanded region of HMBC spectrum (0.5 – 3.0 ppm and 10 – 80 ppm) ($CDCl_3$).

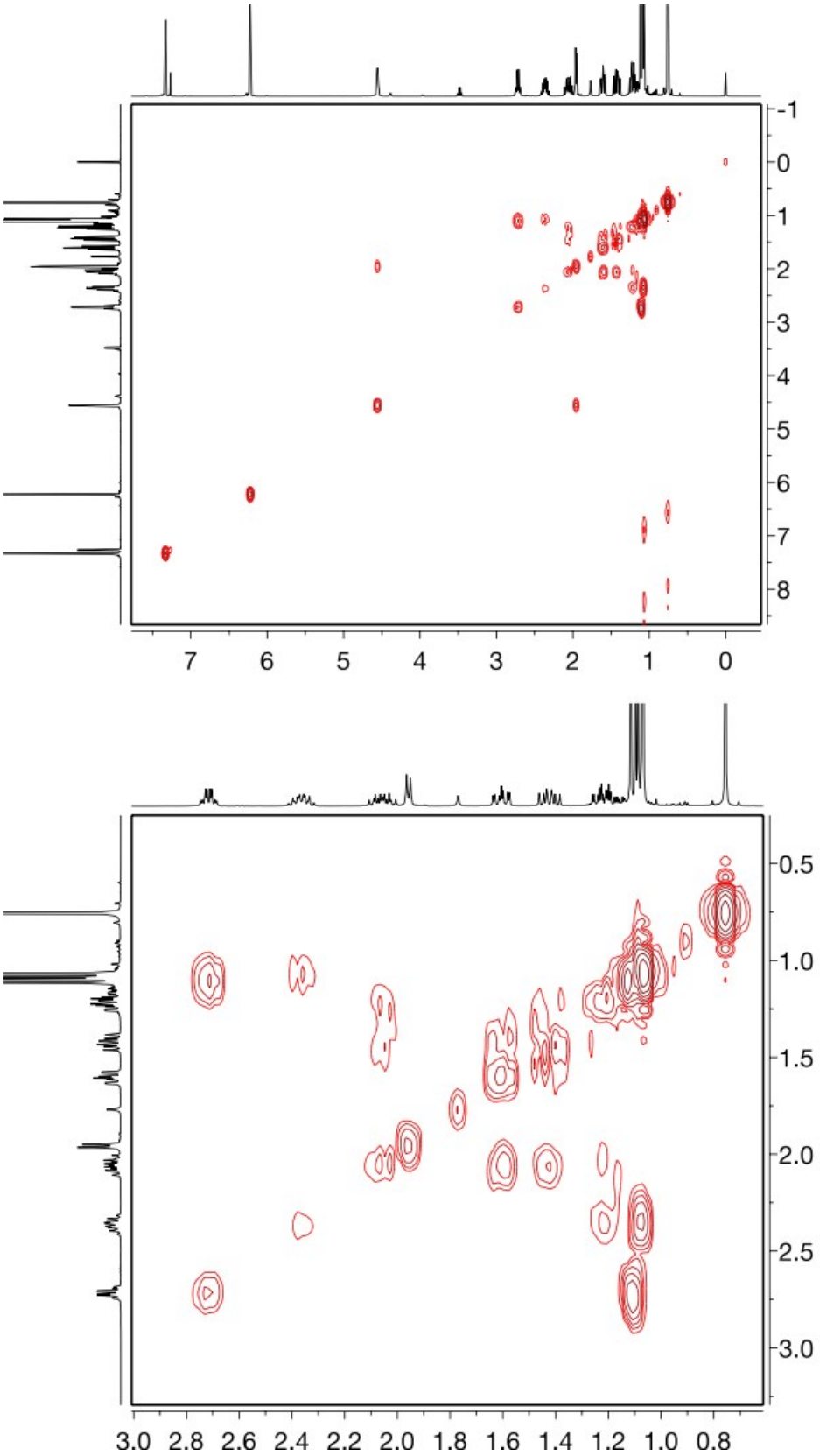


Fig. S5. ^{1}H-^{1}H COSY spectrum of compound **1** and an expanded region of the spectrum (0.5 – 3.5 ppm and 0.5 – 3.5 ppm) ($CDCl_3$).

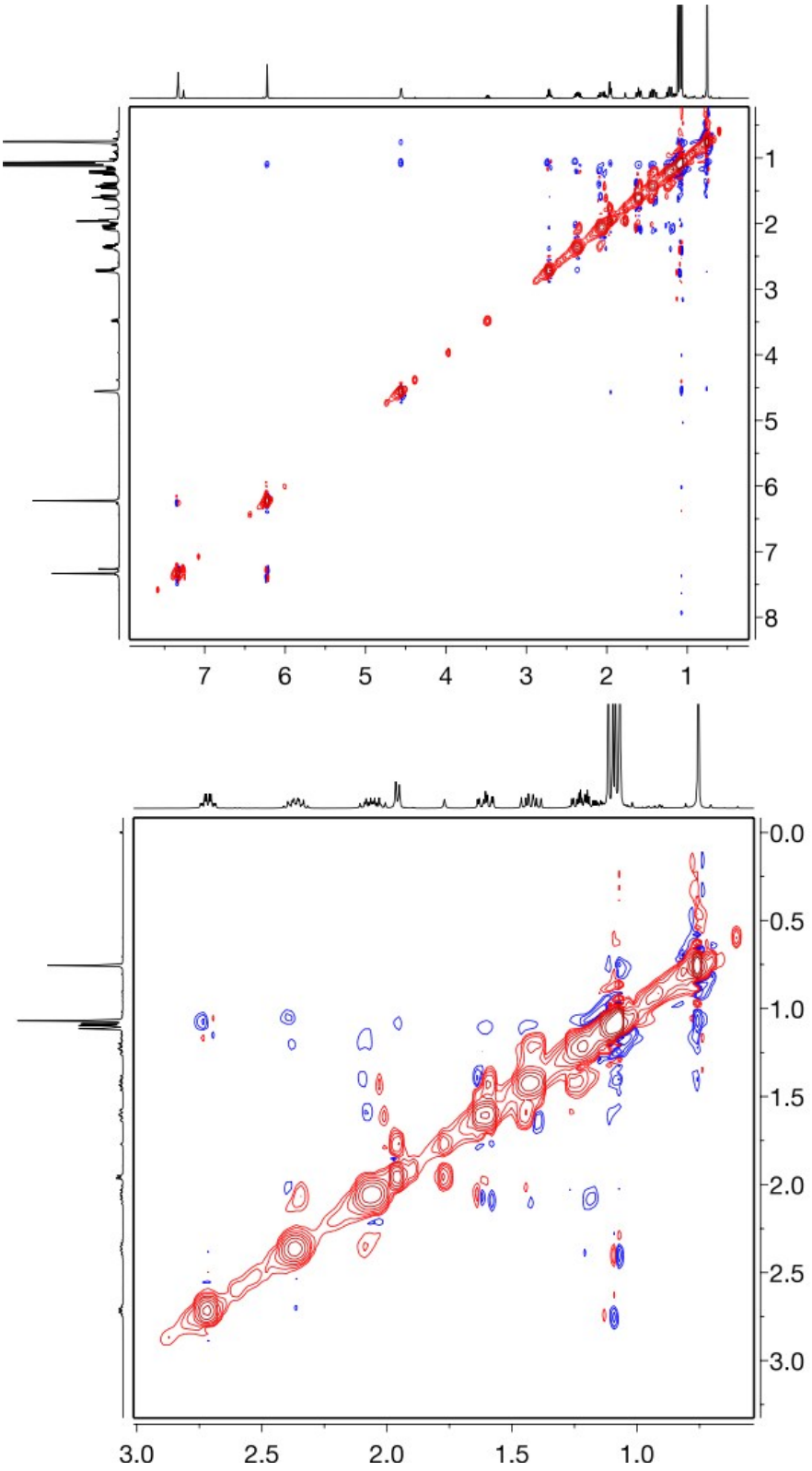


Fig. S6. NOESY spectrum of compound **1** and an expanded region of the NOESY spectrum (0.5 – 3.0 ppm and 0.0 – 3.5 ppm) ($CDCl_3$).

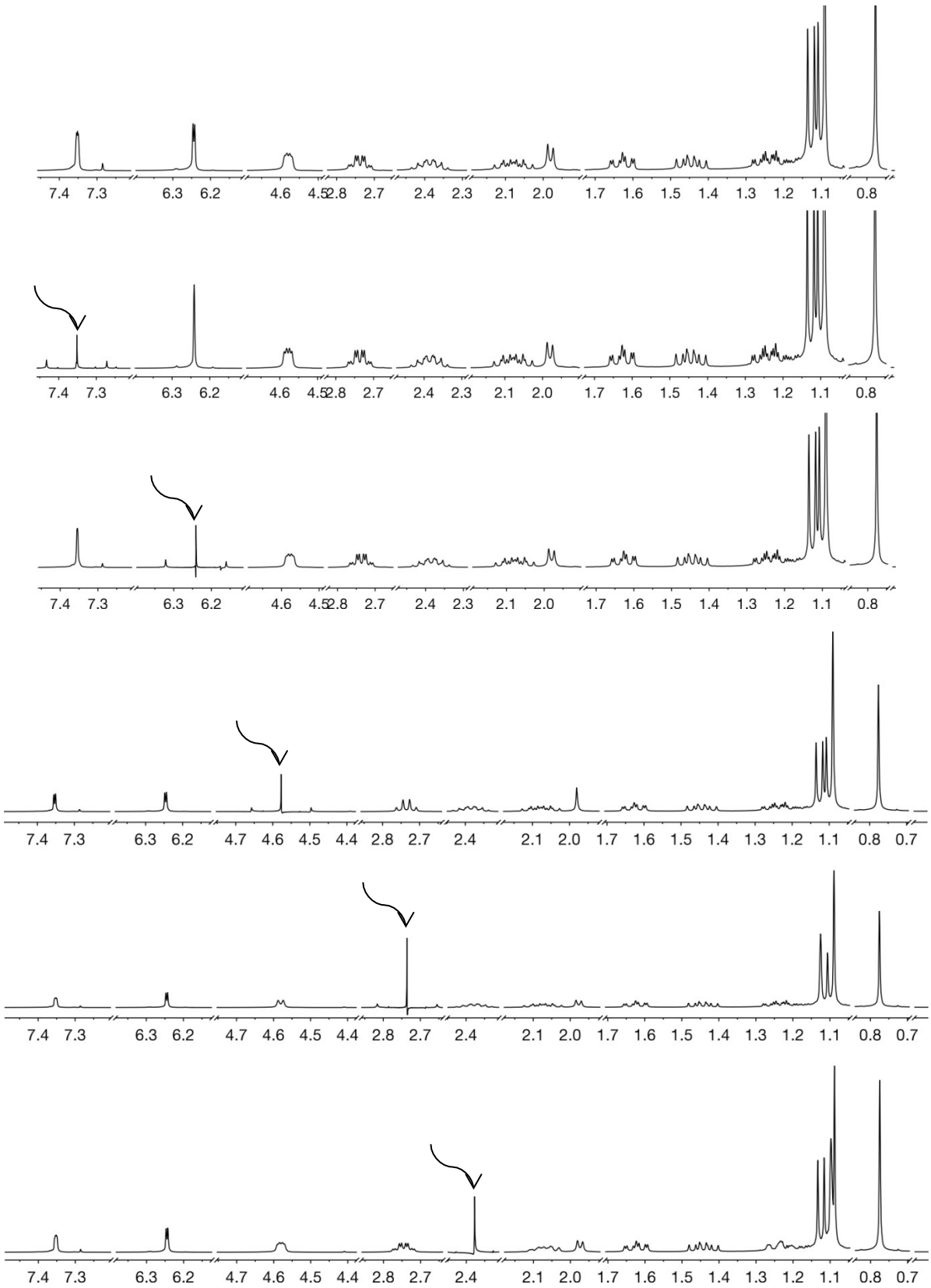
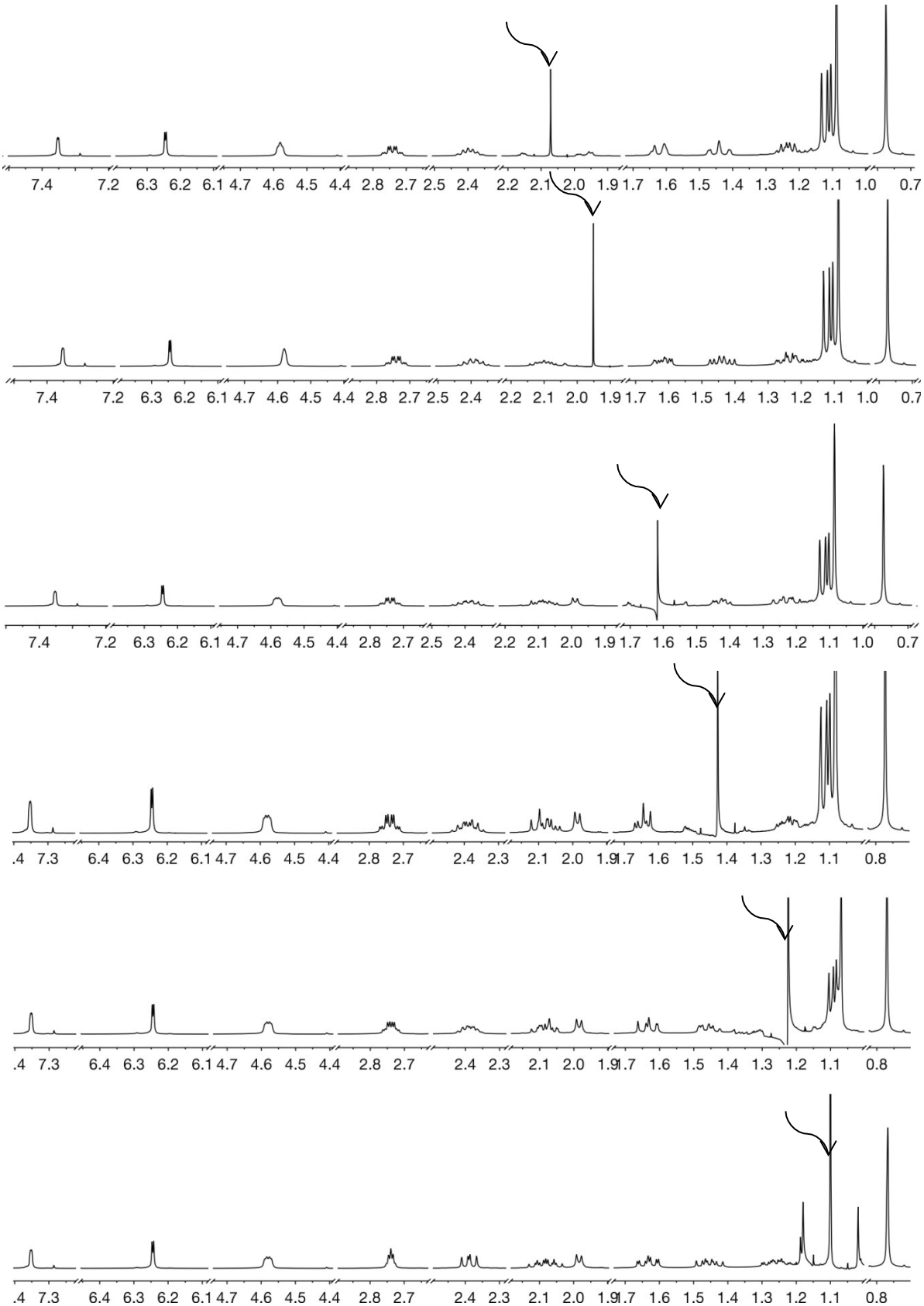


Fig. S7. Homonuclear decoupled proton spectra of compound **1**. Decoupler frequency is marked with an arrow ($CDCl_3$).



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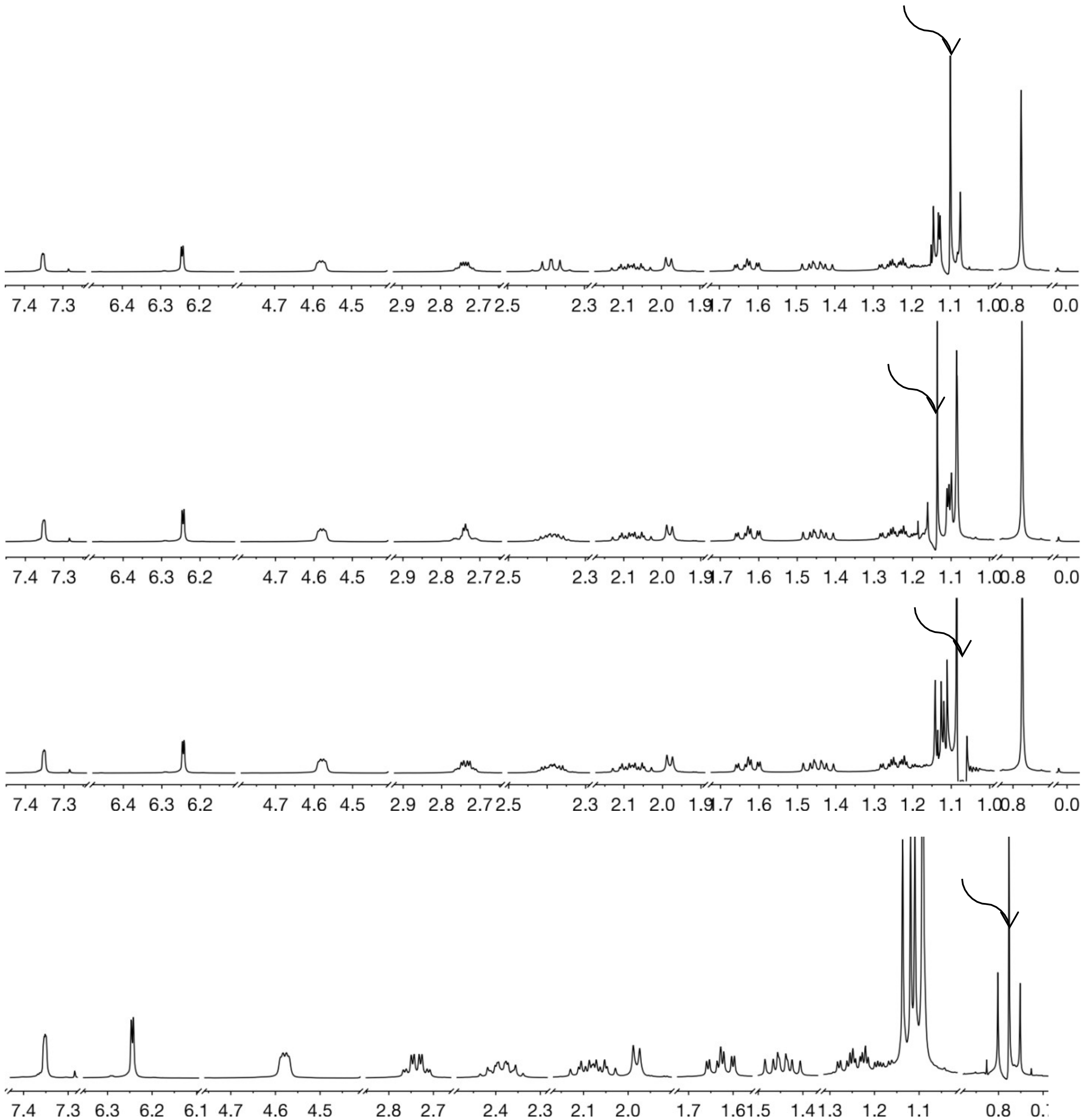
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7.4 7.3 6.4 6.3 6.2 4.7 4.6 4.5 2.9 2.8 2.7 2.5 2.3 2.1 2.0 1.9 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.8 0.0
7.4 7.3 6.4 6.3 6.2 4.7 4.6 4.5 2.9 2.8 2.7 2.5 2.3 2.1 2.0 1.9 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.8 0.0
7.4 7.3 6.3 6.2 6.1 4.7 4.6 4.5 2.8 2.7 2.4 2.3 2.1 2.0 1.7 1.6 1.5 1.4 1.3 1.2 1.1 0.8

Fig. S7. (Contd.)

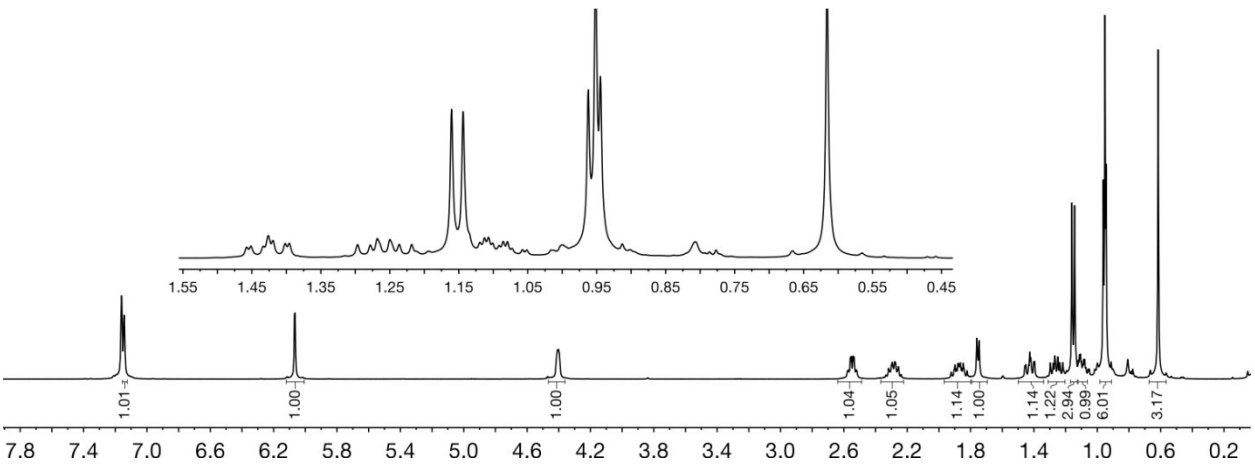


Fig. S8. ^{1}H NMR spectrum of α-furanopinguisanol (**1**) (C_6D_6).

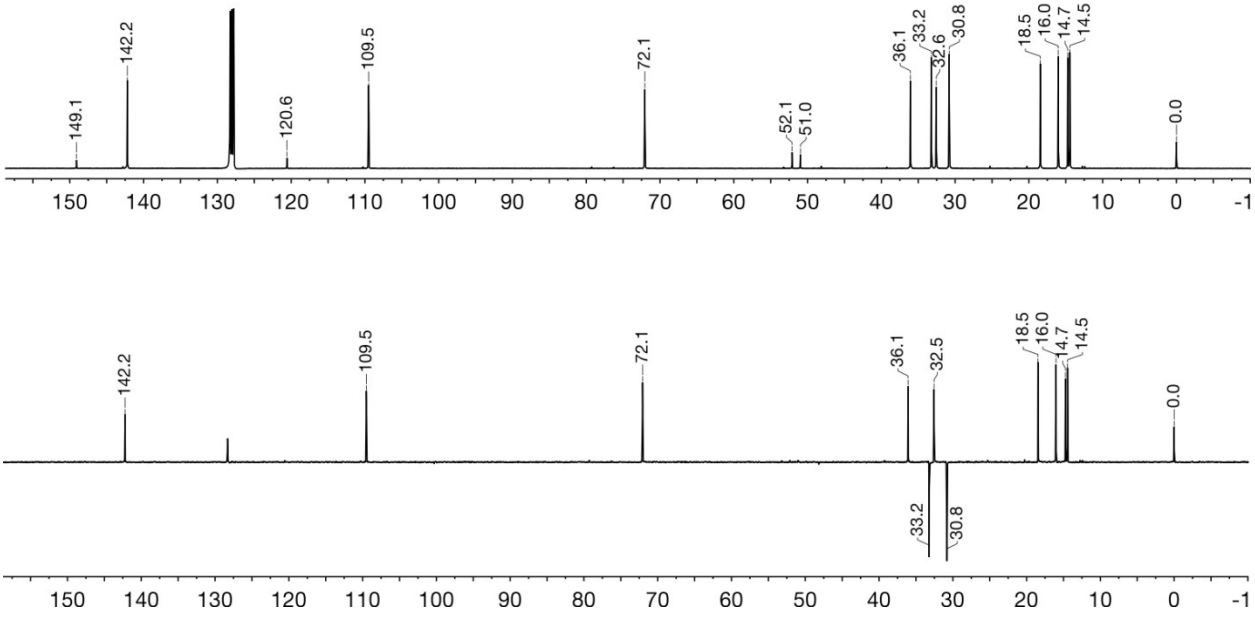


Fig. S9. ^{13}C NMR spectrum of **1** (above), DEPT-135 spectrum (below). CH_3 and CH signals are above, CH_2 signals are below the baseline (C_6D_6).

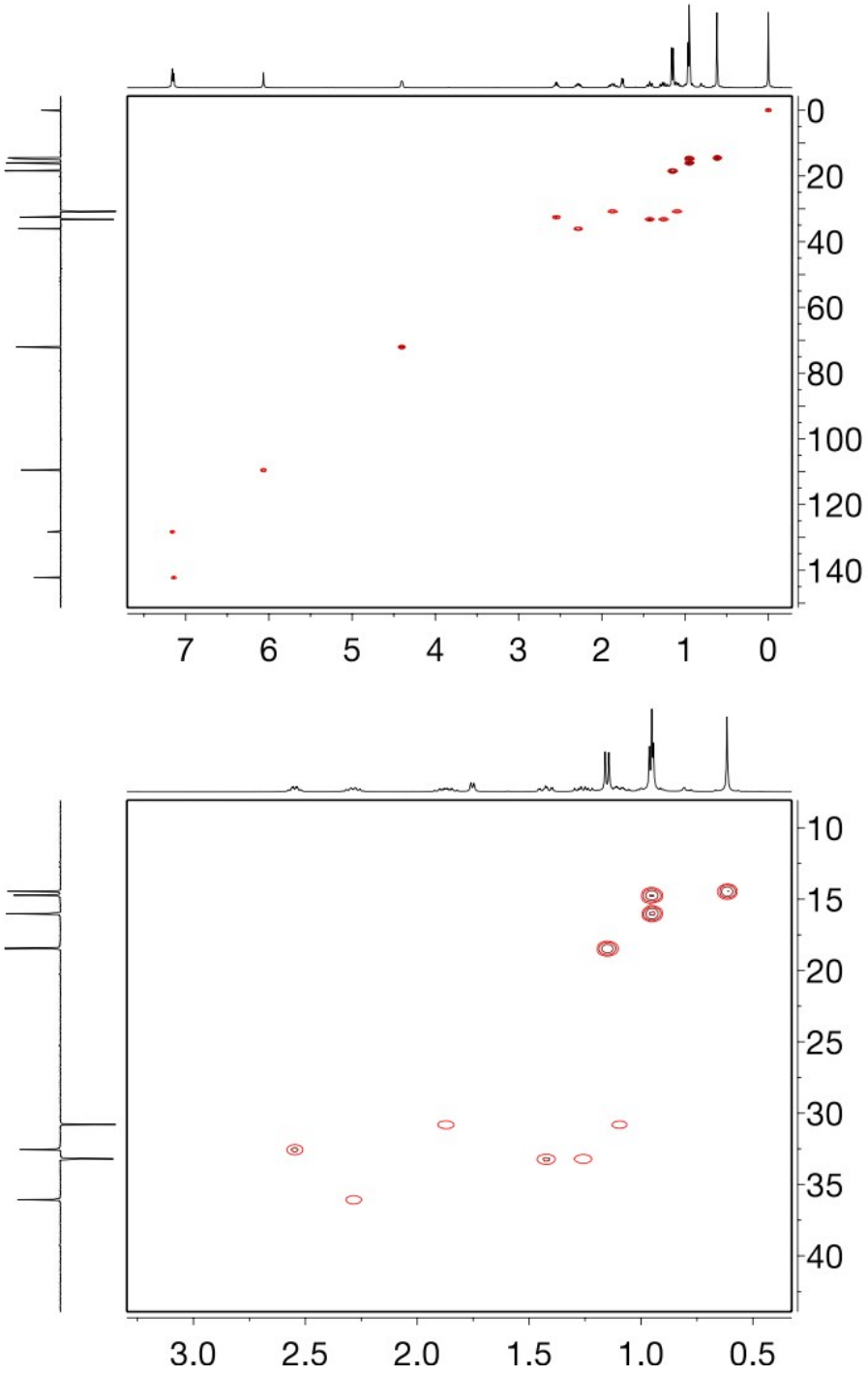


Fig. S10. HSQC spectrum of compound **1** and an expanded region of HSQC spectrum (0.5 – 3.0 ppm and 10 – 45 ppm) (C_6D_6).

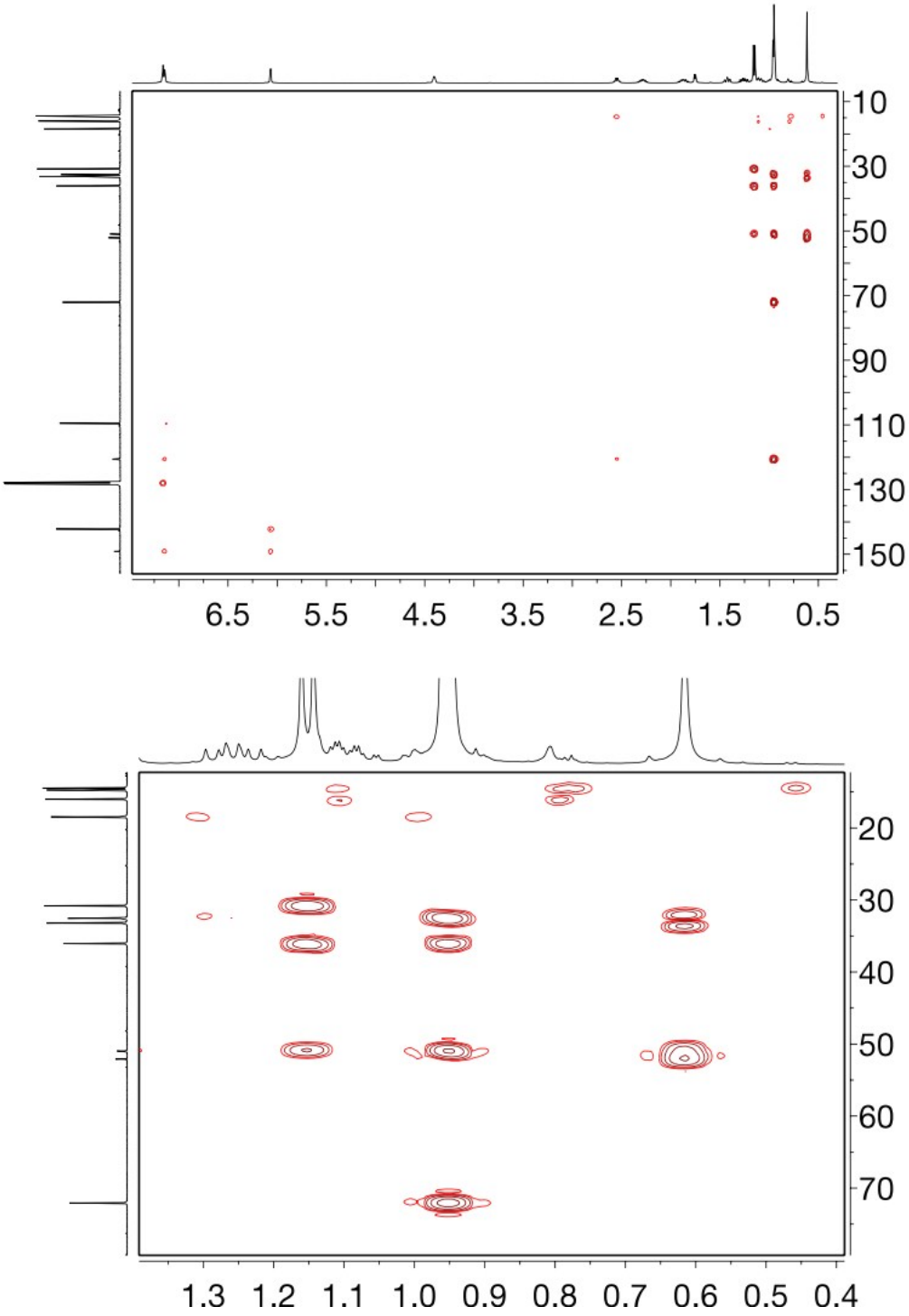


Fig. S11. HMBC spectrum of compound **1** and an expanded region of HSQC spectrum (0.5 – 3.0 ppm and 10 – 45 ppm) (C_6D_6).

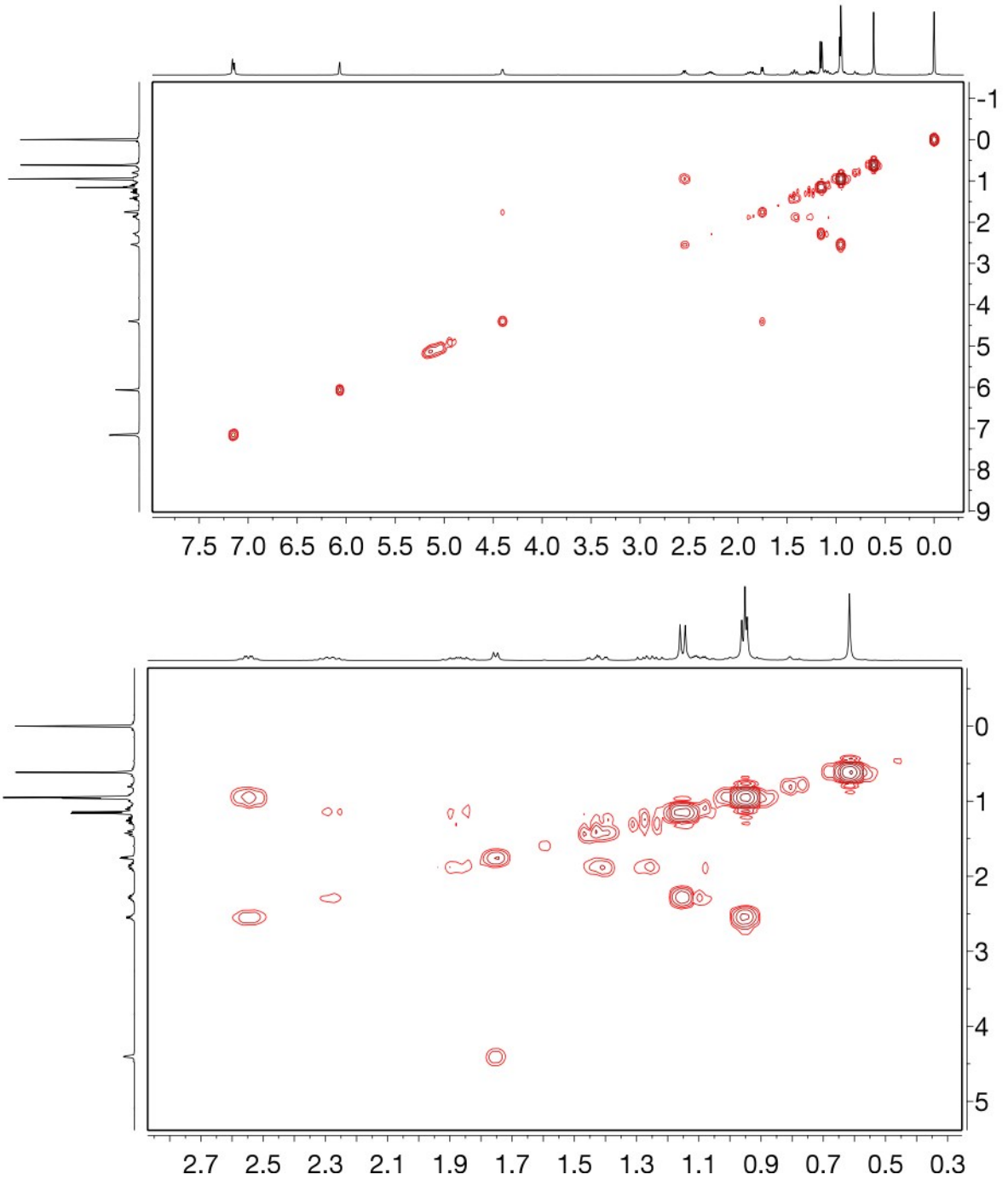


Fig. S12. ^{1}H-^{1}H COSY spectrum of compound **1** and an expanded region of ^{1}H-^{1}H COSY spectrum (0.5 – 5.0 ppm and 0 – 2.5 ppm) (C_6D_6).

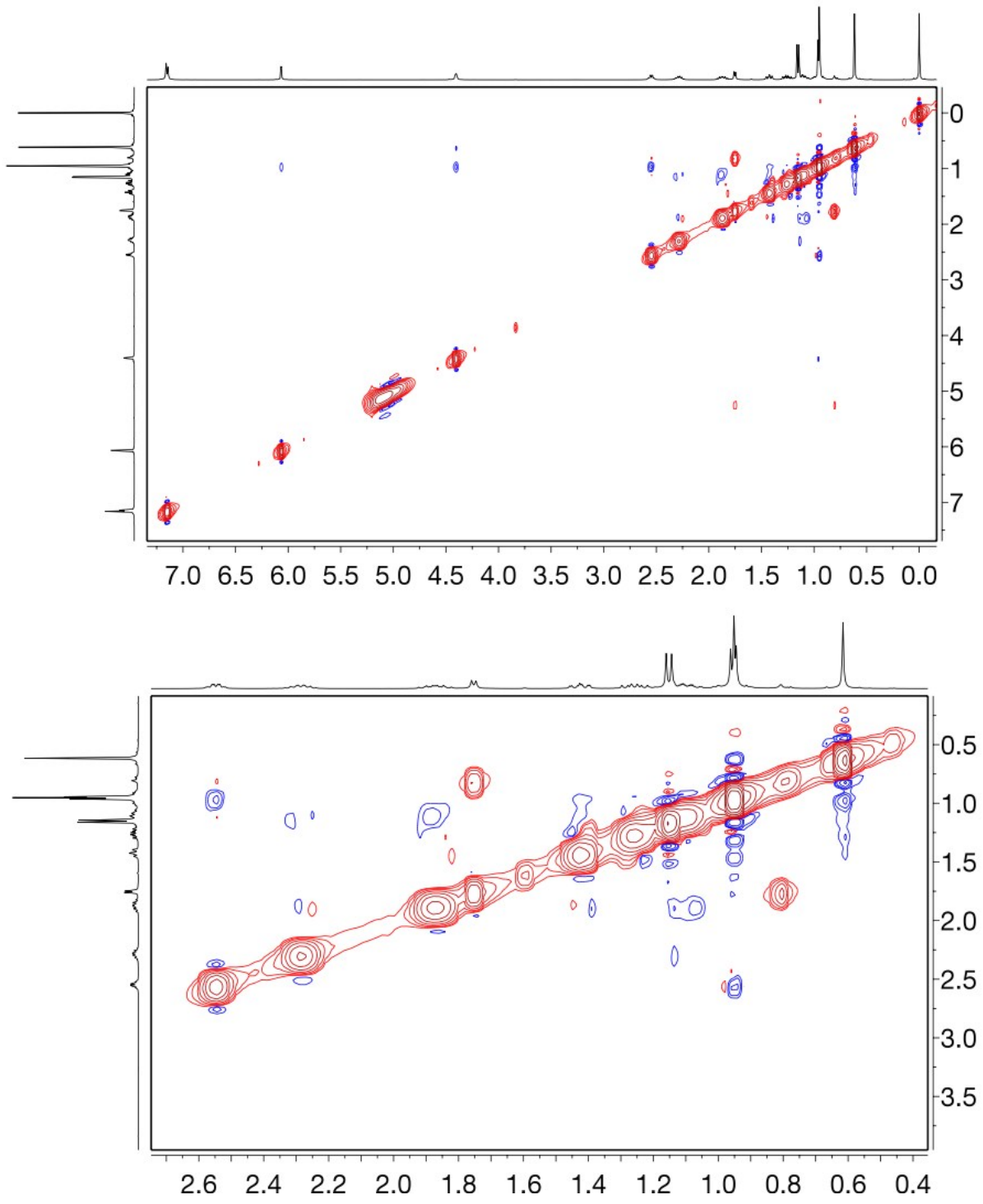


Fig. S13. NOESY spectrum of compound **1** and an expanded region of NOESY spectrum (0.5 – 3.5 and 0.5-3.0 ppm) (C_6D_6).

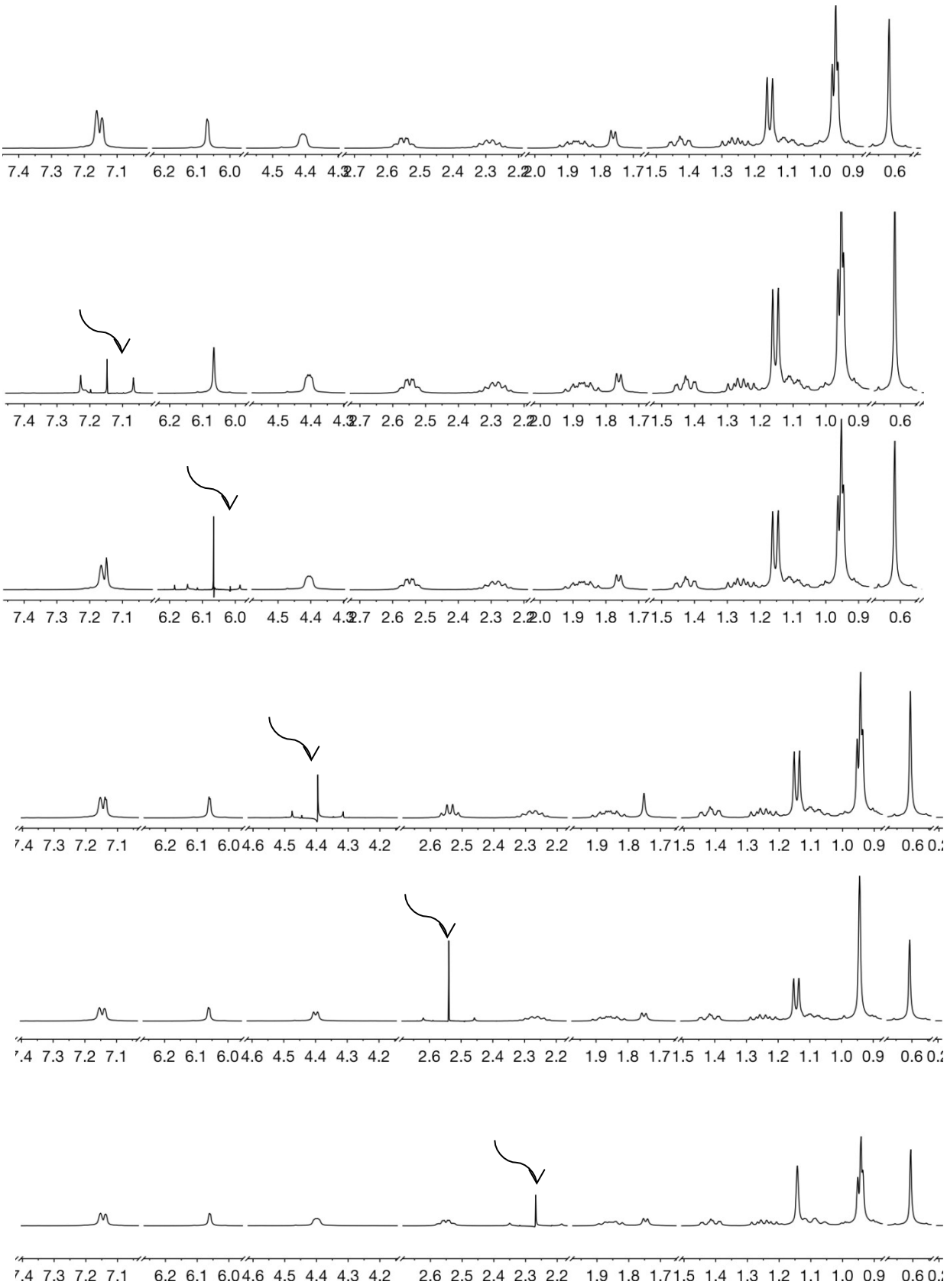


Fig. S14. Homonuclear decoupled proton spectra of compound **1**. Decoupler frequency is marked with an arrow (C_6D_6).

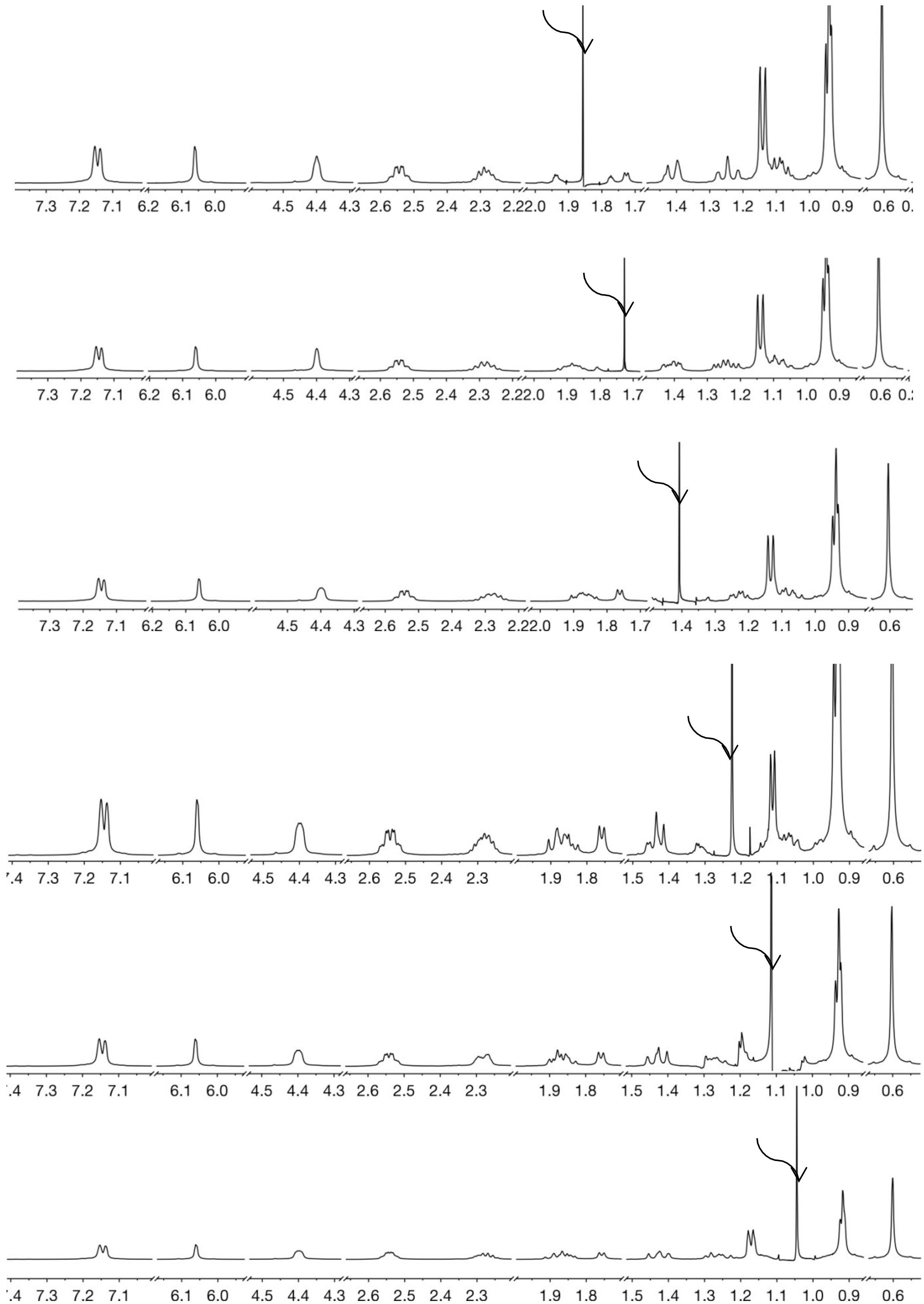
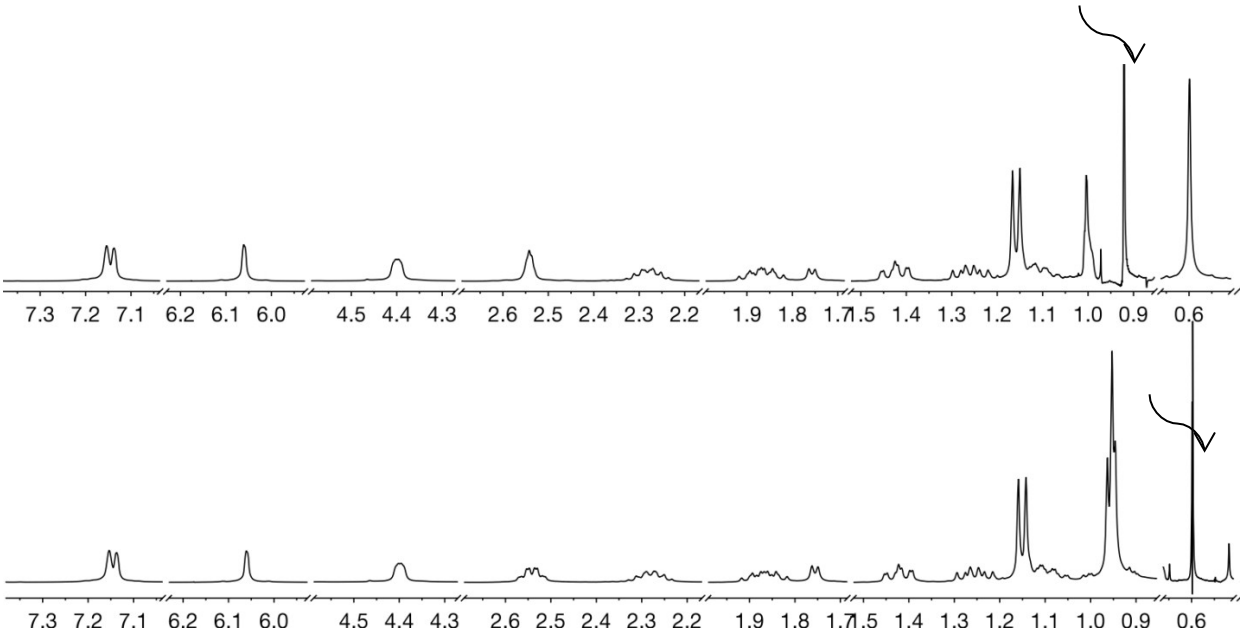


Fig. S14. (Contd.)



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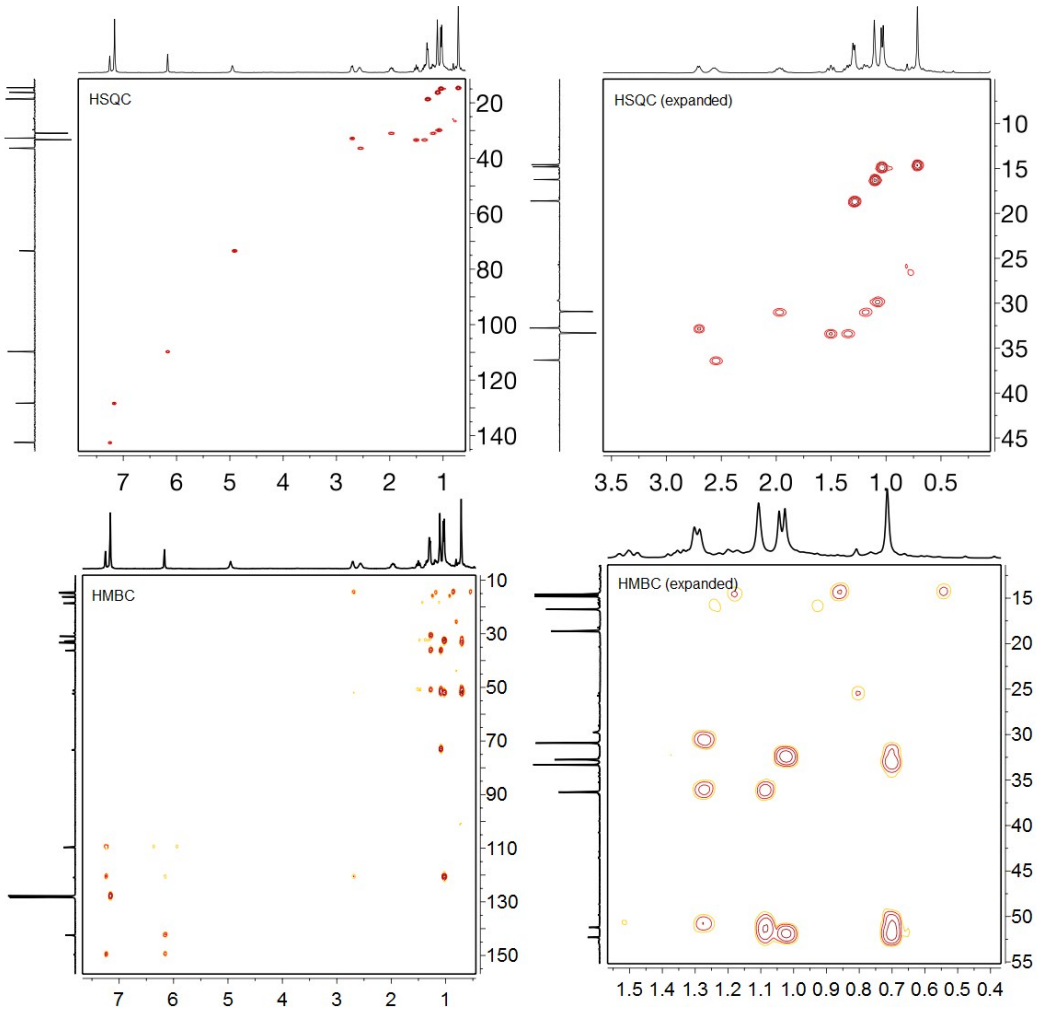


Fig. S15. HSQC (above) and HMBC (below) spectra of compound **1** after the addition of 9 mg of $Eu(fod)_3$.

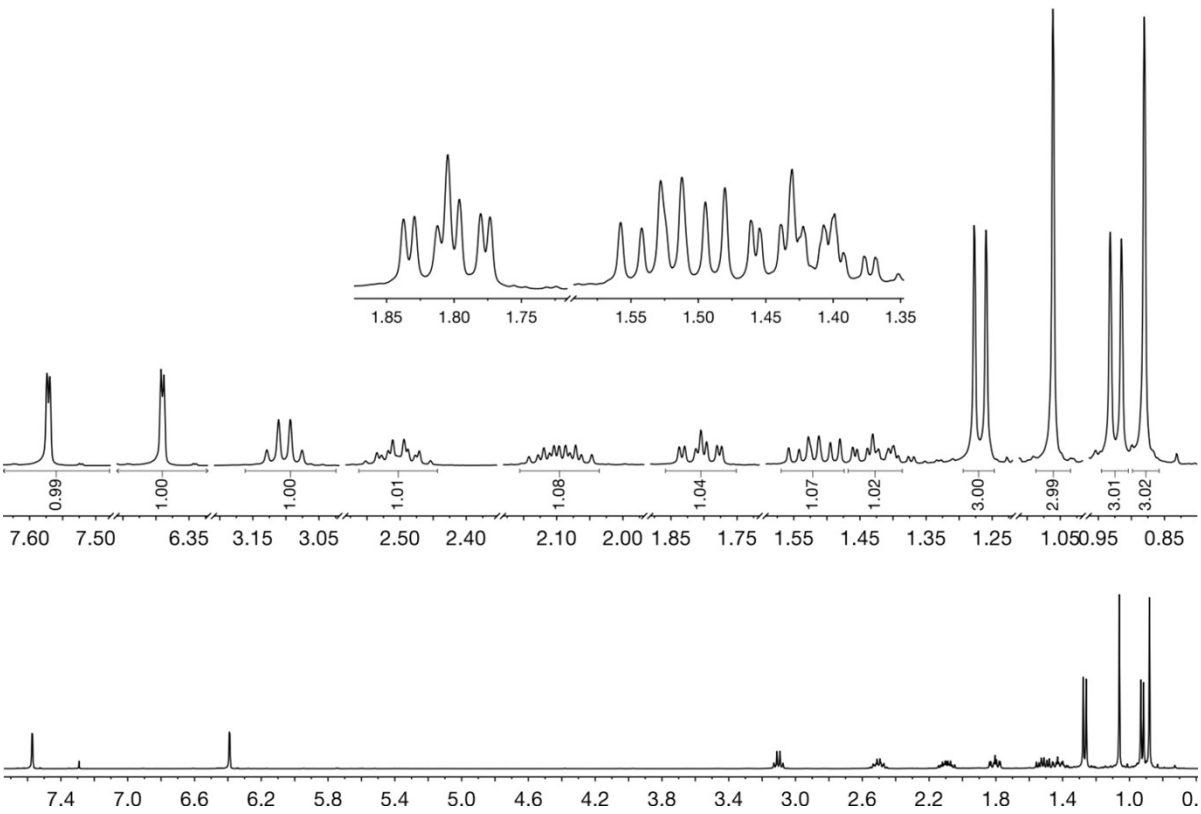


Fig. S16. ^{1}H NMR spectrum of furanopinguisanone (**2**) ($CDCl_3$).

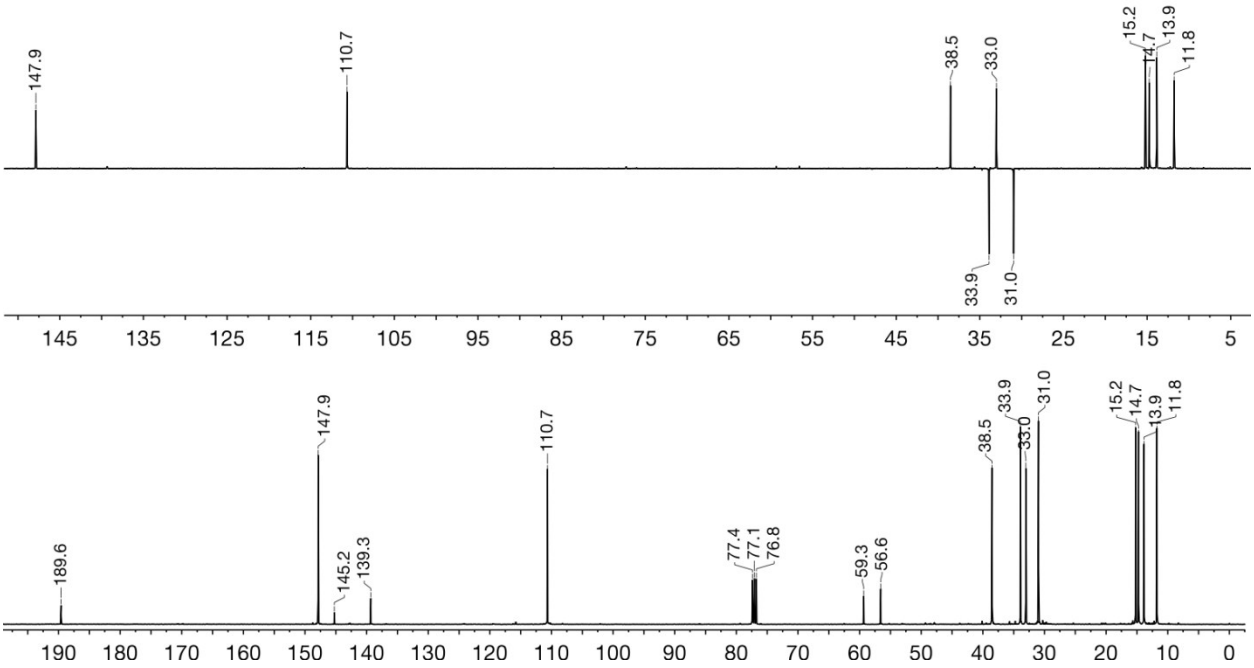


Fig. S17. ^{13}C NMR spectrum of **2** (below), DEPT-135 spectrum (above). CH_3 and CH signals are above, CH_2 signals are below the baseline ($CDCl_3$).

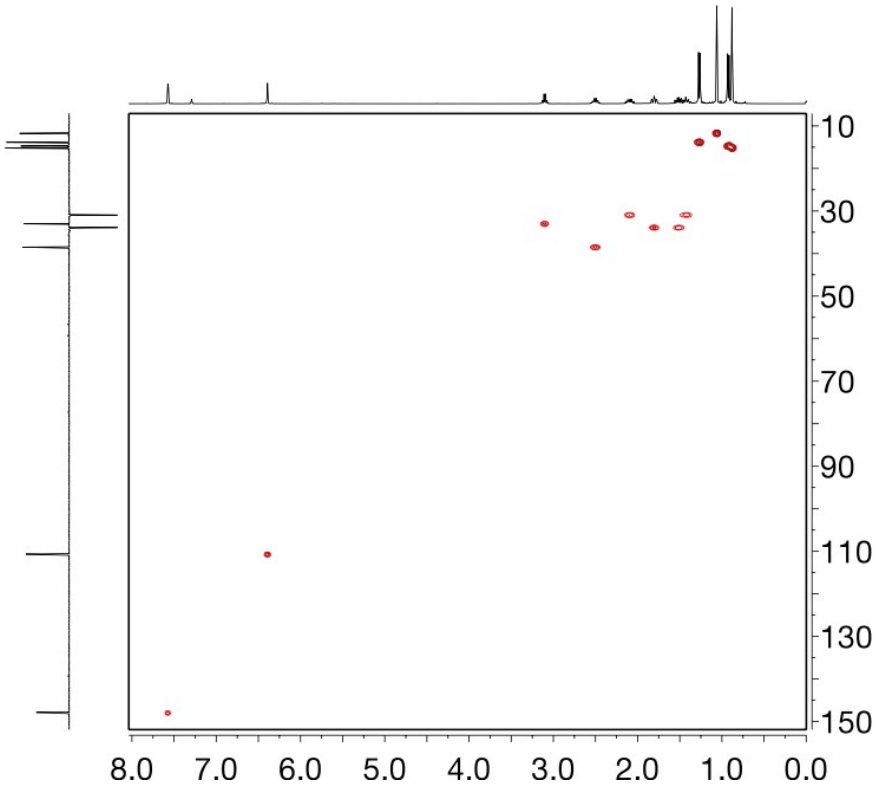


Fig. S18a. HSQC spectrum of **2** ($CDCl_3$).

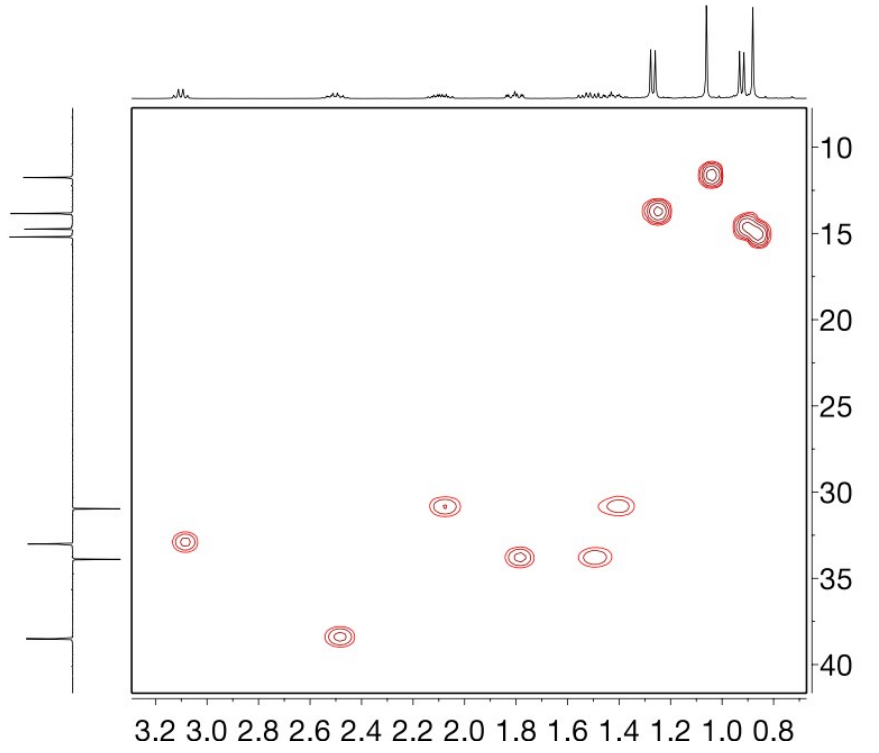


Fig. S18b. Expanded region of HSQC spectrum (0.8 – 3.2 ppm and 10 – 40 ppm) ($CDCl_3$).

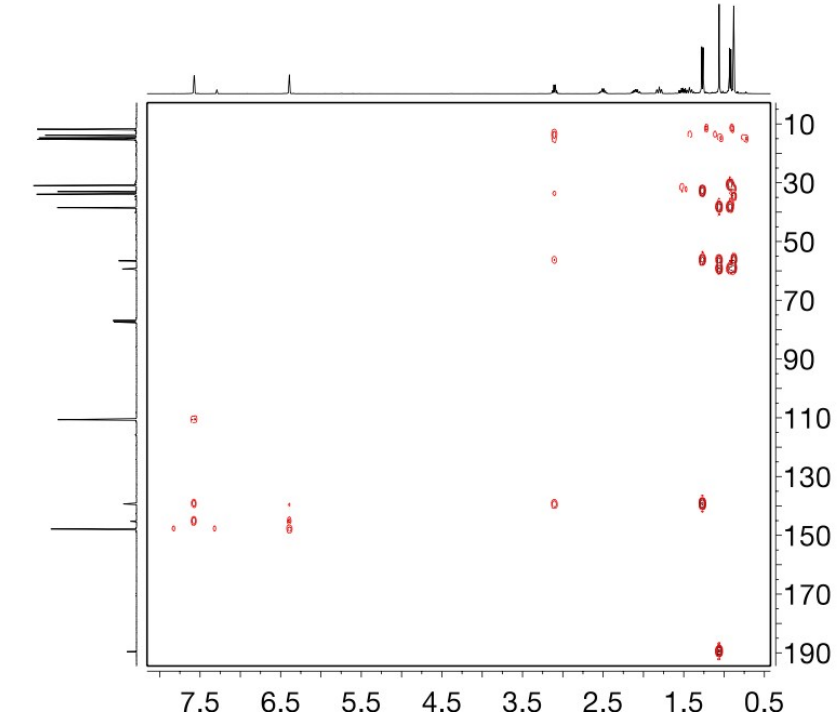


Fig. S19a. HMBC spectrum of **2** ($CDCl_3$).

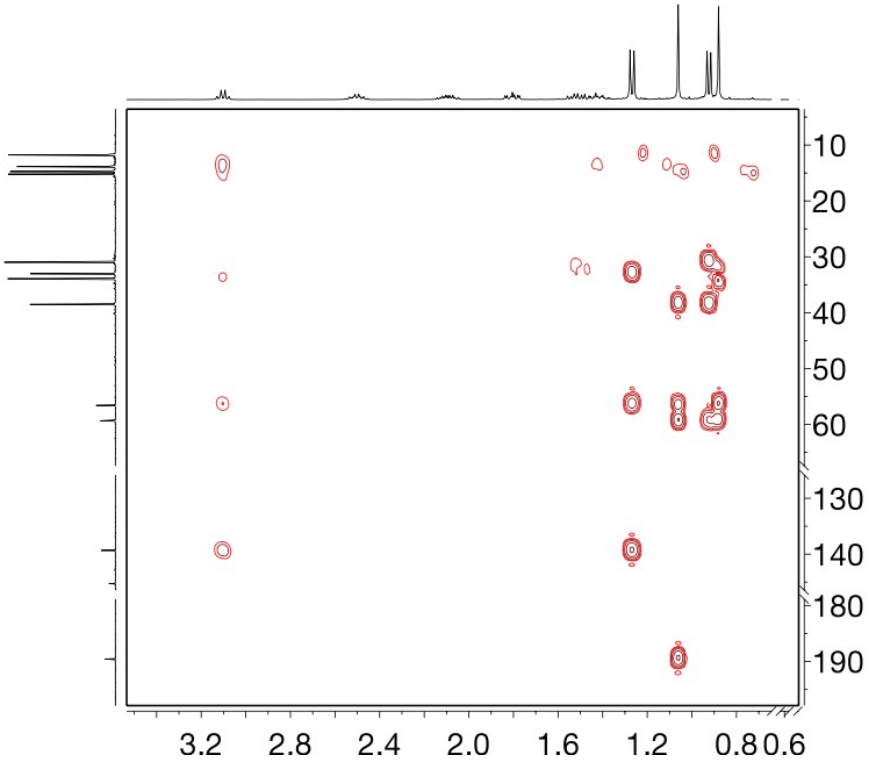


Fig. S19b. Expanded region of HMBC spectrum of **2** ($CDCl_3$).

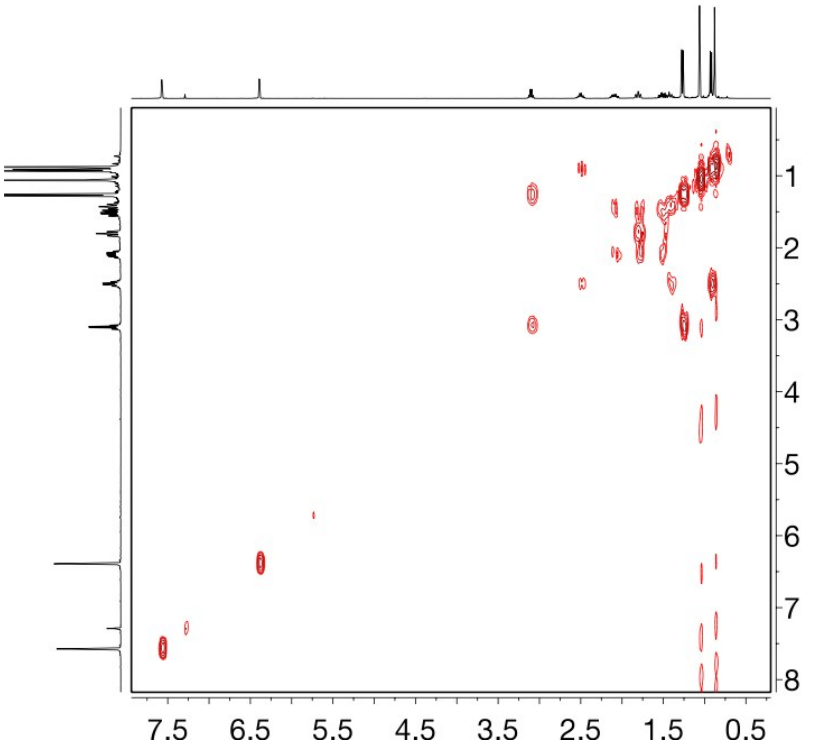


Fig. S20a. ^{1}H-^{1}H COSY spectrum of **2** ($CDCl_3$).

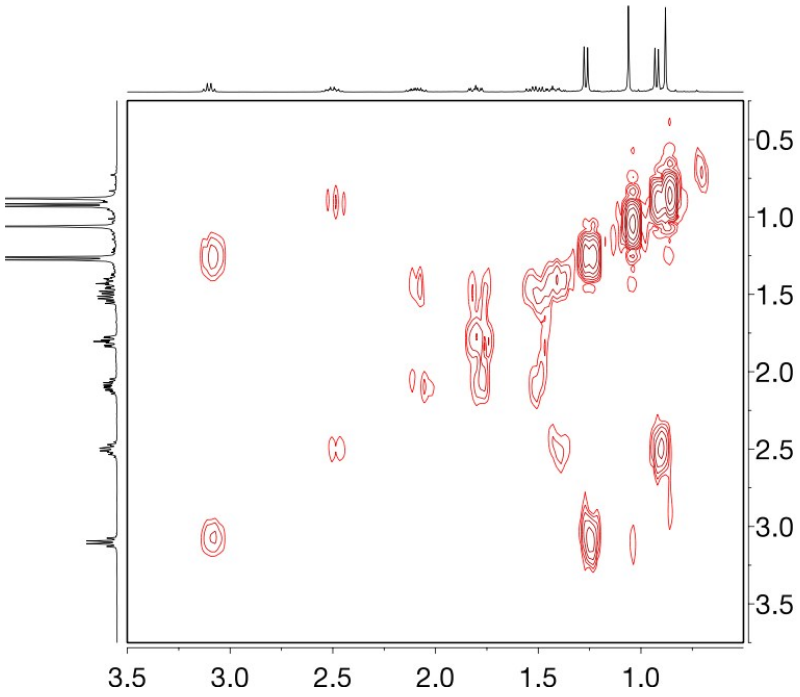


Fig. S20b. Expanded region of ^{1}H-^{1}H COSY spectrum of **2** (0.5– 3.5 ppm) ($CDCl_3$).

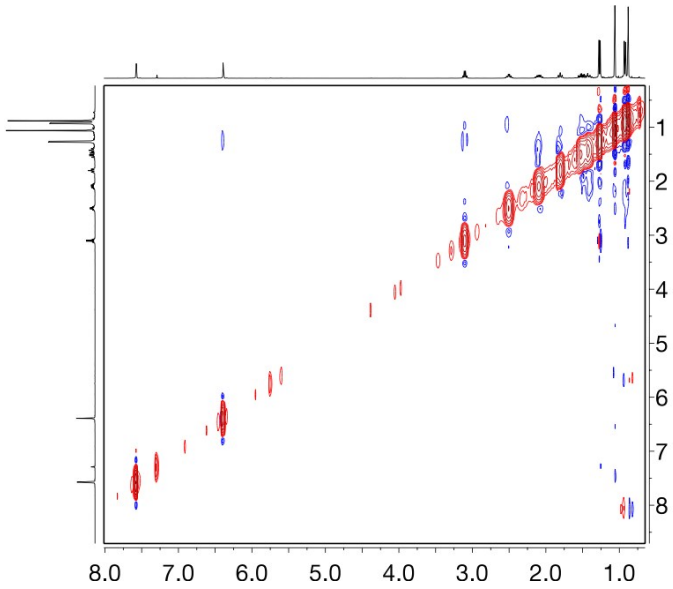


Fig. S21a. NOESY spectrum of **2** ($CDCl_3$).

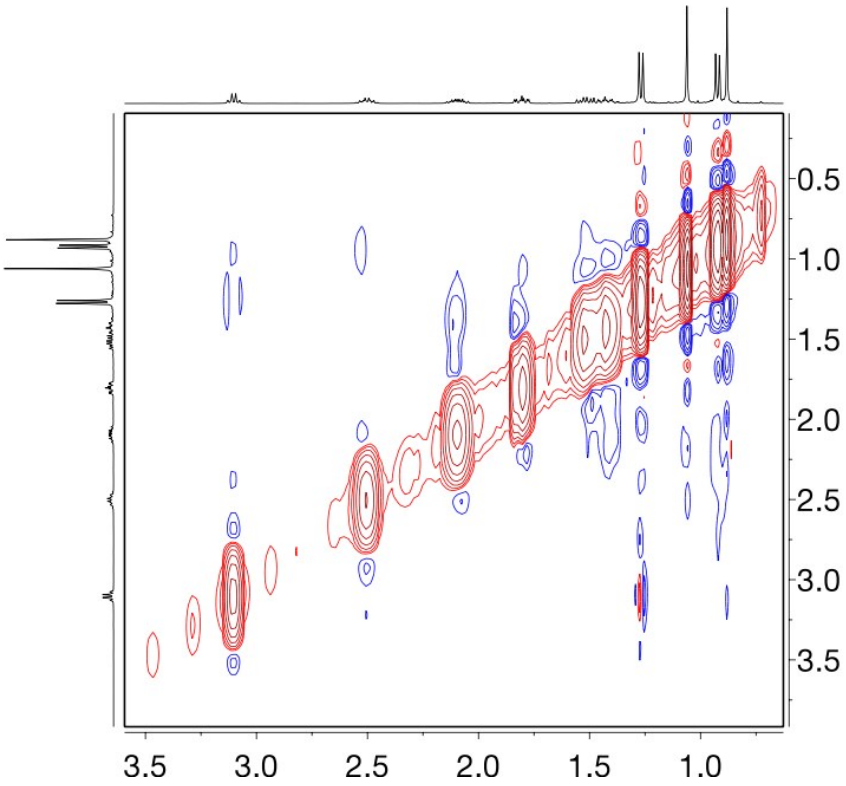


Fig. S21b. Expanded region of NOESY spectrum of **2** (0.5– 3.5 ppm) ($CDCl_3$).

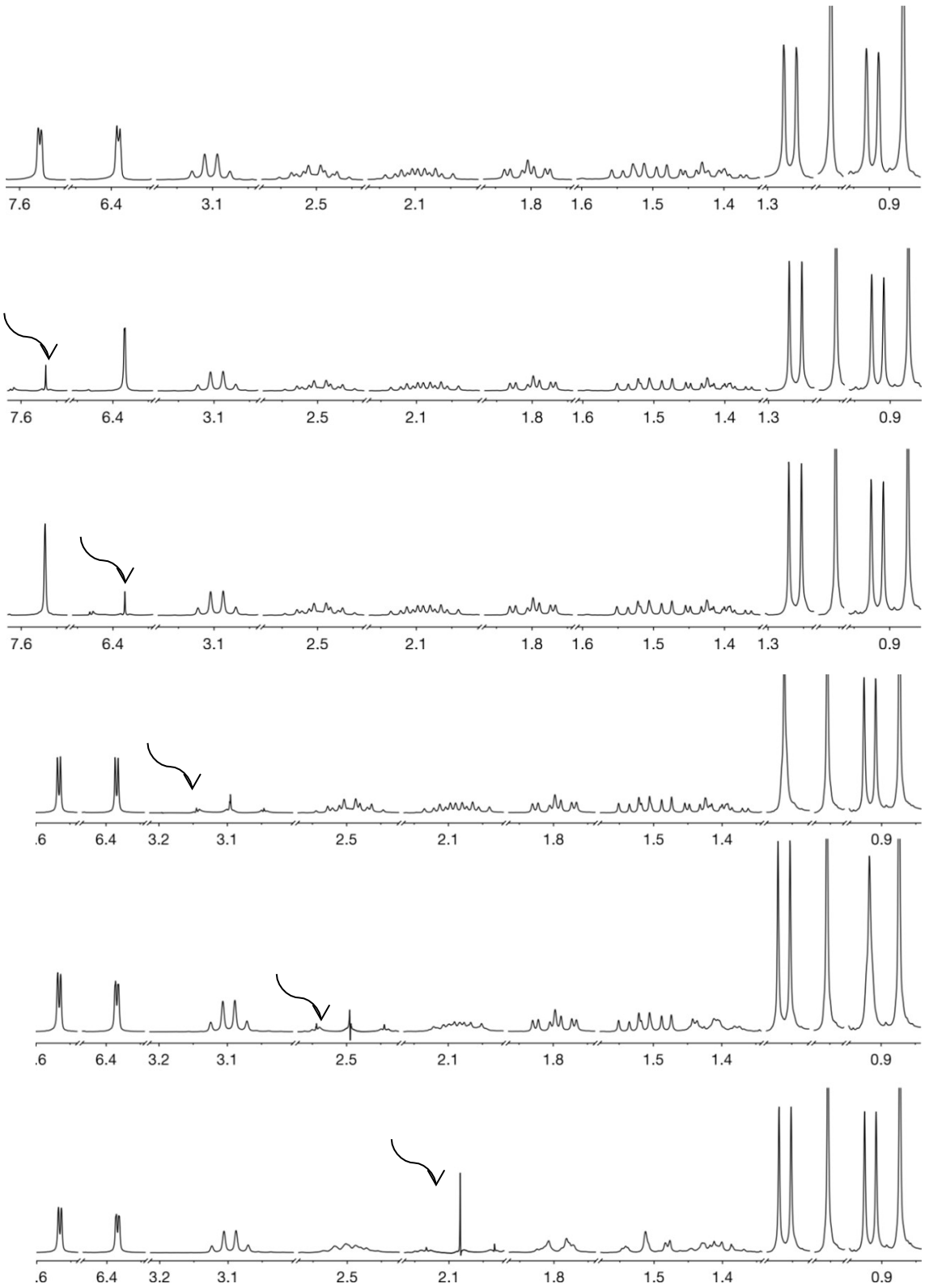
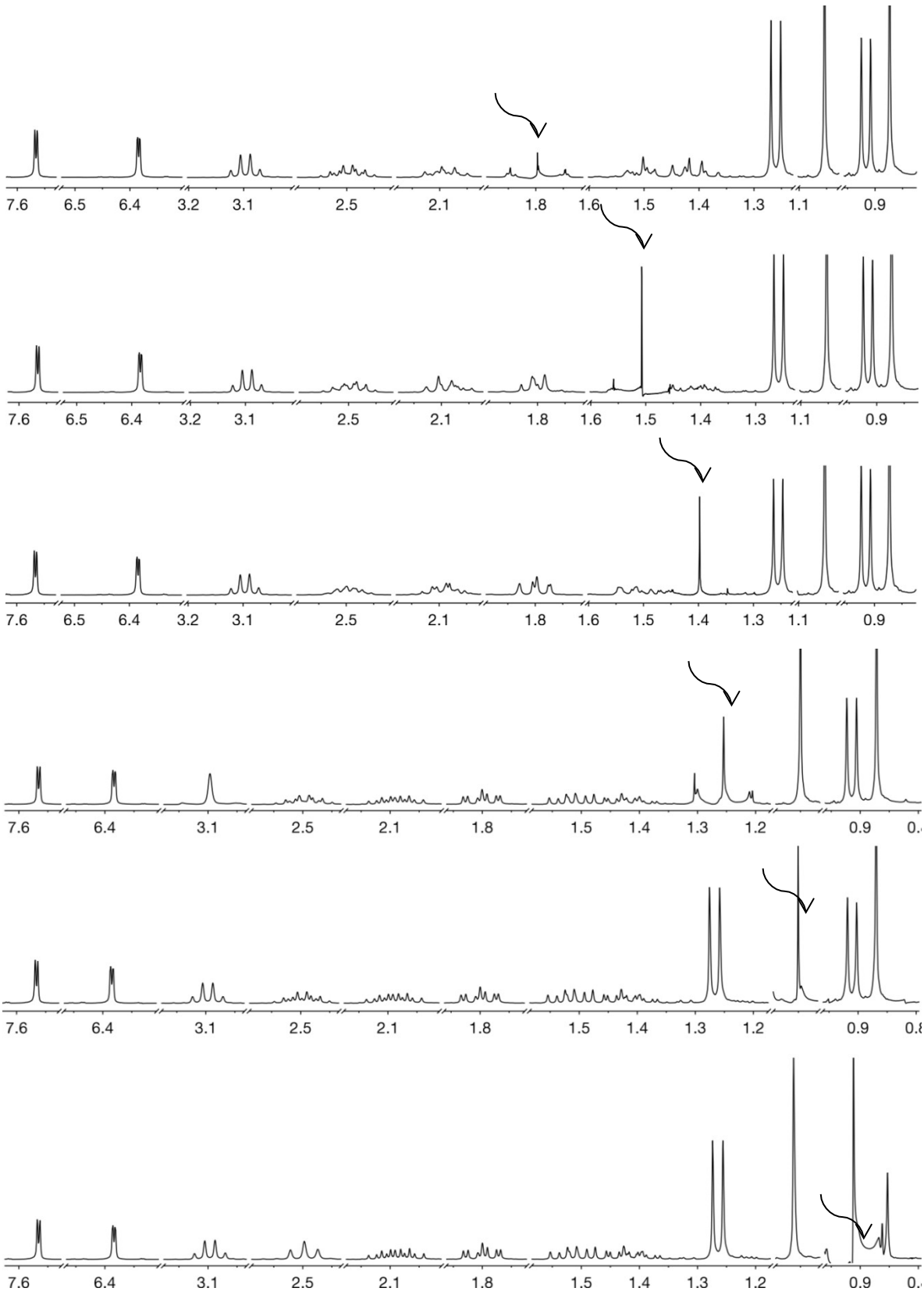


Fig. S22. Homonuclear decoupled proton spectra of compound **2**. Decoupler frequency is marked with an arrow ($CDCl_3$).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7.6 6.5 6.4 3.2 3.1 2.5 2.1 1.8 1.6 1.5 1.4 1.3 1.1 0.9
7.6 6.5 6.4 3.2 3.1 2.5 2.1 1.8 1.6 1.5 1.4 1.3 1.1 0.9
7.6 6.4 3.1 2.5 2.1 1.8 1.5 1.4 1.3 1.2 0.9
7.6 6.4 3.1 2.5 2.1 1.8 1.5 1.4 1.3 1.2 0.9
7.6 6.4 3.1 2.5 2.1 1.8 1.5 1.4 1.3 1.2 0.9

Fig. S22. (Cont.)

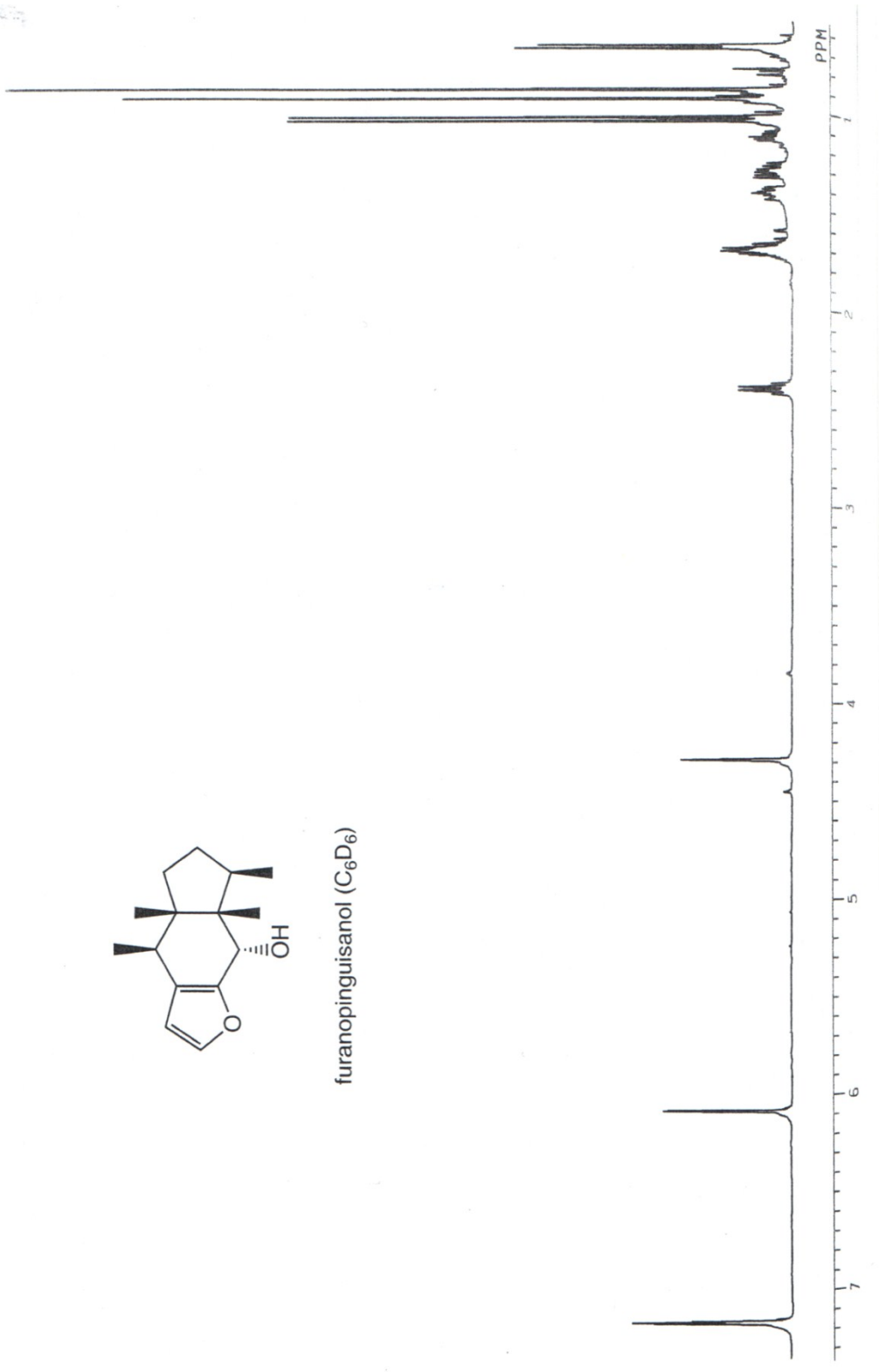

Fig. S23. ^{1}H NMR spectrum of the alleged α-furanopinguisanol from the work of Tori et al. (Phytochemistry, 1993, 2:335–348), as published in Y. Asakawa, M. Tori, The Atlas of 400 MHz NMR Spectra of Natural Products, Hirokawa Publishing Co., Tokyo, Japan, 1993, p. 98. For comparison sake, please, confer to S10 for the ^{1}H NMR of furanopinguisanol isolated in the current work.

Structural elucidation of compound **1**

The molecular formula of compound **1** ($C_{15}H_{22}O_2$) was determined using the elemental composition data and confirmed from the isotopic ratio $[M+1]^+/[M]^+$. The observed ratio of 16.5% agreed with the theoretically predicted value of 16.6%. This was further substantiated from the number of ^{13}C signals in the proton decoupled ^{13}C spectrum (15 signals) and the integration of proton signals (which allowed us to account for all 22 protons).

The chemical shifts and coupling constants measured in $CDCl_3$ were used throughout this section, unless otherwise indicated; full spectral data (in both chloroform-*d* and benzene-d_6) are listed in Table 1 and the carbon numbering used in this work is shown in Fig. 1. Two low-field proton signals (at 6.22 and 7.33 ppm) indicated the presence of an aromatic moiety; in ^{13}C NMR spectrum only 4 carbons could belong to an aromatic ring, thus suggesting that an aromatic system other than benzene. The presence of a furan nucleus was deduced based on the following arguments: 1) no heteroatom other than oxygen was present in the molecule. This fact ruled out the presence of nitrogen- or sulfur-containing heteroaromatic groups; 2) chemical shifts of two CH aromatic carbons (C11, 142.2 and C10, 109.3 ppm, see Fig. 1 for numbering scheme) corresponded well with the literature values of δ_{C2} and δ_{C3} in furan itself (143.0 and 109.9 ppm, respectively; [24]; 3) δ values of protons attached to C10 and C11 (6.22 and 7.39, respectively) are also in accordance with literature values of 6.38 and 7.42 ppm [24]; 4) the value of the observed coupling constant, $^3J_{10,11}$ = 1.9 Hz, is characteristic of H2-H3 coupling in furans [24]; 5) presence of a substituted furan ring was also suggested from the UV spectra of compound **1** ($\lambda_{max}(CH_3CN)$ at 223.5 nm with ε = 3428).

The remaining part of the structure was established through the analysis of 1D and 2D NMR spectra. HMBC spectrum (see Fig. 1 for key HMBC and NOESY correlations) showed a correlation of a non-protonated carbon at 120.8 ppm (C5) with methyl protons at 1.11 ppm (H15) across three bonds and a proton with δ 2.72 (H4) across two bonds. The two proton signals coupled (observable in ^1H-^1H COSY spectrum) with a vicinal coupling constant of 7.1 Hz, i.e. they were located at the same carbon (C4, 32.2 ppm, as determined from the HSQC interaction of H4). A further HMBC correlation of H4 and the second non-protonated "aromatic" carbon (C6) also confirmed that C4 is bonded to the furan ring.

Proton at 4.56 ppm (H7) showed HMBC correlations with both C5 and C6, suggesting that this proton is on a carbon (C7, 72.3 ppm) bonded directly to C6. The chemical shift of C7 indicated oxygenation and indeed H7 coupled with an OH proton (J = 5.6 Hz). Note that H7 appeared as a doublet of doublets (*dd*, the second coupling constant was 2.6 Hz), due to its coupling with H4 through five bonds. H15 and H4 showed cross-peaks in the HMBC spectrum with an aliphatic quaternary carbon (C9, 52.2 ppm), which additionally correlated with methyl protons at 0.76 ppm (H14). This proton signal, in turn, coupled with C4 through three bonds, indicating that C14 was bonded to C9. In the HMBC spectrum, H7 proton correlated with a second aliphatic quaternary carbon (C8) and a methyl carbon next to C8 at 15.9 ppm (C12). Finally, an HMBC correlation between C8 and H14 revealed that C4-C9 carbons were all part of a six-membered ring.

The structure of the remainder of the molecule was solved straightforwardly. C12 correlated with H1 (2.37 ppm) as observed in the HMBC spectrum. This proton coupled to a methyl group at 1.08 ppm (H13, J = 6.7 Hz) and diastereotopic CH_2 protons at 1.20 and 2.06 (H2α and β). These protons also coupled to another pair of CH_2 protons at 1.42 and 1.61 (H3α and β) (for a complete list of the coupling constants, see Table 1). Finally, an HMBC correlation between C3 and H14 was observed, thus the presence of the third ring (5-membered, C1, C2, C3, C8, C9) was established. With this, all of the atoms in the structure were accounted for and the connectivity of the molecule was solved (Fig. 1). The stereochemical assignment of furanopinguisanol (**1**) has been presented in detail in the main text of the paper.

A significant disagreement between the spectral data found in the work by Tori et al. [13] and the data obtained in our study should be recognized. The observed differences include:

1) EI-MS spectrum given by Tori et al. [13] was found to be completely different from the spectrum we recorded. Most notable is the absence of the ion at *m/z* 124, which was the base peak of **1** herein. Instead, Tori and co-workers [13] reported that the base peak of their compound was at *m/z* 125. This ion was also present in our spectrum of **1**, but only as a minor peak (with the intensity of less than 8% compared to the intensity of *m/z* 124, so we could assume that it represents an isotopic ion of the base peak, and not a fragment ion peak).

2) The ^{13}C chemical shifts listed in the paper by Tori et al. [13], on average, differed by 1.42 ppm compared to the shifts of **1** recorded in the same solvent (C_6D_6) herein. The largest difference was noted for the hydroxyl-bearing carbon atom (C7), that differed by 6.6 ppm. ^{13}C NMR shifts are known to be highly reproducible parameters of organic compounds (especially when the compounds are recorded in the same solvent) [28]. According to the mentioned study of Grzonka and Davies [28] a spread of ^{13}C NMR shift values of Δ 0.5 ppm in both directions is reasonable for non-polar compounds and anything beyond this needs closer inspection. The deviation of 6.6 ppm for C7 can thus only be explained by the fact that either we or the Japanese team made an error during the structure elucidation process.

3) Proton shifts also significantly deviated from the one in our spectra. For example, the differences in δ_H values (in deuterated benzene) for H1, H4 and H7 protons were 0.59, 0.14 and 0.12 ppm, respectively. For a comparison of the ^1H NMR spectra of compound **1** from the current work and that from Tori et al. [13], please, refer to the supplementary file (Figs. S10 and S25, respectively).

Evidences that compound **1** from this study and the compound the Japanese researchers isolated were not the same are overwhelming. While Tori and co-workers [13] claimed that the relative stereochemistry of the molecule was established using nOe difference experiments, we were able to obtain the original spectral data from the Japanese work (which we also included it in the Supplementary material, Fig. S10), and we must express our doubt that such conclusions were possible. Poor signal separation observable in the spectra used by Tori et al. [13] hindered a definite determination of stereochemistry. At this moment, we could argue that the compound isolated by the Japanese team does not correspond to structure **1**.

A possible explanation we came up with was that Tori and co-workers [13] actually isolated the diastereomeric compound - β-furanopinguisanol - and incorrectly assigned the stereochemistry at C7 (this would explain the large difference in δ_{C7} shifts). We tried to prove this hypothesis by an inversion of the configuration at C7 of compound **1** through a Mitsunobu reaction [29]. Unfortunately, after several attempts we failed to produce the *β* diastereoisomer.

Structural elucidation of compound **2**

The connectivity and relative stereochemistry of compound **2** was established in an analogous manner as for compound **1**. Here, we list some of the differences between the spectral data of **1** and **2**:

1) The lack of the OH stretch and the appearance of an intensive band at 1675 cm^{-1} in the IR spectrum clearly demonstrated the formation of a conjugated carbonyl group; the conjugation was also observable by a bathochromic shift of the UV maximum ($\lambda_{max}(CH_3CN)$ at 270.5 nm with ε = 3259);

2) The carbonyl group was observed from the ^{13}C NMR signal at 189.6 ppm; the signal of the oxygenated sp^3-carbon at 72.3 ppm was lost following the oxidation;

3) The proton signal at 4.56 ppm (H7) also disappeared; this resulted in the simplification of the H4 signal due to the removal of one of the coupling constant; this *qd* from the spectrum of **1** was transformed into a quartet;

4) The electron-accepting nature of the carbonyl group caused a downfield shift of C5 signal by almost 20 ppm after the conversion;

5) Methyl group signals in the proton spectra were well separated and the determination of the relative stereochemistry of **2** was possible even without the aid of a shifting reagent.