

Supporting Information
for
The myxocoumarins A and B from *Stigmatella*
***aurantiaca* strain MYX-030**

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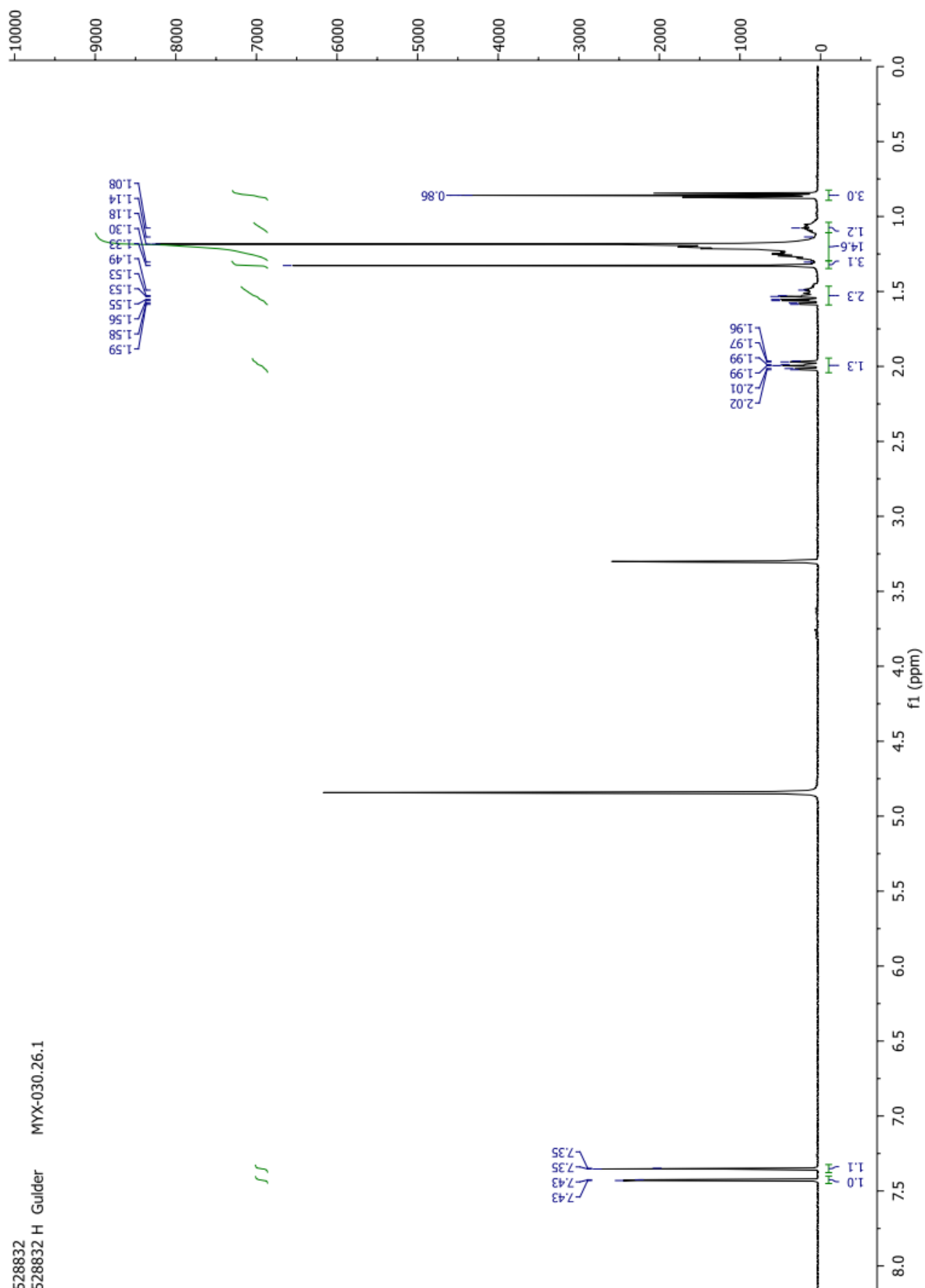
This work is dedicated to Prof. Dr. Gerhard Höfle on the occasion of his 74th birthday.

**NMR and MS spectra of myxocoumarin A (7) and B (9). In-depth
discussion and analysis of chemical shifts for the verification of the
structure of 9**

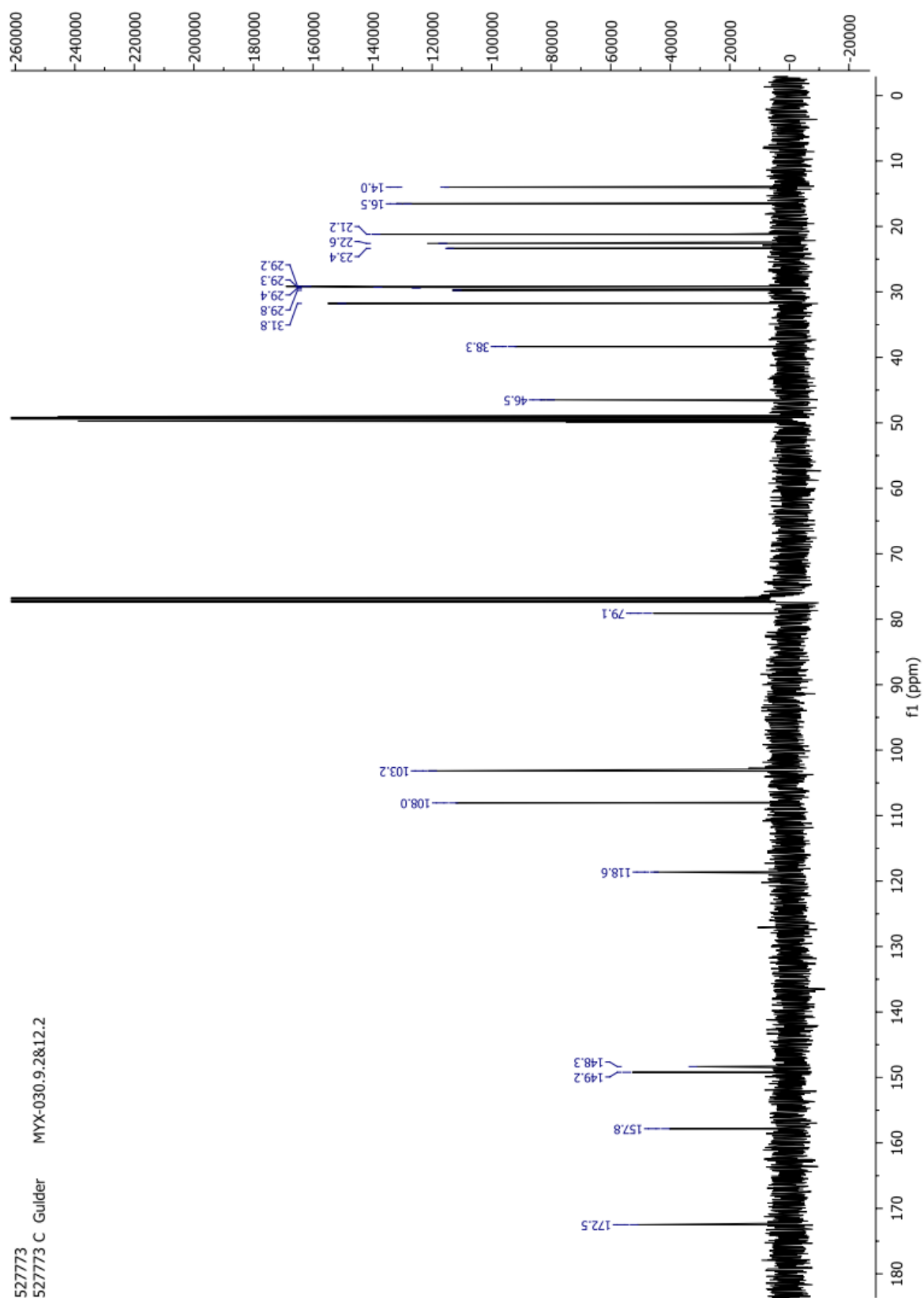
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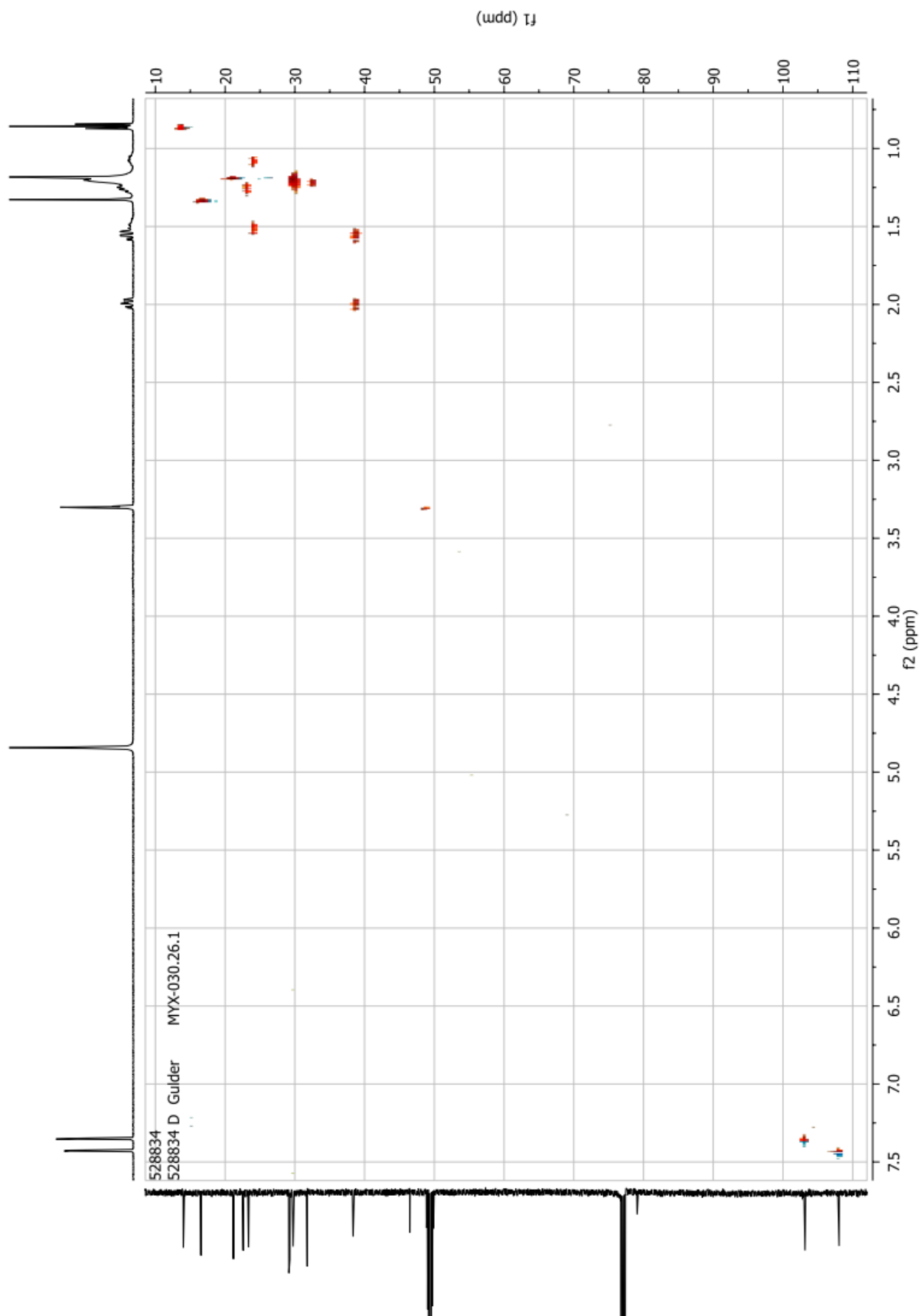
¹H NMR spectrum (CD₃OD) of myxocoumarin A (7)



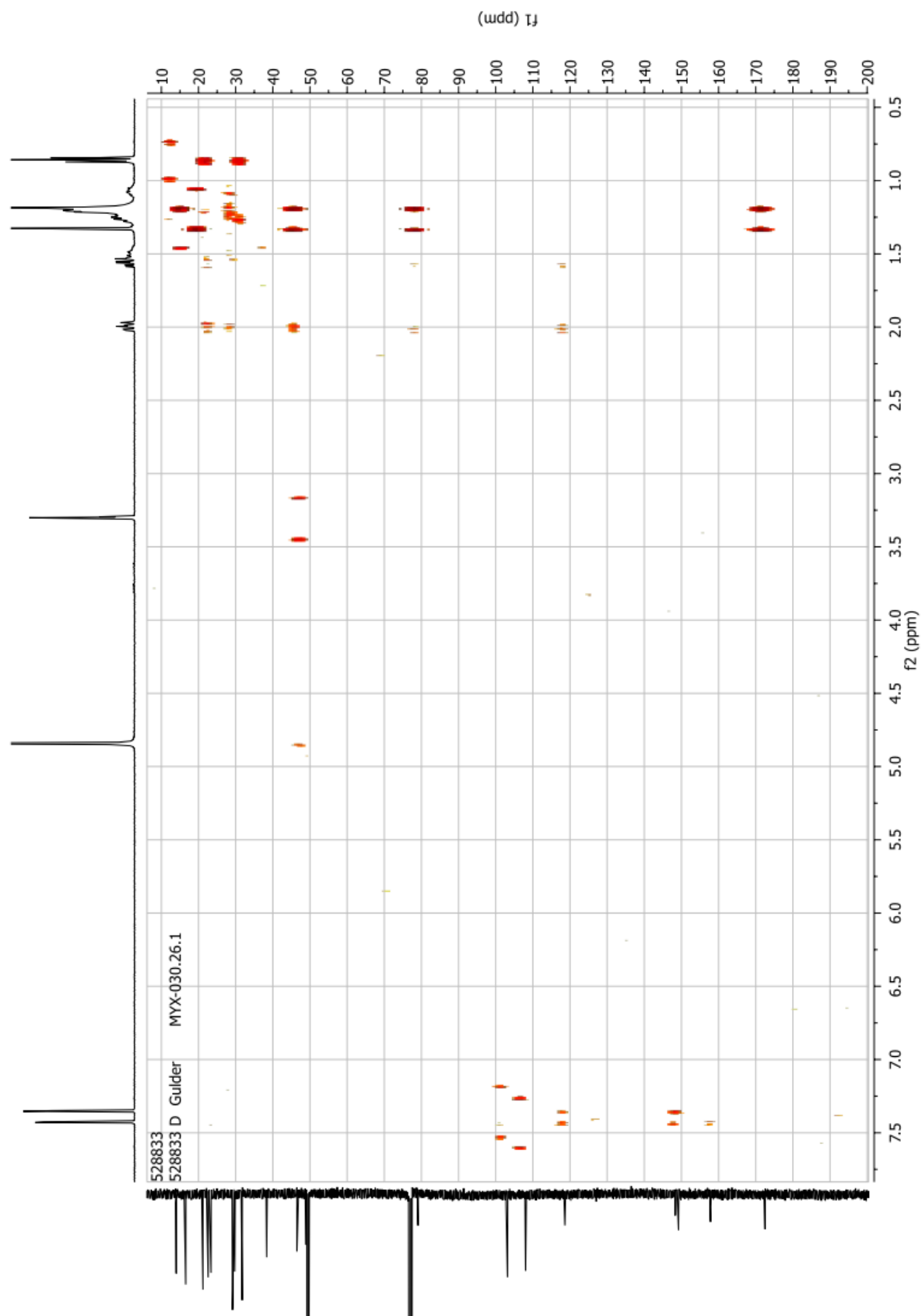
^{13}C NMR spectrum ($\text{CDCl}_3/\text{CD}_3\text{OD}$) of **7**



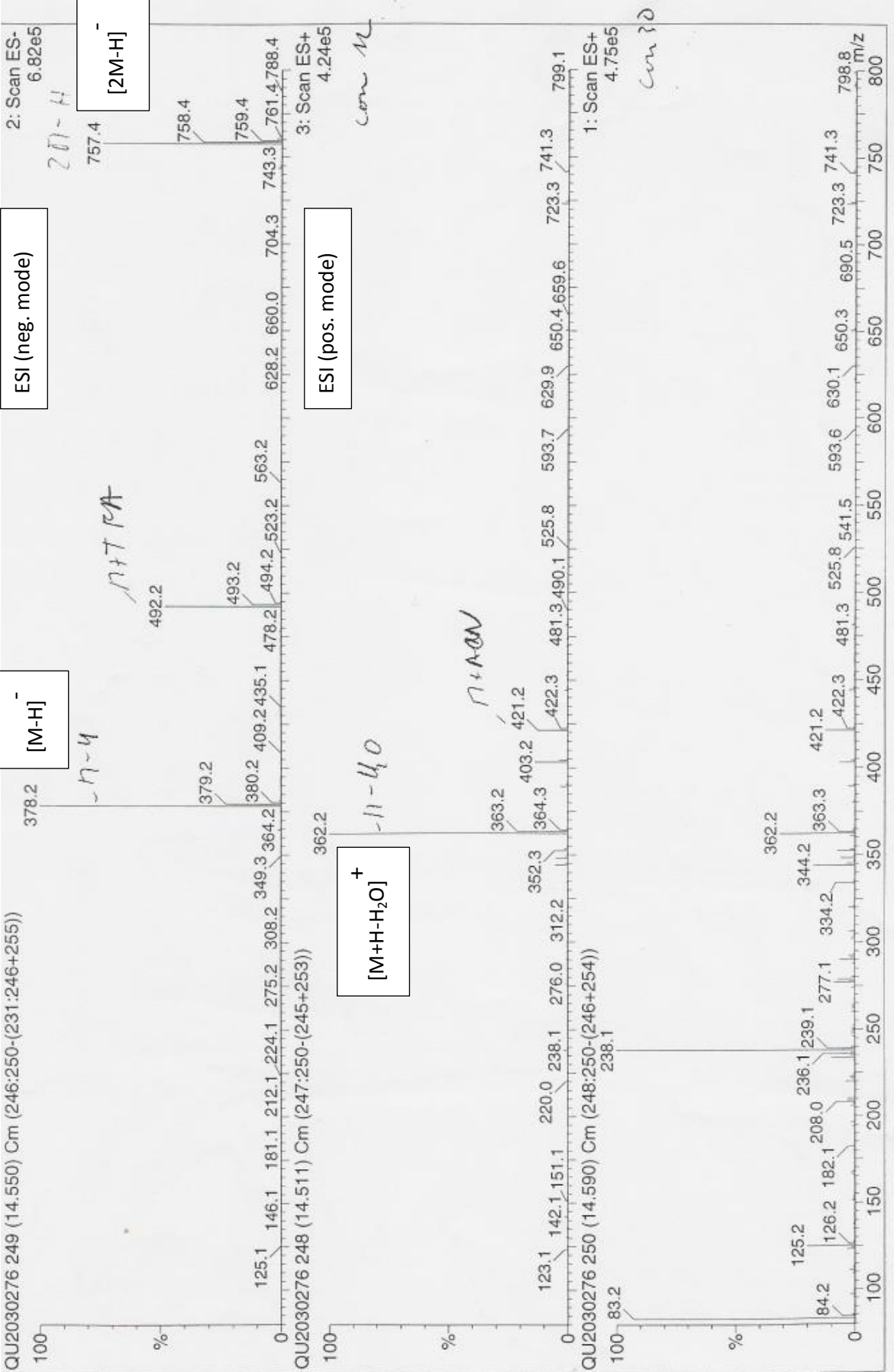
HSQC spectrum (CD₃OD) of 7



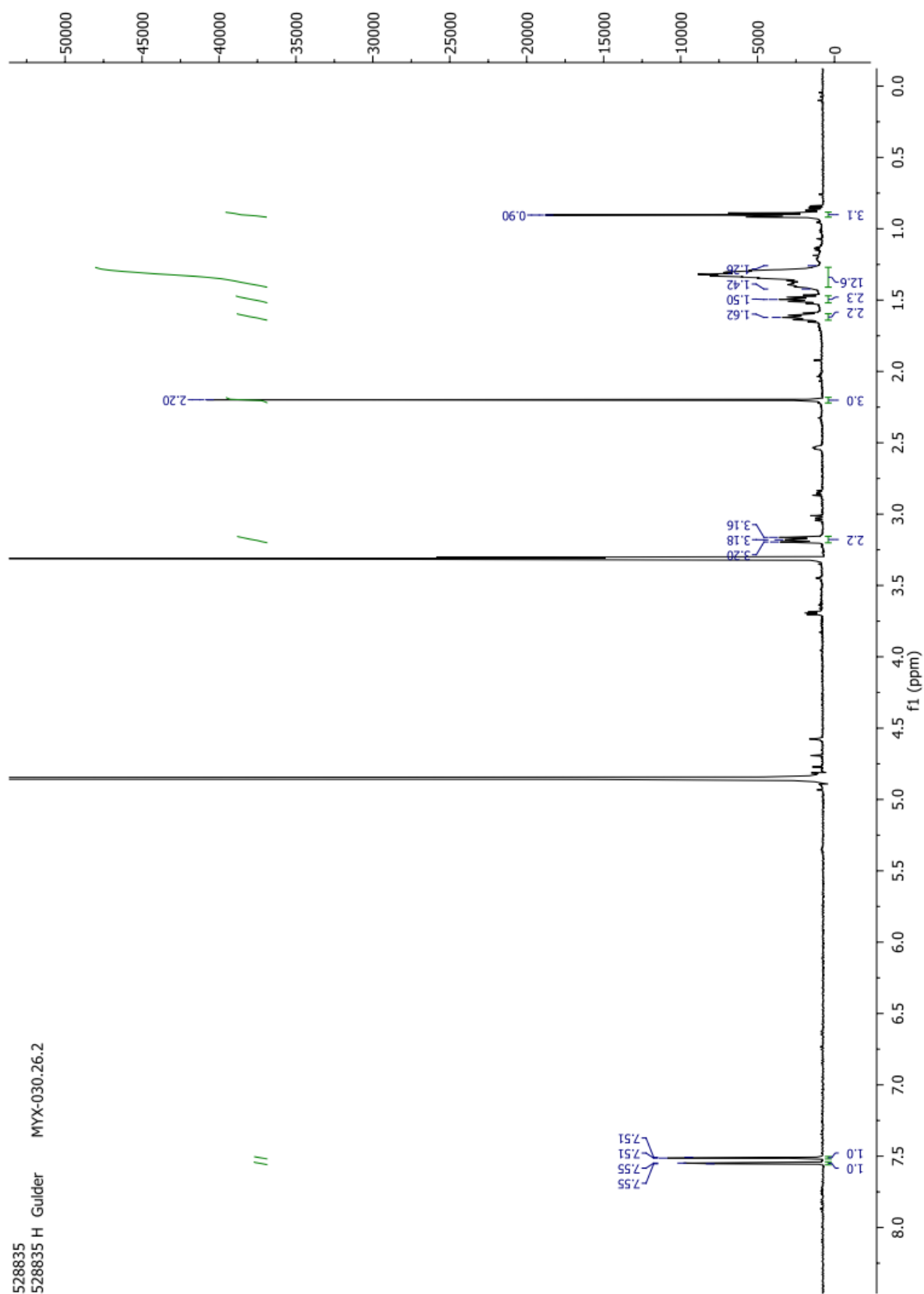
HMBC spectrum (CD₃OD) of 7



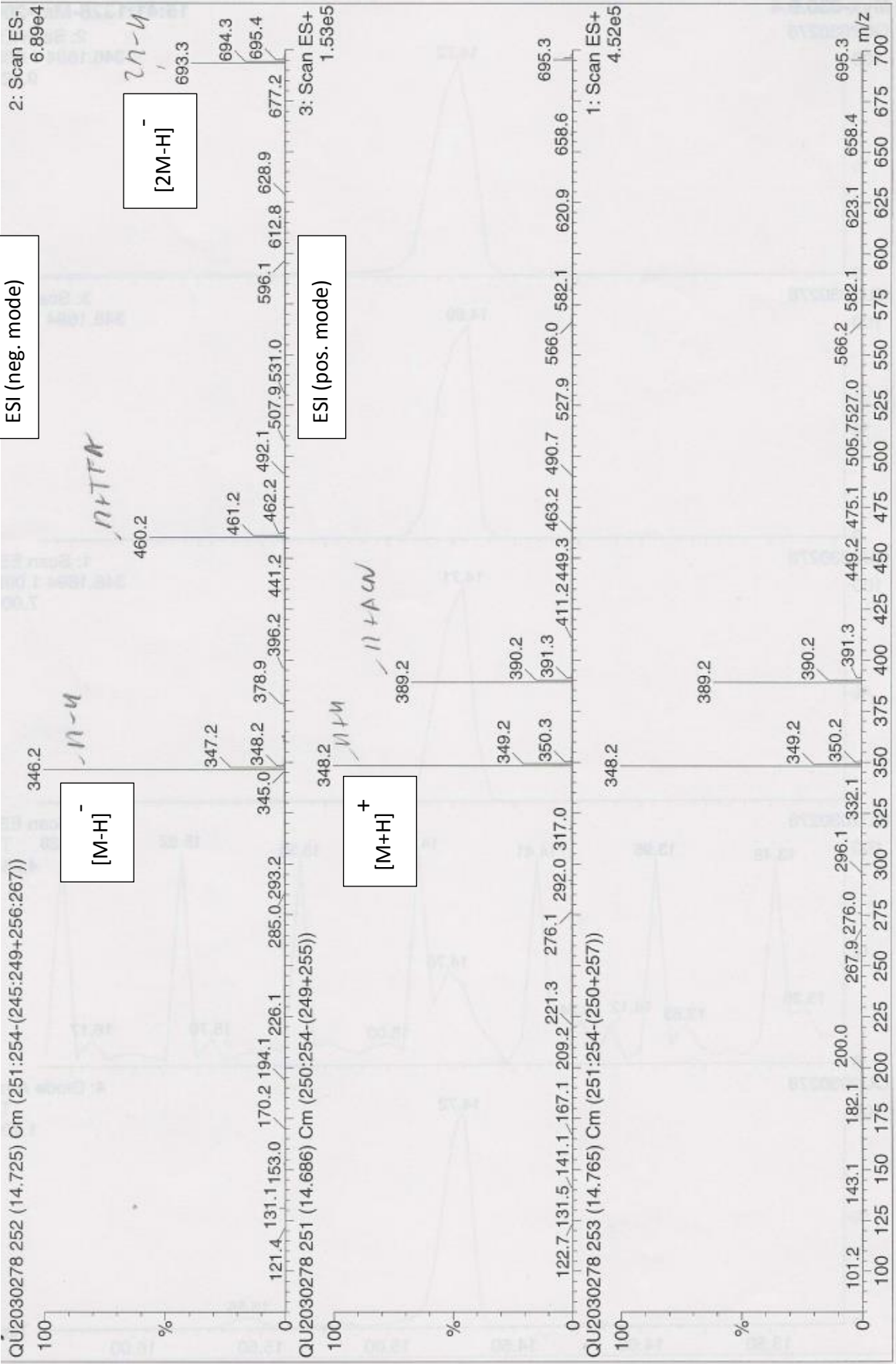
MS spectra of 7



¹H NMR spectrum (CD₃OD) of myxocoumarin B (9)



MS spectra of 9



¹H chemical shift analysis of myxocoumarin B (9)

Since the available amount of **9** did not suffice to obtain high quality ¹³C NMR data, the proposed structure of **9** was further corroborated with ¹H NMR data from the literature as follows:

In a first step, the identity and positions of the two substituents at the heterocyclic ring were ascertained. Since no benzylic coupling between the aromatic protons and the two alkyl substituents was observed, both alkyl groups had to be located at the heterocycle. Literature data [1,2] shows that a methyl group in 3 or 4 position of a coumarin ring appears at 2.2 or 2.4 ppm, respectively, in CDCl₃. Furthermore, a hydroxy group in peri position (at C(5)) to a 4-methyl causes a downfield shift of ca 0.3 ppm of this methyl group, as can be seen by a comparison of such methyl groups in 1-methylnaphthalene (2.65 ppm) [3] and in 8-methyl-1-naphthol (3.00 ppm) [4]. A similar downfield shift is also observed in 4-methyl-5-hydroxycoumarin derivatives [5]. Finally, the chemical shift difference of the benzylic protons in 1-methyl- and 1-ethylnaphthalene [3] (0.43 ppm) as well as 2-methyl- and 2-ethylnaphthalene [3] (0.30 ppm) were used to calculate the influence of the “alkyl chain extension” [6] on the chemical shifts of the protons at the C atoms directly attached to position 3 or 4 of the coumarin system. Using the three increments extracted above from measured reference data, it is possible to predict the chemical shifts of the CH₂ groups at position 3 or 4 of the coumarin system starting from the chemical shifts in 3,4-dimethylcoumarin [1]. The result for the two possible isomers is presented in Figure S1. Compound **9** shows the CH₂ group attached to the heterocycle at 3.18 ppm and a methyl singlet at 2.20 ppm (Table S1).

Table S1: ^1H NMR spectroscopic data of myxocoumarin B (**9**) at 500 MHz in CD_3OD

position	δ_{H} (J in Hz) ^b
6	7.55*, d (2)
8	7.51*, d (2)
9	3.18 m
10	1.62, m
11	1.49, m
12–16	1.26–1.42, m
17	0.90, t (7)
18	2.20, s

*Signal assignment interchangeable

As can be seen in Figure S1, these data are only in agreement with the C_9 alkyl chain in 4-position and the methyl group in 3-position.

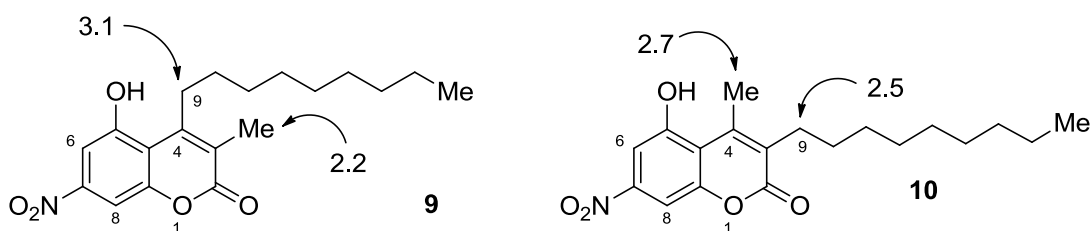


Figure S1: Calculated ^1H NMR chemical shifts [ppm] for the two conceivable myxocoumarin B isomers **9** and **10**.

In a second step, the chemical shifts of the aromatic protons were compared with the ^1H NMR data of the related 5-nitroresorcinol [**7**]: 7.12 (d, $J = 2.1$ Hz, 2H, H-4, H-6), 6.62 (t, $J = 2.1$ Hz, 1H, H-2). The two aromatic protons in **9** absorb at ca. 0.4 ppm lower than H-4 or H-6 in 5-nitroresorcinol, since the upfield shift caused by the acylated O atom is much smaller than that of a hydroxy group (0.1 ppm [2] as compared to ca. 0.6 ppm [8]). The small chemical shift difference between the two aromatic protons (0.05 ppm) shows that the nitro group occupies the 7-position between these two protons, since the difference of the substituent effects of a nitro

group on the chemical shifts of protons in ortho and para position to it is 0.4 ppm, whereas this difference for the hydroxy substituent is only 0.05 ppm [8].

Finally, the chemical shift of the CH₂ group attached to the 4-position (3.18 ppm) proves that the 5-position is substituted (as discussed above). This concludes the assignment of the structure of **9** by ¹H NMR. The resulting substitution pattern given in Figure 3 is thus in full agreement to the structure expected from a joint biosynthetic pathway leading to myxocoumarins A (**7**) and B (**9**) as discussed in the main text of the manuscript.

References

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