2-(Tetrahydrofuran-2-yl)acetic Acid and Ester Derivatives as Long-range Pollinator Attractants in the Sexually Deceptive Orchid *Cryptostylis ovata*

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Supporting Information

Figure S1. ^{1}H NMR spectrum of the isolated compound from *C. ovata* after HPLC purification (d4-MeOH, 1.7 mm probe)

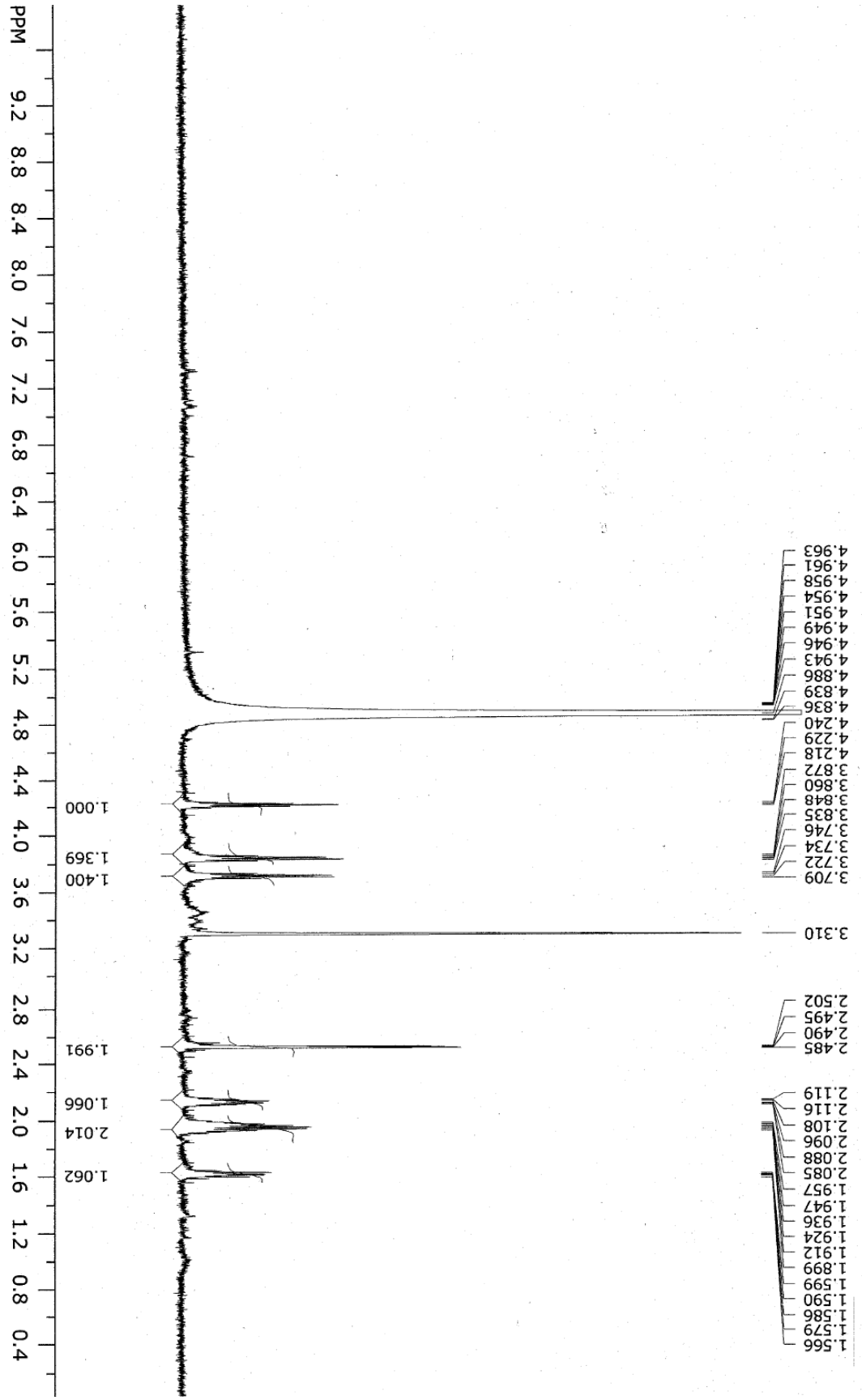


Figure S2. Expansion of the ^{1}H NMR spectrum of the isolated compound from *C. ovata* after HPLC purification (d4-MeOH, 1.7 mm probe)

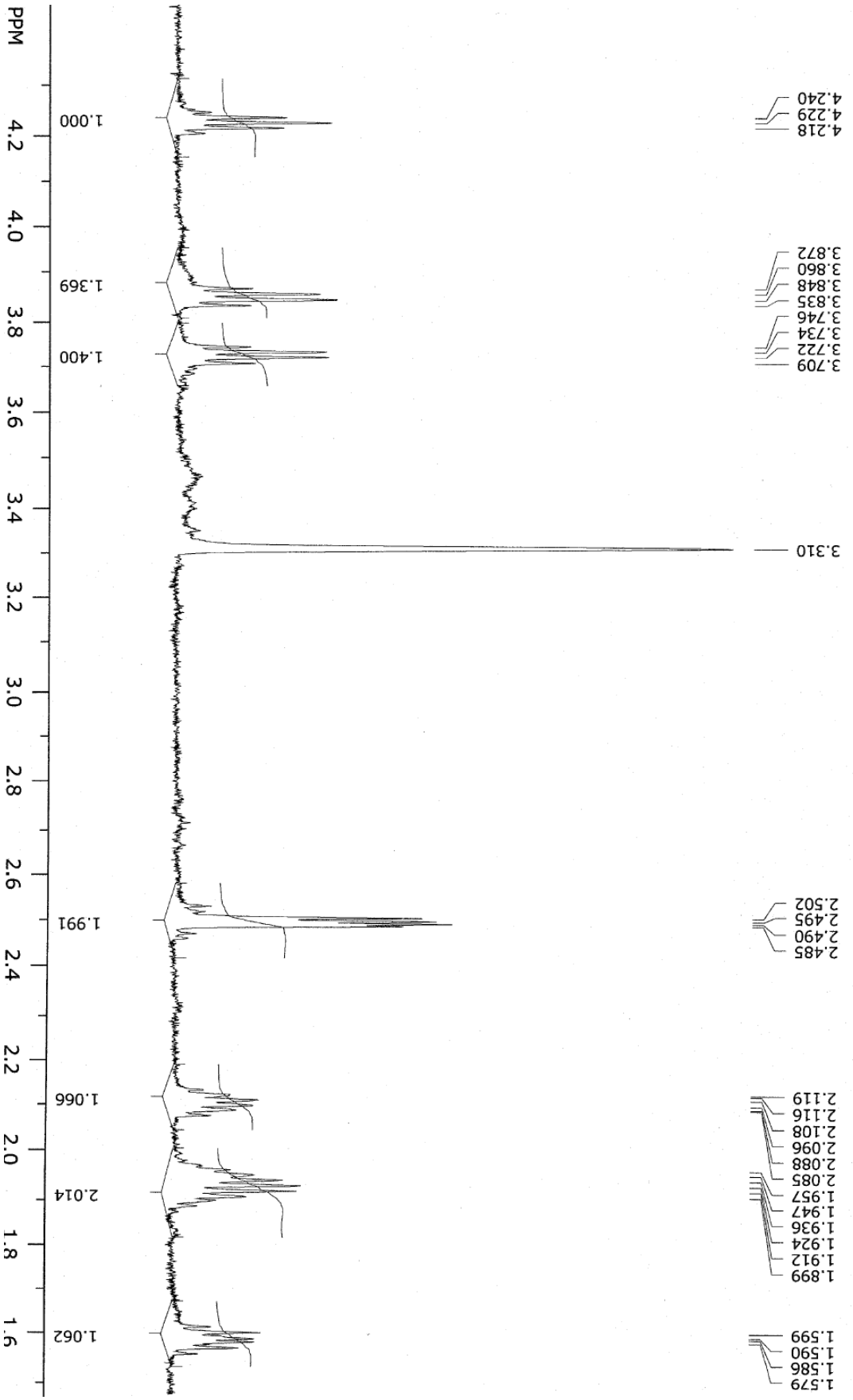


Figure S3. ^{13}C NMR spectrum of the isolated compound from *C. ovata* after HPLC purification (d4-MeOH, 1.7 mm probe)

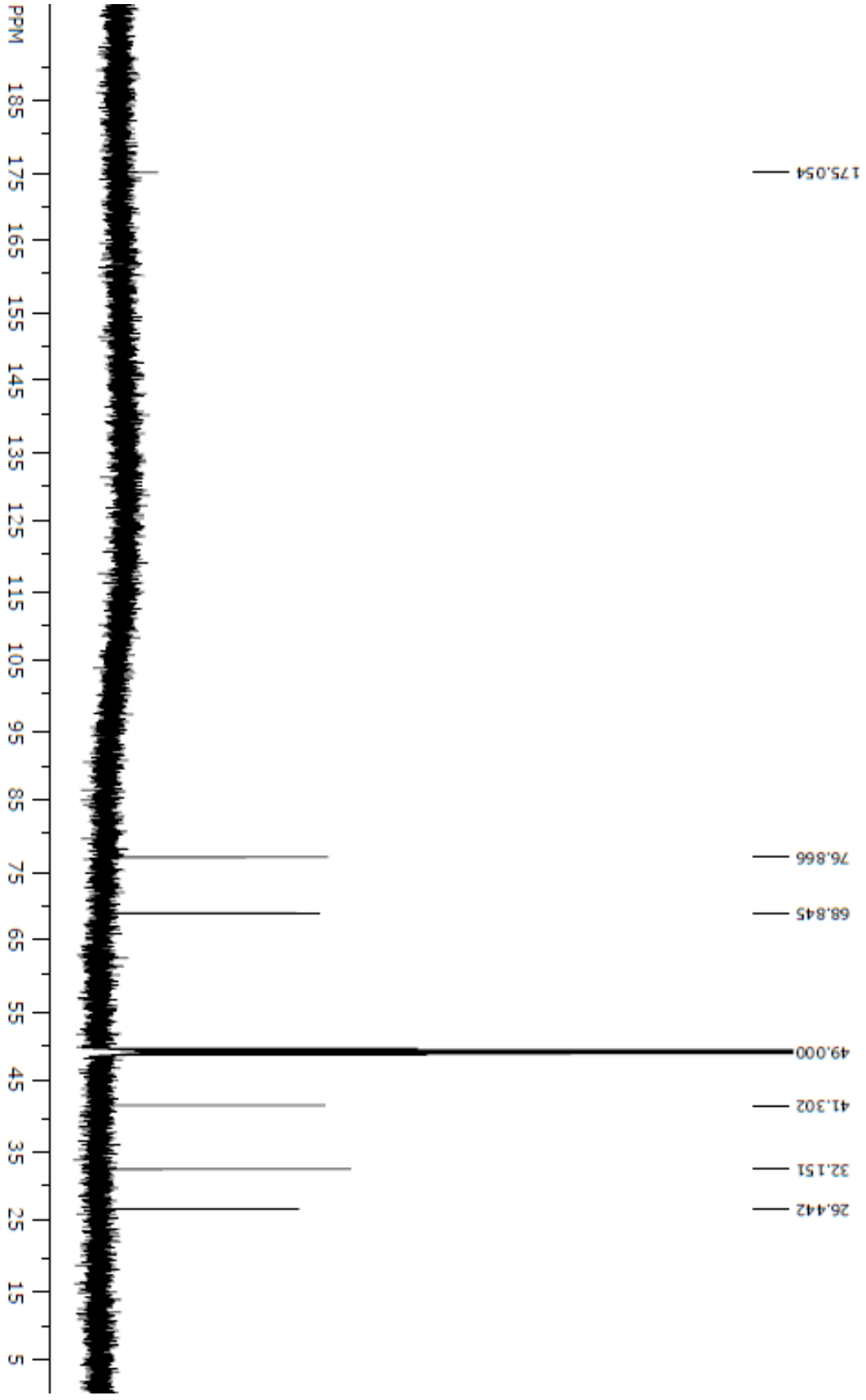


Figure S4. Isolated compound from *C. ovata* after HPLC purification (d4-MeOH, 1.7 mm probe, HSQC)

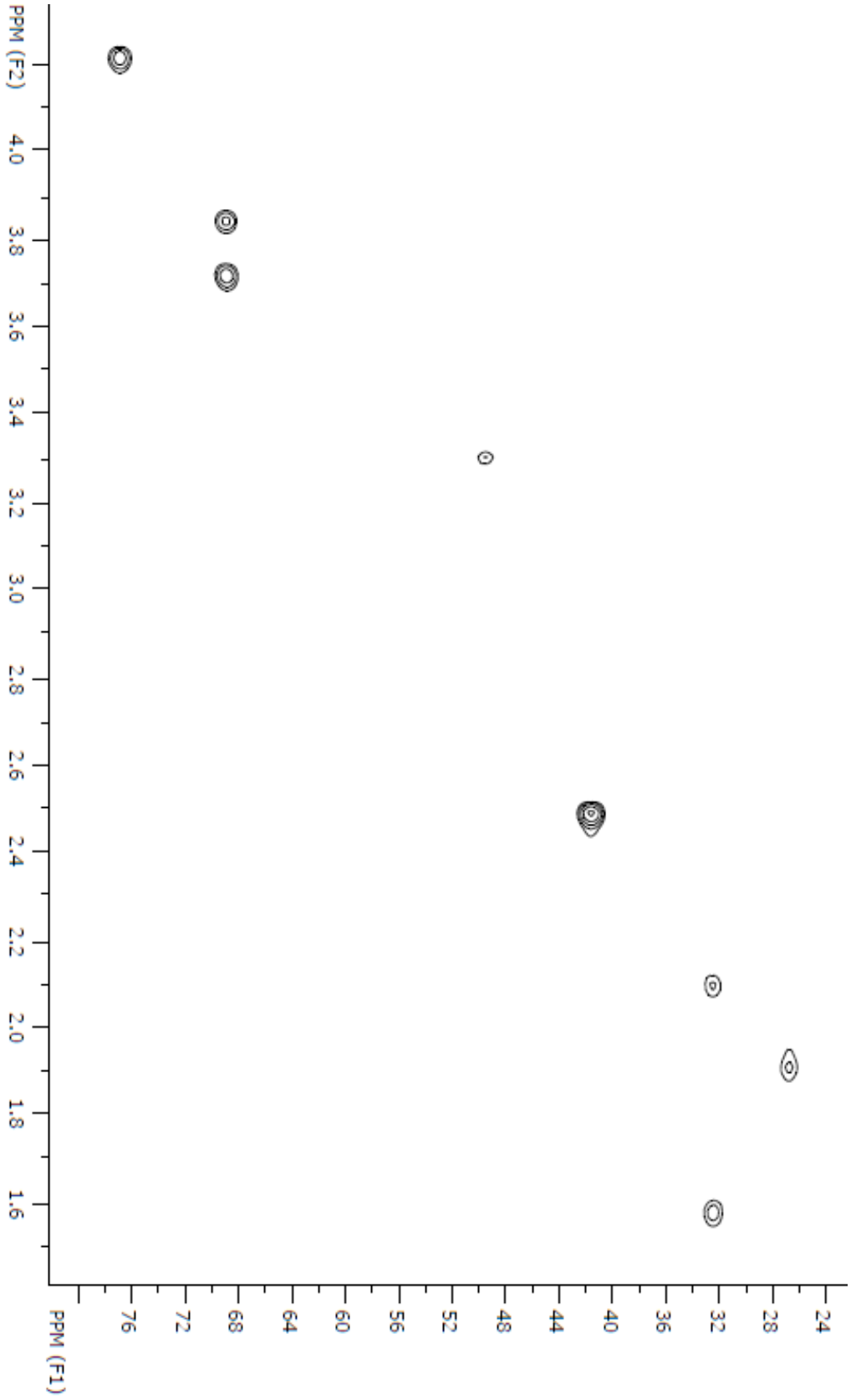


Figure S5. Isolated compound from *C. ovata* after HPLC purification (d4-MeOH,1.7 mm probe, HMBC)

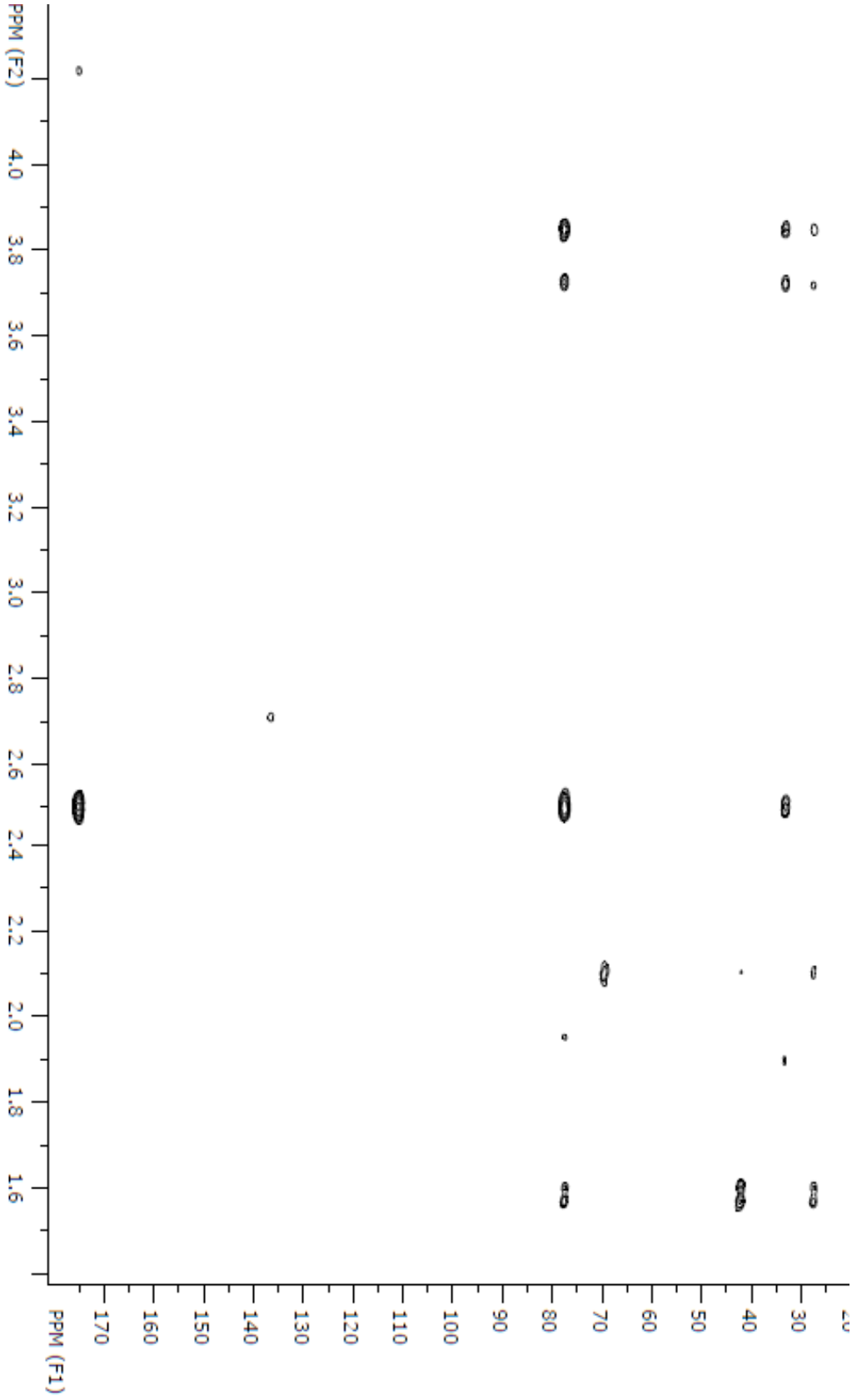


Figure S6. Isolated compound from *C. ovata* after HPLC purification (d4-MeOH, 1.7mm probe, COSY)

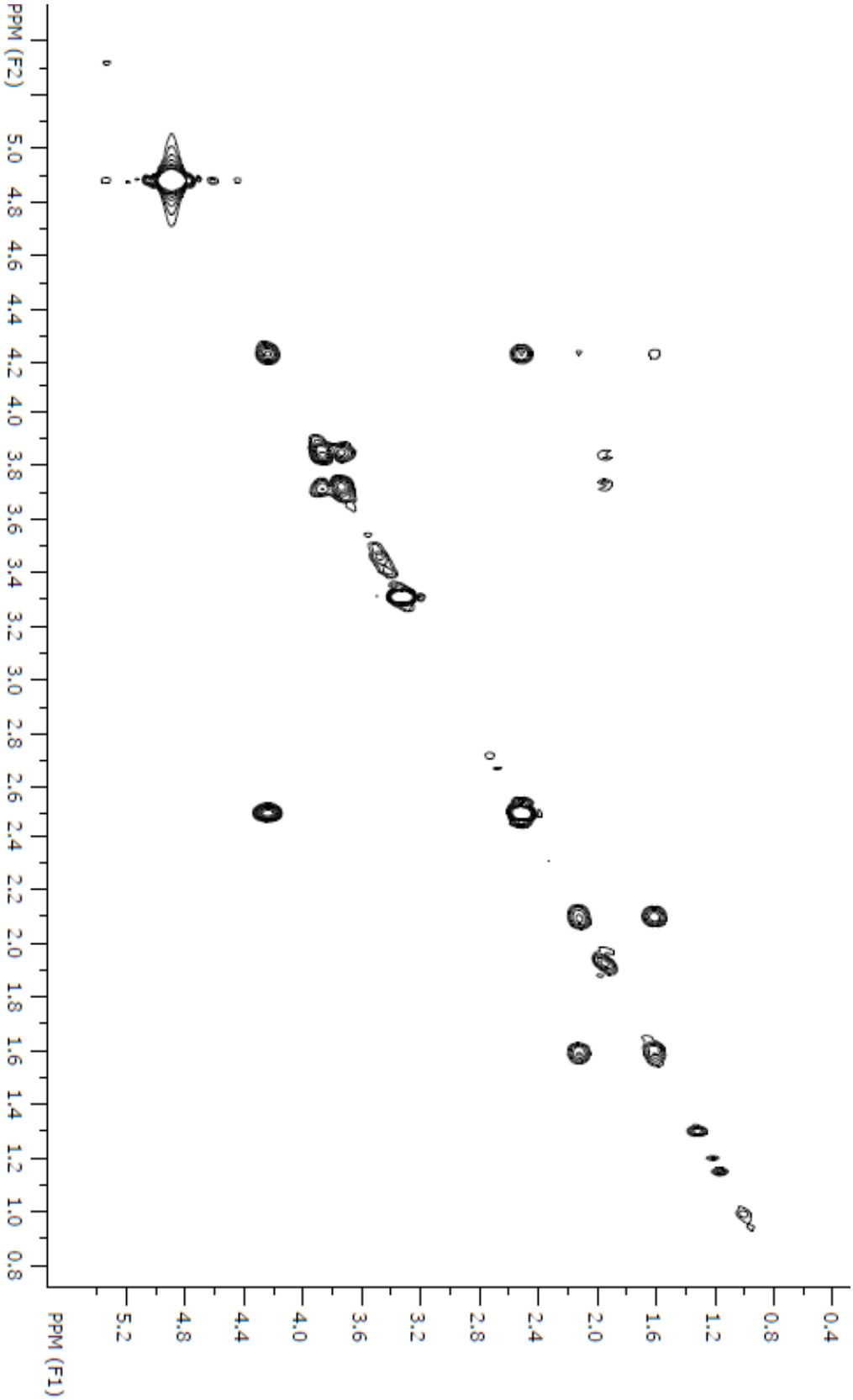
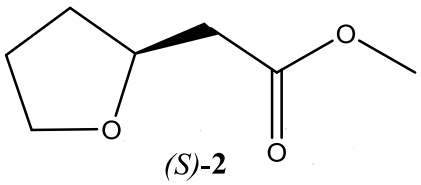


Figure S7. ^{1}H NMR spectrum of (*S*)-**2** ($CDCl_3$)



(S)-**2**

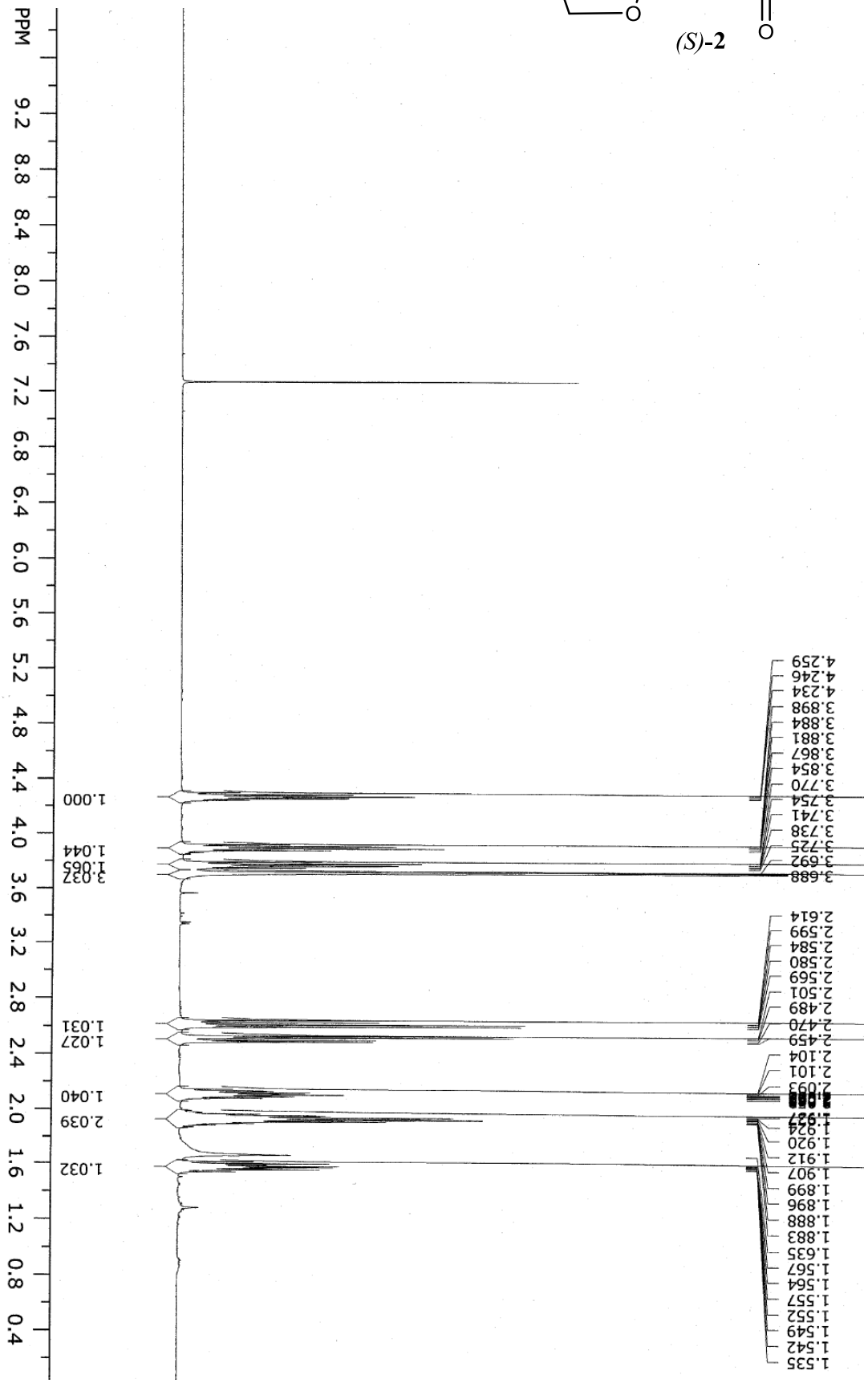
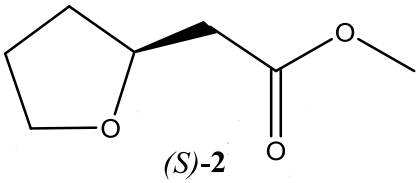


Figure S8. ^{13}C NMR spectrum of (*S*)-**2** ($CDCl_3$)



(S)-**2**

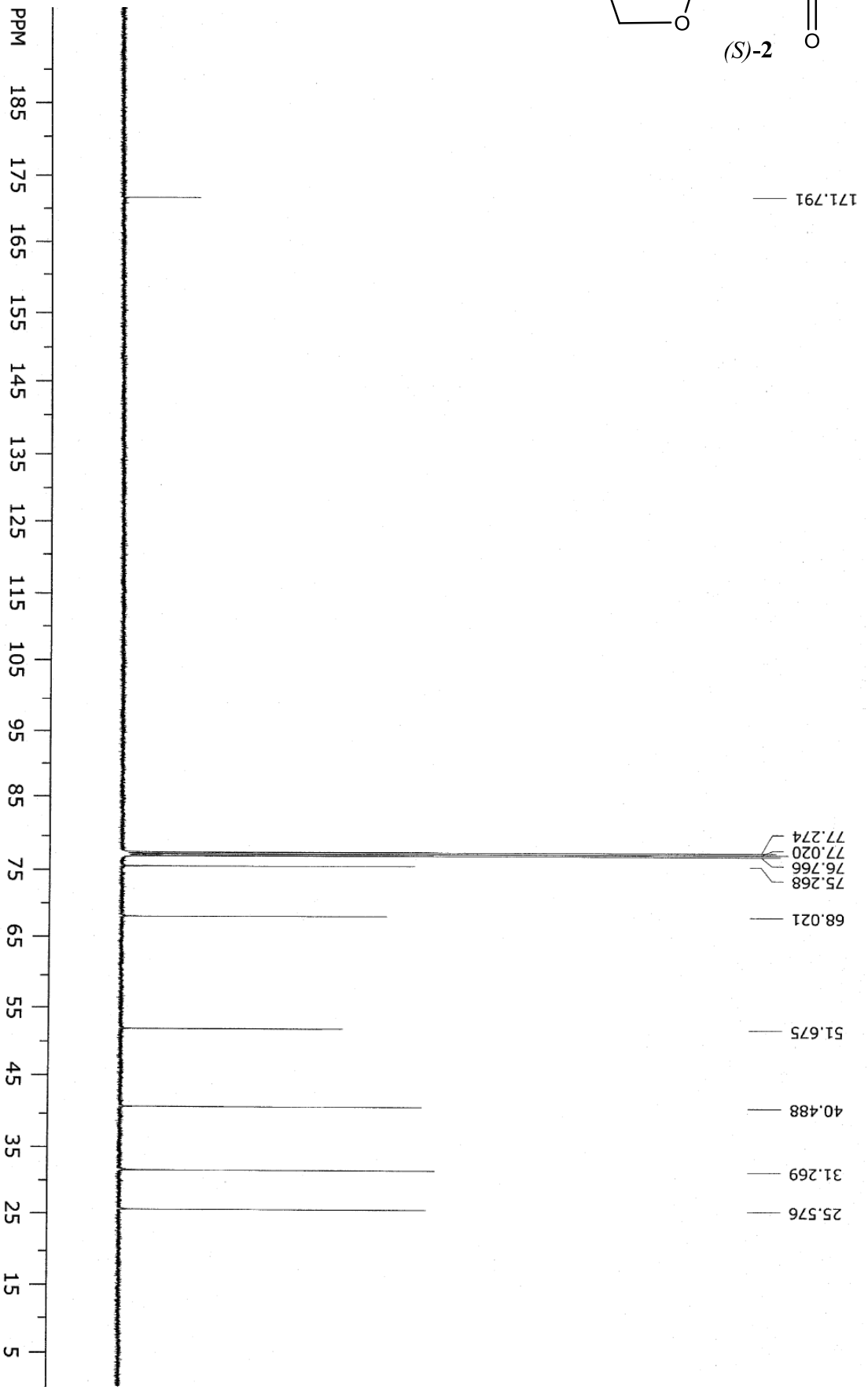
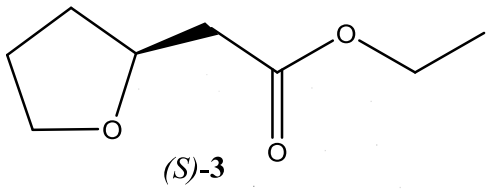
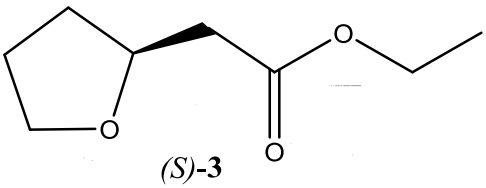


Figure S9. ^{1}H NMR spectrum of (*S*)-**3** ($CDCl_3$)



(S)-**3**

Figure S10. ^{1}H NMR spectrum of (*S*)-**3** ($CDCl_3$)



(S)-**3**

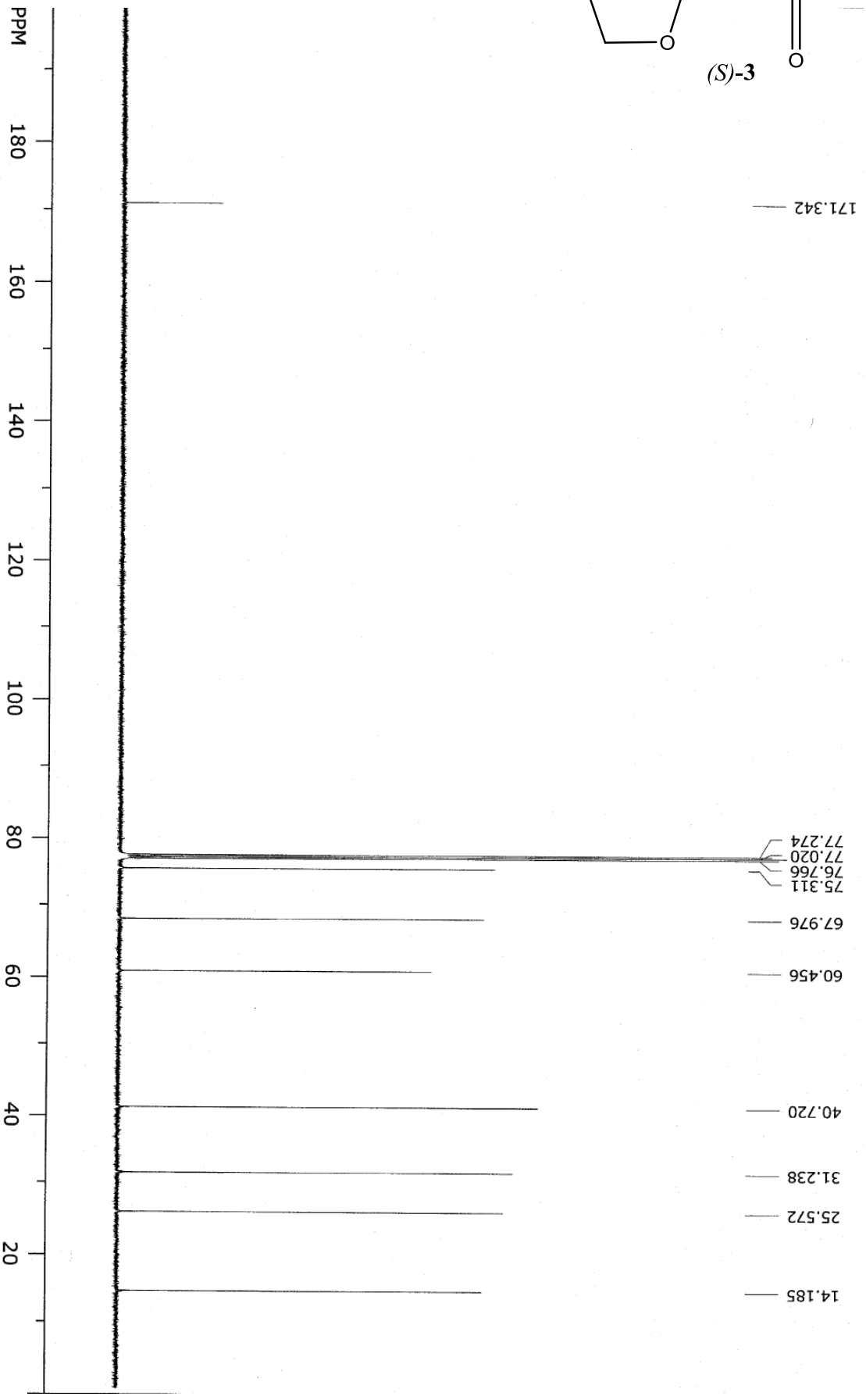


Figure S11.ECD spectra of the two enantiomers of **1**

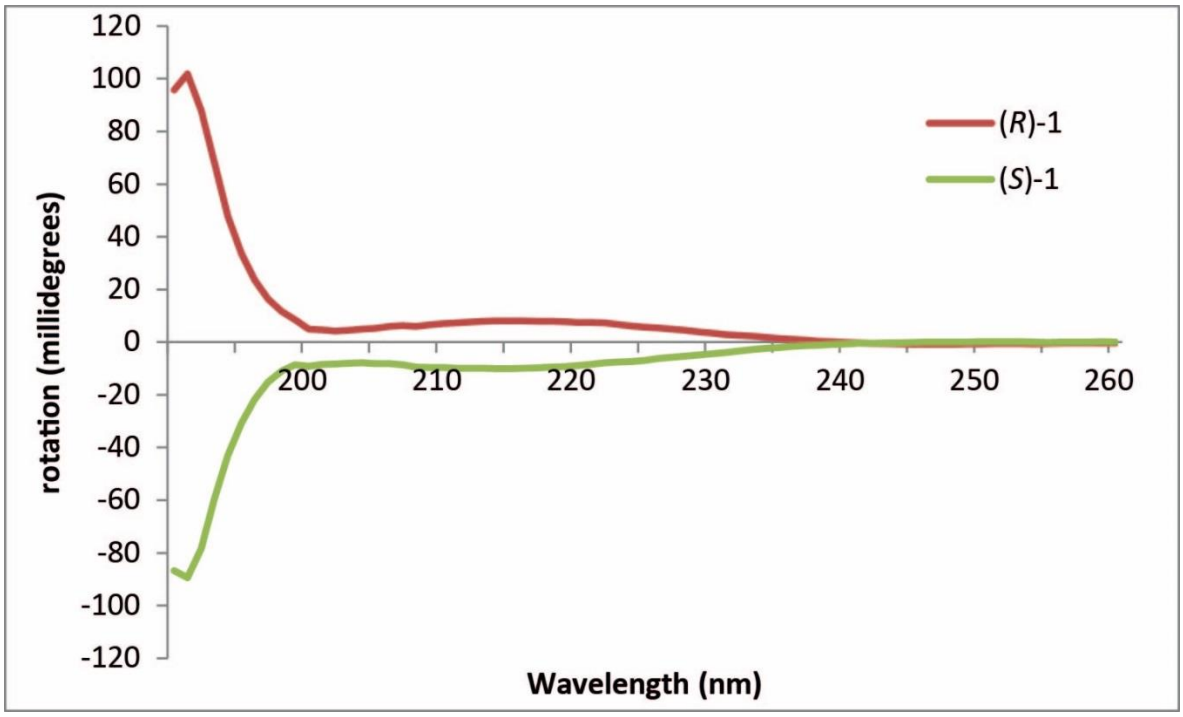
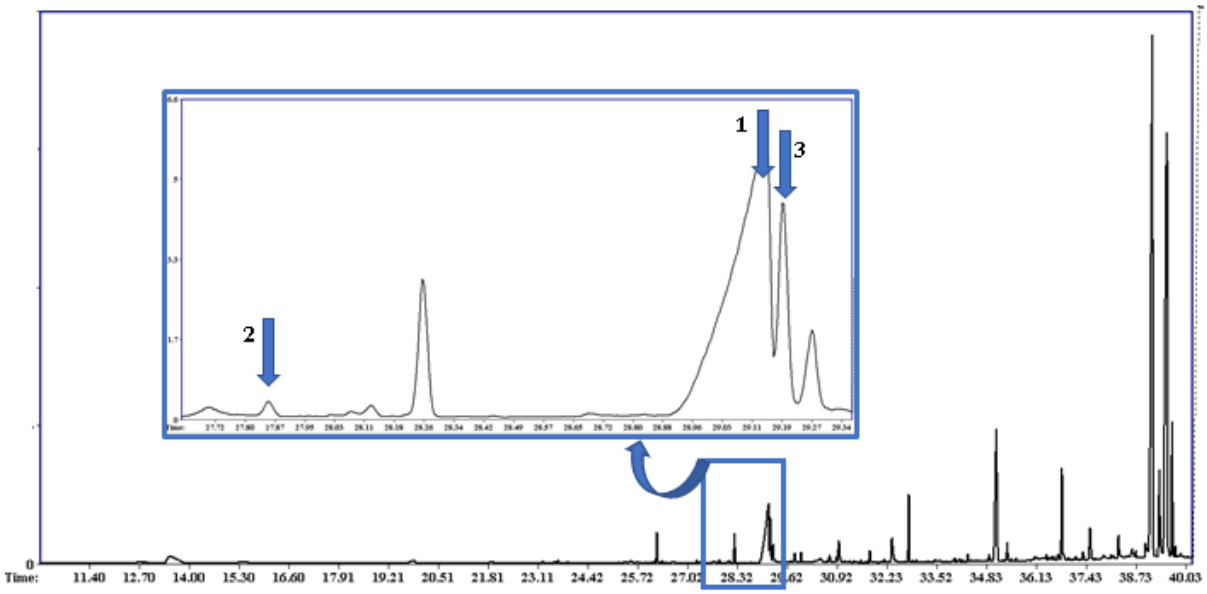


Figure S12. GC-MS total ion chromatograms

a) Trace of *C. ovata* methanol extracts with identified compounds labelled.



b) Enantioselective GC traces showing floral extract (top), synthetic mixture of **1** (middle) and mass spectrum (bottom).

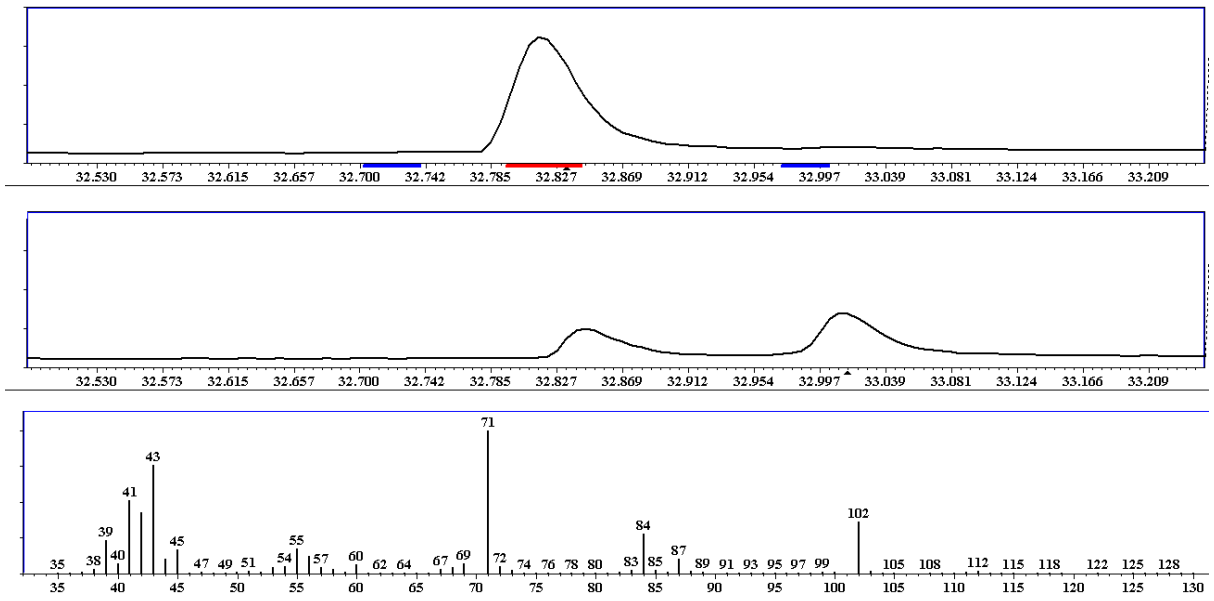


Table S1. Additional bioassay experiments

Bioassays were conducted according to the protocol in the main manuscript.

a) Pollinator attraction to floral extracts in different individual solvents

Cryptostylis ovata flowers (*n*=3) were extracted in 2 mL of solvent for 24 hours, before the extracts were concentrated to ca 100 µL under a gentle stream of nitrogen at roomtemperature. For each solvent (A-D), 10 µL of each extract were suspended on a pin. Apart from the individual solvents, a treatment of a combination of all solvents (A-D), on a single pin, was added (3 µL of each solvent). In total eight trials were conducted over two days.

Solvent	**Approaches**	**Lands**	**Copulations**
Water(A)	68	9	4
Methanol (B)	68	8	1
Dichloromethane (C)	70	6	2
Hexane (D)	7	0	0
Mix of A-D	63	8	3

G-tests revealed no significant difference in proportion of responses observed to the solvent treatments A-C and Mix - no treatment had a greater proportion of lands or copulations.

b) Comparison of individual solvent extracts, *rac*-**1**, and negative control (solvent only)

In additions to comparing different extraction solvents, a direct comparison between solvent extracts (as in **a**), *rac*-**1** (at 50 µg), and pin with solvent (MeOH, 10 µL) only. In total 10 trials were conducted over two days.

Treatment	**Approaches**	**Lands**	**Copulations**
Water(A)	124	15	1
Methanol (B)	156	12	4
Synthetic *rac*-**1** (C)	108	1	0
Blank	3	0	0

c) Dose-response experiment of *rac*-**1** (4 trials) and (*S*)-**1** (5 trials)

Pins were loaded of various amounts of *rac*-**1** and (*S*)-**1** to determine the optimal amount for pollinator attraction.

***Rac*-1**	**Approaches**	**Lands**	**(*S*)-1**	**Approaches**	**Lands**
100 µg	24	1	50 µg	19	0
10 µg	11	0	1 µg	21	1
1 µg	30	0	20 ng	23	0
0.1 µg	32	0	0.4 ng	1	0