INVERTED STEREOCONTROL OF IRIDOID SYNTHASE IN SNAPDRAGON

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Supplemental Data

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Supplemental NMR Data

Supplementary Tables

TABLE S1. **Comparison of ISY binding pockets.***

	% ID	Type	149	246	342	345	346	349	352
CrISY	100	ISY	F	A	F	I	A	S	L
OeISY	72.3	ISY	I	V	F	I	A	L	V
NcISY2	54.5	ISY	V	S	F	T	I	N	R
AmISY	65.6	epiISY	W	W	L	V	V	N	T

*Positions relative to CrISY. Cr: *Catharanthus roseus* (periwinkle, genbank JX974564), Oe: *Olea europaea* (olive, genbank KT954038), Nc: *Nepeta cataria* (catnip, genbank KY882234), Am: *Antirrhinum majus* (snapdragon, genbank Am.18679)

TABLE S2. **Grafting the binding pocket of AmISY on CrISY.***

construct	mutations	*R*-citronellal (%)
CrISY	-	n.d.
CrISY-R1	A246W, F342L	0.97
	CrISY-R1 plus:	
CrISY-R4	F149W	7.27
CrISY-R5	S349N, L352T	1.32
CrISY-R6	I345V, A346V	17.08
CrISY-R7	F149W, I345V, A346V, S349N, L352T	3.42
AmISY	-	88.90

*The fraction of *R*-citronellal is given for reactions with (2*E*)-geranial. Residue numbering is relative to CrISY. N.d.: not detectable.

TABLE S3. **Oligonucleotides for cloning and qRT-PCR.**

Name	**Sequence (5' to 3')**
55_f	ATGAGCTGGTGGTGGGC
55_r	TCAAGGAACTATCTTGTAAGCCTTCACT
66_f	ATGGGCTCCATTGATGC
66_r	TCATGGGATAAATTTCTCGGCTC
79_f	ATGAGCTGGTGGTATAGAAGA
79_r	TTAAGGAATAAACTTGAAATCTCTCATTTTGTTAATTG
85_f	ATGGCGAGCTGGTGGTGG
85_r	TCATGGAACAATTTTGTGAGC
qRT_55_f	CCGTTTAGCATGATGAATTTGGTG
qRT_55_r	GCCTTAGAACCTGGAAACCT
qRT_66_f	TCATAATGGGTTGTTCAAAGAAAACG
qRT_66_r	CCAAACACGAAAGGGAGATTCA
qRT_79_f	CCTTGTAGTATGATGAACACTGTCA
qRT_79_r	GACGTTTCAGTTCCGGTATACAC
qRT_85_f	TAGCATGATGAACATGATGGACTC
qRT_85_r	TAGCAGTTCCAGGAGGCT

Supplementary Figures

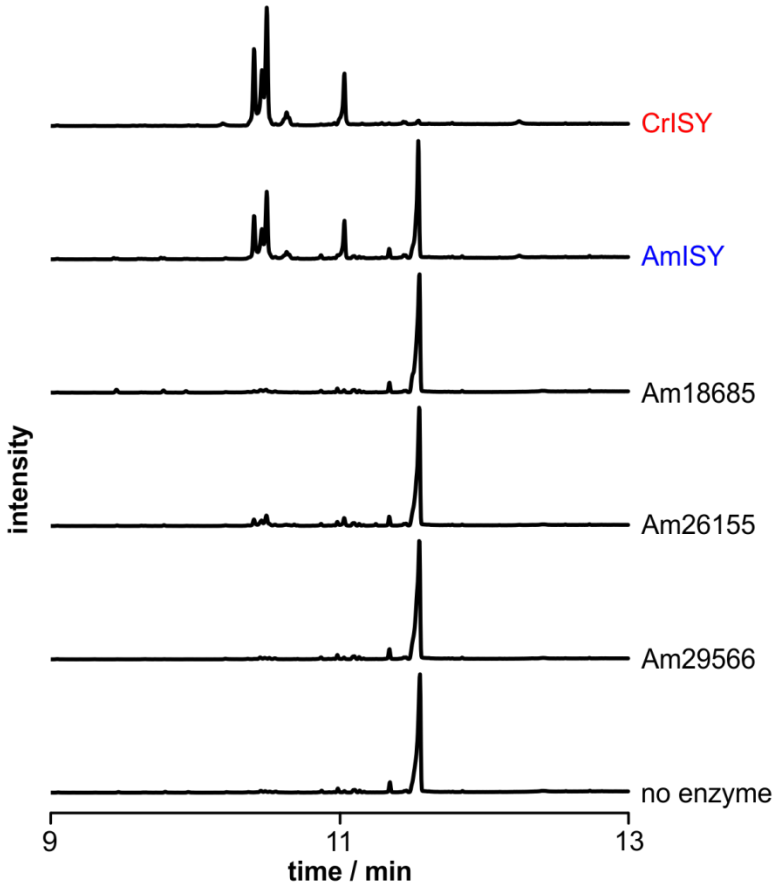


FIGURE S1. **GC-MS of AmISY candidates compared to CrISY.** Although conversion is not complete, candidate Am18679 (AmISY) is the only enzyme showing a product profile similar to CrISY. The other candidates generate very little (Am26155) or no product.

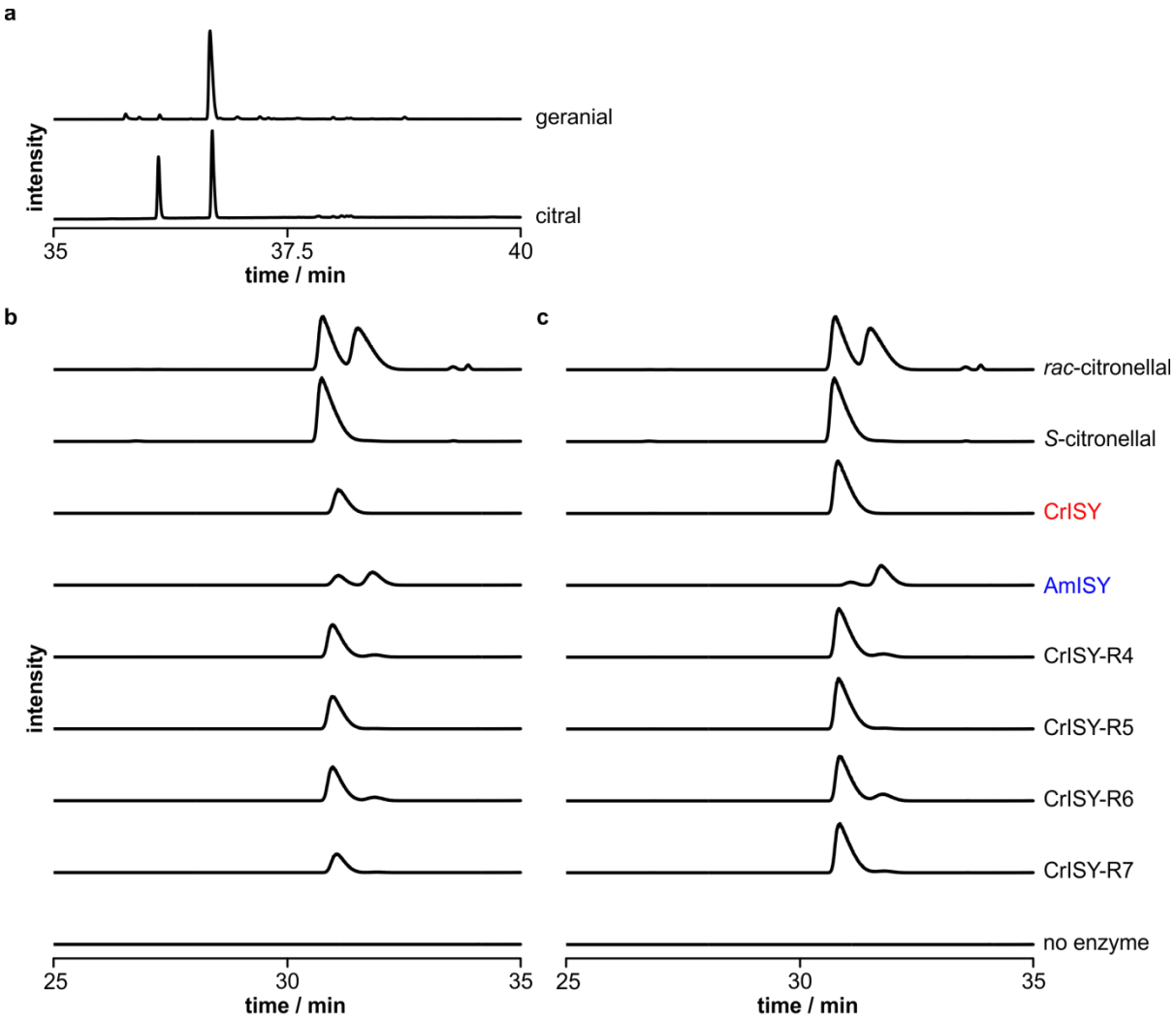
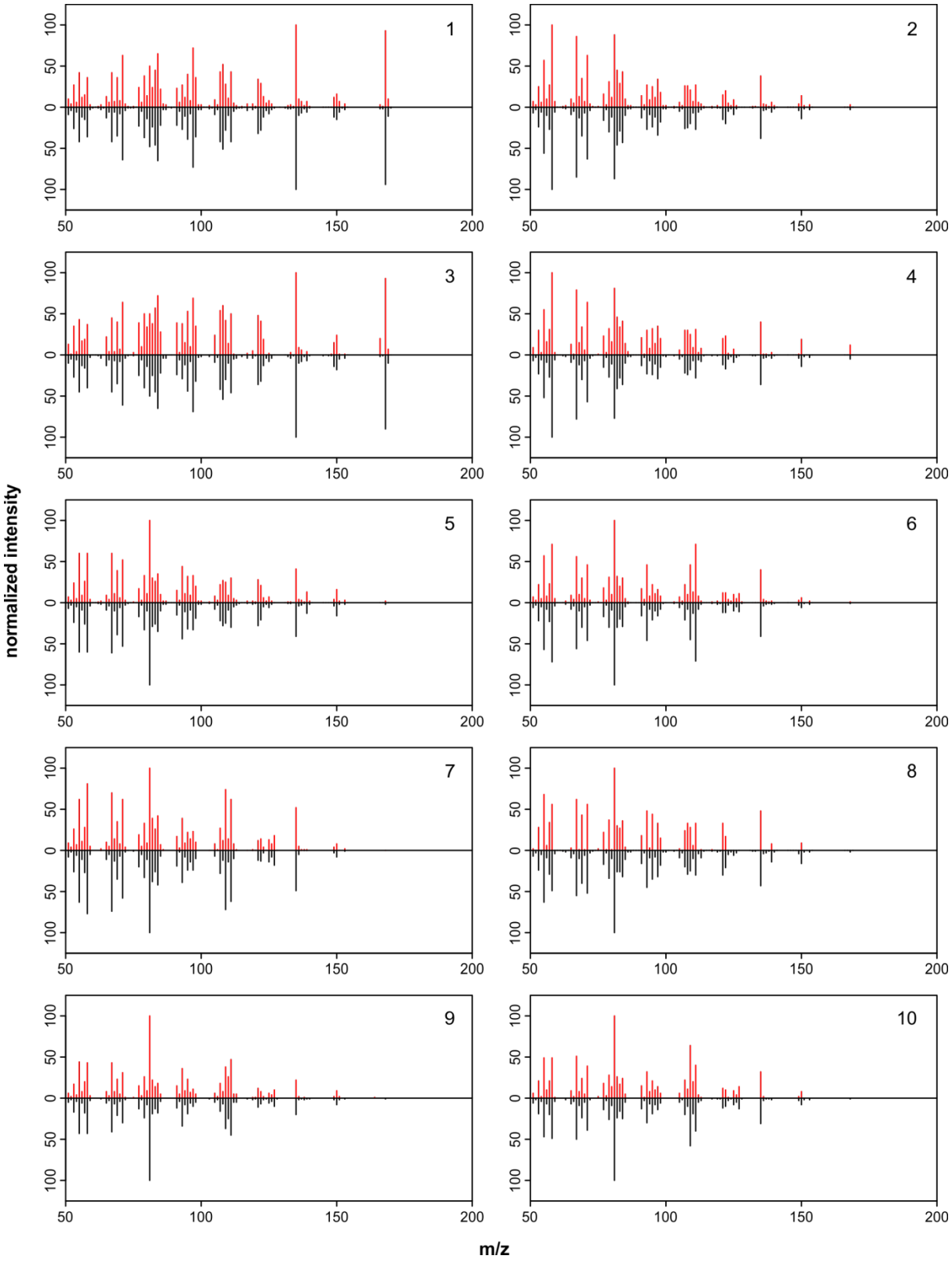


FIGURE S2. **GC-MS analysis of citral and geranial conversion.** a) In comparison to commercial citral (bottom), which is a mixture of geranial and neral, geranial (t_R= 36.675 min) obtained by careful oxidation of geraniol contains smaller quantities of the *2Z* configured neral isomer (2.5%, t_R= 36.138 min). Citral (a) and geranial (b) were reduced to citronellal with CrISY, AmISY and CrISY mutants R1-R4. a) Enzymatic conversion of citral shows stereoconvergence with CrISY (100% *S*-citronellal) and a mixture of *R*- and *S*-citronellal with AmISY (61% *R*- and 39% *S*-citronellal). b) Geranial afforded similar product profiles with most enzymes but with AmISY, the fraction of *R*-citronellal was increased to 89%. Among the CrISY-R mutants, the highest fraction of *R*-citronellal was obtained with CrISY-R6 (17% *R*-citronellal; A246W, F342L, I345V, A346V). Chromatograms in a) are scaled to the largest peak, whereas chromatograms in b) and c) are all scaled to the same intensity.



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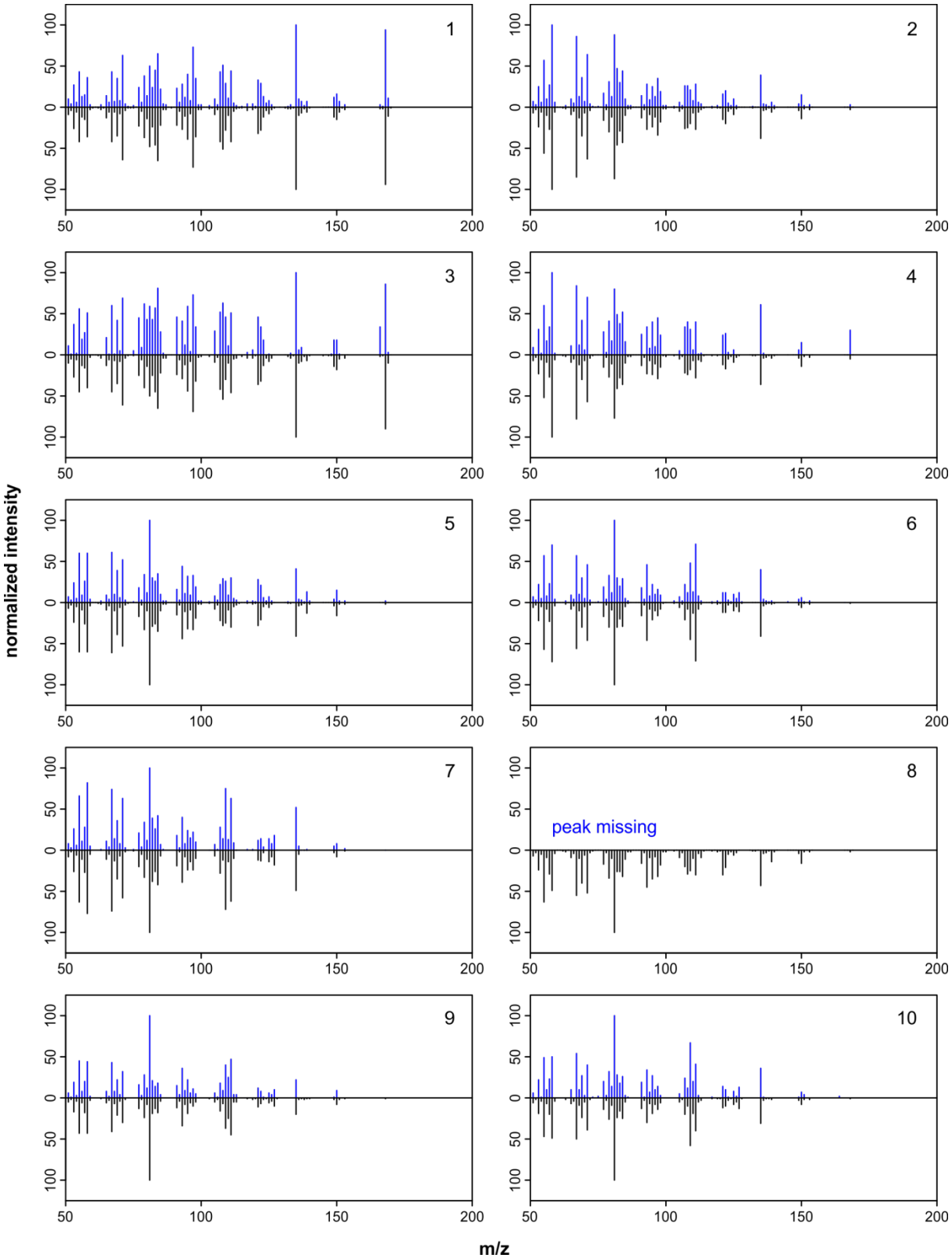
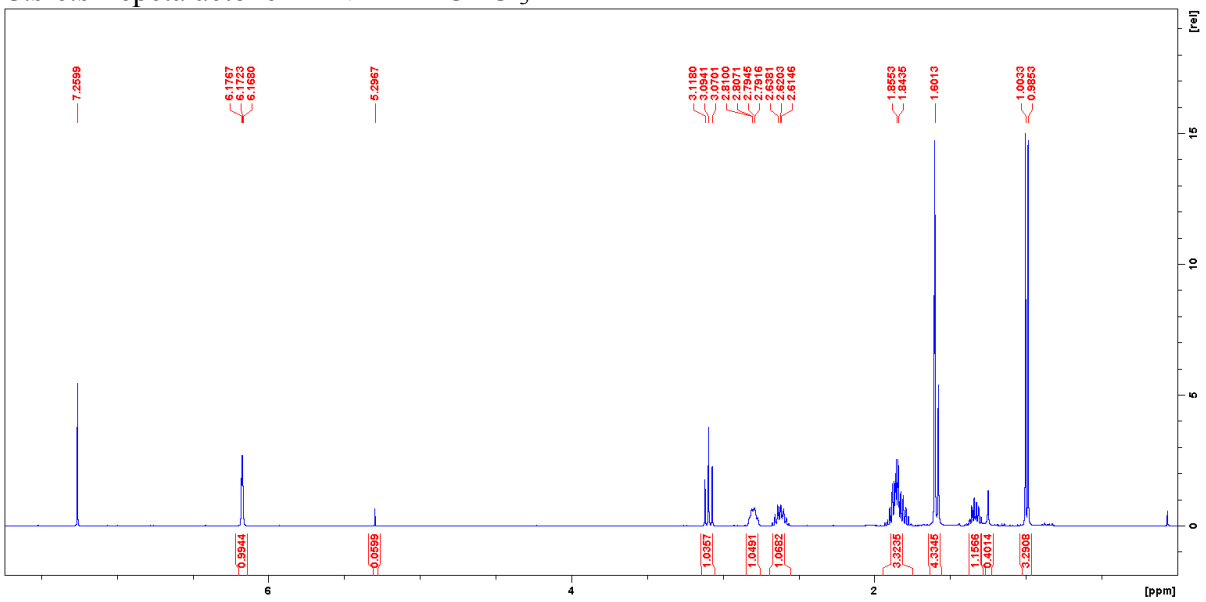


FIGURE S3. **GC-MS spectra of CrISY (red) and AmISY (blue) products compared to authentic standards (black).** Peak numbering (upper right corner) refers to **Fig. 3**.Spectra were calculated in AMDIS32 by manually integrating all scans belonging to one peak and subtracting

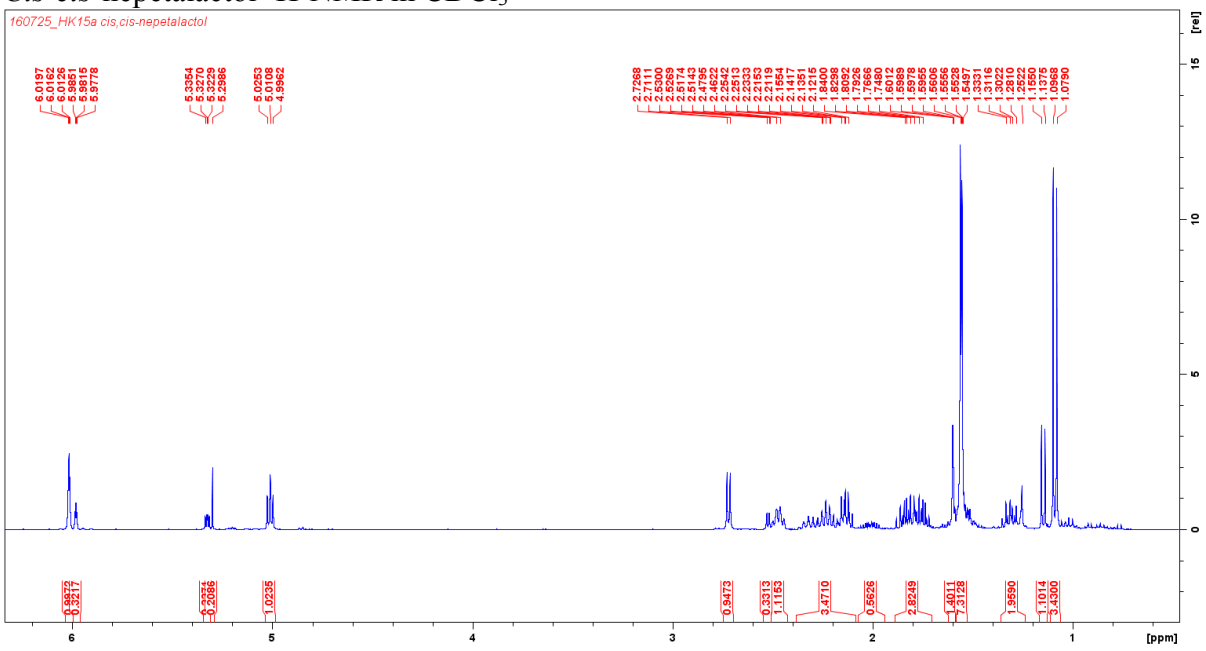
background signal before or after the peak. Compound 8' is missing in the AmISY chromatogram. Since AmISY products are enantiomers of the CrISY products, retention times on the chiral column (**Fig. 3**) are different, but the spectra are identical to those of the standards with opposite chirality.

NMR spectra

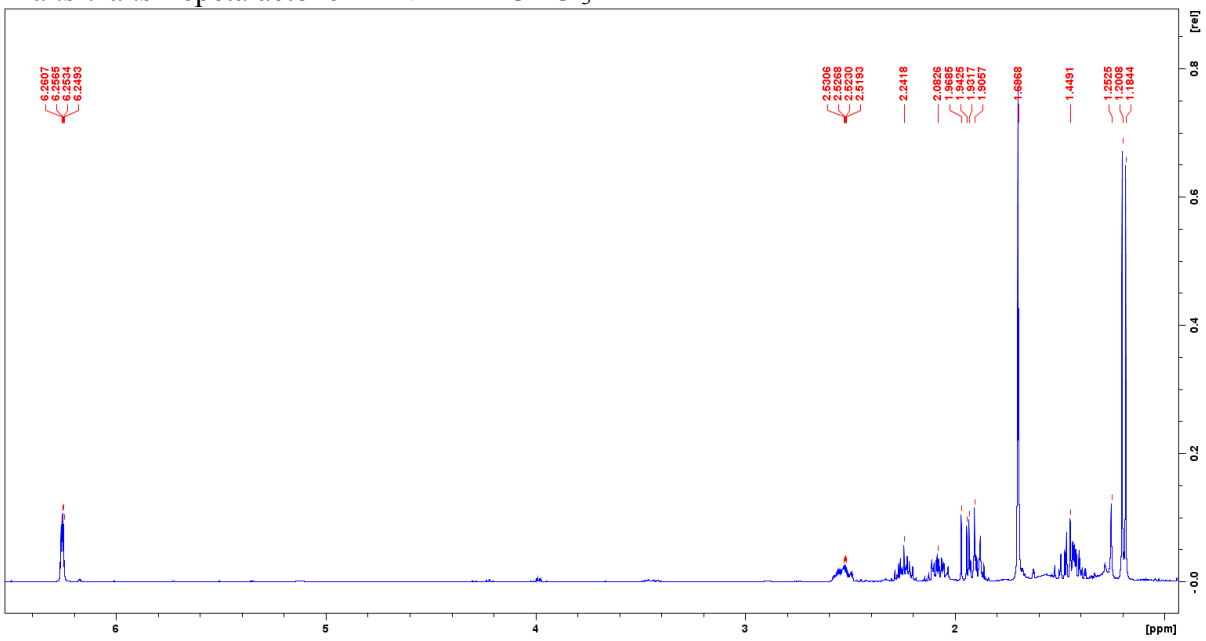
Cis-cis-nepetalactone ^{1}H-NMR in $CDCl_3$



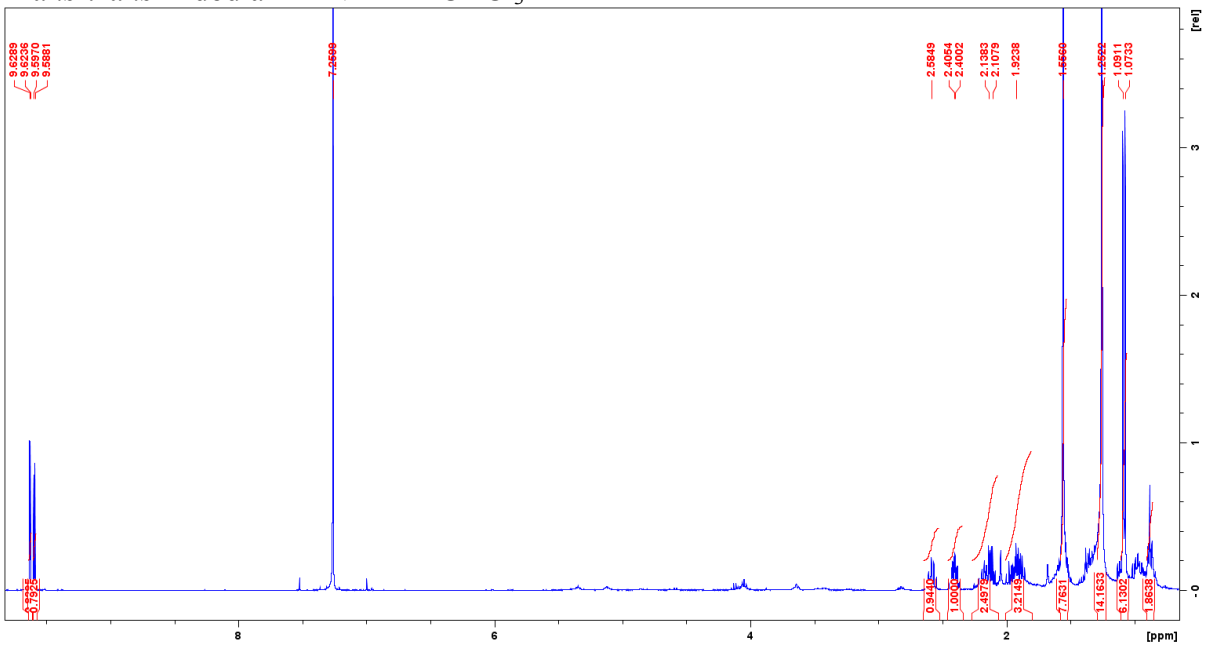
Cis-cis-nepetalactol ^{1}H-NMR in $CDCl_3$



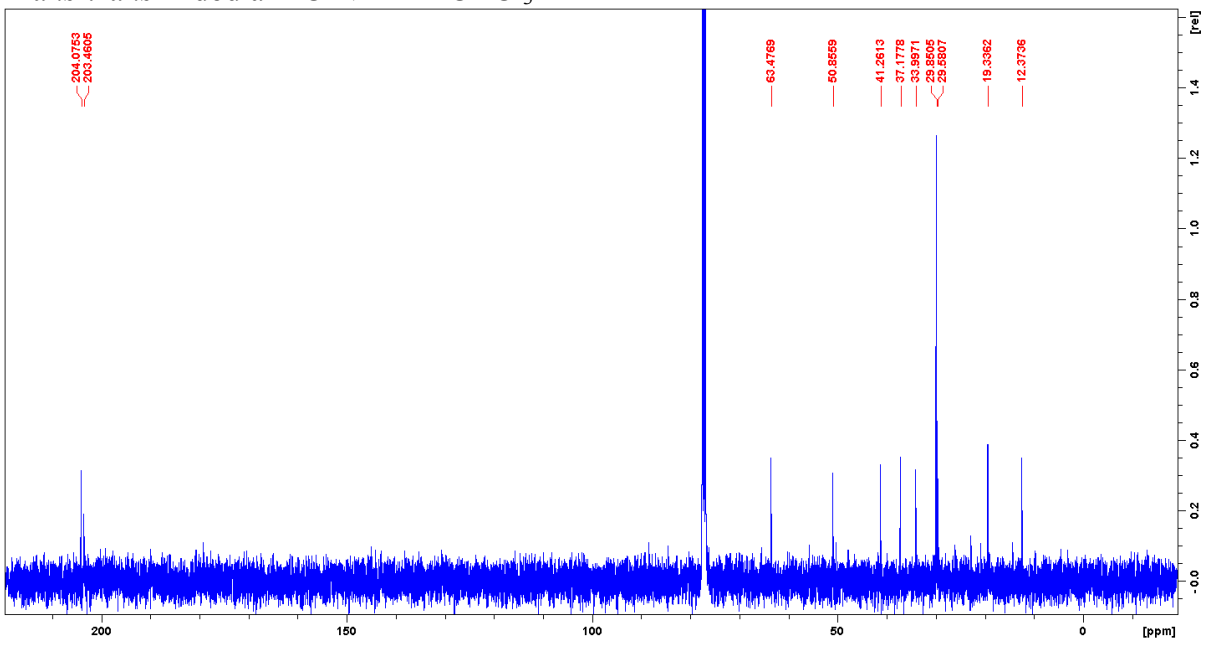
Trans-trans-nepetalactone ^{1}H-NMR in $CDCl_3$



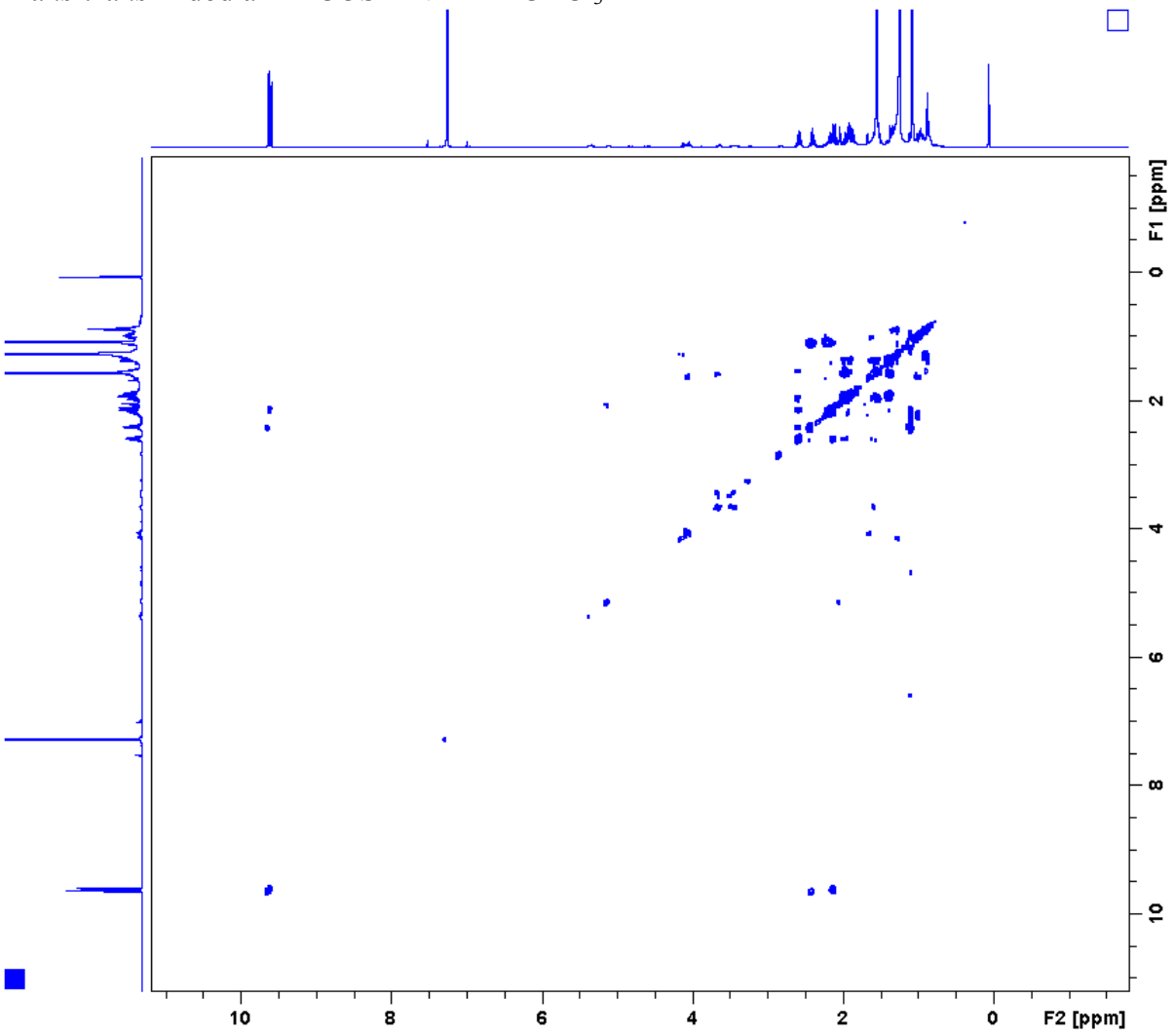
Trans-trans-iridodial ^{1}H-NMR in $CDCl_3$



Trans-trans-iridodial ^{13}C-NMR in $CDCl_3$



Trans-trans-iridodial ^{1}H-COSY-NMR in $CDCl_3$



Trans-trans-iridodial $^{1}H/^{13}C$-HSQC-NMR in $CDCl_3$