

Supporting Information
for
Biomimetic synthesis and HPLC–ECD analysis of the
isomers of dracocephins A and B

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Details of physicochemical measurements

Potentiometric pK_a determination

T3-Sirius automated pK_a analyser (Sirius Analytical Instruments Ltd., Forest Row, UK) fitted with combination Ag/AgCl pH electrode was used for determination of dissociation constants. The pK_a values were calculated by RefinementPro™ software (Sirius Analytical Instruments Ltd., Forest Row, UK). Methodologies used by the software have been described in earlier publications [1,2].

Titration of dracocephins A and B

The titration of dracocephins A and B was carried out in a similar procedure as described in the literature [3]. In each experiment, 1.50 mL of a 1 mM aqueous solution of sample was prealkalified to pH 12.0–12.5 with 0.5 M KOH, and then titrated with 0.5 M HCl to an appropriately low pH, usually 2.0. The titrations were carried out at constant ionic strength ($I = 0.15$ M KCl) and temperature ($T = 25.0 \pm 0.1$ °C), and under nitrogen atmosphere. Three to five parallel measurements were carried out and the pK_a values of samples were calculated by RefinementPro™ software.

Potentiometric determination of partition coefficients

Partition coefficients were determined in a similar manner as described in the literature [4]. Typically, 0.8–2.5 mL of 0.5–10 mM solutions of samples were titrated under the same conditions as in pK_a determinations but in the presence of various amounts of the partitioning solvent, water-saturated *n*-octanol. The phase ratio applied was varied from 1.5 mL water – 0.05 mL *n*-octanol to 1.5 mL water – 1.0 mL

n-octanol. From the *n*-octanol containing titrations the p_oK_a (the apparent ionization constant in the presence of *n*-octanol) and then $\log P$ values were estimated and refined by a weighted non-linear least-squares procedure, where the aqueous pK_a values (taken from aqueous titrations) were used as unrefined contributions. For each compound a minimum of three to six titrations at different phase volume ratios were measured, and the respective average $\log P$ values were calculated. $\log D$ values were also calculated by $\log P$ and pK_a values using the Henderson–Hasselbalch equation. The relevant relationships between $\log P$, pK_a , and p_oK_a , for mono- and multiprotic substances, including cases of ion-pair formation, have been described in detail earlier [5].

Antioxidant activity: The radical scavenging capacity of dracocephins A and B were tested in the microplate format of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [6]. Naringenin and quercetin were obtained from PhytoLab (Vestenbergsgreuth, Germany), and used as controls.

Permeability measurements: The PAMPA-BBB method was previously published [7]. Briefly, a 96-well acceptor plate and a 96-well filter plate are assembled into a sandwich. The hydrophobic filter material of the 96 well filter plate is coated with 5 μ L of a 2.6% (w/v) dodecane/hexane (25:75 v/v %) solution of porcine brain lipid (PBL). Subsequently, the acceptor wells at the bottom of the sandwich are filled with 300 μ L of 10 mM PBS solution with 5% DMSO adjusted to pH 7.4. The donor wells at the top of the sandwich are hydrated with 150 μ L of test compound solution. The test compound solution is prepared by diluting $\times 100$ from a 10 mM stock solution in DMSO using PBS solution at pH 7.4 with 5% DMSO followed by filtration through a MultiScreen Solubility filter plate. The resulting sandwich is then incubated at 37 °C

for 4 h. After the incubation, PAMPA sandwich plates are separated and compound concentrations in donor and acceptor solutions are determined by HPLC-DAD.

Biology

Cell lines

SH-SY5Y neuroblastoma was cultured in EMEM media supplemented with non-essential amino acids, 1 mM Na pyruvate and 10% inactivated fetal bovine serum, nystatin, 2 mM L-glutamine, 100 U penicillin and 0.1 mg streptomycin, purchased from Sigma. Cells were cultured at 37 °C and 5% CO₂.

Two mouse lymphoma cell lines were used to test for the capacity of the compounds to inhibit the function of the ABCB1 transporter: a parental (L5178_{PAR}) cell line, L5178 mouse T-cell lymphoma cells (ECACC catalog no. 87111908, U.S. FDA, Silver Spring, MD, U.S.), and a multi-drug resistant (L5178_{MDR}) cell line derived from L5178 by transfection with pHa MDR1/A retrovirus [8]. Cells were cultured in McCoy's 5A media supplemented inactivated horse serum and antibiotics as above. MDR cell line was selected by culturing the infected cells with 60 µg/L colchicine (Sigma).

Materials and methods:

Compounds (±)-**2a–d** and (±)-**3a–d** were dissolved in DMSO at a stock concentration of 10 mM.

Cytotoxicity on SH-SY5Y cells: Cytotoxicity on SH-SY5Y cells was measured in an analogous manner as described in the literature [9]. 10,000 cells per well were seeded overnight. Serial dilutions of the compounds were prepared and added the following day to the plate. Cells were then incubated for 48 h, after which 10% MTT was added to each well. After 4 h, SDS was added to the medium and the results

were read after o/n incubation. Fifty percent inhibitory concentrations (IC_{50}) were calculated using nonlinear regression curve fitting of log (inhibitor) versus normalized response and variable slope with a least squares (ordinary) fit of GraphPad Prism 5 software, for three independent samples.

Evaluation of compounds effect on rhodamine 123 accumulation: Rd123 concentration inside L5178_{PAR} and L5178_{MDR} cells was determined by flow cytometry. Briefly, 2×10^6 cells/mL were treated with 2 and 20 μ M of each compound and incubated for 10 min at rt. Rhodamine 123 (Sigma, Germany) was added to a final concentration of 5.2 μ M. The samples were incubated for 20 min at 37 °C in water bath and then centrifuged (2000 rpm, 2 min). The pellet was resuspended in 0.5 mL of phosphate buffer saline (PBS) (Sigma, Germany). The washing step was repeated twice. The fluorescence of the samples was measured by flow cytometry (Becton Dickinson FACScan, BD, U.S.). Tariquidar at 2 μ M was used as positive control. Fluorescence activity ratio measures the capacity of inhibition (accumulation of Rd123) and it is equal to the ratio between the FL-1 values of the L5178_{MDR} cells treated and untreated.

Computational section

Mixed torsional/low-frequency mode conformational searches were carried out by means of the Macromodel 9.9.223 software using the Merck Molecular Force Field (MMFF) with an implicit solvent model for $CHCl_3$ [10]. Geometry reoptimizations were carried out at the B3LYP/6-31G(d) level in vacuo, B3LYP/TZVP, B97D/TZVP, CAM-B3LYP/TZVP and M06-2X/TZVP levels with the PCM solvent model for MeCN or $CHCl_3$. TDDFT ECD calculations were run with various functionals (B3LYP, BH&HLYP, CAM-B3LYP, PBE0) and the TZVP basis set as implemented in the Gaussian 09 package with the same or no solvent model as in the preceding DFT

optimization step [11]. ECD spectra were generated as sums of Gaussians with 3000 cm^{-1} widths at half-height (corresponding to ca. 24 nm at 280 nm), using dipole-velocity-computed rotational strength values [12]. Boltzmann distributions were estimated from the ZPVE-corrected B3LYP/6-31G(d) energies in the gas-phase calculations and from the B3LYP/TZVP, B97D/TZVP, CAM-B3LYP/TZVP and M06-2X/TZVP energies in the solvated ones. The MOLEKEL software package was used for visualization of the results [13].

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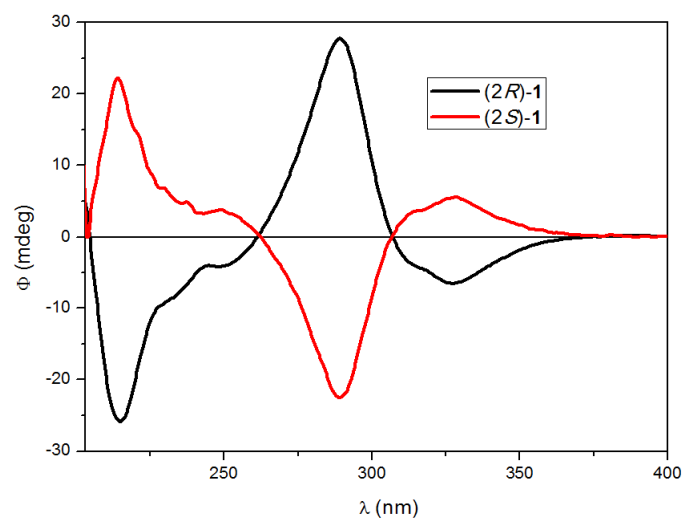


Figure S1: HPLC–ECD spectra of (2*R*)- and (2*S*)-naringenin (**1**).

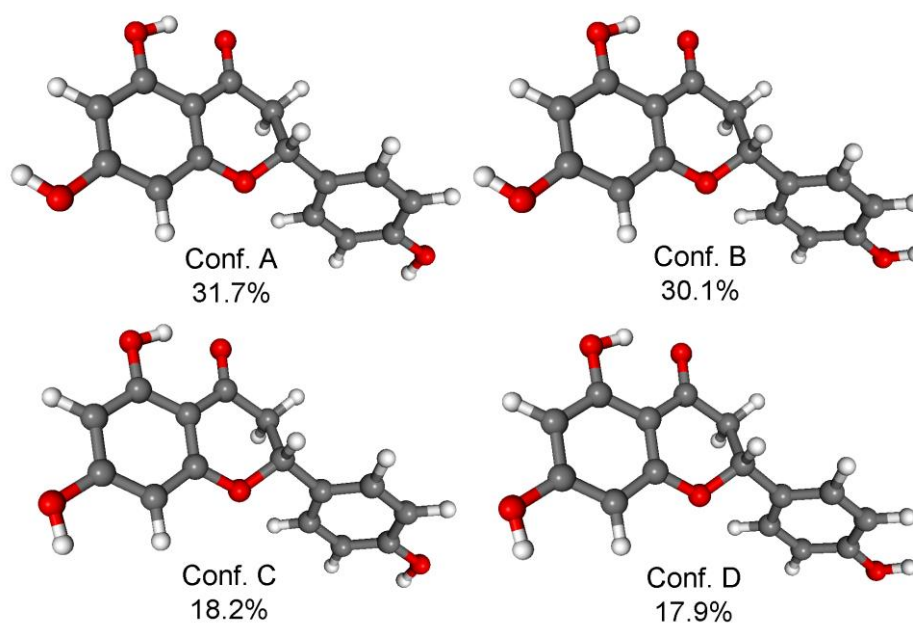


Figure S2: Structure and population of the low-energy B3LYP/6-31G(d) in vacuo conformers (>2%) of (*R*)-**1**.

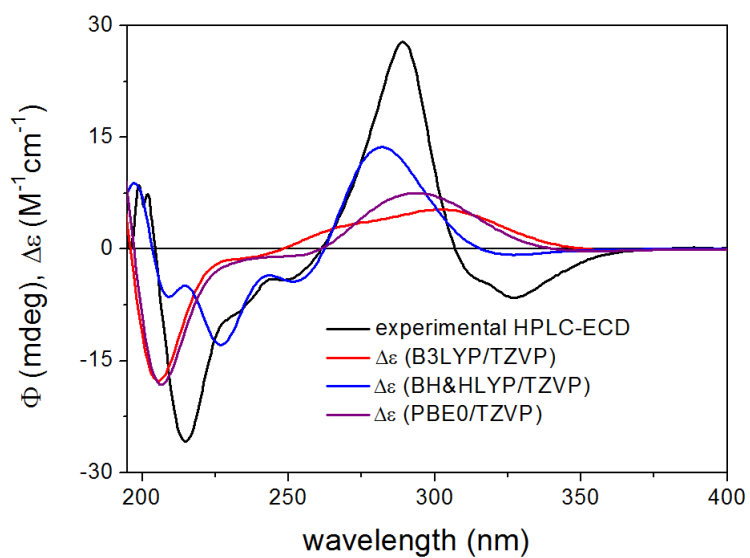


Figure S3: Experimental HPLC–ECD spectrum of (*R*)-**1** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) in vacuo low-energy conformers at various levels.

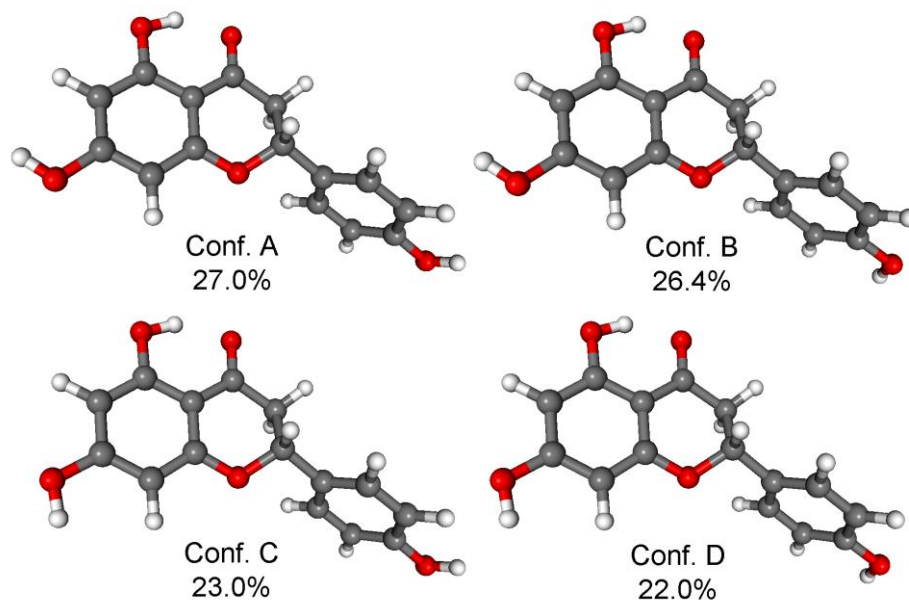


Figure S4: Structure and population of the low-energy B3LYP/TZVP PCM/CHCl₃ conformers (>2%) of (*R*)-**1**.

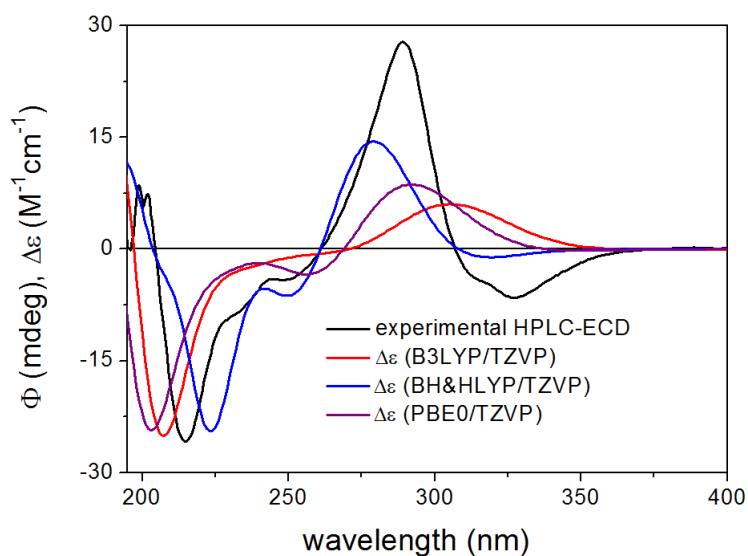


Figure S5: Experimental HPLC–ECD spectrum of (*R*)-**1** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/CHCl₃ low-energy conformers at various levels.

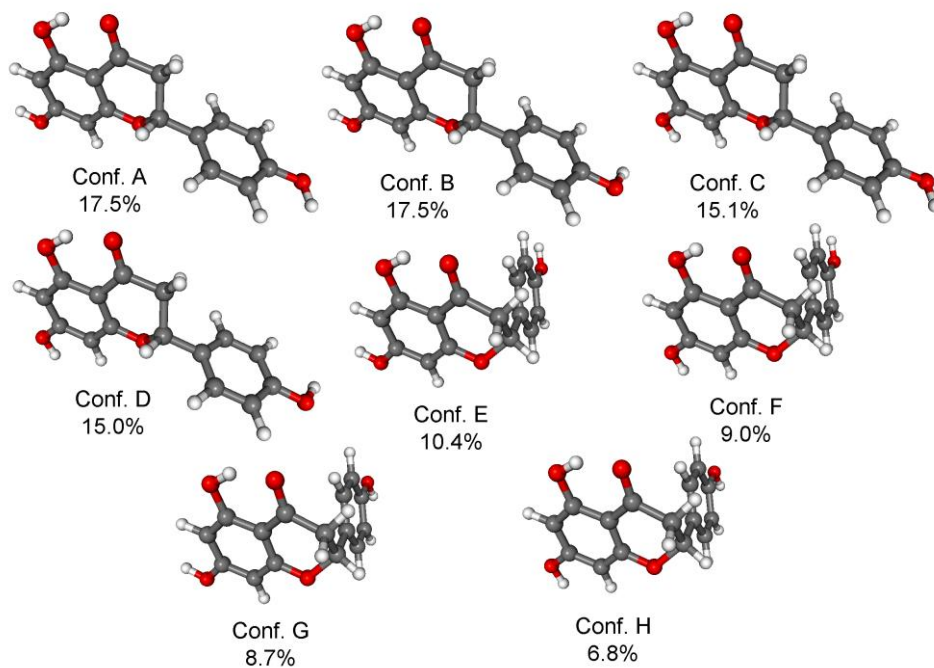


Figure S6: Structure and population of the low-energy B97D/TZVP PCM/CHCl₃ conformers (>2%) of (*R*)-**1**.

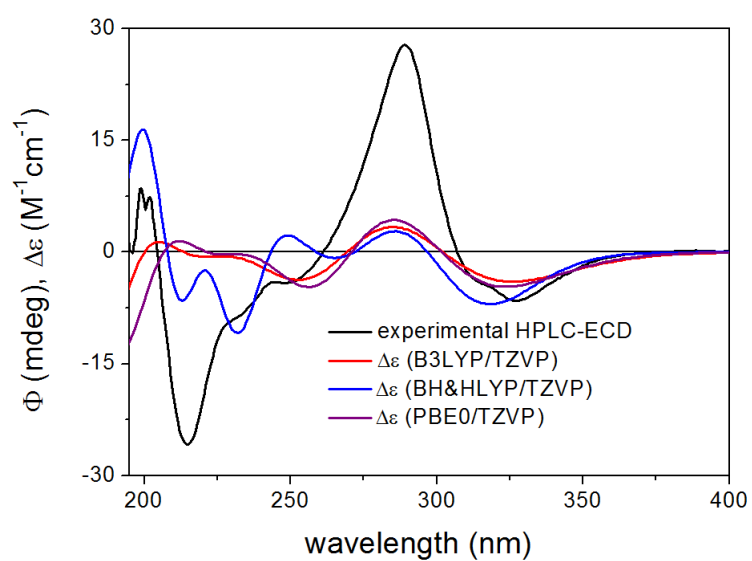


Figure S7: Experimental HPLC–ECD spectrum of (*R*)-**1** compared with the Boltzmann-weighted ECD spectra computed for the B97D/TZVP PCM/CHCl₃ low-energy conformers at various levels.

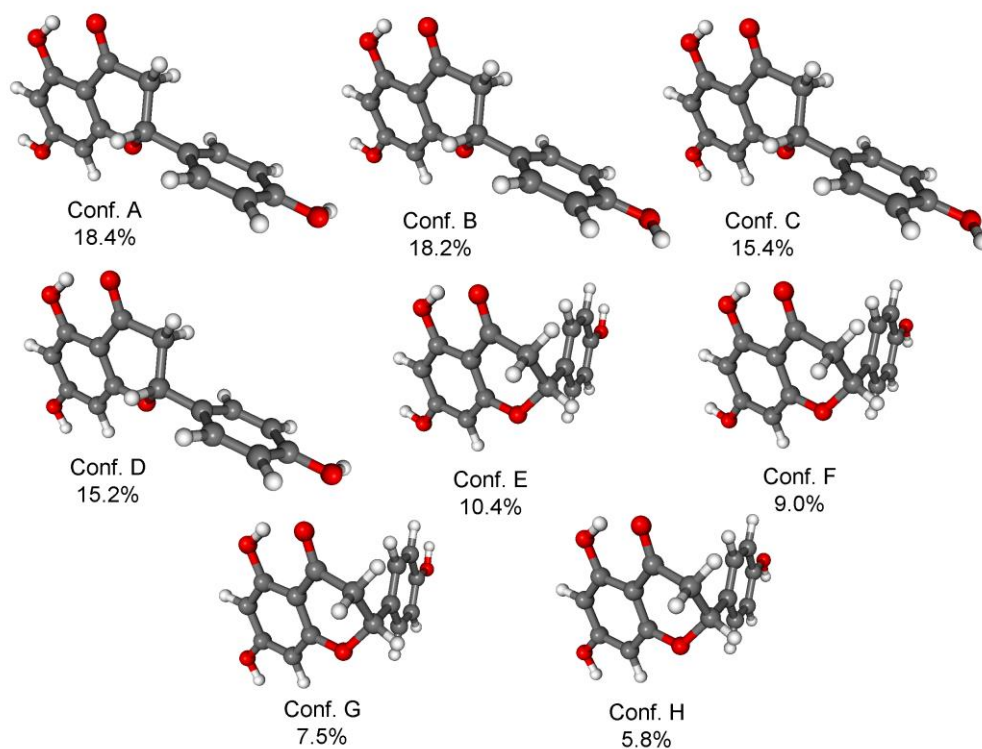


Figure S8: Structure and population of the low-energy M06-2X/TZVP PCM/CHCl₃ conformers (>2%) of (*R*)-**1**.

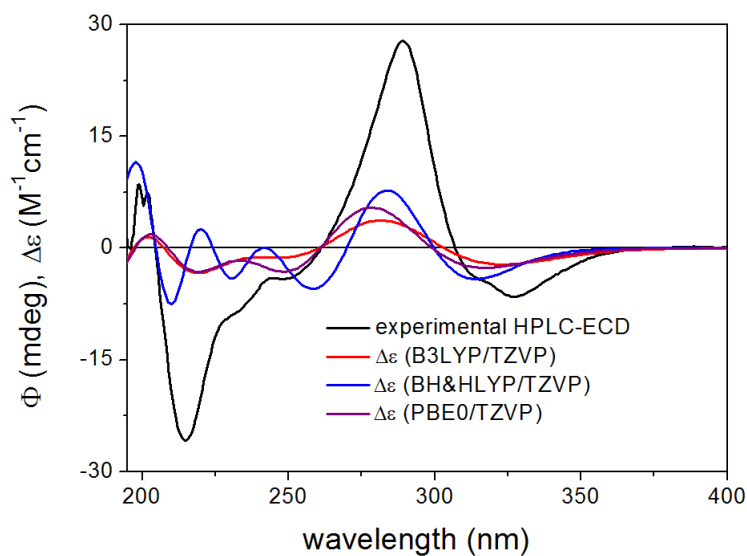


Figure S9: Experimental HPLC–ECD spectrum of (*R*)-**1** compared with the Boltzmann-weighted ECD spectra computed for the M06-2X/TZVP PCM/CHCl₃ low-energy conformers at various levels.

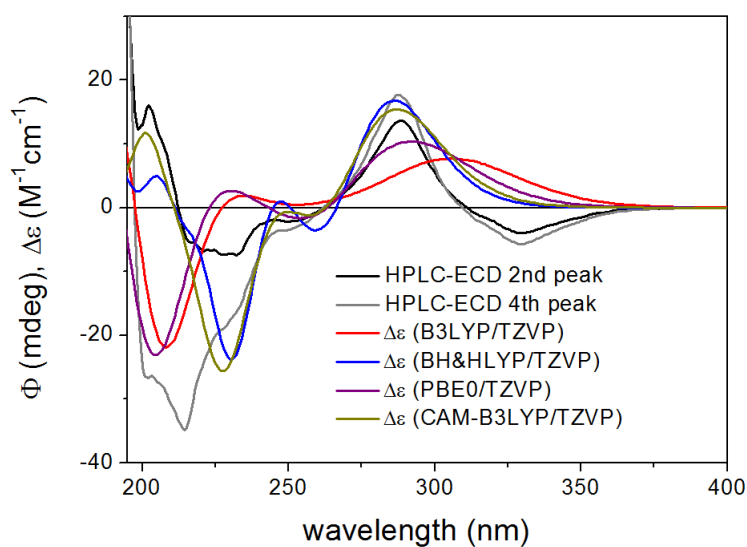


Figure S10: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (*2R,5''R*)-**2** at various levels.

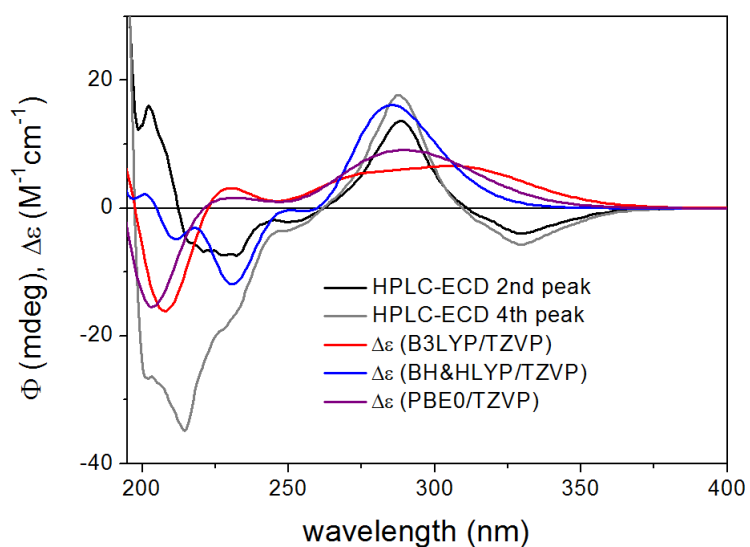


Figure S11: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) in vacuo low-energy conformers of (2*R*,5''*R*)-**2** at various levels.

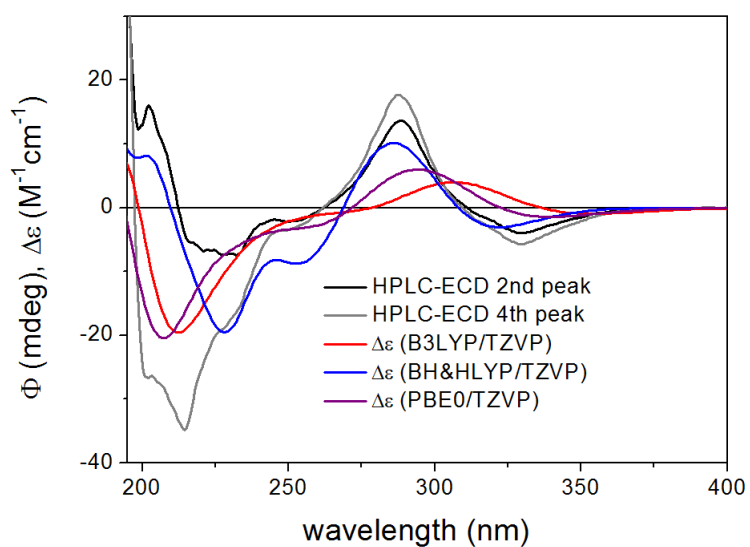


Figure S12: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) in vacuo low-energy conformers of (2*R*,5''*S*)-**2** at various levels.

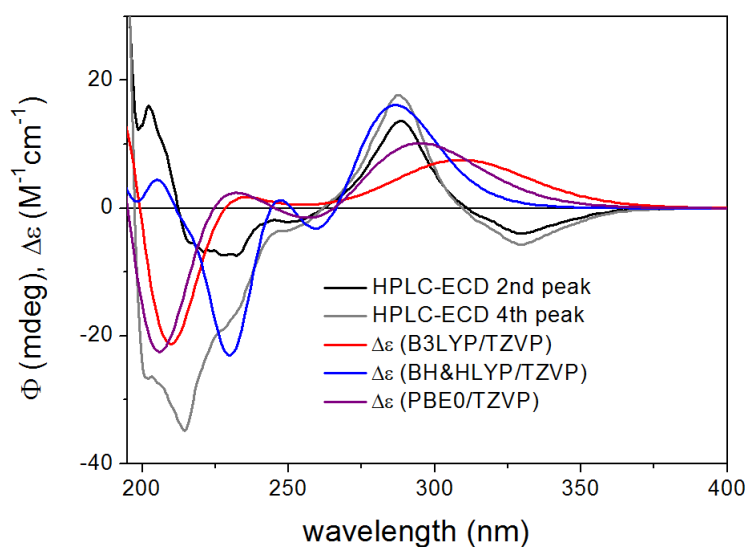


Figure S13: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (2*R*,5''*R*)-**2** at various levels.

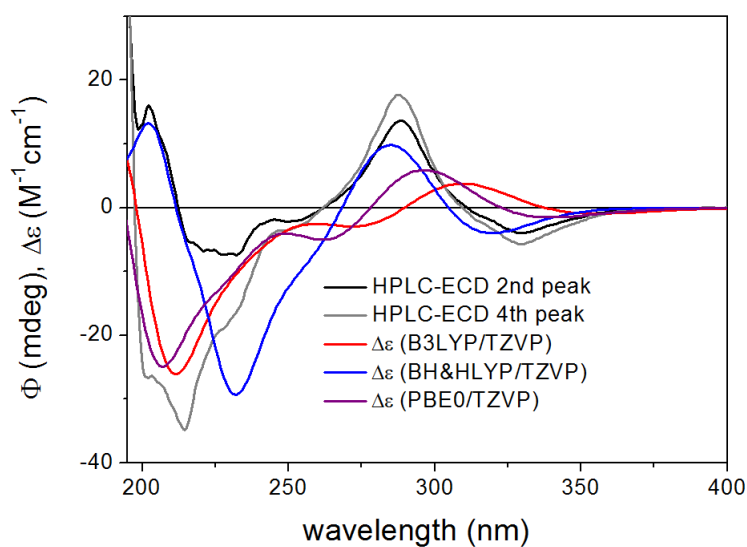


Figure S14: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (2*R*,5''*S*)-**2** at various levels.

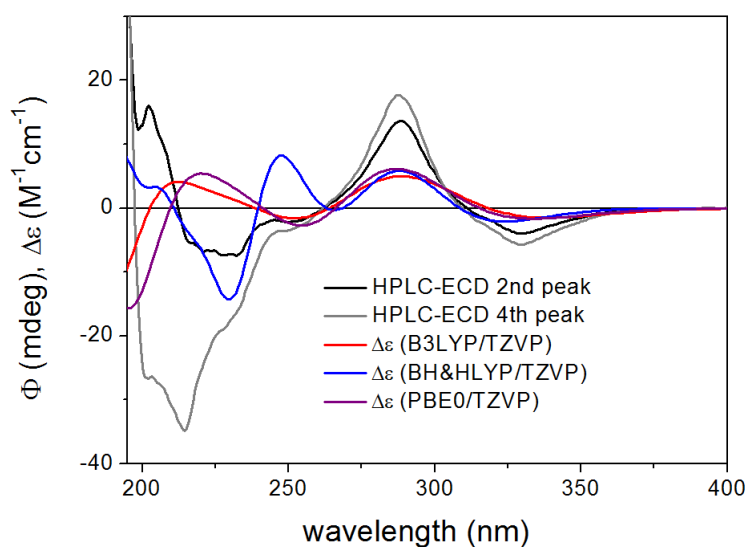


Figure S15: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the B97D/TZVP PCM/MeCN low-energy conformers of $(2R,5'R)$ -**2** at various levels.

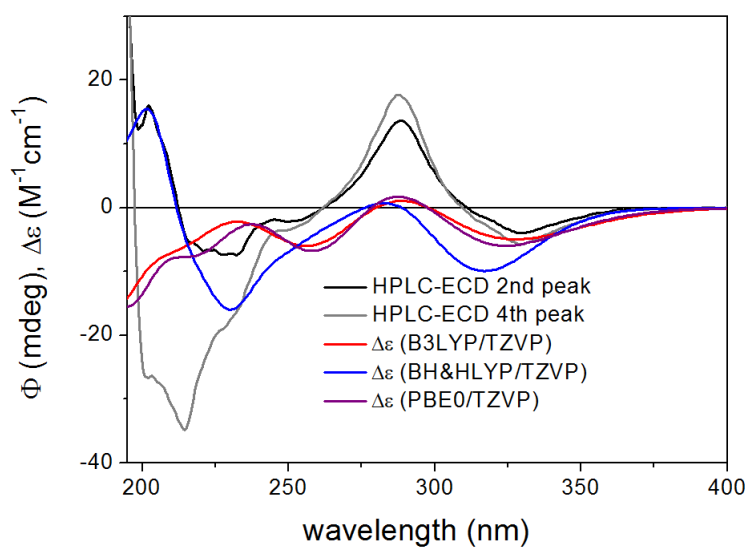


Figure S16: Experimental HPLC–ECD spectra of **2a** and **2d** compared with the Boltzmann-weighted ECD spectra computed for the B97D/TZVP PCM/MeCN low-energy conformers of $(2R,5'S)$ -**2** at various levels.

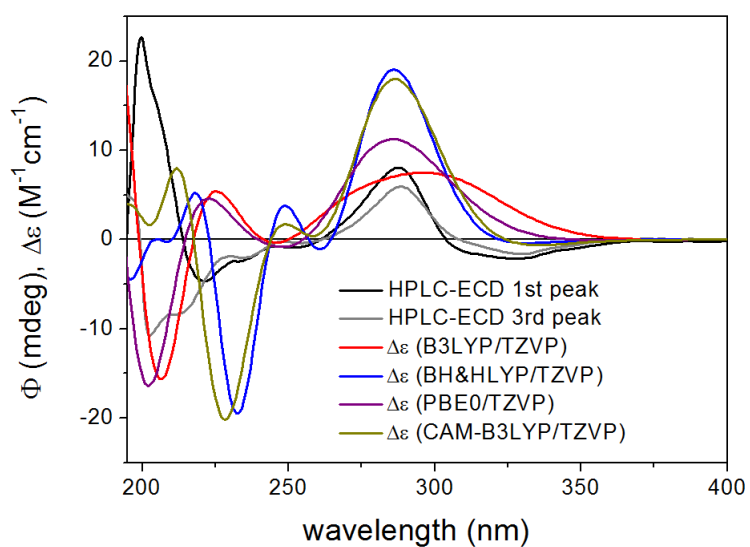


Figure S17: Experimental HPLC–ECD spectra of **3b** and **3c** compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (2*R*,5'*R*)-**3** at various levels.

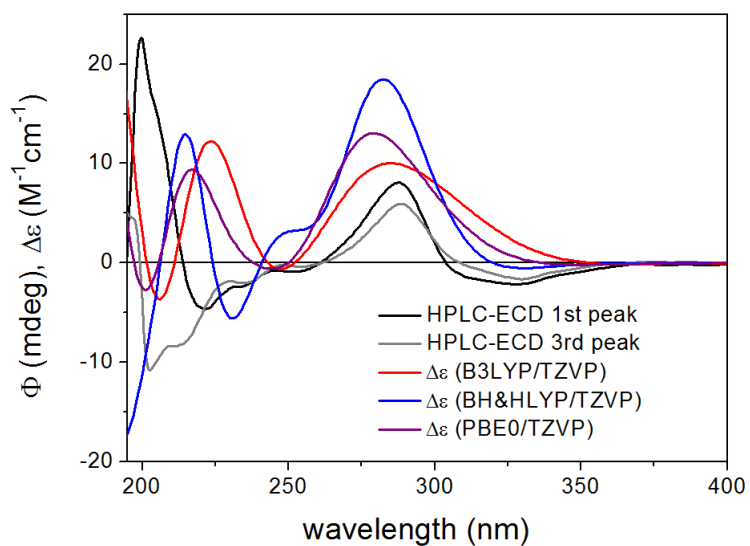


Figure S18: Experimental HPLC–ECD spectra of **3b** and **3c** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) in vacuo low-energy conformers of (2*R*,5'*R*)-**3** at various levels.

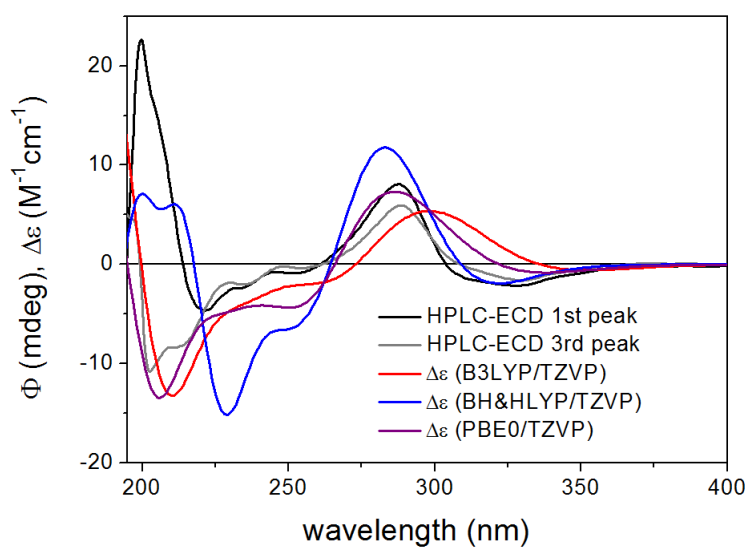


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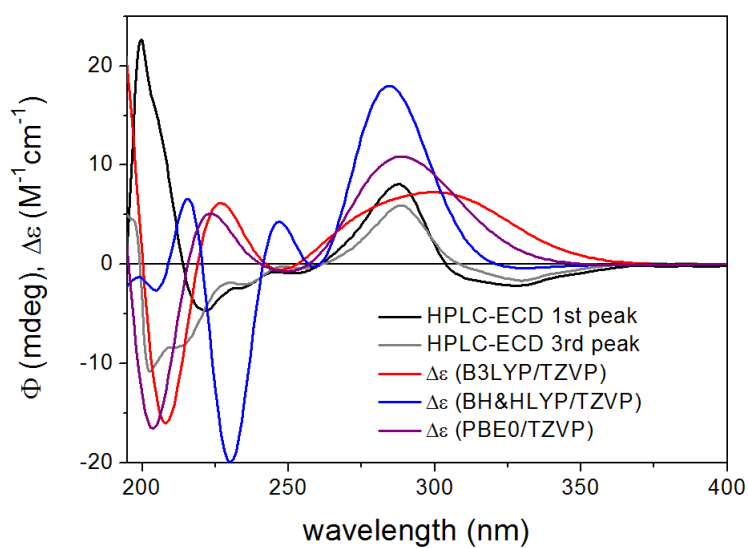


Figure S20: Experimental HPLC–ECD spectra of **3b** and **3c** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (2*R*,5''*R*)-**3** at various levels.

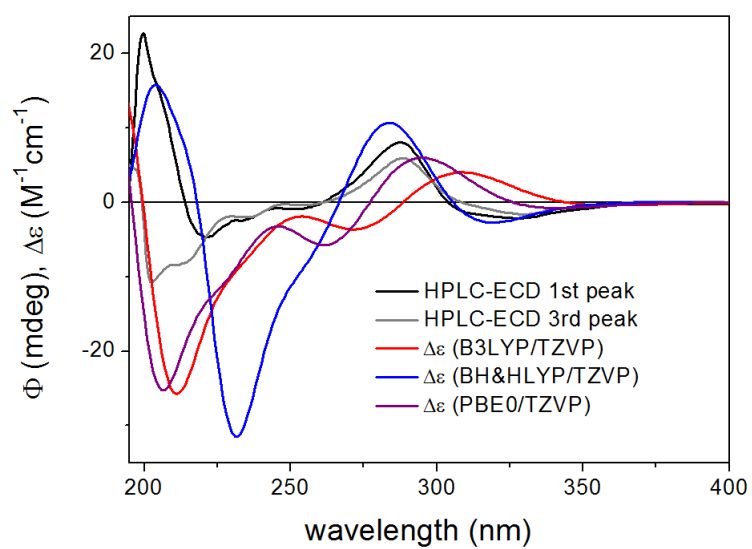


Figure S21: Experimental HPLC–ECD spectra of **3b** and **3c** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of $(2R,5'S)$ -**3** at various levels.

IV-3-1 Hazai Laszlo
Szigetvari Aron
v5h-8650

Spectrometer: vnmrs-500
Date: Feb 5 2015
Solvent: cd3od
Temp. 25.0 C / 298.1 K
Pulse Sequence: s2pu1
Relax. delay 5.000 sec
Pulse 45.0 degrees
Acq. time 2.045 sec
Width 8012.8 Hz
Acq. size 32768 points (Re+Im)
Single scan
OBSERVE H1, 499.9120765 MHz
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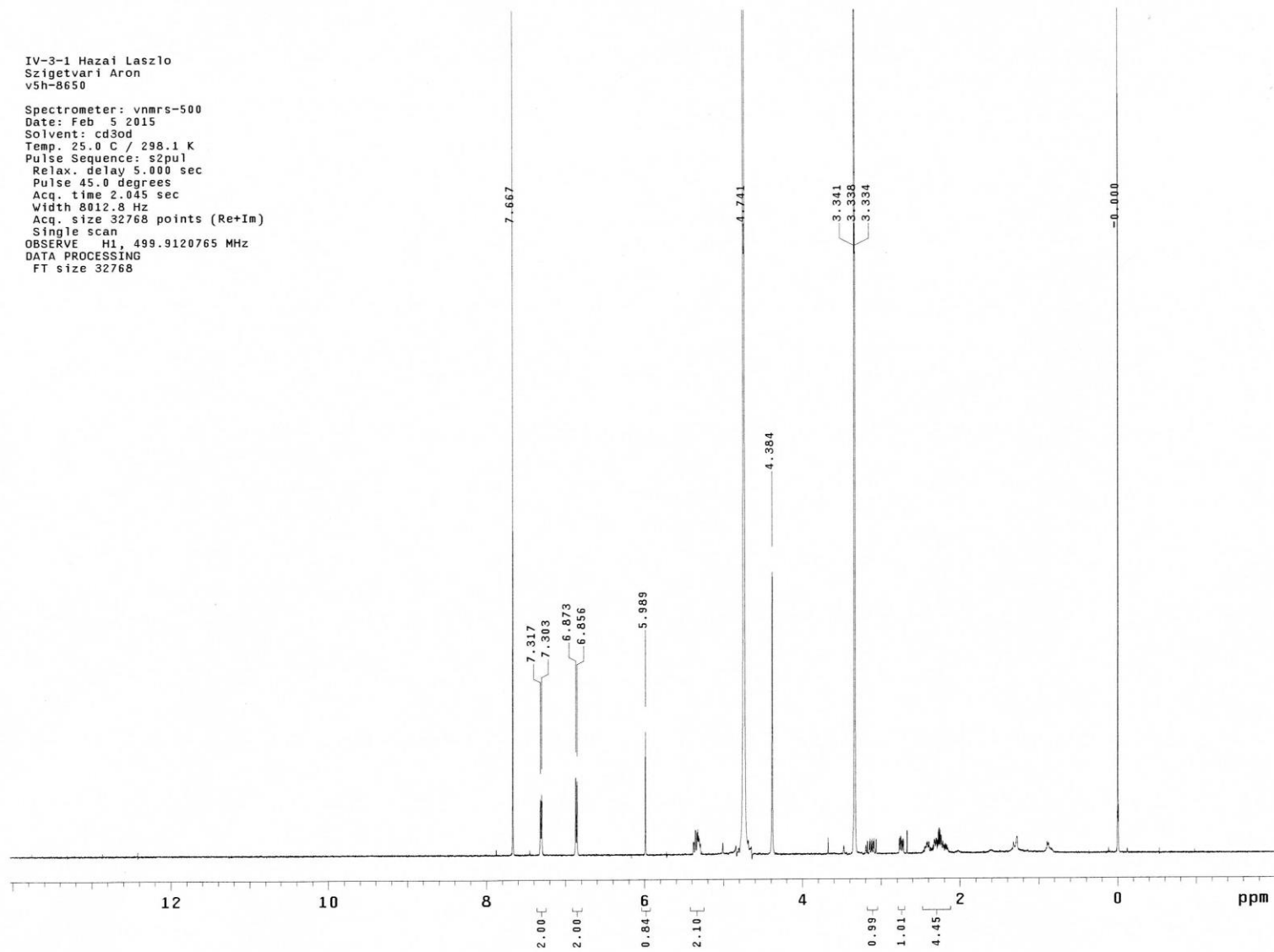


Figure S22: ^1H NMR spectrum of (\pm)-**3a-d** in CD_3OD .

IV-3-1 Hazai Laszlo
 Szigetvari Aron
 v5h-8650
 mixN=800 ms
 CDCl3+MeOD (2:1)

exp85 NOESY

SAMPLE		FLAGS	
date	Feb 5 2015	hs	nn
solvent	cd3od	sspul	y
sample		PFGflg	y
ACQUISITION		hsglvl	5796
sw	3434.1	SPECIAL	
at	0.150	temp	25.0
np	1030	gain	10
fb	4000	spin	not used
ss	32	F2 PROCESSING	
dl	1.000	sb	-0.150
nt	32	sbs	-0.150
2D ACQUISITION		fn	2048
sw1	3434.1	F1 PROCESSING	
ni	200	sb1	-0.058
TRANSMITTER		sbs1	-0.058
tn	H1	proc1	lp
sfrq	499.915	fn1	2048
tof	20.2	DISPLAY	
tpwr	52	sp	815.8
pw	6.500	wp	3430.7
NOESY		sp1	815.8
mixN	0.800	wp1	3430.7
PRESATURATION		rfl	-812.5
satmode	n	rflp	0
wet	n	rfl1	-812.5
DECOUPLER		rflp1	0
dn	C13	PLOT	
dm	nnn	wc	155.0
		sc	0
		wc2	155.0
		sc2	0
		vs	3.80469e+06
		th	2
		al	cdc ph

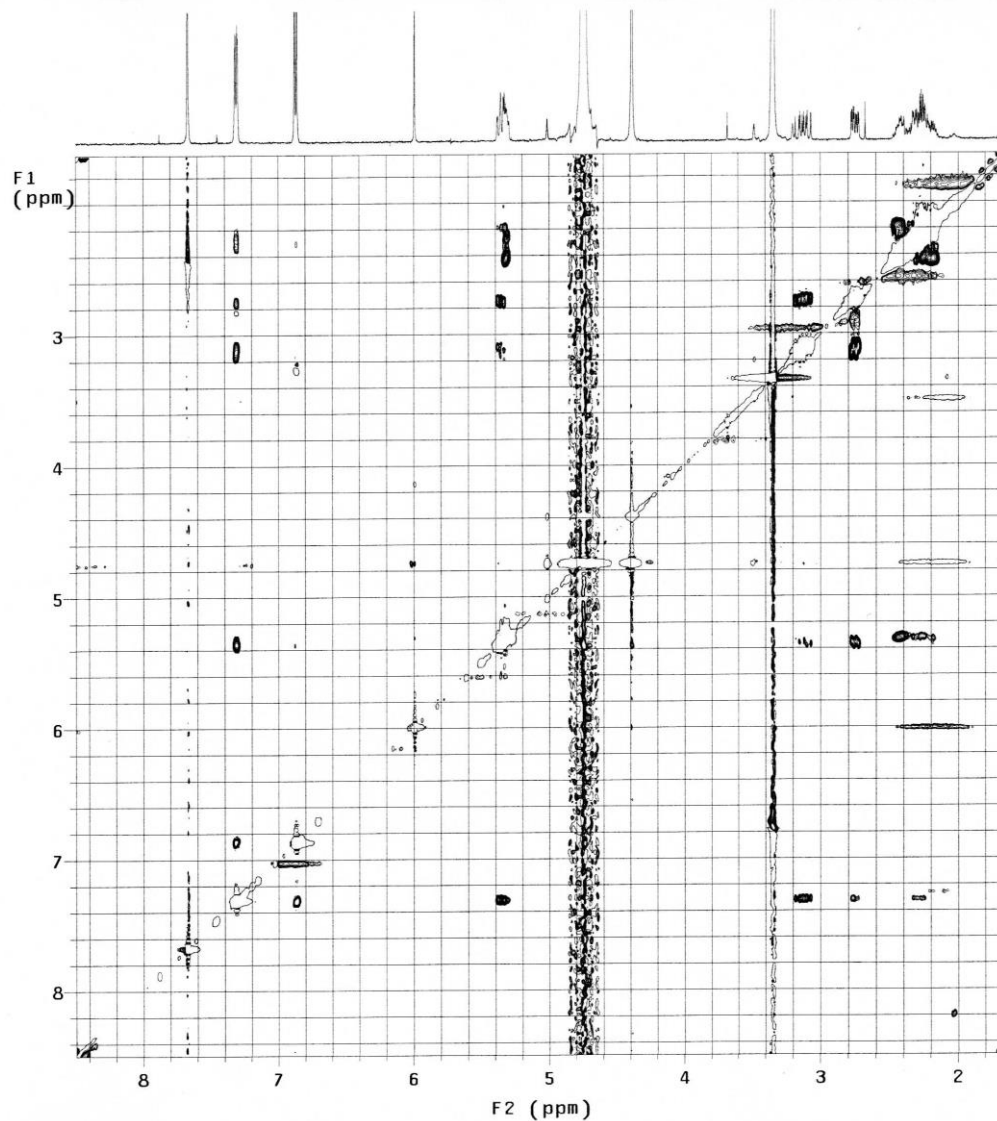


Figure S23: NOESY spectrum of (±)-3a-d (No. 1).

IV-3-1 Hazai Laszlo
 Szigetvari Aron
 v5h-8650
 mixN=200 ms
 CDC13+MeOD (2:1)

exp84 NOESY

	SAMPLE	FLAGS	nn
date	Feb 5 2015	hs	nn
solvent	cd3od	sepul	y
sample		PFGflg	y
ACQUISITION		hsglvt	5796
sw	3434.1	SPECIAL	
at	0.150	temp	25.0
np	1030	gain	10
fb	4000	spin	not used
ss	32	F2 PROCESSING	
d1	1.000	sb	-0.150
nt	32	sbs	-0.150
2D ACQUISITION		fn	2048
sw1	3434.1	F1 PROCESSING	
ni	200	sb1	-0.058
TRANSMITTER		sbs1	-0.058
tn	H1	procl	lp
sfrq	499.915	fn1	2048
tof	20.2	DISPLAY	
tpwr	52	sp	819.2
pw	6.500	wp	3102.1
NOESY		sp1	825.9
mixN	0.200	wp1	3216.1
PRESATURATION		rfl	-812.5
satmode	n	rfp	0
wet	n	rfl1	-812.5
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	155.0
		sc	0
		wc2	155.0
		sc2	0
		vs	1494
		th	2
		ai	cdc ph

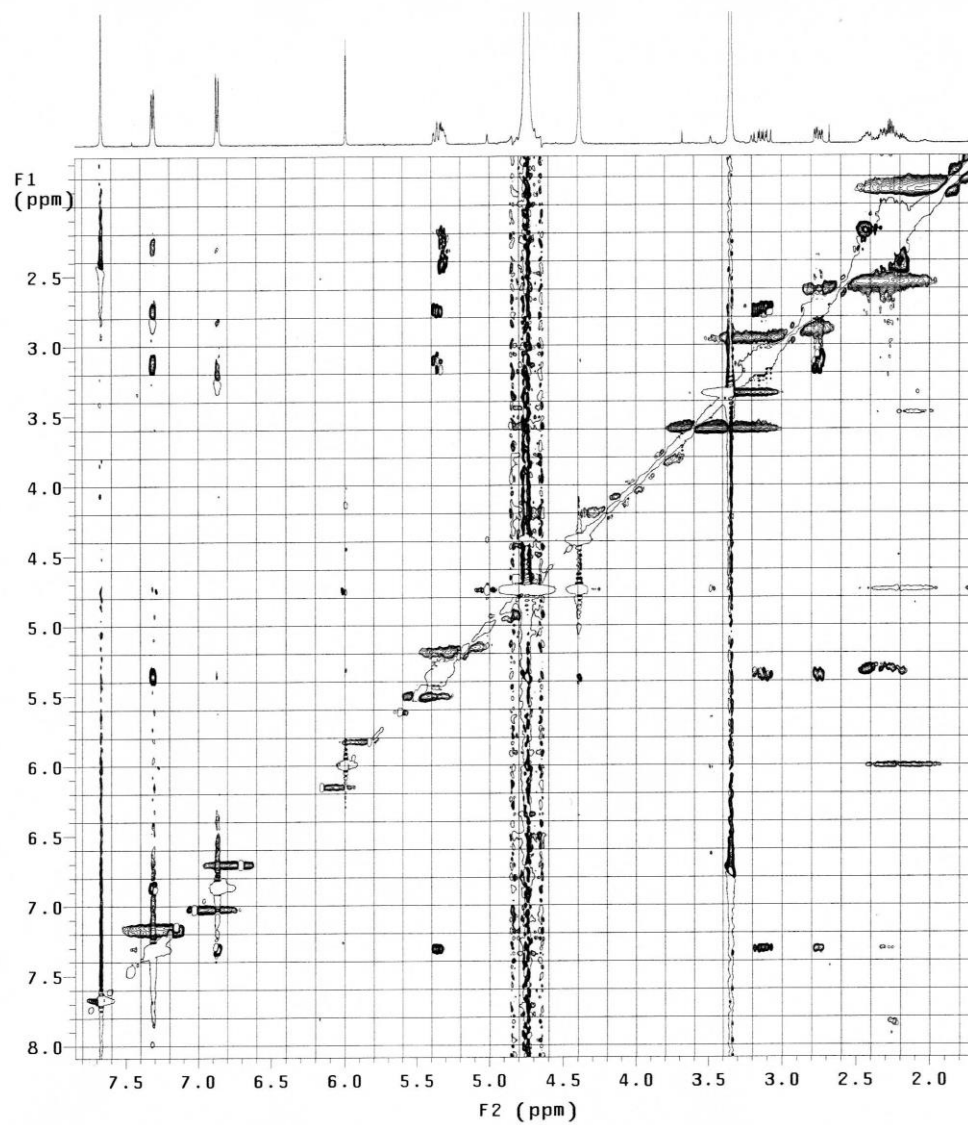


Figure S24: NOESY spectrum of (±)-3a-d (No. 2).

IV-3-1 Hazai Laszlo
Szigetvari Aron
v5h-8650

exp7 gCOSY

	SAMPLE	FLAGS	
date	Feb 5 2015	hs	nn
solvent	cd3od	sspul	y
sample	hsglv1		5796
ACQUISITION		SPECIAL	
sw	2289.4	temp	25.0
at	0.150	gain	10
np	686	spin	not used
fb	4000	F2 PROCESSING	
ss	32	sb	-0.075
d1	1.000	sbs	not used
nt	2	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	2289.4	sb1	-0.056
n1	256	sbs1	not used
d2	0	procl	lp
PRESATURATION		fn1	
satmode	n	fn1	2048
wet	n	sp	1046.1
TRANSMITTER		wp	
tn	H1	sp1	1667.8
sfrq	499.914	wp1	1032.7
tof	-495.1	rf1	1748.3
tpwr	52	rfl	-869.5
pw	6.500	rfl1	0
GRADIENTS		rfl1	
gzlvie	4830	rfl1	-869.5
gtE	0.001000	rfp1	0
EDratio	1.000	sc	0
gstab	0.000500	sc2	155.0
DECOUPLER		sc2	
dn	C13	vs	15722
dm	nnn	th	4
	ai	cdc	av

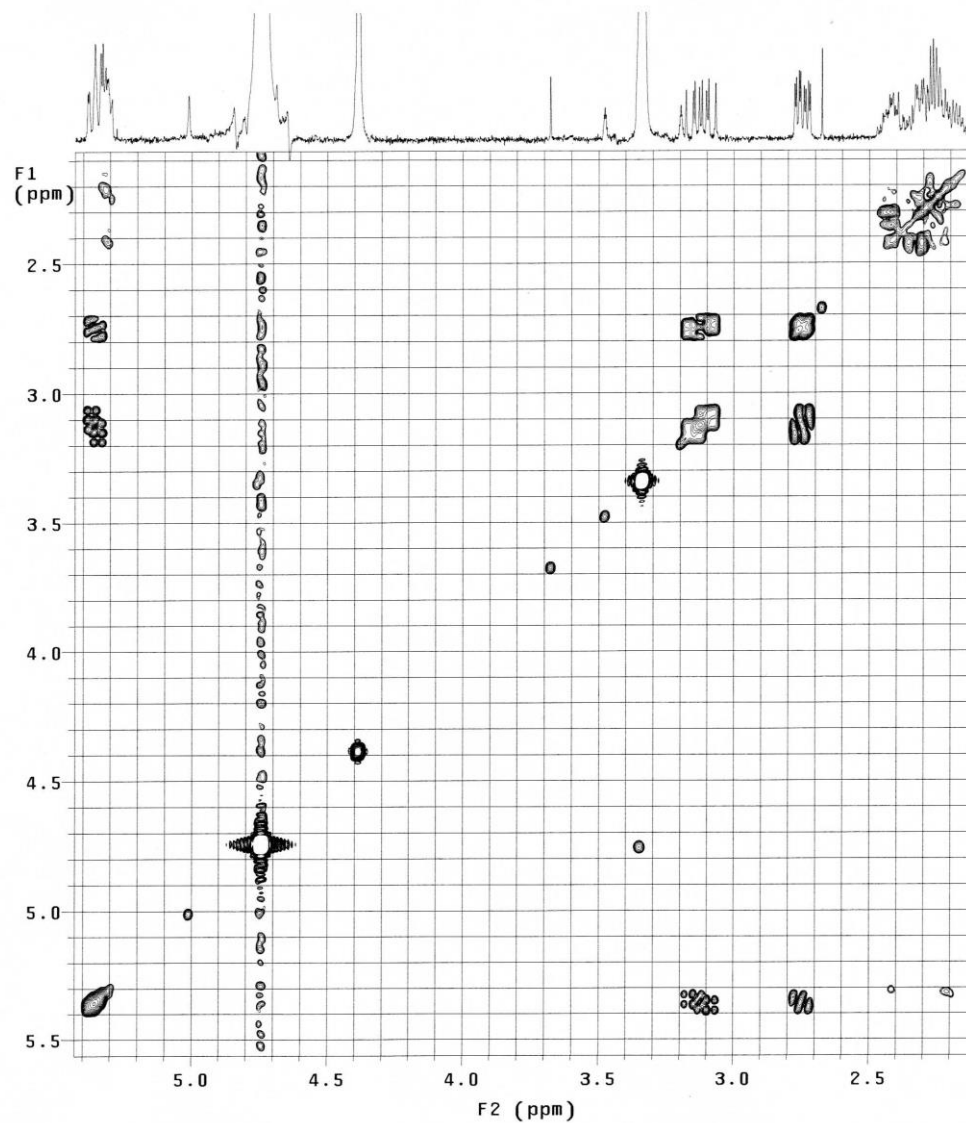


Figure S25: gCOSY spectrum of (±)-3a-d.

IV-3-1 Hazai Laszlo
Szigetvari Aron
v8h-5309
CDCl₃+MeOD (2:1)
Spectrometer: vnmrs-800
Date: Feb 2 2015
Solvent: cd3od
Temp. 30.0 C / 303.1 K
Pulse Sequence: s2pu1
Relax. delay 10.000 sec
Pulse 90.0 degrees
Acq. time 2.569 sec
Width 12755.1 Hz
Acq. size 65536 points
Single scan
OBSERVE H1, 799.6991766 MHz
DATA PROCESSING
FT size 524288

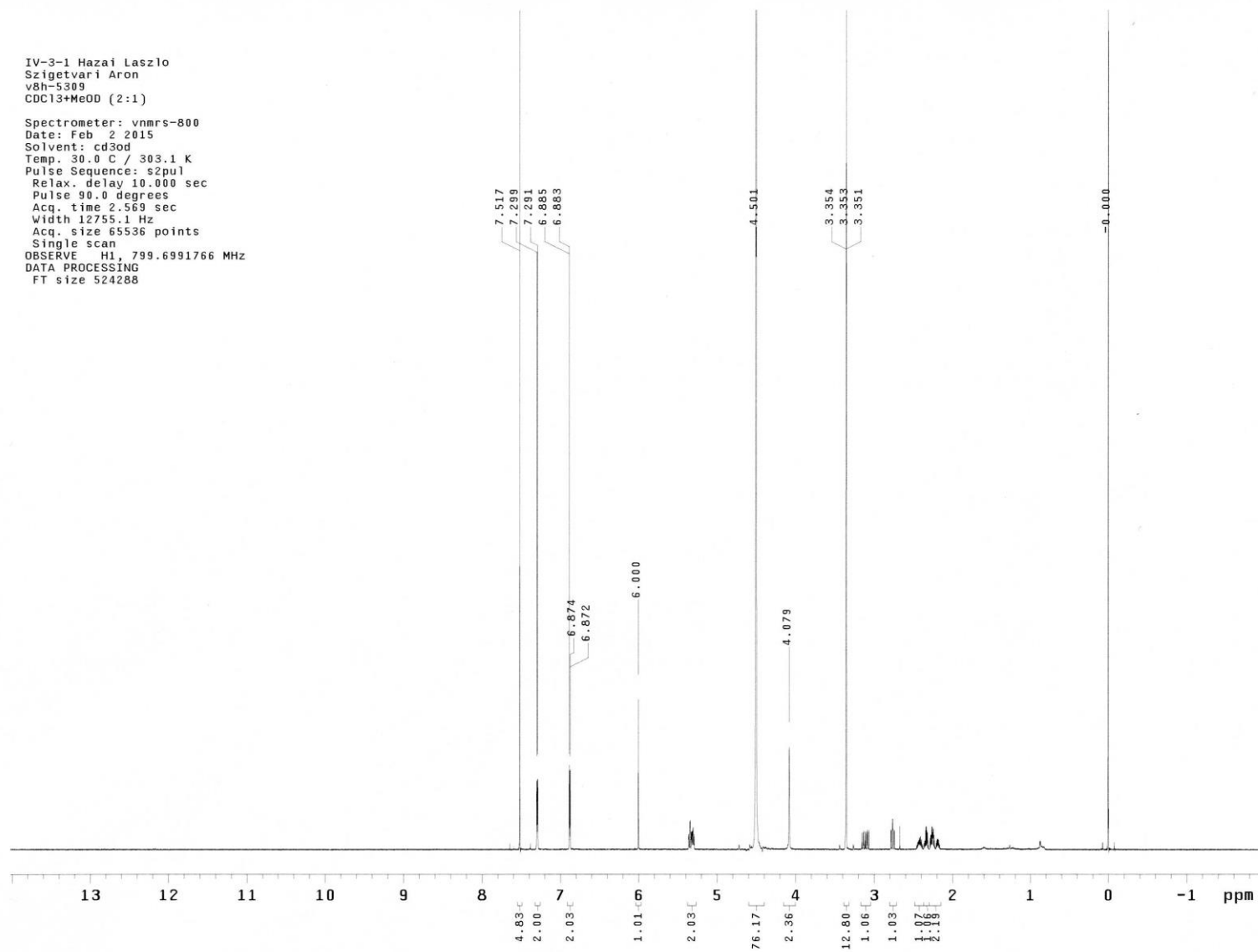


Figure S26: ¹H NMR spectrum of (±)-**3a-d** in CDCl₃ : CD₃OD (2:1).

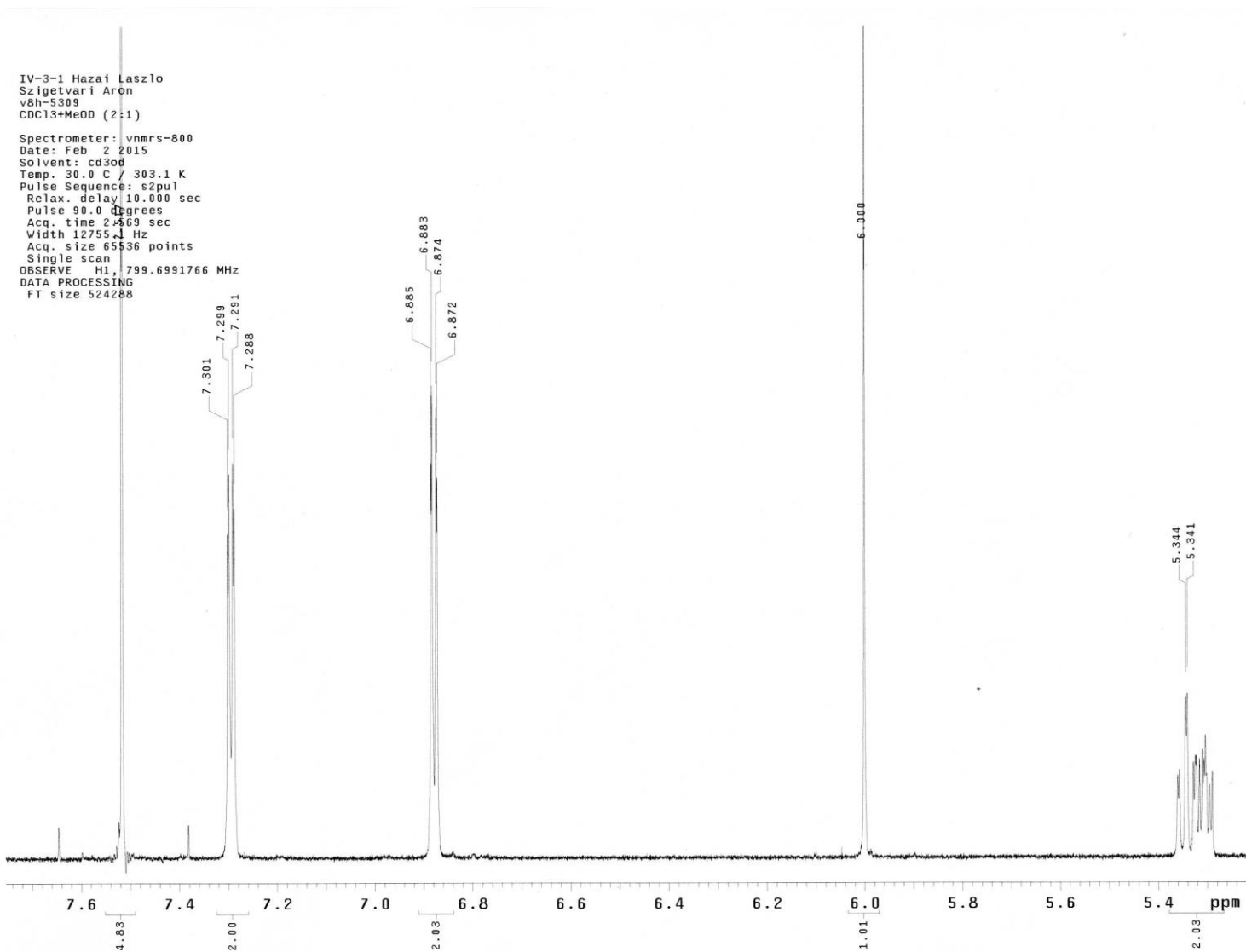


Figure S27: ¹H NMR spectrum of (±)-**3a-d** in CDCl₃ : CD₃OD (2:1) – zoom from 5.24 to 7.74 ppm.

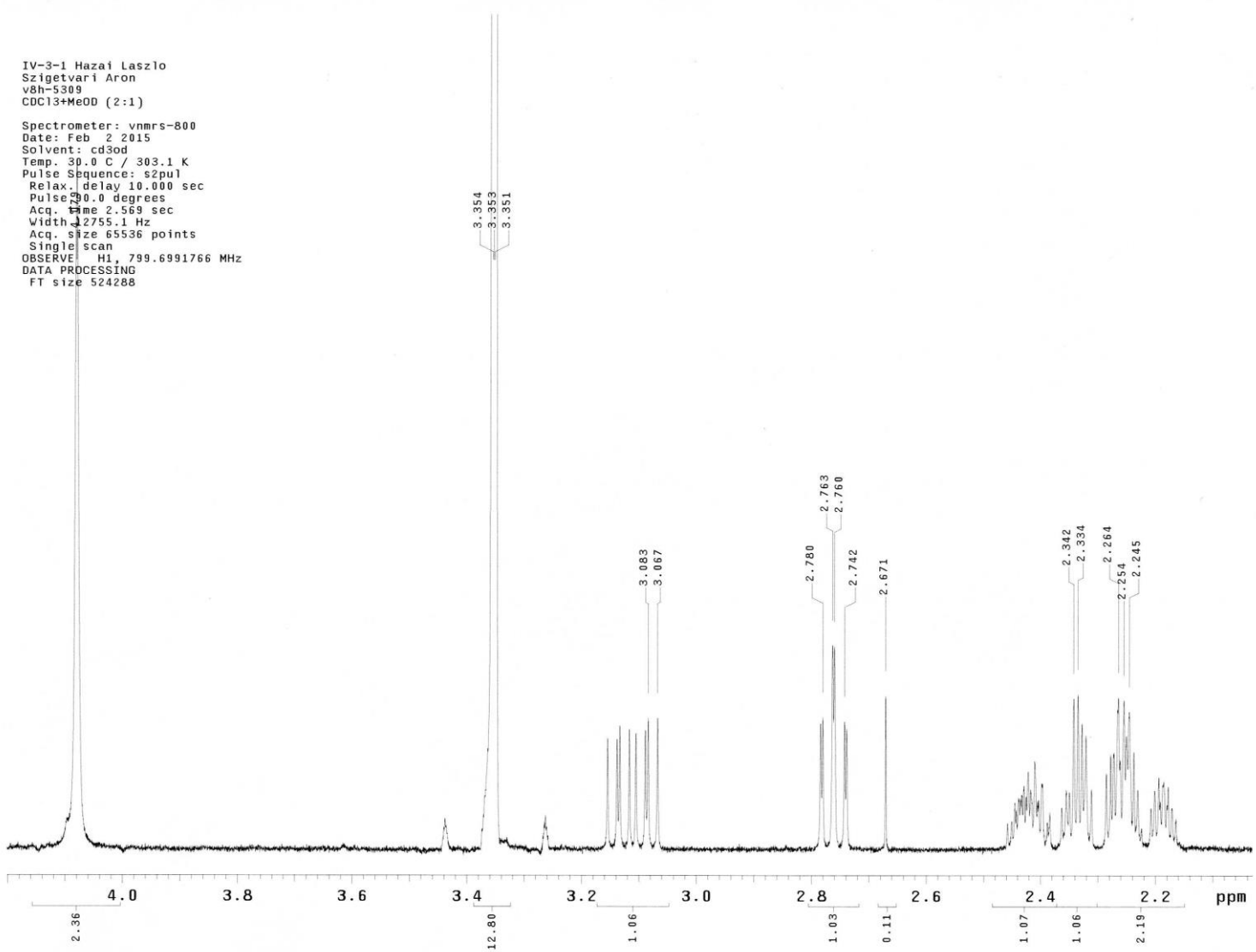


Figure S28: ^1H NMR spectrum of (\pm)-**3a-d** in CDCl_3 : CD_3OD (2:1) – zoom from 2.04 to 4.20 ppm.

IV-3-1 Hazai Laszlo
 Szigetvari Aron
 v8h-5309
 CDCl3+MeOD (2:1)
 exp23 gHSQCAD

SAMPLE	FLAGS	ACQUISITION	ARRAYS
date Feb 2 2015	hs nn	array	phs
solvent cd3od	sspu1 y	arraydim	10
sample	pfgflg		phase
ACQUISITION	hsglv1 5820	i	130
sw 7440.5	SPECIAL	1	2
at 0.200	temp 30.0	2	
np 2976	gain 30		
fb 4000	spin not used		
ss 32	GRADIENTS		
d1 1.000	gzlvIE 4854		
nt 4	gtE 0.002000		
2D ACQUISITION	EDratio 3.974		
sw1 30165.9	gstab 0.000500		
ni 96	F2 PROCESSING		
phase arrayed	sb -0.200		
PRESATURATION	sbs -0.200		
satmode n	fn 4096		
wet n	F1 PROCESSING		
TRANSMITTER	sb1 -0.003		
tn H1	sbs1 -0.003		
sfrq 799.702	procl ip		
tof -986.2	fn1 2048		
tpwr 56	DISPLAY		
pw 6.700	sp 1700.1		
DECOUPLER	wp 4359.7		
dn C13	sp1 4200.6		
dof -4982.4	wp1 22595.0		
dm nny	rfl 668.7		
decwave W40_CP_8880	rfp 0		
dmf 41667	rfl1 925.2		
dpwr 42	rfp1 0		
pxlv1 58	PLOT		
px 14.300	wc 150.0		
HSQC	sc 0		
j1xh 146.0	wc2 155.0		
nullflg y	sc2 0		
mult 2	vs 24774		
ADIABATIC	th 2		
px180ad CP_8880_a~	ai cdc ph		
d300			
px180adR CP_8880~			
ad300R			
px180 400.4			
pxlv1180 56			
px180ref CP_8880~			
ref200			
px180r 2000.8			
pxlv1180r 47			

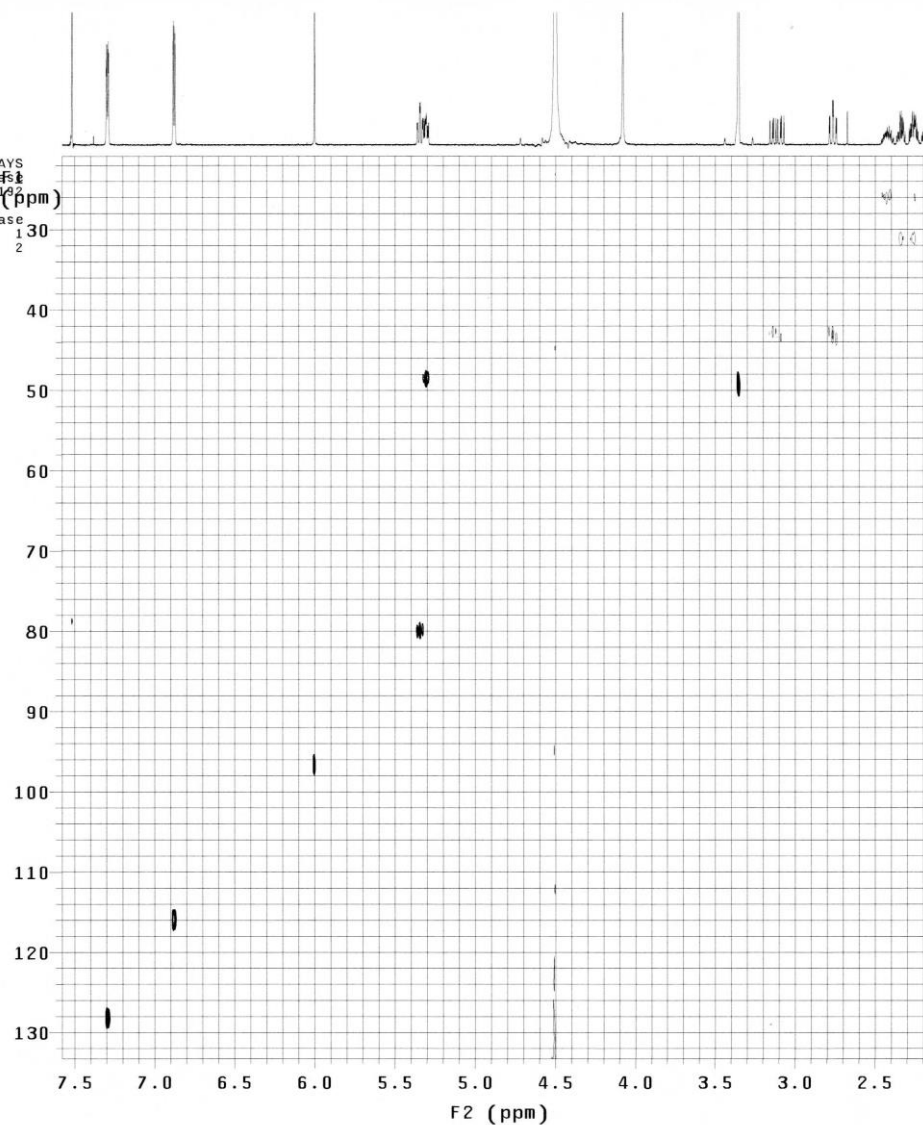


Figure S29: gHSQCad spectrum of (±)-3a-d.

IV-3-1 Hazai Laszlo
 Szigetvari Aron
 v8h-5309
 CDCl3+MeOD (2:1)
 exp25 gHMBCAD

SAMPLE		FLAGS	ACQUISITION	ARRAYS
date	Feb 2 2015	hs	nn	phase
solvent	cd3od	sspu1	y	400
sample		PFGflg		
		hsglv1	5820	
sw	7183.9	SPECIAL	i	phase
at	0.143	temp	1	F1 1
np	2048	gain	2	2
fb	4000	spin		
ss	32	GRADIENTS		
d1	1.000	gz1v11	485	
nt	8	gt1	0.001000	
		gz1v13	1455	
2D ACQUISITION		gt3	0.001000	
sw1	46242.8	gt3	0.001000	
ni	200	gstab	0.000500	
phase	arrayed	F2 PROCESSING		
PRESATURATION	sb	-0.143		
satmode	n	sbs	-0.143	
wet	n	fn	4096	
		F1 PROCESSING		
tn	H1	sb1	-0.004	
sfrq	799.702	sbs1	-0.004	
tof	-1001.4	procl	lp	
tpwr	56	fn1	2048	
pw	6.700	DISPLAY		
DECOUPLER	sp	572.6		
dn	C13	wp	5679.1	
dof	3058.9	sp1	2724.8	
dm	nnn	wp1	42178.5	
decwave	W40_CP_8880	rf1	556.9	
dmf	41667	rfp	0	
dpwr	42	rfl1	933.0	
pxwlv1	58	rflp1	0	
pxw	14.300	PLOT		
HMBC	wc	155.0		
j1xh	146.0	sc	0	
jnxh	8.0	wc2	145.0	
ADIABATIC	sc2	0		
pxw180ad CP_8880_a~	vs	2797		
	th	2		
pxwlv1180	56	ai	cdc	av
pxw180	400.4			

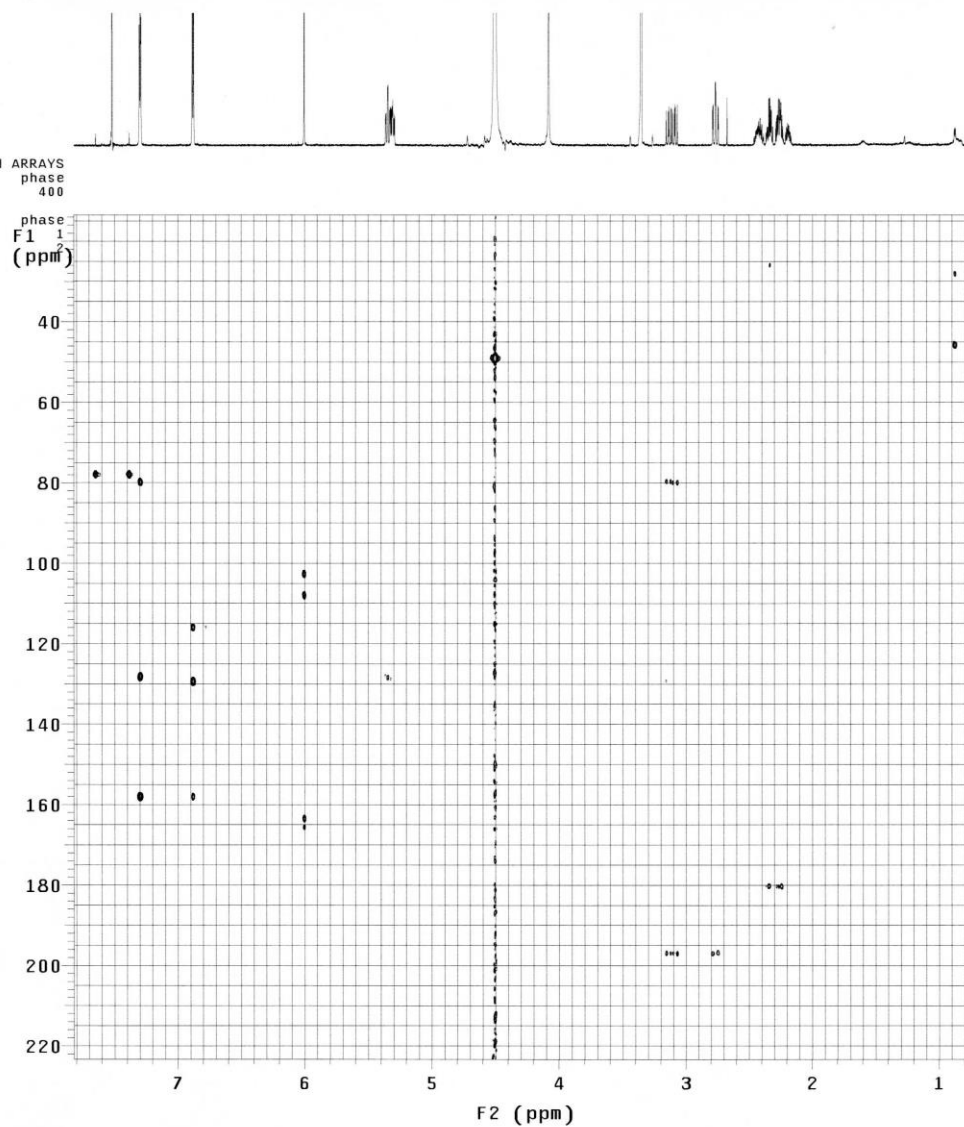


Figure S30: gHMBCad spectrum of (±)-3a-d.

IV-3-2 Hazai Laszlo
Szigetvari Aron
v8h-5310

Spectrometer: vnmrs-800
Date: Feb 2 2015
Solvent: cd3od
Temp. 30.0 C / 303.1 K
Pulse Sequence: s2pul
Relax. delay 10.000 sec
Pulse 90.0 degrees
Acq. time 2.569 sec
Width 12755.1 Hz
Acq. size 65536 points
Single scan
OBSERVE H1, 799.6992133 MHz
DATA PROCESSING
FT size 524288

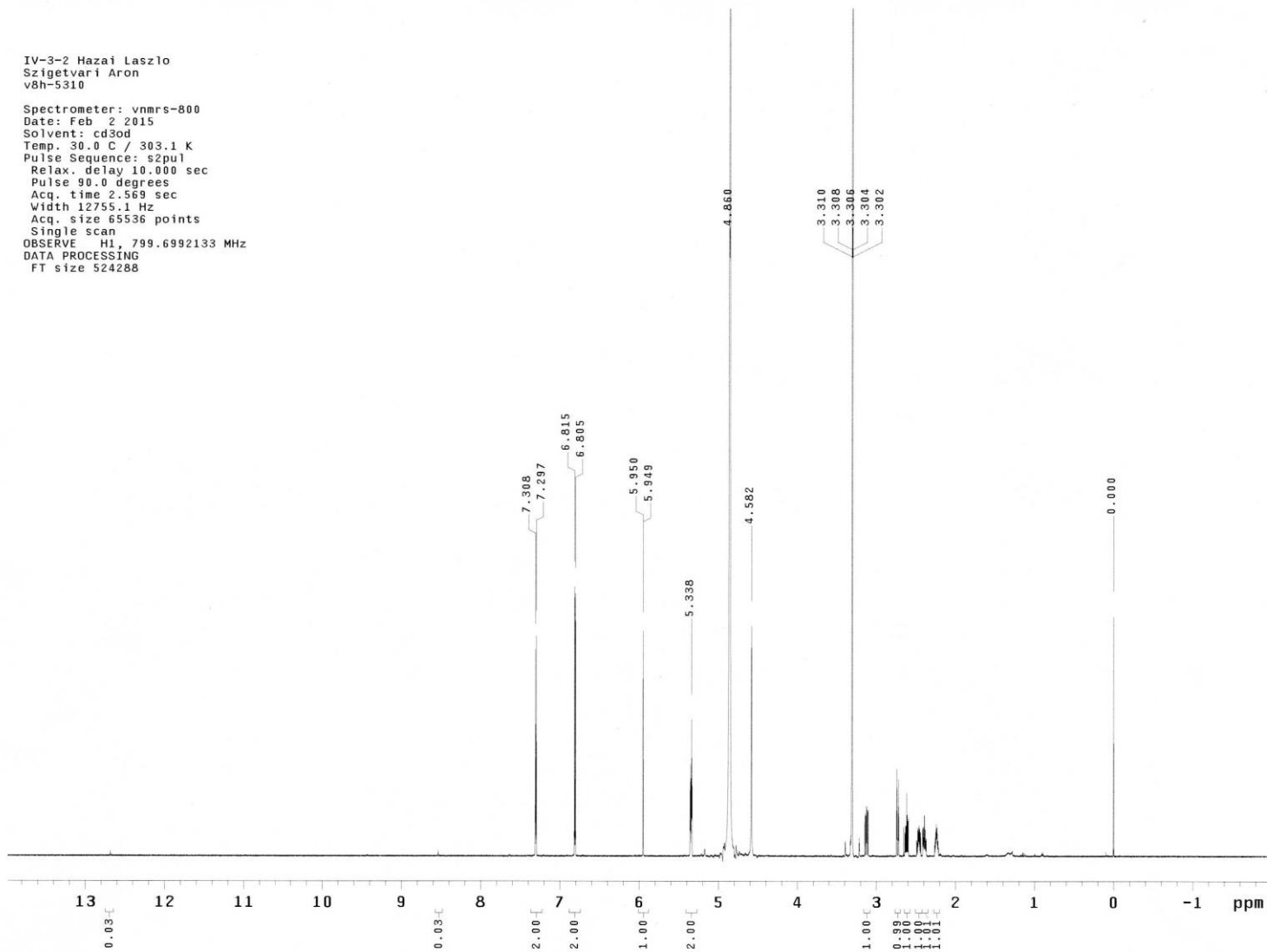


Figure S31: ^1H NMR spectrum of (\pm) -2a-d.

IV-3-2 Hazai Laszlo
Szigetvari Aron
v8h-5310
Spectrometer: vnmrs-800
Date: Feb 2 2015
Solvent: cd3od
Temp. 30.0 C / 303.1 K
Pulse Sequence: s2pul
Relax. delay 10.000 sec
Pulse 90.0 degrees
Acq. time 2.569 sec
Width 12755.1 Hz
Acq. size 65536 points
Single scan
OBSERVE H1, 799.6992133 MHz
DATA PROCESSING
FT size 524288

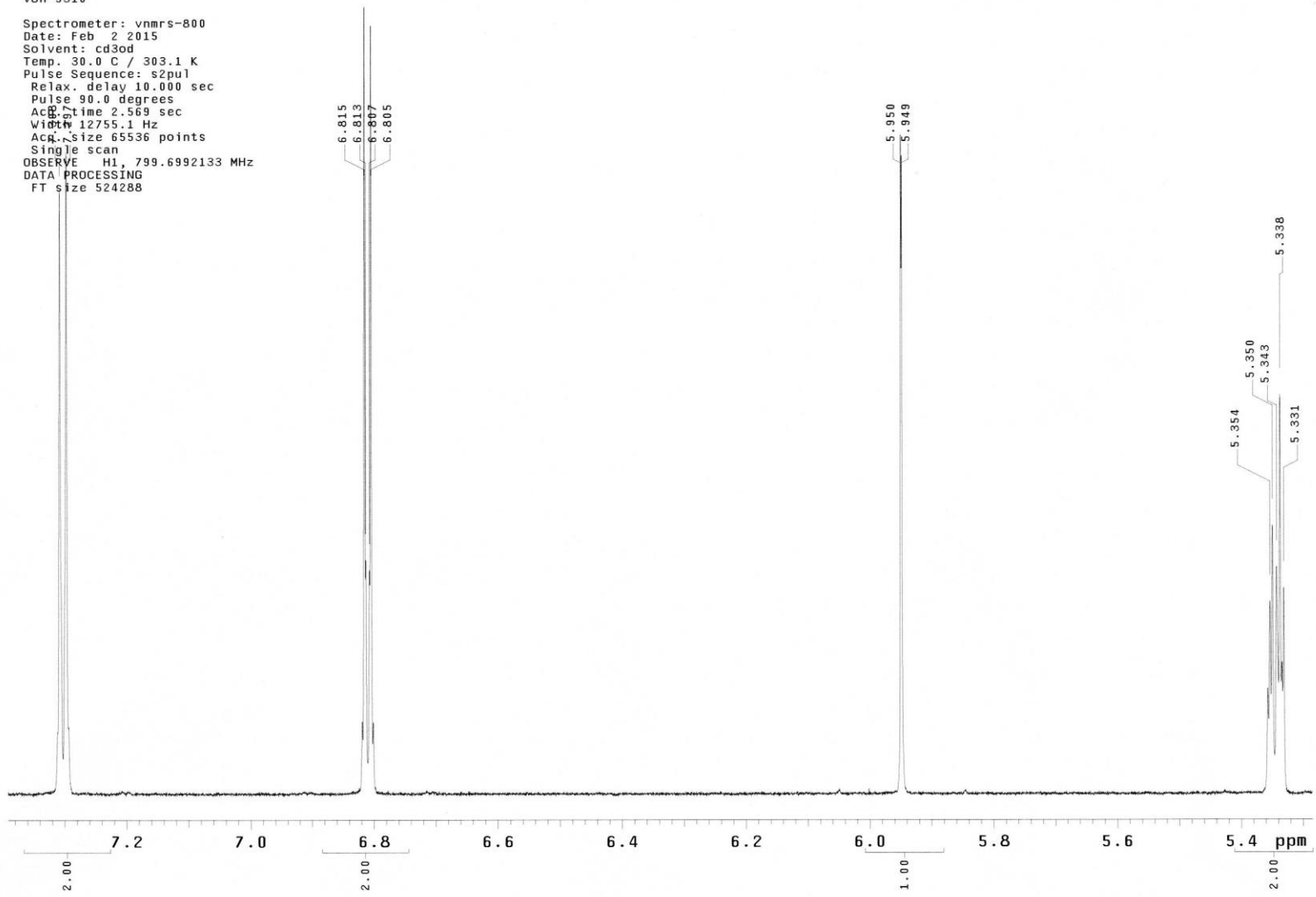


Figure S32: ¹H NMR spectrum of (±)-2a-d – zoom from 5.30 to 7.38 ppm.

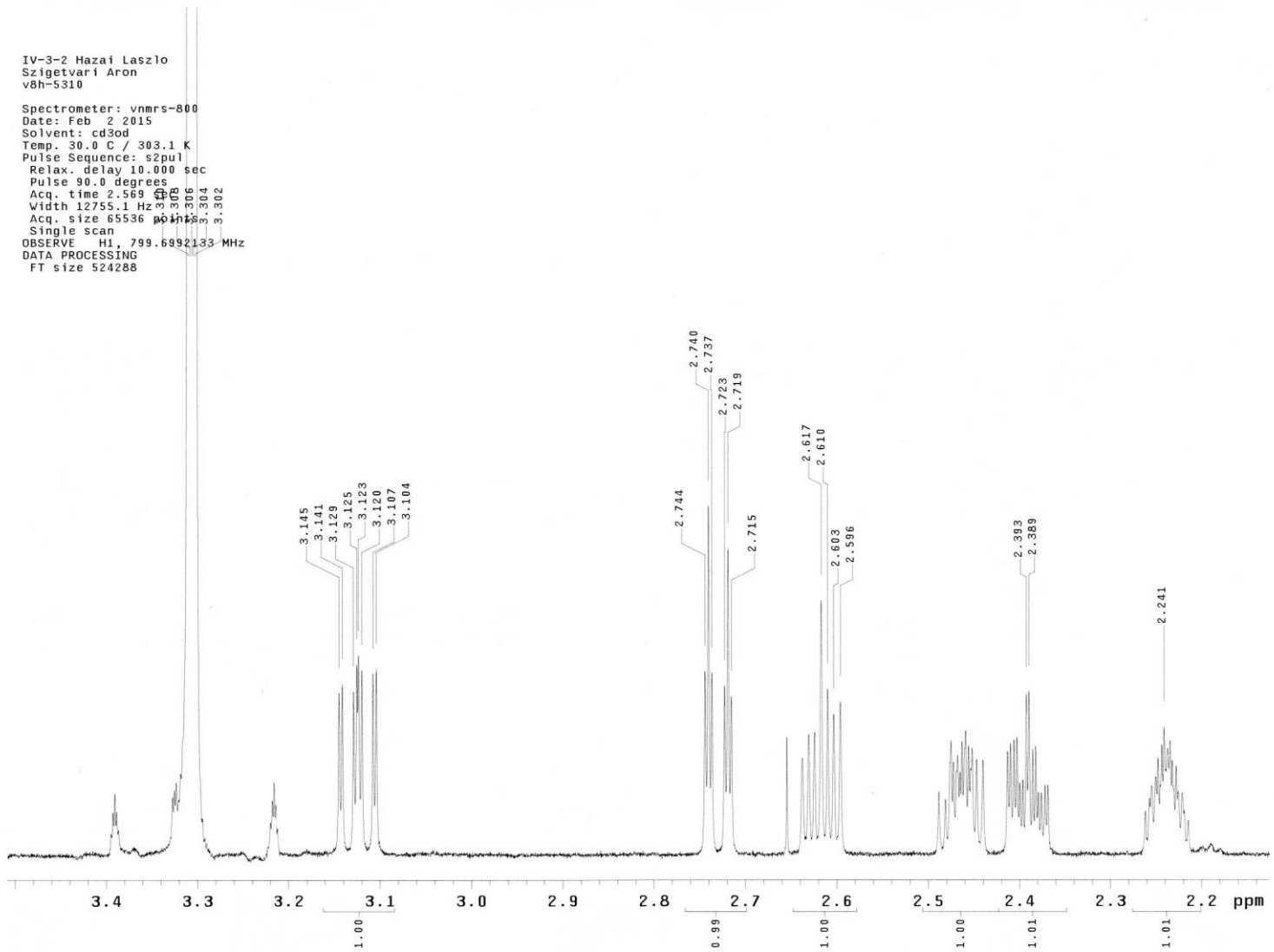


Figure S33: ^1H NMR spectrum of (\pm)-**2a-d** – zoom from 2.14 to 3.50 ppm.

IV-3-2 Hazai Laszlo
Szigetvari Aron
v8h-5310

exp39 gCOSY

SAMPLE		FLAGS	
date	Feb 2 2015	hs	nn
solvent	cd3od	sspul	y
sample	hsglv1		5820
ACQUISITION		SPECIAL	
sw	3306.9	temp	30.0
at	0.150	gain	F1
np	992	spin	not used
fb	4000	F2 PROCESSING	(ppm)
ss	32	sb	-0.075
d1	1.000	sbs	not used
nt	24	fn	1024
2D ACQUISITION		F1 PROCESSING	
sw1	3306.9	sb1	-0.039
ni	256	sbs1	not used
d2	0	proc1	lp
PRESATURATION		DISPLAY	
satmode	n	sp	1642.5
wet	n	wp	2796.6
TRANSMITTER		PLOT	
tn	H1	sp1	1545.6
sfrq	799.702	wp1	2945.2
tof	-1046.2	rfl	-1300.2
tpwr	56	rfp	0
pw	6.700	rfl1	-1300.2
GRADIENTS		PLOT	
gzlvIE	4854	wc	180.0
gtE	0.001000	sc	0
EDratio	1.000	wc2	145.0
gstab	0.000500	sc2	8.5
DECOUPLER		vs	22579
dn	C13	th	2
dm	nnn	ai	cdc av

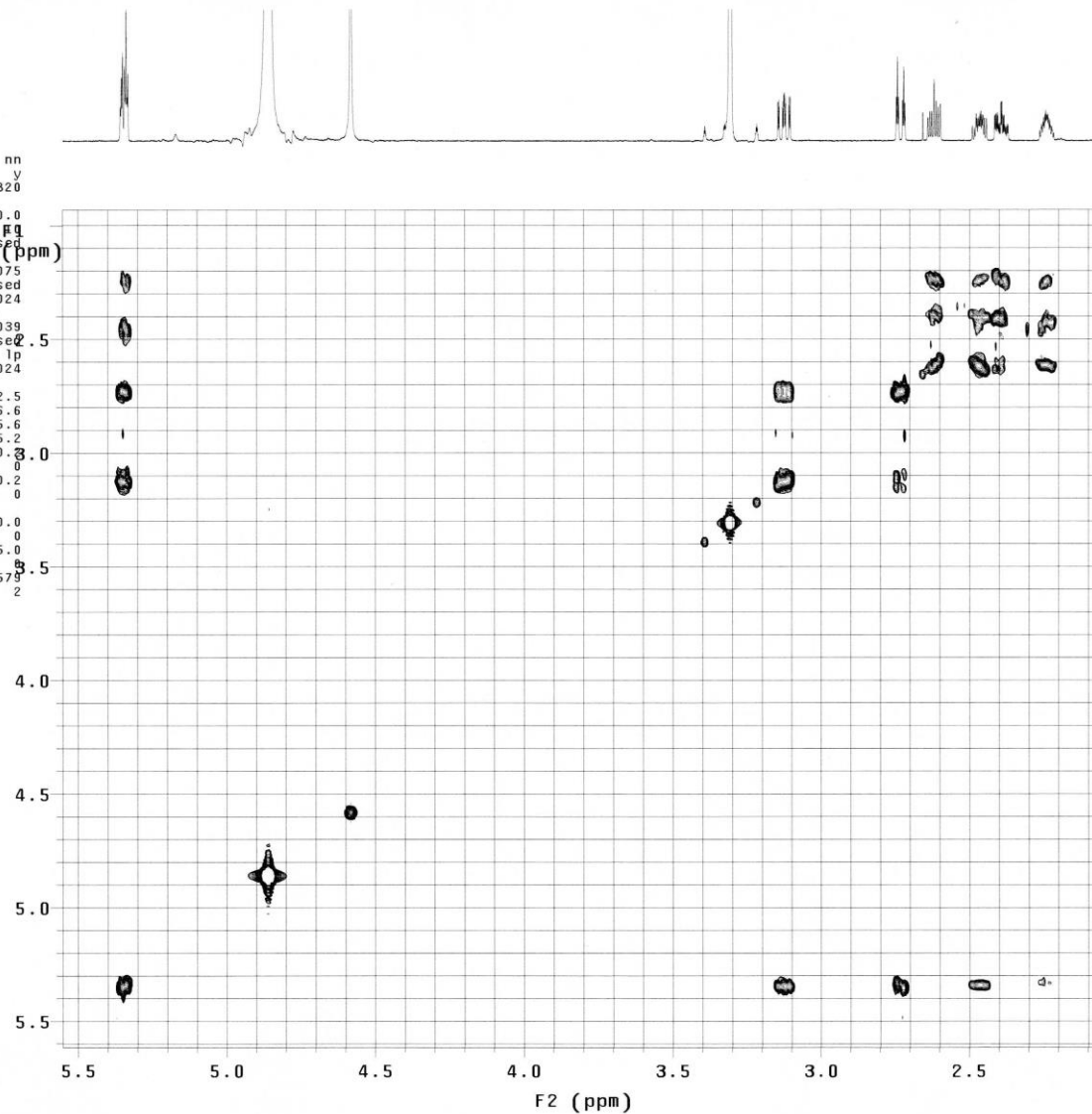


Figure S34: gCOSY spectrum of (±)-2a-d.

IV-3-2 Hazai Laszlo
Szigetvari Aron
v8h-5310

exp38 NOESY

SAMPLE		FLAGS	
date	Feb 2 2015	hs	nn
solvent	cd3od	sspu1	y
sample		PFGflg	y
ACQUISITION		hsglv1	5820
sw	5186.7	SPECIAL	
at	0.150	temp	30.0
np	1556	gain	20
fb	4000	spin	not used
ss	32	F2 PROCESSING	
d1	1.000	sb	-0.150
nt	32	sbs	-0.150
2D ACQUISITION		fn	2048
sw1	5186.7	F1 PROCESSING	2.5
ni	200	sb1	-0.039
TRANSMITTER		sbs1	-0.039
tn		procl	lp
sfrq	799.703	fni	2048
tof	-119.6	DISPLAY	3.0
tpwr	56	sp	1291.9
pw	6.700	wp	5181.7
NOESY		sp1	1291.9
mixN	0.200	wp1	5181.7
PRESATURATION		rfl	-1286.9
satmode	n	rfp	0
wet	n	rfl1	-1286.9
DECOUPLER		rfp1	0
dn	C13	PLOT	4.0
dm	nnn	wc	180.0
		sc	0
		wc2	145.0
		sc2	0
		vs	798.9
		th	2
		ai	cdc ph

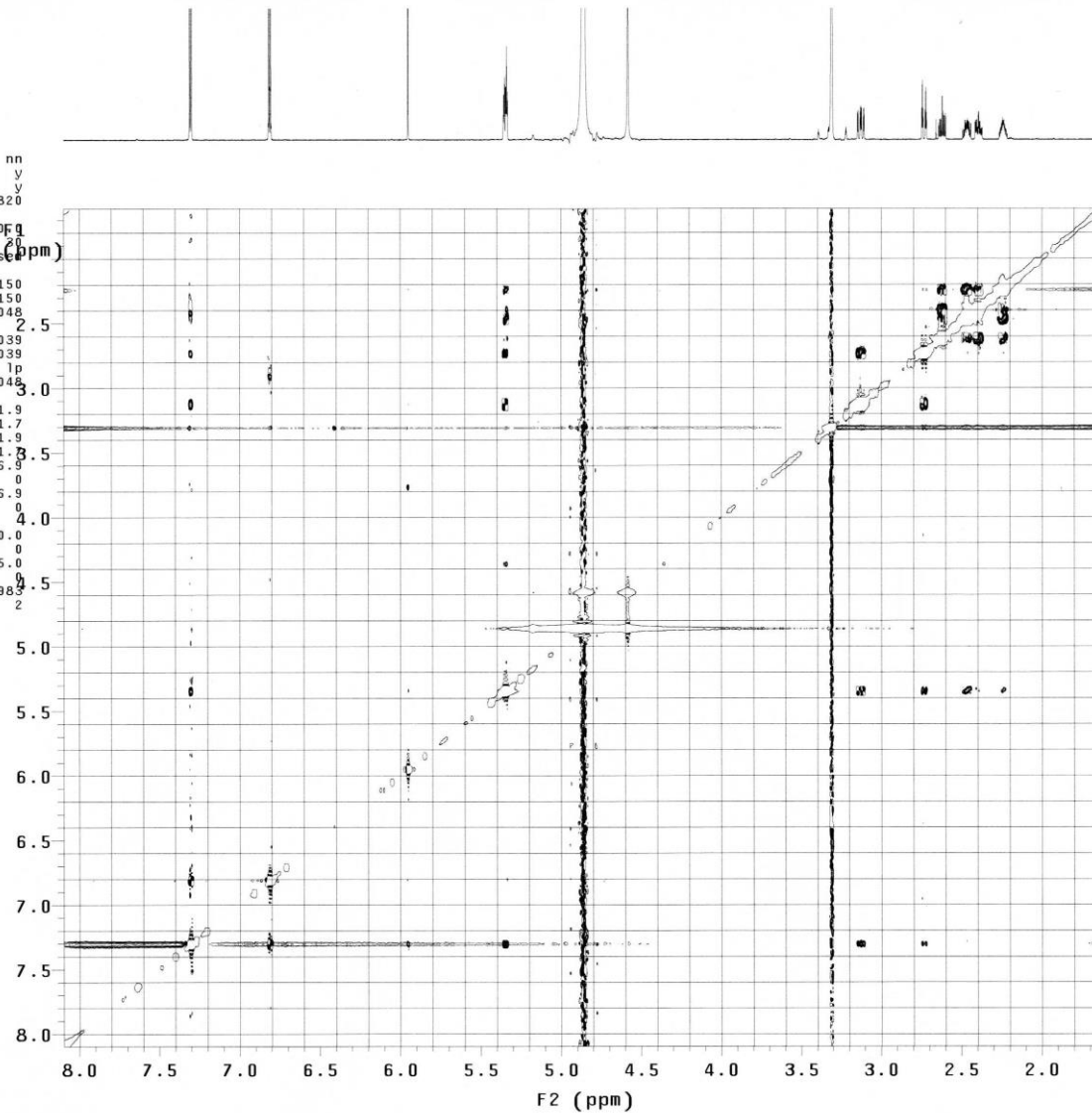


Figure S35: NOESY spectrum of (±)-2a-d.

IV-3-2 Hazai Laszlo
 Szigetvari Aron
 v8h-5310

exp33 gHSQCAD

SAMPLE		FLAGS		ACQUISITION		ARRAYS	
date	Feb 2 2015	hs	nn	array	phase	1	1
solvent	cd3od	sspu1	y	arraydim	1	2	2
sample		PFGflg	5820				
		hsglvt					
ACQUISITION		SPECIAL		i		phase	
sw	7022.5			1	1	30	
at	0.200	temp	30.0	2	2		
np	2808	gain	30				
fb	4000	spin	not used				
ss	32	GRADIENTS					
d1	1.000	gzlv1E	4854				
nt	2	gtE	0.002000				
2D ACQUISITION		EDratio		3.974			
sw1	29154.5	gstab	0.000500				
ni	96	F2 PROCESSING					
phase	arrayed	sb	-0.200				
PRESATURATION		sbs	-0.200				
satmode	n	fn	4096				
wet	n	F1 PROCESSING					
TRANSMITTER		sb1	-0.003				
tn	H1	sbs1	-0.003				
sfrq	799.702	proci	1p				
tof	-1102.9	fni	2048				
tpwr	56	DISPLAY					
pw	6.700	sp	1744.8				
DECOUPLER		wp	4227.9				
dn	C13	sp1	4579.7				
dof	-5484.1	wp1	22463.8				
dm	nny	rf1	614.3				
decwave	W40_CP_8880	rfp	0				
dmf	41667	rf11	716.0				
dpr	42	rfp1	0				
px1vl1	58	PLOT					
pxw	14.300	wc	150.0				
	HSQC	sc	0				
j1xh	146.0	wc2	155.0				
nullflg	y	sc2	0				
mult	2	vs	11189				
ADIABATIC		th	2				
pxw180ad	CP_8880_a~	ai	cdc	ph			
	d300						
pxw180adr	CP_8880~						
	ad300R						
pxw180	400.4						
pxw1vl180	56						
pxw180ref	CP_8880~						
	ref200						
pxw180r	2000.8						
pxw1vl180r	47						

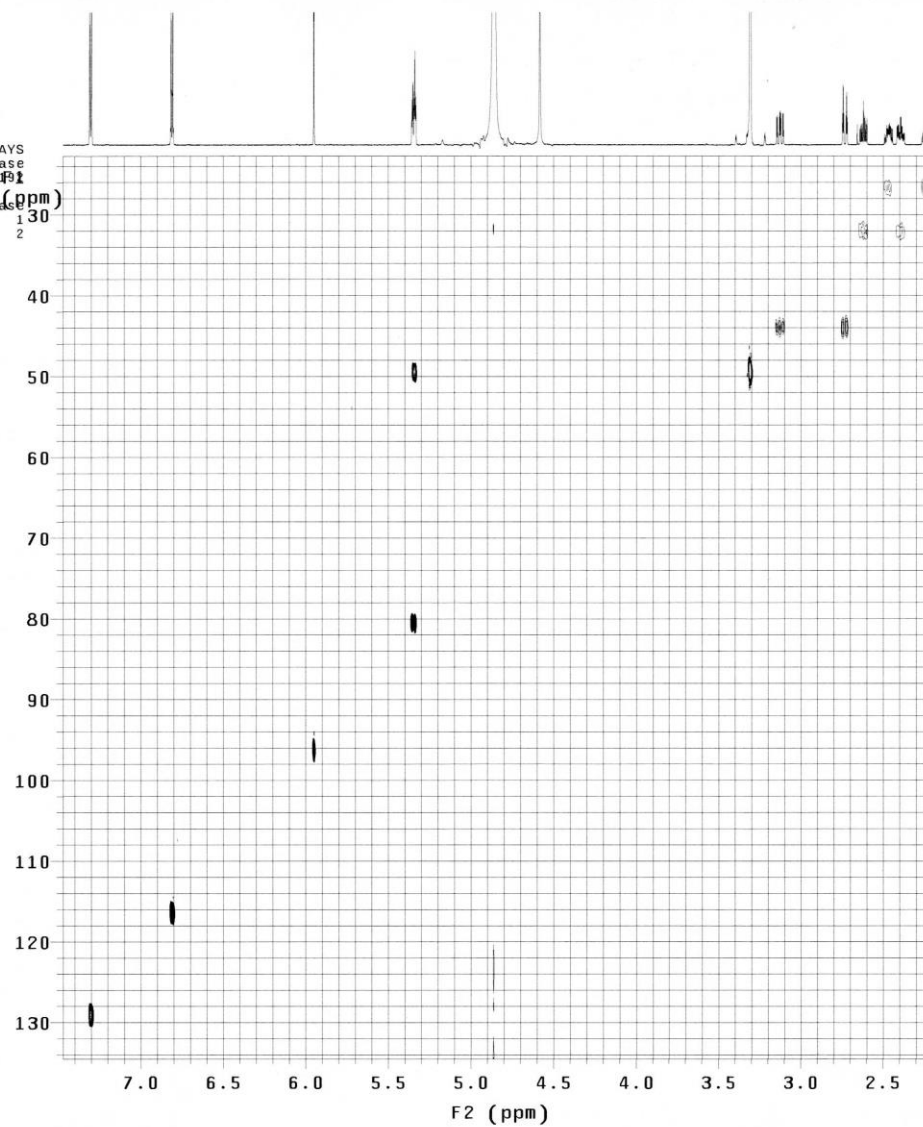


Figure S36: gHSQCad spectrum of (±)-2a-d.

IV-3-2 Hazai Laszlo
Szigetvari Aron
v8h-5310

exp34 gHMBCAD

SAMPLE		FLAGS		ACQUISITION		ARRAYS	
date	Feb 2 2015	hs	nn	array	phase	1	
solvent	cd3od	sspu1	y	arraydim	400		
sample		PFGflg	y				
		hsglv1	5820	i	phase	1	
ACQUISITION		SPECIAL		1			
sw	6983.2			2			
at	0.147	temp	30.0				
np	2048	gain	10				
fb	4000	spin	not used				
ss	32	GRADIENTS					
d1	1.000	gzlv11	485				
nt	4	gt1	0.001000				
2D ACQUISITION		gzlv13	1455				
sw1	43219.9	gt3	0.001000				
n1	200	gstab	0.000500				
phase	arrayed	F2 PROCESSING					
PRESATURATION	sb	sb	-0.147				
satmode	n	sbs	-0.147				
wet	n	fn	4096				
TRANSMITTER		F1 PROCESSING					
tn	H1	sb1	-0.005				
sfrq	799.702	sbs1	-0.005				
tof	-1057.2	procl	lp				
tpwr	56	fn1	2048				
pw	6.700	DISPLAY					
DECOUPLER		sp	1578.7				
dn	C13	wp	4466.8				
dof	1554.8	sp1	3876.1				
dm	nnn	wp1	36762.2				
decwave	W40_CP_8880	rf1	549.0				
dmf	41667	rfp	0				
dpr	42	rf11	724.5				
pxxlv1	58	rfp1	0				
pxx	14.300	PLOT					
HMBC		wc	155.0				
j1xh	146.0	sc	0				
jnxh	8.0	wc2	145.0				
ADIABATIC		sc2	0				
pxx180ad	CP_8880_a~	vs	9408				
	d300	th	2				
pxxlv1180	56	ai	cdc av				
pxx180	400.4						

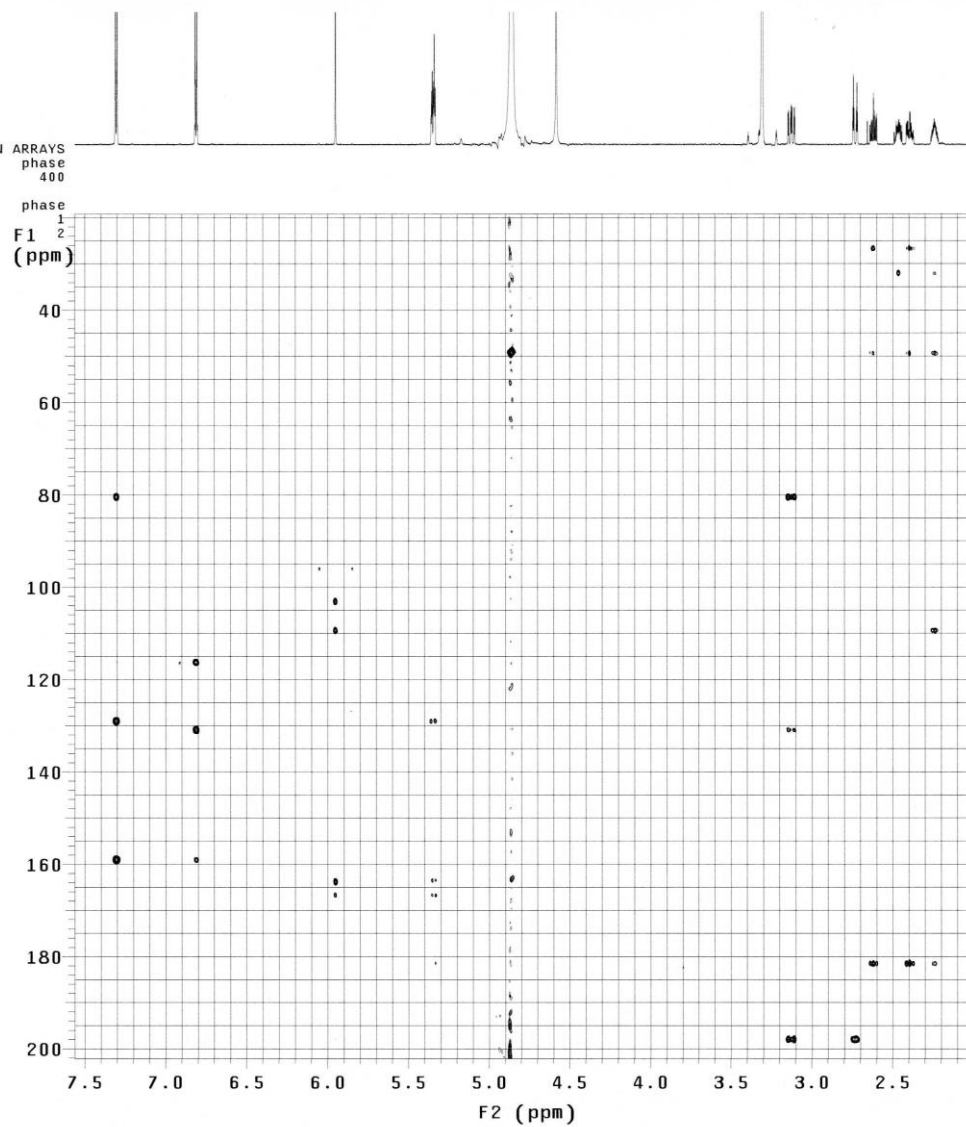


Figure S37: gHMBCad spectrum of (±)-2a-d.

IV-3-2 Hazai Laszlo
Szigetvari Aron
v8c-2114

Spectrometer: vnmrs-800
Date: Feb 2 2015
Solvent: cd3od
Temp. 30.0 C / 303.1 K
Pulse Sequence: s2pul
Relax. delay 1.000 sec
Pulse 90.0 degrees
Acq. time 1.311 sec
Width 50000.0 Hz
Acq. size 131072 points (Re+Im)
12000 repetitions
OBSERVE C13, 201.0842309 MHz
DECOUPLE H1, 799.7032131 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072

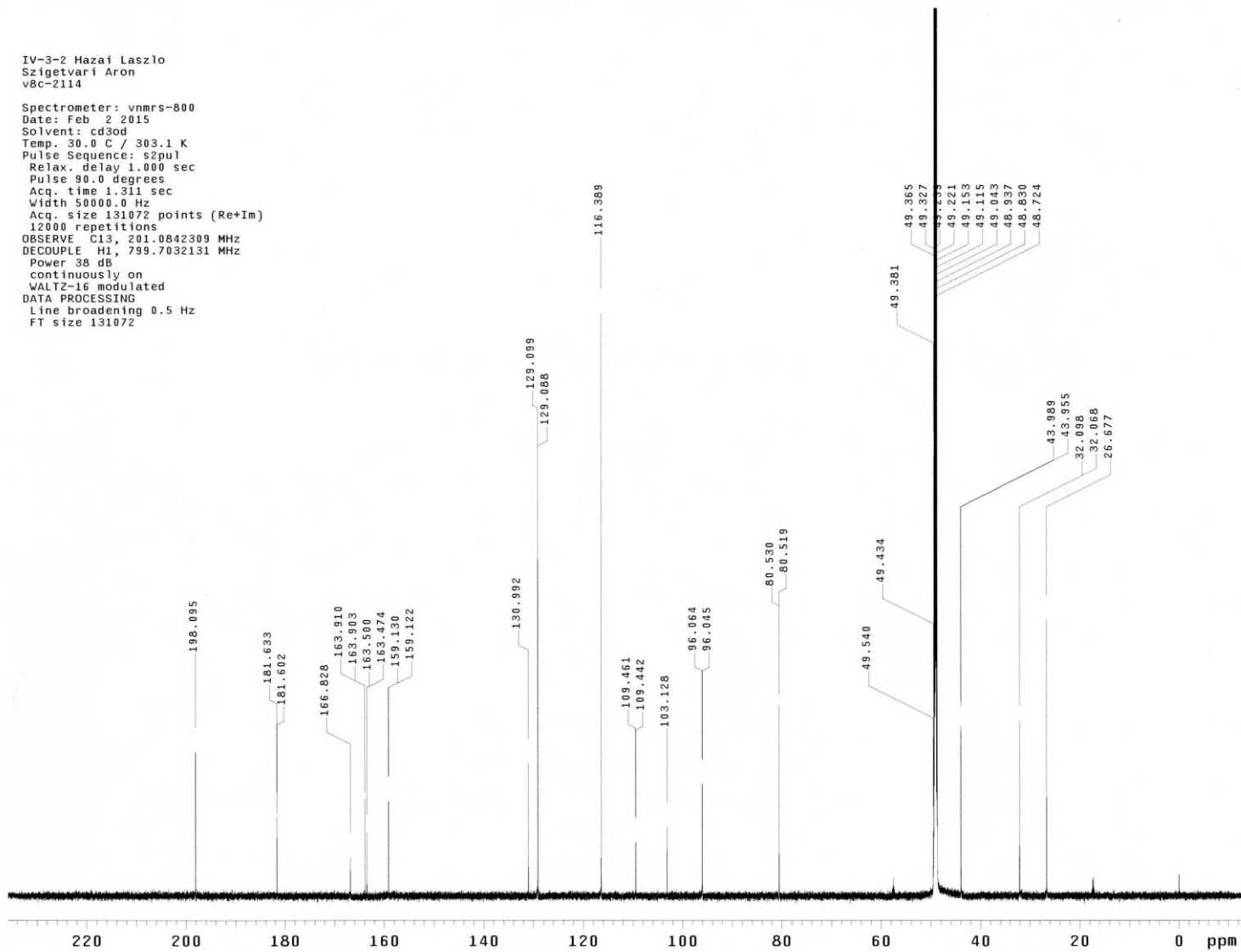


Figure S38: ^{13}C NMR spectrum of (\pm)-2a-d.