"Flurbiprofen: A Study of the Behavior of the Scalemate by Chromatography, Sublimation, and NMR"

Supplementary Material

Magdalena Kwiatkowska[1], Alicja Wzorek[1,*], Anna Kołbus[1], Mariusz Urbaniak[1], Jianlin Han[2], Vadim A. Soloshonok[3,4] and Karel D. Klika[5,*]

[1] Institute of Chemistry, Jan Kochanowski University in Kielce, Uniwersytecka 7, 25-406 Kielce, Poland; magdalena.kwiatkowska@ujk.edu.pl (M.K.); awzorek@ujk.edu.pl (A.W.); anna.kolbus@ujk.edu.pl (A.K.); mariusz.urbaniak@ujk.edu.pl (M.U.)

[2] College of Chemical Engineering, Nanjing Forestry University, Nanjing, 210037 Jiangsu, China; hanjl@njfu.edu.cn

[3] Department of Organic Chemistry I, Faculty of Chemistry, University of the Basque Country UPV/EHU, Paseo Manuel Lardizábal 3, E-20018 San Sebastián, Spain; vadym.soloshonok@ehu.eus

[4] IKERBASQUE, Basque Foundation for Science, Alameda Urquijo 36-5, Plaza Bizkaia, E-48011 Bilbao, Spain

[5] Molecular Structure Analysis, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany; k.klika@dkfz.de

* Correspondence: awzorek@ujk.edu.pl (A.W.); k.klika@dkfz.de (K.D.K.); Tel.: +48-41-349-7019 (A.W.); +49-6221-42-4515 (K.D.K.)

Abstract: 2-(2-Fluoro-4-biphenyl)propionic acid (flurbiprofen), from the phenylalkanoic acid family of nonsteroidal anti-inflammatory drugs (NSAID's), is currently on the pharmaceutical market as a racemate. This racemic compound was tested for its propensity to undergo the self-disproportionation of enantiomers (SDE) phenomenon by various forms of chromatography (SDEvC) such as routine gravity-driven column chromatography, medium-pressure liquid chromatography (MPLC), preparative thin-layer chromatography (PTLC), and size-exclusion chromatography (SEC), and also by sublimation (SDEvS). Furthermore, examination by nuclear magnetic resonance (NMR) in various solvents found that flurbiprofen exhibited the phenomenon of self-induced diastereomeric anisochronism (SIDA). By measurement of the diffusion coefficient (D), the longitudinal relaxation time (T_1), and the transverse relaxation time (T_2) using NMR, as well as by electrospray ionization-mass spectrometry (ESI-MS) examinations, the preferred intermolecular association was found to be solvent dependent, e.g. heterochiral association was preferred in toluene while homochiral association was preferred in more polar solvents. This study also attempted, unsuccessfully, to correlate the NMR measurements of flurbiprofen with chromatographic outcomes for the rationalization and prediction of chromatographic results based on NMR measurements. Since the intermolecular hydrogen bonding of the acid groups in flurbiprofen overwhelmingly predominates over other intermolecular interactions, flurbiprofen seemed to represent a good test case for this idea. The behavior of scalemic samples of flurbiprofen is important as although it is currently dispensed as a racemate, clinical applications of the *R* enantiomer have been investigated. Both SDEvC and SDEvS have ramifications for the preparation, handling, and storage of enantioenriched flurbiprofen, and this concern applies to other chiral drugs as well.

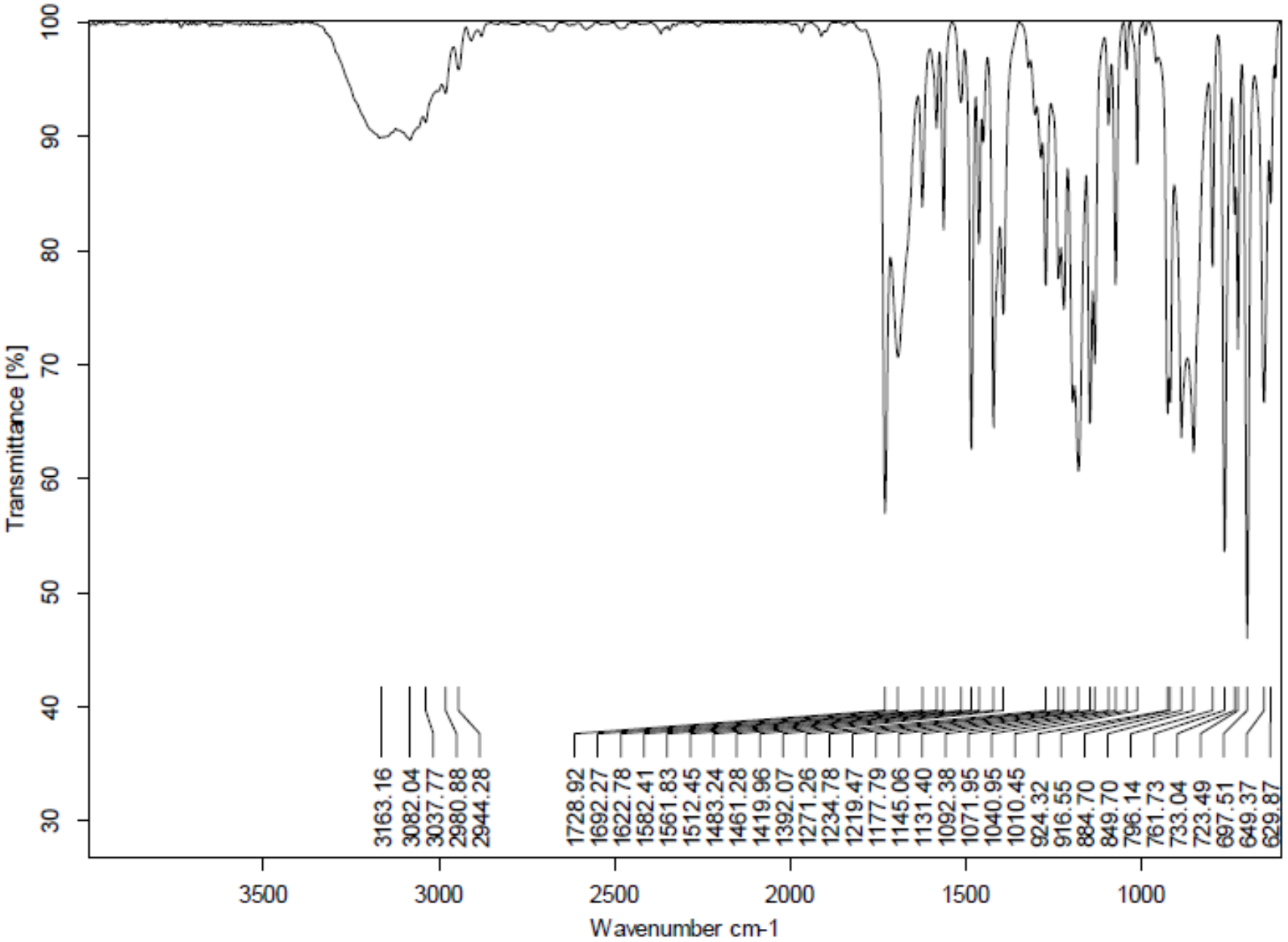


Figure S1. IR spectrum of (*R*)-flurbiprofen.

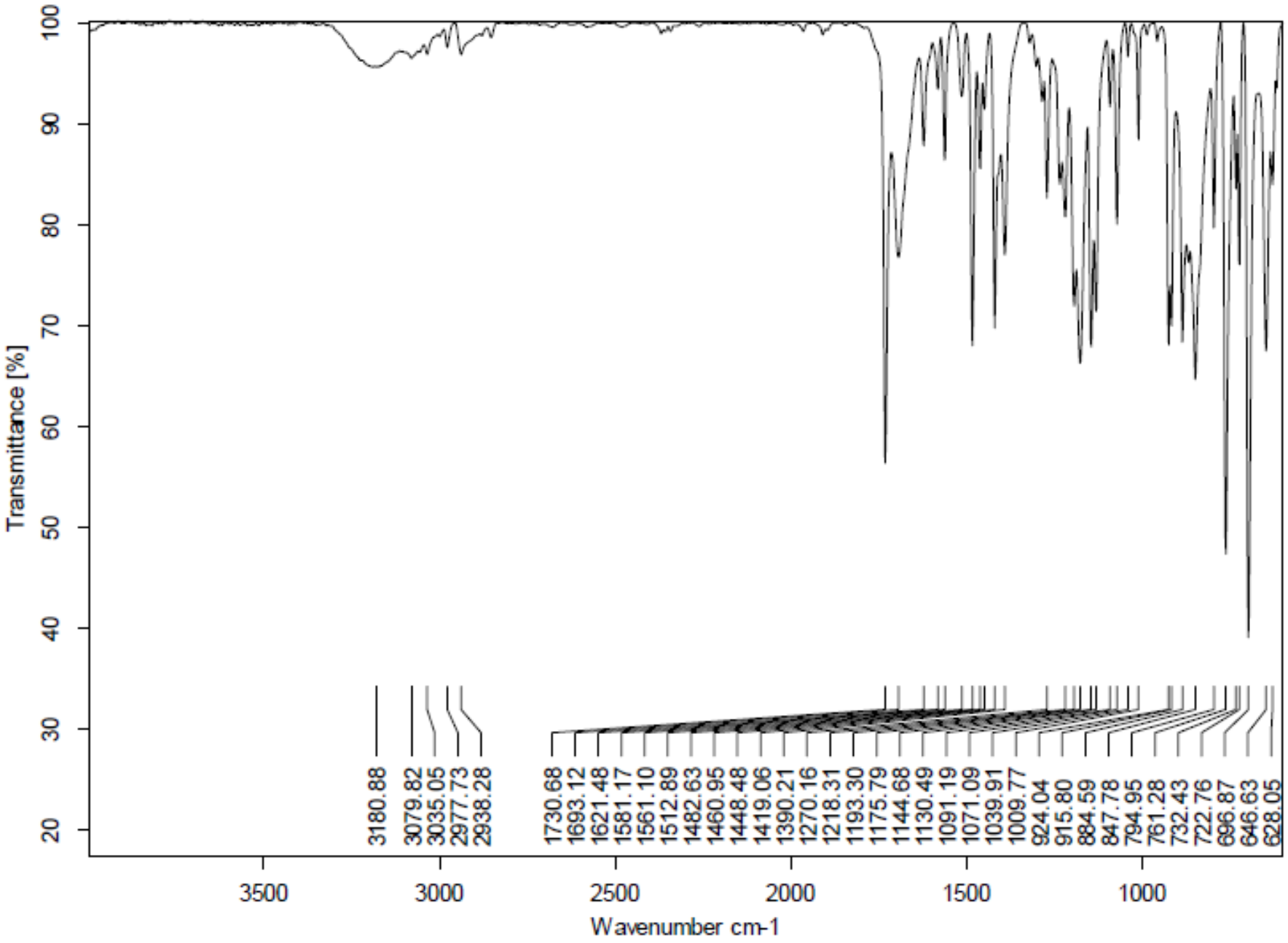


Figure S2. IR spectrum of (*S*)-flurbiprofen.

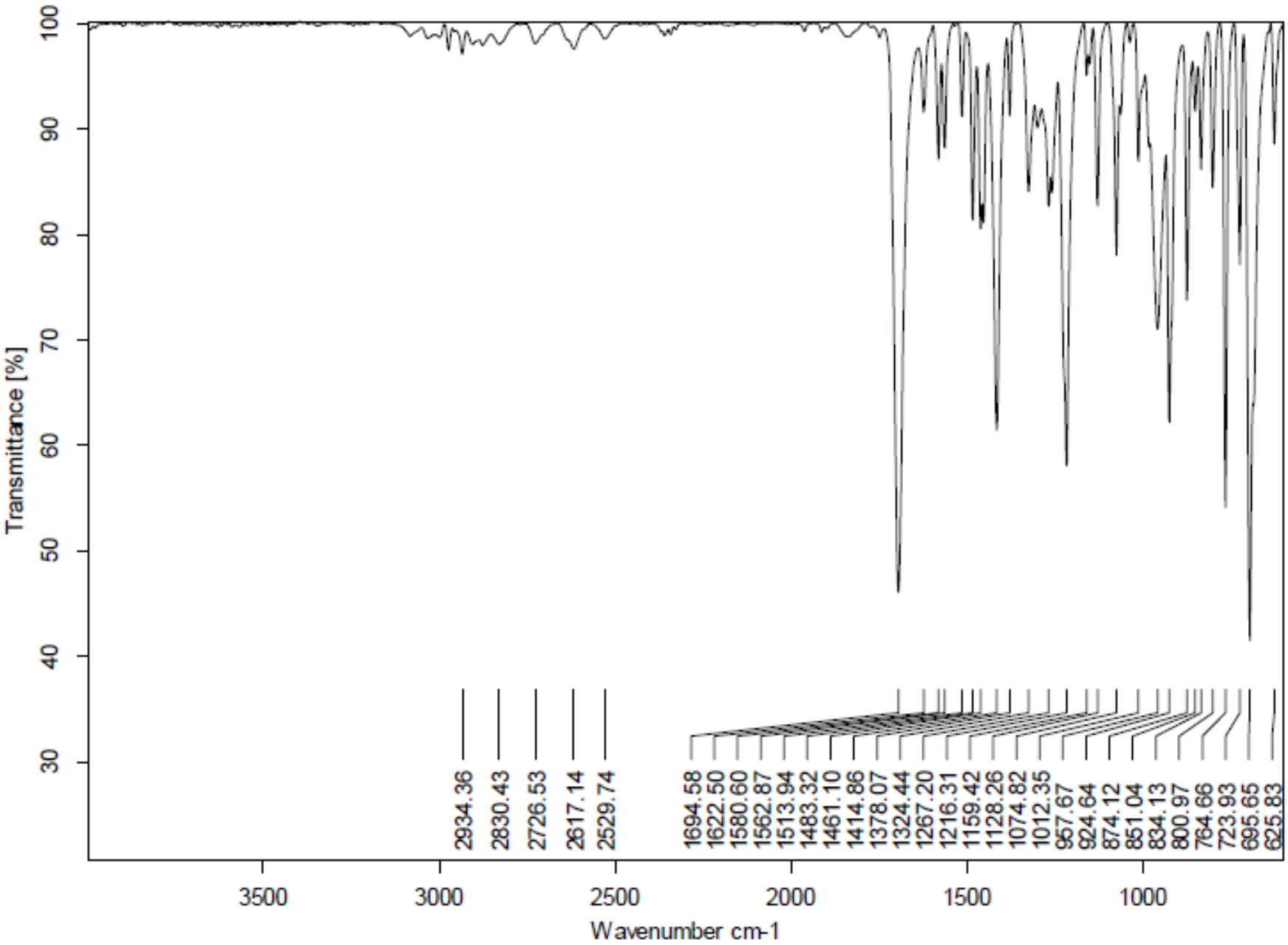


Figure S3. IR spectrum of (*rac*)-flurbiprofen.

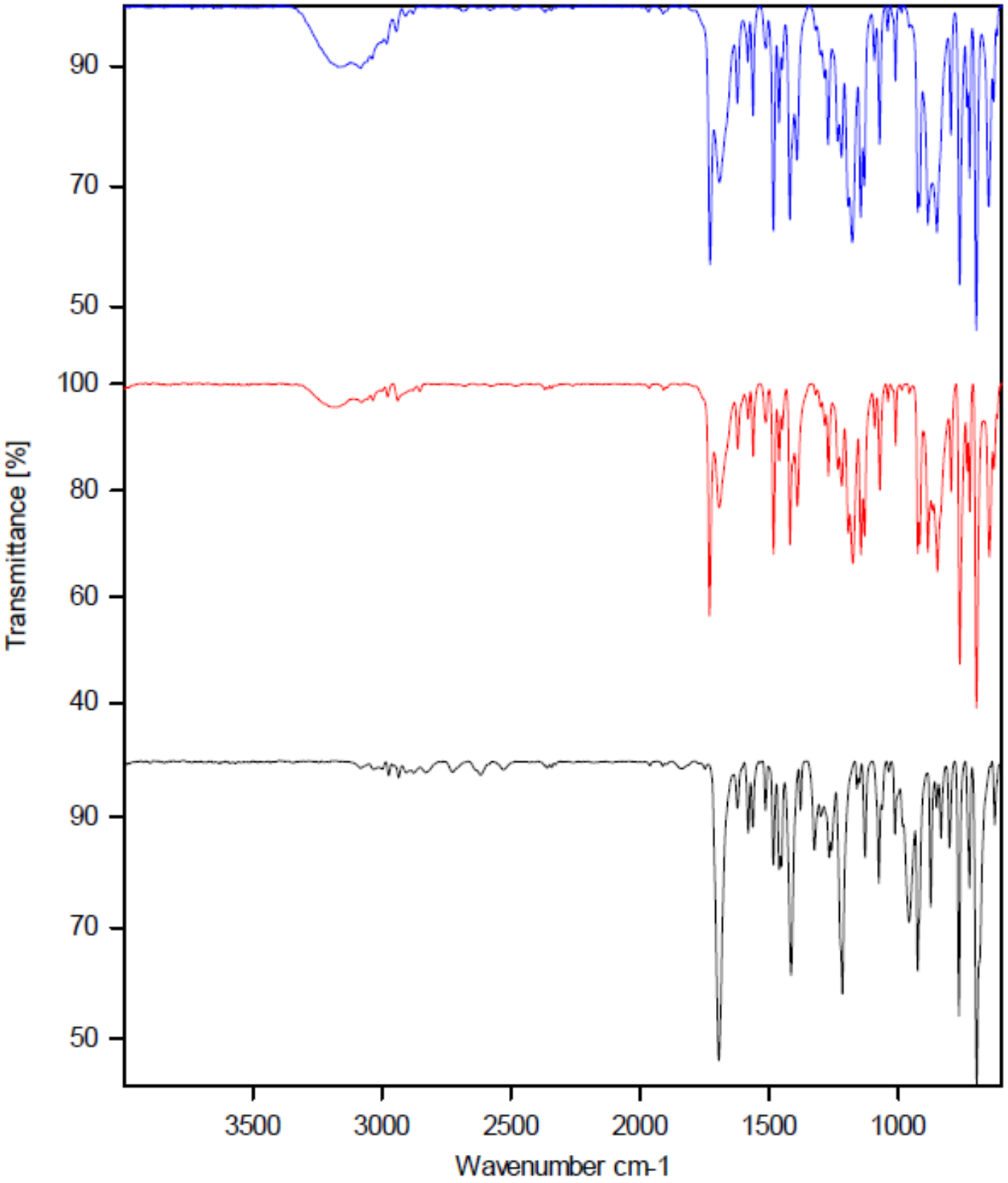


Figure S4. IR spectra of: blue – (*R*)-flurbiprofen, red – (*S*)-flurbiprofen, black – (*rac*)-flurbiprofen.

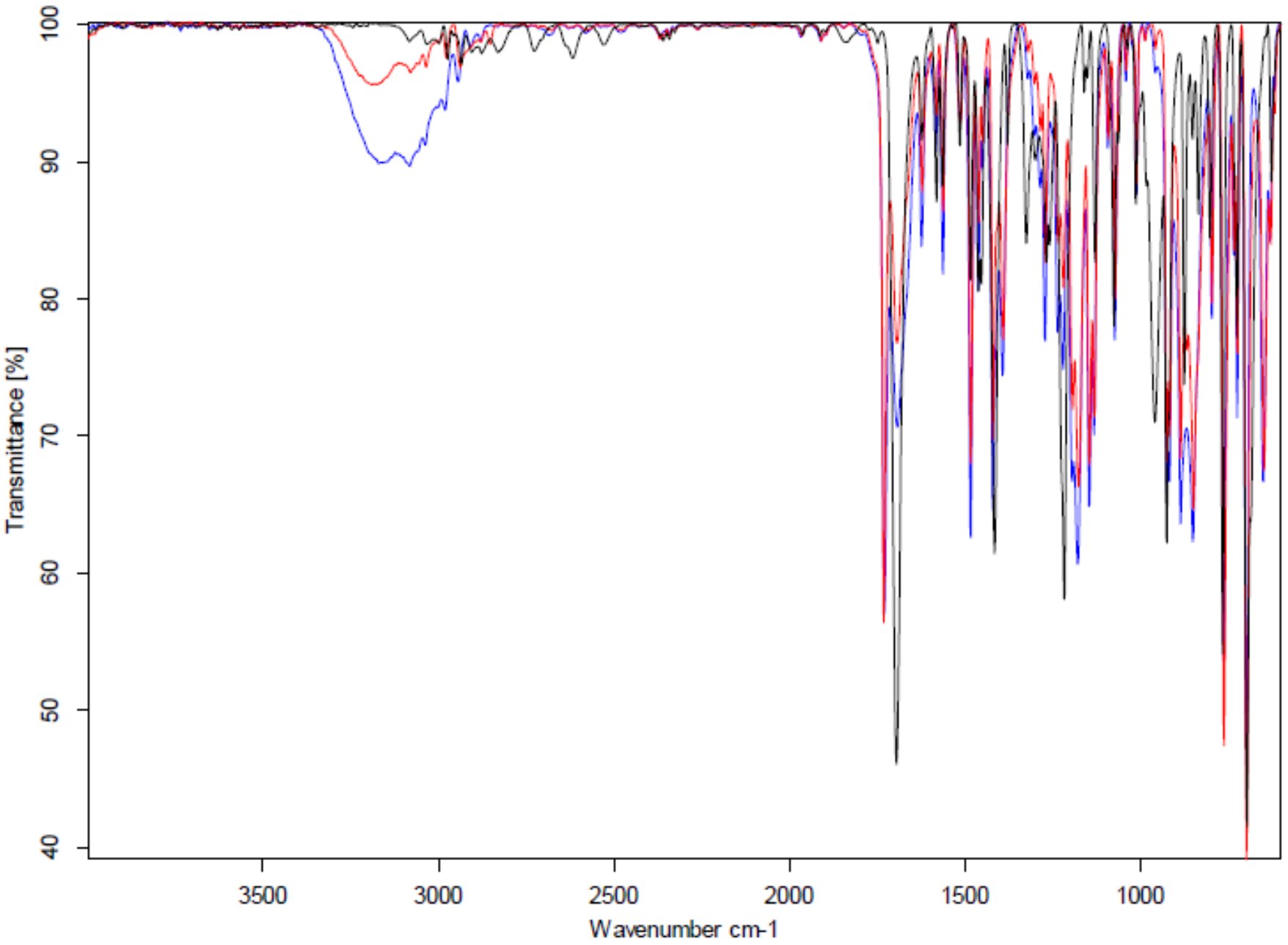


Figure S5. IR spectra of: blue – (*R*)-flurbiprofen, red – (*S*)-flurbiprofen, black – (*rac*)-flurbiprofen.

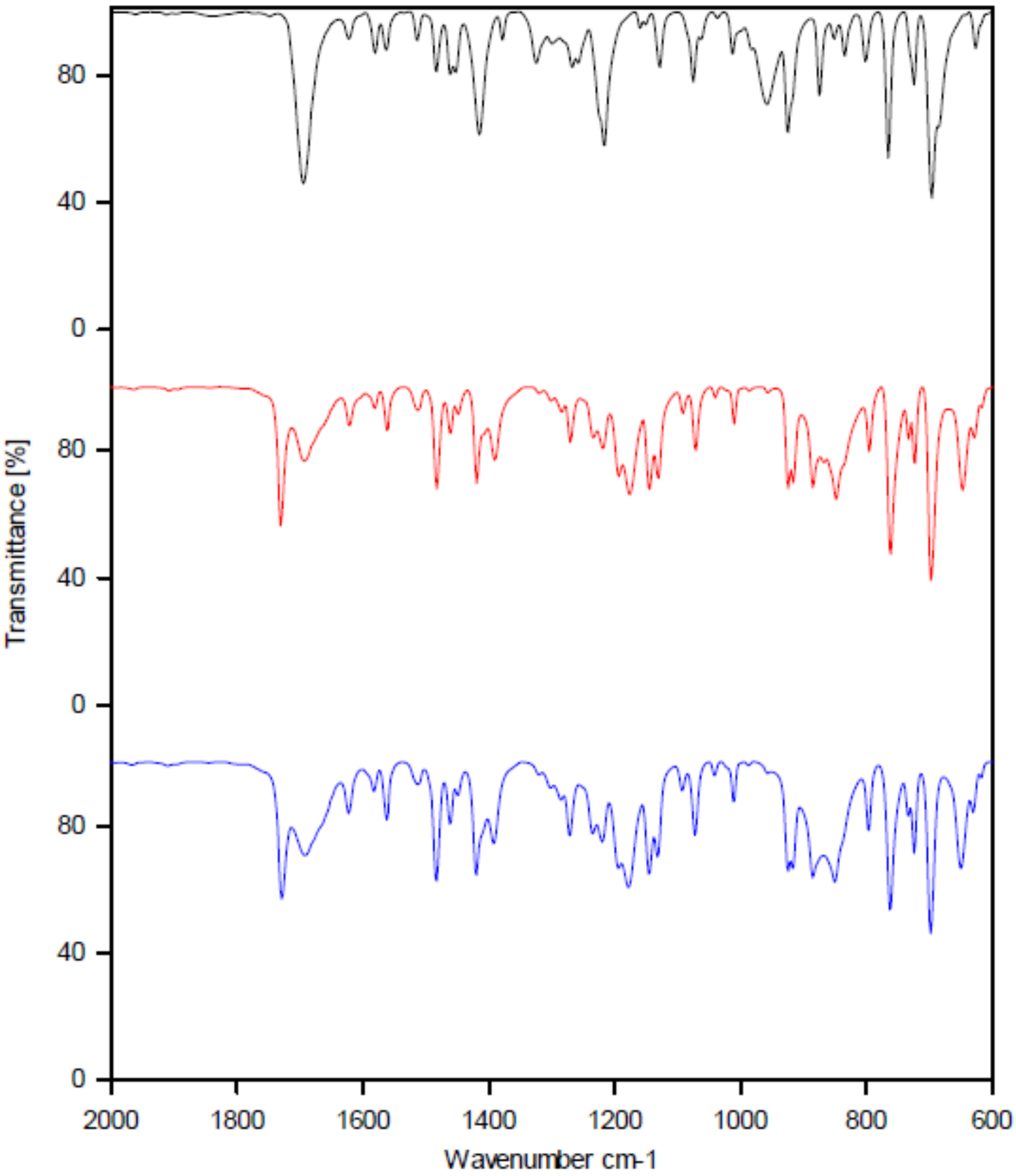


Figure S6. IR spectra of: blue – (*R*)-flurbiprofen, red – (*S*)-flurbiprofen, black – (*rac*)-flurbiprofen.

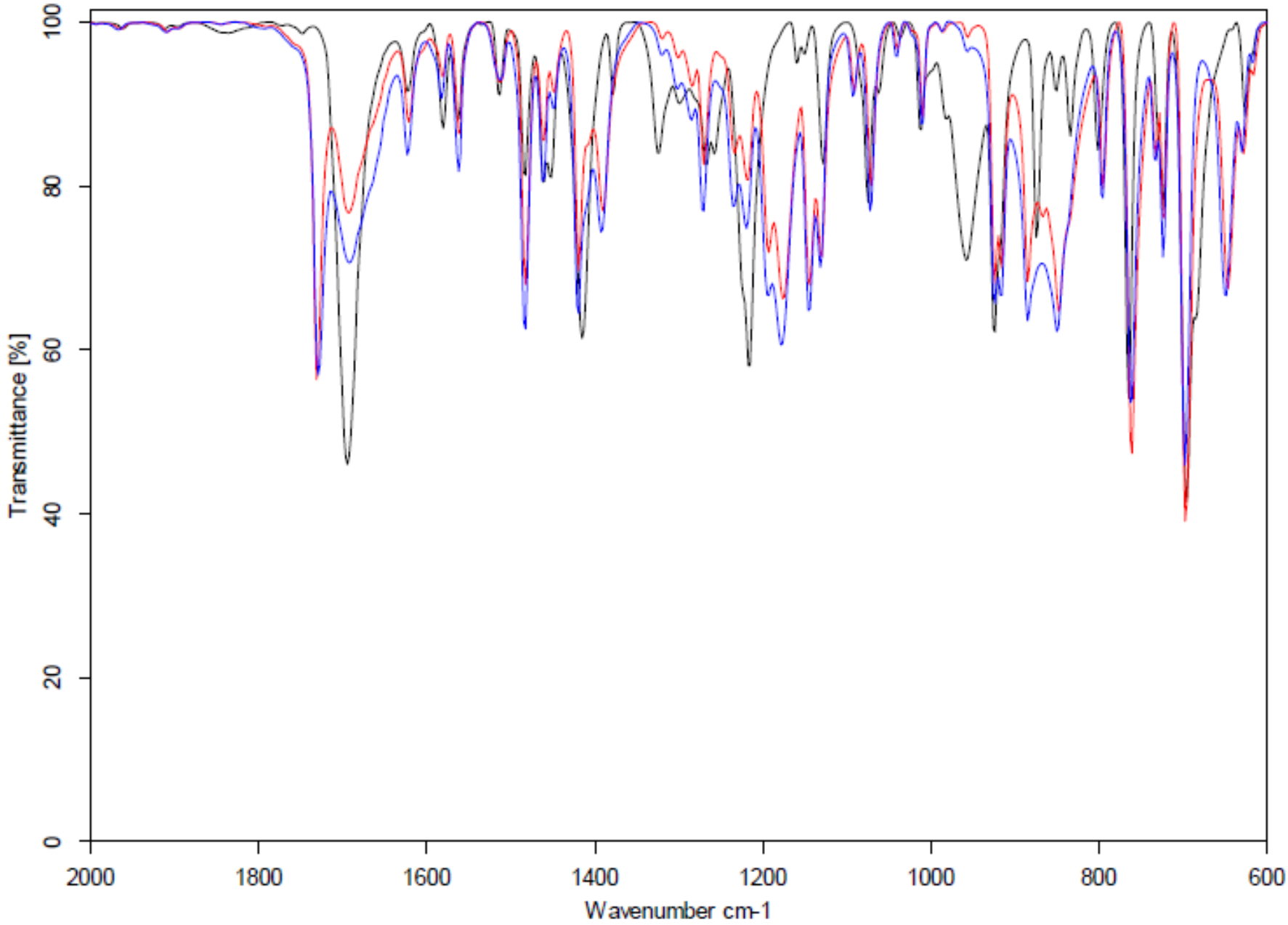


Figure S7. IR spectra of: blue – (*R*)-flurbiprofen, red – (*S*)-flurbiprofen, black – (*rac*)-flurbiprofen.

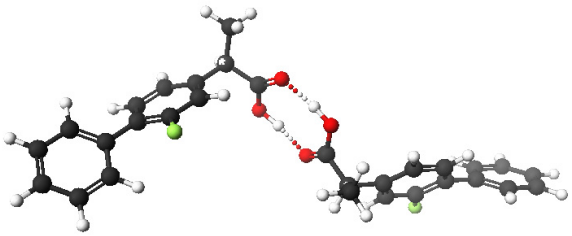
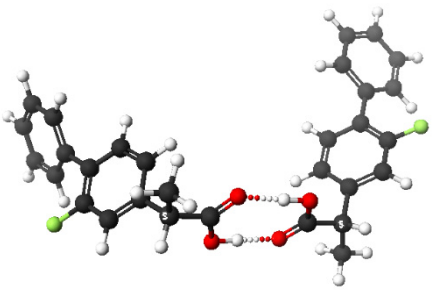
Table S1. Diastereomeric salt crystallization of flurbiprofen with chiral amines in ethanol

run	amine	sample ee, %	sample mass, mg	product ee, %	product mass, mg
1	(*S*)-1-cyclohexyl ethylamine	0.0	200.0	87.0, *S*	20.0
2	(*S*)-1-cyclohexyl ethylamine	77.0, *S*	100.0	95.0, *S*	30.0
3	(*S*)-1-cyclohexyl ethylamine	87.0, *S*	200.0	97.0, *S*	70.0
4	(*R*)-1-cyclohexyl ethylamine	55.0, *R*	150.0	89.0, *R*	60.0
5	(*R*)-1-phenyl ethylamine	55.0, *R*	150.0	80.0, *R*	60.0

Table S2. DFT-calculated energies of geometry optimized dimeric flurbiprofen associates at the B88LYP/GGA level of theory

Structure	Absolute energy, kcal/mol	Relative energy, kcal/mol
RO_RO	−1,041,193.700	1.08
SO_SO	−1,041,194.780	0.00
RO_SO	−1,041,192.140	2.64
RF_RF	−1,041,096.480	98.30
RO_RF	−1,041,180.510	14.27

Table S3. Structures of optimized dimeric associates

Homochiral dimer RO−RO	
Homochiral dimer SO−SO	

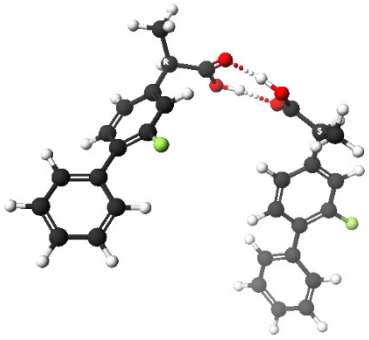
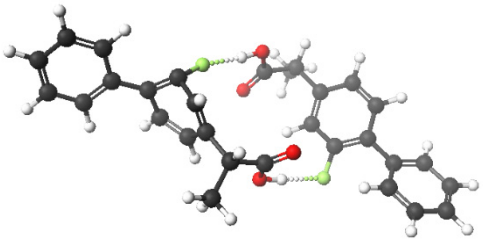
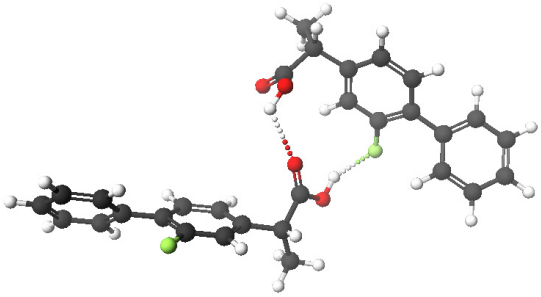
Heterochiral dimer RO−SO	
Homochiral dimer RF−RF	
Homochiral dimer RO−RF	

Table S4. Sublimation of (*scl*)-flurbiprofen

run	*T*, °C	*P*, mbar	time, hours	sample		residue		sublimate		Δee, %
				ee, %	wt., mg	ee, %	wt., mg	ee, %	wt., mg	
1	70	ambient	20	74.8	–	–	–	73.2	–	−1.6*
2	70	0.0–0.2	24	59.8	240.0	58.6	35.0	62.0	205.0	3.4
3	100	0.0–0.2	2	79.6	80.0	80.6	10.0	77.0	70.0	−3.6
4	40	0.0–0.2	76	93.2	27.0	97.8	20.0	85.8	7.0	−12.0
5	40	0.0–0.2	72	59.8	150.0	59.5	–	70.0	6.0	10.5
				continuation:		58.3	–	70.4	–	12.1
6	50	0.0–0.2	44	88.4	70.6	91.0	59.5	84.2	11.1	−6.8
7	60	0.0–0.2	24	88.4	55.0	94.0	41.1	85.8	13.9	−8.2
8	80	0.0–0.2	5	88.4	62.8	93.5	29.0	86.4	33.8	−7.1
9	100	0.0–0.2	5	74.5	–	75.2	–	69.0	–	−6.2
10	70	0.0–0.2	25	75.2	–	77.4	–	–	–	−2.4*
11	90	ambient	20	74.8	–	–	–	73.3	–	−1.5*
12	100	0.0–0.2	5	74.8	–	76.2	–	64.6	–	−11.6

* To the starting material.

Table S5. Summary of NMR experiments

sample, ee %	wt., mg /vol., µL[a]	solvent	^{1}H (400 and 600 MHz)	^{19}F (400 MHz)	^{13}C (100 MHz)	$D^{b} \times 10^{-10}$, m^2s^{-1} (600 MHz)	T_1, T_2 (600 MHz)
rac	21.2/660 + 10-fold dilution	acetonitrile-d_3	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	ditto	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	14.88 i, 14.62 a	–
R						14.97 i, 14.60 a	–
S, 82%	11.3	1,4-dioxane-d_8	only very small or negligible changes between conc. and dilute samples, no SIDA or very, very weak	small change, no SIDA or very, very weak	only very small or negligible changes between conc. and dilute samples, no SIDA or very, very weak	–	–
S, 82%	5.7	1,4-dioxane-d_8				–	–
S, 82%	5.1	$CDCl_3$	SIDA only on CH_3	no SIDA	no SIDA	–	–
rac	10.1	$CDCl_3$	small differences to each other and to the dilute sample generally, but strong association based on $–CO_2H$ proton	little difference to each other, small difference to dilute sample	very little difference to each other and generally to the dilute sample, but strong association based on $–CO_2H$ carbon	12.19 i, 11.71, a 7.24[c] i, 7.19 a[c]	T_1
R						12.10 i, 11.70 a 7.11[c] i, 7.14 a[c]	
S, 82%	5.1	*c*-hexane-d_{12}–MTBE, 4:1	no SIDA	no SIDA	no SIDA	–	–
S, 82%	10.7	*c*-hexane-d_{12}–MTBE, 4:1	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	ditto	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	–	–
rac	8.1	*c*-hexane-d_{12}–MTBE, 4:1	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	ditto	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	7.07 i, 6.11 a	T_1
R	8.1/637					6.81 i, 5.86 a	
rac	10.4/871	*c*-hexane-d_{12}–MTBE, 4:1	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	ditto	conc. and dilute, no SIDA but clear differences between conc. and dilute – aSIDA	–	–
R	10.4/800					–	–
S, 82%	5.3	toluene-d_8	good SIDA on $–CH_3$, no SIDA on –CH, aromatics hard to see	good SIDA	SIDA on $–CH_3$, –CH, $–CO_2H$, and some aromatics	–	–
rac	~10.8	toluene-d_8	small differences to each other and to the dilute sample generally	clearer differences for all 3 samples	very little difference to each other and to the dilute sample but with some exceptions	7.36 i, 7.24 a	T_1, T_2
R						7.49 i, 7.36 a	

[a] Volume 600 µL unless otherwise indicated. [b] Values measured with 5-mm tubes except where indicated, legend: i, intensity; a, area. [c] Measured in 2.5-mm tubes to reduce convection and provide a more accurate assessment due to the closeness of the values.

Table S6. CC: silica gel, start 87% ee, loading 1 mmol/30 g, *n*-hexane/ethyl acetate (2:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	90.2	5.7
2	86.4	59.6
3	88.6	12.3
4	85.8	6.4
5	87.2	4.1
6	87.8	3.0
7	88.4	2.3
8	89.5	1.6
9	88.6	1.3
10	88.6	1.1
11	88.9	1.0
12	88.9	0.6
13	90.4	0.5
14	90.0	0.4

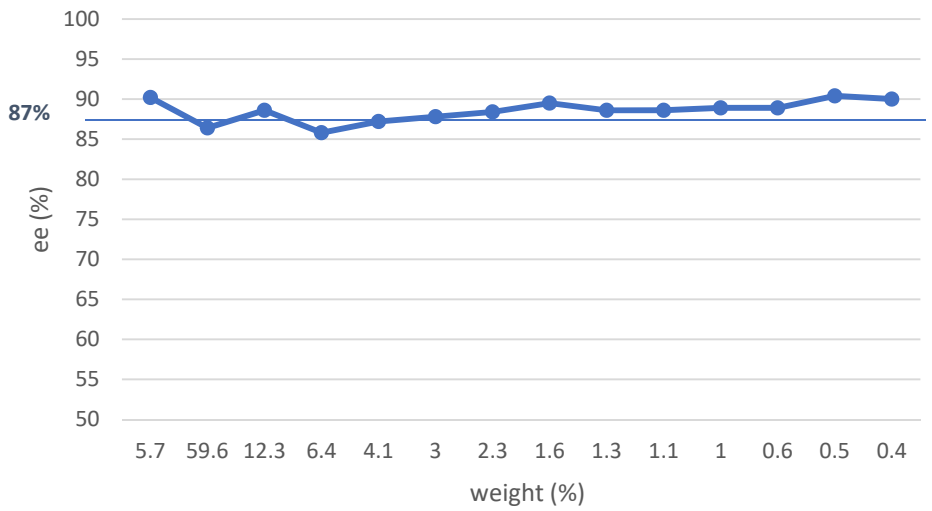


Table S7. CC: silica gel, start 87% ee, loading 1 mmol/30 g, *c*-hexane/ethyl acetate (2:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	87.0	31.9
2	87.0	44.7
3	91.2	7.9
4	86.8	5.0
5	87.6	3.2
6	88.0	2.3
7	88.4	1.6
8	88.6	1.3
9	88.8	0.9
10	88.2	0.7
11	86.6	0.5

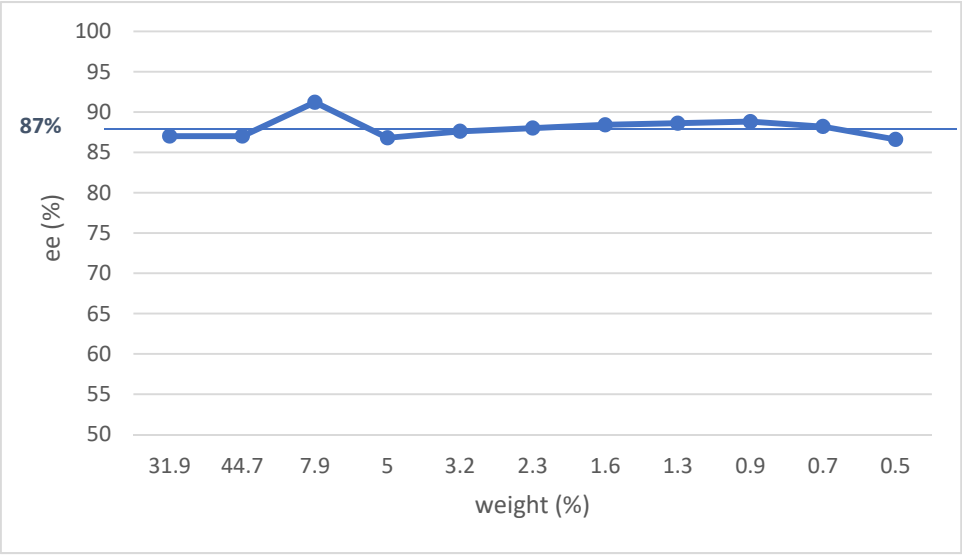


Table S8. CC: silica gel, start 87% ee, loading 1 mmol/30 g, *n*-hexane/MTBE (1:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	85.0	71.6
2	88.6	14.8
3	86.6	5.7
4	87.8	3.2
5	88.6	1.9
6	87.8	1.8
7	87.8	0.7
8	88.4	0.3

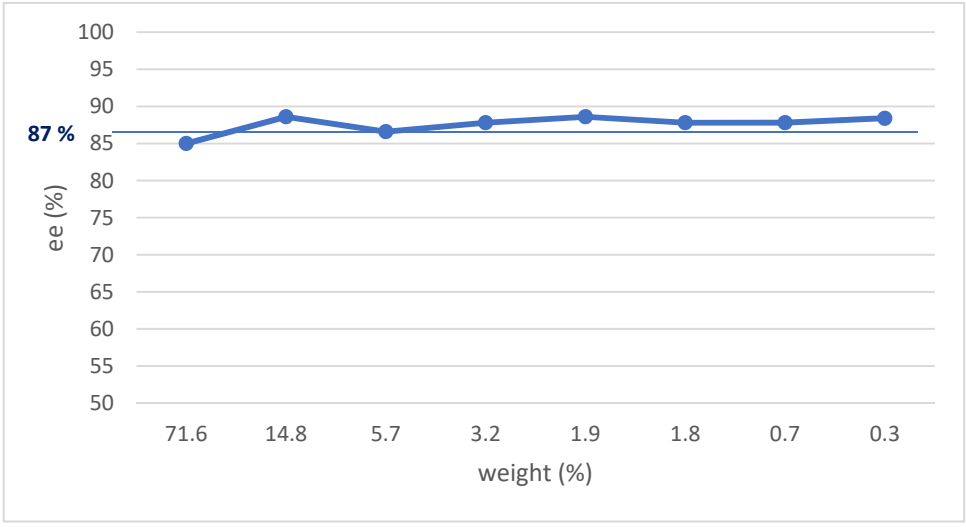


Table S9. CC: silica gel, start 87% ee, loading 1 mmol/30 g, *c*-hexane/ MTBE (1:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	83.8	5.3
2	85.2	52.4
3	86.0	17.2
4	87.2	13.1
5	88.0	7.2
6	89.0	3.8
7	90.4	1.0

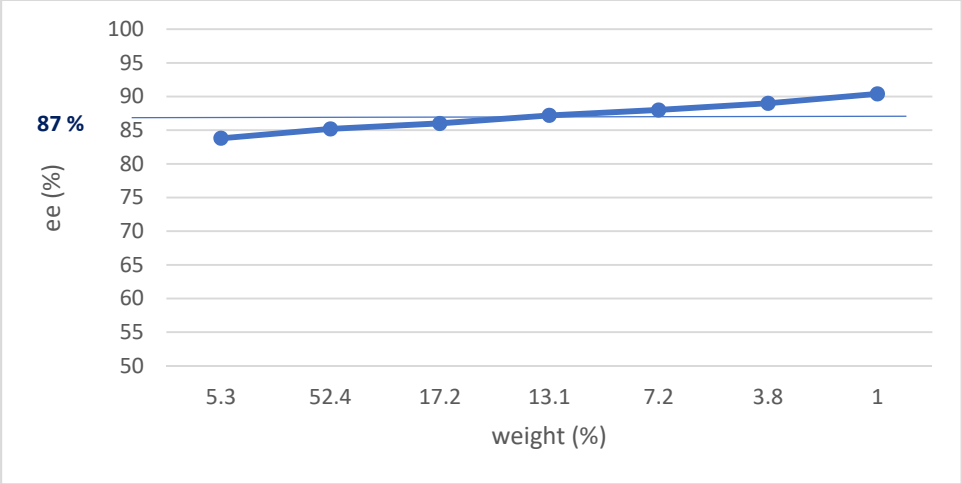


Table S10. CC: silica gel, start 77% ee, loading 1 mmol/30 g, *c*-hexane/ MTBE (4:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	75.6	33.2
2	77.5	26.1
3	79.6	10.1
4	78.0	5.5
5	76.8	8.1
6	78.8	6.1
7	77.6	4.9
8	77.4	3.3
9	79.5	1.7
10	80.6	1.0

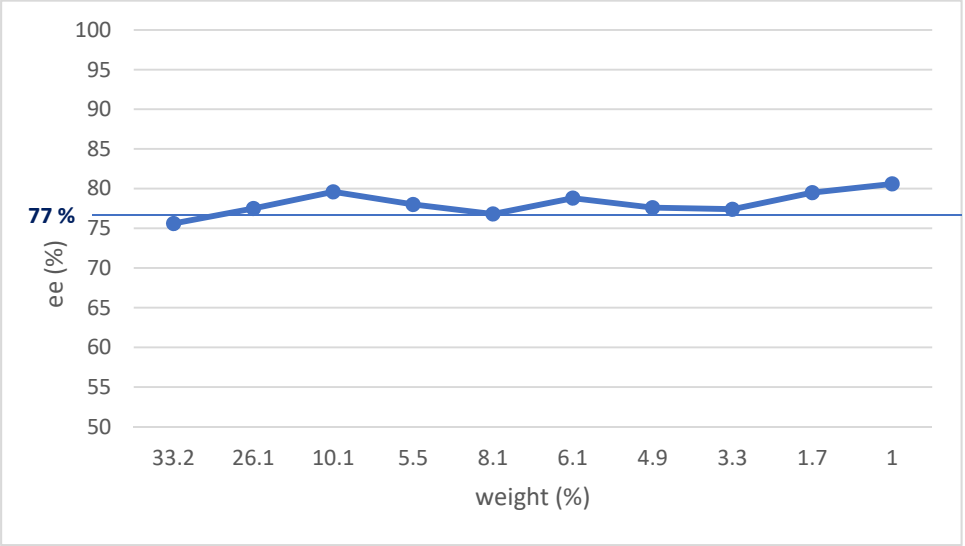


Table S11. CC: silica gel, start 77% ee, loading 1 mmol/30 g, toluene/MTBE (20:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	76.8	10.9
2	77.0	16.6
3	78.8	10.8
4	82.6	5.9
5	76.2	4.8
6	77.8	4.4
7	80.2	2.1
8	81.0	1.8
9	81.0	1.4
10	80.0	1.2
11[a]	79.6	3.8
12[a]	89.4	2.0

[a] 50-mL fractions.

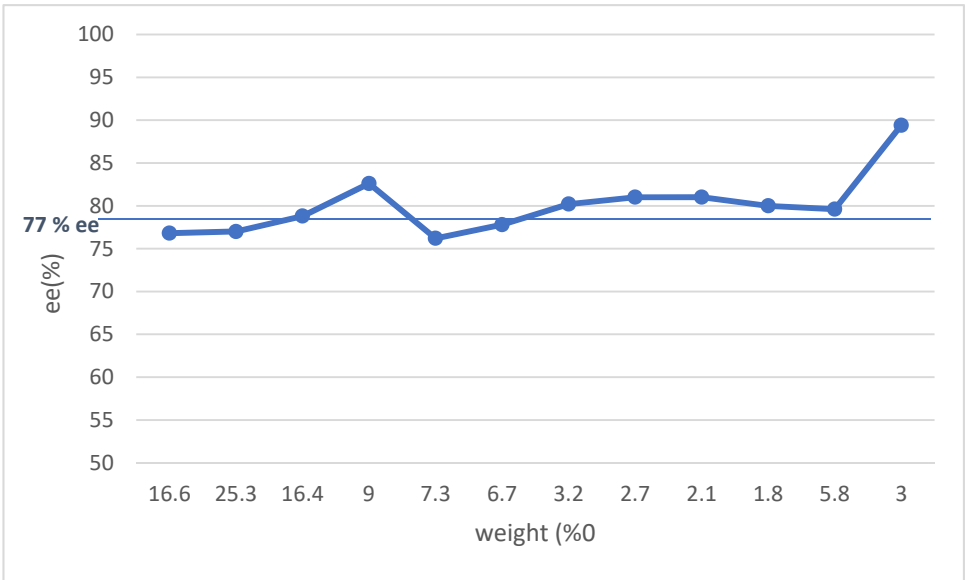


Table S12. MPLC: 20 μm silica gel, flow rate 5 mL/min, start 80% ee (25.8 mg), loading 0.11 mmol/10 g, *n*-hexane/ethyl acetate (4:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	80.4	3.7
2	84.0	12.6
3	78.6	12.7
4	78.8	11.3
5	79.2	9.6
6	80.4	8.5
7	78.3	6.6
8	79.4	8.5
9	80.8	7.3
10	81.0	6.8
11	82.4	4.5
12	83.2	3.9
13	80.6	4.1

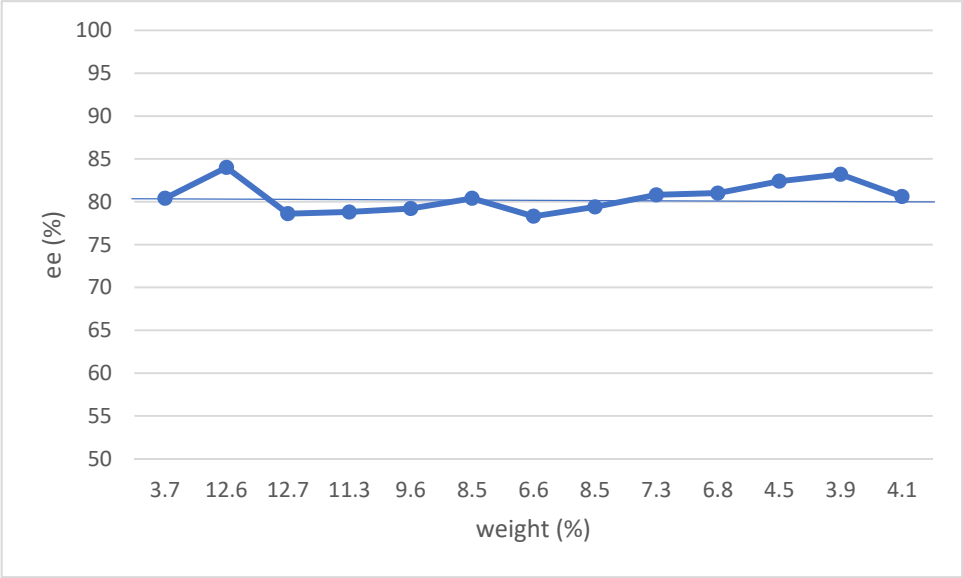


Table S13. MPLC: 20 μm silica gel, flow rate 2 mL/min, start 87% ee (27.0 mg), loading 0.11 mmol/10 g, *n*-hexane/ethyl acetate (4:1), 10 mL fractions

Fraction	ee, %	Weight, %
1	93.0	2.1
2	86.2	13.4
3	85.5	15.6
4	84.2	17.7
5	89.6	13.5
6	84.4	11.2
7	86.6	7.3
8	85.5	8.5
9	88.0	7.2
10	86.2	3.5

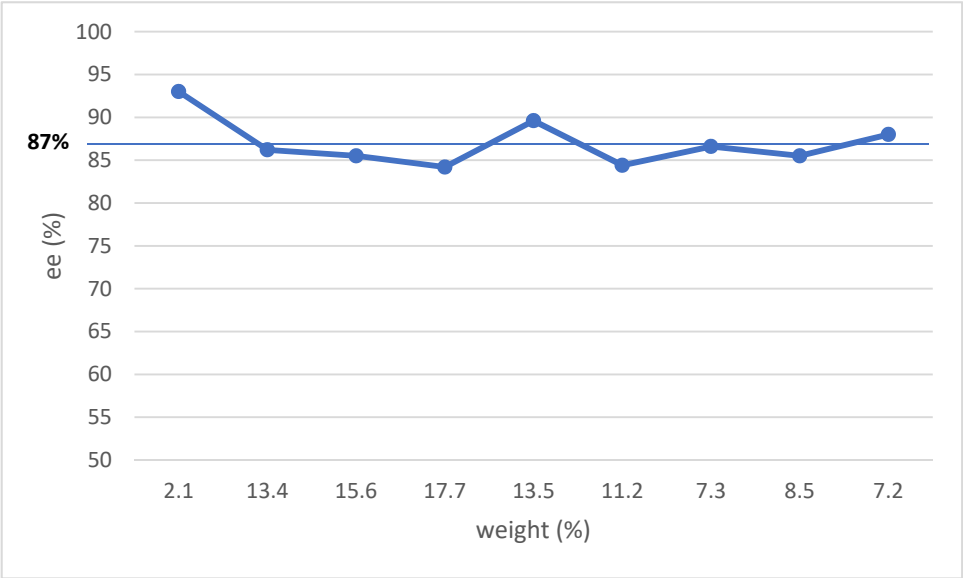


Table S14. PTLC: silica gel

solvent	R_f	sample		1st fraction		2nd fraction		3rd fraction		Δee,[a] %	high Δee,[b] %
		ee, %	wt, mg	ee, %	wt., mg	ee, %	wt., mg	ee, %	wt., mg		
ethyl acetate	0.80	80.0	49.5	80.2	11.2	79.4	15.3	79.5	20.8	0.7	0.8
c-hexane–MTBE, 1:7	0.75	87.0	51.3	86.0	15.1	86.6	18.3	87.2	15.2	−1.2	–
n-hexane–EtOAc, 1:5	0.50	76.0	46.2	78.2	14.4	80.0	16.9	75.0	10.6	3.2	5.0

[a] The Δee is calculated as (ee of the first fraction − ee of the final fraction). [b] If (ee of the fraction with the highest ee − ee of the fraction with the lowest ee) is larger than Δee, then this is reported as high Δee. The sign is determined by the order of the two fractions (earlier − later)

Table S15. SEC: Sephadex LH-20, start 77% ee (100 mg), loading 0.4 mmol/30 g, $CHCl_3$, 10 mL fractions

Fraction	ee, %	Weight, %
1	81.2	1.3
2	79.4	7.2
3	75.0	34.9
4	76.4	34.6
5	79.4	14.1
6	80.0	4.9
7	77.0	3.0

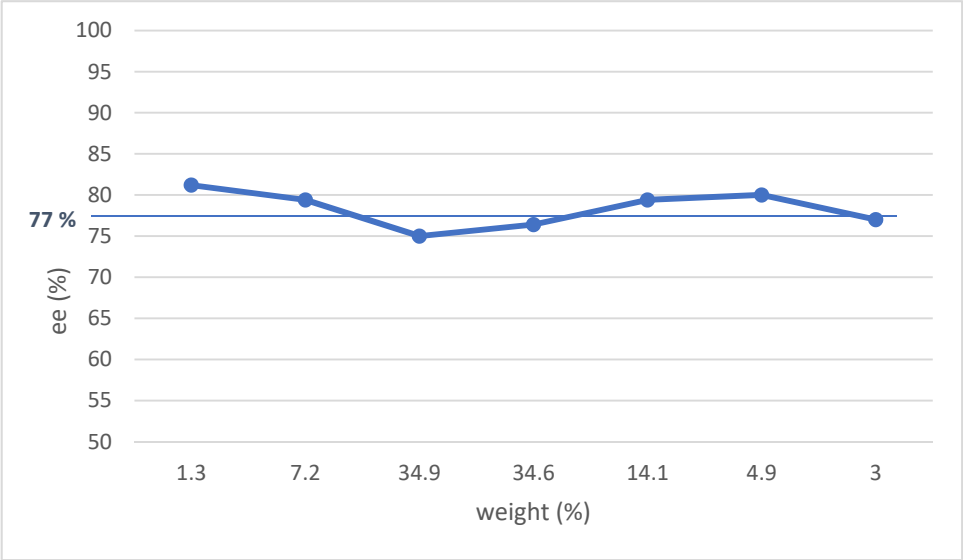


Table S16. SEC: Sephadex LH-20, start 77% ee (200 mg), loading 1.6 mmol/15 g, $CHCl_3$, 10 mL fractions

Fraction	ee, %	Weight, %
1	75.2	12.3
2	75.0	29.2
3	76.8	25.4
4	78.4	18
5	79.4	10.1
6	80.4	3.9
7	79.6	1.1

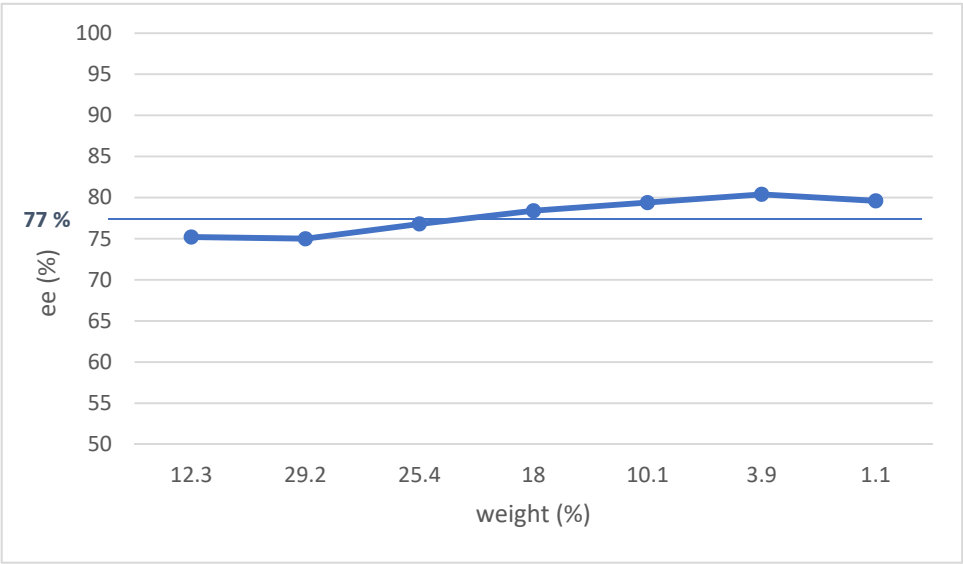


Table S17. SEC: Sephadex LH-20, start 77% ee (200 mg), loading 3.2 mmol/7.5 g, $CHCl_3$, 10 mL fractions

Fraction	ee, %	Weight, %
1	75.4	25.4
2	75.8	36.9
3	78.4	23.2
4	80.4	13.0
5	83.2	1.5

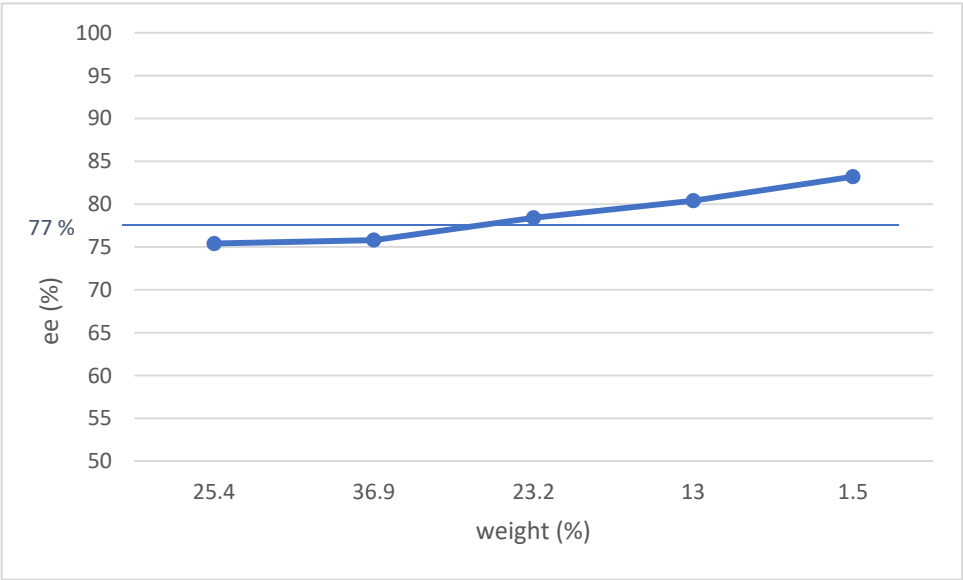


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Table S18. Summary of chromatographic results

run	method	solvent	loading, mmol/30 g	sample ee, %	first fraction ee, %	final fraction ee, %	Δee,[a] %	high Δee,[b] %
1	CC, silica	*n*-hexane–ethyl acetate, 2:1	1.0	87.0	90.2	90.0	0.2	−4.6
2	CC, silica	*c*-hexane–ethyl acetate, 2:1	1.0	87.0	87.0	86.6	0.4	4.6
3	CC, silica	*n*-hexane–MTBE, 1:1	1.0	87.0	85.0	88.4	−3.4	−3.6
4	CC, silica	*c*-hexane–MTBE, 1:1	1.0	87.0	83.8	90.4	−6.6	–
5	CC, silica	*c*-hexane–MTBE, 4:1	1.0	77.0	75.6	80.6	−5.0	–
6	CC, silica	toluene–MTBE, 20:1	1.0	77.0	76.8	89.4	−12.6	−13.2
7	MPLC, silica	*n*-hexane–ethyl acetate, 4:1	0.11[c]	77.0	80.4	80.6	−0.2	5.7
8	MPLC, silica	*n*-hexane–ethyl acetate, 4:1	0.11[c]	87.0	93.0	86.6	6.8	8.8
9	PTLC, silica	ethyl acetate	49.5[d]	80.0	80.2	79.5	0.7	0.8
10	PTLC, silica	*c*-hexane–MTBE, 1:7	51.3[d]	80.7	86.0	87.2	−1.2	–
11	PTLC, silica	*n*-hexane–ethyl acetate, 1:5	46.2[d]	76.0	78.2	75.0	3.2	5.0
12	SEC, sephadex	$CHCl_3$	0.4	77.0	81.2	77.0	4.2	6.2
13	SEC, sephadex	$CHCl_3$	1.6[e]	77.0	75.2	79.6	−4.4	−5.4
14	SEC, sephadex	$CHCl_3$	3.2[f]	77.0	75.4	83.2	−7.8	–

[a] The Δee is calculated as (ee of the first fraction − ee of the final fraction). [b] If (ee of the fraction with the highest ee − ee of the fraction with the lowest ee) is larger than Δee, then this is reported as high Δee. The sign is determined by the order of the two fractions (earlier − later). [c] 10 g of stationary phase. [d] Amount (mg) loaded onto the plate. [e] 15 g of stationary phase. [f] 7.5 g of stationary phase.

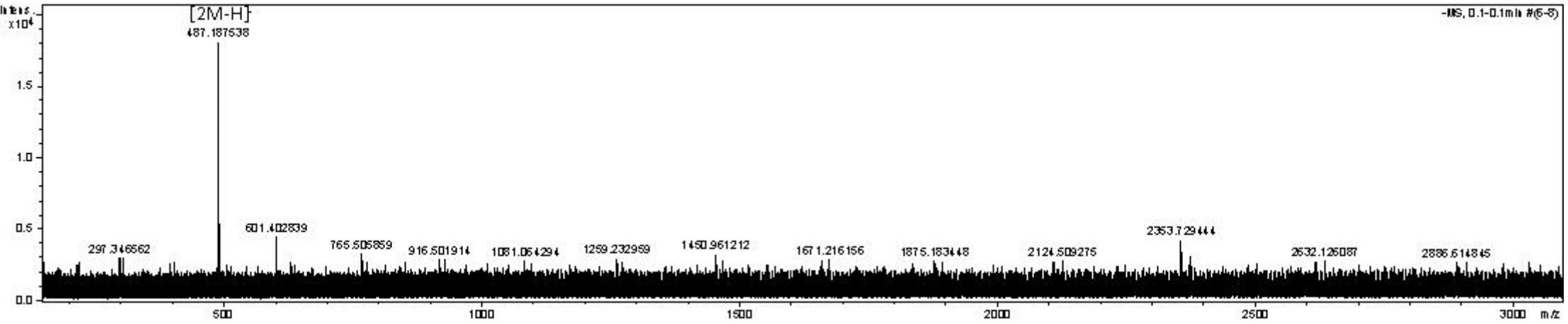

Figure S8. ESI of (*rac*)-flurbiprofen.

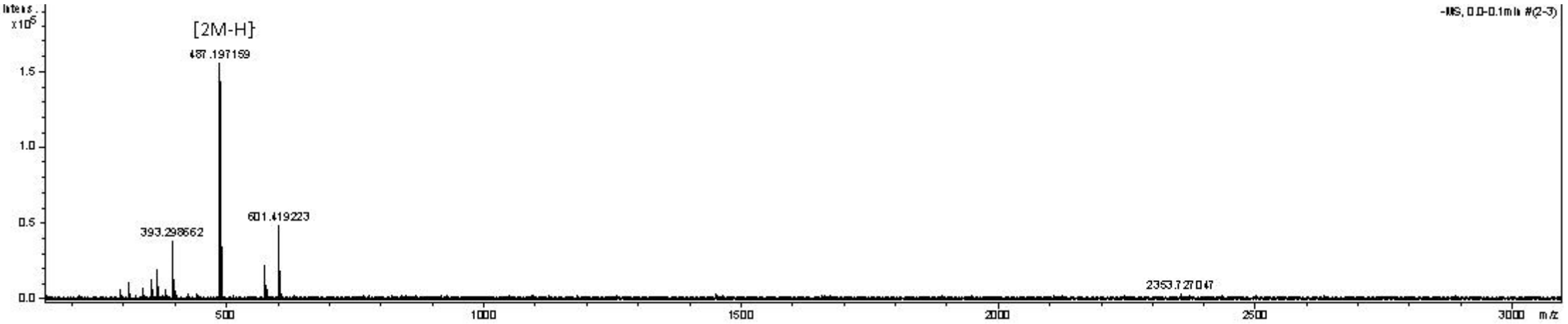

Figure S9. ESI of (*S*)-flurbiprofen (97% ee).

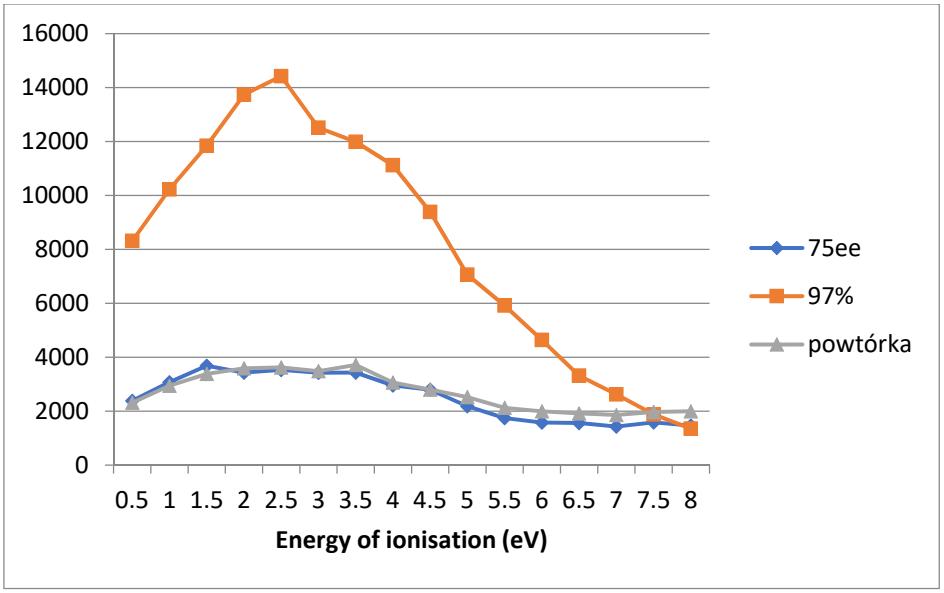

Figure S10. [2M − H]⁻ dimer ion count vs. ionization of energy for (*S*)-flurbiprofen (97% ee), (*scl*)-flurbiprofen (75% ee), and (*rac*)-flurbiprofen solutions at a concentration of 0.3 mg/mL in acetonitrile.

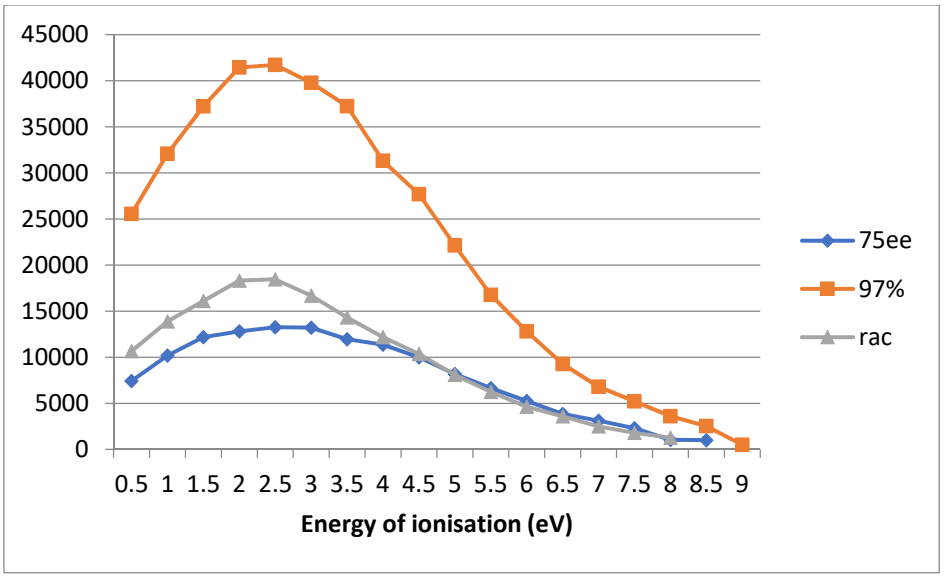

Figure S11. [2M − H]⁻ dimer ion count vs. ionization of energy for (*S*)-flurbiprofen (97% ee), (*scl*)-flurbiprofen (75% ee), and (*rac*)-flurbiprofen solutions at a concentration of 0.4 mg/mL in acetonitrile.

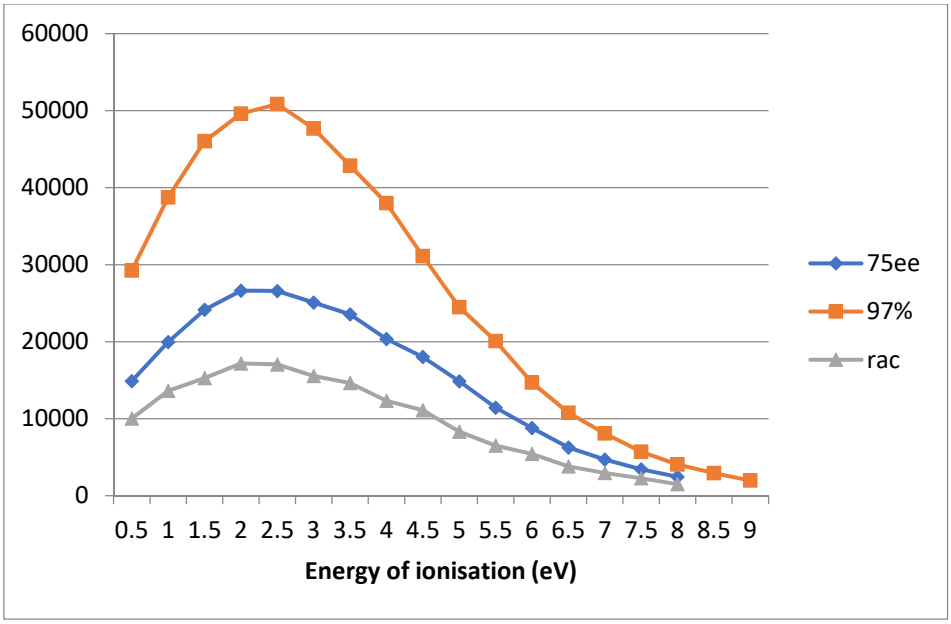

Figure S12. [2M − H]⁻ dimer ion count vs. ionization of energy for (*S*)-flurbiprofen (97% ee), (*scl*)-flurbiprofen (75% ee), and (*rac*)-flurbiprofen solutions at a concentration of 0.5 mg/mL in acetonitrile.

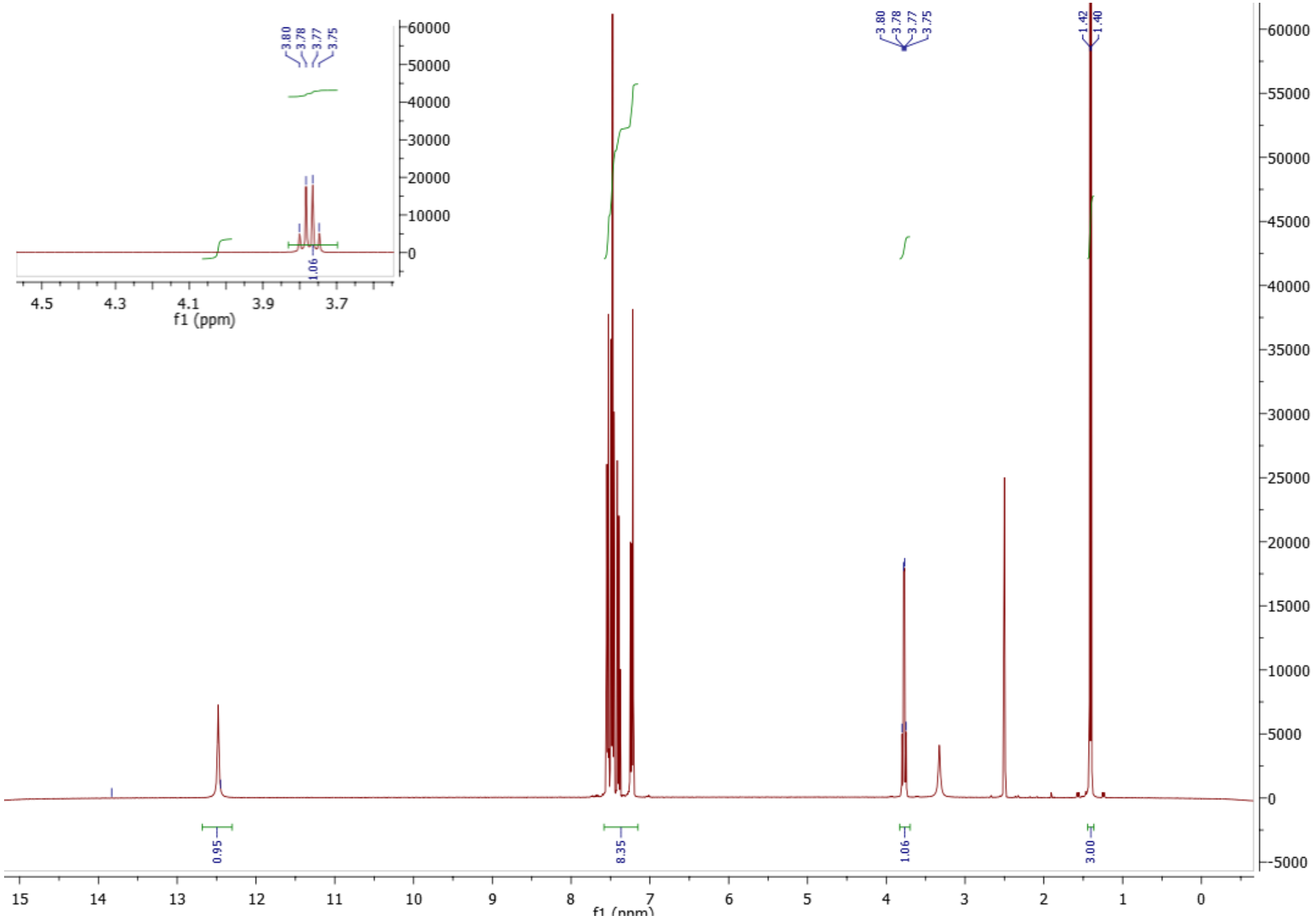

Figure S13. ^{1}H NMR spectrum of (*rac*)-flurbiprofen (400 MHz, DMSO-d_6).

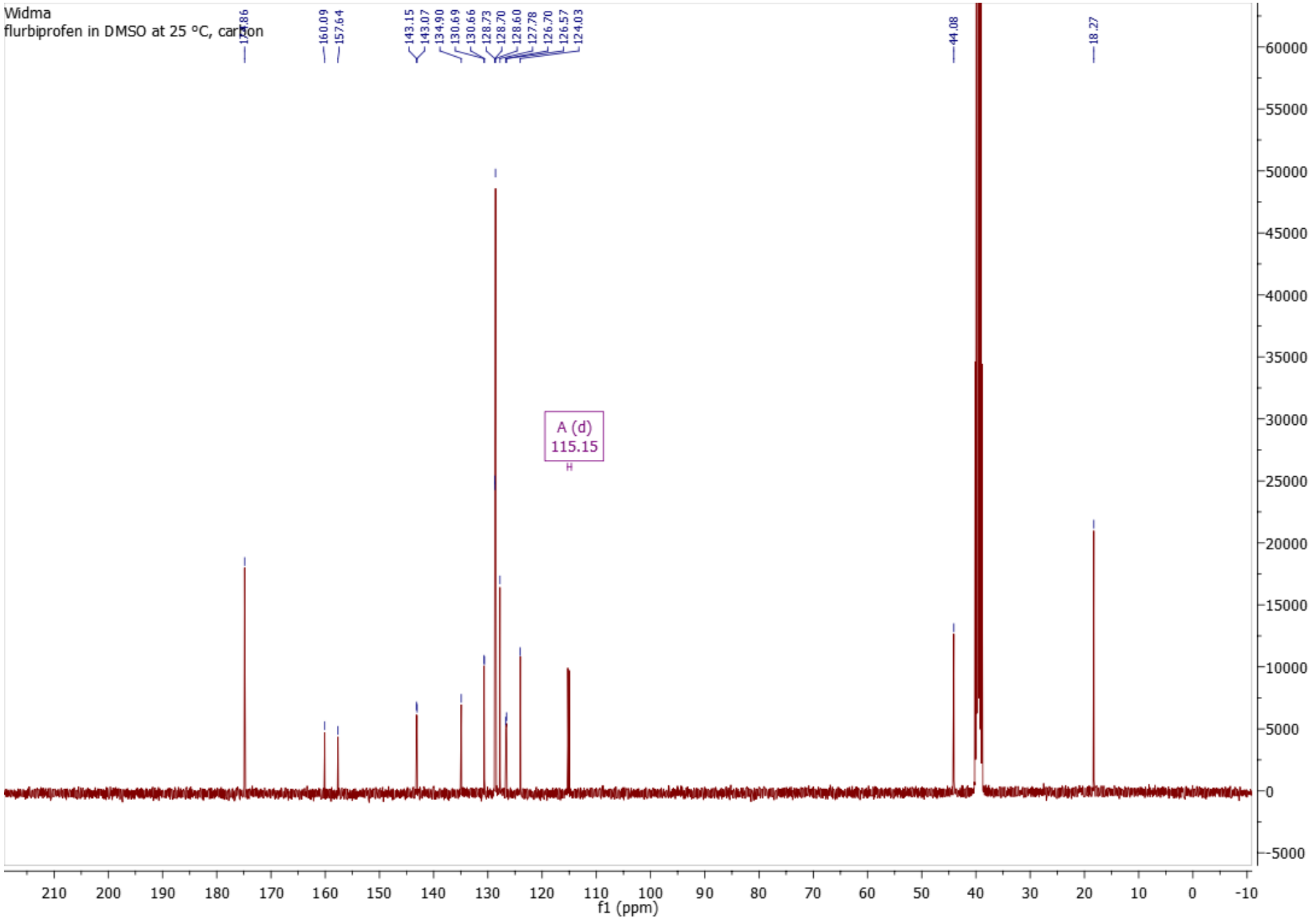

Figure S14. ^{13}C NMR of spectrum of (*rac*)-flurbiprofen (100 MHz, DMSO-d_6).

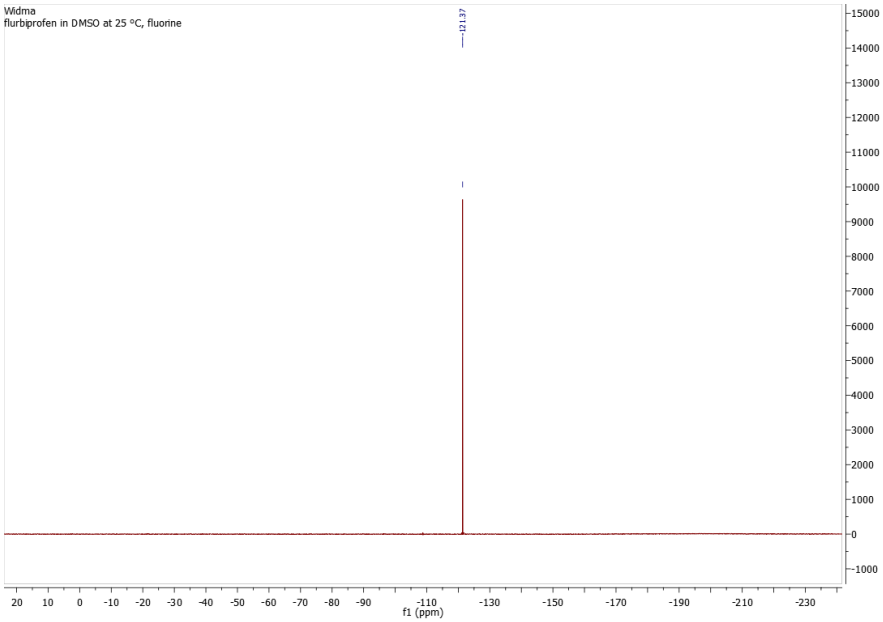

Figure S15. ^{19}F NMR of spectrum of (*rac*)-flurbiprofen (376 MHz, DMSO-d_6).

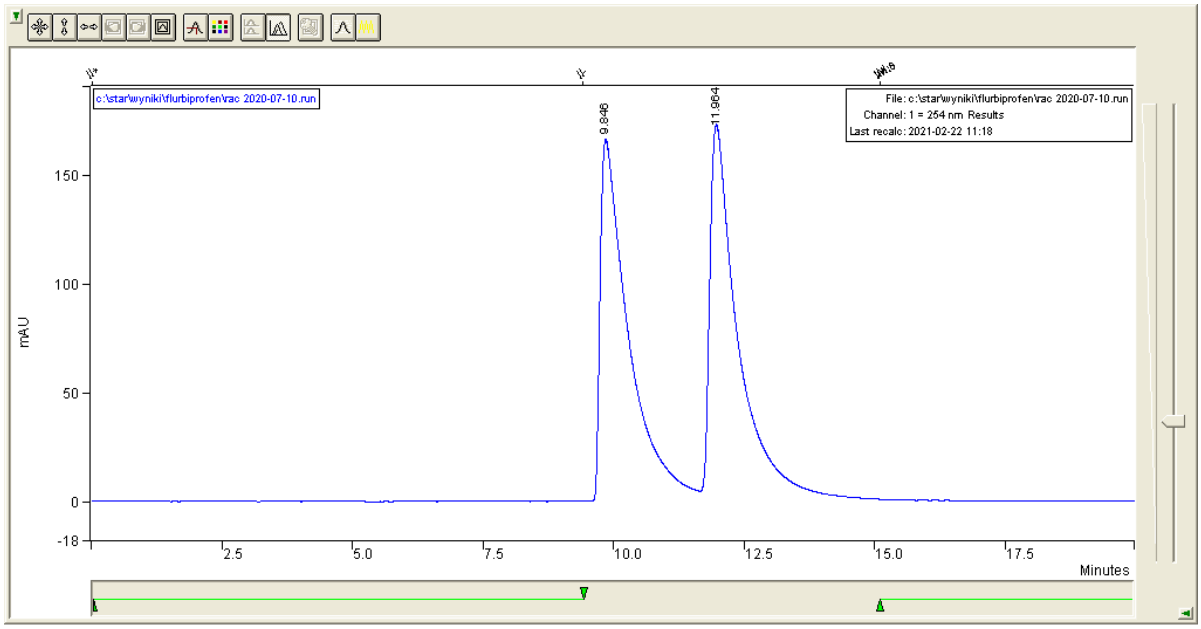

Figure S16. Chiral analysis of (*rac*)-flurbiprofen.

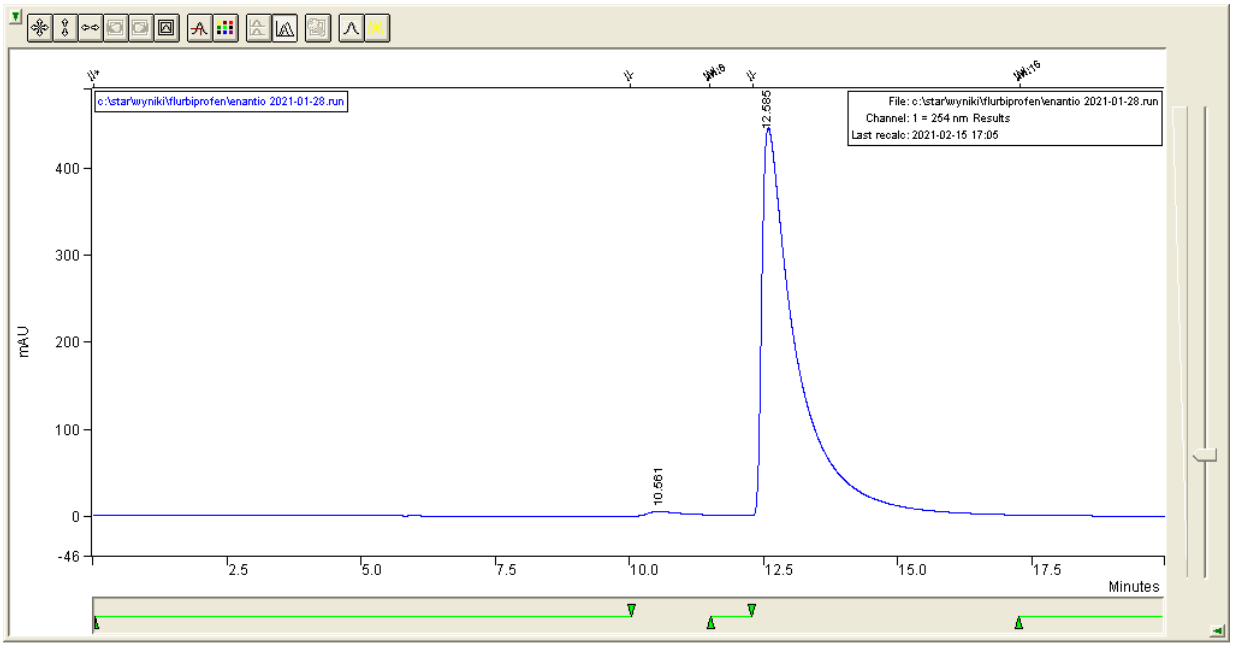

Figure S17. Chiral analysis of (*S*)-flurbiprofen (97% ee).