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Supplementary Material

Chemoenzymatic Stereodivergent Synthesis of all the Possible Stereoisomers of the 2,3-Dimethylglyceric Acid Ethyl Ester

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Abstract: 2,3-dihydroxy-2-methylbutyric acid, also known as 2,3-dimethylglyceric acid, constitutes the acyl and/or the alcoholic moiety of many bioactive natural esters. Herein, we describe a chemoenzymatic methodology which gives access to all the four possible stereoisomers of the 2,3-dimethylglyceric acid ethyl ester. The racemic ethyl α-acetolactate, produced by the *N*-heterocycle carbene (*NH*C)-catalyzed coupling of ethyl pyruvate and methylacetoin was employed as the starting material. The racemic mixture was resolved through (*S*)-selective reductions, promoted by the acetylacetoin reductase (AAR) affording the resulting ethyl (2*R*,3*S*)-2,3-dimethylglycerate; the isolated remaining (*S*)-ethyl α-acetolactate was successively treated with baker's yeast to obtain the corresponding (2*S*,3*S*) stereoisomer. *syn*-2,3-Dimethylgliceric acid ethyl ester afforded by reducing the *rac*-α-acetolactate with $NaBH_4$ in the presence of $ZnCl_2$ was kinetically resolved through selective acetylation with lipase B from *Candida antarctica* (CAL-B) and vinyl acetate to access to (2*S*,3*R*) stereoisomer. Finally, the (2*R*,3*R*) stereoisomer, was prepared by C3 epimerization of the (2*R*,3*S*) stereoisomer recovered from the above kinetic resolution, achieved through the TEMPO-mediated oxidation, followed by the reduction of the produced ketone with $NaBH_4$. The resulting 2,3-dimethylglycertate enriched in the (2*R*,3*R*) stereoisomer was submitted to stereospecicific acetylation with vinyl acetate and CAL-B in order to separate the major stereoisomer. The entire procedure enabled conversion of the racemic α-acetolactate into the four enantiopure stereoisomers of the ethyl 2,3-dihydroxy-2-methylbutyrate with the following overall yields: 42% for the (2*R*,3*S*), 40% for the (2*S*,3*S*), 42% for the (2*S*,3*R*) and 20% for the (2*R*,3*R*).

Keywords: biocatalysis; stereodivergent synthesis; asymmetric synthesis; natural compounds.

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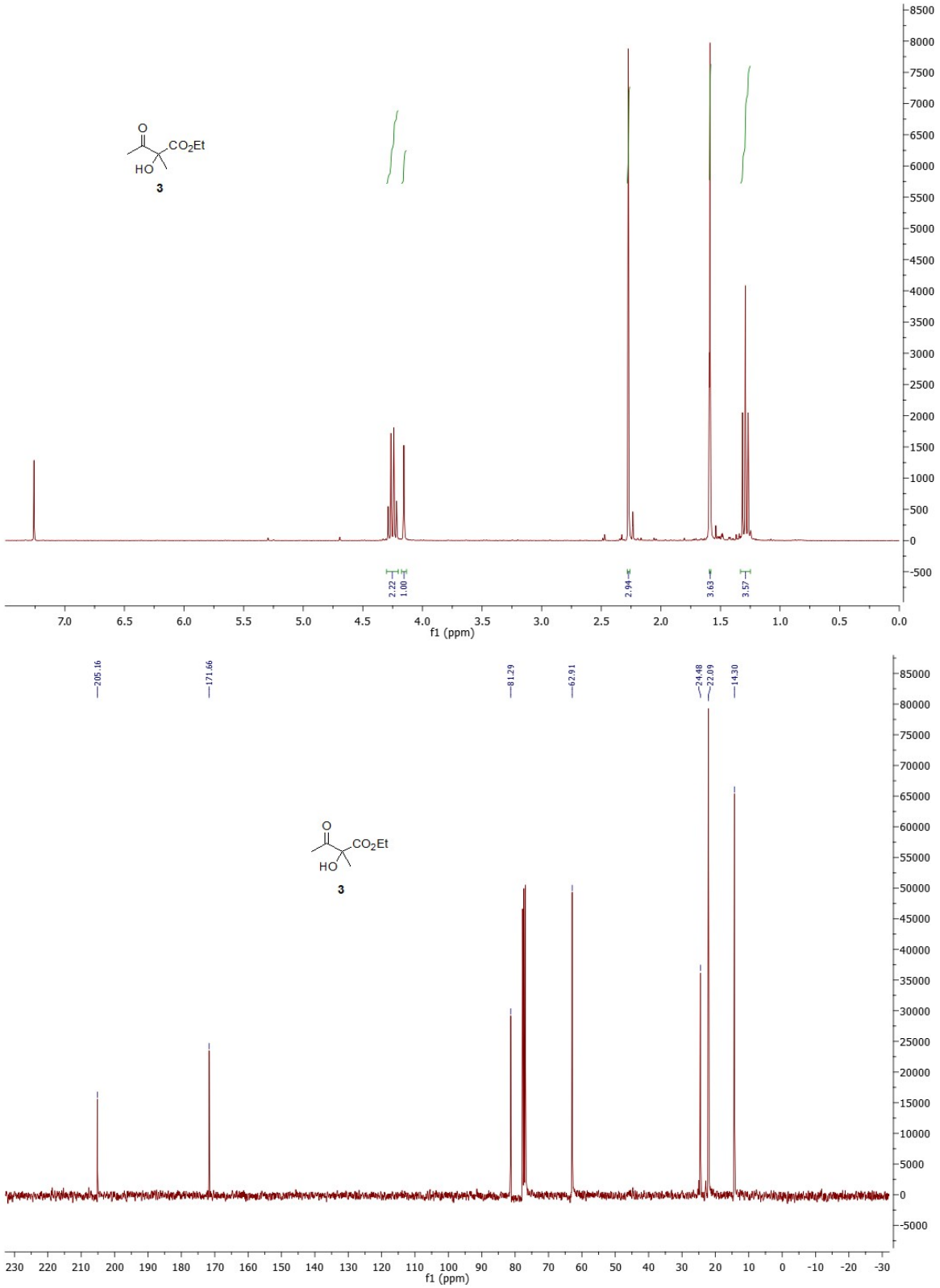


Figure S1. ^{1}H- and ^{13}C-NMR spectra of compound **3**.

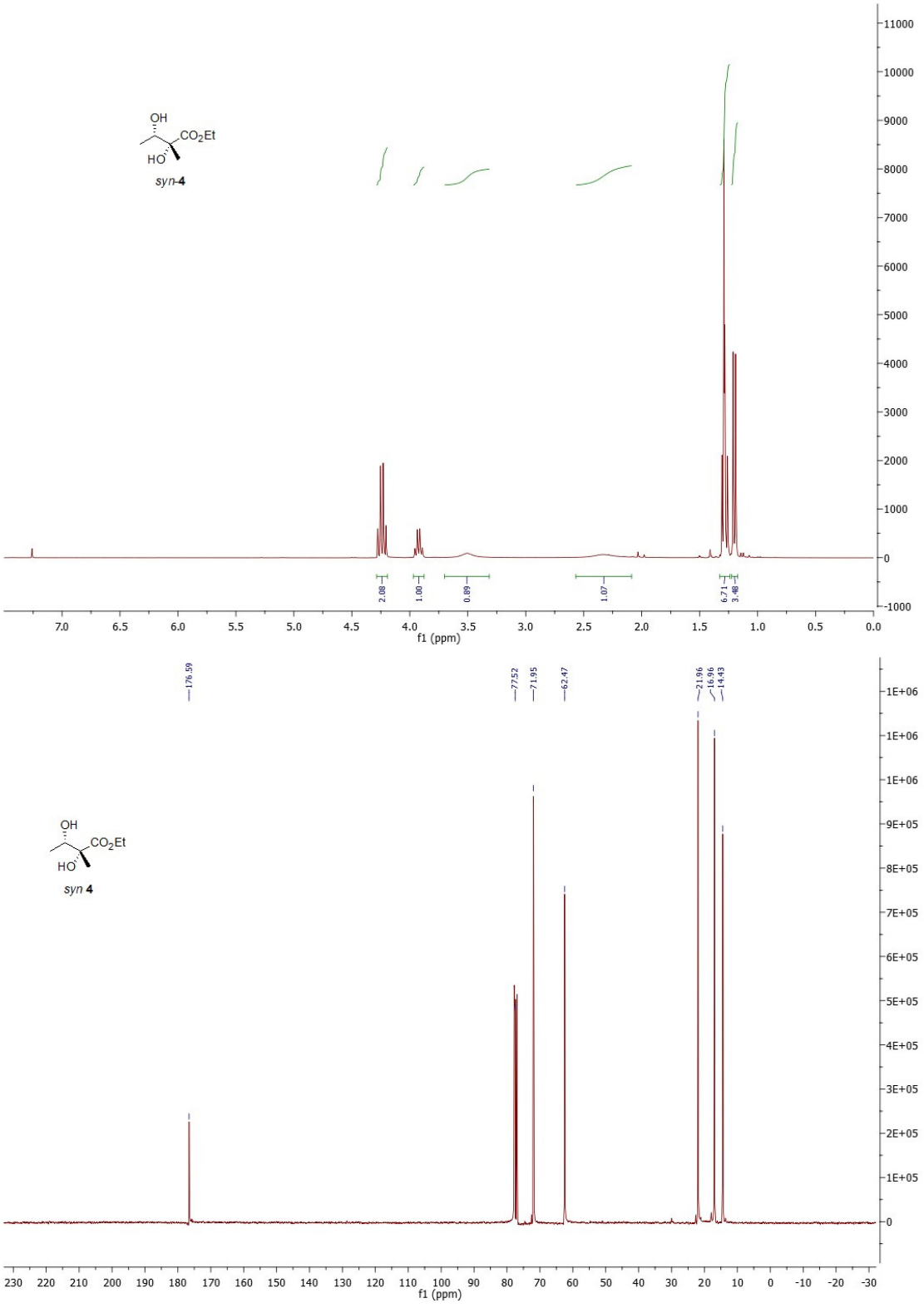


Figure S2. ^{1}H- and ^{13}C-NMR spectra of *syn*-**4** [(2*R*,3*S*)-**4** and (2*S*,3*R*)-**4**].

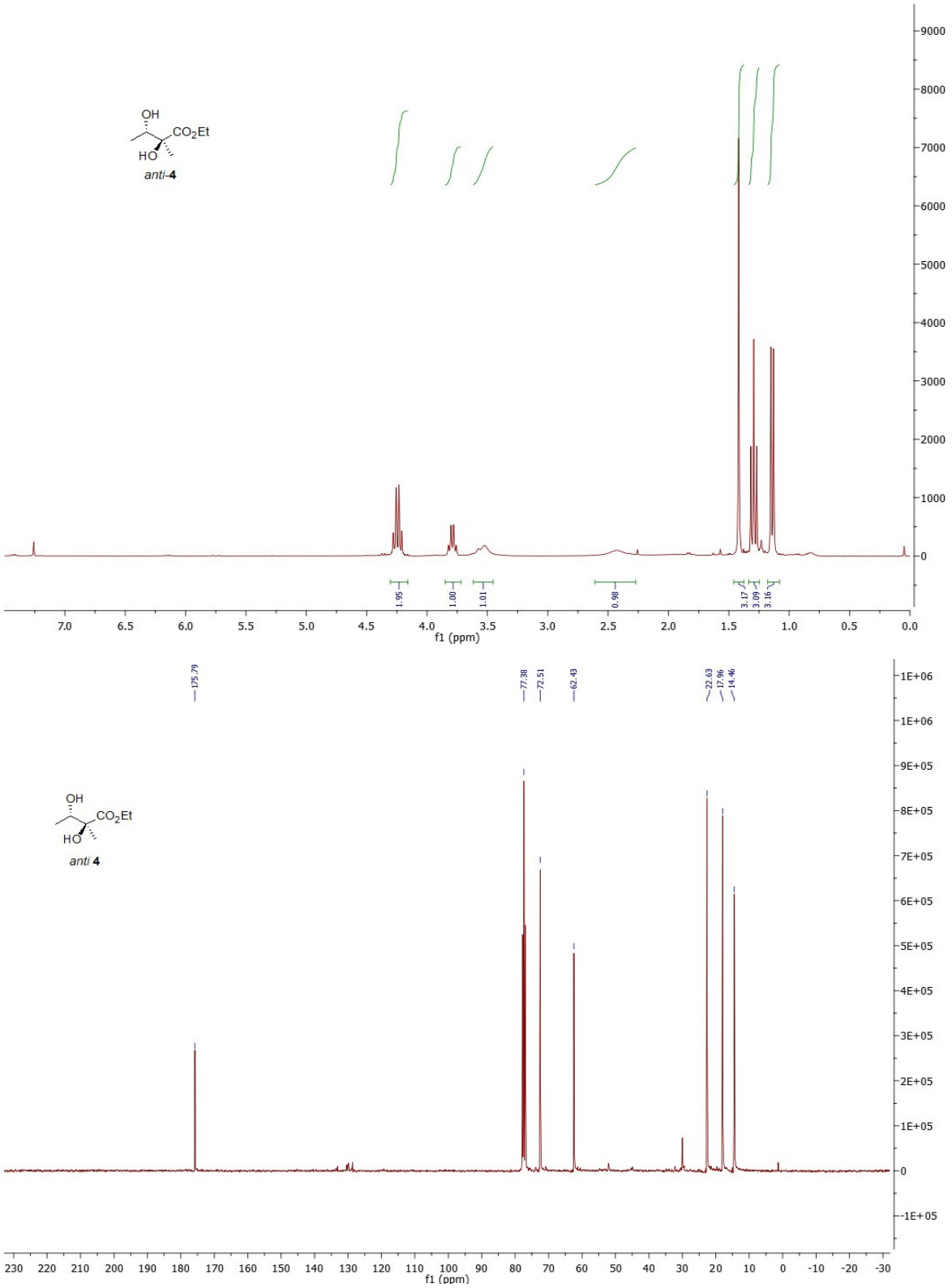


Figure S3. ^{1}H- and ^{13}C-NMR spectra of *anti*-**4** [(2*S*,3*S*)-**4** and (2*R*,3*R*)-**4**].

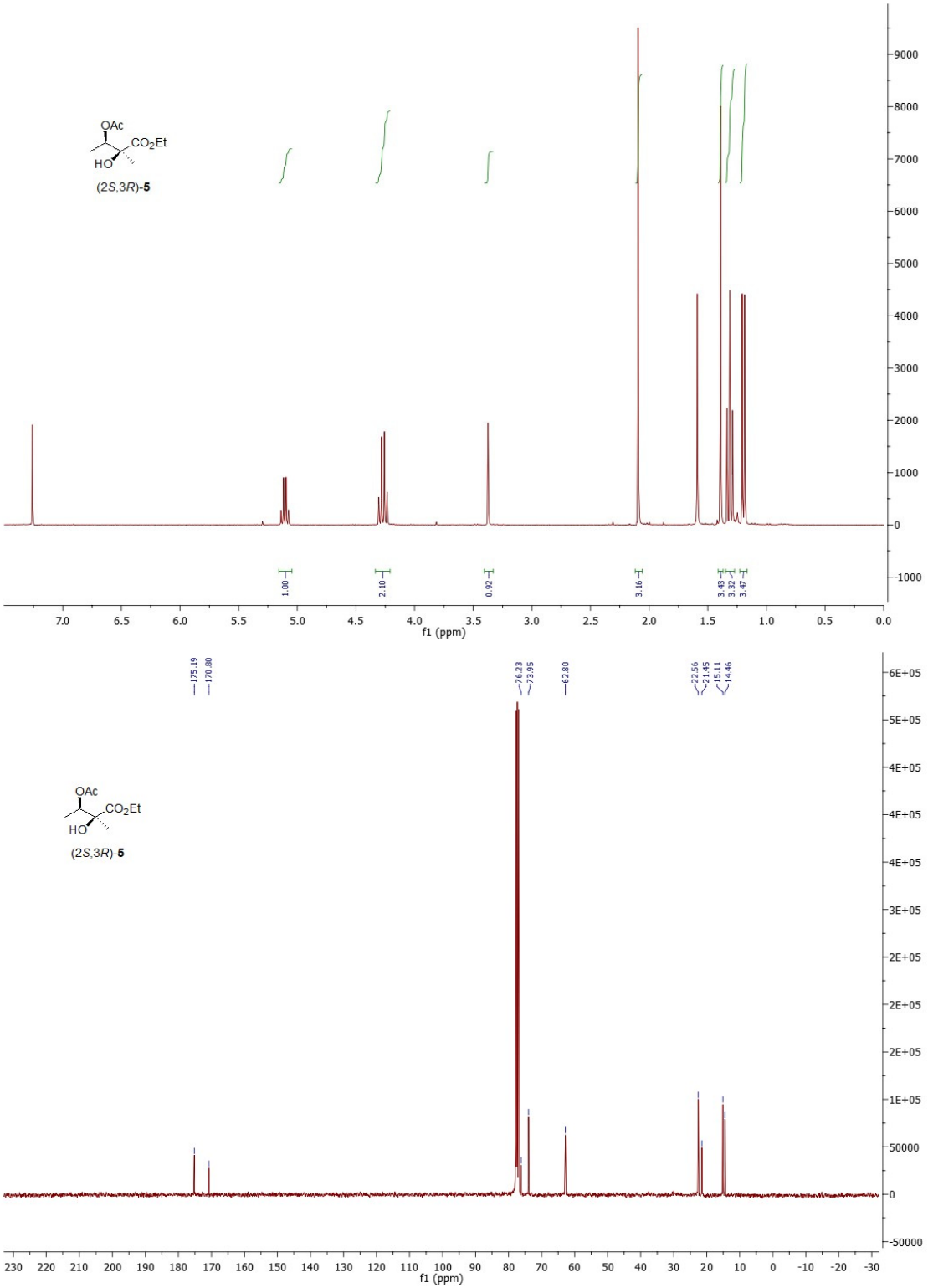


Figure S4. ^{1}H- and ^{13}C-NMR spectra of (2*S*,3*R*)-**5**.

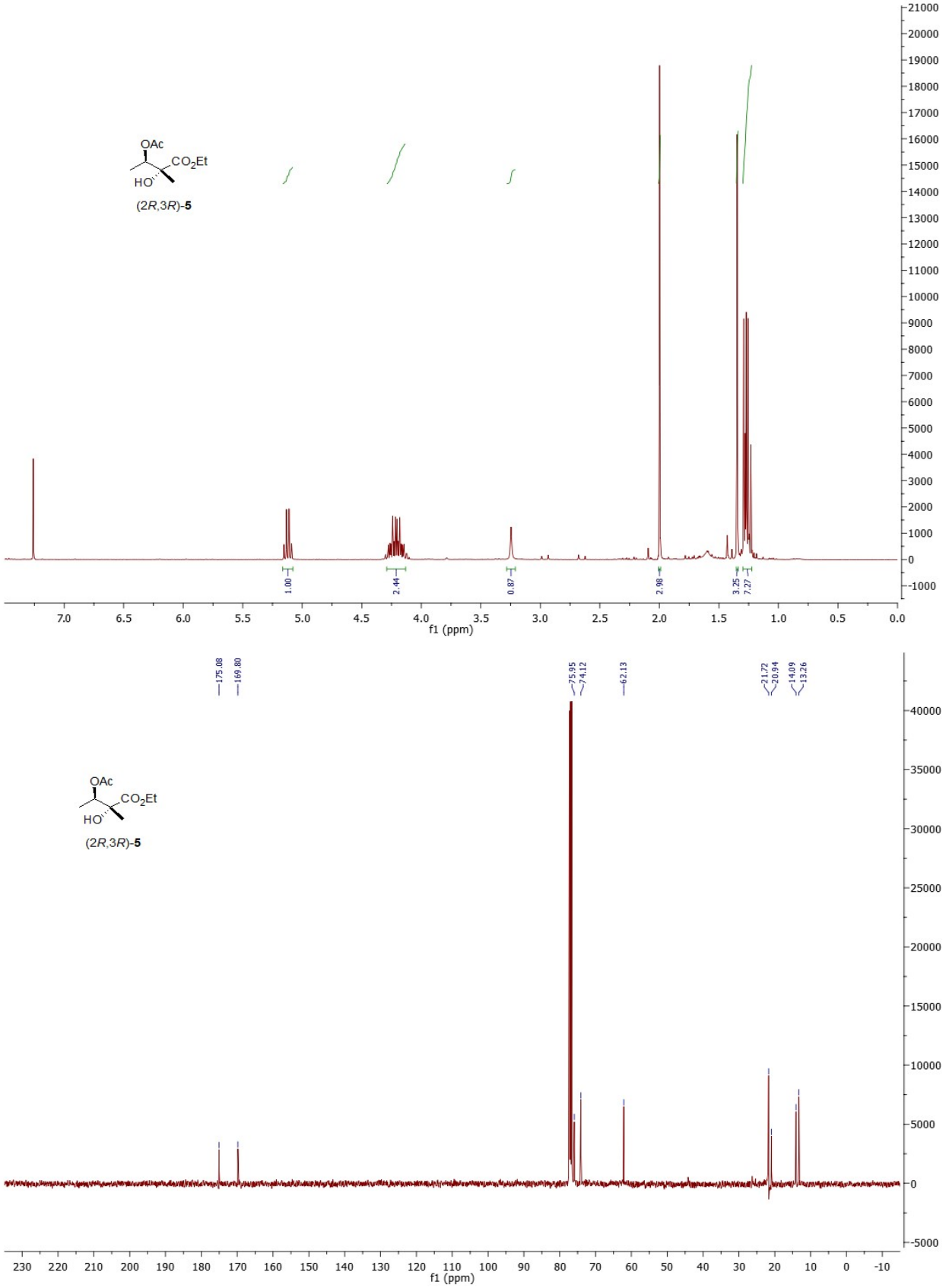


Figure S5. ^{1}H- and ^{13}C-NMR spectra of (2*R*,3*R*)-**5**.

^{1}H-NMR spectrum of the syn/anti mixture of **4** obtained by reduction of racemic **3** with $NaBH_4$ in the absence of $ZnCl_2$.

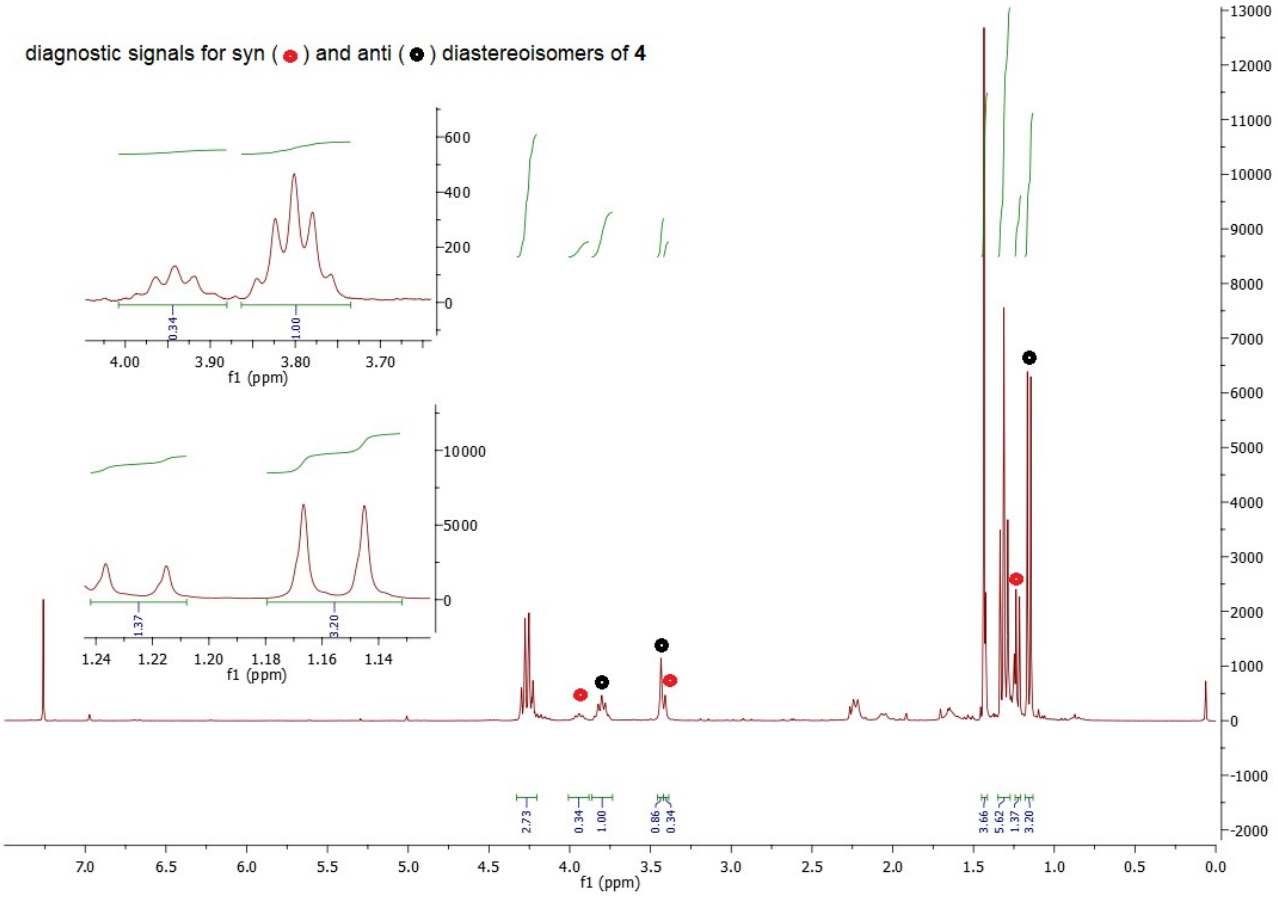


Figure S6. ^{1}H-NMR of the *anti*/*syn* mixture of **4** and chelation model scheme.

For literature data on *syn*- and *anti*-**4** ^{1}H-NMR spectra see:
Greiner A.; Ortholand J-Y. Erythroselective aldol condensation of amine free 2-t-butyl-5-methyl-2phenyl-1,3-dioxolan-4-on3 lithium enolate synthesis of ethyl acetolactate enantiomers. *Tetrahedron letters*, **1992**, *33*, 1897-1900.

Synthesis of the ethyl (2*R*,3*S*)-2-*O*-tosyl-2,3-dimethylglycerate (2*R*,3*S*)-6

A solution of (2*R*,3*S*)-**4** (122 mg, 0.75 mmol) and *p*-dimethylaminopyridine (5 mg, 0.04 mmol) in pyridine (1 mL) was cooled to 0 °C and *p*-toluenesulfonyl chloride (190 mg, 1.0 mmol) was added in four portions over 30 min. The mixture was kept at room temperature for 5 h and then diluted with water (5 mL). The suspension was extracted with ethyl acetate (3 x 4 mL) and dried under vacuum. The residue was chromatographed on silica gel wit cyclohexane-ethyl acetate 4:1 as eluent to afford the compound (2R,3S)-6 as a white solid (208 mg, 0.66 mmol), 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.3 Hz, 2H, Ts), 7.32 (d, *J* = 7.9 Hz, 2H, Ts), 4.92 (q, *J* = 6.6 Hz, 1H, CHOTs), 4.24 – 4.00 (m, 2H, CH_2), 3.28 (br s, 1H, OH), 2.43 (s, 3H, CH_3), 1.32 (d, *J* = 6.6 Hz, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3).

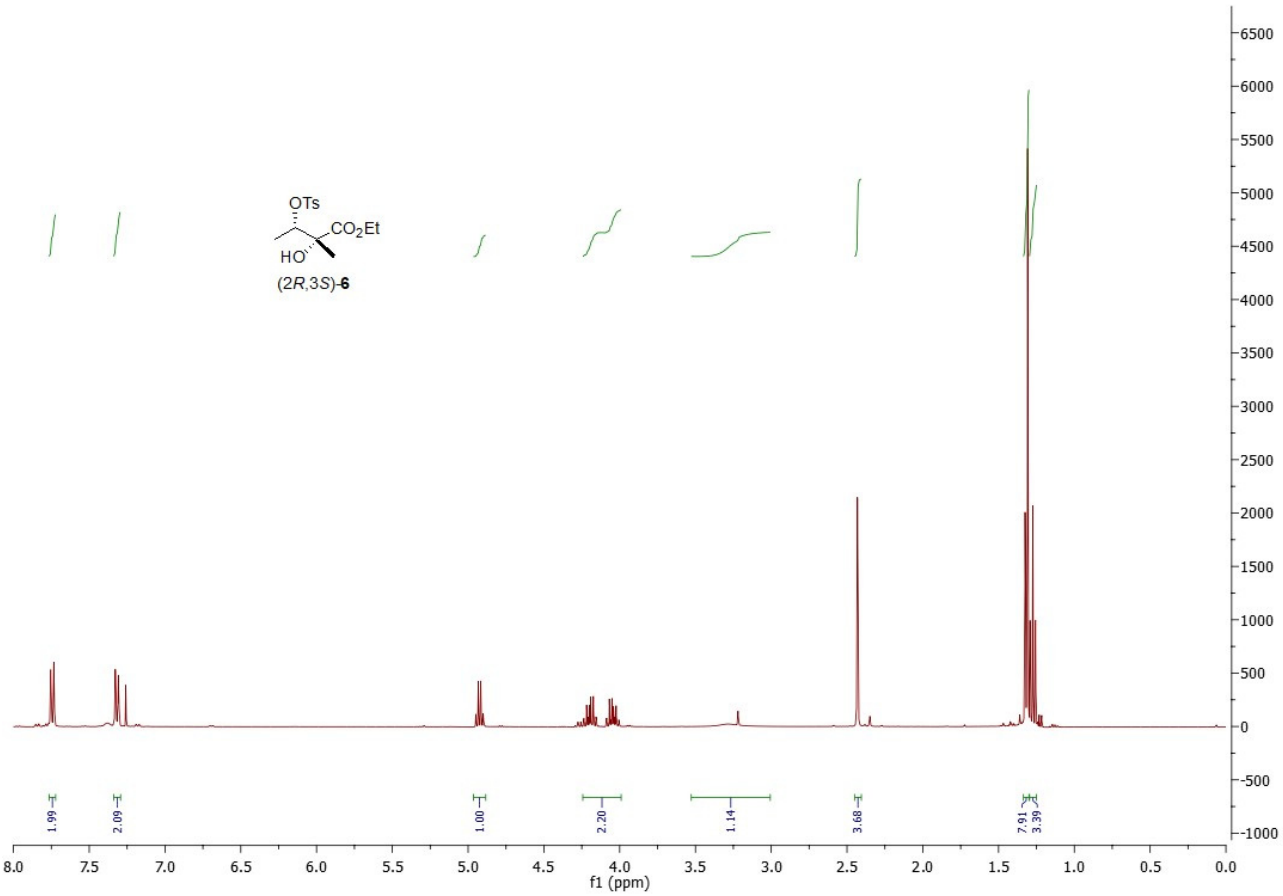


Figure S7. Synthesis and ^{1}H-NMR of compound **6**.

Synthesis of the epoxide (2*R*,3*R*)-7

The compound (2*R*,3S)-**6** (208 mg, 0.66 mmol) (2.06 g, 7.2 mmol) was added to a solution of triethylamine (200 mg, 1.98 mmol) and acetic acid (238 mg, 3.96 mmol) in toluene (4 mL) previously stirred at room temperature for half an hour. The mixture was heated to 80 °C, and stirred at this temperature for 4 h. After cooling to room temperature, the reaction mixture was diluted with toluene (10 mL) and was washed successively with aqueous 2 M HCl solution (5 mL) and 10% (w/v) aqueous K_2CO_3 solution (10 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel with cyclohexane-ethyl acetate 6:1 to afford the epoxide (2*R*,3*R*)-**7** as a colorless oil (62 mg, 0.43 mmol), 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (qd, *J* = 7.1, 2.3 Hz, 2H, CH_2), 3.03 (q, *J* = 5.4 Hz, 1H, CH), 1.55 (s, 3H, CH_3), 1.32 (d, *J* = 5.4 Hz, 3H, CH_3), 1.29 (t, *J* = 7.1 Hz, 3H, CH_3).

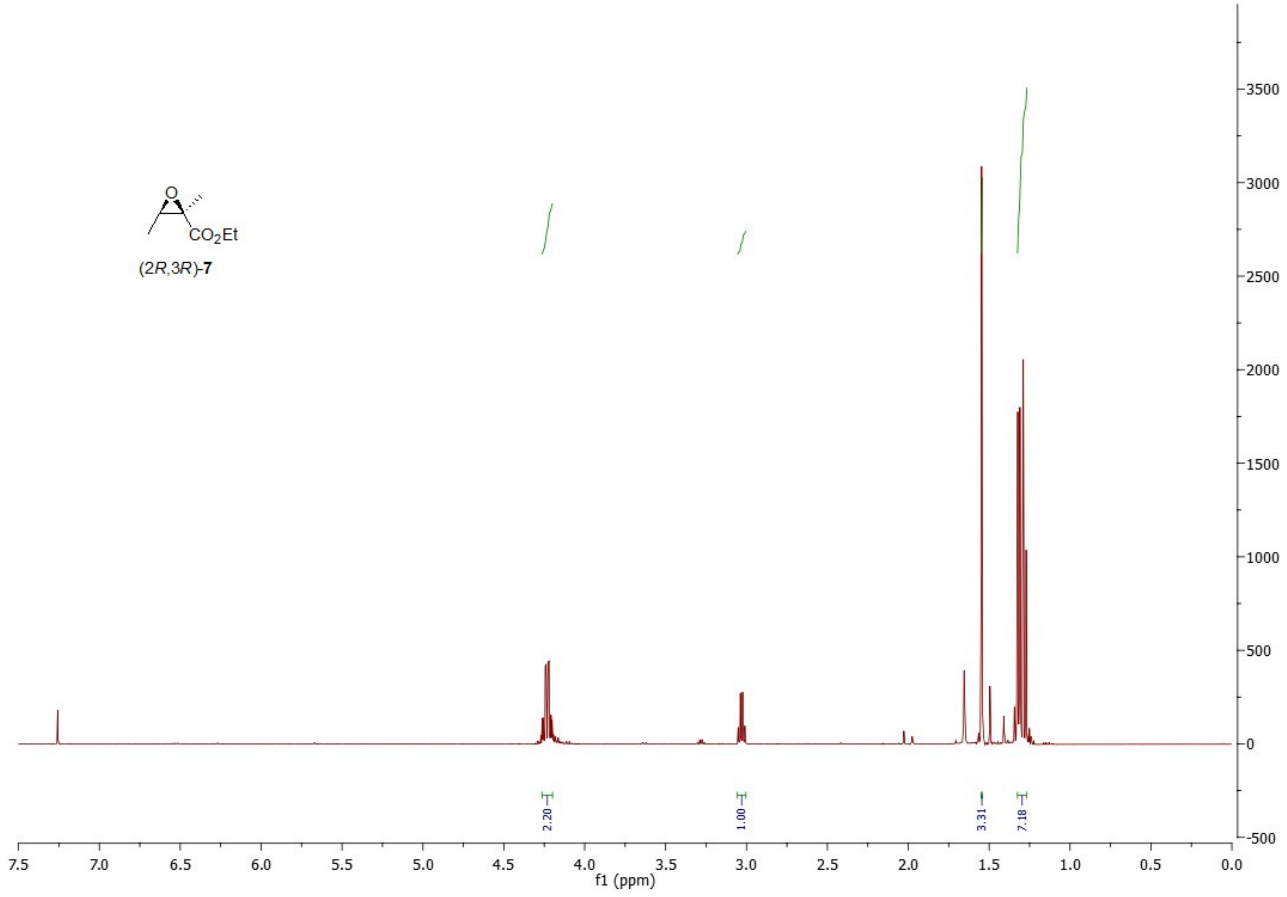
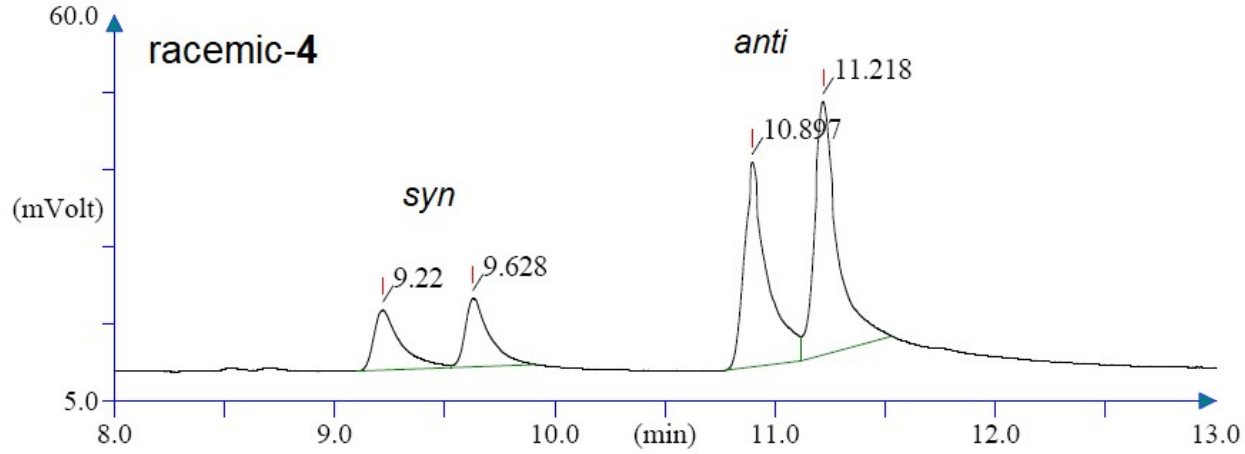
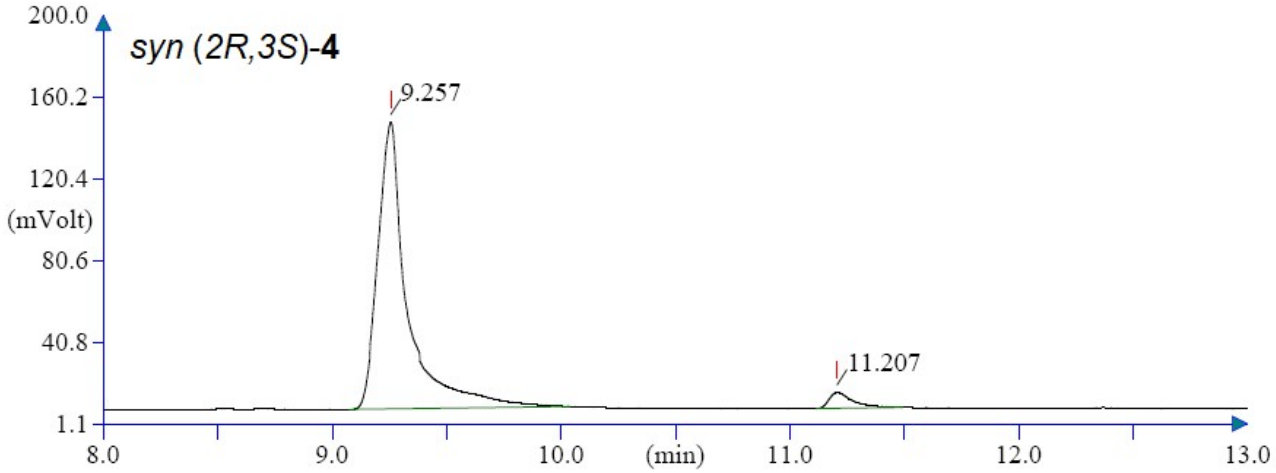


Figure S8. Synthesis and ^{1}H-NMR of compound **7**.

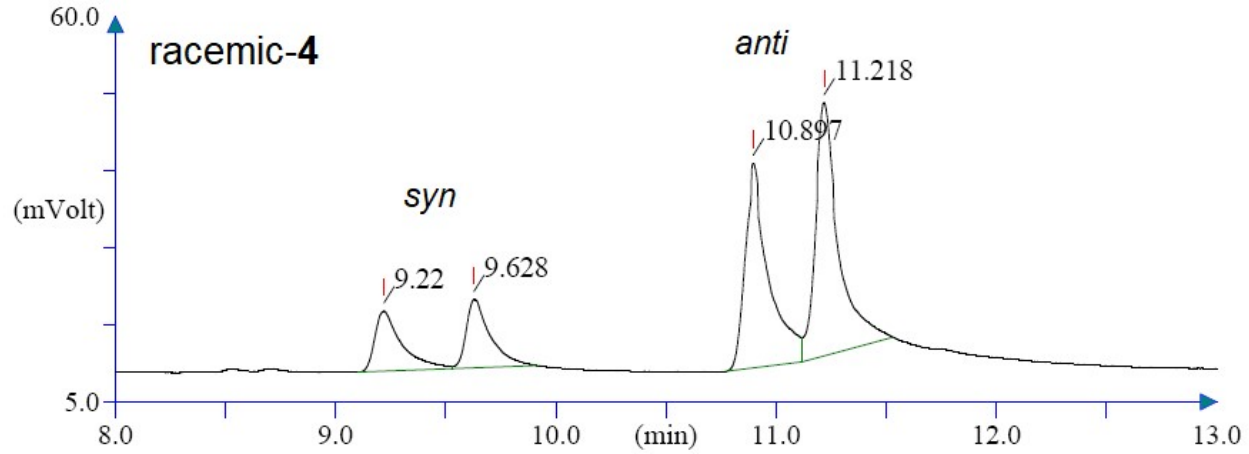


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc
9.220	733601	11.695	11.695
9.628	741352	11.818	11.818
10.897	2151541	34.299	34.299
11.218	2646493	42.189	42.189
	6272986		100.000

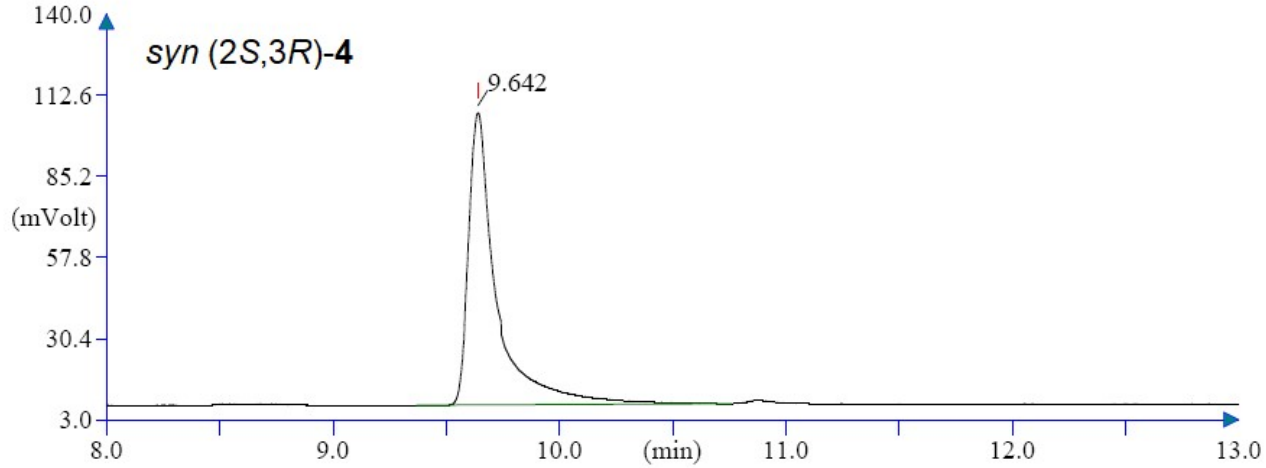


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Cor
9.257	12633240	95.729	95.729	
11.207	563576	4.271	4.271	
	13196820		100.000	
	6272986		100.000	

Figure S9. Chiral phase GC for the trifluoroacetyl derivative of (2R,3*S*)-**4.**

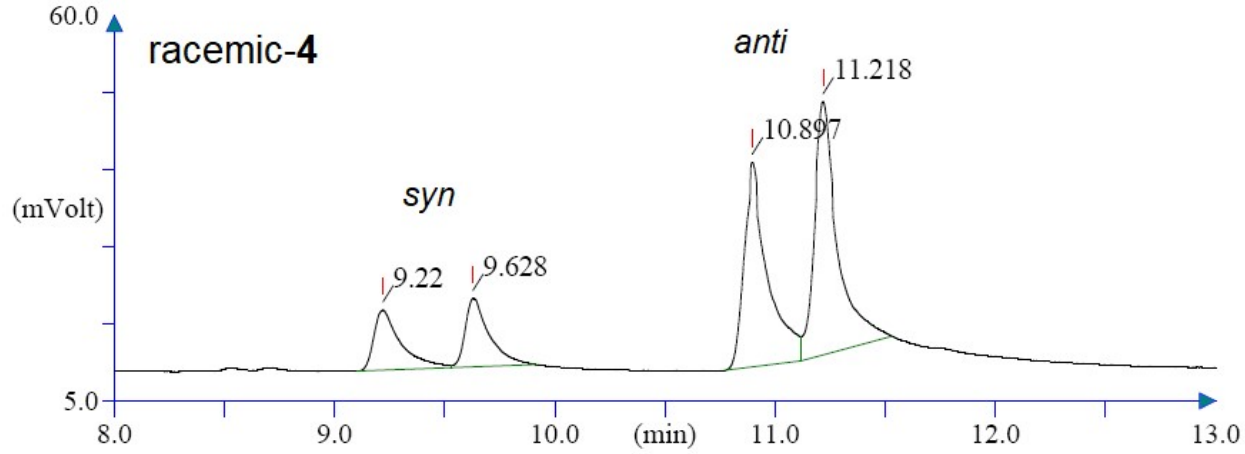


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc
9.220	733601	11.695	11.695
9.628	741352	11.818	11.818
10.897	2151541	34.299	34.299
11.218	2646493	42.189	42.189
	6272986		100.000

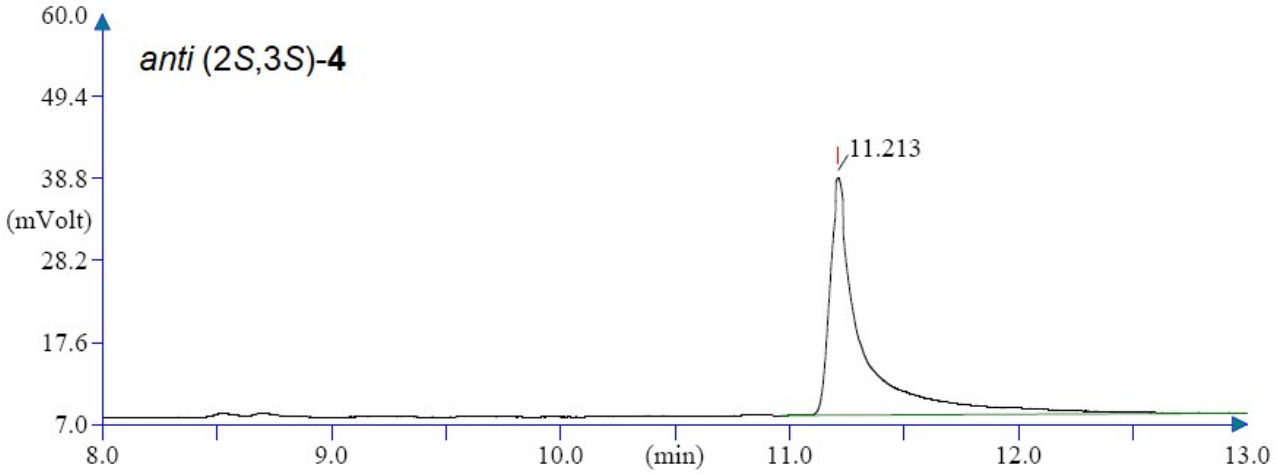


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Com
9.642	8546274	100.000	100.000	
	8546274		100.000	

Figure S10. Chiral phase GC for the trifluoroacetyl derivative of (2*S*,3*R*)-**4.**

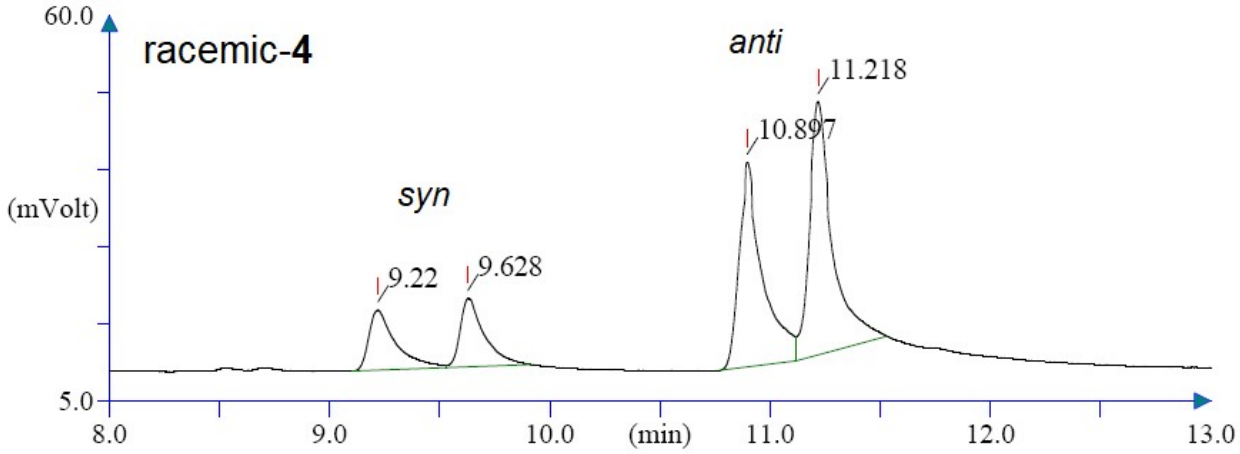


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc
9.220	733601	11.695	11.695
9.628	741352	11.818	11.818
10.897	2151541	34.299	34.299
11.218	2646493	42.189	42.189
	6272986		100.000

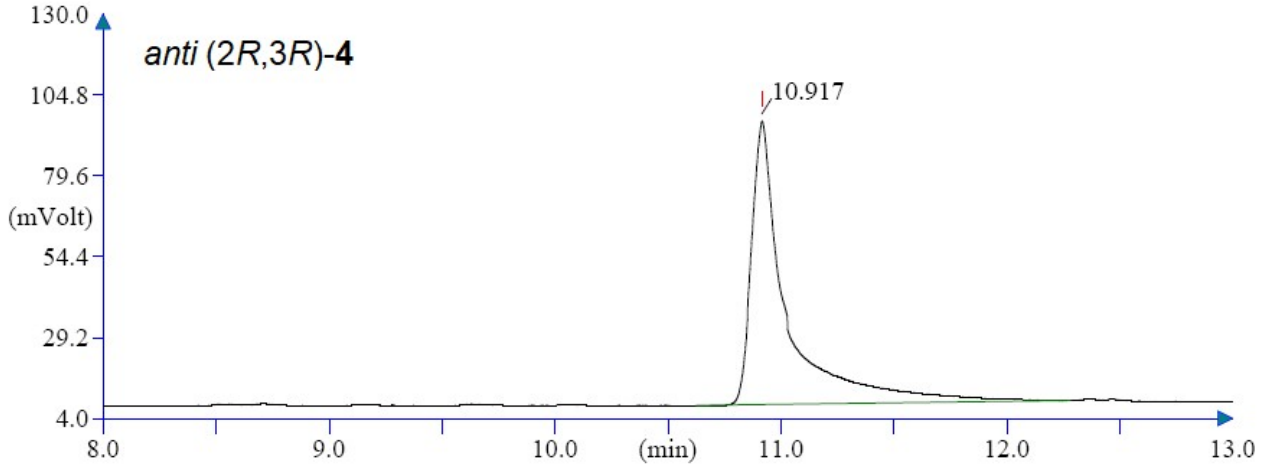


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Cor
11.213	3011497	100.000	100.000	
	3011497		100.000	

Figure S11. Chiral phase GC for the trifluoroacetyl derivative of (2*S*,3*S*)-**4.**

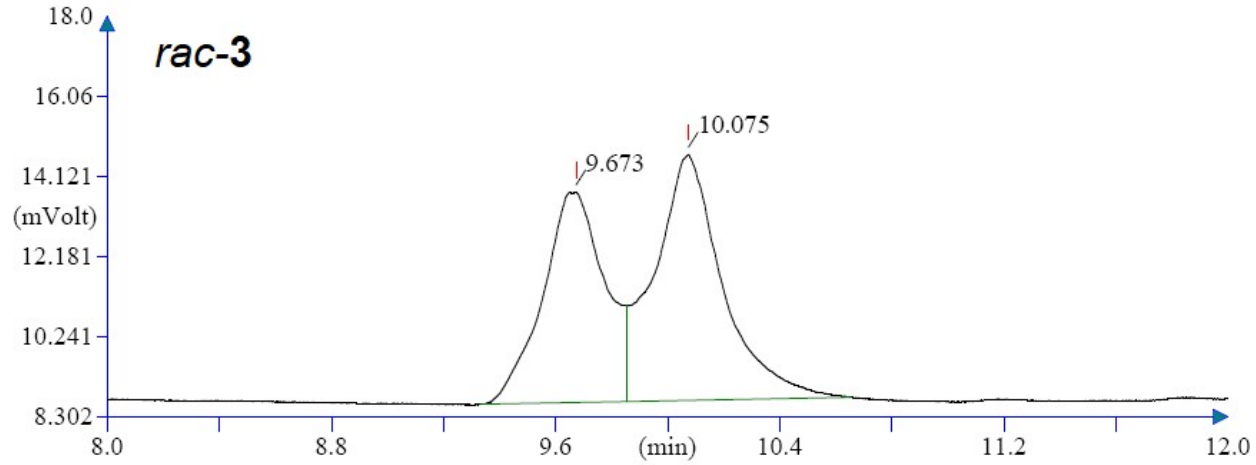


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc
9.220	733601	11.695	11.695
9.628	741352	11.818	11.818
10.897	2151541	34.299	34.299
11.218	2646493	42.189	42.189
	6272986		100.000

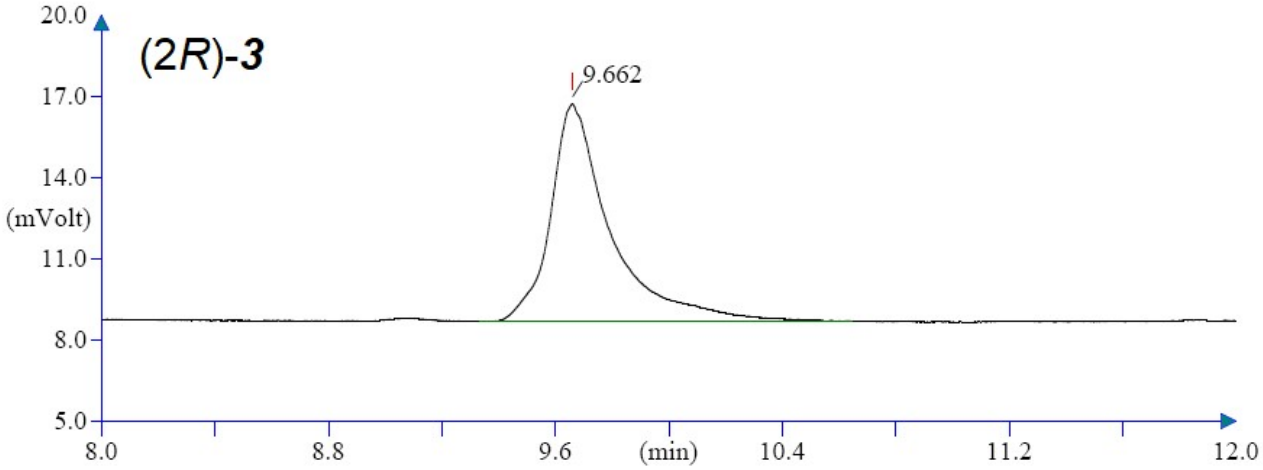


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Com
10.917	9671712	100.000	100.000	
	9671712		100.000	

Figure S12. Chiral phase GC for the trifluoroacetyl derivative of (2*R*,3*S*)-**4.**

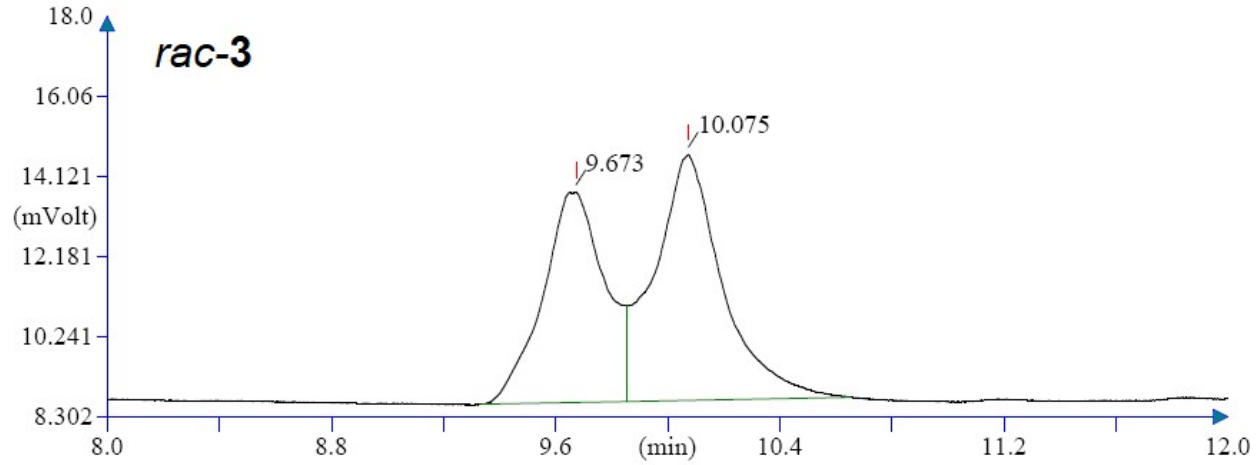


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Com
9.673	785967	43.866	43.866	
10.075	1005780	56.134	56.134	
	1791746		100.000	

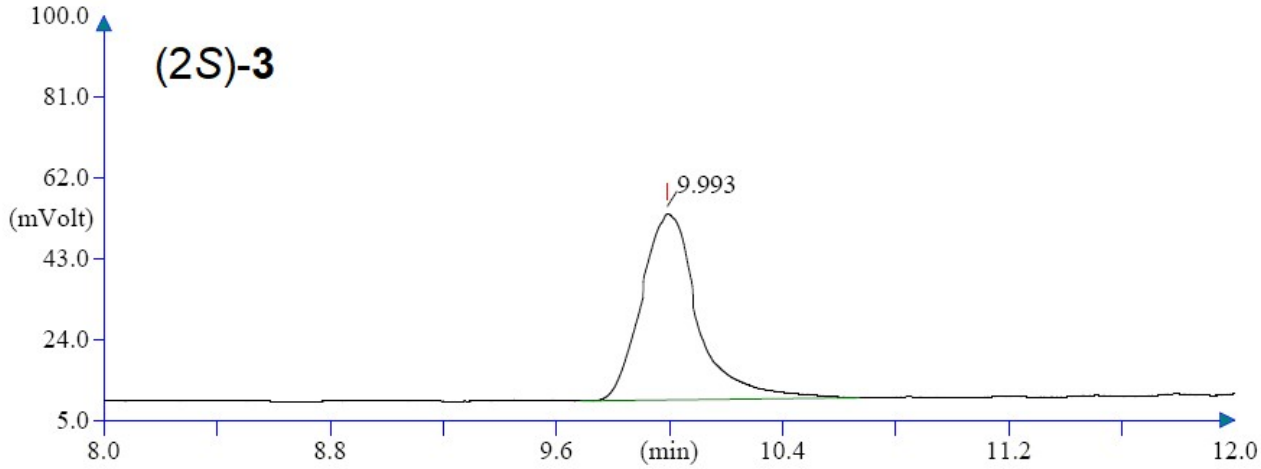


Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Con
9.662	1178508	100.000	100.000	
	1178508		100.000	

Figure S13. Chiral phase GC for the trifluoroacetyl derivative of (2*R*)-**3.**



Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Com
9.673	785967	43.866	43.866	
10.075	1005780	56.134	56.134	
	1791746		100.000	



Retention Time (min)	Area (.1*uV*sec)	Area % (%)	Original Conc	Con
9.993	6072989	100.000	100.000	
	6072989		100.000	

Figure S14. Chiral phase GC for the trifluoroacetyl derivative of (2*S*)-**3.**