Supplementary Materials

A Convenient U-Shape Microreactor for Continuous Flow Biocatalysis with Enzyme-Coated Magnetic Nanoparticles–Lipase-Catalyzed Enantiomer Selective Acylation of 4-(Morpholin-4-yl)butan-2-ol

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1 Materials and methods

1.1 Materials

Iron(III) chloride hexahydrate, sodium acetate trihydrate, tetraethoxysilane (TEOS), 35% ammonia solution, polyethylene glycols (PEG 400, and PEG 4000), hexadecyltrimethoxysilane (HdTMOS), 3-(2-aminoethylamino)propyldimethoxymethylsilane (ApDMOMS), sodium dihydrogen phosphate dihydrate, di-sodium hydrogen phosphate heptahydrate, neopentyl glycol diglycidyl ether (NGDE), sodium phosphate and vinyl acetate were purchased from Merck (Darmstadt, Germany) or Alfa Aesar Europe (Karlsuhe, Germany).
Ethylene glycol, 2-propanol, ethanol and hexane were purchased from Merck (Darmstadt, Germany). Patosolv® (a mixture of 10-15% 2-propanol and 85-90% ethanol) was a product of MolarChemicals (Budapest, Hungary).
CaLB for immobilization experiments (recombinant *Candida antarctica* lipase B as lyophilized powder) was obtained from c-LEcta (Leipzig, Germany).

1.2 Analytical methods

NMR spectra were recorded in the indicated deuterated solvents on Bruker DRX-500 or DRX-300 spectrometers operating at 500 MHz or 300 MHz for ^{1}H, and 126 or 75 MHz for ^{13}C. NMR signals are given in ppm on the δ scale.
Infrared (IR) spectra were recorded on a Bruker ALPHA FT-IR spectrometer (in ATR mode) and wavenumbers (ν) of bands are listed in cm^{-1}.
The gas chromatographic analyses were performed as given in Section 3.2 of the main article. The method and retention times are detailed in Table S1.

Table S1. GC method and retention times for analysis of the kinetic resolution reactions by chiral GC.

Substrate	**Temperature program**	**Retention times (min)**			
		(*S*)-**alcohol** (*S*)-**1**	(*R*)-**alcohol** (*R*)-**1**	(*S*)-**acetate** (*S*)-**2**	(*R*)-**acetate** (*R*)-**2**
(±)-**1**	160-170 °C, 0.8 °C/min, hold on 170 °C	14.18	14.39	17.03	17.55

Since the FID signal area for the alcohol **1** and acetate **2** compounds were not proportional with their molar amounts, the molar response factor (f) of the two different compounds were determined from peak areas (PA), the gas chromatogram of an equimolar mixture of (±)-**1** ($PA_1 = PA_{(S)\text{-}1} + PA_{(R)\text{-}1}$) and (±)-**2** ($PA_2 = PA_{(S)\text{-}2} + PA_{(R)\text{-}2}$), using the $f = PA_2 / PA_1$ equation. In this way, $f = 1.145$ could be determined for the alcohol **1** and acetate **2** compounds.

2 Preparation of MNP carriers for CaLB immobilization

Preparation of the MNP carriers was based on previously published methods [i,ii].

2.1 Preparation of the magnetite core (MNP)

To a 1 L Erlenmeyer flask, iron (III) chloride hexahydrate (20.2 g), polyethylene glycol (PEG 4000, 20.2 g), sodium acetate trihydrate (54 g), and ethylene glycol (600 mL) were added. After pouring the homogenous orange solution—obtained from the mixture by sonication—into an autoclave, the mixture was stirred for 24 h at 200 °C. After cooling, the MNPs from the resulting black suspension were washed (by suspending and anchoring with the aid of a neodymium magnet and decanting) with RO water (3 × 200 mL) and 2-propanol (IPA, 2 × 150 mL). The MNPs were dried in the fume hood at room temperature overnight.

2.2 Silica shell-covered MNPs (MNP-TEOS)

To a 1 L Erlenmeyer flask, MNPs (5.5 g), PEG 400 (5.5 g), ethanol (192.5 mL), and RO water (27.5 mL) were added, and the mixture was sonicated for 20 min. At room temperature and constant shaking at 200 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany), first 35% ammonia solution (13.8 mL) was added and then tetraethoxysilane (TEOS, 8.25 mL) was added to the mixture, which was shaken further for 24 h. The MNP-TEOS from the resulting black suspension was washed (by suspending and anchoring with the aid of a neodymium magnet and decanting) with RO water (4 × 15 mL, until neutral pH), and IPA (3 × 150 mL). The MNPs were dried in the fume hood at room temperature overnight.

2.3 Surface modification for immobilization by adsorption (MNP_A)

Into a 20 ml screw-cap vial, MNPs-TEOS (500 mg), PEG 400 (250 mg) and ethanol (5 mL) were added, and the suspension was sonicated for 10-15 min. At room temperature and constant shaking at 200 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany), first 35% ammonia solution (50 μL) was added and then after 10 min hexadecyltrimethoxysilane (HdTMOS, 195 μL) was added to the mixture which was shaken further for 24 h. The MNP_A from the resulting black suspension was washed (by suspending and anchoring with the aid of a neodymium magnet and decanting) with IPA (2.5 mL), IPA:water=1:1 (2.5 mL), and IPA (2.5 mL). The MNP_A carrier was dried in the fume hood at room temperature overnight.

2.4 Surface modification for covalent immobilization (MNP_C)

The carrier for covalent immobilization of CaLB (MNP_C) was prepared in two steps.

2.4.1 Heterofunctionalization of MNP-TEOS

Into a 20 ml screw-cap vial, MNPs-TEOS (600 mg), PEG 400 (300 mg) and ethanol (6 mL) were added, and the suspension was sonicated for 10-15 min. At room temperature and constant shaking at 200 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany), first 35% ammonia solution (60 μL) was added, then after 10 min hexadecyltrimethoxysilane (HdTMOS, 220 μL) and 3-(2-aminoethylamino)propyl-dimethoxymethylsilane (ApDMOMS, 7.6 μL) were added to the mixture which was shaken further for 24 h. The heterofunctionalized MNP-TEOS from the resulting black suspension was washed (by suspending and anchoring with the aid of a neodymium magnet and decanting) with IPA (2.5 mL), IPA:water=1:1 (2.5 mL), and IPA (2.5 mL), and dried in the fume hood at room temperature overnight.

2.4.2 Activation of the heterofunctionalized MNP-TEOS with NDGE

Into a 20 ml screw-cap vial, heterofunctionalized MNP-TEOS (500 mg), PEG 400 (250 mg) and IPA (9 mL) were added, and the suspension was sonicated for 10-15 min. Then, a solution of neopentyl glycol diglycidyl ether (NPDGE, 20 mg) solution in IPA (1 mL) was added and the mixture was shaken at 60 °C and at 300 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany) for 24 h. The carrier for covalent immobilization (MNP_C) from the resulting black suspension was washed (by suspending

and anchoring with the aid of a neodymium magnet and decanting) with IPA (3 × 2.5 mL) and dried in the fume hood at room temperature overnight.

3 Immobilization of Lipase B from *Candida antarctica* (CaLB) on the MNP carriers

To a screw-capped vial (4 mL), MNPs (40 mg, MNP_A for adsorption, or MNP_C for covalent immobilization) and proper amounts of CaLB solution in sodium phosphate buffer (8.0, 4.0, 2.66, 2.0, 1.0 mg mL^{-1}, in pH 7.5, 100 mM) were added to provide the desired CaLB to MNP mass ratio (1:5,1:10,1:20,1:15,1:40 mg mg^{-1}) and the volume was completed to a final volume of 1 mL with phosphate buffer (pH 7.5, 100 mM). The resulting mixtures were shaken at 300 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany) at room temperature for 2 h; resulting in CaLB-MNP_{An} or CaLB-MNP_{Cm} (where n= 5-40 and m= 5-40, reflecting the CaLB:MNP mass ratio applied).
Protein concentration before the immobilization and after the immobilization from the supernatant was determined by the Bradford assay [80]. Samples (7.5 μL) taken from each CaLB solution before adding to MNPs and from each supernatant of immobilization trials after (30, 60, 90, 120 min) were added to vials of a 96-well plate (UV-Star microplate, half area, clear; Greiner Bio-One, Kremsmünster, Austria) containing sodium phosphate buffer (67.5 μL, pH 7.5, 100 mM) followed by the addition of Bradford reagent (75 μL). The absorbance of the assay mixtures at 595 nm were measured in a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA).
Since $A = \varepsilon \times c \times L$ (where the path length (L) and the molar absorption coefficients (ε) are constants), the immobilization yield (Y) for a certain trial was calculated by using the absorption of the initial CaLB solution (A_0) and of the supernatant from an immobilization after a certain time (A_S), according to Eq. 1.

$$Y = \frac{A_S}{A_0} \times 100 \qquad \text{(Equation 1)}$$

4 Synthesis of the racemic alcohol (±)-1, and the racemic and (*S*)-acetates (±)-2 and (*S*)-2

4.1 4-(Morpholin-4-yl)butan-2-one

Morpholine (1.70 mL, 1.72 g; 19.8 mmol) and methyl vinyl ketone (2.05 mL, 1.73 g, 25 mmol, 1.25 equiv.) were reacted according to the method of Rossi D. et al. [81] to yield 4-(morpholin-4-yl) butan-2-one in 60% yield (1.87 g) as a yellow oil.

IR (film, cm^{1}): 2960–2850, 2810, 1705, 1460–1450, 1360, 1275–1290, 1140, 1115, 1070. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.14 (*s*, 3H, -CH_3), 2.42 (*m*, 4H, NCH_2CH_2C=O), 2.61 (*m*, 4H, NCH_2CH_2O), 3.66 (*t*, *J* = 4.8 Hz, 4H, NCH_2CH_2O). Spectra agreed with those reported [**Error! Bookmark not defined.**].

4.2 Racemic 4-(morpholin-4-yl)butan-2-ol (±)-**1**

To 4-(morpholin-4-yl)butan-2-one (1.80 g, 11.5 mmol), methanol (18.0 mL) was added, and the mixture was cooled down to 0 °C. Then, the sodium borohydride (0.657 g; 17.3 mmol, 1.5 eq.) was added portionwise, and then the mixture was stirred at room temperature for 2 h. The product was obtained after adding dichloromethane (60 mL) followed by washing the organic layer with water (2 × 20 mL) and brine (10 mL). After drying the organic phase over magnesium sulfate, the solvent was evaporated to leave the racemic alcohol (±)-**1** as a yellowish oil (1.47 g, 80.4%).
IR (film, cm^{1}): 3404, 2962, 2853, 2615, 1453, 1448, 1288, 1115, 1031, 900, 856, 733. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 1.16 (*dd*, 3H, -CH_3), 1.50 (*m*, 1H, CH*H*-CH-O), 1.65 (*m*, 1H, C*H*H-CH-O), 2.41 (*br s*, 2H, CHCH_2CH_2), 2.63 (*m*, 4H, NCH_2CH_2O), 3.71 (*br s*, 4H, NCH_2CH_2O), 3.96 (*m*, 1H, C*H*-O), 5.78 (*br s*, 1H, C-O*H*). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 23.5, 33.0, 53.8, 58.3, 66.9, 69.8. The NMR spectra agreed with that reported [82].

4.3 Racemic 4-(morpholin-4-yl)butan-2-yl acetate (±)-**2**

Into a 20 mL vial containing a mixture of racemic 4-(morpholin-4-yl) butan-2-ol (±)-**1** (200 mg, 1.26 mmol) and 2M NaOH solution (125 μL, 2.5 mmol) in ethyl acetate (10 mL), acetyl chloride (196 mg, 2.5 mmol, 178 μL) was added dropwise at room temperature and the resulting mixture was shaken at 300 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany) at room temperature for 24 h. After

washing the organic layer with 10% Na_2CO_3 solution (2 mL), water (2 × 2 mL) and brine (2 mL), and drying over magnesium sulfate, the solvent was evaporated to leave the racemic acetate (±)-**2** as a yellowish oil (182 mg, 72%).
IR (film, cm^1): 2955, 2863, 2810, 1734, 1459, 1448, 1372, 1242, 1118, 1071, 1013, 867. ^1H NMR (500 MHz, $CDCl_3$) δ 1.25 (*d*, *J* = 6.3 Hz, 3H, -C*H*$_3$), 1.70 (*ddt*, *J* = 14.0, 9.0, 5.6 Hz, 1H, CH*H*-CH-O), 1.78–1.89 (*m*, 1H, C*H*H-CH-O), 2.03 (*s*, 3H, -COC*H*$_3$), 2.39 (*m*, 2H, CHCH$_2$C*H*$_2$), 2.45 (*br s*, 4H, NC*H*$_2$CH$_2$O), 3.73 (*t*, *J* = 4.7 Hz, 4H, NCH$_2$C*H*$_2$O), 4.97 (*ddd*, *J* = 7.6, 6.3, 5.1 Hz, 1H, CH-O). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 20.08, 21.33, 32.78, 53.68, 55.04, 66.83, 69.47, 170.65.

4.4 (S)-4-(Morpholin-4-yl)butan-2-yl acetate (*S*)-**2**

Into a 20 mL vial containing a mixture of (*S*)-4-(morpholin-4-yl)butan-2-ol (*S*)-**1** (80 mg, 0.5 mmol; obtained from CaLB-catalyzed KR in batch mode) and 5M NaOH solution (20 µL, 1.0 mmol) in ethyl acetate (5 mL), acetyl chloride (79 mg, 1.0 mmol, 71 µL) was added dropwise at room temperature and the resulting mixture was shaken at 300 rpm in a Vibramax 100 shaker (Heidolph, Schwabach, Germany) at room temperature for 1 h. After washing the organic layer with 10% Na_2CO_3 solution (1 mL), water (2 × 1 mL) and brine (1 mL) and drying over magnesium sulfate, the solvent was evaporated to leave the enantiopure acetate (*S*)-**2** as a yellowish oil (90 mg, 90%).
$[\alpha]^{25}_D$= −1.2 (*c* 3, EtOH) for the sample having *ee* >99.5% by GC. The IR, ^1H NMR and ^{13}C NMR spectra of the produced (*S*)-acetate (*S*)-**2** were indistinguishable from those of the (*R*)-acetate (*R*)-**2**.

5 Original chromatograms and spectra

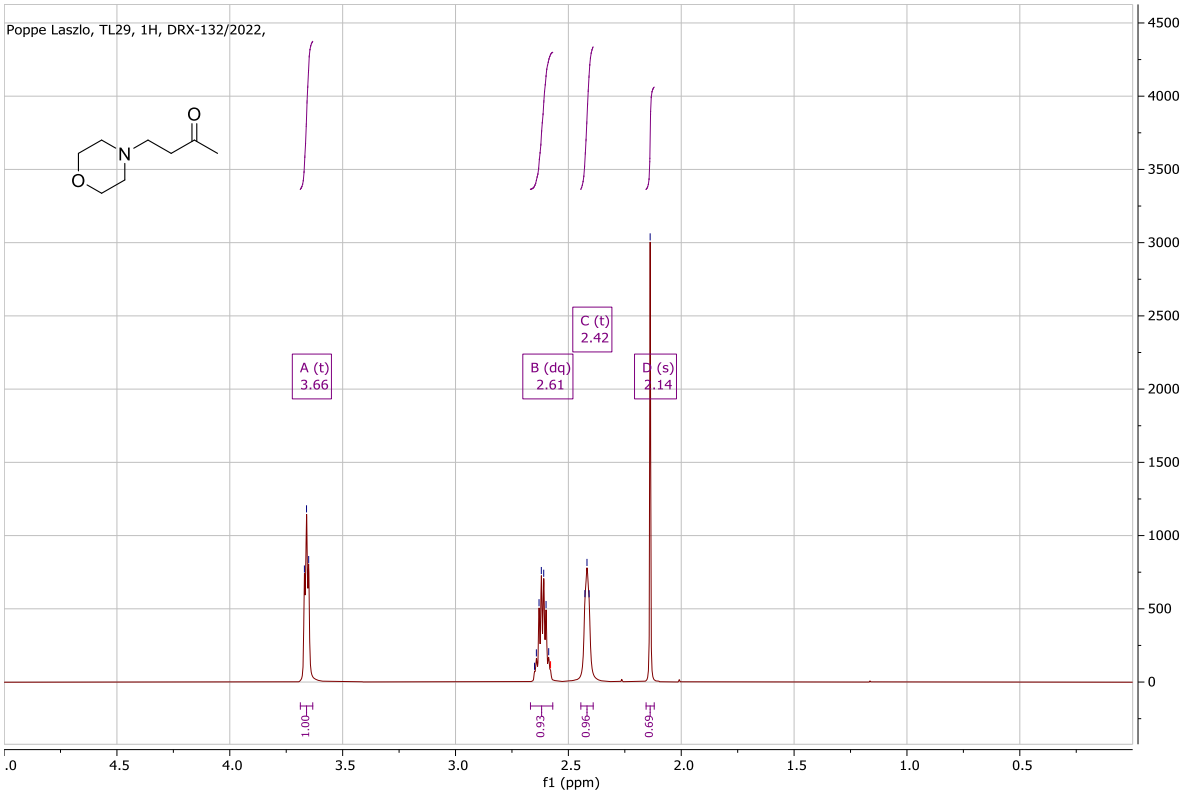

Figure S1. ^1H NMR spectrum of 4-(morpholin-4-yl)butan-2-one.

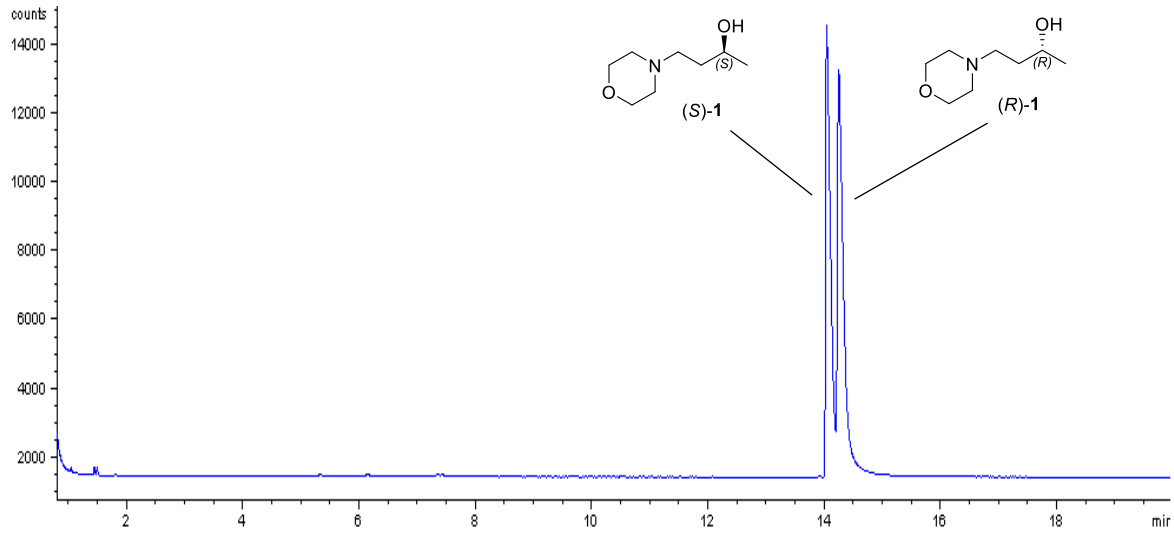

Figure S2. Gas chromatogram of racemic 4-(morpholin-4-yl)butan-2-ol (±)-**1.**

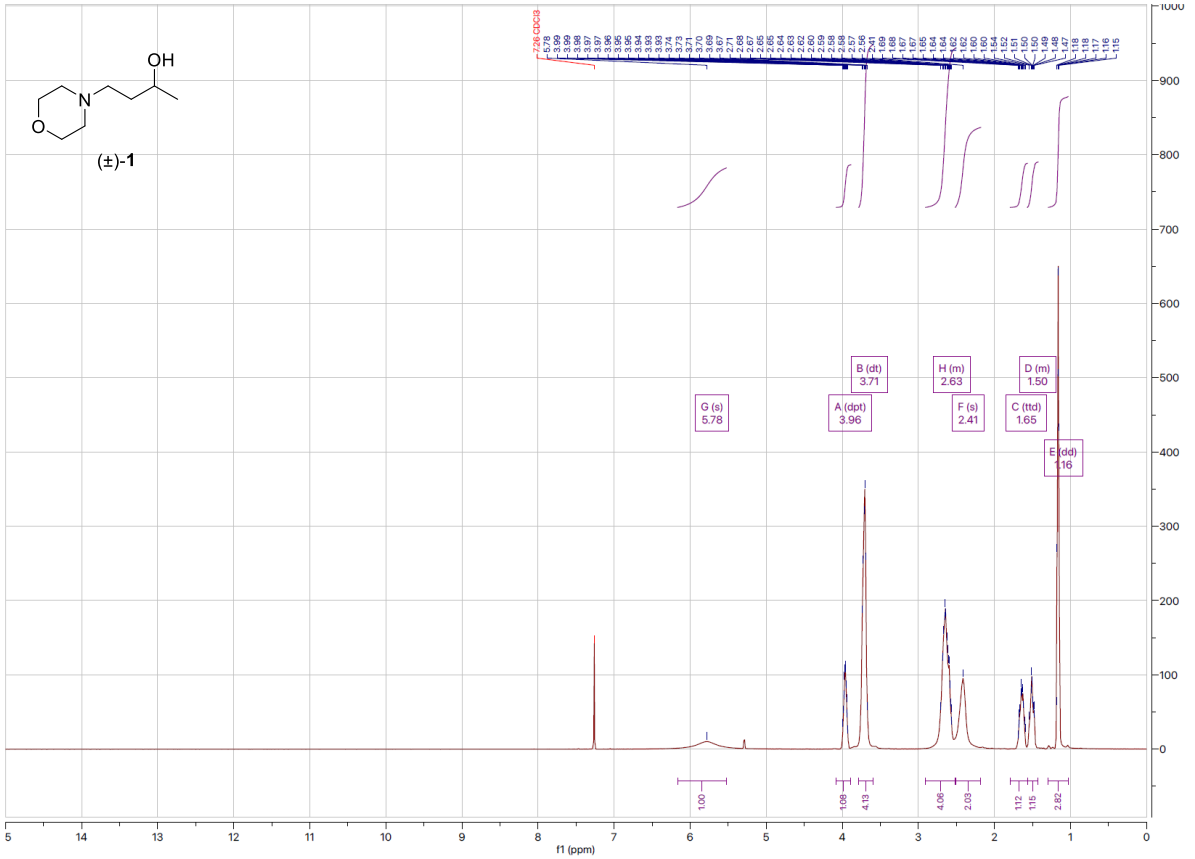

Figure S3. ^{1}H NMR spectrum of racemic 4-(morpholin-4-yl)butan-2-ol (±)-**1.**

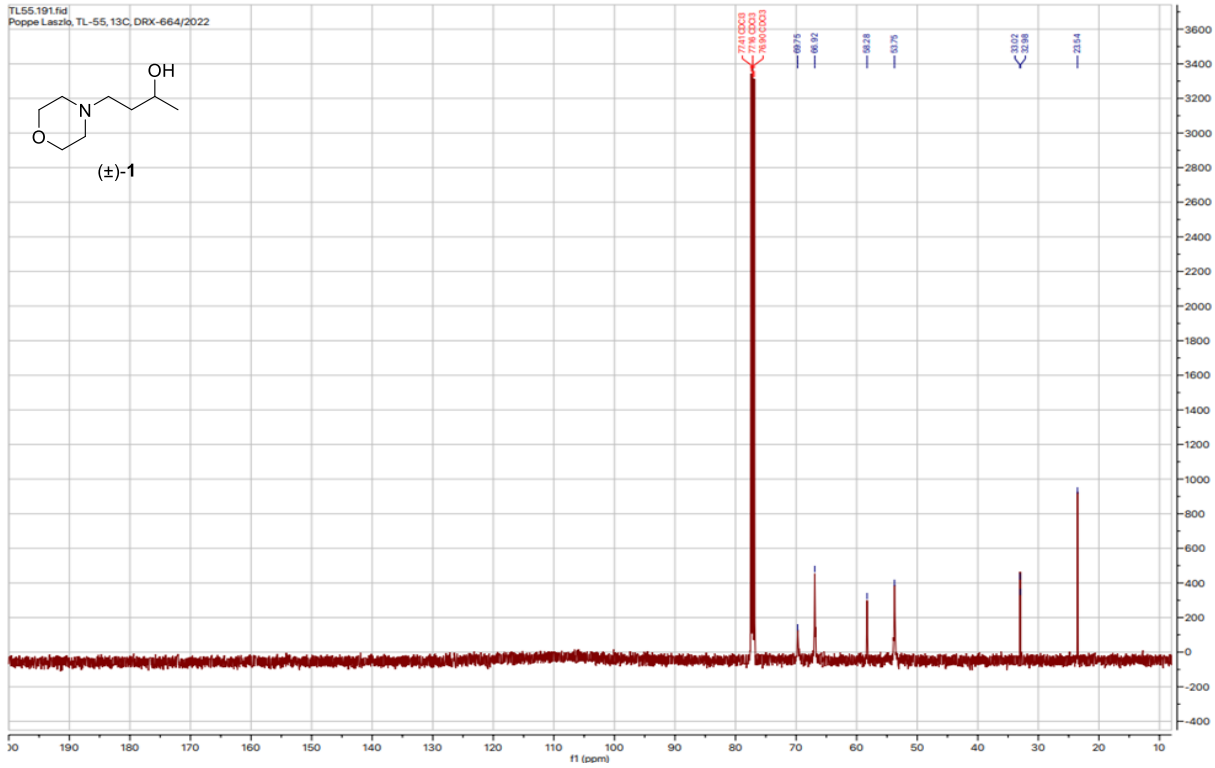

Figure S4. ^{12}C NMR spectrum of racemic 4-(morpholin-4-yl)butan-2-ol (±)-**1.**

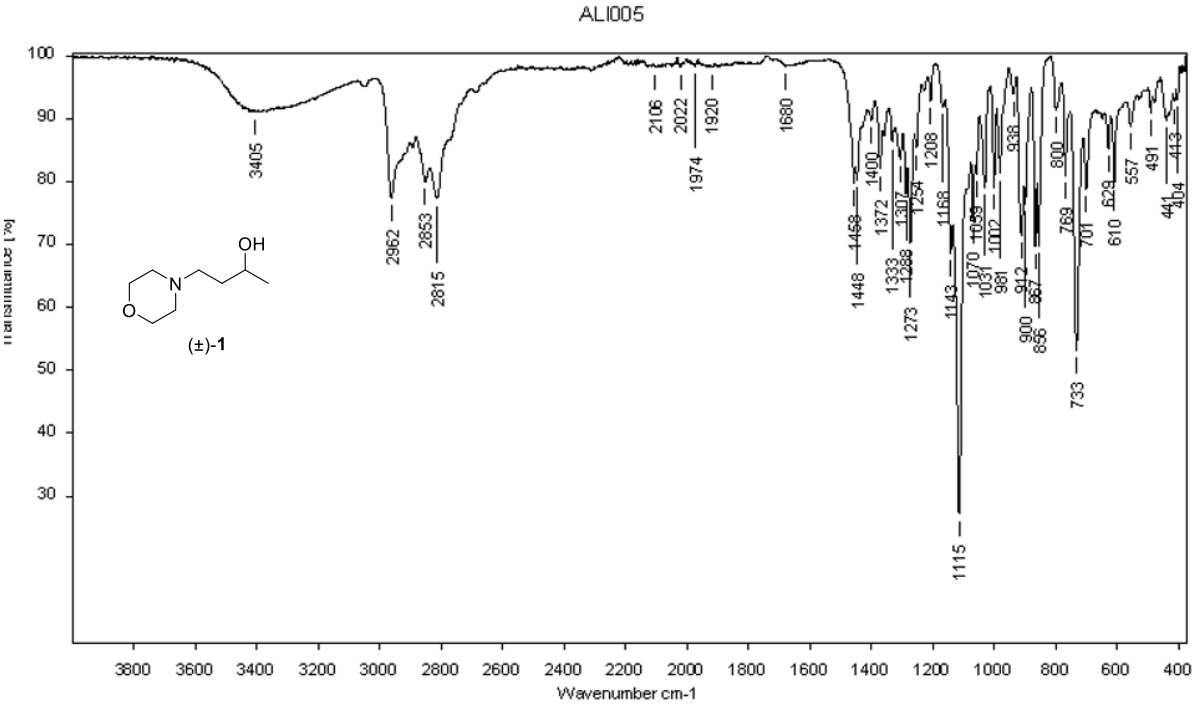

Figure S5. IR spectrum of racemic 4-(morpholin-4-yl)butan-2-ol (±)-**1.**

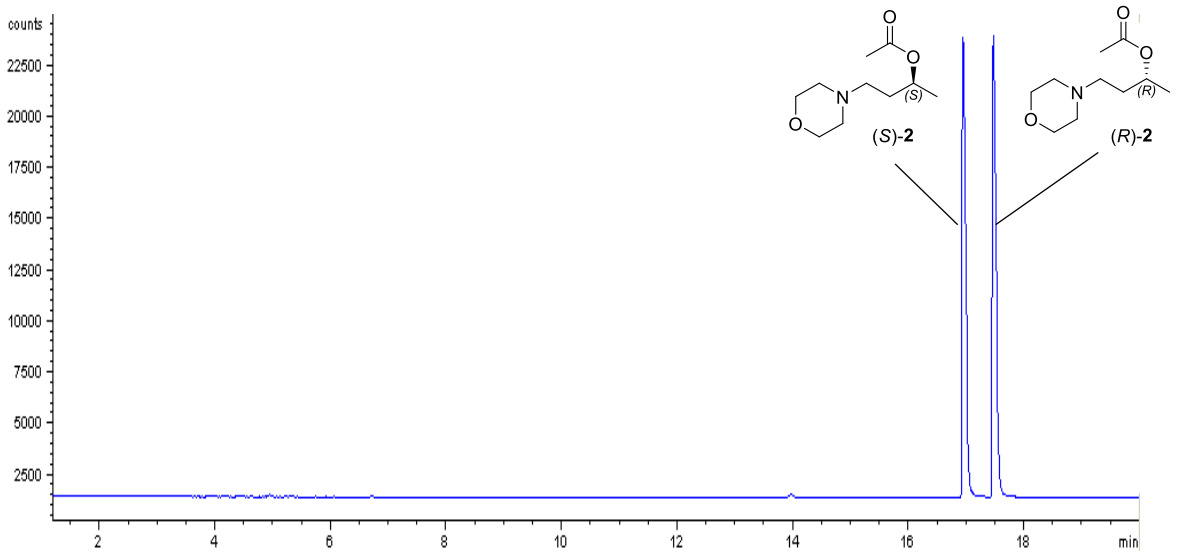

Figure S6. Gas chromatogram of racemic 4-(morpholin-4-yl)butan-2-yl acetate (±)-**2.**

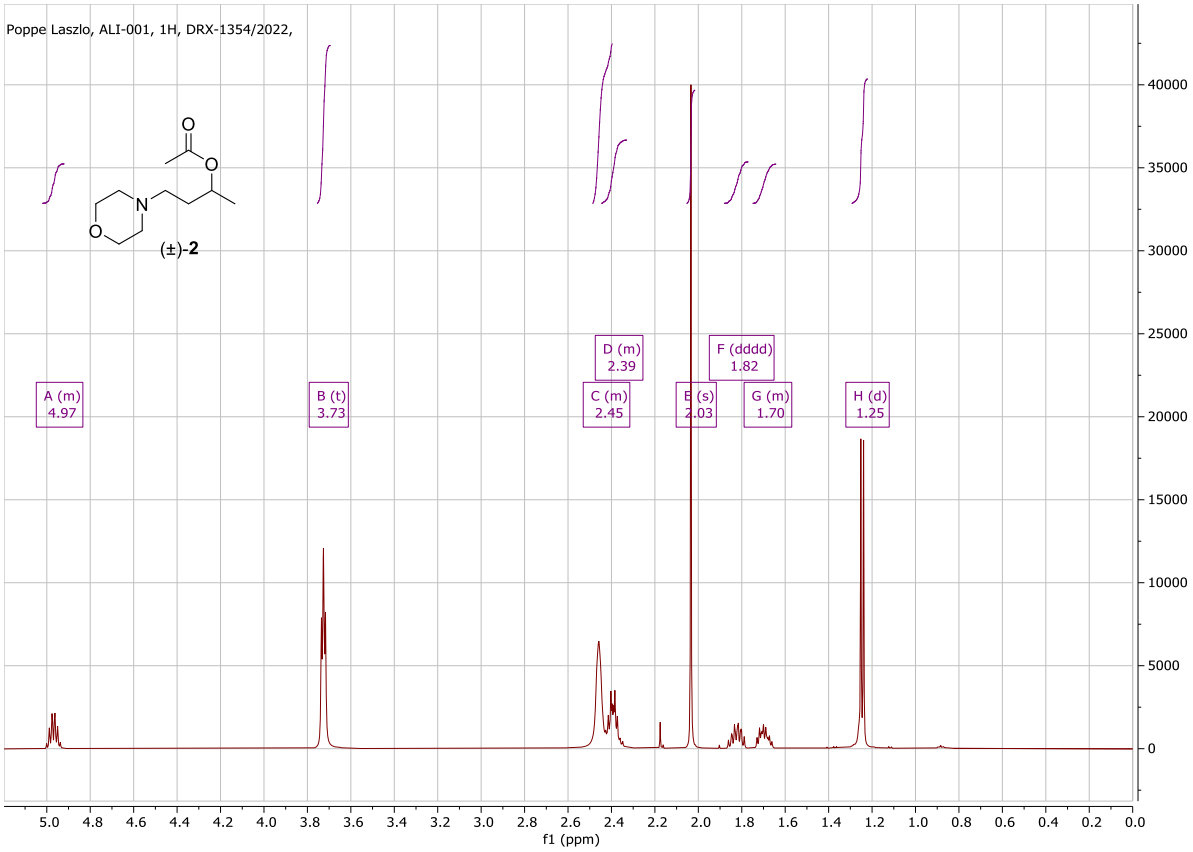

Figure S7. ^{1}H NMR spectrum of racemic 4-(morpholin-4-yl)butan-2-yl acetate (±)-**2.**

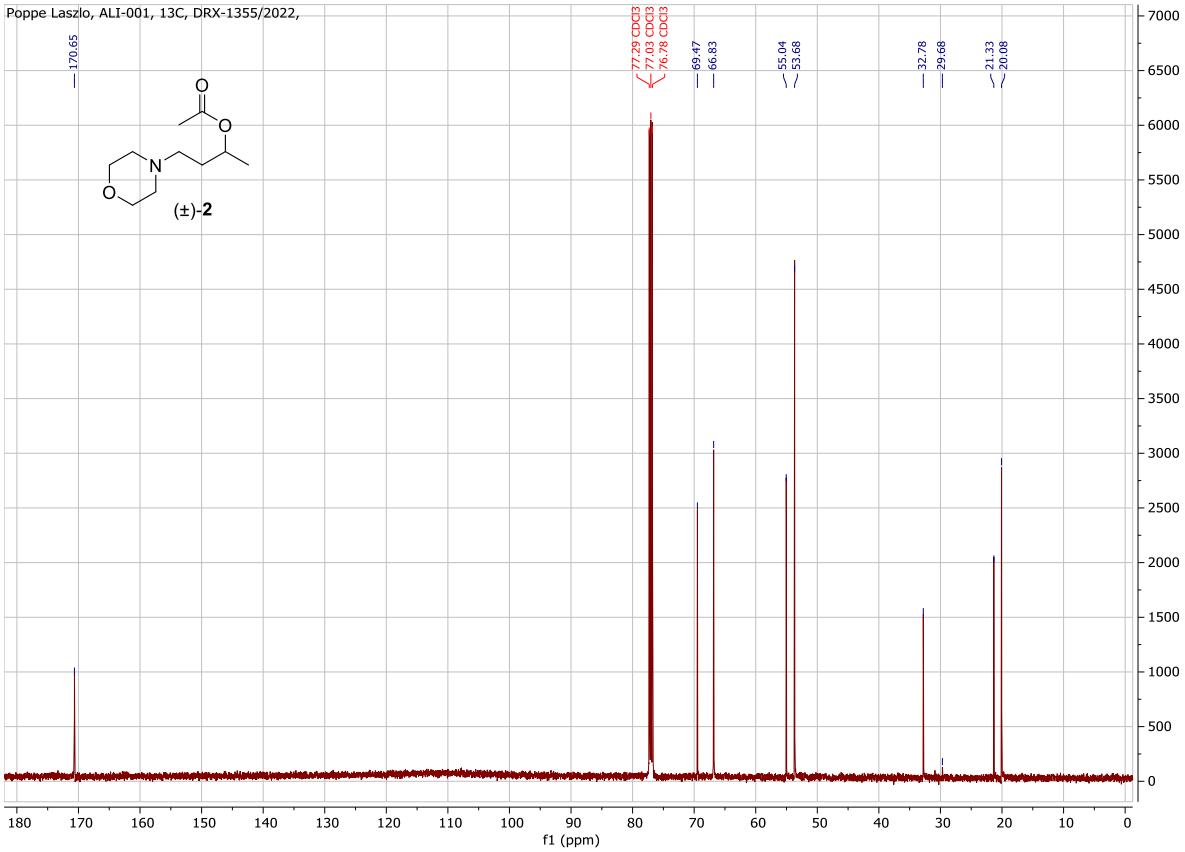

Figure S8. ^{13}C NMR spectrum of racemic 4-(morpholin-4-yl)butan-2-yl acetate (±)-**2.**

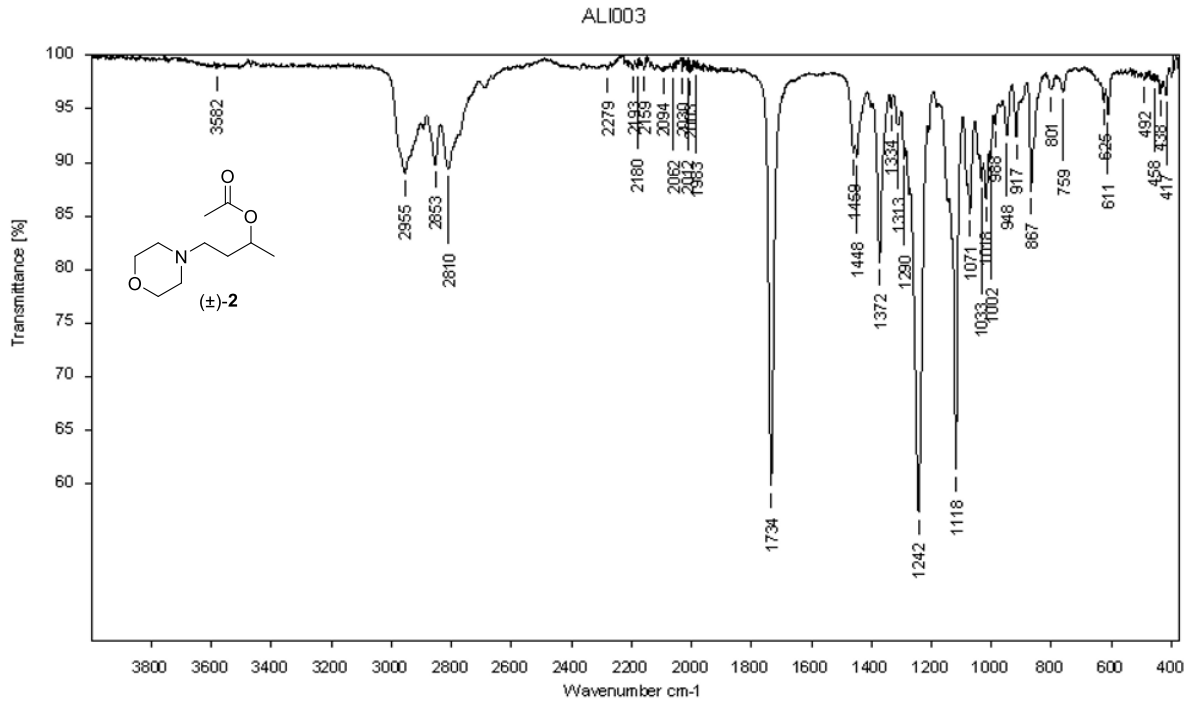

Figure S9. IR spectrum of racemic 4-(morpholin-4-yl)butan-2-yl acetate (±)-**2.**

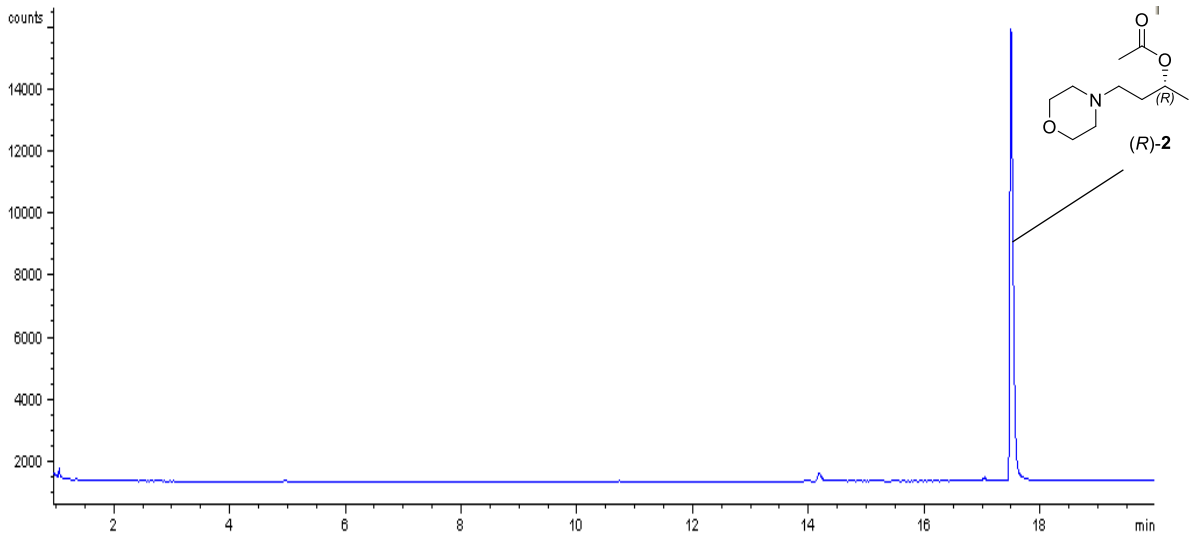

Figure S10. Gas chromatogram of the (*R*)-4-(morpholin-4-yl)butan-2-yl acetate (*R*)-**2** produced by CaLB-MNP$_{C15}$-catalyzed kinetic resolution in batch mode.

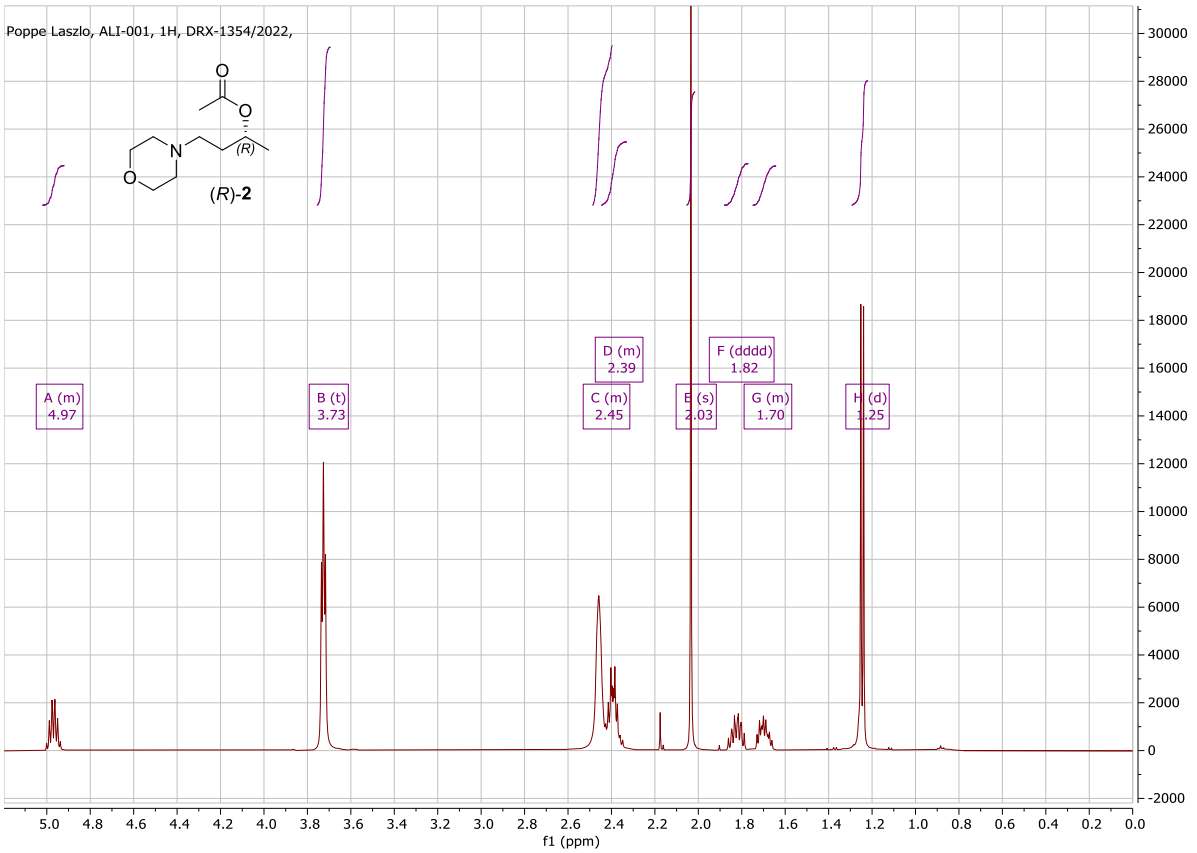

Figure S11. ^{1}H NMR spectrum of the (*S*)-4-(morpholin-4-yl)butan-2-yl acetate (*R*)-**2** produced by CaLB-MNP$_{C15}$-catalyzed kinetic resolution in batch mode.

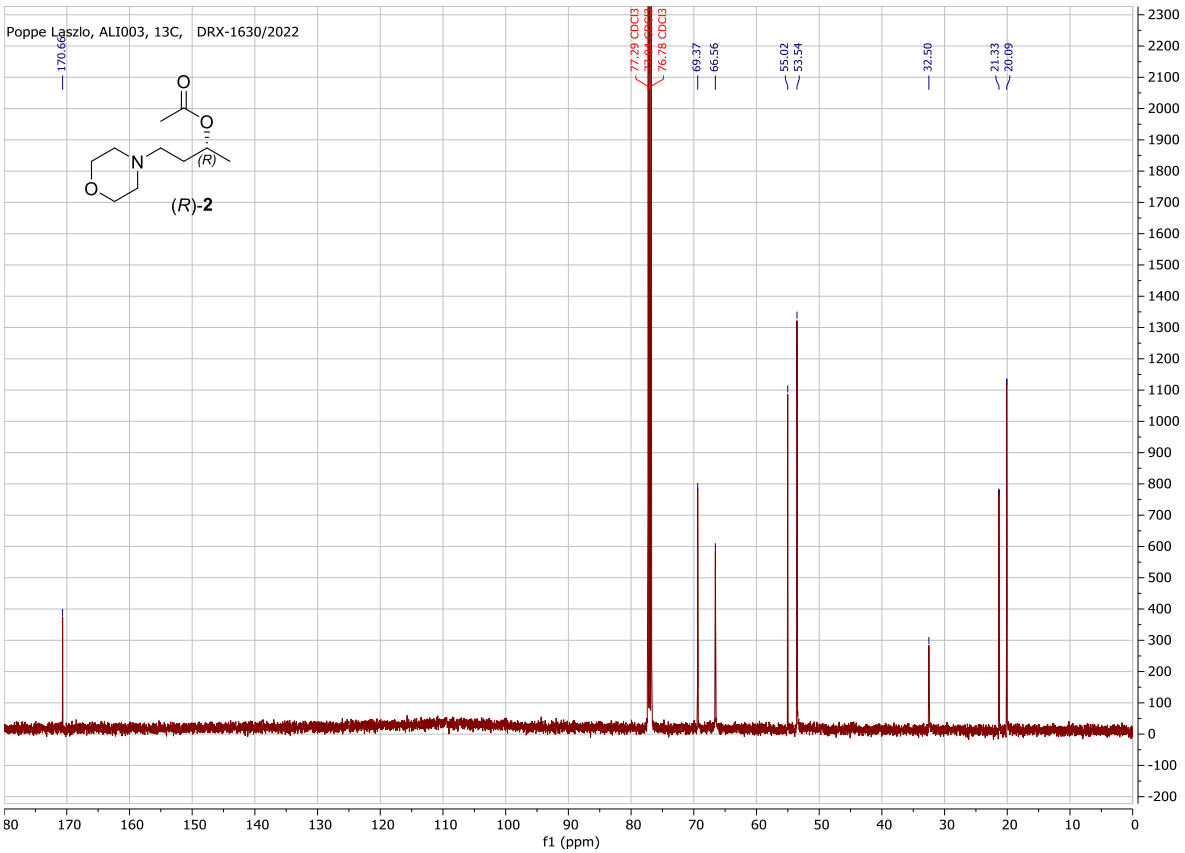

Figure S12. ^{13}C NMR spectrum of the (*R*)-4-(morpholin-4-yl)butan-2-yl acetate (*R*)-**2** produced by CaLB-MNP$_{C15}$-catalyzed kinetic resolution in batch mode.

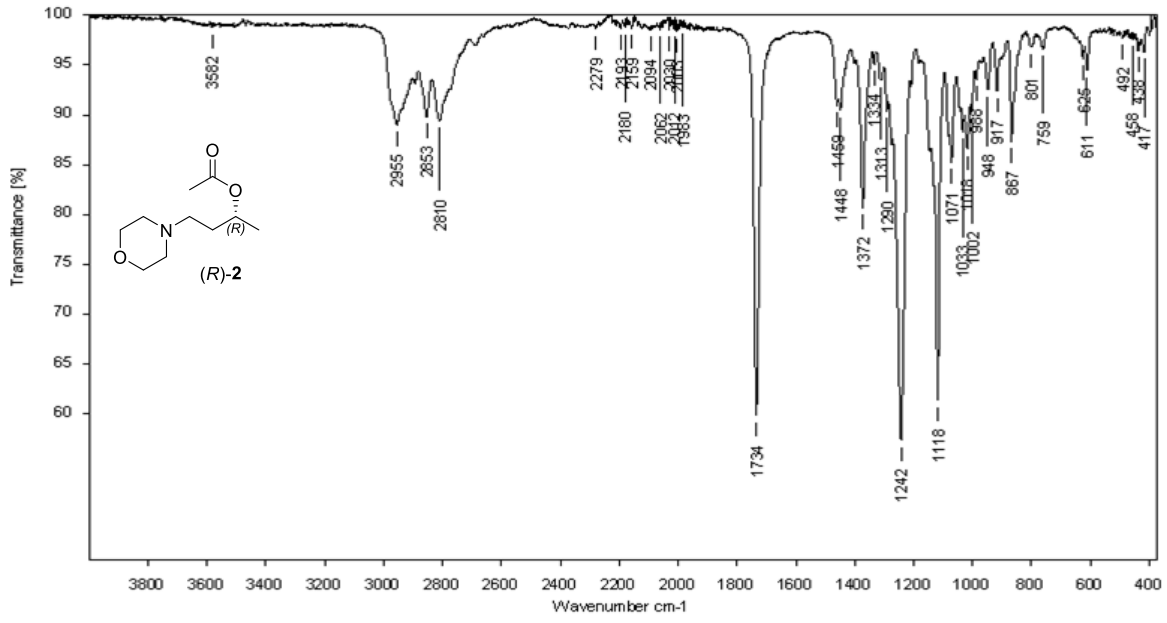

Figure S13. IR spectrum of the (*R*)-4-(morpholin-4-yl)butan-2-yl acetate (*R*)-**2** produced by CaLB-MNP$_{C15}$-catalyzed kinetic resolution in batch mode.

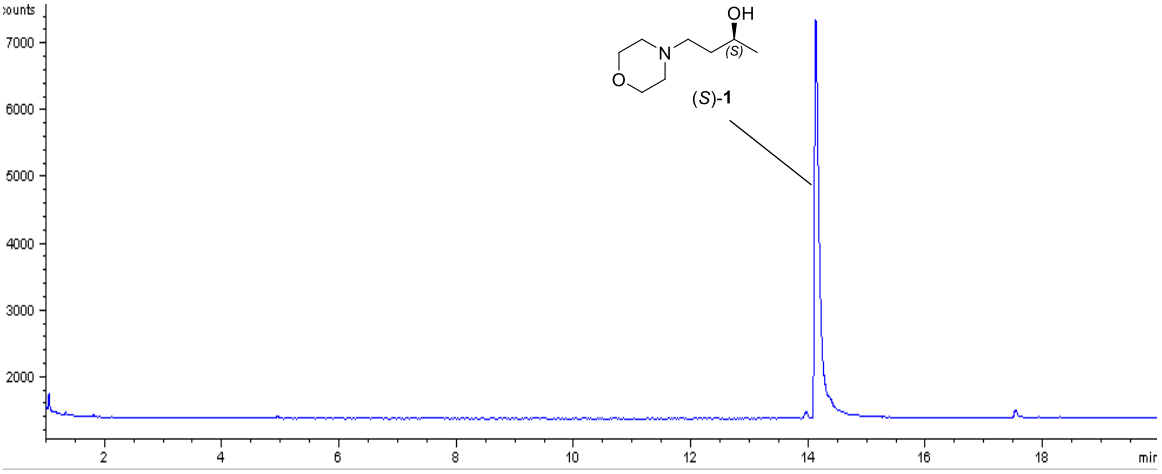

Figure S14. Gas chromatogram of the (*S*)-4-(morpholin-4-yl)butan-2-ol (*S*)-**1** produced by CaLB-MNP$_{C15}$-catalyzed kinetic resolution in batch mode.

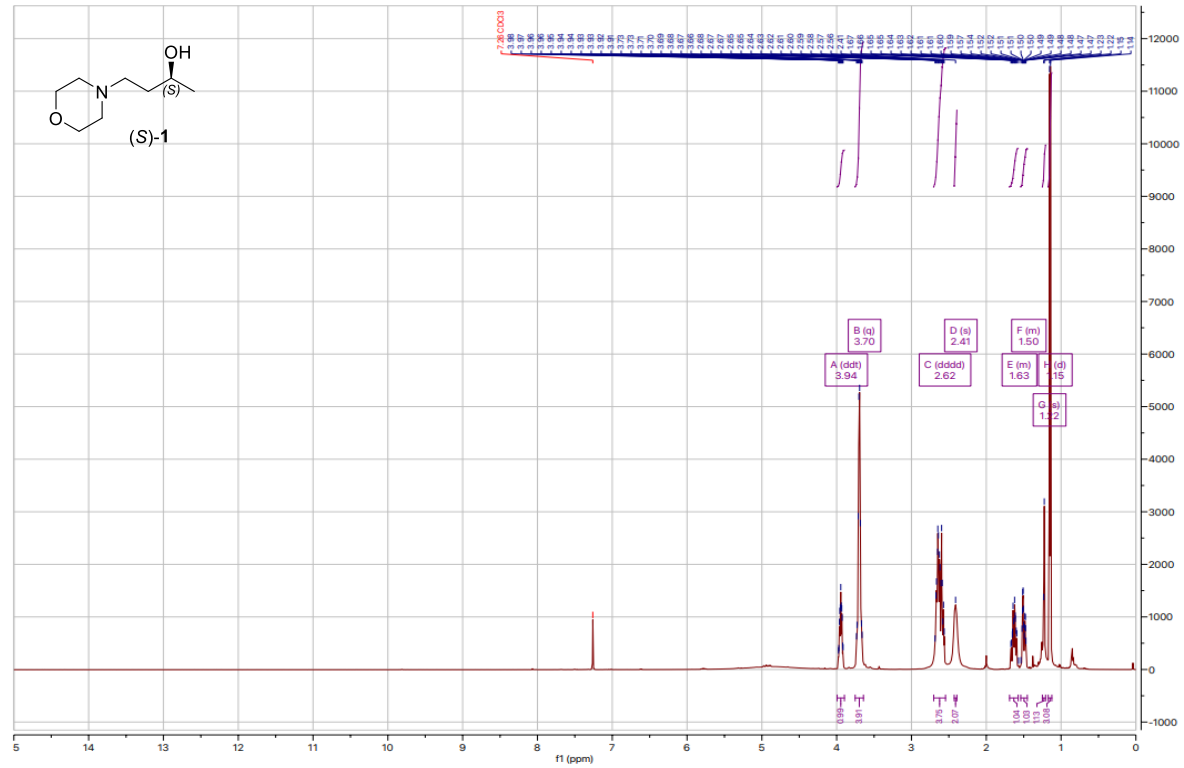

Figure S15. ^{1}H NMR spectrum of the (*S*)-4-(morpholin-4-yl)butan-2-ol (*S*)-**1** produced by CaLB-MNP$_{C15}$-catalyzed kinetic resolution in batch mode.

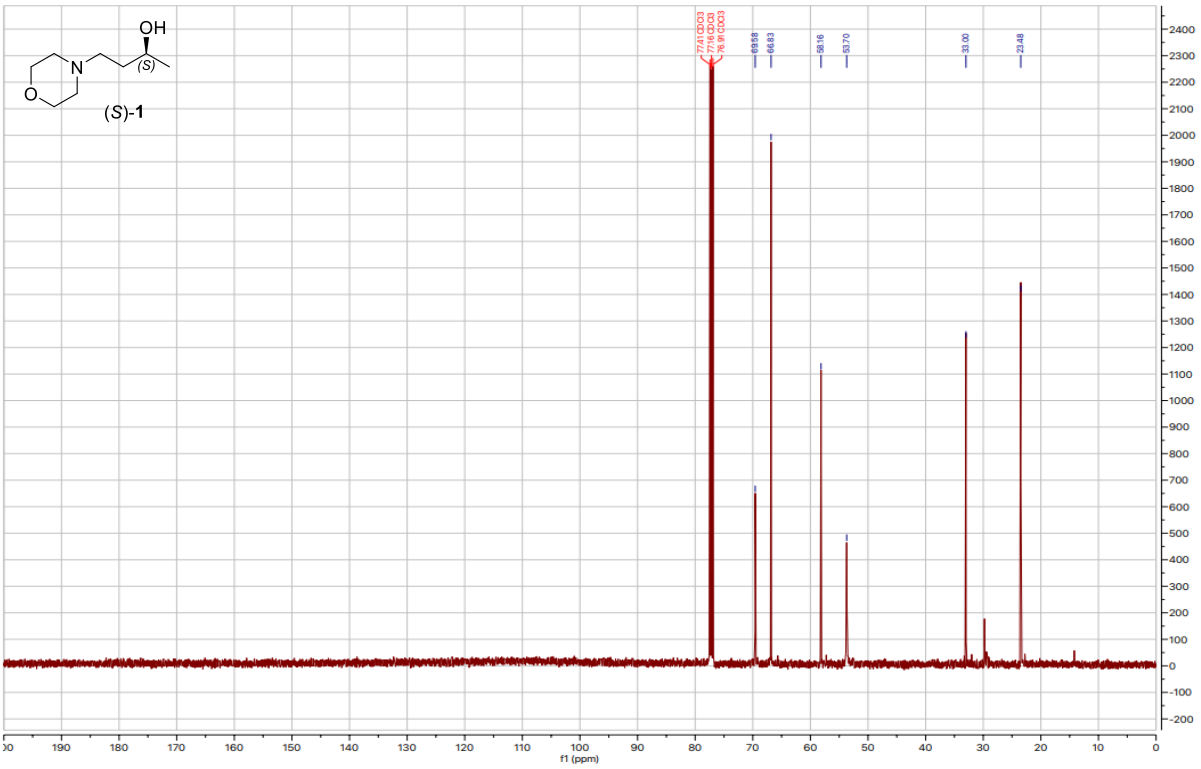

Figure S16. ^{13}C NMR spectrum of the (*S*)-4-(morpholin-4-yl)butan-2-ol (*S*)-**1** produced by CaLB-MNP$_{C10}$-catalyzed kinetic resolution in batch mode.

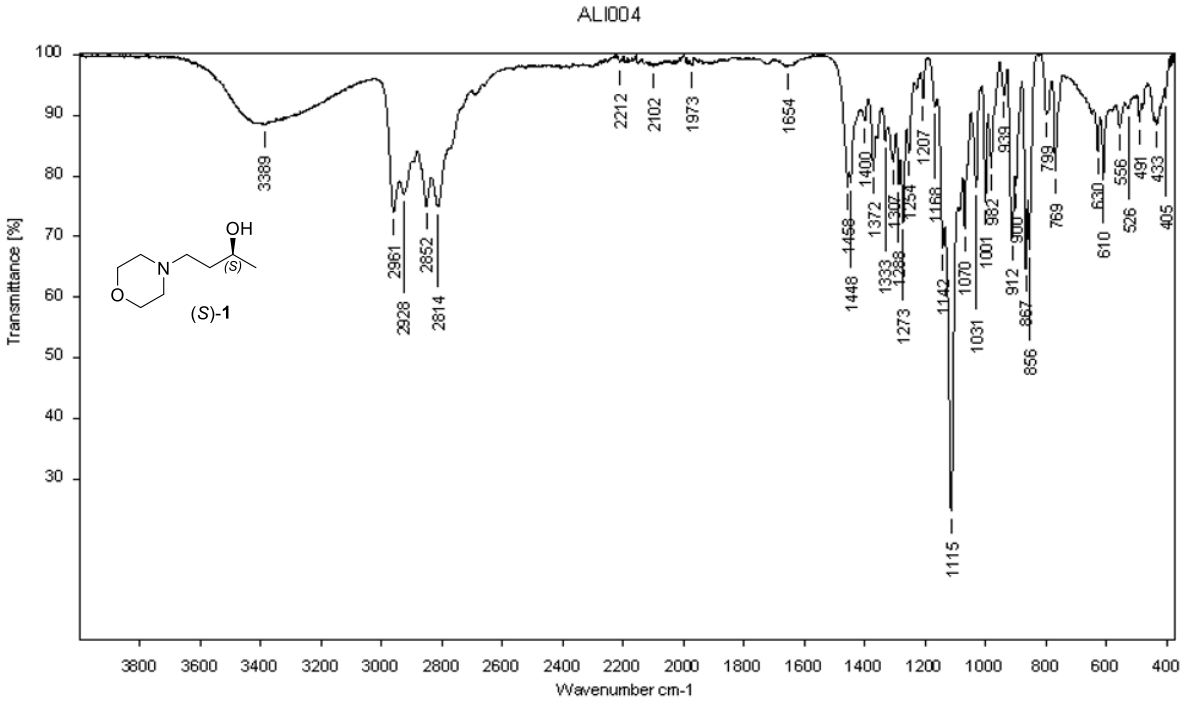

Figure S17. IR spectrum of the (*S*)-4-(morpholin-4-yl)butan-2-ol (*S*)-**1** produced by CaLB-MNP$_{C10}$-catalyzed kinetic resolution in batch mode.