Supporting Information Appendix

Stereoselective reduction of diarylmethanones via ketoreductase@metal-organic framework

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General methods

Unless otherwise specified, all reagents and solvents were purchased from commercial sources and used as received. Chemically competent cells of *E. coli* BL21 (DE3) were purchased from Weidi Biotech (Shanghai, China) and Transgen (China). LB medium contained yeast extract (5 g/L), tryptone (10 g/L), and NaCl (10 g/L). Antibiotics were used at the following concentration, kanamycin: 50 μg/mL. NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. Coupling constant (*J*) values are given in Hz. Optical rotations were measured by a Rudolph AUTOPOL I Automatic Polarimeter. Chiral-HPLC analysis was performed with Daicel Chiralpak AD-H column (25 cm × 4.6 mm × 5 μm), IA column (25 cm × 4.6 mm × 5 μm), Chiracel OD-H column (25 cm × 4.6 mm × 5 μm), Chiracel OJ-H column (25 cm × 4.6 mm × 5 μm), Chiracel OB-H column (25 cm × 4.6 mm × 5 μm).

Expression, and purification of N-terminal-His_6-tagged KmCR2

An approximately 12 h culture of *E. coli* BL21 (DE3) cells freshly transformed with the plasmid pET28a-KmCR2 and grown in LB media supplemented with kanamycin (50 μg/mL) was diluted 1: 100 into 0.5 L of the same medium in a 2 L flask. The culture was shaken at 37 °C until the optical density at 600 nm reached 0.6-0.8, and then the flask was placed in an ice/water bath for 30 minutes before the addition of IPTG to a final concentration of 100 μM. The culture was shaken for an additional 16-18 h at 18 °C. The cells were collected by centrifugation and then were re-suspended in 20 mL of lysis buffer (50 mM NaP_i, 300 mM NaCl, 10 mM imidazole, 10% glycerol (v/v), pH 7.5.). The cells were lysed by sonication on ice, which was operated using an intermittent working mode (55% power, 6 seconds on, 14 seconds off, 15 minutes total working time), and debris was removed by centrifugation at 8500 rpm for 10 minutes at 4 °C. The supernatant was loaded onto a column containing 3 mL of Ni-NTA resin previously equilibrated with lysis buffer. After equilibration of the resin with the lysate in an orbital shaker for ca. 20 min, the flow-through was discarded and the resin was washed with 3 × 15 mL of wash buffer (50 mM NaP_i, 300 mM NaCl, 20 mM imidazole, 10% glycerol (v/v), pH 7.5). Resin-bound protein was eluted with elution buffer (50 mM NaP_i, 300 mM NaCl, 250 mM imidazole, 10% glycerol (v/v), pH 7.5). Fractions of 1 mL were collected and the absorbance at 280 nm was measured by using a NanoDrop One spectrophotometer. 2.5mL of the higher concentration protein solution was selected. Imidazole and excess salt were removed by passing the protein solution through a PD-10 desalting column previously equilibrated with a storage buffer (50 mM NaP_i, 300 mM NaCl, 10% glycerol (v/v), pH 7.5). Protein was eluted with 3.5 mL of the storage buffer, and the protein concentrations were measured by using a NanoDrop One spectrophotometer.

Preparation of pure ZIF-8 and KmCR2@ZIF

Pure ZIF-8： A solution of zinc nitrate hexahydrate (1.17 g, 3.95 mmol) in deionized (DI) water (8 g) was added into a solution of 2-methylimidazole (22.70 g, 276.50 mmol) in DI water (80 g). The resulting mixture was then stirred at room temperature for 5 min (500 rpm), centrifuged to collect the white precipitate, and then washed and centrifuged for three times with DI water. Finally, the sample was collected and dried under vacuum at 45 °C for 12 h to collect the white powder.

KmCR2@ZIF： Add 500 μL of KmCR2 enzyme solution (7 mg/mL) and 400 μL of polyvinylpyrrolidone (PVP) solution (initial concentration of 10 mg/mL) to a 100 mL beaker and leave it to mix well. Then 0.31 mol/L aqueous zinc nitrate hexahydrate solution (2 mL) and 0.1 mol/L aqueous 2-methylimidazole solution (50 mL) were added, and mixed evenly. The resulting mixture was then stirred for 15 min (500 rpm) at room temperature, centrifuged to collect the white precipitate, and then washed and centrifuged for three times with deionized water. Finally, the samples were collected and freeze-dried at -60 ℃ for 24 h, and then collected as white powder.

In order to optimize and arrive at the optimal conditions for enzyme immobilization, various parameters were evaluated: five different temperatures from 15 ℃-55 ℃, four different reaction times from 15 min-60 min, as well as four different PVP concentrations and three different enzyme dosages.

Optimization of conditions for the preparation of immobilized enzymes

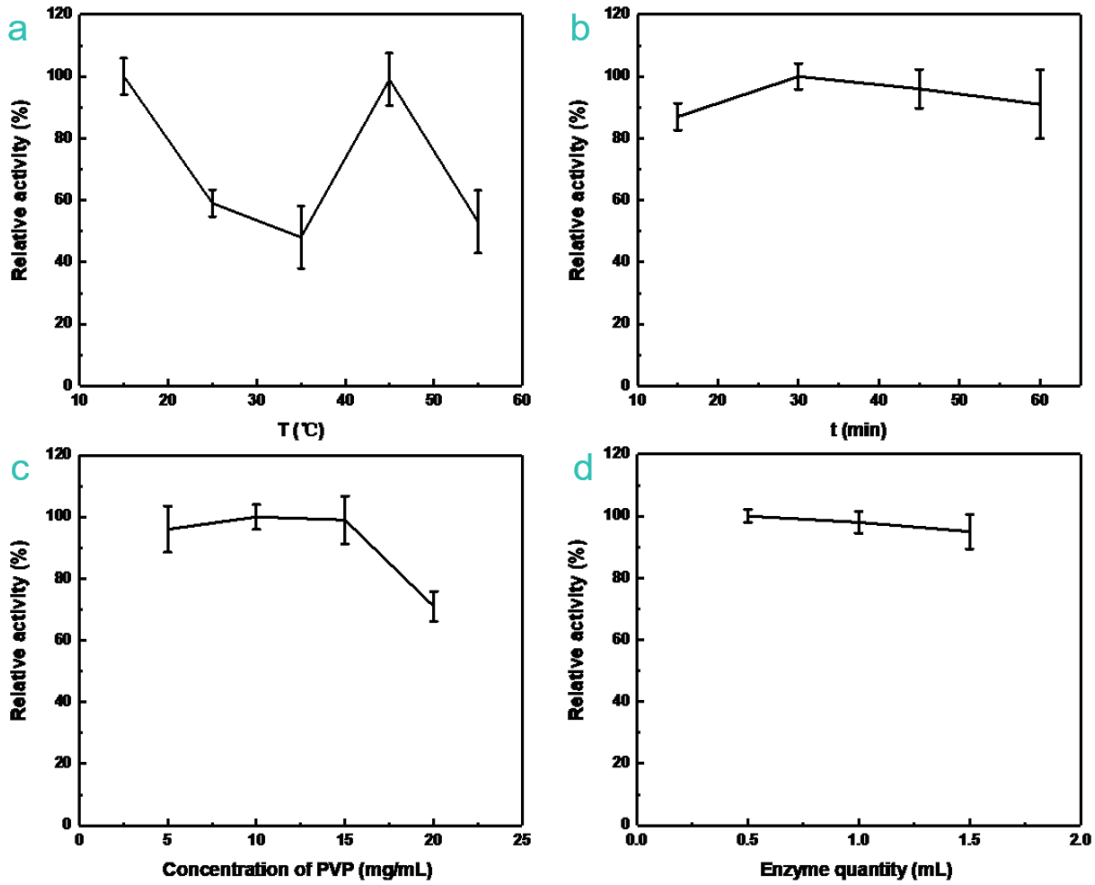


Figure S1. Optimization of the synthesis temperature, synthesis time, PVP concentration and enzyme dosage in the preparation of immobilized enzyme. Each data point represents the mean ± S.D. (range) of triplicate assays.

Characterization of pure ZIF-8 and KmCR2@ZIF materials

The micrographs at various magnifications were analyzed by an SEM (ZEISS Sigma 300, Germany). The PXRD analysis was carried out through a diffractometer (Rigaku-2038, Japan) using Cu-Kα radiation at 2θ from 5° to 90°. Fourier transform infrared spectroscopy (FTIR, Thermo Scientific, USA) was used to characterize the functional groups of the materials by conventional pressing, with wave numbers ranging from 400 cm^{-1} to 4000 cm^{-1}; A thermogravimetric analyzer (Mettler TGA-DSC3+, Switzerland) was used to test the thermal stability of the samples, and the temperature was increased from 30 ℃ to 800 ℃ under nitrogen atmosphere at a rate of 10 ℃/min.

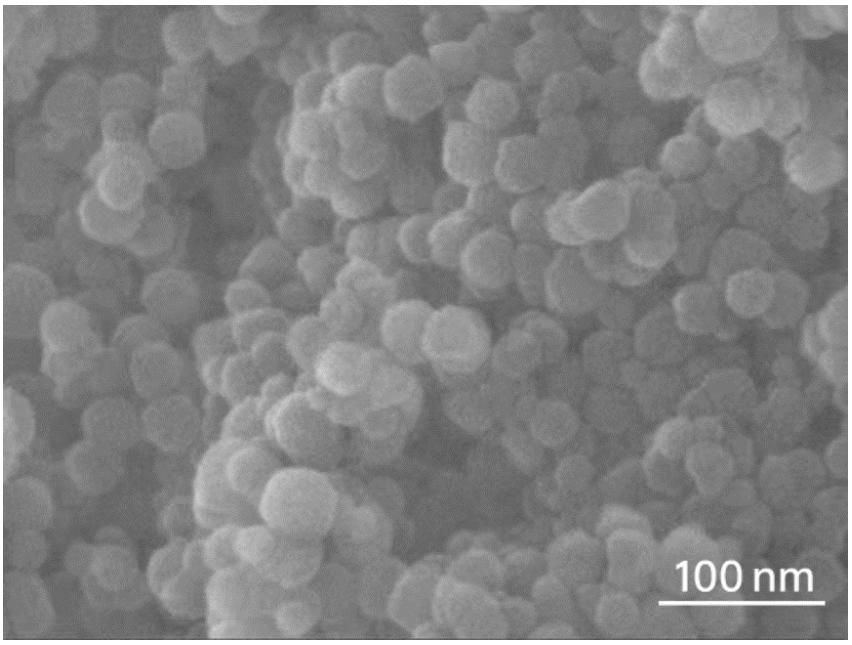
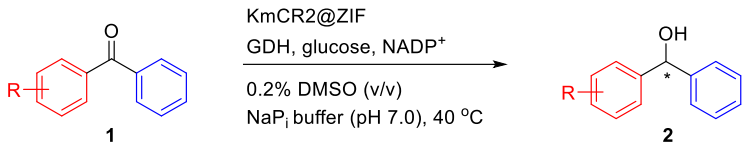


Figure S2. SEM image of pure ZIF-8.

KmCR2@ZIF- and free KmCR2-catalyzed stereoselective reduction of (4-chlorophenyl)(phenyl)methanone (1a) at an analytical scale

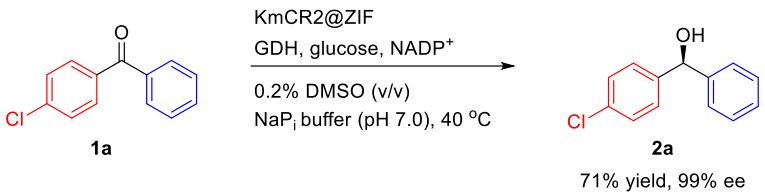
The reaction mixture (1 mL) containing **1a** (2 mM), glucose (4 mM), $NADP^+$ (1 mM), DMSO (0.2%, v/v), KmCR2@ZIF (1 mg), and 19 g/L cell-free extract (CFE) (wet cell weight) of GDH in NaP_i buffer (100 mM, pH 7.0) in the Eppendorf tube was shaken in a temperature-controlled orbital shaker at 200 rpm for 24 h. The mixture was extracted with ethyl acetate and centrifuged for three times, and the combined organic layer was dried with anhydrous Na_2SO_4, and concentrated *in vacuo* to give the crude product, which was subjected to the ^1H NMR and chiral HPLC analysis to determine the conversion as well as the ee value. The assays were run for three times. The determined average enzyme activity was normalized, and the highest conversion was defined as the enzyme activity of 100%

The activity of free enzyme was determined in a similar manner, except by replacing the immobilized enzyme with the free enzyme solution (100 μL).



Scheme S1. KmCR2@ZIF-catalyzed stereoselective reduction of diarylmethanones (**1**) to chiral diarylmethanols (**2**) at an analytical scale.

The reaction mixture (1 mL) containing **1** (2 mM), glucose (4 mM), $NADP^+$ (1 mM), DMSO (0.2%, v/v), KmCR2@ZIF (1 mg), and 19 g/L cell-free extract (CFE) (wet cell weight) of GDH in NaP_i buffer (100 mM, pH 7.0) in the Eppendorf tube was shaken in a temperature-controlled orbital shaker at 40 °C and 200 rpm for 24 h. The mixture was extracted with ethyl acetate and centrifuged for three times, and the combined organic layer was dried with anhydrous Na_2SO_4, and concentrated *in vacuo* to give the crude product, which was subjected to the ^1H NMR and chiral HPLC analysis to determine the conversion as well as the ee value.



Scheme S2. KmCR2@ZIF-catalyzed stereoselective reduction of diarylmethanone **1a** to chiral diarylmethanol **2a** at a semi-preparative scale (0.2 mmol).

The reaction mixture (100 mL) containing **1a** (2 mM), glucose (4 mM), $NADP^+$ (1 mM), DMSO (0.2%, v/v), KmCR2@ZIF (100 mg), and 19 g/L cell-free extract (CFE) (wet cell weight) of GDH in NaP_i buffer (100 mM, pH 7.0) in the conical flask was shaken in a temperature-controlled orbital shaker at 40 °C and 200 rpm for 24 h. The mixture was extracted with ethyl acetate, and centrifuged for three times, and the combined organic layer was washed with brine, dried with anhydrous Na_2SO_4, and concentrated *in vacuo* to give the crude product, which was purified via preparative-TLC to give **2a** as white solids with a yield of 65% (28.2 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.26 (m, 9H), 5.83 (s, 1H), 2.37 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.4, 142.2, 133.3, 128.7, 128.6, 127.9, 126.6, 126.5, 75.6. $[\alpha]_D^{20} = +17.23$ (c = 0.29, $CHCl_3$). (lit.[1] $[\alpha]_D^{20} = +21.0$ (c = 0.40, $CHCl_3$)). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 97/3 hexane/ethanol, flow rate of 1.0 mL/min, 225 nm UV lamp, 25 °C, t_1 = 20.526 min, t_2 = 22.835 min (major); >99% ee.

Recyclability and reusability evaluation for KmCR2@ZIF-catalyzed stereoselective reduction of diarylmethanone 1a to chiral diarylmethanol 2a at an analytical scale

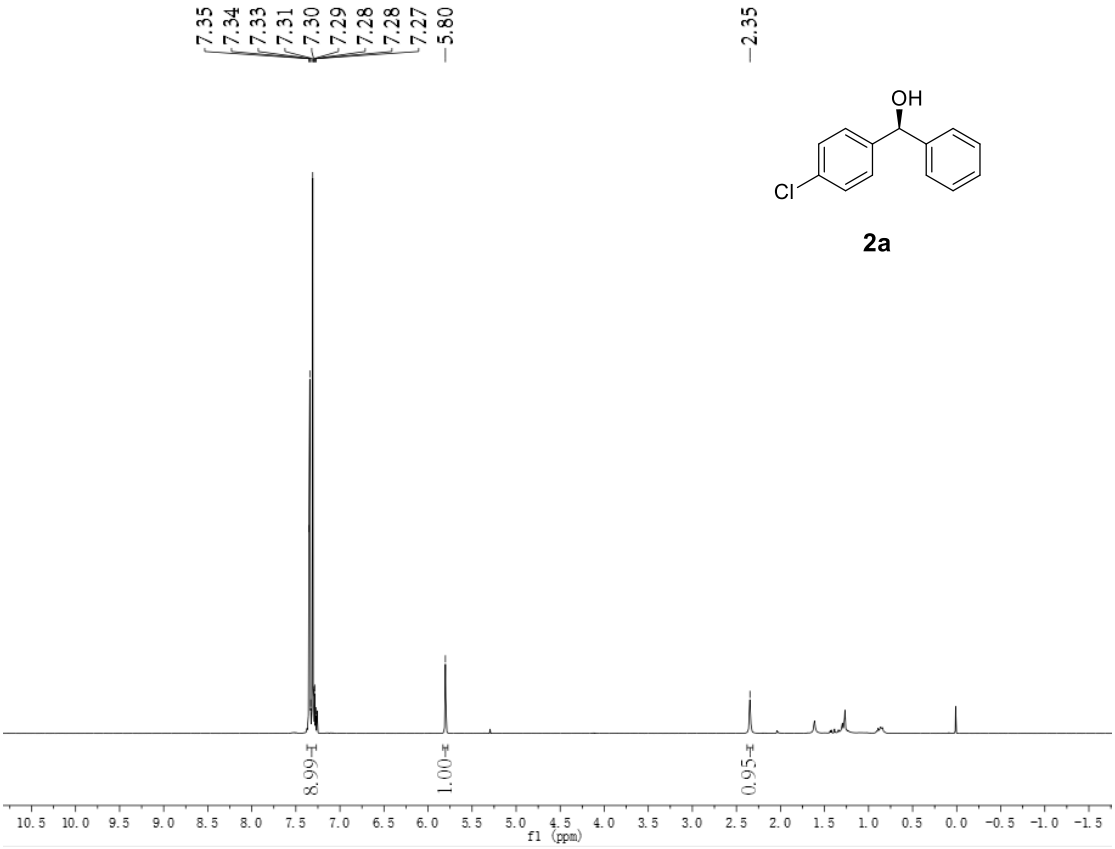
The reaction mixture (1 mL) containing **1a** (2 mM), glucose (4 mM), $NADP^+$ (1 mM), DMSO (0.2%, v/v), KmCR2@ZIF (1 mg), and 19 g/L cell-free extract (CFE) (wet cell weight) of GDH in NaP_i buffer (100 mM, pH 7.0) in the Eppendorf tube was shaken in a temperature-controlled orbital shaker at 40 ℃ and 200 rpm for 24 h. The mixture was extracted with ethyl acetate and centrifuged for three times, and the combined organic layer was dried with anhydrous Na_2SO_4, and concentrated *in vacuo* to give the crude product, which was subjected to the ^1H NMR analysis to determine the conversion. The assays were run for three times. The determined average enzyme activity was normalized, and the conversion rate obtained from the first cycle of the enzyme reaction was defined as the enzyme activity of 100%. The immobilized enzymes were recovered after each cycle and reused in the next reaction under the identical conditions.

References

[1] Li, Z.; Wang, Z.; Wang, Y.; Wu, X.; Lu, H.; Huang, Z.; Chen, F. Substituent Position-Controlled Stereoselectivity in Enzymatic Reduction of Diaryl- and Aryl(heteroaryl)methanones. *Adv. Synth. Catal*. **2019**, *361*, 1859-1865.

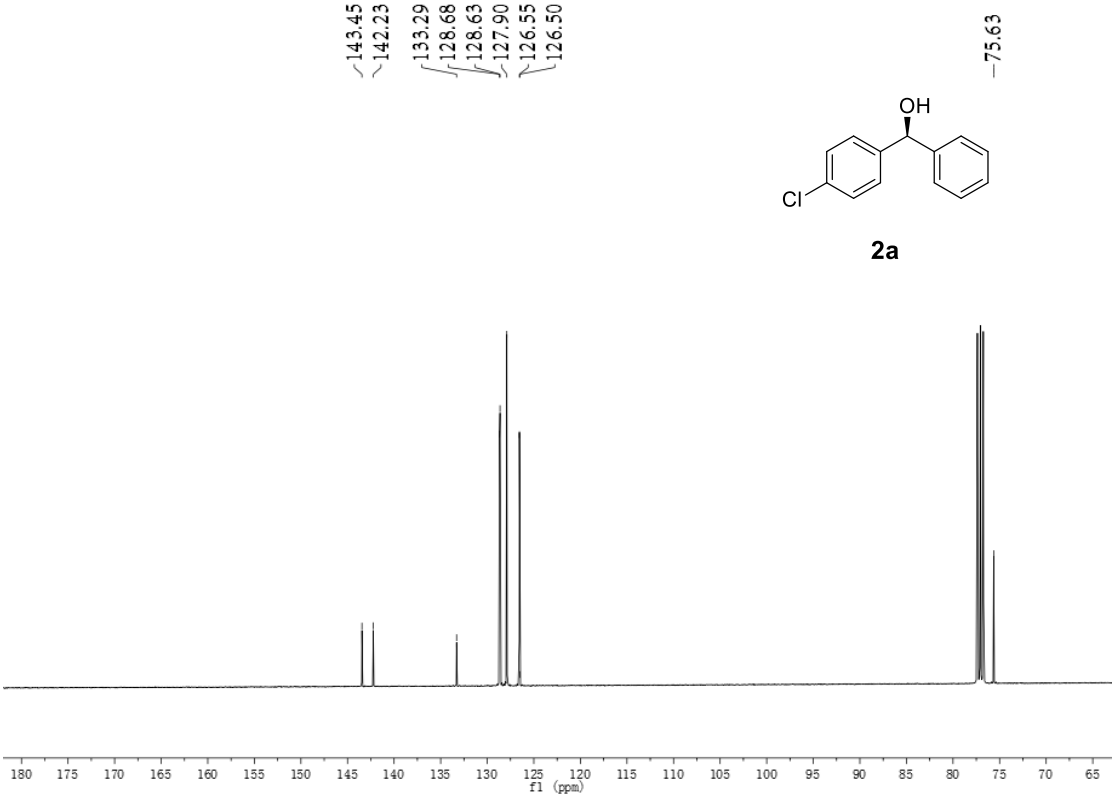
^{1}H-NMR and ^{13}C-NMR Spectra of 2a

The ^{1}H NMR（400 MHz，$CDCl_3$）spectrum of **2a**



2a

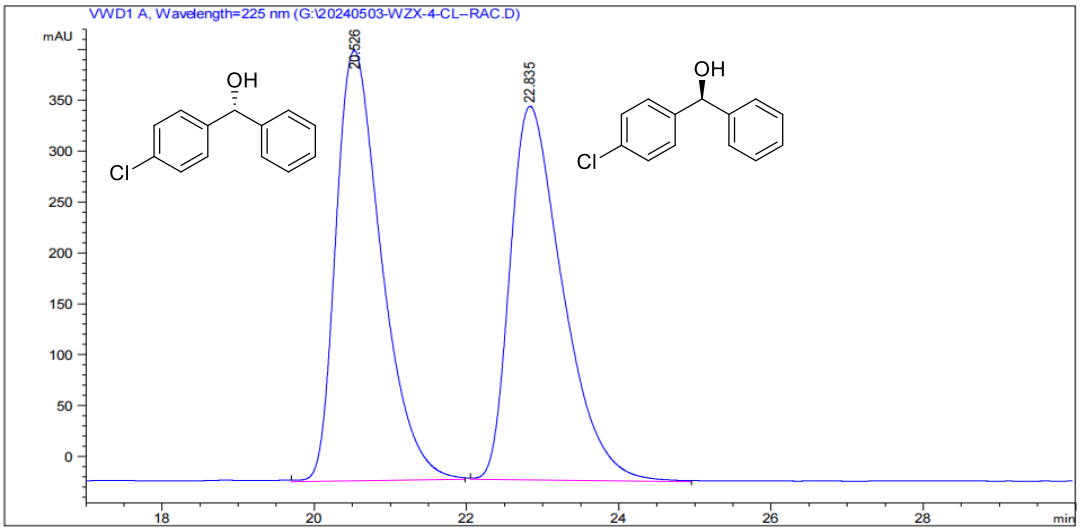
The ^{13}C NMR（400 MHz，$CDCl_3$）spectrum of **2a**



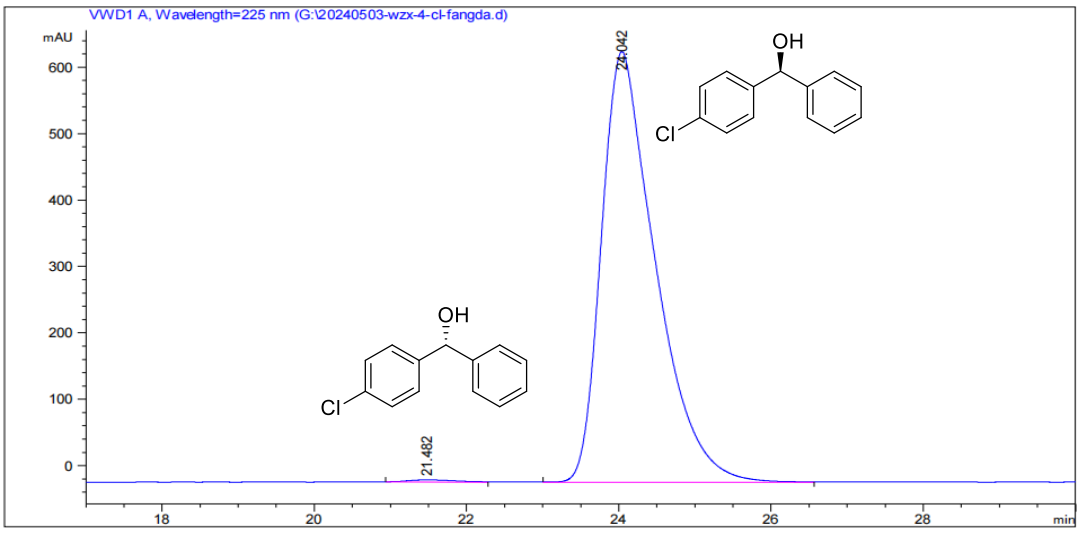
2a

Chiral HPLC Spectra

(S)-(4-chlorophenyl)(phenyl)methanol (**2a**)
Chiracel® AD-H, 250 × 4.6 mm column, hexane/2-propanol 97:3, 1 mL/min flow rate, 225 nm UV lamp



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.526	MM	0.6937	1.76103e4	423.07333	49.9027
2	22.835	MM	0.8018	1.76790e4	367.48370	50.0973

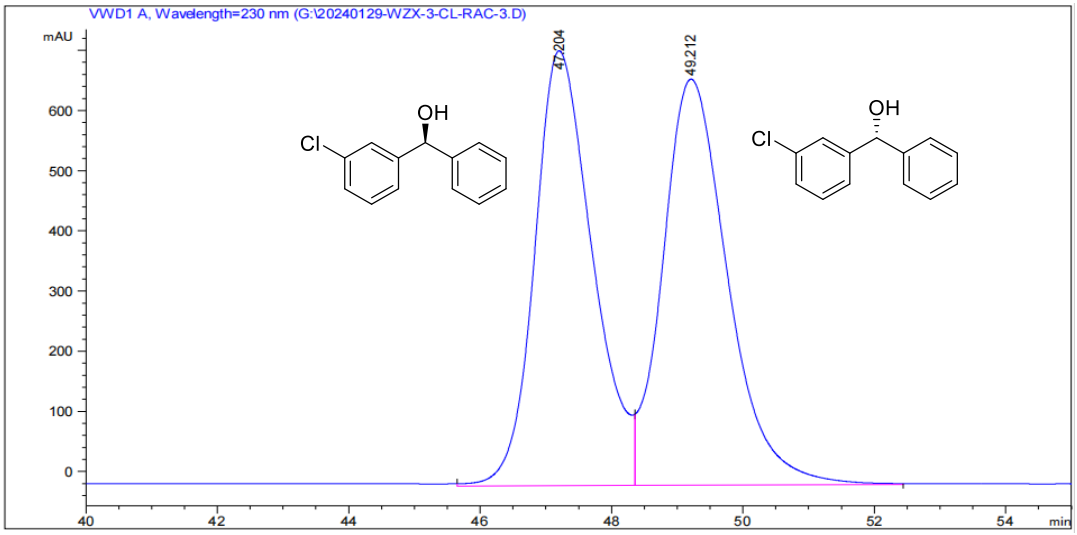


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	21.482	MM	0.7337	142.95787	3.24756	0.4450
2	24.042	MM	0.8218	3.19811e4	648.58905	99.5550

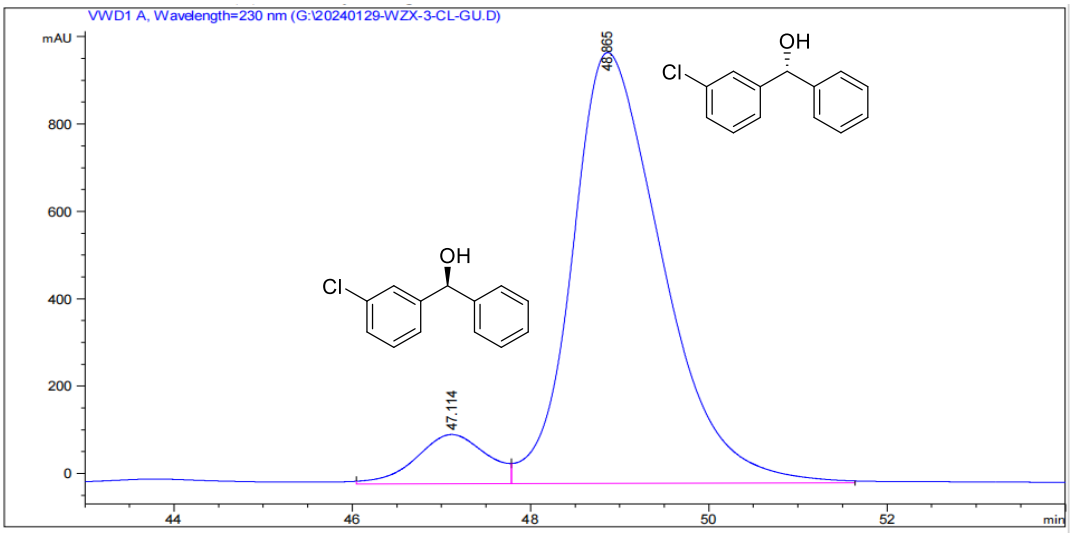
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation.

(R)-(3-chlorophenyl)(phenyl)methanol (**2b**)

Chiracel® IA 250 × 4.6 mm column，hexane/2-propanol 98:2,，0.5 mL/min flow rate, 230nm UV lamp



Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	47.204	1	MF	4.43431e4	722.66577	48.8078
2	49.212	1	FM	4.65094e4	674.94061	51.1922

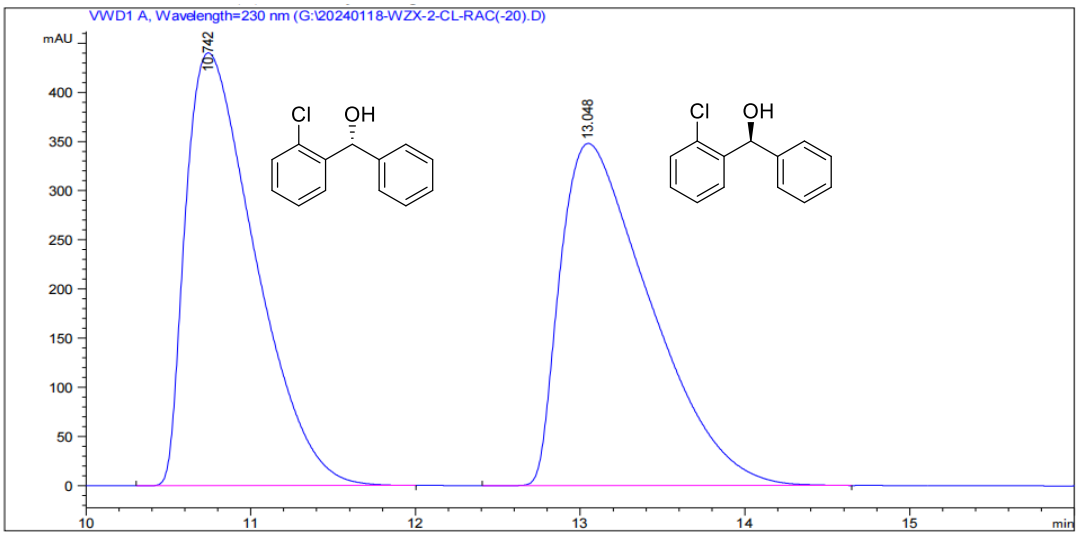


Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	47.114	1	MF	6250.34375	112.75475	8.2892
2	48.865	1	FM	6.91529e4	986.53546	91.7108

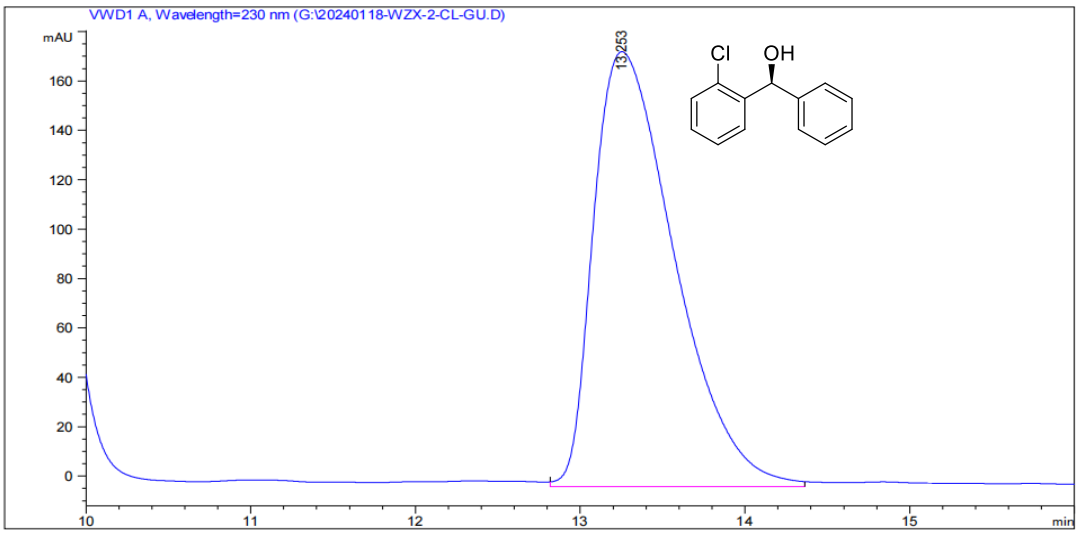
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(S)-(2-chlorophenyl)(phenyl)methanol (**2c**)

Chiracel® OJ-H 250 × 4.6 mm, hexane/2-propanol 80:20, 1 mL/min flow rate, 230nm UV lamp



Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	10.742	1	BB	1.29032e4	439.92017	49.3301
2	13.048	1	BB	1.32536e4	347.79895	50.6699

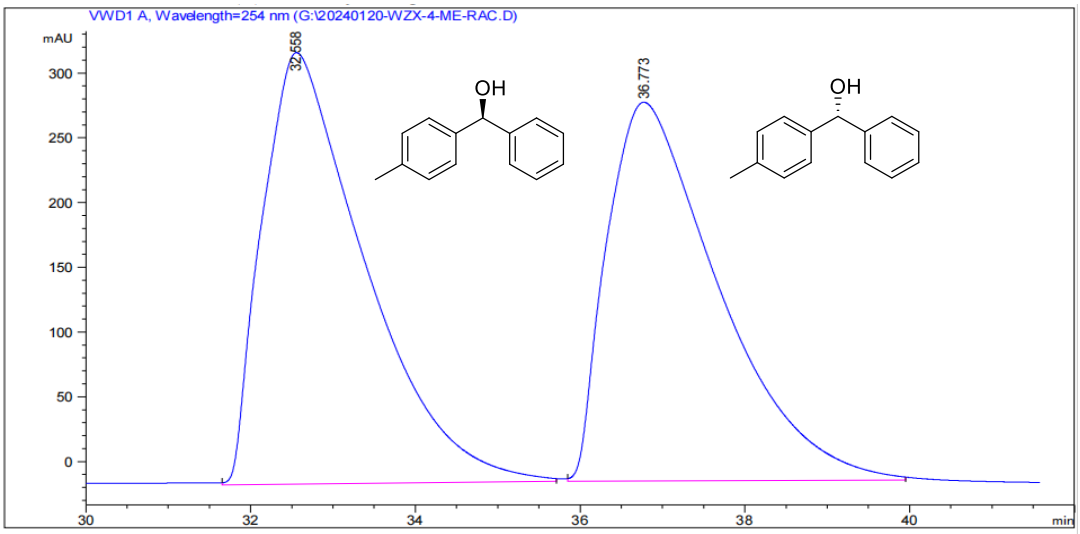


Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	13.253	1	MM	6029.81592	176.10973	100.0000

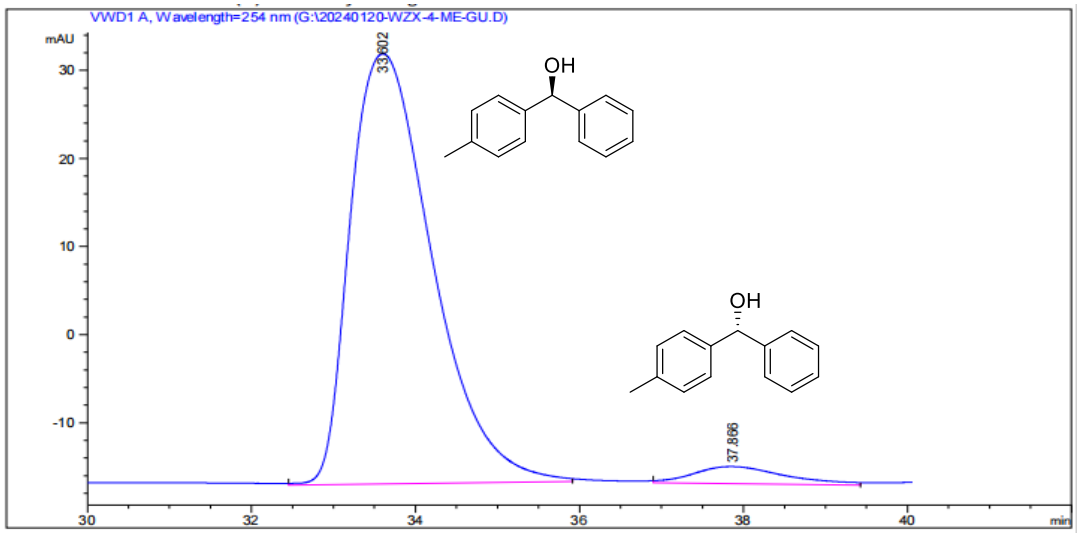
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(S)-phenyl(*p*-tolyl)methanol (**2d**)

Chiracel® OD-H 250 × 4.6 mm column，hexane/2-propanol 95:5，0.5 mL/min flow rate, 254 nm UV lamp



Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	32.558	1	MM	2.88615e4	333.26227	51.1982
2	36.773	1	MM	2.75106e4	292.46170	48.8018

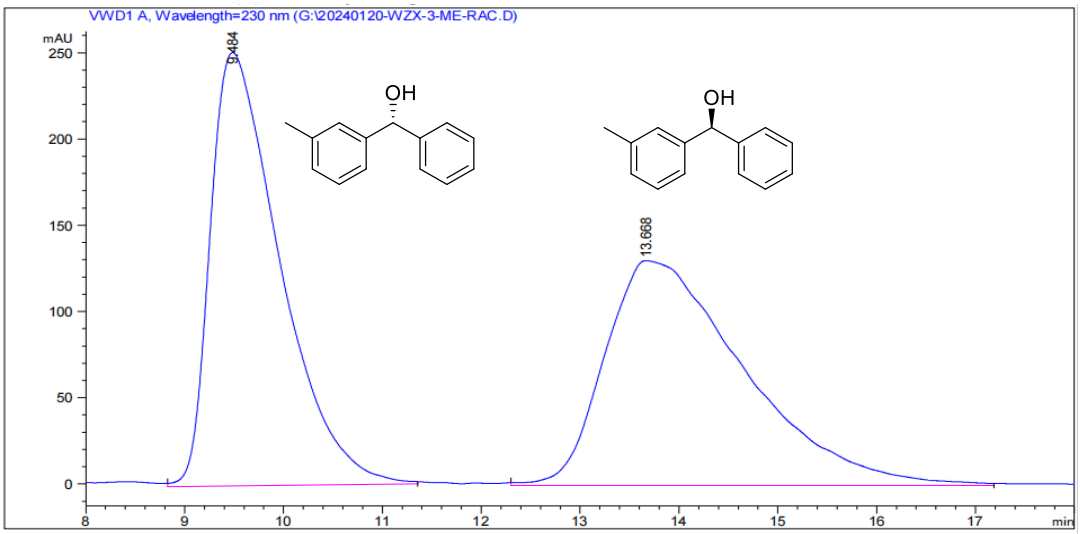


Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	33.602	1	MM	3388.04565	48.82663	95.7422
2	37.866	1	MM	150.66992	1.93208	4.2578

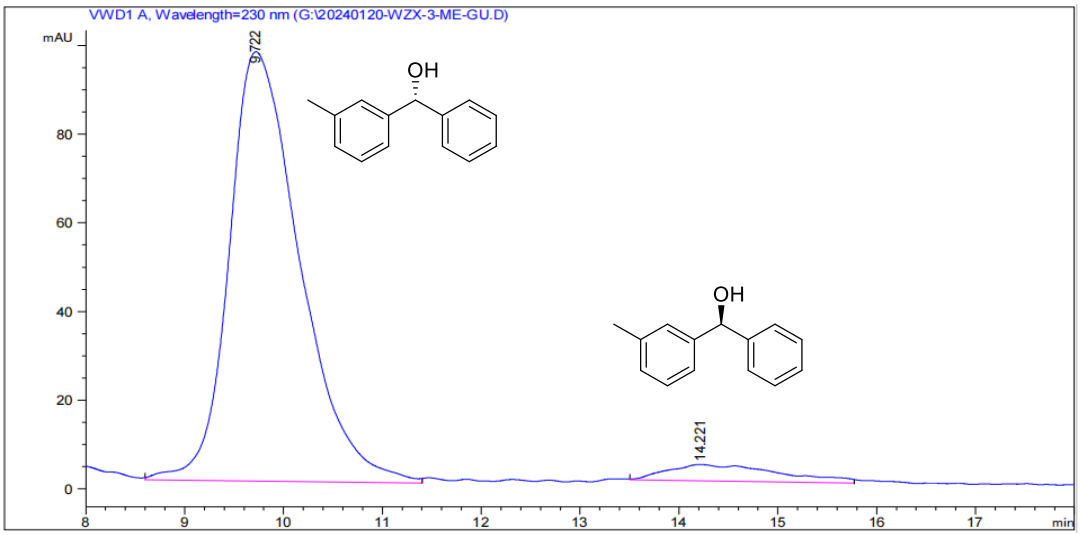
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(R)-phenyl(*m*-tolyl)methanol (**2e**)

Chiracel® OB-H 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 230 nm UV lamp



Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	9.484	1	MM	1.23184e4	251.18817	49.0993
2	13.668	1	MM	1.27704e4	130.19264	50.9007

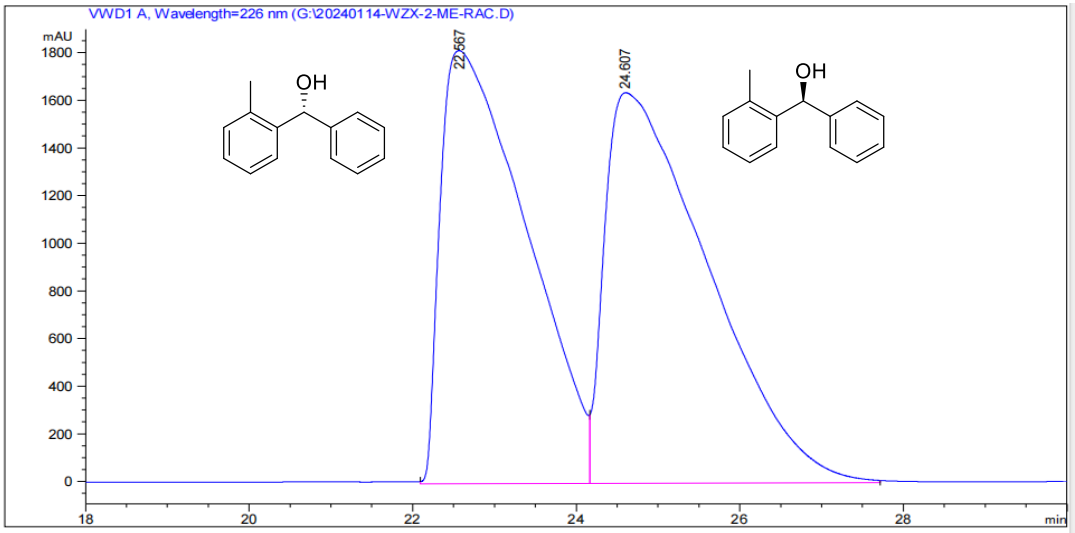


Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	9.722	1	MM	4828.99854	96.84884	94.2859
2	14.221	1	MM	292.65869	3.69232	5.7141

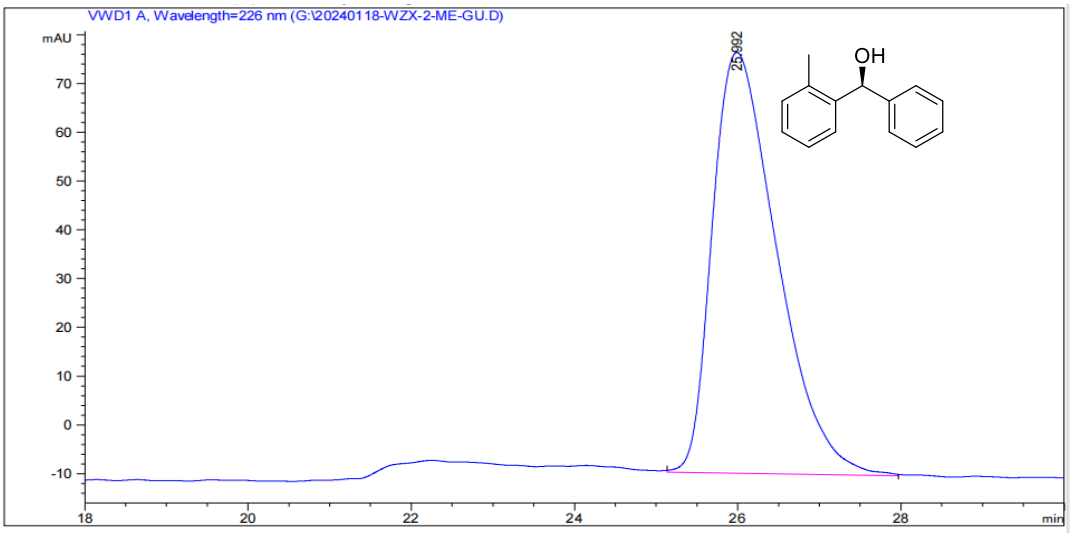
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(S)-phenyl(*o*-tolyl)methanol (**2f**)

Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 95:5, 1 mL/min flow rate, 226nm UV lamp



Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	22.567	1	MF	1.32858e5	1817.76514	47.8938
2	24.607	1	FM	1.44544e5	1639.44519	52.1062

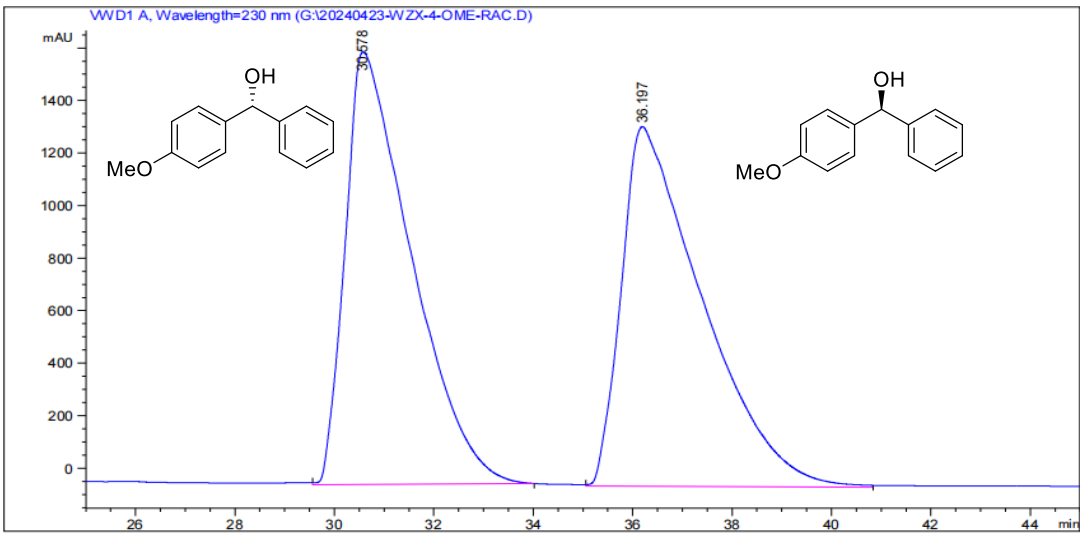


Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	25.992	1	MM	4743.20166	86.25251	100.0000

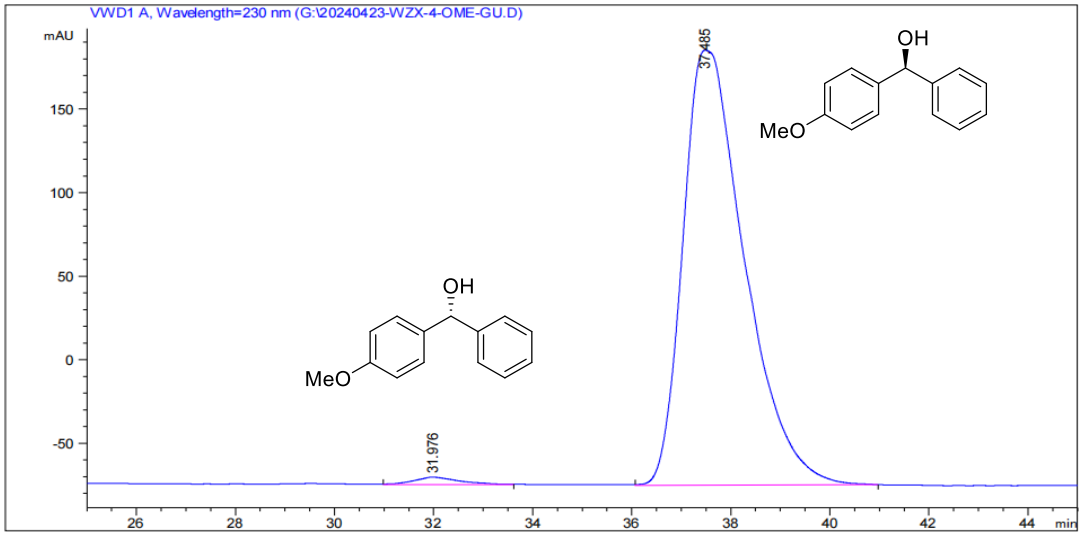
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(S)-(4-methoxyphenyl)(phenyl)methanol (**2g**)

Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1 mL/min flow rate, 230nm UV lamp



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	30.578	MM	1.5388	1.52037e5	1646.71179	49.5710
2	36.197	MM	1.8836	1.54668e5	1368.57507	50.4290

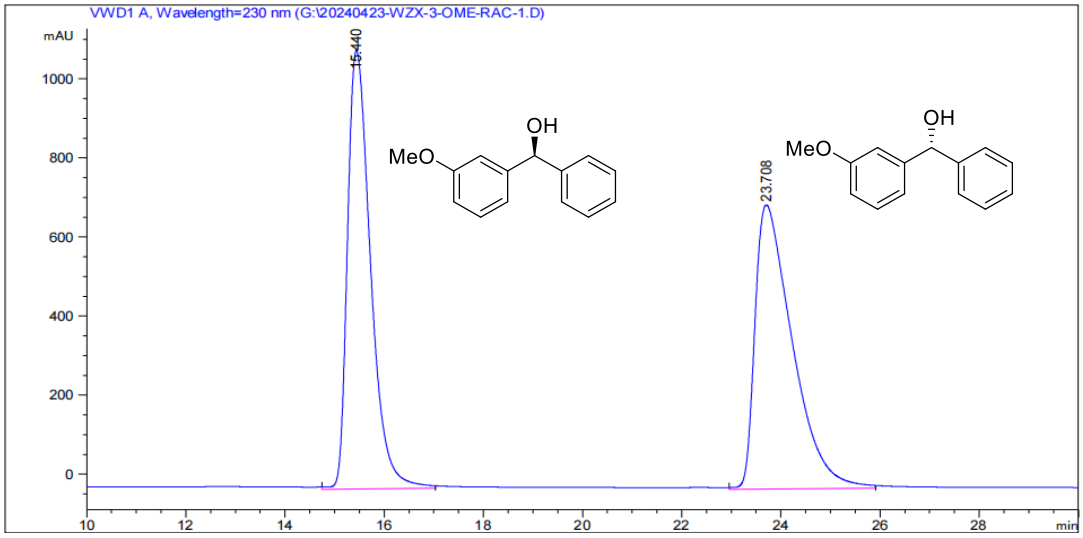


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	31.976	MM	1.1122	304.36743	4.56111	1.3343
2	37.485	MM	1.4407	2.25074e4	260.37491	98.6657

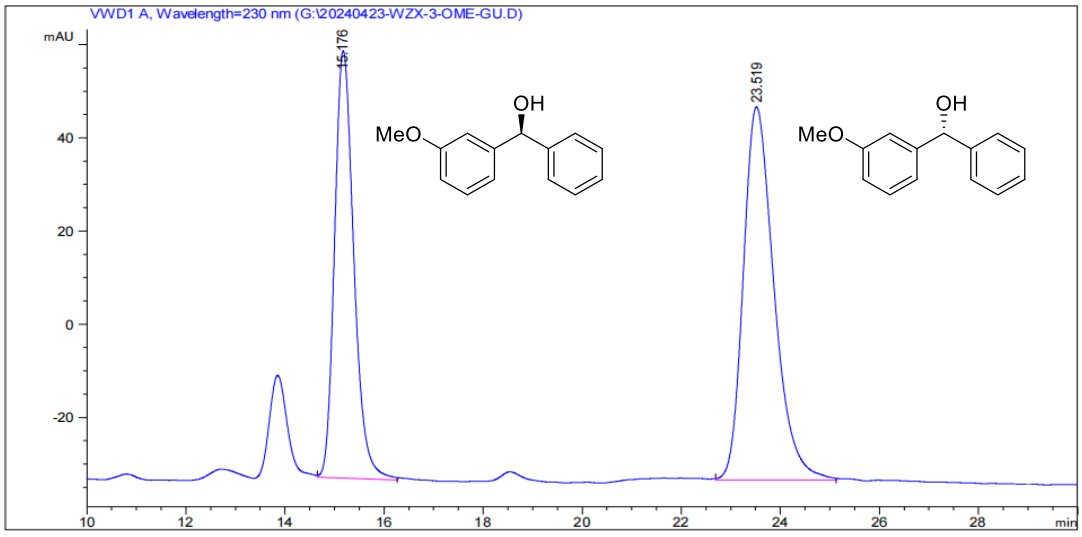
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(R)-(3-methoxyphenyl)(phenyl)methanol (**2h**)

Chiracel® OD-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1 mL/min flow rate, 230nm UV lamp



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.440	MM	0.5384	3.58457e4	1109.73096	48.7628
2	23.708	MM	0.8731	3.76646e4	718.98370	51.2372

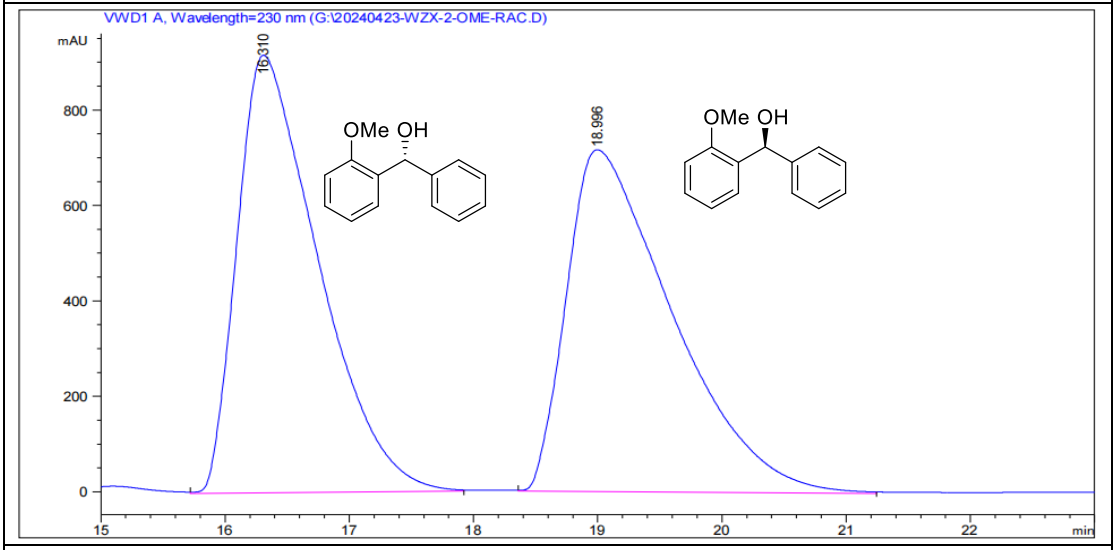


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.176	MM	0.4499	2475.27441	91.68860	41.8168
2	23.519	MM	0.7169	3444.05811	80.07034	58.1832

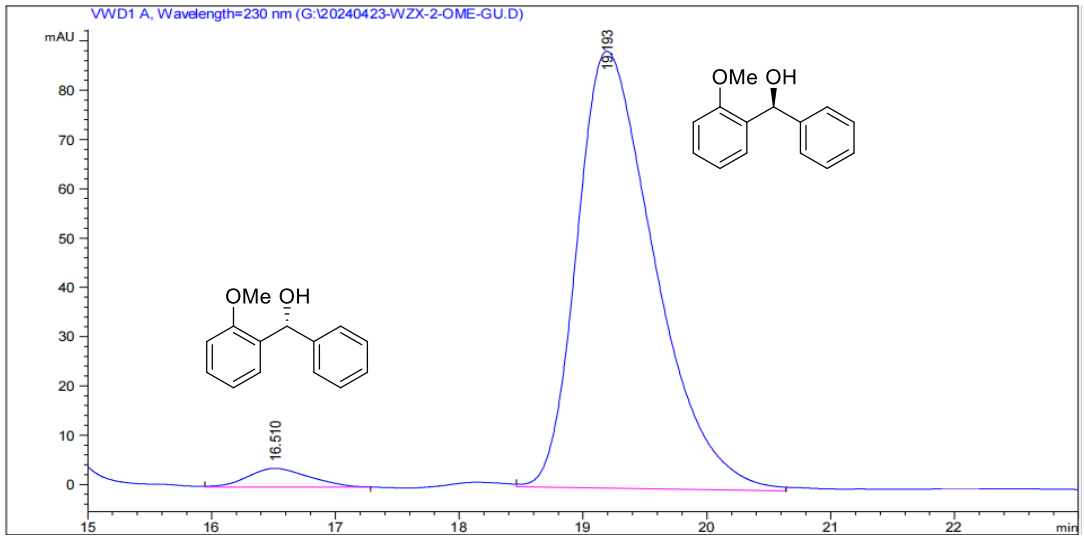
The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation

(S)-(2-methoxyphenyl)(phenyl)methanol (**2i**)

Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1 mL/min flow rate, 230nm UV lamp



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.310	MM	0.7567	4.16267e4	916.81348	49.5885
2	18.996	MM	0.9847	4.23176e4	716.21973	50.4115



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.510	MM	0.5711	129.77672	3.78712	3.2565
2	19.193	MM	0.7253	3855.35669	88.59053	96.7435

The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2@ZIF-catalyzed biotransformation