Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary information

Enantioselective formation of 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate by chiral catalysis using enzymes immobilized magnetic core-shell nanocomposites

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Experimental

Synthesis of monodisperse ultra small magnetite nanoparticles: Synthesis of magnetite (Fe_3O_4) nanoparticles (MNP), with narrow particle size distribution was performed according to procedures previously reported by our group[1]. 8.46 g $FeCl_2$, $4H_2O$ and 22.95 g of $FeCl_3$, $6H_2O$ were dissolved in 500 ml deionised water previously degassed with nitrogen. The mixture kept at 80°C under nitrogen atmosphere and 50mL of aqueous ammonium hydroxide (25% w/v NH_4OH) was added drop wise to the mixture over 30 min under vigorous stirring. The reaction was allowed to proceed for a further 1 hour. The black reaction products were collected and washed several times in deionised water using magnetic separation. The obtained MNPs were then coated with mesoporous silica shell as described in the following section.

Synthesis of core-shell magnetic nanoparticles: 480 mg of magnetite nanoparticles was dispersed in 200 ml of an ethanol/water (4/1, V/V) solution. 14 ml of 28% NH_4OH solution was added to the mixture and stirred for 5 minutes. Afterward 10 g of cetyl trimethyl ammonium bromide (CTAB) was added to the solution and the suspension was homogenized by ultrasonic bath for 15 min. 6 mL of tetraethoxy silane (TEOS) was then added drop-wise under vigorous stirring for 24hrs. The resultant black product was isolated from the reaction mixture and washed several times with acidic ethanol solution in ultrasonic bath to remove the surfactant from the mesopores, followed by subsequent washing with deionised.

Surface functionalization: Surface activation of nanoparticles was performed using the novel tri-phasic reverse emulsion method (TPRE) reported recently by our group[2], 150 mg of mesoporous silica coated nanoparticles were dispersed in 30ml of toluene in the presence of 5g of Triton X100 and the mixture was shaken to form a tri-phasic reverse emulsion. APTS was added to the emulsion to a final concentration of 2% w/v and the mixture kept on a rotator at 40 rpm for 24 hours at 50°C. After 24 hours of reaction, the nanoparticles were collected by magnetic separation and washed 3 times with coupling solution [0.8 % (v/v) glacial acetic acid in methanol]. The surface amine density was measured by an established colorimetric assay[3,4] using 4-nitrobenzaldehyde.

Conversion of surface amine groups to aldehyde: This reaction was performed by using glutaraldehyde. SSC buffer (20×) was prepared by dissolving sodium chloride (175.3 g) and sodium citrate (88.2 g) in 1 litre distilled, deionised water (final pH 7.4). SSC buffer (1×) was prepared by diluting 50 mL 20×SSC buffer to 1 litre with distilled, deionised water. 50 mg of nanoparticles were washed 3 times with 10 mL 1×SSC buffer and the supernatant removed. 4 mL of a 5% w/v glutaraldehyde solution (in 20×SSC buffer) was added and the suspension was incubated for 3 hours at 18°C with end-over-end rotation (40 rpm). After 3 hours, the nanoparticles were collected magnetically, washed 3 times with 5 mL 1×SSC buffer followed by 3 times with 5 mL of PBS buffer.

Immobilisation of lipases on nanoparticles: 50 mg of the nanoparticles (non-functionalised for physical absorption or functionalised for covalent binding with enzyme) were added to 4 ml of lipase solution (1mg/ml lipase in phosphate buffer). The mixture was incubated at 18°C with constant rotation at 40 rpm for 20 h. After incubation, the enzyme adsorbed nanoparticles were separated and the amount of enzyme immobilised was calculated by determining the amount of lipase left in the supernatant by measuring absorption at λ_{595}nm using UV/Vis spectrophotometry by Bradford assay[5].

Table S1: Concentration of enzymes loaded onto the nanoparticles by different routes

Modified nanoparticles	µg of enzyme loaded / mg of nanoparticles	Amount (mg) of modified nanoparticles used (equivalent to 500µg enzyme)
Chemically immobilised CRL	80.0	6.3
Physically immobilised CRL	72.0	6.9
Chemically immobilised PFL	44.0	11.4
Physically immobilised PFL	76.1	6.6

Desymmetrization of meso-cyclopent-2-en-1,4-diacetate by enzymatic hydrolysis: 50µmol of cyclopent-2-en-1,4-diacetate was added to 1mL of solvent mixture (hexane: water ratio is 80% to 20%) followed by the addition of 500 µg of free lipases or equivalent amount of immobilised lipase on nanoparticles support (see table S1). The reaction mixture was incubated at 25°C with end-over-end rotation (40 rpm). During the reaction, a 5 µL aliquot of the reaction mixture (water layer) was withdrawn at different time intervals (1 h, 4 h, 24 h and 48 h) and analyzed by GC for the determination of products such as meso-cyclopent-2-en-1,4-diol*,* 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate and it's enantiomer 4-(*S*)-hydroxycyclopent-2-en-1-(*R*)-acetate as they are soluble in water. The hexane layer was analysed for the determination of concentration of un-reacted meso-cyclopent-2-en-1,4-diacetate.

Characterization:

Small Angle X-ray Scattering (SAXS) measurements of the mesoporous core-shell nanoparticles were performed by using HECUS S3 Micro, GMBH GRAZ with Geni Xenocs software instrument in order to investigate the size and shape of the particles. Scattering curves were monitored in a q-range from 0.01 to 0.5 $Å^{-1}$. A broad peak corresponding to the mesopores size of 3.9 nm (see Figure S2)

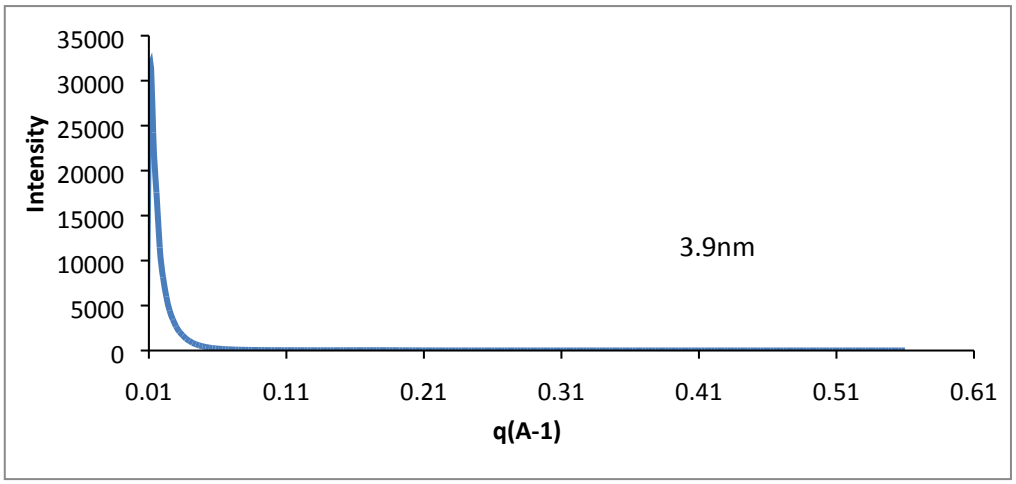


Figure S1: SAXS pattern of mesoporous silica core shell particles.

The powder X-ray diffraction pattern of the iron oxide core nanoparticles was performed using Inel Equinox 2000 powder diffractometer equipment. The samples preparation was done by drying and grinding of nanocomposites into a fine powder. The powder was packed into X-ray sample holder ensuring smooth surface with no visible pits or cracks.

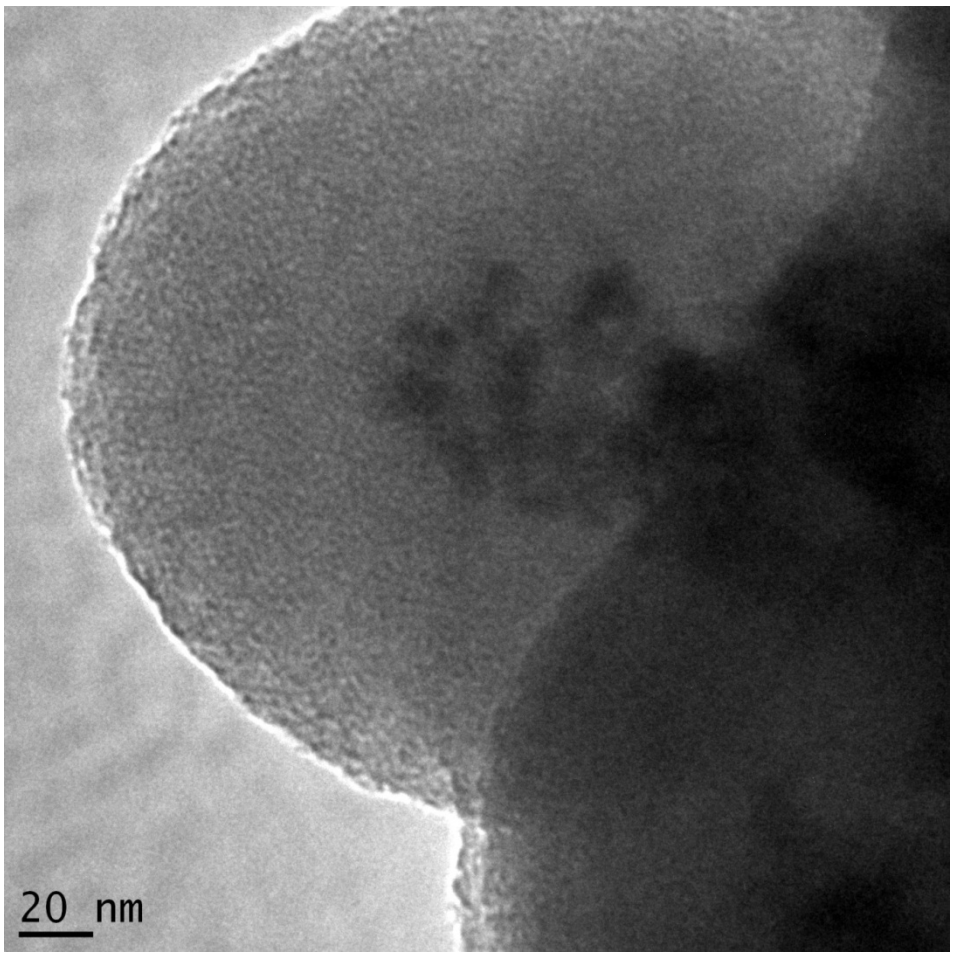


Figure S2: TEM image of core-shell nanocomposites

The materials exhibited various peaks (Figure S1) corresponding to Miller indices of 220, 311, 400, 422, 511, 440. They have been identified as fingerprint peaks of pure magnetite (Fe_3O_4) in the 2θ range of 30 to 75. The low intensity of the peaks is an indication of ultra-small size of magnetite particles. The mean particle diameters were also calculated from the XRD pattern using the line width of most intense peak corresponding to 311 Miller plane by applying the Scherer equation:

$D = k\lambda / (b \times \cos\theta)$ where b is the peak width of the XRD peak at angle θ, λ is the wavelength (1.5418 Å) of the X-ray used, and K is the Scherer constant (a shape factor), about 0.9 for magnetite and maghemite[6]. The mean particles sizes were calculated to be 10.4 nm which is similar to the value obtained from the TEM analysis reported in the manuscript.

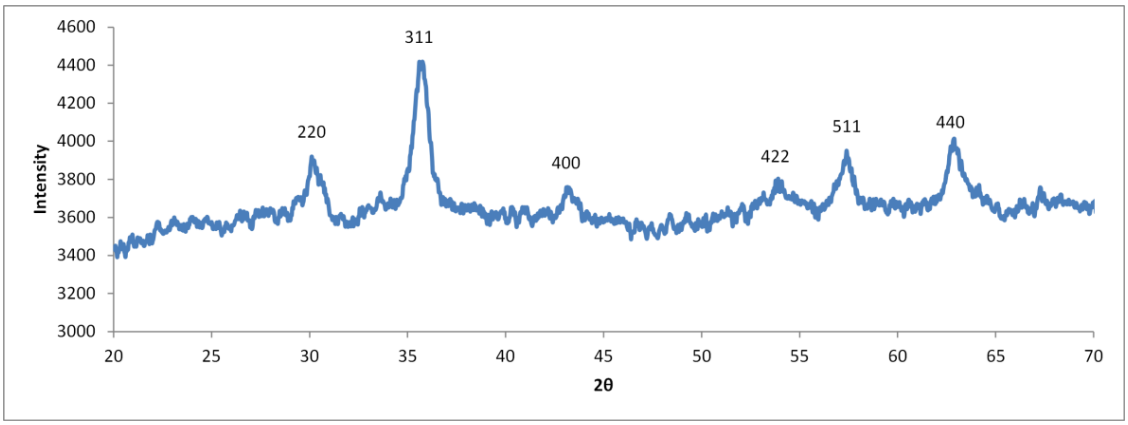


Figure S3: XRD pattern of core magnetite

Transmission electron microscopy (TEM), analysis was conducted on a JEOL JEM-2000 EX electron microscope Operated at 200 kV. Images were processed using Gatan Digital Micrograph Software.

N_2 gas adsorption and desorption isotherms were collected using a Micromeritics ASAP 2010 (Accelerated Surface Area and Porosimetry System) instrument. Brunauer-Emmett-Teller method (BET) was used to calculate the specific surface area of the materials and BJH method was used for average pore size determination.

Sample analysis

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) for the detection of chiral products

Starting material meso-cyclopent-2-en-1,4-diacetate and products such as meso-cyclopent-2-en-1,4-diol, 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate and it's enantiomer 4-(*S*)-hydroxycyclopent-2-en-1-(*R*)-acetate were quantitatively determined using a Varian Chrompack CP-3380 Gas Chromatograph and nitrogen as the carrier gas (California, USA). Chromatograms were interpreted using Varian Star Integrator software version 4.51. The method of analysis involved injecting 1 µL of aliquot (the water layer) of the reaction mixture and analysed with a temperature program starting at 50ºC and finishes at 200ºC with a ramp rate of 10ºC per minute.

Selected reaction products were also analysed (where specified) by GC-MS on a Thermoscientific Trace GC ultra (Milan, Italy), with DSQ II Mass Spectrometer (Texas, USA) and a Triplus AS auto-sampler, using helium as the carrier gas. Chromatograms were interpreted using Xcalibur software version 2.0.7.

Both techniques employed a Supelco BETA DEX™ 110 fused silica capillary column (length = 30 m, internal diameter = 0.25 mm, film thickness 0.25 µm), which is specifically produced to separate chiral compounds for analysis.

Calibration curves were constructed using a series of dilutions of meso-cyclopent-2-en-1,4-diacetate, meso-cyclopent-2-en-1,4-diol, 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate and its enantiomer 4-(*S*)-hydroxycyclopent-2-en-1-(*R*)-acetate.

Calibration curves were constructed in water for 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate and meso-cyclopent-2-en-1,4-diol, as they both are insoluble in hexane. A calibration curve for meso-cyclopent-2-en-1,4-diacetate was constructed in hexane to calculate the exact amount of starting material at time = 0 minutes and to monitor the conversion. In the case of the compounds calibrated in water, the data (absolute peak areas) were plotted against various known concentrations.

The calibration curves constructed for 4-(R)-hydroxycyclopent-2-en-1-(S)-acetate and meso-cyclopent-2-en-1,4-diol are presented in Figure S3. The regression values (R=0.99) of both the linear plots are within the high confidence range.

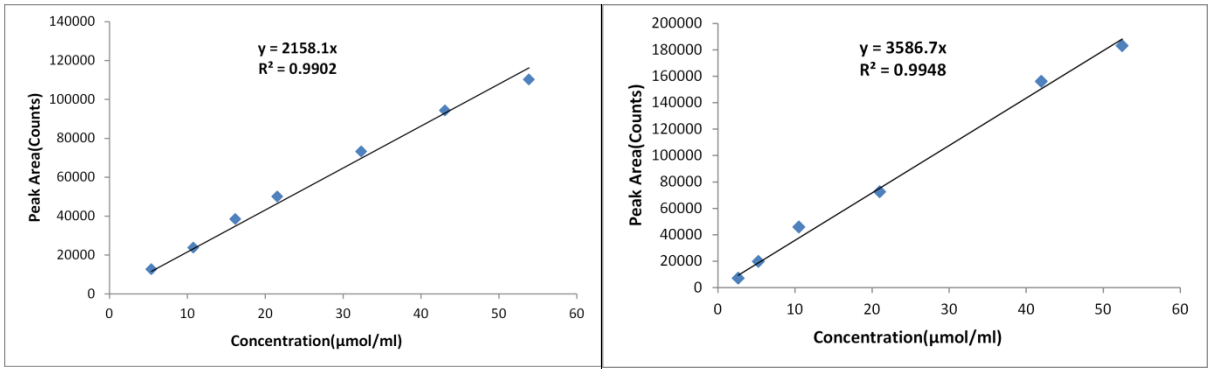


Figure S4: Calibration curves for meso-cyclopent-2-en-1,4-diol(left) and 4-(R)-hydroxycyclopent-2-en-1-(S)-acetate (right) in water.

Figure S4 present the calibration curve of meso-cyclopent-2-en-1,4-diacetate in hexane. The regression value (R=0.99) of the linear plot is within the high confidence range hence used for the determination of unknown concentration in the hexane layer during the experiment.

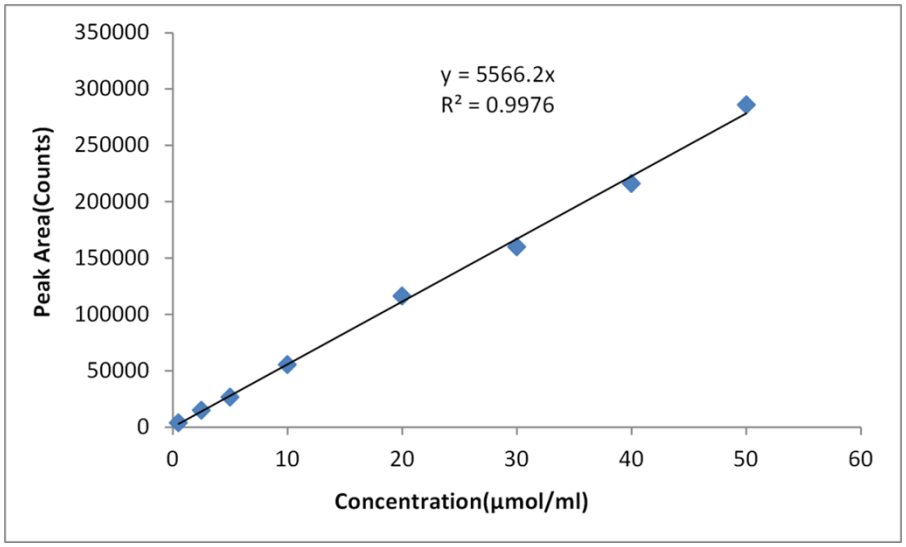


Figure S5: Calibration curve for meso-cyclopent-2-en-1,4-diacetate in hexane.

Figures S5 and S6 present the gas chromatographs of reaction products using two different enzymes. Figure S5 exhibited one optical isomer (retention time R_T = 11.15mins) when PFL was used as an enzyme whereas Figure S6 exhibited both isomers at two different retention times (R_T = 11.15mins and R_T = 11.27mins). The confirmation of the chiral products due to the retention times of 11.15 mins and 11.27 mins were verified by injecting pure commercial (Sigma Aldrich: Cat. No.446041 and Cat. No. 00848) compounds corresponding to the two chiral isomers.

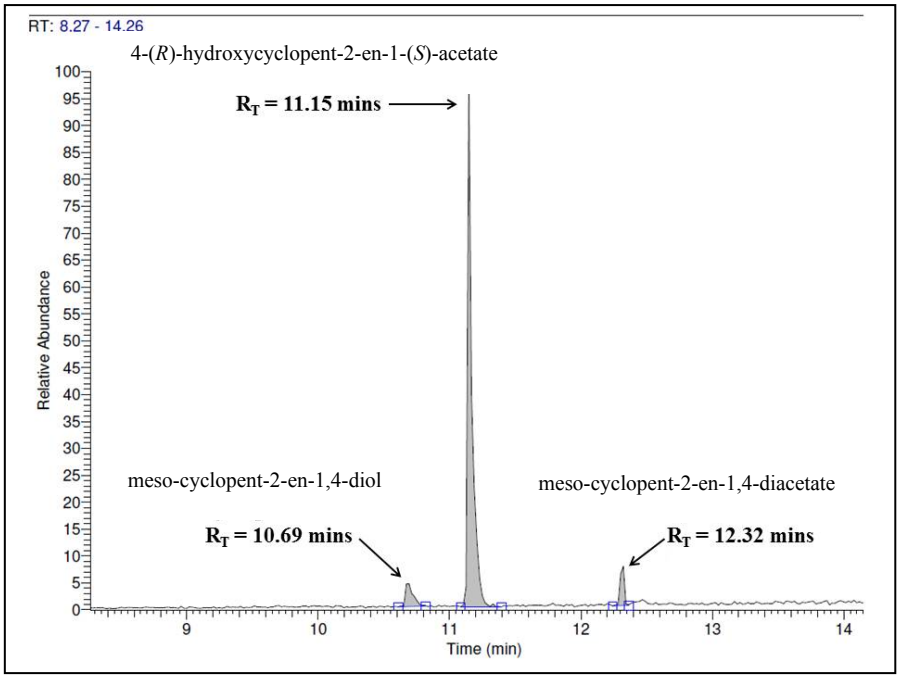


Figure S6: Scanned GC chromatogram of the reaction products after 48 hours using PFL immobilised nanoparticles. Retention time is indicated by R_T.

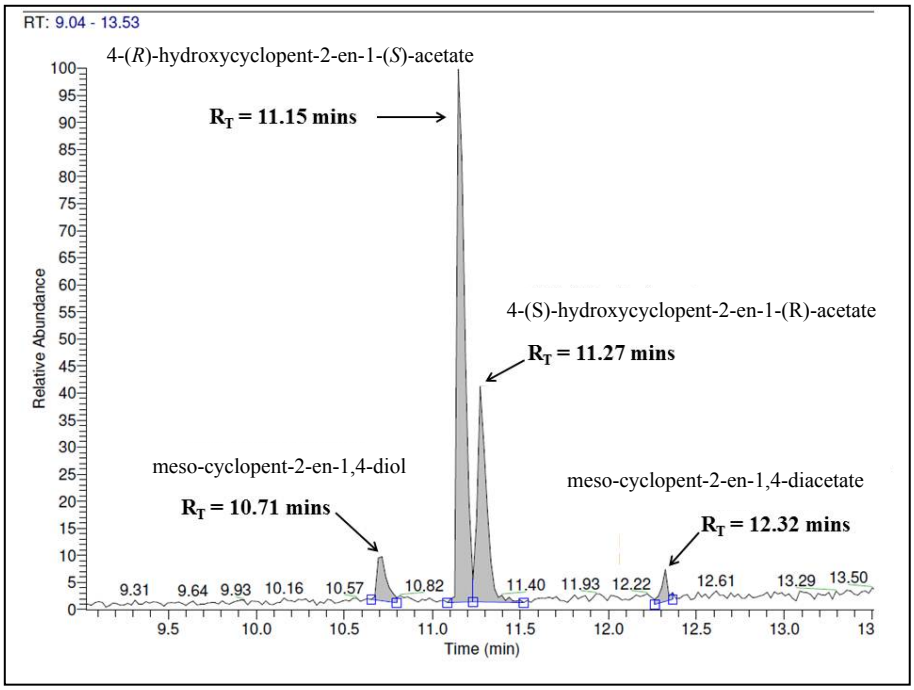


Figure S7: Scanned GC chromatogram of the reaction products after 48 hours using immobilised CRL as catalyst. Retention time is indicated by R_T.

The products were further confirmed by injecting them in GC-MS. It was found that the compound corresponding to the peak at R_T = 11.27 mins was found to have the same mass as 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate obtained from the compound at R_T = 11.15.

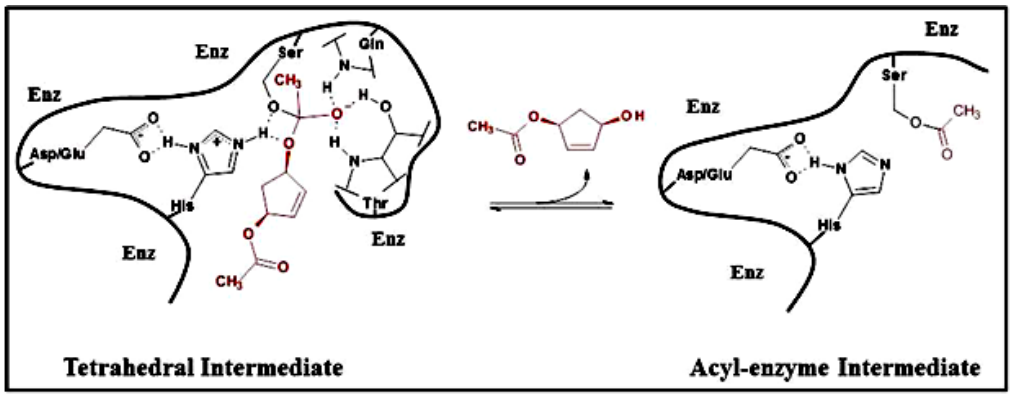
We have further confirmed that the peak at R_T = 11.15 was due to the chiral product (1S,4R)-form by adding a small amount (~5 µmol) of pure 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate (Sigma-Aldrich Cat. No.446041) into the reaction mixture. It was observed that only the peak area corresponding to the peak at R_T 11.15 mins (i.e. 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate) had increase. Similarly, when a small amount (~5 µmol) of pure 4-(*S*)-hydroxycyclopent-2-en-1-(*R*)-acetate (Sigma-Aldrich cat. No. 00848) was added to the mixture and it was observed that only the peak area corresponding to R_T = 11.27 mins (i.e. 4-(*S*)-hydroxycyclopent-2-en-1-(*R*)-acetate) had increase. Hence, it was concluded from this and the GC-MS data, that the peak at 11.27 mins correspond to 4-(*S*)-hydroxycyclopent-2-en-1-(*R*)-acetate.

The enantiomeric excess (*e.e.*) in the reaction mixture was calculated using the following equation reported earlier.[7]

$$e.e. = \frac{\text{moles of major enantiomer - moles of minor enantiomer}}{\text{total moles of both enantiomers}}$$

Table S2: Enantiomeric excess of 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate at different reaction times using PFL and CRL immobilized nanoparticles

Time (hr)	Enzyme PFL		Enzyme CRL	
	Physically adsorbed	Chemically immobilized	Physically adsorbed	Chemically immobilized
1	1	1	0.29	0.44
4	1	1	0.43	0.45
24	1	1	0.51	0.45
48	1	1	0.56	0.43



Reaction Scheme S1: Plausible reaction mechanism with active site of the enzyme[8]

Sample: MANEE ME50 SAMPLE2
Operator:
Submitter:
File Name: C:\ASAP2010\DATA\000-330.SMP

Started: 12/3/13 1:19:11PM Analysis Adsorptive: N2
Completed: 12/3/13 7:52:54PM Analysis Bath: 77.35 K
Report Time: 12/4/13 12:22:19PM Thermal Correction: No
Sample Weight: 0.0390 g Smoothed Pressures: No
Warm Freespace: 26.9656 cm³ Cold Freespace: 84.3376 cm³
MEASURED
Equil. Interval: 5 secs Low Pressure Dose: None

Analysis Log

Relative Pressure	Pressure (mmHg)	Vol Adsorbed (cm³/g STP)	Elapsed Time (HR:MN)	Saturation Press.(mmHg)
		01:08	778.82013	
0.009696153	7.55225	165.5964	01:35	
0.029195433	22.74092	202.6007	01:46	
0.059476162	46.32861	232.8964	01:55	
0.077457418	60.33664	246.6897	02:03	
0.099711744	77.67383	261.8477	02:10	
0.119702483	93.24818	274.4307	02:16	
0.139577189	108.73241	286.4065	02:21	
0.159561731	124.30315	297.9986	02:27	
0.179540750	139.86975	309.4835	02:32	
0.199643700	155.53395	320.5264	02:38	
0.247472692	192.80006	346.0134	02:45	
0.300827520	234.37230	373.3956	02:51	
0.352159091	274.37082	399.4691	02:58	
0.399662336	311.38742	424.2953	03:04	
0.449577198	350.28571	452.3903	03:11	
		03:14	779.15265	
0.499741916	389.37347	483.0664	03:20	
0.549579949	428.20215	517.9254	03:28	
0.600044963	467.51886	557.8402	03:36	
0.650307459	506.67731	601.3031	03:44	
0.701402663	546.48407	643.7252	03:52	
0.751994810	585.89929	676.1357	03:58	
0.800739853	623.87598	695.2524	04:02	
0.835924809	651.28845	703.6163	04:04	
0.866627741	675.20880	709.4619	04:06	
0.873879904	680.85809	713.6445	04:08	
0.901661739	702.50244	717.1811	04:10	
0.941826348	733.79437	722.1762	04:12	
0.949141171	739.49237	726.2590	04:14	
0.975940405	760.37097	730.7147	04:16	
0.994910056	775.14819	741.8629	04:20	
0.995045885	775.24817	750.1886	04:30	
0.973327725	758.32623	745.6572	04:32	
0.933994744	727.68054	738.6444	04:34	
0.908825543	708.07001	732.2299	04:36	
0.900181753	701.33453	727.8881	04:38	
0.873482280	680.53186	723.8119	04:40	
0.833188456	649.13788	719.2430	04:42	

0.808019499	629.52777	715.1793	04:44
0.800200746	623.43524	712.5164	04:46
0.749530069	583.95691	708.3204	04:48

Sample: MANEE ME50 SAMPLE2
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Warm Freespace: 26.9656 cm³ Cold Freespace: 84.3376 cm³
MEASURED
Equil. Interval: 5 secs Low Pressure Dose: None

Analysis Log

Relative Pressure	Pressure (mmHg)	Vol Adsorbed (cm³/g STP)	Elapsed Time (HR:MN)	Saturation Press.(mmHg)
0.684348610	533.17340	699.3829	04:50	
0.650046449	506.44757	691.4480	04:53	
0.601153043	468.35358	675.0970	04:57	
0.551558156	429.71265	648.3128	05:03	
0.502536613	391.51727	602.3699	05:14	
		05:17	779.08032	
0.454570278	354.14676	519.6702	05:36	
0.395617219	308.21759	439.0060	05:52	
0.346726205	270.12756	408.6908	06:00	
0.290958358	226.67993	377.5899	06:08	
0.251024936	195.56859	355.5200	06:15	
0.200444974	156.16273	326.8814	06:24	
0.140533178	109.48663	290.9993	06:34	

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Warm Freespace: 26.9656 cm³ Cold Freespace: 84.3376 cm³
MEASURED
Equil. Interval: 5 secs Low Pressure Dose: None

BET Surface Area Report

BET Surface Area: 1187.7596 ± 6.7348 m²/g
Slope: 0.003604 ± 0.000021
Y-Intercept: 0.000061 ± 0.000003
C: 59.780467
VM: 272.847535 cm³/g STP
Correlation Coefficient: 9.999021e-01

Molecular Cross-section: 0.1620 nm²

Relative Pressure	Vol Adsorbed (cm³/g STP)	1/ [VA*(Po/P - 1)]
0.059476162	232.8964	0.000272
0.077457418	246.6897	0.000340
0.099711744	261.8477	0.000423
0.119702483	274.4307	0.000495
0.139577189	286.4065	0.000566
0.159561731	297.9986	0.000637
0.179540750	309.4835	0.000707
0.199643700	320.5264	0.000778

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Sample Weight: 0.0390 g Smoothed Pressures: No
Warm Freespace: 26.9656 cm³ Cold Freespace: 84.3376 cm³
MEASURED
Equil. Interval: 5 secs Low Pressure Dose: None

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t-Plot Report

Micropore Volume: -0.075912 cm³/g
Micropore Area: -120.7243 m²/g
External Surface Area: 1308.4839 m²/g
Slope: 84.592958 ± 0.248473
Y-Intercept: -49.076590 ± 1.026914
Correlation Coefficient: 9.99978e-01
Thickness Range: 3.5000 to 5.0000 A

t = [13.9900 / (0.0340 - log(P/Po))] 0.5000

Surface Area Correction Factor: 1.00
Density Conversion Factor: 0.001547
Total Surface Area (by BET): 1187.7596

Relative Pressure	Statistical Thickness (A)	Vol Adsorbed (cm³/g)
0.009696153	2.6140	165.5964
0.029195433	2.9864	202.6007
0.059476162	3.3326	232.8964
0.077457418	3.4956	246.6897
0.099711744	3.6761	261.8477
0.119702483	3.8256	274.4307
0.139577189	3.9665	286.4065
0.159561731	4.1029	297.9986
0.179540750	4.2355	309.4835
0.199643700	4.3665	320.5264
0.247472692	4.6737	346.0134
0.300827520	5.0176	373.3956
0.352159091	5.3583	399.4691
0.399662336	5.6887	424.2953
0.449577198	6.0581	452.3903
0.499741916	6.4598	483.0664
0.549579949	6.8986	517.9254
0.600044963	7.3951	557.8402
0.650307459	7.9585	601.3031

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Sample Weight: 0.0390 g Smoothed Pressures: No
Warm Freespace: 26.9656 cm^3 Cold Freespace: 84.3376 cm^3
MEASURED
Equil. Interval: 5 secs Low Pressure Dose: None

Summary Report

Area

Single Point Surface Area at P/Po 0.19964370 : 1116.7492 m^2/g

BET Surface Area: 1187.7596 m^2/g

Langmuir Surface Area: 1665.8753 m^2/g

Micropore Area: -120.7243 m^2/g

External Surface Area: 1308.4839 m^2/g

BJH Adsorption Cumulative Surface Area of pores
between 17.000000 and 3000.000000 A Diameter: 1250.4231 m^2/g

BJH Desorption Cumulative Surface Area of pores
between 17.000000 and 3000.000000 A Diameter: 1426.0217 m^2/g

Volume

Single Point Adsorption Total Pore Volume of pores less than
824.3239 A Diameter at P/Po 0.97594041: 1.130270 cm^3/g

Micropore Volume: -0.075912 cm^3/g

BJH Adsorption Cumulative Pore Volume of pores
between 17.000000 and 3000.000000 A Diameter: 1.250046 cm^3/g

BJH Desorption Cumulative Pore Volume of pores
between 17.000000 and 3000.000000 A Diameter: 1.250506 cm^3/g

Pore Size

Adsorption Average Pore Diameter (4V/A by BET): 38.0639 A

BJH Adsorption Average Pore Diameter (4V/A): 39.9879 A

BJH Desorption Average Pore Diameter (4V/A): 35.0768 A

References

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