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Asymmetric epoxidation of chalcone catalyzed by reusable poly-L-leucine immobilized on hydrotalcite

Ronald-Alexander Miranda [a,b], Jordi Llorca [c], Francisco Medina [a,b], Jesús E. Sueiras [a,b], Anna M. Segarra [a,b,*]

[a] Departament d'Enginyeria Química, Universitat Rovira i Virgili, Av. Països Catalans, 26, Campus Sescelades, Tarragona 43007, Spain
[b] EMaS-Centro de Investigación en Ingeniería de Materiales y Micro/nanoSistemas, Campus Sescelades, Tarragona 4300, Spain
[c] Institut de Tècniques Energètiques and Center for Research in NanoEngineering, Universitat Politècnica de Catalunya (CRnE-UPC), Diagonal 647, ed. ETSEIB, Barcelona 08028, Spain

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ABSTRACT

Nanohybrid materials based on polyamino acids immobilized onto inorganic materials are of interest for their potential applications in protein engineering, biomedicine and catalysis. We developed an efficient and eco-friendly new protocol for the immobilization of synthesized poly-L-leucine (PLL) onto rehydrated hydrotalcite (HT_r). To do this, we synthesized different PLLs containing both *C*-terminal and *N*-terminal groups and compared them with a commercial PLL_c. These synthetic polypeptides were immobilized onto HT_r in water as the liquid medium with less than 30 min of ultrasound treatment. The obtained PLLs/HT_r synzyme showed excellent activity and enantioselectivity when used as a catalyst in the asymmetric Julià–Colonna epoxidation reaction of chalcone. Moreover, these nanohybrid materials based on PLL_S did not require any pre-activation time, which were easily separated from the reaction media and, unlike the commercial PLLc-supported catalyst, were reusable, exhibiting high stability after five consecutive runs without any apparent deactivation.

1. Introduction

Polyamino acids (PAA) are a kind of natural polymer with important applications in biotechnology [1], bio-nanomaterials [2] and catalysis [3]. These biomolecules are usually synthesized by ring-opening polymerization of α-amino acid and *N*-carboxyanhydrides (NCA) initialized by nucleophilic species or bases [4], (Scheme 1). Tertiary amines are able to activate NCA and prepare PAA with high molecular weight without incorporating the initiator in the polymer structure [5,6].

Synthetic PAA such as poly-L-leucine (PLL) are known as excellent catalysts for the asymmetric Julià–Colonna epoxidation reaction of acyclic (*E*)-enones (e.g. chalcone) [3,7] (Scheme 2). The obtained enantiomerically enriched α,β-epoxy ketones are versatile intermediates for the synthesis of several important chiral drugs such as taxol (cancer chemotherapy) [8], (+)-clausenamide (antiamnaesic agent) [9], statine (cholesterol-lowering drug) [10] and (+)-fenoprofen (rheumatoid arthritis drug) [11].

The original triphasic protocol [3] (PAA/aqueous phase/organic phase) had two important problems: the lengthy reaction time and the difficulty of recovering the gel- or paste-like catalyst. To overcome these limitations, Roberts et al. developed a non-aqueous biphasic system using organic bases and anhydrous urea-hydrogen peroxide [12] and a PAA on silica (*PaaSiCat*) as catalyst [13]. Under *PaaSiCat* conditions, the reaction was reduced to 4 h and yielded optically active epoxides with high enantioselectivity (up to 97% ee). In spite of these improvements, the potential industrial application of these protocols still faces some problems related to the time-consuming catalyst pre-activation, which has to be carried out separately and causes difficulties in the workup process. Another important enhancement was done by Geller et al. [14], who presented a modified triphasic protocol whereby the use of a cocatalyst such as tetrabutylammonium bromide (TBAB) accelerates the hydrogen peroxide anion phase transfer through the tri-phase interface. As a result, by using poly-L-leucine-1,3-diaminopropane as catalyst, reaction times were improved by up to 30 min without any separate pre-activation being required. Although the Julià–Colonna epoxidation mechanism is not at all clear, several studies have suggested that the five terminal L-leucine residues of α-helical PLL play an important role in its catalytic activity and enantioselectivity [15]. These results also suggest that the *C*-terminal group does not participate in the catalytic process [7,16].

Different methodologies for immobilizing AAs and oligoamino acids onto hydrotalcite-like compounds (HTs), $M^{2+}_{1-x}M^{3+}_{x}(OH)_2(A^{n-})_{x/n}\cdot yH_2O$, have been developed [17]. However, to the best of our knowledge, there are no examples in the literature of PAA being immobilized onto a HT. The positive charge of HTs layers is the result of the brucite-like layers [$Mg(OH)_2$] with edge-sharing

* Corresponding author at: Departament d'Enginyeria Química, Universitat Rovira i Virgili, Av. Països Catalans, 26, Campus Sescelades, Tarragona 43007, Spain. Fax: +34 977 559621.
E-mail address: anamaria.segarra@urv.cat (A.M. Segarra).

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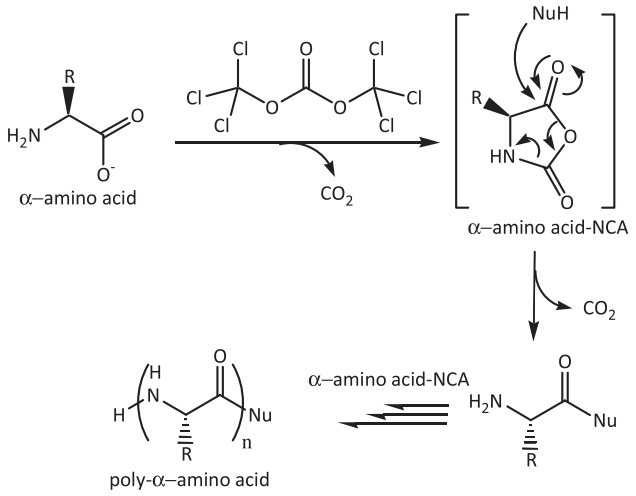


Scheme 1. Synthesis of PAA by ring-opening polymerization of NCA.

octahedral hydroxyl occupied by bivalent (Mg^{2+}) but also trivalent (Al^{3+}) cations and must be balanced by anions located in the interlayer. These can be exchanged by other organic or inorganic anions [18]. Hydrotalcite-like materials can reconstruct the original layered structure after being calcined by rehydration in a CO_2-free atmosphere. Rehydrated hydrotalcite, also called meixnerite, contains interlayer OH^- anions, which provide significant Brønsted basic properties [19,20].

In this context, and with a view to industrial application, we have synthesized different PLL with a *C*-terminal group (PLL_s). After immobilizing those onto meixnerite, we obtained more stable PLL-supported chiral synzymes. The nanohybrid catalysts were tested in the Julià–Colonna epoxidation of chalcone under phase-transfer catalyst/triphasic conditions. In addition, they were recovered by simple filtration and reused in several consecutive runs without any pre-activation or deactivation.

2. Experimental

2.1. General

All reactions and manipulations were conducted under an atmosphere of dry argon. The L-leucine-NCA was prepared as previously reported [21]. This compound was characterized by ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.94–1.05 (m, 6H), 1.66–1.78 (m, 1H), 1.80–1.86 (m, 2H), 4.33–4.38 (m, 1H), 7.05 (s, 1H) ppm, where chemical shifts are reported relative to tetramethylsilane.

Anhydrous 1,4-dioxane and triethylamine from Aldrich were used as precursors for synthesizing the PLL.

Chalcone, tetrabutylammonium bromide (TBAB), hydrogen peroxide, toluene, tetrahydrofurane (THF), NaOH and $MgSO_4$ from Sigma–Aldrich were used as reactants and solvents in the catalytic test.

The commercially available PLL (PLL_c) was poly-L-leucine-1,3-diaminopropane supplied by Across Organics (product No. 327582500) [15].

The precursor salts, $Mg(NO_3)_2{\cdot}6H_2O$ (purity 99%) and $Al(NO_3)_3{\cdot}9H_2O$ (purity 98%), were purchased from Sigma–Aldrich.

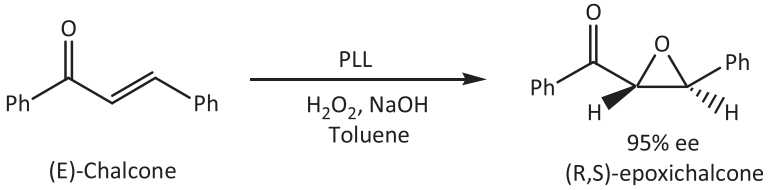
2.2. Analysis and characterization

FTIR spectra were recorded on a Nicolet Nexus Fourier transform instrument equipped with a DTGS KBr detector. For each spectrum, 100 scans in the 4000–400 cm^{-1} range were recorded with a resolution of 4 cm^{-1}, using the standard KBr disk (15 mg of sample in 100 mg of KBr). High-resolution transmission electron microscopy (HRTEM) was performed with a JEOL 2010F instrument equipped with a field emission source and working at an acceleration voltage of 200 kV. The point-to-point resolution of the microscope was 0.19 nm, and the resolution between lines was 0.14 nm. ^{1}H NMR spectra were recorded on a Varian 400 spectrometer. ^{13}C and ^{27}Al-MAS-NMR spectra were recorded at room temperature on a Varian Mercury spectrometer equipped with a 7 mm BB-CP MAS probe at a working frequency of 100.6 and 104.21 MHz, respectively. The spectra were recorded using the cross-polarization pulse sequence at room temperature under magic angle spinning at a spinning rate of 5.5 kHz. A total of 4000 scans were collected with a sweep width of 100 MHz and a pulse width of 0.2 s. Powder X-ray diffraction (XRD) patterns of the samples were obtained using a Siemens D5000 diffractometer with nickel-filtered CuKα radiation. XRD analyses of all the samples were performed in thin films. The patterns were recorded for 2θ angles between 3° and 70°. X-ray diffraction was used to determine the basal spacing of the free supports and the supports with the adsorbed complexes. The basal spacing of each sample was calculated from the (0 0 1) reflection in its X-ray pattern. This basal spacing was associated with the distance between (0 0 1) layers (d_{001}) and was located at angles (2θ) between 5° and 7°. The thermogravimetric analysis (TGA) was performed using a Perkin Elmer TGA7 thermobalance.

MALDI/MS measurements were performed on a Voyager-DE STR, from Applied Biosystems, operating in reflectron positive ion mode. The instrumental conditions were as follows: accelerating voltage 20 kV; reflectron potential 66%; and delay time 185 ns. Spectra were typically the sum of 100 laser shots. Dithranol was used as matrix (saturated solution in THF). After 2 mg of copolymer was dissolved in 1 mL of trifluoroacetic acid:chloroform (1:1), 5 µl of this solution was added to the same volume of the matrix solution and 2.5 µl of a doping agent (1 mg of potassium trifluoroacetate in 1 ml of THF). About 1 µl of the resulting solution was deposited on a stainless steel sample holder and allowed to dry before being introduced into the mass spectrometer. Three independent measurements were carried out for each sample. External mass calibration was done using Calmix 1 and Calmix 2 from a Sequazyme Peptide Mass Standards Kit (Applied Biosystems). The acquisition mass range was 200–50,000 Da.

2.3. Synthesis of PLL

The different varieties of as-synthesized poly-L-leucine (PLL_S) were synthesized by means of the polycondensation method, with triethylamine used as initiator [22]. The L-leucine-NCA (0.4 g) was



Scheme 2. Asymmetric Julià–Colonna epoxidation of chalcone.

dissolved in dry 1,4-dioxane (7 mL) under Ar atmosphere and stirred at the indicated temperature (r.t or 60 °C). After 15 min, the corresponding amount of triethylamine (monomer/initiator ratio (M/I) = 10, 5 or 2.5) was added. The reactor was closed with a freshly prepared calcium chloride drying tube. The reaction was stopped after 4 days. Milli-Q water (14 mL) was added as a workup solvent, and the mixture was stirred for 2 more hours. Finally, the obtained solid was filtered and dried at 60 °C under vacuum. The samples were labeled PLLX_Y (where X is the M/I ratio, X = 1 for M/I = 10, X = 2 for M/I = 5 and X = 3 for M/I = 2.5; and Y is the temperature of synthesis, nothing for room temperature and Y = 60 for 60 °C). All obtained polymers were characterized by ^{13}C-*MAS*-NMR (400 MHz) (δ = 20.09, 35.24, 51.64, 71.540, 111.819, 131.91, 151.52, 171.80, 191.50, 211.11, 230.91 ppm) as well as by MALDI-TOF and ESI-TOF mass spectroscopy.

2.4. Synthesis of HTs

Mg-Al HT (molar ratio 2:1) was prepared by the standard co-precipitation method at room temperature as follows. The appropriate amounts of $Mg(NO_3)_2{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$ were dissolved in 150 cm^{-3} of distilled water and added dropwise into a glass vessel which initially contained 200 cm^{-3} of deionized water. The pH was kept at 10 by adding a 2 M NaOH solution. Both solutions were mixed by means of vigorous stirring. The suspension was stirred overnight at room temperature. The precipitated solid was filtered and washed several times with water and dried at 110 °C to yield the as-synthesized hydrotalcite (HT-as). The solid was calcined in air by heating at 10 °C/min up to 450 °C over 6 h to obtain the corresponding mixed oxides and then reconstructed in water (1 mL/mg HT) decarbonated by sonication for 30 min, stirred for 30 min and sonicated again for 30 min under inert atmosphere [23,24]. After drying at 40 °C, the sample was labeled HT_r. Total rehydration of the meixnerite was confirmed by ^{27}Al-MAS-NMR and DRX.

2.5. Preparation of the chiral synzymes PLL/HT_r

The $PLL2_{60}$ (poly-L-leucine synthesized using an M/I ratio of 5 at 60 °C) and the commercial poly-L-leucine (PLL_C) were immobilized as follows. Water or THF solutions (5 mL) of each PLL (100 mg) were prepared and added to a suspension of the solid HT_r (300 mg) in water or THF, respectively. The immobilization process was then performed using three different protocols. In the first protocol (called method 1), the mixture was stirred for 2 days at a temperature of 80 °C (water) or 60 °C (THF); in the second protocol (called method 2), the mixture was stirred at room temperature for 1 h; and in the third protocol (called method 3), the PLL was immobilized under ultrasound treatment for 30 min. After the immobilization process, the obtained PLL/HT_r nanohybrid materials were separated by filtration, washed several times with THF (the indicated material having been washed with chloroform) and dried under vacuum. The samples obtained were called $IPLL2_{60}$-XY or PLL_C-XY, where X is the method used (A, B or C) and Y is the solvent employed (W for water or T for THF). All nanohybrid materials were characterized by XRD, ^{13}C-*MAS*-NMR and MALDI-TOF MS. Anchored PLL amounts were calculated by TG analysis.

2.6. Standard conditions for the catalytic asymmetric epoxidation of chalcone

The asymmetric epoxidation reaction was performed in a tube of 10 mL. Chalcone (0.036 mmol, 7.7 mg), and H_2O_2 (30 wt.%, 40.6 μL, 11.9 equiv.) was added to a suspension of PLL_C, PLL_S or the immobilized polymers (200 wt.% of PLL_X, 100 mg) with TBAB (0.004 mmol, 1.3 mg) and NaOH 2 M (0.18 mL, 10 equiv.) in toluene (0.5 mL). The reaction mixture was stirred at room temperature for the indicated time. The reaction mixture was quenched with ethyl acetate (1 mL). The liquid phase was separated by centrifugation, and the catalyst (solid phase) was washed several times with toluene. The liquid phase was finally extracted into ethyl acetate. The organic fraction was dried over $MgSO_4$. The solvent was then removed by evaporation under reduced pressure. To study the reusability of the catalyst, the catalytic solid was kept in the tube and the reagents were then added for another run. The product was identified by ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.01 (s, 1H), 4.23 (s, 1H), 7.32–7.45 (m, 7H), 7.54 (d, 1H), 7.94 (d, 2H) ppm. The ee of *trans*-(2R,3S)-epoxy-1,3-diphenyl-propan-1-one formed by L-leu/HT catalyst was determined by chiral HPLC using a ChiralPak IA column. The mobile phase was 25% hexane in ethanol, at a flow rate of 1 mL/min. The wavelength reading was 254 nm. The retention times were t_{major} = 7.6 min and t_{minor} = 10.6 min.

3. Results and discussion

3.1. Catalyst preparation and characterization

Nanohybrid materials based on PLL immobilized onto rehydrated hydrotalcite (HT_r) were synthesized as potential catalysts in an asymmetric Julià–Colonna epoxidation reaction. The immobilization of polyamino acids onto cationic-charged layered materials such as hydrotalcite is favoured by the presence of a carboxylate group in the bioguest. The *N*-terminal group is also important in the catalytic process. The commercially available poly-L-leucine (PLL_C) shows two *N*-terminal groups. For this reason, various poly-L-leucines (PLL_S) with both *N*-terminal and *C*-terminal groups were synthesized. Fig. 1 shows the molecular structures of PLL_C and PLL_S.

PLL_S catalysts were synthesized by means of the polycondensation method using triethylamine as initiator. The influence of the temperature and monomer/initiator molar ratio (M/I) was investigated, and all results were compared with PLL_C. Table 1 summarizes the results.

It was demonstrated that under the studied conditions, different synthesis temperatures and M/I molar ratio variations do not affect the molecular weight (Mw) of PLL_S, which in all cases was close to that of PLL_C. PLL_S synthesized at room temperature had the distribution of degree of polymerization (DP) shown in Table 1. The dimmers, trimmers and tetramers presence in the synthesized polymers (Table 1, entries 2–4) were removed by washing the PLL with chloroform after synthesis (Table 1, entry 5). Similar chemical shifts and vibration frequencies were observed for PLL_C

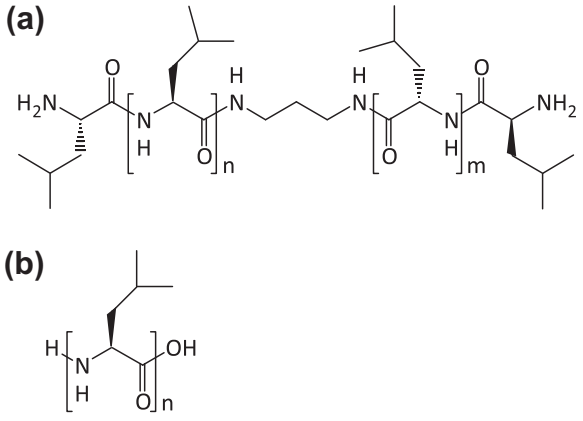


Fig. 1. Molecular structures of (a) PLL_C and (b) PLL_S.

Table 1
Poly-L-leucine synthesis conditions and specifications.

Entry[a]	Material	T (°C)	*M*/*I* ratio	DP[bc]	Mw[c]	PD[c]
1	PLL_C	–	–	5–39	2343.4	1.26
2	PLL1	r.t.	10	2–39	2212.4	1.57
3	PLL2	r.t.	5	2–39	2405.5	1.51
4	PLL3	r.t.	2.5	2–37	2249.1	1.46
5	PLL1[d]	r.t.	10	5–42	2274.7	1.47
6	$PLL1_{60}$	60	10	5–42	2455.8	1.43
7	$PLL2_{60}$	60	5	5–38	2303.4	1.49

[a] See details of the synthesis conditions in Section 2.
[b] Determined by ESI-TOF MS analyses.
[c] Determined by MALDI-TOF analysis with DHB matrix.
[d] PLL was washed several times with chloroform after synthesis.

and PLL_S. These results are in agreement with what was expected. The MALDI-TOF MS spectra of PLL_S showed two sets of peaks that correspond to the Na^+ and K^+ cationized n^*L-Leu + H/OH species. This indicates the presence of a *C*-terminal group in the PLLs instead of the two *N*-terminal groups in the PLL_C.

As Table 1 shows, the PLL_C and the synthesized polyamino acid $PLL2_{60}$ had similar Mw and distribution of DP. For this reason, $PLL2_{60}$ was chosen as a model in the present study.

We studied the immobilization process of the commercial and synthesized PLL onto rehydrated hydrotalcite solids with a 2:1 Mg/Al molar ratio (HT_r). The HT_r was synthesized by the calcination–rehydration method under ultrasounds. Three different protocols were developed to immobilize the PLL onto HTr (see protocols detailed in Section 2). The effect of water and THF as immobilization media was also studied (Table 2).

Method 1, which involves exfoliation of the starting HT_r using water as medium, showed the highest amount of immobilized PLL (Table 2, entry 5), which indicates that nanohybrids based on PLL_C exhibit a low degree of immobilization (Table 2, entry 9). These results demonstrate the important role played by the *C*-terminal group in the immobilization process of PLL onto HT_r. It was also found that the use of ultrasonic treatment increased the amount of immobilized PLL. These results are in agreement with Medina et al., who showed that sonication maximizes accessibility to the OH^- groups of HTs [24].

PXRD patterns of all of the synthesized nanohybrids showed peaks corresponding to (0 0 3) and (0 0 6) reflection planes of meixnerite phase (JCPDS: 22-700) obtained after rehydration of magnesium and aluminum mixed oxides. The same samples, except $IPL2_{60\text{-}2T}$ (Fig. 2f), had new diffraction peaks corresponding to (0 0 3) reflection planes of hydrotalcite with immobilized PLL (Fig. 2). The two types of hydrotalcite structure showed different interlayer spaces. While the interlayer space remained unchanged with respect to the meixnerite in one structure (7.7 Å), the space increased in size in the other one (11.2 Å). This indicates that only a part of the PLL is located between the layers of the HT_r. The PXRD patterns also confirmed that a lower degree of immobilization of PLL_S is obtained with THF used as medium (Fig. 2d, f and h). Moreover, the material crystallinity of the sonicated samples decreased during the immobilization process as the amount of PLL immobilized onto the HT_r increased (Fig. 2b–d).

Fig. 3 shows the HRTEM images of the nanohybrids $IPL2_{60\text{-}1T}$, $IPL2_{60\text{-}1W}$, $IPL2_{60\text{-}3W}$ and $IPL_{C\text{-}3W}$. Nanohybrid materials synthesized by method 1 have indistinguishable morphology (Fig. 3a and b). Nevertheless, the incorporation of the PLL into the hydrotalcite structure was confirmed by increments in the basal spacing by up to 11.1 Å.

Nanohybrids synthesized by method 3 showed similar behavior (Fig. 3c and d). Lattice fringes at 7.5 and 3.7 Å were more intense and corresponded to the (0 0 3) and (0 0 6) crystallographic planes of the hydrotalcite structure, whereas the spacing at 11.1 Å indicates the presence of intercalated polymer.

The largest amount of immobilized PLL was obtained using water as medium. This can be explained by the fact that hydrotalcite-like compounds are hydrophilic materials. This property permits the interaction of the water molecules with both surface and interlayer space of the hydrotalcite and facilitates anionic interchange between the carboxylate end group of PLL and the hydroxide groups of HT_r. When THF was used as immobilization medium, similar behavior was observed, although the interaction with the interlayer space of HT_r was found to be more difficult. Moreover, the absence of *C*-terminal groups in the PLL_C decreases the prevalence of the immobilization process that occurs by interaction between carbonyl groups of the amino acid residues with the OH^- groups on the surface and in the edges of the HT_r layers and which deforms the initial hydrotalcite structure.

3.2. Catalytic activity and selectivity

The commercial (PLL_C), synthesized (PLL_S) and immobilized PLL were used as catalysts in the asymmetric epoxidation reaction of chalcone under the standard conditions reported by Geller et al. with TBAB as a phase transfer cocatalyst (PTC) [14]. Chalcone was chosen as a test compound substrate in order to draw compar-

Table 2
Nanohybrid materials based on PLL_s synthesis conditions.

Entry	Material	Method[a]	PLL	Immobilization medium	Immobilization ratio[b]
1	$IPL2_{60\text{-}1W}$	1	$PLL2_{60}$	Water	0.196
2	$IPL2_{60\text{-}1T}$	1	$PLL2_{60}$	THF	0.082
3	$IPL2_{60\text{-}2W}$	2	$PLL2_{60}$	Water	0.130
4	$IPL2_{60\text{-}2T}$	2	$PLL2_{60}$	THF	0.085
5	$IPL2_{60\text{-}3W}$	3	$PLL2_{60}$	Water	0.225
6	$IPL2_{60\text{-}3T}$	3	$PLL2_{60}$	THF	0.180
7	$IPL_{C\text{-}1W}$	1	PLL_C	Water	0.071
8	$IPL_{C\text{-}2W}$	2	PLL_C	Water	0.037
9	$IPL_{C\text{-}3W}$	3	PLL_C	Water	0.076

[a] Method 1: Magnetic stirring for 2 days with indicated temperature. Method 2: Magnetic stirring for 1 h at room temperature. Method 3: Ultrasound treatment for 30 min.
[b] Immobilization ratio = mg PLL/mg HT_r. Calculated by thermogravimetric (TG) analysis.

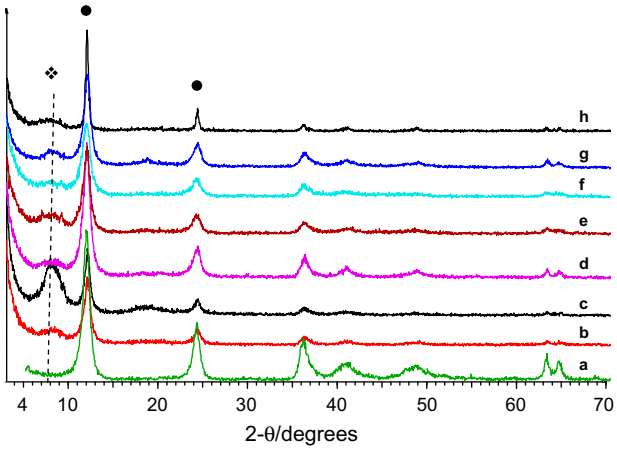


Fig. 2. XRD patterns of nanohybrid PLL/HT_r materials: (a) HT_r, (b) $IPL_{C\text{-}3W}$, (c) $IPL2_{60\text{-}3W}$, (d) $IPL2_{60\text{-}3T}$, (e) $IPL2_{60\text{-}2W}$, (f) $IPL2_{60\text{-}2T}$, (g) $IPL2_{60\text{-}1W}$ and (h) $IPL2_{60\text{-}1T}$. ❖ Basal peaks of (0 0 3) plane with immobilized PLL. ● Basal peaks of (0 0 3) and (0 0 6) planes of the starting HT_r material.

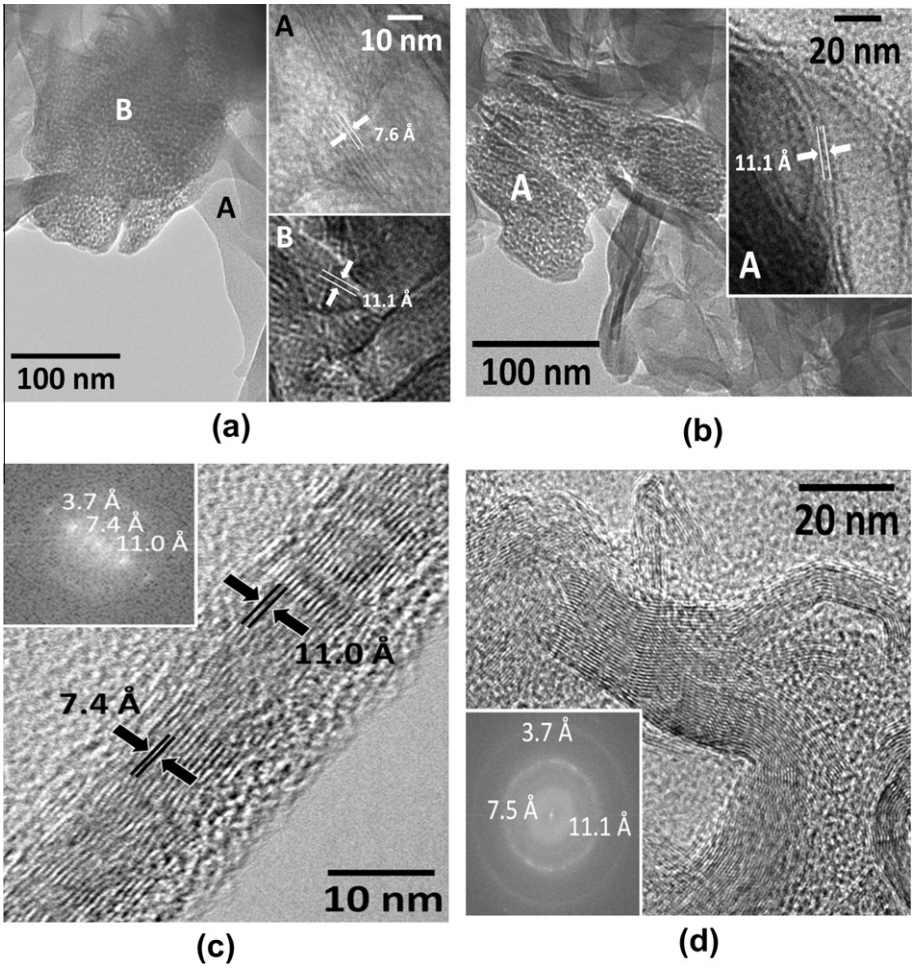


Fig. 3. HRTEM images of (a) $IPL2_{60\text{-}1T}$, (b) $IPL2_{60\text{-}1W}$, (c) $IPL2_{60\text{-}3W}$ and (d) $IPL_{C\text{-}3W}$.

isons with the results published in the literature for the asymmetric epoxidation process.

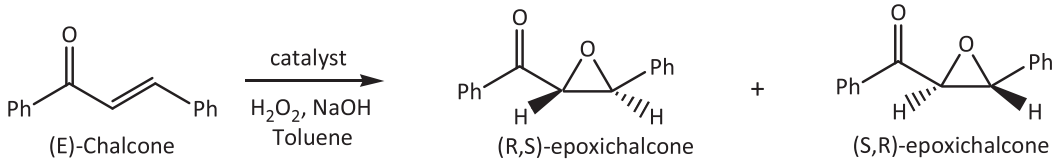
3.2.1. Catalytic test of PLL_S and PLL_C

The manner in which PLL is prepared turned out to be of crucial importance for all of the developed Julià–Colonna protocols [25]. The PLL synthesized by different protocols were therefore tested using the standard test reaction (Scheme 3). The results were compared with those of PLL under the same conditions (Table 3).

In agreement with the Geller studies, the polymers synthesized under thermal treatment ($PLL1_{60}$ and $PLL2_{60}$) were considerably more active in asymmetric epoxidation than the ones synthesized at room temperature and slightly more active than the PLL_C. The PLL_S synthesized at room temperature showed lower activity due to the presence of oligomers with a smaller sized chain (<5 monomers). Nevertheless, after the dimmers, trimmers and tetramers were eliminated by means of chloroform washing, the conversion rate and enantioselectivity increased significantly, up to 99% and 93%, respectively (Table 3, entry 8). This is because the oligomers with fewer than five monomers do not have catalytic properties, as proposed by Berkessel et al. [15a]. In order to optimize the reaction conditions, further epoxidation reactions were carried out to maximize the activity of all PLL_S. Table 4 shows the results.

Interestingly, for all PLL_S, the optimized methodology reduced the reaction time to 30 min. In addition, the synthesized catalysts were highly active yet did not require previous chloroform washing. These interesting results can be explained according to the mechanistic studies of Colonna et al. [26] (Scheme 4).

Colonna et al. found that both hydrogen peroxide and substrate concentration exhibit inhibitory behavior due to the competition of different pathways. Nevertheless, under our conditions, kinetic saturation could be overcome by favouring the pathway that involves the formation of the PLL:HOO^- complex. This rationalization might explain why PLL synthesized at room temperature had very low conversion rates under the Geller conditions while the conversion rate was up to 94% under optimized conditions with 94% enantiomeric excess.



Scheme 3.

Table 3
Asymmetric epoxidation reaction synthesized by poly-L-leucine.

Entry[a]	Catalyst	Conv. (%)[b]	e.e. (%)[c]
1	–	<2	–
2	L-leu	<2	–
3	NCA	<2	–
4	PLL_C	85	95
5	PLL1	27	87
6	PLL2	45	85
7	PLL3	37	91
8[d]	PLL1	99	93
9	$PLL1_{60}$	91	94
10	$PLL2_{60}$	90	92

[a] Standard conditions: Chalcone 7.7 mg (0.036 mmol), catalyst 100 mg (200 wt.% of PLL_X), H_2O_2 97.2 μL (30 wt.%, 28.5 equiv.), NaOH 2 M 75.6 μL (4.2 equiv.), TBAB 1.3 mg (0.004 mmol) in toluene (0.5 mL); 1.5 h reaction time, room temperature. Total diastereoselectivity towards *trans*-1,2-epoxy-1,3-diphenyl-propane-1-ona.
[b] Determined by ^1H NMR.
[c] Enantiomeric excess towards the *trans*-(2R, 3S)-epoxychalcone determined by chiral HPLC.
[d] Using PLL previously washed with chloroform.

Table 4
Optimization of asymmetric epoxidation reaction conditions catalyzed by poly-L-leucine.

Entry[a]	Catalyst	Conv. (%)	ee (%)
1	–	<4	–
2	L-leu	<4	–
3	NCA	<4	–
4	PLL_C	97	92
5	PLL1	98	90
6	PLL2	98	92
7	PLL3	98	93
8	$PLL1_{60}$	94	94
9	$PLL2_{60}$	95	93

[a] Standard conditions: Chalcone 7.7 mg (0.036 mmol), catalyst 100 mg (200 wt.% of PLL_X), H_2O_2 40.6 μL (30 wt.%, 11.9 equiv.), NaOH 2 M 0.18 mL (10 equiv.), TBAB 1.3 mg (0.004 mmol) in toluene (0.5 mL); 0.5 h reaction time, r.t.

3.2.2. Variation of the starting enone

To widen the scope of the new catalysts ($PLL2_{60}$ and $IPL2_{60\text{-}3W}$), some further epoxidation reactions were carried out to broaden the substrate range using the same conditions as chalcone (Table 5). As test compounds substrates were chosen that exhibited low reactivity under standard triphasic conditions [27–29] and much faster conversions under new triphasic/PTC conditions [14].

The new catalysts in the triphasic/PTC conditions show slightly higher activity when the reaction was performed using the non-immobilized catalyst. And, they show similar enantioselectivities in either $PLL2_{60}$ or $IPL2_{60\text{-}3W}$ catalysts.

On the other hand, the *trans*-1,4-diphenyl-2-butene-1,4-dione shows activities comparable with the Geller and coworker results, but the enantioselectivities are significantly lowers (Entry 1 and 2, Table 5). It is important take into account that the reaction times in the epoxidation of phenyl *trans*-styryl sulfone (Entry 3 and 4, Table 5) and phenyl *trans*-4-phenyl-3-buten-2-one (Entry 5 and 6, Table 5) are two and four time lower, respectively, than in the Geller experiment. In addition, the NaOH/TBAB ratio is different. Taken together these facts could explain the poorer activity observed with the $PLL2_{60}$ and $IPL2_{60\text{-}3W}$ catalysts.

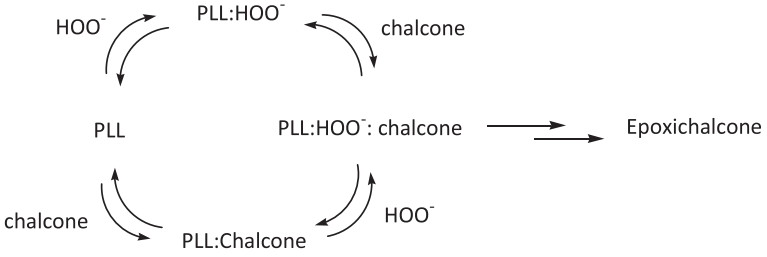
3.2.3. Catalytic test and reusability of nanohybrid materials

We studied the potential of $IPL2_{60\text{-}3W}$ and $IPL_{C\text{-}3W}$ nanohybrid materials in an epoxidation reaction of chalcone in which both materials were synthesized following the same immobilization procedure. We evaluated the new synzymes under optimized conditions at 1 h of reaction (Fig. 4). The activity of the basic catalyst Mg/Al 2:1 dehydrated under ultrasounds (HT_r) was also tested in the epoxidation reaction of chalcone in a previous work [30] presenting a very poor activity (6%).

Fig. 4 shows that the activity and selectivity of the $IPL_{C\text{-}3W}$ and $IPL2_{60\text{-}3W}$ nanohybrid materials decreases slightly in the first run as compared to the corresponding non-immobilized PLL_C and $PLL2_{60}$. In the case of the nanohybrid based on synthesized PLL, comparable values for activity and stereoselectivity were obtained from the second run. These remained constant after the organic phase was removed by simple centrifugation, and epoxidation recycling was repeated for at least five consecutive runs. A remarkable difference in reusability was observed when the nanohybrid material was prepared from PLL_C. The conversion rate and enantiomeric excess decreased significantly upon reuse. This was due to leaching of the PLL_C because no basal peaks of the (0 0 3) and (0 0 6) planes of the PLL intercalated into the hydrotalcite-like compound were observed in the XRD pattern of the $IPL_{C\text{-}3W}$ after five consecutive runs (Fig. 5). The decrease in activity using $PLL_{C\text{-}3W}$ as catalyst confirmed the poor stabilization of PLL_C onto HT_r, probably due to the absence of *C*-terminal groups in the PLL_C.

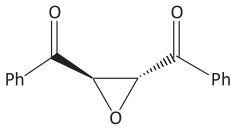
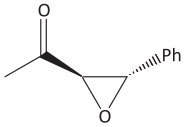
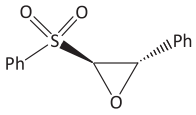
After the final run, used catalysts were characterized by HRTEM. These results show differences between the fresh and reused $IPL2_{60\text{-}3W}$ and $IPL_{C\text{-}3W}$ catalysts (Figs. 3 and 6). The interlayer space of the HT_r in the $IPL2_{60\text{-}3W}$ is better preserved after five consecutive runs (Fig. 6a). Nevertheless, the HRTEM image of reused $IPL_{C\text{-}3W}$ catalyst also reveals that the lattice fringe at 11.1 Å observed in the fresh catalyst disappeared and, interestingly, the (0 0 3) crystallographic plane of the HT structure was centered at 7.6 Å (Fig. 6b). These results are in agreement with the DRX analysis of the reused $IPL2_{60\text{-}3W}$ and $IPL_{C\text{-}3W}$ catalysts (Fig. 5), which indicates that the polymer without any C-terminal groups is no longer intercalated between the HT layers.

In order to understand the lower activity during the first run with the $IPL2_{60\text{-}3W}$ catalyst, TGA-DTA analysis of the pure HT_r, fresh $IPL2_{60\text{-}3W}$, $IPL2_{60\text{-}3W}$ after first run and $IPL2_{60\text{-}3W}$ after second consecutive run has been performed. The pure hydrotalcite shows weight loss between 100 and 200 °C to the loss of interlayer water and at 395 °C, which corresponds to the dehydroxylation of the brucite-like layers. The pure synthesized poly-aminoacid discomposes between 330 and 400 °C. For the fresh $IPL2_{60\text{-}3W}$ compounds,



Scheme 4. Mechanism of PLL-catalyzed asymmetric epoxidation reaction.

Table 5
Epoxidation of different enones.

Entry[a]	Product	Conv. (%)[d]	ee (%)[e]	Time referent experiment (min)
1[b]		>99 (>99)	62 (92)	8
2[c]		99	61	
3[b]		39 (65)	62 (64)	60
4[c]		32	64	
5[b]		30 (82)	19 (68)	120
6[c]		22	13	

[a] Standard conditions: 0.036 mmol substrate, 200 wt.% of catalyst, H_2O_2 40.6 μL (30 wt.%, 11.9 equiv.), NaOH 2 M 0.18 mL (10 equiv.), TBAB 1.3 mg (0.004 mmol) in toluene (0.5 mL); 0.5 h reaction time, r.t.
[b] $PLL2_{60}$.
[c] $IPL_{60\text{-}3W}$.
[d] Determined by ^1H NMR.
[e] Enantiomeric excess towards the *trans*-(2R,3S)-epoxychalcone determined by chiral HPLC and ^1H NMR; results of Ref. [14] experiments are given in parenthesis.

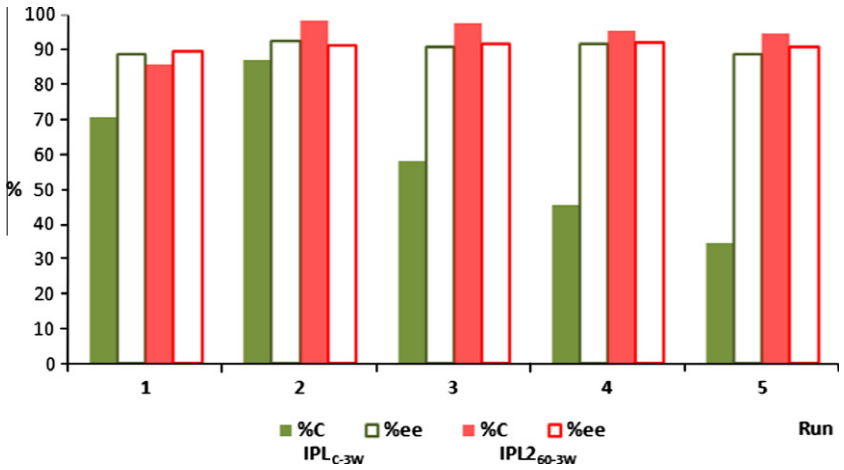


Fig. 4. Reusability of $IPL2_{60\text{-}3W}$ and $IPL_{C\text{-}3W}$. Reaction conditions: 10 equiv. NaOH 2M, 11.9 equiv. H_2O_2 (30 wt.%), 1 h reaction time. Total diastereoselectivity towards *trans*-1,2-epoxy-1,3-diphenyl-propane-1-ona.

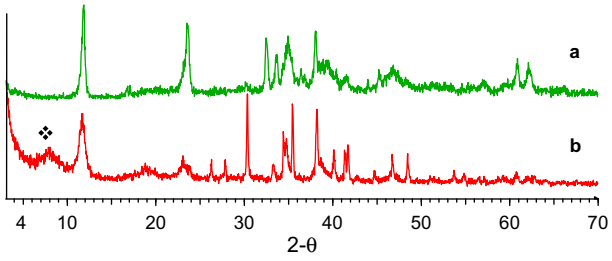


Fig. 5. PDRX patterns of (a) $IPL_{C\text{-}3W}$ and (b) $IPL2_{60\text{-}3W}$ after five consecutive runs. ❖ Basal peaks of (0 0 3) plane of hydrotalcite with immobilized PLL.

the thermal effect is characterized by a three-step decomposition of poly-aminoacid. In TGA of fresh $IPL2_{60\text{-}3W}$, the first weight loss corresponds to the interlayer water and takes place between 100 and 185 °C. The second step involves a gradual weight loss probably due to the polycondensation [31] of the polymer immobilized and dehydroxylation of the brucite-like layers (185–225 °C). And the thirds step shows a weight loss in TGA (225–650 °C) with an endothermic maximum in DTA at 407 °C corresponding to the dehydroxylation of the host layers as well as decomposition of poly-aminoacid located in the interlayers. Weight losses due to reagents or by-products remained in the solid phase was not observed by TGA-DTA analysis.

The Table 6 shows the total weight loss from the TGA analysis for all the compounds. The initial content of PLL in the fresh $IPL2_{60\text{-}3W}$ corresponds to 9.25% w/w. After the first run and several washes with diethyl acetate and toluene, the content of PLL decreases 1.57%, while after the second consecutive run, there are no significant leaching losses of the PLL. This fact could explain the different activity observed during the first run of the

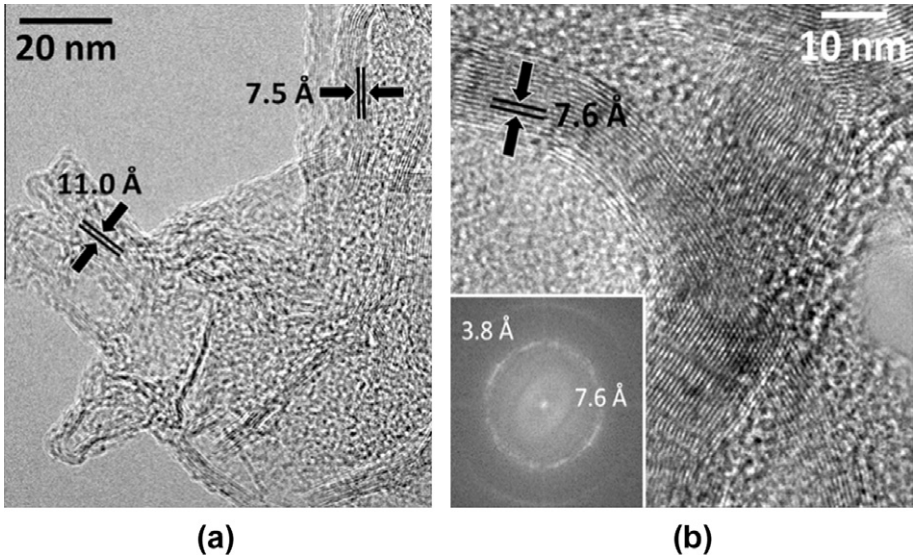


Fig. 6. HRTEM images of (a) $IPL2_{60\text{-}3W}$ and (b) $IPL_{C\text{-}3W}$ after five consecutive runs.

Table 6
Total weight loss by TGA.

Compound	Total weight loss (%w/w)
HT_r	57.09
Fresh $IPL2_{60\text{-}3W}$	47.84
1st run $IPL2_{60\text{-}3W}$	49.41
2nd run $IPL2_{60\text{-}3W}$	50.38

epoxidation of chalcone using the $IPL2_{60\text{-}3W}$ as catalyst due to the necessity of a pre-activation in the first run as also observed Geller et al. [14].

4. Conclusions

The challenge in achieving a well-defined catalytic process is to develop recyclable catalysts that guarantee the expected activity and selectivity for a reasonable number of consecutive runs. We have managed to fulfill this goal in the asymmetric epoxidation of *trans*-chalcone by preparing nanohybrid materials based on synthesized PLL and HT_r. The polymerization of L-leucine-NCA for preparing the PLL_S required tertiary amine as initiator, high temperatures (60 °C) and a workup with water and resulted in a highly active *C*-terminal PLL with chain-lengths similar to those of PLL_C. A simple, efficient and environmentally friendly method of synthesizing nanohybrid materials based on sonication in water was developed. $IPL2_{60\text{-}3W}$ exhibited activity and selectivity comparable to those of $PLL2_{60\text{-}3W}$ and no catalyst pre-activation was necessary. The nanohybrid can be separated from the reaction mixtures by simple centrifugation. In this way, the catalyst can be recovered and reused keeping the same activity and enantioselectivity for at least five consecutive runs. In contrast, the nanohybrid based on the commercial PLL, $IPL_{C\text{-}3W}$, did present a significant leaching of PLL and a notable loss of activity and selectivity upon reuse.

The reusability and reproducibility of the catalytic system in this process makes it viable and practical in economical and technical terms. These nanohybrid materials, being of economic and environmental interest, should therefore receive considerable attention in future scale-up applications and be used as bioactive catalysts in other asymmetric reactions.

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