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Chemoenzymatic preparation of optically active anthracene derivatives

F. Javier Quijada [a], Javier González-Sabín [b], Francisca Rebolledo [a,*], Vicente Gotor [a,*]

[a] *Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33071 Oviedo, Spain*
[b] *Entrechem, SL, Edificio Científico-Tecnológico, Campus de El Cristo, 33006 Oviedo, Spain*

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ABSTRACT

Syntheses and lipase-catalyzed resolutions of a *trans*-2-aminocyclopentanol *rac*-**2** and *trans*-cyclopentane-1,2-diamine *rac*-**3**, bearers of an anthracene unit, have been effectively carried out. *Burkholderia cepacia* lipase catalyzed the transesterification of the β-amino alcohol with very high enantioselectivity ($E > 200$). Lipase B from *Candida antarctica* showed moderate enantioselectivity in the acetylation of the diamine when 1-phenylethyl acetate was used as an acyl donor. In addition, the treatment of the optically active diamine (1*S*,2*S*)-**3** (ee = 95%) with pyridine-2,6-dicarbonyl dichloride yielded the bis(aminoamide) **6**, which was tested as a chiral solvating agent (CSA) of carboxylic acids.

1. Introduction

Optically active β-amino alcohols and vicinal diamines are interesting compounds that have been shown to be effective in the treatment of a wide variety of human disorders.[1] Moreover, they have been widely used as chiral resolving agents and ligands in asymmetric synthesis.[2,3] Amongst the diamines, the commercially available enantiopure *trans*-cyclohexane-1,2-diamine has perhaps been the most used,[4] which is in contrast with the scarcity of applications of its homologous *trans*-cyclopentane-1,2-diamine.[5] Recently, we described a very useful procedure to obtain a variety of optically active *trans*-cyclopentane-1,2-diamine derivatives,[6] such as the bis(aminoamide) **1** (Fig. 1) which is used as chiral solvating agent (CSA) of carboxylic acids.[7] It is worthy to note that apart from chromatographic and capillary electrophoretic methods, NMR spectroscopy using CSAs is a fast and accurate methodology for the determination of the enantiomeric composition of a chiral carboxylic acid.[8] In this sense, we have demonstrated that the presence of the pyridine-2,6-dicarboxamide fragment is a key structural feature for the discriminating ability of the CSA.[9] This fragment ensures a pincer-like conformation for the bis(aminoamide), which is stabilized by two intramolecular H-bonds formed between the NH groups and the pyridine nitrogen.

In connection with this work and with the idea of testing the importance of the aromatic moiety over the tertiary amine of the bis(aminoamide), we herein planned the synthesis of a pyridine-2,6-dicarboxamide derived from the optically active diamine (1*S*,2*S*)-**3** (Fig. 2) bearing an anthracene unit. The presence of this group could increase the efficacy of the resulting bis(aminoamide) as a CSA due to the higher diamagnetic anisotropy of the anthryl ring[10] with respect to the phenyl group of **1**. In addition, due to the interest in non-racemic anthracene derivatives in cycloaddition reactions,[11] and as templates for asymmetric Diels–Alder/retro Diels–Alder strategies,[12] we also carried out the resolution of the β-amino alcohol (±)-**2**, which is a precursor in the synthesis of (±)-**3** (Scheme 1).

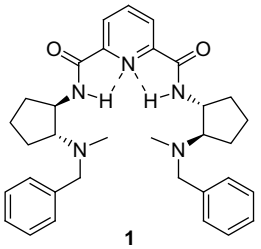

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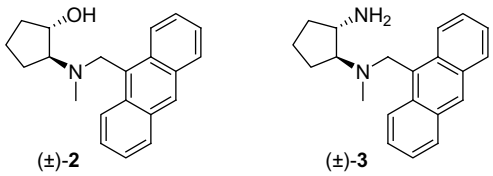
Figure 1. Optically active bis(aminoamide) as a CSA.



(±)-**2** (±)-**3**

Figure 2. β-Amino alcohol and 1,2-diamine bearer of an anthracene unit.

2. Results and discussion

The synthesis of (±)-*trans*-**3** was carried out following the methodology that we recently developed for the preparation of different

* Corresponding authors. Tel./fax: +34 985103448 (V.G.).
E-mail addresses: frv@uniovi.es (F. Rebolledo), vgs@fq.uniovi.es (V. Gotor).

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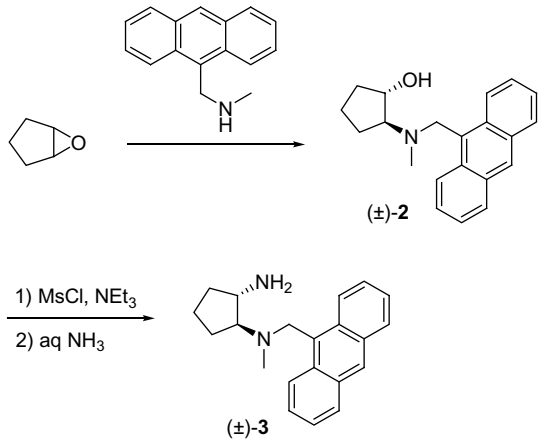
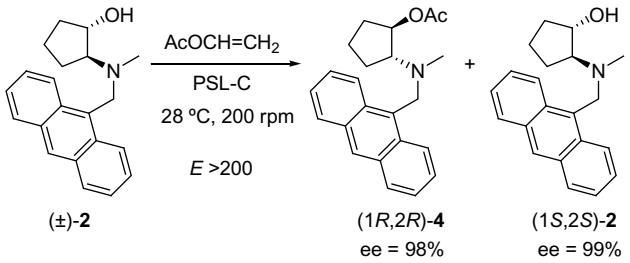


Scheme 1. Synthesis of (±)-**2** and (±)-**3**.

ference of this lipase with a wide variety of cycloalkanols[14] and according to Kazlauskas' rule,[15] we tentatively assigned the (1*R*,2*R*)- and (1*S*,2*S*)-configurations to the isolated product **4** and substrate **2**, respectively.



Scheme 2. Enzymatic kinetic resolution of (±)-**2**.

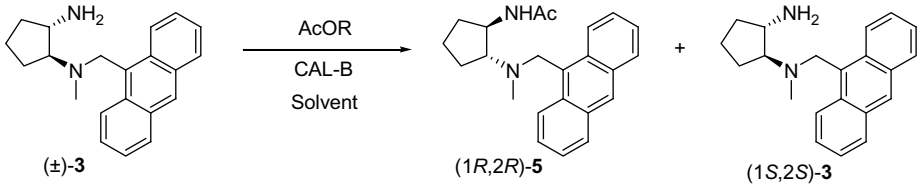
racemic *trans*-*N*,*N*-dialkylcyclopentane-1,2-diamines (Scheme 1). Thus, the reaction of cyclopentene oxide with the commercially available secondary amine 1-(9-anthryl)-*N*-methylmethanamine yielded the racemic β-amino alcohol (±)-*trans*-**2** with very high yield (>90%). After that, the one-pot treatment of (±)-*trans*-**2** with mesyl chloride and aqueous ammonia gave the required diamine (±)-*trans*-**3** with a yield (67%) that was slightly lower than those previously obtained for other *N*,*N*-dialkylcyclopentane-1,2-diamines bearing less bulky substituents. This transformation happens in a stereospecific form because it implies two S_N2 reactions: once the hydroxyl group is mesylated, an aziridinium ion intermediate is formed, which is subsequently attacked by the ammonia thus securing the *trans*-configuration of the resulting diamine.[6a]

Once racemic β-amino alcohol (±)-**2** and diamine (±)-**3** were obtained, we carried out their enzymatic resolutions using the best enzyme in each case. *Pseudomonas cepacia* lipase (PSL-C, recently classified as *Burkholderia cepacia* lipase) was chosen to catalyze the transesterification of the β-amino alcohol (±)-**2** using vinyl acetate as an acyl donor and *tert*-butyl methyl ether (TBME) as solvent. In these conditions, the enzyme catalyzed the acylation of the 2-dialkylaminocyclopentanol with very high enantioselectivity ($E > 200$),[13] product (1*R*,2*R*)-**4** and the remaining substrate (1*S*,2*S*)-**2** were obtained with very high yields and enantiomeric excesses (Scheme 2) after 5 h of reaction. Based on the observed enantiopreference of this lipase with a wide variety of cycloalkanols[14] and according to Kazlauskas' rule,[15] we tentatively assigned the (1*R*,2*R*)- and (1*S*,2*S*)-configurations to the isolated product **4** and substrate **2**, respectively.

On the other hand, the enzymatic resolution of diamine (±)-**3** was performed by means of an acetylation catalyzed by the lipase B from *Candida antarctica* (CAL-B), since the efficiency of this enzyme has widely been demonstrated for the resolution of primary amines.[16] In our first attempt, we tested the simplest reaction conditions, that is, we used ethyl acetate as the acyl donor and solvent (Table 1, entry 1). However, under these conditions, the enzyme catalyzed the acetylation of the diamine but the enantioselectivity was moderate-low ($E = 28$). When the process was conducted at a lower temperature ($T = 10$ °C), the reaction was slower, but contrary to what we expected, that is, the enantioselectivity decreased.

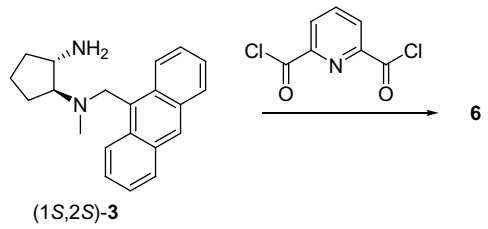
The use of racemic 1-phenylethyl acetate as an acyl donor in aminolysis reactions has proven to have a beneficial effect on the enantioselectivity of the CAL-B.[17] For this reason, we tested this acyl donor in combination with *tert*-butyl methyl ether (TBME) as a solvent for the resolution of (±)-**3** (Table 1, entry 3). As expected, in this case the enantioselectivity increased ($E = 45$), which allowed us to obtain the remaining substrate (1*S*,2*S*)-**3** with very high enantiomeric excess (ee) with a conversion near to 50%. The ees of both compounds isolated from the enzymatic reaction were determined by HPLC with a chiral column (see Section 4). The (1*R*,2*R*)-configuration for the product acetamide **5**, and thus, (1*S*,2*S*)-configuration for the remaining diamine **3** were established by taking into account the enantiopreference observed for CAL-B in

Table 1
CAL-B-catalyzed resolution of (±)-**3**[a]



Entry	Acyl donor (AcOR)	Solvent	T (°C)	Time (h)	(1*S*,2*S*)-**3**		(1*R*,2*R*)-**5**		c[c](%)	E[d]
					Yield[b] (%)	ee_S (%)	Yield[b] (%)	ee_P (%)		
1	AcOEt	AcOEt	28	4	47	40	27	90	31	28
2	AcOEt	AcOEt	10	13	59	12	8	82	13	12
3	AcOCH(Ph)CH_3[e]	TBME	28	60	42	95	48	85	52	45

[a] All the reactions were conducted at 200 rpm.
[b] Isolated yields after flash chromatography.
[c] Conversion degree: $c = ee_S/(ee_S + ee_P)$.
[d] Determined from ee_S and ee_P as in Ref. 13.
[e] A molar ratio ester–diamine of 3:1 was used.



Scheme 3. Synthesis of the optically active bis(aminoamide) **6**.

the acylation of analogous *trans*-cyclopentane-1,2-diamines,[6] and in general, in the acylation of primary amines where the stereogenic centre is the Cα.[14] In all cases, the enzyme catalyzed the acylation of the (*R*)-enantiomer, thus following Kazlauskas' rule.

In order to obtain the required bis(aminoamide), optically active diamine (1*S*,2*S*)-**3** (ee = 95%) was treated with pyridine-2,6-dicarbonyl dichloride (Scheme 3). Thus, compound **6** was isolated in 80% yield. As our previous results with analogous bis(aminoamides) indicated that a pincer-like conformation was necessary for the efficiency of the compound as a CSA,[7,9] we studied the solution structure of **6**. Once the assignation of the most representative ^{1}H NMR signals was achieved, a full set of 1D NOESY experiments was performed. The most significative results were obtained upon irradiation of the amide NH, which produced NOEs on protons of both substituents on the tertiary amino group, in good agreement with the major presence of the intended conformation in solution (Fig. 3).

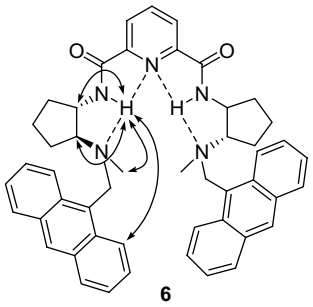


Figure 3. Selected NOEs observed upon irradiation of the amide proton signal of **6**.

Afterwards, we tested the ability of **6** as a chiral solvating agent for some carboxylic acids (Fig. 4). The experiments were carried out by mixing, in the NMR tube, equimolecular amounts of **6** and the carboxylic acid in $CDCl_3$, the final concentration of each component was 10 mM. All the ^{1}H NMR spectra were acquired in a 400 MHz spectrometer at room temperature. In Table 2 the variations of chemical shift ($\Delta\delta$), of the signals of the carboxylic acid experiment in presence of **6**, as well as the splitting between signals corresponding to each enantiomer of the acids ($\Delta\Delta\delta$) are shown.

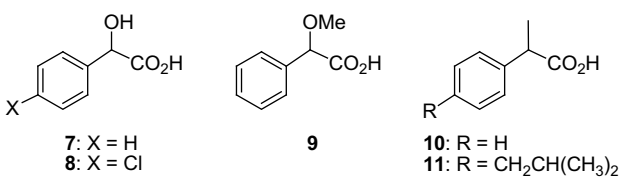


Figure 4. Selected carboxylic acids.

In the case of mandelic acid **7** and its analogous **8**, the signals of the CαH protons of the acids suffer strong shielding ($\Delta\delta$ = −0.58 and −0.78 ppm), which implies an effective deprotonation of the carboxylic group, and so the formation of the complex acid-bisaminoamide. Proof of this is the high efficiency of **6** as CSA with these acids, for which great splitting ($\Delta\Delta\delta$ > 56 Hz) was obtained. However, for the other acids the signals of the CαH experiment suffer much less shielding, and so the splittings obtained were very low. Moreover, the similar shielding observed for other signals of the acids (see Table 2, entries 4, 6 and 8) suggest that this shielding could be caused by the proximity of these to the aromatic groups of the bis(aminoamide), and not due to the transference of the proton between the acid and **6**. This could explain the lower splitting observed for acids **9**–**11** as a consequence of the weaker interaction between the acids and **6**.

Table 2
Induced shift ($\Delta\delta$, ppm) and splitting ($\Delta\Delta\delta$, Hz) for the ^{1}H NMR signals of the carboxylic acids in the presence of **6**[a]

Entry	Acid	Signal	$\Delta\delta$[b] (ppm)	$\Delta\Delta\delta$ (Hz)
1	**7**	CαH	−0.58	73.1
2	**8**	CαH	−0.78	56.3
3	**9**	CαH	−0.16	8.8
4	**9**	OMe	−0.13	9.8
5	**10**	CαH	−0.03	1.9
6	**10**	Me	−0.03	2.6
7	**11**	CαH	−0.03	2.2
8	**11**	Me	−0.03	3.5

[a] Spectra were acquired in a 400 MHz spectrometer.
[b] $\Delta\delta = \delta$ averaged between signals of both enantiomers—δ of the free acid.

On the other hand, the $\Delta\delta$ observed for the signals of the bis(aminoamide) **6** in the presence of the different acids also corroborate that protonation only happens with the mandelic type acids. Thus, in the presence of acids **7** and **8**, the signals of the protons close to the tertiary amino group were strongly de-shielded [$\Delta\delta$ > 1.2 (NCH) and >0.5 ppm (NCH_2)], which is in contrast with the weak de-shielding that the same protons encounter in the presence of acids **10** and **11** ($\Delta\delta$ < 0.1 ppm).

From comparison of the results obtained with **6** acting as CSA (Table 2) with those previously reported with the bis(aminoamide) **1** (see Fig. 1 and Ref. 7), it can be seen that compound **6** is a much more efficient CSA for the mandelic type acids (with **1** $\Delta\Delta\delta$ < 17 Hz were obtained for acids **7** and **8**). However, **1** is a more efficient CSA for acids **9**–**11**, for which splitting greater than 25 Hz were achieved. We propose that when protonation takes place, the anthryl group causes a higher enantiodiscrimination than a phenyl group. However, the higher size of anthryl compared to phenyl avoids the fact that weak acids, such as the 2-arylpropionic acids, protonate the tertiary amino group of **6,** and so a weak complex is formed.

3. Conclusions

In conclusion, we have carried out the chemoenzymatic syntheses of both enantiomers of a *trans*-2-aminocyclopentanol and a *trans*-*N*,*N*-dialkylcyclopentano-1,2-diamine bearer of an anthracene unit. Moreover, from the diamine, we were able to prepare a bis(aminoamide) that showed a great efficiency as a CSA of mandelic type acids.

4. Experimental

4.1. General

Immobilized lipase from *Pseudomonas cepacia* (PSL-C, 783 U/g) recently classified as *Burkholderia cepacia* lipase was purchased from Amano Pharmaceutical Co. *Candida antarctica* lipase B (Novozyme 435, available immobilized on polyacrylamide, 7300 PLU/g) was gifted by Novo Nordisk Co. For the enzymatic reactions, ethyl acetate of spectrophotometric grade (stored with 4 Å molecular

sieves) and the commercially available anhydrous *tert*-butyl methyl ether, vinyl acetate and (±)-1-phenylethyl acetate were used. Melting points were taken on samples in open capillary tubes, and are uncorrected. IR spectra were recorded on an Infrared FT spectrophotometer using solutions in dichloromethane (for solids) or neat (for oils). ^{1}H NMR and proton-decoupled ^{13}C NMR spectra ($CDCl_3$ solutions) were obtained using AC-300 or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), AV-400 MHz, and AV-600 MHz (for 1D-NOESY experiments) spectrometers using the δ scale (ppm) for chemical shifts; calibration was made on the $CDCl_3$ (^{13}C; 76.95 ppm) or the residual $CHCl_3$ (^{1}H; 7.26 ppm) signals.

4.2. Synthesis of (±)-*trans*-2-[(9-anthrylmethyl)(methyl)-amino]cyclopentanol (±)-2

1-(9-Anthryl)-*N*-methylmethanamine (9.04 mmol, 2.00 g) was added to a sealed tube with a solution of cyclopentene oxide (10.0 mmol, 870 μL) in deoxygenated ethanol (25 mL). After 40 h heating at 110 °C, the solvent was evaporated under reduced pressure and the racemic amino alcohol (±)-**2** was purified by flash chromatography (a gradient of 1:1 to 0:1 hexane–ethyl acetate was used as eluent). Yield, 92%; IR: 3417, 1634 cm^{-1}; ^{1}H NMR: δ = 1.42–1.58 (m, 1H), 1.62–2.03 (m, 5H), 2.16 (br s, 1H, OH), 2.21 (s, 3H, N–CH_3), 2.91 (q, ^3J = 7.8 Hz, 1H), 4.16 (q, ^3J = 7.8 Hz, 1H), 4.56 (s, 2H, N–CH_2), 7.43–7.57 (m, 4H), 8.01 (d, ^3J = 8.1 Hz, 2H), 8.41 (s, 1H), 8.48 (d, ^3J = 8.1 Hz, 2H); ^{13}C NMR: δ = 20.2 (CH_2), 22.6 (CH_2), 31.8 (CH_2), 36.9 (CH_3), 51.9 (CH_2), 72.9 (CH), 73.8 (CH), 124.66 (CH), 124.72 (CH), 125.6 (CH), 127.4 (CH), 129.0 (CH), 130.2 (C), 131.2 (C), 131.4 (C). MS (ESI), *m/z* (%) = 306 [(M+H)$^+$, 100], 328 [(M+Na)$^+$, 30]. HRMS (ESI) calcd for $C_{21}H_{24}NO$ [(M+H)$^+$]: 306.1852; found: 306.1851.

4.3. Synthesis of (±)-*trans*-*N*-(9-anthrylmethyl)-*N*-methyl-cyclopentane-1,2-diamine (±)-3

Compound (±)-**2** (4.60 mmol, 1.40 g) was dissolved in anhydrous diethyl ether (19 mL), and triethylamine (13.7 mmol, 1.9 ml) was added. The solution was cooled to 0 °C, and mesyl chloride (5.5 mmol, 365 μl) was added dropwise. A white precipitate was formed that made stirring difficult. The reaction mixture was allowed to warm to room temperature. After 60 min, triethylamine (9.20 mmol, 1.3 mL) and concd aq NH_3 (11 mL) were added, and the resulting two-phase reaction mixture was vigorously stirred for 16 h. The layers were separated, and the aqueous layer was extracted with Et_2O (4 × 20 mL). The combined organic layers were washed with brine (25 mL), dried over Na_2SO_4 and evaporated under reduced pressure to give the crude product (±)-**3**, which was purified by flash chromatography (the following eluents were successively used: hexane–ethyl acetate 6:1; ethyl acetate and ethyl acetate–methanol 2:1). Yellow foam, yield 67%. IR: 3423, 1644 cm^{-1}; ^{1}H NMR: δ = 1.35 (m, 1H), 1.65 (m, 2H), 1.78–1.98 (m, 3H), 2.21 (s, 3H, N–CH_3), 2.36 (br s, 2H, NH_2), 2.73 (q, ^3J = 8.7 Hz, 1H, C*H*–NH_2), 3.17 (q, ^3J = 8.7 Hz, 1H, CH–N), AB system (δ_A = 4.57, δ_B = 4.53, $|^2J_{A,B}|$ = 13.5 Hz), 7.38–7,53 (m, 4H), 7.99 (dd, 2H, ^3J = 8.1 Hz, $|^4J|$ = 0.6 Hz), 8.40 (s, 1H), 8.48 (d, ^3J = 8.7 Hz); ^{13}C NMR: δ = 20.6 (CH_2), 21.1 (CH_2), 32.1 (CH_2), 35.8 (CH_3), 52.0 (CH_2), 53.6 (CH–NH_2), 72.6 (CH–N), 124.65 (CH), 124.74 (CH), 125.4 (CH), 127.2 (CH), 128.9 (CH), 130.5 (C), 131.1 (C), 131.3 (C). MS (EI), *m/z* (%) = 304 (M$^{\cdot+}$, 9), 220 (22), 191 (100). HRMS (EI) calcd for $C_{21}H_{24}N_2$ (M$^{\cdot+}$): 304.1939; found: 304.1942.

4.4. Enzymatic acetylation of (±)-2

To a mixture of PSL-C (165 mg) and 4 Å molecular sieves (50 mg) under a nitrogen atmosphere, TBME (10 mL), (±)-**2** (1.64 mmol, 500 mg) and vinyl acetate (4.90 mmol, 450 μL) were added. The resulting mixture was shaken at 28 °C and 200 rpm for 5 h. Afterwards, the solid was filtered through Celite® and washed with methanol. Evaporation of the solvents and subsequent flash chromatography of the residue (hexane–ethyl acetate 3:1 as eluent) yielded pure β-amino ester (1*R*,2*R*)-**4** and β-amino alcohol (1*S*,2*S*)-**2**.

4.4.1. (1*S*,2*S*)-2-[(9-Anthrylmethyl)(methyl)amino]-cyclopentanol (1*S*,2*S*)-2

Yield, 43%; mp 99–100 °C; $[\alpha]_D^{20}$ = +56.4 (*c* 0.5, $CHCl_3$), ee = 99%.

4.4.2. (1*R*,2*R*)-2-[(9-Anthrylmethyl)(methyl)amino]-cyclopentyl acetate (1*R*,2*R*)-4

Yield, 47%; yellow oil; $[\alpha]_D^{20}$ = −52.5 (*c* 1.0, $CHCl_3$), ee = 98%; IR: 1732, 1244 cm^{-1}; ^{1}H NMR: δ = 1.55–1.90 (m, 4H), 1.98–2.20 [m + 2s, 8H. Singlets correspond to CH_3–CO (1.98 ppm) and N–CH_3 (2.18 ppm)], 3.25 (q, ^3J = 7.8 Hz, 1H), 4.54 (s, 2H, N–CH_2), 5.41 (m, 1H), 7.49 (m, 4H), 8.00 (d, ^3J = 8.3 Hz, 2H), 8.41 (s, 1H), 8.51 (d, ^3J = 8.8 Hz, 2H); ^{13}C NMR: δ = 21.3 (CH_3), 21.8 (CH_2), 26.1 (CH_2), 31.5 (CH_2), 37.4 (CH_3), 51.6 (CH_2), 70.7 (CH), 76.5 (CH), 124,7 (CH), 124.9 (CH), 125.5 (CH), 127.3 (CH), 128.9 (CH), 130.1 (C), 131.2 (C), 131.3 (C), 170.7 (C). MS (ESI), *m/z* (%) = 348 [(M+H)$^+$, 100]. HRMS (ESI) calcd for $C_{23}H_{26}NO_2$ [(M+H)$^+$]: 348.1957; found: 348.1962.

4.5. Enzymatic acetylation of (±)-3

To a mixture of diamine (±)-**3** (1.31 mmol, 400 mg) and CAL-B (131 mg) under a nitrogen atmosphere, TBME (6.5 mL) and 1-phenylethyl acetate (4.00 mmol, 0.64 mL) were added. Then, the mixture was shaken at 28 °C and 200 rpm for 60 h. The enzyme was subsequently filtered through Celite® and was washed with methanol. Solvents were eliminated and the resulting residue was dissolved in dichloromethane (20 mL), after which this solution was washed with 3 M aqueous NaOH (2 × 15 mL). Elimination of the solvents and subsequent flash chromatography of the residue (the following eluents were successively used: hexane–ethyl acetate 1:1; ethyl acetate and ethyl acetate–methanol 4:1) yielded pure aminoamide (1*R*,2*R*)-**5** and diamine (1*S*,2*S*)-**3**.

4.5.1. (1*S*,2*S*)-3

Yield, 42%; yellow foam; $[\alpha]_D^{20}$ = +69.5 (*c* 0.50, $CHCl_3$), ee = 95%.

4.5.2. (1*R*,2*R*)-5

Yield, 48%; yellow solid, mp 176–177 °C; $[\alpha]_D^{20}$ = −39.9 (*c* 0.50, $CHCl_3$), ee = 85%; ^{1}H NMR: δ = 1.05 (m, 1H), 1.45–1.85 [m + s, 7H. Singlet corresponds to CH_3–CO (1.64 ppm)], 2.12 (m, 1H), 2.38 (s, 3H, N–CH_3), 2.63 (q, ^3J = 8.5 Hz, 1H), 3.95 (quintet, ^3J = 8.5 Hz, 1H), AB system (δ_A = 4.50, δ_B = 4.62, $|^2J_{A,B}|$ = 13.5 Hz, N–CH_2), 5.03 (br s, 1H), 7.48 (m, 4H), 8.01 (d, ^3J = 7.6 Hz, 2H), 8.42 (s, 1H), 8.46 (d, ^3J = 9.0 Hz, 2H); ^{13}C NMR: δ = 20.4 (CH_2), 23.1 (CH_3), 29.6 (CH_2), 30.1 (CH_2), 36.6 (CH_3), 51.3 (CH_2), 51.8 (CH), 67.5 (CH), 124.8 (CH), 124.9 (CH), 125.6 (CH), 127.5 (CH), 129.1 (CH), 130.3 (C), 131.1 (C), 131.4 (C),170.2 (C). MS (EI), *m/z* (%) = 346 (M$^{\cdot+}$, 6), 287 (5), 220 (22), 208 (40), 191 (100). HRMS (EI) calcd for $C_{23}H_{26}N_2O$ (M$^{\cdot+}$): 346.2045; found: 346.2044.

4.6. Synthesis of bis(aminoamide) 6

The diamine (1*S*,2*S*)-**3** (0.63 mmol, 193 mg) and pyridine-2,6-dicarbonyl dichloride (0.31 mmol, 65 mg) were dissolved in 4 mL of dry CH_2Cl_2 under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 20 h, after which the mixture was extracted with 3 M aqueous NaOH. The organic layer was dried and evaporated, and the product was purified by flash chromatography (a gradient of hexane–ethyl acetate 3:1 to 1:1 was used as eluent). Yield, 80%; yellow foam; $[\alpha]_D^{20}$ = +141.5 (*c* 1.0, $CHCl_3$), ee = 95%;

^{1}H NMR: δ = 0.88 (m, 2H) 1.42 (m, 2H), 1.62 (m, 4H), 1.80 (m, 2H), 2.0–2.18 (m + s, 8H. Singlet at 2.08 ppm corresponds to 2N–CH_3), 2.51 (bq, ^3J = 8.1 Hz, 2H), 4.22–4.38 (m + d, 4H. Doublet at 4.31 ppm corresponds to 2NC*H*H, $|^2J|$ = 11.2 Hz), 4.44 (d, $|^2J|$ = 11.2 Hz, 2H, 2NCH*H*), 6.75 (br d, 2H, 2NH), 7.02 (t, ^3J = 6.6 Hz, 4H), 7.18 (m, 4H), 7.82 (d, ^3J = 8.1 Hz, 4H), 8.05 (t, ^3J = 7.8 Hz, 1H), 8.22 (d, ^3J = 8.1 Hz, 4H), 8.27 (s, 2H), 8.31 (d, ^3J = 7.8 Hz, 2H); ^{13}C NMR: δ = 20.4 (CH_2), 29.6 (CH_2), 37.3 (CH_3), 49.3 (CH_2), 50.9 (CH), 68.9 (CH), 124.1 (CH), 124.4 (CH), 124.5 (CH), 125.2 (CH), 127.2 (CH), 128.5 (CH), 130.9 (C), 138.4 (CH), 148.6 (C), 163.0 (C). MS (EI), *m/z* (%) = 739 ($M^{\cdot+}$, 6), 548 (36), 191 (100). HRMS (EI) calcd for $C_{49}H_{49}N_5O_2$ ($M^{\cdot+}$): 739.3886; found: 739.3893.

4.7. Determination of the enantiomeric excesses

Enantiomeric excesses of all the optically active compounds isolated from the enzymatic processes were determined by HPLC using a chiral column: (1*S*,2*S*)-**2** and (1*R*,2*R*)-**5** were analyzed directly; (1*R*,2*R*)-**4** was previously saponified (1 N NaOH in MeOH) to the amino alcohol; (1*S*,2*S*)-**3** was transformed into its *N*-Boc derivative **12** [$(Boc)_2O$ in MeOH].

(±)-**2**: Chiralcel OD column, t_R 13.8 (1*S*,2*S*) and 16.3 (1*R*,2*R*) min, Rs 1.5 (hexane–propan-2-ol 90:10, 0.8 mL/min, 20 °C).

(±)-**5**: Chiralcel OD column, t_R 11.2 (1*S*,2*S*) and 16.7 (1*R*,2*R*) min, Rs 3.3 (hexane–ethanol 90:10, 0.8 mL/min, 20 °C).

(±)-**12**: Chiralcel OD column, t_R 39.3 (1*R*,2*R*) and 46.6 (1*S*,2*S*) min, Rs 1.1 (hexane–propan-2-ol 99:1, 0.8 mL/min, 20 °C).

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