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Enzymatic resolution of *N*-protected-β³-amino methyl esters, using lipase B from *Candida antarctica*

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Abstract—Racemic β³-amino methyl esters bearing the amine function protected with Bz, Cbz, Boc, Fmoc and as aminobenzamide, were resolved by enantiospecific transesterifications catalyzed by lipase B from *Candida antarctica*. The reactions proceeded with a high conversion and yielded enantiomerically pure enantiomers.

1. Introduction

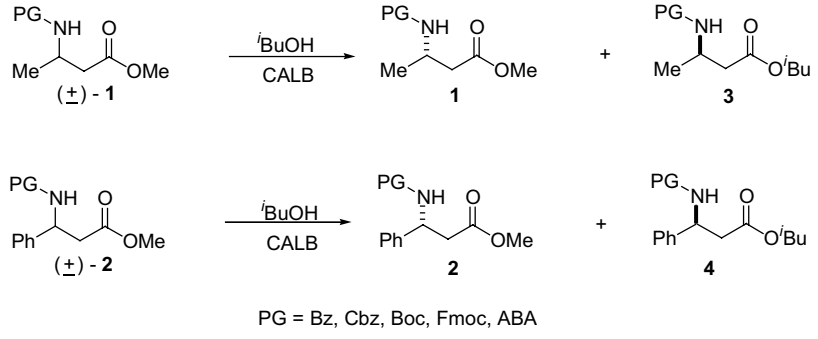
Due to the wide range of applications,[1] the preparation of optically active β³-amino acids is an area of current interest.[2] However, only few enzymatic routes to these compounds have been described.[3] Indeed, few enzymes show activity and stereoselectivity in the presence of β³-amino acids, and it becomes even more difficult if the reactive site is the carboxy function, because the stereocenter is remote from that.[4]

Here we report the enzymatic resolution of methyl 3-aminobutanoate (±)-**1** and methyl 3-amino-3-phenylpropanoate (±)-**2** derivatives, with the amine protected with groups such as Bz, Cbz, Boc, Fmoc and as aminobenzamide (ABA) (Scheme 1). The resolutions were carried out through transesterifications catalyzed by lipase B from *Candida antarctica* (CALB).[5]

2. Results and discussion

2.1. Synthesis of *N*-protected methyl 3-aminobutanoate (±)-1a–e and methyl 3-amino-3-phenylbutanoate derivatives (±)-2a–e

The *N*-protected-β³-amino methyl esters (±)-**1a**–**d** and (±)-**2a**–**d** were obtained from the corresponding racemic



Scheme 1.

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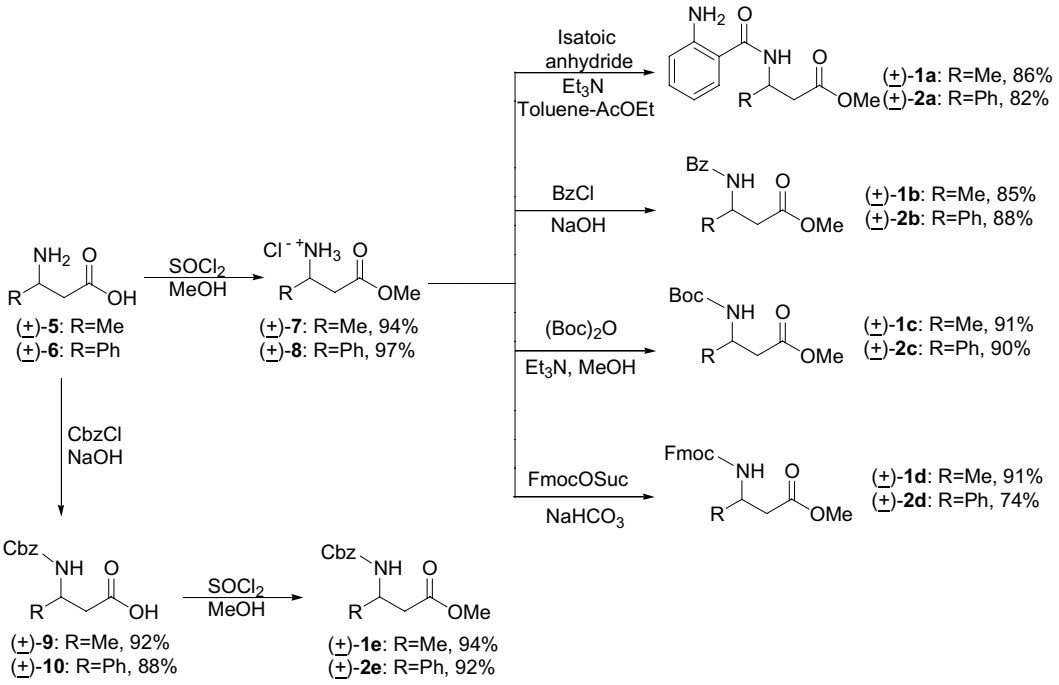


Scheme 2.

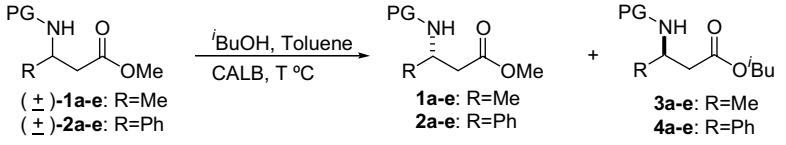
β^3-amino acids (±)-**5** and (±)-**6** (Scheme 2). For compounds (±)-**1e** and (±)-**2e**, we first protected the amine with CbzCl to obtain (±)-**9** and (±)-**10** and then prepared the methyl esters.

2.2. Transesterification of methyl 3-aminobutanoate (±)-1a–e and methyl 3-amino-3-phenylbutanoate (±)-2a–e derivatives, catalyzed by CALB

The enzymatic transesterifications were performed in toluene, using isobutyl alcohol, in presence of CALB. The reactions were monitored by GC and the results are described in Table 1.

At 60 °C we observed slow reactions for compounds (±)-**2a**–**e** where R = Ph (entries 6–10), and after time >192 h, conversion did not reach 50%. This might be due to the steric effect exerted by the Ph group. However, we found that the enzyme could present selectivity against only one of the enantiomers, since, after isolation, we determined the specific rotations of products (+)-**4a**–**e**. In the case of compounds (±)-**1a** and **b**,where R = Me

Table 1. Transesterification of (±)-**1a**–**e** and (±)-**2a**–**e**, catalyzed by CALB



Entry	Product	R	PG	T (°C)	Time (h)	Conversion[a] (%)	Ee[b] (%)	E[c]	$[\alpha]_D$[d]	Absolute configuration[e]
1	**3a**	Me	ABA	60	7	50	>98	>458	+53.4, c = 1.10	*R*
2	**3b**	Me	Bz	60	9	50	>98	>458	+34.9, c = 0.75	*R*
3	**3c**	Me	Boc	45	0.75	50	>98	>458	+19.7, c = 0.60	*R*
4	**3d**	Me	Fmoc	45	0.75	50	>98	>458	+14.9, c = 1.01	*R*
5	**3e**	Me	Cbz	45	0.75	50	>98	>458	+14.6, c = 1.03	*R*
6	**4a**	Ph	ABA	60	240	32	>98	>14	+18.3, c = 0.71	*S*
7	**4b**	Ph	Bz	60	200	30	>98	>13	+22.8, c = 0.70	*S*
8	**4c**	Ph	Boc	60	192	43	>98	>37	−21.1, c = 1.02	*S*
9	**4d**	Ph	Fmoc	45	240	14	>98	>8	−8.2, c = 0.8	*S*
10	**4e**	Ph	Cbz	60	240	28	>98	>12	−9.6, c = 1.00	*S*

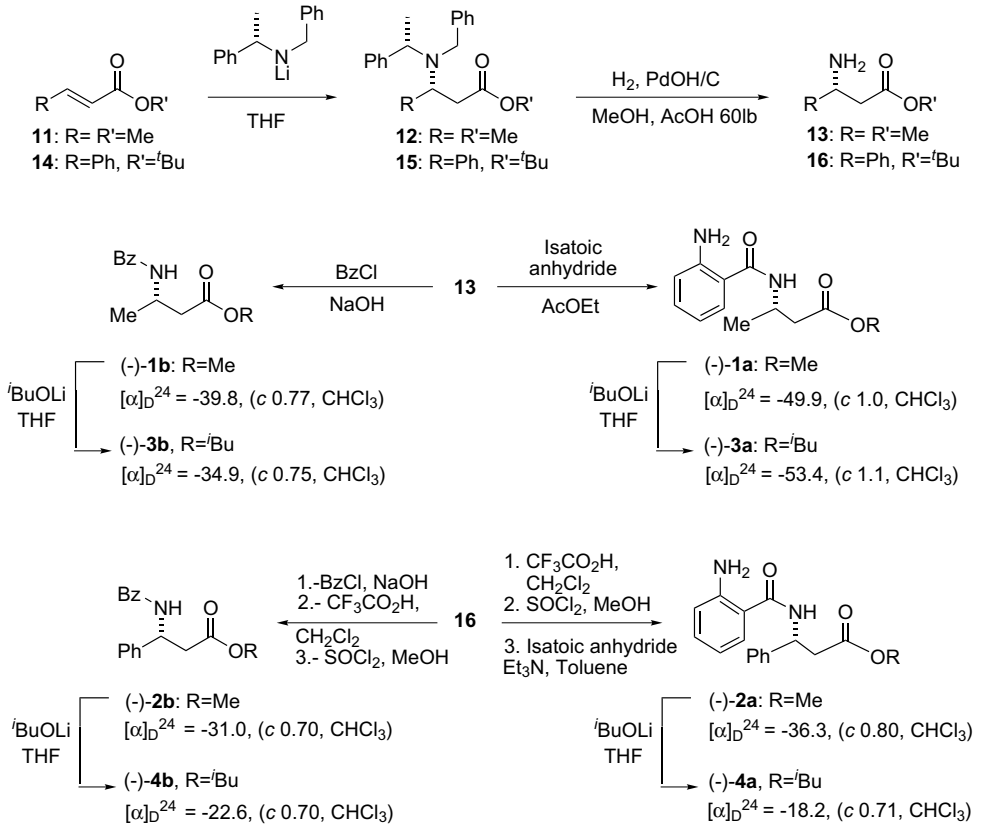
[a] Determined by GC, using a Megabore column, HP 1, 5 m; for PG = ABA, Bz, Boc, Cbz, 140 °C for 1 min → 180 °C (T rise 5 °C/min); for PG = Fmoc, 240 °C for 1 min → 280 °C (T rise 5 °C/min).

[b] Determined by ^{1}H NMR using europium tris[3-(heptafluropropylhydroximethylen)-(+)-camphorate as chiral shift reagent.

[c] The E values were calculated from $E = \ln[(1 - c)(1 - \text{ee})]/\ln[(1 - c)(1 + \text{ee})]$.

[d] Specific rotations were measured with a Perkin–Elmer 341 polarimeter, using $CHCl_3$ as solvent.

[e] Assigned by chemical correlation.



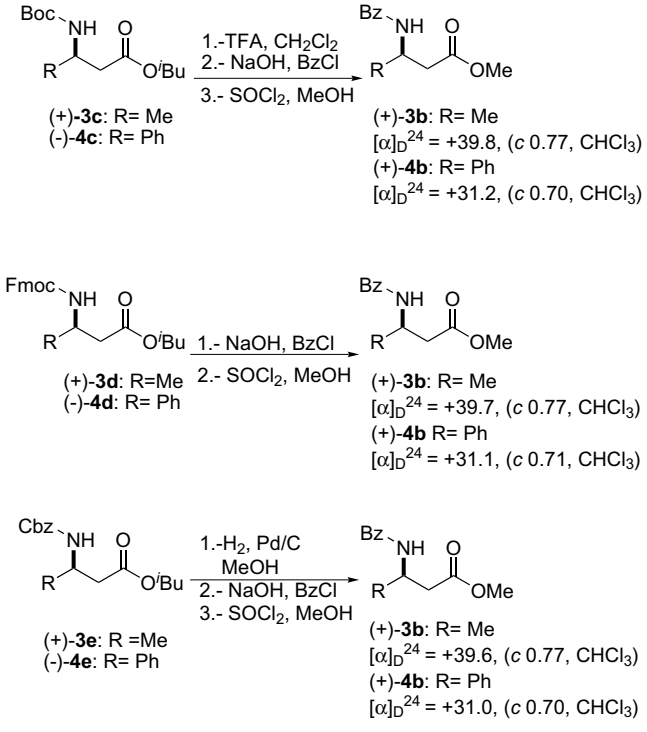
Scheme 3.

(entries 1 and 2), at 60 °C the reactions occurred faster than (±)-**2a**–**e**, and 50% conversion was detected. For compounds (±)-**1c**–**e** (entries 3–5), transesterifications were performed at 45 °C, for 0.75 h at 50% conversion. Products (+)-**3a**–**e** were also optically active.

In order to determine the enantiomeric excesses and the absolute configurations of (+)-**3a** and **b** and (+)-**4a** and **b**, we prepared compounds (−)-**3a** and **b**, and (−)-**4a**, **b** of absolute configuration *S* and *R*, respectively, through the asymmetric synthesis described by Davies and co-workers (Scheme 3).[6] We compared the specific rotations values and in all cases we observed that they were in agreement. On the other hand, the absolute configuration of these compounds was assigned by comparing the sign of their specific rotations; so for (+)-**3a** and **b** is *R* and for (+)-**4a** and **b** is *S*.

The enantiomeric excesses were also determined by ^{1}H NMR, using europium tris[3-(heptafluropropylhydroximethylen)-(+)-camphorate as chiral shift reagent.[7] After the addition of the chiral shift reagent to a solution of (+)-**3a**,**b** and (+)-**4a**,**b** only one diastereoisomeric complex was detected, for each case, confirming the ee to be >98%.

The enantiomeric excesses and the absolute configurations of compounds (+)-**3c**–**e** and (−)-**4c**–**e** were determined by transforming them into *N*-benzoyl-β^3-amino methyl esters (Scheme 4), and then comparing their specific rotations with the ones from enantiomerically pure (−)-**1b** and (−)-**2b**. So, for (+)-**3c**–**e** the absolute configuration is *R*, with ee >98%, and for (−)-**4c**–**e** the absolute configurations is *S*, with ee >98%. As we can see



Scheme 4.

in Scheme 4, CALB was selective for only those enantiomers where the *N*-protected group is pointing toward the observer.[8]

We determine the *E* values for the enzymatic transesterifications finding excellent values ($E > 458$) for (+)-**3a**–**e**, while for (+)-**4a**–**b**, (−)-**3c**–**e**, they were low ($E < 14$).

3. Conclusions

We have reported a new enzymatic method to resolve *N*-protected-β^3-amino methyl ester through transesterification catalyzed by CALB. Despite the stereocenter being remote from the reactive site of the molecules, we observe that CALB presents selectivity for only one enantiomer, to yield enantiomerically pure compounds with a high conversion. The esters resolved can be suitable for β-peptide synthesis in solid phase,[9] or solution techniques;[10] furthermore, after hydrolysis, they would yield to the corresponding β^3-amino acids in enantiomerically pure form.

4. Experimental section

4.1. General

All reagents were purchased from commercial suppliers and used without further purification. Toluene and THF were distilled from sodium. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Gemini 200 or Inova 400 spectrometer and were recorded relative to TMS as internal standard. Microanalyses were performed in Elementar Vario EL III. Optical rotations: 10 cm, 1 mL cell, Perkin–Elmer-341 polarimeter. Melting points were measured in open glass capillaries with a Büchi apparatus and are uncorrected. Conversion was determined by GC, using a Megabore column, HP 1, 5 m; for PG = A-BA, Bz, Boc, Cbz, 140 °C for 1 min → 180 °C (temperature rise 5 °C/min); for PG = Fmoc, 240 °C for 1 min → 280 °C (temperature rise 5 °C/min).

4.2. Synthesis of *N*-protected-β^3-amino methyl ester

N-protected-β^3-amino methyl esters (±)-**1b**–**e** and (±)-**2b**–**e**, were prepared by standard methods.[11] Only preparation and properties of new compounds are described afterwards.

4.2.1. (*rac*)-Methyl 3-(2-aminobenzamido)butanoate, (±)-1a and (*rac*)-methyl 3-(2-aminobenzamido)-phenylpropanoate, (±)-2a. A suspension of methyl esters hydrochlorides **7** and **8** (8 mmol) was treated with triethylamine (9.6 mmol, 1.2 equiv) and isatoic anhydride (8.8 mmol, 1.1 equiv), in a mixture of toluene/ethyl acetate (1:1). The reaction mixture was warmed to 50 °C for 2 h. The triethylamine hydrochloride was removed by filtration. The solvent from the filtrate was removed under reduced pressure and the crude product purified by FC.

4.2.1.1. (*rac*)-Methyl 3-(2-aminobenzamido)butanoate (±)-1a. Yield: 86%. White solid, mp 92–93 °C, ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (3H, d, $J = 6.9$ Hz), 2.62 (2H, d, $J = 5.2$ Hz), 3.70 (3H, s), 4.40–4.60 (1H, m), 5.50 (2H, b), 6.59–6.66, (2H, m) 6.76 (1H, b), 7.18 (1H, $J_{meta} = 1.4$ Hz, $J_{ortho} = 8.1$ Hz), 7.31 (1H, d, $J_{ortho} = 8.4$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.5, 40.1, 42.2, 52.0, 116.1, 116.7, 117.3, 127.2, 132.2, 148.7, 168.3, 172.2. Anal. Calcd for $C_{12}H_{16}NO_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.73; H, 7.06; N, 10.86.

4.2.1.2. (*rac*)-Methyl 3-(2-aminobenzamido)-3-phenylpropanoate, (±)-2a. Yield: 82%. Yellow oil, ^{1}H NMR (200 MHz, $CDCl_3$) 2.97 (2H, t, $J = 5.4$ Hz), 3.63 (3H, s), 5.51–5.61 (3H, m), 6.62–6.70 (4H, m), 7.06–7.44, (6H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 40.2, 49.8, 52.2, 115.4, 116.7, 117.4, 126.1, 127.3,127.6, 128.7, 132.5, 140.6, 149.0, 168.3, 171.6. Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.00; H, 6.23; N, 8.86.

4.2.2. (*rac*)-Methyl 3-[(9*H*-fluren-9-ylmethoxycarbonyl)-3-phenylpropanoate, (±)-2d. Yield: 74%. Methyl ester hydrochloride **8** (5 mmol) was dissolved in 20 ml of distilled H_2O and cool down to 0 °C then $NaHCO_3$ 1.0 M was added (20 mmol, 4 equiv) and the reaction was stirred for 0.3 h. Then, FmocSuc (6 mmol, 1.2 equiv) in acetone. The reaction continued for 3 h. After this time the acetone was evaporated, and reaction mixture extracted with CH_2Cl_2 (3 × 50 ml). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by FC (hexane:ethyl acetate 90:10 → 70:30) to produce (±)-**2d** as a white solid, mp 128 °C, ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.95 (2H, b), 3.88 (3H, s), 4.26 (1H, t, $J = 6.5$ Hz), 4.46 (2H, d, $J = 7.4$ Hz), 5.22 (1H, b), 5.83 (1H, b), 7.30–7.84 (13H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 47.6, 50.7, 52.2, 65.4, 67.1, 120.0, 124.8, 125.1, 126.1, 127.1, 127.6, 127.7, 128.8, 141.3, 146.8, 155.6, 171.5. Anal. Calcd for $C_{25}H_{23}NO_4$: C, 74.79; H, 5.77; N, 3.49. Found: C, 74.51; H, 6.00; N, 3.32.

4.3. General procedure for the enzymatic transesterifications of (±)-1a–e and (±)-2a–e

The enzymatic transesterifications were performed in closed, screw capped 8 ml reaction vessels containing 0.25 mmol of the corresponding *N*-protected-β^3-amino methyl esters, 0.5 ml (0.5 mmol) of anhydrous isobutyl alcohol, 160–200 mg of CALB (Novozym 435) and 7 ml of anhydrous toluene. The reactions were incubated in a thermostated water bath at 45 or 60 °C, and slowly stirred. In order to monitor the reaction progress, samples of 200 μl were withdrawn at different intervals, centrifuged to separate enzyme and analyzed by GC. To finish the reactions, the enzyme was recovered and washed with acetone. Solvent was removed under reduced pressure and the residues were purified by column chromatography on silica gel with hexane–ethyl acetate as eluent (90:10 → 70:30), to obtain *N*-protected-β^3-amino isobutyl esters **3a**–**e** and **4a**–**e**.

4.3.1. (*R*)-Isobutyl 3-(2-aminobenzamido)butanoate, (+)-3a. Yield after isolation 49%. Yellow solid, mp 68 °C, $[\alpha]_D^{24} = +53.1$ (*c* 1.10, $CHCl_3$), ^{1}H NMR (200 MHz,

$CDCl_3$) δ 0.93 (6H, d, $J = 6.9$ Hz), 1.32 (3H, d, $J = 6.6$ Hz), 1.94 (1H, m, $J = 6.6$ Hz), 2.63 (2H, d, $J = 5.2$ Hz), 3.89 (2H, d, 6.6 MHz), 4.41–4.61 (1H, m), 5.52 (2H, b), 6.58–6.66, (2H, m) 6.81 (1H, b), 7.18 (1H, $J_{meta} = 1.4$ Hz, $J_{ortho} = 8.1$ Hz), 7.31 (1H, d, $J_{ortho} = 8.4$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5, 20.5, 28.0, 40.2, 42.2, 71.1, 116.1, 116.7, 117.3, 127.2, 132.2, 148.7, 168.3, 171.9. Anal. Calcd for $C_{15}H_{22}NO_3$: C, 64.73; H, 7.97; N, 10.06. Found: C, 64.56; H, 7.97; N, 9.51.

4.3.2. (*R*)-Isobutyl 3-[(benzoyl)amino]butanoate, (+)-3b. Yield after isolation 49%. White crystals, mp 71–72 °C, $[\alpha]_D^{24} = +34.9$ (c 0.75, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (6H, d, $J = 6.9$ Hz), 1.34 (3H, d, $J = 6.6$ Hz), 1.94 (1H, m, $J = 6.6$ Hz), 2.65 (2H, dd, $J = 5.2$ Hz, $J = 15.0$ Hz), 3.90 (2H, d, 6.8 Hz), 4.49–4.66 (1H, m), 7.02 (1H, b), 7.35–7.54, (3H, m) 7.74–7.78 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5, 20.4, 28.0, 40.0, 42.6, 71.0, 166.3, 172.0. Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.51; H, 7.99; N, 5.23.

4.3.3. (*R*)-Isobutyl 3-[(*tert*-butoxycarbonyl)amino]butanoate, (+)-3c. Yield after isolation 47%. Colorless oil, $[\alpha]_D^{24} = +19.7$ (c 0.60, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (6H, d, $J = 7.0$ Hz), 1.21 (3H, d, $J = 6.8$ Hz), 1.43 (9H, s), 1.92 (1H, m, $J = 6.8$ Hz), 2.51 (2H, d, $J = 6.6$ Hz), 3.88 (2H, d, $J = 6.8$ Hz), 3.92–4.18 (1H, m), 4.95 (1H, b); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5, 20.9, 28.0, 41.1, 43.8, 70.9, 79.5, 154.9, 171.4. Anal. Calcd for $C_{13}H_{25}NO_4$: C, 60.21; H, 9.72; N, 5.40. Found: C, 59.72; H, 9.49; N, 5.21.

4.3.4. (*R*)-Isobutyl 3-[(9*H*-fluren-9-ylmethoxycarbonyl)-amino]butanoate, (+)-3d. Yield after isolation 49%. White crystals, mp 84 °C, $[\alpha]_D^{24} = +14.9$ (c 1.01, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92 (6H, d, $J = 6.6$ Hz), 1.25 (3H, d, $J = 6.6$ Hz), 1.92 (1H, m, $J = 6.6$ Hz), 2.54 (2H, d, $J = 5.6$ Hz), 3.87 (2H, d, 6.6 Hz), 4.1 (1H, m), 4.19 (1H, t, $J = 6.6$ Hz), 4.36 (2H, d, $J = 6.9$ Hz), 7.28 (2H, td, $J_{meta} = 1.6$ Hz, $J_{ortho} = 7.1$ Hz); 7.37 (2H, td, $J_{meta} = 1.2$ Hz, $J_{ortho} = 7.3$ Hz), 7.57 (2H, dd, $J_{meta} = 0.8$ Hz, $J_{ortho} = 7.4$ Hz) 7.73 (2H, d, $J_{ortho} = 7.1$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5, 20.8, 28.0, 40.8, 44.5, 47.5, 66.9, 70.9, 120.0, 125.1, 127.0, 127.7, 141.3, 143.9, 155.5, 171.4. Anal. Calcd for $C_{23}H_{27}NO_3$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.49; H, 7.18; N, 3.65.

4.3.5. (*R*)-Isobutyl 3-[(benzyloxycarbonyl)amino]butanoate, (+)-3e. Yield after isolation 48%. Colorless oil, $[\alpha]_D^{24} = +14.6$ (c 1.03, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92 (6H, d, $J = 7.0$ Hz), 1.24 (3H, d, $J = 6.6$ Hz), 1.91 (1H, m, $J = 6.6$ Hz), 2.53 (2H, d, $J = 6.6$), 3.85 (2H, d, 6.6 Hz), 4.04–4.17 (1H, m), 5.08 (2H, s), 5.20 (1H, b), 7.31–7.35, (5H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5, 20.8, 28.0, 40.8, 44.4, 66.9, 70.9, 128.1, 128.5, 136.5, 155.4, 171.8. Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.08; H, 8.02; N, 4.66.

4.3.6. (*S*)-Isobutyl 3-(2-aminobenzamido)-3-phenylpropanoate, (+)-4a. Yield after isolation 31%. Yellow oil, $[\alpha]_D^{24} = +18.3$ (c 0.71, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.81 (6H, d, $J = 6.8$ Hz), 1.82 (1H, m, $J = 6.6$ Hz), 2.97 (2H, dd, $J = 5.2$ Hz, $J = 14.5$ Hz), 3.80 (2H, d, 6.8 Hz), 5.51-5.61 (3H, m), 6.61–6.69 (4H, m), 7.15–7.45, (6H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.3, 27.91, 40.4, 49.9, 71.3, 115.4, 116.7, 117.4, 126.1, 127.3,127.6, 128.7, 132.5, 140.6, 149.0, 168.3, 171.6. Anal. Calcd for $C_{20}H_{24}N_2O_3$: C, 70.56; H, 7.11; N, 8.23. Found: C, 70.15; H, 7.06; N, 9.29.

4.3.7. (*S*)-Isobutyl 3-[(benzoyl)amino]-3-phenylpropanoate, (+)-4b. Yield after isolation 29%. White crystals, mp 70 °C, $[\alpha]_D^{24} = +22.8$ (c 0.70, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82 (6H, d, $J = 6.9$ Hz), 1.82 (1H, m, $J = 6.6$ Hz), 3.00 (2H, dd, $J = 5.4$ Hz, $J = 15.0$ Hz), 3.80 (2H, d, 6.6 Hz), 5.58–5.67 (1H, m), 7.02 (1H, b), 7.20–7.53 (8H, m), 7.58 (1H, b), 7.84–7.91 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.3 27.9, 40.1, 50.1, 71.3, 126.2, 127.1, 127.6, 128.6, 128.7, 131.6, 134.2, 140.5, 166.3, 171.7. Anal. Calcd for $C_{15}H_{21}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 71.71; H, 7.53; N, 3.68.

4.3.8. (*S*)-Isobutyl 3-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoate, (−)-4c. Yield after isolation 42%. Colorless oil, $[\alpha]_D^{24} = -21.1$ (c 1.02, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.83 (6H, d, $J = 6.6$ Hz), 1.41 (9H, s), 1.43 (9H, s), 1.82 (1H, m, $J = 6.8$ Hz), 2.84 (2H, t, $J = 4.4$ Hz), 3.78 (2H, d, $J = 6.6$ Hz), 5.08 (1H, b), 5.47 (1H, b), 7.21–7.31 (5H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.4, 27.9, 28.7, 41.3, 51.6, 71.0, 79.8, 126.1, 127.5, 128.6, 141.2, 154.9, 170.8. Anal. Calcd for $C_{18}H_{27}NO_4$: C, 67.26; H, 8.47; N, 4.36. Found: C, 66.98; H, 9.04; N, 4.02.

4.3.9. (*S*)-Isobutyl 3-[(9*H*-fluren-9-ylmethoxycarbonyl)-3-phenylpropanoate, (−)-4d. Yield after isolation 12%. Colorless oil, $[\alpha]_D^{24} = -8.2$ (c 0.8, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.83 (6H, d, $J = 7.0$ Hz), 1.82 (1H, m, $J = 6.6$ Hz), 2.89 (2H, d, $J = 5.2$ Hz), 3.79 (2H, d, 6.6 Hz), 4.19 (1H, t, $J = 7.0$ Hz), 4.39 (2H, d, $J = 7.2$ Hz), 5.16 (1H, b), 5.80 (1H, b), 7.24–7.41 (9H, m), 7.56 (2H, d, $J_{ortho} = 7.4$ Hz), 7.73 (2H, d, $J_{ortho} = 7.2$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.4, 27.9, 40.9, 47.6, 51.9, 67.1, 71.2, 120.0, 125.1, 126.1, 127.1, 127.7, 128.8, 141.3, 143.9, 155.5, 172.1.

4.3.10. (*S*)-Isobutyl 3-[(benzyloxycarbonyl)amino]-3-phenylpropanoate, (−)-4e. Yield after isolation 28%. Yellow oil, $[\alpha]_D^{24} = -9.6$ (c 1.00, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.1 (6H, d, $J = 7.0$ Hz), 1.80 (1H, m, $J = 6.6$ Hz), 2.87 (2H, d, $J = 5.4$ Hz), 3.77 (2H, d, 6.6 Hz), 5.10–5.21 (1H, m), 5.08 (2H, s), 7.27–7.31, (5H, m), 5.78 (1H, b); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.3, 20.8, 27.9, 41.0, 52.0, 67.0, 71.1, 126.1, 127.7, 128.1, 128.5, 128.7, 136.3, 140.7, 155.5, 170.7. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.58; H, 6.97; N, 3.06.

4.4. Procedure for the determination of the enantiomeric excess by NMR, using europium tris[3-(heptafluropropylhydroximethylen)-(+)-camphorate Eu(hfc)$_3$

In a typical experiment, racemic mixtures and resolved compounds (0.015 mmol) were weighed into a NMR tube and dissolved in 0.2 ml of $CDCl_3$. Then 0.1 ml of a solution of Eu(hfc)$_3$ (5 mM in $CDCl_3$) was added. The solutions were mixed and then ^{1}H NMR spectra recorded. More solution of Eu(hfc)$_3$ was added and the process repeated until the detection of the diastereoisomeric complexes.

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