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RESEARCH ARTICLE

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Synthesis, resolution, and absolute configuration determination of a vicinal amino alcohol with axial chirality. Application to the synthesis of new box and pybox ligands

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Abstract

New racemic vicinal amino alcohol derivatives with 4-benzylidenecyclohexane skeleton and axial chirality have been prepared. A preparatively easy and efficient protocol for resolution of the *N*-benzoylamino alcohol is described. Using a 250 × 20 mm (L × ID) Chiralpak® IA column, and the appropriate mixture of *n*-hexane/ethanol/chloroform as eluent, both enantiomers of *N*-benzoylamino alcohol **3** are obtained with >99% enantiomeric excess (ee) by successive injections of a solution of the racemic sample in chloroform. The obtained axially chiral vicinal amino alcohol is used to synthesize structurally novel bisoxazoline ligands in high yields.

KEYWORDS

axial chirality, bisoxazolines, chiral stationary phases, enantiomeric separation, vicinal amino alcohols

1 | INTRODUCTION

Chiral 1,2-amino alcohols are essential structural motifs widely found in natural and synthetic biologically active compounds displaying very diverse activities,[1,2] with a tremendous versatility in asymmetric synthesis as building blocks,[3] auxiliaries,[4] or ligands for metal catalyzed reactions[5] and organocatalysis.[6] In most cases, chirality of the 1,2-amino alcohol is due to the presence of a chiral center, but molecular dissymmetry is not restricted to the presence of a chiral center. Compounds featuring a chirality axis or plane are in fact of tremendous importance[7] and are present in many natural compounds,[8–10] ligands or catalysts for asymmetric synthesis, or synthetic intermediates.[11–13] Synthesis, properties, and applications of axially chiral atropoisomers (biphenyls and derivatives) and allenes have been widely studied. However, the preparation and use of chiral alkylidene cycloalkanes has been much less studied, in spite of the fact that alkylidene cycloalkanes are chiral compounds, fully stable at the stereogenic axis.

Considering the value of both chiral 1,2-amino alcohols and axially chiral compounds in asymmetric synthesis, we wondered about the potential application of new axially chiral 1,2-amino alcohols belonging to the alkylidene cycloalkane family.

Focusing on the synthesis and isolation of new α-amino acids and derivatives with unusual structural features,[14–16] we developed a high-performance liquid chromatographic (HPLC) resolution protocol[17] for the enantioseparation of unusual amino acid derivatives

containing a cyclohexylidene moiety on an analytical and semipreparative scale.[18–20] Now we wish to describe the synthesis and resolution of axially chiral 1,2-amino alcohols containing a 4-benzylidenecyclohexane moiety.

2 | MATERIALS AND METHODS

2.1 | General information

Unless otherwise specified, all reagents were obtained from commercial suppliers and used without purification. For anhydrous conditions, reactions were carried out under Ar in solvents dried using a solvent purification system (SPS). *n*-Hexane, ethanol, acetone, and chloroform used for HLPC separations were chromoscan grade from LabScan. Heating was performed using an oil bath mounted on a hot plate magnetic stirrer. Whenever possible, the reactions were monitored by TLC. TLC analysis was performed on precoated silica gel polyester plates with an F_{254} indicator, and products were visualized using ultraviolet (UV) light (254 nm) and ethanolic phosphomolybdic acid solutions followed by heating. Column chromatography was performed on silica gel (Kiesegel 60, 230–400 Mesh) with air pressure or alumina (Aluminum Oxide, 90 Neutral, 50-200 μm). Methyl 1-benzamido-4-oxocyclohexane-1-carboxylate 1 was prepared as previously described.[21]

2.2 | Instrumentation

HPLC separations were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode array detector, and an M-2487 dual wavelength absorbance detector, to monitor analytical and preparative separations, respectively. The chromatographic data were acquired and processed with Millennium® chromatography manager software (Waters). A Rheodyne 7125 syringe-loading sample injector was equipped with 20- and 500-μl loops, respectively, for analytical or semipreparative chromatography. Commercially available polysaccharide chiral stationary phases based on immobilized amylose tris(3,5-dimethylphenylcarbamate), Daicel Chiralpak® IA column, and immobilized cellulose tris (3,5-dimethylphenylcarbamate), Daicel Chiralpak® IB column, were used. Melting points were determined in open capillaries using a Gallenkamp capillary melting point apparatus and are not corrected. The FT-IR spectra of oils were recorded as thin films on NaCl plates; the FT-IR spectra of solids were recorded on pressed KBr pellets using a Thermo Nicolet Avatar 360 FT-IR spectrophotometer. Optical rotations were measured on a Jasco 1020 digital polarimeter at λ 589 nm and 25°C in cells with 1 or 10 cm path length. ^{1}H NMR and ^{13}C NMR spectra were acquired in deuterated solvents on a Bruker AV-400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR using the solvent residual resonance as the internal standard.[22] Spectra were acquired at room temperature unless otherwise stated using a 5-mm probe. High-resolution mass spectra were recorded from methanolic solutions on a Bruker Dalton MICROTOF-Q (quadrupole time-of-flight) microinstrument using the positive electrospray ionization mode (ESI^+).

2.3 | HPLC analytical assays

The HPLC analytical assays were carried out operating under isocratic conditions at room temperature on Chiralpak® IA and Chiralpak® IB 250 × 4.6 mm (L × ID) columns. Different binary and ternary mixtures of solvents were used as eluents. Samples were manually injected. The flow rate was 1 ml/min. The analyte concentration in injected solutions was 5 mg/ml, and the injection volume was 5 μl. Detection was performed at 250 nm. The capacity (k'), selectivity (α), and resolution (R_s) factors were calculated according to the equations $k' = (t_r - t_0)/t_0$, $a = k'_2/k'_1$, $R_s = 1.18(t_{r2} - t_{r1})/(W_{0.5h1} + W_{0.5h2})$. Subscripts 1 and 2 refer to the first and second eluted enantiomer, respectively; t_r are their retention times and $W_{0.5h}$ denote their full width to the half maximum of each peak; t_0 is the dead time.

2.4 | HPLC semipreparative assays

The HPLC semipreparative resolution of compound ***rac*-3** was carried out operating under isocratic conditions at room temperature on a 250 × 20 mm (L × ID) Chiralpak® IA column. A ternary mixture of *n*-hexane/ethanol/chloroform was used as the eluent. Injections and collections were made manually. The flow rate was 0.8 ml/min. The wavelength for UV detection was 270 nm. The column loading capacity, W_s (defined as the maximum sample mass that the column can hold), was experimentally calculated for the analytical 250 × 4.6 mm (L × ID) Chiralpak® IA column by injecting increasing amounts of sample with a concentration of 200 mg/ml.

2.4.1 | Methyl 1-benzamido-4-benzylidenecyclohexane-1-carboxylate (***rac*-2**)

To a stirred suspension of benzyltriphenylphosphonium chloride (7.77 g, 20 mmol) in dry THF (75 ml) under

argon at room temperature, potassium *tert*-butoxide (2.24 g, 20 mmol) was added and stirring was continued for 45 min. A solution of methyl 1-benzamido-4-oxocyclohexane-1-carboxylate **1** (2.75 g, 10 mmol) in dry THF (30 ml) was then added and the reaction mixture was stirred under argon at room temperature for 24 h. On completion, the reaction mixture was acidified to about pH 2 by the addition of 1 N hydrochloric acid solution. The organic solvent was evaporated in vacuo, and the aqueous layer was extracted with diethyl ether (3 × 50 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent: Et_2O/hexanes 4:1) to give 3.11 g (90% yield) of compound ***rac*-2** as a colorless oil that solidified by stirring with a small amount of diethyl ether. White solid; m.p. 160°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.79–7.82 (m, 2H), 7.50–7.55 (m, 1H), 7.42–7.47 (m, 2H), 7.29–7.35 (m, 2H), 7.18–7.24 (m, 3H), 6.35 (s, 2H), 3.75 (s, 3H), 2.81 (dt, J = 13.9, 4.5 Hz, 1H), 2.42–2.49 (m, 2H), 2.16–2.38 (m, 4H), 2.08 (ddd, J = 13.6, 11.5, 4.4 Hz, 1H); ^{13}C{1H} APT NMR (100 MHz, $CDCl_3$, δ): 173.9 (C), 167.2 (C), 139.0 (C), 137.5 (C), 134.1 (C), 131.7 (CH), 128.8 (CH), 128.5 (CH), 128.1 (CH), 127.0 (CH), 126.2 (CH), 123.8 (CH), 59.1 (C), 52.4 (CH_3), 33.8 (CH_2), 33.2 (CH_2), 31.9 (CH_2), 24.2 (CH_2); IR (nujol): ν = 3308 (NH), 1742 (COO), 1638 (CONH), 1579, 1557 (C=C), 1527 (NH δ) cm^{-1}; HRMS (ESI, *m/z*): $[M + Na]^+$ calcd. for $C_{22}H_{23}NNaO_3$, 372.1560; found, 372.1570.

2.4.2 | *N*-(4-Benzylidene-1-(hydroxymethyl) cyclohexyl)benzamide (***rac*-3**)

To a stirred solution of compound ***rac*-2** (1.51 g, 4.32 mmol) in dry diethyl ether (50 ml) under argon at 0°C, a 2 M solution of lithium borohydride in dry THF (4.34 ml, 8.68 mmol) was added and the resulting mixture was stirred for 1 h at room temperature. After completion, the reaction was quenched at 0°C by the slow addition of saturated aqueous ammonium chloride solution (35 ml). The solution was extracted with dichloromethane (3 × 25 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give 1.30 g (94% yield) of compound ***rac*-3**. White solid; m.p. 126°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.74–7.77 (m, 2H), 7.50–7.54 (m, 1H), 7.41–7.46 (m, 2H), 7.30–7.35 (m, 2H), 7.19–7.24 (m, 3H), 6.35 (s, 1H), 6.28 (s, 1H), 4.85 (bs, 1H), 3.81 (s, 2H), 2.68 (dt, J = 14.6, J = 5.2, 1H), 2.28–2.42 (m, 3H), 2.16–2.24 (m, 1H), 2.04–2.13 (m, 1H), 1.82 (ddd, J = 13.5, J = 8.6, J = 6.4, 1H), 1.69 (ddd, J = 13.5, J = 10.6, J = 4.5, 1H); ^{13}C{1H} APT NMR (100 MHz, $CDCl_3$, δ): 168.8 (C), 139.7 (C), 137.5 (C), 134.7 (C), 131.7 (CH), 128.8 (CH), 128.6 (CH), 128.1 (CH), 126.9 (CH), 126.2 (CH), 123.7 (CH), 69.2 (CH_2), 58.5 (C), 33.3 (CH_2), 32.7 (CH_2), 32.0 (CH_2), 24.2 (CH_2); IR (nujol): ν = 3304 (NH), 3218 (OH), 1632 (CONH), 1600, 1577 (C=C), 1535 (NH δ) cm^{-1}; HRMS (ESI, *m/z*): $[M + Na]^+$ calcd. for $C_{21}H_{23}NNaO_2$, 344.1661; found, 344.1633.

2.4.3 | (R_a)-*N*-(4-Benzylidene-1-(hydroxymethyl)cyclohexyl)benzamide [(***R*$_a$)-3**] and (S_a)-*N*-(4-benzylidene-1-(hydroxymethyl) cyclohexyl)benzamide [(***S*$_a$)-3**]

Six hundred milligrams of ***rac*-3** dissolved in $CHCl_3$ (3 ml) were resolved by successive injections of 200 µl of solution (W_s = 40 mg) on a 250 × 20 mm (L × ID) Chiralpak® IA column and using a ternary mixture *n*-Hex/EtOH/$CHCl_3$ (88/6/6) as the eluent (flow rate: 0.8 ml/min). A total of 15 injections were performed, with one injection performed every 8 min. Four separate fractions were collected. The first, second, third, and fourth fractions contained, respectively, 100/0 (235 mg), 75/25 (80 mg), 3/97 (135 mg), and 0.5/99.5 (150 mg) mixtures of **(*R*$_a$)-3** and **(*S*$_a$)-3**. Recrystallization of the fourth fraction from ethanol/ether provided 45 mg of enantiomerically pure **(*S*$_a$)-3**. **(*R*$_a$)-3**: White solid, m.p. = 138°C; $[\alpha]_{25}^{D}$ = −136.6 (c = 0.49 in $CHCl_3$). **(*S*$_a$)-3**: White solid, m.p. = 137°C; $[\alpha]_{25}^{D}$ = 133.2 (c = 0.48 in $CHCl_3$). Spectroscopic data for **(*R*$_a$)-3** and **(*S*$_a$)-3** were identical to those given above for the racemic compound.

2.4.4 | (1-Amino-4-benzylidenecyclohexyl) methanol (***rac*-4**)

To a stirred solution of compound ***rac*-3** (0.90 g, 2.80 mmol) in ethanol (20 ml), a solution of potassium hydroxide (2.01 g, 35.82 mmol) in water (25 ml) was added and the resulting mixture was stirred under reflux conditions for 3 h. Ethanol was evaporated in vacuo, and the aqueous layer was extracted with diethyl ether (2 × 25 ml). The combined organic layers were then extracted with 1 N hydrochloric acid solution (3 × 25 ml). The combined extracts were basified by the addition of 6 N sodium hydroxide aqueous solution. The basic solution was extracted again with dichloromethane (3 × 25 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give 0.51 g (84% yield) of compound ***rac*-4**. White solid; m.p. 117°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.29–7.34 (m, 2H), 7.18–7.21 (m, 3H), 6.29 (s, 1H), 3.41 (s, 2H), 2.54 (dt, J = 14.5, J = 5.7, 1H), 2.32–2.46 (m, 2H),

2.28 (dt, $J = 14.1$, $J = 5.7$, 1H), 2.10 (bs, 3H), 1.54–1.67 (m, 2H), 1.44–1.52 (m, 2H); ^{13}C{1H} APT NMR (100 MHz, $CDCl_3$, δ): 141.2 (C), 137.9 (C), 128.8 (CH), 128.0 (CH), 126.0 (CH), 123.0 (CH), 69.9 (CH_2), 52.2 (C), 36.5 (CH_2), 36.0 (CH_2), 32.3 (CH_2), 24.3 (CH_2); IR (nujol): $\nu = 3335$, 3281 (NH_2), 3155 (OH), 1649, 1591 (C=C) cm^{-1}; HRMS (ESI, *m/z*): $[M + H]^+$ calcd. for $C_{14}H_{20}NO$, 218.1539; found, 218.1535.

2.4.5 | (R_a)-(1-Amino-4-benzylidenecyclohexyl)methanol [(***R*****$_a$**)-**4**] and (S_a)-(1-amino-4-benzylidenecyclohexyl) methanol [(***S*****$_a$**)-**4**]

Hydrolysis of enantiomerically pure (***R*****$_a$**)-**3** and (***S*****$_a$**)-**3** as described above provided (***R*****$_a$**)-**4** and (***S*****$_a$**)-**4**, respectively. (***Ra***)-**4**: White solid, m.p. = 68°C; $[\alpha]_{25}^{D} = -6.9$ ($c = 0.54$ in EtOH). (***S*****$_a$**)-**3**: White solid, m.p. = 68°C; $[\alpha]_{25}^{D} = 7.0$ ($c = 0.31$ in EtOH). Spectroscopic data for (***R*****$_a$**)-**3** and (***S*****$_a$**)-**3** were identical to those given above for the racemic compound.

2.4.6 | (5*R*,5′*R*)-2,2′-(Propane-2,2-diyl)bis(8-((*Z*)-benzylidene)-3-oxa-1-azaspiro[4.5]dec-1-ene) [(***R*****$_a$**,***R*****$_a$**)-**5**]

To a stirred solution of 2,2-dimethyl-malononitrile (51.7 mg, 0.55 mmol) in dry toluene (7 ml) under argon at room temperature, dry[23] zinc triflate (200 mg, 0.55 mmol) was added and the resulting mixture was stirred for 5 min. Then a solution of β-amino alcohol (***R*****$_a$**)-**4** (217 mg, 1 mmol) in dry toluene (5 ml) was added and the solution was heated under reflux for 48 h. The system was allowed to cool to room temperature and the reaction solution was then washed with brine (2 × 20 ml) and 5% aqueous solution of $NaHCO_3$ (3 × 15 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on alumina previously oven-dried for 1 h at 100°C (eluent: hexanes/Et_2O 4:1) to give 135 mg (55% yield) of compound (***R*****$_a$**,***R*****$_a$**)-**5**. Colorless oil; $[\alpha]_{25}^{D} = -36.1$ ($c = 0.39$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.29–7.35 (m, 2H), 7.17–7.22 (m, 3H), 6.32 (s, 1H), 4.05 (s, 2H), 2.76 (ddd, $J = 14.0$, $J = 6.7$, $J = 4.5$, 1H), 2.56 (ddd, $J = 13.8$, $J = 6.8$, $J = 4.9$, 1H), 2.14–2.24 (m, 2H), 1.89 (ddd, $J = 12.6$, $J = 9.8$, $J = 4.5$, 1H), 1.78 (ddd, $J = 13.9$, $J = 9.9$, $J = 4.5$, 1H), 1.65–1.73 (m, 1H), 1.55–1.62 (m, 1H), 1.51 (s, 3H); ^{13}C{1H} APT NMR (100 MHz, $CDCl_3$, δ): 167.6 (C), 140.5 (C), 138.0 (C), 128.8 (CH), 128.0 (CH), 126.0 (CH), 123.1 (CH), 77.2 (C), 70.4 (C), 38.4 (CH_2), 37.7(CH_2), 33.3(CH_2), 25.2(CH_2), 24.4(CH_3); IR (nujol): $\nu = 1658$ (C=N) cm^{-1}; HRMS (ESI, *m/z*): $[M + H]^+$ calcd. for $C_{33}H_{39}N_2O_2$, 495.3006; found, 495.3008.

2.4.7 | 2,6-Bis((*R*)-8-((*Z*)-benzylidene)-3-oxa-1-azaspiro[4.5]dec-1-en-2-yl)pyridine [(***R*****$_a$**,***R*****$_a$**)-**6**]

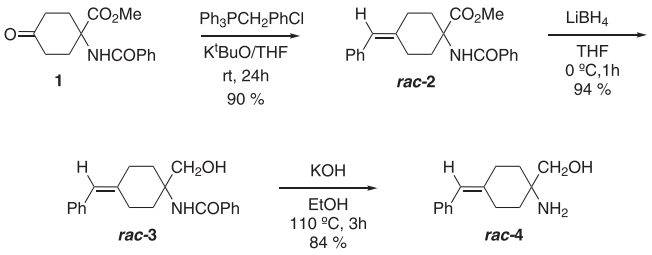
To a stirred solution of pyridine-2,6-dicarbonitrile (71 mg, 0.55 mmol) in dry toluene (7 ml) under argon at room temperature, dry[23] zinc triflate (20 mg, 0.055 mmol) was added and the resulting mixture was stirred for 5 min. Then a solution of β-amino alcohol (***R*****$_a$**)-**4** (217 mg, 1 mmol) in dry toluene (5 ml) was added and the solution was heated under reflux for 24 h. The system was allowed to cool to room temperature, and the reaction solution was diluted by the addition of ethyl acetate (15 ml). The obtained solution was then washed with brine (2 × 20 ml) and aqueous saturated solution of $NaHCO_3$ (3 × 15 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on alumina previously oven-dried for 1 h at 100°C (eluent: gradually form Et_2O/hexanes/4:1 to Et_2O) to give 185 mg (70% yield) of compound (***R*****$_a$**,***R*****$_a$**)-**6**. Colorless oil; $[\alpha]_{25}^{D} = -23.6$ ($c = 0.30$ in $CHCl_3$) [$[\alpha]_{25}^{D} = 24.5$ ($c = 0.10$ in $CHCl_3$) for (***S*****$_a$**,***S*****$_a$**)-**6**]; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.20 (d, $J = 7.9$, 2H), 7.86 (t, $J = 7.9$, 1H), 7.29–7.34 (m, 4H), 7.17–7.23 (m, 6H), 6.35 (s, 2H), 4.31 (s, 4H), 2.80 (ddd, $J = 13.9$, $J = 7.5$, $J = 4.8$, 2H), 2.68 (ddd, $J = 12.7$, $J = 7.5$, $J = 4.4$, 2H), 2.36 (ddd, $J = 12.8$, $J = 8.1$, $J = 4.0$, 2H), 2.27 (ddd, $J = 13.4$, $J = 8.8$, $J = 4.1$, 2H), 2.01 (ddd, $J = 12.9$, $J = 8.7$, $J = 4.7$, 2H), 1.90 (ddd, $J = 13.1$, $J = 8.6$, $J = 4.5$, 2H), 1.80 (ddd, $J = 12.5$, $J = 7.7$, $J = 4.7$, 2H), 1.65–1.73 (m, 2H); ^{13}C{1H} APT NMR (100 MHz, $CDCl_3$, δ): 161.0 (C), 147.0 (C), 140.4 (C), 138.0 (C), 137.3 (CH), 128.9 (CH), 128.1 (CH), 126.1 (CH), 125.8 (CH), 123.4 (CH), 78.1 (C), 71.5 (C), 39.0 (CH_2), 38.3 (CH_2), 33.3 (CH_2), 29.7 (CH_2), 25.2 (CH_2); IR (nujol): $\nu = 1589$ (C=C_{Py}) cm^{-1}; HRMS (ESI, *m/z*): $[M + Na]^+$ calcd. for $C_{35}H_{35}N_3NaO_2$, 552.2621; found, 552.2592.

3 | RESULTS AND DISCUSSION

We started the synthesis from the known 4-substituted cyclohexanone **1**, prepared through Diels–Alder reaction between methyl 2-benzamidoacrylate and 2-trimethylsilyloxy-1,3-butadiene in the presence of ZnI_2 as a catalyst.[21] Wittig olefination of this ketone featuring a symmetry plane with benzyltriphenylphosphonium

bromide led to axially chiral alkene ***rac*-2** in 90% yield, as a racemic mixture. Racemic *N*-benzoyl amino alcohol ***rac*-3** was obtained in 94% yield by reduction of the ester moiety of compound ***rac*-2** with lithium borohydride. Final basic hydrolysis led to the desired axially chiral 1,2-amino alcohol ***rac*-4** in 84% yield, as a racemic mixture (Scheme 1).

With axially chiral compound ***rac*-2**, ***rac*-3**, and ***rac*-4** in hand, we studied their enantiomeric separation by high-performance liquid chromatography using chiral stationary phases based on immobilized 3,5-dimethylphenylcarbamate derivatives of amylose or cellulose at an analytical level using 250 × 4.6 mm (L × ID) columns, namely Chiralpak® IA and Chiralpak® IB. The capacity (k'), selectivity (α), and resolution (R_s) factors for each column in the enantioseparation of all compounds using different eluents were determined (Table 1).



SCHEME 1 Synthesis of axially chiral 1,2-amino alcohol ***rac*-4**

A selectivity value of about 1.15, which allows resolution values higher than 1.5, is required for an easy separation at analytical scale. From results using binary mixtures of *n*-hexane/ethanol as mobile phase, it was concluded that both ***rac*-3** and ***rac*-4** are suitable analytes for enantioseparation with these chiral stationary phases. In both cases, Chiralpak® IA column is the only one that provides selectivity. Nevertheless, the low solubility of both racemates in the binary mixture of solvents, that causes the precipitation of the compounds into the column, hampered its use on a semipreparative scale.

In order to enhance the solubility of racemates and then be able to move from analytical conditions to the semipreparative scale, the addition of acetone or chloroform as a third component to the eluting mixture was evaluated for compounds ***rac*-3** and ***rac*-4**. In all cases, the presence of acetone or chloroform in the eluent led to a decrease in selectivity and resolution, but chloroform mixtures led to better values, some times in the same range as binary mixtures. Unfortunately, even in this ternary mixture, solubility of compound ***rac*-4** remained too low to allow a good loading capacity in semipreparative assays. Analyte ***rac*-3** was then selected for further optimization to extend the study to the semipreparative-scale enantioseparation. Taking into account selectivity, resolution and solubility (improved with increasing percentage of chloroform in the mixture), ternary mixture *n*-hexane/ethanol/chloroform 88/6/6 was chosen

TABLE 1 Chromatographic data for the analytical HPLC resolution of ***rac*-2**, ***rac*-3**, and ***rac*-4** on Chiralpak® IA and Chiralpak® IB using different mobile phases

Compound	Column	Eluent	k'	α	R_s
***rac*-2**	IA	*n*-Hex/EtOH (90/10)	1.54	1.00	-
***rac*-2**	IB	*n*-Hex/EtOH (90/10)	3.32	1.05	0.5
***rac*-3**	IA	*n*-Hex/EtOH (90/10)	1.74	1.21	2.65
***rac*-3**	IB	*n*-Hex/EtOH (90/10)	2.08	1.00	-
***rac*-4**	IA	*n*-Hex/EtOH (90/10)	0.97	1.55	1.90
***rac*-4**	IA	*n*-Hex/EtOH (95/5)	2.07	1.33	2.12
***rac*-4**	IB	*n*-Hex/EtOH (95/5)	2.60	1.00	-
***rac*-3**	IA	*n*-Hex/EtOH/Acetone (92/5/3)	2.57	1.12	1.24
***rac*-3**	IA	*n*-Hex/EtOH/$CHCl_3$ (90/8/2)	2.08	1.19	1.80
***rac*-4**	IA	*n*-Hex/EtOH/Acetone (95/3/2)	2.10	1.18	1.28
***rac*-4**	IA	*n*-Hex/EtOH/$CHCl_3$ (90/8/2)	1.17	1.27	1.30
***rac*-4**	IA	*n*-Hex/EtOH/$CHCl_3$ (92/6/2)	1.78	1.28	1.95
***rac*-3**	IA	*n*-Hex/EtOH/$CHCl_3$ (90/4/6)	3.11	1.12	1.80
***rac*-3**	IA	*n*-Hex/EtOH/$CHCl_3$ (90/6/4)	2.16	1.13	1.94
***rac*-3**	IA	*n*-Hex/EtOH/$CHCl_3$ (85/10/5)	1.22	1.15	1.17
***rac*-3**	IA	*n*-Hex/EtOH/$CHCl_3$ (88/6/6)	2.12	1.12	1.86

Note: Chromatographic conditions: Room temperature, 250 × 4.6 mm (L × ID) columns, injection volume: 5 µl, flow rate 1 ml/min; UV detection 250 nm.

to perform the semipreparative resolution. Figure 1 shows the chromatographic resolution of ***rac*-3**, analytical HPLC with the optimized ternary mixture.

At this point, the column saturation capacity (defined as the maximum sample mass that the column can hold in mg) and the optimum sample volume of the column were determined in an experimental approach. Starting from the previously selected elution conditions on the analytical column and using a solution of 200 mg/ml of compound **3** in chloroform (the most concentrated solution that can be obtained), gradually increased volumes of the sample were injected. The chromatographic data obtained on working in the overload mode on the analytical column are shown in Table 2.

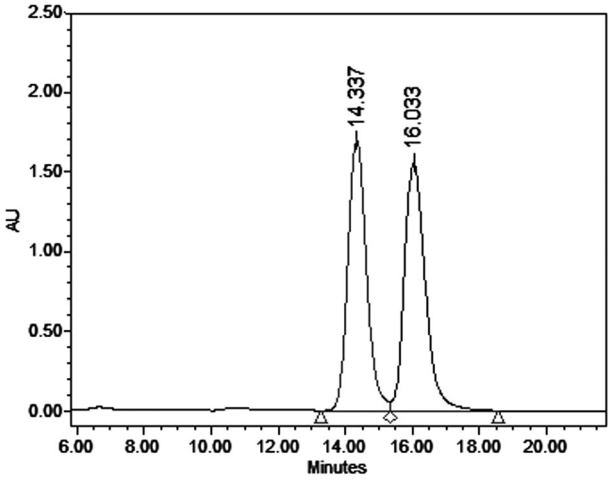


FIGURE 1 HPLC analytical resolution of ***rac*-3** at room temperature on a 250 × 4.6 mm (L × ID) Chiralpak® IA column. Mobile phase composition: *n*-Hex/EtOH/$CHCl_3$ 88/6/6 (*v*/*v*/*v*); flow rate: 1 ml/min; UV detection: 250 nm

TABLE 2 Chromatographic data for the resolution of amino acid derivatives ***rac*-3** on Chiralpak® IA data working in an overload mode in the analytical column at room temperature

V (*μl*)	k'	α	R_s
5	1.90	1.12	1.16
10	1.90	1.14	0.90
15	1.86	1.13	0.80
20	1.86	1.14	0.70

Note: Overload mode, eluent *n*-Hex/EtOH/$CHCl_3$ (88/6/6), c = 200 mg/ml, flow rate 0.8 ml/min; UV detection 270 nm.

Increasing the injected volume, selectivity remained in the same range and resolution was worse even with an injected volume of 5 μl. With this analyte, a significant peak tailing was observed working in overload mode, probably due to the strong interaction of the benzamido group with the carbamate moiety on the selector of the chiral stationary phase. This behavior points out the difficulty in obtaining fractions containing the second eluted enantiomer as single component. That being the case, we decided to work with a large injected volume in a first round to obtain mixtures enriched in either the first or the second eluted enantiomer and perform an additional purification later. We opted to inject 10 μl of a 200 mg/ml solution to work in an overloaded mode on the analytical column (250 × 4.6 mm (L × ID)). That means working with 200-μl injections of the same concentrated solution (40 mg of analyte per injection) on a 250 × 20 mm (L × ID) column and a flow rate of 16 ml/min to keep the same chromatographic parameters (Figure 2).

The semipreparative resolution of compound ***rac*-3** on a 250 × 20 mm (L × ID) Chiralpak® IA column was achieved by successive injections of a solution of the sample in chloroform (Figure 3). In order to enhance throughput, injections were partially overlapped. For each run, four separate fractions were collected and combined with equivalent fractions. The four combined fractions were concentrated and reinjected onto the analytical chiral column to determine their enantiomeric purity; 15 injections

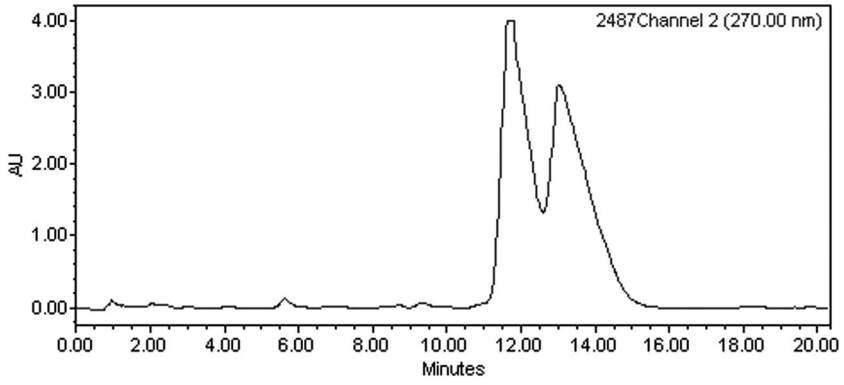


FIGURE 2 Chromatogram for the enantioseparation of ***rac*-3** operating in an overload mode at room temperature on a 250 × 20 mm (L × ID) Chiralpak® IA column. Injection volume: 200 μl, c = 200 mg/ml. Mobile phase composition: *n*-Hex/EtOH/$CHCl_3$ 88/6/6 (*v*/*v*/*v*); flow rate: 16 ml/min; UV detection: 270 nm

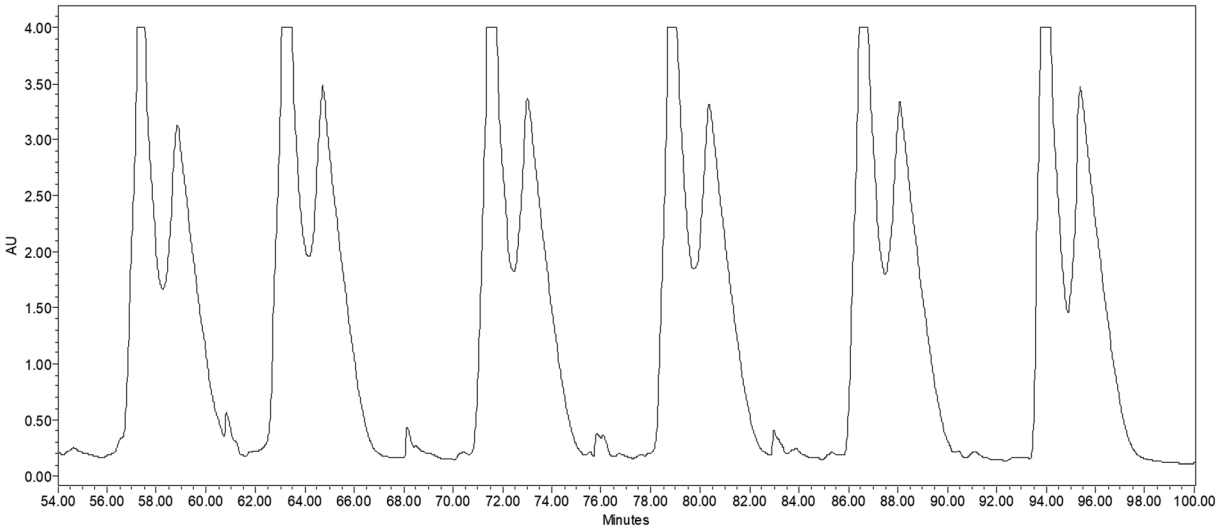


FIGURE 3 Semipreparative chromatogram for the enantioseparation of ***rac*-3** at room temperature on a 250 × 20 mm (L × ID) Chiralpak® IA column. Injection volume: 200 µl, $c = 200$ mg/ml. Mobile phase composition: *n*-Hex/EtOH/$CHCl_3$ 88/6/6 (*v*/*v*/*v*); flow rate: 16 ml/min; UV detection: 270 nm. Repetitive injection every 8 min

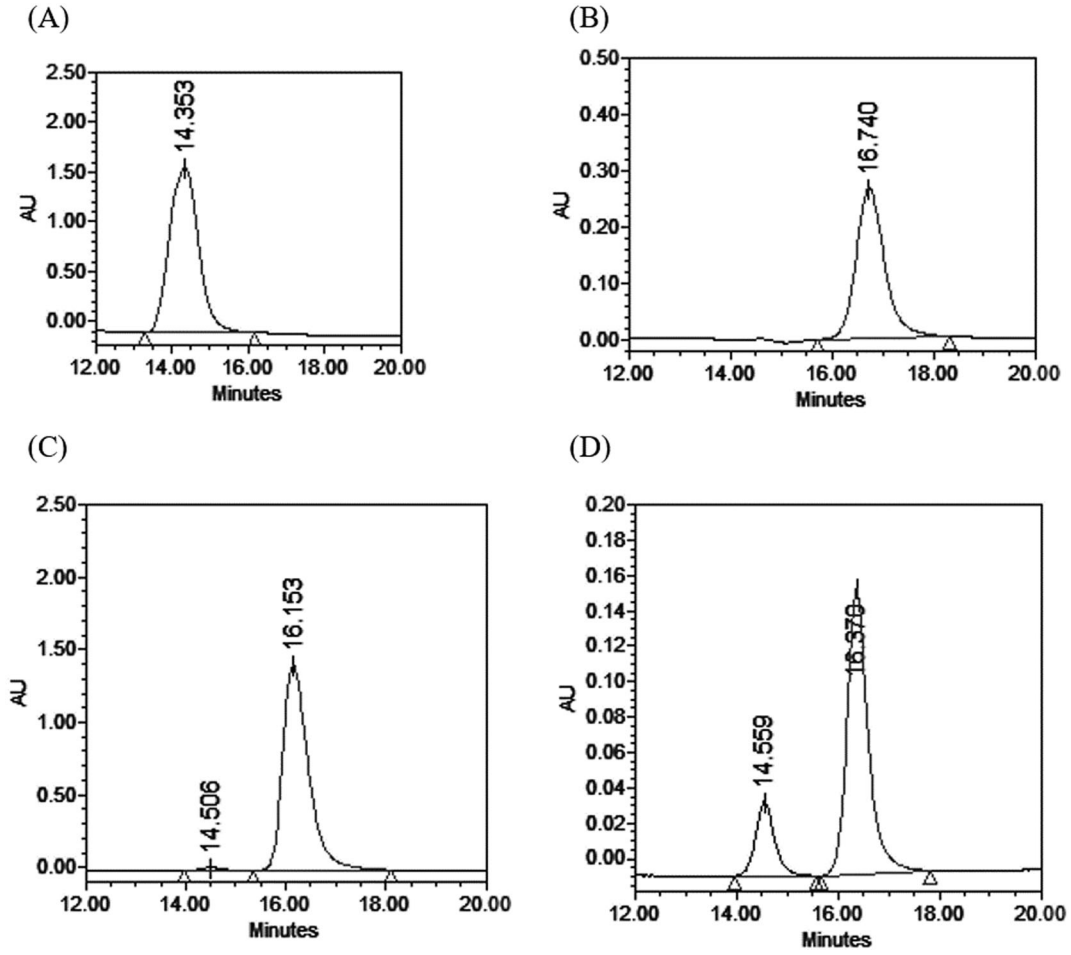


FIGURE 4 Analytical check of fractions collected in the enantioseparation of ***rac*-3** at room temperature on a 250 × 4.6 mm (L × ID) Chiralpak® IA column. Mobile phase composition: *n*-Hex/EtOH/$CHCl_3$ 88/6/6 (*v*/*v*/*v*); flow rate: 1 ml/min; UV detection: 250 nm. (A) First eluted enantiomer in enantiomerically pure form. (B) Second eluted enantiomer in enantiomerically pure form. (C) 99.5/0.5 mixture enriched in the second eluted enantiomer. (D) 87/13 mixture enriched in the second eluted enantiomer

of 200 μl of a 200 mg/ml solution (40 mg of ***rac*-3** per injection) provided the following: First fraction, 235 mg of the first eluted enantiomer in enantiomerically pure form; second fraction, 80 mg of a 75/25 mixture enriched in the first eluted enantiomer; third fraction, 135 mg of a 97/3 mixture enriched in the second eluted enantiomer; and fourth fraction, 150 mg of a 99.5/0.5 mixture enriched in the second eluted enantiomer.

Recrystallization of the last fraction from ethanol/diethyl ether provided 45 mg of the second eluted enantiomer in enantiomerically pure form. A 99/1 mixture of enantiomers was recovered from mother liquor and combined with the third fraction containing a 97/3 mixture enriched in the second eluted enantiomer. To these combined fractions, a similar protocol of semipreparative resolution was applied, and in this case, three separate fractions were collected. In this way, six injections of 200 μl of a 200 mg/ml solution (40 mg of analyte *per* injection) provided the following: First fraction, 29 mg of an 87/13 mixture enriched in the second eluted enantiomer; second fraction, 180 mg of 99.5/0.5 mixture enriched in the second eluted enantiomer; and third fraction, 30 mg of the second eluted enantiomer in enantiomerically pure form.

In summary, from 600 mg of amido alcohol ***rac*-3**, 235 mg of the enantiomerically pure first eluted enantiomer, 180 mg the second eluted enantiomer with a 99% enantiomeric purity, and 75 mg of the second eluted

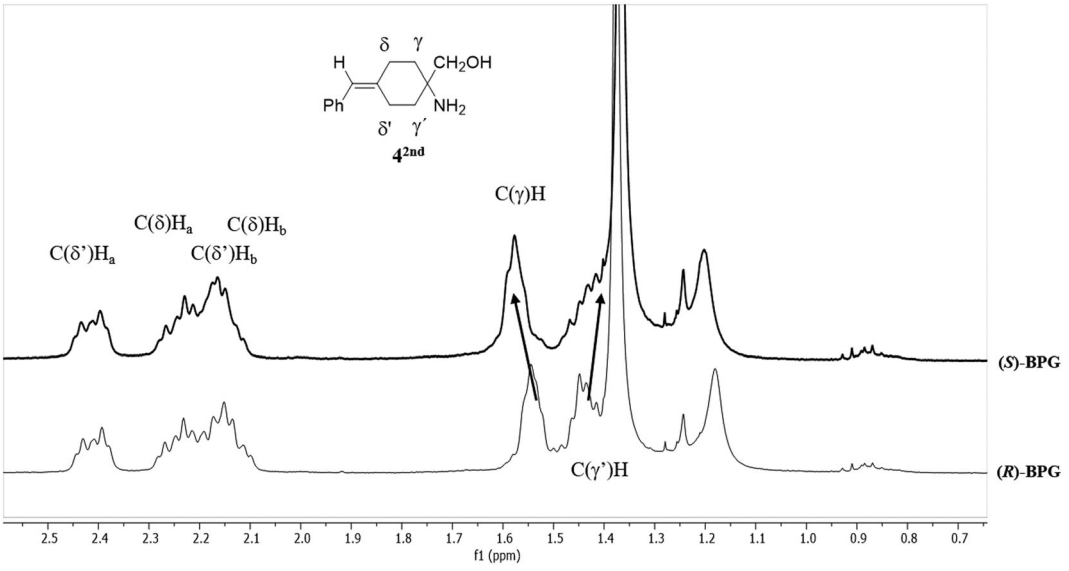


FIGURE 5 Partial ^{1}H NMR spectra (400 MHz) of β-amino alcohol **4^{2}nd** after the addition of 1 equiv. of (*R*)-BPG (bottom line) and (*S*)-BPG (top line) in $CDCl_3$. The upfield and downfield shifts are highlighted

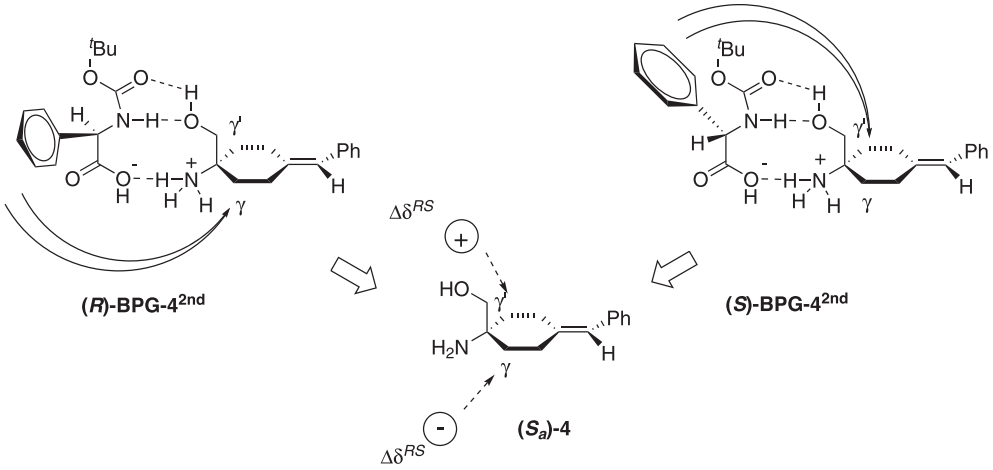
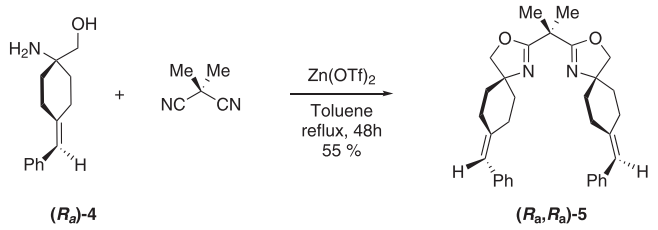
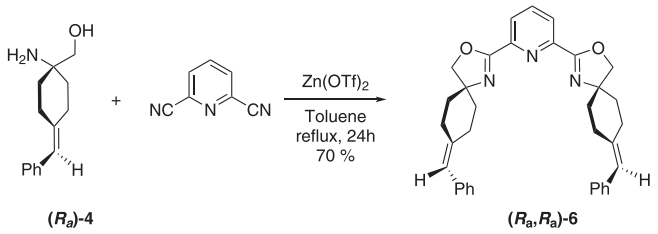


FIGURE 6 Association of (*R*)-BPG and (*S*)-BPG with the S_a enantiomer of β-amino alcohol **4** and sign distribution of $\Delta\delta^{RS}$



SCHEME 2 Synthesis of bis(oxazoline) (R_a,R_a)-**5**



SCHEME 3 Synthesis of pyridine bis(oxazoline) (R_a,R_a)-**6**

enantiomer in enantiomerically pure form, enough to characterization purposes, were obtained. The corresponding analytical check of the several collected fractions in the resolution of ***rac*-3** is shown in Figure 4.

After resolution by semipreparative HPLC, each enantiomer of compound **3** was hydrolyzed as described above and the assignment of the absolute configuration of β-amino alcohol **4²nd** derived from the of second eluted enantiomer of **3** was determined. Absolute configuration determination was performed by ^{1}H using both enantiomers of Boc-β-phenylglycine (BPG) as chiral solvating agent (CSA), as described by Pazos et al.[24] for others β-amino alcohols. Previously, the unambiguous assignment of each signal to the corresponding hydrogens of the cyclohexyl moiety of amino alcohol **4** was performed using bidimensional NMR experiments (COSY and ^{1}H-^{13}C HSQC).

The ^{1}H NMR spectrum of an equimolecular mixture of the enantiopure amino alcohol **4²nd** and (*R*)-BPG performed in $CDCl_3$ was compared with that obtained in a parallel way with a mixture of **4²nd** and (*S*)-BPG. Comparison of (*R*)-BPG/**4²nd** and (*S*)-BPG/**4²nd** ^{1}H-NMR spectra showed that the signals due to the C(γ) protons were shifted downfield in the spectrum of (*S*)-BPG/**4²nd** [$\Delta\delta^{RS} < 0$]. On the other hand, signals due to the C(γ′) protons were shifted upfield in (*S*)-BPG/**4²nd** [$\Delta\delta^{RS} > 0$] (Figure 5).

This different behavior of the shifts of C(γ) and C(γ′) protons in both complexes and the resulting signs of $\Delta\delta^{RS}$ are fully consistent with an association between the enantiopure amino alcohol **4²nd** and the chiral solvating agent according to the model previously proposed by Pazos et al.[24] Noncovalent interactions between the CSA and the β-amino alcohol led to an arrangement where a side of the amino alcohol is located under the shielding cone of the BPG phenyl group. This side is different depending on the configuration of BPG (Figure 6). In such way, for the enantiomer studied, C(γ) hydrogens resonate at a higher field in the presence of (*R*)-BPG than in the presence of (*S*)-BPG [$\Delta\delta^{RS} < 0$], and the opposite occurs for C(γ′) hydrogens. According to this model, the absolute configuration of 1,2-amino alcohol **4²nd** derived from basic hydrolysis of second eluted enantiomer of **3** should be S_a.

One prominent use of chiral amino alcohols is the synthesis of compounds containing a chiral oxazoline ring, which have become one of the most successful, versatile, and commonly used classes of ligands for asymmetric catalysis. As a consequence of their ready accessibility, modular nature, and applicability in metal-catalyzed transformations these ligands have been used with great success in a wide range of asymmetric reactions.[25–29] With both enantiomers of axially chiral amino alcohol **4** in enantiomerically pure form in hand, a new class of bis(oxazoline) and pyridine bis(oxazoline) ligands with a chiral axis at the C_4 position have been prepared from (R_a)-**4** according to Cornejo et al.[30] one-pot procedure. Condensation of the chiral β-amino alcohol (R_a)-**4** (2 mmol) with 2,2-dimethylmalononitrile (1 mmol) using stoichiometric amounts of zinc triflate (1 mmol) under refluxing toluene gave bis(oxazoline) (R_a,R_a)-**5** in 55% yield after 48 h (Scheme 2).

The same reaction using pyridine-2,6-dicarbonitrile required only 24 h to give pyridine bis(oxazoline) (R_a,R_a)-**6** in 70% yield. (Scheme 3). In this reaction, only catalytic amounts of Zn $(OTf)_2$ were needed.

4 | CONCLUSION

In summary, we have described here an easy and efficient protocol for the synthesis and semipreparative HPLC resolution of new axially chiral β-amino alcohols. Box and pybox ligands with new structural features have been obtained in just one step using Zn (OTf)2 to promote condensation of the β-amino alcohol with the corresponding dicarbonitrile.

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DATA AVAILABILITY STATEMENT

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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