Access to Enantiomerically Pure *cis*- and *trans*- β -Phenylproline by HPLC Resolution

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ABSTRACT

The preparation of all four stereoisomers of the proline analogue that bears a phenyl group attached to the β carbon either *cis* or *trans* to the carboxylic acid (*cis*- and *trans*- β -phenylproline, respectively) has been addressed. The methodology developed allows access to multigram quantities of the target amino acids in enantiomerically pure form and suitably protected for use in peptide synthesis. Racemic precursors of *cis*- β -phenylproline and *trans*- β -phenylproline were prepared from easily available starting materials and subjected to HPLC enantioseparation. Semi-preparative columns (250 mm $\times$ 20 mm) containing chiral stationary phases based on amylose (Chiralpak[®] IA) or cellulose (Chiralpak[®] IC) were used, respectively, for the resolution of the *cis*- and *trans*- β -phenylproline precursors.

INTRODUCTION

The stabilization of structural motifs in peptides through the incorporation of residues with well-defined conformational properties is a useful strategy to optimize the pharmacological profile of bioactive peptides.^{1,2} Proline is the only genetically coded amino acid that can be viewed as conformationally constrained. The unique structural features of proline derive from the presence of the five-membered pyrrolidine ring. As a consequence of its cyclic structure, proline acts as a potent turn inductor in peptide chains.^{3,4} Turns are known to be propitious sites for molecular recognition and, indeed, many naturally occurring peptides have been proposed to adopt turns in their bioactive conformation.⁴

The high significance of proline in peptide conformation and biology has stimulated the development of new proline analogues with tailored properties. The addition of functional groups that are present in the side chains of other proteinogenic amino acids is particularly attractive in this regard. It allows the combination of the structural properties of proline with the functionality of other residues. This is the case of β -phenylproline (Figure 1), which results from attaching a phenyl substituent to the pyrrolidine β carbon. Thus, β -phenylproline may be considered as being simultaneously a proline and a phenylalanine analogue. Such combination of structural and functional properties may be synergistic and lead to optimal interaction with the complementary groups in the receptor binding site. Moreover, at variance with phenylalanine, the aromatic side chain in β -phenylproline is anchored in a particular orientation and this may be exploited to investigate the conformational requirements for optimal binding to the receptor. Additionally, for a given configuration at the α carbon, the β -phenyl substituent may exhibit a *cis* or a *trans* configuration with respect to the carbonyl group (in *cis*- and *trans*- β -phenylproline, respectively;

Figure 1), thus increasing the conformational diversity and the opportunities to fine-tune the interaction with the receptor pocket.

<Figure 1>

The combined conformational and functional properties of β -phenylproline have attracted the interest of several researchers and, in fact, some stereoisomers of this amino acid have been incorporated, as a replacement for proline or phenylalanine, into a variety of peptides involved in the regulation of crucial physiological events and considered primary targets for drug development.^{5–13} Some of these studies have met with considerable success, with remarkable improvements in the pharmacological profile of the native sequences being achieved in terms of selectivity, affinity or metabolic stability. Moreover, the large number of patents dealing with biologically active β -phenylproline-containing peptides provides unequivocal proof of the potential that this proline-phenylalanine hybrid amino acid offers in the design of drugable compounds.

In spite of its great potential value, the exploitation of β -phenylproline in the design of peptide-based therapeutically useful compounds is limited by the access to the different stereoisomers (Figure 1) in enantiomerically pure form and sufficient quantities, which is not straightforward. A number of strategies have been described that allow the preparation of one or more β -phenylproline stereoisomers in enantioenriched or optically pure form.^{14–22} Some of them make use of L-proline or L-pyroglutamic acid (5-oxoproline) derivatives as chiral starting materials.^{14–16} In particular, the preparation of the (2*S*,3*R*) stereoisomer has been accomplished by conjugate addition of phenylcuprate to a 3,4-dehydropyroglutamic acid derivative followed by reduction.^{14,15} A 3,4-dehydropyrolidine ester served as a precursor for the preparation of (2*S*,3*S*)- β -phenylproline by incorporation of the β -substituent through of a cross-coupling reaction and

further hydrogenation.¹⁶ Additionally, some β -phenylproline stereoisomers have been obtained by cyclization of open-chain precursors bearing a chiral auxiliary.^{17–20} Such acyclic precursors have been generated, in turn, by Michael addition of a nucleophilic glycine equivalent to a chiral cinnamate,¹⁷ allylation of a chiral phenylglycinol-derived imine,¹⁸ aza-Claisen rearrangement of a cinnamyl amine,¹⁹ or addition of phenylcuprate to an α,β -unsaturated ester derived from Garner's aldehyde.²⁰ Recently, organocatalytic processes have been applied to the synthesis of (2*S*,3*S*)- and (2*R*,3*S*)- β -phenylproline.^{21,22}

Regardless of the synthetic value of the strategies stated above, the operational efficiency of these processes may be compromised by the accessibility of the chiral precursors, the degree of stereochemical control, the large number of steps, or difficulties for a large-scale preparation. Therefore, we sought a more practical access to β -phenylproline in its various stereochemical forms. We describe herein a convenient route for the gram-scale preparation of all four β -phenylproline stereoisomers (Figure 1) in enantiomerically pure form and adequately protected for use in peptide synthesis. The procedure is based on the preparation of racemic precursors of both *cis*- and *trans*- β -phenylproline and their subsequent chromatographic resolution.

MATERIALS AND METHODS

General

All reagents from commercial suppliers were used without further purification. Thin-layer chromatography (TLC) was performed on Macherey-Nagel Polygram syl G/UV precoated silica gel polyester plates. The products were visualized by exposure to UV light (254 nm), iodine vapor or an ethanolic solution of phosphomolybdic acid. Column chromatography was performed using Macherey-Nagel 60Å silica gel. Melting points were determined on a Gallenkamp apparatus. Optical rotations were measured in a 10-cm pathlength cell (7.9 ml volume) using a

JASCO P-1020 polarimeter. High-resolution mass spectra were obtained on a Bruker Microtof-Q spectrometer. IR spectra were registered on a Mattson Genesis or a Nicolet Avatar 360 FTIR spectrophotometer; $\nu_{\max}$ is given for the main absorption bands. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 instrument at room temperature using the residual solvent signal as the internal standard; chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. Duplicate signals were observed for most ^{13}C and several ^1H due to the *cis-trans* isomerism of the amide bond formed by the Boc moiety and the pyrrolidine nitrogen. The percentage of such *cis-trans* species in equilibrium at room temperature for *cis-6* and *trans-6* was determined on a Bruker AV-500 spectrometer at 10 mM concentration. Previously, complete assignment of all ^1H and ^{13}C signals of these compounds was performed through COSY and HSQC experiments. Identification of the species exhibiting a *cis* or a *trans* amide bond for each *cis-6* and *trans-6* was carried out by NOESY experiments (750 ms mixing time) at 233 K to avoid chemical exchange.

High-Performance Liquid Chromatography

HPLC was carried out using a Waters 600 HPLC system equipped with a 2996 photodiode array detector and a 2487 dual wavelength absorbance detector used, respectively, for monitoring analytical and preparative separations. The solvents used as mobile phases were of spectroscopic grade. Analytical assays were performed on Chiralpak[®] IA, IB, and IC columns (Daicel Chemical Industries Ltd., Japan) of 250 mm $\times$ 4.6 mm using different binary and ternary mixtures as eluents and working at flow rates of 0.8–1.0 ml/min. The preparative resolutions were carried out on 250 mm $\times$ 20 mm Chiralpak[®] IA or IC columns (see below for further details). The capacity (k'), selectivity (α) and resolution (R_S) factors are defined as follows: $k' = (t_r - t_0)/t_0$, $\alpha = k'_2/k'_1$, $R_S = 1.18 (t_2 - t_1)/(w_2 + w_1)$, where subscripts 1 and 2 refer to the first and second eluted

enantiomers, t_r ($r = 1, 2$) are their retention times, and w_1 and w_2 denote their half-height peak widths; t_0 is the dead time.

X-ray diffraction

Colorless single crystals were obtained by slow evaporation of a solution of *cis*-**6** in hexanes. The X-ray diffraction data were collected at 295K on an Oxford Diffraction Xcalibur diffractometer provided with a Sapphire CCD detector, using graphite-monochromated Mo- $K\alpha$ radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods using SHELXS-97²³ and refinement was performed using SHELXL-97²⁴ by the full-matrix least-squares technique with anisotropic thermal factors for heavy atoms. Hydrogen atoms were located by calculation and affected by an isotropic thermal factor fixed to 1.2 times the U_{eq} of the carrier atom (1.5 for the methyl protons). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-870467. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystallographic data: monoclinic, space group $P2_1$; $a = 11.8506(9)$ Å, $b = 6.078(4)$ Å, $c = 12.9651(11)$ Å; $\beta = 113.885(9)^\circ$; $Z = 2$; $d_{\text{calcd}} = 1.188$ g·cm⁻³; 15128 reflections collected, 3003 unique ($R_{\text{int}} = 0.050$); data/restraints/parameters: 3003/1/201; final R indices ($I > 2\sigma I$): $R_1 = 0.029$, $wR_2 = 0.052$; final R indices (all data): $R_1 = 0.069$, $wR_2 = 0.056$; highest residual electron density: 0.09 e Å⁻³.

Syntheses

Synthesis of β -phenylproline hydrochloride (5**, mixture of *cis* and *trans* isomers).** A solution of diethyl acetamidomalonate (10.00 g, 46.08 mmol) in anhydrous ethanol (10 ml) kept at 10 °C under an argon atmosphere was treated with sodium ethoxide (1.25 g, 18.43 mmol). *Trans*-cinnamaldehyde (6.4 ml, 50.69 mmol) was added dropwise and the reaction mixture was kept at this temperature for 5 h. The solvent was eliminated and the residue was redissolved in chloroform (60 ml). Triethylsilane (11.0 ml, 69.12 mmol) was added followed by dropwise addition of trifluoroacetic acid (35.5 ml, 461 mmol) over 10 min. After stirring at room temperature for 4 h, the solvent was evaporated. The resulting residue was dissolved in ethyl acetate (200 ml) and a saturated aqueous solution of NaHCO₃ was added in small portions until neutralization was achieved. The aqueous layer was discarded and the organic phase was further washed with saturated aqueous NaHCO₃ (2 $\times$ 50 ml). The organic phase was dried over anhydrous MgSO₄, filtered, and evaporated to dryness. Acetic acid (20 ml) and 6 N HCl (80 ml) were then added and the reaction mixture was heated under reflux for 12 h. The solvent was evaporated to dryness and the residue was redissolved in water. The aqueous solution obtained was washed with ethyl acetate (2 $\times$ 30 ml), concentrated, and lyophilized to afford **5** as a mixture of *cis* and *trans* isomers (9.64 g, 42.28 mmol, 92% overall yield). Spectroscopic data were coincident with those previously reported.²⁵

Synthesis of *cis/trans* methyl *N*-(*tert*-butoxycarbonyl)- β -phenylprolinate (6**, mixture of *cis* and *trans* isomers).** A mixture of **5** (mixture of *cis* and *trans* isomers) (4.03 g, 17.66 mmol) and tetramethylammonium hydroxide pentahydrate (8.00 g, 44.15 mmol) in acetonitrile (80 ml) was treated with di-*tert*-butyl dicarbonate (5.77 g, 26.49 mmol). The mixture was stirred at room temperature for a total of 3 days, with further portions of di-*tert*-butyl dicarbonate (1.92 g, 8.83 mmol) being added after 24 and 48 h. The solvent was evaporated and the residue was partitioned between water (120 ml) and diethyl ether (50 ml). The organic layer was discarded and the

aqueous phase was further washed with diethyl ether (2×30 ml). The aqueous solution was acidified with solid citric acid and extracted with dichloromethane (4×50 ml). The combined organic extracts were dried over anhydrous MgSO_4 , filtered, and evaporated to provide a white solid. The subsequent esterification reaction was carried out in one of the following ways: A) The solid obtained was dissolved in a 2:1 toluene/methanol mixture (100 ml) at room temperature and a 2 M solution of (trimethylsilyl)diazomethane in diethyl ether was added in small portions until a permanently colored solution was obtained. After 10 min, a small quantity of silica gel was added and stirring was continued until the yellow color disappeared. The solvent was eliminated and the residue was purified by column chromatography (eluent: hexanes/ethyl acetate 7:3) to afford **6** as a 7:3 mixture of *cis/trans* isomers (4.99 g, 16.35 mmol, 93% yield from **5**). B) The white solid was dissolved in anhydrous methanol (40 ml) and cesium carbonate (5.88 g, 18.03 mmol) was added. The resulting mixture was stirred at room temperature for 30 min and then evaporated to dryness. The residue was dissolved in *N,N*-dimethylformamide (40 ml) and methyl iodide (1.22 ml, 19.67 mmol) was added. After stirring at room temperature for 4 days, evaporation of the solvent and purification by column chromatography (eluent: hexanes/ethyl acetate 7:3) afforded **6** as a 7:3 mixture of *cis/trans* isomers (4.75 g, 15.57 mmol, 88% yield from **5**). The analytical and spectroscopic characterization of pure *cis*-**6** and *trans*-**6** are given below.

Synthesis of *trans* *N*-(*tert*-butoxycarbonyl)- β -phenylproline (*trans*-7**) and isolation of *cis* methyl *N*-(*tert*-butoxycarbonyl)- β -phenylprolinate (*cis*-**6**).** A 0.5 N aqueous solution of NaOH (64.2 ml, 32.09 mmol) was added to a solution of **6** (7:3 mixture of *cis/trans* isomers) (9.79 g, 32.09 mmol) in methanol (50 ml). After stirring at 25 °C for 22 h, methanol was evaporated and the remaining aqueous solution was diluted with water and extracted with dichloromethane (3×50 ml). The combined organic extracts were dried over anhydrous MgSO_4 , filtered, and evaporated to afford pure *cis*-**6** as a white solid (7.21 g, 23.63 mmol, 74% yield). If necessary,

additional purification can be accomplished by recrystallization from hexanes. The aqueous layer was acidified by the addition of solid citric acid and extracted with dichloromethane (4 × 40 ml). The combined organic extracts were dried over anhydrous MgSO₄, and filtered. Evaporation of the solvent provided *trans*-**7** as a white solid (2.28 g, 7.82 mmol, 24% yield). Combined yield (*cis*-**6** + *trans*-**7**): 98%.

cis-**6**: M.p. 74 °C. IR (nujol) ν 1746, 1702, 1453 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 1.39, 1.47 (two s, 9H); 2.11 (m, 1H); 2.49–2.66 (m, 1H); 3.24, 3.27 (two s, 3H); 3.40–3.50 (m, 1H); 3.60–3.73 (m, 1H); 3.83, 3.92 (two m, 1H); 4.46, 4.55 (two d, 1H, *J* = 8.7 Hz); 7.19–7.33 (m, 5H). ¹³C NMR (CDCl₃, 100 MHz) δ 27.47; 28.31, 28.47; 45.85, 46.21; 47.16, 48.02; 51.26, 51.36; 63.87, 64.35; 80.01, 80.04; 127.46, 127.53; 127.85, 127.92; 128.35, 128.38; 136.69, 136.78; 153.69, 154.32; 171.72, 171.79. HRMS (ESI) C₁₇H₂₄NO₄ [M+H]⁺: calcd 306.1700, found 306.1713.

trans-**7**: M.p. 128 °C. IR (nujol) ν 3320–2560, 1734, 1631 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 1.42, 1.51 (two s, 9H); 1.97–2.10 (m, 1H); 2.28–2.39 (m, 1H); 3.44–3.55 (m, 1H); 3.56–3.70 (m, 1H); 3.72–3.85 (m, 1H); 4.28, 4.44 (two d, 1H, *J* = 6.2, 5.0 Hz, respectively); 7.21–7.37 (m, 5H). ¹³C NMR (CDCl₃, 100 MHz) δ 28.41, 28.54; 32.55, 32.84; 46.11, 46.44; 47.13, 50.02; 65.40, 65.71; 80.85, 81.84; 127.13; 127.32, 127.53; 128.97; 140.55, 141.07; 153.77, 156.28; 174.69, 178.27. HRMS (ESI) C₁₆H₂₂NO₄ [M+H]⁺: calcd. 292.1543, found 292.1555.

Synthesis of *trans* methyl *N*-(*tert*-butoxycarbonyl)- β -phenylprolinate (*trans*-6**).** A 2 M solution of (trimethylsilyl)diazomethane in diethyl ether was added in small portions to a solution of *trans*-**7** (3.06 g, 10.52 mmol) in toluene/methanol 2:1 (60 ml) at room temperature until the yellow color persisted. A small quantity of silica gel was added and stirring was continued until the color disappeared. Evaporation of the solvent and purification by column chromatography (eluent: hexanes/ethyl acetate 7:3) afforded *trans*-**6** as a white solid (3.19 g, 10.45 mmol, 99% yield). M.p. 61 °C. IR (nujol) ν 1747, 1704, 1491 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 1.41, 1.48

(two s, 9H); 1.98–2.08 (m, 1H); 2.25–2.36 (m, 1H); 3.39–3.47 (m, 1H); 3.54–3.65 (m, 1H); 3.69, 3.71 (two s, 3H); overlapped with 3.67–3.78 (m, 1H); 4.24, 4.38 (two d, 1H, $J = 6.7, 6.0$ Hz, respectively); 7.20–7.36 (m, 5H). ^{13}C NMR (CDCl_3 , 100 MHz) δ 28.40, 28.56; 32.42, 33.14; 46.12, 46.28; 48.88, 50.08; 52.13, 52.35; 65.26, 65.94; 80.25, 80.36; 127.05, 127.10; 127.28, 127.40; 128.88, 128.92; 140.66, 141.03; 153.74, 154.39; 173.21, 173.35. HRMS (ESI) $\text{C}_{17}\text{H}_{23}\text{NNaO}_4$ $[\text{M}+\text{Na}]^+$: calcd 328.1519, found 328.1519.

Resolution of *cis*-6: isolation of methyl (2*R*,3*R*)- and (2*S*,3*S*)-*N*-(*tert*-butoxycarbonyl)- β -phenylproline [(2*R*,3*R*)-6 and (2*S*,3*S*)-6]. HPLC resolution of racemic *cis*-6 (5.40 g) dissolved in chloroform (9.00 ml) was carried out by successive injections (one every 12.5 min) of 700 μl on a 250 mm $\times$ 20 mm Chiralpak[®] IA column. A mixture of *n*-hexane/2-propanol 90:10 was used as the eluent working at a flow rate of 16 ml/min and with UV monitoring at 210 nm. Three separate fractions were collected. Optically pure (2*R*,3*R*)-6 (2.690 g) and (2*S*,3*S*)-6 (2.676 g) were respectively obtained by evaporation of the first and third fractions. The second fraction contained 17 mg of (2*R*,3*R*)-6/(2*S*,3*S*)-6 mixture and was discarded.

(2*R*,3*R*)-6: M.p. 99 °C. $[\alpha]_{\text{D}}^{20} -101.2$ (c 0.77, MeOH).

(2*S*,3*S*)-6: M.p. 99 °C. $[\alpha]_{\text{D}}^{20} +102.0$ (c 0.67, MeOH).

Spectroscopic data were identical to those described for *cis*-6.

Synthesis of (2*R*,3*R*)-*N*-(*tert*-butoxycarbonyl)- β -phenylproline [(2*R*,3*R*)-7]. A 2 N solution of lithium hydroxide in methanol/water 1:1 (200 ml) was added to (2*R*,3*R*)-6 (2.20 g, 7.21 mmol) and the reaction mixture was stirred at room temperature for 48 h. After evaporation of the solvent, the residue was taken up in water and extracted with dichloromethane (2 $\times$ 40 ml) (138 mg, 0.45 mmol, of unreacted starting compound were recovered by evaporation of the dichloromethane extracts). Neutralization of the aqueous phase with solid citric acid followed by

1 N HCl resulted in the precipitation of (2*R*,3*R*)-**7**, which was collected by vacuum filtration (1.87 g, 6.42 mmol, 89% yield). M.p. 166 °C. $[\alpha]_D^{20}$ –115.6 (*c* 0.33, MeOH). IR (nujol) ν 3300–2500, 1715, 1699, 1478 cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz) δ 1.38, 1.47 (two s, 9H); 2.07–2.16 (m, 1H); 2.43–2.64 (m, 1H); 3.40–3.50 (m, 1H); 3.60–3.89 (m, 2H); 4.45, 4.56 (two d, 1H, *J* = 8.5, 8.2 Hz, respectively); 7.20–7.33 (m, 5H). ^{13}C NMR (CDCl_3 , 100 MHz) δ 27.53; 28.41, 28.56; 45.84, 46.24; 47.14, 48.04; 63.51, 64.11; 80.49, 80.59; 127.64, 127.73; 127.92, 128.03; 128.60; 136.32, 136.48; 153.85, 154.64; 176.72, 176.76. HRMS (ESI) $\text{C}_{16}\text{H}_{22}\text{NO}_4$ $[\text{M}+\text{H}]^+$: calcd. 292.1543, found 292.1555.

Synthesis of (2*S*,3*S*)-*N*-(*tert*-butoxycarbonyl)- β -phenylproline [(2*S*,3*S*)-7**].** An identical procedure to that described above was applied to transform (2*S*,3*S*)-**6** (2.20 g, 7.21 mmol) into (2*S*,3*S*)-**7** (1.85 g, 6.37 mmol, 88% yield) (unreacted material recovered: 164 mg, 0.54 mmol). M.p. 166 °C. $[\alpha]_D^{20}$ +115.0 (*c* 0.38, MeOH). Spectroscopic data were the same as those given for (2*R*,3*R*)-**7**.

Resolution of *trans*-6**: isolation of methyl (2*S*,3*R*)- and (2*R*,3*S*)-*N*-(*tert*-butoxycarbonyl)- β -phenylproline [(2*S*,3*R*)-**6** and (2*R*,3*S*)-**6**].** HPLC resolution of racemic *trans*-**6** (3.00 g) dissolved in chloroform (6.00 ml) was carried out by successive injections (one every 13.0 min) of 600 μl on a 250 mm $\times$ 20 mm Chiralpak[®] IC column. A mixture of *n*-hexane/2-propanol/chloroform 75:15:10 was used as the eluent working at a flow rate of 17 ml/min and with UV monitoring at 220 nm. Three separate fractions were collected. Optically pure (2*S*,3*R*)-**6** (1.442 g) and (2*R*,3*S*)-**6** (1.421 g) were respectively obtained by evaporation of the first and third fractions. The second fraction contained 61 mg of (2*S*,3*R*)-**6**/(2*R*,3*S*)-**6** mixture and was discarded.

(2*S*,3*R*)-**6**: oil. $[\alpha]_D^{25}$ +61.6 (*c* 0.75, CHCl_3).

(2*R*,3*S*)-**6**: oil. $[\alpha]_{\text{D}}^{25} -60.8$ (*c* 0.74, CHCl₃).

Spectroscopic data were identical to those given for *trans*-**6**.

Synthesis of (2*S*,3*R*)-*N*-(*tert*-butoxycarbonyl)- β -phenylproline [(2*S*,3*R*)-7**].** A 2 N solution of lithium hydroxide in methanol/water 1:1 (90 ml) was added to (2*S*,3*R*)-**6** (1.25 g, 4.10 mmol) and the reaction mixture was stirred at room temperature for 24 h. After evaporation of the solvent, the residue was taken up in water and washed with dichloromethane (2 $\times$ 30 ml). Neutralization of the aqueous solution with solid citric acid followed by 1 N HCl resulted in the precipitation of (2*S*,3*R*)-**7**, which was collected by vacuum filtration (1.16 g, 3.98 mmol, 97% yield). M.p. 157 °C. $[\alpha]_{\text{D}}^{25} +65.3$ (*c* 0.42, MeOH). Spectroscopic data were the same as those given for *trans*-**7**.

Synthesis of (2*R*,3*S*)-*N*-(*tert*-butoxycarbonyl)- β -phenylproline [(2*R*,3*S*)-7**].** In a similar way, (2*R*,3*S*)-**6** (1.25 g, 4.10 mmol) was transformed into (2*R*,3*S*)-**7** (1.14 g, 3.91 mmol, 95% yield). M.p. 157 °C. $[\alpha]_{\text{D}}^{25} -64.8$ (*c* 0.47, MeOH). Spectroscopic data were the same as those given for *trans*-**7**.

RESULTS AND DISCUSSION

Synthesis of racemic compounds

The first step in the synthesis of the target amino acids in enantiomerically pure form involved the preparation of racemic precursors adequate for the subsequent HPLC resolution process. A survey of the different methodologies described in the literature for the synthesis of racemic *cis* and *trans* derivatives of β -phenylproline prompted us to select the route described by Chung et al.²⁶ based on the results previously reported by Cox et al.²⁷ This procedure seemed advantageous because it makes use of easily available inexpensive starting materials and involves high-yielding

transformations. Moreover, it provides access to both *cis* and *trans* isomers from a common compound.

The route starts with the condensation of diethyl acetamidomalonate and *trans*-cinnamaldehyde to form the pyrrolidine derivative **1**²⁷ (Figure 2). Subsequent treatment with triethylsilane in the presence of trifluoroacetic acid results in the reduction of the hydroxyl function.²⁶ In the reported procedure,²⁶ the diester **2** underwent monosaponification and concomitant decarboxylation to yield the *N*-acetyl β -phenylprolinate **3** as a mixture of *cis/trans* isomers (Figure 2), which were separated by selective saponification taking advantage of the larger steric hindrance produced by the phenyl substituent in the *cis* ester. However, we encountered difficulties in cleanly isolating the *cis* and *trans* derivatives following this procedure.²⁶ Thus, saponification of the less hindered *trans* ester was partially accompanied by saponification of the *cis* isomer, so that the *trans* acid (*trans*-**4**) was contaminated with variable percentages of the *cis* acid. Conversely, the unreacted material contained mainly the starting *cis* ester (*cis*-**3**) but also some quantities of the *trans* ester. As a consequence, extensive and laborious purification steps were necessary to eventually isolate the *trans* acid (*trans*-**4**) and *cis* ester (*cis*-**3**) in pure form. Several modifications of the experimental conditions reported were assayed but no substantial improvement was observed.

<Figure 2>

The unexpected difficulties encountered in efficiently separating the *cis* and *trans* mixtures of β -phenylproline derivatives prompted us to replace the *N*-acetyl substituent in **3** by the *tert*-butoxycarbonyl (Boc) group. Chung et al²⁶ observed a superior selectivity in the saponification of the *trans* ester in *cis/trans* mixtures of β -alkylprolinates when the amino group was protected with a Boc instead of an acetyl moiety. They proposed that the substituents at both the nitrogen and β -carbon atoms have an effect on the selectivity of the saponification of *trans* β -alkyl

substituted proline esters in the presence of *cis* esters. Actually, this acetyl-to-Boc exchange was later applied by Mignani et al^{28,29} to the synthesis of *cis*- β -phenylproline, although no reason was given for the modification introduced with respect to the original procedure²⁶ and experimental details were not provided.

Based on these precedents, we decided to explore the benefits of the Boc group in allowing for the selective saponification of the *trans* isomer of the *N*-Boc protected proline **6** (Figure 2). Besides this possible beneficial effect, it was anticipated that the Boc group would be highly advantageous for the subsequent HPLC resolution step, since replacement of the acetyl by the Boc moiety would certainly result in increased solubility of the samples in the mobile phase used for the HPLC enantioseparation (typically containing a high percentage of alkanes). It should be noted that solubility is a major issue to achieve high efficiency in preparative HPLC separations. In addition, the presence of the Boc group in the precursors to be resolved by HPLC methods obviates the need for additional deprotection and protection reactions on the enantiomerically pure material obtained in order to isolate the final amino acids adequately protected for use in peptide synthesis. Moreover, if desired, the amino function of such *N*-Boc amino acids could be eliminated under mild reaction conditions that would not compromise the chiral integrity of the stereogenic centers.

Acid hydrolysis of **2** resulted in simultaneous removal of the acetyl group and decarboxylation to afford the amino acid **5** as a mixture of *cis/trans* isomers (Figure 2). Introduction of the Boc protecting group on the amino function was carried out under the conditions described by Johnson et al³⁰ that involve the use of tetramethylammonium hydroxide to generate a salt soluble in acetonitrile. This method avoids the use of aqueous media and provides superior yields of *N*-Boc protected amino acids when the amino group is sterically hindered, as is the case of proline derivatives. The methyl ester was then formed quantitatively by treatment with a commercial

solution of (trimethylsilyl)diazomethane (Figure 2). Alternatively, the esterification step was carried out by reaction with methyl iodide in the presence of cesium carbonate. Compound **6** was thus isolated as a ~7:3 mixture of *cis/trans* isomers in 93% yield from **5** (88% when using methyl iodide). Following the synthetic scheme outlined in Figure 2, we prepared near 10 grams of **6** (*cis/trans* mixture) in 86% global yield from the starting diethyl acetamidomalonate without carrying out purification of any intermediate compound by either chromatography or recrystallization. The route is adequate for larger scale production.

The selective saponification of the *trans* isomer of **6** in the presence of the *cis* derivative was then undertaken. Different reactions conditions were explored and the optimal selectivity was observed when using a slight excess (with reference to the amount of *trans* isomer in the mixture) of sodium hydroxide in a 1.3:1 mixture of water/methanol at a final concentration of 0.3 N. The solvent composition was crucial to obtain a good selectivity, with aqueous methanol being much more convenient than tetrahydrofuran-based mixtures. Under the conditions mentioned, the carboxylic acid *trans*-**7** was isolated pure after 22 h of reaction at 25 °C while the unreacted material recovered was found to contain exclusively the *cis* ester (*cis*-**6**) (Figure 2). These compounds were isolated pure in 24% and 74% yield, respectively (98% combined yield), from the initial *cis*-**6**/*trans*-**6** mixture with no need for additional purification steps. These results were reproduced working at different scales and slightly different room temperatures (the reaction time needed to be adjusted ± 2 h). When the reaction was stopped before completion (~22 h at temperature <25 °C), a small amount of unreacted *trans* ester was found to contaminate *cis*-**6**. In this case, the latter compound was readily purified by recrystallization from hexanes. These results prove that the Boc group adjacent to the ester function is essential for the selective saponification of *cis/trans* mixtures of β -phenylprolinates, as previously observed²⁶ for β -alkylproline derivatives.

Finally, the carboxylic acid function in *trans*-**7** was quantitatively transformed into the corresponding methyl ester (*trans*-**6**) (Figure 2), as the latter is a more convenient substrate for the subsequent HPLC process. Thus, multigram quantities of racemic *cis*-**6** and *trans*-**6** were ready to be subjected to chromatographic enantioseparation. The inferior amount of *trans* compound isolated obviously derives from the composition of the *cis/trans* mixture of isomers subjected to saponification (~7:3). In case there is a preference for the *trans* derivative, the initial *cis/trans*-**6** mixture can be submitted previously to an epimerization process leading to an increased ratio of *trans* isomer.²⁶

The *cis* stereochemistry of the unreacted ester recovered from the saponification reaction (*cis*-**6**) was initially assigned considering the lower reactivity expected for this isomer as a consequence of the proximity of the β -phenyl substituent. The *cis* relative configuration of the ester and phenyl groups in this compound was supported by the NOE correlation observed between the α and β protons in the NOESY spectrum and was further corroborated by X-ray diffraction analysis of a single crystal (Figure 3). It should be noted that, in the crystalline structure of *cis*-**6**, the amide bond formed by the Boc moiety and the proline nitrogen assumes a *cis* configuration,^s which makes the bulky *tert*-butyl group lie close to the methyl ester. In this arrangement, the ester function of *cis*-**6** is confined between the aromatic ring and the *tert*-butoxy moiety and this situation should hamper strongly the hydroxide-ion attack on the ester carbonyl during the saponification reaction. The steric inaccessibility of this carbonyl carbon is clearly seen in the space-filling model built from the X-ray structure of *cis*-**6** (Figure 3).

^s The terms *cis* and *trans* used to indicate the relative stereochemistry of the phenyl and carbonyl substituents in the five-membered ring should not be confused with those referring to the configuration of the amide bond formed by the pyrrolidine nitrogen and either the acetyl or the Boc group.

<Figure 3>

In comparison, an *N*-acetyl moiety (as in *cis*-**3**) should be much less efficient in shielding the ester carbonyl and, therefore, in protecting it from hydroxide attack. This is so, not only as a consequence of the smaller size of the methyl vs. the *tert*-butoxy group, but also because the ratio of species in which the amide bond involving the pyrrolidine nitrogen exhibits a *cis* configuration is expected to be much lower for **3** than for **6**, taking as a basis that described for the reference compounds methyl *N*-acetyl proline^{31,32} (~20%) and methyl *N*-Boc proline³² (~60%) in chloroform solution. Note that, for a *trans* configuration of this amide linkage, the acetyl and Boc groups produce an identical effect on the contiguous ester because, in this arrangement, it is the carbonyl oxygen atom of the *N*-substituent that points to the proline α carbon (Figure 4). For *cis*-**6** and *trans*-**6**, the *cis/trans* amide ratio was found to be 61:39 and 65:35, respectively, in chloroform (Figure 4). This estimation was made, for each compound, by comparing the resonances corresponding to the α proton in the species exhibiting a *cis* or a *trans* *N*-Boc amide bond, which give rise to separate sets of signals in the ¹H-NMR spectrum. In each compound, the species with a *cis* amide linkage was identified on the basis of the NOE correlation observed between the *tert*-butyl group and both the α and methyl ester protons in the NOESY spectra registered at low temperature to avoid chemical exchange. We also confirmed that the predominance of *cis* amide species is maintained (~70%) for both *cis*-**6** and *trans*-**6** in a mixture of deuterated methanol/water of similar composition to that in which the saponification reaction was carried out.

<Figure 4>

According to the above discussion, the much higher selectivity observed for the saponification of *trans*-**6** in the presence of *cis*-**6** with reference to analogous *N*-acetylated compounds can be

attributed to the more efficient shielding exerted by the Boc group, which, in turn, relies on two factors: the larger volume of the *tert*-butoxy moiety when compared to the methyl group and the much higher ratio of *cis* amide bond associated to *N*-Boc substitution. Needless to say, the Boc group protects the ester moiety from hydroxide attack not only in *cis*-**6** but also in *trans*-**6**. The difference in reactivity observed in the saponification of the two isomers is therefore given by the closer proximity of the phenyl group in the former compound, which hampers nucleophilic attack on the ester carbonyl also from this side of the molecule. In comparison, the hydroxide ions can approach the ester carbonyl of the *trans* isomer from the side of the molecule where the phenyl substituent lies. The much higher proximity of the methyl ester and phenyl group in *cis*-**6** becomes evident when comparing the ¹H-NMR spectra of the two compounds in chloroform. Thus, the signal of the methyl protons appears near 3.7 ppm for *trans*-**6** and is strongly upfield shifted in *cis*-**6** (δ 3.3 ppm).

Isolation of enantiopure compounds by HPLC resolution

In the previous section, we have described the preparation of multigram quantities of *cis*- and *trans*- β -phenylproline derivatives in racemic form, *cis*-**6** and *trans*-**6** (Figure 2). The resolution of such racemic precursors was next addressed in order to isolate the desired amino acids in enantiomerically pure form.

Nowadays, HPLC is recognized as a powerful tool for the production of enantiomerically pure compounds at a preparative scale.^{33–35} Among the chiral stationary phases available, polysaccharide-based ones are particularly useful for this purpose because they combine excellent chiral recognition properties with high loading capacity.^{33–35} In the last years, columns of this type in which the chiral selector is covalently bonded to the silica matrix have become commercially available: Chiralpak[®] IA, IB, and IC, which contain, respectively, tris(3,5-

dimethylphenylcarbamate) of amylose,³⁶ tris(3,5-dimethylphenylcarbamate) of cellulose,³⁷ and tris(3,5-dichlorophenylcarbamate) of cellulose.³⁸ These so-called immobilized phases retain the advantages of coated polysaccharide-based phases while being compatible with a wide variety of organic solvents,^{35–39} a feature of enormous value for preparative-scale resolution. We have successfully applied such chiral stationary phases, either made at the laboratory (prior to their commercialization) or of the Chiralpak[®] type, to the preparative HPLC resolution of non-natural analogues of proline,^{40–42} valine,⁴³ and phenylalanine^{44–52} with diverse structures.

The HPLC resolution of *cis*-**6** and *trans*-**6** was first examined at the analytical level using 250 mm × 4.6 mm Chiralpak[®] IA, IB, and IC columns. Complete baseline separation of peaks was observed for *cis*-**6** on the three stationary phases tested eluting with *n*-hexane/2-propanol mixtures (Table 1), with the best selectivity factor being attained on Chiralpak[®] IA ($\alpha = 3.5$ –4.0). The effect produced by the addition of a third component to the mobile phase was then evaluated. Chloroform and acetone proved detrimental to the resolution factor (R_s) on the IB and IC columns, respectively, whereas *tert*-butyl methyl ether had the opposite effect on the latter stationary phase (Table 1). In spite of this positive result, Chiralpak[®] IA remained the best option to separate the enantiomers of *cis*-**6**. The analytical conditions finally selected to be extended to a preparative scale were elution with a 90:10 *n*-hexane/2-propanol mixture at a flow rate of 0.8 ml/min. The profile obtained under such chromatographic conditions is shown in Figure 5. This choice was made after carrying out further assays on Chiralpak[®] IA operating in an overload mode to establish the loading capacity of the column. These analyses showed that the maximum sample mass that the column was able to hold while keeping baseline separation of the peaks did not increase when adding a third component to the mobile phase.

< Figure 5 >

Regarding *trans*-**6**, Chiralpak[®] IA and IC showed excellent enantiodiscrimination ability eluting with *n*-hexane/2-propanol mixtures, whereas the two enantiomers almost co-eluted on the IB column (Table 2). The latter situation did not change upon addition of chloroform, *tert*-butyl methyl ether or acetone to the mobile phase. Elution with ternary mixtures did not improve the resolution on the IA column, either, whereas chloroform proved positive on Chiralpak[®] IC. Thus, addition of 10% chloroform resulted in a much higher solubility of the sample in the mobile phase and increased substantially the loading capacity of the column while providing selectivity and resolution factors similar to those obtained with *n*-hexane/2-propanol 80:20 (Table 2). Accordingly, the analytical conditions selected for subsequent extension to the preparative resolution of *trans*-**6** were elution with a 75:15:10 mixture of *n*-hexane/2-propanol/chloroform at a flow rate of 0.85 ml/min on Chiralpak[®] IC (Figure 5).

The optimal conditions established at the analytical level in each case were scaled up to perform the preparative enantioseparations. For this purpose, columns of 250 mm × 20 mm size were used. The separation of *cis*-**6** was carried out on Chiralpak[®] IA eluting with 90:10 *n*-hexane/2-propanol at a flow rate of 16 ml/min. Successive injections of 700 µl of a highly concentrated solution of *cis*-**6** in chloroform (600 mg/ml) were performed. Each run was collected into three separate fractions, with equivalent fractions of successive injections being combined. A total of 5.40 g of *cis*-**6** were submitted to resolution following this procedure, which was completed in about 4 h. Evaporation of the first and third fractions provided 2.69 and 2.68 g, respectively, of the first and second eluted enantiomers. Both of them were found to be optically pure by analytical assays (e.e. > 99.5%; in each chromatogram, no trace of the other enantiomer was detected).

The preparative resolution of *trans*-**6** was carried out following a similar protocol working on a 250 mm × 20 mm Chiralpak[®] IC column. Elution was performed with *n*-hexane/2-

propanol/chloroform 75:15:10 at a flow rate of 17 ml/min. In this case, the racemate (3.0 g) was dissolved in chloroform at a concentration of 500 mg/ml and 600 μ l aliquots of this solution were injected consecutively. The separation was completed in about 3 h. From the first and third fractions collected, 1.44 and 1.42 g of the first and second eluted enantiomers of *trans*-**6** were isolated, respectively. The optical purity of the enantiomers separated was assessed at the analytical level (e.e. > 99.5%).

The high efficiency of the enantioseparations performed should be emphasized. In both cases, above 95% of the racemic material injected was recovered in enantiomerically pure form after a single passage through the column (>99% for *cis*-**6**). The productivity of the resolution process is particularly remarkable in the case of *cis*-**6**, for which more than 1.3 g of optically pure material was isolated per hour working on a semi-preparative size column. It is worth noting that the high efficiency of the processes relied, to a large extent, on the compatibility of these immobilized chiral stationary phases with chloroform,^{35–39} at variance with that occurring for coated polysaccharide-derived phases. This allowed injection of the samples at very high concentrations ($\geq$ 500 mg/ml), which had a highly beneficial effect on the loading capacity of the columns. For the *trans* compound, the use of chloroform as a component of the mobile phase was also crucial to the efficiency of the separation.

Finally, the enantiomerically pure compounds obtained by HPLC methods were transformed into the corresponding *N*-Boc amino acids (Figure 6). Saponification of the methyl ester function in each optically pure **6** stereoisomer was accomplished by treatment with excess lithium hydroxide in methanol/water. The reaction conditions had previously been optimized working with racemic material to ensure that no epimerization occurred during the process. The *cis* esters required

longer reaction times but no epimerization was observed for any of the compounds.[‡] This final hydrolysis step allowed the isolation of the target compounds (Figure 6), *i.e.* the four stereoisomers of β -phenylproline in enantiomerically pure form and suitably protected for use in standard peptide synthesis.

<Figure 6>

The absolute configuration of the compounds resolved by HPLC and the *N*-Boc amino acids derived from them (Figure 6) was initially established by comparison with optical rotation values described in the literature.^{17,22,†} This comparison led us to assign (2*R*,3*R*) and (2*S*,3*R*) stereochemistries to the *N*-Boc amino acids obtained from the first eluted enantiomers of *cis*-**6** and *trans*-**6**, respectively (Figures 5,6). Accordingly, the most retained enantiomers and their corresponding *N*-Boc amino acids were (2*S*,3*S*) and (2*R*,3*S*) (Figures 5,6). These assignments were further confirmed by X-ray diffraction analysis of dipeptides containing either (2*S*,3*S*)-**7** or (2*S*,3*R*)-**7** and a phenylalanine residue of known configuration.⁵³

[‡] Note that epimerization can be easily detected even when working with enantiomerically pure material since inversion of the configuration at the α carbon results in a change of the *cis/trans* stereochemistry, that is, in the generation of a diastereoisomer, which is easily detectable in the ¹H-NMR spectrum.

[†] Such values are available for three out of the four stereoisomers of **7**.^{17,22} However, they have been measured in chloroform, a solvent not particularly adequate for carboxylic acids due to the possible formation of dimers at relatively low concentrations (the reported data^{17,22} correspond to *c* > 1.0). Actually, we observed significant variations when measuring the optical rotation of a given **7** stereoisomer in chloroform solution at different concentrations. Accordingly, the optical rotation values of the enantiomerically pure *N*-Boc amino acids prepared in this work (**7**, Figure 6) are given in methanol solution in the belief that they will be more useful for future assignments of absolute configurations or optical purity.

CONCLUSION

A convenient route has been developed for the preparation of all four stereoisomers of β -phenylproline based on the synthesis of racemic precursors of *cis* and *trans* relative stereochemistry and their subsequent chromatographic resolution. The target amino acids have been isolated in gram-scale quantities, enantiomerically pure form and adequately protected for use in peptide synthesis. Their absolute configurations have been determined unambiguously by X-ray diffraction analysis of diastereomeric derivatives.

The synthesis of the racemic precursors relies on a refined version of a previously reported procedure. The conditions for effective discrimination of *cis* and *trans* methyl β -phenylprolinates have been optimized to ensure selective saponification of the *trans* ester during the critical step allowing for the isolation of pure *cis* and *trans* compounds. In this regard, the presence of a Boc group protecting the amino function has proven to be essential and much more effective than an acetyl moiety. The HPLC resolution processes have been carried out on polysaccharide-derived chiral columns, namely Chiralpak[®] IA and IC for the *cis* and *trans* compounds, respectively. The high efficiency of the preparative enantioseparations has allowed the isolation of several grams of optically pure compounds in a few hours. The procedure can be easily scaled-up to the production of larger amounts.

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FIGURE CAPTIONS

Figure 1. Structure of β -phenylproline, which can be viewed as a proline-phenylalanine hybrid. The four possible stereoisomers of β -phenylproline are shown. Note that positions 2 and 3 correspond, respectively, to the α and β carbons.

Figure 2. Synthesis of racemic *cis*-**6** and *trans*-**6**. Abbreviations: Ac, acetyl; Boc, *tert*-butoxycarbonyl; TFA, trifluoroacetic acid.

Figure 3. Left: X-ray crystal structure of *cis*-**6**, where the *cis* stereochemistry of the β -phenyl substituent and the methyl ester group is seen (most hydrogen atoms have been omitted for clarity; heteroatoms are drawn as thermal ellipsoids). Right: Space-filling model built from the X-ray structure of *cis*-**6**, showing the steric shielding produced by the Boc and phenyl groups on the methyl ester (the arrow points to the carbonyl carbon in it).

Figure 4. *Cis-trans* isomerism of the amide bond formed by the pyrrolidine nitrogen and the Boc carbonyl for *cis*-**6** and *trans*-**6**. For each compound, region of the ^1H -NMR spectrum (CDCl_3 , 10 mM, 298 K, 500 MHz) showing the α proton resonance of the *cis* and *trans* amide species and the corresponding *trans/cis* amide ratio.

Figure 5. Analytical HPLC resolution of *cis*-**6** (up) and *trans*-**6** (down). The configuration assigned to each enantiomer is shown. Conditions (*cis*-**6**): column, Chiralpak[®] IA 250 mm $\times$ 4.6 mm; eluent, *n*-hexane/2-propanol 90:10; flow rate, 0.8 ml/min; UV detection, 210 nm. Conditions (*trans*-**6**): column, Chiralpak[®] IC 250 mm $\times$ 4.6 mm; eluent, *n*-hexane/2-propanol/chloroform 75:15:10; flow rate, 0.85 ml/min; UV detection, 259 nm.

Figure 6. Preparation of all four stereoisomers of *N*-Boc protected β -phenylproline in enantiomerically pure form (the synthesis of the racemic precursors *cis*-**6** and *trans*-**6** is presented in Figure 2). Abbreviations: Boc, *tert*-butoxycarbonyl.

Table 1. Selected chromatographic data for the analytical HPLC resolution of *cis*-**6** on Chiralpak[®] IA, IB, and IC columns^a

eluent ^b		k'_1	α	R_S
Chiralpak [®] IA				
<i>n</i> -Hx/ <i>i</i> -PrOH	93:7	0.8	4.0	5.1
<i>n</i> -Hx/ <i>i</i> -PrOH/CHCl ₃	92:5:3	0.7	3.5	5.1
<i>n</i> -Hx/ <i>i</i> -PrOH/ <i>t</i> -BuOMe	90:5:5	1.0	3.9	5.1
<i>n</i> -Hx/ <i>i</i> -PrOH/acetone	95:3:2	1.2	3.7	5.1
Chiralpak [®] IB				
<i>n</i> -Hx/ <i>i</i> -PrOH	93:7	0.9	1.3	3.3
<i>n</i> -Hx/ <i>i</i> -PrOH/CHCl ₃	92:5:3	0.7	1.2	1.9
<i>n</i> -Hx/ <i>i</i> -PrOH/ <i>t</i> -BuOMe	90:5:5	1.0	1.3	3.0
<i>n</i> -Hx/ <i>i</i> -PrOH/acetone	95:3:2	1.1	1.2	3.0
Chiralpak [®] IC				
<i>n</i> -Hx/ <i>i</i> -PrOH	80:20	2.0	1.3	3.0
<i>n</i> -Hx/ <i>i</i> -PrOH/CHCl ₃	75:15:10 ^c	1.0	1.4	3.1
<i>n</i> -Hx/ <i>i</i> -PrOH/ <i>t</i> -BuOMe	70:15:15	1.3	1.5	4.2
<i>n</i> -Hx/ <i>i</i> -PrOH/acetone	80:15:5 ^d	0.6	1.2	1.9

^aColumn size: 250 mm × 4.6 mm ID. Flow rate: 1.0 ml/min (unless otherwise indicated). UV detection at 210 nm (230 nm when the eluent contained chloroform). The chromatographic parameters k'_1 , α , and R_S are defined in the Materials and Methods section.

^b% (v/v); *n*-Hx: *n*-hexane; *i*-PrOH: 2-propanol; *t*-BuOMe: *tert*-butyl methyl ether.

^cFlow rate: 0.9 ml/min.

^dFlow rate: 0.8 ml/min.

Table 2. Selected chromatographic data for the analytical HPLC resolution of *trans*-**6** on Chiralpak® IA, IB, and IC columns^a

eluent ^b		k'_1	α	R_S
Chiralpak® IA				
<i>n</i> -Hx/ <i>i</i> -PrOH	93:7 ^c	0.8	2.1	6.1
<i>n</i> -Hx/ <i>i</i> -PrOH/CHCl ₃	92:5:3	0.6	2.0	5.1
<i>n</i> -Hx/ <i>i</i> -PrOH/ <i>t</i> -BuOMe	90:5:5	0.9	2.0	6.0
<i>n</i> -Hx/ <i>i</i> -PrOH/acetone	95:3:2	0.9	1.8	5.1
Chiralpak® IB				
<i>n</i> -Hx/ <i>i</i> -PrOH	93:7	0.8	1.0	–
<i>n</i> -Hx/ <i>i</i> -PrOH/CHCl ₃	92:5:3 ^d	0.6	1.0	–
<i>n</i> -Hx/ <i>i</i> -PrOH/ <i>t</i> -BuOMe	90:5:5	0.9	1.1	–
<i>n</i> -Hx/ <i>i</i> -PrOH/acetone	95:3:2	0.9	1.0	–
Chiralpak® IC				
<i>n</i> -Hx/ <i>i</i> -PrOH	80:20	1.7	2.9	10.8
<i>n</i> -Hx/ <i>i</i> -PrOH/CHCl ₃	75:15:10 ^d	0.8	3.1	10.3
<i>n</i> -Hx/ <i>i</i> -PrOH/ <i>t</i> -BuOMe	70:15:15	1.3	2.8	9.6
<i>n</i> -Hx/ <i>i</i> -PrOH/acetone	80:15:5 ^c	0.5	2.4	7.9

^aColumn size: 250 mm × 4.6 mm ID. Flow rate: 1.0 ml/min (unless otherwise indicated). UV detection at 210 nm (230 nm when the eluent contained chloroform). The chromatographic parameters k'_1 , α , and R_S are defined in the Materials and Methods section.

^b% (v/v); *n*-Hx: *n*-hexane; *i*-PrOH: 2-propanol; *t*-BuOMe: *tert*-butyl methyl ether.

^cFlow rate: 0.8 ml/min.

^dFlow rate: 0.9 ml/min.

Figure 1 **Figure 2**

Figure 3

Figure 4

Figure 5

Figure 6