Practical Access to the Proline Analogues (*S*,*S*,*S*)- and (*R*,*R*,*R*)-2-Methyloctahydroindole-2-carboxylic Acids by HPLC Enantioseparation

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SHORTENED TITLE: HPLC Resolution of (*S**,*S**,*S**)-(αMe)Oic

KEY WORDS: α-tetrasubstituted amino acid; perhydroindole-2-carboxylic acid; polysaccharide-derived chiral stationary phase; chiral HPLC; HPLC resolution; Chiralpak IC

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Contract grant sponsors: Ministerio de Ciencia e Innovación–FEDER, Gobierno de Aragón. Contract grant numbers: CTQ2007-62245 and CTQ2010-17436; PIP206/2005 and research group E40

ABSTRACT

An efficient methodology for the preparation of the α-tetrasubstituted proline analogue (*S*,*S*,*S*)-2-methyloctahydroindole-2-carboxylic acid, (*S*,*S*,*S*)-(αMe)Oic, and its enantiomer, (*R*,*R*,*R*)-(αMe)Oic, has been developed. Starting from easily available substrates and through simple transformations, a racemic precursor has been synthesized in excellent yield and further subjected to HPLC resolution using a cellulose-derived chiral stationary phase. Specifically, a semi-preparative (250 × 20 mm ID) Chiralpak® IC column has allowed the efficient resolution of more than 4 grams of racemate using a mixture of *n*-hexane/*tert*-butyl methyl ether/2-propanol as the eluent. Multigram quantities of the target amino acids have been isolated in enantiomerically pure form and suitably protected for incorporation into peptides.

INTRODUCTION

Peptides are mediators of key biological functions in our organism and, thereby, provide a great opportunity for therapeutic applications. However, the use of natural peptides as drugs is strongly limited by their rapid metabolism, low bioavailability and poor receptor subtype selectivity.[1] Therefore, much effort is being devoted to endow peptides with enhanced therapeutic properties. In this context, the incorporation of constrained α-amino acids into peptides constitutes a very useful strategy to reduce their flexibility and retard enzymatic degradation.[1,2] Moreover, restricted α-amino acids stabilize particular conformational features, which may lead to improvements in the biological potency if the bioactive conformation is tethered.[1,2]

In particular, α-methylation of α-amino acids is an efficient way to produce surrogates with defined conformational preferences.[3] Indeed, the incorporation of α-methyl amino acids into peptides has shown to reduce their conformational freedom and to prevent the hydrolytic cleavage of neighboring peptide bonds.[1,2,4] Among them, α-methylproline[5] [(αMe)Pro, Fig. 1)] has frequently been used as a replacement for proline to control the secondary structure, and hence, the biological behavior of peptides.[6–8] The great number of patents dealing with bioactive peptides containing (αMe)Pro provides evidence for the enormous value of this amino acid (in general, of α-methylated amino acids) in the development of therapeutic agents.

<Figure 1>

Octahydroindole-2-carboxylic acid (Oic, Fig. 1) is a proline analogue containing a fused cyclohexane ring that has proven extremely useful to optimize the pharmacological profile of bioactive peptides. Particularly notable has been the use of (*S*,*S*,*S*)-Oic for the design of angiotensin-converting enzyme (ACE) inhibitors,[9] antagonists for the B2 receptor of

bradykinin[10,11] and prolyl oligopeptidase (POP) inhibitors.[12] Such (*S*,*S*,*S*)-Oic-containing compounds are useful, among other applications, as antihypertensive drugs, anti-inflammatory agents, for the prevention of heart failure, or the treatment of neurodegenerative diseases and, in fact, a number of them are already on the market or have entered clinical evaluation.

As the α-methylated counterpart of Oic, 2-methyloctahydroindole-2-carboxylic acid [(αMe)Oic, Fig. 1)] is of high intrinsic value as a potential modulator of the conformational constraints induced by proline. Both Oic and (αMe)Oic bear an additional cyclohexane ring with reference to the parent amino acid, proline. This structural feature is appropriate to build up hydrophobic recognition interactions at the binding site of receptors and to endow peptides with greater lipophilicity, which, in turn, may translate into enhanced affinity and bioavailability. Moreover, the stereochemical diversity of Oic and (αMe)Oic greatly increases their potential for the design of peptides. It should be noted that, for a given configuration at the α carbon, four different dispositions of the fused cyclohexane ring are possible. The variety of shapes provided by the different Oic and (αMe)Oic stereoisomers may be of help to fine-tune the interaction with the hydrophobic pocket of a receptor and to elucidate the bioactive conformations of peptides.

In spite of its great potential value, the behavior of (αMe)Oic when included into peptides or other biologically relevant systems remains unexplored. This is due to the lack of synthetic methodologies providing access to the different stereoisomers of (αMe)Oic in enantiomerically pure form. In fact, at variance with Oic, attention on the α-methylated counterpart (αMe)Oic has been focused only recently. Within this context, we have described the preparation of enantiopure (*S*,*S*,*S*)-(αMe)Oic[13] and its epimer at the α carbon (*R*,*S*,*S*)-(αMe)Oic[14] by means of stereoselective alkylation processes. To the best of our knowledge, these are, together with the preparation of (*S*,*S*,*S*)-(αMe)Oic described in a patent,[15] the only synthetic routes to (αMe)Oic

reported to date. Therefore, development of efficient methodologies that ensure accessibility to these and other (αMe)Oic stereoisomers, in optically pure form and significant quantities, remains a challenge. We describe here a convenient route for the multigram-scale preparation of both (*S*,*S*,*S*)-(αMe)Oic and (*R*,*R*,*R*)-(αMe)Oic in enantiomerically pure form. The procedure combines the synthesis of a racemic precursor and a chromatographic resolution procedure.

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 vapour or submersion in cerium molybdate stain [aqueous solution of phosphomolybdic acid (2%), $CeSO_4 \cdot 4H_2O$ (1%) and H_2SO_4 (6%)]. Column chromatography was performed using Macherey-Nagel 60Å silica gel. Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were registered on a Mattson Genesis or a Nicolet Avatar 360 FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H 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. Optical rotations were measured at room temperature using a JASCO P-1020 polarimeter. High-resolution mass spectra were obtained on a Bruker Microtof-Q spectrometer.

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 (respectively used 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 × 4.6 mm ID using different binary and ternary mixtures as eluents and working at flow rates ranging from 0.7 to 1.0 ml/min. The preparative resolution was carried out on a 250 × 20 mm ID Chiralpak® IC column eluting with *n*-hexane/*tert*-butyl methyl ether/2-propanol 50/44/6 (see below for further details). Both the column loading capacity (*i.e.* the maximum sample mass that the column can hold) and the optimum sample concentration had previously been determined on the analytical 250 × 4.6 mm ID Chiralpak® IC column by injecting increasing amounts of sample at different concentrations. 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 of racemic (*S**,*S**,*S**)-**10** were obtained by slow evaporation from a dichloromethane/ethyl acetate solution. The X-ray diffraction data were collected at 100K on an Oxford Diffraction Xcalibur diffractometer provided with a Sapphire CCD detector, using graphite-monochromated Mo-*K*α radiation (λ = 0.71073 Å). The structure was solved by direct methods using SHELXS-97[16] and refinement was performed using SHELXL-97[17] 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 746151. 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: orthorhombic, space group *Pbca*; a = 7.5997(2) Å, b = 17.0955(6) Å, c = 18.1963(6) Å; Z = 8; d_{calcd} = 1.313 g·cm^{-3}; 18156 reflections collected, 3133 unique (R_{int} = 0.0401); data/parameters: 3133/137; final R indices ($I > 2\sigma I$): R_1 = 0.036, wR_2 = 0.089; final R indices (all data): R_1 = 0.062, wR_2 = 0.102; highest residual electron density: 0.39 e Å^{-3}.

Syntheses

Synthesis of methyl *N*-(*tert*-butoxycarbonyl)indole-2-carboxylate (9). To a solution of indole-2-carboxylic acid (**8**) (4.0 g, 24.82 mmol) in methanol (135 ml), sulfuric acid (2.30 ml, 40.77 mmol) was added dropwise. The reaction mixture was heated under reflux for 6 h. The solvent was evaporated and the residue was dissolved in dichloromethane (70 ml) and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and filtered. After evaporation of the solvent, the remaining residue was dissolved in tetrahydrofuran (100 ml) and 4-(dimethylamino)pyridine (361 mg, 2.93 mmol) and di-*tert*-butyl dicarbonate (6.40 g, 29.32 mmol) were added. The reaction mixture was kept at room temperature for 2 h and then the solvent was evaporated. The resulting residue was dissolved in ethyl acetate (70 ml) and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and filtered. The crude product obtained after evaporation of the solvent was purified by column chromatography (eluent: hexanes/ethyl acetate 10/1) to give pure **9** as a white solid (6.36 g, 23.10 mmol, 93% yield). Mp 68–70 ºC. IR (KBr) ν 3019, 2981, 1745, 1712, 1556 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78 (s, 9H), 4.08 (s, 3H), 7.26 (m, 1H), 7.42 (m, 1H), 7.57 (m, 1H), 7.76 (m, 1H), 8.25 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.78, 52.28, 84.55, 114.82, 122.12, 123.25, 126.77,

127.44, 130.34, 137.79, 149.20, 162.31. HRMS (ESI) $C_{15}H_{17}NNaO_4$ $[M+Na]^+$: calcd 298.1050, found 298.1039.

Synthesis of methyl (2*S,3a*S**,7a*S**)-*N*-(*tert*-butoxycarbonyl)octahydroindole-2-carboxylate [(*S**,*S**,*S**)-4].** A solution of **9** (6.0 g, 21.79 mmol) in acetic acid (80 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of PtO_2 (600 mg). After 24 h, the catalyst was filtered off and washed with acetic acid. The solvent was evaporated to dryness and the residue was dissolved in dichloromethane (70 ml) and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and filtered. The solvent was evaporated and the residue purified by column chromatography (eluent: hexanes/ethyl acetate 10/1) to afford pure (*S**,*S**,*S**)-**4** as a colorless oil (5.87 g, 20.72 mmol, 95% yield). HRMS (ESI) $C_{15}H_{25}NNaO_4$ $[M+Na]^+$: calcd 306.1676, found 306.1679. Spectroscopic data are identical to those reported previously[13] for the (*S*,*S*,*S*) enantiomer.

Synthesis of methyl (2*S,3a*S**,7a*S**)-*N*-(*tert*-butoxycarbonyl)-2-methyloctahydroindole-2-carboxylate [(*S**,*S**,*S**)-5].** A 1.8 M solution of LDA in hexanes (26.20 ml, 47.16 mmol) was added dropwise to a stirred solution of (*S**,*S**,*S**)-**4** (5.70 g, 20.11 mmol) in anhydrous tetrahydrofuran (105 ml) kept under an inert atmosphere, at –78 ºC. After stirring at this temperature for 30 min, methyl iodide (5.20 ml, 81.35 mmol) was added dropwise. The resulting solution was warmed to –50 ºC and kept at this temperature overnight. The reaction mixture was quenched with saturated aqueous NH_4Cl (20 ml) and extracted with dichloromethane several times. The organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was eliminated and the residue was purified by column chromatography (eluent: hexanes/ethyl acetate 10/1) to afford pure (*S**,*S**,*S**)-**5** as a colorless oil (4.48 g, 15.06 mmol, 75% yield). IR (neat) ν 1743, 1693 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ 1.06 (m, 1H), 1.14–1.50 (m, 3H) overlapped with 1.30, 1.36 (two s, 9H) and with 1.43, 1.45 (two s, 3H), 1.54–1.68 (m, 4H), 1.94–2.10 (m,

1H), 2.11–2.25 (m, 1H), 2.40–2.48 (m, 1H), 3.60–3.72 (m, 1H) overlapped with 3.60, 3.65 (two s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (duplicate signals are observed for most carbons) 20.10, 20.21; 21.77, 23.13; 23.00, 23.23; 25.03, 25.11; 25.65, 26.46; 27.85, 28.01; 33.62, 34.54; 38.87, 39.97; 51.86, 52.04; 57.52, 57.68; 64.40; 78.41, 78.52; 151.66, 152.32; 174.37, 174.66. HRMS (ESI) $C_{16}H_{27}NNaO_4$ $[M+Na]^+$: calcd 320.1832, found 320.1835.

Synthesis of methyl (2*S,3a*S**,7a*S**)-2-methyloctahydroindole-2-carboxylate hydrochloride [(*S**,*S**,*S**)-10].** A 3N solution of hydrogen chloride in anhydrous ethyl acetate (20 ml) was added to (*S**,*S**,*S**)-**5** (4.40 g, 14.80 mmol) and the reaction mixture was stirred at room temperature for 4 h. The solvent was eliminated and the solid was dissolved in water and lyophilized to afford (*S**,*S**,*S**)-**10** (3.46 g, 14.80 mmol, 100% yield). Mp 167–169 ºC. HRMS (ESI) $C_{11}H_{20}NO_2$ $[M–Cl]^+$: calcd 198.1489, found 198.1485. Spectroscopic data are identical to those reported in our previous work[13] for the (*S*,*S*,*S*) enantiomer.

Synthesis of methyl (2*S,3a*S**,7a*S**)-*N*-(benzyloxycarbonyl)-2-methyloctahydroindole-2-carboxylate [(*S**,*S**,*S**)-11].** To an ice-cooled solution of (*S**,*S**,*S**)-**10** (3.42 g, 14.63 mmol) and *N*,*N*-diisopropylethylamine (10.4 ml, 59.68 mmol) in dry dichloromethane (75 ml), benzyl chloroformate (4.40 ml, 29.28 mmol) was added dropwise. Once the addition was completed, the reaction was kept at room temperature overnight. The mixture was washed with saturated aqueous $NaHCO_3$, and the organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated and the residue was purified by column chromatography (eluent: hexanes/ethyl acetate 10/1) to give (*S**,*S**,*S**)-**11** as a colorless oil (4.47 g, 13.49 mmol, 92% yield). IR (neat) ν 3035, 2950, 1743, 1702 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.00–1.28 (m, 2H), 1.34–1.73 (m, 6H) overlapped with 1.46 and 1.48 (two s, 3H), 1.94, 2.08 (two m, 1H), 2.16–2.27 (m, 1H), 2.44–2.56 (m, 1H), 3.48, 3.62 (two s, 3H), 3.79 (m, 1H), 4.94–5.11 (m, 2H), 7.25–7.45 (m, 5H) ^{13}C NMR (DMSO-d_6, 100 MHz) δ (duplicate signals are observed for most carbons)

19,98, 20.12; 21.63; 22.97, 23.07; 24.89, 24.97; 25.60, 26.61; 33.61, 34.53; 38.80, 40.23; 52.11; 57.66, 58,23; 64.53, 64.96; 65.55, 65.85; 127.21; 127.42; 127.64, 127.69; 128.23; 128.30; 136.49, 136.93; 152.48, 152.80; 174.09, 174.35. HRMS (ESI) $C_{19}H_{25}NNaO_4$ $[M+Na]^+$: calcd 354.1676, found 354.1676.

Resolution of (*S,*S**,*S**)-11: isolation of methyl (2*S*,3a*S*,7a*S*)- and (2*R*,3a*R*,7a*R*)-*N*-(benzyloxycarbonyl)-2-methyloctahydroindole-2-carboxylate [(*S*,*S*,*S*)-11 and (*R*,*R*,*R*)-11].** HPLC resolution of racemic (*S**,*S**,*S**)-**11** (4.142 g) dissolved in chloroform (6.90 ml) was carried out by successive injections (one every 11.5 min) of 600 μl on a 250 × 20 mm ID Chiralpak® IC column. A mixture of *n*-hexane/*tert*-butyl methyl ether/2-propanol 50/44/6 was used as the eluent working at a flow rate of 16 ml/min and with UV monitoring at 220 nm. Three separate fractions were collected. Optically pure (*S*,*S*,*S*)-**11** (2.043 g) and (*R*,*R*,*R*)-**11** (2.006 g) were respectively obtained by evaporation of the first and third fractions. The second fraction contained 38 mg of a 22/78 mixture of (*S*,*S*,*S*)-**11**/(*R*,*R*,*R*)-**11** and was discarded.

(*S*,*S*,*S*)-**11**: colorless oil. $[\alpha]_D$ –9.6 (*c* 0.92, $CHCl_3$).

(*R*,*R*,*R*)-**11**: colorless oil. $[\alpha]_D$ +9.5 (*c* 0.95, $CHCl_3$).

Spectroscopic data for (*S*,*S*,*S*)- and (*R*,*R*,*R*)-**11** are identical to those given above for the racemic compound.

Synthesis of (2*S*,3a*S*,7a*S*)-*N*-(benzyloxycarbonyl)-2-methyloctahydroindole-2-carboxylic acid [(*S*,*S*,*S*)-12]. A 1M solution of potassium hydroxide in methanol (50 ml) was added to (*S*,*S*,*S*)-**11** (2.04 g, 6.16 mmol) and the reaction mixture was heated under reflux for 24 h. After evaporation of the solvent, the residue was taken up in water and washed with diethyl ether. The aqueous phase was acidified with 5% aqueous $KHSO_4$ and extracted with ethyl acetate (3 x 50 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and filtered. Evaporation of

the solvent gave (*S*,*S*,*S*)-**12** as a white foam (1.94 g, 6.11 mmol, 99% yield). $[\alpha]_D$ +11.8 (*c* 0.81, MeOH). IR (KBr) ν 3500–2480, 3064, 2931, 1743, 1697 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.03–1.35 (m, 2H), 1.44–1.78 (m, 6H) overlapped with 1.53, 1.62 (two s, 3H), 2.05, 2.26 (two m, 1H), 2.40–2.55 (m, 2H), 3.88, 3.95 (two m, 1H), 5.05–5.14 (m, 2H), 7.22–7.38 (m, 5H), 9.48 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (duplicate signals are observed for most carbons) 20.37, 20.59; 21.98, 23.05; 23.49, 23.65; 25.53, 25.60; 26.00, 27.05; 34.46, 34.98; 39.63, 41.24; 58.61, 58.98; 64.95, 65.74; 66.89, 66.94; 127.66, 127.70; 127.77; 127.88; 128.29; 128.42; 136.41, 136.58; 153.49, 154.51; 179.24, 180.82. HRMS (ESI neg.) $C_{18}H_{22}NO_4$ $[M–H]^-$: calcd 316.1554, found 316.1554.

Synthesis of (2*R*,3a*R*,7a*R*)-*N*-(benzyloxycarbonyl)-2-methyloctahydroindole-2-carboxylic acid [(*R*,*R*,*R*)-12]. An identical procedure to that described above was applied to transform (*R*,*R*,*R*)-**11** (2.01 g, 6.06 mmol) into (*R*,*R*,*R*)-**12** (1.91 g, 6.03 mmol, 99% yield). $[\alpha]_D$ –12.0 (*c* 0.85, MeOH). HRMS (ESI neg.) $C_{18}H_{22}NO_4$ $[M–H]^-$: calcd 316.1554, found 316.1558. Spectroscopic data are the same as those described for (*S*,*S*,*S*)-**12**.

RESULTS AND DISCUSSION

Synthesis of a racemic precursor

The first stage in the synthesis of enantiomerically pure (*S*,*S*,*S*)- and (*R*,*R*,*R*)-(αMe)Oic involved the preparation of a racemic common precursor to be subsequently subjected to HPLC resolution. The synthesis of such a precursor was addressed by modification of the asymmetric route previously reported[13] for the preparation of enantiopure (*S*,*S*,*S*)-(αMe)Oic, which is presented in Fig. 2. It began with the hydrogenation of commercially available (*S*)-indoline-2-carboxylic acid, (*S*)-**1**, using PtO_2 as a catalyst to afford a 90:10 mixture of the (*S*,*S*,*S*) and (*S*,*R*,*R*)

octahydroindole derivatives. The two epimeric compounds retain the (*S*) configuration at the α carbon present in the starting material and differ in the stereochemistry of the two new chiral centers formed. The desired (*S*,*S*,*S*) stereoisomer was isolated pure by recrystallization and was protected at the carboxylic acid and amino functionalities to give (*S*,*S*,*S*)-**4**. This *N*-Boc amino ester underwent a stereoselective α-methylation reaction and the major diastereoisomer, (*S*,*S*,*S*)-**5**, was isolated by column chromatography. Subsequent deprotection of the carboxylic acid yielded (*S*,*S*,*S*)-(αMe)Oic in the *N*-Boc protected form, that is, adequately protected for use in peptide chemistry.

<Figure 2>

The racemic version of this methodology would require the use of racemic indoline-2-carboxylic acid (*rac*-**1**) as a substrate (Fig. 3). Alternatively, the completely unsaturated compound, indole-2-carboxylic acid (**8**) (Fig. 3), can be envisaged as a less expensive starting material for the preparation of the racemate (*S**,*S**,*S**)-**4**. However, according to the literature,[18,19] the catalytic hydrogenation of the indole system requires harsh reaction conditions (high temperatures and pressures of hydrogen gas). Yet, some ester derivatives of **8** have been reported[20] to undergo hydrogenation under Rh/C catalysis and hydrogen pressure at room temperature.

<Figure 3>

Based on this precedent, we undertook the preparation of the target amino acids, (*S*,*S*,*S*)- and (*R*,*R*,*R*)-(αMe)Oic, starting from an adequately protected derivative of indole-2-carboxylic acid (**8**), namely the *N*-Boc protected ester **9** (Fig. 3). The latter compound was readily obtained by reaction of **8** with methanol in the presence of sulfuric acid followed by treatment with di-*tert*-butyl dicarbonate. The hydrogenation of **9** using PtO_2 as a catalyst was accomplished at room temperature and atmospheric pressure of hydrogen gas. It seems, therefore, that both the amino

and carboxylic acid functions in **8** are to be in the protected form (as are in **9**) for the hydrogenation of the indole system to proceed under such mild conditions. The choice of PtO_2 as the catalyst for this reaction was based on our previous observation[13] that the aromatic ring in (*S*)-**1** is efficiently hydrogenated at atmospheric pressure in the presence of PtO_2 (Fig. 2), whereas the use of Pd/C or Rh requires high pressure of hydrogen.[21,22] Moreover, we were delighted to observe that the hydrogenation of **9** provided racemic (*S**,*S**,*S**)-**4** as a single product. This differential behavior with respect to that described above for (*S*)-**1** (Fig. 2) is due to the fact the three chiral centers are formed at once upon hydrogenation of **9** (Fig. 3). Accordingly, only the compound exhibiting a *cis* relative disposition of the two bridgehead hydrogen atoms and that at the α position, (*S**,*S**,*S**)-**4**, is obtained in this process.

Therefore, the route in Fig. 3 constitutes a highly efficient manner to access racemic (*S**,*S**,*S**)-**4** as a single stereoisomer in high overall yield (88%) starting from inexpensive indole-2-carboxylic acid (**8**). Certainly, this route is much more convenient for the production of racemic Oic derivatives exhibiting an (*S**,*S**,*S**) relative stereochemistry than the racemic version of the asymmetric synthesis reported before[13] (Fig. 2), that would require the use of indoline-2-carboxylic acid (*rac*-**1**) as a substrate. As outlined above, the procedure in Fig. 3 is more advantageous in terms of accessibility of the starting material (**8** is substantially cheaper than *rac*-**1**), global yield, hydrogenation conditions (the process in Fig. 2 requires heating at 60 ºC), and stereoselectivity of the hydrogenation process (complete *vs* 90:10). The synthetic pathway in Fig. 3 is therefore highly convenient for the production of (*S**,*S**,*S**)-Oic derivatives on a large scale.

Once racemic (*S**,*S**,*S**)-**4** was obtained according to the procedure in Fig. 3, the synthetic route proceeded in a similar way to that described in the asymmetric version previously reported[13] and shown in Fig. 2. Thus, the treatment of (*S**,*S**,*S**)-**4** with lithium diisopropylamide generated an intermediate lithium enolate that reacted with methyl iodide to provide an 89:11 mixture of the

two possible diastereoisomers, (*S**,*S**,*S**)-**5** and (*R**,*S**,*S**)-**6** (Fig. 4). It should be noted that the high steric hindrance imposed by the *cis*-fused cyclohexane ring was responsible for the efficient facial stereodifferentiation observed.[13] The major diastereoisomer, (*S**,*S**,*S**)-**5**, was isolated pure from the crude mixture by column chromatography in 75% yield.

<Figure 4>

At this stage, we undertook the exchange of the *N*-Boc protecting group by the Cbz one, as the latter has the advantage of absorbing in the UV range, thus favoring monitoring of the subsequent chromatographic resolution process. This was achieved by treatment of (*S**,*S**,*S**)-**5** with a saturated solution of hydrogen chloride in ethyl acetate followed by reaction with benzyl chloroformate (Fig. 4). In this way, several grams of (*S**,*S**,*S**)-**11** were prepared in 61% overall yield from indole-2-carboxylic acid (**8**). The intermediate amino ester hydrochloride, (*S**,*S**,*S**)-**10**, furnished single crystals suitable for X-ray diffraction analysis. The crystalline structure (Fig. 5) confirmed the expected (*S**,*S**,*S**) relative stereochemistry. As shown in Fig. 5, the cyclohexane ring adopts a chair conformation, which is slightly distorted to accommodate the *cis*-fused five-membered cycle. The latter exhibits an envelope shape, with one of the carbon atoms involved in the fusion (3a) occupying the flap of the envelope. It should be noted that inversion of the reactions sequence carried out to transform (*S**,*S**,*S**)-**4** into (*S**,*S**,*S**)-**11** was examined, but only poor results were obtained. Thus, when the Cbz group was introduced in (*S**,*S**,*S**)-**4** prior to the α-methylation reaction, the latter process afforded only a 25% yield of (*S**,*S**,*S**)-**11** (Fig. 4). Accordingly, this alternative pathway was discarded.

<Figure 5>

HPLC resolution: Isolation of enantiopure amino acids

Once the racemic precursor of the target amino acids, (*S**,*S**,*S**)-**11**, had been obtained, we addressed its resolution by HPLC. The separation of enantiomers by preparative chromatography using chiral stationary phases is nowadays recognized as a powerful tool to produce enantiopure compounds.[23–25] Polysaccharide-based stationary phases are particularly suitable for this purpose due to their excellent chiral recognition ability and high loading capacity.[23–25] Moreover, HPLC columns containing immobilized cellulose and amylose derivatives have recently become commercially available (Chiralpak® IA, IB, and IC).[26–28] In these columns, the chiral support is covalently bonded to the silica matrix, which results in a high stability in the presence of all organic solvents.[25–29] This feature means a great advantage for resolutions on a preparative scale. Chiral stationary phases of this type, either commercial or made at the laboratory (prior to their commercialization), have been used in our group for the preparative HPLC resolution of a wide variety of non-natural protected amino acids.[30–41]

The resolution of racemic (*S**,*S**,*S**)-**11** was firstly tested at the analytical level using 250 × 4.6 mm Chiralpak® IA, IB, and IC columns, that respectively contain tris(3,5-dimethylphenylcarbamate) of amylose,[26] tris(3,5-dimethylphenylcarbamate) of cellulose,[27] and tris(3,5-dichlorophenylcarbamate) of cellulose as the chiral selector.[28] The screening started using mixtures of *n*-hexane/2-propanol as the eluent. The enantiomers of **11** could not be distinguished on the IA column, whereas both Chiralpak® IB and IC showed excellent enantiodiscrimination abilities, with selectivity factors around 1.6 (Table 1). Replacement of the alcohol component in the eluting mixture by acetone was next tested. This change had little effect for the cellulose-based phases, whereas very much improved the chiral recognition ability of Chiralpak® IA. In fact, an almost complete baseline separation of peaks was achieved when eluting with *n*-hexane/acetone 96/4 (v/v) on this stationary phase (Table 1). In spite of this, it remained much

less effective that the cellulose-derived ones and was therefore not considered for further assays. The addition of a third component to the initial *n*-hexane/2-propanol mixture was then evaluated. Among those tested, *tert*-butyl methyl ether provided the best results, particularly for the IC column (Table 1). Thus, when working on this stationary phase, the presence of a high percentage of this solvent in the mobile phase not only improved the selectivity but also led to a substantial enhancement of the resolution factor. Moreover, this change also resulted beneficial to the sample solubility, which is an extremely important issue for the subsequent scaling-up stage. Accordingly, the analytical conditions considered to be optimal for extension to the preparative-scale enantioseparation were elution with a 50/44/6 (v/v/v) mixture of *n*-hexane/*tert*-butyl methyl ether/2-propanol at a flow rate of 0.8 ml/min on Chiralpak® IC. The profile obtained under such chromatographic conditions is shown in Fig. 6. Further assays carried out on the IC analytical column working in an overload mode confirmed that these eluting conditions provided the optimal results in terms of both resolution and column loadability.

<Table 1>

<Figure 6>

These conditions were subsequently scaled-up for the preparative resolution of (*S**,*S**,*S**)-**11** on a 250 × 20 mm ID Chiralpak® IC column. Successive injections of 600 μl of a highly concentrated solution of the sample in chloroform (600 mg per ml of solvent) were performed (Fig. 7). Each run was collected into three separate fractions according to the cut points indicated in Fig. 7, with equivalent fractions of successive injections being combined. Following this protocol, a total of 4.14 g of racemate were submitted to the resolution procedure, which was completed in about 3 h. Evaporation of the first and third fractions provided 2.04 and 2.01 g of the first and second eluted enantiomers, respectively, both of them in optically pure form, as assessed at the analytical level (Fig. 8). This means that 98% of the injected material was recovered in enantiomerically

pure form after a single passage through the column. The high productivity of the enantioseparation process is therefore remarkable since more than 1 g of optically pure material was isolated per hour working on a semi-preparative size column.

< Figure 7>

<Figure 8>

The absolute configuration of the isolated enantiomers was assigned by comparison of their retention times with that of a sample of enantiomerically pure (*S*,*S*,*S*)-**11** prepared separately. Thus, a small amount of (*S*,*S*,*S*)-**5** obtained from (*S*)-indoline-2-carboxylic acid in our previous work[13] following the synthetic procedure in Fig. 2 was transformed into (*S*,*S*,*S*)-**11** by exchange of the Boc-Cbz *N*-protecting groups, and its retention time was found to be coincident with that of the first eluted enantiomer of **11** (Fig. 8). Accordingly, the more strongly retained enantiomer of **11** was assigned an (*R*,*R*,*R*) configuration.

Finally, each enantiomer of **11** was subjected to saponification to provide the corresponding *N*-Cbz amino acid in almost quantitative yield (Fig. 9). Thus, the target compounds, (*S*,*S*,*S*)- and (*R*,*R*,*R*)-(αMe)Oic, were isolated in enantiomerically pure form and suitably protected for further derivatization, including their direct use in peptide synthesis. The Cbz protecting group can be eliminated to liberate the amino function under mild reaction conditions that do not affect the optical integrity of the compounds.

<Figure 9>

CONCLUSION

We have developed an efficient and practical method for the production of multigram quantities of the α-tetrasubstituted proline analogues (*S*,*S*,*S*)- and (*R*,*R*,*R*)-2-methyloctahydroindole-2-

carboxylic acids in enantiomerically pure form and suitably protected for incorporation into peptides. The procedure relies in the use of inexpensive indole-2-carboxylic acid as the starting material, which allows the preparation of a racemic precursor in high overall yield through simple transformations, and the efficiency of the HPLC resolution process carried out on a chiral column (Chiralpak® IC). The procedure has been applied to the isolation of significant quantities of optically pure compounds and can be easily scaled-up to the production of larger amounts. The amino acids thus prepared are proline analogues of great value in the design of peptides with pharmacological applications and other medicinally relevant compounds.

ACKNOWLEDGMENTS

The authors thank Ana Lidia Bernad for assistance with HPLC.

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FIGURE CAPTIONS

Figure 1. Structure of different proline analogues: α-methylproline [(αMe)Pro], octahydroindole-2-carboxylic acid (Oic), and 2-methyloctahydroindole-2-carboxylic acid [(αMe)Oic]. Chiral carbons in the octahydroindole system (corresponding to positions 2, 3a, and 7a) are indicated with asterisks. All throughout the text, a nomenclature without numerical locants will be used for the sake of simplicity; accordingly, a (2*S*,3a*S*,7a*S*) stereochemistry will be denoted as (*S*,*S*,*S*).

Figure 2. Stereoselective synthesis for enantiopure (*S*,*S*,*S*)-(αMe)Oic previously reported (ref. 13). Abbreviations: Boc, *tert*-butoxycarbonyl; LDA, lithium diisopropylamide.

Figure 3. Synthesis of racemic (*S**,*S**,*S**)-**4**.

Figure 4. Synthesis of the racemic precursor of the target amino acids, (*S**,*S**,*S**)-**11**. Abbreviations: Cbz, benzyloxycarbonyl.

Figure 5. X-ray crystal structure of racemic (*S**,*S**,*S**)-**10** [only the (*S*,*S*,*S*) enantiomer is shown]. Heteroatoms are drawn as thermal ellipsoids. Most hydrogens have been omitted for clarity.

Figure 6. HPLC resolution of (*S**,*S**,*S**)-**11** at the analytical level. Column: Chiralpak® IC (250 × 4.6 mm ID). Eluent: *n*-hexane/*tert*-butyl methyl ether/2-propanol 50/44/6. Flow rate: 0.8 ml/min. UV detection: 210 nm.

Figure 7. Preparative HPLC resolution of (*S**,*S**,*S**)-**11**. Profile corresponding to the injection of 600 μl of a solution of **11** in chloroform (concentration: 600 mg solute / ml of solvent). The cut points for the three fractions collected are indicated. Column: Chiralpak® IC (250 × 20 mm ID).

Eluent: *n*-hexane/*tert*-butyl methyl ether/2-propanol 50/44/6. Flow rate: 16 ml/min. UV detection: 220 nm.

Figure 8. HPLC analytical profile of the resolved enantiomers of **11**. Chromatographic conditions as in Fig. 6.

Figure 9. Preparation of *N*-protected enantiomerically pure (*S*,*S*,*S*)- and (*R*,*R*,*R*)-(αMe)Oic.

Table 1. Selected chromatographic data for the analytical HPLC resolution of compound (*S**,*S**,*S**)-**11** on Chiralpak® IA, IB, and IC columns[a]

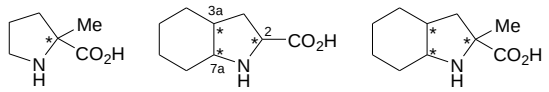
eluent[b]	% (v/v)	k'_1	α	R_S
		Chiralpak® IA		
n-Hx/2-propanol	98/2	2.4	1.0	–
n-Hx/acetone[c]	96/4	2.4	1.1	1.3
		Chiralpak® IB		
n-Hx/2-propanol	98/2	1.6	1.6	5.4
n-Hx/acetone[c]	96/4	2.1	1.5	5.8
n-Hx/*t*-BuOMe/2-propanol	80/19/1	1.6	1.6	5.6
		Chiralpak® IC		
n-Hx/2-propanol	80/20	1.7	1.6	5.2
n-Hx/acetone[c]	90/10[d]	1.0	1.5	5.3
n-Hx/*t*-BuOMe/2-propanol	50/44/6[d]	1.3	1.9	6.7

[a]Column size: 250 × 4.6 mm ID. Flow rate: 1.0 ml/min (unless otherwise indicated). UV detection at 210 nm (unless otherwise indicated). The chromatographic parameters k'_1, α, and R_S are defined in the Materials and Methods section.

[b]*n*-Hx: *n*-hexane; *t*-BuOMe: *tert*-butyl methyl ether.

[c]UV detection at 220 nm.

[d]Flow rate: 0.8 ml/min.



(αMe)Pro **Oic** **(αMe)Oic**

Figure 1

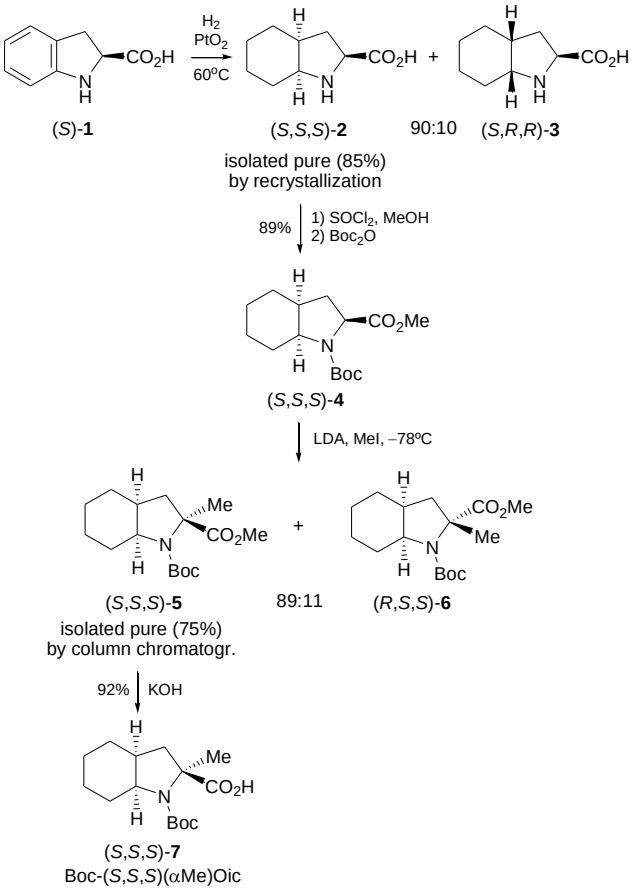

H2
PtO2
60°C
CO2H
(S)-1
(S,S,S)-2
90:10
(S,R,R)-3
isolated pure (85%)
by recrystallization
89%
1) SOCl2, MeOH
2) Boc2O
CO2Me
Boc
(S,S,S)-4
LDA, MeI, −78°C
Me
(S,S,S)-5
89:11
(R,S,S)-6
isolated pure (75%)
by column chromatogr.
92%
KOH
(S,S,S)-7
Boc-(S,S,S)(αMe)Oic

Figure 2

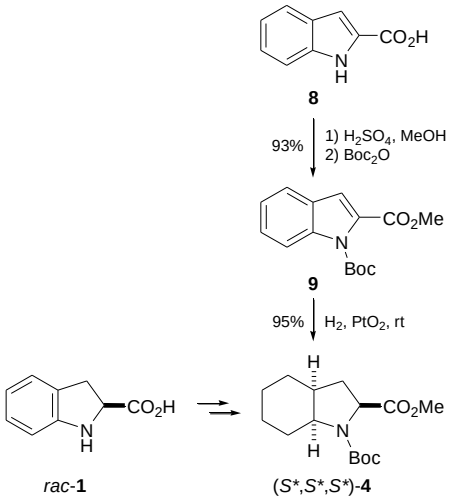

CO2H
N
H
8
93%
1) H2SO4, MeOH
2) Boc2O
CO2Me
N
Boc
9
95%
H2, PtO2, rt
CO2H
N
H
H
CO2Me
N
H
Boc
rac-1
(S*,S*,S*)-4

Figure 3

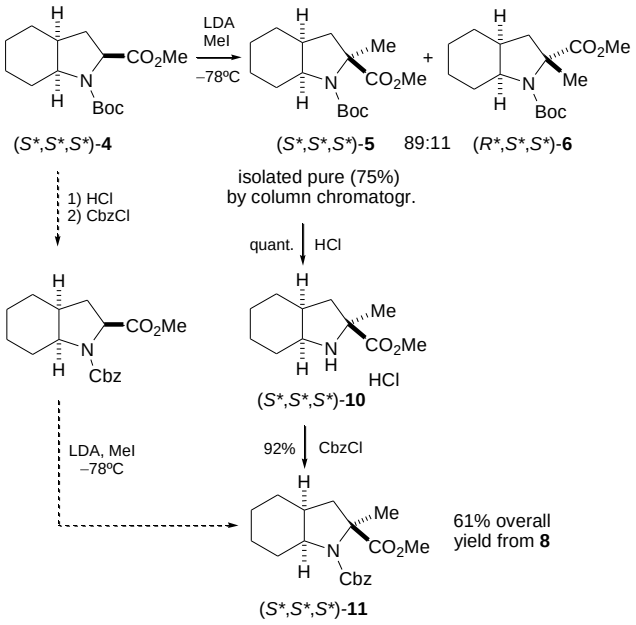

LDA
MeI
–78ºC
(S*,S*,S*)-4
(S*,S*,S*)-5
89:11
(R*,S*,S*)-6
isolated pure (75%)
by column chromatogr.
1) HCl
2) CbzCl
quant.
HCl
(S*,S*,S*)-10
LDA, MeI
–78ºC
92%
CbzCl
61% overall
yield from 8
(S*,S*,S*)-11

Figure 4

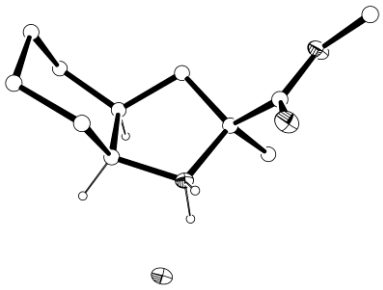


Figure 5

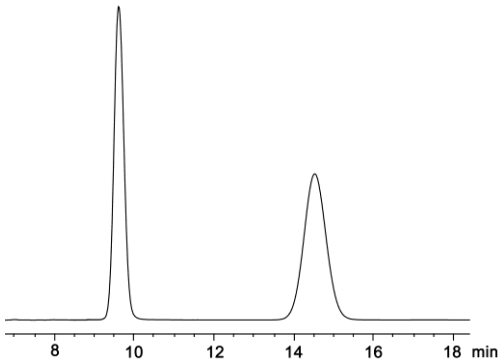


Figure 6

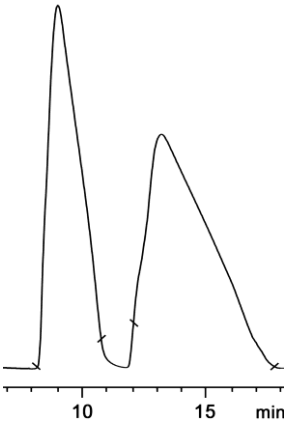


Figure 7

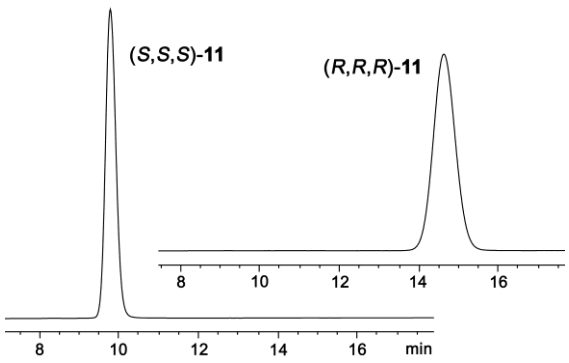


Figure 8

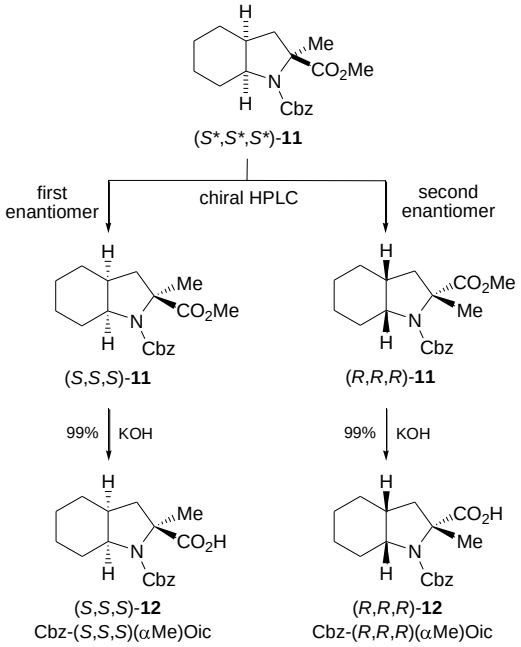

(S*,S*,S*)-11
first enantiomer
chiral HPLC
second enantiomer
(S,S,S)-11
(R,R,R)-11
99% KOH
99% KOH
(S,S,S)-12
Cbz-(S,S,S)(αMe)Oic
(R,R,R)-12
Cbz-(R,R,R)(αMe)Oic

Figure 9