Organic Process Research & Development **2002**, *6*, 762−766

Chemoenzymatic Synthesis of the Four Diastereoisomers of 4-Hydroxypipecolic Acid from *N*-Acetyl-(*R*,*S*)-allylglycine: Chiral Scaffolds for Drug Discovery

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Abstract:
All four diastereoisomers of 4-hydroxypipecolic acid were prepared in a form conveniently protected for drug discovery applications with the use of industrially scaleable methodology. Resolution of the racemic starting material using proprietary acylases followed by an acyliminium ion cyclisation gave diastereomeric mixtures of 4-formyloxypipecolic acid, which were differentiated using an enzyme-catalysed hydrolysis. The products were separated by partition, and by following a sequence of straightforward chemical steps, the individual stereoisomers of the protected 4-hydroxypipecolates were crystallized to optical purity in 100 g quantities.

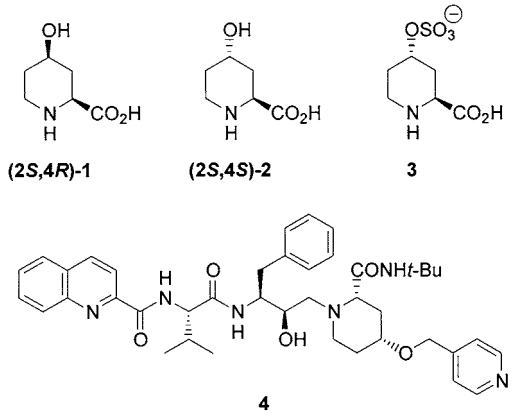

Figure 1.

Introduction

Cyclic molecules bearing three functional groups, which may be elaborated into a wide array of drug-like entities, are potentially valuable as scaffolds in drug discovery. In particular, where the presence of the functional groups creates stereocentres, there may be several stereoisomers of the resultant scaffold. Thus, for example, we have recently described the synthesis and potential utilization of a range of enantiopure 4-(*tert*-butoxycarbonylamino)cyclopent-1-enecarboxylic acid methyl ester scaffolds.[1] In the event that one isomer of such a scaffold should prove valuable as a component of a drug candidate, then it is essential that the methods used in the synthesis are appropriate to large-scale manufacture. In this respect, these cyclopentenecarboxylate scaffolds are accessed through a combination of scaleable bioresolution and catalytic diastereoselective hydrogenation methods. In seeking to develop other useful trifunctional cyclic scaffold structures, we were drawn to the 4-hydroxypipecolic acids **1** and **2** (Figure 1). These are naturally occurring nonproteinogenic amino acids that have been isolated from the leaves of *Calliandra pittieri*, *Strophantus scandeus*, and *Acacia oswaldii*.[2] With their rigid structure and multiple functionality they make ideal candidates for use as scaffolds around which compound libraries may be designed for drug discovery. Indeed, molecules derived from both **1** and **2** have been demonstrated to possess biological activity; the naturally occurring sulphate **3**[3] is an NMDA receptor agonist,[4] and **1** is a constituent of the synthetic HIV protease inhibitor, palinavir **4**.[5] Although many elegant syntheses of the enantiomers of **1** exist,[6] preparations of the *trans*-diastereoisomer **2** are less common, involving either chromatographic separation of the *cis*- and *trans*-diastereomers,[4] or chemistry that is unfavourable on scale.[7] Our objective was thus to identify access to the individual enantiomers of both the *cis*-(**1**) and *trans*-(**2**) 4-hydroxypipecolates, in suitably protected form for library synthesis, via an easily scaleable route from readily available starting materials.

Results and Discussion

Piperidine Ring Synthesis. Preparation of the piperidine ring system is outlined in Scheme 1. This route is based on the known bioresolution of racemic *N*-acetyl amino acids

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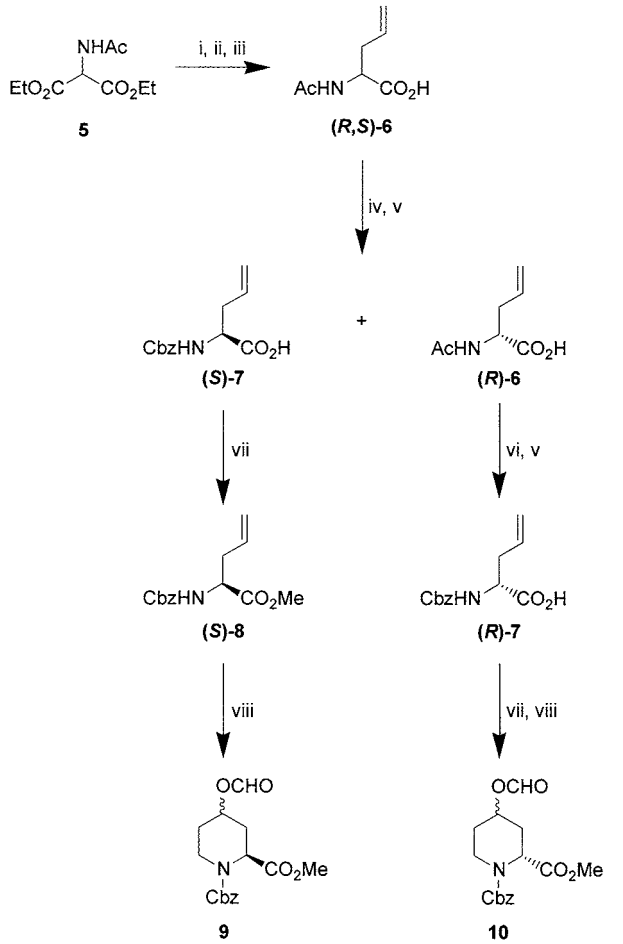
Published on Web 09/19/2002
10.1021/op020017x CCC: $22.00

Scheme 1[a]



[a] Reagents used: (i) *t*-BuOK, allyl bromide; (ii) NaOH; (iii) HCl; (iv) L-acylase, pH 8.0, 65 °C; (v) Cbz-Cl, pH 8.0; (vi) D-acylase, pH 8.0; (vii) $SOCl_2$, MeOH; (viii) paraformaldehyde, HCO_2H.

with aminoacylases[8] and the reported acyliminium ion cyclisation of allylglycine.[9] Condensation of allyl bromide with the diethylacetamidomalonate anion,[10] followed by hydrolysis and decarboxylation, gave the *N*-acetyl amino acid **(*R*,*S*)-6**. Small-scale experiments showed that **(*R*,*S*)-6** was a good substrate for both our recombinant *Thermococcus litoralis* L-aminoacylase[11] (extremophile) and *Alcaligenes sp.* D-aminoacylase[12] enzymes, and in the interests of cost it was decided to use these enzymes in preference to commercially available ones.[13] Hence treatment with L-aminoacylase at 60 °C and pH 8.0 gave a mixture of (***S***)-allylglycine and unreacted **(*R*)-6**. This mixture was reacted with benzyl chloroformate at pH 8.0, and after acidification to pH 2.0, *N*-Cbz-(*S*)-allylglycine **(*S*)-7** was extracted cleanly into toluene (36% yield from racemate, >99% ee). Concentration of the aqueous fraction and extraction into EtOAc allowed recovery of **(*R*)-6** (49%, 80% ee). Treatment of this material

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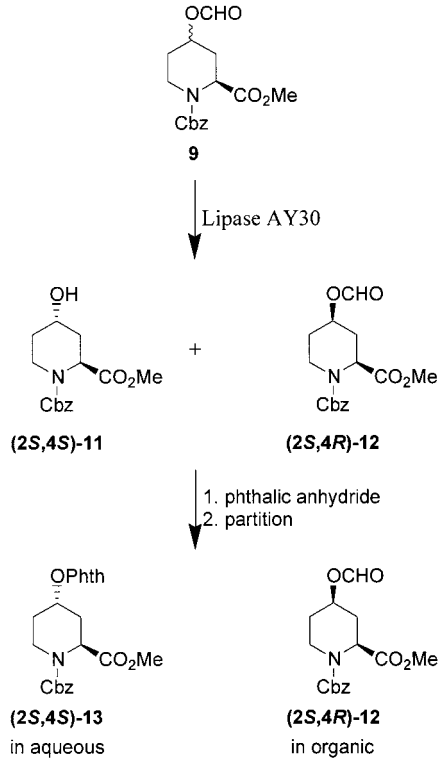
(13) At the time, there was no commercially available D-acylase enzyme.

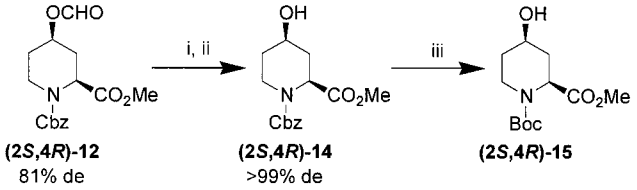
Scheme 2



with D-aminoacylase at pH 8.0 followed by reaction with benzyl chloroformate and extraction as above gave *N*-Cbz-(*R*)-allylglycine **(*R*)-7** (28% overall yield from racemate, >99% ee). The protected amino acids were subsequently esterified in thionyl chloride/methanol. Reaction of the ester **(*S*)-8** in a solution of paraformaldehyde in formic acid using the procedure of Esch et al.[9] gave the 4-formyloxy-(*S*)-pipecolic acid methyl ester **9**, as 1:1 mixture of diastereomers at the 4-position of the piperidine ring, the stereochemistry of the 2-position being previously fixed as that of the amino acid starting material. Compound **(*R*)-8** was elaborated in a similar fashion to give the diastereoisomeric mixture **10**.

Separation of Diastereomers and Synthesis of (2*S*,4*R*)-15 and (2*R*,4*S*)-15. Although TLC distinguished the two diastereomers in mixtures **9** and **10**, on scale their separation by chromatography was unfeasible. An enzyme screen of eight commercial enzymes showed both Lipase AY30 (*Candida rugosa*, Amano) and Chirazyme L1 (*Burkholderia cepacia*, Roche) selectively hydrolysed one of the formate groups of mixture **9**. Interestingly, it was noted these enzymes hydrolysed different diastereoisomers and it was subsequently determined that Lipase AY30 preferentially hydrolysed the *trans*-diastereoisomer, whereas Chirazyme L1 hydrolysed the *cis*-diastereoisomer. However, Lipase AY30 was the more selective enzyme and the reaction was duly scaled up. Thus, treatment of mixture **9** with Lipase AY30 at pH 7.0 yielded the (2*S*,4*S*)-4-hydroxypipecolic acid methyl ester **11** (90% de) and the (2*S*,4*R*)-4-formyloxypipecolic acid methyl ester **12** (81% de) (Scheme 2). Although these two compounds were themselves inseparable, reaction of the mixture with phthalic anhydride converted **(2*S*,4*S*)-11** to the hemiphthalate ester derivative **(2*S*,4*S*)-13**, which could be separated from **(2*S*,4*R*)-12** by partitioning between toluene and saturated

Scheme 3[a]



[a] Reagents used: (i) K_2CO_3, MeOH; (ii) recrystallise; (iii) H_2, 10% Pd/C, Boc_2O.

ammonium carbonate solution.[14] Concentration of the toluene layer gave **(2*S*,4*R*)-12** (51% overall yield from input into biotransformation). The hemiphthalate ester **(2*S*,4*S*)-13** was recovered by acidification of the aqueous layer and extraction into toluene (38% yield from input into biotransformation).

The formate ester **(2*S*,4*R*)-12** was elaborated into the desired product, (2*S*,4*R*)-*N*-Boc-2-carbomethoxy-4-hydroxypiperidine **15** as outlined in Scheme 3. After deformylation of **(2*S*,4*R*)-12** (81% de) using K_2CO_3/MeOH, the resultant **(2*S*,4*R*)-14** (90%, 81% de) was recrystallised to optical purity from EtOAc. Removal of the Cbz group followed by in situ Boc protection gave **(2*S*,4*R*)-15** in 83% yield (>98% ee, >98% purity).

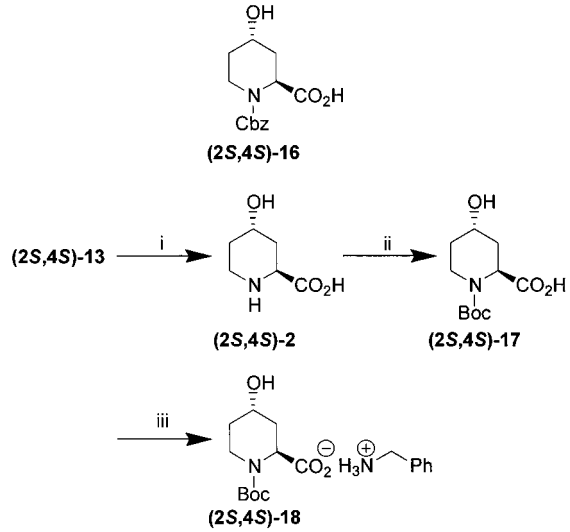
An enzyme screen showed both Chirazyme L9 (*Mucor miehei*, Roche) and Lipase AY30 differentiated the diastereomers present in the mixture **10**. Both enzymes preferentially hydrolysed the *trans*-diastereoisomer, but Chirazyme L9 was the most selective and hence was used in the large-scale biotransformation. After this slow bioresolution, reaction with phthalic anhydride and partitioning as before allowed the separation of **(2*R*,4*S*)-12** from the hemiphthalate ester derivative **(2*R*,4*R*)-13**. **(2*R*,4*S*)-12** was elaborated as in Scheme 3 to give the desired product, **(2*R*,4*S*)-15**.

Elaboration of 13 to *trans*-4-Hydroxypipecolic Acid. Attempts to purify and enhance the de of **(2*S*,4*S*)-13** by crystallisation as an amine salt were unsuccessful. Although hydrolysis of both ester moieties was readily achieved, separation of the Cbz-protected acid **(2*S*,4*S*)-16** from phthalic acid proved difficult. Acid-catalyzed hydrolysis of **(2*S*,4*S*)-13** in HCl, followed by neutralization with Amberlite IRA-93 yielded the free amino acid **(2*S*,4*S*)-2** in 79% yield without any significant epimerisation of either chiral centre (Scheme 4). Boc protection was carried out using triethylamine as the base in a modified Schotten−Baumann procedure, and conveniently, the Boc-acid **(2*S*,4*S*)-17** was crystallised from MeOH/MTBE to >98% de, >95% purity, as the benzylamine salt **18** in which form it was stored. **(2*R*,4*R*)-18** was prepared from hemiphthalate **(2*R*,4*R*)-13** in identical fashion.

Conclusions

From a single racemic starting material, all four stereoisomers of 4-hydroxypipecolic acid have been prepared in suitably protected forms for use as chiral scaffolds in drug discovery. Individual chiral centres have been defined, and optical purity obtained using a powerful combination of biotransformation and crystallisation. The methods used

Scheme 4[a]



[a] Reagents used: (i) 2 M HCl, then Amberlite IRA-93; (ii) Boc_2O, Et_3N; (iii) $BnNH_2$, recrystallise.

should be scaleable for manufacture.

Experimental Section

General. All reagents and solvents were from commercially available sources and used as received. NMR spectra were obtained on a Bruker Avance 400 spectrometer operating at 400 MHz for proton (^1H) and 100 MHz for carbon (^{13}C). Chemical shifts are reported in ppm using Me_4-Si or residual nondeuterated solvent as reference. Coupling constants (*J*) are measured in Hz. GC−MS data were obtained using a HP 5890 series 2 GC fitted with a J&W Scientific DB5 column, attached to a HP 5972 series mass selective detector. De and ee data of final compounds were obtained using a Perkin-Elmer Autosystem Gas Chromatograph fitted with a Varian Chirasil-Dex CB column. All samples were run as the 2-carbomethoxy-4-acetoxy derivative. HPLC data were obtained using a Gilson HPLC system fitted with a Luna phenylhexyl column. Optical rotation data were obtained using a Perkin-Elmer Polarimeter 341 instrument.

***N*-Acetyl-(*R*,*S*)-allylglycine 6.** Sodium methoxide (25 wt % in methanol, 9.0 kg, 9.5 L, 41.5 mol) was added to a suspension of diethylacetamidomalonate (8.0 kg, 36.8 mol) in methanol (15 kg, 19 L), and the mixture was heated to reflux for 30 min. The heat source was removed, and allyl bromide (4.48 kg, 3.2 L, 37.0 mol) was added at a rate sufficient to maintain a gentle reflux. This reflux was maintained for 30 min, after which water (10 L) was added until all solids had dissolved, and the solution was adjusted to pH 10 with 40% aqueous NaOH (7.25 kg), keeping the reaction temperature below 50 °C. After the mixture refluxed for 2 h, the methanol was removed by vacuum distillation and the resultant solution cooled to 40 °C. The pH was adjusted to 3.5 with concentrated HCl (3.7 kg) and stirred for 2 h, after which more concentrated HCl (1.5 L) was added to reduce the solution pH to 2. This was extracted with EtOAc (4 × 9 L), and the combined organic layers were concentrated to yield *N*-acetyl-(*R*,*S*)-allylglycine, **6**, a yellow semisolid (3.25 kg, 56%). ^1H NMR (d_6-DMSO) 8.09 (1H,

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d, *J* 8), 5.73 (1H, m), 5.08 (1H, dd, *J* 17, 2), 5.03, (1H, m), 4.21 (1H, m), 2.42 (1H, m), 2.30 (1H, m), 1.83 (3H, s). ^{13}C NMR (d_6-DMSO) 173.4, 169.5, 134.4, 118.0, 52.1, 35.8, 22.7.

***N*-Benzyloxycarbamoyl-(*S*)-allylglycine (*S*)-7.** To a solution of **(*R*,*S*)-6** (3.2 kg, 20.4 mol) in pH 8.0 Tris-HCl (32 L) at 70 °C was added the cell paste containing the L-aminoacylase (0.32 kg, 3 units/g substrate), and the reaction mixture was stirred at this temperature until GC analysis dictated the reaction was 44% complete. Celite (4.8 kg) was added and the mixture filtered. THF (31 kg, 35 L) was added to the filtrate and the solution cooled to 5 °C. Benzyl chloroformate (1.25 kg, 7.34 mol) was added, keeping the temperature below 10 °C, and using 40% aqueous NaOH, the pH was kept in the 7.5−8.5 range. Stirring was maintained at 10 °C until there was no further pH change, at which point the THF was removed by distillation. The aqueous layer was washed with MTBE (2 × 17 L), acidified to pH 2 using concentrated HCl, and extracted with toluene (3 × 21 L). These toluene layers were combined, washed with 10% NaCl (9 L), and concentrated to minimum volume to yield *N*-benzyloxycarbamoyl-(*S*)-allylglycine, **(*S*)-7**, (1.20 kg, 24%, >99% ee) as a waxy solid. ^{1}H NMR (d_6-DMSO) major rotamer 7.55 (1H, d, *J* 8), 7.34 (5H, m), 5.76 (1H, m), 5.12−5.00 (4H, m), 4.02 (1H, m), 2.44 (1H, m), 2.34 (1H, m). ^{13}C NMR (d_6-DMSO) 173.5, 156.4, 137.3, 134.6, 128.8, 128.4, 128.1, 118.3, 65.7, 54.2, 35.5.The aqueous layer was concentrated to one-third volume and extracted with EtOAc (4 × 11 L). These extracts were combined and concentrated to give the residual **(*R*)-6**, a brown solid (1.17 kg, 36%, 80% ee).

***N*-Benzyloxycarbamoyl-(*R*)-allylglycine (*R*)-7.** A solution of 80% ee **(*R*)-6** (1.17 kg, 7.45 mol) in Tris-HCl pH 8.0 (11.7 L) was heated to 40 °C and D-Aminoacylase enzyme (0.64 L, 50 units/g substrate) added. The mixture was stirred at 40 °C until GC analysis showed the reaction to be 86% complete (24 h), when Celite (1.8 kg) was added and the mixture filtered. THF (14 kg, 16 L) was added to the filtrate and the solution cooled to 5 °C. Benzyl chloroformate (1.05 kg, 6.15 mol) was added, keeping the temperature below 10 °C, and using 40% aqueous NaOH the pH was kept in the 7.5−8.5 range. Stirring was maintained at 10 °C until there was no further pH change, at which point the THF was removed by distillation. The aqueous layer was washed with MTBE (2 × 7.5 L), acidified to pH 2 using concentrated HCl, and extracted with toluene (3 × 10 L). These toluene layers were combined, washed with 10% NaCl (5 L), and concentrated to minimum volume to yield *N*-benzyloxycarbamoyl-(*R*)-allylglycine **(*R*)-7** (0.68 kg, 37%, >99% ee) as a waxy solid. ^{1}H NMR (d_6-DMSO) major rotamer 7.55 (1H, d, *J* 8), 7.34 (5H, m), 5.76 (1H, m), 5.12−5.00 (4H, m), 4.02 (1H, m), 2.44 (1H, m), 2.34 (1H, m). ^{13}C NMR (d_6-DMSO) 173.5, 156.4, 137.3, 134.6, 128.8, 128.4, 128.1, 118.3, 65.7, 54.2, 35.5.

***N*-Benzyloxycarbamoyl-(*S*)-allylglycine Methyl Ester (*S*)-8.** A solution of **(*S*)-7** (1.13 kg, 4.53 mol) in methanol (6 L) was cooled to 0 °C, and thionyl chloride (2.83 kg, 1.74 L, 23.8 mol) was added dropwise, keeping the temperature below 5 °C. The reaction was stirred at this temperature for 1 h and then was allowed to warm to room temperature and was stirred overnight. The excess solvent was removed in vacuo and the residue dissolved in MTBE (6 L). The solution was washed with 1 M HCl (3.4 L) and saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated to yield **(*S*)-8** as a yellow oil (0.86 kg, 73%). ^{1}H NMR (d_6-DMSO) 7.75 (1H, d, *J* 8), 7.33 (5H, m), 5.75 (1H, m), 5.09 (1H, m), 5.05−5.00 (3H, m), 4.10 (1H, m), 3.60, (3H, s), 2.45 (1H, m), 2.34 (1H, m). ^{13}C NMR (d_6-DMSO) 173.5, 156.3, 137.2, 134.2, 128.6, 128.2, 128.0, 118.0, 65.8, 54.0, 52.2, 35.5.

Using an identical procedure, **(*R*)-7** (0.68 kg, 2.73 mol) gave **(*R*)-8** (0.72 kg, 100%).

(2*S*,4*R*,*S*)-*N*-Benzyloxycarbamoyl-2-carbomethoxy-4-formyloxypiperidine 9.[9] Paraformaldehyde (144.0 g, 4.8 mol) was dissolved in hot formic acid (6.5 L) and the resultant solution cooled to 25 °C. **(*S*)-8** (904.3 g, 3.4 mol) was added and the solution stirred for 72 h, at which time GC analysis showed no starting material remained. Excess solvent was removed in vacuo, and the residual oil was dried by azeotroping with toluene (4 × 750 mL) and passed through a silica plug, eluting with EtOAc. Evaporation of the solvent in vacuo left **9** as a yellow oil (1056.3 g, 96%). GC retention times 26.9 min (*trans*-diastereoisomer) 27.6 min (*cis*-diastereoisomer) in a 1:1 ratio. *m*/*z* 321 (M^+), 262, 216, 172, 140, 91.

Using an identical method, **(*R*)-8** (713 g, 2.71 mol) gave **10** (722 g, 83%), which proved spectroscopically identical to **9**.

Separation of Mixture 9. A 10-L jacketed reaction vessel equipped with an overhead stirrer was charged with **9** (1056.3 g), MTBE (3.6 L), and 50 mM potassium phosphate buffer pH 7.0 (4.5 L). Stirring was started to achieve an emulsion and Lipase AY30 (300 g) added. The pH was kept constant at pH 7.0 by the addition of 5 M NaOH. After 4 days at 20 °C the reaction was stopped by filtration through Celite 521. The two layers in the filtrate were separated, and the organic layer was reserved. The Celite was slurried with acetone (500 mL) and filtered. This filtrate was concentrated in vacuo until only aqueous material remained, when it was extracted with MTBE (2 × 500 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo to yield a viscous, cloudy yellow oil (903 g) that was a mixture of (2*S*,4*S*)-*N*-benzyloxycarbamoyl-2-carbomethoxy-4-hydroxypiperidine, **11**, of 90% de and (2*S*,4*R*)-*N*-benzyloxycarbamoyl-2-carbomethoxy-4-formyloxypiperidine, **12**, of 83% de, in an approximate 1:1 ratio. This mixture and DMAP (17.9 g, 0.14 mol) was dissolved in CH_2Cl_2 (6 L) at 20 °C. Et_3N (450 mL, 3.22 mol) was added using a pressure-equalising dropping funnel over a 10-min period. Solid phthalic anhydride (239 g, 1.61 mol) was added batch-wise and stirring continued for 18 h. The reaction mixture was washed with 1 M HCl (3.5 L), and the organic layer concentrated in vacuo. The residue was redissolved in toluene (4 L) and extracted with saturated $(NH_4)_2CO_3$ (3 L). This aqueous layer was washed with toluene (1 L), and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield **(2*S*,4*R*)-12** (545 g, 81% de, 52%). The aqueous layer was acidified to pH 1 with concentrated HCl and extracted with toluene (2 L). The layers were separated, and the

aqueous was extracted once more with toluene (1 L). These two organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo to yield (2*S*,4*S*)-*N*-benzyloxycarbamoyl-2-carbomethoxy-4-hydroxypiperidine, 4-hemiphthalate derivative, **13**, (553 g, 38%). Both products were used directly in the next steps.

Separation of Mixture 10. The separation was carried out in an identical manner, substituting Chirazyme L9 for Lipase AY30. Hence, **10** (722 g, 2.25 mol) was separated into **(2*R*,4*S*)-12** (370 g, 51%) and **(2*R*,4*R*)-11** (80% de, 426 g, 43%).

(2*S*,4*R*)-*N*-Benzyloxycarbamoyl-2-carbomethoxy-4-hydroxypiperidine 14.[15] **(2*S*,4*R*)-12** (545 g, 81% de, 1.70 mol) was dissolved in MeOH (1.5 L) and K_2CO_3 (23.5 g, 0.17 mol) added. The mixture was stirred for 2 h at room temperature, by which time the reaction was complete. MTBE (5 L) was added and the solution washed with H_2O (3 L). The organic phase was dried and concentrated in vacuo. This residue was triturated with EtOAc (600 mL) and heptane (75 mL) to yield (2*S*,4*R*)-*N*-benzyloxycarbamoyl-2-carbomethoxy-4-hydroxypiperidine, **14**, (444.1 g, 90%, 81% de) which was recrystallised from EtOAc (850 mL) to yield optically pure **(2*S*,4*R*)-14** (145.4 g, >99% de). Further crops of identical quality crystals (58.2 g, 33.2 g, both >99% de) were obtained from the liquors (overall yield 48% from **(2*S*,4*R*)-12**). ^{1}H NMR (d_6-DMSO) 7.37 (5H, m) 5.08 (2H, m) 4.64 (2H, m) 3.90 (1H, br s) 3.70 (1H, dt, *J* 8.5, 3.5) 3.59 (3H, br s) 3.47−3.27 (1H, br m) 2.19 (1H, m) 1.82 (1H, dd, *J* 13.5, 6.5) 1.54 (2H, m). ^{13}C NMR (d_6-DMSO) 172.2, 172.0 (rotamer pair), 155.9, 155.5 (rotamer pair), 137.0, 128.6, 128.0, 127.6, 127.5, 66.5, 66.4 (rotamer pair), 51.8, 51.0, 50.8 (rotamer pair), 36.0, 35.9 (rotamer pair), 33.3, 31.0, 30.9 (rotamer pair). GC (material derivatised to acetate) gave retention times 28.2 min (minor diastereoisomer), 29.0 min (major diastereoisomer) in a ratio 1:220. *m*/*z* (as acetate) 335 (M^+), 276, 216, 172, 140, 91.

Using an identical method, 80% de **(2*R*,4*S*)-12** (370 g, 1.15 mol) gave **(2*R*,4*S*)-14** (>99% de, 144 g, 43%).

(2*S*,4*R*)-*N*-*tert*-Butyloxycarbamoyl-2-carbomethoxy-4-hydroxypiperidine 15. (2*S*,4*R*)-14 (144.0 g, 0.49 mol) was dissolved in EtOAc (2.8 L) and Boc_2O (112.8 g, 0.52 mol) added. 10% Pd/C (14 g, 10 wt %) was added and the suspension placed in a 10-L pressure vessel. The vessel was purged with N_2 and charged to 2 bar with H_2. Stirring was maintained until no further H_2 was taken up. The vessel was purged with N_2, and the contents were filtered through Celite and concentrated in vacuo. The residue was purified on a silica plug to yield **(2*S*,4*R*)-15** (106 g, 83%, >98% ee, >99% de, purity (GC) >98%) as a colourless glass. ^{1}H NMR 4.59 (1H, br), 4.00 (1H, m) 3.73 (1H, m), 3.68 (3H, s), 3.41−3.25 (1H br m), 2.37 (1H, br d, *J* 13), 1.87 (1H, dd, *J* 13, 6), 1.62 (2H, m) 1.43 (9H, app d, rotamer pair). ^{13}C NMR (d_6-DMSO) 172.3, 172.2 (rotamer pair), 155.4, 155.0 (rotamer pair), 79.2, 61.2, 51.7, 51.4, 50.2 (rotamer pair), 36.2, 35.3 (rotamer pair), 33.4, 31.2, 31.0 (rotamer pair) 28.1. $[\alpha]^{25}_D$ −24.1 (*c* 2.74, EtOH). GC (material derivatised to acetate) gave retention times 21.8 min (minor diastereoisomer) and 22.3 min (major diastereoisomer) in a ratio of 143:1. *m*/*z* (as acetate) 301 (M^+), 242, 182, 140, 126, 82, 57.

Using an identical method, >99% de **(2*R*,4*S*)-14** (141 g, 0.48 mol) gave **(2*R*,4*S*)-15** (100 g, 80%, >99% ee, >99% de, purity (GC) >98%).

(2*S*,4*S*)-4-Hydroxypipecolic Acid 2. 11 (365 g, 0.82 mol) was mixed with 2 M HCl (1.5 L) and heated to reflux for 5 days. The mixture was cooled and extracted with EtOAc (3 × 1 L). The aqueous layer was concentrated in vacuo to leave a cloudy paste (170 g). This was redissolved in H_2O (500 mL) and the solution neutralised using Amberlite IRA-93. The resin was filtered and washed with H_2O (1.5 L), and the filtrate was concentrated in vacuo and dried by azeotroping with toluene (2 × 500 mL) to leave **(2*S*,4*S*)-2**, a cream solid (94.5 g, 79%). ^{1}H NMR (D_2O) Major diastereoisomer 4.21 (1H, m) 3.90 (1H, dd, *J* 11.5, 3.5) 3.28 (2H, m) 2.20 (1H, m) 1.97−1.84 (3H, m). Minor diastereoisomer 3.95 (1H, m) 3.63 (1H, dd, *J* 13.0, 3.0) 3.47 (1H, ddd, *J* 13.0, 4.5, 2.5) 3.02 (1H, dt, *J* 13.5, 3.5) 2.47 (1H, m) 2.10 (1H, m), 1.58 (2H, m). ^{13}C NMR (D_2O) Major diastereoisomer 174.6, 62.2, 54.6, 38.9, 33.1, 28.3.

Using an identical method, 80% de **(2*R*,4*R*)-11** (400 g, 0.91 mol) gave **(2*R*,4*R*)-2** (130 g, 99%).

(2*S*,4*S*)-*N*-*tert*-Butyloxycarbamoyl-4-hydroxypiperidine-2-carboxylic Acid 17. To a solution of (2*S*,4*S*)-**2** (140 g, 0.97 mol) in H_2O (1 L) and THF (500 mL), Et_3N (135 mL, 0.97 mol) was added dropwise. Boc_2O (317 g, 1.46 mol) in THF (500 mL) was added in a steady stream. As the pH started to drop, a further portion of Et_3N (135 mL, 0.97 mol) was added and the solution stirred at room temperature for 16 h. The THF was removed in vacuo, and the resultant cloudy solution was acidifed to pH 4 with 6 M HCl and then to pH 3 with 1 M HCl and extracted with EtOAc (4 × 1 L). The combined organic extracts were washed with brine (1 L), dried ($MgSO_4$), and concentrated in vacuo to give **(2*S*,4*S*)-17**, a viscous yellow oil (182 g, 76%, 80% de). This was dissolved in EtOAc and the solution cooled on ice. Benzylamine (81.2 mL, 0.74 mol) was added dropwise and stirring maintained for 2 h. After overnight refrigeration, the solid was collected by filtration and dried. This solid (154 g, 60% recovery) was recrystallised from MeOH (150 mL) and MTBE (300 mL). Filtration yielded (2*S*,4*S*)-*N*-*tert*-butyloxycarbamoyl-4-hydroxypiperidine-2-carboxylic acid, benzylamine salt, **18**, as a white solid (104 g, 31% from **2**, >98% ee, >98% de, purity (HPLC) >95%). ^{1}H NMR (CD_3-OD) 7.40 (5H, m) 4.67 (0.4 H, minor rotamer, m) 4.59 (0.6H, d, *J* 5.5, major rotamer) 4.10 (2H, s) 3.94 (1H, br d, *J* 13.0) 3.59 (1H, m) 3.17 (1H, m) 2.48 (1H, m) 1.80 (1H, m) 1.43 (10H, m) 1.25 (1H, m). ^{13}C NMR (CD_3OD) 178.1, 178.0 (rotamer pair) 157.6, 157.3 (rotamer pair) 135.0, 130.2, 130.0, 129.9, 80.8, 67.1, 67.0 (rotamer pair), 57.9, 56.9, 44.3, 41.6, 40.9 (rotamer pair), 37.6, 35.4, 35.2 (rotamer pair) 28.7. $[\alpha]^{25}_D$ −14.6 (*c* 3.16, EtOH).

Using an identical method, **(2*R*,4*R*)-2** (130 g, 0.89 mol) gave **(2*R*,4*R*)-18** (>98% ee, >98% de 111.4 g, 35%, purity (HPLC) >95%).

Received for review January 30, 2002.

OP020017X

(15) Hays, S. J.; Malone, T. C.; Johnson, G. *J. Org. Chem.* **1991**, *56*, 4084−4086.