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Diastereoisomeric Salt Formation and Enzyme-Catalyzed Kinetic Resolution as Complementary Methods for the Chiral Separation of *cis*-/*trans*-Enantiomers of 3-Aminocyclohexanol

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Abstract:

This contribution demonstrates the preparative-scale synthesis of (1*S*,3*S*)-3-aminocyclohexanol by either enzymatic kinetic resolution of Cbz-protected 3-aminocyclohexanols or direct diastereoisomeric salt formation with (*R*)-mandelic acid. The salt formation demonstrates how a single enantiomer, (1*S*,3*S*)-3-aminocyclohexanol (*R*)-mandelate, can be effectively isolated from the *cis*/*trans* racemic mixture and subsequently converted to the free amine, (1*S*,3*S*)-3-aminocyclohexanol, by ion-exchange chromatography. We have also demonstrated how the other three enantiomers of 3-aminocyclohexanol can be prepared by either diastereoisomeric salt formation or enzymatic kinetic resolution.

Introduction

Aminocyclohexanols are important motifs which exhibit a wide range of properties as bioactive molecules.[1] The 2-amino- and 4-aminocyclohexanols are readily available from commercial sources in all the possible enantiomeric and diastereomeric forms. In contrast, the asymmetric synthesis of 3-aminocyclohexanols has received relatively little synthetic attention and only two routes to their enantiomers have been published (Figure 1).[2,3] The two groups describe how enantiomerically pure 3-aminocyclohexanols were prepared by enzyme-catalyzed kinetic resolution of *N*-benzoyl-*O*-acetylated-[2] or Cbz-protected-3-aminocyclohexanols.[3] In the first publication by Bernardelli et al., the enantiomers were separated by a *Candida antarctica* lipase-catalyzed hydrolysis of *trans*-*N*-benzoyl-*O*-acetylated-3-aminocyclohexanol. The addition and removal of *N*-benzoyl- and *O*-acetyl-protecting groups adds four steps to the synthesis, and (1*R*,3*R*)-3-aminocyclohexanol **2** was obtained in 2.2% overall yield over six steps. In the second publication by Levy et al., Cbz-protected *trans*-3-aminocyclohexanol (see **(±)-8**, later in the paper) was prepared by Mitsonobu inversion of the *cis*-phthalimide of 3-aminocyclohexanol, prepared by a slight modification of a method by Sammes et al.[4] After Cbz-protection, the enantiomers were separated by a *Candida antarctica* lipase-catalyzed esterification to give (1*R*,3*R*)-3-aminocyclohexanol **2** in 4.8% overall yield for a 10-step synthesis starting from 2-cyclohexenol. Both of the syntheses include a number of chromatographic purification steps which would add a level of complication should these steps be further scaled up.

Overall, the limited information in the literature regarding the preparation of the *cis* and *trans* racemic 3-aminocyclohexanols[5−12] has restricted the number of approaches towards the individual enantiomers. In this communication we will discuss how it is possible to access all four enantiomers of 3-aminocyclohexanol **1**−**4** by

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(1) In the following examples from 2009, 3-aminocyclohexanol is contained in the core structure of drug-like molecules: (a) Shaw, D.; Leblanc, C.; Lizos, D.; Ritchie, C.; Furminger, V.; Lewis, S.; Hornsperger, B.; Stiefl, N. J.; Weiler, S. PCT Int. Appl. WO 2009050183 A2, 2009. (b) Anandan, S. K.; Webb, H. K.; Do, Z. N.; Gless, R. D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4259–4263. (c) Bruce, I.; Dunstan, A.; Hunt, T. A.; Howsham, C. U.S. Pat. Appl. Publ. 2009163463 A1, 2009; (d) Arnold, W. D.; Bounaud, P.; Chen, C.; Eastman, B.; Gosberg, A.; Gradl, S. N.; Hopkins, S.; Li, Z.; McDonald, I.; Sprengeler, P. A.; Steensma, R. W.; Wilson, M. E. U.S. Pat. Appl. Publ. 2009143352 A1, 2009; (e) Humphries, P. S.; Lafontaine, J. A.; Agree, C. S.; Alexander, D.; Chen, P.; Do, Q. Q. T.; Li, L. L. Y.; Lunney, E. A.; Rajapakse, R. J.; Siegel, K.; Timofeevski, S. L.; Wang, T. L.; Wilhite, D. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2099–2102. (f) Swinnen, D.; Jorand-Lebrun, C.; Grippi-Vallotton, T.; Muzerelle, M.; Royle, A.; Macritchie, J.; Hill, R.; Shaw, J. P. PCT Int. Appl. WO/2009/027283 A1, 2009; (g) Hauel, N.; Ceci, A.; Doods, H.; Kauffmann-Hefner, I.; Konetzki, I.; Schuler-Metz, A.; Walter, R. PCT Int. Appl. WO/2009/021945 A1, 2009; (h) Abbot, S. C.; Boice, G. N.; Gong, L.; Tan, Y.-C., PCT Int. Appl. WO 2009015917 A2, 2009;(i) Roughton, A. L.; Ho, K.-K.; Ohlmeyer, M.; Neagu, I.; Kultgen, S. G.; Ansari, N.; Rong, Y.; Ratcliffe, P. D.; Palin, R., PCT Int. Appl. WO 2009016241 A1, 2009; (j) Bruce, I.; Dunstan, A.; Hunt, T. A.; Howsham, C. PCT Int. Appl. WO/2009/013348 A2, 2009.

(2) Bernardelli, P.; Bladon, M.; Lorthiois, E.; Manage, A. C.; Vergne, F.; Wrigglesworth, R. *Tetrahedron: Asymmetry* **2004**, *15*, 1451–1455.
(3) Levy, L. M.; de Gonzalo, G.; Gotor, V. *Tetrahedron: Asymmetry* **2004**, *15*, 2051–2056.
(4) Sammes, P. G.; Thetford, D. *J. Chem. Soc., Perkin Trans. 1* **1989**, 655–661.
(5) Burford, R. R.; Hewgill, F. R.; Jefferies, P. R. *J. Chem. Soc.* **1957**, 2937–2942.
(6) Burckhardt, U.; Grob, C. A.; Kiefer, H. R. *Helv. Chim. Acta* **1967**, *50*, 231–244.
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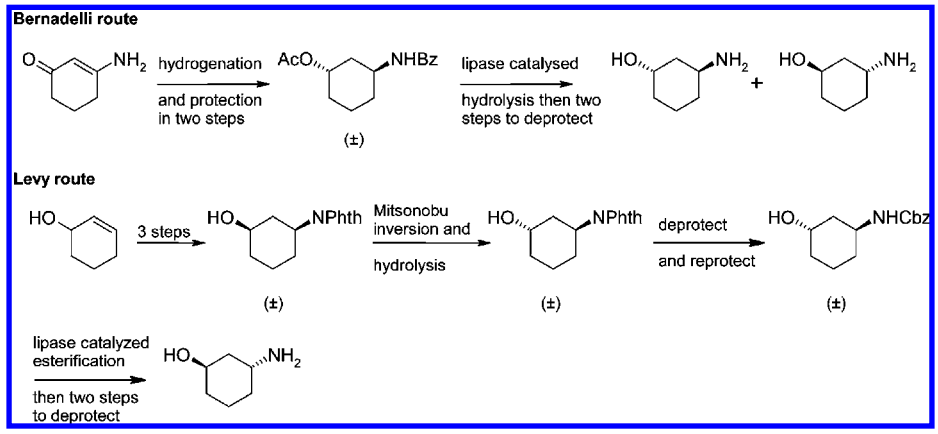
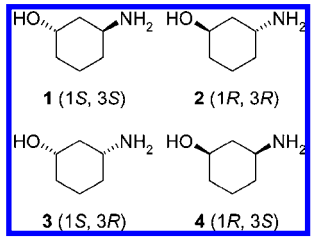


Figure 1. **Previously published routes to chiral 3-aminocyclohexanols.**

either selective salt formation or enzymatic kinetic resolution of *cis*- or *trans*-Cbz-protected 3-aminocyclohexanols.



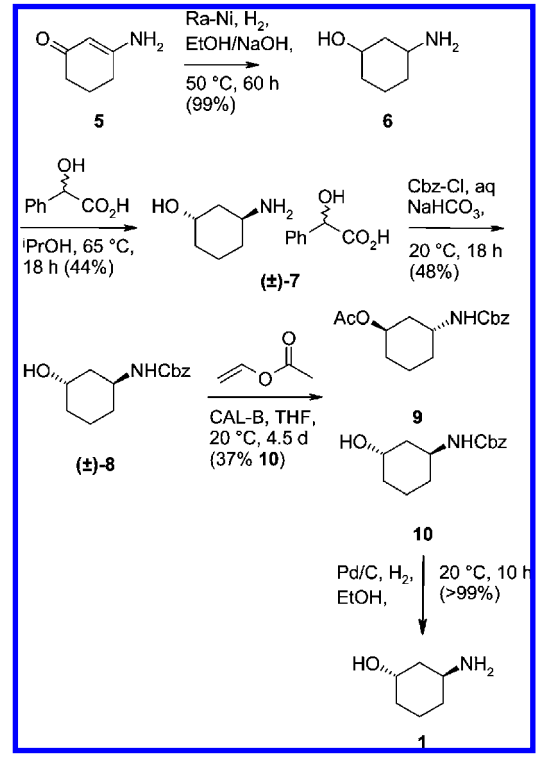
Results and Discussions

In the course of our studies (1*S*,3*S*)-3-aminocyclohexanol **1** was required on preparative scale for lead optimization and profiling purposes. The available chemicals directory contains a number of entries for enantiomerically pure (1*S*,3*S*)-3-aminocyclohexanol **1**; however, after a number of attempts, we were only able to get our hands on mixtures of *cis*- and *trans*-3-aminocyclohexanol, of varying ratios and quality, through the listed commercial sources. For this reason, (1*S*,3*S*)-3-aminocyclohexanol **1** was synthesized on larger scale using the synthesis outlined in Scheme 1.

3-Amino-2-cyclohexen-1-ol **5** was hydrogenated with Raney nickel according to a procedure by Greenhill et al.,[7] and as reported by Bernardelli et al.,[2] the ratio varied between 1:2 and 1:1 for *cis*:*trans* on kilogram scale. After a preliminary screen of chiral acids we found that we were able to obtain clean *trans*-3-aminocyclohexanol mandelate **(±)-7** by crystallization of *cis*-/*trans*-3-aminocyclohexanol **6** with (±)-mandelic acid. Resolution of the corresponding *trans*-2-(*N*-benzyl)aminocyclohexanol by sequential use of (*R*)- and (*S*)-mandelic acid was recently reported by Bolm et al.[13,14]

The kilogram scale batch of the *cis*/*trans* mixture **6** (48:52 *cis*/*trans* by NMR) was crystallized with (±)-mandelic acid to give a 44% yield of the desired *trans*-aminocyclohexanol mandelate **(±)-7**. Mandelate salt **(±)-7** was subsequently protected with a Cbz-group to give racemate **(±)-8**, however, with lower yield than expected. Significant material was lost on crystallization of the protected product and removal of byproduct carried through from the hydrogenation step. High-

Scheme 1. **Synthesis of (1*S*,3*S*)-3-aminocyclohexanol 1**



purity material was required in order to avoid poisoning of the biocatalyst in the next step.

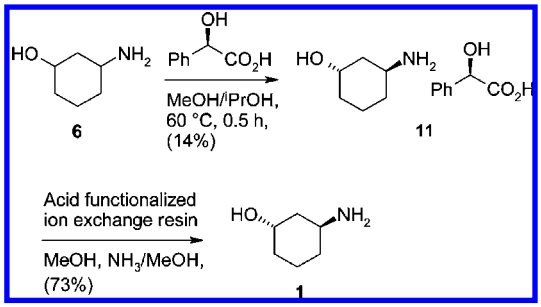
Racemate **(±)-8** was subjected to enzyme-catalyzed kinetic resolution with *Candida antarctica* lipase (CAL-B) according to literature precedence.[3] The desired (1*S*,3*S*)-Cbz protected aminocyclohexanol **10** was obtained in 37% yield, and 99.8% enantiomeric excess by crystallization of the mixture obtained. The structure of intermediate **10** was confirmed by X-ray crystallography. Hydrogenation to remove the Cbz-protecting group gave (1*S*,3*S*)-3-aminocyclohexanol **1** in 7.7% overall yield for five steps from 3-amino-2-cyclohexen-1-ol **5**.

The synthesis outlined above was used to provide sufficient material for our preliminary needs; however, in order to provide more material we sought to simplify and shorten the synthesis, providing chirally pure material by the shortest possible route and avoiding unnecessary protection and deprotection steps wherever necessary.

Selective Diastereoisomeric Salt Formation. The shortened synthesis of (1*S*,3*S*)-3-aminocyclohexanol **1** is shown in Scheme

(13) Schiffers, I.; Rantanen, T.; Schmidt, F.; Bergmans, W.; Zani, L.; Bolm, C. *J. Org. Chem.* **2006**, *71*, 2320–2331.
(14) Schiffers, I.; Bolm, C. *Org. Synth.* **2008**, *85*, 106–117.

Scheme 2. **Direct diastereoisomeric salt formation with (*R*)-mandelic acid and subsequent ion-exchange chromatography to give (1*S*,3*S*)-3-aminocyclohexanol 1**



2. When *cis*/*trans* mixture **6** was subjected to direct diastereoisomeric salt formation with (*R*)-mandelic acid, the required (1*S*,3*S*)-enantiomeric salt **11** was obtained in high diastereoisomeric excess (>95%) and yield comparable to those of the previous syntheses described herein. The structure of mandelate salt **11** was confirmed by X-ray crystallography. The possibility of reaching high diastereoisomeric excess in one single crystallization step compensated for the approximate 50% yield of the theoretical maximum amount of this single isomer. The remainder of the required isomer was lost in the highly dilute crystallization solution, which was required in order to obtain high diastereoisomeric excess. Ion-exchange chromatography with Isolute Separtis SCX-2 resin from Biotage, furnished (1*S*,3*S*)-3-aminocyclohexanol **1** in 10% yield over three steps from 3-amino-2-cyclohexen-1-ol **5**.

Crystallization of *cis*/*trans* mixture **6** with the opposite enantiomer, (*S*)-mandelic acid, led to the opposite (1*R*,3*R*)-enantiomeric salt in high enantiomeric excess (>95%) and 11% yield.

Manipulation of the Mother Liquors To Give *cis*-Enantiomers. Scheme 3 outlines how the mother liquors obtained from crystallization to give *trans*-aminocyclohexanol mandelate **(±)-7** were recycled to give chirally pure *cis*-isomers. The mother liquors from previous salt formations were concentrated and crystallized to remove residual *trans*-mandelate salt to give an oil containing predominantly *cis*-3-aminocyclohexanol **(±)-12**. Unfortunately, it was not possible to further crystallize either *cis*-3-aminocyclohexanol **(±)-12** or Cbz-protected-3-aminocyclohexanol **(±)-13** past a *cis*/*trans* ratio of approximately 85:15. However, when Cbz-protected aminocyclohexanol **(±)-13** was protected as acetate **(±)-14**, the product easily crystallized and gave pure *cis*-racemate **(±)-15** after removal of the acetate group.

cis-Racemate **(±)-15** was subjected to enzyme-catalyzed kinetic resolution with a range of lipases (shown in Table 1) including *Candida antarctica* lipase according to literature precedence (Table 1, entry 1).[3] (1*R*,3*S*)-Cbz protected aminocyclohexanol **16** (whose structure was confirmed by X-ray crystallography) and acetic acid (1*R*,3*S*)-3-benzyloxycarbonylamino-cyclohexyl ester **17** were obtained in high yield and enantiomeric excess after column chromatography of the mixtures was obtained. *Thermomyces lanuginosus* lipase showed superior selectivity over *Candida antarctica* lipase, and by using this enzyme it was possible to stop the reaction at just over

Scheme 3. **Crystallisation of acetate (±)-14 followed by enzymatic kinetic resolution of *cis*-racemate (±)-15**

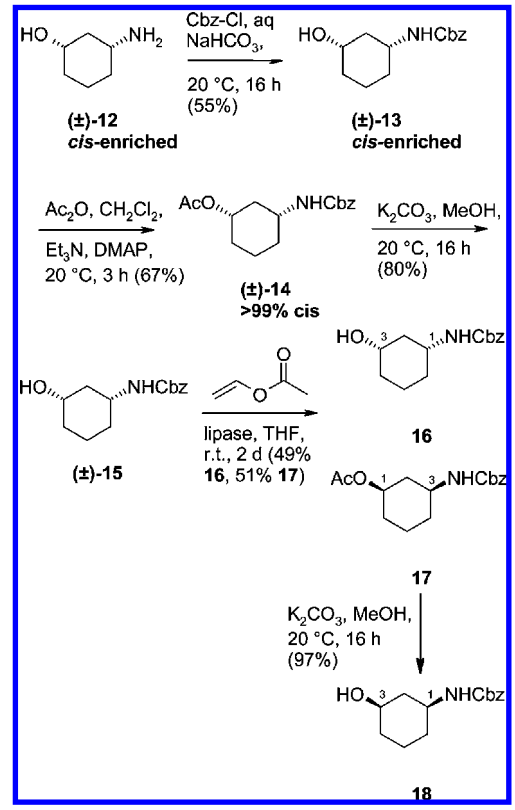


Table 1. **Lipase-catalyzed kinetic resolution of *cis*-racemate (±)-15**

entry	lipase	conversion[a]/%	ee **16**/%	ee **17**/%	*E*[b]
1	*Candida antarctica*	50.6	95.4	93.0	~100
2	*Pseudomonas fluorescens*	50.5	98.6	96.7	~300
3	*Thermomyces lanuginosus*	50.4	99.9	98.4	>1000

[a] As measured by HPLC. [b] Enantiomeric ratio.[15]

50% conversion, giving both enantiomers in high enantiomeric excess (Table 1, entry 3).

Conclusions

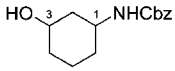
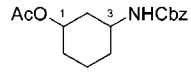
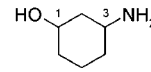
In summary, we have developed a quick and easy method to obtain (1*S*,3*S*)-3-aminocyclohexanol **1** in high enantiomeric excess (>96%) in three steps starting from commercially available 3-amino-2-cyclohexen-1-ol **5**. The method is advantageous compared to previously reported routes because not only is it higher yielding, but it also avoids a chromatographic separation of mixtures from enzyme-catalyzed kinetic resolution and the use of *N*-Cbz-, *O*-acetyl-, or *N*-benzoyl protecting groups. We have also demonstrated how the other three enantiomers of 3-aminocyclohexanol can be prepared by either diastereoisomeric salt formation or enzymatic kinetic resolution.

Experimental Procedures

All chemicals were purchased from Fluka or Sigma-Aldrich unless otherwise mentioned. 3-Amino-cyclohex-2-enone was

(15) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

Table 2. **Chiral HPLC and chiral GC−MS retention times (please note changes in numbering)**

	R_t chiral HPLC / mins		R_t chiral HPLC / mins		R_t chiral GC-MS / mins
trans-(1*S*,3*S*)	23.98	*trans*-(1*S*,3*S*)	8.39	*trans*-(1*S*,3*S*)	15.01
trans-(1*R*,3*R*)	20.71	*trans*-(1*R*,3*R*)	9.96	*trans*-(1*R*,3*R*)	15.12
cis-(1*R*,3*S*)	15.02	*cis*-(1*S*,3*R*)	13.45	*cis*-(1*R*,3*S*)	15.88
cis-(1*S*,3*R*)	16.18	*cis*-(1*R*,3*S*)	18.71	*cis*-(1*S*,3*R*)	15.88

prepared according to a literature procedure for reduction of 3-aminophenol[16] or purchased directly from Apollo Scientific. (*R*)- or (*S*)-Mandelic acid was purchased from AK Scientific. Both *Candida antarctica* lipase (CAL-B) (as immobilized lipase preparation, Novozym 435) and *Thermomyces lanuginosus* lipase (as immobilized lipase preparation, Novozym TL IM) were purchased from Novozymes. *Pseudomonas fluorescens* lipase (Amano AK) was purchased from Amano. Isolute Separtis SCX-2 9536 ion-exchange resin (Si-propylsulfonic) was purchased from Biotage.

Thin layer chromatography (TLC) was performed on Merck silica gel plates 60 F-254, Art. Nr. 5729 with detection by UV (254 nm) or molybdate dip (3 g $KMnO_4$; 20 g K_2CO_2; 5 mL 5% NaOH; 300 mL H_2O). Reverse phase HPLC analyses (for UV-active Cbz-protected analogues) were performed on an Agilent-1100 machine using a Zorbax Eclipse XDB-C18, 4.6 mm × 50 mm, 1.8 μm column, acetonitrile and water as eluent (both containing 0.1% TFA), a column temperature of 35 °C, a flow rate of 1.0 mL/min, and measuring at 216 nm. The standard gradient used was 5−100% MeCN over 6 min, 100% MeCN for 1.5 min, followed by 100 to 5% MeCN over 0.5 min. Normal phase chiral HPLC (for UV-active Cbz-protected analogues) was performed using a Chiralpak AD-H 250 mm × 4.6 mm column, an isocratic mixture of 85:15 hexane/ethanol, a column temperature of 23 °C, with a flow rate of 1.0 mL/min and measuring at 210 nm over 30 min. Non-UV-active 3-aminocyclohexanols were analyzed by GC−MS after derivatization with trifluoroacetic anhydride. Gas chromatographic separation was performed using a Carlo Erba Instruments GC coupled with a Thermo Finnigan TSQ7000 CI/EI mass spectrometer and an on-column injector (data acquisition and evaluation with Excalibur software). Separation was observed using a Beta DEX 120, Supelco GC column (30 m × 0.25 mm, 0.25 μm film thickness) with hydrogen at 2.5 mL/min constant flow and 0.4 μL injection volumes. The column was held at 40 °C for 1 min followed by a ramp of 15 °C/min to 90 °C, 4 °C/min to 150 °C, and 5 °C/min to 200 °C. The TFA derivates of 3-aminocyclohexanol showed no response with FID detection; therefore, it is mandatory to analyze these compounds with GC−MS or GC−ECD. It was possible to derivatize and analyze both the free base as well as the mandelate salts of 3-aminocyclohexanols using this method. For clarity, the retention times for each method are summarized in Table 2 and Figure 2 below.

NMR was performed using a 400 MHz Varian machine, AS 400 Oxford. ^{1}H shifts were referenced to d_6-DMSO at 2.49 ppm and D_2O at 4.79 ppm. ^{13}C shifts were referenced to d_6-DMSO at 39.52 ppm. MS was measured using VG Platform (Fisons Instruments), Spectraflow 783 Detector, HP 1100 series HPLC. Melting points were measured using a Büchi, B-545 machine.

***cis/trans*-3-Aminocyclohexanol 6.** 3-Amino-cyclohex-2-enone **5** (1901 g, 17.1 mol) was dissolved in ethanol (11.5 L) and NaOH (20% aq, 275 mL) in a 20 L autoclave. Raney nickel (366 g, 4.28 mol, 0.25 equiv) was added and the reactor purged with hydrogen. The reaction progress was monitored using TLC (20% MeOH/CH_2Cl_2, $R_{f\ \text{starting material}} = 0.5$, $R_{f\ \text{product}} = 0.14$) until no more starting material was visible, and by hydrogen update which showed that the reaction was complete after 60 h. We noted that hydrogenation of 3-amino-cyclohex-2-enone **2**, prepared in house by hydrogenation of 3-aminophenol,[16] was much slower than when commercially available material was

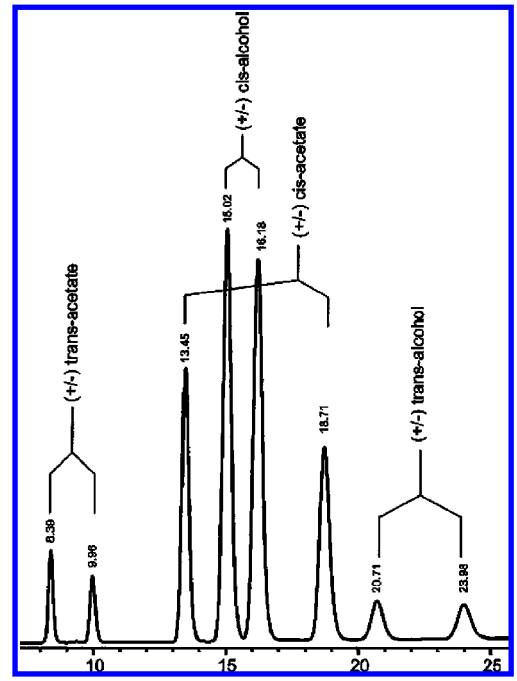


Figure 2. **HPLC trace showing all four isomers of 3-(hydroxycyclohexyl)carbamic acid benzyl ester and all four isomers of acetic acid 3-benzyloxycarbonylamino-cyclohexyl ester.**

(16) Sajiki, H.; Ikawa, T.; Hirota, K. *Org. Process Res. Dev.* **2005**, *9*, 219–220.

used. The reaction mixture was filtered to remove the Raney nickel and concentrated *in vacuo* to give a yellow oil which crystallized on standing to give a hygroscopic yellow solid (1945 g, 99%). ^{1}H NMR (400 MHz, D_2O) δ 0.87−1.79 (8H, m, *cis*/*trans*), 1.79 (0.5H, m, *cis*), 2.52 (0.5H, m, *cis*), 2.87 (0.5H, m, *trans*), 3.49 (0.5H, m, *cis*), 3.97 (0.5H, m, *trans*); *cis*/*trans* 48:52. ^{13}C NMR (101 MHz, D_2O) δ 18.7 (d, *trans*), 21.6 (d, *cis*), 31.7 (d, *trans*), 33.6 (d, *trans*), 33.8 (d, *cis*), 34.1 (d, *cis*), 40.6 (d, *trans*), 43.5 (d, *cis*), 44.8 (s, *trans*), 48.1 (s, *cis*), 66.7 (s, *trans*), 61.2 (s, *cis*), 48:52 *cis*/*trans*. HRMS (FAB) calcd for $C_6H_{13}NO$ + H 116.1070, found 116.1070.

***trans*-3-Aminocyclohexanol (*R*/*S*)-Mandelate (±)-7.** *cis*/*trans*-3-Aminocyclohexanol **6** (1300 g, 1.13 mol) was dissolved in ^{i}PrOH (1.5 M, 7.5 L) at internal temperature 65 °C to give an fine suspension. (±)-Mandelic acid (900 g, 5.92 mol, 0.52 equiv) was added slowly to the hot mixture, and the mixture was heated to 65 °C overnight. The mixture was cooled to 40 °C to give a white precipitate which was removed by filtration (room temperature filter funnel) and washed with ^{i}PrOH (500 mL) to give a white crystalline solid which was dried in the vacuum oven at 40 °C overnight (1333 g, 44.2%). Mp 171.1−171.2 °C. ^{1}H NMR (400 MHz, D_2O) δ 1.14−1.51 (6H, m), 1.77−1.84 (2H, m), 3.23 (1H, m), 3.99 (1H, m), 4.81 (1H, s), 7.19−7.28 (5H, m), 7:93 *cis*/*trans*. ^{13}C NMR (101 MHz, D_2O) δ 18.0 (d), 29.5 (d), 30.3 (d), 35.9 (d), 46.2 (s), 65.4 (s), 74.9 (s), 126.9 (s), 128.1 (s), 128.7 (s), 140.4 (q), 179.3 (q). HRMS (FAB) calcd for $C_6H_{13}NO$ + H 116.1070, found 116.1070 and calcd for $C_8H_8O_3$ − H 151.0408, found 151.0400.

***trans*-3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester (±)-8.** *trans*-3-Aminocyclohexanol (*R*/*S*)-mandelate (±)-7 (1333 g, 4.99 mol) was dissolved in saturated aqueous sodium bicarbonate solution (12 L) and Cbz-Cl (783 mL, 5.49 mol) added dropwise. A white suspension formed, and the resultant mixture was stirred at room temperature overnight, after which time no starting material was visible by TLC. The suspension was dissolved in ethyl acetate (15 L) and the aqueous phase diluted with water (2 L). The organic phase was separated and the aqueous phase washed three more times with ethyl acetate (3 × 5 L). The combined organic phases were washed with brine (4 L) and dried over anhydrous sodium sulfate before concentrating *in vacuo* to give a gummy white solid. The crude solid was triturated in TBME/hexane (1:4, 2 L), the resultant solid was removed by filtration and washed with TBME/hexane (1:4, 500 mL). The crystalline solid was subjected to a second round of crystallization in ethyl acetate/heptane (3:2, 1.5 L) in which the resultant filtered solid was washed with ethyl acetate/heptane (3:2, 500 mL). Two crystallizations were required; otherwise, we observed problems with the subsequent enzyme-catalyzed kinetic resolution step. The bright, white solid formed was dried in the vacuum oven at 40 °C for 3 h (596 g, 47.9%). Mp 130.2−130.4 °C. ^{1}H NMR (400 MHz, DMSO) δ 1.17 (1H, m), 1.33−1.44 (4H, m), 1.58−1.67 (3H, m), 3.71 (1H, m), 3.89 (1H, br s), 4.40 (1H, d, J = 3.3), 4.99 (2H, s), 7.12 (2H, d, J = 8.3), 7.27−7.45 (5H, m). HPLC (Zorbax XDB-C18) r_t = 3.96 min; >95% purity. Chiral HPLC (Chiralpak AD-H) 2.5:97.5 *cis*/*trans*. ^{13}C NMR (101 MHz, D_2O) δ 19.5 (d), 32.6 (d), 32.9 (d), 40.0 (d), 45.8 (s), 65.0 (s), 65.5 (d), 128.2 (s), 128.2 (s), 128.8 (s), 137.8 (q), 155.8 (q). HRMS (FAB) calcd for $C_{14}H_{19}NO_3$ + H 250.1438, found 250.1438, calcd for $C_{14}H_{19}NO_3$ + NH_4 267.1703, found 267.1703 and calcd for $C_{14}H_{19}NO_3$ + Na 272.1257, found 272.1256.

(1*S*,3*S*)-3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester 10. *trans*-3-(Hydroxy-cyclohexyl)carbamic acid benzyl ester **(±)-8** (660 g, 2.65 mol) was suspended in THF (5.44 L) and *Candida antarctica* lipase (Novozym 435, 34 g) was added followed by vinyl acetate (680 mL, 7.38 mol). The mixture was stirred at room temperature. After 4.5 days, the conversion reached 52%, and then the reaction was filtered and the solvent removed under reduced pressure to give a mixture of acetic acid (1*R*,3*R*)-3-benzyloxy-carbonylamino-cyclohexyl ester **9** and (1*S*,3*S*)-3-(hydroxy-cyclohexyl)carbamic acid benzyl ester **10**. The crude mixture was triturated in 10% dichloromethane/ethyl acetate (800 mL) and the solid which formed removed by filtration and dried in the vacuum oven at 40 °C for 1 h to give (1*S*,3*S*)-3-(hydroxy-cyclohexyl)carbamic acid benzyl ester **10** as a bright, white crystalline solid (244 g, 36.8%). The spectroscopic data were identical to those of racemic starting material **(±)-8**. Mp 122.8−122.9 °C [lit. 118−119 °C].[3] $[\alpha]^{20}_{589}$ = +1.4 (*c* 1, $CHCl_3$) [lit. $[\alpha]^{20}_{589}$ = +4.7 (*c* 0.5, $CHCl_3$), 78% ee].[3] Chiral HPLC (Chiralpak AD-H) 99.8% ee, >99% *trans*. Anal. Calcd for $C_{14}H_{19}NO_3$ + 0.068 equiv H_2O C, 67.12; H, 7.70; N, 5.59. Found: C, 67.14; H, 7.71; N, 5.35. Absolute configuration was confirmed by X-ray crystallography.

The mother liquors from the above crystallization were concentrated *in vacuo* to give a gummy solid, a part of which was purified by column chromatography on silica, eluting with ethyl acetate/heptane (2:3) to give acetic acid (1*R*,3*R*)-3-benzyloxycarbonylamino-cyclohexyl ester **9** as an oil which crystallized on standing. Mp 64.8−64.9 °C [lit. 120−125 °C].[3] $[\alpha]^{20}_{589}$ = −8.8 (*c* 1, $CHCl_3$) [lit. $[\alpha]^{20}_{589}$ = −9.6 (*c* 0.13, $CHCl_3$), 98% ee].[31]H NMR (400 MHz, D_2O) δ 1.16 (1H, m), 1.43−1.58 (5H, m), 1.71−1.81 (2H, m), 1.99 (3H, s), 3.62 (1H, m), 4.99 (3H, br m), 7.27−7.37 (5H, m). HPLC (Zorbax XDB-C18) r_t = 4.86 min; >99% purity. Chiral HPLC (Chiralpak AD-H) 97.3% ee, >99% *trans*. ^{13}C NMR (101 MHz, D_2O) δ 19.8 (d), 21.5 (t), 29.4 (d), 31.9 (d), 36.3 (d), 46.0 (s), 65.6 (d), 70.0 (s), 128.2 (s), 128.3 (s), 128.8 (s), 137.7 (q), 155.8 (q), 170.2 (q). HRMS (FAB) calcd for $C_{16}H_{21}NO_4$ + H 292.1543, found 292.1543, calcd for $C_{16}H_{21}NO_4$ + NH_4 309.1809, found 309.1810 and calcd for $C_{16}H_{21}NO_4$ + Na 314.1363, found 314.1361. Anal. Calcd for $C_{16}H_{21}NO_4$ C, 65.96; H, 7.27; N, 4.81. Found: C, 65.92; H, 7.14; N, 4.49.

(1*S*,3*S*)-3-Aminocyclohexanol 1 by Hydrogenation. (1*S*,3*S*)-3-(Hydroxy-cyclohexyl)carbamic acid benzyl ester **9** (241.4 g, 968 mmol) was dissolved in ethanol (2 L) in a shaker, and palladium on carbon (20 g, 18.8 mmol) was added. The flask was charged with hydrogen and shaken at room temperature for 10 h after which time complete consumption of hydrogen was observed and no starting material was observed in the TLC. The catalyst was removed by filtration and the filtrate concentrated *in vacuo* to give a white gummy solid which was dried in high vacuum overnight. The product still contained ethanol and so was dissolved in water/dioxane and freeze-dried to give a gummy hygroscopic white solid (127.4 g, >99%). The analytical data were identical to those of material produced by the following direct crystallization with (*R*)-mandelic acid (see

below). $[\alpha]^{20}_{589} = +8.0$ (*c* 1, MeOH) [lit. $[\alpha]^{20}_{589} = +7.5$ (*c* 1, MeOH), 95.5% ee].[2] GC−MS (Beta DEX 120, Supelco) >99% ee, purity and *trans*.

(1*S*,3*S*)-3-Aminocyclohexanol (*R*)-Mandelate 11. *cis*/*trans*-3-Aminocyclohexanol **6** (441.8 g, 3.84 mol) was dissolved in MeOH/^{i}PrOH (1:1, 2.5 M, 1.53 L) at internal temperature 60 °C to give a fine suspension. (*R*)-Mandelic acid (152 g, 1.00 mol, 0.26 equiv) was added slowly to the hot mixture, and the mixture (now dissolved) was heated to 60 °C for 5 min. The mixture was cooled in an ice bath to 20 °C to give a white precipitate. Seeding crystals (approximately 5 mg) were added at 45 °C during the cooling process. After 10 min at room temperature the solid was removed by filtration (room temperature filter funnel) and washed with ^{i}PrOH (560 mL) and TBME (560 mL) to give a white crystalline solid which was dried under a filter paper at room temperature overnight (138.8 g, 13.5%). The spectroscopic data were identical to those of racemic salt **(±)-7**. Mp 187.7−188.1 °C. $[\alpha]^{20}_{589} = -44$ (*c* 1, MeOH). GC−MS (Beta DEX 120, Supelco) 95.9% ee, 98.2% purity and 1.3:98.7 *cis*/*trans*. Anal. Calcd for $C_{14}H_{21}NO_4$ + 0.305 equiv H_2O C, 61.64; H, 7.98; N, 5.13. Found: C, 61.62; H, 7.80; N, 5.22. Absolute configuration was confirmed by X-ray crystallography.

Crystallization was carried out with (*S*)-mandelic acid at the same concentrations as given above to give (1*R*,3*R*)-3-aminocyclohexanol (*S*)-mandelate (11.3% yield). The spectroscopic data were again identical to those of racemic salt **(±)-7**. Mp 191.8−191.9 °C. $[\alpha]^{20}_{589} = +48$ (*c* 1, MeOH). GC−MS (Beta DEX 120, Supelco) 97.1% ee, 98.4% purity and 1.6:98.4 *cis*/*trans*.

(1*S*,3*S*)-3-Aminocyclohexanol 1 by Ion-Exchange Resin. (1*S*,3*S*)-3-Aminocyclohexanol (*R*)-mandelate **10** (389.7 g, 1.46 mol) was dissolved in MeOH (7 L) with warming to 50 °C on the rotorvap to give a pale-yellow solution. A glass column was filled with Isolute Separtis SCX-2 9536 ion-exchange resin (3 kg, 1.71 mol, resin loading 0.57 mmol/g). The solution of product was added to the column (which was preconditioned with MeOH, 7 L) and pushed onto the resin with nitrogen pressure. The column was washed with MeOH (7 L) until no more mandelic acid was visible in the TLC (80% MeCN/water, $R_{f\ \text{mandelic acid}} = 0.56$, $R_{f\ \text{product}} = 0.21$). The column was flushed with ammonia in MeOH (2N, 23 L) until no more product eluted (as observed by TLC). The product was concentrated *in vacuo* to give a foamy solid which was further dried on the high vacuum for 4 h at room temperature to give a cream-colored powder (123 g, 73.3%). Mp 70.6−70.9 °C. ^{1}H NMR (400 MHz, D_2O) δ 1.06 (1H, m), 1.33 (1H, m), 1.44 (4H, s), 1.60−1.67 (2H, m), 2.89 (1H, m), 3.98 (1H, s). GC−MS (Beta DEX 120, Supelco) 96.4% ee, 98.3% purity and 1.7:98.3 *cis*/*trans*. ^{13}C NMR (101 MHz, D_2O) δ 18.6 (d), 31.6 (d), 33.6 (d), 40.4 (d), 44.7 (s), 66.8 (s). Anal. Calcd for $C_6H_{13}NO$ + 0.162 equiv H_2O + 0.002 equiv MeOH C, 61.01; H, 11.37; N, 11.85. Found: C, 61.02; H, 11.18; N, 11.82.

3-Aminocyclohexanol (±)-12 (*cis*-Enriched). The mother liquors from the crystallization of *cis*/*trans*-3-aminocyclohexanol **6** with (±)-mandelic acid were concentrated *in vacuo* to give a thick oil which crystallized on standing. The solid (*trans*-3-aminocyclohexanol (*R*/*S*)-mandelate **(±)-7**) was removed by filtration to enrich the mother liquors in the *cis*-racemate to a maximum of 84:16 *cis*/*trans* and 9.5% mandelic acid by NMR.

Alternatively, direct hydrogenation of 3-aminophenol to 3-aminocyclohexanol **6** with the Nishimura catalyst (rhodium oxide and platinum oxide)[17] give *cis*-enriched material with a *cis*/*trans* ratio of 72:18.

3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester (±)-13 (*cis*-Enriched). 3-Aminocyclohexanol **(±)-12** (100 g, 786 mmol) was dissolved in saturated aqueous sodium bicarbonate solution (1.57 L) and Cbz-Cl (124 mL, 872 mmol) added dropwise. The orange solution was stirred at room temperature overnight, after which time no starting material was visible by TLC and the product had precipitated out of solution. The mixture was filtered and the solid obtained washed with water (2 × 200 mL) before drying in the vacuum oven at 30 °C (caution: melts above 50 °C). The crude solid was triturated in TBME/hexane (1:3, 300 mL), and the resultant solid wasremoved by filtration and washed with hexane (50 mL). The cream-colored solid formed was triturated a second time in TBME/hexane (1:3, 300 mL), and the resultant solid was removed by filtration and washed with hexane (50 mL). The solid was dried in the vacuum oven at 30 °C for 4 h (120.0 g g, 55.4%). Further crystallizations led the mixture to higher *trans* content. The residual *trans*-product was removed in crystallization after *O*-acetylation in the following step. HPLC (Zorbax XDB-C18) $r_{t\ (cis)} = 3.90$ min; $r_{t\ (trans)} = 3.96$ min; *cis*/*trans* 80:20.

Acetic Acid *cis*-3-Benzyloxycarbonylamino-cyclohexyl Ester (±)-14. To a solution of 3-(hydroxy-cyclohexyl)carbamic acid benzyl ester **(±)-13** (53.1 g, 213 mmol) in dichloromethane (700 mL), containing triethylamine (23.7 g, 234 mmol) and DMAP (100 mg, 0.82 mmol), was added dropwise acetic anhydride (23.9 g, 234 mmol). After addition, the mixture was stirred at room temperature for 3 h before quenching with brine (100 mL). The organic phase was successively washed with 1 N HCl (100 mL), brine (100 mL), and saturated sodium bicarbonate solution (100 mL) before drying over anyhydrous magnesium sulfate. The solvent was removed *in vacuo*, and the resulting solid was recrystallized from diethyl ether/pentane to give a bright, white crystalline solid (50.4 g, 81.2%, 97.5:2.5 *cis*/*trans*). A second recrystallization from diethyl ether/pentane resulted in almost complete removal of the remaining *trans*-material to give a bright, white solid (41.5 g, 66.8%). As an alternative to using highly flammable solvents such as diethyl ether or pentane on larger scale, TBME/heptane mixtures have been shown to be efficient in removing the residual *trans*-material. Mp 85.7−85.9 °C. ^{1}H NMR (400 MHz, D_2O) δ 1.01−1.30 (4H, m), 1.67−1.71 (2H, m), 1.82 (1H, m), 1.96 (3H, s), 2.03 (1H, m), 3.36 (1H, m), 4.59 (1H, m), 4.99 (2H, s), 7.27−7.37 (5H, m). HPLC (Zorbax XDB-C18) $r_t = 4.86$ min; >99% purity. Chiral HPLC (Chiralpak AD-H) 99.75:0.25 *cis*/*trans*. ^{13}C NMR (101 MHz, D_2O) δ 21.4 (d), 21.5 (t), 31.1 (d), 31.9 (d), 38.4 (d), 48.3 (s), 65.6 (d), 71.4 (s), 128.3 (s), 128.6 (s), 128.8 (s), 137.6 (q), 155.7 (q), 170.1 (q). HRMS (FAB) calcd for $C_{16}H_{21}NO_4$ + H 292.1543, found 292.1543 and calcd for $C_{16}H_{21}NO_4$ + NH_4 309.1809, found 309.1810.

(17) Nishimura, S. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 566–567.

***cis*-3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester (±)-15.** A solution of acetic acid *cis*-3-benzyloxycarbonylamino-cyclohexyl ester **(±)-14** (41.8 g, 143.4 mmol), and potassium carbonate (0.5 g, 3.6 mmol) in MeOH (500 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to remove the methanol, and the crude mixture was dissolved in ethyl acetate (300 mL) and brine (300 mL). The organic phase was separated, and the aqueous phase was washed once more with ethyl acetate (100 mL). The combined organic phases were dried over anhydrous sodium sulfate before concentrating *in vacuo*. The crude material was crystallized in dichloromethane/heptane to give a white crystalline solid (28.7 g, 80.2%). Mp 88.7−88.9 °C [lit. 95−97 °C].[31]H NMR (400 MHz, DMSO) δ 0.94−1.19 (4H, m), 1.60−1.75 (3H, m), 1.94 (1H, m), 3.27 (1H, m), 3.36 (1H, m), 4.62 (1H, s), 4.98 (2H, s), 7.22 (2H, d, J = 8.3), 7.28−7.37 (5H, m). HPLC (Zorbax XDB-C18) r_t = 3.90 min; >99% purity. Chiral HPLC (Chiralpak AD-H) >99% *cis*. ^{13}C NMR (101 MHz, D_2O) δ 22.0 (d), 32.3 (d), 35.1 (d), 42.6 (d), 48.7 (s), 65.5 (d), 68.1 (s), 128.2 (s), 128.3 (s), 128.7 (s), 137.7 (q), 155.7 (q). HRMS (FAB) calcd for $C_{14}H_{19}NO_3$ + H 250.1438, found 250.1438, calcd for $C_{14}H_{19}NO_3$ + NH_4 267.1703, found 267.1703 and calcd for $C_{14}H_{19}NO_3$ + Na 272.1257, found 272.1256.

Typical Procedure for Enzymatic Kinetic Resolution of *cis*-3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester (±)-15. *cis*-3-(Hydroxy-cyclohexyl)carbamic acid benzyl ester **(±)-15** (20 g, 80.2 mmol) was suspended in vinyl acetate (200 mL), and *Pseudomonas fluorescens* lipase (Amano AK) (1.05 g) was added. The mixture was stirred at room temperature. After about 2 days, the conversion reached 50.5%; thus, the reaction was filtered, and the vinyl acetate was removed under reduced pressure to give the crude mixture which was purified by column chromatography on silica, eluting with 2% methanol in dichloromethane to yield (1*R*,3*S*)-3-(hydroxy-cyclohexyl)-carbamic acid benzyl ester **16** (9.77 g, 48.8%) and acetic acid (1*R*,3*S*)-3-benzyloxy-carbonylamino-cyclohexyl ester **17** (12.0 g, 51.2%), both as white crystalline solids.

(1*R*,3*S*)-3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester 16. The spectroscopic data were identical to those of racemic starting material **(±)-15**. Mp 99.9−100.5 °C [lit. 95−97 °C].[3] $[\alpha]^{20}_{589}$ = +19.1 (*c* 1, MeOH) [lit. $[\alpha]^{20}_{589}$ = −11.0 (*c* 0.52, $CHCl_3$), 28% ee].[3] Chiral HPLC (Chiralpak AD-H) 98.6% ee and >99% *cis*. Anal. Calcd for $C_{14}H_{19}NO_3$ C, 67.45; H, 7.68; N, 5.62. Found: C, 67.58; H, 7.91; N, 5.79. Absolute configuration was confirmed by X-ray crystallography.

Acetic Acid (1*R*,3*S*)-3-Benzyloxycarbonylamino-cyclohexyl Ester 17. The spectroscopic data were identical to those of racemic material **(±)-14**. Mp 117.6−117.7 °C [lit. 113−115 °C].[3] $[\alpha]^{20}_{589}$ = +8.3 (*c* 1, MeOH) [lit. $[\alpha]^{20}_{589}$ = +9.1 (*c* 0.45, $CHCl_3$), >99% ee].[3] Chiral HPLC (Chiralpak AD-H) 96.7% ee and >99% *cis*. Anal. Calcd for $C_{16}H_{21}NO_4$ C, 65.96; H, 7.27; N, 4.81. Found: C, 65.61; H, 7.19; N, 4.96.

(1*S*,3*R*)-3-(Hydroxy-cyclohexyl)carbamic Acid Benzyl Ester 18. A solution of acetic acid (1*R*,3*S*)-3-benzyloxycarbonylamino-cyclohexyl ester **17** (11.1 g, 38.1 mmol) and potassium carbonate (0.25 g, 1.81 mmol) in MeOH (150 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to remove the methanol, and the crude mixture was dissolved in ethyl acetate (100 mL). The organic phase was separated, and the aqueous phase was washed once more with ethyl acetate (100 mL). The combined organic phases were dried over anhydrous sodium sulfate before concentrating *in vacuo*. The crude material was crystallized in dichloromethane/heptane to give a white crystalline solid (9.25 g, 97.4%). The spectroscopic data were identical to those of racemic starting material **(±)-15**. Mp 98.7−99.1 °C $[\alpha]^{20}_{589}$ = −18.6 (*c* 1, MeOH). Chiral HPLC (Chiralpak AD-H) 97.3% ee and >99% *cis*. Anal. Calcd for $C_{14}H_{19}NO_3$ C, 67.45; H, 7.68; N, 5.62. Found: C, 67.18; H, 7.53; N, 5.81.

Crystallographic Data. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 798573 (**10**), CCDC 798575 (**11**), and CCDC 798574 (**16**). 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 336 033 or email: deposit@ccdc.cam.ac.uk].

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Supporting Information Available

Crystal structures of compounds **10**, **11**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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