Chemoenzymatic Synthesis of 4-Amino-2-hydroxy Acids: A Comparison of Mutant and Wild-Type Oxidoreductases

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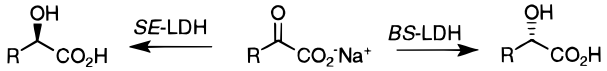
We describe a new chemoenzymatic synthesis of enantiopure 4-amino-2-hydroxy acids using two biotransformations in a single-pot process in aqueous medium. These compounds are valuable as γ-turn mimics for investigations into the secondary structure of peptides. The enzyme substrates are a series of carbobenzyloxy (CBZ)-protected 4-amino-2-keto esters, prepared efficiently from the L-amino acids, alanine, leucine, phenylalanine, and valine. First, the α-amino acids were converted to the corresponding β-amino acids in a simple five-step procedure. A further one-carbon homologation via ozonolysis of the corresponding β-keto cyanophosphoranes gave the required α-keto esters in good yield. The enzyme catalyzed hydrolyses of all the α-keto esters to the corresponding α-keto acids proceeded smoothly with the lipase from *Candida rugosa*. Using the same reaction pot, it was found that wild-type lactate dehydrogenases from either *Bacillus stearothermophilus* (*BS*-LDH) or *Staphylococcus epidermidis* (*SE*-LDH) could be used to specifically reduce the ketone of the alanine-derived α-keto acid **2**, giving the (*S*)- and (*R*)-2-hydroxy acids, respectively, in good yields. However, the more bulky α-keto acids **3**, **4**, and **5** (derived from valine, leucine, and phenylalanine) were not substrates for these enzymes. In contrast, the genetically engineered H205Q mutant of D-hydroxyisocaproate dehydrogenase proved to be an ideal catalyst for the reduction of all the α-keto acids **2**−**5**, giving excellent yields of the CBZ-protected (2*R*,4*S*)-4-amino-2-hydroxy acids as single diastereomers. This genetically engineered oxidoreductase has great potential value in synthesis due to its broad substrate specificity and high catalytic activity. For example, reduction of 1 mmol of N-protected (*S*)-4-amino-2-oxopentanoic acid **2** took just 4 h with the H205Q mutant giving, after esterification, the (*R*)-2-alcohol **25** in 85% yield, whereas with *SE*-LDH the reaction required 4 days to give a 67% yield of **25**.

Introduction

2-Hydroxy acids are valuable building blocks for use in the synthesis of complex molecules and may be prepared in good yields and with excellent enantioselectivities by the lactate dehydrogenase-catalyzed reduction of the corresponding α-keto acids. These reactions are simple to perform on a multigram scale.[1] L- and D-lactate dehydrogenases have been obtained from various mammalian and bacterial sources and exhibit reasonably broad substrate specificities. For example, LDHs from *Bacillus stearothermophilus* (*BS*-LDH) and *Staphylococcus epidermidis* (*SE*-LDH) have been used to prepare a series of (*S*)- or (*R*)-2-hydroxy acids, respectively, which in the main possess either a saturated hydrocarbon side chain or an aromatic ring (Scheme 1).[2,3] The utility of the resultant 2-hydroxy acids as intermediates in synthesis is limited to manipulation of the alcohol and/or the carboxylic acid. Clearly, the utility of these products would be increased by the presence of further functionality in the side chain. Examples of the use of LDHs for the synthesis of functionalized 2-hydroxy acids include the reduction of 2,4-dioxo acids,[4] 3-chloropyruvate,[5] substituted aromatic rings,[6] and unsaturated 2-keto acids.[7] However, in some cases, the reduction was so slow that it is not a viable route for the production of sufficient quantities of the enantioenriched alcohols for use in synthesis. One way to overcome this problem is to genetically modify the enzymes to give enhanced turnover rates and broader substrate specificities.[8]

Scheme 1



R = CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $(CH_3)_2CH$, $PhCH_2$, cyclopropyl

Holbrook and co-workers have genetically altered sites in both the mobile polypeptide chain and other residues around the active site of *BS*-LDH, giving the MVS/GG mutant with an active site that is more hydrophobic and flexible.[9] Casy et al. have shown that reduction of 4-methyl-2-oxopent-3-enoic acid using wild-type *BS*-LDH proceeded very slowly, whereas reduction with this

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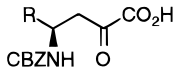
genetically altered MVS/GG mutant proceeded significantly faster, giving the corresponding (*S*)-2-hydroxy acid in 91% yield and 99% ee.[10]

Recently, it has been reported that the D-2-hydroxy-4-methylvalerate dehydrogenase (also known as D-hydroxyisocaproate dehydrogenase[11]) from *Lactobacillus delbrueckii bulgaricus* (*LB*-hicDH) has more favorable kinetic parameters than D-LDHs for certain substrates.[12] Holbrook and co-workers[13] have used genetic engineering to prepare a rational series of mutants of this enzyme and so analyzed the important residues involved in its active site. They found a much enhanced rate for the reduction of 2-oxo-4-phenylbutanoic acid with the H205Q mutant (exchange of the His-205 for Gln) of *LB*-hicDH than with LDHs.

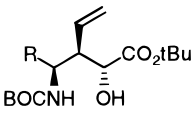
We now describe our investigations on the comparison of this H205Q mutant dehydrogenase with wild-type LDHs for the synthesis of α-hydroxy acids with nitrogen-containing functionality in the side chain. These products are of particular value as γ-turn mimics to investigate the secondary structure of peptides as well as building blocks for use in synthesis.

Results and Discussion

We have shown that carbobenzyloxy (CBZ)-protected 4-amino-2-oxobutanoic acid **1** is reduced by the commercially available wild-type *BS*-LDH and *SE*-LDH, giving the corresponding (*S*)- and (*R*)-2-hydroxy acids, respectively, in good yields and >99% ee.[14] However, the reaction was very slow with *SE*-LDH and, on a 1 mmol scale, took 7 days to reach completion. Hence, we reasoned that the analogous CBZ-protected 4-substituted 4-amino-2-oxobutanoic acids **2**−**5** would be interesting



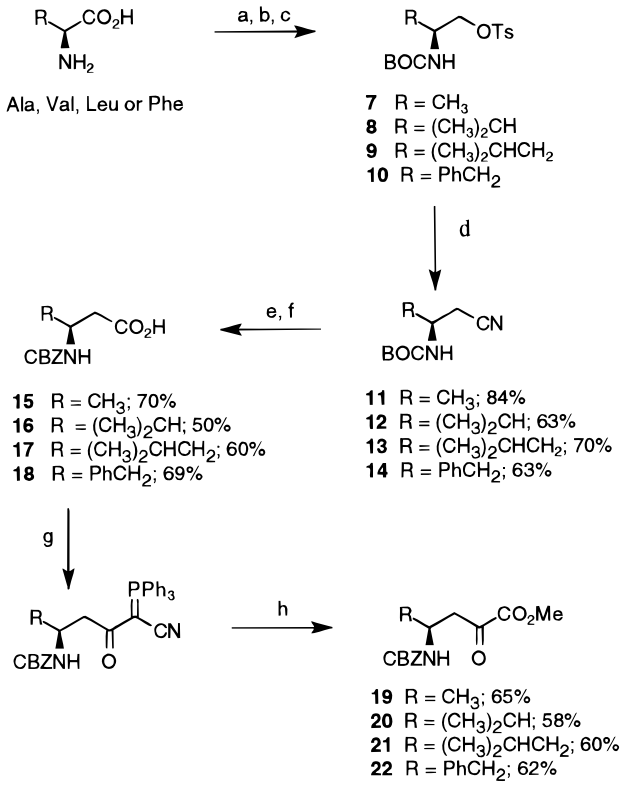
1 R = H
2 R = CH_3
3 R = $(CH_3)_2CH$
4 R = $(CH_3)_2CHCH_2$
5 R = $PhCH_2$



6 R = CH_3, $(CH_3)_2CH$, $(CH_3)_2CHCH_2$ or $PhCH_2$

compounds for our substrate specificity studies with the wild-type and mutant oxidoreductases. Furthermore, the resultant α-hydroxy acids would be of value to examine the γ-turn associated with the secondary structure of proteins.[15] Reetz and co-workers[16] have prepared a series of allyl-substituted 4-amino-2-hydroxy acids **6** via a Wittig rearrangement of 4-amino(allyloxy)acetates (with typical diastereoselectivities of 80%) and shown that when incorporated into peptides these compounds display

Scheme 2[a]



[a] Reagents: (a) BOC_2O, 2 M NaOH; (b) EtOCOCl, Et_3N then $NaBH_4$; (c) TsCl, Et_3N, DMAP; (d) NaCN, DMF; (e) NaCN, DMF; (e) 6 M HCl; (f) BnOCOCl, 2 M NaOH; (g) Ph_3PCHCN, DMAP; (h) O_3, MeOH.

the conformational features of a γ-turn by forming intramolecular hydrogen bonds between the carbonyl group of one residue and the hydroxyl group of the 4-amino-2-hydroxy acid. This appears to be the only reported synthesis of these compounds.

The required α-keto esters **19**−**22** were prepared from the L-amino acids as shown in Scheme 2. There are many examples of the conversion of an α-amino acid to the homologous β-amino acid, including the Arndt−Eistert reaction[17] and the stereoselective ring opening of aziridine 2-carboxylates.[18] We favored an approach involving nucleophilic displacement of a tosylate by cyanide[19] followed by hydrolysis. van den Broek et al.[20] have reported that such tosylates are unstable, and so the tosylates **7**−**10** were immediately treated with sodium cyanide in DMF to give the required nitriles **11**−**14** in 63−84% yields. Under acidic conditions, the nitrile was hydrolyzed and the BOC group removed. The resultant β-amino acids were reacted directly with benzyl chloroformate to give the acids **15**−**18** in 50−70% yields over the two steps. An efficient method for the preparation of α-keto esters from carboxylic acids via ozonolysis of β-ketocyanophosphoranes was described by Wasserman

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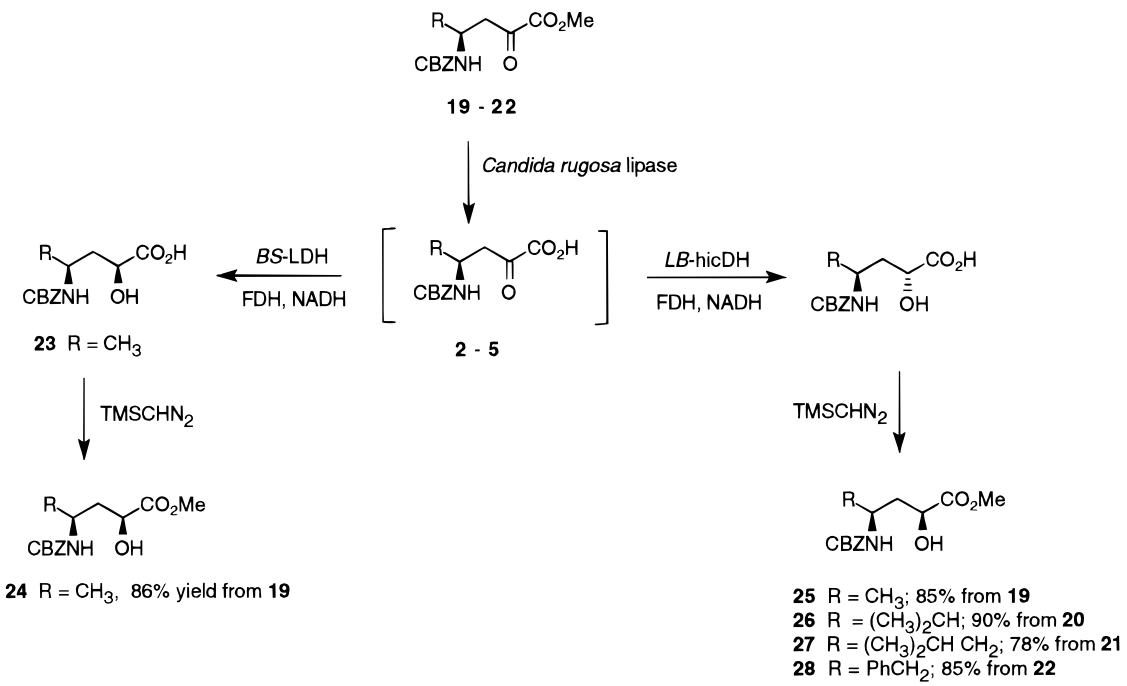
Scheme 3



and Ho,[21] and we used this method to prepare the required α-keto esters. Activation of the acids **15**−**18** with EDCI/DMAP and coupling with (cyanomethylene)-triphenylphosphorane gave the analogous *β*-keto cyano-phosphoranes, which were ozonolyzed in MeOH/CH_2Cl_2 to give the required α-keto esters **19**−**22** in 58−65% yields over the two steps.

For the hydrolysis of the N-protected 4-amino-2-keto esters **19**−**22**, lipase from *Candida rugosa* was used in order to allow mild saponification of the ester at a pH close to 7.0 under aqueous conditions. An advantage of using this method is that the conditions required for the hydrolysis are compatible with the dehydrogenase-catalyzed reduction step, thus allowing the two reactions to be carried out in a one-pot procedure. The oxidoreductases require a stoichiometric amount of the expensive cofactor NADH; however, by use of the formate−formate dehydrogenase (FDH) protocol developed by Shaked and Whitesides,[22] the NADH may be recycled in situ, enabling the economic synthesis of multigram quantities of α-hydroxy acids. For ease of handling and purification, the α-hydroxy acids isolated from the enzyme-catalyzed reactions were directly converted to the corresponding α-hydroxy esters using trimethylsilyldiazomethane (Scheme 3).

The 4-amino-2-oxo esters **19**−**22** were all accepted as substrates by lipase. However, only the alanine-derived α-keto acid **2** was then reduced by *BS*-LDH and *SE*-LDH, giving after esterification the (*S*)- and (*R*)-2-hydroxy esters **24** and **25**, respectively. In the cases of the substrates derived from valine, leucine, and phenylalaine **20**−**22** with more bulky substituents at C-4, only the corresponding α-keto acids **3**−**5** were isolated from the enzyme-catalyzed reactions. In contrast, the mutant H205Q dehydrogenase proved to an excellent catalyst for the reduction of all of the α-keto acids giving the corresponding (*R*)-2-hydroxy esters **25**−**28** as single diastereomers in 78−90% yield over the three steps from the corresponding 2-keto esters **19**−**22**.

The genetically engineered H205Q mutant D-dehydrogenase clearly has great potential value in organic synthesis. This present study indicates that it has a much broader substrate specificity than wild-type LDHs. This may be attributed to the presence of four extra residues in the mobile loop closing the active site, thus allowing accommodation of larger substrates.[12] In addition, the remarkable catalytic activity should enable the efficient synthesis of large quantities of (*R*)-2-hydroxy acids. It has been proposed that the protonated H205 residue forms an ionic interaction with the negative pyrophosphate backbone of NADH allowing tight binding of the cofactor in the active site.[13] Loss of this interaction in the H205Q mutant allows weaker binding of the coenzyme, thus allowing faster release of NAD^+ and therefore resulting in more rapid overall reduction of the α-keto acid. Indeed, our investigations have revealed that on a 1 mmol scale reduction of the CBZ derivative of (*S*)-4-amino-2-oxopentanoic acid **2** with *SE*-LDH required 4 days, giving the (*R*)-2-hydroxy ester **25** in 67% yield over the three steps from the corresponding keto ester **19,** whereas with the H205Q mutant the reduction was complete in 4 h, giving **25** in 85% yield.

In conclusion, we have shown that a combination of two biotransformations (a hydrolysis and specific reduction) in a single-pot process enables the clean and efficient synthesis of novel α-hydroxy acids in excellent yields. The products are to be used as *γ*-turn mimics to explore the secondary structure of peptides. The broad substrate specificity and enhanced turnover rates of the H205Q mutant of *LB*-hicDH gives this enzyme exciting potential as an efficient catalyst for the large-scale production of novel α-hydroxy acids for use in organic synthesis.

Experimental Section

General Methods.[7b] The enzymes used were purchased and stored as follows: Lipase from *C. rugosa* (Sigma Protein)

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(1 000 000 eU) was dissolved in Tris buffer (100 mL, 5 mM) and stored at 4 °C; L-lactate dehydrogenase from *Bacillus stearothermophilus* (*BS*-LDH) (Genzyme) and D-lactate dehydrogenase from *Staphylococcus epidermidis* (*SE*-LDH) (Sigma) were both stored at −20 °C. D-Hydroxyisocaproate dehydrogenase from *Lactobacillus delbrueckii bulgaricus* (*LB*-hicDH) (a gift from Professor J. J. Holbrook, Department of Biochemistry, University of Bristol); formate dehydrogenase from *Candida boidinii* (FDH) (Boerhinger), and β-nicotinamide adenine dinucleotide hydride (NADH) (Genzyme) were all stored at 4 °C.

General Procedure for the Synthesis of *N*-(*tert*-Butoxycarbonyl)-L-amino Acids. The amino acid (0.1 mol) was dissolved in 2 M sodium hydroxide (100 mL) and cooled to 0 °C. Di-*tert*-butyl dicarbonate (1.2 equiv) was slowly added. After 0.5 h, the reaction mixture was warmed to room temperature and allowed to stir for a further 2 h. The reaction mixture was then acidified to pH 2 using concentrated hydrochloric acid and extracted with ethyl acetate (3 × 50 mL). The resulting organic layer was then dried ($MgSO_4$) and concentrated in vacuo. The resulting white solid was then recrystallized using the appropriate solvent.

***N*-(*tert*-Butoxycarbonyl)-L-alanine**: 92% yield; mp 82−84 °C (from ethyl acetate) (lit.[23] mp 82−83 °C); $[\alpha]^{22}_D$ −19.6 (*c* 1.6, CH_2Cl_2) (lit.[23] $[\alpha]^{23}_D$ −19.2 (*c* 2.0, CH_2Cl_2)).

***N*-(*tert*-Butoxycarbonyl)-L-valine**: 94% yield; mp 74−76 °C (from ethyl acetate) (lit.[24] mp 73−79 °C); $[\alpha]^{23}_D$ −7.1 (*c* 1.2, AcOH) (lit.[24] $[\alpha]^{25}_D$ −6.4 (*c* 1.0, AcOH)).

***N*-(*tert*-Butoxycarbonyl)-L-leucine**: 89% yield; mp 81−83 °C (from ethyl acetate and light petroleum) (lit.[24] mp 82−83 °C); $[\alpha]^{23}_D$ −23.6 (*c* 0.9, AcOH) (lit.[24] $[\alpha]^{23}_D$ −24.5 (*c* 2.0, AcOH)).

***N*-(*tert*-Butoxycarbonyl)-L-phenylalanine**: 81% yield; mp 75−78 °C (from ethyl acetate and light petroleum) (lit.[25] mp 76−79 °C (from ethyl acetate and light petroleum)); $[\alpha]^{25}_D$ −6.2 (*c* 1.0, AcOH) (lit.[25] $[\alpha]^{25}_D$ −3.91 (*c* 0.8, AcOH)).

General Procedure for the Reduction of *N*-(*tert*-Butoxycarbonyl)-L-amino Acids Using Ethyl Chloroformate and Sodium Borohydride. The protected amino acid (0.1 mol) was dissolved in THF (100 mL) under a nitrogen atmosphere and cooled to 0 °C. Triethylamine (1.1 equiv) and ethyl chloroformate (1.1 equiv) were then added to form a white precipitate. After 0.25 h, the solution was filtered. The filtrate was added dropwise to a solution of sodium borohydride (1.5 equiv) in water (40 mL) at 0 °C. After 0.5 h, the reaction mixture was warmed to room temperature and allowed to stir for a further 2 h. The reaction mixture was then acidified to pH 2 using 2 M hydrochloric acid and extracted with ethyl acetate (3 × 50 mL). The organic layer was then washed with a saturated solution of sodium hydrogen carbonate, dried ($MgSO_4$), and concentrated in vacuo.

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]propan-1-ol: 69% yield; mp 57−58 °C (from ethyl acetate and light petroleum) (lit.[26] 59−61 °C (from ethyl acetate and light petroleum)); $[\alpha]^{24}_D$ −6.9 (*c* 0.3, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −8.9 (*c* 1.01, $CHCl_3$)).

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylbutan-1-ol: oil; 90% yield; $[\alpha]^{24}_D$ −17.9 (*c* 1.0, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −17.0 (*c* 1.7, $CHCl_3$)).

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-4-methylpentan-1-ol: oil; 70% yield; $[\alpha]^{24}_D$ −26.8 (*c* 0.8, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −22.0 (*c* 1.66, $CHCl_3$)).

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropan-1-ol: 75% yield; mp 93−95 °C (from ethyl acetate and light petroleum) (lit.[26] mp 95−97 °C (from ethyl acetate and light petroleum)); $[\alpha]^{23}_D$ −27.9 (*c* 0.2, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −27.7 (*c* 1.11, $CHCl_3$)).

General Procedure for the Synthesis of the Amino Nitriles 11−14 via the Tosylates 7−10. The amino alcohol (0.1 mol) was dissolved in dichloromethane (100 mL) and cooled to 0 °C under a nitrogen atmosphere. Triethylamine (1.1 equiv), DMAP (0.2 g), and *p*-toluenesulfonyl chloride (1.3 equiv) were all added. The reaction mixture was warmed to room temperature and allowed to stir overnight under a nitrogen atmosphere. The reaction mixture was then acidified to pH 2 with 2 M hydrochloric acid. The two layers were separated, and the aqueous layer was washed with dichloromethane (2 × 50 mL). The combined organic layers were then dried ($MgSO_4$) and concentrated in vacuo.

A solution of the crude tosylate (0.1 mol) in DMF (40 mL) was slowly added to a solution of sodium cyanide (3 equiv) in DMF (50 mL). The reaction mixture was allowed to stir at room temperature for 24 h under a nitrogen atmosphere. The reaction mixture was then diluted with water (70 mL) and extracted with ethyl acetate (3 × 60 mL). The organic layer was then washed with water (3 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting white solid was then purified by recrystallization from the appropriate solvent.

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]butanenitrile (11): 84% over two steps; mp 68−70 °C (from dichloromethane and hexane) (lit.[26] mp 69−71 °C (from dichloromethane and hexane)); $[\alpha]^{25}_D$ −84.3 (*c* 1.0, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −87.0 (*c* 1.0, $CHCl_3$)).

(*R*)-3-[(*tert*-Butoxycarbonyl)amino]-4-methylpentanenitrile (12): 63% yield over two steps; mp 80−82 °C (from dichloromethane and hexane) (lit.[26] mp 82−84 °C (from dichloromethane and hexane)); $[\alpha]^{25}_D$ −83.4 (*c* 0.3, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −81.5 (*c* 0.65, $CHCl_3$)).

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-5-methylhexanenitrile (13): 70% over two steps; mp 75−77 °C (from dichloromethane and hexane) (lit.[26] mp 75−77 °C (from dichloromethane and hexane)); $[\alpha]^{24}_D$ −86.2 (*c* 1.4, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −83.2 (*c* 0.82, $CHCl_3$)).

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-phenylbutanenitrile (14): 63% yield over two steps; mp 118−120 °C (from dichloromethane and hexane) (lit.[26] mp 119−120 °C (from dichloromethane and hexane)); $[\alpha]^{25}_D$ −19.6 (*c* 0.3, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ −18.7 (*c* 0.48, $CHCl_3$)).

General Procedure for the Preparation of CBZ-Protected β-Amino Acids 15−18. The amino nitrile (0.1 mol) was dissolved in 6 M hydrochloric acid and heated under reflux for 12 h. The reaction mixture was then cooled, and 2 M sodium hydroxide was added until a pH of 7 was achieved. The solution was concentrated in vacuo. The crude β-amino acid was then dissolved in 2 M sodium hydroxide (100 mL) and cooled to 0 °C. Benzyl chloroformate (1.2 equiv) was slowly added. After 0.5 h, the reaction mixture was warmed to room temperature and allowed to stir for a further 2 h. The reaction mixture was then acidified to pH 2 using concentrated hydrochloric acid and extracted with ethyl acetate (3 × 50 mL). The resulting organic layer was then dried ($MgSO_4$) and concentrated in vacuo. The resulting white solid was then recrystallized using the appropriate solvent.

(*S*)-3-[(Benzyloxycarbonyl)amino]butanoic acid (15): 70% yield over two steps; mp 104−106 °C (from diethyl ether and light petroleum) (lit.[27] mp 105−107 °C (from diethyl ether and light petroleum)); $[\alpha]^{24}_D$ −24.1 (*c* 0.4, $CHCl_3$) (lit.[27] $[\alpha]^{20}_D$ −26.0 (*c* 1.0, $CHCl_3$)).

(*R*)-3-[(Benzyloxycarbonyl)amino]-4-methylpentanoic acid (16): oil; 50% yield over two steps; $[\alpha]^{23}_D$ −33.6 (*c* 0.2, $CHCl_3$); ν_{max}(film)/cm^{-1} 3327 (NH), 1707 (br, 2 × CO); δ_H (270 MHz) 0.94 (3H, d, J = 7Hz, 5-H$_3$), 0.95 (3H, d, J = 7Hz, 4-CH$_3$), 1.87 (1H, m, 4-H), 2.89 (1H, dd, J = 15, 9Hz, 2-*H*H), 3.07 (1H, dd, J = 15, 4Hz, 2-H*H*), 4.00 (1H, m, 3-H), 5.01 (1H, d, J = 12Hz, PhC*H*H), 5.03 (1H, d, J = 12Hz, PhCH*H*), 7.26−7.36 (5H, m, Ph); Found (CI) $[MH]^+$ 266.1390, $C_{14}H_{19}NO_4$ requires $[MH]^+$ 266.1392; *m*/*z* (CI) 266 ($[MH]^+$, 6), 91 (100).

(*S*)-3-[(Benzyloxycarbonyl)amino]-5-methylhexanoic acid (17): 60% yield over two steps; mp 76−78 °C (from ethyl

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acetate and light petroleum) (lit.[28] mp 80 °C); $[\alpha]^{23}_D$ −31.6 (*c* 1.3, $CHCl_3$) (lit.[28] $[\alpha]^{20}_D$ −29.0 (*c* 2.5, $CHCl_3$)).

(*S*)-3-[(Benzyloxycarbonyl)amino]-4-phenylbutanoic acid (18): 69% yield over two steps; mp 116−118 °C (from ethyl acetate and light petroleum) (lit.[17] mp 118−119 °C); $[\alpha]^{22}_D$ −37.2 (*c* 1.0, AcOH) (lit.[17] $[\alpha]^{20}_D$ −38.0 (*c* 1.0, AcOH)).

General Procedure for the Synthesis of 2-Keto Esters 19−22 via the 3-Keto Cyanophosphoranes. The (cyanomethyl)triphenylphosphorane chloride was prepared according to the method of Trippett and Walker.[29] The salt (1.8 equiv) was dissolved in water (50 mL) and dichloromethane (50 mL). Sodium hydroxide (6 equiv) in water (15 mL) was slowly added. After 0.3 h, the two layers were separated. The dichloromethane layer was dried ($MgSO_4$) and added to a solution of the carboxylic acid (0.1 mol), DMAP (0.2 g), and EDCI (1.8 equiv) in dichloromethane (100 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was then warmed to room temperature and allowed to stir for 16 h. Water (100 mL) was added, and the two layers were separated. The organic layer was washed with a saturated solution of sodium hydrogen carbonate (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude β-keto cyanophosphorane was then dissolved in dichloromethane (100 mL) and methanol (100 mL) and cooled to −78 °C. Ozone was then bubbled through the solution until the reaction mixture had turned blue. Oxygen was then passed through the solution to remove the excess ozone. The solution was warmed to room temperature and concentrated in vacuo. The crude product was purified by recrystallization from the appropriate solvent or by column chromatography, eluting with an increasing ratio of ethyl acetate in light petroleum.

Methyl (*S*)-4-[(Benzyloxycarbonyl)amino]-2-oxopentanoate (19): oil; 65% over two steps; $[\alpha]^{23}_D$ −15.8 (*c* 0.5, $CHCl_3$); ν_{max}(film)/cm^{-1} 3460 (NH), 1732 (br, 3 x CO); δ_H (400 MHz) 1.26 (3H, d, *J* = 8Hz, 5-H$_3$), 3.03 (2H, m, 3-H$_2$), 3.86 (3H, s, OMe), 4.20 (1H, m, 4-H), 5.06 (1H, br m, NH), 5.08 (2H, s, PhC*H*$_2$) and 7.31−7.38 (5H, m, Ph); δ_C (75.5 MHz) 20.6 (5-C), 43.7 (3-C), 45.8 (4-C), 53.1 (OMe), 66.8 (Ph*C*H$_2$), 128.1, 128.2, 128.5, 136.3 (aromatics), 155.5 (1-C), 161 (PhCH$_2$O*C*O), 193.0 (2-C); found (CI) $[MH]^+$, 280.1176, $C_{14}H_{17}NO_5$ requires $[MH]^+$ 280.1185; *m*/*z* (CI) 280 ($[MH]^+$, 7), 91 (100).

Methyl (*R*)-4-[(Benzyloxycarbonyl)amino]-5-methyl-2-oxohexanoate (20): oil; 58% over two steps; $[\alpha]^{23}_D$ −31.7 (*c* 0.3, $CHCl_3$); ν_{max}(film)/cm^{-1} 3388 (NH), 1730 (br, 3 × CO); δ_H (270 MHz) 0.94 (6H, d, *J* = 7Hz, 5-CH_3 and 6-H$_3$), 1.89 (1H, oct, *J* = 7Hz, 5-H), 2.94 (1H, dd, *J* = 16, 8Hz, 3-*H*H), 3.06 (1H, dd, *J* = 16, 4Hz, 3-H*H*), 3.86 (3H, s, OMe), 3.94 (1H, m, 4-H), 4.91 (1H, br d, *J* = 9Hz, NH), 5.05 (2H, s, PhC*H*$_2$), 7.27−7.40 (5H, m, Ph); δ_C (75.5 MHz) 14.2 (6-C), 19.1 (5-CH_3), 32.0 (5-C), 43.0 (3-C), 53.1 (OMe), 61.0 (4-C), 78.0 (Ph*C*H$_2$), 128.2, 128.5, 135.8 (aromatics), 155.0 (1-C), 161.0 (PhCH$_2$O*C*O), 192.0 (2-C); found (CI) $[MH]^+$ 308.1499, $C_{16}H_{21}NO_5$ requires $[MH]^+$ 308.1498; *m*/*z* (CI) 308 ($[MH]^+$, 0.1), 91 (100).

Methyl (*S*)-4-[(benzyloxycarbonyl)amino]-6-methyl-2-oxoheptanoate (21): oil; 60% over two steps; $[\alpha]^{22}_D$ −29.0 (*c* 0.3, $CHCl_3$); ν_{max}(film)/cm^{-1} 3336 (NH), 1732 (br, 3 × CO); δ_H (400 MHz) 0.93 (3H, d, *J* = 6Hz, 7-H$_3$), 0.94 (3H, d, *J* = 6Hz, 6-CH_3), 1.26−1.68 (3H, m, 5-H$_2$ and 6-H), 2.96 (1H, dd, *J* = 17, 7Hz, 3-*H*H), 3.09 (1H, dd, *J* = 17, 5Hz, 3-H*H*), 3.86 (3H, s, OMe), 4.15 (1H, m, 4-H), 4.89 (1H, br d, *J* = 8Hz, NH), 5.06 (2H, s, PhC*H*$_2$), 7.29−7.38 (5H, m, Ph); δ_C (75.5 MHz) 22.0 (7-C), 22.8 (6-CH_3), 25.0 (6-C), 43.7 (5-C), 44.9 (3-C), 53.1 (OMe), 60.0 (4-C), 66.8 (Ph*C*H$_2$), 128.1, 128.2, 128.5, 136.0 (aromatics), 156.0 (1-C), 161.0 (PhCH$_2$O*C*O), 193.0 (2-C); found (CI) $[MH]^+$ 322.1661, $C_{17}H_{23}NO_5$ requires $[MH]^+$ 322.1654; *m*/*z* (CI) 322 ($[MH]^+$, 1), 91 (100).

Methyl (*S*)-4-[(benzyloxycarbonyl)amino]-2-oxo-5-phenylpentanoate (22): 62% yield over two steps; mp 107−109 °C (from ethyl acetate and light petroleum); $[\alpha]^{22}_D$ −15.1 (*c* 0.3, $CHCl_3$); ν_{max}(Nujol mull)/cm^{-1} 3312 (NH), 1750 (ester, CO), 1732 (ketone, CO), 1693 (amide, CO); δ_H (270 MHz) 2.86 (1H, dd, *J* = 14, 7Hz, 5-*H*H), 2.95 (1H, dd, *J* = 14, 7Hz, 5-H*H*), 3.02 (2H, m, 3-H$_2$), 3.83 (3H, s, OMe), 4.33 (1H, m, 4-H), 5.03 (2H, s, PhC*H*$_2$O), 5.07 (1H, d, *J* = 9Hz, NH), 7.13−7.38 (10H, 2 × Ph); δ_C (75.5 MHz) 40.2 and 43.1 (3-C and 5-C), 48.9 (4-C), 53.1 (OMe), 66.7 (Ph*C*H$_2$O), 126.9, 128.0, 128.1, 128.5, 128.7, 129.2, 136.2, 137.0 (aromatics), 155.6, (1-C), 160.8 (PhCH$_2$O*C*O), 192.1 (2-C); *m*/*z* (CI) 356 ($[MH]^+$, 56), 91 (100). Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.6; H, 5.9; N, 3.9. Found C, 67.2; H, 5.9; N, 3.9.

General Procedure for the Synthesis of N-Protected 4-Amino-2-hydroxy Esters. The 2-keto ester (1 mmol) was dissolved in 5 mM tris buffer (40 mL). *C. rugosa* lipase (10 000 eU) was added and the pH adjusted to 7.5 by the addition of 1.0 M hydrochloric acid. The pH was maintained at a value between 7.0 and 7.5 by the addition of 0.1 M sodium hydroxide until the pH had stopped changing or until 1 equiv of base had been added. The solution was deoxygenated by bubbling a stream of nitrogen through for 1 h. DTT (20 μL) was then added followed by the dehydrogenase enzyme (10 mg if *BS*-LDH, 6 mg if *SE*-LDH, and 1 mL if *LB*-hicDH), FDH (10 mg), sodium formate (1 g, 15 equiv), and NADH (10 mg). The reaction was allowed to stir under a nitrogen atmosphere with the pH being kept constant at approximately 6.1 by the addition of 1.0 M hydrochloric acid. After the pH had stopped changing or 1 equiv of acid was added, the reaction mixture was acidified to pH 2 by the addition of 2 M hydrochloric acid and extracted with ethyl acetate (3 × 40 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The resulting 2-hydroxy acid was dissolved in methanol (10 mL) and toluene (10 mL). Trimethylsilyldiazomethane (2.0 M) in hexane (0.6 mL, 1.1 equiv) was slowly added, turning the solution a bright yellow color. The solution was stirred for 0.5 h and then concentrated in vacuo. The resulting residue was purified by recrystallization from the appropriate solvent or by column chromatography, eluting with an increasing ratio of ethyl acetate in light petroleum.

Methyl (2*R*,4*S*)-4-[(Benzyloxycarbonyl)amino]-2-hydroxypentanoate (25). The lipase hydrolysis was complete after 4 days, and the *LB*-hicDH reduction was complete after 4 h. Recrystallization from ethyl acetate and light petroleum gave **25** as a white crystalline solid (85% yield): mp 72−73 °C (from ethyl acetate and light petroleum); $[\alpha]^{22}_D$ +16.7 (*c* 2.0, $CHCl_3$); ν_{max}(Nujol mull)/cm^{-1} 3343 (OH, NH), 1691 (br, 2 × CO); δ_H (270 MHz) 1.24 (3H, d, *J* = 7Hz, 5-H$_3$), 1.81 (2H, m, 3-H$_2$), 3.77 (3H, s, OMe), 4.02 (1H, m, 4-H), 4.30 (1H, dd, *J* = 10, 4Hz, 2-H), 5.03 (1H, d, *J* = 7Hz, NH), 5.09 (2H, s, PhC*H*$_2$), 7.26−7.37 (5H, m, Ph); *m*/*z* (CI) 282 ($[MH]^+$, 50), 264 ($[MH - H_2O]^+$, 29), 91 (100). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.8; H, 6.8; N, 5.0. Found: C, 59.8; H, 7.0; N, 5.2.

The above reaction was repeated using *SE*-LDH, and the reduction was complete after 4 days. Recrystallization from ethyl acetate and light petroleum gave **25** as a white crystalline solid (67% yield).

Methyl (2*S*,4*S*)-4-[(Benzyloxycarbonyl)amino]-2-hydroxypentanoate (24). The lipase hydrolysis was complete after 4 days, and the *BS*-LDH reduction was complete after 3 days. Recrystallization from ethyl acetate and light petroleum gave **24** as a white crystalline solid (86% yield): mp 48−50 °C (from ethyl acetate and light petroleum); $[\alpha]^{22}_D$ −11.4 (*c* 2.0, $CHCl_3$); ν_{max}(Nujol mull)/cm^{-1} 3335 (OH, NH), 1702 (br, 2 × CO); δ_H (270 MHz) 1.21 (3H, d, *J* = 7Hz, 5-H$_3$), 1.94 (2H, m, 3-H$_2$), 3.72 (3H, s, OMe), 4.02 (1H, m, 4-H), 4.27 (1H, t, *J* = 6Hz, 2-H), 4.81 (1H, br m, NH), 5.09 (2H, s, PhC*H*$_2$), 7.27−7.37 (5H, m, Ph); *m*/*z* (CI) 282 ($[MH]^+$, 2), 264 ($[MH - H_2O]^+$, 2), 91 (100). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.8; H, 6.8; N, 5.0. Found: C, 59.4; H, 7.1; N, 5.1.

Methyl (2*R*,4*R*)-4-[(Benzyloxycarbonyl)amino]-2-hydroxy-5-methylhexanoate (26). The lipase hydrolysis was complete after 4 days, and the *LB*-hicDH reduction was complete after 5 h. Purification by flash column chromatography, eluting with 46% ethyl acetate in light petroleum, gave **26** as a viscous oil (90% yield): $[\alpha]^{22}_D$ +9.0 (*c* 2.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2246 (OH, NH), 1694 (br, 2 × CO); δ_H (270 MHz) 0.92 (3H, d, *J* = 7Hz, 6-H$_3$), 0.94 (3H, d, *J* = 7Hz, 5-CH_3), 1.76 (3H, m, 3-H$_2$ and 5-H), 3.73 (1H, m, 4-H), 3.77 (3H, s, OMe), 4.25 (1H, dd, *J* = 8Hz, 6, 2-H), 4.83 (1H, d, *J* = 9Hz, NH),

(29) Trippett, S.; Walker, D. M. *J. Chem. Soc.* **1959**, 3874.

5.10 (2H, s, PhCH_2), 7.26−7.37 (5H, m, Ph); found (CI) $[MH]^+$, 310.1661, $C_{16}H_{23}NO_5$ requires $[MH]^+$ 310.1654; *m*/*z* (CI) 310 ($[MH]^+$, 8), 292 ($[MH - H_2O]^+$, 11), 91 (100).

Treatment of Methyl (*R*)-4-[(Benzyloxycarbonyl)amino]-5-methyl-2-oxohexanoate (20) with Lipase and *BS*-LDH. The reactions were carried out according to the above general procedure using methyl (*R*)-4-[(benzyloxycarbonyl)-amino]-5-methyl-2-oxohexanoate (**20**) (0.31 g, 1 mmol). The lipase hydrolysis was complete after 4 days. No pH change was noted after addition of *BS*-LDH, NADH, sodium formate, and FDH. After 2 weeks, the reaction mixture was worked up to give (*R*)-4-[(benzyloxycarbonyl)amino]-5-methyl-2-oxohexanoic acid (**3**) as a viscous oil (69% yield): $[\alpha]^{26}_D$ −16.3 (*c* 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3335 (OH, NH), 1732 (br, 3 × CO); δ_H (400 MHz) 0.93 (3H, d, *J* = 7Hz, 6-H_3), 0.95 (3H, d, *J* = 7Hz, 5-CH_3), 1.86 (1H, m, 5-H), 2.87 (1H, dd, *J* = 15, 9Hz, 3-*H*H), 3.07 (1H, dd, *J* = 15, 4Hz, 3-H*H*), 3.99 (1H, m, 4-H), 5.01 (1H, d, *J* = 12Hz, PhC*H*H), 5.05 (1H, d, *J* = 12Hz, PhCH*H*), 7.26−7.36 (5H, m, Ph); *m*/*z* (CI) 279 ($[MH]^+ - CH_3$, 2), 91 (100).

Methyl (2*R*,4*S*)-4-[(Benzyloxycarbonyl)amino]-2-hydroxy-6-methylheptanoate (27). The lipase hydrolysis was complete after 4 days, and the *LB*-hicDH reduction was complete after 4 h. Recrystallization from ethyl acetate and light petroleum gave **27** as a white crystalline solid (78% yield): mp 72−73 °C (from ethyl acetate and light petroleum); $[\alpha]^{22}_D$ −1.0 (*c* 2.0, $CHCl_3$); ν_{max}(Nujol mull)/cm^{-1} 3353 (OH, NH), 1682 (br, 2 × CO); δ_H (270 MHz) 0.92 (6H, d, *J* = 6Hz, 6-CH_3 and 7-H_3), 1.24−1.71 (3H, m, 5-H_2 and 6-H), 1.78 (2H, m, 3-H_2), 3.78 (3H, s, OMe), 3.99 (1H, m, 4-H), 4.28 (1H, t, *J* = 7Hz, 2-H), 4.85 (1H, d, *J* = 9Hz, NH), 5.10 (2H, s, PhCH_2), 7.29−7.40 (5H, m, Ph); *m*/*z* (CI) 324 ($[MH]^+$, 1), and 91 (100). Anal. Calcd for $C_{17}H_{25}NO_5$: C, 63.2; H, 7.7; N, 4.3. Found: C, 63.0; H, 7.7; N, 4.1.

Treatment of Methyl (*S*)-4-[(Benzyloxycarbonyl)amino]-6-methyl-2-oxopentanoate (21) with Lipase and *BS*-LDH. The reactions were carried out according to the above general procedure using methyl (*S*)-4-[(benzyloxycarbonyl)amino]-6-methyl-2-oxoheptanoate (**21**) (0.32 g, 1 mmol). The lipase hydrolysis was complete after 4 days. No pH change was noted after addition of *BS*-LDH, NADH, sodium formate, and FDH. After 2 weeks, the reaction mixture was worked up to give (*S*)-4-[(benzyloxycarbonyl)amino]-6-methyl-2-oxoheptanoic acid (**4**) as a viscous oil (0.31 g, 100%): $[\alpha]^{22}_D$ −25.1 (*c* 0.5, $CHCl_3$); ν_{max}(film)/cm^{-1} 3329 (OH, NH), 1694 (br, 3 × CO); δ_H (400 MHz) 0.95 (6H, d, *J* = 6Hz, 6-CH_3 and 7-H_3), 1.28−1.71 (3H, m, 5-H_2 and 6-H), 2.96 (1H, dd, *J* = 17, 7Hz, 3-*H*H), 3.05 (1H, dd, *J* = 17, 5Hz, 3-H*H*), 4.18 (1H, m, 4-H), 5.05 (2H, s, PhCH_2), 7.26−7.41 (5H, m, Ph); found (CI) $[MH]^+$ 308.1480, $C_{16}H_{21}NO_5$ requires $[MH]^+$ 308.1498; *m*/*z* (CI) 308 ($[MH]^+$, 1), 91 (100).

Methyl (2*R*,4*S*)-4-[(Benzyloxycarbonyl)amino]-2-hydroxy-5-phenylpentanoate (28). Lipase hydrolysis was complete after 4 days, and the *LB*-hicDH reduction was complete after 5 h. Purification by recrystallization from ethyl acetate and light petroleum gave **28** as white crystals (0.30 g, 85%): mp 89−91 °C (from ethyl acetate and light petroleum); $[\alpha]^{23}_D$ +2.3 (*c* 0.2, $CHCl_3$); ν_{max}(Nujol mull)/cm^{-1} 3491 (OH), 1732 (CO, ester), 1694 (CO, amide); δ_H (270 MHz) 1.80 (2H, m, 3-H_2), 2.81 (1H, dd, *J* = 13, 7Hz, 5-*H*H), 2.92 (1H, dd, *J* = 13, 7Hz, 5-H*H*), 3.74 (3H, s, OMe), 4.17 (1H, m, 4-H), 4.29 (1H, dd, *J* = 10, 4Hz, 2-H) 4.97 (1H, d, *J* = 9Hz, NH), 5.07 (2H, s, PhCH_2O), 7.15−7.39 (10H, m, 2 × Ph); *m*/*z* (CI) 358 ($[MH]^+$, 1), 91 (100). Anal. Calcd for $C_{20}H_{23}NO_5$: C, 67.2; H, 6.4; N, 3.9. found: C, 67.3; H, 6.5; N, 4.1.

Treatment of Methyl (*S*)-4-[(Benzyloxycarbonyl)amino]-2-oxo-5-phenylpentanoate (22) with Lipase and *BS*-LDH. The reactions were carried out according to the above general procedure using methyl (*S*)-4-[(benzyloxycarbonyl)amino]-2-oxo-5-phenylpentanoate (**22**) (0.35 g, 1 mmol). Lipase hydrolysis was complete after 5 days. No pH change was noted after the addition of *BS*-LDH, NADH, sodium formate, and FDH. After 2 weeks, the reaction mixture was workedup to give (*S*)-4-[(benzyloxycarbonyl)amino]-2-oxo-5-phenylpentanoic acid (**5**) as a white solid (0.28 g, 83%): $[\alpha]^{23}_D$ −17.4 (*c* 2.0, $CHCl_3$); ν_{max}-(Nujol mull)/cm^{-1} 3394 (OH, NH), 1717 (br, 3 × CO); δ_H (400 MHz) 2.87 (2H, m, 5-H_2), 3.01 (2H, m, 3-H_2), 4.36 (1H, m, 4-H), 5.03 (2H, s, PhCH_2O), 5.13 (1H, d, *J* = 8Hz, NH), 7.11−7.48 (10H, m, 2 × Ph); found (CI) $[MH]^+$ 342.1339, $C_{19}H_{19}NO_5$ requires $[MH]^+$ 342.1341; *m*/*z* (CI) 342 ($[MH]^+$, 26), 91 (100).

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