Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 663–669
Tetrahedron

Ketoreductases in the synthesis of valuable chiral intermediates: application in the synthesis of α-hydroxy β-amino and β-hydroxy γ-amino acids

Spiros Kambourakis* and J. David Rozzell

BioCatalytics Inc., 129 North Hill Ave., Suite 103, Pasadena, CA 91106, USA

Received 8 August 2003; revised 5 September 2003; accepted 17 October 2003

Abstract—A general method for the synthesis of β-alkyl α-hydroxy β-amino and α- and γ-alkyl substituted β-hydroxy-γ-amino acids is described. The synthesis of all three classes of amino acids proceeds through a common chiral alcohol intermediate that is generated from a pro-chiral ketone diester via the action of a nicotinamide-dependent ketoreductase. Regioselective chemical or enzymatic hydrolysis followed by rearrangement under Hofmann or Curtius conditions gives the final amino acid products. High yields of single diastereomers of the final amino acids are obtained. Amino acids with both natural and unnatural alkyl substituents can be accessed using this methodology.

1. Introduction

Amino alcohols, and in particular α-hydroxy β-amino acids (**1**, Fig. 1) and β-hydroxy γ-amino acids (**2**, **3**, Fig. 1), also known as statines, are valuable chiral compounds that are present in a variety of natural and synthetic compounds possessing a spectrum of biological activities. These key structural units are found in molecules showing several types of pharmacological activity including protease inhibitors, anti-neoplastic agents, antibacterials and anti-cancer drugs.[1] The significance of these molecules is also evident given the number of research publications and books dedicated to their synthesis.[1–6]

Some methods for the synthesis of such compounds are based on resolutions of ester derivatives using lipases and give low yields.[5] A number of elegant chemical synthetic routes have been reported, however, most require multi-step reaction sequences, chiral catalysts or starting materials, air-sensitive reaction conditions, or unstable intermediates.[1–3] In addition, few if any of these methodologies are generally useful for the synthesis of each of the individual diastereomers of a range of statine analogs, and most are not useful for preparing compounds with alkyl substitutions derived from non-naturally occurring amino acids.[4–6] Gaining control over the stereochemistry of the chiral carbons bearing both the amino and the alcohol groups at reasonable cost and high enantiomeric purity is the key to the successful production of these important chemical intermediates.

Herein we describe a chemo-enzymatic method for the synthesis of individual diastereomers of α-hydroxy β-amino and β-hydroxy γ-amino acids and analogs that are shown in Figure 1. Using non-chiral, readily available starting materials, a range of vicinal amino alcohols can be produced. The key step is a diastereoselective enzymatic reduction that generates two chiral centers in a single step, setting the absolute stereochemistry in the molecule. The remaining chemical steps are straightforward and can be

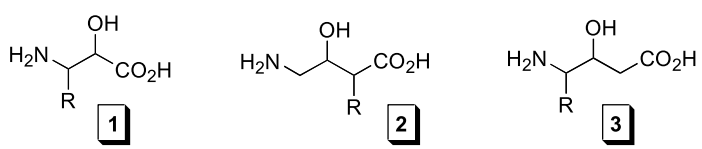


Figure 1. Targeted amino alcohols and amino acids.

* Corresponding author. Fax: +1-626-3563999; e-mail address: skambourakis@biocatalytics.com

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.10.109

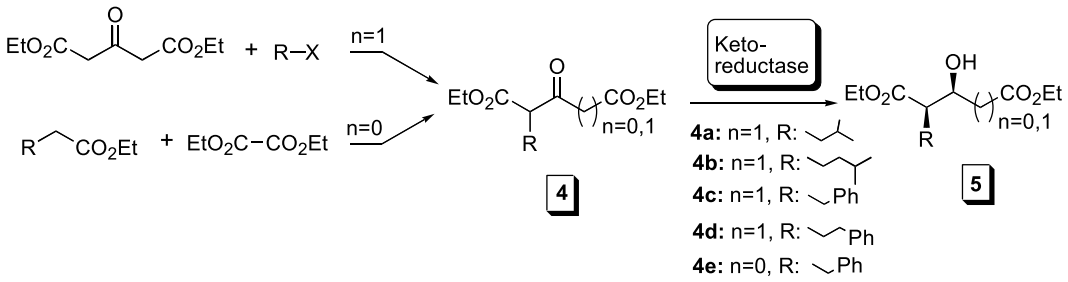


Figure 2. Synthesis of ketones and enzymatic reduction.

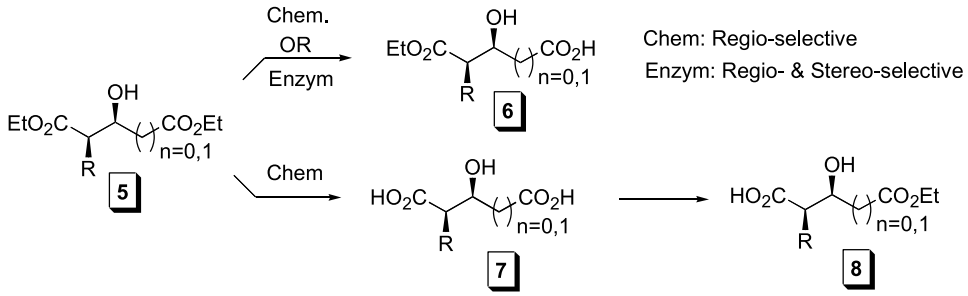


Figure 3. Synthesis of each mono ester can be achieved by either chemical or enzymatic techniques.

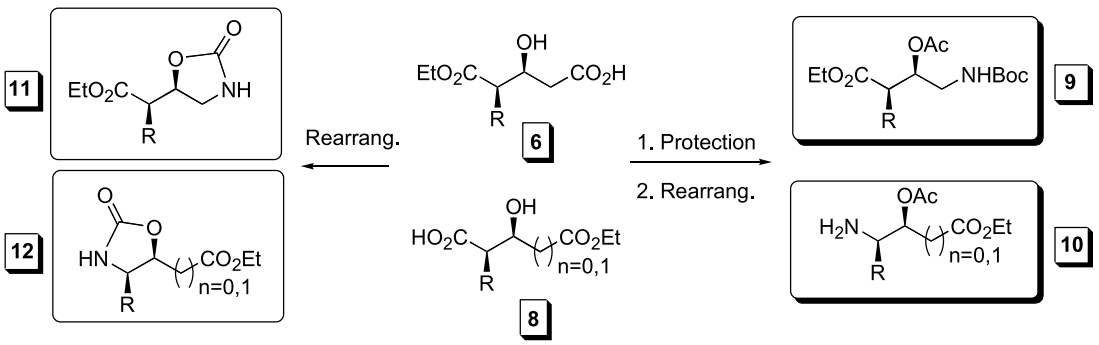


Figure 4. Both the cyclic carbamates or the free amines can be obtained from the rearrangement of the mono-acids.

carried out with readily available reagents. In the basic synthetic method, a ketodiester **4** (Fig. 2) is reduced diastereoselectively to form a key hydroxydiester intermediate **5** in high yield by the action of a ketoreductase enzyme. Both chiral centers present in the final statines and their analogs are generated in this enzymatic step. Regioselective chemical or enzymatic hydrolysis of either ester group of the key intermediate diester, followed by rearrangement under Hofmann or Curtius conditions, gives the final amino alcohol products (Figs. 3 and 4). In a recent report, we described the synthesis of statine and phenylstatine (R=isobutyl, **10a** and R=benzyl, **10c**, Fig. 4) using this methodology.[7] We now expand the method to include the synthesis of a range of statine analogs including α-hydroxy β-amino acids bearing natural and unnatural alkyl substitutions (Fig. 1).

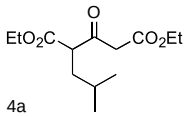
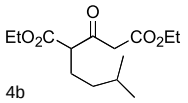
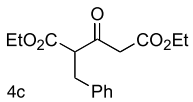
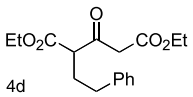
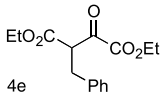
2. Results and discussion

2.1. Synthesis of substituted ketone diesters 4 (Fig. 2) and enzymatic reduction

Synthesis of 2-substituted diethyl ketoglutarates (**4a**–**d**, Fig. 2) is accomplished in a straightforward fashion via the mono-alkylation of diethyl 1,3 acetonedicarboxylate with the appropriate alkyl halide. The homo-statine **4b** and homo-phenylstatine **4d** (Table 1) precursors were synthesized by the alkylation of diethyl 1,3 acetonedicarboxylate with 1-iodo-3-methyl butane and 1-bromo-2-phenyl ethane, respectively, using methods based on previously reported methodology for the synthesis of **4a** and **4c** (Table 1).[7] The analogous compound 2-benzyl diethyl oxaloacetate (**4e**, Fig. 2) was synthesized by a Claisen condensation of ethyl hydrocinnamate and diethyl oxaloacetate using an adaptation of a reported method.[8] As an illustration of the generality of the method, we describe herein two compounds with natural side chains (**4a** and **4c**) and two compounds bearing non-natural alkyl substitutions (**4b**, **4d**, Table 1).

To identify the appropriate ketoreductase for a desired reaction, all ketodiester substrates were screened for reduction in the presence of each of 10 different commercially-available ketoreductases (KRED-10,000 Ketoreductase Screening Set, BioCatalytics Inc, Pasadena, CA, USA) using the protocol provided by the manufacturer. At least one enzyme was shown to be active with every ketone

Table 1. Reductions of ketone substrates using KRED enzymes

Substrate	Yield (A/B/C/D)[a]	
	KRED101	KRED108
 4a	>95% (0/5/0/95)[b]	
 4b	85% (0/13/0/87)	
 4c		95% (0/99/0/0)[b]
 4d	~5%	35% (95/5)[c]
 4e		>90% (0/99/0/0)[d]

[a] A/B/C/D represents the ratio of diastereomers. For statine (**4a**) and phenylstatine (**4c**): A: (3*S*,4*S*); B: (3*R*,4*R*); C: (3*R*,4*S*); D: (3*S*,4*R*).[7]
[b] Reactions described in Ref. 7.
[c] The ratio of diastereomers *SS*+*RR*/*SR*+*RS* is reported. No base-line separation of all diastereomers was achieved by either chiral GC or HPLC.
[d] Major diastereomer: (2*R*,3*S*).

substrate; the results from the best enzyme for each ketodiester are shown in Table 1.

As described earlier, the KRED-catalyzed diastereoselective reduction generates two chiral carbons during this reaction, and as a result, the absolute stereochemistry of the final statines and their analogs is determined at this point. For most reactions, the diastereomeric purity of the products is high, >90%. In cases where the optical purity is lower than desired, it can be further improved by stereoselective enzymatic hydrolysis using a lipase enzyme (vide infra).

For statine and phenylstatine precursors (**4a** and **4c** respectively, Table 1) the absolute stereochemistry of the enzymatic reduction was identified earlier after rearranging to the final amine (**10a** and **10c**) and comparing it with authentic material that was synthesized chemically.[7] For the homo-statine **4b** (Table 1) no standards and no stereo-selective chemical syntheses to either **5b** or **10b** were available. However, based on the chiral GC retention time of the alcohol **5b** (Table 1) and the known diastereoselectivity of the reaction catalyzed by KRED101 on ketodiester **5a** (Table 1), we assume that the major diastereomer of the reduction of **5b** and KRED101 is (3*S*, 4*R*). For the homo-phenylstatine precursor **5d** we were unable to obtain baseline separation of all diastereomers using either chiral GC or HPLC chromatography, and as a result, only the diastereomeric ratio is reported (**4d**, Table 1). Finally, the absolute stereochemistry for the reduction of **4e** by KRED 108 was assigned using chiral HPLC by comparison with authentic material that was synthesized from *R*- and *S*-diethyl malate and benzyl bromide according to reported methodology.[9]

Cell-free enzymatic reaction conditions were developed to carry out the enzymatic reductions. Nicotinamide co-factor was required by all ketoreductases, but with efficient recycling methods, the amount of added co-factor is normally less than 1 mol% relative to starting ketone. All the ketoreductases used in this study require NADPH as the co-factor for hydride transfer. We therefore used a recycling system based on reducing NADP+ to NADPH with glucose and the commercially available enzyme glucose dehydrogenase (GDH-101, BioCatalytics Inc, Pasadena, CA, USA). Glucose is an inexpensive, water-soluble reductant, and the immediate reaction product of the oxidation of glucose, gluconolactone, spontaneously hydrolyses to gluconic acid under the reaction conditions employed, making the overall process essentially irreversible. The pH of the reaction mixture was maintained at 6.7–6.8 using a pH-stat.

2.2. Regioselective hydrolysis and rearrangement

We have previously shown that the two ester groups of **5a** and **5c** (Fig. 3) were hydrolyzed chemically with different reaction rates.[7] As a result, mild basic chemical hydrolysis gave preferably the mono acids **6a** and **6c** (Fig. 3) while complete hydrolysis to the mono acid followed by esterification in ethanol gave mono-acids **8a** and **8c** (Fig. 3).[7] This methodology has been successfully applied to all diethyl 2-alkyl 3-hydroxy glutarates **5a**–**d** as well as the diethyl 2-benzyl 3-hydroxy succinate **5e** (Fig. 3).

Both regio- and stereo-selectivity can also be obtained when a lipase is utilized for the hydrolysis of **5** (Fig. 3). Twenty-four lipases (ICR Screening Set, BioCatalytics, Inc. Pasadena, CA, USA) were screened and enzymes were identified that were active against every diester **5**, giving compounds **6** as the only or the preferred product. In some cases, enzymatic hydrolysis of the second ester yielding the diacid was observed when the reactions were allowed to continue for very long periods or when large excesses of enzyme were used. When a diastereomeric mixture of alcohols was utilized as a starting material for enzymatic hydrolysis, in addition to regioselectivity, stereoselectivity can also be obtained. Such an effect was previously shown in the hydrolysis of a diastereomeric mixture of 2-methyl 3-hydroxy glutarate and ICR 112.[7] Similarly, lipases ICR 113 and ICR 114 selectively hydrolyzed the major component [(2*R*,3*S*)] of a diastereomeric mixture [(2*R*,3*S*)/(2*S*,3*S*), 9:1] of diethyl 2-benzyl-3-hydroxy succinate **5e**, giving mono-acid **6e** (n=0, Fig. 3) in 50–60% yield.

As shown earlier for statine (**5a**) and phenylstatine (**5c**) precursors and now confirmed for the rest of compounds in Table 1, rearrangement of the free alcoholic acids **8** (Fig. 4) under either Curtius or Hofmann conditions gave the cyclic carbamates **12**.[7] Formation of these products was shown to be independent of both the rearrangement reagents and the reaction conditions. The simplest and most straightforward one step synthesis of carbamates **12** was shown to be the heating of the free acid **8** with diphenylphosphoryl azide (DPPA) in toluene.[7] We now show that the opposite cyclic carbamates **11** can be obtained in good yields (50–70%)

when instead of **8**, the less hindered mono-acids **6a–d** were utilized for rearrangement with DPPA. We therefore conclude that this is a general method for the preparation of cyclic carbamates **11** and **12** from the corresponding mono-acids **6** and **8** (Fig. 4).

Formation of the amino acids **9** and **10** was accomplished after the alcohol was protected as an acetate prior to the rearrangement. Under these reaction conditions, the corresponding amides of **8a–d** were prepared and rearranged to the free amine **10a–d** using bis[(trifluoroacetoxy)iodo] benzene [$(CF_3CO_2)_2IPh$]. This methodology was utilized earlier for the synthesis of statine (**10a**) and phenylstatine (**10c**).[7] Besides the β-hydroxy γ-amino acids **10a–d** (n=1, Fig. 4), the 2-benzyl α-hydroxy β-amino acid **10e** (n=0, R=Bz, Fig. 4) was prepared using this methodology in similar overall yield. Therefore, this reaction sequence of acetyl protection of the alcohol followed by rearrangement is a general method for the preparation of statine and analogues **10** (Fig. 4).

The previous rearrangement conditions using bis[(trifluoroacetoxy)iodo]benzene were shown to be inadequate for the preparation of the primary amines **9** (Fig. 4) from the corresponding amides of **6**. This rearrangement was achieved using lead tetracetate [$Pb(OAc)_4$] according to a previously described procedure.[10]

3. Conclusions

This report describes a general method for the synthesis of statines and a number of analogs. An advantage of the methodology reported here is that in most cases each of the four diastereomers can be produced in high stereochemical purity. All compounds are produced from non-chiral, readily available starting materials, and the reactions are performed at room temperature and pressure.

From the cloned commercially-available ketoreductases that we have screened to date against compounds **4a–e**, two commercially available ketoreductases (KRED-101, KRED-108, BioCatalytics, Inc. Pasadena, CA, USA) gave products with good to excellent diastereoselectivity (Table 1). We plan to further broaden the applications for this general method by the development of new ketoreductase enzymes. These will be reported in due course.

4. Experimental

4.1. Synthesis of ketones 4 (Fig. 2)

The methodology that was published earlier for the synthesis of **4a** and **4c** (Table 1) was utilized for the synthesis of homo-statine **4b** and homo-phenylstatine **4d** precursors.[7] These products can be enzymatically reduced to the alcohol without any further purification, or they can be purified using silica gel chromatography (Hex/EtOAc, v/v, 8:2) giving 88% yield (3.6 g) for 1-iodo-3-methylbutane and 65% for 1-bromo-2-phenylethane. NMR verification of the products was performed after enzymatic or chemical reduction (vide infra).

Synthesis of diethyl 2-benzyl diethyl oxaloacetate **4e** (Table 1, Fig. 2) was achieved by a modification of standard methodology.[8] Ethyl hydrocinnamate (1 mL, 5.6 mmol) was dissolved in THF (10 mL) and the solution was cooled at −18 °C before butyl lithium (2.9 mL, 2.5 M in hexanes, 7.3 mmol) was slowly added. After stirring at this temperature for 10 min, diethyl oxalate (0.8 mL, 8.4 mmol) was added through a syringe and the reaction was slowly left to reach room temperature overnight. The reaction mixture was then added to an ice-cold water (50 mL, 0.15 M HCl) solution and extracted twice with diethyl ether (30 mL×2). The combined organic layers were back-extracted with brine, dried with Na_2SO_4 and evaporated to dryness. After silica gel purification a clear oil of 2-benzyl ethyl hydroxy succinate (0.9 g) (**4e**, Table 1) was isolated (yield, 58%). NMR verification of the products was performed after enzymatic or chemical reduction (vide infra).

4.2. Enzymatic reduction to alcohols 5 (Fig. 2)

The Ketoreductase Screening Set (KRED-10,000; Bio Catalytics, Inc., Pasadena, CA, USA) containing 10 different ketoreductases was screened to determine the best enzyme for the diastereoselective reduction of every 2-alkyl-3-ketoglutarate diethyl ester. In addition to the 10 ketoreductases both NADPH co-factor and glucose dehydrogenase (GLDH) are products available from BioCatalytics. Individual reactions containing each ketoreductase (2 mg/mL), NADPH (5 mM), NaCl (100 mM), DMSO (2.5 or 5% v/v) each substrate (25 mM), glucose (100 mM) and glucose dehydrogenase (GLDH, 2 mg/mL) for co-factor recycling were prepared in a phosphate-buffered (1 mL, 300 mM, pH 6.5) solution. The reactions were incubated at 37 °C overnight before they were extracted with ethyl acetate and analyzed by GC or HPLC chromatography.

Larger-scale enzymatic reductions (0.5–2 g of ketone) were prepared according to previously described conditions.[7] The optical purity of each product was determined by chiral GC chromatography using a ChiralDex column (Chiral Technologies, 130 °C, 2 min and then to 180 °C, 0.5 or 1 °C/min) after each enzymatically-produced alcohol was derivatized as the triflate ester. The enantiomeric purity was also examined using chiral HPLC analysis (column: CHIRALCEL OD-RH from Chiral technologies, H_2O/CH_3CN, v/v 60:40, 0.6 mL/min). All products were oily compounds and were analyzed by ^1H NMR.

4.2.1. Compound 5b. From KRED-101 reduction ^1H NMR (400 MHz, $CDCl_3$): δ=0.87+0.88 (d+d, J=3.20 Hz, 6H, $CH_2CH_2CH(CH_3)_2$), 1.17 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 1.27+1.29 (t+t, J=3.60 Hz+J=3.99 Hz, 6H, $CO_2CH_2CH_3$), 1.55 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 1.75 (m, 1H, $CH_2CH_2CH(CH_3)_2$) 2.47+2.51 and 2.55+2.58 (d+d J=9.19 Hz and d+d J=3.99 Hz first d+d overlaps with multiplet, 3H, $CH_2CO_2CH_2CH_3$ and $CHCO_2CH_2CH_3$), 4.2 (pent, J=7.19 Hz, 5H, $CO_2CH_2CH_3$, CHOH).

4.2.2. Compound 5d. From $NaBH_4$ reduction ^1H NMR (400 MHz, $CDCl_3$): δ=1.25 (two overlapping triplets ~2:1 ratio, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 1.31 (two overlapping

triplets ~2:1 ratio, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 1.9+2.05 (m+m, 2H, CH_2CH_2Ph), 2.55 (m, 3H, $CH_2CO_2CH_2CH_3$ and $CHCO_2CH_2CH_3$), 2.7 (m, 2H, CH_2CH_2Ph), 4.15+4.2 (q+q J=7.19 Hz, and a multiplet, 5H, $CO_2CH_2CH_3$, CHOH), 7.18+7.25 (m+m, 5H, CH_2CH_2Ph).

4.2.3. Compound 5e. From KRED-108 reduction ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.20 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 1.27 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 3.00 (m, 1H, $CHCO_2CH_2CH_3$), 2.95+3.23 (m+asymmetric triplet, 2H, CH_2Ph), 4.15+4.22 (m+m, 5H, $CO_2CH_2CH_3$, CHOH), 7.3 (m, 5H, CH_2Ph).

The spectra of **5a** and **5c** have been reported earlier.[7]

4.3. Hydrolysis to the mono acid 6 (Fig. 3)

Hydrolysis to the mono acid **6** (Fig. 3) using mild chemical or enzymatic conditions has been described earlier for the compounds **5a** and **5c**.[7] The same conditions were applied to the rest of the compounds of Table 1 including 2-benzyl-3-hydroxy succinate **5e** which was hydrolyzed to the mono-acid **6e** (R:benzyl, n=0, Fig. 3) by either chemical or enzymatic methods. Under chemical conditions, 2-benzyl-3-hydroxy succinate **5e** (0.15 g, 0.53 mmol) was added to an aqueous/ethanol (2 mL, 8:2, v/v) solution containing NaOH (0.042 g, 1.1 mmol) and the mixture was stirred for 45 min at room temperature until complete reaction to the mono acid was detected by HPLC analysis. Acidification with HCl (pH ~2) and extraction with EtOAc (10 mL, ×2) gave after solvent evaporation the mono-acid **6e** (0.11 g, 0.44 mmol, 83% isolated yield). The same mono-acid was obtained by enzymatic hydrolysis using ICR125, since ^{1}H NMR, GC and HPLC analysis of the chemical and enzymatic hydrolysis products were identical. Under the enzymatic reaction conditions, 2-benzyl-3-hydroxy succinate **5e** (0.15 g, 0.53 mmol) was dissolved in 0.3 mL of DMSO and was mixed with a potassium phosphate solution (200 mM, pH 7, 10 mL) also containing lyophilized ICR125 lipase (10 mg). After stirring at 37 °C for 2–3 h, the mixture was acidified with HCl (2 M) to pH ~2.5 and extracted with EtOAc (5 mL, ×2). Solvent drying with Na_2SO_4 and evaporation to dryness gave pure mono-acid **6e** as an oil in 90% isolated yield.

4.3.1. Compound 6e. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.20 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 3.00+3.05 and 3.21 (d+d, J=9.99 Hz and m, 3H, CH_2Ph and $CHCO_2CH_2CH_3$), 4.15 (m, 3H, $CO_2CH_2CH_3$, CHOH), 7.25 (m, 5H, CH_2Ph).

The hydrolysis of **5b** under mild chemical or enzymatic conditions follows the same regio-selectivity, giving the less hindered acid **6b**. This selectivity was confirmed by ^{1}H NMR analysis after making **8b** by the standard hydrolysis/esterification sequence and rearranging it to **12b** (vide infra). The hydrolysis selectivity and product characterization of **5a** and **5c** has been reported earlier.[7]

4.4. Rearrangement of mono acids 6 to the amines 9 and 11 (Fig. 4)

When rearrangement reactions were performed without prior protection of the alcohol, cyclic carbamates **11** (Fig. 4) were obtained. Following the same rearrangement conditions described earlier,[7] **6a** (reduced by KRED-101, hydrolyzed by ICR116) was mixed in toluene with diphenylphosphoryl azide (1.1 equiv. DPPA) and triethylamine (1.1 equiv.) and the solution was heated at 85 °C for 1 h before the temperature was lowered to 60 °C where it was stirred overnight. Product isolation and purification gave an oily compound **11a** in 55–60% yield.

4.4.1. Compound 11a. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.90+0.92 (d+d, J=4.0 Hz, 6H, $CH_2CH(CH_3)_2$), 1.12 (m, 1H, $CH_2CH(CH_3)_2$), 1.27 (t, J=6.8 Hz, 3H, $CHCO_2CH_2CH_3$), 1.60 (m, 2H, $CH_2CH(CH_3)_2$), 2.78 (m, 1H, $CHCO_2CH_2CH_3$), 3.32+3.34 and 3.61 (d+d and t, J=7.20, 8.79 Hz, 2H, $CH_2NH-CO-OCH$), 4.2 (m, 2H, $CO_2CH_2CH_3$), 4.75 (q, J=7.80 Hz, 1H, $CH_2NH-CO-OCH$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ=14.16 (s, $CHCO_2CH_2CH_3$), 21.55 and 23.36 (s and s, $CH_2CH(CH_3)_2$), 26.06 (s, $CH_2CH(CH_3)_2$), 36.06 (s, $CH_2CH(CH_3)_2$), 43.78 (s, $CHCO_2CH_2CH_3$), 48.02 (s, $CHCO_2CH_2CH_3$), 61.06 (s, $CH_2NH-CO-OCH$), 77.18 (s, $CH_2NH-CO-OCH$), 159.74 (s, $CH_2NH-CO-OCH$), 172.42 (s, $CHCO_2CH_2CH_3$).

Treatment of **6c** with ICR125 and rearrangement with DPPA gave **11c** in 60% yield.

4.4.2. Compound 11c. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.24 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 2.9+3.00 (m+m, 3H, $CHCO_2CH_2CH_3$, CH_2Ph), 3.45+3.62 (t+t, J=7.59+8.79 Hz, 2H, $CH-O-CO-NH-CH_2$), 4.10 (q, J=7.19 Hz, 2H, $CO_2CH_2CH_3$), 4.81 (m, 1H, $CH-O-CO-NH-CH_2$), 6.02 (s, 1H, $CH-O-CO-NH-CH_2$), 7.25 (m, 5H, CH_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.99 (s, $CO_2CH_2CH_3$), 33.62 (s, $CHCO_2CH_2CH_3$), 43.68 (s, CH_2Ph), 51.56 (s, $CO_2CH_2CH_3$), 61.13 (s, $CH-O-CO-NH-CH_2$), 75.95 (s, $CH-O-CO-NH-CH_2$), 128.93+128.67+128.96+137.39 (s, CH_2Ph), 159.33 (s, $CH-O-CO-NH-CH_2$), 171.36 (s, $CO_2CH_2CH_3$).

Protection of the free alcohol prior to the rearrangement was necessary to synthesize the free amines **9**. In an illustration of the methodology **9a** and **9c** were prepared. Protection of the alcohol as an acetate ester was accomplished by reacting it with acetic anhydride (Ac_2O, 1.1 equiv.) in the presence of catalytic amounts (1–2% mol/mol) of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as described previously.[7] Under these conditions, the alcohols of **6a** and **6c** was acetylated in 85–90% isolated yield. In a typical rearrangement reaction, *O*-acetylated carboxylic acid of **6c** (0.3 g, 0.97 mmol) was dissolved in CH_2Cl_2 (4 mL, containing one drop of DMF), oxalyl chloride (0.1 mL, 1.2 mmol) was added and the reaction was stirred at room temperature for 30 min. The solvent was then evaporated; the oily residue was redissolved in THF and cooled in an ice bath for 5 min before ammonia gas was bubbled for 5 min through the solution under vigorous stirring. The reaction was left stirring at 4 °C for 3 more hours in a tightly closed vessel, before it was diluted with 10 mL EtOAc and extracted once with aqueous acid (1 N HCl) until neutral solution was obtained. After washing with brine, the organic layer dried with Na_2SO_4 and evaporated to dryness. The oily amide residue redissolved in 10 mL *tert*-butanol containing

one drop of $SnCl_4$, lead tetraacetate [$Pb(OAc)_4$, 0.53 g, 1.2 mmol] was added and the reaction was stirred at 80 °C for 4 h. At the end of the reaction *tert*-butanol was evaporated, product was redissolved in Et_2O (15 mL) and the solution was filtered through celite. After evaporation of Et_2O and silica gel purification the *O*-acetylated N-Boc protected compound **9c** was obtained (0.22 g, 60% yield from the *O*-acetylated carboxylic acid) as a white solid. The same reaction sequence gave **9a** as a white solid in 55% overall yield.

4.4.3. Compound 9c. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.12 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 1.46 (s, 9H, $C(CH_3)_3$), 2.09 (s, 3H, $CHOCOCH_3$), 2.9 (m broad, 3H, $CHCO_2C_2H_5$ and CH_2Ph), 3.45+3.5 (m broad+m broad, 2H, CH_2-NHBoc), 4.02 (q, J=7.19 Hz, 2H, $CO_2CH_2CH_3$), 4.75 (m, broad, 1H, $CHOCOCH_3$), 5.17 (m, broad, 1H, CH_2NHBoc), 7.2 (m, 5H, CH_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$) δ=14.08 (s, $CO_2CH_2CH_3$), 20.90 (s, $CHOCOCH_3$), 28.35 (s, $CO_2C(CH_3)_3$), 34.41 (s, $CHCO_2C_2H_5$), 41.73 (s, $CO_2C(CH_3)_3$), 49.54 (s, CH_2Ph), 60.68 (s, $CO_2CH_2CH_3$), 73.44 (s, $CHOCOCH_3$), 79.75 (s, CH_2NHBoc), 126.60+128.46+128.89+138.26 (all s, CH_2Ph), 155.89 (s, $CO_2C(CH_3)_3$), 170.11 (s, $CO_2CH_2CH_3$), 171.90 (s, $CHOCOCH_3$).

4.4.4. Compound 9a. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.88+0.90 (d+d, J=4.00 Hz, 6H, $CH_2CH(CH_3)_3$), 1.25 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.52+1.65 (m broad+m broad, 2H, $CH_2CH(CH_3)_3$), 2.05 (s, 3H, $CHOCOCH_3$), 2.75 (m, 1H, $CHCO_2C_2H_5$), 3.35+3.45 (m broad+m broad, 2H, CH_2NHBoc), 4.1 (q, J=7.19 Hz, 2H, $CO_2CH_2CH_3$), 4.7 (s, broad, 1H, $CHOCOCH_3$), 5.1 (m, broad, 1H CH_2NHBoc).

4.5. Hydrolysis to the mono acids 8 and rearrangement to 10 and 12 (Fig. 4)

Synthesis of statine **10a**, phenylstatine **10c** as well as the cyclic carbamates **12a** and **12c** from the corresponding mono-acids **8** has been described earlier.[7] For the formation of **12b** and **12e** the same reaction sequence was applied. Using the same reaction sequence **12e** was synthesized from **5e** (n=0, Fig. 4) in 67% isolated yield. The only difference is that esterification of the di-acid **7e** was performed at room temperature, since a small amount (5–10%) of diester is forming under the esterification conditions.

4.5.1. Compound 12b. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.89+0.91 (d+d, J=1.6 Hz, 6H, $CH_2CH_2CH(CH_3)_2$), 1.22 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 1.28 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 1.60 (m, 3H, $CH_2CH_2CH(CH_3)_2$), 2.65+2.7 and 2.78+2.82 (d+d and d+d, J=6.80, 6.40 Hz, 2H, $CH_2CO_2C_2H_5$), 3.51 (q, J=5.19, 7.19 Hz, 1H, CH–NHCO–O–CH), 4.20 (q, J=7.19 Hz, 2H, $CO_2CH_2CH_3$), 4.58+4.60+4.62 (d+d+d, J=4.80 Hz, 1H, CH–NHCO–O–CH), 5.8 (s, broad, 1H, CH–NHCO–O–CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.14 (s, $CO_2CH_2CH_3$), 22.34 and 22.54 (s and s, $CH_2CH_2CH(CH_3)_2$), 27.92 (s, $CH_2CH_2CH(CH_3)_2$), 33.15 (s, $CH_2CH_2CH(CH_3)_2$), 34.06 (s, $CH_2CH_2CH(CH_3)_2$), 39.42 (s, $CH_2CO_2C_2H_5$), 57.84 (s, $CO_2CH_2CH_3$), 61.13 (s, CH–NHCO–O–CH), 78.02 (s, CH–NHCO–O–CH), 158.58 (s, CH–NHCO–O–CH), 169.32 (s, $CO_2CH_2CH_3$).

4.5.2. Compound 12e. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.28 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 2.89+2.92 and 3.03+3.06 (d+d and d+d, J=8.0, 5.20 Hz, 2H, CH_2Ph), 4.12 (m, 1H, CH–NHCO–O–CH), 4.25 (q, J=7.19 Hz, 2H, $CO_2CH_2CH_3$), 4.68 (d, J=4.80 Hz, 1H, CH–NHCO–O–CH), 5.67 (s, 1H, CH–NHCO–O–CH), 7.2+7.35 (m+m, 5H, CH_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.04 ($CO_2CH_2CH_3$), 41.70 (CH_2Ph), 57.02 ($CO_2CH_2CH_3$), 62.27 (CH–NHCO–O–CH), 77.23 in between the three $CDCl_3$ peaks (CH–NHCO–O–CH), 127.52+129.08+129.21+135.21 (CH_2Ph), 157.33 (CH–NHCO–O–CH), 168.52 ($CO_2CH_2CH_3$).

Protection of the free alcohol of **8e** as an acetate using TMSOTf catalyst and rearrangement to the free amine using [bis(trifluoroacetoxy)iodo]benzene [$(CF_3CO_2)_2PhI$] was performed as described earlier.[7] Under these conditions **8e** (0.3 g, 1.2 mmol) was protected and rearranged giving **10e** (0.15 g, 47% yield from **8e**) as a white solid.

4.5.3. Compound 10e. ^{1}H NMR (400 MHz, D_2O): δ=1.27 (t, J=7.19 Hz, 3H, $CO_2CH_2CH_3$), 2.32 (s, 3H, $CHOCOCH_3$), 3.14 (m, 2H, CH_2Ph), 4.25 (q, overlaps with m, 3H, $CO_2CH_2CH_3$ and $CHNH_2$), 5.17 (d, J=3.20 Hz, 1H, $CHOCOCH_3$), 7.4 (m, 5H, CH_2Ph). ^{13}C NMR (100 MHz, D_2O): δ=15.95 (s, $CO_2CH_2CH_3$), 22.60 (s, $CHOCOCH_3$), 37.96 (s, CH_2Ph), 55.23 (s, $CO_2CH_2CH_3$), 66.98 (s, $CHOCOCH_3$), 73.10 (s, H_2NCH), 130.83+132.13+132.23+136.97 (s, CH_2Ph), 171.25 (s, $CHOCOCH_3$), 174.95 (s, $CO_2CH_2CH_3$).

References and notes

1. Some representative publications: (a) Leanna, M. R.; DeMattei, J. A.; Li, W.; Nichols, P. J.; Rasmussen, M.; Morton, E. *Org. Lett.* **2000**, *2*, 3627. (b) Nicolau, K. C.; Dai, W. M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15. (c) Liang, B.; Richard, D. J.; Portonovo, P. S.; Joullie, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 4469. (d) Rich, D. H.; Sun, E. T. O. *J. Med. Chem.* **1980**, *23*, 27. (e) Li, W. R.; Ewing, W. R.; Harris, B. D.; Joullie, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7659. (f) Marciniszyn, Jr. J.; Hartsuck, J. A.; Tang, J. *J. Biol. Chem.* **1976**, *251*, 7088. (g) Jouin, P.; Poncet, J.; Dufour, M. N.; Pantaloni, A.; Castro, B. *J. Org. Chem.* **1989**, *54*, 617. (h) Sun, C. I.; Chen, C. H.; Kashiwada, Y.; Wu, J. H.; Wang, H. K.; Lee, K. H. *J. Med. Chem.* **2002**, *45*, 4275. (i) Hom, R. K.; Fang, L. Y.; Mamo, S.; Tung, J. S.; Guinn, A. C.; Walker, D. E.; Davis, D. L.; Gailunas, A. F.; Thorsett, E. D.; Sinha, S.; Knops, J. E.; Jewett, N. E.; Anderson, J. P.; Varghese, J. *J. Med. Chem.* **2003**, *46*, 1799.
2. Reviews: (a) Liu, M.; Sibi, P. *Tetrahedron* **2002**, *58*, 7991. (b) In *Enantioselective Synthesis of β-Amino Acids*; Juaristi, E., Ed.; Wiley-VCH: New York, 1997.
3. (a) Kim, B. M.; Bae, S. J.; So, S. M.; Yoo, H. T.; Chang, S. K.; Lee, J. H.; Kang, S. *Org. Lett.* **2001**, *3*, 2349. (b) Adrian, Jr. J. C.; Barkin, J. L.; Fox, R. J.; Chick, J. E.; Hunter, A. D.; Nicklow, R. A. *J. Org. Chem.* **2000**, *65*, 6264. (c) Dinh, T. Q.; Armstrong, R. W. *J. Org. Chem.* **1995**, *60*, 8118. (d) Yuste, F.; Diaz, A.; Ortiz, B.; Sanchez-Obregon, R.; Walls, F.; Ruano, J. L. G. *Tetrahedron: Assymetry* **2003**, *14*, 549.

4. Raddatz, P.; Radunz, H. E.; Schneider, G.; Schwartz, H. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 426.
5. (a) Patel, R. N.; Banerjee, A.; Howell, J. M.; McNamee, C. G.; Brzozowoski, D.; Nanduri, V.; Thottathil, J. K.; Szarka, L. J. *Tetrahedron: Asymmetry* **1993**, *4*, 2069. (b) Gou, D. M.; Liu, Y. C.; Chen, C. S. *J. Org. Chem.* **1993**, *48*, 1287.
6. Patel, R. N.; Banerjee, A.; Ko, R. Y.; Howell, J. M.; Li, W. S.; Comezoglu, F. T.; Partyka, R. A.; Szarka, L. J. *Biotechnol. Appl. Biochem.* **1994**, *20*, 23.
7. Kambourakis, S.; Rozzell, J. D. *Adv. Synth. Catal.* **2003**, *345*, 699.
8. Floyd, D. E.; Miller, S. E. *Organic Syntheses*, Collect. Vol. 4, p 141.
9. Seebach, D.; Aebi, J.; Wasmuth, D. *Organic Syntheses*, Collect. Vol. 63, p 109.
10. Baumgarten, H. E.; Smith, H. L.; Staklis, A. *J. Org. Chem.* **1975**, *40*, 3554.