Free α-Oxiranyl Amino Acids

David B. Berkowitz* and Michelle L. Pedersen

Department of Chemistry, University of Nebraska–Lincoln, Lincoln, Nebraska 68588-0304

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Analogues of natural amino acids, in which the α-proton is replaced by an unsubstituted epoxide ring, are potential mechanism-based inhibitors for pyridoxal phosphate dependent enzymes.[1] Yet, free α-oxiranyl amino acids have remained elusive until now. The synthesis of an α-(phenyl-substituted)oxiranyl amino ester has been reported. However, the accessibility and stability of the corresponding free, zwitterionic α-oxiranyl amino acid remained an open question.[9]

Herein we report the first synthesis of members of this class of unnatural amino acids. These compounds possess an additional β-stereocenter and so can exist as two diastereomers, *erythro* and *threo*, defined as illustrated in Figure 1.[10] Our goal was, therefore, to develop a general synthesis of α-oxiranyl amino acids, that would provide access to each pure diastereomer, for a given R group. Our synthetic strategy was to generate α-oxiranyl amino acids via the epoxidation of suitably protected α-vinyl amino acids, followed by deprotection of the amino and carboxyl groups. We reasoned that benzyl (carboxyl) and benzyloxycarbonyl (amino) protecting groups would be ideal for this purpose as they might be removed in a single, relatively mild, hydrogenolytic operation. Initially, we developed a convenient and quite general procedure for the synthesis of α-vinyl amino acids from the parent amino acids.[11] More recently, we discovered that the free α-vinyl amino acids thereby obtained could be globally protected with hydrogenolytically removable protecting groups in a one pot operation.[12]

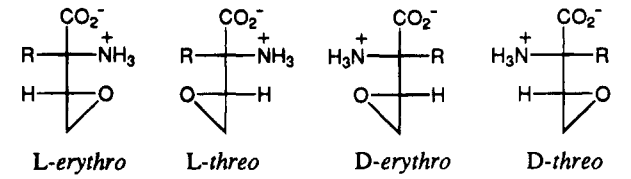
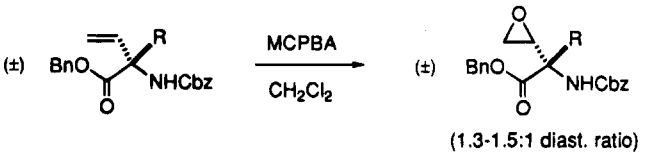


Figure 1.

Scheme 1



	R	Yield	Diastereomers Separable?
1a,b	Me	80%	By HPLC only
2a,b	*i*-Pr	78%	Yes
3a,b	Bn	81%	No

These protected α-vinyl amino acids are easily epoxidized with MCPBA in CH_2Cl_2 (Scheme 1). Both the *erythro* and *threo* diastereomers are obtained. The *threo* diastereomer is marginally favored in each case (1.3–1.5:1 ratios).[13] However, separation of these diastereomeric protected, α-oxiranyl amino acids via conventional chromatography proved possible only for the valine analogues **2a** and **2b**. For the alanine analogues **1a** and **1b**, HPLC provided access to the homogeneous diastereomers. But attempts to separate the diastereomeric α-oxiranyl phenylalanines using HPLC with normal and reverse phase (C-18) columns and a variety of eluents met with little success.

This led us to examine alternative procedures for synthesizing α-oxiranyl amino acids via diastereomeric intermediates that might be more easily separated chromatographically. In fact, dihydroxylation of protected α-vinylphenylalanine leads to two diastereomeric

(1) Among the classes of such compounds that have proven to be effective inhibitors are α-vinyl amino acids,[2] α-ethynyl amino acids,[2a] α-allenyl amino acids,[3] α-(mono,[4] di,[5] and tri[6])-fluoromethyl amino acids and α-chlorofluoromethyl amino acids.[7] With amino acid decarboxylases, the α-halogenomethyl amino acids are believed to function by diverting the α-carbanionic intermediate formed by enzyme-mediated decarboxylation into a β-elimination pathway, as opposed to the usual α-protonation pathway.[5,8] Subsequent events lead to the formation of a covalent enzyme–inhibitor adduct[5] or cofactor–inhibitor adduct.[8] We reasoned that new classes of α-branched amino acids possessing a β-leaving group as part of a small, strained ring would be particularly interesting classes of compounds to synthesize and study in this regard. Presumably, for such compounds, relief of ring strain could serve as a thermodynamic driving force to favor the "β-elimination" (ring-opening) pathway.

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(8) Bhattacharjee, M. K.; Snell, E. E. *J. Biol. Chem.* **1990**, *265*, 6664–6668 and references cited therein.

(9) (a) Neubauer, H.-J.; Baeza, J.; Freer, J.; Schöllkopf, U. *Liebigs Ann. Chem.* **1985**, 1508–1511. (b) Schöllkopf, U. *Pure Appl. Chem.* **1983**, 1799–1806.

(10) Note that the D- or L- designation here follows if one formally treats the α-oxirane analogously to the α-proton that it replaces. However, for clarity, the oxirane ring is placed in a vertical position in the Fischer projection here, in contrast to the horizontal position normally occupied by the α-proton.

(11) (a) Pedersen, M. L.; Berkowitz, D. B. *Tetrahedron Lett.* **1992**, *33*, 7315–7318; (b) Pedersen, M. L.; Berkowitz, D. B. *J. Org. Chem.* **1993**, *58*, 6966–6975.

(12) Berkowitz, D. B.; Pedersen, M. L. *J. Org. Chem.* **1994**, *59*, 5476–5478.

(13) Diastereoselective peracid-mediated epoxidations of protected α-vinylglycine esters and of related alcohols have been reported.[14] However, in all of these cases, an α-hydrogen is present. It is worthy of note that the most commonly accepted model for hydroxyl- or carbamate-directed epoxidations[15] attributes the *threo* diastereoselectivity usually observed to $A^{(1,3)}$ interactions present in the *erythro* transtition state, but absent from the *threo* transition state. In our system, due to the absence of an α-hydrogen, this model would predict substantial $A^{(1,3)}$ strain in both transition states, perhaps accounting for the observed formation of both *threo* and *erythro* products.

(14) (a) Shaw, K. J.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 4515–4523. (b) Ohfune, Y. *Acc. Chem. Res.* **1992**, *25*, 360–366. (c) Askin, D.; Wallace, M. A.; Vacca, J. P.; Reamer, R. A.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1992**, *57*, 2771–2773.

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(16) Modest diastereoselectivities were obtained for these osmylations [*erythro*:*threo* ratios: 1:1.2 (R = Me); 1:2.7 (R = *i*-Pr); 2.9:1 (R = Bn)]. The low diastereoselectivity was of little concern here since both diastereomeric diols were desired and could be conveniently separated by conventional chromatography. For examples of diastereoselective dihydroxylations of protected allylic or homoallylic amines, bearing α-hydrogens and other potential directing groups as well, see: (a) Sames, D.; Polt, R. *J. Org. Chem.* **1994**, *59*, 4596–4601. (b) King, S. B.; Ganem, B. *J. Am. Chem. Soc.* **1991**, *113*, 5089–5090. (c) Hauser, F. M.; Ellenberger, S. R.; Clardy, J. C.; Bass, L. S. *J. Am. Chem. Soc.* **1984**, *106*, 2458–2459.

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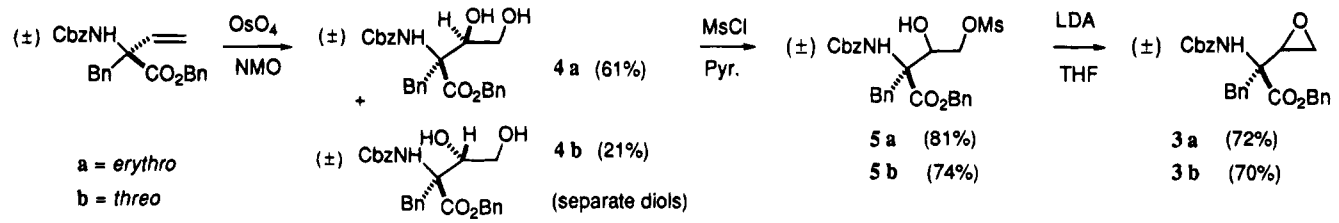
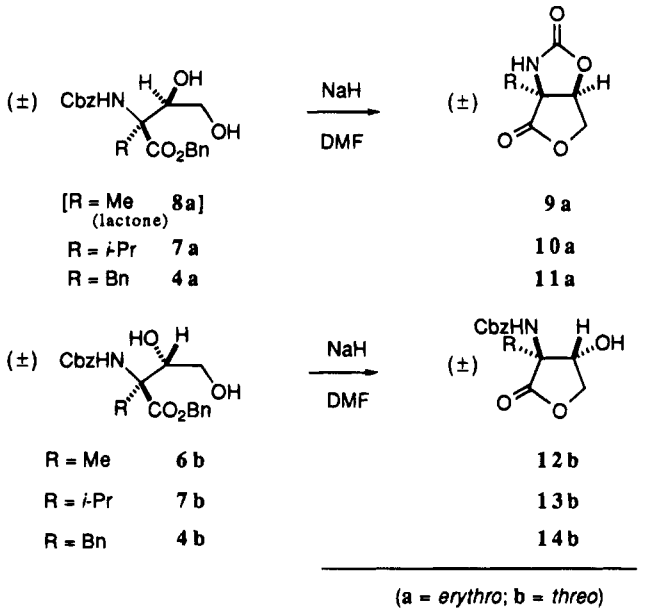
Scheme 2



diols **4a** and **4b**, that are readily separated by flash chromatography.[16] For each diastereomer, selective mesylation of the primary hydroxyl group, followed by deprotonation of the secondary hydroxyl group with LDA, induces nucleophilic cyclization to afford the desired epoxide (Scheme 2). Thus, this indirect epoxidation approach provides a practical solution to the diastereomer separation problem in the phenylalanine series.

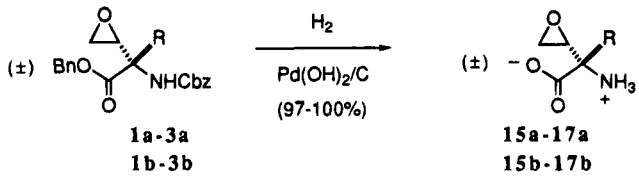
Scheme 3



The relative stereochemistry of each of the α-oxiranyl amino acids synthesized could be deduced from cyclization experiments performed on the protected vicinal diols produced in the osmylation step. Thus, treatment of diols **7a** and **4a** with NaH in DMF yields the bicyclic oxazolidinones **10a** and **11a**, respectively (Scheme 3). Two cyclizations had occurred, leading to the formation of both a γ-lactone and an oxazolidinone ring with the release of two molecules of benzyl alcohol. On the other hand, treatment of the diastereomeric diols **7b** and **4b** with NaH in DMF produced the monocyclic lactones **13b** and **14b**, with the release of one molecule of benzyl alcohol. In the alanine series, at the dihydroxylation step, the *threo*-diol **6b** was isolated, but the *erythro*-diol was obtained in monocyclized form, as the corresponding γ-lactone **8a**. Treatment of **8a** with NaH in DMF produced the bicyclic oxazolidinone **9a**, whereas **6b** produced only monocyclic γ-lactone **12b**, under the same conditions.

From these experiments, one can deduce that the **a** series compounds have the *erythro* relative stereochemistry, which permits formation of the 5,5-*cis*-fused oxazolidinone lactones **9a**–**11a**. The **b** series compounds, on the other hand, must possess the *threo* relative stereochemistry, as bicyclic oxazolidinone lactones are not formed here, because these would necessarily be highly strained, 5,5-*trans*-fused systems.[17] Therefore, one observes only a single cyclization to the monocyclic γ-lactones **12b**–**14b**, in these cases. On the basis of these results, the relative stereochemistry of each of the α-oxiranyl amino acids synthesized can also be deduced, as these have been chemically correlated with the corresponding vicinal diols.[18] We note also that, although all compounds were synthesized in racemic form in this work, all four stereoisomeric α-oxiranyl amino acids (Figure 1) could, in principle, be obtained, by starting from enantiomerically pure α-vinyl amino acids.[19]

Scheme 4



15 R = Me; **16** R = *i*-Pr; **17** R = Bn; (a = *erythro*; b = *threo*)

Most importantly, hydrogenation of each of the protected, diastereomerically homogeneous, *erythro* and *threo*, α-oxiranyl analogues of alanine, valine, and phenylalanine (**1a**–**3a** and **1b**–**3b**) cleanly yields the corresponding free α-oxiranyl amino acid (**15a**–**17a** and **15b**–**17b**, respectively). Additionally, the stability of *threo*-α-oxiranylvaline (**16b**) was studied, as a function of pH (actually pD in these NMR experiments). Interestingly, **16b** is stable (i) in D_2O for weeks; (ii) at pD 3 (DCl/100 mM $NaPO_4$ buffer) for one week; and (iii) at pD 12 (100 mM $NaPO_4$ buffer) for days, as judged by ^1H NMR. Further studies on the properties of these and related α-branched amino acids will be reported in due course.

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Supporting Information Available: Spectral data for all new compounds and experimental procedures for their synthesis; ^1H NMR spectra for compounds **15a**–**17a** and **15b**–**17b**, as well as ^1H NMR spectra for **16b** after incubation: (i) in D_2O for 36 days, (ii) at pD 3 for one week, and (iii) at pD 12 for 2 days (21 pages).

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(17) For example, for the alanine series, molecular mechanics minimizations (PC Model, MMX force field) predict that the observed 5,5-*cis*-fused oxazolidinone-lactone **9a** lies 26 kcal/mol lower in energy than its *trans*-fused isomer (not observed).

(18) In the alanine and valine series, the diastereomeric *erythro* and *threo* diols were synthesized and converted to the corresponding protected, α-oxiranyl amino acids by the same procedure as was employed in the phenylalanine series (Scheme 2). Experimental details are provided in the supporting information.

(19) For approaches to enantiomerically enriched α-vinyl amino acids, see: Berkowitz, D. B.; Pumphrey, J. A. and Shen, Q. *Tetrahedron Lett.* **1994**, *35*, 8743–8746 and references therein.