Preparation of (*R*)-Phenylalanine Analogues by Enantioselective Destruction Using L-Amino Acid Oxidase

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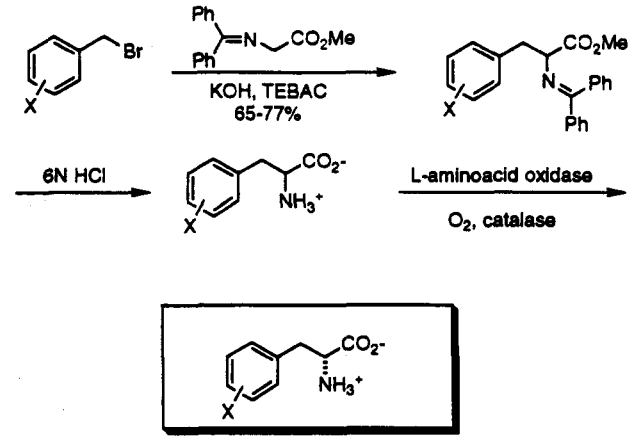
Because of the potent biological activities of naturally-occurring amino acids and peptides and the desire for the development of therapeutics based on natural leads, methods for preparation of unnatural amino acids have acquired great importance in medicinal chemistry.[1] Several of these methods are aimed specifically at the (*R*)-enantiomers;[2] access to such substances provides an avenue to overcome endogenous proteolytic degradation pathways. We have focused on a family of (*R*)-phenylalanine analogues that could be used to develop structure–activity relationships for phenylalanine-containing peptides using modified Hansch analysis[3] and combinatorial peptide synthesis.[4] On the basis of O'Donnell's report of the preparation of (*R*)-*p*-chlorophenylalanine using the base-catalyzed alkylation of a glycine Schiff's base with a chiral catalyst,[5] we initially chose this method for preparation of our targets. The O'Donnell method unfortunately relies on a crystallization step to raise the enantiomeric excess of initial alkylation products (40–68% ee in our substrates) to optical purity. While successful for the para-substituted phenylalanine analogues we desired, the crystallization of molecules with unsymmetrical substitutions was unsuccessful. It is worth noting that, in another recent study,[6] we found that alkylation using the O'Donnell procedure with an allylic halide capable of undergoing electron transfer provides racemic product. This method is thus of more limited scope than would superficially appear.

We turned to resolution as a method to obtain our targets. While Whitesides has reported an excellent method to form (*S*)-amino acids that we used in another study,[6] to obtain the (*R*)-isomers requires additional protection/deprotection steps. We therefore chose a method to directly produce the (*R*)-amino acid from the racemic mixture using L-amino acid oxidase (L-AAOx). This flavoenzyme has been well-studied;[7] phenylalanine is an excellent substrate among the hydrophobic amino acids of 5–8 carbons,[8] so it was expected that phenylalanine analogues would behave well. In fact, a kinetic study of the oxidation of several Phe congeners by L-AAOx has been reported that shows that the reaction is relatively insensitive to substitution in the aromatic ring.[9] No preparative methods using the enzyme are known.

Seven phenylalanine analogues were obtained as the hydrochlorides by phase-transfer alkylation of methyl



N-(diphenylmethylidene)glycinate with the cognate benzylic halides and hydrolysis in 6 N HCl. Addition of propylene oxide to their methanol solutions results in precipitation of the amino acid. The racemic amino acids were dissolved in buffer at a concentration of 0.1 M and 20 units of L-AAOx and catalase were added. After vigorous stirring (for aeration) at 35 °C over 2 d, the reaction mixture was taken to pH 7 and purified by ion-exchange chromatography. The α-keto acid derived from the (*S*)-isomer is removed in the column flowthrough, and the (*R*)-amino acid is obtained on concentration of the aqueous ammonia solution used to elute it from the Dowex resin. The optical purity of these materials was determined by esterification under a standard protocol,[10] conversion to the MTPA amide,[11] and NMR and GC analysis. In all cases, the product is >97% ee. The outcomes of all the reaction sequences are summarized in the Table I. Because both L-amino acid oxidase and D-amino acid oxidase are inexpensive and commercially-available, this methodology should be applicable to production of either enantiomer of a desired amino acid.

Experimental Section

General Procedure for Resolution of Phenylalanine Analogues by Destruction of the L-Isomer. A racemic phenylalanine analogue (15 mmol) was dissolved in Tris-maleate buffer (150 mL, pH 7.8) containing 0.1 M KCl. To this solution was added 40 mg of L-amino acid oxidase (Sigma Type I, activity 0.55 units/mg) and 10 mg of catalase. After 24–48 h, the reaction mixture was brought to pH 7 with HCl and purified by ion-exchange chromatography over Dowex 50, eluting the amino acid with 1 N ammonia.

(*R*)-3′-(Trifluoromethyl)phenylalanine:[12] ^{1}H NMR (300 MHz, D_2O) δ 7.64 (m, 2 H), 7.52 (m, 2 H), 3.96 (dd, J = 7.7, 5.6 Hz, 1 H), 3.29 (dd, J = 14.4, 5.6 Hz, 1 H), 3.16 (dd, J = 14.4, 7.7 Hz, 1 H).

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Table I

entry	aromatic ring substitution	% yield alkylation	% yield hydrolysis	% yield resolution	mp (°C)	α_D deg	ee (%)
1	3'-trifluoromethyl	71	66	92	106.8	-3.40	97
2	3'-methyl	75	72	95	112.7	+4.20	>99
3	3'-methoxy	65	75	95	155.8	+5.05	97
4	2'-methyl	72	76	90	147.3	-2.52	>99
5	2'-chloro	69	70	94	134.5	-2.12	>99
6	3'-chloro	73	78	92	120.6	+1.66	>99
7	2'-fluoro	76	81	95	184.7	-2.70	>98

(*R*)-3'-Methylphenylalanine: ^{1}H NMR (300 MHz, D_2O) δ 7.27 (dd, J = 7.5, 7.5 Hz, 1 H), 7.16 (d, J = 7.5 Hz, 1 H), 7.10 (s, 1 H), 7.06 (d, J = 7.5 Hz, 1 H), 3.92 (dd, J = 7.8, 5.1 Hz, 1 H), 3.21 (dd, J = 14.5, 5.1 Hz, 1 H), 3.03 (dd, J = 14.5, 7.8 Hz, 1 H), 2.29 (s, 3 H). HRMS Calcd. for $C_{10}H_{14}NO_2$: 180.1024; found 180.1023.

(*R*)-3'-Methoxyphenylalanine: ^{1}H NMR (300 MHz, D_2O) δ 7.2 (dd, J = 7.8, 7.8 Hz, 1 H), 6.86 (m, 1 H), 3.76 (s, 1 H), 3.75 (dd, J = 7.8, 5.2 Hz, 1 H), 3.07 (dd, J = 14.0, 5.2 Hz, 1 H), 2.93 (dd, J = 14.0, 7.8 Hz, 1 H). HRMS Calcd. for $C_{10}H_{14}NO_3$: 196.0974; found 196.0975.

(*R*)-2'-Methylphenylalanine:[13] ^{1}H NMR (300 MHz, D_2O) δ 7.23 (m, 4 H), 4.18 (dd, J = 8.8, 6.2 Hz, 1 H), 3.38 (dd, J = 14.6, 6.2 Hz, 1 H), 3.09 (dd, J = 14.6, 8.8 Hz, 1 H), 2.30 (s, 3 H).

(*R*)-2'-Chlorophenylalanine:[13] ^{1}H NMR (300 MHz, D_2O) δ 7.46 (m, 1 H), 7.32 (m, 3 H), 4.02 (dd, J = 8.6, 5.8 Hz, 1 H), 3.42 (dd, J = 14.2, 5.8 Hz, 1 H), 3.14 (dd, J = 14.2, 8.6 Hz, 1 H).

(*R*)-3'-Chlorophenylalanine:[13] ^{1}H NMR (300 MHz, D_2O) δ 7.28 (m, 3 H), 7.15 (m, 1 H), 3.67 (dd, J = 7.4, 5.6 Hz, 1 H), 3.05 (dd, J = 14.0, 5.6 Hz, 1 H), 2.91 (dd, J = 14.0, 7.4 Hz, 1 H).

(*R*)-2'-Fluorophenylalanine:[14] ^{1}H NMR (300 MHz, D_2O) δ 7.31 (m, 2 H), 7.28 (m, 2 H), 3.97 (dd, J = 7.8, 5.7 Hz, 1 H), 3.32 (dd, J = 14.6, 5.7 Hz, 1 H), 3.09 (dd, J = 14.6, 7.8 Hz, 1 H).

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Supplementary Material Available: ^{1}H NMR spectra of (*R*)-3'-methoxyphenylalanine and (*R*)-3'-methylphenylalanine (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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