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Convenient Preparation of All Four Possible Stereoisomers of 2-Benzyl-3,4-epoxybutanoic Acid, Pseudomechanism-based inactivator for Carboxypeptidase A *via* α-Chymotrypsin-Catalyzed Hydrolysis[1]

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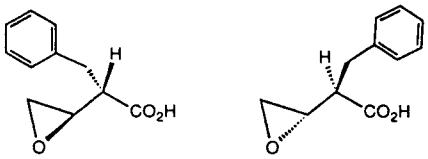
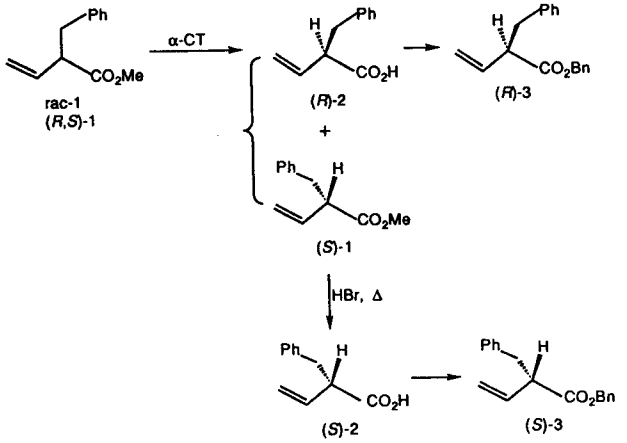
In recent years, interest in using enzymes for preparation of optically active compounds has been growing rapidly.[2] Especially, enzymatic enantioselective hydrolysis of esters using lipases has received much attention.[3] Proteases hydrolyze peptides as well as esters with high specificity and thus they are potentially useful for kinetic resolution of esters to obtain optically active corresponding acids. α-Chymotrypsin is a readily available inexpensive protease of much studied: Its crystal structure, catalytic mechanism, and substrate specificity including streochemistry have been well established.[4,5] The enzyme catalyzes the hydrolysis of amides and esters having a hydrophobic side chain at the α-position with the L-stereochemistry,[4,5] thus racemic esters having an aromatic side chain such as benzyl group at the α position can be resolved using the enzyme. This paper describes the use of α-chymotrypsin in the preparation of (2*S*,3*R*)- and (2*R*,3*S*)-2-benzyl-3,4-epoxybutanoic acid (BEBA), pseudomechanism-based inactivators for carboxypeptidase A.[6,7] The other two stereoisomers of the epoxyacid *i.e.*, (2*S*,3*S*)- and (2*R*,3*R*)-BEBA were also prepared. Previously, these inhibitors were synthesized stereospecifically in six steps starting with optically active malic acid.[7]

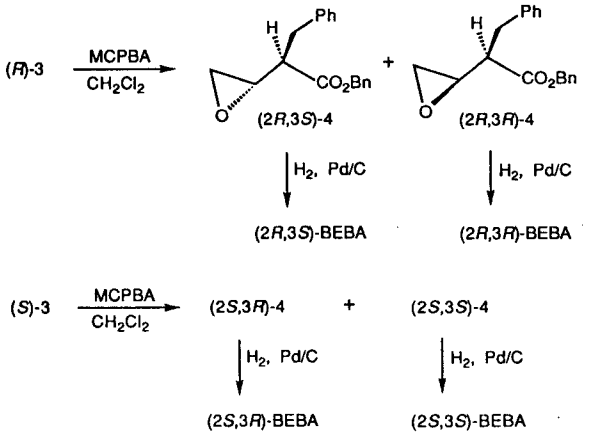


(2*S*,3*R*)-BEBA (2*R*,3*S*)-BEBA

The starting material of the present synthesis, racemic methyl 3-phenyl-2-vinylpropanoate (**1**) was obtained as described in the literature[8] and was subjected to catalytic hydrolysis using α-chymotrypsin in a dilute aqueous buffer solution (0.01 M) of pH 7.8. The pH of the reaction mixture was maintained at 7.8 by slow and continuous addition of 0.1 N sodium hydroxide solution until the theoretical amount of the base is consumed. The enzyme specifically hydrolyzed the (*R*)-**1** to give (*R*)-3-phenyl-2-vinylpropanoic acid (**2**). Unhydrolyzed **1** was recovered from the reaction mixture. The methyl ester of high optical purity (above 98% ee) could be obtained by extending the incubation time with an additional amount (a 10% excess) of the alkaline solution. The



Scheme 1.



Scheme 2.

enantiomeric purity of the product was determined from the NMR resonance peak of α-methyl group in the (*S*)-alanine derivative that was obtained by condensing the resolved acid with (*S*)-alanine.

Previously, we found that diasteroisomers of BEBA benzyl ester (**3**) are readily separable by column chromatography using silica gel,[6b] so that the optically active 3-phenyl-2-vinylpropanoic acids thus obtained were converted to the corresponding benzyl ester by allowing them to react with benzyl bromide in DMF in the presence of potassium carbonate. Treatment of (*S*)-**3** with *m*-chloroperbenzoic acid in methylene chloride afforded benzyl ester of BEBA as a mixture of diastereomers which were separated by column chromatography to give (2*S*,3*R*)- and (2*S*,3*S*)-BEBA benzyl ester (**4**). Similarly, benzyl esters of (2*R*,3*S*)- and (2*R*,3*R*)-BEBA were obtained from (*R*)-**3** by epoxidation with *m*-chloroperbenzoic acid followed by chromatographic separation. BEBA benzyl esters thus obtained were treated with hydrogen in the presence of Pd/C to afford optically active BEBAs. The spectral data as well as the specific rotation of (2*S*,3*R*)-BEBA synthesized in this study agree with those of the authentic sample[7] that was prepared by asymmetric synthesis, confirming the streochemical assignment in the present synthesis. The remaining three stereoisomers of BEBA were similarly obtained.

Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 300 MHz FT-NMR spectrometer in deuteriochloroform and chemical shifts are expressed in ppm relative to tetramethylsilane. IR spectra were recorded on Perkin Elmer spectrophotometer (Model 843), and mass spectra were obtained with KRATOS MS25 RFA spectrometer. Specific rotations were obtained using JASCO DIP-360 polarimeter. Analytical thin-layer chromatography (TLC) was performed on precoated plates (silica gel, $60F_{254}$) purchased from E. Merck, using a mixture of hexane and ethyl acetate (6 : 1 v/v) as eluent. α-Chymotrpsin (EC 3.4.21.1) used is Sigma Type II bovine pancreas obtained from Sigma Chemical Co.

Methyl 3-phenyl-2-vinylpropanoate, (*R,S*)-1. To a stirred methanol solution obtained by dissolving 3-phenyl-2-pentanoic acid[8] (1 g, 5.7 mmol) in methanol (20 mL) was added acetyl chloride (1 mL, 1.4 mmol) under chilling in an ice bath. The resulting mixture was stirred for 12 hr at room temperature, then evaporated under reduced pressure. The residue was dissolved in ether, and the ether solution was washed with 10% aqueous sodium bicarbonate solution three times and dried over anhydrous magnesium sulfate. Evaporation of the ether solution under reduced pressure gave an oil which was purified by column chromatography to give 1.03 g (95%) of (*R,S*)-**1**. IR ($CHCl_3$): 3018, 2961, 1722, and 1212 cm^{-1}; ^{1}H NMR: δ 2.84 (1H, dd), 3.09 (1H, dd), 3.34 (1H, m), 3.63 (3H, s), 5.08 (1H, d), 5.11 (1H, d), 5.80 (1H, ddd), and 7.14-7.29 (5H, m); ^{13}C NMR: δ 38.39, 51.77, 51.93, 117.61, 126.40, 128.31 (2C), 128.99 (2C), 135.33, 138.61, and 173.72.

Enzyme-Catalyzed Hydrolysis of Racemic Methy 3-phenyl-2-vinylpropanoate. Racemic **1** (1 g, 5.3 mmol) was suspended in 20 mL of water containing 2 mL of phosphate buffer (0.1 M, pH 7.8). To the mixture was added α-chymotprysin (200 mg), and was stirred slowly. The pH of the reaction mixture was maintained at 7.8 by addition of aqueous sodium hydroxide solution (0.1 N) using a pH stat. When 2.6 mL of the alkaline solution was consumed, the reaction mixture was extracted with ether, and the combined extracts was dried over anhydrous magnesium sulfate, then evaporated under reduced pressure to give an oily residue which was purified by column chromatography on silica gel to give 0.47 g (47%) of (*S*)-**1**. [α] = +26.4° (*c* = 1, EtOH).

The aqueous layer after extraction with ether was acidified with dilute hydrochloric acid to pH about 2, and the acid that separated was extracted with ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to give (*R*)-3-phenyl-2-vinylpropionic acid, (*R*)-**2** (0.38 g, 41%) as an oil.

IR ($CHCl_3$): 3024, 2928, 1703, 1490, 1449, 1212 cm^{-1}; ^{1}H NMR: 2.94 (2H, m), 3.32 (1H, q), 5.09 (2H, m), 5.81 (1H, m), 7.12-7.27 (5H, m); ^{13}C NMR: 38.39, 51,77, 51.93, 117.61, 126.40, 128.31 (2C), 128.99, (2C), 135.33, 138.61, and 173.72; [α] = −31.7° (*c* = 1, EtOH).

(*S*)-3-Pheny-2-vinylpropanoic acid, (*S*)-2. The unhydrolyzed (*S*)-**1** (1 g, 5.26 mmol) obtained from the above α-chymotrysin-catalyzed hydrolysis of (*R,S*)-**1** was heated with hydrobromic acid (48%, 10 mL) under reflux for 4 hr, then chilled in an ice bath. After dilution with cold water, the reaction mixture was extracted with ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to give (*S*)-**2** (0.65 g, 70%) as an oil. The spectral data are the same as those obtained with (*R*)-**2**.

Benzyl (*R*)-3-phenyl-2-vinylpropanoate, (*R*)-3. To a stirred mixture of (*R*)-**2** (1 g, 5.6 mml), DMF (20 mL), and powdered potassium carbonate (0.94 g, 0.68 mmol, 1.2 mol eq) was added slowly at 0 ℃ benzyl bromide (0.675 mL, 5.5 mmol, 0.98 eq). The stirring was continued for about 40 min, after which the reaction mixture was diluted with ethyl acetate. The solution thus obtained was washed with sodium thiosulfate (20 mL×3), sodium bicarbonate (5%, 20 mL×3), citric acid (10%, 20 mL×3), and brine (20 mL×3), successively, then dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The oily residue was purified by column chromatography to give (*R*)-**3** (1.42 g, 95%) as an oil. IR ($CHCl_3$): 3027, 2949, 1722, 1490, 1449 and 1146 cm^{-1}; ^{1}H NMR: δ 2.86 (1H, q), 3.10 (1H, q), 3,39 (1H, q), 5.05 (2H, d), 5.08 (1H, s), 5.12 (1H, s), 5.88 (1H, m), and 7.11-7.31 (10H, m); MS (m/z): 266 (M^+), 180, 174, 157, 129, 115, 107, 91, and 76. [α] = −17.8° (*c* = 1, EtOH).

Benzyl (*S*)-3-Phenyl-2-vinylpropanoate, (*S*)-3 was prepared from (*S*)-**2** in the same fashion as (*R*)-**3** was prepared. IR ($CHCl_3$): 3027, 2949, 1722, 1490, 1449 and 1146 cm^{-1}; ^{1}H NMR: δ 2.86 (1H, q), 3.10 (1H, q), 3,39 (1H, q), 5.05 (2H, d), 5.08 (1H, s), 5.12 (1H, s), 5.88 (1H, m), and 7.11-7.31 (10H, m); MS (m/z): 266 (M^+), 180, 174, 157, 129, 115, 107, 91, and 76. [α] = 20.7° (*c* = 1, EtOH).

Epoxidation of Benzyl (*R*)-3-Phenyl-2-vinylpropanoate. A solution of *m*-chloroperbenzoic acid (3.89 g, 3 eq) dissolved in methylene chloride was added slowly to a stirred solution of (*R*)-**3** (1 g, 3.7 mmol) at room temperature. The resulting solution was kept stirring for 3 days, then evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the solution was washed successively with aqueous sodium thiosulfate solution (10%, 20 mL×3) then with brine (20 mL×3), and evaporated under reduced pressure to give a mixture of (2*R*,3*R*)-BEBA benzyl ester and (2*R*,3*S*)-BEBA benzyl ester as an oil. TLC R_f values: 3.4 for (2*R*,3*R*)-BEBA benzyl ester and 3.2 for (2*R*,3*S*)-BEBA benzyl ester. The mixture was separated and purified by column chromatography (silica gel) eluting with a mixture of hexane and ethyl acetate (20 : 1 v/v) to give (2*R*,3*R*)-BEBA benzyl ester (0.34 g, 32.5%) and (2*R*,3*S*)-BEBA benzyl ester (0.45 g, 43.5%), both as an oil. **(2*R*,3*R*)-BEBA benzyl ester:** IR ($CHCl_3$) 3019, 2924, 1722, 1490, 1449, and 1212, 861 cm^{-1}; ^{1}H NMR: δ 2.56 (1H, q), 2.67 (1H, q), 2.78 (1H, t), 3.12 (3H, m), 5.05 (2H,t), and 7.13-7.30 (10H, m); ^{13}C NMR ($CDCl_3$): δ 35.9, 46.6, 50.7, 52.2, 66.5, 126.6, 128.1, 128.2, 128.4, 128.5, 129.0, 135.5, 137.9, 171.7; [α] = +2.43° (*c* = 1, EtOH). **(2*R*,3*S*)-BEBA benzyl ester:** IR ($CHCl_3$) 3019, 2924, 1722, 1490, 1449, and 1212, 863 cm^{-1}; ^{1}H NMR: δ 2.30 (1H, dd), 2.52 (1H, dd), 2.69 (1H, t), 2.90 (1H, dd), 3.10 (1H, dd), 3.21 (1H, m), 5.13 (2H, s), and 7.12-7.36 (10H, m); ^{13}C NMR ($CDCl_3$): δ 35.1, 46.3, 50.7, 52.2, 66.6, 126.7, 128.0, 128.2, 128.4, 128.5, 128.8, 135.6, 137.9, 172.5; MS (m/z): 282 (M^+), 251, 236, 223, 205, 197, 191, 180, 161, 147, 129, 117, 91; [α] = −1.50° (*c* = 1, EtOH).

(2*R*,3*S*)-2-Benzyl-3,4-epoxybutanoic Acid, (2*R*,3*S*)-BEBA. To the methol solution obtained by dissolving (2*R*, 3*S*)-BEBA benzyl ester (0.45 g, 1.6 mmol) in anhydrous me-

thanol (10 mL) was added a catalytic amount of Pd/C and the resulting solution was stirred under hydrogen gas (1 atm) for about 1 hr. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to give **(2*R*,3*S*)-BEBA** (0.30 g, 97%) as an oil. IR ($CHCl_3$): 3028, 2925, 1706, 1490, 1449, and 1211, 883 cm^{-1}; ^1H NMR: δ 2.29 (1H, m, 2-H), 2.50 (1H, q, CH-Ph), 2.69 (1H, t, CH-Ph), 2.89-3.17 (2H, dq, 4-H),3.20 (1H, m, 3-H), 7.18-7.32 (5H, m, Ph), and 8.40 (1H, br, CO_2H); ^{13}C NMR: δ 40.0, 46.5, 50.3, 52.0, 126.8, 128.4, 128.7, 137.8 and 177.6; $[\alpha]=+9.80°$ ($c=1$, EtOH), lit[7] $[\alpha]=+9.8°$ ($c=1$, EtOH).

(2*S*,3*R*)-2-Benzyl-3,4-epoxybutanoic Acid, (2*S*,3*R*)-BEBA. This compound was prepared in a similar fashion as described above from (2*S*,3*R*)-BEBA benzyl ester in 95% yield. The spectral data (IR, ^1H NMR) were identical with those obtained for (2*R*,3*S*)-BEBA; $[\alpha]=-10.8°$ ($c=1$, EtOH).

(2*R*,3*R*)-2-Benzyl-3,4-epoxybutanoic Acid, (2*R*,3*R*)-BEBA. This compound was prepared from (2*R*,3*R*)-BEBA benzyl ester in 97% yield in a similar fashion as described for the preparation of (2*R*,3*S*)-BEBA. IR ($CHCl_3$): 3028, 2925, 1706, 1490, 1449, and 1211, 881 cm^{-1}; ^1H NMR: δ 2.55 (1H, m, 2-H), 2.71 (1H, q, CHPh), 2.84 (1H, t, CHPh), 3.10-3.13 (3H, m, 4-H_2 and 3-H), and 7.20-7.31 (5H, m, Ph). ^{13}C NMR: δ 35.5, 46.6, 50.2, 52.7, 126.7, 128.5, 128.9, 137.7, and 176.7; MS (m/z): 192 (M^+), 178, 167, 149, 121, 111, 97, 91, 85, 77, 73, 71, and 60; $[\alpha]=+1.84°$ ($c=1$, EtOH).

(2*S*,3*S*)-2-Benzyl-3,4-epoxybutanoic Acid, (2*S*,3*S*)-BEBA. This compound was prepared from (2*S*,3*S*)-BEBA benzyl ester in 95% yield following the procedure described for the preparation of (2*S*,3*R*)-BEBA. The spectral data (IR, ^1H NMR) of this compound are identical with those of (2*R*,3*R*)-BEBA. $[\alpha]=-1.72°$ ($c=1$, EtOH).

Determination of optical purity of the resolved 2-benzylvinylacetic acid. A mixture of resolved 2-benzylvinylacetic acid (49.3 mg, 0.28 mmol), L-alanine methyl ester hydrochloride (39.2 mg, 0.28 mmol), triethylamine (28.3 mg, 0.28 mmol), and DCC (20.6 mg, 0.28 mmol) in methlene chloride (10 mL) was allowed to stir at room temperature overnight. The reaction mixture was chilled in ice, then filtered to remove dicyclohexylurea. The filtrate was evaporated on a rotary evaporator under reduced pressure, and the residue was treated with ethyl acetate, whereby most of the product was dissolved in methylene chloride. The insoluble residue (dicyclohexylurea) was removed by filtration, and the filtrate was evaporated under reduced pressure. Optical purity of the resolved acid was determined from the relative NMR peak intensity of the methyl proton signals of the product at 1.34 (d) and 1.22 (d). The former signal corresponds to the methyl protons of the product formed with (*R*)-2-benzylvinylacetic acid with L-alanine methyl ester, and the latter to the methyl protons of the product formed with (*S*)-2-benzylvinylacetic acid containing as an impurity resulting from the resolution.

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Fragmentation of Tertiary Alkyl Amine Ions: Mechanism of C-C Bond Cleavage

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The multiphoton ionization of tertiary amines has been previously studied by Parker *et. al.*[1] They showed that on visible laser irradiation trimethylamine produced a parent ion, P^+ and an ion missing an H atom, $[P-H]^+$. Triethylamine formed a parent ion and an ion lacking a methyl radical, $[P-CH_3]^+$. Cyclic amines such as ABCO (quinuclidine)