150 °C for 8 h. After removal of the catalyst by centrifugation and then filtration, the product was isolated by distillation or by MPLC on silica gel (ethyl acetate or chloroform/hexane = 1/5–10 eluent). Spectral data of each product were compared with those of authentic samples and literature values.[6,13,17a,18,19]

2-Butyl-2-cyclohexen-1-one (8a): $[M^+]$ *m/e* 152; IR (NaCl) 2900, 2850, 1700, 1610 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.40 (t, 1 H), 2.40–0.70 (m, 15 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 200.8 (s), 138.8 (d), 136.1 (s), 40.0 (t), 29.9 (t), 26.8 (t), 23.7 (t), 23.5 (t), 21.9 (t), 13.9 (q).

2-Pentyl-2-cyclohexen-1-one (8b): $[M^+]$ *m/e* 166; IR (NaCl) 2900, 2800, 1700, 1610 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.34 (t, 1 H), 2.40–0.66 (m, 17 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 201.1 (s), 139.4 (d), 136.6 (s), 40.2 (t), 29.9 (t), 26.9 (t), 23.8 (t), 23.5 (t), 22.6 (t), 21.7 (t), 13.1 (q).

2-Octyl-2-cyclohexen-1-one (8c): $[M^+]$ *m/e* 208; IR (NaCl) 3000, 2900, 2850, 1700, 1640 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.1 (t, 1 H), 2.2–1.0 (m, 20 H), 0.8 (t, 3 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 203.8 (s), 143.1 (d), 137.2 (s), 40.6 (t), 30.1 (t), 29.6 (t), 29.0 (t), 27.0 (t), 26.4 (t), 23.6 (t), 22.6 (t), 22.3 (t), 20.6 (t), 13.8 (q).

2-Decyl-2-cyclohexen-1-one (8d): $[M^+]$ *m/e* 236; IR (NaCl) 3030–2900, 2860, 1700, 1620 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.0 (t, 1 H), 2.7–1.1 (m, 24 H), 0.8 (t, 3 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 206.1 (s), 144.2 (d), 137.1 (s), 41.7 (t), 31.2 (t), 30.1 (t), 29.8 (t), 29.0 (t), 27.8 (t), 27.0 (t), 26.2 (t), 23.8 (t), 22.4 (t), 22.0 (t), 20.4 (t), 12.1 (q).

2-Hexadecyl-2-cyclohexen-1-one (8e): $[M^+]$ *m/e* 320; IR (NaCl) 3050–2920, 2860, 1710, 1640 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.5 (t, 1 H), 2.8–0.7 (m, 39 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 209.1 (s), 146.8 (d), 138.8 (s), 41.2 (t), 34.6 (t), 30.7 (t), 29.7 (t), 28.9 (t), 27.5 (t), 26.6 (t), 25.2 (t), 24.6 (t), 23.2 (t), 22.9 (t), 22.6 (t), 22.4 (t), 22.1 (t), 21.4 (t), 21.1 (t), 20.7 (t), 19.7 (t), 10.5 (q).

2-Isobutyl-2-cyclohexen-1-one (8f): $[M^+]$ *m/e* 152; IR (NaCl) 3020–2980, 2890, 2850, 1705, 1660 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 5.7 (t, 1 H), 3.1 (m, 1 H), 2.8 (d, 2 H), 2.2–1.1 (m, 12 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 211.6 (s), 144.4 (d), 140.6 (s), 43.2 (t), 30.7 (d), 29.7 (t), 24.2 (t), 22.7 (t), 14.1 (q).

2-Pentyl-2-cyclopenten-1-one (8g): $[M^+]$ *m/e* 152; IR (NaCl) 2950, 2865, 1718, 1645, 1470, 1370, 1300–1120 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.2 (t, 1 H), 2.8–1.9 (m, 12 H), 0.9 (t, 3 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 206.1 (s), 147.4 (d), 137.8 (s), 38.8 (t), 31.4 (t), 29.4 (t), 27.2 (t), 23.6 (t), 19.9 (t), 14.1 (q).

2-Octyl-2-cyclopenten-1-one (8h): $[M^+]$ *m/e* 194; IR (NaCl) 2950, 2870, 1720, 1650, 1470, 1380, 1300–1140 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.42 (t, 1 H), 2.57 (t, 2 H), 2.07 (m, 2 H), 1.87 (t, 2 H), 1.33 (m, 12 H), 0.9 (t, 3 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 195.1 (s), 139.3 (d), 137.5 (s), 39.7 (t), 29.7 (t), 29.4 (t), 28.9 (t), 27.1 (t), 26.8 (t), 23.4 (t), 22.7 (t), 20.1 (t), 14.0 (q).

2-Decyl-2-cyclopenten-1-one (8i): $[M^+]$ *m/e* 222; IR (NaCl) 3000, 2880, 1720, 1650, 1470–1450, 1400, 1300–1120 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 6.53 (t, 1 H), 2.6 (t, 2 H), 2.0 (m, 2 H), 1.9–0.9 (m, 21 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 200.2 (s), 142.6 (d), 138.1 (s), 41.2 (t), 31.6 (t), 29.8 (t), 28.7 (t), 27.4 (t), 24.5 (t), 23.8 (t), 22.6 (t), 21.2 (t), 20.9 (t), 20.1 (t), 12.6 (q).

Cross-Condensation of 3-Methylcyclopentanone (2e) with Pentanol (7b) Catalyzed by Cp_2ZrH_2 (1) and $NiCl_2$. A mixture of 1 (0.2 mmol), $NiCl_2$ (0.2 mmol), **2e** (10 mmol), and **7b** (10 mmol) was placed into an autoclave (50 cm^3) in an atmosphere of argon and heated with shaking at 150 °C for 8 h. After removal of the catalyst by filtration, the reaction mixture was subjected to MPLC on silica gel (benzene/hexane = 2/1 eluent) to give dihydrojasmone (**13**) and its regioisomer **14**.

Dihydrojasmone (13): $[M^+]$ *m/e* 166; IR (NaCl) 3000, 1680, 1650, 1370, 1060 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 2.7–2.0 (m, 9 H), 1.8–1.1 (m, 6 H), 0.87 (t, 3 H) [lit.[18] 2.6–1.9 (m, 6 H), 2.05 (s, 3 H), 1.6–1.0 (m, 6 H), 0.85 (t, 3 H)]; ^{13}C NMR ($CDCl_3/Me_4Si$) δ 206.6 (s), 167.6 (s), 140.3 (s), 37.6 (t), 34.7 (t), 32.1 (t), 28.4 (t), 27.3 (t), 24.5 (t), 19.9 (q), 14.1 (q).

4-Methyl-2-pentyl-2-cyclopenten-1-one (14): $[M^+]$ *m/e* 166; IR (NaCl) 3000, 1680, 1650, 1400, 1020 cm^{-1}; ^1H NMR ($CDCl_3/Me_4Si$) δ 5.2 (d, 1 H), 2.9–1.8 (m, 11 H), 1.2 (d, 3 H), 0.9 (t, 3 H); ^{13}C NMR ($CDCl_3/Me_4Si$) δ 206.0 (s), 146.7 (d), 138.0 (s), 40.2 (t), 35.1 (d), 32.3 (t), 28.4 (t), 27.2 (t), 25.1 (t), 15.4 (q), 14.1 (q).

Registry No. **1**, 12116-83-5; **2a**, 108-94-1; **2b**, 120-92-3; **2c**, 583-60-8; **2d**, 589-92-4; **2e**, 1757-42-2; **3a**, 110-88-3; **3b**, 75-07-0; **3c**, 123-72-8; **3d**, 110-62-3; **3e**, 124-13-0; **3f**, 112-31-2; **3g**, 78-84-2; **4a**, 3045-98-5; **4b**, 1122-25-4; **4c**, 7153-14-2; **4d**, 25677-40-1; **4e**, 72927-86-7; **4f**, 107799-61-1; **4g**, 43108-69-6; **4h**, 16424-35-4; **4i**, 40564-16-7; **4j**, 107799-65-5; **4k**, 107799-66-6; **5a**, 1502-22-3; **5b**, 7027-34-1; **5c**, 13705-82-3; **6a**, 4170-30-3; **6b**, 645-62-5; **6c**, 34880-43-8; **6d**, 13893-39-5; **6e**, 25234-33-7; **7a**, 71-36-3; **7b**, 71-41-0; **7c**, 111-87-5; **7d**, 112-30-1; **7e**, 36653-82-4; **7f**, 78-83-1; **8a**, 34737-39-8; **8b**, 25435-63-6; **8c**, 52914-72-4; **8d**, 107799-62-2; **8e**, 107799-63-3; **8f**, 107799-64-4; **8g**, 25564-22-1; **8h**, 54625-13-7; **8i**, 64351-95-7; **9**, 78091-18-6; **10a**, 100-52-7; **10b**, 98-01-1; **10c**, 104-55-2; **11a**, 897-78-9; **11b**, 893-00-5; **11c**, 18977-40-7; **11d**, 895-80-7; **11e**, 1026-78-4; **11f**, 21856-78-0; **13**, 1128-08-1; **14**, 75359-55-6; $NiCl_2$, 7718-54-9; $Cp_2Zr(H)Cl$, 37342-97-5; Cp_2ZrCl_2, 1291-32-3; Cp_2TiCl_2, 1271-19-8; Cp_2HfCl_2, 12116-66-4; $ZrCl_4$, 10026-11-6; $TiCl_4$, 7550-45-0; $AlCl_3$, 7446-70-0; $4\text{-}H_3CC_6H_4SO_3H$, 104-15-4; $NiBr_2$, 13462-88-9; $Ni(acac)_2$, 3264-82-2; $PdCl_2$, 7647-10-1; $CuCl_2$, 7447-39-4; 2,4,6-trimethyl-1,3,5-trioxane, 123-63-7.

Different Enzymatic Reactions of an Enantiomeric Pair: Simultaneous Dual Kinetic Resolution of a Keto Ester by Bakers' Yeast

Dee W. Brooks,*[1a] Michael Wilson,[1b] and Michael Webb[1b]

Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064, and Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

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An example of different enzymatic reactions of an enantiomeric pair involving a simultaneous dual kinetic resolution by two different enzymes in bakers' yeast on a racemic synthetic substrate is described. The 1*R*,5*S* enantiomer of methyl (±)-3-oxo-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate (**1**) is converted by a dehydrogenase enzyme present in bakers' yeast to methyl (+)-(1*R*,2*R*,3*S*,5*S*)-3-hydroxy-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate (**4**) and the 1*S*,5*R* enantiomer of **1** is converted to 7,7-(ethylenedioxy)bicyclo[3.3.0]octan-3-one (**5**) by a process that likely involves ester hydrolysis by an esterase and subsequent decarboxylation of a proposed intermediate β-keto acid.

The applications of enzymes to effect selective transformations of synthetic substrates is a useful method to provide optically pure intermediates for synthesis.[2] Microorganisms may be simply viewed by an organic chemist

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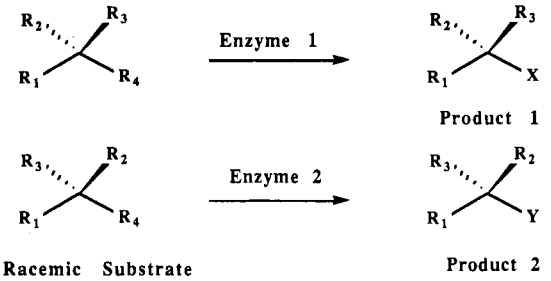
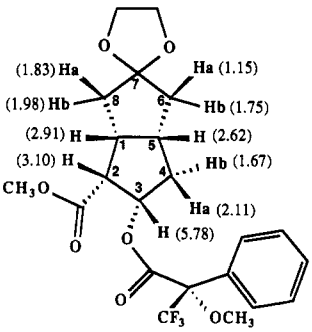
Scheme I. Simultaneous Dual Kinetic Resolution by Two Different Enzymes



as a collection of enzymes in a self-sustaining environment, complete with all necessary co-factors for catalysis. The advantages of enzyme-catalyzed reactions are selectivity (regio, stereo, and enantio) and mild reaction conditions. A major disadvantage, from a synthetic perspective, is the lack of generality of enzymatic transformations due to defined substrate requirements. Elucidating the scope of substrate variability for enzyme-catalyzed processes is an important aspect in expanding the application of these methods in synthesis.

Numerous examples of enzyme-catalyzed reactions of synthetic substrates have been reported that demonstrate (1) asymmetric transformations of prochiral substrates, (2) asymmetric bond-forming reactions, and (3) kinetic resolution of racemates by preferential reaction of one enantiomeric pair. Since a microorganism such as bakers' yeast contains many enzymes, the possibility exists for distinct enzymatic transformations of an enantiomeric pair of a racemic synthetic substrate. A different reaction of each enantiomer of a racemic synthetic substrate by two different enzymes would provide two enantiomerically pure products, thus accomplishing a resolution by different chemical reactivity of an enantiomeric pair. This process is illustrated in Scheme I.

In the course of investigations involved in developing applications of reductions by bakers' yeast for the preparation of chiral intermediates for asymmetric synthesis, we have observed a case of differential enzymatic reactions of an enantiomeric pair which is described as follows. The bicyclo[3.3.0] keto ester **1** has been used as an intermediate in the synthesis of (±)-6a-carbaprostaglandin I_2 (**2**).[3] The keto ester **1** appeared to be an interesting substrate for microbial reduction by bakers' yeast since it contained three asymmetric centers and existed in solution as a mixture of racemic endo and exo isomers at the methoxycarbonyl group, therefore potentially offering the formation of eight discreet chiral hydroxy esters. The conformation of this system leads to stereoselective reduction

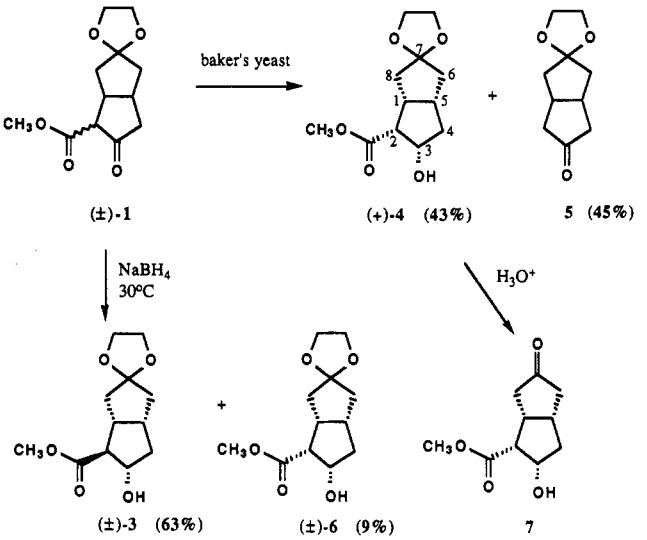


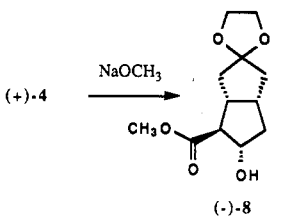
Coupling Constants (Hz)

$J_{1,2}$ = 8.7	$J_{3,4a}$ = 4.6	$J_{5,4a}$ = 9.5	$J_{4a,4b}$ = 13.3
$J_{1,5}$ = 8.8	$J_{3,4b}$ = 0	$J_{5,4b}$ = 0.4	$J_{6a,6b}$ = 10.3
$J_{1,8a}$ = 8.8	$J_{2,3}$ = 4.6	$J_{5,6a}$ = 8.6	$J_{8a,8b}$ = 9.2
$J_{1,8b}$ = 8.6		$J_{5,6b}$ = 8.5	

Figure 1. ^{1}H NMR chemical shifts and spin–spin coupling constants of the (*S*)-(−)-MTPA ester of (+)-**4**.

by synthetic hydride reducing agents and it was of interest to compare the outcome of enzyme-mediated reduction using bakers' yeast. The reduction of **1** with $NaBH_4$ at −20 °C is known to proceed stereoselectively to provide the racemic hydroxy ester **3**.[3a] The keto ester **1** was incubated with fermenting bakers' yeast (*Saccharomyces cerevisiae*) in pH 7 phosphate buffer for 48 h, after which the mixture was continuously extracted with dichloromethane for 48 h, to provide only two products; the hydroxy ester **4** (43%) and the ketone **5** (45%).





Determination of the Absolute Structure of the Yeast-Derived Hydroxy Ester 4. The ^{1}H NMR spectrum of the yeast-derived hydroxy ester **4** was different than that of the hydroxy ester **3** from $NaBH_4$ reduction of **1**. However, conducting the $NaBH_4$ reduction of **1** at 30 °C gave two isomeric hydroxy esters, **3** (63%) and **6** (9%). The minor isomer **6** exhibited an identical ^{1}H NMR spectrum as that of the yeast-derived hydroxy ester **4**. In the ^{1}H NMR spectrum of **3** where the ester group is trans

(1) (a) Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064. (b) Department of Chemistry, Purdue University, West Lafayette, IN 47907.

(2) For pertinent reviews of this topic refer to: (a) *Enzymes as Catalysts in Organic Synthesis*; Schneider, M. P., Ed.; NATO ASI Series C: Vol. 178; D. Reidel: Dordrecht, 1986; Vol. 178. (b) *Enzymes in Organic Synthesis*; Ciba Foundation Symposium 111, Pitman: London, 1985. (c) Whitesides, G. M.; Wong, C. H. *Aldrichimica Acta* **1983**, *16*, 27. (d) Sih, C. J.; Rosaza, J. P. *Applications of Biochemical Systems in Organic Chemistry*; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Part I, Chapter III. (e) Kieslich, K. *Microbial Transformations of Chemical Compounds Excluding Steroids and Noncyclic Structures*; G. Thieme Verlag: Stuttgart, 1976.

(3) (a) Nicolaou, K. C.; Sipio, W. J.; Magolda, R. L.; Seitz, S.; Barnette, W. E. *J. Chem. Soc., Chem. Commun.* **1978**, *1067*. (b) Kojima, K.; Sakai, K. *Tetrahedron Lett.* **1978**, *3743*. (c) Shibasaki, M.; Ueda, J.; Ikegami, S. *Tetrahedron Lett.* **1979**, 433. (d) Barco, A.; Benetti, S.; Pollini, G. P.; Baraldi, P. G.; Gandolfi, C. *J. Org. Chem.* **1980**, *45*, 4776. (e) Review: Aristoff, P. A. *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*; Pike, J. E.; Morton, D. R., Jr., Eds.; Raven Press: New York, 1985; Vol. 14, pp 309–391.

to the hydroxy group and exocyclic, the proton α to the ester group, H2, appeared as a finely split doublet at 2.51 ppm, $J_{1,2}$ = 0.4 Hz, $J_{2,3}$ = 0 Hz. The same proton, H2 in the yeast-derived hydroxy ester **4** appeared as a doublet of doublets at 2.80 ppm, $J_{1,2}$ = 3.0 Hz, $J_{2,3}$ = 5.0 Hz, supporting a structure where the ester group is cis to the hydroxy group and endocyclic. Further support for this assignment of relative configuration was provided by ^{1}H-decoupling experiments which provided chemical shift assignments and spin–spin coupling constants for the protons of the (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester[4] derivative of **4**, as summarized in Figure 1.

The enantiomeric purity of the hydroxy ester **4** was determined by analysis of the ^{1}H NMR spectra at 470 MHz of the (*R*)- and (*S*)-MTPA esters of **4** and the corresponding ketone **7** derived from acid-catalyzed hydrolysis of the ethylene ketal. In each case the chemical shift of the singlet due to the methoxycarbonyl group was clearly resolved. The enantiomeric purity of **4** and **7** was found to be >99%.[5]

Treatment of **4** in methanol with a catalyic amount of sodium resulted in isomerization of the ester group to the trans,exocyclic configuration, providing **8**, $[\alpha]_D$ −27° (*c* 0.5, $CHCl_3$, 23 °C). The ^{1}H NMR spectra of **8** and racemic **3** were identical. A resolution of racemic **3** and chemical correlation of the absolute configuration for (−)-**8** has been reported.[6] The rotation reported for (−)-**8** was $[\alpha]_D$ −28.8° (*c* 1, $CHCl_3$, 23 °C), which agrees well with (−)-**8** prepared from **4**. Thus the absolute configuration of the yeast-derived hydroxy ester **4** is established as methyl (+)-(1*R*,2*R*,3*S*,5*S*)-3-hydroxy-7,7-(ethylenedioxy)bicyclo-[3.3.0]octane-2-carboxylate.

Prior to this report[6] of the resolution and determination of absolute configuration of (+)- and (−)-**3**, we attempted to establish the absolute configuration of **4** using empirical methods based on ^{1}H NMR chemical shift nonequivalence[7] of chiral MTPA[4] and *O*-methylmandelate (OMM)[8] ester derivatives. For the MTPA esters the configurational correlation model[4] positions the trifluoromethyl group eclipsing the carbonyl as shown in Figure 2. The model predicts that the group R_1 of the (*R*)-MTPA ester should appear upfield in the ^{1}H NMR spectrum, presumably as a result of the shielding by eclipsing the phenyl ring, compared to the same group R_1 of the (*S*)-MTPA ester. The opposite holds true for the group R_2. The ^{1}H signal of the methoxycarbonyl group in the (*R*)-MTPA ester of **4** appears at 3.49 ppm and that of the (*S*)-MTPA ester appears at 3.61 ppm. Aqueous hydrolysis of the ketal group of these MTPA esters provided the (*R*)-MTPA ester of **7** with ^{1}H ($COOCH_3$) at 3.49 ppm and the (S)-MTPA ester of **7** with ^{1}H ($COOCH_3$) at 3.64 ppm. These observations support a prediction where group R_1 contains the methoxycarbonyl group as shown in Figure 2. The absolute configuration of the hydroxy group in **4** is thus correctly predicted to be *S*.

The configurational correlation model for *O*-methylmandelate (OMM) esters[8] positions the methoxy group eclipsing the carbonyl as shown in Figure 3. In this model the substituent that eclipses the phenyl ring of the OMM

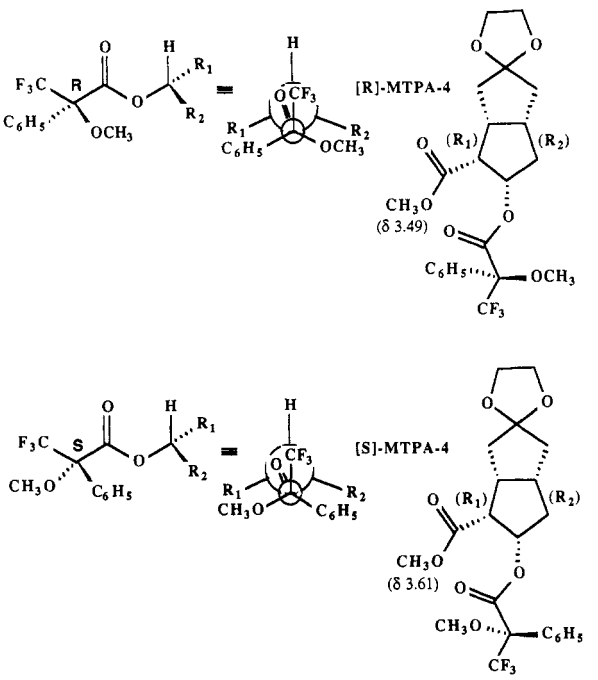


Figure 2. Configurational correlation model for (*R*)- and (*S*)-MTPA esters of **4**.

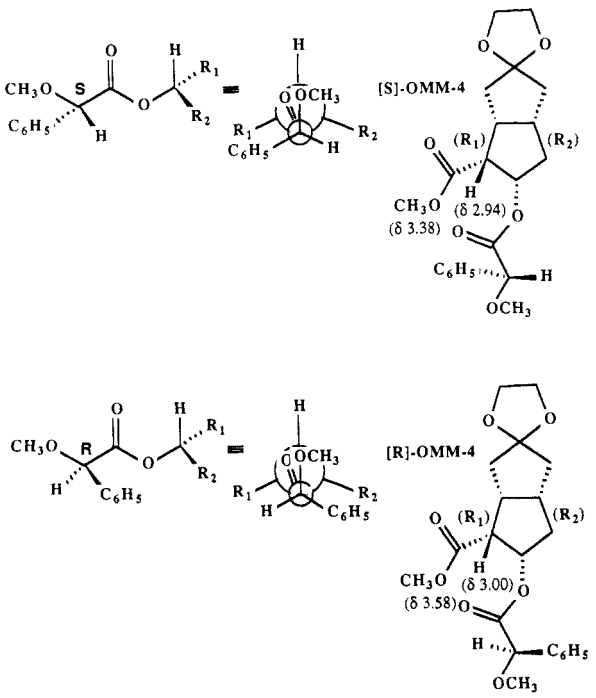


Figure 3. Configurational correlation model for (*S*)- and (*R*)-*O*-methylmandelate esters of **4**.

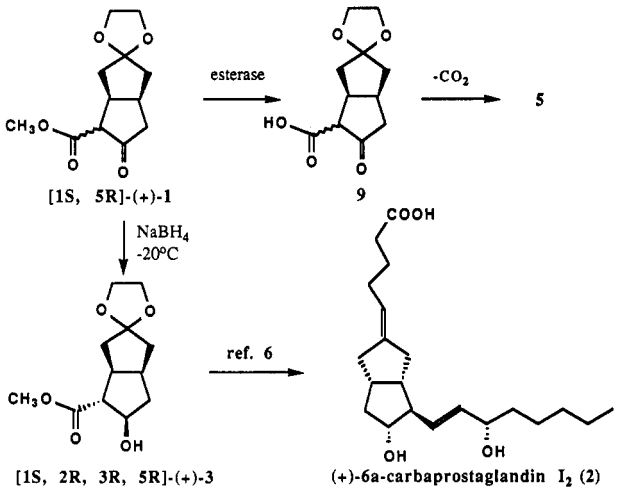
ester is observed upfield. The methoxy ^{1}H signal of the methoxycarbonyl group in the (*S*)-OMM ester of **4** appears at 3.38 ppm and that of the (*R*)-OMM ester of **4** appears at 3.58 ppm. The same trend is evident for the (*S*)-OMM ester of **7**, with ^{1}H ($COOCH_3$) at 3.35 ppm, and (*R*)-OMM ester of **7**, with ^{1}H ($COOCH_3$) at 3.60 ppm. Chemical shift nonequivalence of other ^{1}H signals as predicted by the model are also evident but the methoxycarbonyl signal is particularly diagnostic in predicting the correct absolute structure of **4**.

Rationale for the Formation of Half-Ketal Ketone 5. The fact that the hydroxy ester **4** was enantiomerically pure and of the (+)-1*R*,2*S*,3*S*,5*S* configuration implies that it was derived from the 1*R*,5*S* enantiomer of (±)-**1** by

(4) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2534.
(5) A control experiment established a practical limit of detection of 1% of diastereomer in the ^{1}H NMR spectrum at 470 MHz.
(6) Kojima, K.; Amemiya, S.; Koyama, K.; Sakai, K. *Chem. Pharm. Bull.* **1985**, *33*, 2688.
(7) Review: Rinaldi, P. L. *Progr. NMR Spectrosc.* **1982**, *15*, 291.
(8) Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370.

enzyme-mediated reduction. The 1*S*,5*R* enantiomer of (±)-1 *did not undergo carbonyl reduction* as no trace of another hydroxy ester, MTPA or OMM diastereomer, could be observed. When the yeast reduction was run for 48 h all of the substrate (±)-1 was consumed. The remaining (approximately 50%) mass balance was accounted for by the isolation of the half-ketal ketone **5** (45%). This product is achiral and therefore is not directly traceable as being derived from the 1*S*,5*R* enantiomer of (±)-1. A possible explanation for the formation of **5** is that the 1*S*,5*R* enantiomer of (±)-1 undergoes selective ester hydrolysis via an esterase enzyme to an intermediate β-keto acid **9** which readily decarboxylates (possibly in a nonenzymatic step) to the observed product **5**.

When the yeast reduction was terminated prior to complete conversion of the substrate (±)-1, after 16 h, the products observed were the hydroxy ester **4** (34%), the ketone **5** (30%), and recovered substrate **1** (27%). The recovered unreacted substrate was enriched in (+)-1, which upon stereoselective reduction with $NaBH_4$ gave the trans,exocyclic hydroxy ester **3**, enriched in (+)-**3**. The (+)-**3** enantiomer has been correlated and is of known absolute configuration 1*S*,2*R*,3*R*,5*R* and has also been further elaborated to (+)-**6a**-carbaprostaglandin I_2.[6] This



observation leads to the conclusion that the 1*S*,5*R* enantiomer of (±)-1 does accumulate as the 1*R*,5*S* enantiomer is selectively reduced by a dehydrogenase enzyme in a kinetic resolution process. However, the unreduced 1*S*,5*R* enantiomer is selectively hydrolyzed by an esterase enzyme[9] in a kinetic resolution process, at a slightly slower rate of reaction than the dehydrogenase reaction, to provide a proposed intermediate β-keto acid **9**, which undergoes decarboxylation to provide the observed product **5**. *The above process is an example of a simultaneous dual kinetic resolution of a synthetic racemic substrate by different enzyme-catalyzed reactions.* Applications of this phenomena might prove to be a useful novel strategy for the preparation of chiral intermediates for asymmetric synthesis if suitable substrates can be designed or enzyme systems identified.

Experimental Section

All experiments requiring anhydrous conditions were conducted under a dry nitrogen atmosphere. Reactions were performed at room temperature unless indicated otherwise and with stirring using a magnetically driven stir bar. Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F-254 plates (0.25 mm). The plates were visualized by spraying or dipping with a *p*-anisaldehyde solution (1350 mL of ethanol, 50 mL of concentrated H_2SO_4, 15 mL of glacial acid acid, 37 mL of *p*-anisaldehyde) or a phosphomolybdic acid soluton (5% in methanol) followed by heating the plate (125–150 °C). Chromatography was performed with 230–400-mesh silica gel. Solvents were evaporated on a rotary evaporator at aspirator pressure (ca. 20 mm). Nuclear magnetic resonance (NMR) spectra were acquired on a Perkin-Elmer R-32, Nicolet 470 MHz, QE-300, or Varian XL-200 NMR spectrometer. Chemical shifts are reported in ppm downfield relative to tetramethylsilane as standard. Mass spectra were obtained via electron impact (EI) or chemical ionization (CI) on a Finnigan 4171 mass spectrometer. Optical rotations were measured with a Rudolph Research Autopol III polarimeter and are expressed as $[\alpha]_D$ (concentration in g/100 mL, solvent, temperature).

Reduction of (±)-1 with Bakers' Yeast. Methyl (+)-(1*R*,2*R*,3*S*,5*S*)-3-Hydroxy-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate (4) and 7,7-(Ethylenedioxy)bicyclo[3.3.0]octan-3-one (5). To a vigorously stirred solution of 200 mL of pH 7 phosphate buffer (9.1 g of KH_2PO_4 and 18.9 g of Na_2HPO_4 in 1 L of H_2O), D-glucose (20 g) and yeast extract (1 g) at 30 °C, open to the air, was added dry active bakers' yeast (15 g, Fleischmann's, Standard Brands Inc.). After stirring for 30 min, the keto ester substrate **1** (1.0 g) was added dropwise over 30 min. The mixture was stirred at 25 °C for 48 h and then continuously extracted for 48 h with dichloromethane (250 mL) containing pyridine (1 mL). The organic extract was concentrated and the residue was purified by chromatography (silica gel, 30% ethyl acetate in hexane) to give (+)-**4** (435 mg, 43%) and **5** (340 mg, 45%). (+)-**4**: ^1H NMR ($CDCl_3$, 470 MHz) δ 1.83 (3 H, m), 1.95–2.10 (3 H, m), 2.69 (1 H, m), 2.8 (1 H, dd, *J* = 5.0, 3.0 Hz), 2.93 (1 H, m), 3.65 (1 H, br s, OH), 3.70 (3 H, s, CH_3), 3.89 (4 H, m, OCH_2CH_2O), 4.5 (1 H, m); ^{13}C NMR ($CDCl_3$, 50.3 MHz) 38.8 (CH_2), 39.0 (CH), 39.3 (CH_2), 41.6 (CH), 42.2 (CH_2), 51.6 (CH_3), 51.8 (CH), 63.9 (CH), 64.8 (CH), 75.7 (CH), 118.3 (C), 174.6 (C); IR δ_{max}, cm^{-1} 3550 (w), 1720 (s); $[\alpha]_D$ +18.1 (*c* 3.75, $CHCl_3$, 23 °C); MS, M^+ = 242. Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.49; H, 7.49. Found: C, 59.44; H, 7.52. **5**: ^1H NMR ($CDCl_3$, 470 MHz) δ 1.71 (2 H, dd, *J* = 6.8, 2.3 Hz), 2.11–2.25 (4 H, m), 2.47 (2 H, dd, *J* = 10.4, 6.8 Hz), 2.82 (2 H, m), 3.85 (4 H, s); MS, M^+ = 182. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 65.85; H, 7.81.

Reduction of (±)-1 with $NaBH_4$, Methyl (±)-3-Hydroxy-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate Isomers 3 and 6. To a stirred solution of (±)-1 (300 mg, 1.25 mmol) in methanol (15 mL) at 30 °C was added $NaBH_4$ (12 mg, 0.31 mmol), and the mixture was stirred for 3.5 h. Water (10 mL) was added and the pH of the mixture was adjusted to pH 7 by dropwise addition of 0.5 N HCl. The solution was evaporated at reduced pressure to remove the methanol and then extracted with ether (2 × 20 mL). The ether extract was washed with saturated aqueous NaCl (10 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography (silica gel, 30% ethyl acetate in hexane) to give trans,exocyclic hydroxy ester **3** (189 mg, 63%) and cis,exocyclic hydroxy ester **6** (28mg, 9%). (±)-**3**: ^1H NMR ($CDCl_3$, 470 MHz) δ 1.64 (2 H, m), 1.83 (1 H, d, *J* = 6 Hz), 2.02 (2 H, m), 2.29 (1 H, m), 2.51 (1 H, d, *J* = 0.4 Hz), 2.55 (1 H, m), 2.68 (2 H, m), 3.72 (3 H, s, CH_3), 3.90 (4 H, m, OCH_2CH_2O), 4.22 (1 H, m); ^{13}C NMR ($CDCl_3$, 50.3 MHz) 35.5 (CH), 40.4 (CH_2), 40.5 (CH_2), 41.3 (CH_2), 41.3 (CH), 51.7 (CH_3), 57.8 (CH), 64.0 (CH_2), 64.3 (CH_2), 75.5 (CH), 118.4 (C), 175.2(C); MS, M^+ = 242. Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.49; H, 7.49. Found: C, 59.58; H, 7.62.

(±)-**6**: identical ^1H NMR (470 MHz) and MS as **4**.

(*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetic Acid Ester of 4. To a solution of **4** (120 mg, 0.5mmol) and pyridine (50 mg, 0.6 mmol) in dichloromethane (1 mL) was added (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (150 mg, 0.6 mmol). The mixture was stirred for 16 h, after which dichloromethane (10 mL) was added followed by water (10 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (5 mL) and saturated aqueous NaCl (5 mL), dried over $MgSO_4$, filtered, and evaporated. ^1H NMR analysis of the crude residue showed only one isomer. This was purified by chromatography

(9) Treatment of the corresponding ethyl ester of (±)-1 with bakers' yeast under similar conditions as for **1** gave the (+)-hydroxy ester (40%), >98% ee) and recovered substrate (+)-β-keto ethyl ester (45%, 68% ee) but no half-ketal ketone **5**. Therefore the esterase activity observed for **1** appears to be selective for a methyl ester.

(silica gel, 20% ethyl acetate in hexane) to give the (*S*)-MTPA ester of **4** (224 mg, 98%); ^{1}H NMR ($CDCl_3$, 470 MHz) δ 1.15 (1 H, dd, *J* = 10.3, 8.6 Hz, H6a), 1.67 (1 H, d, *J* = 13.3 Hz, H4b), 1.75 (1 H, m, H6b), 1.83 (1 H, dd, *J* = 9.2, 8.8 Hz, H8a), 1.96 (1 H, m, H8b), 2.11 (1 H, m, H4a), 2.62 (1 H, m, H5), 2.91 (1 H, m, H1), 3.10 (1 H, dd, *J* = 8.7, 4.6 Hz, H2), 3.52 (3 H, MTPA-OCH_3), 3.61 (3 H, s, $COOCH_3$), 3.87 (4 H, m, OCH_2CH_2O), 5.78 (1 H, t, *J* = 4.6 Hz, H3), 7.40 (3 H, m), 7.55 (2 H, m).

(*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetic Acid Ester of 7. To a solution of the (*S*)-MTPA ester of **4** (44 mg, 0.1 mmol) in tetrahydrofuran (1 mL) was added 2 N HCl (1 mL) and the mixture was stirred for 16 h. Ether (10 mL) was added and the organic phase was washed with water (10 mL) and saturated aqueous NaCl (10 mL), dried over $MgSO_4$, filtered, and evaporated to give the (*S*)-MTPA ester of **7** (39 mg, 95%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.56 (1 H, m), 1.74 (1 H, m), 2.2–2.6 (4 H, m), 2.85 (1 H, m), 3.06 (1 H, m), 3.24 (1 H, dd, *J* = 7.5, 4.5 Hz), 3.50 (3 H, MTPA-OCH_3), 3.64 (3 H, s, $COOCH_3$), 5.76 (1 H, dt, *J* = 4.5, 2.0 Hz), 7.4 (3 H, m), 7.47 (2 H, m).

(*R*)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid esters of 4: prepared in the same manner of the (*S*)-MTPA ester; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (1 H, dd, *J* = 10,9 Hz), 1.8–2.4 (5 H, m), 2.65 (1 H, m), 2.90 (1 H, m), 3.10 (1 H, dd, *J* = 9,5 Hz), 3.49 (3 H, s, $COOCH_3$), 3.52 (3 H, MTPA-OCH_3), 3.87 (4 H, m), 5.78 (1 H, t, *J* = 5 Hz), 7.40 (3 H, m), 7.55 (2 H, m).

(*R*)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid ester of 7: prepared by aqueous 2 N HCl hydrolysis of (*R*)-MTPA ester of **4**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.68 (1 H, m), 2.07 (1 H, m), 2.26 (1 H, m), 2.48 (2 H, m), 2.64 (1 H, m), 2.89 (1 H, m), 3.06 (1 H, m), 3.24 (1 H, dd, *J* = 7.5, 5 Hz), 3.45 (3 H, MTPA-OCH_3), 3.49 (3 H, s, $COOCH_3$), 5.73 (1 H, dt, *J* = 4.5, 3.0 Hz), 7.41 (3 H, m), 7.44 (2 H, m).

(*R*)-*O*-Methylmandelic Acid Ester of 4. (*R*)-*O*-Methylmandelic acid (40 mg, 0.24 mmol) was added to a mixture of oxalyl chloride (40 mg, 0.30 mmol) and dimethylformamide (30 mg, 0.40 mmol) in acetonitrile (1 mL) at 0 °C. The mixture was stirred for 10 min and then a solution of **4** (50 mg, 0.20 mmol) in pyridine (0.5 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h after which ether (20 mL) and water (10 mL) was added. The organic phase was washed with saturated aqueous cupric sulfate (2 × 5 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified by preparative TLC (silica gel, 2.0 mm, 20 cm × 20 cm plate, 20% ethyl acetate in hexane) to give the (*R*)-OMM ester of **4** (74 mg, 95%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25–2.2 (6 H, m), 2.62 (1 H, m), 2.92 (1 H, m), 3.00 (1 H, m), 3.42 (3 H, s, OCH_3), 3.58 (3 H, s, $COOCH_3$), 3.92 (4 H, m, OCH_2CH_2O), 4.70 (1 H, s), 5.66 (1 H, t, *J* = 4 Hz), 7.3–7.45 (5 H, m).

(*R*)-*O*-Methylmandelic acid ester of 7: prepared by aqueous 2 N HCl hydrolysis of (*R*)-OMM ester of **4**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.4 (1 H, m), 1.77 (1 H, m), 2.3–2.6 (4 H, m), 2.84 (1 H, m), 3.08 (1 H, m), 3.14 (1 H, m), 3.38 (3 H, s, OCH_3), 3.60 (3 H, s, $COOCH_3$), 4.65 (1 H, s), 5.62 (1 H, m).

(*S*)-*O*-Methylmandelic acid ester of 4: prepared in the same manner as the (*R*)-OMM ester of **4**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.70–2.2 (6 H, m), 2.68 (1 H, m), 2.88 (1 H, m), 2.94 (1 H, m), 3.15 (3 H, s, OCH_3), 3.38 (3 H, s, $COOCH_3$), 3.95 (4 H, s, OCH_2CH_2O), 4.67 (1 H, s), 5.58 (1 H, t, *J* = 4 Hz), 7.3–7.45 (5 H, m).

(*S*)-*O*-Methylmandelic acid ester of 7: prepared by aqueous 2 N HCl hydrolysis of (*S*)-OMM ester of **4**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.69 (1 H, m), 2.1–2.7 (6 H, m), 2.95 (1 H, m), 3.05 (1 H, m), 3.14 (3 H, s, OCH_3), 3.35 (3 H, s, $COOCH_3$), 4.60 (1 H, s), 5.56 (1 H, m), 7.37 (5 H, m).

Methyl (−)-(1*R*,2*S*,3*S*,5*S*)-3-Hydroxy-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate (8). To a solution of **4** (133 mg, 0.5 mmol) in dry methanol (5 mL) was added sodium (2 mg), and the mixture was stirred for 2 h. Solid $NaHCO_3$ (50 mg) was added followed by water (15 mL) and ether (30 mL). The organic phase was washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography (silica gel, 30% ethyl acetate in hexane) to give (−)-**8** (125 mg, 95%): ^{1}H NMR, MS identical with that of (±)-**3**; $[\alpha]_D$ −27° (*c* 0.5, $CHCl_3$, 23 °C).

Methyl (+)-(1*R*,2*R*,3*S*,5*S*)-3-Hydroxy-7-oxobicyclo[3.3.0]octane-2-carboxylate (7): prepared by aqueous 2 N HCl hydrolysis of **4**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.80 (1 H, dt, *J* = 14, 2 Hz), 2.10 (1 H, m), 2.3–2.6 (4 H, m), 2.94 (1 H, dd, *J* = 8.5, 3.5 Hz), 2.99 (1 H, m), 3.19 (1 H, m), 3.38 (1 H, m), 3.75 (3 H, s, $COOCH_3$), 4.54 (1 H, t, *J* = 4 Hz, CHOH); ^{13}C NMR ($CDCl_3$, 75 MHz) 37.47 (CH), 39.96 (CH), 41.46 (CH_2), 41.85 (CH_2), 46.10 (CH_2), 51.91 (CH_3), 53.39 (CH), 74.44 (CHOH), 174.03 ($COOCH_3$), 218.89 (CO); MS, M^+ = 198. Anal. Calcd for $C_{10}H_{14}O_4$: C, 60.59; H, 7.12. Found: C, 60.41; H, 6.99.

First-Order Rate Constants for the Racemization of Each Component in a Mixture of Isomeric Dipeptides and Their Diketopiperazines[1]

Grant Gill Smith* and Rocky Baum

Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300

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L-Alanylglycine (L-Ala-Gly), glycyl-L-alanine (Gly-L-Ala), and c-L-Ala-Gly [diketopiperazine (DKP)] were racemized at 120 °C in aqueous phosphate-buffered solutions at pH 8.0, a pH value near maximum racemization. The kinetics were followed by regression analysis. The racemization of Ala-Gly and Gly-Ala closely followed reversible first-order kinetics. The initial rate of racemization of DKP was fast but soon slowed, likely because of hydrolysis to the dipeptides. The resulting rate was similar to that of the dipeptides. The observed racemization rate constants of the dipeptides and DKP were shown to be independent of the concentration of the peptides and the concentration of buffer. Component isolation studies using preparative TLC and chiral-phase GC analysis, coupled with computer analysis, showed an equilibrium existing between Ala-Gly, Gly-Ala, and DKP and the individual rates of racemization. At equilibrium, the mole fractions are as follows: Ala-Gly, 0.57; DKP, 0.22; Gly-Ala, 0.21. The rate constant for racemization of DKP was only 2 times that of Gly-Ala and 7 times the rate of Ala-Gly. Ala-Gly racemized 20 times and Gly-Ala 66 times faster than free alanine. The results support the influence of neighboring groups in the racemization of dipeptides. Factors that contribute to the rapid racemization (epimerization) are discussed.

Racemization of peptide-bound amino acids is a phenomenon of considerable importance to synthetic peptide chemists[2] and to biogeochemists.[3] Peptide chemists try to avoid conditions that might unfavorably alter the con-

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