obtained by mere condensation of the anhydride acid chloride[6] **6** with histamine in pyridine.

Both structures permit only the more basic lone pairs of the carboxylates to be directed toward one of the histidine nitrogens. In doing so, they are the first model systems that reproduce this structural feature of the serine proteases. The conformation shown for **5** is taken from an energy-minimized structure obtained by using the AMBER[9] force field. The calculations indicate bifurcated hydrogen bonding (ca. 2.65 Å) between the heavy atoms of the carboxylate and the *distal* nitrogen of imidazolium. Aryl stacking between aromatic nuclei is also likely, as the calculated distance between these surfaces is 3.4 Å. Similar analysis of **7** also suggests bifurcated hydrogen bonding, but now the *proximal* nitrogen of the imidazolium is involved with the carboxylate.

What is significance of this arrangement? Poor solubility of **5** in H_2O prevented its titration in that most biorelevant solvent. The pK_a of the acid in **5** is 4.7 (Table I) and that of the imidazolium function is 7.2 in 50% $EtOH/H_2O$ (w/w). However, the protonated *acridine* nitrogen shows a pK_a of 6.2 in this molecule, and its role in modifying the acidity of the other functions is not easily predicted. The corresponding values for Z-His **8a** in the same solvent system suggest that only a slight enhancement of imidazole basicity is due to the syn lone pairs (entry 1 versus 2).

Systems which permit unambiguous assessment of the stereoelectronic effects rather than inductive effects, steric effects, or entropic effects are difficult to find, but **7** is a reasonable candidate. The acidity of **9**[10] or **10** may be used as the standard for the carboxyl function in this skeleton; their pK_a values are unexceptional. In either $EtOH/H_2O$ or H_2O the interplay of acid and base in **7** can be seen. Thus, the nearby carboxylate enhances the basicity of the imidazole, and the imidazolium nucleus enhances the acidity of the carboxylic acid. Indeed **7** is the strongest base of the series.

Relvant comparison are possible with the cis isomer **11** although the alkene bond permits additional forms of communication between the two functions.[11] The saturated **8b** also shows enhanced

9 R = H
10 R = CH_3
11

basicity.[12] The high basicity of the imidazole of these (entries 7, 8, and 9) is most likely due to the intramolecular hydrogen bond involving the anti lone pair. Thus, even the anti lone pair of a carboxylate can offer significant stabilization of an imidazolium function. The additional effect of 0.3 to 0.7 pK_a units (entry 5 versus 7-9) can be attributed to the syn lone pair. This provides modest support for Gandour's hypothesis[4] concerning the stereoelectronics of carboxyl oxygen at the active sites of enzymes. Structure **5** also incorporates a primary hydroxyl group in the vicinity of the acid-base pair, and its reactivity as an elaborated serine protease model is under investigation.

Acknowledgment. We thank the National Institutes of Health for support and Professor Wm. Gordon for helpful advice.

(9) MACROMODEL 1.5, Still, W. C. The AMBER force field is from Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, C.; Chio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.

(10) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Jones, S.; Nemeth, D.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 5033-5035.

(11) Roberts, J. D.; Yu, C.; Flanagan, C.; Birdseye, T. R. *J. Am. Chem. Soc.* **1982**, *104*, 3945-3949.

(12) Martell, A. E.; Smith, R. M. *Critical Stability Constants, Vol 1: Amino Acids*; Plenum Press: New York, NY, 1974; p 79, See especially p xii of the introduction. The original literature values (7.56) are adjusted to concentration constants comparable to our measurements. Chakravarty, A.; Cotton, F. A. *J. Phys. Chem.* **1963**, *678* 2978-2979. Meyer, J. L.; Bauman, J. E., Jr. *J. Am. Chem. Soc.* **1970**, *92*, 4210-4216. Reference 11, however, reports pK_a = 7.7 for this substance.

The Effects of Pressure on Porphyrin *c*-Cytochrome b_5 Complex Formation

Jack A. Kornblatt*

Enzyme Research Group, Department of Biology
Concordia University, 1455 de Maisonneuve
Montreal, P.Q., Canada H3G 1M8

Gaston Hui Bon Hoa

Institut de Biologie Physico-Chimique
13, rue Pierre et Marie Curie, 75005 Paris, France

Lindsay Eltis and A. Grant Mauk*

Department of Biochemistry, University of British Columbia
Vancouver, British Columbia, Canada V6T 1W5

Received January 14, 1988

Cytochrome b_5 forms a reasonably stable 1:1 complex with cytochrome *c* in solution at low ionic strength and at a pH (7.5) that is approximately the average of the isoelectric points of the two proteins.[1] Previous studies of the interaction between these two proteins have employed electronic difference spectroscopy,[1] NMR spectroscopy,[2] specifically modified derivatives of cytochrome *c*,[3] and computer graphics modelling[4] in combination with electrostatics[5] and molecular dynamics calculations.[6] Together, these studies have produced a reasonably detailed model for the mechanism of interaction between these two proteins, many features of which require further experimental evaluation. To obtain complementary hydrodynamic information concerning the cytochrome b_5·cytochrome *c* complex, we have now determined the change in partial specific volume that occurs on formation of this complex by monitoring the quenching of porphyrin cytochrome *c* fluorescence[7] by cytochrome b_5 as a function of pressure under solution conditions known to promote stable 1:1 complex formation at ambient pressure.

Trypsin solubilized cytochrome b_5 was purified from bovine liver microsomes as described previously.[12] Porphyrin cytochrome *c* was prepared from horse heart cytochrome *c* (Type VI, Sigma Chemical Co.) as described previously.[11a-c] The quenching of

(1) Mauk, M. R.; Reid, L. S.; Mauk, A. G. *Biochemistry* **1982**, *21*, 1843.

(2) Eley, C. G. S.; Moore, G. R. *Biochem. J.* **1983**, *215*, 11.

(3) (a) Ng, S.; Smith, M. B.; Smith, H. T.; Millett, F. *Biochemistry* **1977**, *16*, 4975. (b) Stonehuerner, J.; Williams, J. B.; Millett, F. *Biochemistry* **1979**, *18*, 5422.

(4) Salemme, F. R. *J. Mol. Biol.* **1976**, *102*, 563.

(5) Mauk, M. R.; Mauk, A. G.; Weber, P. C.; Matthew, J. B. *Biochemistry* **1986**, *25*, 7085.

(6) Wendoloski, J. J.; Matthew, J. B.; Weber, P. C.; Salemme, F. *Science* (*Washington, D.C.*) **1987**, *238*, 794.

(7) The use of porphyrin *c* in this work is based on the assumption that this derivative of the protein exhibits hydrodynamic and electrostatic properties that are approximately equivalent to those of the native protein. This assumption is clearly imperfect insofar as removal of Fe(III) from cytochrome *c* is expected to change the dipole moment of the protein[8] as well as to affect (in a currently undefined manner) the electrostatic potential surface of the protein. It is also conceivable that the thermal stability of porphyrin *c* may be lower than that of the native cytochrome. Nevertheless, viscosity measurements, circular dichroism spectra, and tryptophan fluorescence studies[9] have provided experimental evidence that the solution structure of porphyrin *c* is virtually unchanged from that of cytochrome *c*. In addition, we note that the distance between the prosthetic groups of the proteins in the porphyrin cytochrome *c*·cytochrome b_5 complex estimated by Forster energy transfer (17-18 Å[10]) is consistent with the distance predicted by the computer graphics model for the complex proposed by Salemme.[4] On the basis of many of these considerations, a substantial literature has developed with the implicit assumption that these two proteins are interchangeable,[11] so the present work has been undertaken, in part with these precedents in mind.

(8) Koppenol, W. H.; Margoliash, E. *J. Biol. Chem.* **1982**, *257*, 4426.

(9) Fisher, W. R.; Taniuchi, H.; Anfinsen, C. B. *J. Biol. Chem.* **1973**, *248*, 3188.

(10) Eltis, L.; Mauk, A. G., manuscript in preparation.

0002-7863/88/1510-5909$01.50/0 © 1988 American Chemical Society

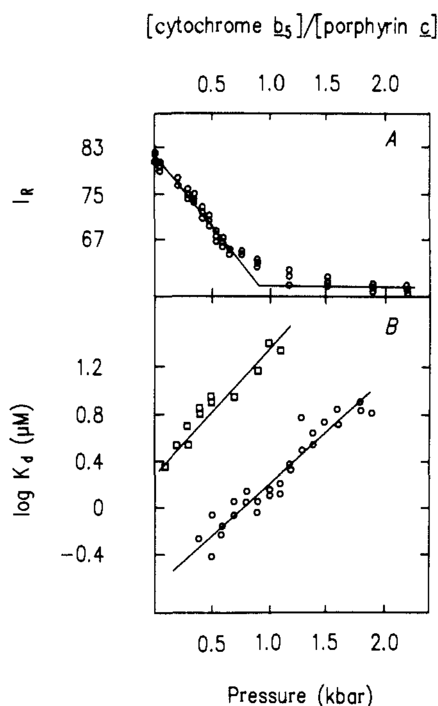


Figure 1. The influence of pressure on complex formation by cytochrome b_5 and porphyrin cytochrome *c*. (A) Titration of porphyrin cytochrome *c* with cytochrome b_5 at atmospheric pressure. Defined mixtures of porphyrin cytochrome *c* and cytochrome b_5 were prepared in 1 mM bisTris (pH 7.0) adjusted to μ = 1 mM with KCl. The fluorescence yield of the porphyrin cytochrome *c* in the mixture was monitored (5 °C) with an SLM Model 4800 fluorometer (8-nm bandwidth) with an excitation wavelength of 500 nm and an emission wavelength of 620 nm. The uncertainty in binding constants measured in this manner is ca. ±20%. (B) 1:1 mixtures of porphyrin cytochrome *c* and cytochrome b_5 (3 μM each) were placed in the pressure bomb that was interfaced to the fluorometer.[11b,15] The fluorescence of the mixture of the bomb was monitored at 1 bar; the pressure was then raised until no further increase in fluorescence was detectable. This value corresponded to the fluorescence yield of the free porphyrin cytochrome *c*; on the basis of percent-free porphyrin in the original 1 bar measurement and the value obtained at the highest pressures, we verified that the fluorescence of the bound porphyrin was 72% that of the free; 72% is the value obtained from Figure 1A. Excitation and emission wavelengths, bandwidths, pH, and temperature were as in A. As in our previous studies,[11b,15] fluorescence changes were completely reversible and independent of the direction from which the pressure was approached: (○), μ = 1 mM ($K_d = 2 \times 10^{-7}$ M); (□), μ = 10 mM ($K_d = 2.2 \times 10^{-6}$ M).

porphyrin cytochrome *c* fluorescence produced by addition of cytochrome b_5 was monitored, and the titration curve shown in Figure 1A was constructed.[11a-g] The results shown in this figure are consistent with a 1:1 binding stiochiometry and a dissociation constant, K_d, of 2×10^{-7} M^{-1} (μ = 1 mM, pH 7.0). The dependence of K_d on ionic strength (data not shown) has been analyzed by extended Debye–Huckel theory to yield a charge product (Z_1Z_2 of −26) and a dissociation constant of 10^{-8} M^{-1} at μ = 0 M. Comparison of these results with those obtained from difference electronic absorption spectroscopy of the two native proteins[1] ($K_d = 10^{-7}$ M^{-1} and $Z_1Z_2 = -60 \pm 10$) indicates that porphyrin cytochrome *c* does not interact with cytochrome b_5 in exactly the same manner as the two native proteins interact with each other.

Changes in volume accompanying the interaction of proteins with substrates, ligands, or other macromolecules may be determined by studying the effects of elevated hydrostatic pressure on the complexes of interest.[13] The effect of pressure on the equilibrium constant for dissociation of the cytochrome b_5·porphyrin cytochrome *c* complex at two ionic strengths as determined from fluorescence intensity is shown in Figure 1B. In both cases, the response of the complex to increased pressure was the same. ΔV° was calculated to be −46 and −56 mL/mol for the upper and lower curves in Figure 1B, respectively, for an average value of about −50 mL/mol. The change in fluorescence intensity observed at elevated pressure does not arise from an affect on cytochrome b_5, and the data have been corrected for the small perturbation in the fluorescence of porphyrin cytochrome *c* observed under pressure.[15] Furthermore, we do not observe the pressure-induced solvation of the cytochrome b_5 heme reported by others using rat liver cytochrome b_5.[14] We attribute this discrepancy in results to differences in the heme binding properties of the two species of apo-protein.

The −50 mL/mol change in partial specific volume resulting from dissociation of the cytochrome b_5·porphyrin cytochrome *c* complex is significantly greater than the values associated with dissociation of the cytochrome *c* peroxidase·porphyrin cytochrome *c*[15] (0 mL/mol), cytochrome *c* oxidase·porphyrin cytochrome *c*[16] (−17 mL/mol), and cytochrome P-450_{LM2}·cytochrome b_5[14] (−23 mL/mol) complexes. We propose that the observed decrease in volume reflects the removal of solvent from the interface of the two molecules on closest approach and the rehydration of the bare surface charges with concomitant decrease in volume (electrostriction) upon separation.[16] This conclusion is fully consistent with our previous thermodynamic data for the interaction between cytochrome b_5 and cytochrome *c* and argues strongly that the removal of water from the interface of cytochrome b_5·cytochrome *c* is the principal contribution to the free energy of complex formation.[1]

As discussed by Fisher et al.,[14] if the separation of charge in a heterologous dimer dissociation is analogous to the ionization of acetic acid[17] and if solvent is completely excluded from the protein–protein interface,[1,4] our observed volume change for the cytochrome b_5·cytochrome *c* pair of −50 mL/mol is consistent with the involvement of roughly four or five ion pairs in the association of these two proteins. This number correlates well with the number of salt bridges that the Salemme model[4] has predicted to form between carboxylate groups on the surface of cytochrome b_5 and lysyl ϵ-amino groups on the surface of native cytochrome *c*. However, similar analysis of the change in partial specific volume for the porphyrin cytochrome *c*·cyanocytochrome *c* peroxidase complex indicates that no electrostatic interaction occurs between these two proteins, a result clearly inconsistent with a large body of work (e.g., ref 18). This apparent anomaly could result from several causes,[19] each of which would complicate the electrostatics

(11) (a) Kornblatt, J. A.; English, A. M. *Eur. J. Biochem.* **1986**, *155*, 505. (b) Kornblatt, J. A.; Hui Bon Hoa, G.; English, A. M. *Biochemistry* **1984**, *23*, 5906. (c) Kornblatt, J. A.; Luu, H. A. *Eur. J. Biochem.* **1986**, *159*, 407. (d) Vanderkooi, J. M.; Landesberg, R.; Hayden, G. W.; Owens, C. S. *Eur. J. Biochem.* **1977**, *81*, 339. (e) Vanderkooi, J. M.; Glatz, P.; Casadei, J.; Woodrow, G. V. *Eur. J. Biochem.* **1980**, *100*, 189. (f) Koloczek, H.; Horie, T.; Yonetani, T.; Annie, H.; Mania, G.; Vanderkooi, J. M. *Biochem.* **1987**, *26*, 3142. (g) Robinson, A. B.; Kamen, M. D. In *Structure and Function of Cytochromes*; Okunuki, K., Ed.; University of Tokyo Press: Tokyo, 1963; p 383.

(12) Reid, L. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1982**, *104*, 841.

(13) Weber, G.; Drickamer, H. *Quart. Rev. Biophys.* **1983**, *16*, 89.

(14) Fisher, T. M.; White, R. E.; Sligar, S. G. *J. Am. Chem. Soc.* **1986**, *108*, 6835.

(15) Kornblatt, J. A.; English, A. M.; Hui Bon Hoa, G. *Eur. J. Biochem.* **1986**, *159*, 39.

(16) Clothia, C.; Janin, J. *Nature (London)* **1975**, *256*, 705.

(17) Neuman, R. C.; Kauzmann, W.; Zipp, A. *J. Phys. Chem.* **1973**, *77*, 2687.

(18) Erman, J. E.; Vitello, L. B. *J. Biol. Chem.* **1980**, *255*, 6224.

(19) Heremans[20] has noted that volume changes of complex formation are frequently smaller than expected on the basis of electrostatic considerations. Hydrophobic interactions in the peroxidase·porphyrin–cytochrome *c* pair may contribute a positive volume change on dissociation.[21] The peroxidase·cytochrome *c* case is further complicated by the fact that at least two intramolecular ion pairs are broken during complex formation and are replaced by intermolecular bridges.[22] If all four charges do not take part in ion pair formation in the complex, the volume change for the four on dissociation would have a positive sign. Finally, the peroxidase in the unligated state shows a large, pressure-sensitive change in spin state. This is not spectroscopically detectable in the cyano derivative (on which the pressure studies were carried out), but the protein may still undergo pressure-induced fluctuations.

(20) Heremans, K. *Ann. Rev. Biophys. Bioeng.* **1982**, *11*, 1.

analysis of Fisher et al.[14] for protein-protein complexes.

Acknowledgment. This research was supported by an Operating Grant from NSERC (to J.A.K.) and by NIH Grant GM-28834 (to A.G.M.). L.E. was the recipient of an MRC Studentship.

Note Added in Proof. After acceptance of this manuscript for publication, Rodgers et al. (*Science*, (*Washington, D.C.*) **1988**, *240*, 1675) published similar studies on the interaction of recombinant rat cytochrome b_5 with horse heart cytochrome *c*. Our results differ from theirs in one important respect: the changes in volume on complex formation that we observe are approximately 50% of the values they report. In the absence of a precise account of the methods employed by Rodgers et al., we temporarily ascribe this difference to our use of different proteins, recombinant rat cytochrome b_5 versus trypsin-solubilized bovine liver microsomal cytochrome b_5 and native cytochrome *c* versus porphyrin cytochrome *c*. We note that the recombinant rat cytochrome b_5 itself is reported to be sensitive to pressure and that the volume changes calculated by Rodgers et al. may include a contribution arising from heme solvation as well as from the cytochrome b_5·cytochrome *c* interaction.

(21) (a) Kauzmann, W. *Adv. Protein. Chem.* **1959**, *14*, 1. (b) Weber, G.; Tanaka, F.; Okamoto, B. Y.; Drickamer, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1264.

(22) (a) Osheroff, N.; Borden, D.; Koppenol, W. H.; Margoliash, E. *J. Biol. Chem.* **1980**, *255*, 1689. (b) Poulos, T. L.; Finzel, B. C. In *Peptide and Protein Reviews*; Hearn, M. T. W., Ed.; Marcel Dekker: New York, 1986; Vol. 4, p 11.

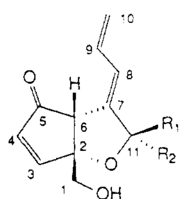
Total Synthesis of (+)-Didemnenones A and B. Absolute Configurations of the Didemnenones

Craig J. Forsyth and Jon Clardy*

Department of Chemistry, Baker Laboratory
Cornell University, Ithaca, New York 14853-1301

Received May 6, 1988

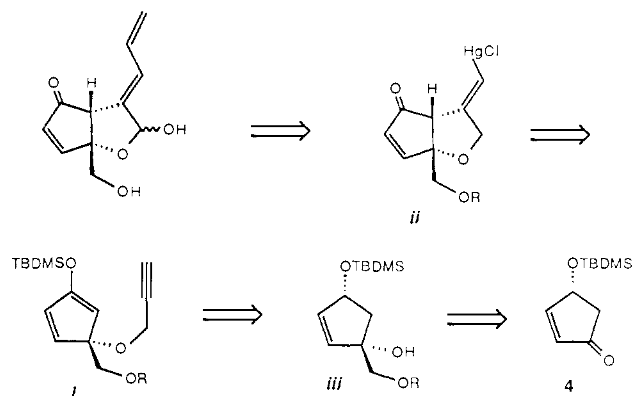
The didemnenones—recently discovered, densely functionalized, and biologically active—are marvelous synthetic targets.[1] We have recently completed an efficient enantiospecific total synthesis of didemnenones A (**1**) and B (**2**) and established the absolute configurations shown in structures **1-3**.



The Caribbean tunicate *Trididemnum* cf. *cyanophorum* produced didemnenones A (**1**) and B (**2**), while halfway around the world, the South Pacific tunicate *Didemnum voeltzkowi* produced didemnenones C and D.[1] The relative stereostructures of the didemnenones were established by an X-ray diffraction analysis of the acetal **3** derived from **1** and **2** followed by chemical and spectral correlations.[1]

Our approach to didemnenones A (**1**) and B (**2**) is shown in Scheme I. The most difficult issues were forming the C6-C7 bond and controlling the stereochemistry at C8. The C6-C7 bond could be formed by using a mercuric chloride induced cyclization[2]

Scheme I



of acetylenic silyl enol ether **i**. This transformation would be followed by the stereospecific conversion of the resultant vinyl mercurial **ii** to the corresponding halide and formation of the C8-C9 bond by using the recently reported palladium-catalyzed coupling of alkenyl halides and vinyl tin reagents.[3] Since replacement of the mercury and formation of the C8-C9 bond were both expected to proceed with retention, the *E*-diene would be formed.[3,4] Allylic oxidation would provide the proper oxidation level at C11. The configuration at C6 in the cis-fused cyclization product **ii** would be defined by the configuration at C2 in cyclization precursor **i**. The configuration at C2, in turn, could be established in **iii** via 1,3-chirality transfer in a diastereofacial selective nucleophilic addition to the chiral enone **4**.[5] Since both antipodes of **4** were available,[6] both enantiomers of **1** and **2** could be prepared.

The synthesis began with the addition of the hydroxymethyl anion equivalent *tert*-butoxymethyllithium[7] (1.4 equiv in tetrahydrofuran(THF)/*tert*-butyl methyl ether, -78 °C, 5 min) to (*R*)-4-(*tert*-butyldimethylsilyloxy)-2-cyclopentenone (**4**)[8] to afford (1*S*,4*R*)-1-(*tert*-butoxymethyl)-4-(*tert*-butyldimethylsilyloxy)-2-cyclopentenol (**5**)[9] (74.5% yield, $[\alpha]_D^{21}$ + 79.6° (*c* 0.950, $CHCl_3$)). The desired (1*S*,4*R*) adduct was readily separated from the (1*R*,4*R*) adduct (7:1 ratio of diastereomers) by silica gel chromatography (hexane-ethyl acetate, 4:1). The stereochemistry of the individual isomers of **5** was established by ^1H NMR analysis (see Supplementary Material). As anticipated, nucleophilic attack occured predominantly from the face trans to the silyloxy group of **4** and established the key stereocenter in **5**. Ether formation (propargyl bromide, NaH, THF, 96.0%) gave **6**, which was desilylated (nBu_4NF, THF, 96.2%) to **7** and oxidized to enone **8** (pyridinium dichromate, CH_2Cl_2, 94.3%). Silyl enol ether for-

(1) Lindquist, N.; Fenical, W. H.; Sesin, D. F.; Ireland, C. M.; Van Duyne, G. D.; Forsyth, C. J.; Clardy, J. *J. Am. Chem. Soc.* **1988**, *110*, 1308-1309.

(2) Drouin, J.; Boaventura, M. A.; Conia, J. M. *J. Am. Chem. Soc.* **1985**, *107*, 1726-1729.

(3) Stille, J. K.; Groh, B. L. *J. Am. Chem. Soc.* **1987**, *109*, 813-817.

(4) Jensen, F. R.; Rickborn, B. *Electrophilic Substitution of Organomercurials*; McGraw-Hill: New York, 1968; pp 64 and 89.

(5) For related additions to 4-hydroxy-2-cyclopentenone and a derivative see: Hua, D. H.; Venkatarama, S. Tetrahedron *Lett.* **1985**, *26*, 3765-3768. Nagoaka, H.; Miyakoshi, T.; Yamada, Y. *Tetrahedron Lett.* **1984**, *25*, 3621-3624.

(6) For some of the methods that are available for the preparation of either enantiomer of **4** see: (a) Kitamura, M.; Kasahara, I.; Manabe, K.; Noyori, R.; Takaya, H. *J. Org. Chem.* **1988**, *53*, 708-710. (b) Noyori, R.; Tomino, I.; Yamada, M.; Nishizawa, M. *J. Am. Chem. Soc.* **1984**, *106*, 6717-6725. (c) Gill, M.; Rickards, R. W. *Tetrahedron Lett.* **1979**, 1539-1542. (d) Okamoto, Y.; Aburatani, R.; Kawashima, M.; Hatada, K.; Okamura, N. *Chem. Lett.* **1986**, 1767-1770. (e) Laumen, K.; Schneider, M. *Tetrahedron Lett.* **1984**, *25*, 5875-5878. (f) Asami, M. *Tetrahedron Lett.* **1985**, *26*, 5803-5806. (g) Ogura, K.; Yamashita, M.; Tsuchihashi, G. *Tetrahedron Lett.* **1976**, 759-762. (h) Additional references cited in: Noyori, R.; Suzuki, M. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 847-876.

(7) Corey, E. J.; Eckrich, T. M. *Tetrahedron Lett.* **1983**, *24*, 3165-3168.

(8) (*R*)-**4** ($[\alpha]_D^{21}$ +62.8° (*c* 2.50 g/100 mL, MeOH, ca. 94% ee) (lit.[6a] $[\alpha]_D^{22}$ +66.6° (*c* 1.0, MeOH) for enantiomerically pure (*R*)-**4**)) was prepared by silylation (TBDMSCl, $(iPr)_2(Et)N$, DMAP, CH_2Cl_2) of (*R*)-4-hydroxy-2-cyclopentenone obtained from (-)-(2*S*,3*S*)-tartaric acid according to ref 6g. (*S*)-**4** ($[\alpha]_D^{24}$ -43.6° (*c* 0.184 g/100 mL, MeOH, ca. 65% ee)) was similarly prepared from (+)-(2*R*,3*R*)-tartaric acid.

(9) All reactions involving air-sensitive compounds were performed under argon or nitrogen. All new compounds gave satisfactory spectral and analytical data, which are included in the supplementary material.

0002-7863/88/1510-5911$01.50/0 © 1988 American Chemical Society