$$\underset{m/z\ 16}{NH_2^-} + CH_2O \rightarrow NH_3 + \underset{m/z\ 29}{HCO^-} \quad (2)$$

$$HCO^- + NH_3 \rightarrow CO + \underset{m/z\ 18}{NH_4^-} \quad (3)$$

of the NH_4^- ions, which indicates that in a subsequent ion/molecule reaction a hydride is transferred from HCO^- to ammonia[10] (eq 3). This is further supported by the observation that in the $ND_2^-/ND_3/CH_2O$ system mainly ND_3H^- is formed (eq 4; see Figure 1). The $ND_2H_2^-$ ion observed is due to incomplete

$$HCO^- + ND_3 \rightarrow CO + \underset{m/z\ 21}{ND_3H^-} \quad (4)$$

labeling of the ND_3 used rather than to the occurrence of a reaction analogous to the one that forms H_3O^- (eq 1). In

$$\underset{m/z\ 18}{ND_2^- + CH_2O} \rightleftharpoons [ND_2^- \cdot CH_2O]^* \rightleftharpoons$$

$$[ND_2H \cdot HCO^-]^* \rightarrow ND_2H + \underset{m/z\ 29}{HCO^-} \quad (5a)$$

$$[ND_2H \cdot HCO^-]^* \not\rightarrow ND_2H_2^- + CO \quad (5b)$$

$$m/z\ 20$$

agreement with this, NH_3D^- is formed exclusively in the $NH_2^-/NH_3/CD_2O$ system.[11]

Information concerning the structure of the NH_4^- ion can be derived from its reaction with formaldehyde:

$$\underset{m/z\ 18}{NH_4^-} + CH_2O \rightarrow NH_3 + \underset{m/z\ 31}{CH_3O^-} \quad (6)$$

The ND_3H^- ion is observed to transfer only a hydride ion, not a deuteride ion, to formaldehyde (eq 7). This is shown by ejection

$$\underset{m/z\ 21}{ND_3H^-} + CH_2O \rightarrow ND_3 + \underset{m/z\ 31}{CH_3O^-} \quad (7a)$$

$$\underset{m/z\ 21}{ND_3H^-} + CH_2O \not\rightarrow ND_2H + \underset{m/z\ 32}{CH_2DO^-} \quad (7b)$$

of the ND_3H^- ions, which results in a partial decrease of the abundance of the CH_3O^- ions.[12] Conversely, the NH_3D^- ion only transfers the D^- ion to formaldehyde.

These observations show that the hydrogen and deuterium atoms in the ND_3H^- and NH_3D^- ions do not become equivalent. The observed ions can therefore best be described as a hydride ion solvated by an ammonia molecule: $H^- \cdot NH_3$. This is in agreement with recent ab initio molecular orbital calculations at the 4-31++G level on NH_4^-, which indicate that the $H^- \cdot NH_3$ structure is more stable than the $H_2 \cdot NH_2^-$ structure[3].

It is possible to bracket the heat of formation of NH_4^- on the basis of the observed reactions. An upper limit of about +85 kJ·mol^{-1} can be calculated from eq 3,[13,14] and a lower limit of −80 kJ·mol^{-1} can be derived from eq 6.[13,15] The calculated[3] heat of formation of the $H^- \cdot NH_3$ structure is about +85 kJ·mol^{-1}, in agreement with the present experiments.

The HCO^- ion has been observed to transfer a hydride ion to methylamine and ethylamine as well (eq 8). No hydride transfer

$$HCO^- + RNH_2 \rightarrow CO + RNH_3^- \quad (8)$$

$$R = H, CH_3, C_2H_5$$

to dimethylamine has been observed, probably due to the competition of a fast proton-transfer reaction leading to $(CH_3)_2N^-$.

Acknowledgment. We thank Prof. R. R. Squires (Purdue University, IN) and Prof. P. v. R. Schleyer (Universität Erlangen-Nürnberg, West Germany) for stimulating discussions and the Netherlands Organization for Pure Research (SON/ZWO) for financial support. J.E.J. also thanks the Ministry of Education of the Netherlands and the Foundation of the University of Turku for a travel grant.

Registry No. NH_4^-, 12325-21-2; HCO^-, 57340-31-5; ND_3H^-, 84809-69-8; ammonia, 7664-41-7; methylamine, 74-89-5; ethylamine, 75-04-7.

(9) Comisarow, M. B.; Grassi, V.; Parisod, G. *Chem. Phys. Lett.* **1978**, *57*, 413–416.

(10) For other examples of gas-phase hydride-transfer reactions, see: (a) Murphy, M. K.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1976**, *98*, 1433–1440. (b) DePuy, C. H.; Bierbaum, V. M.; Schmitt, R. J.; Shapiro, R. H. *Ibid.* **1978**, *100*, 2920–2922. (c) Schmitt, R. J.; Bierbaum, V. M.; DePuy, C. H. *Ibid.* **1979**, *101*, 6443–6445. (d) Bartmess, J. E. *Ibid.* **1980**, *102*, 2483–2484.

(11) CD_2O was generated by depolymerization of perdeuteroparaformaldehyde (purchased from Merck).

(12) The CH_3O^- ions are also formed by a hydride ion transfer from HCO^- to formaldehyde; see ref 4.

(13) Heats of formation of neutral molecules have been taken from the following: Rosenstock, H. M.; Draxl, K.; Steiner, B. W.; Herron, J. T. *J. Phys. Chem. Ref. Data* **1977**, *6*, Suppl. 1.

(14) The heat of formation of HCO^- has been taken from the value calculated by the following: Chandrasekhar, J.; Andrade, J. G.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1981**, *103*, 5612–5614.

(15) The heat of formation of CH_3O^- has been taken from the following: Bartmess, J. E.; McIver, R. T., Jr. In "Gas Phase Ion Chemistry"; Bowers, M. T., Ed.; Academic Press: New York, 1979; Vol. 2, Chapter 11, pp 87–121.

Double Isotope Fractionation: Test for Concertedness and for Transition-State Dominance

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Most chemical transformations involve the making and breaking of more than one bond, and the question of the timing of these events arises frequently. We report here a possible solution to this problem, in which the isotopic fractionation at one site (e.g., the bond-making site) is measured as a function of the isotope at the other (e.g., the bond-breaking site). This approach, a "double fractionation" experiment, can be used not only to test for concertedness but also to discover whether the isotopically sensitive transition state(s) are fully rate determining. The experiment is general, provided that substitution at one site (e.g., of D for H) can be specified while the fractionation at the other site (e.g., of H vs. D, ^{12}C vs. ^{13}C, or ^{16}O vs. ^{18}O) is measured.

We illustrate the method with the enzyme proline racemase,[1] which proceeds by a "two-base" mechanism (Figures 1 and 2).[2] From experiments with D-[2-^{2}H]- and L-[2-^{2}H]proline,[2,3] it is known that the transition state(s) for substrate interconversion involve kinetically significant proton motion at both protonic sites and that both protons are sequestered on their sites until the proline is released.[4] Let us first assume that the reaction is stepwise (Figure 1). In the first step the C-2 proton of D-proline (H″) is abstracted to give a carbanionic intermediate, and in the second step L-proline is formed by delivery of a solvent-derived proton (H′) to this carbanion. If the reaction is run in mixed H_2O–D_2O then because transition-state 2 is kinetically significant, there will be discrimination against deuterium, and the product L-proline will have a lower deuterium content than the solvent. The product

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§ National Science Foundation Predoctoral Fellow.

(1) Belasco, J. G. Ph.D. Thesis, Harvard University, 1980. The enzyme was purified to homogeneity by Dr. L. M. Fisher, from *Clostridium sticklandii*.[2]

(2) (a) Cardinale, G. J.; Abeles, R. H. *Biochemistry* **1968**, *7*, 3970–3978. (b) Rudnick, G.; Abeles, R. H. *Ibid.* **1975**, *14*, 4515–4522.

(3) L. M. Fisher, unpublished experiments.

(4) The observed deuterium isotope effects on k_{cat}/K_m are 3.2 (for L-[2-^{2}H]proline) and 2.7 (for D-[2-^{2}H]proline).

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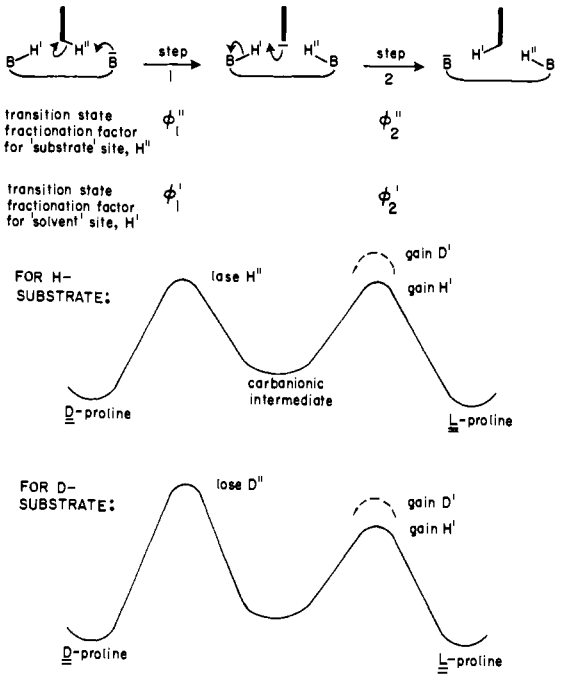

Figure 1. Stepwise pathway for the reaction catalyzed by proline racemase. The consequence of deuterium substitution in the substrate (step 1) and of deuterium rather than proton delievery (step 2) are shown as transition-state differences simply for illustration. All species are enzyme bound. The proline ring is represented, edge on, by the vertical bar. The two enzymic bases are designated by B.

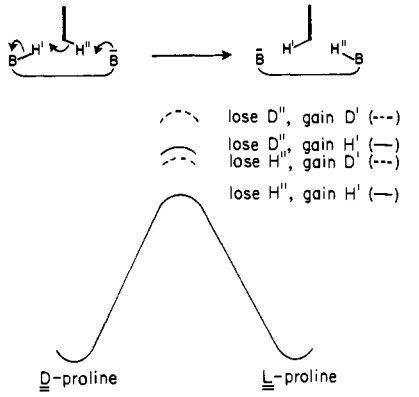

Figure 2. Concerted pathway for the reaction catalyzed by proline racemase. The energy barriers are drawn for a case where the fractionation factors for the two protonic sites are slightly different. For other details, see Figure 1 legend.

deuterium content provides the mixed transition-state fractionation factor, $\phi'_{1,2}$.[5,6] Now, if the reaction is repeated in the same solvent[7] but starting with *deuterated* D-[2-^{2}H]proline, the abstraction step (step 1) will be selectively slowed by the deuterium substitution ($\phi''_1 < 1$), and the proton-delivery step (step 2, in which the discrimination against solvent-derived deuterium is manifest) will be made less rate limiting (see Figure 1). The mixed fractionation factor ($\phi'_{1,2}$) *will therefore rise*[8] to a value nearer to that for the solvent-derived proton at rest in transition-state 1, i.e., toward ϕ'_1.

If, however, the two proton transfers are synchronous (Figure 2), then provided that multiple sites of proton transfer in a single transition state behave independently with respect to isotopic substitution,[9] the observed fractionation factor for the solvent-derived site *will be the same* whatever the isotope at the other site. That is, the deuterium content of the product will be the same whether the substrate was deuterated or not.

We have assumed above that the interconversion of the two enzyme–proline complexes substrate rate limiting, but what if the proline "on–off" steps are kinetically significant? For the stepwise reaction, the mixed fractionation factors will then be modulated by the (now significant) on–off transition state(s), but for the same reasons as before, the mixed factor for the deuterated substrate will still be greater than that for the protonated substrate. For the concerted process, however, use of deuterated substrate will make the catalytic step more cleanly rate limiting, and the mixed fractionation factor *will fall*[8] (i.e., the discrimination against solvent deuterium will increase) toward the unmixed value for a proton in flight.

The relative magnitudes of the mixed fractionation factors observed with the protonated substrate $(\phi'_{1,2})_{H''}$ and the deuterated substrate $(\phi'_{1,2})_{D''}$ can be written

$$\zeta = \frac{(\phi'_{1,2})_{H''}}{(\phi'_{1,2})_{D''}} - 1 = \frac{(\phi''_1 - \phi''_2)(\phi'_1 - \phi'_2)}{(\kappa\phi''_2 + \phi''_1)(\phi'_2 + \kappa^{-1}\phi'_1)} \quad (1)$$

If $(\phi'_{1,2})_{D''}$ is greater than $(\phi'_{1,2})_{H''}$ [$\zeta < 0$], the reaction is stepwise. If $(\phi'_{1,2})_{D''}$ is less than or equal to $(\phi'_{1,2})_{H''}$ [$\zeta \geq 0$], either the reaction is concerted or the reaction is stepwise with equal fractionation factors in transition-states 1 and 2 for at least one of the sites (i.e., $\phi'_1 \approx \phi'_2$ and/or $\phi''_1 \approx \phi''_2$) (see below). Moreover, $\zeta = 0$ implies that the catalytic transition state(s) are cleanly dominant, whereas $\zeta > 0$ indicates that the on–off transition state(s) are also kinetically significant.

The double isotope fractionation experiment has been applied[1] to proline racemase using D-[2-^{1}H]- and D-[2-^{2}H]proline in D_2O:H_2O (3:1, v/v),[10] and the observed mixed fractionation factors are 0.37 ± 0.01 and 0.38 ± 0.01, respectively, whence $\zeta = -0.03 \pm 0.05$. That is, *deuterium substitution on the substrate site (H'') does not affect the isotopic discrimination at the solvent site (H')*. Were the transition-state fractionation factors for the protons at rest on the enzyme's catalytic groups (B^- and BH, in Figures 1 and 2) to be near unity ($\phi'_1 \approx \phi''_2 \approx 1$), a value for ζ of −0.35 would be predicted for the stepwise mechanism.[11] This is clearly outside the experimental limits for ζ, and a stepwise reaction where B^- and BH are oxygen or nitrogen centers is thereby ruled out. However, if, as Rudnick and Abeles[2b] have suggested, the two enzyme functionalities are thiols, then $\phi'_1 \approx \phi''_2 \approx 0.5$,[12] and ζ would be −0.06, which *is* consistent with our experimental findings.

It is therefore evident that the proline racemase reaction either is concerted or, if stepwise, must involve catalytic groups (e.g., thiols) having fractionation factors near 0.5. A method for determining these factors and thereby evaluating the alternatives will be reported in due course.

Acknowledgment. We are greatly indebted to Dr. L. M. Fisher for the evaluation of many of the quantitative aspects of proline

(5) The mixed fractionation factor $\phi_{1,2}$ for two transition states with fractionation factors ϕ_1 and ϕ_2 is $\phi_{1,2} = (1 + \kappa)/(\phi_1^{-1} + \kappa\phi_2^{-1})$, where κ is the partition ratio of the intermediate, k_{-1}/k_2.

(6) Indeed, such a solvent discrimination experiment provides information *only* about the transition state: the discrimination is independent of ground-state fractionation.

(7) Medium effects are avoided by performing both reactions in the same solvent.

(8) Assuming for the moment that the fractionation factors for *bound* protons (i.e., ϕ'_1 and ϕ''_2) are near unity.

(9) Kresge, A. J. *Pure Appl. Chem.* **1964**, *8*, 243–258. Hegarty, A. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1975**, *97*, 7188–7189. Nevertheless, there may be evidence, at least in some instances, for interaction between protonic sites in a single transition state (W. W. Cleland, personal communication).

(10) In 200 mM NH_4HCO_3 buffer, pH 8.0, 30 °C. In this buffer, the reaction rate is limited by the substrate-handling steps and not by the interconversion of the two forms of unliganded enzyme.[2,3] The reaction was quenched when the optical rotation had fallen to 70% of its initial value, the remaining D enantiomer was removed with D-amino acid oxidase, and the L-proline was esterified with methanol/thionyl chloride and acylated with *N*-(trifluoroacetyl)-L-prolyl chloride. The deuterium content of the diastereoisomeric derivative *N*-(trifluoroacetyl)-L-prolyl-L-proline methyl ester was determined by mass spectrometry after purification by preparative gas chromatography.

(11) This value is calculated from the known discrimination against L-[2-^{2}H]proline and D-[2-^{2}H]proline[4] and does not require any assumptions as to the magnitude of κ.[1]

(12) Schowen R. L. In "Isotope Effects on Enzyme-Catalyzed Reactions"; Cleland, W. W., O'Leary, M. H., Northrop, D. B., Eds.; University Park Press: Baltimore, 1977; pp 64–99. Szawelski, R. J.; Wharton, C. W.; White, S. *Biochem. Soc. Trans.* **1982**, *10*, 232–233.

racemase catalysis that made this application of the double isotope fractionation experiment possible. We are also grateful to Dr. W. W. Cleland, who has independently devised and used the double fractionation method, for communicating his work[13] prior to publication.

Registry No. Proline racemase, 9024-09-3.

(13) Hermes, J. D.; Roeske, C. A.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1982**, *21*, 5106–5114.

Stereoselectivity of Intramolecular Dicobalt Octacarbonyl Alkene–Alkyne Cyclizations: Short Synthesis of *dl*-Coriolin

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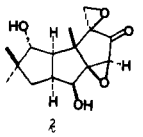
Almost a decade ago Pauson[1] reported the potentially very useful reaction of a strained alkene with an alkyne–dicobalt octacarbonyl complex, to give cyclopentenones, albeit in modest yield.[2] The cyclopentenone annulation is regiospecific; the larger group becomes adjacent to the carbonyl group. An intramolecular version of this reaction has recently been described by Schore,[3] the yield of bicyl[3.3.0]oct-2-en-3-one (**1**) was 30% (Scheme I).

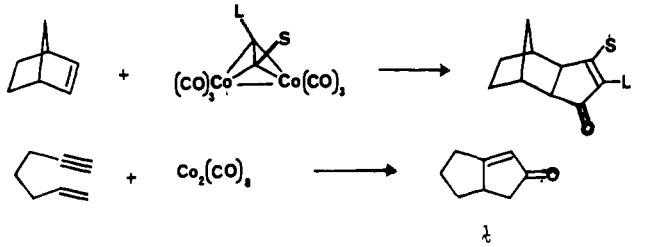
If this reaction is to be of use for the synthesis of natural products, it is essential that it be stereoselective. To date there have been no studies concerned with this point. Furthermore the compatibility of this cyclization with a propargyl functionality is by no means certain.

Concurrent to the above investigations, we have examined the stereoselectivity of intramolecular alkene–alkyne dicobalt octacarbonyl mediated cyclopentenone cyclizations. Here we report a stereoselective synthesis of functionalized bicyclo[3.3.0]enones and illustrate the inherent simplicity of this highly convergent methodology with a concise synthesis of the antitumor sesquiterpene coriolin **2**.[4]



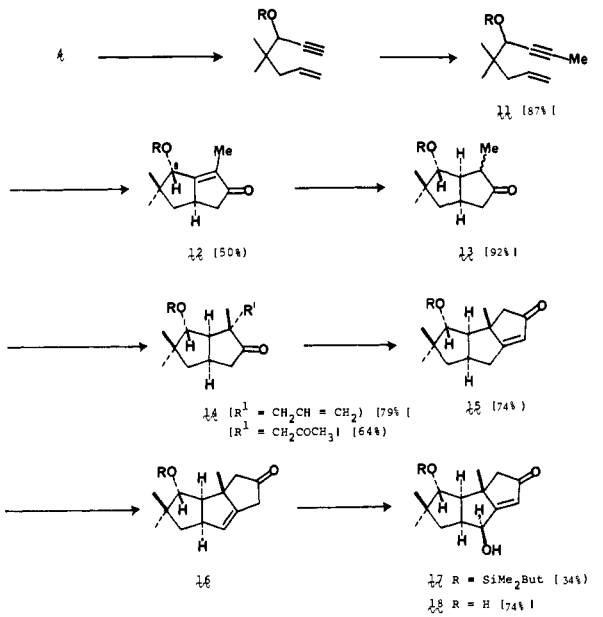
2

Scheme I



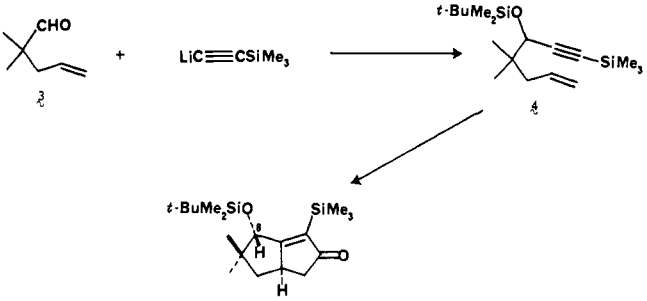
1

Scheme II[a]



[a] R = $SiMe_2$-*t*-Bu

Almost without exception, ring-forming processes benefit from the classical Thorpe–Ingold effect.[5] Consequently, in both conception and reality, it proved useful to exclude unsubstituted model studies. The aldehyde **3**[6] was treated with lithio(trimethylsilyl)acetylene followed by quenching by ClSi-*t*-$BuMe_2$/THF/reflux 20 h to give **4** (86%, bp 96–98 °C (0.9 mmHg)). When the enyne **4** was heated with $Co_2(CO)_8$ (1.0 equiv) in heptane (saturated with CO) at 110 °C (sealed tube), the bicyclo[3.3.0]enone **5** was isolated in 79% yield after chromatography and distillation (bp 128 °C (0.5 mmHg)), along with 3% of the C-8 epimer (26:1).



The proof of the stereochemistry depicted in **5** rests upon the following chemical and physical evidence. Hydrogenation of **5** (10% Pd/C) gave **6** (90%), which upon treatment with HBF_4/THF/H_2O gave the keto alcohol **7** (87%, mp 70.5–71.5 °C). The

(1) Khand, I. U.; Knox, G. R.; Pauson, P. L.; Watts, W. E.; Foreman, M. I. *J. Chem. Soc., Perkin Trans. 1* **1973**, 977. Khand, I. U.; Pausen, P. L. *Ibid.* **1976**, 30. Pausen, P. L.; Khand, I. U. *Ann. N.Y. Acad. Sci.* **1977**, *295*, 2.

(2) Unstrained alkenes require severe conditions, and the yields of cyclopentenones are low, ca. 10–20%: Bladon, P.; Khand, I. U.; Pauson, P. L. *J. Chem. Res. Miniprint* **1977**, 0146. Khand, I. U.; Pauson, P. L. *Ibid.* **1980**, 3501; *J. Chem. Res. Synops.* **1980**, 277. For a general review of the use of organocobalt chemistry in synthesis see: Nicholas, K. M.; Nestle, M. O.; Seyferth, D. "Transition Metal Organometallics in Organic Synthesis"; Alper, H., Ed.; Academic Press: New York, 1978; Vol. II.

(3) Schore, N. E.; Croudace, M. C. *J. Org. Chem.* **1981**, *46*, 5436. Croudace, M. C.; Schore, N. E. *Ibid.* **1981**, *46*, 5357.

(4) For a complete account of the complexities of the problems involved in the synthesis of coriolin see: Danishefsky, S.; Zamboni, R.; Kahn, M.; Etheredge, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 3460. Tatsuta, K.; Akimoto, K.; Kimoshita, M. *J. Antibiot.* **1980**, *33*, 100. Shibasaki, M.; Iseki, K.; Ikegami, S. *Synth. Commun.* **1980**, *10*, 551. Shibasaki, M.; Iseke, K.; Ikegami, S. *Tetrahedron Lett.* **1980**, *21*, 3587. For the synthesis of **18** and its subsequent conversion into coriolin see: Trost, B. M.; Curran, D. P. *J. Am. Chem. Soc.* **1981**, *103*, 7380. The enone **18** has also recently been described: Ito, T.; Tomiyoshi, M.; Nakamura, K.; Azuma, S.; Izawa, M.; Maruyama, F.; Yanagiya, M.; Shirahama, H.; Matsumoto, T. *Tetrahedron Lett* **1982**, *23*, 1721. Mehta, G.; Reddy, A. V.; Murthy, A. N.; Reddy, D. S. *J. Chem. Soc., Chem. Commun.* **1982**, 540. For a collection of references in this area see: Schuda, P. F.; Ammon, H. L.; Heimann, M. R.; Bhattacharjee, S. *J. Org. Chem.* **1982**, *47*, 3434.

(5) DeTar, D. F.; Luthra, N. P. *J. Am. Chem. Soc.* **1980**, *102*, 4505. Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 208. Eliel, E. L. "Stereochemistry of Carbon Compounds"; McGraw-Hill: New York, 1962; pp 106–202.

(6) Magnus, P.; Nobbs, M. *Synth. Commun.* **1980**, *10*, 273.

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