tween the two aldehyde groups. Mild oxidation of the free aldehyde with Tollens' reagent and reduction with $NaBH_4$ gave acid 10, which was lactonized by treatment with toluenesulfonic acid and oxidized under Swern conditions[17] to give the pinacol cyclization substrate 3.

Slow addition of keto aldehyde 3 in dimethoxyethane (DME) at room temperature to a stirred slurry prepared by reduction of $TiCl_3(DME)_{1.5}$[18] with Zn–Cu gave a mixture of four cyclic diols (10:9:5:<1) in a combined yield of 48%. The major diol (20% yield) was readily identified as 11, a substance previously prepared by Marshall[19,20] and epimeric with crassin at C3 and C4. The minor diol (<1% yield) was identified as 2, the substance necessary for conversion into crassin, and the other two diols (18% and 10% yields) were identified as the C4 and C3 epimers[21] of 2, respectively. Thus, the coupling does in fact proceed without destroying the lactone ring, but the wrong stereoisomers are produced.

Fortunately, we were able to solve the stereochemical problem easily by carrying out a double inversion, 11 → 2. Treatment of the major diol product (11) with methanesulfonyl chloride and benzyltrimethylammonium hydroxide gave the known[9] epoxide 12, epimeric with crassin at C4, and mild treatment with aqueous acid opened the epoxide with selective inversion at the tertiary center to provide 2. Isomerization of butyrolactone 2 gave 15, and methylenation then proceeded without incident to give (±)-crassin, mp 174–176 °C, spectroscopically identical with an authentic sample prepared from natural crassin acetate.

We believe that this synthesis is a first step in opening an important new direction for titanium-induced carbonyl-coupling reactions, making possible the synthesis of complex, highly oxygenated macrocycles.

Supplementary Material Available: Experimental details on the preparation and characterization of all synthetic intermediates (10 pages). Ordering information is given on any current masthead page.

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(20) We thank Professor James Marshall, University of South Carolina, for invaluable help in providing copies of the ^{1}H NMR spectra of numerous synthetic cembranoid lactones.

(21) The C4 epimer was identified by X-ray crystallography of the related δ lactone.

Structures of Ciguatoxin and Its Congener

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Ciguatoxin (CTX) is a toxic principle of ciguatera, one of the largest scale food poisonings of nonmicrobial origins, which results from eating coral reef fish. Despite the great deal of effort made by Scheuer's group of the University of Hawaii,[1,2] extreme difficulties in collecting toxic fish coupled with the complexity of
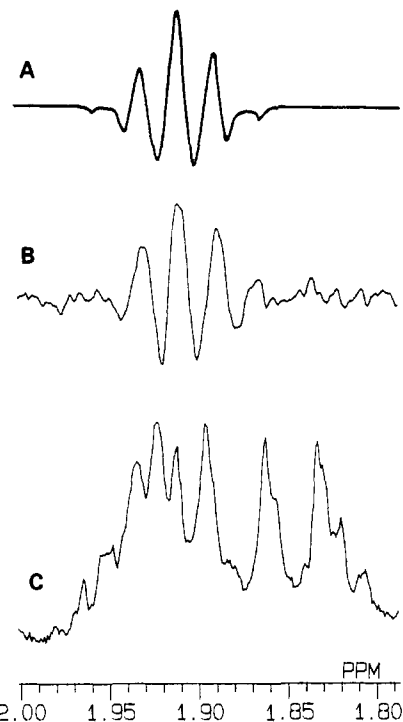
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Figure 1. Simulation of decoupling difference ^{1}H NMR spectrum (A) of H-39 of 2. The difference spectrum (B) was determined by subtracting a nondecoupling spectrum (C) (pyridine-d_5, 25 °C) from a decoupling one with irradiation at δ 0.917 (Me-57). Simulation was done as to an eight-spin system due to H_2-38/H-39(Me-57)/H_2-40 using chemical shifts obtained from cross peaks on COSY and coupling constants of H-39 (qt, 7.7, 8.4 Hz).

the CTX molecule have hampered chemical studies. Its structure has thus become one of the most challenging targets among natural-product chemists. In the previous reports, we have presented a molecular formula, $C_{60}H_{86}O_{19}$, and a partial structure (C1–C22) of the toxin, including the stereochemistry.[3,4] We report here the elucidation of the rest of the molecule (C23–C60), resulting in the determination of the planar structure of the whole molecule.

CTX (0.35 mg) was extracted from the moray eel, *Gymnothorax javanicus*, as reported previously.[3] A less polar congener 2 (0.74 mg) was obtained from the causative epiphytic dinoflagellate, *Gambierdiscus toxicus*, collected in the Gambier Islands. Its HR-FABMS suggested a probable molecular formula of $C_{60}H_{84}O_{16}$ (MH^+, m/z 1061.584; found, 1061.587). ^{1}H NMR chemical shifts and coupling constants of 2 clearly showed 2 to be identical with 1 except for both terminal parts of the molecule.[5-7] Thus the structure elucidation of the common part will be discussed according to the data obtained on 2. The same set of ^{1}H NMR measurements were also made as on 1.

In ^{1}H NMR spectra of 1 or 2 measured at 25 °C, signals due to H-22 through H_2-31 were extremely broadened or missing, probably because of slow conformational perturbation of ring F, as observed with brevetoxin A.[8] The problem was solved by measurements at low temperatures (−20 or −25 °C) in which missing signals appeared and broad signals sharpened. Since we could match each proton with a single signal, which had a chemical shift close to that of the broad signal observed at 25 °C, rings F and G were presumed to take a single conformation at the low temperatures.

The proton connectivities including hydroxy protons were mainly established by ^{1}H-^{1}H COSY data obtained under various con-

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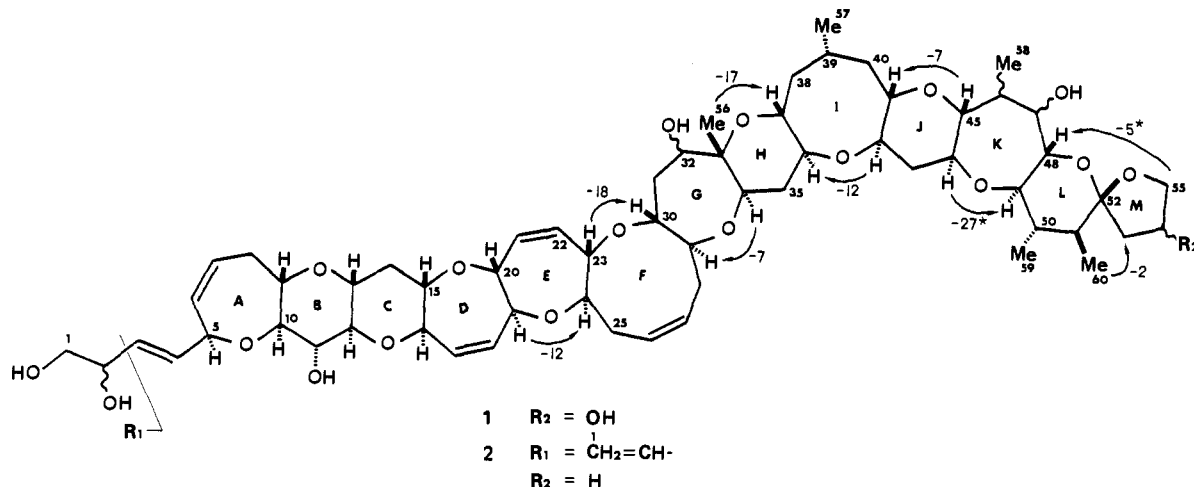
Figure 2. Relative configurations and NOEs of ciguatoxin (**1**) and its analogue **2**. The figures denote NOEs in percentages obtained from NOE difference spectra measured in CD_3CN at -25 °C (400 MHz) except for those with asterisks, which were obtained in pyridine-d_5 at -25 °C. The latter were shown to be approximately 3 times larger negative values than the former, presumably owing to the viscosity of the solvents.

ditions.[9]   The COSY measured at low temperatures clearly revealed connectivities of C1–C32, C34–C38, C40–C51, and C53–C55 but left the skeletal chain in three fragments due to the presence of two quaternary carbons (C33 and C52) and an unassignable methine (C39). As close chemical shifts of H_2-38, H-39, and H_2-40 deterred assignments of their couplings, ^{1}H NMR simulation was carried out for an eight-spin system due to H_2-38/H-39(Me-57)/H_2-40. The simulated spectrum agreed well with a decoupling difference spectrum observed upon irradiation at Me-57 (Figure 1), It was thus revealed that H-39 was

coupled with H_2-38 and H_2-40 with $^3J_{H,H}$ of 8.4, ca. 0 Hz and 8.4, ca. 0 Hz, respectively, as was the 5-axial proton of oxocane in a crown conformer.[10]

Since the sample size was not enough for the measurements of HMBC, which would detect $^2J_{C,H}$ and $^3J_{C,H}$, the connectivities around C33 and C52 were clarified chiefly on the basis of coupling constants and NOEs (Figure 2). Judging from the chemical shifts and $^3J_{H,H}$,[5] H-34 and H-37 were assignable to axial protons of tetrahydropyran. A large NOE due to H-37/Me-56 and a coupling pattern of H-34 (dd, 12.2 and 4.2 Hz) indicated that the methyl was axially substituted on the juncture carbon (C33). Double quantum filter COSY (DQF-COSY) clarified another group neighboring on C33 by giving a cross peak due to a long-range coupling between H-32 and Me-56. ^{13}C NMR spectra of **2**[11] gave a signal at δ 109.2, which was a typical value for a ketal carbon of spirofused five/six-membered rings.[12]   The orientation of C53 on ring L was assigned to be equatorial on the basis of NOEs, which were prominently observed on Me-60/H-53 and on H-48/H-55 (Figure 2) of both **1** and **2**, while no NOE was detected on H-48/H-53, which should come close to each other if C53 were axially substituted.

Ether linkages were elucidated by NOEs observed on protons or a methyl (C56) attached to ring-juncture oxy carbons (Figure 2). All of the ring fusions are trans because coupling constants of angular protons are typical for an antiperiplanar substitution on oxy carbons,[5,6]   The diequatorial orientations of Me-59 and Me-60 were indicated by a decoupling difference experiment in which $^3J_{50-51}$ (11.2 Hz) was typical for 1,2-diaxial protons of cyclohexane,

Structural alterations of **2** from **1** were readily determined on the basis of 2D NMR data, which showed the presence of a *trans*-butadiene moiety and deoxidation at C54 in **2**.[5,7]

The stereochemistry of C2, C32, C46, C47, and C54 was not known chiefly because of ambiguity in the conformation of seven- or five-membered rings, Nevertheless, the planar structure and the fusing manner of all rings of CTX have been elucidated for the first time in this study.

(5) ^{1}H NMR spectrum of **2** at 25 °C (400 MHz, pyridine-d_5): δ 7.34 (1 H, d, 2 Hz, 11-OH), 6.76 (1 H, d, 3 Hz, 47-OH), 6.49 (1 H, dd, 15, 11 Hz, H-3), 6.35 (1 H, dt, 16, 10 Hz, H-2), 5.90 (1 H, dd, 15, 5 Hz, H-4), 5.89 (1 H, m, H-18), 5.86 (1 H, m, H-6), 5.81 (1 H, m, H-7), 5.74 (1 H, dt, 13, 2 Hz, H-17), 5.67 (1 H, dt, 13, 2 Hz, H-21), 5.29 (1 H, d, 1 Hz, 32-OH), 5.11 (1 H, dd, 16, 2 Hz, H-1), 5.04 (1 H, dd, 10, 2 Hz, H-1′), 4.80 (1 H, m, H-5), 4.47 (1 H, ddd, 11, 9, 5 Hz, H-44), 4.22 (1 H, br d, 9 Hz, H-20), 4.20 (1 H, dd, 3, 2 Hz, H-47), 4.16 (1 H, dt, 1, 8 Hz, H-32), 4.11 (1 H, dt, 2, 9 Hz, H-11), 4.08 (1 H, br d, 9 Hz, H-19), 4.03 (1 H, br d, 9 Hz, H-16), 4.03 (1 H, dd, 9, 1 Hz, H-48), 3.94 (1 H, t, 10 Hz, H-49), 3.87 (2 H, m, H-55, H-55′), 3.74 (1 H, t, 9, 9 Hz, H-10), 3.55 (1 H, ddd, 11, 9, 4 Hz, H-15), 3.50 (1 H, m, H-9), 3.50 (1 H, ddd, 10, 9, 4 Hz, H-37), 3.43 (1 H, t, 9 Hz, H-12), 3.36 (1 H, ddd, 12, 9, 4 Hz, H-13), 3.35 (1 H, m, H-36), 3.34 (1 H, dd, 12, 4 Hz, H-34), 3.34 (1 H, m, H-42), 3.20 (1 H, dt, 3, 10 Hz, H-41), 3.19 (1 H, dd, 9, 5 Hz, H-45), 2.73 (1 H, ddd, 15, 7, 4 Hz, H-8), 2.59 (1 H, m, H-43), 2.59 (1 H, m, H-46), 2.58 (1 H, dt, 12, 4 Hz, H-14), 2.55 (1 H, m, H-8′), 2.26 (1 H, dt, 12, 4 Hz, H-35), 2.03 (1 H, m, H-40), 1.94 (1 H, m, H-50), 1.93 (1 H, m, H-53), 1.91 (1 H, q, 12 Hz, H-35′), 1.91 (1 H, m, H-39), 1.90 (1 H, m, H-54), 1.85 (1 H, q, 12 Hz, H-14′), 1.85 (1 H, m, H-53′), 1.84 (1 H, m, H-38), 1.77 (1 H, q, 12 Hz, H-43′), 1.72 (1 H, m, H-40′), 1.68 (1 H, m, H-54′), 1.60 (1 H, qd, 7, 11 Hz, H-51), 1.54 (1 H, m, H-38′), 1.37 (3 H, s, Me-56), 1.29 (3 H, d, 8 Hz, Me-58), 1.28 (3 H, d, 6 Hz, Me-59), 0.97 (3 H, d, 7 Hz, Me-60), 0.92 (3 H, d, 8 Hz, Me-57). Assignments of H-22 through H-31 are given in footnote 6.

(6) ^{1}H NMR spectrum of **2** at -20 °C (400 MHz, pyridine-d_5): δ 6.10 (1 H, br d, 13 Hz, H-22), 6,07 (1 H, m, H-27), 6.05 (1 H, m, H-26), 4.03 (1 H, br d, 8 Hz, H-23), 3.86 (1 H, br d, 9 Hz, H-29), 3.68 (1 H, m, H-24), 3.68 (1 H, m, H-30), 3.01 (1 H, m, H-25), 2.98 (1 H, m, H-28), 2.68 (2 H, m, H-31, H-31′), 2.36 (1 H, m, H-28′), 2.20 (1 H, m, H-25′).

(7) ^{1}H NMR chemical shifts of **1** agreed with those of **2** within 0.02 ppm except for signals due to H-1 through H-7, H-48 through H_2-55, and the substituents in these parts. ^{1}H NMR spectrum of **1** at 25 °C, (400 MHz, pyridine-d_5): δ 6.67 (1 H, d, 4 Hz, 2-OH), 6.53 (1 H, d, 4 Hz, 54-OH), 6.4C (1 H, dd, 6, 4 Hz, 1-OH), 6.38 (1 H, dd, 15, 5 Hz, H-4), 6.37 (1 H, dd, 15, 5 Hz, H-3), 5.90 (1 H, ddd, 11, 3, 2 Hz, H-6), 5.77 (1 H, dddd, 11, 8, 2, 1 Hz, H-7), 4.86 (1 H, m, H-5), 4.86 (1 H, m, H-54), 4.69 (1 H, m, H-2), 4.06 (1 H, dd, 10, 1 Hz, H-48), 3.98 (2 H, m, H_2-1), 3.96 (1 H, t, 10 Hz, H-49), 2.40 (1 H, dd, 13, 8 Hz, H-53), 2.35 (1 H, dd, 13, 5 Hz, H-53′), 2.01 (1 H, m, H-50), 1.67 (1 H, m, H-51), 1.32 (3 H, d, 6 Hz, Me-59), 1.24 (3 H, d, 7 Hz, Me-60).

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(9) COSY spectra of **1** were measured in CD_3OD at -20 °C, in pyridine-d_5 both at 25 °C and at -20 °C, and in CD_3CN-D_2O (2;1) at 25 °C; those of **2** were measured in CD_3CN at -20 °C and in pyridine-d_5 both at 25 °C and at -20 °C. The spectra were recorded either on a 400-MHz spectrometer (JEOL, GSX-400) or on a 600-MHz instrument (Bruker, AM-600).

(10) MM2 calculations as to rings H, I, and J suggested that ring I took a crown conformation; a dihedral angle formed by H_{ax}-38/H-39 was calculated to be 150.3° and that formed by H_{eq}-38/H-39 to be 95.2°. According to Karplus equation with $^3J(180°)$ taken as 11.8 Hz (Muller, N.; Schultz, P. J. *J. Phys. Chem.* **1964**, *68*, 2026), $^3J(H_{ax}$-38/H-39) was shown to be 8.6 Hz, and $^3J(H_{eq}$-38/H-39) to be -0.2 Hz.

(11) Besides the signal at δ 109.2, nine prominent peaks were observed in δ 128.4–137.5, 21 peaks in 68.0–88, and 17 peaks in 13.6–46.7 in a ^{13}C NMR spectrum (100 MHz, CD_3CN, 2 × 10^5 scans).

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and to Prof. M. Hirama of Tohoku University for discussion. This work was supported by a grant-in-aid for overseas research from the Ministry of Education, Science and Culture, Japan; by the French Ministry of Research and Superior Education; by the Pasteur Institute of Paris; and by the French Polynesia Government.

Supplementary Material Available: Table of ^{1}H NMR chemical shifts and coupling constants of **1** and **2**, ^{1}H NMR spectrum and COSY map of **1**, and ^{1}H and ^{13}C NMR spectra, COSY and DQF-COSY maps, and NOE difference spectra of **2** (14 pages). Ordering information is given on any current masthead page.

Proton Inventory of a Bifunctional Ribonuclease Model

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We have described the hydrolysis of 4-*tert*-butylcatechol cyclic phosphate (**1**) catalyzed by β-cyclodextrin 6,6′-bis(imidazoles).[2–4] In our earliest work[2,3] we used mixtures of the A,C, and A,D isomers,[3] but recently we have reported[4] a study of the pure isomers and also of the A,B isomer **2**. Our pH/rate studies[2–4] showed that k_{cat} reached an optimum when the catalyst was partially protonated, so that both a basic imidazole (Im) and an acidic imidazolium (ImH[+]) group were present. We have also described a detailed kinetic study of the cleavage of RNA polymers and dimers by imidazole buffers.[5–7] Again a pH optimum showed that both Im and ImH[+] were involved, but in a bifunctional *sequential* mechanism in which the ImH[+] first protonated the substrate phosphate ion and the Im then promoted attack of a hydroxyl group, forming a phosphorane intermediate. This then cleaved in a subsequent fast step. However, we pointed out[6] that what is sequential with separate buffer species could well become simultaneous if the catalytic groups are attached, as in **2** or in the enzyme ribonuclease A for which **2** is a model.

Our finding that the A,B isomer **2** is better[4,8] than the A,C or A,D analogues supports this; in a truly sequential mechanism, the intermediate could rotate in the cyclodextrin cavity and the relative placement of the two catalytic groups should be irrelevant. However, one could imagine other factors that might favor one catalyst over the other, such as simple hydrogen bonding to phosphate without an actual proton transfer by ImH[+] in the transition state. In our mechanistic proposals,[4,6] there should be a true protonation by ImH[+] as the Im delivers H_2O to the phosphate, forming a phosphorane intermediate (Scheme I).

To check this, we have used the well-known proton inventory method,[9] studying the rate in various mixtures of H_2O and D_2O.

Scheme I
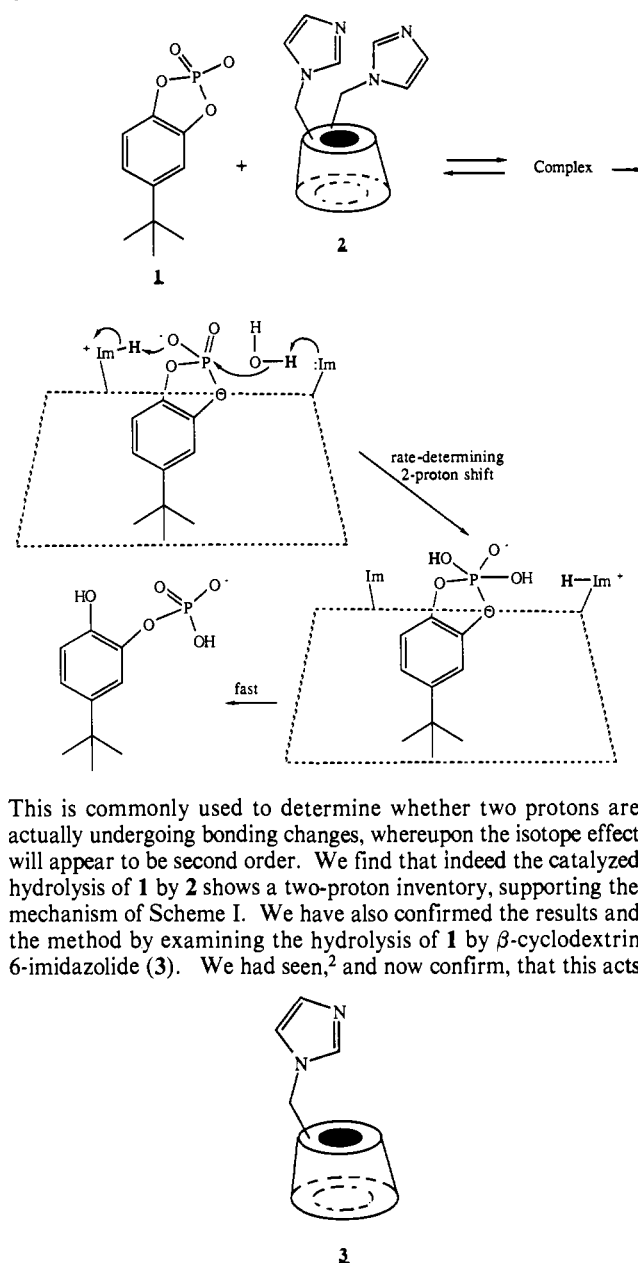
This is commonly used to determine whether two protons are actually undergoing bonding changes, whereupon the isotope effect will appear to be second order. We find that indeed the catalyzed hydrolysis of **1** by **2** shows a two-proton inventory, supporting the mechanism of Scheme I. We have also confirmed the results and the method by examining the hydrolysis of **1** by β-cyclodextrin 6-imidazolide (**3**). We had seen,[2] and now confirm, that this acts

as a simple base catalyst, Im delivering the H_2O to bound substrate. In this case the proton inventory method indicates that only one proton moves in the transition state, as expected. The proton inventory results with bifunctional **2** are similar to those observed[10] when the enzyme ribonuclease A catalyzes a similar reaction.

We have described[4] the pH/rate profile for the cleavage at 25.0 °C of **1** complexed by **2**, a bell-shaped curve with a broad maximum at pH 5.75; in D_2O,[11] a bell-shaped curve was seen with its broad maximum at a pH meter reading of 6.1 (pH 6.5 corrected for the meter isotope effect), and the rate decreased by a factor of 4.0. Thus the proton inventory study was conducted buffered[11] to a pH meter reading of 6.00 (H_2O) in 0.01 increments to 6.10 (D_2O) with nine intermediate mixtures. The substrate was at 1 mM and the catalyst at 5 mM, well in excess of the K_d of 0.18 mM[4] for the complex of **1** with **2**. The results are plotted in Figure 1. The curve shows distinct downward curvature. The points

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(8) We find $\Delta H^* = 6.9 \pm 1.2$ kcal/mol for k_{cat}, while that for cleavage of **1** by the A,C isomer of **2** is 9.2 ± 1.6, and for the A,D isomer, 9.6 ± 1.7 kcal/mol. $\Delta S^* = -49.1 \pm 6.5$ eu for **2**, and -43.9 ± 5.8 and -43.3 ± 5.7 eu for the A,C and A,D isomers, respectively. For catalyzed cleavage of **1** by *N*-methylimidazole, $\Delta H^* = 11.7 \pm 2.1$ kcal/mol and $\Delta S^* = -37.7 \pm 4.9$ eu.

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