by the increase in $\Delta S^{\ddagger}$ but the parameters for Fl(6) and Fl(7) are quite exceptional. As shown in Figure 13, four plots including Fl(Bu) give an excellent linear relationship expressed by eq 2 (r = 0.99). The slope corresponds to the isokinetic temperature (β = 333 K) for this reaction. On the other hand, plots for Fl(6) and Fl(7) deviate from eq 2 to the lower area, indicating that the transition state for these strained flavinophanes is somewhat different from those of others.

$$\Delta H^{\ddagger} = 333\Delta S^{\ddagger} + 18.1 \text{ (kcal mol}^{-1}\text{)} \quad (2)$$

Concluding Remarks. The present paper established on the basis of spectroscopic studies, X-ray structure analysis, theoretical calculations, and kinetic studies that (i) ring strain in (5-deaza)isoalloxazinophanes induced by short-strap bridging is relaxed mainly by the rotation of the C(1')-N(10) bond and slightly (if any) by deformation of the isoalloxazine plane, (ii) reactivities of (5-deaza)isoalloxazinophanes are closely related to ring strain (more strained, more reactive), and (iii) the structural change from the plane in the oxidized form to the bend in the reduced form, a characteristic of the redox reaction of (5-deaza)flavins, plays an important role in the release of steric strain. We believe that these novel structure-reactivity relationships have important implications on biochemical studies of flavoenzymes.

Acknowledgment. We thank Professor M. Ōki and Professor S. Misumi for helpful discussions. We are indebted to A. Kitamura and T. Nakane for technical assistance. This work was supported by a Grant-In-Aid from the Ministry of Education of Japan.

Supplementary Material Available: Listings of bond lengths and bond angles for dFl(6) (2 pages). Ordering information is given on any current masthead page.

Coenzyme Models. 48. Novel Diastereo-Differentiating Hydrogen Transfer and "Rope-Skipping" Racemization in Chiral Flavinophanes and 5-Deazaflavinophanes

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Abstract: Cyclic flavins and 5-deazaflavins with planar chirality [Fl(n) and dFl(n), n = 6, 7, 8, 10, and 12] and their noncyclic analogues [Fl(Bu) and dFl(Bu)] were optically resolved for the first time by a liquid chromatographic method. They did not racemize below 40 °C, but Fl(n) and dFl(n) with a long strap ($n \geq 10$) racemized invariably when they were reduced to the 1,5-dihydro forms. This novel redox-induced "rope-skipping" racemization occurs because the flat (5-deaza)isoalloxazine plane is folded along a line through N(5) [or C(5)] and N(10) like butterfly wings in the reduced forms, and thus, rope-skipping racemization is facilitated. A similar racemization took place via the formation of sulfite adducts at N(5) or C(5), indicating that the adducts also employ the folded conformation. These chiral (5-deaza)flavinophanes could oxidize optically active thiols (62.8% enantiomeric excess) and NADH model compounds (60.0% enantiomeric excess) in an asymmetric manner. When dFl(n) were reduced to $dFl_{red}(n)$, ^1H NMR gave a pair of doublets for the two C(5) protons. This indicates that the central ring in the reduced isoalloxazine employs a boat form and that the flip-flop motion is significantly suppressed by the ring structure. By use of the nuclear Overhauser effect, the two ^1H NMR peaks were assigned to axial proton (higher magnetic field) and equatorial proton (lower magnetic field). The tracer experiments using dFl(n) established for the first time that the hydrogen transfer to dFl(n) and from $dFl_{red}(n)$ occurs exclusively at the "axial" C(5) position. These novel results were found owing to unique characteristics of cyclic (5-deaza)flavins. Furthermore, the high asymmetric discrimination suggests that planar chirality is a promising approach to the design of new flavoenzyme model systems.

Flavins and NAD(P)$^+$ coenzymes are versatile redox "catalysts" in many biological systems.[3-5] Thus, biomimetic studies of these redox coenzymes have been of interest, and in particular, asymmetric reduction of substrates with carbonyl groups by optically active NADH model compounds has been widely investigated.[6,7] In contrast, very little precedent exists for asymmetric redox reactions mediated by flavins. To the best of our knowledge, there are only two examples of flavins with a chiral substituent: one possesses an asymmetric carbon substituent at N(3),[8] and the other has one at N(10).[9] Unfortunately, the optical yields attained in these chiral flavins were relatively low (less than 31% enantiomeric excess).[8,9] We therefore approached the challenge of the synthesis of new flavins with "larger" chiral frames of reference such as axial chirality.[10]

Here, we address new cyclic flavins [flavinophanes: Fl(n)] and 5-deazaflavins [5-deazaflavinophanes: dFl(n)] with n = 6, 7, 8, 10, and 12 which may show another large chiral framework, planar chirality. If the strap length is short enough to suppress the

(1) Nagasaki University.
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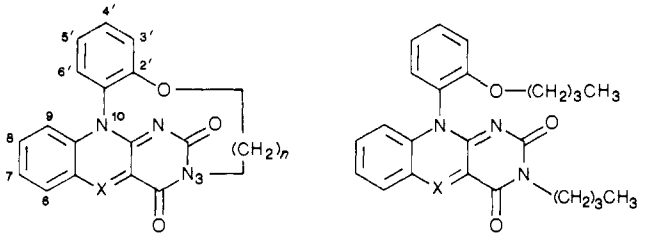
(3) (a) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256. (b) Bruice, T. C. In *Biomimetic Chemistry*; American Chemical Society: Washington, DC, 1980; p 89.
(4) (a) Walsh, C. In *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, 1979; p 358. (b) Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148.
(5) (a) Hemmerich, P.; Nagelschneider, G.; Veeger, C. *FEBS Lett.* **1970**, *8*, 69. (b) Massey, V.; Hemmerich, P. *Biochem. Soc. Trans.* **1980**, *8*, 246.
(6) The following papers have reported the high optical yields (ca. 90%): (a) Ohno, A.; Ikegami, M.; Kimura, T.; Oka, S. *J. Am. Chem. Soc.* **1979**, *101*, 7306. (b) DeVries, J. G.; Kellog, R. M. *J. Am. Chem. Soc.* **1979**, *101*, 2759. (c) Seki, M.; Baba, J.; Oda, J.; Inoue, Y. *J. Am. Chem. Soc.* **1981**, *103*, 4613. (d) Meyers, A. I.; Oppenlaender, T. *J. Am. Chem. Soc.* **1986**, *108*, 1989.
(7) For a comprehensive review see Shinkai, S. In *Enzyme Chemistry-Impact and Applications*; Suckling, C. J., Ed.; Chapman & Hall: London, 1984; p 40.
(8) Tanaka, K.; Okada, T.; Yoneda, F.; Nagamatsu, T.; Kuroda, K. *Tetrahedron Lett.* **1984**, *25*, 1741.
(9) Shinkai, S.; Nakao, H.; Tsuno, T.; Manabe, O.; Ohno, A. *J. Chem. Soc., Chem. Commun.* **1984**, 849.
(10) (a) Shinaki, S.; Nakao, H.; Manabe, O. *Tetrahedron Lett.* **1985**, *26*, 5183. (b) Shinkai, S.; Nakao, H.; Kuwahara, I.; Miyamoto, M.; Yamaguchi, T.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* **1988**, 313.

0002-7863/89/1511-4935$01.50/0

"rope-skipping" racemization, one may be able to resolve optically these racemic (5-deaza)flavinophanes. We have found that the (5-deaza)flavinophanes can be optically resolved by an HPLC method.[11,12] By use of these chiral (5-deaza)flavinophanes, several new redox properties were found for the first time. Thus, this paper reports novel examples for optical resolution and chiral recognition of (5-deaza)flavinophanes.



Experimental Section

Materials. The syntheses of Fl(n), dFl(n), and their noncyclic analogues [Fl(Bu) and dFl(Bu)] were described in an earlier publication.[13] Preparation of 1-benzyl-1,4-dihydronicotinamide (BNAH) has been described previously.[14] 4,4-Dideuterio-BNAH (BNAD) was prepared by repeated oxidation of BNAH with chloranil followed by reduction with $Na_2S_2O_4$ in D_2O.[15] The isotopic purity of BNAD determined by NMR was 96%. (R)- and (S)-N^{α}-(methylbenzyl)-1-propyl-1,4-dihydronicotinamide (MPNAH) were synthesized according to Ohno's method.[16] Among optically active thiols used herein, L-cysteine, N-acetyl-L-cysteine, L-cysteine methyl ester, and 1,4-dithio-L-threitol were commercially available from Wako Pure Chemical Co. or Merck. The syntheses of other optically active thiols were described previously.[17]

Optical Resolution. Racemic (5-deaza)flavinophanes [Fl(n) and dFl(n)] and noncyclic analogues [Fl(Bu) and dFl(Bu)] were optically resolved by an LC method using a chiral packing column (Sumipax OA-2000). The mobile phase was n-hexane–1,2-dichloroethane–ethanol (4:2:1 v/v/v). In most cases the peak separation was complete. We separated the eluent into three fractions and in every case obtained (+) isomers from the first fraction and (−) isomers from the last fraction. We could recover about 40 mg of the (+) isomers and 25 mg of the (−) isomers from 100 mg of racemic compounds. The inferior recovery of (−) isomers is due to "tailing" of the (+) isomers.

Product Analysis for Redox-Induced Racemization. Fl(n) and dFl(n) were once converted to the reduced forms, which were then reoxidized to the oxidized forms. The reduction was carried out in an anaerobic aqueous solution (pH 8.6 with 0.10 M borate) at 30 °C with a Thunberg cuvette, and the progress of the reduction was monitored spectrophotometrically. The reoxidation of $Fl_{red}(n)$ was readily attained by introducing a dioxygen stream into the cuvette. On the other hand, reduced 5-deazaisoalloxazines $dFl_{red}(n)$, which were less sensitive to dioxygen, were reoxidized by potassium ferricyanide ($[K_3Fe(CN)_6]/[dFl_{red}(n)]$ = 4). The aqueous solutions were extracted with chloroform. Products Fl(n) and dFl(n) were recovered almost quantitatively in the chloroform layers. The solutions were subject to HPLC analysis using the chiral packing column (Sumipax OA-2000).

Kinetic Measurements. The rate constants for thermal racemization of Fl(n) and dFl(n) were estimated by the sampling method at 80–140 °C. The reaction was carried out in a flask immersed in a thermostated oil bath. An aliquot was withdrawn at regular intervals from the solution and analyzed by HPLC.

Oxidations of optically active thiols by Fl(n) were carried out anaerobically at 30 °C (water–methanol, 1:2 v/v). The pseudo-first-order rate constants were determined by monitoring the disappearance of the absorption band (445 nm) of Fl(n). The reactions of (R)- or (S)-MPNAH with Fl(n) and dFl(n) were carried out anaerobically at 30 °C in acetonitrile. The progress of the reaction was monitored by the disappearance of the absorption bands of Fl(n) and dFl(n). The reactions satisfied the first-order equation for up to two half-lives. The pseudo-first-order rate constants thus obtained were first order in MPNAH. Therefore, asymmetric discrimination for these reactions was evaluated with the second-order rate constants (k_2).

Table I. Optical Resolution of Fl(n) and dFl(n) by HPLC

(5-deaza)flavin	optical purity/%[a] (+) isomer	optical purity/%[a] (−) isomer	$[\alpha]^{25}_D$ of (+) isomer[b]
Fl(7)	100	100	500
Fl(8)	100	99.6	388
Fl(10)	100	99.6	329
Fl(12)	98.0	96.7	291
Fl(Bu)	99.4	98.3	311
dFl(6)	100	100	196
dFl(7)	100	100	302
dFl(8)	100	97.5	303
dFl(10)	99.6	97.9	236
dFl(12)	99.1	99.5	221
dFl(Bu)	98.5	97.0	194

[a] This denotes the content of each isomer determined by HPLC. [b] C = (2–5) × 10^{-2} g dl^{-1}, methanol.

Table II. Activation Parameters for Thermal Isomerization of Fl(n) and dFl(n)[a]

(5-deaza)flavin	k_{373}/s^{-1}	$\Delta G^{\ddagger}_{373}$/kcal mol^{-1}	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta S^{\ddagger}$/cal mol^{-1} deg^{-1}
Fl(10)	7.02×10^{-7}	32.5	28.6	−10.3
Fl(Bu)	4.64×10^{-6}	31.1	19.6	−30.7
dFl(10)	6.90×10^{-5}	29.1	18.2	−29.3
dFl(Bu)	1.36×10^{-5}	30.3	9.36	−56.1

[a] Solvent N,N-dimethylformamide; temperature 80–140 °C.

Results and Discussion

Optical Resolution of Fl(n) and dFl(n). The results are summarized in Table I. It is seen from Table I that (+) isomers obtained from the first fraction have optical purities of 98–100%. In cases where the peak separation was complete, we could recover 40–45 mg of the (+) isomers with 100% optical purity from 100 mg of the racemic compounds. On the other hand, optical purities of the (−) isomers were inferior to those of the (+) isomers because of "tailing" of the (+) isomers. Table I also shows that these (5-deaza)flavinophanes have relatively large $[\alpha]_D$ values.

Thermal and "Redox-Induced" Racemization. As a prelude to redox-induced racemization, we investigated thermal racemization of the oxidized forms. First, we confirmed that neither cyclic Fl(n) and dFl(n) nor noncyclic Fl(Bu) and dFl(Bu) racemize at room temperature (below 40 °C). We thus measured the rate of racemization at high temperature (80–140 °C) and estimated (when racemization occurred) their activation parameters. The results are summarized in Table II. Fl(n) and dFl(n) with $n \leq 8$ did not racemize even at this temperature range, whereas those with $n \geq 10$ racemized slowly. This indicates that at the high temperature the strap in Fl(n) and dFl(n) with $n \geq 10$ is long enough to racemize in a "rope-skipping" manner but that in Fl(n) and dFl(n) with $n \leq 8$ is too short to skip over the 2-carbonyl group. Thus, the critical length for thermal racemization is n = 9. This conclusion is in accord with prediction based on the Corey–Pauling–Koltun (CPK) molecular models.

Examination of Table II reveals that the rate constants for Fl(10) and dFl(10) are not so different from those for noncyclic Fl(Bu) and dFl(Bu), which are simple atropisomers.[10] Apparently, racemization of Fl(10) and dFl(10) is not subject to a specific mechanism arising from the cyclic structure. However, the careful inspection of the activation parameters suggests that Fl(10) and dFl(10) have $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ greater than Fl(Bu) and dFl(Bu) (the differences are 8.8–9.0 kcal mol^{-1} in $\Delta H^{\ddagger}$ and 20.4–26.8 kcal mol^{-1} deg^{-1} in $\Delta S^{\ddagger}$). This means that in Fl(10) and dFl(10) the unfavorable $\Delta H^{\ddagger}$ increase is compensated by the favorable $\Delta S^{\ddagger}$

(11) Shinkai, S.; Yamaguchi, T.; Nakao, H.; Manabe, O. *Tetrahedron Lett.* **1986**, *27*, 1611.

(12) The synthesis of flavinophanes have also been reported in the following papers, but there is no precedent for optical resolution and asymmetric recognition: (a) Zipplies, M. F.; Krieger, C.; Staab, H. A. *Tetrahedron Lett.* **1983**, *24*, 1925. (b) Seward, E.; Diederich, F. *Tetrahedron Lett.* **1987**, *28*, 5111.

(13) Shinkai, S.; Kawase, A.; Yamaguchi, T.; Manabe, O.; Wada, Y.; Yoneda, F.; Ohta, Y.; Nishimoto, K. *J. Am. Chem. Soc.*, preceding paper in this issue.

(14) Shinkai, S.; Yamada, S.; Kunitake, T. *Macromolecules* **1978**, *11*, 65.

(15) Caughey, W. S.; Schellenberg, K. A. *J. Org. Chem.* **1966**, *31*, 1978.

(16) Ohnishi, Y.; Kagami, M.; Ohno, A. *J. Am. Chem. Soc.* **1975**, *97*, 4766.

(17) (a) Strijtveen, B.; Feringa, B. L.; Kellog, R. M. *Tetrahedron* **1987**, *43*, 123. (b) Strijtveen, B.; Kellog, R. M. *Tetrahedron* **1987**, *43*, 5039.

Table III. HPLC Analysis of Reoxidized Fl(n) and dFl(n)[a]

(5-deaza)flavin	reagent	optical purity of starting (+) isomer/%	optical purity of recovered (5-deaza)flavin/% (+) isomer	(−) isomer
Fl(7)	BNAH	100	100	0
Fl(8)	BNAH	100	100	0
Fl(8)	BDT	100	100	0
Fl(10)	BNAH	100	50.1	49.9
Fl(10)	BDT	100	50.2	49.8
Fl(12)	BNAH	98.0	50.6	49.4
dFl(8)	BNAH	99.6	99.5	0.5
dFl(10)	BNAH	99.6	54.0	46.0
dFl(12)	BNAH	99.1	59.8	40.2

[a] pH 8.6 with 0.10 M borate; [Fl(n) or dFl(n)] = 1.00×10^{-4} M; [BNAH] = [BDT] = 5.00×10^{-4} M.

increase. The negative entropy term indicates that molecular freedom is lost in the transition state. In particular, Fl(Bu) and dFl(Bu) have negatively large $\Delta S^{\ddagger}$ values. This suggests that the molecular motion of cyclic Fl(10) and dFl(10) is fairly restricted even in the initial state and thus the change in $\Delta S^{\ddagger}$ is suppressed to small values compared with those for noncyclic Fl(Bu) and dFl(Bu).

We expected that Fl(n) and dFl(n) would give the same activation parameters as long as the ring size is identical. Contrary to our expectation, Fl(10) gave $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ significantly greater than those of dFl(10). A similar difference was found between Fl(Bu) and dFl(Bu). At present, we cannot explain what is the origin of this unexpected difference. Presumably, some difference in the resonance structure including N(5) reduces the steric crowding of Fl(n) in the transition state.

As described above, none of the chiral (5-deaza)flavinophanes used in this paper racemize below 40 °C: the $[\alpha]_D$ values in *N,N*-dimethylformamide (DMF) were constant for 1 day within the experimental error (4%). After this treatment, the solutions were concentrated under reduced pressure at room temperature and subjected to HPLC analysis on the chiral packing column. The optical purities thus obtained were essentially identical with those of the starting materials. One can conclude, therefore, that the oxidized forms are optically stable below 40 °C. Here, we reduced chiral Fl(n) to $Fl_{red}(n)$ by two different methods: (i) BNAH (1-benzyl-1,4-dihydronicotinamide) reduction which occurs by a "hydride equivalent" transfer[3-7] and (ii) BDT (1,4-butanedithiol) reduction which proceeds via a 4a-adduct intermediate.[18,19] After completion of reduction, the reduced forms were reoxidized (see Experimental Section). The results of the HPLC analysis of the chloroform layers are summarized in Table III.

Table III shows that, irrespective of the reduction methods, optical purities of recovered Fl(n) and dFl(n) with $n \leq 8$ are essentially unaffected by the redox treatments. This means that the strap is too short to allow the rope-skipping racemization not only in the oxidized forms but also in the reduced forms. Very interestingly, recovered Fl(n) and dFl(n) with $n \geq 10$, which are optically stable at room temperature, were racemized completely (within experimental error of HPLC analysis). Why did racemization of these chiral (5-deaza)flavinophanes take place only when they underwent the redox treatments? It has been unequivocally established on the basis of NMR and X-ray crystallographic studies that oxidized flavins are planar, whereas reduced flavins are folded along a line through N(5) and N(10) like butterfly wings.[20-22] The nitrogen atoms N(5) and N(10) in

Scheme I

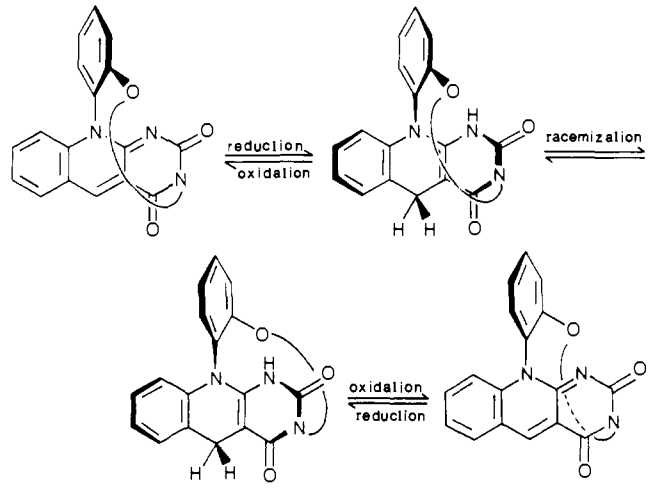


Table IV. HPLC Analysis of Fl(n) and dFl(n) Recovered by Decomposition of the Sulfite Adducts

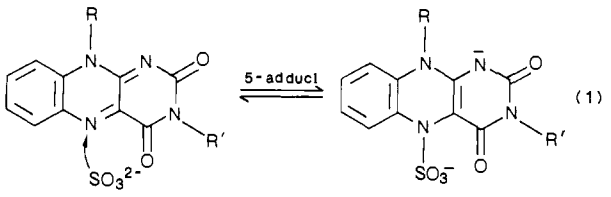
(5-deaza)flavin	optical purity of starting (+) isomer/%	optical purity of recovered (5-deaza)flavin/% (+) isomer	(−) isomer
Fl(8)	100	100	0
Fl(10)	100	41.8	58.2
dFl(8)	100	100	0
dFl(10)	99.6	53.2	46.8

oxidized flavins have planar sp^2 hybridization, whereas those in reduced flavins have a high degree of tetrahedral sp^3 hybridization.[20-22] Inspection of CPK molecular models suggests that the $(CH_2)_n$ chains in the oxidized forms are stretched on the isoalloxazine ring without the motional freedom, whereas those in the reduced forms are relatively flexible owing to the folded isoalloxazine plane. This implies that the redox treatments change the "tense" oxidized flavinophane ring into the "relaxed" reduced flavinophane ring. As a result, rope-skipping racemization becomes possible only when they are reduced [Scheme I: illustrated for dFl(n)]. Precedent exists for the conformational or configurational changes related to the rope-skipping molecular motion.[23-27] However, the present system is novel in that rope-skipping racemization can only occur when the planar ring is converted to the folded ring by redox treatments. This unique character stems from the redox-induced structural change in the isoalloxazine plane.

Racemization via Adduct Formation. It is known that flavins and 5-deazaflavins reversibly form covalent adducts (i.e., 5-adducts) with certain nucleophiles such as CN^- and SO_3^{2-} at the 5-position.[3-5,7] It is generally believed that the 5-adducts would have a structure similar to that of the 1,5-dihydro forms, but this explanation is based only on the similarity of the absorption spectra.[3-5,7] Hence, it would be of great value to examine whether the racemization is also induced through the adduct formation. If they racemize, it provides unambiguous evidence for the bent 5-adduct structure. With this objective in mind, we treated chiral Fl(n) and dFl(n) with Na_2SO_3 (eq 1) and examined whether recovered (5-deaza)flavinophanes racemize. The results are summarized in Table IV.

It is clearly seen from Table IV that the results for racemization via the SO_3^{2-} adducts are quite complementary to those for the

(18) Yokoe, I.; Bruice, T. C. *J. Am. Chem. Soc.* **1975**, *97*, 450.
(19) Loechler, E. L.; Hollocher, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 7312, 7322, 7328.
(20) Tausher, L.; Ghisla, S.; Hemerich, P. *Helv. Chim. Acta* **1973**, *56*, 630 and references cited therein.
(21) Kierkegaard, P.; Norrestam, R.; Werner, P.; Csoregh, I.; Glehn, M.; Karlsson, M.; Leijonmarch, M.; Ronnquist, O.; Sternland, B.; Tillberg, O.; Torbjornsson, L. In *Flavins and Flavoproteins*; University Park Press: Baltimore, 1971; p 1.
(22) Moonen, C. T.; Vervoort, J.; Müller, F. *Biochemistry* **1984**, *23*, 4859, 4868.
(23) Chung, J.; Rosenfeld, S. M. *J. Org. Chem.* **1983**, *48*, 387.
(24) Brown, H. S.; Muenchausen, C. P.; Sousa, L. R. *J. Org. Chem.* **1980**, *45*, 1682.
(25) Marshall, J. A.; Audia, V. H.; Jenson, T. M.; Guida, W. C. *Tetrahedron* **1986**, *42*, 1703.
(26) Chang, M. H.; Dougherty, D. A. *J. Am. Chem. Soc.* **1983**, *105*, 4102.
(27) Brown, A. B.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1986**, *108*, 3509.

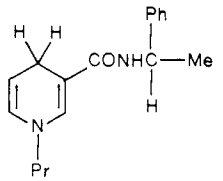


(1)

redox treatments: Fl(10) and dFl(10) racemize when they form the 5-adducts, whereas Fl(8) and dFl(8) retain the optical purities of the starting (+) isomers. The occurrence of racemization in Fl(10) and dFl(10) supports the view that N(5) and N(10) in 1,5-dihydroflavins and C(5) and N(10) in 1,5-dihydro-5-deazaflavins have a high degree of tetrahedral sp^3 hybridization. As a result, the sterically relaxed 5-adducts allow rope-skipping of the $(CH_2)_{10}$ strap. In contrast, the absence of racemization in Fl(8) and dFl(8) indicates that the $(CH_2)_8$ strap is still too short to cause the rope-skipping racemization. One can thus conclude that the 5-adducts employ the bent structure with butterfly-like wings similar to that of the 1,5-dihydro forms.

Chiral Discrimination. Asymmetric reduction of substrates with carbonyl groups by optically active NADH model compounds has been widely investigated.[6,7] Through these studies it was demonstrated that the reduction by chiral dihydropyridines included in a cyclic structure can afford very high optical yields (ca. 90% ee).[6b,c] The findings suggest that planar chirality may be a potential approach to asymmetric reactions mediated by flavins and 5-deazaflavins.

The reactions of (+)-Fl(*n*) and (+)-dFl(*n*) with (*R*)- and (*S*)-MPNAH were first carried out in an aqueous system at 30 °C, but no asymmetric discrimination was observed ($k_{2,R}/k_{2,S}$ = 1.0 ± 0.1). We therefore employed acetonitrile as solvent with



MPNAH

an added metal cation to act as a bridge between (5-deaza)flavin and 1,4-dihydronicotinamide at the transition state.[6,7] In acetonitrile Mg^{2+} ion is expected to interact with both Fl(*n*) [or dFl(*n*)] and MPNAH and control the steric reaction pathway.[6,7] The reaction was apparently first order in Fl(*n*) and MPNAH (for conditions, see footnote *a* to Table V). The second-order rate constants (k_2) for the reaction in the presence of $Mg(ClO_4)_2$ are summarized in Table V. The results were obtained in the presence of 100 mM $Mg(ClO_4)_2$ for Fl(*n*) and 1.0 mM $Mg(ClO_4)_2$ for dFl(*n*). As described in the preceding paper,[13] the rate constants decrease with increasing ring size, and the decrease in $k_{2,S}$ is more pronounced than that in $k_{2,R}$. As a result, $k_{2,R}/k_{2,S}$ increases with increasing ring size. The highest enantiomeric selectivity was observed for less reactive (+)-Fl(12) and (+)-dFl(12) ($k_{2,R}/k_{2,S}$ = 4.0). This difference corresponds to 60% enantiomeric excess [=$(k_{2,R} - k_{2,S})/(k_{2,R} + k_{2,S})$]. It is established that the interco-enzyme hydrogen transfer from NAD(P)H (and its model compounds) proceeds via a face-to-face orientation.[28,29] Examination of Corey–Pauling–Koltun models suggests that the polymethylene strap in these (5-deaza)flavinophanes effectively covers one side of the isoalloxazine plane and Fl(12) and dFl(12), having the longest polymethylene chain, provide the largest covering effect. This explains why the highest enantiomeric selectivity was observed for Fl(12) and dFl(12).

Very high enantiomeric selectivities (39–96% ee) have been observed for transamination catalyzed by pyridoxals appended to cyclodextrins or to cyclophanes.[30–34] In these systems the reaction proceeds via a covalently bonded intermediate (i.e., Schiff base). Expectedly, the adduct formation is very favorable to chiral discrimination. It is known that oxidation of thiols by favins proceeds via the 4a-adduct intermediate.[18,19] One may thus expect that this reaction would result in the higher enantiomeric selectivity. Oxidation of optically active thiols by (+)- and (−)-Fl(*n*) to the corresponding disulfides was carried out anaerobically at 30 °C in water–methanol (1:2 v/v). The reaction pH was set near the pK_a of thiols with 0.05 M carbonate or 0.05 M borate because the oxidation becomes maximal near the pK_a.[18,19] The pseudo-first-order rate constants (k_+ and k_-) are summarized in Table VI.

Among four commercially available thiols (three cysteine derivatives and 1,4-dithio-L-threitol),[35] enantioselective oxidation was observed only for *N*-acetyl-L-cysteine. The highest enantiomeric excess [$(k_+ - k_-)/(k_+ + k_-)$] was attained with Fl(12) (50.6% ee). On the other hand, the thiols having the chiral carbon next to the sulfhydryl group (runs 2–4) invariably showed enantioselective oxidation, and with Fl(8) a higher enantiomeric excess was found than with Fl(12). The highest enantiomeric excess was attained for the oxidation of (*R*)-MeCH(SH)COOEt by Fl(8) (62.8% ee). Of further interest is the sign of k_+/k_-. *N*-Acetyl-L-cysteine has the *S* configuration. Hence, (*S*)-thiols give $k_+/k_- > 1$ whereas (*R*)-thiols give $k_+/k_- \leq 1$.

The foregoing findings are summarized as follows: (i) Fl(8) shows higher enantioselectivity than Fl(12) for thiols C*–SH

Table V. Second-Order Rate Constants (k_2) for the Reaction of (+)-Fl(*n*) and (+)-dFl(*n*) with MPNAH in Acetonitrile[a]

(+)-(5-deaza)flavin	$[Mg(ClO_4)_2]$/mM	k_2 for MPNAH/M^{-1} s^{-1} (*R*)-MPNAH	(*S*)-MPNAH	$k_{2,R}/k_{2,S}$
Fl(7)	100	0.55	0.66	0.83
Fl(8)	100	0.24	0.12	2.0
Fl(12)	100	0.14	0.035	4.0
Fl(8)	2.0	0.36	0.28	1.3
Fl(12)	2.0	0.18	0.18	1.0
dFl(6)	1.0	3.69	2.16	1.7
dFl(7)	1.0	2.63	1.24	2.1
dFl(8)	1.0	1.38	0.59	2.3
dFl(12)	1.0	0.72	0.18	4.0

[a] 30 °C; N_2; [Fl(*n*) or dFl(*n*)] = 5.00 × 10^{-5} M; [MPNAH] = 4.00 × 10^{-4} M.

Table VI. Pseudo-First-Order Rate Constants (k_+ and k_-) for Oxidation of Optically Active Thiols by (+)- and (−)-Fl(*n*)[a]

run	thiol	Fl(*n*)	10^5k_+/s^{-1}	10^5k_-/s^{-1}	k_+/k_-
1	*N*-acetyl-L-cysteine[b]	Fl(7)	5.50	2.18	2.52
		Fl(8)	5.07	2.05	2.47
		Fl(12)	1.70	0.558	3.05
2	(*R*)-MeCH(SH)COOEt[c]	Fl(8)	1.12	4.90	0.229
		Fl(12)	1.15	1.26	0.913
3	(*S*)-$C_6H_5CH(SH)COOMe$[d]	Fl(8)	25.2	6.98	3.61
		Fl(12)	4.28	4.17	1.03
4	(*R*)-$C_6H_5CH_2CH(SH)COOMe$[d]	Fl(8)	1.04	2.27	0.458
		Fl(12)	1.20	1.17	1.03

[a] 30 °C; N_2; [Fl(*n*)] = (2.5–5.0) × 10^{-5} M. [b] pH 9.55; [thiol] = 1.00 × 10^{-2} M. [c] pH 8.00; [thiol] = 1.00 × 10^{-2} M. [d] pH 8.00; [thiol] = 5.00 × 10^{-3} M.

(28) Porter, D. J. T.; Blankenhorn, G.; Ingraham, L. L. *Biochem. Biophys. Res. Commun.* **1973**, *52*, 447.
(29) Blankenhorn, G. *Eur. J. Biochem.* **1975**, *50*, 351.
(30) (a) Breslow, R.; Doherty, J.; Guillot, G.; Lipsy, C. *J. Am. Chem. Soc.* **1978**, *100*, 3227. (b) Breslow, R.; Hammond, R.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421.
(31) (a) Zimmerman, S. C.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694. (b) Breslow, R.; Czarnik, A. W.; Lauer, M.; Leppkes, R.; Winkler, J.; Zimmerman, S. *J. Am. Chem. Soc.* **1986**, *108*, 1969.
(32) Tabushi, I.; Kuroda, Y.; Yamada, M.; Higashimura, H.; Breslow, R. *J. Am. Chem. Soc.* **1985**, *107*, 5545.
(33) Tachibana, Y.; Ando, M.; Kuzuhara, H. *Chem. Lett.* **1982**, 1765.
(34) For a comprehensive review see Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170.
(35) Optically active thiols tested in addition to those recorded in Table VI are L-cysteine, L-cysteine methyl ester, and 1,4-dithio-L-threitol. These thiols showed almost no asymmetric discrimination (k_+/k_- = 1.0 ± 0.1).

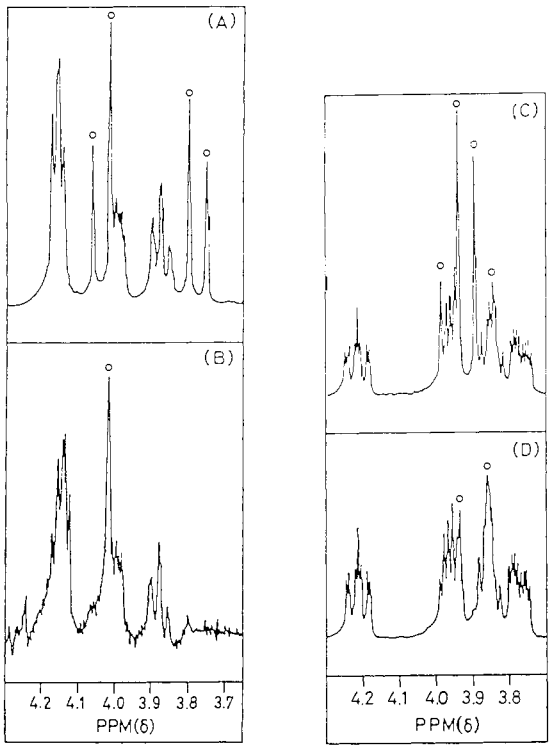


Figure 1. Partial ^1H NMR spectra (400 MHz) of the C(5) protons [labeled by (○)] in $dFl_{red}(n)$ in CD_3OD at room temperature: (A) reduced by $NaBH_4$ ($n = 6$); (B) reduced by $NaBD_4$ ($n = 6$); (C) reduced by $NaBH_4$ ($n = 10$); (D) reduced by $NaBD_4$ ($n = 10$). Chemical shifts in these spectra are given relative to tetramethylsilane.

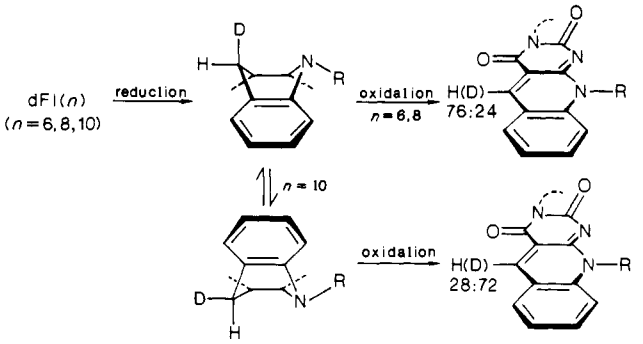
whereas the reverse is true for thiols C*CH_2–SH and (ii) $k_+/k_- > 1$ for (*S*)-thiols and $k_+/k_- \leq 1$ for (*R*)-thiols. Careful examination of Table VI reveals that the high enantiomeric excess values for runs 2–4 are achieved by a conspicuous increase in k_+ (or k_-) for Fl(8) whereas that for *N*-acetyl-L-cysteine is achieved by a conspicuous decrease in k_- for Fl(12). As described in the preceding paper, Fl(8) is more activated than Fl(12) because of the strap-induced small dihedral angle (θ) between the isoalloxazine ring and the 10-phenyl ring. This suggests that discrimination for the thiols C*–SH occurs more favorably in isoalloxazine having a small θ whereas that for the thiols C* CH_2–SH occurs in isoalloxazine having a large θ (near 90°). Since the absolute configuration for Fl(*n*) is not yet established, it is difficult to specify further these selectivities. However, the present findings indicate that planar chirality is a promising approach to flavin-mediated, asymmetric oxidations.

Diastereo-Differentiating Hydrogen Transfer. In NAD(P)H the two protons at C(4) of the 1,4-dihydronicotinamide moiety occupy diastereotopic positions. These two protons can be discriminated by the difference in ^1H NMR chemical shift, but the difference (if any) becomes quite slight in the free coenzymes.[36,37] In an NAD(P)H model system these two protons usually give the same chemical shift, but Rob et al.[38] and de Kok et al.[39] demonstrated that they give quite different chemical shifts in the case where the nicotinamide skeleton is included in a ring structure. By using these NAD(P)H model compounds, they established that hydrogen exchange occurs exclusively via the axial C(4) position.[38,39] In contrast, the hydrogen exchange mechanism of flavin coenzymes is understood less well. This difficulty is related to the fact that the redox reaction occurs at the weakly basic N(5)-position, and therefore, the "labeled" N(5)–H proton is exchangeable with solvent protons.[3,5] 5-Deazaflavin is known to be an essential skeleton in cofactor F_{420}.[3-5,7,40] Since the 5-nitrogen in flavin has been replaced by a carbon, 5-deazaflavin is well suited for studies of the hydrogen transfer mechanism not only in cofactor F_{420} but also in flavin coenzymes.[41] In order to obtain an insight into the diastereo differentiation, we investigated the hydrogen transfers to dFl(*n*) and from $dFl_{red}(n)$, expecting that the reduced forms [i.e., $dFl_{red}(n)$] would give different chemical shifts for the two C(5) protons.

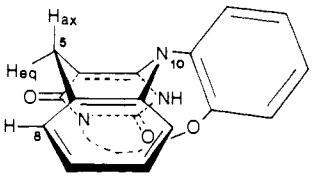
Table VII. Deuterium Content (%) at the Axial C(5) Position in $dFl_{red}(n)$

reducing agent	$dFl_{red}(n)$		
	$n = 6$	$n = 8$	$n = 10$
$NaBD_4$	>95	>95	50
$Na_2S_2O_4$ in D_2O–CD_3OD	>95		
BNAD	>95		

Scheme II



It is known that the C(5) protons in conventional 1,5-dihydro-5-deazaisoalloxazine give a singlet ^1H NMR peak.[42,43] When dFl(n = 6, 8, and 10) were reduced with $NaBH_4$ in CD_3OD, the ^1H NMR spectrum (400 MHz, JEOL GX-400) gave a pair of doublets for the two C(5) protons (Figure 1A). The result indicates that the central ring in $dFl_{red}(n)$ employs a boat-shaped conformation, affording the magnetically nonequivalent protons H_{ax} and H_{eq}. We applied NOE (nuclear Overhauser effect) in



^1H NMR to the assignment of H_{ax} and H_{eq}. The boat-shaped geometry shortens the distance from 6-H to H_{eq} compared to that to H_{ax}. Thus, the NOE spectra of $dFl_{red}(6)$ were measured while the 6-H was saturated (CD_3OD, 20 °C): as expected, a positive NOE was observed for the two C(5) protons, the lower magnetic field one being about two times stronger than the higher magnetic field one. The result establishes that H_{eq} and H_{ax} are assigned to the lower and the higher magnetic fields, respectively.

First, we reduced dFl(n) to $dFl_{red}(n)$ by three different methods: (i) $NaBD_4$ (98% isotope purity) in CD_3OD, (ii) $Na_2S_2O_4$ in D_2O (100% isotope purity)–CD_3OD (99.5% isotope purity) (1:4 v/v), and (iii) 4,4-dideuterio-1-benzyl-1,4-dihydronicotinamide (BNAD, 96% isotope purity) in D_2O–CD_3OD (1:4 v/v). In the ^1H NMR spectra of $dFl_{red}(6)$ (Figure 1B) and $dFl_{red}(8)$, H_{eq} appeared as a singlet peak with an integral intensity of 1 H whereas the peak for H_{ax} disappeared almost completely. The results are sum-

(36) Sarma, R. H.; Kaplan, N. O. *Biochemistry* **1970**, *9*, 539.
(37) Oppenheimer, N. J.; Arnold, L. J.; Kaplan, N. O. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 3200.
(38) Rob, F.; van Ramesdonk, H. J.; van Gerresheim, W.; Bosma, P.; Scheele, J. J.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1984**, *106*, 3826.
(39) de Kok, P. M. T.; Buck, H. M. *J. Chem. Soc., Chem. Commun.* **1985**, *1009*.
(40) Eirich, L. D.; Vogeles, G. D.; Wolfe, R. S. *Biochemistry* **1978**, *17*, 4583 and references cited therein.
(41) Manstein et al. have used 8-hydroxy-5-deaza-FAD as a probe to determine the absolute stereochemistry of flavoenzymes: Manstein, D. J.; Massey, V.; Pai, E. F. In *Flavins and Flavoproteins*; Walter de Gruyter: Berlin, 1987; p 3.
(42) Brüstlein, M.; Bruice, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 6548.
(43) Spencer, R.; Fisher, J.; Walsh, C. *Biochemistry* **1976**, *15*, 1043.

Table VIII. Deuterium Content (%) at the C(5) Position in Reoxidized dFl(n)

method to prepare $dFl_{red}(n)$	recovered dFl(n)		
	$n = 6$	$n = 8$	$n = 10$
$NaBD_4$	20	23	72
$Na_2S_2O_4$ in D_2O–CD_3OD	20		
BNAD	28		

marized in Table VII. This supports the view that the incorporated hydrogen occupies almost exclusively (>95% judging from the accuracy of ^1H NMR) an axial position. In contrast, $dFl_{red}(10)$ prepared by $NaBH_4$ gave a pair of doublets (Figure 1C), but that prepared by $NaBD_4$ gave singlet H_{eq} and H_{ax} signals (intensity 0.5 H each) in the 1:1 intensity ratio (Figure 1D). As described above, $dFl_{red}(n = 6$ and 8) with the small ring size are optically stable whereas $dFl_{red}(10)$ with the large ring size rapidly racemizes in a rope-skipping manner. Conceivably, hydrogen is incorporated primarily into the axial C(5) position of $dFl_{red}(10)$ but is rapidly exchanged through the rope-skipping interconversion. These stereochemical reaction processes can be illustrated as in Scheme II.[44]

$dFl_{red}(n)$ in deuterated solvents was mixed with *N*-methylacridinium iodide (1.2 equiv) in water and stirred for 1 h at room temperature. Reoxidized dFl(n) was extracted with chloroform and purified by preparative TLC. The ^1H NMR data of dFl(n) thus obtained (>90% recovery) are summarized in Table VIII. Table VIII shows that dFl(10) contains 72% of the deuterium at the C(5) position. Since the incorporated deuterium occupies the axial and the equatorial positions in a 1:1 ratio and is exchangeable in $dFl_{red}(10)$, one can estimate the primary isotope effect for $dFl_{red}(10) \rightarrow dFl(10)$ to be $k_H/k_D = 72/28 = 2.6$. This value is comparable with the relatively small primary isotope effect reported for the oxidation of 4,4-dideuterio-1,4-dihydronicotinamide derivatives ($k_H/k_D = 2.8 \pm 1.3$).[45-48] In $dFl_{red}(6)$ and $dFl_{red}(8)$ the H_{ax}–H_{eq} exchange is inhibited, so that one can estimate the reactivity ratio of H_{ax} vs H_{eq} by measuring the concentration of deuterium remaining in reoxidized dFl(n). As shown in Table VIII, an almost constant deuterium content, 24 ± 4% was found. Taking the primary isotope effect into account, the reactivity ratio of H_{ax} vs H_{eq} is calculated to be (76/24) × 2.6 = 8.2. This corresponds to the total diastereo-differentiating ability of dFl(n).

It is extremely important to discuss why H_{ax} is more reactive than H_{eq}. The principle of microscopic reversibility suggests that incorporation of hydrogen into the axial position for $dFl(n) \rightarrow dFl_{red}(n)$ and dehydrogenation from the axial position for $dFl_{red}(n) \rightarrow dFl(n)$ should experience the almost same transition state. Therefore, these two processes should be explained by the same reaction mechanism. The first possible explanation is a steric effect: since the H_{eq} side in $dFl_{red}(n)$ is covered by the $(CH_2)_n$ chain, the approach of the substrate may be more difficult. This may be the case for the reduction of dFl(n) by BNAH, which proceeds via a face-to-face charge-transfer complex.[28,29] However, the steric effect is rather unlikely in small, inorganic reductants such as $NaBH_4$ and $Na_2S_2O_4$. In fact, however, these reductants result in a stereochemical course identical with that of the large organic reductants. The second, more likely, possibility is a stereoelectronic effect;[38] that is, the axial transition state is more stabilized by the π-type overlap (hyperconjugation) between the partially broken (or partially formed) C(5)–H bond and the neighboring benzene or pteridine π-system.[38,49] A similar stereoelectronic effect is propered by Rob et al.[38] to explain the axial selectivity in 1,4-dihydronicotinamide derivatives. We believe that the stereoelectronic effect is mainly responsible for the high degree of diastereo differentiation.

Conclusions. We believe that the present paper demonstrates several new concepts important in flavin chemistry: (i) redox-induced "rope-skipping" racemization, (ii) high asymmetric discrimination, and (iii) hydrogen exchange at the axial C(5) position. Concept i is very novel because this type of racemization is only possible when the ring framework changes reversibly. In (5-deaza)flavinophanes the geometrical change is induced by the sp^2–sp^3 interconversion of N(5) [or Cn5)] and N(10). Concept iii is also novel because discrimination between H_{ax} and H_{eq} in 1,5-dihydro-5-deazaflavins becomes possible for the first time by including the 5-deazaisoalloxazine skeleton in the phane structure. This finding may have important biochemical implications on diastereo differentiation not only in 5-deazaflavin-dependent cofactor F_{420} but more in general in flavin-dependent enzymes. Further characterizations and applications of chiral (5-deaza)-flavinophanes are now under investigation. Of particular interest is, we believe, the application to asymmetric thermal and photooxidation reactions in which Fl(n) and dFl(n) act not only as catalysts but also as host molecules.

(44) Walsh and co-workers[43] have observed scrambling of the chiral hydrogen isotope at the C(5) position of 5-deazaflavins. This is not the case in the present study because the reduction of dFl(n) by $NaBH_4$ was completed in a few minutes and was incomparably faster than the hydrogen transfer between dFl(n) and $dFl_{red}(n)$ (e.g., see Hemmerich, P.; Massey, V.; Fenner, H. *FEBS Lett.* **1977**, *84*, 5). We reduced 3-methyl-5-deuterio-10-phenyl-5-deazaisoalloxazine by $NaBD_4$ in the presence of 1,5-dihydro-3-methyl-10-phenyl-5-deazaisoalloxazine. 1,5-Dihydro-3-methyl-5-deuterio-10-phenyl-5-deazaisoalloxazine (scrambled species) was not found in the products (detected by ^1H NMR).

(45) Verhoeven, J. W.; van Gerresheim, W.; Martens, F. M.; van der Kerk, S. M. *Tetrahedron* **1986**, *42*, 975.

(46) Ohno, A.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2041.

(47) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 5834.

(48) Srinivasan. R.; Fisher, H. F. *J. Am. Chem. Soc.* **1985**, *107*, 4301.

(49) Brounts, R. H. A. M.; Buck, H. M. *J. Am. Chem. Soc.* **1983**, *105*, 1284.