Coenzyme Models. 47. Synthesis and Reactivity Studies of Novel Flavinophanes and 5-Deazaflavinophanes: Correlation between Flavin Reactivity and Ring Strain

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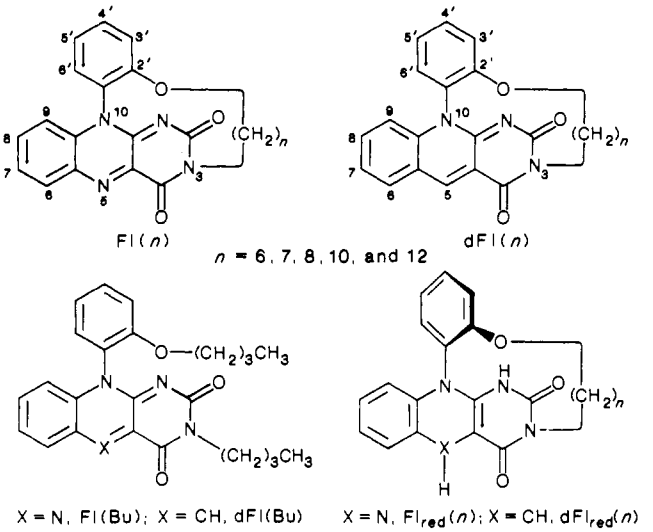
Abstract: New flavinophanes Fl(n) and 5-deazaflavinophanes dFl(n) were synthesized in which N(3) and O(2′) in the 10-(2-hydroxyphenyl) group were linked by a $-(CH_2)_n-$ chain (n = 6, 7, 8, 10, and 12). In the ^{1}H NMR spectra the chemical shift of 9-H in the isoalloxazine ring and 6′-H in the 10-phenyl ring moved to lower magnetic field with decreasing ring size. The change corresponds to the decrease in the dihedral angle between the isoalloxazine ring and the 10-phenyl ring. The NOE (nuclear Overhauser effect) measurement also supported that the distance between 9-H and 6′-H decreases with decreasing ring size. X-ray crystallographic studies of dFl(6) established that the dihedral angle (θ) between the isoalloxazine ring and the 10-phenyl ring is 76.5°, considerably smaller than the 90° that minimizes the steric crowding of conventional 10-phenylisoalloxazine derivatives. The ab initio MO calculation of 10-phenylisoalloxazine indicates that the equilibrium geometry is such that θ = 60–90° and Fl(n) and dFl(n), with the smaller θ, are destabilized because of steric hindrance. The steric strain induced by the short ring strap is reflected by the reactivities of Fl(n) and dFl(n): For the oxidation of 1-benzyl-1,4-dihydronicotinamide (BNAH), sterically strained Fl(6) and dFl(6) have rate constants 11 and 16 times greater than those for Fl(12) and dFl(12). For the reversible 5-adduct formation between Fl(n) and SO_3^{2-}, k_f (forward reaction) for Fl(6) is greater by a factor of 5.2 than that for Fl(12), while k_r (reverse reaction) for Fl(6) is smaller by a factor of 3.6 than that for Fl(12). These results can be explained by destabilization of the initial state due to steric strain induced by the ring strap.

Enzymes catalyze reactions by reducing the free energy difference between ground states and transition states.[5,6] This concept can be extended to molecular design in biomimetic chemistry. In principle, when the structure of the initial state is constrained to be close to that of the transition state (for example, due to steric strain), rate accelerations should be observed. The cyclophane family is a convenient system for evaluating this hypothesis, because the stability of the initial state can be changed systematically by steric strain arising from the ring size.[7-13] For example, [n]anthracenophanes with $n \leq 8$ easily undergo oxidation to yield anthraquinone due to aryl ring deformation.[11]

It is expected that the reactivity of flavin compounds is potentially subject to similar manipulations. Oxidized flavins are almost planar molecules, whereas reduced flavins are folded along a line through N(5) and N(10) like butterfly wings.[15-17] The significant change in structure between the oxidized and reduced forms suggests that the reactivity of the flavin family can be controlled by enforced deformation of the flavin plane; deformation may lead to improvement in oxidizing ability because the bent structure would more closely approximate the transition state. In order to test this hypothesis, we have examined the effect of short-strap bridging on the reactivity of the following compounds: five [n+3](3,10)isoalloxazinophanes [Fl(n)] and five [n+3]-(3,10)-5-deazaisoalloxazinophanes [dFl(n)] (n = 6, 7, 8, 10, and 12) and their open-chain analogues [Fl(Bu) and dFl(Bu)] (Chart I).[18] Indeed, flavin reactivities are profoundly related to the ring size: the smaler the ring size, the more reactive.

Chart I[a]



[a] The numbers in the structures are used for assignment of ^{1}H NMR spectra.

(1) Nagasaki University.
(2) Takeda Chemical Industries.
(3) Kyoto University.
(4) Osaka City University.
(5) Leinhard, G. E. *Science* **1973**, *180*, 149.
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(18) Flavinophanes have also been synthesized by Zipplies et al. and Seward et al., but there is no precedent for the reactivity studies and optical resolution: (a) Zipplies, M. F.; Krieger, C.; Staab, H. *Tetrahedron Lett.* **1983**, *24*, 1925. (b) Seward, E.; Diederich, F. *Tetrahedron Lett.* **1987**, *28*, 5111.

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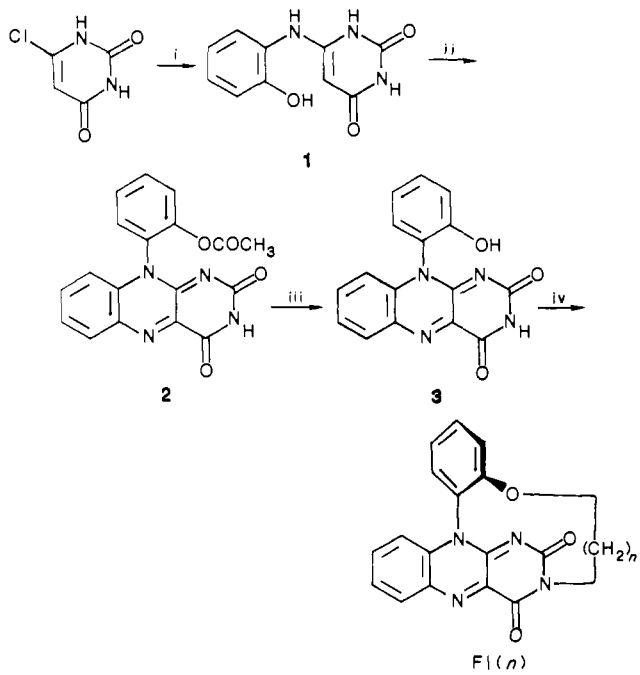
Scheme I[a]



[a](i) o-Aminophenol; (ii) nitrosobenzene in acetic acid/acetic anhydride; (iii) 1-butylamine; (iv) $Br(CH_2)_nBr$.

Experimental Section

Materials. Flavinophanes were synthesized according to Scheme I. The synthetic route for the flavin skeleton is basically analogous to Yoneda's method.[19]

6-(2-Hydroxyanilino)uracil (1). 6-Chlorouracil (3.16 g, 21.5 mmol) and *o*-aminophenol (4.73 g, 43.3 mmol) were dispersed in 20 mL of sulfolane, and the reaction mixture was stirred at 110 °C for 90 min. At the end of the reaction, **1** precipitated as white crystals. After cooling, the reaction mixture was poured into excess water, and the white precipitate was recovered by filtration. This was dissolved in 2 M NaOH and treated with activated charcoal under a nitrogen stream. After filtration the product was reprecipitated by acidification with concentrated HCl. The crystal was recovered by filtration and washed with methanol: mp >310 °C; yield 82%; IR (KBr) $\nu_{C=O}$ 1630 and 1710 cm^{-1}, ν_{NH} 3310 cm^{-1}. Anal. Calcd for $C_{10}H_9N_3O_3 \cdot 0.2H_2O$: C, 53.91; H, 4.25; N, 18.86. Found: C, 53.73; H, 4.07; N, 18.78.

10-(2-Acetoxyphenyl)isoalloxazine (2). **1** (3.14 g, 14.3 mmol) was dissolved in a mixed solvent (210 mL) of acetic acid/acetic anhydride (2:5 v/v). The reaction mixture was refluxed under a nitrogen stream, and nitrosobenzene (1.50 g, 14.0 mmol) in 10 mL of acetic acid was added dropwise. After 30 min, the solution was cooled and filtered to remove insoluble materials. The concentration of the filtrate in vacuo gave the brown oil. This was crystallized from acetone: mp 260 °C dec; yield 25%; IR (KBr) $\nu_{C=O}$ 1660, 1720, and 1760 cm^{-1}. Anal. Calcd for $C_{18}H_{14}N_3O_3$: C, 62.07; H, 3.47; N, 16.08. Found: C, 57.36; H, 3.88; N, 14.95. The elemental analysis and TLC (two spots) suggested that the product contains a small amount of 10-(2-hydroxyphenyl)isoalloxazine (**3**). **2** was further hydrolyzed to **3** during the recrystallization treatment. We therefore abandoned the purification of **2** and used the mixture for the synthesis of Fl(*n*).

10-(2-Hydroxyphenyl)isoalloxazine (3) and 9′-Oxa-10′,11-benzo[11](3,10)isoalloxazinophane [Fl(8)]. **2** (1.03 g, 2.93 mmol) was treated with 1-butylamine (1.12 g, 15.3 mmol) in 100 mL of methanol at room temperature. The progress of the reaction was followed by TLC [silica gel, chloroform/methanol (10:1 v/v)]. After 20 min, 1-butylamine was neutralized with acetic acid, and the solution was concentrated in vacuo. The residue (ca. 5 mL) was diluted with water, and the orange precipitate was recovered by filtration. Since the ester carbonyl peak (1760 cm^{-1}) was not detected in the IR spectrum, the product was directly used for the next reaction.

3 (0.51 g, 1.67 mmol), 1,8-dibromooctane (0.48 g, 1.76 mmol), and Cs_2CO_3 (1.40 g, 4.30 mmol) were dissolved in 50 mL of *N,N*-dimethylformamide (DMF) and heated at 80–90 °C for 6 h. After cooling, the solution was filtered to remove insoluble cesium salt and then acidified by acetic acid. The solution was concentrated in vacuo, and the residue (ca. 5 mL) was diluted with water. The yellow precipitate was recovered by filtration and, after being dried, subjected to separation by preparative TLC (silica gel, chloroform). The highest colored band (R_f = 0.4) was collected: mp 285–286 °C; yield (from **2**) 7.2%; IR (KBr) $\nu_{C=O}$ 1710 and 1760 cm^{-1}; mass spectrum, *m/e* 416 (M^+); ^1H NMR ($CDCl_3$) δ 1.0–1.8 [$-(CH_2)_6-$, m, 12 H], 3.9–4.3 (OCH_2 and NCH_2, m, 4 H), 7.06 (9-H, d, 1 H), 7.16 (3′-H, d, 1 H), 7.20 (5′-H, q, 1 H), 7.33 (6′-H, d, 1 H), 7.5–7.6 (4′- and 7-H, m, 2 H), 7.69 (8-H, q, 1 H), 8.33 (6-H, d, 1 H). Anal. Calcd for $C_{24}H_{24}N_3O_3$: C, 69.21; H, 5.81; N, 13.45. Found: C, 69.00; H, 5.84; N, 13.28.

Other isoalloxazinophanes were synthesized from **3** and the corresponding dibromides in a manner similar to that described above. We record their analytical data.

7′-Oxa-8′,9′-benzo[9](3,10)isoalloxazinophane [Fl(6)]: mp >300 °C; yield (from **2**) 2%; mass spectrum, *m/e* 388 (M^+); ^1H NMR ($CDCl_3$) δ 0.9–1.9 [$-(CH_2)_4-$, m, 8 H], 3.9–4.5 (OCH_2 and NCH_2, m, 4 H), 7.16 (3′-H, d, 1 H), 7.23 (5′-H, q, 1 H), 7.31 (9-H, d, 1 H), 7.49 (6′-H, d, 1 H), 7.54 (4′-H, q, 1 H), 7.59 (7-H, q, 1 H), 7.70 (8-H, q, 1 H), 8.34 (6-H, d, 1 H). Anal. Calcd for $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42. Found: C, 68.26; H, 5.32; N, 14.12.

8′-Oxa-9′,10′-benzo[10](3,10)isoalloxazinophane [Fl(7)]: mp 287–288 °C; yield (from **2**) 5.3%; mass spectrum, *m/e* 402 (M^+); ^1H NMR ($CDCl_3$) δ 0.9–1.9 [$-(CH_2)_5-$, m, 10 H], 3.7–4.4 (OCH_2 and NCH_2, m, 4 H), 7.13 (9-H, d, 1 H), 7.20 (3′-H, d, 1 H), 7.22 (5′-H, q, 1 H), 7.41 (6′-H, d, 1 H), 7.55 (7-H, q, 1 H), 7.59 (4′-H, q, 1 H), 7.69 (8-H, q, 1 H), 8.34 (6-H, d, 1 H). Anal. Calcd for $C_{23}H_{22}N_4O_3$: C, 68.64; H, 5.51; N, 13.92. Found: C, 68.62; H, 5.51; N, 13.64.

11′-Oxa-12′,13′-benzo[13](3,10)isoalloxazinophane [Fl(10)]: mp 255–257 °C; yield (from **2**) 8.1%; IR (KBr) $\nu_{C=O}$ 1650 and 1740 cm^{-1}; mass spectrum, *m/e* 444 (M^+); ^1H NMR ($CDCl_3$) δ 1.0–1.8 [$-(CH_2)_8-$, m, 16 H], 3.9–4.4 (OCH_2 and NCH_2, m, 4 H), 6.95 (9-H, d, 1 H), 7.17 (3′-H, d, 1 H), 7.20 (5′-H, q, 1 H), 7.27 (6′-H, d, 1 H), 7.5–7.6 (4′- and 7-H, m, 2 H), 7.66 (8-H, q, 1 H), 8.33 (6-H, d, 1 H). Anal. Calcd for $C_{26}H_{28}N_4O_3$: C, 70.25; H, 6.35; N, 12.60. Found: C, 70.23; H, 6.34; N, 12.38.

13′-Oxa-14′,15′-benzo[15](3,10)isoalloxazinophane [Fl(12)]: mp 232–235 °C; yield (from **2**) 9.6%; IR (KBr) $\nu_{C=O}$ 1660 and 1730 cm^{-1}; mass spectrum, *m/e* 472 (M^+); ^1H NMR ($CDCl_3$) δ 1.0–1.8 [$-(CH_2)_{10}-$, m, 20 H], 4.0–4.3 (OCH_2 and NCH_2, m, 4 H), 6.92 (9-H, d, 1 H), 7.17 (3′-H, d, 1 H), 7.20 (5′-H, q, 1 H), 7.27 (6′-H, d, 1 H), 7.56 (4′- and 7-H, m, 2 H), 7.65 (8-H, q, 1 H), 8.32 (6-H, d, 1 H). Anal. Calcd for $C_{28}H_{32}N_4O_3$: C, 71.16; H, 6.84; N, 11.86. Found: C, 71.49; H, 6.95; N, 11.63.

10-[2-(Butyloxy)phenyl]-3-butylisoalloxazine [Fl(Bu)]. **3** (1.56 g, 5.09 mmol), *n*-butyl iodide (2.82 g, 15.3 mmol), and Cs_2CO_3 (4.20 g, 12.3 mmol) were dissolved in 100 mL of DMF and heated at 50 °C. The progress of the reaction was followed by TLC. After 1 h, the solution was concentrated in vacuo, and the residue (ca. 5 mL) was diluted with water. The yellow precipitate was recovered by filtration. The product was dissolved in chloroform and treated with activated charcoal. After filtration, the filtrate was concentrated in vacuo, and the residue was recrystallized from benzene: mp 263–264 °C; yield 24%; ^1H NMR ($CDCl_3$) δ 0.67–1.7 ($CH_2CH_2CH_3$, m, 14 H), 3.90 and 3.97 [NCH_2, m and m (each peak should give six lines theoretically), 2 H], 4.09 (OCH_2, t, 2 H), 6.91 (9-H, d, 1 H), 7.15 (3′-H, d, 1 H), 7.18 (5′-H, q, 1 H), 7.26 (6′-H, d, 1 H), 7.55 (4′- and 7-H, m, 2 H), 7.64 (8-H, q, 1 H), 8.31 (6-H, d, 1 H). Anal. Calcd for $C_{24}H_{26}N_4O_3$: C, 68.88; H, 6.26; N, 13.39. Found: C, 69.16; H, 6.29; N, 13.27.

5-Deazaflavinophanes were synthesized according to Scheme II.

10-(2-Hydroxyphenyl)-5-deazaisoalloxazine (4). **1** (4.10 g, 18.7 mmol) and o-chlorobenzaldehyde (3.93 g, 28.0 mmol) were dissolved in 90 mL of DMF, and the solution was heated at 160–180 °C for 5 h. The progress of the reaction was followed by TLC [silica gel, chloroform/methanol (10:1 v/v)]. The solution was concentrated in vacuo, and the residue (ca. 10 mL) was poured into water. The yellow precipitate was recovered by filtration and recrystallized from ethanol: mp >310 °C; yield 54%; IR (KBr) ν_{NH} 3300 cm^{-1}, ν_{OH} 3200 cm^{-1}, $\nu_{C=O}$ 1650 and 1710 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 6.96 (9-H, d, 1 H), 7.0–7.9 (7-, 8-, 3′-, 4′-, 5′-, and 6′-H, m, 6 H), 8.37 (6-H, d, 1 H), 9.29 (5-H, s, 1 H), 10.06 (NH, br, 1 H). Anal. Calcd for $C_{17}H_{11}N_3O_3 \cdot 0.4H_2O$: C, 65.34; H, 3.80; N, 13.45. Found: C, 65.67; H, 3.96; N, 13.29.

9′-Oxa-10′,11′-benzo[11](3,10)-5-deazaisoalloxazinophane [dFl(8)]. **4** (2.24 g, 7.34 mmol), 1,8-dibromooctane (2.03 g, 7.46 mmol), and Cs_2CO_3 (4.52 g, 13.9 mmol) were dissolved in 200 mL of DMF and heated at 80–90 °C. The progress of the reaction was followed by TLC. After 6 h, the reaction solution was cooled and filtered to remove insoluble cesium salts. The solution was acidified by acetic acid and then concentrated in vacuo. The residue (ca. 5 mL) was poured into water, the precipitate

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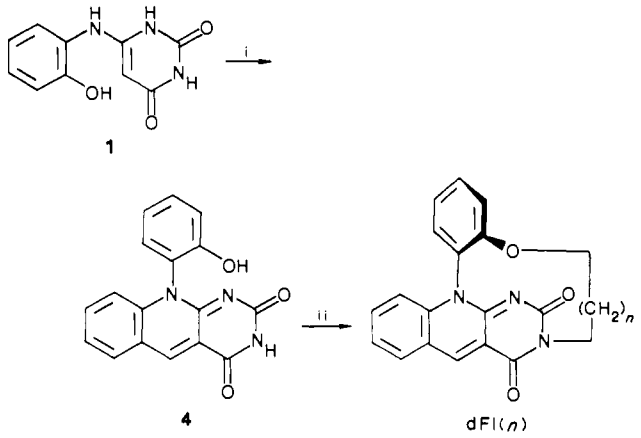
Scheme II[a]



[a] (i) o-Chlorobenzaldehyde; (ii) $Br(CH_2)_nBr$.

being recovered by filtration. The product was purified by preparative TLC (silica gel, chloroform, R_f = 0.4): mp 288–289 °C; yield 10.5%; IR (KBr) $\nu_{C=O}$ 1640 and 1690 cm^{-1}; mass spectrum, m/e 415 (M^+); ^1H NMR ($CDCl_3$) δ 0.8–1.9 [$-(CH_2)_6-$, m, 12 H], 3.9–4.2 (OCH_2 and NCH_2, m, 4 H), 7.04 (9-H, d, 1 H), 7.15 (3′-H, d, 1 H), 7.18 (5′-H, q, 1 H), 7.32 (6′-H, d, 1 H), 7.44 (7-H, q, 1 H), 7.52 (4′-H, q, 1 H), 7.63 (8-H, q, 1 H), 7.93 (6-H, d, 1 H), 8.99 (5-H, s, 1 H). Anal. Calcd for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.05; N, 10.01. Found: C, 72.45; H, 6.07; N, 10.09.

Other 5-deazaisoalloxazinophanes were synthesized from **4** and the corresponding dibromides in a manner similar to that described for dFl(8). We record their analytical data.

7′-Oxa-8′,9′-benzo[9](3,10)-5-deazaisoalloxazinophane [dFl(6)]. As described above the reaction of **3** and 1,6-dibromohexane gave Fl(6) in 2% yield. When **4** and 1,6-dibromohexane were allowed to react at 80–90 °C, they predominantly afforded 3-(6-bromohexyl)-10-[2-[(6-bromohexyl)oxy]phenyl]-5-deazaisoalloxazine and no dFl(6). Probably, the chain length is too short to cyclize. Thus, this compound was synthesized at 100 °C for 6 h under a nitrogen stream and isolated by preparative TLC [silica gel, chloroform/methanol (40:1 v/v), R_f = 0.6 (the highest colored band)]. The yield was relatively low: mp >300 °C; yield 4.0%; IR (KBr) $\nu_{C=O}$ 1640 and 1695 cm^{-1}; mass spectrum, m/e 387 (M^+); ^1H NMR ($CDCl_3$) δ 0.9–1.8 [$-(CH_2)_4-$, m, 8 H], 4.0–4.4 (OCH_2 and NCH_2, m, 4 H), 7.16 (3′-H, d, 1 H), 7.21 (5′-H, q, 1 H), 7.31 (9-H, d, 1 H), 7.45 (7-H, q, 1 H), 7.48 (6′-H, d, 1 H), 7.51 (4′-H, q, 1 H), 7.66 (8-H, q, 1 H), 7.95 (6-H, d, 1 H), 8.96 (5-H, s, 1 H). Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.19; H, 5.48; N, 10.49.

8′-Oxa-9′,10′-benzo[10](3,10)-5-deazaisoalloxazinophane [dFl(7)]: mp 290–295 °C; yield 27%; mass spectrum, m/e 401 (M^+); ^1H NMR ($CDCl_3$) δ 0.9–1.8 [$-(CH_2)_5-$, m, 10 H], 3.7–4.4 (OCH_2 and NCH_2, m, 4 H), 7.11 (9-H, d, 1 H), 7.16 (3′-H, d, 1 H), 7.19 (5′-H, q, 1 H), 7.41 (6′-H, d, 1 H), 7.44 (7-H, q, 1 H), 7.51 (4′-H, q, 1 H), 7.64 (8-H, q, 1 H), 7.94 (6-H, d, 1 H), 8.98 (5-H, s, 1 H). Anal. Calcd for $C_{24}H_{23}N_3O_3$: C, 71.80; H, 5.77; N, 10.47. Found: C, 71.05; H, 6.69; N, 9.96.

11′-Oxa-12′,13′-benzo[13](3,10)-5-deazaisoalloxazinophane [dFl(10)]: mp 260–261 °C; yield 26%; IR (KBr) $\nu_{C=O}$ 1640 and 1695 cm^{-1}; mass spectrum, m/e 443 (M^+); ^1H NMR ($CDCl_3$) δ 0.9–1.9 [$-(CH_2)_8-$, m, 16 H], 3.8–4.3 (OCH_2 and NCH_2, m, 4 H), 6.93 (9-H, d, 1 H), 7.15 (3′-H, d, 1 H), 7.17 (5′-H, q, 1 H), 7.25 (6′-H, d, 1 H), 7.43 (7-H, q, 1 H), 7.52 (4′-H, q, 1 H), 7.62 (8-H, q, 1 H), 7.93 (6-H, d, 1 H), 9.00 (5-H, s, 1 H). Anal. Calcd for $C_{27}H_{29}N_3O_3$: C, 73.11; H, 6.59; N, 9.47. Found: C, 73.11; H, 6.58; N, 9.37.

13′-Oxa-14′,15′-benzo[15](3,10)-5-deazaisoalloxazinophane [dFl(12)]: mp 250–251 °C; yield 10.2%; IR (KBr) $\nu_{C=O}$ 1640 and 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9–1.8 [$-(CH_2)_{10}-$, m, 20 H], 3.9–4.3 (OCH_2 and NCH_2, m, 4 H), 6.91 (9-H, d, 1 H), 7.15 (3′-H, d, 1 H), 7.18 (5′-H, q, 1 H), 7.26 (6′-H, d, 1 H), 7.42 (7-H, q, 1 H), 7.52 (4′-H, q, 1 H), 7.61 (8-H, q, 1 H), 7.92 (6-H, d, 1 H), 8.99 (5-H, s, 1 H). Anal. Calcd for $C_{29}H_{33}N_3O_3$: C, 73.86; H, 7.05; N, 8.91. Found: C, 74.04; H, 7.03; N, 8.95.

10-[2-(Butyloxy)phenyl]-3-butyl-5-deazaisoalloxazine [dFl(Bu)]. This compound was synthesized from **4** (1.56 g, 5.11 mmol) and *n*-butyl iodide (2.76 g, 15.0 mmol) in the presence of Cs_2CO_3 (4.06 g, 12.6 mmol) in 100 mL of DMF. The reaction was continued at 120–130 °C for 8 h under a nitrogen stream. The solution was concentrated in vacuo, and the residue (ca. 10 mL) was subjected to steam distillation to remove unreacted *n*-butyl iodide. The precipitate in a distillation flask was recovered by filtration and recrystallized from benzene: mp 275–276 °C; yield 28%; IR (KBr) $\nu_{C=O}$ 1640 and 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.63–1.8 ($CH_2CH_2CH_3$, m, 14 H), 3.88 and 3.96 [NCH_2, m and m (each peak should give six lines theoretically), 2 H], 4.05 (OCH_2, t, 2 H), 6.90 (9-H, d, 1 H), 7.14 (3′-H, d, 1 H), 7.13 (5′-H, q, 1 H), 7.26 (6′-H, d, 1 H), 7.42 (7-H, q, 1 H), 7.52 (4′-H, q, 1 H), 7.61 (8-H, q, 1 H), 7.92 (6-H, d, 1 H), 8.98 (5-H, s, 1 H). Anal. Calcd for $C_{25}H_{27}N_3O_3$: C, 71.92; H, 6.52; N, 10.06. Found: C, 71.59; H, 6.47; N, 9.94.

Table I. Absorption Maxima (nm) of Fl(*n*) and dFl(*n*)[a]

	λ_{max} (ϵ_{max})	
n	Fl(*n*)	dFl(*n*)
6	443 (6430)	402 (8300)
7	443 (6560)	401 (7450)
8	443 (8690)	398 (8730)
10	443 (8590)	398 (8820)
12	443 (8750)	402 (9860)

[a] Methanol; 30 °C.

Table II. Rate Constants for the Reactions with 1-Benzyl-1,4-dihydronicotinamide (k_{BNAH}), 1,4-Butanedithiol (k_{BDT}), and SO_3^{2-} and CN^- (k_{obs}) at 30 °C

			k_{obs}/s^{-1}	
flavin	$k_{BNAH}/M^{-1}\ s^{-1}$	k_{BDT}/s^{-1}	SO_3^{2-} [a]	CN^- [b]
Fl(6)	24.2	6.08×10^{-2}	6.17×10^{-3}	
Fl(7)	13.0	2.16×10^{-2}	4.13×10^{-3}	
Fl(8)	6.54	1.41×10^{-2}	3.33×10^{-3}	
Fl(10)	2.72	8.05×10^{-3}	1.44×10^{-3}	
Fl(12)	2.20	7.33×10^{-3}	1.22×10^{-3}	
Fl(Bu)	2.78	1.02×10^{-2}	1.53×10^{-3}	
dFl(6)	1.00			1.60×10^{-2}
dFl(7)	0.349			1.19×10^{-2}
dFl(8)	0.244			9.84×10^{-3}
dFl(10)	0.0712			7.94×10^{-3}
dFl(12)	0.0625			4.99×10^{-3}
dFl(Bu)	0.109			8.74×10^{-3}

[a] $[Na_2SO_3] = 3.00 \times 10^{-2}$ M. [b] $[KCN] = 2.00 \times 10^{-3}$ M.

Kinetic Measurements. Oxidation of 1-benzyl-1,4-dihydronicotinamide (BNAH) by Fl(*n*) was carried out at 30 °C under the aerobic conditions. Under these conditions Fl(*n*) is rapidly recycled because reduced flavin $Fl_{red}(n)$ is immediately reoxidized by dioxygen. The progress of the reaction was monitored spectrophotometrically by following the disappearance of the absorption band of BNAH (λ_{max} 357 nm). The decrease obeyed the first-order rate equation for up to three half-lives. On the other hand, oxidation of BNAH by dFl(*n*) was carried out under the anaerobic conditions because the rate of O_2 reoxidations of $dFl_{red}(n)$ is relatively slow.[20] The progress of the reaction was monitored by following the disappearance of the absorption band of dFl(*n*) (λ_{max} around 400 nm). The reaction was first order in BNAH and Fl(*n*) [or dFl(*n*)]. The details of kinetic measurements for oxidation of thiols and adduct formation with SO_3^{2-} and CN^- have been described.[21-23] These reactions were also first order in Fl(*n*) [or dFl(*n*)]. The typical absorption maxima and rate constants are summarized in Tables I and II.

Theoretical Calculations. In order to obtain an insight into the equilibrium geometry of Fl(*n*), the ab initio MO calculation of a model compound, 10-phenylisoalloxazine, has been carried out with the IMSPACK program[24] with the STO-3G minimal basis set of the standard parametrization.[25] The use of more extended basis sets (4-31G or 6-31G) may be recommended for an energy calculation such as potential energy surface or bond energy. It is known, however, that the STO-3G minimal basis set provides reasonable results for the molecular geometry.[26] Since the size of the present molecule is considerably large, we decided to use

(20) Hemmerich, P.; Massey, V.; Fenner, H. *FEBS Lett.* **1977**, *84*, 5.
(21) Shinkai, S.; Kusano, Y.; Manabe, O. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1111.
(22) Shinkai, S. *Makromol. Chem.* **1978**, *179*, 2637.
(23) Shinkai, S.; Harada, A.; Manabe, O.; Yoneda, F. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 928.
(24) Morokuma, K.; Kato, S.; Ktaura, K.; Ohmine, I.; Sakai, S.; Obara, S. Program No. 0372, IMS Computer Center Program Library; The Institute for Molecular Science: Okazaki, Japan, 1980.
(25) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724.
(26) Norrestam, R.; Stensland, B. *Acta Crystallogr.* **1972**, *B28*, 440.

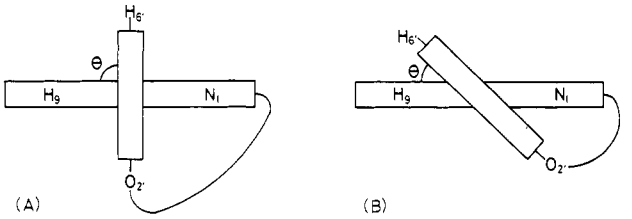


Figure 1. Top views of (5-deaza)isoalloxazinophanes with long-strap bridging (A) and short-strap bridging (B).

the STO-3G minimal basis set for the present calculation.

In the practical calculation, the experimental geometry of 10-phenyllumiflavin[26] was used for the isoalloxazine part of 10-phenylisoalloxazine because the electronic structure of lumiflavin and isoalloxazine is expected to be essentially similar in the ground state. A regular hexagon geometry has been assumed for the phenyl part, where the C–C and C–H bond distances are taken to be 1.40 Å and 1.09 Å, respectively.

Results and Discussion

Comments on the Synthesis of Isoalloxazinophanes. The synthetic study of flavinophanes and 5-deazaflavinophanes has been very limited. To the best of our knowledge there exist only two precedents:[18] for example, Zipplies et al.[18] synthesized [4]metacyclo[3](6,10)isoalloxazinophane via a very complicated route. The object of our investigation is to change the strap length systematically, so that we developed a new method which included the cyclization step at the end of the synthetic route. Thus, (5-deaza)isoalloxazinophanes were synthesized by the reaction of 10-(2-hydroxyphenyl)isoalloxazine (**3**) or 10-(2-hydroxyphenyl)-5-deazaisoalloxazine (**4**) with $Br(CH_2)_nBr$ in the presence of cesium carbonate under the high-dilution conditions. Examination of Corey–Pauling–Koltun molecular models suggests that (5-deaza)isoalloxazinophanes with $n \geq 8$ seem free from steric strain while a strap shorter than $n = 7$ induces some steric strain in the (5-deaza)isoalloxazine structure. In fact, yields for n = 8–12 were relatively good (7.2–26%) while those for $n = 6$ were extremely low, and dFl(6) was obtained only when the reaction temperature was enhanced up to 100 °C.

NMR and UV–Visible Spectroscopic Studies. Ring strain in (5-deaza)isoalloxazinophanes could be relaxed in two different ways: that is, (i) the isoalloxazine ring is deformed out of the plane, or (ii) the dihedral angle (θ in Figure 1) between the isoalloxazine ring and the 10-phenyl ring is changed. As recorded in Table I, the absorption maxima of Fl(n) and dFl(n) are almost constant (regardless of the ring size) and are comparable with those of noncyclic Fl(Bu) and dFl(Bu). Thus, the data in Table I suggest that ring strain is possibly relaxed by the change in the dihedral angle θ (possibility ii).

We found that the conformational change related to the rotation of the C(1′)–N(10) bond is conveniently monitored by ^{1}H NMR (400 MHz; GEOL GX-400). Figure 2 shows chemical shifts of aromatic protons in the 7–8 ppm region. Examination of Figure 2 reveals that Fl(n) and dFl(n) with $n \geq 10$ give chemical shifts comparable with those of noncyclic Fl(Bu) and dFl(Bu), whereas chemical shifts of 9-H and 6′-H with $n \leq 8$ move more and more to lower magnetic field as the strap becomes shorter. The finding supports the view that Fl(n) and dFl(n) with $n \geq 10$ are free from steric strain. In these strain-free (5-deaza)isoalloxazinophanes, 9-H and 6′-H are placed on the 10-phenyl ring and the isoalloxazine ring, respectively (type A in Figure 1). As a result, these protons are strongly affected by the ring current characteristic of the π-conjugate system. When θ is smaller than 90° (type B in Figure 1), these protons are less shielded by the ring current, resulting in a gradual downfield shift. For example, the chemical shift for 9-H moves to lower magnetic field by 0.39 ppm (6.92 → 7.31 ppm) for Fl(n) and by 0.40 ppm (6.91 → 7.31 ppm) for dFl(n) on going from n = 12 to n = 6. We believe that the difference in the chemical shifts is roughly correlated with θ.

Another useful NMR method to probe the bond rotation is the nuclear Overhauser effect (NOE). As the peak intensity in NOE is proportional to r^{-6} (r: distance between two nuclei), the slight change in the distance is sensitively amplified by the peak intensity.

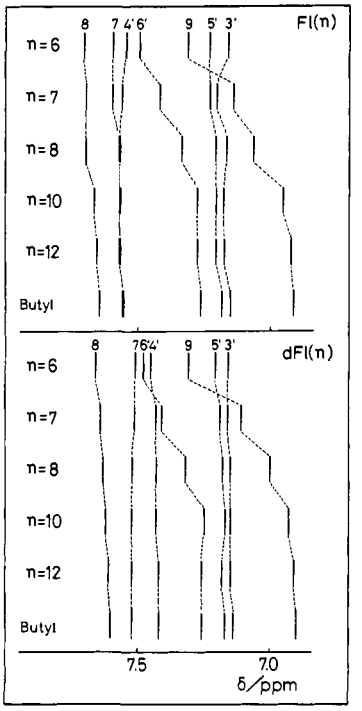


Figure 2. ^{1}H NMR chemical shift of the aromatic protons: $CDCl_3$; room temperature; 400-MHz JEOL GX-400.

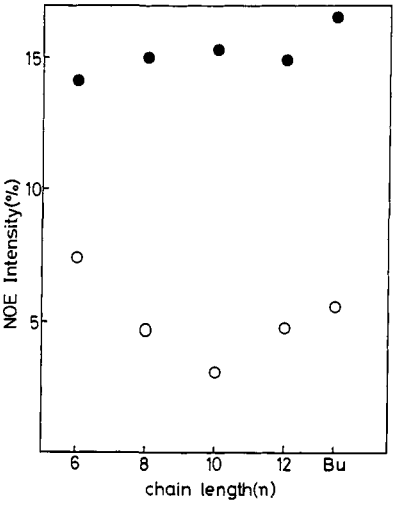


Figure 3. NOE intensities of 8-H (filled circles) and 6′-H (open circles) plotted against the chain length.

We measured ^{1}H NMR spectra of aromatic protons in dFl(n) while 9-H was saturated. As shown in Figure 3, the peak intensity for 8-H is almost constant (0.15 ± 0.01). Under identical conditions, the peak intensity for 6′-H decreases with increasing ring size from n = 6 to n = 10. The result indicates that the distance between 9-H and 8-H is constant while that between 9-H and 6′-H increases with increasing ring size. Clearly, this change is rationalized in terms of the θ change caused by the C(1′)–N(10) bond rotation. On the other hand, it is not clear yet why the NOE intensities of 6′-H for dFl(12) and dFl(Bu) are greater than that for dFl(10). One possible rationale is that θ for dFl(10) is fixed at nearly 90° while dFl(12) and dFl(Bu), the 10-phenyl ring of which has the rotational freedom, give an average θ smaller than 90°.

When dFl(7) was reduced by $NaBH_4$ in CD_3OD, chemical shifts for aromatic protons moved to higher magnetic field. In particular, the peak for 9-H showed an upfield shift (by 0.91 ppm: 7.11 → 6.20 ppm) larger than that of any other aromatic proton. A similar upfield shift was observed for 9-H of other Fl(n) and dFl(n) with $n \leq 8$. The finding suggests that θ is increased upon

Table III. Crystal Data

formula	$C_{23}H_{21}N_3O_3$
M	387.4
crystal system (recrystallized from ethyl acetate)	triclinic
space group	$P\bar{1}$
cell dimensions	
a/Å	10.920 (4)
b/Å	10.662 (4)
c/Å	9.570 (3)
α/deg	100.21 (3)
β/deg	68.30 (3)
γ/deg	113.85 (2)
volume/Å^3	946.4 (6)
Z	2
D_{exptl}/Mg m^{-3}	1.36
R	0.065

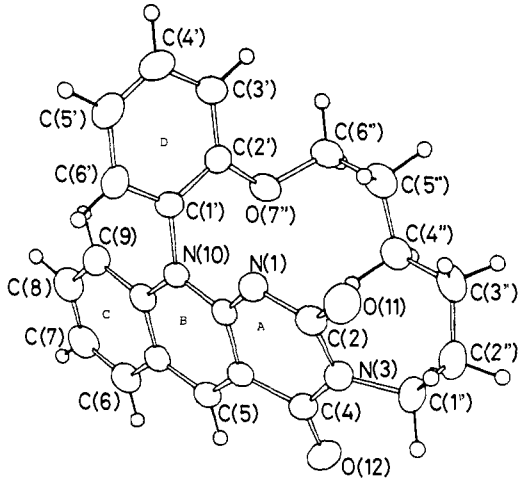


Figure 4. X-ray structure of dFl(6).

reduction of the (5-deaza)isoalloxazine ring. It is known that reduced flavins and 5-deazaflavins employ a folded conformation through N(5)–N(10) or C(5)–N(10)[15-18,27,28] and steric crowding in oxidized (5-deaza)flavins is significantly relaxed in reduced forms.[27-31] This steric relaxation would allow the change in the dihedral angle to 90°, which can minimize steric crowding arising from the 10-phenyl ring.

X-ray Crystallographic Studies. We determined the crystal structure of dFl(6) having the shortest ring strap. The crystal data are summarized in Table III, and the molecular structure is illustrated in Figures 4 and 5 (bond lengths and bond angles are deposited as supplementary material). The remarkable feature of this structure is that the dihedral angle θ between the rings B and D (76.5°) is much smaller than 90°. It is thus clear that ring strain is primarily relaxed by the rotation of the C(1')–N(10) single bond.

It is known that the ring skeleton of the oxidized isoalloxazine is essentially planar:[16] the largest deviation of any atom from a

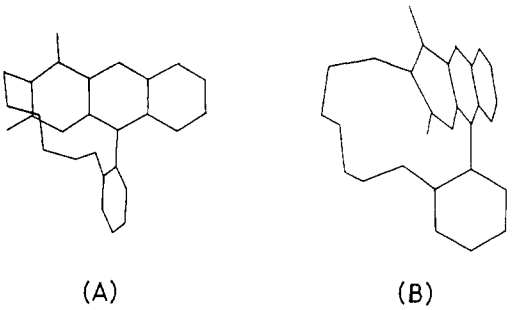


Figure 5. X-ray structure of dFl(6). In (A) the least-squares plane of the isoalloxazine ring is placed in the sheet plane. In (B) the 10-phenyl ring is placed in the sheet plane.

Table IV. Deviation (Å) from the Least-Squares Plane of the Three Rings (A, B, and C)

atom	deviation	atom	deviation
N(1)	−0.079	C(7)	−0.018
C(2)	−0.0174	C(8)	0.109
N(3)	0.234	C(9)	0.139
C(4)	0.065	C(9a)	0.037
C(4a)	−0.110	N(10)	0.074
C(5)	−0.150	C(10a)	−0.053
C(5a)	−0.106	O(11)	−0.135
C(6)	−0.123	O(12)	0.135

least-squares plane through 14 non-hydrogen atoms in the three rings is less than 0.1 Å. We recently succeeded in the X-ray analysis of 3-methyl-10-(2-hydroxyphenyl)isoalloxazine.[32] In this isoalloxazine, the three rings are nearly coplanar: the dihedral angle between the rings A and B is 2.2°, and that between the rings B and C is also 2.2°. On the other hand, the isoalloxazine plane of dFl(6) is not so planar as those in the noncyclic analogues: the dihedral angle between the rings A and B is 9.5°, and that between the rings B and C is 1.3°. The relatively large dihedral angle between rings A and B must be induced by ring strain. The deviation of 16 atoms (including two oxygens) from a least-squares plane is recorded in Table IV. It is seen from Table IV that the nine atoms have a deviation greater than 0.1 Å and the greatest deviation (0.234 Å) is observed for N(3), the bridgehead atom for strap. These X-ray data suggest that the isoalloxazine plane in dFl(6) is significantly deformed by the short $(CH_2)_6$ strap. At present, we cannot specify if the slight deformation of the isoalloxazine plane is also reflected by the reactivities.

Theoretical Calculations. When the isoalloxazine ring and the 10-phenyl ring in 10-phenylisoalloxazine can enjoy coplanarity (i.e., $\theta = 0°$), the maximum delocalization of π-electrons is realized. However, steric hindrance between the isoalloxazine ring and the phenyl ring also becomes maximal at $\theta = 0°$. In contrast, at $\theta = 90°$ there exists no delocalization between the isoalloxazine ring and the phenyl ring, but steric hindrance is expected to be minimal. Therefore, one can expect an equilibrium geometry at $0° < \theta < 90°$.

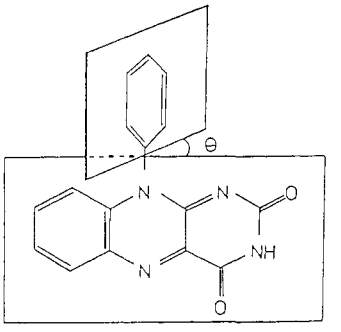


Figure 6 indicates the ab initio MO calculation of 10-phenylisoalloxazine plotted against θ. It is seen from Figure 6

(27) Shinkai, S.; Nakao, H.; Manabe, O. *Tetrahedron Lett.* **1985**, *26*, 5183.

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

(29) Shinkai, S.; Nakao, H.; Kuwahara, I.; Miyamoto, M.; Yamaguchi, T.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* **1988**, 313.

(30) Shinkai, S.; Nakao, H.; Yamaguchi, T.; Manabe, O. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 307.

(31) According to the ^{15}N NMR study by Müller et al.,[17] the reduced neutral isoalloxazine is slightly bent in an apolar solvent whereas the formation of hydrogen bonds in a protic solvent decreases the bend. In the present study, the bent structures can be proposed for 1,5-dihydro(5-deaza)isoalloxazine skeletons in $Fl_{red}(n)$ and $dFl_{red}(n)$ because of several lines of evidence: (i) chemical shifts for 9-H significantly move to higher magnetic field upon reduction to $Fl_{red}(n)$ and $dFl_{red}(n)$, (ii) (5-deaza)isoalloxazines with axial or planar chirality, which do not racemize in the oxidized state, easily racemize when they are reduced to 1,5-dihydro forms,[28-30] and (iii) the X-ray structure analysis of 5-methyl-1,5-dihydro-5-deazaisoalloxazinophane establishes that the central ring adopts a boat-shaped conformation (Shinkai, S.; Nishiyama, N.; Matsuda, T.; Kanazawa, R.; Kawase, A.; Manabe, O. *Chem. Lett.* **1988**, 1861). These results all support the bent structure for $Fl_{red}(n)$ and $dFl_{red}(n)$.

(32) Shinkai, S.; Kawanabe, S.; Kawase, A.; Yamaguchi, T.; Manabe, O.; Harada, S.; Nakamura, H.; Kasai, N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2095.

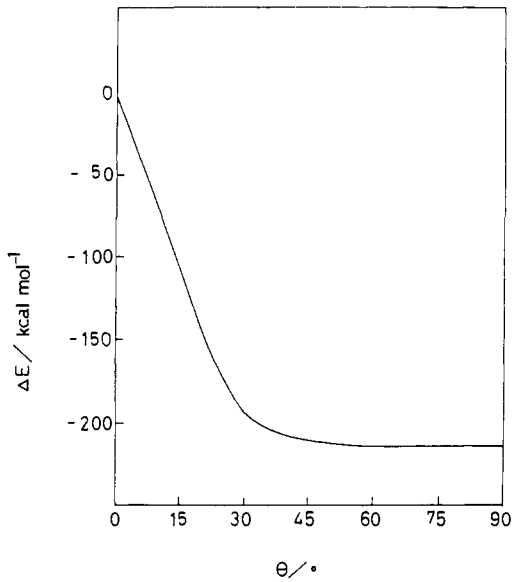


Figure 6. Ab initio calculation of 10-phenylisoalloxazine.

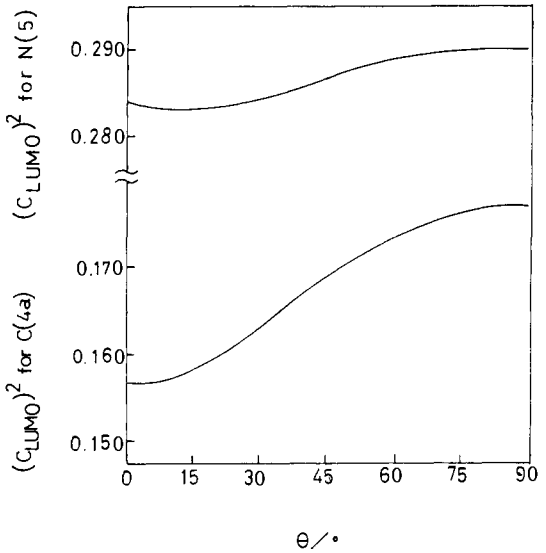


Figure 7. Square of the AO coefficient for C(4a) and N(5) in the LUMO.

that this molecule is more destabilized with decreasing θ and in particular the ΔE conspicuously increases below $\theta = 60°$. The result implies that the equilibrium geometry is expected to be 60–90° and governed mainly by steric hindrance rather than by resonance between the isoalloxazine ring and the 10-phenyl ring. The θ value determined for dFl(6) by the X-ray analysis was 76.5°. This value is in good accord with θ expected for the equilibrium geometry.

Oxidized flavins serve as good electron acceptors in many oxidation/reduction reactions.[14] The electron-accepting MO is, of course, the lowest unoccupied MO (LUMO). Therefore, the reactivity of each atom for the nucleophilic attack or the electron transfer is defined as the square of the AO coefficient (C_{LUMO}^2) in LUMO. In Figure 7, C_{LUMO}^2 values for C(4a) and N(5) are plotted against θ. Interestingly, Figure 7 tells us that the reactivity of C(4a) sensitively changes in response to the θ change while that of N(5) is less affected by θ. Thus, rate constants for reactions involving C(4a) intermediates may be more dependent upon the θ change.

Kinetic Studies. In order to establish a possible relation between ring strain and reactivities of (5-deaza)isoalloxazines, we carried out three different reactions mediated by flavins: (i) oxidation of 1-benzyl-1,4-dihydronicotinamide (BNAH), (ii) oxidation of 1,4-butanedithiol (BDT), and (iii) 5-adduct formation with Na_2SO_3 or KCN. Reactions i, ii, and iii (except KCN) were applied to Fl(n), and reactions i and iii (except Na_2SO_3) were applied to dFl(n).[33]

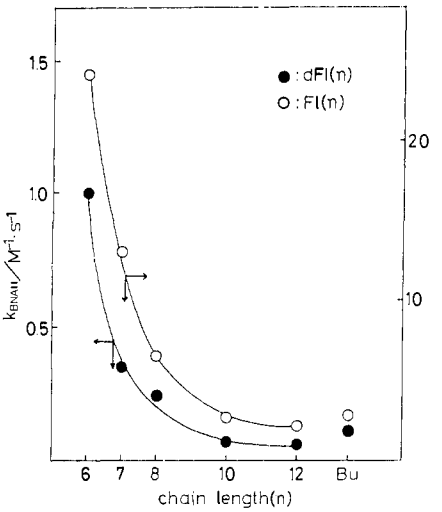


Figure 8. Second-order rate constants (k_{BNAH}) for the reaction of BNAH with Fl(n) and dFl(n): 30 °C, water/MeOH (50.8:49.2 v/v), pH 9.0 with 6 mM borate buffer, and [BNAH] = 1.04×10^{-4} M for Fl(n) [$(2.1–2.6) \times 10^{-5}$ M, aerobic] and 3.50×10^{-4} M for dFl(n) [$(2.2–2.6) \times 10^{-5}$ M, anaerobic].

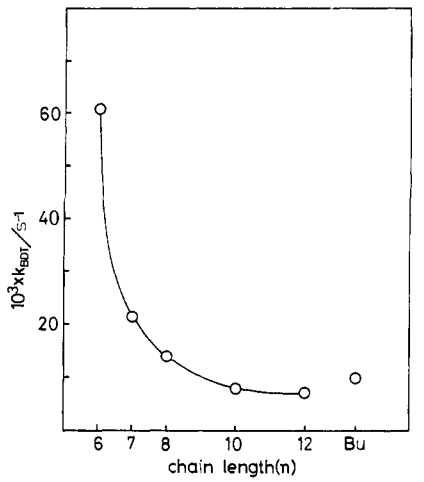


Figure 9. Pseudo-first-order rate constants (k_{BDT}) for the reaction of BDT with Fl(n): 30 °C, anaerobic, water/MeOH (50.8:49.2 v/v), pH 9.0 with 60 mM borate buffer, [BDT] = 5.02×10^{-3} M, and [Fl(n)] = $(2.1–2.6) \times 10^{-5}$ M.

In Figure 8 second-order rate constants (k_{BNAH}) for the reaction of BNAH with Fl(n) and dFl(n) are plotted against n. This reaction proceeds via a transition state with a face-to-face orientation, and N(5) [or C(5)] is included as a reaction center.[34,35] The k_{BNAH} values for Fl(n) and dFl(n) with $n \geq 10$ are almost constant and comparable with those for Fl(Bu) and dFl(Bu) while the k_{BNAH} values for Fl(n) and dFl(n) with $n \leq 8$ increase with decreasing ring size. The k_{BNAH} values for Fl(6) (24.2 M^{-1} s^{-1}) and dFl(6) (1.00 M^{-1} s^{-1}) are greater by 11- and 16-fold than those for Fl(12) and dFl(12), respectively. This trend is well correlated with the ^1H NMR chemical shifts of 9-H and 6′-H (Figure 2). The finding supports the view with the reaction rate is speeded up because of the enforced rotation of the C(1′)–N(10) bond in the initial state. A similar relationship was observed for oxidation of BDT by Fl(n) (Figure 9), k_{BDT} (pseudo-first-order rate con-

(33) The reaction between Na_2SO_3 and dFl(n) was too fast to follow by the conventional method. The reaction between KCN and Fl(n) did not proceed.

(34) Porter, D. J. T.; Blankenhorn, G.; Ingraham, L. L. *Biochem. Biophys. Res. Commun.* **1973**, *52*, 447.
(35) Blankenhorn, G. *Eur. J. Biochem.* **1975**, *50*, 351.

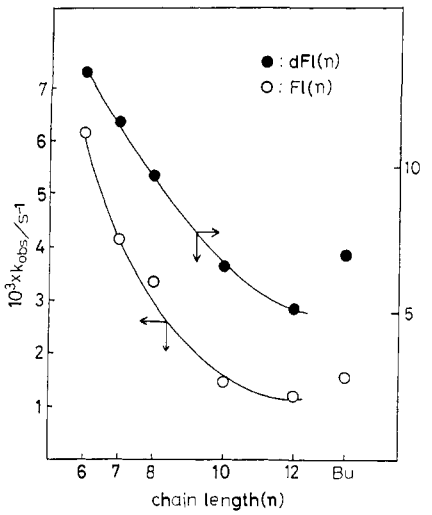


Figure 10. Pseudo-first-order rate constants (k_{obs}) for the reactions of Fl(n) and Na_2SO_3 (open circles) and of dFl(n) and KCN (filled circles): 30 °C, aerobic, water/MeOH (50.8:49.2 v/v), [Fl(n)] = (2.1–2.6) × 10^{-5} M, [dFl(n)] = (2.2–2.6) × 10^{-5} M, [Na_2SO_3] = 3.00 × 10^{-2} M, and [KCN] = 2.00 × 10^{-3} M.

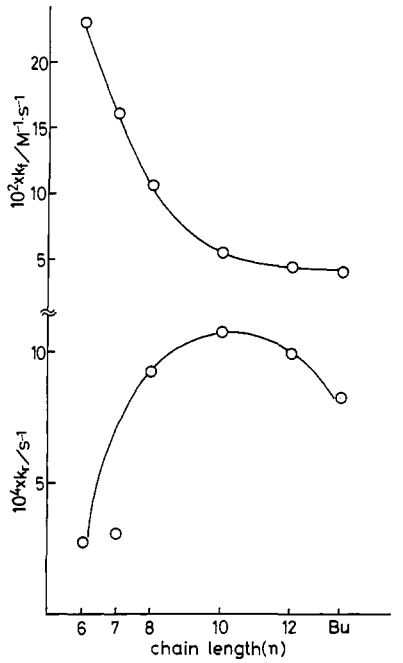


Figure 11. Forward rate constants (k_f) and reverse rate constants (k_r) for the reaction of Fl(n) and Na_2SO_3.

stants) for Fl(6) (6.08 × 10^{-2} s^{-1}) being greater by a factor of 8.3 than that for Fl(12). It is known that this reaction proceeds via the 4a-adduct intermediate.[36-38] Therefore, ring strain in the oxidized form is also released by the 4a-adduct formation.[39]

Further insight into the correlation between ring strain and flavin reactivities could be obtained from the reactions of Fl(n) and dFl(n) with nucleophiles such as SO_3^{2-} and CN^-. The apparent first-order rate constants (k_{obs}) for these nucleophiles showed similar dependence on the ring size (Table II and Figure 10). Since these reactions proceed under equilibria, one can estimate k_f for the forward reaction and k_r for the reverse reaction

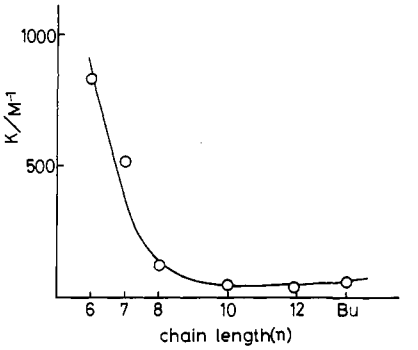


Figure 12. Equilibrium constants (K) for the reaction of Fl(n) and Na_2SO_3.

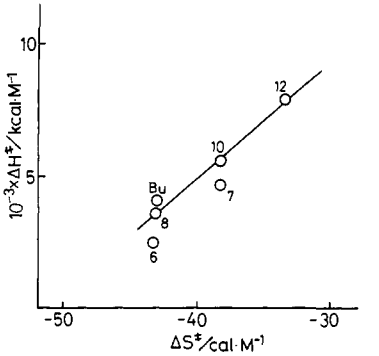


Figure 13. $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ compensation relationship.

Table V. Activation Parameters for the Reaction of Fl(n) and BNAH

Fl(n)	$\Delta G^{\ddagger}_{303}$/kcal mol^{-1}	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta S^{\ddagger}$/eu
Fl(6)	15.8	2.58	−43.5
Fl(7)	16.3	4.71	−38.3
Fl(8)	16.6	3.58	−43.1
Fl(10)	17.2	5.61	−38.3
Fl(12)	17.2	7.09	−33.5
Fl(Bu)	17.1	4.08	−43.0

separately. We thus determined the k_f and k_r for the reaction of Fl(n) and SO_3^{2-} by eq 1,[40,41] where k_{obs} and K denote the

$$k_{obs} = k_f[SO_3^{2-}] + k_r = k_f[SO_3^{2-}] + k_f/K \quad (1)$$

apparent first-order rate constant and the equilibrium constant ($=k_f/k_r$ = [5-adduct]/[Fl(n)][SO_3^{2-}]), respectively. The plots of k_{obs} vs [SO_3^{2-}] [(1–10) × 10^{-2} M] resulted in good linear relationships ($r > 0.99$; data not shown here). From the least-squares computation of the slope and the intercept we obtained k_f, k_r, and K and plotted them against n in Figures 11 and 12. It is seen from Figure 11 that k_f increases with decreasing ring size but the reverse is found for k_r. The k_f value for Fl(6) (0.231 M^{-1} s^{-1}) is greater by a factor of 5.2 than that for Fl(12) (0.0442 M^{-1} s^{-1}), whereas k_r for Fl(6) (2.77 × 10^{-4} s^{-1}) is smaller by a factor of 3.6 than that for Fl(12) (9.90 × 10^{-4} s^{-1}). As a result, K for Fl(6) (834 M^{-1}) is greater by a factor of 19 than that for Fl(12) (44.6 M^{-1}). These results are consistently understood by the view that both the forward reaction and the reverse reaction are affected by destabilization of the oxidized state.[42]

It is interesting to know how ring strain in Fl(n) is reflected by activation parameters. We measured the k_{BNAH} for the reaction of Fl(n) and BNAH at 20, 30, and 40 °C and determined $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. The results are summarized in Table V. Examination of Table V reveals that the increase in $\Delta H^{\ddagger}$ is well compensated

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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.

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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

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