Atropisomeric Flavoenzyme Models with a Modified Pyrimidine Ring: Syntheses, Physical Properties, and Stereochemistry in the Reactions with NAD(P)H Analogs

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Optically active 5-deazaflavin derivatives (3-aryl-10-(4-*tert*-butylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione) with an axial chirality at the pyrimidine ring have been synthesized, and the kinetics of enantiomerization have been measured for some of them. The absolute configurations of these compounds have been determined by X-ray crystallographic analysis and chemical reactions for the first time in atropisomeric flavoenzyme models. Enantioface-differentiating (net) hydride-transfer reactions with 1-benzyl-1,4-dihydronicotinamide (BNAH) have revealed that the selectivity of the reacting face of the 3-[2-(hydroxymethyl)phenyl] derivative **1** changes depending on the presence or absence of Mg^{2+}; the hydroxymethyl group of **1** exerts steric inhibition in the absence of Mg^{2+}, whereas it facilitates the approach of BNAH in the presence of Mg^{2+}. Asymmetric (net) hydride-transfer reactions with chiral 1,4-dihydro-2,4-dimethyl-*N*-(α-methylbenzyl)-1-propylnicotinamide (Me_2PNPH) predict that the most favorable intermolecular arrangement of these two molecules at the transition state is the one in which the pyrimidine ring of **1** and the carbamoyl group of Me_2PNPH tend to face each other and the maximum overlap of their molecular planes is achieved regardless of the presence or absence of Mg^{2+}. The arrangement mimics that of FAD and NADPH in the active site of a flavoenzyme. The present result indicates an energetically favorable overlap of the molecular planes of a flavin and an NAD(P)H coenzyme, as well as a significant influence of functional groups from an apoenzyme in proximity to a flavin coenzyme on the stereochemistry of biological redox reactions.

Introduction

Flavoenzymes require flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as a coenzyme and catalyze oxidation−reduction reactions in biological systems, in which the flavin group is the oxidizing or reducing agent.[4−7] At the active sites of flavoenzymes, flavin coenzymes are covalently bound or tightly held to apoproteins to form chiral environments, and the reactivity of a flavin coenzyme and stereoselectivity in the reactions with substrates are markedly enhanced.[8−14]

To clarify the reaction mechanism of flavoenzymes, optically active flavins (Fl) and 5-deazaflavins (dFl) with a function as apoproteins have been synthesized and investigated widely. For example, Shinkai *et al.*[15−19] synthesized optically active flaviophanes and 5-deazaflavinophanes, which oxidized optically active thiols and NAD(P)H analogs in an asymmetric manner. Yoneda *et al.*[20−22] synthesized an optically active dFl with axial and planar chirality, where one face of the molecule was blocked by an alkyl chain at the N(10) position, and determined its absolute configuration.[22] It has been suggested that a ternary complex composed of dFl, Mg^{2+}, and NAD(P)H analog is involved in the mimetic intercoenzyme reactions. These flavoenzyme models oxidized NAD(P)H analogs asymmetrically, revealing that asymmetry in the face of the flavin significantly influences the stereochemistry of (net) hydride-transfer reactions with an NAD(P)H analog.

Recently, Yoneda *et al.*[23−25] synthesized a series of dFls with an axial chirality at the N(3) position and observed diastereofacial deactivation through steric hindrance[23,24]

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(4) Walsh, C. *Enzymatic Reaction Mechanism*; W. H. Freeman: San Francisco, 1979; pp 358−431.
(5) Massey, V.; Hemmerich, P. *Biochem. Soc. Trans.* **1980**, *8*, 246−257.
(6) Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148−155.
(7) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256−262.
(8) Fisher, J.; Walsh, C. *J. Am. Chem. Soc.* **1974**, *96*, 4345−4346.
(9) Yamazaki, S.; Tsai, L.; Stadtman, T. C.; Jacobson, F. S.; Walsh, C. *J. Biol. Chem.* **1980**, *255*, 9025−9027.
(10) Pai, E. F.; Schulz, G. E. *J. Biol. Chem.* **1983**, *258*, 1752−1757.
(11) Jacobson, F.; Walsh, C. *Biochemistry* **1984**, *23*, 979−988.
(12) Yamazaki, S.; Tsai, L.; Stadtman, T. C.; Teshima, T.; Nakaji, A.; Shiba, T. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1364−1366.
(13) Manstein, D. J.; Pai, E. F.; Schopfer, L. M.; Massey, V. *Biochemistry* **1986**, *25*, 6807−6816.
(14) Manstein, D. J.; Massey, V.; Ghisla, S.; Pai, E. F. *Biochemistry* **1988**, *27*, 2300−2305.
(15) Shinkai, S.; Yamaguchi, T.; Nakao, H.; Manabe, O. *Tetrahedron Lett.* **1986**, *27*, 1611−1614.
(16) Shinkai, S.; Yamaguchi, T.; Kawase, A.; Kitamura, A.; Manabe, O. *J. Chem. Soc., Chem. Commun.* **1987**, 1506−1508.
(17) Shinkai, S.; Kawase, A.; Yamaguchi, T.; Manabe, O. *J. Chem. Soc., Chem. Commun.* **1988**, 457−458.
(18) Shinkai, S.; Kawase, A.; Yamaguchi, T.; Manabe, O.; Wada, Y.; Yoneda, F.; Ohta, Y.; Nishimoto, K. *J. Am. Chem. Soc.* **1989**, *111*, 4928−4935.
(19) Shinkai, S.; Yamaguchi, T.; Kawase, A.; Manabe, O.; Kellogg, R. M. *J. Am. Chem. Soc.* **1989**, *111*, 4935−4940.
(20) Kawamoto, T.; Tanaka, K.; Yoneda, F.; Hayami, J. *Tetrahedron Lett.* **1989**, *30*, 7431−7434.
(21) Kawamoto, T.; Tanaka, K.; Kuroda, Y.; Yoneda, F. *Chem. Lett.* **1990**, 1197−1200.
(22) Kawamoto, T.; Taga, T.; Bessho, K.; Yoneda, F.; Hayami, J. *Tetrahedron Lett.* **1994**, *35*, 8631−8634.
(23) Kawamoto, T.; Tomishima, M.; Yoneda, F.; Hayami, J. *Tetrahedron Lett.* **1992**, *33*, 3169−3172.
(24) Kawamoto, T.; Tomishima, M.; Yoneda, F.; Hayami, J. *Tetrahedron Lett.* **1992**, *33*, 3173−3176.

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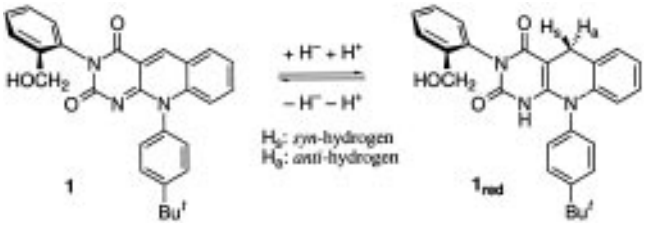
Scheme 1[27]



as well as diastereofacial activation through intramolecular acid catalysis.[25] The results suggest that there is diastereoselective interaction between the pyrimidine ring of a dFl and the carbamoyl group of an NAD(P)H analog.

Here, we designed and synthesized an optically active dFl with a 2-(hydroxymethyl)phenyl group at the N(3) position, **1**, as a novel flavoenzyme model (Scheme 1).[26]

The hydroxymethyl group that occupies one face of the pyrimidine ring has the ability to coordinate onto a metal ion through an electronegative oxygen atom that is absent in an alkyl group. The hydroxymethyl group is expected to mimic a potential functional group of an apoprotein in proximity to a flavin coenzyme. By comparing the physical properties and stereochemical reactivity of **1** with those of analogous dFl derivatives, especially those of **2**, which has a methyl group instead of a hydroxymethyl group, we will discuss the effects of the hydroxymethyl group on the stereochemistry in the reactions with NAD(P)H analogs. The intermolecular arrangement between a flavin model and an NAD(P)H analog at the transition state of (net) hydride-transfer reaction will also be discussed.

Results and Discussion

Syntheses and Optical Resolutions of dFl Derivatives. dFl derivatives were prepared according to the procedure shown in Scheme 2. The synthesis of the dFl skeleton was followed by the procedure reported by Yoneda *et al.*[28] 5-Deuterated derivatives of dFl, **2**-*5-d* and **8**-*5-d*, were synthesized by the reactions of **7** and **13** with 2-fluoro[α-^{2}H]benzaldehyde, respectively. The 5-deuterated counterpart of **1**, **1**-*5-d* was prepared from **8**-*5-d* according to the same synthetic route as that shown in Scheme 2. Since both the 5-deazaisoalloxazine ring and the carboxyl group were reduced in the reduction of **8** to $\mathbf{1_{red}}$, sodium borodeuteride was employed for this reaction instead of sodium borohydride in order to substitute two deuterium atoms at the C(5) position of $\mathbf{1_{red}}$.

Although not only the C(5) position but also the methylene part of the hydroxymethyl group in the substituent at the N(3) position of **1**-*5-d* is deuterated, the deuteration may not affect stereochemical results of the present reactions.

The optical resolutions of **1** and **2** were carried out by HPLC on a chiral stationary phase.[29,30] The (+)-enantiomers of **1** and **2** were obtained as the first fractions, respectively. The specific rotations of the enantiomers of **1** and **2** are shown in Table 1.

Table 1. Specific Rotations of the Enantiomers of 1, 2, and 19[a]

		$[\alpha]_D$ (abs config)	
dFl (*c*)	*T*, °C	1st fraction	2nd fraction
1 (1.00)	16	+59.1° (*S*)	−57.3° (*R*)
2 (1.01)	25	+25.1° (*R*)	−25.9° (*S*)
19 (1.00)	23	+7.5° (*R*)	−8.1° (*S*)

[a] All dFls were obtained in >99% ee.

Table 2. Rate Constants for Enantiomerization ($k_{rot.}$) of 1 and 2 in DMF

	$k_{rot.} \times 10^6$, s^{-1}	
T, °C	**1**	**2**
30	1.12 ± 0.01	1.07 ± 0.01
40	4.37 ± 0.06	4.08 ± 0.04
50	15.1 ± 0.2	14.2 ± 0.1
60	46.7 ± 0.7	55.1 ± 0.6
70	152 ± 3	170 ± 3

Table 3. Activation Parameters for Enantiomerization of 1 and 2

activation param	**1**	**2**
E_a, kcal mol^{-1}	25.0 ± 0.2	26.3 ± 0.4
$\Delta G^{\ddagger}$, kcal mol^{-1}	25.9 ± 0.02	26.0 ± 0.03
$\Delta H^{\ddagger}$, kcal mol^{-1}	24.4 ± 0.2	25.6 ± 0.4
$\Delta S^{\ddagger}$, cal mol^{-1} deg^{-1}	−5.26 ± 0.63	−1.31 ± 1.17

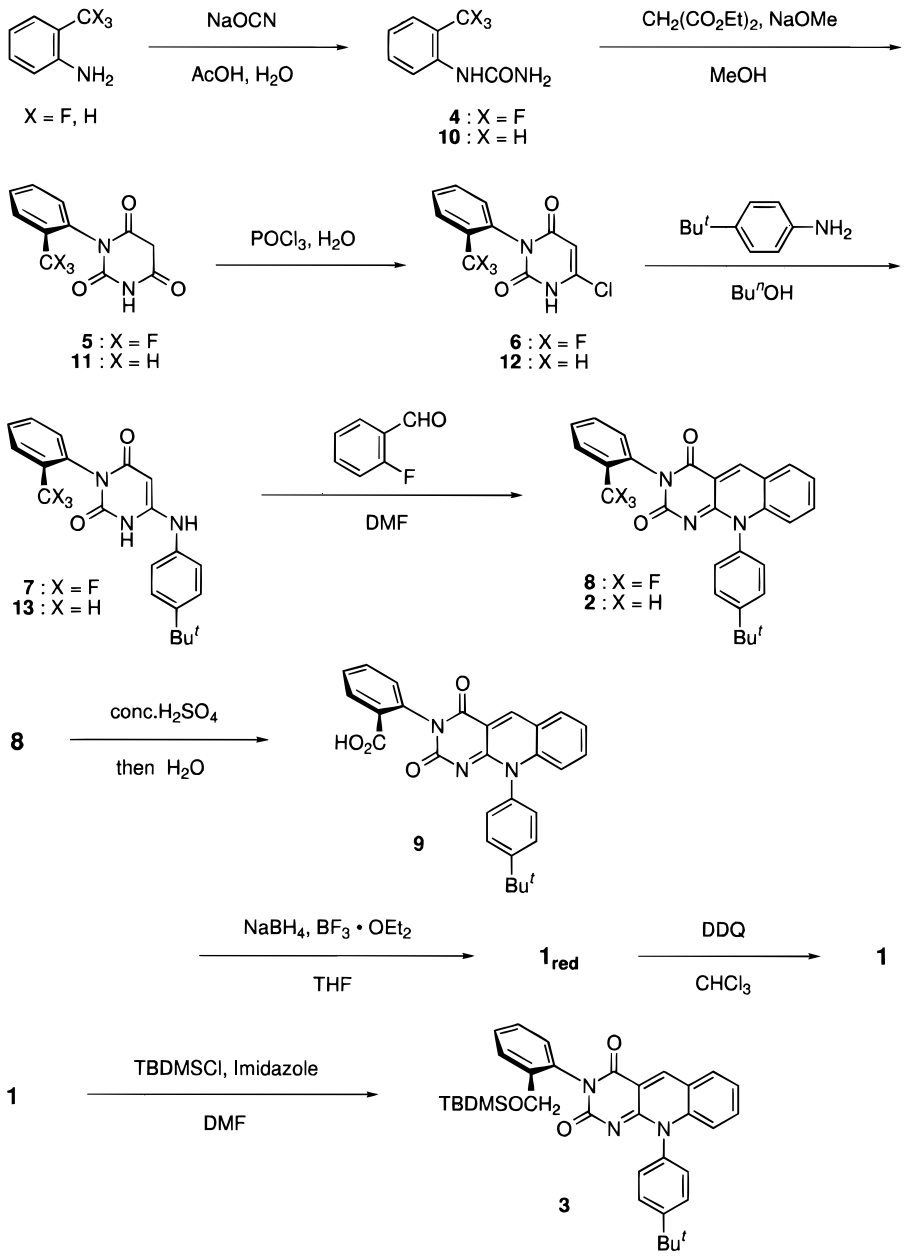
Thermal Enantiomerization. In order to estimate the conformational stability of **1** and **2**, the rate constants ($k_{rot.}$) and activation parameters for the rotation around the N(3)−C(aryl) bond in these compounds have been studied (Scheme 3). The rate constants are listed in Table 2. The Arrhenius and Eyring plots have excellent linear relationships ($r > 0.9996$). The activation parameters obtained from the plots are listed in Table 3. The results indicate that the difference between a hydroxymethyl group and a methyl group has little influence on the energy barrier for rotation around the N(3)−C(aryl) bond and confirm that the racemization of **1** and **2** can be disregarded under the present reaction conditions (within 3 h at 298 K).

X-ray Crystallographic Analyses and Determinations of the Absolute Configurations. Structures of crystals of **1** and **2** obtained by recrystallization from ethanol and toluene, respectively, have been revealed by X-ray crystallography. The ORTEP drawings are illustrated in Figures 1 and 2, and the crystallographic data are summarized in Table 4. There is an intermolecular hydrogen bond between the hydroxy group and the carbonyl group at the C(2) position of an adjacent molecule (Figure 1b).

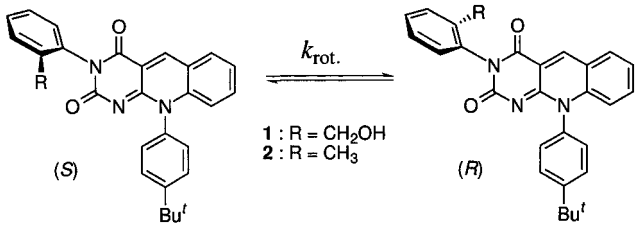
In order to determine the absolute configuration of **1** by X-ray crystallographic analysis, we first prepared several chiral dFls from **1** (Scheme 4)[31] and attempted to confirm the absolute configurations of these enantiomers. However, since these dFls are conformationally unstable or partially racemize before crystals suitable for

(25) Kawamoto, T.; Tomishima, M.; Kunitomo, J.; Yoneda, F.; Hayami, J. *Tetrahedron Lett.* **1992**, *33*, 7173−7176.
(26) Ohno, A.; Kunitomo, J.; Kawamoto, T.; Tomishima, M.; Bessho, K.; Yoneda, F. *Tetrahedron Lett.* **1994**, *35*, 9729−9732.
(27) Only one enantiomer is shown in the scheme for convenience.
(28) Nagamatsu, T.; Hashiguchi, Y.; Yoneda, F. *J. Chem. Soc., Perkin Trans. 1* **1984**, 561−565.
(29) Conditions for preparative chromatography of **1**: column, CHIRALCEL OD (2 cm ø × 25 cm); eluent, ethanol; flow rate, 3.5 mL/min; detection, UV 254 nm; retention times, 55.1 and 83.0 min: conditions for analytical chromatography of **1**: column, CHIRALCEL OD (0.46 cm ø × 5 cm); eluent, ethanol; flow rate, 0.2 mL/min; detection, UV 254 nm; retention times, 11.3 and 16.7 min.
(30) Conditions for preparative chromatography of **2**: column, CHIRALCEL OD (2 cm ø × 25 cm); eluent, ethanol; flow rate, 4.0 mL/min; detection, UV 254 nm; retention times, 78.5 and 156 min: conditions for analytical chromatography of **2**: column, CHIRALCEL OD (0.46 cm ø × 5 cm); eluent, ethanol; flow rate, 0.5 mL/min; detection, UV 254 nm; retention times, 7.6 and 14.1 min.
(31) Structures and purities of all dFl derivatives were confirmed by ^{1}H NMR.

Scheme 2[27]



Scheme 3



X-ray crystallographic analyses are obtained, we failed to determine the absolute configuration of **1** by this method.

Therefore, we designed **19**, which was expected to maintain its conformation for a long time even at room temperature. The synthesis of **19** was carried out according to Scheme 5, and the optical resolution was accomplished by HPLC.[32] The (+)-enantiomer was obtained from the first fraction. The specific rotations of the enantiomers of **19** are also listed in Table 1. It has been confirmed that the conformation of **19** is stable for over 1 month at room temperature.

From the X-ray crystallographic analysis of (−)-**19**, which was recrystallized from methanol, by means of anomalous dispersion effect of the bromine atoms, it has been confirmed that (−)-**19** has the *S* configuration.[33] This is the first successful determination of the absolute configuration of an optically active Fl or dFl derivative that has no chiral substituent in the molecule. The circular dichroism (CD) spectra of the enantiomers of **19** in acetonitrile are shown in Figure 3.

Next, (*S*)-(−)-**19** was debrominated by catalytic hydrogenation, and the resultant **2** was subjected to HPLC from which its conformation was determined to be (*R*)-(+) (Scheme 6).

(32) Conditions for preparative chromatography of **19**: column, CHIRALCEL OD (2 cm ø × 25 cm); eluent, ethanol; flow rate, 1.5 mL/min; detection, UV 254 nm; retention times, 89.5 and 95.6 min: conditions for analytical chromatography of **19**: column, CHIRALCEL OD (0.46 cm ø × 5 cm + 0.46 cm ø × 25 cm); eluent, ethanol; flow rate, 0.5 mL/min; detection, UV 254 nm; retention times, 23.7 and 29.1 min. The second fraction was obtained in >99% ee by repetition of the optical resolution.

(33) Kawai, Y.; Kunitomo, J.; Ohno, A. *Acta Crystallogr., Sect. C*, in press.

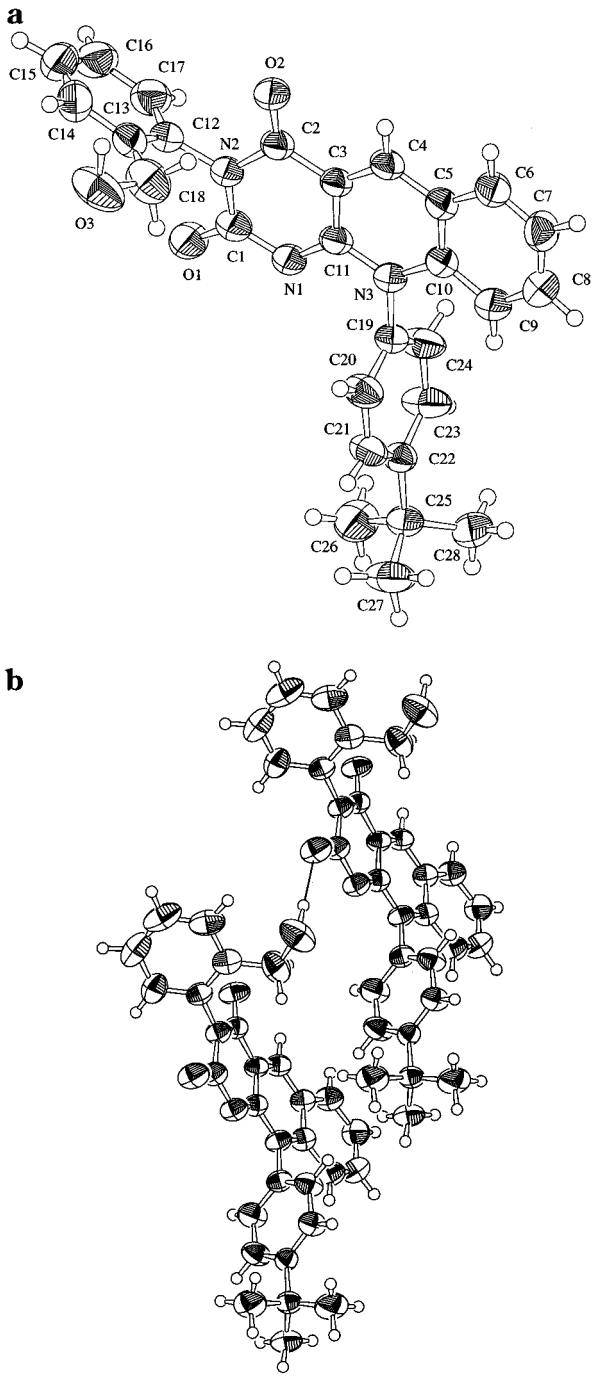


Figure 1. ORTEP drawings of (a) **1** in the front view and (b) two molecules of **1** linked by a hydrogen bond (indicated by a solid line) with displacement ellipsoids at 50% probability level. Only the (*S*)-(+)-enantiomer is illustrated in both drawings.

Finally, the (+)-enantiomer of **1** was converted into **2** according to the procedure shown in Scheme 6. The resultant **2** was subjected to HPLC, which confirmed that the compound was the (−)-enantiomer. Consequently, the absolute configuration of (+)-**1** has been assigned as *S*.

Enantioface-Differentiating (Net) Hydride-Transfer Reactions. In order to investigate the selectivity of the faces in which a (net) hydride is transferred, reductions of various dFl-*5*-*d*s with 1-benzyl-1,4-dihydronicotinamide (BNAH) were studied (Scheme 7). The reactions were carried out in acetonitrile at 298 K in the dark under argon atmosphere and monitored by TLC. *Syn*/*anti* ratios of the reacting faces were determined by ^{1}H NMR spectroscopy based on the area ratio of two diastereotopic protons at the C(5) position of the reduced

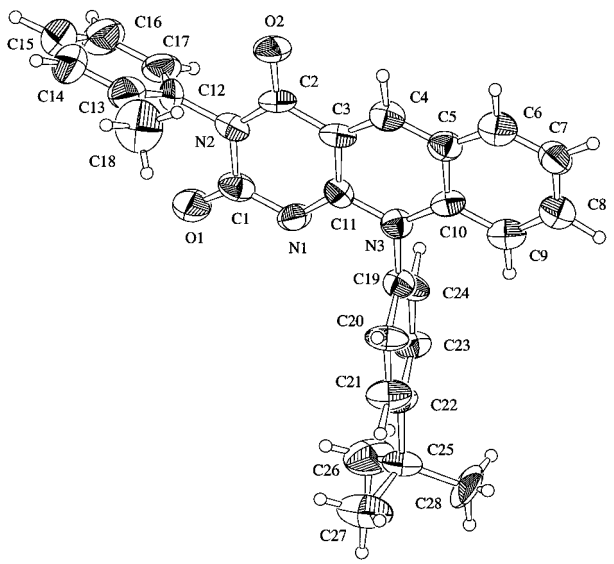


Figure 2. ORTEP drawing of **2** with displacement ellipsoids at 30% probability level. Only the (*S*)-(−)-enantiomer is illustrated here.

Table 4. Summary of Crystallographic Data[a]

dFl	(±)-**1**	(±)-**2**
formula	$C_{28}H_{25}N_3O_3$	$C_{28}H_{25}N_3O_2$
formula wt	451.52	435.52
cryst color, habit	yellow, prismatic	yellow, prismatic
cryst dimens, mm	0.64 × 0.20 × 0.20	0.85 × 0.20 × 0.10
cryst syst	monoclinic	orthorhombic
space grp	$P2_1/n$ (#14)	*Pbca* (#61)
a, Å	22.406(1)	22.340(6)
b, Å	13.200(4)	20.03(1)
c, Å	7.858(2)	10.534(5)
β, deg	94.31(1)	
V, Å^3	2317(1)	4713(6)
Z value	4	8
D_{calc}, g/cm^3	1.294	1.227
$2\theta_{max}$, deg	120.2	120.1
no. total reflns	3918	3967
no. unique reflns	3621	3967
no. observns ($I > 3.00\sigma(I)$)	2047	914
no. variables	408	298
R	0.042	0.061
R_w	0.043	0.072
S	1.64	1.96

[a] Common to all structures: diffractometer, Rigaku AFC7R; radiation, Cu Kα (λ = 1.541 78 Å); temperature, 20.0 °C.

Table 5. Enantioface-Differentiating (Net) Hydride-Transfer Reaction between Racemic dFl-*5*-*d* and BNAH[a]

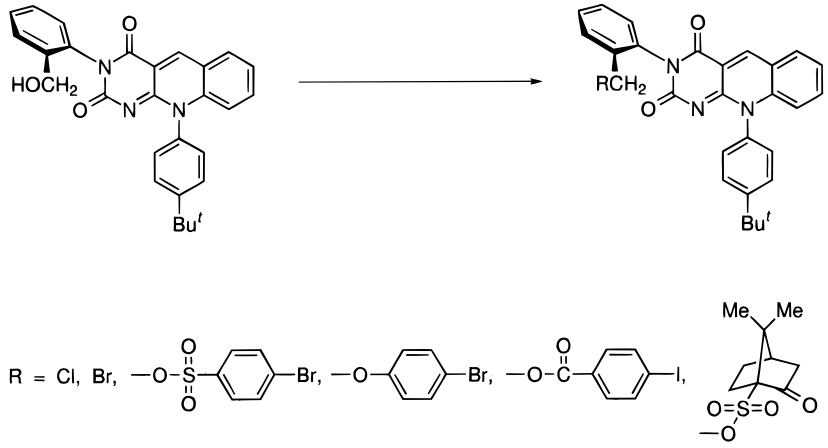
dFl-*5*-*d*	catalyst (equiv)[b]	reaction time, min	ratio of reacting faces, *syn*:*anti*[c]
1	$Mg(ClO_4)_2$ (5)	10	78:22
	CCl_3CO_2H (10)	60	31:69
2	$Mg(ClO_4)_2$ (5)	180	30:70
3	$Mg(ClO_4)_2$ (5)	180	20:80
8	$Mg(ClO_4)_2$ (5)	120	29:71

[a] [dFl-*5*-*d*] = 4.0 × 10^{-2} M, [BNAH] = 2.0 × 10^{-1} M. In the dark under Ar at 298 K. [b] Equivalency to [dFl-*5*-*d*]. [c] Estimated errors are within ±2 for all observed values.

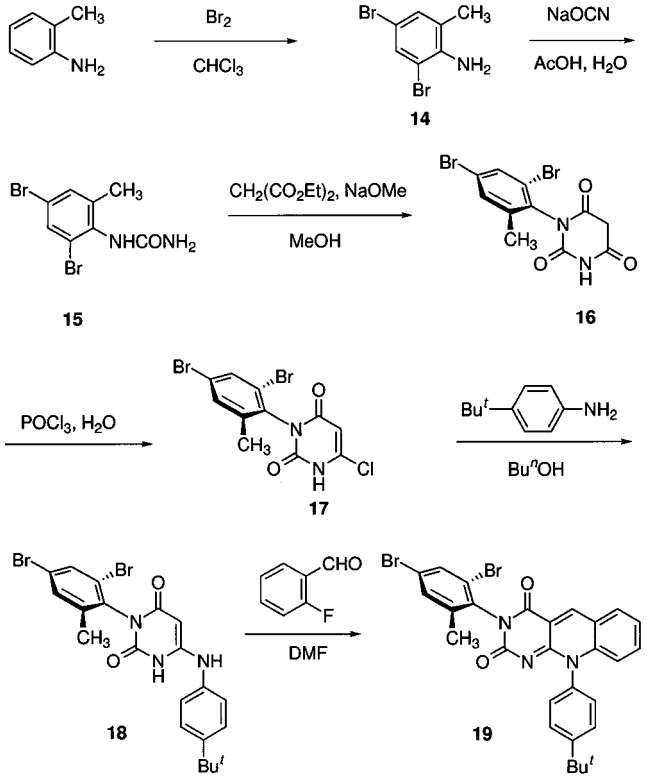
dFl-*5*-*d* thus obtained. *Syn* and *anti* hydrogen atoms were assigned in a similar manner as described previously.[24,25] The results are summarized in Table 5.

In order to distinguish diastereomeric hydrogens at the C(5) position, either the dFl derivative or an NAD(P)H analog must be substituted by deuteration at the C(5) or C(4) position, respectively. We employed dFls deuterated at their C(5) positions (**1**-*5*-*d*, **2**-*5*-*d*, **3**-*5*-*d*, and **8**-*5*-*d*) for

Scheme 4[27]



Scheme 5[27]



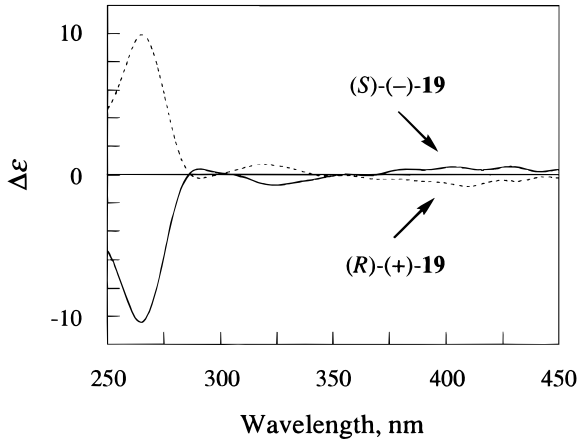


Figure 3. CD spectra of **19** in acetonitrile. The solid and broken lines represent the spectra of (*S*)- and (*R*)-enantiomers, respectively.

this purpose, and these deuterated dFls were reduced by an undeuterated NAD(P)H analog. However, for simplicity, we will denote the deuterated dFls without the term "*-5-d*" hereafter. The deuterated compounds are indicated appropriately in Tables 5 and 8 and the Experimental Section.

In the presence of Mg^{2+}, the (net) hydride transfer from BNAH to **2**, **3**, or **8** takes place predominantly in the *anti* face, which is reasonable from the viewpoint of steric interference by the substituent on the N(3) phenyl ring. On the other hand, the selectivity observed in the reaction of **1** is the opposite of that of **2**, **3**, or **8**. It should also be noted that the reaction of **1** proceeds much faster than that of **2**, **3**, or **8**.

In the absence of the catalyst, however, the reaction of **1** did not proceed at all, which reveals that the proton in the hydroxy group does not play the role of an acid catalyst or the proton in this hydroxy group is not dissociated in contrast to the one in a hydroxynaphthyl group.[25] Therefore, we studied the (net) hydride-transfer reaction in the presence of an acid catalyst in place of Mg^{2+}. Since this type of reaction does not proceed with a weak acid,[25,34] a strong acid is required for this purpose. Indeed, the reaction took place under the acid catalysis of an excess amount of trichloroacetic acid. The result obtained is also listed in Table 5. Although dichloroacetic and chloroacetic acids are too weak to complete the reaction and the reactions are associated by the acid-catalyzed decomposition of BNAH, the *syn*/*anti* ratios in the product obtained, $\mathbf{1}_{red}$-*5-d*, after 60 min of the reactions were the same, within experimental error, as that listed in Table 5.[35] The *syn*/*anti* selectivity in the Brønsted acid-catalyzed reaction is reversed from that observed under the Lewis acid (Mg^{2+})-catalyzed reaction and the same as those observed with **2**, **3**, or **8** in the presence of Mg^{2+}. Thus, there remains no doubt that the reversed stereoselectivity observed for **1** in the presence of Mg^{2+} stems entirely from the interaction between **1** and Mg^{2+}. The interaction does not exist in the reaction with **2**, **3**, or **8**.

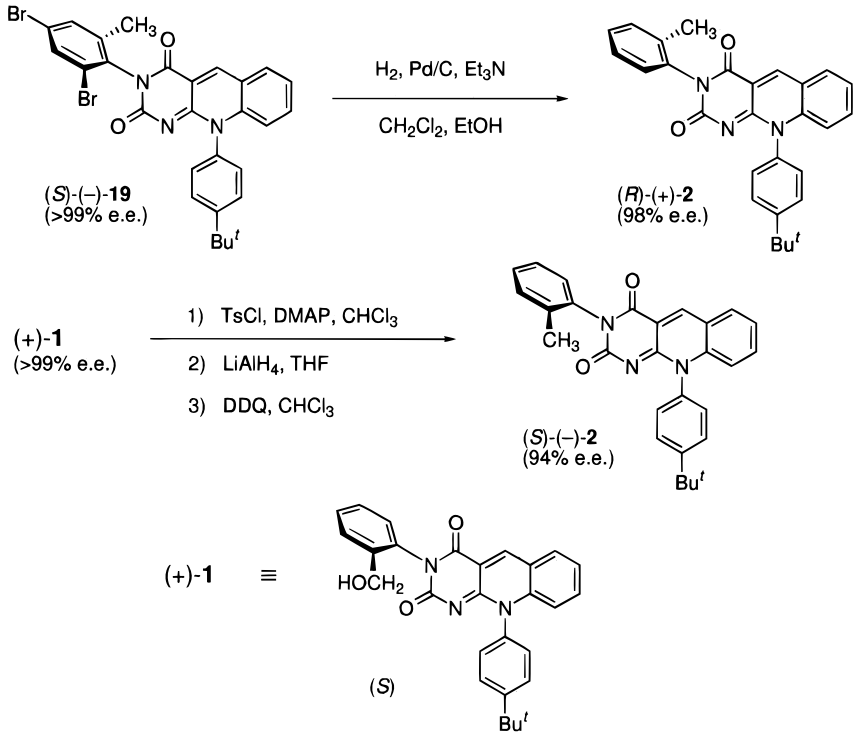
In order to clarify the origin of this interaction, the association constants (*K*) of **1** and **2** with Mg^{2+} in anhydrous acetonitrile at 293 K were measured.[36] Linear plots shown in Figure 4 indicate that a 1:1 complex is

(34) Shinkai, S.; Kawanabe, S.; Kawase, A.; Yamaguchi, T.; Manabe, O. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2095−2102.

(35) Although trifluoroacetic acid was also used as an acid catalyst, *syn*/*anti* ratio was unable to be determined, because BNAH was decomposed by the acid very quickly.

(36) The association constants of several dFl derivatives with Mg^{2+} were reported previously. Fukuzumi, S.; Kuroda, S.; Tanaka, T. *Chem. Lett.* **1984**, 417−420.

Scheme 6



Scheme 7[27]

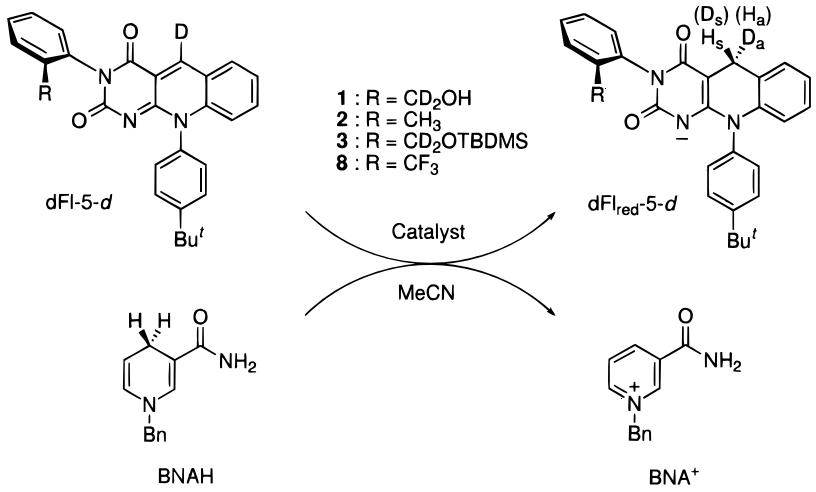


Table 6. Association Constants (*K*) of 1 and 2 with Magnesium Ion[a] in Acetonitrile[b] at 293 K

$$dFl + Mg^{2+} \overset{K}{\rightleftharpoons} dFl{\cdot}Mg^{2+}$$

dFl	R	K, M^{-1}
1[c]	CH_2OH	668 ± 3
2[d]	CH_3	374 ± 2

[a] $Mg(ClO_4)_2$ was used as the source of Mg^{2+}. [b] Water content in acetonitrile used for this measurement was <0.030% v/v (^1H NMR). [c] [**1**] = 5.11×10^{-4} M. [d] [**2**] = 5.96×10^{-4} M.

formed between dFl and Mg^{2+}. As listed in Table 6, the association constant of **1** is about twice as large as that of **2**. Although the factor of 2 of the association constant observed here seems small, the value is acceptable because the isoalloxazine ring has the ability to coordinate onto a metal ion in an organic solvent, as exemplified by **2**,[36] and the coordinating ability of an oxygen atom in an undissociated hydroxy group (*vide supra*) might be small similarly to that of an ethereal oxygen. Thus, on the basis of the variation in enantioface differentiation in the presence and absence of Mg^{2+}, the factor of 2 can be accepted as the indication for the crucial role of the hydroxymethyl group.

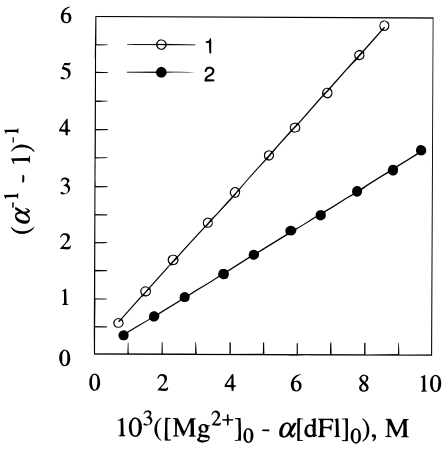


Figure 4. Plots of $(\alpha^{-1} - 1)^{-1}$ against $10^3([Mg^{2+}]_0 - \alpha[dFl]_0)$. $Mg(ClO_4)_2$ was used as the source of Mg^{2+}. Correlation coefficients are >0.9999 for both plots.

Table 7. Diastereoface-Differentiating (Net) Hydride-Transfer Reaction between Racemic 1 and Chiral Me_2PNPH[a]

config of Me_2PNPH	equiv of $Mg(ClO_4)_2$[b]	ratio of **1** reacted *S*:*R*[c,d]
4*S*,9*S*	0	47:53
	10	62:38
	50	74:26
4*R*,9*R*	0	52:48
	10	36:64
	50	28:72

[a] [**1**] = 1.0×10^{-2} M, [Me_2PNPH] = 5.0×10^{-4} M. In the dark under Ar at 298 K. [b] Equivalency to [Me_2PNPH]. [c] Estimated errors are within ±1 for all observed values. [d] The ratio was kept constant meaningfully throughout the reaction.

Taking into account the fact that an NAD(P)H analog can form a complex with Mg^{2+},[37] it is reasonable to propose the formation of a ternary complex (**1**·Mg^{2+}·BNAH)[20,23,38] preceding the transition state of the reaction. The contribution of the hydroxy oxygen for complexation inevitably predicts that the *syn* face has more chance than the *anti* face to form a complex, and then to react, with BNAH under the Mg^{2+} catalysis. Thus, the hydroxymethyl group of **1** has not only a catalytic function in the presence of Mg^{2+} but also a stereocontrolling function, showing the change in stereochemistry from that without Mg^{2+}.

The results predict that the hydroxymethyl group of **1** plays a significant role in coordinating onto Mg^{2+} to form a ternary complex with BNAH rigidly and that stabilization of the system through the formation of a complex in the *syn* face is much more feasible than destabilization through steric repulsion in this face.

On the other hand, the hydroxymethyl group in the absence of Mg^{2+} is not different from other substituents such as methyl, trifluoromethyl, and [(*tert*-butyldimethylsilyl)oxy]methyl groups in terms of interaction with BNAH in the sense that it is nothing but a sterically interfering group. Consequently, these substituents result in the deactivative *anti* preference rather than a *syn* face reaction.

Diastereoface-Differentiating (Net) Hydride-Transfer Reactions. To elucidate the intermolecular arrangement between **1** and an NAD(P)H analog at the transition state of (net) hydride-transfer reactions, a diastereoface-differentiating reaction between **1** and a chiral NAD(P)H analog, 1,4-dihydro-2,4-dimethyl-*N*-(α-methylbenzyl)-1-propylnicotinamide (Me_2PNPH),[39] was studied. Racemic **1** was reduced with (4*S*,9*S*)- or (4*R*,9*R*)-Me_2PNPH in acetonitrile at 298 K in the dark under argon atmosphere, and $\mathbf{1_{red}}$ obtained after 30 min was then oxidized to **1** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The oxidized product was subjected to HPLC to measure the *S*/*R* ratio in **1**. The results are listed in Table 7.

Although this reaction proceeds without Mg^{2+} because the reactivity of Me_2PNPH is much higher than that of BNAH, the *S*/*R* ratio in **1** obtained from the reaction mixture is not large enough to allow detailed discussion. Even for the Mg^{2+}-catalyzed reactions, about 10 times excess Mg^{2+} is required for obtaining a result similar to that obtained from the reaction with BNAH.

Table 8. Asymmetric (Net) Hydride-Transfer Reaction between Chiral 1-5-*d* and Chiral Me_2PNPH[a]

config of **1**-5-*d*	config of Me_2PNPH	$Mg(ClO_4)_2$	reaction time, min	ratio of reacting faces, *syn*:*anti*[b]
S	4*S*,9*S*	yes	<1	62:38
		no	15	49:51
	4*R*,9*R*	yes	<1	44:56
		no	7	29:71
R	4*R*,9*R*	yes	<1	60:40
		no	15	48:52
	4*S*,9*S*	yes	<1	45:55
		no	7	30:70

[a] [**1**-5-*d*] = 2.0×10^{-2} M, [Me_2PNPH] = 5.0×10^{-2} M, [$Mg(ClO_4)_2$]= 5.0×10^{-4} M. In the dark under Ar at 298 K. [b] Estimated errors are within ±2 for all observed values.

The observation stems not only from the fact that Me_2PNPH exhibits lower selectivity due to its higher reactivity than BNAH but also from the fact that the reacting face in BNAH is enantiotopic with respect to its molecular plane, whereas that in Me_2PNPH is diastereotopic and only one of the faces can contribute to the (net) hydride-transfer reaction.

Previously, we proposed, on the basis of the results of enantioface-differentiating (net) hydride-transfer reactions, that the pyrimidine ring of dFl and the carbamoyl group of an NAD(P)H analog must face each other at the transition state in the presence of Mg^{2+}.[24] When the same concept is applied to the present reaction between (*S*)-**1** and (4*R*,9*R*)-Me_2PNPH, for example, the faces of the two reacting molecules cannot overlap each other in order to transfer the (net) hydride in the *syn* face of **1**. The unfavorable molecular arrangement (*vide infra*) may reduce the relative reactivity of **1** in the *syn* face. Consequently, the *syn*/*anti* selectivity of **1** becomes lower in the reaction with Me_2PNPH than that with BNAH.

Asymmetric (Net) Hydride-Transfer Reactions. Since the diastereoface-differentiating selectivity in the reaction of **1** and Me_2PNPH is low, especially in the absence of Mg^{2+}, there exists a limitation to the detailed discussion of the intermolecular arrangement of **1** and an NAD(P)H analog at the transition state when a racemic mixture of **1** is employed for the reaction. Therefore, we studied asymmetric (net) hydride-transfer reactions between chiral **1** and chiral Me_2PNPH. Each enantiomer of **1**, which was optically resolved by HPLC, was reduced by a chiral Me_2PNPH under conditions similar to those described above, and the *syn*/*anti* selectivity was measured. The results are summarized in Table 8.

In the presence of Mg^{2+}, the reduction of **1** with Me_2PNPH proceeds rapidly. It is noteworthy, however, that the reduction of (*S*)-**1** with (4*R*,9*R*)-Me_2PNPH takes place more than twice as fast as that with (4*S*,9*S*)-Me_2PNPH in the absence of Mg^{2+}.

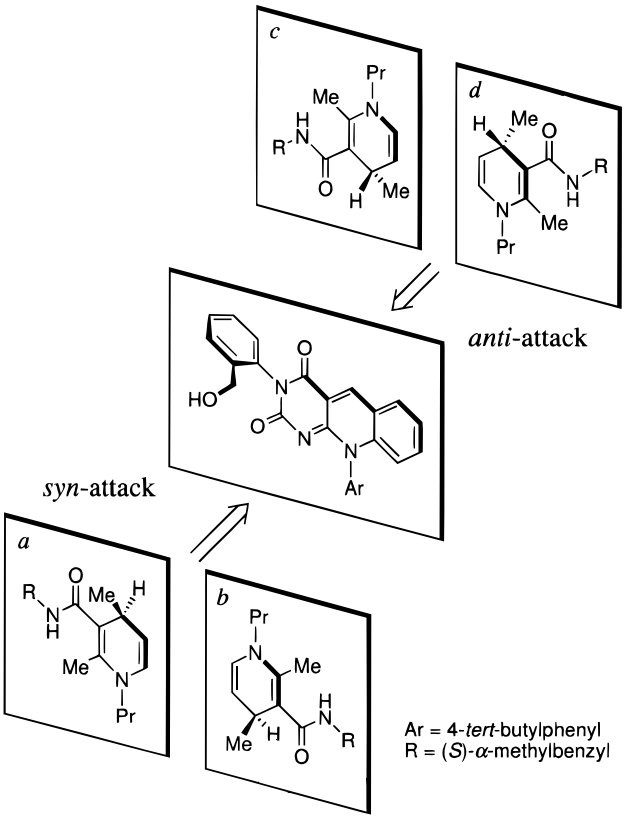
Here, we categorize the reduction of (*S*)-**1**, for example, into four depending on the presence or absence of Mg^{2+} and whether the reducing agent is (4*S*,9*S*)- or (4*R*,9*R*)-Me_2PNPH, and each case will be discussed below.

(1) In the reaction with (4*S*,9*S*)-Me_2PNPH in the presence of Mg^{2+}, two arrangements of Me_2PNPH, a and c shown in Figure 5a, are possible under the assumption that the pyrimidine ring of dFl interacts with the carbamoyl group of an NAD(P)H analog at the transition state.[24] It is plausible to expect that a (net) hydride

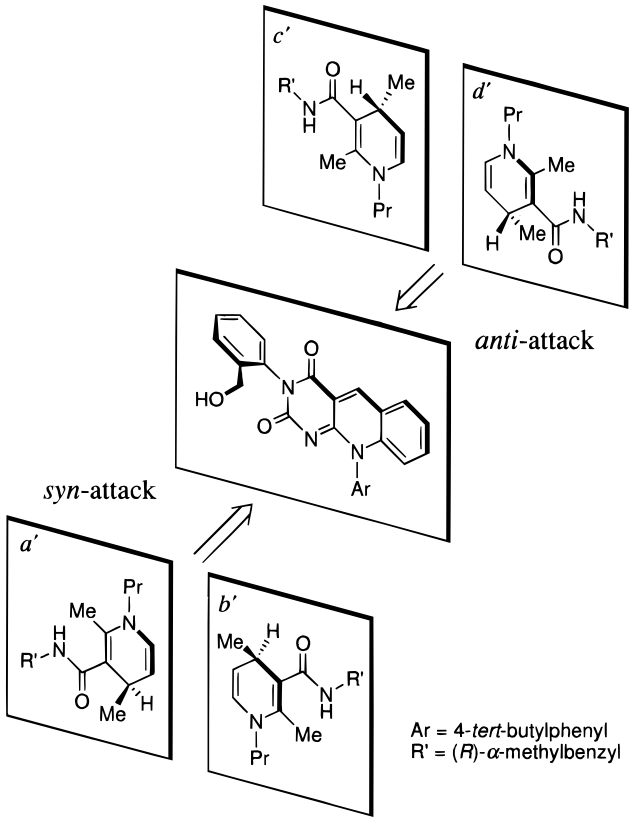
(37) It has been reported that the association constant between 1,4-dihydro-*N*-(α-methylbenzyl)-1-propylnicotinamide, an analog of BNAH, and Mg^{2+} in acetonitrile at 273 K is 1.9×10^3 M^{-1}: Ohno, A.; Yamamoto, H.; Okamoto, T.; Oka, S.; Ohnishi, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2385−2386.

(38) For the contribution of a ternary complex in the reaction, see: Ohno, A.; Yasui, S.; Nakamura, K.; Oka, S. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 290−293.

(39) It has been confirmed that the conformation at the side-chain carbamoyl group does not affect stereochemistry of the reaction largely: Ohno, A.; Ikeguchi, M.; Kimura, T.; Oka, S. *J. Am. Chem. Soc.* **1979**, *101*, 7036−7040.



(a)



(b)

Figure 5. Intermolecular arrangements between (a) (*S*)-(+)-**1** and (4*S*,9*S*)-Me_2PNPH and (b) (*S*)-(+)-**1** and (4*R*,9*R*)-Me_2PNPH. Magnesium ion is omitted from the drawing for simplicity.

transfer in the *syn* face is favored over the one in the *anti* face, because the coordination of the hydroxymethyl group of **1** onto Mg^{2+} overwhelms the steric hindrance in energy due to matched arrangement. However, the *syn*/*anti* selectivity becomes lower than expected with this combination because Me_2PNPH exhibits such a high reactivity in the presence of Mg^{2+} that the reaction proceeds even in an unfavorable conformation as depicted in c.

(2) In the reaction with (4*S*,9*S*)-Me_2PNPH in the absence of Mg^{2+}, four arrangements of Me_2PNPH, a−d shown in Figure 5a, are possible. If the arrangements b and d contributed mainly to the (net) hydride transfer, the results observed in the reaction with (4*S*,9*S*)- and (4*R*,9*R*)-Me_2PNPH (*vide infra*) might be similar in contrast to the observed results. Instead, the arrangements a and c, in which the pyrimidine ring of **1** and the carbamoyl group of Me_2PNPH face each other, are more favorable than the arrangements b and d. In addition, it is recognized that the *syn* attack becomes more important in the reaction with Me_2PNPH (*syn*/*anti* = 49/51) than that with BNAH (*syn*/*anti* = 31/69) in spite of the absence of coordination influence by Mg^{2+} in these reaction systems. This result supports the presence of a certain effect that makes the reaction in the *syn* face more favorable than that in the *anti* face. Thus, it seems likely that conformation a, where the maximum overlap of molecular planes of **1** and Me_2PNPH is achieved, is the most favorable arrangement for the formation of a binary complex (**1**·Me_2PNPH),[40] and stabilization through this conformation is large enough to compensate for energetically unfavorable steric hindrance.

(3) In the reaction with (4*R*,9*R*)-Me_2PNPH in the presence of Mg^{2+}, two arrangements, a′ and c′ shown in Figure 5b, are possible similarly to the system mentioned in case 1. Here, however, the *anti* selectivity predominates slightly over the *syn* selectivity, or the *syn* selectivity is lowered in this combination by the presence of Mg^{2+}.

The result strongly suggests that large overlapping of molecular planes of **1** and Me_2PNPH is important in determining the molecular arrangement at the transition state of the reaction, and this effect overwhelms the favorable electronic interaction through the coordination by a hydroxy group.

(4) In the reaction with (4*R*,9*R*)-Me_2PNPH in the absence of Mg^{2+}, four arrangements, a′−d′ shown in Figure 5b, are possible. For the same reason as described in case 2, arrangements a′ and c′ are more plausible than the arrangements b′ and d′. Since the (net) hydride transfer in the *anti* face is predicted to be more favorable than the *syn* face in this reaction system from the viewpoint of steric hindrance, together with the fact that the reaction with (4*R*,9*R*)-Me_2PNPH proceeds faster than that with (4*S*,9*S*)-Me_2PNPH, arrangement c′ is nominated as the most favorable arrangement at the transition state.

Thus, it is concluded that the most suitable intermolecular arrangement between **1** and NAD(P)H analog at the transition state of (net) hydride-transfer reactions is the one in which two molecules are arranged with maximum overlap of their molecular planes and the pyrimidine ring of **1** is set in front of the carbamoyl group of the analog, regardless of the presence or absence of Mg^{2+} as portrayed in Figure 6.

(40) *Cf.* Ohno, A.; Goto, T.; Nakai, J.; Oka, S. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3478−3481.

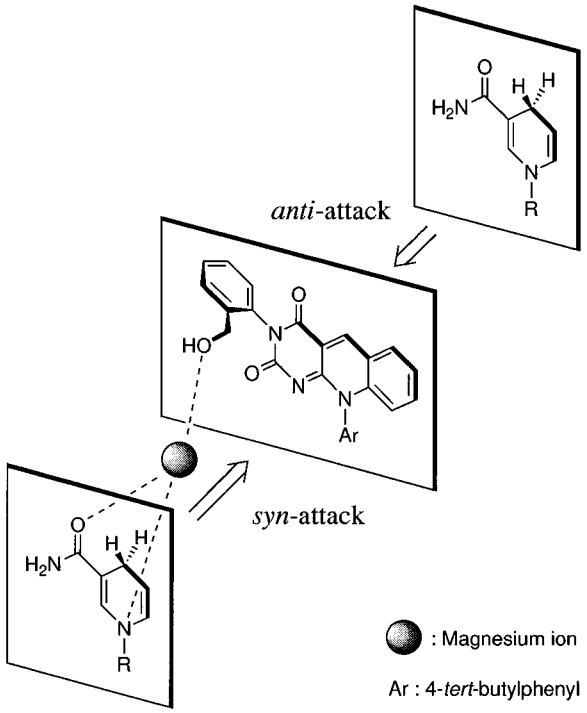


Figure 6. Most predominant intermolecular arrangements between (*S*)-(+)-**1** and an NAD(P)H analog at the transition states of (net) hydride-transfer reactions in the presence (*syn* face) and absence (*anti* face) of magnesium ion, respectively. The conformation of the side-chain carbamoyl group of NAD(P)H analog is drawn arbitrarily.

It should be noted that the hydroxymethyl group of **1** controls both reactivity and stereochemistry in the (net) hydride-transfer reaction; in the presence of Mg^{2+}, the (net) hydride transfer takes place predominantly in the *syn* face of **1** due to the coordination of the hydroxy group onto Mg^{2+} to form a ternary complex, whereas in its absence, the reaction proceeds predominantly in the *anti* face because of the predominance of steric interference by this substituent.

The intermolecular arrangement postulated here is similar to that reported for FAD and NADPH in the active site of glutathione reductase:[10,41] the flavin moiety of FAD is stacked onto the nicotinamide ring of NADPH and the pyrimidine ring of the flavin and the carbamoyl group of the nicotinamide face each other. It is of great interest that the intermolecular arrangements such as those shown in Figure 6 can be seen in a model system even though no steric compulsion exists to arrange them in this order.

The present result strongly indicates not only a possibility that there might exist stabilizing effects due to the overlap of molecular planes of a flavin and an NAD(P)H coenzyme but also a possibility that functional groups in an apoprotein in proximity to a flavin coenzyme in the active site of a flavoenzyme have significant influence on the stereoselective interaction with a substrate.

The effect of stereochemical control by a polar functional group elucidated in the present study may suggest that particular amino acid residues in the flavoprotein might have contributed to establish the stereochemistry of modern and sophisticated biochemical redox reactions during chemical evolution of the enzyme.[42,43]

(41) Karplus, P. A.; Schulz, G. E. *J. Mol. Biol.* **1989**, *210*, 163−180.

Experimental Section

Instruments. Melting points (mp) were obtained using a Yanagimoto micromelting point apparatus and were uncorrected. UV−vis spectra were recorded on a Hitachi U-3210 spectrophotometer equipped with a Hitachi SDR-30 temperature controller. Infrared (IR) spectra were recorded on a JASCO FT/IR-5300 spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian VXR 200 FT-NMR spectrometer with tetramethylsilane as an internal reference, and all shifts are indicated in ppm. High-resolution mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 mass spectrometer. Elemental analyses were performed using a Yanaco MT-3 elemental analyzer. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Circular dichroism (CD) spectra were recorded on a JASCO J-720W spectropolarimeter. Column chromatography was performed using Nacalai Tesque silica gel 60 (70−230 mesh). Preparative TLC was run on 20 cm × 20 cm plates with a 0.5 mm layer of Nacalai Tesque silica gel 60 PF_{254}.

Materials. Organic reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd., Nacalai Tesque, Inc., Tokyo Kasei Kogyo Co., Ltd., and Aldrich Chemical Co., Inc. Methanol and THF were dried by reflux and distillation on sodium. Chloroform, dichloromethane, and DMF were dried by reflux and distillation on calcium hydride.

1-(2-Methylphenyl)urea (**10**),[44] 2,4-dibromo-6-methylaniline (**14**),[45] 1-benzyl-1,4-dihydronicotinamide (BNAH),[46] and 1,4-dihydro-2,4-dimethyl-*N*-(α-methylbenzyl)-1-propylnicotinamide (Me_2PNPH)[39] were synthesized as reported previously. Their mp, IR, ^{1}H NMR, and elemental analysis data were consistent with the structures. 2-Fluoro[α-^{2}H]benzaldehyde was prepared by the oxidation of 2-fluoro[α,α-$^2H_2$]benzyl alcohol with pyridinium chlorochromate in dry dichloromethane. 2-Fluoro[α,α-$^2H_2$]benzyl alcohol was prepared by the reduction of ethyl 2-fluorobenzoate with lithium aluminum deuteride (98 atom % D) in dry ether. Both deuterated compounds exhibited IR, ^{1}H NMR, and HRMS data consistent with the structures.

1-[2-(Trifluoromethyl)phenyl]urea (4). 2-(Trifluoromethyl)aniline (62.0 mL, 500 mmol) was dissolved in acetic acid/water (1:1, 400 mL) and stirred at room temperature. To the solution was slowly added a suspension of sodium cyanate (65.0 g, 1000 mmol) in water (500 mL). A white precipitate of the product appeared quickly. The suspension was stirred for 2.5 h at room temperature and poured into ice−water. The precipitate was collected by filtration, washed with water, and air dried. Recrystallization from ethyl acetate gave **4** (34.0 g, 33% yield) as white plates: mp 201−202 °C; IR (KBr) 3439, 3341, 3218, 1657, 1618, 1524, 1321, 760 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.42 (brs, 2H), 7.18 (t, J = 7.6 Hz, 1H), 7.53−7.63 (m, 2H), 7.82 (brs, 1H), 7.96 (d, J = 8.4 Hz, 1H). Anal. Calcd for $C_8H_7F_3N_2O$: C, 47.07; H, 3.46; N, 13.72. Found: C, 47.28; H, 3.43; N, 13.88.

1-[2-(Trifluoromethyl)phenyl]barbituric Acid (5). Sodium (2.8 g, 120 mmol) was dissolved in dry methanol (100 mL) at 0 °C. To the solution were added **4** (20.4 g, 100 mmol) and diethyl malonate (18.2 mL, 120 mmol), and the mixture was refluxed with stirring for 16 h under argon atmosphere. After being cooled, the solution was poured into ice−water and made strongly acidic with 2 M hydrochloric acid. The precipitate was collected by filtration, washed with water, and air dried. Recrystallization from hot methanol gave **5** (24.5 g, 90% yield) as white plates: mp 270−272 °C; IR (KBr) 3233, 3160, 3102, 2992, 2893, 1686, 1437, 1346, 1318, 775 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.87 (dd, J = 21.2, 75.4 Hz, 2H), 7.52−7.85 (m, 4H), 11.72 (brs, 1H). Anal. Calcd for $C_{11}H_7F_3N_2O_3$: C, 48.54; H, 2.59; N, 10.29. Found: C, 48.77; H, 2.47; N, 10.36.

(42) Pai, E. F.; Karplus, P. A.; Schultz, G. E. *Biochemistry* **1988**, *27*, 4465−4474.

(43) Ohno, A.; Tsutsumi, A.; Kawai, Y.; Yamazaki, N.; Mikata, Y.; Okamura, M. *J. Am. Chem. Soc.* **1994**, *116*, 8133−8137.

(44) Kurzer, F. *Organic Syntheses*; Wiley: New York, 1963; Vol. IV, pp 49−51.

(45) Nevile, R. H. C.; Winther, A. *Ber. Dtsch. Chem. Ges.* **1880**, *13*, 962−973.

(46) Mauzerall, D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2261−2264.

6-Chloro-3-[2-(trifluoromethyl)phenyl]uracil (6). To a mixture of **5** (9.53 g, 35 mmol) in phosphorus oxychloride (65.2 mL, 700 mmol) was added water (3.15 mL, 175 mmol) portionwise. The resulting mixture was heated with stirring at 60 °C for 28 h. Phosphorus oxychloride was removed under reduced pressure, and the residue was poured into ice−water. The mixture was neutralized with 2 M aqueous sodium hydroxide, and the precipitate was collected by filtration, washed with water, and air dried. Recrystallization from ethyl acetate gave **6** (7.33 g, 72% yield) as white needles: mp 227−228 °C; IR (KBr) 3133, 2951, 2799, 1744, 1653, 1487, 1456, 1410, 1318, 812, 770 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.05 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.4 Hz, 1H), 7.77−7.87 (m, 2H), 12.68 (brs, 1H). Anal. Calcd for $C_{11}H_6ClF_3N_2O_2$: C, 45.46; H, 2.08; N, 9.64. Found: C, 45.57; H, 2.06; N, 9.60.

6-(4-*tert*-Butylanilino)-3-[2-(trifluoromethyl)phenyl]uracil (7). A solution of **6** (7.27 g, 25 mmol) and 4-*tert*-butylaniline (8.76 mL, 55 mmol) in butanol (50 mL) was refluxed with stirring for 3 h under argon atmosphere. After the mixture was cooled, the precipitate was collected by filtration and washed with methanol. Recrystallization from hot methanol gave **7** (7.70 g, 76% yield) as white needles: mp 292−293 °C; IR (KBr) 3329, 3081, 2965, 1734, 1599, 1559, 1422, 1318, 766 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29 (s, 9H), 4.83 (s, 1H), 7.20 (d, J = 8.6 Hz, 2H), 7.43 (d, 3H), 7,63 (t, J = 7.4 Hz, 1H), 7.73−7.83 (m, 2H), 8.34 (brs, 1H), 10.76 (brs, 1H). Anal. Calcd for $C_{21}H_{20}F_3N_3O_2$: C, 62.53; H, 5.00; N, 10.42. Found: C, 62.74; H, 5.00; N, 10.44.

10-(4-*tert*-Butylphenyl)-3-[2-(trifluoromethyl)phenyl]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (8). 2-Fluorobenzaldehyde (1.26 mL, 12 mmol) and **7** (4.03 g, 10 mmol) were dissolved in DMF (8 mL), and the solution was heated with stirring at 100 °C for 6 h. After the mixture was cooled, the precipitate was collected by filtration and washed with methanol. Recrystallization from hexane/chloroform gave **8** (3.18 g, 65% yield) as yellow needles. 10-(4-*tert*-Butylphenyl)-3-[2-(trifluoromethyl)phenyl][5-^{2}H]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (**8**-*5-d*, 98 atom % D) was prepared from **7** and 2-fluoro[α-^{2}H]benzaldehyde similarly: mp >300 °C; IR (KBr) 3474, 3081, 2967, 2874, 1717, 1669, 1620, 1566, 1534, 1491, 1454, 1414, 1318, 797, 764 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9H), 6.94 (d, J = 8.0 Hz, 1H), 7.21−7.35 (m, 3H), 7.46 (t, J = 7.0 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.62−7.73 (m, 4H), 7.80 (d, J = 7.8 Hz, 1H), 7.95 (dd, J = 1.6, 7.8 Hz, 1H), 9.07 (s, 1H). Anal. Calcd for $C_{28}H_{22}F_3N_3O_2 \cdot 0.5CHCl_3$: C, 62.33; H, 4.13; N, 7.65. Found: C, 62.62; H, 4.13; N, 7.70.

10-(4-*tert*-Butylphenyl)-3-(2-carboxyphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (9). A solution of **8** (4.90 g, 10 mmol) in concentrated sulfuric acid (15 mL) was heated at 130 °C with stirring for 5 h. After being cooled, the solution was poured into ice−water, and the yellow precipitate was extracted with chloroform. The organic layer was washed with brine and dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness under reduced pressure. The residue was recrystallized from chloroform/methanol to give **9** (4.14 g, 89% yield) as a yellow powder. 10-(4-*tert*-Butylphenyl)-3-(2-carboxyphenyl)[5-^{2}H]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (**9**-*5-d*, 98 atom % D) was prepared from **8**-*5-d* similarly: dec 278−280 °C; IR (KBr) 3410, 3185, 3050, 2965, 2868, 2616, 2504, 1715, 1642, 1618, 1532, 1491, 1453, 1416, 797, 754 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.42 (s, 9H), 6.79 (d, J = 8.4 Hz, 1H), 7.28−7.42 (m, 3H), 7.52 (m, 2H), 7.64−7.82 (m, 4H), 8.03 (dd, J = 1.6, 7.8 Hz, 1H), 8.27 (dd, J = 1.2, 7.8 Hz, 1H), 9.23 (s, 1H), 12.79 (brs, 1H). Anal. Calcd for $C_{28}H_{23}N_3O_4 \cdot CH_3OH$: C, 70.01; H, 5.47; N, 8.45. Found: C, 69.77; H, 5.34; N, 8.45.

10-(4-*tert*-Butylphenyl)-3-[2-(hydroxymethyl)phenyl]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (1). To a suspension of **9** (3.72 g, 8 mmol) in dry THF (30 mL) was added sodium borohydride (0.91 g, 24 mmol), and the mixture was stirred at 0 °C. After the generation of hydrogen had ceased, a solution of boron trifluoride diethyl etherate (*ca.* 47%, 3 mL) in dry THF (15 mL) was added to the suspension, and the mixture was stirred for 1 h at room temperature. Then, the mixture was poured into ice−water, and organic materials were extracted with chloroform three times. The combined organic solution was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. Crude 10-(4-*tert*-butylphenyl)-1,5-dihydro-3-[2-(hydroxymethyl)phenyl]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione ($\mathbf{1}_{red}$), which contained a small amount of **1** due to air-oxidation, was obtained as a pale yellow solid.

The solid was dissolved in chloroform (50 mL), and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.82 g, 8 mmol) was added to the solution with stirring at room temperature. After 15 min, the reaction mixture was washed with saturated aqueous sodium bicarbonate twice as well as with brine once and dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel with chloroform/methanol (50:1) as eluent, and the eluted material was recrystallized from chloroform/methanol to give **1** (3.32 g, 92% yield) as yellow plates. 10-(4-*tert*-Butylphenyl)-3-[2-(hydroxy[^{2}H$_2$]methyl)phenyl][5-^{2}H]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (**1**-*5-d*, 98 atom % D) was prepared similarly from **9**-*5-d* and sodium borodeuteride (98 atom % D) as starting materials: mp 300 °C; UV (CH_3CN) λ_{max} 267 (ϵ 37 800), 319 (ϵ 10 700), 401 nm (ϵ 12 000); IR (KBr) 3493, 3042, 2967, 2874, 1709, 1649, 1612, 1564, 1530, 1487, 1453, 1412, 797, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.60 (t, J = 6.4 Hz, 1H), 4.47−4.51 (m, 2H), 6.95 (d, J = 8.4 Hz, 1H), 7.17−7.31 (m, 3H), 7.43−7.52 (m, 3H), 7.59−7.73 (m, 4H), 7.97 (dd, J = 1.4, 8.0 Hz, 1H), 9.08 (s, 1H); HRMS (EI) m/z calcd for $C_{28}H_{25}N_3O_3$ ($M^{\bullet+}$) 451.1897, found 451.1899. Anal. Calcd for $C_{28}H_{25}N_3O_3$: C, 74.48; H, 5.58; N, 9.31. Found: C, 74.28; H, 5.67; N, 9.14.

1-(2-Methylphenyl)barbituric Acid (11). Sodium (2.8 g, 120 mmol) was dissolved in dry methanol (100 mL) at 0 °C. To the solution were added **10** (15.0 g, 100 mmol) and diethyl malonate (18.2 mL, 120 mmol), and the solution was refluxed with stirring for 16 h under argon atmosphere. After being cooled, the solution was poured into ice−water and made strongly acidic with 2 M hydrochloric acid. The precipitate was collected by filtration, washed with water, and air dried. Recrystallization from hot methanol gave **11** (17.7 g, 81% yield) as white plates: mp 250−252 °C; IR (KBr) 3219, 3162, 3098, 2992, 2892, 1680, 1497, 1435, 820, 774, 725 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.09 (s, 3H), 3.78 (dd, J = 21.2, 42.0 Hz, 2H), 7.14−7.31 (m, 4H), 11.53 (brs, 1H). Anal. Calcd for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.32; H, 4.66; N, 12.79.

6-Chloro-3-(2-methylphenyl)uracil (12). To a mixture of **11** (10.9 g, 50 mmol) in phosphorus oxychloride (46.6 mL, 500 mmol) was added water (2.25 mL, 125 mmol) portionwise. The resulting mixture was heated with stirring at 60 °C for 24 h. Phosphorus oxychloride was removed from the mixture under reduced pressure, and the residue was poured into ice−water. The mixture was neutralized with 2 M aqueous sodium hydroxide, and the precipitate was collected by filtration, washed with water, and air dried. Recrystallization from ethyl acetate gave **12** (6.20 g, 52% yield) as white plates: mp 228−229 °C; IR (KBr) 3102, 2888, 2799, 1736, 1649, 1495, 1408, 1323, 826, 764, 720 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.04 (s, 3H), 6.00 (s, 1H), 7.13−7.34 (m, 4H), 12.56 (brs, 1H). Anal. Calcd for $C_{11}H_9ClN_2O_2$: C, 55.83; H, 3.83; N, 11.84. Found: C, 55.88; H, 3.74; N, 11.88.

6-(4-*tert*-Butylanilino)-3-(2-methylphenyl)uracil (13). A solution of **12** (3.55 g, 15 mmol) and 4-*tert*-butylaniline (5.57 mL, 35 mmol) in butanol (25 mL) was refluxed with stirring for 3 h under argon atmosphere. After the mixture was cooled, the precipitate was collected by filtration and washed with methanol. Recrystallization from hot methanol gave **13** (3.58 g, 68% yield) as white needles: mp 300 °C; IR (KBr) 3310, 3079, 2963, 1726, 1597, 1516, 1300, 725 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (s, 9H), 2.06 (s, 3H), 4.86 (s, 1H), 7.07−7.29 (m, 6H), 7.42 (d, J = 8.6 Hz, 2H), 8.26 (brs, 1H), 10.60 (brs, 1H). Anal. Calcd for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 72.21; H, 6.59; N, 12.11.

10-(4-*tert*-Butylphenyl)-3-(2-methylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (2). 2-Fluorobenzaldehyde (0.63 mL, 6 mmol) and **13** (1.75 g, 5 mmol) were dissolved in DMF (5 mL), and the solution was heated with stirring at 100 °C for 6 h. After the mixture was cooled, the precipitate was collected by filtration and washed with methanol. Re-

crystallization from hexane/chloroform gave **2** (1.31 g, 60% yield) as yellow needles. 10-(4-*tert*-Butylphenyl)-3-(2-methylphenyl)[5-^{2}H]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (**2**-*5*-*d*, 98 atom % D) was prepared from **13** and 2-fluoro[α-^{2}H]benzaldehyde similarly: mp 293−294 °C; UV (CH_3CN) λ_{max} 266 (ϵ 40 200), 318 (ϵ 11 400), 402 nm (ϵ 12 800); IR (KBr) 3461, 3061, 2967, 2868, 1711, 1661, 1618, 1566, 1534, 1491, 1454, 1414, 797, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 2.18 (s, 3H), 6.93 (d, J = 8.6 Hz, 1H), 7.12−7.17 (m, 1H), 7.22−7.34 (m, 5H), 7.45 (t, J = 7.4 Hz, 1H), 7.61−7.70 (m, 3H), 7.94 (dd, J = 1.6, 7.8 Hz, 1H), 9.06 (s, 1H); HRMS (EI) *m*/*z* calcd for $C_{28}H_{25}N_3O_2$ ($M^{\bullet+}$) 435.1948, found 435.1950. Anal. Calcd for $C_{28}H_{25}N_3O_2 \cdot CHCl_3$: C, 62.77; H, 4.72; N, 7.57. Found: C, 62.71; H, 4.76; N, 7.63.

3-[2-[[(*tert*-Butyldimethylsilyl)oxy]methyl]phenyl]-10-(4-*tert*-butylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (3). *tert*-Butyldimethylsilyl chloride (226 mg, 1.5 mmol), **1** (226 mg, 0.5 mmol), and imidazole (204 mg, 3.0 mmol) were dissolved in dry DMF (8 mL), and the solution was stirred for 1 h at room temperature under argon atmosphere. The solution was poured into ice−water, and organic materials were extracted with ethyl acetate. The organic solution was washed with water four times as well as with brine once and dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness under reduced pressure. The yellow residue was recrystallized from hexane/chloroform to give **3** (207 mg, 73% yield) as a yellow powder. 3-[2-[[(*tert*-Butyldimethylsilyl)oxy][^{2}H$_2$]methyl]phenyl]-10-(4-*tert*-butylphenyl)[5-^{2}H]pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (**3**-*5*-*d*, 98 atom % D) was prepared similarly from **1**-*5*-*d*: mp 225−226 °C; IR (KBr) 3451, 3040, 2957, 2859, 1715, 1663, 1618, 1566, 1535, 1491, 841, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 6H), 0.88 (s, 9H), 1.43 (s, 9H), 4.64 (s, 2H), 6.91 (d, J = 8.8 Hz, 1H), 7.13 (dd, J = 1.6, 7.4 Hz, 1H), 7.21−7.48 (m, 5H), 7.61−7.69 (m, 4H), 7.94 (dd, J = 1.4, 7.8 Hz, 1H), 9.06 (s, 1H). Anal. Calcd for $C_{34}H_{39}N_3O_3Si$: C, 72.18; H, 6.95; N, 7.43. Found: C, 72.02; H, 6.87; N, 7.41.

1-(2,4-Dibromo-6-methylphenyl)urea (15). To acetic acid (45 mL) was dissolved **14** (11.9 g, 45 mmol), and the solution was stirred at room temperature. A suspension of sodium cyanate (5.85 g, 90 mmol) in water (45 mL) was added to the solution dropwise until a white precipitate appeared. Then, the rest of the suspension and water (45 mL) were added quickly with vigorous stirring. After 3 min, the precipitate that appeared was collected by filtration, washed with water, and air dried. Recrystallization from ethanol gave **15** (1.85 g, 13% yield) as white needles: mp >300 °C; IR (KBr) 3434, 3281, 1659, 1599, 1535, 1352, 852 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.22 (s, 3H), 5.95 (brs, 2H), 7.45 (d, J = 2.2 Hz, 1H), 7.66 (d, J = 2.2 Hz, 1H), 7.78 (brs, 1H). Anal. Calcd for $C_8H_8Br_2N_2O$: C, 31.20; H, 2.62; N, 9.10. Found: C, 31.26; H, 2.57; N, 9.13.

1-(2,4-Dibromo-6-methylphenyl)barbituric Acid (16). Sodium (0.34 g, 15 mmol) was dissolved in dry methanol (20 mL) at 0 °C. To the solution were added **15** (3.08 g, 10 mmol) and diethyl malonate (1.82 mL, 12 mmol), and the mixture was refluxed with stirring for 16 h under argon atmosphere. After being cooled, the solution was poured into ice−water and made strongly acidic with 2 M hydrochloric acid. The precipitate was collected by filtration, washed with water, and air dried. Recrystallization from hot methanol to gave **16** (2.48 g, 66% yield) as white plates: mp 249−250 °C; IR (KBr) 3544, 3233, 3127, 2886, 1709, 1582, 1557, 1377, 1348, 779 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H), 3.97 (brs, 2H), 7.63 (d, J = 1.8 Hz, 1H), 7.84 (d, J = 1.8 Hz, 1H), 11.83 (brs, 1H). Anal. Calcd for $C_{11}H_8Br_2N_2O_3$: C, 35.14; H, 2.14; N, 7.45. Found: C, 35.04; H, 2.18; N, 7.34.

6-Chloro-3-(2,4-dibromo-6-methylphenyl)uracil (17). To a mixture of **16** (1.50 g, 4 mmol) in phosphorus oxychloride (3.73 mL, 40 mmol) was added water (0.22 mL, 12 mmol) portionwise. The resulting mixture was heated with stirring at 60 °C. After 72 h, the mixture was poured into ice−water, neutralized with 2 M aqueous sodium hydroxide, and extracted with ethyl acetate twice. The organic solution was washed with brine and dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel with hexane/ethyl acetate (1:1) as eluent, and the product was recrystallized from ethyl acetate to give **17** (1.18 g, 75% yield) as a white powder: mp 237−238 °C; IR (KBr) 3179, 3108, 2942, 2801, 1730, 1665, 1462, 1410, 1323, 802, 775 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.12 (s, 3H), 6.12 (s, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.86 (d, J = 1.6 Hz, 1H), 12.86 (brs, 1H). Anal. Calcd for $C_{11}H_7Br_2ClN_2O_2$: C, 33.50; H, 1.79; N, 7.10. Found: C, 33.76; H, 1.86; N, 7.05.

3-(2,4-Dibromo-6-methylphenyl)-6-(4-*tert*-butylanilino)uracil (18). A mixture of **17** (1.97 g, 5 mmol) and 4-*tert*-butylaniline (1.75 mL, 11 mmol) in butanol (12.5 mL) was refluxed with stirring for 3 h under argon atmosphere. After the mixture was cooled, the precipitate was collected by filtration, washed with methanol, and dried to give **18** (2.22 g, 88% yield) as white plates: mp >300 °C; IR (KBr) 3316, 3108, 2961, 2868, 1723, 1601, 1516, 1462, 1416, 1300, 783 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 9H), 2.11 (s, 3H), 4.85 (s, 1H), 7.20 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 2.2 Hz, 1H), 7.81 (d, J = 2.2 Hz, 1H), 8.44 (brs, 1H), 10.86 (brs, 1H). Anal. Calcd for $C_{21}H_{21}Br_2N_3O_2$: C, 49.73; H, 4.17; N, 8.28. Found: C, 49.75; H, 4.10; N, 8.24.

3-(2,4-Dibromo-6-methylphenyl)-10-(4-*tert*-butylphenyl)-pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (19). 2-Fluorobenzaldehyde (0.63 mL, 6 mmol) and **18** (2.54 g, 5 mmol) were dissolved in DMF (15 mL), and the solution was heated with stirring at 120 °C for 6 h. After DMF was removed from the solution under reduced pressure, methanol (50 mL) was added to the residue, and insoluble compounds were removed by filtration. The methanol was removed from the filtrate under reduced pressure, the residue was subjected to column chromatography on silica gel with hexane/ethyl acetate (1:1) as eluent, and the product was recrystallized from chloroform/methanol to give **19** (1.78 g, 60% yield) as yellow plates: mp 224−225 °C; UV (CH_3CN) λ_{max} 266 (ϵ 35 300), 319 (ϵ 10 900), 402 nm (ϵ 11 900); IR (KBr) 3476, 3050, 2965, 1707, 1659, 1613, 1566, 1530, 1489, 1453, 1412, 795, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 2.20 (s, 3H), 6.95 (d, J = 8.8 Hz, 1H), 7.23 (t, J = 8.8 Hz, 1H), 7.29 (s, 1H), 7.42 (s, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.63−7.72 (m, 4H), 7.96 (dd, J = 1.6, 8.0 Hz, 1H), 9.11 (s, 1H). Anal. Calcd for $C_{28}H_{23}Br_2N_3O_2 \cdot CH_3OH$: C, 55.70; H, 4.35; N, 6.72. Found: C, 55.52; H, 4.30 N, 6.65.

(*S*)-(−)-**19**: CD (CH_3CN) λ_{max} 265 ($\Delta\epsilon$ −10.43), 291 ($\Delta\epsilon$ +0.415), 324 ($\Delta\epsilon$ −0.723), 403 nm ($\Delta\epsilon$ +0.560).

(*R*)-(+)-**19**: CD (CH_3CN) λ_{max} 265 ($\Delta\epsilon$ +9.93), 291 ($\Delta\epsilon$ −0.228), 319 ($\Delta\epsilon$ +0.723), 410 nm ($\Delta\epsilon$ −0.800).

Kinetic Measurements for Thermal Enantiomerization. Thermal enantiomerization of **1** or **2** was carried out by immersing a solution of chiral **1** or **2** in DMF (*ca.* 1.0 × 10^{-3} M) in a thermostated oil bath at 30−70 °C. At appropriate intervals, aliquots were withdrawn and subjected to HPLC analysis to measure ee.[29,30]

Crystallographic Studies. The lattice parameters and intensity data were measured on a Rigaku AFC7R diffractometer and an 18 kW rotating anode generator with 8 kW Cu Kα radiation. The structures were solved by the direct method, and non-hydrogen atoms were refined anisotropically. All calculations were performed using a Texsan crystallographic software package developed by Molecular Structure Corporation (1985 and 1992). The crystallographic parameters are listed in Table 4.

Determination of the Absolute Configuration of 2. (*S*)-(−)-**19** (>99% ee, 20.2 mg, 0.034 mmol) was dissolved in dichloromethane (0.2 mL). To the solution were added 10% palladium on carbon (50 mg), triethylamine (0.05 mL), and ethanol (5 mL), and the resulting mixture was stirred for 1 h at room temperature under hydrogen atmosphere. After palladium on carbon was removed by filtration, the solvent was removed from the filtrate under reduced pressure. The residue was subjected to preparative TLC with chloroform/methanol (50:1) as a developing solvent to give (*R*)-**2** (5.7 mg, 28% yield), which was subjected to HPLC to confirm that this is the (*R*)-(+)-enantiomer (98% ee).

Determination of the Absolute Configuration of 1. (+)-**1** (>99% ee, 16.3 mg, 0.036 mmol), *p*-toluenesulfonyl chloride (19.1 mg, 0.10 mmol), and 4-(dimethylamino)pyridine (12.2 mg, 0.10 mmol) were dissolved in dry chloroform (5 mL), and the solution was stirred for 1 h at 0 °C under argon atmosphere. After the solvent was evaporated from the

solution to dryness under reduced pressure, the residue was subjected to column chromatography on silica gel with chloroform as eluent to give 10-(4-*tert*-butylphenyl)-3-[2-[[(*p*-toluenesulufonyl)oxy]methyl]phenyl]pyrimido[4,5-*b*]quinoline-2,4-(3*H*,10*H*)-dione. Dry THF (5 mL) was added to the compound, and the suspension was stirred at 0 °C. Lithium aluminum hydride (3.8 mg, 0.10 mmol) was added portionwise to the suspension, and the resulting mixture was stirred for 1 h at 0 °C. After excess lithium aluminum hydride was decomposed with ethyl acetate, the mixture was poured into water and organic materials were extracted with chloroform twice. The combined organic layers were washed with water and dried over anhydrous magnesium sulfate, and the solvent was evaporated to dryness under reduced pressure to give crude $\mathbf{2}_{red}$. To the residue were added chloroform (1 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (11.4 mg, 0.050 mmol), and the mixture was stirred for 5 min at room temperature. After the chloroform was removed from the solution under reduced pressure, the residue was subjected to column chromatography on silica gel with hexane/ethyl acetate (1:2) as eluent to give **2** (12.7 mg, 81% yield), which was subjected to HPLC to confirm that this is the (*S*)-(−)-enantiomer (94% ee). Consequently, the absolute configuration of (+)-**1** has been determined to be *S*.

Enantioface-Differentiating (Net) Hydride-Transfer Reaction between Racemic dFl-*5-d* and BNAH. To a solution of BNAH (214 mg, 1.0 mmol) and a catalyst (magnesium perchlorate (223 mg, 1.0 mmol) or trichloroacetic acid (327 mg, 2.0 mmol)) in acetonitrile (5 mL) was added racemic dFl-*5-d* (0.2 mmol), and the mixture was stirred in the dark at 298 K under argon atmosphere. The reaction was monitored by TLC. The time required for completion of the reaction is shown in Table 5. After the reaction was complete, the solvent was removed under reduced pressure and chloroform (*ca*. 50 mL) was added to the residue. The suspension was washed with saturated aqueous ammonium chloride and brine and dried over anhydrous magnesium sulfate, and the solvent was evaporated from the solution to dryness under reduced pressure. The residue was subjected to short column chromatography on silica gel with chloroform as eluent to give a mixture of diastereomers of dFl_{red}-*5-d*. The *syn*/*anti* ratios of the reacting faces of **2**, **3**, and **8** were determined with $\mathbf{2}_{red}$-*5-d*, $\mathbf{3}_{red}$-*5-d*, and $\mathbf{8}_{red}$-*5-d* thus obtained, respectively. The ratio in $\mathbf{1}_{red}$-*5-d* was determined through $\mathbf{3}_{red}$-*5-d*, which was derived from $\mathbf{1}_{red}$-*5-d* as follows: $\mathbf{1}_{red}$-*5-d*, *tert*-butyldimethylsilyl chloride (90 mg, 0.6 mmol), and imidazole (82 mg, 1.2 mmol) were dissolved in dry DMF (3 mL), and the solution was stirred in the dark at room temperature under argon atmosphere. After 1 h, the solution was poured into ice−water, and organic materials were extracted with ethyl acetate. The organic layer was washed with water four times as well as with brine once and dried over anhydrous magnesium sulfate, and the solvent was evaporated to dryness under reduced pressure. The residue was subjected to preparative TLC with hexane/ethyl acetate (2:1) as a developing solvent in the dark to give diastereomers of $\mathbf{3}_{red}$-*5-d*. In the same manner as described in previous papers,[24,25] the *syn*/*anti* ratio of the reacting faces was determined from the ratio of peak areas of the two diastereomeric C(5) protons in the ^{1}H NMR spectrum. The C(5) protons of the diastereomers could be discriminated by the difference in ^{1}H NMR chemical shifts in the presence of $Eu(fod)_3$-d_{27} (0.10 mol equiv to dFl_{red}-*5-d*).

Measurements of Association Constants. The formation of a complex between dFl and Mg^{2+} was monitored spectrophotometrically by following the disappearance of the absorbance at 439 nm of dFl. The linearity of the plot shown in Figure 4 reveals that the molar ratio in the complex is 1:1. The association constants were obtained from the slopes of the plots.

Diastereoface-Differentiating (Net) Hydride-Transfer Reaction between Racemic 1 and Chiral Me_2PNPH. Racemic **1** (45 mg, 1.0×10^{-1} mmol) was added to a solution of chiral Me_2PNPH (1.5 mg, 5.0×10^{-3} mmol) and magnesium perchlorate (0−50 mol equiv to Me_2PNPH) in acetonitrile (10 mL), and the solution was stirred in the dark for 30 min at 298 K under argon atmosphere. No products other than $\mathbf{1}_{red}$ were detected on TLC and HPLC. The solution was evaporated to dryness under reduced pressure, and the residue was subjected to preparative TLC with hexane/ethyl acetate (1:3) as a developing solvent in the dark to give $\mathbf{1}_{red}$. To a solution of the $\mathbf{1}_{red}$ in chloroform (3 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (23 mg, 1.0×10^{-1} mmol), and the mixture was stirred at room temperature. After 5 min, the solvent was evaporated from the suspension to dryness under reduced pressure, and the residue was subjected to preparative TLC with ethyl acetate as a developing solvent to give **1**. The *S*/*R* ratio in **1** reduced by Me_2PNPH was then determined by HPLC.

Asymmetric (Net) Hydride-Transfer Reaction between Chiral 1-*5-d* and Chiral Me_2PNPH. To a solution of chiral Me_2PNPH (75 mg, 0.25 mmol) and magnesium perchlorate (0 or 2.5 mol equiv to **1**-*5-d*) in acetonitrile (5 mL) was added chiral **1**-*5-d* (45 mg, 0.1 mmol), and the mixture was stirred in the dark at 298 K under argon atmosphere. The reaction was monitored by TLC, and the results are summarized in Table 8 together with the time required for completion of the reaction. Then, the same procedures as those described for the enantioface-differentiating (net) hydride-transfer reactions were carried out to determine the *syn*/*anti* ratio of the reacting faces.

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