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First Example of "Diastereotopic Face Activation" and Chiral Recognition without Metal Assistance. A Novel 5-Deazaflavin Enzyme Model.

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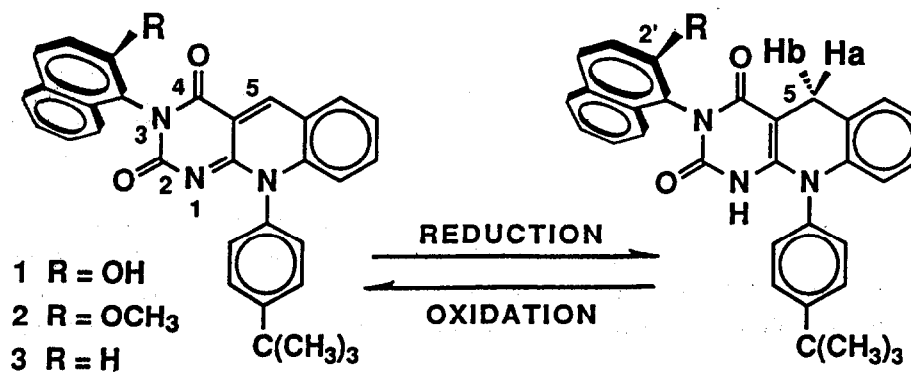
Abstract: A chiral 5-deazaflavin **1** with 2-hydroxynaphthyl group at N(3) position of the pyrimidine ring moiety was synthesized. **1** underwent, in the absence of magnesium ion, a "(net) hydride transfer" from BNAH to C(5) almost exclusively on the face where OH group is present. It was also the case with PNPH, and enantioselectivity for PNPH by **1** was higher in the absence of magnesium ion than in its presence. These results indicate that the "diastereotopic face activation" in **1** is operated as well as chiral recognition for PNPH by **1**.

Flavin and 5-deazaflavin[1] play important roles in the redox reactions in biological systems. It is conceivable that at the active site of the flavoenzymes, one face of flavin molecule is blocked by a "wall" of protein , thus only the other open face is allowed to interact with substrates. Besides this "diastereotopic face deactivation" or blocking, functional groups of the apoproteins present in proximity to a flavin molecule may contribute to "diastereotopic face activation" in the flavin residue through a diastereoface selective protonation or , in some instances, a metal coordination to enhance the face selective interaction with substrates. To affect the "diastereotopic face activation", we have prepared a novel optical active 5-deazaflavin **1** with axial chirality at N(3) position of the pyrimidine ring moiety (Scheme 1.). In **1**, OH group at C(2') position is the potential



Scheme 1[3].

functional group for an intramolecular acid catalyst. Since the rotation about N(3)-C(1') bond is restricted[2a] at room temperature, the phenolic OH group will reside only on one of the 5-deazaflavin faces. Thus the interaction[2b] between a flavin molecule and NAD(P)H models should be facilitated.

In chiral flavoenzyme models described so far[2], "diastereotopic face deactivation" factors through steric hindrance have been introduced into flavins or 5-deazaflavins. And no successful flavoenzyme model endowed with an effective intramolecular device for "diastereotopic face activation" has been reported[4].

The chiral 5-deazaflavin **1** was prepared (95%) by demethylation of chiral **2** with boron tribromide at low temperature without concomitant racemization (checked by HPLC) (Scheme 1.). Compound **2** was synthesized starting from (2-methoxynaphthyl)urea in a similar yield to that in a previous paper[2a]. To study the effect of substituent at C(2') position on the "(net) hydride transfer" reactions , 5-deazaflavin **3** , which lacks functionality at C(2') position, was also prepared in the same way (Scheme 1.). Successful optical resolution of **1-3** was achieved by an HPLC on a chiral stationary phase (CHIRALCEL OD). Specific rotations of the enantiomers are listed in Table 1.

Table 1. Specific Rotation of 5-Deazaflavin Enantiomers (c = 1.00 in chloroform)

5-Deazaflavin	e.e.(%)*		
(1)	>99.5	+94.5	-94.2
(2)**	>99.5	-40.3	+40.1
(3)***	>99.5	+23.9	-23.7

* Analytical HPLC showed no presence of the other enantiomer.
(-)-2** gave (+)-**1**. ***Absolute / relative configuration of **3** is not yet identified.

To discuss the diastereoface differentiating "(net) hydride transfer" reactions in **1-3**, 5-deazaflavin **1-3** were subjected to reactions with NAD(P)H models. As reported in a previous paper[2b], the two diastereotopic protons at C(5) position of the reduced 5-deazaflavins (Scheme 1.) were identifide by ^{1}H-NMR with an aid of Eu(fod)3-d27 (Figure 1a.).

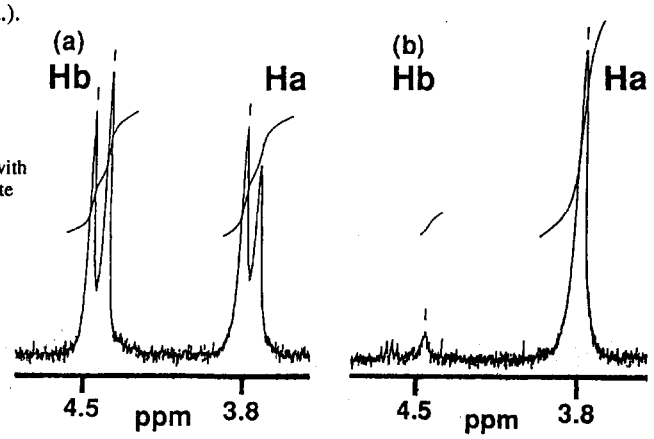


Figure 1. [5] ^{1}H-NMR spectra (300MHz) of C(5)-H in $CDCl_3$ at 298K in the presence of Eu(fod)3-d27 (0.20eq). (a) Product of reduction of **1** with $NaBH_4$ in ethanol. (b) Product of reduction of the deuterated **1** with BNAH in the absence of magnesium perchlorate in dichloromethane (Table 2. Run 3).

5-Deazaflavins **1-3** deuterated at C(5) position (98% isotope purity)[2b] were prepared and they were reduced with BNAH (1-benzyl-1,4-dihydronicotinamide) in the presence and absence[4] of magnesium perchlorate in acetonitrile at 298K. The ratios of the two diastereomers (one having a protium on "**a**" side and deuterium on "**b**" side at C(5) of the reduced form of 5-deazaflavin (Scheme 1.) is designated as **Ha,Db**, and

the other is designated as **Da,Hb**) were determined by ^{1}H-NMR. The results shown in Table 2 reveal that the OH group at C(2') position favors "**a**" side attack on **1** and the "bulky" methoxy group and *tert*-butyldimethylsilyl group[5] at C(2') position retards the "**a**" side attack.

Table 2. Diastereoface Differentiating "(Net) Hydride Transfer" to Deuterated 5-Deazaflavin*

Deuterated 5-Deazaflavin	Solvent	Catalyst	Time(hr)	Ha(%)	Hb(%)
(1)	CH_2Cl_2	-	2	97	3
(1)	CH_3CN	-	2	95	5
(1)[5]	CH_3CN	$Mg(ClO_4)_2$	0.25	75	25
(3)[8]	CH_3CN	$Mg(ClO_4)_2$	1	64	36
(2)[7]	CH_3CN	$Mg(ClO_4)_2$	0.75	50	50
(4)[5]	CH_3CN	$Mg(ClO_4)_2$	1	20	80

at 298K, $[dFl] = 1.0 \times 10^{-2}$ (M), $[BNAH] = 5.0 \times 10^{-2}$ (M), $[Mg(ClO_4)_2] = 5.0 \times 10^{-2}$ (M)

And surprisingly, the "(net) hydride transfer" occurred almost exclusively at **Ha** position ("**a**" face) in the absence of magnesium ion in acetonitrile as well as in dichloromethane (Figure 1b.). These results could be accomodated as follows. In the presence of magnesium ion, BNAH will be activated by the formation of a binary complex[9] ($BNAH{\cdot}Mg^{2+}$), which can lead to a productive ternary complex[2b] on both faces of 5-deazaflavin, with Mg^{2+} being a cramp iron or a jointing union between "(net) hydride" donor and acceptor molecules. On the other hand, in the absence of magnesium ion, cluster formation between **1** and BNAH is facilitated on the face, where the OH group is present , thus "(net) hydride transfer" took place almost exclusively at **Ha** position. Essentially no intermolecular acid catalysis was observed for **2** and **3**.[4,6] An analogue of **1**, which has 2-hydroxyphenyl group at N(3) position, was similarly reduced by BNAH resulting in a "(net) hydride transfer" in the absence of magnesium ion (This model compound allows a free rotation about N(3)-C(1') bond, thus giving rise to no diastereoface differentiation.). These results clearly indicate that the OH group at C(2') position of **1** facilitates the "(net) hydride transfer"reactions furnishing a "diastereotopic face activation" in the reactions with BNAH.

Chiral recognition by 5-deazaflavins **1-3** were investigated in a model reaction of asymmetric intercoenzyme "(net) hydride transfer" reaction. Oxidation reactions of PNPH (1-propyl-N-a-methylbenzyl-1,4-dihydronicotinamide)[2a,d,f,h] by (+)-**1**, (-)-**2**, and (+)-**3** were carried out in the presence of or in the absence of magnesium perchlorate. Pseudo-first-order rate constants[10] were determined by monitoring the

Table 3. Discrimination Factor and Estimated Pseudo-first-order Rate Constans ($k\psi$ min^{-1})

5-Deazaflavin	Solvent	Catalyst	$k\psi$-(R)-PNPH / $k\psi$-(S)-PNPH	$k\psi \times 10^3$ (R)-PNPH	$k\psi \times 10^3$ (S)-PNPH
(+)-(1)*	CH_3CN	$Mg(ClO_4)_2$	1.40	1.82	1.30
(+)-(1)**	CH_3CN	-	2.20	1.04	0.472
(+)-(1)**	CH_2Cl_2	-	1.93	0.943	0.488
(-)-(2)*	CH_3CN	$Mg(ClO_4)_2$	1.00	0.714	0.712
(+)-(3)*	CH_3CN	$Mg(ClO_4)_2$	1.25	0.626	0.499

at 298K, * $[dFl] = 1.0 \times 10^{-4}$ (M), $[PNPH] = 5.0 \times 10^{-4}$ (M), $[Mg(ClO_4)_2] = 5.0 \times 10^{-4}$ (M)

** $[dFl] = 1.0 \times 10^{-4}$ (M), $[PNPH] = 2.0 \times 10^{-3}$ (M)

decrease of absorption at 420nm, which corresponds to the absorption of 5-deazaflavins (Table 3.). As Table 3 shows, (+)-**1** and (+)-**3** oxidized (R)-PNPH more rapidly than its (S) isomer. Higher degree of chiral recognition (k_R/k_S) was observed for (+)-**1** than for (+)-**2** or (+)-**3** in the presence of magnesium ion. However, the degree of chiral recognition by (+)-**1** was appreciably higher in the absence of magnesium ion than in its presence. These results are unique and against those of usual cases[11], where the magnesium bridging brings about a higher chiral recognition.

The results in the present paper show the first successful example of "diastereotopic face activation" in 5-deazaflavin models and imply that the multiple interactions between substrate and reagent are quite crucial in a flavoenzyme system in biological system.

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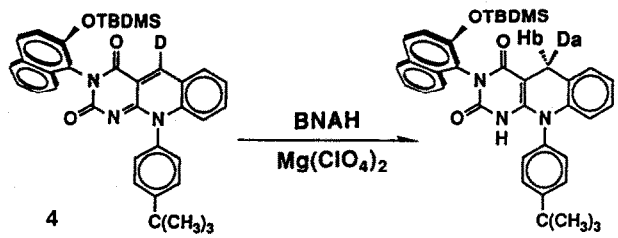
3. One of the enantiomers is depicted throughout the text.
4. Shinkai, S.; Kawanabe, S.; Kawase, A.; Yamaguchi, T.; Manabe, O. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2095-2102.
5. The peaks in the higher magnetic field (Hh) and in the lower (Hl) in this figure were assigned to **Ha** and **Hb** by the following experiments. **4** (TBDMS = *tert*-butyldimethylsilyl) is prepared from deuterated **1** and reduced with BNAH in the presence of magnesium perchlorate in acetonitrile at 298K. The reduced **4** gave the two peaks for diastereomers, Hh : Hl = 80 : 20 in ^{1}H-NMR in the presence of the shift reagent, which is simply due to the steric hindrances of OTBDMS group, meaning that Hh corresponds to **Hb** and Hl to **Ha**. The reduced **1** (Table 2. Run 3) was transformed into the reduced **4** under argon and gave Hh : Hl = 3 : 97, meaning **Ha** : **Hb** = 97 : 3. Thus, in the reduced **1**, Hh and Hl can be assigned to **Ha** and **Hb** respectively.



6. In **2** and **3** the reaction did not proceed at all in the absence of magnesium ion. Even in the presence of p-cyanophenol (See ref 4.), m-trifluoromethylphenol, or acetic acid, where [dFl] = 1.0 x 10^{-2}(M), [BNAH] = 5.0 x 10^{-2}(M), [Acid] = up to 2.0 M (the latter two acids) in acetonitrile or dicloromethane, the reaction was not detected at all at 298K.
7. The peaks for the two protons have not been assigned to **Ha** and **Hb** in ^{1}H-NMR.
8. As is the case with the results in ref 2b, molecular model suggests steric hindrance of 1-naphthyl group should simply retard the approach of "(net) hydride donor" from the "**b**" side in 5 deazaflavin **3**.
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10. k = $[\text{Rate}]_0/[\text{dFl}]_0$, where "0" stands for initial rate and initial concentration. See ref 2h (ref and notes 14).
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