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Diastereoface Differentiating "(Net) Hydride Transfer" in Novel 5-Deazaflavins Modified at Pyrimidine Ring

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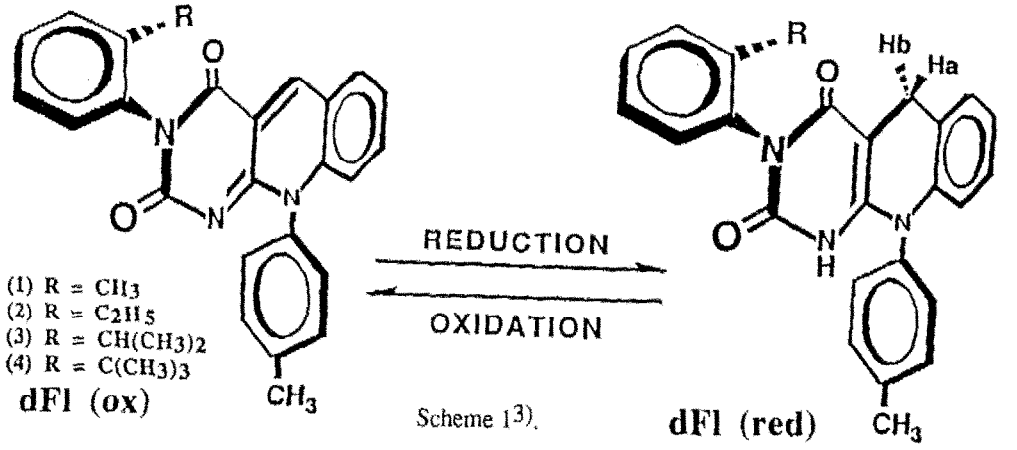
Abstract. In novel 5-deazaflavin models (1)-(5) where one face of the pyrimidine ring moiety is flanked, "(net) hydride transfer" from BNAH ocurred at mainly C(5) on the face which aligns with the open side of the pyrimidine ring. The degree of diastereoface differentiation depends on the bulkiness of the substituent on phenyl group at N(3). The results revealed that the pyrimidine ring moiety of flavin ring system interacts with the carbamoyl group of BNAH in the transition state of the "(net) hydride transfer" reaction. Diastereoface differentiating "(net) hydride abstraction" from the reduced 5-deazaflavin was also investigated.

It is well known that the discrimination of the diastereotopic two protons at C(4) of the nicotinamide moiety of NAD(P)H in redox reactions is essentially absent in the free coenzyme, and relevant when it associates with dehydrogenase family. In cyclic, enzyme bound NAD(P)H models, Rob and Kok[1] demonstrated that "(net) hydride" exchange occurs exclusively at one of the diastereotopic positions at C(4) of dihydronicotinamide moiety.

It should be interesting to investigate "(net) hydride transfer" reaction mechanism in flavin coenzymes as well as in NAD(P)H. Shinkai and co-workers synthesized 5-deazaflavins incorporated into a cyclophane framework and demonstrated that "(net) hydride" exchange occurs exclusively at the axial position of C(5) in the reduced 5-deazaflavin.[2] Using an acyclic, enzyme bound 5-deazaflavin model, we reported that "(net) hydride" incorporation occurs exclusively at the equatorial position at C(5) of the reduced product[4] in contrast



(1) R = CH_3
(2) R = C_2H_5
(3) R = $CH(CH_3)_2$
(4) R = $C(CH_3)_3$

dFl (ox) **dFl (red)**

Scheme 1[3].

to the results observed in cyclic models[1,2]. We have revealed that conformation of the reduced 5-deazaflavin affects significantly the reactivity of the diastereotopic two protons at C(5).[4]

In a preceding paper, we described the synthesis and reaction of novel 5-deazaflavin derivatives (**1**)-(**4**) (Scheme 1) and (**5**) possessing 2-substituted phenyl or 1-naphthyl group at N(3) position where one face of the pyrimidine ring is flanked through the hindered rotation about aryl C-N(3) bond. We revealed that the pyrimidine ring moiety of flavin ring system gives an important effect on the asymmetric "(net) hydride transfer" reaction with NAD(P)H. In the above model reaction, there was an interesting tendency. As the substituent on phenyl group became larger, the degree of chiral recognition ability of 5-deazaflavin enantiomers increased with the concomitant increase of rate constants. Considering that the reaction between flavins and NAD(P)H occurs in face to face exposition, a diastereoface differentiating approach of the NAD(P)H model to 5-deazaflavin derivatives may closely be related to chiral recognition ability of the 5-deazaflavin model compounds. Compared with former model compounds[1,2,4], where one face of the ring containing reactive center of dihydronicotinamide or 5-deazaflavin molecule is flanked, the present model compounds have the substituents on phenyl group at N(3) position giving steric hindrance on one of the pyrimidine faces. However the hydride accepting central ring of the 5-deazaflavin should not be affected directly. In the present paper, we describe diastereoface differentiating "(net) hydride transfer" reactions in hitherto unknown 5-deazaflavin models and the interaction of the pyrimidine ring moiety of flavin ring system with substrates.

When compounds (**1**)-(**5**) were reduced with sodium borohydride, the two protons at C(5) of the products gave a sharp singlet peak in ^{1}H-NMR at 298K. It is well known that the central ring of reduced flavins or 5-deazaflavins adopt (half)boat shape folded along a N(5)- or C(5)-N(10) line[2,4,5], and the two protons at C(5) ocuppy diastereotopic positions which can be discriminated in ^{1}H-NMR when the ring flipping of the central ring is prevented[2,4]. The present result indicates that a fast ring flipping makes the two protons magnetically equivalent. In order to detect diastereoface differentiating "(net) hydride transfer" reactions during the transformation of dFl(ox) into dFl(red), the two protons should be identified in ^{1}H-NMR. At low temperature the singlet peak changed into AB quartet, still not completely resolved at 233K. An addition of Eu(fod)3-d27 to $CDCl_3$ solution of the reduced products from (**1**)-(**5**) gave acceptable separation of the peaks for the two protons[6](Figure 1(a)).

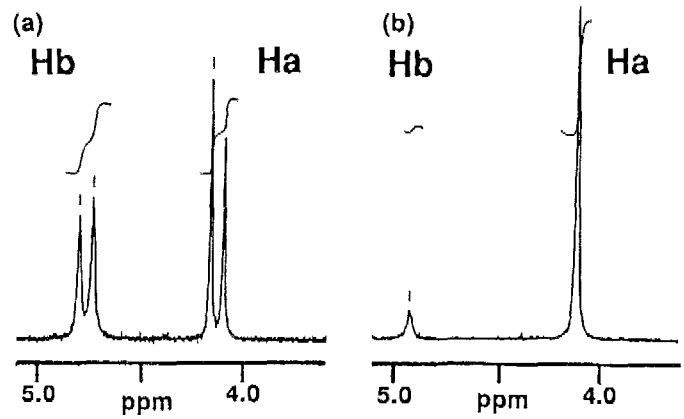


Figure 1.
^{1}H-NMR spectra (300MHz) at C(5) in $CDCl_3$ at 298K in the presence of Eu(fod)$_3$ (0.20eq) in the product of (**4**) reduced with $NaBH_4$ (a), and the product of deuteriated (**4**) reduced with BNAH (b).

5-Deazaflavin derivatives (**1**)-(**5**) deuterated at C(5) position (98% isotope purity) were prepared by the condensation of o-fluorobenzaldehyde-α-d and the corresponding 6-aminouracil derivative and they were

reduced with 1-benzyl-1,4-dihydronicotinamide (BNAH) in acetonitrile in the presence of magnesium perchlorate at 298K. In this reaction, no reverse "(net) hydride transfer" process took place. The ratio of the diastereomers (one having protium on "**a**" side (Scheme 1.) and duterium on "**b**" side at C(5) of the reduced product is designated as **Ha,Db** and the other is designated as **Da,Hb**), which was constant throughout the reaction time, was measured in the presence of the shift reagent and listed in Table 1. As Figure 1 (b) shows, "(net) hydride" incorporation occurred mainly at the proton in the higher magnetic field. As the substituent on phenyl group at N(3) became larger, the intensity of the peak in the higher magnetic field increased and that in the lower decreased. From these results the peak in the higher magnetic field can be assigned to Ha and that in the lower to Hb (Scheme 1). In the deuterated (**4**), the "(net) hydride" incorporation occurred exclusively at Ha position, which is the same degree of the diastereoface differentiation as those observed in former models where one face of the reactive center is flanked[1,2,4].

Table 1. Diastereoface Differentiating "(Net) Hydride Transfer" to the 5-Deuterated-5-Deazaflavins

Deuterated	BNAH*			$NaBH_4$**	
5-Deazaflavin	Ha(%)	Hb(%)	Time(hr)	Ha(%)	Hb(%)
(**1**)	66	34	3	50	50
(**2**)	76	24	2	50	50
(**3**)	80	20	2	50	50
(**4**)	99	1	1	50	50
(**5**)	60	40	3	50	50

*[5-Deazaflavin] = 4.0×10^{-2}(M), [BNAH] = 2.0×10^{-1}(M), [$Mg(ClO_4)_2$] = 2.0×10^{-1}(M)

**[5-Deazaflavin] = 4.0×10^{-2}(M), [$NaBH_4$] = 2.0×10^{-1}(M), The reactions were instantaneous.

In the transition state of the "(net) hydride transfer" reaction, there could be two kinds of orientation of the carbamoyl group of BNAH on an approach to a 5-deazaflavin face; one is that the carbamoyl group resides over the benzene ring of the 5-deazaflavin face, and the other is that it resides over the pyrimidine ring. The former pathway allows a less hindered approach to a reaction center, least affected by *tert*-butyl group on phenyl group at N(3). However the present results imply that this reaction proceeds through the latter pathway where the carbamonyl group of BNAH attracts the pyrimidine ring moiety of 5-deazaflavins most probably intervened by magnesium ion. Sodium borohydride was also employed to reduce the deuterated 5-deazaflavin derivatives (**1**)-(**5**) in ethanol at 298K. As Table 1 shows, no differentiation was observed even in the deuterated (**4**) possessing *tert*-butyl group on phenyl group. These results suggest that a hydride attack by sodium borohydride proceeds essentially with no perturbation from the pyrimidine ring moiety.

It is also important to investigate diastereoface differentiating "(net) hydride transfer" reactions from flavins to other "(net) hydride" acceptors, since flavins serve as mediators of electron transport in biological systems.Two diastereomers, **Ha**, **Db**, and **Da**, **Hb**[7] at C(5) in the reduced form of (**4**), were oxidized with the following three "(net) hydride" acceptors, [1] 3-methyl-10-ethylflavin (MEF), [2] 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), and [3] $CuCl_2$ respectively and the contents of the proton at C(5) was measured in ^{1}H-NMR.

As Table 2 shows, in the oxidation with MEF, Ha is about four times more susceptible to "(net) hydride transfer" than Hb. In the reactions with DDQ and $CuCl_2$, no discrimination of the two protons at C(5) was observed, however significant primary isotope effect[8] was observed. The results indicate that the oxidation reaction with quinones or cupric ion proceeds with no participation of pyrimidine ring moiety.

Table2. Diastereoface Differentiating "Net Hydride Abstraction" from the Reduced Form of (4)[a)]

Reagent	Solvent	Time (hr)	"Hydride" Abstraction from the Reduced (4)				kHa/kHb
			Ha(%)	Db(%)	Da(%)	Hb(%)	
MEF[b)]	$CHCl_3$	72	90	10	66	34	4.2[9)]
DDQ[c)]	Benzene	0.5	83	17	17	83	1.0
$CuCl_2$[d)]	Acetic acid	72	75	25	28	72	1.0

a) All the reaction was carried out under argon at 298K, [reduced (**4**)] = 2.5 x 10^{-2}(M)
b)[MEF] = 1.25 x 10^{-1}(M) c)[DDQ] = 1.25 x 10^{-1}(M) d)[$CuCl_2$] = 5.0 x 10^{-1}(M)

The oxidation reaction with quinones or cupric ion proceeds with no participation of pyrimidine ring moiety. The diastereoface differentiating "(net) hydride" abstraction observed with MEF seems to be due to the steric hindrance by the bulky oxidizing molecule. It is reported that at C(4) of dihydronicotinamide or at C(5) of a reduced 5-deazaflavin of cyclic models, the proton on the "open face" (**Ha**) is about eight times as reactive as that on the "hindered face" (**Hb**)[1a,2], where **Ha** occupies the reactive, axial position and **Hb** occupies the retarded, equatorial position. In the present model, a rapid interconversion in the positions of the two protons (**Ha** and **Hb**) will take place between the axial and the equatorial positions, leading to give a smaller discrimination of the two protons in comparison with those observed in cyclic models.

The results in the present paper and the preceding paper reveal that the pyrimidine ring moiety of flavin ring system interacts with the carbamoyl group of nicotinamide moiety of NAD(P)H and its models in the transition state of "(net) hydride transfer" reactions, thus significantly affecting asymmetric reactions between flavins and NAD(P)H in biological systems.

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2. Shinkai S.; Yamaguchi T.; Kawase A.; Manabe, O.; Kellog, R. M. *J. Am. Chem. Soc.* **1989**, *111*, 4935-4940.
3. In the reduced forms of present models, a rapid interconverisin of the two possible bent conformations will take place, giving rise to a time averaged ^{1}H-NMR spectra for two C(5) protons. Thus for the simplicity, reduced form of the 5-deazaflavin dFl(red) is depicted in a planar form in this scheme.
4. Kawamoto, T.; Tanaka, K.; Kuroda,Y.; Yoneda, F. *Chem. Lett.* **1990**, 1197-1200.
5. Tanscher, L.; Ghisla, S.; Hemmerich, P. *Helv. Chim. Acta.* **1973**, *56* ,630-644.
6. It has been found that the degree of separation of the peaks depends on bulkiness of the substituent on phenyl group at N(3) position. Most probably, this is because the shift reagent is in face selective equilibrium on either side of pyrimidine ring moiety of reduced 5-deazaflavin derivatives. The details will be discussed elsewhere.
7. This diastreomer was prepared by the reduction of (**4**) with 4,4-dideuterio-1-benzyl- 1,4-dihydroniocotinamide (98% isotope purity) in acetonitrile in the presence of magnesium perchlorate at 298K.
8. Goto, M.; Mikata, Y., Ohno, A. *Bull. Chem. Soc. Jpn.*, **1990**, *63*, 2682-2686.
9. In a competitive (pseudo-) first-order reaction, the product ratio is equal to the ratio of the rate constants and

$$\frac{k_H^a}{k_D^b} = \frac{90}{10} \qquad \frac{k_D^a}{k_H^b} = \frac{66}{34} \text{ , thus } \qquad \frac{k_H^a}{k_H^b} \cdot \frac{k_D^a}{k_D^b} = \frac{90 \cdot 66}{10 \cdot 34} .$$

As the relative reactivity of the two diastereomers (**Ha,Db/Da,Hb**) proved the absence of quantum mechanical tunneling effect,

$$\frac{k_H^a}{k_H^b} = \frac{k_D^a}{k_D^b} \text{ is a plausible assumption giving rise to } \frac{k_H^a}{k_H^b} = \sqrt{\frac{90 \cdot 66}{10 \cdot 34}} = 4.2.$$

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