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Variation of Reaction Channel in a Flavoenzyme Model with a Modified Pyrimidine Ring

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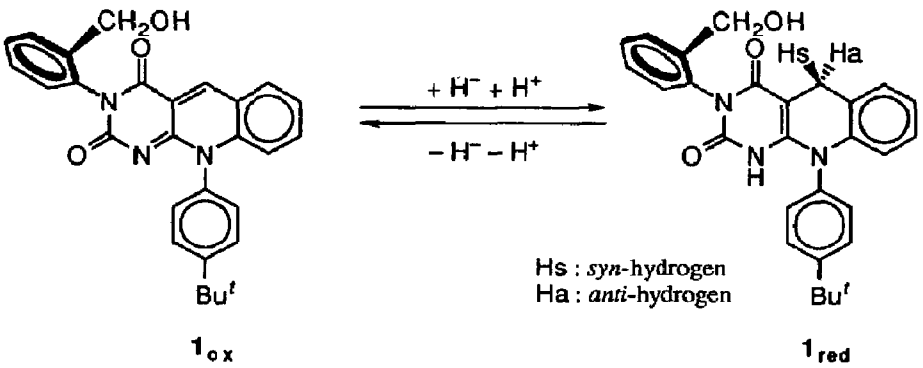
Abstract: A chiral 5-deazaflavin derivative with 2-hydroxymethylphenyl group at N(3) position of the pyrimidine ring has been synthesized. Oxidized form of the 5-deazaflavin derivative is reduced stereospecifically: the hydroxymethyl group exerts steric inhibition in the absence of Mg^{2+}, whereas it facilitates the approach of a reducing agent in the presence of Mg^{2+}.

Flavin and 5-deazaflavin[4-6] play important roles in redox reactions in biological systems. At the active site of flavoenzymes, functional groups of apoproteins in proximity to a flavin coenzyme may affect stereochemistry and facility of the reaction through a diastereoface selective protonation or coordination of a metal ion. To investigate stereochemical as well as catalytic effect of functional groups in apoproteins, we have prepared a novel optically active 5-deazaflavin derivative **1** with axial chirality at N(3) position of the pyrimidine ring (Scheme 1).

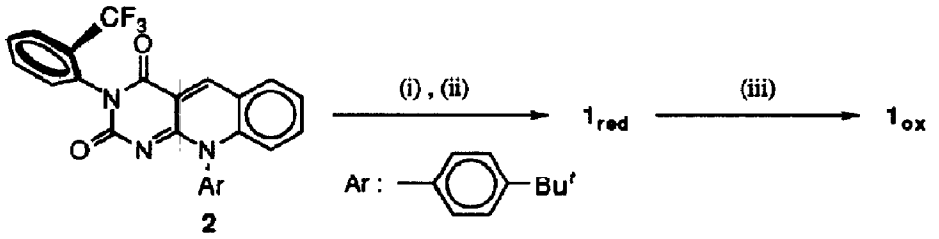


$\mathbf{1}_{ox}$ $\mathbf{1}_{red}$

Scheme 1

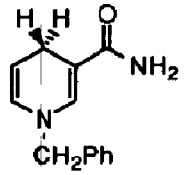
In the model compound, the hydroxymethyl group at C(2') position mimics potential functional group of apoproteins in proximity to a flavin coenzyme. The rotation about N(3)-C(aryl) bond in the model is restricted[7] at room temperature. Flavoenzyme models with "diastereoface deactivation" factor[7-10] through steric hindrance and those with "diastereoface activation" factor[11] through intramolecular acid catalysis have been reported so far.

Racemic $\mathbf{1}_{red}$ and $\mathbf{1}_{ox}$ were prepared according to Scheme 2. Compound **2** was synthesized by Yoneda's method[7] starting from (2-trifluoromethylphenyl)urea which was prepared from 2-trifluoromethylaniline by treatment with sodium cyanate[12]. Optical resolution of $\mathbf{1}_{ox}$ was carried out by HPLC on a chiral stationary phase (CHIRALCEL OD, ethanol). Specific rotations ($[\alpha]_D^{25}$; *c*,1.00; $CHCl_3$) of the enantiomers of $\mathbf{1}_{ox}$ were measured to be +59.1 and –57.3, respectively. Absolute configurations of $\mathbf{1}_{red}$ and $\mathbf{1}_{ox}$ are not yet elucidated (also *cf.* ref. 20).

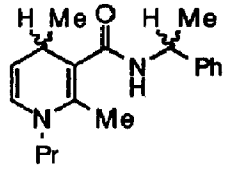


Reagents: (i) conc.H_2SO_4; (ii) $NaBH_4$, BF_3-OEt_2, THF; (iii) DDQ,$CHCl_3$

Scheme 2



BNAH



Me_2PNPH

To investigate enantioface specificity in the reduction of $\mathbf{1}_{ox}$, (net) hydride transfer reactions from *N*-benzyl-1,4-dihydronicotinamide (BNAH) to $\mathbf{1}_{ox}$ deuterated at the C(5) position ($\mathbf{1}_{ox}$-5-*d*)[7] have been studied in the presence and absence of magnesium perchlorate in acetonitrile at 298 K in the dark under Ar atmosphere.[13] The results are summarized in Table 1.

Table 1. Enantioface Differentiating (Net) Hydride Transfer Reaction between $\mathbf{1}_{ox}$ and BNAH[a)]

Catalyst (Equiv.)[b)]	Reaction Time/min	Area Ratio H_{syn}	Area Ratio H_{anti}
$Mg(ClO_4)_2$ (5)	10	78	22
CCl_3CO_2H (10)[c)]	60	31	69

[a)] $[\mathbf{1}_{ox}] = 4.0 \times 10^{-2}$ *M*, [BNAH] = 2.0×10^{-1} *M*. [b)] Equivalency to $[\mathbf{1}_{ox}]$.

Quite reasonably from the viewpoint of steric interference, the reaction without Mg^{2+} prefers the attack of (net) hydride from the open face of $\mathbf{1}_{ox}$.[8] On the other hand, the enantioface specificity observed here in the presence of Mg^{2+} is not only reversed from the result obtained in its absence but also reversed from the results obtained from the reactions with the analog of $\mathbf{1}_{ox}$ without a hydroxyl group.[8] The observation suggests clearly that the hydroxyl group plays an important role to coordinate a Mg^{2+} to form a ternary complex with BNAH;[14] an electronic effect to promote the reaction in the *syn* face[15,16] overwhelms in energy steric retardation in this face. It should be noted that the reaction with $\mathbf{1}_{ox}$ is accelerated tremendously in comparison with the analogs without a hydroxyl group.[8] Thus, the hydroxymethyl group in the side chain of $\mathbf{1}_{ox}$ has a catalytic function as well as stereo-controlling function similarly to the role of hydroxyl group in an apoenzyme.

Steric arrangement between $\mathbf{1}_{ox}$ and an NAD(P)H analog in the complex is elucidated more precisely when a diastereoface differentiating reduction is studied by using (4*S*,9*S*)- and (4*R*,9*R*)-*N*-α-methylbenzyl-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide (Me_2PNPH)[17] as the reducing agents.[18] Here, $\mathbf{1}_{red}$ obtained by the reduction after 30 min in acetonitrile at 298 K in the dark under Ar atmosphere was again oxidized by DDQ to $\mathbf{1}_{ox}$, and this $\mathbf{1}_{ox}$ was subjected to HPLC to measure the (+)-$\mathbf{1}_{ox}$/(−)-$\mathbf{1}_{ox}$ reactivity ratio. The ratio corresponds almost directly to the ratio of *syn*/*anti* reacting face.[19,20] Results are listed in Table 2.

It is surprising that diastereoface is not differentiated in the absence of Mg^{2+}. Although the experiments undoubtedly predict that the yield of one enantiomer is slightly higher than that of the other, such small differences as those listed in Table 2 cannot be appointed as meaningful values for further discussion. At the same time, the result indicates that the reaction with Me_2PNPH requires 10 times as concentrated Mg^{2+} as that required for the reaction with BNAH in order to exert similar stereospecificity. The observation stems from the fact that Me_2PNPH can react with $\mathbf{1}_{ox}$ with a reasonable reactivity without the assistance by Mg^{2+}, whereas the reaction with BNAH in the absence of Mg^{2+} hardly proceeds due to its low reactivity. Note that the presence and absence of Mg^{2+} afford opposite stereochemical results.

It is concluded from the result that molecular arrangement between oxidizing and reducing reagents in the complex is quite strict. Since BNAH has a σ symmetry with respect to its molecular plane, approach of BNAH to $\mathbf{1}_{ox}$ always results in the reaction regardless the attacking face. This is not the case for Me_2PNPH, in which a hydrogen to be transferred resides in one of diastereofaces only. Consequently, only one face of

Table 2. Diastereoface Differentiating (Net) Hydride Transfer Reaction between $\mathbf{1}_{ox}$ and Me_2PNPH[a)]

Me_2PNPH	Equiv. of $Mg(ClO_4)_2$[b)]	$\mathbf{1}_{ox}$ Reacted (+)-$\mathbf{1}_{ox}$	(−)-$\mathbf{1}_{ox}$	Ratio *syn* / *anti*[c)]
(4*S*,9*S*)	0	47	53	1.0 / 1.1
(4*S*,9*S*)	10	62	38	1.6 / 1.0
(4*S*,9*S*)	50	74	26	2.8 / 1.0
(4*R*,9*R*)	0	52	48	1.1 / 1.0
(4*R*,9*R*)	10	36	64	1.8 / 1.0
(4*R*,9*R*)	50	28	72	1.0 / 2.6

a) $[\mathbf{1}_{ox}] = 1.0 \times 10^{-2}$ *M*, $[Me_2PNPH] = 5.0 \times 10^{-4}$ *M*. b) Equivalency to $[\mathbf{1}_{ox}]$. c) The ratio does not change meaningfully throughout the reaction.

Me_2PNPH is responsible to form a reacting complex remaining the other to be abortive. In a combination of (4*S*,9*S*)-Me_2PNPH and (–)-$\mathbf{1}_{ox}$,[20] for example, the carbamoyl side chain of the former has to face against the aromatic ring of the latter in order to proceed the reaction from this complex. This is an unreactive molecular arrangement even though (–)-$\mathbf{1}_{ox}$ provides its "active" face.[19] As a result, difference in reactivity of each face of $\mathbf{1}_{ox}$ becomes smaller in the reaction with Me_2PNPH than that with BNAH as indeed is seen in Table 2.

When Mg^{2+} is present in the reaction system, the hydroxyl group in $\mathbf{1}_{ox}$ plays a catalytic role to form a complex with an NAD(P)H analog by coordinating on Mg^{2+},[15] and the face of $\mathbf{1}_{ox}$ occupied by the hydroxymethyl group now becomes more reactive than the other. Thus, the hydroxyl group controls both reactivity and stereochemistry of the reaction.

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20. It is shown in Table 1 that (4*S*,9*S*)-Me_2PNPH reacts with (+)-$\mathbf{1}_{ox}$ more easily than with (–)-$\mathbf{1}_{ox}$ in the presence of Mg^{2+} and *vice versa*. The results as well as those reported in ref. 18 suggest that (+)-$\mathbf{1}_{ox}$ and (–)-$\mathbf{1}_{ox}$ have *S*- and *R*-configurations, respectively.

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