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Synthesis and Reaction of Novel 5-Deazaflavins with Axial Chirality at Pyrimidine Ring Moiety

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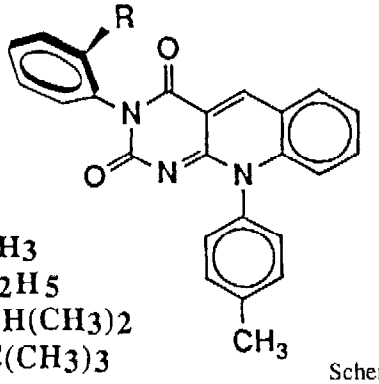
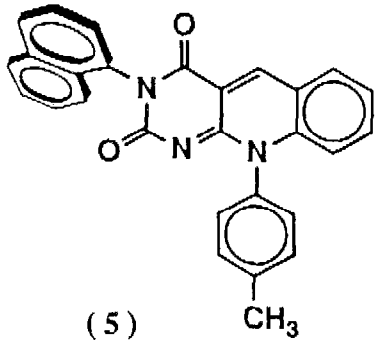
Abstract. A series of novel 5-deazaflavin derivatives possessing axial chirality at pyrimidine ring moiety have been prepared to investigate effects of the pyrimidine site on the stereoselective reactions between flavins and substrates. Successful optical resolution of the racemic compounds has been achieved by HPLC method on a chiral stationary phase and a diastereomer formation method. The chiral recognition ability of the 5-deazaflavin enantiomers was investigated in a model reaction of asymmetric intercoenzyme "(net) hydride transfer" reactions.

Flavin and 5-deazaflavin [1] are the main redox coenzymes which play important roles in biological systems. Among three rings of the flavin or 5-deazaflavin molecule, the most polar pyrimidine ring is well known to participate in the formation of hydrogen bonding with the apoenzyme and in the coordination onto a metal ion at the active site of flavoenzymes[2]. It may be possible to consider that there are some electrostatic interactions between the pyrimidine site of flavin or 5-deazaflavin ring system and the substrates, leading to stereoselective reactions between them. To investigate effects of the pyrimidine site of flavin ring system on the stereoselective reactions with substrates, we have now designed novel 5-deazaflavin derivatives with axial chirality at pyrimidine ring moiety (**1**)-(**5**), as are depicted in Scheme 1.



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



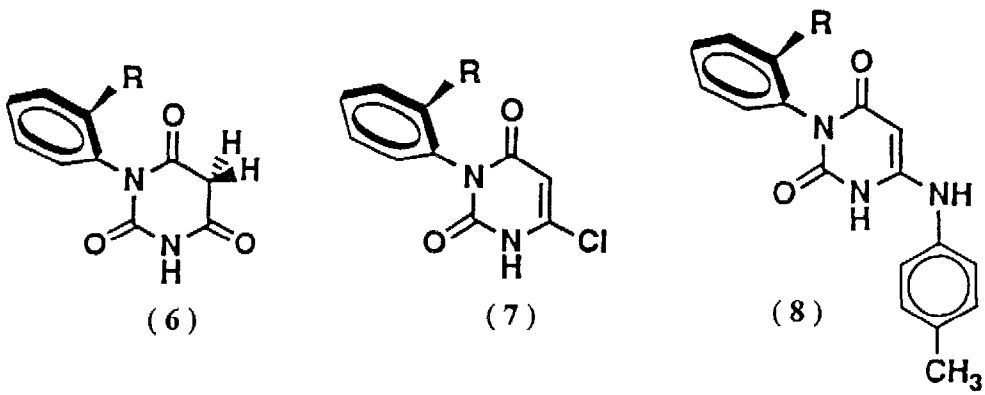
(5)

Scheme 1[3].

In these model compounds, the rotation about the aryl C-N(3) bond on the pyrimidine ring is restricted at room temperature, giving an enantiomeric pair of atropisomers[4]. Since the substituents on phenyl group at

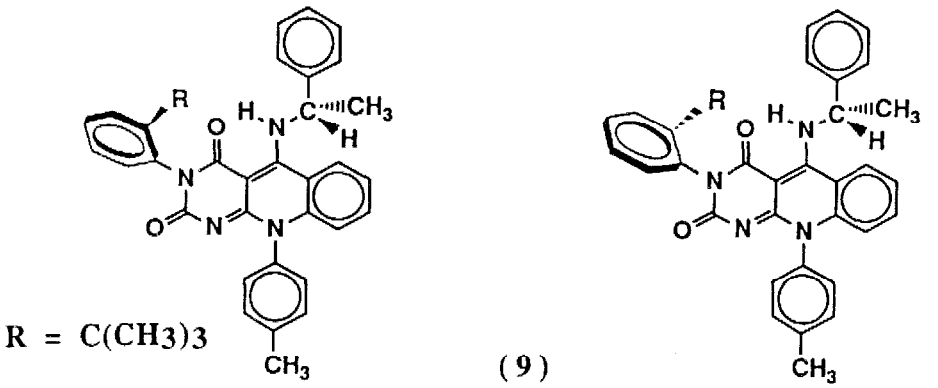
N(3) position will cover a part of one face of the pyrimidine site, the interaction, if any, between the pyrimidine face and substrates will be interferred. In chiral flavin coenzyme models so far reported[5], successful chiral recognition and diastereoface differentiation were achieved with the flavin and 5-deazaflavin derivatives included in a cyclophane structure by Shinkai et al.[5d,e] and the 5-deazaflavin derivative bearing planar chirality by our group[5f,g], both of which possess axial chirality at N(10) position. In those model compounds, one of the (5-deaza)flavin faces containing the reactive central ring is covered, and the reaction was possible only on the other face. While, in the present model compounds, the substituents on phenyl group provide steric hindrance on the pyrimidine ring, however little steric hindrance on the reactive central ring. Therefore, the present model compounds will be well suited to investigate effects of the pyrimidine site of flavin ring system on the stereoselective reactions with substrates. The present paper reports the first example of synthesis and reaction of 5-deazaflavins with axial chirality at pyrimidine ring moiety, as a novel type of flavoenzyme model.

The racemic model compounds (**1**)-(**5**) were prepared according to Yoneda's method; condensation of N-arylureas[6] with dimethyl malonate gave 1-arylbarbituric acids (**6**) in 70-75% yields. A regioselective chlorination of (**6**) with phosphoryl chloride in the presence of small amount of water afforded 3-aryl-6-chlorouracil derivatives (**7**) in 75-80% yields[7], which were treated with p-toluidine to give 3-aryl-6-(4'-methylphenyl)amino uracil derivatives (**8**) in 80-85% yields (Scheme 2). Finally, condensation of (**8**) with o-fluorobenzaldehyde gave the racemic (**1**)-(**5**) in 80-85% yields.



Scheme 2.

The optical resolution of the racemic compounds was carried out by the HPLC method on a chiral stationary phase (CHIRALCEL OD). It has been found that the efficiency of separation of the enantiomers depends on the bulkiness of the substituent on phenyl group at N(3) position; as the substituent becomes bulkier, the degree of separeation of the enantiomers was decreased, and in (**4**), no separation was observed under any available conditions. For optical resolution of (**4**), a diastereomer formation method developed by us[5f] was employed. The racemic (**4**) was transformed into the diastereomers (**9**) (Scheme 3.) using (S)-(-)-α-methylbenzylamine as a chiral auxiliary, which were completely separated in a preparative scale[8].



(9)

Scheme 3.

Table 1. Optical Resolution and Specific Rotations of the Enantiomers*

	Method**	e.e.(%)		
(1)	A	99.8	+31.2°	-28.7°
(2)	A	99.8	+21.7°	-20.6°
(3)	A	99.0	+24.3°	-24.3°
(4)	B	99.9	+ 4.2°	- 4.2°
(5)	A	99.8	+40.1°	-37.3°

* c=1.00 in chloroform

** A; HPLC methd on a chiral stationary phase, B; Diastereomer formation method.

No racemization was observed in the tranformation of (9) into the chiral form of (4) (checked by the ^{1}H-NMR in the presence of (+)-Eu(hfc)$_3$.). The enantiomeric excess and the specific rotations of the 5-deazaflavin enantiomers are listed in Table 1. These compounds has rather smaller specific rotations in comparison with those of known chiral flavin coenzyme models[5]. The absolute configuration of the chiral compounds is under investigation

The chiral recognition ability of the 5-deazaflavin enantiomers was investigated in a model reaction of asymmetric intercoenzyme "(net) hydride transfer" reactions. The oxidation reactions of (R)- and (S)-N-α-methylbenzyl-1-propyl-1,4-dihydronicotinamide (PNPH)[5d,f] with 5-deazaflavin enantiomers ((+)-dFl) (+)-1-(+)-(5) were carried out in acetonitorile in the presence of magnesium perchlorate at 298K. The pseudo-

Table 2. Discrimination Factor and the Estimated Pseudo-first-order Rate Constants ($k\psi$ min^{-1})***

	$k\psi$-(R)-PNPH / $k\psi$-(S)-PNPH	$k\psi$ x 10^{-4} (R)-PNPH	$k\psi$ x 10^{-4} (S)-PNPH
(+)-(1)	1.43	5.55	3.88
(+)-(2)	1.63	6.01	3.70
(+)-(3)	1.73	6.17	3.57
(+)-(4)	2.20	6.92	3.13
(+)-(5)	1.20	5.60	4.64

*** 298K, [(+)-dFl] = 1.00 x 10^{-4}(M), [PNPH] = 5.00 x 10^{-4} (M), [$Mg(ClO_4)_2$] = 5.00 x 10^{-4}(M)

first-order rate constants were measured[9] by monitoring the decrease in the absorption difference at 430nm which corresponds to absorption of 5-deazaflavins and are listed in Table 2.

As Table 2 shows , the dextro rotatory 5-deazaflavin enantiomer ((+)-dFl) oxidizes (R)-PNPH more rapidly than its (S) isomer, and the net chiral recognition ability increases as bulkiness of the substituent on phenyl group at N(3) position increases, although "prochiral H at C(4)" selectivity of PNPH enantiomers remains to be studied. Furthermore, there is an interesting tendency that as the substituent becomes larger, the k_R is increasing , on the other hand, the k_S is decreasing, leading to give a larger discrimination factor (k_R/k_S). As the accompanying paper[10] shows, a "(net) hydride transfer" to the hindered face is essentially inhibited for (4) with *tert* - butyl group on phenyl group, and is facilitated as the substituent becomes less bulky. Thus the rate measured can be expressed as the sum of the rate of the reaction on the open 5-deazaflavin face and that on the hindered face. If this reaction proceeds through a direct attack, the decrease in rate of attack on the hindered face will not affect the reaction flux on the open face, accordingly the total rate constant should decrease. This is not the case in the present reaction. The increase of the rate in the reaction with (R)-PNPH can be well accomodated by a preassociation mechanism, without Mg^{2+} the reaction dose not proceed as is the case with the former studies.[5] In the preassociation mechanism, as the substituent on phenyl group becomes larger, the concentration of the ternary complex[11] (dFl•Mg^{2+}•PNPH) on the hindered face decreases, making the concentration of the ternary complex on the open face higher, leading to give a larger net rate.

The present results revealed that the pyrimidine site of flavin ring system gives appreciable effects on the enantioselective "(net) hydride transfer" reaction with NADH in biological systems. The detailed mechanistic aspects of this "(net) hydride transfer" reaction in the present model compounds will be reported in the near future.

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7. 4 Chloro and 4,6-dichloro derivatives are also formed besides 6-chloro derivatives. It has been found that the ratio of the products depends on the experimental conditions employed, especially on the temperature and the amount of water. The details will be discussed elsewhere.
8. Because of the limited solubility in the solvent for the separation, the diastereomers derived from the compounds other than (4) were not able to be separated in a preparative scale.
9. $k\varphi = [Rate]_0 / [(+)\text{-dFl}]_0$, "0" stands for initial rate and concentration. See ref. 5f (References and Notes 14).
10. Accompanying paper, *Tetrahedron Lett.* **1992**.
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