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5-Deazaflavinocyclophane as a Novel Flavoenzyme Model. Synthesis and Diastereoface-differentiating Reactions

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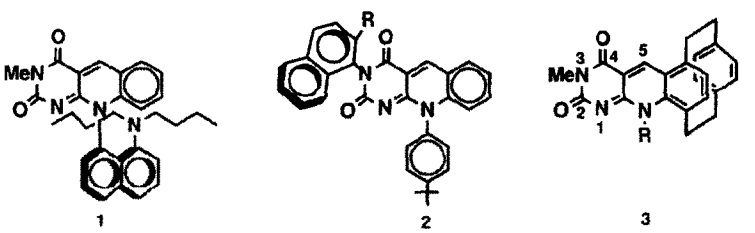
Abstract: New 5-deazaflavinocyclophane with planar chirality, a novel flavoenzyme model, has been synthesized. This compound provides the first example for selective diastereoface-differentiation during its own reduction and oxidation.

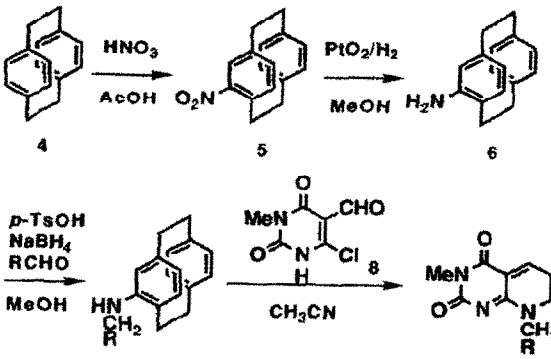
A concept has generally been accepted that a coenzyme is located and fixed at the active site of the enzyme in some geometry, and that one face of the coenzyme is strongly guarded by the wall of the apoprotein. When a substrate approaches, the coenzyme interacts with it from only the other face. Such an environmental effect is important for stereoselectivity and chiral recognition of the enzyme. Flavin and 5-deazaflavin (5-dFl) play important roles in redox reactions in many biological systems.[1] As a part of our continuing studies on coenzyme model compounds, we reported syntheses and reactivities of the 5-dFls having axial and/or planar chirality, (**1**) and (**2**).[2] Now we have designed 5-deazaflavinocyclophanes (**3**) as a novel flavoenzyme model, because it is expected that one face of **3** at the C(5) position, which is the reaction centre of 5-dFl, is blocked completely. Here we would like to report the synthesis and diastereoface-differentiating reactions during reduction and oxidation of compounds (**3**).



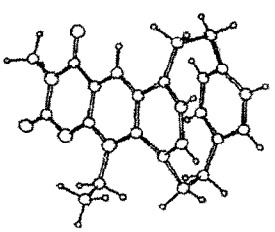
1 **2** **3**

Scheme 1

Scheme 2 shows the synthetic route to **3**. Nitration of [2.2]paracyclophane (**4**) gave mononitro paracyclophane (**5**), which was then reduced with platinum oxide-hydrogen to aminoparacyclophane (**6**).[3] Compound (**6**) was treated with *p*-toluenesulfonic acid, sodium cyanoborohydride, and an aldehyde to afford the monoalkylated compounds (**7**) in moderate yields. The condensation reaction of compounds (**7**) and 6-chloro-5-formyl-3-methyluracil (**8**)[4] gave the corresponding 5-deazaflavinocyclophanes (**3**)[5] in 20-40 %



Scheme 2



molecular structure of **3B**

Fig 1

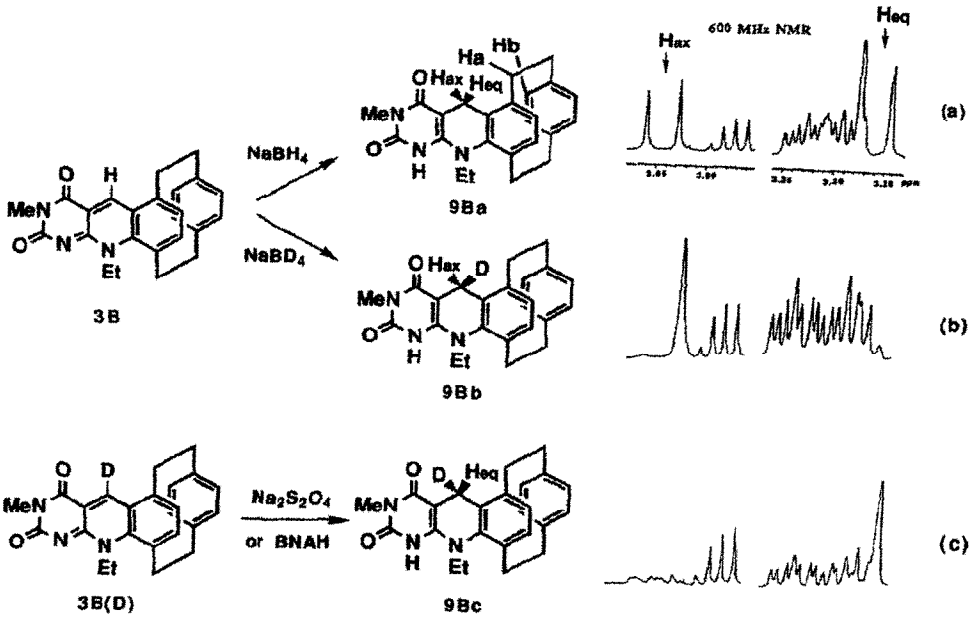


Chart 1

yield. The optical resolution of the racemic **3B** was carried out by the HPLC method on a chiral stationary phase (CHIRALCEL OD). This method resulted in a satisfactory separation of the enantioners: optical purities are >99 % for (+)- and (-)-**3B**. Specific rotations were measured in chloroform: $[\alpha]_D^{23}$= +697, (c=0.50) for (+)-**3B** and $[\alpha]_D^{24}$= -706, (c=0.44) for (-)-**3B**. These compounds did not racemize below 150°C in DMF.

Fig. 1 shows the X-ray crystal structure of compound (**3B**).† It is clear that one face at the C(5) position is completely covered with an aromatic proton of the [2.2]paracyclophane structure. **3B** was reduced with sodium borohydride ($NaBH_4$) in ethanol at room temperature. Chart 1(a) shows the 600 MHz n.m.r. spectrum of reduced product (**9Ba**).[6] It is known that the C(5) protons (H_{ax} and H_{eq}) in conventional 1,5-dihydro-5-dFl give a singlet ^1H NMR peak.[7] The peaks for the two protons at C(5) in **9Ba** appear as a double doublet. This indicates that H_{ax} and H_{eq} are magnetically nonequivalent. We applied a nuclear Overhauser effect difference spectrum (NOEDS) to assign H_{ax} and H_{eq}. Irradiation of the C(5) proton in the lower magnetic field increased the intensities of H_a (6.4 %) and H_b (17.2 %), on the other hand, irradiation of the C(5) proton in the higher magnetic field increased the intensities of one proton of $NC\underline{H}_2CH_3$ (4.0 %). These results show that the doublet in the lower field belongs to H_{ax} and the other one in the higher field belongs to H_{eq}. Moreover they suggest that the central ring of **9Ba** adopts a fixed boat or half boat form and H_{ax} occupies (pseudo) axial position and H_{eq} occupies (pseudo) equatorial position.[2]

In order to make sure that the reduction selectively occurred from open face, **3B** was reduced with $NaBD_4$. Chart 1(b) shows the n.m.r. sperctrum of reduced product (**9Bb**). The doublet of H_{ax} has changed to a singlet and that of H_{eq} has disappeared.[8] It is assumed that the deuteride anion has come from the open face of **3B** and occupied (pseudo) equatorial position. Next the C(5) deuterated compound (**3B(D)**) was likewise synthesized.[9] It was reduced with sodium dithionite ($Na_2S_2O_4$) or 1-benzyl-1,4-dihydronicotinamide (BNAH), a model compound for NAD(P)H, to afford **9Bc**. The NMR spectrum of **9Bc** shows that the doublet of H_{ax} has disappeared and that of H_{eq} has changed to singlet (Chart 1(c)). It means that this reduction also proceeds from the open face. The above results show that the reduction of compound (**3B**) with these reductants proceeds entirely selectively from the open face.

The diastereoface-differentiation was also observed during the oxidation of **9B**. When each compound (**9Ba**~**9Bc**) was left under aerobic conditions at room temperature, **9Ba** and **9Bb** gave the corresponding starting material (**3B**), and **9Bc** gave (**3B(D)**) selectively. When (+)-**3B** was reduced with $NaBH_4$ and then the reduced product was reoxidized by air, no epimerisation was confirmed by HPLC method. These results indicate that open face hydrogen or deuterium at C(5) position is entirely selectively removed with air dioxygen, that is, the (pseudo) axial proton in **9B** has no reactivity. This is different from the results observed in the cyclic models,[10] and is because the "H_{ax}" in **9B** is completely shielded by the wall of the paracyclophane structure.

In conclusion, as predicted, both the reduction of **3B** with some reductants and the oxidation of **9B** with air proceed specifically from the open face. This implies that the paracyclophane structure of compounds (**3**) provides a very efficient wall, and effects completely diastereoface-differentiation for the redox reaction of **3**. Therefore the 5-dFl derivatives (**3**) should be called flavoenzyme models because they have steric function of the apoprotein in their own molecules.

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5. One of the enantiomers is depicted throughout this text.
6. Specific rotations were measured in chloroform: $[\alpha]_D^{23}$= +159, (c=1.00) for (+)-**9Ba** and $[\alpha]_D^{23}$= -157, (c=1.00) for (-)-**9Ba**.
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8. The chemical shifts and coupling constants of n.m.r. spectra of compound (**9B**) were slightly changed according to concentration of the sample.
9. C(5) position of **3B**(**D**) was deuterized about > 99%.
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† *Crystal data for* **3B**: $C_{24}H_{23}N_3O_2$, M=385.47, monoclinic, space group $P2_1/n$, a=14.724(4), b=16.188(6), c=8.100(2) Å, β=103.16(2)°, U=1879.9 Å^3, Z=4, and D_C=1.362 gcm^{-3}. The reflection data of 2903 reflections with $0< \theta<60°$ were collected on a Rigaku AFC-5 diffractometer using monochromated Cu$K\alpha$ radiation and ω-2θ scan technique ($-16\leq h \leq 16$, $0\leq k \leq 18$, $0\leq l \leq 9$). The structure was solved by direct method and refined by full-matrix least-squares method. The final R value was 0.072 for 2653 observed reflections [$F > 3\sigma(F)$].