A New Synthetic Approach to 5-Deazaflavin and 5-Deaza-10-thiaflavin

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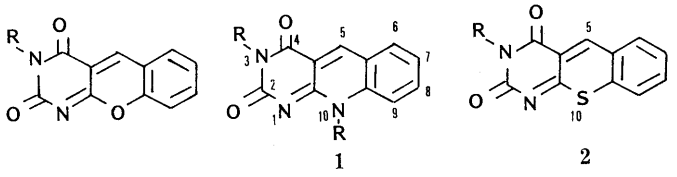
A novel, one-step synthesis of 5-deazaflavin was developed, in which a [4+2]cycloaddition is presumably involved, to give the 5-deazaflavin derivative in moderate yield. This methodology was successfully applied to the stepwise preparation of its thio analogue, 5-deaza-10-thiaflavin, whose 1,5-dihydro derivative was transformed into the corresponding sulfone in good yield.

Keywords 5-deazaflavin; one-step synthesis; coenzyme model; 5-deaza-10-thiaflavin; redox potential; hetero Diels–Alder reaction

Recently, we have reported new and simple synthetic methods for 5-deaza-10-oxaflavin (2*H*-chromeno[2,3-*d*]-pyrimidine-2,4(3*H*)-dione) as a part of our continuing investigation on the development of functional model compounds for coenzymes, and we have also shown that 5-deaza-10-oxaflavin has a high ability to oxidize some kinds of alcohols to give the corresponding carbonyl compounds under acidic conditions in satisfactory yield.[1] A [4+2]cycloaddition mechanism (hetero Diels–Alder reaction) has been suggested to operate in one of those syntheses. Now, this practical and convenient synthetic approach has been successfully applied to the formation of the skeleton of one of the representative redox coenzymes, 5-deazaflavin (**1**), as well as its thio analogue, a 5-deaza-10-thiaflavin (1-benzothiopyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione) derivative (Chart 1). In this paper, we describe a one-pot synthesis of 5-deazaflavin and stepwise preparations of 5-deaza-10-thiaflavin and its analogue in detail.[2]



1 2

5-deaza-10-oxaflavin 5-deazaflavin 5-deaza-10-thiaflavin

Chart 1

5-Deazaflavin coenzyme, such as factor 420, is a particularly interesting compound involved in the reduction of carbon dioxide to methane in biological systems,[3] and extensive chemical and biological research in this field has been done in order to elucidate the biological functions and also to explore a new synthetic mimic molecule from the viewpoint of synthetic organic chemistry.[4] Several synthetic methodologies have been reported and they include a) the condensation of anthranilaldehyde with barbituric acid[5]; b) the cyclization of 6-(*N*-alkylanilino)uracils with a one-carbon reagent, such as Vilsmeier-Haack reagent[6]; c) the condensation of 6-chloro-5-formylpyrimidine with *N*-alkylanilines[6]; d) the oxidative cyclization of aryl bis(6-substituted aminouracil-5-yl)methanes with diethyl azodicarboxylate (DAD)[7]; e) the condensation of 6-(substituted amino)uracil with *o*-halogenobenzaldehydes.[8]

In a similar manner to the efficient synthesis of 5-deaza-10-oxaflavin previously reported,[1] a mixture of 3-methyl-6-chlorouracil (**3**) and *o*-(substituted amino)benzyl alcohol (**4**), which was derived from reduction of the corresponding

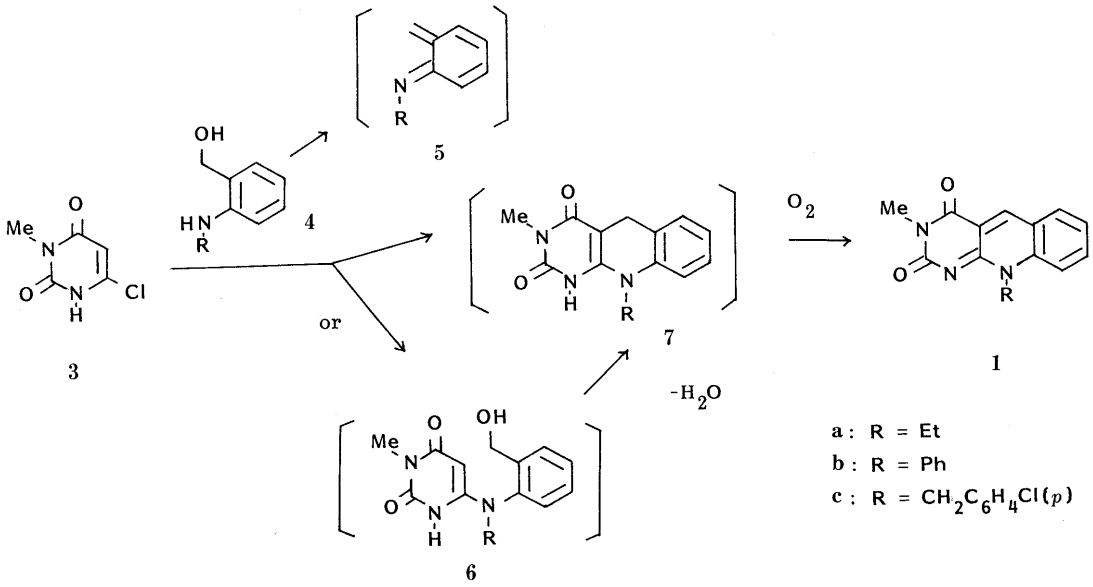


Chart 2. Preparation of 5-Deazaflavin Derivatives

This paper is dedicated to Professor Haruaki Yajima on the occasion of his retirement from Kyoto University in March 1989.

anthranilic acid or its ester derivative with lithium aluminum hydride, was refluxed in *N*,*N*-dimethylformamide (DMF) or heated in nitrobenzene at 200 °C to furnish the desired 5-deazaflavin (**1**) (oxidized form), directly in moderate yield (Chart 2). In contrast to the preparation of 5-deaza-10-oxaflavin, where the 1,5-dihydro derivative was formed and isolated, no 1,5-dihydro-5-deazaflavin was obtained, probably owing to the higher reactivity of the intermediate, 1,5-dihydro-5-deazaflavin (**7**), toward oxidants, especially atmospheric oxygen. Some 5-deazaflavin derivatives were prepared in this way and identified by comparison with authentic samples obtained by alternative synthetic methods.[6,8] Involvement of the hetero diene, 1,2-azabenzoquinone-2-methide (azaxylylene) (**5**) in this reaction, as in that of 1,2-benzoquinone-2-methide (*o*-xylylene) previously reported, might be feasible. Attempted hetero Diels–Alder reaction with rather unreactive dienophiles, such as dihydropyran, cyclohexene, or cyclooctene ended in failure. There are precedents[9] for 1,4-cycloaddition of a dienophile with a hetero-diene, *N*-alkylazaxylylene, though the reaction conditions employed were severer.[9] Alternatively, another reaction pathway might be suggested, in which Michael-type addition of *o*-(substituted amino)benzyl alcohol (**4**) to 6-chlorouracil (**3**) occurs to form 6-(*o*-hydroxymethylanilino)uracil (**6**), which undergoes dehydrative cyclization. At the moment, no definite mechanistic evidence is available, so this subject is still under investigation. We believe this is the simplest method for the preparation of 5-deazaflavin so far reported.

Replacement of a particular hetero atom in a molecule by a sulfur atom might have a significant influence upon the chemical and physical properties of the original compound and this strategic concept is often applied to the field of medicinal chemistry from the biological point of view. 5-Deaza-10-thiaflavin is one candidate, in which the nitrogen atom at the N(10) position in 5-deazaflavin is replaced by a sulfur atom. Therefore, an efficient synthetic method for this sulfur compound was required for our investigation on the redox potentials of a series of 5-deazaflavin derivatives. One of the authors (F.Y.) has already reported a synthesis of 5-deaza-10-thiaflavin consisting of condensation of thiophenol with 6-chlorouracil and one-carbon elongation with the Vilsmeier reagent, and also shown that 5-deaza-10-thiaflavin has a similar redox behavior to that of 5-deazaflavin.[10,11] We applied the present method to the preparation of this interesting molecule.

First of all, *o*-mercaptobenzyl alcohol was heated with the 3-methyl-6-chlorouracil (**3**), but substantial decomposition occurred and an intractable mixture of products was formed. This unsuccessful result led us to use *o*-mercaptobenzoic acid (**9**), which is commercially available and relatively stable to heat, as a starting material. Thus, *o*-mercaptobenzoic acid (**9**) was heated with 3-methyl-6-chlorouracil without using solvent at 230 °C to afford 3-methyl-5-oxo-10-thia-5-deazaflavin (**10**) in 49% yield. In the reaction, either the hetero Diels–Alder pathway involving compound **12** as a reactive intermediate or an ionic Michael addition pathway might participate. The resulting 5-oxo compound **10** was successfully converted to 3-methyl-5-deaza-10-thiaflavin (**2**) through two synthetic sequences involving reduction with lithium aluminum hydride partially decomposed by isopropyl alcohol equimolar to $LiAlH_4$, and subsequent oxidation with DAD[11] in 46% overall yield as shown in Chart 3. It turned out that the 1,5-dihydro derivative (**11**) of 5-deaza-10-thiaflavin (**2**) is also oxidized by atmospheric oxygen to 5-deaza-10-thiaflavin (**2**), but its reaction rate is too slow for a practical synthesis.

A preliminary experiment on the redox potential of 5-deaza-10-thiaflavin in hand was undertaken (Chart 4), but no remarkable oxidizing ability toward alcohols was observed as compared with the 5-deaza-10-oxaflavin. This result is shown in Table I.

Finally, we turned our attention to oxidation of the sulfur atom in 5-deaza-10-thiaflavin in an attempt to find a

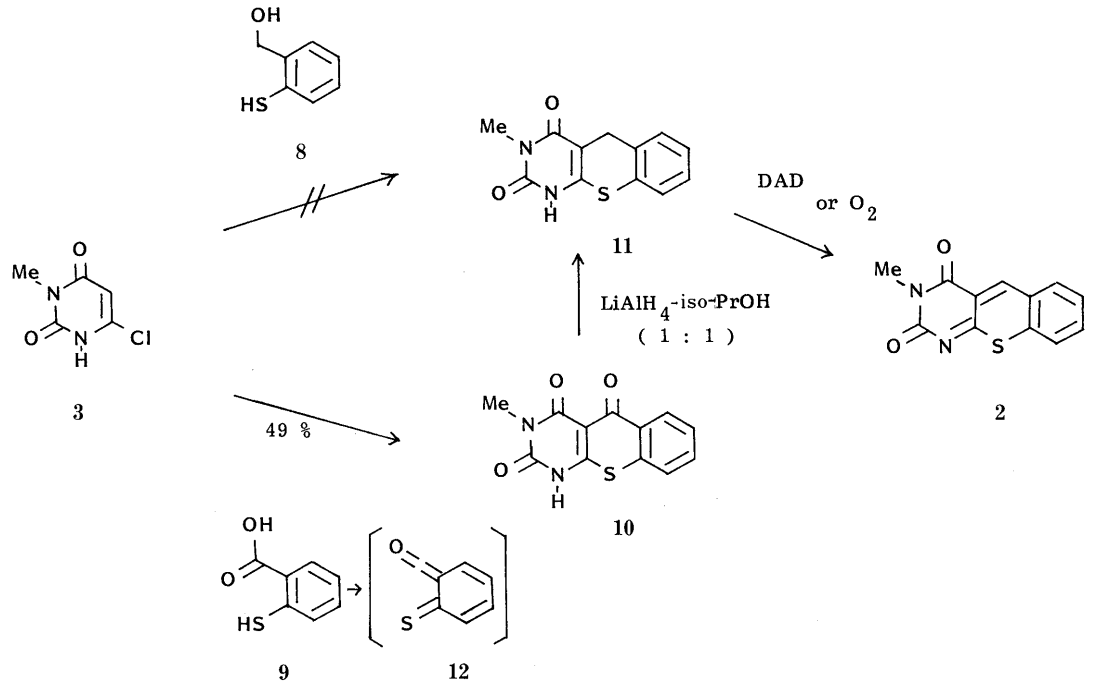


Chart 3. Preparation of 5-Deaza-10-thiaflavin

more efficient organic oxidant. It may be expected that oxidation of sulfur to sulfone or sulfoxide would endow the molecule with more potential oxidizing character due to the inductive effect. A variety of oxidants including metachloroperbenzoic acid, hydrogen peroxide, sodium metaperiodate, peracetic acid, bromine, *N*-bromo- or *N*-chlorosuccinimide (NBS or NCS) and 'oxone', were tried for the oxidation of 5-deaza-10-thiaflavin, but decomposition occurred, probably due to the initial attack of the oxidant on the double bond between C(5) and C(4a). On the other hand, among the oxidizing agents mentioned above, 'oxone' is an only reagent which is effective for the transformation of 1,5-dihydro-5-deaza-10-thiaflavin into the corresponding sulfone (**13**). In this way, the sulfone derivative (**13**) was obtained in fairly good yield as a crystalline compound. However, this compound is very stable toward a variety of oxidizing reagents and it proved impossible to reoxidize it to the desired sulfone (**14**) (Chart 5).

Studies on an alternative route to the sulfone (**14**) or sulfoxide (**15**) and an investigation to compare the redox potentials of these derivatives in relation to the electrostatic situation in the coenzyme model compound are in progress.

In conclusion, the new synthetic method presented in this paper is an most efficient and practical route to the biologically interesting 5-deazaflavin and 5-deaza-10-thiaflavin.

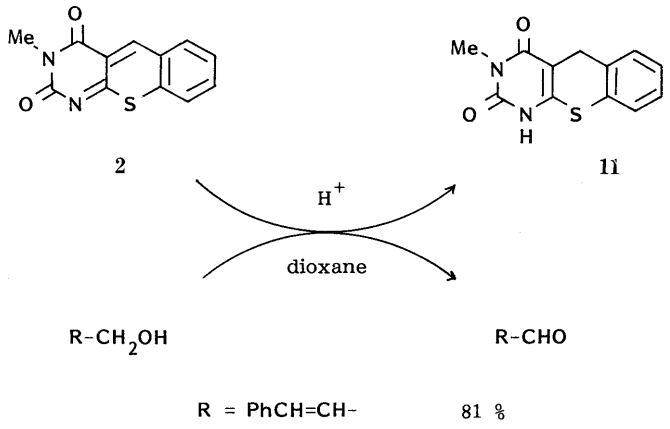
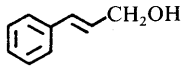
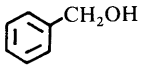
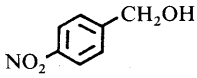
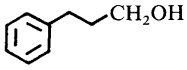
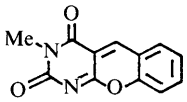
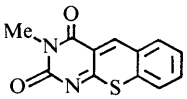


Chart 4. Oxidation of Alcohol with 5-Deaza-10-thiaflavin

Experimental

Melting points were determined with Yanagimoto melting point apparatus and are uncorrected. The infrared (IR ν_{max}) spectra were determined on a Shimadzu IR-400 spectrophotometer in Nujol. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained in dimethyl sulfoxide-d_6 (DMSO-d_6) at 200 MHz on a JEOL FX 200 instrument with chemical shifts being reported in δ units from tetramethylsilane as an internal standard and couplings in hertz. Mass spectra (MS) were taken on a JEOL JMS OlSG-2 instrument by direct insertion at 75 eV. Gas-liquid

TABLE I. Comparison of Oxidation[a] of Alcohols with 5-Deaza-10-thiaflavin and 5-Deaza-10-oxaflavin

Oxidant	Alcohol			
				
	100[b]	90[b]	71[b]	20[b]
	81	60	23	<2

a) The yield (%) of the aldehyde was determined by GLC. b) Reported value.[1]

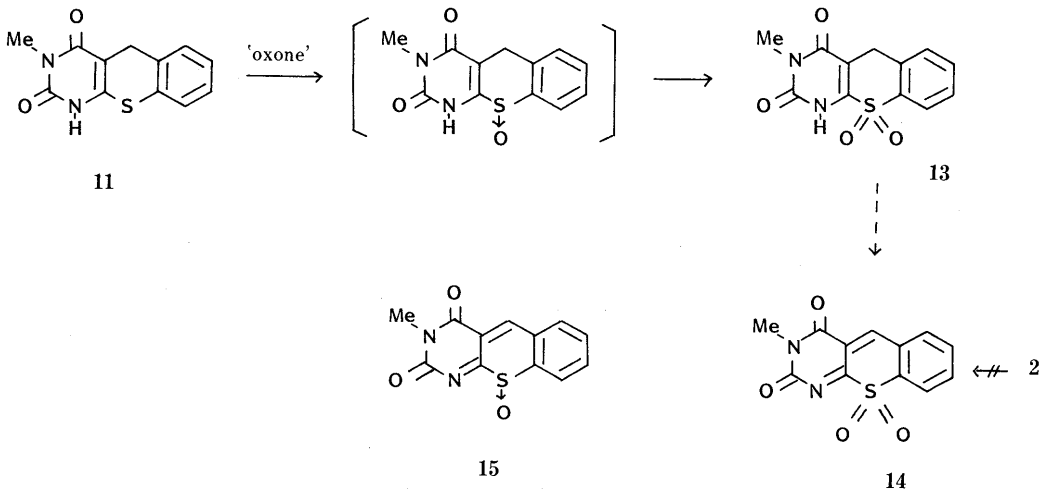


Chart 5. Attempted Synthesis of 5-Deaza-10-dioxothiaflavin (**14**)

chromatography (GLC) was done on a Shimadzu GC-7AG with a glass column (3 m) of 5% FFAP.

Preparation of 5-Deazaflavin Derivatives (1a—c) with *o*-(Substituted amino)benzyl Alcohol (4): General Procedure A mixture of 3-methyl-6-chlorouracil (**3**) (2.0 mmol) and the *o*-(substituted amino)benzyl alcohol (**4**) (4.0 mmol) in DMF (5.0 ml) (or nitrobenzene) was heated under reflux (or at 200 °C) for 4 h. Concentration of the reaction mixture under reduced pressure and purification of the residue by column chromatography on silica gel gave the 3-methyl-5-deazaflavin (**1a—c**) in moderate yields (**1a**, R = Et, 26%; **1b**, R = Ph, 17%; **1c**, R = $CH_2C_6H_4Cl$-*p*, 16%). These samples were identified by comparison with authentic samples prepared by an alternative synthetic method.[6,8]

3-Methyl-5-oxo-5-deaza-10-thiaflavin (10) A mixture of *o*-mercaptobenzoic acid (**9**) (4.0 g, 26.0 mmol) and 6-chlorouracil (**3**) (3.0 g, 18.7 mmol) was heated at 230 °C for 30 min. After cooling to room temperature the residue was crushed into small pieces and washed with methanol. Recrystallization was carried out from acetic acid. Yield was 2.3 g (49%). mp > 300 °C. IR: 2585, 1705, 1660 cm^{-1}. ^{1}H-NMR: 12.65 (1H, s, NH), 8.27 (1H, d, J = 8.0, Ar-H), 7.81 (1H, d, J = 8.0, Ar-H), 7.71 (1H, t, J = 8.0, Ar-H), 7.59 (1H, t, J = 8.0, Ar-H), 3.19 (3H, s, N-Me). *Anal.* Calcd for $C_{12}H_8N_2O_3S$: C, 55.37; H, 3.10; N, 10.76; S, 12.32. Found: C, 55.20; H, 2.98; N, 10.73; S, 12.16.

1,5-Dihydro-3-methyl-5-deaza-10-thiaflavin (11) A suspension of lithium aluminum hydride (2.4 g, 63.2 mmol) and iso-propanol (3.8 g, 63.3 mmol) in 30 ml of dry tetrahydrofuran (THF) was stirred for 30 min at 0 °C, and was added portionwise into a stirred mixture of **10** (5.0 g, 19.2 mmol) and 30 ml of dry THF at the same temperature. The resulting mixture was further stirred for 2 h. A saturated aqueous solution of ammonium chloride was added carefully at 0 °C to decompose the excess reagent and the mixture was filtered through Celite powder. The solid was washed with chloroform. The filtrate and washings were combined and the whole was extracted with chloroform. After washing with water, the organic layer was dried over $MgSO_4$ and concentrated. The crystalline residue was recrystallized from acetic acid to yield 2.3 g of **11** (49%). mp 265—267 °C. IR: 2575, 1705, 1640 cm^{-1}. ^{1}H-NMR: 11.89 (1H, s, NH), 7.45 (2H, m, Ar-H), 7.38 (2H, m, Ar-H), 3.70 (2H, s, $-CH_2-$), 3.15 (3H, s, NMe). *Anal.* Calcd for $C_{12}H_{10}N_2O_2S$: C, 58.52; H, 4.09; N, 11.37; S, 13.02. Found: C, 58.54; H, 3.95; N, 11.31; S, 13.05.

3-Methyl-5-deaza-10-thiaflavin (2) A mixture of **11** (2.0 g, 8.1 mmol) and diethyl azodicarboxylate (6.0 ml, 30.5 mmol) was stirred at 80 °C for 2 h. The mixture was filtered and washed with ethyl ether. The crystalline residue was recrystallized from acetic acid. Yield was 1.67 g (84%). mp > 300 °C. IR: 2585, 1640, 1585 cm^{-1}. ^{1}H-NMR: 8.95 (1H, s, $=CH-$), 8.35 (1H, d, J = 8.0, Ar-H), 8.02 (1H, d, J = 8.0, Ar-H), 7.88 (1H, t, J = 8.0, Ar-H), 7.70 (1H, t, J = 8.0, Ar-H), 3.30 (3H, s, N-Me). *Anal.* Calcd for $C_{12}H_8N_2O_2S$: C, 59.00; H, 3.30; N, 11.47; S, 13.13. Found: C, 58.84; H, 3.05; N, 11.49; S, 13.06.

General Procedure for Oxidation of Alcohols with 3-Methyl-5-deaza-10-thiaflavin (2) A solution of **2** (0.10 mmol), the appropriate alcohol (0.10 mmol) and *p*-toluenesulfonic acid monohydrate (0.10 mmol) in 1,4-dioxane (8.0 ml) was refluxed for 1.0 h. The resulting carbonyl compound (aldehyde) was identified and quantified by GLC comparison with a known amount of commercially available authentic sample.

10,10-Dioxo-3-methyl-5-deaza-10-thiaflavin (13) A solution of "oxone" (potassium peroxymonosulfate, $2KHSO_5$ $KHSO_4$ K_2SO_4) (880 mg, 1.4 mmol) in 5.0 ml of water was added dropwise to a stirred mixture of **11** (60 mg, 0.24 mmol) and 3.0 ml of methanol at 0 °C. The resulting mixture was stirred for 15 h at 5 °C, then concentrated under reduced pressure to afford the residue, which was extracted with chloroform. The chloroform layer was washed with brine, then dried over $MgSO_4$. Concentration of the organic layer gave a crystalline residue which was recrystallized from ethanol. Yield was 55 mg (82%). mp 258—260 °C. IR: 2585, 1725, 1640, 1450 cm^{-1}. ^{1}H-NMR: 8.18 (1H, s, NH), 7.98 (1H, d, Ar-H), 7.65 (3H, m, Ar-H), 4.00 (2H, s, $-CH_2-$), 3.29 (3H, s, NMe). MS *m/z*: 278 (M^+). *Anal.* Calcd for $C_{12}H_{10}N_2O_4S$: C, 51.79; H, 3.62; N, 10.07; S, 11.52. Found: C, 51.80; H, 3.52; N, 9.77; S, 11.42.

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