DE SYNTHESE EN FOTOCHEMIE VAN 8-GESUBSTITUEERDE 5-DEAZAFLAVINES

Aan mijn ouders

Voor Conny

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SYNTHESIS AND PHOTOCHEMISTRY OF 8-SUBSTITUTED 5-DEAZAFLAVINS

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 22 oktober 1986
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

STELLINGEN

1. Het postulaat van Yoneda et al., dat slechts één molecuul diethyl azodicarboxylaat bij de oxidatieve ringsluiting van 5,5'-arylmethyleenbis(6-methylaminouracil) derivaten een rol speelt, is door experimentele gegevens weerlegd.

   F. Yoneda, K. Mori, Y. Sakuma en H. Yamaguchi, J.Chem.Soc., Perkin Trans. I, 978 (1980).
   Dit proefschrift.

2. Het mechanisme, voorgesteld door Bag et al., voor de oxidatie van benzylesters door ceriumammoniumnitraat is niet in overeenstemming met de experimentele gegevens.

   A.K. Bag, S.R. Gupta en D.N. Dhar, Indian J.Chem. 25B, 433 (1986).

3. De door Davis et al. gegeven structuurformule voor 1,3,6-trimethyllumazine-7-sulfenylzuur is onjuist.

   F.A. Davis, L.A. Jenkins en R.L. Billmers, J.Org.Chem. 51, 1033 (1986).

4. De ^{1}H nmr spectra van arylglyoxalen en hun hydraten zijn door Joshi et al. verkeerd geïnterpreteerd.

   K.C. Joshi, V.N. Pathak en M.K. Goyal, J.Heterocyclic Chem. **18**, 1651 (1981);
   K.C. Joshi, K. Dubey en A. Dandia, Heterocycles **16**, 1545 (1981).

5. Yamashita et al. gaan er ten onrechte van uit, dat de door hen uit mout geïsoleerde β(1→4)-D-glucanasen endogene enzymen moeten zijn.

   H. Yamashita, F. Hayase en H. Kato, Agric.Biol.Chem. **49**, 1313 (1985);
   H. Yamashita, T. Hirono, F. Hayase en H. Kato, Agric.Biol.Chem. **50**, 733 (1986);
   S.A.G.F. Angelino en P. Peereboom, 49e NIBEM-jaarboekje 55 (1985).

6. In geval van tri en tetra gesubstitueerde alkenen dient de cis-trans nomenclatuur, zoals vaak gebruikt in de nomenclatuur van zeatines, vervangen te worden door de E-Z nomenclatuur.

   B.A. McGraw, R. Horgan en J.K. Heald, Phytochemistry **24**, 9 (1985);
   J.H. Lister in "The Chemistry of Heterocyclic Compounds. Fused Pyrimidines". Part II; D.J. Brown, Ed., Wiley, New York, 1971, p. 354.

7. De mogelijkheid, dat bij de oxidatie van arylcyclopropanen door cytochroom P-450 uit konijnenlever allereerst de phenyl ring wordt geoxideerd in plaats van de cyclopropyl ring, wordt ten onrechte door Suckling et al. buiten beschouwing gelaten.

   C.J. Suckling, D.C. Nonhebel, L. Brown, K.E. Suckling, S. Seilman en C.R. Wolf, Biochem.J. **232**, 199 (1985);
   K.E. Suckling, C.G. Smellie, I. El Sayeed Ibrahim, D.C. Nonhebel en C.J. Suckling, FEBS Letters **145**, 179 (1982);
   T. Shono en Y. Matsumura, J.Org.Chem. **35**, 4157 (1970).

8. De veronderstelling dat langdurige bestraling van 5-deazaflavines in aanwezigheid van een electronen donor leidt tot de quantitatieve vorming van het 5,5'-dimeer (Bliese et al.), ongeacht het type electronen donor (Duchstein et al.), is onjuist.

M. Bliese, A. Launikonis, J.W. Loder, A.W.-H. Mau en W.H.F. Sasse, Aust.J.Chem. **36**, 1873 (1983);
H.-J. Duchstein, H. Fenner, P. Hemmerich en W.R. Knappe, Eur.J.Biochem. **95**, 167 (1979).
Dit proefschrift.

9. Een toelatingsbeleid van HBO-opleidingen, waarbij belang gehecht wordt aan de aanwezigheid van vakken in de keuzepakketten, die niet relevant zijn voor de te volgen HBO-opleiding, is aan bedenkingen onderhevig.

10. Door de talrijke arbitrale discussies tijdens menig tenniswedstrijd wordt de indruk gewekt dat er dan ook slechts sprake kan zijn van een vermoedelijke winnaar en een waarschijnlijke verliezer.

P.A.J. Link — Wageningen, 22 oktober 1986

Synthesis and photochemistry of 8-substituted 5-deazaflavins.

VOORWOORD

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CONTENTS

The following publications form part of this thesis.

- Synthesis of 8-substituted 5-deazaflavins.
  P.A.J. Link, H.C. van der Plas and F. Muller,
  J. Heterocyclic Chem., **22**, 841 (1985).

- Synthesis of water soluble 8-substituted 5-deazaflavins.
  P.A.J. Link, H.C. van der Plas and F. Muller,
  J. Heterocyclic Chem., **22**, 873 (1985).

- The half-wave potentials of 8-substituted 5-deazaflavins. Polarographic determination of the dissociation constants of some 8-substituted 5-deazaflavosemiquinones.
  P.A.J. Link, H.C. van der Plas and F. Muller,
  J. Org. Chem., **51**, 1602 (1986).

- On the photoreduction of 5-deazaflavins.
  P.A.J. Link, H.C. van der Plas and F. Muller,
  J. Chem. Soc., Chem. Comm., (1986), in press.

- Spectroscopy and photochemistry of 8-substituted 5-deazaflavins.
  P.A.J. Link, H.C. van der Plas and F. Muller,
  Photochem. Photobiol., submitted.

- The photoreduction of 8-substituted 5-deazaflavins and the 5-deazaflavin catalyzed photoreduction of methyl viologen.
  P.A.J. Link, H.C. van der Plas and F. Muller,
  Photochem. Photobiol., submitted.

1 INTRODUCTION

1.1 GENERAL

An important class of respiratory enzymes occurring widely in animals and plants is that of the flavoproteins. In these proteins either riboflavin, flavin-mononucleotide (FMN) or flavin-adenine-dinucleotide (FAD) functions as the coenzyme (Figure 1). The functionality in these coenzymes is located in the

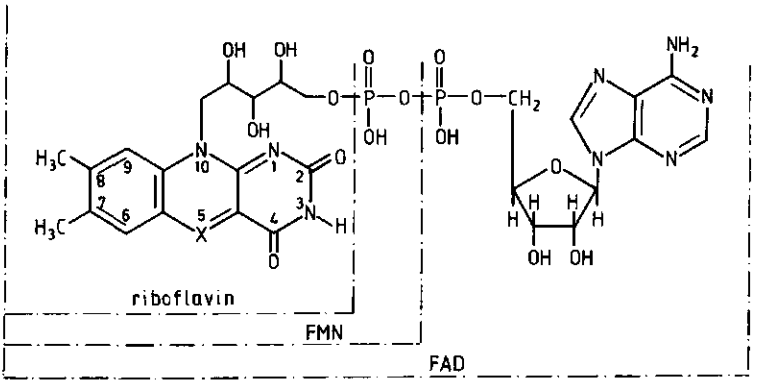
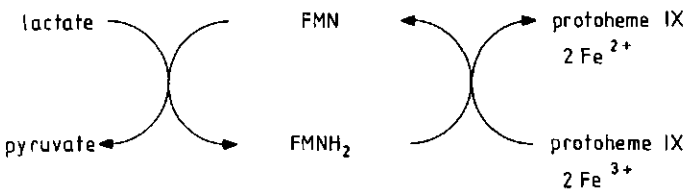


Figure 1. Structural formulae of some common (5-deaza)flavins; X=N, flavin; X = CH, 5-deazaflavin.

isoalloxazine ring structure. Three main types of flavoprotein activities can be distinguished by both their chemical and enzymological characteristics, namely reversible substrate dehydrogenation, electron transfer and activation of molecular oxygen [1].

5-Deazariboflavin (7,8-dimethyl-10-(D-ribityl)pyrimido[4,5-*b*]quinoline-2,4-(3*H*,10*H*)-dione) (Figure 1) has achieved much attention as a tool to elucidate the mechanism of flavin-catalyzed dehydrogenation. In the dehydrogenation the interesting question whether the hydrogen derived from the substrate will first go to position N(5) or to position N(1) of the flavin could not be answered with natural coenzyme due to the fast exchange of hydrogen, bonded to either N(1) or N(5) of the dihydroflavin, with water.

Because the hydrogen atom introduced at position 5 of 5-deazaflavin is not exchangeable, 5-deazaFMN and 5-deazaFAD have been widely used as flavin coenzyme analogs in reconstitution experiments with a number of flavoproteins [2-6]. These 5-deazaflavins were found to bind the labeled itinerant hydrogen at position 5. However, it was strongly criticized that the results of these reconstitution experiments were extrapolated to the mechanism of flavin-catalyzed dehydrogenations, since the chemistry of flavins and 5-deazaflavins has been shown to be fundamentally different [7-10]. The chemical behaviour of 5-deazaflavin resembles that of nicotinamide rather than that of flavin as is exemplified by the fast reduction by borohydride and the slow reduction via $2e^-$-transfer by dithionite, as well as by the very slow reoxidation by oxygen of dihydro-5-deazaflavin and dihydronicotinamide. The opposite is true for flavin. Moreover, an important unique feature of flavoproteins is their ability to accept electron pairs and transfer them as single electrons, or *vice versa*, due to the stability of the flavosemiquinone radical [11]. This is exemplified by the FMN of flavocytochrome b_2 from baker's yeast accepting two electrons from lactate, which is oxidized to pyruvate, and transmitting them one at a time to its built-in acceptor, protoheme IX [12-14] (Scheme 1). When this enzyme was



Scheme 1

reconstituted with 5-deazaFMN, which lacks radical stabilization just as nicotinamide, the ability of transferring electrons to protoheme IX was lost [15]. Hence, these experiments convincingly show that 5-deazaflavin cannot be considered as a flavin analog, but may be referred to as a flavin-shaped nicotinamide, which blocks flavin-dependent $1e^-$-transfer and oxygen-activation and limits transhydrogenation.

In the same period, when 5-deazaflavin was used extensively in reconstitution experiments, 5-deazaflavin has been found in nature as part of F-420, a coenzyme universally present in methane-producing bacteria [16-23]. This

coenzyme has been characterized as a derivative of 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-hydroxy-10-(D-ribityl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione) [16] (Figure 2).

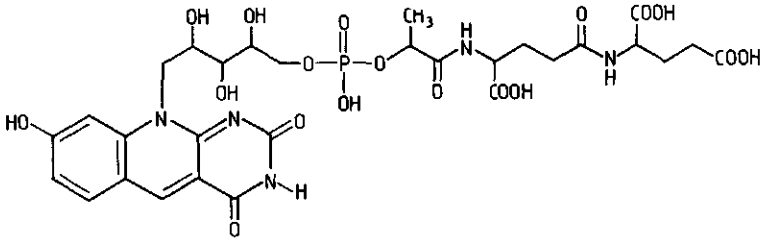
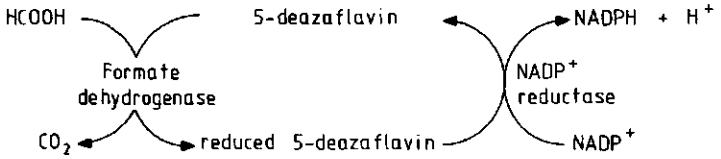


Figure 2. Structure of F-420.

The occurrence of 5-deazaflavin as part of naturally occurring systems is not restricted to methanogens, for it has also been found in cell extracts of *Streptomyces griseus* [24]. The F-420.H_2/F-420 couple has a reduction potential neatly between the $H_2/2H^+$ or $HCOOH/CO_2$ couple and the NADP/NADPH couple [25]. Hence, in methanogen redox metabolism F-420 is used to link hydrogen or formate oxidation to reduction of NADP (Scheme 2) and perhaps to serve as reductant in the last step of methane biosynthesis [21,26-29].

The observation that the dihydro form of F-420 is instantaneously reoxidized by oxygen [18] initiated the idea that the presence of the hydroxy substituent at position 8 would have brought the 5-deazaflavin system out of the straits of

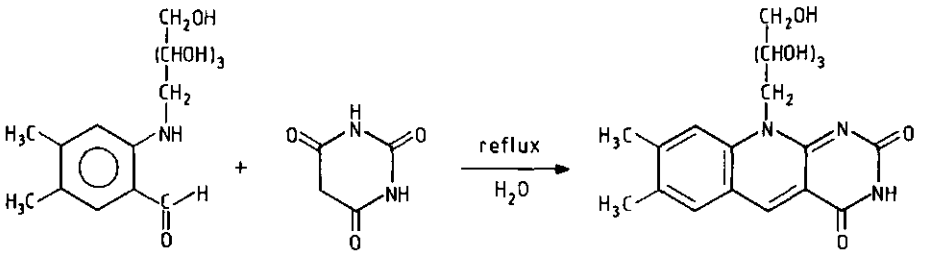


Scheme 2

$2e^-$ chemistry back into the $1e^-/2e^-$ chemistry being the key biological redox characteristic of flavins. Although this reasoning was doubted later [30] it excited new interest in the synthesis of 5-deazaflavins in order to obtain a chromophor that would combine nicotinamide and flavin activity [31].

1.2 SYNTHESIS OF 5-DEAZAFLAVINS

Several synthetic routes were developed to obtain *N*-10-alkyl-5-deazaflavins. 5-Deazariboflavin (**3**) has been first synthesized by O'Brien *et al.* [32] by the condensation of barbituric acid (**1**) and the anthranilaldehyde **2** (Scheme 3). In order to circumvent the laborious preparation of anthranilaldehyde **2** an



2 1 3

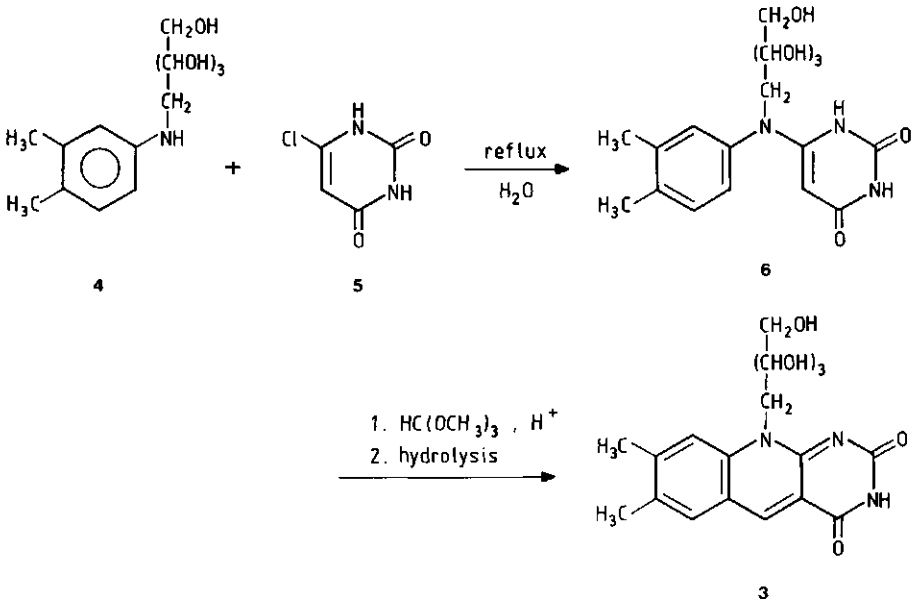
Scheme 3

improved synthesis was published by Ashton *et al.* [33] using *N*-D-ribityl-3,4-xylidine (**4**) as starting material. Condensation of **4** and 6-chlorouracil (**5**) yielded 6-(*N*-D-ribityl-3,4-xylidino)uracil (**6**), which on cyclization with trimethyl orthoformate in the presence of catalytic *p*-toluenesulfonic acid led to the formation of 5-deazariboflavin with a fully protected ribityl chain. Hydrolysis gave the end product **3** (Scheme 4).

By application of this method 7,8-didemethyl-8-hydroxy-5-deazariboflavin, the main component of F-420 (Figure 2), has been prepared from *N*-D-ribityl-3-hydroxyaniline and **5** [34]. A small improvement of the yield of **3** has been achieved by cyclization of the tetraacetyl derivative of **6** with phosphorus oxychloride ($POCl_3$) in dimethylformamide (DMF) followed by deacetylation [35].

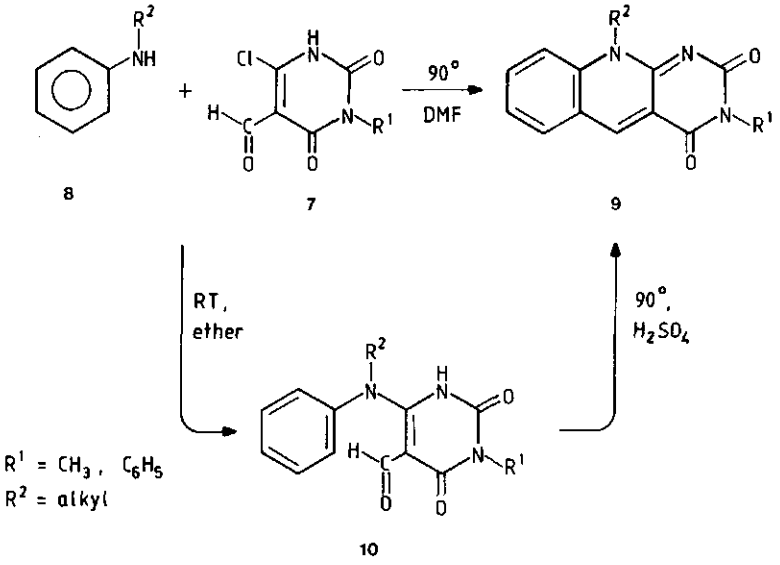
The Yoneda group [36] has generalized the method outlined in Scheme 4 by cyclization of a variety of 6-(*N*-alkylanilino)uracils with the one-carbon reagents ethyl chloroformate, triethyl orthoformate or $POCl_3$/DMF (chapter 2, Scheme 1).

A second method developed by Yoneda [36] involved the synthesis of 6-chloro-5-formyluracil **7** by treatment of 3-*N*-methyl or -phenyl- barbituric acid with $POCl_3$/DMF. Reaction of **7** with *N*-alkylaniline **8** gave 5-deazaflavin **9** (Scheme 5).



4 5 6 3

Scheme 4

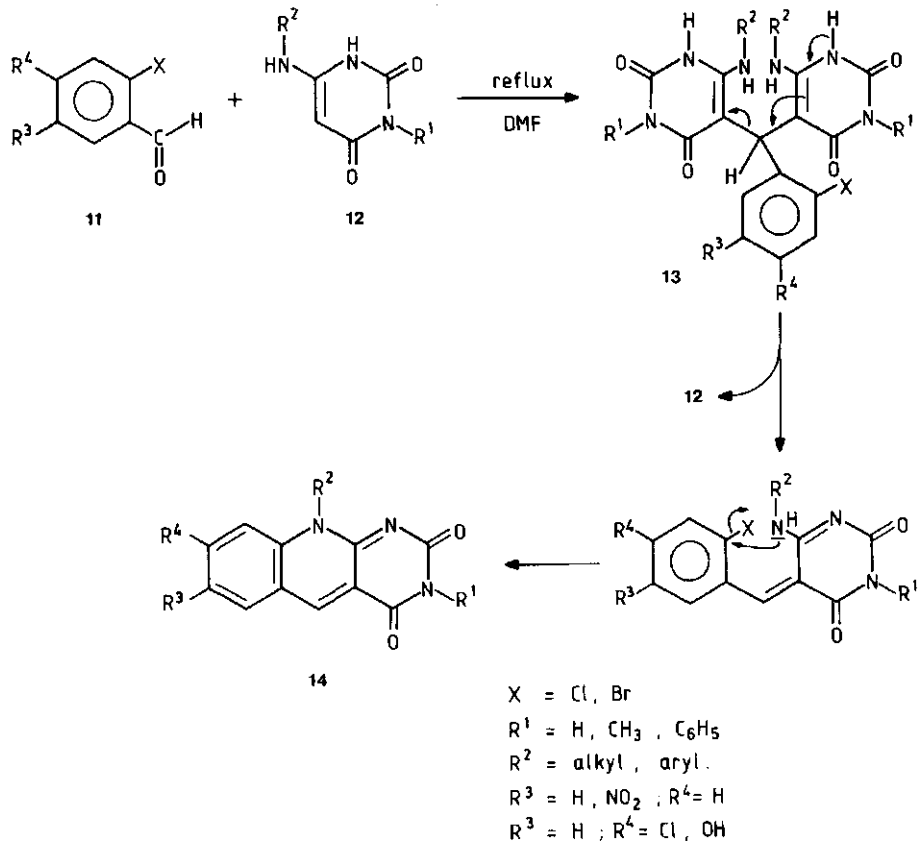


8 7 9 10

Scheme 5

When the reaction was carried out in ether at room temperature 6-(*N*-alkylanilino)-5-formyluracil **10** was obtained. Warming of this product in a small amount of concentrated sulfuric acid gave 5-deazaflavin **9**.

Using these methodologies it was observed that the synthesis of 10-*N*-aryl-5-deazaflavins still encountered difficulties. 6-(*N*-arylanilino)uracils appeared



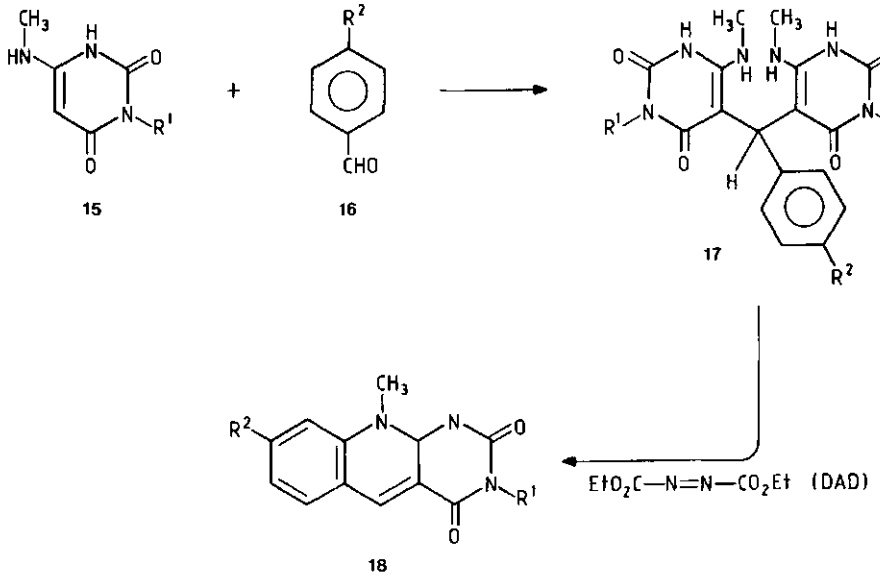
Scheme 6

not to be available by the condensation of 6-chlorouracil with diphenylamines and 6-chloro-5-formyluracils were unreactive towards diphenylamines under the conditions mentioned in Scheme 5. Therefore, a new method has been developed [37], which is based on the condensation of 6-(*N*-substituted amino)uracils **12** with *o*-halogenobenzaldehydes **11** (Scheme 6).

Although these methods have been applied successfully for synthesizing 5-

deazaflavins with a large variety of substituents at N(10) and N(3) [36-40], they are less suitable for obtaining 8-substituted 5-deazaflavins, since introduction of a substituent at position 8 requires *m*-substituted *N*-alkylanilines or *p*-substituted *o*-halogenobenzaldehydes, the synthesis of which is laborious (chapter 2), and the cyclization might be non-regioselective leading to a mixture of products [31].

Based on Yoneda's work [41-44] we elaborated a suitable method for the synthesis of 8-substituted 5-deazaflavins, which is described in detail in chapters 2 and 3. The generalized pathway is depicted in Scheme 7 and starts with the condensation between a 6-methylaminouracil derivative **15** and a



Scheme 7

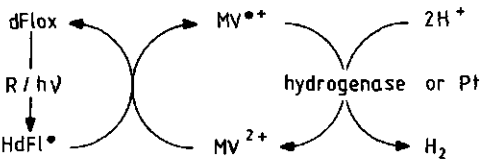
p-substituted benzaldehyde **16** yielding 5,5'-arylmethylenebis(6-methylaminouracil) **17**. Oxidative cyclization with diethyl azodicarboxylate (DAD) leads to the formation of 8-substituted 5-deazaflavin **18**.

1.3 APPLICATION OF 5-DEAZAFLAVINS AS REDUCING PHOTOCATALYSTS

As mentioned before 5-deazaflavin cannot replace flavin in biological $1e^-$-transfer processes due to its lack of radical stabilization. However, it has

been shown that the 5-deazaflavin radical is easily generated photochemically [45-47]. Owing to the low reduction potential and high reactivity of the radicals [9], 5-deazaflavins have been shown to act as very efficient photocatalysts in the photoreduction of a wide variety of redox carriers in the presence of an electron donor. Krasna [48] used 5-deazaflavin as a photocatalyst for the conversion of solar energy into hydrogen production as outlined in Scheme 8, in which dFlox = 3,10-dimethyl-5-deazaflavin, $HdFl^{\bullet}$ = 5-deazaflavin radical, R = electron donor, MV^{2+} = methyl viologen and $MV^{\bullet+}$ = reduced methyl viologen. A large variety of compounds including amines, carboxylic acids, carbohydrates and amino acids could serve as electron donor.

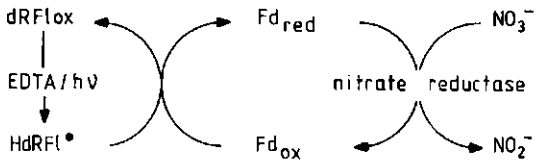
The versatality of 5-deazaflavin acting as a photocatalyst has been shown by



Scheme 8

the photoreduction of a large variety of enzymes including flavoproteins, heme proteins, iron and iron-sulfur proteins by 5-deazariboflavin in the presence of ethylenediaminetetraacetate (EDTA) as electron donor. The photosystem appeared to be effective even with enzymes, which could not be reduced by dithionite [49,50]. The non-generality of the photosystem is exemplified by the fact that at least one iron-sulfur protein in the nitrogenase complex of *Azotobacter vinelandii* is not accessible to reduction by $HdFl^{\bullet}$, but requires as an intermediary catalyst flavodoxin, which is reduced by $HdFl^{\bullet}$ [51]. The greater efficiency of 5-deazariboflavin as a photocatalyst compared to FMN, FAD, lumiflavin or riboflavin is exemplified by the ferredoxin-dependent enzymatic reduction of nitrate [52]. The enzyme nitrate reductase catalyses the reduction of nitrate to nitrite being the first step in the assimilatory reduction of nitrate to ammonia. The nitrate reductase of *Anacystis nidulans* exhibits an absolute dependency of ferredoxin, which is easily photoreduced by the 5-deazaflavin/EDTA photosystem (Scheme 9, dRFlox = 5-deazariboflavin, Fd=ferredoxin).

A serious disadvantage of the 5-deazaflavin photoreducing system is the fact that the light required to generate the 5-deazaflavin radical has an energy high enough to destroy the protein enzyme with time. For instance the electron



Scheme 9

transfer occurring within the system depicted in Scheme 9 stops after 30 minutes of irradiation. The present study was aimed at synthesizing modified 5-deazaflavins, which require lower energy excitation for generating radicals (chapter 2 and 3 [53,54]), and at studying the effect of the modification on the reduction potential of the dFlox/HdFl$^{\bullet}$ couple (chapter 4 [55]), on the spectroscopic and photochemical properties as well as on the activity as a photoreducing catalyst (chapter 5, 6 and 7 [56-58]).

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2 SYNTHESIS OF 8-SUBSTITUTED 5-DEAZAFLAVINS

2.1 INTRODUCTION

In nature, flavins often act as coenzymes in a wide variety of biological reactions. Flavin is characterized by three main modes of action: (de)hydrogenation, oxygen activation, and electron transfer. Unlike flavin, 5-deazaflavin (Figure 1) is rarely found in nature. An example is F-420, the coenzyme

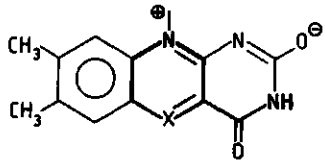


Figure 1. Structural comparison of neutral (deaza)flavin and nicotinamide species; X = N, flavin; X = CH, 5-deazaflavin; nicotinamide (heavy lines, X = CH).

of methane-producing bacteria [1]. 5-Deazaflavin resembles the structure of flavin; the only structural difference is the replacement of N-5 by CH. Based upon theoretical studies, Sun and Song [2] proposed that this structural difference would not considerably change the reactivity of deazaflavin as compared to flavin. Later it was shown [3,4] that this proposal is incorrect; in fact the chemistry of flavin and 5-deazaflavin is fundamentally different.

The chemical behaviour of 5-deazaflavin resembles that of nicotinamide rather than that of flavin. This is the reason why 5-deazaflavin is often referred to as a flavin-shaped nicotinamide. When in flavoenzymes flavin is replaced by 5-deazaflavin, two of the three main modes of action of flavin are lost. Only (de)hydrogenation is retained, although to a rather limited extent.

5-Deazaflavoproteins cannot be involved in one-electron processes mainly because of the unfavourable very low redox potential of 5-deazaflavin. The radical state in free 5-deazaflavin can only be achieved by enforced one-electron reduction, usually by a photochemical process. The 5-deazaflavin radical combines high reactivity with a very low redox potential. Scherings *et al.* [5] and Massey and Hemmerich [6] demonstrated the photoreductive power of

5-deazaflavin in the reduction of a wide variety of biological redox systems using ethylenediaminetetraacetic acid as an electron donor. Krasna [7] showed that in addition many other compounds (*e.g.* carbohydrates, carboxylic acids, amino acids, and proteins) can serve as electron donors.

The application of 5-deazaflavin in the reduction of redox enzymes has a serious drawback. The generation of the reducing 5-deazaflavin radical [4] (Figure 2) starting with a photoreaction between excited 5-deazaflavin and an electron donor and subsequent photodissociation of the photodimer, which is sensitized by 5-deazaflavin, requires continuous uv irradiation to drive the process. The wavelength needed to form the dimer and to photodissociate it (300-400 nm) corresponds to an energy level high enough to destroy the protein enzyme with time.

To avoid this problem of photodestruction of the protein we have synthesized a number of red-shifted 5-deazaflavins to avoid short wavelength irradiation otherwise needed to generate the radical. The aim of the study was therefore to synthesize the 5-deazaflavins absorbing at longer wavelengths.

2.2 SYNTHESIS OF 8-SUBSTITUTED 5-DEAZAFLAVINS.

2.2.1 General procedure

The 5-deazaflavins must meet two requirements before they can be used for the photoreduction of enzymes. i) They must absorb radiation at a wavelength high enough to prevent destruction of the enzyme, and ii) they must be soluble in aqueous media.

To meet the first requirement, we have chosen to introduce a chromophoric group at the C(8) position of the 5-deazaisoalloxazine skeleton. Through tricyclic resonance, such a substituent at C(8) is expected to cause a bathochromic shift in the absorption spectrum.

Yoneda [8] has developed two methods for the synthesis of substituted 5-deazaflavins.

The first method (Scheme 1) starts with a reaction between 6-chlorouracil (1) and a *m*-substituted *N*-methylaniline **2** yielding the 6-*N*-methylanilinouracil **3**. Cyclization of **3** with the one-carbon Vilsmeier reagent forms 5-deazaflavin **4**.

In order to circumvent the possible non-regioselective cyclization of **3** (see arrows, **3**), leading to a mixture of 6- and 8-substituted 5-deazaflavins **4** and **5** [9], a second method (Scheme 2) has been applied.

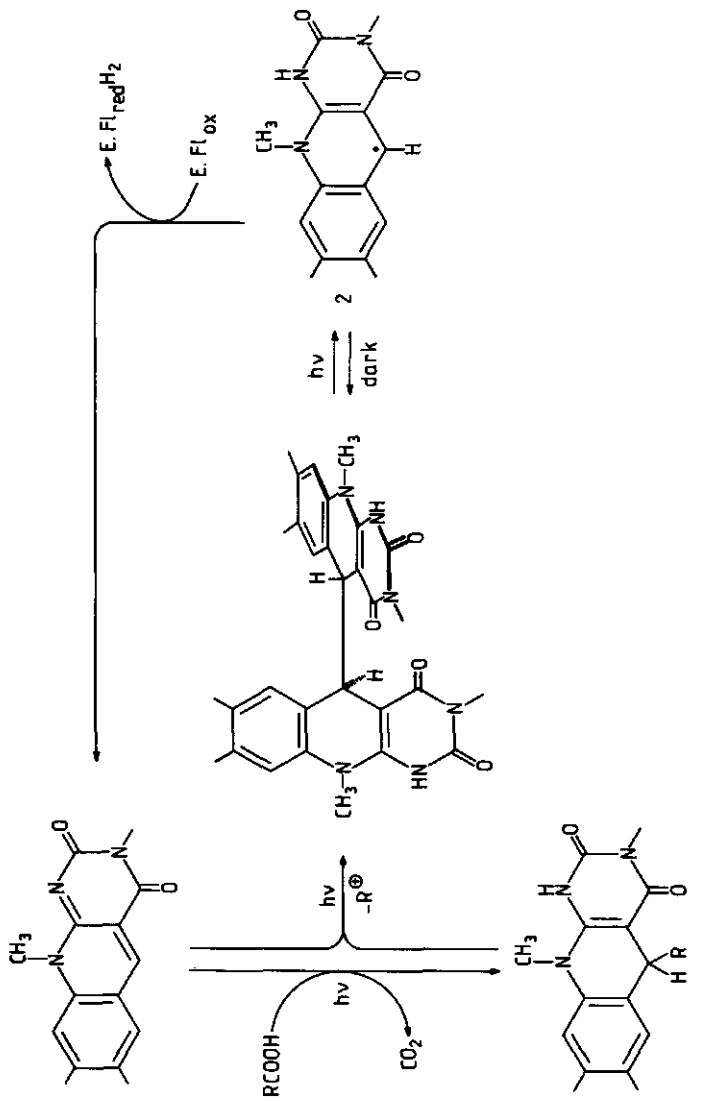
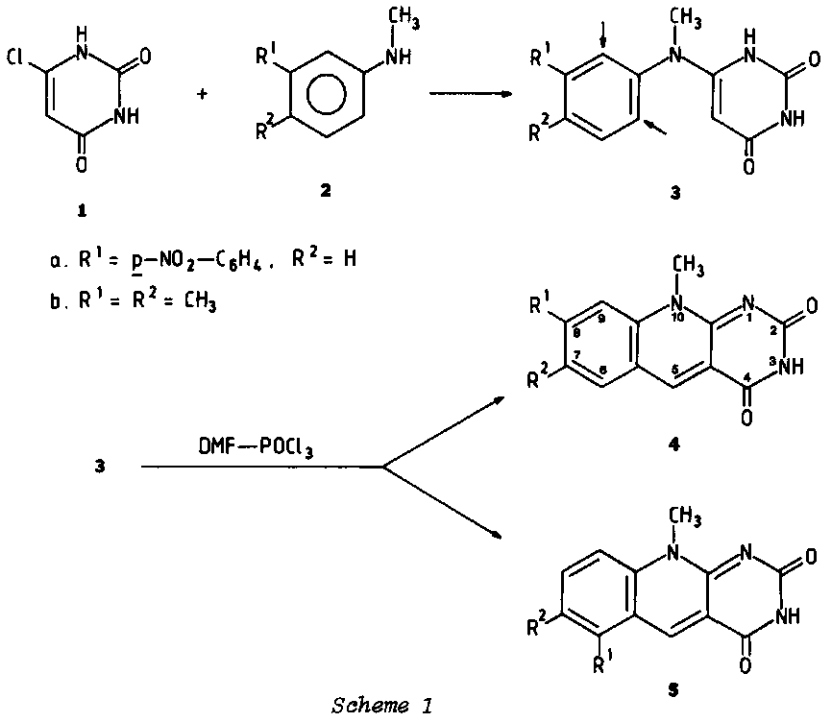


Figure 2. 5-Deazaflavin redox system.



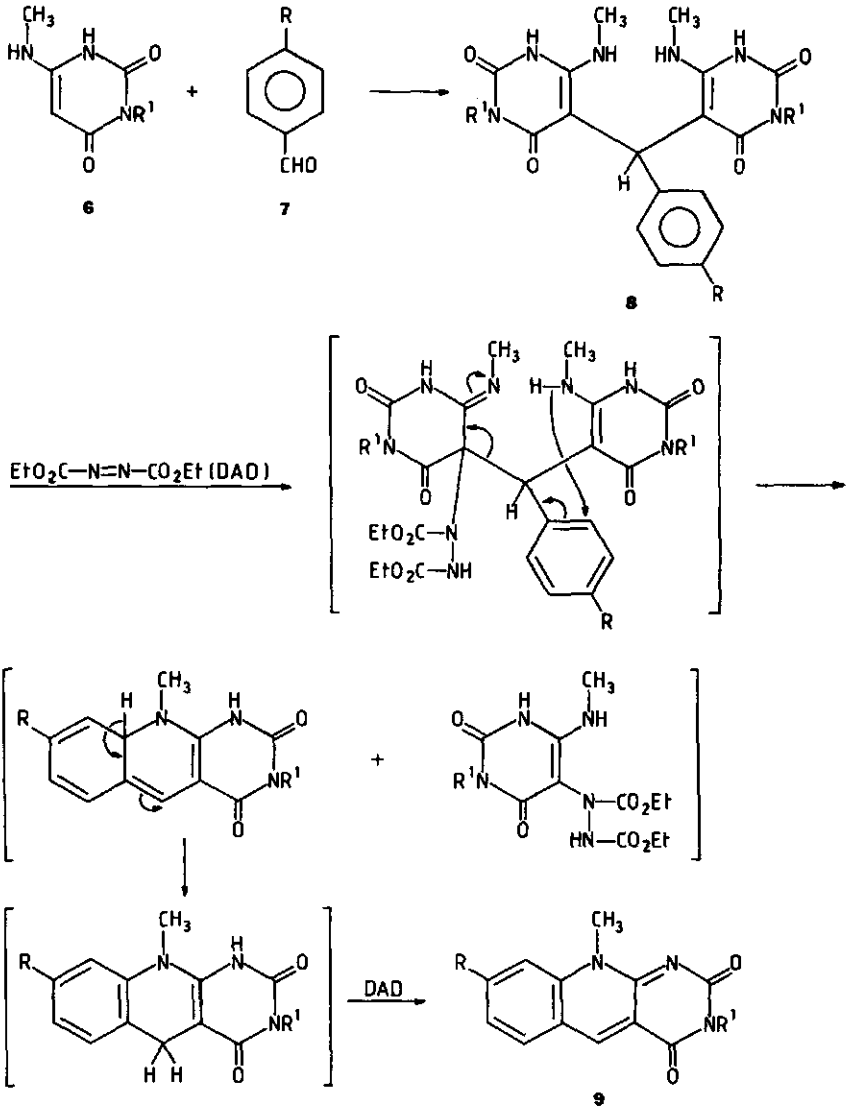
Scheme 1

The method involves condensation between a 6-methylaminouracil derivative **6** and a *p*-substituted benzaldehyde **7** and leads to the formation of 5,5'-arylmethylenebis(6-methylaminouracil) **8**. Addition of diethyl azodicarboxylate (DAD) starts a reaction sequence yielding the 5-deazaflavin **9**, substituted specifically at the C(8) position. The course of the reaction probably involves the intermediates, given in Scheme 2.

Another advantage of the second method is that the starting material **7** contains the substituent to be introduced at the C(8) position of the 5-deazaisoalloxazine skeleton at the *para* position while the first method requires the *m*-substituted *N*-methylaniline derivative 2. The synthesis of **7** is often far easier than that of **2**, as is illustrated by the synthesis of 3-methylamino-4'-nitrobiphenyl (**2a**) being used in the first method. The preparation of **2a** requires a multistep procedure starting from *m*-nitroaniline. Application of the Gomberg-Bachmann reaction gives 3-nitrobiphenyl. Reduction of the nitro group, followed by acetylation, nitration at the C(4') position, methylation of the acetylamino group and subsequent deacetylation finally yields the desired starting material **2a**. The analog in the second method is 4,4'-nitrobiphenylcarboxaldehyde, which is easily synthesized by nitration of 4-biphenylcarbox-

aldehyde.

The introduction of the desired polar substituent or group into the N(3) position of the 5-deazaisoalloxazine was achieved either by replacing the hydrogen of the NH-group in **9** (R^1=H) by this group or alternatively by using **6** as starting material in which such a group is already present [10].



Scheme 2

2.2.2 *Synthesis of 5-deazaflavins by cyclization with a one-carbon reagent (method 1).*

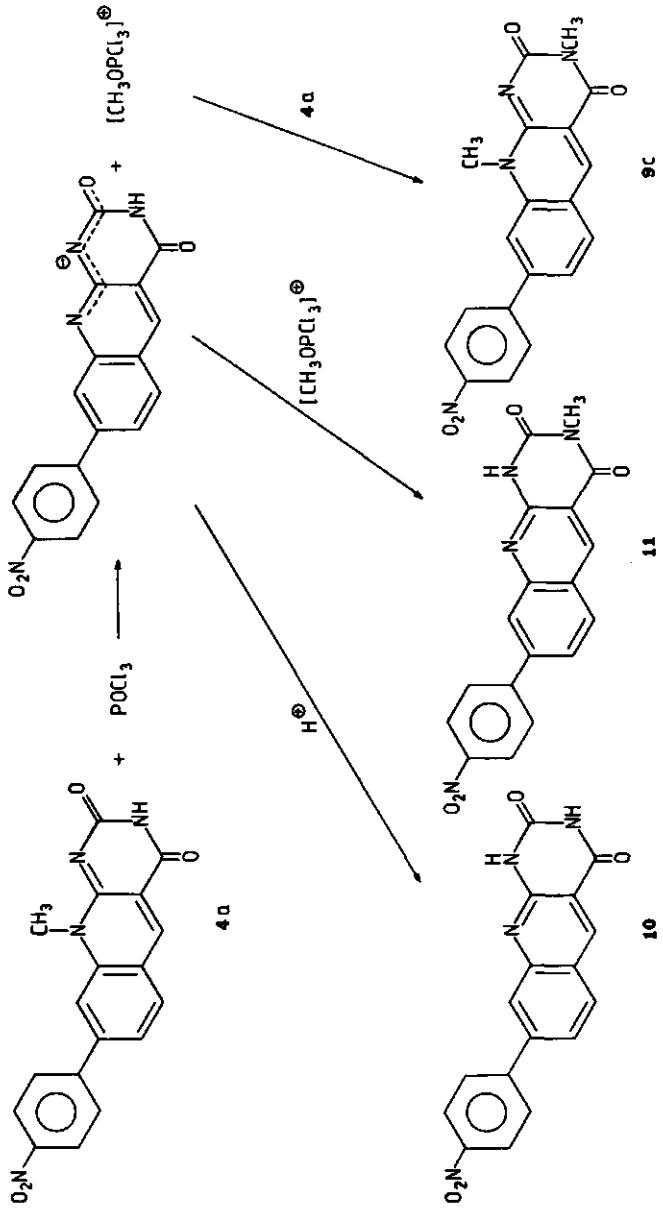
As mentioned above, this method usually provides a mixture of 6- and 8-substituted-5-deazaflavins. In order to avoid this we have tried to introduce a *p*-nitrophenyl substituent that will prevent during the cyclization the formation of the 6-analog due to sterical interference.

For this purpose 6-chlorouracil (**1**) and an excess of 3-methylamino-4'-nitrobiphenyl (**2a**) were refluxed in 1-butanol, leading to the formation of **3a**. This method has several advantages over the fusion reaction, as applied by Yoneda [11]; excess of **2a** is regained in better yield and in less vitiated form.

Cyclization of **3a** with a tenfold excess of phosphorus oxychloride in dimethylformamide [12] at 90° gave decomposition to a large extent. In our hands reaction of **3a** with an equimolar amount of phosphorus oxychloride at 60° yielded yellow crystals. The low-energy mass spectrum of this compound showed, besides the parent peak m/e 348 (**4a**), a peak m/e 362, indicating the presence of 8-(*p*-nitrophenyl)-3,10-dimethyl-5-deazaisoalloxazine (**9c**) (Scheme 3), and a peak m/e 334, suggesting the formation of 8-(*p*-nitrophenyl)-5-deazaalloxazine (**10**). The 300 MHz nmr spectrum showed, besides the signals of **4a** (δ 4.24 (s, 3H, N(10)CH_3), 8.03 (dd, 1H, C(7)H), 8.27 (d, 1H, C(9)H), 8.31(d, 2H, ArH), 8.40 (d, 1H, C(6)H), 8.47 (d, 2H, ArH), 9.12 (s, 1H, C(5)H), 11.21 (br s, 1H, NH)), two small signals of the same intensity at 4.18 ppm and 3.29 ppm agreeing well with the *N*-methyl signals of **9c**. In addition a small signal at 3.17 ppm and a series of small signals in the aromatic region confirm the presence of small quantities of non-methylated and/or mono-methylated 5-deazaalloxazines **10** and **11**. Demethylation has been shown to occur in the synthesis of 10-methylisoalloxazine [13]. It has been shown that demethylation is suppressed when a substituent with a +I effect is present at the C(8) position. This is explained by the fact that the +I effect increases the electron density at the N(10) position and thus strengthens the nitrogen-carbon bond. The opposite is true in our case where the substituent at the C(8) position is electron withdrawing, *i.e.* *p*-nitrophenyl group, thus weakening the nitrogen-carbon bond.

The use of phosphorus oxychloride as demethylating agent has also been demonstrated with *N*-methylated purines [14]. This demethylation reaction is proposed to proceed by conversion of the methyl group into a trichloromethyl group and subsequent hydrolytic fission of the nitrogen-carbon bond. We have however observed that demethylation is also accompanied by remethylation. Thus, the proposed mechanism seems less applicable to our reactions. Therefore we suggest a mechanism, which is based on the slight nucleophilic character of the

phosphoryl bond [15] (Scheme 3). Nucleophilic attack of phosphorus oxychloride on the weakly bonded *N*(10)-methyl group leads to the formation of a demethylated anion, derived from **4a**, and the methoxytrichlorophosphonium ion. Remethylation by this ion leads to the formation of dimethyl- and monomethyl-

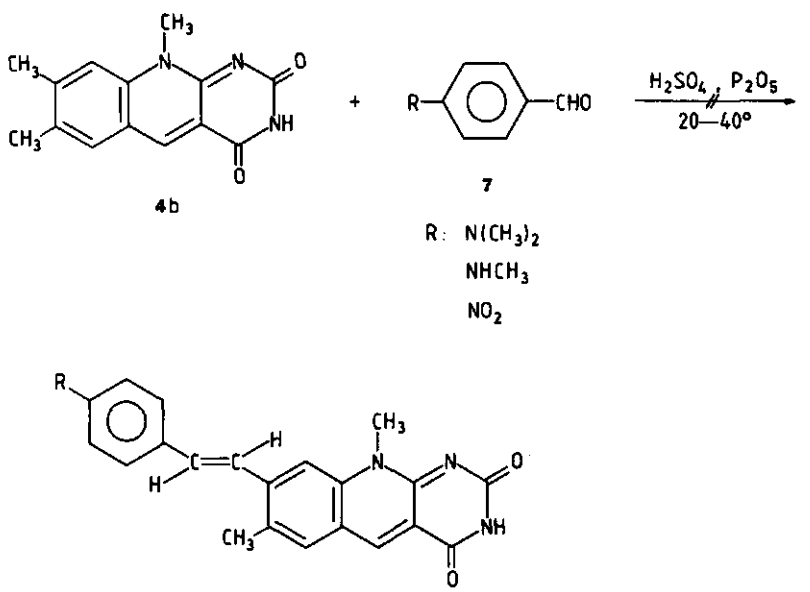


Scheme 3

deaza(iso)alloxazines.

The fact that synthesis of **4a** by cyclization with a one-carbon reagent leads to the formation of an inseparable mixture of products combined with the possibility of obtaining mixtures of 6- and 8-substituted 5-deazaflavins made this method less suitable.

Another attempt to introduce at C(8) substituents with a bathochromic shift effect was to modify the C(8)-methyl group in 5-deazalumiflavin (7,8,10-trimethyl-5-deazaisoalloxazine) (**4b**). It is known that the C(8)-methyl group in the related molecule lumiflavin is π-electron deficient [16] and undergoes aldol-type condensation reactions with aromatic aldehydes [17]. Moreover, the methyl group is easily oxidized to carboxylic acid by either nitric acid or potassium permanganate [17a]. It has been calculated that the π-electron deficiency is also present in 5-deazaflavin [18]. We have applied therefore the aldol-condensation reaction also to 5-deazalumiflavin (**4b**) (Scheme 4) to possibly synthesize C(8)-substituted compounds in this way. Condensation with several *p*-substituted benzaldehydes was not successful. Also the oxidation reaction did



Scheme 4

not occur with 5-deazalumiflavin. Obviously the π-electron deficiency at the C(8) position of 5-deazalumiflavin is not sufficient for these reactions to occur. This is consistent with the fact that the calculated π-electron

deficiency at the C(8) position for lumiflavin [16] is larger than that for 5-deazalumiflavin [18].

2.2.3 Synthesis of 5-deazaflavins by oxidative cyclization (method 2).

Condensation of 6-methylamino-3-methyluracil (**6**, R^1=CH_3) with several aryl-aldehydes (**7**, R=Cl, C_6H_5, *p*-NO_2-C_6H_4) in refluxing ethanol gave the 5,5'-methylenebis(6-methylamino-3-methyluracils) **8a-c** in good yield (Table 1). Spectral and elemental analysis support the structures. The nmr spectra showed the characteristic signal of the methine proton on the carbon carrying the aryl group and two 6-methylamino-3-methyluracil moieties in the 5.5 ppm region (Table 1). Field desorption mass spectra showed the parent peaks and the peaks corresponding to the thermolysis products 6-methylamino-3-methyluracil and 5-benzylidene-6-methylamino-3-methyluracil. Oxidative cyclization with excess DAD at 140° (**8a**), 170° (**8c**), or 170° in sulfolane (**8b**) gave the 5-deazaflavins **9a-c** in moderate yield (Table 1). Refluxing **9a** in 40% aqueous dimethylamine gave **9d** in good yield. This is in contrast to the report [19] that **9a** would not be reactive enough for nucleophilic substitution under these conditions.

Catalytic reduction of **9c** in 6*N* hydrochloric acid yielded **9e**. Condensation of the amino compound **9e** with *p*-dimethylaminobenzaldehyde was unsuccessful because of the deactivating effect of the electron donating dimethylamino group on the formyl function. Refluxing **9e** with a 1:1 mixture of the aldehyde and its diethylacetal (obtained by treatment of the aldehyde with triethoxymethaan) in dimethylformamide gave **9f** in good yield. Spectral and elemental analysis of **9a-f** were in good agreement with the corresponding structures. All showed a characteristic low-field H(5) singlet. Because of the strongly electron donating effect of the dimethylamino group, the H(5) singlet of **9d** is shifted upfield as compared to that of **9a-c,e,f** (Table 1). Low energy mass spectra showed only the parent peaks. The bathochromic effect of the various substituents on the uv spectra is shown in Table 1.

2.3 EXPERIMENTAL

The ^1H nmr spectra were obtained with a Hitachi Perkin Elmer R-24B, a Varian EM 390 or a Bruker CXP-300 spectrometer operating at 300 MHz, using tetramethylsilane as an internal standard. Mass spectra were recorded on a Kratos MS 9 instrument. The uv-visible spectra were obtained with a Aminco DW-2a uv-vis or a Beckman DU-7 spectrophotometer. Melting points were uncorrected. Silica gel GF plates were used for analytical thin layer chromatography.

*Table 1 Yields and some physical properties of 5,5'-methylenebis(6-methylamino-3-methyluracils) **8** (R^1=CH_3) and of the 5-deazaflavins **9** (R^1=CH_3)*

Compd.	R^1	R	Yield %	mp °C	δ *	λ_{max}/nm (log ε)**
8a	CH_3	Cl	85	288-289 dec	5.43	
8b	CH_3	C_6H_5	80	279-281 dec	5.48	
8c	CH_3	p-NO_2-C_6H_4	80	290-292 dec	5.50	
9a	CH_3	Cl	60	314-318 dec	9.70	412 sh (4.07)
9b	CH_3	C_6H_5	50	>350	9.70	424 sh (4.27)
9c	CH_3	p-NO_2-C_6H_4	75	>350	9.87	440 sh (3.38), DMSO
9d	CH_3	$N(CH_3)_2$	88	>350	8.97	441 (4.78)
9e	CH_3	p-NH_2-C_6H_4	60	>350	9.82	442 (4.23)
9f	CH_3	p-$(CH_3)_2N$-C_6H_4-CH=N-C_6H_4	83	345-348 dec	9.75	441 (4.01), DMSO

* Chemical shifts in parts per million relative to TMS of methine protons of **8** in DMSO and of C(5) protons of **9** in trifluoroacetic acid.
** Absorption maximum of **9** in methanol unless otherwise stated; sh = shoulder.

*3-Methylamino-4'-nitrobiphenyl (**2a**).*

A solution of 20 g of 3-acetamidobiphenyl [20] in 240 ml of glacial acetic acid and 160 ml of concentrated sulfuric acid was stirred at 0° as a mixture of 16 ml of fuming nitric acid and 40 ml of glacial acetic acid was added dropwise. The mixture was stirred for 10 hours at 0° and then poured onto ice. The solid was collected on a filter, washed with water, and recrystallized from ethanol to give 11 g (45%) of 3-acetamido-4'-nitrobiphenyl; pale-yellow needles, mp 191-193° (lit. [20a] 192°); nmr (60 MHz, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 7.30-8.07 (m, 4H, ArH), 7.80 (d, 2H, Ar'H), 8.27 (d, 2H, Ar'H), 10.00 (br s, 1H, NH); ms: m/e 256 (M^+).

A solution of 11 g of 3-acetamido-4'-nitrobiphenyl in 150 ml of dimethylformamide was stirred at room temperature as 14 g of potassium carbonate and 7 g of powdered sodium hydroxide was added in small portions. A solution of 9 ml of dimethyl sulfate in 25 ml of dimethylformamide was then added dropwise. After a few minutes of stirring, the mixture was poured onto ice. The solid was collected on a filter, washed with water, and recrystallized from ethanol to give 11 g (95%) of 3-*N*-methylacetamido-4'-nitrobiphenyl; yellow plates, mp 149-150°; nmr (60 MHz, deuteriochloroform): δ 1.96 (s, 3H, CH_3), 3.32 (s, 3H, NCH_3), 7.13-7.57 (m, 4H, ArH), 7.68 (d, 2H, Ar'H), 8.24 (d, 2H, Ar'H); ms: m/e

270 (M^+).

Anal. Calcd. for $C_{15}H_{14}N_2O_3$; C, 66.65; H, 5.22. Found: C, 66.69; H, 5.04.

A mixture of 11 g of 3-*N*-methylacetamido-4'-nitrobiphenyl and 200 ml of 10% ethanolic hydrochloric acid was stirred at reflux for 18 hours. After cooling the solution was concentrated *in vacuo* and neutralized with diluted ammonia. The solid was collected on a filter, washed with water, and recrystallized from ethanol to give 9 g (97%) of **2a**; red plates, m.p. 121-122°; nmr (60 MHz, deuteriochloroform): δ 2.86 (s, 3H, NCH_3), 3.85 (br s, 1H, NH), 6.50-7.42 (m, 4H, ArH), 7.63 (d, 2H, Ar'H), 8.19 (d, 2H, Ar'H); ms: m/e 228 (M^+).

Anal. Calcd. for $C_{13}H_{12}N_2O_2$: C, 68.40; H, 5.30 Found: C, 68.12; H, 5.52

*6-[3-(p-Nitrophenyl)-N-methylanilino]uracil (**3a**).*

A mixture of 1.5 g of 6-chlorouracil [21] and 4.7 g of 3-methylamino-4'-nitrobiphenyl (2a) in 50 ml of 1-butanol was stirred at reflux for 60 hours. After cooling the solid was collected on a filter, washed with chloroform and recrystallized from acetic acid to give 2.8 g (81%) of yellow needles, mp. 330-331° dec.; nmr (60 MHz, DMSO-d_6): δ 3.36 (s, 3H, NCH_3), 4.51 (s, 1H, C(5)H), 7.30-7.87 (m, 4H, ArH), 8.05 (d, 2H, Ar'H), 8.39 (d, 2H, Ar'H), 10.51 (br s, 1H, NH), 10.67 (br s, 1H, NH); ms: m/e 338 (M^+).

Anal. Calcd. for $C_{17}H_{14}N_4O_4 \cdot 1H_2O$: C, 57.30; H, 4.53. Found: C, 57.45; H, 4.66.

*6-(N-methyl-3,4-xylidino)uracil (**3b**).*

A mixture of 2.2 g of 6-chlorouracil and 6 g of *N*-methyl-3,4-xylidine (**2b**) [22] was stirred at 165° (oilbath) for 6 minutes with stirring. After cooling, the reaction mixture was crushed in ether, collected by filtration, washed with water and dried to give 3.3 g (90%) of yellow needles, mp 282-284° (lit. [11] 281°); nmr (60 MHz, DMSO-d_6): δ 2.23 (s, 6H, $2ArCH_3$), 3.20 (s, 3H, $N-CH_3$), 4.23 (s, 1H, C(5)H), 6.80-7.33 (m, 3H, ArH), 10.17 (br s, 1H, NH), 10.42 (br s, 1H, NH); ms: m/e 245 (M^+).

*7,8,10-Trimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (**4b**).*

A mixture of 2,5 g of **3b** and 1.9 ml of phosphorus oxychloride in 30 ml of dimethylformamide was stirred at 90° for 2 hours. After cooling, it was diluted with water and neutralized with sodium hydrogen carbonate. The solid was collected on a filter, washed with water, and dried to give 2.2 g (85%) of yellow needles, mp >350° (lit. [12] >360°); nmr (60 MHz, trifluoroacetic acid) δ 2.60 (s, 3H, $C(7)CH_3$), 2.75 (s, 3H, $C(8)CH_3$), 4.49 (s, 3H, $N(10)CH_3$), 8.05 (br s, 2H, ArH), 9.51 (s, 1H, C(5)H); ms: m/e 255 (M^+).

Anal. Calcd. for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13. Found: C, 65.57; H, 5.21.

4'-Nitrobiphenyl-4-carboxaldehyde (7, R=p-NO_2-C_6H_4).

A solution of 25 g of 4-biphenylcarboxaldehyde in 550 ml of sulfuric acid was stirred at -5° and 14 g of potassium nitrate was added in small portions. The thus formed green solution was stirred for 2 hours while the temperature was kept below 0° and then poured onto 5 kg of crushed ice. The precipitate was collected on a filter, washed with water, and recrystallized from ethanol to give 21 g (68%) of pale yellow needles, mp 130°; nmr (60 MHz, deuteriochloroform, acetone-d_6): δ 7.73 (d, 2H, ArH), 7.77 (d, 2H, Ar'H), 7.93 (d, 2H, ArH), 8.21 (d, 2H, Ar'H), 9.95 (s, 1H, CHO); ms: m/e 227 (M^+).

Anal. Calcd. for $C_{13}H_9NO_3$: C, 68.72; H, 3.99. Found: C, 68.81; H, 4.23.

5,5'-Arylmethylenebis(6-methylamino-3-methyluracils) ***8a-c.***

General procedure.

A mixture of 0.013 mole of 6-methylamino-3-methyluracil (6) [23], 0.013 mole of arylaldehyde and 40 ml of ethanol was stirred at reflux for 48 hours. After cooling, the precipitate was collected on a filter, washed with ethanol, and purified by stirring in ethanol at reflux, followed by hot filtration.

5,5'-p-Chlorophenylmethylenebis(6-methylamino-3-methyluracil) (8a).

Yield 2.40 g (85%) of white needles, mp 288-289° dec. (lit. [8b] 289°); nmr (60 MHz, DMSO-d_6): δ 2.80 (br d, 6H, C(6)NCH_3, C(6')NCH_3), 3.10 (s, 6H, N(3)CH_3, N(3')CH_3), 5.43 (s, 1H, C(5,5')H), 7.04 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.95 (br s, 2H, C(6)NH, C(6')NH), 10.63 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 432/434 (M^+), 277 (cluster), 155.

Anal. Calcd. for $C_{19}H_{21}ClN_6O_4$: C, 52.72; H, 4.89. Found: C, 52.07; H, 5.09.

5,5'-[1,1'-Biphenyl]-4-ylmethylenebis(6-methylamino-3-methyluracil) (8b).

Yield 2.45 g (80%) of white plates, mp 279-281° dec.; nmr (90 MHz, DMSO-d_6): δ 2.82 (d, 6H, C(6)NCH_3, C(6')NCH_3), 3.10 (s, 6H, N(3)CH_3, N(3')CH_3), 5.48 (s, 1H, C(5,5')H), 7.11 (d, 2H, ArH), 7.27-7.71 (m, 5H, Ar'H), 7.49 (d, 2H, ArH), 8.00 (br s, 2H, C(6)NH, C(6')NH), 10.59 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 474 (M^+), 319 (cluster), 155.

Anal. Calcd. for $C_{25}H_{26}N_6O_4$: C, 63.28; H, 5.52. Found: C, 63.11; H, 5.21.

5,5'-[4'-Nitro-1,1'-biphenyl]-4-ylmethylenebis(6-methylamino-3-methyluracil) (8c).

Yield 2.70 g (80%) of pale yellow plates, mp 290-292° dec.; nmr (90 MHz, DMSO-d_6): δ 2.82 (d, 6H, C(6)NCH_3, C(6')NCH_3), 3.10 (s, 6H, N(3)CH_3, N(3')CH_3), 5.50 (s, 1H, C(5,5')H), 7.19 (d, 2H, ArH), 7.63 (d, 2H, ArH), 7.92 (d, 2H, Ar'H), 8.00 (br q, 2H, C(6)NH, C(6')NH), 8.28 (d, 2H, Ar'H), 10.72 (s, 2H, N(1)H, N(1')H); field desorption ms: m/e 519 (M^+), 364 (cluster), 155.

Anal. Calcd. for $C_{25}H_{25}N_7O_6$: C, 57.80; H, 4.85. Found: C, 57.55; H, 4.49.

8-Chloro-3,10-dimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (9a).

A mixture of 2 g of **8a** and 3.7 ml of diethyl azodicarboxylate (DAD) was stirred at 140° (oilbath) for 3 hours. After cooling, the mixture was diluted with ethanol, and allowed to stand overnight at room temperature. The solid was collected on a filter, washed with ethanol, and recrystallized from dimethylformamide to give 0.76 g (60%) of yellow needles, mp 314-318° dec. (lit. [8b] 328°); nmr (90 MHz, trifluoroacetic acid): δ 3.59 (s, 3H, N(3)CH_3), 4.45 (s, 3H, N(10)CH_3), 7.93 (dd, 1H, C(7)H), 8.27 (d, 1H, C(9)H), 8.30 (d, 1H, C(6)H), 9.70 (s, 1H, C(5)H); ms: m/e 275/277 (M^+).

Anal. Calcd. for $C_{13}H_{10}ClN_3O_2$: C, 56.63; H, 3.66. Found: C, 56.33; H, 3.44.

8-Phenyl-3,10-dimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (9b).

A mixture of 0.80 g of **8b**, 2.1 ml of DAD, and 0.1 ml of sulfolane was stirred at 170° (oilbath) for 2 hours. After cooling, the mixture was diluted with ethanol, and allowed to stand overnight at room temperature. The solid was collected on a filter, washed with ethanol and ether, and dried to give 0.27 g (50%) of yellow needles, mp >350°; nmr (90 MHz, trifluoroacetic acid): δ 3.60 (s, 3H, N(3)CH_3), 4.53 (s, 3H, N(10)CH_3), 7.40-7.87 (m, 5H, ArH), 8.24 (d, 1H, C(7)H), 8.37 (s, 1H, C(9)H), 8.41 (d, 1H, C(6)H), 9.70 (s, 1H, C(5)H); ms: m/e 317 (M^+).

Anal. Calcd. for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76. Found: C, 72.01; H, 4.76.

8-p-Nitrophenyl-3,10-dimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (9c).

A mixture of 2.5 g of **8c** and 3.8 ml of DAD was stirred at 170° (oilbath) for 7 hours. After cooling, the mixture was diluted with ethanol and allowed to stand overnight at room temperature. The solid was collected on a filter, washed with ethanol and ether, and dried to give 1.3 g (75%) of yellow plates, mp >350°; nmr (90 MHz, trifluoroacetic acid): δ 3.67 (s, 3H, N(3)CH_3), 4.63 (s, 3H, N(10)CH_3), 8.05 (d, 2H, ArH), 8.29 (d, 1H, C(7)H), 8.40-8.69 (m, 4H, ArH,

C(6)H, C(9)H), 9.87 (s, 1H, C(5)H); nmr (90 MHz, DMSO-d_6): δ 3.30 (s, N(3)CH_3), 4.08 (s, N(10)CH_3), 9.10 (s, C(5)H); ms: m/e 362 (M^+).
Anal. Calcd. for $C_{19}H_{14}N_4O_4$: C, 62.98; H, 3.89. Found: C, 62.99; H, 3.76.

*8-Dimethylamino-3,10-dimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (**9d**).*

A mixture of 0.32 g of **9a** and 20 ml of 40% aqueous dimethylamine was stirred at reflux for 8 hours. After cooling, the solid was collected on a filter, washed with water, and recrystallized from dimethylformamide-1-butanol 3:2 to give 0.29 g (88%) of yellow plates, mp >350° (lit. [8b] >360°); nmr (90 MHz, trifluoracetic acid): δ 3.39 (s, 6H, N(CH_3)$_2$), 3.55 (s, 3H, N(3)CH_3), 4.16 (s, 3H, N(10)CH_3), 6.77 (br s, 1H, C(9)H), 7.35 (br d, 1H, C(7)H), 7.94 (d, 1H, C(6)H), 8.97 (s, 1H, C(5)H); ms: m/e 284 (M^+).
Anal. Calcd. for $C_{15}H_{16}N_4O_2$: C, 63.36; H, 5.67. Found: C, 62.92; H, 5.33.

*8-p-Aminophenyl-3,10-dimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (**9e**).*

Compound **9c** (1.3 g) suspended in 200 ml of 6*N* hydrochloric acid, was hydrogenated in the presence of 0.65 g of Pd/C (10%) as a catalyst during 1 hour. The solution was filtered, and neutralized with sodium hydrogen carbonate. The precipitate was collected on a filter, washed with water, and recrystallized from dimethylformamide to give 0.72 g (60%) of red-orange plates, mp >350°; nmr (90 MHz, trifluoroacetic acid): δ 3.66 (s, 3H, N(3)CH_3), 4.60 (s, 3H, N(10)CH_3), 7.77 (d, 2H, ArH), 8.01 (d, 2H, ArH), 8.25 (d, 1H, C(7)H), 8.49 (s, 1H, C(9)H), 8.54 (d, 1H, C(6)H), 9.82 (s, 1H, C(5)H); ms: m/e 332 (M^+).
Anal. Calcd. for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85. Found: C, 68.73; H, 4.84.

Diethylacetal of p-*dimethylaminobenzaldehyde.*

A mixture of 7.5 g of *p*-dimethylaminobenzaldehyde, 10 ml of triethoxymethane, 0.4 g of ammonium nitrate, and 4 ml of absolute ethanol was stirred at reflux for 1 hour. After cooling, the solution was filtered, taken up in 20 ml of ether, washed several times with diluted ammonia and water, and concentrated by evaporation. To remove traces of triethoxymethane, the residual oil was stirred in water and allowed to stand for a week at room temperature. The solid was collected on a filter and dried to give 1.6 g of gray-yellow plates, m.p. 54-64°; nmr (60 MHz, deuteriochloroform): δ 3.01 (s, 6H, N(CH_3)$_2$), 6.67 (d, 2H, ArH), 7.70 (d, 2H, ArH), 9.72 (s, 1H, CHO) for *p*-dimethylaminobenzaldehyde; δ 1.19 (t, 6H, 2 CH_3), 2.90 (s, 6H, N(CH_3)$_2$), 3.55 (q, 4H, 2 CH_2), 5.41 (s, 1H, CH), 6.67 (d, 2H, ArH), 7.31 (d, 2H, ArH) for the diethylacetal of *p*-dimethylaminobenzaldehyde; integral ratio CHO-CH is 1:1.

8-[p-(p-Dimethylaminophenylmethylene)aminophenyl]-3,10-dimethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione (9f).

A mixture of 0.2 g of **9e**, 1.3 g of a 1:1 mixture of *p*-dimethylaminobenzaldehyde and its diethylacetal (see preparation above), and 10 ml of dimethylformamide was stirred at reflux for 3 hours. After cooling, the solid was collected on a filter, washed with ether and dried to give 0.23 g (83%) of orange needles, mp 345-348° dec.; nmr (90 MHz, trifluoroacetic acid): δ 3.46 (s, 6H, $N(CH_3)_2$), 3.61 (s, 3H, $N(3)CH_3$), 4.56 (s, 3H, $N(10)CH_3$), 7.66-8.55 (m, 11H, ArH), 9.75 (s, 1H, C(5)H), 10.00 (s, 1H, CHN); ms: m/e 463 (M^+).

Anal. Calcd. for $C_{28}H_{25}N_5O_2$: C, 72.54; H, 5.44. Found: C, 72.23; H, 5.32.

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3 SYNTHESIS OF WATER SOLUBLE 8-SUBSTITUTED-5-DEAZAFLAVINS

3.1 INTRODUCTION

5-Deazaflavin has been applied as a photocatalyst in the reduction of a wide variety of biological redox systems [1,2]. The use of 5-deazaflavin as a photocatalyst has serious drawbacks in that continuous uv irradiation is required to drive the process and that the needed wavelength (300-400 nm) corresponds to an energy level high enough to destroy the protein enzyme in time. To avoid photodestruction of the protein we have synthesized a number of 5-deazaflavins, which have a chromophoric group at the C(8) position of the 5--deazaisoalloxazine skeleton, causing the absorption maximum to undergo a red shift.

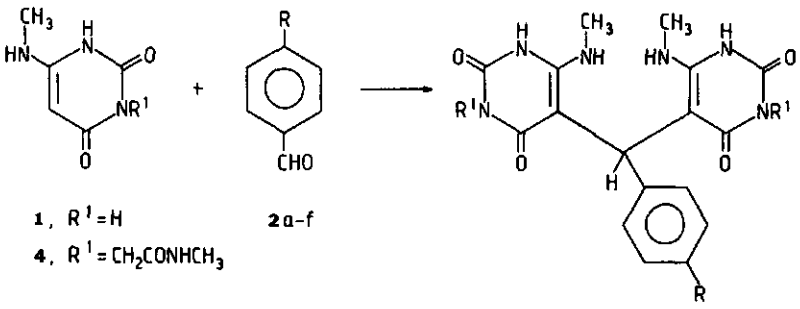
In a recent publication [3] we have described the synthesis of 8-substituted 5-deazaflavins by the following three pathways: i) cyclization of *N*-methylanilinouracil derivatives with a one-carbon reagent, ii) aldol-type condensation of the C(8) methyl group in 5-deazalumiflavin with aromatic aldehydes, and iii) oxidative cyclization of arylmethylenebis(6-methylaminouracil) derivatives. Only the latter method appeared to be suitable for our goal and has been applied in the synthesis of 5-deazaflavins described in this report.

The present report is also concerned with the synthesis of 8-substituted 5-deazaflavins containing a carboxymethyl group at the N(3) position. This polar group increases the solubility of 5-deazaflavins in aqueous media, in which photoreduction of redox enzymes are to be carried out.

3.2 RESULTS AND DISCUSSION

Two methods of preparing 5-deazaflavins were explored.

In the first method (Scheme 1) 6-methylaminouracil (1) was condensed with the arylaldehydes **2b-f** giving the 5,5'-methylenebis(6-methylaminouracils) **3b-f** (Table 1). The oxidative cyclization of **3b-f** with diethyl azodicarboxylate (DAD) was not successful. Variation in reaction temperature, use of larger excess of DAD, longer reaction time, and applying solvents *e.g.* sulfolane, dimethylformamide and hexamethylphosphoramide, led to incomplete conversion and/or formation of a very impure product. The very low solubility and the



	a	b	c	d	e	f
R	F	Cl	NO_2	p-NO_2-C_6H_4	p-Cl-C_6H_4	C_6H_5

Scheme 1

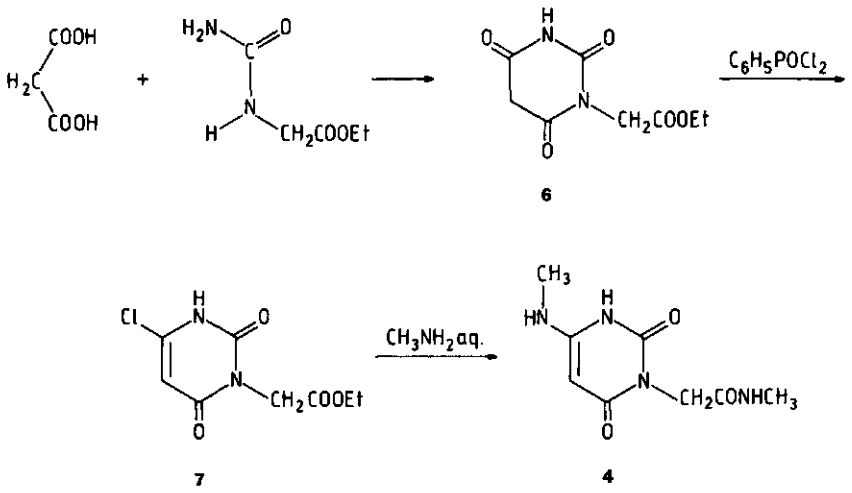
thermolability of **3b-f** might explain the insufficient course of the reaction; with **3c** even complete decomposition occurred.

Based on our previous experience [3] that oxidative cyclization of 5,5'-methylenebis(6-methylaminouracils) with DAD occur successfully when a substituent is present at N(3), we decided to use as starting material 6-methylaminouracil, containing at position 3 a *N*-methylcarbamoylmethyl group, *i.e.* compound **4**. In later stages of the reaction this *N*-methylcarbamoylmethyl group can then be hydrolysed into a carboxymethyl group by acid treatment.

6-Methylamino-3-(*N*-methylcarbamoylmethyl)uracil (4) was prepared as indicated in Scheme 2. *N*-Ethoxycarbonylmethylureum was condensed with malonic acid in acetic anhydride, yielding *N*-ethoxycarbonylmethyl barbituric acid (6). Subsequent treatment with phenylphosphorus oxychloride yielded 6-chloro-3-ethoxycarbonylmethyluracil (7), which on refluxing in 40% aqueous methylamine gave 6-methylamino-3-(*N*-methylcarbamoylmethyl)uracil (4) in an overall yield of 21%.

Reaction of **4** with the arylaldehydes **2a-d** in refluxing ethanol (Scheme 1) gave compounds **5a-d** in excellent yields (Table 1). Oxidative cyclization of **5a-d** with DAD in sulfolane led to the formation of 5-deazaflavins **8a-d** (Scheme 3). Spectral and analytical data are in agreement with the corresponding structures (Table 2) [4].

Hydrolysis of **8b-d** in concentrated hydrochloric acid yielded 5-deazaflavins **9b-d**. Refluxing **8a** in 40% aqueous dimethylamine led to the formation of **8g**,



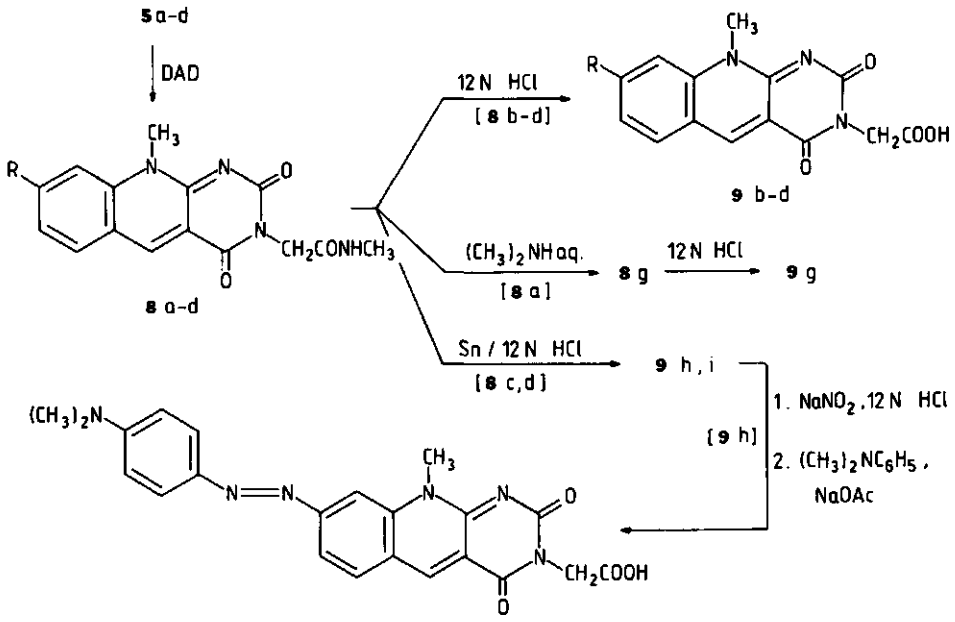
Scheme 2

*Table 1 Yields and some physical properties of 5,5'-methylenebis(6-methylaminouracils) **3** and **5***

Compd.	R	Yield (%)	mp °C	δ*
3b	Cl	92	300-302 dec	5.30
3c	NO_2	88	302-305 dec	5.43
3d	p-NO_2-C_6H_4	90	303 dec	5.39
3e	p-Cl-C_6H_4	70	307-309 dec	5.62 (trifluoroacetic acid)
3f	C_6H_5	84	286-288 dec	5.40
5a	F	86	237-240 > 245 dec	5.40
5b	Cl	99	243-245 dec	5.45
5c	NO_2	88	295-298 dec	5.48
5d	p-NO_2-C_6H_4	94	279-280 dec	5.46

* Chemical shifts (in parts per million relative to TMS) of methine protons of **3** and **5** in DMSO, unless otherwise stated.

which was converted into compound **9g** by hydrolysis. Compounds **9h,i** were obtained by means of reductive hydrolysis of **8c,d**, using tin powder in concentrated hydrochloric acid. By diazotization of **9h** with sodium nitrite in concentrated hydrochloric acid, followed by coupling with *N,N*-dimethylaniline, the highly-coloured compound **10** was obtained. Spectral and analytical data are in agreement with the corrsponding structures (Table 2).



	a	b	c	d	g	h	i
R	F	Cl	NO_2	p-NO_2-C_6H_4	$(CH_3)_2N$	NH_2	p-NH_2-C_6H_4

Scheme 3

3.3 EXPERIMENTAL

The ^{1}H-nmr spectra were obtained with a Hitachi Perkin Elmer R-24B, a Varian EM 390 or a Bruker CXP-300 spectrometer operating at 300 MHz, using tetramethylsilane as an internal standard. Mass spectra were recorded on a Kratos MS 9 instrument. The uv-visible spectra were obtained with a Beckman DU-7 spectrophotometer. Melting points were uncorrected. Silica gel GF plates were used for

analytical thin layer chromatography.

*4'-Chlorobiphenyl-4-carboxaldehyde (**2e**).*

Under stirring 1.3 ml of titanium chloride was added dropwise to a solution of 1 g of 4-chlorobiphenyl in 6 ml of dichloromethane at a temperature of 2°. Over a period of 30 minutes 0.6 ml of dichloromethyl methyl ether was added at the same temperature. The mixture was stirred overnight at room temperature and then poured onto crushed ice. Chromatography on silica gel 60 using toluene as eluent gave a product that by recrystallization from a mixture of ethanol-water (4:1) yielded 0.65 g (57%) of colorless needles, mp >110° dec.; nmr (60 MHz, deuteriochloroform): 7.33 (d, 2H, Ar'H), 7.51 (d, 2H, Ar'H), 7.60 (d, 2H, ArH), 7.87 (d, 2H, ArH), 9.94 (s, 1H, CHO); ms: m/e 216/218 (M^+).
Anal. Calcd. for $C_{13}H_9ClO$: C, 72.06; H, 4.19. Found: C, 71.71; H, 3.95.

*Table 2 Yields and some physical properties of 5-deazaflavins **8, 9 and 10***

Compd.	R	Yield (%)	mp °C	δ*	λ_{max}/nm (log ε)**
8a	F	43	335-340 dec	9.71	
8b	Cl	42	345-350 dec	9.75	
8c	NO_2	70	>350	9.87	
8d	*p*-NO_2-C_6H_4	62	>350	9.82	
8g	$(CH_3)_2N$	85	347-349 dec	9.04	
9b	Cl	70	330-330.5 dec	9.09	420 (4.04)
9c	NO_2	75	320-324 dec	9.16	453 sh (3.85)
9d	*p*-NO_2-C_6H_4	76	348-350 dec	9.12	435 sh (4.18)
9g	$(CH_3)_2N$	92	305-308 dec	8.77	443 (4.74)
9h	NH_2	73	>325 dec	8.53	430 (4.74)
9i	*p*-NH_2-C_6H_4	61	313-315 dec	8.93	455 (4.52)
10	*p*-$(CH_3)_2N$-C_6H_4-N=N	64	>350	9.07	530 (4.64)

* Chemical shifts (in parts per million relative to TMS) of C-5 protons of **8** in trifluoroacetic acid and of **9** and **10** in DMSO.

** Absorption maximum of **9** and **10** in DMSO; sh=shoulder.

5,5'-Arylmethylenebis(6-methylaminouracils) ***3b-f.***

General procedure. A mixture of 0.01 mole of 6-methylaminouracil **(1)** [5], 0.015 mole of arylaldehyde, and 100 ml of acetic acid was stirred at 90° for 5 hours. After cooling, the mixture was concentrated, the solid product obtained was washed with ether or ethanol, and recrystallized from DMSO.

5,5'-p-Chlorophenylmethylenebis(6-methylaminouracil) ***(3b).***

Yield 1.86 g (92%) of white plates, mp 300-302° dec.; nmr (60 MHz, DMSO-d_6): δ 2.78 (br d, 6H, C(6)NCH_3, C(6')NCH_3), 5.30 (s, 1H, C(5,5')H), 6.97 (d, 2H, ArH), 7.19 (d, 2H, ArH), 7.97 (br s, 2H, C(6)NH, C(6')NH), 10.39 and 10.47 (br s, 4H, 4xNH); field desorption ms: m/e 404/406 (M^+), 263 (cluster), 141.
Anal. Calcd. for $C_{17}H_{17}ClN_6O_4$: C, 50.44; H, 4.23. Found: C, 50.16; H, 4.52.

5,5'-p-Nitrophenylmethylenebis(6-methylaminouracil) ***(3c).***

Yield 1.91 g (88%) of pale-yellow plates, mp 302-305° dec; nmr (60 MHz, DMSO-d_6): δ 2.82 (br d, 6H, C(6)NCH_3, C(6')NCH_3), 5.43 (s, 1H, C(5,5')H), 7.35 (d, 2H, ArH), 8.09 (d, 2H, ArH), 8.1 (br s, 2H, C(6)NH, C(6')NH), 10.69 and 10.78 (br s, 4H, 4xNH); field desorption ms: m/e 415 (M^+), 274 (cluster), 141.
Anal. Calcd. for $C_{17}H_{17}N_7O_6 \cdot 1H_2O$: C, 47.11; H, 4.42. Found: C, 47.18; H, 4.26.

5,5'-[4'-Nitro-1,1'-biphenyl]-4-ylmethylenebis(6-methylaminouracil) ***(3d).***

Yield 2.21 g (90%) of pale-yellow needles, mp 303° dec.; nmr (90 MHz, DMSO-d_6): δ 2.81 (br s, 6H, C(6)NCH_3, C(6')NCH_3), 5.39 (s, 1H, C(5,5')H), 7.20 (d, 2H, ArH), 7.64 (d, 2H, ArH), 7.93 (d, 2H, Ar'H), 8.1 (br s, 2H, C(6)NH, C(6')NH), 8.29 (d, 2H, Ar'H), 10.53 (br s, 4H, 4xNH); field desorption ms: m/e 491 (M^+), 350 (cluster), 141.
Anal. Calcd. for $C_{23}H_{21}N_7O_6$: C, 56.21; H, 4.31. Found: C, 56.35; H, 4.62.

5,5'-[4'-Chloro-1,1'-biphenyl]-4-ylmethylenebis(6-methylaminouracil) ***(3e).***

Yield 1.68 g (70%) of white plates, mp 307-309° dec.; nmr (90 MHz, trifluoroacetic acid): δ 3.16 (s, 6H, C(6)NCH_3, C(6')NCH_3), 5.62 (br s, 1H, C(5,5')H), 7.44 (d, 2H, ArH), 7.5 (br s, 2H, C(6)NH, C(6')NH), 7.61 (d, 2H, ArH), 7.81 (d, 2H, Ar'H), 8.09 (d, 2H, Ar'H); field desorption ms: m/e 480/482 (M^+), 339 (cluster), 141.
Anal. Calcd. for $C_{23}H_{21}ClN_6O_4$: C, 57.44; H, 4.40. Found: C, 57.39; H, 4.24.

5,5'-[1,1'-Biphenyl]-4-ylmethylenebis(6-methylaminouracil) ***(3f).***

Yield 1.87 g (84%) of white plates, mp 286-288° dec.; nmr (90 MHz, DMSO-

$-d_6$): δ 2.82 (br d, 6H, C(6)NCH_3, C(6')NCH_3), 5.40 (s, 1H, C(5,5')H), 7.15 (d, 2H, ArH), 7.41 (d, 2H, ArH), 7.47-7.77 (m, 5H, Ar'H), 7.8 (br s, 2H, C(6)NH, C(6')NH), 10.53 and 10.59 (br s, 4H, 4xNH); field desorption ms: m/e 446 (M^+), 305 (cluster), 141.

Anal. Calcd. for $C_{23}H_{22}N_6O_4$: C, 61.87; H, 4.97. Found: C, 61.97; H, 5.07.

N-Ethoxycarbonylmethylbarbituric acid ***(6)***.

209 g of *N*-ethoxycarbonylmethylureum [6] and 167 g of malonic acid were dissolved in 300 ml of glacial acetic acid at 60°. With stirring 294 ml of acetic anhydride was added in 2 hours at 70°. After addition the temperature was raised to 90° in 3 hours and stirring was continued for another 3 hours. The acetic acid and the acetic anhydride were removed *in vacuo*. The solid was recrystallized twice from ethanol to give 152 g (50%) of pale-yellow plates , mp 134.5-135°; nmr (60 MHz, DMSO-d_6): δ 1.21 (t, 3H, ethyl CH_3), 3.82 (br s, 2H, 2C(5)H), 4.14 (q, 2H, ethyl CH_2), 4.44 (s, 2H, NCH_2), 11.72 (br s, 1H, N(1)H); ms: m/e 214 (M^+).

Anal. Calcd. for $C_8H_{10}N_2O_5$: C, 44.86; H, 4.71. Found: C, 44.92; H, 4.46.

6-Chloro-3-ethoxycarbonylmethyluracil ***(7)***.

152 g of **6** were dissolved in 130 ml of phenylphosphorus oxychloride at 150° (oil bath) and the solution was stirred for 20 minutes at the same temperature. After cooling the mixture was poured onto 1 kg of crushed ice and neutralized with 10% ammonia. The precipitate was filtered off and the filtrate was extracted with chloroform. From the chloroform layer an additional amount of **7** was obtained. Both portions were combined and recrystallized from dichloromethane-petroleum ether (60/80) to give 115 g (70%) of pale-yellow plates, mp 152-154°; nmr (60 MHz, deuteriochloroform): δ 1.28 (t, 3H, ethyl CH_3), 4.22 (q, 2H, ethyl CH_2), 4.63 (s, 2H, N(3)CH_2), 5.91 (s, 1H, C(5)H), 10.20 (br s, 1H, N(1)H); ms: m/e 232/234 (M^+).

Anal. Calcd. for $C_8H_9ClN_2O_4$: C, 41.30; H, 3.90. Found: C, 41.32; H, 3.60.

6-Methylamino-3-(N-methylcarbamoylmethyl)uracil ***(4)***.

A mixture of 113 g of **7** and 1 l of 40% aqueous methylamine was stirred at reflux for 48 hours. After cooling, the mixture was concentrated *in vacuo* to a small volume and neutralized with 3*N* hydrochloric acid. The solid was collected on a filter, washed with water and recrystallized twice from methanol to give 63 g (61%) of pale plates, mp 290-293° dec.; nmr (90 MHz, DMSO-d_6): δ 2.57 (d, 3H, $CONCH_3$), 2.67 (d, 3H, C(6)NCH_3), 4.24 (s, 2H, N(3)CH_2), 4.52 (s, 1H,

C(5)H), 6.14 (br q, 1H, C(6)NH), 7.82 (br q, 1H, CONH), 10.12 (br s, 1H, N(1)H); ms: m/e 212 (M^+).

Anal. Calcd. for $C_8H_{12}N_4O_3$: C, 45.28; H, 5.70. Found: C, 45.16; H, 5.59.

5,5'-Arylmethylenebis[6-methylamino-3-(N-methylcarbamoylmethyl)uracils] ***5a-d.***

General procedure. A mixture of 0.05 mole of **4**, 0.075 mole of arylaldehyde, and 250 ml of ethanol was stirred at reflux for 72 hours. A precipitate was obtained, which was collected on a filter, washed with ethanol, and purified by stirring in ethanol at reflux, followed by hot filtration.

5,5'-p-Fluorophenylmethylenebis[6-methylamino-3-(N-methylcarbamoylmethyl)-uracil] ***(5a).***

Yield 12.2 g (86%) of white plates, mp 237-240°, >245° dec.; nmr (90 MHz, DMSO-d_6): δ 2.63 (br s, 6H, 2X $CONCH_3$), 2.80 (br s, 6H, C(6)NCH_3, C(6')NCH_3), 4.35 (s, 4H, N(3)CH_2, N(3')CH_2), 5.40 (s, 1H, C(5,5')H), 6.70-7.27 (m, 4H, ArH), 7.81 (br s, 4H, 4xNH), 10.76 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 530 (M^+), 318 (cluster), 212.

Anal. Calcd. for $C_{23}H_{27}FN_8O_6.2H_2O$: C, 48.76; H, 5.52. Found: C, 48.94; H, 5.57.

5,5'-p-Chlorophenylmethylenebis[6-methylamino-3-(N-methylcarbamoylmethyl)-uracil] ***(5b).***

Yield 13.5 g (99%) of white needles, mp 243-245° dec.; nmr (60 MHz, DMSO--d_6): δ 2.62 (d, 6H, 2x$CONCH_3$), 2.83 (br d, 6H, C(6)NCH_3, C(6')NCH_3), 4.37 (s, 4H, N(3)CH_2, N(3')CH_2), 5.45 (s, 1H, C(5,5')H), 7.25 (s, 4H, ArH), 7.8 (br s, 2H, C(6)NH, C(6')NH), 7.90 (br q, 2H, 2xCONH), 10.93 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 546/548 (M^+), 334 (cluster), 212.

Anal. Calcd. for $C_{23}H_{27}ClN_8O_6$: C, 50.50; H, 4.98. Found: C, 50.74; H, 5.05.

5,5'-p-Nitrophenylmethylenebis[6-methylamino-3-(N-methylcarbamoylmethyl)-uracil] ***(5c).***

Yield 13.9 g (88%) of pale-yellow needles, mp 295-298° dec.; nmr (90 MHz, DMSO-d_6): δ2.60 (d, 6H, 2x$CONCH_3$), 2.80 (br d, 6H, C(6)NCH_3, C(6')NCH_3), 4.35 (s, 4H, N(3)CH_2, N(3')CH_2), 5.48 (s, 1H, C(5,5')H), 7.38 (d, 2H, ArH), 7.8 (br s, 2H, C(6)NH, C(6')NH), 7.84 (br q, 2H, 2xCONH), 8.04 (d, 2H, ArH), 10.84 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 557 (M^+), 345 (cluster), 212.

Anal. Calcd. for $C_{23}H_{27}N_9O_8.4H_2O$: C, 43.87; H, 5.60. Found: C, 43.81; H, 5.38.

5,5'-[4'-Nitro-1,1'-biphenyl]-4-ylmethylenebis[6-methylamino-3-(N-methylcarbamoylmethyl)uracil] ***(5d)***.

Yield 14.9 g (94%) of pale-yellow plates, mp 279-280° dec.; nmr (90 MHz, DMSO-d_6): δ 2.59 (d, 6H, 2x $CONCH_3$), 2.82 (br s, 6H, C(6)NCH_3, C(6')NCH_3), 4.33 (s, 4H, N(3)CH_2, N(3')CH_2), 5.46 (s, 1H, C(5,5')H), 7.24 (d, 2H, ArH), 7.63 (d, 2H, ArH), 7.79 (br s, 4H, 4xNH), 7.91 (d, 2H, Ar'H), 8.28 (d, 2H, Ar'H), 10.79 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 633 (M^+), 421 (cluster), 212.

Anal. Calcd. for $C_{29}H_{31}N_9O_8$: C, 54.97; H, 4.93. Found: C, 54.56; H, 5.22.

8-Fluoro-10-methyl-3-(N-methylcarbamoylmethyl)pyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(8a)***.

A mixture of 1 g of **5a**, 1.1 ml of DAD, and 0.8 ml of sulfolane was stirred at 110° (oilbath) under nitrogen for 20 hours. After cooling, the mixture was diluted with ethanol, and allowed to stand overnight at room temperature. The solid was collected on a filter, washed with ethanol and ether, and recrystallized from DMSO to give 0.24 g (43%) of pale-yellow needles, mp 335-340° dec.; nmr (90 MHz, trifluoroacetic acid): δ 2.96 (br s, 3H, $CONCH_3$), 4.48 (s, 3H, N(10)CH_3), 5.03 (s, 2H, N(3)CH_2), 7.34-8.13 (m, 3H, CONH, C(7)H, C(9)H), 8.30-8.66 (m, 1H, C(6)H), 9.71 (s, 1H, C(5)H); ms: m/e 316 (M^+).

Anal. Calcd. for $C_{15}H_{13}FN_4O_3$: C, 56.96; H, 4.14. Found: C, 56.96; H, 4.43.

8-Chloro-10-methyl-3-(N-methylcarbamoylmethyl)pyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(8b)***.

A mixture of 1.5 g of **5b**, 1.5 ml of DAD, and 1.5 ml of sulfolane was stirred at 120° (oilbath) under nitrogen. After 2 hours 1 ml of DAD and after 5 hours 1 ml of sulfolane was added. Stirring was continued for 10 hours. The work-up was similar to that of **8a**, yielding 0.40 g (42%) of pale-yellow needles, mp 345-350° dec.; nmr (90 MHz, trifluoroacetic acid): δ 3.05 (d, 3H, $CONCH_3$), 4.54 (s, 3H, N(10)CH_3), 5.07 (s, 2H, N(3)CH_2), 7.60 (br s, 1H, CONH), 8.03 (dd, 1H, C(7)H), 8.37 (d, 1H, C(9)H), 8.42 (d, 1H, C(6)H), 9.75 (s, 1H, C(5)H); ms: m/e 332/334 (M^+).

Anal. Calcd. for $C_{15}H_{13}ClN_4O_3 \cdot 1H_2O$: C, 51.36; H, 4.31. Found: C, 51.70; H, 4.31.

8-Nitro-10-methyl-3-(N-methylcarbamoylmethyl)pyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(8c)***.

A mixture of 2 g of **5c**, 4 ml of DAD, and 1 ml of sulfolane was stirred at

150° (oilbath) under nitrogen for 16 hours. The work-up was similar to that of **8a**, yielding 0.76 g (70%) pure **8c**, yellow needles, mp >350°; nmr (90 MHz, trifluoroacetic acid): δ 3.03 (d, 3H, $CONCH_3$), 4.67 (s, 3H, $N(10)CH_3$), 5.07 (s, 2H, $N(3)CH_2$), 7.62 (br s, 1H, CONH), 8.73 (s, 2H, C(6)H, C(7)H), 9.26 (s, 1H, C(9)H), 9.87 (s, 1H, C(5)H); ms: m/e 343 (M^+).

Anal. Calcd. for $C_{15}H_{13}N_5O_5$: C, 52.48; H, 3.82. Found: C, 52.36; H, 4.07.

8-p-Nitrophenyl-10-methyl-3-(N-methylcarbamoylmethyl)pyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(8d)***.

A mixture of 1 g of **5d**, 1.5 ml of DAD, and 0.5 ml of sulfolane was stirred at 150° (oilbath) under nitrogen for 4 hours. The work-up was similar to that of **8c**, yielding 0.41 g (62%) of yellow needles, mp >350°; nmr (90 MHz, trifluoroacetic acid): δ 3.05 (d, 3H, $CONCH_3$), 4.66 (s, 3H, $N(10)CH_3$), 5.09 (s, 2H, $N(3)CH_2$), 7.63 (br s, 1H, CONH), 8.06 (d, 2H, ArH), 8.32 (d, 1H, C(7)H), 8.42-8.70 (m, 4H, ArH, C(6)H, C(9)H), 9.82 (s, 1H, C(5)H); field desorption ms: m/e 419 (M^+).

Anal. Calcd. for $C_{21}H_{17}N_5O_5$: C, 60.14; H, 4.09. Found: C, 59.90; H, 3.91.

8-Dimethylamino-10-methyl-3-(N-methylcarbamoylmethyl)pyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(8g)***.

A mixture of 0.50 g of **8a** and 6 ml of 40% aqueous dimethylamine was stirred at reflux for 2 hours. After cooling, the solid was collected on a filter, washed with water, and recrystallized from DMSO to give 0.46 g (85%) of yellow plates, mp 347-349° dec.; nmr (90 MHz, trifluoroacetic acid): δ 3.03 (br s, 3H, $CONCH_3$), 3.46 (s, 6H, $N(CH_3)_2$), 4.23 (s, 3H, $N(10)CH_3$), 5.04 (s, 2H, $N(3)CH_2$), 6.86 (br s, 1H, C(9)H), 7.42 (br d, 1H, C(7)H), 7.57 (br s, 1H, CONH), 8.02 (d, 1H, C(6)H), 9.04 (s, 1H, C(5)H); field desorption ms: m/e 341 (M^+).

Anal. Calcd. for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61. Found: C, 59.51; H, 5.85.

Refluxing a mixture of **8b** and 40% aqueous dimethylamine for 10 hours gave the same product, although in lower yield (65%).

8-Chloro-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(9b)***.

A solution of 0.40 g of **8b** in 15 ml of concentrated hydrochloric acid was stirred at reflux for 12 hours. After cooling, the mixture was neutralized with 6*N* sodium hydroxide. The solid was collected on a filter, washed with water, and recrystallized from DMSO to give 0.27 g (70%) of yellow plates, mp 330-330.5° dec.; nmr (90 MHz, DMSO-d_6): δ 4.04 (s, 3H, $N(10)CH_3$), 4.55 (s, 2H,

$N(3)CH_2$), 7.61 (dd, 1H, C(7)H), 8.08 (d, 1H, C(9)H), 8.25 (d, 1H, C(6)H), 9.09 (s, 1H, C(5)H); field desorption ms: m/e 319/321 (M^+).

Anal. Calcd. for $C_{14}H_{10}ClN_3O_4.1H_2O$: C, 49.79; H, 3.58. Found: C, 49.58; H, 3.32.

8-Nitro-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(9c)***.

A solution of 0.25 g of **8c** in 8 ml of concentrated hydrochloric acid was stirred at reflux for 12 hours. After cooling, the solution was filtrated and poured on crushed ice. The solid was collected on a filter, washed with water and recrystallized from DMSO to give 0.18 g (75%) of yellow needles, mp 320--324° dec; nmr (90 MHz, DMSO-d_6): δ 4.15 (s, 3H, $N(10)CH_3$), 4.56 (s, 2H, $N(3)CH_2$), 8.26 (br d, 1H, C(7)H), 8.47 (d, 1H, C(6)H), 8.63 (br s, 1H, C(9)H), 9.16 (s, 1H, C(5)H); field desorption ms: m/e 330 (M^+).

Anal. Calcd. for $C_{14}H_{10}N_4O_6$: C, 50.91; H, 3.05. Found: C, 50.67; H, 2.79.

8-p-Nitrophenyl-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(9d)***.

A mixture of 0.35 g of **8d** and 10 ml of concentrated hydrochloric acid was stirred at 100° (oilbath) for 24 hours. After cooling, the solid was collected on a filter, washed with water, and recrystallized from DMSO to give 0.28 g (76%) of yellow needles, mp 348-350° dec.; nmr (90 MHz, DMSO-d_6): δ 4.17 (s, 3H, $N(10)CH_3$), 4.54 (s, 2H, $N(3)CH_2$), 7.96 (d, 1H, C(7)H), 8.13-8.47 (m, 6H, ArH, C(6)H, C(9)H), 9.12 (s, 1H, C(5)H); field desorption ms: m/e 406 (M^+).

Anal. Calcd. for $C_{20}H_{14}N_4O_6.2H_2O$: C, 54.30; H, 4.10. Found: C, 54.11; H, 4.29.

8-Dimethylamino-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(9g)***.

0.42 g of **8g** was hydrolysed as described for **8b**. Recrystallization of the product from DMSO yielded 0.39 g (92%) of yellow prisms, mp 305-308° dec.; nmr (300 MHz, DMSO-d_6): δ 3.27 (s, 6H, $N(CH_3)_2$), 4.04 (s, 3H, $N(10)CH_3$), 4.47 (s, 2H, $N(3)CH_2$), 6.66 (d, 1H, C(9)H), 7.13 (dd, 1H, C(7)H), 7.99 (d, 1H, C(6)H), 8.77 (s, 1H, C(5)H); field desorption ms: m/e 328 (M^+).

Anal. Calcd. for $C_{16}H_{16}N_4O_4.1H_2O$: C, 55.48; H, 5.24. Found: C, 55.66; H, 5.10.

8-Amino-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione ***(9h)***.

A solution of 1.5 g of 8c in 10 ml of concentrated hydrochloric acid was

stirred at room temperature and 1.0 g of tin powder was added in small portions. The mixture was stirred at reflux for 48 hours. After cooling, the solid was collected on a filter, washed with 1*N* hydrochloric acid and water, and recrystallized from DMSO to give 0.96 g (73%) of yellow needles, mp >325° dec.; nmr (90 MHz, DMSO-d_6): δ 3.84 (s, 3H, N(10)CH_3), 4.52 (s, 2H, N(3)CH_2), 6.68 (br s, 1H, C(9)H), 6.82 (br d, 1H, C(7)H), 7.13 (br s, 2H, NH_2), 7.75 (d, 1H, C(6)H), 8.53 (s, 1H, C(5)H); field desorption ms: m/e 300 (M^+).

Anal. Calcd. for $C_{14}H_{12}N_4O_4$: C, 56.00; H, 4.03. Found: C, 55.70; H, 4.28.

*8-*p*-Aminophenyl-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione* ***(9i)***.

A solution of 63 mg of **8d** in 2 ml of concentrated hydrochloric acid was stirred at room temperature and 38 mg of tin powder was added. The mixture was stirred at reflux for 20 hours and worked up as described for **9h** yielding 36 mg (61%) of red needles, mp 313-315° dec.; nmr (90 MHz, DMSO-d6): δ 4.11 (s, 3H, N(10)CH_3), 4.52 (s, 2H, N(3)CH_2), 6.71 (d, 2H, ArH), 7.53-7.98 (m, 4H, ArH, C(7)H, C(9)H), 8.11 (d, 1H, C(6)H), 8.93 (s, 1H, C(5)H); field desorption ms: m/e 376 (M^+).

Anal. Calcd. for $C_{20}H_{16}N_4O_4 \cdot 1H_2O$: C, 60.91; H, 4.60. Found: C, 60.66; H, 4.83.

*8-(*p*-Dimethylaminophenyl)azo-10-methyl-3-carboxymethylpyrimido[4,5-b]quinoline-2,4(3H,10H)-dione* ***(10)***.

A mixture of 1.0 g of **9h** and 20 ml of concentrated hydrochloric acid was stirred at -5° and a solution of 1.5 g of sodium nitrite in 10 ml of water was added dropwise. Stirring was continued for 45 minutes after which excess nitric acid was destroyed with urea. While the temperature was kept between -5° and 0°, a solution of 0.5 ml of *N,N*-dimethylaniline in 5 ml of concentrated hydrochloric acid was added dropwise, after which stirring was continued for 3 hours. Next the pH was adjusted to 6 with sodium acetate and the mixture was allowed to stand overnight at 5°. The solid was collected on a filter, washed with water and ethanol, and recrystallized from DMSO to give 1.0 g (64%) of green-purple needles, mp >350°; nmr (300 MHz, DMSO-d_6): δ 3.12 (s, 6H, N(CH_3)$_2$), 4.15 (s, 3H, N(10)CH_3), 4.49 (s, 2H, N(3)CH_2), 6.89 (d, 2H, ArH), 7.89 (m, 3H, ArH,(7)H), 8.17 (s, 1H, C(9)H), 8.31 (d, 1H, C(6)H), 9.07 (s, 1H, C(5)H); field desorption ms: m/e 432 (M^+).

Anal. Calcd. for $C_{22}H_{20}N_6O_4 \cdot 2H_2O$: C, 56.40; H, 5.16. Found: C, 56.72; H, 5.41.

3.4 REFERENCES AND NOTES

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[4] Synthesis of compounds **8** has been rationalized in terms of the initial addition of one molecule of DAD to **5**, followed by cyclization with concomitant elimination of 6-methylamino-5-(1,2-bisethoxycarbonylhydrazino)-3-(*N*-methylcarbamoylmethyl)uracil. This compound has however never been isolated [3]. In the synthesis of **8** we were able to isolate a compound, which analytical data suggest the structure 6-methylamino-5,5-bis(1,2-bisethoxycarbonylhydrazino)-3-(*N*-methylcarbamoylmethyl)uracil (Figure 1), white needles, mp 200-202°; nmr (90MHz, DMSO-d_6): δ 1.13 (t, 6H, 2xethyl CH_3), 1.22 (t, 6H, 2x ethyl CH_3), 2.56 (d, 3H, $CONCH_3$), 2.89 (br d, 3H, $C(6)NCH_3$), 3.73-4.37 (m, 10H, $N(3)CH_2$, 4x ethyl CH_2); ms: only signals from thermolysis products were observed. Anal. Calcd. for $C_{20}H_{32}N_8O_{11}$: C, 42.85; H, 5.75. Found: C, 43.01; H, 5.66.

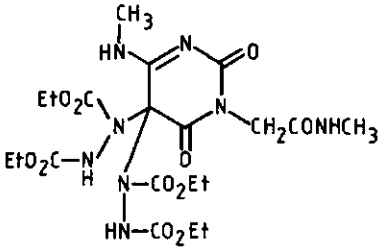

Figure 1.

These findings suggest a cyclization mechanism, in which <u>two</u> molecules of DAD are involved instead of one, as postulated earlier.

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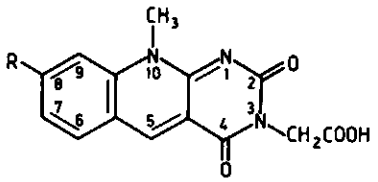
4 THE HALF-WAVE POTENTIALS OF 8-SUBSTITUTED 5-DEAZAFLAVINS. POLAROGRAPHIC DETERMINATION OF THE DISSOCIATION CONSTANTS OF SOME 8-SUBSTITUTED 5-DEAZAFLAVOSEMIQUINONES

4.1 INTRODUCTION

5-Deazaflavin has been applied as a photocatalyst in the reduction of a wide variety of biological redox systems [1,2]. Its application, however, has serious drawbacks since continuous UV irradiation is required to drive the process and the energy of the required wavelength (300-400 nm) destroys the protein with time. To avoid photodestruction of the protein we have synthesized a number of 5-deazaflavins **1a-g**, which feature a chromophoric group at the C(8)



1	R
a	Cl
b	NO_2
c	p-NO_2-C_6H_4
d	$(CH_3)_2N$
e	NH_2
f	p-NH_2-C_6H_4
g	p-$(CH_3)_2N$-C_6H_4-N=N

1a-g

position of the 5-deazaisoalloxazine skeleton, causing the absorption maximum to undergo a red shift, and a carboxymethyl group at the N(3) position increasing the solubility of 5-deazaflavins in aqueous media, in which photoreduction of redox enzymes is carried out [3,4].

Because 8-hydroxy-5-deazaflavin has been found in nature as part of F-420, *i.e.* the coenzyme of methane producing bacteria [5,6], several 8-substituted 5-deazaflavins have been synthesized as model compounds. Their UV spectroscopic

data and redox properties have been examined and found to be very sensitive to the electronic influence of the substituents [7,8].

The present report is concerned with the redox properties of a new set of 8-substituted 5-deazaflavins, *i.e.* 8-chloro-(**1a**), 8-nitro-(**1b**), 8-*p*-nitrophenyl-(**1c**), 8-dimethylamino-(**1d**), 8-amino-(**1e**), 8-*p*-aminophenyl-(1f) and 8-(*p*-dimethylaminophenyl)azo-5-deazaflavin (**1g**). The mechanism proposed [2,9,10] for the photoreduction of enzymes with 5-deazaflavin acting as a photocatalyst, implies the formation of the 5-deazaflavosemiquinone radical. Because of the high reactivity and very low redox potential of the radical, it is possible to reduce a wide variety of enzymes with catalytic quantities of 5-deazaflavin. To investigate the effect of the substituent at the C(8) position on the reducing power of the radical, we wanted to determine the half-wave potentials ($E_{\frac{1}{2}}$) of our 5-deazaflavins by differential pulse polarography.

In addition, the substituent effects on the dissociation contants of the 5-deazaflavosemiquinones have been determined from these polarographic data.

4.2 RESULTS AND DISCUSSION

Determination of $E_{\frac{1}{2}}$ for the radical formation step of 5-deazaflavins containing a reducible group at C(8) as in **1b,c,g** is not possible because reduction of the group at C(8) takes place at a less negative potential than reduction of the 5-deazaisoalloxazine skeleton. This is exemplified by comparison of the electrochemical reduction of **1b** and **1e** at different pH values (Table I).

*Table I Comparison of half-wave potentials of 5-deazaflavins **1b** and **1e** at different pH values (in mV vs NHE)*

pH / Comp.	1.90	7.00	9.00
1b	160	- 165	- 260
	- 435	- 835	- 840
	- 710	- 1005	- 1070
1e	- 715	- 1000	- 1060

As can be seen from Table I, the polarogram of **1b** shows two peaks at less negative potential corresponding to the reduction of the nitro group in two steps, followed by a peak at the same potential as obtained for **1e**. The same reduction sequence is observed with **1c** and **1g**, respectively, *i.e.* primarily reduction of the nitro and azo function, respectively, followed by reduction of the 5-deazaisoalloxazine skeleton with the yet reduced substituent at C(8).

$E_{1/2}$ values for compounds **1a,d,e,f** are obtained directly (Table II). The results show the large effect of the substituent at C(8) on the $E_{1/2}$ value of the 5-deazaflavin/5-deazaflavosemiquinone couple.

Table II Half-wave potentials ($E_{1/2}$) at different pH values, standard potentials (E_0) and pK_1 and pK_2 values of 5-deazaflavins

Compd.	pH	$E_{1/2}$*, mV vs.NHE	E_0*, mV vs.NHE	pK_1	pK_2
1a	1.90	-510	-395	1.0±0.2	5.9±0.2
	7.00	-740			
	9.00	-745			
1d	1.90	-775	-615	2.7±0.3	9.3±0.8
	7.00	-1030			
	9.00	-1135			
1e	1.90	-715	-581	2.2±0.2	8.2±0.3
	7.00	-1000			
	9.00	-1060			
1f	1.34	-635	-513	2.0±0.2	7.5±0.2
	6.22	-880			
	7.00	-915			
	8.14	-950			
	9.65	-960			

* ± 10 mV

The $E_{1/2}$ values shift to more negative values with increasing electron donating character of the substituents. A similar effect has been found in studies on the rate of reduction and reoxidation of 8-substituted 5-deazaflavins [7c]. It was also found that the $E_{1/2}$ values are pH dependent (Table II). This can be explained [11] by the electrode reaction represented by eq 1, in

which "dFlox" stands for oxidized 5-deazaflavin and "HdFl$^{\cdot}$" for 5-deazaflavosemiquinone, protonated at N(1).

$$dFlox + H^+ + 1e^- \longrightarrow HdFl^{\cdot} \qquad (1)$$

The Nernst relationship of this redox couple is expressed in eq 2.

$$E = E_0 + \frac{RT}{F} \ln \frac{[dFlox]\,[H^+]}{[HdFl^{\cdot}]} \qquad (2)$$

Both the oxidized and semiquinone species have a dissociation equilibrium

$$HdFlox^+ \underset{<}{\overset{K_1}{\rightleftharpoons}} H^+ + dFlox \qquad HdFl^{\cdot} \underset{<}{\overset{K_2}{\rightleftharpoons}} H^+ + dFl^{\cdot -}$$

The mass action expressions are given in eq 3, with

$$K_1 = \frac{[H^+]\,[dFlox]}{[HdFlox^+]} \qquad K_2 = \frac{[H^+]\,[dFl^{\cdot -}]}{[HdFl^{\cdot}]} \qquad (3)$$

K_1 and K_2 being dissociation constants. Activity effects are neglected.

According to the dissociation equilibria in eq 3 both the oxidized and semiquinoide substance can be present in the protonated and deprotonated form. This leads to the expressions in eq 4 for the total concentrations of the oxidized and one-electron reduced species,

$$[dFlox]_t = [dFlox] + [HdFlox^+] \qquad [HdFl^{\cdot}]_t = [HdFl^{\cdot}] + [dFl^{\cdot -}] \qquad (4)$$

Values of the concentrations [dFlox] and [HdFl$^{\cdot}$] in eq 2 are obtained from eq 3 and eq 4,

$$[dFlox] = \frac{K_1[dFlox]_t}{K_1 + [H^+]} \qquad [HdFl^{\cdot}] = \frac{[HdFl^{\cdot}]_t[H^+]}{K_2 + [H^+]} \qquad (5)$$

Substitution of eq 5 into eq 2 leads to eq 6 and eq 7,

$$E = E_0 + \frac{RT}{F} \ln \frac{[\mathrm{dFlox}]_t}{[\mathrm{HdFl}^{\cdot}]_t} + \frac{RT}{F} \ln \frac{K_1([H^+] + K_2)}{K_1 + [H^+]} \qquad (6)$$

$$E = E_{1/2} + \frac{RT}{F} \ln \frac{[\mathrm{dFlox}]_t}{[\mathrm{HdFl}^{\cdot}]_t} \text{ with } E_{1/2} = E_0 + \frac{RT}{F} \ln \frac{K_1([H^+] + K_2)}{K_1 + [H^+]} \qquad (7)$$

where E_0 in eq 6 is the pH independent standard reduction potential of the redox couple dFlox/HdFl$^{\cdot}$ and $E_{1/2}$ in eq 7 is the half-wave potential measured for a given 5-deazaflavin and is pH dependent. As can be seen from eq 7, $E_{1/2}$ is not only defined by E_0 and $[H^+]$, but also by the dissociation constants K_1 and K_2. This leads to the conclusion that measurement of $E_{1/2}$ as function of the hydrogen ion concentration provides a method for calculating the dissociation constant K_2. This is exemplified by calculation of K_2 of 5-deazaflavin **1f**. It is easy to understand that eq 7 reduces to eq 8a-c for restricted hydrogen ion concentrations as indicated and with the reasonable assumption that K_1 is much larger than K_2.

$$E_{1/2} = E_0 + \frac{RT}{F} \ln \frac{K_1[H^+]}{K_1 + [H^+]} \qquad [H^+] \cong K_1 \text{ or } [H^+] > K_1 \qquad (8a)$$

$$E_{1/2} = E_0 + \frac{RT}{F} \ln [H^+] \qquad K_1 >> [H^+] >> K_2 \qquad (8b)$$

$$E_{1/2} = E_0 + \frac{RT}{F} \ln ([H^+] + K_2) \qquad [H^+] \cong K_2 \text{ or } [H^+] < K_2 \qquad (8c)$$

From measurements of $E_{1/2}$ at a pH value below pK_1 (the values of pK_1 have been determined spectrophotometrically) and use of eq 8a, a value for E_0 of -512 mV has been calculated for 5-deazaflavin **1f**. To calculate K_2, $E_{1/2}$ is measured at such a pH value that eq 8c can be used. However, as K_2 is unknown, it is difficult to choose the appropriate pH beforehand. By trial and error we have found that in case of **1f** the $E_{1/2}$ value obtained at pH 9.65 differs distinctly from the value calculated according to eq 8b. This is certainly caused by the participation of the dissociation of the 5-deazaflavosemiquinone in the overall electrode reaction, eq 8c being valid as a result. Applying now 8c we have calculated a pK_2 value of 7.6 for **1f**.

Using the measured and calculated values for pK_1, E_0 and pK_2 we have plotted calculated $E_{1/2}$ values against pH according to eq 7 (Figure 1).

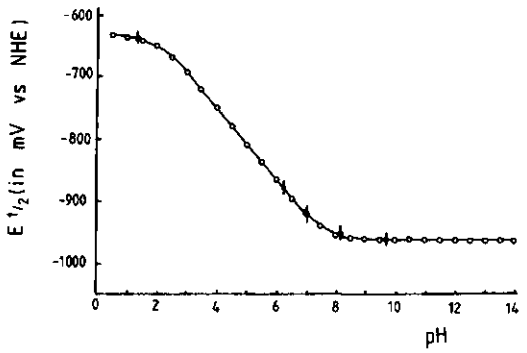


Figure 1. The pH dependence of the half-wave reduction potential ($E_{1/2}$) of 5-deazaflavin ***1f****; (0) calculated $E_{1/2}$ values; (●) experimentally determined $E_{1/2}$ values.*

As can be seen from Figure 1, the experimentally determined $E_{1/2}$ values are well in line with the calculated $E_{1/2}$ vs pH plot showing the validity of our theoretical approach. A more accurate calculation of pK_2, taking all experimentally determined $E_{1/2}$ values into account, finally leads to a value of 7.5. The same procedure has been applied for the 5-deazaflavins **1a,d,e**. The results are shown in Table II.

Since the pK_2 value of the unsubstituted 5-deazaflavin is reported to be very divergent (pK_2 = 5 [9] and pK_2 = 8 [12]), we wanted to establish more accurately its value from a possible correlation between the pK_2 values of compounds **1a,d,e,f** and their respective σ_p values. However, the σ_p value of the *p*-aminophenyl substituent is unknown. As it has been demonstrated [13] that there exists a relationship between reduction potentials and sigma values in organic systems, we determined the σ_p value of that substituent by the relationship found between the E_0 values of compounds **1a,d,e** and their respective σ_p values [14] (Figure 2).

From this relationship we calculated a σ_p (*p*-aminophenyl) value of -0.34. The correlation of the σ_p values of the four substituents with pK_2 is shown in Figure 2, from which we could derive a pK_2 value for the unsubstituted 5-deazaflavin of 6.5 ± 0.2.

As mentioned in the literature [12], kinetic $E_{1/2}$ values rather than thermodynamic potentials have been obtained due to dimerization of the respective radicals. Values for thermodynamic $E_{1/2}$ potentials are either equal to or more negative than the respective values for kinetic $E_{1/2}$ potentials, depending on the rates of radical dimerization.

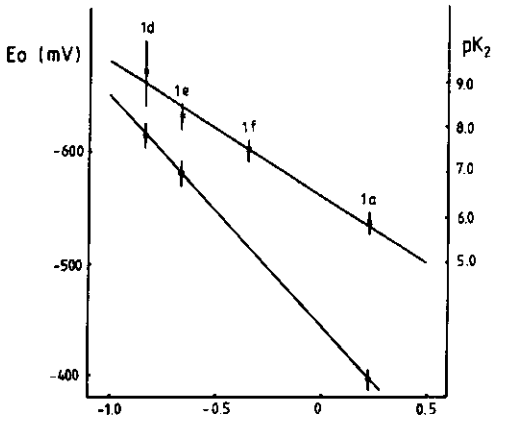


Figure 2. E_o *values of compounds* **1a,d,e** *vs.* σ_p *(●); p*K_2 *values of compounds* **1a,d,e,f** *vs.* σ_p *(X).*

Despite the fact that we have carried out our measurements at rather arbitrary concentrations of **1a-g** we still found a good relationship between $E_{1/2}$ and pH values which points to an apparent lack of a concentration dependence of $E_{1/2}$, *i.e.* the dimerization rate is low compared to the overall electrode reaction rate.

Comparison of the data in Table II with respect to the substituent at C(8) leads to the conclusion that $E_{1/2}$ potentials shift to more negative values and that pK_1 and pK_2 values increase with increasing electron donating character of the substituent. The fact that the E_0 and pK_2 values meet the Hammett relation points at the importance of through resonance in the reduction and dissociation process.

4.3 EXPERIMENTAL

Half-wave potentials for $1e^-$ reduction of 5-deazaflavins were determined by differential pulse polarography with a Quickstep instrument [15]. A saturated Ag/AgCl electrode was used as reference against the static mercury drop electrode (repeating mode). The following buffers were used: 0.1 M KCl/HCl buffer, pH 1.34 and 1.90; 0.1 M phosphate buffer, pH 6.22 and 7.00; 0.1 M Tris buffer, pH 8.14, 9.00 and 9.65. Concentrations of 5-deazaflavin solutions were varied between 30 μM and 100 μM. The temperature was 20 ± 2 °C.

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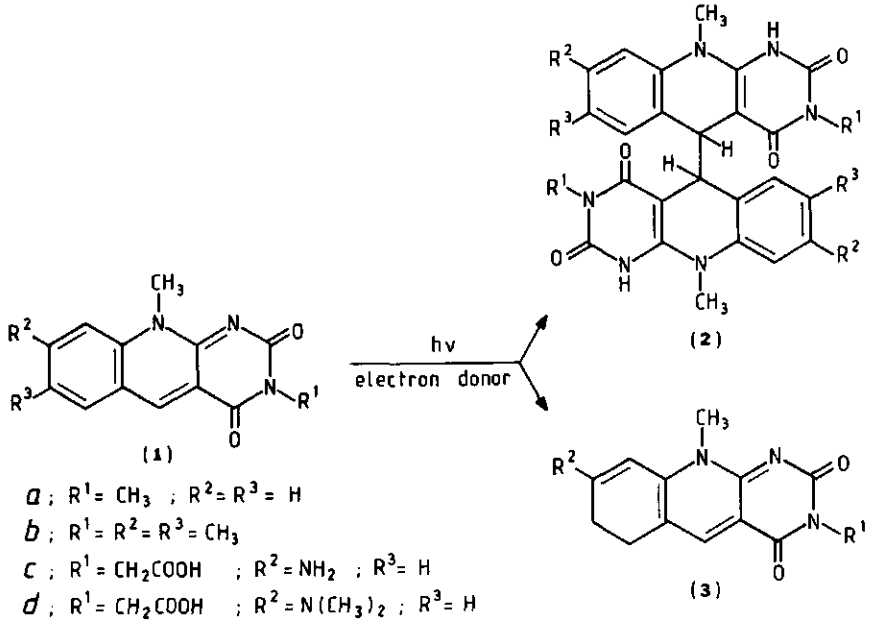
5 ON THE PHOTOREDUCTION OF 5-DEAZAFLAVINS

It has been shown [1] that prolonged illumination of 3-methyl- (**1a**) and 3,7,8 -trimethyl-5-deazaflavin (**1b**) in the presence of oxalate or ethylenediaminetetraacetate (EDTA) as electron donors leads to the formation of dimeric compounds (**2a**, **2b**) as final products of the photoreduction (Scheme 1).



(1)

a ; $R^1 = CH_3$; $R^2 = R^3 = H$
b ; $R^1 = R^2 = R^3 = CH_3$
c ; $R^1 = CH_2COOH$; $R^2 = NH_2$; $R^3 = H$
d ; $R^1 = CH_2COOH$; $R^2 = N(CH_3)_2$; $R^3 = H$

(2)

(3)

Scheme 1

Recently we reported [2] the synthesis of novel water soluble 5-deazaflavins containing at position 8 a strong electron donating group, *i.e.* 8-amino- (**1c**) or 8-dimethylamino-3-carboxymethyl-5-deazaflavin (**1d**). We were interested in the photoreduction of these compounds, since the presence of electron donating groups at position 8 might possibly change the path of the photoreduction. As source of reducing equivalents we used oxalate, EDTA, or triethanolamine (TEA).

A deoxygenated solution of (**1c**) in water (pH 8.0), containing a 200-fold excess of TEA, was exposed to a 20 W blue fluorescent lamp (Sylvania F20T12-B)

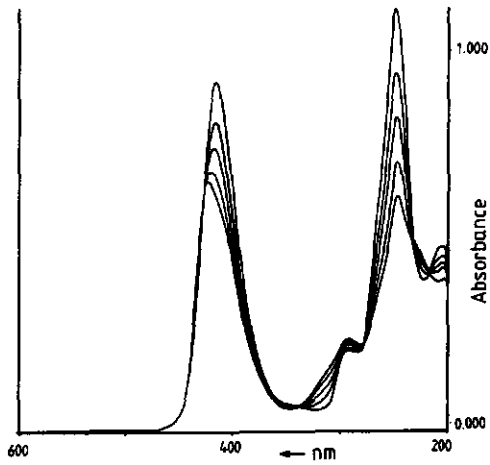


Figure 1. Photoreduction of (1c) ($2.0x10^{-5}$ mol dm^{-3}) by TEA ($4x10^{-3}$ mol dm^{-3}).

Spectra recorded after spectrum 5 have been omitted because the isosbestic points were lost in these spectra apparently due to some photodecomposition appearing after prolonged illumination. The end-spectrum, however, has its first absorption maximum at 426 nm.
All spectra show at 248 nm a constant increment above the theoretical absorbance. This is caused by a concentration enhancement of TEA relative to the reference due to the deaeration procedure.

for several hours. UV/VIS spectroscopic monitoring (Figure 1) showed a decrease in the absorption maxima at 417 and 249 nm and the formation of a species with absorption maxima at 426 and 247 nm. Isosbestic points were found at 426, 340, 278, 231, and 216 nm. When the illumination was finished and air was admitted the spectrum remained unchanged. Since the photodimer, formed in the light-mediated conversion of 5-deazaflavin (**1a**) with oxalate, has its first UV maximum at 312 nm and is slowly reoxidized by air in the dark [1a,b], it is evident that the photoproduct originating from (**1c**) cannot be assigned a dimeric structure. To obtain more specific analytical data of the photoproduct the photoreduction was performed on a preparative scale by illuminating a solution of 30 mg of (**1c**) and 0.3 g of TEA in 75 ml of distilled water, which was continuously flushed with argon, for 4 days. Isolation and characterization of the photoproduct by ^{1}H- and ^{13}C-nmr spectroscopy, mass spectrometry and UV/VIS data showed that it was the dihydro derivative (**3c**). ^{1}H-nmr (300 MHz) in D_2O δ 2.51 (m, 2H, 6-CH_2), 2.75 (m, 2H, 7-CH_2), 3.56 (s, 3H, 10-NCH_3), 4.41 (s, 2H, 3-NCH_2), 5.53 (s, 1H, H-9), 7.59 (s, 1H, H-5); ^{13}C-nmr (300 MHz) in D_2O δ 26.1 (C-6), 27.3 (C-7), 33.4 (10-NCH_3), 45.4 (3-NCH_2), 88.6 (C-9), 101.9 (C-5a), 119.1 (C-4a), 132.2 (C-5), 155.1 (C-8), 157.3 (C-9a), 158.9 (C-10a), 164.8, 170.3, 176.2 (3 x CO); field desorption ms: m/e 302 (M^+); UV/VIS λmax

(borate buffer, pH 8.0), 426 (log ε 4.50), 409 (shoulder), 294 (3.95), 247 (4.18), 229 (4.14).

Although the formation of dihydro compounds has been observed in the photoreduction of a large variety of aromatic compounds [3], hydrogenation across the C6-C7 bond in 5-deazaflavin photochemistry is unprecedented.

This unexpected formation of a 6,7-dihydro compound was also observed when (**1d**) was exposed to light in the presence of TEA under identical conditions; the compound being formed was assigned the structure (**3d**). ^{1}H-nmr (300 MHz) in D_2O δ 2.57 (m, 2H, 6-CH_2), 2.68 (m, 2H, 7-CH_2), 3.14 and 3.20 (6H, 8-$N(CH_3)_2$), 3.48 (s, 3H, 10-NCH_3), 4.35 (s, 2H, 3-NCH_2), 5.09 (s, 1H, H-9), 7.44 (s, 1H, H-5); field desorption ms: m/e 330 (M^+); UV/VIS λmax (borate buffer, pH 8.0), 438 (log ε 4.67), 419 (shoulder), 303 (3.83), 251 (4.26), 231 (4.16). Elemental analysis of both (**3c**) and (**3d**) could not be achieved due to the instability of these compounds during the purification procedures.

From these results it is evident that the presence of the amino- or dimethylamino group at position 8 drastically influences the course of the photoreduction. No dimer formation occurs, but rather exclusive hydrogenation across the C6-C7 bond. It has been shown [4,5] that in roseoflavin (**1**, R^1=H, R^2=$N(CH_3)_2$, R^3=CH_3; C(5) replaced by N(5)) an intramolecular charge transfer from the 8-dimethylamino group to the pteridine moiety occurs, which is even more significant in the triplet state. It is expected that in the triplet state of (**1c**, **1d**) a similar effect would be operative, which enhances the eletrophilicity of the benzene ring and hence favours its reduction.

The use of other photoreducing agents such as oxalate and EDTA was also investigated*. Irradiation of a deoxygenated solution of (**1c**) with blue light using either oxalate or EDTA as electron donors and monitoring the absorption spectrum (Figure 2, for EDTA) showed an absorption maximum at 426 nm, typical for the formation of (**3c**). However, the low intensity of this first absorption band, the hypsochromic shift of the absorption maximum at 249 nm and the bathochromic shift of the absorption maximum at 295 nm, together with the observation of isosbestic points (452, 347, 300, and 236 nm) all show that this spectrum is completely different from that observed during the photoreduction of (**1c**) with TEA, and strongly indicates the formation of a second compound, which does not absorb above 380 nm. This compound is sensitive to air, since reoxidation to (**1c**) slowly occurred in the dark, when air was admitted to the

* Irradiation experiments carried out in the absence of added reducing agents showed that self-reduction is negligible.

solution. These spectroscopic and chemical properties point to the formation of dimer (**2c**). Similar observations were made with compound (**1d**)**. In Table 1 the ratios between the formation of dimers (**2c**, **2d**) and 6,7-dihydro compounds (**3c**, **3d**), obtained in the photoreduction of (**1c**, **1d**) with various electron donors, are summarized.

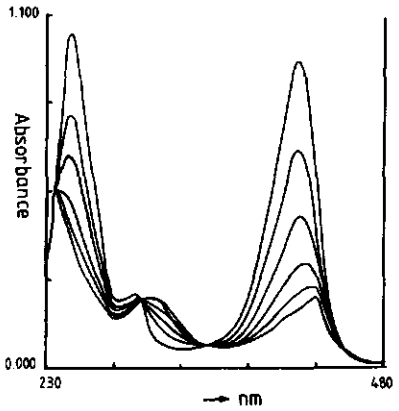


*Figure 2. Photoreduction of (**1c**) ($2.0x10^{-5}$ mol dm^{-3}) by EDTA ($4x10^{-3}$ mol dm^{-3}).*

*Table 1 The dependence of the composition of the end-product on the electron donor used in the photoreduction of (**1c**, **1d**)*

Compound	Electron donor	%(2)	%(3)
(**1c**)	EDTA	67	33
(**1c**)	oxalic acid	22	78
(**1c**)	TEA	0	100
(**1d**)	EDTA	13	87
(**1d**)	oxalic acid	0	100
(**1d**)	TEA	0	100

Table 1 shows that with the strongly electron donating dimethylamino group the reduction of the benzene ring is very strongly favoured. Since the amino group is a weaker electron donating group, the orientation to the benzene ring is less important and, as a result, dimer formation even predominates in the photoreduction of (**1c**) with EDTA.

** No attempts were made to isolate (**2c**, **2d**) from the reaction mixtures in order to achieve a more proper charaterization of these compounds, because reoxidation of (**2c**, **2d**) during the isolation procedure would be inevitable.

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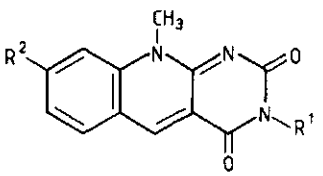
6 SPECTROSCOPY AND PHOTOCHEMISTRY OF 8-SUBSTITUTED 5-DEAZAFLAVINS

6.1 INTRODUCTION

5-Deazaflavins (dFlox's) have received much attention since the discovery that 8-hydroxy-5-deazaflavin is present in nature as part of F-420, *i.e.* the coenzyme of methane producing bacteria [1,2].

The biological relevance of dFlox as substitute for flavin in flavoproteins is limited since among the three principal types of flavoprotein activities, *i.e.* (de)hydrogenation, oxygen activation and electron transfer, only the first is retained in deazaflavoproteins, and even this to a rather limited extent [3].

Of great importance for understanding the chemistry of dFlox is the fact that from dFlox the highly reactive 5-deazaflavin radical (HdFl$^{\bullet}$) can be photochemically generated. The radical state combines high reactivity with a very low reduction potential making it possible to apply it as a photocatalyst in the reduction of a wide variety of biological redox systems [4,5]. However, its application as a photocatalyst has serious drawbacks in that it requires continuous UV irradiation to drive the process and, moreover, that the energy



	R^1	R^2
1a.	CH_2COOH	Cl
1b.	CH_2COOH	NO_2
1c.	CH_2COOH	p-NO_2-C_6H_4
1d.	CH_2COOH	$N(CH_3)_2$
1e.	CH_2COOH	NH_2
1f.	CH_2COOH	p-NH_2-C_6H_4
1g.	CH_2COOH	p-$(CH_3)_2N$-C_6H_4-N=N
1h.	CH_3	H

Figure 1. Structure of 5-deazaflavins.

of the UV light (300-400 nm) is high enough to destroy the protein with time. To avoid photodestruction of the protein we have synthesized a number of water soluble dFlox's (Figure 1, **1a-g**) with a chromophoric group at position 8 of the 5-deazaisoalloxazine skeleton causing a red shift of its first absorption maximum [6,7]. Therefore these compounds need less energy to be excited. The effect of the substituent at position 8 on the reducing power of HdFl$^{\cdot}$ was determined by measuring the half-wave potentials of the dFlox's **1a-g** by differential pulse polarography. It was found that they become more negative with increasing electron donating character of the substituent [8].

The present report deals with the effect of the substituent at C(8) on the spectroscopic properties of the dFlox's **1a-g** and is concerned with the effect of the substituent on the course of the photoreduction of dFlox in the presence of several electron donors. A short communication of a part of this investigation has been published elsewhere [9] (chapter 5).

6.2 MATERIALS AND METHODS

Spectroscopic apparatus

The ^{1}H- and ^{13}C-nmr spectra were obtained with a Bruker CXP-300 spectrometer operating at 300 MHz. Mass spectra were recorded on a Kratos MS 9 instrument. The UV-visible spectra were measured with a Beckman DU-7 or a Varian DMS 90 UV-visible spectrophotometer. Fluorescence emission and excitation spectra were obtained with an Aminco SPF-500^{TM} ratio spectrofluorometer equiped with a Hewlett Packard 9815 A calculator. Phosphorescence emission spectra were recorded on a Perkin Elmer LS-5 luminiscence spectrometer equiped with spectral correction.

Preparative photochemistry

Compounds (**1a-g**) were prepared as described previously [7]. Preparative photoreductions were carried out in a glass tube (l, 55 cm; i.d. 2 cm) equiped with a gas inlet tube. All reaction mixtures were flushed with argon, which was freed from oxygen by passage through a Chrompack gas-clean oxygen no. 7990 filter for several hours prior to irradiation and during irradiation. The light source for the photoreactions was a 20 W blue fluorescent lamp (Sylvania F20T12-B, for the spectral output see Krasna [21]). Due to the instability of the photoreduction products during purification procedures we were not able to achieve accurate yields, melting points and elemental analysis.

Photoreduction of ***1a*** *by triethanolamine (TEA)*

A solution of 18 mg of **1a** and 0.15 g of TEA in 50 ml of distilled water was irradiated overnight. The solution was freeze-dried, washed with aceton and dried *in vacuo* over $CaCl_2$ yielding as established by nmr spectroscopy a mixture of the 6,7-dihydro compound **3a** and reoxidized dimer *i.e.* **1a**; ^{1}H-nmr (300 MHz, D_2O): δ 2.12 (m, 2H, 6-CH_2), 2.28 (m, 2H, 7-CH_2), 3.87 (s, 3H, 10-NCH_3), 4.42 (s, 2H, 3-NCH_2), 5.17 (s, 1H, H-9), 7.61 (s, 1H, H-5) for **3a**; δ 3.95 (s, 3H, 10-NCH_3), 442 (s, 2H, 3-NCH_2), 7.30 (d, 1H, H-7), 7.70 (d, 1H, H-6), 7.76 (s, 1H, H-9), 8.68 (s, 1H, H-5) for **1a**; field desorption ms: m/e 319/321 (M^+ for **1a**) and 321/323 (M^+ for **3a**).

Photoreduction of ***1a*** *by oxalate*

A solution of 16 mg of **1a** and 0.1 g of oxalic acid in 75 ml of distilled water adjusted with 0.1 M NaOH to pH 8 was irradiated for 6 hours. The product was freeze-dried and analyzed without further purification; ^{1}H-nmr (300 MHz, D_2O) showed two sets of signals consistent with a 40:60 mixture of two stereoisomeric dimers **2a** (I and II): δ 2.57 (s, 3H, 10-NCH_3), 4.00 (s, 1H, H-5), 4.48 (s, 2H, 3-NCH_2), 6.30-7.20 (m, 3H, ArH) for I; δ 2.85 (s, 3H, 10-NCH_3), 3.90 (s, 1H, H-5), 4.36 (s, 2H, 3-NCH_2), 6.30-7.20 (m, 3H, ArH) for II. The mixture was contaminated by traces of **1a**.

Photoreduction of ***1d*** *by TEA*

A solution of 30 mg of **1d** and 0.3 g of TEA in 75 ml of distilled water was irradiated for 10 days. The work-up as described above yielded **3d**; ^{1}H-nmr (300 MHz, D_2O): δ 2.57 (m, 2H, 6-CH_2), 2.68 (m, 2H, 7-CH_2), 3.14 and 3.20 (6H, 8-$N(CH_3)_2$), 3.48 (s, 3H, 10-NCH_3), 4.35 (s, 2H, 3-NCH_2), 5.09 (s, 1H, H-9), 7.44 (s, 1H, H-5); field desorption ms: m/e 330 (M^+); UV/VIS λ_{max} (borate buffer, pH 8.0), 438 (log ε 4.67), 419 (shoulder), 303 (3.83), 251 (4.26), 231 (4.16).

Photoreduction of ***1e*** *by TEA*

Photoreduction of **1e** as described for **1d** with an irradiation time of 4 days yielded **3e**; ^{1}H-nmr (300 MHz, D_2O): δ 2.51 (m, 2H, 6-CH_2), 2.75 (m, 2H, 7-CH_2), 3.56 (s, 3H, 10-NCH_3), 4.41 (s, 2H, 3-NCH_2), 5.53 (s, 1H, H-9), 7.59 (s, 1H, H-5); ^{13}C-nmr (300 MHz, D_2O): δ 26.1 (C-6), 27.3 (C-7), 33.4 (10-NCH_3), 45.4 (3-NCH_2), 88.6 (C-9), 101.9 (C-5a), 119.1 (C-4a), 132.2 (C-5), 155.1 (C-8), 157.3 (C-9a), 158.9 (C-10a), 164.8, 170.3, 176.2 (3 x CO); field desorption ms: m/e 302 (M^+); UV/VIS λ_{max} (borate buffer, pH 8.0), 426 (log ε 4.50), 409 (shoulder), 294 (3.95), 247 (4.18), 229 (4.14).

Analytical photochemistry

Analytical photochemical experiments were carried out in a Hellma 193 Suprasil I cuvette. Solutions contained in this cuvette were made anaerobic by flushing with oxygen-free argon for at least 30 minutes followed by repeated cycles of evacuation and flushing with oxygen-free argon. The light source was a 20 W blue fluorescent lamp (Sylvania F20T12-B) or a 250 W slide projector lamp at the low intensity setting.

6.3 RESULTS AND DISCUSSION

6.3.1 UV-visible light absorption, fluorescence and phosphorescence data

When compared with the dFlox **1h** containing no substituent at C(8) the presence of a substituent at position 8 of the 5-deazaisoalloxazine skeleton causes a shift of the first absorption maximum in the UV spectrum, the magnitude of the shift strongly depending on the substituent (Table 1).

Table 1 UV-visible light absorption data of dFlox's in borate buffer pH 8.0

Compound	λ_{max}/nm (log ε); sh = shoulder				
1a	403 (sh)	391 (4.18)	327 (4.08)	261 (4.62)	
1b	417 (3.99)	334 (sh)	325 (4.16)	281 (sh)	261 (4.56)
1c	418 (sh)	404 (4.35)	347 (4.41)	275 (sh)	259 (4.56)
1d	442 (4.65)	310 (4.06)	265 (sh)	253 (4.69)	
1e	417 (4.65)	295 (3.93)	264 (sh)	249 (4.69)	
1f	428 (4.49)	314 (sh)	262 (4.62)		
1g	537 (4.59)	418 (4.04)	280 (sh)	251 (4.41)	
1h*	390 (4.04)	326 (4.01)	258 (4.60)		

* Reference [10]

Selected spectra are shown in Figure 2. With electron donating substituents at position 8 a moderate to large bathochromic shift of the first band is observed. This is caused by the enlargement of the tricyclic resonance (Figure 3a; [11,12]. Although electron withdrawing substituents oppose this tricyclic resonance a bathochromic shift is still observed, which in case of the nitro group even resembles that of the electron donating amino substituent! However, in case of an electron withdrawing substituent the first band is thought to be originated from a transition related to a different electron migration (Figure

3b; [13]. Based on this assumption it is understandable that an electron withdrawing substituent also causes a bathochromic shift.

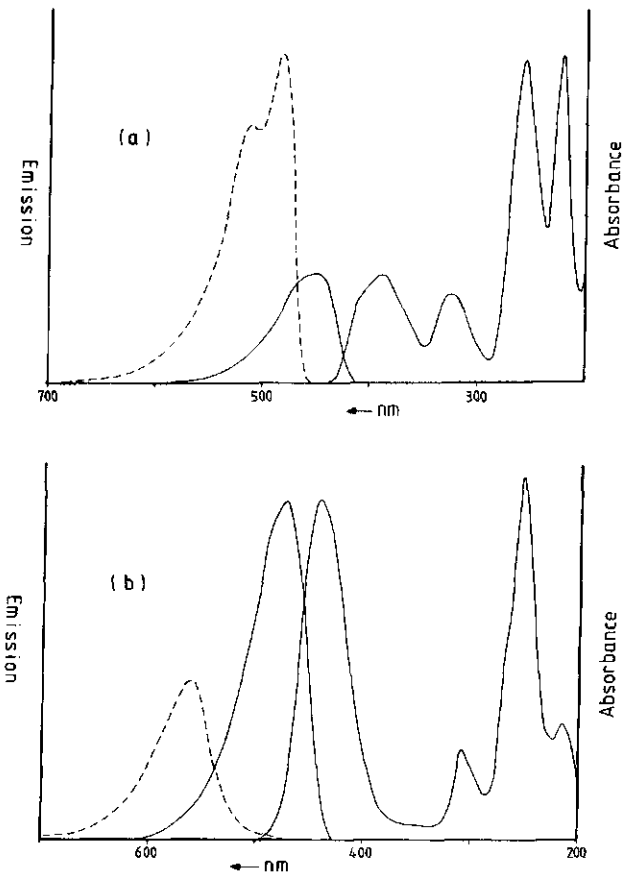


*Figure 2. The absorption (_____; borate buffer, pH 8.0; 298 K), fluorescence emission (_____; borate buffer, pH 8.0; 298 K) and phosphorescence emission (----; borate buffer, pH 8.0 - sucrose glass; 77 K) spectra of (a) **1a** and (b) **1d**.*

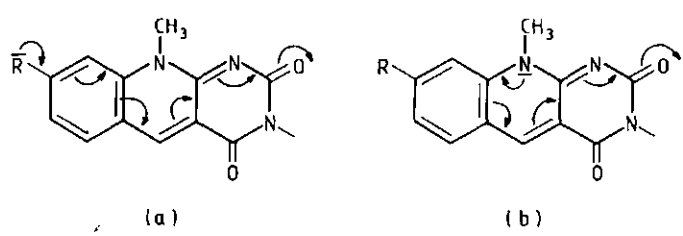


Figure 3. The electron migration for the first transition of dFlox's with (a) an electron donating and (b) an electron withdrawing group at C (8).

As reported earlier [14] the absorption spectrum of the parent compound dFlox has undergone a blue shift in comparision with flavin, but the pattern of absorption bands is very similar. This resemblance extends also to 8-substituted dFlox's and flavins as exemplified by the spectra of 5-deazaroseoflavin **1d** and roseoflavin [15]. Both show a strong band in the 250-260 nm region, a small shoulder in the 300-310 region and the red-shifted main absorption at 442 nm and 500 nm, respectively.

Spectroscopic data of fluorescence and phosphorescence emission, fluorescence excitation as well as fluorescence and phosphorescence quantum yields of compounds **1a-h** are summarized in Table 2.

Table 2 Fluorescence and phosphorescence data of dFlox's

	Fluorescence[a]			Phosphorescence[b]	
Compound	λ_{max}/nm (em.)	λ_{max}/nm (exc.)	Φ_f[c]	λ_{max}//nm (em.)	Φ_p[c]
1a	453	403 (sh), 392, 325, 259	0.068	512, 482	0.88
1b	-	-	0	540 (sh), 516, 490 (sh)	0.034
1c	-	-	0	590 (sh), 548 513	0.21
1d	474	430, 303, 261(sh), 253	0.020	561	0.011
1e	459	418, 296, 265 (sh), 250	0.45	517	0.031
1f	-	-	0	573 (sh), 535, 501	0.058
1g	-	-	0	510, 490	0.011
1h	445[d]		0.37[e]	480[d]	0.24[d]

[a] Borate buffer, pH 8.0; 298 K; quantum yields are given relative to that of 2-aminoanthracene in benzene [16,17].

[b] Borate buffer, pH 8.0 - sucrose glass; 77 K; quantum yields are given relative to that of **1h** in ethanol [14].

[c] Experimental error 10%

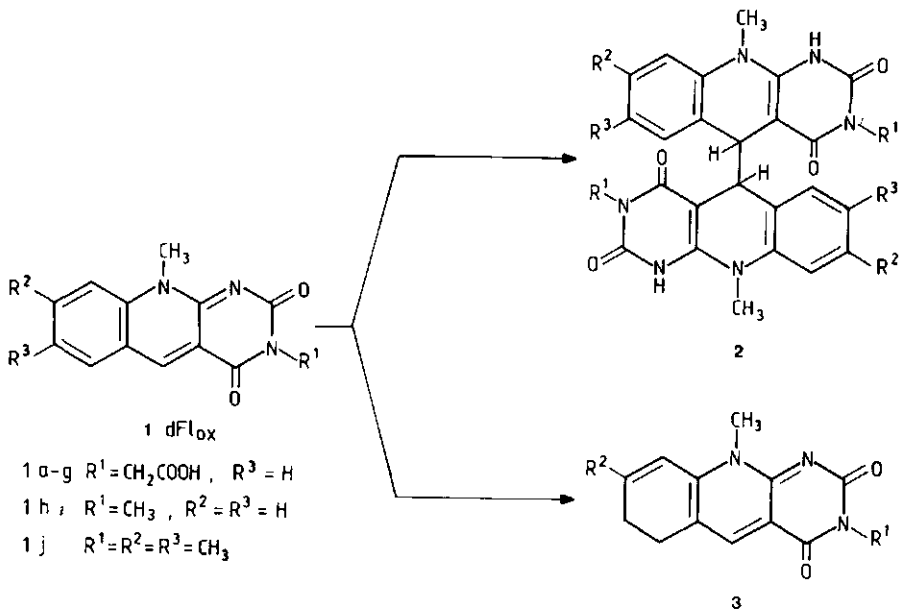
[d] Reference [10].

[e] Reference [14].

Some representative fluorescence and phosphorescence emission spectra are shown in Figure 2. Table 2 clearly shows the effect of the substituent on fluorescence and phosphorescence characteristics. A few key results are summarized: i) the maximum fluorescence red shift of 29 nm is obtained for the 8-dimethylamino compound **1d**, relative to dFlox **1h**; ii) the fluorescence quantum yield varies between 0 and 0.45; iii) the relatively low fluorescence quantum yield and the blue shifted excitation maximum of 5-deazaroseoflavin **1d** are also found for roseoflavin [15] hence showing again the spectroscopic similarity between flavins and 5-deazaflavins; iv) the phosphorescence maximum attains the maximum red shift of 81 nm for **1d** relative to **1h**; v) the phosphorescence quantum yield as compared to the phosphorescence quantum yield of **1h** varies between a value about 20 times smaller (for **1d**) and about 4 times larger (for **1a**); vi) the phosphorescence emission of **1g** seems to be originated from the isolated 5-deazaisoalloxazine ring system with a decreased phosphorescence measurement due to autoabsorption as a result.

6.3.2 Photoreduction of 5-deazaflavins

It has been reported [5,10,18,19] that prolonged illumination of 3-methyl-5--deazaflavin **1h** and 3,7,8-trimethyl-5-deazaflavin **1j** in the presence of the electron donor oxalate or ethylenediaminetetraacetate (EDTA) leads to the formation of dimeric compounds **2h**, **2j** as final products of the photoreduction (Scheme 1).



Scheme 1

In our work we found that the photoreduction of dFlox's is strongly influenced by the substituent at position 8 and the electron donor used [9]. Prolonged illumination of **1d, 1e** with blue light using TEA as electron source leads to the formation of the 6,7-dihydrocompounds **3d, 3e** and not to the dimeric species **2d, 2e**. Using EDTA instead of TEA as electron donor a mixture of **2d, 2e** and **3d, 3e** has been obtained.

In order to broaden the scope of our study on the dimer/dihydro formation we studied the photoreduction of a number of 8-substituted 5-deazaflavins **1a-g** in the presence of several electron donors.

Illumination of **1a** with blue light in the presence of oxalate, and following the reaction by UV-visible spectrometry (Figure 4a), led to a 90% decrease of the absorption at 391 nm and the formation of a set of isosbestic points at 220, 283 and 311 nm. The spectral changes are consistent with predominant formation of dimer **2a** and are not compatible with the formation of the 6,7-dihydro compound **3a** to a large extent, since **3a** would exhibit a pronounced absorption around 390 nm. The formation of **2a** is confirmed by the nmr structure assignment of the isolated photoproduct. In contrast with the 6,7-dihydro compounds dimers are reactive towards oxygen [9]. It was found that 89% of **1a** was regained on admitting air to the reaction mixture. This result unequivocally justified the conclusion that the end-product of the illumination of **1a** consisted of a mixture of **2a** (89%) and **3a** (11%). Nearly identical results were obtained when **1a** was illuminated in the presence of EDTA instead of oxalate: isosbestic points were formed at 223, 284, and 309 nm and the end-product consisted of a mixture of **2a**(91%) and **3a** (9%).

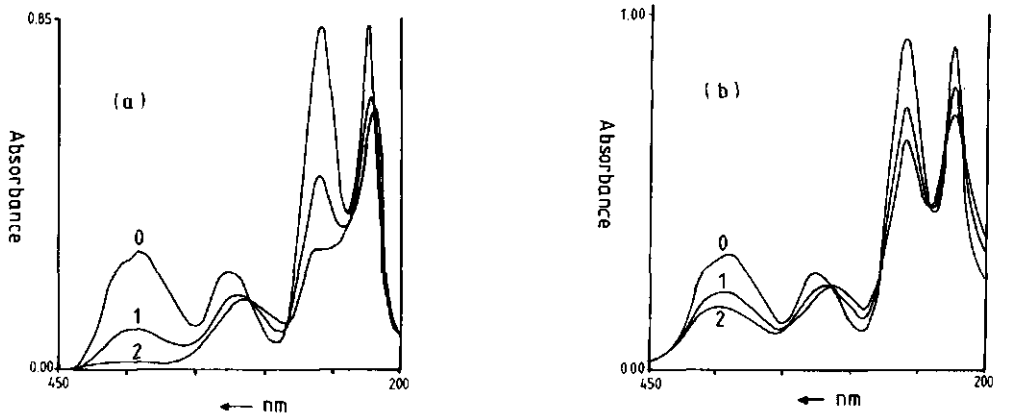


Figure 4. Photoreduction of **1a** *(2.0 x 10^{-5} mol dm^{-3}) by (a) oxalate (4 x 10^{-3} mol dm^{-3}) and (b) TEA (4 x 10^{-3} mol dm^{-3}) in borate buffer, pH 8.0.*

However, a very different result was obtained when **1a** was illuminated in the presence of TEA. Monitoring (Figure 4b) the absorption spectrum of **1a** and TEA during irradiation with blue light revealed a bathochromic shift of the first absorption band up to 5 nm together with a 48% decrease of the absorption at 391 nm. A set of isosbestic poionts was formed very divergent from that with oxalate or EDTA *i.e.* 219, 233, 242, 277, 314, and 429 nm. On admitting air to the reaction mixture reoxidation into **1a** (19%) was found. All these experimental results together with the nmr data of the photoproduct led to the conclusion that photoreduction of **1a** by TEA yields a mixture of **2a** (19%) and **3a** (81%). Products arising from a photonucleophilic substitution of the 8-chloro group by the amine [20] were not detected.

When the photoreduction of **1f** by EDTA was followed by UV-visible spectrometry (Figure 5) we observed a 94% decrease of the absorption maximum at 428 nm, the formation of isosbestic points at 236, 276, and 343 nm and a reoxidation to an extent of 90%, which is fully consistent with the formation of dimer **2f** (90%) and 6,7-dihydro compound **3f** (10%) as end-products of the photoreduction.

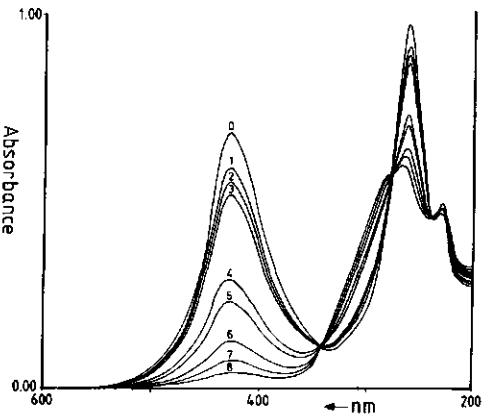


Figure 5. Photoreduction of ***1f*** *(2.0 x 10^{-5} mol dm^{-3}) by EDTA (4 x 10^{-3} mol dm^{-3}) in borate buffer, pH 8.0.*

The ratios between **2** and **3** found in the photoreduction of **1a**, **1d**, **1e**, **1f** in the presence of various electron donors are summarized in Table 3. This table shows the large effect of the electron donor on the course of the photoreduction: reduction across the C6-C7 bond is highly favoured with TEA as electron donor, and dimer formation becomes more important with oxalate

Table 3 The composition of the photochemical reaction mixture of **1** *as dependent on the electron donor used*

	EDTA		oxalate		TEA	
Compound	% **2**	% **3**	% **2**	% **3**	% **2**	% **3**
1a	91	9	89	11	19	81
1d	13	87	0	100	0	100
1e	67	33	22	78	0	100
1f	90	10				

and predominates with EDTA, except in the photoreduction of **1d** with all three electron donors in which formation of 6,7-dihydro-5-deazaflavin is favoured. Concerning the effect of the substituent on the course of the photoreduction it is clear that formation of the 6,7-dihydro compound 3 is promoted by strong electron donating substituents.

Prolonged illumination of dFlox's with a reducible substituent at position 8 (**1b**, **1c**, **1g**) by EDTA showed that all three compounds undergo reduction of the C-8 substituent prior to the reduction of the 5-deazaisoalloxazine skeleton.

Figure 6 shows the photoreduction of the 8-nitro compound **1b** with EDTA. Determination of the absorption spectrum in time revealed that the absorption at 417 nm increased and a set of isobestic points was formed at 231, 255 and 355 nm (curves 0-5, Fig. 6a). On prolonged illumination the absorption at 417 nm kept increasing and a new set of isobestic points at 233, 255, 362 and 435 nm was obtained (curves 5-8, Fig. 6b). The final spectrum was identical to that obtained on irradiation of **1e** with EDTA. These results are fully consistent with an initial two-step photoreduction of **1b** into **1e**, followed by the photoreduction of **1e**. A similar photoreduction pattern was observed on irradiation of **1c** in the presence of EDTA *i.e.* reduction of the *p*-nitrophenyl group in two distinct steps yielding the *p*-aminophenyl compound **1f**, followed by the photoreduction of **1f**.

To examine the photoreduction of **1g** by EDTA the blue fluorescent light source with its maximum output at 440 nm was replaced by a 250 W slide projector lamp in order to obtain a sufficient output of light in the absorption range of **1g**. Monitoring the absorption spectra of a mixture of **1g** and EDTA during irradiation (Figure 7) a concomitant decrease of the absorption at 537 nm and an increase of the absorption at 417 nm and the formation of a set of isosbestic points at 273, 372 and 439 nm were observed. Prolonged illumination caused spectroscopic changes (not shown), which were fully identical to those observed in the photoreduction of **1e** by EDTA. These results prove that the photoreduction of **1g** first leads to

the formation of **1e** as the sole product, followed by the formation of a mixture of **2e** and **3e** being the photoreduction products of **1e**.

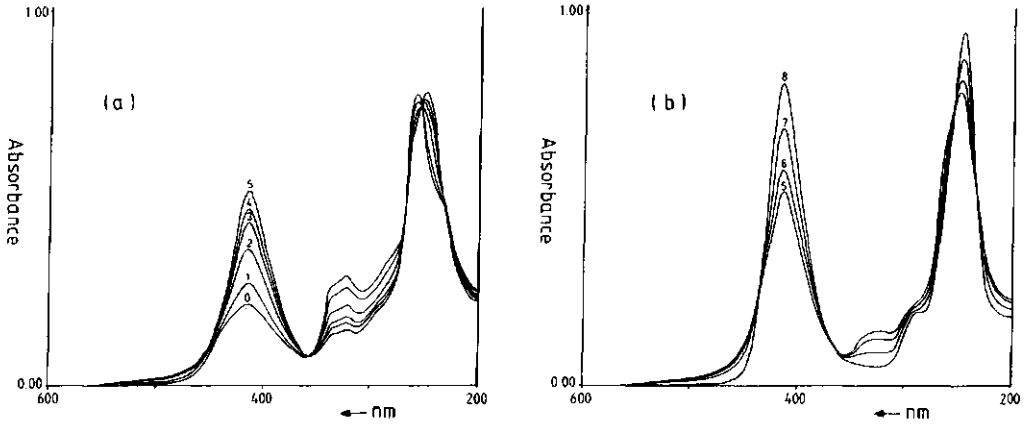


Figure 6. Two-steps (a, b) photoreduction of **1b** *(2.2 x 10^{-5} mol dm^{-3}) by EDTA (4 x 10^{-3} mol dm^{-3}) in borate buffer, pH 8.0.*

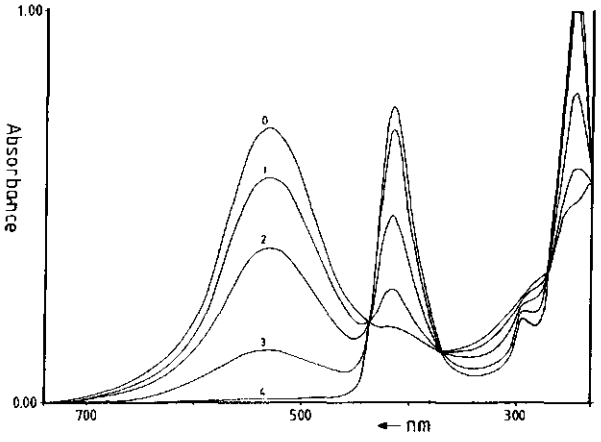


Figure 7. Photoreduction of **1g** *(1.8 x 10^{-5} mol dm^{-3}) by EDTA (4 x 10^{-3} mol dm^{-3}) in borate buffer, pH 8.0.*

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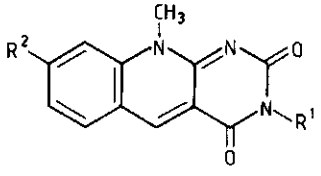
7 THE PHOTOREDUCTION OF 8-SUBSTITUTED 5-DEAZAFLAVINS AND THE 5-DEAZAFLAVIN CATALYZED PHOTOREDUCTION OF METHYL VIOLOGEN

7.1 INTRODUCTION

In context of the search for a 5-deazaflavin (dFlox), which combines a low excitation energy with a high rate of catalysis of the photoreduction of flavoenzymes, we synthesized a number of water soluble 8-substituted 5-deazaflavins (Figure 1, **1a-g**) [1]. The effect of the substituent at position 8 on the



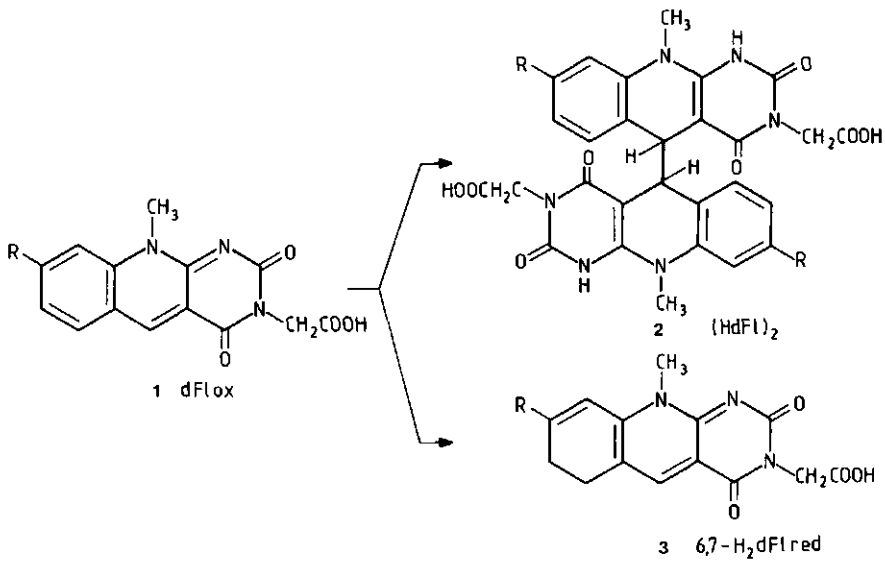
	R^1	R^2
1a.	CH_2COOH	Cl
1b.	CH_2COOH	NO_2
1c.	CH_2COOH	p-NO_2-C_6H_4
1d.	CH_2COOH	$N(CH_3)_2$
1e.	CH_2COOH	NH_2
1f.	CH_2COOH	p-NH_2-C_6H_4
1g.	CH_2COOH	p-$(CH_3)_2N$-C_6H_4-N=N
1h.	CH_3	H

Figure 1. Structures of 5-deazaflavins.

reducing power of the 5-deazaflavosemiquinone radical ($HdFl^{\cdot}$), which is proposed to be the actual reducing species in the photoreduction system [2], was determined by measuring the half-wave potentials of **1a-g** by differential pulse polarography. It was found that the half-wave potentials become more negative with increasing electron donating character of the substituent [3].

Absorption, fluorescence and phosphorescence spectra as well as fluorescence and phosphorescence quantum yields of **1a-g** appeared to be highly influenced by the substituent at position 8 of the 5-deazaisoalloxazine skeleton [4]. The course of the photoreduction of **1a-g** in the presence of electron donors was

studied. It was estabilised that the photoreduction in case of **1a,1d,1e,1f** leads to the formation of the 5,5'-dimer ($(HdFl)_2$, **2**) and/or the 6,7-dihydro-5-deazaflavin (6,7-H_2dFlred, **3**) (Scheme 1). Reduction of the C(6)-C(7) bond



Scheme 1

appeared to be promoted by strong electron donating substituents and electron donors with bulky substituents. The ratio's between **2** and **3**, determined previously [4,5], are summarized in Table 1.

*Table 1 The composition of the photochemical reaction mixture of **1** as dependent on the electron donor used*

	EDTA		oxalate		TEA	
Compound	%2	%3	%2	%3	%2	%3
1a	91	9	89	11	19	81
1d	13	87	0	100	0	100
1e	67	33	22	78	0	100
1f	90	10				

5-Deazaflavins containing a reducible substituent at position 8, *i.e.*

1b,1c,1g, exhibited reduction of the substituent prior to reduction of the 5-deazaisoalloxazine skeleton.

The present report is concerned with a study of the rate of the photoreduction of **1a,1d,1e,1f** by various electron donors and a study of the substituent effect on the photocatalyzing ability of the dFlox's in the photoreduction of methyl viologen (MV^{2+}).

7.2 RESULTS AND DISCUSSION

7.2.1 Mechanistic considerations

To measure the rate of the photoreduction we determined the change of the absorbance of the first absorption maximum (unless otherwise stated) in the presence of several electron donors as a function of time. From the logarithmic relation observed a pseudo first-order decay of dFlox was established (Table 2). This relation was found to be rather accurate for more than 95% conversion of dFlox except for the photoreduction of **1d** by both oxalate and ethylenediaminetetraacetate (EDTA) for reasons outlined below. With triethanolamine (TEA) as electron donor the photoreduction of **1e** only obeyed pseudo first-order kinetics up to 65% conversion and the photoreduction of **1d** went off too fitfully for a reproducible kinetic analysis, despite the distinct course of the reaction. This was probably due to the occurrence of a shift of the protonation

Table 2 Pseudo first-order rate constants (s^{-1}) of the photoreduction of 5-deazaflavins with various electron donors[a]

Compound	EDTA	oxalate	TEA
1a	$(2.1\pm0.1)10^{-2}$	$(1.6\pm0.1)10^{-2}$	$(2.7\pm0.1)10^{-3}$
1d	1×10^{-6}[b]	$(2.6\pm0.1)10^{-7}$[c]	
1e	$(9.1\pm0.2)10^{-4}$	$(3.2\pm0.2)10^{-6}$	$(1.4\pm0.1)10^{-4}$
1f	$(1.2\pm0.1)10^{-5}$		

a [dFlox] 2.0×10^{-5} mol dm^{-3}, [electron donor] 4×10^{-3} mol dm^{-3}, borate buffer pH 8.0.

b This value is mere indicative for the order of magnitude of the photoreduction rate.

c Pseudo second-order rate constant $(7.5\pm0.5)10^{-2}$ dm^3 mol^{-1} s^{-1}.

equilibrium of TEA (pK_a 7.8, [6]) towards the non-oxidizable protonated species during the long irradiation period.

It has been observed [7] that the rate of disappearance of dFlox only obeyes pseudo first-order kinetics up to 70% conversion during irradiation in the presence of oxalate and then decreases. Our observation that in general the photoreduction is not delayed opposes to this report and seems to indicate that the authors [7] have overlooked the formation of some 6,7-H_2dFlred, which exhibits a distinct absorbance at the monitored wavelength.

We found that pseudo first-order kinetics are not applicable to the photoreductions of **1d** by both oxalate and EDTA (Figure 2). The photoreduction of **1d**

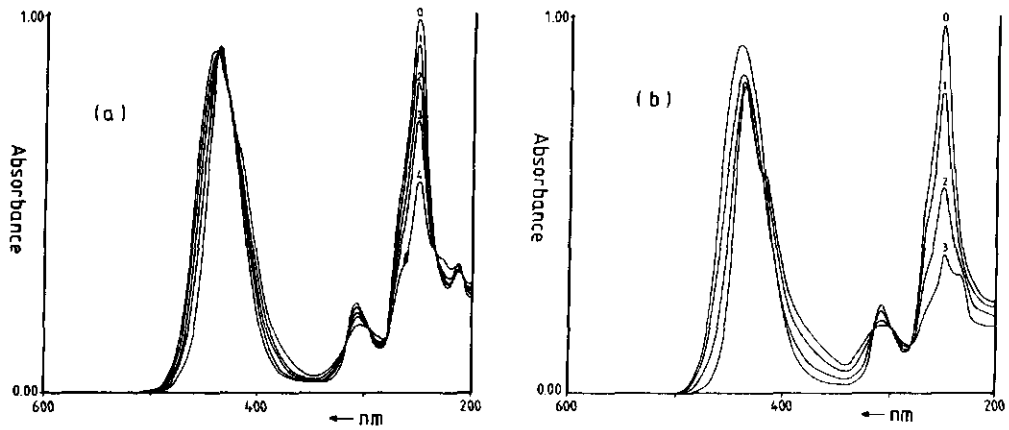


Figure 2. (a) Photoreduction of **1d** *(2.0 x 10^{-5} mol dm^{-3}) by oxalate (4 x 10^{-3} mol dm^{-3}) in borate buffer, pH 8.0. Curves 0-4 correspond to irradiation times of 0, 89, 184, 278 and 444 hours respectively.*
(b) Photoreduction of **1d** *(2.0 x 10^{-5} mol dm^{-3}) by EDTA (4 x 10^{-3} mol dm^{-3}) in borate buffer, pH 8.0. Curves 0-3 correspond to irradiation times of 0, 30, 53 and 77 hours respectively.*

by oxalate exhibited, when spectra were recorded at various irradiation times, the same spectral changes as were observed in the photoreduction of **1d** with TEA. Since the latter reaction leads to the formation of **3d** only [5], it is justified to conclude that the oxalate-mediated photoreduction of **1d** also yields only **3d**. Supporting evidence was obtained from the fact that on admitting air to the reaction mixture reoxidation into **1d**, which points to the presence of dimer **2d** [5], was not observed. This result can be expressed in

quantitave terms in Eq. 1 and 2, being valid at each moment during the photoreduction of **1d** by oxalate.

$$[\text{dFlox}] = [\text{dFlox}]_0 - [6,7\text{-H}_2\text{dFlred}] \quad (1)$$

$$A_{252} = \varepsilon_{252}^{ox}\,[\text{dFlox}] + \varepsilon_{252}^{red}\,[6,7\text{-H}_2\text{dFlred}] \quad (2)$$

A_{252} is the absorbance at 252 nm and ε_{252}^{ox} and ε_{252}^{red} are the molar extinction coefficients of **1d** and **3d** respectively. Combination of Eq. 1 and 2 leads to Eq. 3, in which A_{252}^{∞} is the absorbance at 252 nm at infinite time. When A_{252} was plotted against time a flat S-shaped curve was obtained (Figure 3); this S-

$$A_{252} = (\varepsilon_{252}^{ox} - \varepsilon_{252}^{red})\,[\text{dFlox}] + A_{252}^{\infty} \quad (3)$$

shaped form is typical for the involvement of autocatalysis in the dihydro formation [8]. In our case the pseudo first-order decay of **1d** yielding **3d**

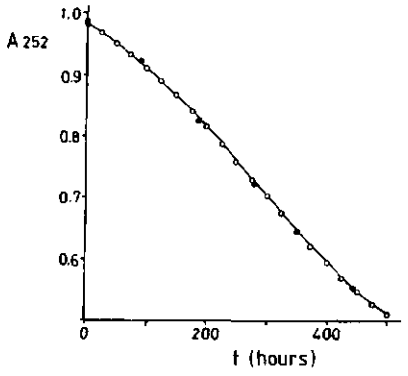


Figure 3. The partly autocatalytic decay of **1d** *in the photoreduction by oxalate as found expression in the A_{252}-dependence on time; (O) values calculated according to Eq. 3 and 8 with $k_1 = 2.6 \times 10^{-7}\ s^{-1}$ and $k_2 = 7.5 \times 10^{-2}\ dm^3\ mol^{-1}\ s^{-1}$; (●) experimentally determined values.*

according to Eq. 4 is thought to be accompanied by a competing reaction, involving **3d** acting as a photocatalyst as expressed in Eq. 5.

$$\text{dFlox} + C_2O_4^{2-} + 2H^+ \xrightarrow[k_1]{h\nu} 6,7\text{-H}_2\text{dFlred} + 2CO_2 \quad (4)$$

$$dFlox + 6,7\text{-}H_2dFlred + C_2O_4^{2-} + 2H^+ \xrightarrow[k_2]{h\nu} 2\ 6,7\text{-}H_2dFlred + 2CO_2 \qquad (5)$$

The rate expression for the decay of **1d** is given by Eq. 6, the concentration of oxalate being in excess.

$$\frac{d[dFlox]}{dt} = -\left(k_1\,[dFlox] + k_2\,[dFlox]\,[6,7\text{-}H_2dFlred]\right) \qquad (6)$$

Substitution of Eq. 1 in Eq. 6 leads to Eq. 7, which on integration gives Eq. 8.

$$\frac{d[dFlox]}{dt} = -\left\{\left(k_1 + k_2\,[dFlox]_0\right)[dFlox] - k_2\,[dFlox]^2\right\} \qquad (7)$$

$$[dFlox] = \frac{\left(k_1 + k_2\,[dFlox]_0\right)[dFlox]_0\, e^{-\left(k_1 + k_2[dFlox]_0\right)t}}{k_1 + k_2\,[dFlox]_0\, e^{-\left(k_1 + k_2[dFlox]_0\right)t}} \qquad (8)$$

By trial and error using Eq. 3 and 8 we were able to estimate values for k_1 and k_2 well in line with the calculated and experimentally determined absorbances at 252 nm (Figure 3, Table 2).

Also in the photoreduction of **1d** with EDTA an autocatalytic effect was found: when the time-dependent concentrations of **1d**, calculated from the recorded spectra (Figure 2b), were plotted against time a flat S-shaped curve was obtained again. The decrease of the absorbance at 442 nm, the loss of the isosbestic point in this region and the observed small formation of **1d** after reoxidation with air are consistent with the formation of **2d** next to **3d**. The formation of this mixture complicates the derivation of the rate law, since it is impossible to express the concentrations of **2d** and **3d** into [dFlox], required to derive a rate expression for the decay of **1d** like Eq. 7. Therefore, from our observations (Figure 2b) it is only justified to conclude that the photoreduction of **1d** by EDTA is several times faster compared to the photoreduction of **1d** by oxalate.

The data about product composition (Table 1) and photoreduction rates (Table 2) both in relation to the substituent present at position 8 and to the nature of the electron donor used in the photoreduction of dFlox offer new elements for elucidating the mechanism of 5-deazaflavin photochemistry. Particularly the element of steric interference appears to be important as will be shown below.

In dye photochemistry the common mechanism is the $1e^-$-transfer to yield the dye semiquinone anion and the substrate radical cation [9]. When the same electron donor is used, rate-differences in the $1e^-$-transfer to various dyes depend on the differences in the reduction potential, *i.e.* the electron affinity in the ground state, and the excitation energy, *i.e.* the energy by which the electron affinity is increased when the acceptor dye molecule is excited [9,10]. In order to investigate the applicability of this $1e^-$-transfer mechanism in our photosystems we determined the reduction potentials at pH 8.0 [3] and excitation energies of the various dFlox's (Table 3). The excitation energies were calculated from the phosphorescence spectra [4], since it has been established that the triplet state of dFlox is the reactive form in the photoreduction of dFlox [11,12]. Because the rate of photoreduction is influenced by the quantum yield of triplet formation, which may be approximated by the term $(1-\Phi_f)$ [4,7,13], values for $(1-\Phi_f)$ are included in Table 3.

Table 3 Reduction potentials ($E^1_{8.0}$), triplet energies ($E_{0,0}$) and approximate quantum yields of triplet formation ($1-\Phi_f$) of 5-deazaflavins

Compound	$E^1_{8.0}$ in V	$E_{0,0}$ in eV	$1-\Phi_f$
1a	-0.74	2.57	0.93
1d	-1.09	2.21	0.98
1e	-1.04	2.40	0.55
1f	-0.95	2.48	1.00

From this Table it is clear that the mechanism of $1e^-$-transfer outlined above does not offer a completely satisfying explanation for the differences in photoreduction rates of dFlox's. This is exemplified by the photoreduction of **1f** by EDTA being about a hundred times slower than the photoreduction of **1e** by EDTA, despite the fact that **1f** exhibits more favourable values for $E^1_{8.0}$, $E_{0,0}$ and $(1-\Phi_f)$ and a larger absorption of incident light with respect to the spectral output of the blue fluorescent lamp [14].

Based on the quantum efficiency of disappearance of dFlox (Φ_{-dFlox}) in the photoreduction of **1h** by oxalate being 1.5, another mechanism has been proposed [7]. This mechanism (Eq. 9-14) includes the formation of a triplet excimer T_E. Since for the maximum quantum yield of triplet formation (*i.e.* $1-\Phi_f$)

$$dFlox + h\nu \xrightarrow{I} {}^3dFlox \qquad (9)$$

$$^3dFlox \xrightarrow{k_T^\circ} dFlox \quad (10)$$

$$^3dFlox + dFlox \xrightarrow{k_E} T_E \quad (11)$$

$$T_E \xrightarrow{k_TE} 2\ dFlox \quad (12)$$

$$T_E + C_2O_4^{2-} + 2H^+ \xrightarrow{k_X} X + 2\ CO_2 \quad (13)$$

$$X \longrightarrow (HdFl)_2 \quad (14)$$

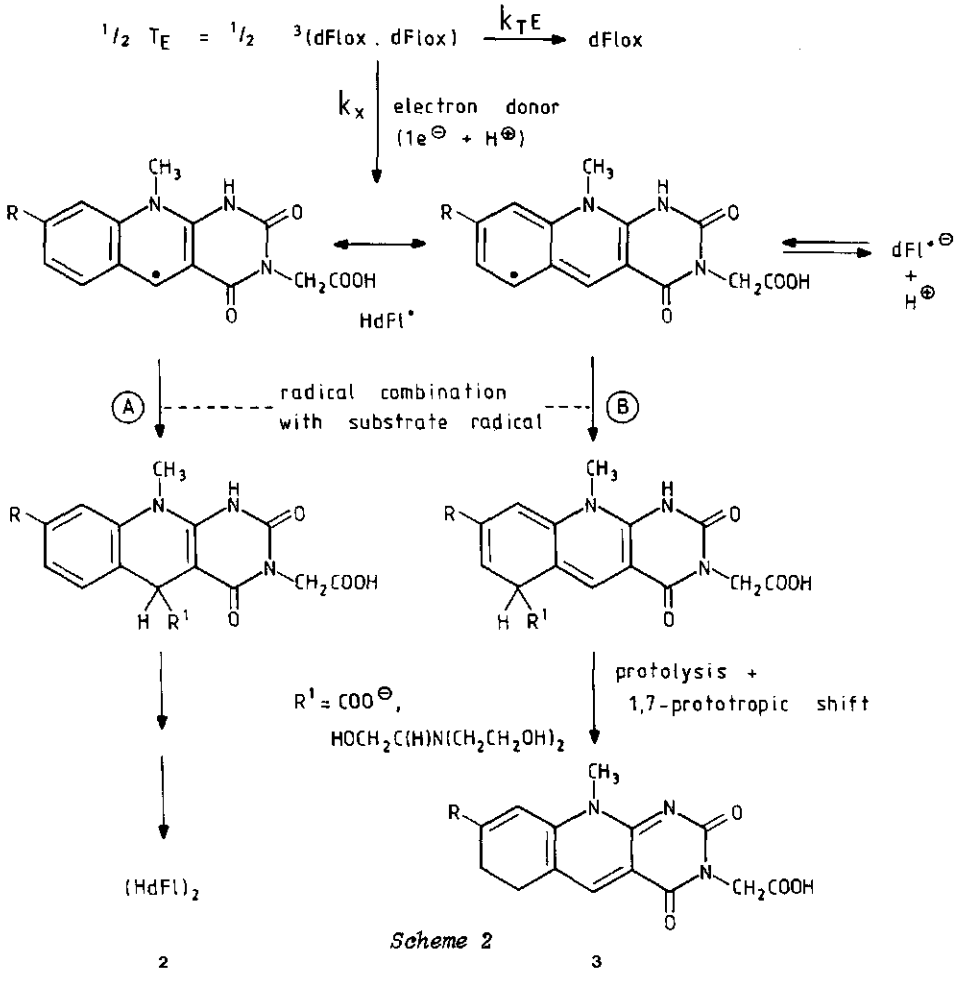
a value of 0.80 has been determined and Φ_{-dFlox} is about twice as large, it is justified to conclude that internal conversion from the first excited singlet state ($S_1 \rightsquigarrow S_0$) is negligible and the efficiency of excimer formation is close to unity *i.e.* k_E [dFlox] >> $k_T°$. This mechanism offers no insights into the structure of X. The alternative formulation in which X incorporates the elements of oxalate so that carbon dioxide is released only when the dimer is formed cannot be dismissed. The rate of disappearance of dFlox derived from this mechanism follows pseudo first-order kinetics as shown in Eq. 15 [7].

$$\frac{d[dFlox]}{dt} = - \frac{I.k_X[C_2O_4^{2-}]}{k_TE + k_X\ [C_2O_4^{2-}]} \quad (15)$$

It seems that the results summarized in Table 1 and 2 can nicely be explained by this mechanism, assuming that the excimer T_E adopts a sandwich structure, like excimers derived from aromatic hydrocarbons [15]. It is obvious that substituents like the *p*-aminophenyl and dimethylamino group will exert a large steric effect with a pronounced enhancement of k_TE in Eq. 15 and, as a result, a decrease of the photoreduction rate of **1d** and **1f**. An analogous explanation may be valid for the relatively low reactivity of **1e** towards oxalate when the steric hindrance is caused by hydrogen bridged complex formation between the amino group and oxalate.

The composition of the reaction product of the various photoreductions (Table 1) points to the involvement of a steric as well as an electronic contribution in the reaction mechanism as outlined in Scheme 2. The present data are insufficient to specify the initial interaction between T_E and the electron donor. The 1e$^-$-transfer from the electron donor to T_E, which in case of oxalate is accompanied by decarboxylation, yields the 5-deazaflavosemiquinone ($HdFl^\bullet$), which is stabilized by electron delocalization. Subsequent

radical combination of HdFl$^{\bullet}$ and the radical of the electron donor may occur following two different pathways. Route A yields a C(5)-adduct, which is well



Scheme 2

established in 5-deazaflavin photochemistry and is thought to be the precursor in dimer formation [12,16]. Route B leads to the formation of the C(6)-adduct, which on protolysis and a 1,7-prototropic shift yields the 6,7-dihydro-5-deazaflavin (6,7-H_2dFlred). Comparison of the mechanisms outlined in Eq. 9-14 and Scheme 2 leads to the conclusion that X represents a number of intermediates including the 5-deazaflavosemiquinone radical and the σ-bonded adduct.

According to Table 1 formation of 6,7-H_2dFlred is favoured with increasing electron donating character of the substituent and when TEA is used as electron donor. This observation may be explained by the mechanism outlined in Scheme 2. Radical combination of HdFl$^{\bullet}$ and TEA-radical yielding the C(5)-adduct is more

sterically hindered as compared to C(6)-adduct formation and hence route B predominates in the photoreduction of dFlox by TEA. With oxalate, encountering little steric hindrance, dimer formation is more favoured. Photoreduction of dFlox by the electron donor EDTA ($RCOO^-$) leads to the formation of $HdFl^\cdot$ and the EDTA radical ($RCOO^\cdot$). After decarboxylation $R^\cdot$ is obtained, which is also able to act as a single electron donor as outlined in Eq. 16-19 in a simplified manner [17].

$$dFlox + RCOO^- + H^+ \xrightarrow{h\nu} HdFl^\cdot + RCOO^\cdot \quad (16)$$

$$RCOO^\cdot \longrightarrow R^\cdot + CO_2 \quad (17)$$

$$dFlox + R^\cdot + H^+ \xrightarrow{h\nu} HdFl^\cdot + R^+ \quad (18)$$

$$2HdFl^\cdot \longrightarrow dFlox + H_2dFlred \quad (19)$$

The 5-deazaflavosemiquinone radical presumably decays by dismutation (Eq. 19) yielding dFlox and H_2dFlred, which in case of the 1,5-dihydro compound finally leads to the formation of the dimer **2** [12]. Hence, with EDTA as electron donor the photoreduction does not involve adduct formation and steric interference is minimized, which is consistent with the observation of dimer formation to a higher extent than obtained in the photoreduction with oxalate or TEA as electron donor.

The preference of photoreduction of the C6-C7 bond of dFlox's with a strong electron donating substituent at position 8 is explained by the following reasoning. It has been shown [18,19] that in roseoflavin (compound **1d**, in which C(5)H is replaced by N(5)) an intramolecular charge transfer from the 8-dimethylamino group to the pteridine moiety occurs, which is even more outspoken in the triplet state. It can be expected that in the triplet state of **1d,1e** a similar effect is operative, which enhances the electrophilicity of the benzene ring and hence favours its reduction following route B. The present data are not sufficient to give any insight into the transformation of the $2e^-$-reduced species into the dimer.

7.2.2 Photochemical reduction of methyl viologen

Common substrates for the dFlox catalyzed photoreductions are EDTA [2] and methyl viologen (MV^{2+}) in combination with a large variety of electron donors [14,20]. In order to investigate the efficiency of the various dFlox's as

photocatalyst we illuminated a mixture of dFlox **1a,1d-1f** and EDTA in the presence of a 20 fold excess (relative to [dFlox]) of MV^{2+} and determined the rate of reduced methyl viologen ($MV^{\bullet+}$) formation by measuring the increase of the absorption at 600 nm ($\varepsilon_{MV^{\bullet+}}$ = 8600 mol^{-1} dm^3 cm^{-1}, [14]) as a function of time. As a representative of the dFlox's with a reducible substituent at position 8 we also included **1g** in the experiments requiring the 250 W slide projector lamp as light source. The rate of $MV^{\bullet+}$ formation appeared to be pseudo first-order in MV^{2+}. Rate constants of MV^{2+} reduction and those of dFlox reduction in the absence of MV^{2+} using EDTA are summarized in Table 4. With all dFlox's a quantitative formation of $MV^{\bullet}$ was observed (2000% yield based on dFlox), which is consistent with the mechanism depicted in Scheme 3.

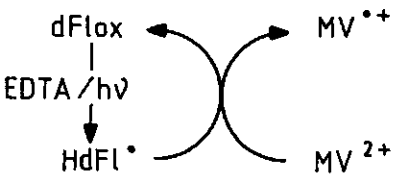
Table 4 Pseudo first-order rate constants (s^{-1}) of the photoreduction of dFlox by EDTA and of the photoreduction of MV^{2+} by EDTA, catalyzed by dFlox, using a 250 W slide projector lamp as ligth source

Compound	dFlox, EDTA[a]	MV^{2+}, dFlox, EDTA[b]
1a	$(6.5\pm0.1)10^{-2}$	$(7.1\pm0.2)10^{-3}$
1d	$3x10^{-6}$[c]	$(3.0\pm0.2)10^{-5}$
1e	$(2.3\pm0.1)10^{-3}$	$(3.8\pm0.2)10^{-4}$
1f	$(1.7\pm0.1)10^{-5}$	$(2.5\pm0.4)10^{-6}$
1g		$(3.8\pm0.2)10^{-4}$

a [dFlox] $2.0x10^{-5}$ mol dm^{-3}; [EDTA] $4x10^{-3}$ mol dm^{-3}; borate buffer, pH 8.0;

b [MV^{2+}] $1.00x10^{-4}$ mol dm^{-3}; [dFlox] $5.0x10^{-6}$ mol dm^{-3}; [EDTA] $1x10^{-3}$ mol dm^{-3}; borate buffer, pH 8.0;

c This value is mere indicative for the order of magnitude of the photoreduction rate.



Scheme 3

When **1g** was used as a photocatalyst we observed first a small decrease of

the absorption at 600 nm accompanied by the disappearance of the purple colour of **1g**. After the purple colour had vanished completely, the absorption at 600 nm started to increase reaching a value of 0.86, which agrees with 100% formation of $MV^{\cdot+}$. Since the rate constant of MV^{2+} photoreduction by **1g** is the same as that found for **1e**, these results seem to indicate that prior to the reduction of MV^{2+} a quantative reduction of the azo function of **1g** occurs yielding the photocatalyst **1e**.

The most striking result mentioned in Table 4 is the fact that the photoreduction of **1f** by EDTA is faster than the photoreduction of **1d** by EDTA, while the reversed reactivity is observed in the 5-deazaflavin mediated photoreduction of MV^{2+}. It can be suggested that the dismutation process of $HdFl^{\cdot}$ in the MV^{2+} photoreduction does not occur. It accounts for the higher rate of photoreduction of MV^{2+} by **1d** relative to **1f**, assuming that the dismutation process in case of **1d**, which leads to the formation of 6,7-H_2dFlred, is slow compared to the formation of 1,5-H_2dFlred in case of **1f**. Alternatively, the electron density in the 8-dimethylamino-5-deazaflavosemiquinone radical may be more centered in the benzene ring (especially at C(6)), making a less sterically hindered encounter between MV^{2+} and $HdFl^{\cdot}$ possible resulting in a decreased activation energy of the $1e^-$-transfer process. Still 20 hours of irradiation were required to reduce 90% of MV^{2+}.

The ability of dFlox to act as a photoreducing catalyst in the presence of EDTA may offer an explanation for the results obtained in the photoreduction of dFlox's with a reducible substituent at position 8 (**1b**,**1c**,**1g**) in the presence of EDTA, *i.e.* reduction of the nitro group in two distinct steps or the azo function prior to the reduction of the 5-deazaisoalloxazine skeleton. This explanation is based on the observation that, when during the photoreduction of **1g** by EDTA the absorbance at 537 nm is monitored and plotted against time, a flat S-shaped curve is obtained. This feature suggests, as we have seen before, an autocatalytic contribution to the rate of disappearance of **1g**, which may be explained by the formation of the initial photoproduct **1e**, acting as a photoreducing catalyst on irradiation in the presence of EDTA; N,N-dimethyl-1,4-phenylenediamine, which is probably formed during the photoreduction, may also contribute to the autocatalysis due to its electron donating ability [21]. The photoreduction of the nitro group in **1b**, **1c** involves two steps, *i.e.* the formation of the hydroxylamino compounds in the first step, which in turn are reduced to the corresponding amino derivatives. In both steps an acceleration in relation to the expected pseudo first-order decay is observed. Apparently, both products, *i.e.* the hydroxylamino and amino compounds, are able to act as

photocatalyst in the corresponding reduction step. This may explain the complete built-up of these products prior to further reduction suggesting that the rate of autocatalysis is higher than the succeeding reduction.

7.3 EXPERIMENTAL

Analytical photochemical experiments were carried out as described previously [4] (chapter 6).

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GENERAL DISCUSSION

Interest in the chemistry of 5-deazaflavin lies mainly in the high reactivity and low reduction potential of its 5-deazaflavosemiquinone radical ($HdFl^{\cdot}$). This radical, which can be generated photochemically in the presence of an electron donor, is capable to donate an electron to a redox system thereby returning to the oxidized state. Hence, 5-deazaflavin in combination with a suitable electron donor offers a photosystem, in which 5-deazaflavin acts as a photoreducing catalyst as has been shown by the photoreduction of a large variety of enzymes [1]. However, the light required for the generation of the reducing radical corresponds to such an high energy that it destroys the protein enzyme in a certain period of time. The aim of the present study is the search for a 5-deazaflavin derivative, which on the one hand exhibits a lower excitation energy than the parent system making it possible to avoid damage to the protein enzyme and on the other hand remains effective as a photoreducing catalyst in the presence of a suitable electron donor.

To meet the first requirement we synthesized 5-deazaflavins containing a chromophoric group at position 8 of the 5-deazaisoalloxazine skeleton (chapters 2 and 3). It can be expected that substituents at this position cause a bathochromic shift in the absorption spectrum. Three methods of preparing 8-substituted 5-deazaflavins were explored.

The first method, based upon the cyclization of *N*-methylanilinouracil derivatives, appears to be less useful, because of the formation of inseparable mixtures due to the de- and remethylation at position 3 and 10, and, more important, the cyclization is non-regioselective leading to a mixture of isomeric 5-deazaflavins.

The second method to introduce a substituent at C(8) is based upon the aldol-type condensation of the C(8) methyl group in 5-deazalumiflavin with aromatic aldehydes. However, this method also fails due to an insufficient π-electron deficiency of the C(8) methyl group.

The third method, which was investigated and later on succesfully applied, involves the oxidative cyclization of 5,5'-arylmethylenebis(6-methylaminouracil) derivatives (**8**, Scheme 2, chapter 2) with diethyl azodicarboxylate (DAD). The utility of this method appears to depend on the solubility of **8** in DAD and/or sulfolane, and on its thermolability. The very low solubility of **8** (R^1=H) requires a long reaction time at a high temperature to achieve cyclization with partly or complete decomposition of **8** as a result. By introducing the *N*-methylcarbamoylmethyl group at N(3) this solubility problem is circumvented.

Moreover, the introduction of this group has the additional advantage that after hydrolysis of the amide group to a carboxymethyl group a water soluble compound is formed. In this way a series of water soluble 8-R-5-deazaflavins (8-RdFlox, R=Cl, NO_2, *p*-NO_2-C_6H_4, NH_2, *p*-NH_2-C_6H_4, $N(CH_3)_2$, *p*-$N(CH_3)_2$-C_6H_4-N=N) were synthesized. They all exhibit a bathochromic shift of the first absorption maximum, varying between 1 nm and 147 nm compared to the parent system 5-deazaflavin (see Table 1).

Table 1 Absorption maxima and reduction potentials ($E'_{8.0}$) of 8-substituted 5-deazaflavins (8-RdFlox) and pseudo first -order rate constants of the photoreduction of methyl viologen by EDTA, catalyzed by 8-RdFlox

R	λ_{max}(nm)	$E'_{8.0}$ (V)	k(s^{-1})
Cl	391	-0.74	7.1×10^{-3}
$N(CH_3)_2$	442	-1.09	3.0×10^{-5}
NH_2	417	-1.04	3.8×10^{-4}
p-NH_2-C_6H_4	428	-0.95	2.5×10^{-6}
p-$N(CH_3)_2$-C_6H_4-N=N	537	-	-
H	390[a]	-0.83[b]	-

[a] Reference 4

[b] Calculated from eq 8c, chapter 4

To measure the effect of the modification at C(8) on the reducing power of the 5-deazaflavin radical, which is the actual reducing species within the photosystem, the half-wave reduction potentials ($E_{½}$) of various 8-R-5-deazaflavins (R=Cl, $N(CH_3)_2$, NH_2, *p*-NH_2-C_6H_4) were determined (chapter 4) (see Table 1). It appears impossible to obtain $E_{½}$ values for those 8-R-5-deazaflavins, which contain a reducible substituent R at position 8 (R=NO_2, *p*-NO_2-C_6H_4, *p*-$N(CH_3)_2$-C_6H_4-N=N) since the substituent is reduced prior to the 5-deazaisoalloxazine skeleton. The observed pH dependency of $E_{½}$ is explained in terms of involvement of the dissociation equilibrium of 5-deazaflavin and its radical in the electron-transfer process and enables the calculation of the dissociation constants of the respective 5-deazaflavin radicals. The fact that the standard reduction potentials (E_0) meet the Hammett relation points to a pure electronic effect of the substituents on the E_0 values and indicate that the formation of radicals follows the same electron-transfer mechanism.

The reducing power of the 8-R-HdFl$^{\cdot}$ radicals when generated photochemically from 8-RdFlox have also been investigated by studying the rate of reduction of methyl viologen (MV^{2+}) in the presence of ethylenediaminetetraacetate (EDTA). The results are also summarized in Table 1.

In Table 1 are summarized the main properties, which determine the applicability of a particular 5-deazaflavin to act as a catalyst in the photoreduction of enzymes: i) the first absorption maximum, which is related to the light energy required by the photosystem, ii) the reduction potential of the dFlox/HdFl$^{\cdot}$ couple at pH 8.0, which is calculated from the relation, found between the reduction potential and pH, and represents the reducing power of the photosystem, and iii) the pseudo first-order rate constant of the 5-deazaflavin mediated photoreduction of methyl viologen in the presence of EDTA as a measure for the rate with which catalytic photoreductions of enzymes will take place.

From this Table it is obvious that the most favourable excitation energy is found with the *p*-(dimethylaminophenyl)azo chromophore at position 8 of the 5-deazaisoalloxazine skeleton. However, irradiation in the presence of an electron donor causes the reduction of this chromophore leaving at position 8 an amino group. This compound requires, however, a higher excitation energy in order to act as a photocatalyst. This feature is observed with all 5-deazaflavins with a reducible substituent (NO_2, *p*-NO_2-C_6H_4) at position 8.

The introduction of an electron donating substituent R at position 8 is attended with a small (R=NH_2) to moderate (R=$N(CH_3)_2$) decrease of the excitation energy, together with an increase of the reducing power. However, despite these favourable effects the photoreduction rate is lower than that of 8-CldFlox.

As conclusion a 8-substituted 5-deazaflavin, which unites the three main properties, required to act as an efficient catalyst in the photoreduction of enzymes, has not been found so far.

The effect of the substituent at position 8 of the 5-deazaisoalloxazine skeleton and of the nature of the electron donor on the course and rate of the photoreduction of 5-deazaflavins has been determined by illuminating 5-deazaflavins in the presence of various electron donors. It has been assumed [3,4] that long-term illumination of 5-deazaflavin yields exclusively the radical dimer independent of the electron donor [5]. The present data (chapters 5, 6 and 7) are fully in disagreement with this assumption, since it is established that the photoreduction not only leads to the formation of the radical dimer, but in addition to the 6,7-dihydro derivative.

From Table 1 in chapter 7 it is clear that the formation of the 6,7-dihydro compound is favoured if the electron donating character of the substituent at position 8 increases, and is promoted by the electron donor in the order triethanolamine (TEA) > oxalate > EDTA. The explanation for these results (Scheme 2, chapter 7) is based on an initial $1e^-$-transfer from the electron donor to excited dFlox yielding the 5-deazaflavosemiquinone radical ($HdFl^{\cdot}$), followed by a recombination of radicals, when TEA and oxalate are used as the electron donor.

Contrary to the assumption of C(5) being the only site of adduct formation [3-5] the results summarized in Table 1 indicate that C(6) competes with C(5) in this respect. The preference for the formation of the 6,7-dihydro compound in the presence of TEA as the electron donor is then explained by the smaller steric effect, experienced by the bulky TEA radical, for adduct-formation at the C(6) position instead of the C(5) position. With EDTA no adduct-formation takes place after the initial $1e^-$-transfer process, but instead dismutation of $HdFl^{\cdot}$ yielding dFlox and dihydro-5-deazaflavin occurs. As a result the steric effect is minimized and the formation of 1,5-dihydro-5-deazaflavin, which finally yields the dimer [3], predominates.

The promotion of the reduction of the C6-C7 bond by electron donating substituents at position 8 is caused by the intramolecular charge transfer from the substituent to the pyrido[2,3-*d*]pyrimidine moiety [6,7], which increases the electrophilicity of the benzene ring and hence the susceptibility to its reduction.

The observed rates of photoreduction (Table 2, chapter 7) are not consistent with a mere electronic effect of the substituent at position 8 as is exemplified by the extremely low rates of photoreduction of 8-RdFlox ($R=N(CH_3)_2$, p-NH_2-C_6H_4) by EDTA and of 8-RdFlox ($R=N(CH_3)_2$, NH_2) by oxalate. However, it has been proposed [4] that a preformed triplet excimer ^{3}(dFlox.dFlox)* is the precursor in 5-deazaflavin photochemistry. With the assumption that this excimer adopts a sandwich structure [8], it is understandable that bulky substituents like the dimethylamino and p-aminophenyl group and, in case of the photoreduction of 8-NH_2dFlox by oxalate, the hydrogen bridged complex between the amino group and oxalate exert a large steric effect, which results in a fast decay of the excimer into ground state dFlox and hence in a decrease of the rate of photoreduction.

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SUMMARY

5-Deazaflavin has been applied as a photocatalyst in the reduction of a wide variety of biological redox systems. The use of 5-deazaflavin as a photocatalyst has serious drawbacks in that continuous UV irradiation is required to drive the process and that the needed wavelength (300-400 nm) corresponds to an energy level high enough to destroy the protein enzyme in time.

To avoid photodestruction of the protein a number of 5-deazaflavins, which have a chromophoric group at position 8 of the 5-deazaisoalloxazine skeleton, causing the absorption maximum to undergo a red shift, were synthesized (chapter 2). This was accomplished i) by the oxidative cyclization of 5,5'-arylmethylenebis(6-methylaminouracil) derivatives and ii) by the cyclization of *N*-methylanilinouracil derivatives with a one-carbon reagent. The latter method led to the formation of impure products. Condensation and oxidation reactions with the π-electron deficient C(8) methyl group in 5-deazalumiflavin did not occur. By the former method a number of 8-substituted 5-deazaflavins with a carboxymethyl group at the N(3) position were synthesized (chapter 3). This polar group was introduced prior to the oxidative cyclization and increased the solubility of 5-deazaflavins in aqueous media, in which photoreduction of redox enzymes are to be carried out. Introduction of substituents at position 8 caused a bathochromic shift that varied between 1 and 147 nm (chapter 6).

Since the 5-deazaflavin radical is the actual reducing species within the photosystem, the effect of the substituents, introduced at position 8, on the reducing power of the radical was determined by measuring the half-wave potentials of the 5-deazaflavins (chapter 4). The dissociation constants of 5-deazaflavosemiquinones were successfully determined on the basis of the polarographic properties of 5-deazaflavins. Both half-wave potentials and dissociation constants appeared to be very sensitive to the substituent at position 8 and to meet the Hammett relation.

The effect of the substituent on the spectroscopic (chapter 6) and photochemical (chapters 5, 6 and 7) properties was investigated. In contrast with the findings of others that irradiation of 5-deazaflavins in the presence of oxalate or ethylenediaminetetraacetate (EDTA) as electron donor exclusively leads to the formation of dimeric compounds as end-products of the photoreduction, illumination of 8-R-5-deazaflavins (R=Cl, $N(CH_3)_2$, NH_2, *p*-NH_2-C_6H_4) in the presence of various electron donors (EDTA, oxalate and triethanolamine) yielded the dimeric species 5,5'-bis(1,5-dihydro-8-R-5-deazaflavin) and/or 6,7-dihydro-8-R-5-deazaflavin. It was found that reduction

of the C(6)-C(7) bond is highly promoted by strong electron donating substituents at position 8 and bulky electron donors. 5-Deazaflavins with a reducible substituent at position 8 (R=NO_2, *p*-NO_2-C_6H_4, *p*-$N(CH_3)_2$-C_6H_4-N=N) exhibited the reduction of the substituent prior to the reduction of the 5-deazaisoalloxazine skeleton.

The course (chapter 6) and rate (chapter 7) of the photoreduction of 8-R-5-deazaflavins were established and explained in terms of electronic and steric effects, exerted by the substituent at position 8 and the electron donor. In some cases the rate of photoreduction appeared to contain an autocatalytic element.

The catalytic effect of 8-R-5-deazaflavins in the photoreduction of methyl viologen by EDTA was investigated (chapter 7). The substituent effect on the rate of the 8-R-5-deazaflavin mediated photoreduction of methyl viologen by EDTA was found to be comparable with that on the photoreduction rate of 8-R-5-deazaflavin in the presence of EDTA with the exception of 8-R-5-deazaflavin (R=$N(CH_3)_2$), which showed a remarkable relative enhancement of the reactivity towards methyl viologen photoreduction.

SAMENVATTING

5-Deazaflavine is toegepast als fotokatalysator in de reductie van een grote verscheidenheid aan biologische redox systemen. Een belangrijk nadeel van het gebruik van 5-deazaflavine als fotokatalysator is gelegen in het feit, dat de energie van het benodigde licht (λ: 300-400 nm) hoog genoeg is om het enzym te vernietigen.

Om vernietiging van het enzym te voorkomen werden een aantal 5-deazaflavines, die een chromophore groep op plaats 8 van het 5-deazaisoalloxazine ringsysteem bezitten, waardoor het absorptie maximum een roodverschuiving ondergaat, gesynthetiseerd (hoofdstuk 2). Dit werd bereikt i) door de oxidatieve ringsluiting van 5,5'-arylmethyleenbis(6-methylaminouracil) derivaten en ii) door de ringsluiting van *N*-methylanilinouracil derivaten. De laatstgenoemde methode leidde tot de vorming van onzuivere producten. Condensatie en oxidatiereacties met de C(8) methyl groep in 5-deazalumiflavine traden, ondanks het enigszins zure karater van deze groep, niet op. Met behulp van de eerstgenoemde methode werden een aantal 8-gesubstitueerde 5-deazaflavines met een carboxymethyl groep aan N(3) gesynthetiseerd (hoofdstuk 3). Deze polaire groep werd ingevoerd voor de oxidatieve ringsluiting en verhoogde de oplosbaarheid van de 5-deazaflavines in waterig milieu, waarin fotoreductie van redox enzymen uitgevoerd dient te worden. De invoering van substituenten op plaats 8 bracht een bathochrome verschuiving teweeg varierend van 1 tot 147 nm (hoofdstuk 6).

Omdat binnen het fotosysteem het 5-deazaflavine radicaal het eigenlijke reducerende agens is, werd het effect van de substituent, ingevoerd op plaats 8, op het reducerend vermogen van het radicaal bepaald door meting van de reductiepotentialen van de 5-deazaflavines (hoofdstuk 4). De dissociatieconstanten van de 5-deazaflavosemichinonen werden met succes vastgesteld op basis van de polarografische eigenschappen van de 5-deazaflavines. Zowel de reductiepotentialen als de dissociatieconstanten bleken zeer gevoelig voor de substituent op plaats 8 en te voldoen aan de Hammett relatie.

Het effect van de substituent op de spectroscopische (hoofdstuk 6) en fotochemische (hoofdstuk 5, 6 en 7) eigenschappen werd onderzocht. In tegenstelling tot de constatering van anderen, dat bestraling van 5-deazaflavines in aanwezigheid van oxalaat of ethyleendiaminetetraacetaat (EDTA) als electronen donor uitsluitend leidt tot de vorming van het 5,5'-dimer, werden bij belichting van 8-R-5-deazaflavines (R=Cl, $N(CH_3)_2$), NH_2, *p*-NH_2-C_6H_4) in aanwezigheid van verschillende electronen donoren (EDTA, oxalaat en triethanolamine) het dimer 5,5'-bis(1,5-dihydro-8-R-5-deazaflavine) en/of 6,7-dihydro-8-R-5-deaza-

flavine gevormd als eindproducten van de fotoreductie. Vastgesteld werd, dat de vorming van de 6,7-dihydro verbinding sterk bevorderd wordt door sterk electronenstuwende groepen op plaats 8 en grote electronen donoren. Reductie van de substituent voorafgaand aan de reductie van het 5-deazaisoalloxazine ringsysteem trad op bij 5-deazaflavines met een reduceerbare substituent ($R=NO_2$, p-NO_2-C_6H_4, p-$N(CH_3)_2$-C_6H_4-N=N) op plaats 8.

De productvorming bij en de reactiesnelheid van de fotoreductie van 8-R-5-deazaflavine werden vastgesteld en verklaard op basis van electronische en sterische effecten, uitgeoefend door de substituent op plaats 8 en de electronen donor. In enkele gevallen bleek de fotoreductiesnelheid een autocatalytisch element te bevatten.

Het catalytische effect van 8-R-5-deazaflavine in the fotoreductie van methyl viologen door EDTA werd onderzocht (hoofdstuk 7). Het effect van de substituent R op de snelheid van bovengenoemde reductie bleek vergelijkbaar met het effect van R op de snelheid van de fotoreductie van 8-R-5-deazaflavine door EDTA. Een uitzondering hierop vormde 8-$N(CH_3)_2$-5-deazaflavine, dat een verhoudingsgewijs hoge reactiviteit jegens de fotoreductie van methyl viologen vertoonde.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 30 oktober 1952 te Utrecht geboren. In 1971 behaalde hij het diploma Gymnasium-β aan de Scholengemeenschap "De Breul" te Zeist. In hetzelfde jaar begon hij met de studie scheikunde aan de Rijksuniversiteit te Utrecht. Het kandidaatsexamen (S1) werd afgelegd in 1975. Het doctoraalexamen met als hoofdvak bio-organische scheikunde (Prof.Dr. J.F.G. Vliegenthart en Dr. W.A.R. van Heeswijk) en als bijvak pedagogiek en didaktiek van de scheikunde volgde in 1978.

Na gedurende een half jaar zijn hoofdvakonderzoek te hebben voortgezet in de functie van "ambtenaar buiten bezwaar van 's rijks schatkist" werd hij als wetenschappelijk ambtenaar aangesteld aan het Laboratorium voor Organische Chemie van de Landbouwhogeschool te Wageningen, waar hij van 1979 tot en met 1985 onder leiding van Prof.Dr. H.C. van der Plas en Prof.Dr. F. Müller het in dit proefschrift beschreven onderzoek verrichtte. Gedurende het promotie-onderzoek was hij betrokken bij het onderwijs aan studenten en HBO-B stagiaires.