Synthesis of 8-Substituted 5-Deazaflavins

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Received September 27, 1984

To obtain 5-deazaflavins exhibiting red-shifted light absorption spectra, a number of C(8) substituted 5-deazaisoalloxazines were synthesized. This was accomplished a) by the oxidative cyclization of 5,5'-arylmethylenebis(6-methylaminouracil) derivatives and b) 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. Introduction of substituents at the C(8) position caused a bathochromic shift that varied between 10 and 40 nm.

J. Heterocyclic Chem., **22**, 841 (1985).

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 F420,

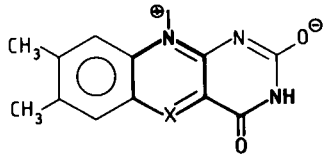


Figure 1. Structural comparison of neutral (deaza)flavin and nicotinamide species; X = N, flavin; X = CH, 5-deazaflavin; nicotinamide (heavy lines, X = CH).

the coenzyme of methane-producing baceteria [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 unfavorable 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 combined 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 wave length needed to form the dimer and to photodissociate it (300-400 nm) corresponds to an energy level high enough to destory 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.

Synthesis of 8-Substituted 5-Deazaflavins.

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.

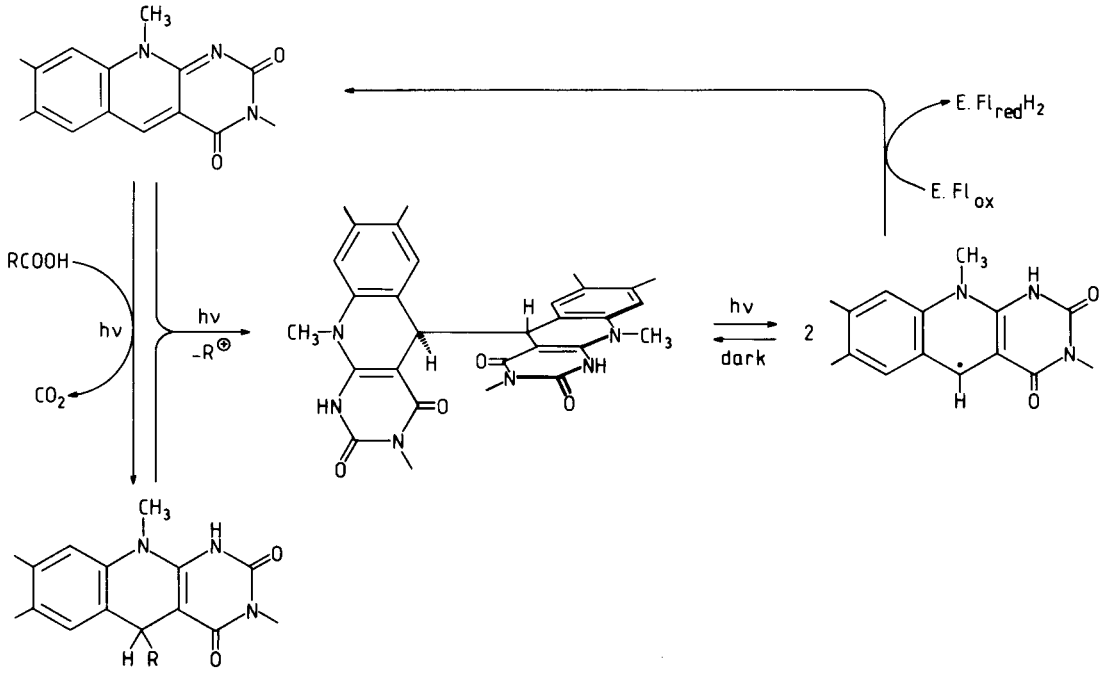
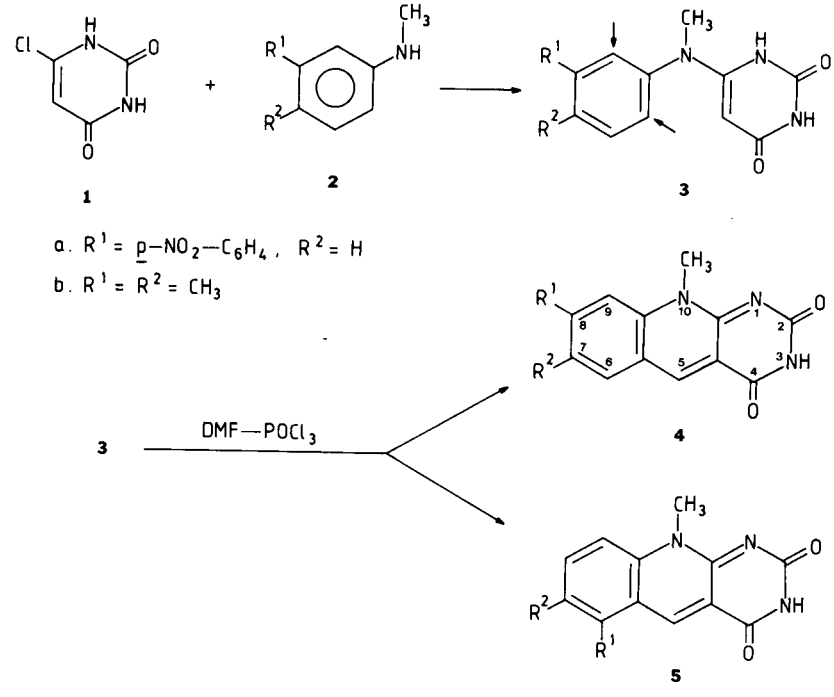


Figure 2. 5-Deazaflavin redox system.

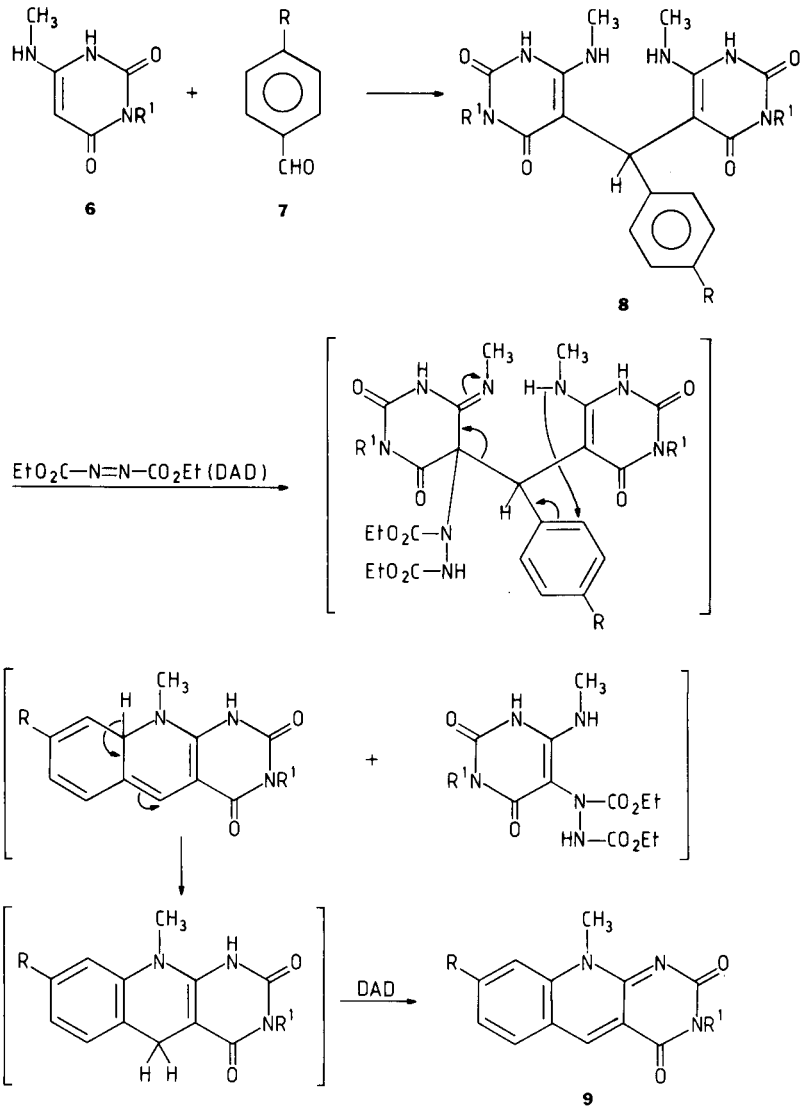
Scheme 1



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.

Scheme 2



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-biphenylcarboxaldehyde.

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 wich such a group is already present [10].

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.

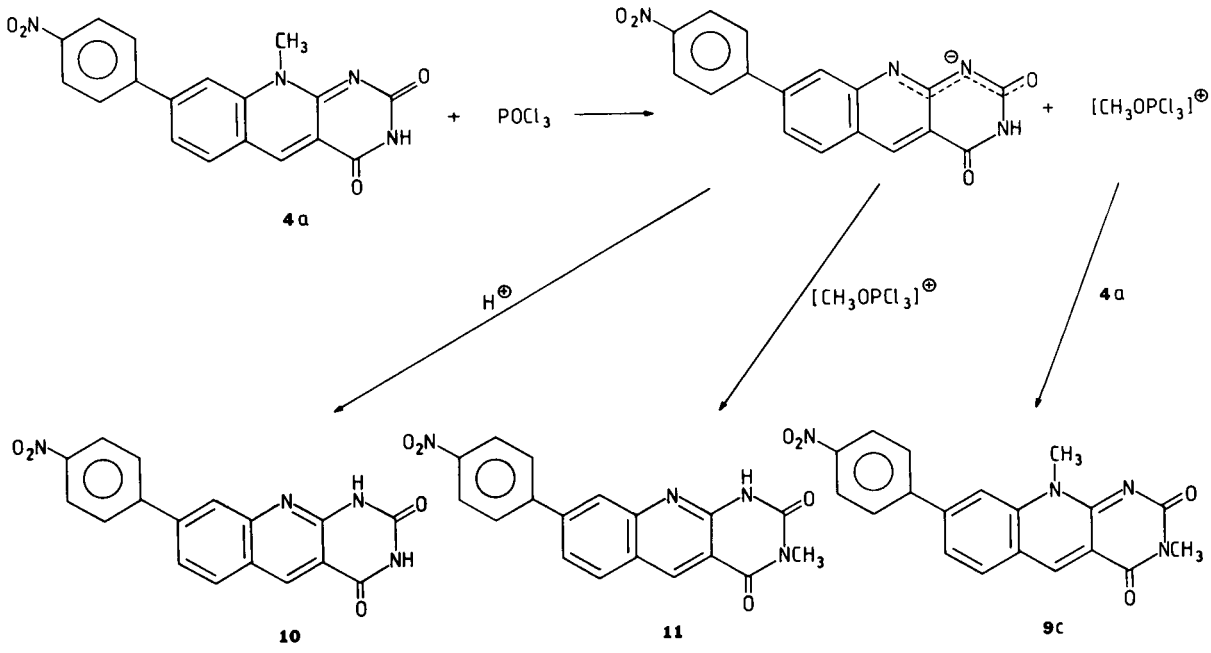
For this purpose 6-chlorouracil (**1**) and an excess of 3-methylamino-4'-nitrobiphenyl (**2a**) were refluxed in 1-butanol, leading to the formation of **3a**. This method has several advantages over the fusion reaction, as applied by Yoneda [11]; excess of **2a** is regained in better yield and in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

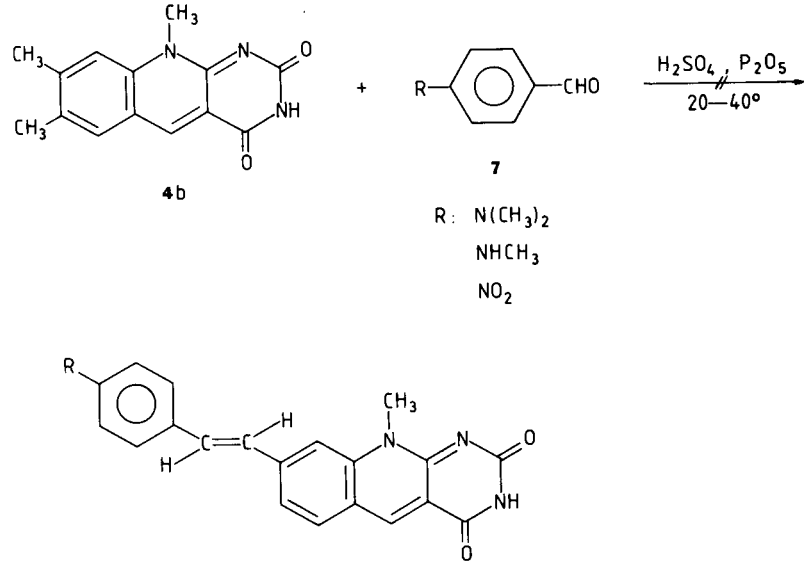
The use of phosphorus oxychloride as demethylating agent has also been demonstrated with *N*-methylated purines [14]. This demethylation reaction is proposed to proceed by conversion of the methyl group into a trichloromethyl group and subsequent hydrolytic fission of the nitrogen-carbon bond. We have 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 monomethyldeaza(iso)alloxazines.

The fact that synthesis of **4a** by cyclization with a one-carbon reagent leads to the formation of an inseparable

Scheme 3



Scheme 4



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 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].

Synthesis of 5-Deazaflavins by Oxidative Cyclization (Method 2).

Condensation of 6-methylamino-3-methyluracil (**6**, R^1 = CH_3) with several arylaldehydes (**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.

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)

Compound No.	R^1	R	Yield %	MP, °C	δ [a]	λ max/nm (log ε) [b]
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

[a] 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. [b] Absorption maximum of **9** in methanol unless otherwise stated, sh = shoulder.

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 are uncorrected. Silica gel GF plates were used for analytical thin layer chromatography.

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** as red plates, mp 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° (oil bath) 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, NCH_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(3*H*,10*H*)-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**).

This compound was obtained in a yield of 85% (2.40 g) 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**).

This compound was obtained in a yield of 80% (2.45 g) white plates, mp 279-281° dec; (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**).

This compound was obtained in a yield of 80% (2.70 g), 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(3*H*,10*H*)-dione (**9a**).

A mixture of 2 g of **8a** and 3.7 ml of diethyl azodicarboxylate (DAD) was stirred at 140° (oil bath) 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(3*H*,10*H*)-dione (**9b**).

A mixture of 0.80 g of **8b** and 2.1 ml of DAD, and 0.1 ml of sulfolane was stirred at 170° (oil bath) 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, C(10)CH$_3$), 7.40-7.87 (s, 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(3*H*,10*H*)-dione (**9c**).

A mixture of 2.5 g of **8c** and 3.8 ml of DAD was stirred at 170° (oil bath) 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(3*H*,10*H*)-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, trifluoroacetic 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(3*H*,10*H*)-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, mp 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(3*H*,10*H*)-dione (**9f**).

A mixture of 0.2 g of **9e**, 1.3 g of a 1:1 mixture of *p*-dimethylaminobenzaldehyde and its diethyl acetal (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 $C_{26}H_{25}N_5O_2$: C, 72.54; H, 5.44. Found: C, 72.23; H, 5.32.

Acknowledgement.

We would like to thank Mr. A. van Veldhuizen and Dr. H. Holterman for obtaining the 300 MHz nmr spectra, Drs. C. Landheer and Mr. C. Teunis for obtaining the mass spectra, and Mr. H. Jongejan for the elemental analyses.

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