Synthesis of Water Soluble 8-Substituted 5-Deazaflavins

P. A J. Link, H. C. van der Plas* and F. Müller [a]

Department of Organic Chemistry and Biochemistry [a], Agricultural University,
De Dreijen 5, 6703 BC Wageningen, The Netherlands

Received October 19, 1984

By oxidative cyclization of 5,5'-arylmethylenebis(6-methylaminouracil) derivatives with diethyl azodicarboxylate a number of 5-deazaisoalloxazines were synthesized having at the C(8) position a substituent that causes a bathochromic shift varying between 20 and 130 nm, depending on the substituent. To increase the solubility in aqueous media 8-substituted 5-deazaflavins were prepared having a carboxymethyl group at the N(3) position. The carboxymethyl group was introduced prior to the oxidative cyclization.

J. Heterocyclic Chem., **22**, 873 (1985).

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.

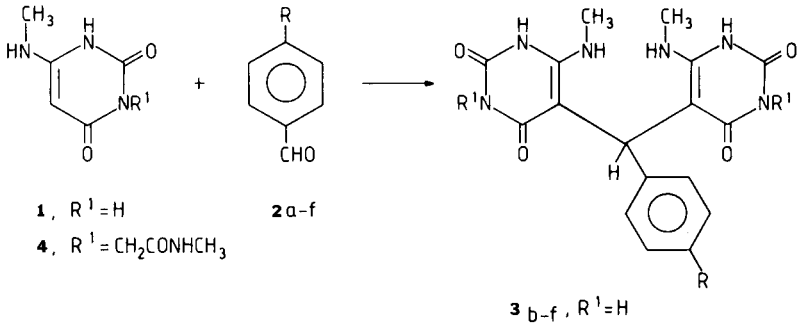
Results and Discussion.

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

Based on our previous experience [3] that oxidative cyc-

Scheme 1



1, R^1 = H

4, R^1 = $CH_2CONHCH_3$

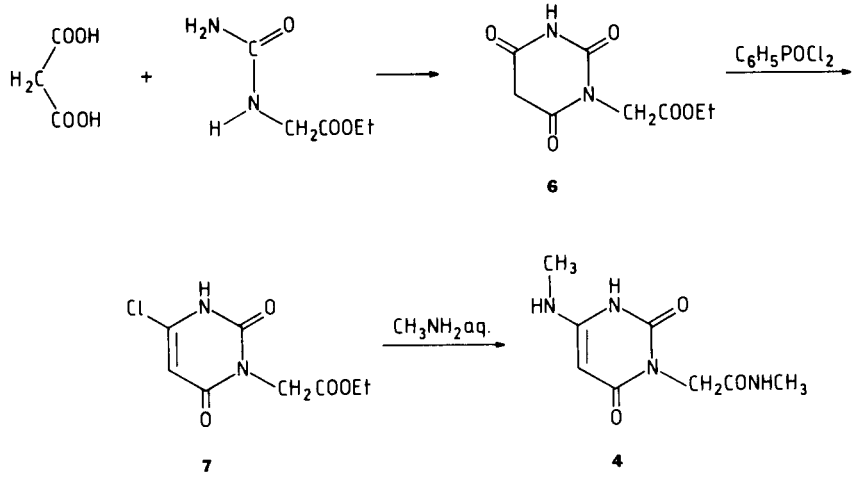
2a-f

3 b-f, R^1 = H

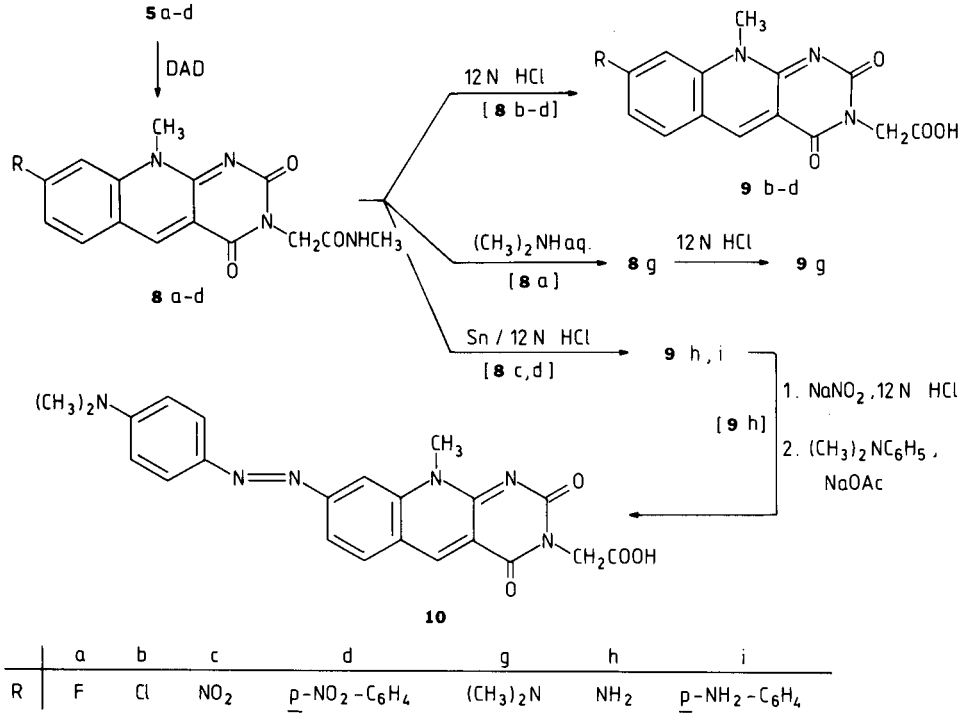
5 a-d, R^1 = $CH_2CONHCH_3$

	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 2



Scheme 3



	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

lization 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].

Table 1

Yields and Some Physical Properties of 5,5'-Methylenebis(6-methylaminouracils) **3** and **5**

Compound	R	Yield (%)	Mp, °C	δ [a]
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

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

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**, 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 corresponding structures (Table 2).

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): δ 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.

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**).

This compound was obtained in a yield of 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, 4 × NH); 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**).

This compound was obtained in a yield of 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, 4 × NH); 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**).

This compound was obtained in a yield of 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,

Table 2

Yields and Some Physical Properties of 5-Deazaflavins **8**, **9** and **10**

Compound	R	Yield (%)	Mp, °C	δ [a]	λ max/nm (log ε) [b]
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)

[a] 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. [b] Absorption maximum of **9** and **10** in DMSO, sh = shoulder.

Ar'H), 10.53 (br s, 4H, 4 × NH); 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-yl-methylenebis(6-methylaminouracil) (**3e**).

This compound was obtained in a yield of 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-yl-methylenebis(6-methylaminouracil) (**3f**).

This compound was obtained in a yield of 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, 4 × NH); 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**).

N-Ethoxycarbonylmethylureum [6] (209 g) 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**).

Compound **6** (152 g) was 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**).

Compound **5a** was obtained in 86% yield, 12.2 g of white plates, mp 237-240°, >245° dec; nmr (90 MHz, DMSO-d_6): δ 2.63 (br s, 6H, 2 × $CONCH_3$), 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, 4 × NH), 10.76 (br s, 2H, N(1)H, N(1')H); field desorption ms: m/e 530 (M^+), 318 (cluster), 212.

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

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

This compound was obtained in a yield of 99%, 13.5 g of white needles, mp 243-245° dec; nmr (60 MHz, DMSO-d_6): δ 2.62 (d, 6H, 2 × $CONCH_3$), 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, 2 × CONH), 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**).

This compound was obtained in a yield of 88%, 13.9 g of pale yellow needles, mp 295-298° dec; nmr (90 MHz, DMSO-d_6): δ 2.60 (d, 6H, 2 × $CONCH_3$), 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, 2 × CONH), 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 $C_{23}H_{27}N_9O_8 \cdot 4H_2O$: 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**).

This compound was obtained in a yield of 94%, 14.9 g of pale yellow plates, mp 279-280° dec; nmr (90 MHz, DMSO-d_6): δ 2.59 (d, 6H, 2 × $CONCH_3$), 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 (s, 2H ArH), 7.63 (d, 2H, ArH), 7.79 (br s, 4H, 4 × NH), 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(3*H*,10*H*)-dione (**8a**).

A mixture of 1 g of **5a**, 1.1 ml of DAD and 0.8 ml of sulfolane was stirred at 110° (oil bath) 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(3*H*,10*H*)-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° (oil bath) 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 $C_{15}H_{13}ClN_4O_5 \cdot 1H_2O$: 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(3*H*,10*H*)-dione (**8c**).

A mixture of 2 g of **5c**, 4 ml of DAD and 1 ml of sulfolane was stirred at 150° (oil bath) under nitrogen for 16 hours. The work-up was similar to that of **8a**, yielding 0.76 g (70%) of 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(3*H*,10*H*)-dione (**8d**).

A mixture of 1 g of **5d**, 1.5 ml of DAD and 0.5 ml of sulfolane was stirred at 150° (oil bath) 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(3*H*,10*H*)-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(3*H*,10*H*)-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 $C_{14}H_{10}ClN_3O_4 \cdot 1H_2O$: 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(3*H*,10*H*)-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(3*H*,10*H*)-dione (**9d**).

A mixture of 0.35 g of **8d** and 10 ml of concentrated hydrochloric acid was stirred at 100° (oil bath) 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 $C_{20}H_{14}N_4O_6 \cdot 2H_2O$: 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(3*H*,10*H*)-dione (**9g**).

Compound **8g** (0.42 g) 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 $C_{16}H_{16}N_4O_4 \cdot 1H_2O$: 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(3*H*,10*H*)-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(3*H*,10*H*)-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-d_6): 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-methylcarboxymethylpyrimido[4,5-*b*]-quinoline-2,4(3*H*,10*H*)-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 *p*H 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, C(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.

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. Theunis for obtaining the mass spectra, and Mr. H. Jongejan for the elemental analyses.

REFERENCES AND NOTES

[1] G. Scherings, H. Haaker and C. Veeger, *Eur. J. Biochem.*, **77**, 621 (1977).

[2] V. Massey and P. Hemerich, *Biochemistry,* **17**, 9 (1978).

[3] P. A. J. Link, H. C. van der Plas and F. Müller, *J. Heterocyclic Chem.*, **22**, (1984).

[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 (90 MHz, DMSO-d_6): δ 1.13 (t, 6H, 2 × ethyl CH_3), 1.22 (t, 6H, 2 × 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, 4 × 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.

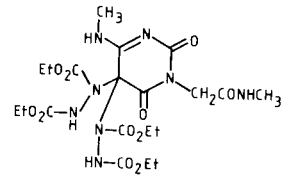


Figure 1

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

[5] H. Goldner, G. Dietz and E. Carstens, *Ann. Chem.*, **691**, 142 (1966).

[6] C. Harries and M. Weiss, *Ber.*, **33**, 3418 (1900).