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REDUCTION OF 5-URACILYLMETHYLENEPYRIDINIUM SALTS BY THIOLS. A MODEL OF THE REDUCTION STEP OF THE THYMIDYLATE SYNTHASE REACTION

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Abstract Reaction of 1-(5-uracilylmethylene)pyridinium salts with thiols leads to the formation of the corresponding thymine derivatives This transformation of a uracil derivative to the corresponding thymine is explained on the basis of the formation of an exocyclicmethylene intermediate, analogous to that proposed in the thymidylate synthase reaction, followed by its reduction by additional thiol, presumably involving a radical mechanism

The enzyme thymidylate synthase (E C 2 1 1 45) mediates the conversion of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP) [3], required for the biosynthesis of DNA The methyl group involved in this transformation is donated by the cofactor 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4-folate) The mechanism of the overall reaction involves the following steps (i) A nucleophilic attack by the thiol residue of Cys-198 (of the apoenzyme) at the C(6) position of the uracil moiety of the substrate, resulting in a nucleophilic centre at C(5), (ii) attack by the latter upon the iminium carbon of the (imidazolidine ring-opened) intermediate, derived from the cofactor, to result in a ternary apoenzyme-substrate-cofactor covalent complex, (iii) fragmentation of the ternary complex into an exocyclicmethylenedihydrouracil intermediate and tetrahydrofolate and (iv) reduction of the exocyclic intermediate by tetrahydrofolate via a "hydride equivalent" transfer, with concomitant release of the Cys-198 thiol and formation of dTMP (Fig 1) [3]

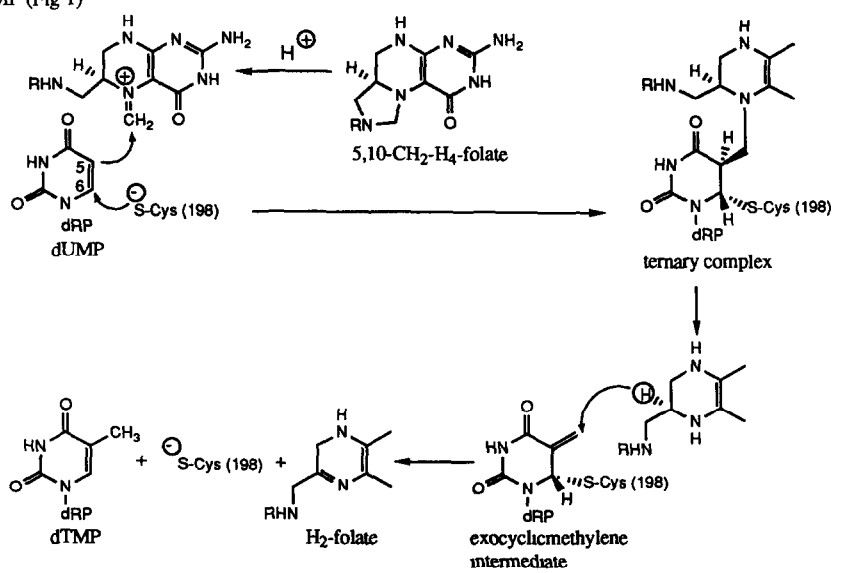
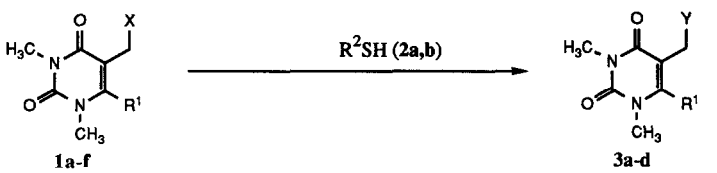
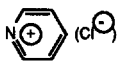
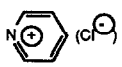
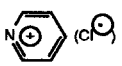


Fig 1

Direct evidence has been recently presented for the formation of the ternary complex [4] However, details of the fragmentation step and aspects of the reduction process require further enquiry [5] In this paper we describe the results of the reaction of uracil derivatives **1a-f** (Scheme I) with thiols (**2a,b**) which, serving as a model reaction, throw light upon the formation of the exocyclicmethylene intermediate and its reduction, via a radical mechanism, to thymine derivatives (**3a-d**)



1a-f **3a-d**

	R^1	X	R^2		R^1	Y
1a	H		**2a** p-(NO_2)-$C_6H_5CH_2$	**3a**	H	H
1b	H		**2b** t-butyl	**3b**	CH_3	H
1c	H	Cl		**3c**	p-(NO_2)-$C_6H_4CH_2S$	H
1d	H	I		**3d**	H	p-(NO_2)-$C_6H_4CH_2S$
1e	CH_3					
1f	SPh					

Scheme I

Results and Discussion

In a typical reaction the uracil derivative (0 3 mmol) and the thiol (0 75 mmol) were refluxed in 10 ml of dry distilled benzene The formation of the corresponding thymine derivative was followed by HPLC, using 3-methyluracil as an internal standard [6] The results are presented in Table 1

Table 1 Reaction of uracil derivatives with thiols

Uracil derivative	thiol	time (hours)	product	yield (%) [6]
1a	**2a**	169	**3a**	97
1a	**2b**	408	**3a**	35
1b	**2a**	96	**3a**	50 *
1c	**2a**	8	**3d**	80
1d	**2a**	8	**3d**	67
1e	**2a**	384	**3b**	18
1e	**2b**	576	**3b**	0
1f	**2a**	48	**3c**	13

* xylene (b p 140 °C) used as solvent

The formation of thymine derivatives **3a** or **3b** by the reaction of **1a** and **1e** with thiols, as a function of time, is presented in Fig 2 The thymine derivatives were isolated and identified by their spectra and by comparison with authentic samples Reaction of

1b with 2a proceeded very slowly to yield 3a, however, this reaction could be significantly expedited by addition of acetic acid Of mechanistic significance (vide infra) is the formation of 3c in the reaction of 1f with thiol 2a In contrast to the aforementioned, reaction of uracil derivatives 1c and 1d with thiol 2a resulted in the formation of thio ether 3d

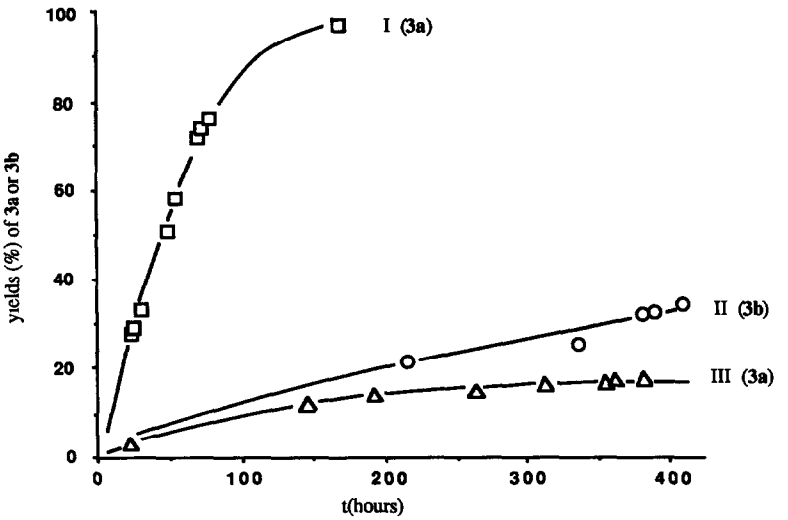
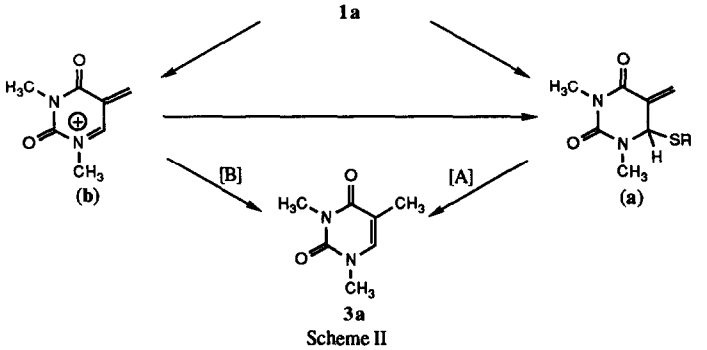


Fig 2 Rate of formation of thymine derivatives upon reaction of pyridinium salts with thiols (benzene, reflux)
I reaction of 1a with 2a (product 3a), II reaction of 1e with 2a (product 3b), III reaction of 1a with 2b (product 3a)

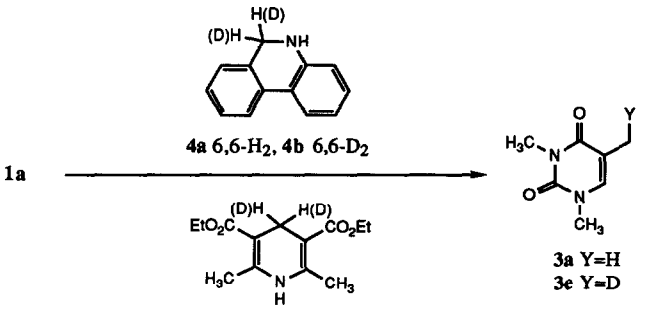
The formation of thymine derivatives from the salts (1a and 1e) can be rationalized on the basis of two mechanistic pathways (A and B) described for 1a in Scheme II According to mechanism A, 1a is converted to exocyclicmethylene intermediate a which is reduced via a radical mechanism - to be discussed later - to thymine 3a The formation of a can proceed by either the attack of a thiolate anion at C(6), followed by expulsion of pyridine, or via a reverse sequence of steps, that is, first the loss of pyridine (leading to b) and a subsequent addition of thiolate In mechanism B, the conjugated iminium ion is directly reduced by a hydride species



Scheme II

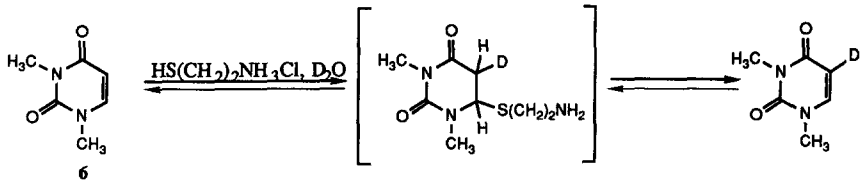
That pathway B can operate in the transformation of 1a to 3a is demonstrated by the experiments in which 1a is converted to 3a by heating with either dihydrophenanthridine (4a) or Hantzsch ester (5a) (Scheme III) When the corresponding deuterated reducing

agents (**4b**,**5b**) were employed, the thymine formed (**3e**) contained one deuterium atom in the C(5) methyl group (Scheme III), as attested by the ^{1}H NMR and mass spectra [7] of the product It is noteworthy, that the latter reactions proceeded at a slower rate than the analogous reductions by thiols Thus, formation of 50% **3a**, by reaction of **1a** with thiol **2a** and Hanzsch ester (**5**), takes place in two and seven days, respectively Furthermore, it was shown that when **1a** was allowed to react with a 1 1 mixture of **5b** and **2a**, the product of the reaction, based upon NMR analysis, was (non-deuterated) **3a** Another interesting and relevant observation is the fact that the perchlorate salt of **1a** is not reduced by tributyltinhydride in the presence of AIBN (typical radical reducing conditions), even after refluxing in benzene for two weeks This is interesting, since it is the second case observed by us [8] which suggests that α,ß-unsaturated iminium salts cannot be reduced by radical reducing agents In the event that this should be a general situation, then the α,ß-unsaturated iminium salts could be regarded as diagnostic substrates for hydride mediated reductions In the context of the present study, the implication would be that thiol mediated (presumably radical) reductions do not utilize an intermediate of type **b**



Scheme III

That the reaction of **1a** with thiols proceeds via mechanism A derives support from the considerations discussed in the sequel The intermolecular and intramolecular addition of nucleophiles to the C(6)-position of uracil derivatives has been widely evidenced [9] The intramolecular addition of thiols to C(6) of uracil derivatives, has been shown by us previously [10] As an example of intermolecular thiol group addition to uracil, it was demonstrated that an exchange of the C(5)-hydrogen took place when 1,3-dimethyluracil (**6**) was allowed to react with $HS(CH_2)_2NH_3Cl$ in D_2O (Scheme IV) The extent of the exchange was pD dependent and exhibited a maximum at about 9 8 [11] There was no exchange in the absence of the thiol This strongly suggests the addition of thiol to the uracil substrate, as expressed in Scheme IV

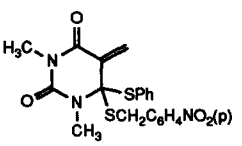


Scheme IV

That an intermediate such as (a) (Scheme II) is involved in the reaction of the pyridinium salts with thiols is supported by the observed differences in the rates of thymine formation when steric hinderance is introduced to the thiol addition step, either via substitution at C(6) of the substrate, or via increase in bulk of the thiol In this context, a comparison of the rates of thymine formation from **1a** and **1e** by reaction with **2a**,**b** is highly informative Inspection of Fig 2 shows that the rate (of **3a** or **3b** formation) de-

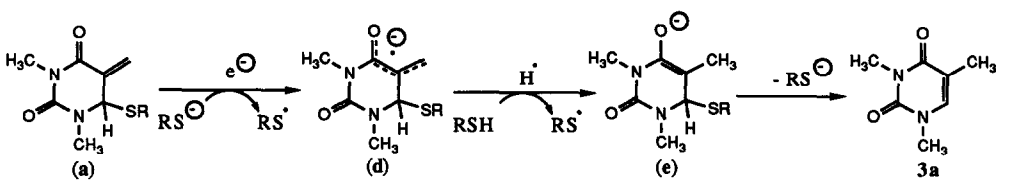
creases with an increase in hinderance to the thiol addition step at C(6) In case of salt **1e**, reaction with t-butylthiol (**2b**), is so highly hindered that the transformation is not measurable The formation of **3a** from **1b**, albeit at a very slow rate (50% conversion after refluxing for four days in xylene; b p 140 °C), is in accord with both, the reduced electrophilic character of C(6) - implicated in the thiol addition reaction - and the sluggishness of the pyrrolidine (pKa = 11 3) elimination step Addition of acetic acid promotes both of these processes In the absence of acetic acid, the thiol itself protonates the pyrrolidine nitrogen, thus accounting for a slow reaction The observation that compounds **1c** and **1d** react with thiol **2a** to yield only thio ether **3d**, also emphasizes the role of thiol addition to electrophilic C(6) in the overall reduction reaction A further decrease in the electropositive character of C(6) such as in **1e** and **1d** suppresses the thiol addition and allows the nucleophilic substitution of the halogen by thiol to become the predominant reaction pathway

The reaction of **1f**, a substrate which incorporates a thiol group at C(6), with thiol **2a**, is a critical case with respect to the evidence for pathway A The reaction leads to the formation of thymine derivative **3c** The exchange of the thiol residue accompanying the reduction process can be rationalized by invoking the formation of intermediate (c) [analogous to (a) in mechanism A, Scheme II] The reduction of this intermediate is accompanied by elimination of the more stable of the two thiol anions, namely, the phenyl mercaptide anion In this context it is highly relevant that there is no exchange of the thiol residue when 1,3-dimethyl-6-thiophenyluracil is allowed to react with p-nitrobenzylthiol (**2a**), under the conditions of the reaction



(c)

Whereas radical reactions of thiols, including reduction processes, are amply documented [12], in only one, somewhat related, case it has been suggested that thiols reduce by the delivery of a "hydride species" [13] The evidence presented for this mechanism however, is not unambiguous [14] In the light of the results obtained in our study we favour the reduction of intermediate (a) according to the sequence described in Scheme V In this mechanism, the thiol anion transfers an electron to (a) with the formation of radical anion (d) Subsequently, a second molecule of thiol delivers a hydrogen radical to intermediate (d), whereby anion (e) is generated The latter undergoes elimination of the thiol anion to give the final product **3a** The question whether the reduction involves a hydride or a radical mechanism is under further investigation in our laboratory



(a) (d) (e) **3a**

Scheme V

Experimental

Melting points were determined on a Leitz Wetzlar apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 257 spectrophotometer Absorptions are given in cm^{-1} NMR spectra were determined with Varian A60 (60 MHz), Bruker AC 200 (200 MHz) and Bruker WM 250 (250 MHz) instruments, using TMS as internal standard. Chemical shifts (∂) are given in ppm and spin-spin coupling constants (J) in Hertz Mass spectra were obtained with a Varian Matt 711 spectrometer (EI = electron impact,

FI = field ionization) Flash chromatography was applied using the method described by Still [15], using silicagel 60 (Merck) as stationary phase All solvents were distilled prior to use

Methylation of N(1) and N(3) of uracil and thymine derivatives

General procedure

A mixture of 3 0 mmol of uracil or thymine derivative, 35 mmol CH_3I and 35 mmol K_2CO_3 in 50 ml of acetone p a was stirred at room temperature for 50 hours The salts were removed by filtration, the solution was evaporated and the residue was suspended in 50 ml CH_2Cl_2 The insoluable salts were filtered and CH_2Cl_2 was evaporated Products were crystallized from ethanol

1,3-Dimethyluracil (6)

yield 378 mg (90 %), m p 120-122 °C (lit [16] 121 5 °C), IR ($CHCl_3$) 3000, 1710, 1660, ^{1}H NMR ($CDCl_3$) 3 32 (s, 3H, NCH_3), 3 37 (s, 3H, NCH_3), 5 70 (d, 2H, H^5, J = 7 8), 7 09 (d, 2H, H^6, J = 7 8)

1,3-Dimethylthymine (3a)

yield 318 mg (69 %), m p 156 °C (lit [17] 155 °C), IR ($CHCl_3$) 3000, 2900, 1685, 1660, 1610, ^{1}H NMR ($CDCl_3$) 1 94 (d, 3H, CH_3, J = 1 1), 3 36 (s, 3H, NCH_3), 3 37 (s, 3H, NCH_3), 6 99 (d, 1H, H^6, J = 1 1), MS (EI) 154 (M^+, 100 %), 97 (21), 68 (84), 56 (13), exact mass found 154 0738, calc for $C_7H_{10}N_2O_2$ 154 0743

1,3,6-Trimethylthymine (3b)

yield 328 mg (65 %), m p 128-130 °C (lit [18] 123-125 5 °C), IR ($CHCl_3$) 3000, 2950, 1685, 1640, 1420, 1350, ^{1}H NMR ($CDCl_3$) 1 96 (s, 3H, CH_3), 2 22 (s, 3H, CH_3), 3 33 (s, 3H, NCH_3), 3 40 (s, 3H, NCH_3), MS (EI) 168 (M^+, 100 %), 110 (7), 96 (11), 82 (52), 68 (15), 56 (75), 53 (7), 42 (14), 39 (7), 31 (11), 28 (20), 18 (30), exact mass found 168 0902, calc for $C_8H_{12}N_2O_2$ 168 0899

1,3-Dimethyl-5-chloromethyluracil (1c)

To a stirred solution of 6 3 g (45 mmol) of **6** in 150 ml of 30 % HCl was added 3 0 g (100 mmol) of paraformaldehyde The mixture was heated to 80 °C for 2 hours, while HCl gas was lead through After cooling to room temperature, the half of the solvent was evaporated and the remaining solution was extracted with $CHCl_3$ (3x) The $CHCl_3$ fractions were collected, washed with 50 ml H_2O, dried over $MgSO_4$ and evaporated, yielding 7 36 g (87 %) of **1c**, m p 146-147 °C (lit [19] 148-149 °C), IR ($CHCl_3$) 3000, 1710, 1660, 1640, 1460, 1440, ^{1}H NMR ($CDCl_3$) 3 36 (s, 3H, NCH_3), 3 42 (s, 3H, NCH_3), 4 39 (d, 2H, CH_2, J = 0 5), 7 34 (s, 1H, H^6)

1,3-Dimethyl-5-(N-pyrrolidino)methyluracil (1b)

A solution of 940 mg (5 0 mmol) of **1c** in 10 ml of pyrrolidine was stirred at room temperature for 48 hours The excess of pyrrolidine was evaporated and the residue was separated by flash chromatography (SiO_2, CH_2CL_2/CH_3OH 90 10), yielding 342 mg (31 %) of **1b** (after crystallization from ethanol), m p 198-200 °C, IR ($CHCl_3$) 2960, 2680, 1710, 1660, ^{1}H NMR ($CDCl_3$) 2 14 (m, 4H, 2x CH_2 pyrrolidine moiety), 3 35 (s, 6H, 2x NCH_3), 3 49 (m, 4H, 2x NCH_2 pyrrolidine moiety), 4 08 (s, 2H, CH_2), 8 62 (s, 1H, H^6), MS (EI) 223 (M^+, 23 %), 194 (56), 181 (6), 167 (12), 166 (7), 153 (29), 140 (12), 96 (46), 84 (8), 70 (100), 67 (5), 55 (30), 42 (47), 39 (7), exact mass found. 223 1319, calc for $C_7H_{17}N_3O_2$ 223 1321

1,3-Dimethyl-5-iodomethyluracil (1d)

A mixture of 566 mg (3 0 mmol) of **1c** and 600 mg (4 0 mmol) NaI in 35 ml CH_3CN was refluxed for 3 hours After cooling to room temperature the salts were removed by filtration and the solvent was evaporated, yielding 776 mg (92 %) of **1d**, m p 158-160 °C, IR ($CHCl_3$) 3000, 1700, 1660, 1480, 1460, ^{1}H NMR ($CDCl_3$) 3 33 (s, 3H, NCH_3), 3 36 (s, 3H, NCH_3), 4 16 (s, 2H, CH_2), 7 33 (s, 1H, H^6)

p-Nitrobenzylthiol (2a)

A mixture of 3 24 g (15 mmol) of p-nitrobenzylbromide and 1 71 g (15 mmol) $KSCOCH_3$ in 200 ml of absolute ethanol was stirred at room temperature for 24 hours The formed precipitate (KBr) was removed by filtration and the filtrate was evaporated The residue was dissolved in 150 ml 0 1N $NaOCH_3$ and stirred under N_2 for 15 min The mixture was acidified with 15 ml CH_3COOH and almost evaporated to dryness 250 ml of $CHCl_3$ was added and the mixture was basified with a saturated $NaHCO_3$ solution The organic layer was separated, washed with 50 ml H_2O, dried over Na_2SO_4 and evaporated, yielding 2 52 g (99 %) of **2a**, m p 48-50 °C (lit [20] 58 °C), IR ($CHCl_3$) 3000, 2830, 1580, 1510, 1330, ^{1}H NMR ($CDCl_3$) 1 82 (t, 1H, SH, J = 7 8), 3 79 (d, 2H, CH_2, J = 7 8), 7 47 (d, 2H, Ar, J = 8 7), 8 15 (d, 2H, Ar, J = 8 7)

1,3-Dimethyl-5-p-nitrobenzylthiomethyluracil (3d)

A mixture of 94 mg (0 5 mmol) of **1c**, 85 mg (0 5 mmol) of **2a** and 1 ml Et_3N in 50 ml CH_3CN was refluxed under Ar for 6 hours The solution was evaporated and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/EtOAc 50 50), yielding 124 mg (77 %) of **3d**, m p 105-106 °C, IR ($CHCl_3$) 3000, 2800, 1700, 1660, 1640, 1600, 1520, 1340, ^{1}H NMR ($CDCl_3$) 3 35-3 40 (3x s, 8H, CH_2, 2x NCH_3), 3 86 (s, 2H, CH_2), 7 08 (s, 1H, H^6), 7 51 (d, 2H, Ar, J = 8 6), 8 16 (d, 2H, Ar, J = 8 7), MS (EI) 321 (M^+, 16 %), 185 (42), 169 (11), 153 (100), 136 (14), 128 (8), 121 (6), 96 (71), 89 (8), 78 (9), 69 (7), 63 (5), 55 (28), exact mass found 321 0788, calc for $C_{14}H_{15}N_3O_4S$ 321 0783

5,6-Dihydrophenanthridine-6,6-d_2 (4b)
To a stirred suspension of 200mg (4 76 mmol) $LiAlD_4$ in 200 ml dried ether was added in portions 1 0 g (5 12 mmol) of phenanthridone The mixture was refluxed for 18 hours After cooling to room temperature 10 ml H_2O was added and the organic layer was separated, dried over $MgSO_4$ and evaporated, yielding 623 mg (67 %) of **4b** (after crystallization from hexane), m p 123-124 °C (m p 5,6-dihydrophenanthridine 123 °C [21]), IR ($CHCl_3$) 3400, 3000, 1600, 1500, ^1H NMR ($CHCl_3$) 3 56 (s, 1H, H^5), 6 69 (dd, 1H, Ar, J = 0 9, J = 7 9), 6 86 (dt, 1H, Ar, J = 1 1, J = 7 6), 7 20 (m, 4H, Ar), 7 70 (m, 2H, Ar), MS (FI) 183 (M^+)

5-Uracilylmethylenepyridinium salt 1a
To a stirred solution of 2 0 g (10 6 mmol) of **1c** in 60 ml CH_3CN was added 2 ml of pyridine The mixture was refluxed fot 16 hours After cooling to room temperature the solution was evaporated and the residue was washed with 15 ml ether twice, yielding 2 50 g (88 %) of **1a**, m p 208-209 °C, IR (KBr) 3050, 2980, 1700, 1650, 1630, 1475, ^1H NMR (DMSO-d_6) 3 14 (s, 3H, NCH_3), 3 39 (s, 3H, NCH_3), 5 54 (s, 2H, CH_2), 8 16 (t, 2H, pyridine moiety, J = 6 7), 8 44 (s, 1H, $H^{6'}$), 8 61 (t, 1H, pyridine moiety, J = 7 8), 9 19 (d, 2H, pyridine moiety, J = 6 0) Elemental analysis showed the presence of water of crystallization anal found C, 46 90, H, 5 89, N, 13 77, calc for $C_{12}H_{14}N_3O_2Cl$ 2 25 H_2O C, 46 75, H, 6 01, N, 13 64 The perchlorate salt of **1a** was obtained by exchange of the chloride ion of **1a** with perchlorate from $AgClO_4$, carried out in CH_3CN

5-Uracilylmethylenepyridinium salt 1e
To a stirred solution of 1 66 g (10 8 mmol) of 1,3,6-trimethyluracil in 75 ml 30 % HCl was added 750 mg (25 mmol) of paraformaldehyde The mixture was heated to 80 °C for 2 hours, while HCl gas was lead through After cooling to room temperature the half of the solvent was evaporated and the remaining solution was extracted with $CHCl_3$ (3x) The collected $CHCl_3$ fractions were dried over Na_2SO_4 and evaporated to dryness The residue was dissolved in 90 ml CH_3CN, 5 ml of pyridine was added and the mixture was refluxed for 48 hours After cooling to room temperature, the solution was evaporated and the crude product was washed with 15 ml of ether twice, yielding 816 mg (27 %) of **1e**, m p 212-214 °C, IR (KBr) 3020, 2950, 1685, 1625, 1470, 1350, ^1H NMR (D_2O) 2 58 (s, 3H, CH_3), 3 28 (s, 3H, NCH_3), 3 54 (s, 3H, NCH_3), 5 71 (s, 2H, CH_2), 8 06 (t, 2H, pyridine moiety, J = 6 8), 8 56 (t, 1H, pyridine moiety, J = 7 8), 8 85 (d, 2H, pyridine moiety, J = 5 7) Elemental analysis showed the presence of water of crytallization anal found C, 49 23, H, 6 30, N, 13 51, calc for $C_{13}H_{16}N_3O_2Cl$ 2 H_2O C, 49 13, H, 6 30, N, 13 23

5-Uracilylmethylenepyridinium salt 1f
A solution of 2 47 g (8 0 mmol) of 1,3-dimethyl-6-thiophenyluracil and 2 0 g (67 mmol) of paraformaldehyde in 150 ml 30 % HCl was stirred at room temperature for 2 hours, while HCl gas was lead through The half of the solution was evaporated and the residue was extracted with $CHCl_3$ (3x) The collected $CHCl_3$ fractions were washed with 50 ml H_2O and evaporated The resulting emulsion was dissolved in 200 ml CH_3CN, 20 ml of pyridine was added and the mixture was refluxed for 48 hours After cooling to room temperature the solution was evaporated and the residue was washed with 20 ml of ether twice, yielding 2 65 g (89 %) of a pale brown foam, IR (KBr) 3000, 1700, 1650, 1580, 1480, ^1H NMR (CD_3CN) 3 23 (s, 3H, NCH_3), 3 38 (s, 3H, NCH_3) 5 78 (s, 2H, CH_2), 7 30 (m, 5H, Ar), 7 90 (t, 2H, pyridine moiety, J = 7 0), 8 40 (t, 1H, pyridine moiety, J = 7 8), 8 87 (d, 2H, pyridine moiety, J = 5 6)

Reaction of **1f** with **2a**
A mixture of 356 mg (1 0 mmol) of **1f** and 423 mg (2 5 mmol) of **2a** in 10 ml of benzene was refluxed for 2 days After cooling to room temperature the solvent was evaporated and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 95 5), yielding 42 mg (13 %) of a pale yellow oil (**3d**), IR ($CHCl_3$) 3010, 2800, 1700, 1670, 1580, 1510, 1330, ^1H NMR ($CDCl_3$) 2 07 (s, 3H, CH_3), 3 36 (s, 3H, NCH_3), 3 37 (s, 3H, NCH_3), 3 90 (s, 2H, CH_2), 7 53 (d, 2H, Ar, J = 8 7), 8 15 (d, 2H, Ar, J = 8 7), MS (EI) 321 (M^+, 46 %), 261 (18), 218 (7), 185 (76), 166 (15), 150 (18), 136 (100), 128 (22), 120 (14), 106 (63), 99 (22), 90 (29), 78 (40), 66 (17), 56 (23), 42 (27), 28 (83)

Reaction of **1a** with **4a**
A mixture of 53 5 mg (0 2 mmol) of **1a** and 91 0 mg of **4a** in 10 ml of benzene was refluxed under Ar for 7 days After cooling to room temperature the solvent was evaporated and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/EtOAc 50 50, yielding 14 8 mg (48 %) of **3a**

Reaction of **1a** with **5a**
A mixture of 53 5 mg (0 2 mmol) of **1a** and 127 mg of **5a** in 10 ml of benzene was refluxed under Ar for 7 days After cooling to room temperature the solvent was evaporated and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/EtOAc 50 50, yielding 16 2 mg (53 %) of **3a**

References and Notes

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2 Taken in part from the research project carried out by G A Rood, in fullfilment of the requirements of the Drs diploma, University of Amsterdam

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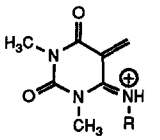
6 HPLC analyses were carried out on a Perkin Elmer 3D HPLC system (UV detection at 254 nm), using a Polygosil 60 C18 (4 6 x 125 mm) reversed phase column and CH_3OH/H_2O 50 50 as mobile phase The flow rate was set at 1 5 ml/min 3-Methyluracil was used as internal standard
7 Exact mass found. 155 0790, calc for $C_7H_9DN_2O_2$ 155 0805
8 α,ß-iminium salt **8** is reduced by Hantzsch Ester but not by tetrahydropterin (van der Meij, P F C , Doctorate Dissertation, University of Amsterdam, 1989, p 33)



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11 A solution of 1 0 mmol of **6** and 2 5 mmol of $HS(CH_2)_2NH_3Cl$ was heated in D_2O to 51 °C The experiment was carried out at several pD values A blank experiment (without thiol) was carried out to show the influence of the thiol H/D exchanges were calculated from ^1H NMR spectra of the solutions if the C(5) hydrogen is exchanged for deuterium, the intensity of the doublet from this hydrogen decreases and a singlet (∂ = 7 09 ppm) appears between the doublet The results are shown in table 2

sample no	reactants	pD	formation of **7** (%) 26 hours	100 hours
1	**6**	8 8	0	0
2	**6**, $HS(CH_2)_2NH_3Cl$	6 7	5	9
3	**6**, $HS(CH_2)_2NH_3Cl$	7 7	11	24
4	**6**, $HS(CH_2)_2NH_3Cl$	8 3	18	38
5	**6**, $HS(CH_2)_2NH_3Cl$	9 1	34	62
6	**6**, $HS(CH_2)_2NH_3Cl$	10 5	47	77
7	**6**, $HS(CH_2)_2NH_3Cl$	11 3	37	65

table 2

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