Stereochemistry of the Formation of 4-Alkoxyimino-5,6-dihydro-6-alkoxyaminopyrimidin-2(1*H*)-ones from Cytosines and Hydroxylamines

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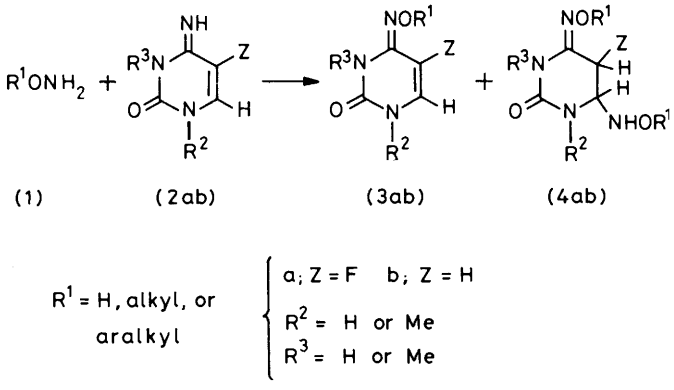
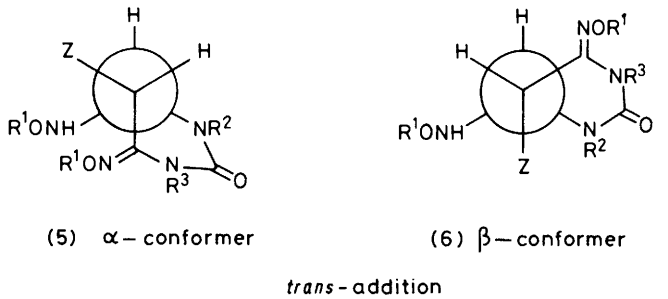
High resolution ^1H and ^{19}F n.m.r. data together with deuterium labelling studies are presented which reveal that the addition of hydroxylamines across the 5,6-double bond of cytosines is predominantly *trans*. The 4-alkoxyimino-5,6-dihydro-6-alkoxyaminopyrimidin-2(1*H*)-one products show *syn/anti* isomerism about the 4-alkoxyimino-group dependent on the substituent at N(3) (H or Me, respectively) and conformational changes throughout the molecules which are dependent on the substituents at N(1) (H or Me) and C(5) (H or F).

It is now well established that the reactions of hydroxylamines (1) with cytosine and its derivatives (2) may give rise to two products (3) and (4), the ratio of which depends upon the structure of (2) and the pH of the reaction medium.[1,2] The latter, in turn, depends to some extent upon (1) since maximum rate coefficients for the reaction are observed at a pH which is close to the pK_a of (1).[3-5] Thus for Z = Me the only detectable product is (3) whereas with Z = F and R^1 = H product (4) appeared to be unique. With (2a) and R^1 = Me or $PhCH_2$ however, small quantities of (3) were detected, especially at low pH (<4) [6] and it is well known that (2b) gives mixtures of (3) and (4) for all R^1. This paper seeks to focus attention on the stereochemistry associated with the formation of (4) since a systematic study of this topic in terms of variations in R^1,R^2, R^3, and Z has not yet been published.

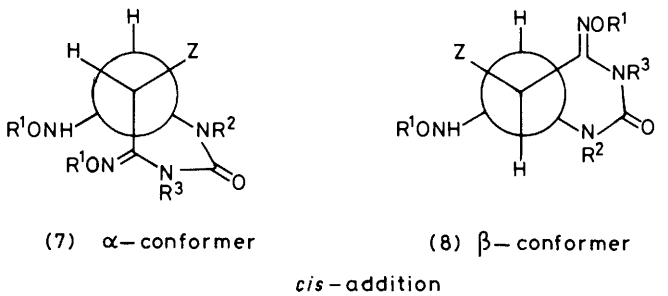


(1) (2ab) (3ab) (4ab)



(5) α–conformer (6) β–conformer

trans-addition



(7) α–conformer (8) β–conformer

cis-addition

Results and Discussion

The three fundamental problems associated with the stereochemistry of (4) are as follows. (i) Whether the addition of (1) across the 5,6-double bond of (2) is *cis* or *trans*. This gives rise to (ii) a conformational problem associated with the six-membered ring. For instance if Z = H, (4) may exist as two conformational isomers designated α or β whereas if Z = F (or D) *trans* addition may, in principle, be distinguished from *cis* but each diastereoisomer may exist in either the α- or β-conformation (5)—(8). (iii) Whether the hydroxyimino-group at C(4) is situated *syn* or *anti* to N(3).

The first problem was resolved for (4a; R^1 = H, Me, or $PhCH_2$ and $R^2 = R^3$ = H) by ^1H and ^{19}F n.m.r. which showed that coupling between fluorine and the proton at C(6) (Table 1) was uniquely consistent with *trans*-addition to form the β-conformer. This is understandable since the β-conformer allows hydrogen bonding between the fluorine and the NH of the 6-alkoxyamino-group whilst at the same time permitting hydrogen bonding between N(1)-H and the oxygen of the 6-alkoxyamino-group and between N(3)-H and the 4-alkoxyimino-group in its *syn*-configuration as shown in (9). The assignment is confirmed under resolution-enhanced conditions at 250 MHz by the fact that for R^1 = H or $PhCH_2$ and $R^2 = R^3$ = H, four-bond (W) couplings [7] of *ca.* 1.2 Hz were observed between the C(5) hydrogen and both N(1)-H and N(3)-H, thus placing the C(5) hydrogen essentially in the plane of the alkoxyiminopyrimidine ring structure (*i.e.* 'equatorial'). With R^2 = Me, however, $^3J_{HF}$ is decreased to 5.0 Hz and the four-bond coupling between C(5)-H and N(3)-H disappears suggesting that these two hydrogens are no longer coplanar. Hence the *trans*-β-isomer and the *cis*-α-isomer are eliminated and since the $^3J_{5H6H}$ coupling for this molecule is too small (5.0 Hz) for a *trans*-antiperiplanar disposition of the hydrogens, the *cis*-β-isomer also fails to fit. One is left with the *trans*-α-conformer in which the steric interference between the N(1)-CH_3 and the 6-alkoxyamino-group is minimised whilst maintaining hydrogen bonding between N(6)-H and fluorine and between N(3)-H and the 4-alkoxyimino-group (*syn*) as shown in (10). This analysis receives further support from the observation in the ^{19}F spectrum of four-bond coupling (J 5.3 Hz) between fluorine and the N(3)-H which places fluorine in the plane of the ring. Incidentally

Table 1. Coupling constants in the ^1H n.m.r. spectra of (4a) at 250 MHz

Assignment	Coupling (±0.1 Hz) for compounds: (4a; $R^1 = R^2 = R^3 = H$) ($[^2H_6]$DMSO)	(4a; R^1 = Me, $R^2 = R^3 = H$) ($[^2H_6]$DMSO)	(4a; $R^1 = PhCH_2$, $R^2 = R^3 = H$) ($CDCl_3$)	(4a; $R^1 = PhCH_2$, R^2 = Me, $R^3 = H$) ($CDCl_3$)
5-H ^2J_F	49.5	48.6	50.2	47.8
$^3J_{6H}$	2.9	3.2	2.5	5.1
$^4J_{N(3)H}$	1.1		1.25	0
$^4J_{N(1)H}$	1.1		1.25	0
6-H ^3J_F	19.0	16.0	19.6	5.0
$^3J_{6NH}$	8.0	6.8	11.0	3.0
$^3J_{N(1)H}$	1.1	2.3	0.9	0
6-NH ^4J_F			0.8	1.4
N(3)H ^4J_F	0	0	0	3.3

Table 2. Coupling constants in the ^1H n.m.r. spectra of (4b) at 250 MHz

Assignment		Coupling (±0.1 Hz) for compounds: (4b; $R^1 = R^2 = R^3 = H$) ($[^2H_6]$DMSO)	(4b; R^1 = Me, $R^2 = R^3 = H$) ($[^2H_6]$DMSO)	(4b; $R^1 = PhCH_2$, $R^2 = R^3 = H$) ($CDCl_3$)	(4b; $R^1 = PhCH_2$, R^2 = Me, $R^3 = H$) ($CDCl_3$)
5-H_A (low field, axial	$^2J_{5H_AH_B}$	15.6	15.6	15.6	15.8
	$^3J_{6H}$	4.5	5.1	5.1	5.0
5-H_B (high field, equatorial)	$^2J_{5H_AH_B}$	15.4	15.6	15.5	15.8
	$^3J_{6H}$	2.3	1.2	4.2	2.2
	$^4J_{N(1)H}$	†		1.1	
	$^4J_{N(3)H}$	†	1.2	0.9	1.1
6-H	$^3J_{6NH}$	3.6	3.5	6.7	4.9
	$^3J_{N(1)H}$	†	4.5	3.0	

† Signal broad due to exchange, therefore no small couplings observed.

Table 3. ^1H N.m.r. data of products from the reactions of (2b; R^2 = H, R^3 = H or Me) and 5-deuterio-(2b) with (1; $R^1 = PhCH_2$) and dideuterio-(1)

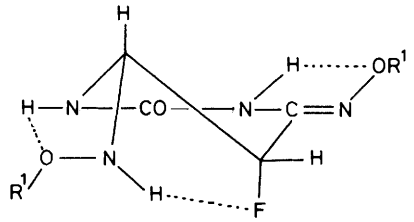
Reactants	δ (5-H_AH_B)	J/Hz	
(2b; $R^2 = R^3 = H$) + (1)	2.73—2.48	$^3J_{5H_A,6H}$	5.0
		$^3J_{5H_B,6H}$	3.9
[5-^{2}H]-(2b; $R^2 = R^3 = H$) + (1)	2.69	$^3J_{5H_A,6H}$	5.2
(2b; $R^2 = R^3 = H$) + $[^2H_2]$-(1)	2.51	$^3J_{5H_B,6H}$	3.8
[5-^{2}H]-(2b; $R^2 = R^3 = H$) + $[^2H_2]$-(1)	None		
(2b; R^2 = H, R^3 = Me) + (1)	3.04—2.64	$^3J_{5H_A,6H}$	5.8
		$^3J_{5H_B,6H}$	4.4
(2b; R^2 = H, R^3 = Me) + $[^2H_2]$-(1)	3.00	$^3J_{5H_B,6H}$	4.1

the ^{19}F chemical shift of the α-isomer is 4.5 p.p.m. upfield of the β-isomer.

Examination of the n.m.r. spectra of the residues of reaction mixtures from which the above products crystallised showed that no other isomers of (4) had been formed. Thus the reaction is highly stereoselective with *trans*-stereochemistry about the double bond and produces the β-isomer provided N(1) is bound to hydrogen but the α-isomer for R^2 = Me.

With Z = H [*i.e.* (4b)] and R^1 = H, Me or $PhCH_2$, R^2 = H or Me and R^3 = H, the data (Table 2) are consistent with either the *trans*-α-isomer (5) or the *cis*-α-isomer (7) but the *cis*- or *trans*-β-isomers are excluded by the fact that the vicinal ($^3J_{5H6H}$) coupling constants never exceed 5.1 Hz and would be expected to be much larger for *trans*-antiperiplanar hydrogens.

The hydrogens at C(5) [of (4b)] form the AB part of an ABX system and high resolution n.m.r. reveals that the high field signal of the AB system is a quartet of triplets. Homonuclear decoupling established that the triplets were due to four-bond (W) coupling between one hydrogen at C(5) and the N(1) and N(3) hydrogens of the ring. Hence the high field proton of the AB system must be in the plane of the pyrimidinone ring, *i.e.* in an ' equatorial ' position. In the case of *trans*-α-addition to the 5,6-double bond, the equatorial proton at C(5) originates from the substrate (2) whereas with *cis*-addition the equatorial proton would arise from hydroxylamine. A proof of the stereochemistry of (4b) was therefore provided by analysis of the ^1H n.m.r. spectra of products from reaction of cytosine and 5-deuteriated cytosine with both deuteriated and non-deuteriated *O*-benzylhydroxylamine. The results are summarised in Table 3 which shows that with 5-deuteriocytosine



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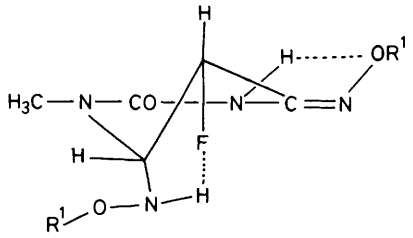

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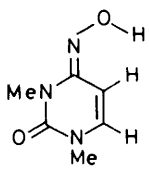
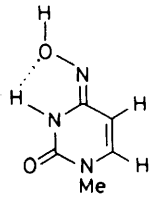
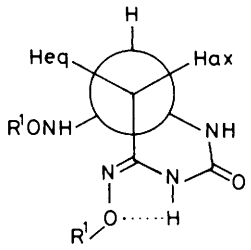


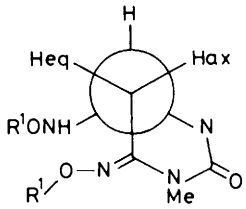
(11)– *anti*



(12)– *syn*



(13)– *trans*-α-*syn*



(14)– *trans*-α-*anti*

and *O*-benzylhydroxylamine a doublet is observed at δ 2.69 with vicinal coupling ^3J 5.2 Hz which corresponds to the low field section of the AB spectrum. Thus the equatorial proton is occupied by deuterium which indicates that the addition is stereoselective and *trans*, a result which is confirmed by the observation of a doublet (^3J 3.8 Hz) at δ 2.51 from the reaction of cytosine with deuterio-*O*-benzylhydroxylamine. No long range coupling could be observed in these experiments due to signal broadening. Thus we may conclude that for (2b) all the above reactions to form (4) are stereoselectively *trans* about the 5,6-bond and proceed to form the α-conformer. What is not clear however is why the 6-alkoxyamino-group prefers to be axial rather than equatorial in every case.

With 3-methylcytosine a slightly different picture emerged. In the AB region of the spectrum of (4b; R^1 = $PhCH_2$, R^2 = H and R^3 = Me) although all the coupling constants were still consistent with the *trans*-α-isomer the low field signal at δ 3.0 appeared as a quartet of doublets due to four-bond coupling with N(1)-H (confirmed by decoupling) and the high field signal remained as a quartet. Thus the equatorial proton has moved downfield by *ca.* 0.5 p.p.m. which is analogous to the work reported by Brown [8] who noted that the C(5) proton in 1,3-dimethyl-4-hydroxyiminopyrimidin-2-one (11) appeared at lower field (δ 6.15) than the corresponding proton (δ 5.69) of the 1-methyl analogue (12). The difference was attributed to a difference in configuration between the hydroxyimino-group in (12)-*syn* due to intramolecular hydrogen bonding and (11)-*anti*, imposed by steric factors.* Hence the change in chemical shift of the equatorial proton in (4b; R^3 = Me) is also probably due to a change in configuration of the 4-alkoxyimino-group from *syn* [as in (13)] to *anti* [as in (14)] directed by loss of hydrogen bonding to N(3)-H.

Thus the formation of (4b) is stereoselective *trans*-α and if the substituent at N(3) is hydrogen, the *syn*-form of the hydroxyimino-function is preferred but with an alkyl group at N(3) the *anti*-form is obtained, the choice being dictated by intramolecular hydrogen bonding.

When the reaction of (2a) with *O*-methylhydroxylamine at pH 3.4 was monitored by h.p.l.c., two products of type (4a) were isolated. Both had the same analysis but different m.p.s and the isomer with the lower retention time had an identical

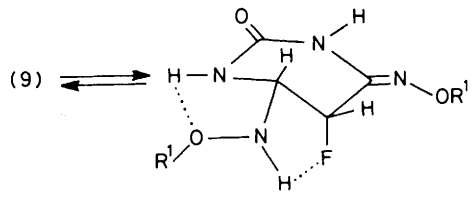
* We observed a similar phenomenon for (3b; R^1 = Me, R^3 = H or Me).

Table 4. Comparison of ^{1}H n.m.r. data (in [$^2H_6$]DMSO) on the two products of type (4a) isolated from the reaction of (2a) with (1; R^1 = Me) by h.p.l.c.

Assignment		Coupling (±0.1 Hz) for compounds: (9)	(15)
5-H	^2J_F	δ 5.1—5.3	δ 4.82—5.02
	$^3J_{6H}$	48.6	47.6
	$[J_{N(3)H}$	3.2	2.2
	$[J_{N(1)H}$		1.1
			0
6-H	^3J_F	δ 4.80	δ 4.36
	$^3J_{5H}$	16.0	9.0
	$^3J_{N(1)H}$	3.0	2.4
		2.3	4.4
6-NH	$^3J_{6H}$	δ 6.90	δ 6.8
	^4J_F	6.9	3.3
			3.1

n.m.r. spectrum to that reported in Table 1 for (4a; R^1 = Me, R^2 = R^3 = H) as isolated by crystallisation and hence was identified as the β-isomer of *trans*-addition. The second product showed proton resonance signals in precisely the expected ratios but significant changes in both chemical shifts and coupling constants (Table 4).

The fall in $^3J_{HF}$ from *ca.* 16 to 9 Hz is not sufficient to diagnose the material as a *trans*-α-conformer [*cf.* (4a; R^1 = $PhCH_2$, R^2 = Me) for which $^3J_{HF}$ 5.0 Hz] but it does denote a decrease in the dihedral angle between F and C(6)-H. Furthermore, the ^{19}F n.m.r. of the second fraction showed a single, long range coupling (J 3.1 Hz) to N(6)-H which disappeared from the spectrum in [$^2H_4$]MeOH by exchange and which was confirmed by the ^{1}H n.m.r. data on N(6)-H. The α-isomer would have been expected to show four-bond coupling to both N(1)-H and N(3)-H and hence it seems likely that the new compound is still a *trans*-β-isomer but modified in some way. The decrease in $^2J_{6HF}$ is accompanied by a slight decrease in $^3J_{5H6H}$, a slight increase in $^3J_{6HN(1)H}$, a considerable decrease in $^3J_{6NH6H}$, and the appearance of a four-bond coupling between C(5)-H and N(3)-H of 1.1 Hz. It is sugges-



ted that the best way to accommodate these facts within the *trans*-β-system is a slight adjustment in ring geometry from conformation (9) to conformation (15) brought about by a change from *syn*- to *anti*-isomerism about the 4-hydroxyimino-bond. The significant downfield shift of the ^{19}F resonance (by *ca.* 20 p.p.m.) denotes a considerable decrease in the shielding at fluorine which appears (at least in the ^1H spectra) to be linked with a change in configuration of the 4-hydroxyimino-group from *syn* to *anti*. Incidentally, as noted earlier, the *trans*-α-*syn* isomer of (4a; R^2 = Me) has a ^{19}F signal *ca.* 4 p.p.m. upfield of its *trans*-β-*syn* isomer. It now only remains to define the origin of (15). A careful examination of h.p.l.c. data and n.m.r. spectra revealed that (15) was not obtained as a product from the reaction of (2a) with *O*-methylhydroxylamine at pH 4.5. At pH 3.5, however, *ca.* 20% of the addition–substitution product (4a; R^1 = Me, R^2 = R^3 = H) was in fact (15) rather than (9) but at this lower pH substantial quantities of the substitution product (3a) were also formed. This suggested that (15) was derived from (3a) rather than (2a) and a series of h.p.l.c. experiments confirmed this hypothesis. At pH 4.5, reaction of (3a; R^1 = Me, R^2 = R^3 = H) gave (15) and a barely detectable quantity of (9) and a similar result was obtained at pH 3.5 with, in this case, an additional peak at low retention time corresponding to a small quantity (*ca.* 10%) of 5-fluorouracil from the acid-catalysed hydrolysis of (3a). Since it is unlikely that simple addition of *O*-methylhydroxylamine across the 5,6-double bond of (3a) would change the configuration of the hydroxyimino-group from *syn* to *anti*, the isolation of (15) implies, yet again, that addition at C(4) is involved in the mechanism of addition to the 5,6-double bond.

Experimental

^1H and ^{19}F n.m.r. spectra were recorded on Bruker HFX 90, WM 250, and WH 400 (Queen Mary College, London) spectrometers with Me_4Si and $CFCl_3$ as the respective internal standards.

M.p.s (all uncorrected) were obtained using a Kofler hot-stage microscope and elemental analyses were carried out at University College, London or the School of Pharmacy, Department of Chemistry, University of London.

The various commercial sources of materials were as follows: cytosine (B.D.H.); 5-fluorocytosine and 3-methylcytosine (Sigma); L-(+)-cysteine (Aldrich); methoxyamine hydrochloride (Eastman; recrystallised twice from ethanol before use); hydroxylamine hydrochloride (Fisons). Benzyloxyamine hydrochloride was prepared by a standard procedure from *N*-hydroxyphthalimide.[9]

4-Amino-5-deuteriopyrimidin-2(1H)-one (*5-Deuteriocytosine*).—L-(+)-Cysteine (2.33 g, 1.92×10^{-2} mol) was suspended in D_2O (15 ml) and warmed until all the cysteine dissolved. The solution was then cooled and the process repeated twice more, after which the solution was evaporated to dryness under reduced pressure to yield L-(+)-[^2H]cysteine.

L-(+)-[^2H]Cysteine was then suspended in D_2O (20 ml), and on warming dissolved. To this was added cytosine (230 mg, 2.67×10^{-3} mol), which dissolved with stirring. The mixture was adjusted to pH 8.0 and left at 90 °C for 16 h. The mixture was then concentrated (to *ca.* 10 ml), adjusted to pH 6.5, filtered, and the filtrate applied to a preparative scale reverse-phase h.p.l.c. column. The eluant used was a 0.02M solution of ammonium formate in aqueous methanol (4% MeOH in H_2O, v/v) at a flow rate of 3 ml min^{-1}. The detection system operated at 254 nm and the [5-^2H]cytosine fraction had a retention time of *ca.* 240 s. The eluant fraction was accumulated by a series of repeat injections until all the filtrate had been applied to the column. The fraction was then freeze-dried to give 110 mg (48%) of solid; δ_H (CD_3OD; 250 MHz) 7.45 (1 H, s, 6-H) and 5.9 (d, 5-H, integration indicates <5% H at C-5).

1-Methyl-4-aminopyrimidin-2(1H)-one (*1-Methylcytosine*).—Cytosine (1.0 g, 9.0×10^{-3} mol) was placed in a round-bottom flask (50 ml) under nitrogen. To this was added hexamethyldisilazane (HMDS) (20 ml) and trimethylchlorosilane (TMCS) (1 ml) and the mixture was heated under reflux until all the cytosine had dissolved (*ca.* 2.5 h). The mixture was allowed to cool, methyl iodide (10 ml) was introduced slowly, and the mixture was then reheated and monitored periodically by t.l.c. When no cytosine remained [*ca.* 5 h, R_F ($CHCl_3$–EtOH 3 : 1, v/v) 0.34] the mixture was cooled and evaporated to dryness under reduced pressure to give a residue. This was treated with acetic acid (2N, 20 ml) and the yellow solution was evaporated to dryness under reduced pressure. The resulting pale yellow residue was recrystallised from ethanol to give 855 mg (76%) of the title compound as crystals, m.p. 296 °C (decomp.) [lit.,[10] 285 °C (decomp)]; δ_H (D_2O; 250 MHz) 7.75 (1 H, d, *J* 7.5 Hz, 6-H), 6.09 (1 H, d, 5-H), and 3.42 [3 H, s, N(1)CH_3].

1-Methyl-4-amino-5-fluoropyrimidin-2(1H)-one (*1-Methyl-5-fluorocytosine*).—This compound was obtained from 5-fluorocytosine (489 mg, 3.8×10^{-3} mol) by employing a similar procedure to that used above [5-fluorocytosine R_F ($CHCl_3$–MeOH, 2 : 1, v/v) 0.4] to give 511 mg (94%) of the title compound, m.p. >300 °C, δ_H (D_2O; 250 MHz) 7.76 (1 H, d, *J* 5.9 Hz, 6-H) and 3.36 [3 H, s, N(1)CH_3].

4-Hydroxyimino-5-fluoro-5,6-dihydro-6-hydroxyaminopyrimidin-2(1H)-one (4a; R^1 = R^2 = R^3 = H).—5-Fluorocytosine (1.5 g, 1.16×10^{-2} mol) was suspended in methanol (10 ml) and a solution of hydroxylamine hydrochloride (8.08 g, 1.16×10^{-1} mol) in water (20 ml) was added. The pH of the mixture was adjusted to *ca.* 6.0 [NaOH (10N)] and the mixture was stirred at room temperature until all the 5-fluorocytosine had dissolved (3 h). After 36 h a 90% decrease in the absorbance at 280 nm was observed and a precipitate was collected to give 1.76 g (88.2%) of the *title compound*, m.p. 270 °C (Found: C, 26.4; H, 4.0; N, 30.7. $C_4H_7FN_4O_3$ requires C, 26.95; H, 3.95; N, 31.45%), δ_H ([$^2H_6$]DMSO; 250 MHz) 10.7 (1 H, s, 4-NOH), 8.93br [1 H, s, N(3)H], 7.77 [1 H, d, N(1)H], 7.2 (1 H, s, 6-NOH), 5.84 (1 H, dd, 6-NH), 5.11 (1 H, m, 5-H), and 4.47 (1 H, m, 6-H); δ_F ([$^2H_6$]DMSO; 235.63 MHz) −197.61 (dd, 5-F); δ_C ([$^2H_6$]DMSO; 22.62 MHz) 152.81 (s, C-2), 141.05 (d, $^2J_{CF}$ 17.2 Hz, C-4), 80.86 (d, $^1J_{CF}$ 179.6 Hz, C-5), and 68.57 (d, $^2J_{CF}$ 19.9 Hz, C-6).

4-Methoxyimino-5-fluoro-5,6-dihydro-6-methoxyaminopyrimidin-2(1H)-one (4a; R^1 = Me, R^2 = R^3 = H).—This compound was prepared from 5-fluorocytosine (0.67 g, 5.2×10^{-3} mol) and *O*-methylhydroxylamine hydrochloride (8.44 g, 1.0×10^{-1} mol) by a similar method except that the reaction was buffered at pH 4.5. The product was collected as crystals and recrystallised from methanol to give 0.64 g (65%) of the

title compound, m.p. 163 °C (Found: C, 33.6; H, 5.35; N, 26.25. $C_6H_7FN_4O_3$ requires C, 34.95; H, 5.4; N, 27.1%), δ_H ([$^2H_6$]DMSO; 250 MHz) 9.24 [1 H, s, N(3)H], 7.49 [1 H, s, N(1)H], 6.91 (1 H, d, 6-NH), 5.19 (1 H, dd, 5-H), 4.61 (1 H, m, 6-H), 3.74 (3 H, s, 4-$NOCH_3$), and 3.43 (3 H, s, 6-$NOCH_3$); δ_F ([$^2H_6$]DMSO; 235.16 MHz) −199.71 (dd, 5-F); δ_C([$^2H_6$]-DMSO; 62.85 MHz) * 150.58 (s, C-2), 117.07 (d, $^2J_{CF}$ 31.6, C-4), 80.63 (d, $^1J_{CF}$ 183.1, C-5), 65.99 (d, $^2J_{CF}$ 19.6, C-6), 61.80 (s, 4-OCH_3), and 61.4 (s, 6-OCH_3).

4-*Benzyloxyimino*-5-*fluoro*-5,6-*dihydro*-6-*benzyloxyamino*-*pyrimidin*-2(1H)-*one* (4a; R^1 = $PhCH_2$, R^2 = R^3 = H).—This product was prepared from 5-fluorocytosine (0.74 g, 5.77 × 10^{-3} mol) and *O*-benzylhydroxylamine hydrochloride (3.58 g, 2.9 × 10^{-2} mol) in aqueous methanol (4:1; 50 ml) at pH 4.1. The precipitated product was recrystallised from methanol to give 1.05 g (51%) of *crystals*, m.p. 127 °C (Found: C, 60.4; H, 5.35; N, 15.7. $C_{18}H_{19}FN_4O_3$ requires C, 60.35; H, 5.35; N, 15.65%), δ_H ($CDCl_3$) 7.73 [1 H, s, N(3)H], 7.33, 7.32 (10 H, 2 × s, C_6H_5), 6.05 [1 H, s, N(1)H], 5.84 (1 H, dd, 6-NH), 5.08 (2 H, s, 4-OCH_2), 5.03 (1 H, dd, 5-H), 4.70 (2 H, s, 6-OCH_2), and 4.56 (1 H, oct, 6-H); δ_F ($CDCl_3$) −100.2 (dd, 5-F).

1-*Methyl*-4-*benzyloxyimino*-5-*fluoro*-5,6-*dihydro*-6-*benzyl*-*oxyaminopyrimidin*-2(1H)-*one* (4a; R^1 = $PhCH_2$, R^2 = Me, R^3 = H).—This compound was formed by the reaction of *O*-benzylhydroxylamine (234 mg, 1.92 × 10^{-3} mol) with 1-methyl-5-fluorocytosine (30.6 mg, 2.14 × 10^{-4} mol) in 50% aqueous methanol (3 ml) at pH 4.0. After 4 days the precipitated product was collected and recrystallised from methanol to give 35.6 mg (44.7%) of pale yellow *crystals*, m.p. 169 °C (Found: C, 61.5; H, 5.8; N, 14.2. $C_{19}H_{21}FN_4O_3$ requires C, 61.3; H, 5.65; N, 15.0%); δ_H ($CDCl_3$; 250 MHz) 7.52 [1 H, s, N(3)H], 7.32 (10 H, m, C_6H_5), 6.08 (1 H, d, 6-NH), 5.34 (1 H, dd, 5-H), 5.06 (2 H, s, 4-OCH_2), 4.65 (2 H, s, 6-OCH_2), 4.45 (1 H, m, 6-H), and 3.02 [3 H, s, N(1)CH_3]; δ_F ($CDCl_3$; 235.16 MHz) −204.92 (dt, 5-F); δ_C ($CDCl_3$; 62.89 MHz) 150.7 (s, C-2), 141.5 (d ^2J_F 17.4 Hz, C-4), 136.7 (s, *ipso* C), 128.4 (phenyl C), 79.6 (d, ^1J_F 188.5 Hz C-5), 77.0 (s, 2 × OCH_2), 72.8 (d, ^2J_F 21.8 Hz, C-6), and 33.7 p.p.m [s, N(1)-CH_3].

4-*Hydroxyimino*-5,6-*dihydro*-6-*hydroxyaminopyrimidin*-2(1H)-*one* (4b; R^1 = R^2 = R^3 = H).—Cytosine (1.0 g, 9.0 × 10^{-3} mol) was suspended in methanol (20 ml) and added to a solution of hydroxylamine hydrochloride (6.26 g, 9.0 × 10^{-2} mol) in water (20 ml). The pH was adjusted to *ca.* 6.0 [NaOH (10N), 5 ml] and the pyrimidinone dissolved. After 12 h a solid had precipitated which was collected, and a u.v. spectrum of the filtrate revealed *ca.* 20% of the initial absorbance value at λ 270 nm. After 36 h a further examination revealed no change in the u.v. spectrum and no more solid had precipitated. The solid was recrystallised from methanol to give 0.78 g (54%) of the title compound, m.p. 151 °C (decomp.); [lit.,[11] 157—158 °C (decomp.)], which showed a single spot on silica t.l.c., R_F 0.46 (MeOH) and 0.22 (water-saturated butanol), δ_H ([$^2H_6$]DMSO; 250 MHz) 9.86 (1 H, s, 4-NOH), 8.22 [1 H, s, N(3)H], 7.53 [1 H, s, N(1)H], 7.36 (1 H, s, 6-NOH), 5.68br (1 H, s, 6-NH), 4.18 (1 H, m, 6-H), and 2.47 (2 H, m, ABq of ABX system, 5-H_AH_B); δ_C ([$^2H_6$]DMSO; 22.62 MHz) 151.81 (s, C-2), 142.96 (s, C-4), 64.56 (s, C-6), and 26.21 (s, C-5).

4-*Methoxyimino*-5,6-*dihydro*-6-*methoxyaminopyrimidin*-2(1H)-*one* (4b; R^1 = Me, R^2 = R^3 = H).—Cytosine (0.48 g, 4.32 × 10^{-3} mol) was suspended in a solution of *O*-methylhydroxylamine hydrochloride (3.98 g, 4.76 × 10^{-2} mol) in water (20 ml). The mixture was adjusted to pH 4.5 [NaOH (10N)] and stirred until all the cytosine dissolved (4 h). After 6 days at 38 °C two u.v. absorbing spots could be seen by t.l.c. (R_F 0.32, 0.53; water-saturated butanol). The mixture was adjusted to pH 8.5, evaporated to dryness under reduced pressure, and the resulting residue was dissolved in a minimum of water-saturated butanol. This solution was then applied to a silica gel column, and eluted with water-saturated butanol, monitoring the eluant by u.v. The band of the title compound was collected (R_F 0.53) and evaporated to dryness under reduced pressure to give a residue which was recrystallised from methanol–n-pentane to give (4b; R^1 = Me) (0.37 g, 45.5%), m.p. 134 °C (lit.,[11] 136 °C); δ_H ([$^2H_6$]DMSO; 250 MHz) 8.62 [1 H, s, N(3)H], 7.55 [1 H, s, N(1)H], 6.71 (1 H, d, 6-NH), 4.24 (1 H, m, 6-H), 3.64 (3 H, s, 4-$NOCH_3$), 3.40 (3 H, s, 6-$NOCH_3$), and 2.56 (2 H, m, ABq of ABX system, 5-H_AH_B).

The preparation of 4-benzyloxyimino-5-deuterio-5,6-dihydro-6-benzyloxyaminopyrimidin-2(1*H*)-one (4b; R^1 = $PhCH_2$, R^2 = R^3 = H) has been described previously.[12]

1-*Methyl*-4-*benzyloxyimino*-5,6-*dihydro*-6-*benzyloxyamino*-*pyrimidin*-2(1H)-*one* (4b; R^1 = $PhCH_2$, R^2 = Me, R^3 = H).—1-Methylcytosine (33 mg, 2.64 × 10^{-4} mol) was dissolved in water (1.5 ml), added to a solution of *O*-benzylhydroxylamine (281.5 mg, 2.3 × 10^{-3} mol) in methanol (1.5 ml) and the mixture adjusted to pH 4.0 with concentrated HCl. After 72 h at room temperature a crystalline solid had precipitated which was filtered off to give 40.4 mg (43.2%) of the *title compound*, m.p. 158 °C (Found: C, 62.25; H, 6.45; N, 14.95. $C_{19}H_{22}N_4O_3$ requires C, 64.14; H, 6.25; N, 15.8%), δ_H ($CDCl_3$; 250 MHz) 7.63 [1 H, s, N(3)H], 7.35 (10 H, m, C_6H_5), 5.63 (1 H, d, 6-NH), 4.99 (2 H, s, 4-OCH_2), 4.62 (2 H, s, 6-OCH_2), 4.34 (1 H, m, 6-H), 3.02 [3 H, s, N(1)CH_3], and 2.76 (2 H, m, ABq of ABX system, 5-H_AH_B).

3-*Methyl*-4-*benzyloxyimino*-5,6-*dihydro*-6-*benzyloxyamino*-*pyrimidin*-2(1H)-*one* (4b; R^1 = $PhCH_2$, R^2 = H, R^3 = Me).—This compound was prepared by a method analogous to that above but using 3-methylcytosine hydrochloride (22 mg, 1.35 × 10^{-4} mol). After 4 days the title compound was obtained as a solid (37.5 mg, 78%), m.p. 93—94 °C (lit.,[13] 93 °C), δ_H ($CDCl_3$; 400 MHz) 7.30 (10 H, m, C_6H_5), 5.90br [1 H, s, N(1)H], 5.40vbr (1 H, s, 6-NH), 5.01 (2 H, s, 4-OCH_2), 4.63 (2 H, s, 6-OCH_2), 4.41 (1 H, m, 6-H), 3.15 [3 H, s, N(3)CH_3], and 2.91 (2 H, m, ABq of ABX system, 5-H_AH_B).

Reactions analysed by H.p.l.c.—5-Fluorocytosine–O-methylhydroxylamine hydrochloride. 5-Fluorocytosine (311 mg, 2.4 × 10^{-3} mol) was added to a solution of *O*-methylhydroxylamine hydrochloride (4.30 g, 5.1 × 10^{-2} mol) in water (40 ml). The mixture was stirred, adjusted to pH 3.4 [NaOH (10N)], and left at 38 °C for 40 h. The solution was then adjusted to pH *ca.* 10.0 and evaporated to dryness under reduced pressure, to give a pale yellow residue. This was dissolved in a minimum of aqueous methanol (10% MeOH in H_2O, v/v; 20 ml) and adjusted to pH 6.0, filtered and applied to a preparative scale reverse-phase h.p.l.c. column.

With a flow rate of 4 ml min^{-1}, an eluant of ammonium formate (0.02M) in aqueous methanol (10% MeOH in H_2O, v/v), and the detector set at 254 nm, four peaks were observed. The reaction mixture was applied by repeated injections and three of the four eluant fractions were collected and freeze-dried. The four fractions are assigned as follows.

Fraction 1 had retention time 120—225 s and was unchanged 5-fluorocytosine. It was not collected.

Fraction 2, retention time 370—450 s, was a solid (134 mg,

* Shifts relative to [$^2H_6$]DMSO at δ 39.5 p.p.m.

27.1%). This compound showed identical n.m.r. spectra (^1H and ^{19}F) and m.p. as 4-methoxyimino-5-fluoro-5,6-dihydro-6-methoxyaminopyrimidin-2(1*H*)-one (4a; R^1 = Me, R^2 = R^3 = H).

Fraction 3, retention time 500—550 s, was a solid (76.8 mg, 20.1%), m.p. 178 °C, identified as 4-*methoxyimino*-5-*fluoropyrimidin*-2(1H)-*one* (3a; R^1 = Me, R^2 = R^3 = H) (Found: C, 35.8; H, 4.05; N, 24.8. $C_5H_6FN_3O_2$ requires C, 37.75; H, 3.8; N, 26.4%), δ_H ([$^2H_6$]DMSO; 250 MHz) 9.7 [1 H, s, N(1)H], 7.09 (1 H, d, *J* 7.3 Hz, 6-H), and 3.71 (3 H, s, 4-$NOCH_3$); δ_H (CD_3OD; 250 MHz) 6.87 (1 H, d, *J* 6.6 Hz, 6-H), and 3.84 (3 H, s, 4-$NOCH_3$); δ_F (CD_3OD; 235.36 MHz) − 172.04 (d, *J* 6.1 Hz, 5-F).

Fraction 4, retention time 630—750 s, was a pale yellow solid, (44 mg, 8.9%), m.p. 137—138 °C. Fraction 4 had the same C : H ratio (0.153) as fraction 2, but the m.p. and n.m.r. spectra (^1H and ^{19}F) were different, δ_H ([$^2H_6$]DMSO; 250 MHz) 9.34br [1 H, s, N(3)H], 7.74 [1 H, d, N(1)H], 6.96 (1 H, t, 6-NH), 4.9 (1 H, dd, 5-H), 4.36 (1 H, m, 6-H), 3.75 (3 H, s, 4-$NOCH_3$), and 3.37 (3 H, s, 6-$NOCH_3$); δ_F ([$^2H_6$]-DMSO; 235.36 MHz) −180.1 (dq, 5-F); δ_F (CD_3OD; 235.36 MHz) −182.4 (dd, 5-F). There was no evidence for the formation of the product from fraction 4 in experiments conducted at pH 4.5.

4-*Methoxyimino*-5-*fluoropyrimidin*-2(1H)-*one*-*O*-*methylhydroxylamine* (3b; R^1 = Me, R^2 = R^3 = H). 4-Methoxyimino-5-fluoropyrimidin-2(1*H*)-one (5.8 mg, 3.6×10^{-5} mol) was dissolved in a solution of *O*-methylhydroxylamine (132 mg, 1.58×10^{-3} mol) in water (1 ml). The solution was divided into two equal portions which were adjusted to pH 3.5 and 4.5 using sodium hydroxide (1N). After 1 week at 25 °C the mixtures were analysed by reverse-phase h.p.l.c. using a Waters 8C18 10μ column (10 cm) with an eluant of aqueous ammonium formate (0.02M)–methanol (5 : 1, v : v), a flow rate of 2 ml min^{-1} and the u.v. detector set at 254 nm. Substantial quantities of the material corresponding to fraction 4 of the previous experiment were observed in both samples. The peak corresponding to fraction 2 was barely detectable (<10% of fraction 4) but at pH 3.5 an extra peak with almost the same retention as fraction 1 was observed which is assigned to the hydrolysis product of (3a), 5-fluorouracil.

It is clear therefore that fraction 4 originates from (3a) and not from the reaction of 5-fluorocytosine with (1; R^1 = Me).

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