# SYNTHESIS OF DERIVATIVES OF 3,4-DIHYDRO-6H,8H-PYRIMIDO[4,5-c][1,2]OXAZIN-7-ONE

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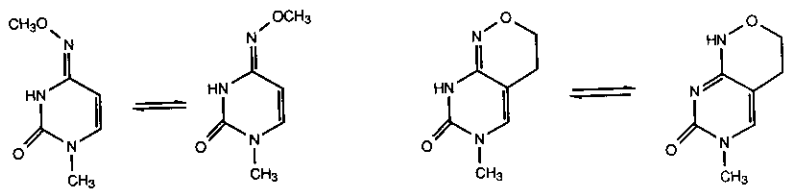
Abstract — In model experiments seeking a pyrimidine which had the hydrogen-bonding potential of both thymine and cytosine synthetic routes to the bicyclic 3,4-dihydro-6H,8H-pyrimido[4,5][1,2]oxazin-7-one 2 ring system have been investigated. 1-Methyl-5-(2-bromoethyl)uracil 3 was converted to the 5-(2-phthalimido-oxyethyl) derivative 9 and then to the corresponding 4-triazolo derivative 10. Ammonia in dioxan afforded 6-methyl-3,4-dihydro-6H,8H-pyrimido[4,5-c][1,2]oxazin-7-one 11. The ring closure of 4-oxyimino-5-(2-chloroethyl)pyrimid-2-ones was also investigated, yielding 1,6-dimethyl- and 1-benzyl-6-methylpyrimido[4,5-c][1,2]oxazin-7-one, but not the parent structure 11.

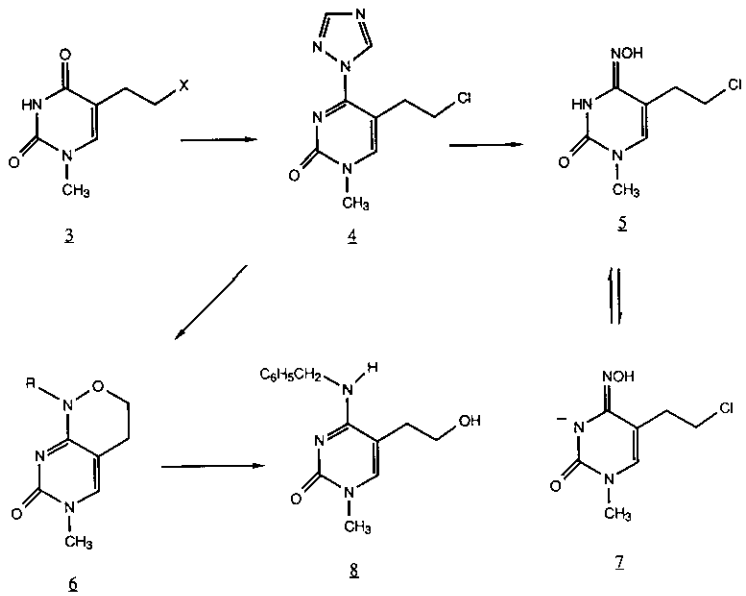
Much earlier work has demonstrated that bases $N^4$-hydroxy- and $N^4$-methoxycytosine when introduced into DNA can base-pair with both adenine (A) and guanine (G) and can lead to replication errors and mutagenic changes [1,2]. In explanation it is held that this is due to the fact that their tautomeric constants are around 10-30 (favouring the imino form) compared with the normal bases whose $K_T$ values are $10^4$-$10^5$; they can therefore hydrogen-bond either in the imino- or amino-form[3,4]. In experiments designed to investigate this degeneracy oligo-deoxynucleotides were constructed carrying an $N^4$-methoxycytosine ($mo^4c$) residue. Melting profiles of duplexes with their oligomer complements showed that an $mo^4c$ residue when hydrogen-bonded to either A or G gave duplexes of comparable stability[5], although additional $mo^4c$ residues led to a progressive depression of the $T_m$ value[6]. Others have given evidence that the favoured conformer of $mo^4c$ is syn- 1a whereas hydrogen bonding with A or G requires the anti- 1b conformation. The drastic reduction in $T_m$ in a duplex containing $N^4$-methoxy-5-methylcytosine gave credence to this view, steric hindrance, in this instance further stabilising the syn- conformer[5].




1a 1b 2

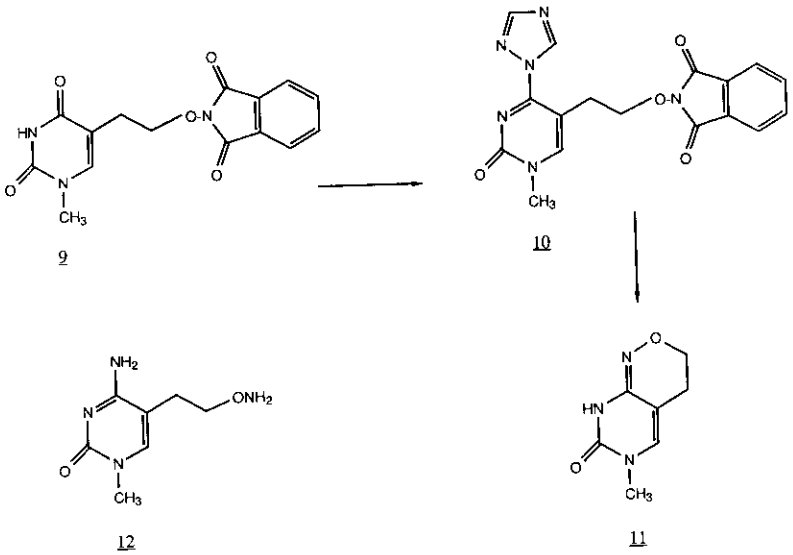
Here we describe synthetic routes to the novel pyrimido-oxazin-7-one 2 ring system in which the oxy-amino function is held in an orientation corresponding to the anti- conformer 1b. Experiments directed to the synthesis and properties of oligonucleotides containing the base 2 are in progress.

5-(2-Hydroxyethyl)uracil[8] was converted to the 5-(2-chloroethyl)uracil by treatment with triphenylphosphine and tetrachloromethane[9] and using tetrabromomethane, the corresponding 5-(2-bromoethyl)uracil was obtained. Each on silylation and treatment with methyl iodide gave the 1-methyl derivative 3 (X=Cl,Br). The chloro derivative reacted quantitatively with phosphoryl tristriazolide[10] affording the 4-triazolopyrimid-2-one 4 and the latter with hydroxylamine hydrochloride in pyridine gave $N^4$-hydroxycytosine 5. This compound proved to be unexpectedly stable to a variety of basic reagents and we were unable to induce it to ring close. However when 4 was treated at room temperature in pyridine with N-methylhydroxylamine hydrochloride, displacement of the triazolo group and ring closure occurred to give the pyrimido-oxazin-7-one 6 (R=Me). We surmise that base catalysed ring closure of 5 is inhibited by the preferential formation of the N(3)-anion 7. By use of N-benzylhydroxylamine, 6 ($R=CH_2C_6H_5$) was also obtained, although in much poorer yield. In initial experiments to remove the benzyl protecting group, palladium-catalysed hydrogen transfer led to rapid reduction by monitoring the mobility of the product on tlc. This was confirmed by examining the $^1$H-nmr spectrum of the isolated product which showed a doublet at $\delta$ 4.52 (J=5.4 Hz) (which gave a singlet on shaking with $D_2O$) coupled with a triplet at $\delta$ 7.80 (J=5.4Hz) (which disappeared on shaking with $D_2O$) indicating the presence of a $-CH_2-N-H$ group. Therefore the N-O bond cleavage had evidently occurred to afford 1-methyl-$N^4$-benzyl-5-(2-hydroxyethyl)cytosine 8.




3 4 5 6 8 7

As an alternate method of ring closure the 5-(2-bromoethyl)uracil 3 (X=Br) was converted to the phthalimido-oxyethyl derivative 9 and then to the 4-triazolopyrimid-2-one 10. When the latter was treated with ammonia in anhydrous dioxan the desired 6-methyldihydropyrimido-oxazin-7-one 11 was produced directly. Tlc experiments suggested that the initial displacement of the triazolo residue occurred to give the cytosine derivative; subsequent removal of the phthalimido group by ammonia[11] then led via 12, to the expected displacement of the 4-amino group with ring formation.



Although we have not yet studied the tautomeric equilibrium in the 3,4-dihydro-6H,8H-pyrimido-[4,5-c][1,2]oxazin-7-one ring system, a uv spectral comparison of compound 11 with that of the N-1 methyl derivative 6 ($R=CH_3$) was obtained. In 95% ethanol, compounds 11 and 6 ($R=CH_3$) showed absorption maxima at 305.0 and 298.4 nm respectively. However in 0.1M HCl/95% ethanol their respective uv spectrum profiles was almost identical both showing a maximum absorption at 306.9 nm and thus implying that on protonation both compounds formed similar cations. The above evidence suggests the preponderance of compound 11 to be in the imino-tautomer form as in the case of $N^4$-hydroxy- and $N^4$-methoxycytosines.

## EXPERIMENTAL

$^1$H-N.m.r. spectra were obtained with Varian HA100 and CFT-20 instruments with tetramethylsilane as internal standard. Unless otherwise stated values given on an δ scale refer to singlet absorption and integration values and signal assignment are in parentheses. For multiplet d = doublet, t = triplet, q = quartet and m = complex multiplet. Mass spectra were recorded with a Kratos MS30 instrument. Ultraviolet spectra were recorded on a Beckman DU-65 Spectrophotometer. Light absorption data refer to solutions in ethanol unless otherwise stated, inflections are given in parentheses. Tlc was carried out on precoated silica plates in dichloromethane-methanol, 90:10 (A) or 80:20 (B) and column chromatography was performed with Merck kieselgel 60H. Melting points were measured with a Kofler hot stage apparatus.

5-(2-Chloroethyl)uracil- A solution of 5-(2-hydroxyethyl)uracil (0.5 g, 3.2 mmol), triphenylphosphine (1.12 g, 4.32 mmol), anhydrous carbon tetrachloride (1.6 ml, 16 mmol) in anhydrous dimethylformamide (16 ml) was kept stirring at room temperature for 24 h. Evaporation of the deep yellow solution, in vacuo, and recrystallisation of the residue from methanol gave the product (0.41 g, 73%) as colourless crystals: mp 273-274°C; $^1$H-nmr $(CD_3)_2SO$:2.61(2H, t, J=7.1 Hz, $CH_2$), 3.67(2H, t, J=7.1 Hz, $CH_2$), 7.36 (1H, 6-H), 10.96 (2H, broad, N-H).

Anal. Calcd for $C_6H_7N_2O_2Cl$: C, 41.3, H, 4.0, N, 16.1. Found: C, 41.5, H, 4.0, N, 16.0.

5-(2-Bromoethyl)uracil- This was synthesised as above but using carbon tetrabromide. The crude product was washed with methanol and crystallised from ethanol to give a colourless powder (42%): mp 255-260°C (decomp.); $^1$H-nmr $(CD_3)_2SO$: 2.71 (2H, t, J=7.0 Hz, $CH_2$), 3.56 (2H, t, J=7.0 Hz, $CH_2$), 7.36 (1H, d, J=5.5 Hz, 6-H), 10.81 (1H, d, J=4.0 Hz, N-H), 11.13 (1H, N-H).
Anal. Calcd for $C_6H_7N_2O_2Br$: C, 32.9; H, 3.2; N, 12.8. Found: C, 32.6; H, 2.9; N, 12.6.

1-Methyl-5-(2-chloroethyl)uracil 3 (X=Cl)- To 5-(2-chloroethyl)uracil (150 mg, 0.86 mmol) was added hexamethyldisilazane (2.0 ml, 12 mmol) and trimethylchlorosilane (0.02 ml). The white suspension was left stirring for 15 h at 150°C to form a clear solution. The excess reagents were evaporated *in vacuo* to give a colourless oil to which methyl iodide (4 ml) was added. The solution was then gently refluxed for 6 h. Excess methyl iodide was distilled off and the viscous liquid obtained was dissolved in methanol (15 ml) and refluxed for another 12 h. After evaporation of excess methanol, column chromatography in $CHCl_3$-$CH_3OH$ (100:3) gave the product as colourless crystals (120 mg, 74%):mp 174-175°C; $^1$H-nmr($CDCl_3$) 2.74 (2H, t, J=6.1 Hz, $CH_2$), 3.38 (3H, $CH_3$), 3.71 (2H, t, J=6.1 Hz, $CH_2$), 7.10 (1H, 6-H), 9.44 (1H, broad, N-H).
Anal. Calcd for $C_7H_9N_2O_2Cl$: C, 44.6; H, 4.8; N, 14.9. Found: C, 44.1; H, 4.5; N, 14.4; ms: *Found, m/z* 188.0360 ($M^+$, 7.5). *Calcd* for $C_7H_9N_2O_2Cl$, 188.0352.

1-Methyl-5-(2-bromoethyl)uracil 3 (X=Br) was synthesised as above from 5-(2-bromoethyl)uracil. It gave colourless crystals (56%) from methanol: mp 184-185°C; H-nmr$(CD_3)_2SO$:2.70 (2H, t, J=7.4 Hz, $CH_2$), 3.22 (3H, N-$CH_3$), 3.32 (2H, t, J=7.4 Hz, $CH_2$), 7.62 (1H, 6-H), 11.34 (1H, N-H).
Anal. Calcd for $C_7H_9N_2O_2Br$: *C, 36.0; H, 3.9; N, 12.0. Found: C, 36.1; H, 3.7; N, 12.2; ms:* Found, m/z 153.0666 ($M^+$ - Br, 100). Calcd for $C_7H_9N_2O_2$, 153.0664.

1-Methyl-4-triazolo-5-(2-chloroethyl)pyrimid-2-one 4 - Phosphorus oxychloride (4 ml) was added to a suspension of triazole (12.8 g, mmol) in dry acetonitrile (300 ml) at 0°C followed by addition of anhydrous triethylamine (30 ml) and the suspension was left stirring for 0.5 h. A solution of 3 (X=Cl) (2.06 g, 11 mmol) in acetonitrile (25 ml) was added slowly to the above suspension and the reaction mixture was left stirring for another 5 h. Excess solvent was removed under vacuum and saturated bicarbonate solution (100 ml) was added, followed by extraction with chloroform (3 x 25 ml). Evaporation of the solvent yielded a crude which was purified by column chromatography to afford pale yellow crystals (1.75 g, 60%): mp 122-123°C; $^1$H-nmr($CDCl_3$): 3.31 (2H, t, J=6.0 Hz, $CH_2$), 3.65 (3H, N-$CH_3$), 3.82 (2H, t, J=6.0 Hz, $CH_2$), 7.76 (1H, 6-H), 8.10 (1H, triazole-H), 9.31 (1H, triazole-H).
Anal. Calcd for $C_9H_{10}N_5OCl$: C, 45.1; H, 4.2; N, 29.2. Found: C, 44.7; H, 4.2; N, 29.4; ms: Found, m/z 239.0588 ($M^+$, 2.18). Calcd for $C_9H_{10}N_5OCl$, 239.0574.

1-Methyl-$N^4$-hydroxy-5-(2-chloroethyl)cytosine 5 - The above triazolo compound (0.1 g; 0.42 mmol) and hydroxylamine hydrochloride (0.058 g; 0.82 mmol) were stirred in dry pyridine at room temperature for 12 h. Working up by washing a chloroform solution of the product with sodium bicarbonate solution and crystallisation from acetonitrile gave colourless needles (0.15 g, 90%): mp 148-149°C; $^1$H-nmr $(CD_3)_2SO$: 2.57 (2H, t, J= 7.0 Hz, $CH_2$), 3.08 (3H, N-$CH_3$), 3.73 (2H, t, J= 7.0 Hz, $CH_2$), 6.85 (1H, 6-H), 9.36 (1H, N-H), 10.05 (1H, N-OH).

Anal. $C_7H_{10}N_3O_2Cl$: C, 41.3; H, 4.9; N, 20.6. Found: C, 41.0; H, 4.9; N, 20.5; ms: Found, m/z 203.0465 ($M^+$, 7.44). Calcd for $C_7H_{10}N_3O_2Cl$, 203.0461.

Reaction of 5 under a variety of conditions with (a) triethylamine in acetonitrile, (b) saturated methanolic ammonia and (c) tetramethylguanidine in dimethylformamide all gave back starting material.

1,6-Dimethyl-3,4-dihydro-6H,8H-pyrimido[4,5-c][1,2]oxazin-2-one 6 (R=$CH_3$) - Compound 4 (0.31 g, 1.29 mmol) was stirred with N-methylhydroxylamine hydrochloride (0.54 g, 6.47 mmol) in anhydrous pyridine (10 ml) for 12 h at room temperature. After removal of solvent the residue was worked up the usual way. The crude (0.20 g) after column chromatography and recrystallisation from dioxan afforded the product as colourless needles (160 mg, 68%): mp 155-156°C; H-nmr ($CDCl_3$): 2.69 (2H, t, J=5.7 Hz, $CH_2$), 3.35 (3H, N-$CH_3$), 3.38 (3H, N-$CH_3$), 4.14 (2H, t, J=5.7 Hz, $CH_2$), 7.06 (1H, 6-H); uv (0.1 M HCl/95% $C_2H_5OH$) $\lambda_{max}$: (257.5), 306.9; log ε: 3.26, 3.89; (95% $C_2H_5OH$) $\lambda_{max}$: (250.6), 298.4; log ε: 3.43, 3.95.
Anal. Calcd for $C_8H_{11}N_3O_2$: C, 53.0; H, 6.1; N, 23.2. Found: C, 53.3, H, 6.1; N, 23.4; ms: Found, m/z 181.0854 ($M^+$, 24.39). Calcd for $C_8H_{11}N_3O_2$, 181.0851.

1-Benzyl-6-methyl-3,4-dihydro-6H,8H-pyrimido[4,5-c][1,2]oxazin-2-one 6 (R=$CH_2C_6H_5$) was prepared in the same way as 6 (R=$CH_3$) from 4 (0.36 g, 1.5 mmol) and N-benzylhydroxylamine hydrochloride (1.19 g, 7.5 mmol) in anhydrous pyridine (10 ml). The major product (54 mg, 13%) was isolated by column chromatography. $^1$H-Nmr ($CDCl_3$): 2.73 (2H, t, J= 5.5 Hz, $CH_2$), 3.47 (3H, N-$CH_3$), 4.10 (2H, t, J= 5.5 Hz, $CH_2$), 5.10 (2H, N-$CH_2$), 7.20-7.40 (6H, m, H-6 and aromatic protons).

Reduction of the N-benzyl derivative (57 mg, 0.22 mmol) by 10% Pd/C (57 mg) and ammonium formate (69 mg) in anhydrous methanol (5 ml) under reflux for 10 min gave complete conversion to a product slow running on tlc (solvent A). Only a small amount of the product was isolated for $^1$H-nmr analysis: $^1$H-Nmr ($(CD_3)_2SO$: 2.46 (2H, t, J=6.3 Hz, $CH_2$), 3.19 (3H, N-$CH_3$), 3.51 (2H, t, (J=6.3 Hz), $CH_2$), 4.52 (2H, d, J=5.4 Hz, $CH_2$), 7.16-7.32 (5H, m, aromatic protons), 7.42 (1H, 6-H), 7.80 (1H, t, J=5.4 Hz, N-H). The nmr spectrum is consistent with the structure 1-Methyl-$N^4$-benzyl-5-(2-hydroxyethyl)cytosine 8.

1-Methyl-5-(2-phthalimido-oxyethyl)uracil 9 - A solution of 5-bromoethyluracil 3 (X=Br) (4.35 g, 18.7 mmol), N-hydroxyphthalimide (4.56 g, 28 mmol) and dry triethylamine (0.7 ml) in anhydrous dimethylformamide (200 ml) was kept at 80°C for 12 h. After removal of the solvent under reduced pressure, the thick oil was triturated with ethanol and a few drops of water. The pale brown precipitate gave cream crystals (2.0 g, 35%) from ethanol: mp 211-212°C; $^1$H-nmr ($(CD_3)_2SO$: 2.50 (2H, t, J=6.6 Hz, $CH_2$), 3.22 (3H, $CH_3$), 4.23 (2H, t, J=6.6 Hz, $CH_2$), 7.71 (1H, H-6), 7.86 (4H, aromatic protons), 11.32 (1H, broad, N-H).
Anal. Calcd for $C_{15}H_{13}N_3O_5$: C, 57.1; H, 4.1; N, 25.4. Found: C, 57.3; H, 4.4; N, 25.6; ms: Found, m/z 315.0849 ($M^+$, 0.48). Calcd for $C_{15}H_{13}N_3O_5$, 315.0855.

1-Methyl-4-triazolo-5-(2-phthalimido-oxyethyl)pyrimid-2-one 10 - Phosphoryl tristriazolide was prepared as above from phosphorus oxychloride (0.4 ml), triazole (1.28 g, 18.5 mmol) and triethylamine (3 ml). To a stirred solution of the latter was added a solution of 9 (0.3 g,

0.95 mmol) in acetonitrile (10 ml). The reaction was completed in 4 h. Working up in the usual way gave a solid which was recrystallised from dioxan to give colourless needles (0.14 g, 42%): mp 255-256°C; $^1$H-nmr $(CD_3)_2SO$: 3.20 (2H, t, J=6.6 Hz, $CH_2$, 3.51 (3H, $CH_3$), 4.28 (2H, t, J=6.6 Hz, $CH_2$), 7.87 (4 H, aromatic protons), 8.18 (1H, 6-H), 8.57 (1H, triazolo proton), 9.31 (1H, triazolo proton).

Anal. Calcd for $C_{17}H_{14}N_6O_4$: C, 55.7: H, 3.8; N, 23.0. Found: C, 55.5; H, 3.9 N, 23.2; ms: Found, m/z 366.1101 ($M^+$, 0.24). Calcd for $C_{17}H_{14}N_6O_4$, 366.1076.

6-Methyl-3,4-dihydro-6H,8H-pyrimido[4,5-c][1,2]oxazin-7-one 11 - Compound 10 (166 mg, 0.47 mmol) was dissolved in hot anhydrous dioxan (3 ml) followed by addition of saturated dioxan/ammonia (4 ml). The sealed reaction bottle was kept at 70°C in an oil bath for 4 h. Cooling, evaporation of the solvent and chromatography gave colourless crystals of the product (47 mg, 60%): mp 252-253°C; $^1$H-nmr $(CD_3)_2SO$: 2.50 (2H, t, H=5.5 Hz, $CH_2$), 3.07 (3H, N-$CH_3$), 3.79 (2H, t, J=5.5 Hz, $CH_2$), 6.82 (1H, 6-H), 10.55 (1H, N-H); uv (0.1 M HCl/95% $C_2H_5OH$) $\lambda_{max}$: (263.2), 306.9; log ε: 3.35, 3.88; (95% $C_2H_5OH$) $\lambda_{max}$: (264.3); 305.0; log ε: 3.20, 3.75.

Anal. Calcd for $C_7H_9N_3O_2$: C, 50.3; H, 5.4; N, 25.1. Found: C, 50.4; H, 5.6; N, 22.2; ms: Found, m/z 167.0700 ($M^+$, 100). Calcd for $C_7H_9N_3O_2$, 167.0691.

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