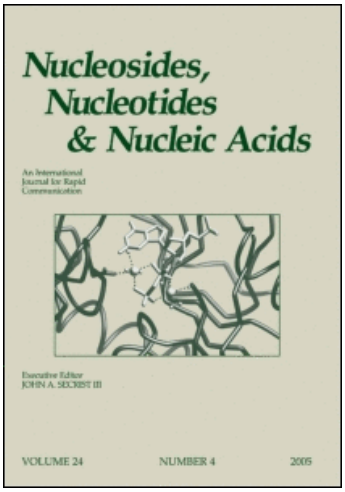

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Synthesis of Bicyclic N^4-Oxycytidine Derivatives

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SYNTHESIS OF BICYCLIC N^4-OXYCYTIDINE DERIVATIVES. †

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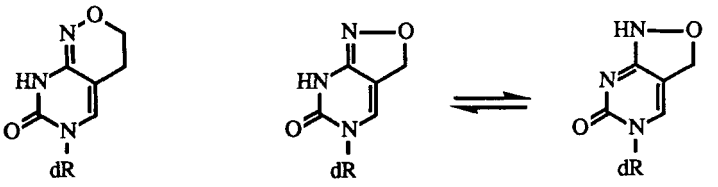
Abstract. 5-ß-D-Ribofuranosyl-3H,5H,7H-pyrimido[4,5-c][1,2]oxazol-6-one, **13**, a fixed anti-conformer of N^4-alkoxycytidines was synthesised to investigate its hydrogen-bonding potential.

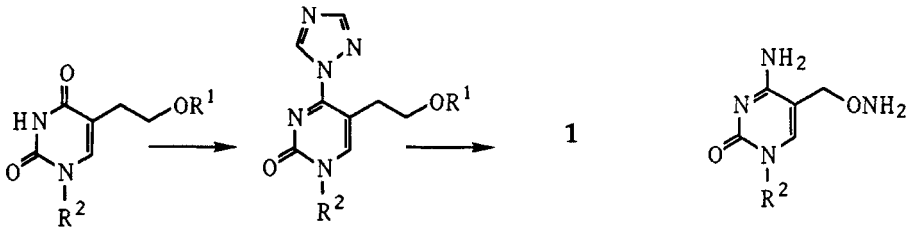
In earlier work we have been interested in the synthesis and application of nucleosides in which the amino-imino tautomeric constants (K_T) were nearer to unity than the natural nucleosides, which are of the order of 10^4-10^5. In the pyrimidine series, cytosines with electronegative O- and N- substituents on N^4 have the desired characteristics.[1,2] For work with such analogues in oligonucleotides good stability to deprotection conditions during machine synthesis is essential and N^4-alkoxy derivatives satisfy this requirement. In particular the 2'-deoxynucleoside **1** has been studied in detail and although the K_T is not known the tautomer shown is the favoured one. However, when present in duplexes the base forms stable hydrogen bonds with both adenine and guanine. We took the view that the tautomeric state might be fine-tuned. By contracting the oxazino ring in **1** to that in **2** and thus reducing the bond angle N^4-C^4-C^5 the C^4-N^4 bond order might be reduced and the K_T might in this way be shifted towards the amino tautomer of **2**.

† This paper is dedicated to the memory of the late Professor Roland K. Robins.



1 2

For the synthesis of **2** our initial experiments utilised a route similar to that for **1**.[3] For the latter the 5-hydroxyethyldeoxyuridine derivative **3** (R^1 = H) was converted first by introduction of a phthalimido group **3** (R^1 = phthalimido) then triazolylation to the corresponding 4-triazolonucleoside **4**. Ammonia in methanol or dioxan removed the phthaloyl residue and simultaneously led to ring closure, either directly or via the intermediate cytosine. It is well known that cytosines may react with alkoxyamines by direct displacement of the amino group.[4] Turning to the synthesis of **2**, corresponding experiments with the 3',5'-di-O-p-toluoyl-ß-D-ribofuranosyl-5-hydroxymethyl-2'-deoxyuridine as starting material led to **5**, with very little evidence of ring closure. We will discuss this work and a variety of other attempted cyclisation routes elsewhere but the evidence and general considerations suggested that the stereochemistry of the transition state for such a ring closure was very unfavourable. The present experiments relate to the formation of the desired ring system, in the ribo- series.

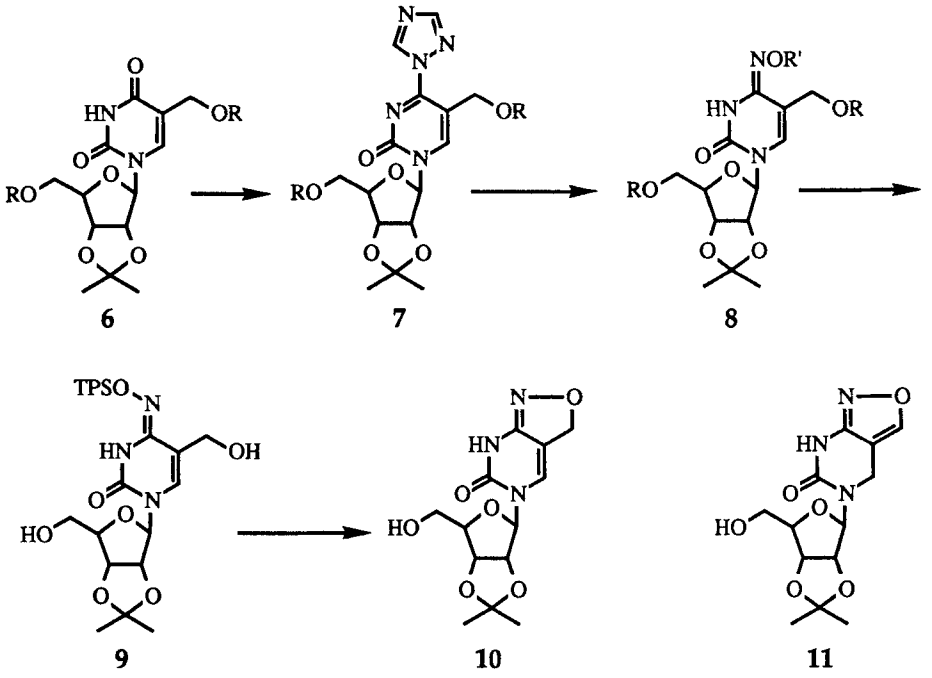


3 4 5

R^2 = 3,5-Di-O-p-toluoyl-2-deoxy-ß-D-ribofuranosyl

The 5-hydroxymethylation of 2',3'-O-isopropylideneuridine is facile, giving **6** (R = H) in high yield.[5] Protection of the two primary hydroxyl functions by dimethoxytritylation or preferably by t-butyldimethylsilylation (R = DMT or TBDMS) was followed by conversion to the 4-triazolo-derivatives **7** and thence, with hydroxylamine hydrochloride in pyridine to the N^4-hydroxycytosines **8** (R' = H, R = DMT or TBDMS). From the latter a variety of N^4-sulphonyloxy derivatives were prepared, though the 2,4,6-triisopropylbenzenesulphonyl (TPS)

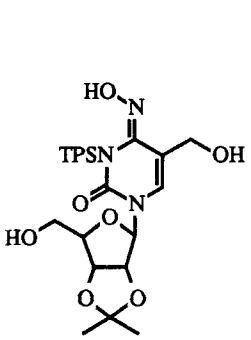
derivative was found to be best. Although the subsequent synthetic steps were effective in both the 5,5'-di-O-dimethoxytrityl- and the 5,5'-di-O-TBDMS series only the latter, more convenient and higher yielding series will be discussed.



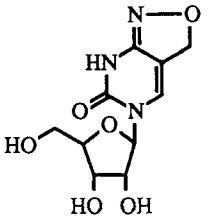
The triisopropylbenzenesulphonyl derivative **8** (R' = TPS, R = TBDMS) on treatment with ammonium fluoride in methanol gave **9** in high yield. On reaction with potassium t-butoxide in t-butanol ring closure proceeded very rapidly (< 1 minute) and in excellent yield.

Ring closure had occurred to give a product of the correct mass (MH^+ 312) and an ^1H-n.m.r. spectrum consistent with the desired product. In particular, the latter showed a triplet resonance at 6.88 ppm (allylic coupling, J = 2.7Hz) in the expected region for C^6-H (pyrimidine numbering), coupling to the CH_2 at 4.94 ppm. However, the u.v-spectra under differing pH conditions did not correspond to those found for the six-ring analogue **1**.[6] Thus at pH 1.5 no shift occurred, under conditions where the latter compound was converted to its cation. Indeed, it appeared to have a pk_a in the region of -1 to -2. Additionally, it showed a bathochromic shift in base, in contrast to **1**. These properties suggested to us that the compound might in fact be the isomeric isoxazole derivative **11** and that the function of the powerful base used in the ring closure was to effect the allylic

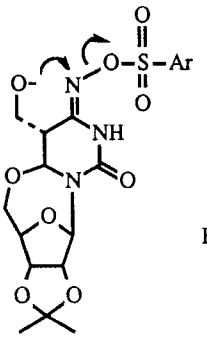
proton transfer (**10** to **11**). Indeed, isoxazoles may be prepared by the displacement of sulphate from an oxime-O-sulphonate under basic conditions,[7] and 3-acylaminoisoxazoles have u.v. absorption spectra similar to those obtained for the ring closed product.[8]



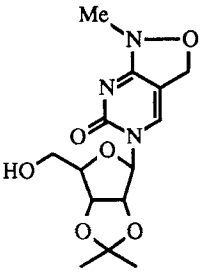
12



13



14



15

In this connection we observed that with mild base, such as triethylamine or sodium methoxide in methanol, there was no evidence of ring closure. Instead an isomeric sulphonyl derivative was formed with a ^{1}H-n.m.r. spectrum virtually identical to that of **9**, except that the exchangeable resonance at 11.27 ppm was absent. Such a spectrum would be consistent with the N^3-sulphonylated derivative **12**. The possibility that the sulphonyl group had migrated to one of the alcohol groups was eliminated as the ^{1}H-n.m.r. spectrum showed two exchangeable triplets in the same position as those of the OH groups in the starting material **9**, and additionally because the product showed a negative ferric chloride reaction implying that either the N^3 or the N^4-OH proton was absent. However, if this is indeed the case, the 1,4-migration must be reversible as *both* sulphonyl derivatives undergo ring closure with potassium t-butoxide to give the same product, **10**. The structure of the two isomeric TPS-derivatives have been tentatively assigned as described, but further work is being carried out to clarify these assignments.

The decision as to the assignment of the structure of the ring-closed product was made in favour of the originally expected product **10** by NOE experiments. Irradiation of the methylene protons at 4.94 ppm gave rise to a single NOE to the vinylic proton signal at 6.88 ppm. However, irradiation of the vinylic signal at 6.88 ppm gave rise to strong NOE signals due to the methylene group and to the 1'- and 2'-protons of the sugar, and a weaker signal to the 3'-proton. These results eliminate the isoxazole structure **11** from consideration. In addition we are

attempting to obtain final confirmation of **10** by X-ray crystallography. Finally, acid-catalysed deprotection of **10** afforded 5-ß-D-ribofuranosyl-3H,5H,7H-pyrimido[4,5-c][1,2]oxazol-6-one, **13**.

In corresponding experiments using the same synthetic route, but in the 2'-deoxyribosyl-series (to be elaborated on elsewhere) we found that the ring closure reaction did not proceed cleanly as in the ribo- series, rather it gave multiple products; disappearance of the starting material was very slow and the isolated yield of the desired product was less than five percent. Since it is well known that rapid reversible addition of the 5'-hydroxyl group to the C^5-C^6 double bond occurs in isopropylideneuridine (catalysing *inter alia* hydroxymethylation at C^5) but very poorly with 2'-deoxyuridine we suggest that such an adduct, **14**, with consequent alteration of the stereochemistry at C^5 is responsible for the easy ring closure.

In an attempt to clarify which tautomeric state the ring-closed product, **10**, adopts we prepared the fixed tautomer **15**. This was prepared by displacement of the triazolo group in **7** (R = TBDMS) by N-methylhydroxylamine hydrochloride in pyridine, followed by removal of the two silyl groups as previously. This intermediate is unlikely to undergo ring-closure as before since the N^4-amino group is fully substituted, and therefore we attempted ring closure by alternative methods. Mitsunobu conditions were employed to affect cyclisation to give the desired compound **15** in low yield as a crystalline product. The ^{1}H-n.m.r. spectrum was consistent with the desired structure. The u.v. spectrum showed a λmax at 283nm (ε ,13000) with a bathochromic shift in acid (λmax, 287nm). The bicyclic compound **10** however, had a λmax at 305nm and showed no bathochromic shift until the pH was lowered to the order of -1. From this we conclude that the bicyclic compound **10** does not exist in the amino form, but we defer further discussion of this question until further information is available.

EXPERIMENTAL:

General: ^{1}H-n.m.r. spectra were obtained on a Bruker WM-250, ^{13}C-n.m.r. on a Bruker AM-400 and NOE experiments on a Bruker AMX-500. N.m.r. spectra were obtained in d^6-DMSO. Mass spectra were recorded on a Kratos MS890. Ultraviolet spectra were recorded on a Perkin-Elmer Lambda 2 spectrophotometer in methanol unless otherwise stated. Tlc was carried out on pre-coated F_{254} silica plates and column chromatography with Merck kieselgel 60. Melting points were measured with a Gallenkamp melting point apparatus

and are un-corrected. Unless otherwise stated reactions were worked up as follows: After removal of the solvent, the product was dissolved in chloroform and washed with aqueous sodium bicarbonate solution. The combined organic fractions were dried over sodium sulphate and evaporated.

5'-O-t-butyldimethylsilyl-2',3'-O-isopropylidene-5-t-butyldimethylsilyloxy-methyluridine **6** (R = TBDMS). 5-Hydroxymethyl-2',3'-O-isopropylideneuridine (10g, 31.8mmol) was dissolved in 100ml dry pyridine and t-butyldimethylsilyl chloride (14.4g, 95.5mmol) added and the solution stirred at room temp. overnight. The reaction was worked up, chromatographed ($CHCl_3$) and dried to a white foam. Yield 13.57g, 79%. ^{1}H-n.m.r. ∂ (ppm) , 0.02, 0.07 (12H, 2 x s, 4 x $SiCH_3$), 0.84, 0.88 (18H, 2 x s, 2 x t-butyl), 1.29, 1.48 (6H, 2 x s, isopropylidene), 3.74 (2H, m, H5', H5''), 4.13 (1H, m, H4'), 4.31 (2H, d, J=2Hz, CH_2), 4.66 (1H, m, H3'), 4.93 (1H, m, H2'), 5.77 (1H, d, J=2Hz, H1'), 7.56 (1H, s, H6), 11.45 (1H, s, NH). m/z 527 $(M\text{-}Me)^+$, Accurate mass for $(M\text{-}Me)^+$ 527.2632, $C_{24}H_{43}Si_2N_2O_7$ requires 527.2597.

1-(5-O-t-butyldimethylsilyl-2,3-O-isopropylidene-ß-D-ribofuranosyl)-4-(1,2,4-triazolo)-5-t-butyldimethylsilyloxymethylpyrimid-2-one **7**. 1,2,4-Triazole (17.2g, 249mmol) was suspended in 200ml acetonitrile and cooled to 0°C. Phosphoryl chloride (4.7ml, 50mmol) was added over 2 minutes and the solution stirred for 10 minutes at 0°C and then triethylamine (42ml, 300mmol) added over 15 minutes and the solution stirred for 20 minutes. The above silylated product **6** (R = TBDMS) (9g, 16.6mmol) in 30ml acetonitrile was added and the solution stirred at room temp. for 6 hours. The reaction was worked up and chromatographed ($CHCl_3$, 1% MeOH) and dried to a white foam. Yield 7.68g, 78%. ^{1}H-n.m.r. ∂ (ppm), -0.04, -0.05 (6H, 2 x s, 2 x Si-CH_3), 0.07, 0.09 (6H, 2 x s, 2 x Si-CH_3), 0.71, 0.88 (18H, 2 x s, 2 x t. butyl), 1.32, 1.51 (6H, 2 x s, isopropylidene), 3.83 (2H, m, H5', H5''), 4.55 (1H, t, H4'), 4.66 (1H, d, H3'), 4.88 (1H, d, H2'), 4.90 (2H, s, CH_2), 5.79 (1H, s, H1'), 8.41, 9.40 (2H, 2 x s, 2 x triazolo CH), 8.43 (1H, s, H6). m/z 578 $(M\text{-}Me)^+$, Accurate mass for $(M\text{-}Me)^+$ 578.2883, $C_{26}H_{44}Si_2N_5O_6$ requires 578.2819.

N^4-Hydroxy-5'-O-t-butyldimethylsilyl-2',3'-O-isopropylidene-5-t-butyl-dimethylsilyloxymethylcytidine **8** (R' = H, R = TBDMS). The above triazolo compound (7g, 11.8mmol) was dissolved in dry pyridine (50ml) and to this was added hydroxylamine hydrochloride (4g, 58mmol) and the solution stirred at room temp. overnight. The reaction was worked up and chromatographed

($CHCl_3$) forming a white foam. Yield 5.89g, 90%. ^{1}H-n.m.r. ∂ (ppm), 0.04, 0.08 (12H, 2 x s, Si-Me), 0.86, 0.89 (18H, 2 x s, Si-t-butyl), 1.29, 1.48 (2 x s, isopropylidene), 3.71 (2H, m, H5', H5''), 3.98 (1H, m, H4'), 4.25 (2H, s, CH_2), 4.62 (1H, m, H3'), 4.85 (1H, m, H2'), 5.75 (1H, d, J = 2.6Hz, H1'), 6.75 (1H, s, H6), 9.68 (1H, s, OH), 10.17 (1H, s, NH). m/z 542 $(M-Me)^+$, Accurate mass for $(M-Me)^+$ 542.2758, $C_{24}H_{44}Si_2N_3O_7$ requires 542.2706.

N^4-Triisopropylbenzenesulphonyloxy-5'-O-t-butyldimethylsilyl-2',3'-O-isopropylidene-5-t-butyldimethylsilyloxymethylcytidine **8** (R' = TPS, R = TBDMS). The above N^4-Hydroxycytidine **8** (R = TBDMS, R' = H) (5g, 9mmol) was dissolved in 50ml pyridine and triisopropylbenzene sulphonyl chloride (4g, 13.2mmol) added and the solution stirred at room temp. overnight. The reaction was worked up and chromatographed ($CHCl_3$) and dried to a white foam/gum. Yield 5.38g, 73%. ^{1}H-n.m.r. ∂ (ppm), -0.09, -0.02 (12H, 2 x s, Si-Me), 0.78 (18H, s, Si-t-butyl), 1.17-1.22 (18H, m, 3 x H_3C-CH-CH_3), 1.28, 1.47 (2 x s, isopropylidene), 2.93 (1H, septet, isopropyl CH), 3.70 (2H, m, H5', H5''), 4.03 (2H, s, CH_2), 4.12-4.20 (3H, m, 2 x ortho isopropyl CH, H4'), 4.62 (1H, m, H3'), 4.85 (1H, m, H2'), 5.68 (1H, d, H1'), 7.13 (1H, s, H6), 7.27 (2H, s, 2 x Ar-H), 11.30 (1H, s, NH). m/z 808 $(M-Me)^+$, 766 $(M-t\text{-}butyl)^+$.

N^4-Triisopropylbenzenesulphonyloxy-5-hydroxymethyl-2',3'-O-isopropylidenecytidine **9**. The above N^4-tipsyloxycytidine **8** (R = TBDMS, R' = TPS) (5g, 6.1mmol) was dissolved in 100ml absolute ethanol, ammonium fluoride (2.25g, 61mmol) added and the solution heated at 50°C for 6 hours. Evaporation and chromatography ($CHCl_3$/2% MeOH) gave a white foam. Yield 3.34g, 92%. ^{1}H-n.m.r. ∂ (ppm), 1.18-1.22 (18H, m, 3 x H_3C-CH-CH_3), 1.28, 1.48 (6H, 2 x s, isopropylidene CH_3), 2.94 (1H, septet, isopropyl CH), 3.54 (2H, t, H5', H5''), 3.89 (2H, d, 5-CH_2), 4.02 (1H, m, H4'), 4.16 (2H, m, ortho isopropyl CH), 4.72 (1H, m, H3'), 4.82 (1H, m, H2'), 4.93 (1H, t, OH), 5.01 (1H, t, OH), 5.83 (1H, d, H1'), 7.25 (1H, s, H6), 7.28 (2H, s, 2 x Ar-H), 11.27 (1H, s, NH). u.v. λmax (nm) pH 7, 233 (ε = 17900), 271 (ε = 16900). λmin 250.

5-(2,3-O-isopropylidene-ß-D-Ribofuranosyl)-3H,5H,7H-pyrimido[4,5-c][1,2]-oxazol-6-one **10**. The above N^4-tipsyloxycytidine **9** (2g, 3.36mmol) was dissolved in 30ml dry t-butanol and potassium t-butoxide (1.13g, 10.1mmol) added and the solution stirred at room temp. for 1 hour. In later experiments it was noted that the cyclisation reaction had gone to completion within one minute, and that

prolonged reaction times led eventually to degradation of the product. The solution was neutralised with glacial acetic acid and evaporated to dryness. The solid was dissolved in chloroform and extracted with aqueous sodium bicarbonate, dried (sodium sulphate), evaporated and chromatographed ($CHCl_3$/3% MeOH) to give a white foam. Yield 0.80g, 77%. ^{1}H-n.m.r. ∂ (ppm), 1.27, 1.47 (6H, 2 x s, isopropylidene), 3.55 (2H, m, H5', H5''), 3.93 (1H, m, H4'), 4.68 (1H, m, H3'), 4.77 (1H, m, H2'), 4.94 (2H, d, J=2.7 Hz, CH_2), 5.03 (1H, t, OH), 5.77 (1H, d, H1'), 6.88 (1H, t, J=2.7 Hz, H6), 11.12 (1H, s, NH). ^{13}C-n.m.r. 100 MHz ∂ (ppm), 25.31, 27.19 (isopropylidene), 61.43 (C5'), 68.38 (C5), 80.39 (C4'), 82.90 (C3'), 85.27 (C2'), 90.02 (C1'), 113.28 (Me-C-Me), 114.32 (C4), 121.61 (C6), 149.02 (C5), 153.04 (C=O). m/z (+ve FAB), 312 (MH^+). Accurate mass 311.1123, $C_{13}H_{17}N_3O_6$ requires 311.1111. u.v. λmax (nm) pH 7, 1.5, 242 (ε = 5000), 305 (ε = 4500), λmin 272. pH 12, λmax 238 (ε = 5300), 263 (ε = 5100), 311 (ε = 3800), λmin 253, 288, (reversible).

N^4-Hydroxy-N^3-triisopropylbenzenesulphonyl-5-hydroxymethyl-2',3'-O-isopropylidenecytidine **12.** The N^4-tipsyloxycytidine **9** (200mg, 0.34mmol) was stirred at room temp. in 2ml triethylamine for 30 minutes. The solvent was evaporated and the product dissolved in chloroform and extracted with aqueous sodium carbonate, dried over sodium sulphate and evaporated to a white foam which was chromatographed ($CHCl_3$/2% MeOH) to give a white powder. Yield 196mg, 98%. M. pt. 98-100°C. ^{1}H-n.m.r. ∂ (ppm), 1.20 (18H, m, 3 x H_3C-CH-CH_3), 1.28, 1.48 (6H, 2 x s, isopropylidene CH_3), 2.94 (1H, septet, isopropyl CH), 3.54 (2H, t, H5', H5''), 3.88 (2H, d, 5-CH_2), 4.05 (1H, m, H4'), 4.15 (2H, m, ortho isopropyl CH), 4.72 (1H, m, H3'), 4.81 (1H, m, H2'), 4.93 (1H, t, OH), 5.02 (1H, t, OH), 5.82 (1H, d, H1'), 7.25 (1H, s, H6), 7.28 (2H, s, 2 x Ar-H). u.v. λmax (nm) 232 (ε = 15000), 271 (ε =12000). λmin 250.

5-ß-D-Ribofuranosyl-3H,5H,7H-pyrimido[4,5-c] [1,2]oxazol-6-one **13.** The above bicyclic compound **10** (0.7g, 2.2mmol) was stirred at room temp. in 25ml 80% formic acid for 4 hours. TLC showed a mixture of formate esters. The solution was evaporated and the gum stirred with 10ml methanolic ammonia for 2 hours. The solution was evaporated to dryness and chromatographed ($CHCl_3$/15%MeOH) to give a white solid. Yield 0.43g, 71%. M. pt. 219-220°C (darkening above 210°C). ^{1}H-n.m.r. ∂ (ppm), 3.52 (2H, m, H5', H5''), 3.75 (1H, m, H4'), 3.92 (2H, m, H3', H2'), 4.89 (2H, d, J = 2.7Hz, CH_2), 4.94 (1H, t, C5'-OH), 5.04 (1H, d, OH), 5.25 (1H, d, OH), 5.71 (1H, d, H1'), 6.95 (1H, t, J = 2.7Hz, H6), 11.03

(1H, s, NH). ^{13}C-n.m.r. 100 MHz ∂ (ppm), 61.46 (C5'), 68.41 (C5), 70.35 (C4'), 72.52 (C3'), 84.64 (C2'), 87.16 (C1'), 114.03 (C4), 120.72 (C6), 149.43 (C5), 153.03 (C=O). m/z 139 (Base)H$^+$, 133 (ribosyl)$^+$, Accurate mass on (Base)H$^+$, 139.0394 $C_5H_5N_3O_2$ requires 139.0381. u.v. λmax (nm) pH 7, pH 1.5, 244 (ε = 6200), 307 (ε = 6200), λmin 272. pH 12, λmax (nm) 240 (ε = 6600), 263 (ε = 6300), 318 (ε = 5300), reversible.

N^4-Hydroxy-N^4-methyl-5'-O-t-butyldimethylsilyl-2',3'-O-isopropylidene-5-t-butyldimethyl-silyloxymethylcytidine. The above triazolo compound 7 (R = TBDMS) (3g, 5.1mmol) was dissolved in 40ml pyridine and to this was added N-methylhydroxylamine hydrochloride (1.26g, 15.1mmol) and the solution stirred at room temp. overnight. The reaction was worked up and chromatographed ($CHCl_3$/1%MeOH) and dried to a white foam. Yield 1.20g, 42%. ^{1}H-n.m.r. ∂ (ppm), 0, 0.07 (12H, 2 x s, Si-Me), 0.80, 0.90 (18H, 2 x s, Si-t-butyl), 1.27, 1.47 (6H, 2 x s, isopropylidene), 3.22 (3H, s, N-Me), 3.73 (2H, m, H5', H5''), 4.14 (1H, m, H4'), 4.64 (3H, m, H3', CH_2), 4.84 (1H, m, H2'), 5.70 (1H, d, H1'), 7.61 (1H, s, H6), 10.16 (1H, s, OH). m/z 554 (M-OH)$^+$, Accurate mass for (M-OH)$^+$ 554.3046, $C_{26}H_{48}Si_2N_3O_6$ requires 554.3069.

N^4-Hydroxy-N^4-methyl-2',3'-O-isopropylidene-5-hydroxymethylcytidine. N^4-Methyl-N^4-hydroxy-5'-O-t-butyldimethylsilyl-2',3'-O-isopropylidene-5-t-butyldimethylsilyloxymethylcytidine (1.1g, 1.9mmol) was dissolved in 25ml absolute ethanol and ammonium fluoride (0.71g, 19mmol) added and the solution heated at 50°C for 16 hours. The solution was evaporated and chromatographed ($CHCl_3$/10% MeOH) and dried to give a white powder. Yield 0.45g, 68%. M. pt. 142-147°C (collapsing above 60°C). ^{1}H-n.m.r. ∂ (ppm), 1.28, 1.48 (6H, 2 x s, isopropylidene), 3.26 (3H, s, N-Me), 3.35 (1H, s, OH), 3.56 (2H, m, H5', H5''), 4.04 (1H, m, H4'), 4.42 (2H, s, CH_2), 4.74 (1H, m, H3'), 4.86 (1H, m, H2'), 4.99 (1H, t, OH), 5.80 (1H, d, H1'), 7.72 (1H, s, H6). m/z 325 (M-H_2O)$^+$, Accurate mass for (M-H_2O)$^+$ 325.1286 $C_{14}H_{19}N_3O_6$ requires 325.1269. u.v. λmax (nm) pH 7, 280 (ε = 13000). λmin 242; pH 1.5, λmax, 290 (ε = 14300). λmin 250; pH 12, λmax, 210, 250, 277, 312, irreversible.

1-Methyl-5-(2,3-O-isopropylidene-ß-D-riboruranosyl)-3H,5H,7H-pyrimido[4,5-c][1,2]-oxazol-6-one **15**. N^4-Methyl-N^4-hydroxy-2',3'-O-isopropylidene-5-hydroxymethylcytidine (150mg, 0.4mmol) was dissolved in 15ml dry DMF and to the solution was added triphenyl phosphine (138mg, 0.53mmol) then DEAD

(83µl, 0.53mmol) and the solution stirred at room temp. for one hour. The solution was evaporated and chromatographed ($CHCl_3$/5% MeOH) to give a yellow solid which was recrystallised from ethanol. Yield 41mg, 29%. M.Pt. 268-269°C. (Darkening above 230°C). ^{1}H-n.m.r. ∂ (ppm), 1.28, 1.48 (6H, 2 x s, isopropylidene), 3.43 (3H, s, N-Me), 3.58 (2H, m, H5', H5''), 4.13 (1H, m, H4'), 4.70-4.91 (2H, m, H3', H2'), 4.82 (2H, s, CH_2), 5.13 (1H, t, OH), 5.77 (1H, d, H1'), 8.10 (1H, d, J = 3.6Hz, H6). m/z 325 (M^+), 310 $(M-Me)^+$. Accurate mass 325.1266, $C_{14}H_{19}N_3O_6$ requires 325.1269. u.v. λmax (nm) pH 7, 283 (ε = 13000). λmin 243; pH 1.5, λmax, 212, 287.

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