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The Synthesis of 2'-Homouridine, its Incorporation into a Dinucleoside Monophosphate and Hydrolytic Behaviour of the Dimer

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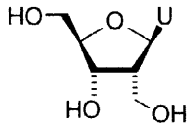
Abstract: An efficient route to 2'-homouridine (**1**), a new nucleoside analogue, is reported that is based on an ene reaction. This nucleoside has been incorporated into a dinucleoside monophosphate and hydrolytic studies on the dimer show that it does not behave like a ribonucleotide. © 1998 Elsevier Science Ltd. All rights reserved.

Naturally occurring *C*-branched nucleosides such as the oxetanocins[1] and their synthetic analogues (2',3'-dideoxy-3'-α-*C*-hydroxymethyl nucleosides[2,3]) have been shown to exhibit potent antiviral activity. *C*-Branched nucleosides have also attracted considerable attention as precursors for backbone-modified antisense oligonucleotides.[4] Recently we reported that diuridine monophosphate analogues containing functionalised 2'-α-*C*-substituents, namely the carboxymethyl, acetamido, hydroxyethyl and 2,3-dihydroxypropyl groups, show considerably increased resistance to enzymatic hydrolysis in comparison to UpU.[5] The hydroxyethyl-modified dimer was particularly resistant to snake venom phosphodiesterase-catalysed hydrolysis (relative half-life of 129 in comparison to UpU) and was totally refractory to hydrolysis by nuclease P1. It was of interest to discover how dimers containing the closely related 2'-deoxy-2'-α-*C*-hydroxymethyluridine (2'-homouridine, **1**), which is a simple homologue of uridine, would behave. This was particularly intriguing given that the only other report on this type of nucleoside analogue had suggested that oligonucleotides containing 2'-α-*C*-hydroxymethylthymidine may be susceptible to hydrolysis by RNA degrading enzymes, because of the RNA-like structure of this modification.[6] However, the susceptibility of these substrates to hydrolysis by specific ribonucleases was not examined. To address this question more closely we now describe an efficient synthesis of 2'-homouridine (**1**), its incorporation into a dinucleoside monophosphate and a preliminary investigation into the hydrolytic stability of this modification.



U = uracil-1-yl

1

Several approaches to the direct synthesis of 2'-homouridine were investigated, however the method of choice involved a double bond migration on the readily available allyl nucleoside[7] (**2**) through an ene reaction (**Scheme 1**). Thus, treatment of **2** with 4-methyl-1,2,4-triazoline-3,5-dione in CH_2Cl_2 in the dark and overnight, gave a very clean reaction which afforded the required ene product (**3a**) in 82% yield after column chromatography. In subsequent reactions column chromatography was not necessary as precipitation of the crude reaction mixture from CH_2Cl_2 into hexane afforded the pure compound as a white powder (91% yield). The ene reaction was also repeated using less expensive phenyltriazoline dione which also proceeded to give the

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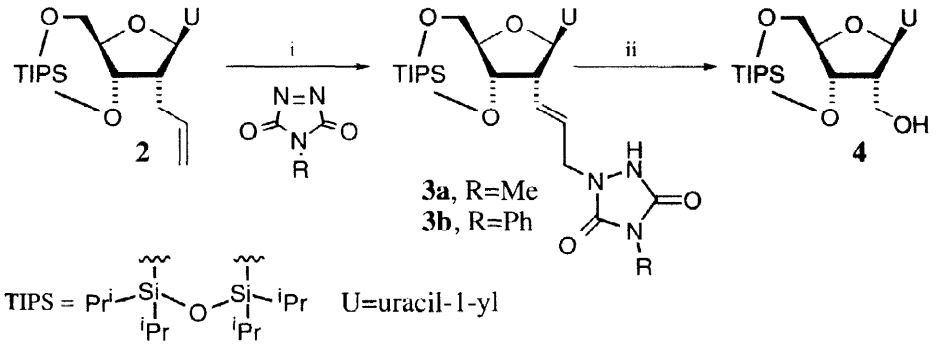
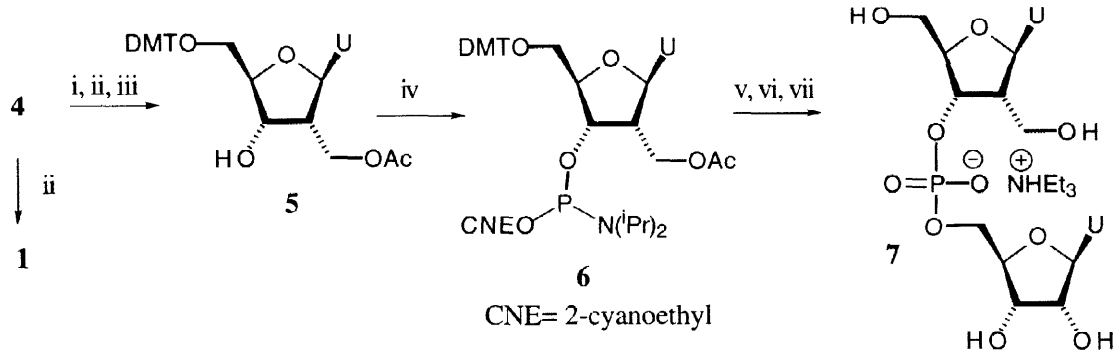
ene product (**3b**), but in a reduced yield of 56%. Also, it was no longer possible to purify the product simply by precipitation as the product was partially soluble in hexane.



Scheme 1. *Reagents and conditions*; i) methyl- or phenyl-triazoline dione (1.2 eq.), CH_2Cl_2, 16h, 82% (**3a**) 58% (**3b**); ii) a) O_3, CH_2Cl_2, -78 °C; b) $NaBH_4$ (8 eq.), MeOH, 0 °C, 90%.

Initial attempts at osmium tetroxide-mediated cleavage of the alkene (**3a**) proved to be inefficient, however it was found that the double bond could be selectively cleaved by ozone. Although the 5,6-double bond in uracil is known to react with ozone[9] the more reactive exocyclic double bond could be selectively cleaved by carefully controlled treatment with ozone.[8] The resulting ozonide was not isolated but immediately subjected to reductive work-up using an excess of sodium borohydride to afford the required hydroxymethyl derivative (**4**) in excellent yield. This combination of an ene reaction followed by ozonolysis provides a very efficient route to an otherwise difficult target. A previously reported synthesis of the closely related 2'-α-*C*-hydroxymethylthymidine used a relatively indirect approach based on glycosylation of a 2-α-*C*-hydroxymethyl sugar derivative.[10] Whilst relatively inefficient radical reactions using styryl stannane derivatives have been used to prepare the related 2',3'-dideoxy-3'-α-*C*-hydroxymethyl nucleosides.[11]

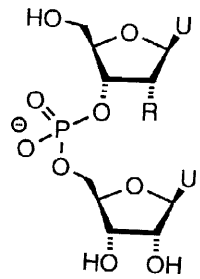


Scheme 2. *Reagents and conditions*; i) Ac_2O, pyridine; ii) $NEt_3 \cdot 3HF$, THF; iii) DMTCl, pyridine, CH_2Cl_2; iv) 2-cyanoethyl-bis-*N,N'*-diisopropylaminophosphoramidite, diisopropylammonium tetrazolide, CH_2Cl_2; v) 2',3'-di-*O*-acetyluridine, tetrazole, CH_3CN; vi) I_2, 2,6-lutidine, water, THF; vii) (a) NH_3, MeOH, (b) AcOH/water (4:1).

Scheme 2 shows the subsequent standard manipulations performed on the TIPS-protected homouridine **4**, using experimental conditions analogous to those previously described.[5] The DMT-protected nucleoside (**5**), was prepared for solid-phase-style coupling by conversion into the 3'-*O*-phosphoramidite (**6**), using 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite and coupled with 2',3'-di-*O*-acetyluridine in acetonitrile in the presence of 1*H*-tetrazole under conditions analogous to those used in solid-

phase synthesis.[12] Deprotection of the dimer was accomplished by sequential treatment with methanolic ammonia and 80% acetic acid. Purification was carried out by HPLC on a Nucleosil C_{18} reverse phase column, using a gradient of 0→10% MeCN in 50 mM triethylammonium bicarbonate (pH 8.0) over 20 min, with a flow rate of 1 mL/min, to give ($U^{OHMe}pU$, **7**) as the triethylammonium salt. The spectroscopic data obtained for dimer $U^{OHMe}pU$ was absolutely consistent with the proposed structure.[13] Digestion with SVPD and HPLC analysis of the hydrolysate, showed the presence of the two expected hydrolysis products, namely uridine-5'-monophosphate (UMP) and 2'-homouridine (**1**), which was obtained by desilylation of **4** (**Scheme 2**).

The characteristic property of RNA in comparison to DNA, is the ease with which it undergoes base-catalysed hydrolysis resulting from attack of the vicinal hydroxyl group and proceeding through the formation of a strained five-membered cyclic phosphate.[14] The **Table** summarises hydrolysis data obtained for the $U^{OHMe}pU$ (entry 2) in comparison to UpU (entry 1) and together with data for the previously reported[5] hydroxyethyl-containing dimer ($U^{OHEt}pU$, entry 3). The half-life for the hydrolysis of $U^{OHMe}pU$ in 2 M NaOH at 80 °C, was determined to be 220 min. In comparison, hydrolysis of $U^{OHEt}pU$ under identical conditions was extremely slow and a half-life was not determined. However, the observation that hydrolysis is considerably faster for $U^{OHMe}pU$ in comparison to $U^{OHEt}pU$, suggests that for the former dimer, the reaction proceeds through a six-membered cyclic phosphate. The half-life for the hydrolysis of UpU in 0.1 M NaOH was determined to be 440 min. at 20 °C and 33 min. at 50 °C and thus over this temperature range a 10 °C rise in temperature increases the rate by 2.4 fold. This equates to an increase in stability towards base of 1840 fold for $U^{OHMe}pU$ in comparison to UpU. Given this very large rate difference and the fact that base- or buffer-catalysed cleavage of RNA is often examined as a model for the action of ribonucleases,[15] it seems unlikely that oligonucleotides containing 2'-homouridine will be substrates for RNases. In support of this we found that $U^{OHMe}pU$ was untouched by ribonuclease A after incubation for two days, under conditions that gave total cleavage of UpU in less than 30 min.



entry	R	Relative half-life with HO^-	Hydrolysis with ribonuclease A[c]	Relative half-life with SVPD[c]
1	OH	1[a]	very fast	1
2	CH_2OH	1840[b]	no	39
3	CH_2CH_2OH	very slow[b]	no	129

Table. Summary of hydrolysis data for UpU, $U^{OHMe}pU$ and $U^{OHEt}pU$

[a]Hydrolysis performed in 0.1 M NaOH at 50 °C; [b]Hydrolysis performed in 2 M NaOH at 80 °C; [c]Enzymatic hydrolyses performed as previously described[5].

The relative half-lives for hydrolysis catalysed by snake venom phosphodiesterase (SVPD) were found to be 1, 39 and 129 for UpU, $U^{OHMe}pU$ and $U^{OHEt}pU$, respectively, and this trend shows increasing hydrolytic resistance with increasing length of the 2'-alkyl chain. Hydrolysis by SVPD is known to proceed through a covalent phosphoryl intermediate involving an active site threonine residue[16] and based on this mechanism it is not unexpected that the introduction of substituents that are bulky, in comparison to the hydroxyl group of the wild-type substrate, result in a reduced rate of hydrolysis.

In conclusion, we have developed an efficient synthesis of 2'-homouridine and believe it to be a very useful analogue of uridine that maintains all the original functionality and yet confers significant resistance to chemical and enzymatic hydrolysis of dinucleotides containing this analogue. It is expected that longer oligomers containing this modification will be also show increased resistance to hydrolytic cleavage. Significantly, preliminary transcription studies using T7 RNA polymerase with the 2'-homouridine 5'-triphosphate, show that this analogue can be incorporated into RNA enzymatically and details of this work will be reported shortly.

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References and notes:

1. Hoshino, H.; Shimizu, N.; Shimada, N.; Takita, T.; Takeuchi, T. *J. Antibiot.* **1987,** *40,* 1077-1078.
2. Svansson, L.; Kvarnström, I.; Classon, B.; Sammuelson, B. *J. Org. Chem.* **1991,** *56,* 2993-2997.
3. Tseng, C. K.-H.; Marquez, V. E.; Milne, G. W. A.; Wysocki, R. J.; Mitsuya, H.; Shirasaki, T.; Driscoll, J. S. *J. Med. Chem.* **1991,** *34,* 343-349.
4. De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995,** *28,* 366-374.
5. Lawrence, A. J.; Pavey, J. B. J.; Cosstick, R.; O'Neil, I. A. *J. Org. Chem.* **1996,** *61,* 9213-9222.
6. Schmit, C.; Bevierre, M.-O.; De Mesmacker, A.; Altmann, K.-H. *Bioorg. and Med. Chem. Lett.* **1994,** *4,* 1969-1974.
7. De Mesmaeker, A.; Lebreton, J.; Hoffmann, P.; Freier, S. M. *Synlett* **1993,** 677-679.
8. The ene product **3a** (500 mg, 0.8 mmol) was dissolved in dry CH_2Cl_2 (10 mL), cooled to -78 °C, and a stream of ozone passed through the solution for 1 min. The resulting solution was seen (as judged by tlc) to still contain starting material and therefore ozonolysis was continued for a further 4.5 min until all the starting material was consumed. The resulting solution was allowed to warm to room temperature and then added over 2 min into a solution of sodium borohydride (237 mg, 6.4 mmol) in methanol (40 ml) at 0 °C. After 10 min the resulting solution was neutralised with solid citric acid, the solvent evaporated and the residue dissolved in ethyl acetate (50 mL) and washed with saturated sodium bicarbonate solution (2x30 mL) and brine (2x20 mL). The organic layer was separated, dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Column chromatography over silica gel eluting with CH_2Cl_2/MeOH (0-4%) afforded the required product as a white hygroscopic foam (350 mg, 87%). Anal. Found: C, 51.88, H, 8.14, N, 5.59, $C_{22}H_{40}N_2O_7Si_2.1/2H_2O$ requires C, 51.84, H, 8.11, N, 5.50; Found FAB HRMS *m/z* $(M + H)^+$, 501.2441. $C_{22}H_{41}N_2O_7Si_2$ requires $(M + H)^+$, 501.2452; ^{1}H NMR (400 MHz) δ 10.20 (1H, br s, NH), 7.67 (1H, d, *J* = 8.0 Hz, H6), 5.94 (1H, d, *J* = 2.4 Hz, H1'), 5.72 (1H, d, *J* = 8.0 Hz, H5), 4.59 (1H, t, *J* = 8.4 Hz, H3'), 4.12 (1H, dd, *J* = 3.2, 13.0 Hz, H5'), 4.07-3.93 (4H, m, H5", H4' and 2 x H6'), 3.10-2.92 (1H, br s, OH), 2.53-2.50 (1H, m, H2'), 1.26-1.02 (28H, m, TIPS); ^{13}C NMR (75.5 MHz) δ 163.97 (C4), 150.64 (C2), 139.66 (C6), 102.14 (C5), 87.36 (C1'), 83.58 (C4'), 69.57 (C3'), 60.73, 59.49 (C6' + C5'), 49.91 (C2'), 17.19, 17.11, 17.02, 16.86, 16.74, 16.63, 13.14, 12.75, 12.65, 12.32 (TIPS).
9. Matsui, M.; Kamiya, K.; Shibata, K.; Muramatsu, H. *J. Org. Chem.* **1990,** *55,* 1396-1399.
10. Schmit, C. *Synlett* **1994,** 238-240.
11. Sanghvi, Y. S.; Ross, B.; Bharadwaj, R.; Vasseur, J. J. *Tetrahedron Letters* **1994,** *35,* 4697-4700.
12. Kierzek, R.; Caruthers, M. H.; Longfellow, C. E.; Swinton, C. E.; Turner, D. H.; Frier, S. M. *Biochemistry* **1986,** *25,* 7840-7846.
13. **2'-Deoxy-2'-α-*C*-(hydroxymethyl)uridylyl-(3'-5')-uridine** (**7**) triethylammonium salt. ^{1}H NMR (400 MHz; D_2O) δ 7.84 (1H, d, *J* = 8.1 Hz, H6), 7.82 (1H, d, *J* = 8.2 Hz, H6), 6.08 (1H, d, *J* = 8.4 Hz, H1'), 5.89 (1H, d, *J* = 4.8 Hz, H1'), 5.87 (1H, d, *J* = 8.1 Hz, H5), 5.85 (1H, d, *J* = 8.2 Hz, H5), 4.32-4.14 (6H, m, 2 x H6', 2 x H3' and 2 x H4'), 4.08-4.04 (1H, m, UH2'), 3.92 (1H, dd, *J* = 6.5, 11.6 Hz, H5'), 3.77-3.75 (2H, m, 2 x H5"), 3.70 (1H, d, *J* = 8.2 Hz, H5'), 3.14 (6H, q, J = 7.4 Hz, CH_3CH_2), 2.70-2.64 (1H, m, $U^{HM}H2'$), 1.22 (9H, t, *J* = 7.4 Hz, CH_3CH_2); ^{31}P NMR (101 MHz; $CDCl_3$) δ -0.47; *m/z* (FAB^-) 563 (M^-, 100%), 451 ([*M*-uracil]$^-$, 6).
14. Westheimer, F. H. *Acc. Chem. Res.* **1968,** *1,* 70.
15. Kirby, A. J.; Marriott, R. E. *J. Am. Chem. Soc.* **1995,** *117,* 833-834.
16. Culp, J. S.; Butler, L. G. *Arch. Biochem. Biophys* **1986,** *246,* 245-249.