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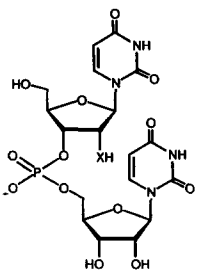
3' - Thiouridylyl - (3' → 5') - uridine

Xiaohai Liu and Colin B. Reese*

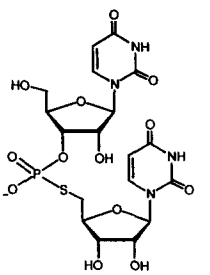
Department of Chemistry, King's College London, Strand, London WC2R 2LS, England

Abstract: 3'-Thiouridylyl-(3'→5')-uridine **3** undergoes base-catalysed hydrolysis more rapidly than UpU **1a**; it also undergoes cleavage more rapidly than UpU in glacial acetic acid solution, but shows much less (if any) tendency to isomerize.

The possible development of antisense chemotherapy[1] has encouraged organic chemists to undertake the synthesis of oligodeoxyribo- and oligoribo-nucleotide analogues, and particularly analogues in which the sugar residues and internucleotide linkages are modified. Studies directed towards the elucidation of the mechanism of ribozyme action[2] have also led to the synthesis of modified oligoribonucleotides. Our own studies in the synthesis of oligonucleotide analogues have been further stimulated by a long-standing intrinsic interest[3] in the chemistry of ribonucleic acids (RNA). As part of these studies, we have examined the effects of replacing the 2'-hydroxy function of uridylyl-(3'→5')-uridine (UpU) **1a** by a thiol function[4] (as in **1b**) and the 5'-bridging oxygen atom in the internucleotide linkage of UpU by a sulfur atom[5] (as in **2**). In order to complete what we consider to be a fundamental study in RNA chemistry, we have now[6] synthesized 3'-thiouridylyl-(3'→5')-uridine **3** and have thereby examined the effect of replacing the 3'-bridging oxygen atom in the internucleotide linkage of UpU by a sulfur atom.



1 a; X = O
b; X = S



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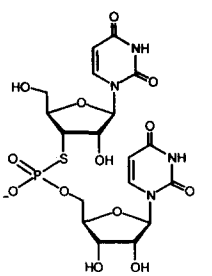
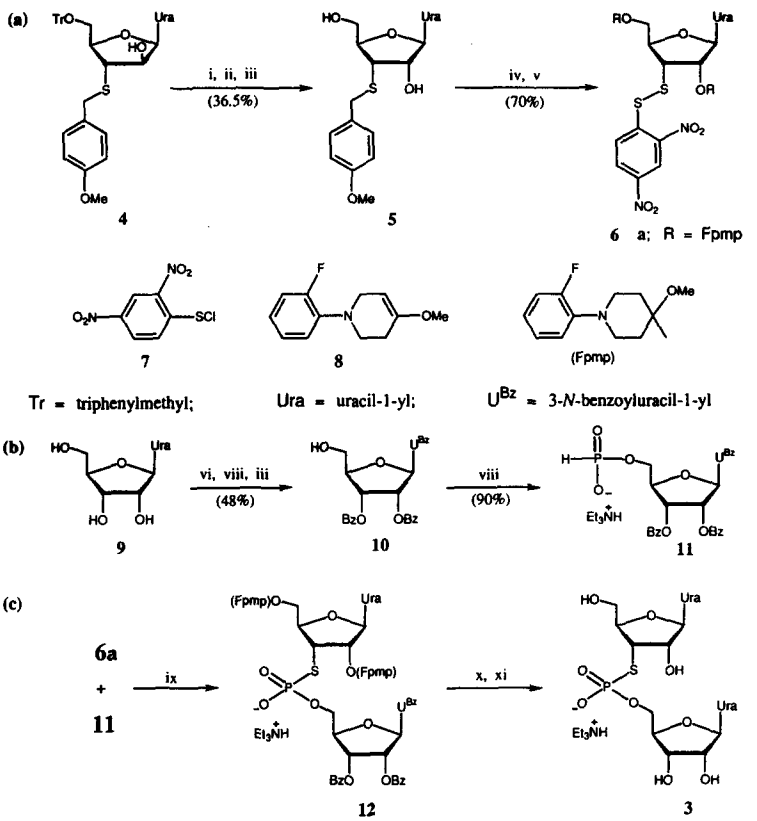


3

3'-Thiouridylyl-(3'→5')-uridine **3** was prepared (Scheme 1) from the building blocks **6a** and **11**. The thioether[7] **4** was subjected (Scheme 1) to the Mitsunobu reaction[8] in the presence of 4-nitrobenzoic acid. Saponification of the resulting 4-nitrobenzoate ester and removal of the 5'-*O*-trityl group gave the *S*-(4-methoxybenzyl) derivative[9] **5** of 3'-thiouridine in 36.5% overall yield. Treatment of the latter compound with 2,4-dinitrophenylsulfanyl chloride[11] **7** in the presence of trifluoroacetic acid, followed by reaction of the product with the enol ether[12] **8**, also in the presence of acid, gave the fully-protected disulfide building block **6a** in 70% overall yield. The *H*-phosphonate building block[13] **11** was prepared (Scheme 1b) in the usual way[5] from 2',3'-di-*O*-benzoyl-3-*N*-benzoyluridine[14] **10** in 90% yield. The three benzoyl

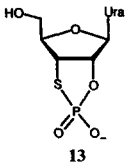


Tr = triphenylmethyl; Ura = uracil-1-yl; U^{Bz} = 3-*N*-benzoyluracil-1-yl

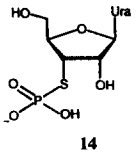
Scheme 1 *Reagents and conditions:* i, 4-$(O_2N)C_6H_4$-CO_2H, Ph_3P, EtO_2C-N=N-CO_2Et, MeCN, 0 °C to RT; ii, $MeNH_2$, EtOH, RT; iii, AcOH-H_2O (4 : 1 v/v), reflux; iv, 7, CF_3CO_2H, CH_2Cl_2, 0 °C; v, 8, CF_3CO_2H, CH_2Cl_2, RT; vi, TrCl, C_5H_5N, 100 °C; vii, BzCl, C_5H_5N, RT; viii, a, reagent prepared from PCl_3, Et_3N, 1,2,4-1*H*-triazole, THF, -35 °C, 15 min, b, Et_3N-H_2O (1 : 1 v/v), -35 °C to RT; ix, Me_3SiCl, Et_3N, CH_2Cl_2, RT, 17 hr; x, NH_3, MeOH, RT, 17 hr; xi, AcOH-H_2O (2 : 98 v/v), RT, 17 hr.

protecting groups were introduced to facilitate the isolation both of the *H*-phosphonate **11** and the intermediate dinucleoside phosphorothioate **12**. The two building blocks **6a** and **11** were coupled together (Scheme 1c) by essentially the same modification of the procedure of Li *et al*[15] that was previously used in the preparation of uridylyl-(3'→5')-(5'-thiouridine)[5] **2**. Following the removal of the benzoyl and Fpmp protecting groups (steps x and xi) and chromatography of the products on DEAE-Sephadex A-25, 3'-thiouridylyl-(3'→5')-uridine **3** was isolated as its pure triethylammonium salt[16] (δp [D_2O] 18.69).

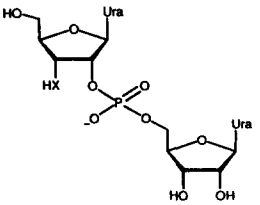
When 3'-thiouridylyl-(3'→5')-uridine **3** was heated at 50°C in 0.05 mol dm^{-3} sodium glycinate buffer solution (pH 10.06), it was converted into uridine **9**, 3'-thiouridine 2',3'-cyclic phosphorothioate **13** and 3'-thiouridine 3'-phosphorothioate[17] **14**. The reaction displayed pseudo first order kinetics with $t_{1/2}$ = 25 min[19]. Under the same conditions, $t_{1/2}$ for the hydrolysis of UpU **1a** was found to be 80 - 90 hr. Thus at pH 10.06 and 50°C, the 3'-thio-analogue **3** underwent base-catalyzed hydrolysis at a rate *ca.* 200 times faster than that of UpU **1a**. This result is perhaps surprising in that both reactions involve the formation and the cleavage of a phosphorus-oxygen bond. A possible contributing factor to the increased base lability of **3** relative to that of **1a** is the presence of a larger 3'-sulfur atom in **3** which presumably leads to a lower energy transition state in the hydrolysis reaction and to a less strained five-membered cyclic ester product **13**. The latter cyclic



13



14



15 a; X = O
b; X = S

Ura = uracil-1-yl

phosphorothioate **13** was most probably the only primary nucleotide product in the above base-catalysed hydrolysis of **3**; in a separate study carried out in 0.05 mol dm^{-3} sodium glycinate buffer solution (pH 9.87) at 30°C, 3'-thiouridine 2',3'-cyclic phosphorothioate **13** was found to undergo hydrolysis ($t_{1/2}$ = 165 min) to give 3'-thiouridine 3'-phosphorothioate **14** as the sole product.

3'-Thiouridylyl-(3'→5')-uridine **3** was rapidly converted mainly into uridine **9** and 3'-thiouridine 2',3'-cyclic phosphorothioate **13** in glacial acetic acid solution at 30°C; after 6 min, only *ca.* 25% of substrate **3** remained and after 30 min, virtually none was left. Small quantities of 3'-thiouridine 3'-phosphorothioate[20] **14** and another product [t_R = 7.51 min (programme A)[19], < 5% of the total absorbance at 260 nm] that may have been 3'-thiouridylyl-(2'→5')-uridine **15b** or the corresponding dimeric disulfide[4] were observed after 30 min. UpU **1a** was found to be somewhat more stable in glacial acetic acid at 30°C, but it showed more tendency to isomerize to give uridylyl-(2'→5')-uridine **15a**; after 60 min, the products[21] consisted of substrate **1a** (49.8%), its 2'→5'-isomer **15a** (15.7%), uridine 2'- and 3'-phosphates (4.6%), uridine 2',3'-cyclic phosphate (12.3%) and uridine (17.6%).

Finally, the action of various hydrolytic enzymes on 3'-thiouridylyl-(3'→5')-uridine **3** was investigated. The latter dinucleoside phosphorothioate **3** was found to be a good substrate for *Crotalus adamanteus* snake venom phosphodiesterase, but it did not undergo digestion in the presence of bovine spleen phosphodiesterase. 3'-Thiouridylyl-(3'→5')-uridine **3** also proved to be a good substrate for ribonuclease A: in the presence of the latter enzyme, it underwent digestion to give first a mixture of uridine **9**, 3'-thiouridine 2',3'-cyclic phosphorothioate **13** and 3'-thiouridine 3'-phosphorothioate **14**, and finally a 1:1 mixture of uridine and 3'-thiouridine 3'-phosphorothioate. In confirmation of the latter observation, 3'-thiouridine 2',3'-cyclic phosphorothioate **13** was independently found to be a good substrate for ribonuclease A; in the presence of the latter enzyme it underwent quantitative digestion to give 3'-thiouridine 3'-phosphorothioate **14** as the sole product.

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9. Found : C, 53.51, H, 5.30, N, 7.29. $C_{17}H_{20}N_2O_6S$ requires : C, 53.67; H, 5.30; N, 7.36%, m.p. 144-146°C; δ_H [$(CD_3)_2SO$] 3.19 (1 H, dd, *J* 4.9 and 9.3), 3.63 (1 H, m), 3.72 (3 H, s), 3.73-3.83 (3 H, m), 4.01 (2 H, m), 5.27 (1 H, t, *J* 4.7), 5.58 (1 H, d, *J* 8.1), 5.65 (1 H, d, *J* 1.7), 5.92 (1 H, d, *J* 5.2), 6.84 (2 H, d, *J* 8.6), 7.24 (2 H, d, *J* 8.6), 8.02 (1 H, d, *J* 8.1), 11.29 (1 H, br s). The identical compound **5** was obtained, albeit in low yield, by the acidic hydrolysis of the products of the reaction between the conjugate base of 4-methoxybenzyl mercaptan and 3'-*O*-mesyl derivative of 1-(2,5-di-*O*-trityl-β-D-xylofuranosyl)uracil[10].

10. Yung, N. C. ; Fox, J. J. *J. Am. Chem. Soc.*, **1961**, *83*, 3060-3066.

11. The acid-catalysed reaction between 4-methoxybenzyl thioethers and 2,4-dinitrophenylsulfanyl chloride **7** proceeds under similar conditions to those reported previously[7] for 2-nitrophenylsulfanyl chloride.

12. Reese, C. B.; Thompson E. A. *J. Chem. Soc., Perkin Trans. 1*, **1988**, 2881-2885.

13. δ_P[$(CD_3)_2SO$] 2.1 (d, $J_{P,H}$ 602.8).

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16. In an experiment starting from disulfide **6a** (0.1 mmol) and *H*-phosphonate **11** (0.2 mmol), the isolated yield of pure dinucleoside phosphorothioate **3** was 387 A_{260} units.

17. 3'-Thiouridine 3'-phosphorothioate **14** was prepared by a modification of the procedure of Müller and Roth[18]: 3'-deoxy-3'-(2,4-dinitrophenyldisulfanyl)uridine **6**, R = H (0.1 mmol) was treated first with Me_3SiCl (0.5 mmol) and Et_3N (0.55 mmol) and then with $P(OSiMe_3)_3$ (0.15 mmol) in CH_2Cl_2 solution. Aqueous work-up and fractionation of the products on DEAE-Sephadex A-25 gave the triethylammonium salt of 3'-thiouridine 3'-phosphorothioate **14** (507 A_{260} units), δ_P[D_2O] 16.1. Treatment of a vigorously stirred solution of the latter material (210 A_{260} units) in water (0.5 ml) with n-Bu_3N (0.5 mmol) and $ClCO_2Et$ (0.2 mmol), and fractionation of the products on DEAE-Sephadex A-25 gave the triethylammonium salt of 3'-thiouridine 2',3'-cyclic phosphorothioate **13** (168 A_{260} units), δ_P[D_2O] 37.1.

18. Müller, C. E.; Roth, H. J. *Tetrahedron Lett.*, **1990**, *31*, 501-502.

19. The progress of the reaction was monitored by HPLC. Reverse phase HPLC was carried out on a 5μ Jones APEX ODS column, following programme A [linear gradient of CH_3CN - 0.1 mol dm^{-3} aqueous triethylammonium acetate (pH 7.0) (3:97 to 20:80 v/v) over 10 min, followed by a linear gradient (20:80 to 30:70 v/v) over a further 5 min]. Retention times for uridine **9**, 3'-thiouridine 2',3'-cyclic phosphorothioate **13**, 3'-thiouridine 3'-phosphorothioate **14** and 3'-thiouridylyl-(3'→5')-uridine **3** were 3.37, 4.83, 6.27 and 7.76 min, respectively.

20. The formation of 3'-thiouridine 3'-phosphorothioate **14** was presumably due to the presence of traces of moisture in the acetic acid.

21. This reaction was monitored by reverse phase HPLC, following programme B [linear gradient of CH_3CN - 0.1 mol dm^{-3} aqueous triethylammonium acetate (pH 7.0) (3:97 to 7:93 v/v) over 10 min]. The numbers in parentheses represent percentages of the total absorbance at 260 nm.

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