The Synthesis of 2′-Thiouridylyl-(3′ → 5′)-uridine

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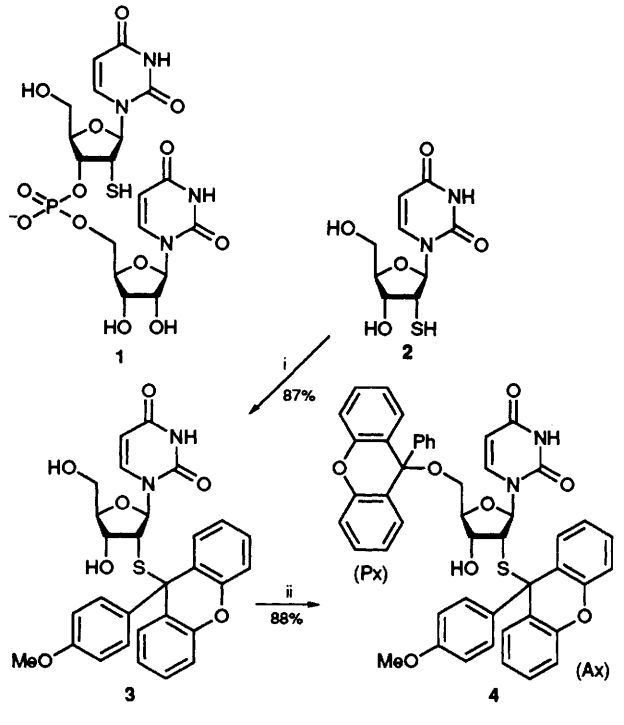
The synthesis of 2′-thiouridylyl-(3′ → 5′)-uridine **1** and some of its properties are described.

The concept of antisense chemotherapy has encouraged organic chemists to undertake the synthesis of a variety of oligodeoxyribo- and oligoribo-nucleotide analogues, and especially those analogues in which the sugar residues and internucleotide linkages are modified. One oligoribonucleotide modification which is of particular interest, both in the context of antisense[1] and ribozyme[2] research, is that obtained by replacing the crucial 2′-hydroxy functions by thiol groups. We now report the synthesis and discuss some of the properties of 2′-thiouridylyl-(3′ → 5′)-uridine **1**, which we believe to be the first described oligonucleotide analogue of this type.

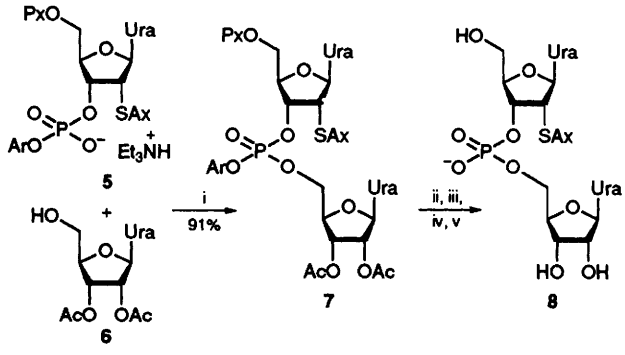
The key nucleoside building block **4** was prepared in two steps (Scheme 1) from 2′-thiouridine[5] **2** in 76% overall yield and isolated as a crystalline solid, mp 157–159 °C; this compound **4** was converted by the standard procedure[6] into the corresponding triethylammonium 3′-(2-chlorophenyl) phosphate **5** which was isolated as a colourless solid precipitate {δ_P[$(CD_3)_2SO$] −6.0} in 96% yield. The latter material was coupled (Scheme 2) with 2′,3′-di-*O*-acetyluridine[7] **6** (0.84 equiv.) in the presence of MSNT[8] (2.4 equiv.) in anhydrous pyridine solution to give the fully-protected dinucleoside phosphate **7** {δ_P[$(CD_3)_2SO$] −7.0, −7.1} in 91% isolated yield. The latter material was unblocked by a four-step process (Scheme 2) to give 2′-[9-(*p*-anisyl)xanthen-9-yl]thiouridylyl-(3′ → 5′)-uridine **8**. This material was isolated as an HPLC-homogeneous triethylammonium salt [356 A_{260} units from 0.235 g (*ca.* 0.18 mmol) of **7**; δ_P(CD_3OD + D_2O) 0.2].

When a solution of the above triethylammonium salt **8** (100 A_{260} units) and pyrrole (2 mm^3, 0.03 mmol) in 0.1 mol dm^{-3} hydrochloric acid was allowed to stand at room temperature for 16 h, triethylammonium 2′-thiouridylyl-(3′ → 5′)-uridine **1** (60 A_{260} units) was obtained. The latter compound was characterized on the basis of ^{1}H, ^{13}C [δ (0.1 mol dm^{-3} HCl in D_2O) 45.0,† 2′-C-SH], ^{31}P [Fig. 1(*a*), δ (0.1 mol dm^{-3} HCl in D_2O) −0.3] NMR spectroscopic data; it was found to be *ca.* 97% pure by HPLC (Fig. 1(*b*)]. Only partial removal of the 2′-[9-(*p*-anisyl)xanthen-9-yl] protecting group occurred in the absence of pyrrole.[3,5,9]

2′-Thiouridylyl-(3′ → 5′)-uridine **1** does not display the equivalent of what is arguably the most characteristic property of a ribooligonucleotide in that its 2′-thiol function does not interact with the internucleotide phosphodiester linkage under either acidic or basic conditions; furthermore, it is not a substrate for ribonuclease A. When 2′-thiouridylyl-(3′ → 5′)-uridine **1** is heated in 0.1 mol dm^{-3} hydrochloric acid (pH 1.0) at 100 °C for 1 h, it is partially (*ca.* 33%) converted into its dimer **9** [δ_C (D_2O) 54.7,† 2′-C-S_2-; δ_P (D_2O) −0.6]. Unlike that of uridylyl-(3′ → 5′)uridine,[11] the internucleotide linkage



Scheme 1 *Reagents and conditions*: i, 9-(*p*-anisyl)xanthen-9-ol (AxOH),[3] $MeOCH_2CO_2H$, MeCN, room temp., 30 min; ii, 9-chloro-9-phenylxanthene (PxCl),[4] C_5H_5N, room temp., 30 min



Scheme 2 *Reagents and conditions*: i, 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-1*H*-triazole (MSNT),[8] C_5H_5N, room temp., 45 min; ii, Cl_2CHCO_2H, pyrrole,[9] CH_2Cl_2, room temp., 2 min; iii, Ac_2O, C_5H_5N, room temp., 7 h; iv, (*E*)-2-nitrobenzaldoxime,[10] N^1,N^1,N^3,N^3-tetramethylguanidine, MeCN, room temp.,1.5 h; v, aq. NH_3 (*d* 0.88), 6 h, room temp.: Ura = uracil-l-yl, Px = phenylxanthen-9-yl, Ax = 4-(*p*-anisyl)xanthen-9-yl, Ar = 2-chlorophenyl

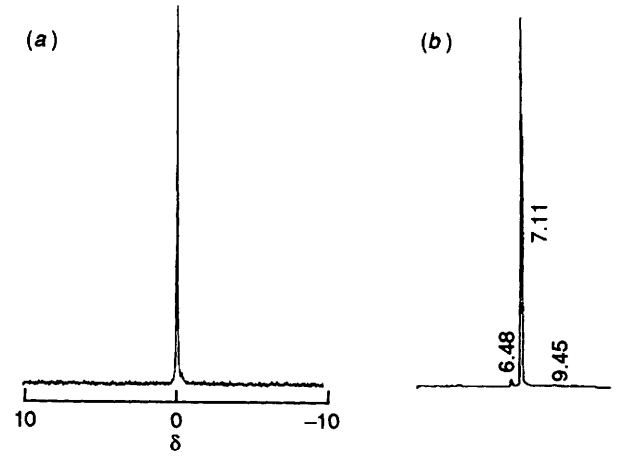
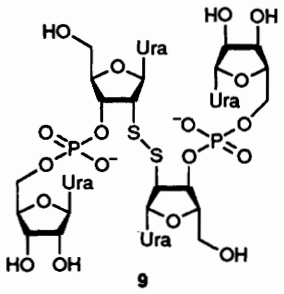


Fig. 1 (*a*) ^{31}P NMR spectrum (145.8 MHz, 0.1 mol dm^{-3} HCl in D_2O) of 2′-thiouridylyl-(3′ → 5′)-uridine **1**; (*b*) reverse phase HPLC profile [Jones APEX OS 10 μ column, eluted with 0.1 mol dm^{-3} aqueous triethylammonium acetate–MeCN (95:5–50:50 v/v)] of 2′-thiouridylyl-(3′ → 5′)-uridine **1** (R_t 7.11 min)



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of the 2′-thio analogue **1** shows no tendency whatsoever to undergo cleavage or to migrate under the latter conditions. Dimer **9** is also slowly formed as the main product when 2′-thiouridylyl-(3′ → 5′)-uridine **1** is allowed to stand in neutral (pH 7.0) aqueous solution at room temperature. Under more basic conditions (*i.e.* at pH 9.0), appreciable cleavage of glycoside linkage of the 2′-thiouridine residue also occurs, leading to the formation of uracil[12] and uridine 5′-phosphate. The latter compound is presumably a further decomposition product of the resulting apyrimidinic acid.

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Footnote

† The chemical shifts of the C-2′ resonance signals of 2′-thiouridine[5] **2** and its dimer[5] occur at δ 44.9 and 55.5, respectively, in $(CD_3)_2SO$ solution.

References

1 *Oligonucleotides: Antisense Inhibitors of Gene Expression*, ed. J. S. Cohen, Macmillan, London, 1989.
2 W. P. Jencks, *Nature*, 1992, **358**, 543.
3 J. H. Marriott, M. Mottahedeh and C. B. Reese, *Carbohydr. Res.*, 1991, **216**, 257.
4 J. B. Chattopadhyaya and C. B. Reese, *J. Chem. Soc., Chem. Commun.*, 1978, 640.
5 K. J. Divakar, A. Mottoh, C. B. Reese and Y. S. Sanghvi, *J. Chem. Soc., Perkin Trans. 1*, 1990, 969.
6 J. M. Brown, C. Christodoulou, A. S. Modak, C. B. Reese and H. T. Serafinowska, *J. Chem. Soc., Perkin Trans 1*, 1989, 1751.
7 G. W. Kenner, A. R. Todd, R. F. Webb and F. W. Weymouth, *J. Chem. Soc.*, 1954, 2288.
8 S. S. Jones, B. Rayner, C. B. Reese, A. Ubasawa and M. Ubasawa, *Tetrahedron*, 1980, **36**, 3075.
9 C. B. Reese, H. T. Serafinowska and G. Zappia, *Tetrahedron Lett.*, 1986, **27**, 2291.
10 C. B. Reese and L. Zard, *Nucleic Acids Res.*, 1981, **9**, 4611.
11 B. E. Griffin, M. Jarman and C. B. Reese, *Tetrahedron*, 1968, **24**, 639.
12 M. Imazawa, T. Ueda and T. Ukita, *Chem. Pharm. Bull. Jpn.*, 1975, **23**, 604.