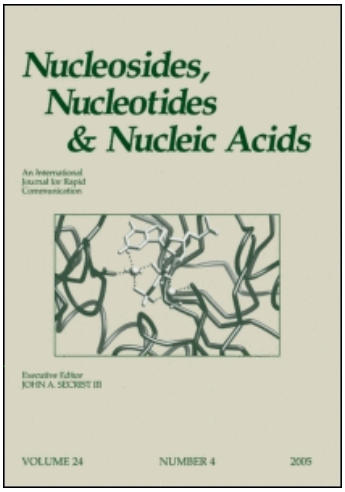

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NUCLEOSIDES & NUCLEOTIDES, 8(7), 1179-1188 (1989)

NUCLEOSIDES. 145. SYNTHESIS OF 2,5'-ANHYDRO-2-THIOURIDINE AND ITS CONVERSION TO 3'-O-ACETYL-2,2'-ANHYDRO-5'-CHLORO-5'-DEOXY-2-THIOURIDINE. STUDIES DIRECTED TOWARD THE SYNTHESIS OF 2'-DEOXY-2'-SUBSTITUTED arabino NUCLEOSIDES (7).[1]

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Abstract: In an attempt to introduce a substituent at C-2' in the ''up'' arabino configuration directly by nucleophilic displacement reaction of a preformed pyrimidine ribonucleoside, we synthesized 2,5'-anhydro-5'-deoxy-2-thiouridine (**6**) in three steps from uridine. Compound **6** was converted into the 3'-O-acetyl derivative **7**. Upon treatment of **7** with triflyl chloride in methylene chloride in the presence of triethylamine and p-dimethylaminopyridine, 2,2'-anhydro-1-(3-O-acetyl-5-chloro-2,5-dideoxy-β-D-arabinofuranosyl)-2-thiouracil (**9**) was obtained as the only isolable product. Obviously, the intermediate 3'-O-acetyl-2,5'-anhydro-2'-O-triflyl-2-thiouridine (**8**) was attacked by the chloride nucleophile at C-5' first giving the 2'-O-triflyl-2-thiouridine intermediate from which **9** was formed by intramolecular nucleophilic reaction.

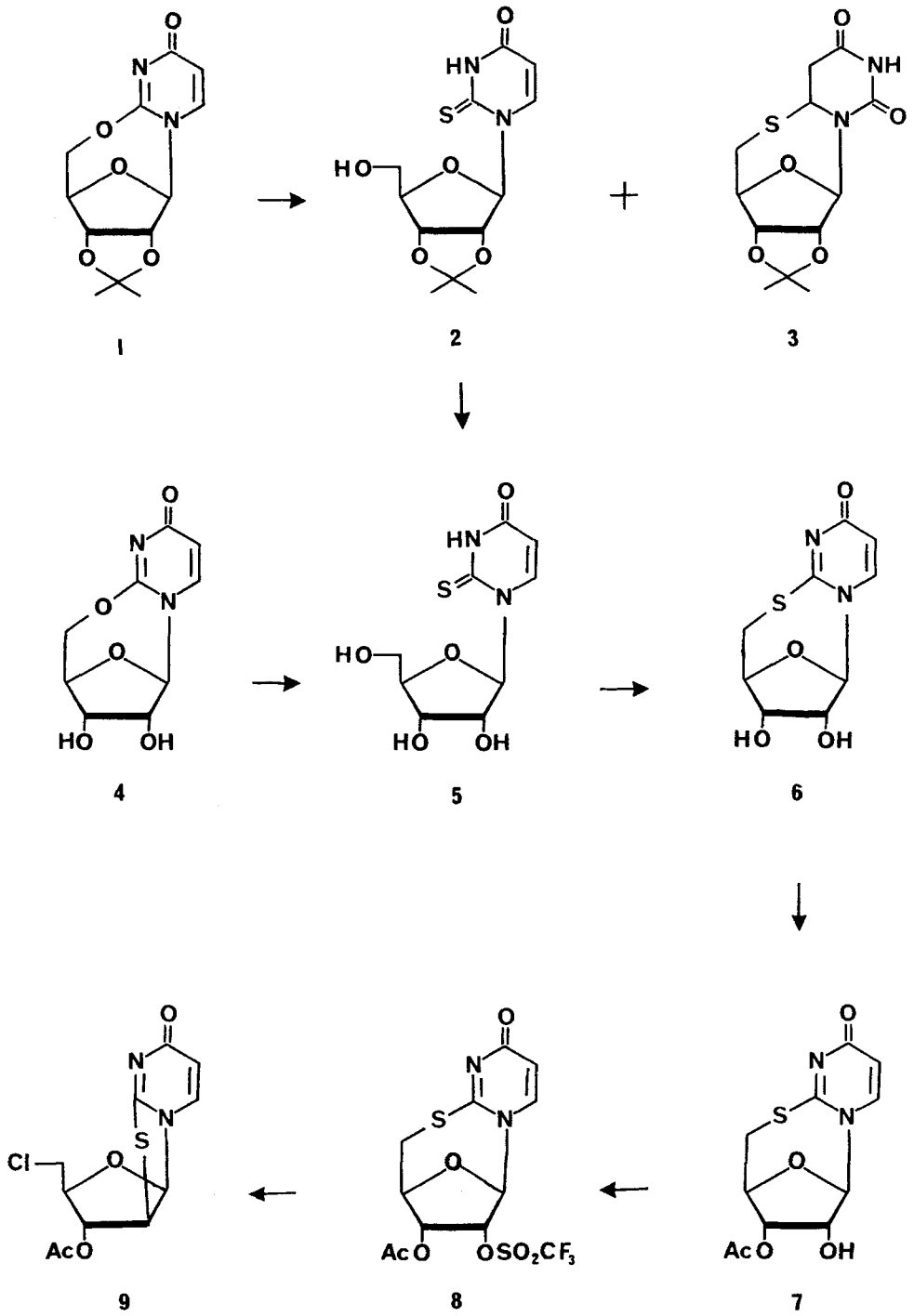
Many 1-(2-deoxy-2-substituted-β-D-arabinofuranosyl)pyrimidines have shown antitumor[3-6] and/or antiviral[7-9] activities. All of these nucleosides have been synthesized by condensation of an appropriately protected 2-substituted arabinose derivative with a pyrimidine and, if necessary, followed by modification on the heterocyclic base.[3-16] We have recently attempted to introduce an halogen substituent into the 2'-''up'' position on a preformed pyrimidine ribonucleoside by nucleophilic

displacement reaction by way of anhydronucleoside intermediates.[17-21] This strategy was successfully executed in the area of C-nucleosides.[17] Thus, the triflate group of 4,5'-anhydro-3'-O-acetyl-2'-O-triflyl-1-methylpseudouridine was displaced relatively smoothly with a nucleophile, such as acetate, chloride, bromide or azide ion, to give rise to the 2'-''up''-substituted-4,5'-anhydro-C-nucleoside. Mild acid hydrolysis of the anhydro linkage of the product afforded the desired 5-(2-substituted-β-D-arabinofuranosyl)-1-methyluracil.

The above procedure, however, was found not to be applicable to pyrimidine N-nucleosides. When 2,5'-anhydro-3'-O-acetyl-2'-O-triflyl-uridine was treated with various nucleophiles, the only products obtained were the corresponding 5'-substituted 2,2'-anhydrouridines.[20] On the other hand, 6,5'-anhydro-3-N-benzyl-1-(2'-O-triflyl-β-D-ribofuranosyl)barbituric acid was converted into the 5'-chloro-6,3'-anhydro-xylo derivative through triflyl migration upon treatment with LiCl.[21] Treatment of 2,3'-anhydro-1-(5-O-acetyl-2-O-triflyl-β-D-xylofuranosyl)uracil with LiCl afforded the 2'-''up''-chloro-2,3'-anhydro-lyxo nucleoside, i.e., the 2'-triflate group was directly displaced by the chlorine nucleophile. All attempts to hydrolyze the 2,3'-anhydro linkage of the lyxo product, however, resulted in the formation of 2'-chloro-2',3'-didehydrouridine.[22]

Since sulfur is larger in size but less electronegative than oxygen, the 2,5'-S-anhydro linkage (Chart 1) might be more stable than the 2,5'-O-anhydro linkage. We hoped, therefore, that the 2'-triflate in **8** might be displaced directly by a nucleophile. We synthesized 2-thiouridine (**5**) as the key intermediate by the published methods[23] starting from 2,5'-anhydro-2',3'-O-isopropylideneuridine (**1**). Treatment of **1** with H_2S in dry pyridine afforded, as described in the

CHART 1



literature, the desired 2',3'-O-isopropylidene-2-thiouridine (**2**) only as the minor product (35 % yield) and 5'-deoxy-5',6-epithio-5,6-dihydro-2',3'-O-isopropylideneuridine (**3**, 60 % yield) as the major product. De-O-isopropylidenation of **2** afforded **5** in 82 % yield (or 28 % overall from **1**). We found, however, that treatment of 2,5'-anhydrouridine (**4**)[24] with H_2S in pyridine directly afforded **5** in 64 % yield. Compound **5** prepared was identical with 2-thiouridine prepared by the procedure of Niedballa and Vorbruggen[25] via condensation of 2-thiouracil and 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose.

Conversion of **5** into 2,5'-S-anhydro-2-thiouridine (**6**) was accomplished by application of Mitsunobu's procedure[26] with modification:[24a] namely, by treatment of **5** with Ph_3P and diethyl azodicarboxylate (DEAD) in aqueous dioxane. Butyltin catalyzed acetylation of **6** afforded 3'-O-acetyl-2,5'-S-anhydro-2-thiouridine (**7**). The structure of **7** was readily established by ^1H NMR spectroscopy in Me_2SO-d_6. A multiplet (of H-2') at δ 5.05 in **7** collapsed upon addition of D_2O to a double doublet coupling with H-1' and H-3' ($J_{1',2'}$ = 0.4, $J_{2',3'}$ = 6.6 Hz). These data together with the presence of a narrow doublet for H-1' ($J_{1',2'}$ = 0.5 Hz) established the presence of the free 2'-OH function in **7**.

Upon treatment of **7** with triflyl chloride in methylene chloride in the presence of triethylamine and p-dimethylaminopyridine (DMAP), 2,2'-S-anhydro-1-(3-O-acetyl-5-chloro-5-deoxy-β-D-arabinofuranosyl)-2-thiouracil (**9**) was obtained in 20 % yield as the major isolable product. The ^1H NMR spectrum showed that there was no dissociable proton in this product. The large $J_{1',2'}$ value of 7.1 Hz and small coupling between H-2' and H-3' ($J_{2',3'}$ = 2.2 Hz) established the arabino configuration of the carbohydrate moiety of the product. The UV spectrum of this

product showed the maximum absorbance at 227.6 nm whereas the λmax for **7** was 242.0 nm. It has been reported that the λmax of 2,2'-S-anhydro-1-(β-D-arabinofuranosyl)-2-thiouracil was 230 nm[27] whereas that for **6** was 244 nm.[24a] These data together with elemental analyses fully established the 5'-chloro-2,2'-S-anhydro structure **9** for the product.

The TLC analyses of the reaction mixture showed the presence of two major spots of about equal amounts one of which (less polar) corresponded to the spot of **9**. A small amount of the more polar component was separated from the column. The structure of the latter was established as 3'-O-acetyl-2'-O-triflyl-2,5'-S-anhydro-2-thiouridine (**8**) by ^{1}H NMR analyses and chemical conversion to **9**. The overall pattern of the ^{1}H NMR spectrum was very similar to that of **7**, except for the H-2' signal which appeared as a double doublet at much lower field (δ 8.00) than that of **7**. When this compound was treated with LiCl in DMF, **9** was obtained in 50 % yield.

The above results clearly show that even C-5' of the S-anhydro linkage of **8** is more susceptible to nucleophilic attack than the triflate group at C-2'. Apparently, the chloride nucleophile attacked C-5' to generate sulfur anion at C-2 which subsequently attacked C-2' to form the 2,2'-S-anhydro linkage with concomitant loss of the triflate, resulting in the formation of **9**.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX90Q spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in ppm (δ) and signals are described as s (singlet), d

(doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), brs (broad singlet). Values given for coupling constants are first order. Column chromatography was conducted under low pressure using flash grade silica gel (Merck #9385, 0.040-0.063 nm). TLC was performed on silica gel Analtech Uniplates and visualized using short wave-length UV light. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

2-Thiouridine (5) from 2,5'-Anhydrouridine (4).

To a solution of **4**[24] (300 mg, 1.33 mmol) in a mixture of water (5 mL) and pyridine (10 mL) was added liquid H_2S (15 mL). The mixture was kept at room temperature in a sealed tube for 4 days. After removal of the solvent in vacuo, the residue was chromatographed on a silica gel column ($CHCl_3$-MeOH, 5:1 v/v) to give **5** (220 mg, 64 %), mp 214 °C. The mixture's melting point with an authentic sample[27] was undepressed. Also, this compound was identical to an authentic 2-thiouridine with respect to IR and ^1H NMR spectral characteristics.

2,5'-S-Anhydro-2-thiouridine (6).

To a stirred suspension of **4** (4.0 g, 15.4 mmol) and Ph_3P (12.1 g, 46.1 mmol) in 1,4-dioxane (50 mL) was added dropwise DEAD (8.03 g, 46.1 nmmol), and the mixture was stirred for 3 h. Water (4 mL) was added and the mixture was heated at reflux for 30 min. After concentration of the mixture, the residue was dried by azeotropic distilation with toluene (50 mL). The residue was suspended in benzene (100 mL) and heated at reflux for 10 min. Upon cooling to room tempoerature, crude crystals of **6** deposited and were collected by filtration and recrystallized from EtOH to give pure **6** (2.84 g, 76 %), mp 164-165 °C (lit.[24a] mp 164-165 °C).

3'-O-Acetyl-2,5'-S-anhydro-2-thiouridine (7).

A suspension of **6** (3 g. 12.4 mmol) and n-Bu_2SnO (3.06 g, 12.4 mmol) in MeOH (240 mL) was heated under reflux until a clear solution was obtained. After concentration of the solution in vacuo, the residue was dissolved in DMF (150 mL). The solution was cooled in an ice-bath, and Ac_2O (1.35 g, 13.2 mmol) was added. The mixture was stored overnight at 4 °C, and then concentrated in vacuo. The residue was triturated with EtOH, and the solid was recrystallized from EtOH to give pure **7** (2.46 g, 70 %), mp 121-122 °C. ^1H NMR (Me_2SO-d_6) δ 2.08 (3H, s, OAc), 3.12 (1H, dd, H-5', $J_{5',5''}$ = 14.6, $J_{4',5'}$ = 3.0 Hz), 3.50 (1H, dd, H-5'', $J_{5',5''}$ = 14.6, $J_{4',5''}$ = 2.5 Hz), 4.91 (1H, narrow m, H-4'), 5.05 (1H, dt, H-2', collapsed to dd upon D_2O exchange, $J_{1',2'}$ = 0.2, $J_{2',3'}$ = 6.6 Hz), 5.34 (1H, d, H-3', $J_{2',3'}$ = 6.6, $J_{3',4'}$ = 0 Hz), 5.66 (1H, d, H-1', $J_{1',2'}$ = 0.2 Hz), 5.95 (1H, d, OH, exchangeable), 6.00 (1H, d, H-5, $J_{5,6}$ = 7.7 Hz), 8.03 (1H, d, H-6). UV (H_2O) λmax 242.0 nm, λmin 203.0 nm.

Anal. Calcd. for $C_{11}H_{12}N_2O_5S$: C, 46.47: H, 4.26: N, 9.85: S, 11.28. Found: C, 46.42: H, 4.21: N, 9.73: S, 11.63.

Treatment of 7 with Triflyl Chloride. Isolation of 2,2'-S-anhydro-1-(2-O-acetyl-5-chloro-5-deoxy-β-D-arabinofuranosyl)-2-thiouracil (9) and 3'-O-Acetyl-2,5'-S-anhydro-2'-O-triflyl-2-thiouridine (8).

To a stirred suspension of **7** (2.0 g, 7.04 mmol), DMAP (0.86 g, 7.04 mmol) and Et_3N (1.42 g, 14.07 mmol) in CH_2Cl_2 (250 mL) was added dropwise a solution of triflyl chloride (1.5 mL, 14.07 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred overnight at room temperature, and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$-MeOH, 5:1 v/v). The first fraction from the column

contained **9** which, after recrystallization from EtOH, was isolated as colorless crystals (425 mg, 20 %), mp 210-211 °C. ^{1}H NMR (Me_2SO-d_6) δ 2.09 (3H, s, OAc), 3.73 (1H, dd, H-5', $J_{4',5'}$ = 6.3, $J_{5',5''}$ = 12.0 Hz), 3.85 (1H, dd, H-5'', $J_{4',5''}$ = 4.1, $J_{5',5''}$ = 12.0 Hz), 4.48 (1H, m, H-4'), 4.66 (1H, dd, H-2', $J_{1',2'}$ = 7.1, $J_{2',3'}$ = 2.2 Hz), 5.17 (1H, dd, H-3', $J_{2',3'}$ = 2.2, $J_{3',4'}$ = 3.8 Hz), 5.93 (1H, d, H-5, $J_{5,6}$ = 7.7 Hz), 6.43 (1H, d, H-1', $J_{1',2'}$ = 7.1 Hz), 7.88 (1H, d, H-6, $J_{5,6}$ = 7.7 Hz). UV (H_2O) λmax 262.8 (sh) and 227.6 nm, λmin 217.2 nm.

Anal. Calcd. for $C_{11}H_{11}ClN_2O_4S$: C, 43.64: H, 3.66: Cl, 11.71: N, 9.25. Found: C, 43.62: H, 3.61: Cl, 11.57: N, 9.14.

Compound **8** (80 mg) was then eluted from the column. This compound, however, was too unstable and resisted purification. ^{1}H NMR (Me_2SO-d_6) δ 2.07 (3H, s, OAc), 3.20 (1H, dd, H-5', $J_{4',5'}$ = 3.0, $J_{5',5''}$ = 12.0 Hz), 3.52 (1H, dd, H-5'', $J_{4',5''}$ = 1.6, $J_{5',5''}$ = 12.9 Hz), 5.03 (1H, narrow m, H-4'), 5.55 (1H, d, H-3', $J_{2',3'}$ = 6.3, $J_{3',4'}$ = 0 Hz), 5.98 (1H, d, H-5, $J_{5,6}$ = 7.7 Hz), 6.06 (1H, d, H-1', $J_{1',2'}$ = 0.5 Hz), 6.50 (1H, dd, H-2', $J_{1',2'}$ = 0.5, $J_{2',3'}$ = 6.3 Hz), 8.00 (1H, d, H-6, $J_{5,6}$ = 7.7 Hz).

Reaction of 8 with LiCl.

To a solution of **8** (50 mg, 0.12 mmol) in DMF (5 mL) was added LiCl (51 mg, 0.12 mmol). The mixture was stirred at 100 °C for 30 min, and then concentrated. The residue was chromatographed on a silica gel column ($CHCl_3$-MeOH, 5:1 v/v) to give **9** (18 mg, 50 %) after recrystallization from EtOH, mp 210-211 °C, undepressed upon admixture of an authentic sample.

References and Footnotes

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