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SIMPLE AND EFFECTIVE METHOD FOR THE SYNTHESIS OF 3′,5′-SUBSTITUTED 1-β-*D*-ARABINOFURANOSYLURACIL

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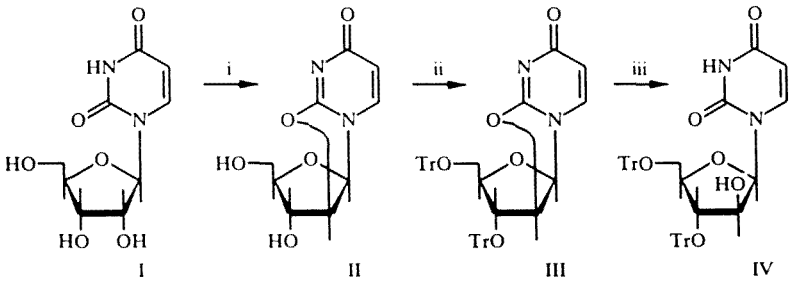
3′,5′-Substituted arabinofuranosyluracil is a starting compound in 2′-modifications. A convenient and effective method is proposed for the synthesis of 1-(3′,5′-di-o-trityl-β-D-arabinofuranosyl)uracil by successive reactions of 2,2′-cyclization of uridine, 3′,5′-tritylation of the 2,2′-anhydrouridine, and hydrolytic cleavage of the 2,2′-anhydro bond.

Modified 1-β-*D*-arabinofuranosylpyrimidines have antineoplastic activity and are used in antineoplastic therapy [1-10]. The volume and polarity of the substituent in the 2′-arabino position of the carbohydrate moiety have a considerable influence on the biological activity, most probably through their effects in changing the conformation of the carbohydrate fragment and the reactivity of the 3′-hydroxyl group. Moreover, oligonucleotides containing modified nucleosides are finding extensive applications in "antisense therapy" [11]. One of the approaches to the synthesis of "antisense therapeutics" is the construction of a monomer unit (modified nucleoside) by introducing into the molecule a "sticky group," either nucleophilic or electrophilic in nature, followed by the introduction of the modified nucleoside into the oligonucleotide. Such an approach requires a conveniently protected nucleoside with a reactive group that will subsequently provide for the addition of an intercalating or chelating agent.

Scheme 1



i) $(C_6H_5O)_2CO$, HMFA, $NaHCO_3$, 150 °C, 20 min; ii) TrCl, Py, 100 °C, 25 h; iii) NaOH/EtOH, refluxing, 5h

One of the starting compounds that meets these requirements is 1-(3′,5′-substituted-β-*D*-arabinofuranosyl)uracil, the modification of which at position 2′ can be accomplished either by 2′-O-(amino)alkylation [12] or by 2′-C-alkyl/arylation through the corresponding keto derivative [13].

As the protective groups for the 3′- and 5′-hydroxyls, we used triphenylmethyl (trityl), since trityl ethers, which are stable in alkaline and weakly acidic media and under certain other conditions [14], are preferred in some cases because of the low cost of trityl chloride in comparison with organosilicon reagents.

Previously, 1-(3′,5′-di-O-trityl-β-*D*-arabinofuranosyl)uracil had been obtained by several comparatively low-efficiency methods [15-17]. Two of these methods consisted essentially of inversion of the 2′-hydroxyl group by forming a 2,2′-cyclointer-

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mediate; this was accomplished in four stages as a minimum [15, 16]. In the first of these methods, the starting compound was uridine; in the second method, the starting material was a previously prepared 2′-deoxy-2′-fluorouridine. In a third method, 1-(3′,5′-di-O-trityl-β-*D*-arabinofuranosyl)uracil was obtained successfully as a result of borohydride reduction of the 3′,5′-di-O-trityl-2′-keto group [17]. When using these methods, the yield of the desired product was no greater than 20%.

We are proposing a convenient and highly effective method for the synthesis of 1-(3′,5′-di-O-trityl-β-*D*-arabinofuranosyl)uracil (see Scheme 1), consisting of three successive reactions: 1) cyclization of the original uridine (I) to 2,2′-anhydrouridine (II); 2) tritylation of the 2,2′-anhydrouridine to form the 3′,5′-bistritylated product (III); and 3) decyclization to form the arabinonucleoside (IV).

Anhydronucleosides are important starting compounds in the synthesis of arabinonucleosides; they can be obtained through the corresponding activated intermediates such as tosyl [15], mesyl [18], carbonyl [19], and thiocarbonyl [20] derivatives. However, most of these methods are very laborious and give low yields. We have carried out the 2,2′-cyclization of uridine by interaction of uridine I with diphenyl carbonate by a method given in [19], in hexamethylphosphoric triamide [21], which simultaneously plays the role of an effective catalyst of the S_N2 reaction that takes place.

3′,5′-Di-O-tritylation was accomplished by trityl chloride in pyridine [23]. However, the introduction of the trityl group into position 3′ of the 2,2′-anhydronucleoside II required rather severe reaction conditions (25 h at 100°C).

The 2,2′-anhydro bond was cleaved with nearly quantitative yield by refluxing the 2,2′-anhydro-1-(3′,5′-di-O-trityl-β-*D*-arabinofuranosyl)uracil in an alkaline water—ethanol solution, following a procedure given in [23]. As a result, the desired 1-(3′,5′-di-O-trityl-β-*D*-arabinofuranosyl)uracil was obtained with a total yield of 60% (on original uridine).

EXPERIMENTAL

Capillary melting points were determined in a Thomas Hoover instrument; the values were not corrected. The ^1H NMR and ^{13}C NMR spectra were taken in a Bruker 25FT NMR instrument. The individuality of the products that were obtained was monitored by TLC on Merck Kieselgel F254 plates. The compounds were detected in ultraviolet light and/or by spraying with a solution of sulfuric acid in methanol, with subsequent combustion. The substances were separated by column chromatography on Merck silica gel, 35-70 mesh, 40 Å.

2,2′-Anhydro-1-(β-*D*-arabinofuranosyl)uracil (II) was synthesized by a method of [19] as modified in [21], with a 92% yield, mp 240-242°C. Literature data: mp 238-244°C [19], 246-248°C [22], 234-236°C [20]. PMR spectra (DMSO-d_6), ppm: 7.85 (1H, d, 6-H), 6.34 (1H, d, 1′-H), 6.0 (1H, br.s, 3′-OH), 5.87 (1H, d, 5-H), 5.23 (1H, d, 2′-H), 5.04 (1H, br.s, 5′-OH), 4.41 (1H, s, 3′-H), 4.11 (1H, t, 4′-H), 3.24 mp (2H, m, 5′-H). ^{13}C spectra (DMSO-d_6), ppm: 171.54 (C-4), 159.91 (C-2), 137.98 (C-6), 108.62 (C-5), 88.87, 89.32 and 90.11 (C-1′, C-2′ and C-4′), 74.81 (C-3′), 60.88 mp (C-5′)

2,2′-Anhydro-1-(3′,5′-di-O-trityl-β-*D*-arabinofuranosyl)uracil (III). A mixture of 2,2′-anhydro-1-(β-*D*-arabinofuranosyl)uracil (II) (11.3 g, 50 mmoles) and triphenylchloromethane (41.8 g, 150 mmoles) in 150 ml of absolute pyridine was stirred for 25 h at 100°C. In 5 h, a homogeneous cherry-colored solution was formed. The course of the reaction was followed by TLC (system consisting of 10% methanol in methylene chloride, double development). When the reaction was completed, the mixture was treated as follows [14]: The solution was diluted with 20 ml of ethanol; after 10 min, it was poured into 3 liters of ice water, while stirring vigorously. The product was extracted three times with 300 ml of chloroform, and the chloroform layer was washed with water. This operation was repeated three times. The combined chloroform extracts were vacuum-evaporated to a minimum volume; the product was isolated by column chromatography (eluent 0.5% methanol in methylene chloride). Yield of compound III 23.1 g (67%), mp 143-145°C. Literature data [24]: mp 141-143°C. PMR spectrum ($CDCl_3$), ppm: 7.62 (1H, d, 6-H), 7.12-7.29 (30H, m, Ar), 6.16 (1H, d, 1′-H), 5.0 (1H, d, 5-H), 4.31 (1H, t, 3′-H), 3.93 (1H, m, 2′-H), 3.59 (1H, dd, 4′-H), 3.29 (2H, m, 5′-H). ^{13}C spectrum ($CDCl_3$), ppm: 162.48 (C-4), 149.68 (C-2), 143.31 (C-6), 127.50-128.93 (Ar), 89.39 (C-1′), 81.90 (C-4′), 72.35 (C-3′), 62.34 (C-2′), 61.40 mp (C-5′).

1-(3′,5′-Di-O-trityl-β-*D*-arabinofuranosyl)uracil (IV). Compound III (10.4 g, 13.8 mmoles) was dissolved in a mixture of 200 ml of 70% methanol and 50 ml of 1 N NaOH, and was refluxed for 5 h. The course of the reaction was followed by TLC (system consisting of 3% methanol in methylene chloride). After the reaction had been completed, the solution was neutralized with acetic acid and evaporated in a rotary evaporator. The still residue was dissolved in methylene chloride, and the solution was washed three times with distilled water. The organic phase was dried over sodium sulfate and evaporated to dryness. Yield of compound IV 9.8 g (98%), mp 149-150°C. Literature data [15]: mp 145-160°C. PMR spectrum ($CDCl_3$), ppm: 8.65-8.95 (1H, br.s, NH), 7.57 (1H, d, 6-H), 7.18-7.36 (30H, m, Ar), 6.11 (1H, d, 1′-H), 5.54 (1H,

d, 5-H), 5.17 (1H, m, 2'-OH), 4.0 (1H, m, 3'-H), 3.93 (1H, m, 4'-H), 3.64 (1H, d, 2'-H), 3.42 (1H, dd 5'-H), 3.28 (1H, dd, 5'-H). ^{13}C spectrum ($CDCl_3$), ppm: 163.38 (C-4), 149.98 (C-2), 143.64 (C-6), 127.0-128.67 (Tr), 100.40 (C-5), 74.71-88.36 (Cl'−C4'), 63.35 mp (C-5').

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