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STEREOSELECTIVE SYNTHESIS OF NOVEL ANALOGUES OF 2'-DEOXY- AND 2',3'-DIDEOXYNUCLEOSIDES WITH POTENTIAL ANTIVIRAL ACTIVITY

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ABSTRACT: The stereoselective synthesis of new 2'-deoxy- and 2',3'-dideoxy-2'-C-alkylnucleosides with potential antiviral activity is presented. The compounds here described were tested for their antiproliferative property against human tumor cell lines and none showed any significant antitumor activity.

Sugar modified nucleosides belong to a large family of compounds, which includes many of the most active antitumor and antiviral drugs licensed so far[1]. Interesting members of this family are the 2'-modified nucleosides.

The most representative examples of this group are 1-β-D-arabinofuranosylcytosine (ara-C)[2], and 1-β-D-arabinofuranosyladenine (araA)[3]. Another subfamily of sugar modified nucleosides, 2',3'-dideoxy nucleosides, includes the most active antiviral drugs against HIV[4].

Among all the possible substitutions for the 2'-position of a nucleoside that can be explored, there is an increasing interest in nucleosides bearing branched-chain sugars, as these compounds have already demonstrated interesting biological activities[5]. The introduction of a 2'-C-alkyl substituent can also provide intermediates for the synthesis of a new family of 2',3'-dideoxynucleoside analogs[6,7] with potential antiviral activity.

The synthesis of 2'-deoxy-2'-C-alkylpyrimidines reported up to now[5] involved the addition of an organometallic reagent to the 2'-ketonucleoside, which provided a mixture of 2'R and 2'S diasteromers, and subsequent deoxygenation of the 2'-hydroxyl group[8].

Only two preparations of 2',3'-dideoxy-2'-C-alkylnucleosides have been reported so far, and both deal with the *de novo* synthesis of the sugar and subsequent coupling with the base. In one case, the alkyl group was introduced stereospecifically into the sugar[7,9], while in the other a mixture of diasteromers was obtained[6]. In both cases the coupling reaction afforded a mixture of the α- and β-nucleosides.

In the present paper we describe the stereoselective synthesis of 2'-deoxy-2'-C-alkylnucleosides. Additionally, their antitumoral activity is reported.

Two different routes were followed, in which, by means of simplified chemistry, either stereoselectively or stereospecifically modifications of the ribonucleoside were introduced. In the first approach, a stereoselective synthesis of (2'S)-2'-deoxy-2'-C-methylpyrimidines was accomplished, taking advantage of the fact that the α-face of the 3',5'-protected nucleosides is the least hindered (Fig.1).

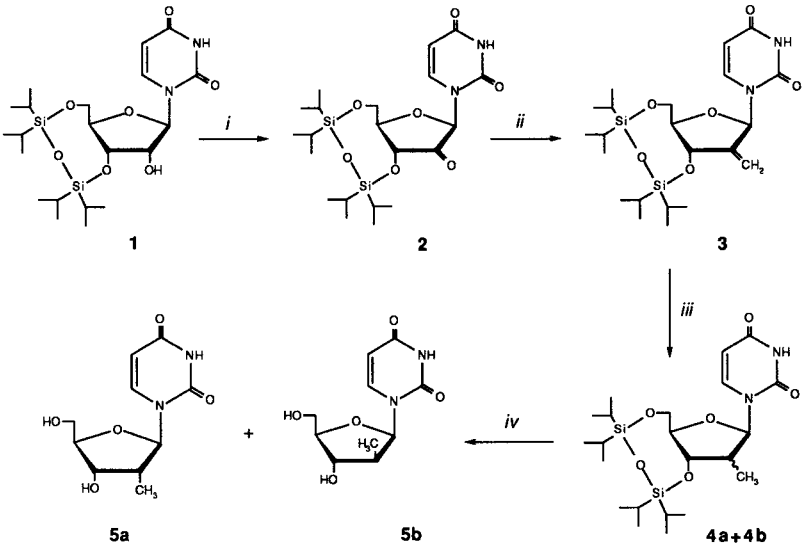

Figure 1. *i*- CrO_3, Py, Ac_2O; *ii*- Ph_3PCH_3Br, BuLi; *iii*- H_2/Pd; *iv*-TBAF.

The reaction of the protected 2'-ketouridine[10] **2** with methyltriphenyl phosphonium bromide produced the already known 2'-deoxy-2'-C-methyleneuridine[11] **3**, which was subjected to catalytic hydrogenation to generate a mixture of the protected (2'R)- and (2'S)-2'-deoxy-2'-C-methyluridine (**4a** and **4b**) in quantitative yield.

By selecting different catalysts for the hydrogenation step, it is possible to vary the 2'S/2'R ratio. For example, 10% Pd on charcoal provided a ca. 1:1 mixture of diasteromers, while the use of 5% Pd on $CaCO_3$ gave a 3/1 2'S/2'R ratio, as estimated by integration of the ^{1}H-NMR spectrum. The assignment of the (2'R)- and

(2'S)-2'-deoxy-2'-C-methyluridine was achieved by comparison with the NMR data reported for the thymidine analogs[5]. The mixture of the protected nucleosides could not be separated at this stage of the synthesis. The disiloxane bridge was removed, and the unprotected nucleosides **5a** and **5b** were separated by silica gel column chromatography (dichloromethane/methanol 15:1) to afford the pure isomers.

In the second approach, the stereospecific synthesis of (2'R)-2'-deoxy-2'-C-alkylnucleosides was attempted (Fig. 2).

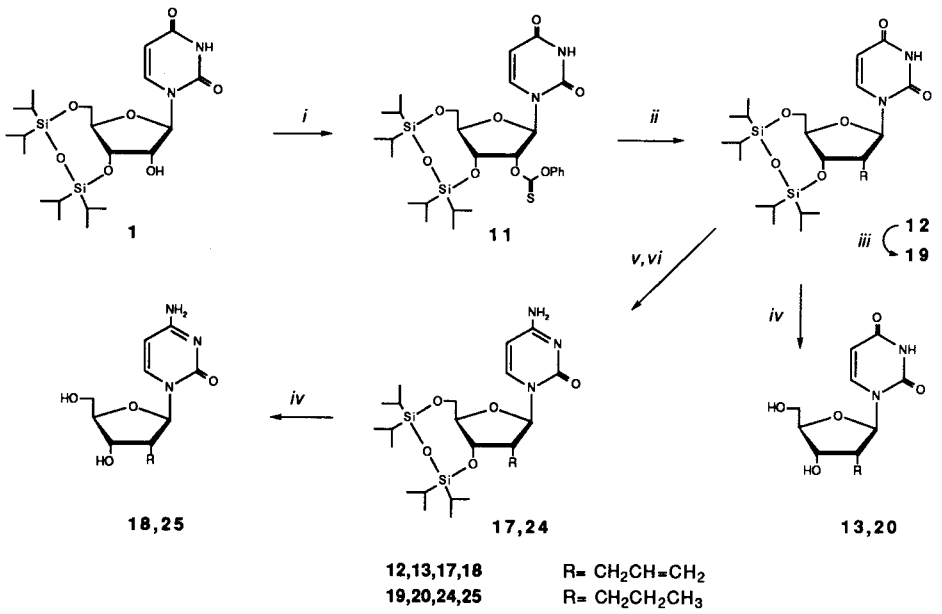

Figure 2. *i*- PhOCSCl; *ii*- $Bu_3SnCH_2CH{=}CH_2$, AIBN; *iii*- H_2/Pd; *iv*- TBAF; *v*- 1,2,4-triazole, $POCl_3$; *vi*- NH_3

It has been previously reported that sterically hindered derivatives of mannose and xylose gave C-allyl products with retention of configuration, making use of a radical reaction between the 2'-O-phenoxythiocarbonyl derivative and allyltributyltin, with AIBN as initiator[12]. The same stereospecificity was also observed in the synthesis of 3'-C-allyluridine[13]. These results, together with the fact that the α-face of the 3',5'-O-tetraisopropyldisiloxane-1,3-diyl-nucleosides is the least hindered, as corroborated by stereoselective hydrogenation, prompted us to attempt the radical reaction of the 2'-thionoester **11** with allyltributyltin. In this way the 2'-deoxy-2'-C-allyl derivative **12** was obtained in 67% yield. Subsequent desilylation gave compound **13,** which 1D-NOE analysis confirmed to have the expected R configuration at C-2', since irradiation of H-2' caused a NOE at H-6 (10.0 %) and irradiation of H-1 (proR) of the allyl group produced a NOE at H-1' (7.9 %), and H-4' (0.7 %).

Further hydrogenation of **12** with 10% Pd on charcoal as catalyst afforded 2'-C-propyl derivative **19** in 96% yield. Deprotection of **19** with tetrabutylammonium fluoride in THF gave (2'R)-2'-deoxy-2'-C-propyluridine (**20**).

The cytidine analogs were obtained from the protected allyl (**12**) and propyl (**19**) nucleosides by reaction with 1,2,4-triazole and phosphoryl chloride, and subsequent displacement with ammonia. Removal of the disiloxane bridge afforded pure (2'R)-2'-deoxy-2'-C-allylcytidine (**18**) and (2'R)-2'-deoxy-2'-C-propylcytidine (**25**) in 55 and 14% overall yield from **12** and **19**, respectively.

With the purpose of synthesizing 2',3'-dideoxy analogs, the 3'-hydroxyl groups of compounds **5a**, **5b**, **13** and **20** were removed.

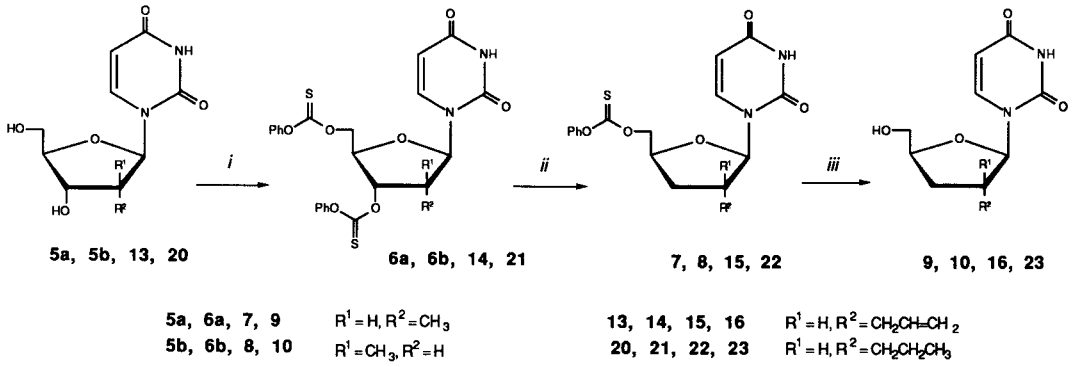

Figure 3. *i*- PhOCSCl; *ii*- Bu_3SnH, AIBN; *iii*- NaOH, Py, EtOH.

Deoxygenation was achieved following the route proposed by Barton, which involves a radical chain reaction of thionocarbonte derivatives with tributyltin hydride[14]. Recent results[15] indicate that it is possible to selectively deoxygenate a secondary hydroxyl in the presence of a primary one, and therefore, as shown in Figure 3, the additional protection of 5'-hydroxyl was unnecessary. Compounds **7**, **8**, **15** and **22** were obtained from their precursors in 40, 59, 33 and 32% yield respectively. This strategy also provided an alternative way to resolve the diasteromeric mixture, since the 3',5'-dithioacyl derivatives **6a** and **6b** showed differential chromatographic behaviour. The stereochemistry of C-2' was determined by NOE difference spectroscopy after deoxygenation and deprotection of the 5'-acyl function. Irradiation of 2'-C-methyl hydrogens of compound **9** produced a NOE at H-1' (12.0 %), at H-3" (2.8 %) and at H-4' (3.6 %), and on irradiation of H-2', a NOE at H-6 (6.3 %) was observed. These results indicate a (2'R) configuration for this compound and its precursors **6a** and **7**. For compound **10** a NOE at H-1' (13.8 %), at H-3" (3.8 %) and at H-4' (4.9 %) was observed when H-2' was irradiated, and at H-6 (1.1 %) when the protons of the methyl group were irradiated. Therefore, compounds **6b**, **8** and **10** were assigned as the respective (2'S)-2'-C-methyl derivatives.

The deoxygenation of cytidine analogs could not be achieved by using the same strategy. Upon reaction of 3',5'-di-O-phenylthionocarbonate-(2'R)-2'-deoxy-2'-C-allyl-N-benzoylcytidine with tributyltin hydride under the same conditions tried for compounds **7** and **8**, no deoxygenation product was observed and the starting material was recovered unaltered. An alternative route, involving the ammonia displacement of 4-(1,2,4-triazolyl) derivatives did not prove to be successful either.

Selected ^{1}H-NMR data for the final compounds are shown in table I. All the modified nucleosides reported in the present work showed analytical data in agreement with the assigned structures[16].

Table I. Chemical shifts for the new 2'-deoxy- and 2',3'-dideoxy-2'-C-alkyl-nucleosides synthesized.

	δ*, ppm								
Comp.	H-1'	H-2'	H-3'	H-4'	H-5'	H-5"	H-5	H-6	Others
5a	5.79	2.21	4.00	3.83	←3.55[a]→		5.65	7.83	0.91(CH_3)
5b	6.09	2.42	←3.74[a]→		←3.61[a]→		5.60	7.98	0.82(CH_3)
9	5.67	2.39	2.14	4.25	3.81	3.63	5.68	8.05	1.15(CH_3), 1.75(H-3")
10	6.16	2.78	1.66	4.11	3.92	3.70	5.65	8.21	0.94(CH_3), 1.99(H-3")
13	6.04	2.35	4.26	3.97	3.73	3.71	5.72	7.92	2.12(H-1a[b]),2.45(H-1b[b]), 4.93(H-3a[b]),5.06(H-3b[b]), 5.75(H-2[b])
16	5.76	2.46	2.16	4.28	3.95	3.68	5.74	7.76	2.21(H-1a[b]),2.37(H-1b[b]), 5.08[a](H-3[b]),5.75(H-2[b]), 1.84(H-3")
18	6.12	2.30	4.26	3.96	3.73	3.70	5.90	7.90	2.12(H-1a[b]),2.46(H-1b[b]), 4.91(H-3a[b]),5.05(H-3b[b]), 5.76(H-2[b])
20[c]	5.80	2.08	4.14	3.82	←3.58[a]→		5.58	7.64	0.78(CH_3[d]), 1.35[a](H-1,2[d])
23	5.74	2.33	2.18	4.27	3.95	3.68	5.73	7.71	0.92(CH_3[d]), 1.38[a](H-1,2[d]) 1.80(H-3")
25[c]	6.10	2.19	4.25	3.96	←3.68[a]→		5.91	7.92	0.88(CH_3[d]), 1.45[a](H-1,2[d])

*Measured at 500 MHz, unless otherwise stated. [a]The centre of the complex multiplet is reported. [b]Numbering for the allyl moiety. [c]Measured at 200 MHz. [d] Numbering for the propyl moiety.

Biological activity. All the compounds were tested for their antiproliferative activity against human tumor cell lines (K562 erythromyeloid stem cells, U937 and HL60 myeloid cells, Molt-4 and MT-2 T-lymphoid cells and DAUDI EBV-immortalized B-

lymphoid cells). Of all these cell lines, MT-2 cells have the peculiarity of being immortalized by the human leukemic retrovirus HTLV-1. This cell line can actively produce infecting virion particles in vitro. None of the compounds tested (**5a**, **5b**, **9**, **10**, **13**, **16**, **18**, **20**, **23** and **25**) could significantly inhibit the growth (cell count and viability) and proliferation (^{3}H-thymidine incorporation) of these cell lines up to 72 hours after treatment with increasing nucleoside concentrations (1.25, 2.5, 5, 10 μg/ml). Asynchronous and synchronous cell populations were equally insensitive to inhibition of cell growth under these experimental conditions.

The antiviral activity of these novel nucleoside analogues is currently under investigation.

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