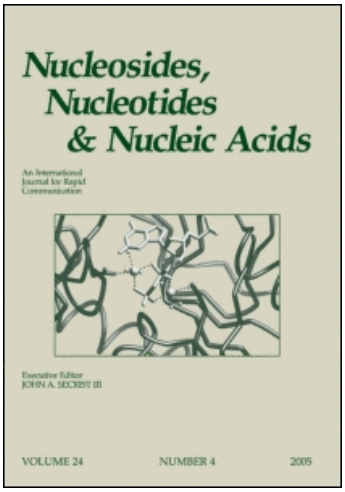

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3'/5'-Regioselectivity of Introduction of the 9-Fluorenyl-Methoxycarbonyl Group to 2'-o-Tetrahydropyran-2-YL-and 2'-O-(4-Methoxytetrahydropyran-4-YL-)-Nucleosides: Useful Intermediates for Solid-Phase-Rna-Synthesis

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3'/5'-REGIOSELECTIVITY OF INTRODUCTION OF THE 9-FLUORENYL-METHOXYCARBONYL GROUP TO 2'-O-TETRAHYDROPYRAN-2-YL- AND 2'-O-(4-METHOXYTETRAHYDROPYRAN-4-YL-)-NUCLEOSIDES: USEFUL INTERMEDIATES FOR SOLID-PHASE-RNA-SYNTHESIS

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ABSTRACT: X-ray structure analysis of the more laevorotatory isomers of 2'-O-tetrahydropyranyl-4N-benzoylcytidine (4b) and of 2'-O-tetrahydropyranyluridine (5b) confirmed their chirality at the satellite anomeric centre C2'' to be S. The other diastereomers (4a resp. 5a) exhibited an unexpected reversal of 3'/5'-regio-selectivity when treated with 9-fluorenylmethoxycarbonyl chloride in pyridine. The X-ray crystallographic results form the basis for a mechanistic proposal.

1. INTRODUCTION

In the context of our efforts towards the development of a reliable method for solid-phase synthesis of oligoribonucleotides, we re-investigated the acid-labile tetrahydropyranyl group [1] for permanent 2'-O-protection in combination with the 5'-O-fluorenylmethoxycarbonyl group, cleavable under basic conditions after each chain elongation step [2]. Here we wish to report our observations on the 3'/5'-regioselectivity

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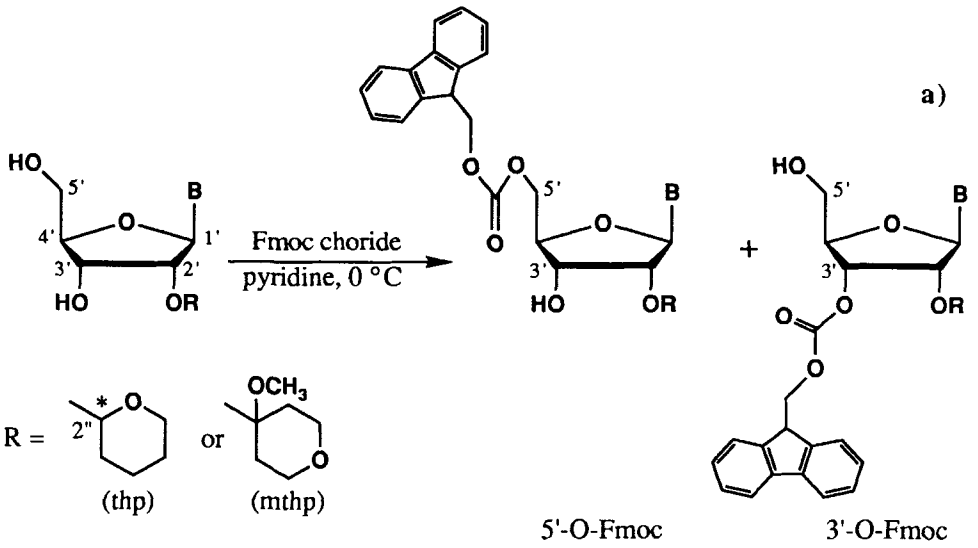
SCHEME 1: 3'/5'-Regioselectivity of Introduction of the Fmoc group



Thymidine (**1**)				24	(77%)	:	(3%)	1	
A^{Bz}	(*high* R_f)-thp	(**2a**)		5	(24%)[b]	:		1	c)
	(*low* R_f)-thp	(**2b**)		5	(58%)	:		1	c)
	mthp	(**2c**)		10	(65%)	:		1	c)
G^{iBu}	(*high* R_f)-thp	(**3a**)		5	(41%)	:	(8%)	1	
	(*low* R_f)-thp	(**3b**)		4	(40%)	:	(10%)	1	
	mthp	(**3c**)		10	(52%)	:		1	c)
C^{Bz}	(*high* R_f)-thp	(**4a**)	C2"-**R**	1	(17%)[d]	:	(56%)	4	
	(*low* R_f)-thp	(**4b**)	C2"-**S**	4	(60%)	:	(16%)	1	
	mthp	(**4c**)		8	(61%)	:		1	c)
U	(*high* R_f)-thp	(**5a**)	C2"-**R**	1	(11%)	:	(34%)	3	
	(*low* R_f)-thp	(**5b**)	C2"-**S**	3	(37%)	:	(12%)	1	e)
	mthp	(**5c**)		10	(64%)	:		1	c)

a) separated individually from unreacted starting material as well as from 3',5'-O-di-Fmoc nucleoside; isolated yields are given in brackets; yields are optimized for mthp derivatives **2c** - **5c** (cf. lit.2).
b) two chromatographies performed on main product
c) amount of side product estimated from t.l.c. (UV at 254 nm)
d) side product contaminated (probably with 5'- and/or 3'-O-fluorenylmethylether)
e) 23 % of 3',5'-O-di-Fmoc derivative collected

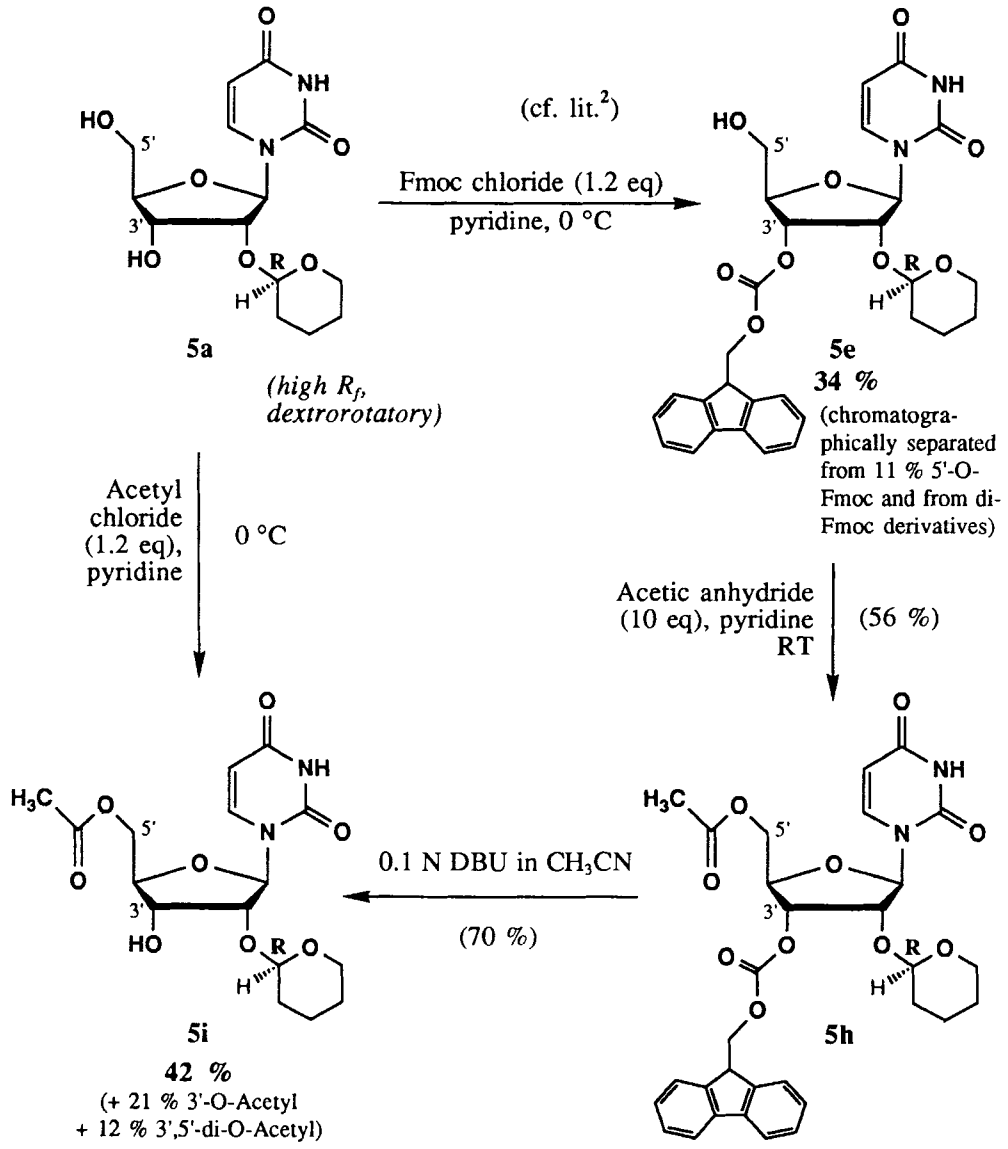
of acylation of the 2'-O-protected nucleoside substrates and to correlate chemical reactivity with absolute configuration at the C2" of the tetrahydropyranyl group, as determined by X-ray crystal structure analysis.

2. REGIOCHEMISTRY IN ACYLATION REACTIONS

SCHEME 1 gives the 3'/5'-regioselectivities observed for acylation of the various nucleoside substrates with 9-fluorenylmethoxycarbonyl (Fmoc) chloride in pyridine solution at 0 °C. This temperature is appropriate, because at -40 °C Fmoc chloride decomposes in the reaction medium to dibenzofulvene, carbon dioxide and pyridinium chloride faster than it attacks the nucleoside hydroxyl groups. The best preparative results were generally obtained by adding 1.2 equivalents of crystalline Fmoc chloride to a cooled solution of the nucleoside in anhydrous pyridine [2], yet the very same selectivities were obtained by adding solutions of the chloroformate in acetonitrile dropwise [3] or in one concentrated lot to the cooled substrate. Thymidine (**1**) served as a starting point, where we were able to confirm the reported [3] high yield and selectivity of introduction onto the 5'-hydroxyl group. The described 2'-O-protected derivatives in the *ribo* series however are characterised by a considerably lower selectivity for the primary hydroxyl group. Moreover, the fact that for both pyrimidines the isomer with higher mobility on silica gel (**4a** resp. **5a**) gave predominantly the undesired 3'-carbonate was clearly unanticipated and prompted the present investigation. Fmoc-acylation selectivities for the case of 2'-O-(4-methoxytetrahydropyran-4-yl)-nucleosides are included in Scheme 1 for comparison; here we isolated the desired 5'-O-Fmoc-nucleosides as the main products throughout and in satisfactory yields of 52-65 % (cf. lit. [2]).

Is such irregularity in the case of diastereomeric *thp*-derivatives observed for any acylation reaction or for the described carbonate diester formation only? SCHEME 2 illustrates that the reversal of selectivity is observed only in the case of Fmoc-carbonate formation, but not for ordinary acetylation with acetyl chloride under otherwise identical conditions. The preference here is 2 : 1 in favour of the primary hydroxyl group. If the acetylation is carried out at -40 °C, we observe a ratio of 6 : 1 in favour of the 5'-acetate **5i**, as determined by integration of the resonances of the with D_2O exchangeable hydroxyl protons (5.20 ppm as a broad doublet for 3'-OH, resp. 5.14 ppm as a broad triplet for 5'-OH). In addition, acetylation (or carbonate diester formation) leads to a downfield shift of the corresponding sugar ring or 5'-methylene protons of 0.7 - 1.0 ppm. After isolation of the major 2'-O-(R)-thp-uridine Fmoc-carbonate diester **5e** by silica gel chromatography, the latter was acetylated to the acetyl fluorenylmethoxy-

SCHEME 2: Correlation of 3'/5'-Regioselectivity of Acylations for 2'-O-(**R**)-thp-Uridine



carbonyl nucleoside **5h**, which in turn on treatment with 0.1 N DBU in acetonitrile (cf. lit. [2]) gave 5'-O-acetyl-2'-O-(R)-thp-uridine (**5i**). The 250-MHz-^{1}H-NMR-spectrum of the material **5i** obtained *via* the route **5a** → **5e** → **5h** was identical with the one observed for the main product after direct acetylation of **5a**, thus providing a coherent structural proof by ^{1}H-NMR-spectroscopy.

The constitutions of the other Fmoc-nucleoside carbonate diesters are evidenced by 250-MHz-^{1}H-NMR-spectra of the main isolated products, and in the case of guanosine the spectra of the side products (3'-O-Fmoc-2'-O-(R resp. S)-thp-2N-isobutyroyl-guanosine, **3e** resp. **3g**) are recorded as well.

3. X-RAY-STRUCTURE ANALYSIS OF THE PYRIMIDINE DERIVATIVES 4b AND 5b

For our synthetic purposes we simply characterised the C2"-diastereomers of the tetrahydropyranyl ethers by their relative mobility on silica gel. W. Klyne and co-workers [4] first established an empirical correlation of the molecular rotation of tetrahydropyranyl ethers of six related 17β-hydroxy-steroids with their absolute configuration at the acetal carbon centre. They assigned the **R** absolute configuration to the less laevorotatory and the **S** absolute configuration to the more laevorotatory diastereomer by comparison with α-D-pyranosides, which are generally less laevorotatory than their β-anomers [5]. This correlation was confirmed in the literature by X-ray crystal structure analysis for the case of two nucleoside derivatives (2'-O-(S)-thp-adenosine [6] and 2'-O-(R)-thp-uridine **5a** [7]); in this work we further determined the structures of 2'-O-(S)-thp-4N-benzoylcytidine (**4b**) and of 2'-O-(S)-thp-uridine (**5b**), easily obtained by crystallisation from acetonitrile / ether (isothermal diffusion), thus providing independent and complete structural information on the pyrimidine derivatives.

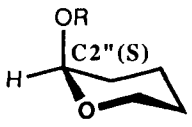


FIGURE 1 gives the crystal structures of **4b** and **5b**, as determined in this work. With respect to the pyranose ring, none of the examples violates the anomeric effect [8], according to which, however bulky, the more electronegative substituent on the anomeric centre (R = 4N-benzoylcytidin-2'-yl in **4b** or uridin-2'-yl in **5b**) is axially disposed.

The structures confirm that the more laevorotatory isomer has the **S** configuration at the C2" satellite anomeric centre. To compare the conformations of the four molecules 2'-O-(S)-thp-adenosine, **5a**, **5b** and **4b**, a best molecular fit was undertaken, using the

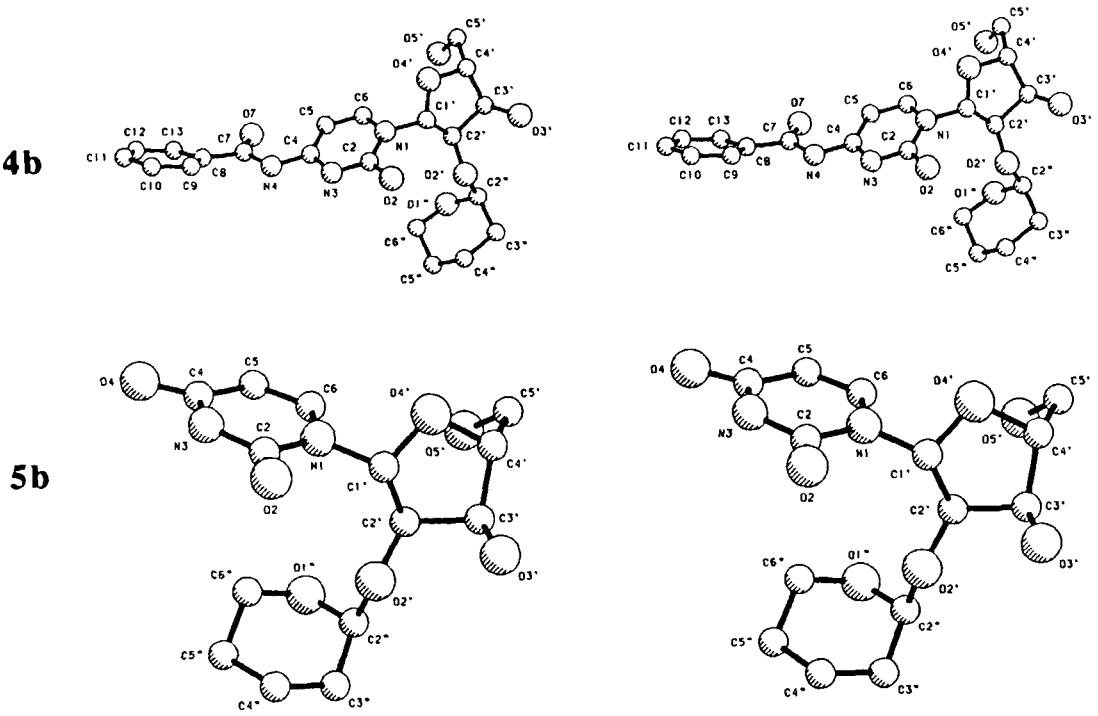


FIGURE 1: Stereographic projections of the crystal structures of 2'-O-(S)-tetrahydropyranyl-4N-benzoylcytidine (**4b**) and 2'-O-(S)-tetrahydropyranyl-uridine (**5b**) with atom numbering

sugar carbon and oxygen atoms including the glycosidic C1'-N-bond. As can be seen from FIGURE 2, the purine as well as two of the pyrimidine derivatives (diastereomers **5a** and **5b**) possess a **C2'-endo** ribose puckering, whereas the benzoylcytidine derivative **4b** adopts a somewhat unusual **C1'-exo** puckering. The latter however is quite close on the pseudorotation cycle [9] with a calculated phase angle P = 130° compared to P = 162° for C2'-endo. All derivatives are in the **anti** conformational range around the glycosidic bond (-ac; $\chi(A_{thp(S)}$ [6]) = -136.5°, χ(**5a** [7]) = -131.9°, χ(**5b**) = -134.8°, χ(**4b**) = -138.9°). Considering the very rapid equilibrium of furanose ring puckering in solution [10], we find difficulty in establishing a distinctive direct effect of the nature of the base on sugar ring conformation. How does the base residue then influence reactivity of 3'- and 5'-hydroxyl groups depending on the configuration at the C2" satellite anomeric centre?

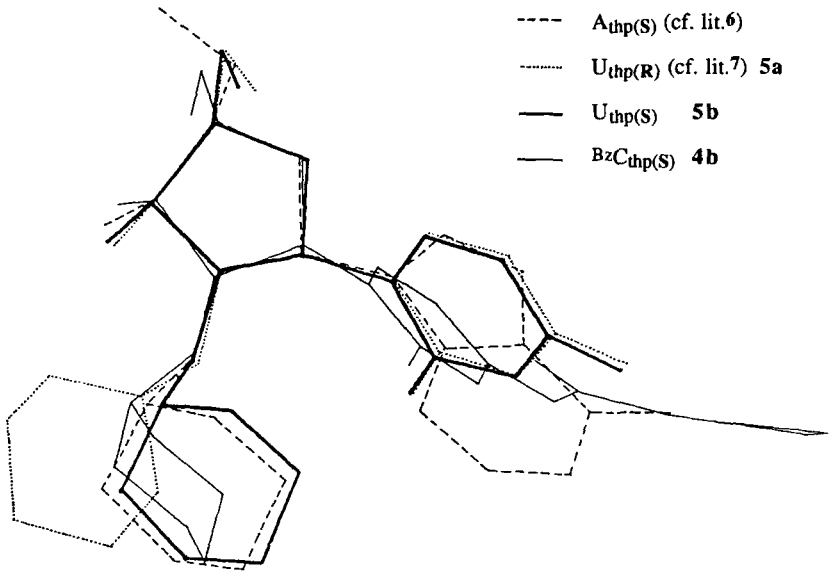
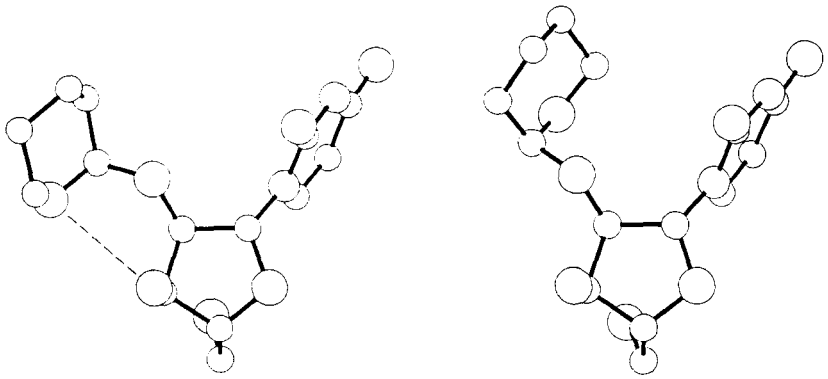


FIGURE 2: Best molecular fit using the fragment O(5')-C(5')-C(4')-O(4')-C(3')-O(3')-C(2')-O(2')-C(1')-N of the crystal structures of the four nucleoside derivatives

4. DISCUSSION: MODEL FOR DIFFERENTIAL REACTIVITY

From comparison of the two crystal structures of 2'-O-(S resp. R)-tetrahydropyranyluridine (**5a** resp. **5b**, cf. FIGURES 2 and 3 as well as Fig. 6 in lit.[7]) we find that only the conformation of the R-form favours the formation of an intramolecular hydrogen bond of the 3'-hydroxyl group to the O1" tetrahydropyranyl oxygen atom. Repulsive Van der Waals interactions as well as the anomeric effect operate as conformation determinating factors. This intramolecular hydrogen bond not only reduces the polarity of the R-form (as discussed in lit.[7]), but also enhances the nucleophilicity of the adjacent 3'-oxygen atom, thus rendering it more prone to electrophilic attack by an acylium intermediate formed under the present reaction conditions.

Recent studies on the reactivity of nucleoside and nucleotide derivatives under similar reaction conditions (cf. lit.[11,12]) give correlations of acylation selectivities with the pK_a-values of the respective hydroxyl groups. Predominant 2'-O-acylation is observed throughout as a consequence of a reduced pK_a-value of the 2'-OH group by at least 2 units with respect to the 5'-OH group (cf. lit.[13,14]). Although weaker, similar



5a 5b

FIGURE 3: Comparing structures **5a** and **5b**; a possible intramolecular hydrogen bond in the case of **5a** is indicated by a broken line (cf. as well Fig. 6 in lit.[7]).

inductive effects may be held responsible for increased acylation of the 3'-OH group in this present case; however, the formation of an intramolecular hydrogen bond should increase its pK_a-value, since the proton is bound more firmly this way. A highfield shift of about 0.3 ppm for the 3'-hydroxyl proton of **5a** (**4a**) with respect to **5b** (**4b**) may reflect this shielding effect by hydrogen bonding in solution as it is observed in the crystal. Nevertheless, both arguments point in the same direction, namely enhanced nucleophilic reactivity for the 3'-hydroxyl group.

How can we rationalise the pyrimidine specific reversal of selectivity as outlined in SCHEMES 1 and 2? FIGURE 4 gives an explanation: Only the pyrimidines are susceptible to a transient internal 5'-OH protection by cyclic carbonate diester formation with the base oxygen atom O2. Such an intermediate can be formed by consumption of one molecule of chloroformate through intramolecular transacylation either from initially formed 5'-O-Fmoc-diester or from an activated O2-pyrimidine-Fmoc-diester under the reaction conditions. Conformationally, this structure is easily accessible by an *anti* → *syn* rotation around the glycosidic bond with an energy barrier well below 6 kcal/mole for the relevant puckering modes [15] - this being valid certainly for the R isomers. The 5'-oxygen atom is confirmed to be preferentially above the furanose ring for pyrimidine nucleosides regardless of sugar pucker (+sc with respect to torsional angle γ, cf. FIGURE 2 and lit.[16]). Moreover is this cyclic structure most favourably stabilised by $n_{O4'} \rightarrow \pi_{C=O}^*$ donation; in fact it is found from a large number of crystal structural data that for O····C=O distances shorter than 3 Å the carbon atom is displaced from the plane

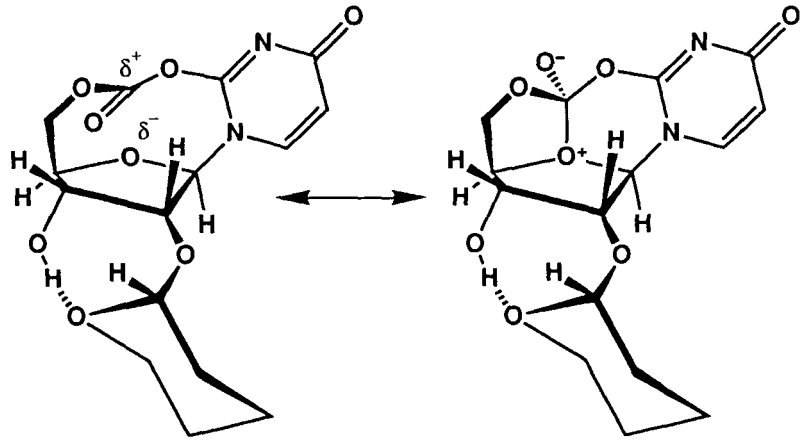


FIGURE 4: Proposed transient internal 5'-O-2O-carbonate diester formation in the case of pyrimidines. The cyclic intermediate structure is stabilised by internal $n_{O4'} \rightarrow \pi_{C=O}^*$ donation, as indicated by a mesomeric ortho carbonate structure.

of the carbonyl group towards the nearby oxygen atom [17,18]. Subsequent acylation of the intermediate can take place only at the free 3'-OH group, whereafter the hydrolytic conditions during work-up set free again the carbonyl-O2-oxygen of the base as well as the 5'-OH group. Monomeric derivatives of orthocarbonic acid have been isolated occasionally in the literature, e. g. as part of a bis-chelate complex of malonenamide oxime with Ni(II) [19].

We thus propose two factors responsible for the observed reversal of selectivity in the case of the R-diastereomers of 2'-O-tetrahydropyranyl-4N-benzoylcytidine (**4a**) and 2'-O-tetrahydropyranyluridine (**5a**): first, enhancement of nucleophilicity of the 3'-hydroxyl group by internal hydrogen bonding (as outlined in FIGURE 3), and second, transient blocking of the 5'-hydroxyl group by cyclic carbonate diester formation under the reaction conditions (cf. FIGURE 4), the latter exclusively being possible for pyrimidine base residues.

EXPERIMENTAL PART

General: The nucleoside derivatives were prepared as described and referenced in lit.[2] for the case of 2'-O-(4-methoxytetrahydropyran-4-yl)-nucleosides. Melting points and specific molecular rotations of **4a/b** and **5a/b** were in agreement with *literature values* [6].

^{1}H-NMR-Spectra of 5'-(resp. 3')-O-(9-Fluorenylmethoxycarbonyl)-2'-O-(tetrahydropyran-2-yl)-ribonucleosides (cf. SCHEME 1)

^{1}H-NMR-spectra (250 MHz) were measured on a Bruker WM 250 instrument in DMSO-d_6; chemical shifts δ are given in ppm downfield from TMS = 0, coupling constants J in Hz; D_2O-exchange was performed on all samples.

5'-O-Fmoc-2'-O-thp(*high R_f*)-6N-benzoyladenosine (**2d**; 24 % isolated as the main product; two chromatographies performed): 1.2-1.8 (mm, 6 H; -CH_2-(3",4",5") of thp), 3.0-3.3 (m, 2 H; -CH_2-(6") of thp), 4.18-4.50 (mm, 7 H; -CH-(4',3',2') of ribose ring, -CH_2-(5') of ribose, -O-CH_2-fluorenyl), 4.72 (m, 1 H; -CH-(2") of thp), 4.88 (t, J=5.0, 1 H; fluorenyl-9H), 5.43 (d, J=6, 1 H; exchangeable; 3'-OH), 6.23 (d, J=4.9, 1 H; -CH-(1') of ribose), 7.2-8.1 (mm, 13 H; fluorenyl- resp. benzoyl-(aromatic H)), 8.68, 8.75 (2s, 2 H; adenyl-2H resp. 8H), 11.3 (sbr, 1 H; exchangeable; adenyl-N6-H).

5'-O-Fmoc-2'-O-thp(*low R_f*)-6N-benzoyladenosine (**2f**; 58 % isolated as the main product): 1.2-1.8 (mm, 6 H; -CH_2-(3",4",5") of thp), 3.0-3.3 (m, 2 H; -CH_2-(6") of thp), 4.15-4.51 (mm, 7 H; -CH-(4',3',2') of ribose ring, -CH_2-(5') of ribose, -O-CH_2-fluorenyl), 4.76 (m, 1 H; -CH-(2") of thp), 4.92 (t, J=5.3, 1 H; fluorenyl-9H), 5.55 (d, J=5, 1 H; exchangeable; 3'-OH), 6.22 (d, J=4.5, 1 H; -CH-(1') of ribose), 7.2-8.1 (mm, 13 H; fluorenyl- resp. benzoyl-(aromatic H)), 8.68, 8.74 (2s, 2 H; adenyl-2H resp. 8H), 11.2 (sbr, 1 H; exchangeable; adenyl-N6-H).

5'-O-Fmoc-2'-O-thp(*high R_f*)-2N-isobutyroylguanosine (**3d**; 41 % isolated as the main product): 1.12 (2d, J=6.8, 6 H; -CH($\underline{CH_3}$)$_2$), 1.3-1.7 (mm, 6 H; -CH_2-(3",4",5") of thp), 2.75 (septet, J=6.8, 1 H; -$\underline{CH}$(CH_3)$_2$), 3.4, 3.9 (m, 2H; -CH_2-(6") of thp), 4.10-4.40 (mm, 5 H; -CH-(4',3',2') of ribose ring, -CH_2-(5') of ribose), 4.51 (d, J=5.5, 2 H; -O-CH_2-fluorenyl), 4.60 (m, 1 H; -CH-(2") of thp), 4.66 (t, J=5.5, 1 H; fluorenyl-9H), 5.32 (d, J=5, 1 H; exchangeable; 3'-OH), 5.97 (d, J=6.0, 1 H; -CH-(1') of ribose), 7.28-7.91 (mm, 8 H; fluorenyl-(aromatic H)), 8.22 (s, 1 H; guanyl-8H), 11.5-12.3 (2sbr, 2 H; exchangeable; guanyl-1N-H resp. 2N-H).

3'-O-Fmoc-2'-O-thp(*high R_f*)-2N-isobutyroylguanosine (**3e**; 8 % isolated as side product): 1.12 (2d, J=6.8, 6 H; -CH($\underline{CH_3}$)$_2$), 1.2-1.5 (mm, 6 H; -CH_2-(3",4",5") of thp), 2.71 (septet, J=6.8, 1 H; -$\underline{CH}$(CH_3)$_2$), 3.2-3.6 (mm, 4 H; -CH_2-(6") of thp, -CH_2-(5') of ribose), 4.06-4.52 (mm, 4 H; -CH-(4') of ribose ring, -O-CH_2-fluorenyl, -CH-(2") of thp), 4.75 (t, J=5.4, 2 H; fluorenyl-9H), 4.82 (dxd, $J_{2'-1'}$=8.0, $J_{2'-3'}$=5.2, 1 H; -CH-(2') of ribose ring), 5.17 (dbr, $J_{3'-2'}$=5.2, 1 H; -CH-(3') of ribose ring), 5.33 (t, J=3, 1 H; exchangeable; 5'-OH), 5.88 (d, $J_{1'-2'}$=8.0, 1 H; -CH-(1') of ribose ring),

7.28-7.94 (mm, 8 H; fluorenyl-(aromatic H)), 8.26 (s, 1 H; guanyl-8H), 12.5-13.2 (2sbr, 2 H; exchangeable; guanyl-1N-H resp. 2N-H).

5'-O-Fmoc-2'-O-thp(*low* R_f)-2N-isobutyroylguanosine (**3f**; 40 % isolated as the main product): 1.12 (2d, J=6.8, 6H; -CH($\underline{CH_3}$)$_2$), 1.2-1.7 (mm, 6 H; -CH_2-(3",4",5") of thp), 2.75 (septet, J=6.8, 1 H; -$\underline{CH}$(CH_3)$_2$), 3.18-3.32 (m, 2 H; -CH_2-(6") of thp), 4.10-4.41 (mm, 5 H; -CH-(4',3',2')-, -CH_2-(5') of ribose), 4.51 (d, J=6, 2 H; -O-CH_2-fluorenyl), 4.67 (t, J=6, 1 H; fluorenyl-9H), 4.75 (m, 1 H; -CH-(2") of thp), 5.46 (d, J=5, 1 H; exchangeable; 3'-OH), 6.00 (d, J=6.2, 1 H; -CH-(1') of ribose ring), 7.29-7.92 (mm, 8 H; fluorenyl-(aromatic H)), 8.21 (s, 1 H; guanyl-8H), 11.5-12.2 (2sbr, 2 H; exchangeable; guanyl-1N-H resp. 2N-H).

3'-O-Fmoc-2'-O-thp(*low* R_f)-2N-isobutyroylguanosine (**3g**; 10 % isolated as side product): 1.12 (2d, J=6.8, 6 H; -CH($\underline{CH_3}$)$_2$), 1.16-1.49 (mm, 6 H; -CH_2-(3",4",5") of thp), 2.75 (septett, J=6.8, 1 H; -$\underline{CH}$(CH_3)$_2$), 2.75-3.05 (m, 2 H; -CH_2-(6") of thp), 3.61 (m, 2 H; -CH_2-(5') of ribose), 4.15 (m, 1 H; -CH-(4') of ribose), 4.35 (t, J=5, 1 H; fluorenyl-9H), 4.60 (m, 1 H; -CH-(2") of thp), 4.73 (d, J=5, 2 H; -O-CH_2-fluorenyl), 4.88 (dxd, $J_{2'\text{-}1'}$=8.2, $J_{2'\text{-}3'}$=5.1, 1 H; -CH-(2') of ribose ring), 5.25 (dbr; $J_{3'\text{-}2'}$=5.1, 1 H; -CH-(3')- of ribose ring), 5.32 (t, J=5, 1 H; exchangeable; 5'-OH), 5.87 (d, J=8.2, 1 H; -CH-(1') of ribose ring), 7.32-7.95 (mm, 8 H; fluorenyl-(aromatic H)), 8.24 (s, 1 H; guanyl-8H), 11.6-12.2 (2sbr, 2 H; exchangeable; guanyl-1N-H resp. 2N-H).

3'-O-Fmoc-2'-O-(**R**)-thp(*high* R_f)-4N-benzoylcytidine (**4e**; 56 % isolated as the main product): 1.2-1.7 (mm, 6 H, -CH_2-(3",4",5") of thp), 3.2-3.8 (mm, 4 H, -CH_2-(6") of thp, -CH_2-(5') of ribose), 4.18-4.21 (m, 1 H, -CH-(4') of ribose ring), 4.33 (t, J=5, 1 H, fluorenyl-9H), 4.43 (dxd, $J_{2'\text{-}1'}$=3.6, $J_{2'\text{-}3'}$=5.2, 1 H; -CH-(2') of ribose ring), 4.62 (d, J=5, 2 H; -O-CH_2-fluorenyl), 4.88 (m, 1 H; -CH-(2") of thp), 5.00 (tbr, $J_{3'\text{-}2'}$=5.2, 1 H; -CH-(3') of ribose ring), 5.43 (t, J=5, 1 H; exchangeable; 5'-OH), 5.91 (d, $J_{1'\text{-}2'}$=3.6, 1 H; -CH-(1') of ribose ring), 7.30-8.02 (mm, 14 H; cytidyl-5H, fluorenyl- resp. benzoyl-(aromatic H)), 8.47 (d, J=8, 1 H; cytidyl-6H), 11.3 (sbr, 1 H; exchangeable; cytidyl-4N-H).

5'-O-Fmoc-2'-O-(**S**)-thp(*low* R_f)-4N-benzoylcytidine (**4f**; 60 % isolated as the main product): 1.2-1.7 (mm, 6H; -CH_2-(3",4",5") of thp), 3.3-3.7 (m, 2 H; -CH_2-(6") of thp), 4.06-4.17 (m, 2 H; -CH_2-(5') of ribose), 4.28-4.38 (mm, 3 H; -CH-(4',3',2') of ribose ring), 4.42-4.61 (mm, 3 H; fluorenyl-9H-CH_2-O-), 4.85 (m, 1 H; -CH-(2") of thp), 5.44 (d, J=5, 1 H; exchangeable; 3'-OH), 6.00 (d, J=4.2, 1 H; -CH-(1') of ribose

ring), 7.30-8.13 (mm, 15 H; cytidyl-5H and -6H, fluorenyl- resp. benzoyl-(aromatic H)), 11.3 (sbr, 1 H; exchangeable; cytidyl-N4-H).

3'-O-Fmoc-2'-O-(**R**)-thp(*high* R_f)-uridine (**5e**; 34 % isolated as the main product): 1.2-1.6 (mm, 6H; -CH_2-(3",4",5") of thp), 3.26-3.62 (mm, 4 H; -CH_2-(6") of thp, -CH_2-(5') of ribose), 4.08 (m, 1 H; -CH-(4') of ribose ring), 4.33 (tbr, $J_{2'-1'}$=5.0, 1 H; -CH-(2') of ribose ring), 4.43 (t, J=6, 1 H; fluorenyl-9H), 4.60 (d, J=6, 2 H; -O-CH_2-fluorenyl), 4.64 (m, 1 H; -CH-(2") of thp), 5.06 (dxd, $J_{3'-2'}$=5.3, $J_{3'-4'}$=3.5, 1 H; -CH-(3') of ribose ring), 5.39 (br, 1 H; exchangeable; 5'-OH: assignment according to additional data, *vide infra*), 5.71 (d, J=8, 1 H; uridyl-5H), 5.88 (d, J=5.0, 1 H; -CH-(1') of ribose ring), 7.31-7.92 (mm, 9H; uridyl-6H, fluorenyl-(aromatic H)), 11.4 (sbr, 1 H; exchangeable; uridyl-3N-H).

5'-O-Fmoc-2'-O-(**S**)-thp(*low* R_f)-uridine (**5f**; 43 % isolated as the main product, 37 % crystalline from ethylacetate/pentane): 1.4-1.7 (mm, 6 H; -CH_2-(3",4",5") of thp), 3.3-3.6 (m, 2 H; -CH_2-(6") of thp), 4.00-4.16 (m, 2 H; -CH_2-(5') of ribose), 4.16-4.35 (mm, 4 H; -CH-(4',3',2') of ribose ring, fluorenyl-9H), 4.55 (d, J=6.2, 2 H; -O-CH_2-fluorenyl), 4.74 (m, 1 H; -CH-(2") of thp), 5.40 (d, J=5.5, 1 H; exchangeable; 3'-OH), 5.55 (d, J=7, 1 H; uridyl-5H), 5.91 (d, J=5.8, 1 H; -CH-(1') of ribose ring), 7.29-7.92 (mm, 9 H; uridyl-6H, fluorenyl-(aromatic H)), 11.4 (sbr, 1 H; exchangeable; uridyl-3H).

Chemical Correlation of Regioselectivity according to SCHEME 2

5'-O-Acetyl-3'-O-Fmoc-2'-O-(**R**)-thp-(*high* R_f)-uridine (**5h**): 200 mg (0.36 mmoles) of 3'-O-Fmoc-2'-O-(**R**)-thp-(*high* R_f)-uridine (**5e**) were treated with 340 µl (3.6 mmoles) of acetic anhydride in 2 ml of dry pyridine for 2 hours at room temperature. (TLC: complete conversion to a less polar compound). The reaction mixture was quenched by addition of 0.5 ml of water and evaporated at high vacuum. The residue was partitioned between chloroform and saturated aqueous sodium hydrogen carbonate, the organic phase dried over sodium sulfate and evaporated after addition of toluene to remove small amounts of pyridine. The crude was chromatographed twice on 12 g of silica gel 60 H (Merck, Kieselgel 7736; eluent: chloroform containing 2 % of ethanol): 119 mg (0.20 mmoles, 56 %) as a white precipitate from chloroform/pentane. ^{1}H-NMR-spectrum of **5h**: 1.2-1.6 (mm, 6 H; -CH_2-(3",4",5") of thp), 2.03 (s, 3H; -$COCH_3$), 3.2-3.6 (m, 2 H; -CH_2-(6") of thp), 4.00-4.35 (mm, 4 H; -CH-(4',2'), -CH_2-(5') of ribose), 4.52-4.63 (mm, 3 H; fluorenyl-9H-CH_2-O-), 4.69 (m, 1 H; -CH-(2") of thp), 5.03 (m, 1 H; -CH-(3') of ribose ring), 5.71 (d, J=8, 1 H; uridyl-5H), 5.80 (d, J=5,

1 H; -CH-(1') of ribose ring), 7.30-7.92 (mm, 9 H; uridyl-6H, fluorenyl-(aromatic H)), 11.4 (sbr, 1 H; exchangeable; uridyl-3N-H).

5'-O-Acetyl-2'-O-(**R**)-thp-(*high R_f*)-uridine (**5i**): The above material (**5h**) was treated with 1 ml of a 0.1 N solution of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) in acetonitrile (cf. lit.[2]) for 30 min., evaporated after addition of toluene and subjected to chromatography as described above: 79 mg (0.14 mmoles, 70 %) as a white precipitate from chloroform/pentane. ^{1}H-NMR-spectrum of **5i**: 1.4-1.8 (mm, 6 H; -CH_2-(3",4",5") of thp), 2.06 (s, 3 H; -$COCH_3$), 3.4-3.9 (m, 2 H; -CH_2-(6") of thp), 4.04 (m, 2 H; -CH_2-(5') of ribose), 4.17-4.32 (mm, 3 H; -CH-(4',3',2') of ribose ring), 4.80 (m, 1 H; -CH-(2") of thp), 5.2 (br, 1 H; exchangeable; 3'-OH), 5.67 (d, J=8, 1 H; uridyl-5H), 5.80 (d, J=4, 1 H; -CH-(1') of ribose ring), 7.67 (d, J=8, 1 H; uridyl-6H), 11.4 (sbr, 1 H; exchangeable; uridyl-3N-H).

Direct acetylation of **5a**: 65 mg (0.20 mmoles) of 2'-O-(**R**)-thp-(*high R_f*)-uridine (**5a**) in 1.0 ml of anhydrous pyridine were cooled to 0 °C. Thereafter 17 µl (0.24 mmoles) of acetyl chloride were injected. After 30 min. the reaction mixture was quenched by the addition of water, concentrated at reduced pressure, taken up in 20 ml of chloroform and washed with saturated aqueous sodium hydrogen carbonate solution. The organic phase was concentrated to an oil and coevaporated successively from toluene, ethanol and chloroform. Unreacted starting material **5a** was removed by chromatography on 10 g of flash Silicagel, using chloroform (stabilised with 2 % of ethanol) as the eluent. All product containing fractions were combined, concentrated and precipitated from chloroform/pentane (57 mg after drying at high vacuum). ^{1}H-NMR-spectrum (Bruker, WM-300): All the resonances of the main product coincide with the ones observed for **5i**; in addition, the resonances for two minor products were observed, namely 3'-O-acetyl-2'-O-(**R**)-thp-(*high R_f*)-uridine (**5k**) and 3',5'-di-O-acetyl-2'-O-(**R**)-thp-(*high R_f*)-uridine (**5l**). From the integral ratio of the exchangeable protons (5.20 ppm, broad doublet for 3'-OH of main product; 5.14 ppm, broad triplet for 5'-OH of side product; integral ratio 2:1) the content of di-acetylated material was estimated by comparison with the integral of the -CH-(2")-acetal proton resonances at 4.80 ppm (**5i**), 4.66 ppm (**5k**) and 4.43 ppm (**5l**) amounting to 56:28:16 % respectively.

Crystal structure determination of 2'-O-(S)-thp-4N-benzoylcytidine (**4b**) *and 2'-O-(S)-thp-uridine* (**5b**)

Compounds **4b** and **5b** crystallize from acetonitrile / ether. Intensities were measured with an Enraf-Nonius CAD4-diffractometer using graphite monochromatized

TABLE 1: Crystal data, structure determination and refinement of **4b** and **5b**

	4b	**5b**
System	monoclinic	orthorhombic
Space group	$P2_1$	$P2_12_12_1$
Unit cell		
a (Å)	5.433(3)	6.655(1)
b (Å)	16.954(4)	12.817(8)
c (Å)	11.180(4)	18.604(12)
β (°)	97.99(5)	
μ (Mo K_α) (cm^{-1})	1.15	1.20
Number of reflections		
unique	2539	1308
observed (I>3σ(I))	1817	518
Resolution	MULTAN-76 [22]	SHELXS-86 [23]
Refinement	SDP-package [24]	SHELX-76 [25]
Hydrogen atoms	difference Fourier	calculated
Final R-value	0.044	0.087

Mo K_α-radiation (λ = 0.71069 Å, ω-2θ scan, $(\sin\theta/\lambda)_{max}$ = 0.55). No correction for absorption effects was made. As a check on the stability of the instrument and the crystal, three reflections were measured every 120 min. to check the intensity stability and every 200 reflections to check the crystal orientation stability. Crystal data, structure determination and refinement are summarized in TABLE 1. Hydrogen atoms were not refined and were assigned isotropic temperature factors 10 % greater than those of the atoms to which they are bound. Atomic scattering factors used were those of Cromer and Mann [20] for C, N and O, and of Stewart [21] for H. Atomic co-ordinates are deposited with the Cambridge Crystallographic Database.

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