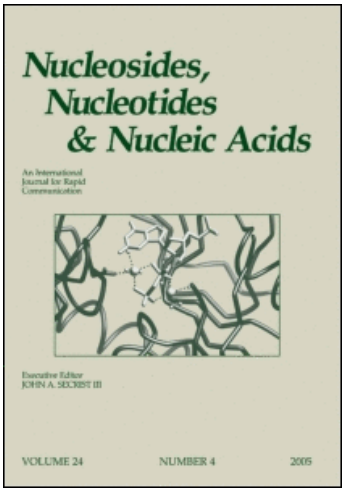

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Glycosylation Reaction of Unprotected Sugars with Hydroxyalkylthymine

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Glycosylation Reaction of Unprotected Sugars with Hydroxyalkylthymine

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ABSTRACT

Under mild conditions (Lewis acid/solvent/room temperature), the reaction of unprotected glucose, deoxyribose or xylose with hydroxylalkylthymine gives selectively nucleoside analogs with a spacer arm between sugar and base moiety. Experimental conditions (Lewis acid, solvent) for this new strategy leading to nucleoside analogs synthesis are discussed.

Key Words: Nucleoside; Carbohydrate; Glycosylation.

INTRODUCTION

Since the discovery of the human immunodeficiency virus (HIV) known as the etiological agent of AIDS,[1] increasing efforts have been devoted to design compounds that can block replication of retroviruses. In our laboratory, we have developed a new family of nucleoside analogs with a spacer arm.[2–4] It is now known that an aliphatic chain not only serves as neutral linkage but also affects the coiling of DNA. Increasing the length of spacer arm linkage would thus enhance bending of DNA, leading to base

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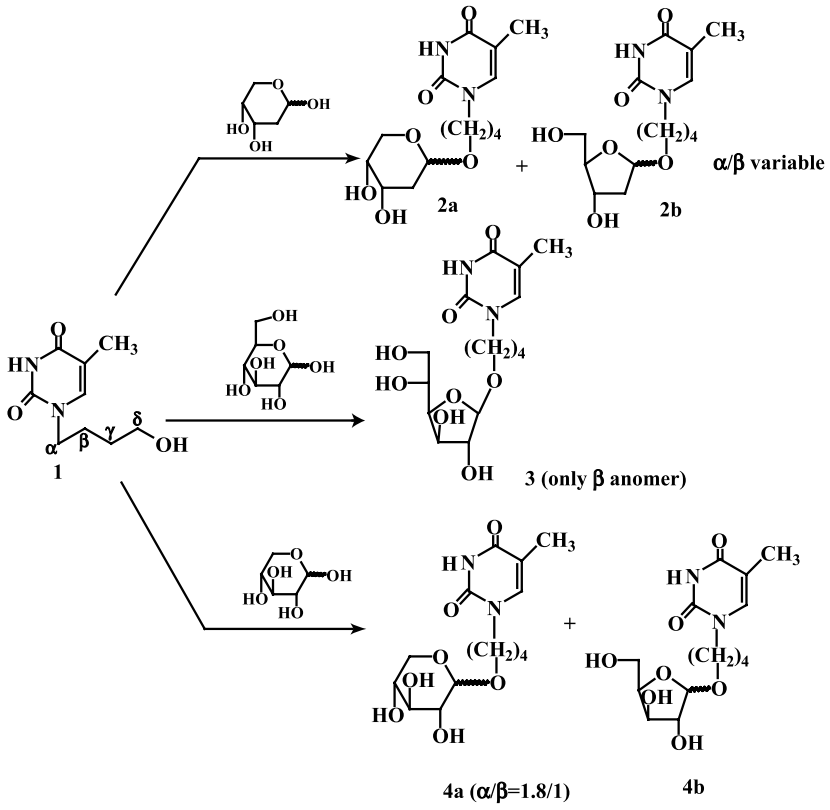
pair opening where the bases then become susceptible to attack by reactive groups.[5] Spacer arms have also been used in oligomer nucleoside antisense synthesis for preventing the enzymatic degradation by nucleases.[6] Synthesis of such compounds needs protection and deprotection sequences of hydroxyl groups. In addition, glycosylation reaction with acetylated or benzoylated carbohydrate leads to an important amount of secondary product (40%) resulted from trans-esterification of alcohol. In this context, direct synthesis from totally-O-unprotected sugar would be highly desirable.

Plusquellec et al.[7–9] showed that glycosylation of unprotected sugars with long chain alcohols was possible by using appropriate solvents and catalysts. Thus this strategy has been applied to the preparation of glycosylated alkylthymine **2**, **3**, **4** from hydroxylalkylthymine **1** (Scheme 1).

Synthesis of hydroxyalkylated thymine **1** was carried out by reaction of bromobutylacetate in DMF in the presence of sodium hydride under inert atmosphere as described in our previous paper[10] followed by a deacetylation using sodium methylate in dry methanol.

Using $FeCl_3$, $AlCl_3$ or BF_3OEt_2 as catalysts in various solvents, (CH_3CN, THF), glycosylation of **1** with 2-deoxyribose, glucose or xylose led to the corresponding



Scheme 1. Preparation of glycosylated alkylthymine **2**, **3**, **4** from hydroxylalklthymine.

Table 1. Reaction conditions for the synthesis of glycosylated alkylthymine.

Starting sugar	Catalyst/ solvent	Reaction[a] time (h)	Overall yield[b] (%)	Obtained compounds	α/β ratio of major product[c]
2-deoxyribose	$FeCl_3/CH_3CN$	2	54	2a/2b (95/5)	4/1
	$FeCl_3$/THF	24	61	2a/2b (95/5)	1/1.8
	$AlCl_3/CH_3CN$	20	25	2a/2b (95/5)	1.6/1
	BF_3OEt_2/THF	24	20	2a/2b (95/5)	–
Glucose	$FeCl_3/CH_3CN$	4	60	3 (100 furanose form)	0/1
	BF_3OEt_2/THF	48	traces	–	–
Xylose	$FeCl_3/CH_3CN$	168	20	4a/4b (95/5)	1.8/1[b]

[a]Conditions, [sugar] = 1 mmol, [**1**] = 1 mmol, [Lewis acid] = 2,4 mmol, solvent = 8 mL.
[b]Acetylated products.
[c]This ratio was determined by ^{1}H NMR.

glycoside in pyranose or furanose form, in 20 to 61% yields (Table 1). The reactions were performed at room temperature with different reaction times. For analytical purpose the crude mixtures were acetylated and purified by flash chromatography and then analyzed by NMR. The results are summarized in Table 1.

2-Deoxyribose, glucose and xylose gave the best yields using $FeCl_3$ as catalyst in acetonitrile. In the case of 2-deoxyribose the use of $FeCl_3$ leads nearly exclusively to the pyranose form. The anomeric ratio α/β varies from 4/1 to 1/2 according to solvent used acetonitrile and THF respectively (Table 1). Xylose led also nearly exclusively to the pyranose form with a poorer yield, its anomeric ratio α/β is 1.8/1. In contrast

Table 2. Effect of $FeCl_3$ concentration and reaction time.

Entry	$FeCl_3$ (eq)	Reaction time (h)	Yield[a] (%)
1	0,4	48	0
2	2,15	2,5	9
3	2,4	1	20
		2	54
		4	12
		24	degradation
4	3	1,5	10
5	8	1	degradation

[a]Yield of mixture of α and β anomers.
Conditions: sugar = 1 mmol, [1] = 1 mmol, CH_3CN = 8 mL.

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Table 3. ^{13}C NMR data of acetylated derivatives of **2a**, **3** and **4a** in $CDCl_3$ (50 MHz).

	2a (α anomer)	2a (β anomer)	3	4a (α anomer)	4a (β anomer)
Thymine					
C4	164.08	164.27	164.23	164.03	164.03
C2	150.79	150.90	150.82	150.72	150.72
C6	140.31	140.38	140.55	140.43	140.43
C5	110.57	110.74	110.63	110.73	110.73
$C(CH_3)$	12.28	12.24	12.33	12.3	14.12
Alkyl					
Cα	48.17	48.38	48.12	48.4	48.24
Cβ	26.03	26.14	26.05	25.99	25.88
Cγ	26.41	26.39	26.32	26.18	26.26
Cδ	68.44	67.13	67.91	67.85	68.86
Carbohydrate					
$C_{1'}$	99.37	97.63	106.49	95.86	100.78
$C_{2'}$	32.51	30.94	80.37	71.07	71.01
$C_{3'}$	67.87	65.78	73.44	69.59	71.57
$C_{4'}$	66.69	67.68	78.33	69.34	68.92
$C_{5'}$	63.15	60.96	68.61	58.43	62.19
$C_{6'}$	–	–	63.53		
Acetyl					
(*C*O)	170.34,	170.51,	170.75	170.16	170.16
	170,11	170,2	169.75	170.09	170.09
			169.49	169.94	169.94
		21.04	169.39		
(CH_3)	20.94	20.98	20.81	20.78	20.78
	20.88		20.77	20.73	20.73
			20.64	20.69	20.69
			20.53		

glucose in acetonitrile gives only the β furanose with acceptable yield (60%). This good stereoselectivity was also observed in literature for the system glucose/fatty alcohol.[7]

We also studied the effect of Lewis acid concentration using 2-deoxyribose and **1** as starting materials (Table 2).

A catalytic amount of Lewis acid gives no reaction even after 48 hours. An excess of $FeCl_3$ is necessary but beyond 2.5 eq degradation is observed. The best result is obtained by using 2.4 eq after 2 hours. The increase of reaction time gives also degradation.

All products were derivatised by acetylation (Ac_2O/pyridine, room temperature) and after standard work up the pure compound were isolated as analytical sample.

In a typical experiment, compound **2a,b** were synthesised by reaction of hydroxylalkylthymine **1** (198 mg, 1 mmol) in 8 mL of dry acetonitrile containing $FeCl_3$ (389 mg, 2.4 mmol) with 2-deoxy-D-ribose (134 mg, 1 mmol) at room temperature for 2 hours. Pyridine (10 mL) and acetic anhydride (2 mL) were then added. After 12 hours and usual work up, the product was purified by flash chromatography on SiO_2 (petroleum ether/acetone as eluent).

Structures were established by spectroscopic methods (^{1}H NMR, ^{13}C NMR, Cosy and HMQC)[a] and were based on previous results on methyl furanosides.[11] ^{13}C NMR data of acetylated **2a**, **3**, **4a** are presented on Table 3.

In summary, this paper presents an efficient and new route for the synthesis of nucleosides with a spacer arm linking the sugar and the base moiety. This approach could be applied to other pyrimidines or purines. New compounds in this series are currently under investigation in our laboratory.

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[a]Selected values of ^{1}H NMR for acetylated derivatives of 2a (α) and 3 compound 2a (α) : thymine: 8.98 s (1H) NH, 7.01 q (1.2) (1H) H6, 1.91 d (1.2) (3H) CH_3, alkyl : 3.74 t (7.1) (2H) Hα, 1.78 m (2H) Hβ, 1.65 dt (6.1) (2H) Hγ, 3.91 dt (6.1) (1H)Hδ1, 3.49 dt (6.1) (1H) Hδ2, carbohydrate : 4.51 dd (5.4–5.3) (1H) H1′, 2.0 dd (2.9–8.4) (1H) H2′, 2.0 dd (2.9–6.7) (1H) H2″, 5.03 ddd (3.2–7.8–6.4) (1H) H3′, 5.08 ddd (1.9–3.4–3.4) (1H) H4′, 4.04 dd (12.9–3.6) (1H) H5′, 3.57 dd (12.9–1.9) (1H) H5′, CH_3 acetyl : 2.12 s, 2.08 s, 2.03 s, 2.01 s (12H). Compound 3 : thymine : 8.81 s (1H) NH, 7.01 d (1.1) (1H) H6, 1.93 d (1.1) (3H) CH_3, alkyl : 3.76 t (7.2) (1H) Hα, 1.81 m (2H) Hβ, 1.66 m (2H) γ, 3.72 m (1H) Hδ1, 3.49 dt (5.8) (1H) Hδ2, carbohydrate : 4.98 s (1H) H1′, 4.98 s (1H) H2′, 5.3 d (5.1) (1H).H3′, 4.48 dd (5.1–9.4) (1H) H4′, 5.28 ddd (2.3–5.6–9.4) (1H) H5′, 4.64 dd (2.3–12.2) (1H) H6′, 4.11 dd (5.6–12.2) (1H) H6′, CH3 acetyl : 2.12 s, 2.08 s, 2.03 s, 2.01 s (12H).

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