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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

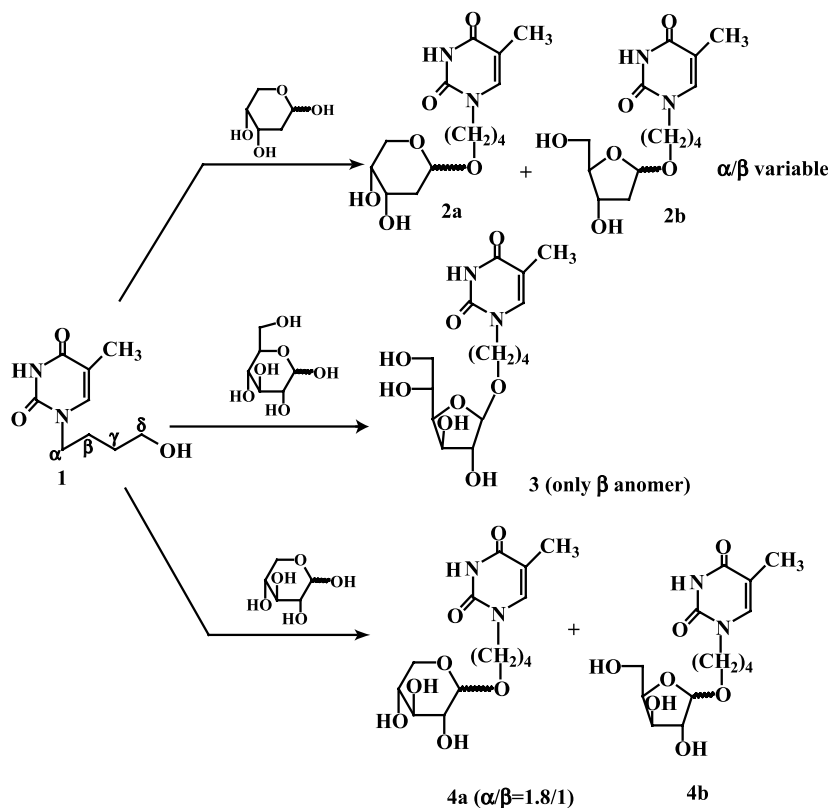
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pair opening where the bases then become susceptible to attack by reactive groups.^[5] Spacer arms have also been used in oligomer nucleoside antisense synthesis for preventing the enzymatic degradation by nucleases.^[6] Synthesis of such compounds needs protection and deprotection sequences of hydroxyl groups. In addition, glycosylation reaction with acetylated or benzoyleated 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 hydroxylalkylthymine.



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 ₃ CN	2	54	2a/2b (95/5)	4/1
	FeCl ₃ /THF	24	61	2a/2b (95/5)	1/1.8
	AlCl ₃ /CH ₃ CN	20	25	2a/2b (95/5)	1.6/1
	BF ₃ OEt ₂ /THF	24	20	2a/2b (95/5)	–
Glucose	FeCl ₃ /CH ₃ CN	4	60	3 (100 furanose form)	0/1
	BF ₃ OEt ₂ /THF	48	traces	–	–
Xylose	FeCl ₃ /CH ₃ CN	168	20	4a/4b (95/5)	1.8/1 ^b

^aConditions, [sugar] = 1 mmol, [1] = 1 mmol, [Lewis acid] = 2,4 mmol, solvent = 8 mL.

^bAcetylated products.

^cThis ratio was determined by ¹H 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 FeCl₃ as catalyst in acetonitrile. In the case of 2-deoxyribose the use of FeCl₃ 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 FeCl₃ 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

^aYield of mixture of α and β anomers.

Conditions: sugar = 1 mmol, [1] = 1 mmol, CH₃CN = 8 mL.



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)
<i>Thymine</i>					
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
<i>Alkyl</i>					
C α	48.17	48.38	48.12	48.4	48.24
C β	26.03	26.14	26.05	25.99	25.88
C γ	26.41	26.39	26.32	26.18	26.26
C δ	68.44	67.13	67.91	67.85	68.86
<i>Carbohydrate</i>					
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		
<i>Acetyl</i>					
(CO)	170.34, 170.11	170.51, 170.2	170.75 169.75 169.49 169.39	170.16 170.09 169.94	170.16 170.09 169.94
(CH ₃)	20.94 20.88	20.98	20.81 20.77 20.64 20.53	20.78 20.73 20.69	20.78 20.73 20.69

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 (^1H 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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^aSelected values of ^1H 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) CH₃, alkyl : 3.74 t (7.1) (2H) H α , 1.78 m (2H) H β , 1.65 dt (6.1) (2H) H γ , 3.91 dt (6.1) (1H) H δ 1, 3.49 dt (6.1) (1H) H δ 2, 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', CH₃ 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) CH₃, alkyl : 3.76 t (7.2) (1H) H α , 1.81 m (2H) H β , 1.66 m (2H) γ , 3.72 m (1H) H δ 1, 3.49 dt (5.8) (1H) H δ 2, 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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