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Synthesis and Biological Evaluation of Thymine Nucleosides Fused with 3′,4′-Tetrahydrofuran Ring

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Abstract—The pyrimidine nucleosides fused with 3′,4′-tetrahydrofuran ring were successfully synthesized, starting from 1,2;5,6-di-*O*-isopropylidene-D-glucose and assayed for antiviral activities against HIV-1, HIV-2, EMCV, Cox. B3 and VSV. Thymine analogue (**5**) and its corresponding 2′-deoxy analogue (**6**) exhibited high cytotoxicity instead of giving antiviral activities.

Introduction

A number of 2′,3′-dideoxy nucleosides such as 3′-azido-3′-deoxythymidine (AZT), dideoxycytidine (ddC) and dideoxyinosine (ddI) have been discovered to possess significant antiviral activity against HIV[1] and have stimulated considerable interest as potential antiviral agents.[2] It has been suggested that proper conformation of the dideoxynucleosides is required for them to exhibit antiviral activity.[3] Unmodified nucleosides exist in either S-type (2′-*endo*/3′-*exo*) or N-type (2′-*exo*/3′-*endo*) conformation, but due to the low energy barrier between this two dominating conformers a fast equilibrium between states exists in solution state.[4] Therefore, many approaches to lock the puckering of the furanose ring into N-type or S-type have been made since HIV-1 reverse transcriptase is able to discriminate between two conformationally locked carbocyclic AZT triphosphate analogues.[5]

Nowdays, a number of nucleoside analogues with fixed sugar-ring puckering have been synthesized and evaluated for antiviral activity. Among them, bicyclic nucleoside analogues containing a fused methylene group **1**,[6] oxirane **2**[7] and oxethane **3**[8] have been synthesized and reported to have anti-HIV activity through inhibition of HIV reversetranscriptase, but 3′,4′-cyclopentane fused pyrimidine nucleoside **4** did not show antiviral activity[9] (Fig. 1).

Therefore, based on these finding, novel 3′,4′-tetrahydrofuran fused pyrimidine nucleosides were designed and synthesized to obtain further information regarding the correlation between sugar ring conformation and antiviral activity. Here, we report the synthesis of

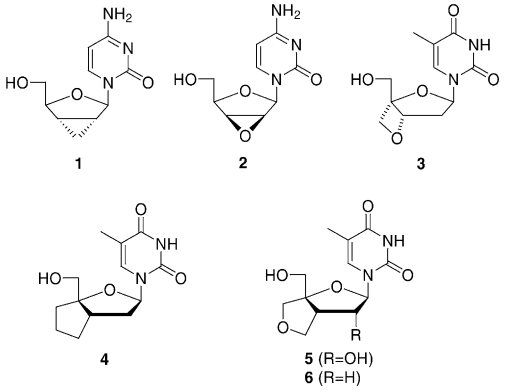

Figure 1. The rationale to the target nucleosides.

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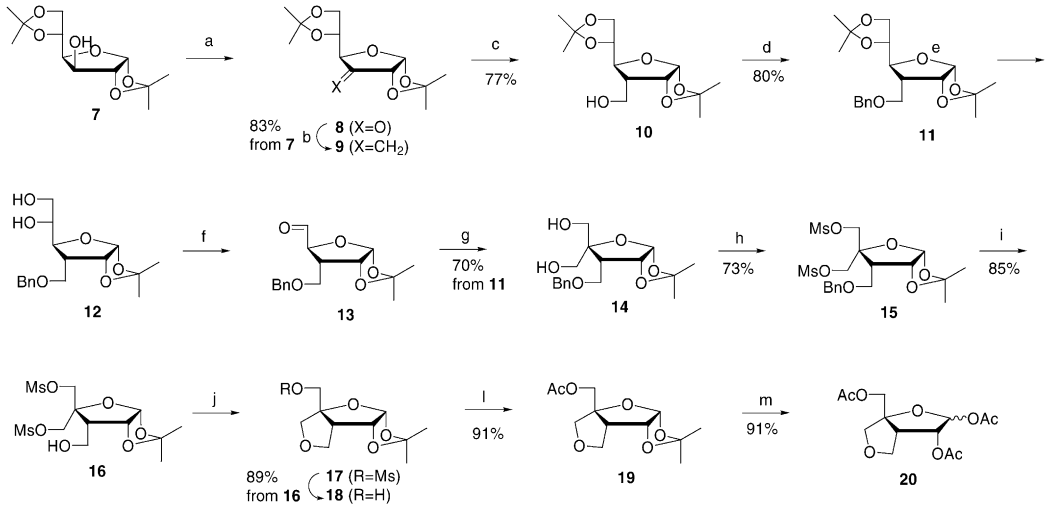
pyrimidine nucleosides fused with 3′,4′- tetrahydrofurn ring, starting from 1,2;5,6-di-*O*-isopropylidene-D-glucose as potential antiviral agents.

Results and Discussion

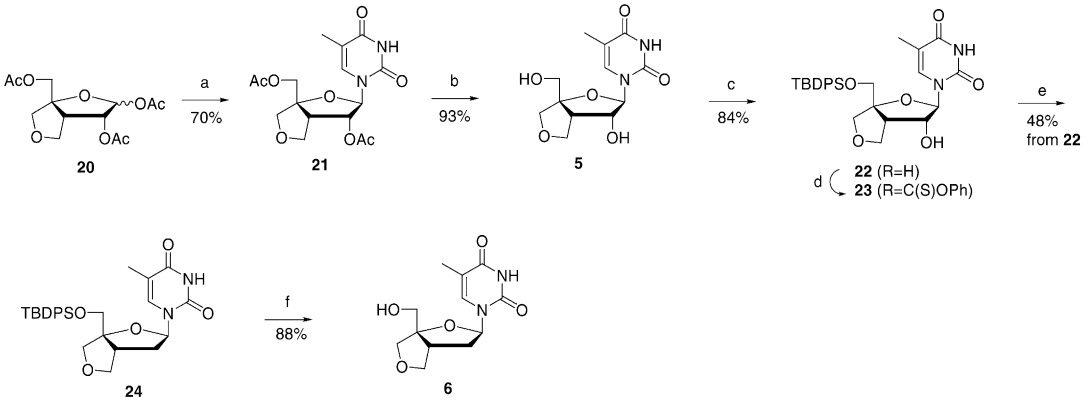
Our synthetic strategy to the desired 3′,4′-tetrahydrofuran fused pyrimidine nucleosides (**5** and **6**) is to synthesize the bicyclo furo[3,4-*b*]furan derivative (**20**) as a glycosyl donor and then to condense with nucleosidic base. Synthesis of the key glycosyl donor **20** from 1,2;5,6-di-*O*-isopropylidene-D-glucose (**7**) is shown in Scheme 1.

1,2;5,6-Di-*O*-isopropylidene-D-glucose was oxidized with PDC to give the ketone **8**, which was treated with methyl triphenylphosphonium bromide in the presence of *n*-BuLi to give the olefin **9** in 83% overall yield. Hydroboration followed by oxidation of the methylene **9** yielded the hydroxymethyl derivative **10** in 77% yield, which was treated with benzyl bromide gave the benzyl ether **11** in 80% yield. 5,6-*O*-isopropylidene group of **11** was selectively removed using aqueous 75% acetic acid to give the diol **12**. Oxidative cleavage of **12** with sodium periodate afforded the corresponding aldehyde **13,** which was immediately alkylated using 37% aqueous formaldehyde followed by in situ Cannizarro reaction to give the diol **14** in 70% overall yield. **14** was treated with methanesulfonyl chloride to give the dimesylate **15** in 73% yield, which was debenzylated by Pd-catalyzed dehydrogenolysis to afford **16** in 85% yield. The intramolecular cyclization of **16** with sodium hydride followed by hydroxylation at 5-carbon of the resulting tricyclic derivative **17** with aqueous sodium hydroxide gave the bicyclic derivative **18** in 89% overall yield. Treatment of **18** with acetic anhydride gave the acetate **19** in 91% yield, which was hydrolyzed with aqueous 85% formic acid and then successively acetylated to give the glycosyl donor **20** in 91% yield as an anomeric mixture.

Condensation of the acetate **20** with silylated thymine by modified Vorbrügen method[10] gave the protected



Scheme 1. Reagents and Conditions: (a) PDC, Ac_2O, CH_2Cl_2, reflux, 2 h; (b) Ph_3PCH_3Br, BuLi, THF, rt to 55 °C, 3 h; (c) (i) $BH_3{\cdot}SMe_2$, THF, rt, 3h; (ii) H_2O_2, 2N NaOH, rt, 2 h; (d) BnBr, NaH, TBAl, THF, rt, 16 h; (e) 75% AcOH, 55 °C, 2 h; (f) $NaiO_4/H_2O$, EtOH, 0 °C, 30 min; (g) 37% HCHO, 2N NaOH, dioxane, rt, 3 days; (h) MsCl, pyridine, rt, 14 h; (i) H_2, $Pd(OH)_2$, EtOH, rt, 14 h; (j) NaH, THF, 55 °C, 1 h; (k) 0.5N NaOH, reflux, 12 h; (l) Ac_2O, pyridine, 12 h; (m) (i) 85% HCO_2H, 55 °C, 2 h; (ii) Ac_2O, pyridine, rt, 16 h.



Scheme 2. Reagents and Conditions: (a) Thymine, BSA, TMSOTf, CH_3CN, 60 °C, 4 h; (b) NH_4OH/MeOH, rt, 14 h; (c) TBDPSCl, imidazole, DMF, rt, 16 h; (d) PhOC(S)Cl, DMAP, Et_3N, CH_3CN, 0 °C, 2 h; (e) Bu_3SnH, AlBN, toulene, 100 °C, 3 h; (f) TBAF, THF, rt, 1.5 h.

Table 1. Antiviral activities of the synthesized nucleosides[a,b]

Compd	Anti-HIV (EC_{50})		Cytotoxicity CC_{50}	Anti-PC (EC_{50})			Cytotoxicity CC_{50}
	IIIB	ROD		EMCV	Cox, B3	VSV	
5	95.80	>0.54	0.54	100.00	100.00	100.00	100.00
6	>100.00	>100.00	<0.80	>4.11	>4.11	3.45	4.11
AZT	0.0012	0.01	1.64	—	—	—	–
Rib	—	—	—	20.94	181.36	14.41	300.00

[a]EC_{50} (μg/mL) is the concentration of test compound required to inhibit virus-induced cytopaticity by 50%.
[b]Cytotoxicity is expressed as the percentage of cell death of MT-4 cells or HeLa cells cultured with test compounds. The number of viable cells was determined by the MTT assay. The CC_{50} (μg/mL) is the concentration of test compound which cause 50% cytotoxicity to uninfected cells.

nucleosides **21** in 70% yield. Deprotection of ester group of **21** with NH_4OH/MeOH gave the desired bicyclic nucleosde **5**[11] in 93% yield. Primary hydroxyl group of **5** was protected to give TBDPS ether **22** in 84% yield. Treatment of **22** with phenoxythiocarbonyl chloride yielded **23,** which upon treatment with tributyltin hydride in the presence of AIBN furnished the 2′-deoxy derivative **24** in 48% overall yield.[12] Deblocking of 5′-silyl group of **24** with tetrabutylammonium fluoride (TBAF) in THF afforded the final 2′-deoxy analogue **6**[13] in 88% yield (Scheme 2).

All synthesized bicyclic nucleosides (**5** and **6**) were evaluated for their inhibitory effects on the replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells and encephalomyocaditis virus (EMCV), Coxasakie virus B3 (Cox. B3) and the vesicular stomatitis virus (VSV) in HeLa cells cultures. Compound **5** exhibited weak antiviral activities against HIV and compound **6** exhibited moderate and weak antiviral activities against EMCV, Cox. B3 and VSV as shown in Table 1. But, the activity of compounds **5** and **6** against tested viruses occurred at cytotoxic concentration.

In summary, we have successively synthesized novel bicyclic nucleosides fused with 3′,4′-tetrahydrofuran ring, starting from 1,2;5,6-di-*O*-isopropylidene-D-glucose via intramolecular cyclization. All synthesized nucleosides exhibited high cytotoxicity without any significant antiviral activities. The difference concerning anti-HIV activity observed between the oxetane fused **3** and the structurally closely related **6** may be due to the ring size of the fused ring. It is concluded that this class of conformationally rigid nucleosides can be a new template for antitumor agents. Synthesis of 3′,4′-methylene and oxirane fused nucleosides that are ring contracted analogues of **3** and antitumor assays of **5** and **6** are in progress.

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11. Selected data for **5**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (3H, d, J=0.72 Hz, CH_3), 2.72 (1H, m, H-3′), 3.44–3.73 (5H, m, H-5′, H-4″ and H-3″a), 4.22–4.29 (2H, m, H-2′ and H-3″b), 5.20 (1H, t, J=5.34 Hz, OH), 5.56 (1H, d, J=4.62 Hz, OH), 5.76 (1H, d, J=8.07, H-1′), 7.64 (1H, d, J=0.99, H-6), 11.28 (1H, brs, NH). FAB-MS m/z: 285 $[M+H]^+$.
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13. Selected data for **5**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77 (3H, d, J=0.99 Hz, CH_3), 2.08–2.13 (2H, m, H-2′), 2.78 (1H, m, H-3′), 3.46–3.78 (6H, m, H-5′, H-3″ and H-4″), 5.19 (1H, t, J=5.43 Hz, OH), 6.16 (1H, t, J=7.30, H-1′), 7.70 (1H, m d, J=1.2 Hz, H-6), 11.27 (1H, brs, NH). FAB-MS m/z: 269 $[M+H]^+$.