Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2403–2406

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Evaluation of Novel D-2′-Azido-2′,3′-dideoxyarabinofuranosyl-4′-thiopyrimidines and Purines

Hea Ok Kim,[a] Yong Hee Park,[b] Hyung Ryong Moon[b] and Lak Shin Jeong[b,*]

[a]*Division of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-742, Republic of Korea*
[b]*Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Republic of Korea*

Received 27 March 2002; accepted 22 May 2002

Abstract—Novel D-2′-azido-2′,3′-dideoxyarabinofuranosyl-4′-thiopyrimidines and purines have been synthesized, starting from L-xylose via azidation at the 2′-position as a key step. Most of the final nucleosides exhibited toxicity-dependent anti-HIV-1 activity, among which D-α-adenine analogue **3h** was found to be the most cytotoxic.

Introduction

Substitution of the 2′- or 3′-position of the D-2′,3′-dideoxynucleosides with fluorine or azide has led to the development of potent anti-HIV agents.[1] Among them, 2′-'up'-fluoro-2′,3′-dideoxyadenosine (**1**, 2′-F-ddA)[2] has been undergoing clinical trials for the treatment of AIDS and AIDS-related complex (ARC) and 2′-'up'-azido-2′,3′-dideoxyadenosine (**2**)[3] has also been reported to show potent anti-HIV-1 activity (Fig. 1).

4′-Thionucleosides in bioisosteric relationships with 4′-oxonucleosides have also been paid great attention by medicinal chemists for the development of new antiviral and antitumor agents since they are metabolically stable as well as biologically active.[4] Recently, along with the development of the efficient synthetic procedures of 4-thiosugar,[5] 2′-deoxy-2′-substituted-[6] and 2′,3′-dideoxy-2′-fluoro-4′-thionucleosides[7] have been synthesized and reported to show promising antiviral and antitumor activities. More recently, our laboratory has reported the structure–activity relationships of D- and L-2′-'down'-azido-2′,3′-dideoxy-4′-thionucleosides as antiviral and antitumor agents.[8] Based on these findings, it was of interest to design and synthesize the target 4′-thionucleosides **3** shown in Fig. 1, since sulfur of the 4′-thionucleosides is in bioisosteric relationship with oxygen of the corresponding 4′-oxonucleosides. Herein, we report the synthesis and antiviral activity of novel D-2′-'up'-azido-2′,3′-dideoxy-4′-thiopyrimidine and purine nucleosides (**3**), starting from L-xylose via azidation as a key step.

Results and Discussion

For the synthesis of the desired pyrimidine and purine nucleosides **3**, the glycosyl donor **9** (Scheme 1) was first prepared and then condensed with nucleobases.

L-Xylose was converted to compound **4** according to an efficient procedure developed by our laboratory.[8] The standard Mitsunobu reaction was employed to invert the stereochemistry of the hydroxyl group of **4**, giving the benzoate **5** in 70% yield without the participation of sulfur atom.[9] Treatment of **5** with methanolic ammonia gave **6**, which was subjected to another Mitsunobu reaction using diphenylphosphoryl azide (DPPA) as a nucleophile to afford the 'up' azido derivative **7** in good yield. As in the case of the synthesis of 'down' azido sugar,[8] no sulfur participation was observed in Mitsunobu reaction with DPPA, proceeding in a pure S_N2 reaction. It is also interesting to note that in the case of the preparation of 'down' azido derivative, the same Mitsunobu reaction failed to give the desired azido derivative, requiring two-step conversion (mesylation and substitution with sodium azide).[8] Oxidation of **7** with *m*CPBA followed by heating the resulting sulfoxide **8** with acetic anhydride yielded the key intermediate **9**, which is ready for the condensation with nucleobases.

*Corresponding author. Tel.: +82-2-3277-3466; fax: +82-2-3277-2851; e-mail: lakjeong@ewha.ac.kr

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00394-3

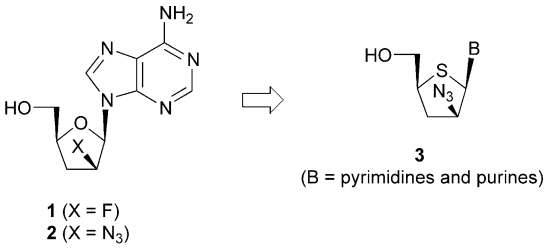
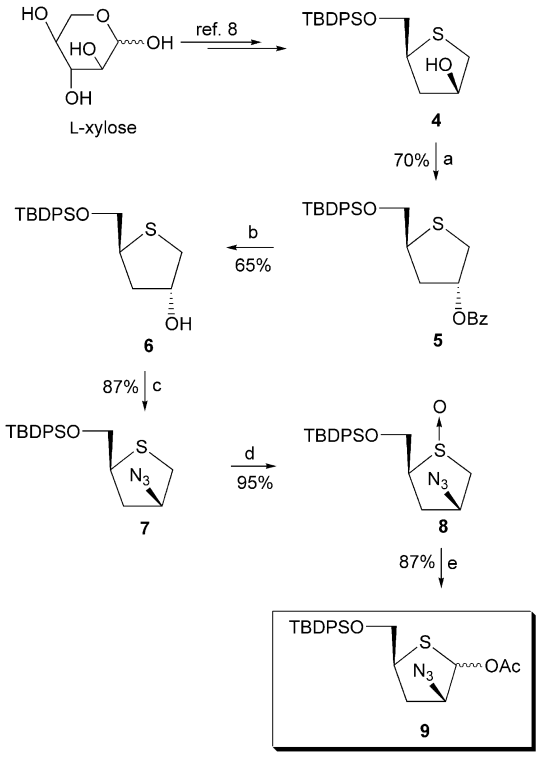
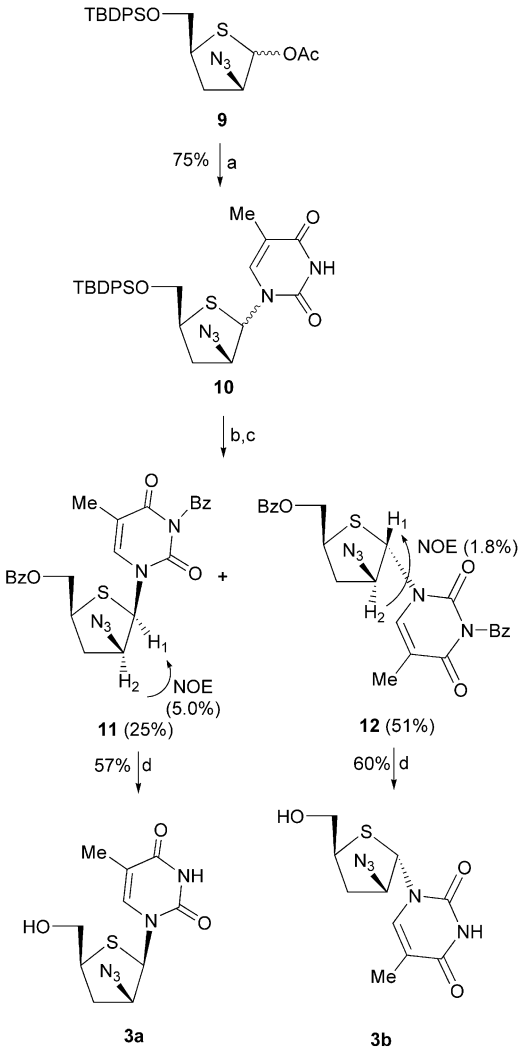


Figure 1. The rationale for the design of the desired nucleosides **3**.



Scheme 1. Reagents and conditions: (a) Ph_3P, DEAD, BzOH, THF, 50 °C, 15 h; (b) NH_3, MeOH, rt, 15 h; (c) Ph_3P, DEAD, DPPA, THF, 0 °C, 1 h; (d) *m*CPBA, CH_2Cl_2, −78 °C, 2 h; (e) Ac_2O, 100 °C, 15 h.



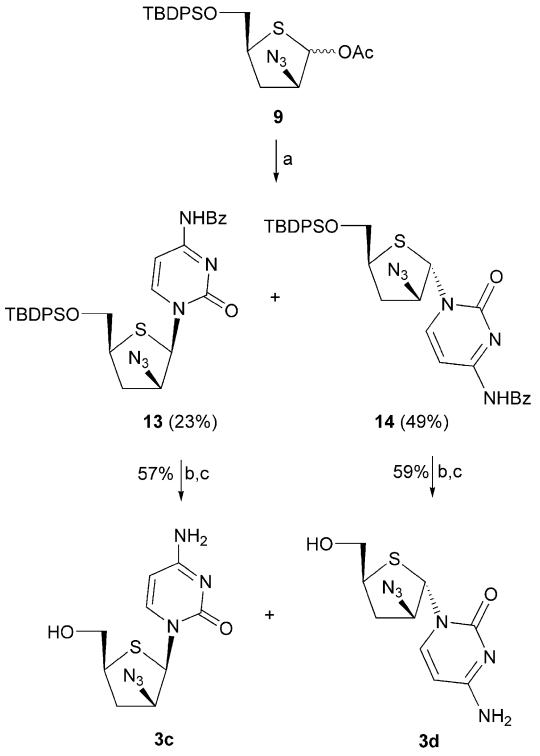
Scheme 2. Reagents and conditions: (a) silylated thymine, TMSOTf, $ClCH_2Cl_2Cl$, rt, 12 h; (b) *n*-Bu_4NF, THF, rt, 1.5 h; (c) BzCl, pyridine, 70 °C, 48 h; (d) NaOMe, MeOH, rt, 15 h.

For the synthesis of thymine derivatives, **3a** and **3b** (Scheme 2), acetate **9** was condensed with silylated thymine in the presence of TMSOTf as a Lewis acid to give **10** as an inseparable anomeric mixture (75%). Since desilylation of **10** with *n*-tetrabutylammonium fluoride also produced an inseparable anomeric mixture, the desilylated product was treated with excess benzoyl chloride in pyridine at 70 °C to afford β-anomer **11** and α-anomer **12** in 1:2 ratio after the purification on silica gel. The major formation of α-anomer **12** over β-anomer **11** during the condensation is believed to be attributed to the presence of bulky azido group on the β-side since condensation of 2′-azido-2′,3′-dideoxyribosyl acetate with silylated thymine in the presence of TMSOTf produced β-anomer and α-anomer in 3:1 ratio.[8] Anomeric configurations of **11** and **12** were readily assigned by ^{1}H NMR NOE experiments as shown in Scheme 2. Irradiation of 2′-H of compound **11** gave a large NOE effect (5.0%) on its 1′-H, indicating β-anomer, while a small NOE effect (1.8%) was observed on the same experiment in the case of compound **12**, resulting in α-anomer. In addition to NOE effect, similar ^{1}H NMR patterns of **11** and **12** were observed to those of 2′-azido-2′,3′-dideoxy-4′-thioribofuranosylnucleosides.[8] For example, the 4′-proton of α-anomer **12** appeared further downfield than that of β-anomer **11** because of the deshielding effect by cytosine and the 5′-protons of **11** appeared further downfield than those observed for **12** due to the same deshielding effect. These typical ^{1}H NMR patterns and similar ^{1}H NOE effects were consistently maintained in other pyrimidine and purine nucleoside derivatives synthesized in Schemes 3 and 4. Treatment of each of **11** and **12** with sodium methoxide in methanol yielded the final nucleosides, **3a** and **3b**, respectively.

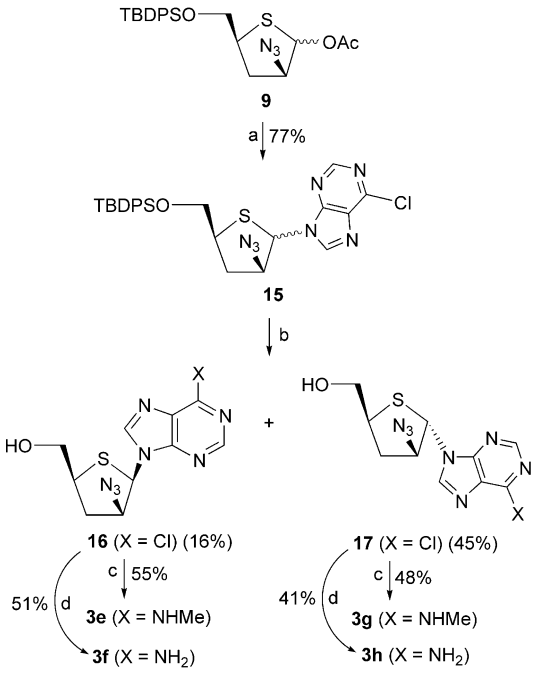
Other pyrimidine nucleosides, cytosine derivatives **3c** and **3d**, were synthesized as depicted in Scheme 3. Condensation of acetate **9** with silylated N^4-benzoylcytosine in the presence of TMSOTf gave the β-anomer **13** (23%) and α-anomer **14** (49%) after the purification on

silica gel. Both anomers **13** and **14** were deproteced under the standard conditions to afford **3c** and **3d**, respectively.

The synthetic route to the purine nucleoside analogues, **3e**–**3h**, is shown in Scheme 4. Condensation of the acetate



Scheme 3. Reagents and conditions: (a) silylated N^4-benzoylcytosine, TMSOTf, $ClCH_2Cl_2Cl$, rt, 12 h; (b) *n*-Bu_4NF, THF, rt, 1.5 h; (c) NH_3, MeOH, rt, 15 h.



Scheme 4. Reagents and conditions: (a) silylated 6-chloropurine, TMSOTf, $ClCH_2Cl_2Cl$, rt to 40 °C, 15 h; (b) *n*-Bu_4NF, THF, AcOH, rt, 1 h; (c) $MeNH_2$, MeOH, 80 °C, 24 h; (d) NH_3, MeOH, 80 °C, 24 h.

9 with silylated 6-chloropurine in the presence of TMSOTf yielded **15** as an inseparable anomeric mixture. As in the case of 2′-'down' azido derivative,[8] the N^3-isomer formed initially during the condensation cleanly migrated to the N^9-isomer on heating. Desilylation of **15** with *n*-tetrabutylammonium fluoride gave the β-anomer **16** and α-anomer **17** in about 1:3 ratio after the isolation by silica gel column chromatography. The β-anomer **16** was converted to N^6-methyladenine derivative **3e**[10] and adenine derivative **3f** by heating with methanolic methylamine and methanolic ammonia, respectively. Similarly, N^6-methyladenine derivative **3g**[11] and adenine derivative **3h** were obtained from the α-anomer **17**.

All the synthesized nucleosides **3a**–**3h** were tested against several viruses such as HIV-1, HSV-1, and HSV-2.

As shown in Table 1, most of the compounds were found to exhibit toxicity-dependent anti-HIV-1 activity in MT-4 cells, among which α-adenine derivative **3h** was the most cytotoxic. Unlike the nontoxic and potent antiviral activity of 2′-azido-2′,3′-di-deoxy-4′-oxonucleosides, all the corresponding 4′-thionucleosides exhibited cytotoxicity, indicating that small change from oxygen to sulfur led to a big difference in biological activity (antiviral versus cytotoxic), despite their bioisosteric relationship. On the basis of the cytotoxicity of **3h**, its anticancer assay is in progress in our laboratory and the results will be reported elsewhere. However, all the synthesized compounds did not exhibit antiviral activity against HSV-1 and HSV-2.

In summary, we have completed the synthesis of novel D-2′-azido-2′,3′-dideoxyarabinofuranosyl-4′-thiopyrimidines and purines, starting from L-xylose via azidation at the 2′-position as a key step. Since most of the final nucleosides exhibited toxicity-dependent anti-HIV-1 activity, it is believed that in terms of medicinal chemistry, this class of nucleosides can act as a new template for the design of anticancer agents, instead of antiviral agents.

Table 1. Antiviral activities and cytotoxicity of the synthesized nucleosides

Compd	HIV-1[a] EC_{50} (μg/mL)	Cytotoxicity[b] CC_{50} (μg/mL)	HSV-1 EC_{50} (μg/mL)	HSV-2 EC_{50} (μg/mL)
3a	> 56.5	56.1	> 100	> 100
3b	> 63.5	63.5	> 100	> 100
3c	> 41.7	41.7	> 100	> 100
3d	> 100	> 100	> 100	> 100
3e	> 48.1	48.1	> 100	> 100
3f	> 66.6	66.6	> 100	> 100
3g	> 61.5	61.5	> 100	> 100
3h	> 17.3	17.3	> 100	> 100
AZT	0.0005	0.52	0.52	> 100
Acyclovir	ND	> 76.6 (HSV-1) > 11.4 (HSV-2)	0.13	0.88

[a]Indicative of 50% cytopathic concentration in virus-infected MT-4 cells.
[b]Indicative of 50% survival concentration in virus-uninfected MT-4 cells.

Acknowledgements

This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6–01NA01–0002). The authors thank Dr. C.-K. Lee (KRICT) for the antiviral testing.

References and Notes

1. Nasr, M.; Litterst, C.; McGowan, J. *Antiviral Res.* **1990**, *14*, 125.
2. Marquez, V. E.; Tseng, C. K.-H.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H., Jr.; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S. *J. Med. Chem.* **1990**, *33*, 978.
3. Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.* **1987**, *30*, 1270.
4. (a) Bobek, M.; Whistler, R. L. *J. Med. Chem.* **1972**, *15*, 168. (b) Dyson, M. R.; Coe, P. L.; Walker, R. T. *J. Chem. Soc., Chem. Commun.* **1991**, *741*. (c) Ototani, N.; Whistle, R. L. *J. Med. Chem.* **1974**, *17*, 535. (d) Secrest, J. A., III; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A. *J. Med. Chem.* **1991**, *34*, 2361. (e) Dyson, M. R.; Coe, P. L.; Walker, R. T. *J. Med. Chem.* **1991**, *34*, 2782.
5. (a) Uenishi, J.; Motoyama, M.; Nishiyama, Y.; Wakabayashi, S. J. *J. Chem. Soc., Chem. Commun.* **1991**, 1421. (b) Uenishi, J.; Takahashi, K.; Motoyama, M.; Akashi, H.; Sasaki, T. *Nucleosides Nucleotides* **1994**, *13*, 1347.
6. (a) Yoshimura, Y.; Kitano, K.; Satoh, H.; Watanabe, M.; Miura, S.; Sakata, S.; Sasaki, T.; Matsuda, A. *J. Org. Chem.* **1996**, *61*, 822. (b) Yoshimura, Y.; Kitano, K.; Yamada, K.; Satoh, H.; Watanabe, M.; Miura, S.; Sakata, S.; Sasaki, T.; Matsuda, A. *J. Org. Chem.* **1997**, *62*, 3140. (c) Jeong, L. S.; Moon, H. R.; Choi, Y. J.; Chun, M. W.; Kim, H. O. *J. Org. Chem.* **1998**, *63*, 4821. (d) Yoshimura, Y.; Kitano, K.; Yamada, K.; Sakata, S.; Miura, S.; Ashida, N.; Machida, H. *Bioorg. Med. Chem.* **2000**, *8*, 1545.
7. (a) Jeong, L. S.; Nicklaus, M. C.; George, C.; Marquez, V. E. *Tretrahedron Lett.* **1994**, *35*, 7569. (b) Jeong, L. S.; Nicklaus, M. C.; George, C.; Marquez, V. E. *Tetrahedron Lett.* **1994**, *35*, 7573.
8. Kim, H. O.; Kim, Y. H.; Suh, H.; Jeong, L. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 599.
9. Jeong, L. S.; Yoo, S. J.; Moon, H. R.; Kim, Y. H.; Chun, M. W. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3325.
10. Compound **3h**: UV (MeOH) λ_{max} 260 nm; IR (KBr) 2115 cm^{-1} (N_3); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.40 (s, 1H, H-8), 8.08 (s, 1H, H-2), 5.99 (d, 1H, $J=6.0$ Hz, 1′-H), 5.58 (br s, 2H, NH_2), 4.78 (td, 1H, $J=5.6$, 8.0 Hz, 2′-H), 4.11 (quintet, 1H, $J=6.0$ Hz, 4′-H), 3.85 (dd, 1H, $J=4.8$, 11.2 Hz, 5′-H_a), 3.78 (dd, 1H, $J=6.0$, 11.2 Hz, 5′-H_b), 3.16 (s, 1H, OH), 2.50 (m, 1H, 3′-H_a), 2.19 (m, 1H, 3′-H_b). Anal. calcd for $C_{10}H_{12}N_8OS$: C, 41.09; H, 4.14; N, 38.33; S, 10.97. Found: C, 41.45; H, 4.33; N, 38.69; S, 10.73.
11. Compound **3f**: UV (MeOH) λ_{max} 260 nm; IR (KBr) 2114.50 cm^{-1} (N_3); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.38 (s, 1H, H-8), 8.32 (s, 1H, H-2), 6.21 (d, 1H, $J=6.0$ Hz, 1′-H), 5.54 (br s, 2H, NH_2), 4.40 (td, 1H, $J=5.2$, 10.8 Hz, 2′-H), 4.01 (d, 1H, $J=4.0$ Hz, 5′-H_a), 4.00 (d, 1H, $J=4.0$ Hz, 5′-H_b), 3.83 (m, 1H, 4′-H), 3.50 (br s, 1H, OH), 2.53–2.46 (m. 2H, 3′-H). Anal. calcd for $C_{10}H_{12}N_8OS$: C, 41.09; H, 4.14; N, 38.33; S, 10.97. Found: C, 41.21; H, 4.10; N, 38.06; S, 10.89.