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Synthesis and antitumor activity of novel 2′,3′-dideoxy-2′,3′-diethane thionucleosides bearing 1,2,3-triazole residues

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ABSTRACT

A series of novel 2′,3′-dideoxy-2′,3′-diethanethioribonucleosides and those modified with a triazole ring were prepared in excellent yields and their antitumor activity was evaluated. Nucleosides with a triazole ring, **16a**–**16c**, showed significantly improved activity towards a broad range of tumor cell lines.

Cancer is one of the leading causes of death in the world. Nucleoside analogues are rationally designed anticancer drugs used in clinical therapy for both solid tumors and hematological malignancies. These include capecitabine (5′-deoxy-5-*N*-[(pentoxy)carbonyl]-cytidine),[1] cladribine (2-chloro-2′-deoxyadenosine),[2] fludarabine (9-β-D-arabinofuranosyl-2-fluoroadenosine),[3] clofarabine (2-chloro-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine, Fig. 1),[4] a next generation nucleoside analogue which is under clinical investigation as well as gemcitabine (2′,2′-difluorodeoxycytidine)[5] used for treating broad kinds of tumors such as pancreatic, metastatic bladder, lung, breast, ovarian, head and neck cancers.

With an improved understanding of the genes and pathways responsible for cancer initiation and progression, cancer drug development has undergone a paradigm change in recent years, from predominantly cytotoxic agent-based therapy to therapy aimed at molecular and genetic targets. Nucleoside transporters are important for the cellular uptake of anticancer nucleoside mimetic drugs.[6,15] Some nucleoside mimetics, such as NBMPR (Fig. 1), which act as potent nucleoside transporter inhibitors, have been reported for use in cancer therapy.[7] Ribonucleotide reductase (RDPR), which is responsible for DNA synthesis via free radical intermediates, is also an attractive target for antitumor chemotherapy.[8] A number of 2′-position modification nucleoside analogues have been shown to be effective mechanism-based inhibitors of this enzyme and may be potential anticancer agents[9] such as 2′-methylene-2′-deoxy-cytidine and 2′-methylene-2′-deoxy-uridine,[10] 2′-*O*-allyl-D-arabifuranosylcytosine[11] as well as 3′-*C*-methyladenosine.[12]

It is worthwhile to note that replacement of the 2′,3′-hydroxy groups by a mercapto group which can then react with the active site of RDPR was found to be a potent inhibitor of RDPR in vitro[13] and some 2′,3′-dideoxy-3′-thionucleosides have shown good anti-HIV activity.[14] Furthermore, many nucleosides have proven to be inhibitors of multienzymes.[15] Cladribine 5′-triphosphate,[16] clofarabine[17] and gemcitabine 5′-diphosphate[18] inhibit both DNA polymerase and ribonucleotide reductase. This work summarizes a number of novel nucleosides with ethanethio groups which are potent inhibitors of multienzyme targets.

1,2:3,5-Di-*O*-isopropylidene-α-D-xylose **1** prepared from D-xylose was effectively transformed into the corresponding diacetate **2** in a large scale via a one-pot procedure as previously reported.[19] Treatment of **2** with ethanethiol in the presence of $ZnBr_2$ generated the key intermediate **3** in good yield. Compound **3** contains two ethanethiol groups at C-2 and C-3 with inversed configuration at C-3.[20] Removal of the acetyl groups in **3** was performed with

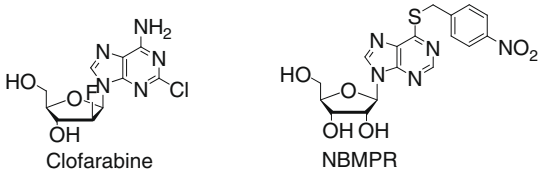

Figure 1. Clofarabine and NBMPR.

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methylamine to give **4** in 94% isolated yield, which was subsequently cyclized in the presence of NBS to afford the expected ribofuranose derivative **5** in 83% isolated yield without selectively protecting the primary alcohol as previously described by Miljkovic and co-workers.[21] Protecting the primary hydroxyl group with an acetyl group afforded the anticipated intermediator **6** in 56% overall yield for 5 steps (steps from a to e, Scheme 1).

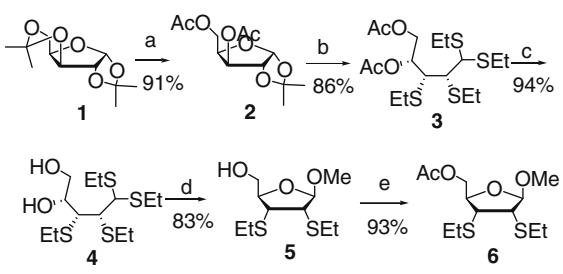
Under Silyl-Hilbert-Johnson nucleoside synthetic conditions,[22] 2,3-dideoxy-D-ribofuranose derivative **6** was treated with trimethylsilylated N^4-acetylcytosine in the presence of trimethylsilyl trifluoromethanesulfate (TMSOTf) to give the corresponding nucleosides as an anomeric mixture (β/α = 2.5:1), which was isolated using a short column to afford the β-isomer **7** in 51% yield (Scheme 2). After a series of attempts at improving the yield, we found that displacement of TMSOTf with ethylaluminum dichloride ($EtAlCl_2$) led to **7** (76% isolated yield) as the sole product without the counterpart α-isomer. Ethylaluminum dichloride possibly leads to the formation of the key intermediate **A** (Fig. 2) directing nucleophilic base attack from only the top face rather than from either face of the sugar ring. Whereas oxonium intermediate **B** (Fig. 2) generated by the strong Lewis acid, TMSOTf, could be attacked from both faces to give both α- and β-isomers. Removal of the acetyl protecting group was performed with methylamine in ethanol to generate the desired nucleoside **10** in nearly quantitative yield. Under similar conditions, the expected nucleosides **11** and **12** were also synthesized in good yields.

The molecular structure of **7** was further confirmed to be the β-anomer by a NOESY spectrum. In the ^1H-^1H dimensional NMR spectrum of compound **7**, two cross-peaks are observed between the H-6 (at δ 8.18) and H-3′ (at δ 3.58) as well as H-2′ (at δ 3.87), respectively. Furthermore, it was also observed that the resonance signal of the anomeric proton (at δ 5.99) interacts with the methylene proton of 2′-S$CH_2$$CH_3$ (at δ 2.79) (Fig. 3). These NOESY spectroscopic data provide strong evidence for a β-D-ribofurano assignment.

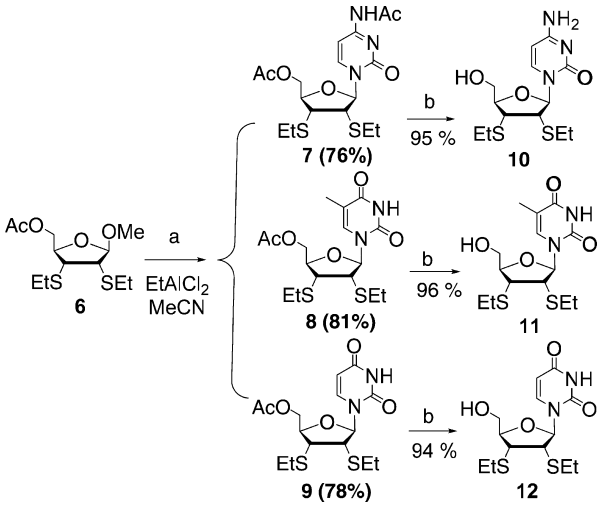
1,2,3-Triazoles recently have gained significant interest in various fields of drug discovery and bioconjugation.[23] Introduction of a triazole ring into nucleosides to improve bioactivity in antitumor and/or antivirus agents[24] has become widespread in drug design practices since the first synthetic nucleoside drug, ribavirin,[25] showed a broad spectrum of antiviral activity against many RNA and DNA viruses.

Therefore, we next concentrated on further modifications of the nucleosides by introducing a triazole group expecting that this type of aromatic system will provide the triazole nucleoside with advantageous binding properties to the corresponding biological target.

Treatment of D-ribose derivative **5** with benzoyl chloride provided **13** (Scheme 3), which was subsequently treated with trimethylsilyl azide and stannic chloride to form, exclusively, the desired azidoribofuranoside **14** in 74% isolated yield in 2 steps (steps a and b, Scheme 3). Compound **14** was identified to be β-



Scheme 1. Reagents and conditions: (a) Ac_2O, $HClO_4$–SiO_2, CH_2Cl_2, rt, 91%; (b) EtSH, $ZnBr_2$, CH_2Cl_2, rt, 86%; (c) CH_3NH_2, C_2H_5OH, 94%; (d) NBS, CH_3OH, 83%; (e) Ac_2O, Et_3N, CH_2Cl_2, 93%.



Scheme 2. Reagents: (a) $EtAlCl_2$, MeCN; (b) CH_3NH_2, C_2H_5OH.

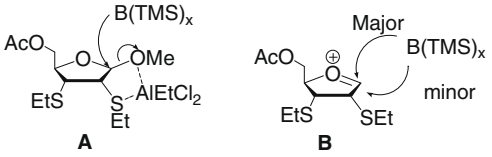


Figure 2. Proposed intermediates.

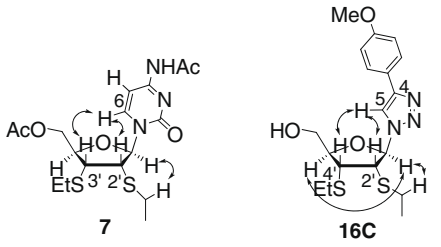
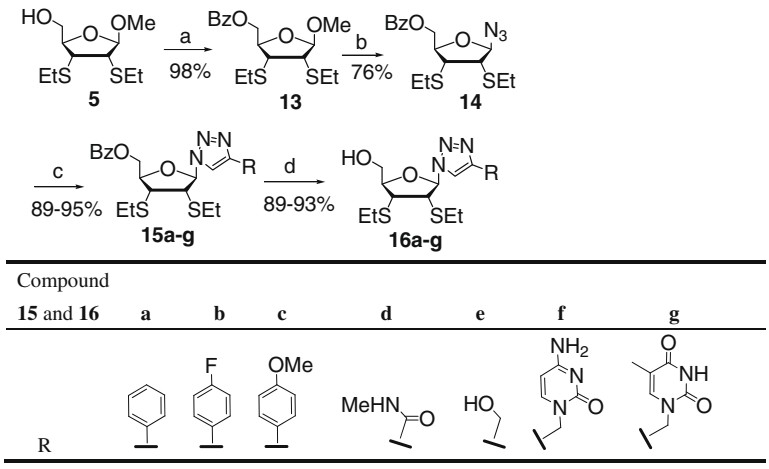


Figure 3. Through space proton–proton interactions indicated by the ^1H–^1H NOESY spectra of compound **7** and **16c**.

anomeric by the NOESY spectrum of the following nucleoside products. Huisgen cycloaddition of the triazole group with a series of alkynes was successfully carried out in the presence of Cu–$CuSO_4$ at rt without sodium ascorbate[23e] to give the corresponding triazole nucleosides in excellent yields (89–95%). Cleavage of the protecting groups in **15** was performed with methylamine in ethanol to afford the final products **16** in approximately 90% yield. The triazole structure was identified to be an unambiguous 1,4-disubstituted cycloaddition product according to the ^1H–^1H NOESY spectrum data of the deprotected product **16c** (Fig. 3).

The molecular structure of **16c** was further elucidated to be a β-isomer by a NOESY spectrum (Fig. 3). In the ^1H–^1H dimensional NMR spectrum of compound **16c**, two cross-peaks are observed between the resonance signals of the anomeric proton (at δ 6.26) and the methylene proton of 2′-S$CH_2$$CH_3$ (at δ 2.59) and 4′-H (at δ 4.07), respectively (Fig. 3). Moreover, it was also observed that the proton in the triazole ring (at δ 8.72) couples with H-3′ (at δ 3.89) as well as H-2′ (at δ 4.21). Additionally, these two cross-peaks also indicated that the substitute groups are at the 1,4-positions in the 1,2,3-triazole ring. These NOESY spectroscopic data provide strong evidence for the β-anomeric configuration assignments and the triazole ring structure.



Scheme 3. Reagents and conditions: (a) BzCl, Et_3N, CH_2Cl_2, rt, 98%; (b) $TMSN_3$, $SnCl_4$ (5 mol %), CH_2Cl_2, 76%; (c) alkyne, Cu, $CuSO_4$, THF/BuOH/H_2O (2:1:1); (d) CH_3NH_2, C_2H_5OH.

These novel nucleosides were evaluated for their cytotoxicity in vitro towards the following human cancer cell lines: human hepatocellular liver carcinoma cell line ($HepG_2$), human lung adenocarcinoma cell line (A549), human pulmonary carcinoma cell (LAC), and human cervical carcinoma cell line (Hela). Cytotoxic activity was determined using the MTT assay, after exposure of cells to the test compounds for 72 h. Floxuridine was used as the reference compound and the results are presented in Table 1.

Compounds **10**–**12** showed moderate activity against the three tested tumor cells, with EC_{50} values ranging from 29.47 to 177.13 μM (Table 1). The thymidine analogue **11** showed the best activity against Hela cells. Remarkably, the triazole nucleosides **16a**, **16b** and **16c** exhibit activity against $HepG_2$ cells with EC_{50} ranging from 9.6 to 10.98 μM, about 10-fold better than those for **10**–**12** and around twofold higher than those for the reference drug floxuridine, EC_{50} = 18.84 μM. Towards A549 cells, nucleosides **10**–**12** showed much lower cytotoxicity than **16a**, **16b** and **16c**. Nucleosides **16b** and **16c** were also found to be more active than the reference drug and compounds **10**–**12** against both the LAC and Hela cell lines. However, nucleosides **16d**–**16g** were found to be inactive against these 4 types of cancer cell lines (EC_{50} >100 μM, Table 1). The higher cytotoxic activity exhibited by nucleosides **16a**–**16c** than nucleosides **10**–**12** towards all four tested tumor cells might stem from the triazole ring. On the other hand, nucleosides **16d**–**16g** were inactive against these cancer cell lines perhaps because of the methylene group between the triazole ring and the nucleobase, which interrupts the conjugation of the triazole ring with the aromatic system.

Table 1
Cytotoxicity of nucleosides against four cancer cell lines in vitro

Compound	EC_{50}[a] (μM)			
	$HepG_2$	A549	LAC	Hela
10	115.76	86.41	n/a	177.13
11	n/a	101.40	n/a	29.47
12	74.74	87.35	n/a	75.69
16a	9.60	44.15	20.66	11.17
16b	9.62	20.22	16.22	6.92
16c	10.98	23.36	14.51	5.69
16d	>100	>100	>100	>100
16e	>100	>100	>100	>100
16f	>100	>100	>100	>100
16g	>100	>100	>100	>100
Floxuridine	18.84	9.74	32.09	10.26

[a] EC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared with an untreated control.

The possible conjugation between the triazole ring and the benzene ring in nucleosides **16a**, **16b** and **16c** may offer improved binding potential to a biological target, but further studies will be needed to address this hypothesis.

In conclusion, a series of novel 2′,3′-dideoxy-2′,3′-diethanethioribonucleosides, **10**–**12**, were prepared in 40–44% overall yields in 7 steps (Schemes 1 and 2) and those nucleosides containing a triazole ring, **16a**–**16g**, were obtained in 59–65% overall yields in four steps (Scheme 3). Their antitumor activity against four human cancer cell lines was evaluated in vitro. Nucleosides **10**–**12** exhibited only moderate antitumor activity. In contrast, the series of triazole modified nucleosides **16a**–**16c** showed significantly improved antitumor activity towards $HepG_2$, A549 and Hela cell lines and higher cytotoxicity towards $HepG_2$, LAC and Hela cell lines than the control drug floxuridine. Moreover, compounds **16b** and **16c** showed similar antitumor activity towards the four cell lines and may be potential inhibitory agents towards a broader range of tumor cell lines. However, triazole nucleosides **16d**–**16g** were inactive to all four tested cancer cell lines. These results might suggest that the conjugation effects of the triazole ring with the aromatic system is important for bioactivity. Further structure–activity studies with similar nucleosides are currently underway in our laboratory.

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