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Synthesis and anti-HIV activity of 1,3-dithiolane nucleosides

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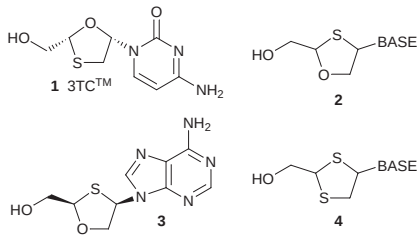
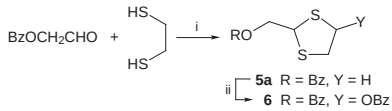
The potent activity displayed by 3′-azido-3′-deoxythymidine (AZT)[1] against human immunodeficiency virus (HIV) provides impetus for the development of novel nucleoside analogues.[2] Unfortunately, those compounds with the natural stereochemistry possess undesirable pharmacological properties[3] and are susceptible to the development of resistant stains of HIV.[3,4] In an attempt to overcome some of these detrimental side effects, the carbohydrate moiety of 2′,3′-dideoxynucleoside analogs has been replaced by other five membered rings.[3d,4] It has been demonstrated that hetero-substitution of these rings has a profound effect on the biological activity of the resulting nucleoside analogue[5] as displayed by (−)-2′-deoxy-3′-thiacytidine (3TC, Epivir) **1**.[5c,6]



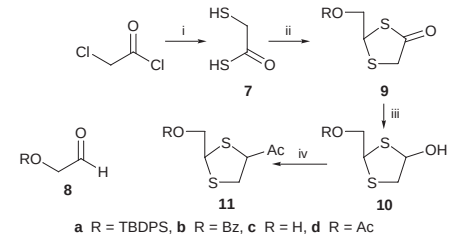
As part of an ongoing search for new anti-AIDS leads, we further explored this class of thioribonucleosides. These compounds possess improved metabolic stability to phosphorylases which cleave the glycosidic bond in nucleosides.[7] Recently, we reported the anti-HIV activity of 2,4-disubstituted 1,3-oxathiolane nucleosides **2** by transposing the sulfur and oxygen atoms of **1**.[4a] In this series, the (−)-adenine derivative **3** with the natural configuration was found to be twice as active as ddI in MT-4 cells. We further modified the oxathiolane ring by replacement of the oxygen atom of **2** with sulfur. This is exemplified by the general structure **4**. Here we report the synthesis and anti-HIV activity of this class of compounds.

The synthetic route to (±)-1,3-dithiolane compounds is based upon coupling a persilylated heterocyclic base with a dithiolane moiety **5** bearing a suitable leaving group Y at the 4-position under Vorbruggen's conditions.[8] Two approaches were considered for the preparation of the ring **5**. The first route is the introduction of a leaving group at position 4 using peroxide derivatives. For example, dithiolane **6** was prepared by treating **5a** with benzoyl peroxide in refluxing benzene (Scheme 1).[9]

The second approach offers a more general route for the synthesis of the key intermediate 1,3-dithiolane **11**† with a variety of displaceable leaving groups at C-4. Our synthetic strategy was based on the preparation of 1,3-dithiolan-4-one **9**, followed by reduction and acylation to give 4-acyloxy-1,3-dithiolane **11** (Scheme 2). Thus, reaction of freshly distilled $ClCH_2COCl$ with excess NaSH (3 equiv.) in absolute ethanol at −10 °C gave **7**[10] in quantitative yield. The crude product was



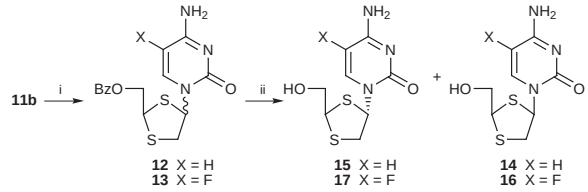
Scheme 1 *Reagents and conditions*: i, C_6H_6, TSA; ii, BzOOH, C_6H_6, heat.



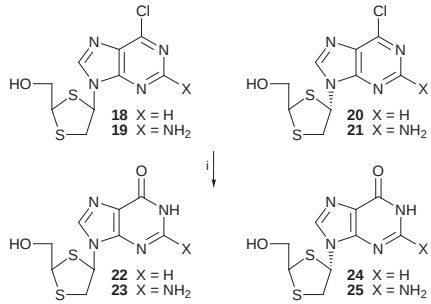
Scheme 2 *Reagents and conditions*: i, NaSH, absolute EtOH, −10 °C; ii, **8**, ZnI_2, CH_2Cl_2; iii, DIBAL-H, PhMe or BH_3·THF, $B(MeO)_3$; iv, AcOCl, Py.

immediately treated with aldehyde **8** in the presence of ZnI_2 as catalyst in CH_2Cl_2[11] to give the desired (±)-1,3-dithiolan-4-one **9** in moderate yield (50–60%). The initial synthetic procedure was based upon using the TBDPS protecting group for the hydroxy function of **9**. Reduction of **9a** with DIBAL-H (1.1 equiv.) in toluene gave thiolactol **10a** which was subsequently acylated to give the key intermediate **11a** in high yield. The silyl protecting group was later replaced by a benzoate group in order to facilitate the separation of the *cis* and *trans* nucleoside isomers. Applying the same conditions to reduce the benzoate **9b** did not result in any reduction product. However, using excess of DIBAL-H (3 equiv.) was successful and both the thiolactone and the benzoate function were reduced to give diol **10c** in 40% yield. Compound **10c** was then bis-acylated giving intermediate **11d** in high yield. Efforts were then directed to scale-up this procedure. Unfortunately, the DIBAL-H reduction proved particularly intractable. We therefore investigated other reducing agents that are selective and require little work-up. Only BH_3·THF (1.2 equiv.) catalyzed by $B(OMe)_3$ (1 equiv.) gave satisfactory results. The reduction was completed in 16 h and the product **10b** was obtained in 95% yield. This compound was then acylated to give the expected product **11b**. Following the same procedure, a number of different leaving groups (Bz, *m*-$ClC_6H_4CH_2$ and *p*-$O_2NC_6H_4CH_2$) were successfully introduced at C_4 of the sugar moiety **11**.

Compound **11b** is suitable for coupling with silylated cytosine or 5-fluorocytosine under refluxing conditions in CH_2Cl_2 and in the presence of $SnCl_4$ (Scheme 3). This gave the desired nucleoside analogue **12** or **13** as a 1 : 2 mixture of *cis* and *trans* isomers in moderate yields. Replacement of $SnCl_4$ with TMSI altered the ratio of the isomers. For example, compound **6** reacted with silylated N-acetylcytosine to give a mixture of the *cis* and *trans* nucleosides **12** in 62% yield with a slight predominance of the *cis* isomer.[9] Similar results were obtained using other leaving groups at C_4. This did not improve the yield



Scheme 3 *Reagents and conditions*: i, 2,4-Bis(trimethylsilyloxy)pyrimidine, $ClCH_2CH_2Cl$, $SnCl_4$, reflux, 16 h; ii, NH_3, MeOH.



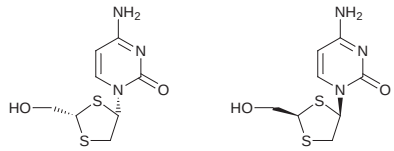
Scheme 4 *Reagents and conditions*: i, 25% aq. Me_3N–H_2O, 55–65%.

or the *cis*:*trans* ratio. The next step was the separation of the isomers **12** or **13**. This was achieved by flash chromatography on silica gel by prior acetylation of the amino group (cytosine) or by reverse chromatography HPLC after deprotection (5-fluorocytosine). The protecting groups were then removed by treatment with methanolic ammonia to give the desired nucleosides **14**–**17** in high yields. The relative stereochemistry of these products was assigned by difference NOE spectra.

Similarly, uracil, thymine, adenine and guanine derivatives were produced from **11b** using the same conditions. However, in the case of hypoxanthine and guanine analogs **22**–**25**, synthesis was undertaken by treating the corresponding 6-chloropurine or 2-amino-6-chloropurine derivatives **18**–**21** with 20 equiv. of an aqueous Me_3N solution in water (Scheme 4).

The anti-HIV activity of (±)-1,3-dithiolane nucleoside analogues was evaluated in MT-4 (human T helper) cells at concentrations up to 100 μg ml^{-1} and compared with 3TC™ (Epivir)[12] and the 5-fluoro derivative (FTC).[13] In this assay, only *cis* cytosine and 5-fluorocytosine derivatives **14** and **16** displayed inhibitory activity at ID_{50} of 9.3 and 4.8 μg ml^{-1} and were not cytotoxic at 100 μg ml^{-1}, whereas 3TC™ and FTC showed anti-HIV activity at 0.3 and 0.14 μg ml^{-1}, respectively. All the other nucleosides did not exhibit antiviral activity with no cytotoxicity up to 100 μg ml^{-1}. In contrast, *cis* and *trans* 6-chloropurine derivatives **18** and **20** showed cytotoxicity at CD_{50} of 10 μg ml^{-1}.

Described herein is a novel class of anti-HIV (±)-1,3-dithiolane nucleoside analogues. The biological results demonstrate that replacement of an oxygen atom of the oxathiolane with sulfur causes reduction in antiviral activity. It should be noted that compounds **14** and **16** are racemic. Resolution of the enantiomers may improve the activity. Therefore, enantiomeric separations of the racemic *cis* **14** was undertaken by chiral HPLC.[14] This gave the two enantiomers **26** and **27** in a



26 $[\alpha]_D^{25}$ +75 (*c* 0.10, H_2O) **27** $[\alpha]_D^{25}$ –70 (*c* 0.10, H_2O)

reasonable yield. It was found that isomer **27** possesses the natural configuration as evidenced by enzymatic resolution of the racemic mixture. Thus treatment of the mixture of the two enantiomers with cytidine deaminase converted only **27** to its corresponding uracil derivative. However, the unnatural enantiomer **26** was recovered and characterized by comparison of HPLC retention time and optical rotation with the previously isolated isomer. Both enantiomers were submitted for anti-HIV evaluation. Neither of the two compounds displayed improved antiviral activity.

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Notes and references

† *Selected data* for **10b**: colorless oil; δ_H($CDCl_3$): 8.03 (m, 2H), 7.57 (dt, 1H, *J* 7, 1), 7.44 (t, 2H, *J* 7), 5.85 (m, 1H), 4.84 and 4.76 (t's, 1H, *J* 7), 4.68 and 4.39 (m's, 1H), 4.48 and 4.27 (m's, 1H), 3.30 (m, 2H), 2.85 (m, 1H). For **16**: δ_H(DMSO-d_6) 8.34 (d, 1H, *J* 7.5), 7.83 (br s, 1H), 7.59 (br s, 1H), 6.39 (m, 1H), 5.57 (t, 1H, *J* 5.5), 4.62 (t, 1H, *J* 6), 3.75 (t, 2H, *J* 6), 3.59 (m, 2H). For **17**; δ_H(DMSO-d_6) 8.10 (d, 1H, *J* 7 Hz), 7.78 (br s, H), 7.54 (br s, 1H), 6.41 (t, 1H, *J* 2), 5.36 (t, 1H, *J* 5.5), 4.81 (t, 1H, *J* 7 Hz), 3.45 (m, 4H). For **19**: δ_H(DMSO-d_6) 8.45 (s, 1H), 7.03 (br s, 2H), 6.40 (t, 1H, *J* 4), 5.53 (t, 1H, *J* 6), 4.73 (t, 1H, *J* 7), 3.80 (dd, 1H, *J* 13, 4.5), 3.74 (t, 2H, *J* 6), 3.63 (dd, 1H, *J* 13, 5); δ_C(DMSO-d_6) 159.80, 153.49, 150.01, 141.95, 123.61, 65.57, 64.52, 56.23, 42.93; HRMS (FAB): M^+ calc. for $C_9H_{11}ClN_5OS_2$ 304.00937, found 304.00880. For **21**: δ_H(DMSO-d_6) 8.34 (s, 1H), 7.02 (br s, 2H), 6.44 (t, 1H, *J* 3), 5.41 (t, 1H, *J* 6), 4.86 (t, 1H, *J* 7), 3.58 (m, 3H), 3.49 (m, 1H); HRMS (FAB): M^+ calc. for $C_9H_{11}ClN_5OS_2$ 304.00937, found 304.00840. For **26**: mp 108–110 °C; δ_H(DMSO-d_6) 8.06 (d, 1H, H-6′, *J* 7.63), 7.20 (br d, 2H, NH_2), 6.47 (t, 1H, *J* 4.4), 5.74 (d, 1H, *J* 7.42) 5.50 (t, 1H), 4.61 (t, 1H, *J* 6.5 Hz), 3.74 (t, 2H, *J* 6.00 Hz), 3.46 (dd, 1H, *J* 4.30, 12.9), and 3.36 (dd, 1H, *J* 4.1, 10.4); HRMS (FAB): M^+ calc. for $C_8H_{12}N_3O_2S_2$ 246.03709, found 246.03610. For **27**: mp 200–202 °C (decomp.); δ_H(DMSO-d_6) 7.90 (d, 1H, *J* 7.35), 7.17 (br d, 2H), 6.48 (d, 1H, *J* 3.72), 5.69 (d, 1H, *J* 7.47), 5.38 (t, 1H), 4.74 (t, 1H, *J* 6.83), 3.43 (m, 4H); HRMS (FAB): M^+ calc. for $C_8H_{12}N_3O_2S_2$ 246.03709, found 246.034640.

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14 Chiral Column: Cyclobond I 2000 Beta-RSP 4.6 mm ID × 250 mm; mobile phase 10% MeCN–0.05% (AcOH–Et_3N, pH 6.74); pressure 965 psi and flow rate 0.50 ml min^{-1}. For **26**: t_R = 19.880 min; for **27**: t_R = 22.201 min.

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