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Synthesis and Antiviral Activities of *N*-9-Oxypurine 1,3-Dioxolane and 1,3-Oxathiolane Nucleosides

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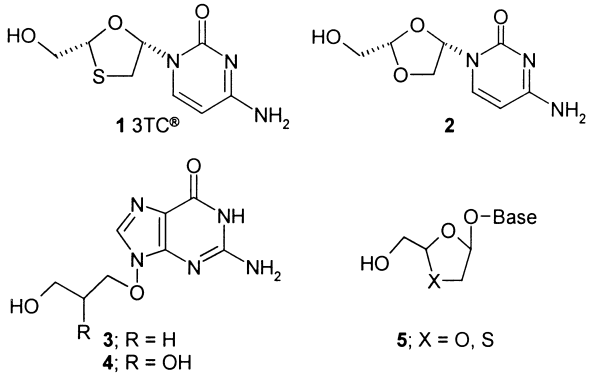
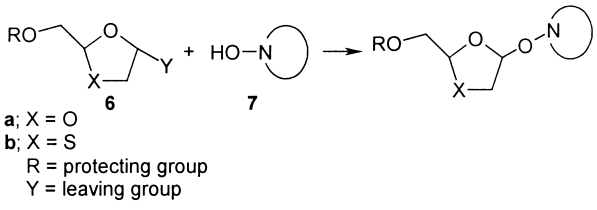
Abstract—Two series of 1,3-dioxolanes and 1,3-oxathiolane nucleosides containing *N*-9-oxypurine were synthesized as potential antiviral agents. These compounds were prepared by reacting the sugar moieties with iodo- or bromotrimethylsilane, followed by treatment with a mixture of sodium hydride and the desired *N*-hydroxy purine base. The preparation of these *N*-hydroxybases was also described. No significant antiviral activity was observed against HIV, HBV, HSV-1, HSV-2, or HCMV. © 2000 Elsevier Science Ltd. All rights reserved.

Nucleoside antimetabolites play an important role in the field of cancer chemotherapy and treatment of viral diseases. 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] One approach is to replace the carbohydrate moiety of 2′,3′-dideoxynucleoside analogues[3a] by other five-membered rings.[3b,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] and (+)-2′-deoxy-3′-oxacytidine **2**.[7]



As part of an ongoing search for new antiviral leads, we further explored this class of 3′-heterosubstituted nucleosides. It has been reported that compounds in which the acyclic substituent or the sugar moiety is linked to the heterocyclic base through a nitrogen–oxygen bond, possess useful biological properties.[8] For example, the guanine derivative **3** showed potent and selective activity against HSV-1, HSV-2, and VZV and is superior to that of acyclovir.[8c,d] Therefore, we replaced the acyclic portion or the ribose moiety by 1,3-oxathiolane and 1,3-dioxolane rings. This is exemplified by the general structure **5**.

The synthetic route to (±)-1,3-dioxolane and 1,3-oxathiolane nucleoside analogues **5** is based upon reaction of *N*-hydroxyheterocycles **7** with a dioxolane or oxathiolane moiety **6** bearing a suitable leaving group Y (Scheme 1).



Scheme 1.

Our strategy was to build stepwise the *N*-hydroxypurine base since its direct synthesis by oxidation of the base has not yet been achieved. *N*-9-Hydroxy-6-chloropurine **12** was selected as the key base in this series since it provides a versatile intermediate for the synthesis of several purine derivatives. Scheme 2 describes the preparation of this key compound **12** from 4,6-dichloro-2-

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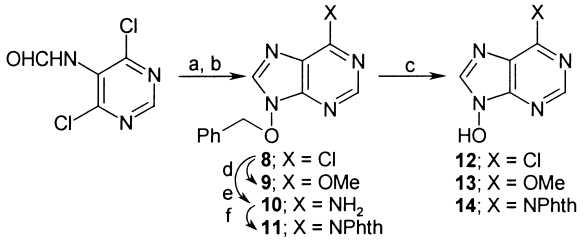


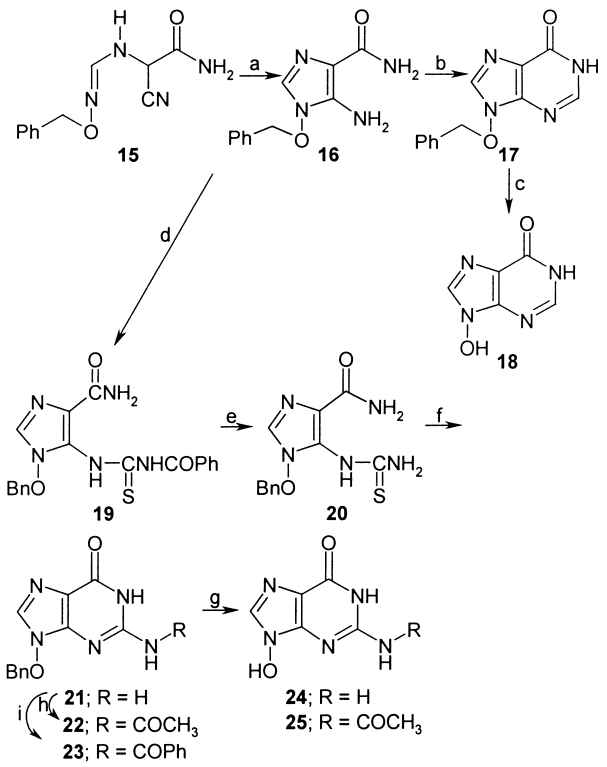
Scheme 2. (a) $PhCH_2ONH_2$, *i*-Pr_2 EtN, diglyme; (b) $(EtO)_3CH$, 12 M HCl, DMF; (c) BCl_3, CH_2Cl_2, 0 °C; (d) NaOMe, MeOH; (e) NH_3, MeOH; (f) O-$C_6H_4(COCl)_2$, DMAP, Et_3N, THF, 0 °C.

formamidopyrimidine.[9] The latter was treated with benzyloxyamine to give **8**, followed by cyclization to the imidazole using triethylorthoformate.

Attempts to remove the benzyl group of **8** using TMSI or Me_2BBr resulted in degradation of the purine ring. However, hydroxypurine **12** has been successfully prepared in almost quantitative yield by treatment of chloro-derivative **8** with BCl_3 in CH_2Cl_2 at 0 °C. This procedure is amenable to scale-up. This chloro compound was also converted to other 6-substituted purine derivatives. For example, methoxypurine **13** was prepared by reaction of chloro **8** with methanolic sodium methoxide followed by catalytic hydrogenation.

Adenine **10** was also prepared from chloro **8**.[9] The amino function was then protected as phthalimido (Phth) **11** before hydrogenation to the free *N*-hydroxyl compound. Removal of the benzyl group afforded the *N*-hydroxy adenine derivative **14**. The synthesis of 9-hydroxy hypoxanthine **18** was performed through the key intermediate 5-amino-4-aminocarbonyl-1-benzyloxy-imidazole **16**[10] (Scheme 3). This compound is the precursor for a number of antiviral purine derivatives and its preparation was based upon cyclization of the formamidine **15**.[11] Closure of the aminoimidazole ring **16** by heating with triethylorthoformate followed by hydrogenation gave *N*-9-hydroxyhypoxanthine **18** in a good yield.

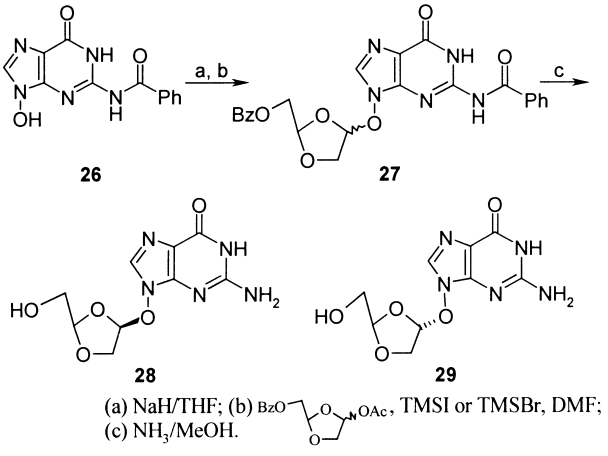
Efforts were then directed to the synthesis of *N*-9-benzyloxy-2-amino-6-chloropurine, in particular guanine. Our synthetic strategy was based on the cyclodesulfurization of the amino carbonyl **20** (Scheme 3). Thus, treatment of **16** with benzoyl isothiocyanate in acetone afforded the thiocarbamoyl **19**, which was hydrolyzed to the thiourea **20**.[12] Cyclodesulfurization of the latter under alkaline conditions gave protected guanine **21** in high yield. This was hydrogenated to give hydroxy-guanine **24**. Due to the high polarity of the latter, we decided to protect the 2-amino function of **24** as the acetate or benzoate. This was achieved by treating **21** with acetic anhydride or benzoyl chloride in pyridine to give **22** and **23**, respectively. Unlike the 1-(benzyloxy) imidazole which is unstable under alkaline conditions,[8c] the *N*-benzyloxy purines **8**–**11**, **17** and **21**–**23** are stable under a variety of conditions. These include acidic and basic conditions, as well as temperatures (< 90 °C) and catalytic hydrogenation. In addition, the final free *N*-hydroxy compounds **12**–**14**, **18** and **24**–**26** can be stored for months at 0 °C without decomposition.



Scheme 3. (a) Et_2O, BF_3/DME, 60 °C; (b) $HC(OEt)_3$, reflux; (c) HCl/MeOH; Pd/C, H_2; (d) PhCONCS, acetone; (e) K_2CO_3/aq MeOH–acetone; (f) $Cu(OCOCH_3)_2$, aq NaOH; (g) Pd/C, EtOH, cyclohexane, reflux; (h) Ac_2O, pyridine; (i) C_6H_5COCl, pyridine.

Two approaches were considered for the preparation of purine nucleosides. The first route was based upon coupling of a suitably functionalized *N*-hydroxy purine base with 1,3-dioxolane or oxathiolane sugars under Mitsunobu conditions. Unfortunately, this reaction appeared to be ineffective and resulted in low yield. In fact, the Mitsunobu reaction between acylated furanose and 1-hydroxybenzotriazole gave similar results.[13]

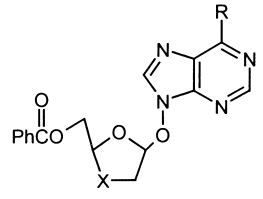
The second approach offers a more general route for the synthesis of these nucleosides. Scheme 4 illustrates a representative example where the sugar moiety of 1,3-



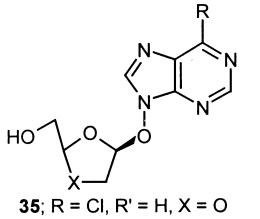
Scheme 4.

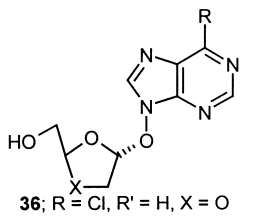
dioxolane was reacted with iodo- or bromotrimethylsilane then the solution was treated with a mixture of sodium hydride and protected *N*-9-hydroxy guanine **26** in DMF. This gave the desired nucleoside **27** as a 1:1 mixture of *cis* and *trans* isomers in high yields. Replacement of halotrimethylsilane with trimethylsilyltriflate or the base sodium hydride with triethylamine did not alter the ratio of isomers but reduced the yield. Separation of the isomers of **27** was achieved by flash chromatography on silica gel or by reverse chromatography HPLC after deprotection. The protecting groups where then removed by treatment with methanolic ammonia to give the expected nucleosides **28** and **29** in good yields.[14]

Similarly, 6-methoxy purine **39–42** and hypoxanthine **43–46** derivatives were produced using the same conditions. However, in the case of 6-chloro **35–38** and adenine **47–50** nucleosides, the yield was low with undesirable byproducts. We therefore decided to investigate a number of other synthetic routes to prepare these compounds. For example, the syntheses of chloro **35–38** were achieved by treating the hypoxanthine **32** or **33** with either phosphorous oxychloride in DMF or with carbon tetrachloride–triphenylphosphine (3:1) in acetonitrile. In the case of adenine **47–50**, two approaches were considered for their preparation. The first route was to react the 6-methoxypurine derivative **34** with ethanolic ammonia in a bomb for 48 h. This method resulted in low yield. An alternative approach was by a halogen amino group interchange of the appropriate 6-chloropurine precursor. For example, treatment of the chloro derivative **30** or **31** with ethanolic ammonia in a bomb gave high yields of the expected adenine **47–50**.



30; R = Cl, X = O
31; R = Cl, X = S
32; R = OH, X = O
33; R = OH, X = S
34; R = OCH_3, X = S





35; R = Cl, R' = H, X = O
37; R = Cl, R' = H, X = S
39; R = OCH_3, R = H, X = O
41; R = OCH_3, R' = H, X = S
43; R = OH, R' = H, X = O
45; R = OH, R' = H, X = S
47; R = NH_2, R' = H, X = O
49; R = NH_2, R' = H, X = S

36; R = Cl, R' = H, X = O
38; R = Cl, R' = H, X = S
40; R = OCH_3, R' = H, X = O
42; R = OCH_3, R' = H, X = S
44; R = OH, R' = H, X = O
46; R = OH, R' = H, X = S
48; R = NH_2, R' = H, X = O
50; R = NH_2, R' = H, X = S

The anti-HIV activity of (±)-1,3-dioxolane and 1,3-oxathiolane nucleoside analogues **35–50** was evaluated in MT-4 (human T helper) cells at concentrations up to 100 μg/mL and compared with 3TC® (Epivir) and AZT. In this assay, none of the nucleosides displayed any inhibitory activity or cytotoxicity up to 100 μg/mL except hypoxanthine **45**, which showed cytotoxicity at CD_{50} of 10 μg/mL. The anti-HBV activity of these nucleosides was assessed in hepatoma cell line 2.2.15 transfected with human HBV. None of these compounds showed activity against extracellular HBV. The anti-herpetic activities and cytotoxicities of these compounds were determined in plaque reduction assays in vero cells and Flow 2002 (human fibroblast) cells infected with HSV-1 (KOS strain), HSV-2 (186 strain) and HCMV (WFI strain), respectively. The *cis* and *trans* guanine derivatives **28** and **29** were weakly inhibitory to HSV-1 and HSV-2 replication with no cytotoxicity up to 100 μg/mL.

Described herein is a novel class of (±)-1,3-dioxolane and 1,3-oxathiolane nucleoside analogues. The biological results demonstrate that linking the sugar to the heterocyclic base through an oxygen causes a dramatic reduction in antiviral activity.

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14. Selected data for **28**: mp >220 °C; δ_H (DMSO-d_6) 11.68 (bs, 1H), 7.86 (s, 1H), 6.57 (bs, 2H), 5.87 (d, 1H, $J=3.8$ Hz), 5.23 (t, 1H, $J=6.0$ Hz), 5.13 (t, 1H, $J=3.9$ Hz), 4.38 (d, 1H, $J=10.4$ Hz), 4.04 (dd, 1H, $J=4.1$, 10.4 Hz), 3.57 (m, 2H). LRMS (FAB) m/z 270 (MH^+). For **29**: mp >240 °C (d); δ_H (DMSO-d_6) 11.71 (bs, 1H), 7.87 (s, 1H), 6.62 (bs, 2H), 5.96 (dd, 1H, $J=2.7$, 4.1 Hz), 5.42 (t, 1H, $J=3.3$ Hz), 4.98 (t, 1H, $J=6.0$ Hz), 4.23 (dd, 1H, $J=4.3$, 9.9 Hz), 4.08 (dd, 1H, $J=1.3$, 10.0 Hz), 3.46 (dd, 2H, $J=3.4$, 5.9 Hz); LRMS (FAB) m/z 270 (MH^+). For **41**: mp 162–164 °C; δ_H (DMSO-d_6) 8.65 (s, 1H), 8.56 (s, 1H), 6.20 (dd, 1H, $J=2.8$, 4.4 Hz), 5.60 (t, 1H, $J=6.0$ Hz), 5.45 (t, 1H, $J=4.9$ Hz), 3.84 (m, 1H), 3.70 (m, 1H), 3.57 (m, 2H); δ_C (DMSO-d_6) 160.89, 152.66, 147.90, 140.78, 112.21, 106.61, 89.43; HRMS (FAB) M^+ calcd for $C_{10}H_{12}N_4O_4S$ 285.065752, found 285.068300. For **42**: mp 178 °C, δ_H (DMSO-d_6) 8.66 (s, 1H), 8.56 (s, 1H), 6.30 (dd, 1H, $J=2.9$, 4.2 Hz), 5.86 (t, 1H), 5.17 (t, 1H, $J=5.0$ Hz), 3.55 (m, 1H), 3.48 (m, 1H), 3.47 (m, 2H); δ_C (DMSO-d_6) 160.86, 152.74, 148.29, 140.69, 117.12, 111.13, 87.09, 64.07, 54.54, 36.16; HRMS (FAB) M^+ calcd for $C_{10}H_{12}N_4O_4S$ 285.065752, found 285.068600. For **45**: mp 238 °C (d); δ_H (DMSO-d_6) 12.21 (s, 1H), 8.36 (s, 1H), 8.09 (s, 1H), 6.12 (dd, 1H, $J=2.8$, 4.1 Hz), 5.52 (t, 1H, $J=5.8$ Hz), 5.46 (t, 1H, $J=4.9$ Hz), 3.88 (m, 1H), 3.68 (m, 1H), 3.53 (m, 2H); δ_C (DMSO-d_6) 156.87, 146.99, 144.61, 137.30, 120.25, 112.28, 89.26, 65.22, 35.98; HRMS (FAB) M^+ calcd for $C_9H_{10}N_4O_4S$ 271.050102, found 271.052970. For **46**: mp 147 °C; δ_H (DMSO-d_6) 12.22 (b, 1H), 8.35 (s, 1H), 8.08 (s, 1H), 6.26 (dd, 1H, $J=2.8$, 4.3 Hz), 5.83 (t, 1H, $J=5.2$ Hz), 5.17 (t, 1H, $J=5.0$ Hz), 3.64 (m, 1H), 3.56 (m, 1H), 3.45 (m, 2H); HRMS (FAB) M^+ calcd for $C_9H_{10}N_4O_4S$ 271.050102, found 271.052970.