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Synthesis and Biological Evaluation of L- and D-Configurations of 2′,3′-Dideoxy-4′-C-methyl-3′-oxacytidine Analogues

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Abstract—Novel L- and D-configuration 2′,3′-dideoxy-4′-C-methyl-3′-oxacytidine and their 5-fluoro analogues have been synthesized from 1-benzyloxy-2-propanone and L-ascorbic acid in eight steps and evaluated for biological activity.

In the search for novel nucleosides as anticancer and antiviral agents, modifications of the sugar moiety have led to the discovery of 1,3-dioxane and 1,3-dioxathiolane nucleoside, in which the 3′-carbon has been replaced by an oxygen or a sulfur atom, respectively.[1–6] Among these sugar-modified nucleosides, (−)-L-β-2′,3′-dideoxy-3′-thiacytidine (3TC, Lamivudine) is being clinically used as an anti-AIDS and anti-hepatitis B virus (HBV) drug and L-2′,3′-dideoxy-3′-oxacytidine (**1**) showed significant activity against solid and lymphoid tumors both in vitro and in vivo,[6] and also exhibited potent anti-HIV and anti-HBV activity.[5] Recently, some 4′-substituted nucleosides have also been reported to show anticancer and antiviral activities.[7–12] Among these compounds, 2′-deoxy-4′-C-methylcytidine (**2**) showed significant activity against murine L1210 leukemia cells (IC_{50}, 0.16 μM)[10] and human T-cells, CCRF-HSB-2 cells (IC_{50}, 0.12 μg/mL),[11] and also exhibited potent antiviral activity against HIV-1 in MT-4 cells (IC_{50}, 0.072 μM).[12]

Based on these findings, we designed and synthesized the L- and D-configurations of 2′,3′-dideoxy-4′-C-methyl-3′-oxacytidine (**3**, **4**), in which the C–OH of the 3′-position in compound **2** was replaced with a bioisosteric oxygen atom, thereby combining the structural features of compounds **1** and **2**. Since the replacement of the hydrogen in the 5-position with a fluoro atom in cytidine analogues might increase biological activity,[13,14] the 5-fluoro derivatives **5** and **6** were also synthesized. Herein, we report the synthesis and biological evaluation of the L- and D-configurations of 2′,3′-dideoxy-4′-C-methyl-3′-oxacytidine **3** and **4**, and their 5-fluoro derivatives **5** and **6** (Fig. 1).

The key intermediates (2*S*,4*RS*)-4-acetoxy-2-[(benzyloxy)methyl]-2-methyldioxolane (**7**) and (2*R*,4*RS*)-4-acetoxy-2-[(benzyloxy)methyl]-2-methyldioxolane (**8**) were synthesized as described in Scheme 1. Condensation[15] of 1-benzyloxy-2-propanone (**9**)[16] with L-ascorbic acid (**10**) in acetonitrile in the presence of *p*-toluenesulfonic acid afforded a mixture of diastereomers of the dioxolane derivatives **11** and **12** as a white solid in moderate yield (55%). By ^{1}H NMR spectroscopy, the ratio of **11** to **12** was about 1:1; however, the mixture could not be readily separated into single isomers at this stage. The mixture of **11** and **12** gave only one spot on a silica gel plate in various solvent systems and could not

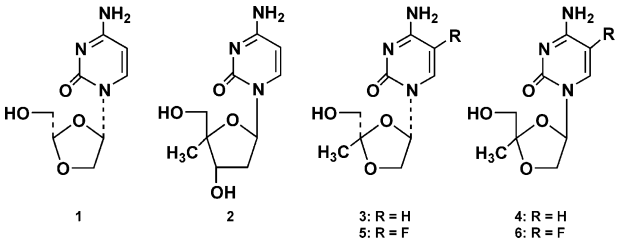

Figure 1.

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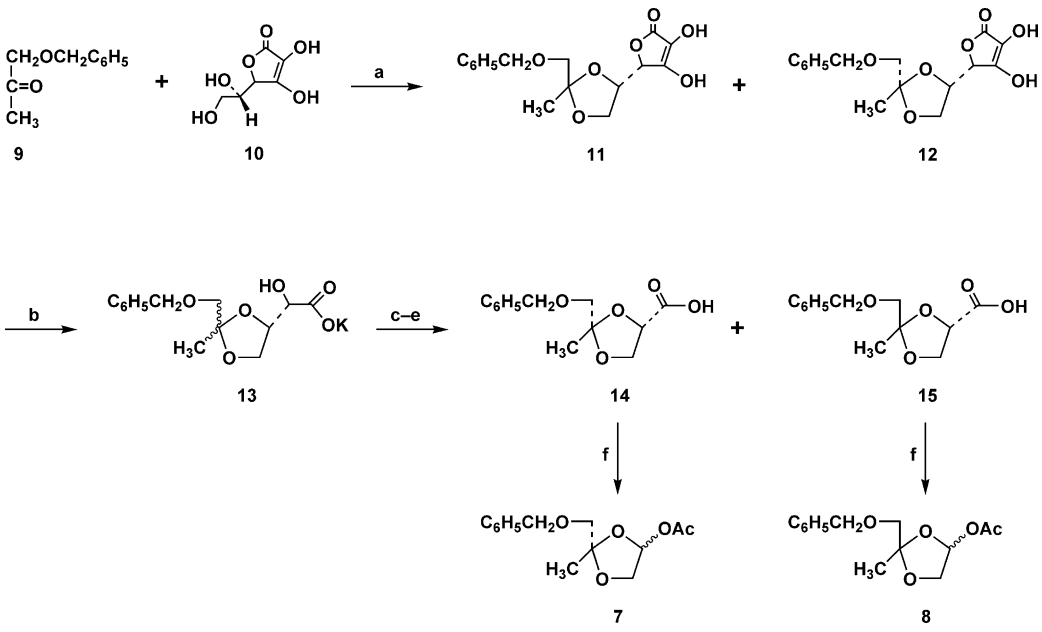


Scheme 1. Reagents and conditions: (a) TsOH, acetonitrile; (b) 30% H_2O_2, K_2CO_3, H_2O, EtOH; (c) NaOCl, $RuCl_3$ hydrate, benzyltriethylammonium chloride, H_2O/dichloroethane/acetonitrile, pH 8; (d) HCl, dichloromethane; (e) flash chromatography; (f) $Pb(OAc)_4$, pyridine, acetonitrile.

be separated by crystallization using different solvents. Oxidative degradation of the lactone ring of the diastereomers with 30% hydrogen peroxide and potassium carbonate in ethanol, followed by further oxidation of the resulting potassium salt (**13**) with sodium hypochlorite, catalyzed by ruthenium trichloride and benzyltriethylammonium chloride under controlled pH conditions[15,17] in a mixture of dichloroethane, acetonitrile and water, furnished the two dioxolane carboxylic acid isomers **14** and **15**. The two isomers were isolatable by silica gel column chromatography (CH_2Cl_2/EtOH, 30:1),[15,18] since the less polar acid **14** moved faster than **15**. Conversion of the carboxyl group to the acetoxy group was achieved by oxidative decarboxylation[19] of **14** and **15** with lead tetraacetate in acetonitrile in the presence of pyridine to give the respective key intermediates **7** and **8**.[20]

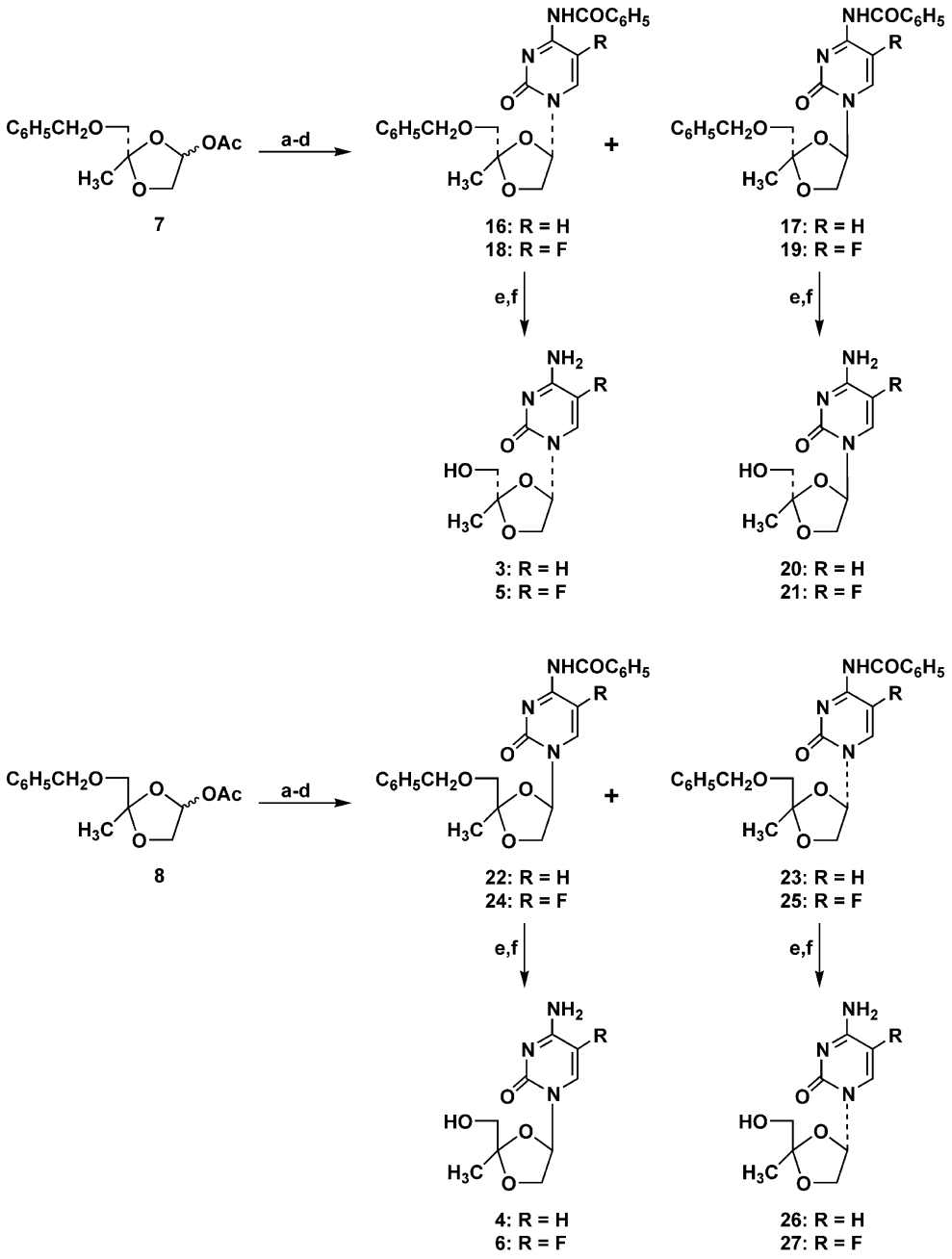
Coupling of the acetate **7** (Scheme 2) with silylated *N*-benzoylcytosine or *N*-benzoyl-5-fluorocytosine in the presence of the Lewis acid, trimethylsilyl trifluoromethanesulfonate in dichloroethane afforded the respective mixture of α- and β-nucleosides **16** and **17**, and **18** and **19**, which were separated by silica gel column chromatography (CH_2Cl_2/EtOAc/EtOH, 10:10:0.5). Deprotection of the nucleoside derivatives separately with NH_3 in methanol, followed by palladium oxide and cyclohexene in refluxing ethanol furnished the compounds with L-configuration **3** and **5**, and the corresponding α-isomers **20** and **21**.[21] Similarly, coupling of the acetate **8** with silylated *N*-benzoylcytosine or *N*-benzoyl-5-fluorocytosine, followed by separation and deprotection afforded the corresponding compounds with *R*-configuration **4** and **6**, and the α-isomers **26** and **27**.[21]

The assignment of the L- and D-configurations of the sugar and the nucleosides were determined by comparison of their physical and optical properties with those of similar 1,3-dioxolane analogues reported in the literature.[15,19,22] The assignment of the anomeric configurations of these nucleosides was made on the basis of the characteristics of the proton NMR spectra. The 4′-CH_3 protons of the α-anomers appeared at a lower field than those of the β-anomers. Conversely, the 5′-H protons of the α-anomers appeared at a higher field than those of the β-anomers (Table 1). These shifts were attributed to the fact that protons at the *syn*-position relative to the base are more deshielded than those in an *anti*-position to the base. The 4′-CH_3 protons of the α-anomers and the bases are on the same side of the sugar ring and those of the β-anomers are on the opposite side. In contrast, the 5′-H protons of the α-anomers and the bases are on the opposite side of the sugar ring, whereas those of the β-anomers are on the same side. The findings are similar to reports of others with pyrimidine nucleosides, that the 4′-H protons of the α-anomers appeared at a lower field than those of the β-anomers and the 5′-H protons of the α-anomers appeared at a higher field than those of the β-anomers.[19,22,23]

Table 1. Proton NMR chemical shifts δ (ppm)

Compd	4′-CH_3[a]	Δ δ	5′-H[a]	Δ δ
3 (β)	1.27 (*anti*)	0.14	3.49 (*syn*)	0.10
20 (α)	1.41 (*syn*)		3.39 (*anti*)	
5 (β)	1.24 (*anti*)	0.18	3.53 (*syn*)	0.13
21 (α)	1.42 (*syn*)		3.40 (*anti*)	

[a]Stereochemistry relative to the base.



Scheme 2. Reagents and conditions: (a) cytosine or 5-fluorocytosine; (b) HMDS, reflux 6 h; (c) TMSOTf, dichloroethane; (d) flash chromatography; (e) NH_3, CH_3OH; (f) PdO hydrate, cyclohexene, EtOH.

The synthesized compounds **3**–**6** and **20**, **21**, **26**, and **27** were evaluated in vitro for their cytotoxicities against the L1210 and P388 leukemias, the CCRF-CEM lymphoblastic leukemia, and the $B_{16}F_{10}$ melanoma cell lines and the results are shown in Table 2. The L-configuration analogue (**3**) produced IC_{50} values of 45, 5, 30 and 100 μM and its D-isomer (**4**) produced IC_{50} values of 50, 10, 30 and 100 μM against L1210, P388, CCRF-CEM and $B_{16}F_{10}$ cells, respectively. The corresponding α-isomers **20** and **26** and 5-fluorocytosine analogues **5** and **6** and **21** and **27** showed weak or no activity up to 100 μM against these neoplastic cell lines.

Antiviral assays were performed against herpes simplex Type-1 virus (KOS strain), herpes simplex Type-2

Table 2. Evaluation of L- and D-configurations of 2′,3′-dideoxy-4′-C-methyl-3′-oxacytidine analogues against L1210, P388, CCRF-CEM and $B_{16}F_{10}$ neoplastic cell lines in vitro

	IC_{50} (μM)[a]			
Compd	L1210	P388	CCRF-CEM	$B_{16}F_{10}$
3	45	5	30	100
4	50	10	30	100
20	>100	>100	>100	100
26	>100	>100	>100	>100
5	>100	50	>100	>100
6	>100	>100	>100	>100
21	>100	>100	>100	>100
27	>100	>100	>100	>100

[a]IC_{50} values represent the drug concentration (μM) required to inhibit cancer cell replication by 50%. The compounds were tested up to a concentration of 100 μM.

virus (333 strain), hepatitis B virus, and human immunodeficiency virus (HIV-IIIB) in vitro. None of the compounds showed toxicity or activity against these respective virus strains up to the maximum tested concentrations of 50, 10, 100 μM.

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18. Compound **14**: oil $[\alpha]_D^{22}$ +4.5° (*c* 0.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 3H, CH_3), 3.60 (s, 2H, CH_2), 4.20 (dd, 1H, J=8.0 Hz, 2.4 Hz, 5-H_A), 4.30 (t, 1H, J=5.7 Hz, 5-H_B), 4.55 (t, 1H, J=4.5 Hz, 4-H), 4.61 (s, 2H, $ArCH_2$), 7.22–7.30 (m, 5H, ArH), 9.30 (bs, 1H, COOH, D_2O exchangeable). Compound **15**: oil $[\alpha]_D^{22}$ −15.7° (*c* 0.1, MeOH; ^{1}H NMR (DMSO-d_6) δ 1.48 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 4.15 (dd, 1H, J=8.1, 2.7 Hz, 5-H_A), 4.32 (t, 1H, J=5.4 Hz, 5-H_B), 4.55 (s, 2H, $ArCH_2$), 4.60 (t, 1H, J=3.6 Hz, 4-H), 7.20–7.32 (m, 5H, ArH), 8.20 (bs, 1H, COOH, D_2O exchangeable).
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20. Compound **7**: oil, ^{1}H NMR ($CDCl_3$) δ 1.48, 1.55 (s, 3H, CH_3), 2.00, 2.10 (s, 3H, CH_3), 3.45, 3.55 (s, 2H, CH_2), 4.05–4.20 (m, 2H, 5-H), 4.64, 4.67 (s, 2H, $ArCH_2$), 6.40–6.47 (m, 1H, 4-H), 7.20–7.32 (m, 5H, ArH). Compound **8**: oil, ^{1}H NMR ($CDCl_3$) δ 1.46, 1.53 (s, 3H, CH_3), 2.00, 2.10 (s, 3H, CH_3), 3.43, 3.53 (s, 2H, CH_2), 4.07–4.22 (m, 2H, 5-H), 4.62, 4.65 (s, 2H, $ArCH_2$), 4.41–6.47 (m, 1H, 4-H), 7.20–7.32 (m, 5H, ArH).
21. Compound **3**: mp 190–192 °C; $[\alpha]_D^{22}$ −17.6° (*c* 0.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 3H, 4′-CH_3), 3.49 (d, 2H, J=6.0 Hz, 5′-H), 3.96 (dd, 1H, J=3.0, 6.6 Hz, 2′-H_A), 4.32 (dd, 1H, J=3.9, 5.7 Hz, 2′-H_B), 5.19 (t, 1H, 5′-OH, D_2O exchangeable), 5.71 (d, 1H, J=7.5 Hz, 5-H), 6.16 (dd, 1H, J=3.3, 6.2 Hz, 1′-H), 7.20, 7.50 (s, 2H, NH_2, D_2O exchangeable), 7.87 (d, 1H, J=7.5 Hz, 6-H). Compound **20**: mp 212–214 °C; $[\alpha]_D^{22}$ +10.2° (*c* 0.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 3H, 4′-CH_3), 3.35 (d, 2H, J=6.0 Hz, 5′-H), 3.95 (dd, 1H, J=2.4, 6.2 Hz, 2′-H_A), 4.37 (dd, 1H, J=3.6, 8.0 Hz, 2′-H_B), 5.06 (t, 1H, 5′-OH, D_2O exchangeable), 5.78 (d, 1H, J=7.4 Hz, 5-H), 6.16 (dd, 1H, J=1.8, 3.2 Hz, 1′-H), 7.05, 7.15 (s, 2H, NH_2, D_2O exchangeable), 7.54 (d, 1H, J=7.5 Hz, 6-H). Compound **5**: mp 192–194 °C; $[\alpha]_D^{22}$ −20.2° (*c* 0.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.24 (s, 3H, 4′-CH_3), 3.52 (d, 2H, 5′-H, J=6.0 Hz), 4.02 (dd, 1H, J=2.7, 9.6 Hz, 2′-H_A), 4.34 (dd, 1H, J=5.7, 9.6 Hz, 2′-H_B), 5.35 (t, 1H, 5′-OH, D_2O exchangeable), 6.14 (dd, 1H, J=2.7, 5.7 Hz, 1′-H), 7.55, 7.80 (s, 2H, NH_2, D_2O exchangeable), 8.21 (d, 1H, J=7.2 Hz, 6-H). Compound **21**: mp 198–200 °C; $[\alpha]_D^{22}$ +10.2° (*c* 0.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 3H, 4′-CH_3), 3.40 (d, 2H, J=6.0 Hz, 5′-H), 4.03 (dd, 1H, J=2.1, 6.2 Hz, 2′-H_A), 4.34 (dd, 1H, J=3.2, 7.6 Hz, 2′-H_B), 5.08 (t, 1H, 5′-OH, D_2O exchangeable), 6.19 (dd, 1H, J=2.1, 4.7 Hz, 1′-H), 7.61, 7.82 (s, 2H, NH_2, D_2O exchangeable), 7.61 (d, 1H, J=6.9 Hz, 6-H). Compound **4**: mp 191–192 °C; $[\alpha]_D^{22}$ +18.4° (*c* 0.1, MeOH). Compound **26**: mp 210–212 °C; $[\alpha]_D^{22}$ −12.4° (*c* 0.1, MeOH). Compound **6**: mp 194–196 °C; $[\alpha]_D^{22}$ +17.2° (*c* 0.1, MeOH). Compound **27**: mp 198–200 °C; $[\alpha]_D^{22}$ −13.2° (*c* 0.1, MeOH).
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