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Synthesis of 5′-*C*-methyl-1′,3′-dioxolan-4′-yl nucleosides

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Abstract—Novel racemic 5′-*C*-methyl-1′,3′-dioxolan-4′-yl nucleosides were synthesized from the key intermediate, 2-benzoyloxymethyl-4-oxo-5-*C*-methyl-1,3-dioxolane, which was prepared from racemic lactic acid.

Hepatitis C virus (HCV) has infected an estimated 170 million people worldwide.[1] More than 50% of patients with acute HCV infection will progress to chronic hepatitis. Currently the only FDA approved treatments for HCV infection are interferon-α mono-therapy and combination of interferon-α with ribavirin. Therefore, there is an urgent need to develop new and more effective therapies for the treatment of HCV infections.

Since Norbeck first reported a synthesis of (±)-*cis*-1-[2′-(hydroxymethyl)-1′,3′-dioxolan-4′-yl]thymine with moderate anti-HIV activity in ATH8 cells in 1989,[2] many dioxolane nucleosides have been synthesized as potent antiviral agents. Among them, (−)-(2′*R*,4′*R*)-2,6-diamino-9-[2′-(hydroxymethyl)-1′,3′-dioxolan-4′yl]purine (DAPD),[3] (−)-(2′*S*,4′*R*)-1-[2′-(hydroxymethyl)-1′,3′-dioxolan-4′-yl]cytosine (L-OddC),[4] and (−)-(2′*S*,4′*R*)-1-[2′-(hydroxymethyl)-1′,3′-dioxolan-4′-yl]-5-iodouracil (L-IOddU)[5] are currently in pre-clinical or clinical studies to assess their value as antiviral or anticancer agents.

Recently, Carroll et al. described 2′-*C*-methyl *ribo*-nucleosides with potent activity against HCV.[6] Storer has reported that 1′,3′-dioxolan-4′-yl nucleoside triphosphates showed potent anti-HCV activity.[7] We herein report the synthesis of novel racemic 5′-*C*-methyl-1′,3′-dioxolan-4′-yl nucleosides as potential antiviral agents.

We prepared the key intermediates, *trans*-lactone **2**[8] and *cis*-lactone **3**,[9] by condensation of 2-benzoyloxyacetaldehyde diethyl acetal with racemic lactic acid in the presence of BF_3 etherate in 18% and 34% yields, respectively (Scheme 1).[10] Selective reduction of **3** with LiAl(*t*-BuO)$_3$H followed by in situ acetylation with Ac_2O in the presence of 4-dimethylaminopyridine (DMAP) gave the epimeric (anomeric) 4-acetoxydioxolanes (**4**). The target nucleosides **7**[11] and **8**[12] were prepared by coupling of **4** with silylated 5-fluorocytosine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) followed by deprotection with butylamine in methanol in good yields.[13]

The *trans*-lactone **2** was converted to the corresponding nucleosides in a similar fashion (Scheme 2). Interestingly, the *trans*-dioxolane **9** epimerized during the coupling reaction to give an inseparable mixture of 5′-*C*-Me (up), 5′-*C*-Me (down), as well as a mixture of anomers. This was supported by the ^{1}H NMR of the mixture which contained 4 sets of doublets for 6-H [δ 8.48 (J = 7.2 Hz), 8.20 (J = 7.2 Hz), 7.95 (J = 6.8 Hz) and 7.60 (J = 6.8 Hz)].

A possible mechanism for this epimerization is illustrated in Scheme 3. Treatment of **2** with Lewis acid would give the intermediate oxonium ion **i**. Ring opening of **i** would produce the proposed intermediate **ii** which, after nonselective ring closure, would give a mixture of **iii** and **i** (*cis*- and *trans*-isomers). Attack of the base on both the alpha and beta face of intermediates **iii** and **i** would then give the observed mixture of 4 diastereomers. Epimerization is not observed with the *cis*-isomer **4**, possibly because treatment with Lewis acid would directly result in the more stable intermediate **iii** (both 2 and 5 substituents are in quasi-equatorial), compared to intermediate **i** (at least one of

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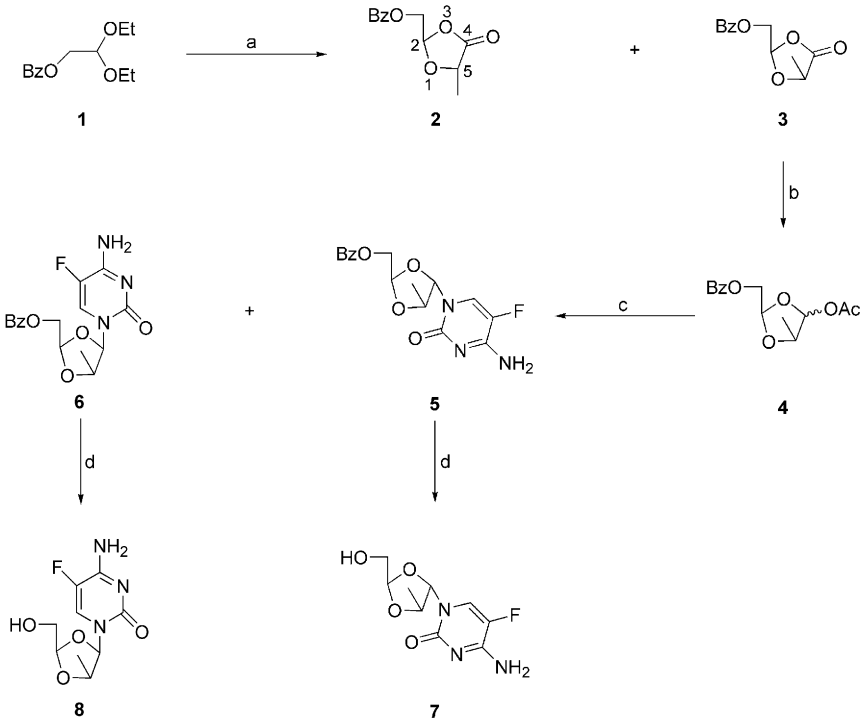
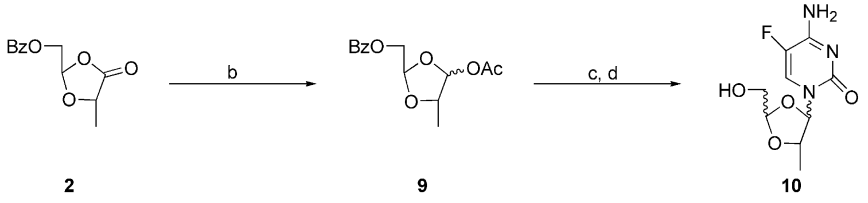
the substituents is in quasi-axial). Reaction of this more stable intermediate with base before ring opening would prevent epimerization.

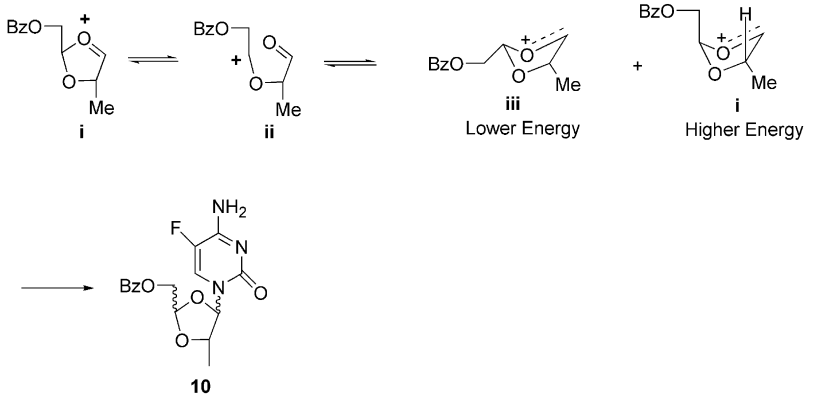
Structures of the synthesized nucleosides were confirmed by ^{1}H NMR and high resolution MS. Relative stereochemical assignments were determined based on NOE difference spectra, where transfer of magnetization between 2-H and 5-*C*-CH_3 of the *trans*-isomer **2** was observed. Additionally, a correlation between 2-H and 5-H of *cis*-isomer **3** was observed. In NOE difference spectra for the final nucleosides, transfer of magnetization between 5′-H and 2′-H as well as 5′-H and 6-H of α-**7** were found. For the β-nucleoside **8**, NOE enhancements between 2′-H and 4′-H as well as 2′-H and 5′-H were observed (Fig. 1).



Scheme 1. Reagents and conditions: (a) (±)-lactic acid, $BF_3{\times}Et_2O$, CH_3CN, rt; (b) (1) $LiAl(t\text{-}BuO)_3H$, THF, −20 °C; (2) Ac_2O, DMAP, −20 °C; (c) (1) 5-F-cytosine, HMDS, $(NH_4)_2SO_4$, reflux; (2) TMSOTf, CH_2Cl_2, rt; (d) *n*-$BuNH_2$, MeOH, rt.



Scheme 2.



Scheme 3.

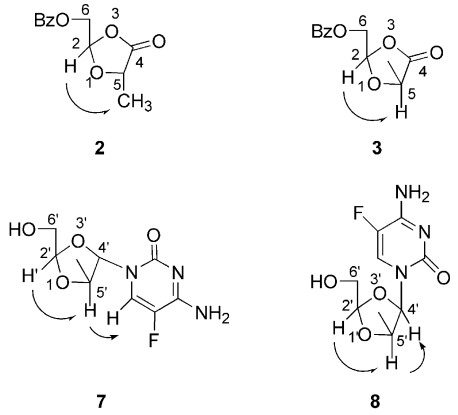


Figure 1.

In preliminary biological evaluation, these nucleosides were found to have weak activity against HIV-1 in primary human lymphocytes and were eventually inactive against HBV[14] and HCV.[15] None of the compounds were toxic in human lymphocytes, HepG2 or Huh7 cells up to 100 μM.

In conclusion, we have developed a synthetic method for the 5′-*C*-methyl(up)-1′,3′-dioxolan-4′-yl nucleosides from the key intermediate **3** prepared by condensation of 2-benzoyloxyacetaldehyde with racemic lactic acid.

Acknowledgements

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8. Compound **2**: Solid, mp 47.0–48.5 °C. ^{1}H NMR ($CDCl_3$) δ 8.06–7.44 (m, 5H, Bz), 5.96 (m, 1H, 2-H), 4.54 (m, 3H, CH_2, 5-H), 1,51, 1.49 (d, $J=7.2$ Hz, 3H, 5-CH_3). Anal. calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C, 60.99; H, 5.15. HRMS (FAB) obsd, *m*/*z* 237.0769, calcd for $C_{12}H_{14}O_5$, *m*/*z* 237.0763 $(M+H)^+$.
9. Compound **3**: Solid, mp 50.5–52 °C. ^{1}H NMR ($CDCl_3$) δ 8.02–7.44 (m, 5H, Bz), 5.82 (m, 1H, 2-H), 4.57 (d, $J=2.8$ Hz, 2H, CH_2), 4.56 (m, 1H, 5-H), 1.51 (d, $J=6.4$ Hz, 3H, 5-CH_3). Anal. calcd for $C_{12}H_{12}O_5$: C, 61.00; H, 5.10. Found: C, 60.99; H, 5.15. HRMS (FAB) obsd, *m*/*z* 237.0769, calcd for $C_{12}H_{14}O_5$, *m*/*z* 237.0763 $(M+H)^+$.
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11. Compound **7**: Solid, mp 123–125 °C. ^{1}H NMR (DMSO-d_6) δ 7.97 (d, $J=6.8$ Hz, 1H, 6-H), 7.87, 7.61, (ss, 2H, NH_2, D_2O exchangeable), 5.59 (dd, $J=1.2$, 5.2 Hz, 1H, 4′-H), 5.37 (t, $J=3.6$ Hz, 1H, 2′-H), 5.01 (t, $J=6.0$ Hz, 1H, OH, D_2O exchangeable), 4.14 (m, 1H, 5′-H), 3.46 (dd, $J=4.0$, 6.0 Hz, 2H, 6′-CH_2), 1.31 (d, $J=6.4$ Hz, 3H, 2-CH_3). Anal. calcd for $C_9H_{12}N_3O_4F+1/4H_2O$: C, 43.28; H, 5.05; N, 16.83. Found: C, 43.44; H, 5.07; N, 16.57. HRMS (FAB) obsd, *m*/*z* 246.0896, calcd for $C_9H_{13}FN_3O_4$, *m*/*z* 246.0890 $(M+H)^+$.
12. Compound **8**: Solid, mp 143–145 °C. ^{1}H NMR (DMSO-d_6) δ 8.40 (d, $J=7.6$ Hz, 1H, 6-H), 7.80, 7.57, (ss, 2H, NH_2, D_2O exchangeable), 6.16 (d, $J=4.4$ Hz, 1H, 4′-H), 5.50 (t, $J=5.6$ Hz, 1H, OH, D_2O exchangeable), 4.90 (s, 1H, 2′-H), 4.32 (m, 1H, 5′-H), 3.73 (m, 2H, 6′-CH_2), 1.03 (d, $J=6$ Hz, 3H, 2-CH_3). Anal. calcd for $C_9H_{12}N_3O_4F+1/4H_2O$: C, 43.28; H, 5.05; N, 16.83. Found: C, 43.39; H, 5.06; N, 16.47. HRMS (FAB) obsd, *m*/*z* 246.0893, calcd for $C_9H_{13}FN_3O_4$, *m*/*z* 246.0890 $(M+H)^+$.
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