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1,3-Dihydrobenzo[*c*]furan Nucleoside Analogues: Additional Studies of the Thymine Derivative

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Abstract—The detailed study of the 1,3-dihydrobenzo[*c*]furan derivative of thymine is reported. The lack of anti-HIV activity of this compound in cell culture experiments is shown to be related to the inability of the corresponding 5′-triphosphate derivative to interact efficiently with the reverse transcriptase.

D4T (2′,3′-didehydro-2′,3′-dideoxythymidine, Fig. 1) is used in the treatment of human immunodeficiency virus (HIV) infection.[1,2] Its mechanism of action is similar to that of other nucleoside HIV-reverse transcriptase (RT) inhibitors, and requires intracellular metabolization into the corresponding 5′-triphosphate derivative, which acts as a competitive inhibitor for the RT reaction and incorporation into viral DNA. In contrast to other pyrimidine nucleoside analogues, the initial phosphorylation of d4T, catalyzed by thymidine kinase, is the rate-limiting step in this metabolic process. Moreover, the introduction of the 2′,3′-double bond results in increasing the lability of the glycosidic bond and decreasing the lipophilicity compared to the corresponding saturated 2′,3′-dideoxynucleoside series. Thus, d4T penetrates into the central nervous system, an important site of HIV replication, to a limited extent.[3]

Following several chemical strategies, we have previously reported the synthesis of 1,3-dihydrobenzo[*c*]furan nucleoside analogues.[4–6] In this new series, the fusion of the benzene ring to the 2′,3′-positions of the sugar residue might preserve the biological activity related to restricted nucleoside analogues such as 2′,3′-didehydro-2′,3′-dideoxy-nucleoside derivatives, and also increase the chemical stability and lipophilicity of the resulting compound. Herein, the synthesis, physicochemical parameters and in vitro anti-HIV evaluation of the 1,3-dihydrobenzo[*c*]furan derivative of thymine **1** (Fig. 1) are reported. Additional studies have been carried out in order to explain the lack of anti-HIV activity of this compound in cell culture experiments.

Initially investigated in the case of uracil analogues,[4,6] the most efficient synthesis of the 1,3-dihydrobenzo[*c*]furan nucleoside **1** employed the Sharpless asymmetric dihydroxylation methodology (Scheme 1). Briefly, selective protection of one aldehyde function of the *o*-phthalaldehyde **2** followed by homologation using Wittig chemistry gave the styrene derivative **4**. Asymmetric dihydroxylation of **4** with AD-mix α afforded the diol **5**. Esterification of the primary hydroxyl group of **5** followed by deprotection of the aldehyde functionality afforded the cyclic acetal **7** (α and β forms). This mixture was then condensed with silylated thymine, and deprotection of the resulting intermediates **8** and **9** gave rise to the target nucleoside **1** and its α anomer **10** which were separated by silica gel column chromatography.

The chemical stability of compound **1** was studied in comparison to d4T (Table 1). In the three studied buffers, hydrolysis of **1** and d4T gave rise to the formation of thymine but the disappearance of the nucleoside analogue **1** was significantly slower than that of d4T.

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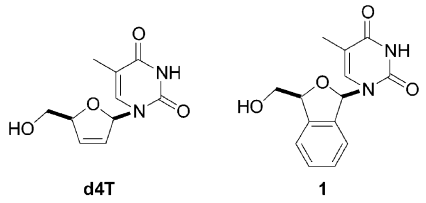
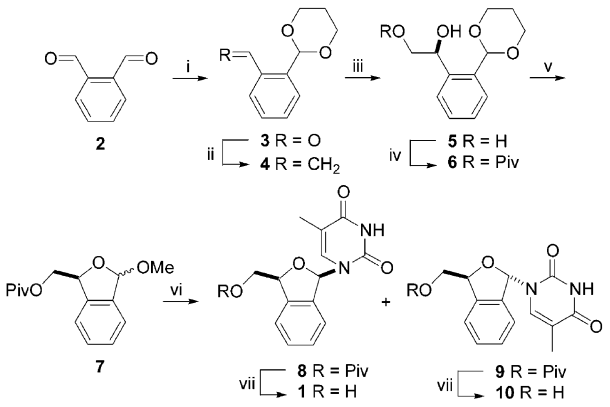


Figure 1.

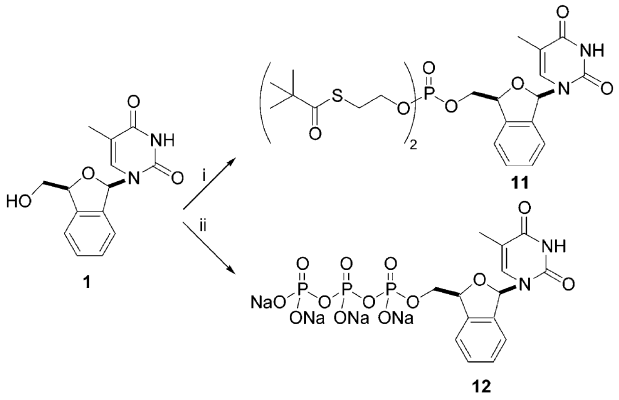


Scheme 1. Reagents and conditions: (i) propane-1,3-diol, PTSA, toluene; (ii) $(C_6H_5)_3CH_3PBr$, *t*BuOK, toluene; (iii) AD-mix α, *t*BuOH, H_2O; (iv) PivCl, $(C_2H_5)_3N$, toluene; (v) HCl, MeOH; (vi) silylated thymine, $SnCl_4$, $C_2H_4Cl_2$; (vii) NaOH, dioxane, H_2O.

Thus, the presence of the benzene ring as electron-withdrawing group stabilizes the glycosidic bond compared to the olefinic analogue. Moreover, introduction of the aromatic residue in the 2′,3′-positions of d4T increases the liphophilicity of the resulting structure to a positive logarithmic value of the partition coefficient (Table 1).

The 1,3-dihydrobenzo[*c*]furan derivative **1** was evaluated for its inhibitory effect on the replication of HIV-1 in human T4-lymphoblastoid cells, CEM-SS and MT-4. This compound has been found inactive against HIV-1 replication at concentrations up to 10 μM. In order to explain this lack of antiviral activity, the corresponding mononucleoside phosphotriester **11**[7] (Scheme 2) bearing two *S*-pivaloyl-2-thioethyl (*t*BuSATE) groups has been synthesized according to a published procedure.[8] We have previously demonstrated that in vitro a dependence on kinase-mediated phosphorylation could be overcome by the use of such kind of mononucleotide prodrugs (pronucleotides). Thus, applied to d4T such pronucleotide approach led to a 10-fold increase of the anti-HIV activity of the parent nucleoside in cell culture experiments.[9] Unfortunately, the bis(*t*BuSATE) phosphotiester **11** did not display in vitro anti-HIV activity.

Consequently, the absence of antiviral activity of the 1,3-dihydrobenzo[*c*]furan derivative **1** could be due to inadequate second and/or third phosphorylation steps, or to inefficient inhibition of RT by its 5′-triphosphate form. In order to discriminate between these hypotheses, the 5′-triphosphate derivative **12**[11] was obtained using the Ludwig–Eckstein methodology (Scheme 2).[10] In an in vitro assay using poly(rA).oligo$(dT)_{12\text{-}18}$ as template primer and [^{3}H]-TTP as substrate, compound



Scheme 2. Reagents and conditions: (i) bis(*S*-pivaloyl-2-thioethyl) *N*,*N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_3CN then *t*BuOOH, toluene; (ii) 2-chloro-4*H*-1,2,3-dioxaphosphorin-4-one, pyridine, dioxane then bis(tri-*n*-butylammonium) pyrophosphate, DMF then classical work-up.[10]

Table 1. Calculated half-lives (day) in three aqueous buffers[a] and partition coefficients (log *P*)[b] of compound **1** in comparison to d4T

Compd	pH 7.3	pH 2.0	pH 1.2	log *P*
1	34	31	18	0.55±0.42
d4T	6	5	3	−0.77±0.18

[a]Concentration studied: 20 mM in Tris–HCl (pH 7.3), glycine-HCl (pH 2.0) and KCl-HCl (pH 1.2).
[b]Log *P* determination was performed using log *P* dB 4.5 calculations (ACD, Toronto, Canada).

12 was found to be inactive against wild HIV-1 RT up to 100 μM, while d4T-TP, used as a positive control, displayed a IC_{90} value of 0.1 μM. One explanation for this result may be related to the steric effect of the benzene ring which does not allow the access of the triphosphate derivative to the enzyme nucleotide binding site.

In conclusion, despite better chemical stability and lipophilicity of the benzo[*c*]furan derivative **1** compared to d4T, the lack of activity of **1** cannot be overcome by using a bis(SATE) pronucleotide approach. Furthermore, the inability of the triphosphate **12** to inhibit HIV-1 RT restricts the utility of such 1,3-dihydrobenzo[*c*]furan nucleosides as potential anti-HIV agents.

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phosphate (**11**): 60% yield; ^{31}P NMR (121 MHz, DMSO-d_6) δ −0.66, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.4 (bs, 1H, Thy–NH), 7.53–7.32 (m, 4H, aromatic H), 7.30 (d, 1H, 1′-H, $J_{1'-3'}$ = 1.4 Hz), 6.90 (d, 1H, 6-H, J = 1.0 Hz), 5.44 (m, 1H, 3′-H), 4.53–4.39 (m, 2H, 8′-H and 8″-H), 3.97–3.89 (m, 4H, CH_2O), 3.07–2.94 (m, 4H, CH_2S), 1.67 (d, 3H, Thy–CH_3, J = 0.7 Hz), 1.16, 1.15 (2s, 18H, *t*Bu), ^{13}C NMR (75 MHz, DMSO-d_6) δ 205.5, 205.7 (2s, C=O), 163.7 (4-C), 151.0 (2-C), 138.4, 136.3 (2s, 4′-C and 5′-C), 135.7 (6-C), 129.7, 129.2, 122.7, 122.2 (4s, aromatic C), 110.2 (5-C), 86.4 (1′-C), 81.4 (d, 3′-C, J_{P-C} = 8.4 Hz), 68.8 (d, 8′-C, J_{P-C} = 5.7 Hz), 65.5, 65.4 (2d, CH_2O, J_{P-C} = 5.6 Hz), 46.0 (s, $(CH_3)_3C$), 28.0, 27.9 (2d, CH_2S, J_{P-C} = 8.2 Hz), 26.8 ($(CH_3)_3C$), 11.9 (Thy–CH_3), MS FAB > 0 *m*/*z* 1285 $(2M+H)^+$, 643 $(M+H)^+$, 145 $(t\text{BuCOSCH}_2\text{CH}_2)^+$, MS FAB < 0 *m*/*z* 641 $(M-H)^-$, 497 $(M-t\text{BuSATE})^-$, 385 $(M-Nu)^-$, 125 $(B)^-$, Anal calcd for $C_{28}H_{39}N_2O_9PS_2$: C 52.33, H 6.12, N 4.36. Found: C 51.83, H 6.07, N 4.30. HRMS calcd for $C_{28}H_{40}N_2O_9PS_2$ $(M+H)^+$ 643.1913, Found 643.1946, UV (ethanol 95): λ_{max} 263 nm, λ 232 nm, HPLC rt = 23.9 min (gradient from 0 to 80% acetonitrile in 20 mM triethylammonium acetate buffer (pH 6.6), programmed over a 30 min period with a flow rate of 1 mL/min).

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10. Ludwig, J.; Eckstein, F. *J. Org. Chem*. **1989**, *54*, 631.
11. Selected data for triphosphate **12**: 10% yield; ^{31}P NMR (121 MHz, D_2O) δ −22 (dd, P_{β},$J_{P\beta P\alpha}$ = 18.2 Hz, $J_{P\beta Pg}$ = 19.4 Hz), −11.1 (d, P_{α},$J_{P\beta P\alpha}$ = 18.2 Hz), −8.2 (d, $P_{\gamma?}$,$J_{P\beta Pg}$ = 19.4 Hz), ^{1}H NMR (300 MHz, D_2O) δ 7.57–7.28 (m, 5H, aromatic-H, 1′-H), 7.09 (s, 1H, 6-H), 5.56 (m, 1H, 3′-H), 4.40–4.24 (m, 2H, 8′-H and 8″-H), 1.73 (s, 3H, Thy–CH_3), ^{13}C NMR (75 MHz, D_2O) δ 166.7 (4-C), 152.6 (2-C), 138.8–134.6 (2s, 9′-C,10′-C), 138.0 (6-C), 130.4, 129.4, 122.8, 122.7 (4s, aromatic C), 112.1 (5-C), 87.7 (1′-C), 83.3 (d, 3′-C, J_{P-C} = 9.1 Hz), 67.4 (d, 8′-C, J_{P-C} = 6.0 Hz), 11.5 (Thy-CH_3), MS FAB < 0 *m*/*z* 579 $(M-Na)^-$, 557 $(M-2Na+H)^-$, 535 $(M-3Na+2H)^-$, 513 $(M-4Na+3H)^-$, HPLC rt = 9.1 min (see ref 8 for conditions).