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Design, synthesis, and anti-HIV activity of 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U), 2′,3′-dideoxyuridine (ddU) phosphoramidate 'ProTide' derivatives

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Abstract—We report the synthesis of 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) and 2′,3′-dideoxyuridine (ddU) phosphoramidate 'ProTide' derivatives and their evaluation against HIV-1 and HIV-2. In addition, we conducted molecular modeling studies on both d4U and ddU monophosphates to investigate their second phosphorylation process. The findings from the modeling studies provide compelling evidence for the lack of anti-HIV activity of d4U phosphoramidates, in contrast with the corresponding ddU phosphoramidates.

Nucleoside analogues lacking the 3′-hydroxyl group, for example, 2′,3′-dideoxy 2′,3′-didehydrothymidine (d4T) and 2′,3′-dideoxycytidine (ddC), have proved to be effective in treating the Acquired Immunodeficiency Syndrome (AIDS)[1] which is caused by Human Immunodeficiency Virus (HIV).[2] These agents produce their effects as HIV reverse transcriptase (HIV-RT) inhibitors and/or DNA chain terminators after being converted to their corresponding 5′-triphosphates.[3] However, the use of these drugs has been limited due to the emergence of resistance and inherent toxicity as well as dependence on kinase mediated nucleoside activation to generate the bioactive triphosphates. Bearing in mind the success of d4T as an anti-HIV agent and with the aim of finding alternative anti-HIV drugs with high potency and acceptable toxicity profiles, we decided to investigate the anti-HIV activity of 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) and 2′,3′-dideoxyuridine (ddU) (Fig. 1).

Both (d4U) and (ddU) are known to have poor anti-HIV activity.[4] For d4U, we hypothesized that this poor activity is due to its inefficient phosphorylation to its active triphosphate form as this was proved to be the case for ddU.[5] Thus, we decided to explore these possibilities further by applying the 'ProTide' approach to both d4U and ddU in order to bypass the first phosphorylation step and to estimate the effects that phosphoramidate prodrugs have on the antiviral activity. In addition, we carried out some molecular modeling investigations to study the possible second phosphorylation step that is required for the activation of these agents at their 5′-monophosphate level.

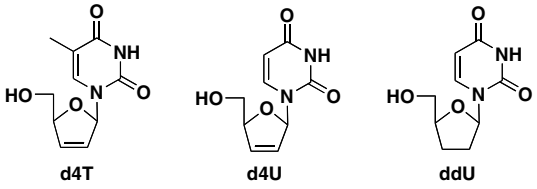

Figure 1. Structures of d4T, d4U, and ddU.

In the 'ProTide' approach, the phosphate group is masked to improve the poor membrane permeability seen when the free nucleotides are used, coupled with the inherent lability of free monophosphates to dephosphorylation. Upon entering the cell, the group masking the phosphate moiety may undergo enzymatic conversion to release the nucleoside analogue monophosphate, which may be subsequently phosphorylated to the di- and triphosphates of the nucleoside analogue.[6]

Keywords: HIV; Phosphoramidates; ddU; ProTide.
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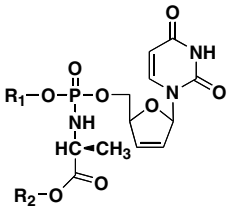
d4U was prepared via the same procedure adopted by McGuigan et al.[7] Thus, 2′-deoxyuridine was first 3′,5′-dimesylated and then reacted with aqueous sodium hydroxide to afford **2** in a 52% yield. Treatment of **2** with sodium hydride and DMF gave the 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) in a moderate yield, 43%. Hydrogenation of d4U using 10% palladium on carbon yielded ddU in a 69% yield (Scheme 1).

In our previous work on nucleoside analogue phosphoramidates, particularly d4T, we found that phenyl-L-alanine ester phosphoramidates were efficacious in enhancing the anti-HIV activity of d4T.[8] More recently Congiatu et al.[9] reported the superiority of naphthyl-L-alanine esters in improving the anticancer activity profile of BVDU compared to the phenyl analogues. Thus, we decided to make a small family of phenyl and naphthyl-alanine ester phosphoramidates of both d4U and ddU, and then examine their anti-HIV activity. The selection of d4U and ddU phosphoramidates synthesized for this study is summarized in Table 1.

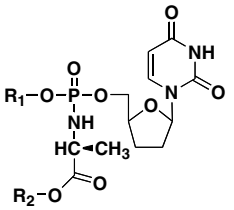
The 'ProTide' phosphoramidates were synthesized according to the previously reported synthetic routes.[10] Aryl phosphochloridates were prepared by the reaction of phenyl/naphthyl dichlorophosphates with the appropriate amino acid ester hydrochloride. The resulting phosphorochloridates were allowed to react with the nucleoside analogue in THF and *t*-BuMgCl to give the target phosphoramidates in moderate yields (Scheme 2).

The synthesized nucleosides and ProTides were tested against both HIV-1 and HIV-2 with data shown in Tables 2 and 3, and d4T (Stavudine) included as a positive control. The biological data for d4U derivatives revealed that neither of the parent nucleosides nor any of the phosphoramidate derivatives was found to be significantly active against HIV-1 or HIV-2 (Table 2), although the naphthyl benzyl compound (**1d**) showed slight activity. This highlighted the inability of the parent nucleoside to inhibit HIV which is in agreement with the observation made by Balzarini et al.[4] In addition, the biological data indicated the failure in general of the tested phosphoramidate derivatives to improve the anti-HIV activity of d4U. All the tested compounds, including d4U, were shown to exert some toxicity. This poor anti-HIV activity of d4U and its phosphoramidates could be attributed to three reasons. First, the inability of the d4U ProTides to efficiently deliver d4U monophosphate into cells. Second, the failure of d4U-monophosphate to be further phosphorylated to its triphosphate and/or third, the triphosphate of d4U being inactive as an inhibitor of HIV RT.

Table 1. The structures of potential anti-HIV ProTides



1a-d



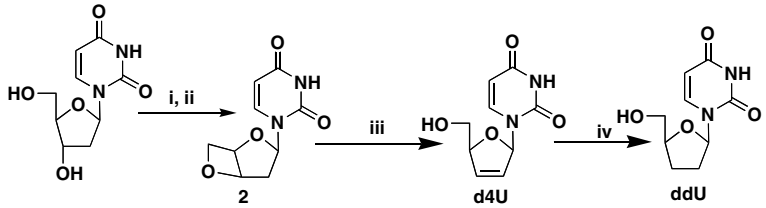
2a-d

Compound	R_1	R_2
1a	Phenyl	Me
1b	Phenyl	Et
1c	Phenyl	*t*-Bu
1d	1-Naphthyl	Bn
2a	Phenyl	Me
2b	Phenyl	Et
2c	Phenyl	*t*-Bu
2d	1-Naphthyl	Bn

By contrast, although the nucleoside analogue ddU was similarly found not to possess any anti-HIV activity, its phosphoramidates exerted moderate activities (Table 3). This indicated that using phosphoramidates to bypass the first phosphorylation step of ddU resulted in a boost of anti-HIV activity. This highlights the success of phosphoramidates as a tool for the intracellular delivery of nucleoside analogue monophosphates. Therefore, we concluded that the first phosphorylation step is the reason for the poor anti-HIV activity of ddU. As for d4U, none of the tested ddU phosphoramidates exerted significant toxicity (Table 3).

It is also of note that a significant SAR emerged for the ddU ProTides. The *t*-butyl ester **2c** was inactive, while the methyl and ethyl esters showed some activity. The naphthyl benzyl ester **2d** was most active of all.[9]

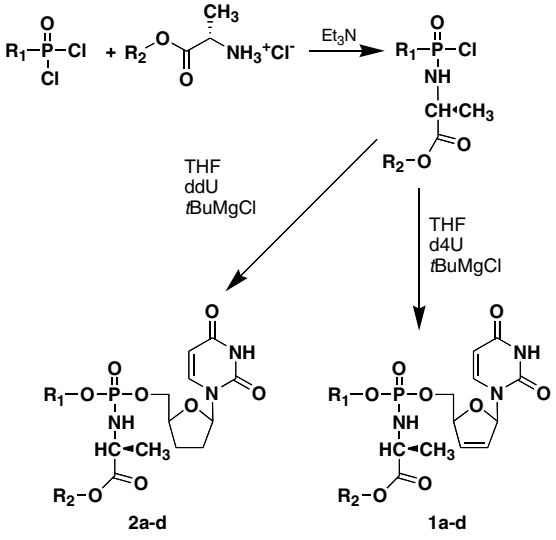
As a result of the differences in activities between d4U and ddU as well as the failure of d4U phosphoramidates to improve the anti-HIV activity unlike that for ddU, we decided to conduct molecular modeling studies investigating the second phosphorylation step. This was done by docking[11] both d4U and ddU monophosphates into the active site of thymidylate kinase, which was shown to be the enzyme responsible for the phosphorylation of numerous clinically proven antiviral nucleosides. In this study, we used thymidine monophosphate as refer-



Scheme 1. Synthetic route to d4U and ddU. Reagents and conditions: (i) $MeSO_2Cl$, Pyr, rt; (ii) aq. NaOH, MeOH, reflux; (iii) NaH, DMF, 100 °C; (iv) H_2, 10% Pd/C, overnight.

ence when analyzing the docking results of both d4U and ddU monophosphates. The resulting structures for the two nucleotide analogues were ranked according to the relative position of the uracil base with the corresponding position of the thymine base in the crystal structure of TMP, and only the results with a RMSD values below 1.0 Å were further analyzed. The best results, shown in Figure 2, revealed that the phosphorus group of ddU-monophosphate was placed in a very similar position to that of the naturally occurring thymidine monophosphate. This therefore permits a good second phosphorylation of ddU monophosphate, and thus allows the activation of inactive ddU on ProTide bypass of the limiting initial phosphorylation.

However, by contrast the phosphorus group of d4U monophosphate was placed significantly far from that of thymidine monophosphate. In fact, it was placed further out from the active site of thymidylate kinase and, more importantly, closer to the ATP molecule. As the monophosphate group bears oxygen atoms that are negatively charged like that of ATP, a putative repulsion could occur between d4U monophosphate and ATP which would most likely limit the second phosphorylation step of d4U monophosphate if not prevented it at all. Thus, even a successful ProTide bypass of the initial phosphorylation of d4U would not be able to confer antiviral activity on this nucleoside. Thus, the conclusions of these modeling studies on the monophosphates of ddU and d4U are entirely consistent with the differential outcomes of the ProTide approach in each case.



Scheme 2. General synthetic scheme of d4U/ddU ProTides.

Table 2. Anti-HIV data of d4U and its 'ProTide' phosphoramidates

Compound	EC_{50} (μM) CEM/0		CC_{50} (μM) CEM/0
	HIV-1	HIV-2	
1a	>50	>10	96.6 ± 13.3
1b	>50	>50	212 ± 53.9
1c	>250	>250	>250
1d	25	25	100
d4U	>50	>50	207 ± 60.8
d4T	0.65	0.77	174

EC_{50}: The effective concentration (μM) required to protect CEM cells against the cytopathogenicity of HIV by 50%. CC_{50}: The cytostatic concentration (μM) required to reduce CEM cell viability by 50%.

Table 3. Anti-HIV data of ddU and its ProTides

Compound	EC_{50} (μM) CEM/0		CC_{50} (μM) CEM/0
	HIV-1	HIV-2	
2a	36.7 ± 11.5	⩾50	121 ± 1.4
2b	43.3 ± 20.8	93.3 ± 32.1	>250
2c	>250	>250	>250
2d	15.0 ± 0.0	20.0 ± 7.1	96.9 ± 0.85
ddU	>250	>250	>250
d4T	0.65	0.77	174

EC_{50}: The effective concentration (μM) required to protect CEM cells against the cytopathogenicity of HIV by 50%. CC_{50}: The cytostatic concentration (μM) required to reduce CEM cell viability by 50%.

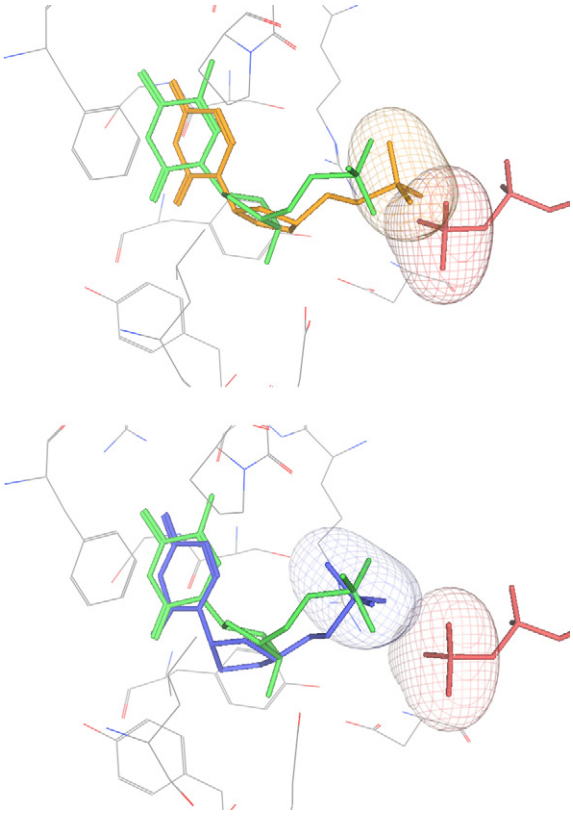


Figure 2. Docking results of both ddUMP (bottom) and d4UMP (top) with TMP in thymidylate kinase.

In conclusion, we have synthesized and studied the anti-HIV activity of d4U, ddU, and some of their phosphoramidate ProTides. The results showed that neither d4U nor ddU possessed significant anti-HIV activity. As for their phosphoramidates, d4U phosphoramidates did not exert significant anti-HIV activity, whereas ddU phosphoramidates showed markedly improved anti-HIV activity. This indicated that the first phosphorylation

step is the one responsible for the inability of ddU to exert any significant anti-HIV activity. Regarding d4U, we have shown that bypassing the first phosphorylation step does not improve its anti-HIV activity and using molecular modeling, we have suggested that the inefficient second phosphorylation step may in part be responsible for the poor anti-HIV activity of d4U. Overall, we are currently synthesizing more ddU phosphoramidates with different amino acids and esters to explore whether we can tune its anti-HIV activity to sub-micromolar levels.

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References and notes

1. De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491.
2. (a) Barre-Sinoussi, F.; Cherman, J. C.; Rey, R.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868; (b) Border, S.; Gallo, R. C. *New Eng. J. Med.* **1984**, *311*, 1292.
3. (a) Balzarini, J.; Herdewijn, P.; De Clercq, E. *J. Biol. Chem.* **1989**, *264*, 6127; (b) Gao, W. Y.; Agbaria, R.; Driscoll, J. S.; Mitsuya, H. *J. Biol. Chem.* **1994**, *269*, 12639.
4. Balzarini, J.; Kang, G.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. *Mol. Pharmacol.* **1987**, *32*, 162.
5. Hao, Z.; Cooney, D. A.; Farquhar, D.; Perno, C. F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N. R.; Balzarini, J.; Johns, D. *Mol. Pharmacol.* **1989**, *37*, 157.
6. Cahard, D.; McGuigan, C.; Balzarini, J. *Mini-Rev. Med. Chem.* **2004**, *4*, 371.
7. McGuigan, C.; Pathirana, R. N.; Snoeck, R.; Andrei, G.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2003**, *47*, 1847.
8. McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1996**, *39*, 1748.
9. Congiatu, C.; Brancale, A.; Mason, M. D.; Jiang, W. G.; McGuigan, C. *J. Med. Chem.* **2006**, *49*, 452.
10. (a) McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1993**, *36*, 1048; (b) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1996**, *39*, 1748; (c) McGuigan, C.; Tsang, H. W.; Cahard, D.; Turner, K.; Velazquez, S.; Salgado, A.; Bidois, L.; Naesens, L.; De Clercq, E.; Balzarini, J. *Antiviral Res.* **1997**, *35*, 195.
11. Docking studies were performed using the FlexX module of SYBYL7.2 (Tripos Inc., 1699 South Hanley Rd., St. Louis, MO 63144, USA. http://www.tripos.com) and the results were analyzed using MOE 2006.08 (Molecular Operating Environment (MOE 2006.08). Chemical Computing Group, Inc., Montreal, Que., Canada. http://www.chemcomp.com).