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Crown Nucleoside Monophosphate Diesters: a New Class of Nucleoside Prodrugs

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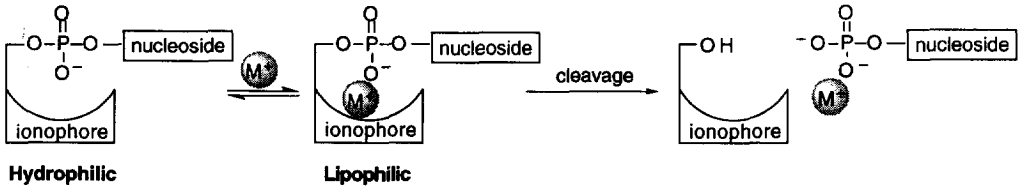
Abstract: *An ionophore-nucleotide conjugate is proposed as a new class of nucleoside prodrug. Specifically, 5'-phosphate diester derivatives of the anti-HIV agents AZT (3'-azido-3'-deoxythymidine) and DDU (2',3'-dideoxyuridine) were prepared with a crown ether as the phosphate masking group. Biological testing of these prodrugs revealed they had moderate anti-HIV activity.*

Most of the currently licensed antiviral drugs and many anticancer drugs are nucleoside analogues. To become biologically active, these compounds have to enter the cell and be converted to nucleotides by viral or cellular kinases. The kinases involved in activating the nucleosides are usually substrate specific, which places a limitation on the deviation of analogue structure. Thus, it is thought that in many cases direct introduction of the nucleotide would provide a distinct therapeutic advantage.[1,2] To directly use nucleotides, however, two major problems have to be overcome. First, nucleotides are readily dephosphorylated by non-specific serum phosphatases. Second, the anionic nucleotides cannot penetrate cellular membranes, so even if degradation is avoided their therapeutic efficacy is still very low. Consequently, there has been a heavy focus, particularly with antiviral drugs over the past decade, on introducing nucleotides into cells using various prodrug strategies. Most of the prodrugs have been nucleoside 5'-monophosphate esters. A range of phosphate masking groups have been examined including alkyl,[3] aryl,[4] lipid,[5] amino acid,[6] saccharide,[7] and steroid.[8] Nucleoside monophosphate diester dimers have also been reported.[9]

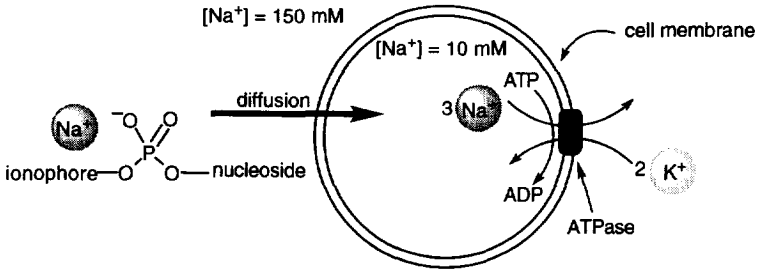
While the nucleotide prodrug strategy remains promising there are a number of associated problems that have hindered progress. The initial prodrug is often the neutral phosphate triester, which is hydrolytically labile. The subsequent diester, however, is up to a million times less reactive, and most strategies are dependent on this cleavage step being achieved by cellular phosphatases. When an anionic nucleoside 5'-phosphate diester diffuses through a lipophilic membrane it presumably must be accompanied by a metal cation which is an energetically demanding process. Prodrug strategies have attempted to overcome this unfavorability by making the prodrug very lipophilic. However, extreme lipophilicity is not always desirable as it may result in poor bioavailability.[10] To our knowledge, there has been no report of a nucleotide prodrug with ionophoric masking groups. Herein, we describe a prodrug strategy that is based on the ionophore-nucleoside 5'-phosphate diester conjugate shown in Scheme 1. Because of its anionic charge, the conjugate is reasonably hydrophilic; however, association with a metal cation produces a lipophilic ion-pair that is capable of diffusion through a bilayer membrane. Subsequent intracellular cleavage of the phosphate diester releases the nucleoside 5'-monophosphate which can be converted enzymatically into bioactive nucleoside 5'-triphosphate.

Scheme 1



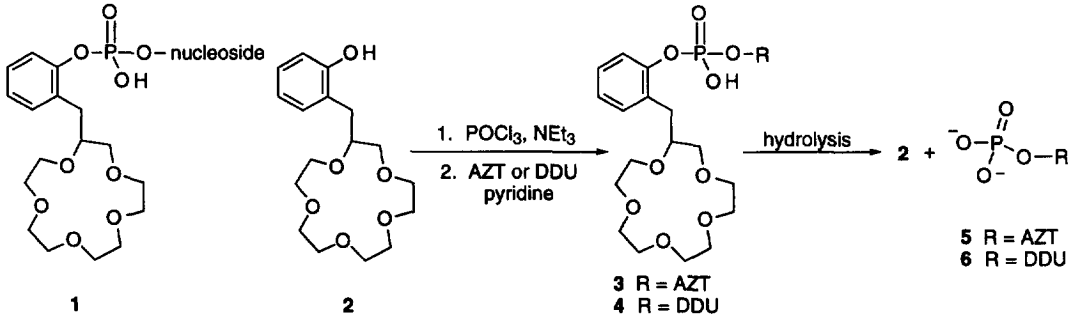
If diffusion of the metal cation/prodrug ion-pair through the cell membrane is fast, then the amount of intracellular prodrug at thermodynamic equilibrium will be determined by the various transmembrane cation concentration differences, as well as the prodrug's cation binding selectivity. Most cells have an intracellular Na^+ concentration that is about fifteen times lower than the extracellular concentration.[11] This is maintained by a ubiquitous Na^+-K^+ antiport which is driven by the hydrolysis of ATP by the enzyme Na^+,K^+-ATPase (Scheme 2). We postulate that this inward-directed Na^+ concentration gradient will force anionic, Na^+ binding compounds, such as the idealized prodrug conjugate shown in Schemes 1 and 2, to accumulate within cells. In the absence of other factors, an intracellular concentration factor of up to fifteen-fold may be possible.

Scheme 2



Ideally, the ionophoric masking group should incorporate the following features; (i) The combined ionophore-nucleotide conjugate should be a Na^+ selective membrane carrier; (ii) the prodrug should undergo ready and selective phosphate ester cleavage to release the desired nucleoside 5'-monophosphate; (ii) the by-products of prodrug cleavage should be of low toxicity.[12]

Inspiration for our first generation design was provided by the work of Bartsch and coworkers, who showed that lipophilic crown phosphonic acid monoalkyl esters were able to selectively extract sodium ions into an organic phase.[13] With this knowledge in mind, we designed the 15-crown-5 nucleotide conjugate, **1**, as a new class of nucleoside prodrug. At physiological pH, compound **1** will be anionic and water soluble, but upon association with an alkali metal cation, a neutral and lipophilic complex will be formed that should be capable of diffusing across a cell membrane. The phosphate aryl ester in **1** ensures that the preferred cleavage pathway produces the nucleoside 5'-monophosphate, along with the crown ether **2**.[12]



Here we disclose the synthesis and preliminary biological activity of the first compounds examined within this series, namely, **3** and **4** which are prodrugs of the anti-HIV agents AZT (3'-azido-3'-deoxythymidine) and DDU (2',3'-dideoxyuridine), respectively. Crown **2**[14] was coupled with AZT[15] to give **3** in 19 % yield after HPLC purification, and with DDU[16] to give **4** in 5 % yield after HPLC purification. In both cases the final coupling step involved treatment of crown **2** with phosphorus oxychloride, followed by subsequent condensation with the appropriate nucleoside.[17]

Prodrugs **3** and **4** were tested for anti-HIV activity at the US National Cancer Institute. Two assays were performed, anti-HIV activity in infected CEM-SS cells (50 % effective concentration against HIV cytopathic effects, EC_{50}) and cell toxicity (50 % inhibitory concentration of uninfected cell growth, IC_{50}). Compared to literature reports, the activity of **3** is approximately fourteen times weaker than AZT (Table 1).[18] However, the activity of **4** is approximately eleven times greater than DDU.[18] This latter result is noteworthy. While the triphosphate derivative of DDU is a potent inhibitor of HIV reverse transcriptase, DDU itself is essentially ineffective at blocking HIV infection in cultured cells as it is a poor substrate for cellular nucleoside kinases.[19] The fact that **4** has better activity than DDU suggests that at least some of the prodrug is diffusing into the cells and degrading to produce DDU monophosphate, **6**, which is then anabolized to bioactive DDU triphosphate. To further validate this theory, biological testing is required using nucleoside kinase deficient cell lines. A final point is that the IC_{50} values for prodrugs **3** and **4** are quite high, indicating that the crown ether masking group introduced little, if any, cell toxicity.[12]

Table 1.

Compound	EC_{50} (M)	IC_{50} (M)
3	7×10^{-8}	$> 2 \times 10^{-6}$
AZT[a]	5×10^{-9}	$> 5 \times 10^{-7}$
4	5×10^{-5} (9×10^{-6})[b]	$> 2 \times 10^{-4}$
DDU[a]	($> 10^{-4}$)[b]	($> 10^{-4}$)

[a]Reference 17. [b]EC_{18}

It is known that certain dideoxynucleosides, such as DDU, exhibit weak anti-HIV activity because they are poor substrates for nucleoside kinases. As noted by others,[19] and suggested by the results reported here, the use of dideoxynucleotide prodrugs is a potential method for overcoming this problem.

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17. Compound **3** was obtained as a white solid. ^{1}H NMR [600 MHz]: δ 1.25 (s, 1H), 1.31 (s, 1H), 1.83 (s, 3H), 1.90 (s, 1H), 2.32 (bm, 2H), 2.77 (bd, 1H, J = 6), 3.50 (bs, 2H), 3.51 - 3.71 (bs, 16H), 3.81 (s, 1H), 4.03 (s, 1H), 4.25 (s, 1H), 4.30 (s, 1H), 4.42 (s, 1H), 6.27 (t, 1H, J = 6), 7.00 (t, 1H, J = 7.2), 7.10 (m, 1H) 7.16 (m, 1H) 7.36 (d, 1H, J = 7.2), 7.68 (s, 1H), 8.9 (s, 1H), ppm; ^{13}C NMR [150 MHz]: δ 8.5, 12.2, 29.6, 32.2, 37.3, 45.7, 60.9, 65.7 (d, J = 6.6), 83.0 (d, J = 8), 69.5 -71.5 (m), 84.6, 111.2, 119.9, 123.9, 127.5, 129.2, 130.9, 135.9, 150.3, 150.7 (d, J = 5.5), 163.7, ppm; ^{31}P NMR [202 MHz, external trimethyl phosphate]: δ -8.263 (bs, 1P), ppm; HRMS (FAB): calcd for $C_{27}H_{38}O_{12}N_5PNa$ = 678.2152 found 678.2153; IR 3853, 3744, 3443, 2924, 2361, 2105, 1695, 1457, 1267, 1105 cm^{-1}. Compound **4** was obtained as a waxy solid. ^{1}H NMR [300 MHz]: δ 1.29 (1H, t, J = 7.2), 2.06 (m, 3H), 2.31 (m, 1H), 2.47 (dd, 1H, J = 14.1, 3.9), 3.02 (q, 1H, J = 7.2), 3.2 - 3.8 (m, 19H), 4.15 (m,1H), 4.23 (m, 1H), 5.59 (dd, 1H, J = 8,1, 2.4), 6.04 (t, 1H, J = 4.5) 6.95 (t, 1H, J = 6.6), 7.12 (m, 2H), 7.48 (d, 1H J = 7.8), 8.03 (m, 1H), 8.81 (bs, 1H), ppm; ^{13}C NMR [150 MHz]: δ 8.5, 25.2, 32.4, 32.7, 45.5, 66.4, 68.3, 68.5, 68.8, 69.2, 69.3, 69.4, 70.6, 70.9, 80.4, 86.3, 101.6, 119.9, 122.7, 127.7, 129.1, 129.9, 147.1, 150.5, 152.2, 163.4, ppm; ^{31}P NMR [121 MHz, external trimethyl phosphate]: δ -7.137 (s, 1P), ppm; HRMS (FAB): calcd for $C_{26}H_{37}O_{12}N_2PNa$ = 623.1982 found 623.2001.
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