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# Enhancing the Aqueous Solubility of d4T-based Phosphoramidate Prodrugs

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**Abstract**—A range of polyether *para*-substituted phosphoramidates were synthesised and found to have substantially elevated aqueous solubilities compared to the underivatised parent prodrug. A 30-fold increase in aqueous solubility could be achieved without a substantial decrease of in vitro activity against HIV-1. Replacement of the aryl (i.e. phenolic) moiety by tyrosine led to a substantial enhancement in aqueous solubility but also to a decrease in antiviral potency. A previously unobserved trend was identified, relating increased aryl substituent steric bulk to decreased antiviral activity. 

The nucleotide prodrug ("protide") concept continues to be a subject of intensive research.[1] Principal objectives are to improve the activity of existing antiviral nucleoside analogues and to confer activity upon those nucleoside analogues that express poor activity due to inefficient intracellular phosphorylation.

We have shown that our approach using aryloxy phosphoramidates can lead to elevated levels of anti-HIV potency for a range of antiviral nucleoside analogues and does successfully by-pass dependency on nucleoside kinases.[2–4] Recently, we demonstrated that simple mono-substitution of the aryl ring of the phosphoramidates based on d4T, ddA and d4A significantly enhances antiviral activity relative to the parent phosphoramidates.[5–7] In addition to those substituents that markedly enhanced antiviral potency, we were able to show that for d4T-based phosphoramidates a variety of aryl substituents could be tolerated. Antiviral data for 21 various aryl-substituted d4T-based phosphoramidates (**1a**–**u**, Fig. 1) were subjected to a Hansch-type QSAR analysis, which revealed activity to be principally dependent on lipophilicity as measured by compound logP (eq 1).[7] Compared to other parts of the phosphoramidate structure, chemical modification of the aryl group appears to result in no loss of activity, thus we proposed that appropriate aryl substituents might be used to optimise the pharmacological profile of these types of prodrugs, without compromising antiviral potency.

An important parameter that might be modified by the choice of aryl substituents is prodrug aqueous solubility. Consequently, we sought to synthesize a series of polyether *para*-substituted phosphoramidates **2a**–**2d** (Fig. 2). These substituents should have the dual effect of enhancing prodrug aqueous solubility without compromising optimum or near-optimum lipophilicity (and thus antiviral potency). Additionally, they should allow the investigation of the effects of substituent steric bulk beyond the range of those substituents previously studied.[8]

$$\log 1/EC_{50}[HIV-1] = -2.440 + 5.157(1.117)\log P - 1.499(0.395)(\log P)^2 \quad (1)$$

Intracellular activation of the parent prodrug (**1a**) leads to the release of three major by-products; L-alanine (a naturally occurring amino acid), methanol and phenol. Studies in vitro suggest that replacement of the methyl ester by an ethyl ester—leading to the release of a notably less toxic alcohol—is not detrimental to antiviral activity.[9] The release of phenol may represent a greater challenge.
Although a full toxicological appraisal of the effects of phenols on humans is incomplete, (particularly with

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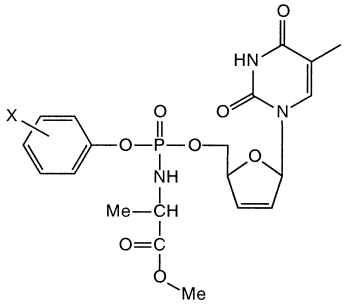
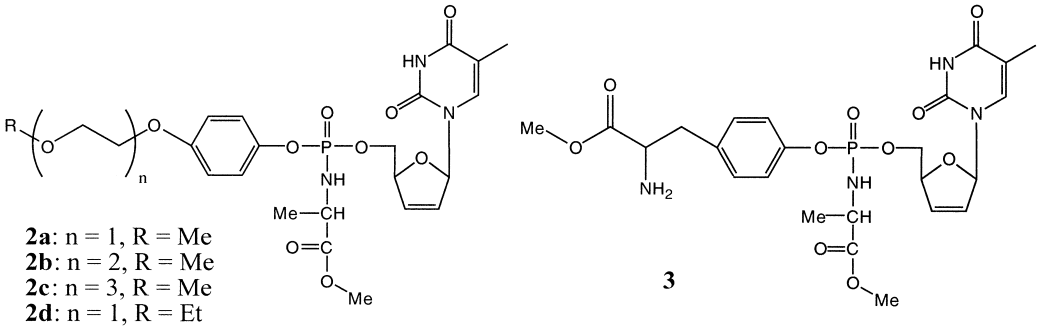


**1a**: X = H
**1b**: X = *p*-$NO_2$
**1c**: X = *p*-CN
**1d**: X = *p*-COOMe
**1e**: X = *p*-COMe
**1f**: X = *p*-Cl
**1g**: X = *p*-F
**1h**: X = *p*-Me
**1i**: X = *p*-OMe
**1j**: X = *m*-COMe
**1k**: X = *p*-$CF_3O$
**1l**: X = 3,4-$Cl_2$
**1m**: X = *m*-F
**1n**: X = *o*-Cl
**1o**: X = *p*-Br
**1p**: X = *p*-I
**1q**: X = *m*-Cl
**1r**: X = *m*-Br
**1s**: X = *m*-I
**1t**: X = *p*-$CF_3$
**1u**: X = *m*-$CF_3$

**Figure 1.**



**2a**: n = 1, R = Me
**2b**: n = 2, R = Me
**2c**: n = 3, R = Me
**2d**: n = 1, R = Et

**3**

**Figure 2.**

regards to mutagenicity and carcinogenicity, for example) many of the effects of phenol toxicity are well characterized.[10] In addition to non-specific modes of toxicity, phenols (particularly those which are both lipophilic and acidic) are known to be potent uncouplers of oxidative phosphorylation.[11,12] Therefore, the replacement of the phosphoramidate phenol moiety with compounds that might be less toxic would seem worthy of investigation. Thus, we replaced the phenol moiety of **1a** with the phenolic moiety of tyrosine (e.g. **3**, Fig. 2). The free amino group of tyrosine should also lead to an enhanced prodrug aqueous solubility but, conversely, a diminished lipophilicity.

The phenolic precursors for the synthesis of compounds **2a–d** were prepared by refluxing hydroxyquinone with the corresponding polyether alcohol in the presence of phosphomolybdic acid. Synthesis of the corresponding phosphoramidates was accomplished via conventional phosphorochloridate chemistry (described elsewhere[13]). Synthesis of **3** was achieved starting from commercially available L-tyrosine methyl ester hydrochloride salt (**4**). Protection of the amino function with BOC gave compound (**5**), which was subsequently converted to the phosphoramidate (**6**) via conventional phosphorochloridate chemistry. Final deprotection of the amino group, under acidic conditions, liberated the desired compound as its trifluoroacetyl salt. Compounds **1a**, **1i**, **2a–d**, **3** were isolated as diastereomeric mixtures resulting from mixed stereochemistry about the phosphorus centre, with the diastereomeric ratio, determined by both $^{31}P$ NMR and HPLC, typically of the order 1:1. Diastereomeric splittings were observed in the $^{1}H$, $^{13}C$ and $^{31}P$ NMR spectra and additional splitting patterns from phosphorus coupling, where appropriate, were seen in the $^{13}C$ and $^{1}H$ NMR spectra.[14]

The molar extinction coefficients and aqueous solubility of compounds **1a**, **1i**, **2a-d**, **3** and **6** were determined by standard methods (Table 1). Introduction and progressive elongation of the polyether side-chain on the aryl moiety led to a clear, stepwise increase in the aqueous solubility of the prodrugs. From a saturation concentration of 6.2 mmol/l for the parent prodrug **1a** the solubility could be increased to a maximum of 375.4 mmol/l for **2c** (a ca. 60-fold increase). By contrast, the lipophilicity of compounds **2a–c** remained constant as measured by logP (Table 1), an important result for the retention of antiviral activity. Compound **2d** showed comparable aqueous solubility to compound **1i** (having a simple methoxy substituent) but, as expected, was less soluble than compound **2a**, which bears a smaller terminal alkyl group on the aromatic side-chain.

Replacement of the phenolic moiety of **1a** by tyrosine methyl ester, led to at least a 100-fold increase in aqueous solubility. However, the low value of the measured logP (Table 1) suggested that the in vitro antiviral potency might be less than observed for the parent prodrug, a premise that was shown to be correct (Table 2).

The anti-HIV data (HIV-1 and HIV-2) for compounds **2a–d**, **3** and **6** are given in Table 2 (data for **1a** and **1i** are also given for reference). Predicted values for activity against HIV-1 using eq (1) are also displayed along with substituent *MR* values—used as an approximation of substituent steric bulk (noting the comments of Hansch et al.[15]). Compounds **2a–d** and **6**, all displayed activity against HIV-1 and HIV-2 that was comparable to that of the nucleoside analogue d4T. Additionally (and unlike d4T), potency was retained in a thymidine kinase deficient ($TK^-$) cell-line, indicating sucessful release of the nucleoside monophosphate analogue. Compared to

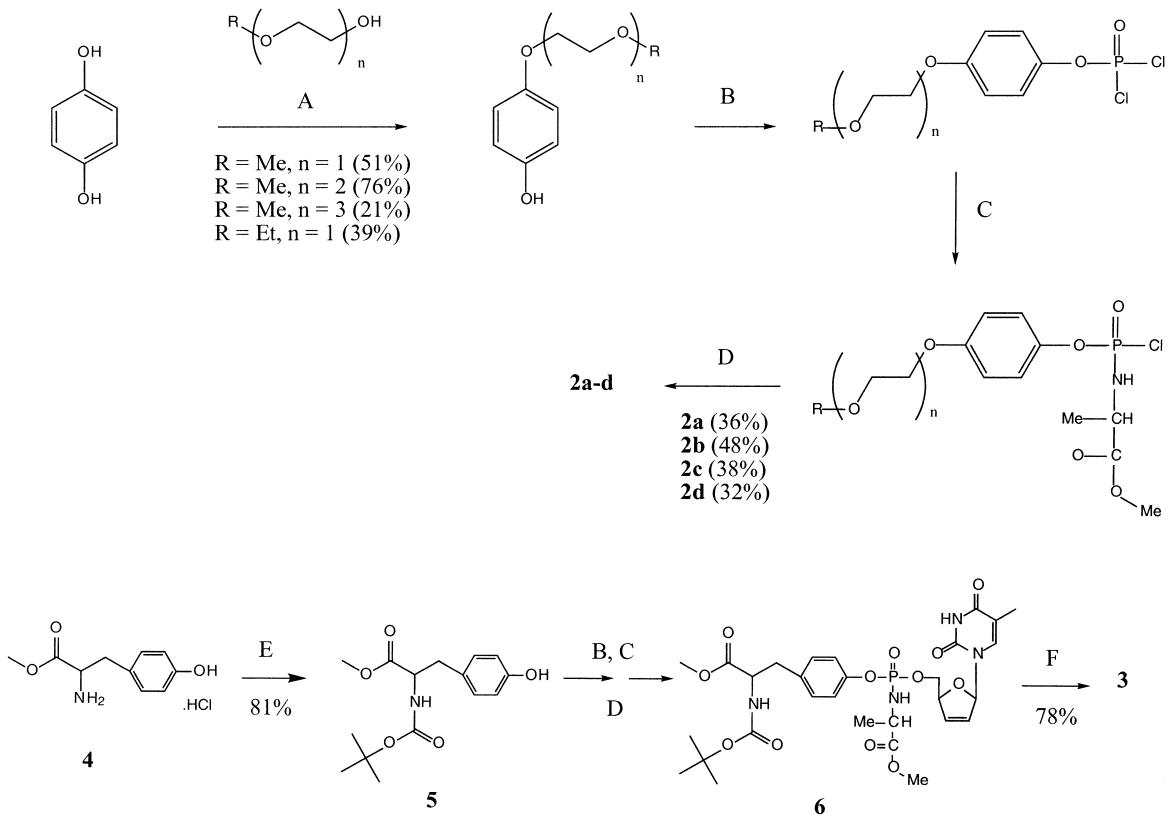



**Figure 3.** A: Phosphomolybdic acid (cat.), reflux, 6 h; B: $POCl_3$, $Et_3N$, −78 °C, 16 h; C: alanine methyl ester hydrochloride, $Et_3N$, −78 °C, 16 h; D: d4T, NMI, RT, 16 h; E: BOC-anhydride, $Et_3N$, RT, 1 h; F: TFA:DCM (1:1), RT, 1 h.

**Table 1.**

| Compound | Molar extinction coefficient (ϵ) (l $mol^{-1}$ $cm^{-1}$) | Saturation conc. (mmol/L) | logP (1-octanol/water) | Esterase lability[a] (%) |
|---|---|---|---|---|
| **1a** | 7640 | 6.2 | 1.04 | 5.4 |
| **1i** | 8503 | 66 | 1.09 | 79 |
| **2a** | 8181 | 184 | 0.86 | 10 |
| **2b** | 8277 | 259 | 0.86 | 10 |
| **2c** | 8846 | 375 | 0.86 | 10 |
| **2d** | 8864 | 86 | 1.10 | 10 |
| **3** | 8182 | >794 | 0.09 | 0 |
| **6** | 9194 | 6.3 | 1.07 | 5.4 |

[a]% of compound hydrolysed after incubation with pig liver esterase for 24 h (pH 7.6, 37 °C).[5]

the parent phosphoramidate (**1a**), only compound **2a** expressed a similar potency against HIV-1. Against HIV-2, **2a** was markedly less potent than **1a**, expressing a potency comparable to that of d4T. By a comparison of the *actual* $EC_{50}$ values against HIV-1 and those predicted using the Hansch-type equation (eq 1), it is apparent that an increased substituent steric bulk leads to a smaller antiviral activity than might be predicted from using logP alone. Indeed, an analysis of the polyether series (**2a–d**) reveals a step-wise deviation between the observed and predicted activity values for each additional ethoxy unit present in the structure. Similarly, the activity displayed by the N-BOC protected phosphoramidate **6** is also less than might be expected on the basis of logP alone. Taken together, these results suggest a clear and negative correlation between substituent steric bulk and antiviral activity—which is apparently most evident when the substituent *MR* value is near 20 or above. Compound **3**, the most water soluble of the series, is the least active, a result predicted by the QSAR equation. This is consistent with the theory that lipophilicity is a necessary property for adequate diffusion of the prodrug across cellular membranes. The numerical discrepancy between the observed and predicted values may reflect the far lower lipophilicity of **3** compared to those compounds used to construct the original QSAR.

In an attempt to explain why a substituent of large steric bulk might display a decreased antiviral activity we subjected all of the compounds from Table 1 to a pig liver esterase (PLE) assay, which we have used previously to model the putative esterase-driven hydrolysis of the amino acid ester, believed to be the first step in intracellular prodrug activation.[5] The results are presented in Table 1 as the percentage of compound hydrolysed after 24 h. It can be seen from these values that no differences exist between any of the compounds in the polyether series, **2a–d**. Additionally, whilst compounds **2a–d**, **1a**, **1i** and **6** all serve as esterase substrates, no observable hydrolysis of compound **3** occurred over the experimental time-scale. Although we have previously seen that quantitative comparisons between antiviral activity and the results of the PLE assay can be unsuccessful, we have noted that processesing by PLE is a necessary requirement for a prodrug to express in

**Table 2.**

| Compound | $EC_{50}$ (μM)[a] CEM (HIV-1) | $EC_{50}$ (μM) Predicted[c] (HIV-1) | $EC_{50}$ (μM) CEM (HIV-2) | $EC_{50}$ (μM) CEM/TK$^-$ (HIV-2) | $CC_{50}$[b] | *MR*[e]p-X |
|---|---|---|---|---|---|---|
| **1a** | 0.075 | 0.049 | 0.075 | 0.075 | >100 | 1.03 |
| **1i** | 0.057 | 0.040 | 0.053 | 0.047 | 35 | 7.87 |
| **2a** | 0.09 | 0.13 | 0.6 | 0.34 | 47 | 19.28[f] |
| **2b** | 0.55 | 0.13 | 1 | 1.2 | 114 | 30.69[f] |
| **2c** | 0.75 | 0.13 | 1.3 | 1.35 | 64 | 42.10[f] |
| **2d** | 0.2 | 0.04 | 0.25 | 0.4 | 100 | 23.93[f] |
| **3** | 4 | 97.28 | 10 | 5 | [d] | 26.56[f] |
| **6** | 0.65 | 0.043 | 0.7 | 0.8 | 217 | 52.37[f] |
| d4T | 0.651 | — | 0.770 | 33 | 174 | — |

[a]$EC_{50}$ is the concentration required to protect CEM cells against the cytopathicity of HIV by 50%. Data are the mean of two to four independent experiments.
[b]$CC_{50}$ is the concentration required to inhibit CEM cell proliferation by 50%.
[c]Using eq (1).
[d]Not determined.
[e]From Hansch and Leo.[16]
[f]Calculated from fragment values.[16]

vitro antiviral activity.[5,17] It is unclear as to whether the inactivity of **3** under the PLE assay conditions indicates that poor processing by cellular esterase(s) contributes to the low observed antiviral activity, but it is clear that **3** is the least active compound in this study.

In conclusion, we have demonstrated that the aqueous solubility of phosphoramidate prodrugs based on d4T can be enhanced by substitution of polyether side-chains on the aryl ring. We have shown that replacement of the phenol moiety with that of tyrosine can substantially enhance aqueous solubility but at the cost of antiviral activity, confirming the importance of controlling prodrug lipophilicity. Additionally, we report a newly observed trend correlating substituent steric bulk with decreased antiviral activity.

## Acknowledgements

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14. Selected data for **2a**, 2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(4-methoxyethoxyphenyl methoxyalaninyl) phosphate: $\delta_P$[$CDCl_3$] 4.17, 4.71; $\delta_H$[$CDCl_3$] 1.38 (3H, m, Ala-Me), 1.90 (3H, d, 5-Me), 3.50 (3H, d, OMe), 3.76 (6H, m, $CO_2$Me, $CH_2$-2″, NH), 4.03 (1H, m, Ala-CH), 4.15 (1H, m, $CH_2$-1″), 4.38 (2H, m, H-5′), 5.10 (1H, m, H-4′), 5.96 (1H, m, H-3′), 6.39 (1H, m, H-2′), 6.88 (2H, m, *meta*-Ph), 7.05 (1H, m, H-1′), 7.12 (2H, m, *ortho*-Ph), 7.32 (1H, m, H-6), 8.81 (1H, d, NH); $\delta_C$[$CDCl_3$] 15.08, 15.14 (5-Me), 23.79 (t, J 3.5, Ala-Me), 52.81, 52.94 (Ala-CH), 55.39 (COOMe), 62.00 (OMe), 69.22, 69.85 (d, J 5.2, C-5′), 70.42, 70.42, 70.46 ($CH_2$-2″), 73.77, 73.79 ($CH_2$-1″), 87.33, 87.38, 87.44, 87.49 (C-4′), 92.34, 92.60 (C-1′), 111.69, 111.82 (C-5), 118.08, 118.22 (*meta*-Ph), 123.66, 123.73, 123.87, 123.93 (*ortho*-Ph), 130.05, 130.22 (C-2′), 135.84, 136.14 (C-3′), 138.36, 138.64 (C-6), 146.52, 146.61, 146.75, 146.83 (*para*-Ph), 153.46, 153.54 (C-2), 158.77 (Ph), 166.34, 166.43 (C-4), 176.58, 176.68, 176.75, 176.85 (Ala-CO); MS *m/e* FAB 540.1747 ($MH^+$, $C_{23}H_{31}N_3O_{10}P$ requires 540.1747); HPLC $t_R$ 30.24, 30.73 min (grad. I); $t_R$ 31.32, 31.91 min (grad. II). HPLC (Shimadzu) was conducted on an SSODS2 reverse phase column using a water/acetonitrile (Fisher: HPLC grade) eluent; gradient I (standard gradient): 0–80% $CH_3CN$ (0–60 min), 80–0% $CH_3CN$ (60–65 min), flow rate: 1 ml/min, UV detection at 265 nm; gradient II: 0–10% $CH_3CN$ (0–5 min), 10–70% $CH_3CN$ (5–55 min), 70–0% $CH_3CN$ (55–60 min), flow rate: 1 ml/min, UV detection at 265 nm.
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