Synthesis and Anti-HIV-1 Activity of Bis-ketol AZT Monophosphates

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The synthesis of five bis-ketol AZT monophosphates and their anti-HIV-1 activity in CEM-SS host cells are reported.

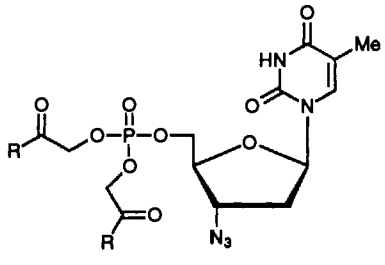
We report the synthesis of a series of bis-ketol AZT monophosphates, **1**, representatives of a new sub-class of masked nucleotides.[1] These novel phosphotriesters have been evaluated at the National Cancer Institute (NCI) and are quite active against HIV-1 in human cells.[2]

The preparation of the first examples, **2**, of tris-ketol phosphates *via* the 'exhaustive ketolization' of anhydrous H_3PO_4 with *p*-(difluoroiodo)toluene (DFIT, 4-$MeC_6H_4IF_2$) and various silyl enol ethers (SEEs) in *tert*-butyl alcohol has recently been described.[3] The synthesis of **1** was made possible by application of this methodology to anhydrous pyrophosphoric acid (PPA) and hydrolysis of the resulting tetrakis-ketol pyrophosphates, **3**, to bis-ketol hydrogen phosphates, **4** [eqn. (1)]. Phosphorylation of AZT with **3** (R = Ph, Bu^t, 2-furyl) or **4** (R = C_6H_{13}, Me) affords the bis-ketol AZT monophosphates **1** [eqn. (2)].

More specifically, the tetrakis-ketol pyrophosphates were generated by the sequential treatment of PPA (Aldrich, 97%) with (1) a four-fold molar excess of (diacetoxyiodo)benzene (or DFIT for **3a**) and (2) an excess of the appropriate SEE in dry *tert*-butyl alcohol (room temp., N_2). They are sensitive to moisture and, once isolated, were generally employed without further purification. Compound **3a** (white solid) separated from the reaction medium, with or without added pentane, and was collected by filtration, while **3b** (white solid) was precipitated from the concentrated reaction mixture with pentane. Of the tetrakis-ketol pyrophosphates prepared, only **3a** and **3b** have been obtained in a near analytically pure state (C, H analyses consistent with **3a**·$2H_2O$ and **3b**·H_2O). The crude tetrakis-ketol pyrophosphates **3d** and **3e**, isolated by vacuum evaporation of *tert*-butyl alcohol, were taken up in dichloromethane (10 cm^3) and treated with water (*ca.* 0.1 and 0.2 cm^3) whereupon they were converted into the bis-ketol hydrogen phosphates **4d** and **4e**. Conditions and yields for selected preparations of **3** and **4** are given in Table 1.

Compounds **3** and **4** can be readily distinguished by ^1H NMR analysis (300 MHz, $CDCl_3$). In particular, the methylene hydrogens of **4** afford a moderately lowfield doublet, while the diastereotopic methylene hydrogens of **3**, unequally coupled with phosphorus, give rise to a complex multiplet best understood by simulation studies as the AB sub-spectrum of an ABXX′ spin system (*i.e.*, the phosphorus atoms in **3** are isochronous but not isogamous). For example, the ^1H spectrum of **4b** (made from **3b**·H_2O) exhibits a doublet at δ 4.93 (J_{HP} 10 Hz). The spectrum of **3b**·H_2O, on the other hand, shows a nearly symmetrical 15-line multiplet centred at δ 5.12.

The bis-ketol AZT monophosphates, **1a–c**, were prepared by the condensation of AZT with **3a–c** in the presence of triethylamine (room temp., CH_2Cl_2) and purified by column chromatography. For example, a mixture of AZT (1.0 mmol),



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	R
1a·H_2O	Ph
1b	Bu^t
1c·H_2O	
1d	C_6H_{13}
1e	Me

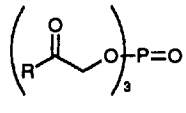

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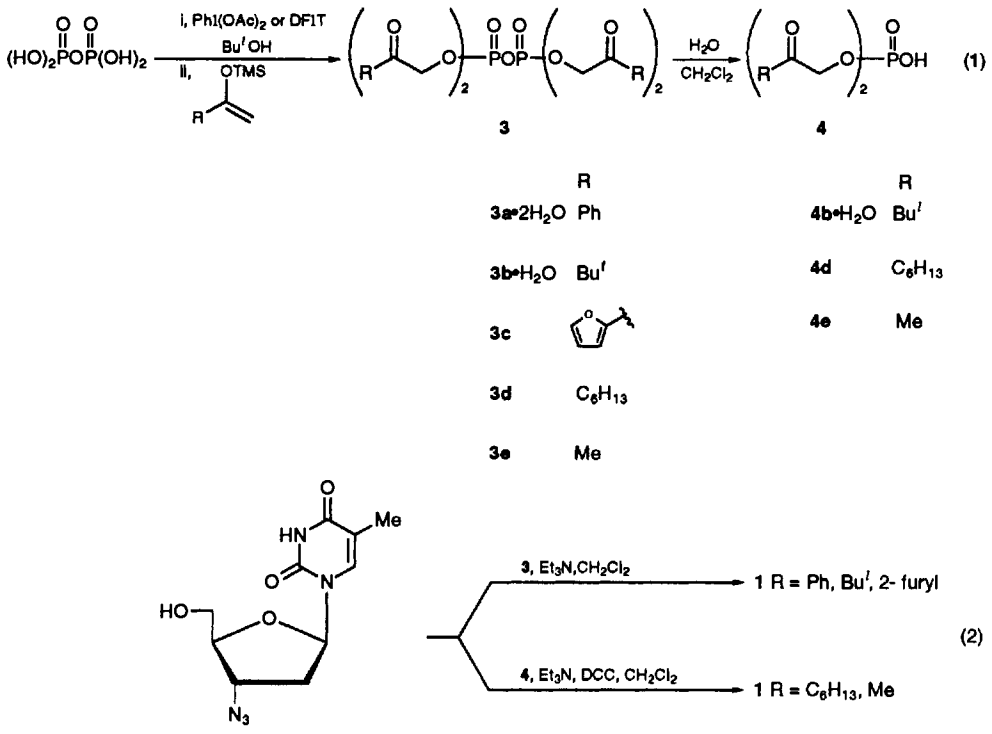
Table 1 Conditions and yields for selected preparations of tetrakis-ketol pyrophosphates and bis-ketol hydrogen phosphates

Compd.[a]	Reactants (mmol) PPA	Phl(OAc)$_2$	SEE[c]	Vol (cm^3) Bu^{t}OH	Time (h)	Mp (°C)	Isolated yield (%)
3a·$2H_2O$	5.0	20.0 (DFIT)	42	50	14.2	127–129[d]	50
3b·H_2O	2.4	9.7	19	12	24	92–93	56[e]
3c	5.1	21.3	37	20	4	Oil	32[f]
4d	5.1	20.8	42	17	4	57–58[d]	25[g]
4e[b]	3.2	13.3	26	20	5	Oil	80[g]

[a] C,H (Calc: Found): **3a**·$2H_2O$ (55.98, 4.7; 56.3, 5.1), **3b**·H_2O (48.98, 7.88; 49.3, 7.5), **4d** (54.84, 8.92; 55.1, 9.1). [b] Minor impurities by NMR, mono- and tris-ketol phosphates among them. [c] Added volumetrically, mmol not corrected for impurities in SEE. [d] Mp and C,H analyses on samples from other preps. [e] Phl(OAc)$_2$ recovered (4.16 mmol), yield based on unrecovered Phl(OAc)$_2$. [f] Two fractions collected, yield based on first fraction (*ca.* 80 mass % pure) and corrected for purity. [g] Based on starting PPA.



(1)

(2)

Table 2 Conditions and yields for selected preparations of bis-ketol AZT monophosphates

Compd.[a]	Reactants (mmol) AZT	3 or 4	Et_3N	DCC	Vol (cm^3) CH_2Cl_2	Time (h)	Mp (°C)	Isolated yield (%)
1a•H_2O	1.0	1.2	1.3	—	22	7.33	61–63	72
1b	0.72	0.73	1.1[b]	—	10	46	55–56.5	28
1c•H_2O	1.0	1.6	1.5	2.3[c]	15	Overnight	59.5–61	85
1d	1.0	2.8	2.1(5)	3.9	15	16[d]	Oil	66
1e	1.0	~4	3.6	6.1(5)	20	3.6[d]	Syrup	71

[a] C,H analyses within ±0.29%, N analyses within ±0.49%. Except for **1b**, elemental analyses and mp on samples from other preps. [b] DMAP (23 mg) added after 22 h. [c] DCC added to convert any **4c** present in the sample into product. [d] After Et_3N was introduced.

3a•$2H_2O$ (1.2 mmol) and Et_3N (1.3 mmol) in CH_2Cl_2 (22 cm^3) was stirred at room temperature for 7.33 h and then concentrated to afford a thick yellow oil. Chromatography of the oil on silica gel with hexanes–EtOAc gave a 'foamy' solid comprising **1a** and unchanged AZT (*ca.* 15 mol%). Extraction of this material (in CH_2Cl_2) with H_2O (*i.e.*, to remove AZT) gave **1a**•H_2O in 72% yield.

The bis-ketol AZT monophosphates **1d** and **1e** were prepared by the treatment of AZT–**4d** and AZT–**4e** mixtures first with dicyclohexylcarbodiimide (DCC) and then with triethylamine (room temp., CH_2Cl_2). For example, a mixture of AZT (1.0 mmol), **4d** (2.8 mmol) and DCC (3.9 mmol) in CH_2Cl_2 (15 cm^3) was stirred for 4 h at room temperature. Et_3N (2.15 mmol) was then introduced, and stirring was continued for 16 h (*i.e.*, no AZT left by TLC). A solid component was filtered off and the filtrate was concentrated. Chromatography of the residual material (silica gel, hexanes–EtOAc) gave a yellow oil identified as **1d** (66% yield).

Yields and conditions for selected preparations of **1a–e** are given in Table 2. All of these compounds have been characterized by IR, NMR (^1H, ^{13}C, ^{31}P) and elemental (CHN) analysis.

The bis-ketol AZT monophosphates were examined at the NCI for *in vitro* anti-HIV activity with susceptible T4 lymphocytes. Testing was done by the formazan assay method[4] on a CEM-SS host cell line infected with a cell-free preparation of HIV-1 (RF variant). With this system, AZT, the parent nucleoside, is reported[4] to exhibit an EC_{50} of 5×10^{-8} mol dm^{-3} and an $IC_{50} > 1 \times 10^{-3}$ mol dm^{-3} ($TI_{50} > 2 \times 10^4$). The EC_{50} values of **1a–e**, given in Table 3, were determined from plots of percent cellular protection *versus* $\log_{10}$ [**1**] for eight concentrations (*i.e.*, ranging from 2.0×10^{-6} to 6.3×10^{-10} mol dm^{-3}) of each masked nucleotide.‡ The IC_{50} values for uninfected cells were determined similarly (*i.e.*, for eight concentrations of each masked nucleotide ranging from 2.0×10^{-4} to 6.4×10^{-6} mol dm^{-3}).

The bis-ketol AZT monophosphates show excellent anti-HIV-1 activity [EC_{50}'s from 8.0×10^{-9} to $< 6.3 \times 10^{-10}$ mol

‡ Two preliminary anti-HIV assays for each bis-ketol AZT monophosphate, with the same set of concentrations employed for the IC_{50} determinations, were also conducted. In 8 of 10 cases, the results were consistent with EC_{50} values $< 6.4 \times 10^{-8}$ mol dm^{-3} (the lowest concentration of **1a–e** tested). In one of the plots for **1d**, the cellular protection corresponding to [**1d**] = 6.4×10^{-8} mol dm^{-3} fell below 50% ($EC_{50} = 9.8 \times 10^{-8}$ mol dm^{-3}). One of the plots for **1a**•H_2O contains an obviously deviant point (slightly less than 50% protection) at $\log$[**1a**•H_2O] = −5.7, but otherwise indicates an EC_{50} value $< 6.4 \times 10^{-8}$ mol dm^{-3}. The EC_{50}'s given in Table 3 are more reliable.

Table 3 Anti-HIV-1 activity of bis-ketol AZT monophosphates

Compound	IC_{50} (mol dm^{-3})[a,b]	EC_{50} (mol dm^{-3})[c]	TI_{50}
1a·H_2O	1.1×10^{-4}	7.3×10^{-9}	1.5×10^{4}
		2.5×10^{-9}	4.4×10^{4}
1b	$> 2.0 \times 10^{-4}$	8.0×10^{-9}	$> 2.5 \times 10^{4}$
		1.1×10^{-9}	$> 1.8 \times 10^{5}$
1c·H_2O	8.6×10^{-5}	4.5×10^{-9}	1.9×10^{4}
		1.7×10^{-9}	5.1×10^{4}
1d	$> 1.4 \times 10^{-4}$	1.8×10^{-9}	$> 7.8 \times 10^{4}$
		$< 6.3 \times 10^{-10}$	$> 2.2 \times 10^{5}$
1e	$> 2.0 \times 10^{-4}$	5.4×10^{-9}	$> 3.7 \times 10^{4}$
		$< 6.3 \times 10^{-10}$	$> 3.2 \times 10^{5}$

[a] The concentration of **1** at which 50% of uninfected cells are killed. This is related to the growth inhibitory properties of the compound. [b] Average of two determinations. [c] The concentrations of **1** at which 50% of HIV-1 treated cells survive; *i.e.*, the *in vitro* anti-HIV-1 activity of the compound.

dm^{-3}], and, although they appear to be more cytotoxic than AZT, exhibit high therapeutic indices (*ca.* 10^4 to 10^5). The origin of their anti-HIV activity has not yet been ascertained. However, it seems plausible that intracellular hydrolytic unmasking of the bis-ketol nucleotides might generate AZT monophosphate, perhaps more efficiently than the enzymatic phosphorylation of AZT.[5] This mode of action has been proposed in the literature for other nucleoside phosphotriesters.[6]

Acknowledgements

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