New and Efficient Approach to Aryl Phosphoramidate Derivatives of AZT/d4T as Anti-HIV Prodrugs

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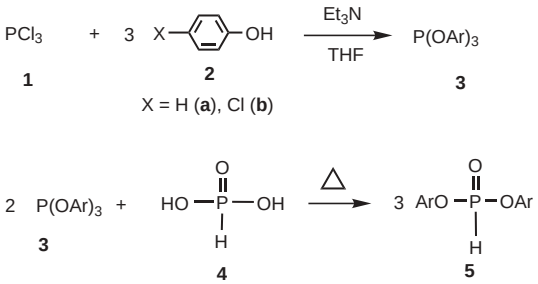
Abstract: Ester exchange of diaryl phosphite with AZT or d4T produced aryl AZT/d4T *H*-phosphonate, and the following reaction with amino acid esters in the presence of hexachloroethane and triethylamine yielded membrane-soluble anti-HIV prodrugs aryl phosphoramide derivatives of AZT and d4T in good yields.

Key words: aryl phosphoramidate, anti-HIV, AZT, d4T

Some dideoxynucleosides (ddNs) have emerged as efficient drugs against human immunodeficiency virus (HIV),[1–8] and the anti-retroviral effects of these compounds involve their conversion, through cellular enzymes, to the corresponding 5′-triphosphates (ddNTPs) which interact with HIV-associated reverse transcriptase. The ddNTPs may act as competitive inhibitors, preventing the incorporation of the natural substrates (dNTPs), or as alternate substrates incorporated in the growing DNA chain, leading to termination of the newly synthesized viral nucleic acid.[9] However, in many cases the unnatural ddNs have poor affinity for nucleoside kinases,[10] one possibility to improve the efficiency of ddNs could be to bypass the phosphorylation steps. Unfortunately, these polar nucleotides are not able to cross the cell membrane efficiently,[11] and they are readily dephosphorylated in extracellular fluids and on cell surfaces by nonspecific phosphohydrolases.[12–15] Hence, the strategies of temporarily masking or reducing the phosphate negative charges of nucleoside 5′-monophosphates (NMPs) with neutral substituents to prodrugs are used, these prodrugs would be freed to the corresponding NMPs once inside the cell. McGuigan et al. have designed and synthesized aryl phosphoramidate derivatives of AZT and d4T which show good efficacy against HIV.[16–18] The typical synthesis of the phosphoramidate derivatives is from sequential reactions of phosphoryl chloride with phenol or *p*-substituted phenol, amino acid methyl ester and nucleoside,[16] however, the method usually needs a long reaction time and absolute water-free condition, and sometimes yields are very low. So it is necessary to develop a simple, rapid and general approach to aryl phosphoramidate derivatives.

We realize that Atherton–Todd reaction is an efficient method for the construction of P-N bond from phosphite-containing P-H bond, however, to our knowledge, it was only used for synthesis of dialkoxylphosphoramidates from *H*-dialkoxylphosphonates, there are no reports about preparation of aryl phosphoramidates from aryl *H*-phosphonates. We attempted the following procedure: esterchange reaction of diphenyl phosphite with AZT produced phenyl AZT 5′-*H*-phosphonate diester, and the following Atherton–Todd reaction with phenylalanine methyl ester in the presence of tetrachloromethane and triethylamine only yielded trace amount of phenyl methoxyphenylalaninyl phosphate of AZT. When hexachloroethane replaced tetrachloromethane in the Atherton–Todd reaction above, a target product was obtained in good yield. The result encouraged us to develop a general method for synthesis of aryl phosphoramide derivatives of AZT and d4T.



Scheme 1 Synthetic route of diaryl phosphites

The synthetic route of diaryl phosphite is shown in Scheme 1 according to known procedures.[19,20] Reaction of 3.0 equivalents of phenol or *p*-chlorophenol with phosphonyl chloride in THF at room temperature almost quantitatively yielded triphenyl or tri-*p*-chlorophenyl phosphite in the presence of triethylamine. After two hours, triethylamine hydrochloride was filtered, the solution was concentrated by rotary evaporation, and the corresponding triphenyl phosphite was obtained. The amount of 0.5 equivalents of phosphoric acid was added to triaryl phosphite, the mixture was heated at 70 °C for one hour and at 150 °C for three hours. The diaryl phosphite was quantitatively transferred, and it could be used for the following reaction without any purification. AZT or d4T in dry THF was added dropwise to 1.5 equivalents of diaryl

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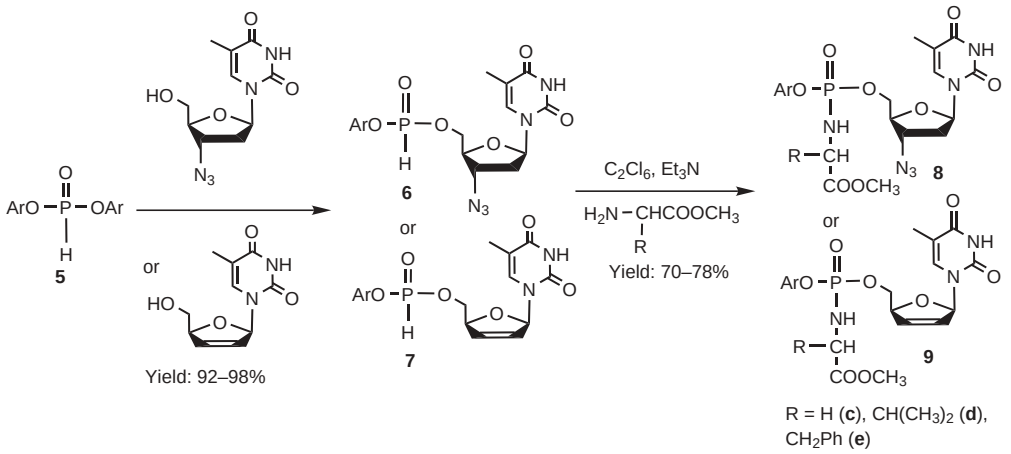


Scheme 2 Synthetic route of aryl phosphoramidate derivatives of AZT and d4T

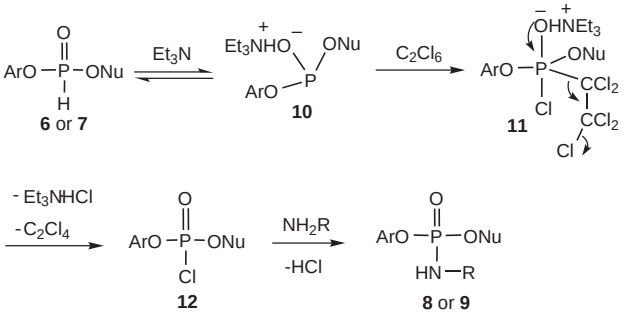
phosphite and a catalytic amount of triethylamine (about 0.2 equiv) in dry THF at –5 °C. The ester-change reaction of diaryl phosphite with nucleoside completed within 20 minutes. Amino acid methyl ester, triethylamine and CH_2Cl_2 solution of hexachloroethane were sequentially added to the reaction solution above, and the Atherton–Todd reaction produced the target products, aryl phosphoramides derivatives of AZT and d4T, in good yields as shown in Table 1. The synthetic route to the target products is shown in Scheme 2, and their structure was identified by ^{31}P NMR, ^{1}H NMR, ^{13}C NMR and ESI-MS[21] after isolation by silica gel column chromatography using EtOAc–MeOH (40:1) as eluent.

Table 1 ^{31}P NMR and Yields of the Synthesized Compounds

Compound	Ar	NuOH	R	^{31}P NMR (δ/ppm)	Yield (%)[a]
8ac	Ph	AZT	H	4.11, 4.34	64
8ad	Ph	AZT	*iso*-Propyl	4.29, 4.56	72
8ae	Ph	AZT	Benzyl	3.99, 4.13	67
9ac	Ph	d4T	H	4.37, 4.98	65
9ad	Ph	d4T	*iso*-Propyl	4.23, 4.77	71
9ae	Ph	d4T	Benzyl	3.30, 3.70	67
8bc	*p*-Cl-Ph	AZT	H	4.63, 4.99	63
8bd	*p*-Cl-Ph	AZT	*iso*-Propyl	4.49, 4.75	68
8be	*p*-Cl-Ph	AZT	Benzyl	3.45, 3.89	60
9bc	*p*-Cl-Ph	d4T	H	4.31, 4.72	69
9bd	*p*-Cl-Ph	d4T	*iso*-Propyl	4.42, 4.90	67
9be	*p*-Cl-Ph	d4T	Benzyl	3.36, 3.91	72

[a] Isolated yield of the last two steps.

A possible formation mechanism of aryl phosphoramidate derivatives of AZT/d4T was proposed in Scheme 3, and it could be similar to the pathway of the classical Atherton–Todd reaction using tetrachloromethane as chlorination reagent.[22] Aryl nucleoside 5′-*H*-phosphonate diester could transferred into tricoordinated phosphite (**10**) in the presence of triethylamine, reaction of **10** with hexachloroethane formed pentacoordinated phosphane intermediate **11**, and then **11** changed into the corresponding chlorophosphate **12** with releasing tetrachloroethylene and triethylamine hydrochloride. The following reaction of **12** with amino acid methyl ester yielded aryl phosphoramidate derivatives of AZT/d4T.



Scheme 3 Proposed formation mechanism of aryl phosphoramidate derivatives

In summary, ester-change reaction of diaryl phosphite with AZT or d4T in THF–CH_2Cl_2 almost quantitatively yielded asymmetric nucleoside *H*-phosphonate diesters, the following Atherton–Todd reaction using hexachloroethane as chlorination agent produced aryl phosphoramidate derivatives of AZT/d4T in good yields under mild conditions. The method is rapid, convenient and efficient, and it could be used for preparation of other phosphoramidates.

General Procedure for Preparation of Aryl Phosphoramidate Derivatives.

Diaryl phosphite (1.5 mmol; about 85%, 0.41 g) and a catalytic amount of Et_3N (about 3 drops) were dissolved in 5 mL of dry THF and cooled to –5 °C. AZT (1.0 mmol, 0.27 g) in 5 mL of dry THF was added dropwise to the solution at this temperature under the

nitrogen atmosphere. The solution was stirred about 20 min and warmed from –5 °C to r.t. during this process. At –5 °C, 1.1 mmol of amino acid methyl ester hydrochloride, Et_3N (TEA, 0.31 g, 3.0 mmol) and CH_2Cl_2 solution (3 mL) of hexachloroethane (0.85 g, 3.6 mmol) were sequentially added to the resulting solution, and the solution was stirred about 2 h. The solvent was removed under reduced pressure, and the remaining gum was dissolved in 10 mL of $CHCl_3$, washed with sat. $NaHCO_3$ solution (10 mL), 1 M HCl (10 mL) and distilled H_2O (2 × 15 mL). The organic phase was dried over anhyd Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc–MeOH (40:1), the solvent was evaporated by rotary distillation, and the target product was obtained as white foam.

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(21) Analytical data of representative compound **9ae** [2′,3′-dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyphenylalaninyl phosphate)]: ^{13}P NMR (121 MHz, $CDCl_3$): δ = 3.30, 3.70 (a pair of diastereomers, ratio of peak area = 4:6) ppm. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.78 (s, 3 H, 5-Me), 2.94–2.99 (m, 2 H, CH_2Ph), 3.64 (s, 3 H, OMe), 3.84–4.19 (m, 4 H, Phe-C*H*, Phe-N*H*, 2H-5′), 4.89 (m, 1 H, H-4′), 5.82–5.88 (m, 1 H, H-3′), 6.20–6.29 (m, 1 H, H-2′), 7.12–7.27 (m, 12 H, 2 Ph, H-1′, H-6), 9.43 (s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.21 (C5), 40.30 (*C*H_2Ph), 52.29 (OCH_3), 55.45, 55.73 (Phe-*C*H), 66.20, 66.87 (C-5′), 84.36, 84.58 (C-4′), 89.42, 89.67 (C-1′), 111.20, 111.31 (C5), 119.91, 119.98 (OPh-*ortho*), 125.06 (OPh-*para*), 127.11, 127.21 (C-2′), 128.49, 129.34, 129.63 (CH_2*Ph*), 129.34, 129.63 (OPh-*meta*), 133.18–133.50 (C-3′), 135.46 (6-C), 150.18, 150.29 (OPh-*ipso*), 150.86 (C2), 163.84 (C4), 172.72 (Phe-CO). ESI-MS: *m/z* = 542 $[M + H]^+$, 564 $[M + Na]^+$.
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