One-Pot Synthesis of Aryl Phosphoramidate Derivatives of AZT/d4T as Anti-HIV Prodrugs

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Abstract: Arbuzov reaction of aryl phosphorodichloridite with mixture of one equivalent of AZT or d4T and one equivalent of *tert*-butyl alcohol led to the corresponding AZT/d4T aryl *H*-phosphonate diesters, and the following reaction of the *H*-phosphonate diesters with amino acid methyl esters in the presence of *N*-chlorosuccinimide (NCS) produced membrane-soluble anti-HIV prodrugs AZT/d4T aryl phosphoramidate derivatives in good yields.

Key words: aryl phosphoramidate derivatives, anti-HIV prodrugs, *N*-chlorosuccinimide (NCS), AZT, d4T

Some dideoxynucleosides (ddNs) including 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine)[1] and 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T, stavudine)[2] have emerged as efficient drugs against human immunodeficiency virus (HIV),[3] 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. Specifically, the first phosphorylation step for d4T is the rate-limiting step, whereas for AZT phosphorylation to the nucleoside 5′-diphosphate is the slowest step in the phosphorylation pathway.[4] However, in many cases the unnatural ddNs have poor affinity for nucleoside kinases,[5] 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,[6] and they are readily dephosphorylated in extracellar fluids and on cell surfaces by nonspecific phosphohydrolases.[7] 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 developed the phosphoramidate diester prodrugs of 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A), 2′,3′-dideoxyadenosine (ddA)[8a] and d4T,[8b–d] which exhibited greatly enhanced activity against HIV compared to their parent nucleoside analogues in vitro, and full activity was retained in kinase-deficient cell lines.[8] So the development of nucleoside prodrugs capable of undergoing intracellular activation to the corresponding nucleotides has become an area of intense interest.[9] Recently, we have developed the methods for synthesis of the nucleoside phosphporamidate monoesters using nucleoside di-, triphosphates[10,11] or fluorenylmethyl nucleoside 5′-*H*-phosphonates[12] as starting materials. The typical synthesis of the nucleoside phosphoramidate diesters is from sequential reactions of phosphoryl chloride with phenol or *p*-substituted phenol, amino acid methyl ester and nucleoside,[13] however, the intermediates aryl chlorophosphoramidates are of low reactivity, the following reaction with nucleosides usually needs a long reaction time, and sometimes yields are very low. Here, we would like to report a new method for synthesis of aryl phosphoramidate derivatives.

Atherton–Todd reaction is a convenient and efficient method for synthesis of phosphoramidates from *H*-phosphonates, however, to the best of our knowledge, it is only suitable for synthesis of dialkyl phosphoramidates from dialkyl *H*-phosphonates. More recently, we reported a method for the preparation of aryl phosphoramidates through the reaction of aryl *H*-phosphonates with amino acid methyl esters in the presence of hexachloroethane (C_2Cl_6) and triethylamine.[14] However, nucleoside aryl *H*-phosphonates were from the ester-exchange reaction of diaryl phosphite with nucleosides in pyridine, in which an excess of diaryl phosphite (1.5 equiv) was needed, otherwise, more di-substituted products, the dinucleoside *H*-phosphonates, were observed. The following in situ reaction of two kinds of *H*-phosphonates (nucleoside aryl *H*-phosphonate and diaryl phosphite) with an excess of amino acid methyl ester (1.1 equiv) in the presence of an excess of C_2Cl_6 (3.6 equiv) produced two kinds of phosphoramidates and complex side-products containing phosphates. Here, we have developed an improved and cheaper procedure for synthesis of aryl phosphoramidates of nucleosides. We attempted the following procedure: three-component reaction of aryl phosphorodichloride[15] at –5 °C with one equivalent of nucleoside (AZT or d4T) and one equivalent of *tert*-butyl alcohol yielded nucleoside aryl *H*-phosphonate in about 86% yield (^{31}P NMR determination) while small amount of dinucleoside phosphite (^{31}P NMR: δ = ca. 9.50 ppm, 2–7%) and the other unknown phosphate side product (^{31}P NMR: δ = ca. 0.30 ppm, 5–10%) appeared. Reaction of phenyl AZT 5′-

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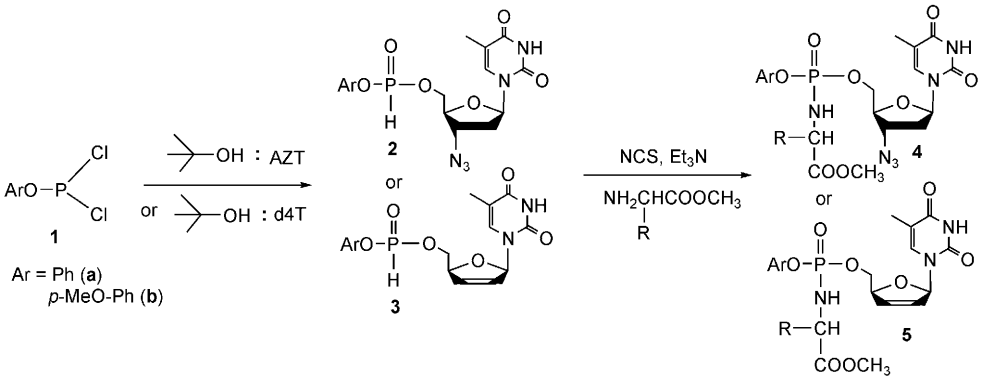
H-phosphonate diester with phenylalanine methyl ester in the presence of *N*-chlorosuccinimide (NCS) and triethylamine yielded phenyl methoxyphenylalaninyl phosphoramidate of AZT in 70% yield. Encouraged by the good result, we developed a general method for synthesis of aryl phosphoramidate derivatives of AZT and d4T (Scheme 1, Table 1). The ^{31}P NMR spectroscopy recorded the overall progress of the reactions. For example, reaction of *p*-methoxyphenyl phosphorodichloridite (^{31}P NMR: δ = 178.40 ppm) with a mixture of one equivalent of AZT and one equivalent of *tert*-butyl alcohol yielded the AZT *p*-methoxyphenyl *H*-phosphonate diester (^{31}P NMR: δ = 6.33, 5.90 ppm, a pair of diastereomers 1:1, $^{1}J_{P-H}$ = ca. 720 Hz) in about 86% yield (^{31}P NMR determination), and the following reaction of the *H*-phosphonate diester with alanine methyl ester in the presence of NCS almost quantitatively produced AZT *p*-methoxyphenyl phosphoramidate (^{31}P NMR: δ = 4.88, 4.10 ppm, a pair of diastereomers 1:1).

In summary, the three-component Arbuzov reaction of aryl phosphorodichloridite with mixture of one equivalent of AZT or d4T and one equivalent of *tert*-butyl alcohol yielded the corresponding AZT/d4T aryl *H*-phosphonate diesters, and the following treatment of the *H*-phosphonate diesters with different amino acid methyl esters in the presence of NCS produced AZT/d4T aryl phosphoramidate derivatives in good yields under mild conditions. The method is rapid, convenient and efficient, and could be used for the preparation of other phosphoramidates.

General Procedure for the Preparation of Aryl Phosphoramidate Derivatives

To 1 mmol of aryl phosphorodichloridite in CH_2Cl_2 (5 mL) was added dropwise 1 mmol of AZT/d4T, 1 mmol of *t*-BuOH and 1 mmol of Et_3N in THF (25 mL) at –5 °C within 40 min under nitrogen atmosphere, and the solution was stirred at 0 °C for another 30 min. To this solution were added 1 mmol of amino acid methyl ester



Scheme 1 Synthetic route of aryl phosphoramidate derivatives of AZT/d4T

Table 1 ^{31}P NMR and Total Yields of the Synthesized Compounds **4** and **5**

Compd	Ar	NuOH	R	^{31}P NMR (δ/ppm)	Yield (%)[a]
4ac	Ph	AZT	CH_3	3.73, 3.41	70
4ad	Ph	AZT	*i*-Pr	4.56, 4.29	71
4ae	Ph	AZT	H	4.67,4.34	63
5ac	Ph	d4T	CH_3	3.87, 3.19	72
5ad	Ph	d4T	*i*-Pr	4.77, 4.23	69
5ae	Ph	d4T	Benzyl	3.70, 3.30	70
4bc	*p*-MeO–Ph	AZT	CH_3	4.08,3.81	78
4bd	*p*-MeO–Ph	AZT	H	5.14, 4.85	69
5bc	*p*-MeO–Ph	d4T	CH_3	4.35, 3.70	77
5be	*p*-MeO–Ph	d4T	Benzyl	4.08, 3.06	79

[a] Isolated overall yield from **1** to **4** or **5**.

and 1 mmol of NCS, respectively, 4 mmol of Et_3N in CH_2Cl_2 (15 mL) was added dropwise at –5 °C within 1 h, and then the solution was warmed to r.t. and stirred for another 2 h at r.t. The solvents were removed by rotary distillation. Then, 5 mL of EtOAc was added to the residue, and the triethylamine hydrochloride was filtered. The filtrate was concentrated, and the residue was purified by silica gel column chromatography using EtOAc–MeOH (40: 1) as eluent. The corresponding aryl phosphoramidate derivative of AZT/d4T was obtained as white or yellow foam in moderate to high yield.

Characterization data of two representative compounds are shown as follows:

Compound 4ac

Yield 70%. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 3.73, 3.41 ppm (a pair of diastereomers, 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ = 9.73 (br, 1 H, NH-3), 7.36–7.20 (m, 6 H, H-6 and phenyl), 6.21–6.13 (m, 1 H, H-1′), 4.35–4.20 (m, 4 H, H-3′, H-4′ and H-5′), 4.10–3.95 (m, 2 H, NH and CH of Ala), 3.71 (d, 3 H, OCH_3), 2.39–2.31 (m, 1 H, H-2′), 2.24–2.19 (m, 1 H, H-2′), 1.89 (d, 3 H, CH_3-5), 1.37 (m, 3 H, CH_3 of Ala) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 164.1, 150.5, 135.5, 129.9, 125.4, 120.1, 111.5, 85.0, 82.4, 65.8, 60.3, 52.7, 50.4, 37.4, 21.0, 12.5 ppm. ESI-MS: *m*/*z* = 509.2 $[M + H]^+$. HRMS: *m*/*z* calcd $[M+H]^+$: 509.1550; found: 509.1558.

Compound 5bc

Yield 77%. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 4.35, 3.70 ppm (a pair of diastereomers, 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ = 9.25 (d, 1 H, NH-3), 7.34–7.28 (m, 1 H, H-6), 7.13–7.07 (m, 3 H, H-1′ and phenyl), 6.84–6.80 (m, 2 H, phenyl), 6.37–6.27 (m, 1 H, H-2′), 5.89 (t, 1 H, H-3′), 5.02 (br, 1 H, H-4′), 4.37–4.29 (m, 2 H, H-5′), 4.01–3.97 (m, 1 H, NH of Ala), 3.87–3.84 (m, 1 H, CH of Ala), 3.77 (s, 3 H, OCH_3), 3.71 (d, 3 H, OCH_3), 1.84 (d, 3 H, CH_3-5), 1.34 (m, 3 H, CH_3 of Ala) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.2, 164.0, 156.9, 151.0, 143.9, 135.9, 133.3, 127.5, 121.2, 114.7, 111.4, 89.8, 84.8, 66.8, 55.7, 52.7, 50.2, 21.0, 12.4. ESI-MS: *m*/*z* = 496.1 $[M + H]^+$. HRMS: *m*/*z* calcd $[M + H]^+$: 496.1485; found: 496.1473.

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