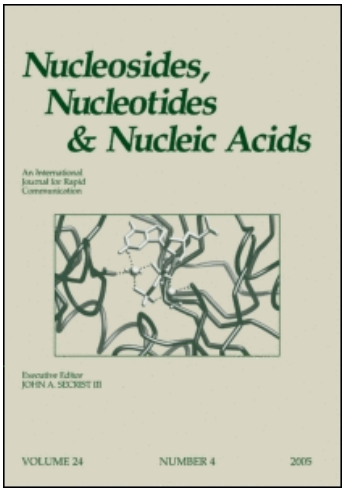

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5′-CARBAMOYLPHOSPHONYL-[6-^{3}H]-AZT AS A TOOL FOR STUDYING METABOLIC TRANSFORMATIONS OF THE NONRADIOACTIVE COUNTERPART, AN INHIBITOR OF HIV REPLICATION

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□ *An effective synthesis of 5′-carbamoylphosphonyl-[6-^{3}H]-AZT was developed from [6-^{3}H]-AZT. For the synthesized compound, chemical and enzymatic stability were determined and its penetration across HL-60 cell membranes was studied.*

Keywords 3′-Azido-3′-deoxythymidine; human immunodeficiency virus; nucleoside phosphonates; 5′-carbamoylphosphonyl-3′-azido-3′-deoxythymidine

At present 3′-azido-3′-deoxythymidine (AZT, Retrovir) is one of the most popular nucleoside-based drugs used in anti-HIV therapy. For the prevention of AZT 5′ glucuronidation, which is one of the major elimination pathways of AZT, numerous AZT derivatives containing various protective groups at the 5′ position were designed.[1] A good example of modifications of this type is AZT 5′-H-phosphonate (phosphazide, Nikavir), which has been approved in Russia as an anti-HIV drug. Following oral administration, it slowly releases AZT thus being an AZT depot form.[2]

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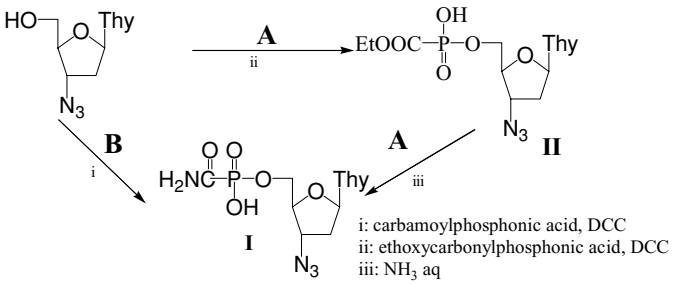

SCHEME 1

The goal of our work was the design of 5′-modified AZT derivatives with increased stability towards blood and hepatic hydrolyzing enzymes and the ability to be metabolized to give the active nucleoside in the cells. We drew our attention on the group of AZT 5′-carbamoylphosphonates, which bear a negative charged phosphonate residue similarly to phosphazide, manifest anti-HIV activity comparable to that of phosphazide but lower toxicity[3] and are supposed to be more stable in organism than phosphazide. For the study of metabolic transformations of AZT 5′-carbamoylphosphonate (**I**) we developed the synthesis of its counterpart labeled with ^{3}H at the 6-position of thymine. The use of the labeled compound allowed reliable detection of metabolites at the concentrations of about 10^{-9} M. In this work we also present the results of stability studies and preliminary data on penetration of [^{3}H]-**I** into HL-60 cells.

As a starting compound, we used [6-^{3}H]AZT with a specific activity of 20 Ci/mmol (obtained from the Institute of Molecular Genetics, Russian Academy of Sciences). Two synthetic routes can be used for the preparation of phosphonate **I** (Scheme 1). From the point of view of the radioactive synthesis, the major drawback of method A previously used for the synthesis of the nonradioactive counterpart[3,4] is the involvement of the radioactive component as early as at the first stage of the two-stage synthesis. Therefore, we started from method **B** based on the coupling of [6-^{3}H] AZT with the preliminarily prepared carbamoylphosphonic acid.

According to method **B**, commercial triethyl phosphonoformate was successively treated with 32% aqueous ammonia, bromotrimethylsilane, and water to give carbamoylphosphonic acid. The coupling of the latter with [6-^{3}H]AZT in the presence of DCC (DMF, 20°C, 18 hours) resulted in 16–18% of target [6-^{3}H]-**I**. With the aim to increase the yield of the nucleoside coupling with the phosphonic component, we tried to adjust method A[3] to the conditions of radioactive syntheses (low concentrations of the radioactive component, and large excesses of coupling agents and the phosphonic component).

TABLE 1 The effect of NH_3 concentration on product ratio (20°C, 18 hours)

Product,%[a]	32% NH_3 aq	25% NH_3 aq	10% NH_3 aq
AZT 5′-carboxyphosphonate	5	9.5	16
[6-^{3}H]-**I**	91	86	50

[a]Total radioactivity (100%) is the summary of radioactivities of unreacted [6-^{3}H]-**II** and products.

The variation of coupling agents (Piv-Cl, TPS-Cl and BrCN) did not result in satisfactory product yields. Therefore, for the synthesis of target [6-^{3}H]-**I** we developed a one-pot procedure involving the interaction of [6-^{3}H] AZT and free ethoxycarbonylphosphonic acid in the presence of DCC followed by aminolysis. Under optimal conditions (DMF, 40°C, 12 hours, 10 eq. ECPA, 100 eq. DCC) the yield of nucleotide [6-^{3}H]-**II** achieved 45–50% (HPLC data). Aminolysis of [6-^{3}H]-**II** was carried out with aqueous ammonia, the product yield and product composition being considerably affected by the ammonia concentration (Table 1) and reaction temperature (Table 2).

As a result, the aminolysis reaction was carried out in the presence of 32% ammonia at 0°C for 18 hours. The overall yield of target [6-^{3}H]-**I** after isolation by reversed-phase HPLC was 17–25%, radioactive purity 96%, and specific activity 20 Ci/mmol.

Chemical stability of the synthesized phosphonate **I** at varied pH is shown in Table 3.

Enzymatic stability of compound **I** (4 μM) was studied at 37°C in whole dog blood, rabbit liver homogenate, and rabbit liver clarified lyzate. The experiments showed that phosphonate **I** is not hydrolyzed in blood for at least 24 hours. The half-life in the clear lyzate was 16 hours (versus 2.5 hours for phosphazide), whereas in the homogenate it was only 3 hours (versus 35 minutes for phosphazide).

Compound [^{3}H]-**I** was used for studying intracellular uptake in human HL-60 cells. It was found that the penetration rate of phosphonate [^{3}H]-**I** only achieved about one-tenth of that of parent AZT. It also was demonstrated that phosphonate [^{3}H]-**I** is effectively hydrolyzed by cellular enzymes after penetration. Particularly, after 24 hours incubation, the ratio of the intact phosphonate [^{3}H]-**I** and the metabolites in the cells achieved 16:84. A

TABLE 2 The effect of temperature on product ratio (32% NH_3, 20 hours)

Product,%[a]	−18°C	0°C	18°C	40°C
AZT 5′-carboxyphosphonate	< 1	2.1	4.5	8
[6-^{3}H]-**I**	45	85	89	92

[a]Total radioactivity (100%) is the summary of radioactivities of unreacted [6-^{3}H]-**II** and products.

TABLE 3 Half-lives of phosphonate **I**[a] and the product ratio at varied pH (37°C)

pH	2.0	7.2	8.5
$T_{1/2}$	28	>48	>48
AZT/phosphazide	60/40	—	—

[a] 4 μM.

major intracellular metabolite was AZT 5′-monophosphate, likely resulted from degradation of **I** to AZT followed by kinase-catalyzed phosphorylation of the latter.

To summarize, we developed an effective method of synthesis of 5′-carbamoylphosphonyl-[6-^{3}H]AZT of high radioactive purity and high specific activity. It was stable in different biological media and was hydrolyzed in the HL-60 cell line to give the parent AZT. Thus, similarly to phosphazide, phosphonate **I** is an AZT prodrug, but more enzymatically stable. Considerably lower anti-HIV activity and toxicity of compound **I** compared to those of AZT are in good agreement with low efficacy of its penetration across cell membranes.

REFERENCES

1. Parang, K.; Wiebe, L.I.; Knaus, E.E. Novel approaches for designing 5′-O-ester prodrugs of 3′-azido-2′,3′-dideoxythymidine (AZT). *Curr. Med. Chem.* **2000**, 7(10), 995–1039.
2. Skoblov, Yu.S.; Karpenko, I.L.; Shirokova, E.A.; Popov, K.E.; Andronova, V.A.; Galegov, G.V.; Kukhanova, M.K. Intracellular metabolism and pharmacokinetics of 5′-hydrogenphosphonate of 3′-azido-2′,3′-dideoxythymidine, a prodrug of 3′-azido-2′,3′-dideoxythymidine, *Antiviral Res.* **2004**, 63, 107–113.
3. Jasko, M.V.; Shipitsyn, A.V.; Khandazhinskaya, A.L.; Shirokova, E.A.; Sol′ev, P.N.; Plyasunova, O.A.; Pokrovsky, A.G. New AZT-containing derivatives of alkyl- and aminocarbonylphosphonic acids. *Bioorg. Khim.* **2006**, 32(6), 542–546.
4. Jasko, M.; Shipitsin, A.; Shirokova, E.; Krayevsky, A.; Polsky, B.; Baron, P.; MacLow, C.; Ostrander, M.; O'Hara, B. 5′-Phosphonates of ribonucleosides and 2′-deoxyribonucleosides: Synthesis and antiviral activity. *Nucleosides Nucleotides*, **1993**, 12, 879–893.