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Research Article

Synthesis of tritium-labelled 3′-azido-3′-deoxythymidine

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Summary

New approaches to the synthesis of 3′-azido-3′-deoxythymidine labelled with tritium in the heterocyclic base have been developed. With this aim, enzymatic transribosylation with [^{3}H]thymine using the enzyme preparation from rat liver and a three-step chemical synthesis with use of the tritium labelled precursor were studies. The enzyme preparation did not catalyse the transfer of the 3′-azido-3′-deoxyribosyl fragment to the [^{3}H]thymine residue. 5′-*O*-Benzoyl-2,3′-anhydrothymidine was taken as a precursor for the tritium labelling by the chemical methods. The resulting [^{3}H]3′-azido-3′-deoxythymidine was obtained with a specific radioactivity of 18.3 Ci/mmol, the tritium is located in the C-6 position of the thymine residue.

Key Words: tritium-labelling; solid and liquid state catalytic hydrogen exchange; 3′-azido-3′-deoxy-[6-^{3}H]thymidine

Introduction

The mechanisms of action, metabolic pathways, and cellular uptake of 3′-azido-3′-deoxythymidine have been intensively studied in recent

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years. These studies require 3′-azido-3′-deoxythymidine labelled in different parts of the molecule and at high specific radioactivity ($A_{mol} > 10$
Ci/mmol).

The synthesis of tritium labelled 3′-azido-3′-deoxythymidine has been achieved by several methods. For example, [5′-^{3}H]3′-azido-3′-deoxythymidine (14 Ci/mmol) was synthesized by the reduction of the corresponding 3′-azido-5′-oxo-3′,5′-dideoxythymidine with tritium labelled sodium borohydride.[1] For the preparation of [methyl-^{3}H]3′-azido-3′-deoxythymidine (35.9 mCi/mmol), a four-step procedure has been used.[2] The starting [methyl-^{3}H]thymidine was protected at position 5′ by a trityl group, transformed to the corresponding [methyl-^{3}H]5′-*O*-trityl-2,3′-anhydrothymidine, treated with sodium azide, and deprotected.

Tritium labelled 3′-azido-3′-deoxythymidine can also be synthesized using the reaction of solid-state catalytic hydrogenation (SSCH).[3,4] It was shown,[5] that catalytic reactions of 3′-azido-3′-deoxythymidine with gaseous tritium in solution result mainly in the formation of the corresponding 3′-aminonucleoside. This process also took place in SSCH reactions to yield 20–70% of 3′-amino-3′-deoxythymidine. It has been shown that the specific radioactivity of the 3′-azido-3′-deoxythymidine obtained in the reaction of solid state catalytic isotope exchange with gaseous tritium and in the reaction of isotope exchange with tritiated water did not exceed 0.5 Ci/mmol. About 90% of the tritium was localized in the thymine part of the molecule.[5]

The present study is concerned with the development of new approaches to the effective synthesis of tritium labelled 3′-azido-3′-deoxythymidine, in which the tritium is localized in the heterocyclic base. Two routes were chosen for the study:

1. Enzymatic synthesis of 3′-azido-3′-deoxythymidine by transdeoxyribosylation reaction of thymine using an enzyme preparation isolated from rat liver[6]:

$$[^3\mathrm{H}]\text{thymine} + 3' - \text{azido} - 3' - \text{deoxythymidine} \leftrightarrow$$
$$[^3\mathrm{H}]3' - \text{azido} - 3' - \text{deoxythymidine} + \text{thymine}$$

2. Chemical synthesis of [^{3}H]3′-azido-3′-deoxythymidine similar to the method described in Zaitseva *et al.*[7] 5′-*O*-Benzoyl-2,3′-anhydrothymidine (I) was chosen as a precursor for the tritium labelling (Figure 1).

We could not find any published data on the enzyme transfer of the 3′-azido-3′-deoxyribosyl residue from one base to another. Therefore,

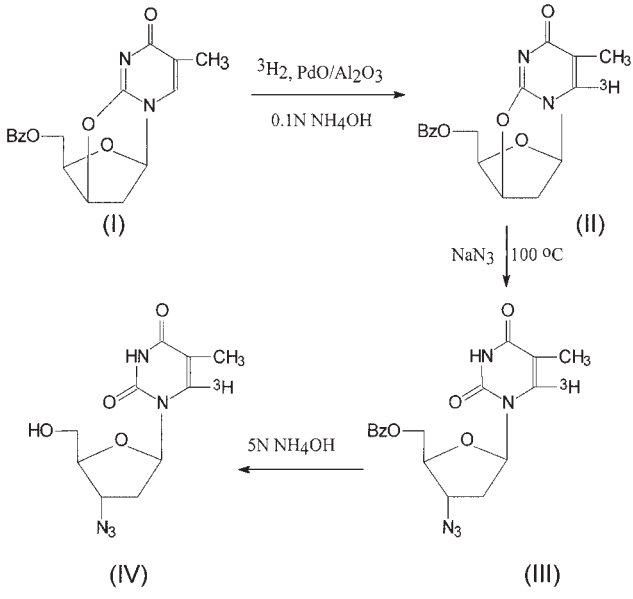

Figure 1. The synthesis of tritium labelled 3′-azido-3′-deoxythymidine. (I) – 5′-*O*-benzoyl-2,3′-anhydrothymidine, (II) – [6-^{3}H]5′-*O*-benzoyl-2,3′-anhydrothymidine, (III) – [6-^{3}H]5′-*O*-benzoyl-3′-azido-3′-deoxythymidine, (IV) – [6-^{3}H]3′-azido-3′-deoxythymidine

we tried to find out whether this reaction can take place and, if the result is positive, optimize the conditions.

The chemical synthesis performed by us was based on the isotopic exchange of 5′-*O*-benzoyl-2,3′-anhydrothymidine (I) with gaseous tritium both in solution and in the solid phase. Further studies were concerned with the optimization of transformation conditions for tritium labelled 5′-*O*-benzoyl-2′,3′-anhydrothymidine (II) to tritium labelled 3′-azido-3′-deoxythymidine taking into consideration the microquantities of labelled compounds involved.

Results and discussion

The use of transribosylation enzyme for the preparation of labelled compounds has been developed into a routine procedure.[6] However, in most cases it has involved the synthesis of natural nucleosides, but for various nucleoside analogues the procedure method needs to be optimized.

The results of the transdeoxyribosylation reaction of thymine with the enzymatic preparation from rat liver are shown in Table 1. The accuracy

Table 1 Some characteristics of the transdeoxyribosilation reaction of [^{3}H]thymine (48 Ci/mmol)

Donor of ribose residue	Molar ratio thymine/donor	A_{mol} of target compound (Ci/mmol)	Conversion (%)
3′-Azido-3′-deoxythymidine	1:4	0.0014	0.012
5-Hydroxymethyl-3′-azido-2′, 3′-dideoxyuridine	1:6	0.0007	0.005
2′-Deoxyuridine	1:4	48	81.0
Thymidine	1:4	7.8	65.0

Table 2 Characteristics of the SSCH reaction of 5′-*O*-benzoyl-2,3′-anhydrothymidine using tritium–protium (1:250) mixture

Solvent	Catalyst	T(°C)	Duration	5′-*O*-benzoyl-2,3′-anhydrothymidine	
				Ci/mol	Yield (%)
No	5%PdO/Al_2O_3	160	30 min	20	47
		170		21	31
		180		22	15
NH_4OH 0.1 N	5%PdO/Al_2O_3	25	20 Hrs	95	33
	5%Pd/$BaSO_4$	25		25	37
	5%Pd/$CaCO_3$	25		75	28
	10%Pd/$CaCO_3$	25		64	30

of the method allowed us to detect less than 0.001% of the maximal enzymatic activity. The data show that the enzymatic preparation is sensitive to the structure of the sugar residue, and in the case of 3′-azido-3′-deoxyribosyl its transfer to another base did not proceed.

The first stage of the chemical synthesis was the preparation of tritium labelled 5′-*O*-benzoyl-2,3′-anhydrothymidine (II, Figure 1) by the SSCH reaction in the presence of gaseous tritium (Table 2). Evidently, an increase in the reaction temperature caused a considerable decrease in the product yield, whereas A_{mol} remained practically constant and did not exceed 21 Ci/mol. The A_{mol} of the preparation (II) obtained by the reaction of catalytic isotope exchange with gaseous tritium in solution was considerably higher and reached a maximum of 95 Ci/mol (or about 20 Ci/mmol for 95% tritium, Table 2).

The next step was the optimization of the synthesis of [^{3}H]5′-*O*-benzoyl-3′-azido-3′-deoxythymidine (III). The kinetic curves for the azidation of [^{3}H]5′-*O*-benzoyl-2,3′-anhydrothymidine (II) with sodium

azide are presented in Figures 2 and 3. Equilibrium had been reached after 15 h and a maximum yield of 36% was achieved when the molar ratio of NaN_3/compound (II) was 16:1 (Figure 3).

In the last stage [^{3}H]5′-*O*-benzoyl-3′-azido-3′-deoxythymidine (III) was treated with 5 M ammonia solution to remove the benzoyl group. After heating at 60°C for 1 h, the reaction was complete to give (IV); the specific radioactivity of the product had decreased by 24% which may be because some of the tritium was located in the benzoyl group.

On the basis of the above described model experiments, we performed a preparative synthesis of tritium labelled 3′-azido-3′-deoxythymidine (IV). Tritium was introduced by the reaction of catalytic isotopic exchange with gaseous tritium in solution. The resulting (IV) was prepared with a specific radioactivity of 18.3 Ci/mmol and radiochemical purity of more than 99%. The chemical yield was 13.5% starting from 5′-*O*-benzoyl-2,3′-anhydrothymidine (I). The product was stored in ethanol-water (1:1 v/v) solution at −20°C, at a radioactive concentration of 1 mCi/ml.

Comparison of the ^{1}H and ^{3}H NMR spectra for compound (IV) show that the singlet at δ 7.63 in the latter spectrum is associated with the H-6 position of the thymine base.

To conclude, unlike the enzymatic reaction of transdeoxyribosylation of 3′-azido-3′-deoxythymidine, the chemical approach allowed the

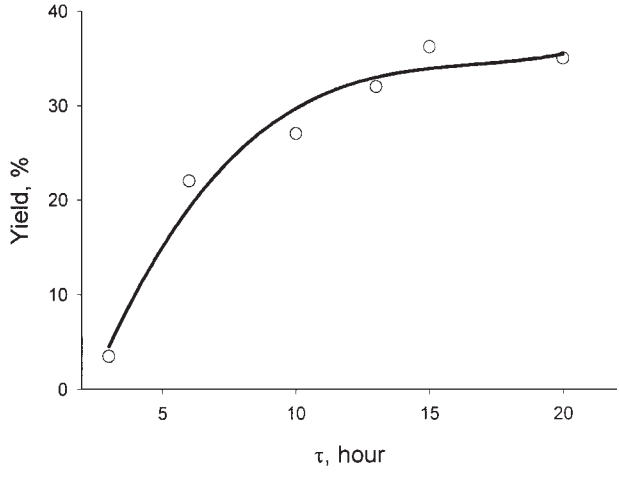

Figure 2. Yield of 5′-*O*-benzoyl-3′-azido-3′-deoxythymidine in a reaction mixture (tenfold surplus NaN_3) as a function of time

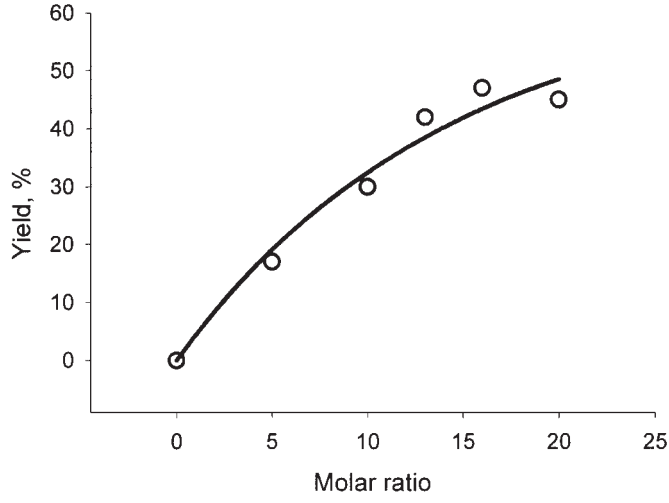

Figure 3. Yield of 5′-*O*-benzoyl-3′-azido-3′-deoxythymidine as a function of NaN_3/compound (II) ratio (reaction time 3 h)

synthesis of [6-^{3}H]3′-azido-3′-deoxythymidine at a high specific radioactivity. The radiochemical purity of the target compound exceeded 99% (by TLC analysis). The radioactive yield was 6.7%.

Experimental

The catalysts used were 5% PdO/Al_2O_3 (RSS 'Applied Chemistry', St. Petersburg), 5% $Pd/BaSO_4$ (Aldrich) and 10% $Pd/CaCO_3$ (Fluka).

5′-*O*-Benzoyl-2,3′-anhydrothymidine (I) was prepared according to Kimuva *et al.*[8] UV absorption spectra were obtained using a Specord M-40 spectrophotometer. The NMR spectra were obtained using D_2O as solvent and a AMX III-400 (Bruker) spectrometer with the 400 MHz operating frequency for ^{1}H and 426 MHz for ^{3}H NMR. Sample radioactivity was measured on a RLC-20 scintillation counter using EcoLM TM scintillation liquid (ICN). Reactions involving gaseous tritium were performed on an installation suitable for manipulations with gaseous tritium.[9] TLC was carried out on Silufol plates (Czech Republic) in chloroform-ethanol (95:5 v/v) solvent systems. The R_f values for reference 5′-*O*-benzoyl-3′-azido-3′-deoxythymidine (I) and 3′-azido-3′-deoxythymidine were 0.35 and 0.15, respectively. For the analysis of 3′-azido-3′-deoxythymidine, plates with PEI-cellulose

(Merck), water solvent (R_f 0.63) were also used. HPLC was conducted using a 13-μm (10 × 250 mm) Nucleosil C18 (Macherey-Nagel) column. The mobile phase for isolation of 5′-*O*-benzoyl-2,3′-anhydrothymidine was 60% methanol in water, elution rate 1.5 ml/min, retention time 13.1 min; for isolation of 3′-azido-3′-deoxythymidine: 15% acetonitrile in 0.1 mol/l TEAB, pH 7.3, elution rate 1.5 ml/min, retention time 19.5 min.

Enzymatic transdeoxyribosylation reactions were carried out with trans-*N*-glycosidase (EC 2.4.2.6) isolated from rat liver as previously described.[6] The reaction mixture contained 1 μmol of thymine, a donor of a ribosyl residue (see Table 1) 4–6 μmol; $MgCl_2$ 14 μmol; potassium phosphate buffer pH 7.0 – 400 μmol; enzyme 800 μl (with the activity of 37.5 nmol min^{-1} ml^{-1}); total volume was 4.0 ml. The reaction mixture was incubated at 37°C for 2 h. After the incubation, the protein was denatured by heating at 100°C for 5 min and then separated by centrifugation.

Synthesis of tritium labelled 5′-O-benzoyl-2,3′-anhydrothymidine: 3.3 mg of 5′-*O*-benzoyl-2,3′-anhydrothymidine was placed in a reaction glass vial, and 400 μl of 0.1 N ammonia in 50% water ethanol and 50 mg of 5% PdO/Al_2O_3 catalyst were added. The vial was then frozen in liquid nitrogen, evacuated, and filled with gaseous tritium up to pressure of 400 mm Hg. Upon defrosting, the reaction mixture was stirred at room temperature for 21 h. After the reaction was complete, tritium was removed from the vial and the catalyst separated by centrifugation. Labile tritium was removed by evaporation with 10 ml of 50% ethanol. Isolation of the product achieved by HPCL. As a result, 110 mCi of product was obtained with a specific radioactivity of 24.6 Ci/mmol and 45% yield.

Synthesis of tritium labelled 5′-O-benzoyl-3′-azido-3′-deoxythymidine: 1.5 mg of [^{3}H]5′-*O*-benzoyl-2,3′-anhydrothymidine was dissolved in 0.9 ml of absolute dimethylformamide, then 5 mg of NaN_3 was added and the solution inserted in a glass vial that was then sealed. The vial was thermostated at 100°C for 15 h.

To extract [^{3}H]5'-O-benzoyl-3′-azido-3′-deoxythymidine, the reaction mixture was placed in a tube, to which a 0.1 M solution of $NaHCO_3$ (pH 9.9 to precipitate the excess of NaN_3) and 2 ml of distilled chloroform were added. The solution was centrifuged, and the product allowed to precipitate over 15 min and the choloroform layer separated with a pipette. This operation was repeated two times to achieve the maximum extraction. The chloroform extractions were combined and

evaporated in vacuum with ethanol for complete removal of traces of the solvent. The radioactivity yield of the generated compound, estimated by TLC, was 36%.

Removal of benzoyl protection and isolation of tritium labelled 3′-azido-3′-deoxythymidine: The dry remainder after extraction was dissolved in 2 ml of 5 N ammonia solution. The solution was then placed in a glass vial which was sealed and thermostated at 60°C for 1 h. After completion of the reaction the contents of the vial were evaporated in vacuum, then 5 ml of ethanol was added to the remainder and it was evaporated again. HPLC was used for the isolation of [^{3}H]3′-azido-3′-deoxythymidine from the reaction mixture. The result was 25 mCi of tritium labelled 3′-azido-3′-deoxythymidine with a specific radioactivity of 18.3 Ci/mmol and 13.5% chemical yield. The radiochemical purity of the product, estimated by thin layer chromatography in the above mentioned systems, was over 99%. [6-^{3}H]3′-azido-3′-deoxythymidine was stored at −20°C as a solution of 1 mCi/ml in 1:1 (v/v) water–ethanol. ^{1}H-NMR (D_2O, δ, ppm; *J*, Hz): 7.65s (0.8H, H-6), 6.22t (1H, *J* 6.7, H-1′), 4.39m (1H, H-3′), 4.04m (1H, H-4′), 3.85m (2H, H-5′), 2.55m (2H, H-2′), 1.92s (3H, 5-CH_3). ^{3}H-NMR (D_2O, δ, ppm): 7.65s (^{3}H-6).

References

1. Hill JA, Freeman GA. *J Label Compd Radiopharm* 1988; **25**: 277–280.
2. Aggarwal SK, Shalinsky DR, Argawal KC. *J Label Compd Radiopoharm* 1988; **25**: 1055–1060.
3. Sidorov GV, Myasoedov NF. *Bioorganicheskaya Khimia* 1993; **19**: 1220–1225.
4. Sidorov GV, Myasoedov NF, Yasko MV, *J Label Compd Radiopoharm* 1994; **34**: 339–351.
5. Sidorov GV, Zverkov YuB, Myasoedov NF. *Radiokhimia* 2003; **3**, in press.
6. Ivankova EK, Sidorov GV, Myasoedov NF. *Radiokhimia* 1981; **23**: 296–300.
7. Zaitseva VE, Dyatkina NB, Krayevsky AA, Skaptsova NV, Turina OV, Gnuchev NV, Gottikh BP, Azhayev AV. *Bioorganicheskaya Khimia* 1984; **10**: 670–680.
8. Kimura J, Fujisawa Y, Yoshizawa T, Fukuda K, Mitsunobu O. *Bull. Chem. Soc. Jpn* 1979; **52**: 1191–1196
9. Mikhailov KS, Lavrov OV, Myasoedov NF. *Organicheskie soedinenia mechennye padioacrivnymi izotopami* [*Organic compounds labelled by radioactive isotopes*]. 1977, 253–258.

