PREPARATION OF α-5-AZA-2′-DEOXY-[6-^{3}H]CYTIDINE

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α-5-Aza-2′-deoxy cytidine was labeled by tritium on the C-6 of the heterocyclic triazine ring. The structure of the α-5-aza-2′-deoxy-[6-^{3}H]cytidine and the position of the label was proved by ^{3}H and ^{1}H NMR. The specific activity was 0.71 TBq mmol^{-1} (19.2 Ci mmol^{-1}) and radiochemical purity was >99%. The long term stability of the product during the storage at –21 and –72 °C was followed by radio-HPLC.

Keywords: α-5-Aza-2′-deoxy-cytidine; CESG; Tritium; ^{3}H NMR.

α-5-Aza-2′-deoxy-cytidine (**1**; α-ADC) is nucleoside anologue known for its cancerostatic activity against leukemic cells[1]. Its antileukemic activity is given by its conversion to β-5-aza-2′-deoxy-cytidine in the solution, which is the actual active agent. It is believed that cancerostatic activity of β-5-aza-2′-deoxy-cytidine is based on its ability to interfere with the DNA methylation in cells[2–4]. The potential advantage of an administration of the α-anomer as a prodrug is based on a one hundred times lower toxicity of the α-anomer to healthy cells as compared to toxicity of the β-anomer. For the application distribution metabolism excretion (ADME) studies on laboratory animals the compound labeled with radionuclide ^{3}H was required. One of us reported[5] the labeling of β-anomer by tritium by catalytic exchange solution gas (CESG). More recently 1-(β-D-arabinofuranosyl)-5-aza-cytosine was labeled by tritium by CESG and by ^{3}H NMR it was confirmed that the tritium was introduced exclusively on the carbon 6 of triazine ring[6].

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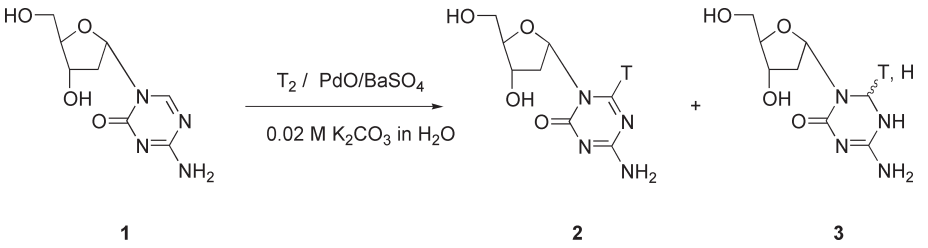
RESULTS AND DISCUSSION

α-ADC **1** was dissolved in water containing 0.02 M K_2CO_3 and exposed to tritium gas at the presence of palladium on barium sulfate catalyst. After the removal of labile radioactivity by repeated evaporation of the reaction mixture from water solution 8.47 GBq (229 mCi) of crude product was obtained. The first HPLC purification on a short preparative reverse phase (RP) column (50 × 21.2 mm) in water–acetonitrile mixture yielded 2.61 GBq (70.5 mCi) of product with only 87% radiochemical purity. The isolated byproducts were conserved for ^{3}H NMR examination. An 888 MBq (24 mCi) aliquot of the α-[6-^{3}H]ADC **2** was repurified on semipreparative RP column (250 × 10 mm) yielding 662 MBq (17.9 mCi) of product with radiochemical purity >97%. It was realized that after the evaporation of pooled fractions and their redissolution, the radiochemical purity droped by about 5%. To provide a product with >97% radiochemical purity suitable for biochemical experiments and in vivo experiments the α-[6-^{3}H]ADC **2** was always freshly repurified on semipreparative column using pure water as mobile phase thus avoiding the need to process the pooled fractions (only dilution to the 1 mCi ml^{-1} concentration was performed). This procedure repeatedly gave the product with >99% radiochemical purity. The specific activity of the α-[6-^{3}H]ADC **2** was calculated from the activity of the solution and mass assay by HPLC and was 0.71 TBq mmol^{-1} (19.2 Ci mmol^{-1}). The pure product was further characterized by ^{3}H NMR – only a singlet at δ 8.3 corresponding to ^{3}H at C-6 of the triazine ring was detected. The specific activity, calculated from the decrease of the ^{1}H signal at δ 8.3 in ^{1}H NMR spectrum, was 0.72 TBq mmol^{-1} (19.4 Ci mmol^{-1}). In the ^{3}H NMR spectrum of the principal byproducts fraction two singlets at δ 4.61 and 4.45 were observed overlapping with multiplet 4.2–4.7. These two singlets were split to doublets with J = 12.5 Hz when ^{1}H broad band decoupling was switched off. This observation confirmed that the principal side product (beside products of hydrolysis) is α-5-aza-5,6-dihydro-2′-deoxy-[6-^{3}H]cytidine (**3**)



1 **2** **3**

SCHEME 1

(Scheme 1). The dihydro side product **3** represented 25% of the total activity of the reaction mixture.

The stability of α-[6-^{3}H]ADC **2** was followed during a 15 week period. The 0.1 ml aliquots of the solution of α-[6-^{3}H]ADC **2** in water at a concentration 37 MBq ml^{-1} (1 mCi ml^{-1}) were pipetted to 1.5 ml Eppendorf tubes. The aliquots were quickly frozen in liquid nitrogen and then stored either at –20 or –72 °C. For analysis a new sample was defrosted and analysed by radio-HPLC. The results are summarized in Table I. It is clearly seen that the lower storage temperature provides a longer shelf life for the product.

TABLE I
Radiochemical purity of α-5-aza-2′-deoxy-[6-^{3}H]cytidine stored in water at different temperatures; concentration 37 MBq ml^{-1} (1 mCi ml^{-1})

Temperature °C	Starting purity %	Radiochemical purity (in %) at time of storage (weeks)						
		1	4	7	9	11	13	15
–20	>99	98	95.1	90.7	89.3	84.4	80.5	82.2
–72	>99	98	95.4	95.8	94.9	94.0	94.2	92.2

EXPERIMENTAL

^{3}H and ^{1}H NMR spectra (in ppm, δ-scale) were taken on Bruker Avance II 300 MHz spectrometer in dimethylsulfoxide-d_6. The HPLC was performed on a system consisting of a WATERS Delta 600 Pump and Controler, WATERS 2487 UV detector and a RAMONA radiochromatographic detector from Raytest (Germany) with interchangeable fluid cells. For preparative runs the cell with a single small crystal of solid scintillator was used, for analytical runs the column effluent was mixed with Zinsser Quickszint Flow 302 cocktail in 1:3 ratio. Data were collected and processed using Empower 2.0 software. The radioactivities were measured on Perkin–Elmer Tricarb 2900 liquid scintillation counter (LSC) in Zinsser Quicksafe A cocktail. The evaporations were done using a CentriVap Concentrator from Labconco. Unlabeled α-ADC **1** was supplied by Dr Otmar from Institute of Organic Chemistry and Biochemistry[7].

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-5-aza-[6-^{3}H]cytosine (**2**)

A reaction mixture consisting of 2.5 mg of α-ADC **1**, 12.3 mg of 10% PdO/$BaSO_4$ and 0.5 ml of 0.02 M K_2CO_3 in a small flask equipped with a magnetic stirrer was connected to a tritiation manifold, frozen by liquid nitrogen and the air was pumped off by vacuum pump. The mixture was left to thaw, then it was again frozen by liquid nitrogen and the residual gases were pumped off. The freeze-thaw cycle was repeated three times for complete degasing of the reaction mixture. After the last thawing the tritium gas was admitted to the

reaction flask at a pressure of 600 torr and the reaction mixture was stirred for 1 h at laboratory temperature. The labile activity was removed by repeated freeze drying of the reaction mixture from water on a vacuum manifold. The catalyst was then filtered off through a 0.45 micron PTFE filter. The reaction mixture was concentrated and applied onto preparative column Luna 5 μ Phenyl-Hexyl 100A, 50 × 21.2 mm (Phenomenex). The column was eluted by water–acetonitrile 95:5 mixture in isocratic mode. Compound **2** (70.5 mCi) with radiochemical purity 87% was obtained. Repurification of **2** (24 mCi) on a Synergi 4 μ Fusion-RP 80, 250 × 10 mm (Phenomenex), elution with pure water, gave 17.9 mCi of **2** with radiochemical purity >99% and specific radioactivity 19.2 Ci $mmol^{-1}$ (mass assayed by HPLC, activity by LSC).

^3H NMR: singlet at δ 8.3. ^1H NMR: intensity decrease of the singlet at δ 8.3; specific activity calculated from intensity decrease of ^1H signal at C-6 was 19.4 Ci $mmol^{-1}$.

CONCLUSIONS

α-5-Aza-2′-deoxy-[6-^{3}H]cytidine with specific activity 0.71 TBq $mmol^{-1}$ (19.2 Ci $mmol^{-1}$) and radiochemical purity >99% suitable for ADME studies on laboratory animals was prepared. The conditions of a HPLC purification were optimalized to eliminate the need of evaporation of pooled chromatographic fractions. In this way the high radiochemical purity of the freshly purified compound was preserved and the reasonably long shelf life of the tritiated compound was achieved.

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