# SYNTHESIS OF [2-$^{14}$C]-2'-DEOXYCYTIDINE-$^{3}$N-CYANOBORANE

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## SUMMARY

Synthetic nucleosides (e.g., 5-fluorouracil, 6-mercaptopurine) are used extensively as antineoplastic drugs. Recent research in boron chemistry has lead to the development of a series of amine-boranes that possess significant antineoplastic activity. The agents may also be useful in boron neutron capture therapy (BNCT), therefore giving the compounds a dual mechanism of cytotoxic action. Herein is a report of the synthesis of one of the more active boronated nucleosides, 2'-deoxycytidine-$^{3}$N-cyanoborane with a $^{14}$C-label in the 2-position of the pyrimidine ring.

Key words: Nucleoside, deoxycytidine, cyanoborane, 2'-Deoxycytidine-$^{3}$N-cyanoborane, amineborane

## INTRODUCTION

Synthetic nucleosides (e.g., 5-fluorouracil, 6-mercaptopurine and ara C) are used clinically extensively as antineoplastic drugs today (1-3). Though very effective in cytotoxic assays and against selected tumor lines, the overall success of these agents has been limited mainly due to lack of efficacy and to resistance by many tumor types (4-5). Because of their limited effectiveness against certain tumors, there is a continued search for more active nucleosides. Recent developments in boron chemistry has lead to the synthesis of a series of amine-boranes where the boron atom is coordinated to the nitrogen atom of various heterocyclic amines, including the purine and pyrimidine rings of nucleosides, that possess antineoplastic activity (6-7). Hypolipidemic and anti-inflammatory activity has been noted for some of the boron nucleosides (7). Since these compounds contain a boron atom, these agents may also be useful in boron neutron capture therapy (BNCT), thus affording compounds with a dual mechanism of cytotoxic activity (8). Boron peptides, e.g. $Me_3NBH_2C(O)NHCH(R)\text{-}CO_2Me$, where $R = CH_2CH_2SCH_3$, $CH(CH_3CH_2CH_3)$, or $CH_2CH(CH_3)_2$ and $Me_3NBH_2C(O)NH_2CH_2CO_2Et$, have been shown to be selectively

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taken up in small quantities (0-33.9 $\mu g^{n}B/g$ tissue) into a murine melanoma at a ratio of approximately 2:1 relative to the blood and brain tissue (9). In order for BNCT to be successful, therapeutic compounds which accumulated in the tumor tissue with a higher ratio are required. Over the last several years we have searched for such derivatives.

2'-Deoxycytidine-$^{3}$N-cyanoborane is one of the most active derivatives *in vitro* (7) possessing significant activity against murine $L_{1210}$ lymphoid leukemia, P388 lymphocytic leukemia, human $Tmolt_3$ lymphoblastic leukemia, SW480 colorectal adenocarcinoma, lung bronchogenic MB-9812, osteosarcoma TE418, KB epidermoid nasopharynx, Hela-$S^3$ cervical carcinoma, and glioma EH118MG growth and *in vivo* in Ehrlich ascites carcinoma, Lewis Lung, P388 and $L_{1210}$ leukemias (7). The boronated nucleoside has been shown to be stable in aqueous media (i.e. 0.1 M $Et_3NHOAc$, pH 7.0 at 25°C) with a half-life of 2,428 hours (10). Here we report the synthesis of [2-$^{14}$C]-2'-deoxycytidine-$^{3}$N-cyanoborane to be used in tissue distribution and elimination studies in rodents.

## DISCUSSION

The synthesis of 2'-deoxycytidine-$^{3}$N-cyanoborane (11) proceeds by protecting the 3' and 5' hydroxyl groups with a triisopropyl silyl group which also decreases the polarity of the nucleoside so the boron exchange reaction can occur (12, 13). The next step involves exchanging the cyanoborane group off of a weak base, triphenylphosphine (14), onto the more basic ring nitrogen. The final step is the deprotection of the 3' and 5' hydroxyl groups by fluoride (15). The synthesis of [2-$^{14}$C]-2'-deoxycytidine-$^{3}$N-cyanoborane was accomplished from commercially available [2-$^{14}$C]-2'-deoxycytidine, New England Nuclear, Boston, Mass.

[2-$^{14}$C]-2'-Deoxycytidine was diluted with 2'-deoxycytidine hydrochloride. Then the 3' and 5' hydroxyl groups were protected with triisopropylsilyl groups by stirring the diluted material with excess triisopropylsilyl chloride and imidazole in dry DMF under nitrogen. The cyanoborane group was exchanged off of triphenylphosphine cyanoborane onto the nitrogen-$^{3}$ of the protected nucleoside by refluxing the two compounds in dry THF under nitrogen. The exchange reaction was observed to be an equilibrium process, so when equilibrium was obtained after 2.5 hours, the reaction was stopped and the starting material was recycled through the exchange process to give a 70% yield for the exchange. The 3' and 5' triisopropylsilyl protecting groups were removed using tetrabutylammonium fluoride

in THF. The final product, [2-$^{14}$C]-2'-deoxycytidine-$^{3}$N-cyanoborane, had a specific activity of 1.67 mCi/mmol and was obtained in a 29% overall yield.

2-Deoxycytidine-$^{3}$N-cyanoborane was shown to be selectively taken up by a human $Tmolt_3$ leukemia cells in vitro at ratios of 2.7 at 2 hours to 3.6 at 6 hous compared to human Bg-9 fibroblast cell line (7). In vivo administration of 2-deoxycytidine-$^{3}$N-cyanoborane to murine Ehrlich Ascites carcinoma bearing $CF_1$ mice demonstrated tumor to blood ratios of 4.2 at 2 hours and 8.5 at 4 hours (16). Preliminary metabolism and distribution studies indicate that there is at least one major metabolite which is in the process of being isolated and identified (16).

## EXPERIMENTAL PROCEDURES

All chemicals were used as received from the manufacturers except for DMF and THF which were distilled prior to use. [2-$^{14}$C]-2'-deoxycytidine was obtained from New England Nuclear, Boston, Massachusetts. Triphenylphosphine cyanoborane was synthesized as previously reported (14). 2'-Deoxycytidine hydrochloride was obtained from Sigma Chemical Co. All other chemicals were obtained from Aldrich Chemical Co. Radiopurity was determined using a Bioscan BID-100 Image Analyser. $^{14}$C was counted using a Packard Tricarb 4000 liquid scintillation spectrometer using Scintiverse$^{R}$ counting solution. Silica gel G plates were used for TLC analyses, Fischer Scientific.

[2-$^{14}$C]-3',5'-O-Bis-(triisopropylsilyl)-2'-deoxycytidine (2). [2-$^{14}$C]-2'-Deoxycytidine with a specific activity of 20 mCi/mmol (1.27 mCi/0.062 mmol) was diluted with unlabelled 2'-deoxycytidine hydrochloride (180mg, 0.683 mmol) to a specific activity of 1.67 mCi/mmol (1.27 mCi/0.762 mmol). The diluted nucleoside and excess imidazole (355 mg, 5.21 mmol) was dissolved in 3mL of dry DMF. Triisopropylsilylchloride (2.0 mL, 1.8g, 9.3 mmol) was added and the solution was stirred under nitrogen for 22 hours. The excess triisopropylsilyl chloride was removed under reduced pressure. The residue was taken up in ether then washed five times with a saturated solution of sodium chloride. The ether phase was dried over sodium sulfate, filtered, and the ether was removed under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:acetone 4:6 as a solvent system, $R_f$ - 0.33 to yield 211 mg (51%) of colorless product.

[2-$^{14}$C]-3',5'-O-Bis-(triisopropylsilyl)-2'-deoxycytidine-$^{3}$N-cyanoborane (3). Compound 2 (211 mg, 0.39 mmol) and triphenylphosphine cyanoborane (320 mg, 1.06

mmol) were stirred at reflux in dry THF under nitrogen for 2.5 hours. The solvent was removed under reduced pressure. The residue was washed with three portions of 2 mL of ether. The ether phases were combined and the solvent was removed under reduced pressure. The product and unreacted starting material were separated by column chromatography on silica gel using dichloromethane:acetone 9.25:0.75 as a solvent system, $R_f$ - 0.4. The unreacted starting material was resubjected to the above procedure. The combined products afforded 159 mg (70%) of colorless solid.

[2-$^{14}$C]-2'-deoxycytidine-$^{3}$N-cyanoborane (4). A solution of 159 mg (0.274 mmol) of 3 in 3mL THF and 0.8 mL (0.8 mmol) of a 1M solution of tetrabutylammonium fluoride in THF was stirred at room temperature for 0.5 hours. The solvent was removed under reduced pressure and the residue was stirred in 5mL of ether for 2 min., then allowed to stand for 10 min. The ether was decanted and the oily residue was purified by column chromatography on silica gel using dichloromethane:methanol 8.5:1.5 as solvent system, $R_f$ - 0.16, to afford 58.8 mg (81%) of yellow solid. The specific activity was 1.67 mCi/mmol.

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