APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 10, 279–282 (1996)

Synthesis of Trimethylamine-[^{14}C]carboxyborane and Trimethylamine-[^{14}C]carboxymethoxyborane and L1210 Leukemia Cell Uptake

S. Y. Chen,* Bruce S. Burnham,* Bernard F. Spielvogel,† A. Sood,† Steven D. Wyrick* and Iris H. Hall*

* Radiosynthesis Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, CB 7360, Beard Hall, Chapel Hill, NC 27599-7360, USA and † Boron Biological Inc., 620 Hutton St, Raleigh, NC 27606-1490, USA

The anti-neoplastic agents trimethylamine-carboxyborane and its corresponding methyl ester have successfully been radiolabeled with carbon-14 in the carboxyl group. Using the radiolabeled agents we have shown that their L1210 leukemia cell uptake appeared to be by a passive process and binding of the agents to DNA, RNA and protein over 24 h was minimal.

Keywords: radiolabeling; trimethylamine-carboxyborane; trimethylamine-carboxymethoxyborane; leukemic cell uptake; binding to nucleic acids and protein

INTRODUCTION

The amine-carboxyboranes have interesting pharmacological activities. At relatively low doses of 8–20 mg kg^{-1} day^{-1}, trimethylamine-carboxyborane and the corresponding methyl ester have been observed to have potent antineoplastic,[1] anti-inflammatory,[2] antiosteoporosis[3] and hypolipidemic[4] activities with improved activity over currently used clinical agents. These agents also have potential in boron neutron capture therapy in patients who have gliomas, thus reducing tumor growth. They afforded no acute toxicity in mice and rats based on tissue weights and morphology, clinical chemistry values and hematopoietic values at one, two and five times the therapeutic dose after seven days' dosing.[5] the mean survival dose for trimethylcarboxymethoxyborane is 225 mg kg^{-1} i.p. and for trimethylamine-carboxyborane it is >1800 mg kg^{-1} i.p. in mice, which demonstrates a good therapeutic index compared with boronic acids. Thus, this agent appears worthy of radiolabeling for tissue studies in rodents.

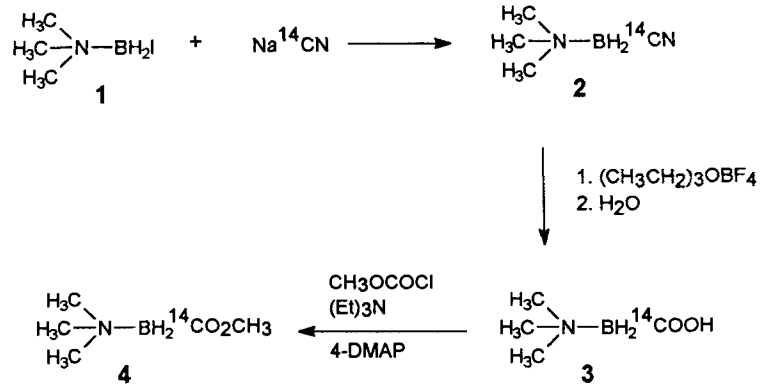
METHODS

Chemistry

The usual most convenient method of preparing trimethylamine carboxyborane is by first preparing trimethylamine-cyanoborane from trimethylamine hydrochloride and sodium cyanoborohydride, then converting the cyanoborane to the carboxyborane triethyloxonium tetrafluoroborate and water.[6] An alternative method of synthesizing the trimethylamine-cyanoborane intermediate is via S_N2 displacement of iodide by cyanide from trimethylamine-iodoborane.[7] This latter method was chosen as the route for preparing the radiolabeled products by using Na^{14}CN as the radiolabeled substance. Use of the crown ether 15-crown-5[8] afforded good uptake of the radiolabeled starting material in the anhydrous environment of the air-sensitive reaction. In order to label trimethylamine-carboxyborane with carbon-14, trimethylamine iodoborate (**1**) was treated with sodium [^{14}C]cyanide in dry tetrahydrofuran (THF) containing a small amount of 15-crown-5. The product, trimethylamine-[^{14}C]cyanoborane (**2**), was obtained in a yield identical to that obtained for the unlabeled compound during the developmental synthesis. Compound **2** was converted to trimethylamine-[^{14}C]carboxyborane (**3**) by treatment with triethyloxonium tetrafluoroborate to form the *N*-alkylated adduct followed by hydrolysis, and was purified by recrystallization. The acid was converted to the methyl ester (**4**) using methyl formate, triethylamine and 4-dimethylaminopyridine (Scheme 1).

CCC 0268–2605/96/030279–04

Received 16 January 1995
Accepted 19 June 1995



Scheme 1

Experimental procedures

All chemicals and reagents were used as received from the manufacturers. $Na^{14}CN$ (53 mCi mmol^{-1}) was obtained from New England Nuclear/Dupont. Column chromatography was performed using silica gel 60, and thin-layer chromatography was performed using silica gel 60 glass plates with fluorescent indicator (Fisher Scientific). ^1H NMR spectra were obtained on a Bruker AC300 300 MHz NMR spectrometer. Radiochemical purity was assessed using a Bioscan BID-200 image analyzer and carbon-14 was counted using a Packard Tricarb 4000 liquid scintillation spectrometer using Scintiverse BD counting solution.

Trimethylamine-[^{14}C]cyanoborane (2)
Sodium [^{14}C]cyanide (53 mCi mmol^{-1}) was diluted with 92.5 mg of sodium cyanide to a specific activity of 2–4 mCi mmol^{-1}. Trimethylamine-iodoborane (**1**; 417 mg, 2.1 mmol) was transferred to a flame-dried two-necked round-bottom flask in a glove bag under nitrogen. The diluted sodium cyanide was then transferred to the flask and the container rinsed with a total of 10 ml of dry THF containing four drops of 15-crown-5. The flask was fitted with a nitrogen inlet and outlet and flushed with nitrogen. An additional 10 ml of THF containing four drops of 15-crown-5 was added and the reaction stirred at reflux for 8 h. The volatiles were removed *in vacuo* and the colorless solid residue was washed thoroughly with dichloromethane (CH_2Cl_2) and the washings added to a column of silica gel 60. The column was then eluted with 75 ml of CH_2Cl_2–hexane (2:1) followed by CH_2Cl_2 to afford 109 mg (53%) of pure colorless solid; $R_f = 0.2$ (CH_2Cl_2).

Trimethylamine-[^{14}C]carboxyborane (3)
Compound **2** (109 mg, 1.1 mmol) was dissolved in 5 ml of dry CH_2Cl_2 and 1.1 ml (2.2 mmol, 100% excess) of a 2.0 M solution of triethyloxonium tetrafluoroborate in THF was added. The reaction solution was stirred at reflux under nitrogen for 24 h. The solvent was removed *in vacuo*, 1.0 ml of water was added to the residue and the mixture was stirred at room temperature for 2.5 days. The aqueous phase was extracted with CH_2Cl_2; the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to afford the crude product as a solid. This solid was recrystallized from CH_2Cl_2–hexane (9:1) to afford 17.5 mg (52%) of the pure product (>99% by TLC–radioscan) as a colorless solid; $R_f = 0.33$ [CH_2Cl_2–acetone (9:1)]. The specific activity was determined to be 4.2 mCi mmol^{-1}.

Trimethylamine-[^{14}C]carboxymethoxyborane (4)
Compound **3** [50 mg (crude from above reaction), 0.43 mmol] was dissolved in 5 ml of dry CH_2Cl_2 and cooled to 0 °C in an ice bath; then 0.05 ml (61 mg, 0.65 mmol) of methyl chloroformate, 0.06 ml (44 mg, 0.43 mmol) of triethylamine, followed by 13 mg of 4-dimethylaminopyridine (DMAP), were added to the solution. The solution was stirred at 0 °C for 1 h. The organic phase was washed with distilled water, dried ($MgSO_4$) and evaporated *in vacuo*. The crude residue was column-chromatographed on silica gel. The column was prepared with ethyl acetate:hexane (7:3) containing 1% triethylamine. The triethylamine was washed off the column with ethyl acetate:hexane (7:3) and then the crude material was loaded onto the column and eluted with ethyl acetate:hexane (7:3) to afford 26 mg of product as a colorless solid (45%) which was >99% pure

by TLC–radioscan; $R_f = 0.55$ (ethyl acetate:hexane [7:3]).

Pharmacology

To measure the time course of uptake, L-1210 lymphoid leukemia cells (10^6) which had been grown in RMPI 1640 + 10% fetal calf serum and antibiotics were incubated with 1 μCi of the trimethylamine-carboxyborane or its methyl ester at 37 °C. After 5, 10, 15, 30, 45, 60, 90, 120, 240, 360 and 480 min, the cells were harvested by centrifugation at 3500 rpm for 10 min. The cells were washed twice in growth medium, homogenized and counted in a packard scintillation beta-counter corrected for quenching. Results were expressed as dpm per 10^6 cells.

The incorporation of trimethylamine-carboxyborane and its methyl ester was determined by incubating 1 μC of the drug with L-1210 cells [10^6] in growth medium for 24 h. For the DNA and RNA experiments the reaction was stopped with 1 M perchloric acid + Na P ~ P. The DNA was trapped on GF/F filters and the RNA was trapped on GF/B filter disks, which were washed twice and counted. The protein reaction was stopped with 10% trichloroacetic acid and the labeled protein was trapped on Whatman no. 1 filters by vacuum suction. The filters were washed twice and counted. All values were expressed as dpm per 10^6 cells.

RESULTS AND DISCUSSION

The trimethylamine-carboxyborane and its methyl ester were successfully ^{14}C-labeled with good purity and specific activity. The labeled drugs were taken up into L-1210 lymphoid leukemia cells by a passive diffusion process (Fig. 1). Trimethylamine-carboxyborane levels reached their highest level, i.e. 0.857%, within 15 min, and slowly declined for the next 8 h. Trimethylamine-carboxymethoxyborane was taken up at its highest level of 2.70% at 10 min, then declined over the next 8 h. One would expect the methyl ester to cross cell membranes more readily than the free acid. The partition coefficient in octanol–water was given by $\log P = -0.416$ for the methyl ester and $\log P = -0.600$ for the acid.

The methyl ester was less lipid-soluble than the acid derivative. Trimethylamine-carboxyborane incorporation into L-1210 cells after 24 h showed 0.39% bound to DNA, 0.03% to RNA and 0.80% to protein. Trimethylamine-carboxymethoxyborane incorporation was 0.13% into DNA into 0.19% RNA and 2.38% into protein. These data suggest that the cytotoxicity of the drugs was not due to direct interaction with the DNA molecule to afford the DNA strand scission observed previously with these compounds.[9,10]

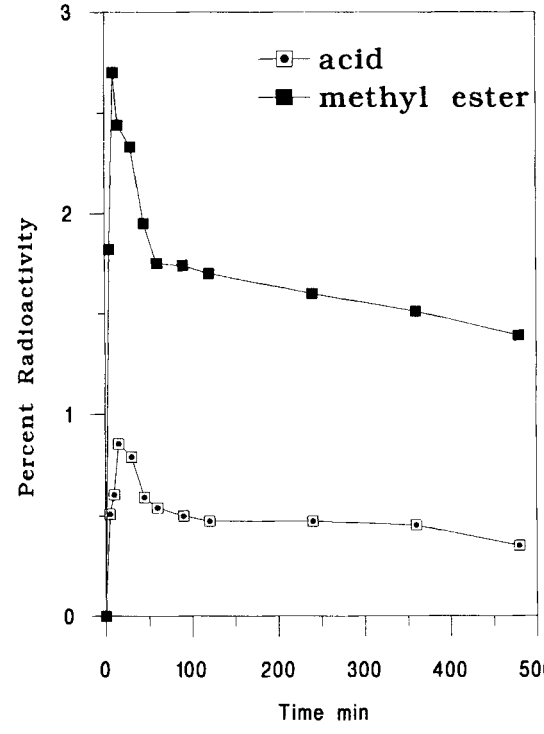


Figure 1 Time course of uptake of trimethylamine-carboxyborane and its methyl ester by L-1210 leukemia cells.

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