RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Synthesis of "No Carrier Added" 1,3-Bis-(2-chloroethyl)nitrosourea (BCNU)

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A chemotherapeutic agent, C-11-labeled BCNU, has been prepared by nitrosation of C-11-labeled BCU, which was synthesized by reacting C-11-labeled phosgene with ethylenimine. Two methods of nitrosation are outlined and the results of both are discussed. The specific activity of C-11 BCNU was about 85 Ci/mmole at the end of bombardment and 30 Ci/mmole at the time of administration. Chemical and radiochemical purity of the final material was at least 98%.

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It has been known for some time that 1,3-bis-(2-chloroethyl)nitrosourea (BCNU) is one of the more effective chemotherapeutic agents for gliomas of the brain, (*1*) but the pharmacokinetics and method of action of this substance have not yet been established (*3*). In order to carry out tracer studies of its distribution and breakdown, BCNU has so far been labeled with N-13 (*2*) and C-14 (*3*). The C-14-labeled BCNU was prepared with the label on the ethylene and carbonyl carbon atoms. The authors did not give details about labeling procedures (*4*). Labeled BCNU with C-14 at the ethylene carbon atoms is of limited use for in vivo study of the metabolites, because several different fragments of the BCNU molecule carry the C-14 label and the limited amount of the labeled material makes separation and identification of those fragments difficult. The carbon atoms of the BCNU molecule are symmetrical on both sides of the carbonyl group, which makes it especially difficult to label it in different positions or to study its biological behavior. This problem is eliminated by placing a C-11 label at the carbonyl and an N-13 at the nitroso position (not at the same time), thus providing a means of analyzing both the pharmacokinetics and the mode of degradation in vivo. By having a label in these positions, but not in the same molecule, and taking advantage of BCNU's route of decomposition into 2-chloroethyl diasomium hydroxide and 2-chloroethylisocyanate, one should be able to study in vitro behavior of the BCNU molecule. BCNU labeled with these two positron-emitting nuclides was accordingly prepared for use with positron emission tomography (PET) to study in vivo the localization pharmacokinetics of human brain tumors.

In this paper we describe the methods used to prepare C-11-labeled BCNU of high specific activity—"no carrier added." The synthesis yields BCNU labeled at the carbonyl carbon. Two different nitrosation procedures were investigated with nonradioactive materials. The optimum conditions for both procedures are described.

MATERIAL AND METHODS

Ethylenimine, used in the synthesis of BCU, was prepared from β-aminoethylsulfonic acid by the method described in Ref. 5, and was stored above NaOH pellets in a refrigerator. We find that the compound cannot be stored for more than three months.

Specific activity of BCNU was determined by HPLC by measuring absorption at 236 nm. The response of the detector was calibrated using a solution of BCNU containing a known amount of the compound. Figure 1 shows recordings of the uv responses from a standard (a) and a sample (b), obtained in the determination of specific activity.

1,3-Bis-(2-chloroethyl)-[2-^{11}C]nitrosourea (C-11 BCNU). This compound was prepared by the nitrosation of BCU [1,3-bis(2-chloroethyl)-urea], which was ob-

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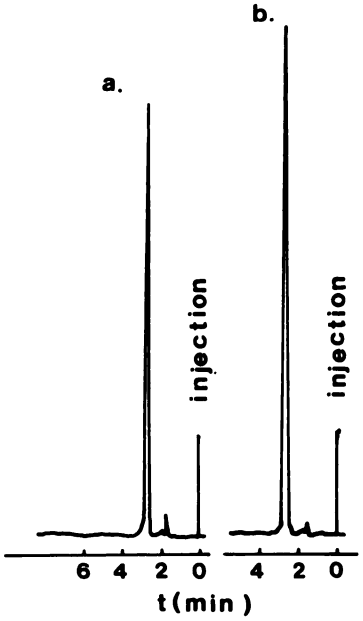

FIG. 1. HPLC chromatograms of BCNU on silicon-gel column, with ether/hexane (70:30) as solvent, at 2 ml/min. (a) 7 μl of standard having 3.18 mg/ml of BCNU; (b) 10 μl out of a 0.5 ml sample containing about 20 mCi C-11 BCNU.

tained from the reaction of ethylenimine with "no carrier added" C-11 phosgene by the reaction sequence shown in Fig. 2. The C-11-labeled phosgene was obtained by passing a mixture of ^{11}CO and chlorine in a quartz spiral around a mercury uv lamp* (*6*,*7*). The [^{11}C]carbon monoxide used in this reaction was produced by irradiating research purity nitrogen (which always includes small amounts of oxygen) with protons, and passing irradiated gases through Zn-oven at 400°C (details in Ref. *6*). Unreacted chlorine was then removed from the stream of gases by antimony powder (*6*,*7*). The C-11 phosgene was collected for 10–30 min in acetone, while cooled to the temperature of dry ice (*6*). A solution of 200 μl ethylenimine in 100 μl of acetone was added to the C-11 phosgene in acetone and stirred for 5 min in a water bath at +5°C. The temperature was raised to 30–40°C and the mixture stirred for an additional 5 min. The solvent, along with unreacted ethylenimine was evaporated under reduced pressure. After evaporation, the residue was dissolved in formic acid and C-11-labeled BCU was nitrosated in the same vessel with the $NaNO_2$.The solution of $NaNO_2$ (200 μl of 0.1 *M*) was slowly added to the solution of C-11 BCU in 0.5 ml of formic acid. The blue color of N_2O_3 appeared immediately and disappeared in 1–2 min.

The nitrosation reaction was done in an ice-salt bath for 5 min with continuous stirring. The final product, BCNU, was extracted into chloroform and purified on a high-performance liquid chromatography (HPLC) column using an ether/hexane (50:50) mixture as solvent at 2 ml/min. In this case a Partisil PAC column was used. C-11 labeled BCNU was eluted in the first 10 ml. The BCNU fraction was collected and identified by its R_f value, using TLC on silica gel with ether/hexane (50:50) and chloroform as solvents.

1,3-Bis(2-chloroethyl)-nitrosourea(nonradioactive BCNU). A sample of BCNU was prepared from 1,3-bis-(2-chloroethyl)-urea (BCU), which was prepared according to the method described by Bestion (*8*) and identified by NMR, and ir and mass spectroscopy. The BCU spectra were compared with those obtained for a BCU sample kindly provided to us.† Nitrosation of BCU was done by sodium nitrite in formic acid as described above, as well as by use of an appropriate concentration of HNO_3 in 1 ml of glacial acetic acid containing 150 mg of powdered copper.

In both syntheses, the product was purified by HPLC using $CHCl_3$ as an eluting solvent and the Partisil PAC column. After evaporation of the solvent, the material was tested for purity and identified by the analytical methods described above.

The purity was tested by regularly examining the TLC plates under ultraviolet, staining the plates in iodine vapor, and developing the plates by an *in situ* nitrosation. The latter was necessary because BCU is not visible otherwise (See Ref. *9* for details).

Work to increase the level of activity and specific activity of C-11 BCNU is in progress. The material is used to study the pharmacokinetics of BCNU in animals and humans; it is also a potential tumor-seeking agent.

RESULTS AND DISCUSSION

The procedures described have been selected to produce labeled material; they would not necessarily produce the best chemical yields. In our work special restrictions were put on reaction time and specific activity, restrictions that drastically reduced available synthetic options. Since the synthesis described is a two-step procedure, an attempt was made to optimize both steps.

Carbon-11-labeled BCU was prepared first. In this preparation small amounts of other products with R_f greater than that of BCU in all solvent systems were always present. We did not identify this product because it was easily removed from the final material. Since our synthesis was with "no carrier added," it was impossible

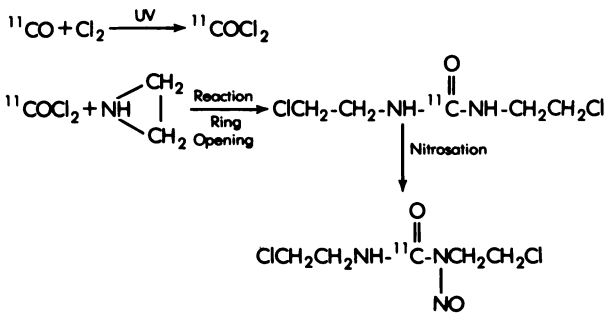

FIG. 2. Reaction sequences in the synthesis of [2-^{11}C]BCNU.

to reproduce the same conditions with an inactive material. The optimum conditions, described in "Materials and Methods," were established by assessing the level of radioactivity obtainable within a certain period. The average radiochemical yield for fifteen preparations, without correction for decay, deriving from this step (preparation of C-11 BCU) amounted to (28 ± 4)%.

To increase the yield of C-11 BCNU from nitrosation of BCU, Table 1 gives the results for nitrosation with copper dust in nitric and glacial acetic acids, and Table 2 with sodium nitrite in anhydrous formic acid.

Nitrosation in glacial acetic acid. This approach to the synthesis of N-13 BCNU was described in Ref. 2, but no reasons for the use of these particular mixtures were offered. Table 1 shows the results obtained for the different concentrations of HNO_3. This reaction was carried out for five minutes at room temperature in 1 ml of glacial acetic acid. The yields are expressed relative to the amount of BCU. In all experiments the concentration of BCU was 0.08 mmole/ml. The maximum yields were obtained by having the concentration of HNO_3 above 0.5 mmole/ml, with the yield about 70%. This yield is for the molar ratio (NO_3^-/BCU) of ~6 and up (see Table 1), which is approximately the maximum yield obtained with "no carrier added" C-11 BCU, even though the ratio of NO_3^-/BCU in these syntheses is much greater. After nitrosation there are three compounds having R_f values 0.40, 0.55, and 0.70 in ether/hexane (70:30) as developing solvent, and 0.12, 0.3, and 0.4 in chloroform. In experiments with C-11-labeled BCU, all three compounds had a C-11 label in their structure. The compound with R_f = 0.40 in ether/hexane has been identified as C-11 BCU.

The compound has the same R_f values (0.4 and 0.1 in the ether/hexane and the chloroform, respectively) as inactive BCU prepared by us and identified by NMR and ir and mass spectrometry, as well as BCU kindly supplied to us. Another positive identification was made by scraping the spot, extracting BCU with ether, and preparing BCNU from it. Carbon-11-labeled BCNU was identified by comparing the TLC R_f value and the HPLC elution volume with those of an inactive sample prepared from BCU and identified by the same methods as mentioned earlier for BCU. Radiochemical yields for the nitrosation of BCU in glacial acetic acid have been from 50 to 80%.

TABLE 1. PREPARATION OF BCNU IN GLACIAL ACETIC ACID*

Concentration HNO_3 nmole/ml	Yield† %
10	4
20	12
30	16
50	40
100	53
500	69
1000	71
1600	69
2000	78

* In all measurements 0.08 mmole of BCU was used.
† From three measurements; uncertainty in the yield determination is estimated to be about 5%.

TABLE 2. PREPARATION OF BCNU IN FORMIC ACID*

Molar ratio ($NaNO_2$/BCU)	Yield† %
5	1.8
10	11.5
15	18.5
20	91
25	95
30	96
40	92

* Concentration of BCU was 0.05 mmole.
† From three independent measurements; uncertainty in the yield is estimated to be about 5%.

The second component (R_f = 0.55) has not been identified because it is not produced when the inactive product is made on a microscale. We can only speculate on the reasons for this compound's absence with a nonradioactive material; probably it is made only when the ratio between phosgene and ethylenimine becomes very small. The third spot (R_f = 0.70) is that of BCNU.

Nitrosation in formic acid. Nitrosation with the $NaNO_2$ in formic acid of nonradioactive BCU (details are given earlier) gave yields of 90% for the concentrations of $NaNO_2$ above 1 mmole/ml and higher, as given in Table 2. In the work with "no carrier added" C-11 BCNU, this nitrosation method gave almost quantitative yields. After nitrosation there were only two spots on TLC plates: R_f = 0.55 and 0.70 in ether/hexane, and 0.3 and 0.4 in chloroform. The first spot comes from unidentified components, as mentioned earlier, whereas the second one belongs to C-11 BCNU.

Purification, quality control, and use of C-11 BCNU. In the preparation the final purification was done by HPLC as described in the "Material and Methods." The BCNU fraction was collected and the solvent evaporated under reduced pressure. The labeled material was dissolved in 2–3 ml of a saline solution and sterilized by filtration through a 0.25-μm Millipore membrane. Usually 10 mCi of C-11 labeled BCNU in a 2–3 ml of saline were used in human PET studies. C-11 BCNU isolated from the reaction mixture by HPLC with

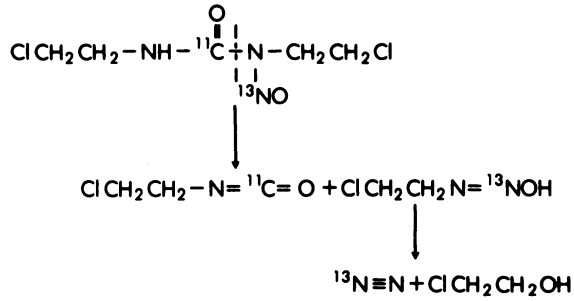

FIG. 3. A mode of decomposition for [2-^{11}C]- and [^{13}N]nitroso BCNU.

ether/hexane (70:30) had chemical and radiochemical purity better than 98%; it contained small (1:2%) admixtures of unidentified material with $R_f = 0.55$ on silicone gel with ether/hexane (70:30) as developing solvent.

We think that a substance with a label in the carbonyl carbon atom is of value in the pharmacokinetic studies of BCNU. This is evident if one considers the probable mode of BCNU decomposition. The fragments of the BCNU, 2-chloroethylisocyanate and 2-chloroethyl diasominium hydroxide (*10,11*), are shown in Fig. 2. If there is a label on the carbonyl carbon atom, the fragments of BCNU that originate from the break of the carbonyl carbon-nitrogen bond will carry a C-11 label. This should permit the identification of 2-chloroethylisocyamate by thin-layer radiochromatography. On the other hand, if the label were on ethyl carbon atoms, both fragments (Fig. 3) would carry the label, and the study of in vivo degradation of BCNU with PET would be complicated. As seen from the decomposition scheme, the use of C-11 and N-13 labels (not in the same molecule) would enable us to study the in vivo behavior of BCNU, because the differently labeled products will follow different in vivo pathways.

CONCLUSION

C-11-labeled BCNU should be a valuable tracer for pharmacokinetical studies of BCNU in brain tumors. We have been using this agent to study its in vivo behavior in humans with PET, and in tumor-bearing animals. In general, C-11 BCNU is ready for intravenous injection 30 min after the end of $^{11}COCl_2$ collection, and has a specific activity of about 30 Ci/mmole at the time of administration to the patient (usually about 30 min after the end of the phosgene collection). This specific activity is about 1/10,000 of the theoretical one. Even though we used chlorine of high purity, we consider this the source of most of the inactive CO or $COCl_2$ (*6*). The cyclotron must run for about 30 min to get 15–20 mCi of the final product for human use. The average overall radiochemical yield at the end of synthesis of C-11 BCNU for fifteen preparations, without correction for decay, is (18 ± 2) % when nitrosation in formic acid is used. The end of the synthesis is considered the time when BCNU fraction is collected from the HPLC, which is about 22 min after the end of the C-11 phosgene collection.

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FOOTNOTES

* Ace Glass Inc., Cat. No. 7840-175

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