Synthesis of 2-Bromo-[1-^{14}C]ethanamine Hydrobromide

Peter H. BACH and James W. BRIDGES

The Robens Institute of Industrial and Environmental Health and Safety and Department of Biochemistry, University of Surrey, Guildford, Surrey, GU2 5XH United Kingdom.

SUMMARY

2-Bromo-[1-^{14}C]ethanamine hydrobromide (BEA) was synthesized from [2-^{14}C]ethan-1-ol-2-amine hydrochloride, by reaction with HBr in a specially designed chamber at an elevated temperature. The synthetic product was purified in the reaction chamber by fractional vacuum sublimation to give a yield of up to 90% of BEA having a purity greater than 95%.

KEY WORDS

2-Bromo-[1-^{14}C]ethanamine hydrobromide, [2-^{14}C]ethan-1-ol-2-amine, Ethylenimine, Fractional vacuum sublimation, Toxicology.

INTRODUCTION

2-Bromoethanamine hydrobromide (BEA) has found applications commercially and industrially and as a synthetic intermediate *eg.* in ethylenimine production (1). BEA is also of great interest because it produces an organ-specific toxic lesion in rodents, namely, renal papillary necrosis (2). None of the described methods for the synthesis of BEA in the laboratory (3) are amenable to a micro-scale, high yield and high purity radiosynthesis from a commercially available starting product. Further, the purification of BEA is hampered by its cyclization to ethylenimine in neutral and basic aqueous solutions and handling may be hazardous because ethylenimine is toxic, being a powerful alkylating agent known to be mutagenic and possibly carcinogenic (4).

In a pilot study we found that the industrially patented method of Groves (5) (used to prepare megagram quantities) could be scaled down to a micro-synthesis

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(< 100mg) of high yield. We designed a reaction chamber that allowed us to synthesize BEA and then purify it *in situ* by fractional vacuum sublimation, thus avoiding purification losses and unnecessary handling hazards.

MATERIAL AND METHODS

Synthesis of labelled BEA

The apparatus is shown schematically in Figure 1. The borosilicate reaction chamber *(f)* was constructed to the specifications shown in Figure 2. The chamber was loaded with 500μCi(18.5 MBq) 44mCi/mmol of [2-^{14}C]ethan-1-ol-2-amine HCl (stated radiochemical purity 99%, Amersham International, Amersham) dissolved in 0.5 mL of water, containing 52mg of carrier ethan-1-ol-2-amine HCl (Aldrich Chemicals, Gillingham), through a pasteur pipette inserted through the opening at *(v)*. The label and carrier were evaporated in a gentle stream of dry nitrogen at 25°C. The opening *(v)* was flame sealed and the chamber was incorporated into the apparatus housed in a fume cupboard.

HBr was generated from freshly redistilled dry tetralin [tetrahydronaphthalene] (BDH, Poole) in a flask (*a*) by the addition of a slow stream of Br_2 from a dropping funnel (*b*). Sodium thiosulphate solution (to quench unreacted bromine) or additional tetralin could be added through the dropping funnel (*c*). The gas was passed through a splash head (*d*) then a washbottle (*e*) containing tetralin to remove any unreacted Br_2 vapour, and thence to the reaction vessel (*f*) which was immersed in a continuously stirred, heated oil bath, fitted with a contact thermometer (*g*). A water-cooled copper coil between *e* and *f* served to condense any tetralin vapour in the HBr flow. The pressure was kept slightly greater than atmospheric (0.5 cms water) using a gas absorption trap (*i*), the outflow of which was cowled by a funnel (*j*) connected to a 'fast-flowing' water vacuum line, to remove and dissolve the unreacted HBr. The empty washbottle (*h*) served to prevent water 'suck-backs' into the reaction chamber. All fittings were Quickfit or Tygon tubing.

The complete system was flushed with pure dry N_2 at room temperature for 10 minutes through the dropping funnel *c*, then purged with HBr for 10 minutes.

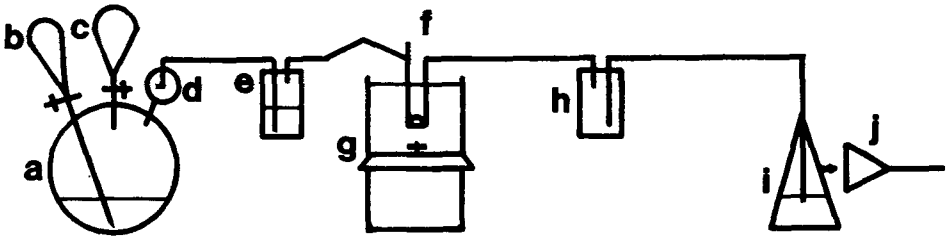

Figure 1. Schematic representation of apparatus used for the synthesis of BEA.

a Round bottom flask

b Dropping funnel,PTFE tap

c Dropping funnel,PTFE tap

d Splash head

e Drechsel bottle

f Micro-reaction chamber

g Heated oil bath

h Drechsel bottle

i Gas absorption trap

j Funnel connected to water vacuum line

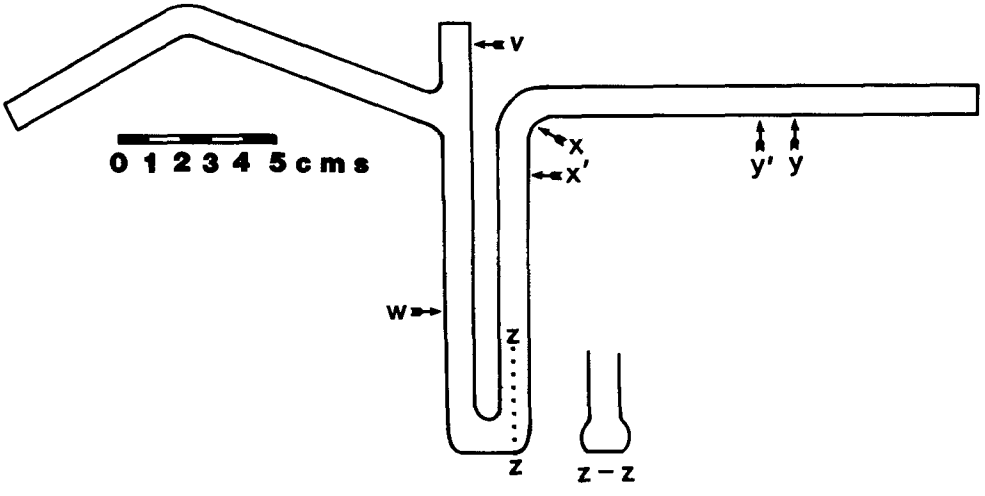

Figure 2. Micro-reaction chamber used for the synthesis and purification of BEA. Side elevation shown as *z*-*z*. See text for other details.

The slow addition of Br_2 through a constricted tip in the dropping funnel *below the surface* of the tetralin gave a steady stream of HBr for the duration of the synthesis. The oil bath was heated to 140°C and increased in increments of 10°C every hour to 190°C at which it was maintained for 1 hour.

Once all the Br_2 had reacted the system was purged with pure dry N_2 for 1 hour. The reaction vessel was removed from the oil bath, cooled to room temperature and the adhering oil washed off with methanol:chloroform 1:1 (v/v). During this time the liquid in the reaction chamber solidified (off white).

Purification by fractional vacuum sublimation

The reaction vessel was flame sealed at *w*, and after cooling the purification vessel was returned to the oil bath submerged to *y* (Figure 2). A water-cooled copper coil was placed on the non-immersed portion and a vacuum line connected to the outlet *via* a trap immersed in solid CO_2-acetone. The vessel was evacuated to 0.05 mm Hg and heated at 100°C for 16 hours. After cooling, washing *etc.*, the limb was broken at *y'*. This was to remove trace amounts of unreacted ethan-1-ol-2-amine HCl and other low temperature sublimates. The low temperature fraction contained a small amount of BEA and an unidentified trace component. BEA was sublimed as a white solid at a vacuum of 0.05 mm Hg and heated at 140°C for 24 hours, with the vessel immersed to *x* and a cooling coil on the limb out of the oil. After cooling, washing *etc.*, the limb was broken at *x'* and the BEA removed from it and stored at 0-5°C. The reaction vessel contained a brown residue of unknown composition.

There was no detectable radioactivity carried over from the reaction vessel during synthesis or beyond the cooling coils used in any of the purification steps. This suggests that ethylenimine (which is relatively volatile) was not formed at any of these stages.

The authenticity of non-labelled synthesized material was confirmed by its IR spectrum, melting point and mixed melting point and by its behaviour on the four tlc systems listed below, visualised with either ninhydrin or fluorescamine and

compared to commercially available BEA (BDH, Poole).

Yield and Purity

The weight of BEA synthesized was determined twice using 'cold' ethan-1-ol-2-amine HCl and yields of 74% and 71% were obtained. Based on the incorporation of radioactivity, however, the yield was in excess of 89%. The discrepancy between the chemical and the radiochemical yields most likely arose from using a sublimation time of 16 hours for the purification of BEA for the 'cold' syntheses. Using this condition residues of BEA remained in the reaction chamber. Impurities in the carrier ethan-1-ol-2-amine HCl would also contribute to such differences.

The specific activity was 210.2 kBq/mg; 44.4 kBq/mmol (5.7 μCi/mg; 1.2mCi/mmol).

BEA purity was assessed by tlc radiochromatography after co-chromatographing with 4 mg/mL of carrier BEA (this had been synthesized and purified as described above) as suggested by Sheppard (6). The tlc supports were:- I) Silica Gel 60 (Merck 5553) and II) Cellulose MN 300 (Anachem, Luton). The solvent systems were a) propan-2-ol:ammonium hydroxide (30% m/v):water::90:8:2 (v/v), b) methanol saturated with NH_3 vapour, c) chloroform:methanol::9:1 (v/v) and d) propan-2-ol:ethanol:1N-HCl (aq)::3:3:2 (v/v). Plates were autoradiographed against Kodak blue brand X-ray film.

Neither the low temperature sublimate nor the reaction product contained ethan-1-ol-2-amine. Purified BEA chromatographed as a single component in systems Id and IId and as a major component (with five trace components) in system Ic. The alkaline systems gave one major component, with a trace substance leading and tailing it (systems Ia and Ib). The two-way elution technique (6) suggested that both the tailing and leading component associated with the alkaline chromatographic systems were due to chemical instability of BEA at an elevated pH (1).

Purity was assessed by liquid scintillation counting of transverse areas of support material and found to be 95% on the alkaline chromatographic system and 98% on the other systems.

DISCUSSION

We have described a micro-modification of a patented method for the industrial synthesis of BEA. The radiosynthesis (from a commercially available starting material) is manipulatively easy, as is the subsequent purification, and both yield and purity of the final product are high.

The method may be useful, for example, in the synthesis of other members of the halogen alkylamine group or their N- or C- substituted products (5) subject to suitable modifications being introduced. This 'synthetic' approach could obviously be applied to molecules labelled on either or both carbon atoms, and to other isotopically labelled starting material.

The *containerised* approach may find other applications where either the starting material or the final product is toxic or carcinogenic. We are of the opinion that fractional vacuum sublimation as a means of purifying radiochemicals has a greater potential than has hitherto been reported.

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