Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 269-274 (1999)

A convenient synthesis of [^{14}C]1, 1'-thiobis(2-chloroethane), [^{14}C]sulfur mustard

A. Fidder, D. Noort* and H.P. Benschop

Department of Chemical Toxicology

TNO Prins Maurits Laboratory

P.O. Box 45, 2280 AA Rijswijk

The Netherlands

Summary

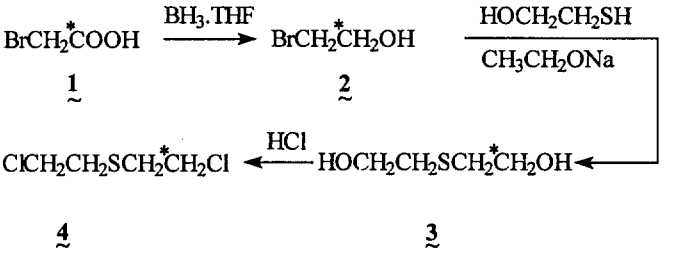
The synthesis of [^{14}C]sulfur mustard is described, starting from bromo[1-^{14}C]acetic acid.

Keywords: sulfur mustard, carbon-14, synthesis

We are engaged in the development of retrospective detection methods to assess exposure to the vesicant 1,1'-thiobis(2-chloroethane) (sulfur mustard), which has been used as a chemical warfare agent since World War I. For this purpose, radioactively labelled sulfur mustard has advantageously been used to identify adducts of the agent with DNA and proteins (1-4). Initially, we used the sulfur-35 labelled agent, which was synthesized by reaction of hydrogen [^{35}S]sulfide with ethylene oxide followed by chlorination of resulting thiodiglycol, according to a method described by Boursnell (5). However, this procedure was often thwarted by complications, which probably resulted from the presence of impurities in the hydrogen [^{35}S]sulfide. Moreover, an inherent disadvantage of using [^{35}S]sulfur mustard is the relatively short half life time

Received 4 September 1998
Accepted 24 September 1998

of the sulfur-35 isotope. We therefore initiated the synthesis of carbon-14 labelled sulfur mustard (4; see Scheme). The synthesis of this compound has been described by Ott et al. (6). Unfortunately, the origin of the labelled starting compound, i.e., 2-bromo[1-^{14}C]ethanol, is not mentioned in this report and, in addition, no full experimental details were given. We here report the synthesis of 4, providing full experimental details.



1 2 3 4

Scheme

Our synthetic route is shown in the Scheme. We reasoned that commercially available, relatively inexpensive bromo[1-^{14}C]acetic acid (1) could function as the radiolabelled precursor. Reduction of 1 with borane tetrahydrofuran complex solution in THF (7) and subsequent work-up of the reaction mixture afforded 2-bromo[1-^{14}C]ethanol (2), which was used without further purification. Reaction of the latter with Na_2S, as described by Ott (6), afforded [^{14}C]thiodiglycol (3) in moderate yield. A major disadvantage of this procedure was the concomitant formation of the carbon-14 labelled disulfide of mercaptoethanol, which could not easily be removed by silica gel column chromatography since this compound has a similar retention as thiodiglycol. Moreover, the formation of the disulfide obviously results in serious losses of radioactivity. This problem could be circumvented by reaction of 2 with 2-mercaptoethanol, under the agency of sodium ethylate. In this case the disulfide was formed in smaller amounts and was not radioactive. After purification on silica gel, 3 was obtained in good radiochemical yield (79%). Treatment of 3 with concentrated

HCl, as reported by Ott (6) and Bent (8), resulted in the formation of 4 in good yield (70%; 56% overall, starting from bromo[1-^{14}C]acetic acid), having a chemical and radiochemical purity of 99%, as assessed by GC analysis. It should be remarked that conversion of 3 into 4 with thionyl chloride (9) was accompanied by the formation of radioactive side-products (10-15%).

In conclusion, we report a convenient synthesis of [^{14}C]sulfur mustard with high radiochemical and chemical purity, starting from the relatively inexpensive bromo[1-^{14}C]acetic acid. Furthermore, it can be envisaged that the intermediate [^{14}C]thiodiglycol can be used as precursor for the synthesis of various carbon-14 labelled metabolites of sulfur mustard (10).

Experimental

Caution. 1,1'-Thiobis(2-chloroethane) (sulfur mustard) is a primary carcinogenic, vesicant, and cytotoxic agent. This compound should be handled only in fume cupboards by experienced personnel.

General procedures. Bromo[1-^{14}C]acetic acid (specific activity 57 mCi/mmol) was purchased from Amersham Pharmacia Biotech. Borane tetrahydrofuran complex solution (ca. 1 M), sodium ethylate in ethanol (21 %), 2-mercaptoethanol and hydrochloric acid (12 M) were purchased from Fluka. Carbosorb and Hionic-Fluor LSC cocktail were purchased from Packard, CT. GC analysis was performed on a Chrompack CP 9001 GC equipped with an FID, using a CPSil 5CB column (10 m, 0.53 mm i.d.). Silica gel column chromatography was performed manually, using silica gel 60 (Fluka) in glass columns.

2-Bromo[1-^{14}C]ethanol (2). To a cooled (0°C) and stirred solution of 1 (ca. 100 mCi; specific activity 57 mCi/mmol) in THF (1.5 mL) was added borane tetrahydrofuran

complex solution (2.6 mL, 1 M) in the course of 30 min. Next, the ice bath was removed and the reaction mixture was stirred overnight at room temperature. The reaction was quenched with water (0.5 mL) and potassium carbonate (500 mg) was added. The organic layer was removed, while the aqueous layer was washed with diethyl ether (5 × 1 mL). The organic layers were collected, dried with $MgSO_4$, filtrated and concentrated to a small volume under normal pressure using a short distillation bridge, affording 2 as a colourless liquid.

[^{14}C]Thiodiglycol (3). A mixture of ethanol (0.5 mL), sodium ethylate (2.1 mmol, 0.79 mL, 21 % solution in ethanol) and 2-mercaptoethanol (147 μL, 2.1 mmol) was added to 2 and the solution was stirred for 2 h at 50 °C. TLC analysis (8% methanol/dichloromethane) indicated almost complete disappearance of 2-mercaptoethanol and the appearance of 3, as was concluded from coelution with cold 3. A small amount of the non-radioactive disulfide was also visible, having a slightly larger Rf value than 3. Ethanol was evaporated under normal pressure and in order to remove the disulfide, the residue was chromatographed on silica gel applying a gradient from 0 to 5 % methanol in dichloromethane in steps of 0.5 % (100 mL each). Fractions containing pure 3 (TLC; detection with I_2 vapour) were combined and concentrated under normal pressure. Fractions contaminated with the disulfide were re-chromatographed.

Radiochemical yield: 79 mCi (79%, based on 100 mCi of 1). The chemical yield was not determined since the solvent had not been removed completely. TLC analysis (8 % methanol/dichloromethane) with radiometric detection showed one radioactive compound. Detection with I_2 colorization showed one spot.

[^{14}C]Sulfur mustard (4). To 3 (39.5 mCi) in a 4 mL vial was added concentrated hydrochloric acid (0.5 mL). The vial was sealed with a screw cap and left at 60 °C for

2 h. A two layer system had formed. Water (0.5 mL) and dichloromethane (1 mL) were added to the cooled vial. After thorough mixing, the two layers were separated by centrifugation. The organic layer was collected and the water layer was washed with dichloromethane (3 × 0.5 mL). Next, the combined dichloromethane layers were washed with water (2 × 0.5 mL) and dried over $MgSO_4$. GC analysis showed, in addition to the solvent peak, a single peak which coincided with cold sulfur mustard. After removal of dichloromethane by evaporation under normal pressure, 4 was obtained in 70% yield (79 mg, 28 mCi) (56% starting from 1). The radiochemical purity was checked by trapping the carbon dioxide evolved from the GC in Carbosorb (3 ml) in 90 second fractions. After addition of Hionic-Fluor LSC cocktail (17 ml), radioactivity was determined by liquid scintillation counting. The main activity (99%) was found in the fraction corresponding with the [^{14}C]sulfur mustard peak. The chemical purity, determined with GC, was 99%. Specific activity: 56.4 mCi/mmol.

Acknowledgements

This work was supported partly by grant no. DAMD17-97-2-7002 from the U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Md., and partly by the Directorate of Military Medical Services of the Ministry of Defence, The Netherlands.

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