Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 5

6-Bis-(2-chloroethyl)amino-6-deoxy-D-[^{14}C(U)]-glucose

H. Jeanette Thomas and John A. Montgomery
Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35255 USA

SUMMARY

^{14}C-Labeled 6-bis-(2-chloroethyl)amino-6-deoxy-D-glucose hydrochloride was prepared from D-[^{14}C(U)]-glucose for DNA binding studies.

Key Words: Nitrogen mustard, 6-bis-(2-Chloroethylamino-6-deoxy-D-[^{14}C(U)glucose hydrochloride, D-[^{14}C(U)]glucose

INTRODUCTION

Cantrell et al. have shown that conjugation of nor nitrogen mustard [bis-(2-chloroethyl)amine] to C-6 of glucose produces an analog (**11**) that has significantly greater activity against the P388 leukemia in mice than nitrogen mustard (HN2), but with significantly less bone marrow toxicity, a potentially important finding to the development of more useful alkylating agents for cancer treatment[1].

Preparation of ^{14}C-labeled 6-bis-(2-chloroethyl)amino-6-deoxy-D-glucose hydrochloride (**11**) was necessary for quantitative studies on drug binding to DNA and protein in normal and neoplastic cells[2].

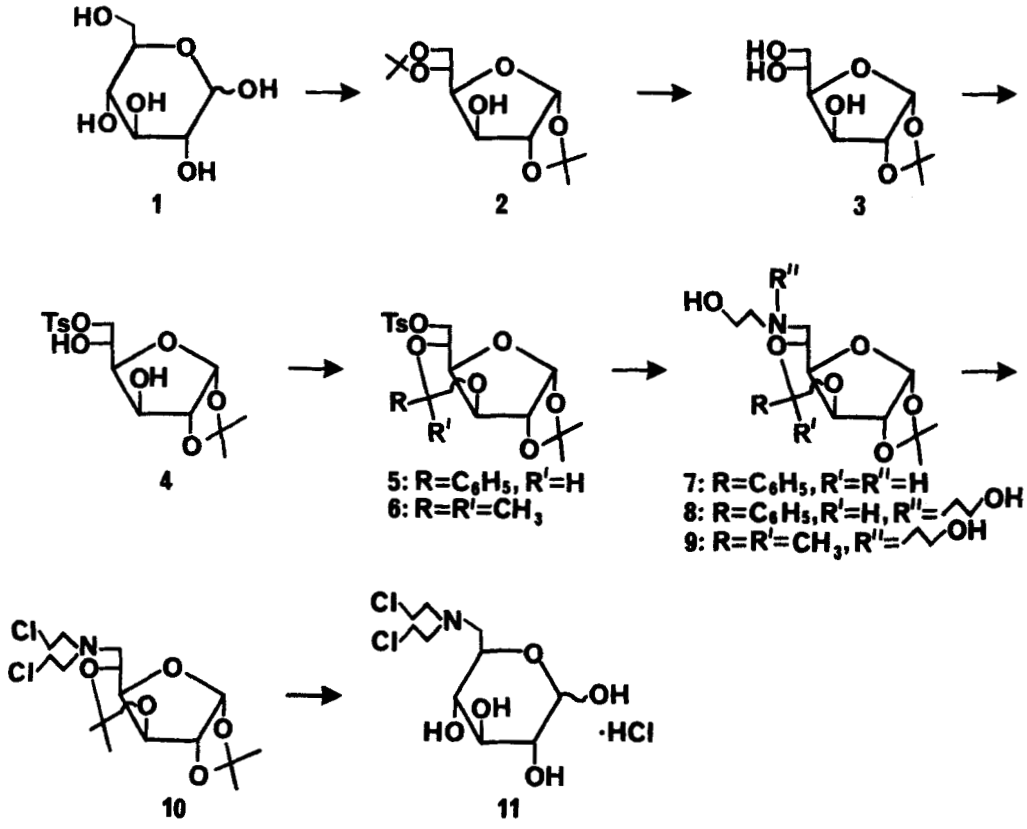
To conserve the ^{14}C-label we prepared 3,5-O-benzylidene-6-deoxy-6-(2-hydroxylethyl)amino-1,2-isopropylidene-D-glucofuranose (**7**) from **5**[3] to react with ^{14}C-2-chloroethanol. The resulting ^{14}C-labeled 3,5-O-benzylidene-6-deoxy-6-[bis-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (**8**) could then be converted to the ^{14}C glucose mustard. This strategy failed, however, because a large excess of 2-chloroethanol was necessary to convert **7** to **8**.

Although the route is longer, we were forced to prepare the ^{14}C- compound from uniformly labeled ^{14}C-glucose. We modified the literature procedure for the preparation of **11**[3] to conserve the ^{14}C-glucose, using 1,2:5,6-di-O-isopropylidene-D-glucofuranose

0362-4803/87/050493-05$05.00

Received June 2, 1986
Revised August 22, 1986

(2) as an intermediate. Selective removal of the 5,6-O-isopropylidene group was followed by tosylation of O-6 to give **4**, which was converted to 1,2:3,5-di-O-isopropylidene-6-O-tosyl-D-glucofuranose **6**. Displacement of the tosyloxy group of **6** by 2,2'-iminodiethanol followed by treatment of **9** with thionyl chloride gave the blocked ^{14}C-glucose mustard (**10**). Treatment of **10** with 6N hydrochloric acid removed the isopropylidene groups giving 6-[bis-(2-chloroethyl)amino]-6-deoxy-D-glucose as the hydrochloride salt (**11**).



EXPERIMENTAL

1,2:5,6-Di-O-isopropylidene-α-D-[^{14}C(U)]-glucofuranose (**2**).

A solution of 5.8 mg (0.03 mmol, 10 mL) of D-[^{14}C(U)]glucose (**1**) (specific activity: 315 mCi/mmol)[4] in 10 mL of ethanol-water (9:1) was diluted with 174.4 mg (0.97 mmol) of glucose (total 1.00 mmol). The resulting solution was evaporated to dryness in vacuo

at 40 °C. The residue was triturated with absolute ethanol and evaporated to dryness three times. The residue was dried for 20 h at ambient temperature and 0.07 mm over phosphorus pentoxide before it was suspended in 9 mL of anhydrous acetone. The suspension was treated with 54 mg (0.33 mmol) of anhydrous ferric chloride, refluxed for 5 h, diluted with 1.8 mL of 10% aqueous potassium bicarbonate, and evaporated in vacuo to remove the acetone. The aqueous mixture was washed with chloroform (3 x 5 mL). The combined chloroform extract was washed with water (2 x 5 mL), dried over magnesium sulfate, and evaporated to dryness in vacuo to give **2** as a crystalline solid: yield 254 mg (98%).

1,2-O-Isopropylidene-α-D-[^{14}C(U)]glucofuranose (**3**).

A solution of 254 mg (0.98 mmol) of **2** in 2.5 mL of 30% aqueous acetic acid (v/v) was kept for 20 min at 55 °C and evaporated to dryness in vacuo. An aqueous solution of the residue was evaporated to dryness in vacuo. An ethyl acetate solution of the resulting solid was dried over magnesium sulfate and evaporated to dryness in vacuo to give **3** as a white solid: yield 172 mg (80%).

1,2-O-isopropylidene-6-O-tosyl-α-D-[^{14}C(U)]-glucofuranose (**4**).

p-Toluenesulfonyl chloride (162 mg, 0.84 mmol) was quickly added to a chilled solution (dry ice-acetone) of **3** in 6 mL of anhydrous pyridine. The resulting solution was stored for 48 h at -20 °C, diluted with 47 mL of water, kept at ambient temperature for 1 h, and evaporated to dryness in vacuo. A chloroform solution of the residue was washed with water, then ice-cold dilute sulfuric acid until the aqueous layer remained acidic, then with water until the aqueous layer was neutral, dried over magnesium sulfate, and evaporated to dryness in vacuo to give **4** as a white solid: yield 243 mg (83%).

1,2:3,5-Di-O-isopropylidene-6-O-tosyl-α-D-[^{14}C(U)]-glucofuranose (**6**).

Concentrated sulfuric acid (12 mL) was added dropwise to a stirred mixture of **4** in 4.52 mL of 2,2-dimethoxypropane containing 695 mg of 4A molecular sieves (Linde). After 4 h the solution was neutralized with calcium oxide, filtered, and evaporated to dryness in vacuo. The residue was partitioned between ethyl acetate and water. The ethyl acetate layer was dried over magnesium sulfate and evaporated to give **6** as an orange syrup: yield 261 mg (97%).

6-Deoxy-6-bis(2-hydroxyethyl)amino-1,2:3,5-di-O-isopropylidene-α-D-[^{14}C(U)]-glucofuranose (**9**).

A solution of **6** in 2.87 mL of 2,2'-iminodiethanol was heated under anhydrous

conditions at 155 °C for 3 h, diluted with 29 mL of chloroform, and washed with water (3 x 12 mL). The combined aqueous wash was back washed with chloroform (3 x 17 mL). The combined organic extract was dried over magnesium sulfate and evaporated to dryness in vacuo giving a syrup. A methanol solution of the syrup was applied to two 8-in. Brinkmann Silica Gel 60 (2 mm) plates which were developed in chloroform-methanol (5:1). The product band, detected by observing under the 365 nm UV light and with a Geiger counter, was scraped off and extracted with methanol. Evaporation of the methanol extract gave **9** as a glass: yield 83 mg (35%).

6-Bis(2-chloroethyl)amino-6-deoxy-1,2:3,5-di-O-isopropylidene-α-D-[^{14}C(U)]-glucofuranose (**10**).

Thionyl chloride (102 μL, 1.40 mmol) was added slowly to a solution of **9** in anhydrous dichloromethane (11 mL). This solution was refluxed for 1 h and evaporated to dryness in vacuo. A solution of the residue in dichloromethane (10 mL) was washed with 10 mL of saturated aqueous sodium bicarbonate, then 10 mL of water, dried over magnesium sulfate, and evaporated to a syrup. An ethyl acetate solution of the syrup was applied to an 8-in. Brinkmann Silica Gel 60 (2 mm) plate, which was developed in cyclohexane-ethyl acetate (1:1). The product band, detected by a Geiger counter, was scraped off and extracted with ethyl acetate. Evaporation of the ethyl acetate extract gave **10** as a syrup: yield 30 mg (29%).

6-Bis-(-2chloroethyl)amino-6-deoxy-D-[^{14}C(U)]-glucose hydrochloride (**11**).

A solution of **10** in 6N hydrochloric acid (0.3 mL) was stirred at 95 °C for 1 h and evaporated to dryness in vacuo. An aqueous solution of the residue was treated with charcoal, filtered, and lypholized to give **11** as a cream colored glass: yield 24 mg (100%); mass spectrum (m/z) 304 $(M + 1)^+$; specific activity determined by liquid scintillation counting 29.4 μCi/mg or 10 mCi/mmol. The compound was homogenous as determined by thin-layer chromatography [analtech silica gel GF, butanol-acetic acid-water (5:2:3); the spots were detected by sulfuric acid char]. The radiochemical purity, determined by radioactivity scans of the thin-layer chromatogram with a Packard radiochromatogram scanner, Model 7201, was >99%.

REFERENCES

1. Cantrell, Jr. J. E., Gruen D., and Schein, P. S. - Cancer Res. **46**: 2340 (1986).

2. Schein P. S., Dean S. W., and Green D. C. - Proc. Am. Assoc. Cancer Res. **27**: 238 (1986).

3. Reist E. J., Spencer R. R., and Baker B. R. - J. Am. Chem. Soc. **82**: 2025 (1960).

4. New England Nuclear, Boston, MA.