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RADIOLABELING KIT/GENERATOR FOR 5-RADIOHALOGENATED URIDINES

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Abstract: A rapid, simple and inexpensive synthesis of 5-radiohalogenated-2'-deoxyuridine from 5-trimethylstannyl-2'-deoxyuridine is described. The total reaction and purification time including thin layer chromatography (tlc) for quality control is less than 30 min. This method produces excellent yields (>95%) of ^{123}I-, ^{125}I-, ^{131}I-UdR. The radiochemical purity of all tested preparations (>20) was determined to be greater than 99%. This new method is the basis of a radiolabeling kit/generator for preparation of radiohalogenated nucleosides. 2'-Deoxyuridine (UdR) halogenated with a stable isotope of bromine was also synthesized indicating that the method can be applied to the preparation of 5-radiobromo-2'-deoxyuridine (BUdR).

Key Words: 5-iodo-2'-deoxyuridine, iodine, radioisotopes, radiolabeling kit/generator.

INTRODUCTION

Synthesis of radioiodinated 5-iodo-2'-deoxyuridine was first described by Prusoff *et al.* (1); this method has been used extensively with and without modifications (2-6). It involves the reaction of UdR with sodium radioiodide in nitric acid or in the presence of other strong oxidants. In addition to IUdR several radiolabeled byproducts are generated reducing the overall yield of IUdR to about 50%. The specific activity

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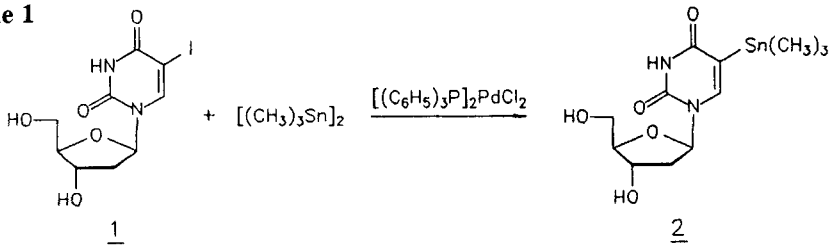
is frequently lowered by uv-absorbing unidentified impurities (4,6). A newer method of IUdR synthesis is based on the radiohalodemercuration reaction (7). This procedure provides excellent yields of no-carrier-added products. However, because of the nature of the precursor, IUdR recovered from the reaction mixture is contaminated with trace amounts of mercury compounds of unspecified nature (up to 200 ppm [7]). It cannot be used in humans without further purification.

The increasing demand for clinically acceptable $^{123/125}$IUdR preparations (8-13) prompted the development of a simple and rapid synthetic method which would serve as a basis of a radiolabeling kit. Such a kit would allow production, on demand and on site, of a high quality, sterile, and no-carrier-added radio-IUdR. Two of the main considerations were: (i) the reaction time compatible with short-lived radioisotopes (i.e. ^{123}I), and (ii) the ease of purification of the final product. The application of trimethylstannyl precursors establishes a rapid, novel synthetic method leading to no-carrier-added, high specific activity nucleosides and nucleotides.

Results and Discussion

5-Trimethylstannyl-2'-deoxyuridine **2** was synthesized from IUdR **1** using the modified method of Wigerinck *et al.* (14) as shown in Scheme 1. The stannyl precursor was purified on a flash silica gel column. Analytical samples were further purified on a normal phase high pressure liquid chromatography (hplc) column. The stability of the stannyl precursor was tested under variety of conditions. The $CHCl_3$ solution of **2** is stable for up to 6 months (longest period tested) when stored at -20°C under

Scheme 1

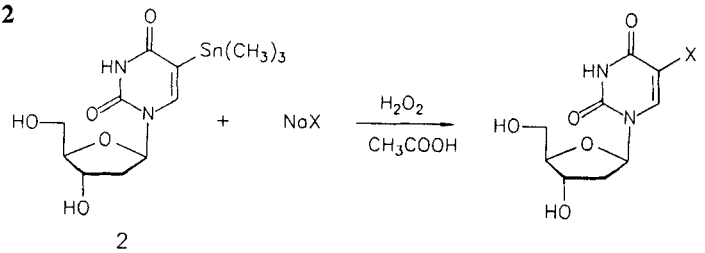


1 2

nitrogen atmosphere. The precursor 2 also appears to be stable when held in a solid form in a tightly capped test tube. It provides excellent yields (>95%) of radio-IUdR even after prolonged storage (7 months).

The reaction conditions for the preparation of the halogenated derivatives were perfected using sodium [^{127}I]iodide. The synthesis is outlined in Scheme 2. The reaction was conducted in either chloroform as a solvent or in a heterogenous mixture using test tubes coated with 2. The complete depletion of iodine was observed in both cases. The reaction time for the heterogenous mixture was just a few seconds longer than for the reaction run in chloroform (60 sec versus 15 sec). The use of 25% hydrogen peroxide in glacial acetic acid (v/v) as the oxidant simplified the purification

Scheme 2



2

1a X=^{123}I
1b X=^{125}I
1c X=^{131}I
1d stable isotopes of iodine & bromine

of the final halogenated deoxyuridine (radio-IUdR, BUdR). The product was isolated on a small, reversed-phase cartridge (about 0.1 mL dry volume of a C_{18} packing in a 1-mL syringe). Filtration through a 0.2 μm sterilization filter also removed all detectable uv-absorbing contaminants. The hplc analysis of the filtered reaction mixture (either ^{123}I or ^{125}I labeling; aliquots from reactions with 1 mCi to 15 mCi of radioiodide) using dual detection at 254 and 280 nm with the sensitivity set at 0.05 absorbance units (AU) failed to reveal any products other than the desired IUdR (radioactivity detected in fractions with the retention time [R_T] corresponding to that of IUdR standard). The tlc analysis (uv at 254 nm and radioactivity detection)

indicated the presence of a single radioactive spot co-migrating with the authentic sample of ^{127}IUdR (Figure 1). More than 20 lots of no-carrier-added ^{123}I-, ^{125}I-, and ^{131}I-UdR were prepared with about 95% yield. The analysis of crude reaction mixtures revealed that in all cases the conversion of radioiodide into radio-IUdR went to completion (100%). The yield of recovery of IUdR was always about 95% due to the losses during transfer of the reaction mixture and the sterilization process. Similar results were obtained during the preparation of 5-bromo-2'-deoxyuridine. The reaction of molar equivalents of **2** and sodium bromide produced pure BUdR with 92 % yield.

The efficiency of this method of making radio-IUdR allowed us to establish a set of radiolabeling conditions compatible with a rapid and facile "kit" preparation of IUdR in a clinical setting. This is particularly important in the case of ^{123}IUdR because of the short half-life (13 h) of ^{123}I. The "kit" contains a test tube coated with 100 μg of 5-trimethylstannyl-2'-deoxyuridine, a vial with the oxidant (H_2O_2/CH_3COOH, 1:3, v/v), a syringe-C_{18}-cartridge equipped with a 0.2 μm sterile filter, tlc plates pre-loaded with a standard (^{127}IUdR), and a vial with tlc developing solvents for quality control testing. The reliability and simplicity of this method allow for routine preparations of high activities of IUdR with the minimal radioactive exposure.

The potential for development of an "IUdR generator" was also explored. The reaction mixture containing 10 mCi of ^{123}IUdR was loaded onto a C_{18} Sep-Pak® cartridge (1mL of dry packing) and eluted daily with 1 mL of saline. Each collected fraction was analyzed for IUdR. The recovery of IUdR was

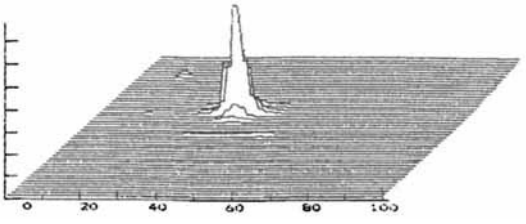


Figure 1. Radioactivity scan of a two-dimensional tlc silica gel plate of the reaction mixture containing ^{125}IUdR. Sample of ^{127}IUdR spotted with the reaction mixture indicated that the radioactivity co-migrates with the uv-absorbing spot. Origin at 0 mm horizontal, solvent front at 90 mm in both directions. Eluted in 8:1 CH_2Cl_2/CH_3OH (v/v).

91% with about 2.2 mCi collected in the first elution and from 1.7 to 1.8 mCi in each of the four consecutive elutions (corrected for decay). Isolated fractions contained only radiolabeled IUdR. The elution of a similarly prepared cartridge containing 5 mCi ^{125}IUdR yielded over 90% of the product collected in 0.25 mL of saline daily for 9 days (about 0.5 mCi per elution).

This novel method of IUdR synthesis is now being generalized to extend its scope to other nucleosides and nucleotides.

EXPERIMENTAL

Materials: All chemicals and solvents were from Aldrich Chemical Company (Milwakee, WI). Iodine-123 was purchased from Nordion (Kanata, Canada), iodine-125 and iodine-131 from either Amersham (Arlington Heights, ILL), ICN (Costa Mesa, CA), or Du Pont NEN Research Products (Boston, MA). ^{123}I and ^{125}I were no-carrier-added with specific activities of about 230,000 Ci/mmol and 2,100 Ci/mmol, respectively. ^{131}I had a specific activity of 790-1570 Ci/mmol. Na^{123}I was provided as a solid containing known amounts of NaOH, other radioisotopes were provided as sodium radioiodide solutions in NaOH. Hplc analyses of radioactive products were made using a C_{18} column (4.6 x 250 mm; Vaydac, Hesperia, CA) with either isocratic 80/20 H_2O/CH_3OH (15 min) followed by linear gradient to 100% CH_3OH (30 min) or isocratic 95/5 H_2O/CH_3CN as the elution solvents. The normal phase columns were from Phenomenex (4.6 x 250 mm and 22.5 x 250 mm; Maxsil 10 Silica; Torrance, CA). Tlc plates were silica gel on plastic backing with uv indicator (EM Science, Gibbstown, NJ). The radioactivity of hplc fractions was measured in a Packard Cobra II gamma counter. Tlc plates were scanned using a gas-flow Vista 100 analytical, digital imaging system (Radiomatic, Meriden, CT). Proton nmr spectra were recorded using a Varian XL 300 spectrometer.

5-(Trimethylstannyl)-2'-deoxyuridine: 5-Iodo-2'-deoxyuridine **1** (1g, 2.8 mmol) was dissolved in 45 mL anhydrous dioxane at about 60°C. The mixture was cooled to room temperature and 50 mg of bis(triphenylphosphine)palladium(II) dichloride and 2 g (6.1 mmol) of hexamethylditin were added. The mixture was refluxed until tlc indicated that all of IUdR reacted (about 5 h). The solution was cooled to 40°C and the solvent evaporated to dryness on a rotary evaporator. The dark brown, solid residue was loaded on a silica flash column and eluted with a 92:8 (v/v) mixture of $CHCl_3/CH_3OH$. Fractions containing the product were combined and evaporated to dryness. The trimethylstannyl derivative **2** was recovered in 54% yield (0.6 g) as a colorless oil. ^{1}Hnmr ($CDCl_3$/DMSO-d_6) 0.68 (s, 9 H, $[CH_3]_3Sn$); 2.57 (m, 1H, *H*C2'); 4.05 (t, 2H, *H*C5'); 4.27 (m, 1H, *H*C4'); 5.43 (t, 1H, C5'-O*H*); 5.66 (d, 1H, C3'-O*H*); 6.64 (t, 1H, *H*C1'); 8.15 (s, 1H, *H*C6); 11.54 (s, 1H, *H*N3).

5-Iodo-2'-deoxyuridine: The same procedure was used for all radioactive isotopes. The reactions with ^{127}I or a carrier-added ^{125}I were carried out on about 100 times larger scale to allow detailed analysis of the reaction mixture and final products. All syntheses involving radioisotopes of iodine were conducted behind a lead-lined screen in a well-ventilated fume hood equipped with charcoal filters. To a solution of 100 μg **2** in 100 μL chloroform was added 1-30 mCi of sodium radioiodide in 0.1 N NaOH (up to 50μL). The mixture was briefly mixed and 5 μL of H_2O_2/CH_3COOH (1:3; v/v) was added. The two-layer reaction mixture was sonicated for 15 sec and three 0.5-μL portions were spotted on silica gel plates to determine the progress of radioiodination. The radioactive spots were measured with a Vista 100 radioactivity scanner; the uv absorbing spots were visualized with a hand-held uv lamp (254 nm). In all cases all of radioiodine was converted into IUdR in less than 15 sec.

The work-up of the reaction mixture was as follows: chloroform was evaporated to dryness under a stream of nitrogen and 1 mL of the desired solvent (saline, 0.05 M phosphate-buffered saline, pH 7.2 [PBS]; double-distilled water) was added to the

residue. The radioactive content of this solution was measured in a dose calibrator (Capintec). In the initial studies each 1-mL solution was divided into two portions to determine the efficiency of purification process and to identify any source of radioactivity losses. One part was passed through a C_{18} cartridge and a 0.2 μm filter whereas the second fraction was only filtered through a 0.2 μm sterile filter. The C_{18} cartridge was washed prior to the purification step with methanol (the equivalent of 10 void volumes of the cartridge), followed by 10 void volume equivalents of distilled water and 3 of the elution solvent (saline, PBS, or water). The radioactive content of collected filtrates was determined in a gamma counter and the mixture was analyzed on tlc plates (CH_2Cl_2/CH_3OH 8:1, v/v, R_f: free iodide 0.1, IUdR 0.45, UdR 0.3, SnUdR 0.7; or 1-butanol saturated with concentrated ammonia, R_f free iodide 0.7, IUdR 0.5) and on a C_{18} reversed phase column (flow rate 1 mL/min; CH_3OH/H_2O isocratic 80/20 [v/v] for 10 min with the linear gradient to 100% CH_3OH at 10 min, retention times [R_T]: free iodide 3 min, UdR 4.5 min, IUdR 8 min, SnUdR 22 min; or CH_3CN/H_2O 95:5 [v/v], R_T free iodide 3 min, UdR 12 min, IUdR 20 min). For radioactive preparations the uv detector was set at 0.05 AU and 1-mL fractions were collected. The radioactive content of each fraction was determined in a gamma counter. To verify the identity of the radioactive product hplc and tlc analyses were performed using samples containing known quantities of ^{127}IUdR, ^{127}I, UdR, and SnUdR. The reactions conducted in the absence of chloroform were treated as described above but the sonication of the reaction mixture was extended to 60 sec. In all cases the conversion of iodide into IUdR was complete. The yield of IUdR recovery was always over 90% (usually 95% or more).

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