DIRECT PREPARATION OF ^{14}C-LABELED 5-ALLYL- AND 5-PROPYL-2'-DEOXYURIDINE FROM [2-^{14}C]2'-DEOXYURIDINE

Jerry L. Ruth[1], Steven K. White, and Donald E. Bergstrom[2]
Department of Chemistry, University of California, Davis, CA 95616 USA

SUMMARY

[2-^{14}C] 5-Allyl-2'-deoxyuridine was synthesized directly from [2-^{14}C]2'-deoxyuridine using mercury, palladium, and 3-chloropropene. [2-^{14}C] 5-Propyl-2'-deoxyuridine was obtained by hydrogenation of the [^{14}C]5-allyl-2'-deoxyuridine. Advantages of the synthetic method and its application to the preparation of other radiolabeled 5-alkyl/alkenyl-2'-deoxyuridines are discussed.

Key words: radiolabeled 5-propyl-2'-deoxyuridine; radiolabeled 5-alkyl-2'-deoxyuridines.

INTRODUCTION

Many 5-alkyl/alkenyl pyrimidine nucleosides exhibit significant bioactivity, particularly as antiviral agents (1). Several, including 5-propyl-dUrd[3] (2,3), E-5-propenyl-dUrd (4,5), 5-(3,3,3-trifluoropropenyl)-dUrd (4,5), and E-5-(2-halovinyl)-dUrds (6) are potent and quite selective against

---

[1]current address: Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27514 USA.

[2]current address: Department of Chemistry, University of North Dakota, Grand Forks, ND 58202 USA, and to whom reprint requests should be addressed.

[3]abbreviations used are: dUrd, 2'-deoxyuridine; dCyd, 2'-deoxycytidine; dUMP, 2'-deoxyuridine 5'-monophosphate; RP-HPLC, reverse phase high pressure liquid chromatography; TLC, thin layer chromatography.

0362-4803/82/070861-06$01.00

Received February 25, 1982
Revised March 18, 1982

herpes simplex and other viruses. In recent years many reports have documented pronounced activity in cell culture and even clinical results, but descriptions of detailed metabolic studies have been lacking, largely due to the unavailability of radiolabeled compound. Ideally, the synthetic preparation of labeled compound should require as few steps and physical manipulation as possible, due to both physiologic hazard and the very small amounts of material normally desired. The syntheses of a few radiolabeled 5-alkyl-dUrd analogs have been reported, most notably in a well-conceived and thorough study by Szabolcs and colleagues (7). However, such traditional methods, which condense a preformed base with a suitably-protected and activated sugar, have required complex reactions and rigorous purification, particularly to separate mixtures of α and β anomers. This has necessarily resulted in the expenditures of large amounts of radiolabel, time, and effort, and in addition has required the synthesis of a different radiolabeled precursor for each analog.

We have earlier reported a non-traditional synthetic approach to unlabeled 5-alkyl/alkenyl uracil and cytosine nucleosides (8-12). The basic method has since been used by others in their preparation of numerous analogs, including E-5-(2-bromovinyl)-dUrd (13) and 5-substituted dUMP analogs directly from dUMP[3] (14), and has proven to be the shortest, simplest, and most efficient route to many such compounds. The method has appeared particularly amenable to the syntheses of radiolabeled pyrimidine nucleosides since it can tolerate a wide variety of functional groups and sugar moieties, utilizes unprotected readily-available dUrd or dCyd as starting materials, can use water or methanol as solvents, and minimizes handling and purification. In this paper we will describe the application of the method to the syntheses of [^{14}C]-labeled 5-allyl- and 5-propyl-dUrd as models for small scale radiosynthesis of this class of analogs.[4]

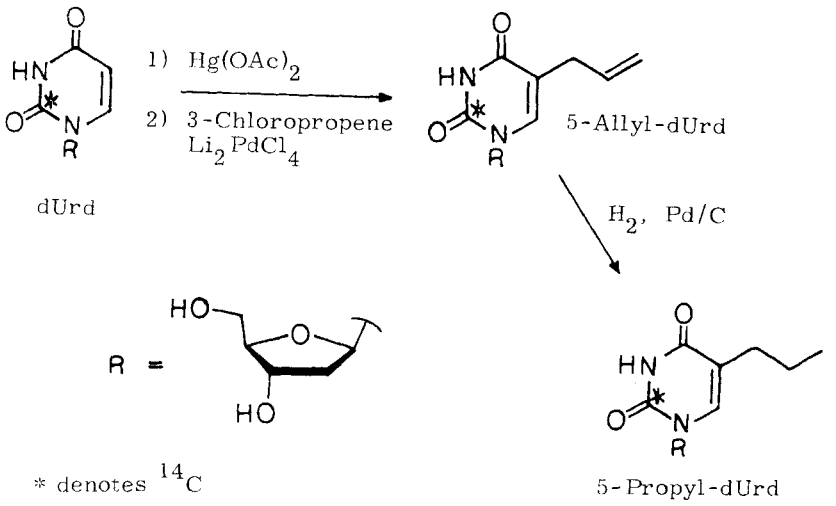
---

[4] We are not aware of any application of this methodology to radiolabeled nucleosides, and felt a brief description was necessary since the compounds are currently being reported in biological studies (J. Ruth and Yung-chi Cheng, unpublished results).

DISCUSSION

The one-pot method of preparing 5-allyl-2'-deoxyuridine directly from dUrd has been described (9), and is illustrated in Scheme I. With little modification, we applied the procedure to less than 9 mg [2-^{14}C] 2'-deoxyuridine; the crude 5-allyl-dUrd obtained was hydrogenated directly over palladium-on-carbon.

Scheme I



Upon purification, a 20% isolated yield (overall from dUrd) of [2-^{14}C]5-propyl-dUrd was obtained in 99.7% radiopurity. No separation of anomers or protection of the sugar was necessary.

Other methods of preparing ^{14}C-radiolabeled 5-propyl-dUrd have required more than seven separate reactions involving numerous transfers and extractions, and vigorous, often painstakingly anhydrous conditions to give overall yields, for example, of less than 12% from ethyl pentanoate (7). In contrast the method described here offers increased yield, greatly reduced physical manipulation, and much simplified purification. In analogy with similar syntheses of unlabeled compounds (8-13), the preparation of longer-chain radiolabeled 5-alkyl-dUrd

analogs by this approach should be equally facile; the radiosyntheses of other antivirals such as ^{14}C-labeled E-5-(2-bromovinyl)-dUrd (13), E-5-(3,3,3-trifluoropropenyl)-dUrd (10), and 5-ethyl-dUrd (8) should be straightforward as well. The preparation of E-5-propenyl-dUrd, for example, requires only a single isomerization step from 5-allyl-dUrd (9).

Reactions as described here and in earlier work are particularly amenable to small scale preparations. Unlike the majority of other carbon-carbon bond-forming reactions, these syntheses do not require anhydrous conditions at any point, and have been carried out entirely in water, methanol, dimethylformamide, or, in the case of acetylenic couplings, trialkylamines or tetrahyrofuran (15-17). The coupling may be accomplished from the 5-mercurated uracil or cytosine nucleosides as described here and elsewhere (8-13), or from 5-iodopyrimidines (17) and 5-iodopyrimidine nucleosides (8,11,15,16) using different conditions. The direct attachment of olefins to the unprotected parent nucleosides will also allow the synthesis of analog radiolabeled in the sugar, the base, or even in the side chain; the approach can also be used in the direct alkylation of nucleoside triphosphates (18). Any or all of these applications could prove uniquely advantageous in studies of biological metabolism.

EXPERIMENTAL

Cold 2'-deoxyuridine was purchased from Sigma Chemical Co; [2-^{14}C] 2'-deoxyuridine (59 mCi/mmol) was from Moravek. Mercury (II) acetate, 3-chloropropene, lithium chloride, 10% palladium on activated carbon, copper (II) chloride, and sodium borohydride were from Aldrich Chemical Co. Palladium (II) chloride was from Matthey Bishop, Inc. Preparative thin layer chromatography plates (silica 60, 0.05x20x20 cm) were from E. Merck. Reverse phase high pressure liquid chromatography (RP-HPLC) was done using a Knauer RP-8 (7 μ C_8 resin) analytic column from Unimetrics Corp, eluting methanol/0.03 N acetic acid (3:7) at 1.0 ml/min detecting at 270 nm; in this system, the retention time of 5-propyl-dUrd was 11.5 min. The 0.10 N solution of lithium tetrachloropalladate (Li_2PdCl_4) in

methanol was prepared by stirring 10 mmol lithium chloride and 5 mmol palladium (II) chloride in 50 ml methanol for 18 h at ambient temperature.

[2-^{14}C]5-Allyl-2'-deoxyuridine. In a 10 ml flask were combined 2.1 umol [2-^{14}C]-dUrd (124 uCi), 8.1 mg dUrd (35 umol), 16 mg mercury (II) acetate (50 umol), and 6 ml water. The mixture was heated to 50°C for 6 h with stirring, then lyophilized. To the residue was added consecutively 6 ml methanol, 40 ul 3-chloropropene (490 umol), and 40 ul 0.10 N Li_2PdCl_4 in methanol. The reaction mixture was stirred 48 h at ambient temperature, then 0.6 mg copper (II) chloride and a second 40 ul portion of 0.10 N Li_2PdCl_4 were added. After an additional 24 h, four 2 mg portions of $NaBH_4$ were added. The reaction was concentrated under reduced pressure to give a solid residue, and repeatedly evaporated to dryness from methanol to remove borates. The residue was suspended in five ml methanol and filtered to give crude [2-^{14}C]5-allyl-dUrd in the methanolic filtrate.

[2-^{14}C]5-Propyl-2'-deoxyuridine. Half (by volume) of the crude 5-allyl-dUrd was hydrogenated using 0.8 mg 10% Pd/C and 30 psig H_2 pressure while stirring for 3 h at ambient temperature. To ensure maximum reduction, the hydrogenation was repeated twice using fresh methanol (5 ml) and catalyst (0.8 mg 10% Pd/C). The reaction mixture was filtered, concentrated under reduced pressure, and chromatogrammed on preparative TLC plates eluting with CH_3CN/n-BuOH/0.1 N NH_4OH (1:6:2:1) using authentic 5-propyl-dUrd as a marker to give 3.8 umol [2-^{14}C]5-propyl-dUrd (3.3 mCi/mmol) in 20% isolated yield from dUrd (yield determined by both total activity and UV). The product was characterized by UV and by comparison to authentic marker (9) using TLC and RP-HPLC; radiopurity was 99.7% by RP-HPLC with no impurities detectable by TLC.

ACKNOWLEDGEMENT This investigation was funded by National Cancer Institute grant CA 21493 awarded to D.E. Bergstrom.

REFERENCES

1. De Clercq, E. - Arch. Internat. Physiol. Biochim. 87:353 (1979).
2. De Clercq, E., Descamps, J., and Shugar, D. - Antimicrob. Agents Chemother. 13:545 (1978).

3. Cheng, Y.C., Domin, B., Sharma, R., and Bobek, M. - Antimicrob. Agents Chemother. 10:119 (1976).
4. Cheng, Y.C., Grill, S., Ruth, J., and Bergstrom, D. - Antimicrob. Agents Chemother. 18:957 (1980).
5. De Clercq, E., Descamps, J., Barr, P., Jones, A., Serafinowski, P. Walker, R, Huang, G., Torrence, P., Schmidt, C., Mertes, M., Kulikowski, T., and Shugar, D. in "Antimetabolites in Biochemistry, Biology, and Medicine", J. Skoda and P. Langen, eds., Pergamon Press, Elmsford, NY, 1979, p 275.
6. De Clercq, E., Descamps, J., De Somer, P., Barr, P., Jones, A., and Walker, R. - Proc. Natl. Acad. Sci. USA 76:2947 (1979).
7. Szabolcs, A., Kruppa., G., Sági, J., and Ötvös, L. - J. Labelled Compounds and Radiopharmaceuticals 14:713 (1977).
8. Bergstrom, D. and Ruth, J. - J. Amer. Chem. Soc. 98:1587 (1976).
9. Ruth, J., and Bergstrom, D.- J. Org. Chem. 43:2870 (1978).
10. Wataya, Y., Matsuda, A., Santi, D., Bergstrom, D., and Ruth, J. - J. Med. Chem. 22:339 (1979).
11. Bergstrom, D., and Ogawa, M. -J. Amer. Chem. Soc. 100:8106 (1978).
12. Bergstrom, D., Ruth, J., and Warwick, P. - J. Org. Chem. 46:1432 (1981).
13. Jones, A., Verhelst, G., and Walker, R. - Tet. Letters 4415 (1979).
14. Bigge, C., Kalaritis, P., and Mertes, M. - Tet. Letters 1653 (1979).
15. Robins, M., and Barr, P. - Tet. Letters 22:421 (1981).
16. Vincent, P., Beaucourt, J.-P., and Pichat, L. - Tet. Letters 22:945 (1981).
17. Edo, K., Sakamoto, T., and Yamanaka, H. - Chem. Pharm. Bull. (Jpn) 22:193 (1979).
18. Langer, P., Waldrop, A., and Ward, D. - Proc. Natl. Acad. Sci. USA 78:6633 (1981).