AN EFFICIENT, HIGH SPECIFIC ACTIVITY RADIOIODINATION OF

5-(1-HYDROXY/METHOXY-2-IODOETHYL)-2'-DEOXYURIDINE BY

ISOTOPE EXCHANGE LABELLING IN PIVALIC ACID MELT

Takashi Iwashina, Rakesh Kumar, Edward E. Knaus and Leonard I. Wiebe[1]

Faculty of Pharmacy and Pharmaceutical Sciences,

University of Alberta, Edmonton, Alberta, Canada   T6G 2N8

SUMMARY

Radioiodinated 5-(1-hydroxy-2-iodoethyl)-2'-deoxyuridine (**1a**) and 5-(1-methoxy-2-iodoethyl)-2'-deoxyuridine (**2a**) were prepared from the corresponding unlabelled nucleosides (**1** and **2**) by isotope exchange in a pivalic acid melt. Compound **1a** was obtained in 71.3% to 26.3% radiochemical yield with a specific activity range of 77 to 2486 GBq/mmol, and **2a** was prepared in 70.3% radiochemical yield at a specific activity of 100.6 GBq/mmol. Low temperatures and the mild reaction conditions encountered in the pivalic acid melt method, which reduce the extent of nucleoside decomposition, were essential for successful exchange radioiodination. Even under optimal conditions (70°C; 100 min), the order in which the reagents were mixed was critical to obtain high radiochemical yields and to minimize chemical decomposition of the nucleosides.

Keywords: exchange radioiodination, pivalic acid melt, pyrimidine nucleosides,

5-(1-hydroxy-2-iodoethyl)-2'-deoxyuridine,

5-(1-methoxy-2-iodoethyl)-2'-deoxyuridine

INTRODUCTION

A number of radiolabelled 5-substituted pyrimidine nucleoside analogs have

been developed as potential tumor localization agents (1-3). Radioiodinated

compounds are particularly suitable for biological investigations. It is often

possible to radioiodinate test compounds by simple electrophilic or

nucleophilic substitution, by addition reactions or by exchange labelling of

cold iodoprecursors. ^{125}I and ^{131}I are suitable for *in vitro* and *in vivo*

---

[1] Author for Correspondence

animal studies, and ^{123}I is ideal for in vivo human studies. 5-(1-Hydroxy/
methoxy-2-iodoethyl)-2'-deoxyuridine (Compounds 1 and 2) have demonstrated
antiviral and antitumor activity in preliminary in vitro tests (4). In order
to further evaluate their potential as non-invasive diagnostic agents, it was
necessary to radioiodinate these novel nucleosides. Substitution or addition
reactions were considered inappropriate because of the requirement for
subsequent isolation and purification after introducing the radioiodine
substituent. However, isotope exchange methods were expected to be suitable
for the radioiodination of 1 and 2, both of which have iodoalkyl substituents.
For example, radioiodinated 19-iodocholesterol has been prepared by exchange in
refluxing acetone solution (5). (E)-5-(2-Iodovinyl)-2'-deoxyuridine (IVDU), a
pyrimidine nucleoside with potent antiviral activity against HSV-1 (TK[+])
(6,7), has been labelled by radioiodide exchange using cuprous ion in dry DMF
at 70-80°C for 20 hr (8), or in 0.05 N hydrochloric acid in ethanol at
100°C for 20 min. (9).

Radioisotope exchange in the melt has been effective for the labelling of
many iodoaryl compounds under high temperature reaction conditions using
acetamide (10), benzoic acid (11), and pivalic acid (12) as molten reaction
media.   Pivalic acid, with its low melting point and weak acidity, appeared to
be suitable for radiodination of compounds 1 and 2. We now report a procedure
for the radioiodination of compounds 1 and 2 using a melt isotope exchange
reaction in pivalic acid.   This procedure provided the mild isotope exchange
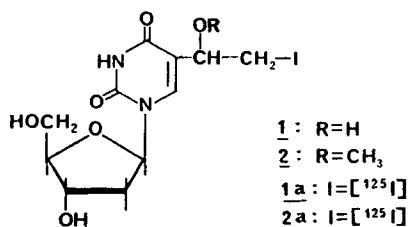conditions necessary for radioiodination of these thermolabile compounds.

1 : R=H
2 : R=CH$_3$
1a : I=[^{125}I]
2a : I=[^{125}I]

Figure 1.   5-(1-Hydroxy/methoxy-2-iodoethyl)-2'-deoxyuridines

EXPERIMENTAL

The synthesis of unlabelled 5-(1-hydroxy-2-iodoethyl)-2'-deoxyuridine (1) and 5-(1-methoxy-2-iodoethyl)-2'-deoxyuridine (2) will be described elsewhere. All chemicals used were of reagent grade quality. "No-carrier-added" sodium [^{125}I] iodide in dilute aqueous NaOH solution was supplied by the Edmonton Radiopharmacy Centre, Edmonton, Canada.

HPLC purifications were carried out with a Waters system consisting of a Model U6K injector, dual 501 pumps and a Model 680 gradient controller, combined with a Hewlett Packard Model 1040A Diode Array UV detector and Model 9133 data system. Analytical HPLC was performed on Radial-Pak uBondapak C_{18} (10u, 7mm x 10cm; Waters Associates Inc.) reverse-phase columns. Mobile phase-I (MeOH:H_2O = 1:3 v/v) was used for purification of compound 1, and mobile phase-II (MeOH:H_2O = 35:65 v/v) was used for purification of compound 2. A NaI(Tl) -ray detector coupled in series to the HPLC UV detector and connected to the data system provided HPLC radiochromatograms. The amount of radioactivity in column effluent fractions was determined with a radioisotope dose calibrator (Capintec Instruments Inc. Model CRC-30RC Radioisotope calibrator).

The decomposition of compounds 1 and 2 at various temperatures in pivalic acid was determined. The test nucleoside solution (50ug 1 or 2 in 50uL ethanol) was introduced into a 0.3 mL Reacti-Vial^{TM} (Pierce Chemical Company) and the solvent was removed under a stream of N_2 gas. Pivalic acid (1 mg) was added to the vial and the mixture was heated at selected temperatures for 1 hr. After cooling, the reaction mixture was dissolved in 30% aqueous MeOH (200uL) and analyzed by HPLC. The % decomposition was determined by quantitative HPLC analyses. The results are illustrated in Figure 2.

For exchange radiolabelling, Na[^{125}I]I (no-carrier-added) in ethanol (20 uL) and pivalic acid (1 mg) were introduced into a 0.3 mL reaction vial. The solvent was removed under a stream of nitrogen, and a solution of the
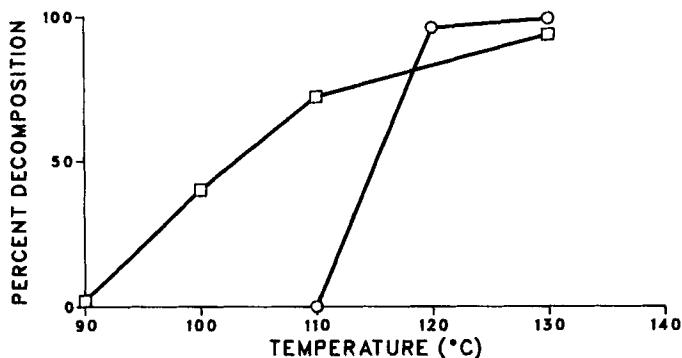
Figure 2. Decomposition of compounds <u>1</u> (-) and <u>2</u> (-) (50 ug) upon heating
at various temperatures for 1 hr in pivalic acid (1 mg).

unlabelled nucleoside (<u>1</u> or <u>2</u>) in ethanol (25 uL) was added to the vial. The
mixture was dried under a stream of nitrogen, the vial was sealed with a
teflon-rubber septum and then heated at 70^{0}C for times ranging from 0.5 to 2
hr.   After cooling, the residue was dissolved in 30% aqueous MeOH (200 uL) and
analyzed by HPLC using the appropriate mobile phase at a flow rate of 1.0
mL/min.   Radioactive fractions which corresponded in retention time (10.5 and
9.5 min for <u>1</u> and <u>2</u>, respectively) to an authentic unlabelled sample were
pooled to afford the radiolabelled compounds. Reaction times and radiochemical
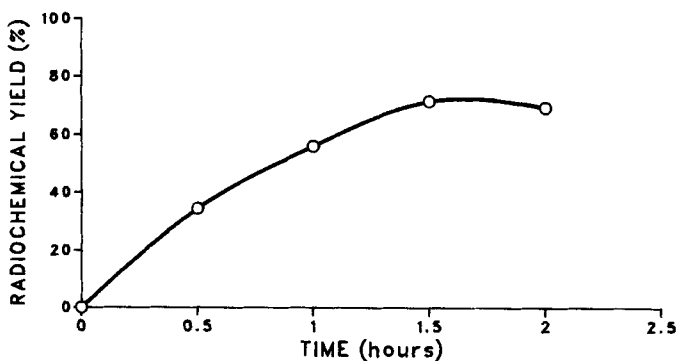yields for <u>1a</u> are shown in Figure 3.

Figure 3. Radiochemical yield of compound <u>1</u> as a function of reaction time at
70^{0}C, for the radioiodine exchange reaction in pivalic acid.

The range of specific activities, radiochemical yields and % decomposition of compound 1 are shown in Table 1. Radiolabelled 2a was similarly prepared by heating at 70°C for 100 min. The specific activity of 2a, starting with 25 ug of 2, was 100.6 GBq/mmol, with a 70.3% radiochemical yield.

TABLE 1. Specific activity and radiochemical yield for the exchange reaction at various concentrations of compound 1 using Method B, with 1 mg pivalic acid and heating for 100 min at 70°C.

Quantity (ug)	Na[125I]I (MBq)	Specific Activity (GBq/mmol)	Radiochemical Yield (%)	Decomposition[a] (%)
50	6.14	35.9	71.3	0.9
5	2.96	152.1	56.4	14.3
0.5	4.75	2486.0	26.3	60.0

[a] Based on starting amount of nucleoside.

RESULTS AND DISCUSSION

A melt isotope exchange reaction in pivalic acid has been developed to radiolabel 1 and 2, in order to investigate their potential as non-invasive probes for localizing tumors and focal viral infections. Initial attempts to radiolabel these nucleosides by exchange in ethanol solution under neutral, acidic or basic conditions resulted in decomposition, failure to label or, usually, both. Although this reaction was not investigated in detail, it was concluded that the relatively high temperatures required (120°) (5) for effective exchange was incompatible for use with this class of nucleoside.

Because compounds 1 and 2 melt with decomposition at relatively low temperatures, (123°C and 115°C, respectively), their chemical stability under melt conditions had to be established. The thermal decomposition of compounds 1 and 2 on heating for 1 hr in molten pivalic acid, was examined at temperatures ranging from 90°C to 130°C (Figure 2). Compound 1, although relatively stable at 90°C, decomposed rapidly at higher temperatures, with 93% decomposition when heated at 130°C. Compound 2 was stable at

temperatures up to 110°C, after which decomposition increased rapidly and was virtually quantitative (98.2%) at 130°C. These results indicated that the high reaction temperatures usually required for isotope exchange reactions, for example 155°C for aryl iodides (12) in pivalic acid, could not be used with compounds 1 and 2. It was ascertained that efficient exchange labelling must be effected at much lower temperatures. A reaction temperature of 70°C, with a reaction time of 100 min, was subsequently found to be optimal.

Na[^{125}I]I was obtained from the supplier as a solution, in aqueous sodium hydroxide. The small quantity of sodium hydroxide present was found to have an appreciable effect on the radiochemical yield when the melt isotope exchange reaction was carried out using pivalic acid, depending on the order in which the reagents were mixed. In Method A, pivalic acid was added after mixing compound 1 or 2 with Na[^{125}I]I, whereas in Method B, pivalic acid and Na[^{125}I]I were mixed and dried prior to adding the nucleoside. The results obtained with the two Methods are summarized in Table 2.

TABLE 2. Radiochemical yield and decomposition of compounds 1 and 2 for two Methods; A) mixing nucleoside with Na^{125}I prior to adding pivalic acid and B) mixing pivalic acid with Na^{125}I prior to adding nucleoside. All reactions were performed with 25 ug of nucleoside in 1 mg of pivalic acid. Data are for reaction times of 1 hr.

Method	Compound	Na[^{125}I]I (MBq)	Temperature (°C)	Radiochemical Yield (%)	Decomposition[a] (%)
A	1	10.55	70	N.D.	80.8
	2	6.62	90	48.4	39.3
B	1	8.07	70	56.0	0
	2	4.88	90	47.5	65.0

[a] Based on starting amount of nucleoside.

Method B afforded 1a and 2a in 56% and 47.5% radiochemical yield, respectively, with no decomposition of compound 1 and 65% decomposition of 2. When Method A

was used for radioiodination, compound <u>2a</u> was obtained in 48.4% radiochemical yield, whereas the labelling of compound <u>1</u> was unsuccessful. The extensive decomposition of compound <u>1</u> (80.8%) using Method A is most likely due to its instability in strongly alkaline conditions. The failure to label compound <u>1</u> (Method A) was therefore attributed to decomposition of <u>1</u> in the presence of sodium hydroxide prior to addition of pivalic acid and initiation of the isotope exchange reaction. In Method B, mixing the aqueous alkaline radioiodide solution with pivalic acid, prior to addition of <u>1</u>, neutralizes the sodium hydroxide present. Consequently, the nucleoside is exposed only to the mildly acidic conditions of the pivalic acid melt.

The optimum time for the exchange labelling of compound <u>1</u> was examined using the reaction conditions which gave the least evidence of decomposition of compound <u>1</u>. As shown in Figure 3, the maximum radiochemical yield (71.3%) was obtained after 1.5 hr. The use of reaction times longer than 2 hr for radioiodination of compound <u>1</u> were not expected to increase net radiochemical yields because of accelerated decomposition of the radiolabelled product.

The radiochemical yield of compound <u>1a</u> was determined under conditions which were expected to produce a 100-fold increase in specific activity, using the most suitable melt isotope exchange reaction conditions (100 min at 70°C, Method B). The results are shown in Table 1. The specific activity of <u>1a</u> was increased to 2.49 TBq/mmol by decreasing the quantity of starting compound <u>1</u> to 0.5 ug in the reaction mixture. However, the radiochemical yield decreased from 71.3% to 26.2%, while decomposition of compound <u>1</u> increased from 0.9% to 60% under these conditions. Thus, it appears possible to obtain even higher specific activity with this procedure but only by sacrificing radiochemical yield.

Although optimization studies were not performed with compound <u>2</u>, an acceptable radiochemical yield (70.3%) with low decomposition (33.8%) was obtained at 70°C with a reaction time of 100 min using Method B.

In summary, radioiodine exchange labelling conditions in pivalic acid melt were developed for the radioiodination of thermally-labile nucleosides 1 and 2. By mixing the alkaline radioiodide solution with pivalic acid prior to addition of the nucleoside to be labelled, and by limiting temperatures to 70^{0}C, radiochemical yields up to 70% and specific activities of up to 2.49 TBq/mmol were attainable within a 100 min reaction time.

REFERENCES

1. Mercer J.R., Knaus E.E. and Wiebe L.I. - J. Med. Chem. 30: 670 (1987).

2. Abrams D.N., Knaus E.E. and Wiebe L.I. - Int. J. Nucl. Med. Biol. 6: 97 (1979).

3. Abrams D.N., Knaus E.E., Lentle B.C. and Wiebe L.I. - Int. J. Nucl. Med. Biol. 6: 103 (1979).

4. Kumar R., Knaus E.E. and Wiebe L.I. - J. Med. Chem. (in press).

5. Counsell R.E., Ranade V.V., Blair R.J., Beierwaltes W.H. and Weinhold P.A. - Steroids 16: 317 (1970).

6. De Clercq E., Descamps J., De Somer P., Barr P.J., Jones A.S. and Walker R.T. - Proc. Natl. Acad. Sci. USA 76: 2947 (1979).

7. Gill M.J., Samuel J., Wiebe L.I., Knaus E.E. and Tyrrell D.L. Antimicrob. Agents Chemother. 25: 476 (1984).

8. Samuel J., Knaus E.E., Wiebe L.I. and Tyrrell D.L. - Int. J. Appl. Radiat. Isotopes 35: 1049 (1984).

9. Verbruggen A., Julien C., De Clercq E. and De Roo M. - Appl. Radiat. Isot. 37: 355 (1986).

10. Seevers R.H.,   Schwender S.W.,   Swayze S.L.   and Counsell R.E. - J. Med. Chem. 25: 618 (1982).

11. Eisenhut M. - Int. J. Appl. Radiat. Isot. 33: 499 (1982).

12. Weichert J.P, Van Dort M., Groziak M.P. and Counsell R.E. - Appl. Radiat. Isot. 37: 907 (1986).