SYNTHESIS OF [2-^{14}C]-1-(2-DEOXY-2-FLUORO-β-D-ARABINOFURANOSYL)-5-FLUORO(CHLORO)URACIL

Hemant K. Misra, Leonard I. Wiebe and Edward E. Knaus*

Faculty of Pharmacy and Pharmaceutical Sciences

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

SUMMARY

The reaction of [2-^{14}C]-2,4-bis-O-(trimethylsilyl)uracil (2) with 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (3) yielded the 3-O-acetyl-5-O-benzoyl nucleoside 4 which was hydrolyzed with methanolic ammonia to afford [2-^{14}C]-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (5) in 55% radiochemical yield with a specific activity of 2 mCi/mmol (74 MBq/mmol). The reaction of 5 with molecular fluorine and chlorine in glacial acetic acid at 25°C afforded the 5-fluoro-6a and 5-chloro-6b analogues of [2-^{14}C]-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil in 85.6 and 87.9% isolated radiochemical yield, respectively. Similar radiochemical yields were obtained when [2-^{14}C]uracil having a specific activity of 12 mCi/mmol (444 MBq/mmol) was utilized.

Key words: Silyl Hilbert-Johnson coupling reaction, electrophilic halogenation, [2-^{14}C]-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-halouracils

INTRODUCTION

A group of 5-halo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosines and uracils were recently developed that exhibit significant antiviral and/or antileukemic activity (1-2). Structure-activity studies indicated that the 2-fluoro substituent in the arabino (up) configuration is essential for potent antiviral activity (1-3) and provides high chemical and metabolic stability to the N-glycosyl linkage thereby providing resistance to extensive deglycosylation *in vivo* (4). Their selective antiviral activity against HSV viruses, and their low toxicity to uninfected cells, is dependent primarily on specific phosphorylation by virus-encoded deoxycytidine-deoxythymidine kinase (3,5).

*Author to whom correspondence should be addressed.

0362-4803/87/091107-10$05.00

Received November 25, 1986
Received February 2, 1987

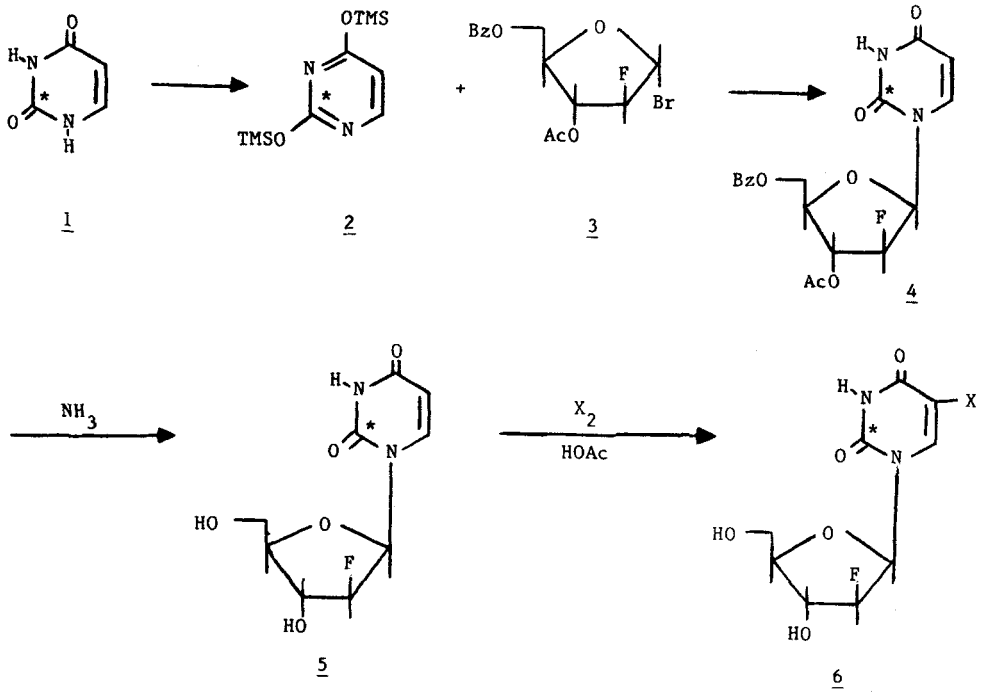
5-Iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cystosine (FIAC) undergoes metabolic deamination by cytosine nucleoside deaminase to produce 5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FIAU) as the major metabolite (4,6) which is phosphorylated and then incorporated into DNA of normal proliferating tissue and of leukemic cells (7).

In an earlier study we reported the synthesis of "carrier-added" and "no-carrier added" [^{123}I, ^{125}I, ^{131}I]-5-iodo- and [^{82}Br]-5-bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (8). We now report a facile procedure for the synthesis of [2-^{14}C]-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (5, [2-^{14}C]FAU), the 5-fluoro analogue 6a ([2-^{14}C]FFAU) and the 5-chloro analogue 6b ([2-^{14}C]FClAU), which were required for preliminary in vitro and in vivo studies for our ongoing program to develop γ-emitting radiotracers for use in scintigraphic in vivo diagnosis of viral and oncological disease. The fluorination reaction described is suitable for the synthesis of [5-^{18}F]FFAU.

RESULTS AND DISCUSSION

In selecting a synthetic route for the synthesis of the required [2-^{14}C]FFAU (6a) and FClAU (6b) we decided to use the silyl Hilbert-Johnson coupling reaction of 2,4-bis-O-(trimethylsilyl)uracil 2 with 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro- α -D-arabinofuranosyl bromide 3. The direct introduction of a fluorine atom into the 2-arabino configuration of a preformed pyrimidine nucleoside would be difficult, if not impossible, due to the ease with which the 2-carbonyl group of the pyrimidine ring participates in nucleophilic displacement of 2-ribofuranosyl substituents (9-10). [2-^{14}C]FAU (5) is a versatile product which could then the elaborated to a wide variety of 5-substituted analogues (8, 11, 12) including 6a and 6b. The synthesis of [2-^{14}C]FIAC (6) and FIAU (13) starting from 5-iodocytosine and 5-iodouracil, respectively have been reported.

Practical syntheses of 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabino-furanosyl bromide (3) and 3,5-di-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide have been reported by Fox et al (14) and Tann et al (15), respectively. Anomeric 3 was separated by silica gel column chromatography prior to use since



reaction of the α-anomer 3 with 2 would be expected to yield predominately or exclusively the β-nucleoside 4, thereby increasing the radiochemical yield of the desired β-conformer. Thus, reaction of [2-^{14}C]-2,4-bis-O-(trimethylsilyl) uracil (2), obtained from the reaction of [2-^{14}C]uracil (1) with hexamethyl-disilazane, with 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide 3 in methylene chloride at 25°C for 7 days yielded [2-^{14}C]-3-O-acetyl-5-O-benzoyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (4). Treatment of 4 with a saturated solution of ammonia in methanol afforded the deblocked nucleoside [2-^{14}C]FAU 5 in 55% isolated radiochemical yield with a specific activity of 2 mCi/mmol. Similar results were obtained utilizing [2-^{14}C]-uracil having a specific activity of 12 mCi/mmol. The reaction of [2-^{14}C]FAU (5) with molecular fluorine and chlorine in glacial acetic acid at 25°C afforded [2-^{14}C]FFAU (6a) and [2-^{14}C]FClAU (6b) in 85.6 and 87.9% isolated radiochemical yield, respectively. The electrophilic halogenation of deoxyuridine and uridine pyrimidine nucleoside analogues using similar conditions has been reported (16-18).

The quantitative in vitro cellular uptake of [2-^{14}C]FAU (5), FFAU (6a) and FClAU (6b) by HSV-1 (TK^+), (TK^-) and mock-infected cells is being studied as a measure of their selective phosphorylation by HSV-1 encoded thymidine kinase (TK), and their nucleoside transport inhibition constants (Ki) are being determined to evaluate their interaction with a nucleoside transporter. Their stability toward the phosphorolytic enzyme thymidine phosphorylase (TP) present in human platelets will be employed as an indicator of their metabolic stability and their octanol/water partition coefficients will be determined as an indicator of their ability to cross the blood-brain barrier (BBB). These in vitro tests can be used to assess the potential utility of antiviral nucleoside analogs, labelled with a gamma or positron emitting radionuclide, as radiopharmaceuticals for the non-invasive diagnosis of suspected herpes simplex encephalitis (HSE) using brain scintigraphy. The quantitative tissue uptake of 6a-b in tumor-bearing mice is underway.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer. Mass spectra (MS) were measured with a Hewlett-Packard 5995A mass spectrometer using the direct insertion probe (DIP) method. All chemicals were of reagent grade quality, unless otherwise noted. Solvents were dried using routine methods, fractionally distilled and stored over molecular sieves. Fluorine and chlorine gas were purchased from Matheson Gas Products and were of the highest quality available. The fluorine gas was manipulated via a manifold system using monel components approved for fluorine gas use. Analytical thin layer chromatography (TLC) was performed on Whatman MK6F microslides (25 x 75 mm, 200u thickness, Whatman Inc., New Jersey, U.S.A.) using solvent systems A, chloroform:methanol (10:1 v/v); B, chloroform:methanol (10:2 v/v) and C, chloroform:acetone (25:1 v/v). Column chromatography was effected using Merck Kieselgel 60 (230-400 mesh). Radio-thin layer chromatography (R-TLC) was performed on a Berthold LB 2832 automatic TLC linear analyzer equiped with a Berthold LB 2821 proportional counter and a Canberra Series 40 multichannel

analyzer. Radioactivity determinations were perfomed by liquid scintillation counting on a Beckman LS 9000 liquid scintillation spectrometer using ACS^{R} II cocktail, using appropriate dilutions or fractions collected from HPLC separations. High pressue liquid chromatography (HPLC) separations and analysis were effected on a Whatman M-9 ODS-3 reverse phase stainless steel column (25 cm length x 9.4 mm i.d.) using a solvent gradient starting with HPLC grade water:methanol (90:10 v/v) and changing to water:methanol (50:50 v/v) during 10 min using the Water's gradient program (Solvent system IV) or an isocratic solvent program using water:methanol (90:10 v/v) at a flow rate of 4 mL min^{-1} with UV detection at 264 nm using a Water's model 481 detector. [2-^{14}C]Uracil, specific activity 52 mCi/mmol (1.92 GBq/mmol) was purchased from Amersham, Canada Ltd.

[2-^{14}C]-2,4-Bis-O-(trimethylsilyl)uracil (2)

Dry hexamethyldisilazane (5mL) was added to a mixture of [2-^{14}C]uracil (1) (44.95 mg, 0.40 mmol, 0.802 mCi) and ammonium sulfate (1.65 mg, 0.012 mmol), and the reaction mixture was heated at 120°C for 5 h under an atmosphere of dry nitrogen in a 10 mL round bottom flask. The reaction mixture was cooled and concentrated under reduced pressue to give 2 as a viscous oil which was used immediately for the synthesis of 4.

2-Deoxy-2-fluoro-3-O-acetyl-5-O-benzoyl-α-D-arabinofuranosyl bromide (3)

Reaction of 1,3-di-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose (0.178 g, 0.524 mmol) with 30% HBr in acetic acid (0.5 mL) at 25°C for 16 h yielded the title compound 3 as an anomeric mixture in 98% yield (α:β = 9:1) using a literature procedure (15). Separation of this anomeric mixture on a Merck Kieselgel column using chloroform:acetone (25:1 v/v) as eluant afforded the α-anomer 3 (0.164 g) which was dried *in vacuo* for 6 h prior to use. Continued elution gave the β-anomer of 3 (0.018 g).

[2-^{14}C]-1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (5)

A solution of the dry α-anomer 3 (0.153 g, 0.426 mmol) in dry methylene chloride (5 mL) was added to [2-^{14}C]-2 prepared above and the mixture was stirred at 25°C for 7 days. Methanol (5 mL) was added and the resulting precipitate was

removed by filtration through a celite pad. The celite pad was washed with methylene chloride (10 mL) and the solvent from the combined filtrates was removed in vacuo. TLC analysis of this material (Solvent system A) indicated the presence of 1, 3 and 4 with R_f values of 0.19, 0.90 and 0.70, respectively. Separation on a silica gel column (1.2 x 23 cm) with chloroform:methanol (17:1 v/v) as eluant afforded [2-^{14}C]-4 which was found to have a radiochemical (R-TLC), anomeric (HPLC) and chemical (HPLC) purity > 99%. A saturated solution of ammonia in methanol (9.5 mL) was added to [2-^{14}C]-4 obtained above and the hydrolysis reaction was allowed to proceed at 25°C for 10 h prior to removal of the solvent in vacuo. HPLC analysis (gradient program IV) indicated 5, retention time 6.16 min, was the major product present. The reaction mixture was purified by elution from a silica gel column (2.5 x 10 cm) using a gradient of chloroform:methanol (20:3 v/v to 10:2 v/v) to yield 5 (0.444 mCi, 55% radiochemical yield) with a chemical (HPLC) and radiochemical (R-TLC) purity > 99%

A larger scale cold synthesis of 4 was carried out using the literature procedure (1) to confirm the chemical identity of [2-^{14}C]-4. The reaction mixture was purified on a silica gel column with chloroform:methanol (90:10 v/v) as eluant to yield 4 (48.3%) as fine white crystals; mp 175-176°C [lit.(1) mp 179-180°C]; ^{1}H NMR ($CDCl_3$) δ: 8.4 (s,1H, N-3H), 8.06 (m, 2H, ortho-phenyl hydrogens), 7.56 (m, 4H, meta- and para-phenyl hydrogens, H-6), 6.23 (d, $J_{1,2F}$=22.2 Hz of d, $J_{1,2}$=2.7 Hz, 1H, H-1'), 5.68 (d, $J_{5,6}$=8.5 Hz, 1H, H-5), 5.38 (m, $J_{2F,3}$=17.1 Hz of d, $J_{3,4}$=2.67 Hz, 1H, H-3'), 5.15 (m, $J_{2,2F}$=51.2 Hz of d, $J_{1,2}$=2.7 Hz of d, $J_{2,3}$=0.8 Hz, 1H, H-2'), 4.69 (m, 2H, H-5', H-5''), 4.0 (complex m, 1H, H-4'), 2.22 (s, 3H, COMe). Mass calcd. for $C_{18}H_{17}N_2O_7F$: 392.10; found: 392.25 (M^+, 0.3% relative intensity).

A larger scale cold synthesis of 5, using the literature procedure (1), was carried out to confirm the chemical identity of [2-^{14}C]-5. The product 5 was obtained in 77% yield; mp 161-162°C [lit. (1) mp 162°C]; ^{1}H NMR (DMSO-d_6) δ: 11.4 (br s, 1H, NH), 7.75 (d, $J_{5,6}$=8.12 Hz, 1H, H-6), 6.15 (d, $J_{1,2F}$=16.25 Hz of d, $J_{1,2}$=4.12 Hz, 1H, H-1'), 5.68 (d, $J_{5,6}$=8.12 Hz, 1H, H-5), 5.14 (d, $J_{2,2F}$=52.8 Hz of d, $J_{1,2}$=4.12 Hz, 1H, H-2'), 4.4 (d,

$J_{2F,3}$=20 Hz of d, $J_{3,4}$=4.5 Hz, 1H, H-3'), 3.82 (m, $J_{3,4}$=4.5 Hz, 1H, H-4'), 3.58 (complex m, 2H, H-5', H-5''). Mass calcd. for $C_9H_{11}N_2O_5F$: 246.06; found: 246.20 (M^+, 5.9% relative abundance).

[2-^{14}C]-1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-fluorouracil (6a)

A solution of fluorine in glacial acetic acid (8.17 mL of a 0.0058 M solution, 0.047 mmol) was prepared (19) and immediately added to [2-^{14}C]-5 (10.6 mg, 0.043 mmol, 141.6 μCi) in a 25 mL round bottomed flask. The reaction was allowed to proceed for 30 min at 25°C with stirring, the solvent was removed in vacuo at 35°C and the product was co-evaporated twice with absolute ethanol (5 mL) to afford a white residue. A saturated solution of ammonia in methanol (10 mL) was added and the mixture was allowed to stir at 25°C for 10 h prior to removal of the solvent in vacuo. The product exhibited a single UV visible spot. Combined TLC-LSC indicated > 89% of the activity on the plate corresponded to [2-^{14}C]-6a. HPLC analysis (water:methanol, 95:5 v/v) indicated a chemical purity > 89.5% (retention time 8.3 min). Purification on a silica gel column (2.5 x 10 cm) with chloroform:methanol (10:2 v/v) as eluant yielded [2-^{14}C]-6a (121.2 uCi, 85.6% radiochemical yield, specific activity 2 mCi/mmol) with a chemical (HPLC) and radiochemical (R-TLC) purity > 99%. The product was identical (HPLC, TLC) to an authentic sample described below.

A larger scale cold synthesis of 6a, using the procedure described above, afforded 6a as a white foam (78.6%) which resisted all attempts at recrystallization; mp 166-167°C [lit.(1) 167-168°C]; ^{1}H NMR (CD_3OD) δ: 8.1 (d, $J_{5F,6}$=6.8 Hz, 1H, H-6), 6.2 (d, $J_{1,2F}$=16 Hz of d, $J_{1,2}$=3.7 Hz, 1H, H-1'), 5.06 (d, $J_{2,2F}$=52.2 Hz of d, $J_{1,2}$=3.7 Hz, 1H, H-2'), 4.36 (d, $J_{2F,3}$=19.6 Hz of d, $J_{2,3}$=4.7 Hz, 1H, H-3'), 3.94 (m, 1H, H-4'), 3.74-3.93 (complex m, 2H, H-5', H-5''). Mass calcd. for $C_9H_{10}N_2O_5F_2$: 264.05;found: 264.10 (M^+, 17.3% relative abundance).

[2-^{14}C]-1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-chlorouracil (6b)

A solution of chlorine gas in glacial acetic acid (0.135 mL of a 0.357 M solution, 0.045 mmol) was prepared and immediately added to [2-^{14}C]-5 (10.2 mg, 0.041 mmol, 137 μCi) in a 25 mL round bottomed flask. The reaction was

allowed to proceed at 25°C for 10 min with stirring, the solvent was removed in vacuo and the product was co-evaporated with absolute ethanol (5 mL) twice to give a white residue. A saturated solution of ammonia in methanol (10 mL) was added and the mixture was allowed to stir at 25°C for 10 h prior to removal of the solvent in vacuo. The product was composed of a single UV visible spot. Combined TLC-LSC indicated > 80% of the activity on the plate corresponded to [2-^{14}C]-6b. HPLC analysis (water:methanol, 90:10 v/v) indicated a chemical purity > 77.5% (retention time 8.73 min). Purification on a silica gel column (2.5 x 10 cm) with chloroform:methanol (10:2 v/v) as eluant afforded [2-^{14}C]-6b (120.5 μCi, 87.9% radiochemical yield, specific activity 2 mCi/mmol) with a chemical (HPLC) and radiochemical (R-TLC) purity > 99%. The product was identical (HPLC, TLC) to an authentic sample described below.

A larger scale cold synthesis of 6b, using the procedure described above, and purification of the product on a silica gel column (2.5 x 15 cm) with chloroform:methanol (80:20 v/v) as eluant gave 6b as a white solid after recrystallization from ethanol (97 %); mp 196-197°C [lit(1) mp 195-196°C] with a chemical purity (HPLC) > 99%; ^{1}H NMR (CD_3OD) δ: 8.18 (s, 1H, H-6), 6.2 (d, $J_{1,2F}$=16.0 Hz of d, $J_{1,2}$=4.0 Hz, 1H, H-1'), 5.06 (d, $J_{2,2F}$≈52.3 Hz of d, $J_{1,2}$=4.0 Hz, 1H, H-2'), 4.3-4.44 (complex m, $J_{2F,3}$=19.5 Hz, $J_{3,4}$=3.1 Hz, $J_{2,3}$=4.1 Hz, 1H, H-3'), 3.95 (complex m, $J_{3,4}$=3.1 Hz, 1H, H-4'), 3.74-3.88 (complex m, J_{gem}=17.4 Hz, $J_{4,5}$=5.12 Hz, 2H, H-5', H-5''). Mass calcd. for $C_9H_{10}N_2O_5F^{37}Cl$: 282.02; found: 282.10 (M^+, 4.1% relative abundance); $C_9H_{10}N_2O_5F^{35}Cl$: 280.02; found: 280.10 (M^+, 11.8% relative abundance).

ACKNOWLEDGEMENTS

We are grateful to the Medical Research Council of Canada (Grant MT-5965) for financial support of this research and to the Alberta Heritage Foundation for Medical Research for a Fellowship to one of us (H.K.M.).

REFERENCES

1. Watanabe K.A., Reichman U., Hirota K., Lopez C. and Fox J.J. - J. Med. Chem. 22:21 (1979).

2. Watanabe K.A., Su T-L.S., Klein R.S., Chu C.K., Matsuda A., Moon W.C., Lopez C. and Fox J.J. - J. Med. Chem. 26:152 (1983).

3. Fox J.J., Lopez C. and Watanabe K.A. - Medicinal Chemistry Advances (Ed. De Las Heras F.G.), p. 27, Pergamon Press, New York, 1981.

4. Philips F.S., Feinberg A., Chou T-C., Vidal P.M., Su. T-L., Watanabe K.A. and Fox J.J. - Cancer Res. 43:3619 (1983).

5. Chou T-C., Burchenal J.H., Schmid F.A., Braun T.J., Su T-L., Watanabe K.A., Fox J.J. and Philips F.S. - Cancer Res. 42:3957 (1982).

6. Chou T-C., Feinberg A., Grant A.J., Vidal P., Reichman U., Watanabe K.A., Fox J.J. and Philips F.S. - Cancer Res. 41:3336 (1981).

7. Grant A.J., Feinberg A., Chou T-C., Watanabe K.A., Fox. J.J. and Philips F.S. - Biochem. Pharmacol. 31:1103 (1982).

8. Misra H.K., Knaus E.E., Wiebe L.I. and Tyrrell D.L. - Appl. Radiat. Isot.; Part A 37:901 (1986).

9. Fox J.J. - Pure Appl. Chem. 18:223 (1969).

10. Watanabe K.A. and Fox J.J. - Chem. Pharm. Bull. 17:211 (1969).

11. Giziewicz J., Gati. L.J., Knaus E.E., Mercer J.R., Flanagan R.J. and Wiebe L.I. - Int. J. Appl. Radiat. Isot. 36:227 (1985).

12. Mercer J.R., Knaus E.E. and Wiebe L.I. - Appl. Radiat. Isot.; Part A 37:613 (1986).

13. Swigor J.E. and Pittman K.A. - J. Labeled Compd. Radiopharm. 22:931 (1985).

14. Reichman U., Watanabe K.A. and Fox J.J. - Carbohydr. Res. 42:233 (1975).

15. Tann C.H., Brodfuehrer P.R., Brundidge C.S. and Howell G.G.- J. Org. Chem. 50:3644 (1985).

16. Vine E.N., Young D., Vine W.H. and Wolf W. - Int. J. Appl. Radiat. Isot. 30:401 (1979).

17. Cech D. and Holy A. - Coll. Czech. Chem. Commun. 41:3335 (1976).

18. Shiue C.-Y., Wolf A.P. and Friedkin M. - J. Labeled Compd. Radiopharm. 21:865 (1984).

19. The fluorine solution was prepared by bubbling nitrogen-diluted fluorine gas (2.5% v/v F_2) into a solution of glacial acetic acid (100 mL) at 25°C for 45 min. The molar concentration of this solution was determined by

treating an aliquot with excess KI and titrating the liberated I_2 with a standard solution of thiosulfate.