Synthesis of 3′-fluoro-3′-deoxythymidine and studies of its ^{18}F-radiolabeling, as a tracer for the noninvasive monitoring of the biodistribution of drugs against AIDS

I. K. Wilson, S. Chatterjee and W. Wolf

Radiopharmacy Program, University of Southern California, 1985 Zonal Ave., Los Angeles, CA 90033 (USA)

(Received May 24, 1991; accepted September 29, 1991)

Abstract

3′-Fluoro-3′-deoxy-thymidine (FDT), a fluorinated analog of 3′-azido-thymidine (AZT), is both more active against the HIV virus but also more toxic than AZT. Because of its fluorine atom, it can be labeled with ^{18}F to be used to monitor this drug's biodistribution and targeting. A new synthesis for FDT, suited for ^{18}F labeling, has been developed. After protecting the 5′-hydroxy group with a trityl group, the 3′-hydroxy group was subtituted with a mesyl group in the lyxo configuration. Treatment with ^{18}F potassium fluoride and crown-18 ether yielded the ^{18}F-labeled fluoro derivative which on detritylation afforded ^{18}F FDT with 7% labeling efficiency. This is the first reported synthesis of 3′-fluoro-5′-O-trityl deoxythymidine using potassium fluoride and preparing its ^{18}F labeled analog. The time required to incorporate ^{18}F in the intermediate compound and isolate the end-product is reasonably short (approximately 2 h) which will allow sufficient time to conduct biological studies with this short-lived radionuclide.

Introduction

3′-Azido-thymidine (AZT) (Fig. 1; $R^1 = R^2 = H$; $R^3 = N_3$) is currently the antiviral drug most widely used [1–4] in the management and control of the HIV virus, believed to be the cause of a variety of clinical conditions referred to as acquired immunodeficiency syndrome (AIDS) [5–7]. For a drug to be effective, it is necessary for that drug to be able to reach its desired target site at the required concentration and rate, thereby allowing it to exercise its therapeutic effect. Because the brain is a major target of the AIDS virus, it is of major interest to know whether an agent that may be effective against the AIDS virus in other parts of the body, is also able to cross the blood/brain barrier. The noninvasive methods that are being developed in our laboratory [8–11] have proven their ability to monitor the degree of targeting of a number of anticancer drugs.

3′-Fluoro-deoxythymidine (FDT) (**VII**, Fig. 1) is an analog of AZT that has been reported, from *in vitro* assays, to offer even greater protection against the effects of the virus than AZT [12]. Because of its narrower selectivity index and higher toxicity, the use of FDT in place of AZT as a therapeutic regimen has not been considered practical [13]. In addition, FDT

0022-1139/91/$3.50

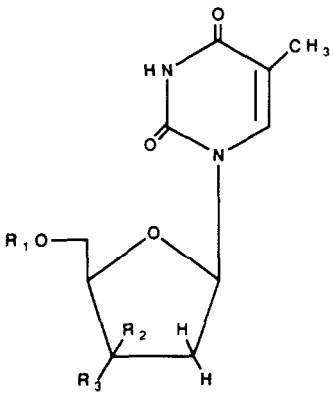

Fig. 1. AZT structure.

	R^1	R^2	R^3	Name
I	Tr	H	OH	5-O-trityl thymidine
II	Tr	H	Ms	3′-mesyl-5′-O-trityl thymidine
III	Tr	H	Ts	3′-tosyl-5′-O-trityl thymidine
IV	Tr	OH	H	1-(5′-O-trityl-β-D-lyxofuranosyl) thymine
V	Tr	Ms	H	3′-mesyl-(5′-O-trityl-β-D-lyxofuranosyl) thymine
VI	Tr	H	F	3′-fluoro-5′-O-trityl-deoxythymidine
VII	H	H	F	3′-fluoro-deoxythymidine
VIII	H	H	OH	thymidine

Tr = $-CPh_3$; Ms = CH_3SO_2-; Ts = p-CH_3PhSO_2-

is also stereochemically similar to AZT; the N_3 and the F substituents are in the 3′ 'down' position. It is interesting to note that the stereochemical isomer of FDT, with fluorine in the 'up' position, is not biologically active, [14] since it fails to meet the requirements necessary to be identified as a substrate for the enzyme reverse transcriptase. Such lack of retroviral activity is also true for the 3′ epimer of AZT with N_3 in the 'up' position [14]. However, with the availability of fluorine-18, FDT labeled with ^{18}F would provide a noninvasive procedure for establishing the location and extent by which this drug may target the tissues and organs infected by the HIV virus. Because of the high sensitivity of radionuclide imaging and measurements, the amount of ^{18}F-FDT that would be administered would be well below any radiotoxic doses and yet allow for monitoring of the *in vivo* biodistribution of this drug. The methods described in this study have been developed to determine which fluorination method could be readily used with ^{18}F. This would require a reasonable radiochemical yield under rapid synthesis conditions.

Experimental

5′-O-Trityl thymidine (I)

Thymidine (7.38 g) was suspended in anhydrous pyridine (100 ml) and trityl chloride (9.20 g) was added to the flask and heated on a steam bath for 30 min. The solution was poured into 4 l of ice water with vigorous stirring. The powdery material that separated was collected by suction filtration. The material was dried overnight in a dessicator charged with phosphorus

pentoxide. Crystallization from acetone/benzene yielded a pure compound having a melting point of 127–128 °C (lit.m.p. [15], 128–130 °C).

3'-Mesyl-5'-O-trityl thymidine (II)

To 5'-O-trityl thymidine (5.0 g), dissolved in anhydrous pyridine (50 ml), was added methanesulfonyl chloride (2.5 ml). The mixture was kept overnight at 0 °C under anhydrous conditions. Ice water (1 ml) was added to the reaction mixture and allowed to stand for 1 h; it was then poured into ice water (500 ml) and stirred vigorously. The resulting precipitate was collected by suction filtration and dried under vacuum (m.p., 111–114 °C; lit. value [16], 111–114 °C).

3'-Tosyl-5'-O-trityl thymidine (III)

5'-O-Trityl thymidine (970 mg, 2 mmol) was dissolved in anhydrous pyridine (10 ml) and stirred on an ice bath. *p*-Tosyl chloride (1.14 g), dissolved in anhydrous pyridine (10 ml), was added dropwise to this solution. The reaction mixture was stirred at room temperature for 32 h during which the reaction was monitored by thin layer chromatography (SiO_2 gel, chloroform/methanol 6:1). After 32 h, water (30 ml) was added to the reaction mixture with stirring, and then extracted with dichloromethane (3×50 ml). The organic layer was dried with sodium sulfate and evaporated under vacuum; residual pyridine was removed by co-evaporation with toluene. Crystallization from ethanol–water yielded a white compound (m.p., 108–110 °C, yield 85%). ^{1}H NMR ($CDCl_3$): δ 1.64 (s, tosyl CH_3), δ 7.80 (m, aromatic tosyl H) ppm.

1-(5'-O-Trityl-β-D-lyxofuranosyl) thymine (IV)

3'-Tosyl-5'-O-trityl thymidine (311 mg) was dissolved in ethanol (4.8 ml). Sodium hydroxide (1 M, 1.95 ml) was added and the mixture was refluxed for 1 h. The mixture was cooled by adding ice chips and ice water (150 ml) dropwise with stirring. The pH was carefully reduced to a value of 2 by adding hydrochloric acid (1 M). The precipitate was collected by suction filtration, washed with large quantities of water and dried overnight (m.p., 254–256 °C, lit. value [17], 254–256 °C; yield, 89%).

3'-Mesyl-5'-trityl-β-D-lyxofuranosyl thymine (V)

5'-Trityl-1-(β-D-lyxofuranosyl)-thymine (5.0 g, 0.01 mol) was dissolved in dry pyridine (30 ml). The solution was cooled in a freezing ice mixture. Under positive nitrogen pressure, methanesulfonyl chloride (2.5 ml, 3.7 g, 0.032 mol) was added dropwise; the reaction mixture was kept in the freezer for 22 h. The flask was kept at room temperature for 3 h, cooled in an ice bath and ice water (1 ml) added. The flask was kept in the refrigerator for an additional hour. The reaction mixture was then poured dropwise into 1 l of ice water. The precipitate was collected and dried (TLC silica gel 60F254; chloroform/methanol 9:1; R_f, 0.54). The compound was crystallized from ethanol (m.p., 118–120 °C, lit. m.p. [18], 116–118 °C; yield, 86%).

Fluorination to 3'-fluoro-5'-O-trityl-deoxythymidine (VI)

Method I: Using the hydroxy compound (IV) with DAST

Compound **IV** (0.485 g) was dissolved in dichloromethane (20 ml) and stirred for 1 h with diethylamino sulfur trifluoride (DAST; 0.5 ml) at room temperature [14]. Then the mixture was poured into sodium bicarbonate solution (5%, 20 ml) and extracted with EtOAc (3×20 ml). The organic layer was dried (sodium sulfate) and purified on a silica gel column (Fig. 1, R^1=Tr; R^2=H; R^3=F).

In an attempted radiofluorination, the solution containing the ^{18}F-fluoride was first dried with anhydrous benzene (1 ml). Compound **IV** dissolved in dichloromethane was added to this dry residue, and from here onwards the procedure was followed exactly as above.

Method II: Using the mesyl compound (V) with potassium fluoride

Potassium fluoride (22 mg, 0.38 mmol) was placed in a flask equipped with a septum and dissolved in dry methanol (1 ml). The solution was evaporated to dryness. The residue was redissolved in dry methanol (1 ml); 18-crown-6 ether (3.15 M, 23 μl, 0.7 mmol) in methanol was added to the solution. It was evaporated to dryness once again. Compound **V** (55.8 mg, 0.10 mmol) dissolved in anhydrous DMF (1 ml) was injected into the flask. The flask was heated in an oil bath (100 °C) for 1 h under nitrogen; at the end of this period no starting material could be detected by TLC (**VI**: yield, 57%; m.p., 104–106 °C).

For radiofluorination, potassium carbonate (1 M, 50 μl), potassium fluoride (0.5%, 0.1 ml) and ^{18}F-KF solution (0.1 ml) were placed in a flask and dried under vacuumn. The residue was dissolved in dry methanol (1 ml) and the procedure then followed was exactly as described above.

Detritylation of compound VI to 3'-fluoro-deoxythymidine (VII)

Method I

To 3'-fluoro-5'-O-trityl thymidine (195 mg) was added 80% acetic acid (2.67 ml); the mixture was then refluxed for 6 min. [16]. The acetic acid was removed by evaporation under vacuum; residual acetic acid was removed by co-evaporation with ethanol. Water was added to the mixture which was then heated at 100 °C for 5 min. The water-insoluble portion was filtered off and the filtrate was evaporated to dryness to yield a crystalline material melting at 178–179 °C. A ^{19}F NMR analysis of this material revealed one peak at −173 ppm relative to $CFCl_3$ (R^1=R^2=H; R^3=F).

Method II

To 3'-fluoro-5'-O-trityl thymidine (0.48 g) was added 50 ml of 2% *p*-toluenesulfonic acid in chloroform/methanol (7:3). The reaction mixture was stirred for 20 min at 40 °C [21]. Sodium hydroxide (1 M, 5.5 ml) was added to the mixture and the latter was stirred an additional 5 min and evaporated under vacuum. After washing the residue with ethanol, it was evaporated under vacuum and resuspended in ethanol. The supernatant was removed

by aspiration and the residual ethanol removed by evaporation. The residue was then applied to a silica gel column. The pure compound was separated by column chromatography using chloroform initially followed by chloroform/methanol (98:2) and analyzing the eluate with chloroform/methanol (6:1) on TLC. The appropriate fractions were combined and evaporated to provide a solid (**VII**) (yield, 64%) melting at 178–179 °C (lit. m.p. [14, 19], 176–178 °C).

The labeling efficiency was 7% for the final product (FDT, **VII**) when using potassium fluoride as the fluorinating agent and 2% *p*-toluenesulfonic acid as the detritylation reagent.

Results and discussion

Starting from thymidine, we synthesized 5′-O-trityl thymidine (**I**). Reaction of this intermediate with *p*-toluenesulfonyl chloride afforded 3′-tosyl-5′-O-trityl thymidine (**III**) in 85% yield. In contrast to 3′-mesyl-5′-trityl thymidine (**II**), which yields the lyxofuranosyl compound (**IV**) in 4 h [17], the 3′-tosyl analog yielded the same compound after refluxing in excess alkali for 1 h. The reaction sequence described above allowed the incorporation of the hydroxy group in the 3′ 'up' position. This compound on fluorination would undergo a Walden inversion to yield a compound substituted with fluorine in the 3′ 'down' position. As mentioned earlier, FDT with fluorine in the 'down' configuration provides anti-HIV activity while such biological activity is not seen in FDT with fluorine in the 3′ 'up' position.

Subsequent treatment of compound **IV** with DAST replaced the hydroxy group at the 3′-position with fluorine along with inversion of configuration under very mild conditions [20].

Our efforts to duplicate this method of fluorination using ^{18}F-fluoride (radioactive fluoride obtained by the ^{18}O(p,n)^{18}F reaction) and DAST (diethylamino sulphur trifluoride) [20] did not yield good results. Attempts to utilize ^{18}F-fluoride as an external nucleophile during fluorination with DAST were unsuccessful and hence there was no incorporation of ^{18}F during the fluorination of 1-(5′-O-trityl-β-D-lyxofuranosyl) thymine (**IV**). Failure of this method necessitated a modification of our synthetic scheme. The OH group at the 3′-carbon was converted to a good leaving group in the mesyl derivative (**V**). This group could easily be replaced by the fluoride ion using potassium fluoride as the fluorinating agent and yielded satisfactory results, both with nonradioactive fluoride and upon fluorination in the presence of traces of ^{18}F. A detailed description of this method of fluorination (method II) has been provided. The ^{18}F-fluoride was obtained as aqueous hydrogen fluoride. Use of this acidic solution caused undesirable cleavage of the trityl group and the glycosidic bond during fluorination. The ^{18}F-fluoride solution was therefore made basic by treatment with potassium carbonate prior to use in the radiofluorination reactions.

Several attempts to obtain 3′-fluoro-deoxythymidine and its C-3′ fluoro epimer were hampered by the detritylation procedure reported by De Clercq

et al. [14]. The procedure which called for heating the tritylated derivatives in 80% acetic acid for 15 min was accompanied by extensive destruction of the nucleoside [14]. We therefore searched for less damaging methods. Initially we used zinc bromide in methanol, which has been reported not to attack the glycosidic bond [21, 22]. Besides insufficient solubility in methanol, our experience with this reagent and 5′-trityl thymidine always led to a reaction mixture which when analyzed in a chromatographic system [TLC on silica gel 60WF254$_s$; upper phase of EtOAc/EtOH/H_2O (4:1:2)] revealed a spot that consistently migrated slower than authentic thymidine. Subsequent trials of detritylation with boron trichloride (1 M in dichloromethane), on the basis that it is a Lewis acid and therefore might possess detritylation potential, suggested the presence of thymine after 10 min reaction with 5′-trityl thymidine, indicating that it might be a too agressive reagent for our purpose. We therefore decided to try 80% acetic acid again, this time heating the reaction mixture for 6 min instead of the 15 min reported by De Clercq *et al.* [14]. Analysis on TLC as above indicated that thymidine (**VIII**) had been formed. However, the difficulty associated with the removal of acetic acid made it necessary to look for a milder medium for detritylation.

Detritylation using 2% toluenesulfonic acid in chloroform/methanol did not cause any cleavage of the nucleoside. This method therefore was advantageous over the previous method using acetic acid [21]. The eluate from the column was analyzed by fluorine-19 NMR spectroscopy, prior to and following detritylation. Analysis of the spectra of the fluorination mixture prior to detritylation revealed three peaks at −96, −99 and −173 ppm relative to $CFCl_3$. However, a similar analysis of the detritylated material revealed only one fluorine absorption at −173 ppm. The absorptions at −173 ppm for both compounds can be further resolved into identical patterns of 16 peaks.

Inasmuch as ^{18}F is a radionuclide with the very short half-life of 109 min, the time required for any radiolabeling is a key consideration in such a synthesis. The procedure described in this study required approximately 2 h from the moment that ^{18}F reacted with the 3′-mesyl-5′-trityl-β-D-lyxofuranosyl (**V**) intermediate until the isolation of ^{18}F-FDT (**VII**). Such a relatively rapid synthesis is critical if one wishes to use such a radiolabeled compound to conduct biological studies. Although other methods of fluorination had been used before, they were not suitable for rapid ^{18}F labeling. However, the efficiency of radiolabeling was only 7%, and further work needs to be undertaken to improve the yield from this reaction.

Acknowledgements

This work was performed with the support of funds provided by the State of California and allocated on the recommendation of the Universitywide Task Force on AIDS. We would like to thank Prof. George Olah, University of Southern California, for his advice on aspects of the fluorination studies,

Prof. Jorge Barrio, University of California at Los Angeles, Dr James Ropchan, US Veterans Administration West Los Angeles Medical Center and Dr Gunther Firnau, McMaster University, Hamilton, Ontario, for their advice on aspects of the radiofluorination reactions.

References

1 E. Oksenhendler, *Nouv. Rev. Fr. Hematol.*, *31* (1989) 69.
2 G. W. Cameiner, *Biochem. Pharmacol.*, *16* (1967) 1691.
3 M. S. Hirsh, *J. Infect. Dis.*, *161* (1990) 845.
4 M. A. Fischel, D. D. Richman, D. M. Causey, M. H. Grieco, Y. Bryson, D. Mildvan, O. L. Laskin, J. E. Groopman, P. A. Volberding and R. T. Schooley, *J. Am. Med. Assoc.*, *262* (1989) 2405.
5 R. C. Gallo, S. Z. Salahuddin and M. Popovic, *Science*, *224* (1984) 500.
6 A. Scott, *New Sci.*, (1987) 36.
7 R. C. Gallo, *J. Acqd. Imm. Def. Syndr.*, *1* (1988) 521.
8 J. Shani, J. Bertram, C. Russel, R. Dahalan, D. C. P. Chen, R. Parti, J. Ahmadi, R. A. Kempf, T. Kawada, F. M. Muggia and W. Wolf, *Cancer Res.*, *49* (1989) 1877.
9 W. Wolf, C. Presant, K. L. Servis and A. El-Tahtawy, *Proc. Nat. Acad. Sci. USA*, *87* (1990) 492.
10 A. El-Tahtawy and W. Wolf, *J. Can. Res. Clin. Oncol.*, (1990) 556.
11 A. El-Tahtawy, T. Johnson, K. L. Servis and W. Wolf, *J. Nucl. Med.*, *30* (1989) 814.
12 E. Matthes, C. Lehman, D. Scholz, H. A. Rosenthal and P. Langen, *Biochem. Biophys. Res. Commun.*, *153* (1988) 825.
13 J. Balazarini, M. Baba, R. Pauwels, P. Herdiwijn and E. De Clercq, *Biochem. Pharmacol.*, *37* (1988) 2847.
14 P. Herdewijn, J. Balazarini, E. De Clercq, R. Pauwels, M. Baba, S. Broder and H. Vanderhaeghe, *J. Med. Chem.*, *30* (1987) 1270.
15 J. P. Horwitz, J. A. Urbanski and J. Chua, *J. Org. Chem.*, *27* (1962) 3300.
16 A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, (1955) 816.
17 J. P. Horwitz, J. Chua, J. A. Urbanski and M. Noel, *J. Org. Chem.*, *28* (1963) 942.
18 J. P. Horwitz, J. Chua and M. Noel, *J. Org. Chem.*, *29* (1964) 2076.
19 P. Langen, G. Etzold, R. Hintsche and G. Kowollik, *Acta Biol. Med. Ger.*, *23* (1969) 759.
20 M. G. Straatmann and M. J. Welch, *J. Nucl. Med.*, *18* (1977) 151.
21 M. D. Matteucci and M. H. Caruthers, *Tetrahedron Lett.*, *21* (1980) 3243.
22 V. Kohli, H. Blocker and H. Koster, *Tetrahedron Lett.*, *21* (1980) 2683.