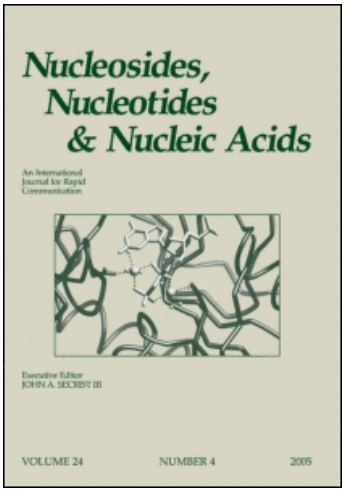

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APPLICATION OF SOLID-PHASE CHEMISTRY FOR THE SYNTHESIS OF 3′-FLUORO-3′-DEOXYTHYMIDINE

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□ *Reported solution-phase methods for the synthesis of 3′-fluoro-3′-deoxythymidine (FLT) are cumbersome, require purification of intermediates, and include several protecting/deprotecting steps. To circumvent these problems, a solid-phase strategy was designed for the synthesis of FLT. Thymidine was immobilized on trityl resin via the 5′-hydroxyl group. The subsequent mesylation of the free 3′-hydroxyl group in the presence of methanesulfonyl chloride afforded the polymer-bound 3′-O-mesylthymidine. Nucleophilic substitution of the mesyl moiety by hydroxyl group in the presence of sodium hydroxide produced the polymer-bound threothymidine. Fluorination with diethylaminosulfur trifluoride followed by acidic cleavage of the polymer-bound FLT in the presence of trifluoroacetic acid afforded FLT.*

Keywords FLT; Solid-phase synthesis; Trityl resin

INTRODUCTION

3′-Fluoro-3′-deoxythymidine (FLT, alovudine) is a nucleoside analogue structurally related to 3′-azido-3′-deoxythymidine (AZT), a commercially available anti-human immunodeficiency virus type 1 (HIV-1) drug. FLT has a substitution of fluorine for the hydroxyl group at the 3′ position of the ribose ring of thymidine, and has been reported to be one of the most active inhibitors of HIV in vitro. FLT is up to 10-fold more potent than AZT in vitro[1,2] and is at least 10 times more active than AZT in monkeys infected with simian immunodeficiency virus.[3] Further investigations of this compound[4,5] showed that FLT-5′-triphosphate (FLT-TP) is a potent and selective inhibitor of HIV-1 reverse transcriptase.

HIV isolates with mutations resulting in multidrug resistance against all currently available reverse transcriptase inhibitors including AZT had

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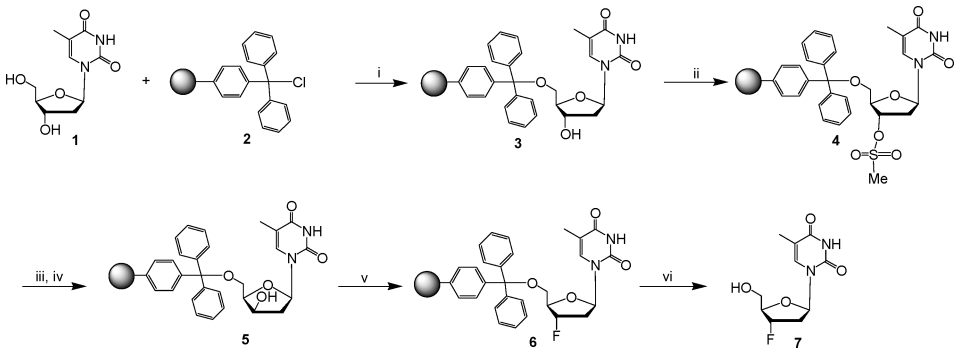
no evidence of cross-resistance to FLT.[6] American Cyanamid Co. (Wayne, NJ, USA) discontinued the development of FLT in 1994 because of the observed hematological toxicity.[7] However, Medivir (Stockholm, Sweden and Cambridge, UK) continued to test FLT for the treatment of patients with multidrug-resistant HIV infection. The Phase IIa clinical trials of FLT were successfully completed in July 2002. All patients underwent treatment without any serious side effects.[8,9] FLT currently is undergoing further clinical tests.

Recently 3′-[^{18}F]fluoro-3′-deoxythymidine ([^{18}F]FLT) has been proposed as a new marker for imaging tumor proliferation by positron emission tomography (PET).[10,11] The introduction of ^{18}F at the ribose rather than labelling the nucleotide with ^{18}F enhanced the metabolic stability of the marker.[12] [^{18}F]FLT was predominantly taken up by proliferating cells. Further phosphorylation of [^{18}F]FLT by thymidine kinase 1 (TK-1) resulted in intracellular trapping of the metabolite, [^{18}F]FLT-monophosphate.[12,13]

The synthesis of FLT in solution phase has been carried out by using several protecting and deprotecting steps.[14–18] These reactions are cumbersome and the intermediates need to be purified in each step. Furthermore, the overall yield is not satisfactory. Because of revival of research interest for using FLT as anti-HIV agent or as a marker in tumor imaging by PET, there is a need for an alternative facile and effective synthesis of this compound. We designed a solid-phase strategy for the synthesis of FLT using unprotected thymidine to circumvent some of the problems associated with the solution-phase methods.

CHEMISTRY

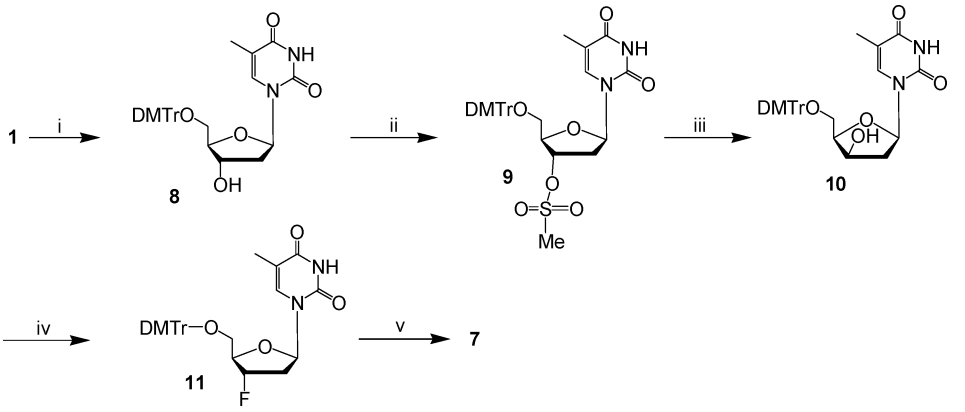
Scheme 1 displays the solid-phase synthesis of FLT. 5′-Hydroxyl group of thymidine (**1**) was immobilized on trityl chloride resin (**2**) in the presence of pyridine to yield polymer-bound thymidine (**3**, 91%). Resin **3**



SCHEME 1 Solid-phase synthesis of FLT. Reagents: (i) pyridine, 48 hours; (ii) MsCl, pyridine, 48 hours; (iii) NaOH (1N), DMF, H_2O, 24 hours; (iv) NaOH (1N), reflux, 24 hours; (v) DAST, benzene, THF, 72 hours; (vi) TFA/DCM (3%), 1 hour.

was subjected to reaction with methanesulfonyl chloride in the presence of pyridine to afford polymer-bound 3′-*O*-mesylthymidine (**4**, 98%). The reaction of **4** with sodium hydroxide in DMF for 48 hours gave polymer-bound threothymidine (**5**, 98%). Diethylaminosulfur trifluoride (DAST) was added to the suspension of resin **5** in anhydrous benzene and THF to produce polymer-bound FLT (**6**, 95%). The reaction was continued for 72 hours. Washing and acidic cleave of trityl resin with TFA/DCM (3%) afforded FLT (**7**, 55%) (overall yield 45%) (Scheme 1).

Scheme 2 shows the solution-phase synthesis of FLT according to the modified reported procedure.[18] Thymidine was converted to 5′-*O*-(4,4′-dimethoxytrityl)thymidine **8** (90%) in the presence of 4,4′-dimethoxytrityl chloride and dry pyridine. Subsequent mesylation of 3′-hydroxyl group in **8** with methanesulfonyl chloride in the presence of pyridine afforded 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-mesylthymidine **9** (94%). Basic hydrolysis of **9** with ethanolic sodium hydroxide afforded 5′-*O*-(4,4′-dimethoxytrityl)threothymidine **10**, which was fluorinated with DAST and sequential acidic deprotection to afford FLT (**7**, 23%) (overall yield 19%).



SCHEME 2 Solution-phase synthesis of FLT. Reagents: (i) pyridine, 4,4′-dimethoxytrityl chloride (DMTrCl), 3 hours; (ii) MsCl, pyridine, 3 hours; (iii) NaOH (1N), EtOH, 12 hours (room temperature), 3 hours (reflux); (iv) DAST, benzene, THF, 2 hours; (vi) CH_3COOH (80%), 15 minutes, reflux.

RESULTS AND DISCUSSION

Recent Phase IIa clinical trials of FLT for the treatment of patients with multidrug-resistant human immunodeficiency virus infection showed promising results without any serious side effects. Furthermore, [^{18}F]FLT has been proposed as a new marker for imaging tumor proliferation by PET. Thus, there is an increased research interest in studying the biological properties of FLT. We designed a solid-phase method for the synthesis of FLT.

The novelty of the method lies in its simplicity. Thymidine is mixed with trityl chloride and is thereby "captured" as an immobilized compound through the 5′-hydroxyl group. Washing the support allows for the recovery of an excess of thymidine and removal of unreacted reagents, and guarantees that no unreacted starting materials remain. This makes the method very economical and cost-effective. Trityl chloride resin has a hindered structure, thereby allowing for the regioselective reaction. The most reactive hydroxyl group (5′-hydroxyl group) of thymidine reacts selectively with hindered resin when an excess amount of thymidine (4 eq) is used.

This solid-phase strategy allowed the synthesis of FLT in a short time when compared to the solution-phase approaches. The synthesis was accomplished without the need for purification of intermediates. The intermediates and the final compound remained trapped on the resin, which facilitated the separation of any unreacted reagent by washing and filtration. The solid-phase method allowed facile isolation and recovery of the final product.

Reported solution-phase approaches to the synthesis of FLT include a variety of protecting groups.[14–17] For example, in a parallel modified solution-phase method,[18] 5′-hydroxyl group of thymidine was protected by 4,4′-dimethoxytrityl group to afford **8**. Subsequent mesylation, basic hydrolysis, fluorination, and acidic cleavage reactions afforded FLT (**7**). All the intermediates were purified by silica gel column chromatography in a time consuming process.

FLT was synthesized in a higher overall yield (45% overall yield) by the solid-phase method when compared to that for the solution-phase method[18] carried out in parallel (19% overall yield). The successful application of the solid-phase strategy for the synthesis of FLT provides insight for the synthesis of other 3′-substituted nucleosides using a similar methodology.

EXPERIMENTAL

General. All reactions were carried out in Bio-Rad polypropylene columns by shaking and mixing using a Glass-Col small tube rotator in dry conditions at room temperature unless otherwise stated. Trityl chloride resin (1.6 mmol/g) was purchased from Novabiochem (Switzerland). Other chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). The chemical structure of FLT was confirmed by nuclear magnetic resonance spectrometry (^{1}H NMR, ^{13}C NMR) on a NMR spectrometer (400 MHz) and a high-resolution PE Biosystems Mariner API time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA, USA).

Polymer-bound thymidine (3). The reaction vessel containing trityl chloride resin (**2**, 1.6 mmol/g, 0.36 mmol, 225 mg), thymidine (**1**, 350 mg,

1.44 mmol) and anhydrous pyridine (10 mL) was shaken at room temperature for 48 hours. The resin was collected by filtration and washed with DMF (2 × 25 mL), DCM (2 × 25 mL), and anhydrous MeOH (2 × 25 mL), respectively, and dried under vacuum to give **3** (272 mg, 91% yield). IR (cm^{-1}): 3382 (O–H), 3056 (N–H), 3019 (N–H), 1683 (C=O amide), 1654 (C=O amide).

Polymer-bound 3′-*O*-mesylthymidine (4). Methanesulfonyl chloride (166 μL, 2.14 mmol) was added to swelled resin **3** (272 mg) in dry pyridine (10 mL). The reaction mixture was shaken for 48 hours at room temperature. The resin was collected by filtration and washed with DMF (2 × 25 mL), DCM (2 × 25 mL), and anhydrous MeOH (2 × 25 mL), respectively. The resin was dried under vacuum to afford **4** (290 mg, 98% yield). IR (cm^{-1}): 3056 (N–H), 3019 (N–H), 1687 (C=O amide), 1654 (C=O amide), 1356 (SO_2), 1173 (SO_2).

The completion of reaction was confirmed by cleaving a small amount of resin **4** with 2% TFA in DCM. The spectral properties were identical with those of 3′-*O*-mesylthymidine. HR-MS (ESI-TOF) (*m/z*): $C_{11}H_{16}N_2O_7$ S calcd, 320.3189; found, 321.2275 $[M + H]^+$, 343.2270 $[M + Na]^+$.

Polymer-bound threothymidine (5). Sodium hydroxide solution (0.5 mL, 1 N) was added to the swelled resin **4** (290 mg) in DMF (20 mL). The mixture was shaken for 24 hours. Additional amount of NaOH solution (1 mL, 1 N) was added, and the reaction mixture was refluxed for 24 hours. The resin was collected by filtration and washed with water (2 × 25 mL), DMF (2 × 25 mL), DCM (2 × 25 mL), and anhydrous MeOH (2 × 25 mL), respectively, and dried under vacuum to yield **5** (262 mg, 98%). IR (cm^{-1}): 3407 (O–H), 3056 (N–H), 3019 (N–H), 1686 (C=O amide).

Polymer-bound 3′-fluoro-3′-deoxythymidine (6). Completely dried resin **5** (262 mg) was swelled in dry benzene (10 mL) and THF (1 mL) and cooled to 0°C. DAST (135 μL, 1.02 mmol) was added to the reaction vessel. The reaction mixture was shaken for 72 hours at room temperature. The resin was collected by filtration and washed with water (2 × 25 mL), DMF (2 × 25 mL), DCM (2 × 25 mL), and anhydrous MeOH (2 × 25 mL), and dried under vacuum to afford **6** (248 mg, 95%). IR (cm^{-1}): 3056 (N–H), 3017 (N–H), 1683 (C=O amide), 1659 (C=O amide).

3′-Fluoro-3′-deoxythymidine (7). Resin **6** (248 mg) was suspended in DCM containing 3% TFA (10 mL) and was shaken at room temperature for 1 hour. The resin was collected by filtration. The filtrate was concentrated under reduced pressure and purified by silica-gel column chromatography using DCM and methanol as eluents (98:2, v/v) to afford FLT (39 mg, 55%). ^{1}H NMR, ^{13}C NMR, and high resolution time-of-flight electrospray mass spectrometry confirmed the structure of the compound. Melting point (177–178°C) (reported m.p. 176–177°C)[14] and the NMR data corresponded to those reported in the literature.[14–18] High resolution ESI-MS for FLT **(7)** ($C_{10}H_{13}FN_2O_4$) cacld, 244.2196; found, 267.2051 $[M + Na]^+$.

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