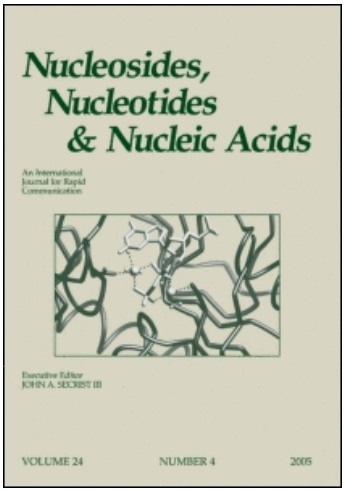

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SYNTHESIS OF A DIFLUOROPHOSPHONATE ANALOG OF THE OXATHIOLANYL NUCLEOSIDE (-)-β-L-(2*R*,5*S*)-1,3-OXATHIANYL-5-FLUOROCYTOSINE (FTC)

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Abstract: A difluorophosphonate analog of (-)-β-L-(2*R*,5*S*)-1,3-oxathianyl-5-fluorocytosine (FTC) was prepared as part of an effort to probe the possibility of obviating the need for endogenous kinases in the activation of nucleosides as potential therapeutic agents.

It is apparent that any nucleoside which requires phosphorylation to become functional *in vivo* must be recognized by endogenous kinases. During the continuing effort to develop nucleosides as therapeutic agents, many new analogs are found to be incapable of undergoing phosphorylation *in vitro*, presumably owing to a widening structural dissimilarity relative to natural substrates. Often nucleoside analogs previously found to be inactive as inhibitors of cellular diseases may be activated by simple chemical phosphorylation.[1] This idea of transforming biologically inert nucleosides into active species has been pursued by many researchers for many years.[2]

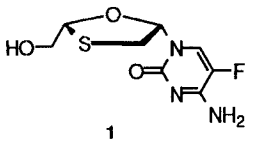
Phosphorylation of nucleosides allows one to indirectly correlate the effects of endogenous kinases on bioactivity via *in vitro* assays against a putative intracellular target when compared to the potency of the parent unphosphorylated nucleoside in an analogous whole cell assay. Since phosphates suffer from an inherent instability to phosphatases and simple hydrolysis it would be advantageous to employ nonhydrolyzable mimics of phosphorylated species if one is interested in discovering viable therapeutic agents.[3] Nonhydrolyzable mimics of phosphorylated nucleosides increase the possibility of discovering a more stable drug candidate if the employed phosphate surrogate could be rendered membrane permeable.[4]

Some time ago, Blackburn and coworkers proposed the use of difluorophosphonates as nonhydrolyzable mimics of phosphates.[5] Aside from negating the problem of hydrolytic instability, the rationale was twofold: steric and electronic. The second pK_a (the more biologically relevant) of a difluorophosphonate ($pK_{a2} \cong 5.5$) is significantly lower than that of an unsubstituted phosphonate ($pK_{a2} \cong 7.1$).[6] This difference ensures that at physiological pH a difluorophosphonate will be doubly ionized as would be a corresponding phosphate ($pK_{a2} \cong 6.5$). The resident fluorines serve not only to lower the pK_a of a difluorophosphonate via their electron-withdrawing capability but also, by virtue of being centers of high electron density, imitate the lone pairs of the replaced oxygen atom.

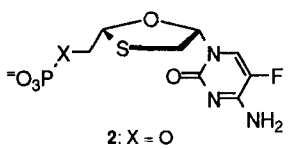
Blackburn's strategy has been implemented by several investigators with varied results. Some have found the difluorophosphonate moiety to be a useful replacement for the natural phosphate. Of note is work by Wright and coworkers in which a difluorophosphonate analog of a guanine nucleotide was found to function as efficiently as the natural triphosphate as a substrate for *Bacillus Subtilis* DNA polymerase III.[7] Danzin and coworkers prepared a difluorophosphonate analog of a nucleoside-based inhibitor of human purine nucleoside phosphorylase and found it to be nearly equipotent to its phosphate equivalent.[8] In the realm of non-nucleosides, Burke and coworkers have recently discovered that the difluorophosphonate analog of phosphorylated tyrosine incorporated in an SH2 recognition peptide displayed equal affinity with respect to the parent compound.[9]

Oxathiolanyl nucleosides have received much attention for their potent antiviral properties.[10] Among these is (-)-β-L-(2*R*,5*S*)-1,3-oxathianyl-5-fluorocytosine (BW524W91, FTC, **1**), which is currently in clinical trials as an anti-HIV agent.[11] Given the importance of **1** and similar compounds as antiviral agents and the fact that the therapeutically most interesting members of this series are of the unnatural L configuration, oxathiolanyl nucleosides provide a good arena for assessing the kinase bypass concept with a nonhydrolyzable phosphate mimic.

We chose as our synthetic target the difluorophosphonate **3**, an analog of monophosphorylated FTC (**2**), a putative intermediate in the bioactivation of **1**. We believed that the incorporation of the difluorophosphonyl moiety could be done in an analogous fashion to known procedures for the assemblage of oxathiolanyl derived nucleosides. While **3** is doubly ionized at physiological pH and thus would have difficulty crossing membranes,[12] our primary goal was to establish a viable synthetic route to difluorophosphonate analogs of oxathiolanyl nucleosides.

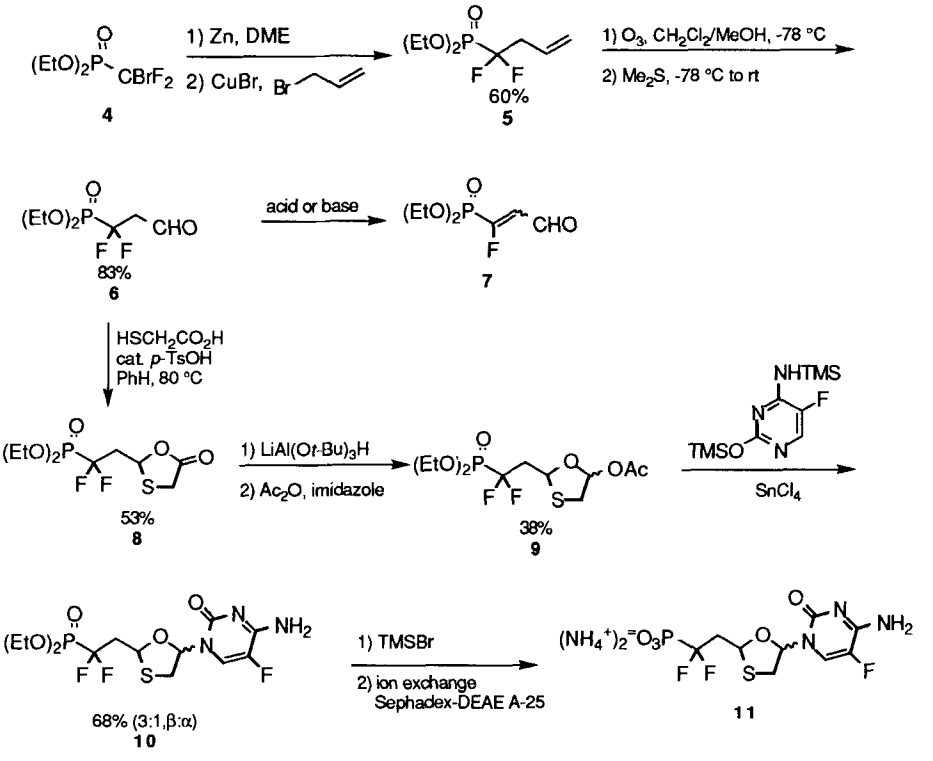


1



2: X = O
3: X = CF_2

As described in Scheme 1, the synthesis began with the preparation of the difluorophosphonyl aldehyde **6** in two steps employing literature procedures.[13] This aldehyde was found to be somewhat unstable and eliminated HF under mildly acidic or basic conditions.[14] Accordingly, exposure of **6** to triethylamine in CH_2Cl_2 cleanly afforded the isomeric unsaturated aldehydes **7** in a 3:1 E:Z ratio. However, treatment of the freshly prepared aldehyde with mercaptoacetic acid and catalytic *p*-toluenesulfonic acid in refluxing benzene resulted in the formation of the desired oxathiolanone **8** in acceptable yields along with products originating from the elimination process. Monoreduction of **8** to a lactol by LiAl(O*t*-Bu)$_3$H was followed by *in situ* acylation employing acetic anhydride and imidazole.[15] The resultant acetal **9**, when treated under standard Vorbrüggen conditions with $SnCl_4$ and persilylated fluorocytosine, was converted to nucleoside **10** as a 3:1 mixture of stereoisomers.[11] NOE studies performed on **10** revealed that the



SCHEME 1

predominant product had the predicted β stereochemistry. Deblocking of the phosphonate was accomplished in the presence of TMSBr in DMF. Purification by ion exchange chromatography provided **11** as the ammonium salt maintaining a 3:1 mixture of stereoisomers.

As anticipated when assayed against HIV in MT4 cells (HIV-1, strain 3B), difluorophosphonate **11** was inactive presumably due to its increased hydrophilicity relative to **1**. Further evaluation of this novel chemical species in light of kinase obviation, enhanced chemical stability, and membrane permeability will be reported as events warrant.

EXPERIMENTAL

General. Unless otherwise noted, all starting materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) was distilled from potassium under nitrogen immediately prior to use. Reactions involving air or moisture sensitive reagents or intermediates were performed under an inert atmosphere of nitrogen in glassware that had been oven and/or flame dried. Flash

chromatography was performed according to published methods.[16] NMR spectra were obtained on either a Varian Gemini 200 (200 MHz) or Varian Unity 300 (300 MHz) spectrometer. Elemental analyses were obtained by Atlantic Microlab, Inc.

Diethyl 1,1-difluoro-2-(5-oxo-1,3-oxathiolan-2-yl)ethylphosphonate (8). A 100 mL flask equipped with a Dean-Stark trap and reflux condenser was charged with freshly prepared aldehyde **6**[9] (621 mg, 2.70 mmol), mercaptoacetic acid (225 μL, 298 mg, 3.23 mmol), *p*-TsOH·H_2O (25.6 mg, 0.135 mmol), and benzene (50 mL). The mixture was heated at reflux for 20 h. The brown heterogeneous solution was cooled to room temperature and concentrated to a volume of about 5 mL under reduced pressure. The residue was partitioned between diethyl ether (100 mL) and aqueous $NaHCO_3$ (50 mL). The organic fraction was washed with water (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography (3:2 hexanes:EtOAc), affording the oxathiolanone **8** as a clear oil (418 mg, 1.37 mmol, 53% yield). The product was somewhat unstable at room temperature, but refrigeration lengthened its shelf life to several days. ^1H NMR [200 MHz] ($CDCl_3$) δ 5.87 (t, 1 H, J = 6.0 Hz), 4.30 (m, 4 H, J = 7.2 Hz), 3.73 (d, 1 H, J = 16.5 Hz), 3.62 (d, 1 H, J = 16.5 Hz), 2.94-2.47 (comp, 2 H), 1.40 (t, 6 H, J = 7.1 Hz); ^{31}P NMR [121 MHz] ($CDCl_3$) δ 5.33 (t, J_{PCF} = 103 Hz); ^{19}F NMR [282 MHz] ($CDCl_3$) δ 17.2-16.7 (comp); Elemental analysis: calculated C, 35.91; H, 5.05. Found C, 36.31; H, 5.20.

(2*RS*,5*RS*)-Diethyl 2-(5-(4-amino-5-fluoro-1,2-dihydro-2-oxo-1-pyrimidinyl)-1,3-oxathiolan-2-yl)-1,1-difluoroethylphosphonate (10). To a 0 °C solution of the lactone **8** (1.10 g, 3.62 mmol) in dry THF (36 mL) was added Li(Al(O*t*-Bu)$_3$H (1.0*M* in THF, 4.70 mL, 4.70 mmol) via syringe pump over the course of 1 h. Two hours after the addition was complete, tlc indicated total consumption of the lactone. A 0 °C solution of fractionally distilled acetic anhydride (3.75 mL, 4.06 g, 39.8 mmol) and imidazole (370 mg, 5.43 mmol) in THF (18 mL) was added via cannula over 5 min. The reaction mixture was warmed to room temperature and stirred for 40 h. The solution was diluted with EtOAc (150 mL) and washed with aqueous $NaHCO_3$ (1 x 75 mL) and H_2O (1 x 75 mL). The combined washes were back extracted with CH_2Cl_2 (1 x 150 mL), and the organic phase was washed with brine (1 x 75 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified immediately by flash chromatography (4:3 hexanes:EtOAc) to recover the acetate **9** as a mixture of diastereomers (3:2 ratio, 490 mg, 1.41 mmol, 39% yield). ^1H NMR [200 MHz] ($CDCl_3$) δ 6.66 (d, 0.6 H, J = 4.5 Hz), 6.58 (d, 0.4 H, J = 3.5 Hz), 5.74-5.63 (comp, 1 H), 4.28 (m, 4 H), 3.35 (dt, 1.2 H, J = 4.3, 11.4 Hz), 3.18 (t, 0.8 H, J = 12.0 Hz), 2.95-2.43 (comp, 2 H), 2.09 (s, 3 H), 1.38 (t, 6 H, J = 7.1 Hz). The isolated material was subjected to the following reaction conditions within 24 h of purification.

A solution of 5-fluorocytosine (467 mg, 3.62 mmol) and ammonium sulfate (47.8 mg, 0.362 mmol) in freshly distilled hexamethyldisilazane was heated at reflux for 16 h. Excess amine was removed by simple distillation, and the silylated pyrimidine was stored under high vacuum for 20 h. Dry CH_2Cl_2 (8.0 mL) and $SnCl_4$ (1.0 M in CH_2Cl_2, 4.0 mL, 3.98 mmol) were added and the mixture was stirred for 30 min. A portion of this solution (11.8 mL, 3.10 mmol) was added in two equal aliquots to the acetate **6** (490 mg, 1.41 mmol) dissolved in 2.8 mL of CH_2Cl_2. After 2 h, concentrated ammonium hydroxide (3.1 mL) was

added and the resulting slurry was filtered through silica gel washing with 4:1 (EtOAc:EtOH). The filtrate was concentrated and further purified by flash chromatography (9:1 EtOAc:EtOH) to recover a single fraction that contained the nucleoside **10** as an off-white solid (399 mg, .956 mmol, 68% yield). An NOE experiment revealed that a 3:1 mixture of diastereomers favoring the β isomer was present. ^{1}H NMR [200 MHz] ($CDCl_3$) δ 7.94 (s, 1 H), 7.91 (s, 1 H), 7.68-7.66 (comp, 1 H), 6.31 (m, .25 H), 6.20 (m, .75 H), 5.93 (m, .25 H), 5.42 (t, .75 H, J = 5.8 Hz), 4.25 (m, 4 H), 3.60-3.17 (comp, 2 H), 2.98-2.67 (comp, 2 H), 1.32 (t, 6 H, J = 7.1 Hz); Elemental analysis: calculated C, 37.41; H, 4.59; N, 10.07. Found C, 37.50; H, 4.62; N, 9.98.

(2*RS*,5*RS*)-2-(5-(4-amino-5-fluoro-1,2-dihydro-2-oxo-1-pyrimidinyl)-1,3-oxathiolan-2-yl)-1,1-difluoroethylphosphonate (11). To a solution of the diethyl phosphonate **10** (185 mg, 0.444 mmol) in DMF (2.1 mL) was added TMSBr (710 μL). The reaction mixture was capped, stirred for 40 h at room temperature, and concentrated *in vacuo*. The residue was purified by ion exchange chromatography on DEAE Sephadex A-25 resin eluting with a gradient of 0 to 0.5 mmol ammonium bicarbonate while the effluant was monitored by uv detection at 254 nm. After liophilization, the phosphonate **11** was recovered as a floculant white powder in the form of an ammonium salt (250 mg). Proton NMR revealed that a 3:1 mixture of diastereomers was present. ^{1}H NMR [300 MHz] (D_2O) δ 7.81 (d, .75 H, J = 6.3 Hz), 7.78 (d, .25 H, J = 6.6 Hz), 6.26 (m, .25 H), 6.10 (m, .75 H), 5.79, (t, .25 H, J = 6.2 Hz), 5.42 (t, .75 H, J = 5.9 Hz), 3.55-3.43 (comp, 1 H), 3.13 (dt, 1 H, J = 2.6, 12.6 Hz), 2.81-2.45 (comp, 2 H); ^{31}P NMR [121 MHz] (H_2O) δ 5.54 (t, J_{PCF} = 103 Hz), 5.46 (t, J_{PCF} = 103 Hz); Elemental analysis: calculated C, 22.51; H, 5.47; N, 15.20. Found C, 22.38; H, 5.07; N, 15.56.

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