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Synthesis of enantiomerically pure D-FDOC, an anti-HIV agent

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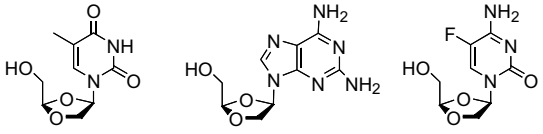
Abstract—The β-D-enantiomer of FDOC (2′,3′-dideoxy-5-fluoro-oxacytidine) exhibits potent anti-HIV-1 activity. It was obtained in optically pure form by employing a tandem kinetic resolution/chiral salt crystallization protocol. In addition, conditions were developed that allowed the unwanted butyrate ester of the L-enantiomer of FDOC to be racemized. This material could then be recycled in future resolutions.

The potential of 1′,3′-dioxolanyl nucleosides as anti-HIV-1 drugs has been recognized for some time. Over a decade ago, β-D-dioxolane-T was synthesized and shown to possess modest anti-HIV activity in vitro without acute cellular toxicity.[1–3] In the mid 1990s, (−)-β-D-2,6-diaminopurine dioxolane (DAPD), a water soluble prodrug of (−)-β-D-dioxolane guanine (DXG), was found to exhibit potent activity against HIV-1 and is now in Phase II clinical trials.[4–6] In the same time frame, both enantiomers of 2′,3′-dideoxy-5-fluoro-oxacytidine (FDOC) were synthesized by us. Although both enantiomers exhibited good potency against HIV, they both appeared to be too toxic to be used clinically.[7,8]



Subsequent to those reports, we discovered that the sample of the less toxic D-enantiomer that was tested actually contained 3–5% of its significantly more toxic L-counterpart. This then raised the interesting question as to whether the observed toxicity was inherent to D-FDOC or resulted from the presence of small quantities of its more toxic enantiomer. To answer this question, we used preparative chiral chromatography to obtain several grams of optically pure D- and L-FDOC, respectively.[9] With these two enantiomers in hand, we could, for the first time, unambiguously evaluate their anti-HIV-1 activity, cytotoxicity and resistance profile. The results of these studies indicated that D-FDOC not only showed excellent potency (EC_{50} and EC_{90} values in primary human lymphocytes infected with HIV-1_{LAI} are 0.04 and 0.26 μM, respectively) and low toxicity (>100 μM in uninfected primary human lymphocytes), but also exhibited no cross resistance to 3TC, AZT or nevirapine. In addition, molecular infectious clones containing M184V or M41L/D67N/K70R/T215Y/K219Q were as susceptible as wild type virus (WT-pNL4-3) to D-FDOC. In primary mouse bone marrow cells, D-FDOC showed no increase in lactic acid production even at 300 μM. In contrast, treatment with either L-FDOC or ddC resulted in a >300-fold increase in lactic acid production relative to untreated control.[10] Furthermore, in HepG2 cells (5 day assay), D-FDOC displayed no toxicity when tested up to 100 μM, whereas its L-counterpart demonstrated significant toxicity at 1.4 μM. While these data, taken in aggregate, clearly indicate that our original toxicity determinations were compromised by the presence of small quantities of the toxic L-enantiomer, they also suggest that clinical evaluations of D-FDOC should only be performed with materials that contain very little, if any, of the

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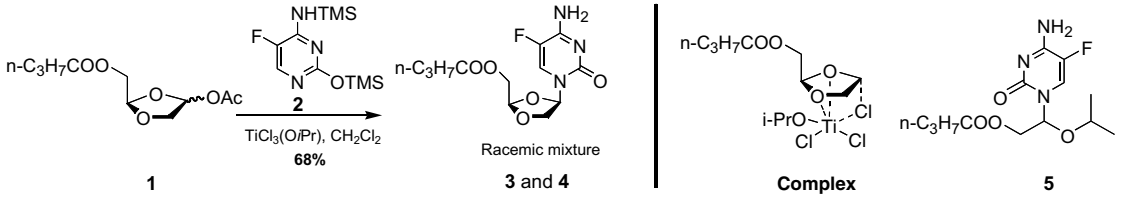
L-enantiomer. Herein, we describe a synthetic approach that employs a tandem kinetic resolution/chiral salt crystallization protocol for preparing the D-enantiomer of FDOC in high enantiopurity. In addition, conditions that allow for the racemization and recycling of the unwanted butyrate ester of the L-enantiomer of FDOC were developed.

The key step in our previously reported synthesis of FDOC was a β-selective glycosylation that was uniquely mediated by titanium trichloroisopropoxide ($TiCl_3(OiPr)$) (Scheme 1).[11,12] Thus, when racemic acetate **1** was allowed to react with silylated 5-fluorocytosine, **2**, in the presence of the oxaphilic Lewis acid, $TiCl_3(OiPr)$, under Vorbrüggen conditions,[13] we observed β:α selectivity of >20:1, albeit only in moderate yield. More recently, we have found that the efficiency of this coupling reaction could be markedly improved (68% yield, β:α selectivity of >20:1) by using 2 equiv $TiCl_3(OiPr)$. The cause of this low yield in the original report was largely due to the competitive formation of an isomeric, ring opening byproduct **5**.[14] An insidious aspect of **5** is that it possesses the same exact mass as FDOC butyrate. In addition, its two most diagnostic resonances, the anomeric proton and the proton on C6 of the cytosine ring, exhibit the same chemical shifts as those of α isomer. Since this material was likely formed by reaction of the product with HCl and isopropanol, generated from $TiCl_3(OiPr)$ hydrolysis, scrupulous exclusion of moisture should and did improve the yield.

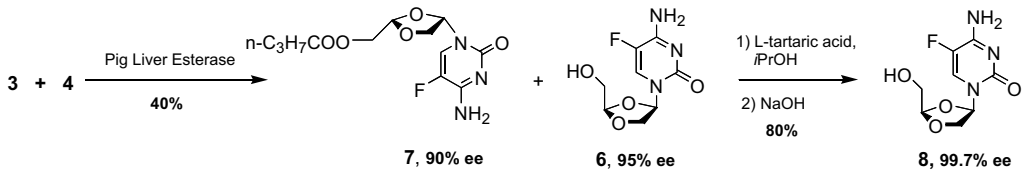
Based on our earlier results we assumed that if an enantiopure oxolactone had been employed, each of the enantiomers of FDOC could be obtained in high enantiopurity. Towards this end the chiral oxolactone **10**[15] (ee > 99%) was converted to the desired nucleoside using the same process. However, chiral HPLC analysis[16] showed the product to be completely racemic (ee = 0%). In retrospect, this result is consistent with the complexation hypothesis described above.[7,11,17] Indeed, Lewis acid complexation, while ensuring the good selectivity, simultaneously renders the C–O bond labile, resulting in the racemization of the chiral lactol acetate prior to glycosylation. In an effort to substantiate our complexation hypothesis, we employed a less oxaphilic Lewis acid catalyst in the glycosylation reaction. Thus, when **10** was allowed to react with the silylated base and TMSOTf, under the standard Vorbrüggen conditions, we obtained a 1:1 β:α mixture. Analysis of the β-product by chiral HPLC indicated low levels of racemization (the observed ee were 90% and 97% when 1.0 and 0.5 equiv of TMSOTf were used, respectively). Consistent with this trend, when the *N*-glycosylation reaction was carried out with 1.5 equiv of TMSOTf, the observed ee dropped to 85%. Even though this approach allowed us to obtain D-FDOC in 97% ee, the tedious chromatography required to separate the α- and β-isomers, as well as the lower-than-desired enantiopurity of the product, caused us to continue our search for more attractive alternatives.

We next attempted to further refine the enantiopurity of the product obtained from our previously developed kinetic resolution [Pig Liver Esterase (PLE) from Sigma] by using a subsequent fractional crystallization (Scheme 2). Thus, exposure of **3** and **4** to PLE in 20% acetonitrile/water produced D-FDOC in 95% ee.[18,19] Incubation of this material with 1 equiv L-tartaric acid in isopropanol at room temperature for about 24 h, resulted in the formation of a crystalline precipitate. The crystals were filtered, dissolved in water and neutralized with sodium hydroxide. Evaporation of the water and subsequent chiral HPLC analysis showed that the enantiomeric excess of D-FDOC to be 99.7%.[19] Interestingly, direct resolution of the butyrates **3** and **4** by chiral salt crystallization technique proved unsuccessful due to the small solubility differences between the two chiral tartrates. Other acids such as (*R*)-(−)-10-camphorsulfonic acid and (*R*)-(−)-mandelic acid were also examined, but L-tartaric acid gave the most satisfactory result.

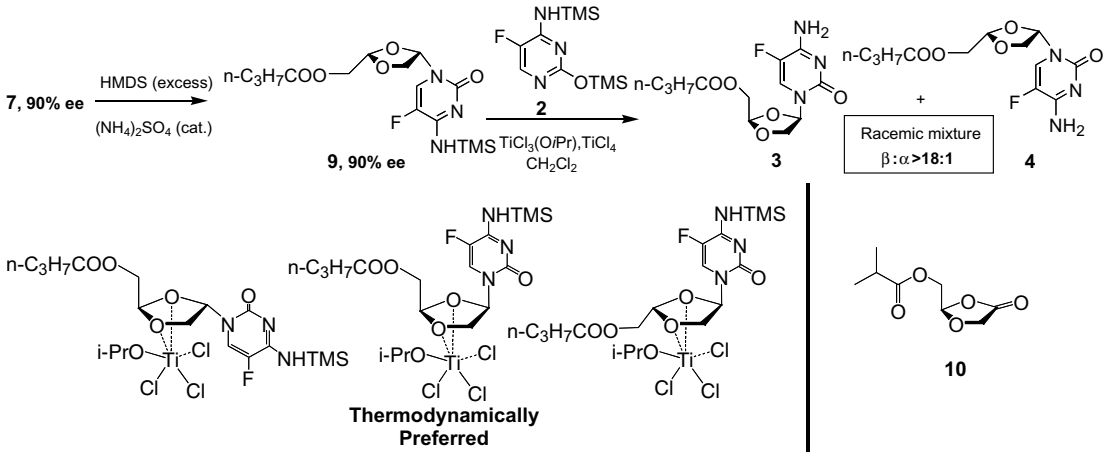
Using this approach, we, for the first time, had in hand a method for producing D-FDOC in 99.7% ee that did not require the use of chiral preparative HPLC. However,



Scheme 1.



Scheme 2.



Scheme 3.

while we considered this to be an important achievement, we noted that during the kinetic enzymatic resolution step, half of the butyrate-FDOC, for example, the unwanted L-enantiomer **7**, was being wasted. We, therefore, sought to develop a method for racemizing **7**, thereby allowing it to be recycled in subsequent kinetic resolution/fractional crystallization runs without producing any α-isomers. Stating this another way, racemization requires the simultaneous scrambling of the **1′S** and **4′S** stereocenters of **7** exclusively to produce an equal population of its enantiomeric **1′R**,**4′R** counterpart. We reasoned that this could be achieved by forming an all-*trans* titanium complex similar to the putative complex produced in the glycosylation of **1** by **2** (vide infra). Towards this end, the butyryl ester of L-FDOC, **7**, was silylated with hexamethyldisilazane (HMDS) and then allowed to react with 1 equiv of **2** in the presence of 2 equiv of $TiCl_3(OiPr)$. After 3 h the reaction was quenched and the optical purity of the products was determined using chiral HPLC.[20] No change was observed! We hypothesized that, since the silylated fluorocytosine was probably a poorer leaving group than the acetate in **1**, we might need a higher degree of Lewis acidity to catalyze the desired process. After several attempts we found that exposing silylated **9** to 1 equiv of **2**, 0.7 equiv of $TiCl_4$ and 2 equiv of $TiCl_3(OiPr)$ for 7 h resulted in complete racemization of the starting L-isomer with maintenance of good β-selectivity (β:α > 18:1) (see Scheme 3).

In conclusion, enantiomerically pure D-FDOC (>99.7% ee) was obtained using a tandem kinetic enzymatic resolution/chiral salt crystallization technique. In addition, the unwanted L-butyrate-FDOC could be completely racemized while maintaining excellent β selectivity. Based on these approaches, we believe that optically pure D-FDOC can be produced on a large scale, thereby permitting more advanced pharmacological and toxicological studies.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.07.016.

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9. Preparative SFC separation was achieved with a Chiralpak AS column using 30% MeOH isocratic elution. Using this approach we obtained D-FDOC > 99% ee (retention time: 4.05 min) and L-FDOC > 99% ee (retention time: 3.48 min).
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12. The observed selectivity differed markedly from the results obtained with other Lewis acids. For example, trimethylsilyl triflate (TMSOTf) gave no stereoselectivity, titanium tetrachloride ($TiCl_4$) caused significant product epimerization and titanium dichlorodiisopropoxide ($TiCl_2(OiPr)_2$) lacked sufficient Lewis acidity to catalyze the glycosylation.
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14. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H), 1.20 (d, 3H), 1.13 (d, 3H), 1.60 (sextet, 2H), 2.27 (t, 2H), 3.72 (m, 1H), 4.10 (q, 1H), 4.20 (q, 1H), 6.01 (dt, 1H), 7.46 (d, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.76, 18.41, 21.59, 22.92, 36.01, 63.97, 72.09, 80.93, 124.77, 136.96, 154.57, 158.45, 173.02. FAB MS $(M + H)^+$: 302.3.
15. A generous sample of chiral, nonracemic oxolactone **10** was kindly provided by Dr. George Painter of Triangle Pharmaceuticals, Inc (now Gilead Science).
16. Chiral HPLC analysis conditions: Chiralpak AS, 25×0.46 cm, detection at 271 nm, mobile phase: EtOH/hexane = 15:85, flow rate: 1.2 mL/min. The retention times for D- and L-isobutyrate-FDOC are 17.8 and 20.3 min.
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19. Chiral HPLC analysis conditions: Chiralpak AS, 25×0.46 cm, detection at 271 nm, mobile phase: EtOH/hexane = 20:80, flow rate: 0.9 mL/min. The retention times for D- and L-FDOC are 18.1 and 15.8 min.
20. Chiral HPLC analysis conditions: Chiralpak AS, 25×0.46 cm, detection at 271 nm, mobile phase: EtOH/hexane = 15:85, flow rate: 1.2 mL/min. The retention times for D- and L-butyrate-FDOC are 18.9 and 21.8 min.