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Synthesis and antiviral evaluation of 2′,2′,3′,3′-tetrafluoro nucleoside analogs

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ABSTRACT

Herein, we report the synthesis of novel 2′,2′,3′,3′-tetrafluorinated nucleoside analogs along with their phosphoramidate prodrugs. A tetrafluoro ribose moiety was coupled with different Boc/benzoyl-protected nucleobases under Mitsunobu conditions. After deprotection, tetrafluorinated nucleosides **13b**, **14b**, **20b**-**22b** were reacted with phenyl-(isopropoxy-L-alaninyl)-phosphorochloridate to afford corresponding monophosphate prodrugs **24b**–**28b**. All synthesized compounds were evaluated against several DNA and RNA viruses including HIV, HBV, HCV, Ebola and Zika viruses.

Incorporation of fluorine(s) into potential drug candidates continues to be investigated by medicinal chemists and has shown the potential to confer favorable biological properties.[1] Due to the similarity in size of fluorine (147 pm) and hydrogen (120 pm), the substitution of a single hydrogen atom by a fluorine atom induces only a minor change in steric factors. However, being the most electronegative atom in the periodic table, its incorporation results in considerable electronic changes in the molecule through inductive electron withdrawing effects. Furthermore, introduction of one or more atoms of fluorine (*e.g.*, CF_2) into a molecule is a viable bioisosteric replacement for a diverse set of functional groups such as carbonyl, ether and hydroxyl groups. Finally, a C-F bond is much stronger than a C-H bond, which can increase the chemical and biological stability of the fluorine-containing compounds, compared to their hydrogen counterparts.

In the field of nucleoside analogs,[2] introduction of fluorine has proven to be successful and has led to several very widely prescribed drugs such as (-)-FTC (HIV), gemcitabine (cancer), or more recently sofosbuvir (hepatitis C virus, HCV) (Fig. 1). In addition to the approved fluorinated nucleosides discussed above, several fluorine-containing analogs have been reported in the literature to have attractive antiviral profiles, although some proved too toxic for human use. Thus, 2′-β-fluoro nucleosides analogs such as FIAC, FEAU, FMAU exhibit potent activities against herpes simplex virus (HSV), hepatitis B virus (HBV), varicella zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV).[3]

Based on these precedents, we turned our attention toward a unique series of 2′,2′,3′,3′-tetrafluorinated nucleoside analogs. Even though this type of highly fluorinated compounds is claimed in a patent published in 2000,[4] their preparation is only described in a generic scheme and with no mention of any biological evaluation. Therefore, we report herein the detailed synthesis and antiviral evaluation of, not only these compounds, but also their phosphoramidate prodrugs.

2,2,3,3-Tetrafluorodideoxy ribose derivative **7** was prepared in four steps from commercially available 4-bromo-3,3,4,4-tetrafluorobut-1-ene **4** following a procedure reported by Linclau et al.[5] (Scheme 1). It is noteworthy that the Sharpless dihydroxylation of the perfluorinated substrate **4** is not entirely enantioselective (reported *ee* 78%, observed 60%). Consequently, the 4′-position of the lactol **7**, obtained after cyclization of the formylated intermediate **6**, is isolated as a 8:2 mixture at the 4′-position while the α/β anomers ratio is 1:1.5. The separation of the diastereomers was not attempted at this stage. Hence, with **7** in hand, the glycosylation reaction was studied and different approaches were evaluated in order to identify the most efficient coupling conditions. Vorbrüggen type coupling of 1′-benzoyl, 1′-mesyl or 1′-triflate tetrafluorinated ribose derivative **7** with either silylated uracil or cytosine in presence of $SnCl_4$ (or TMSOTf) only led to the complete

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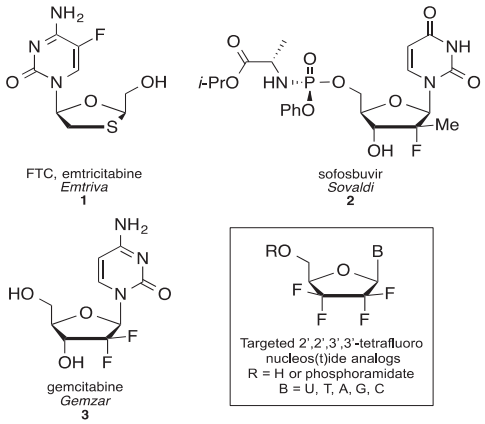
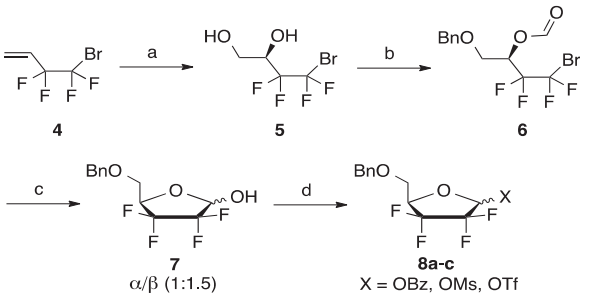


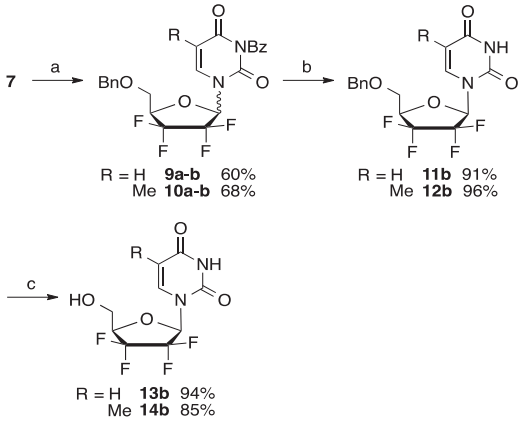
Figure 1. Structures of FDA approved fluorine containing nucleosides **1**–**3** and targeted 2′,2′,3′,3′-tetrafluoro derivatives.



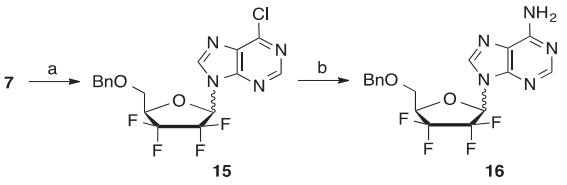
Scheme 1. Reagents and conditions: (a) $K_2OSO_4{\cdot}2H_2O$, $(DHQ)_2Pyr$, $K_3Fe(CN)_6$, *t*-BuOH/H_2O, 4 °C, 9 days, 81%; (b) (i) Bu_2SnO, TBAI, BnBr, toluene, reflux, 82%; (ii) Formic acid, DCC, DMAP, CH_2Cl_2, rt, 16 h, 95%; (c) MeLi, THF, −78 °C, 3 h, 62%; (d) BzCl, Et_3N, CH_2Cl_2, rt, 1.5 h, 92% or MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 16 h, 74% or Tf_2O, pyr, 0 °C to rt, 16 h, quant.

recovery of starting material **8**. Attempts to directly react the 1′-triflate derivative **8c** with the sodium salt of N^3-benzoyluracil was unsuccessful as well.

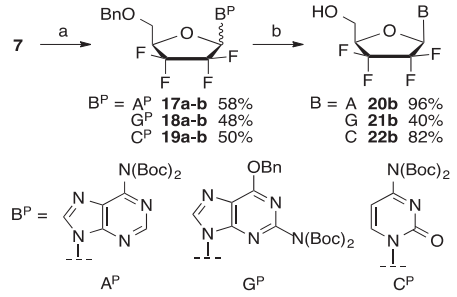
In contrast, treatment of compound **7** with N^3-benzoyluracil or N^3–benzoylthymine under classical Mitsunobu conditions (DIAD and PPh_3 in THF) afforded the desired coupling products **9a**–**b** (60%) and **10a**–**b** (68%) respectively as a silica gel separable mixture of α/β anomers (ratio: 1.5:1) (Scheme 2). Remarkably, the desired coupling products **9** and **10** were only observed when DIAD was added slowly at −20 °C. Performing this reaction at higher



Scheme 2. Reagents and conditions: (a) N^3-BzU or N^3-BzT, DIAD, PPh_3, THF, −20 °C to rt, 2 h (overall yields for both anomers); (b) NH_3, MeOH, 4 °C, 2 h; (c) BCl_3, DCM, −20 °C to rt, 3.5 h.



Scheme 3. Reagents and conditions: (a) 6-chloropurine, DIAD, PPh_3, THF, 2 h, rt, 34%; (b) NH_3/MeOH, 80 °C, 16 h, 86%.



Scheme 4. Reagents and conditions: (a) Protected base (B^P), DIAD, PPh_3, THF, −20 °C to rt, 2 h (overall yields for both anomers); (b) BCl_3, DCM, −20 °C to rt, 3.5 h.

temperatures led to the formation of several unidentified side products as determined by LC-MS. The isolated β anomers **9b** and **10b** were debenzoylated using methanolic ammonia to afford intermediates **11b** (91%) and **12b** (96%), which were subsequently treated with BCl_3 to give uracil and thymine analogs **13b** (94%) and **14b** (85%), respectively. Preparation of the adenine analog was initially attempted by coupling of protected lactol **7** with 6-chloropurine using the Mitsunobu conditions described above (Scheme 3). However, even though the desired coupling product **15** was obtained in 34% yield, separation of the α/β isomers, at this stage, or after 6-amination of the purine ring (compound **16**), was found to be challenging.

Therefore, we then examined the coupling of lactol **7** with *bis*-Boc adenine.[6] Under classical Mitsunobu conditions, the reaction afforded products **17a**–**b** in 58% yield (Scheme 4) as a silica gel separable mixture of α/β anomers (1.5:1). Further treatment of the β-anomer **17b** with BCl_3 afforded adenine nucleoside analog **20b** in 96% yield. Likewise, the coupling with *bis*-Boc-2-amino-6-benzolyloxypurine[7] afforded a mixture of anomers **18** (48%) that were separable by silica gel chromatography. The β-isomer **18b** was then fully deprotected in one step to give **21b** in 40% yield using BCl_3. Finally, the preparation of cytosine analogs **19a–b** was performed in a similar manner using *bis*Boc-cytosine.[8] The separated β-anomer **19b** was subsequently deprotected to afford nucleoside analog **22b** in 82% yield.

Identification of both α and β anomers was determined by ^1H NMR and 2D-NOE experiments after Mitsunobu coupling. In all cases, clear correlations were observed between 1′-protons and 4′-protons of β anomers while α anomers exhibited correlations between 1′-protons and 5′-protons. Additionally, in the case of purine analogs **17** and **18**, correlations between 8-protons and 5′-protons were also noted (Fig. 2).

In order to express their therapeutic effect, nucleoside analogs usually rely on cellular kinases to be phosphorylated onto their active 5′-triphosphate forms. Among the three consecutive phosphorylations, the first phosphate addition has often been identified as the limiting step which led to the development of "protected" monophosphate nucleosides, or nucleoside prodrugs, that can bypass this first activation step.[9] Unlike, 5′-mono, di or triphosphate nucleosides, theses prodrugs are capable of crossing the cell membrane to deliver a monophosphate nucleoside after enzymatic

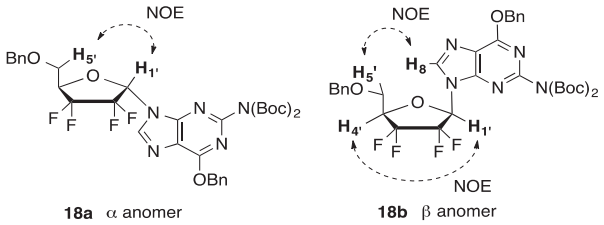


Figure 2. Anomer assignment for compound **18a** and **18b** *via* NOE experiments.

degradation of their biolabile group. The concept of monophosphate or phosphonate prodrugs has been clinically validated with the FDA-approval of drugs such as sofosbuvir **3** (HCV) or tenofovir disoproxyl fumarate (TDF) (HIV and HBV).

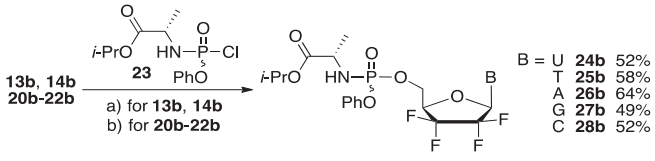
Thus, 5′-monophosphate prodrugs of nucleosides **13b**, **14b** and **20b**-**22b** were prepared by reaction with the chloro phosphoramidate **23** *via* two different strategies, depending on the nature of the nucleobase (Scheme 5).[10] For free-NH_2 containing analogs, such as adenine, guanine and cytosine derivatives **20b**, **21b** and **22b**, *t*-BuMgCl was used to favor alkoxide formation and minimize side reactions with the amino group while the pyrimidine prodrugs **24b** and **25b** were prepared by treatment of **13b** and **14b** with *N*-methylimidazole (NMI). Full conversions were usually observed after 3 h at room temperature affording prodrugs **24b**–**28b**, as a R_P and S_P mixture, in yields ranging from 49% to 64%. Due to the fact that the synthesis of the tetrafluorinated sugar **7** is not completely diastereoselective at the 4′-position, ^{31}P NMR spectra for these compounds indicated the presence of four non separable diastereoisomers in a 4:1:1:4 ratio.

To determine the effects of the tetrafluoro substitution, the energetics of ring conformations were investigated using quantum mechanical calculations (Fig. 3).

Consistent with previous data, the ribose and 2′-deoxyribose rings exhibited lower energies in the *3′-endo* and *2′-endo* conformers, respectively.[11] The *3′-endo* and *2′-endo* conformers were equal in energy for the *2′,3′*-dideoxyribose U and A analogs. The tetrafluoro-2′,3′-dideoxyribose U and A analogs preferred the *3′-endo* conformation by 0.4–1.2 kcal/mol. This result suggests that the tetrafluorinated ribose moiety might be a closer mimic of a ribose rather than a 2′,3′-dideoxyribose.

Consequently, nucleosides **13b**, **14b**, **20b**–**22b** and their corresponding phosphoramidate prodrugs **24b**–**28b** were evaluated against a panel of DNA and RNA viruses. Hence, the screening was performed against HIV-1[12] up to 100 μM and HBV[12] (HepAD38), HCV[13] (clone B replicon), Ebola[14] (Zaire ebolavirus replicon) and Zika[15] (cytopathic effect reduction assay) viruses up to 20 μM. In addition, cytotoxicity was determined in primary human peripheral blood mononuclear (PBM) cells, human lymphoblastoid CEM, African Green monkey Vero cells and HepG2 cells.[16] Except for compound **26b** that exhibited slight toxicities in human PBM (CC_{50} = 27 μM) and CEM (CC_{50} = 14 μM) cells, none of these derivatives exhibited significant activity against these viruses nor toxicities in PBM, CEM and Vero cells up to 100 μM.

In conclusion, five new 2′,2′,3′,3′-tetrafluorodideoxy nucleoside analogs along with their corresponding phosphoramidate prodrugs were synthesized from tetrafluorinated lactol **7** using a key Mitsunobu reaction. We demonstrated that the use of Boc or Bz-protected bases as glycosylation partners in this reaction facilitated the separation of both α and β anomers. None of the synthesized compounds showed marked activity when tested against HIV-1, HBV, HCV, Ebola or Zika viruses.



Scheme 5. Reagents and conditions: (a) NMI, THF, rt, 3 h; (b) *t*-BuMgCl, THF, rt, 3 h.

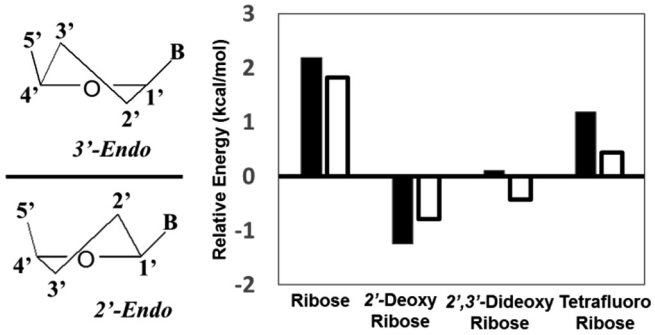


Figure 3. Energetics of ring conformations for differently substituted nucleoside analogs. The y-axis is the relative energy of E[2′-*endo*] – E[3′-*endo*] for each analog. The dark bars represent analogs with an uracil base, and white bars are analogs with an adenine base. Nucleoside analogs that display positive values favor the 3′-*endo* conformation and negative values indicate that the 2′-*endo* conformer is more stable.

Acknowledgments

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.01.006.

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