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Synthesis of 5′-Methylene-Phosphonate Furanonucleoside Prodrugs: Application to D-2′-Deoxy-2′-α-fluoro-2′-β-*C*-methyl Nucleosides

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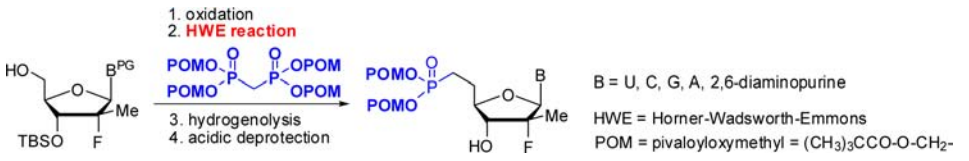
ABSTRACT



A new and facile synthetic pathway to metabolically stable 5′-methylene-bis(pivaloyloxymethyl)(POM)phosphonate furanonucleoside prodrugs is reported. The key step involves a Horner–Wadsworth–Emmons reaction of a tetra(pivaloyloxymethyl) bisphosphonate salt with appropriately protected 5′-aldehydic nucleosides. This efficient approach was applied for the synthesis HCV related 2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl nucleosides.

Over the past 40 years, nucleoside analogues have become essential agents for the treatment of various infectious diseases such as human immunodeficiency virus (HIV), herpes simplex virus (HSV), hepatitis C virus (HCV) and hepatitis B virus (HBV). Their mechanism of action generally involves the direct or delayed chain termination of viral DNA or RNA elongation by incorporation of nucleoside triphosphate analogues. The biotransformation of nucleosides into their corresponding triphosphates involves three successive phosphorylation steps catalyzed by viral and/or cellular kinases, and in many cases, the limiting step in this process is the conversion to the corresponding 5′-monophosphate.[1] Within the nucleoside analogues family, nucleoside phosphonates have emerged as an important class since the discovery of compounds such as tenofovir, adefovir and cidofovir.[2] Because a carbon–phosphorus bond replaces the 5′-oxygen–phosphorus bond, nucleoside phosphonates are more stable analogues of nucleoside monophosphates and can, in theory, bypass the first limiting phosphorylation step. However, as with their phosphate counterparts, the ionic character of these phosphonic acid derivatives is generally an obstacle for absorption and/or cellular penetration, which often translates into a lack of *in vivo* activity for these compounds. In order to mask the negative charges, phosphonate prodrugs bearing biolabile protecting groups, such as pivaloyloxymethyl (POM) and *iso*propyloxymethyl groups (POC), have been developed and incorporated into the FDA approved adefovir dipivoxil **2** and tenofovir diisoproxil fumarate **3**, which are utilized clinically for the treatment of HBV and HIV infections, respectively (Figure 1).[2] The apparently simple replacement of the oxygen atom by a carbon atom, to form 5′-methylene nucleoside phosphonates prodrugs, is actually a low yielding multistep sequence. Synthesis of such compounds involves (1) introduction of a simple phosphonate moiety; (2) a trimethylsilyl bromide driven cleavage of the phosphonate esters; (3) purification of highly polar phosphonic acids; and (4) variable yields when coupling phosphonic acids with the prodrug portion. A recently published exception to this sequence is the approach developed by Agrofoglio

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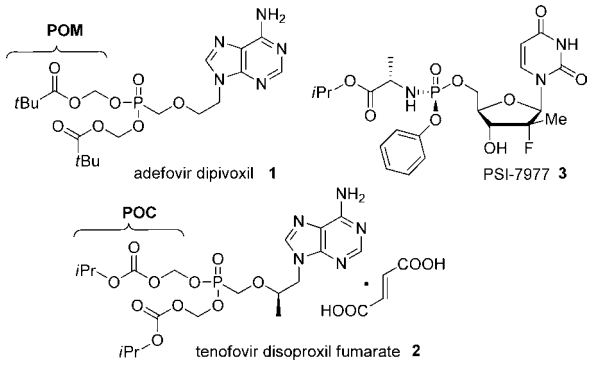

Figure 1. Biologically active phosphonate and phosphoramidate nucleosides.

in which butenyl acylic nucleoside phosphonate prodrugs are prepared using an olefin cross metathesis reaction with POM/POC protected allylphosphonates.[3]

Simple 5′-methylene-alkylphosphonates are most often prepared via an initial Wittig reaction between 5′-aldehydic nucleosides and dialkyl ((triphenylphosphoranylidene)-methyl)phosphonate reagents.[4,5] Alternative synthetic pathways include (1) Arbuzov condensation on a 5′-deoxy-6′-halo-sugar, subsequent glycosylation and deprotection;[6] (2) 3′,4′-*β*-oxetane ring-opening with alkylphosphonate anions followed by 3′-hydroxy inversion;[7] and (3) 4′-*C*-radical generation by photolysis of the 4′-(2-thiopyridone) ester (Barton reaction) *via* protection and 5′-oxidation to the carboxylate.[8] An attractive but surprisingly underexploited synthetic pathway for the synthesis of 5′-methylene-nucleoside phosphonates reported by Montgomery, involves a modified Horner–Wadsworth–Emmons (HWE) olefination by addition of dialkyl bisphosphonate salt on a 5′-aldehydic sugar and its subsequent glycosylation.[9] A similar approach was used later by Blackburn and Rashid for the synthesis of 3-phospho-D-glyceric acid analogues starting from a *β*-D-ribopentodialdehydo-l,4-furanoside[10] and by Van Calenbergh for the synthesis of 6-substituted uridine phosphonic acid analogues.[11]

We sought to extend the approach of Montgomery and Hewson[9] by preparing a dialkyl bisphosphonate where the alkyl portion was the desired final prodrug substituent. Utilization of such a reagent in a modified HWE reaction followed by reduction would eliminate the traditional[4,12] and often problematic phosphate ester cleavage and also make the overall synthesis more convergent by shifting the coupling of the phosphonic acid with the prodrug portion to the HWE reagent synthesis.

Herein, we report a general method to prepare nucleoside 5′-methylene bis(POM)-phosphonate prodrugs that allows (1) direct introduction of the phosphonate moiety bearing the biolabile POM leaving group and (2) apparent compatibility with most 5′-aldehydic nucleosides, independent of the nucleobase by condensation of tetra(POM)-bisphosphonate **B** *via* a modified HWE olefination (Figure 2). As part of our HCV research program, we applied this strategy to the 2′-deoxy-2′-α-fluoro-2′-*β*-*C*-methyl sugar derivatives whose most advanced member, PSI-7977 (GS-7977),[13,14] is currently in phase III clinical trials as a safe and effective anti-HCV agent (Figure 1).[15] The required tetra(POM)-bisphosphonate (TPBP, **5**) was prepared by a reported method from tetramethyl bisphosphonate **4** and chloromethyl pivalate in presence of sodium iodide (Scheme 1).[16] Previous studies involving **5** have been limited to its use as a methylenebisphosphonate prodrug for bone resorption disorders[16] and the closely related mimics of farnesyl diphosphate.[17] The use of tetra substituted bisphosphonates in modified HWE reactions have, to date, been limited to CH_3-, CH_3CH_2-, CF_3CH_2-, *i*-Bu-, Ph-, and Bn-substitutions.

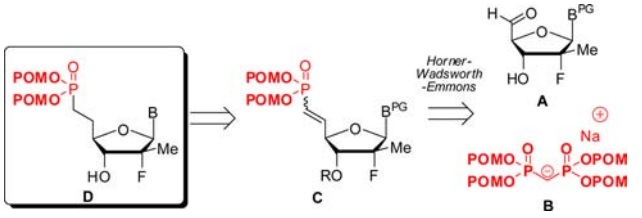

Figure 2. Retrosynthetic analysis.

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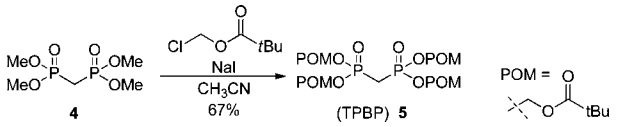
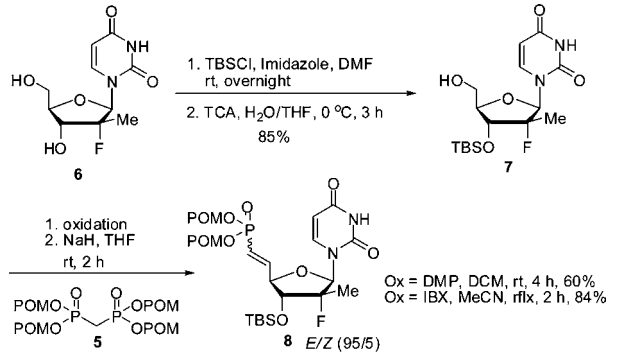
Scheme 1. Synthesis of Tetra(POM)-bisphosphonate (TPBP) **5**



In consideration of the chemical stability of POM groups, the selection of appropriate protective groups for our strategy was crucial and required investigation. We sought a protecting group that (1) can be selectively introduced on the 3′-position of the sugar in high yield; (2) is stable under oxidative and bisphosphonate salt addition conditions; and (3) can easily be removed without affecting the POM groups. *t*-Butyldimethylsilyl (TBS) groups appeared to fit these criteria. Therefore, 3′-OTBS-2′-C-Me-2′F-uridine derivative **7** was prepared as outlined in Scheme 2, by persilylation of **6**[18] with TBSCl followed by selective 5′-desilylation in presence of aqueous trichloroacetic acid (TCA).[19]

Scheme 2. Introduction of 3′-TBS Group and Oxidation Suitability



Oxidation of the 3′-OTBS derivative **7** was conducted with both Dess–Martin periodinane (DMP) at room temperature and 2-iodoxybenzoic acid (IBX)[20] at 80 °C (Scheme 2). The IBX oxidation of **7** afforded clean aldehyde in almost quantitative yield after simple filtration, while aldehyde resulting from DMP oxidation was mixed with several DMP byproducts. Finally, reaction between the crude aldehyde and 2.2 equiv of TPBP **5** led to the formation of desired bis(POM)-vinylphosphonate **8**. Interestingly, the use of a cleaner crude intermediate aldehyde obtained from IBX oxidation had an impact on the efficiency of the overall reaction sequence (60% compared to 84%). As expected with a HWE reaction mechanism, *trans*-bis(POM)-vinylphosphonate **8** was obtained as the major isomer (*E*/*Z* ratio of 95:5 as determined by ^{1}H NMR).

In order to generalize our strategy, this route was applied to the preparation of synthetically more challenging cytosine (C), adenine (A), guanine (G), and 2,6-diamino purine 2′-F-2′-*C*-Me nucleoside bis(POM)-phosphonate analogues **22**–**25**. For each nucleoside base, selective protections were utilized to be compatible with the key oxidation/HWE reaction sequence. Thus, cytosine derivative **9** was first treated with TBSCl and then with 2 equiv of Boc_2O to give the fully protected compound **10**. Finally, selective deprotection using trichloroacetic acid (TCA) afforded the 3′-OTBS-N^4-Boc nucleoside **11** in 81% overall yield with no observed Boc cleavage. A similar sequence was applied to prepare 3′-OTBS-N^6-diBoc adenine nucleoside **14**. The exocyclic amine was bis-Boc protected in high yield by treatment with excess Boc_2O in presence of *N*,*N*-dimethylaminopyridine (DMAP).[21] The TCA mediated deprotection gave the corresponding 5′-hydroxyl product **14** in an overall 74% yield with both Boc groups intact. In order to prepare guanosine and 2,6-diaminopurine phosphonate derivatives **19** and **20**, we started from the 6-chloro precursor **16**, which was synthesized from **15**[22] in 89% yield by persilylation of the sugar hydroxy groups using 5 equiv of TBSCl in presence of imidazole. Substitution of the 6-chloro of nucleoside analogue **16** with either sodium benzyloxide or sodium azide followed by 2-amino protection with Boc_2O led to compounds **17** and **18**. Selective 5′-deprotection with TCA afforded the desired protected intermediates **19** and **20** readily available for conversion to bis(POM)-phosphonate prodrugs (Scheme 3).

Scheme 3. Synthesis of Protected Nucleosides **11**, **14**, **19**, and **20**

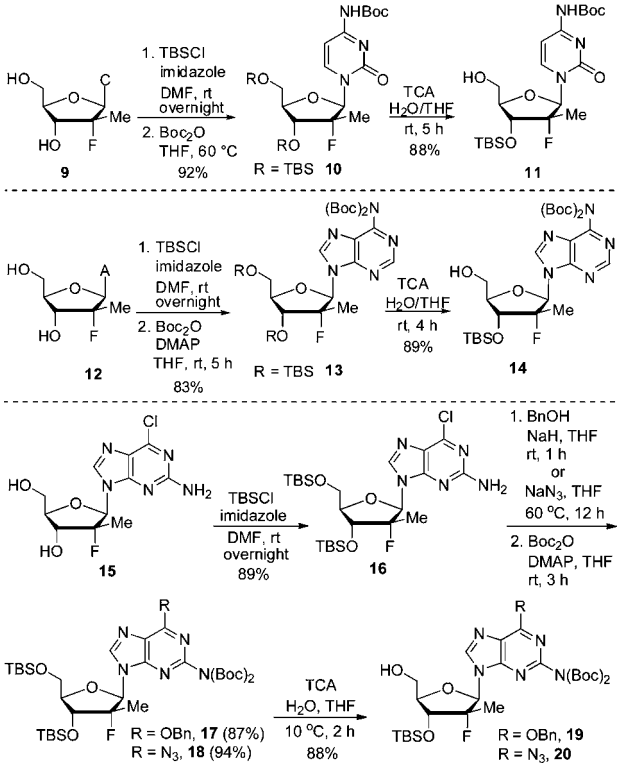


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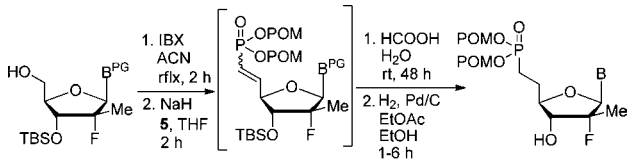
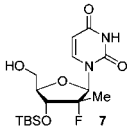
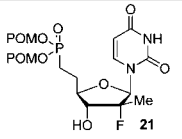
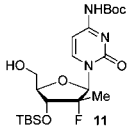
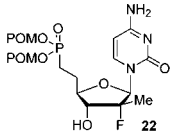
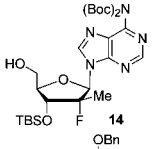
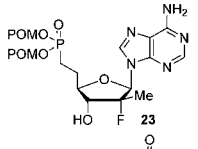
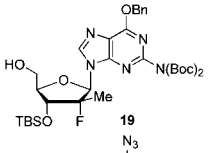
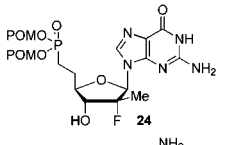
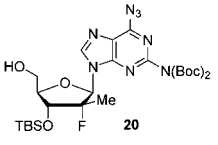
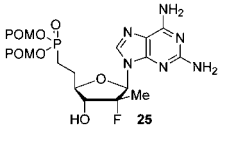
Table 1. Conversion of Protected 5′-Hydroxyl Nucleosides to 5′-Methylene-bis(POM)-phosphonate Prodrugs **21**–**25**



entry	starting material	deprotection product	yield[a] (%)
1	 **7**	 **21**	71
2	 **11**	 **22**	60
3	 **14**	 **23**	64
4	 **19**	 **24**	67
5	 **20**	 **25**	60

[a] Isolated yield over 4 steps.

The IBX oxidation/TPBP sodium salt addition sequence was successfully applied to the protected 5′-OH nucleosides **11**, **14**, **19** and **20** (Table 1). In the oxidation of the purine analogues **14**, **19** and **20**, the *N*,*N*-bis(Boc) protection provides enough steric and/or electron-withdrawing effects to avoid potential decomposition.[23] It is noteworthy that after the modified HWE reaction, we were unable to separate the bisPOM-vinylphosphonate nucleosides from excess tetra(POM)-bisphosphonate **5** by flash silica gel column chromatography (except in the case of the U analogue; entry 1) and thus had to achieve subsequent deprotection with an 80% HCOOH/water solution to facilitate isolation. All attempts to use more traditional desilylation conditions (TBAF, TBAF/HOAc, $HF \cdot Et_3N$ or 1 M HCl in dioxane) led to significant or complete degradation of the substrates. Final hydrogenation using H_2 and Pd/C[24] provided the desired 5′-methylene bis(POM)-phosphonate nucleosides **21**–**25** in yields that ranged from 60 to 71% over 4 steps and 44 to 60% from the starting nucleosides. All five final phosphonate prodrugs were devoid of anti-HCV,[25] anti-HIV, or cytotoxic activity.[26,27]

In conclusion, we report herein the first highly efficient synthesis of 5′-methylene bis(POM)-phosphonate prodrugs through a modified HWE reaction. The key HWE reaction of the sequence involves the addition of a tetra(POM) bisphosphonate sodium salt to Boc/TBDMS protected 5′-aldehydic nucleosides. The 2′-deoxy-2′-α-fluoro-2′-*β*-*C*-methyl-5′-methylene bis(POM)-phosphonate nucleoside prodrugs **21**–**25** were obtained with excellent overall yields that ranged from 60 to 71% over 4 steps from the corresponding protected starting materials. Through this work we have extended the scope of tetra-substituted bisphosphonates that take part in a modified HWE reaction. The versatility of this approach could be utilized for the rapid synthesis of new and diverse phosphonate prodrugs with superior biological activity.

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Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) A reviewer has pointed out to us that generation of the 5′-aldehyde with purine nucleobases is problematic because of cyclonucleoside formation with the *N*-3 amino group, which leads presumably to deglycosylation and other degradation products.

(24) While 1 h completely reduced the vinylphosphonate, several hours were required for one pot removal of the benzyl group and the reduction of the azido group.

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(27) No significant antiviral activities were found for compounds **21**–**25** when tested up to 100 μM against HIV in peripheral blood mononuclear cells and up to 10 μM against HCV in a subgenomic RNA replicon system. No cytotoxicity was observed in Huh7 cells up to 10 μM or in PBM, human lymphoblastoid CEM, or African Green monkey Vero cells up to 100 μM.

The authors declare the following competing financial interest(s): Dr. Schinazi is the founder and a major shareholder of RFS Pharma, LLC. Emory received no funding from RFS Pharma, LLC to perform this work and vice versa.