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(*R*)-9-[2-(Hydroxyphosphinylmethoxy)propyl]adenine as the precursor molecule for antivirals

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ABSTRACT

The biological importance and practical significance of phosphonates have been a major driving force for antiviral research in the past two decades. We describe in this Letter the potential of the H-phosphinate derivative (*R*)-9-[2-(hydroxyphosphinylmethoxy)propyl]adenine as the versatile synthetic intermediate in the preparation of *N*-alkylphosphonamidate and alkylphosphonate series.

Phosphorus-containing compounds became key in various medicinal techniques such as antisense strategy, antigene or gene silencing technique, and in drug therapy making use of modified nucleotides in antiviral or antitumoral treatments.[1] During the past decades, a significant interest has emerged in the preparation and study of phosphonic acids and their derivatives. As it was presumed that the carbon—phosphorus bond was unable to be hydrolyzed by enzymes involved in natural phosphate bond cleavage, they were considered as more stable analogues than natural phosphates. Amongst phosphonate derivatives, acyclic nucleoside phosphonates (ANPs)[1] are outstanding molecules in the fight against DNA virus and retrovirus infections. Three of such compounds have been licenced for the treatment of HCMV infections in AIDS patients (cidofovir, Vistide®), HIV, and chronic HBV infections (Adefovir dipivoxil, Hepsera®, and Tenofovir disoproxil fumarate, Viread®). In addition to these indications, there are various other clinical conditions in which ANPs were found to be of therapeutic interest.[2]

In previous articles, we focused our researches on the impact of the modification of the phosphorus environment in (9-[2-(phosphonomethoxy)ethyl]adenine [PMEA] and (*R*)-9-[2-(phosphonomethoxy)propyl]adenine [PMPA] derivatives to improve their efficacy as antivirals.[3–7] Some of these modifications as thiophosphonate derivatives [S-PMPA] and [S-PMEA] turned out to produce desired therapeutic effects, as it is apparent from recent studies on the inhibition of HIV and HBV infections.[5,8]

In this Letter, we explore new series of analogues. (*R*)-9-[2-(Hydroxyphosphinylmethoxy)propyl]adenine **1** (Scheme 1), H-phosphinate precursor in PMPA series offers a unique opportunity to introduce various modifications of the phosphorus center by changing the oxidation conditions. We do not intend to give an exhaustive coverage of all possible modifications leading to new potential therapeutics, but explore the flexibility of the H-phosphinate chemistry whose exploration lags somehow behind that of H-phosphonate chemistry.[9]

We developed an original method to synthesize H-phosphinate intermediate **1**[3,6] that can serve as precursor to generate modified acyclic nucleoside phosphonates (Scheme 1). This key precursor was then used to synthesize α-boranophosphonate **3**[3] and α-thiophosphonates **4**[5,8] derived from PMPA.

The phosphorus atom in H-phosphinate **1** is an electrophilic center, however, it can be transformed into a nucleophilic center under certain conditions. Indeed, H-phosphinate exists as an equilibrium mixture of two tautomeric forms:[10] a tetra-coordinated phosphinate form and a tri-coordinated phosphonite form. The tervalent form, due to the presence of lone electron pair on the phosphorus center, reacts readily with various electrophiles. On the contrary, in a tetra-coordinated form, the lack of the lone of electron pair on the phosphorus center reduces the reactivity to electrophiles. Nevertheless, by using Atherton–Todd conditions[11] (silylating agent, carbon tetrachloride and pyridine), the phosphinate form can be transformed into phosphonamidate P(V) species

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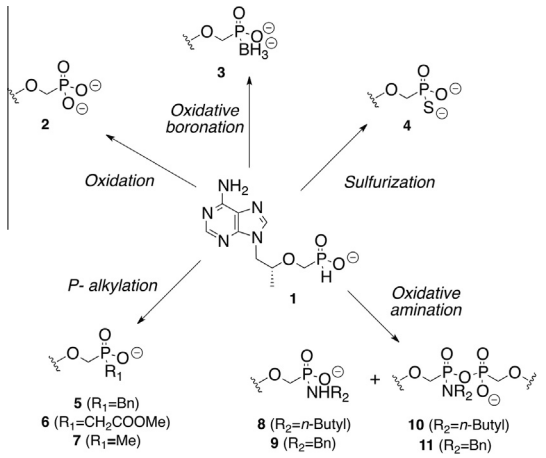


Scheme 1. General structure of H-phosphinate **1** as the versatile precursor, and some oxidative transformations.

(pyrimidium adduct) which can be efficiently attacked by nucleophiles. In order to obtain an effective activation, *N*,*O*-bis (trimethylsilyl)acetamide (BSA) was chosen as the silylating agent[12] and H-phosphinate **1** was treated with BSA in pyridine, or DCM with Et_3N, and the reaction mixture was stirred at room temperature for 16 h (Scheme 2).

Reactions with electrophiles. Synthesis of alkylphosphonates 5, 6, and 7

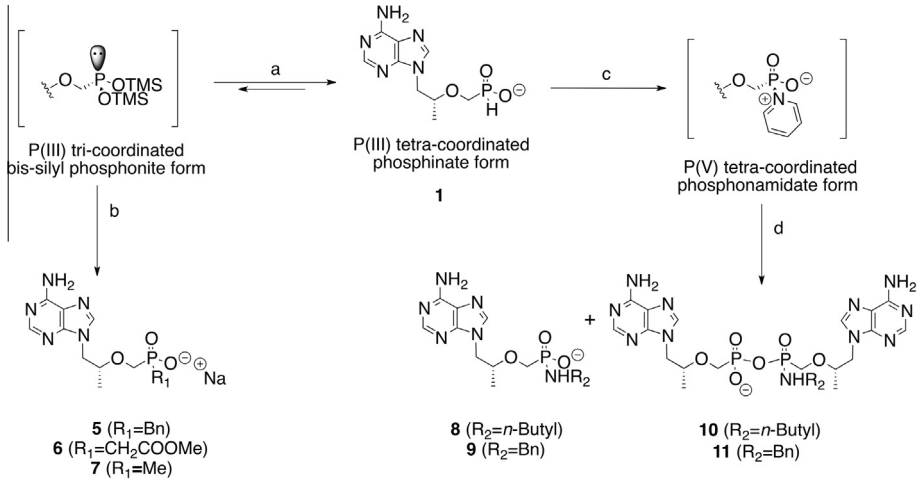
For the formation of P—C bonds, a variety of metal catalyzed cross-coupling reactions have been developed.[13] To perform oxidative addition of H-phosphinate substrates, palladium-catalyzed additions to alkenes[14] and base-promoted addition to alkenes, carbonyls, and alkyl halides as Arbuzov-like reaction have been described.[15,16]

Compounds **5**, **6**, and **7** were prepared by reaction of the bis-silyl phosphonite of **1** with corresponding alkyl halides/benzyl bromide, ethyl 2-bromoacetate, or methyl iodide, during 2 h. Reactions led to expected compounds **5**, **6**, and **7** in 53%, 39%, and 57% yield, respectively. ^{31}P NMR spectra of compounds **5**, **6**, and **7** revealed resonance signals between 29 and 37 ppm.

Reactions with nucleophiles. Synthesis of compounds 8 and 9

Nucleos(t)ides, phosphoramidates, and phosphonamidates have been extensively studied,[17] due to their prodrug activity against viruses and in cancer therapy. In the course of our studies on the chemistry of nucleoside H-phosphinates, we have investigated the synthesis of *N*-alkyl phosphonamidates. The bis-silyl phosphonite of **1** was treated under mild Atherton–Todd reaction conditions[11] (pyridine and carbon tetrachloride) to produce the pyridinium phosphonamidate intermediate, which was subsequently substituted by *n*-butylamine or benzylamine, leading to *N*-alkyl phosphonamidates **8** and **9**[18] (Scheme 2). Using Atherton–Todd conditions, an important amount of the side product identified as the trichloromethylphosphinate was obtained. To minimize its formation, the quantity of carbon tetrachloride has been reduced from 50 to 12 equiv. Inspection of the ^{31}P NMR spectra during the monitoring of the reaction revealed resonance signals at 20 ppm characteristics of the formation of compounds **8** and **9**.[19] Unfortunately isolation of *n*-butyl phosphonamidate **8** and benzyl phosphonamidate **9** was impossible due to their partial instability during workup and chromatography under aqueous conditions. As outlined in Figure 1, chromatogram of the purification under aqueous conditions (Fig. 1–Panel A) of compound **8** revealed the presence of three compounds. HPLC-MS coupling analysis (Fig. 1–Panel B and C–S3′ gradient) allowed us to identify the compound with retention time $^tR = 0.35$ min, ESI-MS [M+H] $m/z = 287.94$ as phosphonate **2**; the compound with retention time $^tR = 1.52$ min, ESI-MS [M+H] $m/z = 342.0$ as *n*-butyl phosphonamidate **8**; the compounds with retention times; $^tR = 2.01$ and 2.09 min, ESI-MS [M+H] $m/z = 611.7$ as compound **10** as a diastereoisomeric mixture. The same results were observed with benzyl phosphonamidate **9** ($^tR = 6.85$ min, ESI-MS [M+H] $m/z = 376.8$) which decomposed rapidly in phosphonate **2** and compound **11** ($^tR = 9.36$ and 9.71 min, ESI-MS [M+H] $m/z = 646.0$).

It has to be noted that the lability of P—N bonds in phosphonamidates has been explored.[20] The P—N bond in a phosphonamidate monoester, is labile and hydrolyzed faster than that in a phosphoramidate diester,[20] so the observed decomposition should be predictable. However the formation of compounds **10** and **11** is more surprising. The SPARC pK_a calculator allowed us to determine pK_a values of compounds **8** and **9**, respectively 3.40 and 3.30.[21] We may hypothesize that under aqueous conditions, the *N*-alkyl



Scheme 2. Synthesis of compounds **5**, **6**, **7**, **8**, **9**, **10**, and **11**. Reagents and conditions: (a) BSA, TEA in DCM, rt, 16 h; (b) R_1X (X = Br for **5** and **6** or X = I for **7**), 2 h then H_2O, then dowex Na^+ exchange after purification; (c) BSA in pyridine, rt, 16 h; (d) CCl_4 and NH_2R_2 (R_2 = *n*-butyl for **8** and R_2 = Bn for **9**), 2 h then H_2O.

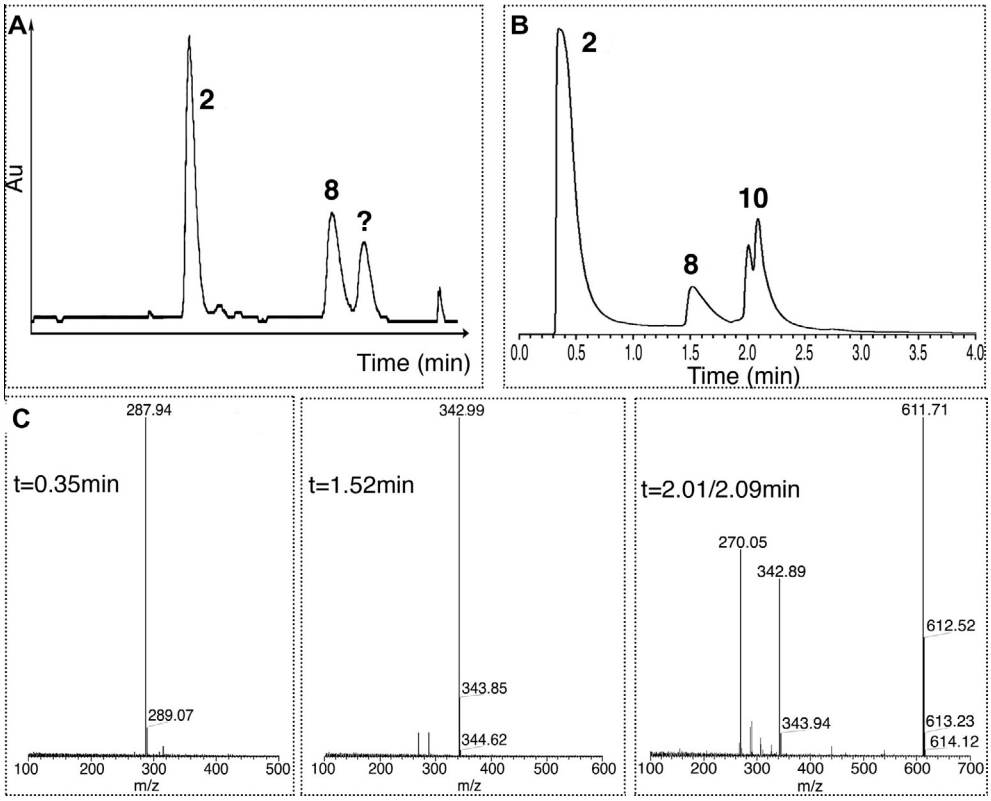


Figure 1. Analysis of *N*-alkylphosphonamidate **8**. Panel A: chromatogram of compound **8** before purification (MPLC analysis). Panel B: chromatogram of compound **8** after purification (HPLC–MS analysis, S3′ gradient). Panel C: Mass fingerprints (ESI^+) of pics observed in panel B.

moiety is a good living group and the nucleophilicity of the oxygen negatively charged is enough to attack phosphorus atom and leads to the formation of the dimeric species.

The ^{31}P NMR chemical shifts of phosphinate **1** and modified-phosphonates **2**, **3**, **4**, **5**, **6**, and **7** are reported in Figure 2. These data should be useful for synthesis monitoring and qualitative identification of these specific compounds and, to some extent, related compounds by extrapolation. For example, ^{31}P NMR signal of **1** around 25 ppm with a huge P—H coupling constant (J_{PH} = 528 Hz) are replaced by characteristic downfield resonance signal around 115 ppm, due to the displacement of electron density from the phosphorus center to the negatively charged coordinated borane for boranophosphonate **3** or by resonance signal at 60 ppm (t, J = 5 Hz), characteristic of predominant thiono form for thiophosphonate **4**.

H-phosphinate chemistry offers a versatile method for synthesis of various phosphonate compounds, combining advantages of tricoordinated phosphorus(III) and tetracoordinated phosphorus(V). Although the focus of our research has been nucleotide chemistry, the H-phosphinate chemistry is not, by any means, confined to this class of compounds. It is likely that the demand will grow regarding other bioactive phosphorus compounds to be used as potential therapeutic agents. In this context, H-phosphinate chemistry might provide necessary tools for modification of phosphorus compounds by means of introducing multiple modifications and even controlling the stereochemistry.

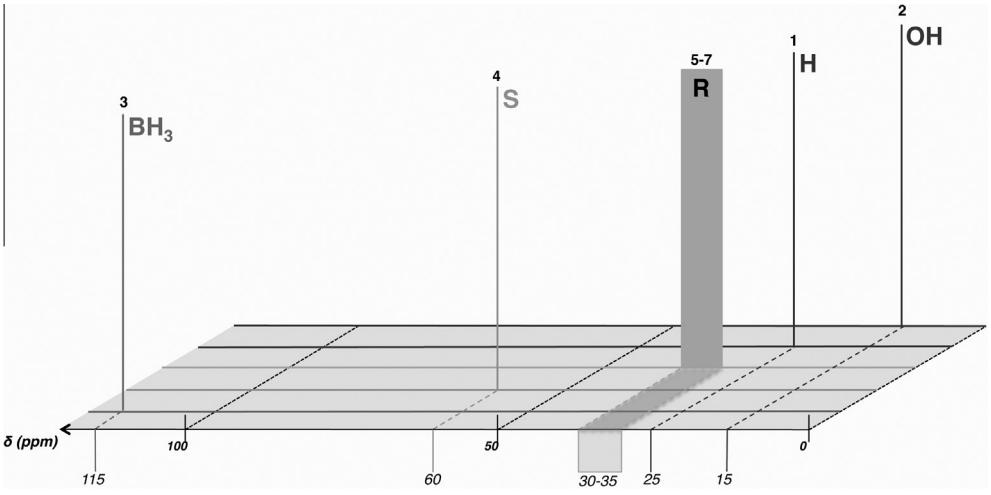


Figure 2. ^{31}P RMN chemical shifts of compounds **1**, **2**, **3**, **4**, **5**, **6**, and **7**.

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Supplementary data

Supplementary data (detailed experimental procedures and spectroscopic data for compounds **5**, **6**, **7**, **8**, **9**, **10** and **11**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.05.061.

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