Synthesis and biological evaluation of inosine phosphonates†‡

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The 4′-phosphonomethoxy analogs of inosine and 2′,3′-dideoxyinosine were synthesized and tested for their activity against HCV and HIV, but found to be inactive. During the course of this synthetic investigation, an unexpected oxidative cleavage of the 2′,3′-bond of 2′-deoxyinosine was observed.

The chemistry used for the synthesis of 5′-modified (2′-deoxy)-adenosine nucleosides[1,2] does not always work for the synthesis of inosine congeners, and in some cases the hypoxantine base needs to be protected. We present here the synthesis of two inosine derivatives with a phosphonomethoxy substituent at the 4′-position. This kind of isostere/isoelectronic replacement has proven in the past to lead to bioactive compounds.[3,4] A particular problem encountered was the oxidation of the 5′-hydroxyl function of the 2′-deoxyinosine to a carboxylic acid group. This conversion could be carried out using the *N*-oxoammonium salt of 2,2,6,6-tetramethylpiperidine-1-oxyl. Given the interest in phosphonate nucleosides in the antiviral field, the obtained compounds were tested against HIV, RSV and in a HCV replicon assay.

Two possible procedures for the synthesis of phosphonate analogs of nucleotides are known. A Lewis acid-mediated glycosidation of a phosphonate alcohol onto an anomeric acetate could be used to introduce the desired phosphonate, as reported previously.[3,5] Phosphonate isoesters of nucleoside monophosphates could also be prepared using a highly stereoselective asymmetric oxyselenenylation of a furanoid glycal or a subsequent electrophilic addition of iodine monobromide to glycal onto the least hindered α-face of the ribose, followed by the introduction of the protected hydroxymethylphosphonate ester onto the β-face.[2]

The phosphonomethoxy derivatives of inosine, **1**, and 2′,3′-dideoxyinosine, **2**, were prepared starting from 2′-deoxyinosine (**3**) in 11 steps, as illustrated in Scheme 1. There were two key transformations in the sequence: firstly, the regiospecific oxidation of the 5′-hydroxyl group into a 4′-carboxylic acid, and secondly, the regiospecific and stereoselective introduction of the phosphonate group into the β-configuration. Direct oxidation of **3** with chromium trioxide in pyridine or potassium permanganate in basic aqueous solution did not result in the desired 4′-carboxylic acid, as described for the oxidation of 2′-deoxyadenosine[1,6] and 2′,3′-*O*-isopropylideneinosine.[7] The product formed under these conditions was the result of oxidative cleavage of the 2′–3′ carbon–carbon bond of the deoxyribose sugar, with the formation of the dicarboxylic acid (Scheme 2). This reaction is rather unexpected for 2′-deoxyribonucleosides and might be explained by the oxidative cleavage of an enol intermediate. When we tried to oxidize unprotected 2′-deoxyadenosine with potassium permanganate under basic conditions, mass spectrometry showed the formation of a compound with a molecular mass that may correspond to 2-(carboxy(adenin-9-yl)methoxy)-3-hydroxypropanoic acid. The oxidation of ribo- or 2-hexenepyranosyl nucleosides, with cleavage of the 2′–3′ carbon–carbon bond of the sugar moiety, resulting in dialdehyde formation, is known.[8]

The platinum-catalyzed oxidation by air, following the procedure of Moss *et al.*,[9] also failed. Finally, the oxidation of the N^1-protected 2′-deoxyinosine, **5**, utilizing the *in situ*-generation of the *N*-oxoammonium salt derived from TEMPO[10] and following deprotection with methanolic ammonia, gave key acid **6**. Furthermore, the reaction generated only acetic acid and iodobenzene as by-products, which were easily removed from the precipitated product to yield acid **6** in analytical purity. Decarboxylative dehydration of acid **6** into furanoid glycal **7** was carried out with *N*,*N*-dimethylformamide dineopentyl acetal in good yield.[1] The haloetherification reaction of glycal **7** with dibenzyl (hydroxymethyl)phosphonate[11] mediated by iodine monobromide, followed by the base (DBU) promoted elimination of hydrogen bromide[1] or oxyselenenation with the aid of phenylselenyl chloride, and then oxidative (hydrogen peroxide) elimination of the phenylselenyl group[1,12] gave rise to olefin **8**.

Saturated phosphonate **2** was prepared from **8** by hydrogenation using catalytic Pd/C with simultaneous removal of the phosphonate benzyl ester and the 2-*N*-benzyloxymethyl protecting groups. Bis-hydroxylation of the double bond in **8** was accomplished using catalytic osmium tetraoxide and *N*-methylmorpholine oxide[13] as the oxidizing reagent. The one step deprotection of the generated single diol isomer[1] resulted in phosphonate isostere **1** of inosine monophosphate in high yield.

In conclusion, a synthetic scheme has been developed leading to the 4′-phosphonomethoxy derivatives of inosine and of 2′,3′-dideoxyinosine. Both compounds showed activity when tested again HIV-1, RSV and in a HCV replicon assay.[14]

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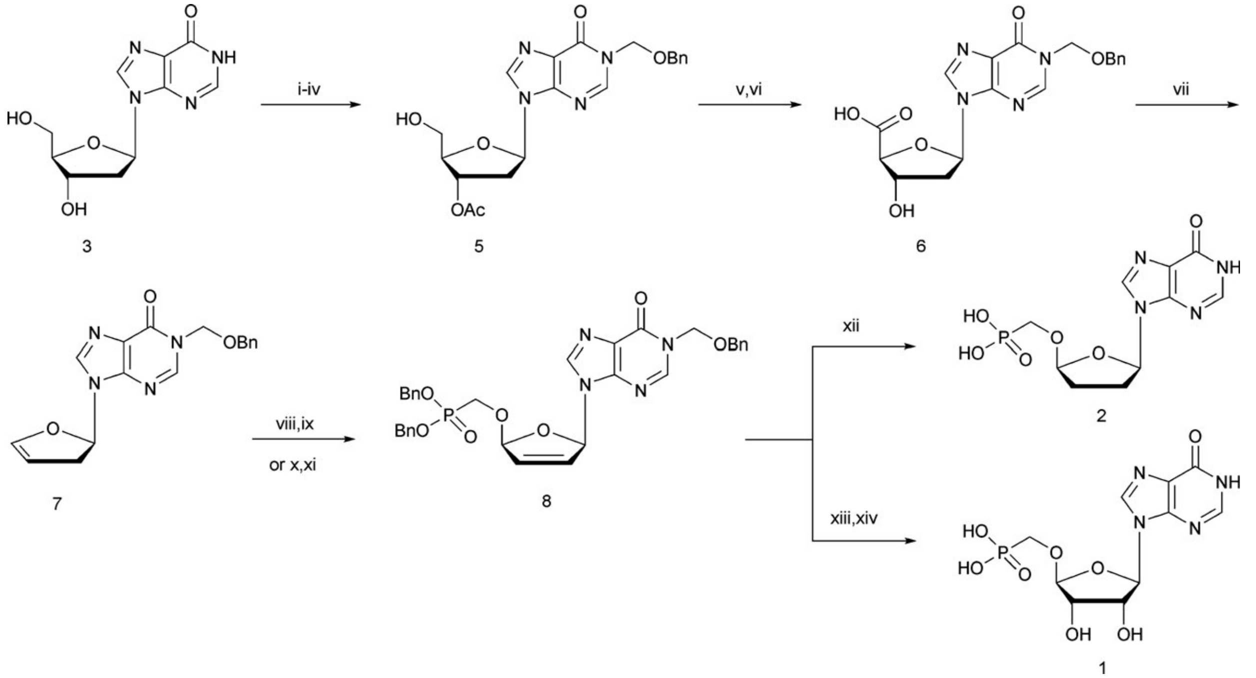
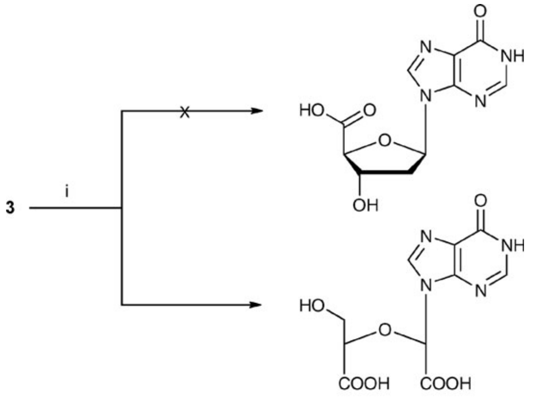


Scheme 1 Synthesis of the 4′-phosphonomethoxy derivatives **1** and **2** of inosine and 2′,3′-dideoxyinosine. Reagents and conditions: (i) MMTrCl, Py; (ii) Ac_2O, Py, 90%; (iii) $BnOCH_2Cl$, DIPEA, DCM, 70%; (iv) TsOH, MeOH–DCM, TEA, 0 °C, 83%; (v) TEMPO, iodobenzene diacetate, $MeCN–H_2O$, 97%; (vi) NH_3, MeOH, 92%; (vii) *N*,*N*-dimethylformamide dineopentyl acetal, DMF, 130 °C, 76%; (viii) PhSeCl, dibenzyl hydroxymethylphosphonate, $LiClO_4$, DCM, −70 °C, 42%; (ix) 30% H_2O_2, dioxane, 0 °C, 45%; (x) IBr, dibenzyl hydroxymethylphosphonate, DCM, −25 °C, 37%; (xi) DBU, dioxane, 65 °C, 80%; (xii) H_2, Pd/C, MeOH, 95%; (xiii) OsO_4, *N*-methylmorpholine *N*-oxide, 70%; (xiv) H_2, Pd/C, MeOH, 95%.



Scheme 2 Direct oxidation of 2′-deoxyinosine **3**. Reagents and conditions: (i) $KMnO_4$, KOH, H_2O, 42%.

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