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Efficient Synthesis of Highly Active Phospha-Isosteres of the Influenza Neuraminidase Inhibitor Oseltamivir

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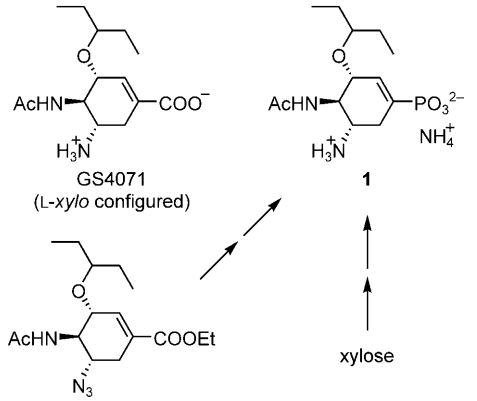
The persistent threat of an influenza pandemic caused by highly pathogenic avian influenza viruses has led to an intense search for improved treatments of the infection.[1] Besides M2 ion channel inhibitors, neuraminidase (NA) inhibitors that mimic the carbenium–oxonium transition state of NA-mediated sialic acid cleavage, such as oseltamivir (GS4071), have so far been most successful.[2] Tamiflu, the phosphate salt of the oseltamivir ethyl ester, first synthesised by Gilead Sciences and marketed by F. Hoffman-La Roche Ltd., is currently dominating the market.[3] Not surprisingly, the search for improved and industrially applicable routes for its total synthesis as well as the development of a new generation of NA inhibitors to combat emerging resistances have become hot topics in organic synthesis.[2,4]

Sialic acid mimicry and high activity in this type of inhibitor is achieved by the cyclohexene-1-carboxylic acid scaffold that carries a glycerol side chain mimic at position 3, an essential acetamide at position 4, and a basic substituent at position 5, in an L-*xylo* configuration (Scheme 1).[5]

We have been promoting the idea that replacement of the carboxylate in such structures by a phosphonate or phosphonate monoester would open up space for structural variation whilst retaining the negative charge under physiological conditions which is essential for activity.[5] Our work has led to a variable synthetic approach to L- and D-*xylo* cyclohexenephosphonates from L- and D-xylose, respectively, as sialidase inhibitors, and a modification of this approach has recently been used to synthesise the phosphonate congener **1** of Tamiflu (Scheme 1).[6]



Scheme 1. Oseltamivir (GS4071) and synthetic approaches to its phospha-isostere **1**.

Herein we report an efficient alternative, taking advantage of the availability of acetamidoazide **2** as the industrial precursor of Tamiflu, which uses the Hunsdiecker–Barton iododecarboxylation methodology to replace the carboxylate by iodine followed by palladium-mediated coupling with dimethylphosphonate as key steps.[7] We thus provide access not only to the potent inhibitor **1** but also to its monoesters, allowing for the presentation of the Tamiflu motif on an unlimited variety of carrier structures (Scheme 2).[5a]

In brief, acetamidoazide **2**[8] was converted into the free acid **3**, which was then subjected to the radical iododecarboxylation procedure to give **4**.[9] This has some significance in itself, as there are very few examples of the radical iododecarboxylation of vinylcarboxylic acids.[10] The azide in vinyl iodide **4** is first converted into the Boc-protected amine **5**, followed by palladium-promoted coupling with dimethylphosphonate to furnish cyclohexenephosphonate **6**. The monoester **7** serves as the key intermediate for the synthesis of all monoesters, employing a previously established mixed diester strategy.[5a,b,11] The distinct advantage that methyl esters and benzyl esters have[5a] over ethyl esters[5–7] was shown by us before.

To demonstrate the versatility of the approach and to obtain a structurally diverse set of exemplary inhibitors, we have chosen the methyl ester **8** as proof of principle, the hexyl ester **12** having a hydrophobic aglycone mimetic, and the galactosyl ester **13** with the natural sialic acid aglycone galactose. The latter two compounds are synthesised in a straightforward manner through alkylation with the corresponding triflates to give mixed diesters **10** and **11**, respectively, followed by cleavage of the methyl ester and subsequent removal of the protecting groups under standard conditions (Scheme 3).

Phosphonates **1**, **8**, **12**, and **13** were investigated for inhibitory activity in a whole-virus assay using allantoic fluid from infected eggs (Table 1).[4d] Our data show that both the unmodified phosphonate and its three monoesters display inhibitory activity against influenza virus NA in the same range as oseltamivir. This was further confirmed by the inhibition of NA from oseltamivir-resistant virus (A/Norway/1735/07, K_M for MUNANA = 11.1 ± 0.6 μM) by compound **1**, with a K_i value of 48 ± 8 nM, compared with 36 nM for oseltamivir. These findings are important as they allow the conclusion that the inhibitory

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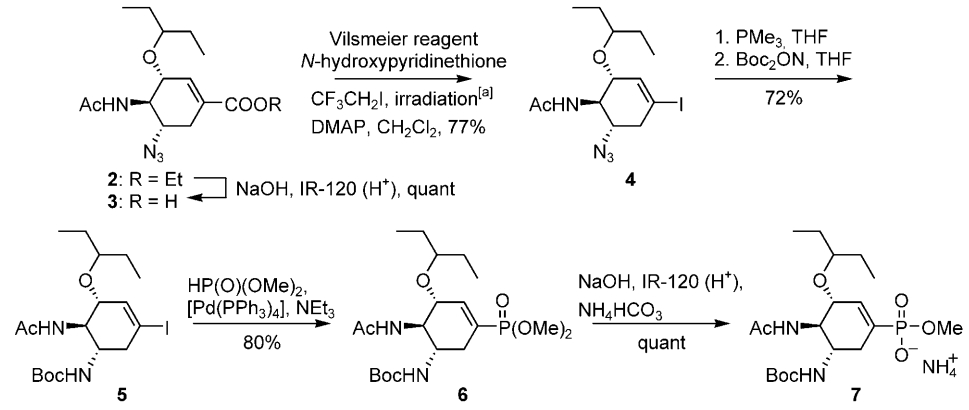
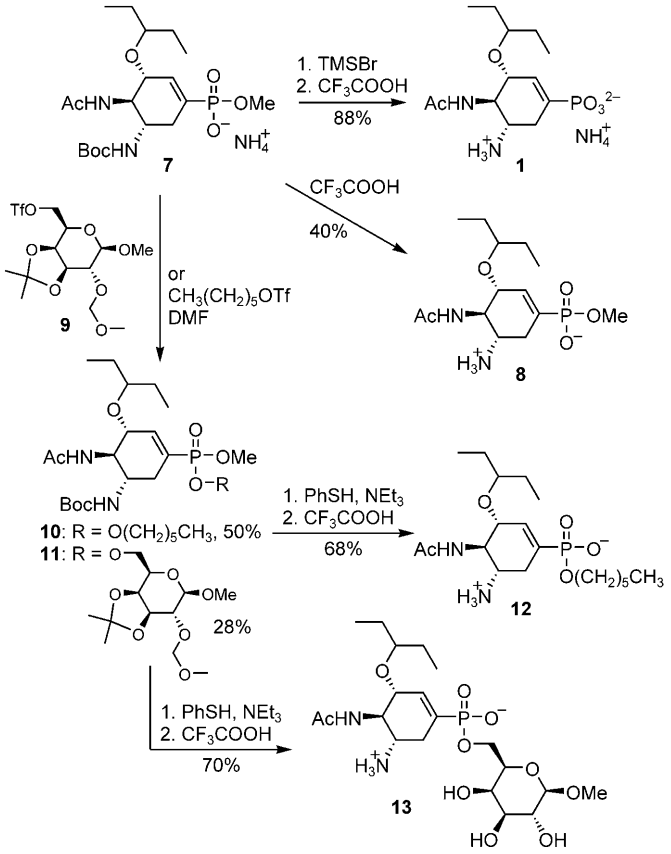


Scheme 2. Replacement of the carboxylate in precursor **2** by a phosphonate via a Hunsdiecker–Barton iododecarboxylation route. [a] 250 W electric flood lamp; see Supporting Information.



Scheme 3. Synthesis of **1** and selected monoesters **8**, **12**, and **13** via a mixed diester route.

Table 1. Inhibition constants (K_i [nM]) for target phosphonates.[a]

1	8	12	13	Oseltamivir
0.17 ± 0.03	0.30 ± 0.04	0.22 ± 0.03	0.85 ± 0.12	0.15

[a] Inhibition of MUNANA hydrolysis catalysed by neuraminidase from influenza virus A/Norway/1758/07; inhibition constants were determined as described in the Supporting Information; K_M for MUNANA = 6.4 ± 0.6 μM.

properties of oseltamivir are essentially retained when the carboxylate is substituted by a monoalkyl phosphonate, which retains a negative charge under physiological conditions. Consequently, our 'phospha-oseltamivir' approach allows the stable display of the oseltamivir motif on natural sialic acid aglyca such as galactose or sialic acid, on mimetics thereof, or on other supports, if attached as a monoester. This may well lead to new, very strongly binding and selective inhibitors and materials which could help combat H5N1 and other pathogenic influenza viruses.

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Keywords: antiviral agents · influenza · inhibitors · oseltamivir · sialic acid · sialylmimetics

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