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Bioorganic & Medicinal Chemistry Letters 14 (2004) 361–364
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation as sialidase inhibitors of *xylo*-configured cyclohexenephosphonates carrying glycerol side-chain mimics

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Received 22 August 2003; revised 29 October 2003; accepted 3 November 2003

Abstract—Based on a strategy previously reported by us, we have synthesized D-*xylo* configured cyclohexenephosphonates designed to mimic the transition state of the sialidase reaction. The double bond orientation corresponds to the benchmark inhibitor Neu5Ac2en and we could selectively introduce hydroxyalkyl substituents in order to simulate the glycerol side-chain of neuraminic acid. The inhibitory activity of a set of compounds towards bacterial sialidases was tested and interesting differences in activity were found.

1. Introduction

Sialidases release sialic acids, a family of nine carbon sugars the most prominent member of which is *N*-acetylneuraminic acid, from their terminal position on cell surface glycoproteins or glycolipids.[1,2] This is exploited by various pathogenic microorganisms which utilize the action of their respective sialidases to promote infection.[3] For instance, influenza virus progeny can only be efficiently released from infected cells when the density of sialic acids on the debris of the infected cell surface is lowered, hence preventing multivalent binding to the viral surface lectin.[4] Another example would be the cleavage of epithelial cell surface sialic acid moieties by the sialidase of *Vibrio cholerae*, thus unmasking the GM1 pentasaccharide which is the ligand for the bacterial toxin responsible for the pathogenesis of cholera infection.[3,5]

In contrast to the influenza enzyme, for which highly potent inhibitors such as GS 4071 or GG 167 (Fig. 1) have been developed, inhibition of bacterial or protozoal sialidases has proven to be far less straightforward.[6] Some of the latter sialidases have been shown to have a modular structure, consisting of a catalytic domain and one or more additional carbohydrate-binding lectin domains, in the case of the *V. cholerae* enzyme also referred to as sialidase-wings.[7]

We have proposed cyclohexenephosphonates such as **1** or **2** (Fig. 1) as a class of sialidase inhibitors which allow attachment of additional sugar moieties, with or without spacer, at the phosphonate group thus generating a monoester which retains the negative charge known to be required for recognition by the enzyme.[8–10] Thus, bifunctional inhibitors[11] could be obtained which bind both the catalytic centre and the lectin-like binding site of the enzyme leading to a putative increase in affinity and selectivity. The feasibility of such a concept has recently been confirmed by the inhibition of the action

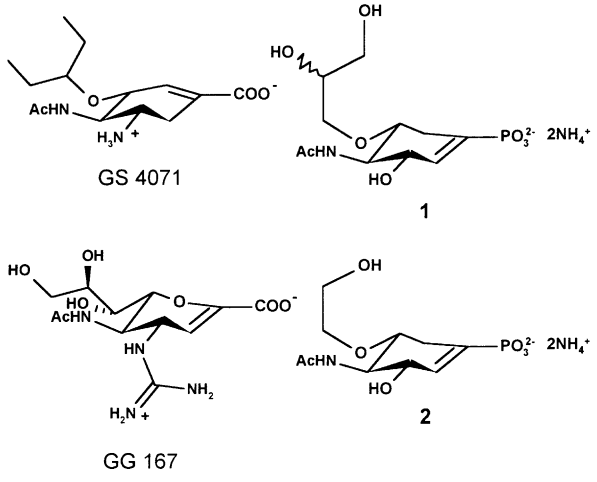

Figure 1. Prominent influenza sialidase inhibitors and 5-substituted cyclohexenephosphonates **1** and **2** reported in this contribution.

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doi:10.1016/j.bmcl.2003.11.003

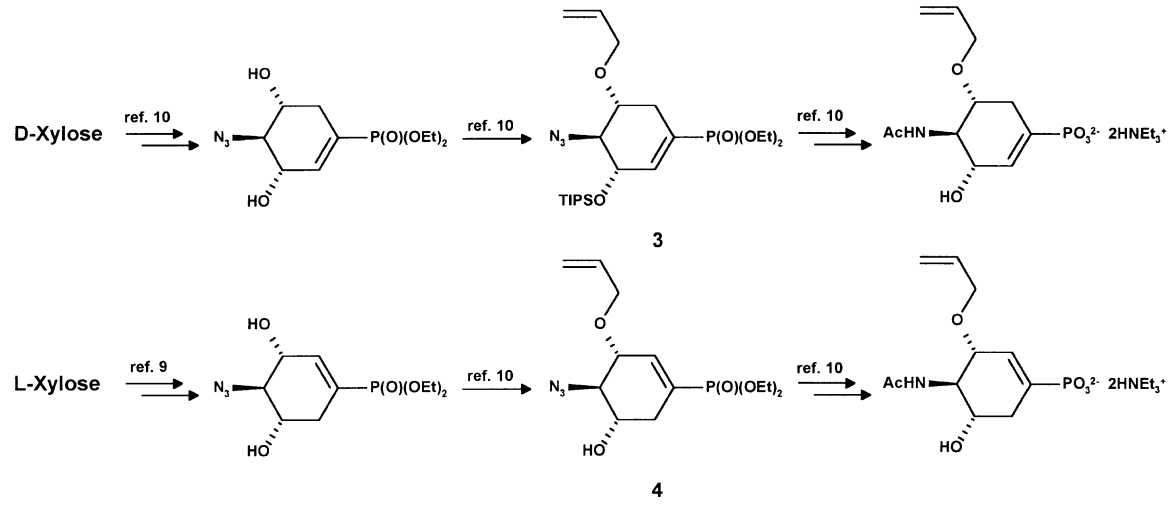


Figure 2. Synthetic approach to D-*xylo* (**3**) and L-*xylo* (**4**) configured cyclohexenephosphonates from D- and L-xylose, respectively.

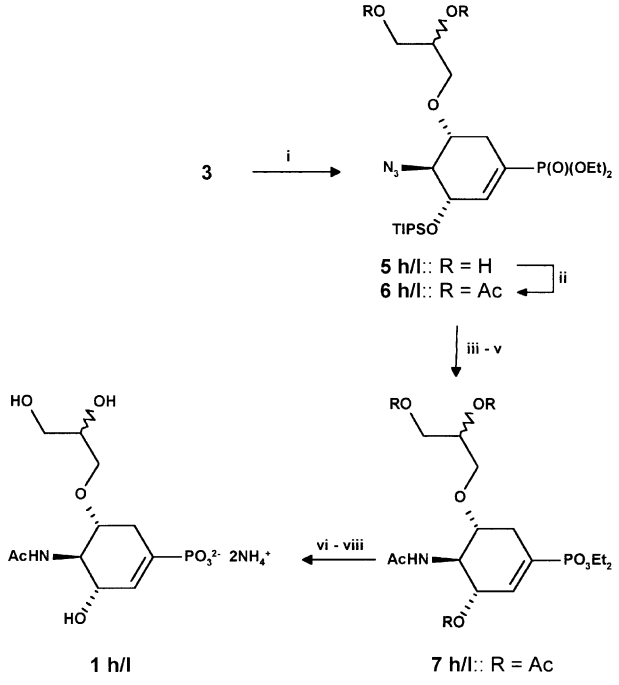


Figure 3. Reagents and conditions: (i) OsO_4, NMO (75%); (ii) Ac_2O, pyridine (qu); (iii) TBAF, THF (qu); (iv) H_2S, pridine, H_2O; (v) Ac_2O, pyridine (60%); (vi) TMSBr, $CHCl_3$ (qu); (vii) NH_4OH, H_2O; (viii) Biogel P2, NH_4HCO_3, H_2O.

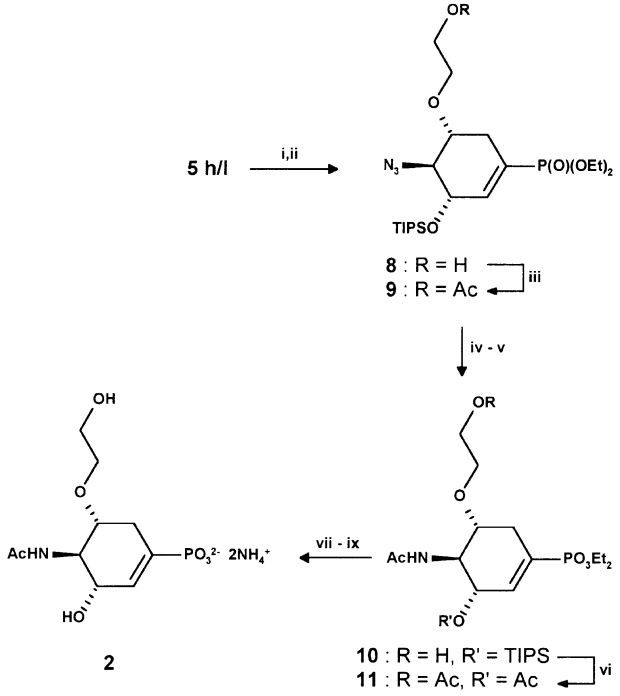


Figure 4. Reagents and conditions: (i) $NaIO_4$, EtOH (qu); (ii) $NaBH_4$, EtOH (95%); (iii) Ac_2O, pyridine (qu); (iv) H_2S, pyridine, H_2O; (v) Ac_2O, pyridine (55%); (vi) TBAF, THF then Ac_2O, pyridine (92%); (vii) TMSBr, $CHCl_3$ (qu); (viii) NH_4OH, H_2O; (ix) Biogel P2, NH_4HCO_3, H_2O.

of modular bacterial sialidases on polyvalent substrates through addition of the lectin-domain ligand, in these cases galactose.[12]

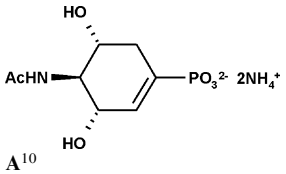
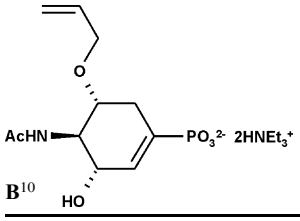
In this paper, we report on the modification of the side chain of previously synthesized cyclohexene-phosphonate **3** (Fig. 2),[10] and its impact on the inhibition of three bacterial sialidases.

2. Synthesis

Starting from **3**, we dihydroxylated[13] the double bond of the allyl substituent selectively to afford a 1:1 mixture of diastereomeric diols **5 h/l** which were acetylated to give **6 h/l** but no attempt to separate them was made (Fig. 3). Removal of the triisopropylsilyl group with tetrabutylammonium fluoride followed by reduction of the azide with hydrogen sulfide and subsequent acetylation led to **7 h/l**.[14] Deprotection was achieved by conversion of the ethyl phosphonate into the corresponding trimethylsilyl ester, hydrolysis with aqueous ammonia and purification of the ammonium phosphonate on Biogel P2 to give **1 h/l**[15] in quantitative yield (Fig. 3).

In order to resemble the glycerol side chain of neuraminic acid more closely in length, the dihydroxypropyl substituent had to be converted into a hydroxyethyl

Table 1. Inhibition of three bacterial sialidases by **1 h/l**[a] and **2** and comparison with previously synthesized compounds **A** and **B**. IC_{50} values are given in M[b]

Compd	Sialidase from		
	V. cholerae	*S. typhimurium*	*C. perfringens*
 **A**[10]	1.8×10^{-3}	1×10^{-3}	n.a.
1 (h/l)	$>4\times10^{-3}$	$>4\times10^{-3}$	n.a.
2	1.6×10^{-3}	1.8×10^{-3}	n.a.
 **B**[10]	1.5×10^{-3}	$<2\times10^{-3}$	n.a.

[a] A 1:1 mixture of diastereomers was investigated.
[b] Values are means of three experiments with a deviation of less than 20%; n.a., not active in the concentration range tested (0.01–10 mM).

moiety. This was achieved by glycol cleavage of **5 h/l** with sodium metaperiodate and sodium borohydride reduction of the resulting aldehyde to give **8** which was acetylated to afford **9** for the purpose of structure confirmation (Fig. 4). Conversion of the azide into the acetamide led to **10** the triisopropylsilyl group of which was removed and the hydroxyl group acetylated to yield the protected target molecule **11**.[16] Cleavage of all ester functions as described above followed by a gel filtration step gave target cyclohexenephosphonate **2**[17] in good yield (Fig. 4).

3. Sialidase inhibition

Inhibition by **1 h/l** and **2** alongside with previously reported[9,10] molecules **A** and **B** of three commercially available bacterial sialidases (IC_{50} values) was determined as described previously (Table 1).[10,18]

V. cholerae sialidase: The enzyme was inhibited by hydroxyethyl-substituted cyclohexenephosphonate **2** and the allyl-derivative **B** in the same order of magnitude as the by the parent compound **A**. An increase in side-chain length resulted in decreased binding although this must be viewed with caution as **1 h/l** was investigated as mixture of diastereomers.

Salmonella typhimurium sialidase: The results were similar to the *V. cholerae* enzyme and indicated that there is some tolerance towards side-chain modification, a fact we try to exploit in our efforts to obtain optimized inhibitors.[19]

Clostridium perfringens sialidase: Our previous finding[10] that cyclohexenephosphonate **A**, which has a double bond orientation comparable to inhibitors DANA[20] or GG 167, does not inhibit the *C. perfringens* sialidase was confirmed by the inability of **1 h/l**, **2** and **B** to inhibit the enzyme.

Among these three enzymes, the *C. perfringens* sialidase seems to be the most selective regarding the double bond orientation in our cyclohexenephosphonates as the corresponding GS 4107 analogous molecules do bind significantly.[10] The only exception we found so far was a monoester,[10] this is not surprising as it is known that the *C. perfringens* sialidase is inhibited effectively by C-glycosides of neuraminic acid carrying hydrophobic aglycones.[21] The combination of a GS 4071-analogous double bond orientation and hydrophobic phosphonate monoesters is currently being investigated in our laboratory.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Mr. C. Röhrig for his help in recording the mass spectra.

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14. Selected physical data for **7 h**/**l**: $R_f = 0.2$ (ethyl acetate/methanol, 10:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.40 (d, 1H, $J_{2,P} = 21.3$ Hz, H-2), 5.69 (m, 1H, NH), 5.56 (m, 1H, H-3), 5.12 (m, 1H, H-2′), 4.43 and 4.20 (2m, 1H, H-3′a), 4.30 and 4.20 (2m, 1H, H-3′b), 4.16 (m, 1H, H-4), 4.16–4.02 (m, 6H, 2 CH_2CH_3), 3.73 and 3.45 (2m, 1H, H-1′a), 3.72 and 3.53 (2m, 1H, H-1′b), 3.67 (m, 1H, H-5), 2.82 (m, 1H, H-6a), 2.26 (m, 1H, H-6b), 2.08–1.99 (4s, 12H, 4 $COCH_3$), 1.36–1.32 (m, 6H, 2 CH_2CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 138.5 (C-2), 75.1 (C-5), 71.8 (C-3), 69.9 (C-2′), 53.9 (C-4), 30.1 (C-6). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ 16.90 (s, 1P, $P(O)(OEt)_2$). $C_{21}H_{34}NO_{11}P$ (M 507.48). MALDI-MS (pos. mode, DHB): 545.9 $(M+K)^+$, 529.9 $(M+Na)^+$, 507.9 $(M+H)^+$.
15. Selected physical data for **1 h**/**l**: ^{1}H NMR (600 MHz, D_2O): δ 5.88 (d, 1H, $J_{2,P} = 17.7$ Hz, H-2), 4.09 (m, 1H, H-3), 3.87 (m, 1H, H-2′), 3.62, 3.44, 3.00, 2.86 (4m, 4H, H-1′a, H-1′b, H-3′a, H-3′b), 3.72 (m, 1H, H-4), 3.42 (m, 1H, H-5), 2.67 (m, 1H, H-6a), 2.09 (m, 1H, H-6b), 1.91 (s, 3H, $NHCOCH_3$). ^{13}C NMR (150.9 MHz, D_2O): δ 130.1 (C-2), 76.2 (C-5), 70.3 (C-3), 70.3, 65.7, 41.3 (C-1′, C-2′, C-3′), 56.2 (C-4), 30.4 (C-6). ^{31}P NMR (242.9 MHz, D_2O): δ 12.34 (s, 1P, PO_3^{2-}). $C_{11}H_{20}NO_8P$ (M 325.25) MALDI-MS (pos. mode, DHB): 347.1 $(M+Na)^+$, 325.1 $(M+H)^+$.
16. Selected physical data for **11**: $R_f = 0.25$ (ethyl acetate/methanol, 10:1). $[\alpha]_D^{20} = 29$ (c 0.2, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.38 (d, 1H, $J_{2,P} = 21.1$ Hz, H-2), 5.59–5.54 (m, 2H, H-3, NH), 4.26, 4.11, 3.78, 3.65 (4m, 4H, H-1′a, H-1′b, H-2′a, H-2′b), 4.12–4.09 (m, 4H, 2 CH_2CH_3), 4.11, (m, 1H, H-4), 3.74 (m, 1H, H-5), 2.80 (m, 1H, H-6a), 2.29 (m, 1H, H-6b), 2.08–1.98 (4s, 12H, 4 $COCH_3$), 1.35–1.32 (m, 6H, 2 CH_2CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 138.5 (C-2), 74.8 (C-5), 71.7 (C-3), 66.9, 63.2 (C-1′, C-2′), 53.9 (C-4), 30.6 (C-6). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ 16.90 (s, 1P, $P(O)(OEt)_2$). $C_{18}H_{30}NO_9P$ (M 435.41). MALDI-MS (pos. mode, DHB): 473.8 $(M+K)^+$, 457.8 $(M+Na)^+$, 435.8 $(M+H)^+$.
17. Selected physical data for **2**: ^{1}H NMR (600 MHz, D_2O): δ 5.91 (d, 1H, $J_{2,P} = 18.1$ Hz, H-2), 4.09 (d, 1H, $J_{3,4} = 9.0$ Hz, H-3), 3.73 (dd, 1H, $J_{4,5} = 9.0$ Hz, H-4), 3.60–3.50 (m, 4H, H-1′a, H-1′b, H-2′a, H-2′b), 3.47 (m, 1H, H-5), 2.67 (m, 1H, H-6a), 2.09 (m, 1H, H-6b), 1.91 (s, 3H, $NHCOCH_3$). ^{13}C NMR (150.9 MHz, D_2O): δ 174.2 ($NHCOCH_3$), 135.4 (d, $J_{1,P} = 165$ Hz, C-1), 131.0 (C-2), 75.9 (C-5), 71.0 (C-3), 70.2, 60.6 (C-1′, C-2′), 56.8 (C-4), 31.2 (C-6), 21.7 ($NHCOCH_3$). ^{31}P NMR (242.9 MHz, D_2O): δ 12.33 (s, 1P, PO_3^{2-}). $C_{10}H_{18}NO_7P$ (M 295.23) MALDI-MS (pos. mode, DHB): 318.1 $(M+Na)^+$, 296.1 $(M+H)^+$.
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