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A Sulfonozanamivir Analogue has Potent Anti-influenza Virus Activity

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Abstract: Influenza virus infection continues to cause significant, often severe, respiratory illness worldwide. A validated target for development of anti-influenza agents is the viral surface protein sialidase. In the current study, we have discovered a highly potent inhibitor of influenza virus sialidase, based on a novel sialosyl sulfonate template. The synthesised 3-guanidino sialosyl α -sulfonate, a sulfonozanamivir analogue, inhibits virus replication *in vitro* at a nanomolar level, comparable to that of anti-influenza drug zanamivir. Using protein X-ray crystallography we show that the sialosyl α -sulfonate template binds within the sialidase active site in a 1C_4 chair conformation. The C1-sulfonate moiety forms crucial and strong-binding interactions with the active site's triarginyl cluster, while the 3-guanidino moiety interacts significantly with conserved active site residues. This sulfonozanamivir analogue provides a new direction in anti-influenza virus drug development.

Influenza virus is a significant human pathogen, causing annual epidemics, and the threat of worldwide pandemics from strains significantly different to those already encountered by the human immune system.^[1-3] A number of influenza virus proteins have been investigated for anti-influenza drug development,^[4-6] with the viral surface protein sialidase (neuraminidase, NA) recognised as a validated target.^[7,8] Inhibition of the viral sialidase hinders the release of new virus progeny from an infected host cell, thereby mitigating virus spread.^[8] Structure-based design against the sialidase in the 1990s led to the development of the influenza virus-specific sialidase inhibitor drug zanamivir (**1**, Relenza[®]) and subsequently oseltamivir carboxylate (the active form of the drug Tamiflu[®]).^[7] Emergence of in particular oseltamivir-resistant virus strains,^[3,9,10] however, provides impetus for ongoing development of new anti-influenza therapeutics,^[5] including new sialidase inhibitors.^[11,12]

Both zanamivir (**1**) and oseltamivir carboxylate were developed on mono-unsaturated six-membered ring core templates, to mimic a transition-state intermediate of the sialidase catalytic mechanism.^[7] Zanamivir was based on Neu5Ac2en **2**, an unsaturated analogue of the natural sialidase substrate, oseltamivir carboxylate on a cyclohexene ring. Subsequently it was postulated^[13,14] that the central ring of the inhibitor needs 'simply' to appropriately position key functional groups to interact strongly with conserved amino acid residues in four pockets of the sialidase active site, a theory validated in the development of the potent sialidase inhibitor drug peramivir, on a cyclopentane template.^[13]

Key among the interacting substituents is a charged group, represented by the carboxylate of the natural substrate, and of **1**, which contributes significantly ($\geq 50\%$ of the interaction energy^[15]) to binding of the substrate or inhibitor to the enzyme active site. We,^[16] and others,^[17] have recently reported that the sialosyl α -sulfonate **4**, with a central, saturated pyranose ring, and in which the natural carboxylate is replaced by a sulfonate group, is an inhibitor of both viral^[16] and bacterial^[17] sialidases. The incorporation of the sulfonate as the acidic group led to significantly greater inhibitory potency when compared to the corresponding carboxylate^[16] and phosphonate^[17] congeners. Sulfonate derivative **4** showed micromolar level inhibition of influenza virus sialidase activity and viral infection *in vitro*, and was more effective than the benchmark unsaturated sialidase inhibitor Neu5Ac2en **2**.^[16] The sialidase inhibitory activity produced by **4** led us to explore the sialosyl α -sulfonate template for development of new, high-potency influenza virus sialidase inhibitors. Herein, we report the elaboration of this template through to the sulfonozanamivir analogue (Figure 1), and provide biological and structural evidence of strong binding interactions of this new class of inhibitor with the sialidase.

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Figure 1. Sialidase inhibitors: zanamivir (4-deoxy-4-guanidino-Neu5Ac2en) **1**, Neu5Ac2en **2**, 4-amino-4-deoxy-Neu5Ac2en **3**, and sialosyl α -sulfonate **4**.

Scheme 1. Synthesis of 3-amino sialosyl sulfonates **12**, and **13**. *Reagents and Conditions:* a) NIS, NaOAc, AcOH, rt, 1.5 h; b) i. Bu_3SnH , 1,4-dioxane, AIBN, 65 °C, 6 h; ii. benzyl chloroformate, NaHCO_3 , $\text{CHCl}_3/\text{H}_2\text{O}$, rt, 2 h; c) i. NaOH 1M, $\text{MeOH}/\text{H}_2\text{O}$, 0 °C, o/n; ii. Ac_2O , py, DMAP, rt, o/n; d) MW, 160 °C, 3 min; e) BzSH, $\text{BF}_3\text{Et}_2\text{O}$, CH_3CN , rt, 2 h; f) H_2O_2 , AcOH, NaOAc, 6 h, 50 °C; g) i. NaOMe, MeOH, 0 °C-rt, o/n; ii. H_2 , 10% Pd/C, $\text{MeOH}/\text{H}_2\text{O}$, rt, o/n.

The development of zanamivir (**1**)^[7,18] centred on structure-guided optimisation of the C-4 substituent on the Neu5Ac2en (**2**) template, to engage strong binding interactions with conserved negatively-charged residues in the influenza virus sialidase active site. Replacement of the C-4 hydroxyl group of **2** with an amino group (giving 4-amino-4-deoxy-Neu5Ac2en **3**), and subsequently with the larger, more basic guanidino group (giving **1**), led to exceptional enhancement of binding affinity and sialidase inhibitory activity.^[18] This sequence of modifications was therefore introduced on the new sialosyl sulfonate template.

Synthetic feasibility led us to choose readily accessible^[19] 4-azido-4-deoxy-Neu5Ac2en derivative **5** as the precursor for the nitrogen-substituted sialosyl sulfonates. The synthetic route began with electrophilic addition across the 2,3-double bond of **5** using NIS/AcOH,^[20] leading to **6** as a single isomer (Scheme 1). Reduction of both the C-4 azide and C-3 iodide of **6** using Bu_3SnH ,^[21] followed by protection of the resulting C-4 amine as the benzyl carbamate, afforded derivative **7** in excellent yield.

Based on our previously reported method of microwave (MW)-mediated decarboxylation,^[16] the synthesis of glycosyl acetate derivative **9** was achieved through a three step reaction sequence. Saponification of the ester groups of **7**, followed by global reintroduction of the acetates under standard conditions ($\text{Ac}_2\text{O}/\text{py}$)^[22] yielded derivative **8**, which was then subjected to MW irradiation to give **9** as an anomeric mixture (α/β 1:5).

Conversion of the glycosyl acetate **9** into the corresponding thioacyl derivative, was initially carried out following our previously reported procedure using thioacetic acid under Lewis acid catalysis in CH_3CN .^[16] In contrast to reaction on the C-3 acetate derivative,^[16] in the presence of the C-3 carbamate, formation of the axial β -thioacetate was found to predominate (α/β 1:7). As optimal sialidase inhibition was achieved with the equatorially-substituted α -sulfonate **4**,^[16,17] alternative reaction conditions and reagents were investigated towards improving the α -stereoselectivity. The best result was achieved through reaction with thiobenzoic acid, giving glycosyl thiobenzoate **10** with a more favourable 1:2 α/β ratio.

Oxidation of the thiobenzoyl moiety of **10**, by treatment with hydrogen peroxide in acetic acid at 50 °C, afforded the glycosyl sulfonate **11** in 78% yield. From this point, a two-step deprotection of the sulfonate mixture **11**, involving hydrogenolysis of the benzyl carbamate with Pd/C, and subsequent Zemplén deacetylation, gave the target 3-amino β - and α - sulfonate derivatives, **12** and **13**, in 98% combined yield.

Introduction of the guanidino moiety at C-3 on the sialosyl sulfonate template was next achieved through removal of the benzyl carbamate of **11** and subsequent reaction of the resulting free amine with *N,N*-di-Boc-1H-pyrazole-1-carboxamide in the presence of Et_3N (Scheme 2). This sequence provided the protected 3-guanidino sialosyl sulfonates **14** in 73% yield over two steps. Finally, Zemplén deprotection, and acidolysis of the Boc groups gave the target 3-guanidino β - (**15**) and α - (**16**) sialosyl sulfonates in 84% combined yield.

Scheme 2. Synthesis of 3-guanidino sialosyl sulfonates **15**, and **16**. *Reagents and Conditions:* a) i. H_2 , 10% Pd/C, $\text{MeOH}/\text{H}_2\text{O}$, AcOH, rt, o/n; ii. *N,N*-di-Boc-1H-pyrazole-1-carboxamide, MeOH, Et_3N , 72 h, rt; b) i. NaOMe, MeOH, 0 °C-rt, 40 h; ii. HCl 1M, H_2O , rt, 12 h.

The 3-amino (**12**, **13**) and 3-guanidino (**15**, **16**) sialosyl sulfonate derivatives were evaluated for their ability to inhibit the activity of a range of influenza A virus type N1 and N2 sialidases. Fifty percent inhibitory concentration (IC_{50}) values were determined by fluorometric assay^[16,23] using 2- α -(4-methylumbelliferyl) *N*-acetyl-D-neuraminic acid^[24] (MUN) as substrate, with purified virus or recombinant protein as the source of enzyme. In line with our findings for the 3-hydroxy sialosyl β -sulfonate,^[16] the 3-*N*-substituted β -sulfonate derivatives **12** and **15** were not inhibitory at 1 mM and 1 μ M, respectively (data not shown).

The 3-amino α -sulfonate derivative **13** inhibited influenza virus sialidases with potencies in the range of 3.2 to 18.9 μ M (Table 1). We observed no improvement in potency against the N2 sialidase upon replacement of the 3-hydroxy moiety of **4** with the 3-amino moiety as displayed in **13** (IC_{50} 2.0 $\pm$ 0.3 μ M;^[16] **13** IC_{50} 3.2 $\pm$ 1.7 μ M). The 3-guanidino α -sulfonate derivative **16**, however, showed very strong activity against influenza A virus sialidases with potencies of 7.9 to 65.2 nM. The addition of the guanidino moiety at the C-3 position of the sialosyl sulfonate significantly improved activity over the amino derivative. This is in line with the increased potency seen on the unsaturated Neu5Ac2en template^[18] when replacing the C-4 amino group (**3**) with the guanidino group (**1**) (Table 1), but the increase in potency is of greater magnitude on the new sialosyl sulfonate template. Importantly, the oseltamivir-resistant N1 (H274Y) sialidase was completely susceptible to inhibition by both the 3-amino **13** and 3-guanidino **16** α -sulfonates.

Table 1. Inhibitory activity (IC_{50} , nM)^[a] of sialosyl α -sulfonate derivatives **13** (3-amino) and **16** (3-guanidino), and the corresponding Neu5Ac2en derivatives **3** and **1**, against influenza A virus and human sialidases.

Sialidase ^[b]	13	16	3	1 (zanamivir)
N2 (Perth) ^[c]	3245 $\pm$ 1678	19.9 $\pm$ 13.4	443.6 $\pm$ 23.1	5.1 $\pm$ 0.8
N1 (Hong Kong) ^[c]	18907 $\pm$ 8620	65.2 $\pm$ 5.8	n.d.	1.0 $\pm$ 0.04
N1 (Hiroshima) ^[c]	9572 $\pm$ 1902	12.8 $\pm$ 3.6	n.d.	1.0 $\pm$ 0.04
N1 (H274Y) ^[c]	17903 $\pm$ 5111	7.9 $\pm$ 1.0	n.d.	1.7 $\pm$ 0.6
N1 (pdm California) ^[d]	16926 $\pm$ 2571	18.4 $\pm$ 3.3	586.9 $\pm$ 58.3	1.0 $\pm$ 0.2
Human Neu2 ^[d,e]	> 1 mM	> 1 mM	n.d.	49300 $\pm$ 300

[a] Sialidase activity was measured using a fluorometric enzymatic assay with MUN as substrate. Compounds and virus or recombinant enzyme were co-incubated for 20 min at room temperature before addition of substrate. Reactions were incubated for 20 min at 37 °C. Inhibition values for influenza virus sialidases are the mean $\pm$ SD of triplicate experiments ($n = 3$). n.d. = not determined. [b] N2 (Perth): A/Perth/16/2009 (H3N2); N1 (Hong Kong): A/Hong Kong/1870/2008 (H1N1); N1 (Hiroshima): A/Hiroshima/48/2008 (H1N1); N1 (H274Y): A/Anhui/1/2005 (H5N1–H274Y); N1 (pdm California): A/Columbia/04/2009 (H1N1). [c] Whole virus particles used as the source of sialidase. [d] Recombinant enzyme. [e] Results are mean $\pm$ SD of duplicate experiments ($n = 2$).

Importantly, neither of the α -sulfonate derivatives (**13**, **16**) showed inhibitory activity against the representative human sialidase Neu2 (Table 1). This is in contrast to sialidase inhibitors based on the unsaturated Neu5Ac2en template which are known to inhibit human sialidases.^[25] For example, zanamivir **1** showed micromolar inhibition of Neu2, in line with activities previously reported.^[25]

To validate the results obtained in the enzyme inhibition assay, sialosyl α -sulfonate derivatives **13** and **16** were also evaluated for their inhibitory effect on influenza A H3N2 (Perth) virus *in vitro* infection of MDCK-II cells (Figure 2). IC_{50} values were determined by immuno-detection of the viral nucleoprotein (NP) in infected cells, and compounds were tested at post-adsorption stage. The 3-amino derivative **13** had a potency of 47.2 ± 21.9 nM against influenza virus in cell-based assay, a 69-fold improvement compared to its activity in the enzymatic assay. The 3-guanidino α -sulfonate derivative **16**, inhibited *in vitro* infection of influenza virus with a high potency of 5.0 ± 2.1 nM, in the same order of magnitude as zanamivir **1** ($IC_{50} = 2.4 \pm 0.8$ nM).

Figure 2. *In vitro* potency of sialosyl α -sulfonate derivatives **13** and **16** against influenza A virus H3N2 (Perth) infection of MDCK-II cells. Dose-dependent inhibition at post-adsorption stage of influenza A/Perth/16/2009 H3N2 virus infection of MDCK-II cells by 3-amino α -sialosyl sulfonate **13**, 3-guanidino α -sialosyl sulfonate **16** and zanamivir **1**. Cells and virus were incubated for 1 h at 35 °C, after which the inoculum was replaced with compound dilutions. Cells were further incubated for 24 h at 35 °C. Infection was measured by *in situ* ELISA, by immuno-staining of the influenza virus NP in infected cells. Data points represent the mean $\pm$ SD of 3 independent experiments ($n = 3$).

Crystallographic studies were carried out to determine the binding mode of 3-amino **13** and 3-guanidino **16** α -sulfonate derivatives in complex with N2 sialidase (H3N2 A/Perth/16/2009). Preformed apo N2 crystals soaked in a 5 mM solution of inhibitor for 48 hours produced N2–inhibitor complexes for each compound, with structure resolution of 2 Å. In both complexes, the inhibitor's central pyranose ring was found in a 1C_4 chair conformation (Figure 3: a, N2–**16**; b, N2–**13**). As we had anticipated,^[16] the anionic sulfonate moiety at C-1 shows extensive electrostatic interaction with the triarginyl cluster (Arg118, Arg292, Arg371).

The C-4 acetamido substituent makes interactions through its amide NH and carbonyl group, and is oriented towards a hydrophobic pocket that nicely accommodates its methyl group, consistent with other sialic acid derivatives in complex with influenza virus sialidases.^[26,27] The C-7 and C-8 hydroxyl groups of the glycerol side-chain are engaged in a bidentate interaction

with the carboxylate of Glu276 that is also observed in influenza virus sialidase–sialic acid derivative complexes,^[26,27] while the terminal C-8 hydroxyl group makes a hydrogen bond with Arg224 (NE).

In each N2–inhibitor complex, the C-3 substituent makes electrostatic and hydrogen bond interactions with negatively-charged residues of the active site. In the N2–3-amino sulfonate **13** complex (Figure 3b), both salt bridge and hydrogen bond interactions are made to the side-chain carboxylate groups of Glu119 and Asp151. In the 3-guanidino sulfonate **16**–N2 complex (Figure 3a), all three of the guanidinyll nitrogens are involved in binding with key active site residues. The interactions that are additional to those seen for the 3-amino group – hydrogen bonds to Glu227 and to the backbone carbonyl group of Asp151, and a bidentate interaction between the two terminal amines and the backbone carbonyl group of Trp178 (Figure 3c and Supplementary Table 2) – provide a rationale for the 160-fold stronger inhibitory potency of **16** compared to **13** against the N2 sialidase (Table 1).

Comparison of the N2–**16** complex and the A/R1/5+/1957(H2N2) N2–zanamivir (**1**) complex (PDB: 3TIC) revealed highly similar binding modes for **16** and zanamivir, with **16** engaging all of the important active site residues as previously reported for zanamivir^[27] (Figure 3d). The sulfonate anionic moiety at C-1 of **16** forms electrostatic interactions with the triarginyl cluster in a similar manner to the C-1 carboxylate of zanamivir, although the interactions between Arg118 and the C-1 carboxylate of zanamivir (bond distances: 2.8 Å and 3.1 Å) are slightly stronger compared to the sulfonate group of **16** (3.1 Å and 3.3 Å) (Supplementary Table 1).

In summary, in the current study we have demonstrated that the inhibitory potency of our reported^[16] sialosyl sulfonate template can be significantly enhanced through appropriate functional group modification. We exploited opportunities for binding in a predominantly negatively-charged region of the influenza virus sialidase active site, by introducing 3-amino and 3-guanidino substituents on this new inhibitor template. Both of the α -configured sulfonates, amino derivative **13** and guanidino derivative **16**, show significant inhibition of influenza virus sialidase activity.

Crystallographic studies of **13** and **16** have confirmed the binding mode of the sialosyl sulfonate template, showing that the saturated pyranose template in its ¹C₄ chair conformation is capable of accurately positioning substituents to interact with the key, conserved, residues of the influenza virus sialidase active site. As a consequence, 3-guanidino α -sulfonate **16** binds tightly within the active site, exhibiting virtually identical interactions to those of the anti-influenza drug zanamivir **1**. This provides potent nanomolar inhibition of both viral sialidase activity and viral replication. Moreover, the sulfonozanamivir analogue appears to be very selective for influenza virus sialidase, showing no inhibition of the mammalian sialidase Neu2 at a concentration 10⁵-fold greater than that at which viral replication is inhibited.

Thus, we have developed a potent novel inhibitor of influenza virus sialidase that efficiently blocks viral replication *in vitro*. We have detailed the binding mode of the sulfonozanamivir analogue through structural studies that now provides further opportunity for lead optimisation.

Figure 3. Crystal structures of influenza A virus N2 (Perth) sialidase in complex with sialosyl α -sulfonate derivatives **13** and **16**. (a) N2 (Perth) (cyan) in complex with 3-guanidino α -sulfonate **16** (green) (PDB: 6BR5). (b) N2 (Perth) (magenta) in complex with 3-amino α -sulfonate **13** (green) (PDB: 6BR6). The electron density maps of the inhibitors (2mFo-DFc, grey mesh) are contoured at 1.5 σ . (c) Comparison of the binding modes of 3-amino sulfonate **13** (magenta) and 3-guanidino sulfonate **16** (cyan) in complex with N2 (Perth). (d) Comparison of the binding modes of 3-guanidino sulfonate **16** complexed with N2 (Perth) (cyan) and zanamivir **1** complexed with A/R1/5+/1957(H2N2) N2 (yellow) (PDB: 3TIC). In panels c and d, interactions between ligands and residues of the sialidase active site of ≤ 3.6 Å are shown as dashed lines in the same colour as the carbon of each ligand. In panels c and d, residue E227, which interacts with the guanidino groups of **16** and zanamivir **1**, is omitted for clarity. N2 (Perth): A/Perth/16/2009.

Experimental Section

Synthetic procedures, spectral characterisation, biological assays and crystallographic details are provided in the Supporting Information.

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Keywords: influenza virus • sialic acid • antiviral agent • sialidase • sulfonate

References:

- [1] C. W. Potter, *J. Appl. Microbiol.* **2001**, *91*, 572-579.
- [2] T. Horimoto, Y. Kawaoka, *Nature Rev. Microbiol.*, **2005**, *3*, 591-600.
- [3] M. Zambon, *Curr. Opin. Infect. Dis.* **2014**, *27*, 560-565.
- [4] K. Das, *J. Med. Chem.* **2012**, *55*, 6263-6277.
- [5] F. G. Hayden, *Influenza Other Respir. Viruses* **2013**, *7*, 63-75.
- [6] M. G. Ison, *Expert Rev. Anti Infect. Ther.* **2015**, *13*, 417-425.
- [7] M. von Itzstein, *Nat. Rev. Drug Discov.* **2007**, *6*, 967-974.
- [8] E. Garman, G. Laver in *Viral Membrane Proteins: Structure, Function, and Drug Design* (Ed.: W. Fischer), Kluwer Academic / Plenum, New York, **2005**, pp 247-267.
- [9] T. G. Sheu, V. M. Deyde, M. Okomo-Adhiambo, R. J. Garten, X. Xu, R. A. Bright, E. N. Butler, T. R. Wallis, A. I. Klimov, L. V. Gubareva, *Antimicrob. Agents Chemother.* **2008**, *52*, 3284-3292.
- [10] C. W. Seibert, S. Rahmat, F. Krammer, P. Palese, N. M. Bouvier, *J. Virol.* **2012**, *86*, 5386-5389.
- [11] J.-H. Kim, R. Resende, T. Wennekes, H.-M. Chen, N. Bance, S. Buchini, A. G. Watts, P. Pilling, V. A. Streltsov, M. Petric, R. Liggins, S. Barrett, J. L. McKimm-Breschkin, M. Niikura, S. G. Withers, *Science*, **2013**, *340*, 71-75.
- [12] C.-K. Cheng, C.-H. Tsai, J.-J. Shie, J.-M. Fang, *Future Med. Chem.* **2014**, *6*, 757-774.
- [13] Y. S. Babu, P. Chand, S. Bantia, P. Kotian, A. Dehghani, Y. El-Kattan, T.-H. Lin, T. L. Hutchison, A. J. Elliott, C. D. Parker, S. L. Ananth, L. L. Horn, G. W. Laver, J. A. Montgomery, *J. Med. Chem.* **2000**, *43*, 3482-3486.
- [14] G. T. Wang, *Expert Opin. Ther. Patents* **2002**, *12*, 845-861.
- [15] N. R. Taylor, M. von Itzstein, *J. Med. Chem.* **1994**, *37*, 616-624.
- [16] Á. Hadházi, M. Pascolutti, B. Bailly, J. C. Dyason, A. Borbás, R. J. Thomson, M. von Itzstein, *Org. Biomol. Chem.* **2017**, *15*, 5249-5253.
- [17] C.-J. Vavricka, C. Muto, T. Hasunuma, Y. Kimura, M. Araki, Y. Wu, G. F. Gao, H. Ohri, M. Izumi, H. Kiyota, *Sci. Rep.* **2017**, *7*, 8239. DOI:10.1038/s41598-017-07836-y
- [18] M. von Itzstein, W. Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. Van Phan, M. L. Smythe, H. F. White, S. W. Oliver, *Nature* **1993**, *363*, 418-423.
- [19] M. von Itzstein, B. Jin, W.-Y. Wu, M. Chandler, *Carbohydr. Res.*, **1993**, *244*, 181-185.
- [20] K. Okamoto, T. Kondo, T. Goto, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 631-636.
- [21] S. Ciccotosto, M. von Itzstein, *Tetrahedron Lett.* **1995**, *36*, 5405-5408.
- [22] A. Marra, P. Sinay, *Carbohydr. Res.* **1989**, *190*, 317-322.
- [23] M. Potier, L. Mameli, M. Bélisle, L. Dallaire, S. B. Melançon, *Anal. Biochem.* **1979**, *94*, 287-296.
- [24] R. W. Myers, R. T. Lee, Y. C. Lee, G. H. Thomas, L. W. Reynolds, Y. Uchida, *Anal. Biochem.* **1980**, *101*, 166-174.
- [25] Hata, K. Koseki, K. Yamaguchi, S. Moriya, Y. Suzuki, S. Yingsakmongkon, G. Hirai, M. Sodeoka, M. von Itzstein, T. Miyagi, *Antimicrob. Agents Chemother.* **2008**, *52*, 3484-3491.
- [26] J. N. Varghese, J. L. McKimm-Breschkin, J. B. Caldwell, A. A. Kortt, P. M. Colman, *Proteins: Struct., Funct., Genet.* **1992**, *14*, 327-332.
- [27] J. N. Varghese, V. C. Epa, P. M. Colman, *Protein Sci.* **1995**, *4*, 1081-1087.

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A highly potent influenza virus sialidase inhibitor has been developed on a novel sialosyl sulfonate template. X-Ray crystallography shows substituents of the sulfonozanamivir analogue interact with key, conserved, residues of the influenza virus sialidase active site, leading to nanomolar level inhibition of both sialidase activity and viral replication.