Dihydropyrancarboxamides Related to Zanamivir: A New Series of Inhibitors of Influenza Virus Sialidases. 1. Discovery, Synthesis, Biological Activity, and Structure−Activity Relationships of 4-Guanidino- and 4-Amino-4*H*-pyran-6-carboxamides

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4-Amino- and 4-guanidino-4*H*-pyran-6-carboxamides **4** and **5** related to zanamivir (GG167) are a new class of inhibitors of influenza virus sialidases. Structure−activity studies reveal that, in general, secondary amides are weak inhibitors of both influenza A and B viral sialidases. However, tertiary amides, which contain one or more small alkyl groups, show much greater inhibitory activity, particularly against the influenza A virus enzyme. The sialidase inhibitory activities of these compounds correlate well with their in vitro antiviral efficacy, and several of the most potent analogues displayed useful antiviral activity in vivo when evaluated in a mouse model of influenza A virus infection. Carboxamides which were highly active sialidase inhibitors in vitro also showed good antiviral activity in the mouse efficacy model of influenza A infection when administered intranasally but displayed modest activity when delivered by the intraperitoneal route.

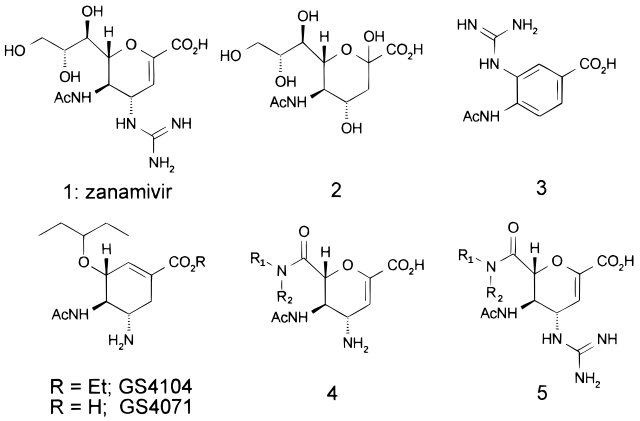
Introduction

Influenza virus is a major cause of respiratory disease and produces significant morbidity and mortality.[1,2] During infection, the highly contagious virus is readily transmitted by aerosol and can spread very rapidly, producing characteristic epidemics of disease. Although widely recognized for many years, there are currently only a few drugs available for influenza treatment. The only licensed existing drugs are the adamantanes, amantadine and rimantadine, which act specifically against influenza A virus by blocking the ion channel of the M2 protein.[3] However, these compounds are not widely used owing to their limited spectrum of activity and adverse side effects and also because of the rapid emergence of resistant virus during treatment. It is therefore apparent that new drugs to treat and control the spread of influenza virus infection are required.

The surface of the influenza virus contains two glycoproteins, hemagglutinin and sialidase, which play key roles in the virus replicative cycle, facilitating the entry and exit of virus from the host cell. It has been postulated that agents which interfere with the function of either of these glycoproteins should have antiviral properties.[4−6]

Zanamivir (GG167, **1**) (Chart 1) is a potent inhibitor of sialidases from both influenza A and B viruses.[7−15]

Chart 1. Sialidase Inhibitor Structures



It displays potent antiviral activity both in vitro and in vivo[16] and is currently undergoing phase III clinical evaluation for the treatment of influenza. It was originally conceived through the application of modeling techniques to the crystal structure of influenza virus sialidase complexed with sialic acid (**2**).[17,18] In several recent papers we and others have described detailed structure−activity relationship (SAR) studies based around zanamivir.[11,15,19−24] This work has demonstrated that each of the four substituents around the dihydropyran ring is critical in the binding of zanamivir and that significant modification or removal of any one of these groups results in a dramatic loss of inhibitory activity. Other groups have reported that **3**, a simple aromatic analogue of zanamivir, is a micromolar inhibitor of influenza virus sialidases. Although the sialidase

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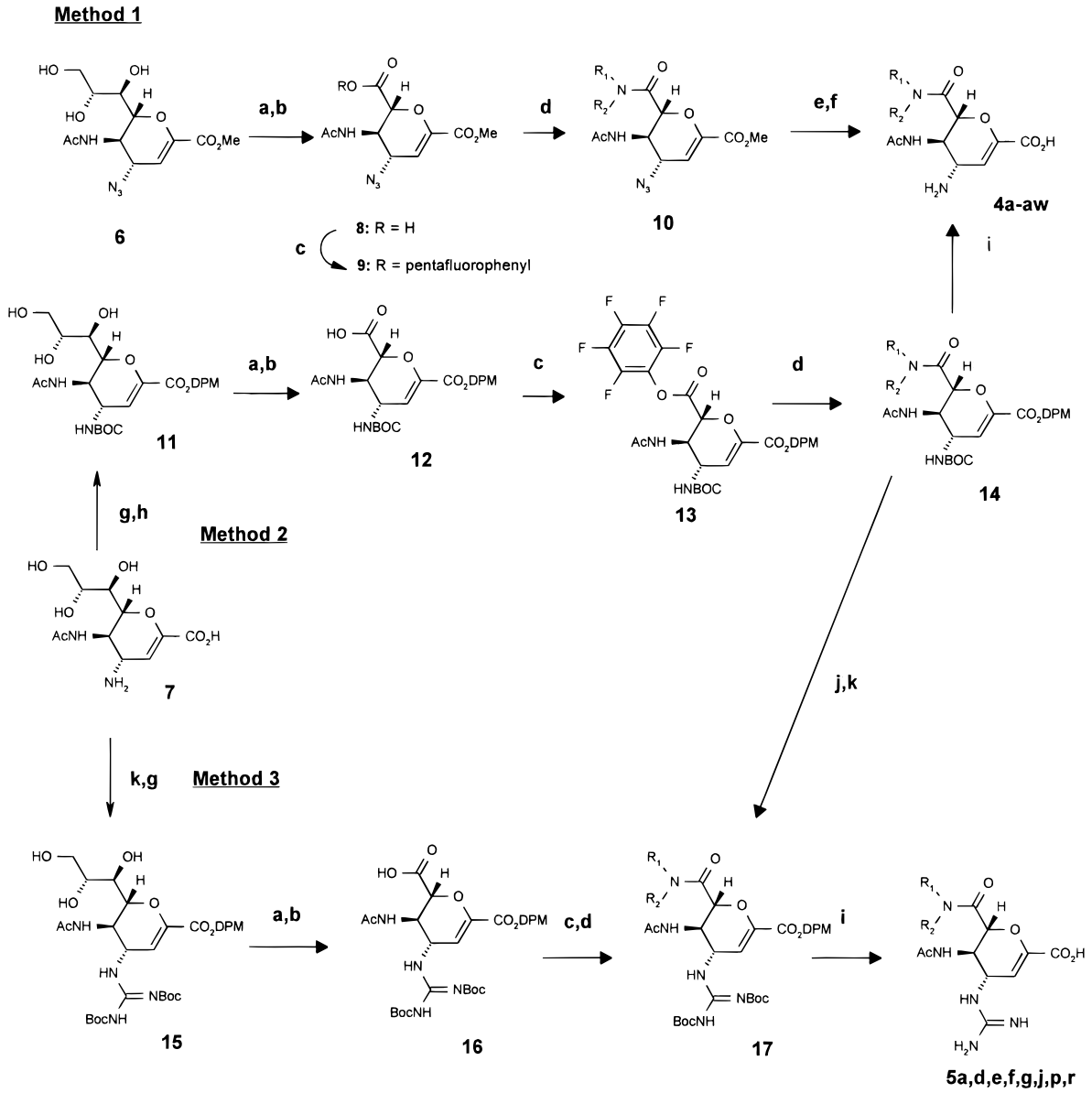
Scheme 1[a]



[a] (a) $NaIO_4$; (b) $NaClO_2$; (c) pentafluorophenyl trifluoroacetate; (d) amine, R_1R_2NH; (e) Ph_3P; (f) Et_3N, aq; (g) Ph_2CN_2; (h) Boc_2O; (i) CF_3CO_2H; (j) HCl dioxane; (k) bisBocPCH.

affinity of **3** is approximately 1000-fold lower than that of zanamivir, the most interesting feature of this compound is that it adopts an alternative binding orientation.[25−27]

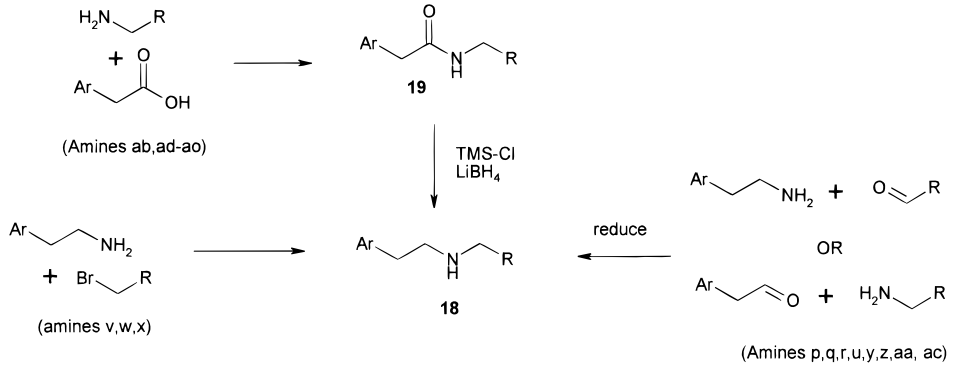
In animal models of influenza virus infection, zanamivir displays excellent antiviral activity when administered intranasally, but it is less effective when delivered systemically. It has very low oral bioavailability (~3% in mice) and is rapidly eliminated from circulation by renal excretion.[9,12] For this reason, there is still a need to identify further influenza virus sialidase inhibitors which may be more effective following systemic administration. Toward this goal, in two recent communications we disclosed a preliminary account of a new series of 4*H*-pyrancarboxamide influenza sialidase inhibitors derived from zanamivir.[28,29] More recently workers from Gilead have claimed GS4104 (the ethyl ester prodrug of GS4071) to be an orally active influenza sialidase inhibitor in mouse models of efficacy.[30,31] Herein we describe further details of our discovery of the carboxamide inhibitors (of general formulas **4** and **5**) and disclose further SAR. In vitro and in vivo antiviral properties of representative compounds of this new series are also reported. The accompanying article describes crystallographic and modeling work which has been used to rationalize the observations herein.

Chemistry

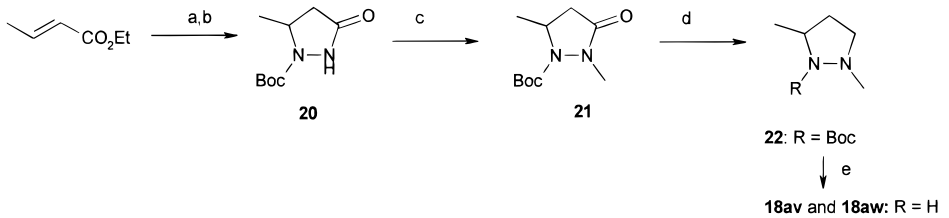
(For simplicity, compound numbers refer to general structures with additional letter codes identifying specific side chains and intermediates involved in their synthesis.)

(a) Synthesis of Precursor Acids and Coupling Reactions. Target compounds **4a**−**aw** and **5a,d**−**g,j,p,r** were prepared by three general methods from protected 4-azido-Neu5Ac2en (**6**) and 4-aminoNeu5Ac2en (**7**) as outlined in Scheme 1 and reported previously.[7,28,29] The initial set of analogues **4a**−**e** was prepared from the azide **6**[11,19] by method 1 via the

Scheme 2



Scheme 3[a]



[a] (a) Hydrazine hydrate, ethanol; (b) $(tBuOCO)_2O$, Na_2CO_3, aq dioxane; (c) MeI, K_2CO_3, DMF; (d) $BH_3 \cdot Me_2S$; (e) HCl, ether.

intermediate acid **8** and amides **10a**−**e**. However, this route was later superseded by methods 2 and 3 starting from compound **7** since in the latter routes all protecting groups could be removed in a single deprotection step. Illustrative procedures for all of these methods are described in the Experimental Section. In all cases amide bond formation was achieved via a conventional coupling reaction at room temperature between the appropriate amine and the pentafluorophenyl ester derived from acid **8**, **12**, or **16**.[32] Direct coupling of acids **8, 12**, and **16** with amines generally produced lower yields of the desired amides under a range of conditions.

(b) Synthesis of Amines 18 for Coupling Reactions. A range of methods used for the preparation of amines for coupling are outlined below and in Schemes 2 and 3. (Amines not specifically mentioned were obtained from commercial suppliers.) Target compounds **4a** and **5a** were prepared from *N*-Boc-ethylenediamine, compound **4c** from glycine *tert*-butyl ester. *N*-(Arylalkyl)-*N*-alkylamines **18ab,ad**−**ao** for the synthesis of targets **4ab**,**ad**−**ao** were made in two stages by coupling the appropriate commercially available alkylamine with the appropriate arylacetic acid followed by reduction of the intermediate amides (**19**) with lithium borohydride and trimethylsilyl chloride (Scheme 2).[33] Amines **18p**−**r,u,y,z,aa,ac** for the preparation of compounds **4p**−**r, 5r, 4u,y,z,aa,ac** were synthesized by reductive amination of a primary amine with propionaldehyde or phenylacetaldehyde. Amines **18v**−**x** for the preparation of compounds **4v**−**x** were prepared by alkylation of phenylethylamine with the appropriate alkyl halide.

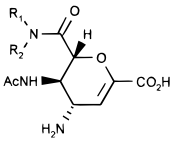
(*R*,*R*)- and (*S*,*S*)-2,5-dimethylpyrrolidine and *cis*-2,5-diethylpyrrolidine for the pyrrolidine amides **4as**−**au** were prepared in a straightforward manner from (2*S*,5*S*)- and (2*R*,5*R*)-hexanediol and 3,6-octanediol,[34] respectively, via initial conversion to the dimesylates followed by cyclization with benzylamine and hydrogenolysis with palladium black and formic acid in methanol. Racemic 1,3-dimethylpyrazolidine (**18av,aw)** for the synthesis of **4av,aw** was prepared from ethyl crotonate by the route outlined in Scheme 3. Thus treatment of ethyl crotonate with hydrazine in ethanol and subsequent treatment with di-*tert*-butyl dicarbonate afforded **20**. Methylation of the pyrrolidinone N-1 nitrogen in **20** produced intermediate **21**. Hydrolysis of the Boc group and borane reduction of **21** then produced the required racemic amine **18av,aw**.

Results and Discussion

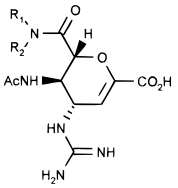
1. Discovery of Carboxamides and Initial Optimization of Activity. Our initial notion which led to the discovery of the carboxamides was to replace the stereochemically complex glycerol side chain of zanamivir (**1**) with a much simpler achiral substituent which could also potentially make interactions in the glycerol binding pocket. We envisaged that such a modification might eventually facilitate the synthesis of analogues by approaches other than from carbohydrate starting materials and would also permit further manipulation of the new 6-substituent to allow modification of the physicochemical properties of new inhibitors. In addition to these thoughts, the preparation of a common intermediate capable of undergoing simple chemical modification would also facilitate rapid exploration of SAR in the glycerol binding pocket through automation of the coupling and deprotection chemistry.

The carboxylic acids **8** and **12** were accessible via conventional carbohydrate methodology and thus were ideal candidates for the above strategy. Having secured the preparation of **8**, we initially decided to individually synthesize the small set of representative amides **4a**−**e** (Table 1). As previous experience had revealed little difference in the SAR patterns between 4-amino and 4-guanidino derivatives, we decided to synthesize the less potent 4-amino derivatives in the first instance since these could be prepared more rapidly. The primary amides **4a,b** contained terminal amino and hydroxyl groups which we envisaged could potentially

Table 1. Sialidase Inhibitory Activities of Initial Carboxamides (IC_{50}, nM)[a]



4



5

			4		**5**	
R_1	R_2	compd	Flu A	Flu B	Flu A	Flu B
$CH_2CH_2NH_2$	H	**a**	>390000		20000	
CH_2CH_2OH	H	**b**	420000			
CH_2CO_2H	H	**c**	>480000			
$CH_2CH_2CH_3$	H	**d**	19000	50000	500	44000
CH_3	CH_3	**e**	2400	61000	25	11000
$CH_2CH_2CH_3$	CH_3	**f**	180	23000	4	4500

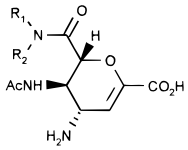
[a] Cf. zanamivir (**1**): Flu A, 5 nM; Flu B, 4 nM.

make interactions with the carboxyl group of Glu-276 in a fashion similar to the 8,9-hydroxyl groups in zanamivir.[20] Amides **4c**−**e** were included to complete a small representative set of targets designed to probe the glycerol binding pocket with a range of different groups. At the outset we anticipated that these latter compounds, which did not contain hydrogen bond donor or charged side-chain amide groups, would have lower sialidase affinity. However, when compounds **4a**−**e** were evaluated as sialidase inhibitors,[8] the propylamide **4d** and dimethylamide **4e** were found to be the most active, while the others produced significantly weaker inhibition (Table 1). This initial surprising set of results prompted us to prepare the propylmethylamide **4f** and also the 4-guanidino analogues **5a,d**−**f**.

Remarkably, our results revealed that compound **5f** had equivalent inhibitory activity to zanamivir against influenza A virus sialidase. However, while its activity against influenza B sialidase had improved (compared to the secondary amide **5d**), the improvement was much less pronounced, and the tertiary amides **5e,f** exhibited approximately 1000-fold selectivity for influenza A virus sialidase.

Compound **5f** represented a highly promising lead molecule for further investigation, and so we set out to rapidly establish the factors responsible for its remarkable activity and selectivity. To achieve preliminary exploration of the SAR for this new series of inhibitors, which clearly had to be interacting with the sialidase in a novel fashion, the synthesis outlined in method 2 (Scheme 1) was utilized in the automated formation of an 80-component library of 4-amino derivatives. The amine set for coupling was made up of a random collection of 40 amines, together with a further 40 selected on the basis of their similarity to methylpropylamine (i.e., other small and lipophilic primary and secondary amines) used for preparing **4f** and **5f**. All the amines used in this exercise were commercially available or selected from our in-house archive. Following the coupling reaction with **13**, the pentafluorophenyl ester of **12**, the crude intermediate amides **14** were deprotected to targets **4** using trifluoroacetic acid. The resulting samples were then evaporated to dryness and tested directly in the sialidase inhibition assay without any purification. (The byproducts from the sequence

Table 2. IC_{50}s (nM) for Pure Compounds Prepared from Initial Library



R_1	R_2	compd	Flu A	Flu B
$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	**4g**	12	2000
$-CH(Me)-(CH_2)_2-CH(Me)-$		**4h**	6	2800
$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$	**4i**	450	>430000
CH_2CH_3	CH_2CH_3	**4j**	3	360
$CHCH{=}CH_2$	$CHCH{=}CH_2$	**4k**	40	8400
cyclohexyl	H	**4l**	19000	>450000

were also prepared and tested separately in the inhibition assay, and they showed no effect on sialidase activity.) The 80 samples were tested at nominal concentrations of 120, 12, 1.2, and 0.12 μg/mL against both influenza A and B virus sialidases (raw data available as Supporting Information). Those samples which showed promising activity were then prepared as pure compounds and retested (Table 2).

Analysis of the results obtained from evaluating the library immediately revealed several activity trends. First, it was apparent that the enhanced inhibitory activity and selectivity for influenza A virus sialidase observed with the compounds **5e,f** was quite general for tertiary amides. Thus, while some of the tertiary amides were highly active against influenza A virus sialidase, none of the 80 analogues were significantly active against influenza B virus sialidase below 5 μg/mL. It was also apparent that exceptional inhibitory activity against influenza A virus sialidase could be achieved even without the requirement for the analogues to possess a 4-guanidino group. Thus the diethylamide **4j**, dipropylamide **4g**, and dimethylpyrrolidine **4h** (isomer mixture) all displayed similar activity to zanamivir against influenza A virus sialidase. Of all the compounds prepared in the initial library, only the cyclohexylamide **4l** proved to be substantially less active when synthesized and isolated pure.

More detailed analysis of the results shows that the two substituents on the carboxamide nitrogen make different interactions with the sialidases. The amide C(O)−N bond is planar, and in the secondary amides the lowest energy conformations in solution occur when the single *N*-alkyl substituent is trans to the dihydropyran ring (i.e., occupies position R_1, cis to the carbonyl oxygen atom). In support of this, solution NMR of the secondary amides in all cases showed only a single rotamer species, and AM1 semiempirical quantum mechanics calculations predict the trans-isomer to be preferred over the cis by 1−2 kcal/mol. (AM1 calculations were performed using Vamp v6.1, available from Oxford Molecular, Oxford, U.K. Energies in solution were computed using a single-point SCRF calculation at the optimized gas-phase geometries.) Since the dramatic improvement in activity against influenza A virus only occurred within a subset of tertiary amides, we therefore deduced that this enhancement in binding was due to the effect of introducing an amide N-substituent into the cis-position relative to the dihydropyran ring (i.e., R_2). AM1 calculations support this view, suggesting that the enhanced binding of the

tertiary amides is due to an increased preference for the observed binding conformation.

For a given *cis*-amide group, further increasing the size of the *trans*-amide substituent did not dramatically affect sialidase inhibitory activity, while the enhancement in influenza A virus inhibitory activity only occurred when the *cis*-amide substituent was small. Thus the dibutyl **4i** and larger amides were less active than the dipropylamide **4g** and diethylamide **4j**. This suggested that there is a size limit for the cis-substituent in achieving optimum sialidase binding.

2. Further SAR and Inhibition of Sialidases from Other Strains of Influenza Virus. Having prepared the library described above and made some preliminary deductions regarding the SAR of the series, we then proceeded to prepare a range of further compounds to investigate these deductions in greater detail. In vitro results from these investigations are summarized in Tables 3−7.

The simple propyl- and ethylamides **4g,j,m−r** and **5g,j,p,r** (Table 3) all displayed extremely potent activity against influenza A virus sialidase with only the benzylpropylamide **4o** showing poor activity. These results confirmed the earlier conclusion from the library study that one of the alkylamide substituents (trans) could be modified extensively without any adverse effect on inhibitory activity. The inhibitory activities against influenza B sialidase were all much lower than against influenza A. Optimum activity occurs against both enzymes when the cis-substituent is an ethyl group, but all the analogues are highly selective.

Table 3. Simple Ethyl- and Propylamides

			enzyme inhibition (IC_{50}, nM)		plaque reduction (IC_{50}, ng/mL)	
R_2	R_1	compd	Flu A	Flu B	Flu A	Flu B
propyl	propyl	**4g**	12	2000	2	700
		5g	2	540	0.1	320
ethyl	ethyl	**4j**	3	360	160	>1000
		5j	1	490	0.1	180
propyl	ethyl	**4m**	3	410	4	280
propyl	butyl	**4n**	4	950	5	>1000
propyl	$PhCH_2$	**4o**	4200	180000		
propyl	$PhCH_2CH_2$	**4p**	2	3600	30	280
		5p	5	840	6	1000
propyl	$PhCH_2CH_2CH_2$	**4q**	18	46000	1	>1000
propyl	$(CH_2)_8Me$	**4r**	23	37000	5	32000
		5r	7	>3000	2	2700

Since the phenethylpropylamide **4p** displayed excellent inhibitory activity, a range of phenethylamides (**4s−ae**) with modified *cis*-amide groups were prepared in order to try and improve activity against the influenza B enzyme (Table 4). In most cases the inhibitory activity of compounds was either little affected or significantly reduced when either the size of the group was increased or polar functionality was introduced. Overall no improvement in activity over the propylamide **4p** was achieved.

Substitution of the phenyl ring in compound **4p** also had little effect on either the sialidase inhibitory activity (compounds **4af−ao**, Table 4) or the selectivity of compounds. However, several compounds with exceptional in vitro antiviral activity in the plaque reduction assay against influenza A were identified in this series (compounds **4ag−ak,am−ao**).

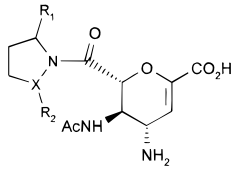
The 2,5-dimethylpyrrolidine **4h** (as a mixture of stereoisomers) had emerged as a lead compound from the initial library. Both the methyl groups on the pyrrolidine appear to be critical for activity since compounds **4aq,ap** are progressively weaker inhibitors of sialidases (Table 5). Synthesis of the individual diastereoisomers of **4h** revealed that the activity was mainly due to the cis-isomer **4ar** with the trans-compounds both displaying much weaker inhibitory activity. Increasing the size of the pyrrolidine substituents from methyl to ethyl (compound **4au**) also significantly reduced activity. The azapyrrolidines **4av,aw** were also less active than **4ar**.

Thus, despite synthesis of numerous carboxamide analogues, the general feature of selectivity for influenza A sialidase occurs consistently throughout the series.

Table 4. 4-Amino Phenethylpropylamides: Aryl Substituents and Cis-Pocket Modifications

			enzyme inhibition (IC_{50}, nM)		plaque reduction (IC_{50}, ng/mL)	
R_{trans}	R_{cis}	compd	Flu A	Flu B	Flu A	Flu B
$PhCH_2CH_2$	H	**4s**	12000	67000	36000	>100000
$PhCH_2CH_2$	methyl	**4t**	320	51000	400	>10000
$PhCH_2CH_2$	ethyl	**4u**	5	8200	2	7500
$PhCH_2CH_2$	propyl	**4p**	2	3600	30	280
$PhCH_2CH_2$	CH_2CO_2H	**4v**	130000	75000	950	11500
$PhCH_2CH_2$	CH_2CONH_2	**4w**	19000	31000		
$PhCH_2CH_2$	$CH_2CH_2NMe_2$	**4x**	>300000	>300000		
$PhCH_2CH_2$	$CH_2CH_2N_3$	**4y**	9	28000	1	23000
$PhCH_2CH_2$	CH_2CH_2OH	**4z**	12	100000		
$PhCH_2CH_2$	CH_2CHMe_2	**4aa**	14	110000	7	
$PhCH_2CH_2$	$CHMe_2$	**4ab**	8	7900		
$PhCH_2CH_2$	$CH_2CH_2NH_2$	**4ac**	47000	260000		
$PhCH_2CH_2$	cyclopropyl	**4ad**	46	36000		
$PhCH_2CH_2$	$CH_2CH_2CH_2OH$	**4ae**	3000	>360000		
X-$PhCH_2CH_2$	propyl					
p-OMe		**4af**	8	3300	3	3600
p-OH		**4ag**	9	1900	0.1	2000
m-OMe		**4ah**	5	3200	0.4	1200
m-OH		**4ai**	3	2300	0.2	2900
p-Ph		**4aj**	2	1500	0.01	1600
o,*p*-di-Cl		**4ak**	6	16000	0.01	3200
p-$CHMe_2$		**4al**	12	6200		
p-CH_2Ph		**4am**	270	5200	<0.1	3100
o-OMe		**4an**	9	12000	0.04	9000
m-Ph		**4ao**	2	4500	0.3	300

Table 5. Pyrrolidines: Activity Resides in Cis-Isomer, Azapyrrolidines



					enzyme inhibition (IC_{50}, nM)		plaque reduction (IC_{50}, ng/mL)	
R_1	R_2	compd	X	pyrrolidine isomer	Flu A	Flu B	Flu A	Flu B
H	H	**4ap**	CH		2900		15000	>100000
Me	H	**4aq**	CH	1:1 *R*:*S*	310	210000	>10000	>10000
Me	Me	**4ar**	CH	cis	5	340	50	230
Me	Me	**4as**	CH	*RR*	110	4700		
Me	Me	**4at**	CH	*SS*	110	53000	17	
Et	Et	**4au**	CH	cis	290	26000	75	
Me	Me	**4av**	NH	isomer 1	210	13000		
Me	Me	**4aw**	NH	isomer 2	52	20000		

Table 6. Activity of Representative Carboxamides vs a Wider Range of Sialidases (IC_{50}, NM)[a]

compd	A/Aichi (N2)	A/Victoria (N2)	A/PR (N1)	A/USSR (N1)	B/Lee	B/Beijing	B/Victoria
4f	180	110	3700	4700	23000	>100000	ND
5f	3	28	5.6	26	4700	13200	4400
4g	3	20	8	30	4600	ND	2000
4h	6	35	1600	120	3400	1600	ND
4i	450	100	11000	5900	>560000	>100000	>560000
4j	5	20	40	85	1600	900	730
4p	2	22	ND	ND	13000	8500	ND
zanamivir (**1**)	2	10	1	2	5	3	2

[a] A/Aichi/2/68; A/Victoria/3/75; A/PR/8/34; A/USSR/90/77; B/Lee/40; B/Victoria/102/85; B/Beijing/1/87. ND, not determined.

Table 7. iv Pharmacokinetics of Selected Carboxamides in Mouse[a]

R_1	R_2	compd	dose (mg/kg)	Clp (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)
propyl	methyl	**5f**	50	17.8	0.3	0.21
propyl	propyl	**4g**	50	12.1	0.4	0.35
		5g	10	16.0	0.5	0.37
nonyl	propyl	**4r**	10	17.9	1.3	0.87
		5r	10	21.8	1.2	0.92
zanamivir		**1**	50	24.9	0.3	0.16

[a] Clp, (plasma clearance, the volume of plasma that can be cleared of drug per unit time; V_{ss}, volume of distribution at steady state, the theoretical volume that the body would have to occupy if the concentration throughout the body were the same as that in the plasma, under steady-state pharmacokinetic conditions; $t_{1/2}$, plasma elimination half-life, the time taken for the plasma concentrations to fall by one-half, once distribution is complete.

Evaluation of several of the carboxamides against a wider range of influenza A and B sialidases also confirmed that this trend is widespread across many isolates (Table 6). A full discussion and explanation of the selectivity of carboxamides for influenza A sialidase is presented in the following article.[37]

3. Pharmacokinetics and in Vivo Efficacy of Carboxamides (Tables 7 and 8). The iv pharmacokinetics of representative amides were measured in the mouse, together with in vivo efficacy in a mouse model of influenza A virus infection. Compounds were administered by both the intranasal (in) and intraperitoneal (ip) routes (detailed protocols for these experiments are outlined in the Experimental Section).

In mice, the highly polar zanamivir is very rapidly cleared from the systemic circulation by renal excretion. The mouse iv pharmacokinetic data for the carboxamides show that, in general, increasing the lipophilicity of compounds increases their serum elimination half-life. This is due to an increase in volume of distribution rather than reduced clearance (Table 7).

Table 8. Efficacy of Selected Carboxamides in the Mouse Model of Influenza Infection (A/Singapore/1/57)

R_1	R_2	compd	dose (mg/kg)	dosing schedule	route	% reduction in virus titer	ED_{90}[d]	*p* value
propyl	methyl	**5f**	1	*a*	in	95.00		ND
			10	*a*	in	99.90	<1	ND
			50	*b*	ip	χ 74.50		≤0.05
propyl	propyl	**4g**	1	*a*	in	82.00		ND
			10	*a*	in	99.84	2.1	ND
propyl	propyl	**5g**	0.1	*a*	in	99.30		ND
			1	*a*	in	99.93	<0.01	ND
			50	*b*	ip	χ 86.00		≤0.01
nonyl	propyl	**4r**	10	*a*	in	96.00	>1	ND
			100	*b*[c]	ip	71.00		ND
nonyl	propyl	**5r**	1	*a*	in	72.00	ND	ND
			10	*a*	in	>99.99		ND
			50	*b*	ip	50.00		≥0.05
ethyl	ethyl	**5j**	50	*b*	ip	89.00	ND	≤0.01
zanamivir		**1**	0.5	*a*	in	99.50	0.03	ND
			50	*b*	ip	χ 96.20		≤0.01

[a] −17 h, b.i.d. ×4. [b] −1, +3, +7 h. [c] Uranyl nitrate not used in this test. [d] Concentration of intranasally administered drug (mg/kg) which reduces virus titer by 90%. χ = mean of two values. ND, not determined.

Following intranasal administration, zanamivir (**1**) and the polar 4-guanidino carboxamides **5f,g** all display useful antiviral activity. At 1 mg/kg all three compounds reduced virus levels by ≥95%. Weaker antiviral activity was observed with **5r**, and **4g,r** which all required intranasal doses of 10 mg/kg in order to achieve similar high reductions in virus titers. When administered by the intraperitoneal route, substantial reductions in virus titers were only observed at much higher doses with the more active and polar carboxamides (**5g**,**j**). Thus, despite modifying the pharmacokinetic properties, none of the carboxamides in this study proved to be more effective than zanamivir at comparable systemic doses.

4. Comparison between Zanamivir and the Dipropylamide 4g. A detailed kinetic evaluation of the dipropylamide **4g** showed it to be a slow-binding inhibitor of influenza A sialidase but a rapid-binding inhibitor of influenza B sialidase. By contrast, zanamivir is a slow-binding inhibitor of both influenza A and B sialidases. The K_i values for **4g** and zanamivir against influenza A sialidase determined in this experiment are very similar (0.53 ± 0.07 and 0.46 nM, respectively). However, despite this similarity in enzyme affinity, the intranasal efficacy of the two compounds differs markedly in a direct comparison of the drug concentration required to reduce viral titers by 90% (ED_{90}, Table 8). Thus, zanamivir achieved this reduction at 0.03 mg/kg, while the dipropylamide **4g** only achieved the same efficacy at 2.1 mg/kg^{-1}. Our present interpretation of this difference in efficacy is that it must reflect a different distribution of the two drugs within the respiratory tract. A lower concentration of **4g** is presumably achieved and maintained at the site of virus replication as compared with zanamivir administered at the same dose.

Conclusion

4-Amino- and 4-guanidino-4*H*-pyran-6-carboxamides **4** and **5** related to zanamivir are a new class of inhibitors of influenza virus sialidases. While secondary amides are weak inhibitors of both influenza A and B virus sialidases, tertiary amides, containing one or more small alkyl groups, are highly potent inhibitors, particularly of the influenza A virus enzyme. The sialidase inhibitory activities of these compounds correlate well with their in vitro antiviral efficacy. Several of the most potent analogues showed good antiviral activity in a mouse efficacy model of influenza A infection when delivered by the intranasal route.

Experimental Section

(i) Chemistry. FTIR spectra were recorded using a Nicolet 20SXB or a Bio-Rad FTS-7 spectrometer. ^{1}H NMR spectra were recorded either at 250 MHz using a Bruker AC or AM 250 spectrometer or at 400 MHz with a Varian VXR 400 spectrometer; values are reported in ppm (δ). Mass spectra were measured on a HP Engine (thermospray positive) or VG Autospec Q (LSIMS) instrument. Routine microanalyses were performed on a Leco CHNS-932 or Carlo-Erba instrument. Water analyses were performed using a Mitsubishi CA-05 instrument. Flash chromatography was performed with Merck Kieselgel 9385. Analytical HPLC was performed using a Rainin C18 8-μM column, eluting with 0.1% aqueous trifluoroacetic acid/acetonitrile at a flow rate of 1 mL/min.

Method 1. (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-amino-6-(propylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid (4d). (a) (2*R*,3*R*,4*S*)-3-(Acetylamino)-4-azido-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic Acid 6-Methyl Ester (8). (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-azido-6-((1*R*,2*R*),3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic acid methyl ester (**6**) (5.2 g, 15.8 mmol) was dissolved in methanol/water (3:1, v/v, 200 mL). To this was added sodium periodate (6.8 g, 31.6 mmol), and the reaction mixture was stirred at 23 °C for 30 min. The solid was removed by filtration, and the filtrate was evaporated in vacuo to give a white solid. The solid obtained by evaporation of the filtrate was suspended in *tert*-butyl alcohol (100 mL) and cyclohexene (10 mL) and stirred rapidly at 23 °C. To this was added a solution of sodium chlorite (10.4 g, 115 mmol) and potassium dihydrogen orthophosphate (10.4 g, 76 mmol) in water (50 mL). After 3 h a pale-yellow solution was obtained which was partitioned between ethyl acetate (150 mL) and water (300 mL). The aqueous layer was acidified to pH 1 with dilute aqueous hydrochloric acid and extracted with further ethyl acetate (5 × 200 mL). The combined organic extracts were dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give the title compound **8** as a white foam (4.5 g, quantitative) which was used directly without further purification: υ_{max} 2097, 1734, 1673 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) 8.21 (1H, d, NH, J = 8.1 Hz), 6.04 (1H, d, H-5, J = 5 Hz), 4.82 (1H, d, H-2, J = 2.5 Hz), 4.39, 4.13 (2H, 2 × m, H-3,4), 3.78 (3H, s, methyl ester), 1.84 (3H, s, CH_3CO).

(b) (2*R*,3*R*,4*S*)-3-(Acetylamino)-4-azido-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic Acid 6-Methyl Ester 2-(2,3,4,5,6-Pentafluorophenyl) Ester (9). Compound **8** (2.9 g, 10.2 mmol) was dissolved in dry dimethylformamide (7 mL), and pyridine (0.9 mL, 11.2 mmol) was added under nitrogen at 23 °C. To this was added pentafluorophenyl trifluoroacetate (1.9 mL, 11.2 mmol). After 24 h the dark-brown reaction mixture was diluted with ethyl acetate (500 mL) and washed with dilute hydrochloric acid (2 × 50 mL), dilute sodium bicarbonate solution (2 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to give the title compound **9** as a brown oil which was recrystallized from ethyl acetate/petroleum ether to afford a light-brown solid (2.2 g, 47%): ^{1}H NMR (250 MHz, DMSO-d_6) 8.67 (1H, d, NH, J = 8 Hz), 6.37 (1H, d, H-3, J = 5 Hz), 5.78 (1H, d, H-6, J = 2.5 Hz), 4.74 (1H, m), 4.48 (1H, m, H-4,5), 3.96 (3H, s, methyl ester), 2.04 (3H, s, CH_3CO); MS 468 (M + NH_4^+), 451 (M + H^+). Anal. Found: C, 42.74; H, 2.81; N, 11.85. $C_{16}H_{11}F_5N_4O_6$ requires: C, 42.68; H, 2.46; N, 12.44.

(c) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-azido-6-(propylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Methyl Ester (10d). To a solution of pentafluorophenyl ester **9** (0.29 g, 0.65 mmol) in dry tetrahydrofuran (3 mL) was added propylamine (0.06 mL, 0.72 mmol), and the reaction mixture was stirred at 23 °C for 1 h. The reaction was diluted with ethyl acetate and washed with dilute hydrochloric acid (2 M, 25 mL) and saturated sodium bicarbonate (25 mL). The organic phase was dried; the solvent was removed in vacuo to give the title compound **10d** as an off-white solid (0.15 g, 64%): υ_{max} 2100, 1734, 1685 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) 6.46 (1H, br t, propyl NH), 6.08 (1H, d, H-3, J = 3.75 Hz), 6.05 (1H, br d, acetamide NH, J = 7 Hz), 4.62 (1H, d, H-6, J = 6.3 Hz), 4.49 (1H, q, H-5), 4.28 (1H, dd, H-4, J = 3.8, 6.3 Hz), 3.87 (3H, s, methyl ester), 3.25 (2H, m), 2.02 (3H, s, CH_3CO), 1.54 (2H, m), 0.92 (3H, t, J = 7.5 Hz). Anal. Found: C, 48.05; H, 5.78; N, 20.80. $C_{13}H_{19}N_3O_5$ requires: C, 48.0; H, 5.9; N, 21.5.

(d) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-amino-6-(propylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (4d). Azide **10d** (0.10 g, 0.31 mmol) and triphenylphosphine (0.08 g, 0.31 mmol) were dissolved in THF (6 mL) and left to stand at 23 °C for 3 h. The solvent was removed in vacuo to afford a light-brown solid. This was dissolved in methanol/water (1:1, 10 mL) and aqueous potassium hydroxide (1 M, 2 mL) added. After 18 h the solution was acidified with dilute aqueous hydrochloric acid and the solvent evaporated. The residue was purified by reverse-phase

preparative HPLC to give compound **4d** (0.06 g, 49%) as a white solid: ^{1}H NMR (250 MHz, DMSO-d_6) 8.28 (1H, t, propylamide NH), 8.25 (3H, br s, NH_3^+), 8.13 (1H, d, acetamide NH, J = 7.5 Hz), 5.87 (1H, d, H-3, J = 2 Hz), 4.47 (1H, d, H-6, J = 9 Hz), 4.15 (1H, q, H-5, J = 9 Hz), 4.08 (1H, m, H-4), 3.11, 2.99 (2H, m, CH_2N), 1.82 (3H, s, CH_3CO), 1.41 (2H, m, CH_2-CH_2N), 0.84 (3H, t, CH_3, J = 7.5 Hz). Anal. ($C_{12}H_{19}N_3O_5$·H_2O·CF_3CO_2H) C, H, N.

Similarly prepared by method 1 were compounds **4a**−**c,e**. Compounds **5a,d,e** were prepared by guanidinylation of **10a**,**d**,**e** respectively, by a method analogous to that described in method 2 below for the preparation of compound **5f** and also from intermediate **7** by method 2.

Method 2. (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-amino-6-(methylpropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (4f). (a) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-[(*tert*-butoxycarbonyl)amino]-6-((1*R*,2*R*),3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Benzhydryl Ester (11). To a suspension of (4*S*,5*R*,6*R*)-5-(acetylamino)-4-amino-6-((1*R*,2*R*),3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic acid trihydrate (**7**) (8.3 g, 24 mmol) in dioxane/water (2:1, v/v, 75 mL) were added sodium bicarbonate (2.6 g, 31 mmol) and di-*tert*-butyl pyrocarbonate (6.8 g, 31 mmol), and the reaction mixture was stirred at 23 °C for 18 h. The resulting solution was acidified to pH 6 using 2 M hydrochloric acid, and to this was added a solution of diphenyldiazomethane in dichloromethane (125 mL of a 0.29 M solution). This was stirred rapidly for 24 h while maintaining the pH at approximately 6 using 2 M hydrochloric acid. The resulting suspension was filtered, and the solid was dried in vacuo to give the title compound **11** (10.9 g, 82%): ^{1}H NMR (250 MHz, DMSO-d_6) 8.13 (1H, d, NH, J = 8 Hz), 7.49−7.25 (10H, m, Ph), 7.15 (1H, d, NH, J = 8.7 Hz), 6.87 (1H, s, CHPh), 6.06 (1H, d, H-3, J = 3 Hz), 5.88 (1H, m), 4.63 (2H, m), 4.47 (1H, m), 4.34 (1H, m), 4.18 (1H, m), 3.91 (1H, m), 3.67 (2H, m), 3.40 (2H, m, H-4,5,6,7,8,9, OH), 1.88 (3H, s, CH_3CO), 1.41 (9H, s, tBu); MS 557 ($M + H^+$), 457 ($M + H^+ -$ Boc). Anal. ($C_{29}H_{36}N_2O_9$·1.5H_2O) C, H, N.

(b) (2*R*,3*R*,4*S*)-3-(Acetylamino)-4-[(*tert*-butoxycarbonyl)amino]-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic Acid 6-Benzhydryl Ester (12). Triol **11** (8.0 g, 14.3 mmol) was dissolved in methanol/water (5:1, v/v, 180 mL). To this was added sodium periodate (6.9 g, 32.2 mmol), and the reaction mixture was stirred at 23 °C for 3 h. The solid was removed by filtration, and the filtrate was evaporated in vacuo to give a white solid. This was suspended in *tert*-butyl alcohol (70 mL) and cyclohexene (10 mL) and stirred rapidly at 23 °C. To this was added a solution of sodium chlorite (10.7 g, 118 mmol) and potassium dihydrogen orthophosphate (10.7 g, 79 mmol) in water (50 mL). After 18 h a pale-yellow solution was obtained which was acidified using 2 M hydrochloric acid. This was extracted into ethyl acetate (3 × 200 mL) and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo, and the residue was triturated with diethyl ether. The solid was collected by filtration and dried to give **12** (5.1 g, 70%): ^{1}H NMR (250 MHz, DMSO-d_6) 13.1 (1H, br s, CO_2H), 7.96 (1H, d, NH, J = 8.7 Hz), 7.48−7.26 (10H, m, PhH), 6.92 (1H, s, $CHPh_2$), 6.83 (1H, d, NH, J = 6.2 Hz), 6.06 (1H, d, H-3, J = 3 Hz), 4.57 (1H, d, H-6, J = 6.2 Hz), 4.28 (1H, m), 4.15 (1H, m, H-4,5), 1.79 (3H, s, CH_3CO), 1.38 (9H, s, tBu); MS 511 ($M + H^+$). Anal. ($C_{27}H_{30}N_2O_8$·0.25H_2O) C, H, N.

(c) (2*R*,3*R*,4*S*)-3-(Acetylamino)-4-[(*tert*-butoxycarbonyl)amino]-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic Acid 6-Benzhydryl Ester 2-(2,3,4,5,6-Pentafluorophenyl) Ester (13). Acid **12** (3.4 g, 6.7 mmol) was dissolved in dry dimethylformamide (10 mL) and pyridine (0.6 g) under nitrogen and stirred at 23 °C. To this was added pentafluorophenyl trifluoroacetate (2.0 g, 7.1 mmol). After 3 h the reaction mixture was diluted with ethyl acetate (250 mL) and washed with dilute hydrochloric acid (3 × 50 mL), dilute sodium bicarbonate solution (3 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to give the title compound **13** as an off-white foam which was used directly without further purification (4.4 g, 97% crude): ^{1}H NMR (250 MHz, DMSO) 8.22 (1H, d, NH, J = 8 Hz), 7.50−7.27 (10H, m, PhH), 7.06 (1H, m, NH), 6.94 (1H, s, $CHPh_2$), 6.18 (1H, d, H-3, J = 3.8 Hz), 5.25 (1H, d, H-6, J = 6.8 Hz), 4.42 (1H, m), 4.29 (1H, m, H-4,5), 1.82 (3H, s, CH_3CO), 1.38 (9H, s, tBu).

(d) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-[(*tert*-butoxycarbonyl)amino]-6-(methylpropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Benzhydryl Ester (14f). To pentafluorophenyl ester **13** (1.18 g, 1.7 mmol) in dry tetrahydrofuran (13 mL) was added *N*-methylpropylamine (0.15 g, 2 mmol), and the reaction mixture was stirred at 23 °C for 4 h. The solvent was removed in vacuo, and the residue was chromatographed over silica gel (ethyl acetate eluant). The required fractions were combined, and the solvent was removed in vacuo to give the title compound **14f** as a white foam (0.85 g, 88%): ^{1}H NMR (250 MHz, DMSO-d_6) (complicated by rotamers) 8.10−7.98 (1H, m, NH), 7.48−7.26 (10H, m, PhH), 6.91 (1H, s, $CHPh_2$), 6.58 (1H, m, NH), 6.03 (1H, m, H-3), 5.13 (1H, m), 4.34 (1H, m), 4.03 (1H, m), 3.37 (2H, m, CH_2N, H-4,5,6), 3.07, 2.81 (3H, 2 × s, NMe), 1.77 (3H, s, CH_3CO), 1.64−1.22 (2H, m, CH_2), 1.37 (9H, s, tBu), 0.81 (3H, m, CH_3); MS 566 ($M + H^+$), 466 ($M + H^+ -$ Boc). Anal. ($C_{31}H_{39}N_3O_7$·0.5C_6HF_5O) C, H, N.

(e) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-amino-6-(methylpropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (4f). Benzhydryl ester **14f** (0.10 g, 0.18 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (1 mL) and left to stand at 23 °C for 3 h. The solvent was removed in vacuo, and the residue was triturated using diethyl ether (30 mL). The resulting solid was collected by filtration and dried to give the title compound **4f** as a white solid (0.06 g, 85%): ^{1}H NMR (250 MHz, D_2O) (complicated by rotamers) 5.99 (1H, m, H-3), 5.24 (1H, m, H-6), 4.52 (1H, m), 4.25 (1H, m, H-4,5), 3.59−3.18 (2H, m, CH_2N), 3.18, 2.97 (3H, 2 × s, NMe), 2.02 (3H, s, CH_3CO), 1.72−1.46 (2H, m, CH_2), 0.87 (3H, m, CH_3); MS 300 ($M + H^+$). Anal. ($C_{13}H_{21}N_3O_5$·CF_3CO_2H·0.25H_2O) C, H, N.

(4*S*,5*R*,6*R*)-5-(Acetylamino)-4-guanidino-6-(methylpropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (5f). (a) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-6-(methyl-propylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Benzhydryl Ester (17f). Benzhydryl ester **14f** (0.83 g, 1.5 mmol) was dissolved in a solution of hydrogen chloride in dioxane (10 mL of a 4.0 M solution) and stirred under nitrogen for 30 min. The solvent was removed in vacuo to give an off-white foam. This was suspended in tetrahydrofuran (10 mL) and dimethylformamide (5 mL). To this were added triethylamine (0.42 mL) and [(*tert*-butoxycarbonyl)imino]pyrazol-1-ylmethylcarbamic acid *tert*-butyl ester (0.68 g, 2.2 mmol), and the reaction mixture was stirred at 23 °C for 18 h. The reaction mixture was partitioned between ethyl acetate and 1 M hydrochloric acid. The organic phase was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo. The residue was chromatographed (cyclohexane/ethyl acetate, 1:1, eluant). The required fractions were combined, and the solvent was removed in vacuo to give the title compound **17f** as a white foam (0.61 g, 58%): ^{1}H NMR (250 MHz, DMSO-d_6) (complex due to rotamers) 11.41 (1H, s, NH), 8.63 (1H, m, NH), 7.40−7.29 (10H, m, PhH), 6.99 (1H, s, $CHPh_2$), 6.12 (1H, m, H-3), 5.85 (1H, m, N-H), 5.16 (1H, m, H-6), 5.04 (1H, m), 4.33 (1H, m, H-4,5), 3.45 (2H, m, CH_2N), 3.17, 2.89 (3H, 2 × s, NMe), 1.98 (3H, s, CH_3CO), 1.69−1.42 (2H, m, CH_2), 1.49 (18H, s, tBu), 0.87 (3H, m, CH_3); MS 708 ($M + H^+$), 608, 508. Anal. ($C_{37}H_{49}N_5O_9$·0.75H_2O) C, H, N.

(b) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-guanidino-6-(methylpropylcarbamoyl)-5,6-dihydo-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (5f). Benzhydryl ester **17f** (0.11 g, 0.16 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (1 mL) and left to stand for 3 h. The solvent was removed in vacuo, and the residue was triturated using diethyl ether. The solid was collected by filtration and dried to give **5f** as a white solid (0.06 g, 84%): ^{1}H NMR (250 MHz, D_2O) 6.00 (1H, d, H-3, J = 4 Hz), 5.34 (1H, d, H-6, J = 6.3

Hz), 4.43−4.29 (2H, m, H-4,5), 3.56−3.13 (2H, m, CH_2N), 3.13, 2.93 (3H, 2 × s, NMe), 2.00 (3H, s, CH_3CO), 1.79−1.44 (2H, m, CH_2), 0.88 (3H, m, CH_3); MS 342 ($M + H^+$). Anal. ($C_{14}H_{23}N_5O_5 \cdot CF_3CO_2H$) C, H, N.

The following compounds were similarly prepared by method 2: **4b,d,g,h−aw** and **5d,e,j,r.**

Method 3. (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-guanidino-6-(methylpropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (5g). (a) (4*S*,5*R*,6*R*)-5-(acetylamino)-4-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-6-((1*R*,2*R*),3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Benzhydryl Ester (15). To a suspension of (4*S*,5*R*,6*R*)-5-(acetylamino)-4-amino-6-((1*R*,2*R*),3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic acid trihydrate (**7**) (7.8 g, 22.7 mmol) in methanol (60 mL) were added [(*tert*-butoxycarbonyl)imino]pyrazol-1-ylmethylcarbamic acid *tert*-butyl ester (7.1 g, 23 mmol) and triethylamine (4.2 mL). The reaction mixture was stirred at 23 °C for 18 h. Diethyl ether (200 mL) was added, and the resultant solid was collected by filtration to give a white solid. This was suspended in a solution of diphenyldiazomethane in dichloromethane (48.0 mL of a 0.47 M solution), acidified using 2 M hydrochloric acid, and stirred rapidly for 18 h. The organic phase was separated and the solvent removed in vacuo to give a purple foam. This was chromatographed (ethyl acetate eluant), the required fractions were combined, and the solvent was removed in vacuo to give the title compound **15** as a white foam (11.1 g, 70%): ^{1}H NMR (250 MHz, DMSO-d_6) 11.40 (1H, s, NH), 8.28 (1H, d, NH, $J = 7.5$ Hz), 8.21 (1H, d, NH, $J = 8$ Hz), 7.49−7.27 (10H, m, PhH), 6.88 (1H, s, $CHPh_2$), 5.97 (1H, d, H-3, $J = 2.5$ Hz), 4.88 (1H, m), 4.68 (1H, d, $J = 6$ Hz), 4.63 (1H, d, $J = 6$ Hz), 4.36 (1H, t, $J = 6$ Hz), 4.25 (1H, m), 4.12 (1H, m), 3.69 (2H, m), 3.44 (2H, m, H-4,5,6,7,8,9, OH), 1.87 (3H, s, CH_3CO), 1.48 (9H, s, tBu), 1.42 (9H, s, tBu).

(b) (2*R*,3*R*,4*S*)-3-(Acetylamino)-4-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic Acid 6-Benzhydryl Ester (16). To a solution of triol **15** (9.2 g, 13.2 mmol) in methanol/water (5:1, v/v, 120 mL) was added sodium periodate (6.4 g, 29.9 mmol), and the reaction mixture was stirred at 23 °C for 1.5 h. The solid was removed by filtration, and the filtrate was evaporated in vacuo to give a white solid. This was suspended in *tert*-butyl alcohol (55 mL) and cyclohexene (7.7 mL). To this was added a solution of sodium chlorite (8.1 g, 89 mmol) and potassium dihydrogen orthophosphate (8.1 g, 60 mmol) in water (44 mL), and the reaction mixture was stirred at 23 °C for 2 h. The reaction mixture was acidified and extracted using ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to give a tan foam. This was dissolved in diethyl ether, and petroleum ether (40−60) was added to precipitate the title compound **16** as a white solid, which was collected by filtration (6.0 g, 69%): ^{1}H NMR (250 MHz, DMSO-d_6) 11.39 (1H, s, CO_2H), 8.27−8.17 (2H, m, NH), 7.50−7.27 (10H, m, PhH), 6.96 (1H, s, $CHPh_2$), 6.19 (1H, d, H-3, $J = 5$ Hz), 4.82 (1H, d, H-6, $J = 4$ Hz), 4.53 (1H, m), 4.43 (1H, m, H-4,5), 1.83 (3H, s, CH_3CO), 1.47 (9H, s, tBu), 1.41 (9H, s, tBu); MS 653 ($M + H^+$).

(c) (2*R*,3*R*,4*S*)-3-(Acetylamino)-4-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic Acid 6-Benzhydryl Ester 2-(2,3,4,5,6-Pentafluorophenyl) Ester. To a solution of acid **16** (4.4 g, 6.8 mmol) in dry dimethylformamide (10 mL) and pyridine (0.7 mL) was added pentafluorophenyl trifluoroacetate (1.3 mL, 7.4 mmol), and the reaction mixture was stirred at 23 °C for 1 h. More pyridine (0.7 mL) and pentafluorophenyl trifluoroacetate (1.3 mL) were added, and the reaction mixture was stirred for a further 2 h. The reaction mixture was diluted using ethyl acetate and washed consecutively using 1 M hydrochloric acid, saturated sodium bicarbonate solution, and brine. The organic phase was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to give the crude title compound as a tan foam (8.1 g), which was contaminated with pentafluorophenol and used directly without further purification: ^{1}H NMR (250 MHz, DMSO-d_6) 9.97 (1H, br s, NH), 7.49−7.27 (11H, m, PhH, NH), 6.91 (1H, s, $CHPh_2$), 6.52 (1H, m, NH), 6.1 (1H, d, H-3), 4.93 (1H, m, H-6), 4.58 (1H, m), 4.47 (1H, m, H-4,5), 1.88 (3H, s, CH_3CO), 1.42 (18H, s, tBu).

(d) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-6-(dipropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Benzhydryl Ester (17g). To a solution of crude (2*R*,3*R*,4*S*)-3-(acetylamino)-4-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-3,4-dihydro-2*H*-pyran-2,6-dicarboxylic acid 6-benzhydryl ester 2-(2,3,4,5,6-pentafluorophenyl) ester prepared as above (0.70 g) in dry tetrahydrofuran (5 mL) was added dipropylamine (0.14 g, 1.4 mmol), and the reaction mixture was stirred at 23 °C for 4 h. The solvent was removed in vacuo, and the residue was chromatographed (cyclohexane/ethyl acetate, 1:1, eluant). The required fractions were combined, and the solvent was removed in vacuo to give the title compound **17g** as a pale-yellow foam (0.34 g, 78% over two stages): ^{1}H NMR (250 MHz, DMSO-d_6) 11.46 (1H, s, NH), 8.27 (1H, d, NH, $J = 6.3$ Hz), 8.16 (1H, d, NH, $J = 7.5$ Hz), 7.48−7.27 (10H, m, PhH), 6.97 (1H, s, $CHPh_2$), 6.14 (1H, d, H-3, $J = 5$ Hz), 5.18 (1H, m, H-6), 4.67 (1H, m), 4.10 (1H, m, H-4,5), 3.61−3.24 (3H, m, CH_2N), 3.14 (1H, m, CH_2N), 1.84 (3H, s, CH_3CO), 1.60−1.34 (4H, m, 2 × CH_2), 1.46 (9H, s, tBu), 1.40 (9H, s, tBu), 0.85 (3H, t, Me, $J = 7$ Hz), 0.74 (3H, t, Me, $J = 7$ Hz).

(e) (4*S*,5*R*,6*R*)-5-(Acetylamino)-4-guanidino-6-(methylpropylcarbamoyl)-5,6-dihydro-4*H*-pyran-2-carboxylic Acid Trifluoroacetate Salt (5g). Benzhydryl ester **17g** (0.32 g, 0.43 mmol) was dissolved in dichloromethane (2 mL) and trifluoroacetic acid (2 mL) and left to stand at 23 °C for 2 h. The solvent was removed in vacuo and the residue triturated with diethyl ether (20 mL). The resulting solid was collected by filtration and dried to give the title compound **5g** as an off-white solid (0.16 g, 77%): ^{1}H NMR (250 MHz, D_2O) 5.97 (1H, d, H-3, $J = 3.8$ Hz), 5.29 (1H, d, H-6, $J = 6.3$ Hz), 4.41 (1H, dd, H-4, $J = 3.8, 6.3$ Hz), 4.30 (1H, t, H-5, $J = 6.3$ Hz), 3.63−3.08 (4H, m, CH_2N), 1.99 (3H, s, CH_3CO), 1.66−1.51 (4H, m, 2 × CH_2), 0.9 (6H, m, 2 × CH_3); MS 370 ($M + H^+$). Anal. ($C_{16}H_{27}N_5O_5 \cdot CF_3CO_2H$) C, H, N.

The following example was similarly prepared using method 3: **5p**.

Library Synthesis of Carboxamides. A solution of the pentafluorophenyl ester **13** (0.4 mL of a 3 mM solution in acetonitrile, 0.025 mmoL) was dispensed into 80 vials (3-mL volume). To each vial was added a solution of amine (0.025 mmol) in acetonitrile/DMF (0.4 mL). All solutions were dispensed by a Tecan 5072 two-arm dispensing robot modified by Cambridge Consultants Ltd. to allow automated solvent removal by blow-down with nitrogen. After 16 h at ambient temperature the solvent was removed at 50 °C. After cooling, trifluoroacetic acid/acetonitrile (1:4, v/v) solution (1 mL/vial) was added to each vial, and the mixtures were allowed to stand for a further 2 h at ambient temperature. The samples were finally blown-down for over 2 h at 50 °C and dried in vacuo for 18 h. The crude samples were used for biological testing without any further purification.

Illustrative Procedures for the Formation of Amines: Cyclopropylphenethylamine Hydrochloride (18ad). (a) *N*-Cyclopropyl-2-phenylacetamide (19ad). Phenylacetic acid (4.1 g, 30 mmol), cyclopropylamine (2.1 mL, 30 mmol), and 1-hydroxybenzotriazole hydrate (4.1 g, 30 mmol) were dissolved in acetonitrile (50 mL) at room temperature. To this was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (5.8 g, 30 mmol), and the reaction mixture was stirred for 72 h. The reaction mixture was diluted with ethyl acetate and washed with 2 M hydrochloric acid and 2 M sodium hydroxide. The organic phase was dried over anhydrous magnesium sulfate and the solvent removed in vacuo to give the title compound **19ad** as on off-white solid (3.4 g, 65%): ^{1}H NMR (250 MHz, $CDCl_3$) 7.39−7.20 (5H, m, PhH), 5.50 (1H, m, NH), 3.54 (2H, s, CH_2Ph), 2.67 (1H, m, CHN), 0.73 (2H, m), 0.40 (2H, m); MS 176 ($M + H^+$), 351 ($2M + H^+$).

(b) Cyclopropylphenethylamine Hydrochloride (18ad). Trimethylsilyl chloride (9.4 mL, 75 mmol) was added to a stirred 2 M solution of lithium borohydride in tetrahydrofuran

(18.7 mL) under nitrogen at room temperature, and the reaction mixture was stirred for 25 min to give a white precipitate. A solution of the amide **19ad** (3.0 g, 17 mmol) in tetrahydrofuran (40 mL) was added and stirred for 20 h. The reaction mixture was cooled, and methanol (30 mL) was added cautiously. After 20 min the solvent was removed in vacuo and the residue treated with 20% potassium hydroxide solution. This was diluted with water (30 mL) and extracted using ethyl acetate (2 × 80 mL). The combined organics were washed with brine and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give a colorless oil. This was dissolved in methanolic hydrogen chloride, and the solvent was removed in vacuo. The residue was triturated with diethyl ether and the solid collected by filtration and dried in vacuo to give the title compound **18ad** (2.4 g, 71%): ^{1}H NMR (250 MHz, DMSO-d_6) 9.40 (2H, m, NH_2^+), 7.42−7.23 (5H, m, PhH), 3.22 (2H, m, CH_2N), 3.01 (2H, m, CH_2Ph), 2.72 (1H, m, CHN), 0.95 (2H, m), 0.75 (2H, m). Anal. ($C_{11}H_{15}N$·HCl) C, H, N.

1,3-Dimethylpyrazolidine (18av,aw). (a) 5-Methyl-3-oxopyrazolidine-1-carboxylic Acid *tert*-Butyl Ester (20). To a solution of hydrazine hydrate (20.0 mL, 0.42 mol) in ethanol (150 mL) was added a solution of ethyl crotonate (46.5 mL, 0.38 mol) in ethanol (150 mL). The solution was stored at room temperature for 30 min and then refluxed for 2 h. The yellow solution was then cooled and concentrated to an oil which was dissolved directly in water (300 mL) with sodium carbonate (39.7 g, 0.38 mol) and dioxane (50 mL). A solution of di-*tert*-butyl dicarbonate (81.0 g, 0.38 mol) in dioxane (150 mL) was then added, and the mixture stirred at room temperature for 1 h. The resulting suspension was filtered and the precipitate washed with chloroform (200 mL). The combined organics were then concentrated to a small volume and partitioned between water (500 mL) and dichloromethane (10 × 500 mL). The organic extracts were dried and evaporated to afford a yellow syrup which was triturated with ether to afford the title compound **20** (34.8 g, 46% over two stages) as a white solid: υ_{max} (film) 2978, 1703; ^{1}H NMR (250 MHz, $CDCl_3$) 8.7 (1H, br s, NH), 4.45 (1H, ddq, H-5), 3.0 (1H, dd, H-4, J = 9, 17 Hz), 2.2 (1H, dd, H-4′, J = 3, 17 Hz), 1.5 (9H, s, tBu), 1.35 (3H, d, CH_3, J = 6.4 Hz); MS 401 ($2M + H^+$), 345, 301, 201 ($M + H^+$). Anal. ($C_9H_{16}N_2O_3$) C, H, N.

(b) 2,5-Dimethyl-3-oxopyrazolidine-1-carboxylic Acid *tert*-Butyl Ester (21). Pyrazolidine **20** (4.16 g, 20.8 mmol) in DMF (40 mL) was treated with methyl iodide (4 mL) and potassium carbonate (3.45 g, 25 mmol), and the suspension stirred at room temperature for 2 h. The reaction mixture was partitioned between ether (300 mL) and water (300 mL) and the aqueous layer washed with further ether. The combined organic extracts were dried, evaporated, and purified by flash chromatography (25% ethyl acetate in petroleum ether) to afford the title compound **21** as a waxy solid (4.20 g, 94%): υ_{max} (film) 2980, 1709; ^{1}H NMR (250 MHz, DMSO-d_6) 4.45 (1H, m, H-5), 3.1 (3H, s, N-Me), 3.0 (1H, dd, H-4, J = 17, 7 Hz), 1.95 (1H, d, H-4′, J = 17 Hz), 1.45 (9H, s, tBu), 1.15 (3H, d, CH_3, J = 8 Hz); MS 429 ($2M + H^+$), 329, 215 ($M + H^+$), 159. Anal. ($C_{10}H_{18}N_2O_3$) C, H, N.

(c) 2,5-Dimethylpyrazolidine-1-carboxylic Acid *tert*-Butyl Ester (22). Amide **21** (1.0 g, 4.7 mmol) in dichloromethane (5 mL) was treated with borane−dimethyl sulfide complex (1.0 mL, 10.3 mmol) at room temperature. After 18 h the reaction was quenched by cautious addition of methanol until no further effervescence was observed. Water (5 mL) was added, and the solution refluxed for 1 h to destroy the amine−borane complex. The mixture was cooled, concentrated to an oil, and partitioned between water and ethyl acetate. The organic extracts were dried, evaporated, and purified by flash chromatography (ethyl acetate) to afford the title compound **22** as a colorless oil (0.4 g, 44%): ^{1}H NMR (250 MHz, $CDCl_3$) 4.0 (1H, m, H-5), 3.1, 2.95 (2H, 2 × ddd, H-3,3′), 2.6 (3H, s, NMe), 2.25, 1.95 (2H, m, H-4,4′, J = 17 Hz), 1.5 (9H, s, tBu), 1.3 (3H, d, CH_3, J = 8 Hz); MS 401 ($2M + H^+$), 201 ($M + H^+$), 145. Anal. ($C_{10}H_{20}N_2O_2$·0.5H_2O) C, H, N.

(d) (±)-1,3-Dimethylpyrazolidine Hydrochloride (18av,aw). Compound **22** (0.5 g, 2.5 mmol) was stirred at room temperature for 24 h in a 1:1 mixture of trifluoroacetic acid/dichloromethane (5 mL). The solution was evaporated to dryness and triturated from a solution of hydrogen chloride in ether (1 M) to afford thet racemic amine **18av,aw** (0.38 g, 88%) as a highly hygroscopic white solid used directly in method 2 coupling: ^{1}H NMR (250 MHz, D_2O) 3.72 (1H, m, H-3), 3.50 (2H, dd, H-5), 3.02 (3H, s, NMe), 2.48 (1H, dddd, H-4), 1.80 (1H, dddd, H-4′), 1.25 (3H, d, H-6, J = 7.5 Hz).

(ii) Biology. In Vitro Biology. In vitro influenza sialidase inhibition and antiviral activities in plaque reduction assays were measured by the previously reported methods.[8] *Sialidase assay:* The IC_{50} values quoted are calculated from the percent inhibition of enzyme activity in the presence of inhibitor relative to a positive (no inhibitor) control. All reactions were carried out in triplicate, and the mean values of these replicates were used in the analysis of data. *Plaque assay:* The percent inhibition of plaque formation relative to controls was calculated for each inhibitor concentration used. At each concentration, data from three experiments were pooled in order to accurately determine the 50% inhibitory concentration (IC_{50}) for each compound. In most cases corresponding IC_{50}s from different experiments differed by a factor of no more than 2−5.

Influenza A sialidase kinetics assays were carried out as previously described,[13] with data fitted to the generalized integrated rate equation. Values of v_o were independent of [**4g**], and the plot of k_{trans} against [**4g**] was linear, indicating that the data were consistent with a single-step binding mechanism. From the replot of k_{trans} against [**4g**], the k_{on} was determined to be (2.9 ± 0.1) × 10^5 M^{-1} s^{-1} and k_{off} was determined to be (1.5 ± 0.4) × 10^{-4} s^{-1}. From these on and off rates, a K_d value of (5.2 ± 1.4) × 10^{-10} M was calculated. A K_i value was determined from a Dixon plot of $1/v_i$ against [**4g**], giving a value of 0.53 ± 0.07 nM. The K_d comparison for zanamivir is 0.46 nM.[13]

In contrast to the results with the influenza A sialidase, progress plots of product formation against time were linear at all **4g** concentrations when influenza B/Beijing/1/87 sialidase was assayed.

Michaelis−Menten plots in the presence of different fixed concentrations of inhibitor were constructed. V_{max} values were independent of inhibitor concentration; from a replot of K_m against [**4g**], a K_i value of 220 ± 30 nM was determined. The K_d comparison for zanamivir is 1.4 nM.[37]

In Vivo Biology. (a) Influenza Virus Infection in Mice. The protocol for infecting mice has been described previously.[9] Mice were challenged on day 0 by the intranasal instillation of either $10^{3.5}$ $TCID_{50}$/mL of influenza virus A/Singapore/1/57 (where animals were treated by the intranasal route) or $10^{4.5}$ $TCID_{50}$/mL (where animals were treated by the intraperitoneal route), while under light ether anesthesia.

(b) Pretreatment with Uranyl Nitrate. Mice to be dosed by the intraperitoneal route were pretreated intravenously with 5 mg/kg uranyl nitrate (Fluka) 5 days prior to infection, to facilitate a protracted elimination half-life of drug.[35]

(c) Treatment Protocol. Compounds were formulated in 5% DMSO, 1% Tween 80, and 94% phosphate-buffered saline (pH 7.4). Two treatment protocols were used as follows: (1) For groups of 12 mice treated by the intranasal route, treatment was initiated 17 h prior to infection, and then dosages were given twice daily on the day of infection (day 0), the following 2 days, and the morning of day 3. Lungs were removed from each of four mice for virus titer estimation, at midday on days 1−3. Antiviral activities are expressed as percentage reduction in virus titer (AUC) for treated animals, as compared to control mice lung virus titers (AUC). (2) For groups of 10 mice treated by the intraperitoneal route, treatment was initiated 1 h prior to infection, with repeated doses 3 and 7 h postinfection. Animals were culled 24 h postinfection and lungs removed for virus titer estimation. Antiviral activities are expressed as percentage reduction in virus titer for treated animals, as compared to controls. Statistical significance was determined using the Krushall−Wallis mean rank sum test.

Virus titers were determined by ELISA, by a modification of methods described previously.[36]

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Supporting Information Available: Table of IC_{50} values from library experiment and spectroscopic and analytical data for all final compounds (17 pages). Ordering information is given on any current masthead page.

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