Syntheses and Neuraminidase Inhibitory Activity of Multisubstituted Cyclopentane Amide Derivatives

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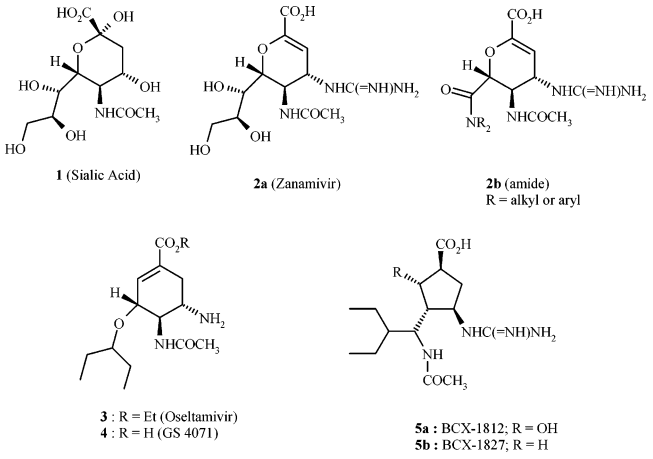
In further studies aimed toward identifying effective and safe inhibitors of influenza neuraminidases, we synthesized a series of multisubstituted cyclopentane amide derivatives. Amides prepared were 14 examples of N-substituted alkyl or aralkyl types from primary amines, 13 examples of the N,N-disubstituted alkyl, aralkyl, or substituted-alkyl type from secondary amines, and 12 examples from cycloaliphatic or substituted cycloaliphatic secondary amines. These compounds bearing two chiral centers, at position-1 in the ring and position-1′ in the side chain attached at position 3, were tested for their ability to inhibit A and B forms of influenza neuraminidase. The 1-ethylpropylamide, diethylamide, dipropylamide, and 4-morpholinylamide showed very good inhibitory activity ($IC_{50} = 0.015-0.080\ \mu M$) vs the neuraminidase A form, but modest activity ($IC_{50} = 3.0-9.2\ \mu M$) vs the neuraminidase B form. Since the parent amides bear two chiral centers (C-1 and C-1′), three of the better inhibitors were tested at higher levels of diastereomeric purity. The diastereomers corresponding to the active forms of the 1-(ethyl)propylamide, the diethylamide, and the dipropylamide (all of the same configuration at the C-1′ chiral center), and the diastereomer of the diethylamide representing the active form at both C-1′ and C-1 were isolated or synthesized from precursors that were isolated as diastereomers. These diastereomers showed some improvement in neuraminidase inhibition over the parent diastereomeric mixtures. 1-Carboxy-1-hydroxy derivatives of the best active compounds, the diethylamide and the dipropylamide, were also prepared. These compounds were not as active as the compounds without the 1-hydroxy group. In an in vivo study, the C-1′ active isomer of the diethylamide from the 1-carboxy series was tested in influenza-infected mice by oral and intranasal administration and found to be very effective only intranasally in preventing weight loss at doses as low as 0.1 (mg/kg)/day.

Introduction

Advances in understanding of the molecular and cellular biology of influenza have led to identification of several molecular targets for the design of agents against this disease. The most advanced clinical agents described to date are inhibitors of neuraminidase, a glycoprotein on the surface of the influenza virus. Neuraminidase cleaves α-ketosidic linkages between sialic acid (**1**, Chart 1) and the adjacent sugar residues. The removal of sialic acid lowers the viscosity of the virus particle, thus permitting entry of the virus into epithelial cells. Neuraminidase also destroys hemagglutinin on the surface of the virus, thereby allowing emergence of progeny virus particles from infected cells.[1,2] Studies with neuraminidase-deficient influenza virus have shown that the mutant virus is still infective but the budding virus particles form aggregates or remain bound to the infected cell surface.[2] Compounds that inhibit neuraminidase can protect the host from viral infection and retard its propagation by preventing spread of the virus among cells and from the site of infection.

Chart 1



Two influenza neuraminidase inhibitors, zanamivir (**2a**, Chart 1, from GlaxoSmithKline and Biota)[3−5] and oseltamivir (**3**, Chart 1, from Hoffman La Roche and Gilead Sciences),[6] recently received FDA approval for use in the treatment and prevention of influenza. Zanamivir is an effective inhibitor of both A and B forms of neuraminidase, but this highly polar compound

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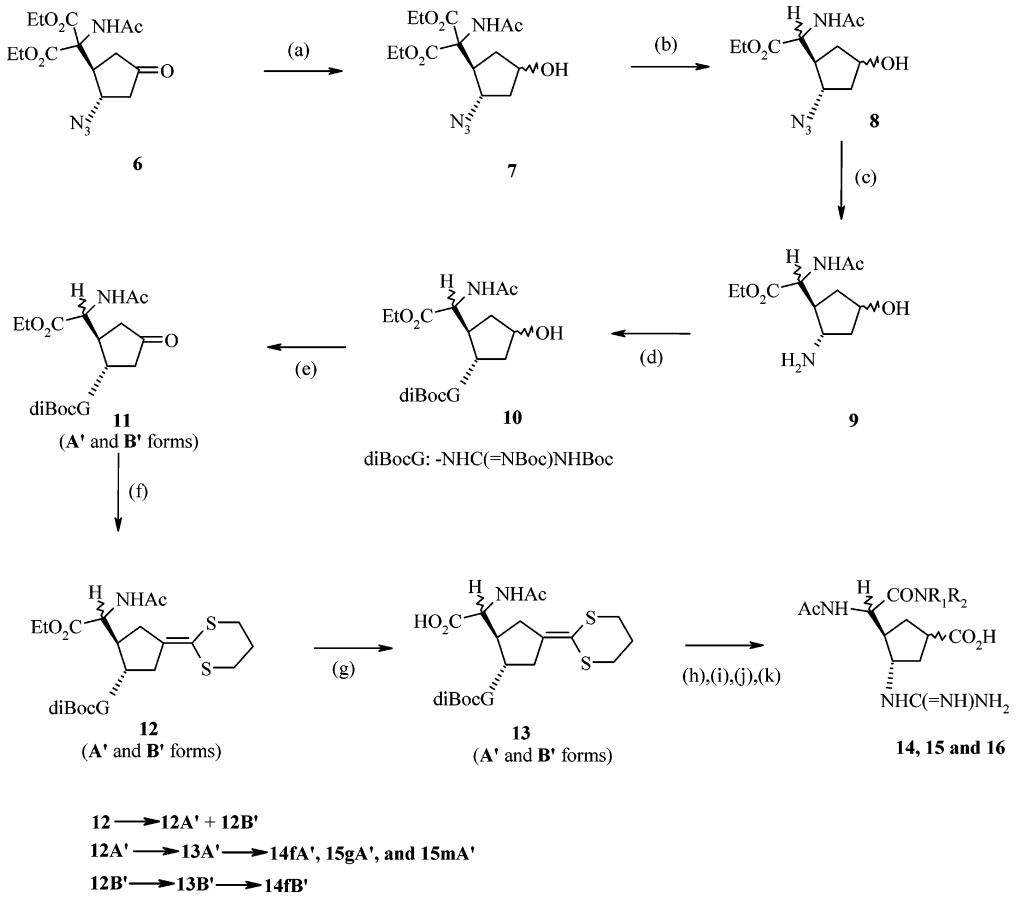
Scheme 1. Synthetic Routes to the Carboxamides of Structural Types **14**−**16**[a]



[a] Reagents: (a) $NaBH_4$; (b) (*i*) NaOH, (*ii*) AcOH; (c) Pd/C, H_2; (d) MeSC(=NBoc)NHBoc, $HgCl_2$, Et_3N; (e) pyridinium chlorochromate; (f) trimethyl-1,3-dithiane, *n*-BuLi; (g) NaOH; (h) MeOCOCl, Et_3N, R_1R_2NH; (i) HCl/MeOH; (j)NaOH; (k) CF_3CO_2H (TFA).

requires administration by oral inhalation. The ester oseltamivir is a prodrug converted after oral intake to its active form, the carboxylic acid (**4**, Chart 1, GS4071).[6] Oseltamivir is also an effective inhibitor of both A and B forms of neuraminidase, but it has been reported to cause nausea and vomiting. A new agent, BCX-1812 (**5**), recently reported from these laboratories, has been shown to be a potent inhibitor of both A and B forms of neuraminidase.[7−11] It is safe without side effects but did not show statistical efficacy in phase III trials, probably because of lack of bioavailability. A number of reports have recently appeared in the literature, which describe the amide derivatives **2b** of zanamivir and these compounds were found very effective against neuraminidase A but have modest activity against neuraminidase B.[12,13] In addition to these amides, a number of other modifications of zanamivir and oseltamivir also have resulted into potent inhibitors of neuraminidase.[14−20] A number of reports have appeared from Abbott Laboratories also on neuraminidase inhibitors.[21−24]

In this report, we describe another study aimed toward identifying neuraminidase inhibitors. In the current work, we studied the multisubstituted cyclopentane system represented by the target compounds shown in Schemes 1−4. The Scheme 1 compounds, consisting of various N-substituted carboxamides derived from the carboxyl group of the side chain attached to position 3, were synthesized as mixtures of isomers at C-1 and C-1′ and tested for their efficacy as inhibitors of A and B forms of neuraminidase. One of the most effective inhibitors of this group was selected for X-ray crystallographic work, and the bound isomer was selected for further preparation or purification. These compounds were then modified as shown in Schemes 3 and 4 by introduction of a 1-hydroxy substituent in order to explore the effect of this variation.

Chemistry

The synthetic route used to prepare the amide series **14a**−**n**, **15a**−**m**, and **16a**−**l** is outlined in Scheme 1. Starting compound **6**, prepared as described in the literature,[11] was reduced by $NaBH_4$ to hydroxy compound **7** in theoretical yield. Partial hydrolysis of the malonate diester group of **7** to the monoester followed by heating in AcOH solution caused decarboxylation and gave **8** in 63% overall yield. The preparation of **8** introduces a chiral carbon, designated C-1′, in the 3-position side chain. Hydrogenation of the azido group of **8** produced amino compound **9**, which, without isolation, was converted to the Boc-protected guanidino derivative **10** with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea in 89% overall yield. Oxidation of **10** with pyridinium chlorochromate regenerated the 1-carbonyl group affording compound **11** in 73% yield (reduction of the 1-carbonyl with later regeneration avoided complications encountered in early experiments in which transformations of azido to protected guanidino were attempted while retaining the 1-oxo group). Carbonyl compound **11**, as a mixture of two diastereomers (60% **A**′ and 40% **B**′) due to the aforementioned chiral

Table 1. Amides **14a**−**n**, **15a**−**m**, **16a**−**l**, Mass Spectral Results Compared with Molecular Weight of Assigned Structures, and in Vitro Inhibitory Activity vs Neuraminidase Forms A and B

						neuraminidase inhibition[a] IC_{50} (μM)	
compd	R_1	R_2	molecular formula	molecular wt	MS (M + 1), m/z^+	A	B
14a	$-CH_2CH_3$	H	$C_{13}H_{23}N_5O_4$	313.36	314.2	0.33	3.1
14b	$-CH(CH_3)_2$	H	$C_{14}H_{25}N_5O_4$	327.39	328.4	1.34	4.6
14c	$-CH_2CH{=}CH_2$	H	$C_{14}H_{23}N_5O_4$	325.37	326.2	0.72	4.3
14d	$-(CH_2)_3CH_3$	H	$C_{15}H_{27}N_5O_4$	341.41	342.2	9.9	46.8
14e	$-CH(CH_3)CH_2CH_3$	H	$C_{15}H_{27}N_5O_4$	341.41	342.3	0.41	16.5
14f	$-CH(CH_2CH_3)_2$	H	$C_{16}H_{29}N_5O_4$	355.44	356.3	0.08	3.0
14g	$-CH(CH_3)(CH_2)_2CH_3$	H	$C_{16}H_{29}N_5O_4$	355.44	356.0	3.2	12.2
14h	$-CH(CH_3)CH_2CH(CH_3)_2$	H	$C_{17}H_{31}N_5O_4$	369.46	370.4	8.4	11.4
14i	$-CH(CH_3)(CH_2)_3CH_3$	H	$C_{17}H_{31}N_5O_4$	369.46	370.5	8.7	37
14j	$-CH(CH_2CH_3)(CH_2)_3CH_3$	H	$C_{18}H_{33}N_5O_4$	383.49	384.0	1.8	16
14k	$-(CH_2)_2C_6H_5$	H	$C_{19}H_{27}N_5O_4$	389.41	390.3	6.4	70.5
14l	$-CH(CH_3)(CH_2)_2C_6H_5$	H	$C_{21}H_{31}N_5O_4$	417.51	418.5	0.23	12.5
14m	$-CH(CH_3)CH_2OCH_3$	H	$C_{15}H_{27}N_5O_5$	357.41	358.0	4.4	25.1
14n	$-CH(CH_2CH_3)CH_2OCH_3$	H	$C_{16}H_{29}N_5O_5$	371.44	372.0	2.2	20.0
15a	$-CH_3$	$(CH_2)_2CH_3$	$C_{15}H_{27}N_5O_4$	341.41	342.3	0.94	6.2
15b	$-CH_3$	$-CH(CH_3)_2$	$C_{15}H_{27}N_5O_4$	341.41	342.3	3.21	6.8
15c	$-CH_3$	$-CH_2CH{=}CH_2$	$C_{15}H_{25}N_5O_4$	339.39	340.3	0.65	11.7
15d	$-CH_3$	$-(CH_2)_3CH_3$	$C_{16}H_{29}N_5O_4$	355.44	356.4	2.4	18.4
15e	$-CH_3$	$-(CH_2)_5CH_3$	$C_{18}H_{33}N_5O_4$	383.49	384.4	12.4	>30
15f	$-CH_3$	$-(CH_2)_2C_6H_5$	$C_{20}H_{29}N_5O_4$	403.48	404.4	8.0	26.7
15g	$-CH_2CH_3$	$-CH_2CH_3$	$C_{15}H_{27}N_5O_4$	341.41	342.3	0.015	3.0
15h	$-CH_2CH_3$	$-(CH_2)_2CH_3$	$C_{16}H_{29}N_5O_4$	355.44	356.3	0.13	7.0
15i	$-CH_2CH_3$	$-(CH_2)_3CH_3$	$C_{17}H_{31}N_5O_4$	369.47	370.4	0.43	
15j	$-CH_2CH_3$	$-CH_2C_6H_5$	$C_{20}H_{29}N_5O_4$	403.48	404.4	0.67	>30
15k	$-CH_2CH_3$	$-CH_2CH_2OH$	$C_{15}H_{27}N_5O_5$	357.41	358.5	0.72	>30
15l	$-(CH_2)_2CH_3$	$-CH_2-CH(CH_2)_2$cyclo	$C_{18}H_{31}N_5O_4$	381.48	382.4	0.20	24.0
15m	$-(CH_2)_2CH_3$	$-(CH_2)_2CH_3$	$C_{17}H_{31}N_5O_4$	369.47	370.4	0.06	9.2
16a	$-(CH_2)_3-$		$C_{14}H_{23}N_5O_4$	325.37	326.4	0.92	8.4
16b	$-(CH_2)_4$		$C_{15}H_{25}N_5O_4$	339.39	340.5	0.47	24.0
16c	$-(CH_2)_5$		$C_{16}H_{27}N_5O_4$	353.42	354.0	0.15	6.9
16d	$-(CH_2)_6$		$C_{17}H_{29}N_5O_4$	367.45	368.1	0.99	25.3
16e	$-CH(CH_3)(CH_2)_4-$		$C_{17}H_{29}N_5O_4$	367.45	368.0	0.24	10.5
16f	$-CH(CH_2CH_3)(CH_2)_4$		$C_{18}H_{31}N_5O_4$	381.47	382.0	0.18	3.1
16g	$-CH(CH_2CH_2CH_3)(CH_2)_4-$		$C_{19}H_{33}N_5O_4$	395.50	396.1	0.15	14.5
16h	$-CH_2CH(CH_3)(CH_2)_3-$		$C_{17}H_{29}N_5O_4$	367.45	368.1	2.55	>10
16i	$-(CH_2)_2CH(CH_3)(CH_2)_2-$		$C_{17}H_{29}N_5O_4$	367.45	368.1	8.2	42.5
16j	$-CH_2CH(CH_3)CH_2C(CH_3)CH_2-$		$C_{18}H_{31}N_5O_4$	381.47	382.0	3.5	>100
16k	$-(CH_2)_2O(CH_2)_2$		$C_{15}H_{25}N_5O_5$	355.39	356.0	0.04	7.8
16l	$-(CH_2)_2S(CH_2)_2-$		$C_{15}H_{25}N_5O_4S$	371.45	372.6	0.23	29.1

[a] Test procedure described under Experimental Section. The typical solution prepared for the IC_{50} test was of 8.0 mL volume and 20 μM concentration.

center, was subjected to condensation (promoted by *n*-BuLi) with 2-trimethylsilyl-1,3-dithiane[25−27] to produce the dithiane derivative **12**, also a mixture of diastereomers. Mild basic hydrolysis of **12** led to the pivotal intermediate **13**, still a mixture of diastereomers, but now bearing a free carboxyl attached to the chiral carbon C-1′.

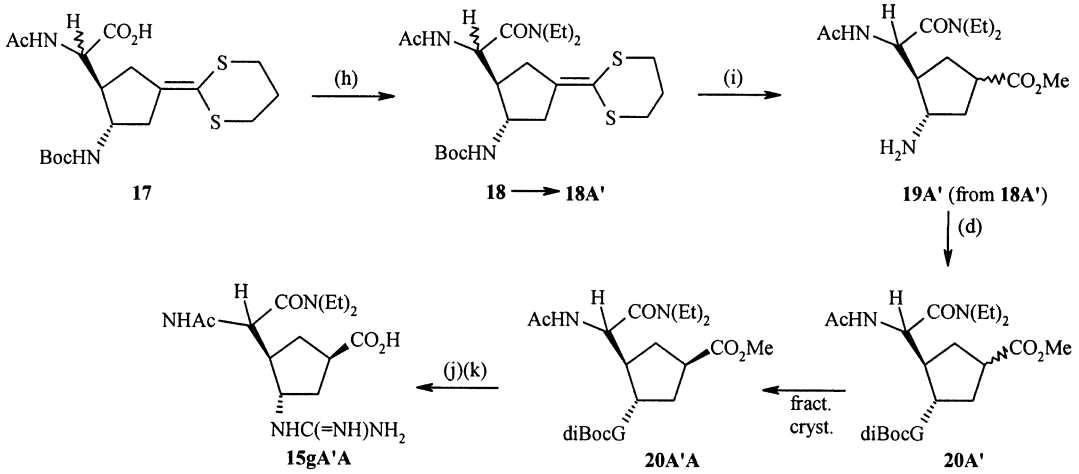
The amides of Table 1 were derived from **13** in four sequential steps: (1) activation of the C-1′ carboxyl group as the mixed anhydride derived from methyl chloroformate and subsequent reaction with appropriate amine, (2) transformation of the cyclopentylidinedithiane group to the 1-methyl ester, (3) hydrolysis of 1-methyl ester to acid with NaOH, and (4) removal of the Boc protecting groups from the 4-guanidino group by treatment with trifluoroacetic acid (TFA). The final reaction solutions were evaporated, and the residues were dissolved in aqueous solutions appropriate for the enzyme inhibition assays whose results are listed in Table 1.

The only stereochemical restraint present in the Scheme 1 route as described is that the 3-position side chain and the 4-guanidino group are trans to one another as initiated in the starting compound **6**. In precursor **8**, the first spatially unrestricted chiral center is introduced, thus allowing the existence of two C-1′ diastereomers. When the carboxyl group at the 1-position is formed in later steps, a second unconstrained chiral center is introduced (at C-1). Thus, each of the target structures, with no constraints at positions C-1 and C-1′, may have four diastereomers. All the compounds are racemic mixtures.

On the basis of X-ray crystallographic studies with one of the potent compounds and in anticipation that some of the diastereomeric mixtures of target types **14**−**16** might show even better neuraminidase inhibitory potency to warrant testing at higher levels of diastereomeric purity, we undertook the tasks of isolating pure samples of each of the two diastereomers of precursor type **12**. This was readily achieved using silica gel chromatography. The pure diastereomers of **12** thus obtained were designated as forms **A′** and **B′** (the prime designations denote connection with the C-1′ chiral center). The separate diastereomers **13A′** and **13B′**, were individually derived from isolated samples **12A′** and **12B′**.

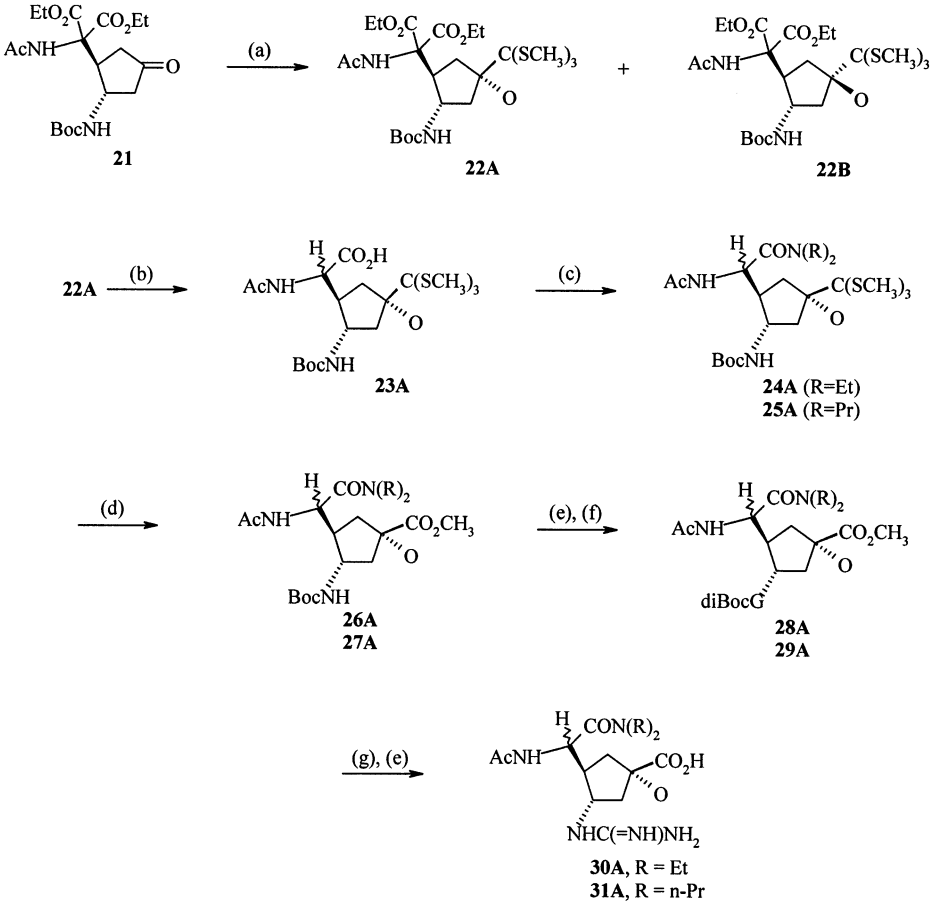
Compound **14f** was selected to prepare both **14fA′** and **14fB′** isomers through the same sequence from **13A′** and **13B′** as described earlier. Once the activity data con-

Scheme 2. Synthesis of Diastereomer **15gA′A**[a]



[a] Reagents: (d) MeSC(=NBoc)NHBoc, $HgCl_2$, Et_3N; (h) MeOCOCl, Et_3N, Et_2NH; (i) HCl/MeOH; (j)NaOH; (k) CF_3CO_2H (TFA).

Scheme 3. Synthetic Routes to the 1-Carboxy-1-hydroxy Derivatives **30A** and **31A**[a]

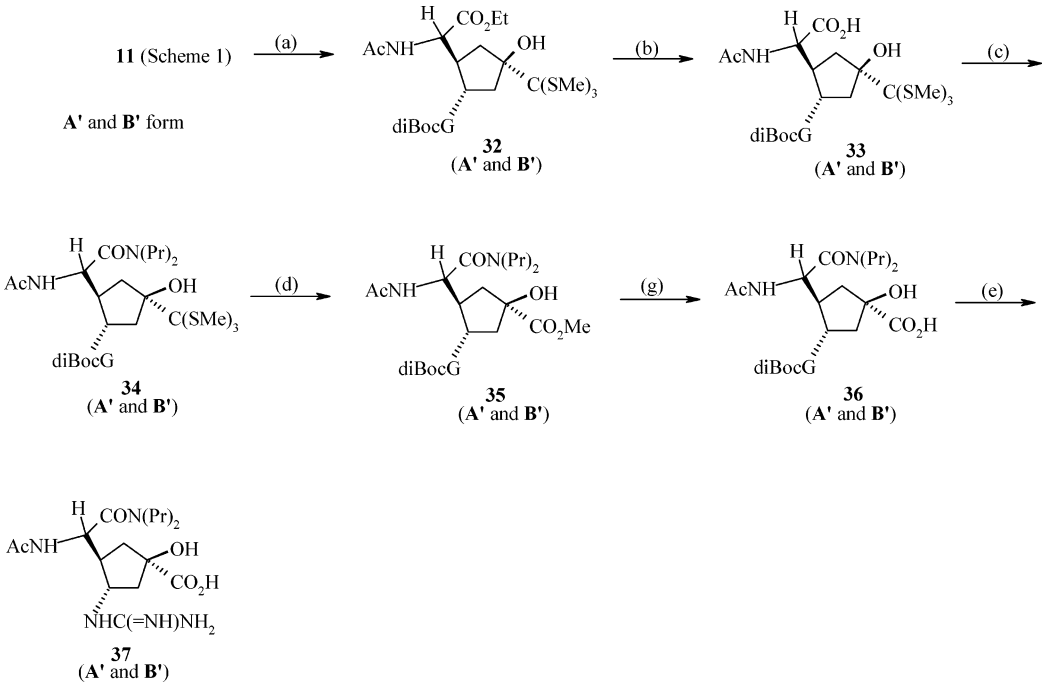


[a] Reagents: (a) $HC(SMe)_3$, *n*-BuLi in THF; (b) (*i*) NaOH, boiling H_2O-EtOH, (*ii*) AcOH, reflux; (c) EtOCOCl, Et_3N; Et_2NH or Pr_2NH; (d) MeOH−H_2O containing $HgCl_2$ and HgO; (e) TFA in CH_2Cl_2; (f) MeSC(=NBoc)NHBoc, Et_3N, $HgCl_2$ in DMF; (g) (*i*) NaOH, H_2O−THF−EtOH, (*ii*) AcOH.

firmed that the **A′** isomer is the active isomer, **15gA′** and **15mA′** were also prepared from **12A′** through the same sequence.

The X-ray crystallographic data from **15g** and the inhibition data on **15g** as well as its diastereomeric form **15gA′** (see Table 1) prompted us to seek a third diastereomeric variant of the **15g** type. We explored the route shown in Scheme 2 with the aim of obtaining the diastereomer incorporating the **A** configuration at both C-1 and C-1′. Beginning with previously reported dithiane derivative **17**,[11] the diethylamide intermediate **18** was prepared using the mixed anhydride method. Repeated recrystallizations (from ethyl acetate) of the diastereomeric product mixture allowed isolation of a pure sample of the **A′** isomer, **18A′**, stemming from the C-**1′** center. The 3-NHBoc group of **18A′** was deprotected, and the resulting amine **19A′** was converted to the diBoc-protected guanidino derivative, which, after repeated recrystallizations (from ether−hexane), was obtained as the pure diastereomer **20A′A** whose configuration as

Scheme 4. Synthetic Route to the C-1′ Diastereomers of **37**[a]



[a] Reagents: (a) $HC(SMe)_3$, *n*-BuLi in THF; (b) (*i*) NaOH, boiling H_2O-EtOH, (*ii*) AcOH, reflux; (c) EtOCOCl, Et_3N; Et_2NH or Pr_2NH; (d) MeOH−H_2O containing $HgCl_2$ and HgO; (e) TFA in CH_2Cl_2; (g) (*i*) NaOH, H_2O−THF−EtOH, (*ii*) AcOH.

indicated was confirmed by NOE results. Standard ester hydrolysis and Boc removal then afforded the sought diastereomer **15gA′A**.

1-Hydroxy-1-carboxy analogues of the most active inhibitors of the 1-carboxy series (the diethyl and dipropylamides **15g** and **15m**) were also prepared for evaluation using appropriate transformations outlined in Scheme 3. Addition of tris(methylthio)methane to previously reported **21**[11] led to purified adduct **22A** in 35% yield and some impure **22B** bearing the carboxyl-group-to-be in the 1-position in the configuration shown and confirmed by NOE methodology. The 1-hydroxy group is trans with reference to the 3-position side chain and cis with the group at position 4 in **22A**. Thus, subsequent products in Scheme 3 were single diastereomers with respect to C-1. The 1-carboxy-to-be precursor **22A** was then converted, using the usual hydrolytic and decarboxylating procedures, to **23A**, thereby introducing a chiral center at C-1′. The C-1′ diastereomeric mixtures were left as such in the remaining steps of Scheme 3. Next, the general procedures for amide formation proved to be satisfactory for preparing the diethylamide precursor **24A** and the dipropylamide precursor **25A**, which were then converted to the 1-carboxy methyl esters **26A** and **27A**. The Boc-protected 4-amino precursors were then converted, in two steps indicated, to the corresponding diBoc-protected 4-guanidino derivatives **28A** and **29A**. These homologous dialkylamide precursors required only final ester hydrolysis and Boc removal to give the target compounds **30A** and **31A**.

To explore the effect of the other isomer at C-1, where the 1-hydroxy group is cis with respect to the 3-position side chain and trans to the 4-position guanidine group, on the neuraminidase inhibitory activity of the dipropylamide type **31A**, the C-1′ diastereomers **37A′** and **37B′** were synthesized as shown in Scheme 4. The diastereomers **11A′** and **11B′** were used for this preparation. It was hoped that changing the Boc-amino group (as used in Scheme 3) to the diboc-guanidino group would influence the addition of tris(methylthio)methane and would go in favor of the desired isomer. It was also hoped that the presence of this bulky group would be favorable for the separation of isomers at C-1. The diastereomers **11A′** and **11B′** were separated using a combination of column chromatography and fractional recrystallization. The addition of tris(methylthio)-methane to **11A′** and **11B′** led to the isomeric mixtures at C-1 and was separated by chromatography to give pure products **32A′** and **32B′**, with each having the configuration as shown in Scheme 4. The sequence of ester hydrolysis to give **33A′** and **33B′** amide formation through mixed anhydride to give **34A′** and **34B′** and tris(methylthio)methyl hydrolysis with $HgCl_2$ and HgO to methyl ester led to the separate diastereomers **35A′** and **35B′**. The final targets **37A′** and **37B′** were obtained through the standard reaction of base hydrolysis of esters **35A′** and **35B′** to acid **36A′** and **36B′** and Boc deprotection with TFA.

Results and Discussion

a. Inhibition in Vitro of Neuraminidase Forms A and B. The 39 amide types listed in Table 1 whose syntheses are outlined in Scheme 1 were selected to provide representative types from primary, secondary, and cycloaliphatic amines in order to probe the binding sites of neuraminidase. These candidates were tested for their ability to inhibit neuraminidase forms A and B. The in vitro test data included in Table 1 suggest very good activity toward the A form of neuraminidase by compounds **14f**, **15g**, **15m**, and **16k**, but activity toward the neuraminidase B form was poor in comparisons with recognized neuraminidase inhibitors. This

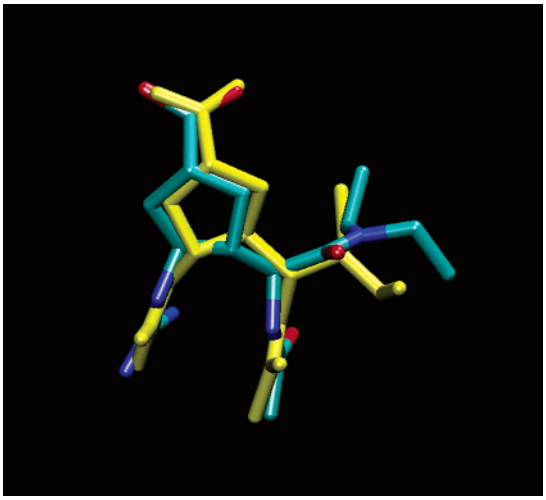


Figure 1. Superimposition of **15g** with BCX-1827 (**5b**).

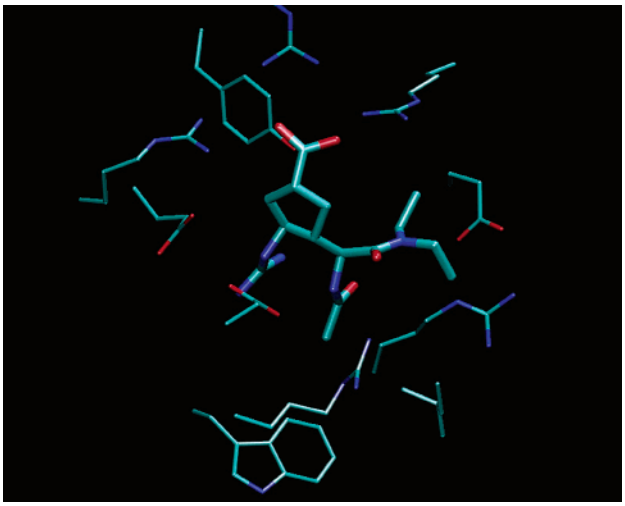


Figure 2. Compound **15g** in the active site of neuraminidase N9.

activity is very much comparable to the amides of zanamivir type as reported in the literature.[12,13]

Compound **15g** (racemic mixture of stereoisomers at C-1 and C-1′) was selected for X-ray crystallographic studies and showed differences and similarities to the binding of compound **5b** (BCX-1827, which has same potency as BCX-1812) in the active site of influenza neuraminidase N9 (Figures 1 and 2). The electron density in the active site revealed unambiguously the stereochemistry of the active isomer of **15g**. The carboxylic acid and the guanidino group are trans to each other, while the carboxylic acid and the side chain bearing amide group are cis to each other. The absolute configuration at C-4 having the guanidine group is *S* in compound **15g** while it is *R* in compound **5b** from the active site structures of the bound complexes. As a result of these differences in the stereochemistry, the guanidino group and the acetamido groups of compound **15g** bind differently in the active site compared to compound **5b**. These differences in the binding of these compounds might explain the differences in the potencies of these two compounds. However, the orientation of the guanidino group of **15g** in the active site is similar to the one observed in the dihydropyran compounds.[12,13] Also, the conformation of the carboxamide linker group in both of these compounds was found to be similar.

Table 2. Inhibitory Activity in Vitro of Some Amides on Neuraminidase A and B

	neuraminidase inhibition IC_{50} (μM)	
compd	A	B
14fA′	0.06	3.0
14fB′	25.0	
15gA′	0.007	3.0
15mA′	0.043	8.6
15gA′A	0.007	1.5
30A	1.6	
31A	0.47	
37A′	4.9	33
37B′	2300	

To confirm the crystallographic data on **15g**, we decided to prepare both isomers at C-1′ for compound **14f**. The inhibitory data showed that the isomer that bound in the active site of neuraminidase was more active compared to the other isomer. This is the case in BCX-1812 and BCX-1827 also, but in this case the different isomer at C-1′ is active. Table 2 shows the difference in the activity of both isomers of **14fA′** and **B′**. The analogues of the active isomer of **14f**, **15mA′**, and **15gA′** were also prepared, and these were also found to possess little better neuraminidase inhibitory activity than the corresponding mixture of isomers. Since these compounds (**14fA′**, **15mA′**, and **15gA′**) have a mixture of isomers at C-1 also and only one isomer was found in the active site, we decided to prepare this isomer (**15gA′A**), hoping that it would have better activity. This isomer of **15gA′** when prepared in pure form was found to have little improvement on neuraminidase inhibition activity on A. These results are presented in Table 2. In this case also we identified an active isomer from the mixture of isomers as we did in our previous work on neuraminidase inhibitors.[11] Since these compounds were not found to have good inhibitory activity on neuraminidase B compared to BCX-1812 and 1827, further attempts were not made to prepare the single enantiomer.

We also explored 1-carboxy-1-hydroxy derivatives of the best of the active types, the diethylamide (**15g**) and the dipropylamide (**15m**). We prepared the mixture of isomers at C-1′ but with the single isomer at C-1 having the same stereochemistry of carboxylic group as in the most active compound in the series without the hydroxy group (**15gA′A**). The compounds of this type (**30A** and **31A**) were found to possess poor inhibitory activity on both neuraminidases A and B. We also explored the other isomer at C-1, where the carboxyl group was trans to the side chain having amide, and prepared both isomers at C-1′ (**37A′** and **37B′**). In this case also, there was poor activity on both neuraminidases A and B. The results are given in Table 2.

b. Influenza A Mouse Model. The better neuraminidase inhibitors of this study include the diethylamide types **15g**, **15gA′**, and **15gA′A**. Compound **15gA′** was selected as representative and was tested in vivo against influenza-infected mice.

In the mouse influenza model, viral infection leads to loss of body weight, and the decrease in body weight correlates with pulmonary viral titer and pulmonary lesion score.[28] Decrease in body weight is also directly correlated to the dose used for viral challenge. In general, the higher the viral challenge dose, the higher

Table 3. % Maximum Weight Loss in Influenza A (H6N2) Infected Mice Treated with **15gA′**

dose, (mg/kg)/day b.i.d.	% max wt loss (±SEM)
0	27.2 ± 1.75
0.1	12.7 ± 3.80[a]
1	8.7 ± 2.34[a]
10	3.1 ± 0.40[a]

[a] $P < 0.0001$ vs no drug.

the weight loss, which could result in mortality. In our studies the viral challenge dose used caused a maximum of 27% weight loss, and no mortality was observed. The efficacy of intranasally administered **15gA′** was evaluated on the basis of the weight loss measured each day for 15 days postinfection for treated, infected animals relative to untreated infected (control) animals. The maximum weight loss generally occurred on day 8 or 9 in the infected group. Vehicle-treated influenza-infected animals lost approximately 27% of their body weight. Treatment of the infected mice with **15gA′** prevented weight loss in a dose-dependent manner. In the 0.1 mg/kg dose group, the weight loss was 12.7%; in the 1.0 mg/kg dose group, the weight loss was 8.7%; and in the 10 mg/kg dose group, the weight loss was 3.1% of their body weight. These data (summarized in Table 3) indicate that **15gA′** administered intranasally is effective in the influenza-infected mouse model at doses as low as 0.1 (mg/kg)/day. However, the compound **15gA′** when tested orally in mice at 10 and 100 mg/kg dose demonstrated no decrease in weight loss compared to untreated animals.

Conclusion

Our studies have discovered a new series of cyclopentane amides that have high selectivity for influenza A neuraminidase. Diethyl grouping on amide was found to be the most appropriate for the activity. The introduction of the hydroxyl group at C-1 had an adverse effect on the activity. The IC_{50} value of the most active compound in this series is very comparable to the IC_{50} value in the dihydropyran series of amides. The binding of these compounds in the active site of neuraminidase A was also similar to the dihydropyran series but different from the cyclopentane alkane series. The compounds were not found to be effective orally but were significantly effective when administered intranasally.

Experimental Section

Commercially available solvents and reagents were used as received. All reactions were conducted under a dry nitrogen atmosphere. Melting points were obtained in open capillary tubes in a Mel-Temp II melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Mass spectra were obtained on a Fison Trio 2000 quadrupole mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM400 or Bruker AM360 spectrometer using tetramethylsilane as the internal standard. IR spectra were run on a Biorad FTS-7 FTIR spectrometer. Flash column chromatography was carried out using 230−400 mesh silica gel. Thin-layer chromatography was used as an indicator for the completion of the reactions and was performed on K 6 F silica gel 60A plates. The spots on TLC were visualized by UV and/or spraying the plate with 1 M ammonium sulfate in 1 N sulfuric acid and heating the plate on a hot plate. Organic solvent extracts in the isolation procedures were dried over anhydrous magnesium sulfate. Abbreviations used are the following: Bn, benzyl; THF, tetrahydrofuran; DMF, dimethylformamide; TFA, trifluoroacetic acid.

General Methods. A. Conversion of Ketone to Alcohol. To a mixture of a ketone (25.0 mmol) in methanol (100 mL) was added sodium borohydride (12.5 mmol) in portions over a period of 5 min at room temperature. The reaction mixture was further stirred for 10 min, and then acetic acid (1 mL) was added and the mixture was concentrated. The residue was dissolved in ethyl acetate (100 mL), washed with water (1 × 100 mL) and brine (1 × 100 mL), and dried. After filtration, the filtrate was concentrated to give the desired alcohol.

B. Conversion of Diester to Monoester. A mixture of the diester (25.0 mmol) and sodium hydroxide (1 N, 75.0 mmol) was stirred for 16 h at room temperature. Acetic acid (glacial, 20 mL) was added, and the mixture was heated at gentle reflux for 2 h. The mixture was then extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (1 × 100 mL) and dried. After filtration, the filtrate was concentrated and the residue was passed through a column of silica gel using ethyl acetate as an eluent to give the desired monoester.

C. Conversion of Azide to Amine. A mixture of azido compound (25.0 mmol) in ethyl acetate (200 mL) was hydrogenated in the presence of 10% palladium-on-carbon (0.5 g) at 40 psi and room temperature for 16 h. The catalyst was removed by filtration and the filtrate was concentrated to give the desired amine.

D. Conversion of Amine to DiBoc-Protected Guanidine. To a mixture of amine (25.0 mmol) in dimethylformamide (60 mL) was added triethylamine (75.0 mmol). The mixture was cooled in an ice bath and bis(N-*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (25.0 mmol) was added, the mixture was stirred for 10 min, and mercury(II) chloride (25.0 mmol) added. The reaction mixture was stirred at ice bath temperature for 1 h, diluted with ethyl acetate (250 mL), and filtered through Celite. The filtrate was washed with water (1 × 200 mL) and brine (1 × 200 mL) and dried. After filtration, the filtrate was concentrated and the residue was passed through a column of silica gel (200 g) using ethyl acetate as an eluent to give the desired protected guanidine.

E. Conversion of Alcohol to Ketone. To a mixture of alcohol (25.0 mmol) in dichloromethane (225 mL) was added pyridinium chlorochromate (75.0 mmol), and the mixture was stirred at room temperature for 16 h. The mixture was diluted with ether, the precipitate was filtered through Celite, and the filtrate was concentrated. The residue was passed through a column of silica gel (500 g) using ethyl acetate/hexane (1:1) as an eluent to give the desired ketone. When the isomers were separated, a column with more silica gel or the repeated chromatography and fractional crystallization were used.

F. Conversion of Ketone to 1,3-Dithiane. To a mixture of 2-trimethylsilyl-1,3-dithiane (175.0 mmol) in THF (300 mL) at 0 °C was added *n*-butyllithium (1.6 M, 175.0 mmol), and the mixture was stirred at 0 °C for 1 h. After the mixture was cooled to −42 °C, a solution of ketone (25.0 mmol) in THF (100 mL) was added over a period of 0.5 h and the mixture was further stirred at −42 °C for 3 h. The mixture was then quenched with saturated ammonium chloride (100 mL) and brought to room temperature. The organic layer was separated, and water (200 mL) was added to the aqueous layer and extracted with ethyl acetate (2 × 400 mL). The combined organic layers were washed with water (1 × 500 mL) and brine (1 × 500 mL) and dried. After filtration, the filtrate was concentrated and the residue was passed through a column of silica gel using ethyl acetate and hexane as eluent to give the desired dithiane compound. When the isomers were separated, a column with more silica gel or the repeated chromatography and fractional crystallization were used.

G. Conversion of Monoester to Acid. To a solution of monoester (25.0 mmol) in ethanol (125 mL) and THF (250 mL) was added 0.5 N NaOH (100 mL), and the mixture was stirred at room temperature for 5 h. Most of the organic solvent was removed under vacuum, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The aqueous layer was

acidified with acetic acid and the solid obtained was collected by filtration, washed with water, and dried to give the desired acid.

H. Conversion of Acid to Amide. To a mixture of acid (1.0 mmol) in THF (8 mL) at 0 °C was added triethylamine (1.1 mmol) and methyl chloroformate (1.1 mmol), and the mixture was stirred for 0.5 h. To this mixture was then added the appropriate amine (2.3 mmol), and the mixture was further stirred at 0 °C for 1 h and at room temperature for 16 h. It was partitioned between ethyl acetate (25 mL) and water (25 mL). The organic layer was washed with water (25 mL) and brine (25 mL) and dried. After filtration, the filtrate was concentrated and, if required, was purified on a silica gel column to give the desired amide.

I. Conversion of Dithiane to Methyl Ester or Acid. To a solution of dithiane compound (1.0 mmol) was added methanolic hydrochloric acid (0.5 N, 25 mL), and the mixture was stirred for 16 h at room temperature and then carefully neutralized with methanolic sodium hydroxide and stirred for 0.5 h. After concentration, the residue was used as such for further reaction or was purified on silica gel column using appropriate eluent. If the required compound is acid, the solution of methanolic HCl hydrolysis is basified with NaOH and the mixture was stirred for 2 h and then neutralized with HCl and concentrated. If the compound contains Boc-protected amine or guanidine, it also get deprotected.

J. Conversion of Boc-Protected Amine or Guanidine to the Corresponding Amine or Guanidine. To a solution of Boc-protected guanidine (1.0 mmol) in dichloromethane (25 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 16 h at room temperature and then concentrated to give the desired guanidino compound as trifluoroacetic acid salt.

K. Conversion of Ketone to Tris(methylthio)methyl and Hydroxy Derivative. To a mixture of tris(methylthio)-methane (50.0 mmol) in THF (80 mL) at −78 °C was added *n*-butyllithium (1.6 M, 55.0 mmol). After the mixture was stirred at −78 °C for 1 h, a solution of ketone (10.0 mmol) in THF (60 mL) was added over a period of 0.5 h and the mixture was further stirred at −78 °C for 3 h. The mixture was then quenched with saturated ammonium chloride (60 mL) and brought to room temperature. The organic layer was separated, and water (100 mL) was added to the aqueous layer and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with water (1 × 200 mL) and brine (1 × 200 mL) and dried. After filtration, the filtrate was concentrated and the residue was passed through a column of silica gel using ethyl acetate and hexane as eluent to give the desired compound. The isomers were separated on a silica gel column.

L. Conversion of Diester to Monoacid. A mixture of diester (3.0 mmol), ethanol (15.0 mL), and 1 N NaOH (15.0 mL) was heated at reflux for 2 h, acidified with acetic acid, and again heated at reflux for 1 h. After concentration, water (50 mL) was added and extracted with dichloromethane (2 × 50 mL). The combined organic extracts were dried, filtered, and concentrated to give the desired acid, which was used as such for further reactions.

M. Conversion of Tris(methylthio)methyl to Methyl Ester. To a solution of tris(methylthio)methyl compound (1.0 mmol) in methanol (23 mL) and water (2 mL) were added mercury(II) chloride (3.6 mmol) and mercury(II) oxide (1.6 mmol), and the mixture was stirred for 2 h. The solids were removed by filtration and washed with dichloromethane (50 mL). To the filtrate was added water (50 mL), and the organic layer separated. The aqueous layer was extracted again with dichloromethane (50 mL), and the combined organic layers were dried. After filtration, the filtrate was concentrated and purified on a silica gel column.

N. Conversion of Methyl Ester to Acid. A mixture of the ester (1.0 mmol) in THF (10 mL) and ethanol (5 mL) was treated with 1 N NaOH (3 mL), and the mixture was stirred for 2 h. After neutralization with acetic acid, the mixture on concentration yielded a solid, which was collected by filtration, washed with water, and dried to give the desired acid.

Compound 7 was prepared from **6** following general procedure A, and the product was obtained as a light-brown oil (yield 100%). ^{1}H NMR ($CDCl_3$): δ 8.30 and 8.35 (two s, 2H, D_2O exchangeable), 4.71−5.00 (m, 2H, D_2O exchangeable), 3.99−4.18 (m, 10H), 3.63−3.69 (m, 1H), 2.94−3.01 (m, 1H), 2.65−2.72 (m, 1H), 2.06−2.16 (m, 3H), 1.8−1.9 (2s, 6H) 1.70−1.77 (m, 2H), 1.54−1.59 (m, 1H), 1.32−1.48 (m, 3H), 1.11−1.19 (m, 12H). IR (neat): 3397 (br), 2984, 2098, 1764, 1505 cm^{-1}. MS (ES$^+$), *m*/*z*: 343.5. Anal. ($C_{14}H_{22}N_4O_6$) C, H, N.

Compound 8 was prepared from **7** following general procedure B, and the product was obtained as a colorless syrup (yield 63%). ^{1}H NMR (DMSO-d_6): δ 8.26 and 8.29 (two s, 2H, D_2O exchangeable), 4.42−4.46 (m, 2H), 4.04−4.13 (m, 7H), 3.80−3.95 (m, 1H), 3.60−3.66 (m, 2H), 2.48−2.56 (m, 2H), 2.20−2.28 (m, 2H), 1.86 and 1.87 (2s, 6H), 1.64−1.70 (m, 2H), 1.44−1.55 (m, 2H), 1.37−1.43 (m, 2H), 1.17−1.22 (m, 6H). IR (neat): 3302 (br), 2980, 2100, 1743, 1664, 1542, 1374 cm^{-1}. MS (ES$^+$), *m*/*z*: 271.2. Anal. ($C_{11}H_{18}N_4O_4$) C, H, N.

Compound 10 was prepared from **8** following general procedures C and D, and the product was obtained as a white foam (yield 89%), mp 75−80 °C. Since it is a mixture of four isomers, it is very difficult to separate cyclopentane ring protons using ^{1}H NMR spectrometry. The broad range of the protons is the following. ^{1}H NMR ($CDCl_3$): δ 1.4−3.0, 4.0−4.9 (cyclopentene ring, CH, and OH), 7.2−11.5 (NH, D_2O exchangeable), 4.04−4.21 ($CO_2CH_2CH_3$), 2.00−2.20 ($NHCOCH_3$), 1.48−1.59 (*tert*-butoxy), 1.19−1.28 ($CO_2CH_2CH_3$). IR (KBr): 3301, 2981, 1735, 1648, 1613, 1416, 1369 cm^{-1}. MS (ES$^+$), *m*/*z*: 487.8. Anal. ($C_{22}H_{38}N_4O_8$) C, H, N.

Compound 11 was prepared from **10** following general procedure E, and the product was obtained as a white foam (yield 73%). Compounds **11A′** and **11B′** were separated by silica gel chromatography and fractional crystallization from the mixture of isomers of compound **11**.

11A′. ^{1}H NMR (DMSO-d_6): δ 11.5 (s, 1H, D_2O exchangeable), 8.36−8.39 (bs, 1H, D_2O exchangeable), 8.09 (d, J = 9.0 Hz, 1H, D_2O exchangeable), 4.54−4.63 (m, 2H), 3.98 (q, J = 7.1 Hz, 2H), 2.96−2.99 (m, 1H), 2.35−2.55 (m, 3H), 1.97−2.05 (m, 1H), 1.94 (s, 3H), 1.41 and 1.47 (2 s, 18H), 1.15 (t, J = 7.1 Hz, 3H). IR (KBr): 3328, 2986, 1730, 1648, 1612, 1515 cm^{-1}. MS (ES$^+$), *m*/*z*: 485.7. Anal. ($C_{22}H_{36}N_4O_8$) C, H, N.

11B′. ^{1}H NMR (DMSO-d_6): δ 11.5 (s, 1H, D_2O exchangeable), 8.34 (d, J = 8.7 Hz, 1H, D_2O exchangeable), 8.20 (d, J = 7.7 Hz, 1H, D_2O exchangeable), 4.64−4.69 (m, 1H), 4.40−4.44 (m, 1H), 3.99−4.07 (m, 2H), 2.87−2.91 (m, 1H), 2.42−2.55 (m, 1H), 2.20−2.33 (m, 3H), 1.86 (s, 3H), 1.37 and 1.48 (two s, 18H), 1.16 (t, J = 6.9 Hz, 3H). IR (KBr): 3328, 2984, 1733, 1648, 1613 cm^1. MS (ES$^+$), *m*/*z*: 485.7. Anal. ($C_{22}H_{36}N_4O_8$) C, H, N.

Compound 12 was prepared from **11** following general procedure F, and the product was obtained as a white foam (yield 70%). Compounds **12A′** and **12B′** were separated by silica gel chromatography and fractional crystallization from the mixture of isomers of compound **12.**

12A′. ^{1}H NMR (DMSO-d_6): δ 11.46 (s, 1H, D_2O exchangeable), 8.24−8.26 (m, 1H, D_2O exchangeable), 8.10 (d, J = 8.8 Hz, 1H), 4.52−4.55 (m, 1H), 4.17−4.19 (m, 1H), 3.97 (q, J = 7.2 Hz, 2H), 2.74−2.88 (m, 6H), 2.54−2.66 (m, 2H), 1.97−2.02 (m, 3H), 1.93 (s, 3H), 1.41 and 1.46 (two s, 18H), 1.15 (t, J = 7.0 Hz, 3H). IR (KBr): 3282, 2982, 1747, 1722, 1676, 1649, 1613, 1532 cm^{-1}. MS (ES$^+$), *m*/*z*: 587.8. Anal. ($C_{26}H_{42}N_4O_7S_2$) C, H, N.

12B′. ^{1}H NMR (DMSO-d_6): δ 11.46 (s, 1H, D_2O exchangeable), 8.20−8.22 (m, 2H, D_2O exchangeable), 4.20−4.50 (m, 2H), 4.05 (q, J = 7.0 Hz, 2H), 2.80−2.95 (m, 6H), 2.29−2.36 (m, 3H), 2.02−2.04 (m, 2H), 1.85 (s, 3H), 1.38 and 1.48 (two s, 18H), 1.17 (t, J = 7.0 Hz, 3H). IR (KBr): 3282, 2982, 1747, 1722, 1676, 1649, 1613, 1532 cm^{-1}. MS (ES$^+$), *m*/*z*: 587.8. Anal. ($C_{26}H_{42}N_4O_7S_2$) C, H, N.

Compound 13 was prepared from **12** following general procedure G, and the product was obtained as a white foam (yield 70%).

13A′ was prepared from **12A′** following general procedure G, and the product was obtained as a white solid (yield 80%). ^{1}H NMR (DMSO-d_6): δ 12.70 (br s, 1H), 11.5 (s, 1 H), 8.29 (d, J = 8.0 Hz, 1 H), 8.07 (d, J = 9.0 Hz, 1 H), 4.45 (dd, J = 9.0 and 3.0 Hz, 1 H), 4.13 (m, 1 H), 2.82 (m, 5 H), 2.57 (m, 2 H), 2.35 (m, 1 H), 2.01 (m, 3 H), 1.91 (s, 3 H), 1.47 (s, 9 H), 1.40 (s, 9 H). IR (KBr): 3323, 2980, 1732, 1713, 1639, 1137 cm^{-1}. MS (ES$^+$), *m/z*: 559.5. Anal. ($C_{24}H_{38}N_4O_7S_2 \cdot 0.75C_2H_6O$) C, H, N.

13B′ was prepared from **12B′** following general procedure G, and the product was obtained as a white solid (yield 81%). ^{1}H NMR (DMSO-d_6): δ 12.67 (br s, 1H), 11.45 (s, 1 H), 8.29 (d, J = 8.1 Hz, 1 H), 8.06 (d, J = 9.0 Hz, 1 H), 4.47 (m, 1H), 4.44 (m, 1H), 4.10−4.15 (m, 1 H), 3.30−3.43 (m, 1H), 2.75−2.88 (m, 4 H), 2.47−2.60 (m, 2 H), 2.35 (m, 1 H), 2.00 (m, 2H), 1.91 (s, 3 H), 1.46 (s, 9 H), 1.40 (s, 9 H). IR (KBr): 3323, 2980, 1732, 1713, 1639, 1137 cm^{-1}. MS (ES$^+$), *m/z*: 559.5. Anal. ($C_{24}H_{38}N_4O_7S_2 \cdot 0.75C_2H_6O$) C, H, N.

Compounds 14−16. These compounds were prepared from **13** following general procedures H−J and D. Compound **13** having same isomeric ratio at C-1′ (60% **A′** and 40% **B′**) was used for the preparation of these compounds. All prepared compounds were characterized by LC/MS and were found having purity >80% (all desired isomers collectively). The compounds were constituted to have concentration of 20 μM and given for biological screening in solution to obtain comparative IC$_{50}$. Mass spectral data are reported in Table 1.

Compounds 14fA′ and **15mA′** were prepared from **13A′** following methods H−J and D. Compounds were characterized by LC/MS and submitted for biological testing.

Compound 15gA′ was prepared from **13A′** following methods H−J and D. ^{1}H NMR (DMSO-d_6 + D_2O): δ 4.75−4.76 (m, 1H), 3.75−3.77 (m, 1H), 3.32−3.39 (m, 3H), 3.12−3.15 (m, 1H), 2.79−2.81 (m, 1H), 1.95−2.32 (m, 3H), 1.82−1.83 (2s, 3H), 1.69−1.71 (m, 1H), 1.37−1.41 (m, 1H), 1.00−1.10 (m, 6H). MS (ES$^+$), *m/z*: 342.3. Anal. ($C_{15}H_{27}N_5O_4 \cdot CF_3CO_2H \cdot H_2O$) C, H, N.

Compound 14fB′ was prepared from **13B′** following methods H−J and D and was characterized by LC/MS and submitted for biological testing.

Compound 19A′. Compound **17** was converted to **18** following method H, and the isomers at C-1′ were separated by repeated fractional crystallization from ethyl acetate to give compound **18A′**, which was further converted to **19A′** following method I. ^{1}H NMR (DMSO-d_6): δ 8.15−8.19 (m, 1H, D_2O exchangeable), 4.63−4.70 (m, 1H), 3.56 (s, 3H), 3.35−3.50 (m, 3H), 3.30 (br s, 2H, D_2O exchangeable), 2.68−3.15 (m, 3H), 1.84−2.08 (m, 3H), 1.83 (s, 3H), 1.34−1.74 (m, 2H), 1.11 (t, J = 7.0 Hz, 3H), 0.99 (t, J = 7.0 Hz, 3H). IR (KBr): 3265, 2971, 1722, 1673, 1614 cm^{-1}. MS (ES$^+$), *m/z*: 314.0. Anal. ($C_{15}H_{27}N_3O_4$) C, H, N.

Compound 20A′A. Compound **19A′** was converted to compound **20A′** following method D, and **20A′** on fractional crystallization from ether/hexane yielded **20A′A** in 13% yield as a single isomer. ^{1}H NMR ($CDCl_3$): δ 11.44 (s, 1H, with D_2O exchangeable), 8.41 (d, J = 7.5 Hz, 1H, D_2O exchangeable), 8.11 (d, J = 8.6 Hz, 1H, D_2O exchangeable), 4.80 (m, 1H), 4.20 (m, 1H), 3.68 (s, 3H), 3.18−3.57 (m, 4H), 2.98 (m, 1H), 2.58 (m, 1H), 2.28 (m, 1H), 2.18 (m, 1H), 2.11 (s, 3H), 1.90 (m, 1H), 1.58 (m, 1H), 1.48 and 1.49 (two s, 18H), 1.22 (t, J = 7.0 Hz, 3H), 1.06 (t, J = 7.0 Hz, 3H). IR (KBr): 3331, 2978, 1732, 1658, 1418 cm^{-1}. MS (ES$^+$), *m/z*: 556.5. Anal. ($C_{26}H_{45}N_5O_8$) C, H, N.

Compound 15gA′A was prepared from compound **20A′A** following methods N and J. ^{1}H NMR (DMSO-d_6): δ 12.24 (s, 1H, D_2O exchangeable), 8.12 (d, J = 9.3 Hz, 1H, D_2O exchangeable), 7.69 (d, J = 7.8 Hz, 1H, D_2O exchangeable), 6.4−7.4 (br m, 3H, D_2O exchangeable), 4.75 (m, 1H), 3.77 (br s, 1H), 3.32−3.38 (m, 3H), 3.15 (m, 1H), 2.81 (m, 1H), 1.97−2.31 (m, 3H), 1.83 (s, 3H), 1.69 (m, 1H), 1.37−1.41 (m, 1H), 1.09 (t, J = 7.0 Hz, 3H), 1.00 (t, J = 7.0 Hz, 3H). IR (KBr): 3425, 2983, 1675, 1627, 1541, 1203 cm^{-1}. MS (ES$^+$), *m/z*: 342.3. Anal. ($C_{15}H_{27}N_5O_4 \cdot CF_3CO_2H$) C, H, N.

Compound 22A was prepared from compound **21**[11] following general method K and was separated by silica gel chromatography using ethyl acetate/hexane from **22B** (yield 35%). ^{1}H NMR ($CDCl_3$): δ 7.57 (s, 1H), 5.40 (d, J = 9.0 Hz, 1H), 4.31−4.21 (m, 4H), 3.95−3.90 (m, 1H), 3.17 (s, 1H), 3.0−2.96 (m, 1H), 2.53−2.49 (m, 1H), 2.43−2.40 (m, 1H), 2.25 (s, 9H), 2.14−2.12 (m, 1H), 2.03 (s, 3H), 1.76 (d, J = 17.0 Hz, 1H), 1.43 (s, 9H), 1.30−1.26 (m, 6H). IR (NaCl): 3383, 2981, 1738, 1688, 1526, 1369, 1274, 1206, 1168 cm^{-1}. MS (ES$^+$), *m/z*: 569.3. Anal. ($C_{23}H_{40}N_2O_8S_3$) C, H, N.

Compound 30A. Was prepared from compound **22A** following general methods L, H, M, J, D, N, and J. ^{1}H NMR (DMSO-d_6): δ 12.60 (br s, 1H, D_2O exchangeable), 8.10−8.28 (m, 1H, D_2O exchangeable), 5.46−7.50 (br m, 4H, D_2O exchangeable), 5.46 (br s, 1H, D_2O exchangeable), 4.84−4.91 (m, 1H), 3.03−3.93 (m, 6H), 2.49−2.59 (m, 1H), 1.81−1.88 (m, 5H), 1.50−1.55 (m, 1H), 0.98−1.23 (m, 6H). IR (KBr): 3363, 1664, 1625, 1202 cm^{-1}. MS (ES$^+$), *m/z*: 358.5. Anal. ($C_{15}H_{27}N_5O_5 \cdot {}_5/_3CF_3COOH$) C, H, N.

Compound 31A was prepared from compound **22A** following general methods L, H, M, J, D, N, and J. ^{1}H NMR (DMSO-d_6): δ 8.30 and 8.10 (d, J = 9.0 Hz, 1H), 7.53−6.81 (m, 5H, D_2O exchangeable), 4.90−4.80 (m, 1H), 3.88 and 3.67 (m, 2H), 3.38−3.18 (m, 3H), 3.05−2.95 (m, 2H), 1.89 and 1.82 (s, 3H), 1.71−1.67 (m, 2H), 1.54−1.43 (m, 6H), 0.84−0.78 (m, 6H). IR (KBr): 3384, 2972, 1670, 1625, 1436, 1203, 1139 cm^{-1}. MS (ES$^+$), *m/z*: 386.3. Anal. ($C_{17}H_{31}N_5O_5 \cdot 1.25CF_3COOH$) C, H, N.

Compound 32A′ was prepared from compound **11A′** following method K. The separation of isomers was achieved by silica gel chromatography, and **32A′** was obtained in 66% yield as a white solid, mp 155 °C. ^{1}H NMR ($CDCl_3$): δ 11.44 (s, 1H, D_2O exchangeable), 8.29 (d, J = 8.8 Hz, 1H, D_2O exchangeable), 8.14 (d, J = 9.5 Hz, 1H, D_2O exchangeable), 4.84 (dd, J = 1.8 Hz, 9.5 Hz, 1H), 4.50 (dd, J = 11.0 Hz, 18.0 Hz, 1H), 3.97−4.12 (m, 2H), 3.45−3.51 (m, 1H), 2.95 (s, 1H, D_2O exchangeable), 2.57−2.70 (m, 2H), 2.23−2.37 (m, 10H), 2.08−2.19 (m, 4H), 1.49 and 1.51 (two s, 18H), 1.20 (t, J = 7.2 Hz, 3H). IR (KBr): 3462, 3279, 1746, 1723, 1652, 1614 cm^{-1}. MS (ES$^+$), *m/z*: 639.4. Anal. ($C_{26}H_{46}N_4O_8S_3$) C, H, N.

Compound 32B′ was prepared from compound **11B′** following method K. The separation of isomers was achieved by silica gel chromatography, and **32B′** was obtained in 34% yield as a white solid, mp 94−96 °C. ^{1}H NMR ($CDCl_3$): δ 11.43 (s, 1H, D_2O exchangeable), 8.39 (d, J = 8.2 Hz, 1H, D_2O exchangeable), 7.25 (d, J = 5.6 Hz, 1H, D_2O exchangeable), 4.68 (m, 1H), 4.46 (dd, J = 7.1, 4.1 Hz, 1H), 4.18 (q, J = 7.0 Hz, 2H), 3.36 (s, 1 H), 2.48 (m, 2H), 2.27 (s, 9H), 2.14 (m, 2H), 2.03 (s, 3H), 1.84 (m, 1H), 1.50 (s, 9H), 1.48 (s, 9H), 1.28 (t, J = 7.0 Hz, 3H). IR (KBr): 3322, 2981, 1723, 1641, 1615, 1142 cm^{-1}. MS (ES$^+$), *m/z*: 639.5. Anal. ($C_{26}H_{46}N_4O_8S_3$) C, H, N.

Compound 35A′ was prepared from compound **32A′** following methods G, H, and M. ^{1}H NMR ($CDCl_3$): δ 11.43 (s, 1H, D_2O exchangeable), 8.58 (d, J = 8.0 Hz, 1H, D_2O exchangeable), 7.53 (d, J = 9.0 Hz, 1H, D_2O exchangeable), 5.01−5.04 (m, 1H), 4.54−4.58 (m, 1H), 3.86 (s, 3H), 3.34−3.47 (m, 2H), 3.34 (s, D_2O exchangeable, 1H), 3.25 (dd, J = 5.5 Hz, 9.5 Hz, 1H), 3.03−3.09 (m, 1H), 2.62−2.65 (m, 1H), 2.33−2.43 (m, 2H), 2.07−2.21 (m, 4H), 1.51−1.70 (m, 5H), 1.47 and 1.49 (two s, 18H), 0.83−0.93 (m, 6H). IR (KBr): 3319, 2971, 1725, 1644, 1625, 1420 cm^{-1}. MS (ES$^+$), *m/z*: 600.5. Anal. ($C_{28}H_{49}N_5O_9$) C, H, N.

Compound 35B′ was prepared from compound **32B′** following methods G, H, and M. ^{1}H NMR ($CDCl_3$): δ 11.39 (s, 1H, D_2O exchangeable), 8.62 (d, J = 8.2 Hz, 1H, D_2O exchangeable), 7.59 (d, J = 9.0 Hz, 1H, D_2O exchangeable), 5.02−5.11 (m, 2H), 4.18 (s, D_2O exchangeable, 1H), 3.82 (s, 3H), 3.24−3.34 (m, 4H), 2.40−2.49 (m, 3H), 2.04−2.15 (m, 1H), 1.86−1.94 (m, 4H), 1.47−1.67 (m, 22H), 0.84−0.96 (m, 6H). IR (KBr): 3287, 2977, 1727, 1624 cm^{-1}. MS (ES$^+$), *m/z*: 600.6. Anal. ($C_{28}H_{49}N_5O_9$) C, H, N.

Compound 37A′ was prepared from compound **35A′** following methods N and J. ^{1}H NMR (DMSO-d_6): δ 8.04 (d, J = 8.2 Hz, 1H, D_2O exchangeable), 7.64 (br s, 1H, D_2O exchangeable), 6.5−7.5 (br hump, 4H, D_2O exchangeable), 4.80−5.00 (m, 1H), 3.90−4.00 (m, 1H), 3.20−3.50 (m, 5H), 2.90−3.10 (m, 1H), 2.05−2.20 (m, 2H), 1.80−1.95 (m, 4H), 1.30−1.60 (m, 4H), 0.70−0.90 (m, 6H). IR (KBr): 3363, 1672, 1624, 1457 cm^{-1}. MS (ES$^+$), *m/z*: 386.6. Anal. ($C_{17}H_{31}N_5O_5 \cdot CF_3COOH$) C, H, N.

Compound 37B′ was prepared from compound **35B′** following methods N and J. ^{1}H NMR (DMSO-d_6): δ 12.64 (br s, 1H, D_2O exchangeable), 8.12 (d, J = 8.9 Hz, 1H, D_2O exchangeable), 7.57 (d, J = 9.0 Hz, 1H, D_2O exchangeable), 6.60−7.50 (br hump, 4H, D_2O exchangeable), 5.28 (br s, 1H), 4.87 (dd, J = 4.2 Hz, 8.9 Hz, 1H), 3.78−3.83 (m, 1H), 2.93−3.40 (m, 3H), 2.36−2.49 (m, 1H), 2.04−2.15 (m, 2H), 1.89−1.93 (m, 4H), 1.76 (dd, J = 8.4 Hz, 13.5 Hz, 1H), 1.42−1.52 (m, 4 H), 0.76−0.84 (m, 6H). IR (KBr): 3356, 2970, 1668, 1608, 1205 cm^{-1}. MS (ES$^+$), *m/z*: 386.2. Anal. ($C_{17}H_{31}N_5O_5 \cdot CF_3$-COOH) C, H, N.

Neuraminidase Inhibition Assay. A standard fluorimetric assay was used to measure influenza virus neuraminidase activity.[29] The substrate 2′-(4-methylumbelliferyl)-α-D-acetylneuraminic acid is cleaved by neuraminidase to yield a fluorescent product that can be quantified. The assay mixture contained inhibitor at various concentrations and neuraminidase enzyme or virus suspension in 32.5 mM MES, 2-(*N*-morpholino)ethanesulfonic acid, and buffer (4 mM calcium chloride at pH 6.5) and incubated for 10−30 min. The reaction was started by the addition of the substrate. After incubation for 0.5−2 h (different times for different viruses), the reaction was terminated by adding 0.2 M glycine−NaOH, pH 10.2 or 0.034 M NaOH in water. Fluorescence was recorded (excitation: 360 nM and emission: 450 nM), and substrate blanks were subtracted from the sample readings. The IC_{50} was calculated by plotting percent inhibition of neuraminidase activity versus the inhibitor concentration. The results are reported as the average of two to seven experiments. For slow-binding inhibition studies, the neuraminidase activity was followed over time either with preincubation with inhibitor for 20 min or with inhibitor added at the same time as the substrate to initiate the reaction. The initial rates determined from the slopes (increase in fluorescence with time) were plotted against the substrate concentration to obtain the IC_{50} values.

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