Structure–Activity Relationship Studies of Novel Carbocyclic Influenza Neuraminidase Inhibitors

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A series of influenza neuraminidase inhibitors with the cyclohexene scaffold containing lipophilic side chains have been synthesized and evaluated for influenza A and B neuraminidase inhibitory activity. The size and geometry of side chains have been modified systematically in order to investigate structure–activity relationships of this class of compounds. The X-ray crystal structures of several analogues complexed with neuraminidase revealed that the lipophilic side chains bound to the hydrophobic pocket consisted of Glu276, Ala246, Arg224, and Ile222 of the enzyme active site. The structure–activity relationship studies of this series have also demonstrated remarkably different inhibitory potency between influenza A and B neuraminidase. This indicated that the lipophilic side chains had quite different hydrophobic interactions with influenza A and B neuraminidase despite their complete homology in the active site. Influenza B neuraminidase appeared to be much more sensitive toward the increased steric bulkiness of inhibitors compared to influenza A neuraminidase. From the extensive structure–activity relationship investigation reported in this article, GS 4071 emerged as one of the most potent influenza neuraminidase inhibitors against both influenza A and B strains.

Introduction

Influenza neuraminidase (NA) catalyzes the cleavage of the terminal sialic acid attached to glycoproteins and glycolipids.[1,2] This process is believed to be necessary for the release of newly formed virus from infected cells and for efficient spread of virus in the respiratory tract.[3,4] Therefore, NA is recognized as a potential target for developing agents against influenza infection. It has been proposed that the sialic acid cleavage by NA might proceed via the oxonium cation transition state **3** (Scheme 1).[5,7] Zanamivir (**5**) is a transition state analogue of **3** which exhibits the potent NA inhibitory activity[8] and is currently being evaluated in phase III clinical trials via the topical delivery to the respiratory tract.[9,10] Aiming at developing oral agents against influenza infection, we have designed new carbocyclic NA inhibitors **6** with various lipophilic alkyl side chains (R_1) in order to examine NA inhibitory activity and oral bioavailability. In this series, it was found that the size and geometry of the alkyl side chains significantly influenced NA inhibitory activity. In fact, X-ray crystallographic studies of several analogues in this series complexed with NA revealed that the alkyl groups (R_1 in **6**) bound into a hitherto unreported hydrophobic pocket in the NA active site.[11] The structure–activity relationship (SAR) studies of this novel series identified GS 4071 (**7**) as a potent NA inhibitor with IC_{50} of 1 nM.[11] Subsequently, GS 4104 (**8**, the ethyl ester prodrug of **7**) was found to be highly orally bioavailable in several animal species and efficacious in both the mouse and ferret models of influenza infection by oral administration.[12] In recent phase II clinical trials, oral efficacy of GS 4104 has been demonstrated both in prophylaxis and treatment of human influenza infection.[13] GS 4104 is currently in phase II/III clinical trials for the prophylaxis and treatment of influenza virus infection.

Scheme 1

1 R = H
2 R = sugars
3
4 X = NH_2
5 X = $C(=NH)NH_2$-NH (Zanamivir)
6 R_1 = alkyls, R_2 = H
7 R_1 = 3-pentyl, R_2 = H (GS 4071)
8 R_1 = 3-pentyl, R_2 = Et (GS 4104)

Herein are described the SAR studies of carbocyclic NA inhibitors against influenza A and B viral neuraminidases. In addition to the series of the C_3 modification, several C_2 and C_6 substituted analogues of GS 4071 (**7**) were examined in order to define further structural features for optimal NA inhibitory activity.

Chemistry

The carbocyclic analogues **6** were prepared according to Scheme 2 from a common intermediate, *N*-tritylaziri-

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Scheme 2

Scheme 3[a]

12a R_1 = cyclohexyl
12b R_1 = cyclohexyl methyl
12c R_1 = benzyl
12d R_1 = n-hexyl
12e R_1 = vinyl

13a R_1 = cyclohexyl
13b R_1 = cyclohexyl methyl
13c R_1 = benzyl
13d R_1 = n-hexyl
13e R_1 = vinyl

14a R_1 = cyclohexyl
14b R_1 = cyclohexyl methyl
14c R_1 = benzyl
14d R_1 = n-hexyl
14e R_1 = vinyl

[a] Reagents: (a) R_1MgBr, Li_2CuCl_4; (b) K_2CO_3, MeOH; (c) MeMgBr, CuI.

Scheme 4[a]

[a] Reagents: (a) *N,N'*-bis(*tert*-butoxycarbonyl)thiourea, $HgCl_2$, Et_3N, DMF; (b) KOH, THF/H_2O; (c) CF_3CO_2H, CH_2Cl_2.

dine **9** which was prepared from quinic acid by a previously described procedure.[11]

Enantiomerically pure alcohols **14a**–**e** were synthesized from (2*R*)-(−)-glycidal tosylate **11** according to Scheme 3. Regioselective ring opening of **11** with the corresponding Grignard reagent (R_1MgBr) under lithium tetrachlorocuprate catalysis provided hydroxy tosylate **12** which is cyclized to epoxide **13** with potassium carbonate in anhydrous methanol.[14] Epoxide **13** is converted to alcohol **14** by the copper(I) iodide catalyzed addition of methylmagnesium bromide. The (3*R*)-enantiomer of **14e** was prepared from the corresponding (2*S*)-(+)-glycidal tosylate in a similar manner.

The guanidino analogues **17** were prepared from the corresponding amino methyl esters **15** as shown in Scheme 4. Treatment of **15** with *N,N'*-bis(*tert*-butoxy carbonyl)thiourea[15] using mercury(II) chloride and triethylamine in DMF provided bis-BOC protected guanidine **16**. Saponification of **16** followed by removal of the BOC groups with trifluoroacetic acid furnished the guanidino analogues **17**.

The C_6-methyl analogue **22** was prepared according to Scheme 5. The 3-pentyl ethyl ester **8** was treated with di-*tert*-butyl dicarbonate followed by osmium tetraoxide to give diol **18**. Compound **18** was converted to allylic acetate **20** stereospecifically via a three-step

Scheme 5[a]

[a] Reagents: (a) $(Boc)_2O$, Et_3N; (b) OsO_4, NMO; (c) SO_2Cl_2, pyridine; (d) DBU, THF; (e) Ac_2O, pyridine; (f) Me_2CuLi, Et_2O; (g) HCl, EtOAc; (h) aqueous KOH, MeOH.

Scheme 6[a]

23 R = H, R' = MOM
24 R = CH_3, R' = H
DMB = 2,5-dimethoxy benzyl

25 R = H
26 R = CH_2OCH_3

27 Z = $CH_2OCH_2OCH_3$
28 Z = CHO
29 Z = $CO_2CH_2CH_3$

[a] Reagents: (a) *p*-TsOH, MeOH; (b) *p*-TsOH, 3-pentanone; (c) MsCl, Et_3N; (d) $BH_3{\cdot}Me_2S$, TMSOTf; (e) $KHCO_3$; (f) MOMCl, *i*-Pr_2EtN; (g) *p*-TsOH, MeOH; (h) oxalyl chloride, DMSO; (i) $NaClO_2$; (j) EtOH, 1,3-diisopropylcarbodiimide; (k) PPh_3, THF/H_2O; (l) KOH, THF.

sequence involving formation of the cyclic sulfate with sulfuryl chloride, elimination of the cyclic sulfate to the allylic alcohol with 1,8-diazabicyclo[5.4.0]undec-7-ene, and acetylation with acetic anhydride. Introduction of the C_6-methyl group was accomplished by stereo- and regioselective 1,4-addition of Me_2CuLi to **20** with concomitant elimination of the acetate group to provide **21**. Deprotection of **21** with anhydrous hydrochloric acid in ethyl acetate followed by saponification provided the C_6-methyl analogue **22**.

The C_2-fluoro analogue **30** was prepared according to Scheme 6. Shikimic acid was converted into fluoro alkene **23** by the procedure reported by Bartlett and

Rich.[16] Ketal exchange of **23** with 3-pentanone with catalytic *p*-toluenesulfonic acid provided **24**. Treatment of **24** with methanesulfonyl chloride followed by regioselective reductive cleavage of the ketal ring with $BH_3 \cdot Me_2S$ and treatment with potassium bicarbonate provided the epoxy alcohol **25**. The MOM protected epoxide **26** was converted to azide **27** in a stereo- and regioselective manner with sodium azide in the presence of ammonium chloride. Conversion of **27** into ethyl ester **29** was accomplished in four steps: (1) deprotection of the MOM group with catalytic *p*-toluenesulfonic acid in methanol, (2) Swern oxidation to aldehyde **28**, (3) oxidation of the aldehyde to the carboxylic acid with $NaClO_2$, (4) and esterification with ethanol and diisopropylcarbodiimide to give **29**. Reduction of the azide group of **29** with triphenylphosphine followed by saponification furnished the C_2-fluoro analogue **30**.

Results and Discussion

A main objective of the present research was to develop new, orally active NA inhibitors as anti-influenza agents. In the case of an influenza epidemic, oral administration may be a convenient and economical method for treatment and prophylaxis. As reported in our preliminary account of a new series of carbocyclic neuraminidase inhibitors, a new hydrophobic pocket in the region corresponding to the glycerol subsite of sialic acid in the NA active site was discovered.[11] The X-ray crystal structures of several carbocyclic inhibitors bound to NA revealed that this hydrophobic pocket consisted of Glu276, Ala246, Arg224, and Ile222.[11,17] The lipophilicity of inhibitors is an important factor for designing orally active drugs since balancing lipophilicity and water solubility could be as critical as the size of the molecule for its absorption from the intestinal tract.[18] Although the hydrophobic effect is still poorly understood on the molecular level,[19] hydrophobic interactions between enzymes and inhibitors or receptors and ligands often resulted in increased binding affinity. By exploring the newly identified hydrophobic pocket in the NA active site, we were able to increase lipophilicity and potency of the carbocyclic NA inhibitors.

To examine the influence of lipophilic groups (R_1 in **6**) on the magnitude of the hydrophobic interaction between inhibitors and NA, we undertook SAR studies by making systematic changes in the length, size and branching of alkyl chains. To establish the limit of the alkyl chain length which can fit in the hydrophobic pocket, linear alkyl analogues were prepared as shown in Table 1. While the methyl and ethyl analogues (**32** and **33**) had steadily increased potency compared to the unsubstituted analogue **31**, as measured by their IC_{50} against influenza NA, compound **34** gave a significant increase in inhibitory activity, which is likely a reflection of added hydrophobic interactions by the terminal methyl of the *n*-propyl group. Further extension of the alkyl chain up to *n*-nonyl (**34** to **40**) resulted in similar potency with IC_{50} values of 150−300 nM, suggesting the additional hydrocarbons up to *n*-nonyl were insufficient to impart higher potency. The X-ray crystal structure of the *n*-hexyl analogue **37** bound to NA revealed that the binding energy gained by the hydrophobic interactions of the *n*-hexyl chain was offset by the partial exposure of the side chain into water outside of the active site. The poor NA inhibitory potency of **41** implies that additional hydrocarbons beyond the *n*-nonyl length may result in the unfavorable hydrophobic binding interaction. Although active-site amino acid residues are conserved across influenza A and influenza B NA subtypes,[1] differences in the binding region of the glycerol side chain of Zanamivir (**5**), particularly in the position and side chain conformation of Glu276 have been reported.[20] The large activity difference in the inhibitory activity of compounds **37** and **39** against influenza A and B NA also suggests that the large alkyl groups in these compounds had less favorable hydrophobic interactions in the active site of the influenza B NA.

Table 1. Influenza Neuraminidase Inhibitory Activity of Carbocyclic Analogues with Linear Alkyl Chains

RO, CO_2H, AcHN, NH_2

compound	R	neuraminidase (IC_{50}, nM) influenza A[a]	influenza B[b]
31	H	6300	ND[c]
32	CH_3	3700	ND
33	CH_3CH_2	2000	185
34	$CH_3(CH_2)_2$	180	ND
35	$CH_3(CH_2)_3$	300	215
36	$CH_3(CH_2)_4$	200	ND
37	$CH_3(CH_2)_5$	150	1450
38	$CH_3(CH_2)_6$	270	ND
39	$CH_3(CH_2)_7$	180	3500
40	$CH_3(CH_2)_8$	210	ND
41	$CH_3(CH_2)_9$	600	ND

[a] A/PR. [b] B/Lee. [c] ND = not determined.

The geometry and conformational mobility of the alkyl chains would also be expected to have great impact on the hydrophobic interactions. Therefore, we next investigated various branched alkyl chain analogues as shown in Table 2. Since the *n*-propyl analogue **34** displayed similar NA inhibitory activity with longer alkyl chains (Table 1), various alkyl groups were added on the *n*-propyl group as branching analogues. Addition of the methyl at the β position of the *n*-propyl chain (compound **42**) did not influence the influenza NA inhibitory activity. However, the additional methyl at the α position (compounds **43** and **44**) resulted in a 20-fold increase in NA inhibitory activity. This indicated that there is opportunity to increase the strength of binding by tailoring the structure of the branched alkyl chains around the α position of the *n*-propyl group. Interestingly, both diastereomers **43** and **44** exhibited almost equal NA inhibitory activity. This result led us to speculate that the methyl and the ethyl groups in the *sec*-butyl analogues **43** and **44** contribute almost the same binding energy by an equal degree of interaction with amino acids in the active site. Therefore, the chirality of the side chains of **43** and **44** had little influence in overall hydrophobic interactions. This result was also consistent with the further improved NA inhibitory activity of **7** (GS 4071), in which both ethyl groups bound into the two different hydrophobic pockets and contributed significantly for the hydrophobic interaction with NA as shown by the X-ray crystal structure.[11] Relatively small activity differences between two allylic diastereomers (**45** and **46**) also supported the idea

Table 2. Influenza Neuraminidase Inhibitory Activity of Carbocyclic Analogues with Branched Alkyl Chains

compound	R	Neuraminidase (IC_{50}, nM) influenza A[a]	influenza B[b]
42	$(CH_3)_2CHCH_2$	200	ND[c]
43	$CH_3CH_2(CH_3)CH^*$ (R)	10	7
44	$CH_3CH_2(CH_3)CH^*$ (S)	9	2
7	$(CH_3CH_2)_2CH$ (GS 4071)	1	3
45		1	3
46		3	24
47		1	4
48		60	120
49		16	6500
50		1	2150

[a] A/PR; [b] B/Lee; [c] ND = not determined.

Table 3. Influenza Neuraminidase Inhibitory Activity of Carbocyclic Analogues with Aryl and Aryl Alkyl Side Chains

compound	R	Neuraminidase (IC_{50}, nM) influenza A[a]	influenza B[b]
51		530	ND[c]
52		620	ND
53		0.3	70
54		12	35
55		>1000	ND
56		>1000	ND
57		90	ND

[a] A/PR; [b] B/Lee; [c] ND = not determined.

of two different directional hydrophobic bindings for the chiral side chains. The almost identical influenza A and B NA inhibitory activity of **7** and **47** agreed with the observation that the chain length of the linear alkyl analogues could be extended considerably without losing the influenza A NA inhibitory activity as discussed above. However, the potent influenza B inhibitory activity exhibited by **47** is quite puzzling in light of the greatly reduced influenza B NA inhibitory activity of **39**, which possesses the same carbon chain length as **47**. To evaluate the importance of steric bulkiness on the alkyl chain for the hydrophobic interaction, cyclohexyl analogues (**48**, **49**, and **50**) were prepared. Compound **48**, with the large cyclohexyl ring attached close to the cyclohexene scaffold, exhibited significantly reduced NA inhibitory activity when compared to **7**. The X-ray crystal structure of **48** bound to NA revealed that the cyclohexyl ring of **48** is far from Glu276 and resulted in the limited hydrophobic interactions. However, when the cyclohexyl ring was extended by an additional one or two methylenes from the chiral center (compounds **49** and **50**), influenza A NA inhibitory activity was improved considerably. In contrast with this, compounds **49** and **50** had greatly reduced influenza B NA inhibitory activity, suggesting that the binding affinity toward influenza B NA could be influenced significantly by the increased steric bulkiness of the C_3 side chain.

In the aryl and aryl alkyl series of compounds (Table 3), the phenyl and the benzyl analogues (**51** and **52**) exhibited poor NA inhibitory activity. However, the biphenyl analogue **57** was more potent when compared to **51** in NA inhibition, suggesting hydrophobic interactions may extend from the para position of the phenyl ring in **51**. Two diastereomers **53** and **54** exhibited very potent influenza A NA inhibitory activity. In this case, the (*S*) isomer **53** is more potent than the (*R*) isomer for influenza A NA, but the influenza B NA inhibitory activities of **53** and **54** were comparable. The X-ray crystal structures of **53** bound to NA revealed that a portion of the phenyl ring (yellow in Figure 1) is exposed to water, similar to the X-ray structure of the *n*-hexyl analogue **37**. The decreased NA inhibitory activities of analogues **55** and **56** are likely due to result from the unfavorable hydrophobic interactions caused by two phenyl rings at each terminal position of the branched chains.

It was reported that the conversion of the C_4-amino group in **4** to the guanidino functionality (compound **5**) imparted over a 100-fold increased potency in NA inhibitory activity.[8] This increased potency is believed to be a consequence of the additional charge−charge interaction brought by the guanidino group with Glu227 located near the NA active site. In the series of carbocyclic NA inhibitors, several guanidino analogues were also prepared, and their NA inhibitory activities were compared with those of corresponding amino analogues (Table 4). Although the guanidino analogues in Table 4 exhibited consistently more potent NA inhibitory activity compared to their amino counterparts, activity differences ranged from 2 to 100-fold. Amino analogues (**43**, **44**, and **7**) which exhibit already potent NA inhibitory activity appeared to have a lesser increase in their NA inhibitory activity upon conversion

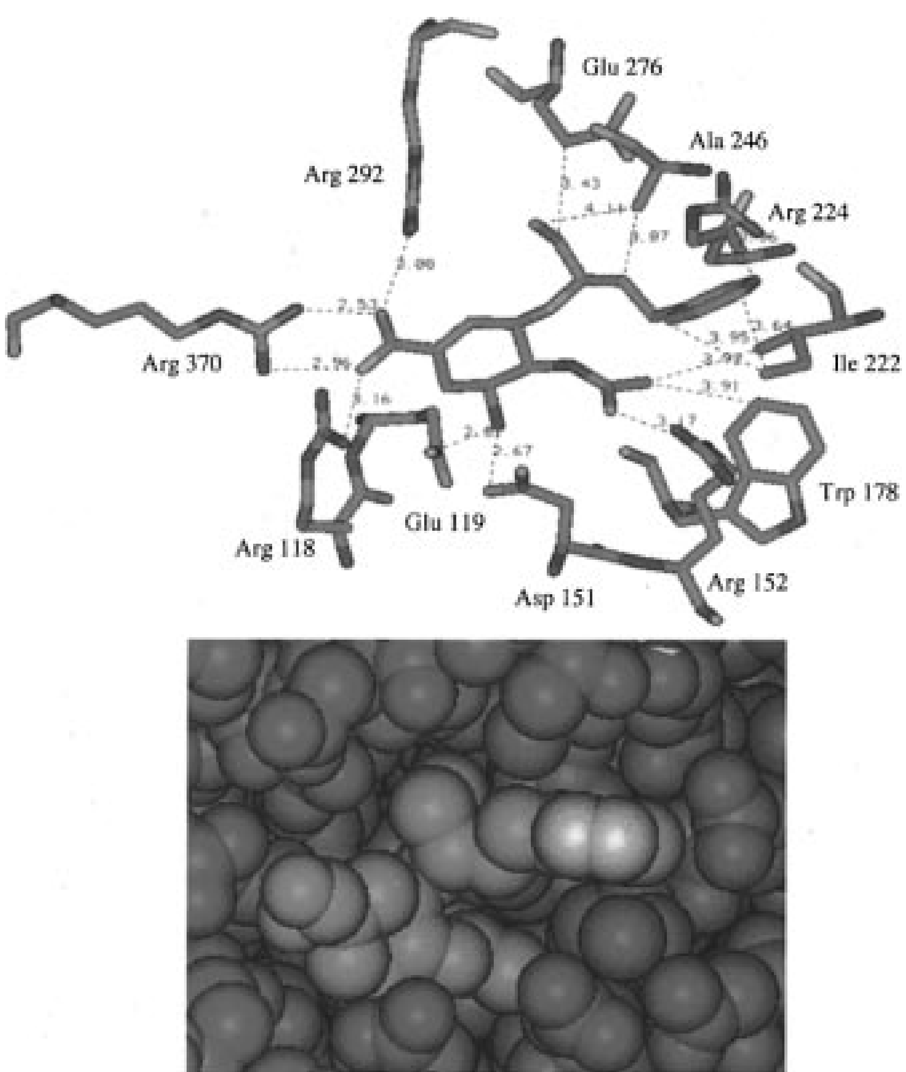

Figure 1. X-ray structure of **53** bound to influenza neuraminidase. A part of the phenyl ring (yellow) is exposed to water. Dash lines indicate close contacts; numbers are distances in angstroms.

Table 4. Influenza Neuraminidase Inhibitory Activity of Carbocyclic Guanidino Analogues

compound	R	neuraminidase (IC_{50}, nM) influenza A[a]
58	H	100 (6300)[b]
59	$CH_3(CH_2)_2$	2 (180)
60	$CH_3(CH_2)_3$	3 (300)
61	$CH_3CH_2(CH_3)CH^*$ (R)	0.5 (10)
62	$CH_3CH_2(CH_3)CH^*$ (S)	0.5 (9)
63	$(CH_3CH_2)_2CH$	0.5 (1)

[a] A/PR. [b] IC_{50} (nM) of corresponding amino compound **6** in parentheses.

of the amino group to the guanidino group (**61**, **62**, and **63**). The different degree of the binding enhancement observed in the guanidino series might suggest that the hydrophobic interactions of the C_3-alkyl groups influenced the hydrogen bonding interaction of the guanidino groups differently through the amino acid network of the NA active site. Other objectives in this SAR study were to determine how C_2 and C_6 substituents on the cyclohexene scaffold would affect NA inhibitory activity.

Table 5. Influenza Neuraminidase Inhibitory Activity of C_2 and C_6 Substituted GS 4071 (**7**) Analogues

compound	X	Y	neuraminidase (IC_{50}, nM) influenza A[a]	influenza B[b]
7	H	H	1	3
64	CH_3	H	2300	ND[c]
30	F	H	3	90
22	H	CH_3	1500	36000

[a] A/PR. [b] B/Lee. [c] ND = not determined.

For this purpose, C_2-methyl, C_2-fluoro, and C_6-methyl analogues of GS 4071 (**7**) were prepared (Table 5). The C_2-methyl analogue **64** exhibited an over 1000-fold decrease in the NA inhibitory activity compared to the parent compound **7**. The poor NA inhibitory activity exhibited by **64** may be due to the steric clash between the C_2-methyl and the nearby Arg292. With the widely held view that the fluorine atom may be regarded as an isostere of the hydrogen atom,[21] the C_2-fluoro analogue **30** retained potent NA inhibitory activity of **7**

against influenza A. However, to our surprise, the influenza B NA activity of **30** was considerably lower than that of **7**. The greatly reduced NA activity of the C_6-methyl analogue **22** might suggest that the lipophilic substituents at the C_6 position could result in considerably less favorable hydrophobic interactions in the NA active site.

Conclusions

On the basis of the structural information available from the X-ray crystal structures of sialic acid and its analogues complexed with influenza NA, a new series of potent carbocyclic NA inhibitors has been identified. The discovery of a new hydrophobic pocket in the NA active site allowed us to investigate various C_3-alkyl and aryl analogues for optimization of NA inhibition. X-ray crystal structures of several analogues in this series complexed with NA revealed that the alkyl chains could bind in two directions for optimal hydrophobic binding interactions. From the SAR described in this report, compound **7** (GS 4071) emerged as one of the most potent influenza A and B NA inhibitor in this series. The ethyl ester prodrug of **7** (**8**, GS 4104) proved to be safe and efficacious for the oral treatment and prophylaxis of human influenza infection in phase I and phase II clinical trials. GS 4104 is currently being evaluated in phase II/III human clinical trials.

Experimental Section

General. All reactions were conducted under a dry atmosphere of argon unless otherwise noted. All reaction solvents were anhydrous grade obtained from Aldrich Chemical Co. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. High-resolution mass spectra were performed by the Mass Spectrometry Lab, University of California, Berkeley. Column chromatography was carried out using 230−400 mesh silica gel. NMR spectra were recorded at 300 MHz unless otherwise indicated.

Alcohols used in the opening of aziridine **9** were obtained from either commercial sources or prepared from literature methods unless otherwise specified in the experimental.

Compounds **7**, **31**, **32**, **33**, **34**, **35**, **42**, **43**, **44**, **48**, **51**, and **58** have been previously reported.[11,17]

Representative Procedure: Epoxide Opening of 11. (2*R*)-Toluene-4-sulfonic Acid 2-Hydroxynonyl Ester (12d). To a solution of Li_2CuCl_4 (11.0 mL of a 0.10 M solution in THF, 1.1 mmol) in dry THF (132 mL) at −35 °C was added *n*-hexylmagnesium bromide (11 mL of a 2.0 M solution in ether, 22 mmol) dropwise. After 40 min, a precooled solution of (2*R*)-glycidyl tosylate **11** (5.02 g, 22 mmol) in dry THF (66 mL) was added via cannula. After 3 h the reaction was quenched with saturated NH_4Cl and diluted with ether. The organic layer was separated, washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography; eluting with EtOAc/hexane (1:4) gave **12d** (4.3 g, 62%).

Representative Procedure: Epoxide Formation of 12d. (2*R*)-1,2-Epoxynonane (13d). To a solution of **12d** (4.3 g, 13.64 mmol) in dry methanol (70 mL) was added solid K_2CO_3 (2.2 g, 15.92 mmol). After being stirred at room temperature for 1 h, the reaction mixture was filtered, concentrated, and partitioned between ether and water. The organic layer was separated, washed with brine, and dried ($MgSO_4$). Concentration followed by distillation gave **13d** (1.6 g, 82%) as an oil.

Representative Procedure: Epoxide Opening of 13d. (3*S*)-Decan-3-ol (14d). To a slurry of CuI (1.05 g, 5.5 mmol) in dry THF (25 mL) at −40 °C was added methylmagnesium bromide (9.2 mL of a 3.0 M solution in ether, 27.6 mmol) dropwise. After 10 min, a solution of **13d** (1.95 g, 13.7 mmol) in dry THF (10 mL) was added via cannula. The reaction mixture was stirred at −40 °C for 1 h, quenched with saturated NH_4Cl, and diluted with ether. The organic layer was separated, washed sequentially with 1 M HCl, water, saturated sodium bicarbonate, and brine, dried ($MgSO_4$), and filtered through a thin pad of silica gel. Concentration in vacuo gave **14d** (2.04 g, 94%) as an oil: ^1H NMR ($CDCl_3$) δ 3.55 (m, 1H), 1.30−1.45 (m, 14H), 0.95 (m, 6H).

1,7-Diphenylheptan-4-ol (65). To a solution of 3-phenylpropionaldehyde (2.2 g, 14.85 mmol) in dry THF (80 mL) at −78 °C was added (3-phenylpropyl)magnesium bromide (25 mL of a 1.3 M solution in ether, 32.5 mmol) dropwise. The reaction mixture was stirred for 1.5 h at −78 °C and then at 0 °C for an additional 30 min. The reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was separated, washed with 1 N HCl, water, saturated sodium bicarbonate, and brine, and dried (Na_2SO_4). Concentration in vacuo followed by flash chromatography of the residue on silica gel with EtOAc/hexane (3:1) afforded the alcohol (2.82 g, 71%): ^1H NMR ($CDCl_3$) δ 7.20 (m, 10H), 3.60 (s, 1H), 2.64 (m, 4H), 1.32−1.80 (m, 8H).

Representative Procedure: Alcoholysis of 9. Synthesis of Ethyl (3*R*,4*R*,5*S*)-4-Acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (10, R = 3-pentyl). To a solution of **9** (15 g, 34 mmol) in 3-pentanol (230 mL) was added $BF_3 \cdot Et_2O$ (6.27 mL, 51 mmol). The solution was heated at 70−75 °C for 2 h and then evaporated to give a residue which was dissolved in dry pyridine (2.0 mL) and treated with acetic anhydride (16 mL, 170 mmol) and (dimethylamino)pyridine (200 mg, 1.6 mmol). [Note: In cases where evaporation of excess alcohol was not possible, the reaction mixture was directly treated with excess acetic anhydride and pyridine. Separation and purification by flash chromatography on silica gel furnished the desired product.] The reaction was stirred at room temperature for 18 h and evaporated, and the residue was partitioned between ethyl acetate and 1 M HCl. The organic phase was washed with saturated sodium bicarbonate and brine and dried. The solvent was evaporated, and the residue was purified by chromatography eluting with hexane/ethyl acetate (1:1) to give **10** (R = 3-pentyl) (7.66 g, 69%). An analytical sample was recrystallized from hexane/ethyl acetate to afford **10** (R = 3-pentyl) as needles: mp 121−123 °C; ^1H NMR ($CDCl_3$) δ 6.79 (t, 1H, J = 2.1 Hz), 5.92 (d, J = 7.5 Hz, 1H), 4.58 (bd, J = 8.7 Hz, 1H), 4.35−4.25 (m, 1H), 3.77 (s, 3H), 3.36−3.25 (m, 2H), 2.85 (dd, J = 5.7, 17.4 Hz, 1H), 2.29−2.18 (m, 1H), 2.04 (s, 3H), 1.60−1.45 (m, 4H), 0.91 (t, J = 3.7 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.1, 166.2, 138.4, 127.7, 82.1, 73.8, 57.5, 57.2, 52.0, 30.4, 26.1, 25.5, 23.4, 9.5, 9.1. Anal. ($C_{15}H_{24}N_4O_4$) C, H, N.

Representative Procedure: Synthesis of 7 from 10 (R = 3-pentyl). (3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic Acid (7). To a solution of azide **10** (R = 3-pentyl) (1 g, 3.1 mmol) in THF (30 mL) were added triphenylphosphine (1.21 g, 4.6 mmol) and water (5.6 mL). The solution was heated at 50 °C for 10 h, THF was evaporated, and the aqueous oily residue was partitioned between ethyl acetate and brine. The organic phase was dried, filtered, and evaporated. Purification of the residue by chromatography eluting with methanol/ethyl acetate (1:1) gave 830 mg of an oil which was dissolved in THF (15 mL) and was treated with 1 N potassium hydroxide (4 mL, 4.16 mmol). The reaction mixture was stirred at room temperature for 40 min and acidified to pH 6 with Dowex 50WX8. The resin was filtered and washed with water and methanol. Solvents were evaporated, and the residue was purified by C_{18} chromatography, eluting with water and then with 5% acetonitrile in water. Fractions containing the desired product were pooled and lyophilized to afford **7** (600 mg, 75%) as a white solid: ^1H NMR (D_2O) δ 6.50 (t, J = 2.1 Hz, 1H), 4.30−4.26 (m, 1H), 4.03 (dd, J = 9.0, 11.7 Hz, 1H), 3.58−3.48 (m, 2H), 2.88 (dd, J = 5.4, 16.8 Hz, 1H), 2.53−2.41 (m, 1H), 1.62−1.40 (m, 4H), 0.90

(t, J = 7.5 Hz, 3H), 0.85 (t, J = 7.5 Hz, 3H). Anal. ($C_{14}H_{24}N_2O_4$·1.5H_2O) C, H, N.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(hexyloxy)-1-cyclohexene-1-carboxylic Acid (37). Prepared in 41% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.60 (s, 1H), 4.29 (d, J = 8.8 Hz, 1H), 4.13 (dd, J = 9.0, 11.0 Hz, 1H), 3.82 (m, 1H), 3.61 (m, 1H), 3.54 (m, 1H), 2.93 (dd, J = 17.0, 5.0 Hz, 1H), 2.52 (m, 1H), 2.16 (s, 3H), 1.62 (m, 2H), 1.38 (m, 6H), 0.95 (t, J = 7.0 Hz, 3H). Anal. ($C_{15}H_{26}N_2O_4$·1.8H_2O) C, H, N.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(heptyloxy)-1-cyclohexene-1-carboxylic Acid (38). Prepared in 26% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.64 (s, 1H), 4.33 (d, J = 8.2 Hz, 1H), 4.21 (t, J = 11.0 Hz, 1H), 3.66 (m, 1H), 3.64–3.85 (m, 2H), 2.99 (dd, J = 17 Hz, 5 Hz, 1H), 2.60 (m, 1H), 2.19 (S, 3H), 1.67 (m, 2H), 1.40 (m, 8H), 0.97 (m, 3H); HRMS (FAB) calcd for $C_{16}H_{29}N_2O_4$ (MH^+) 313.2127, found 313.2131.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(octyloxy)-1-cyclohexene-1-carboxylic Acid (39). Prepared in 29% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 7.02 (s, 1H), 4.37 (d, J = 8.0 Hz, 1H), 4.22 (dd, J = 9.0, 12.0 Hz, 1H), 3.87 (m, 1H), 3.71 (m, 1H), 3.65 (m, 1H), 3.05 (dd, J = 5.5, 17.0 Hz, 1H), 2.63 (m, 1H), 1.64 (m, 2H), 1.37 (m, 10H), 0.95 (t, J = 7.0 Hz, 3H); HRMS (FAB) calcd for $C_{17}H_{31}N_2O_4$ (MH^+) 327.2284, found 327.2279.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(nonyloxy)-1-cyclohexene-1-carboxylic Acid (40). Prepared in 35% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.99 (s, 1H), 4.38 (d, J = 8 Hz, 1H), 4.21 (dd, J = 9.0, 11.0 Hz, 1H), 3.83 (m, 1H), 3.73 (m, 1H), 3.63 (m, 1H), 3.06 (dd, J = 5.5, 17.0 Hz, 1H), 2.62 (m, 1H), 2.19 (s, 3H), 1.64 (m, 2H), 1.37 (s, 12H), 0.90 (t, J = 7.0 Hz, 3H); HRMS (FAB) calcd for $C_{18}H_{33}N_2O_4$ (MH^+) 341.2440, found 341.2434.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(decyloxy)-1-cyclohexene-1-carboxylic Acid (41). Prepared in 37% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.71 (s, 1H), 4.11 (d, J = 8 Hz, 1H), 4.06 (dd, J = 8.0, 10.4 Hz, 1H), 3.69 (m, 1H), 3.51 (m, 1H), 3.42 (m, 1H), 2.89 (dd, J = 6.0, 17.0 Hz, 1H), 2.46 (m, 1H), 2.05 (s, 3H), 1.57 (m 2H), 1.32–1.38 (m, 14 H), 0.92 (t, J = 7.0 Hz, 3H); HRMS (FAB) calcd for $C_{19}H_{35}N_2O_4$ (MH^+) 355.2597, found 355.2599.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-[(3*S*)-decyloxy]-1-cyclohexene-1-carboxylic Acid (47). Prepared in 34% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.89 (s, 1H), 4.44 (d, J = 8.0 Hz, 1H), 4.10 (dd, J = 9.0, 12.0 Hz, 1H), 3.65 (m, 2H), 3.00 (dd, J = 5.5, 17.0 Hz, 1H), 2.65 (m, 1H), 2.14 (s, 3H), 1.61 (m, 4H), 1.33 (m, 10H), 0.92 (m, 6H); HRMS (FAB) calcd for $C_{19}H_{35}N_2O_4$ (MH^+) 355.2597, found 355.2604.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(1(*S*)-(cyclohexylmethyl)propoxy)-1-cyclohexene-1-carboxylic Acid (49). Prepared in 30% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.68 (s, 1H), 4.19 (d, J = 8.0 Hz, 1H), 3.96 (dd, J = 8.0, 11.0 Hz, 1H), 3.62 (m, 1H), 3.46 (m, 1H), 2.89 (dd, J = 5.5, 17.4 Hz, 1H), 2.45 (m, 1H), 2.07 (s, 3H), 1.28–1.70 (m, 5H), 0.94 (t, J = 7.0 Hz, 3H); HRMS (FAB) calcd for $C_{19}H_{33}N_2O_4$ (MH^+) 353.2440, found 353.2441.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(1(*S*)-(2-cyclohexylethyl)propoxy)-1-cyclohexene-1-carboxylic Acid (50). Prepared in 35% yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.64 (s, 1H), 4.15 (d, J = 8.0 Hz, 1H), 3.96 (dd, J = 8.0, 10.7 Hz, 1H), 3.48 (m, 1H), 3.41 (m, 1H), 2.86 (dd, J = 5.7, 17.6 Hz, 1H), 2.44 (m, 1H), 2.05 (s, 3H), 1.18–1.76 (m, 17H), 0.94 (t, J = 7 Hz, 3H); HRMS (FAB) calcd for $C_{20}H_{35}N_2O_4$ (MH^+) 367.2597, found 367.2599.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(bis(phenylmethyl)methoxy)-1-cyclohexene-1-carboxylic Acid Hydrochloride (55). Prepared in 12% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 7.21–6.96 (m, 10H), 6.11 (br s, 1H), 4.12 (br d, J = 7.5 Hz, 1H), 3.90–3.82 (m, 2H), 3.47–3.38 (m, 1H), 2.75–2.50 (m, 5H), 2.34–2.25 (m, 1H), 1.87 (s, 3H).

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(bis(3-phenylpropyl)methoxy)-1-cyclohexene-1-carboxylic Acid (56). Prepared in 15% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 7.15–7.25 (m, 10H), 6.62 (s, 1H), 4.13 (d, J = 8.0 Hz, 1H), 3.94 (dd, J = 8.0, 11.0 Hz, 1H), 3.60 (m, 1H), 3.38 (m, 1H), 2.85 (m, 1H), 2.63 (m, 4H), 2.44 (m, 1H), 1.62 (m, 4H), 1.55 (m, 4H); HRMS (FAB) calcd for $C_{28}H_{37}N_2O_4$ (MH^+) 465.2753, found 465.2755.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(3-biphenylylpropoxy)-1-cyclohexene-1-carboxylic Acid (57). Prepared in 29% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 7.30–7.60 (m, 9H), 6.74 (s, 1H), 4.15 (m, 1H), 4.10 (m, 1H), 3.73 (m, 1H), 3.57 (m, 1H), 3.44 (m, 1H), 2.89 (dd, J = 5.5, 17.0 Hz, 1H), 2.75 (t, J = 6.0 Hz, 2H), 2.50 (m, 1H), 2.05 (s, 3H), 1.93 (m, 2H); HRMS (FAB) calcd for $C_{24}H_{29}N_2O_4$ (MH^+) 409.2127, found 409.2131.

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-[[(1*S*)-1-ethylbut-3-enyl]oxy]-1-cyclohexene-1-carboxylic Acid (45). Prepared in 26% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.43 (s, 1H), 5.76–5.61 (m, 1H), 5.04–4.90 (m, 2H), 4.21–4.17 (m, 1H), 3.92 (dd, J = 8.7, 11.7 Hz, 1H), 3.56–3.50 (m, 1H), 3.48–3.38 (m, 1H), 2.77 (dd, J = 5.4, 17.1 Hz, 1H), 2.42–2.30 (m, 1H), 2.24–2.10 (m, 1H), 1.96 (s, 3H), 1.50–1.40 (m, 2H), 0.79 (t, J = 7.5 Hz, 3H).

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-[[(1*R*)-1-ethylbut-3-enyl]oxy]-1-cyclohexene-1-carboxylic Acid (46). Prepared in 24% overall yield from aziridine **9**: ^{1}H NMR (D_2O) δ 6.32 (s, 1H), 5.79–5.65 (m, 1H), 5.09–4.90 (m, 2H), 4.10 (bd, J = 9.1 Hz, 1H),3.74 (dd, J = 8.7, 11.3 Hz, 1H), 3.51–3.46 (m, 1H), 3.15–3.05 (m, 1H), 2.63 (dd, J = 5.4, 17.3 Hz, 1H), 2.25–2.10 (m, 3H), 1.91 (s, 3H), 1.46–1.26 (m, 2H), 0.70 (t, J = 7.5 Hz, 3H).

Representative Procedure: Guanylation of 15 (R = *n*-propyl). (3*R*,4*R*,5*S*)-4-Acetamido-5-[N^2,N^3-Bis(*tert*-butoxycarbonyl)guanidino]-3-propoxy-1-cyclohexene-1-carboxylic Acid (16; R = *n*-propyl). To a solution of amine 15 (R = *n*-propyl; 529 mg, 1.97 mmol), *N,N'*-bis(*tert*-butoxycarbonyl)thiourea (561 mg, 2.02 mmol), and triethylamine (930 mL) in dry DMF (5.0 mL) cooled to 0 °C was added $HgCl_2$ (593 mg, 2.18 mmol) in one portion. The heterogeneous reaction mixture was stirred for 45 min at 0 °C and then at room temperature for 15 min, after which the reaction was diluted with EtOAc and filtered through a pad of Celite. Concentration in vacuo followed by flash chromatography of the residue on silica gel with EtOAc/hexane (9:1) gave **16** (R = *n*-propyl; 904 mg, 90%) as a pale oil: ^{1}H NMR ($CDCl_3$) δ 11.39 (s, 1H), 8.63 (d, J = 7.8 Hz, 1H), 6.89 (t, J = 2.4 Hz, 1H), 6.46 (d, J = 8.7 Hz, 1H), 4.43–4.32 (m, 1H), 4.27–4.17 (m, 1H), 4.13–4.06 (m, 1H), 3.77 (s, 3H), 3.67–3.59 (m, 1H), 2.83 (dd, J = 5.1, 17.7 Hz, 1H), 2.45–2.33 (m, 1H), 1.95 (s, 3H), 1.65–1.50 (m, 2H), 1.45 (s, 18H), 0.90 (t, J = 7.5 Hz, 3H).

Representative Procedure: Saponification and Deblocking of 16 (R = *n*-propyl). (3*R*,4*R*,5*S*)-4-Acetamido-5-guanidinyl-3-propoxy-1-cyclohexene-1-carboxylic Acid (59). To a solution of **16** (R = *n*-propyl; 904 mg, 1.77 mmol) in THF (10 mL) was added aqueous KOH (3.45 mL of a 1.039 N solution). The reaction mixture was stirred at room temperature for 17 h, cooled to 0 °C, and acidified to pH 4.0 with Amberlite IR-120 (H^+) exchange resin. The resin was filtered and washed with water and methanol. Concentration in vacuo gave the free acid as a pale foam which was dissolved in CH_2Cl_2 (40 mL) and treated with neat trifluoroacetic acid (25 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. Concentration in vacuo gave a pale orange solid which was purified by C_{18} chromatography eluting with water. Fractions containing the desired product were pooled and lyophilized to give **59** (R = *n*-propyl; 495 mg, 68%) as a pale solid: ^{1}H NMR (D_2O) δ 6.66 (s, 1H), 4.29 (bd, J = 9.0 Hz, 1H), 4.01 (dd, J = 10.8, 10.8 Hz, 1H), 3.87–3.79 (m, 1H), 3.76–3.67 (m, 1H), 3.60–3.50 (m, 1H), 2.83 (dd, J = 5.1, 17.4 Hz, 1H), 2.47–2.36 (m, 1H), 2.06 (s, 3H), 1.65–1.50 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H). Anal. ($C_{15}H_{23}O_6N_4F_3$) C, H, N.

(3*R*,4*R*,5*S*)-4-Acetamido-5-guanidinyl-3-butoxy-1-cyclohexene-1-carboxylic acid (60): ^{1}H NMR (D_2O) δ 6.65 (s, 1H), 4.24 (br d, J = 8.9 Hz, 1H), 3.99–3.92 (m, 1H), 3.81–3.68 (m, 2H), 3.57–3.50 (m, 1H), 2.79 (dd, J = 5.1, 17.4 Hz, 1H) 2.42–2.31 (m, 1H), 2.00 (s, 3H), 1.55–1.45 (m, 2H), 1.36–1.24 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H).

(3*R*,4*R*,5*S*)-4-Acetamido-5-guanidinyl-3-(1(*R*)-methylpropoxy)-1-cyclohexene-1-carboxylic acid (61): ^{1}H NMR (D_2O) δ 6.47 (s, 1H), 4.28 (bd, *J* = 8.4 Hz, 1H), 3.93−3.74 (m, 2H), 3.72−3.63 (m, 1H), 2.78 (dd, *J* = 4.8, 17.4 Hz, 1H), 2.43−2.32 (m, 1H), 2.04 (s, 3H), 1.58−1.45 (m, 2H), 1.13 (d, *J* = 6.0 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).

(3*R*,4*R*,5*S*)-4-Acetamido-5-guanidinyl-3-(1(*S*)-methylpropoxy)-1-cyclohexene-1-carboxylic acid (62): ^{1}H NMR (D_2O) δ 6.81 (s, 1H), 4.31 (br d, *J* = 8 Hz, 1H), 3.94−3.76 (m, 2H), 3.71−3.61 (m, 1H), 2.83 (dd, *J* = 5, 17.5 Hz, 1H), 2.43−2.32 (m, 1H), 2.06 (s, 3H), 1.58−1.45 (m, 2H), 1.19 (d, *J* = 6.1 Hz, 3H), 0.82 (t, *J* = 7.2 Hz, 3H).

(3*R*,4*R*,5*S*)-4-Acetamido-5-guanidinyl-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid (63): ^{1}H NMR (D_2O) δ 6.72 (s, 1H), 4.23 (br d, *J* = 8.4 Hz), 3.84−3.66 (m, 2H), 3.46−3.38 (m, 1H), 2.73 (dd, *J* = 4.8, 17.4 Hz, 1H), 2.33−2.23 (m, 1H), 1.91 (s, 3H), 1.48−1.27 (m, 4H), 0.76 (t, *J* = 7.5 Hz, 3H), 0.71 (t, *J* = 7.5 Hz, 3H).

Ethyl 4*β*-Acetamido-5α-(*tert*-butoxycarbamido)-1,2-dihydroxy-3α-(1-ethylpropoxy)-1-cyclohexane-1-carboxylate (18). To a solution of **8** (14.0 g, 44.8 mmol) in dry THF (120 mL) was added a solution of di-*tert*-butyl dicarbonate (11.7 g, 53.8 mmol) in dry THF (30 mL) followed by triethylamine (0.3 mL, 2.15 mmol) at 0 °C. The reaction was stirred for 10 min at 0 °C and for 30 min at room temperature, concentrated, and filtered through a plug of silica gel eluting with EtOAc. After evaporation the residue was dissolved in 2-methyl-2-propanol (200 mL) and treated with *N*-methylmorpholine *N*-oxide (7.75 g, 66.2 mmol) and osmium tetraoxide (1% solution in water, 15 mL). The mixture was stirred for 3 h at 70 °C, cooled to room temperature, and quenched with sodium thiosulfate pentahydrate (5 g in 20 mL of water). The mixture was concentrated in vacuo and partitioned between EtOAc and water. The organic phase was separated and the aqueous phase extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and filtered through a plug of silica gel. Recrystallization from hexane/EtOAc afforded diol **18** (18.4 g, 92%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 6.18 (d, *J* = 9.0 Hz, NH), 4.88 (d, *J* = 9.0 Hz, NH), 4.3 (q, *J* = 7.0 Hz, 2H), 3.96 (m, 2H), 3.88 (m, 1H), 3.8 (s, OH), 3.5 (m, 2H), 2.5 (d, *J* = 5.0 Hz, OH), 2.02 (s, 3H), 1.8−2.1 (m, 2H), 1.45−1.7 (m, 4H), 1.45 (s, 9H), 1.36 (t, *J* = 7.0 Hz, 3H), 0.88 (m, 6H).

Ethyl 4*β*-Acetamido-3α-(*tert*-butoxycarbamido)-5α-(1-ethylpropoxy)-6*β*-hydroxy-1-cyclohexene-1-carboxylate (19). To a solution of **18** (8.5 g, 19.0 mmol) in pyridine (4.2 mL, 52 mmol) and CH_2Cl_2 (100 mL) at −78 °C was added sulfuryl chloride (1.9 mL, 23.8 mmol) dropwise. The reaction was stirred for 10 min at −78 °C, slowly warmed to room temperature, and concentrated. The residue was dissolved in EtOAc, washed with brine, and concentrated. The crude product was recrystallized from hexane/EtOAc to give the cyclic sulfate as a white solid (7.2 g, 75%, 14.2 mmol). The sulfate was dissolved in dry THF (60 mL), and 1,8-diazabicyclo-[5.4.0]undec-7-ene (2.54 mL, 17.0 mmol) was added at 0 °C. After 16 h of stirring at room temperature the reaction was cooled to 0 °C, and water (0.25 mL) and H_2SO_4 (1 N in THF, 18 mL) were added. The mixture was stirred for 40 min at room temperature followed by the addition of saturated sodium bicarbonate and dilution with EtOAc. The organic phase was separated, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine and dried (Mg_2SO_4). Concentration in vacuo followed by flash chromatography of the residue on silica gel EtOAc/hexane (2:1) gave **18** (3.4 g, 56%) as a colorless solid: mp 118−119 °C; ^{1}H NMR ($CDCl_3$) δ 7.0 (d, *J* = 4.0 Hz, 1H), 6.5 (d, *J* = 8.0 Hz, NH), 5.4 (d, *J* = 9.0 Hz, NH), 4.5 (m, 1H), 4.25−4.42 (m, 4H), 3.8 (m, 1H), 3.45 (m, 1H), 3.28 (s, 1H), 1.98 (s, 3H), 1.5 (m, 4H), 1.44 (s, 9H), 1.38 (t, *J* = 7.0 Hz, 3H), 0.9 (m, 6H). Anal. ($C_{21}H_{36}N_2O_7$) C, H, N.

Ethyl 4*β*-Acetamido-6*β*-acetoxy-3α-(*tert*-butoxycarbamido)-5α-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (20). To a solution of **19** (857 mg, 2.0 mmol) in CH_2Cl_2 (10 mL) were added pyridine (242 mL, 3.0 mmol) and (dimethylamino)pyridine (5 mg) followed by acetic anhydride (226 mL, 2.4 mmol). The mixture was stirred for 2.5 h at room temperature, diluted with CH_2Cl_2, washed with water, and dried (Mg_2SO_4). Concentration in vacuo followed by flash chromatography of the residue on silica gel gave **20** (900 mg, 96%): mp 150−151 °C; ^{1}H NMR ($CDCl_3$) δ 7.2 (d, *J* = 4.0 Hz, 1H), 5.78 (brs, 2H), 5.42 (d, *J* = 9.0 Hz, NH), 4.4 (m, 2H), 4.25 (m, 2H), 3.8 (m, 1H), 3.55 (m, 1H), 2.08 (s, 3H), 1.96 (s, 3H), 1.55 (m, 4H), 1.45 (s, 9H), 1.36 (t, *J* = 7.0 Hz, 3H), 0.90 (m, 6H). Anal. ($C_{23}H_{38}N_2O_8$) C, H, N.

Ethyl-4*β*-Acetamido-5α-(*tert*-butoxycarbamido)-3α-(1-ethylpropoxy)-6*β*-methyl-1-cyclohexene-1-carboxylate (21). To a suspension of copper(I) iodide (486 mg, 2.55 mmol) in dry ether (40 mL) at −10 °C was added methyllithium (1.4 M, 3.64 mL, 5.1 mmol) dropwise. The mixture was stirred between −10 and 0 °C for 15 min and cooled to −45 to 40 °C, and a solution of **20** (800 mg, 1.70 mmol) in dry THF (10 mL) was added. The reaction was stirred for 30 min at room temperature, quenched with saturated ammonium chloride, washed with water, and dried (Mg_2SO_4). Concentration in vacuo followed by flash chromatography of the residue on silica gel gave **21** (180 mg, 25%) as a crystalline solid: mp 225−226 °C; ^{1}H NMR ($CDCl_3$) δ 6.63 (s, 1H), 5.8 (d, *J* = 10.0 Hz, NH), 4.8 (d, *J* = 10.0 Hz, NH), 4.24 (m, 2H), 4.05 (ddd, $J_1 = J_2 = J_3$ = 10.0 Hz, 1H), 3.97 (d, *J* = 10.0 Hz, 1H), 3.46 (ddd, $J_1 = J_2 = J_3$ = 10.0 Hz, 1H), 3.35 (m, 1H), 2.56 (m, 1H), 1.99 (s, 3H), 1.52 (m, 4H), 1.45 (s, 9H), 1.3 (t, *J* = 7.5 Hz, 3H), 1.2 (d, *J* = 7.5 Hz, 3H), 0.9 (m, 6H). Anal. ($C_{22}H_{38}N_2O_6$) C, H, N.

4*β*-Acetamido-5α-amino-3α-(1-ethylpropoxy)-6*β*-methyl-1-cyclohexene-1-carboxylic acid (22). To a solution of **21** (80 mg, 0.188 mmol) in CH_2Cl_2 (500 mL) and EtOAc (1.0 mL) was added HCl (1 N in EtOAc, 3 mL), and the mixture was stirred for 1.5 h at room temperature. Solid sodium bicarbonate was added, and after an additional 2 h the mixture was filtered through a plug of silica gel eluting with EtOAc/methanol (7:3). The filtrate was then evaporated to afford the free amine (58 mg, 95%). A portion of this material (36 mg, 0.11 mmol) was dissolved in methanol (1.0 mL) and water (500 mL) and treated with KOH (340 mL of 0.97 N solution). After 16 h of stirring at room temperature, the mixture was acidified to pH 6 with Dowex 50WX8. The resin was filtered and washed with water and methanol. Concentration in vacuo gave a pale solid which was purified by C_{18} chromatography, eluting with water. Fractions containing the desired product were pooled and lyophilized to afford **22** (22 mg, 67%): ^{1}H NMR (D_2O) δ 6.21 (s, 1H), 4.28 (d, *J* = 11.0 Hz, 1H), 4.05 (dd, J_1 = 11.0, J_2 = 11.5 Hz, 1H), 3.55 (m, 1H), 3.29 (dd, *J* = 11.5, 11.0 Hz, 1H), 2.9 (m, 1H), 2.12 (s, 3H), 1.45−1.65 (m, 4H), 1.25 (d, *J* = 7.5 Hz, 3H), 0.9 (m, 6H). Anal. ($C_{15}H_{26}N_2O_4$·2.1H_2O) C, H, N.

1-[[(2,5-Dimethoxybenzyl)oxy]methyl]-3α,4α-(dimethylmethylenedioxy)-2-fluoro-5*β*-methoxymethoxycyclohexene (23). Vinyl fluoride **23** was prepared from shikimic acid in 29% overall yield by a modified procedure of Bartlett:[16] ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 1H), 6.8 (s, 2H), 4.75 (S, 2H), 4.74 (brs, 1H), 4.5 (s, 2H), 4.3 (m, 1H), 4.2 (m, 2H), 3.96 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.4 (s, 3H), 2.6 (m, 1H), 2.32 (m, 1H), 1.52 (s, 3H), 1.45 (s, 3H); ^{19}F NMR ($CDCl_3$, $CFCl_3$) −120.3.

3α,4α-(Diethylmethylenedioxy)-1-[[(2,5-dimethoxybenzyl)oxy]methyl]-2-fluoro-5*β*-hydroxycyclohexene (24). A mixture of **23** (6.0 g, 14.6 mmol) and *p*-toluenesulfonic acid monohydrate (140 mg, 0.73 mmol) in methanol (100 mL) was refluxed for 6 h. The reaction mixture was evaporated to dryness and then coevaporated with 3-pentanone. The crude residue was dissolved in 3-pentanone (60 mL), stirred over molecular sieves (4 Å, 6 g) for 6 h at room temperature, and filtered. Concentration in vacuo followed by flash chromatography of the residue on silica gel with hexane/EtOAc (2:1) gave **24** (4.4 g, 77%): ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 1H), 6.8 (s, 2H), 4.75 (m, 1H), 4.52 (d, *J* = 12.5 Hz, 1H), 4.48 (d, *J* = 12.5 Hz, 1H), 4.05−4.3 (m, 4H), 3.84 (s, 3H), 3.83 (s, 3H), 2.63 (m, 1H), 2.33 (m, 1H), 2.1 (d, *J* = 2.5 Hz, 1H), 1.72 (m, 4H), 0.98 (m, 6H).

4α,5α-Epoxy-3α-(1-ethylpropoxy)-2-fluoro-1-(hydroxymethyl)cyclohexene (25). To a solution of **24** (4.4 g, 11.1 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added triethylamine (3.1 mL, 22.2 mmol) followed by methanesulfonyl chloride (1.1 mL, 14.3 mmol). The reaction mixture was stirred for 10 min at 0 °C and then for an additional 30 min at room temperature. The reaction was diluted with CH_2Cl_2 and quenched with ice−water. The organic phase was separated, washed with brine, and dried ($MgSO_4$). To the filtrate (ca. 250 mL) cooled to −78 °C was added a solution of $BH_3{\cdot}Me_2S$ (5.5 mL of a 10 M solution in THF, 55.0 mmol) followed by trimethylsilyl trifluoromethanesulfonate (4.9 mL, 27.5 mmol). The reaction mixture was stirred for 10 min at −78 °C and then warmed slowly to 0 °C. The reaction was then hydrolyzed by the addition of saturated sodium bicarbonate (250 mL) and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried ($MgSO_4$). Concentration in vacuo gave a residue which was dissolved in methanol (50 mL) and treated with potassium bicarbonate (2.2 g, 22 mmol) and water (35 mL). After 2 h of stirring at 50 °C, the reaction was evaporated and extracted with EtOAc. Concentration in vacuo followed by flash chromatography of the residue on silica gel gave epoxide **25** (1.43 g, 57%) as a colorless solid: mp 72−73 °C; ^1H NMR ($CDCl_3$) δ 4.38 (m, 1H), 4.1−4.35 (m, 2H), 3.45−3.55 (m, 3H), 2.88 (m, 1H), 2.6 (m, 1H), 1.63 (m, 4H), 1.38 (t, J = 7.5 Hz, OH), 1.0 (m, 6H). Anal. ($C_{12}H_{19}FO_3$) C, H, N, F.

4α,5α-Epoxy-3α-(1-ethylpropoxy)-2-fluoro-1-[(methoxymethoxy)methyl]cyclohexene (26). To a solution of epoxide **25** (1.4 g, 6.1 mmol) in CH_2Cl_2 (20 mL) at room temperature was added diisopropylethylamine (2.1 mL, 12.0 mmol) followed by chloromethyl methyl ether (0.7 mL, 9.1 mmol). After 1 h of refluxing, the reaction was diluted with water and extracted with CH_2Cl_2. Concentration in vacuo followed by flash chromatography of the residue on silica gel gave **26** (1.6 g, 96%) as an oil: ^1H NMR ($CDCl_3$) δ 4.64 (s, 2H), 4.35−4.45 (m, 2H), 3.92 (m, 1H), 3.45−3.55 (m, 3H), 3.4 (s, 3H), 2.8 (m, 1H), 2.62 (m, 1H), 1.63 (m, 4H), 1.0 (m, 6H); ^{19}F NMR ($CDCl_3$, $CFCl_3$) −125.2.

4*β*-Acetamido-5α-azido-3α-(1-ethylpropoxy)-2-fluoro-1-[(methoxymethoxy)methyl]cyclohexene (27). Prepared from **26** in 58% over yield by a previously described procedure:[11] mp 98−99 °C; ^1H NMR ($CDCl_3$) δ 5.85 (d, J = 9.5 Hz, NH), 4.65 (s, 2H), 4.5 (m, 1H), 4.2−4.35 (m, 2H), 4.95 (m, 1H), 3.58 (m, 1H), 3.48 (m, 1H), 3.42 (s, 3H), 2.6 (m, 1H), 2.2 (m, 1H), 2.08 (s, 3H), 1.55 (m, 4H), 0.95 (m, 6H); ^{19}F NMR ($CDCl_3$, $CFCl_3$) −119. Anal. ($C_{16}H_{27}FN_4O_3$) C, H, N, F.

4*β*-Acetamido-5α-azido-3α-(1-ethylpropoxy)-2-fluoro-1-cyclohexene-1-carboxaldehyde (28). A solution of **27** (1.15 g, 3.21 mmol) and *p*-toluenesulfonic acid monohydrate (0.61 g, 3.21 mmol) in methanol (40 mL) was refluxed for 4 h. After evaporation the residue was dissolved with EtOAc, washed with sodium bicarbonate, and dried ($MgSO_4$). Concentration in vacuo gave a solid which was recrystallized from hexane/EtOAc to give the alcohol (0.94 g, 93%) as a white solid. In a separate flask dimethyl sulfoxide (0.69 g, 8.8 mmol) in dry CH_2Cl_2 (30 mL) cooled to −60 to −50 °C was treated with oxalyl chloride (2.2 mL of a 2 M solution in CH_2Cl_2, 4.4 mmol). The mixture was stirred for 20 min at this temperature after which a solution of the above alcohol (0.92 g, 2.93 mmol) in dry THF (10 mL) was added. After the mixture was stirred for 20 min at this temperature triethylamine (2.0 mL, 14.4 mmol) was added, and the resultant mixture was allowed to slowly warm to room temperature and then evaporated. The residue was partitioned between EtOAc and water. The organic phase was washed with water and dried ($MgSO_4$). Concentration in vacuo gave a solid which was recrystallized from hexane/EtOAc acetate to afford the aldehyde **28** (0.8 g, 87%) as a white solid: ^1H NMR ($CDCl_3$) δ 10.15 (s, 1H), 6.0 (d, J = 9.0 NH), 4.88 (m, 1H), 4.35 (m, 1H), 3.52 (m, 1H), 3.4 (m, 1H), 2.82 (m, 1H), 2.1 (m, 1H), 2.08 (s, 3H), 1.55 (m, 4H), 0.95 (m, 6H).

Ethyl 4*β*-Acetamido-5α-azido-3α-(1-ethylpropoxy)-2-fluoro-1-cyclohexene-1-carboxylate (29). A mixture of aldehyde **28** (0.8 g, 2.56 mmol), sodium chlorite (0.92 g, 10.2 mmol), and potassium phosphate monobasic (0.84 g, 6.2 mmol) in THF/H_2O/DMSO (15:15:1 mL) was stirred for 6 h at room temperature. The reaction mixture was poured into 2 N HCl (10 mL) at 0 °C and extracted with EtOAc. The organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give the carboxylic acid. The crude carboxylic acid was dissolved in CH_2Cl_2 (10 mL) and THF (10 mL). Ethanol (0.36 mL, 6 mmol), 4-(dimethylamino)pyridine (40 mg), and 1,3-diisopropylcarbodiimide (0.8 mL, 5.2 mmol) were added then added sequentially. After the mixture was stirred for 4 h at room temperature, volatiles were evaporated and the residue was diluted with hexane/EtOAc (1:2). The mixture was filtered and concentrated in vacuo to give a solid which was purified by flash chromatography on silica gel to afford **29** (0.55 g, 60%) as colorless solid: mp 103−104 °C; ^1H NMR ($CDCl_3$) δ 5.85 (d, J = 7.5 Hz, NH), 4.68 (m, 1H), 4.35 (m, 1H), 4.3 (q, J = 7.2 Hz, 2H), 3.4−3.55 (m, 2H), 2.84 (m, 1H), 2.32 (m, 1H), 2.08 (s, 3H), 1.55 (m, 4H), 1.55 (t, J = 7.2 Hz, 3H), 0.93 (t, J = 6.6 Hz, 6H); ^{19}F NMR ($CDCl_3$, $CFCl_3$) −98.2. Anal. ($C_{16}H_{25}FN_4O_4$) C, H, N, F.

4*β*-Acetamido-5α-amino-3α-(1-ethylpropoxy)-2-fluoro-1-cyclohexene-1-carboxylic acid (30). Prepared from **29** in 40% yield following the previously described procedure:[11] ^1H NMR (D_2O) δ 4.42 (m, 1H), 4.24 (m, 1H), 3.58−3.66 (m, 2H), 2.84 (m, 1H), 2.62 (m, 1H), 2.1 (s, 3H), 1.58 (m, 4H), 0.88 (m, 6H); ^{13}C NMR 8.5, 8.7, 22.6, 25.2, 25.3, 27.8 (J = 4.7 Hz), 48.7, 53.6 (J = 8.0 Hz), 74.2 (J = 23.0 Hz), 85.7, 113.4, 153.9 (J = 267.0 Hz), 172.0, 175.3; ^{19}F NMR (D_2O, $CFCl_3$) −114.8. Anal. ($C_{14}H_{23}FN_2O_4{\cdot}1.4H_2O$) C, H, N, F.

Neuraminidase Enzyme Assay. Neuraminidase enzyme activity was determined using minor modifications to the literature method.[22] Influenza A/PR/8/34 (H1N1), purified on sucrose density gradients, was used as the source of enzyme, and 4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid was used as substrate in a reaction buffer containing 33 mM MES, pH 6.5, and 4 mM calcium chloride. Virus was mixed with various inhibitor concentrations and incubated at room temperature for 30 min before substrate was added to a final concentration of 10 μM. Reactions were stopped after 8 min at 37 °C with the addition of 1.5 volumes of 14 mM sodium hydroxide in 83% ethanol. Fluorescence was quantitated in a Perkin-Elmer fluorimeter (Model LS50B) with an excitation wavelength of 360 nm, emission wavelength of 448 nm, and slit widths of 2.5 nM.

Crystallization and Data Collection. Isolation, purification, and crystallization of N9 neuraminidase has previously been reported.[23] Crystals of the neuraminidase−inhibitor **53** complex were obtained by soaking the neuraminidase crystals overnight in 5 mM inhibitor **53** solution (5 mM inhibitor, 2 volumes of 1.4 M KH_2PO_4, 1 volume of 3 M K_2HPO_4). Cubic shaped crystals with approximate dimensions 0.2 × 0.2 × 0.2 mm were used to collect room temperature data at the Stanford Synchrotron Radiation Laboratory, beamline 7-1 (λ = 1.08 Å) using a MAR30 image plate system. The crystals belong to the cubic space group *I*432, a = 182.9 Å. A complete data set to 2.7 Å resolution was collected using 1° oscillations and a total of 30°. The reflections were indexed using DENZO 1.5.11 and merged/scaled using the program SCALEPACK. A total of 51 334 reflections were collected; 12 383 reflections were unique. The reduced data set was 98% complete to 2.7 Å resolution with an R_{merge} of 7.1%.

Structure Solution and Refinement. The neuraminidase complexed with inhibitor **53** structure was solved by molecular replacement using the influenza A subtype N9 neuraminidase crystal structure containing residues 82−469 (PDB ID 1INY).[24] Alternate rounds of model building with the molecular graphics program O version 6.0.3[25] and refinement of the atomic coordinates using X-PLOR 3.1[26] were performed until convergence of the free-R and R-factor was achieved. Examination of F_o-F_c maps showed unambiguous density for the orientation of inhibitor **53**, sugar molecules, and calcium ion. Addition of four *N*-acetyl-D-glucosamine sugars, five α-D-mannose sugars, one calcium ion, and one

inhibitor **53** molecule reduced the free-R and R factor further. The model was refined through positional and overall B factor refinement in X-PLOR until the minimization was complete. The final model has an R factor of 16.3% (R_{free} = 22.8) in the 6 to 2.7 Å resolution range, with rms deviations of 0.012 Å and 1.83° for the bond lengths and angles, respectively.

Structure Analysis. Visual inspection of the structures was aided with the programs O[25] and INSIGHTII[27] on an INDIGO Silicon Graphics workstation. The stereochemistry of the models were examined with PROCHECK 2.1.4.[28]

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