Systematic Structure-Based Design and Stereoselective Synthesis of Novel Multisubstituted Cyclopentane Derivatives with Potent Antiinfluenza Activity

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The design and synthesis of novel, orally active, potent, and selective inhibitors of influenza neuraminidase differing structurally from existing neuraminidase inhibitors are described. X-ray crystal structures of complexes of neuraminidase with known five- and six-membered ring inhibitors revealed that potent inhibition of the enzyme is determined by the relative positions of the interacting inhibitor substituents (carboxylate, glycerol, acetamido, hydroxyl) rather than by the absolute position of the central ring. This led us to design potential neuraminidase inhibitors in which the cyclopentane ring served as a scaffold for substituents (carboxylate, guanidino, acetamido, alkyl) that would interact with the four binding pockets of the neuraminidase active site at least as effectively as those of the established six-membered ring inhibitors such as DANA (**2**), zanamivir (**3**), and oseltamivir (**4**). A mixture of the isomers was prepared initially. Protein crystallography of inhibitor−enzyme complexes was used to screen mixtures of isomers in order to identify the most active stereoisomer. A synthetic route to the identified candidate **50** was developed, which featured (3 + 2) cycloaddition of 2-ethylbutyronitrile oxide to methyl (1*S*,4*R*)-4[(*tert*-butoxycarbonyl)amino]cyclopent-2-ene-1-carboxylate (**43**). Structures of the synthetic compounds were verified by NMR spectroscopy using nuclear Overhauser effect methodology. Two new neuraminidase inhibitors discovered in this work, **50** and **54**, have IC_{50} values vs neuraminidase from influenza A and B of <1 and <10 nM, respectively. These IC_{50} values are comparable or superior to those for zanamivir and oseltamivir, agents recently approved by the FDA for treatment of influenza. The synthetic route used to prepare **50** and **54** was refined so that synthesis of pure active isomer **54**, which has five chiral centers, required only seven steps from readily available intermediates. Further manipulation was required to prepare deoxy derivative **50**. Because the activities of the two compounds are comparable and **54** [RWJ-270201 (BCX-1812)] is the easier to synthesize, it was selected for further clinical evaluation.

Introduction

Despite advances in the understanding of molecular and cellular aspects of influenza, the disease remains the major cause of mortality and morbidity among patients with respiratory diseases.[1−3] Until the approval in 1999 of new agents (discussed below), there were only two options available: (i) vaccines, which are limited in effectiveness because influenza viruses undergo antigenic variation in the two surface antigens (hemagglutinin and neuraminidase), requiring a new formulation each year; (ii) the antiviral drugs amantadine and rimantadine, which are of limited usefulness because they lack activity against influenza virus B and cause unwanted side effects. Furthermore, resistant viral strains rapidly emerge.[4]

Studies of the replication cycle of influenza virus indicate that there are several potential molecular targets for drug design; these include hemagglutinin,[5,6] neuraminidase,[7] M2 protein,[8] and endonuclease.[9] Neuraminidase has been extensively targeted for drug design.[10] In the influenza virus, NA exists as a tetramer consisting of four roughly spherical subunits and a centrally attached stalk containing a hydrophobic region by which it is embedded in the host target cell membrane. Several roles have been suggested for neuraminidase. The enzyme catalyzes the cleavage of the α-ketosidic linkage between sialic acid (**1**, Chart 1) and the adjacent sugar residue. Removal of sialic acid lowers the membrane viscosity and permits entry of the virus into the epithelial cells. Neuraminidase also destroys the HA receptor on host cells, thus allowing the emergence of progeny virus particles from infected cells.[11,12] 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.[12] In general, the neuraminidase is thought to permit the spread of the virus from cell to cell and from the site of infection. Therefore, compounds that inhibit neuraminidase can protect the host from viral infection and retard its propagation.

Analogues of neuraminic acid, such as 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (**2**, DANA; Chart 1) are known to inhibit neuraminidase in vitro with a K_i value of approximately 4 μM.[13] von Itzstein showed that the 4-guanidino analogue of DANA, zanamivir (**3**, Chart 1),

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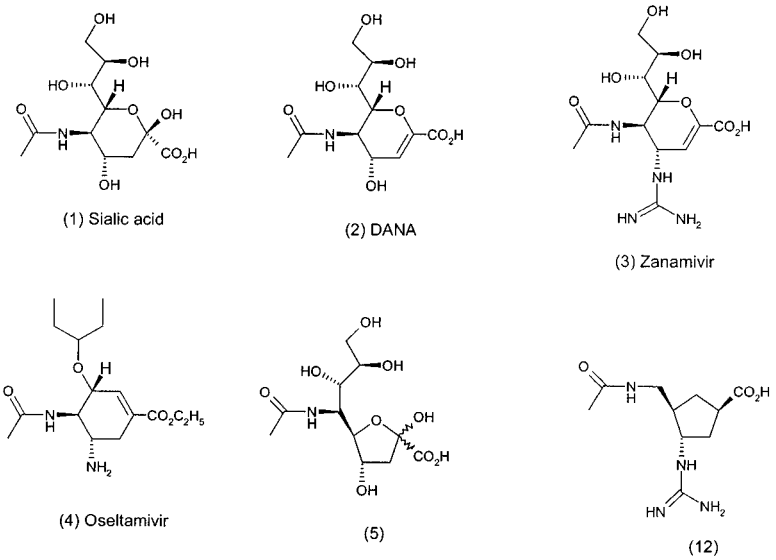
Chart 1



is a potent neuraminidase inhibitor.[14−16] This compound received FDA approval in 1999 and is effective against both influenza A and B. Because of its highly polar nature, **3** requires administration by oral inhalation. Another compound, oseltamivir carboxylate [**4**, ethyl (3*R*,4*R*,5***S***)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexen-1-carboxylate; Chart 1] reported by Kim et al., was also approved by the FDA in 1999.[17] This compound is effective against both A and B neuraminidases and is orally active, but it has been reported to cause vomiting and nausea. Neuraminidase inhibitors can also be used as prophylactic agents (oseltamivir carboxylate was recently approved by the FDA for this use).

Therefore, it is clinically necessary to have a drug that is effective against both types A and B viruses, does not rapidly select for resistance, is orally active, and is safe without side effects. A preliminary report on the structure-based drug design of a new class of neuraminidase inhibitors having those desired properties has been published.[18] In this paper, the detailed synthesis and characterization of these new and promising agents for combating influenza are described.

Rational Design of a New Class of Neuraminidase Inhibitors

The crystal structures of influenza A neuraminidase complex with sialic acid (**1**) and DANA (**2**) have been reported previously.[19] The interactions of DANA with the enzyme consist of strong charge−charge interactions and also some hydrophobic contacts. The active site consists of four binding pockets:[18] (1) an acid pocket where the carboxy group of DANA has hydrogen bond interactions with a triad of arginine residues (residues 118, 292, and 371), (2) an acetamido binding pocket that has a hydrophobic patch formed by Trp 178 and Ile 222 and a buried water molecule, (3) the glycerol binding pocket, and (4) the fourth pocket where C-4 hydroxyl of DANA is positioned. A water molecule surrounded by negatively charged residues characterizes the fourth pocket.

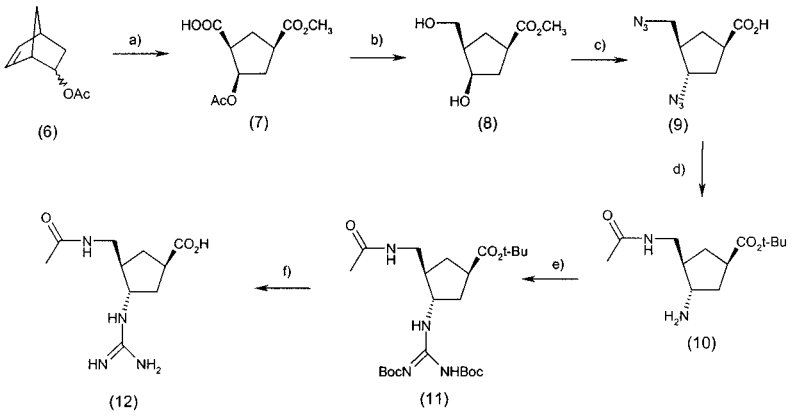
With this information, we envisioned candidates other than six-membered ring compounds such as **1**−**4** but with similar functionalities that would afford interactions with neuraminidase. We also sought to achieve potent binding without the glycerol side chain whose presence might diminish bioavailability, as it does in **3**.

The plan to explore other ring systems was also prompted by the finding of Yamamoto et al.[20] who reported a furanose ring compound, α/*β*-6-acetylamino-3,6-dideoxy-D-glycero-altro-2-nonulofuranosonoic acid (**5**, Chart 1), having neuraminidase inhibitory activity comparable to that of DANA. This compound has the same four substituents for interaction as DANA, but the arrangement of the substituents is distinctive. Although the central ring system of compound **5** is different from that of DANA, its equal potency and the presence of all four binding groups in the molecule prompted us to investigate the crystal structure of the complex of **5** with N9 influenza neuraminidase. This analysis revealed some interesting and important features of binding. The superpositioning of the neuraminidase complexes containing DANA and compound **5** shows that the ring system in **5** is significantly displaced from the pyranose ring of DANA.[18] However, all four main functional groups in both complexes have the same relative positions in the active site and have similar interactions with the enzyme. This crystal structure demonstrates that the positioning of the interacting groups is more important than the central ring system. This observation encouraged us to explore other five-membered ring systems, such as cyclopentane.

Cyclopentane Scaffold

The synthesis of the trisubstituted cyclopentane derivative (**12**, Chart 1) started with 5-norbornen-2-yl acetate (**6**), which was converted to methyl 4*β*-acetyloxy-3*β*-carboxycyclopentane-1*β*-carboxylate (**7**) by published procedures.[21] The carboxyl group of **7** was reduced to hydroxymethyl (via its mixed carboxylic−carbonic anhydride) with sodium borohydride.[22] During this conversion, the *O*-acetyl group was also removed partially, and transfer of the acetyl from 4-hydroxyl to 3-hydroxymethyl was observed as well. Therefore, the complete removal of acetyl groups was achieved with

Scheme 1[a]



[a] Reagents: (a) ref 21; (b) (i) $C_2H_5OC(O)Cl$, Et_3N, (ii) $NaBH_4$, MeOH, (iii) NaOMe, MeOH; (c) (i) TPP, DEAD, N_3H, (ii) NaOH; (d) (i) isobutylene, H_3PO_4, (ii) H_2, Pd/C, (iii) Ac_2O; (e) S−Me−C(=NBoc)(NHBoc), $HgCl_2$; (f) CF_3CO_2H.

sodium methoxide in methanol, resulting in compound **8**. Both hydroxyl groups of **8** were replaced by azido through the Mitsunobu reaction[23] with triphenylphosphine (TPP), diethylazodicarboxylate (DEAD), and hydrazoic acid to give the methyl ester of **9**. Catalytic reduction (Pd/C) of the azido groups of **9** methyl ester resulted in intramolecular 3-azabicyclo[3.2.1]oct-2-one formation due to the cis configuration of methoxycarbonyl group relative to the aminomethyl group. This problem was avoided by first converting the methyl ester to the carboxylic acid **9**, followed by conversion of **9** to its *tert*-butyl ester as shown in the Scheme 1; hydrogenation then gave the expected diamine, and acetylation at 0 °C with 1 equiv of acetic anhydride resulted in selective acetylation of the aminomethyl group, leading to compound **10**. Guanylation of the remaining amino group with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea in the presence of triethylamine and mercuric chloride afforded **11**, which was purified by silica gel column chromatography.[24] The *tert*-butyl ester and *tert*-butoxycarbonyl groups of **11** were removed concomitantly with trifluoroacetic acid to give the sought trisubstituted cyclopentane derivative **12** as its trifluoroacetate.

This compound was found to have an IC_{50} value of 28 μM on N2 and 115 μM on N9, which is superior to the DANA series compound having the same functional groups.[25] This compound was soaked with the N9 neuraminidase, and the structure of the resulting complex revealed that (i) the carboxyl and $NHCOCH_3$ groups have the same interactions as found in **5** and (ii) the guanidino group occupies the fourth binding pocket replacing the existing water molecule and is involved in charge-based interactions with residues Asp 151, Glu 119, and Glu 227 (Figure 1). This mode of binding for the guanidino group is analogous to that observed in the crystal structure of zanamivir (**3**) with influenza A neuraminidase.

Subsequent modeling suggested that other isomers of this compound **12** could also be potentially active compounds. Further, we wanted to explore the small hydrophobic pocket formed by Ala 246, Ile 222, and the aliphatic part of the side chain of Arg 224. The *n*-butyl group was initially chosen to explore the hydrophobic pocket, and a plan to synthesize structure **25** as a mixture of isomers was undertaken (see Scheme 2). It should be noted that **25** has chiral centers at C1, C3, and C4 of the cyclopentyl ring and at C1′ of the external chain attached to C3. In the synthesis of the mixture of isomers, the positions of the guanidino group and the 1-acetylaminopentyl group were fixed as trans while positions C1 and C1′ lacked steric specificity. In this plan, we envisioned that the crystal of neuraminidase would bind with the isomer of **25** having the greatest affinity for the enzyme. Once the isomer of highest binding affinity was identified, we planned a synthetic route leading directly to that compound.

The synthesis of **25** (Scheme 2) began with 4-bromocyclopenten-2-one (**13**),[26] which was converted to 4-azidocyclopenten-2-one (**14**) by reaction with sodium azide. Reaction of **14** with the sodium salt of diethyl acetamidomalonate in ethanol at −40 °C gave the 1,4 adduct **15**.[27,28] Structure **15** is the key intermediate because it contains the three properly positioned precursor groups needed to prepare the trisubstituted **25**; in **25**, the keto group will have been converted to carboxyl, the azido to guanidino, and the diethyl acetamidomalonate to 1-acetylaminopentyl.

Further conversion of these functionalities to the desired groups proved to be challenging. Initially, we attempted conversion of the keto group to a carboxylic in the presence of azido. This was followed by attempted hydrolysis of the ester groups in the presence of keto and azido. Then we attempted conversion of azido to the free amino in the presence of keto. All of these efforts were unsuccessful. To avoid this problem, the azido group was converted to a *tert*-butoxycarbonyl-protected amino by catalytic (Pd/C) hydrogenation of **15** in ethyl acetate in the presence of di-*tert*-butyl dicarbonate to give **16**. The trans relationship of the *tert*-butoxycarbonyl amino and diethyl acetamidomalonate groups was established by NMR spectral data. The next step was conversion of the keto group of **16** to a carboxylic acid precursor. This was accomplished by the reaction of **16** with trimethysilyl 1,3-dithiane and *n*-butyllithium,[29−31] which gave the cyclopentylidene 1,3-dithiane **17**. Hydrolysis of the ester groups followed, and the resulting diacid was decarboxylated to give the monocarboxylic acid **18**. Compound **18** was treated with ethyl chloroformate and triethylamine to give the corresponding mixed carboxylic−carbonic anhydride, which was treated with *N*,*O*-dimethylhydroxylamine to give the *N*-methoxy-*N*-methylamide **19**. Reduction of **19** with lithium tri-*tert*-butoxyaluminohydride (LTBA) gave the aldehyde

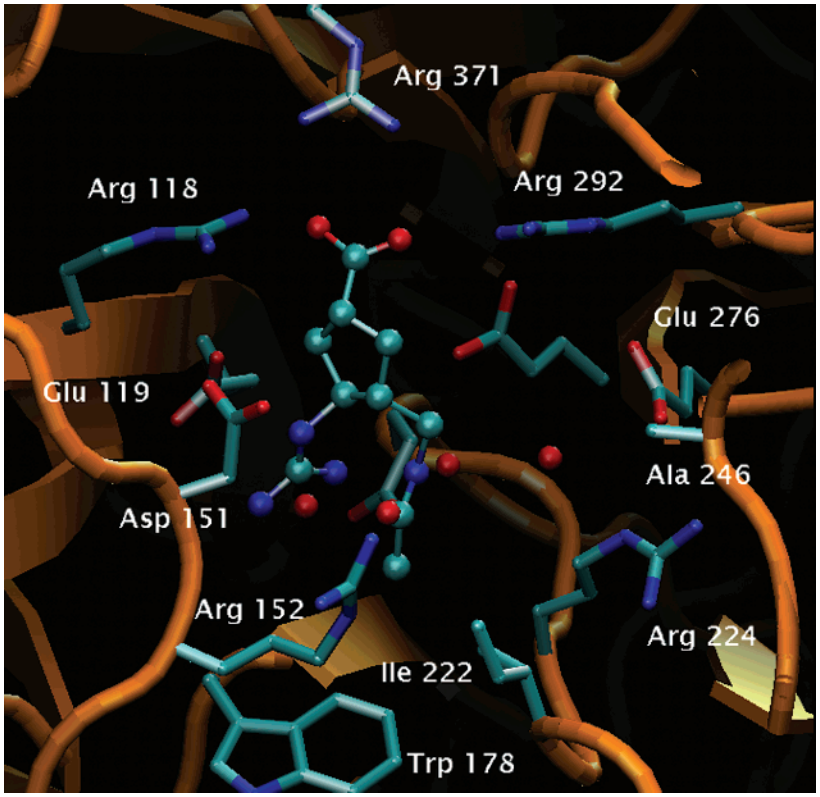
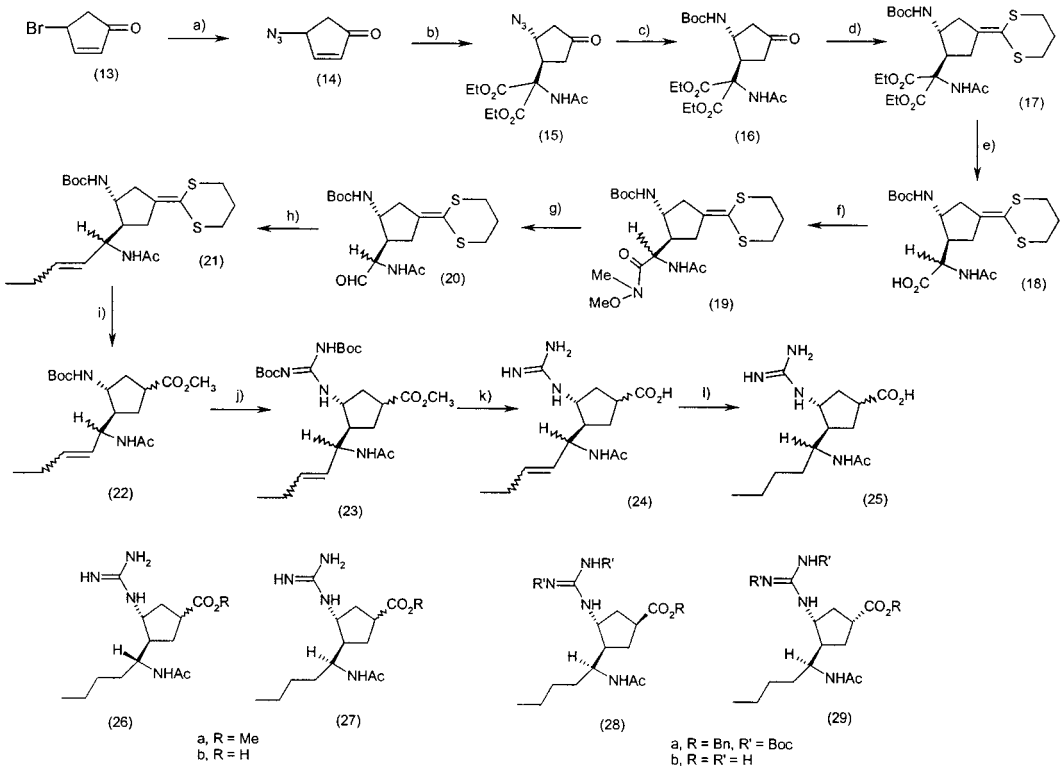


Figure 1. Binding of compound **12** to influenza A neuraminidase N9 active site.

Scheme 2[a]



[a] Reagents: (a) NaN_3; (b) $HC(NHAc)(CO_2Et)_2$, Na; (c) H_2, Pd/C, $(Boc)_2O$; (d) 2-trimethylsilyl-1,3-dithiane, *n*-BuLi; (e) (i) NaOH, (ii) AcOH; (f) (i) ClC(O)OEt, Et_3N, (ii) methoxymethylamine; (g) LTBA; (h) NaHMDS, $Ph_3P{=}CH{-}CH_2{-}CH_3$; (i) MeOH/HCl; (j) (i) CF_3CO_2H, (ii) $MeS{-}C(=NBoc)(NHBoc)$; (k) (i) NaOH, (ii) CF_3CO_2H; (l) PtO_2, H_2.

20. The Wittig reaction of **20** with propyltriphenylphosphonium bromide and sodium hexamethyl disilane (NaHMDS) gave **21** as the expected cis/trans olefin. The next step was conversion of the 1,3-dithiane group to the methyl ester **22**, which was achieved by treatment of **21** with methanolic HCl (0.5 N) at room temperature. These conditions did not affect the NHBoc group. The *tert*-butoxycarbonyl group of **22** was removed by treat-

ment with trifluoroacetic acid in methylene chloride, and the resulting amine was treated with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea in the presence of triethylamine and mercuric chloride to give **23**. Hydrolysis of the methyl ester was followed by removal of the *tert*-butoxycarbonyl groups of **23** to give **24**, and the hydrogenation of the double bond of **24** in the presence of PtO_2 gave **25** as the expected mixture of C-1 and C-1′ diastereoisomers. The mixture was tested as such in order to obtain an IC_{50} value for neuraminidase from both neuraminidase enzymes A and B, with resulting values of 50 and 900 nM for neuraminidase A and B, respectively. This mixture was also used as such in the soak studies on N9 neuraminidase enzyme described below.

Identification of the Active Isomer by Crystallography

The active isomer of **25** was identified by soaking a crystal of influenza N9 neuraminidase in a solution of the isomers for 1 day and then collecting X-ray diffraction data from the crystal at a resolution of 2.5−2.0 Å. The isomer bound to the active site was found to have carboxy and guanidino groups cis to each other and carboxy and 1-acetylaminopentyl trans to each other. The stereochemistry of this isomer bound in the active site differs from the stereochemistry of compound **12** in the active site. As a result, the guanidino group of compound **25** is oriented differently in the fourth pocket compared to the guanidino group of compound **12** and zanamivir **3**.[18]

The mixture of isomers was also soaked with influenza B neuraminidase, and the same isomer was found to bind to the active site of neuraminidase B, although the *n*-butyl side chain of the active isomer adopts two different binding modes in the two structures. In influenza B, the side chain is positioned against a hydrophobic surface formed by Ala 246, Ile 222, and Arg 224. However, in influenza A, the side chain occupies a region formed by the orientation of Glu 276. In its new position, Glu 276 forms hydrogen bonds with the guanidino group of Arg 224. It also shows some rearrangement of the water structure in the acetamido binding pocket, involving interactions with the NH of the *N*-acetyl group. These differences are small but very critical in the design of the molecules that are active for both influenza A and B.

Isolation, Purification, and Confirmation of the Active Isomer from the Mixture of Isomers of Structure 25

When **23** (Scheme 2) was subjected to careful purification by column chromatography on silica gel, both C-1′ diastereomers were isolated. The double bond of each was hydrogenated, and deprotection (*tert*-butoxycarbonyl removal and ester hydrolysis) gave **26b** and **27b**. The enzyme activity assay showed **27b** to be more than 100-fold more active than **26b**, thereby proving that the correct stereochemistry at C-1′ is important.

We then turned to separation of the C-1 diastereomers of **27a**, the protected precursor of the active **27b**. Repeated attempts using silica gel column chromatography failed. The methyl ester was then converted to the benzyl ester in two steps: hydrolysis to the carboxylic acid followed by treatment with benzyl chloroformate. The resulting benzyl ester diastereomers proved to be separable by silica gel column chromatography, affording the diastereomers **28a** and **29a**, which on deprotection gave **28b** and **29b**, respectively. The stereochemical assignments were made from NMR spectral data. Enzyme neuraminidase assay of both compounds **28b** and **29b** showed the values 360 and 13 nM, respectively, on the N2 neuraminidase. It is evident from this assay that the compound identified by crystallography and by enzyme assay is the same. We believe that this is the first example in the literature where the structure of a protein−ligand complex has been used to identify the most active isomer from a mixture of isomers.

Synthesis of Compound 29b

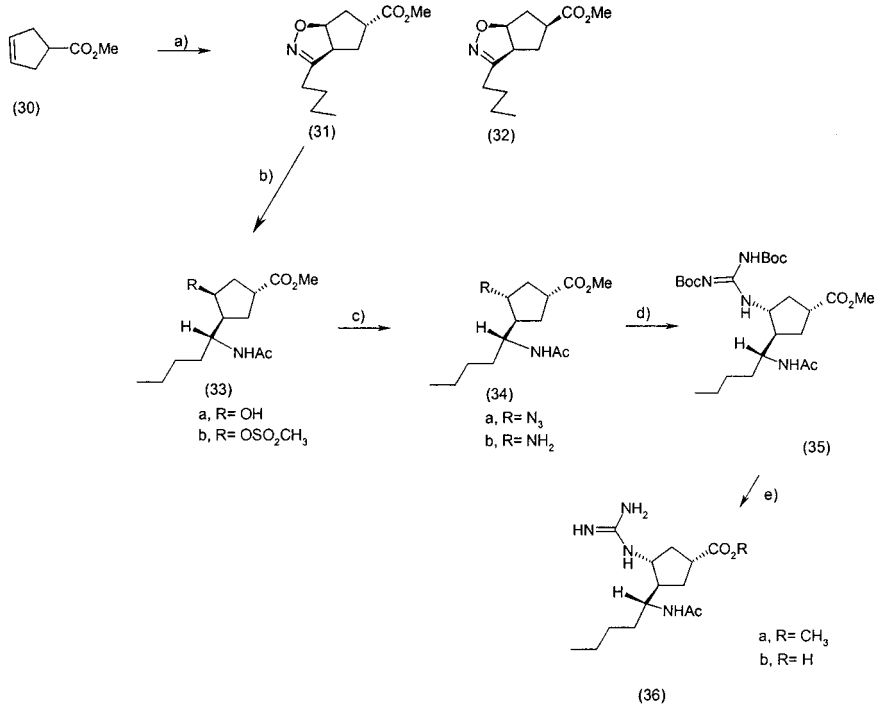
After identification of test compound **29b** as the active isomer, the next challenge was to devise a more direct synthetic route circumventing the difficult and tedious separations of the diastereomers.

The first route explored (see Scheme 3) began with methyl 3-cyclopenten-1-carboxylate (**30**), which was prepared according to published procedures.[32,33] Our planned route has the following stereochemical features: (i) 3 + 2 cycloaddition of **30** with valeronitrile oxide (produced in situ from nitropentane, phenyl isocyanate, and triethylamine) to produce a compound in which the carboxylate and the resulting isoxazoline ring are trans to each other; (ii) that hydrogenolytic opening of the isoxazoline ring followed by N-acetylation would produce the desired C-1′ isomer; (iii) the guanidino group would be obtained from hydroxyl with inversion.

In fact, the 3 + 2 cycloaddition reaction of **30** with the nitrile oxide gave both isomers (**32**, cis, and desired **31**, trans) in the ratio of 1:4, and the two were easily separated by silica gel chromatography. Opening of the isoxazoline ring in **31** was attempted with various reagents (Raney Ni/H_2; $PdCl_2/H_2$; Pd/C, H_2; PtO_2/H_2; $NaBH_4$; $Na(CN)BH_3$; $Mo(CO)_6$, $NaBH_4$; $PdCl_2$, triphenylphosphine, $NaBH_4$). All these experiments produced an undesired isomer *R* involving the C-1′ position. The isoxazoline **31** after PtO_2/H_2 reduction was acetylated to give **33a**, and the planned synthesis was continued as shown in Scheme 3. The subsequent conversions of hydroxyl to mesylate, azido, and amino through standard reactions gave the product **34b**. Compound **34b** was converted to the target **36b** via **35** by treatment with the 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, which is used throughout this work. The IC_{50} value for **36b** as an inhibitor of the N2 enzyme was 14 μM, which is the wrong value for the desired isomer at C-1′. The IC_{50} of 14 μM is approximately 1000-fold greater than that of desired isomer **29b**.

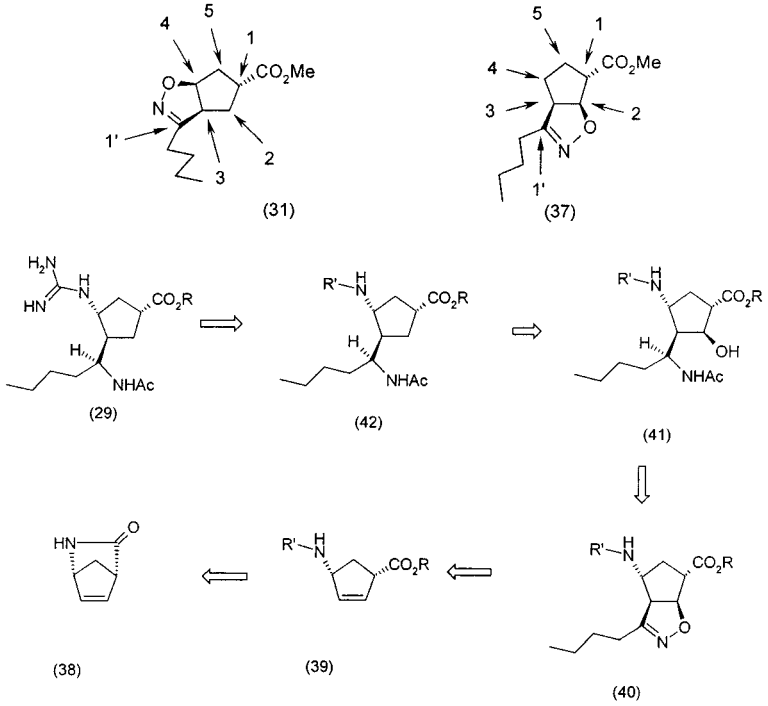
Although the route as described to **36b** did not provide the required stereochemical relationships, our experiences with this synthesis taught us that the cycloaddition approach using a nitrile oxide is a good way to introduce the aminoalkyl side chain into the molecule.

Scheme 3[a]



[a] Reagents: (a) nitropentane, PhNCO, Et_3N; (b) (i) PtO_2, H_2, HCl, (ii) Ac_2O, Et_3N, (iii) CH_3SO_2Cl, Et_3N; (c) (i) NaN_3, (ii) Pd/C, H_2; (d) MeS−C(=NBoc)(NHBoc), $HgCl_2$; (e) (i) CF_3CO_2H, (ii) NaOH.

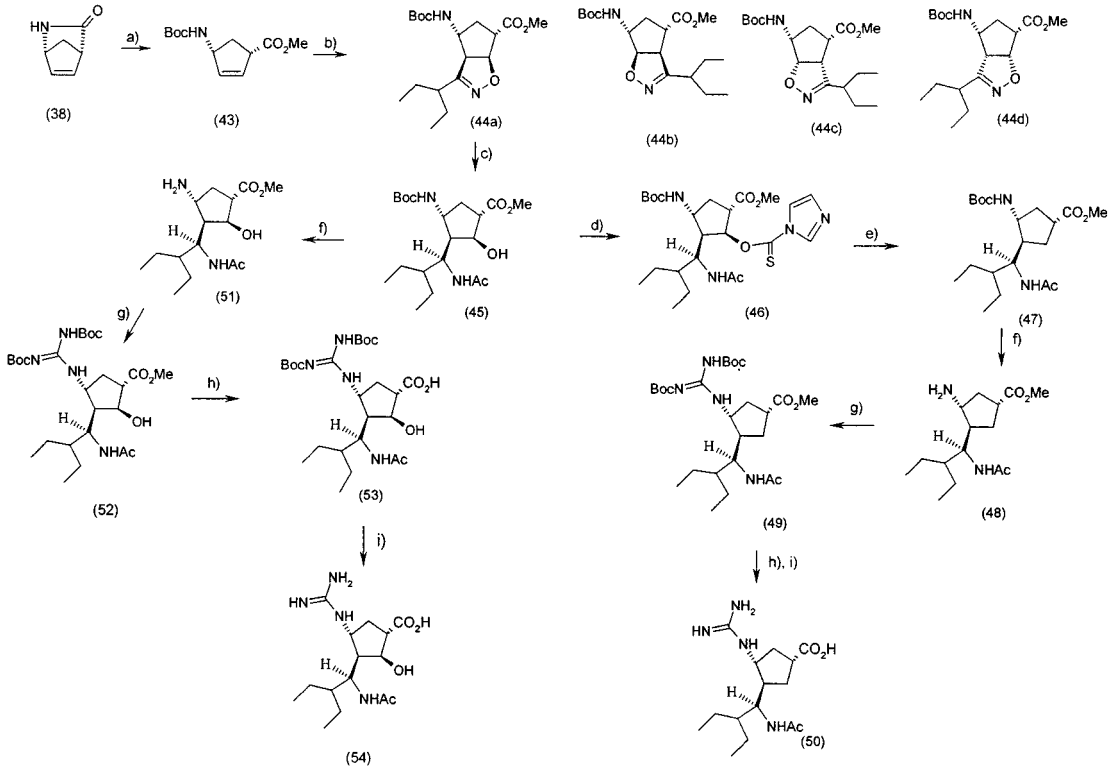
Scheme 4



Design of a Stereoselective Synthesis for 29b, the Desired *S* Isomer at C-1′: Analysis of the Opening of Isoxazoline

In compound **31**, the position of the isoxazoline ring was between C-3 and C-4 with respect to the carboxyl group, and opening of the isoxazoline ring produced the wrong isomer. In planning the new strategy, we envisioned a compound with the isoxazoline ring at C-2 and C-3 as shown in structure **37** (Scheme 4) as a viable alternative intermediate whose stereochemical features favored the required mode of ring opening by catalytic hydrogenolysis. Opening of the isoxazoline of hypothetical intermediate **37** by the method used for **31** should give the desired *S* isomer at the C-1′ position. However, from **31**, the guanidino group-to-be of **36b** was derived from the hydroxyl group produced when the isoxazoline ring was opened. In **37**, however, the hydroxyl group would be at the 2-position in the ring; thus, we needed a group at position-4 of the cyclopentane ring that could be converted into a guanidino group. Later in the

Scheme 5[a]



[a] Reagents: (a) (i) HCl/MeOH, (ii) $(Boc)_2O$, Et_3N; (b) $O_2N-CH_2-CH(C_2H_5)_2$, PhNCO, Et_3N; (c) (i) PtO_2, H_2, HCl, (ii) Ac_2O, Et_3N; (d) thiocarbonyldiimidazole; (e) $(Bu)_3SnH$; (f) HCl, ether; (g) $H_3CS-C(=NBoc)(NHBoc)$, $HgCl_2$; (h) NaOH; (i) CF_3CO_2.

synthesis, the 2-hydroxyl group obtained on opening of the isoxazoline could be deoxygenated. Retroactive analysis (see Scheme 4) of a synthesis suitable for generating a molecule like **37** showed that we needed a compound like **39**, having a double bond between C-2 and C-3 that would undergo the addition reaction with the nitrile oxide and, furthermore, having the adduct bearing an amino functionality at C-4 that could be converted to guanidino. The literature search showed that compounds of the type **39** were well-known and were easily obtained from the 2-azabicyclo[2.2.1]hept-5-en-3-one **38**, which was commercially available in both (+) and (−) forms and also as the racemic mixture.

The cycloaddition reaction of **39** with a nitrile oxide could give four products. For our desired product, addition of nitrile oxide needed to occur on the side of the ring opposite the two cis substituents, and further, the isoxazole oxygen must be attached in position-2 of the cyclopentane ring as in structural type **40**.

Design and the Selection of 50

As reported in our previous publication,[18] from the crystallographic studies, it was found that branched compound **50** (Scheme 5), having two ethyl groups in the side chain, should be the best fit in the active site. One ethyl group of the side chain points into the hydrophobic pocket created by repositioning of the Glu 276 side chain, while the other ethyl group points toward the hydrophobic surface formed by the hydrocarbon chains of Arg 224 and Ile 222. The crystal structure of the neuraminidase complex with **25** suggested that the ideal compound was (1*R*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-ethylbutyl]-4-{[amino(imino)methyl]-amino}cyclopentanecarboxylic acid **(50).** This compound then became a target for an efficient and stereoselective synthesis.

Stereoselective Synthesis of 50

The commercial 2-azabicyclo[2.2.1]hept-5-en-3-one, **38**, was treated with methanolic HCl, and the resulting amino ester was converted by di-*tert*-butyl dicarbonate to **43**. The synthesis described for **36** (Scheme 3) was adapted for **50**; compound **43** (used in place of **30**) underwent 3 + 2 cycloaddition with the nitrile oxide derived from 2-ethyl-1-nitrobutane (used in place of nitropentane) prepared from 2-ethyl-1-bromobutane[34] to give cycloadduct **44a** and other isomers (**44b**, **44c**, and **44d**, 15% total yield). The desired isomer (**44a**) was the major product (60% isolated yield), which was isolated by silica gel chromatography. The isoxazoline ring was then opened by hydrogenolysis in methanol in the presence of PtO_2 and an equivalent amount of HCl at 100 psi followed by acetylation to give **45**. The opening of this isoxazoline favored the desired C-1′ isomer, as we had envisioned with the aid of molecular models during the planning of this synthesis. The desired isomer dominated in a ratio of 94:6. The next step in the synthesis was deoxygenation of the 2-hydroxyl group of **45**. The presence of the adjacent ester group complicated this conversion. Several efforts led to elimination of water. Success was achieved, however, by the reaction of thiocarbonyl diimidazole to give **46** followed by free radical reaction of **46** with tributyltin hydride in toluene at 100 °C to give deoxygenated product **47**. Compound **47** was treated with hydrochloric acid in ether for removal of the *tert*-butoxycarbonyl group, and the resultant amine (**48**) was reacted with 1,3-bis(*tert*-

butoxycarbonyl)-2-methyl-2-thiopseudourea in DMF in the presence of triethylamine and mercuric chloride to give the protected guanidino derivative **49**. Compound **49**, after purification by silica gel column chromatography, was deprotected (ester was hydrolyzed with sodium hydroxide and *tert*-butoxycarbonyl groups were removed with trifluoroacetic acid in dichloromethane) to give the target molecule **50** isolated as its trifluoroacetate.

Compound **45** was also converted to the corresponding guanidino derivative **52** through the same reaction sequence used for **49**. Compound **52** gave the target molecule **54** under the same conditions used for **50**.

With the availability of (−) and (+) forms and also the racemic mixture of 2-azabicyclo[2.2.1]hept-5-en-3-one **38**, we first performed the syntheses of target compounds **50** and **54** beginning with (±)-**38** (Scheme 5). After development of the route, we repeated the synthesis beginning with each optical isomer of **38**. The products **50** and **54** with biological activities as reported in this paper and the earlier paper[18] were derived from (−)-**38**. The product derived from (+)-**38** did not cause any inhibition of neuraminidase, even at 1 mM concentration.

IC_{50} values of both compounds **50** and **54** on various strains of neuraminidase A and B were found to be similar. Because of the fewer steps required for the synthesis, compound **54** was selected for further biological evaluations and is in phase III clinical studies at present. Comparative biological data of compound **54** with the existing drugs zanamivir and oseltamivir have been reported.[35,36]

Conclusion

The structure-based drug design and the synthetic work reported in this paper has led to novel, potent, selective, and orally active influenza neuraminidase inhibitors (structures **50** and **54**). These agents differ structurally from existing six-membered ring neuraminidase inhibitors in that they bear the appropriately positioned enzyme-binding functionalities in the correct stereochemical relationship bound to a cyclopentane scaffold; the trisubstituted **50** with four chiral centers and tetrasubstituted **54** with five presented possibilities of numerous combinations of diastereoisomers. Mixtures of such isomers were separable using chromatography on silica gel. NMR spectroscopy (with nuclear Overhauser effect (NOE) methodology) served to establish the correct stereochemistry of isolated compounds. Protein crystallography was used to screen mixtures of isomers in order to identify the isomer binding to neuraminidase with the greatest avidity. This research may represent the first successful application of this technique to identify the active compound in such mixtures. This method for analyzing ligands bound to protein crystals may have wide applicability for screening mixtures of compounds in order to identify the tightest binding component.

The developmental synthetic work involved studies of several model compounds using lengthy multistep routes requiring tedious separations of diastereoisomers. The final developed synthetic route of choice to prepare pure **54**, however, required only six steps from available intermediate **43**. The first step produced four products (**44a**−**d**), but each was readily isolated with the desired **44a** obtained in 60% yield, while the yields of the other three (**44b**−**d**) totaled about 15%. Hydrogenolytic ring opening of **44a** produced almost exclusively the desired diastereoisomer **45**.

Compounds **54** [RWJ-270201 (BCX-1812)] and **50** proved to be very similar in biological activity, but compound **54** required fewer steps for synthesis. This compound became the agent of choice for further biological evaluation and is now in phase III clinical trials.

Experimental Section

General Methods. 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; CMA-80, chloroform (80), methanol (18), and ammonium hydroxide (2) mixture.

(1*R,3*S**,4*R**)-3-Azido-4-(azidomethyl)cyclopentanecarboxylic Acid (9).** Ethyl chloroformate (10.2 mL, 106.7 mmol) was added to a mixture of (1*R**,3*R**,4*R**)-methyl 4-acetyloxy-3-carboxycyclopentanecarboxylate [**7**, 23.4 g, 101.7 mmol, prepared from norbornen-2-yl acetate (**6**)[21]] and triethylamine (14.96 mL, 106.7 mmol) in tetrahydrofuran (200 mL) over a period of 0.25 h at 0 °C. After being stirred for an additional 0.5 h at the same temperature, the mixture was filtered and the cake was washed with tetrahydrofuran (3 × 10 mL).

Sodium borohydride (15.7 g, 415 mmol) was added to the filtrate in one portion at 10 °C. Then methanol (64 mL) was added dropwise over a period of 1 h. After being stirred an additional 0.5 h, the reaction mixture was neutralized with 1 N HCl. The solvent was removed, and the residue was taken up in water (50 mL). The pH of the reaction was then adjusted to about 6 with 1 N HCl. The mixture was extracted with ethyl acetate (4 × 150 mL). The organic layers were combined, washed with brine (150 mL), and dried. After filtration, the solvent was removed and the residue was stirred with ether (200 mL). The insoluble material was removed by filtration, and the filtrate was concentrated to furnish a white solid. The solid was dissolved in methanol (200 mL), and the solution was treated dropwise at room temperature with a sodium methoxide solution (7 mL, 25 w/v in methanol). The reaction mixture was stirred for 1.5 h, and the solvent was removed. The residue was purified by flash chromatography [20−40% CMA-80 in methylene chloride] to furnish 13.2 g (76%) of **8** as a white solid.

To a mixture of the above compound **8** (13.2 g, 76.0 mmol) in benzene (800 mL) were added triphenylphosphine (44.5 g, 169.0 mmol) and diethyl azodicarboxylate (27.8 mL, 176.0 mmol) followed by dropwise addition of hydrazoic acid (1 M solution in toluene, 190.7 mL, 190.7 mmol) over a period of 0.5 h. The reaction mixture was stirred overnight at room temperature and concentrated to half the original volume. The solid obtained on standing was removed by filtration. The

filtrate was concentrated, and to the residue was added ethyl acetate/hexane. The solid material was removed by filtration. The filtrate was concentrated, and the residue was purified by flash chromatography (5−25% ethyl acetate in hexane) to furnish 8.8 g (52%) of the methyl ester of **9**.

To the methyl ester (8.4 g, 37.6 mmol) was added 1 N NaOH (112.5 mL, 112.5 mmol), and the mixture was stirred for 16 h at room temperature before it was extracted with chloroform (3 × 20 mL). The aqueous layer was acidified to pH 4 using concentrated HCl and then extracted with chloroform (4 × 25 mL). The combined chloroform extracts from the acidic mixture were dried and filtered. The filtrate was concentrated to obtain 7.58 g (96%) of compound **9** as a colorless oil. ^{1}H NMR (DMSO-d_6): δ 12.25 (br s, 1H), 3.80 (m, 1H), 3.40 (m, 2H), 2.81 (m, 1H), 2.15 (m, 3H), 1.85 (m, 1H), 1.45 (m, 1H). IR (KBr): 2941, 2103, 1706, 1254 cm^{-1}. Anal. ($C_7H_{10}N_6O_2$) C, H, N.

(1*R,3*R**,4*S**)-*tert*-Butyl 3-[(Acetylamino)methyl]-4-({-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)-imino]methyl}amino)cyclopentanecarboxylate (11).** To a stirred mixture of **9** (4.0 g, 19.05 mmol) in methylene chloride (40 mL) was rapidly added liquified isobutylene (20 mL) followed by dropwise addition of a mixture of phosphoric acid (prepared by saturating 2.5 mL of 85% H_3PO_4 with P_2O_5) in methylene chloride (2.5 mL) and boron trifluoride etherate (0.9 mL). After the mixture was stirred at −78 °C for 2 h and at room temperature for 16 h, ice water and saturated aqueous $NaHCO_3$ were added until the mixture was basic. The organic layer was separated, and the aqueous layer was extracted with methylene chloride (2 × 20 mL). The combined organic layers were washed with water and brine and were dried. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography (5−10% ether in hexane) to give 1.2 g (24%) of the *tert*-butyl ester of **9**.

The ester was dissolved in methanol (20 mL) and hydrogenated at 50 psi in the presence of 10% Pd/C (0.1 g) for 1 h. The catalyst was removed by filtration. The filtrate was concentrated to furnish 0.94 g (96%) of *tert*-butyl 4-amino-3-aminomethylcyclopentanecarboxylate.

To a solution of above amine (0.63 g, 2.95 mmol) in dichloromethane (30 mL) at − 5 °C was added acetic anhydride (0.25 mL, 2.65 mmol) dropwise with stirring over a period of 5 min. The mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The solvent was removed, and the residue was purified by flash column chromatography [30−100% CMA-80 in methylene chloride] to furnish 0.33 g (44%) of **10**.

To a mixture of **10** (0.33 g, 1.29 mmol) in dimethylformamide (5 mL) were added triethylamine (0.63 mL, 4.52 mmol), 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.4 g, 1.42 mmol), and mercuric chloride (0.39 g, 1.42 mmol). The reaction mixture was stirred at room temperature for 16 h. Ethyl acetate (50 mL) was added, and the slurry was filtered through Celite. The filtrate was washed with water (2 × 20 mL) and brine (20 mL) and was dried. The filtered solution was then concentrated, and the residue was purified by flash column chromatography (30−50% ethyl acetate in hexane) to give 0.51 g (79%) of **11** as a white solid, mp 145−146 °C. ^{1}H NMR (DMSO-d_6): δ 11.50 (br s, 1H), 8.19 (d, J = 7.6 Hz, 1H), 7.85 (m, 1H), 4.0 (m, 1H), 3.2 (m, 1H), 2.95 (m, 1H), 2.80 (m, 1H), 2.1 (m, 3H), 1.8 (s, 3H), 1.7 (m, 2H), 1.5 (s, 9H), 1.4 (s, 18H). IR (KBr): 2976, 1726, 1647, 1366, 1153 cm^{-1}. Anal. ($C_{24}H_{42}N_4O_7$) C, H, N.

(1*R,3*S**,4*R**)-3-[(Acetylamino)methyl]-4-{[amino-(imino)methyl]amino}cyclopentanecarboxylic Acid (12).** A solution of **11** (0.44 g, 0.88 mmol) in methylene chloride (9 mL) and trifluoroacetic acid (1.7 mL, 22.1 mmol) was stirred at room temperature for 1 h. The solvent was evaporated several times after repeated additions of methylene chloride (3 × 5 mL). The residue was triturated with ether. The solid was collected by filtration and dried to give 0.21 g (67%) of **12**. ^{1}H NMR (DMSO-d_6): δ 12.20 (br s, 1H), 7.95 (m, 1H), 7.85 (m, 1H), 7.08 (m, 3H), 3.4 (m, 2H), 3.1 (m, 1H), 2.95 (m, 1H), 2.25 (m, 1H), 2.15 (m, 2H), 1.95 (s, 3H), 1.6 (m, 1H), 1.45 (m, 1H). IR (KBr): 3346, 2945, 2832, 1679, 1028 cm^{-1}. Anal. ($C_{10}H_{18}N_4O_3$·1.25CF_3CO_2H) C, H, N.

(±)-4-Azidocyclopent-2-en-1-one (14). To a mixture of sodium azide (2.12 g, 32.6 mmol) in dimethylformamide (15 mL) at 0 °C was added a solution of 4-bromocyclopent-2-en-1-one[26] (3.5 g, 21.0 mmol) in dimethylformamide (5 mL) over a period of 5 min. The reaction mixture was further stirred at 0 °C for 0.5 h and diluted with ethyl acetate (20 mL). The mixture was washed with water and brine and was dried. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography using 10−15% ethyl acetate in hexane to give 1.9 g (71%) of **14** as a light-yellow oil. ^{1}H NMR ($CDCl_3$): δ 2.35 (dd, J = 18.6 and 2.4 Hz, 1H), 2.77 (dd, J = 18.6 and 6.6 Hz, 1H), 4.67 (dd, J = 4.9 and 2.6 Hz, 1H), 6.35 (dd, J = 5.6 and 1.5 Hz, 1H), 7.54 (dd, J = 5.5 and 2.4 Hz, 1H).

Diethyl 2-(Acetylamino)-2-[(1*R,2*R**)-2-azido-4-oxocyclopentyl]malonate (15).** To a solution of diethyl acetamidomalonate (1.25 g, 5.7 mmol) in ethanol (10 mL) was added freshly cut sodium metal (0.03 g, 1.4 mmol). The reaction mixture was stirred at room temperature until all the sodium had dissolved. The reaction mixture was cooled to −40 °C, and a solution of **14** (0.7 g, 5.7 mmol) in ethanol (5 mL) was added dropwise over a period of 10 min. The reaction mixture was further stirred at −40 °C for 0.5 h, then quenched with trifluoroacetic acid (0.1 mL, 1.4 mmol). The solvent was removed, and the residue was purified by flash column chromatography (60% ether in hexane) to give 1.2 g (63%) of **15** as a white solid, mp 121−122 °C. ^{1}H NMR ($CDCl_3$): δ 6.78 (br s, 1H), 4.38 (m, 1H), 4.30 (m, 4H), 3.26 (m, 1H), 2.78 (ddd, J = 18, 8, and 1 Hz, 1H), 2.54 (dd, J = 18 and 8 Hz, 1H), 2.27 (m, 2H), 2.05 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H). IR (KBr): 3331, 2981, 2107, 1744, 1605, 1525 cm^{-1}. MS (ES^+), m/z: 341.2. Anal. ($C_{14}H_{20}N_4O_6$) C, H, N.

Diethyl 2-(Acetylamino)-2-{(1*R,2*R**)-2-[(*tert*-butoxycarbonyl)amino]-4-oxocyclopentyl}malonate (16).** A mixture of **15** (0.5 g, 1.5 mmol), $(Boc)_2O$ (0.39 g, 1.77 mmol), and 10% Pd/C (0.14 g) in ethyl acetate (25 mL) was hydrogenated at 45 psi for 1 h. The catalyst was removed by filtration, the filtrate was concentrated, and the residue was purified by flash column chromatography (75% EtOAc in hexane) to give 0.31 g of solid, which upon recrystallization from ether/hexane provided 0.27 g (45%) of **16** as a white solid, mp 135−136 °C. ^{1}H NMR ($CDCl_3$): δ 7.0 (s, 1H), 4.86 (m, 1H), 4.33−4.23 (m, 4H), 4.05 (m, 1H), 3.25 (m, 1H), 2.77 (m, 1H), 2.73 (m, 1H), 2.32 (m, 1H), 2.25 (m, 1H), 2.10 (s, 3H), 1.45 (s, 9H), 1.31−1.22 (m, 6H). IR (NaCl): 3365, 2980, 1739, 1689, 1519, 1394, 1369, 1275, 1170 cm^{-1}. MS (CI^-): 413 (M − 1). Anal. ($C_{19}H_{30}N_2O_4$) C, H, N.

Diethyl 2-(acetylamino)-2-{(1*R,2*R**)-2-[2-[(*tert*-butoxycarbonyl)amino]-4-(1,3-dithian-2-ylidene)cyclopentyl]}-malonate (17).** To a mixture of 2-trimethylsilyl-1,3-dithiane (7.88 g, 41.5 mmol) in THF (100 mL) at 0 °C was added *n*-BuLi (1.6 M solution in hexane, 28.6 mL, 45.7 mmol) over a period of 10 min. The mixture was further stirred at 0 °C for 0.75 h and then cooled to −40 °C, and a solution of **16** (4.3 g, 10.4 mmol) in THF (50 mL) was added over a period of 0.25 h. Stirring at −40 °C was continued for 5 h before the mixture was brought to −20 °C and treated with saturated aqueous NH_4Cl (50 mL). The mixture was warmed to room temperature, ether (20 mL) was added, and the organic layer was separated. The aqueous layer was extracted with ether (2 × 25 mL). The combined organic layers were dried. After filtration, the filtrate was concentrated and the residue was purified by flash column chromatography (30−35% ethyl acetate in hexane) to give 3.16 g (59%) of **17** as a colorless oil that solidified on drying, mp 66−68 °C. ^{1}H NMR ($CDCl_3$): δ 6.95 (br s, 1H), 4.85 (m, 1H), 4.23 (m, 4H), 3.77 (m, 1H), 2.98 (m, 2H), 2.84 (m, 5H), 2.22 (m, 2H), 2.11 (m, 2H), 2.05 (s, 3H), 1.44 (s, 9H), 1.26 (m, 6H). IR (KBr): 3388, 2979, 2934, 1743, 1690, 1512, 1368, 1242, 1169 cm^{-1}. MS (ES^+), m/z: 517.7. Anal. ($C_{23}H_{36}N_2O_7S_2$) C, H, N.

(Acetylamino)[(1*R,2*R**)-2-[(*tert*-butoxycarbonyl)amino]-4-(1,3-dithian-2-ylidene)cyclopentyl]ethanoic Acid**

(18). To a solution of **17** (7.5 g, 14.5 mmol) in ethanol (75 mL) was added 1 N NaOH (50.9 mL, 50.9 mmol) and water (25 mL). The solution was heated at reflux for 2 h, then cooled and treated with glacial acetic acid (4.6 mL, 76.3 mmol). The resulting solution was heated at gentle reflux for 1 h and then stirred at room temperature for 16 h. The solid that separated was collected by filtration, washed with water, and dried to furnish 1.63 g (27%) of **18**. The filtrate was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined, dried, and concentrated to furnish another 3.5 g (58%) of **18**. An analytical sample was prepared by recrystallization from ethanol as a white solid, mp 174−176 °C. ^{1}H NMR (DMSO-d_6): δ 7.65 (d, J = 8 Hz, 1H), 6.98 (d, 0.4H), 6.81 (d, J = 6.0, 0.6H), 4.22 (m, 0.4H), 4.06 (m, 0.6H), 3.71 (m, 0.6H), 3.54 (m, 0.4H), 2.80 (m, 4H), 2.27 (m, 2H), 2.08 (m, 4H), 1.82 (s, 3H), 1.36 (s, 5.4H),1.35 (s, 3.6H). IR (KBr): 3371, 2977, 1689, 1530, 1172 cm^{-1}. Anal. ($C_{18}H_{28}N_2O_5S_2$·0.75H_2O) C, H, N.

***tert*-Butyl (1*R**,2*R**)-2-{1-(Acetylamino)-2-[methoxy(methyl)amino]-2-oxoethyl}-4-(1,3-dithian-2-ylidene)cyclopentylcarbamate (19).** To a solution of **18** (5.1 g, 12.3 mmol) in THF (120 mL) cooled to 0 °C was added methyl chloroformate (1.0 mL, 13.5 mmol) and triethylamine (2.2 mL, 15.4 mmol). The reaction mixture was stirred at 0 °C for 0.75 h, and a cold solution of *N*,*O*-dimethylhydroxylamine hydrochloride (1.8 g, 18.5 mmol) and triethylamine (3.5 mL, 24.6 mL) in THF (5 mL) that had been stirred at 0 °C for 0.5 h was added. The reaction mixture was further stirred at room temperature for 16 h and filtered through Celite, and the cake was washed with THF (10 mL). To the filtrate was added again a cold solution of *N*,*O*-dimethylhydroxylamine hydrochloride (1.8 g, 18.5 mmol) and triethylamine (3.5 mL, 24.6 mmol) in THF (5 mL) that had been stirred at 0 °C for 0.5 h and stirred for another 16 h at room temperature. The solvent was removed, and to the residue were added NaOH (0.1 M, 100 mL) and ethyl acetate (100 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 75 mL). The combined organic layers were washed with brine (100 mL), dried, and concentrated. The residue was purified by flash column chromatography (90% ethyl acetate in hexane followed by 25% CMA-80 in methylene chloride) to give 4.2 g (74%) of **19**. An analytical sample was prepared by recrystallization from ether/hexane to give a white solid, mp 122−126 °C. ^{1}H NMR ($CDCl_3$): δ 6.89 (br s, 0.5H), 6.34 (br s, 0.5H), 5.16 (m, 0.5H), 5.01 (br s, 0.5H), 4.89 (br s, 0.5H), 4.60 (d, J = 8 Hz, 0.5H), 3.90 (m, 0.5H), 3.78 (s, 3H), 3.57 (m, 0.5H), 3.22 (s, 1.5H), 3.19 (s, 1.5H), 2.62−2.96 (m, 4.5H), 2.58 (m, 2H), 2.35 (m, 0.5H), 2.07−2.26 (m, 4H), 2.03 (m, 1.5H), 2.02 (s, 1.5H), 1.45 (s, 4.5H), 1.44(s, 4.5H). IR (KBr): 3341, 3269, 2978, 2936, 1715, 1681, 1653, 1521, 1156, 1171 cm^{-1}. MS (ES$^+$), *m*/*z*: 460.5. Anal. ($C_{20}H_{33}N_3O_5S_2$) C, H, N.

***tert*-Butyl (1*R**,2*R**)-2-[1-(Acetylamino)-2-oxoethyl]-4-(1,3-dithian-2-ylidene)cyclopentylcarbamate (20).** To a solution of **19** (0.23 g, 0.5 mmol) in THF (5 mL) at 0 °C was added lithium tri-*tert*-butoxyaluminohydride (1 M solution in THF, 1.1 mL, 1.1 mmol), and the mixture was stirred at room temperature for 16 h. The reaction mixture was carefully quenched with 1 N HCl (1 mL, to pH 4), stirred for 5 min, then treated with ether (10 mL) and 1.0 M aqueous sodium potassium tartrate (10 mL). After this mixture had been stirred at room temperature for 0.5 h, the organic layer was separated and the aqueous layer was extracted further with ether (2 × 10 mL). The combined organic layers were dried and concentrated, and the residue was purified by flash chromatography (50−80% ethyl acetate in hexane) to furnish 0.08 g (40%) of **20** as a white solid, mp 188−192 °C. ^{1}H NMR ($CDCl_3$): δ 9.49 (s, 1H), 7.45 (d, J = 9.6 Hz, 1H), 4.77 (dd, J = 9.6 and 2.1 Hz, 1H), 4.54 (d, J = 8.8 Hz, 1H), 3.73 (m, 1H), 2.83 (m, 5H), 2.69 (dd, J = 17.5 and 7.7 Hz, 1H), 2.52 (m, 1H), 2.16 (s, 3H), 2.10 (m, 4H), 1.41 (s, 9H). IR (KBr): 3337, 2982, 1729, 1681, 1535, 1166 cm^{-1}. MS (ES$^+$), *m*/*z*: 401.1. Anal. ($C_{18}H_{28}N_2O_4S_2$) C, H, N.

***tert*-Butyl (1*R**,2*R**)-2-[-1-(Acetylamino)pent-2-enyl]-4-(1,3-dithian-2-ylidene)cyclopentylcarbamate (21).** To a suspension of propyltriphenylphosphonium bromide (0.28 g, 0.73 mmol) in THF (10 mL) at −78 °C was added hexamethyldisilazane, sodium salt (1 M in THF, 0.73 mL, 0.73 mmol) dropwise. The cold mixture was stirred for 0.25 h, then allowed to warm to 0 °C, stirred for 0.5 h, then cooled again to −78 °C. To this mixture was added **20** (0.097 g, 0.24 mmol) in THF (6 mL), and the resulting mixture was stirred for 1 h at −78 °C. Water (10 mL) was added, and the layers were separated. The aqueous layer was extracted with ether (4 × 10 mL). The combined organic extracts were washed with brine and dried and were concentrated. The residue was purified by radial PLC (50−75% ethyl acetate in hexane) to furnish 0.093 g (91%) of **21** as a white solid, mp 175−177 °C. ^{1}H NMR ($CDCl_3$): δ 6.61−6.54 (m, 1H), 5.75−5.58 (m, 1H), 5.44−5.33 (m, 1H), 4.72 (m, 1H), 3.88−3.82 (m, 1H), 2.86−2.82 (m, 5H), 2.72−2.56 (m, 1H), 2.27−2.00 (m, 8H), 1.97 (s, 3H), 1.45 (s, 9H), 1.0−0.95 (m, 3H). IR (KBr): 3342, 2970, 2935, 1683, 1646, 1537, 1367, 1296, 1170 cm^{-1}. MS (ES$^+$), *m*/*z*: 427.5. Anal. ($C_{21}H_{34}N_2O_3S_2$) C, H, N.

(3*R,4*R**)-3-[1-(Acetylamino)pent-2-enyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic Acid (24).** To a mixture of **21** (0.640 g, 1.5 mmol) in methanol (44 mL) at room temperature was added 6 N HCl (3.8 mL, 22.8 mmol), and the reaction mixture was stirred for 24 h. After careful neutralization with sodium hydroxide, the mixture was extracted with EtOAc (4 × 15 mL). The combined organic extracts were washed with brine, dried, filtered, and concentrated to give 0.37 g (66%) of **22**. The residue (0.28 g) was dissolved in methylene chloride (5.0 mL). Trifluoroacetic acid (1.0 mL, 13.0 mmol) was added, and the solution was stirred at room temperature for 5 h. The solution was concentrated, and the residue was dissolved in DMF (7 mL). To this mixture were added triethylamine (3.0 mL, 21.5 mmol), 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.24 g, 0.81 mmol), and $HgCl_2$ (0.22 g, 0.81 mmol). This mixture was stirred at room temperature for 16 h, then diluted with ethyl acetate (50 mL), and filtered through Celite. The filtered solution was washed with water and brine, and the organic layer was dried and concentrated. The residue was purified by radial PLC (50−75% EtOAc/hexane) to give 0.21 g (64%) of **23**. To a mixture of **23** (0.116 g, 0.23 mmol) in THF (3.5 mL) and water (2 mL) at room temperature was added 1 N NaOH (0.6 mL, 0.6 mmol). The reaction mixture was stirred for 2 h and concentrated. The residue was dissolved in water, and the solution was acidified with glacial acetic acid, then extracted with EtOAc (4 × 10 mL). The combined organic extracts were washed with brine, dried, and concentrated. The residue was dissolved in methylene chloride (8.0 mL), and CF_3CO_2H (0.35 mL, 4.5 mmol) was added. After being stirred at room temperature for 24 h, the reaction solution was concentrated to give the crude product. Trituration with ether afforded 0.064 g (59%) of **24** as a tan solid, mp 62−64 °C. ^{1}H NMR (DMSO-d_6): δ 12.22 (s, 1H), 7.97−7.57 (m, 3H), 7.54−6.97 (m, 4H), 5.60−5.16 (m, 2H), 4.63 and 4.33 (m, 1H), 2.81−2.65 (m, 1H), 2.25−1.99 (m, 6H), 1.97−1.10 (m, 3H), 0.95−0.81 (m, 3H). IR (KBr): 3421, 2972, 1654, 1559, 1542, 1437, 1377, 1203, 1139 cm^{-1}. MS (ES$^+$), *m*/*z*: 297.0. Anal. ($C_{14}H_{24}N_4O_3$·1.5CF_3CO_2H) C, H, N.

(3*R,4*R**)-3-[1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic Acid (25).** A mixture of **24** (0.021 g, 0.045 mmol) and PtO_2 (0.05 g) in EtOH (6 mL) was hydrogenated at 50 psi for 16 h. The reaction mixture was filtered, and the filtrate was concentrated to give the crude product. Trituration with ether gave 0.020 g (95%) of **25** as a tan solid, mp 65−67 °C. ^{1}H NMR (DMSO-d_6): δ 12.20 (s, 1H), 7.81−7.57 (m, 2H), 7.54−6.80 (m, 4H), 3.77−3.40 (m, 1H), 2.78−2.60 (m, 1H), 2.40−2.05 (m, 4H), 1.86 (s, 3H), 1.80−1.23 (m, 7H), 1.10−0.82 (m, 3H). IR (KBr): 3347, 2974, 2927, 2886, 1676, 1455, 1380, 1090, 1050 cm^{-1}. MS (ES$^+$), *m*/*z*: 299.0. Anal. ($C_{14}H_{26}N_4O_3$·1.5CF_3CO_2H) C, H, N.

Methyl (3*R,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylate (26a) and Methyl (3*R**,4*R**)-3-[(1*S**)-1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylate (27a).** The chromatographic separations of compound **23** followed by *tert*-butoxycarbonyl depro-

tection and catalytic reduction as described for **25** provided **26a** and **27a**.

26a. ^{1}H NMR (DMSO-d_6): δ 7.8−7.0 (m, 5H), 3.84 (m, 1H), 3.6 (s, 3H), 2.9 (m, 1H), 2.12−1.95 (m, 3H), 1.86 and 1.85 (s, 3H), 1.79−1.48 (m, 2H), 1.32−1.20 (m, 5H), 0.86−0.82 (m, 3H). IR (NaCl): 3338, 3186, 2958, 1675, 1202, 1137, 1032 cm^{-1}. MS (ES$^+$), *m*/*z*: 313.2. Anal. ($C_{15}H_{28}N_4O_3$·1.5CF_3CO_2H) C, H, N.

27a: ^{1}H NMR (DMSO-d_6): δ 7.55−6.96 (m, 5H), 3.71 (m, 1H), 3.6 (s, 3H), 2.92−2.76 (m, 2H), 2.29−1.90 (m, 3H), 1.80 (s, 3H), 1.77−1.44 (m, 3H), 1.40−1.18 (m, 5H), 0.86−0.83 (m, 3H). IR (NaCl): 3370, 2947, 2833, 1679, 1448, 1203, 1140, 1031 cm^{-1}. MS (ES$^+$), *m*/*z*: 313.3. Anal. ($C_{15}H_{28}N_4O_3$·1.75CF_3CO_2H) C, H, N.

(3*R,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic Acid (26b) and (3*R**,4*R**)-3-[(1*S**)-1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic Acid (27b).** The saponification of the esters **26a** and **27a** provided **26b** and **27b**.

26b. MS (ES$^+$), *m*/*z*: 299.2.

27b. MS (ES$^+$), *m*/*z*: 299.2.

Benzyl (1*S,3*R**,4*R**)-3-[(1*S**)-1-(Acetylamino)pentyl]-4-({-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl}amino)cyclopentanecarboxylate (28a) and Benzyl (1*S**,3*R**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-({-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl}amino)cyclopentanecarboxylate (29a).** These compounds were obtained from **23** by chromatographic separation of isomers at C-1′, further reducing the alkene of the isomer A (active) to alkane, converting methyl ester to benzyl ester through acid, and again separating the isomers by chromatography at C-1.

28a. ^{1}H NMR ($CDCl_3$): δ 11.4 (s, 1H), 8.4 (d, J = 9.0 Hz, 1H),), 8.2 (d, J = 9.0 Hz, 1H), 7.47−7.26 (m, 5H), 5.1 (q, J = 12 Hz, 2H), 4.12 (m, 1H), 3.91 (m, 1H), 2.99 (m, 1H), 2.27 (m, 1H), 2.20 (m, 1H), 2.14 (m, 1H), 1.86 (m, 1H), 1.80 (m, 1H), 1.63−1.51 (m, 24H), 0.93−0.83 (m, 6H). IR (KBr): 3437, 3317, 3283, 2931, 1736, 1727, 1654, 1616, 1411, 1369, 1061 cm^{-1}. MS (ES$^+$), *m*/*z*: 589.7. Anal. ($C_{31}H_{48}N_4O_7$) C, H, N.

29a. ^{1}H NMR ($CDCl_3$): δ 11.41 (s, 1H), 8.49 (d, J = 6.7 Hz, 1H), 7.95 (d, J = 9.3 Hz, 1H), 7.33−7.36 (m, 5H), 5.13 (q, J = 10 Hz, 1H), 4.13 (m, 1H), 3.96 (m, 1H), 2.87 (m, 1H), 2.30 (m, 1H), 2.05−2.14 (m, 4H), 1.94 (m, 1H), 1.77 (m, 1H), 1.44−1.56 (m, 20H), 1.23−1.35 (m, 4H), 0.84−0.86 (m, 4H).

(1*S,3*R**,4*R**)-3-[(1*S**)-1-(Acetylamino)pentyl]-4-({-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl}amino)cyclopentanecarboxylic Acid (28b) and (1*S**,3*R**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-({-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl}amino)cyclopentanecarboxylic Acid (29b).** Hydrogenolysis and trifluoroacetic acid treatment of **28a** and **29a** gave **28b** and **29b**, respectively.

28b. MS (ES$^+$), *m*/*z*: 299.4.

29b. MS (ES$^+$), *m*/*z*: 299.3.

Methyl (3a*S,5*R**,6a*S**)-3-Butyl-4,5,6,6a-tetrahydro-3aH-cyclopenta[*d*]isoxazole-5-carboxylate (31).** A solution of nitropentane (10.8 mL, 87.8 mmol) and Et_3N (20 drops) in dry benzene (30 mL) was added to a refluxing solution of methyl 3-cyclopentenecarboxylate **30** (10.21 g, 80.9 mmol) and phenyl isocyanate (17.5 mL, 161.0 mmol) in dry benzene (50 mL) over a period of 1 h. The mixture was boiled under reflux for an additional hour, the diphenylurea was filtered off and washed with Et_2O (20 mL), and the combined filtrates were concentrated to yield an orange oil. This crude product was distilled (Kugelrohr) to remove unreacted phenyl isocyanate. The remaining mixture was purified by flash chromatography using a gradient of Et_2O/hexane to provide fractions homogeneous in **31** (8.1 g, 45%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 5.02 (m, 1H), 3.65 (m, 1H), 3.63 (s, 3H), 2.74 (m, 1H), 2.32 (m, 1H), 1.98 (m, 1H), 1.85−2.20 (m, 4H), 1.39−1.58 (m, 2H), 1.24−1.38 (m, 2H), 0.83 (t, J = 7.2 Hz, 3H). ^{13}C NMR: δ 13.61, 22.31, 25.95, 28.14, 33.53, 39.24, 41.30, 51.78, 53.71, 84.39, 159.50, 174.54. IR (neat): 1729, 1215 cm^{-1}. MS (ES$^+$), *m*/*z*: 225.9. Anal. ($C_{12}H_{19}NO_3$) C, H, N.

Methyl (3a*S,5*S**,6a*S**)-3-Butyl-4,5,6,6a-tetrahydro-3aH-cyclopenta[*d*]isoxazole-5-carboxylate (31).** Further elution of the reaction mixture from **31** gave 2.0 g (11%) of **32** as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 5.00 (m, 1H), 3.62 (s, 3 H), 3.55 (m, 1H), 2.84 (m, 1H), 2.13−2.45 (m, 5H), 2.02 (m, 1H), 1.41−1.63 (m, 2H), 1.27−1.40 (m, 2H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR: δ 13.67, 22.40, 25.98, 28.11, 31.93, 38.21, 42.76, 51.88, 54.10, 84.94, 160.49, 173.88. IR (neat): 1729, 1216 cm^{-1}. MS (ES$^+$), *m*/*z*: 225.8. Anal. ($C_{12}H_{19}NO_3$) C, H, N.

Methyl (1*R,3*S**,4*S**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-hydroxycyclopentanecarboxylate (33a).** To **31** (1.5 g, 6.7 mmol) in methanol (50 mL) were added concentrated HCl (0.6 mL, 7.2 mmol) and PtO_2 (0.2 g). The mixture was stirred very vigorously at 100 psi hydrogen pressure for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated to give (±)-methyl 3β-(1-aminopentyl)-4β-hydroxycyclopentane-1α-carboxylate hydrochloride, which was used for acetylation. To this amine hydrochloride in dichloromethane (20 mL) were added triethylamine (0.93 mL, 6.7 mmol) and acetic anhydride (0.67 g, 6.7 mmol) at room temperature. The mixture was stirred for 2 h, then was washed with water. The water layer was back-extracted with dichloromethane (20 mL). The combined organic layers were washed with water and brine and were dried. The filtered solution was then concentrated, and the residue was purified by flash column chromatography using hexane/ethyl acetate (1:1) as the eluent. The appropriate fractions were pooled together and concentrated to give 1.1 g (61%) of **33a** as a white solid, mp 137−139 °C. ^{1}H NMR ($CDCl_3$): δ 5.40 (d, J = 9.4 Hz, 1H), 4.29 (m, 1H), 4.15 (m, 1H), 3.68 (s, 3H), 3.15 (m, 1H), 2.90 (m, 1H), 1.90−2.10 (m, 7H), 1.80 (m, 1H), 1.70 (m, 1H), 1.45−1.20 (m, 5H), 0.80 (t, J = 6.0 Hz, 3H). MS (ES$^+$), *m*/*z*: 272.1. Anal. ($C_{14}H_{25}NO_4$) C, H, N.

Methyl (1*R,3*S**,4*S**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-methanesulfonyloxycyclopentanecarboxylate (33b).** Methanesulfonyl chloride (1.0 mL, 12.9 mmol) and Et_3N (2.7 mL, 19.4 mmol) were added to a mixture of **33a** (2.13 g, 7.85 mmol) and 4-(dimethylamino)pyridine (106 mg, 0.87 mmol) in dry CH_2Cl_2 (38 mL) at 4 °C. The mixture was stirred at 4 °C for 16 h, then treated with H_2O (50 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried, filtered, and concentrated. The residue was crystallized from Et_2O/hexane to give 1.1 g (41%) of **33b** as a white solid, mp 128−129 °C. ^{1}H NMR ($CDCl_3$): δ 5.15−5.25 (m, 1 H), 4.00−4.15 (m, 1H), 3.65 (s, 3H), 3.04−3.10 (m, 1H), 3.02 (s, 3H), 2.48−2.51 (m, 1H), 2.01−2.21 (m, 4H), 1.98 (s, 3H), 1.53−1.80 (m, 2H), 1.20−1.45 (m, 5H), 0.91 (t, J = 6 Hz, 3H). IR (KBr): 3303, 1722, 1649, 1556 cm^{-1}. MS (ES$^+$), *m*/*z*: 350.4. Anal. ($C_{15}H_{27}NO_6S$) C, H, N.

Methyl (1*R,3*S**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-azidocyclopentanecarboxylate (34a).** Sodium azide (2.3 g, 35.4 mmol) was added to a solution of **33b** (2.4 g, 6.8 mmol) in dry DMF (75 mL), and the resulting mixture was heated at 80 °C for 3 h. Water (100 mL) was added to the cooled mixture, and extraction with EtOAc (3 × 50 mL) followed. The combined extracts were washed with water (2 × 50 mL) and brine (50 mL) and were dried. After filtration, the filtrate was concentrated and the residue was purified by flash column chromatography using 20% CMA-80 in methylene chloride to give 1.74 g (86%) of **34a** as a white solid, mp 67−68 °C. ^{1}H NMR ($CDCl_3$): δ 5.20 (d, J = 6 Hz, 1 H), 4.05−4.10 (m, 1H), 3.65 (s, 3H), 3.50−3.61 (m, 1H), 2.75−2.80 (m, 1H), 2.29−2.39 (m, 1H), 2.01 (s, 3H), 1.95−2.13 (m, 3H), 1.58−1.69 (m, 2H), 1.20−1.40 (m, 5H), 0.90 (t, J = 6 Hz, 3H). IR (KBr): 3200, 3085, 2091, 1737, 1645 cm^{-1}. MS (ES$^+$), *m*/*z*: 297.4. Anal. ($C_{14}H_{24}N_4O_3$) C, H, N.

Methyl (1*R,3*R**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-aminocyclopentanecarboxylate (34b).** Compound **34a** (95 mg, 0.32 mmol) was hydrogenated in methanol (10 mL) in the presence of PtO_2 (25 mg) at 48 psi for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated to give a crude oil, which was purified by flash column chromatography using ethyl acetate (50 mL) followed by CMA-

80 to give an oil. The oil was dissolved in methanol (1 mL), and Et_2O/HCl (1.0 N, 1 mL) was added. Evaporation then gave 45 mg (50%) of **34b** as a yellow solid, mp 91−92 °C. ^{1}H NMR (DMSO-d_6): δ 7.95−8.15 (m, 3H), 3.90−4.01 (m, 1H), 3.65 (s, 3H), 2.92−3.05 (m, 1H), 2.75−2.85 (m, 1H), 2.15−2.28 (m, 1H), 2.00−2.09 (m, 1H), 1.91 (s, 3H), 1.71−1.90 (m, 4H), 1.10−1.45 (m, 5H), 0.85 (t, J = 6 Hz, 3H). IR (KBr): 3400, 3240, 1733, 1645 cm^{-1}. MS (ES$^+$), *m*/*z*: 271.4. Anal. ($C_{14}H_{26}N_2O_3$·HCl) C, H, N.

Methyl (1*R,3*R**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-({[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)-imino]methyl}amino)cyclopentanecarboxylate (35).** A solution of **34b** (1.45 g, 4.72 mmol) in DMF (20 mL) was treated sequentially with triethylamine (2.5 mL, 17.94 mmol), 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thioseudourea (1.5 g, 5.17 mmol), and $HgCl_2$ (1.4 g, 5.15 mmol). The resulting mixture was stirred at room temperature for 1.5 h. After dilution with ethyl acetate (50 mL), it was filtered through Celite, the filtrate was washed with H_2O (3 × 50 mL), and the organic layer was dried. The filtered solution was concentrated, and the residue was purified by flash column chromatography using 50% ethyl acetate in hexane to give 1.6 g (64%) of **35** as a white solid, mp 129−130 °C. ^{1}H NMR ($CDCl_3$): δ 11.30 (br s, 1H), 8.25 (br s, 1H), 7.10 (br s, 1H), 4.42−4.51 (m, 1H), 3.91−4.00 (m, 1H), 3.70 (s, 3H), 2.75−2.85 (m, 1H), 2.15−2.30 (m, 2H), 1.95 (s, 3H), 1.71−1.85 (m, 1H), 1.50 (s, 18H), 1.25−1.65 (m, 8H), 0.90 (t, J = 6 Hz, 3H). IR (KBr): 3323, 1721, 1716, 1612 cm^{-1}. MS (ES$^+$), *m*/*z*: 513.7. Anal. ($C_{25}H_{44}N_4O_7$) C, H, N.

Methyl (1*R,3*R**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylate (36a).** A solution of **35** (0.3 g, 0.59 mmol) in methylene chloride (5.0 mL) containing trifluoroacetic acid (0.8 mL) was kept at room temperature for 16 h. Evaporation followed, and the residue was triturated with ether to give 0.17 g (70%) of **36a**. ^{1}H NMR (DMSO-d_6): δ 7.8 (m, 2H), 7.0 (br s, 3 H), 3.8 (m, 1 H), 3.6 (s, 3H), 3.5 (m, 1H), 2.7 (m, 1H), 2.2 (m, 1H), 1.8 (m, 6H), 1.5 (m, 1H), 1.2 (m, 6H), 0.80 (m, 3H). IR (KBr): 3365, 3182, 2958, 2873, 1675, 1655, 1552 cm^{-1}. MS (ES$^+$), *m*/*z*: 313. Anal. ($C_{15}H_{28}N_4O_3$·1.15CF_3CO_2H) C, H, N.

(1*R,3*R**,4*R**)-3-[(1*R**)-1-(Acetylamino)pentyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic Acid (36b).** A mixture of the methyl ester of **36a** (13 mg, 0.03 mmol), 1 N sodium hydroxide (0.15 mL, 0.15 mmol), and water (0.2 mL) was stirred at room temperature for 2 h. The solution was then neutralized with 1 N HCl, and the volume was adjusted to 1.0 mL with water to give a 30 mM solution of **36b**. MS (ES$^+$), *m*/*z*: 299.1.

(−)-Methyl (1*S*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]cyclopent-2-ene-1-carboxylate (43). A mixture of (−)-(1*R*,4*S*)-2-azabicyclo[2.2.1]hept-5-en-3-one **38** (Aldrich, 5.0 g, 45.8 mmol) and 1 N HCl in methanol (100 mL) was heated at reflux for 10 h. The solution was then evaporated under reduced pressure, and the residue was stirred with ether (70 mL). The solid obtained was collected, washed with ether, and dried to give 7.0 g (86%) of (1*S*,4*R*)-(−)-methyl-4-aminocyclopent-2-en-1-carboxylate hydrochloride as a white crystalline solid, mp 106−108 °C. To a mixture of this compound (7.0 g, 39.6 mmol) and di-*tert*-butyl dicarbonate (9.5 g, 43.6 mmol) in dichloromethane (200 mL) at 0 °C was added triethylamine (6.1 mL, 43.6 mmol) over a period of 2.5 h, and the reaction mixture was stirred for 1 h. Purification by flash column chromatography with hexane/ethyl acetate gave 8.2 g (86%) of **43.** ^{1}H NMR ($CDCl_3$): δ 5.86 (m, 2H), 4.88 (br s, 1H), 4.78 (m, 1H), 3.71 (s, 3H), 3.47 (m, 1H), 2.51 (m 1H), 1.85 (m, 1H), 1.44 (s, 9H). MS (ES$^+$), *m*/*z*: 242.25. Anal. ($C_{12}H_{19}NO_4$) C, H, N.

(+)-Methyl (3a*R*,4*R*,6*S*,6a*S*)-4-[(*tert*-Butoxycarbonyl)-amino]-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-carboxylate (44a), (−)-Methyl (3a*S*,4*S*,-6*R*,6a*R*)-6-[(*tert*-Butoxycarbonyl)amino]-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-carboxylate (44b), (+)-Methyl (3a*R*,4*S*,6*R*,6a*S*)-6-[(*tert*-Butoxycarbonyl)amino]-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-carboxylate (44c), and (+)-Methyl (3a*S*,4*R*,6*S*,6a*R*)-4-[(*tert*-Butoxycarbonyl)-amino]-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-carboxylate (44d). A mixture of **43** (10 g, 41.4 mmol) and phenyl isocyanate (9.8 g, 82.3 mmol) in dry benzene (100 mL) was heated under reflux. To this mixture was added a mixture of 2-ethyl-1-nitrobutane (9.5 g, 62 mmol, 85% pure by ^{1}H NMR) and triethylamine (10 drops) in dry benzene (25 mL) over a period of 2 h. The stirred mixture was then refluxed for 24 h. When the mixture cooled, the solids were removed by filtration, the filtrate was concentrated, and ethyl ether (200 mL) was added to the residue. The mixture was allowed to stand overnight, the solids were again removed by filtration, and the filtrate was evaporated. The residue was purified by repeated flash column chromatography to give **44a**−**d**.

44a. Yield 9.0 g (61%), mp 66 °C. ^{1}H NMR ($CDCl_3$): δ 5.59 (br s, 1H), 5.20 (d, J = 8.8 Hz, 1H), 4.22 (br s, 1H), 3.76 (s, 3H), 3.58 (d, J = 8.8 Hz, 1H), 3.21 (d, J = 8.1 Hz, 1H), 2.50 (br s, 1H), 2.10 (m, 1H), 2.02 (m, 1H), 1.58−1.78 (m, 4H), 1.44 (s, 9H), 0.87−0.95 (m, 6H). MS (ES$^+$), *m*/*z*: 355.6. Anal. ($C_{18}H_{30}N_2O_5$) C, H, N.

44b. Yield 1.70 g (11%), mp 79−80 °C. ^{1}H NMR ($CDCl_3$): δ 5.20 (br s, 1H), 4.83 (d, J = 9 Hz, 1H), 4.17 (br s, 1H), 3.89 (dd, J = 4.8 and 2.8 Hz, 1H), 3.76 (s, 3H), 2.91 (m, 1H), 2.30 (m, 1H), 2.18 (br s, 1H), 2.08 (m, 1H), 1.55−1.70 (m, 4H), 1.44 (s, 9H), 0.88−0.92 (m, 6H). MS (ES$^+$), *m*/*z*: 355.64. Anal. ($C_{18}H_{30}N_2O_5$) C, H, N.

44c. Yield 0.6 g (4%), oil. ^{1}H NMR ($CDCl_3$): δ 5.13 (d, J = 9.2 Hz, 1H), 4.83 (m, 1H), 4.06 (m, 1H), 3.87 (m, 1H), 3.70 (s, 3H), 2.89 (m, 1H), 2.17 (m, 1H), 1.51−1.91 (m, 6H), 1.44 (s, 9H), 0.89 (t, J = 7.6 Hz, 3H), 0.83 (t, J = 7.6 Hz, 3H). MS (ES$^+$), *m*/*z*: 355.6. Anal. ($C_{18}H_{30}N_2O_5$) C, H, N.

44d. Yield 0.2 g (1.3%), mp 199−201 °C. ^{1}H NMR ($CDCl_3$): δ 5.13 (m, 1H), 4.66 (m, 1H), 4.22 (m, 1H), 3.79 (m, 1H), 3.73 (s, 3H), 3.01 (m, 1H), 2.25 (m, 1H), 2.15 (m, 1H), 1.90 (m, 1H), 1.45−1.65 (m, 4H), 1.44 (s, 9H), 1.0 (t, J = 7.6 Hz, 3H), 0.80 (t, J = 7.6 Hz, 3H). MS (ES$^+$), *m*/*z*: 355.6. Anal. ($C_{18}H_{30}N_2O_5$) C, H, N.

(−)-Methyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-[(*tert*-butoxycarbonyl)amino]-2-hydroxycyclopentanecarboxylate (45). To **44a** (8.0 g, 22.6 mmol) in methanol (160 mL) were added concentrated HCl (1.9 mL, 22.6 mmol) and PtO_2 (0.8 g). The mixture was stirred very vigorously at 100 psi hydrogen pressure for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated to give (−)-methyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(amino)-2-ethylbutyl]-4-[(*tert*-butoxy-carbonyl)amino]-2-hydroxycyclopentanecarboxylate hydrochloride, which was used for acetylation. To this amine hydrochloride (6.62 g, 16.8 mmol) in dichloromethane (60 mL) were added triethylamine (2.3 mL, 16.8 mmol) and acetic anhydride (1.75 mL, 18.4 mmol) at room temperature. The mixture was stirred for 2 h, then was washed with water (60 mL). The water layer was back-extracted with dichloromethane (20 mL). The combined organic layers were washed with water and brine and were dried. The filtered solution was then concentrated, and the residue was purified by flash column chromatography using hexane/ethyl acetate (1:1) as the eluent. The appropriate fractions were pooled together and concentrated to give 4.5 g (67%) of **45**. ^{1}H NMR ($CDCl_3$): δ 7.55 (d, J = 10.0 Hz, 1H), 4.78 (d, J = 9.3 Hz, 1H), 4.23 (d, J = 5.2 Hz, 1H), 4.11−4.15 (m, 1H), 3.99−4.04 (m, 1H), 3.70 (s, 3H), 2.80−2.84 (m, 1H), 2.45−2.53 (m, 1H), 2.08 (s, 3H), 1.96−2.00 (m, 1H), 1.66−1.72 (m, 1H), 1.19−1.44 (m, 15H), 0.77−0.87 (m, 6H). MS (ES$^+$), *m*/*z*: 401.75. Anal. ($C_{20}H_{36}N_2O_6$) C, H, N.

(−)-Methyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-[(*tert*-butoxycarbonyl)amino]-2-[(1*H*-imidazol-1-ylcarbonothioyl)oxy]cyclopentanecarboxylate (46). A mixture of **45** (10.0 g, 25 mmol) and 1,1′-thiocarbonyldiimidazole (9.0 g, 50 mmol) in anhydrous THF (130 mL) was heated at reflux temperature for 16 h. The solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), and the solution was washed with 0.5 N HCl (3 × 100 mL). The ethyl acetate layer was dried,

filtered, and concentrated. The residue was recrystallized from ethyl acetate/hexane to give 7.6 g (59.6%) of **46**. The filtrate was concentrated, and the residue was purified by flash column chromatography using ethyl acetate/hexane as the eluent to give an additional 1.4 g (11%) of **46**. ^{1}H NMR ($CDCl_3$): δ 8.4 (s, 1 H), 7.7 (s, 1 H), 7.05 (s, 1 H), 6.4 (m, 1 H), 6.0 (m, 1 H), 5.0 (m, 1 H), 4.5 (m, 1 H), 4.25 (m, 1 H), 3.75 (s, 3 H), 3.1 (m, 1 H), 2.5 (m, 2 H), 2.0 (s, 3 H), 1.9 (m, 2 H), 1.4 (m, 10 H), 1.15 (m, 3 H), 0.9 (m, 3 H), 0.75 (m, 3 H). Anal. ($C_{24}H_{38}N_4O_6S$) C, H, N.

(−)-Methyl (1*R*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-[(*tert*-butoxy-carbonyl)amino]cyclopentanecarboxylate (47). A solution of **46** (5.0 g, 9.8 mmol) in dry toluene (130 mL) was treated at 100 °C with tributyltin hydride (3.4 mL, 12.6 mmol) followed by azobisisobutyronitrile (AIBN, 0.1 g, 0.06 mmol), and the mixture was stirred at 100 °C for 10 min. The solvent was removed in vacuo, and the residue was dissolved in acetonitrile (100 mL) and washed with hexanes (3 × 100 mL). The acetonitrile layer was concentrated, and the residue was purified by flash column chromatography using ethyl acetate/hexanes (0−50% mixture) as eluent. The appropriate fractions were pooled together and concentrated to give 3.6 g (95%) of **47**. ^{1}H NMR (DMSO-d_6): δ 7.20 (d, J = 9.9 Hz, 1H), 6.72 (d, J = 7.5 Hz, 1H), 3.8 (m, 1H), 3.7 (m, 1H), 3.55 (s, 3H), 2.7 (m, 1H), 2.1 (m, 2H), 1.9 (m, 1H), 1.85 (s, 3H), 1.6 (m, 2H), 1.4 (s, 9H), 1.2 (m, 5H), 0.8 (m, 6H). Anal. ($C_{20}H_{36}N_2O_5$·0.75H_2O) C, H, N.

(−)-(1*R*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic Acid (50). A solution of **47** (0.15 g, 0.375 mmol) in ether (8 mL) was treated with stirring with 1 N HCl in ether (1.1 mL, 1.1 mmol). Stirring at room temperature was continued for 24 h. The solid that separated was collected by filtration, washed with ether, and dried to give 0.12 g of **48**. To a mixture of **48** (0.1 g, 0.31 mmol) in DMF (5.0 mL) were added Et_3N (0.19 mL, 1.4 mmol), 1,3-bis(*tert*-butoxycarbony)-2-methyl-2-thiopseudourea (0.11 g, 0.37 mmol), and $HgCl_2$ (0.1 g, 0.37 mmol). The reaction mixture was stirred at room temperature for 16 h, diluted with EtOAc (20 mL), and filtered. The EtOAc solution was washed with water and brine and was dried. The filtered solution was concentrated, and the residue was purified by flash chromatography to yield 0.13 g (78%) of **49**.

To a solution of **49** (0.1 g, 0.19 mmol) in ethanol/THF (1:1, 2.0 mL) was added 1 N sodium hydroxide (0.5 mL, 0.5 mmol), and the mixture was stirred at room temperature for 3 h. The solvents were removed, the residue was dissolved in water (2.0 mL), and the solution was then acidified with acetic acid. The precipitate that formed was collected by filtration to give 0.08 g (78%) of the acid of **49** as white solid. A solution of this acid in methylene chloride (5 mL) containing trifluoroacetic acid (0.5 mL) was kept at room temperature for 4 h. The solvent was removed, and the residue was triturated with ether to give **50** as trifluoroacetate. ^{1}H NMR (DMSO-d_6 + D_2O): δ 3.88 (m, 1H), 3.60 (m, 1 H), 2.68 (m, 1H), 2.28 (m, 1 H), 2.10 (m, 1H), 1.99 (m, 1H), 1.80 (s, 3 H), 1.53 (m, 2 H), 1.40 (m, 1H), 1.30 (m, 1H), 0.99−1.14 (m, 3H), 0.81−0.86 (m, 6H). MS (ES^+), m/z: 313.4. Anal. ($C_{15}H_{28}N_4O_3$·CF_3CO_2H·0.5H_2O) C, H, N.

(−)-Methyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-amino-2-hydroxycyclopentanecarboxylate (51). A solution of **45** (1.50 g, 3.75 mmol) in ether (8.0 mL) was treated with 1 N HCl in ether (12 mL, 12 mmol). The mixture was stirred at room temperature for 24 h, then heated at reflux for 2 h, and cooled to room temperature. The solid was collected to give 1.26 g of **51**. This was used as such for the next step.

(−)-Methyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-({[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl}amino)-2-hydroxycyclopentanecarboxylate (52). A solution of **51** (1.9 g, 5.0 mmol) in DMF (20 mL) was treated successively with Et_3N (2.4 mL, 17.5 mmol), 1,3-bis(*tert*-butoxycarbony)-2-methyl-2-thiopseudourea (1.45 g, 5.0 mmol), and $HgCl_2$ (1.36 g, 5.0 mmol). The reaction mixture was stirred at room temperature for 16 h. The mixture was diluted with EtOAc (200 mL) and filtered through Celite. The filtrate was washed with water and brine and was dried. The filtered solution was concentrated, and the residue was purified by flash chromatography using ethyl acetate/hexanes as eluent to yield 2.0 g (74%) of **52** as a foam. ^{1}H NMR ($CDCl_3$): δ 11.39 (s, 1H), 8.71 (d, J = 10.0 Hz, 1H), 8.59 (d, J = 9.2 Hz, 1H), 4.44−4.48 (m, 1H), 4.29 (d, J = 4.5 Hz, 1H), 4.23 (s, 1H), 3.97 (t, J = 10.0 Hz, 1H), 3.71 (s, 3H), 2.81−2.84 (m, 1H), 2.50−2.60 (m, 1H), 2.1 (s, 3H), 2.08−2.10 (m, 1H), 181−1.87 (m, 1H), 1.49 (s, 9H), 1.47 (s, 9H), 1.20−1.41 (m, 5H), 0.80 (t, J = 7.3 Hz, 3H), 0.75 (t, J = 7.3 Hz, 3H). Anal. ($C_{26}H_{46}N_4O_8$) C, H, N.

Table 1. Influenza Neuraminidase Inhibition by Cyclopentane Derivatives and Other Known Inhibitors

	IC_{50} [a] (μM)	
compound	influenza A[b]	influenza B[c]
12	27.4 ± 2.2	26.7 ± 0.4
25	0.050 ± 0.009	0.893 ± 0.05
27b	0.025 ± 0.001	0.043 ± 0.011
28b	0.359 ± 0.008	5.29 ± 0.44
29b	0.013[d]	0.412 ± 0.007
36b	14.6 ± 2.13	300[d]
50	<0.001	0.002 ± 0.001
54	<0.001	0.005 ± 0.001
zanamivir	0.002 ± 0.001	0.017 ± 0.001
oseltamivir	<0.001	0.005[d]

[a] Values are mean values ± standard deviations of two to four determinations except where indicated. [b] Influenza A: A/Singapore/157. [c] Influenza B: B/Victoria/70. [d] Determined only once.

(−)-(1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-({-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)-imino]methyl}amino)-2-hydroxycyclopentanecarboxylic Acid (53). A solution of **52** (1.5 g, 2.76 mmol) in ethanol/THF (1:1, 20 mL) was treated with 1 N sodium hydroxide (10 mL, 10 mmol), and the mixture was stirred at room temperature for 3 h. The resulting solution was evaporated, the residue was dissolved in water (20 mL), and the solution was then acidified with acetic acid. The precipitate thus obtained was collected by filtration to give 1.3 g (90%) of **53** as white solid. ^{1}H NMR ($CDCl_3$): δ 11.39 (s, 1H), 8.74 (d, J = 8.8 Hz, 1H), 8.59 (d, J = 8.0 Hz, 1H), 4.36−4.47 (m, 2H), 3.99 (t, J = 9.5 Hz, 1H), 2.81 (br s, 1H), 2.50−2.53 (m, 1H), 2.12 (s, 3H), 2.05−2.10 (m, 1H), 1.83−1.86 (m, 1H), 1.49 (s, 9H), 1.47 (s, 9H), 1.42−1.47 (m, 2H), 1.23−1.29 (m, 4H), 0.85−0.90 (m, 1H), 0.80 (t, J = 7.0 Hz, 3H), 0.76 (t, J = 7.0 Hz, 3H). Anal. ($C_{25}H_{44}N_4O_8$·0.75H_2O) C, H, N.

(−)-(1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-ethylbutyl]-4-{[amino(imino)methyl]amino}-2-hydroxycyclopentanecarboxylic Acid (54). A solution of **53** (2.1 g, 4.0 mmol) in methylene chloride (100 mL) containing trifluoroacetic acid (6.0 mL) was kept at room temperature for 16 h. The solvent was removed, and the residue was triturated with ether to give **54** as trifluoroacetate. ^{1}H NMR (DMSO-d_6 + D_2O): δ 4.36 (m, 2H), 3.84 (m, 1H), 2.81 (m, 1H), 2.67 (m, 1H), 2.57 (m, 1H), 2.02 (s, 3H), 0.79−1.81 (m, 13H). MS (ES^+), m/z: 329.5. Anal. ($C_{15}H_{28}N_4O_4$·1.5CF_3CO_2H) C, H, N.

Neuraminidase Inhibition Assay. A standard fluorimetric assay was used to measure influenza virus neuraminidase activity.[37] 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 2-(*N*-morpholino)-ethanesulfonic acid (MES) and buffer (4 mM calcium chloride at pH 6.5) and was 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; emission, 450 nm), and substrate blanks were subtracted from the sample readings. The IC_{50} was calculated by plotting the percent inhibition of neuraminidase activity versus the inhibitor concentration. The results are reported as the aver-

age of two to seven experiments (Table 1). 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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Supporting Information Available: NMR and NOE experimental results for compounds **16**, **28a**, **31**, **32**, **44a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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