Synthetic Studies on Mannostatin A and Its Derivatives: A New Family of Glycoprotein Processing Inhibitors

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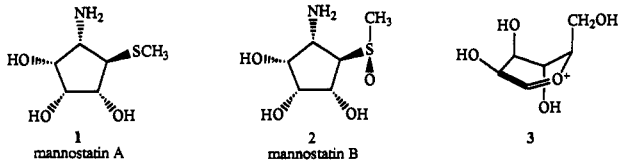
Abstract: Mannostatin A (**1**) is a naturally-occurring α-mannosidase inhibitor whose carbocyclic structure represents a significant departure from known alkaloid-based glycosidase inhibitors. The total synthesis of **1** [(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(methylthio)-2,3,4-trihydroxy-5-aminocyclopentane], as well as those of several analogs and derivatives, was devised in order to probe structure–activity relationships in this family of N-linked glycoprotein biosynthesis inhibitors. The synthetic strategy features an asymmetric hetero Diels–Alder reaction and a highly *syn*-stereoselective alkene dihydroxylation using OsO_4. Both *N*-benzylmannostatin and mannostatin sulfone exhibit good competitive inhibition of jack bean α-mannosidase (K_I = 380 ± 81 and 126 ± 16 nM, respectively), although not as potent as that of **1**. Interestingly, enantiomerically pure 3,4-bis-*epi*-mannostatin is also a modest competitive inhibitor of jack bean α-mannosidase (K_I of 16 ± 2 μM), comparable in activity to 1-deoxymannojirimycin. Synthetic samples of both diastereomeric sulfoxides of **1** exhibit activity comparable to that of **1**. Finally, epoxide **41**, the 3,4-anhydro derivative of **1**, was synthesized and shown to inactivate jack bean α-mannosidase in a time-dependent manner (K_I = 153 ± 26 μM; k_{inact}/K_I = 160 min^{-1} M^{-1}).

Introduction

Glycoproteins are ubiquitous in nearly all life forms and play important roles in molecular recognition,[1] adhesion,[2] and transport.[3] This extraordinary range of function results, in part, from the great diversity in carbohydrate structures found at glycosylated sites. The construction of fully functional N-linked glycoproteins begins with the synthesis and attachment of a common oligosaccharide to a specifically targeted asparagine residue. Then various glycosidase-catalyzed enzymatic trimming and processing steps generate the desired overall oligosaccharide domain. It has been largely through the development of specific glycosidase inhibitors that the detailed molecular events following glycosylation have been unraveled.[4]

Until recently, most glycosidase inhibitors were naturally-occurring polyhydroxylated mono-[5,6] or bicyclic[7,8] alkaloids resembling either D-glucose or D-mannose.[9] However, in 1989, extracts of the soil microorganism *Streptoverticillium verticillus* were found to contain an unusual pentasubstituted cyclopentane named mannostatin A.[10] A corresponding sulfoxide of mannostatin A was also isolated and designated mannostatin B. Both

Chart 1



1 and **2** were potent competitive inhibitors of rat epididymal α-mannosidase, with inhibition constants (K_I) of 48 nM. Mannostatin A also competitively inhibited jack bean, mung bean, and rat liver lysozomal α-mannosidases with IC_{50} values of 70, 450, and 160 nM, respectively.[11] Mannostatin A was also a potent inhibitor of Golgi processing mannosidase II (IC_{50} = 10–15 nM) but was inactive against processing mannosidase I.[11] Studies on cell cultures demonstrated that **1** blocked glycoprotein processing with concomitant increases in hybrid oligosaccharides, as would be consistent with mannosidase II inhibition.

The structure of mannostatin A (**1**, Chart 1) was first established by nuclear magnetic resonance and mass spectrometry.[10a] This assignment was later confirmed by X-ray diffraction, and the absolute stereochemistry of the inhibitor was determined to be that shown in **1**.[10b] X-ray analysis also established the *R*-configuration of the additional stereocenter in mannostatin B, as indicated in **2**.

The potent activity of **1** is intriguing since its carbocyclic structure represents a significant departure from known alkaloid-based inhibitors and bears little resemblance either to D-mannose or to the mannopyranosyl cation **3**, the purported intermediate in hydrolysis.[12] It is even more intriguing to note that the mirror image of **1** (not shown) more closely resembles the putative transition structure for mannopyranoside hydrolysis than does enantiomer **1** itself.[13]

From a biochemical perspective, interest in mannostatin A was heightened by preliminary reports that glycosidase inhibitors exerted immunoregulatory effects and showed promising activity

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in a variety of antitumor and antiviral screens.[14,15] Together with its noteworthy biological activity, the unusual and complex stereostructure of **1** made mannostatin A a worthwhile target for synthesis.

The first total syntheses of **1** were reported simultaneously in 1991 by us[16] and by Knapp and Dhar at Rutgers.[13] The Cornell route employed an acylnitroso cycloaddition to prepare both enantiomerically pure and racemic **1**, while the Rutgers team synthesized the individual enantiomers of **1** separately from D- or L-ribonolactone. Both efforts confirmed the assignment of absolute stereochemistry and further demonstrated that the levorotatory enantiomer of **1** had no inhibitory effect on mannosidases.[13,16] Since then, two additional syntheses of racemic mannostatin A have been reported.[17,18]

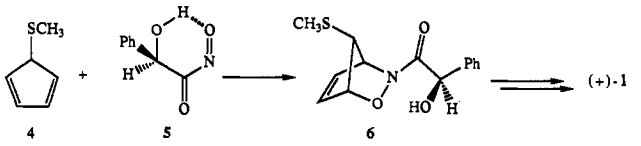
While interest in mannostatin A continues to grow, the mechanism of action of this unusual mannosidase inhibitor remains unknown. Moreover, the absence of activity in (−)-**1** is perplexing and further clouds any understanding of structure–activity relationships which might lead to the synthesis and development of other representatives of this new family of carbocyclic glycosidase inhibitors. We decided to address these issues in the larger context of a mechanistic investigation by designing and synthesizing several analogues, deoxy derivatives, and stereoisomers of mannostatin A. That initiative, besides resulting in a more efficient synthesis of **1**, has led to the discovery of several new congeners of **1** exhibiting potent mannosidase inhibition and inactivation.

Results and Discussion

Stereocontrolled assembly of the cyclopentane ring in mannostatin A, with its five chiral centers and imposing array of functionality, poses a significant challenge to the synthetic chemist. While a variety of [3 + 2] cycloaddition processes are known to provide access to functionalized cyclopentanes,[19] we preferred a [4 + 2] cycloaddition strategy that would generate three of the five chiral centers in **1** while simultaneously installing two of the heteroatoms as part of an appropriate N=O dienophile. The cycloaddition of acylnitroso compounds with dienes was especially attractive,[20] not only because the high reactivity of the nitroso dienophile would accommodate densely functionalized or relatively unreactive dienes but also because a successful asymmetric version of the diene/nitroso cycloaddition reaction has been developed for enantioselective synthesis.[21]

Asymmetric cycloaddition of the acylnitroso compound **5** derived from (*R*)-mandelic acid[21a] with the known 1-(methylthio)-cyclopenta-2,4-diene (**4**) (Scheme 1)[22] should afford predominantly bicyclic adduct **6** with the correct absolute configuration for (+)-mannostatin A. Both relative and absolute stereocontrol in forming the three contiguous O-, S-, and N-containing chiral centers would be assured by (a) *anti* orientation of the methylthio group in **4** as it approaches the heterodienophile and (b)

Scheme 1



intramolecular hydrogen bonding in **5**, which would direct face-selective *endo* cycloaddition *anti* to the bulky phenyl group in **5**. The former effect is well-precedented in the chemistry of 5-substituted cyclopentadienes, while the latter has been documented in prior cycloadditions of **5** itself.[21a] Subsequent osmylation of the remaining double bond was expected to complete the efficient assembly of substituents in **1**.

Both **4** and **5** are highly reactive species, which complicated the desired cycloaddition. The reported synthesis of **4** invoived addition of methanesulfenyl chloride to a solution of cyclopentadienylthallium in CCl_4 at room temperature, whereupon the precipitated salts were filtered and the product was distilled at reduced pressure.[22] Acylnitroso compound **5** was customarily generated by *in situ* oxidation of the corresponding (*R*)-mandelohydroxamic acid[23] which was then immediately trapped by diene. A 2.6:1 ratio of diastereomers was formed, with **6** as the major component. Since our preliminary communication appeared, several experimental observations have led to a significantly improved procedure.

Preparation of **4** by the published method[22] also led to bis-sulfenylated byproducts; however, by using CH_3SCl as the limiting reagent (typically 0.8 equiv), bis-sulfenylation could be minimized. Nevertheless yields of cycloadduct **6** never exceeded 35%. Since commercial samples of cyclopentadienylthallium (the best quality available is listed as 97% pure by Aldrich) were slightly colored, the material was purified by sublimation.[24] Fine white crystals of **5** were obtained in this manner, leaving behind a large residue of nonvolatile material. Gratifyingly, the yields of **6** rose using pure **5**, and by further limiting the quantity of CH_3SCl to 0.6 equiv, multigram scale cycloaddition reactions routinely afforded **6** and its diastereomer (not shown; now as a 3.3:1 ratio) in 45–50% overall yield. Flash chromatography and recrystallization gave pure **6** (mp 89–90 °C). With a more efficient route to our key intermediate at hand, the synthesis of **1** could be pursued on a preparative scale.

It was anticipated that mannostatin's two remaining *cis*-hydroxyl groups might be introduced stereoselectively by vicinal hydroxylation of **6** from the less hindered endo face of the bicyclic alkene. However, this reaction proved more difficult than expected. For example, attempted catalytic osmylation using the published procedure with *N*-methylmorpholine *N*-oxide[25] formed mixtures of the corresponding sulfoxides and sulfones of **6**. In fact, S-oxidation in the presence of co-oxidant has been observed previously.[26] On the other hand, stoichiometric amounts of OsO_4 led to preferential oxidation of the chiral auxiliary group, producing α-keto amide **7** (Scheme 2). Clearly, *endo*-approach to the heterobicyclic [2.2.1] ring system was less favorable than several competing undesirable oxidations.

As an alternative approach which seemed ideally suited to the system at hand, we decided to exploit the high *syn*-stereoselectivity which has recently been observed in the vicinal hydroxylation of

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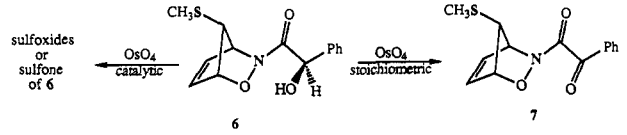
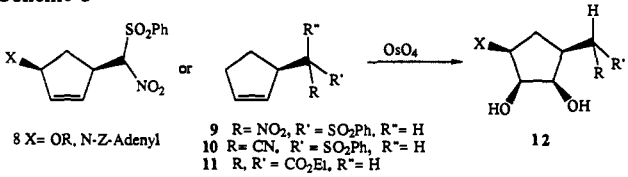
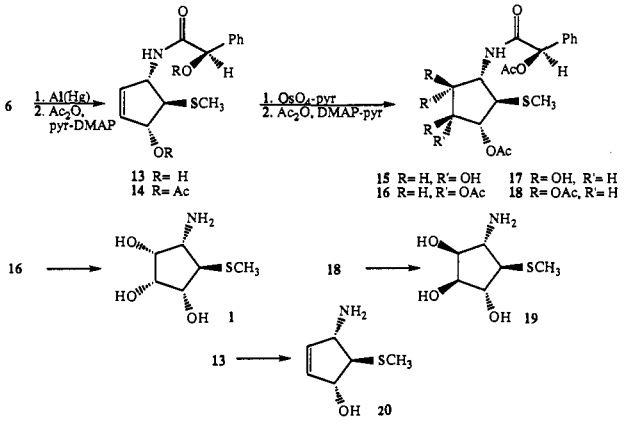
Scheme 2



Scheme 3



Scheme 4

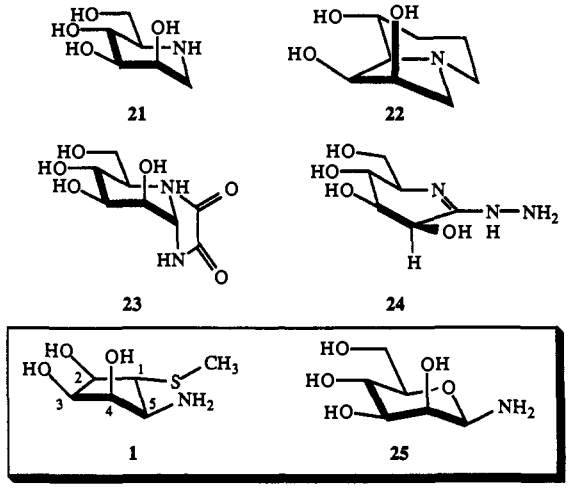


certain bis-allylically substituted cyclopentenes. In 1988, Trost *et al.* reported that osmylation of nitro sulfone **8** (Scheme 2) occurred with complete *syn*-stereoselectivity.[27] A subsequent investigation by Poli further noted a marked *syn* preference for hydroxylations of the monoallylically substituted nitro sulfone **9**, cyano sulfone **10**, and malonate **11** (Scheme 3).[28] *Syn*-stereoselectivity leading to **12**, which was highly solvent-dependent (nonpolar solvents favored *syn*-osmylation), was documented in both stoichiometric and catalytic osmylations.

As yet, no clear-cut mechanistic explanation of this phenomenon has emerged. While directed osmylations have been noted previously,[26b,c] Poli has suggested that conformational effects along the reaction coordinate leading to a late transition state may be responsible for the preferential *syn*-osmylation of **8–11**.[28] Poli noted that neither ^1H, ^{13}C, and ^{17}O NMR nor IR measurements indicate any complexation between OsO_4 and either nitro or sulfonyl groups. Furthermore, when the corresponding methylated analogues of **9–11** (R" = CH_3) were osmylated, predominantly *anti*-selectivity was observed.[28]

The requisite bis-allylically substituted cyclopentene **14** (Scheme 4) was prepared by reductive cleavage of the key bicyclic intermediate **6** using aluminum amalgam in THF–H_2O.[29] Reduction smoothly formed cyclopentenol **13**, and the yield (originally 41%)[16] was improved to 78% by using a large excess of amalgam (30–40 equiv). Complete characterization of **13** was facilitated by acetylation to diester **14** (94%). As with Trost's N,O-bis-allylically substituted cyclopentene **8**, stoichiometric osmylation of **14** (1.5 equiv of OsO_4, pyridine, room temperature, 24 h) proceeded with high facial selectivity to afford diols **15** and **17** in a 20:1 ratio (60%). The mixture was acetylated, and the resulting tetraacetates **16** and **18** were easily separable by silica gel flash column chromatography.

Chart 2



Exhaustive deacylation of **16** by exposure to acid (0.4 M HCl–CH_3OH, 60 °C, 24 h) quantitatively formed optically active mannostatin A hydrochloride (100%), whose physical properties were identical in every respect with those of an authentic sample.[30] With several improvements in the synthesis as noted, enantiomerically pure (+)-**1** could now be obtained in 7–8% overall yield from **4**.

Two new mannostatin congeners, bis-*epi* analogue **19** and dideoxy alkene **20**, were also synthesized at this stage. Acid methanolysis of **18**, the peracetylated minor product of osmylation (Scheme 4), required 5 days to go to completion under the same conditions which produced mannostatin from **16**. Apparently the rate-determining amide methanolysis in **16** was anchimerically assisted by a *cis*-hydroxyl group, an observation that would prove significant in later work (*vide infra*). Nevertheless, more vigorous conditions (6 N HCl–CH_3OH, reflux, 2–3 h) smoothly transformed **18** into 3,4-bis-*epi*-mannostatin A (**19**) in 77% yield. The synthesis of **20** by hydrolysis of mandelamide **13** proceeded smoothly in refluxing sodium hydroxide to afford the target aminoalkene in 71% yield.

In tests against various glycosidases, synthetic and naturally-occurring (+)-**1** exhibited the same inhibitory activity against Golgi processing mannosidase II. Moreover, a synthetic sample of racemic **1** possessed one-half the potency of (+)-**1**, indicating that the unnatural antipode of mannostatin A had no effect on the activity of processing mannosidase II. In addition, synthetic (+)-mannostatin A was a potent competitive inhibitor of jack bean α-mannosidase, with $K_I = 25 \pm 4$ nM. As reported, natural (+)-**1** was a very weak inhibitor of almond β-glucosidase and had no effect on amyloglucosidase.

Interestingly, enantiomerically pure 3,4-bis-*epi*-mannostatin (**19**) proved to be an inhibitor of jack bean α-mannosidase. Inhibition was of the competitive type, with **19** displaying a K_I of 16 ± 2 μM, making it slightly more potent than 1-deoxymannojirimycin (K_I = 68 μM).[6] Alkene **20**, however, which possessed only the contiguous N,O,S heteroatom triad of **1**, exhibited almost no activity against glycosidases. On jack bean mannosidase, relatively little effect was noted in the presence of 1 mM **20**.

As was noted in the Introduction, the structure of **1** little resembles other well-known mannosidase inhibitors such as 1-deoxymannojirimycin (**21**),[6] swainsonine (**22**),[8] kifunensine (**23**),[31] and mannoamidrazone (**24**) (Chart 2),[32] all of which show a clear similarity to D-mannose and are thought to mimic the

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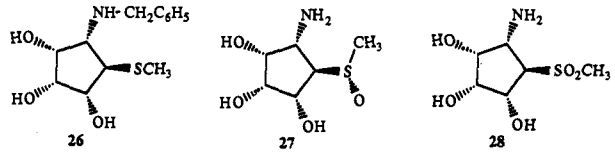
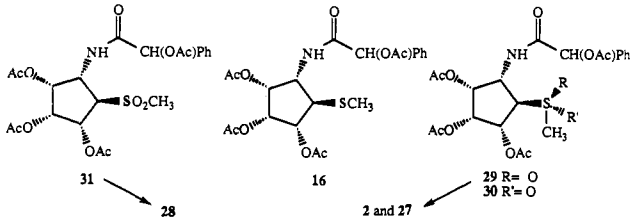
Chart 3



Scheme 5



transition state leading to the conformationally flattened D-mannopyranosyl cation 3.[12] Neither the carbocyclic framework nor the exocyclic amine group of **1** maps easily onto any of these heterocyclic systems, making it difficult to design rational analogues to probe the essential features of enzyme–inhibitor binding. However, mannostatin A does resemble D-mannopyranosylamine (**25**) (box, Chart 2), one of the earliest-known competitive inhibitors of mannosidases.[33] Foremost in the correlation is the alignment of the cyclopentylamine in **1** with the glycosylamine group of **25**, thus positioning mannostatin's amine at the anomeric position. Consistent with the importance of cationic charge at this site[33] is the observation that acetylation of **1** abrogates competitive inhibition.[11] By juxtapositioning structures **1** and **25**, the OH groups at C2, C3, and C4 of the cyclopentane ring correlate rather well with the C6, C3, and C2 hydroxyls, respectively, of the pyranose ring. Moreover, the methylthio substituent of mannostatin A (and the methylsulfinyl group of mannostatin B) maps rather loosely in the vicinity of the pyranose oxygen.

On the basis of this hypothesis, several new mannostatin analogues were predicted to be good mannosidase inhibitors. Glycosidases are thought to have a hydrophobic "pocket" which accommodates the aglycone moiety of typical substrates and enhances the binding of certain inhibitors. Since K_I values of certain hexopyranosylamines increase significantly by incorporating an *N*-benzyl substituent,[34] we predicted that *N*-benzylmannostatin A (**26**) (Chart 3) would also show enhanced inhibition of mannosidases. We further noted that, despite its enhanced potential for noncovalent binding, mannostatin B (**2**) was no more active than **1**, suggesting that some variations in the size of C1-substituents in **1** may be tolerated. Accordingly, the unnatural (*S*)-sulfoxide **27** as well as mannostatin sulfone **28** was added to the list of potential inhibitors for synthesis.

N-Benzylmannostatin **26** was synthesized directly from synthetic (+)-**1** by reductive amination with benzaldehyde ($NaBH_3CN$, CH_3OH–H_2O, 89% yield). To prepare (*S*)-sulfoxide **27**, amide **16** was oxidized to a mixture of stereoisomeric sulfoxides using sodium metaperiodate. The resulting diastereomers **29** and **30** were readily separated by chromatography; however, methanolysis of either diastereomeric amide furnished a 1:1 mixture of **2** and **27**, apparently by epimerizing the chiral configuration at sulfur (Scheme 5).

Alternatively, mannostatin A could be oxidized directly with 1 equiv of dimethyldioxirane to an inseparable 1:1 mixture of **2** and **27**. One-half of the resonances in the ^{13}C NMR spectrum of that mixture matched the carbon NMR spectrum of authentic mannostatin B. Exhaustive oxidation of **16** with dimethyldioxirane (2 equiv, in acetone) cleanly produced amido sulfone **31** which in HCl–methanol formed sulfone **28** in 95% overall yield.

Chart 4

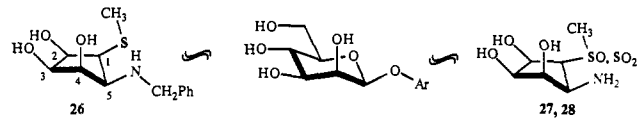
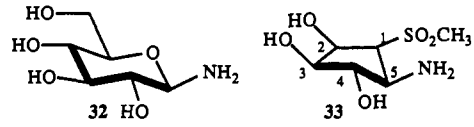


Chart 5



In enzymatic bioassays, *N*-benzylmannostatin **26** did indeed exhibit good competitive inhibition of jack bean mannosidase (K_I = 380 ± 81 nM), although not as potent as that of **1**. Failure to observe enhanced binding may indicate relatively little opportunity for hydrogen bonding or electrostatic interactions in that region of the active site cavity; however, the fact that a bulky benzyl substituent was tolerated by the enzyme might best be explained by positioning the amine group of **26** so as to orient the aromatic ring in the glycoside-binding domain of the active site.

The 1:1 mixture of mannostatin B (**2**) and its sulfoxide diastereomer **27** inhibited jack bean α-mannosidase with the same potency as mannostatin A. Since **2** has also been shown independently to possess the same level of activity as **1** against rat epididymal α-mannosidase,[10] it follows that **1**, **2**, and the diastereomeric sulfoxide **27** are comparable in potency as mannosidase inhibitors. Interestingly, the corresponding sulfone **28** of mannostatin A was nearly as potent an inhibitor of jack bean α-mannosidase as **1** itself. Inhibition by **28** was competitive, with K_I = 126 ± 16 nM.

Taken as a whole, the data on mannostatin *S*-oxides suggest that a considerable range of functionality is tolerated at the C1 position. The relative insensitivity of jack bean α-mannosidase to oxidation state changes at C1 is also consistent with the juxtapositioning of structures shown in Chart 4, whereby the methylthio, methylsulfinyl, and methylsulfonyl groups all map approximately to the hydroxymethyl locus in a corresponding hexopyranoside.

Since D-mannose and D-glucose are epimeric at C2 of the hexose framework, we wondered whether mannostatin might be transformed into a glucosidase inhibitor by changing the relative stereochemistry of the appropriate hydroxyl group. Inspection of model alignments between **1** and mannopyranosylamine **24** (box, Chart 2) suggested that epimerizing the C4 hydroxyl group of **1** would correspond to epimerizing C2 of mannose and would thus create a close structural mimic of β-D-glucopyranosylamine (**32**), itself an effective glucosidase inhibitor (Chart 5).[33] Accordingly, we undertook a synthesis of 4-*epi*-mannostatin sulfone **33**, an oxidized mannostatin analogue which embodies the desired stereochemical change.

One approach to sulfone **33** (Scheme 6) involved acidic hydrolysis of epoxy sulfone **34** to the desired C3,C4-*trans*-diol grouping as in **35**, followed by exhaustive deprotection. Epoxy sulfone **34** might be generated by oxidation of cyclopentene **14**, a key intermediate in the synthesis of mannostatin A. In fact, reaction of **14** with dimethyldioxirane (3–4 equiv, acetone)[35] produced a single epoxy sulfone identified as **34** in 97% yield. Exclusive *syn*-stereoselectivity in this process, established by correlation with the epoxide derived from **13** (Scheme 4) and by NMR nuclear Overhauser effects (NOEs), once again manifested the unusual stereoelectronic effects in this system.

Not surprisingly, hydrolysis of the epoxide ring in **34** required rather severe conditions, as has been noted when an oxirane ring

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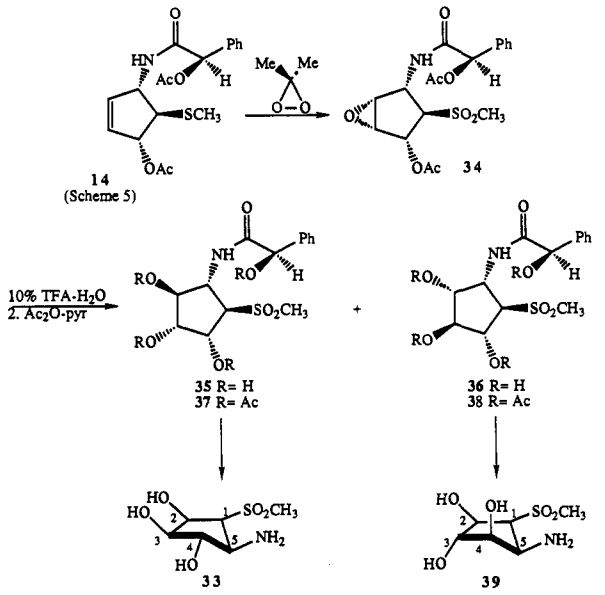
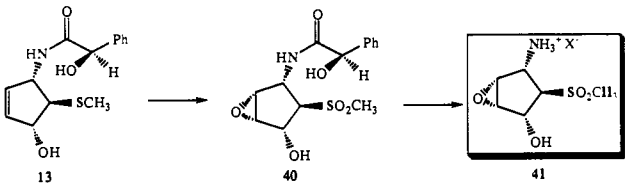
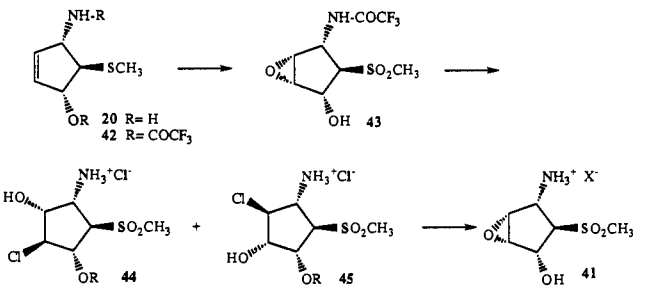
Scheme 6



Scheme 7



Scheme 8



is flanked by numerous electron-withdrawing groups. With methanolic aqueous HCl, a mixture of products, including chlorohydrins, was formed. However, with aqueous trifluoroacetic acid at elevated temperature (1:9 CF_3CO_2H:H_2O, 65 °C, 3 days), a slow, but relatively clean transformation leading to two major products was observed, apparently the result of nonregioselective *trans*-opening of the epoxide. Triols **35** and **36** were difficult to purify but could be separated as their triacetates **37** and **38**, respectively, by careful HPLC. Assignments of relative stereochemistry (Chart 5) were based on unambiguous NMR NOEs. Specifically, the C2 and C3 methine hydrogens in **37** exhibited an NOE enhancement of 10.1%, whereas no significant NOE was observed between the C2 and C3 methines in **38**. In a complementary experiment, the C4 and C5 methines in **37** displayed no measurable NOE while the same protons in **38** gave rise to a 10.9% enhancement.

The stereochemical assignments in **37** and **38** gained further support from the relative rates of acid-catalyzed amide methanolysis, in which anchimeric assistance from the C4-hydroxyl greatly enhanced reaction rates. Exposure of **38** to 0.4 N HCl–CH_3OH for 12 h at 65 °C furnished **39**; however, **37** under the same conditions produced only amide **35**. This dramatic difference in reactivity helped to simplify the epoxide opening reaction, since the product mixture of diastereomeric tetrols **35** and **36** could be hydrolyzed directly (0.4 N HCl–CH_3OH, 65 °C) and the putative "D-gluco" stereoisomer (44% yield as the amide **35**) could easily be separated from amine **39** (34%) on a Dowex ion exchange column. Hydrolysis of amide **35** required more vigorous conditions (6 N HCl, reflux, 3 h) to afford 4-*epi*-mannostatin sulfone **33** in 82% yield.

To determine whether epimerization at C4 of mannostatin produced a change in enzyme specificity, sulfone **33** was assayed against almond β-glucosidase, *Aspergillus niger* amyloglucosidase, and jack bean α-mannosidase. However, neither **33** nor its diastereomer **39** exhibited significant levels of inhibition against any of these enzymes. Clearly, the relative configurations at C3 and C4 of the mannostatin framework play central roles in defining mannosidase inhibition. It would, moreover, appear that the interaction of these cyclopentane-based inhibitors with glycosidases is not adequately explained by the mannopyranosylamine paradigm presented earlier.

One final synthetic target was designed as a mechanistic probe. Since epoxide-containing monosaccharide analogues such as the conduritols[9] and epoxyalkylglycosides[36] have proven to be useful affinity labelling reagents for identifying active site nucleophiles, epoxide **41** (box, Scheme 7) seemed like a promising candidate for active-site directed covalent modification of mannosidase.

In the most direct approach to **41**, oxidation of alkene **13** with 3 equiv of dimethyldioxirane produced epoxy sulfone **40**. Several methods were then investigated for hydrolyzing the mandelamide protecting group in **40** while preserving its epoxide under nonacidic conditions. Unfortunately, neither aqueous base nor any of the commonly used protease enzymes[37] were effective deamidating agents. Even after exposure to HCl–CH_3OH for 2 days at 65 °C, which transformed the epoxide ring into a mixture of diastereomeric chlorohydrins, hydrolysis of the mandelamide in **40** was incomplete. Eventually, treatment of mandelamide **40** with ceric ammonium nitrate (4 equiv, 60 °C)[38] did produce epoxide **41**, although its purification from residual **40** and inorganic byproducts proved difficult.

An alternate synthetic route was therefore devised from alkene **20** (Scheme 8) employing a different N-protecting group that furnished the desired epoxide. Treatment of **20** with trifluoroacetic anhydride furnished amidoester **42** (87%), which reacted with excess dimethyldioxirane (6 equiv) to produce epoxy sulfone **43** (91%). Although resistant to the typical conditions for base cleavage, the trifluoroacetamide group of **43** was cleanly removed in acid (HCl–CH_3OH, 65 °C, 24 h) to afford a mixture of diastereomeric chlorohydrins **44** and **45** as amine hydrochlorides. When treated with excess Amberlite IRA-900 (HO^-) ion exchange resin, the mixture of chlorohydrins recyclized to generate (after acidification with CF_3CO_2H) a single epoxy amine **41** (TFA salt) whose NMR spectrum matched the earlier synthetic sample.

Epoxide **41** inhibited jack bean α-mannosidase in a time-dependent fashion (pH 5.0, 37 °C), as would be expected for an irreversible inactivator. Inactivation was pseudo-first-order with respect to time (rate constants for different inactivator concentrations are presented in Table 1), and loss of enzyme activity was accelerated with increasing concentrations of **41** (Figure 1). Moreover, when jack bean α-mannosidase and **41** were incubated in the presence of mannostatin A, loss of enzymatic activity was retarded by higher concentrations of the competitive inhibitor, indicating that irreversible inactivation by **41** was active site directed (Figure 2). A plot of observed rate constants versus

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Table 1. Rates of Inactivation of Jack Bean α-Mannosidase by **41** as a Function of Concentration

[**41**] (incubation) (mM)	k_{obs} (min^{-1})
0.1	9.8×10^{-3}
0.2	1.3×10^{-2}
0.5	2.0×10^{-2}
1.0	2.1×10^{-2}

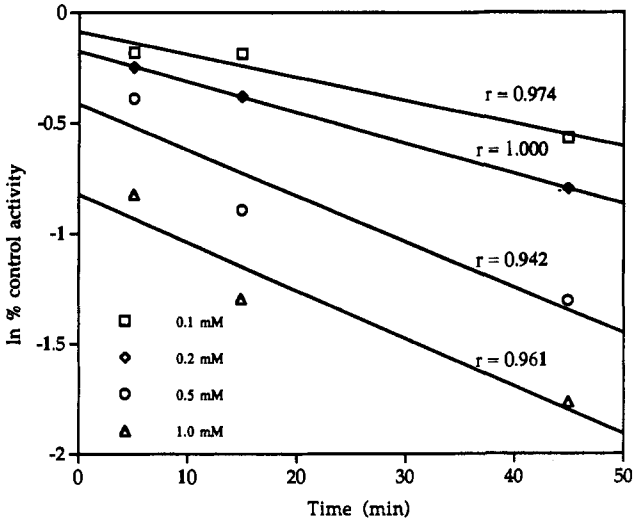

Figure 1. Effect of inactivator concentration on inactivation of jack bean mannosidase by **41**.

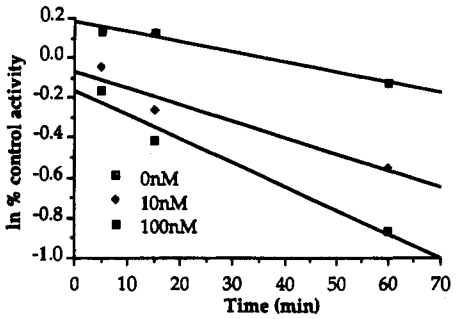

Figure 2. Active site protection of jack bean mannosidase against epoxide **41** by mannostatin A.

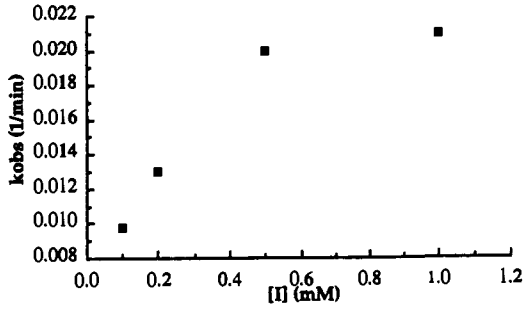

Figure 3. Apparent first-order rate constants versus concentration of epoxide **41**.

inactivator concentration exhibited saturation kinetics (Figure 3), consistent with the formation of an initial noncovalent EI complex which subsequently inactivated the enzyme by covalent modification. Double-reciprocal plots ($1/k_{obs}$ versus $1/[I]$) gave the dissociation constant of the noncovalent (*E*)-**41** complex (K_I = 153 ± 26 μM) as well as the first-order rate constant with which the complex was converted into inactivated enzyme (k_{inact} = (2.45 ± 0.21) × 10^{-2} min^{-1}).

Although many irreversible inactivators of gluco- and galactosidases have been developed, relatively few active site directed inactivators of other glycosidases are known. Conduritol F *trans*-epoxide has been reported to inactivate α-mannosidase from both jack beans and almonds,[9] with second-order rate constants for the association of the free enzyme and inhibitor [k_{inact}/K_I] of 0.8 and <0.02 min^{-1} M^{-1}, respectively. However, judging from the apparent activity of **41** [k_{inact}/K_I = 160 min^{-1} M^{-1}], this epoxide is a potent inactivator of α-mannosidase.

The synthetic approach to mannostatin A described here employs an asymmetric hetero Diels–Alder reaction and subsequent *syn*-selective osmylation to fix all five stereocenters in **1** and several of its derivatives. Moreover, *syn*-selective epoxidation and subsequent hydrolysis provide access to two other stereodefined tetrols in the mannostatin series. This strategy could prove useful in the synthesis of other naturally-occurring pentasubstituted cyclopentanes such as allosamidin,[39] trehalostatin,[40] and keruffaride,[41] some of which display potent biological activity as glycosidase inhibitors. Variations of the approach might also work with several families of readily available 1-heterosubstituted cyclopenta-2,4-dienes[42-45] which should also give cycloadducts arising from *anti*-approach of the dienophile. Examples of the latter obviously include heterodienophiles embodying N=O, N=N, and O=O arrays. Moreover, 1,3-dipoles may also undergo cycloaddition with the above-mentioned cyclopentadienes to produce stereodefined patterns of trisubstituted cyclopentenes whose osmylation and other functionalization is of interest.

Experimental Section

General Procedure. Proton and carbon-13 NMR spectra were obtained on Varian XL-400, Varian VXR-400, Bruker WM-300, Bruker AF-300, and Varian XL-200 spectrometers. Chemical shifts were reported as δ scale in parts per million downfield from tetramethylsilane. Spectra obtained in $CDCl_3$ were referenced to residual $CHCl_3$ (7.24 ppm) for proton NMR or $CDCl_3$ (77.0 ppm) for carbon-13 NMR. Spectra obtained in D_2O were referenced to HOD (4.67 ppm) for proton NMR or an internal standard for carbon-13 NMR. Spectra obtained in d_6-acetone were referenced to residual CHD_2COCD_3 (2.04 ppm) for proton NMR or to the carbonyl carbon of CD_3COCD_3 (206.0 ppm) for carbon-13 NMR. Spectra obtained in CD_3OD were referenced to residual CHD_2-OD (3.30 ppm) for proton NMR or to CD_3OD (49.0 ppm) for carbon-13 NMR. Infrared spectra were obtained on a Mattson Instruments Galaxy Series FT-infrared spectrometer. Ultraviolet spectra were obtained on a Hewlett-Packard HP 8451A diode array spectrophotometer. Optical rotations were measured on a Perkin Elmer 241 Polarimeter. Sample concentrations were expressed in grams of sample per 100 mL of solvent. Mass spectra were acquired using an AEI-MS 902, a Kratos MS-890 or a Finnegan 3300 mass spectrometer. Chemical ionization spectra were obtained using isobutane or methane as the reagent gas. Electron impact spectra were run at a 70-eV ionizing voltage. Some mass spectra were obtained at the Illinois Mass Spectrometry Laboratory using a VG ZAB-SE or VG 70-VSE instrument. Chemical ionization spectra were obtained as described above and fast atom bombardment spectra were obtained in a 3-nitrobenzyl alcohol matrix unless otherwise noted. Uncorrected melting points were determined with a Thomas–Hoover melting point apparatus and expressed in degrees centigrade (°C). High-pressure liquid chromatography (HPLC) was performed using an Eldex Model 9600 instrument equipped with a Waters Model 450 variable wavelength detector.

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium benzophenone ketyl. Benzene, pyridine, and methylene chloride (CH_2Cl_2) were distilled from calcium hydride. Methanol (CH_3OH) was distilled from magnesium methoxide.

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(*R*)-(−)-Mandelohydroxamic Acid (5). A solution of KOH in CH_3OH (6.6 M, 40 mL, 0.26 mmol) was added under Ar to a warm solution of hydroxylamine hydrochloride (18.7 g, 0.27 mol) in CH_3OH (100 mL). A white solid (KCl) precipitated, and the suspension was cooled in an ice bath to ensure complete precipitation. After 15 min, (*R*)-(−)-methyl mandelate (15.0 g, 0.09 mol) was added and the suspension was stirred at room temperature under Ar with TLC monitoring ($FeCl_3$ stain). After 96 h, the solids were filtered off and $CHCl_3$ (600 mL) was added. The precipitate was filtered off, and the supernatant was concentrated to leave 18.2 g of a white solid. This solid was crystallized (1:8 hexane:EtOAc, 450 mL) to give hydroxamic acid **5** (11.2 g, 74%) as a white solid: mp 127–128 °C, lit. 137–138 °C;[21a] R_f 0.36 (9:1:0.1 CH_2Cl_2:CH_3OH:AcOH); $[\alpha]_D$ −63° (c = 1.0, H_2O), −49.2° (c = 1.0, CH_3OH), lit. −63.0° (c = 1.6, H_2O);[21f] ^1H NMR (300 MHz, CD_3COCD_3) 10.25 (s, 1 H), 8.05 (s, 1 H), 7.47–7.26 (m, 5 H), 5.10 (s, 1 H); IR (film) 3400, 3300, 3250, 3000, 1650, 1450, 1075 cm^{-1}.

(1*R*,4*S*,7*R*)-3-(2-(*R*)-Hydroxy-2-phenylacetyl)-7-(methylthio)-2-oxa-3-azabicyclo[2.2.1]hept-5-ene (6). A solution of methanesulfenyl chloride in CCl_4 (1.48 M, 5.0 mL, 7.43 mmol) was added under Ar to a stirred suspension of freshly sublimed cyclopentadienylthallium (135 °C, 0.07 mmHg, 3.25 g, 12.08 mmol) in CCl_4 (30 mL) at room temperature. After addition, the suspension was filtered through Celite and rinsed with CCl_4 (10 mL) to give a yellow solution of 1-(methylthio)cyclopenta-2,4-diene (**4**) which was used without further purification. To this solution was added a solution of (*R*)-(−)-mandelohydroxamic acid (**5**) (1.86 g, 11.2 mmol, $[\alpha]_D$ −63°, c = 1.0, H_2O) in CH_3OH (25 mL) under Ar at 0 °C. Then a solution of $Bu_4N^+IO_4^-$ (0.36 M, 11.0 mmol) in CH_3OH (30 mL) was added under Ar. After 1 h, the ice bath was removed and the solvent removed in vacuo. The residue was dissolved in EtOAc (100 mL) and washed with 10% $NaHSO_3$ (2 × 50 mL), and the organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (3:2 hexane:EtOAc) produced 0.98 g (48%) of a 3.3:1 mixture of diastereomeric cycloadducts as a yellow solid. This solid was crystallized (5:1 hexane:EtOAc) to give the desired major diastereomer **6** (0.50 g, 24%) as a white solid: mp 89–90 °C; R_f 0.32 (3:2 hexane:EtOAc); $[\alpha]_D$ +10.4° (c = 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.21 (br. s, 5 H), 6.22, 5.60, 5.24, 5.18, 5.01, 3.99, 3.25 (br. s, each 1 H), 2.07 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.8, 137.1, 134.1, 131.1, 128.2, 128.1, 127.9, 86.2, 72.6, 65.8, 63.8, 15.8; IR (film) 3400, 3050, 2950, 1670, 1320, 850 cm^{-1}; CIMS m/z 278 (M + 1, 5%), 69 (100%).

The supernatant from crystallization was concentrated to give an oil which upon repeated (two times) flash chromatography (3:2 hexane:EtOAc) yielded the minor diastereomer, (1*S*,4*R*,7*S*)-3-(2-(*R*)-hydroxy-2-phenylacetyl)-7-(methylthio)-2-oxa-3-azabicyclo[2.2.1]hept-5-ene (0.036 g, 2%, not shown) as a clear oil: R_f 0.40 (1:1 hexane:EtOAc); $[\alpha]_D$ −33.5° (c = 1.7, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.38–7.28 (m, 5 H), 6.59, 6.36, 5.20, 5.12, 5.02, 4.00, 3.08 (br. s, each 1 H), 2.01 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.1, 139.1, 135.5, 131.7, 128.5, 128.3, 127.3, 86.5, 72.9, 66.0, 63.8, 15.7; IR (film) 3450, 3050, 2950, 1650, 1325, 840 cm^{-1}; CIMS m/z 278 (M + 1, 97%), 70 (100%).

(1*R*,4*S*,7*R*)-7-(Methylthio)-3-(oxophenylacetyl)-2-oxa-3-azabicyclo-[2.2.1]hept-5-ene (7). A solution of OsO_4 in pyridine (1.0 M, 0.112 mL) was added under Ar to a stirred solution of cycloadduct **6** (0.0254 g, 0.092 mmol) in pyridine (0.150 mL) at room temperature. After 24 h, a $NaHSO_3$ solution (0.45 M) in 1.5:1 pyridine:H_2O (1.25 mL) was added, and the resulting mixture was stirred at room temperature for 4 h and extracted with CH_2Cl_2 (6 × 1 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated in vacuo to give an oil which was flash chromatographed (3:2 hexane:EtOAc) to give the α-keto amide **7** (0.008 g, 32%) as a white solid: R_f 0.63 (3:2 hexane:EtOAc, (2,4-dinitrophenyl)hydrazine positive stain); ^1H NMR (300 MHz, $CDCl_3$) 8.04 (d, 2 H, J = 7.6 Hz), 7.85 (d, 2 H, J = 7.4 Hz), 7.64–7.51 (m, 2 H), 7.46 (t, 4 H, J = 7.5 Hz), 6.74 (m, 1 H), 6.54–6.52 (m, 1 H), 6.43–6.39 (m, 2 H), 5.41 (s, 1 H), 5.38 (s, 1 H), 5.15 (s, 1 H), 5.09 (s, 1 H), 3.41–3.39 (m, 2 H), 2.13 (s, 3 H), 2.10 (s, 3 H); IR (film) 3050, 2900, 1690, 1650, 1230, 830 cm^{-1}; CIMS m/z 276 (M + 1, 49%), 105 (100%); EIMS 275 (M, 2%), 105 (100%).

(1*R*,2*R*,5*S*)-1-(Methylthio)-2-hydroxy-5-(*N*-((*R*)-hydroxyphenylacetyl)-amino)cyclopent-3-ene (13). Aluminum amalgam (0.977 g, 36.2 mmol), prepared according to Keck,[29] was added under Ar to a stirred solution of cycloadduct **6** (0.302 g, 1.81 mmol) in 10:1 THF:H_2O (16.5 mL) at 0 °C. After 3 and 6 h, additional portions of Al–Hg (0.50 g, 18.5 mmol) were added and the reaction mixture was allowed to warm to room temperature for 16 h and was worked up by diluting with THF (50 mL), stirring at room temperature (30 min), and filtering through Celite. Toluene (30 mL) was added, and the reaction mixture was concentrated at the rotary to give an oil which was flash chromatographed (1:3 hexane:EtOAc) to give diol **13** (0.395 g, 78%) as a white solid: mp 118–119 °C; R_f 0.19 (1:2 hexene:EtOAc); $[\alpha]_D$ −51.4° (c = 0.81, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.33–7.30 (m, 5 H), 6.89 (d, 1 H, J = 8.5 Hz), 5.89 (dt, 1 H, J = 5.7, 1.7 Hz), 5.67 (dt, 1 H, J = 5.8, 0.8 Hz), 4.97 (s, 1 H), 4.56 (m, 1 H), 4.49 (br. s, 1 H), 3.84 (br. s, 1 H), 3.55 (br. s, 1 H), 2.82 (t, 1 H, J = 4.0 Hz), 2.06 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) 174.5, 141.5, 136.6, 133.6, 129.3, 129.0, 127.9, 81.8, 75.6, 61.0, 60.5, 14.3; IR (film) 3300, 2920, 1650, 1540, 1100, 1080, 700 cm^{-1}; EIMS m/z 261 (M − H_2O, 20%), 81 (100%); CIMS m/z 262 (M + H − H_2O, 7%), 69 (100%).

(1*R*,2*R*,5*S*)-1-(Methylthio)-2-acetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopent-3-ene (14). Acetic anhydride (0.101 mL, 2.14 mmol) and (*N*,*N*-dimethylamino)pyridine (DMAP, 0.008 g, 0.07 mmol) were added under Ar at room temperature to a stirred solution of diol **13** (0.099 g, 0.36 mmol) in pyridine (4 mL). After 1 h, ether (10 mL) was added and the solution was extracted with saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to give an oil which was flash chromatographed (3:2 hexane:EtOAc) to give diacetate **14** (0.122 g, 94%) as a clear oil: R_f 0.25 (3:2 hexane:EtOAc); $[\alpha]_D$ −59° (c = 0.69, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.43–7.32 (m, 5 H), 6.22 (d, 1 H, J = 8.9 Hz), 6.04 (s, 1 H), 5.98 (dt, 1 H, J = 5.8, 1.7 Hz), 5.91 (dt, 1 H, J = 7.1, 0.8 Hz), 5.42 (m, 1 H), 4.88 (m, 1 H), 2.95 (t, 1 H, J = 4.2 Hz), 2.16 (s, 3 H), 2.11 (s, 3 H), 2.06 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) 172.1, 171.7, 170.7, 136.8, 136.4, 132.8, 129.9, 129.6, 128.5, 83.6, 77.1, 60.9, 56.4, 20.8, 20.6, 14.2; IR (film) 3350, 3050, 2950, 1750, 1680, 1560, 1400, 1240, 1060 cm^{-1}; CIMS m/z 304 (M + 1 − AcOH, 100%).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylthio)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (16) and (1*R*,2*R*,3*S*,4*S*,5*S*)-1-(Methylthio)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (18). A solution of OsO_4 in pyridine (1.0 M, 1.22 mL) was added under Ar to diacetate **14** (0.32 g, 0.88 mmol) in pyridine (2.5 mL) at room temperature. After 4 h, a second portion of OsO_4 in pyridine (1.0 M, 0.35 mL) was added and the solution stirred for 1 h. A $NaHSO_3$ solution (0.65 M) in 1.5:1 pyridine:H_2O (20 mL) was added, and after 4 h, the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give an oil. Acetic anhydride (0.50 mL, 5.30 mmol) and DMAP (0.021 g, 0.17 mmol) were added under Ar at room temperature to a solution of this oil in pyridine (6 mL). After 1 h, Et_2O (30 mL) was added and the solution was extracted with saturated $NaHCO_3$ (2 × 20 mL) and brine (10 mL). The combined aqueous washes were extracted with Et_2O (20 mL), and the organic fractions were combined, dried over $MgSO_4$, and concentrated in vacuo to give an oil. Flash chromatography (1:1 hexane:EtOAc) gave the minor diastereomer **18** (0.010 g, 2.4%) as a clear oil: R_f 0.27 (1:1 hexane:EtOAc); $[\alpha]_D$ −8.7° (c = 0.52, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) 7.45–7.29 (m, 5 H), 6.34 (d, 1 H, J = 8.8 Hz), 6.05 (s, 1 H), 5.27–5.16 (m, 2 H), 5.08 (dd, 1 H, J = 7.2, 3.1 Hz), 4.37 (q, 1 H, J = 8.8 Hz), 2.88 (dd, 1 H, J = 10.4, 7.1 Hz), 2.18 (s, 3 H), 2.08 (s, 6 H), 2.05 (s, 3 H), 1.97 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.4, 169.6, 169.4, 169.3, 168.8, 134.9, 129.1, 128.7, 127.6, 75.4, 72.6, 72.1, 54.6, 49.6, 21.0, 20.8, 20.5, 12.2; ^{13}C NMR (100 MHz, CD_3COCD_3) 169.6, 169.5, 169.3, 168.8, 136.2, 129.0, 128.7, 128.0, 77.2, 75.9, 72.9, 72.8, 55.3, 48.9, 20.3, 20.2, 19.9, 19.8, 11.9; IR (film) 3300, 1750, 1670, 1530, 1360, 1220, 1050 cm^{-1}; CIMS m/z 482 (M + 1, 100%).

The major diastereomer **16** (0.18 g, 43%) was also obtained as a white solid: mp 133–135 °C; R_f 0.19 (1:1 hexane:EtOAc); $[\alpha]_D$ = −6.8° (c = 0.84, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.45–7.35 (m, 5 H), 6.29 (d, 1 H, J = 9.2 Hz), 6.06 (s, 1 H), 5.41 (t, 1 H, J = 4.6 Hz), 5.25 (dd, 1 H, J = 6.3, 4.2 Hz), 5.05 (t, 1 H, J = 6.3 Hz), 4.55 (dt, 1 H, J = 9.3, 6.3 Hz), 3.09 (t, 1 H, J = 6.5 Hz), 2.16, 2.11, 2.03, 2.01, 1.89 (s, each 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) 169.4, 169.0, 168.8, 168.6, 167.4, 135.1, 129.3, 128.9, 127.6, 75.4, 73.6, 71.3, 69.8, 53.4, 53.1, 20.8, 20.5, 20.4, 20.1, 14.1; IR (film) 3400, 1750, 1700, 1530, 1370, 1250 cm^{-1}; CIMS m/z 482 (M + 1, 14%), 69 (100%); EIMS m/z 421 (M − AcOH, 4%), 301 (M − 2AcOH, 10%), 43 (100%).

(+)-Mannostatin A (1). A stirred solution of tetraacetate **16** (0.060 g, 0.125 mmol) in anhydrous HCl–CH_3OH (0.36 M, 3.5 mL) was heated at 70 °C under Ar for 22 h. The solvent was removed in vacuo and the remaining oil dissolved in H_2O (3 mL) and extracted with ether (2 × 2 mL) and EtOAc (2 mL). The aqueous layer was lyophilized to afford (+)-mannostatin A·HCl ((+)-**1**·HCl) (0.027 g, 100%) as a clear oil: R_f 0.25 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +4.5° (c = 0.24, CH_3OH), +3.6° (c = 0.23, H_2O); ^1H NMR (300 MHz, D_2O) 4.18 (dd, 1 H, J = 6.5, 3.9

Hz), 3.99 (t, 1 H, J = 4.5 Hz), 3.90 (dd, 1 H, J = 7.6, 4.8 Hz), 3.44 (t, 1 H, J = 6.7 Hz), 3.01 (t, 1 H, J = 7.4 Hz), 2.05 (s, 3 H); ^{13}C NMR (75 MHz, D_2O, $CDCl_3$ external reference) 76.5, 74.7, 70.9, 57.7, 54.4, 14.6; FABMS m/z 180 (M + 1, 100%).

(1*R*,2*R*,3*S*,4*S*,5*S*)-1-(Methylthio)-2,3,4-trihydroxy-5-aminocyclopentane (19). A stirred solution of tetraacetate **18** (0.0041 g, 0.0085 mmol) in 6 N HCl (1 mL) was heated at reflux under Ar for 3 h. Upon cooling, the solution was extracted with ether (2 × 1 mL). The organic layers were extracted with H_2O (1 mL), and the aqueous layers were combined, frozen, and lyophilized to give an oil which was purified by ion exchange chromatography (Dowex 50X8-200, H^+ form, eluted with H_2O then 0.3 N HCl) to give the hydrochloride salt of **19** (0.0014 g, 77%) as an oil: R_f 0.28 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +10.3° (c = 0.09, H_2O); ^{1}H NMR (300 MHz, D_2O) 3.99 (t, 1 H, J = 5.6 Hz), 3.78–3.76 (m, 2 H), 3.20 (dd, 1 H, J = 10.3, 6.8 Hz), 2.56 (dd, 1 H, J = 10.3, 8.2 Hz), 1.95 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) 77.6, 74.6, 71.3, 57.3, 49.2, 11.1; IR (film) 3270, 2900, 1500, 1425, 1120 cm^{-1}; FABMS m/z 180 (M + 1).

(1*R*,2*R*,5*S*)-1-(Methylthio)-2-hydroxy-5-aminocyclopent-3-ene (20). A solution of amide **13** (0.082 g, 0.29 mmol) in an aqueous 15% NaOH solution (5 mL) under Ar was heated at reflux for 23 h. Upon cooling, the aqueous solution was extracted with Et_2O (3 × 3 mL) and EtOAc (3 × 3 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give the amine **20** (0.030 g, 71%) as a clear oil: R_f 0.33 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +19.5° (c = 0.15, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) 5.85 (s, 2 H), 4.59 (d, 1 H, J = 5.3 Hz), 3.64 (d, 1 H, J = 5.9 Hz), 2.36 (t, 1 H, J = 5.6 Hz), 2.20 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) 137.4, 133.8, 82.0, 64.1, 62.3, 14.1; IR (film) 3400, 3250, 2900, 1570, 1450, 1330, 1100 cm^{-1}; FABMS m/z 146 (M + 1).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylthio)-2,3,4-trihydroxy-5-(*N*-benzylamino)cyclopentane (26). A solution of NaOH in CH_3OH (0.10 M, 0.075 mL) was added under Ar at room temperature to a solution of mannostatin A·HCl (**1**·HCl) (0.007 g, 0.031 mmol) in CH_3OH (0.20 mL) to give a solution having pH 7. To this solution was added a CH_3OH solution of benzaldehyde (2.5 M, 0.013 mL, 0.031 mmol) and two 3-Å molecular sieves. A CH_3OH solution of $NaCNBH_3$ (1.75 M, 0.011 mL, 0.019 mmol) was added, and after 20 h, the reaction mixture was diluted with CH_3OH (1 mL) and filtered through Celite. The solution was acidified to pH 1 with 1 N HCl and concentrated in vacuo. The residue was dissolved in H_2O (2 mL) and extracted with Et_2O (1 mL). The aqueous layer was lyophilized to afford the hydrochloride salt of **26** (0.0089 g, 89%) as a sticky white solid: R_f 0.47 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +20.1° (c = 0.43, CH_3OH); ^{1}H NMR (300 MHz, D_2O) 7.45–7.36 (m, 5 H), 4.36–4.25 (m, 3 H), 4.00 (t, 1 H, J = 5.0 Hz), 3.94 (t, 1 H, J = 5.9 Hz), 3.38 (t, 1 H, J = 7.8 Hz), 3.12 (t, 1 H, J = 7.9 Hz), 1.97 (s, 3 H); ^{13}C NMR (75 MHz, D_2O, $CDCl_3$ external reference) 132.8, 132.4, 132.2, 132.1, 76.8 73.7, 70.9, 61.8, 53.5, 52.8, 14.2; IR (film) 3270, 2910, 1080 cm^{-1}; FABMS m/z 270 (M + 1).

Mannostatin B (2) and (1*R*,2*R*,3*R*,4*R*,5*S*)-1-((*S*)-Methylsulfinyl)-2,3,4-trihydroxy-5-aminocyclopentane (27). A solution of dimethyldioxirane in acetone (0.088 M, 0.070 mL, 0.0061 mmol) was added under Ar at room temperature to a solution of mannostatin A (**1**) (0.0011 g, 0.0051 mmol) in 2:1 acetone:H_2O (0.150 mL). After 1 h, the solution was concentrated in vacuo to give a 1:1 mixture of sulfoxides **2** and **27** (0.0011 g, 93%) as a clear oil: R_f 0.13 (10:2:1 CH_3CN:H_2O:HOAc); ^{1}H NMR (300 MHz, D_2O) 4.39 (br. s, 1 H), 4.03–3.85 (m, 6 H), 3.67 (br. s, 1 H), 3.27–3.19 (m, 2 H), 2.67 (s, 3 H), 2.64 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) 74.7, 74.5, 70.3, 69.9, 69.6, 68.7, 66.6, 66.0, 53.6, 51.4, 36.8, 35.3; IR (film) 3250, 2900, 1610, 1500, 1130, 1030 cm^{-1}; FABMS m/z 196 (M + 1).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylsulfonyl)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (31). A solution of dimethyldioxirane in acetone (0.085 M, 2.9 mL, 0.25 mmol), prepared by the method of Murray,[35] was added under Ar at room temperature to a solution of tetraacetate **16** (0.054 g, 0.11 mmol) in acetone (1 mL). After 30 min, the solution was concentrated in vacuo to give the sulfone **31** (0.054 g, 95%) as a white solid: mp 189–190 °C; R_f 0.47 (1:2 hexane:EtOAc); $[\alpha]_D$ = –13.6° (c = 2.59, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) 7.48–7.31 (m, 5 H), 6.34 (d, 1 H, J = 8.4 Hz), 5.99 (s, 1 H), 5.68 (dd, 1 H, J = 6.7, 4.6 Hz), 5.56 (t, 1 H, J = 3.9 Hz), 5.17 (dd, 1 H, J = 7.0, 3.6 Hz), 4.93 (dt, 1 H, J = 7.6, 3.6 Hz), 3.53 (dd, 1 H, J = 6.8, 3.6 Hz), 3.07, 2.18, 2.09, 2.03, 1.85 (s, each 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.4, 168.6, 168.2, 168.1, 134.5, 129.6, 129.1, 127.3, 75.3, 72.4, 70.6, 69.2, 67.2, 49.9, 40.6, 20.8, 20.5, 20.3, 20.0; (75 MHz, CD_3COCD_3) 170.2, 169.6, 169.5, 169.4, 169.0, 136.6, 129.8, 129.5, 128.6, 76.4, 72.2, 71.5, 70.6, 69.1, 49.8, 40.7, 20.8, 20.3, 20.2; IR (film) 3350, 2950, 1750, 1700, 1550, 1375, 1220 cm^{-1}; CIMS m/z 514 (M + 1, 2%), 62 (100%).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylsulfonyl)-2,3,4-trihydroxy-5-aminocyclopentane (28). A stirred solution of sulfone **31** (0.047 g, 0.091 mmol) in anhydrous HCl–CH_3OH (0.36 M, 2.5 mL) was heated at 65 °C under Ar for 24 h. The solvent was removed in vacuo and the remaining oil dissolved in H_2O (2 mL) and extracted with Et_2O (2 × 1 mL) and EtOAc (1 mL). The aqueous layer was lyophilized to afford the hydrochloride salt of **28** (0.025 g, 95%) as a white solid: mp 197–199 °C (dec); R_f 0.20 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ –6.3° (c = 1.78, CH_3OH); ^{1}H NMR (300 MHz, D_2O) 4.35 (dd, 1 H, J = 7.4, 4.2 Hz), 4.18 (dd, 1 H, J = 6.7, 3.3 Hz), 4.06 (t, 1 H, J = 3.6 Hz), 3.98 (t, 1 H, J = 5.4 Hz), 3.85 (dd, 1 H, J = 7.3, 5.3 Hz), 3.12 (s, 3 H); ^{13}C NMR (75 MHz, D_2O, $CDCl_3$ external reference) 76.2, 73.4, 72.5, 71.4, 52.7, 42.6; IR (film) 3250, 2930, 1610, 1500, 1290, 1130 cm^{-1}; FABMS m/z 212 (M + 1, 100%).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-((*S*)-Methylsulfinyl)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (29) and (1*R*,2*R*,3*R*,4*R*,5*S*)-1-((*R*)-Methylsulfinyl)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)-amino)cyclopentane (30). Sodium periodate (0.012 g, 0.055 mmol) was added under Ar to a stirred solution of sulfide **16** (0.024 g, 0.050 mmol) in 1:1 CH_3OH:H_2O (1 mL) at room temperature. After 20 h, H_2O (1 mL) was added and the CH_3OH removed in vacuo. The aqueous solution was extracted with CH_2Cl_2 (3 × 2 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give a 1:1 mixture of diastereomeric sulfoxides **29** and **30** (0.023 g, 93%) as a white solid. This mixture was separated by flash chromatography (EtOAc) to give one sulfoxide (0.0038 g, 15%) as a clear oil: R_f 0.21 (EtOAc); $[\alpha]_D$ –54.1° (c = 0.185, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.41–7.37 (m, 5 H), 6.45 (d, 1 H, J = 9.4 Hz), 5.95 (s, 1 H), 5.50 (t, 1 H, J = 4.1 Hz), 5.41 (dd, 1 H, J = 7.0, 4.3 Hz), 5.23–5.16 (m, 1 H), 5.01 (dd, 1 H, J = 7.2, 3.5 Hz), 2.96 (dd, 1 H, J = 6.6, 3.8 Hz), 2.61, 2.18, 2.07, 2.03, 1.80 (s, each 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.9, 169.5, 168.9, 168.8, 167.2, 135.0, 129.9, 129.4, 127.8, 75.9, 73.3, 70.6, 70.5, 68.9, 45.5, 37.0, 21.2, 21.0, 20.8, 20.4; IR (film) 2900, 1750, 1700, 1550, 1380, 1230, 1050 cm^{-1}; CIMS m/z 498 (M + 1, 100%).

The stereoisomeric sulfoxide (0.0058 g, 23%) was also obtained as a clear oil: R_f 0.09 (EtOAc); $[\alpha]_D$ –40.3° (c = 0.29, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.40–7.29 (m, 5 H), 6.51 (d, 1 H, J = 6.3 Hz), 6.02 (s, 1 H), 5.73 (br. s, 1 H), 5.52 (t, 1 H, J = 3.9 Hz), 5.09 (dd, 1 H, J = 6.7, 3.3 Hz), 4.67 (br. s, 1 H), 3.12 (br. s, 1 H), 2.51, 2.18, 2.07, 2.02, 1.87 (s, each 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.7, 169.1, 168.9, 168.8, 168.2, 135.1, 129.9, 129.5, 127.8, 73.7, 73.0, 70.1, 68.4, 67.1, 50.0, 37.5, 21.3, 20.9, 20.8, 20.5; IR (film) 1750, 1690, 1540, 1360, 1220, 1030 cm^{-1}; CIMS m/z 498 (M + 1, 100%).

(1*R*,2*R*,3*R*,4*S*,5*S*)-1-(Methylsulfonyl)-2,3,4-trihydroxy-5-aminocyclopentane (33). A stirred solution of amide **35** (0.0043 g, 0.013 mmol) in 6 N HCl (1 mL) was heated at reflux for 3 h. Upon cooling, the solution was extracted with ether (2 × 1 mL). The combined organic layers were extracted with H_2O (1 mL), and the aqueous layers were combined and lyophilized to give an oil which was purified by ion exchange chromatography (Dowex 50X8-200, H^+ form, elution with H_2O then 0.3 N HCl) to give **33**·HCl (0.0026 g, 82%) as an oil: R_f 0.33 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +8.0° (c = 0.13, D_2O); ^{1}H NMR (300 MHz, D_2O) 4.37 (t, 1 H, J = 5.1 Hz), 4.01 (t, 1 H, J = 5.0 Hz), 3.80 (t, 1 H, J = 5.0 Hz), 3.72 (t, 1 H, J = 7.1 Hz), 3.65 (t, 1 H, J = 4.9 Hz), 3.07 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) 76.2, 75.3, 71.2, 67.9, 52.6, 39.7; IR (film) 3310, 2900, 1610, 1520, 1290, 1140 cm^{-1}; FABMS m/z 212 (M + 1).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylsulfonyl)-2-acetoxy-3,4-epoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (34). A solution of dimethyldioxirane in acetone (0.088 M, 7.65 mL, 0.67 mmol) was added under Ar at room temperature to olefin **14** (0.082 g, 0.22 mmol). Two additional aliquots of dimethyldioxirane (0.088 M, 2.45 mL each, 0.22 mmol) were added until complete oxidation was achieved as judged by TLC. At this point, the solution was concentrated in vacuo to yield the epoxide **34** (0.089 g, 97%) as a white solid: mp 229–231 °C (dec); R_f 0.40 (1:3 hexane:EtOAc); $[\alpha]_D$ = –73.8° (c = 0.24, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) 7.44–7.34 (m, 5 H), 6.65 (d, 1 H, J = 8.9 Hz), 6.01 (s, 1 H), 5.57 (dd, 1 H, J = 7.3, 1.6 Hz), 4.98 (ddd, 1 H, J = 9.1, 7.7, 1.7 Hz), 3.89 (dd, 1 H, J = 2.8, 1.7 Hz), 3.69 (dd, 1 H, J = 2.7, 1.7 Hz), 3.12 (t, 1 H, J = 7.5 Hz), 2.69, 2.19, 2.13 (s, each 3 H); ^{13}C NMR (100 MHz, CD_3COCD_3) 170.3, 170.1, 169.3, 136.5, 129.7, 129.4, 128.6, 76.3, 71.9, 64.2, 57.3, 55.8, 50.9, 40.8, 20.7, 20.6; IR (film) 3350, 3000, 1740, 1675, 1250, 1140 cm^{-1}; CIMS m/z 412 (M + 1, 2%), 43 (100%).

(1*R*,2*R*,3*R*,4*S*,5*S*)-1-(Methylsulfonyl)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (37) and (1*R*,2*R*,3*S*,4*R*,5*S*)-1-(Methylsulfonyl)-2,3,4-triacetoxy-5-(*N*-((*R*)-acetoxyphenylacetyl)amino)cyclopentane (38). A stirred solution of epoxide **34** (0.032 g, 0.078 mmol) in 2:1 dioxane:1:9 trifluoroacetic acid:H_2O (6 mL) was heated at 65 °C under Ar for 72 h. The solvent was removed in vacuo and the remaining oil dissolved in H_2O (6 mL) and extracted with EtOAc (2 × 3 mL). The aqueous layer was lyophilized to afford an oil. Acetic anhydride (0.090 mL, 0.95 mmol) and DMAP (0.002 g, 0.016 mmol) were added under Ar at room temperature to a stirred solution of this oil in pyridine (1 mL). After 1 h, Et_2O (6 mL) was added and this solution was extracted with saturated $NaHCO_3$ (3 mL) and brine (3 mL). The combined aqueous washes were extracted with Et_2O (3 mL), and the organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to give an oil. Flash chromatography (2:3 hexane:EtOAc) gave a 1:1 mixture of tetraacetates **37** and **38** (0.016 g, 40%) as a clear oil. This mixture was purified by preparative high-pressure liquid chromatography (Supelco C_{18} Reverse Phase Prep Column, 45:55 CH_3OH:H_2O, 2.5 mL/min) to give (in this order of elution) tetraacetate **38** as a white solid: R_f 0.15 (2:3 hexane:EtOAc); retention time = 21.4 min; $[\alpha]_D = -28.8°$ ($c = 0.045$, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.45–7.36 (m, 5 H), 6.31 (d, 1 H, J = 6.5 Hz), 6.00 (s, 1 H), 5.53–5.46 (m, 2 H), 5.17 (t, 1 H, J = 5.8 Hz), 4.72 (q, 1 H, J = 6.4 Hz), 3.80 (t, 1 H, J = 5.9 Hz), 2.98, 2.19, 2.07, 2.06, 1.83 (s, each 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) 169.9, 169.6, 169.3, 169.0, 168.9, 134.5, 129.4, 129.01, 127.3, 75.4, 73.3, 72.4, 67.6, 49.2, 40.0, 20.8, 20.7, 20.6, 20.1; (75 MHz, CD_3COCD_3) 169.8, 169.6, 169.4, 136.1, 129.5, 129.2, 128.3, 78.1, 76.1, 73.7, 72.1, 68.2, 49.4, 40.3, 20.5, 20.4, 20.4, 20.0; IR (film) 2950, 2800, 1740, 1690, 1350, 1220, 1040 cm^{-1}; FABMS m/z 514 (M + 1).

Tetraacetate **37** also was obtained as a white solid: R_f 0.15 (2:3 hexane:EtOAc); retention time = 23.3 min; $[\alpha]_D = -11.3°$ ($c = 0.05$, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) 7.44–7.34 (m, 5 H), 6.70 (d, 1 H, J = 6.6 Hz), 5.97 (s, 1 H), 5.61–5.57 (m, 2 H), 5.22 (dd, 1 H, J = 7.8, 5.3 Hz), 4.32 (q, 1 H, J = 7.1 Hz), 3.83 (dd, 1 H, J = 6.7, 3.6 Hz), 3.00, 2.19, 2.09, 2.04, 2.03 (s, each 3 H); ^{13}C NMR (75 MHz, CD_3COCD_3) 170.1, 170.0, 169.6, 169.6, 169.5, 136.0, 129.4, 129.1, 128.3, 76.4, 76.3, 72.6, 67.7, 67.4, 51.2, 39.9, 20.5, 20.3, 20.3, 20.1; IR (film) 2900, 1750, 1690, 1550, 1360, 1225 cm^{-1}; FABMS m/z 514 (M + 1).

(1*R*,2*R*,3*R*,4*S*,5*S*)-1-(Methylsulfonyl)-2,3,4-trihydroxy-5-(*N*-((*R*)-hydroxyphenylacetyl)amino)cyclopentane (35) and (1*R*,2*R*,3*S*,4*R*,5*S*)-1-(Methylsulfonyl)-2,3,4-trihydroxy-5-aminocyclopentane (39). A stirred solution of tetraacetates **37** and **38** (0.017 g, 0.032 mmol) in anhydrous HCl–CH_3OH (0.36 M, 1 mL) was heated at 65 °C under Ar for 14 h. The solvent was removed in vacuo and the remaining oil dissolved in H_2O (1 mL) and extracted with ether (2 × 1 mL). The combined ether layers were extracted with water (1 mL), and the aqueous portions were combined and lyophilized to yield a clear oil which was purified by ion exchange chromatography (Dowex 50X8-200, H^+ form). Elution with water provided the amide **35** (0.0043 g, 44%) as an oil: R_f 0.28 (9:1 EtOAc:CH_3OH); $[\alpha]_D$ −14.1° ($c = 0.135$, H_2O); ^1H NMR (300 MHz, D_2O) 7.26–7.22 (m, 5 H), 5.02 (s, 1 H), 4.30–4.22 (m, 2 H), 3.89 (t, 1 H, J = 7.6 Hz), 3.66 (dd, 1 H, J = 7.7, 5.7 Hz), 3.54 (dd, 1 H, J = 8.3, 4.2 Hz), 2.47 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) 175.2, 138.6, 129.3, 129.2, 127.2, 77.9, 74.7, 74.2, 69.4, 68.3, 51.6, 39.2; IR (film) 3300, 2900, 1660, 1520, 1300, 1150, 1080 cm^{-1}; CIMS m/z 386 (M + 1 + C_4H_{10} − H_2O, 5%), 328 (M + 1 − H_2O, 10%), 80 (100%).

Elution with 0.3 N HCl gave the hydrochloride salt of amine **39** (0.0027 g, 34%) as an oil: R_f 0.27 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +6.6° ($c = 0.085$, CH_3OH); ^1H NMR (300 MHz, D_2O) 4.09–3.98 (m, 3 H), 3.85 (t, 1 H, J = 5.3 Hz), 3.75 (t, 1 H, J = 7.4 Hz), 3.08 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) 80.1, 74.6, 71.7, 67.7, 48.2, 40.1; IR (film) 3250, 2960, 1600, 1520, 1290, 1140 cm^{-1}; FABMS m/z 212 (M + 1).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylsulfonyl)-2-hydroxy-3,4-epoxy-5-(*N*-((*R*)-hydroxyphenylacetyl)amino)cyclopentane (40). A solution of dimethyldioxirane in acetone (0.044 M, 30 mL, 1.27 mmol) was added under Ar at room temperature to alkene **13** (0.107 g, 0.384 mmol). After 16 h, an additional aliquot (0.044 M, 7.3 mL) of dimethyldioxirane in acetone was added to achieve complete oxidation as judged by TLC. At this point, the solution was concentrated in vacuo to yield a clear oil which was flash chromatographed (1:3 hexane:EtOAc) to give epoxy sulfone **40** (0.054 g, 43%) as a white solid: R_f 0.30 (EtOAc) ^1H NMR (200 MHz, CD_3COCD_3) 7.92 (br. s, 1 H), 7.52–7.46 (m, 2 H), 7.35–7.24 (m, 3 H), 5.40 (d, 1 H, J = 4.0 Hz), 5.11 (d, 1 H, J = 4.5 Hz), 4.79–4.58 (m, 2 H), 4.52 (t, 1 H, J = 1.2 Hz), 3.67–3.62 (m, 2 H), 3.34 (t, 1 H, J = 7.0 Hz), 2.71 (s, 3 H).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylsulfonyl)-2-hydroxy-3,4-epoxy-5-aminocyclopentane (41). Amberlite IRA-900 ($^-$OH) ion exchange resin was prepared by sequential rinsing with H_2O, CH_3OH, H_2O, 2 N NaOH, and H_2O. This resin (0.015 g) was added under Ar at room temperature to a stirred solution of chlorohydrins **44** and **45** (0.006 g, 0.021 mmol) in CH_3OH (1 mL). After 5 h, the solution was filtered through Celite and the filtrate acidified with 1:9 trifluoroacetic acid:CH_3OH and concentrated in vacuo to leave the trifluoroacetate salt of epoxide **41** (0.0054 g, 84%) as an oil: R_f 0.34 (10:2:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ = +20.0° ($c = 0.18$, H_2O); ^1H NMR (300 MHz, D_2O) 4.48 (dd, 1 H, J = 7.5, 1.5 Hz), 4.11 (dd, 1 H, J = 8.7, 1.3 Hz), 3.80 (dd, 1 H, J = 3.0, 1.2 Hz), 3.74 (dd, 1 H, J = 3.0, 1.4 Hz), 3.33–3.27 (m, 1 H), 3.01 (s, 3 H); ^{13}C NMR (100 MHz, D_2O, CH_3OH internal reference) 71.7, 64.1, 57.8, 53.7, 49.3, 40.4; IR (film) 3450, 1675, 1275, 1200, 1130 cm^{-1}; FABMS m/z 194 (M + 1).

(1*R*,2*R*,5*S*)-1-(Methylthio)-2-(trifluoroacetoxy)-5-(*N*-(trifluoroacetyl)amino)cyclopent-3-ene (42). Pyridine (0.050 mL, 0.62 mmol) and trifluoroacetic anhydride (0.064 mL, 0.46 mmol) were added under Ar at 0 °C to a stirred solution of amino alcohol **20** (0.030 g, 0.21 mmol) in CH_2Cl_2 (1.5 mL). After 1 h, this solution was extracted with saturated NaCl (3 × 1 mL). The combined aqueous washes were extracted with CH_2Cl_2 (1 mL), and the organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to give trifluoroacetate **76** (0.061 g, 87%) as an oil: R_f 0.77 (7:3 hexane:EtOAc); $[\alpha]_D = -1.2°$ ($c = 0.46$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) 6.82 (d, 1 H, J = 7.8 Hz), 6.11–6.06 (m, 2 H), 5.64 (d, 1 H, J = 3.8 Hz), 4.91 (dd, 1 H, J = 8.7, 4.6 Hz), 3.15 (t, 1 H, J = 4.4 Hz), 2.21 (s, 3 H); IR (film) 3300, 3100, 2900, 1770, 1720, 1570, 1170 cm^{-1}; CIMS m/z 224 (M + 1 − CF_3COOH, 100%).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1-(Methylsulfonyl)-2-hydroxy-3,4-epoxy-5-(*N*-(trifluoroacetyl)amino)cyclopentane (43). A solution of dimethyldioxirane in acetone (0.053 M, 9.05 mL, 0.48 mmol) was added under Ar at room temperature to the olefin **42** (0.041 g, 0.12 mmol). Two additional aliquots of the dimethyldioxirane solution in acetone (0.053 M, 2.26 mL each, 0.12 mmol) were added until complete oxidation was achieved as judged by TLC and NMR. At this point, the solution was concentrated in vacuo to yield epoxide **43** (0.028 g, 91%) as a white solid: mp 210–215 °C (dec); R_f 0.23 (1:3 hexane:EtOAc); $[\alpha]_D$ = +3.3° ($c = 0.44$, acetone); ^1H NMR (300 MHz, CD_3COCD_3) 5.10 (d, 1 H, J = 6.5 Hz), 4.80 (t, 1 H, J = 8.3 Hz), 4.54 (m, 1 H), 3.76 (dd, 1 H, J = 2.9, 1.5 Hz), 3.70 (dd, 1 H, J = 2.9, 1.5 Hz), 3.29 (dd, 1 H, J = 8.9, 7.6 Hz), 2.96 (s, 3 H); ^{13}C NMR (100 MHz, CD_3COCD_3) 156.8 (q, 1C, J_{C-F} = 37.3 Hz), 116.3 (q, 1C, J_{C-F} = 286.9 Hz), 72.3, 65.1, 65.0, 57.4, 55.2, 41.0; IR (film) 3450, 3350, 2950, 1710, 1560, 1290, 1170, 1150 cm^{-1}; CIMS m/z 290 (M + 1, 100%).

(1*R*,2*S*,3*S*,4*R*,5*S*)-1-(Methylsulfonyl)-2,4-dihydroxy-3-chloro-5-aminocyclopentane (44) and (1*R*,2*R*,3*S*,4*S*,5*R*)-1-(Methylsulfonyl)-2,3-dihydroxy-4-chloro-5-aminocyclopentane (45). A stirred solution of epoxide **43** (0.008 g, 0.026 mmol) in anhydrous HCl–CH_3OH (0.36 M, 1.0 mL) was heated at 65 °C under Ar for 24 h. The solvent was removed in vacuo, and the remaining oil was dissolved in H_2O (1 mL) and extracted with ether (2 × 1 mL). The aqueous layer was lyophilized to afford a mixture of chlorohydrins **44** and **45** (0.007 g, 100%) as an oil: R_f 0.55 (10:2:1 CH_3CN:H_2O:HOAc); ^1H NMR (300 MHz, D_2O) 4.59–3.83 (m, 10 H), 3.12, 3.08 (s, each 3 H); ^{13}C NMR (100 MHz, D_2O, CH_3OH internal reference) 76.1, 75.1, 72.7, 70.6, 68.3, 67.8, 65.1, 60.3, 53.2, 48.3, 40.0, 39.5; IR (film) 3300, 1620, 1300, 1150 cm^{-1}; FABMS 230/232 (M + 1, Cl doublet, 2.7:1).

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Supplementary Material Available: Text describing general biological procedures, tables of representative kinetic data, and plots from enzymatic assays for K_I determinations (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.