Synthesis of Natural Product-Inspired Inhibitors of *Mycobacterium tuberculosis* Mycothiol-Associated Enzymes: The First Inhibitors of GlcNAc-Ins Deacetylase

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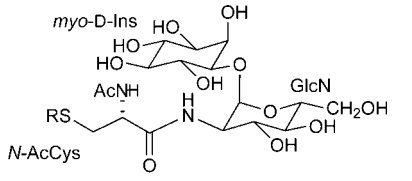
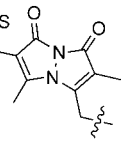
Synthesis and evaluation of a chemical library of inhibitors of the mycothiol biosynthesis enzyme GlcNAc-Ins deacetylase (MshB) and the mycothiol-dependent detoxification enzyme mycothiol-*S*-conjugate amidase (MCA) from *Mycobacterium tuberculosis* are reported. The library was biased to include structural features of a group of natural products previously shown to competitively inhibit MCA. Molecular docking studies that reproducibly placed the inhibitors in the active site of the enzyme MshB reveal the mode of binding and are consistent with observed biological activity.

Introduction

Mycothiol (MSH,[a] **1**) is a small molecular weight thiol[1–3] that is found exclusively in a subset of Gram-positive bacteria, or actinomycetes, that encompass mycobacteria.[4] Like its eukaryotic and Gram-negative counterpart, glutathione, MSH is present in reduced (**1**) and oxidized forms (**2**, MSSM),[5] and plays a critical role in maintaining a reductive intracellular environment by functioning as a redox reagent in its own right, by functioning as a cofactor for enzymes involved in redox reactions, and by facilitating detoxification of electrophiles and toxins via nucleophilic addition to the sulfhydryl group of cysteine (exemplified by the *S*-conjugate MSH bimane, **3**, MsmB).[6]



1 MSH R = H
2 MSSM R = MS
3 MSmB R =



MSH comprises one unit each of 1-*myo*-D-inositol (*myo*-D-Ins), glucosamine (GlcN), and *N*-acetyl-cysteine. MSH biosynthesis is carried out by four biosynthetic enzymes, MshA−D, as depicted in Scheme 1. MSH-assisted detoxification is facilitated by a fifth enzyme known as mycothiol-*S*-conjugate amidase (MCA), which cleaves the scissile *N*-AcCys-GlcN amide bond of mycothiol-*S*-conjugates to yield mercapturic acids, or AcCys-*S*-conjugates, that can be excreted from the cell.[7] Independent studies have demonstrated that chemically or genetically altered mycobacterial strains that are deficient in MSH production become hypersensitive to most currently used antitubercular antibiotics,[8,9] that exposure to these antibiotics results in upregulation of MSH biosynthesis genes in *Mycobacterium tuberculosis*, BCG,[10] and that MSH is indeed essential for viability of *M. tuberculosis*,[11–13] the strain that causes tuberculosis. The persisting need for extensive treatment regimens, which currently consist of 6–12 months of multiple drug therapy, combined with the now common place emergence of drug resistant mycobacterial strains in tuberculosis patients emphasizes the continuing need for discovery of new classes of antibiotics and new antitubercular.[14] Collectively, the above observations suggest that small molecules that can interfere with MSH biosynthesis or MSH-assisted detoxification may have therapeutic potential, and this hypothesis is being explored by an increasing number of researchers in the way of identification and synthesis of inhibitors of MSH-associated enzymes.[15]

In previous studies we identified several novel and known natural products[16–18] and synthetic compounds[19,20] that inhibit the mycobacterial detoxification enzyme MCA with sub- to low micromolar IC_{50} values and demonstrated that a series of bromotyrosine-derived molecules, exemplified by natural products **4**−**6**, are competitive inhibitors of MCA.[17] MCA has been shown to be a zinc metalloenzyme.[17,21] On the basis of NMR structural studies of competitive inhibitors of MCA of the substrate mycothiol-*S*-bimane (**3**, MsmB) and biosynthetic intermediates such as GlcN-Ins, we proposed that competitive inhibitors such as **4**−**6**, each of which possess amide bonds and moieties known to chelate metals such as oximes and oxazolines, mimic conformations of natural substrates when bound to MCA.[22] These cumulative findings led us to speculate that a small molecule incorporating features of the natural substrates, namely, the pseudo disaccharide of MSH, together with those of the competitive natural product inhibitors, may be able to inhibit not only MCA but also the homologous biosynthetic enzyme 1-D-*myo*-inosityl 2-acetamido-2-deoxy-α-D-glucopyranoside (GlcNAc-Ins) deacetylase (MshB). Here we describe the synthesis and characterization of a new set of inhibitors that

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[a] Abbreviations: TFA, trifluoroacetic acid; MSH, mycothiol; MSmB, mycothiol-*S*-bimane; GlcNAc-Ins, *N*-acetyl-1-D-*myo*-inosityl-2-amino-2-deoxy-α-D-glucopyranoside; MCA, mycothiol-*S*-conjugate amidase; MshB, *N*-acetyl-1-D-*myo*-inosityl-2-amino-2-deoxy-α-D-glucopyranoside deacetylase.

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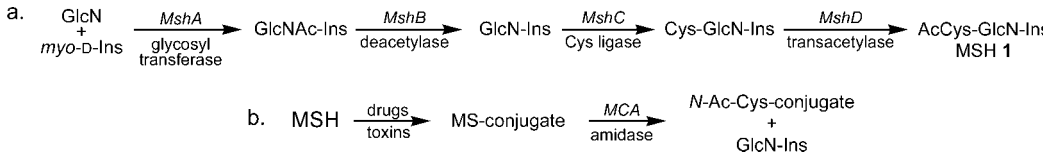
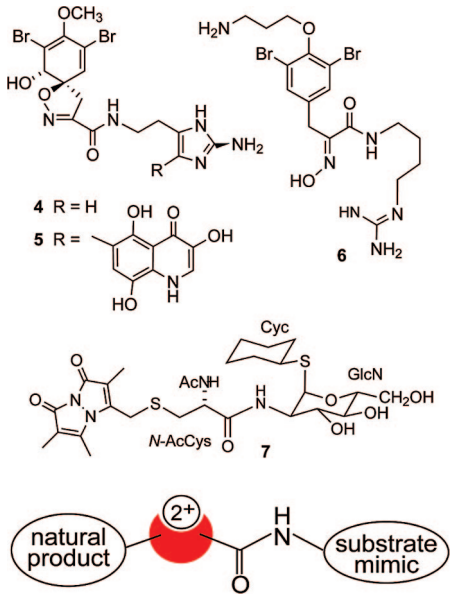
Scheme 1. (a) MSH Biosynthesis and (b) MSH-Mediated Detoxification



meet these criteria and are the first reported inhibitors of a MSH biosynthetic enzyme, namely, MshB.

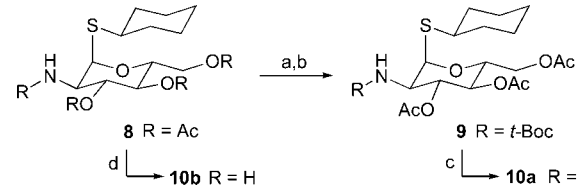


Results and Discussion

Chemical Synthesis and Rationale. MSH is composed of one unit each of 1-*myo*-D-inositol (*myo*-D-Ins), GlcN, and *N*-acetyl-cysteine and is assembled as shown in Scheme 1. Earlier, Knapp and co-workers synthesized an *S*-bimane derivative of a simplified MSH analog, namely, the cyclohexyl thioglycoside analog **7**, which was shown to be a practical substrate for the detoxification enzyme MCA with rates about 2-fold less than those measured for a well-studied substrate MSmB (**3**).[23] More recently, we reported a series of inhibitors wherein the cyclitol is replaced by a quinic acid moiety.[20] Together, our results indicate that the presence of the cyclitol *myo*-D-inositol, at least for the amidase MCA, is not strictly required and that MCA likely binds or tolerates the cyclohexyl and quinic acid derived groups. In terms of meeting the criteria for our notion of the ideal inhibitor for MCA and homologues described above, incorporation of the cyclohexyl thioglycoside unit was most attractive. Building on this scaffold, we synthesized 23 compounds that incorporated a selection of substructures that would allow us to explore the effects on enzyme inhibition of substituents coupled to the amino group of GlcN. Mindful of the information gleaned from our earlier studies of natural product and synthetic inhibitors, we chose to include phenyl and sulfonyl groups, oxazole or oxazine units, and cysteine isosteres such as aspartic acid and homoserine.

Synthesis of the analogs began with the preparation of cyclohexyl thioglycoside tetraacetate **8** (Scheme 2) following the route published by Knapp et al.[24] Conversion of **8** to 2-amino-3,4,6-triacetoxy cyclohexyl thioglycoside **10a**, which allows coupling to the thioglycoside scaffold under a variety of conditions compared to unprotected aminotriol **10b**, was accomplished in two steps. Treatment of **8** with Boc-anhydride

Scheme 2. Preparation of Triacetyl Thioglycoside[a]



[a] Reagents and conditions: (a) DMAP, Boc_2O, THF, heat, 6 h; (b) MeOH, H_2NNH_2, 0 °C, 2 h; (c) TMSOTf, CH_2Cl_2, 0 °C, 4 h, 65%; (d) H_2NNH_2 (neat), reflux, 24 h, 78%.

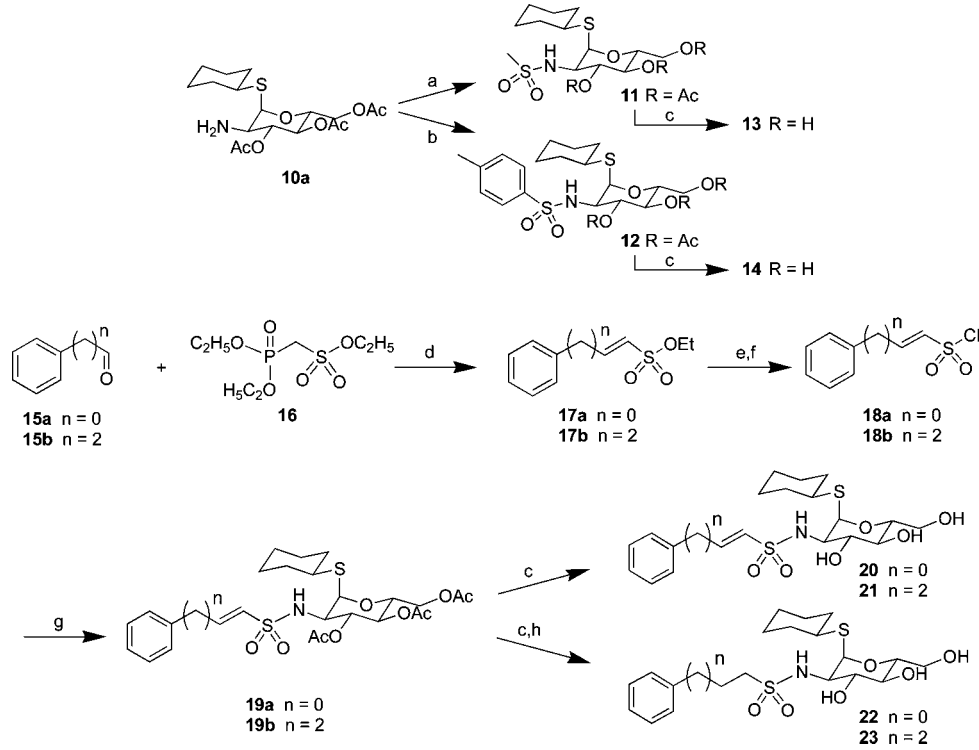
and DMAP followed by hydrazine afforded *N*-Boc protected amine **9**,[25] and free amine triacetate **10a** was obtained in quantitative yield upon treatment with TMSOTf in methylene chloride.

Sulfonamide analogs shown in Scheme 3 were prepared by the addition of the appropriate sulfonyl chloride to **10a** in the presence of triethylamine, followed by deprotection with sodium methoxide in methanol. Thus, treatment of **10a** with mesyl or tosyl chloride furnished triacetoxy sulfonamides **11** and **12** in greater than a 90% yield, and quantitative deprotection gave triols **13** and **14**. Horner−Wadsworth−Emmons olefination of benzaldehye (**15a**) or 3-benzyl propanal (**15b**) with sulfonyl phosphonate **16**[26] gave exclusively (*E*)-olefins **17a** and **17b**, and activation with thionyl chloride, TBAI, and triphenylphosphine provided sulfonyl chlorides **18a**,**b** for coupling to amine **10a** to give **19a** and **19b**. Deprotection of a portion of **19a** and **19b** provided α,*β*-unsaturated sulfonamides **20** and **21**, and reduction of the remaining material with palladium on carbon followed by hydrolytic deprotection gave saturated sulfonamides **22** and **23**.

To test the effect of a trifluoroacetate group on the amide suspected to be near to the active site, amine **10a** was treated with trifluroacetic anhydride to give analog **24**. In a previous study on a library of synthetic psammaplin analogs, we showed that compounds containing a phenyl ring separated by two to four carbon atoms from the cyclic portion of the structure were among the best inhibitors.[19] As an extension of this finding, we sought to generate a few analogs with this moiety. Coupling of *N*-Boc-L-homophenylalanine and **10a** gave protected intermediate **25**, and removal of the acetates afforded compound **26**. Intermediate **25** was in turn treated with TFA followed by acetylation, trifluoroacetylation, or sulfonylation of the amine to give triacetates **27**−**29**; global deprotection of each provided triols **30**−**32**, respectively (Scheme 4).

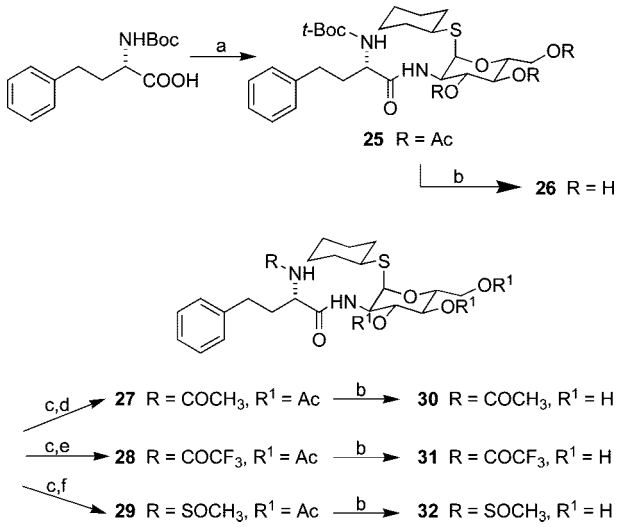
Inspired by the activity of a series of bromotyrosine-derived natural products containing spiroisooxazoline moieties, we were especially interested in analogs containing similar units. Synthesis of such analogs began with D/L-serine (**33a**) or D/L-homoserine (**33b**; Scheme 5). Conversion of the primary hydroxyl to its silyl ether followed by esterification using trimethylsilyldiazomethane[27] in THF gave the desired methyl esters **34a**,**b** in acceptable yields (49% over two steps). Amidation with 3-fluorobenzoic acid through activation by diethyl cyanophosphonate at 0 °C gave amides **35a**,**b**. Efficient cyclization of **35a** to oxazole **36a** and of **35b** to oxazine **36b**

Scheme 3. Preparation of Sulfonamides[a]



[a] Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, rt, 1 h; (b) TosCl, Et_3N, CH_2Cl_2, rt, 1 h; (c) NaOMe, MeOH; (d) *n*-BuLi, THF, −78 °C to rt, 18 h; (e) TBAI, acetone, heat, 24 h; (f) $SOCl_2$, triphenylphosphine, CH_2Cl_2, 0 °C to rt, 1 h; (g) **10a**, Et_3N, CH_2Cl_2, rt, 4 h; (h) H_2, Pd-C, EtOH.

Scheme 4. Preparation of Homophenylalanine Analogs[a]



[a] Reagents and conditions: (a) **10a**, DIEA, EDCI/HOBt, DMF, 0 °C−rt, 3 h; (b) NaOMe/MeOH; (c) TFA, CH_2Cl_2; (d) acetic anhydride, pyridine; (e) trifluoroacetic anhydride, pyridine; (f) mesyl chloride, pyridine.

was accomplished by treatment with DAST ((diethylamino)sulfur trifluoride).[28a,b] Basic hydrolysis of the methyl esters gave acids **37a** and **37b** that were in turn coupled to **10a** to provide thioglycoside amides **38a** and **38b**. Deacetylation of **38a** and **38b** gave the desired target compounds **39** and **40** in quantitative yields. Compounds in which the cysteine unit is replaced by a similar amino acid were obtained by coupling *N*-Boc-L-homoserine to **10a** using the same coupling and deprotection protocols as described above to give analog **41** (Scheme 6). A portion of this homoserine coupled triacetate intermediate was subjected to $RuCl_3$ oxidation and deacetylation to provide yet another type of analog, **42**, in which the thioether sulfur atom has been oxidized to a sulfone.

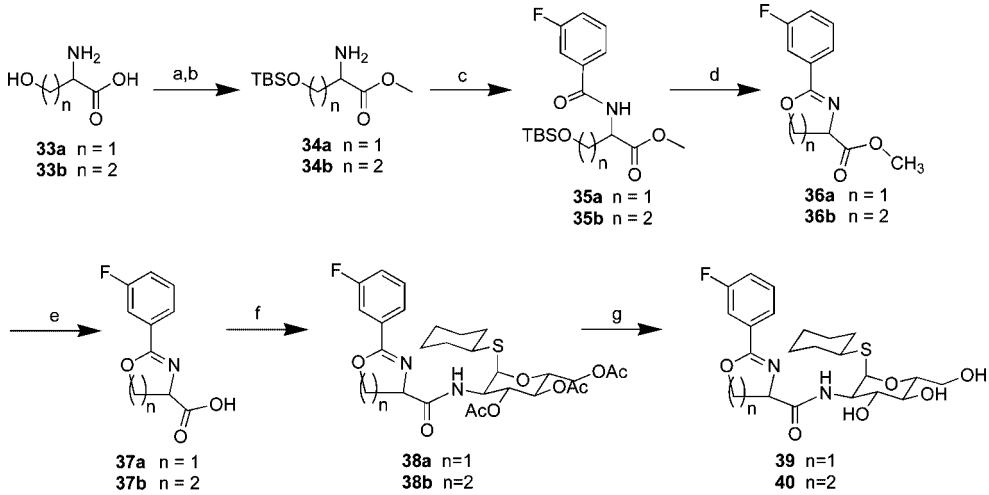
At this point, preliminary screening results indicated that the heterocycle-containing analogs **39** and **40**, generated from racemic serine and homoserine, were among the most potent of the analogs described above. With the protected amine **10a** in hand and the methodology now optimized, we proceeded to synthesize the optically pure diastereomers of **39** and **40** starting with D- or L-serine (to give **39D** and **39L**) and D- or L-homoserine (to give **40D** and **40L**). In addition, three other related and hydrolytically stable analogs were prepared using commercially available acids to give analogs **43**−**45** in good yields (Scheme 7).

Last, to test whether the cyclohexyl group is necessary for inhibition and binding to MCA or MshB, three analogs built upon GlcN alone were prepared (Scheme 6). 1,3,4,6-Tetraacetoxy GlcN **46** was prepared according to a literature procedure and EDCI/HOBt coupling to acids used for inhibitors **43**−**45**, followed by deacetylation, provided analogs **47**−**49** (Scheme 8).

Biological Characterization. A total of 23 analogs, 18 of which are built on the thioglycoside scaffold and three upon GlcN, were tested for their ability to inhibit two homologous, MSH-associated enzymes from *M. tuberculosis*, namely, the MSH biosynthetic enzyme MshB and the detoxification enzyme MCA. Previously described fluorescence-detected HPLC assays[7,17] were used for screening against both enzymes. The results of initial screening at two concentrations (200 and 50 μM) are presented in Figure 1. Dose–response curves were subsequently generated for compounds that reproducibly inhibited one or both of the enzymes by at least 50% when tested at 200 μM and 20% when tested at 50 μM (Table 1).

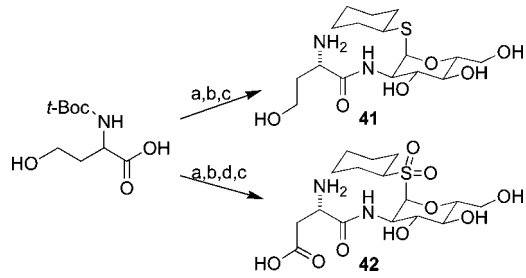
Sulfonamides **21** and **23**, homophenylalanine trifluoroacetate **31**, homoserine analog **41**, and heterocycles **39**, **40**, **43**, and **44**

Scheme 5. Preparation of Oxazole and Oxazine Heterocycles[a]



[a] Reagents and conditions: (a) TBDMSCl, DBU, CH_3CN, rt; (b) $TMSCHN_2$, MeOH/benzene; (c) 3-fluorobenzoic acid, DIEA, DECP, DMF, 0 °C, 3 h; (d) DAST, CH_2Cl_2; (e) LiOH (3:1, MeOH/water); (f)**10a**, DIEA, DEPC, 0 °C−rt; (g) NaOMe, MeOH, rt.

Scheme 6. Preparation of **41** and **42**[a]



[a] Reagents and conditions: (a) TFA; (b) **10a**, DIEA, EDCI/HOBt, DMF, 0 °C−rt, 3 h; (c) NaOMe/MeOH; (d) aq NaIO(a), CH_3CN/CCl_4, $RuCl_3$ (2 mol%).

were the strongest inhibitors of the biosynthetic enzyme MshB. For MCA, a similar pattern emerged where sulfonamides **20**−**23**, trifluoroacetate **31** and its *N*-Boc analog **26**, and the same set of heterocycles (**39**, **40**, **43**, **44**) stood out as the best inhibitors, while analog **41** was only weakly inhibitory at 50 μM. Somewhat surprisingly, the optically pure forms of compounds **39** and **40**, incorporating D- and L-serine and D- and L-homoserine, respectively, (namely, **39D** and **39L** and **40D** and **40L**) were found to inhibit MshB with very similar IC_{50} values as the diastereomeric mixtures first synthesized.

Modeling. Crystal structures of the deacetylase MshB were solved contemporaneously by the groups of James[29] and Baker[30] in 2003. In the structure solved by McCarthy et al.,[30] β-octyl glucoside, a component of the crystallization buffer cocrystallized in the active site giving rise to a reliable model of the true substrate GlcNAc-Ins bound to MshB. To gain insight into the mode of binding of our inhibitors to MshB, docking calculations were performed for three of the most potent inhibitors (Autodock 4.0[31]). We first tested whether docking the substrate GlcNAc-Ins to MshB would duplicate the model generated from the crystal structure. Starting from random initial positions and orientations, GlcNAc-Ins was docked to MshB in two steps (see Supporting Information). Multiple runs defining the entire surface of the protein with Zn^{2+} in the active site[32] produced a highly populated cluster of solutions (1.0 Å cutoffs) that also exhibited the lowest binding energies and occupied a cleft-like binding site formed by 10 residues, including His13, Asp15, Asp16, Glu45, Arg68, Asp95, His144, His147, Gln247, and Ser260. The refinement step (defining a region slightly larger

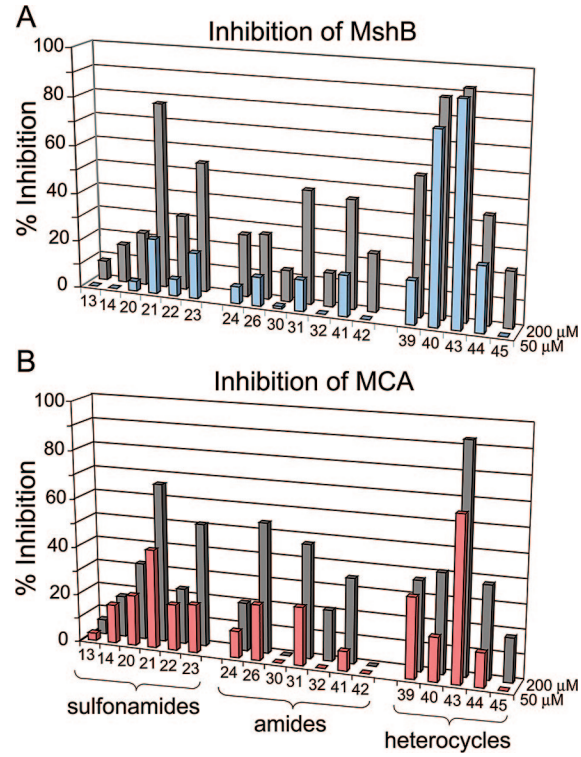


Figure 1. Inhibition of MshB and MCA. A total of 23 compounds were tested at 200 μM and 50 μM for inhibition of (A) MshB and (B) MCA using fluorescence-detected HPLC assays. Grey bars indicate % inhibition at 200 μM in both graphs and blue and pink bars indicate % inhibition at 50 μM against MshB and MCA, respectively. Bars represent the average of at least two independent assays run in duplicate or triplicate; standard errors averaged ±15% of indicated values for both enzymes.

than and centered around the binding site) likewise produced a low-energy cluster of solutions that showed nearly identical docked geometries relative to one another and to the X-ray model, with the same atoms participating in hydrogen bonding interactions with MshB and Zn^{2+} (Figure 2a).

The same protocol was used to dock heterocycles **39**, **40**, and **43** to MshB, with results for **40L** and **43** shown in Figure 2b and Figure 2c, respectively. For all three inhibitors, the most highly populated cluster of conformers occupied the same low-

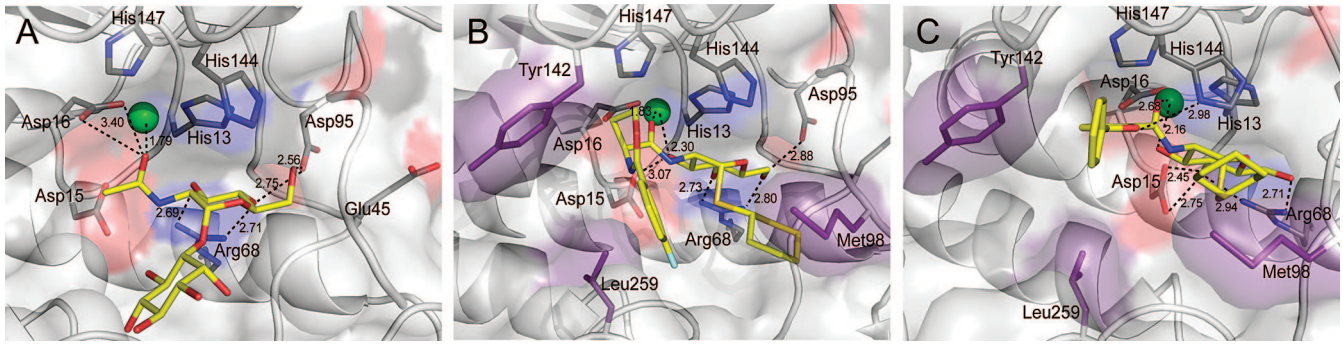
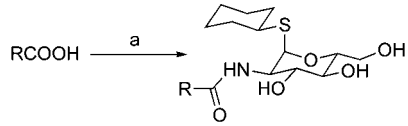
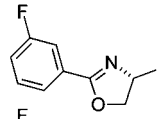
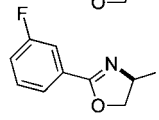
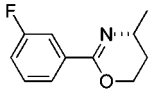
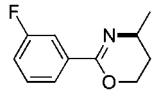
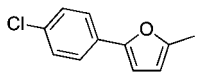
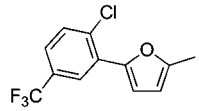
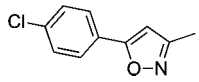


Figure 2. Results of docking GlcNAc-Ins (A) and inhibitors **40L** (B) and **43** (C) to MshB. MshB residues in contact with the ligands are displayed as rods with N and O atoms colored blue and red, respectively. Ligands are shown in yellow, the Zn^{2+} ion is shown as a green sphere, and hydrophobic residues and their surfaces are colored purple.

Scheme 7. Heterocyclic Analogs and Yields from Couplings[a]



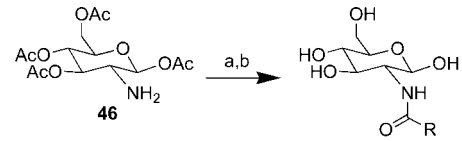
(a) **10b**, DIEA, EDCI/HOBt, DMF, 0 °C-rt, 3 h

Product	R	% yield
39L		72
39D		65
40L		61
40D		64
43		71
44		60
45		65

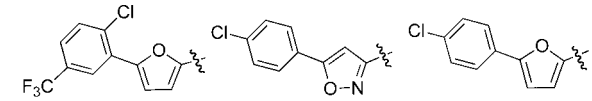
[a] Reagents and conditions: (a) **10b**, DIEA, EDCI/HOBt, DMF, 0 °C−rt, 3 h.

energy binding site as that of GlcNAc-Ins, located at the active site of the enzyme, and the inhibitors fit well into the cleft formed by residues lining the active site. The amide O atom of each inhibitor ligates the zinc (average distance of 2.25 Å), and in each complex, the cyclohexyl ring packs against the hydrophobic residues Met98 and Gly46 (Figure 2b,c). Although the average positions of the GlcN rings in the docked structures are shifted slightly relative to one another, each is positioned to allow formation of at least two hydrogen bonds between either the C3, C4, or C6 hydroxyl groups of the ligand and the Nη atoms of Arg68 or the Oδ atoms of Asp 15 or Asp95. In addition, the C-3 hydroxyls in **39** (not shown) and **40** are bound to zinc (average distance of 2.10 Å). The hydrophobic groove

Scheme 8[a]



R =



47 **48** **49**

[a] Reagents and conditions: (a) DIEA, EDCI/HOBt, RCOOH, CH_2Cl_2; (b) NaOMe, MeOH.

Table 1. IC_{50} Values for the most Potent Inhibitors of MshB or MCA

	IC_{50} (μM)	
cmpd	MshB	MCA
21	105 ± 27	90 ± 17
23	101 ± 14	120 ± 26
26	ND[a]	145 ± 14
39	28 ± 6	ND[a]
40	10 ± 2	ND[a]
43	7 ± 1	33 ± 4

[a] Not determined; IC_{50} values greater than 200 μM.

whose walls are formed by Leu259 on one side and Tyr142 and Gly143 on the other accommodates the aromatic and heterocyclic portion of the inhibitors. The docked positions and hydrogen bonding patterns were the same for the D- and L-isomers of **39** and **40** (not shown). The lowest energy conformation of furan **43** makes an additional hydrogen bond between the furan oxygen and His144 (Figure 2c), which is absent in **39** and **40**.

Concluding Remarks. In recent years, MSH has been shown to be essential for growth of Mtb, supporting the validity of targeting MSH biosynthetic enzymes for the development of new classes of antimycobacterials.[11–13] To date, inhibitors of MSH-associated enzymes have been limited to those of the detoxification enzyme MCA and have included natural products, natural product-like libraries, and substrate mimics. Interestingly, although we have tested many of these compounds for their ability to inhibit MshB (unpublished data, C.A.B., S.R., Lisa Eckman), none of our previously reported MCA inhibitors have displayed notable activity toward that biosynthetic enzyme. This was surprising given that the active sites of these two enzymes are predicted to be nearly identical on the basis of sequence alignments, and it prompted us to attempt to design and synthesize a small molecule that would inhibit MshB, or ideally, both MshB and MCA.

In this study, we have constructed a small library of compounds built upon GlcN or a thioglycoside scaffold shown

previously to bind to MCA and modestly inhibit the growth of *M. tuberculosis*.[33] The diversity of structures appended to these scaffolds was selected in part on the basis of structures of competitive, natural product inhibitors. Although inhibitory concentrations remain in the low micromolar range, this approach has been successful in that it has allowed us to construct the first reported inhibitors of a MSH biosynthetic enzyme, namely, MshB, and supports the notion that natural product−substrate chimeras might act as dual inhibitors toward MshB and MCA. These results should provide the basis for further development of more potent inhibitors of MSH biosynthesis.

Experimental Section

Materials and Reagents. Recombinant forms of MshB[34] and MCA[7] were expressed and prepared using published protocols,[7,34] and fluorescent-detected HPLC assays were carried out as described previously.[17] All chemicals were of analytical grade and used without further purification.

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-5-(*tert*-butoxycarbonylamino)-6-(cyclohexylthio)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (9). To a solution of tetracetate **8** (1.5 g, 3.37 mmol) and DMAP (83 mg, 0.67 mmol) in 6 mL of THF was added Boc_2O (1.46 g, 6.73 mmol), and the solution was refluxed at 60 °C for 6 h. The mixture was cooled to 0 °C, diluted with MeOH (10 mL), and treated with hydrazine (0.42 mL, 13.5 mmol) for 2 h. The mixture was diluted with CH_2Cl_2, washed with 0.5 N HCl, $CuSO_4$, and $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo, and the residue was purified over Si gel (25/75 EtOAc/CH_2Cl_2) to give the desired carbamate **9** in 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (d, J = 5.4 Hz, 1H), 4.99 (m, 2H), 4.71 (d, J = 9.6 Hz, 1H), 4.37 (m, 1H), 4.21 (dt, J = 4.8, 7.5 Hz, 1H), 4.06 (dd, J = 1.8, 10.2 Hz, 1H), 2.83 (m, 1H), 2.09 (s, 3H), 2.02 (s, 6H), 1.75 (m, 2H), 1.56 (m, 2H), 1.42 (s, 9H), 1.30–1.42 (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 170.8, 169.6, 84.4, 80.2, 71.9, 68.7, 62.3, 53.5, 45.2, 34.4, 33.8, 28.4, 26.0, 25.7, 20.8; LRMS $C_{23}H_{38}NO_9S$ ($M + H^+$) 504.2.

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-5-amino-6-(cyclohexylthio)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (10a). To a solution of carbamate **9** (0.20 g, 0.40 mmol) in CH_2Cl_2 (7 mL) at 0 °C was added TMSOTf (137 μL, 0.80 mmol), and the mixture was stirred for 4 h, after which time the mixture was quenched with cold saturated aq $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, the solvent was removed in vacuo, and the crude product was purified on a short Si gel column (5/95 MeOH/CH_2Cl_2) to give the free amine **10a** in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (d, J = 5.4 Hz, 1H), 4.94 (m, 2H), 4.46 (m, 1H), 4.30 (dd, J = 5.1, 7.2 Hz, 2H), 4.05 (dd, J = 2.1, 10.2 Hz, 2H), 3.22 (br, 2H), 2.85 (m, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.75 (m, 2H), 1.59 (m, 1H), 1.27–1.47 (m, 8H); LRMS $C_{18}H_{30}NO_7S$ ($M + H^+$) 404.2.

General Procedure for Coupling Sulfonyls and Amine 10a (Scheme 3). To a solution of **10a** (0.31 mmol) and triethylamine (0.15 mL, 1.11 mmol) in CH_2Cl_2 (2.2 mL) at 0 °C was added the desired sulfonyl chloride (0.63 mL of a 0.47 M solution in CH_2Cl_2, 0.28 mmol). The reaction was allowed to go to completion (2–4 h), as determined by consumption of starting material **10a** by TLC (50/50 EtOAc/CH_2Cl_2).

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-(methylsulfonamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (11). The title compound **11** was obtained following the standard coupling procedure described above. Purification of the crude product by preparative TLC (1/4 MeOH/CH_2Cl_2) afforded **11** as a white solid (93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.44 (d, J = 5.1 Hz, 1H), 5.00 (m, 2H), 4.82 (d, J = 10.2 Hz, 1H), 4.35–4.41 (m, 1H), 4.28 (dd, J = 4.5, 12.3 Hz, 1H), 4.06 (dd, J = 2.1, 12.3 Hz, 1H), 3.86 (dt, J = 10.5, 5.4 Hz, 1H), 2.96 (s, 3H), 2.84–2.92 (m, 1H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.73–1.80 (m, 2H), 1.56–1.60 (m, 1H), 1.22–1.49 (m, 5H).

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-(4-methylphenylsulfonamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (12). The title compound **12** was obtained following the standard coupling procedure described above. Purification of the crude product by preparative TLC (15/85 MeOH/CH_2Cl_2) afforded **12** as a white solid (93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 5.15 (d, J = 5.1 Hz, 1H), 4.87–5.01 (m, 3H), 4.23–4.34 (m, 2H), 4.01 (dd, J = 1.8, 4.2 Hz, 1H), 3.76 (dt, J = 10.2, 5.4 Hz, 1H), 2.59–2.66 (m, 1H), 2.42 (s, 3H), 2.08 (s, 3H), 1.99 (s, 3H), 1.56–1.93 (m, 9H), 1.23–1.40 (m, 6H).

General Procedure for Global Deacetylation. In the final deprotection step, 1 mL of sodium methoxide (1 M solution in MeOH) was added to each triacetate (ca. 0.074 mmol in 1 mL of MeOH), and the mixtures were stirred for 1 h at rt. The solutions were concentrated in vacuo prior to purification by preparative TLC or HPLC.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)methanesulfonamide (13).** The title compound **13** was obtained following the deacetylation procedure described above. Purification of the crude product by preparative TLC (15/85 MeOH/CH_2Cl_2) afforded **13** as a white solid (93%). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$) δ 5.17 (d, J = 4.5 Hz, 1H), 3.97 (m, 1H), 3.68 (m, 2H), 3.29 (m, 1H), 3.18 (m, 1H), 2.81 (s, 3H), 2.59 (m, 1H), 1.75 (m, 2H), 1.49 (m, 2H), 1.34 (m, 1H), 1.09 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 85.5, 72.6, 72.2, 70.9, 61.2, 57.7, 55.9, 44.4, 41.1, 34.0, 33.4, 25.7, 25.4; IR (cm^{-1}) 3373 (br), 2927, 1729, 1455, 1311, 1154; solvent A, 11.9; solvent B, 15.6 min; HRMS (ES+) m/z calcd for $C_{13}H_{25}NO_6S_2Na$ ($[M + Na]^+$), 378.1021; found, 378.1013; Δ = −2.1 ppm.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-4-methylbenzenesulfonamide (14).** The title compound **14** was obtained following the deacetylation procedure described above. Purification of the crude product by preparative TLC (15/85 MeOH/CH_2Cl_2) afforded **14** as a white solid (95%). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$) δ 7.82 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 5.02 (d, J = 4.8 Hz, 1H), 3.91 (m, 1H), 3.74 (m, 1H), 3.69 (dd, J = 5.4, 6.7 Hz, 1H), 3.47 (dd, J = 5.4, 6.0 Hz, 1H), 3.40 (m, 1H), 3.31 (m, 1H), 2.44 (s, 3H), 2.41 (m, 1H), 1.87 (m, 1H), 1.71 (m, 3H), 1.58 (m, 1H), 1.26 (m, 3H), 1.18 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) 144.6, 140.6, 130.7 (2C), 128.2 (2C), 85.9, 74.3, 72.9, 72.3, 62.4, 59.3, 45.4, 35.5, 34.5, 27.0, 26.9, 21.5; IR (cm^{-1}) 3280, 2923, 2615, 1597, 1451, 1315, 1158; solvent A, 24.4; solvent B, 26.0 min; HRMS (ES+) m/z calcd for $C_{19}H_{29}NO_6NaS_2$ ($[M + Na]^+$), 454.1334; found, 454.1334; Δ = 0.0 ppm.

General Procedure for Horner−Wadsworth−Emmons Olefination. To a solution of triethyl-α-phosphonomethanesulfonate (**16**; 0.26 g, 1.01 mmol) in THF (10.0 mL) at −78 °C, *n*-BuLi (0.40 mL of a 2.5 M solution in hexanes, 1.01 mmol) was added dropwise. The resulting pale yellow solution was maintained for 15 min, after which benzaldehyde (**15a**) or 3-phenyl-propionaldehyde (**15b**; 1.0 mmol) was added and the mixture was allowed to warm to rt in 1 h. After 4 h, the solution was concentrated in vacuo, the crude residue was suspended in water, the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were dried over $MgSO_4$, concentrated in vacuo, and purified by column chromatography (Si Gel, 15/85 EtOAc/hexanes) to give **17a** (78%) and **17b** (82%) as white solids.

(*E*)-Ethyl 2-Phenylethenesulfonate (17a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.58 (s, 1H), 7.50–7.54 (m, 2H), 7.43–7.47 (m, 2H), 6.74 (d, J = 15.3, 1H), 4.24 (q, J = 7.2 Hz, 2H), 1.41 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.7, 132.0, 131.5, 129.3, 128.6, 121.3, 67.0, 15.0.

(*E*)-Ethyl 4-Phenylbut-1-ene-1-sulfonate (17b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.35 (m, 5H), 6.91 (dt, J = 6.9, 11.5 Hz, 1H), 6.17 (d, J = 11.5 Hz, 1H), 3.97–4.09 (m, 2H), 2.79–2.86 (m, 2H), 2.62 (m, 2H), 1.32 (m, 3H).

General Procedure for the Synthesis of Thionyl Chlorides. To a solution of sulfone ethylester **17a** or **17b** (0.38 mmol) in 3.8 mL of acetone, tetrabutylammonium iodide (0.23 g, 0.62 mmol)

was added, and the mixture was heated at reflux and monitored by TLC for complete consumption of starting material, after which the solution was cooled to rt, concentrated in vacuo, and the crude product was used directly in the next step. To this crude product in CH_2Cl_2 (3 mL) at 0 °C was added triphenylphosphine (0.2 g, 0.76 mmol). The mixture was stirred for 10 min and thionyl chloride (0.84 mL of a 1.0 M solution in CH_2Cl_2, 0.84 mmol) was added slowly. The mixture was allowed to warm to rt, stirred for 1 h, and concentrated in vacuo, and the crude sulfonyl chlorides **18a** and **18b** were used directly in the next coupling step.

2-Phenyl-ethene-1-sulfonyl Chloride (Crude 18a). Yield, 74% (two steps); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.15–7.31 (m, 5H), 6.98 (s, 1H).

4-Phenyl-but-1*E*-ene-1-sulfonyl Chloride (Crude 18b). Yield, 69% (two steps); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25–7.45 (m, 5H), 7.08 (m, J = 15 Hz, 1H), 6.78 (d, J = 15 Hz, 1H), 2.95 (t, J = 7.8 Hz, 2H), 2.76 (q, J = 7.2 Hz, 2H).

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-((*E*)-2-phenylvinylsulfonamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (19a). The title compound **19a** was obtained by following the standard sulfonyl coupling procedure described above. Purification of the crude product by preparative TLC (Si gel, 2/3 EtOAC/hexanes) provided **19a** as a white solid (83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.51 (m, 5H), 6.74 (d, J = 15.6 Hz, 1H), 5.45 (d, J = 5.4 Hz, 1H), 5.01 (m, 2H), 4.86 (d, J = 10.2 Hz, 1H), 4.35–4.40 (m, 1H), 4.28 (dd, J = 4.8, 12.3 Hz, 1H), 4.04 (dd, J = 2.4, 12 Hz, 1H), 3.80–3.89 (m, 1H), 2.77–2.86 (m, 1H), 2.08 (s, 3H), 2.02 (s, 3H), 1.90 (s, 3H), 1.69–1.70 (m, 2H), 1.57 (bs, 1H), 1.23–1.46 (m, 7H).

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-((*E*)-4-phenylbut-1-enylsulfonamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (19b). The title compound **19b** was obtained by following the standard sulfonyl coupling procedure described above. Purification of the crude product by preparative TLC (Si gel, 2/3 EtOAC/hexanes) provided **19b** as a white solid (79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.17 (m, 5H), 5.96–6.09 (m, 1H), 5.58–5.64 (m, 1H), 5.48 (d, J = 5.4 Hz, 1H), 5.03 (q, J = 7.8 Hz, 2H), 4.71 (d, J = 102 Hz, 1H), 4.38–4.40 (m, 1H), 4.92 (dd, J = 4.5, 12.6 Hz, 1H), 4.08–4.16 (m, 2H), 3.81–3.96 (m, 2H), 3.49 (d, 7.2 Hz, 2H), 2.82–2.95 (m, 1H), 2.09 (s, 3H), 2.02–2.04 (m, 6H), 1.74 (bs, 2H), 1.37 (bs, 1), 1.23–1.43 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.6, 169.6, 137.6, 128.7, 128.6, 128.5, 126.5, 116.5, 85.8, 71.2, 68., 68.6, 62.0, 56.0, 52.9, 45.6, 34.3, 33.9, 33.6, 25.9, 25.6, 20.9, 20.8, 20.7.

(*E*)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-phenylethenesulfonamide (20). The title compound **20** was obtained by following the standard deacetylation procedure described above. Purification of the crude product by preparative TLC (Si gel, 10/90 MeOH/CH_2Cl_2) provided **20** as a white solid (96%). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$) δ 7.61 (m, 1H), 7.48 (d, J = 16.3 Hz, 1H), 7.44 (m, 4H), 5.45 (d, J = 5.1 Hz, 1H), 3.98 (m, 1H), 3.79 (dd, J = 2.5, 9.4 Hz, 1H), 3.73 (dd, J = 5.4, 6.8 Hz, 1H), 3.53 (dd, J = 5.3, 5.5 Hz, 1H), 3.48 (dd, J = 8.6, 8.5 Hz, 1H), 3.37 (m, 1H), 3.33 (m, 1H), 2.79 (m, 1H), 1.97 (m, 6H), 1.70 (m, 2H), 1.57 (m, 1H), 1.26 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD), 140.7, 134.7, 131.7, 130.3(2C), 129.4(2C), 128.9, 86.3, 74.3, 73.2, 72.4, 62.7, 59.4, 45.3, 35.4, 34.9, 27.1, 26.9; IR (cm^{-1}) 3456, 3023, 2929, 1740, 1366, 1217, 1149; solvent A, 26.3; solvent B, 27.3 min; HRMS (ES+) m/z calcd for $C_{20}H_{29}NO_6NaS_2$ ([M + Na]$^+$), 466.1334; found, 466.1320; Δ = −3.0 ppm.

(*E*)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-4-phenylbut-1-ene-1-sulfonamide (21). The title compound **21** was obtained by following the standard deacetylation procedure described above. Purification of the crude product by preparative TLC (Si gel, 10/90 MeOH/CH_2Cl_2) provided **21** as a white solid (91%). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$) δ 7.27 (m, 5H), 5.95 (m, 1H), 5.74 (m, 1H), 5.44 (d, J = 5.2 Hz, 1H), 4.17 (m, 1H), 4.02 (m, 2H), 3.79 (m, 2H), 3.60 (m, 1H), 2.89 (m, 1H), 2.03 (m, 5H), 1.77 (m, 2H), 1.65 (m, 1H), 1.47 (m, 6H), ^{13}C NMR (125 MHz, CD_3OD) δ 141.4, 137.6, 129.8 (2C), 127.3, 120.4, 118.8, 87.1, 74.6, 72.6, 62.3, 59.6, 53.4, 45.3, 39.8, 35.7, 34.6, 27.0; IR (cm^{-1}) 3318, 2971, 2927, 1739, 1449, 1321, 1217; solvent A, 31.5; solvent B, 33.6 min; HRMS (ES+) m/z calcd for $C_{22}H_{33}NO_6NaS_2$ ([M + Na]$^+$), 494.1647; found, 494.1632; Δ = −3.0 ppm.

General Procedure for Hydrogenation of Sulfonamide Triacetates. An aliquot of each of the α,β-unsaturated sulfonamides **19a** or **19b** (0.12 mmol) in EtOH (1.2 mL) was subjected to hydrogenation (1 atm) in the presence of 10% Pd/C for 8 h. The heterogeneous suspension was filtered through a pad of Celite, washed with EtOAc (40 mL), and concentrated in vacuo. The crude products were purified by preparative TLC (SiO_2, 40/60 EtOAc/hexanes) to provide quantitatively the corresponding reduction products **22** (88%) and **23** (90%) as white solids.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-phenylethanesulfonamide (22).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.18 (m, 5H), 5.42 (d, J = 5.7 Hz, 1H), 3.96 (t, J = 12.0 Hz, 2H), 3.75–2.69 (m, 2H), 3.59–3.28 (m, 3H), 3.17–3.10 (m, 2H), 2.79–2.72 (m, 1H), 1.91 (bs, 2H), 1.68–1.55 (m, 3H), 1.36–1.18 (m, 5H); HRMS (ES) m/z calcd for $C_{20}H_{31}NO_6NaS_2$ ([M + Na]$^+$), 468.1491; found, 468.1483; Δ = −1.6 ppm.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-4-phenylbutane-1-sulfonamide (23).** ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$) δ 7.26 (m, 2H), 7.20 (m, 2H), 5.39 (d, J = 5.2 Hz, 1H), 3.99 (m, 1H), 3.80 (d, J = 2.0, 9.6 Hz, 1H), 3.73 (d, J = 5.3, 6.6 Hz, 1H), 3.47 (m, 1H), 3.17 (m, 2H), 2.89 (m, 1H), 2.66 (m, 2H), 2.02 (m, 2H), 1.89 (m, 2H), 1.75 (m, 5H), 1.59 (m, 2H), 1.35 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 142.4, 129.6 (2C), 129.5 (2C), 127.0, 87.0, 74.6, 73.5, 72.9, 62.7, 59.4, 54.5, 45.3, 36.6, 35.6, 35.0, 31.5, 27.8, 27.4, 27.0, 24.6; solvent A, 32.4; solvent B, 34.7 min; IR (cm^{-1}) 3342 (br), 3026, 2927, 1738, 1447, 1366, 1217; HRMS (ES+) m/z calcd for $C_{22}H_{35}NO_6NaS_2$ ([M + Na]$^+$), 496.1804; found, 496.1796; Δ = −1.5 ppm.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2,2,2-trifluoroacetamide (24).** To a solution of **10a** (0.077 mmol) in CH_2Cl_2 (0.77 mL) was added pyridine (0.31 mmol), and the mixture was cooled to 0 °C. Trifluoroacetic anhydride (0.092 mmol) was added to the solution and the mixture was allowed to warm to rt and stirred overnight. The mixture was concentrated in vacuo and the crude oil was purified via prep TLC (40/60 EtOAc/Hex) to provide **24** as a white solid (88%). ^{1}H NMR (600 MHz, CD_3OD/$CDCl_3$), δ 5.56 (d, J = 5.5 Hz, 1H), 4.04 (dd, J = 5.4, 5.9, 1H), 4.00 (m, 1H), 3.82 (d, J = 9.9 Hz, 1H), 3.75 (dd, J = 5.3, 6.7 Hz, 1H), 3.69 (t, J = 8.9 Hz, 1H), 3.39 (t, J = 9.2 Hz, 1H), 2.82 (m, 1H), 1.98 (m, 5H), 1.33 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 159.3, 118.5, 83.4, 74.6, 72.7, 72.2, 62.6, 56.1, 45.1, 35.4, 35.1, 27.0, 26.5, 26.4; solvent A, 20.4; solvent B, 24.3 min; IR (cm^{-1}) 3456, 3016, 2977, 1739, 1442, 1366, 1228; HRMS (ES−) m/z calcd for $C_{14}H_{21}NO_5F_3S$ ([M − H]$^-$), 372.1093; found, 372.1091; Δ = −0.4 ppm.

General Procedure for Coupling of Acids to Amine 10a (Schemes 4, 6, 7). To a stirred solution of carboxylic acid (0.19 mmol) and **10a** (0.21 mmol) at 0 °C in DMF (2.0 mL) was added sequentially DIEA (40 μL, 0.23 mmol) and DEPC (40 μL, 0.26 mmol). The mixture was warmed to rt over a 1 h period and maintained for an additional 1 h. The now light yellow solution was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). Purification by preparative TLC (SiO_2, 40/60 EtOAc/hexanes) provided the UV-active products.

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-5-(2-(*tert*-butoxycarbonylamino)-4-phenylbutanamido)-6-(cyclohexylthio)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (25). The title compound **25** was obtained by following the standard acid coupling procedure described above to provide **25** as a white solid (74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.31 (m, 3H), 7.15–7.19 (m, 2H), 6.27 (d, J = 7.8 Hz, 1H), 5.50 (d, J = 5.1 Hz, 1H), 5.12 (m, 2H), 4.90 (m, 1H), 4.42 (m, 2H), 4.27 (dd, J = 4.8, 12.3 Hz, 1H), 4.09 (m, 1H), 2.81 (m, 1H), 2.63 (t, J = 7.5 Hz, 1H), 2.09 (s, 3H), 2.02 (s,

3H), 1.92 (s, 3H), 1.70 (m, 2H), 1.58 (m, 1H), 1.46 (s, 9H), 1.24–1.40 (m, 10H); LRMS $C_{33}H_{49}N_2O_{10}S$ ($M + H^+$) 665.3.

***tert*-Butyl 1-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-ylamino)-1-oxo-4-phenylbutan-2-ylcarbamate (26).** The title compound **26** was obtained by following the standard deacetylation procedure described above to provide **26** as a white solid (97%). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$), δ 7.31 (m, 2H), 7.26 (d, $J = 7.0$ Hz, 2H), 7.22 (t, $J = 8.2$ Hz, 1H), 5.62 (d, $J = 4.7$ Hz, 1H), 4.14 (t, $J = 5.6$ Hz, 1H), 4.07 (m, 2H), 3.86 (m, 2H), 3.59 (t, $J = 8.3$ Hz, 1H), 3.48 (t, $J = 9.0$ Hz, 1H), 2.86 (m, 1H), 2.78 (m, 1H), 2.72 (m, 2H), 2.13 (m, 2H), 1.99 (m, 4H), 1.56 (s, 9H), 1.28–1.45 (m, 5H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.3, 170.8, 158.0, 142.7, 129.8, 129.7 (2C), 127.4, 84.6, 81.2, 74.5, 73.4, 72.6, 62.7, 56.0, 45.5, 35.6, 35.4, 35.2, 33.2, 29.2 (3C), 27.1; solvent A, 35.0; solvent B, 40.2 min; IR (cm^{-1}) 3342, 3016, 2970, 1739, 1447, 1366, 1217; HRMS (ES+) *m*/*z* calcd for $C_{27}H_{42}N_2O_7NaS$ ($[M + Na]^+$), 561.2610; found, 561.2615; $\Delta = 0.8$ ppm.

General Procedure for Removal of the *t*-Boc Group with TFA. Cold anhydrous TFA (2.5 mL) was added to *N*-Boc protected amine (0.054 mmol) at 0 °C. The yellow mixture was stirred for 5 min, warmed to rt with stirring, and concentrated in vacuo. The acid was quenched with pyridine (2 mL), stirred for 30 min, and concentrated in vacuo. The crude products were passed through a plug of Si gel (10/90 MeOH/ CH_2Cl_2), concentrated in vacuo, and treated with desired acid, anhydride, or acid chloride.

(2*R*,3*S*,4*R*,5*R*,6*R*)-5-(2-Acetamido-4-phenylbutanamido)-2-(acetoxymethyl)-6-(cyclohexylthio)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (27). Title compound **27** was obtained following treatment with TFA, as desribed above. To a solution of **10a** (0.077 mmol) in CH_2Cl_2 (0.77 mL) was added pyridine (0.31 mmol), and the mixture was cooled to 0 °C. Acetic anhydride (0.092 mmol) was added to the solution and the mixture was allowed to warm to rt and stirred overnight. The mixture was concentrated in vacuo and the crude oil was purified via prep TLC (10/90 MeOH/CH_2Cl_2) to provide **27** as a white solid (93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.32 (m, 5H), 6.37 (d, $J = 8.1$ Hz, 1H), 5.98 (d, $J = 7.5$ Hz, 1H), 5.58 (d, $J = 5.4$ Hz, 1H), 5.12 (m, 2H), 4.43–4.49 (m, 3H), 4.32 (dd, $J = 4.8$, 7.5 Hz, 1H), 4.13 (m, 1H), 2.84 (m, 1H), 2.67 (m, 2H), 2.14 (s, 3H), 2.08 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.92 (m, 2H), 1.75 (m, 2H), 1.61 (m, 1H), 1.28–1.46 (m, 8H); LRMS $C_{30}H_{43}N_2O_9S$ ($M + H^+$) 607.3.

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-(4-phenyl-2-(2,2,2-trifluoro-acetamido)butanamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (28). Title compound **28** was obtained following treatment with TFA, as described above. To a solution of **10a** (0.077 mmol) in CH_2Cl_2 (0.77 mL) was added pyridine (0.31 mmol), and the mixture was cooled to 0 °C. Trifluoroacetic anhydride (0.092 mmol) was added to the solution and the mixture was allowed to warm to rt and stirred overnight. The mixture was concentrated in vacuo and the crude oil was purified via prep TLC (10/90 MeOH/CH_2Cl_2) to provide **28** as a white solid (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14−7.32 (m, 5H), 7.05 (d, $J = 6.6$ Hz, 1H), 6.18 (d, $J = 7.8$ Hz, 1H), 5.50 (d, $J = 5.4$, 1H), 5.09 (m, 2H), 4.39–4.51 (m, 3H), 4.28 (dd, $J = 4.8$, 7.8 Hz, 1H), 4.08 (dd, $J = 2.4$, 9.9 Hz, 1H), 2.81 (m, 1H), 2.58–2.65 (m, 2H), 2.18 (m, 1H), 2.09 (s, 3H), 2.03 (s, 3H), 1.90 (s, 3H), 1.70 (m, 2H), 1.25–1.30 (m, 10H); LRMS $C_{30}H_{40}F_3N_2O_9S$ ($M + H^+$) 661.3.

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-(2-(methylsulfonamido)-4-phenylbutanamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (29). Title compound **29** was obtained following treatment with TFA, as described above. To a solution of **10a** (0.077 mmol) in CH_2Cl_2 (0.77 mL) was added pyridine (0.31 mmol), and the mixture was cooled to 0 °C. Methanesulfonic acid anhydride (0.1 mmol) was added to the solution and the mixture was allowed to warm to rt and stirred overnight. The mixture was concentrated in vacuo and the crude oil was purified via prep TLC (10/90 MeOH/CH_2Cl_2) to provide a white solid (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.29 (m, 5H), 6.41 (d, $J = 8.4$ Hz, 1H), 5.58 (d, $J = 5.1$ Hz, 1H), 5.23 (d, $J = 8.1$ Hz, 1H), 5.10 (m, 2H), 4.45 (m, 2H), 4.27 (dd, $J = 5.1$, 12.3 Hz, 1H), 4.07 (dd, $J = 2.1$, 10.8 Hz, 1H), 3.90 (m, 1H), 2.91 (s, 3H), 2.72 (m, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.93 (s, 3H), 1.92 (m, 2H), 1.70 (m, 3H), 1.56 (m, 1H), 1.25–1.28 (m, 6H); LRMS $C_{29}H_{43}N_2O_{10}S_2$ ($M + H^+$) 643.3.

***N*′-acetyl-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-4-phenylbutanehydrazide (30).** Title compound **30** was obtained following the standard deacetylation procedure described above. Purification of the crude product by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **30** as a white solid (95%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.27 (m, 2H), 7.21 (d, $J = 7.5$ Hz, 2H), 7.17 (t, $J = 7.1$ Hz, 1H), 5.53 (d, $J = 5.3$ Hz, 1H), 4.41 (dd, $J = 5.7$, 3.0 Hz, 1H), 4.00 (m, 2H), 3.80 (m, 1H), 3.73 (dd, $J = 5.3$ Hz, $J = 6.7$ Hz, 1H), 3.54 (m, 1H), 3.36 (t, $J = 9.5$ Hz, 1H), 2.82 (m, 1H), 2.69 (m, 4H), 2.09 (m, 2H), 2.06 (s, 3H), 1.95 (m, 3H), 1.33 (m, 6H); ^{13}C NMR (125 MHz, $CD_3OD/CDCl_3$), 174.5, 173.4, 142.8, 138.2, 130.0, 127.2, 84.3, 74.7, 73.1, 72.7, 62.7, 56.0, 54.6, 45.2, 35.5, 35.1, 35.0, 33.2, 27.0, 22.7; solvent A, 21.6; solvent B, 27.3 min; IR (cm^{-1}) 3457, 3026, 2971, 1739, 1435, 1366, 1229; HRMS (ES+) *m*/*z* calcd for $C_{24}H_{36}N_2O_6NaS$ ($[M + Na]^+$), 503.2192; found, 503.2177; $\Delta = -2.9$ ppm.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-4-phenyl-2-(2,2,2-trifluoroacetamido)butanamide (31).** Title compound **31** was obtained following the standard deacetylation procedure described above. Purification of the crude product by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **31** as a white solid (91%). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 6.97 (m, 3H), 6.88 (m, 2H), 5.20 (d, $J = 5.3$, 1H), 4.30 (bs, 1H), 4.18 (m, 1H), 3.67 (dt, $J = 10.0$, 2.9 Hz, 1H), 3.49 (d, $J = 3.4$ Hz, 2H), 3.22 (dd, $J = 3.4$, 8.9 Hz, 1H), 3.14 (t, $J = 9.7$ Hz, 1H), 2.47 (m, 1H), 2.39 (m, 2H), 1.85 (m, 2H), 1.75 (m, 2H), 1.63 (m, 6H), 1.41 (m, 2H), 1.27 (m, 1H), 0.95 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD), 172.6, 142.1, 129.9, 129.8 (2C), 127.6, 84.5, 74.2, 73.1, 72.6, 62.8, 56.0, 54.9, 45.7, 35.6, 35.1, 34.8, 33.1, 27.0; solvent A, 31.4; solvent B, 35.5 min; IR (cm^{-1}) 3456, 3026, 2971, 1739, 1447, 1366, 1229; HRMS (ES+) *m*/*z* calcd for $C_{24}H_{33}N_2O_6F_3NaS$ ($[M + Na]^+$), 557.1909; found, 557.1911; $\Delta = 0.3$ ppm.

***N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-(methylsulfonamido)-4-phenylbutanamide (32).** Title compound **32** was obtained following the standard deacetylation procedure described above. Purification of the crude product by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **32** as a white solid (89%). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$), 7.27 (m, 2H), 7.21 (d, $J = 7.5$ Hz, 2H), 7.17 (t, $J = 7.1$ Hz, 1H), 5.58 (d, $J = 5.3$ Hz, 1H), 3.99 (m, 2H), 3.80 (m, 2H), 3.59 (dd, $J = 3.2$, 8.8 Hz, 1H), 3.42 (t, $J = 3.6$ Hz, 1H), 2.97 (s, 3H), 2.78 (m, 2H), 2.71 (m, 1H), 2.06 (m, 3H), 1.94 (m, 5H), 1.69 (m, 2H), 1.57 (m, 1H), 1.26 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD), 174.0, 142.1, 129.2, 126.9, 83.6, 78.9, 73.6, 72.4, 72.0, 62.2, 57.9, 55.4, 44.5, 41.2, 36.2, 35.1, 34.6, 32.6, 26.5; solvent A, 23.7; solvent B, 26.8 min; IR (cm^{-1}) 3456, 3017, 2971, 1739, 1456, 1366, 1229; HRMS (ES+) *m*/*z* calcd for $C_{23}H_{36}N_2O_7NaS_2$ ($[M + Na]^+$), 539.1862; found, 539.1866; $\Delta = 0.8$ ppm.

General Procedure for Preparation of *tert*-Butyldimethylsilyl Methyl Esters. To a stirred solution of *tert*-butyldimethylsilyl chloride (3.80 g, 25.2 mmol) in CH_3CN (16 mL) was added **33a** or **33b**.[27] Upon cooling to 0 °C, DBU (3.8 mL, 25.2 mmol) was added to the mixture, and stirring was maintained at rt overnight. The white heterogeneous mixture was filtered, and the precipitate was crystallized from MeOH/CH_3CN to give silyl ethers **34a** or **34b** as white powders in 70–85% yield. To a stirred solution of **34a** or **34b** (8.55 mmol) in benzene (80 mL) and MeOH (24 mL) at rt was added trimethylsilyldiazomethane (2.0 M in hexanes, 22 mL, 44 mmol), and the mixture was stirred overnight at rt. The yellowish solution was concentrated in vacuo to give the desired *O*-*tert*-butyldimethylsilyl methyl ester quantitatively to be used in the next step without further purification.

3-(*tert*-Butyl-dimethyl-silanyloxy)-2-(3-fluoro-benzoylamino)-propionic Acid (34b). ^{1}H NMR (300 MHz, CD_3OD) δ 4.89 (bs, 2H), 3.85 (t, $J = 3.7$ Hz, 2H), 3.68 (dd, $J = 4.5$, 7.8 Hz, 1H), 3.29

(m, 2H), 2.15 (m, 1H), 1.97 (m, 1H), 0.93 (s, 9H), 0.13 (s, 6H); HRMS (APCI+) *m/z* calcd for $C_{10}H_{24}NO_3Si$ ($[M + H]^+$), 234.1525; found, 234.1517; $\Delta = -3.6$ ppm.

General Procedure for Coupling Benzoic Acids to Amines. To a stirred solution containing 3-fluorobenzoic acid (0.64 g, 4.62 mmol), HOBt (0.70 g, 4.62 mmol), EDCI (0.89 g, 4.62 mmol), and DIEA (1.7 mL, 9.62 mmol) in anhydrous DMF (20 mL) was added **34a** or **34b** (3.85 mmol), and the mixture was maintained at rt for 12 h with stirring. Upon dilution with ethyl acetate (60 mL) and water (40 mL), the organic layer was separated and washed with saturated sodium bicarbonate solution (3 × 35 mL) and brine (35 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude products were purified by column chromatography (Si gel, gradient 15–45% EtOAc/hexanes) to give the amides **35a** (71%) and **35b** (69%).

Methyl 3-(*tert*-Butyldimethylsilyloxy)-2-(3-fluorobenzamido)propanoate (35a).. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.57 (m, 2H), 7.42 (m, 1H), 7.22 (m, 1H), 6.95 (d, $J = 7.8$ Hz, 1H), 4.83 (dt, $J = 2.4$, 2.7 Hz, 1H), 4.14 (dd, $J = 2.4$, 7.8 Hz, 1H), 3.95 (dd, $J = 3.0$, 7.2 Hz, 1H), 3.78 (s, 3H), 0.86 (s, 9H), 0.03 (s, 6H); HRMS (APCI+) *m/z* calcd for $C_{17}H_{27}NO_4SiF$ ($M + H^+$), 356.1693; found, 356.1682; $\Delta = -3.2$ ppm.

Methyl 4-(*tert*-Butyldimethylsilyloxy)-2-(3-fluorobenzamido)butanoate (35b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.56 (m, 3H), 7.33–7.40 (m, 1H), 7.13–7.20 (m, 1H), 4.81 (m, 1H), 3.80 (m, 2H), 3.74 (s, 3H), 2.13 (m, 2H), 0.83 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 166.1, (164.5, 161.3 F−C coupling), 130.3, 136.5, 122.8, 118.7, 114.7, 60.9, 52.5, 52.2, 33.5, 26.1 (3C), −5.3 (2C); HRMS (APCI+) *m/z* calcd for $C_{18}H_{29}NO_4SiF$ ($[M + H]^+$), 370.1850; found, 370.1848; $\Delta = -0.5$ ppm.

Methyl 2-(3-Fluorophenyl)-4,5-dihydrooxazole-4-carboxylate (36a). To a stirred solution containing amide **35a** (0.81 mmol) in CH_2Cl_2 (3.5 mL) was added DAST (0.6 mL, 3.24 mmol) at rt, and the solution was maintained for 7 h after which it was quenched slowly with saturated aq $NaHCO_3$. The aqueous layer was extracted three times with CH_2Cl_2, and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography (Si gel, 15–30% EtOAc/hexanes) provided oxazole methyl ester **36a** as a clear oil (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75–7.79 (m, 1H), 7.65–7.70 (m, 1H), 7.35–7.42 (m, 1H), 7.20 (m, 1H), 4.96 (dd, $J = 2.7$, 8.1 Hz, 1H), 4.71 (dd, $J = 0.6$, 8.1 Hz, 1H), 4.61 (dd, $J = 2.1$, 8.7 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ Hrms (APCI+) *m/z* calcd for $C_{11}H_{11}NO_3F$ ($M + H^+$), 224.0723; found, 224.0728; $\Delta = 2.2$ ppm.

Methyl 2-(3-Fluorophenyl)-5,6-dihydro-4*H*-1,3-oxazine-4-carboxylate (36b). Title compound **36** was obtained following the same procedure as described above for **36a**. Purification by column chromatography (Si gel, 15–30% EtOAc/hexanes) provided oxazine methyl ester **36b** as a clear oil (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71–7.74 (m, 1H), 7.61–7.66 (m, 1H), 7.26–7.35 (m, 1H), 7.07–7.14 (m, 1H), 4.30–4.47 (m, 3H), 3.78 (s, 3H), 2.12–2.22 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 164.4, 161.1, 155.9, 135.9, 129.8, 123.2, 117.9, 114.6, 63.7, 54.6, 52.6, 24.2; HRMS (APCI+) *m/z* calcd for $C_{12}H_{13}NO_3F$ ($M + H^+$), 238.0879; found, 238.0886; $\Delta = 2.7$ ppm.

General Procedure for Preparation of Acids. To an ice-cooled solution of either oxazine methyl ester **36a** or oxazoline methyl ester **36b** in MeOH/water (3:1) was added LiOH (2.3 equiv) with stirring. The mixture was allowed to warm to rt in 1 h, concentrated in vacuo, and the white solid was dissolved in water/CH_2Cl_2. The layers were separated and the aqueous layer was acidified with 1 N HCl to pH 2.0 and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to provide the respective acids **37a** and **37b** in quantitative yields.

2-(3-Fluorophenyl)-4,5-dihydrooxazole-4-carboxylic Acid (37a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, $J = 8.1$ Hz, 1H), 7.65–7.69 (m, 1H), 7.49 (dt, $J = 5.7$, 8.1 Hz, 1H), 7.31 (dt, 2.4, 8.7 Hz, 1H), 4.94 (q, $J = 9.0$ Hz, 1H), 4.69 (d, $J = 8.7$ Hz, 2H).

2-(3-Fluorophenyl)-5,6-dihydro-4*H*-1,3-oxazine-4-carboxylic Acid (37b). ^{1}H NMR (300 MHz, CD_3OD) δ 7.71 (bd, $J = 7.8$ Hz, 1H), 7.62 (bd, $J = 9.6$ Hz, 1H), 7.37–7.47 (m, 1H), 7.20–7.27 (m, 1H), 4.48 (t, $J = 5.7$ Hz, 2H), 4.30 (t, $J = 5.4$ Hz, 1H), 2.24–2.35 (m, 1H), 2.07–2.18 (m, 1H).

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-(2-(3-fluorophenyl)-4,5-dihydrooxazole-4-carboxamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (38a). The title compound **38a** was obtained following the standard coupling procedure described above. Purification of the crude product by preparative TLC (40/60EtOAc/hexanes) afforded **38a** as a white solid (79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, $J = 8.1$ Hz, 1H), 7.71 (dt, $J = 2.4$, 9.3, 1H), 7.42 (dt, $J = 5.7$, 8.1 Hz, 1H), 7.23 (dd, 2.4, 8.4 Hz, 1H), 7.05 (d, $J = 9$ Hz, 1H), 5.35 (d, $J = 5.7$ Hz, 1H), 5.04–5.21 (m, 2H), 4.78 (dd, $J = 6.9$, 11.1 Hz, 1H), 4.59–4.71 (m, 2H), 4.45–4.54 (m, 1H), 4.34–4.38 (m, 1H), 4.27 (dd, $J = 4.8$, 12.6 Hz, 1H), 4.04–4.16 (m, 1H), 2.08 (s, 3H), 2.03–2.05 (m, 6H), 1.05–1.86 (m, 11H).

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(cyclohexylthio)-5-(2-(3-fluorophenyl)-5,6-dihydro-4*H*-1,3-oxazine −4-carboxamido)-tetrahydro-2*H*-pyran-3,4-diyl Diacetate (38b). The title compound **38b** was obtained following the standard coupling procedure described above. Purification of the crude product by preparative TLC (40/60EtOAc/hexanes) afforded **38b** as a white solid (71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (m, 2H), 7.36 (m, 1H), 7.15 (m, 1H), 5.56 (d, J= 5.4 Hz, 0.5H), 5.48 (d, J= 5.4 Hz, 0.5H), 5.18 (m, 2H), 5.10 (m, 1H), 4.54–4.25 (m, 5H), 4.13–4.00 (m, 2H), 2.95–2.88 (m, 1H), 2.80–2.73 (m, 1H), 2.48 (m, 1H), 2.08 (m, 9H), 1.35–1.95 (m, 10H).

(*R*)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-(3-fluorophenyl)-4,5-dihydrooxazole-4-carboxamide (39D). Title compound **39D** was obtained following the standard deacetylation protocol described above. Purification of triol by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **39D** as a white solid (90%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.75 (d, $J = 5.1$ Hz, 1H), 7.76 (m, 1H), 7.43 (m, 1H), 7.25 (m, 1H) 5.51 (d, $J = 5.4$ Hz, 1H), 4.70 (m, 2H), 4.88 (dd, $J = 8.4$, 2.4 Hz, 1H), 4.07 (dd, $J = 5.1$, 5.4 Hz, 1H), 3.95 (m, 1H), 3.77 (m, 2H), 3.46 (m, 2H), 2.82 (m, 1H), 1.96 (m, 2H), 1.74 (m, 2H), 1.58 (m, 1H), 1.22–1.42 (m, 5H); ^{13}C NMR (75 MHz, CD_3OD) δ 172.9, 166.6, 164.8 (doublet due to ^{19}F−^{13}C coupling), 131.0, 124.9, 119.9 (doublet), 116.2, 115.9 (doublet), 83.9, 73.5, 73.0, 71.5, 71.3, 69.6, 62.0, 54.8, 45.1, 34.9, 34.5, 26.5, 26.4, 26.3; solvent A, 26.7; solvent B, 33.9 min; HRMS (ES−) *m/z* calcd for $C_{22}H_{28}FN_2O_6S$ ($[M - H]^-$), 467.1652; found, 467.1642; $\Delta = -2.2$ ppm.

(*S*)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-(3-fluorophenyl)-4,5-dihydrooxazole-4-carboxamide (39L). Title compound **39L** was obtained following the standard deacetylation protocol described above. Purification of triol by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **39L** as a white solid (78%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.79 (d, $J = 5.8$ Hz, 1H), 7.67 (d, $J = 6.0$ Hz, 1H), 7.45 (m, 1H), 7.27 (m, 1H), 5.27 (d, $J = 5.4$ Hz, 1H), 4.91 (dd, $J = 8.4$, 1.5 Hz, 1H), 4.66 (m, 1H), 4.13 (dd, $J = 5.1$, 5.4 Hz, 1H), 3.91 (m, 1H), 3.77 (m, 2H), 3.45 (m, 2H), 2.67 (m, 1H), 1.84 (m, 1H), 1.67 (m, 1H), 1.58 (m, 1H), 1.47 (m, 2H) 1.03–1.21 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.4, 166.7, 165.1, 161.8, 131.3, 125.2, 120.1, 116.3, 84.7, 74.2, 73.5, 71.6, 69.4, 62.2, 54.5, 45.3, 34.8, 34.5, 26.5, 26.4, 26.3; solvent A, 25.8; solvent B, 32.4 min; HRMS (ES−) *m/z* calcd for $C_{22}H_{28}FN_2O_6S$ ($[M - H]^-$), 467.1652; found, 467.1653; $\Delta = 0.2$ ppm.

(*R*)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-(3-fluorophenyl)-5,6-dihydro-4*H*-1,3-oxazine-4-carboxamide (40D). Title compound **40D** was obtained following the standard deacetylation protocol described above. Purification of triol by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **40D** as a white solid (91%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.02 (d, $J = 8.7$ Hz, 1H), 7.76 (dd, $J = 7.8$, 10.2 Hz, 1H), 7.35 (m, 1H), 7.16 (dt, $J = 2.7$, 5.7 Hz, 1H), 5.45 (d, $J = 5.1$ Hz, 1H), 4.51 (m, 1H), 4.41 (m, 1H), 4.39 (m, 1H), 4.30 (m, 1H), 4.21 (m, 1H), 3.97 (m, 1H), 3.76 (m,

1H), 3.70 (t, $J = 9.0$ Hz, 1H), 3.52 (m, 1H), 2.86 (m, 1H), 2.47 (m, 2H), 2.02 (m, 2H), 1.75 (m, 1H), 1.59 (m, 1H), 1.23–1.46 (m, 8H); solvent A, 31.7; solvent B, 37.6 min; HRMS (ES−) *m/z* calcd for $C_{23}H_{30}FN_2O_6S$ ([M − H]$^-$), 481.1809; found, 481.1808; $\Delta = -0.1$ ppm.

(*S*)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylthio)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl)-2-(3-fluorophenyl)-5,6-dihydro-4*H*-1,3-oxazine-4-carboxamide (40L). Title compound **40L** was obtained following the standard deacetylation protocol described above. Purification of triol by preparative TLC (10/90 MeOH/CH_2Cl_2) provided **40L** as a white solid (90%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.96 (d, $J = 9.0$ Hz, 1H), 7.74 (dd, $J = 7.8$, 10.2 Hz, 1H), 7.33 (m, 1H), 7.13 (dt, $J = 2.7$, 6.0 Hz, 1H), 5.30 (d, $J = 5.1$ Hz, 1H), 4.42 (m, 1H), 4.30 (m, 3H), 3.99 (m, 2H), 3.77 (m, 2H), 3.61 (m, 1H), 2.74 (m, 1H), 2.47 (m, 2H), 1.85 (m, 2H), 1.55 (m, 2H), 1.06–1.33 (m, 6H); solvent A, 29.2; solvent B, 35.7 min; HRMS (ES−) *m/z* calcd for $C_{23}H_{30}FN_2O_6S$ ([M − H]$^-$), 481.1809; found, 481.1817; $\Delta = 1.7$ ppm.

(2*S*)-2-Amino-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(cyclohexylthio)-tetrahydro-4,5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl)-4-hydroxybutanamide (41). Title compound **41** was obtained by following the standard procedures for removal of the *N*-Boc group with TFA, coupling, and deacetylation described above. Purification of the crude product by preparative TLC (15% MeOH/CH_2Cl_2) provided **41** as a white solid (69% yield over two steps). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 5.54 (d, $J = 5.1$ Hz, 1H), 5.50 (t, $J = 4.2$ Hz, 2H), 4.62 (m, 1H), 4.39 (m, 1H), 4.27 (m, 1H), 4.00 (m, 2H), 3.79 (m, 1H), 3.65 (m, 1H), 2.82 (m, 1H), 2.07 9m, 1H), 1.86 (m, 1H), 1.68–1.75 (m, 5H), 1.36–1.46 (m, 6H); solvent A, 16.2; solvent B, 18.8 min; HRMS (ES+) *m/z* calcd for $C_{16}H_{31}N_2O_6S$ ([M + H]$^+$), 379.1916; found, 379.1913; $\Delta = -0.8$ ppm.

(*S*)-3-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(Cyclohexylsulfonyl)-tetrahydro-4,5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-ylcarbamoyl)-3-aminopropanoic Acid (42). Title compound **42** was obtained in four steps described previously. Following the standard procedures for removal of the *N*-Boc group and acid coupling to **10a**, as described above, the intermediate was passed through a plug of Si gel (15/85 MeOH/CH_2Cl_2) and dried in vacuo. To a stirred solution of intermediate (0.11 mmol) in 1:1 CH_3CN/CCl_4 (2 mL) was added $NaIO_4$ (3 equiv) in water (1.5 mL), followed by $RuCl_3$ (2 mol%). The mixture was stirred for 2 h, filtered through a pad of Celite, concentrated in vacuo, and purified by column chromatography (Si gel, 1/1 MeOH/CH_2CL_2). Global deacetylation following the general procedure described above afforded **42** as a white solid (35% yield in four steps). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 5.29 (d, $J = 5.4$ Hz, 1H), 4.29 (m, 2H), 4.13 (t, $J = 5.1$ Hz, 1H), 3.92 (d, $J = 10$ Hz, 1H), 3.65 (dd, $J = 5.7$, 6.9 Hz, 1H), 3.49 (m, 2H), 3.10 (br, 2H), 2.87 (m, 1H), 2.66 (dd, $J = 2.8$, 6.2 Hz, 2H), 2.13 (m, 2H), 1.93 (m, 1H), 1.83 (m, 1H), 1.72 (m, 1H), 1.21–1.48 (m, 6H); solvent A, 14.8; solvent B, 16.4 min; HRMS (ES+) *m/z* calcd for $C_{16}H_{29}N_2O_9S$ ([M + H]$^+$), 425.1594; found, 425.1591; $\Delta = -0.7$ ppm.

5-(4-Chlorophenyl)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(cyclohexylthio)-tetrahydro-4,5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl)furan-2-carboxamide (43). Title compound **43** was obtained following the standard coupling procedure described above with coupling to **10b**. Purification of the crude product by RP-HPLC (20–80% MeOH in 0.05% aq TFA) provided **43** as a white solid (71%). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 7.94 (d, $J = 9.8$ Hz, 2H), 7.54 (d, $J = 9.1$ Hz, 2H), 7.35 (d, $J = 4.6$ Hz, 1H), 7.04 (d, $J = 3.8$ Hz, 1H), 5.74 (d, $J = 5.6$ Hz, 1H), 4.34 (dd, $J = 4.9$, 6.8 Hz, 1H), 4.13 (m, 1H), 3.94 (dd, $J = 2.6$, 11.8 Hz, 1H), 3.87 (dd, $J = 3.7$, 5.4 Hz, 1H), 3.84 (m, 1H), 3.55 (t, $J = 8.8$ Hz, 1H), 2.95 (m, 1H), 2.06 (m, 1H), 1.82 (m, 2H), 1.65 (m, 1H), 1.30–1.54 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.4, 159.4, 155.1, 146.2, 134.8, 129.3 (2C), 128.1, 125.9 (2C), 117.9, 108.0, 84.6, 73.9, 72.9, 70.8, 61.7, 53.9, 45.4, 34.6, 34.5, 26.6, 26.5, 26.3; solvent A, 26.8; solvent B, 34.4 min; HRMS (ES+) *m/z* calcd for $C_{23}H_{29}NO_6ClS$ ([M + H]$^+$), 482.1404; found, 482.1387; $\Delta = -3.6$ ppm.

5-(4-Chlorophenyl)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(cyclohexylthio)-tetrahydro-4,5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl)isoxazole-3-carboxamide (44). Title compound **44** was obtained following the standard coupling procedure described above with coupling to **10b**. Purification of the crude product by RP-HPLC (20–80% MeOH in 0.05% aq TFA) provided **44** as a white solid (60%). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 7.91 (d, $J = 9.2$ Hz, 2H), 7.60 (d, $J = 8.3$ Hz, 2H), 7.15 (s, 1H), 5.71 (d, $J = 5.4$ Hz, 1H), 4.12 (m, 1H), 3.92 (m, 2H), 3.75 (dd, $J = 3.2$, 8.7 Hz, 1H), 3.59 (t, $J = 9.5$ Hz, 1H), 2.93 (m, 1H), 2.06 (m, 2H), 1.84 (m, 2H), 1.67 (m, 1H), 1.30–1.53 (6H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.4, 159.7, 131.6, 130.2 (2C), 128.0 (2C), 126.0, 100.0, 84.0, 73.8, 72.3, 71.6, 62.1, 55.2, 45.2, 34.9, 34.5, 26.6, 26.5, 26.3; solvent A, 27.8; solvent B, 36.4 min; HRMS (ES−) *m/z* calcd for $C_{22}H_{26}N_2O_6ClS$ ([M − H]$^-$), 481.1200; found, 481.1183; $\Delta = -3.6$ ppm.

5-(5-Chloro-2-(trifluoromethyl)phenyl)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2-(cyclohexylthio)-tetrahydro-4,5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl)furan-2-carboxamide (45). Title compound **45** was obtained following the standard coupling procedure described above with coupling to **10b**. Purification of the crude product by RP-HPLC (20–80% MeOH in 0.05% aq TFA) provided **45** as a white solid (65%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.02 (s, 1H), 7.29–7.41 (m, 2H), 7.05 (dd, $J = 3.6$, 8.4 Hz, 2H), 5.30 (d, $J = 5.1$ Hz, 1H), 4.06 (dd, $J = 5.4$, 5.3 Hz, 1H), 3.77 (m, 1H), 3.57 (m, 2H), 3.40 (dd, $J = 1.8$, 9.0 Hz, 1H), 3.27 (dd, $J = 9.6$ Hz, 1H), 2.58 (m, 1H), 1.72 (m, 2H), 1.45 (m, 2H), 1.29 (m, 1H), 0.96–1.17 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.3, 158.7, 150.5, 146.7, 134.2, 131.5, 129.7, 129.3, 128.6, 125.2, 116.3, 113.8, 83.5, 72.7, 72.0, 70.7, 61.3, 53.8, 44.4, 33.9, 33.6, 25.6, 25.5, 25.3; solvent A, 27.8; solvent B, 35.3 min; HRMS (APCI+) *m/z* calcd for $C_{24}H_{28}NO_6F_3SCl$ ([M + H]$^+$), 550.1278; found, 550.1279; $\Delta = 0.2$ ppm.

General Procedure for Synthesis and Purification of GlcN Analogs. GlcN amides were prepared in two consecutive steps by following the standard coupling and global deacetylation procedures described above. Crude products were purified by preparative TLC (SiO_2, 15/85 MeOH/CH_2Cl_2) to provide each analog as a white solid in 60–65% yield in two steps.

5-(2-Chloro-5-(trifluoromethyl)phenyl)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl) furan-2-carboxamide (47). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 8.46 (bs, 1H), 7.75 (d, $J = 8.3$ Hz, 1H), 7.65 (d, $J = 8.3$ Hz, 1H) 7.32 (d, $J = 3.8$ Hz, 1H), 7.29 (d, $J = 3.8$ Hz, 1H), 5.19 (br, 1H), 4.11 (m, 1H), 3.88 (m, 2H), 3.74 (m, 1H), 3.46 (t, $J = 9.5$ Hz, 1H), 3.38 (t, $J = 8.4$ Hz, 1H); solvent A, 20.8; solvent B, 22.4 min; HRMS (ES+) *m/z* calcd for $C_{18}H_{18}NO_7ClF_3$ ([M + H]$^+$), 452.0724; found, 452.0705; $\Delta = -4.2$ ppm.

5-(4-Chlorophenyl)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl)isoxazole-3-carboxamide (48). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 7.89 (d, $J = 9.1$ Hz, 2H), 7.55 (d, $J = 8.4$ Hz, 2H), 7.16 (s, 1H), 5.23 (d, $J = 7.6$ Hz, 1H), 4.08 (dd, $J = 4.2$, 6.4 Hz, 1H), 3.85 (m, 2H), 3.74 (dd, $J = 4.3$, 5.7 Hz, 1H), 3.44 (t, $J = 9.2$ Hz, 1H), 3.37 (t, $J = 7.8$ Hz, 1H); solvent A, 21.3; solvent B, 23.1 min; HRMS (ES+) *m/z* calcd for $C_{16}H_{17}N_2O_7ClNa$ ([M + Na]$^+$), 407.0622; found, 407.0604; $\Delta = -4.4$ ppm.

5-(4-Chlorophenyl)-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl)furan-2-carboxamide (49). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 7.97 (d, $J = 7.5$ Hz, 2H), 7.54 (d, $J = 6.6$ Hz, 2H), 7.25 (d, $J = 7.3$ Hz, 1H), 7.15 (d, $J = 8.4$ Hz, 1H); solvent A, 20.3; solvent B, 21.2 min; HRMS (ES+) *m/z* calcd for $C_{17}H_{18}NO_7ClNa$ ([M + Na]$^+$), 406.0669; found, 406.0680; $\Delta = 2.6$ ppm.

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Supporting Information Available: General experimental methods, details of the docking protocols using AutoDock 4.0, and

a figure showing the overlay of docked inhibitors and substrate. This material is available free of charge via the Internet at http://pubs.acs.org.

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