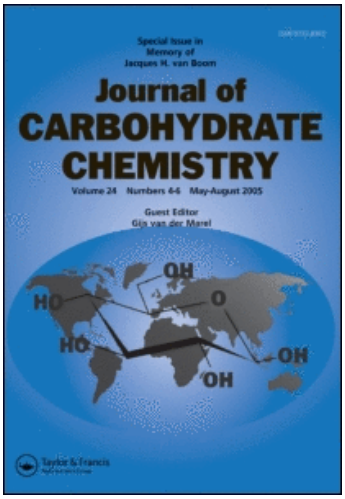

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A Family of Mycothiol Analogues

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A Family of Mycothiol Analogues

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A thioglycoside aminotriol scaffold has been elaborated by acylation, reductive alkylation, sulfonation, phosphorylation, and other procedures to produce a library of 40 functionalized thioglycosides that superficially resemble the enzyme-binding portions of the *Mycobacterium tuberculosis* detoxifier mycothiol and its metabolic congeners. To the extent that these analogues mimic the transition states derived from substrates of the mycothiol-associated enzymes, they might prove useful as inhibitors and, ultimately, as drug leads.

Keywords D-glucosaminide, Metalloprotein, Ligase, Glycosyltransferase, Acetyltransferase, Deacetylase, Reductase, Amidase, Mycothiol, Thioglycoside aminotriol scaffold

INTRODUCTION

Most actinomycetes, including *Mycobacterium tuberculosis*, biosynthesize and maintain high levels of mycothiol (MSH, **1**)[1] as chemical protection against oxidative stress[2–6] and electrophilic agents.[7–11] The unusual three-component structure of **1** arises from a biosynthetic sequence commencing formally with *myo*-inositol and *N*-acetylglucosamine precursors (Sch. 1).[12] A glycosyl transferase,[13] designated MshA, couples two components, not yet identified, to give GlcNAc-Ins (**3**), which in turn is deacetylated by a second enzyme (MshB)[14] to the aminoglycoside GlcN-Ins (**4**). An ATP-dependent ligase (MshC)[6,10,15] joins **4** with cysteine, and the resulting amide **5** is acetylated in an acetyl-CoA mediated process catalyzed by an acetyltransferase (MshD).[17,18]

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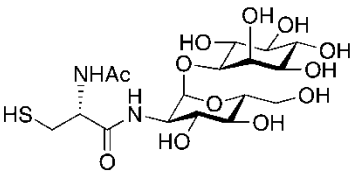
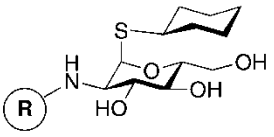
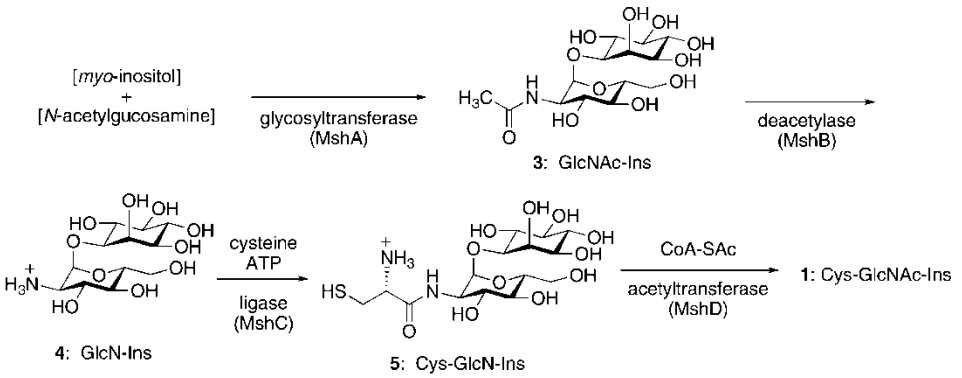


1: mycothiol (MSH)



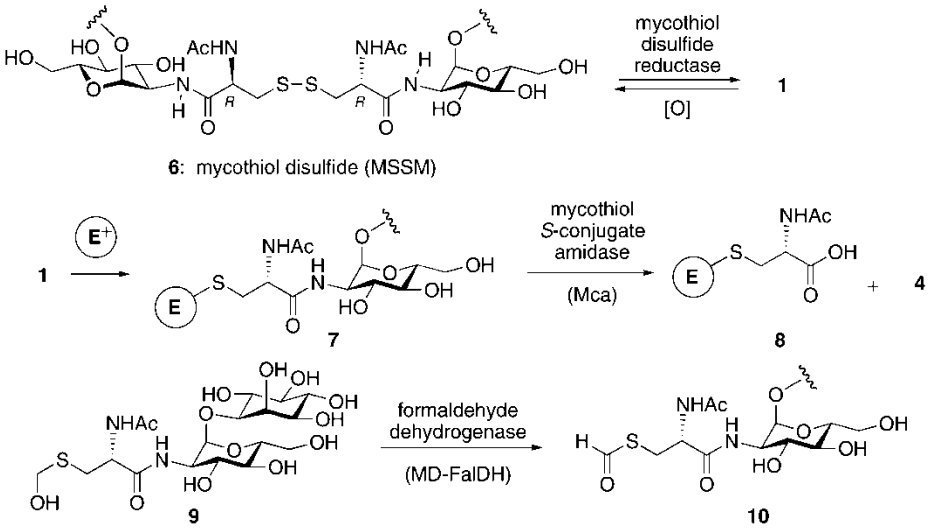
2: MSH analogues



Scheme 1: Biosynthesis of mycothiol.

Several MSH-processing enzyme systems have also been studied (Sch. 2). Oxidative dimerization of **1** gives the mycothiol disulfide **6**, and enzyme mediated or spontaneous coupling of **1** with alkylating or otherwise electrophilic species (represented by "E^+") gives rise to the *S*-substituted MSH derivatives **7**. These metabolites are respectively processed by mycothiol disulfide reductase, which converts **6** back to **1**, and mycothiol conjugate amidase, which cleaves the *S*-conjugated *N*-acetylcysteine unit **8** from **7** for exportation from the cell. The formaldehyde adduct of MSH (**9**) is oxidized to the *S*-formyl derivative **10** by a mycothiol-dependent formaldehyde dehydrogenase.[7,19]

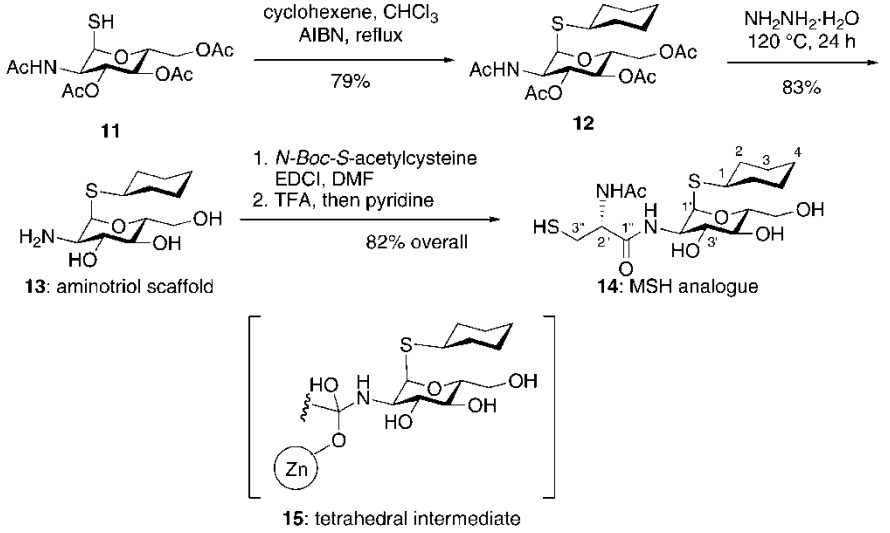
Drug-resistant tuberculosis infection is rampant globally: an estimated 16 million people were ill with the disease in 2002, 1.8 million died, and the numbers are increasing.[20] The mycothiol-associated pathways offer an opportunity for counteracting resistance to current antitubercular drugs. MSH-deficient mutants of *M. smegmatis* survive and grow under laboratory conditions, suggesting that MSH may not be critical for protection against *endogenous* oxidizing or electrophilic agents in some mycobacteria. However, direct correlation was found between MSH depletion and enhanced sensitivity to *exogenous* toxins and antibiotics, including erythromycin, azithromycin,



Scheme 2: Mycothiol-processing enzymes.

vancomycin, penicillin G, rifamycin, and rifampin.[21–24] Developing inhibitors of the nonmammalian MSH biosynthetic or processing enzymes (Schemes 1 and 2) may therefore constitute an important approach toward improving tuberculosis treatments.[25]

In order to establish the substrate requirements for the mycothiol *S*-conjugate amidase (Mca, Sch. 2), we earlier prepared and evaluated the mycothiol *S*-cyclohexyl thioglycoside analogue **14** (Sch. 3).[26] The fact that



Scheme 3: An aminotriol scaffold for construction of MSH analogues.

the bimane derivatives of MSH and **14** were cleaved by Mca at comparable rates (14200 vs. 7500 nmol min^{-1} mg-protein^{-1}, respectively) indicates that the inositol ring and linking oxygen atom of MSH contribute little to substrate binding, and thus might be dispensed with in the design of inhibitors of Mca.[26] Likewise, the inositol ring of MSH was also shown to be noncritical for substrate binding by mycothiol disulfide reductase (Sch. 2).[2] As most of the enzymes in Schemes 1 and 2 involve attaching, cleaving, or maintaining an acyl group at the nitrogen of a 2-amino-2-deoxy-α-D-glucopyranoside, the thioglycoside aminotriol **13** seemed to be a promising starting point for the preparation of inhibitors. Thioglycosides are generally more resistant to enzymatic degradation than are *O*-glycosides,[27–30] and are often, as is the case with **13**, easier to prepare in anomerically pure form.[31,32] Furthermore, by attaching *N*-substituents to **13**, we have the opportunity to mimic structural and charge features of possible tetrahedral intermediates such as **15** or their associated transition states. Evidence has been presented that both MshB and Mca are zinc-containing metalloproteins that might catalyze amidolysis through such intermediates.[33–35]

CONVERSION OF THE SCAFFOLD TO MSH ANALOGUES

The scaffold aminotriol **13** was efficiently prepared in gram quantities by our previously published route,[26] and coupling reactions of **13** with a variety of carboxylic acids and other commercially available or readily synthesized partners were carried out. In this way, a family of 40 mycothiol analogues (“**MA**”) featuring a 2-amino-2-deoxy-1-thio-α-D-glucopyranoside core was generated, as shown in Charts 1 and 2 (structures **MA-1** through **MA-40**). The details of the preparation of the analogues are given in the experimental section, and their ^{1}H and ^{13}C NMR spectra are listed in Tables 1 and 2. All compounds were stable to storage in the freezer for several months.

The coupling agent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) was used in DMF solution to join **13** (=**MA-1**) with carboxylic acids in 13 cases (**MA-3**, **MA-4**, **MA-5**, **MA-6**, **MA-10**, **MA-11**, **MA-12**, **MA-13**, **MA-14**, **MA-37**, **MA-38**, **MA-39**, and **MA-40**). The acids are mostly commercially available, but for a few analogues the acids were made by literature procedures (**MA-11**, **MA-12**, and **MA-38**) or by additions to (*E*)-3-iodoacrylic acid (**MA-13** and **MA-14**). The EDCI coupling of *unprotected* oxime carboxylic acids with **13** failed, but the coupling was successful with a DCC/HOBT procedure to give **MA-34**, **MA-35**, and **MA-36**. Reductive alkylation and direct alkylation were used for **MA-18** and **MA-19**, respectively. Sulfonylation of **13** with the appropriate sulfonyl chloride led to **MA-21** and **MA-22**. Similarly, acylation of **13** gave **MA-20** and **MA-32**, and acetimidation led to **MA-24**. Phosphorylation gave **MA-25**, and phosphonation produced **MA-26**. Condensation of **13** with various isocyanates and isothiocyanates gave **MA-27**, **MA-28**, **MA-29**,

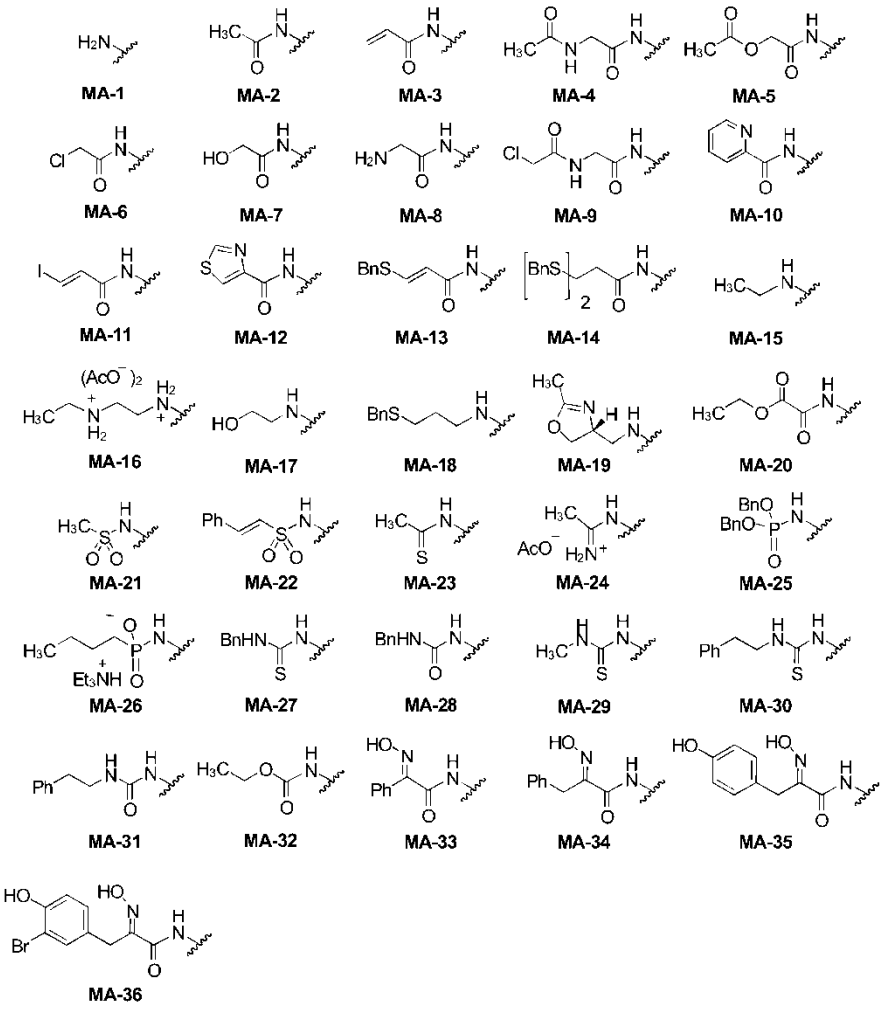


Chart 1: Mycothiol analogues prepared from the aminotriol scaffold.

MA-30, and **MA-31**. Ester hydrolysis of **12** gave the acetamidotriol **MA-2**. The remaining analogues were made from other family members by acylation (**MA-9**), reduction (**MA-15**, **MA-16**, and **MA-17**), hydrolysis (**MA-7**), oximation (**MA-33**), thionation (**MA-23**), and displacement (**MA-8**) reactions, respectively.

CHARACTERIZATION OF THE MSH ANALOGUES

The mycothiol analogues **MA-1** through **MA-40** were characterized by their ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), by their ESI-mass spectra (see experimental section), and by spectral comparisons within the group and with similar compounds. For example, all members showed characteristic

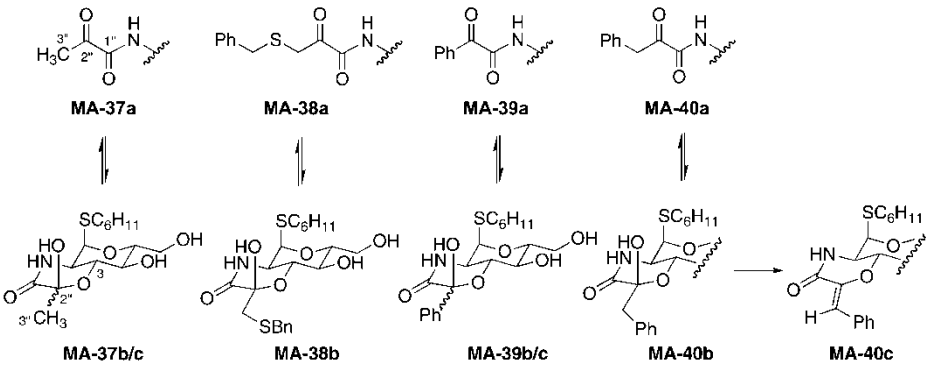


Chart 2: Mycothiol analogues subject to cyclization.

NMR signals for the cyclohexyl 2-amino-2-deoxy-1-thio-α-D-glucopyranoside core, with expected differences attributable to the *N*-substituent.[31] The oximes **MA-33**, **MA-34**, **MA-35**, and **MA-36** were formed as single imino stereoisomers that we assign as *E* based on analogy to similar compounds.[36]

The α-ketoamides **MA-37**, **MA-38**, **MA-39**, and **MA-40** showed a varying tendency to cyclize with O-3' to give hemiacetals (Chart 2).[37] The benzoylformamide **MA-39** is mostly ($\sim$80%) in the keto form **MA-39a**, as indicated by the C=O resonance at 190.7 ppm. Its hemiacetal exists as an approximately equal mixture of presumed anomers **MA-39b** and **MA-39c**, $\sim$10% each, according to integration of the respective anomeric protons. No evidence for $-OCH_3$ or $-OCD_3$ mixed acetals[37] was seen by ESI-MS. The pyruvamide **MA-37** exists as a mixture of keto and two presumed hemiacetal forms, **MA-37a**, **MA-37b**, and **MA-37c**, respectively, in a ratio of approximately 40:30:30, based on integration of the respective anomeric protons at 5.49, 5.54, and 5.58 ppm. In contrast, the 3-(*S*-benzylthio)-2-oxopropionamide **MA-38** exists entirely in the cyclized form **MA-38b** (no keto carbon). The stereochemistry at the acetal carbon C-2" is tentatively assigned as (*S*), with axial hydroxyl, based on the anomeric effect. The 3-phenyl-2-oxopropionamide **MA-40** also cyclizes completely to the hemiacetal **MA-40b** (no keto carbon, anomeric proton at 5.82 ppm, acetal carbon at 96.9 ppm). The stereochemistry is again assumed to be mostly (*R*) at C-2" (with axial hydroxyl), although a trace amount of another isomer with anomeric proton at 5.84 ppm is present. Unlike **MA-38**, however, **MA-40** is accompanied by a single product of dehydration, alkene **MA-40c**, to varying extent, commonly 50% or more. The alkene proton at 6.60 ppm[38] and the alkene carbon at 113.9 ppm are diagnostic. Stereochemistry of **MA-40c** is assigned as (*Z*) based on the expected lower steric interactions between the phenyl ring and the amide carbonyl oxygen. Dehydration of **MA-40b** might be favored relative to **MA-38b** because the C=C forms in conjugation with phenyl in the former case. In contrast to the ketoamides,

Table 1: ^{1}H NMR data for mycothiol analogues.*a*

Cpd	H-1′	H-2′	H-5′	H-6′a	H-6′b	H-3′	H-4′
MA-2	5.53, d; 5.2	4.01, dd; 5.6, 11.2	3.97, ddd; 2.0, 5.2, 9.6	3.80, dd; 2.4, 12.0	3.72, dd; 5.2, 11.6	3.54, dd; 8.8, 10.8	3.35, dd; 8.8, 9.6
MA-3	5.57, d; 5.3	4.10, dd; 5.2, 11.0	3.99, ddd; 2.5, 5.0, 9.7	3.82, dd; 2.5, 12.0	3.73, dd; 5.1, 12.1	3.60, dd; 8.6, 11.0	3.38, app t; 9.4
MA-4	5.49, d; 5.4	4.05, dd; 5.1, 10.5	3.98, ddd; 2.7, 5.4, 9.9	3.80, dd; 2.4, 12.3	3.73, dd; 5.4, 12.3	3.53, dd; 8.7, 10.8	3.36, app t; 9.6
MA-5	5.48, d; 5.1	4.07, dd; 5.4, 11.1 7.92, d; 7.2 (NH)	3.97, ddd; 2.1, 5.1, 9.6	3.80, dd; 2.7, 12.0	3.72, dd; 5.1, 12.0	3.55, dd; 9.0, 11.1	3.36, dd; 9.0, 9.6
MA-6	5.52, d; 5.2	4.04, dd; 5.2, 10.8	3.97, ddd; 2.4, 5.2, 9.6	3.80, dd; 2.4, 12.0	3.73, dd; 5.2, 12.0	3.55, dd; 8.8, 10.8	3.37, dd; 8.8, 9.6
MA-7	5.50, d; 5.4	4.08, dd; 5.1, 10.5	4.00–3.95, m	3.81, dd; 2.4, 12.0	3.73, dd; 5.4, 12.3	3.53, dd; 8.7, 10.8	3.38, app t; 9.9
MA-8	5.51, d; 5.4	4.07, dd; 5.4, 11.1	3.99, ddd; 2.4, 4.8, 9.9	3.81, dd; 2.7, 12.0	3.73, dd; 4.8, 12.0	3.55, dd; 8.7, 11.1	3.40–3.34, m
MA-9	5.49, d; 5.4	4.06, dd; 5.7, 11.1	3.98, ddd; 2.4, 5.1, 9.9	3.80, dd; 2.7, 12.3	3.72, dd; 5.4, 12.3	3.54, dd; 8.7, 11.1	3.36, app t; 9.9
MA-10	5.59, d; 5.4	4.27, dd; 5.4, 10.5	4.03, ddd; 2.7, 5.1, 9.6	3.84, dd; 2.7, 12.0	3.77, dd; 4.8, 11.7	3.65, dd; 8.7, 10.8	3.45, app t; 9.6
MA-11	5.54, d; 5.2	4.05, dd; 5.2, 11.2	3.98, ddd; 2.4, 5.6, 10.0	3.80, dd; 2.8, 12.4	3.72, dd; 5.2, 12.0	3.57, dd; 8.4, 10.8	3.36, dd; 8.8, 9.6
MA-12	5.59, d; 5.2	4.25, dd; 4.8, 10.8	4.01, ddd; 2.8, 5.2, 10.0	3.83, dd; 2.4, 12.0	3.76, dd; 5.2, 12.0	3.62, dd; 8.8, 10.8	3.43, app t; 9.6
MA-13	5.70, d; 5.5	4.07, dd; 5.4, 11.1	3.96, ddd; 3.0, 5.7, 9.9	3.79, dd; 2.4, 12.0	3.74, dd; 4.5, 12.0	3.54, dd; 8.4, 10.8	3.40, app t; 9.6
MA-14	5.53, d; 5.7	4.01, dd; 5.4, 11.1	3.97, ddd; 2.1, 5.4, 9.3	3.80, dd; 2.7, 12.0	3.72, dd; 5.4, 12.0	3.54, dd; 8.4, 10.5	3.38, dd; 9.0, 9.6
MA-15	5.54, d; 5.4	2.87, dd; 5.1, 10.2	3.99, ddd; 2.4, 4.8, 9.3	3.82, dd; 2.4, 11.7	3.74, dd; 5.4, 12.0	3.45, app t; 8.7	under MeOH

(*continued*)

Table 1: Continued

Cpd	H-1′	H-2′	H-5′	H-6′a	H-6′b	H-3′	H-4′
MA-16	5.54, d; 4.8	3.10–2.99, m	4.00–3.91, m	3.79, dd; 2.7, 12.3	3.73, dd; 5.4, 12.3	3.42–3.30, m	3.42–3.30, m
MA-17	5.53, d; 5.1	2.86, dd; 5.1, 10.2	3.97, ddd; 2.4, 5.4, 9.3	3.80, dd; 2.4, 11.7	3.72, dd; 4.8, 11.7	3.75–3.57, m	3.41, dd; 9.0, 9.9
MA-18	5.52, d; 4.8	2.95, dd; 5.2, 10.4	3.96, ddd; 2.8, 5.2, 9.6	3.79, dd; 2.4, 11.6	3.73, dd; 5.2, 11.6	3.46, dd; 8.8, 10.0	3.33, app t; 9.6
MA-19	5.52, d; 5.1	3.01–2.91, m	4.04–3.96, m	3.93–3.86, m	3.86–3.73, m	3.86–3.73, m	3.45, dd; 8.1, 9.9
MA-20	5.54, d; 5.6	4.06, dd; 5.2, 10.8	3.98, ddd; 2.4, 5.2, 10.0	3.81, dd; 2.4, 12.0	3.74, dd; 5.2, 12.0	3.62, dd; 8.8, 10.8	3.39, dd; 8.8, 9.6
MA-21	5.36, d; 5.1	3.51, dd; 5.4, 10.5	3.96, ddd; 2.7, 4.8, 9.9	3.79, dd; 2.4, 11.7	3.72, dd; 5.1, 12.3	3.44, dd; 8.1, 10.5	3.37, dd; 8.1, 9.6
MA-22	5.42, d; 4.5	3.54–3.42, m	3.96, ddd; 2.4, 4.8, 9.6	3.77, dd; 2.4, 11.7	3.70, dd; 5.1, 12.0	3.54–3.42, m	under MeOH
MA-23	5.94, d; 5.4	4.59, dd; 5.4, 11.1	3.99, ddd; 2.4, 4.8, 9.9	3.81, dd; 3.0, 12.3	3.83–3.71, m	3.83–3.71, m	3.39, dd; 8.7, 9.9
MA-24	5.53, d; 5.4	3.84, dd; 5.1, 10.5	3.99, br dt; 3.6, 9.6	3.79, d; 3.6	3.79, d; 3.6	3.68, dd; 8.4, 10.2	3.40, dd; 8.7, 9.6
MA-25	5.34, d; 4.5	3.45–3.30, m	3.96, ddd; 2.4, 4.8, 9.3	3.78, dd; 2.7, 12.0	3.71, dd; 5.1, 12.0	3.45–3.30, m	3.45–3.30, m
MA-26	5.44, d; 5.2	3.77–3.70, m	4.00, ddd; 2.4, 4.8, 10.0	3.81, dd; 2.4, 12.4	3.77–3.67, m	3.42–3.35, m	3.38, app t; 9.6
MA-27	5.79, d; 5.4	4.57, dd; 4.8, 10.5	3.95, ddd; 2.4, 4.8, 9.6	3.81, dd; 2.4, 12.0	3.73, dd; 5.4, 12.0	3.51, dd; 8.7, 10.8	3.39, app t; 9.9
MA-28	5.51, d; 4.8	3.92, dd; 5.2, 10.4	3.97, ddd; 2.4, 5.2, 9.6	3.80, dd; 2.4, 11.7	3.72, dd; 5.1, 11.7	3.43, app t; 8.8, 10.4	3.37, app t; 8.8, 9.6
MA-29	5.73, d; 4.8	4.57–4.48, br m	3.96, ddd; 2.4, 4.8, 9.6	3.80, dd; 2.4, 11.6	3.73, dd; 5.2, 12.0	3.59–3.49, br m	3.39, app t; 10.0
MA-30	5.76, d; 5.1	4.59–4.50, m	3.95, ddd; 2.4, 4.8, 9.6	3.81, dd; 2.7, 12.0	3.73, dd; 5.4, 12.3	3.51, br t, 9.0, 10.5	3.38, app t; 9.9
MA-31	5.49, d; 5.1	3.89, dd; 5.1, 10.2	3.95, ddd, 2.7, 5.1, 9.3	3.80, dd; 2.4, 12.0	3.72, dd; 5.1, 12.0	3.45–3.27, m	3.45–3.27, m

MA-32	5.48, d; 5.4	3.79–3.72, m	3.95, ddd; 2.4, 5.1, 9.9	3.79, dd; 2.7, 12.0	3.71, dd; 5.1, 12.0	3.46, app t; 8.7	3.34, app t; 9.6
MA-33	5.70, d; 5.4	4.26, dd; 5.7, 11.4	4.03, ddd; 2.7, 5.1, 9.9	3.83, dd; 2.4, 12.0	3.75, dd; 5.1, 12.0	3.59, dd; 8.7, 10.8	3.42, dd; 9.0, 9.9
	5.58, d, 5.4	4.14, dd; 5.1, 10.8					
MA-34	5.47, d; 5.6	4.07, dd; 5.2, 10.8 7.47, d; 8.4 (NH)	3.94, ddd; 2.4, 5.2, 9.6	3.79, dd; 2.4, 12.0	3.72, dd; 5.2, 12.0	3.49, dd; 8.8, 10.4	3.37, app t; 9.6
MA-35	5.46, d; 5.2	4.06, ddd; 5.6, 8.0, 10.8 7.45, d; 8.0 (NH)	3.93, ddd; 2.4, 5.2, 9.6	3.81–3.69, m	3.81–3.69, m	3.48, dd; 8.8, 10.8	3.36, app t; 9.6
MA-36	5.47, d; 5.1	4.06, dd; 5.1, 10.8 7.47, d; 8.4 (NH)	3.93, ddd; 2.4, 4.8, 9.6	3.79, dd; 2.4, 12.0	3.72, dd; 5.1, 12.0	3.49, dd; 9.0, 10.5	3.36, app t; 9.6
MA-37a	5.49, d; 5.1	4.05–3.95, m	4.05–3.95, m	3.81, dd; 2.4, 12.3	3.74, dd; 4.8, 12.3	3.58, app t; 9.0	3.39, app t; 9.9
MA-37b	5.54, d; 5.4				3.73, dd; 5.1, 12.0	3.52, dd; 8.4, 11.1	3.38, app t; 9.6
MA-37c	5.58, d; 5.1						
MA-38b	5.86, d; 5.6	3.88–3.76, m	4.10–4.02, m	3.68, dd; 2.4, 14.0	3.88–3.76, m	4.41, dd; 9.2, 11.6	4.10–4.02, m
MA-39	5.69, d; 5.1	4.19, dd; 5.4, 11.1	4.02, ddd; 2.4, 4.8, 9.6	3.83, dd; 2.7, 12.3	3.76, dd; 5.1, 12.3	3.63, dd; 8.7, 11.7	3.42, dd; 9.3, 9.9
MA-39a	5.45, d; 5.1						
MA-39b	5.37, d; 5.1						
MA-40b	5.82, d; 5.6	3.49–3.42, m	4.08–3.97, m	4.08–3.97, m	3.90–3.77, m	4.28, dd; 9.2, 11.6	3.90–3.77, m
MA-40c	5.70, d; 5.4	3.58, dd; 5.1, 9.9	4.05, dt; 3.9, 9.9	3.88–3.78, m	3.88–3.78, m	4.42, app t; 9.0	3.72, app t; 9.3

(*continued*)

Table 1: Continued

Cpd	H-1	H-2,3,4,5,6	H-1″	H-2″	H-3″	H-4″	Ar-H's
MA-2	2.86–2.78, m	2.01–1.26, m		1.97, s			
MA-3	2.90–2.76, m	2.04–1.20, m		6.35, dd; 9.0, 17.0	trans: 6.23, dd; 2.8, 17.0 cis: 5.68, dd; 2.8, 9.0		
MA-4	2.89–2.78, m	2.04–1.29, m		3.92, 3.85, ABq; 17.1		2.01, s	
MA-5	2.84–2.79, m	2.05–1.29, m		4.61, 4.55, ABq; 15.0		2.14, s	
MA-6	2.88–2.80, m	2.20–1.25, m		4.11, 4.10, ABq; 13.6			
MA-7	2.88–2.79, m	2.01–1.25, m		4.00, s			
MA-8	2.84–2.79, m	2.15–1.29, m		3.40–3.34, m			
MA-9	2.84–2.78, m	2.01–1.26, m		3.98, 3.92, ABq, 17.7		4.13, s	
MA-10	2.88–2.80, m	2.00–1.22, m					8.65, br d; 4.8 8.11, d; 7.8 7.97, td; 1.5, 7.5 7.57, ddd; 1.5, 5.1, 7.8
MA-11	2.83–2.79, m	1.99–1.24, m		7.08, d; 14.8	7.72, d; 14.8		
MA-12	2.84–2.81, m	2.10–1.25, m					9.03, d; 2.0
MA-13	2.81–2.72, m	2.00–1.25, m		7.54, d; 15.0	6.01, d; 15.0	4.05, s	7.38–7.22, m
MA-14	2.86–2.70, m	1.82–1.29, m		2.97, d; 6.9	4.15, t; 6.9	3.83, s	7.32–7.21, m
MA-15	2.90–2.76, m	2.14–1.30, m	2.90–2.76, m 2.61, dq; 7.2, 11.1	1.15, t; 6.9			
MA-16	2.86–2.82, m	2.13–1.29, m	2.86–2.82, m 2.81–2.76, m	3.10–2.99, m	3.10–2.99, m	1.32, t; 7.2	1.92, s ($^{-}$OCOC$\underline{H}_3$)

MA-17	2.92–2.83, m	2.09–1.23, m	2.92–2.83, m 2.70, ddd; 4.5, 7.2, 11.7	3.75–3.57, m			
MA-18	2.93–2.87, m	2.05–1.30, m	2.74, ddd; 6.4, 8.0, 14.4 2.93–2.87, m	1.83–1.75, m	2.49, t; 7.2	3.73, s	7.34–7.20, m
MA-19	3.01–2.91, m	2.05–1.26, m	3.67, dd; 3.9, 11.4 3.56, dd; 5.4, 11.4	4.31–4.21, m	4.04–3.96, m		2.37, s (-OCNC$\underline{H}_3$)
MA-20	2.88–2.80, m	2.20–1.26, m			4.34, q; 6.8	1.36, t; 7.2	
MA-21	2.91–2.83, m	2.10–1.27, m	3.03, s				
MA-22	2.80–2.72, m	2.01–1.20, m	7.10, d; 15.3	7.45, d; 15.9			7.60–7.57, m 7.43–7.41, m
MA-23	2.86–2.77, m	1.99–1.25, m		2.48, s			
MA-24	2.94–2.84, m	2.04–1.29, m		2.26, s			1.91, s ($^{-}$OCOC$\underline{H}_3$)
MA-25	2.90–2.84, m	1.97–1.15, m	5.18–5.03, m				7.43–7.31, m
MA-26	2.94–2.85, m	2.08–1.29, m	3.30–3.10, m	1.77–1.56, m	1.77–1.56, m	0.93, t; 7.2	3.19, q; 7.2 (NC$\underline{H}_2$CH$_3$)$_3$ 1.31, t; 6.8 (NCH$_2$C$\underline{H}_3$)$_3$
MA-27	2.86–2.79, m	2.15–1.22, m		4.66, d; 15.0 d; under HOD			7.34–7.24, m
MA-28	2.86–2.79, m	2.01–1.25, m		4.38, 4.27, ABq; 15.0			7.30–7.20, m
MA-29	2.86–2.80, m	2.01–1.27, m		2.97, s			
MA-30	2.93–2.79, m	2.03–1.28, m		3.83–3.63, m	2.91, dd; 2.1, 6.9 2.83, dd; 6.9, 10.5		7.32–7.17, m
MA-31	2.86–2.79, m	2.20–1.22, m		3.45-3.27, m	2.77, t; 7.2		7.30–7.16, m
MA-32	2.88–2.78, m	2.04–1.22, m		4.09, q; 6.9	1.24, t; 6.9		
MA-33	2.96–2.86, m	2.09–1.29, m					7.76–7.72, m 7.51–7.46, m

(*continued*)

Table 1: Continued

Cpd	H-1	H-2,3,4,5,6	H-1″	H-2″	H-3″	H-4″	Ar-H's
							7.42–7.32, m
MA-34	2.80–2.72, m	1.96–1.21, m			3.96, 3.88, ABq; 13.2		7.27, d; 7.6 7.21, t; 7.2
							7.14, t; 6.8
MA-35	2.78–2.65, m	1.96–1.20, m			3.87, 3.76, ABq; 13.2		7.09, d; 8.4 6.64, d; 8.4
MA-36	2.79–2.50, m	1.97–1.20, m			3.86, 3.74, ABq; 13.2		7.37, d; 2.1 7.09, dd; 2.1, 8.4 6.76, d; 8.1
MA-37a,b,c	2.84–2.80, m	2.01–1.28, m			2.40, s 1.47, s		
MA-38b	3.05–2.96, m	2.07–1.26, m			3.88–3.76, m	3.88–3.76, m 3.10, d; 13.6	7.46, d; 7.2 7.32–7.24, m
MA-39a,b,c	2.94–2.86, m 2.78–2.64, m	2.05–1.26, m					8.16–8.13, m 7.71–7.64, m 7.53, t; 7.8 7.34–7.31, m
MA-40b	2.96–2.87, m	2.14–1.09, m			3.90–3.77, m		7.33–7.27, m
MA-40c	2.96–2.87, m	2.14–1.26, m			6.60, s		7.57, d; 7.2 7.36, t; 7.5 7.22, t; 7.2

[a]Spectra were taken at 400 or 300 MHz (^{1}H) and 100 or 75 MHz (^{13}C) in CD_3OD solutions. Chemical shifts, reported in ppm downfield from TMS, are followed by multiplicities and then coupling constants, in hertz. Assignments are tentative and are based on related compounds and on first-order coupling constants.

Table 2: ^{13}C NMR for mycothiol analogues.[a]

Cpd	C-1′	C-3′	C-4′	C-5′	C-6′	C-2′	C-1	C-2	C-3	C-4	C-1″	C-2″	C-3″	C-4″	Ar-C's
MA-2	84.01	74.36	72.79	72.62	62.60	55.92	44.71	35.30, 34.96	27.09, 26.90	26.83	173.58	22.55			
MA-3	87.09	77.45	75.77	75.64	65.61	59.03	47.86	38.31, 37.93	30.06, 29.89	29.81	171.29	134.94	130.01		
MA-4	84.63	74.75	73.34	72.77	62.84	55.98	45.35	35.66, 35.22	27.33, 27.15	27.15	172.02	43.77	172.02	22.69	
MA-5	84.32	74.57	72.89	72.45	62.54	55.56	45.16	35.38, 34.94	27.02, 26.86	26.86	170.25	63.29	179.47	20.48	
MA-6	84.10	74.59	72.96	72.44	62.53	56.09	45.15	35.35, 34.96	27.03, 26.85	26.85	169.32	43.15			
MA-7	84.53	74.71	73.42	72.27	62.54	55.11	45.27	35.39, 34.94	27.04, 26.86	26.86	175.23	62.47			
MA-8	84.06	74.49	72.96	72.74	62.61	55.11	44.97	35.53, 35.09	27.24, 27.11	27.04	176.47	44.62			
MA-9	84.28	74.47	73.01	72.54	62.58	55.78	45.05	35.39, 34.95	27.08, 26.90	26.90	171.12	43.08	174.39	43.55	
MA-10	84.73	74.88	73.63	72.25	62.59	55.78	45.40	35.35, 34.92	26.99, 26.81	26.81	166.53				150.54, 149.80, 138.88, 127.98, 123.13
MA-11	83.97	74.46	72.52	72.52	62.52	55.95	44.98	35.30, 34.93	27.07, 26.93	26.93	166.82	140.01	94.70		
MA-12	84.83	74.99	73.61	72.40	62.72	55.90	45.53	35.50, 35.07	27.16, 26.97	26.97	166.97				183.05, 155.68, 125.36
MA-13	84.07	73.82	72.52	72.02	62.22	55.42	44.74	34.98, 34.54	26.74, 26.52	26.52	166.90	142.97	117.53	37.14	137.20, 129.61, 129.43, 128.26
MA-14	83.99	74.35	72.77	72.60	62.59	55.91	45.01	35.30, 34.96	27.08, 26.90	26.90	173.57	27.63	57.77	36.20	139.58, 130.18, 129.59, 128.13
MA-15	84.37	75.06	74.31	72.61	63.56	62.88	44.14	35.66, 35.17	27.39, 27.23	27.17	42.58	15.44			
MA-16	85.41	75.28	74.45	72.57	63.38	62.79	44.78	35.81, 35.34	27.39, 27.27	27.18	under MeOH	43.87	43.65	11.89	179.67 ($^{-}$O<u>C</u>OCH$_3$) 23.98 ($^{-}$OCO<u>C</u>H$_3$)

(*continued*)

Table 2: Continued

Cpd	C-1′	C-3′	C-4′	C-5′	C-6′	C-2′	C-1	C-2	C-3	C-4	C-1″	C-2″	C-3″	C-4″	Ar-C's and others
MA-17	84.57	75.13	74.40	72.57	63.59	62.14	44.41	35.70, 35.19	27.39, 27.28	27.20	under MeOH	62.83			
MA-18	83.54	74.23	74.09	72.19	62.72	62.42	44.62	35.47, 34.85	27.04, 26.89	26.89	46.61	29.16	29.61	36.78	139.96, 129.99, 129.45, 127.94
MA-19	83.89	74.56	72.77	62.21	61.03	58.59	46.10	35.57, 35.00	27.11, 27.06	26.90	50.70	70.60	63.65	170.14	12.50 ($-OCN\underline{C}H_3$)
MA-20	83.88	74.67	72.83	72.18	62.47	56.28	45.28	35.34, 34.91	26.99, 26.83	26.83	159.20	161.26	64.05	14.25	
MA-21	87.06	74.63	73.69	72.83	62.65	59.57	45.34	35.56, 34.97	27.22, 27.06	27.06	41.89				
MA-22	84.21	72.36	71.06	70.49	60.47	57.32	43.12	33.45, 32.78	25.08, 24.86	24.84	132.64	126.89			138.41, 129.53, 128.08, 127.36
MA-23	81.78	74.71	72.74	72.74	62.68	61.58	45.12	35.39, 35.25	27.21, 27.04	26.94	203.04	33.28			
MA-24	81.83	74.73	72.79	72.79	62.72	61.62	45.17	35.42, 35.27	27.24, 27.06	26.96	161.49	33.32			179.67 ($^{-}O\underline{C}OCH_3$) 23.98 ($^{-}OCO\underline{C}H_3$)
MA-25	87.07	74.31	74.31	72.32	62.50	57.81	45.09	35.38, 34.75	26.97, 26.85	26.76	69.49, d; 4.5 69.26, d; 4.5				140.53, 129.51, 129.31, 128.90, 128.81
MA-26	85.72	74.58	72.34	72.18	62.31	53.57	45.37	35.44, 34.88	27.04, 26.91	26.83	27.63, d; 114.6	25.42, app t; 10.7	21.48	14.25	47.33 ($(N\underline{C}H_2CH_3))_2$ 9.68 ($(NCH_2\underline{C}H_3))_2$
MA-27	84.78	74.81	73.95	72.68	62.72	59.86	45.44	35.53, 35.26	27.21, 27.03	27.03	167.44	under MeOH			140.18, 129.62, 128.74, 128.38
MA-28	85.59	74.50	73.84	72.56	62.62	56.17	45.03	35.41, 35.02	27.07, 26.89	26.89	160.62	44.59			141.21, 129.42, 128.16, 127.95

MA-29	84.66	74.63	73.60	72.53	62.59	59.71	45.25	35.40, 35.05	27.07, 26.89	26.89					
MA-30	84.67	74.64	73.80	72.61	62.62	59.57	45.28	35.40, 35.09	27.06, 26.89	26.89	167.32	46.65	36.26		140.54, 129.88, 129.50, 127.32
MA-31	85.66	74.50	73.90	72.65	62.68	56.11	45.03	35.43, 35.00	27.07, 26.91	26.91	160.66	42.59	37.46		140.79, 129.80, 129.46, 127.24
MA-32	84.77	74.35	73.04	72.35	62.56	57.15	44.82	36.36, 34.96	27.11, 26.94	26.94	156.32	61.95	15.05		
MA-33	84.39	74.54	72.68	72.62	62.62	56.05	45.23	35.56, 34.85	26.96, 26.96	26.87	167.19	154.09			133.49, 130.63, 129.44, 127.64
MA-34	84.63	74.83	73.40	72.20	62.57	55.70	45.38	35.32, 34.93	26.99, 26.82	26.82	165.67	152.76	29.79		138.12, 130.11, 129.27, 127.20
MA-35	84.60	74.82	73.37	72.18	62.56	55.69	45.39	35.31, 34.92	26.99, 26.82	26.77	165.79	153.28	28.87		156.79, 131.15, 128.77, 116.01
MA-36	84.40	74.73	73.24	72.07	62.49	55.67	45.31	35.27, 34.91	27.05, 26.84	26.84	165.27	152.48	28.55		153.54, 134.32, 130.35, 130.30, 116.79, 110.32
MA-37a,b,c	84.31, 84.22	74.71, 74.63	73.18	72.32, 72.15	62.55	55.83, 55.67, 55.60	45.34, 45.19, 45.07	35.40, 35.05, 34.91, 34.90	27.01, 26.84	26.84	171.49	186.08	26.04, 25.74, 24.52		
MA-38b	84.40	81.41	74.55	68.67	61.90	60.88	45.17	35.40, 35.29	26.87, 26.87	26.70	159.64	98.80	34.65	32.19	139.22, 130.65, 129.35, 128.12
MA-39a,b,c	83.96, 84.39, 84.26	74.57, 74.73, 74.66	72.87, 73.14, 73.13	72.49, 72.33	62.55	55.85, 55.64	45.15, 45.33	35.41, 34.98, 34.75	27.00, 26.88, 26.91	26.83	167.01	190.65			135.59, 134.54, 131.44, 129.73, 129.52, 129.51, 128.96, 128.89, 127.7, 127.61
MA-40b,c	84.38, 82.79	81.64, 81.06	74.51, 73.98	68.58, 68.52	61.96, 61.87	60.75, 54.55	45.00, 43.78	35.39, 35.19, 34.88, 34.75	27.16, 27.05, 26.94, 26.89	26.74, 26.05	165.31, 163.09	96.86, 156.98	41.15, 113.87		136.72, 136.65, 130.52 129.78, 129.68, 129.58 128.30, 127.98

[a]Assignments of the carbons are tentative and based on analogous compounds. Data for carbons C-2′, C-3′, C-4′, C-5′, and C-6′ are presented in arbitrary order; the assignments may be interchanged.

the hydroximinoamides **MA-33**, **MA-34**, **MA-35**, and **MA-36** do not cyclize onto O-3'.

FUTURE BIOLOGICAL EVALUATION

Although only 40 analogues are described here, it is clear from the straightforward nature of the various coupling reactions that the same procedures (alkylation, amide coupling, condensation, etc.) could be used to generate many new members if enzymatic or biological evaluation indicates the need. Such studies are presently underway and will be reported as results warrant. At this date 27 of the mycothiol analogues have been screened for inhibition against *M. tuberculosis* by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility of the Southern Research Institute, and four of them show weak activity as evaluated in an Alamar blue assay (Table 3).

EXPERIMENTAL SECTION

General

Melting points (mp) were determined with a Electrothermal mp apparatus and are uncorrected. TLC was performed on Merck glass silica gel 60 plates; column chromatography employed 230–400 mesh flash silica. NMR spectra were recorded with a Varian 300- or 400-MHz spectrometer on CD_3OD solutions. Mass spectra were obtained with a Finnigan LCQ_{DUO} LC/MS/MS spectrometer.

Procedure A—Typical Amide Formation

A solution of aminotriol[26] **13** (= **MA-1**, 0.10 mmol, azeotropically dried with 3 × 2 mL of toluene) and the appropriate carboxylic acid (0.20 mmol) in 1 mL of dimethylformamide was cooled to 0°C and then treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 0.21 mmol). The

Table 3: Inhibitory activity of mycothiol analogues against *M. tuberculosis*.[a]

Compound	Inhibition (%)
MA-6	51
MA-18	27
MA-23	4
MA-28	12

[a]Compounds **MA-3**–**MA-13**, **MA-15**–**MA-25**, **MA-37**, and **MA-39** were evaluated against *M. tuberculosis strain* $H_{37}RV$ at a concentration of 6.25 μg/mL using an Alamar blue assay.

reaction mixture was allowed to warm to 23°C over a 3-hr period, quenched with 3 drops of water, concentrated, and then purified by column chromatography.

Procedure B—Coupling Unprotected Hydroxyimino Acids

A solution of aminotriol **13** (= **MA-1**, 0.10 mmol, azeotropically dried with 3 × 2 mL of toluene), *N*-hydroxybenzotriazole (0.10 mmol), and hydroxyimino acid (0.10 mmol) in 1 mL of dimethylformamide was cooled to 0°C and then treated with dicyclohexylcarbodiimide (DCC, 0.15 mmol). The mixture was allowed to warm to 23°C, and then was stirred for an additional 16 hr. The white precipitate (dicyclohexylurea) was removed by filtration, the supernatant was concentrated, and then the crude product was purified by column chromatography.

Cyclohexyl 2-Acetamido-2-deoxy-1-thio-α-D-glucopyranoside (MA-2)

De-*O*-acetylation of **12** (783 mg, 1.75 mmol) with methanolic sodium methoxide as for **MA-7** gave the acetamidotriol **MA-2**: 515 mg, 92%, colorless oil; R_f 0.40 (9 : 1 dichloromethane/methanol); ESI-MS m/z 342 MNa^+.

Cyclohexyl 2-Acrylamido-2-deoxy-1-thio-α-D-glucopyranoside (MA-3)

Commercial acrylic acid was coupled with **MA-1** by procedure A: 23 mg, 72%, colorless oil; R_f 0.54 (3 : 3 : 14 methanol/ethyl acetate/dichloromethane); ESI-MS m/z 354 MNa^+.

Cyclohexyl 2-(2-N-Acetylglycinamido)-2-deoxy-1-thio-α-D-glucopyranoside (MA-4)

Commercial *N*-acetylglycine was coupled with **MA-1** by procedure A: 65 mg, 86%, colorless oil; R_f 0.30 (3 : 3 : 14 methanol/ethyl acetate/dichloromethane); ESI-MS m/z 399 MNa^+.

Cyclohexyl 2-(2-Acetoxyacetamido)-2-deoxy-1-thio-α-D-glucopyranoside (MA-5)

Commercial acetoxyacetic acid was coupled with **MA-1** by procedure A: 33 mg, 81%, colorless oil; R_f 0.23 (1 : 9 methanol/dichloromethane); ESI-MS m/z 400 MNa^+.

Cyclohexyl 2-(2-Chloroacetamido)-2-deoxy-1-thio-α-D-glucopyranoside (MA-6)

Commercial chloroacetic acid was coupled with **MA-1** by procedure A: 21 mg, 72%, white solid, mp 183–185°C; R_f 0.33 (1 : 9 methanol/dichloromethane); ESI-MS m/z 376 MNa^+.

Cyclohexyl 2-Deoxy-2-(2-hydroxyacetamido)-1-thio-α-D-glucopyranoside (MA-7)

A solution of the acetoxyglycinamide **MA-5** (27 mg, 0.072 mmol) in 0.5 mL of methanol was treated at 0°C with methanolic sodium methoxide (2 drops, 25% w/w solution in MeOH), stirred at 23°C for 30 min, and then concentrated. Purification by column chromatography with 1 : 9 methanol/dichloromethane as the eluant afforded 22 mg (92%) of **MA-7** as a colorless oil: R_f 0.85 (3 : 7 methanol/dichloromethane); ESI-MS m/z 358 MNa^+.

Cyclohexyl 2-Deoxy-2-glycinamido-1-thio-α-D-glucopyranoside (MA-8)

Aqueous ammonia (approximately 0.75 mL) was added dropwise over a period of 6 hr to a solution of the chloroacetamide **MA-6** (58 mg, 0.150 mmol) in 1.5 mL of methanol, by which time the reaction was complete according to TLC analysis. The product was purified by column chromatography with 1 : 4 methanol/dichloromethane as the eluant to afford 52 mg (95%) of **MA-8** as a colorless oil: R_f 0.15 (3 : 17 methanol/dichloromethane); ESI-MS m/z 335 MH^+.

Cyclohexyl 2-[2-N-(2-Chloroacetamido)glycinamido]-2-deoxy-1-thio-α-D-glucopyranoside (MA-9)

MA-8 was coupled with chloroacetic acid by procedure A: 20 mg, 72%, colorless oil; R_f 0.15 (1 : 4 methanol/dichloromethane); ESI-MS m/z 433 MNa^+.

Cyclohexyl 2-Deoxy-2-(2-picolinamido)-1-thio-α-D-glucopyranoside (MA-10)

Commercial 2-picolinic acid was coupled with **MA-1** by procedure A: 28 mg, 82%, white solid, mp 179–181 °C; R_f 0.60 (1 : 19 methanol/dichloromethane); ESI-MS m/z 405 MNa^+.

Cyclohexyl 2-Deoxy-2-(3-E-iodoacrylamido)-1-thio-α-D-glucopyranoside (MA-11)

(*E*)-3-Iodoacrylic acid was prepared by the literature method[39] and then was coupled with **MA-1** by procedure A: 29 mg, 76%, white solid, mp 212–214°C; R_f 0.40 (3 : 17 methanol/dichloromethane); ESI-MS m/z 480 MNa^+.

Cyclohexyl 2-Deoxy-2-(4-thiazolamido)-1-thio-α-D-glucopyranoside (MA-12)

4-Thiazolecarboxylic acid was prepared by the literature method[40] and then was coupled with **MA-1** by procedure A: 25 mg, 83%, colorless oil; R_f 0.60 (3 : 17 methanol/dichloromethane); ESI-MS m/z 411 MNa^+.

3-(S-Benzylthio)acrylic acid and 3,3-Bis(S-benzylthio)propionic acid

A solution of 3-Iodoacrylic acid (500 mg, 2.53 mmol), and *N, N*-diisopropylethylamine (Hünig's base, 1.32 mL, 7.59 mmol) in 1.0 mL of THF was treated with benzyl mercaptan (0.33 mL, 3.04 mmol). A white solid precipitated

after 1.5 hr. The reaction mixture was stirred for an additional 1.5 hr and then diluted with 100 mL of ethyl ether. The resulting solution was washed with saturated aqueous sodium bicarbonate (3×50 mL), and the combined organic layer was dried over sodium sulfate, filtered, and then concentrated to a residue containing both acid products (1 : 1 mixture respectively by ^{1}H NMR analysis). The products were purified by chromatography with 1 : 9 ethyl acetate/dichloromethane as the eluant to provide 245 mg (80%) of 3-(*S*-benzylthio)acrylic acid and 322 mg (80%) of 3,3-bis(*S*-benzylthio)propionic acid as colorless oils: R_f 0.38 and 0.45, respectively (3 : 7 ethyl acetate/dichloromethane); ^{1}H NMR for 3-(*S*-benzylthio)acrylic acid (300 MHz, CD_3OD) 7.73 (d, $J = 15.0$, 1H), 7.41–7.25 (m, 5 H), 5.81 (d, $J = 15.0$, 1H), 4.11 (s, 2 H); ^{1}H NMR for 3,3-bis(*S*-benzylthio)propionic acid (300 MHz, CD_3OD) 3.98 (t, $J = 7.5$, 1H), 3.84 (d, $J = 13.2$, 2H), 3.78 (d, $J = 13.2$, 2H), 2.72 (d, $J = 7.2$, 2 H).

Cyclohexyl 2-[(3-E-(S-benzylthio)acrylamido]-2-deoxy-1-thio-α-D-glucopyranoside (MA-13)

3-(*S*-Benzylthio)acrylic acid (synthesized by the procedure above) was coupled with **MA-1** by procedure A: 16.4 mg, 64%, colorless oil; R_f 0.30 (1 : 4 methanol/dichloromethane); ESI-MS m/z 454 MH^+.

Cyclohexyl 2-[3,3-Bis(S-benzylthio)propionamido]-2-deoxy-1-thio-α-D-glucopyranoside (MA-14)

3,3-Bis(*S*-benzylthio)propionic acid (synthesized by the procedure above) was coupled with **MA-1** by procedure A: 13.5 mg, 65%, colorless oil; R_f 0.15 (1 : 4 methanol/dichloromethane); ESI-MS m/z 474 $(MNa\text{-}BnSH)^+$.

Cyclohexyl 2-Deoxy-2-ethylamino-1-thio-α-D-glucopyranoside MA-15)

A solution of **MA-2** (46 mg, 0.151 mmol) and lithium aluminum hydride (40 mg, 1.05 mmol) in 2.0 mL of dry THF was heated at reflux for 2 d. The reaction was concentrated to half volume, cooled to 0°C, and then quenched by dropwise addition of 1 mL of aqueous sodium sulfate. Methanol (2.0 mL) was added and the solution was stirred for 6 hr, filtered, and then concentrated. Chromatography with 3 : 3 : 7 methanol/ethyl acetate/dichloromethane as the eluant afforded 27 mg (61%) of **MA-15** as a colorless oil: R_f 0.35 (5 : 1 : 14 methanol/ethyl acetate/dichloromethane); ESI-MS m/z 306 MH^+.

Cyclohexyl 2-Deoxy-2-(2-ethylamino)ethylamino-1-thio-α-D-glucopyranoside, bis(acidic acid) salt (MA-16)

Reduction of **MA-4** (38 mg, 0.085 mmol) as described for **MA-15** (procedure directly above) followed by chromatography with 1 : 2 : 5 acetic acid/methanol/dichloromethane as the eluant afforded 24 mg (63%) of the salt **MA-16** as a colorless oil: R_f 0.18 (3 : 2 methanol/dichloromethane); ESI-MS m/z 349 MH^+.

Cyclohexyl 2-Deoxy-2-(2-hydroxyethylamino)-1-thio-α-D-glucopyranoside (MA-17)

Reduction of **MA-5** (31 mg, 0.082 mmol) as described for **MA-15** followed by chromatography with 3 : 17 methanol/dichloromethane as the eluant afforded 15 mg (55%) of **MA-17** as a colorless oil: R_f 0.20 (3 : 17 methanol/dichloromethane); ESI-MS m/z 322 MH^+.

Cyclohexyl 2-[3-(S-benzylthio)propylamino]-2-deoxy-1-thio-α-D-glucopyranoside (MA-18)

3-(*S*-Benzylthio)propionaldehyde[41] (46 mg, 0.260 mmol) and **MA-1** (51 mg, 0.185 mmol) in dichloromethane (1.0 mL) and DMF (0.10 mL) was treated with sodium triacetoxyborohydride (55 mg, 0.260 mmol). The solution became cloudy within 15 min. The reaction mixture was stirred for 1.5 hr and then concentrated. Chromatography with 1 : 19 methanol/dichloromethane as the eluant provided 64 mg (78%) of **MA-18** as a colorless oil: R_f 0.44 (3 : 17 methanol/dichloromethane); ESI-MS m/z 442 MH^+.

Cyclohexyl 2-Deoxy-2-[(4-R-2-methyl-2-oxazolin-4-yl)methylamino]-1-thio-α-D-glucopyranoside acetic acid salt (MA-19)

(*R*)-4-Hydroxymethyl-2-methyl-2-oxazoline was prepared by the literature method,[42] and then converted to its *O*-methanesulfonyl derivative as follows. Methanesulfonyl chloride (357 μL, 1.05 equiv) was added to a solution of (*R*)-4-hydroxymethyl-2-methyl-2-oxazoline (504.8 mg, 4.39 mmol) in 8 mL of dichloromethane at 0°C, followed by triethylamine (673 μL, 1.1 equiv.). The solution was allowed to slowly come to room temperature over a 2-hr period, and then was concentrated under vacuum. The unstable crude mesylate was used for the subsequent alkylation reaction without further purification. A solution of crude (4-*R*-2-methyl-2-oxazolin-4-yl)methyl methanesulfonate (100 mg, 0.518 mmol), **MA-1** (46 mg, 0.166 mmol), and potassium carbonate (30 mg, 0.217 mmol) in 2.0 mL of DMF was heated at 70°C for 12 hr. The reaction mixture was concentrated and then chromatographed with 1:1 methanol/dichloromethane as the eluant to give 56 mg (78%) of **MA-19** as a colorless oil: R_f 0.10 (1 : 4 methanol/dichloromethane); ESI-MS m/z 375 MH^+.

Cyclohexyl 2-Deoxy-2-(2-ethoxy-2-oxoacetamido)-1-thio-α-D-glucopyranoside (MA-20)

Commercial ethyl chlorooxoacetate (14 μL, 0.121 mmol) was added to a solution of **MA-1** (28 mg, 0.101 mmol) and Hünig's base (21 μL, 0.117 mmol) in 1.0 mL of DMF at 0°C. The reaction mixture was stirred at 23°C for 12 hr and then concentrated. Chromatography with 4 : 1 ethyl acetate/dichloromethane as the eluant provided 30 mg (79%) of **35** as a colorless white solid:

mp 164–165°C; R_f 0.50 (1 : 9 methanol/dichloromethane); ESI-MS m/z 401 MNa^+.

Cyclohexyl 2-Deoxy-2-methanesulfonamido-1-thio-α-D-glucopyranoside (MA-21)

Methanesulfonyl chloride (12 μL, 0.155 mmol) was added to a solution of **MA-1** (40 mg, 0.144 mmol) in 2.0 mL of dioxane at 0°C. Triethylamine (22 μL, 0.157 mmol) was then added, and the solution was stirred for 2 hr at 23°C. Concentration and then chromatography with 1 : 19 methanol/dichloromethane as the eluant gave 39 mg (77%) of the sulfonamide **MA-21** as a colorless oil: R_f 0.20 (1 : 9 methanol/dichloromethane); ESI-MS m/z 378 MNa^+.

Cyclohexyl 2-Deoxy-2-(2-phenylethenylsulfonamido)-1-thio-α-D-glucopyranoside (MA-22)

Commercial (*E*)-2-styrenesulfonyl chloride (19 mg, 0.095 mmol) was added to a solution of **MA-1** (22 mg, 0.079 mmol) and Hünig's base (17 μL, 0.095 mmol) in 1.0 mL of 1 : 1 DMF/THF at 0°C, whereupon the mixture turned from clear to yellow. The reaction mixture was stirred at 23°C for 4 hr, and then concentrated. Chromatography with 13 : 7 ethyl acetate/dichloromethane as the eluant provided 28 mg (86%) of **MA-22** as a colorless oil: R_f 0.83 (9 : 41 methanol/dichloromethane); ESI-MS m/z 466 MNa^+.

Cyclohexyl 2-Amino-2-N-thioacetamido-2-deoxy-1-thio-α-D-glucopyranoside (MA-23)

Lawesson's reagent (52 mg, 0.130 mmol) was added to a solution of **12** (68 mg, 0.153 mmol) in 2 mL of THF, and the suspension was stirred at 23°C for 12 hr under an argon atmosphere. The reaction mixture was cooled to 23°C and then chromatographed directly with 3 : 7 ethyl acetate/dichloromethane as the eluant to afford 60 mg (100%) of the thioamide triacetate as a yellow oil. This product was dissolved in 2.0 mL of methanol, and then was treated with sodium methoxide (4 drops, 25% w/w solution in MeOH) under an argon atmosphere. After 30 min the solution was concentrated and then chromatographed with 1 : 19 methanol/dichloromethane as eluant to afford 44 mg (98%) of **MA-23** as a light yellow oil: R_f 0.50 (1 : 9 methanol/dichloromethane); ESI-MS m/z 358 MNa^+.

Cyclohexyl 2-Acetamidino-2-deoxy-1-thio-α-D-glucopyranoside acetic acid salt (MA-24)

A solution of **MA-1** (30 mg, 0.108 mmol), ethylacetimidate hydrochloride (14 mg, 0.113 mmol), and triethylamine (16 μL, 0.113 mmol) in 1.5 mL of 1 : 1 dioxane/methanol was stirred at 23°C for 6 hr. The reaction mixture was concentrated and then chromatographed with 3 : 7 : 90 methanol/acetic acid/dichloromethane as the eluant to give 31 mg (75%) of the acetate salt

MA-24 as a colorless oil: R_f 0.10 (1 : 4 methanol/dichloromethane); ESI-MS m/z 319 MH^+.

Cyclohexyl 2-Deoxy-2-(O,O′-dibenzylphosphoramido)-1-thio-α-D-glucopyranoside (MA-25)

Tetrabenzylpyrophosphate[43] (77 mg, 0.143 mmol) was added to a solution of **MA-1** (33 mg, 0.119 mmol) and Hünig's base (93 μL, 0.179 mmol) in 1.0 mL of 1 : 1 DMF/THF at 0°C. The mixture was allowed to stir at 23°C for 6 hr. Concentration and chromatography with 17 : 3 ethyl acetate/dichloromethane as the eluant provided 38 mg (60%) of **MA-25** as a colorless oil: R_f 0.38 (1 : 9 methanol/dichloromethane); ESI-MS m/z 560 MNa^+.

Cyclohexyl 2-(1-butanephosphonamido)-2-deoxy-1-thio-α-D-glucopyranoside triethylamine salt (MA-26)

Commercial *n*-butylphosphonic dichloride (45 μL, 0.320 mmol) was added to a solution of **MA-1** (59 mg, 0.213 mmol) and Hünig's base (78 μL, 0.447 mmol) in 1.0 mL of DMF at 0°C. The mixture was allowed to stir at 23°C for 4 hr. Concentration and then chromatography with 13 : 7 ethyl acetate/dichloromethane as the eluant provided 29 mg (27%) of the triethylamine salt **MA-26** as a colorless oil: R_f 0.20 (3 : 2 : 15 methanol/triethylamine/dichloromethane); NI-ESI-MS m/z 498 MH^-.

Cyclohexyl 2-[N(3)-Benzylthioureido]-2-deoxy-1-thio-α-D-glucopyranoside (MA-27)

Commercial benzylisothiocyanate (29 μL, 0.217 mmol) was added to a solution of **MA-1** (50 mg, 0.181 mmol) in 1.5 mL of 1 : 1 DMF/THF at 0°C. The reaction mixture was stirred at 23°C for 2 hr, concentrated, and then chromatographed with 1 : 19 methanol/dichloromethane as the eluant to afford 65 mg (85%) of **MA-27** as a white solid: mp 185–187°C; R_f 0.30 (1 : 9 methanol/dichloromethane); ESI-MS m/z 449 MNa^+.

Cyclohexyl 2-[N(3)-Benzylureido]-2-deoxy-1-thio-α-D-glucopyranoside (MA-28)

Commercial benzylisocyanate (27 μL, 0.220 mmol) was added to a solution of **MA-1** (50 mg, 0.183 mmol) in 1.5 mL of 1 : 1 DMF/THF at 0°C. The reaction mixture was stirred at 23°C for 2 hr, concentrated, and then chromatographed with 1 : 9 methanol/dichloromethane as the eluant to afford 59 mg (79%) of **MA-28** as a white solid: mp 167–169.5°C; R_f 0.20 (1 : 9 methanol/dichloromethane); ESI-MS m/z 433 MNa^+.

Cyclohexyl 2-Deoxy-2-[N(3)-methylthioureido]-1-thio-α-D-glucopyranoside (MA-29)

Commercial methylisothiocyanate (13 μL, 0.192 mmol) was added to a solution of **MA-1** (44 mg, 0.159 mmol) in 1.5 mL of 1 : 1 DMF/THF at 0°C. The reaction mixture was stirred at 23°C for 2 hr, concentrated, and then chromatographed with 9 : 1 ethyl acetate/dichloromethane as the eluant to afford 41 mg (73%) of **MA-29** as a colorless oil: R_f 0.26 (ethyl acetate); ESI-MS m/z 373 MNa^+.

Cyclohexyl 2-Deoxy-2-[N(3)-2-phenylethylthioureido]-1-thio-α-D-glucopyranoside (MA-30)

Commercial phenethylisothiocyanate (27 μL, 0.178 mmol) was added to a solution of **MA-1** (41 mg, 0.149 mmol) in 1.5 mL of 1 : 1 DMF/THF at 0°C. The reaction mixture was stirred at 23°C for 2 hr, concentrated, and then chromatographed with 7 : 3 ethyl acetate/dichloromethane as the eluant to afford 46 mg (73%) of **MA-30** as a colorless oil: R_f 0.70 (3 : 17 methanol/dichloromethane); ESI-MS m/z 425 MH^+.

Cyclohexyl 2-Deoxy-2-[N(3)-2-phenylethylureido]-1-thio-α-D-glucopyranoside (MA-31)

Commercial phenethylisocyanate (24 μL, 0.176 mmol) was added to a solution of **MA-1** (41 mg, 0.149 mmol) in 1.5 mL of 1 : 1 DMF/THF at 0°C. The reaction mixture was stirred at 23°C for 2 hr, concentrated, and then chromatographed with ethyl acetate as the eluant to afford 59 mg (91%) of **MA-31** as a colorless oil: R_f 0.71 (3 : 17 methanol/dichloromethane); ESI-MS m/z 447 MH^+.

Cyclohexyl 2-Deoxy-2-(ethoxyformamido)-1-thio-α-D-glucopyranoside (MA-32)

Ethyl chloroformate (20 μL, 0.212 mmol) was added to a solution of **MA-1** (56 mg, 0.202 mmol) and Hünig's base (39 μL, 0.216 mmol) in 1.5 mL of 1 : 1 dioxane/DMF at 0°C. The reaction mixture was stirred at 23°C for 6 hr, concentrated, and then chromatographed with 4 : 1 ethyl acetate/dichloromethane as the eluant to provide 54 mg (74%) of **MA-32** as a white solid: mp 144–146°C; R_f 0.42 (ethyl acetate); ESI-MS m/z 372 MNa^+.

Cyclohexyl 2-Deoxy-2(2-hydroxyimino-2-phenylacetamido)-1-thio-α-D-glucopyranoside (MA-33)

Hydroxylamine hydrochloride (36 mg, 0.059 mmol) was added to a solution of **MA-39** (20 mg, 0.049 mmol) in 0.75 mL of methanol at 0°C. The solution was allowed to warm to 23°C over a 4-hr period, and then was concentrated. Chromatography with ethyl acetate as the eluant provided 16 mg (76%) of

MA-33 as a colorless oil: R_f 0.22 (1 : 9 methanol/dichloromethane); ESI-MS m/z 447 MNa^+.

2-Hydroxyimino-3-phenylpropionic Acid

Phenylpyruvic acid (300 mg, 1.83 mmol) and sodium hydroxide (73 mg, 1.83 mmol) were dissolved in 4.6 mL of water at 0°C. In a separate flask, hydroxylamine hydrochloride (127 mg, 1.83 mmol) and sodium bicarbonate (153 mg, 1.83 mmol) were dissolved in 3.7 mL of water at 0°C. The hydroxylamine solution was then added to the phenylpyruvate solution, and the resulting solution was allowed to warm to 23°C and was stirred for 6 h. During this period the pH was maintained at 5–6 by dropwise addition of concentrated aqueous hydrochloric acid. The reaction mixture was acidified to pH 2, extracted with ethyl acetate (3 × 50 mL). The organic extract was dried over magnesium sulfate, filtered, and then concentrated to afford 294 mg (90%) of 2-hydroxyimino-3-phenylpropionic acid as a white solid: 164–165°C (dec.); ^{1}H NMR (200 MHz, DMSO-d_6) 7.31–7.17 (m, 5 H), 3.81 (s, 2 H), 3.32 (br s, 1 H); ^{13}C NMR (50 MHz, DMSO-d_6) 165.2, 150.2, 136.8, 128.6, 128.4, 126.2, 29.9; NI-ESI-MS m/z 178 MH^-.

Cyclohexyl 2-Deoxy-2(2-hydroxyimino-3-phenylpropionamido)-1-thio-α-D-glucopyranoside (MA-34)

2-Hydroxyimino-3-phenylpropionic acid (prepared by the procedure above) was coupled with MA-1 by procedure B: 50 mg, 79%, white solid, mp 144–146°C; R_f 0.33 (1 : 9 methanol/dichloromethane); ESI-MS m/z 461 MNa^+.

2-Hydroxyimino-3-(4-hydroxyphenyl)propionic Acid

Commercial (4-hydroxyphenyl)pyruvic acid (670 mg, 3.74 mmol) was converted to its oxime as for 2-hydroxyimino-3-phenylpropionic acid to afford 725 mg (94%) of 2-hydroxyimino-3-(4-hydroxyphenyl)propionic acid as an off-white solid: 170–172°C (dec.); ^{1}H NMR (400 MHz, CD_3OD) 7.10 (d, $J = 8.4$, 2 H), 6.68 (d, $J = 8.4$, 2 H), 3.82 (s, 2H); ^{13}C NMR (50 MHz, CD_3OD) 167.0, 156.8, 152.7, 131.0, 128.6, 116.1, 30.0; NI-ESI-MS m/z 194 MH^-.

Cyclohexyl 2-Deoxy-2[2-hydroxyimino-3-(4-hydroxyphenyl)propionamido]-1-thio-α-D-glucopyranoside (MA-35)

2-Hydroxyimino-3-(4-hydroxyphenyl)propionic acid (prepared by the procedure above) was coupled with **MA-1** by procedure B: 56 mg, 76%, white solid, mp 214–216°C; R_f 0.30 (3 : 17 methanol/dichloromethane); ESI-MS m/z 477 MNa^+.

2-Hydroxyimino-3-(3-bromo-4-hydroxyphenyl)propionic Acid

(3-Bromo-4-hydroxyphenyl)pyruvic acid[44] (300 mg, 1.16 mmol) was converted to its oxime by using the procedure described above for

2-hydroxyimino-3-phenylpropionic acid, affording 265 mg (84%) of 2-hydroxyimino-3-(3-bromo-4-hydroxyphenyl)propionic acid as a white solid: 147–148°C (dec.); ^{1}H NMR (400 MHz, CD_3OD) 7.37 (d, $J = 2.0$, 1 H), 7.08 (dd, $J = 2.0$, 8.0, 1 H), 6.78 (d, $J = 8.0$, 1 H), 3.80 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD) 166.7, 153.8, 152.0, 134.4, 130.4, 130.3, 117.0, 110.5, 29.7; NI-ESI-MS m/z 273 and 275 MH^-.

Cyclohexyl 2-Deoxy-2[2-hydroxyimino-3-(3-bromo-4-hydroxyphenyl) propionamido]-1-thio-α-D-glucopyranoside (MA-36)

2-Hydroxyimino-3-(3-bromo-4-hydroxyphenyl)propionic acid (prepared by the procedure above) was coupled with **MA-1** by procedure B: 96 mg, 86%, white solid, mp 218–220°C; R_f 0.42 (3 : 17 methanol/dichloromethane); ESI-MS m/z 555 and 557 MNa^+.

Cyclohexyl 2-Deoxy-2-(2-oxopropionamido)-1-thio-α-D-glucopyranoside (MA-37a, MA-37b/c)

Commercial pyruvic acid was coupled with **MA-1** by procedure A: 41 mg, 82%, 2 : 3 mixture respectively by ^{1}H NMR analysis, colorless oil; R_f 0.30 (1 : 9 methanol/dichloromethane); ESI-MS m/z 370 MNa^+.

Cyclohexyl 2-Deoxy-2-[3-(S-benzylthio)-2-oxopropionamido]-1-thio-α-D-glucopyranoside (MA-38b)

3-(S-Benzylthio)pyruvic acid was prepared by the literature method[45] and then was coupled with **MA-1** by procedure A: 48 mg, 62%, completely cyclized as one isomer by ^{1}H NMR analysis, colorless oil; R_f 0.35 (1 : 4 ethyl acetate/dichloromethane); ESI-MS m/z 474 $(MNa\text{-}H_2O)^+$.

Cyclohexyl 2-Deoxy-2(2-oxo-2-phenylacetamido)-1-thio-α-D-glucopyranoside (MA-39a, MA-39b, MA-39c)

Commercial benzoylformic acid was coupled with **MA-1** by procedure A: 46 mg, 77%, 8 : 1 : 1 respectively by ^{1}H NMR analysis, colorless oil; R_f 0.64 (3 : 17 methanol/dichloromethane); ESI-MS m/z 432 MNa^+.

Cyclohexyl 2-Deoxy-2(2-oxo-3-phenylpropionamido)-1-thio-α-D-glucopyranoside (MA-40b, MA-40c)

Commercial phenylpyruvic acid was coupled with **MA-1** by procedure A: 22 mg, 84%, 1 : 1 respectively by ^{1}H NMR analysis, colorless oil; R_f 0.58 (4 : 1 ethyl acetate/dichloromethane); ESI-MS m/z 428 MNa^+.

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