Family of Thiomercuric Derivatives of Sugars: Synthesis, Fungicidal/Herbicidal Activity, and Application to the X-Ray Structure Determination of the Corresponding Enzymes

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Abstract. A series of thiomercuric derivatives of mono- and disaccharides **1–7**, in which methylmercury or phenylmercury is covalently attached to anomeric thioglycosides, were synthesized for structure–function studies of glycosidases. Thiomethylmercuryl xylobiosides **5** and **6** were found to inhibit intracellular xylanase-T6 in a competitive manner, with K_i values of 0.35 mM and 0.01 mM, respectively. These inhibitors have been co-crystallized with the enzyme and are being used for X-ray analysis. 1-(Thiomethylmercuric)-β-D-xyloside (**3**) affords crystals belonging to the orthorhombic space group $P2_12_12_1$ and at 293(2) K: a = 6.7510(2), b = 9.7140(2), c = 29.4770(9) Å, V = 1933.08(9) Å, Z = 8, $R(F^2)$ = 0.0329, and $R_w(F^2)$ = 0.0626. There are two molecules (A and B) in the asymmetric unit, and each one shows an almost linear S–Hg–C arrangement. Biological tests on **1**–**7** indicated that they exhibit potent fungicidal and herbicidal activities.

INTRODUCTION

To date, structure–function studies on glycosidases have been hampered by difficulties either with the protein itself or with its specific substrate/inhibitor. Thus, although a number of glycosidases have been either structurally or mechanstically studied in some detail, only in a few cases have the two approaches been satisfactorily combined.[1] The most commonly used methodology for crystallographic phase determination is the preparation of heavy-atom derivatives (with mercury, lead, etc.) of protein crystals. However, although such an approach, if successful, can finally lead to the three-dimensional structure determination of a protein, the information gained on the active-site architecture and the catalysis is still very limited. In order to provide insight into interactions at the active site, which is crucial for substrate specificity and catalysis, there is a need to further examine the X-ray diffraction of either the enzyme–substrate or enzyme–inhibitor complexes.

In attempts to combine these two important steps in structure–function studies of both glycosidases and many other carbohydrate-binding proteins, we have designed and synthesized thiomercuric derivatives of carbohydrates **1–7**. In this set of compounds, the methylmercury or phenylmercury moiety is covalently attached to anomeric thioglycosides. It was predicted that the examination of these compounds, in the form of stable binary complexes with appropriate enzymes, should provide both the specific binding and the heavy-atom derivative suitable for complete X-ray analysis. The choice of anomeric thiomercuric derivatives follows the

Dedicated to the memory of Raymond U. Lemieux.

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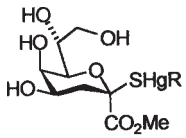

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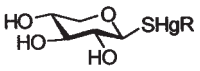

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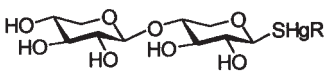

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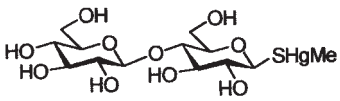

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results obtained by Shigeta et al.[2] and Fitz et al.,[3] who used thiomercuric analogues of sialic acid to determine the crystallographic phases for pertussis toxin, and 9-*O*-acetylsialic acid esterase from influenza C virus, respectively. These groups also showed that the replacement of anomeric C–O linkage in the inhibitor with a C–S–Hg linkage does not significantly affect binding to the enzyme.

Thiomercuric derivatives **1** and **2** were designed as model compounds for a structure–function study of enzymes involved in the biosynthetic formation and utilization of 3-deoxy-D-manno-2-octulosonic acid (Kdo). This unique 8-carbon-atom sugar is a specific constituent of the lipopolysaccharide of most Gram-negative bacteria and is required by them for growth and virulence.[4] Several groups have therefore pursued inhibition of Kdo metabolism as a strategy for the development of novel antibacterial drugs. Indeed, some of the potent inhibitors of CMP-Kdo synthetase have shown in vivo antibacterial activity.[5] These results have prompted us to further design synthetic molecules exhibiting selective activity against Gram-negative bacterial cells.[6]

The derivatives of xylose (**3**-**4**), xylobiose (**5**-**6**), and cellobiose (**7**) were designed for the structural study of xylanases and cellulases. Xylanases (1,4-β-D-xylan xylanhydrolases; E.C. 3.2.1.8) hydrolyze the 1,4-β-D-xylopyranosyl linkage of xylans. Xylan is a major component of the plant cell wall, and serves as the backbone of D-xylopyranosyl units linked by β-(1-4) glycosidic bonds. The structural characterization of xylanase-active sites is of great interest, since it can lead to a better understanding of their catalytic mechanism and contribute significant knowledge to the rational design of specific oligosaccharide-binding sites through protein engineering. Much of the interest in xylanases stems from their potential biotechnological applications, which include biobleaching in the pulp and paper industry,[7a,b] bioconversion of lignocellulose material to fermentative products, partial hydrolysis of animal feedstock to improve digestibility, and enzymatic organic synthesis.[7c,d]

It is noteworthy that, in addition to their scientific importance, they might be expected to exhibit pesticidal activity since compounds **1–7** represent organic mercury derivatives. Thus, although the use of mercury compounds as pesticides has virtually ceased over the past decade due to their toxic effect,[8] increased resistance of bacteria, fungi, and plants to existing pesticides requires a continuous search for new compounds exhibiting potent biological activities.[9] Since compounds **1–7** are unique structures that combine a sugar skeleton with a stable mercury linkage, they might have some important advantages over other families of currently used pesticides. In the fields of pesticides and other agricultural or pharmaceutical chemicals, no compounds of this class have been reported in the literature, to the best of our knowledge. Here, we report the synthesis and various biological tests of a series of thiomercuric derivatives of mono- and disaccharides.

RESULTS AND DISCUSSION

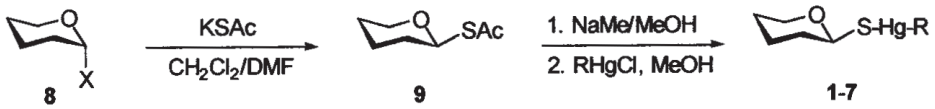
Synthesis of Thiomercuric Derivatives

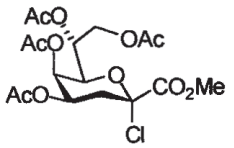
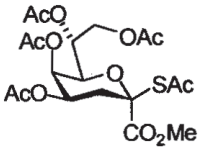
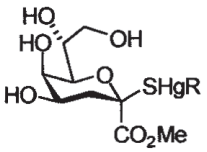
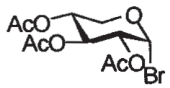
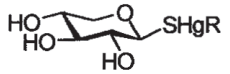
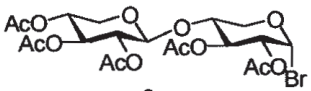
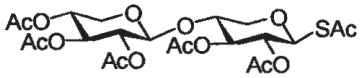
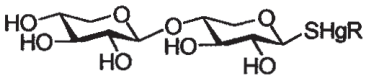
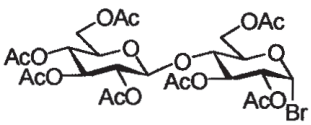
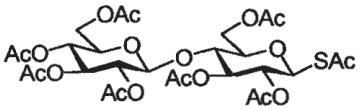
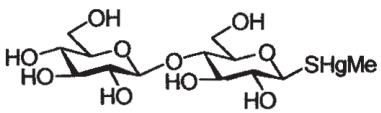
All thiomercuric derivatives were prepared according to the general procedure shown in Table 1. Anomeric chlorides or bromides of the peracetylated saccharide (**8**a–d) were treated with potassium thioacetate in a dichloromethane/DMF mixture to produce the corresponding thioacetate (**9**a–d) according to published procedures.[4,10] Treatment of the thioacetate (**9**a–d) with MeONa/MeOH resulted in the S- and O-deacetylated products. The resulting crude material was treated in situ with either methylmercury(II)chloride or phenylmercury(II)chloride to produce the corresponding thiomercuric derivatives **1–7**. The purity and structure of each product were established by a combination of ^{1}H, ^{13}C NMR, 2D-COSY, and mass spectral analysis. The observed large coupling constants (^{3}J~9 Hz) between anomeric and C2 protons confirm the desired β-configuration of thiomercuric linkages in compounds **2–7**.

In attempts to learn more about the conformation of these unique sugar derivatives, a single-crystal X-ray analysis of **3** was made (Fig. 1). It is noted that no similar structure of any carbohydrate derivative linked to a S–Hg–C fragment is yet to be found in the Cambridge Crystallographic Structural Database (CCSD).[11]

The X-ray analysis of **3** reveals a chair conformation of the sugar ring, while the thiomercuryl moiety is oriented *trans* to the oxygen substitution at carbon 2, indi-

Table 1. General Scheme for the preparation of thiomercuric derivatives **1–7**



Starting Material	Thioacetate (yield)	Final Compounds (yield)
 **8a**	 **9a** (70%)	 **1**: R=Me (69%); **2**: R=Ph (76%)
 **8b**	 **9b** (72%)	 **3**: R=Me (71%); **4**: R=Ph (53%)
 **8c**	 **9c** (65%)	 **5**: R=Me (65%); **6**: R=Ph (51%)
 **8d**	 **9d** (58%)	 **7** (67%)

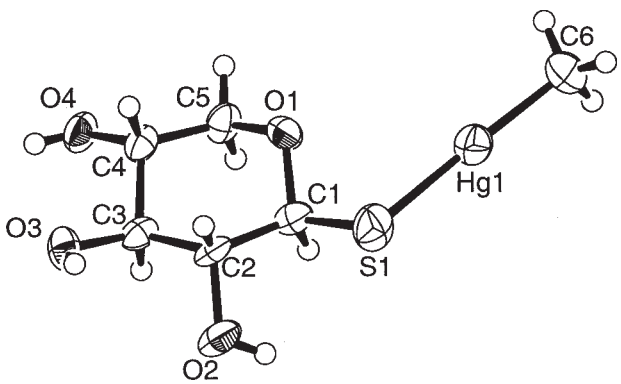


Fig. 1. ORTEP drawing of Compound **3** from single-crystal X-ray analysis. Selected crystallographic data: Average bond lengths (Å): Hg(1)–C(6) = 2.06(1), Hg(1)–S(1) = 2.349(3), S(1)–C(1) = 1.804(9), C(1)–O(1) = 1.43(1), C(1)–C(2) = 1.52(1), C(2)–O(2) = 1.419(9), C(2)–C(3) = 1.52(1), C(3)–O(3) = 1.43(1), C(3)–C(4) = 1.54(1), C(4)–O(4) = 1.44(1), C(4)–C(5) = 1.49(1), C(5)–O(1) = 1.43(1). Average bond angles (°): S(1)—Hg(1)–C(6) = 176.5(3), Hg(1)–S(1)–C(1) = 98.7(3), S(1)–C(1)–C(2) = 118.8(6), S(1)–C(1)–O(1) = 108.0(7), C(1)–O(1)–C(5) = 112.0(8), O(1)–C(1)–C(2) = 110.0(6), O(2)–C(2)–C(3) = 109.4(7), C(1)–C(2)–O(2) = 108.9(6), C(1)–C(2)–C(3) = 111.4(7), C(2)–C(3)–O(3) = 109.9(7), C(4)–C(3)–O(3) = 110.2(6), C(2)–C(3)–C(4) = 110.4(8), C(5)–C(4)–O(4) = 108.0(7), C(3)–C(4)–O(4) = 109.5(8), C(3)–C(4)–C(5) = 110.4(7), C(4)–C(5)–O(1) = 110.3(7). The esd in parentheses is in the unit of least significant digit.

cating the β-anomeric configuration (Fig. 1). The mercury atom has an almost linear stereochemistry with normal Hg–S and Hg–C distances and an S–Hg–C angle of 175.7(3)°. These values are in agreement with those found for the CH_3–Hg–S moieties in the CCSD.[11] Interestingly, an analysis of the crystallographic data also reveals that compound **3** exists as two symmetry-independent structures, A and B, within the unit cell.[12] As illustrated in Fig. 2, most of the bond distances and angles in A and B are very similar, although a marked difference exists in the side-chain conformation. This difference is clearly seen by comparing torsion angles O1–C1–S–Hg (see insert in Fig. 2), which were found to be 47.4(6)° and 145.2(6)° in A and B, respectively. The presence of two similar independent structures, A and B, was also reported for other thiomercury derivatives.[13] This phenomenon was attributed to a low residual Lewis acidity of mercury in these complexes, leading to the formation of "secondary bonds" that are intermediate in strength between covalent and van der Waals bonds.[12a,b,13] Indeed, the intramolecular Hg···O1 distances in structures A and B of **3** were found to be 2.965 Å and 3.127 Å, respectively. These distances are smaller than the sum of the van der Waals radii for the atoms concerned, confirming the presence of a secondary interaction in this part of the molecule.

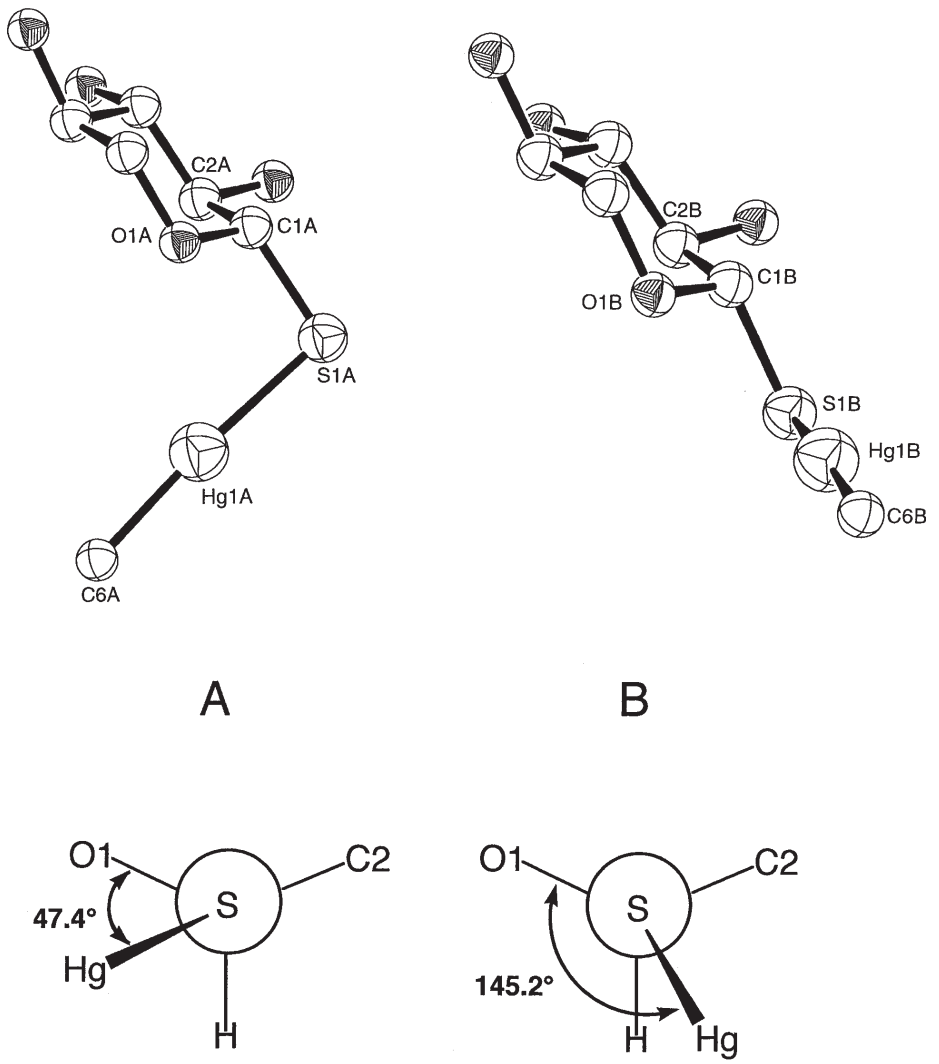


Fig. 2. View of the two independent structures, A and B, of Compound **3** as they appear in the unit cell. The inserts show Neuman projections through the C1–S bond for A and B, highlighting the difference in torsion angle O1–C1–S–Hg between structures A and B.

*Interaction of **5** and **6** with intracellular xylanase*

An intracellular xylanase (IXT6) from *Bacillus stearothermophilus* T-6 has recently been cloned, sequenced, and overexpressed in our laboratory (Technion).[14] The purified enzyme exhibited different substrate specificity towards synthetic substrates compared to that of the extracellular xylanase (XT6). Crystallographic studies of IXT6 were initiated in order to study the specificity and catalysis of this unique xylanase, as well as to provide a structural basis for the rational introduction of enhanced thermostability by site-specific mutagenesis. We recently reported a preliminary X-ray analysis of IXT6,[14b] and molecular replacement studies and multiple anomalous dispersion (MAD) experiments are currently in progress in order to determine the detailed three-dimensional structure of this enzyme. However, in attempts to ascertain the actual active site architecture, we designed and synthesized mercury-substituted xylobioside inhibitors of the enzyme, **5** and **6**, which can be used to form an isomorphous heavy-atom derivative.

Compounds **5** and **6** were evaluated as inhibitors of homogeneous IXT6. Inhibition was measured by using hydrolysis of *p*-nitrophenyl xylobioside (PNPX2) as a synthetic substrate, for which the K_m and k_{cat} values were determined to be 0.1 mM and 6.9×10^3 min^{-1}, respectively. The inhibition constants were measured from initial velocity studies, and inhibition patterns were found to be competitive (Fig. 3). Both **5** and **6** were found to be inhibitors with K_i values of 0.35 mM and 0.01 mM, respectively. The observed 10-fold enhanced binding of **6** to the PNPX2 substrate (based on the comparison of K_i of **6** with the K_m of PNPX2) indicates that replacing the aglycon with the thiomercury group seems to be a useful strategy, not just for preparing heavy-atom derivatives of crystals of IXT6, but also as a general tool for crystallographic studies of glycosidases. The inhibitors **5** and **6** have been co-crystallized with IXT6 and are being used for X-ray analysis.

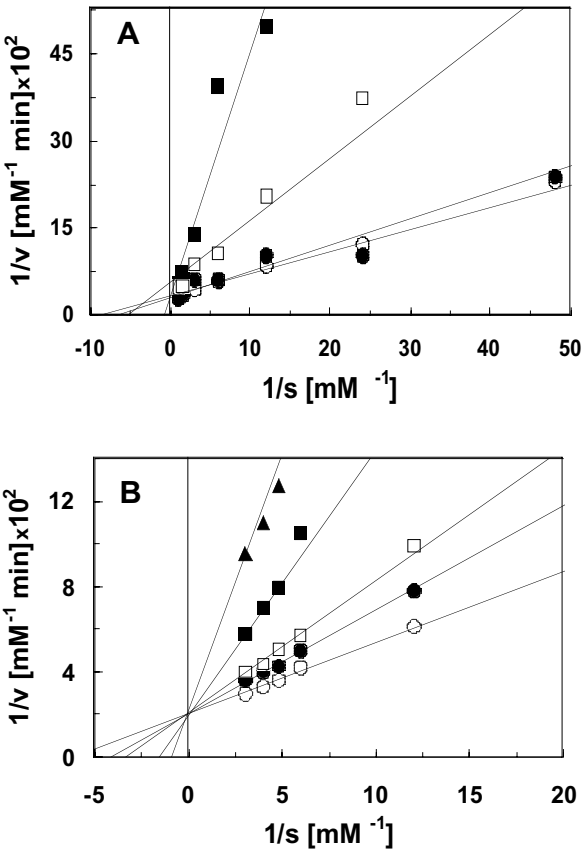


Fig. 3. Inhibition of IXT6 by **5** (A) and **6** (B). Double-reciprocal plots of initial velocities are given as a function of PNPX2 concentrations. (A) The concentrations of **5** were none (○), 0.5 mM (●), 1.0 mM (□), and 2.0 mM (■). For the assays, the enzyme (1.9 nM) was first preincuabted with various concentrations of **5** (0–2.0 mM) at 50 °C for 15 min, and the reactions were initiated by adding PNPX2 (0.02–1.0 mM). (B) The concentrations of **6** were none (○), 4.6 mM (●), 9.3 mM (□), 27.7 mM (■) and 55.4 mM (▲). For the assays, the enzyme (1.9 nM) was first preincuabted with various concentrations of **6** (0–55.4 mM) at 50 °C for 15 min, and the reactions were initiated by adding PNPX2 (0.082–0.33 mM).

*Examination of **1–7** for Herbicidal and Fungicidal Activities*

Compounds **1–7** were tested for various biological activities, and while displaying only moderate bactericidal activity against *Bacillus* sp. (data not shown), they exhibited very potent herbicidal and fungicidal activities. Herbicidal activity was tested for seed germination and shoot elongation using the *Amaranthus palmeri* weed, the *Cuscuta campestris* parasitic plant, and *Corchorus olitorius* (a plant grown for agriculture) (see Table 2). The data in Table 2 show different activity levels for the various thiomercuric derivatives. In addition, a high degree of selectivity among different plants was achieved. For example, compounds **3**, **5,** and **7** were highly inhibitory toward *A. palmeri* and *Corchorus olitorius* seed germination, whereas compounds **2–7** were active against *Cuscuta campestris* seeds. It should be noted that compounds that failed to inhibit complete seed germination demonstrated a high activity level on shoot development and elongation. Therefore, for potential herbicidal use, the combined effect of each compound on seed germination and shoot elongation should be considered. The observed high selectivity of this set of compounds is very intriguing especially in terms of their potential for further practical applications. From

this standpoint, of all the above compounds, derivative **4** is perhaps the most interesting due to its high inhibitory activity (100% inhibition at a concentration of 0.02 mg/mL) on the parasitic weed *Cuscuta campestris*, its very high selectivity, and the low cost of its preparation from commercial xylose.

Fungicidal activity was tested against phytopathogenic fungi *Pythium aphanidermatum*, *Phytophthora cytrophthora*, *Alternaria alternata, Macrophomina phaseolina,* and *Fusarium oxysporum* f. sp. *basilici*. For comparison, three commercial fungicides that are very common today in agriculture, Delsene [2-(methoxycarbonylamino)-benzimidazole],[15a] Rubigan [α-(2-chlorophenyl)-α-(4-chlorophenyl)-5-pyrimidinemethanol],[15b] and Vectra (bromoconazole) [15c], were used as references under the same experimental conditions. Following preliminary tests of all new compounds (**1**–**7**), we selected the most active thiosaccharides, **3**, **4**, and **7**, and studied them in greater detail. The results of these experiments are illustrated in Table 3. The following points may be noted from these data. First, all three thiomercuric derivatives, **3**, **4**, and **7**, show higher activity than the reference fungicides. Secondly, concentrations as low as 10 ppm of compounds **3** and **4** caused a 100% inhibition of all five phytopathogenic fungi tested in our experiments. For example, *Alternaria alternata,* the causal agent of many distructive diseases of numerous plant crops, is almost 50% inhibited at 1 ppm concentration of **3**, **4,** and **7**.

The data in Tables 2 and 3 clearly illustrate the power of these newly prepared thiomercuric derivatives of sugars as potent herbicides and fungicides. Although the reasons for these activities are not yet clear, the stability tests of **1**–**7** at 50 °C and a pH range of 6–8 (followed by TLC) showed that they are completely stable for 24 h. Thus, the thermal degradation of these compounds and the release of alkyl mercury as a biologically active compound may be ruled out. This, of course, cannot rule out their biological decomposition within plant or fungal cells.

In summary, a series of thiomercuric derivatives of mono- and disaccharides, in which alkyl(aryl)mercury is covalently attached to anomeric thioglycosides, were successfully prepared and their various biological functions examined. The data obtained from the inhibition studies of intracellular xylanase enzyme (IXT6) as a case study reveals that anomeric thiomercuric derivatives of sugars can be successfully used as a general tool for structure–function studies of glycosidases. Various tests of this set of compounds for herbicidal and fungicidal activities reveal that they all exhibit potent activities. Although the reasons for these activities are not yet clear, the observed high herbicidal/fungicidal activity, in addition to the observed selectivity of some of the tested derivatives, provide evidence of their potential commercial applications in agriculture.

EXPERIMENTAL SECTION

Chemical

General. ^{1}H NMR spectra were recorded on a Bruker AM-400 spectrometer, and chemical shifts reported (in ppm) relative to internal Me_4Si (δ 0.0) with $CDCl_3$ as solvent, and to HOD (δ = 4.63) with D_2O as solvent. ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer at 100.61 MHz,

Table 2. Inhibitory effect of thiomercuric derivatives of sugars **1–7** on seed germination[a]

	Amaranthus palmeri		*Corchorus olitorius*		*Cuscuta campestris*	
compound	germination (%)	shoot length (mm)	germination (%)	shoot length (mm)	germination (%)	shoot length (mm)
Water	75.2	40.9 ± 6.2	93.0	34.4 ± 4.0	76.6	50.0 ± 8.8
1% DMSO	2.4	13.3 ± 9.8	98.2	35.7 ± 4.3	47.4	22.9 ± 5.7
1	32.2	3.6 ± 0.8	28.2	3.1 ± 0.9	57.1	7.2 ± 2.6
2	0.0	—	81.1	4.1 ± 1.5	6.9	1.0 ± 0.0
3	1.6	5.5 ± 2.1	0.0	—	0.0	—
4	0.0	—	85.7	8.3 ± 2.0	0.0	—
5	11.6	2.3 ± 2.0	5.0	1.5 ± 0.9	13.8	3.9 ± 0.7
6	0.0	—	77.6	5.1 ± 1.9	0.0	—
7	0.0	—	0.0	—	0.0	—

[a]For details see Experimental Section. Concentrations of inhibitors used: **1** (1 mg/mL), **2** (0.1 mg/mL), **3** (1 mg/mL),**4** (0.02 mg/mL), **5** (1 mg/mL), **6** (0.1 mg/mL), and **7** (1 mg/mL). Compounds **2**, **4**, and **6** were dissolved in 1% DMSO in sterile water, compounds **1**, **3**, **5**, and **7** were dissolved in sterile water. Percent germination and schoot length (in mm) were determined in relation to those in control experiments (sterile water or 1% DMSO in sterile water) after 4 days.

Table 3. Inhibitory effect of the most active thiomercuric derivatives of sugars **3**, **4**, and **7** on phytopathogenic fungi[a]

compound	concentration (ppm)	Colony diameter (mm)				
		Pythium aphanidermatum	*Phytophthora cytrophthora*	*Alternaria alternata*	*Macrophomina phaseolina*	*Fusarium oxisporum* f.sp.*basilici*
Water		68.0 ± 3.5	50.0 ± 1.7	62.5 ± 1.2	75.3 ± 2.1	64.7 ± 1.5
3	0.1	62.2 ± 2.0	46.7 ± 3.8	47.0 ± 5.2	57.0 ± 0.0	61.0 ± 1.7
	1	31.3 ± 8.1	21.3 ± 1.5	27.3 ± 1.2	12.0 ± 4.0	46.7 ± 3.5
	10	0.0	0.0	0.0	0.0	0.0
	50	0.0	0.0	0.0	0.0	0.0
4	0.1	43.0 ± 4.4	24.3 ± 1.5	48.7 ± 4.0	47.7 ± 1.5	47.0 ± 1.0
	1	0.0	14.3 ± 1.2	26.3 ± 2.9	10.3 ± 4.0	18.0 ± 0.0
	10	0.0	0.0	0.0	0.0	0.0
7	0.1	67.3 ± 0.6	48.3 ± 1.5	50.0 ± 0.0	56.7 ± 2.5	62.0 ± 2.6
	1	47.0 ± 2.6	24.3 ± 0.6	29.7 ± 4.7	27.7 ± 4.0	50.3 ± 1.5
	10	0.0	0.0	0.0	0.0	7.3 ± 0.6
	50	0.0	0.0	0.0	0.0	0.0
Delsene	0.1	67.3 ± 1.5	49.3 ± 1.2	52.0 ± 1.0	26.3 ± 3.2	60.7 ± 0.6
	1	65.7 ± 1.2	47.0 ± 3.5	49.7 ± 1.5	12.3 ± 1.5	56.0 ± 1.0
	10	64.0 ± 1.7	32.0 ± 7.1	54.0 ± 3.0	0.0	0.0
	50	64.0 ± 1.0	35.3 ± 9.0	58.5 ± 2.5	0.0	0.0
Vectra	0.1	72.7 ± 0.6	48.3 ± 1.5	41.3 ± 5.5	66.7 ± 1.2	46.7 ± 1.5
	1	70.7 ± 2.3	40.7 ± 1.2	34.0 ± 1.0	51.3 ± 2.1	20.0 ± 2.0
	10	64.7 ± 0.6	22.0 ± 1.7	15.7 ± 1.2	31.0 ± 3.0	0.0
	50	45.7 ± 3.0	19.7 ± 3.8	0.0	11.3 ± 0.6	0.0
Rubigan	0.1	70.3 ± 0.6	49.0 ± 0.0	44.0 ± 2.0	62.3 ± 2.1	54.3 ± 1.5
	1	68.7 ± 1.2	37.7 ± 2.5	22.7 ± 5.5	52.0 ± 1.7	42.3 ± 2.1
	10	44.0 ± 1.7	21.1 ± 1.8	14.7 ± 2.5	39.0 ± 5.3	28.3 ± 2.1
	50	15.7 ± 2.3	12.3 ± 0.6	0.0	17.3 ± 6.1	27.7 ± 3.2

[a]For details see Experimental Section. The standard fungicides and compounds **3** and **7** were dissolved in sterile water, compound **4** was dissolved in 1% DMSO in sterile water.

and chemical shifts (in ppm) reported relative to solvent resonance (δ = 77.00, secondary chemical shift reference) for $CDCl_3$ solutions, or to sodium 2,2-dimethyl-2-silapentane sulfonate (δ = 0.0, external primary chemical shift reference) for D_2O solutions. Mass spectra were obtained on a TSQ-70B mass spectrometer (Finnigan Mat) under fast-atom bombardment (FAB) conditions in glycerol matrices or by negative chemical ionization (NCI) in isobutane. Reactions were monitored by TLC on silica gel 60 F_{254} (0.25 mm, Merck), and spots were visualized by charring with a yellow solution containing $(NH_4)Mo_7O_{24}.4H_2O$ (120 g) and $(NH_4)_2Ce(NO_3)_6$ (5 g) in 10% H_2SO_4 (800 mL). Flash column chromatography was performed on silica gel 60 (70–230 mesh). Melting points were determined using an Electrothermal IA-9300 melting point apparatus in open capillary tubes. Optical rotations were measured at 20 °C using a Jasco DIP-370 digital polarimeter.

Compounds **8a–d** were prepared according to published procedures.[16] 1,2,3,4-Tetra-*O*-acetate-β-D-xylose and β-D-cellobiose octaacetate were provided by Sigma. All other chemicals were provided by Aldrich or Sigma and used without further purification, unless otherwise specified. All solvents were dried over standard drying agents[17] and freshly distilled prior to use.

Methyl 4,5,7,8-tetra-O-*acetyl-2*-S-*acetyl-2,3-dideoxy-D-manno-2-octulosonate* (**9a**). The solution of **8a** (0.61 g, 1.39 mmol) in 10 mL of CH_2Cl_2–DMF (4:1) was added to a suspension of potassium thioacetate (0.32 g, 2.78 mmol) in 5 mL of CH_2Cl_2–DMF (4:1). After stirring for 12 h at room temperature, the reaction mixture was diluted with cold ethyl acetate (100 mL), and ice-cold water (25 mL) was added. The organic layer was washed twice with water (2 × 25 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel column (EtOAc/hexane, 1:9) to give **9a** (0.47 g, 70%) as an oil: $[\alpha]^{20}_D$ = 6.5° (*c* 1, $CHCl_3$); R_f = 0.47 (1:1 EtOAc–hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (t, 1H, $J_{3a\text{-}3eq}$ =12.7 Hz, $J_{3a\text{-}4}$ = 12.5 Hz, H-3a), 2.41 (dd, 1H, $J_{3a\text{-}3eq}$ = 12.7 Hz, $J_{3eq\text{-}4}$ = 4.7 Hz, H-3eq), 4.97 (ddd, 1H, *J* = 2.9, 3.2, and 11.9 Hz, H-4), 5.30

(d, 1H, J = 2.7 Hz, H-5), 4.57 (d, 1H, J = 9.7 Hz, H-6), 5.10 (ddd, 1H, J = 2.7, 2.2, and 8.1 Hz, H-7), 4.21 (dd, 1H, J = 12.3 and 4.7 Hz, H-8), 4.39 (d, 1H, J = 12.3 Hz, H-8'), 3.60 (s, 3H, CO_2CH_3), 2.24 (s, 3H, S-Ac), 2.00, 2.02, 2.06, 2.07 (4s, 12H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.67 (4 OAc), 30.20 (S-Ac), 31.90 (CO_2CH_3), 52.79 (C-3), 62.50 (C-8), 64.04 (C-5), 66.50 (C-4), 67.93 (C-7), 72.45 (C-6), 85.40 (C-2), 169.15, 169.65, 170.27, 170.54 (4 OAc), 191.32 (CO_2CH_3). FABMS: m/z 479.1 (M^++H, $C_{19}H_{26}O_{12}S$, Calcd 478.5).

Methyl 2-methylthiomercuric-β-D-manno-2-octulosonate (**1**). A 0.5 M solution of NaOMe in methanol (1.2 mL) was added to a stirred solution of **9a** (0.64 g, 1.34 mmol) in MeOH (15 mL) at 0 °C. After 3.5 h at 0 °C, the reaction mixture was neutralized by the addition of Dowex (H^+), the resin was filtered, and resulting filtrate evaporated to dryness. The residue was dissolved in MeOH (15 mL), and methylmercury(II)chloride (0.37 g, 1.48 mmol) was added at 0 °C. After 1.5 h at 0 °C, the reaction mixture was neutralized with NH_4OH (dilute solution in MeOH), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on silica gel column (MeOH/$CHCl_3$, 1:9) and then crystallized from MeOH to give **1** (450 mg, 69%): mp 172–173 °C (decomp.); $[\alpha]_D^{20}$ = 58.5° (c 1, MeOH); R_f = 0.35 (1:4, MeOH-$CHCl_3$). ^{1}H NMR (400 MHz, D_2O): d 1.92 (t, 1H, $J_{3a\text{-}3eq}$ = 12.6 Hz, $J_{3eq\text{-}4}$ = 12.4 Hz, H-3a), 2.36 (dd, 1H, $J_{3a\text{-}3eq}$ = 12.6 Hz, $J_{3eq\text{-}4}$ = 4.6 Hz, H-3eq), 3.51 (ddd, 1H, J = 3.4, 4.5, and 12.2 Hz, H-4), 3.78 (d, 1H, J = 2.6 Hz, H-5), 3.01 (d, 1H, J = 9.0 Hz, H-6), 3.67-3.70 (m, H-7 and H-8′), 3.47 (dd, 1H, J = 12.0 and 6.4 Hz, H-8), 3.65 (s, 3H, CO_2CH_3), 0.66 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O): δ 11.12 (CH_3), 41.73 (C-3), 56.22 (CO_2CH_3), 66.07 (C-8), 67.48 (C-5), 69.94 (C-4), 71.54 (C-7), 79.23 (C-6), 86.56 (C-2), 178.17 (CO_2CH_3). CIMS: m/z 483.3 (MH^+, $C_{10}H_{18}HgO_7S$, Calcd 482.9). Anal. Calcd for $C_{10}H_{18}HgO_7S$: C, 24.87; H 3.76. Found: C 25.04; H 3.77.

Methyl 2-phenylthiomercuric-β-D-manno-2-octulosonate (**2**). The title compound was prepared by using the same procedure described for **1**. Treatment of thioacetate **9a** (0.42 g, 0.87 mmol) with NaOMe (0.5 M, 0.90 mL) was followed by treatment with phenylmercury(II)chloride (0.30 g, 0.96 mmol), which, after chromatography of the crude material on silica gel (MeOH/$CHCl_3$, 1:9) and lyophylization, produced 357 mg (76%) of pure **2**: $[\alpha]_D^{20}$ = 58.5° (c 1, MeOH); R_f = 0.41 (MeOH/$CHCl_3$, 1:4). ^{1}H NMR (400 MHz, D_2O) δ 1.97 (t, 1H, $J_{3a\text{-}3eq}$ = 12.5 Hz, $J_{3a\text{-}4}$ = 12.4 Hz, H-3a), 2.43 (dd, 1H, $J_{3a\text{-}3eq}$ = 12.5 Hz, $J_{3eq\text{-}4}$ = 4.6 Hz, H-3eq), 3.54 (ddd, 1H, J = 3.2, 4.3 and 12.0 Hz, H-4), 3.81 (d, 1H, J = 2.7 Hz, H-5), 3.12 (d, 1H, J = 8.9 Hz, H-6), 3.67-3.72 (m, H-7 and H-8′), 3.43 (dd, 1H, J = 11.7 and 6.7 Hz, H-8), 3.69 (s, 3H, CO_2CH_3), 7.18-7.33 (m, 5H, aromatic protons). ^{13}C NMR (100 MHz, D_2O): δ 41.30 (C-3), 56.51 (CO_2CH_3), 66.28 (C-8), 67.56 (C-5), 70.01 (C-4), 71.62 (C-7), 79.57 (C-6), 86.72 (C-2), 131.59, 131.77, 139.35 (aromatic carbons). CIMS: m/z 545.2 (MH^+, $C_{15}H_{20}HgO_7S$, Calcd 544.9). Anal. Calcd for $C_{15}H_{20}HgO_7S$: C, 33.06; H 3.70. Found: C 32.88; H 3.90.

2,3,4-Tri-O-acetyl-1-S-acetyl-β-D-xyloside (**9b**). The title compound was prepared by using the same procedure as described for **9a**. Treatment of **8b** (3.45 g, 10.18 mmol) with potassium thioacetate (2.32 g, 20.35 mmol) and chromatography of the crude mixture (EtOAc/hexane, 1:9) produced pure **9b** (2.45 g, 72%): mp 95–98 °C $[\alpha]^{20}_D$ = –8.4° (c 1, $CHCl_3$); R_f = 0.56 (EtOAc/hexane, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.99 (t, 1H, $J_{2,3}$ = 7.8 Hz, H-2), 5.18 (t, 1H, $J_{3,4}$ = 7.8 Hz, H-3), 4.91 (ddd, 1H, $J_{4,5a}$= 8.0 Hz, $J_{4,5b}$ = 4.8 Hz, H-4), 3.52 (dd, 1H, $J_{5a,5b}$ = 11.9 Hz, H-5a), 4.12 (dd, 1H, H-5b), 2.36 (s, 3H, S-Ac), 2.02, 2.03, 2.04 (3s, 9H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.62 (3OAc), 30.81 (S-Ac), 65.69 (C-5), 68.25 (C-4), 69.08 (C-3), 71.64 (C-2), 80.34 (C-1), 169.30, 169.58, 169.71 (3 OAc). CIMS: m/z 335.2 (MH^+, $C_{13}H_{18}O_8S$, calcd 334.3). Anal. Calcd for $C_{13}H_{18}O_8S$: C, 46.70; H, 5.43; S, 9.59. Found: C, 47.28; H, 5.62; S, 9.24.

1-(Thiomethylmercuric)-β-D-xyloside (**3**). The title compound was prepared by using the same procedure as described for **1**. Treatment of thioacetate **9b** (0.35 g, 1.06 mmol) with NaOMe (0.5 M, 0.6 mL) was followed by treatment with methylmercury(II)chloride (0.29 g, 1.16 mmol), which, after chromatography of the crude material on silica gel (MeOH/$CHCl_3$, 1:9) and crystallization from MeOH, produced 280 mg (71%) of pure **3**: mp 150–151°C; $[\alpha]_D^{20}$ = –20.8° (c 1, MeOH); $[a]_D^{20}$ = –1.8° (c 1, DMSO); R_f = 0.44 (1:4, MeOH-$CHCl_3$). ^{1}H NMR (400 MHz, D_2O): d 4.79 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 2.99 (t, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.20 (t, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.48 (ddd, 1H, $J_{4,5a}$ = 9.5 Hz, $J_{4,5b}$ = 5.5 Hz, H-4), 3.14 (t, 1H, $J_{5a,5b}$ = 11.5 Hz, H-5a), 3.80 (dd, 1H, H-5b), 0.62 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O): δ 12.52 (CH_3), 71.88 (C-5), 72.01 (C-4), 79.85 (C-3), 80.92 (C-2), 86.14 (C-1). CIMS: m/z 380.3 (MH^+, $C_6H_{12}HgO_4S$, Calcd 380.8). Anal. Calcd for $C_6H_{12}HgO_4S$: C,18.92; H 3.18. Found: C 19.20; H 3.06.

1-(Thiophenylmercuric)-β-D-xyloside (**4**). The title compound was prepared by using the same procedure as described for **1**. Treatment of thioacetate **9b** (2.87 g, 8.5 mmol) with NaOMe (0.5 M, 10 mL) was followed by treatment with phenylmercury(II)chloride (2.69 g, 8.60 mmol), which, after chromatography of the crude material on silica gel (MeOH/$CHCl_3$, 1:9) and crystallization from MeOH, produced 1.85 g (53%) of pure **4**: mp 157–158 °C (decomp.); $[\alpha]_D^{20}$ = 8.6° (c 1, DMSO); R_f = 0.56 (MeOH/$CHCl_3$,1:4). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.62 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 2.87 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 4.99 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.07 (ddd, 1H, $J_{4,5a}$ = 10.3 Hz, $J_{4,5b}$ = 5.7 Hz, H-4), 3.03 (t, 1H, $J_{5a,5b}$ = 11.6 Hz, H-5a), 3.72 (dd, 1H, H-5b), 7.18–7.41 (m, 5H, aromatic protons). ^{13}C NMR (100 MHz, DMSO-d_6): δ 69.71 (C-5), 70.14 (C-4), 78.00 (C-3), 78.15 (C-2), 84.04 (C-1), 127.55, 127.75, 128.15, 136.79 (aromatic carbons). CIMS: m/z 443.1 (MH^+, $C_{11}H_{14}HgO_4S$, Calcd 442.9). Anal. Calcd for $C_{11}H_{14}HgO_4S$: C, 29.83; H, 3.19. Found: C, 29.59; H, 3.47.

2,3,2′,3′,4′-Penta-O-acetyl-1-S-acetyl xylobioside (**9c**). The title compound was prepared by using the same procedure as described for **9a**. Treatment of **8c** (1.17 g, 2.11 mmol) with potassium thioacetate (0.48 g, 4.22 mmol) and chromatography of the crude mixture (EtOAc/hexane, 1:9) produced pure **9c** (0.76 g, 65%): mp 200–201°C $[\alpha]^{20}_D$ = –61.8° (c 1, $CHCl_3$); R_f = 0.33 (1:1 EtOAc–hexane). ^{1}H NMR (400 MHz, $CDCl_3$):

δ 5.22 (d, 1H, $J_{1,2}$ = 9.1 Hz, H-1), 4.93 (t, 1H, $J_{2,3}$ = 8.4 Hz, H-2), 5.13 (t, 1H, $J_{3,4}$ = 8.4 Hz, H-3), 4.86 (ddd, 1H, $J_{4,5a}$ = 7.7 Hz, $J_{4,5b}$ = 5.0 Hz, H-4), 3.45 (t, 1H, $J_{5a,5b}$ = 11.0 Hz, H-5a), 4.01 (dd, 1H, H-5b), 4.52 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1′), 4.78 (t, 1H, $J_{2',3'}$ = 7.8 Hz, H-2′), 5.07 (t, 1H, $J_{3',4'}$ = 7.8 Hz, H-3′), 3.80 (ddd, 1H, $J_{4',5'a}$ = 8.6 Hz, $J_{4',5'b}$ = 5.2 Hz, H-4′), 3.38 (dd, 1H, $J_{5'a,5'b}$ = 11.6 Hz, H-5′a), 4.08 (dd, 1H, H-5′b), 2.34 (s, 3H, S-Ac), 1.99, 2.00, 2.01, 2.02, 2.04 (5s, 15H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.61 (3 OAc), 20.70 (OAc), 20.76 (OAc), 30.83 (S-Ac), 61.68 (C-5′), 66.55 (C-2), 69.17 (C-2′), 70.42 (C-3), 70.60 (C-3′), 72.85 (C-4′), 74.30 (C-4), 80.52 (C-1), 99.63 (C-1'), 169.06, 169.48, 169.54, 169.72, and 169.90 (5 OAc). FABMS: *m*/*z* 551.0 (M^+ + H, $C_{22}H_{30}O_{14}S$, Calcd 550.5). Anal. Calcd for $C_{22}H_{30}O_{14}S$: C, 47.99; H, 5.49; S, 5.83. Found: C, 48.20; H, 5.64; S, 5.71.

1-(Thiomethylmercuric)-β-D-xylobioside (**5**). The title compound was prepared by using the same procedure as described for **1**. Treatment of thioacetate **9c** (0.26 g, 0.47 mmol) with NaOMe (0.5 M, 1.5 mL) was followed by treatment with methylmercury(II)chloride (0.12 g, 0.48 mmol), which, after chromatography of the crude material on silica gel (MeOH/$CHCl_3$, 1:9) and lyophylization, produced 0.15 g (65%) of pure **5**: $[\alpha]_D^{20}$ = –24.6° (*c* 1, MeOH); R_f = 0.38 (1:3, MeOH-$CHCl_3$). ^{1}H NMR (400 MHz, D_2O): δ 4.81 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 3.26 (t, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.33 (t, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.47 (ddd, 1H, $J_{4,5a}$ = 9.7 Hz, $J_{4,5b}$ = 5.5 Hz, H-4), 3.08 (t, 1H, $J_{5a,5b}$ = 11.5 Hz, H-5a), 3.95 (dd, 1H, H-5b), 4.29 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1′), 3.14 (t, 1H, $J_{2',3'}$ = 7.6 Hz, H-2′), 3.22 (t, 1H, $J_{3',4'}$ = 7.6 Hz, H-3′), 3.64 (ddd, 1H, $J_{4',5a'}$ = 9.7 Hz, $J_{4',5'b}$ = 5.3 Hz, H-4′), 3.03 (t, 1H, $J_{5'a,5'b}$ = 11.5 Hz, H-5′a), 3.81 (dd, 1H, H-5′b), 0.63 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O): δ 12.44 (CH3), 67.89 (C-5'), 69.71 (C-5), 71.87 (C-2), 75.45 (C-2'), 77.90 (C-3), 78.30 (C-3'), 79.21 (C-4′), 80.93 (C-4), 86.00 (C-1), 104.47 (C-1′). FABMS: *m*/*z* 512.9 (M^+ + H, $C_{11}H_{20}HgO_8S$, Calcd 513.1). Anal. Calcd for $C_{11}H_{20}HgO_8S$: C, 25.76; H 3.93. Found: C 25.64; H 4.22.

1-(Thiophenylmercuric)-β-D-xylobioside (**6**). The title compound was prepared by using the same procedure as described for **1**. Treatment of thioacetate **9c** (0.26 g, 0.47 mmol) with NaOMe (0.5 M, 1.5 mL) was followed by treatment with phenylmercury(II)chloride (0.15 g, 0.48 mmol), which, after chromatography of the crude material on silica gel (MeOH/$CHCl_3$, 1:9) and crystallization from MeOH, produced 0.15 g (51%) of pure **6**: mp 174–175 °C (decomp.); $[\alpha]_D^{20}$ = –12.9° (*c* 1, MeOH); R_f = 0.47 (MeOH-$CHCl_3$, 1:3). ^{1}H NMR (400 MHz, D_2O) *d* 4.76 (1H, $J_{1,2}$ = 9.3 Hz, H-1), 3.21 (t, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.31 (t, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.43 (ddd, 1H, $J_{4,5a}$ = 9.6 Hz, $J_{4,5b}$ = 5.6 Hz, H-4), 3.07 (t, 1H, $J_{5a,5b}$ =11.5 Hz, H-5a), 3.93 (dd, 1H, H-5b), 4.24 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 3.13 (t, 1H, $J_{2',3'}$ = 8.0 Hz, H-2′), 3.19 (t, 1H, $J_{3',4'}$ = 8.0 Hz, H-3′), 3.61 (ddd, 1H, $J_{4',5'a}$ = 9.8 Hz, $J_{4',5'b}$ = 5.5 Hz, H-4′), 3.03 (t, 1H, $J_{5'a,5'b}$ = 11.5 Hz, H-5′a), 3.77 (dd, 1H, H-5′b), 7.14-7.26 (m, 5H, aromatic protons). ^{13}C NMR (100 MHz, D_2O): δ 67.74 (C-5′), 69.67 (C-5), 71.69 (C-2), 75.27 (C-2′), 77.73 (C-3), 78.12 (C-3′), 79.03 (C-4′), 80.73 (C-4), 85.86 (C-1), 104.30 (C-1′), 131.26, 131.47, 139.21 (aromatic carbons). FABMS: *m*/*z* 575.1 (M^+ + H, $C_{16}H_{22}HgO_8S$, Calcd 575.0). Anal. Calcd for $C_{16}H_{22}HgO_8S$: C, 33.42; H 3.63. Found: C 33.71; H 3.88.

2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-2S-acetyl cellobioside (**9d**). The title compound was prepared by using the same procedure as described for **9a**. Treatment of **8d** (1.88 g, 2.69 mmol) with potassium thioacetate (0.61 g, 5.38 mmol) and chromatography of the crude mixture (EtOAc/hexane, 1:9) produced pure **9d** (1.08 g, 58%): mp 209–210 °C, $[\alpha]_D^{20}$ = –14.3 (*c* 1, $CHCl_3$); R_f = 0.54 (EtOAc/hexane, 3:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 3.76 (t, 1H, $J_{2,3}$ = 8.6 Hz, H-2), 5.20 (t, 1H, $J_{3,4}$ = 8.6 Hz, H-3), 5.02 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.70 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ =5.1 Hz, H-5), 4.33 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.1 Hz, H-6a), 5.10 (t, 1H, H-6b), 4.46 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.88 (t, 1H, $J_{2',3'}$ = 8.0 Hz, H-2'), 4.07 (dd, 1H, $J_{3',4'}$ = 8.0 Hz, H-3'), 4.45 (t, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 3.62 (ddd, 1H, $J_{4',5'}$ = 9.7 Hz, $J_{5',6'a}$ = 4.9 Hz, H-5′), 4.02 (dd, 1H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{5',6'a}$ = 4.9 Hz, H-6′a), 4.99 (t, 1H, H-6′b), 2.33 (s, 3H, S-Ac), 1.94, 1.97, 1.98, 1.99, 2.00, 2.06, 2.08 (7s, 21H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.43 (6OAc), 20.70 (OAc), 30.71 (SAc), 61.81 (C-5′), 61.95 (C-5), 68.17 (C-2), 69.49 (C-2′), 71.72 (C-3), 72.11 (C-3′), 73.02 (C-4′), 73.63 (C-4), 75.97 (C-6′), 77.31 (C-6), 80.18 (C-1), 100.54 (C-1′), 168.82, 169.13, 169.36, 169.42, 170.00, 170.05, 170.29 (7OAc). CIMS: m/z 695.1 (MH^+, $C_{28}H_{38}O_{18}S$, Calcd 694.6). Anal. Calcd for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found: C, 48.43; H, 5.65; S, 4.64.

2-(Thiomethylmercuric)-β-D-cellobioside (**7**). The title compound was prepared by using the same procedure as described for **1**. Treatment of thioacetate **9d** (0.49 g, 0.72 mmol) with NaOMe (0.5 M, 5.0 mL) was followed by treatment with methylmercury(II)chloride (0.19 g, 5.0 mmol), which, after chromatography of the crude material on silica gel (MeOH/$CHCl_3$, 1:9) and crystallization from MeOH, produced 0.41 g (67%) of pure **7**: mp 159–162 °C (decomp.); $[\alpha]_D^{20}$ = 23.8 (*c* 1, DMSO); R_f = 0.62 (1:1 MeOH-$CHCl_3$). ^{1}H NMR (400 MHz, D_2O): δ 4.86 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1), 3.05 (t, 1H, $J_{2,3}$ = 8.8 Hz, H-2), 3.38 (t, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 3.60 (t, 1H, $J_{4,5}$ = 7.3 Hz, H-4), 3.41 (ddd, 1H, $J_{4,5}$ = 7.3 Hz, $J_{5,6a}$ = 6.0 Hz, H-5), 3.55 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 6.0 Hz, H-6a), 3.76 (t, 1H, H-6b), 4.31 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1′), 3.12 (t, 1H, $J_{2',3'}$ = 8.3 Hz, H-2′), 3.31 (t, $J_{3',4'}$ = 8.3 Hz, H-3′), 3.47 (t, 1H, $J_{4',5'}$ = 9.2 Hz, H-4′), 3.29 (ddd, 1H, $J_{4',5'}$ = 9.2 Hz, $J_{5',6a'}$ = 5.2 Hz, H-5′), 3.52 (dd, 1H, $J_{6'a,6'b}$ = 12.7 Hz, $J_{5',6'a}$ = 5.2 Hz, H-6′a), 3.74 (t, 1H, H-6′b), 0.61 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O): δ 12.57 (CH_3), 63.14 (C-5′), 63.38 (C-5), 72.24 (C-2), 75.91 (C-2′), 78.29 (C-3), 78.42 (C-3′), 78.72 (C-4′), 80.85 (C-4), 81.61 (C-6′), 81.73 (C-6), 85.16 (C-1), 105.18 (C-1′). FABMS: m/z 573.2 (M^+ + H, $C_{13}H_{24}O_{10}S$, Calcd 572.9). Anal. Calcd for $C_{13}H_{24}O_{10}S$: C, 27.25; H, 4.22. Found: C, 27.12; H, 4.33.

Crystallographic

1-(Thiomethylmercuric)-β-D-xyloside (**3**) was recrystallized from methanol. Intensity data from clear colorless transparent plate crystals were collected at 293(2) K on a Nonius KappaCCD diffractometer. Table 4 provides crystallographic

and data collection details, and Table 5 contains the coordinates of the non-hydrogen atoms. Data reduction and space group determination were performed using the DENZO[18a] program. The SHELXS-97[18b] program was used for crystal structure solution by application of direct methods. The SHELXL-97[18c] program was used for refinement by full-matrix least squares. The numerical absorption correction was performed using the maXus[18d] program. Flack parameter (Table 4) was determined according to Flack.[19] All hydrogen atoms were geometrically placed and refined isotropicaly as constrained to the corresponding carbon atoms.

Inhibition Study

The homogeneous IXT6 (specific catalytic activity 179 U/mg) was isolated from the overproducing strain *Escherichia coli* [BL21(DE3)(pET9d-*xynA2*)], as previously described.[14] Steady-state kinetic studies were performed by following changes in visible absorbance using a Biochrom 4060 (Pharmacia) spectrophotometer equipped with a circulating water bath. Initial hydrolysis rates were determined by incubating appropriate concentrations of the substrate PNPX2[16c] in a 100 mM phosphate buffer (pH 7.0) at 50 °C within the spectrophotometer until thermally equilibrated. Following equilibration, IXT6 (200 µL, at a final concentration of 1.9 nM) was added, and the increase in the absorbance at 420 nm was monitored as a function of time. To determine K_i values, stock solutions of inhibitors **5** and **6** were prepared by dissolving in small amounts of DMSO, followed by adjusting the resulting homogeneous solution with a 100 mM phosphate buffer (pH 7.0) to final concentrations of 0.1–1.01% DMSO in the assay mixture. The enzyme was first preincubated with various inhibitor concentrations at 50 °C for 15 min, and the reactions were initiated by addition of PNPX2 substrate. Initial rates were determined by a least-squares fitting of the first 10% of the progress curve to a straight line. All samples were assayed in triplicate, and analogous results were obtained in two to four different experiments. The data were fitted to the competitive model using the equation: $Y = V[S]/[K(1+[I]/K_i) + [S]]$, employing the commercial GraFit software program. The K_i values were calculated either from the above treatment or from secondary replots of the slopes from initial double-reciprocal plots (1/v versus 1/[S]) versus inhibitor concentration.

Pesticidal Activity

Herbicidal activity. Herbicidal activity of tested compounds was studied with the weed *Amarantus palmeri*, the

Table 4. Selected experimental details for **3**

1. Crystal data	
$C_6H_{12}HgO_4S$	Mo $K\alpha$ radiation
M_r = 380.81	λ = 0.71070 Å
Orthorhombic	Cell parameters from 10373 reflections
$P2_12_12_1$	θ = 2.2–20.5°
a = 6.7510 (2) Å	µ = 16.115 mm^{-1}
b = 9.7140 (2) Å	T = 293 (2) K
c = 29.4770 (9) Å	Plate
V = 1933.08 (9) Å^3	Transparent colourless
Z = 8	0.19 × 0.16 × 0.05 mm
D_x = 2.617 Mg m^{-3}	
D_m not measured	
2. Data collection	
KappaCCD Nonius diffractometer	2930 reflections with I > 2 sigma (I)
Phi scan	R_{int} = 0.0540
Absorption correction: numerical	θ_{max} = 25.32°
T_{min} = 0.060, T_{max} = 0.447	h = –7→ 8
8559 measured reflections	k = –11→ 9
3286 independent reflections	l = -35→ 27
none standard reflections	
3. Refinement	
Refinement on F^2	$(\Delta/\sigma)_{max}$ = 0.001
R[F^2 > 2σ (F^2)] = 0.0329	Dr_{max} = 1.066 e Å^{-3}
wR (F^2) = 0.0655	Dr_{max} = 1.066 e Å^{-3}
S = 1.037	Scattering factors from *International Tables for Crystallography* (Vol. C)
3286 reflections	
223 parameters	Flack parameter = –0.010 (10)

Table 5. Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for **3**.

	x	y	z	U(eq)*
Hg(1A)	1885(1)	–6902(1)	4689(1)	44(1)
S(1A)	1464(4)	–8636(2)	4142(1)	45(1)
O(1A)	4930(8)	–7524(6)	3995(2)	43(2)
O(2A)	1059(8)	–7653(6)	3138(3)	39(2)
O(3A)	3630(10)	–5549(6)	2790(2)	40(2)
O(4A)	7770(8)	–5998(7)	3078(3)	46(2)
C(1A)	3327(11)	–8090(9)	3741(3)	34(2)
C(2A)	2595(11)	–7046(8)	3395(3)	31(2)
C(3A)	4287(11)	–6625(8)	3085(3)	30(2)
C(4A)	6074(12)	–6148(8)	3367(4)	36(2)
C(5A)	6625(13)	–7225(10)	3712(4)	47(3)
C(6A)	2110(16)	–5424(9)	5193(4)	54(3)
Hg(1B)	2357(1)	–1562(1)	4750(1)	49(1)
S(1B)	2745(4)	–2948(3)	4103(1)	55(1)
O(1B)	–822(8)	–1905(6)	3988(2)	39(2)
O(2B)	2337(8)	–3030(6)	3051(2)	46(2)
O(3B)	–1710(9)	–3486(5)	2711(2)	42(2)
O(4B)	–4358(8)	–1367(6)	3069(3)	41(2)
C(1B)	976(12)	–2103(8)	3740(4)	37(2)
C(2B)	591(11	–2992(9)	3323(3)	35(2)
C(3B)	–1126(12)	–2471(8)	3033(4)	33(2)
C(4B)	–2906(12)	–2082(8)	3336(3)	33(2)
C(5B)	–2249(12)	–1210(8)	3716(4)	38(2)
C(6B)	2163(16)	–490(10)	5345(4)	59(3)

*U(eq) is defined as one third of the trace of the orthogonalized Uij tensor**.**

agriculture plant *Corchorus olitorius,* and the parasitic weed *Cuscuta campestris*. Solutions of **1**, **3**, **5,** and **7** were prepared by dissolving an appropriate amount of the tested compound in sterile water to a final concentration of 1 mg/mL. Solutions of **2**, **4,** and **6** were prepared in 1% DMSO with final concentrations of 0.1, 0.1, and 0.02 mg/mL, respectively. The solution of tested compound (1 mL) was applied on a sterile filter disk located in the middle of a petri dish containing surface-sterilized seeds. Two petri dishes, each containing 100 seeds, were used for each compound. The dishes were sealed with Parafilm covered with aluminum foil, and placed in a growth chamber at 26 °C. The number of germinating seeds and shoot length were determined after four days. Water or 1% DMSO solution in water served as controls.[20a]

Fungicidal activity. Fungicidal activity of tested compounds was studied with phytophogenic fungi *Pythium aphanidermatum* (Phymycetes, Peronosporales, Phytiaceae), *Phytophora cytrophthora* (Phymycetes, Peronosporales, Phytiaceae), *Alternaria alternata* (Fungi Imperfecti, Moniliales, Sphaeriodeceae), *Macrophomina phaseolina* (Fungi Imperfecti, Sphaeropsidales, Sphaeriodeceae), and *Fusarium oxysporum* f. sp. *basilici* (Fungi Imperfecti, Moniliales, Tubercullariaceae). Sterile aqueous solutions of **3**, **4,** and **7** were mixed with a potato dextrose agar medium in petri dishes to give final concentrations of 0.1, 1, 10, and 50 ppm. An 8-mm agar disk with fungal mycelia from a 7-day-old culture was placed in the center of the petri dishes containing the compound-amended agar. The dishes were incubated under continuous fluorescent light at 26 °C for 1–7 days according to the tested fungus, after which the colony diameter was measured in two perpendicular directions.[20b] Petri dishes containing potato dextrose agar alone, or embedded with the fungicides Delsene, Vectra, and Rubigan, served as control or reference, respectively.

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