Synthesis of 1,2- and 1,3-*N*-Linked Disaccharides of 5-Thio-α-D-mannopyranose as Potential Inhibitors of the Processing Mannosidase Class I and Mannosidase II Enzymes

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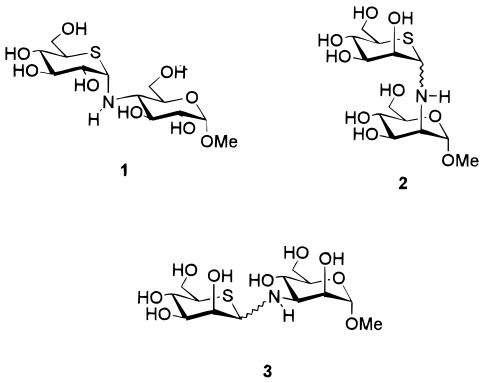
The syntheses of S/N acetal heteroatom analogues of 1,2- and 1,3-linked mannopyranose disaccharides are described. The compounds are analogues of the Man-α-(1→2)-Man and Man-α-(1→3)-Man disaccharide components of oligosaccharides found in *N*-glycoproteins that are cleaved by trimming mannosidases during glycoprotein processing. Glycosylamine formation, without the necessity of hydroxyl group protection, proceeded through acid-catalyzed condensation reactions of 5-thio-D-mannose with either methyl 2-amino-2-deoxy- or 3-amino-3-deoxy-α-D-mannopyranoside to give methyl 2-amino-2-deoxy-2-*N*-(5-thio-α/*β*-D-mannopyranosyl)-α-D-mannopyranoside (**2**) or methyl 3-amino-3-deoxy-3-*N*-(5-thio-α/*β*-D-mannopyranosyl)-α-D-mannopyranoside (**3**), respectively. The superiority of mercuric chloride over acetic acid as a catalyst for this reaction is reported. Acetylation of the anomeric mixtures gave the heptaacetates from which the major *β*′-isomers could be separated by chromatography and/or crystallization.

Introduction

Glycosidases are necessary for the processing of oligosaccharide portions of glycoproteins that can ultimately yield complex structures. The presence of these complex structures on cell surfaces has been associated with cellular recognition and has implications in tumor metastasis and the infectivity of viruses.[1] Inhibition of oligosaccharide processing by glycosidase inhibitors might constitute, therefore, an effective therapeutic strategy. As part of a program for investigating the potential of heteroatom analogues of disaccharides as glycosidase inhibitors, we have reported the synthesis of maltoside and kojibioside derivatives in which the ring and/or the interglycosidic oxygen atoms have been replaced with sulfur or selenium.[2−5] Most recently, a new class of disaccharides in which the ring oxygen of the nonreducing sugar is replaced by sulfur and the interglycosidic oxygen atom is replaced by NH was synthesized.[6] The S/N acetal analogue of methyl maltoside **1** was found to bind to glucoamylase as a competitive inhibitor of maltose binding with a K_i of 4 μM. In an extension of this approach, we have now chosen the class I mannosidase and mannosidase II enzymes as suitable targets for inhibition.

Our previous report demonstrated that S/N acetal disaccharide analogues could be prepared in moderate yields by gentle heating of a methanol solution of unprotected 5-thio-α-D-glucose and excess methyl 2-amino-2-deoxy- or 4-amino-4-deoxy-α-D-glucopyranoside with a catalytic amount of acetic acid. Presumably the reaction proceeded through the open chain imine intermediate (Scheme 1). Closure of this intermediate by intramolecular 6-*exo-trig* nucleophilic attack of the thiol must be preferred over reversion to starting materials. The reaction was, however, slow, and attempts to increase the rate by heating to higher temperatures resulted in decreased yields due to the Amadori rearrangement of the product. We hypothesized that the slow rate reflected the inefficient activation of the sulfur atom (a soft Lewis base) by protic acids (hard Lewis acids). Mercuric salts are known to complex strongly with thiols and should catalyze the formation of the required open chain intermediate.



We report herein the preparation of S/N analogues of α/*β*-Man-(1→2)-α-Man-OMe and α/*β*-Man-(1→3)-α-Man-OMe disaccharides, **2** and **3**, in which the nonreducing portion is 5-thiomannopyranose and the interglycosidic atoms are NH. In addition, we describe an improved method for the condensation of 5-thio sugars with deoxyamino sugars using mercuric chloride catalysis.

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Scheme 1

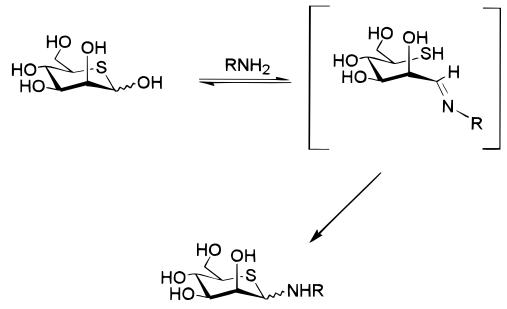
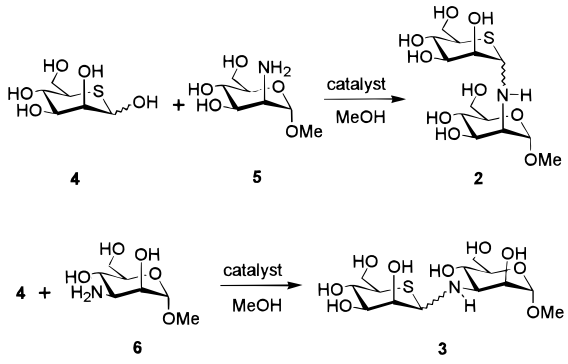


Table 1. Comparison of HOAc- and $HgCl_2$-Catalyzed Reactions

reaction	catalyst	mole ratio[a]	time (h)/ temp (°C)	yield (%)	β:α
4 + **5**	AcOH	1:1.5:0.05	26/65	67%	1.5:1
	$HgCl_2$	1:1.0:0.015	4/45	82%	1.7:1
4 + **6**	AcOH	1:3.0:0.05	42/65	57%	3.3:1
	$HgCl_2$	1:1.0:0.015	4.5/45	74%	2.7:1

[a] **4**:**5**:catalyst or **4**:**6**:catalyst.

Scheme 2



Results and Discussion

The required monosaccharide derivatives 5-thio-D-mannose (**4**),[7] methyl 2-amino-2-deoxy-α-D-mannopyranose (**5**),[8] and methyl 3-amino-3-deoxy-α-D-mannopyranose (**6**)[9] were synthesized by the literature methods. Reactions of **4** with either of **5** or **6** were performed using the reaction conditions specified in Table 1 and either HOAc or $HgCl_2$ as catalysts (see Scheme 2). The $HgCl_2$ catalyzed reactions gave higher yields, in less time, under milder conditions, and only required a stoichiometric amount of the amino sugar. Products **2** and **3** were produced as α/β mixtures with the β-isomer predominating in all cases. The products were stable toward hydrolysis in neutral water for at least 2 weeks, although slow mutarotation over a period of 2−3 days could be observed by ^{1}H NMR until an equilibrium β:α ratio of 3.7:1 for **2** and 4.0:1 for **3** was reached. The isomers were not separable by column chromatography but were characterized by NMR spectroscopy using ^{1}H−^{1}H and ^{1}H−^{13}C correlated experiments. Assignment of anomeric stereochemistry to the isomers of the 5-thiomannopyranose ring was based on the observed γ-gauche upfield shifts observed for C3 and C5 of the minor isomer for both **2** and **3**. This is typically observed for α-pyranosides relative to β-pyranosides as a result of steric compression at C3 and C5 caused by the gauche heteroatom in the α-isomers. The usual technique for assigning stereochemistry of mannopyranosides is by measurement of J_{C1-H1} and comparison to the typical values of 170 Hz for α-isomers and 160 Hz for β-isomers. We have previously noted that such general trends are not observed for S/N acetals,[6] and, in the present case, both **3**α and **3**β were observed to have identical $J_{C1'-H1'}$ coupling constants of 152 Hz. The isomers of **2** and **3** exhibited the typical ^{1}H−^{1}H coupling constants of D-mannopyranosides in the usual $^4C_1$ conformation with the exception of slightly larger than normal $J_{1',2'}$ values (**2**α, $J_{1',2'}$ = 3.8 Hz; **2**β, $J_{1',2'}$ = 1.5 Hz; **3**α, $J_{1',2'}$ = 3.7 Hz; **3**β, $J_{1',2'}$ = 1.3 Hz). Typically α-D-mannopyranosides have $J_{1,2}$ values of 1−2 Hz, while β-D-mannopyranosides have $J_{1,2}$ values of <1 Hz. In the case of **2,** selective crystallization from CH_2Cl_2−MeOH and recrystallization from $CHCl_3$−MeOH gave >95% isomerically pure samples of **2**β. Analysis of these samples by ^{1}H NMR spectroscopy showed persistent contamination by CH_2Cl_2 or $CHCl_3$, which must be due to the inclusion of solvent molecules in the crystals. This was reflected in the broad, variable melting point range observed. Both **2** and **3** were further characterized by the observation of M + H ions in the high-resolution FAB mass spectra.

Conventional acetylation of **2** and **3** gave heptaacetate derivatives **7** and **8**, respectively. These α/β mixtures were more amenable to separation by chromatography and/or crystallization, and pure crystalline samples of the major isomers **7**β or **8**β could be secured. Fractions of the α-isomers, **7**α and **8**α, containing small amounts of the corresponding β-isomers, were also readily characterized by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectra for each of the four heptaacetate isomers showed clear NH coupling to H1′ and H2 in the case of the isomers of **7** and to H1′ and H3 in the case of the isomers of **8**. This may be attributed to slow exchange of the NH protons due to intramolecular H-bonding. In the case of the β-isomers **7**β and **8**β, hydrogen-bonding of the NH to the 2′-*O*-acetate carbonyl oxygen would enforce a NH−H1′ dihedral angle of approximately 180° leading to the large $J_{NH,H1'}$ values observed (11.7 Hz for **7**β and 10.9 Hz for **8**β). Alternatively, for the α-isomers, **7**α and **8**α, this mode of hydrogen bonding is not possible, and smaller $J_{NH,H1'}$ coupling constants resulting from the preferred orientation about the C1′-NH−C2/C3 linkage governed principally by the *exo*-anomeric effect are observed (8.0 Hz for **7**α and 5.0 Hz for **8**α). Similar arguments which invoke preferential hydrogen bonding between the NH and either the 3-*O*-acetate in **7**α or the 2-*O*-acetate in **8**α would explain the intermediate $J_{NH,H2}$ value for **7**α (8.0 Hz) and the large $J_{NH,H3}$ value for **8**α (10.3 Hz) compared to the small $J_{NH,H2}$ (2.5 Hz) and $J_{NH,H3}$ (3.7 Hz) coupling constants observed for the β-isomers **7**β and **8**β, respectively. A NOESY spectrum of the **8**α/β mixture showed clear NOE contacts of H1′ with H3′ and H5′ for the major isomer but no such contacts for the minor isomer, thus confirming the assignment of the β-configuration to the major isomer of **8** and, hence, **3**. Some of the isomers of **7** and **8** exhibited other anomalous coupling constants. In particular, compound **7**α showed $J_{1',2'}$ of 7.1 Hz and $J_{3'4'} = J_{4'5'}$ of 6.6 Hz, coupling constants which are not typical for α-mannopyranosides. We attribute these observations to substantial deviation from

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$^4C_1$ stereochemistry in the 5-thio sugar. The NH hydrogen bonding mentioned above may be the origin of the distortions from the expected conformation in the 5-thiomannopyranose moiety. It is noteworthy that there is literature evidence[10] for an atypical conformation of 5-thio-β-D-mannopyranose pentaacetate, for which smaller than expected $J_{3,4}$ and $J_{4,5}$ values were observed. A more definitive conformational study of these derivatives is in progress.

In summary, an efficient method for the production of *N*-linked disaccharide derivatives having 5-thiomannopyranose at the nonreducing end has been developed. The method is simple and high-yielding and requires no protection/deprotection steps. The occurrence of compounds **2** and **3** as anomeric mixtures is not a serious concern since we expect the enzymes to bind the α-anomer preferentially, as was the case with glucoamylase binding of **1** but not its anomer.[6]

Experimental Section

General Methods. Chromatographic and spectroscopic techniques were as previously described.[6] Optical rotations were measured at 21 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz for proton and carbon, respectively. All assignments were confirmed with the aid of two-dimensional ^{1}H,^{1}H (COSYDFTP) or ^{1}H,^{13}C (INVBTP) experiments using standard Bruker pulse programs. High-resolution fast atom bombardment mass spectra (FAB HRMS) were recorded from a thioglycerol matrix at 8 kV.

General Procedure for HOAc-Catalyzed Reactions. A mixture of 5-thio-D-mannopyranose (**4**) and the deoxyamino sugar **5** or **6** (1.5−3.0 mmol/mmol of **4**) in MeOH (5−20 mL/mmol of **4**) containing a catalytic amount of HOAc was refluxed until TLC analysis (4:2:1 EtOAc−MeOH−H_2O) indicated that little further change was occurring. Solvents were removed in vacuo and the residue purified by column chromatography on silica gel using 4:2:1 EtOAc−MeOH−H_2O as the eluent.

General Procedure for $HgCl_2$-Catalyzed Reactions. An equimolar mixture of **4** and the deoxyamino sugar **5** or **6** in MeOH (6.0 mL/mmol of **4**), containing a catalytic amount of $HgCl_2$, was refluxed until TLC analysis (4:2:1 EtOAc−MeOH−H_2O) indicated that little further change was occurring. The mixture was cooled to room temperature and saturated with H_2S by bubbling gas from a cylinder into the mixture for approximately 30 s. This produced a black precipitate of mercuric sulfide. The mixture was filtered through Celite and the filter cake rinsed thoroughly with additional methanol. The colorless filtrate was concentrated in vacuo and the residue purified by column chromatography on silica gel using 4:2:1 EtOAc−MeOH−H_2O as the eluent. The purified products (**2** or **3**) were dissolved in water and lyophilized to give amorphous powders. Crystallization of **2** from MeOH−CH_2Cl_2 and recrystallization from $CHCl_3$−MeOH gave **2**β (mp 127−140 °C), which was >95% isomerically pure by ^{1}H NMR but which was contaminated by $CHCl_3$ that could not be removed by application of high vacuum.

General Procedure for Acetylations. A solution of **2** or **3** (50−100 mg) in pyridine (2 mL) and Ac_2O (1.5 mL) containing a single crystal of *N*,*N*-(dimethylamino)pyridine was kept at room temperature for 1−2 h. Volatile material was removed by application of vacuum. The residue was applied to a silica gel column with a minimum amount of EtOAc and eluted with 3:2 EtOAc−hexanes to give an α/β mixture of the heptaacetate derivative **7** or **8** in quantitative yield. Pure samples of the major β-isomers were obtained by selective crystallization from Et_2O−hexanes for **7**β or EtOAc−hexanes for **8**β.

Methyl 2-Amino-2-deoxy-2-*N*-(5-thio-α/β-D-mannopyranosyl)-α-D-mannopyranoside (2). Ratio of β:α = 1.7:1 by ^{1}H NMR. FAB HRMS. Calcd for M + H: 372.1328. Found: 372.1333. Anal. Calcd for $C_{13}H_{25}NO_9S$: C, 42.04; H, 6.78; N, 3.77. Found: C, 41.79; H, 6.77; N, 3.77 (for the α/β mixture).

Methyl 2-Amino-2-deoxy-2-*N*-(5-thio-β-D-mannopyranosyl)-α-D-mannopyranoside (2β). ^{1}H NMR (D_2O): δ 4.77 (obscured by HOD resonance, H1), 4.33 (1H, d, $J_{1,2'}$ = 1.5 Hz, H1′), 4.11 (1H, dd, $J_{2',3'}$ = 2.9 Hz, H2′), 3.93 (1H, dd, $J_{5',6a'}$ = 3.2, $J_{6a',6b'}$ = 11.9 Hz, H6a′), 3.90 (1H, dd, $J_{2,3}$ = 5.3, $J_{3,4}$ = 9.4 Hz, H3), 3.84 (1H, dd, $J_{5,6a}$ = 1.9, $J_{6a,6b}$ = 12.1 Hz, H6a), 3.74 (1H, dd, $J_{5,6b}$ = 5.3 Hz, H6b), 3.72 (1H, dd, $J_{5',6b'}$ = 5.6 Hz, H6b′), 3.68 (1H, dd, $J_{3',4'}$ = 9.7, $J_{4',5'}$ = 10.4 Hz, H4′), 3.60−3.51 (2H, m, H4 and H5), 3.43 (1H, dd, H3′), 3.37 (3H, s, OCH_3), 3.28 (1H, dd, $J_{1,2}$ = 1.2 Hz, H2), 2.82 (1H, ddd, H5′); ^{13}C NMR (D_2O): δ 101.70 (C1), 77.98 (C3′), 76.76 (C2′), 75.08 (C4), 72.97 (C4′), 72.03 (C3), 69.27 (C5), 65.05 (C1′), 63.78 (C6′), 63.65 (C6), 60.74 (C2), 57.67 (OCH_3), 49.04 (C5′).

Methyl 2-Amino-2-deoxy-2-*N*-(5-thio-α-D-mannopyranosyl)-α-D-mannopyranoside (2α). ^{1}H NMR (D_2O): δ 5.00 (1H, d, $J_{1,2}$ = 1.5 Hz, H1), 4.18 (1H, dd, $J_{1,2'}$ = 3.8, $J_{2',3'}$ = 2.6 Hz, H2′), 4.02 (1H, d, H1′), 3.92 (1H, dd, H6a′), 3.75 (2H, m, H4′, H6b′), 3.70 (1H, dd, H3), 3.14 (1H, dd $J_{2,3}$ = 3.6 Hz, H2), 3.36 (br s, 4H, H2′, OCH_3), 2.99 (1H, ddd, $J_{5',6a'}$ = 3.6, $J_{5',6b'}$ = 7.0, $J_{4',5'}$ = 10.0 Hz, H5′), other resonances not assignable due to overlap with signals of β-isomer **2**β. ^{13}C NMR (D_2O): δ 103.65 (C1), 75.57 (C2′), 74.92, 74.67, 73.02, 72.92, 70.07(C1′), 69.86 (C6′), 63.9, 63.40 (C2), 63.05, 56.40 (OCH_3), 47.10 (C5′).

Methyl 2-Amino-2-deoxy-2-*N*-(2,3,4,6-tetra-*O*-acetyl-5-thio-β-D-mannopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (7β). Colorless crystalline solid, mp 97−98 °C (Et_2O−hexanes). ^{1}H NMR ($CDCl_3$): δ 5.57 (1H, dd, $J_{1',2'}$ = 2.2, $J_{2',3'}$ = 2.9 Hz, H2′), 5.36 (1H, t, $J_{3',4'}$ = $J_{4',5'}$ = 9.4 Hz, H4′), 5.30 (1H, dd, $J_{2,3}$ = 4.4, $J_{3,4}$ = 9.8 Hz, H3), 5.09 (1H, t, $J_{4,5}$ = 9.8 Hz, H4), 4.90 (1H, dd, H3′), 4.61 (1H, d, $J_{1,2}$ = 1.6 Hz, H1), 4.27 (1H, dd, $J_{1',NH}$ = 11.7 Hz, H1′), 4.23−4.18 (2H, m, H6a′, H6b′), 4.18 (1H, dd, $J_{5,6a}$ = 4.8, $J_{6a,6b}$ = 12.2 Hz, H6a), 4.09 (1H, dd, $J_{5,6}$ = 2.4 Hz, H6b), 3.86 (1H, ddd, H5), 3.38 (4H, br s, OCH_3, H2), 3.12(1H, ddd, $J_{5',6a'}$ = $J_{5',6b'}$ = 3.3 Hz, H5′), 2.21, 2.06 (6H), 2.03 (6H), 2.00, 1.99 (21H, 5s, 7 COCH_3), 1.87 (1H, dd, $J_{2,NH}$ = 2.5 Hz). ^{13}C NMR ($CDCl_3$): δ 170.59, 170.37, 170.16, 169.64, 169.56 (3C), (7 *C*OCH$_3$), 99.20 (C1), 72.49 (C3′), 72.31 (C2′), 70.16 (C3), 69.14 (C4′), 68.31 (C5), 66.24 (C4), 62.77 (C6), 62.42 (C6′), 60.34 (C1′), 56.66 (C2), 55.21 (OCH_3), 41.58 (C5′), 20.84, 20.70, 20.59 (3C), 20.56, 20.47 (7 COCH_3). Anal. Calcd for $C_{27}H_{39}NO_{16}S$: C, 48.72; H, 5.90; N, 2.10. Found: C, 49.09; H, 6.12; N, 2.08.

Methyl 2-Amino-2-deoxy-2-*N*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-mannopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (7α). ^{1}H NMR ($CDCl_3$): δ 5.38 (1H, dd, $J_{1',2'}$ = 7.1, $J_{2',3'}$ = 2.8 Hz, H2′), 5.39 (1H, t, $J_{3',4'}$ = $J_{4',5'}$ = 6.6 Hz, H4′), 5.28 (1H, dd, H3′), 5.20(1H, dd, $J_{2,3}$ = 3.8, $J_{3,4}$ = 9.8 Hz, H3), 5.14 (1H, t, $J_{4,5}$ = 9.8 Hz, H4), 4.66 (1H, d, $J_{1,2}$ = 1.7 Hz, H1), 4.34 (1H, dd, $J_{5',6a'}$ = 6.5, $J_{6a',6b'}$ = 11.6 Hz, H6a′), 4.21 (1H, dd, $J_{5,6a}$ = 4.6, $J_{6a,6b}$ = 12.3 Hz, H6a), 4.17 (1H, dd, $J_{5',6b'}$ = 7.0 Hz, H6b′), 4.13 (1H, dd, $J_{1',NH}$ = 8.0 Hz, H1′), 4.09 (1H, dd, $J_{5,6}$ = 2.4 Hz, H6b), 3.85 (1H, ddd, H5), 3.38 (3H, s, OCH_3), 3.36 (1H, m, H2), 3.32(1H, ddd, H5′), 2.16, 2.14, 2.08, 2.07, 2.05, 2.04, 2.02 (21H, 7s each 3H, 7 COCH_3), 1.60 (1H, br t, $J_{1',NH}$ = $J_{2,NH}$ 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 170.84, 170.44 (2C), 170.16, 169.80, 169.49, 169.15 (7 *C*OCH$_3$), 101.07 (C1), 71.42 (C3), 70.94 (C2′), 70.68 (C3′), 69.02 (C4′), 68.13 (C5), 66.48 (C4), 63.04 (C6′), 62.14 (C6), 60.88 (C1′), 57.02 (C2), 55.24 (OCH_3), 41.55 (C5′), 20.83 (2C), 20.77 (2C), 20.68 (2C), 20.59 (7 COCH_3).

Methyl 3-Amino-3-deoxy-3-*N*-(5-thio-α/β-D-mannopyranosyl)-α-D-mannopyranoside (3). Ratio of β:α = 2.7:1 by ^{1}H NMR. FAB HRMS. Calcd for M + H: 372.1328. Found: 372.1351. Anal. Calcd for $C_{13}H_{25}NO_9S$: C, 42.04; H, 6.78; N, 3.77. Found: C, 41.91; H, 6.89; N, 3.59 (for the α/β mixture).

Methyl 3-Amino-3-deoxy-3-*N*-(5-thio-β-D-mannopyranosyl)-α-D-mannopyranoside (3β). ^{1}H NMR (D_2O): δ 4.73 (d 1H, $J_{1,2}$ = 1.4 Hz, H1), 4.34 (d, 1H, $J_{1,2'}$ = 1.3 Hz, H1′), 4.11 (1H, dd, $J_{2',3'}$ = 2.9 Hz, H2′), 3.98 (1H, dd, $J_{2,3}$ = 3.0 Hz, H2), 3.93 (1H, dd, $J_{5',6a'}$ = 3.4, $J_{6a',6b'}$ = 11.9 Hz, H6a′), 3.84 (1H, dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 12.1 Hz, H6a), 3.71 (1H, dd, $J_{5',6b'}$ = 5.9 Hz, H6b′), 3.69 (1H, dd, $J_{5,6b}$ = 7.6 Hz, H6b), 3.68 (1H, dd, $J_{3',4'}$ = 9.6, $J_{4',5'}$ = 10.3 Hz, H4′), 3.60 (1H, ddd, H5), 3.50 (1H,

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t, $J_{3,4}$ = 10.0 Hz, H4), 3.44 (1H, dd, H3′), 3.38 (3H, s, OCH_3), 3.06 (1H, dd, H3), 2.86 (1H, ddd, H5′). ^{13}C NMR (D_2O): δ 102.93 (C1), 77.80 (C3′), 76.54 (C2′), 75.26 (C5), 72.79 (C4′), 68.38 (C2), 67.60 (C4), 63.62, 63.56 (C6 and C6′), 62.50 (C1′), 58.74 (C3), 57.42 (OCH_3), 48.85 (C5′).

Methyl 3-Amino-3-deoxy-3-*N*-(5-thio-α-D-mannopyranosyl)-α-D-mannopyranoside (3α). ^{1}H NMR (D_2O): δ 4.69 (d, 1H, $J_{1,2}$ = 1.5 Hz, H1), 4.17 (1H, dd, $J_{1,2'}$ = 3.7 Hz, $J_{2',3'}$ = 2.8 Hz, H2′), 4.11 (2H, m, H1′ and H2), 3.90 (1H, dd, $J_{5',6a'}$ = 3.5, $J_{6a',6b'}$ = 11.8 Hz, H6a′), 3.84 (1H, dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 12.0 Hz, H6a), 3.78 (1H, dd, $J_{5',6b'}$ = 6.8 Hz, H6b′), 3.74 (1H, dd, $J_{3',4'}$ = 9.2 H4′), 3.70 (1H, dd, $J_{5,6b}$ = 3.1 Hz, H6b), 3.69 (1H, dd, H3′), 3.60 (1H, ddd, $J_{4,5}$ = 10.0 Hz H5), 3.49 (1H, t, $J_{3,4}$ = 10.0 Hz, H4), 3.36 (3H, s, OCH_3), 3.03 (1H, ddd, H5′), 2.98 (1H, dd, H3). ^{13}C NMR (D_2O): δ 103.17 (C1), 75.59, 75.26, 74.46, 72.63 (C4′), 71.86 (C2′), 69.26 (C4), 68.76 (C1′) 63.7, 63.6 (C6 and C6′), 61.24 (C3), 57.26 (OCH_3), 46.76 (C5′).

Methyl 3-Amino-3-deoxy-3-*N*-(2,3,4,6-tetra-*O*-acetyl-5-thio-*β*-D-mannopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (8*β*). ^{1}H NMR ($CDCl_3$): δ 5.38 (1H, dd, $J_{1',2'}$ = 1.9, $J_{2',3'}$ = 3.0 Hz, H2′), 5.33 (1H, t, $J_{3',4'}$ = $J_{4',5'}$ = 10.2 Hz, H4′), 5.07 (1H, dd, $J_{1,2}$ = 1.6, $J_{2,3}$ = 3.5 Hz, H2), 4.89 (1H, dd, H3′), 4.80 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 10.2 Hz, H4), 4.70 (d, 1H, H1), 4.29 (1H, dd, $J_{5,6a}$ = 5.1, $J_{6a,6b}$ = 12.2 Hz, H6a), 4.26 (1H, dd, $J_{1',NH}$ = 10.9 Hz, H1′), 4.20 (1H, dd, $J_{5',6a'}$ = 6.0, $J_{6a',6b'}$ = 11.8 Hz, H6a′), 4.14 (1H, dd, $J_{5',6b'}$ = 4.3 Hz, H6b′), 4.09 (1H, dd, $J_{5,6b}$ = 2.3 Hz, H6b), 3.95 (1H, ddd, H5), 3.39 (3H, s, OCH_3), 3.37 (1H, dt, $J_{3,NH}$ = 3.7 Hz, H3), 3.11 (1H, ddd, H5′), 2.19, 2.14, 2.11, 2.09, 2.08, 2.02, 1.95 (21H, 7s each 3H, 7 COCH_3), 1.78 (1H, br dd, NH). ^{13}C NMR ($CDCl_3$): δ 170.70, 170.62, 170.48, 170.41 (2C), 169.79, 169.50 (7 *C*OCH$_3$), 98.39 (C1), 72.51 (2C, C2′ and C3′), 69.61 (C2), 69.12 (C2), 68.22 (C1), 67.15 (C4), 62.67, 62.55 (C6 and C6′), 60.25 (C1′), 55.48 (C3), 55.21 (OCH_3), 41.71 (C5′), 21.17, 20.73 (4C), 20.64, 20.51 (7 COCH_3). Anal. Calcd for $C_{27}H_{39}NO_{16}S$: C, 48.72; H, 5.90; N, 2.10. Found: C, 48.84; H, 5.84; N, 2.14.

Methyl 3-Amino-3-deoxy-3-*N*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-mannopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (8α). ^{1}H NMR ($CDCl_3$): δ 5.33 (1H, m, H4′), 5.22 (2H, m, H2′ and H3′), 5.08 (1H, dd, $J_{1,2}$ = 1.6, $J_{2,3}$ = 3.4 Hz, H2), 4.98 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H4), 4.74 (1H, d, H1), 4.30 (1H, dd, $J_{5',6a'}$ = 6.3, $J_{6a',6b'}$ = 12.0 Hz, H6a′), 4.28 (1H, dd, $J_{5,6a}$ = 5.4, $J_{6a,6b}$ = 12.1 Hz, H6a), 4.17 (1H, dd, $J_{5',6b'}$ = 5.0 Hz, H6b′), 4.08 (1H, dd, $J_{5,6}$ = 2.4 Hz, H6b), 3.98 (1H, br t, $J_{1',2'}$ = $J_{1',NH}$ = 5.0 Hz, H1′), 3.95 (1H, ddd, H5), 3.40 (1H, ddd, H5′), 3.38 (3H, s, OCH_3), 3.35 (1H, td, $J_{3,NH}$ = 10.3 Hz, H3), 2.18, 2.14, 2.13, 2.10, 2.08, 2.05, 2.00 (21H, 7s each 3H, 7 COCH_3), 1.67 (1H, br dd, NH). ^{13}C NMR ($CDCl_3$): δ 170.69, 170.67, 170.51, 170.33, 170.28, 169.49, 169.44 (7 *C*OCH$_3$), 97.97 (C1), 71.26 (C3′), 70.27 (C2), 70.17 (C2′), 69.11 (C4′), 68.56 (C5), 67.38 (C4), 62.52, 62.34 (C6, C6′), 58.93 (C1′), 55.16 (2C, C3 and OCH_3), 40.32 (C5′), 20.97, 20.95, 20.72 (2C), 20.62 (2C), 20.59 (7 COCH_3).

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