Extracellular Carbohydrate Metabolites from *Streptomyces coelicolor* A3(2)

Stanislav Pospíšil,* Petr Sedmera, Petr Halada, and Miroslav Petříček

Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic

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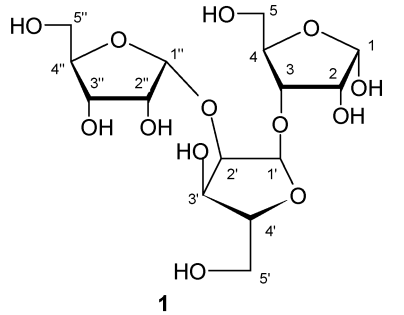
A new ribose trisaccharide, α-Rib*f*-(1→2)-α-Rib*f*-(1→3)-α-Rib*f* (**1**), was isolated together with 5-*O*-(α-mannosyl)-*myo*-inositol (**2**), 2-*O*-(α-mannosyl)-*myo*-inositol (**3**), trehalose (**4**), and D-ribulose (**5**) from a submerged cultivation of *Streptomyces coelicolor* A3(2). The structures of these compounds were elucidated by spectroscopic and chemical methods. Concentrations of these compounds in the medium were in the range from 0.04 (**1**) to 0.5 (**4**) mg/mL.

Streptomycetes are soil-dwelling, filamentous, Gram-positive saprophytic bacteria. They are responsible for over 50% of the known microbial metabolites, including many antibiotics used in human and veterinary medicine. *Streptomyces coelicolor* A3(2) is the most thoroughly studied member of the genus.[1] The sequencing of its genome was completed 5 years ago.[2] This strain is known to produce polyketides (actinorhodins,[3−5] kalafungin,[3] phenocyclinone,[3] 5-hydroxyaloesaponarin[4]), butanolides,[6,7] siderophores,[8−10] alkylprodiginines,[11] cyclic depsipeptides,[12] aminocoumarins,[13] α- and γ-pyrone derivatives,[14,15] and various terpenic compounds.[16−18] However, no carbohydrates have been described among its metabolites. This paper reports the isolation, identification, and structure elucidation of several extracellular carbohydrates found in the fermentation broth of *S. coelicolor* A3(2). These compounds include trisaccharide α-Rib*f*-(1→2)-α-Rib*f*-(1→3)-α-Rib*f* (**1**), 5-*O*-(α-mannosyl)-*myo*-inositol (**2**), 2-*O*-(α-mannosyl)-*myo*-inositol (**3**), trehalose (**4**), and D-ribulose (**5**).



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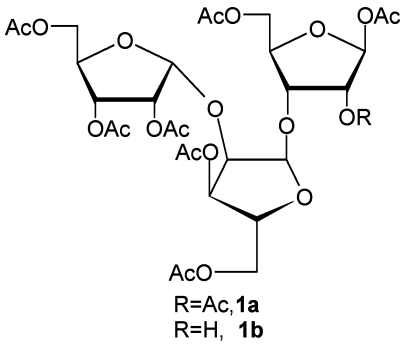
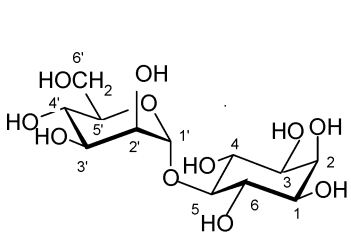


R=Ac, **1a**
R=H, **1b**

Results and Discussion

Submerged cultivations of *S. coelicolor* A3(2) in the semisynthetic medium containing $(NH_4)_2SO_4$, yeast extract, glycerol, and mineral salts afforded the best production of the carbohydrates, which were easily detected directly in the fermentation broth by TLC and orcinol reagent.[19] Upon removal of cells by centrifugation, the concentrated supernatant was acidified, and pigments together with nonpolar substances were extracted with chloroform. Chromatography of the neutralized aqueous phase on Toyopearl HW40F yielded compounds **1**, **4**, **5**, and a mixture of **2** and **3**. Column chromatography on Si gel in EtOAc−AcOH−MeOH−H_2O was further needed to separate the mixture of **2** and **3** from impurities. Chromatography on Sephadex LH-20 in MeOH represented the last purification step.



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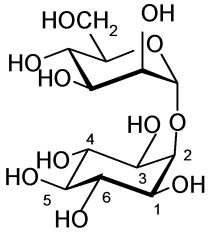

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The molecular formula of the most polar compound (**1**) was determined as $C_{15}H_{26}O_{13}$ by a combination of ESIMS (ions $[M + Na]^+$ and $[M + K]^+$ at *m*/*z* 437 and 453) and ^{13}C NMR (15 signals, three −OCHO−, nine −OCH−, and three $-OCH_2-$; Table 1). An HMBC experiment (Figure 1) indicated the presence of three pentofuranose rings (intra-ring couplings H-1 to C-4 and H-4 to C-1) in the molecule linked together by α(1→2) and α(1→3) bonds. The comparison of the ^{13}C NMR data with those of all methyl furanosides[20,21] suggested a *ribo*-configuration. The $[M + Na]^+$ ion of the octaacetate **1a** obtained by peracetylation of **1** (besides a small amount of heptaacetate **1b**) had a *m*/*z* value of 773, corresponding to the molecular formula $C_{31}H_{42}O_{21}Na$. The absence of an acetylation shift at H-3 and H-2′ in the ^1H NMR spectrum of **1a** and **1b** confirmed the above-made deduction concerning the linkages. Changes in $J_{1,2}$ indicated an inverted configuration (i.e., 1-β) at C-1 (Table 2). The comparison of both ^1H and ^{13}C NMR data with those of all eight possible tetra-*O*-acetyl-D-aldopentofuranoses[22] unambiguously determined the *ribo*-configuration of all three units. Thus, the peracetates have structures **1a** and **1b**, and the parent compound is represented by the structure **1**, i.e., α-Rib*f*-(1→2)-α-Rib*f*-(1→3)-α-Rib*f*.

The next chromatographic fraction produced a $[M + Na]^+$ ion at *m*/*z* 365 consistent with the summary formula $C_{12}H_{22}O_{11}Na$. According to the ^{13}C NMR spectrum, the building blocks were an anomeric carbon, one oxymethylene, and 10 oxymethines. NMR spectroscopy revealed two species (4:1), each containing an anomeric center with a *manno*-configuration (based on the extracted set of vicinal couplings).[23] Both of the direct couplings of the anomeric carbons (175.8 and 170.9 Hz) indicated they were α-anomers;[24] thus this compound could not be a reducing sugar.

* Corresponding author. Phone: +420-296-442-335. Fax: +420-296-442-347. E-mail: spospis@biomed.cas.cz.

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Table 1. ^{1}H and ^{13}C NMR Data of **1** (400 and 100 MHz, D_2O, 30 °C)

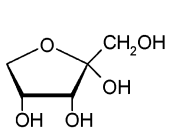
atom	δ_C	δ_H, multiplicity, *J* [Hz]	HMBC[a]
1	97.20	5.286 d (4.7)	3, 4
2	70.39	4.189 dd (6.8, 4.7)	1
3	80.32	4.028 dd (6.8, 1.6)	1, 2, 4, 5, 1′
4	86.11	4.242 ddd (3.4, 2.9, 1.6)	3
5	61.37	3.554 dd (12.5, 3.4)	3,4
		3.506 dd (12.5, 2.9)	3,4
1′	104.72	5.261 d (4.5)	3, 2′, 3′, 4′
2′	75.47	4.356 dd (5.8, 4.5)	1′, 3′, 1″
3′	70.24	4.117 dd (5.8, 0.9)	1′, 2′, 4′, 5′
4′	88.13	4.194 ddd (2.8, 2.6, 0.9)	3′
5′	61.73	3.484 dd (12.6, 2.6)	3′, 4′
		3.450 dd (12.6, 2.8)	3‘,4‘
1″	101.58	5.263 d (4.4)	2′, 2″, 4″
2″	71.65	4.245 dd (6.6, 4.4)	1″, 3″
3″	70.00	4.011 dd (6.6, 2.1)	1″, 4″, 5″
4″	87.78	4.130 ddd (4.4, 3.4, 2.1)	
5″	61.69	3.530 dd (12.4, 3.4)	3″, 4″
		3.468 dd (12.4, 4.4)	3‘‘,4‘‘

[a] Carbons showing couplings to the indicated proton.

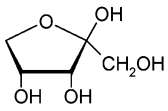
Furthermore, the protons at the mannose attachment position had vicinal couplings of different magnitude. Therefore, this sample is probably a mixture of regioisomers of α-mannosyl cyclitol. Indeed, two compounds, **2a** and **3a**, of the same molecular formula, $C_{30}H_{40}O_{20}$, were obtained by acetylation. Complete analysis of the ^{1}H NMR spectrum (Table 3) showed that all cyclitol protons present in the molecule were axial except one that was equatorial; that is, it is the *myo*-inositol. The cyclitol protons not exhibiting a downfield shift upon acetylation identified the site of attachment, C-5 with **2a** and C-2 with **3a**; therefore the native compounds are 5-*O*-(α-mannosyl)-*myo*-inositol (**2**) and 2-*O*-(α-mannosyl)-*myo*-inositol (**3**).

The ^{13}C NMR spectrum of **4** contained only six signals (OCHO, four OCH, OCH_2). A seven-spin system corresponding to a α-Glc*p* moiety[23] was found in the ^{1}H NMR spectrum. The autocorrelation cross-peak (H-1, C-1) in the HMBC indicated the trehalose. This identity was confirmed by ^{1}H and ^{13}C NMR data comparison with an authentic sample.

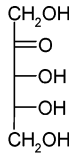
The ^{13}C NMR spectrum of the compound **5** indicated a ketose (no anomeric OCH's, one keto group, two signals of carbons of the −O−C−O− type). The presence of three −CH(O−)CH(O−)-CH_2O− and three isolated AB systems due to CH_2OH groups (COSY, TOCSY) was consistent with the structure of a pentulose (three major components, **5a**−**5c**; interpreted similarly to the recently described spectra of lactulose[25]). This conclusion was



5a

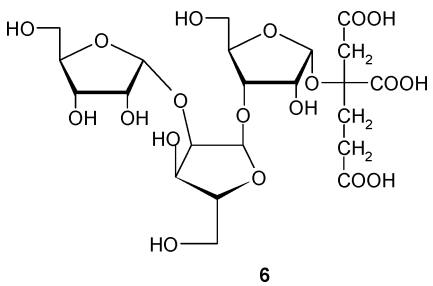


5b



5c

confirmed by comparison with available NMR data of D-*erythro*- and D-*threo*-2-pentuloses[26] and with NMR spectra (^{1}H, ^{13}C) of the authentic sample. According to the sign of optical rotation, **5** is D-ribulose.



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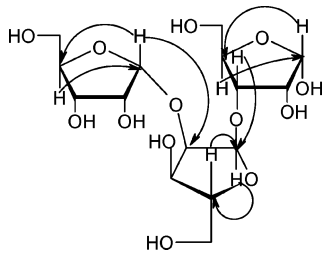

Figure 1. Important HMBC correlations for **1**.

The tririboside **1** is identical with the oligosaccharide part of ribocitrin (**6**), a dextransucrase inhibitor[27,28] effective against dental caries.[29] Ribocitrin is produced by *Streptomyces neygawaensis* MF 980-CF-1.[28] Despite the widespread occurrence of ribose in nature, compounds containing ribose to ribose bond are rather scarce. One example is poly(ADP-Rib),[30] having α (1″→2′) bonds, and a second one is the capsular polysaccharide from *Haemophilus influenzae* (ribose−ribitol, 1↔1 bond).[31] Both 2-*O*- and 2,6-di-*O*-α-mannosyl-*myo*-inositols are a part of bacterial phosphatidylinositol mannosides.[32−34] The 2-*O*- and 6-*O*-α-mannosyl derivatives were synthesized.[35,36] Trehalose (**4**) is quite widespread throughout various organisms including *S. coelicolor*.[37] It is a multifunctional molecule serving as a source of energy and carbon and as an osmolyte.[38] The excretion of **4** by *Streptomyces* has not yet been reported, and therefore, the example of *S. coelicolor* given here is probably the first one. Ribulose (**5**) is engaged in cellular metabolism as ribulose-5-phosphate in the pentose−phosphate cycle, which carries out the interconversion of pentoses and hexoses. Accumulation of ribulose and xylulose by *Brevibacterium* and *Corynebacterium* strains had been previously reported.[39] Excretion of **5** in its free form into the medium is a unique phenomenon and promising from the point of view of possible biotechnological applications.

Experimental Section

General Experimental Procedures. Optical rotations were determined using a Perkin-Elmer 241 polarimeter. NMR spectra were measured on a Varian INOVA-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) in D_2O or $CDCl_3$ at 30 °C. Nalorac 3 mm microprobes were used for the study of acetylated derivatives. Chemical shifts (δ; in ppm) were referenced to internal acetone (δ_H 2.030, δ_C 30.50) or to the residual solvent signal (δ_H 7.265, δ_C 77.00), respectively. Standard software was used for all 2D NMR experiments; the pulse sequence for 1D-TOCSY[40] was obtained through Varian User Library. The shaped pulses were generated using the Pandora' s Box shaping program,[41] which is a part of the Varian software package. Positive-ion mass spectra were recorded on an LCQ Deca ion trap mass spectrometer (Finnigan, San Jose, CA) equipped with a nanoelectrospray ion source. Samples dissolved in 30% aqueous CH_3CN were sprayed directly from EconoTipTM emitters (New Objective, Inc., Woburn, MA). The spray voltage was set at 1.2 kV, and the heated capillary was kept at 150 °C. Full scan spectra were acquired over the *m*/*z* range 100−1000 Da. Positive-ion electrospray mass spectra of the samples dissolved in 30% CH_3CN−0.1% HCOOH were acquired on an APEX-Qe FTMS instrument equipped with a 9.4 T superconducting magnet and a Combi ESI/MALDI ion source (Bruker Daltonics, Billerica, MA). The instrument was externally calibrated using triply and doubly charged ions of angiotensin I and sextuply and quintuply charged ions of insulin, which results in a typical mass accuracy below 1 ppm.

TLC was performed on Si gel 60 plates (Merck; 0.2 mm) with EtOAc−AcOH−MeOH−H_2O (4:3:3:1) as the mobile phase.[42] The spots were visualized by spraying with an orcinol reagent[19] followed by heating to 150 °C. Analyses of peracetylated carbohydrates were also performed by TLC on Si gel 60 plates (Merck; 0.2 mm). Mobile phase was heptane−EtOAc−MeOH (5:4:1). The concentrations of the carbohydrates in the fermentation broth were measured on TLC Si gel plates (Merck; 0.2 mm) prepared as described above. Densitometric measurement was accomplished on a Shimadzu CS 930 scanner. The

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **1a** and **1b** (400 and 100 MHz, $CDCl_3$, 30 °C)

	1a		**1b**	
atom	δ_C	δ_H, multiplicity, *J* [Hz]	δ_C	δ_H, multiplicity, *J* [Hz]
1	98.45	6.164 s[a]	100.97	6.125 d (1.0)
2	73.03	5.249 d (4.4)	73.35	4.172 dd (3.1, 1.9)
3	71.98	4.462 dd (8.2, 4.4)	76.94	4.283 m
4	79.34	4.298 ddd (8.2, 5.9, 2.6)	80.25	4.304 m
5	63.68	4.407 dd (12.0, 2.6)	64.41	4.362 dd (11.4, 4.3)
		4.160 dd (12.0, 5.9)		4.164 dd (11.4, 3.6)
1′	98.88	5.228 d (4.0)	100.69	5.183 d (4.5)
2′	72.95	4.282 dd (6.6, 4.0)	75.32	4.156 dd (6.9, 4.5)
3′	70.62	5.186 dd (6.6, 4.1)	70.68	5.219 dd (6.9, 2.6)
4′	79.36	4.255 ddd (4.1, 4.1, 3.4)	81.48	4.309 ddd (4.3, 3.9, 2.6)
5′	63.20	4.345 dd (12.0, 3.4)	63.46	4.303 dd (11.9, 3.9)
		4.150 dd (12.0, 4.1)		4.184 dd (11.9, 4.3)
1″	98.80	5.277 d (4.4)	100.40	5.418 d (4.5)
2″	70.56	4.991 dd (7.4, 4.4)	71.12	4.923 dd (7.3, 4.5)
3″	69.57	5.122 dd (7.4, 4.4)	69.46	5.232 dd (7.3, 3.7)
4″	79.50	4.268 ddd (4.4, 4.3, 3.2)	80.07	4.291 ddd (4.4, 3.7, 3.0)
5″	63.15	4.348 dd (12.0, 3.2)	63.17	4.353 dd (11.9, 3.0)
		4.187 dd (12.0, 4.3)		4.172 dd (11.9, 4.4)

[a] $J > 0$. Additional signals: **1a**: ^{1}H NMR 2.084s, 2.089 s, 2.095 s, 2.097 s, 2.102 s, 2.106 s, 2.135 s, 2.172 s (8 × Ac); ^{13}C NMR 20.30, 20.56, 20.63, 20.70, 20.73, 20.76 (2 C), 21.05 (8 × Ac), 168.89, 169.34, 168.86 (2 C), 169.96, 170.26, 170.47, 170.52 (8 × C═O); **1b**: ^{1}H NMR 2.047 s, 2.095 s, 2.098 s (6 H), 2.102 s, 2.127 s, 2.153 s (7 × Ac); ^{13}C NMR 20.34, 20.51, 20.72 (2 C), 20.74, 20.79, 21.14 (7 × Ac), 169.26, 169.99, 170.15, 170.38, 170.40, 170.51, 170.60 (7 × C═O).

Table 3. ^{1}H and ^{13}C NMR Data of **2a** and **3a** (400 and 100 MHz, $CDCl_3$, 30 °C)

	2a		**3a**[a]
atom	δ_C	δ_H, multiplicity, *J* [Hz]	δ_H, multiplicity, *J* [Hz]
1	68.29	5.097 dd (10.5,2.9)	5.094 dd (10.5,2.5)
2	68.26	5.601 dd (2.9,2.8)	4.315 dd (2.6,2.5)
3	69.22	5.064 dd (10.3,2.8)	5.006 dd (10.7,2.6)
4	69.88	5.388 dd (10.3,10.0)	5.509 dd (10.7,9.5)
5	77.74	4.118 dd (10.0,8.6)	5.197 dd (9.8,9.8)
6	71.83	5.186 dd (10.5,8.6)	5.532 dd (10.5,9.8)
1′	99.53	4.994 d (2.0)	4.971 d (2.0)
2′	69.33	5.160 dd (3.3,2.0)	5.406 dd (3.3,2.0)
3′	68.74	5.132 dd (9.9,3.3)	5.423 dd (9.9,3.3)
4′	65.27	5.326 (10.1,9.9)	5.375 dd (9.9,9.4)
5′	69.77	3.969 ddd (10.1,3.9,2.3)	4.191 ddd (9.4,4.5,2.4)
6′	61.80	4.214 dd (12.5,3.9)	4.306 dd (12.4,4.5)
		4.126 dd (12.5,2.3)	4.091 dd (12.4,2.4)

[a] No ^{13}C NMR was measured because of the small amount available. Additional signals: **3a**: ^{1}H NMR 1.967, 1.988, 2.015, 2.034, 2.084, 2.099, 2.126, 2.153, 2.188 (each 3 H, s); ^{13}C NMR 20.40, 20.47, 20.53, 20.54, 20.65, 20.68, 20.71, 20.77, 20.86, 169.23, 169.53 (2 C), 169.57 (2 C), 169.66, 169.92, 170.04, 170.57; **4a**: ^{1}H NMR 2.006, 2.018, 2.034, 2.039, 2.078, 2.088, 2.095, 2.097 (each 3 H, s).

visualized spots of ribose trisaccharide (**1**), mannosyl-*myo*-inositols (**2**, **3**), trehalose (**4**), and D-ribulose (**5**) were scanned at 575, 520, 520, and 625 nm, respectively. Commercially available D-ribulose (Sigma, St. Louis, MO), trehalose (Sigma), purified mannosyl-*myo*-inositols (**2**, **3**), and ribose trisaccharide (**1**) served as standards. Concentrations of the products were calculated from a calibration curve obtained by regression analysis. Glycerol was detected by TLC as a brown spot after reaction with the orcinol reagent.[19]

Bacterial Cultivation. Submerged cultivations of *S. coelicolor* A3-(2) were carried out in 500 mL flat-bottom flasks containing 50 mL of the medium (grams per liter): yeast extract 2 (Difco); $(NH_4)_2SO_4$ 2; $CaCO_3$ 5; NaCl 5; K_2HPO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.1; glycerol 30.0 for inoculum, 70.0 for the production medium. After 30 h of cultivation, the seed culture was used as an inoculum (15%, v/v) for the production medium. Cultivations were performed on an orbital shaker (162 rpm) at 28 °C for 7 days until complete utilization of the carbon source. During the cultivation, the pH of the culture was checked at 12 h intervals and 2 M NaOH was added to reach a mild alkaline value (pH 9).

Isolation of Compounds 1−5. The cells from two cultivation flasks were separated by centrifugation, and supernatant (100 mL) was lyophilized. The dry matter was resuspended in water to reach 15% of the original volume. The insoluble substances were removed by centrifugation. The supernatant was acidified with 1 N HCl to pH 2.5. Extraction with one-fourth the volume of $CHCl_3$ removed pigments and other nonpolar substances. The neutralized H_2O phase (2.2 g dry matter in 15 mL) was subjected to column chromatography (3 × 47 cm) on Toyopearl HW40F (Tosoh Corp., Japan). Typically, 10 fractions (15 mL each) were collected, and the content of carbohydrates was monitored by TLC. Fractions containing carbohydrate metabolites were pooled, lyophilized, and repeatedly purified on Toyopearl HW40F. Ribose trisaccharide (**1**), trehalose (**4**), and ribulose (**5**) were further purified by column chromatography (1.7 × 40 cm) on Sephadex LH-20 (Pharmacia, 0.025−0.1 mm) in MeOH. Fractions (6 mL each) were checked by UV spectrophotometry at 350 to 200 nm and TLC followed by detection with the orcinol reagent. MeOH from carbohydrate-positive fractions was removed *in vacuo*. These procedures afforded **1** (2 mg), **4** (10 mg), and **5** (6 mg). Additional column chromatography (1.7 × 28 cm) on Si gel 60 (Merck, 0.040−0.063 mm) with EtOAc−AcOH−MeOH−H_2O (4:3:3:1) as a mobile phase was needed for the purification of mannosyl-*myo*-inositols (**2**, **3**) from the Toyopearl fractions. Solvents were removed *in vacuo*, and the final separation step was column chromatography on Sephadex LH-20 with MeOH as described above. In this procedure we obtained 5 mg of a mixture of **2** and **3**.

Acetylation of Ribose Trisaccharide (1) and Mannosyl-*myo*-inositols (2, 3). The carbohydrate (10 mg) was dissolved in acetic anhydride (10 mL) and pyridine (3 mL); 4-dimethylaminopyridine (20 mg; Fluka Chemie) was added as a catalyst. The reaction mixture was stirred at room temperature overnight, and then the remaining acetic anhydride was hydrolyzed by ice. Acetylated products were extracted from the reaction mixture with $CHCl_3$. The combined extracts were dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. Products were further purified by the preparative TLC on Si gel plates (Kieselgel 60; 0.5 mm, Merck). Plates were developed in heptane−EtOAc−MeOH (5:4:1), and the products were visualized on small parts of the plates with the orcinol reagent. The appropriate bands were scraped off, and the desired compounds were eluted with MeOH. Final purification was achieved by column chromatography on Sephadex LH-20 in MeOH.

Noncrystalline compounds **1a**, **1b**, **2a**, and **3a** were prepared in this way (4, 1.0, 2.7, and 0.8 mg, respectively).

Tririboside (1): white solid; $[\alpha]_D$ −58.57 (*c* 0.14, H_2O); ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100 MHz, D_2O), see Table 1; HRESIFTMS 437.1260 $[M + Na]^+$, calcd 437.1266 for $C_{15}H_{26}O_{13}Na$.

Octaacetate of tririboside (1a): amorphous, white solid; $[\alpha]_D$ +125.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; HRESIFTMS 773.2116, $[M + Na]^+$, calcd 773.2111 for $C_{31}H_{42}O_{21}Na$.

Heptaacetate of triribose (1b): amorphous, white solid; $[\alpha]_D$ +119.0 (*c* 0.24, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; HRESIFTMS 731.2008 $[M + Na]^+$, calcd 731.2005 for $C_{29}H_{40}O_{20}Na$.

Mannosyl-*myo*-inositols (2 + 3): amorphous, white solid; ^{1}H NMR (D_2O, 30 °C), major component (i.e., **2**), mannose part, δ 3.456 (1 H, dd, J = 12.2, 5.0, H-6′b), 3.490 (1 H, dd, J = 10.1, 9.9, H-4′), 3.624 (1 H, dd, J = 12.2, 2.4, H-6′a), 3.631 (1 H, dd, J = 9.9, 3.3, H-3′), 3.766 (1 H, ddd, J = 10.1, 5.0, 2.4, H-5′), 3.826 (1 H, dd, J = 3.3, 1.6, H-2′), 4.968 (1 H, d, J = 1.6, H-1′); cyclitol part, δ 3.121 (1 H, dd, J = 9.3, 9.3), 3.293 (1 H, dd, J = 10.0, 3.1), 3.426 (2 H, m), 3.526 (1 H, J = 9.5, 9.3, the site of attachment), 3.804 (1 H, dd, J = 3.1, 2.7); ^{13}C NMR (D_2O, 30 °C) δ 61.02 (CH_2), 66.87 (CH), 70.58 (CH), 70.71 (CH), 71.21 (CH), 71.81 (CH), 72.79 (CH), 72.82 (CH), 73.06 (CH), 73.17 (CH), 80.10 (CH), 101.25 (CH); minor component (i.e., **3**), mannose part, δ 3.631 (1 H, dd, J = 9.8, 3.3, H-3′), 3.871 (1 H, dd, J = 3.3, 1.8, H-2′), 4.905 (1 H, d, J = 1.8, H-1′); cyclitol part, δ 3.059 (1 H, m, H-3′), 3.341 (1 H, dd, J = 10.2, 2.5, H-2′), 3.896 (1 H, dd, J = 2.5, 2.1, the site of attachment); ^{13}C NMR (D_2O, 30 °C) δ 61.11 (CH_2), 66.90 (CH), 70.48 (CH), 72.87 (CH), 75.06 (CH), 80.27 (CH), 101.73 (CH); HRESIFTMS 365.1053 $[M + Na]^+$, calcd 365.1054 for $C_{12}H_{22}O_{11}Na$.

Peracetylated 5-*O*-(α-mannosyl)-*myo*-inositol (2a): amorphous, white solid; $[\alpha]_D$ +80.0 (*c* 0.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 3; HRESIFTMS 743.2008 $[M + Na]^+$, calcd 743.2005 for $C_{30}H_{40}O_{20}Na$.

Peracetylated 2-*O*-(α-mannosyl)-*myo*-inositol (3a): amorphous, white solid; $[\alpha]_D$ +37.5 (*c* 0.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), see Table 3; HRESIFTMS 743.1999 $[M + Na]^+$, calcd 743.2005 for $C_{30}H_{40}O_{20}Na$.

Trehalose (4): amorphous solid, identified by spectroscopic comparison with a standard sample (Sigma, St. Louis, MO).

D-Ribulose (5): syrupy liquid; $[\alpha]_D$ −8.91 (*c* 0.86, H_2O); identified by spectroscopic comparison with a standard sample (Sigma, St. Louis, MO).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**, **2** + **3**, **5**; ^{1}H NMR spectra of compounds **1a**, **1b**, **2a**, and **3a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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