Two Bioactive Saponins from *Albizia subdimidiata* from the Suriname Rainforest[1]

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Bioassay-guided fractionation of a methanol extract of *Albizia subdimidiata* using the engineered yeast strains 1138, 1140, 1353, and Sc7 of *Saccharomyces cerevisiae* as the bioassay tool resulted in the isolation of the two active saponins **1** and **2**; one of these, albiziatrioside A (**1**), is described for the first time. The structures of **1** and **2** were established on the basis of HRMS, 1D and 2D NMR spectral data, and GC−MS analysis of the sugar units. Both isolated compounds showed significant cytotoxicity against the A2780 cell line.

In recent years we have been engaged in a program to discover bioactive agents from the Suriname rainforest using a mechanism-based bioassay involving genetically engineered mutants of the yeast *Saccharomyces cerevisiae*;[2] this work has been reviewed.[3] As a part of this program the plant *Albizia subdimidiata* (Splitg.) Barneby & J. W. Grimes (Fabaceae) was collected in the Paramaribo district on the Backboord property, Suriname, in April 1998, and its methanol extract was found to show reproducible activity in the yeast assay.

The genus *Albizia* comprises about 150 species widely distributed in the tropics, with the greatest diversity in Africa and Central and South America. Historically, some authors have considered the New World species distinct from the African and Asian members of the genus.[4] In Africa several *Albizia* species are used in folk medicine for the treatment of rheumatism, stomach trouble, cough, diarrhea, scabies, and wounds and as an anthelmintic.[5] In traditional Chinese medicine *Albizia julibrissin,* known as the silk tree, is used for the treatment of insomnia, irritability, poor memory, and injuries.[6] *Albizia lebbeck* is reported to have antiseptic, antidysenteric, and antitubercular activity.[7]

A MeOH extract of *A. subdimidiata* stems and infructescence had IC_{12} values of 1500, 1800, 2475, and 16 000 μg/mL, respectively, against the 1138, 1140, 1353, and Sc7 yeast strains.[2] Fractionation of these extracts by liquid−liquid partition caused the bioactivity to partition successively into the aqueous MeOH phase of a hexane−80% aqueous MeOH partition and then into the $CHCl_3$ phase of a $CHCl_3$−60% aqueous MeOH partition. Purification of the organic layer using Sephadex LH-20 and Si gel columns, followed by preparative TLC, resulted in the isolation of the new saponin **1** and the known saponin **2**.

Compound **1** showed IR absorption bands at ν_{max} 1642 and 1550 cm^{-1} and two characteristic signals in the ^{13}C NMR spectrum (Table 2) at 23.16 and 173.47 ppm, diagnostic for $NHCOCH_3$. In the ^1H NMR spectrum of **1** (Table 1) signals for three anomeric protons at δ 4.44 (d, J = 7.5 Hz, 2H) and 4.52 (d, J = 5.5 Hz) were correlated with signals for three carbons at 104.85, 106.47, and 103.32 ppm by an HMQC experiment and were diagnostic for the presence of three sugar molecules. The chemical shift of the anomeric carbons and of C-3 of the triterpenoid ring moiety at 90.27 ppm indicated that the three sugars were attached to C-3. After complete acid hydrolysis the aglycon was identified as oleanolic acid through comparison of ^1H and ^{13}C NMR and mass spectral data with the literature data.[8]

Compound **1** showed a sodiated molecular ion $(M + Na)^+$ at m/z 946.514 in its HRFABMS, consistent with the

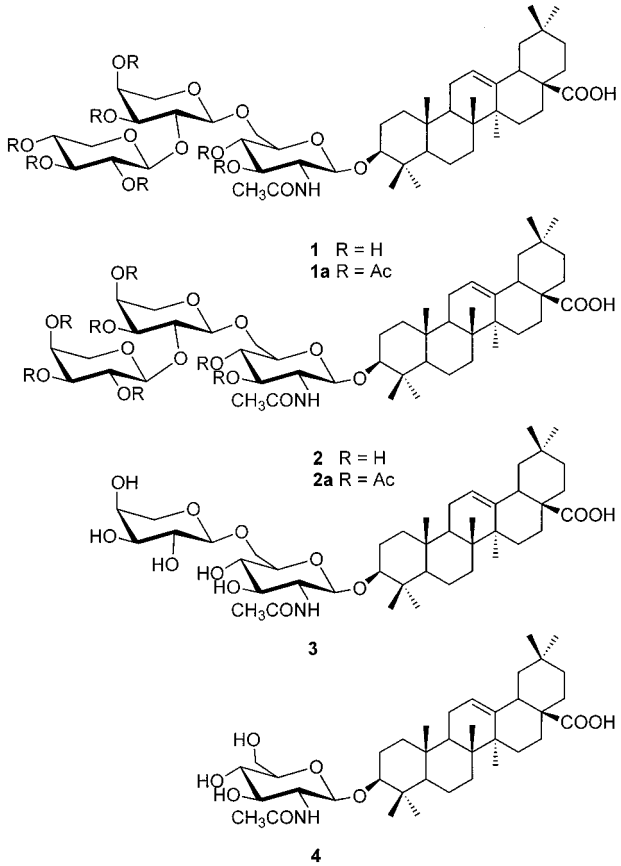

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Table 1. Selected ^{1}H NMR Data for Compounds **1**−**3**, **1a**, and **2a**[a]

positions	1	2	1a	2a	3	4
12	5.21 (1H, s)	5.21 (1H, s)	5.21 (1H, s)	5.22 (1H, s)	5.22 (1H, s)	5.23 (1H, s)
23	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.95 (3H, s)	1.03 (3H, s)
24	0.94 (3H, s)	0.93 (3H, s)	0.94 (3H, s)	0.94 (3H, s)	0.92 (3H, s)	0.93 (3H, s)
25	0.86 (3H, s)	0.85 (3H, s)	0.83 (3H, s)	0.83 (3H, s)	0.89 (3H, s)	0.86 (3H, s)
26	0.75 (3H, s)	0.75 (3H, s)	0.76 (3H, s)	0.77 (3H, s)	0.76 (3H, s)	0.81 (3H, s)
27	1.15 (3H, s)	1.15 (3H, s)	1.16 (3H, s)	1.17 (3H, s)	1.16 (3H, s)	1.15 (3H, s)
29	0.88 (3H, s)	0.88 (3H, s)	0.89 (3H, s)	0.92 (3H, s)	0.90 (3H, s)	0.90 (3H, s)
30	0.94 (3H, s)	0.94 (3H, s)	0.94 (3H, s)	0.93 (3H, s)	0.96 (3H, s)	0.97 (3H, s)
GluNAc H-1	4.44 (d, J = 7.5 Hz)	4.43 (d, J = 8.4 Hz)	4.57 (d, J = 6.0 Hz)	4.65 (d, J = 8.4 Hz)	4.43 (d, J = 8.5 Hz)	4.42 (d, J = 7.6 Hz)
Ara H-1	4.52 (d, J = 5.5 Hz)	4.55 (d, J = 5.8 Hz)	4.80 (d, J = 7.2 Hz)	4.80 (d, J = 6.8 Hz)	4.34 (d, J = 6.5 Hz)	
term. xyl H-1	4.44 (d, J = 7.5 Hz)		4.63 (d, J = 8.0 Hz)			
term. ara H-1		4.49 (d, J = 6.7 Hz)		4.49 (d, J = 5.6 Hz)		
CH_3CONH	1.95 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	1.94 (3H, s)	1.97 (3H, s)

[a] Obtained in CD_3OD. Chemical shifts in ppm from internal TMS, coupling constants in Hz.

Table 2. ^{13}C NMR Data for Compounds **1** and **2**[a] in CD_3OD

position	1	2	position	1	2
1	39.99	39.93	27	26.56	26.53
2	27.05	27.00	28	184.68	184.70
3	90.27	90.64	29	33.80	33.82
4	39.65	39.69	30	24.22	24.22
5	56.92	56.85	GluNAc		
6	19.35	19.38	1	104.85	104.93
7	29.05	29.05	2	57.72	57.63
8	40.53	40.53	3	76.59	76.34
9	49.13	49.11	4	72.24	72.96
10	37.93	37.92	5	75.68	75.67
11	24.57	24.59	6	69.59	69.47
12	122.99	122.96	Ara		
13	145.96	146.02	1	103.32	103.37
14	42.91	42.94	2	81.26	80.43
15	35.24	35.25	3	73.10	74.16
16	24.53	24.54	4	68.49	68.75
17	47.71	47.72	5	65.40	65.72
18	43.11	43.12	Terminal sugar	Xyl	Ara
19	47.76	47.78	1	106.47	105.84
20	31.71	31.72	2	75.69	71.95
21	34.06	34.08	3	77.50	73.41
22	34.10	34.91	4	71.05	69.65
23	28.57	28.56	5	67.20	67.11
24	15.97	16.00	CH_3CONH	23.16	23.19
25	18.02	18.06	CH_3CONH	173.47	173.47
26	17.07	17.11			

[a] Assignment made by combination of DEPT, HMQC data, and comparison with literature data.[10]

molecular formula $C_{48}H_{77}NO_{16}$. Both ^{13}C NMR and MS data indicated that the sugar moiety contained two pentoses and *N*-acetylglucosamine. The sugars present were positively identified by hydrolysis with methanolic HCl, followed by reduction and acetylation to form the corresponding alditol acetates. GC analysis of the alditol acetates and direct comparison with standard sugars[9] indicated the presence of arabinose, xylose, and *N*-acetylglucosamine. The glucosamine was assigned as D-glucosamine on the basis of the conversion of **1** and **2** to the known monoside **4**,[10] and the arabinose and xylose were identified as L-arabinose and D-xylose by the procedure described below.

Compound **2** had spectroscopic data that were very similar to those of **1**, and the sugars were identified as two units of arabinose and one of *N*-acetyl-D-glucosamine by GC−MS analysis as described above. Its structure was confirmed as **2** by comparison of its spectroscopic data with those of the previously isolated compound from *Calliandra anomala*[11] and *Pithecellobium racemosum*.[12]

Partial acid hydrolysis of both **1** and **2** yielded the same bioside (**3**) and monoside (**4**). Bioside **3** was isolated and characterized by negative-ion FABMS (M − 1 at *m*/*z* 790) and by comparison of the ^{1}H NMR data of its anomeric protons to those of the known saponin prosapogenin 10.[13] Monoside **4** was also isolated and characterized by ^{1}H NMR (Table 1) and FABMS (M^+ at *m*/*z* 659) as 3-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid (**3**). These data indicate that **1** and **2** differ only in the terminal sugar, with xylose in **1** and arabinose in **2**.

Acetylation of **1** resulted in a downfield shift of all sugar protons except those attached to carbons involved in the sugar−aglycon and sugar−sugar linkages, which were then present in a much less overlapped part of the ^{1}H NMR spectrum (Table 1). A COSY experiment on the acetylated product **1a** showed a correlation between H-5 of *N*-acetylglucosamine (δ 4.22, dd, J = 2.1, 5.7 Hz) and both of its unshifted H-6 protons (δ 3.512, dd, J = 11.8, 9.2 Hz; 4.18, dd, J = 5.9, 11.8 Hz). A second important correlation was observed between H-1 of arabinose (δ 4.57, d, J = 5.9 Hz) and its unshifted H-2 (δ 3.82, m, overlapped with other protons), indicating that the terminal xylose is attached to C-2 of arabinose linked to C-6 of *N*-acetylglucosamine.

The absolute configurations of the pentose sugars of **1** were determined by preparing their thiazolidine derivatives by treatment with L-cysteine methyl ester hydrochloride, followed by conversion into their acetates and comparison with standards by GC−EIMS.[14,15] The standards from L-arabinose and D-xylose gave overlapping peaks on our column, but these were clearly separated from the peaks from D-arabinose and L-xylose. The thiazolidine acetates prepared from **1** gave a single peak corresponding to D-xylose and/or L-arabinose derivatives, thus indicating the absence of L-xylose and D-arabinose in **1**. Since the previously reported GC−MS data proved the presence of xylose and arabinose, the absolute configurations of the pentoses were assigned as L-arabinose and D-xylose. A similar control experiment with **2** confirmed that both its sugars were L-arabinose.

Further evidence for the nature of the sugar linkages in **1** was derived from analysis of the MS fragmentation of the sugars after methylation, hydrolysis, reduction, acetylation, and GC separation (see Supporting Information).[16] The ^{13}C NMR signals of the sugar moieties in **1** were also comparable to those of saponins having similar sugar types and linkage sequences.[11,12,17]

Compounds **1** and **2** showed weak activity in our yeast bioassay (Table 3). In a cytotoxicity test using the A2780 cell line both compounds showed significant cytotoxicity,

Table 3. Biological Activities of Compounds **1** and **2**

	yeast activity[a]				
compound	1138	1140	1353	Sc7	cytotoxicity[b]
1	30	35	35	30	0.9
2	25	40	30	45	0.8

[a] Activity against the indicated *S. cerevisiae* strains (IC_{12} values, μg/mL). [b] Mammalian cytotoxicity to the A2780 cell line (IC_{50} values, μg/mL).

with IC_{50} values of 0.9 and 0.8 μg/mL for compounds **1** and **2**, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a MIDAC M-series FTIR instrument. NMR spectra were recorded in CD_3OD at 399.951 MHz for ^1H and100.578 MHz for ^{13}C NMR, using standard Varian pulse sequence programs. FAB and GC mass spectra were obtained on a VG 7070 E-HF mass spectrometer. HRFAB mass spectra were obtained on a Kratos MS50 mass spectrometer, and MALDI-TOF spectra were obtained on a Kratos Kompact SEQ instrument. Melting points are uncorrected.

Yeast Bioassay. The bioassay was carried out as previously described.[2c]

Cytotoxicity Bioassay. The in vitro antitumor cytotoxicity assays were performed at Bristol-Myers Squibb Pharmaceutical Research Institute as previously described.[18]

Plant Material. The stems and infructescences of *A. subdimidiata* were collected in the Paramaribo district on the Backboord property, Suriname, in April 1998. Voucher specimens are deposited in the National Herbarium of Suriname, Paramaribo, Suriname, and the Missouri Botanical Garden, St. Louis, MO.

Extraction and Isolation. Extracts of the stems and infructescences for screening were prepared with EtOAc and MeOH by Bedrijf Geneesiddelen Voorziening, Suriname, and sent for bioassay and isolation work to Virginia Polytechnic Institute and State University; the methanol extracts were supplied to VPISU as BGVS M970039 and BGVS M970088, respectively. The MeOH extracts described above were active against the mutant strains 1138, 1140, 1353, and Sc7 of *S. cerevisiae*. The four strains were used to determine the IC_{12} values of the total extract and pure isolates, while the activity was monitored during the fractionation procedures using the 1138 mutant strain. The MeOH extracts of the stems (1.3 g) and infructescences (0.9 g) showed the same level of activity (IC_{12} values of 1500, 1800, 2475, and 16 000 μg/mL against the 1138, 1140, 1353, and Sc7 yeast strains). The separate extracts were dissolved in 80% aqueous MeOH and extracted with hexane (200 mL × 3). The activity was retained in the aqueous MeOH fraction (1.1, 0.6 g, respectively), which was diluted with H_2O to 60% aqueous MeOH and extracted with $CHCl_3$ (200 mL × 3). The $CHCl_3$ fractions (620, 312 mg) retained the activity (IC_{12} values of 150, 90 μg/mL) and were purified by chromatography on Sephadex LH-20 (3 × 45 cm) using solvents hexane−CH_2Cl_2 (1:1, 300 mL), CH_2Cl_2−Me_2CO (9:1, 200 mL), CH_2Cl_2−Me_2CO (1:1, 200 mL), CH_2Cl_2−Me_2CO−MeOH (2:2:1, 200 mL), and MeOH (100 mL). The active fractions eluted with CH_2Cl_2−Me_2CO−MeOH (2:2:1) (410, 200 mg) were combined and refractionated using a flash Si gel column eluting with 15% MeOH in $CHCl_3$. The active fractions (150, 62 mg) were subjected to preparative TLC (Si gel, EtOAc−MeOH−H_2O, 30:5:4, double development) to obtain **1** (20, 10 mg, R_f value 0.49) and **2** (35, 14 mg, R_f value 0.45).

Albiziatrioside A (3-*O*-β-D-Xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid, 1): amorphous powder, mp 272−274 °C; $[\alpha]^{26}_D$ + 30° (*c* 1.0, MeOH); IR (film) ν_{max} 3373 (OH), 2942, 1658 (COOH), 1642, 1550 (NHCO) cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m/z* 946.514 $(M + Na)^+$ (calcd for $C_{48}H_{77}NO_{16}Na$, 946.514).

3-*O*-α-L-Arabinopyranosyl(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid (2): amorphous powder, dec at 275 °C; $[\alpha]^{26}_D$ +39°(*c* 1.0, MeOH); IR (film) ν_{max} 3376 (OH), 2940, 1670 (COOH), 1639, 1551 (NHCO) cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m/z* 946.515 $(M + Na)^+$ (calcd for $C_{48}H_{77}NO_{16}Na$, 946.514).

Acid Hydrolysis of 1 and 2. Methanolic solutions of 2 mg each of **1** and **2** were treated separately with 1 mL of 3% H_2SO_4 in dry MeOH under reflux for 5 h. The solutions were then neutralized by Na_2CO_3 and extracted with EtOAc to give an aqueous fraction containing the sugar(s) and an EtOAc fraction containing the aglycone or the partially hydrolyzed products. Purification of the EtOAc-soluble components by preparative TLC yielded oleanolic acid (^1H, ^{13}C NMR, MS) from both **1** and **2**.

Partial Hydrolysis of 1 and 2. Compounds **1** and **2** (4 mg each) were treated separately with 10 mg of oxalic acid in 1 mL of MeOH−H_2O (1:1) at 60 °C with reaction monitoring by TLC. After 48 h the products were extracted into EtOAc and purified by preparative TLC (Si gel, EtOAc−MeOH−H_2O, 30:5:4) to give bioside **3** (1.0 mg from **1** and 0.9 mg from **2**) and monoside **4** (detected but not isolated). In a separate experiment a mixture of **1** and **2** (4 mg) was hydrolyzed under similar conditions to give **3** (1.1 mg) and **4** (0.9 mg).

3-*O*-α-L-Arabinopyranosyl(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid (3): ^1H NMR, see Table 1; FABMS$^-$ *m/z* 790 (M − H, 82), 686 (16), 658 (M − ara-1, 55), 640 (23), 483 (34), 455 ($C_{30}H_{47}O_3$, 100).

3-*O*-2-Acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid (4): ^1H NMR, see Table 1; ^1H NMR spectrum in pyridine-d_5 matches literature data;[19] FABMS *m/z* 660 (M^+ + H, 10), 659 (M^+, 4), 658 (M^+ − H, 6), 455 ($C_{30}H_{47}O_3$, 16), 454 (36), 453 (100), 439 ($C_{30}H_{47}O_2$, 32%), 437 (42).

Acetylation of 1 and 2. Compounds **1** and **2** (5 mg each) in pyridine (0.5 mL) were treated separately with Ac_2O (0.2 mL) for 24 h at room temperature. Evaporation of the resulting solutions under a stream of argon yielded chromatographically homogeneous acetates **1a** and **2a**.

3-*O*-β-D-Xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid peracetate (1a): ^1H NMR, see Table 1; MALDI-TOF *m/z* 1257 $(M + K)^+$ (47), 1241 $(M + Na)^+$ (100).

3-*O*-α-L-Arabinopyranosyl(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyloleanolic acid peracetate (2a): ^1H NMR, see Table 1; MALDI-TOF *m/z* 1241 (M^+) (100).

Sugar Identification by GC−MS. Inositol (200 μg) as internal standard was added to 1.3 mg each of **1** and **2** in separate vessels, and the samples were freeze-dried. To each sample 1 mL of methanolic HCl was added, and the mixture was allowed to react for 20 h at 80 °C and then dried under a stream of nitrogen at 40 °C. Aliquots of 1 M NH_4OH and 2% $NaBH_4$ (1 mL each) were added to each sample and the solutions incubated at 40 °C for 90 min. The reaction mixtures were quenched with glacial HOAc (0.1 mL) and treated with 1-methylimidazole (0.2 mL) followed by Ac_2O (2 mL) for 10 min. The reaction mixtures were then quenched by the addition of water and extracted with CH_2Cl_2. The CH_2Cl_2 layers were then analyzed by GC using a GC-17A (Shimadzu) equipped with a Rtx-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film; Restek) with an initial temperature of 150 °C for 2 min and then programmed to 195 °C at a rate of 15 °C/min. The temperature was kept at 195 °C for 1 min, then increased to 225 °C at a rate of 3 °C/min, and kept at 225 °C for 1 min, then increased to 240 °C at a rate of 15 °C/ min, and finally the temperature was kept at 240 °C for 2 min. Retention times of 10.6, 10.9, and 15.6 min were observed for the alditol acetates of arabinose, xylose, and *N*-acetylglucosamine, respectively.

Determination of the Absolute Configuration of Sugars by GC−MS. Standard methyl 2-(D- and L-arabinotetrahydroxybutyl)thiazolidine-4(*R*)-carboxylates and methyl 2-(D- and

l-ribotetrahydroxybutyl)thiazolidine-4(*R*)-carboxylates were synthesized as previously reported.[14] The acetates of these compounds were prepared by treating 100 mg of a thiazolidine with 0.5 mL of acetic anhydride and 0.5 mg of pyridine overnight at room temperature, followed by dilution with 50 mL each of water and $CHCl_3$. The mixture was shaken, the $CHCl_3$ layer was collected, washed, dried, and evaporated, and the product was purified by preparative TLC (Si gel, hexanes−EtOAc, 1:1). The compositions of the products were confirmed by FABMS. The corresponding derivatives of **1** and **2** were prepared by first hydrolyzing 1−2 mg each of **1a** and **2a** overnight at 100 °C in MeOH−1 N HCl (1:1, 500 μL), followed by extraction with H_2O−$CHCl_3$ and evaporation of the water-soluble fraction. This fraction was then treated with pyridine (500 μL) and L-cysteine methyl ester hydrochloride (6 mg) and the mixture stirred overnight at room temperature. Ac_2O (0.3 mL) was then added and the mixture allowed to react overnight at room temperature. The solvent was removed in a stream of argon. Both standards and samples were analyzed by GC−MS using a HP5 capillary column (60 m × 0.25 mm i.d., 0.32 μM film) with an initial temperature of 75 ° C, which was programmed to 250 °C at 10 °C/min. Retention times of 26.50 (D-arabinose), 26.57 (L-arabinose and D-xylose), and 26.93 min (L-xylose) were observed for the standards. The chromatograms were monitored in the positive ion mode both by TIC and by selective ion monitoring at *m*/*z* 146, a major fragment ion.

Linkage Analysis. A solution of 1 mg of **1** and **2** in dry DMSO was treated separately with 100 mg of finely powdered dry NaOH, and the mixture stirred for 1 h. A total of 400 μL of CH_3I was added in portions to each sample, and the mixtures were allowed to react for 40 min. The reactions were then quenched by the addition of 10 mL of $Na_2S_2O_3$ solution in H_2O (100 mg/mL) and extracted with 2 mL of CH_2Cl_2. The organic layers were washed four times with H_2O and dried under a stream of N_2 at 40 °C. To each sample 1 mL of 0.25 M H_2SO_4 in aqueous HOAc was then added, and the solutions were incubated at 80 °C for 2.5 h. The solutions were then neutralized with 350 μL of 2 M NH_4OH and dried. Reduction was then achieved using 500 μL of 2.5 M NH_4OH and 500 μL of 1 M freshly prepared $NaBD_4$ in 2 M NH_4OH, with incubation at 60 °C for 1 h. The reaction mixtures were quenched by the addition of 150 μL of HOAc, and the samples were dried. The samples were then treated with 200 μL of 1-methylimidazole and 2 mL of Ac_2O for 10 min, quenched with H_2O, and extracted with CH_2Cl_2 (2 × 1 mL). The combined organic layers were then washed with water, dried under a stream of N_2, and dissolved separately in MeOH. The MeOH solutions were subjected to GC−MS analysis using a 30 m × 0.32 mm i.d. HP5 column (5% phenyl methyl silicone) connected to the VG7070 E-HF mass spectrometer with an initial temperature of 95 °C and then temperature programming to 285 °C at a rate of 10 °C/min.

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Supporting Information Available: Mass spectrometric fragmentation schemes of methylated, hydrolyzed, reduced, and acetylated carbohydrates from **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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