Antibacterial, Partially Acetylated Oligorhamnosides from *Cleistopholis patens*

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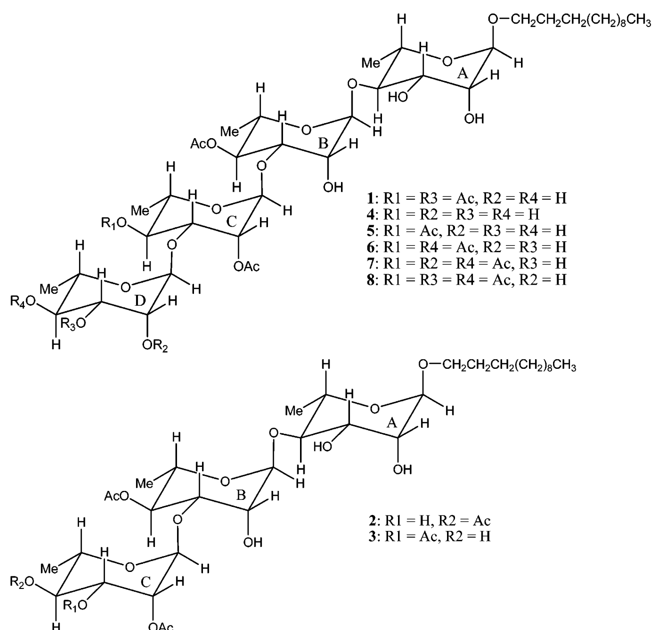
Three new (**1**−**3**) and five known (**4**−**8**) partially acetylated oligorhamnoside derivatives were obtained from *Cleistopholis patens* via high-throughput natural products chemistry procedures. The rapid structure elucidation and dereplication were performed primarily utilizing a capillary-scale NMR probe and LR-/HRESIMS spectroscopic methods. Compounds **1**, **2**, and **6** were found to possess significant in vitro antibacterial activity against the Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* ATCC 33591 and *S. aureus* 78-13607A with MICs of ≤16 μg/mL. Furthermore, **2** and **6** were found to show significant in vitro antibacterial activity against an expanded panel of Gram-positive pathogens including either ATCC strains or well-characterized clinical isolates from the global SENTRY Antimicrobial Surveillance Program.

The genus *Cleistopholis* (Annonaceae) is a small genus with only three or four species, all in tropical Africa. *C. patens* (Benth.) Engl. et Diels is a large tree whose leaves and bark are commonly used in folk medicines.[1−3] The methanolic extract of the leaves was found to have antiplasmodial activity.[2] This plant has been reported to yield terpenoids,[4,5] alkaloids,[6] and partially acetylated oligorhamnoside derivatives.[3,4] As part of our high-throughput natural products program directed toward the discovery of novel antibacterial agents from plants,[7] eight partially acetylated oligorhamnosides (**1**−**8**) with significant in vitro antibacterial activity were obtained from *C. patens*. Complete HSQC, HMBC, and NOESY NMR spectra are generally needed to elucidate a new structure and its relative stereochemistry. However, at least 70 μg of a sample is needed for HSQC/HMBC even using the advanced capillary-scale NMR technologies.[8] Compared to conventional isolation, it was hard to generate more physical-chemical data for the mass-limited compounds previously obtained via high-throughput natural products chemistry procedures.[7,8] In the case of compounds **1**−**8**, multiple semipreparative HPLC collections were performed and the combination of the corresponding evaporative light scattering detection (ELSD) peaks (Figure 1) was achieved to obtain pure active compounds in sufficient quantity to measure their optical rotations and FT-IR data.



Results and Discussion

The molecular weight of compound **1** and its chemical formula of $C_{44}H_{74}O_{21}$ were deduced from the negative mode high-resolution electrospray ionization mass spectrum (HRESIMS), which showed the $[M - H]^-$ ion peak at *m/z* 937.4642. The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) data showed general features similar to those of the known partially acetylated dodecanyl tetrarhamnosides **4**−**8**.[3,9,10] In the low field of the HSQC NMR spectrum, four anomeric protons were easily confirmed at δ 4.64 (1H, brs, H-1A; δ_C: 101.5), 5.22 (1H, brs, H-1B; δ_C: 103.0), 4.87 (1H, brs, H-1C; δ_C: 100.6), and 4.81 (1H, brs, H-1D; δ_C: 101.1). Therefore, four separated proton spin systems could be readily distinguished as the respective rhamnoside rings in the gCOSY spectrum. Similar to the known dodecanyl tetrarhamnosides **5**−**8**,[3,9] the terminal methyl group at δ 0.90 (3H, t, J = 6.7 Hz) and the signals at δ 1.31−1.37 (18H, overlapped) and 1.59 (2H, m) indicated the presence of a saturated long-chain hydrocarbon. The coupling between the nonequivalent oxygenated methylene protons at δ 3.68 and 3.40 (each 1H, m) and the methylene protons at δ 1.59 was also observed in the gCOSY spectrum, which together with the molecular formula suggested the presence of an *n*-dodecanyl ether moiety. Four acetyl methyl groups were found at δ 2.14 (3H, s), 2.11 (3H, s), 2.07 (3H, s), and 2.06 (3H, s) in the ^{1}H NMR spectrum. The linkage positions of the ether side chain and the acetoxyl substituents as well as the interglycosides were all unambiguously determined by HMBC correlations (Figure 2), which showed that the *n*-dodecanyl moiety identified as the aglycon was linked to the C-1A position, the interglycosidic positions were sequentially at (1→3), (1→3), and (1→4), and the four acetoxyl moieties were linked to C-4B, C-2C, C-4C, and C-3D, respectively. The α anomeric configuration in each rhamnose unit was deduced from a 2D $^1J_{C-1,H-1}$ NMR experiment. From the completely resolved multiplets for the four anomeric ^{13}C atoms, at δ 101.5 (C-1A), 103.0 (C-1B), 100.6 (C-1C), and 101.1 (C-1D), all four of the $^1J_{C-1,H-1}$ values were found to be around 169−170 Hz.[3,9,11] The α configuration was further supported by the small coupling constant (less than 1 Hz) of the anomeric proton.[12] Thus, **1** was elucidated to be 1-*O*-dodecanyl-3-*O*-acetyl-α-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside.

The molecular weight of compound **2** and its chemical formula of $C_{36}H_{62}O_{16}$ were determined from the positive mode high-

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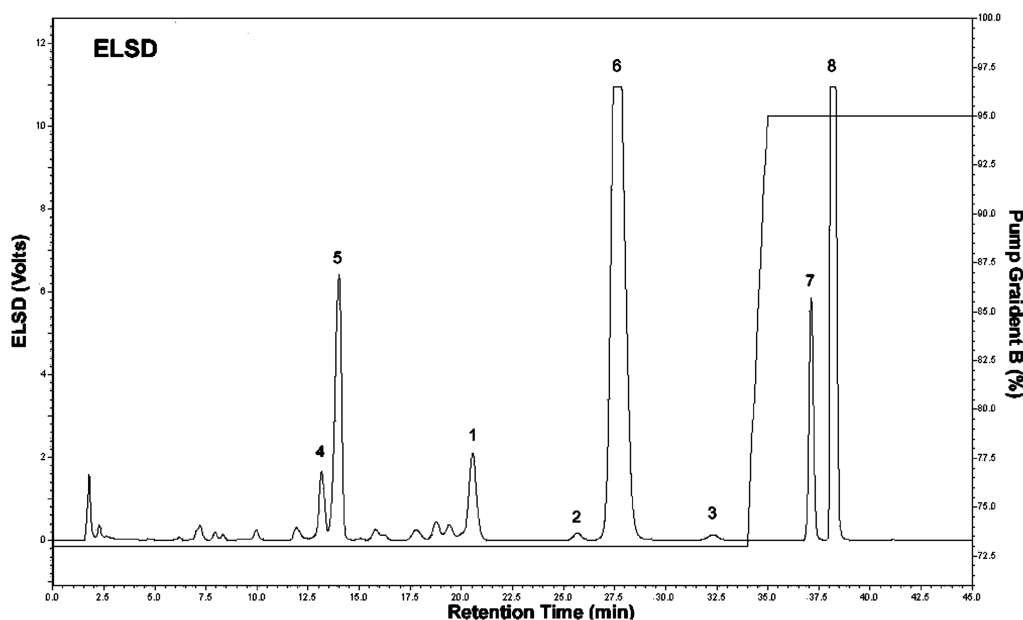

Figure 1. Semipreparative HPLC purification of the tetrarhamnoside (**1**, **4**−**8**) and trirhamnoside (**2**, **3**) dodecanyl ether derivatives. The above ELSD chromatogram is represented for only one of the collections. The other five ELSD chromatograms are very similar to this one. Quantities of each compound was estimated by its own ELSD peak integration area.[8] The total amount of each compound resulted from the combination of the corresponding ELSD peaks from all six collections.

Table 1. ^{1}H NMR Spectral Data for **1**−**3** (in CD_3OD at 600 MHz, *J* values in Hz)[a]

position	1	2	3
1A	4.64 (1H, brs)	4.65 (1H, d, 1.1)	4.65 (1H, brs)
2A	3.72 (1H, brd, 3.5)	3.73 (1H, dd, 3.4, 1.1)	3.73 (1H, brd, 3.4)
3A	3.76 (1H, dd, 9.9, 3.5)	3.77 (1H, dd, 9.9, 3.4)	3.76 (1H, dd, 9.9, 3.4)
4A	3.54 (1H, t, 9.9)	3.52 (1H, t, 9.9)	3.53 (1H, t, 9.9)
5A	3.64 (1H, m)	3.64 (1H, m)	3.64 (1H, m)
Me-6A	1.28 (3H, d, 6.2)	1.29 (3H, d, 6.2)	1.28 (3H, d, 6.2)
1B	5.22 (1H, brs)	5.23 (1H, d, 1.4)	5.23 (1H, d, 1.2)
2B	4.07 (1H, brd, 3.4)	4.07 (1H, dd, 3.5, 1.4)	4.08 (1H, dd, 3.5, 1.2)
3B	3.88 (1H, dd)[b]	3.89 (1H, dd, 9.9, 3.5)	3.91 (1H, dd)[b]
4B	5.10 (1H, t, 9.8)	5.10 (1H, t, 9.9)	5.12 (1H, t, 9.9)
5B	3.90 (1H, m)[b]	3.92 (1H, m)	3.88 (1H, m)[b]
Me-6B	1.15 (3H, d, 6.1)	1.14 (3H, d, 6.0)	1.15 (3H, d, 6.1)
1C	4.87 (1H, brs)[c]	4.93 (1H, brs)	4.87 (1H, brs)[c]
2C	5.01 (1H, brd, 3.4)	4.95 (1H, brd, 3.4)	5.08 (1H, brd, 3.5)
3C	4.24 (1H, dd, 9.9, 3.4)	4.13 (1H, dd, 9.9, 3.4)	5.14 (1H, dd, 9.9, 3.5)
4C	4.98 (1H, t, 9.9)	4.89 (1H, t)[c]	3.50 (1H, t, 9.9)
5C	4.04 (1H, m)	3.98 (1H, m)	3.92 (1H, m)[b]
Me-6C	1.17 (3H, d, 6.4)	1.16 (3H, d, 6.4)	1.30 (3H, d, 6.5)
1D	4.81 (1H, brs)[d]		
2D	3.87 (1H, brd)[b]		
3D	4.80 (1H, dd)[d]		
4D	3.68 (1H, t, 9.9)		
5D	3.50 (1H, m)		
Me-6D	1.22 (3H, d, 6.3)		
MeCO	2.14 (3H, s) [4B]	2.13 (3H, s) [4B]	2.09 (3H, s) [4B]
	2.11 (3H, s) [2C]	2.12 (3H, s) [2C]	2.08 (3H, s) [2C]
	2.07 (3H, s) [4C][e]	2.09 (3H, s) [4C]	2.02 (3H, s) [3C]
	2.06 (3H, s) [3D][e]		
OCH_2	3.68/3.40 (m)	3.67/3.41 (m)	3.67/3.41 (m)
$CH_2\ \beta$	1.59 (2H, m)	1.56 (2H, m)	1.57 (2H, m)
$CH_2\ \gamma$	1.37 (2H, m)	1.38 (2H, m)	1.38 (2H, m)
$(CH_2)_8$	1.31 (16H, brs)	1.29 (16H, brs)	1.30 (16H, brs)
Me	0.90 (3H, t, 6.7)	0.91 (3H, t, 6.7)	0.91 (3H, t, 6.8)

[a] Assignments were made by a combination of 1D- and 2D-NMR techniques (^{1}H−^{1}H COSY, HSQC, and HMBC). [b] Proton signals in the same column with the same superscript were overlapped. [c] Proton signals were hidden within the residual HOD signal in the solvent of CD_3OD. [d] Proton signals in the same column with the same superscript were overlapped. [e] Assignments in the same column with the same superscript are interchangeable.

Table 2. ^{13}C NMR Spectral Data for **1**−**3** (in CD_3OD at 150 MHz)[a]

position	1	2	3
1A	101.5	101.6	101.5
2A	72.6	72.8	72.7
3A	73.3	73.2	73.3
4A	81.2	81.3	81.3
5A	68.1	68.2	68.0
Me-6A	18.9	18.5	18.5
1B	103.0	103.3	103.2
2B	72.0	71.8	71.8
3B	78.5	78.6	78.6
4B	73.9	74.1	74.2
5B	69.9	70.0	70.2
Me-6B	18.0	18.1	18.2
1C	100.6	100.5	100.5
2C	73.6	73.9	71.8
3C	76.5	68.4	73.9
4C	74.1	74.8	69.2
5C	70.2	69.1	68.2
Me-6C	18.2	17.1	17.5
1D	101.1		
2D	72.3		
3D	74.5		
4D	70.7		
5D	69.2		
Me-6D	17.8		
*Me*CO	21.2 [4B]	20.9 [4B]	21.0 [4B]
	20.9 [2C]	20.9 [2C]	20.9 [2C]
	20.9 [4C][b]	20.4 [4C]	20.6 [3C]
	20.8 [3D][b]		
*C*OMe	171.4 [4B]	171.4 [4B]	171.4 [4B]
	171.3 [2C]	171.2 [2C]	171.2 [2C]
	171.0 [4C]	170.8 [4C]	171.2 [3C]
	171.0 [3D]		
OCH_2	68.4	68.5	68.5
$CH_2\ \beta$	30.5	30.4	30.4
$CH_2\ \gamma$	27.3	27.3	27.3
$(CH_2)_6$	30.9−30.3	30.9−30.3	30.9−30.3
CH_2	32.8	32.7	32.7
CH_2	23.2	23.2	23.2
Me	14.2	14.1	14.1

[a] Assignments were made by a combination of 1D- and 2D-NMR techniques (^{1}H−^{1}H COSY, HSQC, and HMBC). [b] Assignments in the same column with the same superscript are interchangeable.

resolution electrospray ionization mass spectrum (HRESIMS). The MS, ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) data indicated **2** was a partially acetylated dodecanyl trirhamnoside derivative, which had only three anomeric protons and carbons in the low field of the HSQC NMR spectrum, which resonated at δ 4.65 (1H, d, J = 1.1 Hz, H-1A; δ_C: 101.6), 5.23 (1H, d, J = 1.4 Hz, H-1B; δ_C:

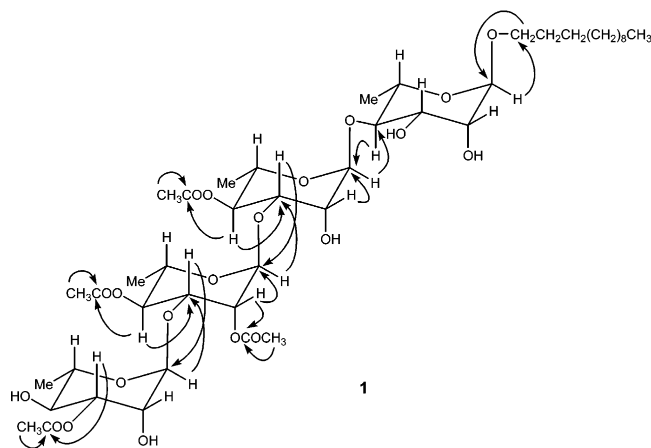

Figure 2. Key $^{n}J_{C,H}$ ($n = 2$ or 3) HMBC correlations of **1** to construct the linkage positions of the ether side chain and the acetoxyl substituents as well as the interglycosides.

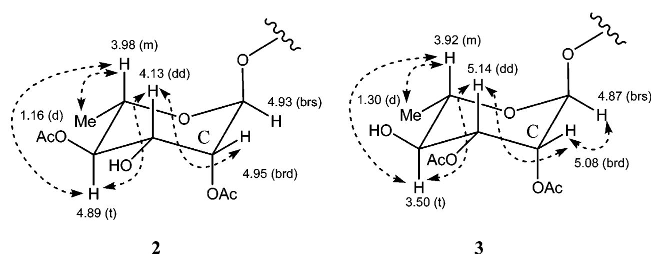

Figure 3. $^{1}H-^{1}H$ COSY correlations observed in the terminal rhamnose unit of **2** and **3**.

103.3), and 4.93 (1H, brs, H-1C; δ_C: 100.5). Except for a proton spin system of the *n*-dodecanyl ether moiety, three separated proton spin systems were revealed in the gCOSY spectrum attributable to three rhamnoside units. Three acetyl methyl groups were found at δ 2.13 (3H, s), 2.12 (3H, s), and 2.09 (3H, s) in the ^{1}H NMR spectrum. The linkage positions of the *n*-dodecanyl moiety at C-1A, the interglycosides at (1→3) and (1→4), and an acetoxyl at C-4B in these three rhamnoside rings were established by a combination of gCOSY, HSQC, and HMBC correlations, which were totally identical with those in **1**. The locations of two other acetoxyl moieties at C-2C and C-4C in the terminal rhamnose unit were determined by the observed correlations in the gCOSY (Figure 3), and the downfield chemical shifts of H-2C and H-4C resonated at δ 4.95 and 4.89, respectively. The α configuration in each rhamnose unit was deduced from biogenetic consideration and also from the small coupling constant (<1.4 Hz) of the anomeric proton. Therefore, **2** was elucidated to be 1-*O*-dodecanyl-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside.

The HRESIMS data showed that compounds **3** and **2** both have the same chemical formula ($C_{36}H_{62}O_{16}$). From its ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), and HSQC NMR spectral data, **3**, containing three acetates, showed features similar to those of **2**. The only difference was the chemical shifts in the terminal rhamnose moiety, which required different locations of the two acetoxyl groups (Figure 3). Analysis of the gCOSY spectrum for ring C showed the existence of an eight-proton-spin-system coupling between the anomeric H-1C at δ 4.87 (1H, brs) and H-2C at δ 5.08 (1H, brd, $J = 3.5$ Hz), between H-2C and H-3C at δ 5.14 (1H, dd, $J = 9.9$, 3.5 Hz), between H-3C and H-4C at δ 3.50 (1H, t, $J = 9.9$ Hz), between H-4C and H-5C at δ 3.92 (1H, m), and between H-5C and Me-6C at δ 1.30 (3H, d, $J = 6.5$ Hz). The downfield chemical shifts of H-2C and H-3C revealed that the acetoxyl group was located at C-2C and C-3C, respectively. Consequently, **3** was

Table 3. Antibacterial Activity of Compounds **1**, **2**, and **6**[a]

compound/ antimicrobial agent[b]	MIC (μg/mL)	
	S. aureus ATCC 33591[c]	*S. aureus* 78-13607A[c]
1	8	16
2	8[d]	8
6	0.5[d]	1
oxacillin	>8	>8
erythromycin	>8	0.25
clindamycin	>8	0.12
tetracycline	>8	≤ 4
trimethoprim/sulfamethoxazole	≤ 0.5	≤ 0.5
ciprofloxacin	0.5	>4
gentamicin	≤ 2	≤ 2
chloramphenicol	>16	
rifampin	≤ 0.25	≤ 0.25
vancomycin	1	1

[a] Vancomycin (MIC, 1 μg/mL) was utilized as a test compound control and was tested concurrently during each test day. An ethanol control was utilized to determine the significant antimicrobial effect at the highest test concentration of test compound (alcohol carryover). [b] MIC data for these antimicrobial agents were shown here for the antimicrobial susceptibility profile. [c] Both strains of methicillin-resistant *Staphylococcus aureus* were susceptible to Synercid, linezolid, and mupirocin. [d] See ref 13.

elucidated as 1-*O*-dodecanyl-2,3-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside.

Partially acetylated dodecanyl tetra- and trirhamnoside derivatives cleistetroside-1 (**8**),[3,10] -2 (**6**),[3,10] -3,[9] -4 (**5**),[9,10] -6 (**7**),[3,4,9] and -7,[9] as well as cleistrioside-1,[9,10] -2,[3,4] -3,[3,4] and -4,[3] were previously obtained from *Cleistopholis* spp. On the basis of the previous works by Waterman,[3,9] the trivial names for the above three new isolates were assigned as cleistetroside-8 (**1**), cleistrioside-5 (**2**), and cleistrioside-5 (**3**). To the best of our knowledge, the trivial name of cleistetroside-5 is missing in the series; therefore, the known compound **4**[10] is herein named as cleistetroside-5.

Compounds **1** and **2** were found to possess significant in vitro antibacterial activity against the Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* ATCC 33591 and *S. aureus* 78-13607A with MICs of ≤ 16 μg/mL. During this same study phase, the major component **6** was found to have significant MICs of 0.5 and 1 μg/mL (Table 3).[13] Compounds **2** and **6** were tested against a more extensive panel of Gram-positive bacterial pathogens including strains with well-characterized resistance phenotypes. The tests were broken into phase I and phase II. Phase I organisms (Table 4) were either ATCC strains or well-characterized clinical isolates from the global SENTRY Antimicrobial Surveillance Program. Phase II organisms (Table 5) were well-characterized clinical isolates with differing resistance profiles tested to determine potential co-resistances to these two compounds. Compounds **2** and **6** exhibited significant activity when tested against these Gram-positive pathogens, with the exception of some *Corynebacterium* spp. Equivalent potencies for compounds **2** and **6** were demonstrated for *S. aureus*, *Enterococcus* spp., and *Bacillus* spp. A slight potency advantage (2-fold lower) of compound **6** against *S. pneumoniae* was noted. Compound **2** was more potent against coagulase-negative staphylococci (equivalent to that against *S. aureus*) than compound **6**. Compound **6** showed a significant decrease in growth at equivalent MIC values; growth, however, persisted in the wells at ≥ 32 μg/mL. Further studies should be considered including minimum bactericidal concentration (MBC) determinations, synergy studies, mutational rates to resistance, the same bioassays applied to the rest of the pure partially acetylated oligorhamnosides for structure−activity relationship (SAR) consideration, etc., to better characterize compounds **2** and **6** during the early preclinical phase of development.

Table 4. Phase I Antibacterial Activity of Compounds **2** and **6**[a]

organism	MIC (μg/mL) 2	MIC (μg/mL) 6
Staphylococcus aureus ATCC 29213 (oxacillin-susceptible)	2	4
Staphylococcus aureus ATCC 33591 (oxacillin-resistant)	2[b]	2[b]
Staphylococcus epidermidis clinical isolate 12−1788A (oxacillin-susceptible)[c]	4	>32
Staphylococcus haemolyticus clinical isolate 107−1240C (oxacillin-resistant)[d]	4	32
Enterococcus faecalis ATCC 29212 (ampicillin-susceptible, vancomycin-susceptible)	4	2
Enterococcus faecium clinical isolate 24-45A (ampicillin-resistant, vancomycin-resistant)	2	2
Streptococcus pneumoniae ATCC 49619 (penicillin-intermediate)	8	4
Streptococcus pneumoniae clinical isolate 33-50B (penicillin-resistant)	8	4
Corynebacterium jeikium clinical isolate 15-196A (wild-type)[e]	>32	32
Bacillus cereus clinical isolate 24-59A (wild-type)	2	2
Streptococcus pyogenes clinical isolate 91-8747 (erythromycin-resistant)	8	4
Streptococcus mitis clinical isolate 61-2900C (penicillin-resistant)	8	4
Pseudomonas aeruginosa ATCC 27853[f]	>32	32
Escherichia coli ATCC 25922[f]	>32	>32

[a] Phase I organisms are either American Type Culture Collection (ATCC) strains or well-characterized isolates from the global SENTRY Antimicrobial Surveillance Programs. Vancomycin (MIC, 1 μg/mL) and ethanol (growth ≥ 7.2% ETOH) controls provided valid internal quality control and assurance of study methods and results. [b] See ref 13. [c] Most prevalent involved in infections. [d] Second most prevalent involved in infections; bad actor. [e] This strain is resistant to most drugs. [f] Gram-negative control.

Table 5. Phase II Antibacterial Activity of Compounds **2** and **6**[a]

organism	MIC (μg/mL) 2	MIC (μg/mL) 6
*Staphylococcus*aureus 4-90A (mupirocin-resistant)	2	2−4
*Staphylococcus*aureus 300-12053A (quinupristin/dalfopristin-resistant)	2	2−4
*Staphylococcus*aureus 106-12591A (linezolid-resistant)	2	2−4
Coagulase-negative staphylococci 63-18A (mupirocin-resistant)	2−4	>32
Coagulase-negative staphylococci 90-10840A (quinupristin/dalfopristin-resistant)	2−4	>32
Coagulase-negative staphylococci 82−1645A (linezolid-resistant)	2−4	>32
Enterococcus faecalis 48-22D (vancomycin-resistant, *van*A phenotype)	2	2−4
Enterococcus faecalis 82-876D (vancomycin-resistant, *van*B phenotype)	2	2−4
Enterococcus faecalis 15-534I (linezolid-resistant)	2	2−4
Enterococcus faecalis 39-11D (high-level gentamicin and streptomycin-resistant)	2	2−4
Enterococcus faecium 15-206A (vancomycin-resistant, *van*A phenotype)	2	2−4
Enterococcus faecium 103-1232D (vancomycin-resistant, *van*B phenotype)	2	2−4
Enterococcus faecium 11-4103A (linezolid-resistant)	2	2−4
Enterococcus faecium 65-1208A (quinupristin/dalfopristin-resistant)	2	2−4
Bacillus spp. 107-1461D (tetracycline-resistant)	2	2
Bacillus spp. 67-1057A (trimethoprim/ sulfamethoxazole-resistant)	2	2
Bacillus spp. 32−2406A (erythromycin-resistant)	2	2
Streptococcus pneumoniae 68-2307B (multidrug-resistant)	4−16	4
Streptococcus pneumoniae 4−38B (erythromycin-resistant, clindamycin-resistant)	4−16	4
Streptococcus pneumoniae 35−405B (erythromycin-resistant, clindamycin-susceptible)	4−16	4
Streptococcus pneumoniae 2-2784A (levofloxacin-resistant)	4−16	4
Corynebacterium spp. 105-1513C (doxycycline-resistant)	8	16
Corynebacterium spp. 58-9161A (rifampin-resistant)	8	16
Corynebacterium spp. 61-5454A (high-level streptomycin-resistant)	8	16
β-haemolytic streptococci 91-526D (erythromycin-resistant, clindamycin-resistant)	4	4
β-haemolytic streptococci 109-820D (erythromycin-resistant, clindamycin-susceptible)	4	4
β-haemolytic streptococci 19-756A (levofloxacin-resistant)	4	4
viridans group streptococci 32-2395A (mupirocin-resistant)	4	4
viridans group streptococci 107-6380A (levofloxacin-resistant)	4	4
viridans group streptococci 27-2832A (linezolid-resistant)	4	4
viridans group streptococci 58-1602D (multidrug-resistant)	4	4

[a] Phase II organisms are well-characterized clinical isolates with differing resistance profiles tested to determine potential co-resistances to compounds **2** and **6**. Vancomycin (MIC, 0.5 μg/mL) and ethanol (growth ≥ 7.2% ETOH) controls provided valid internal quality control and assurance of study methods and results.

Experimental Section

General Experimental Procedures. For instrumentation and general automated flash chromatography, solvent evaporation, and capillary-scale NMR see the preceding papers.[7a,8] IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrometer (Nicolet Instruments Corporation, Madison, WI). Optical rotations were measured in MeOH using an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, Flanders, NJ).

To generate more pure compounds for biological confirmation and the structure elucidation, one preparative HPLC system in the former parallel four-channel preparative HPLC system[7a] was switched to a semipreparative HPLC system. The modified parallel three-channel preparative HPLC system was assembled and consisted of three Beckman System Gold 126 gradient HPLC pumps (Beckman Coulter Inc., Fullerton, CA) with system controllers and three-way solvent delivery modules, three Beckman System Gold 166 single wavelength UV detectors with preparative flow cells, a Gilson 215/849 multiple probe autosampler (Gilson Inc., Middleton, WI), and three Gilson 204 fraction collectors. Each flash fraction (flash fractions 2−5)[7a] was separated into 40 fractions (20 mL/min, 1 min per collection per tube) using the parallel three-channel preparative HPLC system. A different 40 min gradient was applied to each flash fraction for adequate separation: 60−100% acetonitrile in H_2O for flash fraction 2, 30−100% acetonitrile in H_2O for flash fraction 3, and 5−100% acetonitrile in H_2O for flash fractions 4 and 5. The system was controlled by Beckman 32 Karat chromatography software.

A modified parallel eight-channel HPLC-ELSD-MS system was assembled and consisted of a LCT time-of-flight mass spectrometer with an eight-way MUX electrospray interface (Micromass Ltd, Manchester, UK), a Waters 1525EF multisolvent delivery system (Waters Corporation, Milford, MA) to pump solvents through an eight-way manifold, which splits the flow to eight HPLC columns (4.6 × 100 mm, 4 μm, Phenomenex Fusion-RP C-18), a Gilson 215/889 multiple probe autosampler, and eight Alltech 500 ELSD detectors (Alltech Associates Inc., Deerfield, IL). HPLC chromatographic conditions were 5% acetonitrile in H_2O modified with 0.1% HCOOH for the first 2.0 min, a linear gradient of acetonitrile from 5% to 40% in 8.0 min, then to 85% in 1.0 min, hold at 85% acetonitrile for 2.0 min, then a linear gradient from 85% to 5% acetonitrile over 1.0 min, hold 5% acetonitrile for 3 min to equilibrate. The system was controlled by Waters MassLynx software version 4.0. The instrument was operated in both positive and negative electrospray modes with the capillary voltage set to 3.1 kV in positive electrospray mode and 2.7 kV in negative electrospray mode. Desolvation temperatures of 375 °C with

a source temperature of 120 °C were used for all experiments. The nitrogen desolvation and nebulizer gas flow rates were set to 1200 and 6 L/h, respectively.

Semipreparative HPLC isolation for individual compounds was modified and performed on a single-channel Beckman HPLC system consisting of a Keystone BetaMax Neutral C18 column (8 × 250 mm i.d., 5 μm), a Beckman 168 diode array UV detector, an Alltech 500 ELSD detector, and a Gilson 204 fraction collector equipped with minitubes loaded in a 96-well plate (flow rate: 3 mL/min, 0.33 min per collection time per minitube). A QuickSplit adjustable flow splitter (Analytical Scientific Instruments, El Sobrante, CA) was used to split the flow into 10:90 to ELSD and fraction collector, respectively. For ELSD Alltech 500 conditions: Set the gas flow to achieve a pressure of 3.44−3.46 bar, attenuation 5, and temperature 80 ± 1°C.

Plant Material. The leaves of *C. patens* were collected from the Tchimbele region of Gabon in November 2000. Plant samples were dried on site, then shipped to Sequoia Sciences. The plant was identified by Gretchen Walters (Missouri Botanical Garden Herbarium, St. Louis, MO). A voucher specimen (No. 831) was deposited at the Herbarium of the Missouri Botanical Garden.

Extraction and Isolation. Dried leaves (400 g) were extracted with EtOH/EtOAc (50:50) followed by H_2O/MeOH (30:70), to obtain 18 and 26 g of dry organic and aqueous extracts, respectively. The organic extracts were loaded on the Flash Master II automated chromatographic system using our standard elution gradient to generate the flash fractions.[7a,8] The flash fraction 4 (75% EtOAC, 25% MeOH) totaled 300 mg; a 50 mg aliquot was fractionated by preparative C18 HPLC from 5% to 100% acetonitrile in H_2O, collecting 40 1-min fractions. Compounds 1−8 resided in preparative HPLC fraction 35, which was primarily antibacterial against Gram-positive methicillin-resistant *Staphylococcus aureus*. Review of the HPLC-ELSD-MS data acquired on all of the preparative fractions from the flash fraction 4 suggested preparative HPLC fraction 35 contained compounds with molecular weights less than 1000, which could readily be isolated using reversed-phase chromatography. The initial mobile phase gradient applied to isolating compounds **1**−**8** from fraction 35 was based on the elution profile observed during the preparative HPLC separation that created the fraction. A semipreparative HPLC method was developed that resulted in an isocratic gradient of 73% acetonitrile in H_2O acidified with 0.05% TFA for 34 min, then followed by a linear gradient of acetonitrile from 73% to 95% over 1.0 min, and finally followed by an isocratic gradient of 95% B for 10.0 min. The corresponding ELSD peaks (Figure 1) from six collections were combined to afford compounds **1** (1.1 mg, t_R = 20.5 min), **2** (0.4 mg, t_R = 25.6 min), **3** (0.4 mg, t_R = 32.2 min), **4** (0.8 mg, t_R = 13.1 min), **5** (2.6 mg, t_R = 14.0 min), **6** (4.8 mg, t_R = 27.8 min), **7** (1.9 mg, t_R = 37.1 min), and **8** (2.9 mg, t_R = 38.3 min).

Cleistetroside-8 (1): gummy solid; $[\alpha]_D^{20}$ (*c* 0.10, MeOH) −45.5; IR ν_{max} (film) 3465 (brs), 2977, 2921, 2855, 1745 (brs), 1376, 1233, 1134, 1077, 1041 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ESIMS *m/z* 937 [M − H]$^-$, 983 [M + HCOO$^-$]$^-$; HRESIMS *m/z* 937.4642 ($C_{44}H_{73}O_{21}$ requires 937.4644).

Cleistrioside-5 (2): gummy solid; $[\alpha]_D^{20}$ (*c* 0.04, MeOH) −41.0; IR ν_{max} (film) 3471 (brs), 2977, 2925, 2852, 1745 (brs), 1377, 1235, 1137, 1076, 1043 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ESIMS *m/z* 749 [M − H]$^-$, 795 [M + HCOO$^-$]$^-$, 751 [M + H]$^+$; HRESIMS *m/z* 751.4120 ($C_{36}H_{63}O_{16}$ requires 951.4116).

Cleistrioside-6 (3): gummy solid; $[\alpha]_D^{20}$ (*c* 0.04, MeOH) −47.5; IR ν_{max} (film) 3468 (brs), 2975, 2924, 2853, 1745 (brs), 1377, 1236, 1133, 1077, 1044 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ESIMS *m/z* 795 [M + HCOO$^-$]$^-$, 751 [M + H]$^+$, 773 [M + Na]$^+$; HRESIMS *m/z* 773.3939 ($C_{36}H_{62}O_{16}$Na requires 773.3936).

Cleistetroside-5 (4):[10] gummy solid; $[\alpha]_D^{20}$ (*c* 0.05, MeOH) −49.5; IR ν_{max} (film) 3460 (brs), 2976, 2923, 2853, 1744 (brs), 1376, 1235, 1135, 1077, 1042 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 4.64 (1H, brs, H-1A), 3.73 (1H, brd, J = 3.5 Hz, H-2A), 3.76 (1H, dd, J = 9.9, 3.5 Hz, H-3A), 3.52 (1H, t, J = 9.9 Hz, H-4A), 3.64 (1H, m, H-5A), 1.29 (3H, d, J = 6.2 Hz, Me-6A), 5.22 (1H, brs, H-1B), 4.08 (1H, brd, J = 3.4 Hz, H-2B), 3.89 (1H, dd, overlapped, H-3B), 5.11 (1H, t, J = 9.8 Hz, H-4B), 3.90 (1H, m, overlapped, H-5B), 1.15 (3H, d, J = 6.1 Hz, Me-6B), 4.87 (1H, brs, H-1C), 5.04 (1H, brd, J = 3.4 Hz, H-2C), 4.25 (1H, dd, J = 9.9, 3.4 Hz, H-3C), 3.83 (1H, t, J = 9.9 Hz, H-4C), 3.99 (1H, m, H-5C), 1.17 (3H, d, J = 6.4 Hz, Me-6C), 4.81 (1H, brs, H-1D), 3.69 (1H, brd, J = 3.5 Hz, H-2D), 3.50 (1H, dd, J = 9.8, 3.5 Hz, H-3D), 3.34 (1H, t, J = 9.8 Hz, H-4D), 3.49 (1H, m, H-5D), 1.21 (3H, d, J = 6.3 Hz, Me-6D), 2.13 (3H, s, *Me*CO-4B), 2.09 (3H, s, *Me*CO-2C), 3.66/3.40 (each 1H, m, −OCH_2), 1.59 (2H, m), 1.36 (2H, m), 1.31 (16H, brm), and 0.90 (3H, t, J = 6.7 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 101.5 (C-1A), 72.6 (C-2A), 73.3 (C-3A), 81.4 (C-4A), 68.2 (C-5A), 18.5 (Me-6A), 103.1 (C-1B), 72.1 (C-2B), 78.7 (C-3B), 73.9 (C-4B), 69.8 (C-5B), 18.0 (Me-6B), 100.6 (C-1C), 73.7 (C-2C), 76.2 (C-3C), 73.8 (C-4C), 70.2 (C-5C), 17.9 (Me-6C), 104.3 (C-1D), 72.7 (C-2D), 72.4 (C-3D), 73.9 (C-4D), 70.8 (C-5D), 18.3 (Me-6D), 21.1 and 170.9 (MeCO-4B), 20.9 and 171.1 (MeCO-2C), 68.7 (−OCH_2), 30.6 (−CH_2), 27.3 (−CH_2), 30.9−30.3 (6 × −CH_2), 32.8 (−CH_2), 23.3 (−CH_2), and 14.4 (−CH_3); ESIMS *m/z* 853 [M − H]$^-$.

Cleistetroside-4 (5):[9,10] gummy solid; $[\alpha]_D^{20}$ (*c* 0.25, MeOH) −71.5; IR ν_{max} (film) 3465 (brs), 2977, 2923, 2854, 1745 (brs), 1376, 1235, 1136, 1076, 1041 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 4.65 (1H, brs, H-1A), 3.72 (1H, brd, J = 3.4 Hz, H-2A), 3.76 (1H, dd, J = 9.9, 3.4 Hz, H-3A), 3.54 (1H, t, J = 9.9 Hz, H-4A), 3.64 (1H, m, H-5A), 1.29 (3H, d, J = 6.2 Hz, Me-6A), 5.23 (1H, brs, H-1B), 4.07 (1H, brd, J = 3.5 Hz, H-2B), 3.88 (1H, dd, overlapped, H-3B), 5.12 (1H, t, J = 9.8 Hz, H-4B), 3.90 (1H, m, overlapped, H-5B), 1.15 (3H, d, J = 6.1 Hz, Me-6B), 4.87 (1H, brs, H-1C), 5.05 (1H, brd, J = 3.5 Hz, H-2C), 4.24 (1H, dd, J = 9.9, 3.5 Hz, H-3C), 4.99 (1H, t, J = 9.9 Hz, H-4C), 4.03 (1H, m, H-5C), 1.17 (3H, d, J = 6.4 Hz, Me-6C), 4.80 (1H, brs, H-1D), 3.68 (1H, brd, J = 3.5 Hz, H-2D), 3.52 (1H, dd, J = 9.8, 3.5 Hz, H-3D), 3.34 (1H, t, J = 9.8 Hz, H-4D), 3.49 (1H, m, H-5D), 1.21 (3H, d, J = 6.2 Hz, Me-6D), 2.14 (3H, s, *Me*CO-4B), 2.12 (3H, s, *Me*CO-2C), 2.09 (3H, s, *Me*CO-4C), 3.67/3.40 (each 1H, m, −OCH_2), 1.59 (2H, m), 1.38 (2H, m), 1.32 (16H, brm), and 0.90 (3H, t, J = 6.6 Hz); ^{13}C NMR (CD_3OD) data in agreement with literature;[9] ESIMS *m/z* 895 [M − H]$^-$, 941 [M + HCOO$^-$]$^-$.

Cleistetroside-2 (6):[3,10] gummy solid; $[\alpha]_D^{20}$ (*c* 0.40, MeOH) −59.5; IR ν_{max} (film) 3469 (brs), 2975, 2924, 2853, 1746 (brs), 1375, 1236, 1134, 1077, 1042 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 4.65 (1H, brs, H-1A), 3.72 (1H, brd, J = 3.4 Hz, H-2A), 3.76 (1H, dd, J = 9.9, 3.4 Hz, H-3A), 3.53 (1H, t, J = 9.9 Hz, H-4A), 3.64 (1H, m, H-5A), 1.29 (3H, d, J = 6.2 Hz, Me-6A), 5.23 (1H, brs, H-1B), 4.07 (1H, brd, J = 3.5 Hz, H-2B), 3.88 (1H, dd, overlapped, H-3B), 5.12 (1H, t, J = 9.8 Hz, H-4B), 3.90 (1H, m, overlapped, H-5B), 1.15 (3H, d, J = 6.2 Hz, Me-6B), 4.88 (1H, brs, H-1C), 5.05 (1H, brd, J = 3.4 Hz, H-2C), 4.25 (1H, dd, J = 9.9, 3.4 Hz, H-3C), 4.99 (1H, t, J = 9.9 Hz, H-4C), 4.04 (1H, m, H-5C), 1.18 (3H, d, J = 6.3 Hz, Me-6C), 4.83 (1H, brs, H-1D), 3.71 (1H, brd, J = 3.5 Hz, H-2D), 3.68 (1H, dd, J = 9.8, 3.5 Hz, H-3D), 4.90 (1H, t, J = 9.8 Hz, H-4D), 3.66 (1H, m, H-5D), 1.10 (3H, d, J = 6.3 Hz, Me-6D), 2.14 (3H, s, *Me*CO-4B), 2.12 (3H, s, *Me*CO-2C), 2.08 (3H, s, *Me*CO-4C), 2.10 (3H, s, *Me*CO-4D), 3.67/3.39 (each 1H, m, −OCH_2), 1.59 (2H, m), 1.37 (2H, m), 1.32 (16H, brm), and 0.90 (3H, t, J = 6.7 Hz); ^{13}C NMR (CD_3OD) data in agreement with literature;[3] ESIMS *m/z* 937 [M − H]$^-$, 983 [M + HCOO$^-$]$^-$.

Cleistetroside-6 (7):[3,9] gummy solid; $[\alpha]_D^{20}$ (*c* 0.15, MeOH) −57.0; IR ν_{max} (film) 3467 (brs), 2976, 2924, 2851, 1744 (brs), 1377, 1235, 1137, 1076, 1044 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 4.66 (1H, brs, H-1A), 3.72 (1H, brd, J = 3.5 Hz, H-2A), 3.76 (1H, dd, J = 9.8, 3.5 Hz, H-3A), 3.53 (1H, t, J = 9.8 Hz, H-4A), 3.64 (1H, m, H-5A), 1.29 (3H, d, J = 6.2 Hz, Me-6A), 5.23 (1H, brs, H-1B), 4.07 (1H, brd, J = 3.5 Hz, H-2B), 3.88 (1H, dd, overlapped, H-3B), 5.12 (1H, t, J = 9.8 Hz, H-4B), 3.90 (1H, m, overlapped, H-5B), 1.16 (3H, d, J = 6.1 Hz, Me-6B), 4.87 (1H, brs, H-1C), 5.04 (1H, brd, J = 3.5 Hz, H-2C), 4.26 (1H, dd, J = 9.8, 3.5 Hz, H-3C), 4.99 (1H, t, J = 9.8 Hz, H-4C), 4.04 (1H, m, H-5C), 1.18 (3H, d, J = 6.4 Hz, Me-6C), 4.82 (1H, brs, H-1D), 4.84 (1H, brd, J = 3.4 Hz, H-2D), 3.86 (1H, dd, J = 9.8, 3.4 Hz, H-3D), 4.85 (1H, t, J = 9.8 Hz, H-4D), 3.70 (1H, m, H-5D), 1.12 (3H, d, J = 6.3 Hz, Me-6D), 2.15 (3H, s, *Me*CO-4B), 2.09 (3H, s, *Me*CO-2C), 2.14 (3H, s, *Me*CO-4C), 2.11 (3H, s, *Me*CO-2D), 2.12 (3H, s, *Me*CO-4D), 3.66/3.39 (each 1H, m, −OCH_2), 1.59 (2H, m), 1.38 (2H, m), 1.31 (16H, brm), and 0.91 (3H, t, J = 6.7 Hz); ^{13}C NMR (CD_3OD) data in agreement with literature;[3,9] ESIMS *m/z* 979 [M − H]$^-$, 1025 [M + HCOO$^-$]$^-$, 981 [M + H]$^+$, 1003 [M + Na]$^+$.

Cleistetroside-1 (8):[3,10] gummy solid; $[\alpha]_D^{20}$ (*c* 0.25, MeOH) −58.5; IR ν_{max} (film) 3463 (brs), 2976, 2925, 2851, 1745 (brs), 1376, 1236, 1135, 1077, 1043 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 4.64 (1H, brs, H-1A), 3.72 (1H, brd, J = 3.5 Hz, H-2A), 3.76 (1H, dd, J = 9.9, 3.5 Hz, H-3A), 3.52 (1H, t, J = 9.9 Hz, H-4A), 3.64 (1H, m, H-5A), 1.28 (3H, d, J = 6.2 Hz, Me-6A), 5.23 (1H, brs, H-1B), 4.08 (1H, brd, J = 3.5 Hz, H-2B), 3.89 (1H, dd, overlapped, H-3B), 5.12 (1H, t, J = 9.8 Hz, H-4B), 3.91 (1H, m, overlapped, H-5B), 1.15 (3H, d, J = 6.2

Hz, Me-6B), 4.90 (1H, brs, H-1C), 5.03 (1H, brd, $J = 3.4$ Hz, H-2C), 4.26 (1H, dd, $J = 9.9$, 3.4 Hz, H-3C), 4.99 (1H, t, $J = 9.9$ Hz, H-4C), 4.04 (1H, m, H-5C), 1.17 (3H, d, $J = 6.4$ Hz, Me-6C), 4.85 (1H, brs, H-1D), 3.86 (1H, brd, $J = 3.4$ Hz, H-2D), 4.94 (1H, dd, $J = 9.8$, 3.4 Hz, H-3D), 5.08 (1H, t, $J = 9.8$ Hz, H-4D), 3.81 (1H, m, H-5D), 1.13 (3H, d, $J = 6.3$ Hz, Me-6D), 2.16 (3H, s, *Me*CO-4B), 2.13 (3H, s, *Me*CO-2C), 2.03 (3H, s, *Me*CO-4C), 2.02 (3H, s, *Me*CO-3D), 2.09 (3H, s, *Me*CO-4D), 3.66/3.39 (each 1H, m, $-OCH_2$), 1.58 (2H, m), 1.37 (2H, m), 1.32 (16H, brm), and 0.90 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (CD_3OD) data in agreement with literature;[3] ESIMS *m/z* 979 $[M - H]^-$, 1025 $[M + HCOO^-]^-$, 981 $[M + H]^+$, 1003 $[M + Na]^+$.

Antibacterial Activity. Compounds **1** (20 μg), **2** (30 μg), and **6** (30 μg)[13] were bioassayed for their in vitro antibacterial activity against Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* ATCC 33591 and *S. aureus* 78-13607A, a clinical isolate from France isolated from a patient with a documented bloodstream infection (Table 3). Compounds **2** (300 μg) and **6** (1.1 mg)[13] were bioassayed for their in vitro antibacterial activity against an expanded panel of Gram-positive bacteria (Tables 4 and 5). The assays were performed at the Jones Group/JMI Laboratories (North Liberty, IA). Organisms were inoculated into broth microdilution panels at concentrations suggested by the National Committee for Clinical Laboratory Standard Methods (M7-A6, 2003). Vancomycin was used as a control and tested concurrently. Ethanol controls were used to determine the significance of antimicrobial effect at the highest test concentration. Isolates were aseptically transferred into panel wells using an autoinoculating device. Cation-adjusted Mueller-Hinton broth was used as the growth medium. Isolates were incubated overnight to 24 h at 35°C in ambient air. Panels were read manually, and no visual growth of organisms was recorded as the endpoint MIC.

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