JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Halogenated Spirotetronates from *Actinoallomurus*

Carlo Mazzetti,*[,†,‡] Mirko Ornaghi,[†] Eleonora Gaspari,[†] Silvia Parapini,[§] Sonia Maffioli,[†] Margherita Sosio,*[,†] and Stefano Donadio[†]

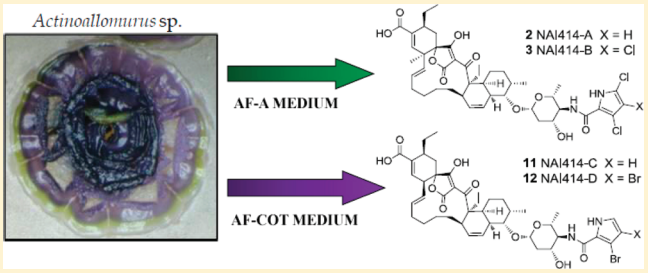
[†]NAICONS, Via G. Fantoli 16/15, 20138, Milan, Italy

[‡]ITB-CNR, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy

[§]Dipartimento di Sanità Pubblica, Microbiologia, Virologia, Università degli Studi di Milano, Via Carlo Pascal 36, 20133 Milan, Italy

Supporting Information

ABSTRACT: Two new members of the spirotetronate class, nai414-A and nai414-B, were discovered and isolated from an *Actinoallomurus* sp. Their structures were established by 1D and 2D NMR, UV, and MS analyses and by chemical degradation. They showed antimicrobial and antitumor activity against Gram-positive bacteria and against human microvascular endothelial cells, respectively. Substituting bromide for chloride ions in the growth medium afforded mono- and dibrominated derivatives.



The spirotetronates are a class of secondary metabolites that feature an unusual aglycone (**1**) that contains a characteristic tetronic acid spiro-linked to a cyclohexene ring and connected to a *trans*-decalin system either by a ketone or by a carboxylic ester (Figure 1). Tetrocarcin A, chlorothricin, and kijanimicin are three representative members of this class, and they show broad biological activities such as antibacterial[1] and antitumor[2] activity and cholesterol biosynthesis inhibition.[3]

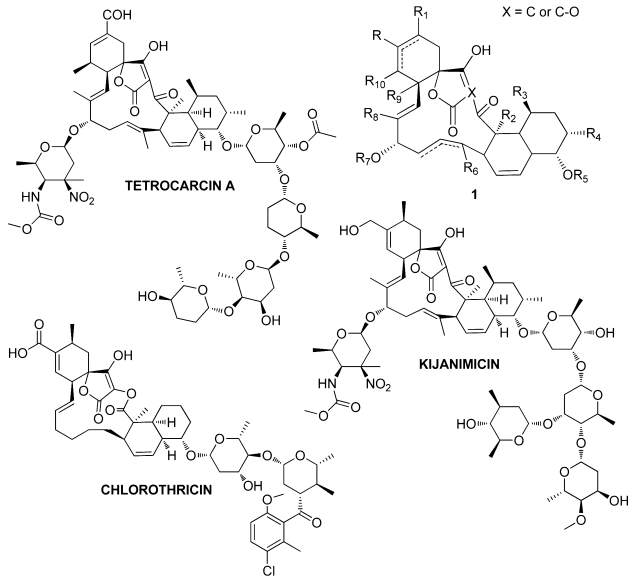
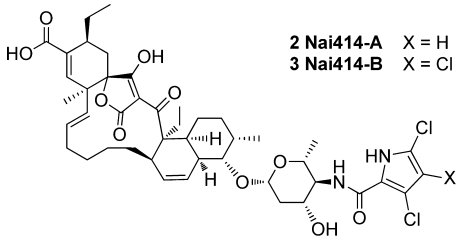


Figure 1. Members of the spirotetronate class.

During our screening for new bioactive compounds from the genus *Actinoallomurus*,[4] we have discovered two new molecules belonging to this class, designated nai414-A (**2**) and nai414-B (**3**), which showed interesting antistaphyloccocal activity.



RESULTS AND DISCUSSION

The producing strain ID 145414 was isolated from a soil sample collected in Avigliano (Potenza, Italy). The phylogenetic analysis of its 16S rRNA sequence assigned it to the phylotype Alp64 within the genus *Actinoallomurus* (data not shown).[5] The strain was cultured in AF-A medium at 30 °C, where it reached the stationary phase after about seven days. Under these conditions, the inhibition of *Staphylococcus aureus* growth was observed after six to eight days.

For isolation of the active compounds, the producing strain was cultured at 30 °C for eight days. The mycelium was then separated from the supernatant and extracted with MeOH. The supernatant was extracted with EtOAc, and the organic phase was combined with the methanolic extract of the mycelium. The solvent was then removed *in vacuo* to give the crude mixture, which was purified by reversed-phase chromatography to give **2** and **3** in a relative ratio of 15:1.

Compound **2** was obtained as a white solid with a chemical formula of $C_{44}H_{56}Cl_2N_2O_{10}$, as established by high-resolution

Received: January 3, 2012
Published: May 22, 2012

ESI analysis. The isotopic pattern of the molecular peak clearly indicated the presence of two chlorine atoms, and the UV spectrum showed an absorption maximum (λ_{max}) at 268 nm.

The COSY and TOCSY data showed the presence of a major spin system belonging to the backbone of the molecule, three minor spin systems, and three single signals (Figure 2). The

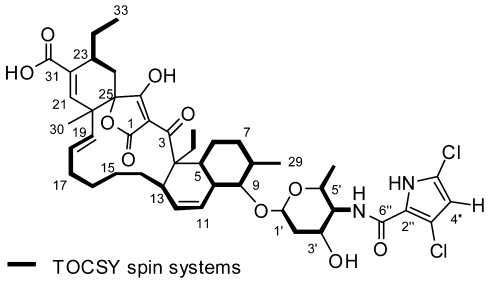
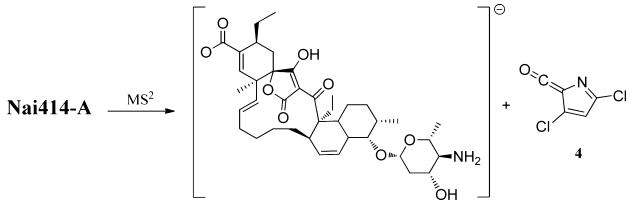


Figure 2. Spin systems based on TOCSY and COSY analyses of nai414-A (**2**).

backbone spin system of the molecule contained 16 carbons, four of which were incorporated into double bonds, one in a *Z* configuration and the other one in an *E* configuration assigned on the basis of their vicinal coupling constants, 10 and 15 Hz, respectively. The $^3J_{CH}$ HMBC correlations of H-5 with C-13, C-11, and C-9; H-11 with C-9 and C-5; H-12 with C-4 and C-10; and H-9 with C-5, C-11, and C-7 strongly suggested a decalin unit, which contained the *cis*-double bond and a quaternary carbon (C-4). The COSY correlations allowed the full assignment of this spin system, which contained four additional linear carbons, C-14, C-15, C-16, and C-17, and the C_{18}–C_{19} *trans*-double bond. The $^3J_{CH}$ HMBC correlations of the singlet signals H_3-30 with C-19, C-21, and C-25, and H-21 with C-19 and C-25, allowed the identification of the linking region between the C-19 and the cyclohexene ring, which contained the spin system C-33, C-32, C-23, and C-24 (Figure 2). The HMBC and COSY data for the C_{27}–C_{28} fragment, which showed cross-peaks between H_2-27 and C-3, C-13, and C-5, and H_3-28 with C-4, revealed the position of this small spin system on the C-4 carbon of the decalin ring.

The elucidation of the sugar moiety began by assigning the anomeric proton H-1′. In particular, the HMBC data, which showed cross-peaks between H-1′ and C-9, and a coupling constant $^3J_{H\text{-}1',\ Hax\text{-}2'}$ of 9 Hz readily indicated a *β*-glycosidic bond between the sugar and the backbone of the molecule. The relative *trans*-diaxial relationship between protons H-3′, H-4′, and H-5′ was suggested by their vicinal coupling constants, all above 9 Hz. The chemical shift of C-4′ (58.7 ppm) and the HMBC data of proton H-4′, which showed a correlation with carbon C-6″, indicated an amide linkage. A sharp singlet at 6.21 ppm in the ^{1}H NMR spectrum linked to a carbon that resonated at 108 ppm suggested the presence of a pyrrole ring. Moreover, the HMBC data showed correlations between H-4″ and carbons C-5″, C-3″, and C-4″, whereas by applying a low pass filter ($J \geq 10$ Hz) the only $^3J_{CH}$ correlation observed was between H-4″ and C-2″. This suggested a 2,3,5-trisubstituted pattern with two chlorine atoms in positions C-3″ and C-5″ and an amide carbonyl at position C-2″, which linked to the nitrogen at C-4′ of the sugar. Furthermore, the MS^2 spectrometric analysis of the molecule showed the formation of a single fragment with an *m*/*z* of 680 amu (100%) that had lost the characteristic isotopic pattern for two chlorine atoms. This was consistent with a loss of a neutral molecule with a mass of 161 amu, likely corresponding to ketene **4** in Scheme 1. The remaining four carbons of the molecule observed in the

Scheme 1. MS^2 Fragmentation in Negative Mode of the Molecular Ion of nai414-A (2)



^{13}C NMR spectrum, C-1, C-2, C-3, and C-26, were assigned to the tetronic acid moiety considering the HMBC correlations between H_2-27 and C-3 and of H_2-24 with C-26 and by comparing the ^{13}C NMR data with those available in the literature for decatromicins B.[6]

Analysis of the NOESY data (Figure 3) allowed the assignment of the relative configuration of **2**. In particular, the decalin ring showed NOESY interactions between H-9 and

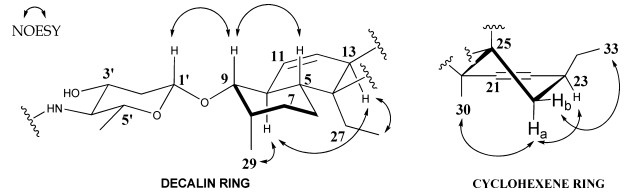


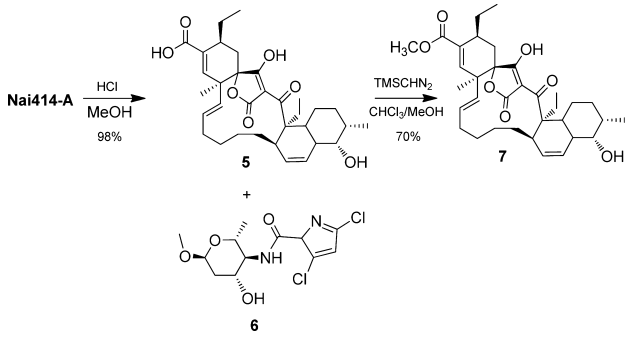
Figure 3. NOESY correlations of the decalin and cyclohexene rings of nai414-A (**2**).

H-1′ and H-5; H-10 and H_3-29; and H-13 and H_3-29 and H-10, suggesting a chair and a half-chair conformation of the fused rings. This was further confirmed by the vicinal coupling constant between H-9 and H-10 (11.4 Hz), which clearly indicated their *trans*-diaxial relationship.

The cyclohexene ring showed NOESY interactions between H-24b and H_3-33, H-23 and H-24a, and H_3-30 and H-24a, which were consistent with the half-chair conformation shown in Figure 3. The large coupling constant observed between the pseudoaxial proton H_a-24 and the pseudoequatorial proton H-23 (8 Hz) could be explained by the steric hindrance offered by the tetronic acid moiety on the C-23 ethyl group, which pushed it away from its pseudoaxial position, reducing the dihedral angle between H-23 and H-24a.

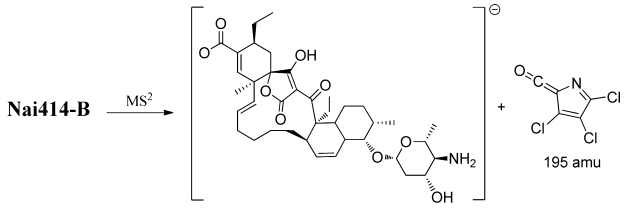
In order to establish the absolute configuration of nai414-A (**2**), we carried out degradation studies (Scheme 2). In particular,

Scheme 2. Methanolysis of nai414-A (2) in 0.1 M HCl



dx.doi.org/10.1021/np300003n | J. Nat. Prod. 2012, 75, 1044–1050

Scheme 3. MS^2 Fragmentation in Negative Mode of the Molecular Ion of nai414-B (3)



the molecule underwent a methanolysis in 0.1 M HCl in MeOH to give aglycone **5** and pyrrolaminosugar **6** in 98% yield. The spectroscopic data of **6** were then compared with those previously reported in the literature, and a match was found, indicating identical compounds.[7]

The absolute configuration of aglycone **5** was determined by applying the modified Mosher's method[8] to the C-9 secondary alcohol. This was achieved by methylating **5** with $TMSCHN_2$ to give **7** in 70% yield. Alcohol **7** was then used in the esterification reaction with (*S*)- and (*R*)-MTPA acid chlorides to give the two diastereomers **8** and **9**, respectively. Hence, the absolute configuration of the stereogenic center C-9 was assigned by measuring the $\Delta\delta_{(S-R)}$ between esters **8** and **9**, which established an *S* configuration (Figure 4).

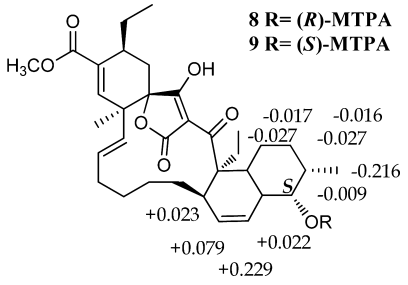
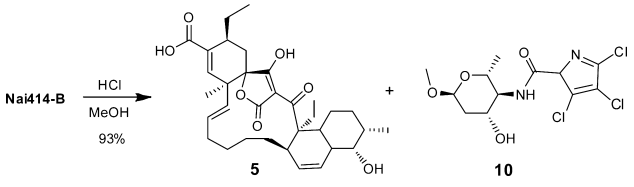


Figure 4. $\Delta\delta_{(S-R)}$ between the two esters **8** and **9**.

The minor congener **3** was obtained as a white solid with a chemical formula of $C_{44}H_{55}Cl_3N_2O_{10}$, as established by high-resolution ESI analysis. The MS^2 analysis of the molecular ion of **3** showed the formation of a single fragment with an *m/z* of 680 amu (100%) with loss of a neutral molecule with a mass of 195 amu together with the characteristic isotopic pattern. This suggested that the additional chlorine was on the pyrrole ring.

This was further confirmed by the absence, in the ^1H NMR spectrum, of the sharp singlet at δ_H 6.21 observed in the spectrum of **2**. Finally, degradation studies of **3**, carried out in 0.1 M HCl in MeOH, resulted in the formation of aglycone **5** and aminosugar **10** (Scheme 4). The absolute configuration of

Scheme 4. Degradation Studies on nai414-B (3)



10 is under investigation and will be determined by comparison with a synthetic analogue.

Nai414-A (**2**) is differentiated from other members of the spirotetronate class by the saturation of the $C_{15}-C_{16}$ bond in the backbone of the molecule, whereas nai414-B (**3**) has the additional feature of the unique trichlorinated pyrrole moiety.

Modulating the Halogenation Pattern. The halogenation of secondary metabolites has been of great interest over the past years, and there are still many biosynthetic aspects that have yet to be addressed such as the different types of enzymes and mechanisms involved in this challenging reaction.[9] The rare, highly chlorinated structure of **3** and the intriguing 2,5-substituted chloride pattern of **2** prompted us to further investigate the halogenation in this recently described genus of *Actinobacteria*. With this regard, we set up a new fermentation substituting the NaCl present in the AF-A medium with an equal amount of KBr (AF-A-Br medium). This resulted in the formation of a complex mixture of derivatives whose *m/z* values indicated the formation of a dichloro- (*m/z* $[M - H]^-$ 841 (100%)), dibromo- (*m/z* $[M - H]^-$ 929 (100%)), or bromochloropyrrole (*m/z* $[M - H]^-$ 885 (100%)) moiety with a loss of specificity. Mixed mono- and trisubstituted derivatives were also produced albeit in lower amount (Figure 5, AF-A-Br).

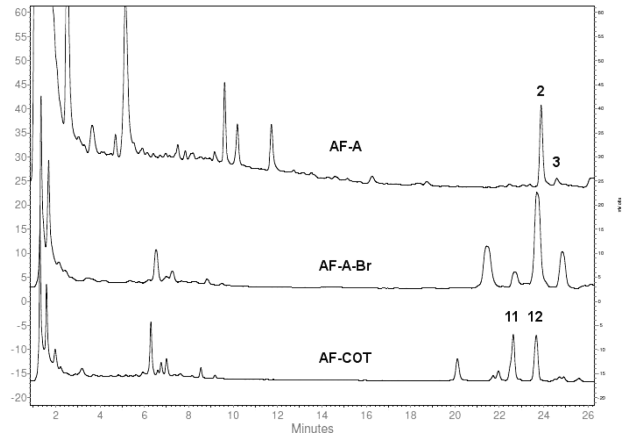
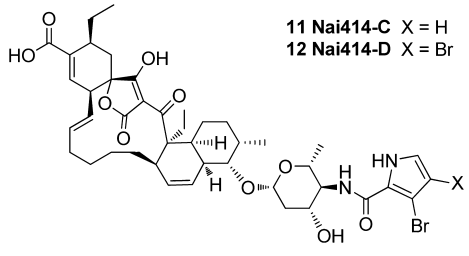


Figure 5. Different media used for bromine incorporation.

Our *in vivo* results are consistent with those published by Walsh and co-workers, which showed that bromide ions compete *in vitro* with chloride in the PltA-mediated chlorination of pyoluteorin in an unspecific way.[11]

In an effort to minimize any chloride source, the yeast extract was removed from the AF-A medium and the soybean meal substituted with cotton flour. Under these new growth conditions (Figure 5, AF-COT medium), the two major compounds produced were the monobromo and the dibromo derivatives **11** and **12**, as indicated by the high-resolution ESI analyses, which showed chemical formulas of $C_{44}H_{57}BrN_2O_{10}$ and $C_{44}H_{56}Br_2N_2O_{10}$, respectively.



HMBC studies on **11** revealed that bromination took place at position C-3″ of the pyrrole, as indicated by the $^3J_{CH}$

dx.doi.org/10.1021/np300003n | J. Nat. Prod. 2012, 75, 1044−1050

correlations of H-4″ with C-2″ and of H-5″ with C-2″ and C3″. Interestingly, the second bromide in **12** was inserted at position C-4″ with a shift in regioselectivity with respect to **2**. These results show that, in the presence of an excess of bromide ions, halogenation still occurs in a highly regioselective fashion with a switch from a 3,5- to a 3,4-halogenated pattern.

Biological Activities. The biological activities of **2**, **3**, **11**, and **12** are consistent with those previously reported in the literature for this class. Using 15 different Gram-positive bacterial strains, **2** showed minimal inhibitory concentrations (MICs) comparable to those of vancomycin (VAN), against all strains. Particularly interesting was the activity against *S. aureus* strains irrespective of their resistance phenotype (MIC range: 0.25−1 μg/mL). Streptococci and enterococci tested were quite sensitive to the compounds with the exception of the *Streptococcus pneumoniae* strain BAA1402, which expresses the *mefE* macrolide efflux system, for which an MIC of 64 μg/mL was observed. There was no detectable activity of **2** against Gram-negative strains or *Candida albicans* (data not shown). Compound **3** showed a similar profile with a reduced potency of 2- to 4-fold for all the tested strains.

Table 1. Antimicrobial Activities of Compounds 2, 3, 11, and 12

	MIC (μg/mL)				
strain	**2**	**3**	**11**	**12**	VAN
Staphylococcus aureus Met-S ATCC6538P	0.5	2			1
Staphylococcus aureus Met-S ATCC25923	0.5	2			1
Staphylococcus aureus Met-S ATCC29213	0.25	2	2	8	0.5
Staphylococcus aureus Met-R L1400	1	4	4	16	1
Staphylococcus aureus GI L3798	1	4			8
Staphylococcus aureus GI Met-R, L3797	0.5	4			>128
Staphylococcus hemolyticus Met-R, L1729	1	4			2
Staphylococcus hemolyticus Met-S, L1730	1	4			1
Streptococcus pneumoniae Mef-E BAA1402	64	>128			4
Streptococcus pneumoniae Pen-S, L44	4	8	8	4	0.5
Streptococcus pyogenes L49	2	8	4	1	1
Enterococcus faecalis Van-S L559	0.5	2	16	32	1
Enterococcus faecalis Van-A L560	0.5	2	16	16	>128
Enterococcus faecium Van-S L568	8	16	64	32	2
Enterococcus faecium Van-A L569	4	8	64	32	>128

The brominated derivatives **11** and **12** showed higher MIC values against *S. aureus* strains and *Enterococcus* sp., whereas activities against *S. pneumoniae* were comparable to those of the chlorinated compounds.

The molecules described here were also evaluated for cytotoxicity against human and murine cell lines (Table 2): **2** was about 250-fold less cytotoxic than adriamycin against human microvascular endothelial cells, whereas **3** appeared slightly more cytotoxic than **2**. Both compounds were inactive against murine bone marrow macrophages. The brominated derivative **12** possessed a cytotoxicity against HMEC-1 cells comparable with those of the chlorinated compounds, while **11** was inactive.

Table 2. Cytotoxicities Expressed as IC_{50} (μM) of Compounds 2, 3, 11, and 12

	2	**3**	**11**	**12**	adriamycin
HMEC-1[a]	9	2	43	3	0.06
BMDM[a]	50	47	143	109	4.6

[a]HMEC-1: human microvascular endothelial cells; BMDM: bone marrow derived macrophages.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. NMR spectra were measured in $CDCl_3$, $(CD_3)_2CO$, or CD_3OD on a Bruker 400 MHz instrument using tetramethylsilane as an internal reference. ESIMS and UV data were recorded on a MS Bruker Ion Trap ESQUIRE 3000 Plus spectrometer, with an LC Agilent 1100 DAD. HRESIMS were recorded on a Exactive spectrometer (Thermo Fisher Scientific) with Orbitrap technology. HPLC separations were carried out using a Shimadzu LC-2010AHT equipped with a Merck LiChrosphere RP18 column, 5 μm (125 × 4 mm). Reversed-phase medium-pressure liquid chromatography was performed on a Combiflash ISCO (Teledyne ISCO) RP18 with 12 and 30 g columns.

Growth Conditions. *Actinoallomurus* ID145414 (GenBank accession number JQ926984) was maintained on S1 5.5 plates.[4] The strain was grown in shakeflasks containing AF-A medium (dextrose 10 g/L, soybean meal 4 g/L, yeast extract 1 g/L, NaCl 0.5 g/L, MES 1.5 g/L, adjusted to pH 5.6 before sterilization) for 7−10 days at 30 °C on a rotatory shaker at 200 rpm. Additional media used for the bromo incorporation studies were AF-A-Br, consisting of AF-A in which NaCl was substituted with 0.5 g/L KBr, and AF-COT (dextrose 20 g/L, cotton flour 10 g/L, MES 1.5 g/L, KBr 1 g/L).

Purification of the Metabolites. Ten flasks containing 100 mL each of *Actinoallomurus* ID145414, grown as described above, were reunited and filtered to separate the mycelium from the cleared broth. The former was extracted with 1 L of MeOH for 2 h at 37 °C, whereas the cleared broth was extracted with EtOAc (3 × 300 mL). The methanolic and the EtOAc phases were combined, and the solvent was removed *in vacuo*. The crude solid was purified by reversed-phase medium-pressure chromatography using 0.1% TFA and MeCN as eluents (MeCN concentration: 20% for 0−3 min; 20−60% for 10 min; 60−90% for 5 min) to give **2** (45 mg) and **3** (3 mg) as white solids. Compounds **11** and **12** were isolated in the same manner, obtaining 5 and 4 mg, respectively. The HPLC analyses were carried out injecting 10 μL of the different samples (1 mg/mL in MeOH) using 0.1% TFA and MeCN as eluents (MeCN concentration: 10% to 90% linear gradient in 35 min).

Degradation of Compound 3. Compound **3** (2.0 mg, 0.08 mmol) was dissolved 1 mL of 0.1 M HCl in MeOH solution, and the reaction was monitored by HPLC. The mixture was left for 2 h and quenched with saturated $NaHCO_3$ solution (1 mL). The solvent was removed *in vacuo*, and the crude product was purified by reversed-phase liquid chromatography with 0.1% TFA/MeCN to give **5** (1.3 mg, 93%) and **10** (0.8 mg, 93%) as a white solid and a colorless oil, respectively.

*Nai414-A (**2**):* $[\alpha]_D^{24}$ −24.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 268 (2.53) nm; ^{1}H NMR (400 MHz, acetone-d_6) δ_H 6.79 (1H, d, *J* = 9.1 Hz, NH-4′), 6.62 (1H, d, *J* = 0.9 Hz, H-21), 6.21 (1H, s, H-4″), 5.91 (1H, ddd, *J* = 10.0, 5.4, 1.8 Hz, H-12), 5.74 (1H, d, *J* = 10.0 Hz, H-11), 5.58 (1H, ddd, *J* = 15.5, 10.0, 3.8 Hz, H-18), 5.38 (1H, d, *J* = 15.5 Hz, H-19), 4.59 (1H, d, *J* = 9.1 Hz, H-1′), 3.88 (1H, ddd, *J* = 11.4, 9.1, 4.4 Hz, H-3′), 3.62 (1H, app. q, *J* = 9.1 Hz, H-4′), 3.57 (1H, m, H-5′), 3.45 (1H, dd, *J* = 10.7, 5.0 Hz, H-9), 2.77−2.73 (2H, m, H-13 and H-23), 2.65 (1H, dd, *J* = 14.6, 7.3 Hz, H-27a), 2.56 (1H, dd, *J* = 14.6, 8.4 Hz, H-24a), 2.44 (1H, m, H-8), 2.31 (1H, dd, *J* = 12.6, 4.4 Hz, H_{eq}-2′), 2.22 (1H, m, H-10), 2.19 (1H, m, H-17a), 2.07 (1H, m, H-24b), 1.94 (1H, m, H-17b), 1.92 (1H, m, H-27b), 1.75 (1H, m, H-32a), 1.70 (1H, m, H-5), 1.68 (1H, m, H-32b), 1.63 (3H, m, H-16a and H_2-7), 1.60 (1H, m, H_{ax}-2′), 1.58 (1H, m, H-16b), 1.40 (1H, m, H-6a), 1.35 (3H, s, H_3-30), 1.30 (1H, m, H-6b), 1.29 (1H, m, H-15a), 1.28 (1H, m, H-15b), 1.23 (3H, d, *J* = 6.0 Hz, H_3-6′), 1.17 (1H, m, H-14a), 1.06 (3H, d, *J* = 7.1, H_3-29), 0.96 (6H, t, *J* = 7.3, H_3-28 and H_3-33), 0.90 (1H, m, H-14b); ^{13}C NMR (100 MHz, acetone-d_6) δ_C 202.0 (C-3),

dx.doi.org/10.1021/np300003n | J. Nat. Prod. 2012, 75, 1044−1050

200.3 (C-26), 167.4 (C-31), 165.9 (C-1), 158.8 (C-6″), 141.9 (C-21), 132.2 (C-18), 131.9 (C-22), 131.4 (C-19), 129.9 (C-12), 124.6 (C-11), 121.9 (C-2″), 117.6 (C-3″), 112.6 (C-5″), 108.1 (C-4″), 104.7 (C-2), 101.6 (C-1′), 85.4 (C-9), 84.5 (C-25), 70.9 (C-5′), 68.9 (C-3′), 58.7 (C-4′), 55.9 (C-4), 44.2 (C-20), 41.9 (C-13), 40.8 (C-5), 40.3 (C-2′), 38.8 (C-10), 36.1 (C-23), 34.0 (C-8), 32.4 (C-14), 31.9 (C-7), 30.4 (C-17), 29.3 (C-24), 25.9 (C-32), 25.45 (C-16), 25.40 (C-15), 23.6 (C-27), 23.5 (C-6), 20.6 (C-30), 18.1 (C-6′), 12.6 (C-29), 12.5 (C-33), 11.7 (C-28); HRESIMS m/z 841.3260 $[M - H]^-$ (calcd for $C_{44}H_{55}Cl_2N_2O_{10}$, 841.3239).

*Nai414-B (**3**):* UV (MeOH) λ_{max} (log ε) 270 (2.54) nm; ^{1}H NMR (400 MHz, acetone-d_6) δ_H 6.84 (1H, d, J = 9.1 Hz, NH-4′), 6.63 (1H, d, J = 0.9 Hz, H-21), 5.92 (1H, ddd, J = 10.0, 5.4, 1.8 Hz, H-12), 5.74 (1H, d, J = 10.0 Hz, H-11), 5.59 (1H, ddd, J = 15.5, 10.0, 3.8 Hz, H-18), 5.38 (1H, d, J = 15.5 Hz, H-19), 4.61 (1H, d, J = 9.1 Hz, H-1′), 3.91 (1H, ddd, J = 11.4, 9.1, 4.4 Hz, H-3′), 3.64 (1H, app. q, J = 9.1 Hz, H-4′), 3.56 (1H, m, H-5′), 3.45 (1H, dd, J = 10.7, 5.0 Hz, H-9), 2.77−2.73 (2H, m, H-13 and H-23), 2.65 (1H, dd, J = 14.6, 7.3 Hz, H-27a), 2.56 (1H, dd, J = 14.6, 8.4 Hz, H-24a), 2.44 (1H, m, H-8), 2.33 (1H, dd, J = 12.6, 4.4 Hz, H_{eq}-2′), 2.22 (1H, m, H-10), 2.19 (1H, m, H-17a), 2.07 (1H, m, H-24b), 1.94 (1H, m, H-17b), 1.92 (1H, m, H-27b), 1.75 (1H, m, H-32a), 1.70 (1H, m, H-5), 1.68 (1H, m, H-32b), 1.63 (3H, m, H-16a and H_2-7), 1.59 (1H, m, H_{ax}-2′), 1.58 (1H, m, H-16b), 1.40 (1H, m, H-6a), 1.35 (3H, s, H_3-30), 1.30 (1H, m, H-6b), 1.29 (1H, m, H-15a), 1.28 (1H, m, H-15b), 1.23 (3H, d, J = 6.0 Hz, H_3-6′), 1.17 (1H, m, H-14a), 1.06 (3H, d, J = 7.1 Hz, H_3-29), 0.96 (6H, t, J = 7.3 Hz, H_3-28 and H_3-33), 0.90 (1H, m, H-14b); HRESIMS m/z 875.2871 $[M - H]^-$ (calcd for $C_{44}H_{54}Cl_3N_2O_{10}$, 875.2850).

*Aminosugar (**6**).* Nai414-A (20 mg, 0.024 nmol) was added to a 0.1 M HCl (1 mL) solution in MeOH. The reaction was left overnight and quenched with saturated $NaHCO_3$ solution (0.5 mL). The solvent was removed *in vacuo*, and the crude product was purified by reversed-phase liquid chromatography with 0.1% TFA/MeCN to give **5** (12.9 mg, 98%) and **6** (7.5 mg, 98%) as a white solid and a colorless oil, respectively. **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.48 (1H, d, J = 9.0 Hz, NH-4′), 6.00 (1H, s, H-4″), 4.70 (1H, d, J = 3.0 Hz, H-1′), 3.97 (1H, m, H-3′), 3.80 (1H, app. q, J = 9.0 Hz, H-4′), 3.69 (1H, m, H-5′), 3.27 (3H, s, OCH_3), 2.19 (1H, m, H-2′a), 1.64 (1H, td, J = 11.7, 2.8 Hz, H-2′b), 1.23 (3H, d, J = 6.2 Hz, H_3-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 160.7 (C-6″), 119.9 (C-2″), 119.3 (C-3″), 113.5 (C-5″), 109.2 (C-4″), 98.5 (C-1″), 68.8 (C-3′), 66.4 (C-5′), 58.8 (C-4′), 54.9 (OCH_3), 38.3 (C-2′), 18.0 (6′-H_3); ESIMS m/z 323 (100%) $[M + H]^+$.

*26-O-Methyl Ester (**7**).* $TMSCHN_2$ in hexane (2.0 M, 0.02 mL, 0.044 mmol) was added to a solution of **5** (12.0 mg, 0.022 mmol) in $CHCl_3$/MeOH (4:1) (0.5 mL) at room temperature (rt). The reaction was left overnight and the solvent removed *in vacuo*. The crude product was purified by column chromatography using $CHCl_3$/MeOH (20:1) to give **7** (8.8 mg, 70%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.577 (1H, d, J = 1.9 Hz, H-21), 5.974 (1H, ddd, J = 10.0, 5.9, 2.3 Hz, H-12), 5.793 (1H, dd, J = 10.0, 1.4 Hz, H-11), 5.515 (1H, ddd, J = 15.4, 9.8, 3.4 Hz, H-18), 5.221 (1H, dd, J = 15.4, 1.2 Hz, H-19), 3.812 (3H, s, OCH_3), 3.759 (3H, s, CO_2CH_3), 3.475 (1H, dd, J = 10.1, 4.7 Hz, H-9), 2.916 (1H, t, J = 6.2 Hz, H-13), 2.706 (1H, t, J = 9.1 Hz, H-23), 2.490 (1H, dd, J = 14.8, 8.9 Hz, H-24a), 2.239 (1H, dq, J = 15.3, 7.4 Hz, H-27a), 2.137 (1H, m, H-8), 2.128 (1H, m, H-17a), 2.119 (1H, m, H-10), 1.983 (2H, m, H-17b), 1.931 (1H, m, H-27b), 1.858 (1H, d, J = 14.8 Hz, H-24b), 1.710 (1H, m, H-32a), 1.641 (1H, m, H-14a), 1.632 (1H, m, H-7a), 1.615 (2H, m, H-6a and H-5), 1.573 (1H, m, H-15a), 1.496 (1H, m, H-7b), 1.487 (1H, m, H-6b), 1.470 (1H, m, H-16a), 1.287 (3H, s, 30-H_3), 1.273 (1H, m, H-15b), 1.256 (1H, m, H-32b), 1.154 (1H, m, H-16b), 0.990 (6H, t, J = 7.3 Hz, H_3-33 and H_3-28), 0.980 (3H, d, J = 7.8 Hz, H_3-29), 0.906 (1H, m, H-14b); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 201.6 (C-3), 187.4 (C-26), 167.6 (C-31), 167.5 (C-1), 142.8 (C-21), 132.7 (C-18), 132.62 (C-12), 132.59 (C-22), 130.7 (C-19), 123.9 (C-11), 109.5 (C-2), 84.5 (C-25), 76.2 (C-9), 63.3 (OCH_3), 58.0 (C-4), 51.7 (CO_2CH_3), 45.0 (C-20), 41.0 (C-5), 39.9 (C-10), 39.8 (C-13), 35.9 (C-23), 34.3 (C-8), 33.1 (C-14), 31.7 (C-7), 30.9 (C-24), 30.2 (C-17), 27.4 (C-32), 26.4 (C-15), 25.5 (C-16), 25.0 (C-27), 22.3 (C-6), 21.7 (C-30), 13.4 (C-29), 11.7 (C-28), 11.5 (C-33); ESIMS m/z 581 (100%) $[M + H]^+$.

*(R)-MTPA Ester of 7 (**8**).* (*S*)-MTPA acid chloride (2 mg, 0.011 mmol) was added to a solution of **7** (3.0 mg, 0.005 mmol), Et_3N (0.011 mmol), and DMAP (cat.) in dry CH_2Cl_2 (0.5 mL) at rt. The reaction was left overnight and quenched with a saturated $NaHCO_3$ solution (0.5 mL). The two phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography using $CHCl_3$/MeOH (20:1) to give **8** (3.5 mg, 85%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.545 (2H, m, Ar), 7.411 (3H, m, Ar), 6.578 (1H, d, J = 1.9 Hz, H-21), 5.821 (1H, ddd, J = 10.0, 6.3, 2.3 Hz, H-12), 5.510 (1H, ddd, J = 15.0, 9.8, 3.8 Hz, H-18), 5.215 (1H, dd, J = 15.0, 0.9 Hz, H-19), 5.120 (1H, d, J = 10.0 Hz, H-11), 4.909 (1H, dd, J = 11.4, 5.0 Hz, H-9), 3.822 (3H, s, OCH_3), 3.760 (3H, s, CO_2CH_3), 3.566 (3H, s, 2′-OCH_3), 2.881 (1H, t, J = 6.3 Hz, H-13), 2.707 (1H, t, J = 9.0 Hz, H-23), 2.490 (1H, dd, J = 14.7, 9.0 Hz, H-24a), 2.372 (1H, m, H-8), 2.365 (1H, m, H-10), 2.243 (1H, dq, J = 15.4, 7.4 Hz, H-27a), 2.136 (1H, m, H-17a), 1.991 (1H, m, H-17b), 1.876 (1H, m, H-27b), 1.853 (1H, m, H-24b), 1.716 (1H, m, H-5 and H-32a), 1.624 (1H, m, H-7a), 1.593 (1H, m, H-14a), 1.586 (1H, m, H-7b), 1.578 (1H, m, H-15a), 1.570 (1H, m, H-6a), 1.494 (1H, m, H-6b), 1.440 (1H, m, H-16a), 1.303 (1H, m, H-15b), 1.280 (3H, s, 30-H_3), 1.224 (1H, m, H-32b), 1.127 (1H, m, H-16b), 0.989 (3H, t, J = 7.4 Hz, H_3-33), 0.952 (3H, d, J = 7.4 Hz, H_3-29), 0.942 (3H, t, J = 7.3 Hz, H_3-28), 0.920 (1H, m, H-14b); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 200.8 (C-3), 188.0 (C-26), 167.6 (C-31), 167.4 (C-1), 166.3 (C-1′), 142.6 (C-21), 133.1 (C-12), 132.6 (C-18), 132.56 (C-22), 132.4 (Ar), 130.6 (C-19), 129.5 (Ar), 128.4 (Ar), 127.4 (Ar), 122.2 (C-11), 109.2 (C-2), 85.6 (2′-CF_3), 84.5 (C-25), 81.4 (C-9), 63.3 (OCH_3), 57.8 (C-4), 55.4 (2′-OCH_3), 51.7 (CO_2CH_3), 45.0 (C-20), 41.2 (C-5), 40.0 (C-13), 37.0 (C-10), 35.8 (C-23), 32.6 (C-14), 31.6 (C-8), 31.5 (C-7), 30.8 (C-24), 30.2 (C-17), 29.6 (C-2′), 27.4 (C-32), 26.1 (C-15), 25.5 (C-16), 24.7 (C-27), 21.9 (C-6), 21.6 (C-30), 13.3 (C-33), 12.1 (C-29), 12.0 (C-28); ESIMS m/z 797 (100%) $[M + H]^+$.

*(S)-MTPA Ester of 7 (**9**).* (*R*)-MTPA acid chloride (2 mg, 0.011 mmol) was added to a solution of **7** (3.0 mg, 0.005 mmol), Et_3N (0.011 mmol), and DMAP (cat.) in dry CH_2Cl_2 (0.5 mL) at rt. The reaction was left overnight and quenched with saturated $NaHCO_3$ solution (0.5 mL). The two phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography using $CHCl_3$/MeOH (20:1) to give **9** (3.4 mg, 82%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.533 (2H, m, Ar), 7.400 (3H, m, Ar), 6.571 (1H, d, J = 1.8 Hz, H-21), 5.900 (1H, ddd, J = 9.8, 5.8, 2.1 Hz, H-12), 5.504 (1H, ddd, J = 15.0, 9.7, 3.7 Hz, H-18), 5.349 (1H, d, J = 9.8 Hz, H-11), 5.206 (1H, dd, J = 15.0, 1.9 Hz, H-19), 4.842 (1H, dd, J = 11.4, 4.9 Hz, H-9), 3.811 (3H, s, OCH_3), 3.751 (3H, s, CO_2CH_3), 3.571 (3H, s, 2′-OCH_3), 2.904 (1H, t, J = 6.4 Hz, H-13), 2.698 (1H, t, J = 9.0 Hz, H-23), 2.482 (1H, dd, J = 14.8, 8.9 Hz, H-24a), 2.387 (1H, m, H-10), 2.363 (1H, m, H-8), 2.233 (1H, dq, J = 15.2, 7.8 Hz, H-27a), 2.141 (1H, m, H-17a), 1.994 (1H, m, H-17b), 1.887 (1H, m, H-27b) 1.854 (1H, m, H-24b), 1.723 (1H, m, H-5), 1.715 (1H, m, H-32a), 1.608 (1H, m, H-7a), 1.592 (1H, m, H-14a), 1.584 (1H, m, H-15a), 1.559 (1H, m, H-7b), 1.543 (1H, m, H-6a), 1.477 (1H, m, H-6b), 1.444 (1H, m, H-16a), 1.297 (1H, m, H-15b), 1.280 (3H, s, 30-H_3), 1.247 (1H, m, H-32b), 1.141 (1H, m, H-16b), 0.979 (3H, t, J = 7.3 Hz, H_3-33), 0.940 (3H, t, J = 7.8 Hz, H_3-28), 0.911 (1H, m, H-14b), 0.736 (3H, d, J = 7.0 Hz, H_3-29); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 200.9 (C-3), 188.0 (C-26), 167.6 (C-31), 167.5 (C-1), 166.7 (C-1′), 142.7 (C-21), 133.4 (C-12), 132.7 (C-22), 132.6 (C-18), 132.4 (Ar), 130.6 (C-19), 129.5 (Ar), 128.4 (Ar), 127.4 (Ar), 122.4 (C-11), 109.2 (C-2), 84.6 (C-25), 84.5 (2′-CF_3), 81.6 (C-9), 63.3 (OCH_3), 57.9 (C-4), 55.6 (2′-OCH_3), 51.7 (CO_2CH_3), 45.0 (C-20), 41.3 (C-5), 39.9 (C-13), 36.8 (C-10), 35.8 (C-23), 32.7 (C-14), 31.3 (C-7), 31.2 (C-8), 30.8 (C-24), 30.2 (C-17), 29.6 (C-2′), 27.4 (C-32), 26.1 (C-15), 25.5 (C-16), 24.8 (C-27), 21.9

(C-6), 21.6 (C-30), 13.3 (C-28), 11.9 (C-29), 11.6 (C-33); ESIMS *m/z* 797 (100%) $[M + H]^+$.

Nai414-C (11): $[\alpha]_D^{24}$ +13.5 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 270 (2.53) nm; ^{1}H NMR (400 MHz, CD_3OD) δ_H 6.78 (1H, d, *J* = 3.5 Hz, H-5″), 6.61 (1H, s, H-21), 6.15 (1H, d, *J* = 3.5 Hz, H-4″), 5.90 (1H, m, H-12), 5.70 (1H, d, *J* = 10.0 Hz, H-11), 5.56 (1H, m, H-18), 5.36 (1H, d, *J* = 15.0 Hz, H-19), 4.54 (1H, d, *J* = 9.3 Hz, H-1′), 3.71 (1H, td, *J* = 11.0, 4.6 Hz, H-3′), 3.57 (1H, t, *J* = 9.7 Hz, H-4′), 3.42 (2H, m, H-5′ and H-9), 2.73 (2H, m, H-13 and H-23), 2.55 (1H, m, H-27a), 2.53 (1H, dd, *J* = 13.8, 8.2 Hz, H-24a), 2.41 (1H, m, H-8), 2.29 (1H, dd, *J* = 11.7, 3.8 Hz, H_{eq}-2′), 2.22 (1H, m, H-10), 2.18 (1H, m, H-17a), 2.00 (1H, m, H-24b and H-17b), 1.92 (1H, m, H-27b), 1.76 (1H, m, H-32a), 1.64 (3H, m, H-16a and H_2-7), 1.62 (1H, m, H-32b), 1.60 (1H, m, H-5), 1.59 (1H, m, H_{ax}-2′), 1.56 (1H, m, H-6a), 1.50 (1H, m, H-16b), 1.46 (1H, m, H-6b), 1.33 (3H, s, H_3-30), 1.29 (2H, m, H_2-15), 1.23 (3H, d, *J* = 5.9 Hz, H_3-6′), 1.22 (1H, m, H-14a), 1.06 (3H, d, *J* = 6.9 Hz, H_3-29), 0.98 (6H, t, *J* = 6.7 Hz, H_3-28 and H_3-33), 0.92 (1H, m, H-14b); ^{13}C NMR (100 MHz, CD_3OD) δ_C 205.6 (C-3), 200.0 (C-26), 168.9 (C-31), 166.3 (C-1), 161.8 (C-6″), 143.0 (C-21), 133.6 (C-18), 131.7 (C-22), 132.3 (C-19), 131.2 (C-12), 125.5 (C-11), 128.8 (C-2″), 113.4 (C-5″), 112.4 (C-4″), 104.3 (C-3″), 104.2 (C-2), 102.9 (C-1′), 87.4 (C-9), 86.5 (C-25), 72.1 (C-5′), 70.0 (C-3′), 59.4 (C-4′), 57.3 (C-4), 45.2 (C-20), 41.8 (C-13), 41.0 (C-2′), 40.2 (C-5), 38.5 (C-10), 37.1 (C-23), 35.1 (C-8), 33.5 (C-14), 33.2 (C-7), 31.7 (C-17), 30.4 (C-24), 27.2 (C-32), 26.7 (C-15), 25.9 (C-16), 25.8 (C-6), 25.2 (C-27), 21.7 (C-30), 18.6 (C-6′), 13.4 (C-29), 12.5 (C-33 and C-28); HRESIMS *m/z* 851.3134 $[M - H]^-$ (calcd for $C_{44}H_{56}BrN_2O_{10}$, 851.3123).

Nai414-D (12): $[\alpha]_D^{24}$ +39.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 270 (2.53) nm; ^{1}H NMR (400 MHz, CD_3OD): δ_H 6.86 (1H, s, H-5″), 6.58 (1H, s, H-21), 5.89 (1H, m, H-12), 5.72 (1H, d, *J* = 10.0 Hz, H-11), 5.56 (1H, m, H-18), 5.38 (1H, d, *J* = 15.0 Hz, H-19), 4.53 (1H, d, *J* = 9.3 Hz, H-1′), 3.71 (1H, td, *J* = 11.0, 4.6 Hz, H-3′), 3.57 (1H, t, *J* = 9.7 Hz, H-4′), 3.42 (2H, m, H-5′ and H-9), 2.73 (2H, m, H-13 and H-23), 2.57 (1H, m, H-27a), 2.55 (1H, dd, *J* = 13.8, 8.2 Hz, H-24a), 2.41 (1H, m, H-8), 2.31 (1H, dd, *J* = 11.7, 3.8 Hz, H_{eq}-2′), 2.22 (1H, m, H-10), 2.18 (1H, m, H-17a), 2.00 (1H, m, H-24b and H-17b), 1.92 (1H, m, H-27b), 1.76 (1H, m, H-32a), 1.66 (3H, m, H-16a and H_2-7), 1.62 (1H, m, H-32b), 1.61 (1H, m, H-5), 1.59 (1H, m, H_{ax}-2′), 1.56 (1H, m, H-6a), 1.52 (1H, m, H-16b), 1.46 (1H, m, H-6b), 1.33 (3H, s, H_3-30), 1.29 (2H, m, H_2-15), 1.23 (3H, d, *J* = 5.9 Hz, H_3-6′), 1.20 (1H, m, H-14a), 1.04 (3H, d, *J* = 6.9 Hz, H_3-29), 0.99 (6H, t, *J* = 6.7 Hz, H_3-28 and H_3-33), 0.92 (1H, m, H-14b); ^{13}C NMR (100 MHz, CD_3OD) δ_C 205.8 (C-3), 200.0 (C-26), 170.0 (C-31), 166.3 (C-1), 163.0 (C-6″), 143.0 (C-21), 133.6 (C-18), 131.7 (C-22), 132.3 (C-19), 131.2 (C-12), 125.5 (C-11), 128.8 (C-2″), 113.2 (C-5″), 104.3 (C-3″), 104.2 (C-2), 102.9 (C-1′), 99.2 (C-4″), 87.4 (C-9), 86.5 (C-25), 72.1 (C-5′), 70.0 (C-3′), 59.4 (C-4′), 57.3 (C-4), 45.2 (C-20), 41.8 (C-13), 41.0 (C-2′), 40.2 (C-5), 38.5 (C-10), 37.1 (C-23), 35.1 (C-8), 33.5 (C-14), 33.2 (C-7), 31.7 (C-17), 30.4 (C-24), 27.2 (C-32), 26.7 (C-15), 25.9 (C-16), 25.8 (C-6), 25.2 (C-27), 21.7 (C-30), 18.6 (C-6′), 13.4 (C-29), 12.5 (C-33 and C-28); HRESIMS *m/z* 929.2241 $[M - H]^-$ (calcd for $C_{44}H_{55}Br_2N_2O_{10}$, 929.2229).

Antimicrobial Assays. All MICs were determined by broth microdilution in sterile 96-well microtiter plates according to CLSI guidelines,[12] using Mueller Hinton broth (Difco Laboratories) containing 20 mg/L $CaCl_2$ and 10 mg/L $MgCl_2$ for all strains except for *Streptococcus* sp., which were grown in Todd Hewitt broth (Difco Laboratories). Strains were inoculated at 5×10^5 cfu/mL and incubated at 37 °C for 20−24 h. All strains (with the exception of the ATCC strains) were clinical isolates from the NAICONS strain collection. *S. aureus* strains included Met^R and Met^S isolates resistant and sensitive to beta-lactams, respectively, and GI (glycopeptide intermediate) strains with decreased susceptibility to vancomycin. *Enterococcus* sp. Van^S and Van-A are sensitive and resistant to vancomycin, respectively, while *S. pneumoniae* Pen^S is sensitive to penicillin. Test compounds were dissolved in DMSO at 10 mg/mL and diluted with the culture medium immediately prior to testing.

Cytotoxicity Assay. Cytotoxicity was evaluated on human microvascular endothelial cells (HMEC-1, provided by the Centers for Disease Control, Atlanta, GA, USA) and on a murine bone marrow macrophage cell line (BMDM). HMEC-1 were maintained in MCDB 131 medium (GIBCO-BRL) supplemented with 10% fetal calf serum (HyClone), 10 ng/mL epidermal growth factor (PreproTech), 1 μg/mL hydrocortisone, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 20 mM Hepes buffer. BMDM were maintained in DMEM (Lonza) supplemented with 10% heat-inactivated FBS (Superior Quality, Euroclone), 2 mM glutamine, 25 mM HEPES, and 10 μg/mL ciprofloxacin (for the cytotoxicity determinations, medium did not contain ciprofloxacin).

For the cytotoxicity, cells were seeded in 96-well flat-bottom tissue culture clusters (Costar) at 10^4 and 5×10^4 cells/well for HMEC-1 and BMDM, respectively. After 24 h, cells were treated with serial dilutions of test compounds (up to 250 μg/mL), in a final volume of 200 μL/well. Cell proliferation was evaluated after 72 h by adding 20 μL of 5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma) in PBS.[13] After an additional 3 h at 37 °C, plates were centrifuged, the supernatants discarded, and the dark blue formazan crystals dissolved using 100 μL of 20% (w/v) SDS and 40% *N*,*N*-dimethylformamide, pH 4.7 with acetic acid. Plates were read on a microplate reader (Molecular Devices Co.) at a test wavelength of 550 nm and a reference wavelength of 650 nm. The results were expressed as IC_{50} values, which are the concentrations of compounds inhibiting cell growth by 50%. All the tests were performed three times in triplicate.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Selected 1D and 2D NMR spectra of compounds **2**, **3**, **7**, **8**, **9**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: dr.carlo.mazzetti@tiscali.it; msosio@naicons.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge A. Guindani, P. Guglierame, and C. Brunati for microbiological support and R. Rossi and Prof. F. Cozzi for analytical support. This work was partially supported from grants by Regione Lombardia.

■ REFERENCES

(1) (a) Lam, K. S.; Hesler, G. A.; Gustavson, D. R.; Berry, R. L.; Tomita, K.; Macbeth, J. L.; Ross, J.; Miller, D.; Forenza, S. *J. Antibiot.* **1996**, *49*, 860−864. (b) Tomita, F.; Tamaoki, T. *J. Antibiot.* **1980**, *33*, 940−945.

(2) (a) Nakashima, T.; Miura, M.; Hara, M. *Cancer Res.* **2000**, *60*, 1229−1235. (b) Anether, G.; Tinholfer, I.; Senfter, M.; Greil, R. *Blood* **2003**, *101*, 4561−4568. (c) Tamaoki, T.; Kasai, M.; Shirahata, K.; Ohkubo, S.; Morimoto, M.; Mineura, K.; Ishii, S.; Tomita, F. *J. Antibiot.* **1980**, *33*, 946−950.

(3) Kawashima, A.; Nakamura, Y.; Ohta, Y.; Akama, T.; Yamagishi, M.; Hanada, K. *J. Antibiot.* **1992**, *45*, 207−212.

(4) Pozzi, R.; Simone, M.; Mazzetti, C.; Maffioli, S.; Monciardini, P.; Cavaletti, L.; Bamonte, R.; Sosio, M.; Donadio, S. *J. Antibiot.* **2011**, *64*, 133−139.

(5) Tamura, T.; Ishida, Y.; Nozawa, Y.; Otoguro, M.; Suzuki, K. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 1867−1874.

(6) Momose, I.; Hirosawa, S.; Nakamura, H.; Naganawa, H.; Iinuma, H.; Ikeda, D.; Takeuchi, T *J. Antibiot.* **1999**, *52*, 787−796.

(7) Schroeder, D. R.; Colson, K. L.; Klohr, S. E.; Mike, S. L.; Matson, J. A. *J. Antibiot.* **1996**, *49*, 865−872.

(8) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(9) (a) Holzer, M.; Burd, W.; Reibig, H. U.; van Pée, K. H. *Adv. Synth. Catal.* **2001**, *343*, 591−595. (b) Lang, A.; Polnick, S.; Nicke, T.; William, P.; Patallo, E. P.; Naismith, J. H.; van Pée, K. H. *Angew. Chem., Int. Ed.* **2011**, *50*, 2951−2953. (c) Clark, B. R.; Murphy, C. D. *Org. Biomol. Chem.* **2009**, *7*, 111−116.
(10) Thomas, G. M.; Burkart, M. D.; Walsh, C. T. *Chem. Biol.* **2002**, *9*, 171−184.
(11) Dorrestein, P. C.; Yeh, E.; Garneau-Tsodikova, S.; Kelleher, N. L.; Walsh, C. T. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 13843−13848.
(12) National Committee for Clinical Laboratory Standards, 7th ed.; Approved Standards, NCCLS document M7-A7; NCCLS: Wayne, PA, 2006.
(13) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.