A Madagascar Sponge *Batzella* sp. as a Source of Alkylated Iminosugars

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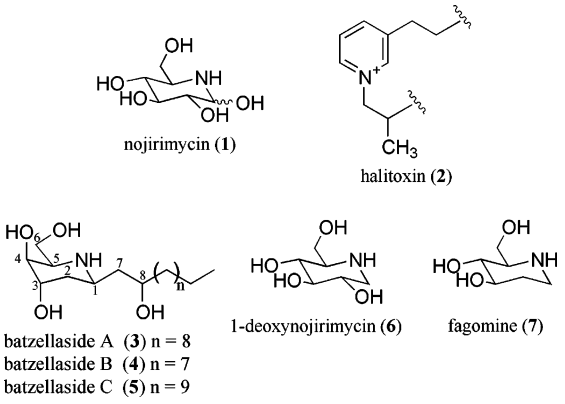
Three new C-alkylated iminosugars, batzellasides A (**3**), B (**4**), and C (**5**), along with the known halitoxin (**2**) polymer were isolated from a *Batzella* sp. sponge, collected off the west coast of Madagascar. Although this class of azasugars is well known from terrestrial sources, our report represents the first examples of iminosugars from a marine organism. Comparison with the properties of known natural and synthetic iminosugars assisted in the structure determinations. Compounds **3**−**5** inhibited the growth of *Staphylococcus epidermidis* with MICs of ≤6.3 μg/mL.

Naturally occurring imino- or azasugars are monosaccharides with the heterocyclic oxygen replaced by nitrogen. These structures are of therapeutic importance as they selectively inhibit carbohydrate-degrading enzymes.[1] Nojirimycin (**1**), a 5-amino-5-deoxyglucose antibiotic obtained from several *Streptomyces* species, was the first member of this class to be isolated and characterized in the 1960s.[2] Subsequently, more than 25 additional analogues of **1** have been described from both plant and microbial sources (see Supporting Information Figure S1 structures).[3] Total synthesis efforts, also beginning in the 1960s, have generated almost all conceivable structural variants of this class, and its literature has been comprehensively reviewed.[4] In light of such history it is surprising that iminosugars have not previously been reported from marine sources. In this short report we now describe the isolation and structure elucidation of three new alkylated iminosugars from a Madagascar *Batzella* sponge.



A sponge (coll. no. 00216) obtained during our 2000 expedition to Madagascar and later identified as *Batzella* sp. was targeted for study because the MeOH(aq) partition fraction of the crude extract, coded as FM (see Figure S16 in Supporting Information), exhibited activity in three mechanism-based assays.[5] These included positive responses against Raf kinase (IC_{50} = 2.8 μg/mL), Smac/IAP (38% enhancement at 20 μg/mL), and MetAP1 (IC_{50} = 0.8 μg/mL) assay targets. Eight Sephadex (S) fractions were obtained, and the first fraction (S1), responsible for the Raf kinase activity, contained the known polymer halitoxin (**2**).[6] These are large molecular weight (500 to >25 000 Da) pyridinium polymers made up of central structure **2**[7] and have been widely isolated from sponges. In addition, **2** is considered to be a nuisance substance because it is nonselective in many enzyme assays, with Raf kinase being particularly sensitive. Further LC screening of the remaining Sephadex fractions using both evaporative light scattering (ELSD) and UV detection pinpointed S3 as containing nonhalitoxin components. This was separated by HPLC to afford H5, H4, and H6, identified as new compounds batzellasides A (**3**), B (**4**), and C (**5**), respectively.

Structure elucidation of these new metabolites began with batzellaside A (**3**), as it was the most abundant of the three compounds isolated. The molecular formula of $C_{18}H_{37}NO_4$ (HRMS *m/z* 332.2784 $[M + H]^+$), requiring one degree of unsaturation, was established for **3**. Using this formula as a dereplication search seed revealed seven known synthetic compounds containing an iminosugar core with an attached long alkyl chain. These structures (listed in Supporting Information Figure S2) included six *N*-alkylated iminosugars related to 1-deoxynojirimycin (**6**):[8] *N*-dodecyl- and *N*-10-methylundecyl-1-deoxynojirimycin,[9,10] *N*-dodecyl-, *N*-dodecyl-5(*S*)-, and *N*-10-methylundecyl-1-deoxygalactonojirimycin,[11−13] and *N*-dodecyl-1-deoxymannojirimycin[14] plus the C-alkylated compound 11′(*R*)-leptophylline A.[15] Comparison of the $C_6H_{12}NO_4$ molecular formula of 1-deoxynojirimycin (**6**) with that of **3** was useful and suggested that the latter might contain a similar iminosugar moiety. However, the ^1H, ^{13}C, and $^1H-^1H$ gCOSY data, shown in Table 1, indicated one of the hydroxyl groups present in **3** was attached to a long saturated alkyl chain. A revised $C_6H_{12}NO_3$ subformula was considered for the iminosugar residue, and additional literature searching revealed the trihydroxy iminosugar fagomine (**7**).[16] The $^1H-^1H$ gCOSY and gHMBC correlations of Figure 1, including two unusual four-bond gHMBC correlations from H-1 to C-6 and from H-7 to C-5, confirmed the iminosugar core shown in **3**, possessing additional alkyl substitution at C-1.

Attention shifted next to defining the position of the hydroxyl group on the alkyl chain. The $^1H-^1H$ gCOSY NMR correlations from H-1 to H-7 and from H-7 to H-8 verified the hydroxyl group was β to the heterocyclic ring. The remaining atoms, consisting of $C_{10}H_{21}$, were assigned to an unbranched alkyl chain attached to C-8, which was confirmed with gCOSY and gHMBC correlations and by the nine ^{13}C signals between δ 22 and 38. The structures of the two remaining compounds, batzellasides B (**4**) and

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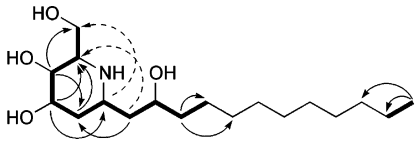
Table 1. NMR Data[a] for Batzellasides A (**3**), B (**4**), and C (**5**) in MeOH-d_4

	3					**4**	**5**
position	δ_C	δ_H (J in Hz)	gCOSY	gHMBC	NOESY	δ_H (J in Hz)	δ_H (J in Hz)
1	57.0	3.55 dddd (12.2, 8.1, 4.0, 4.0)	2, 7	6	2eq, 7, 8	3.58 dddd (12.6, 8.4, 4.1, 4.0)	3.59 dddd (12.8, 8.4, 4.1, 4.0)
2	31.1	1.98 ddd (14.5, 12.6, 2.5) 1.81 dt (14.3, 2.7, 2.7)	1, 2, 3	3, 4, 5	3, 2eq 1, 2ax, 3	2.00 ddd (14.8, 12.3, 2.5) 1.82 dt (14.5, 3.0, 3.0)	2.00 ddd (15.0, 12.3, 2.5) 1.82 dt (14.8, 2.9, 2.9)
3	65.8[b]	3.89 ddd (3.0, 3.0, 3.0)	2, 4	1, 4, 5	2eq, 2ax, 4	3.90 ddd (3.2, 3.2, 3.2)	3.89 ddd (3.2, 3.2, 3.2)
4	65.7[b]	3.73 dd (3.1, 1.5)	3, 5	1, 2, 3, 6	3	3.73 dd (3.1, 1.2)	3.73 dd (3.1, 1.2)
5	51.9	3.48 ddd (8.9, 5.4, 1.2)	4, 6	6	4, 6	3.50 ddd (9.2, 5.0, 1.5)	3.50 ddd (9.3, 5.1, 1.5)
6	59.4	3.73 dd (11.5, 9.0) 3.70 dd (11.5, 5.5)	5	1, 3, 4	5	3.75 dd (11.5, 9.0) 3.70 dd (11.5, 5.5)	3.75 dd (11.5, 9.0) 3.72 dd (11.5, 5.5)
7	38.1	1.68 ddd (13.0, 9.5, 8.1) 1.64 ddd (13.0, 4.0, 3.4)	1, 8	1, 2, 5, 8, 9	1, 8	1.71 ddd (13.0, 9.5, 8.1) 1.67 ddd (13.0, 4.0, 3.4)	1.71 ddd (13.0, 9.5, 8.1) 1.67 ddd (13.0, 4.0, 3.4)
8	70.5	3.82 dddd (9.5, 6.2, 6.2, 3.4)	7, 9		1, 7, 9, 10	3.82 dddd (9.5, 6.2, 6.2, 3.4)	3.83 dddd (9.5, 6.2, 6.2, 3.4)
9	38.0	1.44 m	10	10, 11	8	1.47 m	1.47 m
10	24.8	1.28 bs				1.29 bs	1.30 bs
11	29.3	1.28 bs				1.29 bs	1.30 bs
12	29.3	1.28 bs				1.29 bs	1.30 bs
13	29.0	1.28 bs				1.29 bs	1.30 bs
14	29.3	1.28 bs				1.29 bs	1.30 bs
15	29.3	1.28 bs				1.29 bs	1.30 bs
16	31.6	1.28 bs				1.29 bs	1.30 bs
17	22.3	1.28 bs				0.88 t (6.9)	1.30 bs
18	13.0	0.88 t (6.9)	17	16, 17	17		1.30 bs
19							0.88 t (6.9)

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Assignments can be switched.



gHMBC (J = 8 Hz) (⟶), gHMBC (J = 4 Hz) (- - ➝), and gCOSY (▬) correlations

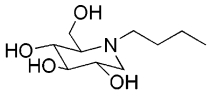
Figure 1. Selected gCOSY and gHMBC correlations of **3**.

C (**5**), were based on comparing their molecular formulas and ^{1}H NMR data with those of **3**. The formulas of **4**, $C_{17}H_{35}NO_4$, and **5**, $C_{19}H_{39}NO_4$, differed from that of **3** by the loss and gain of a CH_2, respectively. Thus, **4** and **5** varied from **3** only in the length of the alkyl chain.

The assignment of the relative stereochemistry of the iminosugar core of **3**−**5** was deduced from a combination of proton J values and ^{1}H and ^{13}C NMR shifts. The equatorial orientation of the alkyl chain of **3** was based on the large J (12.2 Hz) observed between H-1*ax* and H-2*ax*. The coupling patterns of H-2*eq* (δ 1.81, ddd, $J_{1,2eq}$ = 2.7 Hz, $J_{2ax,2eq}$ = 14.3 Hz, and $J_{2eq,3eq}$ = 2.7 Hz) and H-2*ax* (δ 1.98, ddd, $J_{1ax,2ax}$ = 12.6 Hz, $J_{2ax,2eq}$ = 14.5 Hz, and $J_{2ax,3eq}$ = 2.5 Hz) indicated an equatorial orientation of H-3. The relatively small coupling constants, $J_{3,4}$ = 3.0 Hz and $J_{4,5}$ = 1.5 Hz, along with the NOE observed between H4 and H5 were consistent with three possibilities: H-4*eq*/H-5*ax*, H-4*eq*/H-5*eq*, and H-4*ax*/H-5*eq*. A decision among the possibilities was made by evaluating the NMR shifts of H-4, C-4, and C-6 of **3** along with comparing $J_{4,5}$ with those of related model compounds shown in Figure 2. The NMR shift of H-4 (δ 3.73) agreed with an equatorial placement based on comparison with morusimic acids F (**8**, δ_{eq} 3.80), D (**9**, δ_{ax} 3.35), and E (**10**, δ_{ax} 3.16).[17] The two remaining possibilities, H-4*eq*/H-5*ax* and H-4*eq*/H-5*eq*, were distinguished by the following considerations. The former was in agreement with the $J_{4,5}$ = 1.5 Hz of **8**, but there is no model for the latter. The next step involved looking at the agreement in the C-6 shifts observed for both **3** (δ 59.4) and **10** (δ 60.1) in comparison with an upfield shift δ 50−55 ppm calculated for an axial hydroxymethyl such as in **11a**.[18] The H-4*eq*/H-5*eq* stereochemistry was also inconsistent with the configurational mobility expected for **11a**. Figure 2 shows that confirmation **11b** would be favored by 1.0 kcal. Furthermore, comparing the δ_H and δ_C values of **3** with 3,4-di-*epi*-fagomine (**12**)[19] shows very good agreement for H-3*eq*, δ 3.89 vs 3.93, and H-4*eq*, δ 3.73 vs 3.75. However, comparison of C-5, δ 51.9 vs 58.2, and H-5*ax*, δ 3.48 vs 3.16, shows differences that may be due to the substituent at C-1 in **3** versus the lack of one in **12**. Additionally, the lack of cross ring NOE correlations between H-6 and H-1 supports the axial orientation of H-5.[20] These data support the aza-ring stereochemical arrangement shown, with that at C-8 remaining undefined.

One final point can be made about the conformation of the iminosugar core. It is well known that piperidine rings are stabilized by hydrogen bonding between the nitrogen and the axial β-hydroxyl group such as that found in **3**.[21] This observation along with the distinct J values observed for **3** confirms that the ring exists as a single conformer.

Iminosugars derived from terrestrial bacteria and plants, along with those prepared synthetically, include a variety of structural motifs such as piperidines, pyrrolidines, indoizidines, pyrrolizidines, and *nor*tropanes.[1] Their biological properties have centered on glycosidase inhibition in relation to antiviral,[22] insecticidal,[23] nematicidal,[24] and anticancer[25] activities and lysosomal storage disorders.[26] Perhaps the most significant member of this extended class is *N*-butyl-1-deoxynojirimycin (Zavesca, **13**), an agent effective against type 1 Gauchers disease.[27] Although the work outlined above was initiated on the basis of the potency of the extract fractions against numerous enzymes, follow-up testing on **3**−**5** was not performed due to the discontinuation of the assays. Alternatively, **3**−**5** were tested against *Staphylococcus epidermidis*, and all exhibited MICs of ≤6.3 μg/mL.



N-butyl-1-deoxynojirimycin (Zavesca™, **13**)

The structures of **3**−**5** provide a nice extension of the molecular frameworks for a simple iminosugar. A variety

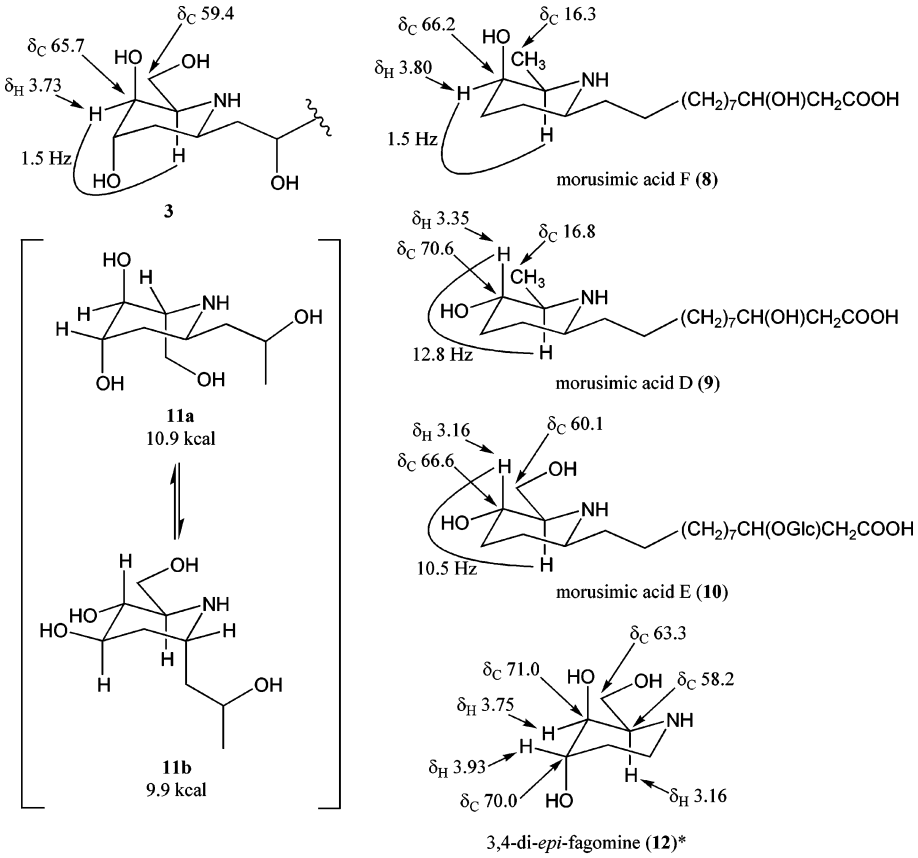


Figure 2. Comparison of NMR data (MeOH-d_4) of **3** with models.

of plant-derived alkaloids are known that are biosynthetically related to **3**−**5** (see Supporting Information Figure S3 structures), yet none exhibit the same theme of hydroxylation and akyl substitution. Numerous alkaloids unrelated to **3**−**5** have been reported from sponges of the genus *Batzella* including tricylic guanidines[28] and pyrroloiminoquinones,[29] plus a sulfolane reported from an Australian sponge/tunicate composite, *Batzella* sp./*Lissoclinum* sp.[30] Of additional note, the new compounds reported above exhibit a very distant biosynthetical relationship to other marine-derived aza heterobicyclics such as the clavepictines,[31] lepadins,[32] and pictamine[33] (see Supporting Information Figure S4).

Experimental Section

General Experimental Procedures. The NMR spectra were recorded at 500 MHz (^{1}H, MeOH-d_4) and 125 MHz (^{13}C, MeOH-d_4). Final NMR assignments were based on 2D NMR data derived from gHMQC, gHMBC, COSY, and NOESY. The normal gradient HMBC experiment used a coupling of 8 Hz (65 ms dephasing) for three-bond correlations, and the long-range experiment used a coupling of 4 Hz (127 ms dephasing) for four-bond correlations. Coupling constants for complex first-order spin systems were obtained using computer-generated models.[34] LCMS was performed with a reversed-phase 5 μm analytical column using photodiode array (PDA) and evaporative light scattering (ELSD) detection with an electrospray ionization time of fight (ESITOF) mass spectrometer. Sephadex LH-20 was used for separation of the crude fractions. HPLC was performed with a reversed-phase 5 μm column using a ELS detector. An ESITOF mass spectrometer was employed for HRESITOFMS. The optical rotation was acquired using a digital polarimeter.

Biological Material, Collection, and Identification. A specimen of *Batzella* sp. (class Demospongiae, order Poecilosclerida, family Demoacidonidae,[35] UCSC coll. no. 00216) was collected from Madagascar using scuba, at depths of 9−24 m: NMST01 (S 12°51.185′, E 48°25.153′). The specimen was thick (>1 cm) encrusting with a smooth surface and a soft consistency that came off like sheets when torn. Both the inside and outside color was brownish. The skeletal elements consisted of two spicule types: oxeas (140−160 μm × 2.3 μm) and styles (100−120 μm × 2.0 μm).

Extraction and Isolation. The sponge was preserved according to our standard procedure as described previously[36] and then transported to the home laboratory at ambient temperature. The organism was soaked three successive times for 24 h in 100% MeOH. The sponge was additionally soaked two successive times for 24 h in 100% CH_2Cl_2. The resulting oil was partitioned as described elsewhere.[36]

Pure compounds were obtained as follows: a 350 mg portion of the FM extract was fractionated using Sephadex LH-20 with 100% MeOH to yield six fractions. The first fraction was identified by ^{1}H NMR and MS as halitoxin (**2**). The third Sephadex fraction (100.2 mg) was separated using reversed-phase HPLC with a gradient of 100% water to 100% MeOH (0.1% formic acid in both solvents) to yield **3** (3.8 mg), **4** (1.7 mg), and **5** (1.9 mg).

Antibacterial Assay. Compounds **3**−**5** were tested against *Staphylococcus epidermidis* (ATCC 12228) following the procedure published elsewhere.[37]

Batzellaside A (3): colorless oil; $[\alpha]^{25}_D$ +8.1° (*c* 0.44, MeOH); HRESITOFMS *m*/*z* 332.2784 (calcd for $C_{18}H_{38}NO_4$ $[M + H]^+$ 332.2773 Δ −1.1 mmu); ^{1}H NMR (500 MHz) in Figure S5 and Table 1; ^{13}C NMR (125 MHz) in Figure S6 and Table 1.

Batzellaside B (4): colorless oil; $[\alpha]^{25}_D$ +10° (*c* 0.5, MeOH); HRESITOFMS *m*/z 318.2658 (calcd for $C_{17}H_{36}NO_4$ $[M + H]^+$ 318.2678 Δ 2.0 mmu); ^{1}H NMR (500 MHz) in Figure S13 and Table 1.

Batzellaside C (5): colorless oil; $[\alpha]^{25}_D$ +12° (*c* 0.4, MeOH); HRESITOFMS *m*/z 346.2980 (calcd for $C_{19}H_{40}NO_4$ $[M + H]^+$ 346.3008 Δ 2.8 mmu); ^{1}H NMR (500 MHz) in Figure S14 and Table 1.

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Supporting Information Available: The isolation scheme, known iminosugars of microbial and plant sources, structures of database hits for $C_{18}H_{37}NO_4$, plant-derived alkaloids related to **3**−**5**, marine natural products related to **3**−**5**, ^{1}H, ^{13}C, COSY, gHMQC, gHMBC (J = 8 Hz), gHMBC (J = 4 Hz), and NOESY NMR spectra data of **3**, ^{1}H NMR spectral data of **4** and **5**, and above-water photograph of 00216. This material is available free of charge via the Internet at http://pubs.acs.org.

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