J. Nat. Prod. **2006,** *69,* 211−218

Cytotoxic Isomalabaricane Triterpenes from the Marine Sponge *Rhabdastrella globostellata*

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Received September 14, 2005

Fourteen isomalabaricane triterpenes were isolated from the marine sponge *Rhabdastrella globostellata*. In addition to the known compounds globostellatic acids A (**1**) and D (**4**) and stelliferin riboside (**13**), 11 of the compounds were new natural products, which included globostelletin (**3**), eight new globostellatic acid congeners, F to M (**2**, **5**−**11**), and two new stelliferin ribosides (**12** and **14**). The isolated compounds were tested against three different cancer cell lines, L5178Y (mouse lymphoma), HeLa (human cervix carcinoma), and PC-12 (rat pheochromocytoma). The isomalabaricane derivatives were found to be selectively active toward the mouse lymphoma cell line L5178Y. The structures were determined by 1D and 2D NMR data and by comparison with spectroscopic data of known related compounds.

The malabaricanes are yellow pigments that were first isolated from the wood of the *Alianthus malabarica* tree and are structurally characterized by a tricyclic terpenoid core and a conjugated, polyene side chain.[1,2] Malabaricane, the trivial name of this group of compounds, was coined for the hypothetical hydrocarbon system (3*S**,3a*R**,5a*S**,9a*S**,9b*S**)-3a,6,6,9a-tetramethyl-3-(1,5,9-trimethyldecyl)perhydrobenz[*e*]indene,[2] where the tricyclic nucleus has a *trans*−*anti*−*trans* ring junction. The rather rare isomalabaricane framework is embedded in a 4,4,8,10-tetramethylperhydrobenz[*e*]indene system with a *trans*−*syn*−*trans* ring junction, which forces the central ring into an unfavorable twist−boat conformation. Isomalabaricane triterpenes have been reported from several genera of marine sponges belonging to the order Astrophorida.[3−17] In the marine environment, this rare class of triterpenoids has been reported from sponge species of the genera *Stelletta*,[13,14,17] *Jaspis*,[15,16] *Geodia*, and *Rhabdastrella*.[10] The nomenclature of the isomalabaricane triterpenoids, which contain a polyene conjugated system, could be classified into three groups: (1) stelletins, (2) stelleferins, and (3) globostellatic acids. Stelletins basically consist of the γ-pyrone functionality, which could be open in some of its congeners such that the olefinic chain terminates as a free carboxylic acid. Stelleferins are oxygenated at position 19, while globostellatic acids are carboxylated at C-4. Their trivial names were sometimes modified according to their sponge origin.

Isomalabaricanes have been reported to significantly inhibit the growth of tumor cells. The cytotoxic isomalabaricane triterpenes, stelletins A, B, C, D, E, and F, were isolated from the Australian sponge *Stelletta* and were found to be active at a low-to-mid nanomolar range of concentration in the 60 human tumor cell line in vitro assay of the National Cancer Institute.[20] In our continuing search for biologically active metabolites from marine invertebrates, we isolated 11 new cytotoxic triterpenes (**2**, **3**, **5**−**12**, and **14**) from the Indonesian sponge *Rhabdastrella globostellata* in addition to the known compounds globostellatic acids A (**1**) and D (**4**)[12] and stelleferin riboside (**13**).[5] Here we describe the isolation and structure elucidation of the new metabolites.

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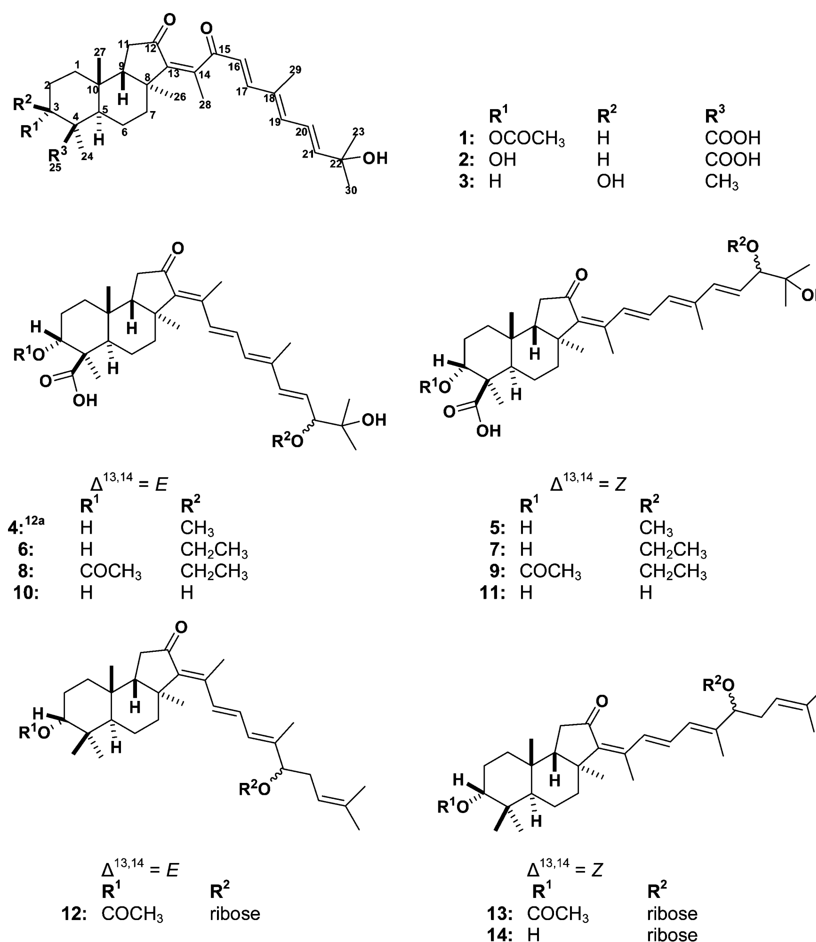
Results and Discussion

The MeOH extract of the frozen sponge (20 g) was partitioned between H_2O and EtOAc to give about 5.2 g of the EtOAc extract. The EtOAc fraction was subjected to vacuum liquid chromatography (VLC) on silica gel with increasing polarity from 100% hexane to 100% EtOAc followed by 100% EtOAc to 100% MeOH. Purification steps were accomplished through normal-phase flash chromatography and reversed-phase HPLC to afford 14 isomalabaricane triterpenes. The presence of the isomalabaricane triterpenes was indicated by the HPLC-DAD chromatogram of the total crude sponge extract. The detected HPLC peaks exhibited UV absorption maxima at 300 to 400 nm, suggesting the presence of a conjugated polyene system, which is typical for the isomalabaricanes.[3−17] Isomerization occurs at the olefinic C-13 bond, which in the presence of light undergoes a rapid interconversion. The *E*/*Z*-isomeric pairs proved difficult to separate and were usually isolated as a mixture in a ratio of 1:1 or 2:1, as previously observed.[3,4,7,8,13,16,20] Congeners with a keto group at C-15, as in compounds **1**−**3**, and a ribose substituent at C-20, like in derivatives **12**−**14**, seem to be more stable in maintaining their $C_{13}-C_{14}$ geometry as was also observed for stelliferin riboside.[5]

The ^{1}H NMR spectra of the isolated compounds were informative particularly when combined with the ^{13}C NMR data. Their ^{1}H NMR spectra showed olefinic proton doublets in the 6.00−8.00 ppm region with coupling constants of ca. 11.0 and 15.0 Hz, which indicated the occurrence of a conjugated double bond system in all the isolated derivatives. This was confirmed by a COSY experiment establishing the connectivity of the olefinic protons. The ^{13}C NMR data of the tricyclic part of the isomalabaricanes and malabaricane tritepenoids differentiated the two families of compounds. Comparison of ^{13}C NMR data of the previously reported malabaricane[6] with those of isomalabaricane triterpenoids[5] revealed that the carbon resonance assigned to C-5 was shifted 6−9 ppm upfield in the isomalabaricanes, while the carbon signal for C-9 resonated 4−5 ppm downfield when compared with that of the malabaricanes. On the other hand, the chemical shifts for the methyl groups in both groups were very similar. The relative configuration of the isolated triterpenoids was further elucidated from ROESY data. NOE effects were observed between H-5/CH_3-26 and H-9/CH_3-27, which confirmed the presence of a *trans*−*syn*−*trans* ring junction typical of the isomalabaricane nucleus. The major differences between the isolated isomalabaricane congeners occur on the side chain (C-13 to C-22) and/or the substitutents at C-3 which were unambiguously established by COSY and HMBC.

10.1021/np050346t CCC: $33.50 © 2006 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/20/2006

Chart 1



Compound **2** showed a negative pseudomolecular ion peak in the ESIMS at m/z 497 $[M - H]^+$, and the molecular formula $C_{30}H_{43}O_6$ $[M + H]^+$ was established by HRESIMS ($C_{30}H_{43}O_6$, m/z 499.3069, calcd 499.3061). It has UV absorptions at λ_{max} 234 and 329 nm in MeOH, the latter being indicative of the presence of a highly conjugated polyene chromophore. The UV maximum at 329 nm suggested the length of the conjugated polyene chain in **2** was shorter than in globostellatic acids B and C[12] and more similar to that of globostellatic acid A (**1**),[12] being compatible with the fact that the higher the conjugation, the greater the bathochromic shift. The NMR data of compound **1** in MeOD were identical to those reported.[12] To compare the NMR data of congeners **1** and **2**, the ^{1}H NMR spectrum of **1** was again recorded in $CDCl_3$, in which congener **2** was more soluble. Indeed the ^{1}H NMR data of **2** were comparable to those of globostellatic acid A (**1**).[12] The ^{1}H NMR spectrum of **2** showed the presence of five aliphatic methyl units at 0.95, 1.25, 1.36 (6H), and 1.47 ppm, two olefinic methyls at 1.91 and 1.96 ppm, an oxygenated methine proton at 4.18 ppm, and five olefinic protons, which were resonances characteristic of an isomalabaricane triterpene. The only difference is the absence of the acetyl methyl singlet at 2.06 ppm positioned at C-3 evident from the upfield shift of H-3 at 4.18 from that in **1** at 5.41 ppm. This was also supported from the respective ^{13}C NMR data and compatible with the 42 mass unit differences in molecular weight between **2** and its known congener **1**. The ^{13}C NMR data for the tricyclic moiety of **2** supported the presence of an isomalabaricane skeleton.[6] Complete and unambiguous assignments of protons and carbons of **2** were established through 2D NMR experiments which included ^{1}H−^{1}H COSY, HMQC, and HMBC (see Table 1). The HMBC correlations (Table 1) of the seven methyl protons with their neighboring carbons unequivocally determined their positions and designated the attachment of the functional groups in the basic framework of the triterpene. The HMBC data were again very similar to those of the known congener **1** except for the differences caused by the change at C-3. The position of the hydroxyl function at C-3 was then confirmed through the long-range correlations of the methyl proton signal at δ 1.25 (CH_3-24) with the methine signal at δ_{C-3} 70.4 and carbonyl signal at δ_{C-4} 181.6, which is also indicative of the carboxyl function at C-4. The relative configuration of **2** was evident from a ROESY experiment that confirmed the presence of a *trans*−*syn*−*trans* ring junction. The 13*Z* configuration in **2** was evident from the NOE between CH_3-26 and CH_3-28, which was identical to that observed in globostellatic acid A (**1**).[12] The equatorial disposition of H-3 was evident from its broad singlet appearance, indicative of small vicinal couplings, and the NOEs with both δ 1.73 (H-2A) and 2.21 (H-2B). Compound **2** is thus the 3-de-*O*-acetyl congener of globostellatic acid A (**1**) and was named globostellatic acid F.

The MS and NMR data for **3** indicated this was closely related to **2** with a methyl group replacing a carboxylic acid function from the difference of 30 mass units and changes in chemical shifts. The presence of a geminal pair of methyl groups was evident from the long range *W*-coupling between the methyl singlets at δ 1.05 and 0.84 for CH_3-24 and -25, respectively. The substitution pattern in ring A was confirmed by an HMBC experiment that showed ^3J correlations of the geminal methyl pair CH_3-24/25 with C-3 at δ 79.8 and C-5 at δ 46.2 along with the ^2J correlations with C-4 at δ 39.8. The double doublet for H-3 of **3** with coupling constants of 11.4 and 4.7 Hz, instead of the broad singlet of congener **2**, indicated an axial orientation of H-3 and consequently a 3β-hydroxyl group.[8] The ^{1}H and ^{13}C NMR data of the tricylic moiety were comparable with those reported for stelliferin D.[15] The relative configuration at C-1, C-3, C-4, and C-5 was confirmed by ROESY. NOEs were detected between H-3 and the Me-24 singlet at δ 1.05 and the H-1A

Table 1. NMR Data of Compounds **2** and **3** in $CDCl_3$[a]

	2				**3**			
position	δ_C	m	HMBC C to H	δ_H	δ_C[b]	m	HMBC C to H	δ_H
1	30.1	CH_2	C*H*$_3$-27	1.18 (1H, m)		CH_2		1.43 (1H, m)
				1.90 (1H, m)				1.56 (1H, m)
2	27.5	CH_2		1.73 (1H, m)		CH_2		1.70 (1H, m)
				2.21 (1H, m)				1.84 (1H, m)
3	70.4	CH	C*H*$_3$-24	4.18 (1H, bs)	79.8	CH	C*H*$_3$-24, C*H*$_3$-25	3.52 (1H, dd, 11.4, 4.7 Hz)
4	47.6	C	C*H*$_3$-24		39.8	C	C*H*$_3$−24, C*H*$_3$−25	
5	39.9	CH	C*H*$_3$-24, C*H*$_3$-27	2.33 (1H, m)	46.2	CH	C*H*$_3$-24, C*H*$_3$-27, C*H*$_3$-25	1.73 (1H, m)
6	19.5	CH_2		1.88 (2H, m)		CH_2		1.53 (1H, m)
								1.78 (1H, m)
7	36.7	CH_2	C*H*$_3$-26	2.09 (1H, m)	42.1	CH_2	C*H*$_3$-26	2.07 (1H, m)
				2.23 (1H, m)				2.22 (1H, m)
8	42.9	C	C*H*$_3$-26					
9	50.1	CH	C*H*$_3$-27, C*H*$_3$-26	1.94 (1H, m)	50.2	CH	C*H*$_3$-27	1.90 (1H, m)
10	36.9	C	C*H*$_3$-27		35.0	C	C*H*$_3$-27	
11	34.7	CH_2		2.13 (1H, m)		CH_2		2.15 (2H, m)
				2.20 (1H, m)				
12		C				C		
13	146.3	C	C*H*$_3$-26, C*H*$_3$-28		145.2	C	C*H*$_3$-26, C*H*$_3$-28	
14	142.9	C	C*H*$_3$-28		142.5	C	C*H*$_3$-28	
15	204.0	C	C*H*$_3$-28		200.2	C	C*H*$_3$-28	
16	124.5	CH		6.18 (1H, d, 16.1 Hz)		CH		6.17 (1H, d, 15.8 Hz)
17	147.7	CH	C*H*$_3$-29	6.93 (1H, d, 16.1 Hz)	147.1	CH	C*H*$_3$-29	6.92 (1H, d, 15.8 Hz)
18	133.9	C	C*H*$_3$-29		134.5	C	C*H*$_3$-29	
19	138.4	CH	C*H*$_3$-29	6.30 (1H, d, 11.0 Hz)	137.0	CH	C*H*$_3$-29	6.31 (1H, d, 11.35 Hz)
20	122.4	CH		6.61 (1H, dd, 11.0, 14.8 Hz)		CH		6.67 (1H, dd, 11.0, 15.1 Hz)
21	146.2	CH	C*H*$_3$-23, C*H*$_3$-30	6.06 (1H, d, 15.1 Hz)	145.1	CH	C*H*$_3$-23, C*H*$_3$-30	6.05 (1H, d, 15.1 Hz)
22	71.1	C	C*H*$_3$-23, C*H*$_3$-30		70.0	C	C*H*$_3$-23, C*H*$_3$-30	
23	29.8	CH_3	C*H*$_3$-30	1.36 (3H, s)		CH_3		1.37 (3H, s)
24	23.5	CH_3	C*H*-3, C*H*-5	1.25 (3H, s)		CH_3		1.05 (3H, s)
25	181.6	C	C*H*$_3$-24			CH_3		0.84 (3H, s)
26	24.8	CH_3	C*H*-7A	1.47 (3H, s)		CH_3		1.42 (3H, s)
27	19.6	CH_3	C*H*-5, C*H*-9	0.95 (3H, s)		CH_3		1.01 (3H, s)
28	17.1	CH_3		1.96 (3H, s)		CH_3		1.94 (3H, s)
29	12.4	CH_3	C*H*-19	1.91 (3H, s)		CH_3		1.91 (3H, s)
30	29.8	CH_3	C*H*$_3$-23	1.36 (3H, s)		CH_3		1.37 (3H, s)

[a] ^{13}C NMR data were obtained from the HMBC and HMQC spectra. [b]Due to the very small yield of the compound isolated, only partial ^{13}C NMR data could be reported.

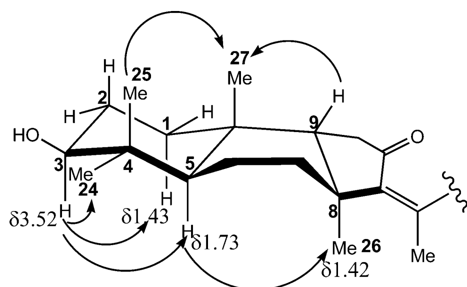


Figure 1. NOE correlations for globostelletin showing relative configurations at C-1, C-3, C-4, C-5, C-8, and C-9.

and H-5 multiplets at δ 1.43 and 1.73, respectively, which implied that C*H*$_3$-24, H-1A, and H-5 are α-oriented like H-3 (Figure 1). Compound **3** is thus a 4-dimethyl derivative of **2** and is also a 3-epimer congener of globostellatic acid F (**2**). Thus, the structure of **3** was unambiguously determined, and the name globostelletin is proposed for this new natural product.

Compound **5** was isolated together with the known compound globostellatic acid D (**4**) as a 1:1 mixture. Globostellatic acid D (**4**) and congener **5** are 13-*E* and 13-*Z* isomers. The positive ESIMS of the isomer mixture showed a single pseudomolecular ion peak at *m*/*z* 515 $[M + H]^+$, and the molecular formula for both isomers was established to be $C_{31}H_{47}O_6$ $[M + H]^+$ (*m*/*z* 515.3351, calc 515.3367) from HRESIMS. The ^{1}H NMR data for the isolated globostellatic acid D (**4**) in MeOD were identical to those reported.[12] However, part of the doublet resonances for C*H*-21 for both isomers were overlapped by the solvent signal. The NMR spectra were then recorded in $CDCl_3$ to be able to differentiate the signals of the two congeners. On the other hand, the ^{1}H and ^{13}C NMR data for the second isomer **5** were comparable to those of **4** (see Tables 2 and 3) and exhibited either overlapping resonances or distinct signals with relatively small differences in chemical shifts. The signals for both isomers were unequivocally differentiated by 2D NMR. The most discernible difference between the two isomers was the chemical shift of the olefinic signal for H-15 at δ 8.05 in **5** and at δ 6.67 in **4**. This indicated that H-15 in **5** was located in the deshielding environment of the carbonyl group, which suggested the 13-*Z* configuration for **5** compared to the 13-*E* configuration of **4**. A similar phenomenon was observed for C*H*$_3$-28 (δ 2.32 in **4** and δ 2.08 in **5**).[10,17,18] Through ROESY, the proton resonances for the C-15 methine and the C-28 methyl unit for both geometrical isomers were distinguished by their respective NOE correlations with C*H*$_3$-26. As observed for both compounds, coupling constants of 15.0 Hz for $J_{15,16}$ and 15.8 Hz for $J_{19,20}$ indicated the 15*E* and 19*E* geometry. Besides establishing the *trans*−*syn*−*trans* orientation in the tricyclic moiety through ROESY, the strong NOE cross-peak between H-5/C*H*$_3$-24 revealed an α-configuration of the methyl group at C-4. Like globostellatic acid F (**2**), H-3 of compound **5** gave a broad singlet signal at δ 4.08, which implied its equatorial orientation. This was also confirmed by ROESY through its similar NOE correlations with H-2A at δ 1.69 and H-2B at δ 2.21 as found in congener **2** (Figure 2). An NOE between H-3 and C*H*$_3$-24 was also observed, which implied the α-configuration of the hydroxyl group, as previously described in stelliferin G[4] and aurorals,[9] all possessing a 3α-hydroxy/acetoxy substituent. This led to the elucidation of structure **5** as the *Z*-isomer of globostellatic acid D (**4**), and it was assigned the name globostellatic acid G.

Compounds **6** and **7** were also obtained as an inseparable mixture (2:1) of *E* and *Z* geometrical isomers. The MS and NMR data

Table 2. ^{1}H NMR Data of Compounds **5**−**11**

H no.	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** ($CDCl_3$)	**8** ($CDCl_3$)	**9** ($CDCl_3$)	**10** (CD_3OD)	**11** (CD_3OD)
1	1.17 (1H, m)	1.19 (1H, m)	1.19 (1H, m)	1.20 (1H, m)	1.20 (1H, m)	1.11(1H, m)	1.11 (1H, m)
	1.95 (1H, m)	1.87 (1H, m)	1.87 (1H, m)	1.79 (1H, m)	1.79 (1H, m)	1.91 (1H, m)	1.91 (1H, m)
2	1.69 (1H, m)	1.70 (1H, m)	1.70 (1H, m)	1.78 (1H, m)	1.78 (1H, m)	1.67 (1H, m)	1.67 (1H, m)
	2.21 (1H, m)	2.22 (1H, m)	2.22 (1H, m)	2.18 (1H, m)	2.18 (1H, m)	2.23 (1H, m)	2.23 (1H, m)
3	4.08 (1H, br s)	4.19(1H, bs)	4.19 (1H, bs)	5.43 (1H, bs)	5.40 (1H, bs)	4.08 (1H, bs)	4.08 (1H, bs)
5	2.51 (bd, 11.8 Hz)	2.49 (1H, m)	2.49 (1H, m)	2.46 (1H, m)	2.46 (1H, m)	2.43 (1H, bd, 12.0 Hz)	2.43 (1H, bd, 12.0 Hz)
6	1.89 (2H, m)	1.82 (2H, m)	1.82 (2H, m)	1.84 (2H, m)	1.84 (2H, m)	1.84 (2H, m)	1.84 (2H, m)
7	2.09 (1H, m)	2.11 (1H, m)	2.11 (1H, m)			2.14 (1H, m)	2.14, 2.21 m
	2.23 (1H, m)	2.19 (1H, m)	2.19 (1H, m)			2.21 (1H, m)	
9	1.87 (1H, m)	1.88 (1H, m)	1.88 (1H, m)	1.90 (1H, m)	1.90 (1H, m)	1.85 (1H, m)	1.85 (1H, m)
11	2.19, 2.23 m	2.21 (1H, m)	2.21 (1H, m)			2.13 (1H, m)	2.13, 2.26 m
		2.28 (1H, m)	2.28 (1H, m)			2.26 (1H, m)	
15	8.05 (1H, d, 15.0 Hz)	6.66 (1H, d, 14.5 Hz)	8.08 (1H, d, 15.0 Hz)	6.67 (1H, d, 15.0 Hz)	8.00 (1H, d, 15.0 Hz)	6.72 (1H, d, 14.9 Hz)	8.05 (1H, d, 15.0 Hz)
16	7.05 (1H, dd, 15.5, 11.4 Hz)	6.99 (1H, dd, 11.5, 14.5 Hz)	6.94 (1H, dd, 11.5, 14.5 Hz)	7.01 (1H, dd, 11.3, 15.1 Hz)	6.96 (3H, s)	7.11 (1H, dd, 11.0, 14.8 Hz)	7.05 (1H, dd, 11.7, 15.5 Hz)
17	6.25 (1H, d, 10.7 Hz)	6.27 (1H, d, 11 Hz)	6.27 (1H, d, 11.0 Hz)	6.28 (d, 11.3 Hz)	6.28 (d, 11.35 Hz)	6.31 (1H, d, 11.35 Hz)	6.22 (1H,d, 11 Hz)
19	6.39 (1H, d, 15.8 Hz)	6.33 (1H, d, 15.4 Hz)	6.33 (1H, d, 15.4 Hz)	6.32 (1H, d, 15.3 Hz)	6.32 (1H, d, 15.3 Hz)	6.43 (1H, d, 15.8 Hz)	6.39 (1H,d, 15.9 Hz)
20	5.70 (1H, dd, 8.0, 16.0 Hz)	5.69 (1H, dd, 8.8, 15.8 Hz)	5.62 (1H, dd, 8.8, 15.8 Hz)	5.7 (dd, 8.2, 15.8 Hz)	5.63 (dd, 8.2, 15.8 Hz)	5.96 (dd, 6.9, 15.8 Hz)	5.90 (1H,dd, 15.8, 6.9 Hz)
21	3.45 (1H, d, 8.0 Hz)	3.55 (1H, d, 8.2 Hz)	3.55 (1H, d, 8.2 Hz)	3.54 (1H, d, 7.6 Hz)	3.54 (1H, d, 7.6 Hz)	3.92 (1H, d, 7.2 Hz)	3.92 (1H, d, 7.2 Hz)
23	1.16 (3H, s)	1.17 (3H, s)	1.17 (3H, s)	1.18 (3H, s)	1.18 (3H, s)	1.18 (3H, s)	1.16 (3H, s)
24	1.25 (3H, s)	1.35 (3H, s)	1.35 (3H, s)	1.26 (3H, s)	1.26 (3H, s)	1.25 (3H, s)	1.24 (3H, s)
26	1.43 (3H, s)	1.43 (3H, s)	1.41 (3H, s)	1.44 (3H, s)	1.42 (3H, s)	1.45 (3H, s)	1.43 (3H, s)
27	0.98 (3H, s)	0.92 (3H, s)	0.92 (3H, s)	0.95 (3H, s)	0.95 (3H, s)	1.01 (3H, s)	1.01 (3H, s)
28	2.08 (3H, s)	2.33 (3H, s)	2.04 (3H, s)	2.34 (3H, s)	2.05 (3H, s)	2.30 (3H, s)	2.07 (3H, s)
29	1.97 (3H, s)	1.97 (3H, s)	1.93 (3H, s)	1.98 (3H, s)	1.94 (3H, s)	1.99 (3H, s)	1.95 (3H, s)
30	1.14 (3H, s)	1.15 (3H, s)	1.14 (3H, s)	1.15 (3H, s)	1.14 (3H, s)	1.16 (3H, s)	1.15 (3H, s)
31		3.33, 3.59 (2H, m)	3.33, 3.59 (2H, m)	3.34, 3.59 (2H, m)	3.34, 3.59 (2H, m)		
32	3.30 (3H, s)	1.19 (3H, t, 7.0 Hz)	1.19 (3H, t, 7.0 Hz)	1.19 (3H, t, 7.0 Hz)	1.19 (3H, t, 7.0 Hz)		
$COCH_3$				2.09 (3H, s)	2.09 (3H, s)		

indicated these were closely related to **4** and **5** except for the presence of additional multiplet resonances at δ_H 3.33 and 3.59 and a methyl triplet at δ_H 1.19 with a coupling constant of 7.0 Hz, indicating the occurrence of an *O*-ethyl instead of the *O*-methyl moiety. The point of attachment at C-21 was confirmed from the appropriate correlations in the HMBC spectrum (Table 3). Identical NOE results were observed in both compounds, which showed correlation of H-3 with H_2-2A/B and CH_3-24, as the previous congeners (Figure 2). The structures of compounds **6** and **7** were unequivocally determined to be the *O*-ethyl congeners of globostellatic acids D (**4**) and G (**5**). Compounds **6** and **7** were assigned the names globostellatic acids H and I, respectively.

An analogous MS and ^{1}H NMR analysis indicated compounds **8** and **9**, obtained as a 2:1 *E*/*Z*-isomeric mixture, which were related to globostellatic acids H (**6**) and I (**7**) (Table 2). The ^{1}H NMR data indicated the presence of the same set of *O*-ethyl proton signals discernible in **6** and **7**. The increased molecular mass of 42 mass units and the detection of an additional methyl singlet at δ 2.09 suggested the presence of an acetoxy moiety plausibly at C-3, which is commonly encountered in many isomalabaricane triterpenoids. This was in agreement with the downfield shift of H-3 in compounds **8** and **9**, which was observed at δ 5.43 and 5.40, respectively, similar to that in globostellatic acid A (**1**).[12] Analysis of 1D ^{1}H NMR and 2D COSY and ROESY spectra showed that the rest of the NMR resonances of the isomeric mixture were analogous to those of **6** and **7** (see Table 2) and possessed the same relative configuration. Hence **8** and **9** were the acetyl congeners of globostellatic acids H (**6**) and I (**7**) and were designated the names globostellatic acids J and K, respectively.

A full analysis of compounds **10** and **11**, also isolated as a 13-*E*/*Z*-mixture in a ratio of 2:1, indicated the presence of unsubstituted hydroxyl groups at C-3 and C-21. The complete carbon assignments were performed indirectly from their HMQC and HMBC spectra (see Tables 2 and 4). Congeners **10** and **11** are the 21-hydroxyl derivatives of globostellatic acids D (**4**) and G (**5**) and were named globostellatic acids L and M, respectively.

Globostellatic acids D (**4**) and G (**5**) are *O*-methylated at C-21, while globostellatic acids H to K (**6**−**9**) have an *O*-ethyl substituent at the same position. It was speculated that these compounds may be artifacts and not natural products since the sponge sample had been preserved in EtOH, while the crude total extract was prepared with MeOH. To examine this possibility, samples of the isomeric pair, globostellatic acids L (**10**) and M (**11**), were dissolved in MeOH or EtOH, respectively, and left at room temperature with stirring for 2 days. Another set of samples were incubated at 40 °C for 24 h. After this stability test, the respective solvents were evaporated, the samples were dried and their ^{1}H NMR spectra were obtained in both $CDCl_3$ and CD_3OD to allow comparison of their NMR data with those of the isolated compounds, respectively. Their ^{1}H NMR spectra did not show any evidence of the 21-*O*-methyl or -ethyl congener. On the basis of these experiments, we assume that compounds **4**−**9** are probably natural products.

Compound **12** was obtained as a yellow oily substance and, unlike the previous derivatives, was isolated as a pure compound. Its isomeric counterpart, the known compound stelliferin riboside (**13**),[5] was also isolated as a homogeneous compound. The molecular formula of **12**, established by HRESIMS as $C_{37}H_{56}O_8$, is identical to that of **13**, and comparison of their ^{1}H NMR spectra confirmed that they were stereoisomers at C-13. The same data (Table 4) indicated the occurrence of an α-ribose moiety from the chemical shifts and coupling signals in the region δ 3.50 to 4.50.[5]

Table 3. ^{13}C NMR Data of Compounds **5**−**7**, **10**, and **11**[a]

	4 (in $CDCl_3$)			**5** (in $CDCl_3$)			**6** (in $CDCl_3$)			**7** (in $CDCl_3$)			**10** (in CD_3OD)			**11** (in CD_3OD)		
pos.	δ_C	m	HMBC (C to H)	δ_C	m	HMBC (C to H)	δ_C	m	HMBC (C to H)	δ_C	m	HMBC (C to H)	δ_C	m	HMBC (C to H)	δ_C	m	HMBC (C to H)
1	30.3	CH_2		30.3	CH_2		28.5	CH_2		28.4	CH_2		29.9	CH_2	CH_3-27	29.9	CH_2	CH_3-27
2	28.7	CH_2		28.7	CH_2		26.9	CH_2		26.9	CH_2		28.5	CH_2		33.5	CH_2	
3	70.7	CH		70.7	CH		70.7	CH		69.8	CH		71.7	CH	CH_3-24	71.7	CH	CH_3-24
4		C			C		48.0	C	CH_3-24		C	CH_3-24	49.3	C	CH_3-24	49.3	C	CH_3-24
5	40.2	CH	CH_3-27	40.2	CH	CH_3-27	40.2	CH	CH_3-24	39.9	CH	CH_3-24	41.5	CH	CH_3-24, CH_3-27	45.4	CH	CH_3-24, CH_3-27
6	20.4	CH_2		20.4	CH_2		20.0	CH_2			CH_2		21.5	CH_2		21.4	CH_2	
7		CH_2			CH_2		40.2	CH_2			CH_2		41.0	CH_2	CH_3-26	39.7	CH_2	CH_3-26
8	45.0	C	CH_3-26	45.0	C	CH_3-26	45.4	C	CH_3-26		C	CH_3-26	46.1	C	CH-7A, CH-9	46.0	C	CH-7A, CH-9
9	49.4	CH		49.4	CH		49.1	CH	CH_3-27	49.1	CH	CH_3-27	50.8	CH	CH_3-26, CH_3-27	50.8	CH	CH_3-26, CH_3-27
10	35.9	C	CH_3-27	35.9	C	CH_3-27	37.0	C	CH_3-27		C	CH_3-27	37.0	C	CH_3-27	37.0	C	CH_3-27
11	36.8	CH_2		37.2	CH_2		36.5	CH_2	CH_3-28	36.7	CH_2	CH_3-28	37.6	CH_2	CH-9	37.8	CH_2	CH-9
12	207.7	C	CH-11A	207.7	C	CH-11A	207.7	C	CH-11A		C	CH-11A	209.9	C		208.9	C	
13	146.3	C	CH_3-26, CH_3-28	146.3	C	CH_3-26, CH_3-28	147.2	C	CH_3-26		C	CH_3-26	148.1	C	CH_3-26, CH_3-28	147.6	C	CH_3-26, CH_3-28
14	141.5	C	CH_3-28	141.5	C	CH_3-28	142.0	C	CH_3-28		C	CH_3-28	143.4	C	CH_3-28	143.9	C	CH_3-28
15	133.3	CH	CH_3-28	133.3	CH	CH_3-28	133.3	CH	CH_3-28	133.4	CH	CH_3-28	133.7	CH	CH_3-28	134.0	CH	CH_3-28
16	131.8	CH		130.9	CH		131.6	CH		131.7	CH		133.3	CH		132.0	CH	
17	132.1	CH	CH_3-29	132.1	CH	CH_3-29	132.0	CH	CH_3-29	131.9	CH	CH_3-29	132.5	CH	CH_3-29	133.5	CH	CH_3-29
18	137.7	C	CH_3-29	137.0	C	CH_3-29	138.1	C	CH_3-29	138.0	C	CH_3-29	139.8	C	CH_3-29	139.2	C	CH_3-29
19	138.8	CH		138.8	CH		138.2	CH		138.1	CH		137.4	CH	CH_3-29	138.0	CH	CH_3-29
20	126.8	CH		127.2	CH		127.8	CH		127.9	CH		131.1	CH		130.3	CH	
21	89.8	CH	CH_3-23, CH_3-30	88.8	CH	CH_3-23, CH_3-30	88.3	CH	CH_3-23	88.2	CH	CH_3-23	80.4	CH	CH_3-23, CH_3-30	81.3	CH	CH_3-23, CH_3-30
22	72.5	C	CH_3-23, CH_3-30	72.5	C	CH_3-23, CH_3-30	73.2	C	CH_3-23	73.2	C	CH_3-23	73.5	C	CH_3-23, CH_3-30	72.8	C	CH_3-23, CH_3-30
23	24.1	CH_3	CH_3-30	25.9	CH_3	CH_3-30	26.1	CH_3		26.0	CH_3		25.2	CH_3	CH_3-30	25.2	CH_3	CH_3-30
24	23.5	CH_3		24.9	CH_3		23.7	CH_3			CH_3		24.4	CH_3		24.4	CH_3	
25		C			C		182.7	C	CH_3-24	182.6	C	CH_3-24	182.7	C	CH_3-24	182.7	C	CH_3-24
26	26.2	CH_3		25.1	CH_3		25.1	CH_3		26.1	CH_3		25.7	CH_3		24.5	CH_3	
27	18.8	CH_3		20.0	CH_3		19.3	CH_3		20.0	CH_3		20.4	CH_3		20.4	CH_3	
28	15.1	CH_3		16.2	CH_3		14.2	CH_3		15.9	CH_3		14.7	CH_3		16.0	CH_3	
29	13.5	CH_3		13.4	CH_3		13.3	CH_3		13.3	CH_3		12.9	CH_3		12.9	CH_3	
30	24.9	CH_3	CH_3-23	24.8	CH_3	CH_3-23	24.1	CH_3	CH_3-23	23.9	CH_3	CH_3-23	25.2	CH_3	CH_3-23	25.4	CH_3	CH_3-23
31	56.0	CH_3	CH-21	57.0	CH_3	CH-21	64.6	CH_2	CH_3-32	65.1	CH_2	CH_3-32						
32							15.2	CH_3	CH_2-31A	15.8	CH_3	CH_2-31A						

[a] Obtained from HMBC and HMQC spectra.

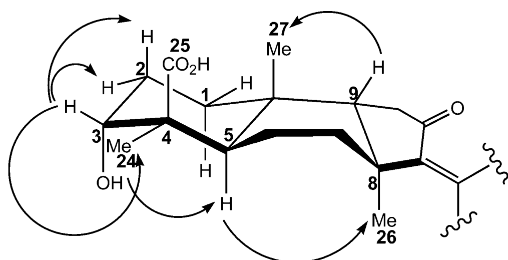


Figure 2. NOE correlations for compounds **2**, **4**, and **11** showing relative configurations at C-3, C-4, C-5, C-8, C-9, and C-10.

Compound **12** was unambiguously identified from 2D NMR experiments as the 13*E*-isomer of stelliferin riboside (**13**).

The related compound **14** was obtained as a yellow amorphous powder. The HRESIMS and NMR data (Table 4) showed that the only difference between **14** and 13*Z*-stelliferin riboside **13**[5] is the absence of an *O*-acetyl moiety at C-3, which was in accordance with the upfield shift of H-3 in **14** at δ 3.52 compared to δ 4.77 in **13**. According to the structural model described by Tsuda et al.,[15] based on the stelliferins, the carbon chemical shift at δ 21.8 for the 4β-Me (CH_3-25) is indicative of the presence of a 3α-hydroxyl group, while a value of δ 17.0 is generally associated with a 3β-hydroxyl group. In **14**, CH_3-25 was found at δ 21.8, indicative of a 3α-hydroxyl group. Thus, **14** is 3-*O*-deacetyl-13*Z*-stelliferin riboside.

The isolated compounds from the sponge *R. globostellata* were tested against different Gram-positive and Gram-negative bacteria. Globostelletin (**3**) and globostellatic acids D (**4**) and G−I (**5**−**7**) showed moderate activity toward *Escherichia coli,* exhibiting inhibition zones of 10 mm at a loading concentration of 10 μg. At a similar concentration, the gylcoside 3-OH-stelleferin riboside (**14**) showed stronger antibacterial activity toward *E. coli* (12 mm zone). For the Gram-positive bacterium *Bacillus subtilis*, only globostelletin (**3**) was found to be active, with zones of 12 and 13 mm at loading concentrations of 5 and 10 μg, respectively. As positive controls, 5 μg of streptomycin and gentamycin were used, which exhibited zones of inhibitions of 20 and 18 mm for *B. subtilis*, respectively, while toward *E. coli*, the antibiotics displayed activities of 13 and 14 mm, respectively. The isolated isomalabaricane derivatives were inactive toward the yeast *Saccharomyces cerevisiae*, and as positive control, 5 μg of nystatin was used, which showed a zone of inhibition of 12 mm.

Several isomalabaricanes have been reported to significantly inhibit the growth of L1210 and KB cancer cells.[11,17] The 14 isomalabaricane triterpenoids showed strong activity against the mouse lymphoma cell line, L5178Y. In contrast, globostellatic acids D/G (**4/5**), H/I (**6/7**), and L/M (**10/11**) and the riboside congeners **13** and **14** were weakly active against HeLa and PC-12 cells, while globostellatic acids A (**1**), F(**2**), and J/K (**8/9**) and globostelletin were inactive (Table 5).

For the activity toward the mouse lymphoma cell line (L5178Y), among the derivatives **4**−**11**, the most active one is the congener pair **6** and **7**, the 3-*O*-deacetyl, 21-*O*-ethyl derivative, with an ED_{50} of 0.31 nmol. However, acetylation of the C-3 hydroxyl group decreases its bioactivity abruptly, as in compounds **8** and **9,** with an ED_{50} of 8.28 nmol. The reverse is found for the stelleferin glycosides, as 13*Z*-stelliferin riboside (**13**) was found to be more active than its deacylated congener **14**, with ED_{50} values of 0.22 and 2.40 nmol, respectively. Thus, cytotoxicity tests of the isolated

Table 4. NMR Data of Compounds **12** and **14** in $CDCl_3$

	12	**14**			
position	δ_H	δ_H	δ_C	m	HMBC (C to H)
1	1.20 (2H, m)	1.79 (1H, m) 1.16 (1H, m)	28.8	CH_2	CH_3-27
2	1.72 (1H, m) 1.99 (1H, m)	1.69 (1H, m) 1.98 (1H, m)	26.9	CH_2	
3	4.77 (1H, bs)	3.52 (1H, bs)	75.8	CH	
4			38.1	C	CH-5
5	2.35 (1H, m)	2.30 (1H, m)	40.2	CH	CH_3-24, CH_3-27
6	1.42 (1H, m) 1.59 (1H, m)	1.47 (1H, m) 1.59 (1H, m)	18.3	CH_2	
7	2.08 (2H, m)	2.10 (2H, m)	38.5	CH_2	CH_3-26
8			44.7	C	CH-9, CH_2-11A/B, CH_3-26
9	1.83 (1H, m)	1.79 (1H, m)	50.4	CH	CH_3-26, CH_3-27
10			35.6	C	CH-5, CH_3-27
11	2.20 (2H, m)	2.20 (2H, m)	36.8	CH_2	
12			207.3	C	CH_2-11A/B
13			146.9	C	CH_3-26, CH_3-28
14			142.0	C	CH_3-28
15	6.63 (d, 15.1 Hz)	8.01 (d, 15.4 Hz)	133.2	CH	CH_3-28
16	6.86 (dd, 11.0, 15.4 Hz)	6.80 (dd, 11.0, 15.4 Hz)	129.2	CH	
17	6.19 (d, 11.0 Hz)	6.40 (d, 11.0 Hz)	130.6	CH	
18			137.9	C	CH-17, CH_3-29
19	4.19 (1H, t, 7.0 Hz)	4.18 (1H, t, 7.0 Hz)	81.9	CH	CH-20, CH_3-29
20	2.24 (1H, m) 2.43 (1H, m)	2.25 (1H, m) 2.40 (1H, m)	32.4	CH_2	
21	5.09 (1H, t, 7.0 Hz)	5.04 (1H, t, 7.0 Hz)	119.4	CH	
22			134.3	C	
23	1.72 (3H, s)	1.69 (3H, s)	25.7	CH_3	CH_3-30
24	0.89 (3H, s)	0.97 (3H, s)	27.8	CH_3	CH_3-25
25	0.93 (3H, s)	0.89 (3H, s)	21.8	CH_3	CH_3-24
26	1.45 (3H, s)	1.40 (3H, s)	24.3	CH_3	
27	1.02 (3H, s)	1.01 (1H, s)	22.5	CH_3	CH-5
28	2.33 (3H, s)	2.02 (3H, s)	16.0	CH_3	
29	1.82 (3H, s)	1.76 (3H, s)	11.8	CH_3	
30	1.64 (3H, s)	1.61 (3H, s)	17.9	CH_3	CH_3-23
$COCH_3$	2.09 (3H, s)				
1′	4.51 (d, 5.0 Hz)	4.41 (d, 5.0 Hz)	97.9	CH	
2′	3.57 (1H, dd, 5.0, 6.0 Hz)	3.54 (1H, dd, 5.0, 6.0 Hz)	71.2	CH	
3′	3.69 (1H, t, 6.0 Hz)	3.63 (1H, t, 6.0 Hz)	72.8	CH	
4′	3.78 (1 H, bm)	3.75 (1 H, bm)	69.7	CH	
5′	3.46 (dd, 6.3, 12.3 Hz) 4.10 (dd, 3.2. 12.3 Hz)	3.40 (dd, 6.3, 12.3 Hz) 4.06 (dd, 3.5, 12.3 Hz)	63.0	CH	

Table 5. Cytotoxicity Data of Isolated Isomalabaricane Derivatives toward L5178Y, HeLa, and PC-12 Cell Lines

	L5178Y % of inhibition			HeLa % of inhibition			PC-12 % of inhibition		
compound	10 μg/mL	3 μg/mL	ED_{50}, nmol	10 μg/mL	3 μg/mL	ED_{50}, nmol	10 μg/mL	3 μg/mL	ED_{50}, nmol
globostellatic acid A (**1**)	100	100	n.t.[a]	0	0	i.a.[b]	0	0	i.a.
globostellatic acid F (**2**)	100	42	10.36	0	0	i.a.	0	0	i.a.
globostelletin (**3**)	94	66	5.34	0	0	>60	0	0	i.a.
globostellatic acid D, G (**4**, **5**)	100	100	0.39	25	4	46.69	42	18	30.33
globostellatic acd H, I (**6**, **7**)	100	100	0.31	23	8	46.05	41	16	28.07
globostellatic acid J, K (**8**, **9**)	100	24	8.28	0	0	i.a	0	0	i.a.
globostellatic acid L, M (**10**, **11**)	100	100	0.92	42	0	27.87	30	0	27.52
stelliferin riboside (**13**)	100	100	0.22	56	0	22.76	38	0	21.54
3-*O*-deacetyl-13,14-*Z*-stelliferin riboside (**14**)	100	100	2.40	100	26	8.14	54	33	27.63

[a] Compound was not further tested (n.t.) to determine the ED_{50} due to sample unavailability. [b] i.a. = inactive; no inhibition observed at 30 μg/mL.

isomalabaricane triterpenes from the sponge *R. globostellata* against different cancer cells showed that all compounds have a strong and specific activity against mouse lymphoma cells. On the other hand, these compounds showed only moderate or even no activity against either human cervix carcinoma (HeLa) or rat pheochromocytoma (PC-12) cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer Model 341 LC polarimeter. ^{1}H NMR and ^{13}C NMR experiments were performed on a Bruker Unity 500 and 125 MHz, respectively, spectrometer using either CD_3OD or $CDCl_3$ as solvent. ESI mass spectra were obtained on a ThermoFinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped with a photodiode array detector. HRESIMS were determined on a Micromass Q-Tof 2 mass spectrometer. Semipreparative HPLC separations were performed on a LaChrom-Merck Hitach, pump L-7100, UV detector L-7400 using a C-18 column (Eurospher 100, 8 mm length, flow rate 5.0 mL/min, UV detection at 340 nm). Column chromatography was performed on silica gel (0.040−0.063 mm; Merck, Darmstadt, Germany) or by flash chromatography (Biotage). For HPLC analysis, samples were injected into an HPLC system equipped with a

photodiode array detector (Dionex, München, Germany). Routine detection was at 235 and 330 nm. The separation column (125 × 4 mm i.d.) was prefilled with Eurospher 100-C18, 5 μm (Knauer, Berlin, Germany). Separation was achieved by applying a linear gradient from 90% H_2O (pH 2.0) to 100% MeOH over 40 min. TLC analysis was carried out using aluminum sheet precoated silica gel 60 F_{254} (Merck, Darmstadt, Germany).

Animal Material. The sponge *R. globostellata* Carter (phylum Porifera, class Demospongiae, order Astrophorida, family Acorinidae) was collected in 1997 from Kapoposang Island, Indonesia, at a depth of 45 ft. It is shaped like a handball, hard, and compressible. Its external surface is red-brown with characteristic warty surface and rounded protrusions in which the oscules are concentrated. The interior has a striking yellow color, which sticks to the skin when touched. The consistency is hard, but easy to cut. Megasclere spicules include orthotriaenes of up to 800 × 30 pm, cladome up to 280 pm, cladium up to 130 × 20 pm, and oxeas of up to 1100 × 20 pm. The microscleres include diverse types of asters, large spherasters (megasters) of 20−55 pm with thick conical rays, smaller multirayed spherasters of 10−15 pm, oxyasters/tylasters with thin rugose rays, and trichodragmas of up to 100 × 5−12 pm, often a bit wavy in outline. The skeleton radiates, with a thick crust of spherasters of 150−300 pm thick, carried by a cortical skeleton of orthotriaenes mixed with oxeas. The choanosomal skeleton is a pulpy mass of oxeas with numerous asters. The combination of characters conforms to descriptions of *R. globostellata.* The subgenus *Rhabdastrella* is distinguished from most other *Stelletta* by the possession of megasters; all species apparently have the typical yellow choanosome. A voucher specimen has been deposited in the Zoological Museum, Amsterdam, under registration number ZMA POR. 17166. The sponge sample was immediately immersed in EtOH after collection and transported to the University of Düsseldorf, Germany, for further isolation work.

Extraction and Isolation. The sponge was extracted with MeOH and then with acetone, and the solvent was removed under reduced pressure. The crude extract was partitioned between H_2O and EtOAc. The EtOAc layer was then subjected to VLC and eluted using a gradient system from 100% *n*-hexane to 100% EtOAc, then 50% MeOH in EtOAc, and finally with 100% MeOH. Each fraction was purified by normal-phase flash chromatography (CH_2Cl_2−MeOH, 95:5 or 9:1) followed by semipreparative reversed-phase HPLC (C_{18} Eurospher 100) using the appropriate ratios of MeOH:H_2O to afford 3.6 mg of compound **1**, 3.2 mg of compound **2**, 2.1 mg of compound **3**, 2.8 mg of compounds **4** and **5**, 1.3 mg of compounds **6** and **7**, 1.6 mg of compounds **8** and **9**, 1.1 mg of compounds **10** and **11**, 1.3 mg of compound **12**, 7.5 mg of compound **13**, and 8.6 mg of compound **14**. For derivatives **4**−**11**, which were isolated as *E*/*Z* mixtures, no further attempts were made to separate the isomers due to their very small yield.

Globostellatic acid F (2): yellow amorphous powder; $[\alpha]^{20}_D$ −19 (*c* 0.182, MeOH); UV λ_{max} (MeOH) 234 and 329 nm; ^{1}H and ^{13}C NMR in $CDCl_3$, see Table 1; HRESIMS *m*/*z* 499.3063 [M + H]$^+$, 100 (calc for $C_{30}H_{43}O_6$, 499.3061).

Globostelletin (3): yellow amorphous powder; $[\alpha]^{20}_D$ +65 (*c* 0.052, MeOH); UV λ_{max} (MeOH) 231 and 329 nm; ^{1}H and ^{13}C NMR in $CDCl_3$, see Table 1; HRESIMS *m*/*z* 469.3290 [M + H]$^+$, 100 (calc for $C_{30}H_{45}O_4$, 469.3320).

Globostellatic acids D (4) and G (5): yellow amorphous powder; $[\alpha]^{20}_D$ was not recorded since the compounds were isolated as an *E*:*Z* mixture (1:1); UV λ_{max} (MeOH) 258 and 371 nm; ^{1}H NMR in $CDCl_3$, see Table 2; ^{13}C NMR in $CDCl_3$, see Table 3; HRESIMS *m*/*z* 515.3351 [M + H]$^+$, 100 (calc for $C_{31}H_{47}O_6$, 515.3367).

Globostellatic acids H (6) and I (7): yellow amorphous powder; $[\alpha]^{20}_D$ was not recorded since the compounds were isolated as an *E*:*Z* mixture (2:1); UV λ_{max} (MeOH) 258 and 370 nm; ^{1}H NMR in $CDCl_3$, see Table 2; ^{13}C NMR in $CDCl_3$, see Table 3; HRESIMS *m*/*z* 551.3357 [M + Na]$^+$, 100 (calc for $C_{32}H_{48}O_6Na$, 551.3349).

Globostellatic acids J (8) and K (9): yellow amorphous powder; $[\alpha]^{20}_D$ was not recorded since the compounds were isolated as an *E*:*Z* mixture (2:1); UV λ_{max} (MeOH) 258 and 370 nm; ^{1}H NMR in $CDCl_3$, see Table 2; HRESIMS *m*/*z* 593.3450 [M + Na]$^+$ 100 (calc for $C_{34}H_{50}O_7Na$, 593.3449).

Globostellatic acids L (10) and M (11): yellow amorphous powder; $[\alpha]^{20}_D$ was not recorded since the compounds were isolated as an *E*:*Z* mixture (2:1); UV λ_{max} (MeOH) 257 and 373 nm; ^{1}H NMR in CD_3OD, see Table 2; ^{13}C NMR in CD_3OD, see Table 3; HRESIMS *m*/*z* 523.3063 [M + Na]$^+$, 100 (calc for $C_{30}H_{44}O_6Na$, 523.3036).

13*E*-Stelliferin riboside (12): yellow amorphous powder; $[\alpha]^{20}_D$ +150 (*c* 0.019, MeOH); UV λ_{max} (MeOH) 258 and 344 nm; ^{1}H NMR in $CDCl_3$, see Table 4; HRESIMS *m*/*z* 651.3865 [M + Na]$^+$ 100 (calc for $C_{37}H_{56}O_8Na$, 651.3867).

3-*O*-Deacetyl-13*Z*-stelliferin riboside (14): yellow amorphous powder; $[\alpha]^{20}_D$ −42 (*c* 0.2, MeOH); UV λ_{max} (MeOH) 234 and 344 nm; ^{1}H and ^{13}C NMR in $CDCl_3$, see Table 4; HRESIMS *m*/*z* 609.3777 [M + Na]$^+$ 100 (calc for $C_{35}H_{54}O_7Na$, 609.3770).

Cytotoxicity Assay. The cytotoxicity against L5178Y mouse lymphoma cells, HeLa human cervix carcinoma cells, and PC-12 rat pheochromocytoma cells was determined using the microculture tetrazolium (MTT) assay and compared to that of untreated controls.[19] Of the test samples, stock solutions in EtOH 96% (v/v) were prepared. Exponentially growing cells were harvested, counted, and diluted appropriately. Of the cell suspension, 50 μL containing 3750 cells was pipetted into 96-well microtiter plates. Subsequently, 50 μL of a solution of the test samples containing the appropriate concentration was added to each well. The concentration range was 3−10 μg/mL. The small amount of EtOH present in the wells did not affect the experiments. The test plates were incubated at 37 °C with 5% CO_2 for 72 h. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at 5 mg/mL in phosphate-buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4), and from this solution, 20 μL was pipetted into each well. The yellow MTT penetrates the healthy living cells, and in the presence of mitochondrial dehydrogenases, MTT is transformed to its blue formazan complex. After an incubation period of 3 h 45 min at 37 °C in a humidified incubator with 5% CO_2, the medium was centrifuged (15 min, 20 °C, 210*g*) with 200 μL of DMSO, and the cells were lysed to liberate the formed formazan product. After thorough mixing, the absorbance was measured at 520 nm using a scanning microtiter-well spectrophotometer. The color intensity is correlated with the number of healthy living cells.

Cell survival was calculated using the formula

$$\text{survival (\%)} = \frac{100 \times (\text{absorbance of treated cells} - \text{absorbance of culture medium})}{(\text{absorbance of untreated cells} - \text{absorbance of culture medium})}$$

All experiments were carried out in triplicate and repeated three times. As controls, media with 0.1% EGMME/DMSO were included in the experiments.

Agar Diffusion Assays. Susceptibility disks (5 mm in diameter) were impregnated with 10 μg of the isolated compounds dissolved in MeOH and placed on LB agar plates inoculated with the test bacteria *B. subtilis* DSM2109 and *E.* coli DSM10290. The plates were observed for zones of inhibition[21] after 24 h of incubation at 37 °C. The compounds were also assayed using *S. cerevisiae* as test organism, which was inoculated on YPD agar plates, and zones of inhibition were recorded after 24 h of incubation at 27 °C. In all cases, controls containing only the respective amount of solvent showed no zones of inhibition.

Acknowledgment. M.F. wishes to acknowledge the Egyptian Government for a scholarship. The authors are grateful to Dr. R. van Soest, Zoological Museum, Amsterdam, for the taxonomical identification of the sponge, and Dr. M. Nimtz, GBF, Braunschweig, for the HREIMS measurements. We would also like to acknowledge Prof. Dr. S. Wiryowidagdo of UI, Jakarta, for the collection of the sponge samples.

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NP050346T