Asterosaponins Isolated from the Starfish *Asterias amurensis*

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Three new asterosaponins 1—3 and four known saponins 4—7 have been isolated from the starfish *Asterias amurensis* Lütken. By means of high magnetic field 1D- and 2D-NMR (^{1}H–^{1}H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY)) and MS analyses, the chemical structures of new compounds were determined to be 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-galactopyranosyl]-5α-chol-9(11)-en-23-one-3β-yl sodium sulfate (1), 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-galactopyranosyl]-5α-cholesta-9(11),24-dien-23-one-3β-yl sodium sulfate (2), and 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-galactopyranosyl]-5α-cholest-9(11)-en-23-one-3β-yl sodium sulfate (3). In addition, the NMR data for known saponins 4—7 were completely assigned by extensive 2D-NMR analysis without chemical degradation.

Key words asterosaponin; *Asterias amurensis*; NMR assignment

In the globalized trading system, invasion of exotic species has become a major concern in the marine environment as the number of human-mediated introductions has increased.[1—3] The *Asterias amurensis* Lütken, commonly called the northern Pacific starfish, originally comes from northeast Asia including Korea, China, Japan, and Russia where it causes considerable damage to commercial shellfishes.[4—6] In the early 1990s, *A. amurensis* was discovered as an invasive species in Australia and, as voracious predators, remain a major threat to benthic marine ecosystem and commercial fisheries in east and southeast Tasmania where the species is conspicuously dispersed.[5,6] In addition, a recent study demonstrated that *A. amurensis* attacks native assemblages that support populations of large surface bivalves, particularly those that live on or just under the sediment surface.[7] Because it damages the marine ecosystem and the fishing industry, interest in identifying profitable secondary metabolites from the species has greatly increased.

The steroidal saponins of starfish have been reported to have various bioactivities including cytotoxic, hemolytic, antiviral, antibacterial, anti-inflammatory, and antifungal activities.[8—12] The starfish saponins can be categorized into three main groups: asterosaponins possessing a sulfated steroidal moiety; steroidal cyclic glycosides, which to date have only been found in two species of the genus *Echinaster*; and polyhydroxysteroidal saponins.[8] Due to the widespread interest in their biological and structural significance, we have undertaken structural studies on the saponins of *A. amurensis*. Our recent investigation on the *n*-BuOH soluble fraction of *A. amurensis* resulted in the isolation of three new asterosaponins **1**—**3**, and four known saponins, thornasteroside A (**4**),[12—18] versicoside A (**5**),[18,19] anasteroside B (**6**),[11] and asteronyl pentaglycoside sulfate (**7**).[15,20] Although the chemical structures of asterosaponins **4**—**7** have been already determined using hydrolyzed molecules, there are few reports on the full NMR assignment for undegraded saponins. Indeed, only partial ^{1}H-NMR data for distinctive methyl protons and the ^{13}C-NMR data for hydrolyzed aglycone and sugars have been published to date.[16,18] By utilizing a high magnetic field (600 MHz) 2D-NMR technique including ^{1}H–^{1}H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY), we could elucidate the structures and complete the NMR assignments for complex saponins without chemical degradation. In this paper, we report on the isolation and structure determination of new asterosaponins **1**—**3** and known saponins **4**—**7** isolated from *A. amurensis*.

Results and Discussion

Compound **1**, a white amorphous powder, showed a pseudo-molecular ion peak at *m/z* 1261.4910 $[M+Na]^+$ in the high resolution (HR)-FAB-MS, consistent with the molecular formula $C_{54}H_{87}NaO_{28}S$. The ^{1}H-NMR spectrum of **1**, in pyridine-d_5, displayed signals of a typical saponin, where the signals for one olefinic proton at δ_H 5.17 (H-11), two oxygenated methine protons at δ_H 4.91 (H-3) and 3.82 (H-6), one acetyl group at δ_H 2.08 (H-24), two tertiary methyl groups at δ_H 0.94 (H-19) and 0.56 (H-18), and one secondary methyl group at δ_H 0.92 (H-21) were observed as steroidal aglycone. The signals for five anomeric protons at δ_H 5.24 (1H, d, J=6.5 Hz), 5.06 (1H, d, J=7.7 Hz), 4.96 (1H, d, J=7.7 Hz), 4.92 (2H, br d, J=8.2 Hz), and three methyl groups as doublets at δ_H 1.77 (3H, d, J=6.0 Hz), 1.73 (3H, d, J=5.9 Hz), and 1.49 (3H, d, J=6.2 Hz) were distinguished as oligosaccharide moiety. Unlike most asterosaponins with a cholestane skeleton, the structure of the steroid moiety of **1** was identified as the cholane skeleton of 3β,6α-dioxy-

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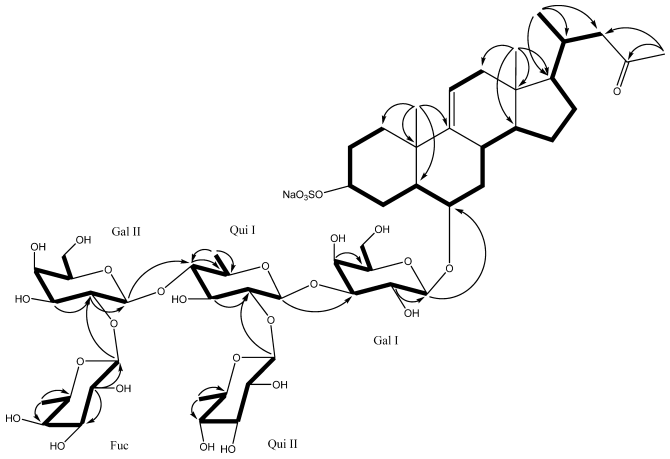

Fig. 1. ^{1}H–^{1}H COSY (——) and Key HMBC (⟶) Correlations of **1**

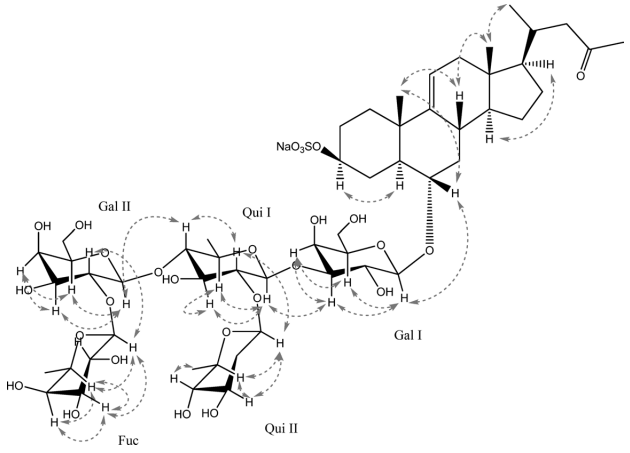

Fig. 2. Selected NOE (◂⋯▸) Correlations of **1**

genated cholen-23-one by 2D-NMR analyses.[21] The HMBC correlations from H-24 (δ_H 2.08) to C-23 (δ_C 208.24) and C-22 (δ_C 50.63), and from H-21 (δ_H 0.92) to C-22 (δ_C 50.63) indicated the cholane skeleton (Fig. 1). In addition, the characteristic carbon signals at δ_C 145.68 (C-9) and 116.48 (C-11) revealed the chol-9(11)-en-23-one skeleton.[11,14,21] The stereochemistry of 3α-H (3β-sulfate) was determined on the basis of the chemical shift at δ_H 4.91 ($W_{1/2}$=20 Hz)[14,16] (*vs.* δ_H 5.31, $W_{1/2}$=7.5 Hz for 3β-H)[22] and the nuclear Overhauser effect (NOE) correlation between H-3 and H-5 (Fig. 2). As shown in Figs. 1 and 2, comprehensive interpretation of the ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC, NOESY, and HR-FAB-MS data suggested that the steroidal aglycone of **1** should be 5α-chol-9(11)-en-23-one-3β,6α-diol 3-sulfate. Although some of the anomeric protons overlapped with the water peak from the solvent, they could clearly be assigned using the HMQC data,[23] where the five anomeric protons at δ_H 5.24 (1H, d, *J*=6.5 Hz), 5.06 (1H, d, *J*=7.7 Hz), 4.96 (1H, d, *J*=7.7 Hz), and 4.92 (2H, br d, *J*=8.2 Hz) correlated with the carbon signals at δ_C 104.95, 107.10, 102.98, 105.45, and 103.91, respectively. The structure of each sugar residue was identified by virtue of the ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC, and NOESY data, which resulted in the identification of two galactose, two quinovose, and one fucose (Table 1). The HMBC and NOE correlations between δ_H 4.92 (GalI-1′) and δ_C 80.97 (C-6)/δ_H 3.82 (H-6) revealed an ether linkage between GalI-1′ and C-6 (Figs. 1, 2). The sequence of oligosaccharide moiety was confirmed by analyses of HMBC and NOESY data. The HMBC correlations from δ_H 4.92 (QuiI-1″) to δ_C 91.69 (GalI-3′) indicated that QuiI-1″ should be linked to GalI-3′ (Fig. 1), which was further confirmed by a NOESY correlation between δ_H 4.92 (QuiI-1″) and δ_H 3.85 (GalI-3′) (Fig. 2). Another HMBC correlation from δ_H 5.24 (QuiII-1‴) to δ_C 82.26 (QuiI-2″) as well as the NOE correlation between δ_H 5.24 (QuiII-1‴) and δ_H 4.07 (QuiI-2″) proved that QuiII-1‴ was linked to QuiI-2″. Through the HMBC correlation between δ_H 4.96 (GalII-1⁗) and δ_C 86.50 (QuiI-4″), and the NOESY cross-peak between δ_H 4.96 (GalII-1⁗) and δ_H 3.58 (QuiI-4″), a (1→4) linkage from GalII to QuiI was identified. Finally, a (1→2) linkage from Fuc to GalII was deduced not only from the HMBC correlation of δ_H 5.06 (Fuc-1⁗) with δ_C 84.37 (GalII-2⁗) but also from the NOESY correlation of δ_H 5.06 (Fuc-1⁗) with δ_H 4.07 (GalII-2⁗) (Figs. 1, 2). The coupling constant values of anomeric protons of GalI (*J*=8.2 Hz) and GalII (*J*=7.7 Hz), QuiI (*J*=8.2 Hz) and QuiII (*J*=6.5 Hz), and Fuc (*J*=7.7 Hz) suggested β-orientations for the sugars, corresponding with the analysis of NOESY data. The absolute configuration of the sugar residues was found to be D-galactose, D-quinovose, and D-fucose, respectively, by GC analysis of their trimethylsilylated L-cysteine methyl ester derivatives. Consequently, the structure of **1** was determined to be 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-galactopyranosyl]-5α-chol-9(11)-en-23-one-3β-yl sodium sulfate. Compounds **2** and **3** were obtained as a mixture (1 : 1). The ratio 1 : 1 was deduced from the integral analysis on the distinctive carbon signals (*e.g.* C-21 and C-23) in the ^{13}C-NMR data. They were recognized as two molecules, the molecular weights of which differed by 2 mass units in the positive ion mode electrospray ionization (ESI)-MS; the pseudo-molecular ion peaks at *m/z* 1279 $[M+H]^+$ and *m/z* 1301 $[M+Na]^+$ were assigned to compound **2**, while the pseudo-molecular ion peaks at *m/z* 1281 $[M+H]^+$ and *m/z* 1303 $[M+Na]^+$ were assigned to compound **3**. The pattern of mass fragmentation in the MS/MS data further supported the assignment of molecular ions to compounds **2** and **3**.[23] The HR-FAB-MS data showed the pseudo-molecular ion peaks at *m/z* 1301.5204 $[M+Na]^+$ and at *m/z* 1303.5361 $[M+Na]^+$, corresponding to molecular formulas of $C_{57}H_{91}NaO_{28}S$ (**2**) and $C_{57}H_{93}NaO_{28}S$ (**3**). The overall ^{1}H- and ^{13}C-NMR data suggested that the mixture of **2** and **3** is a typical steroidal glycoside. Detailed analysis of 2D-NMR data enabled us to identify that the steroidal side chains differed. The side chain for **2** was confirmed by HMBC data displaying the correlations from an olefinic proton at δ_H 6.14 (H-24) to a ketone signal at δ_C 202.39 (C-23), and to both the methyl carbons at δ_C 20.63 (C-26), and 27.40 (C-27). Moreover, the HMBC correlations from a doublet signal at δ_H 0.93 (H-21) to δ_C 56.36 (C-17) and δ_C 51.44 (C-22) as well as from the signals at δ_H 2.13/2.46 (H-22) to δ_C 202.39 (C-23), implied a 3β,6α-dioxygenated cholesta-9(11),24-dien-23-one moiety (Fig. 3). The proton signal of H-3 at δ_H 4.77 ($W_{1/2}$=22 Hz) as well as the NOE correlation between H-3 and H-5 suggested the α-configuration of H-3 (alternative 3β-sulfate).[14,16,22] On the basis of the difference of 2

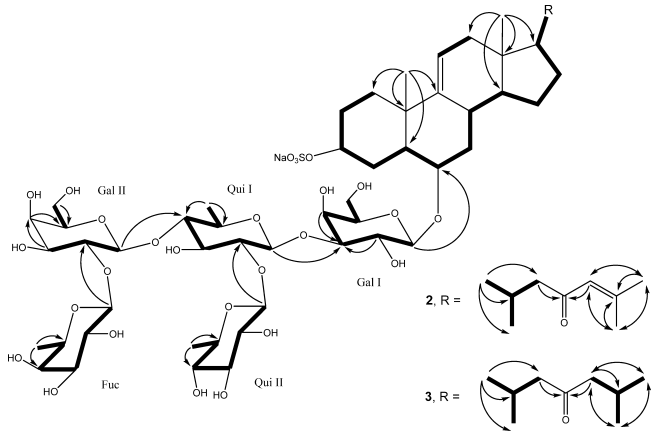

Fig. 3. ^{1}H–^{1}H COSY (——) and Key HMBC (⟶) Correlations of **2** and **3**

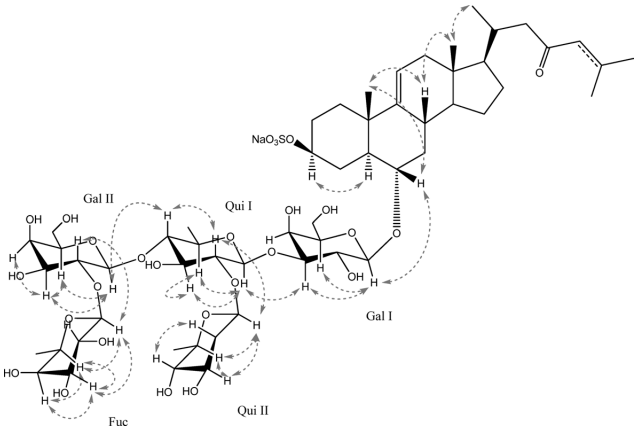

Fig. 4. Selected NOE (◂----▸) Correlations of **2** and **3**

mass units, the structure of **3** was easily estimated by saturation of one of the double bonds in **2**. A 3β,6α-dioxygenated cholest-9(11)-en-23-one skeleton for **3** was determined using the HMBC data, in which the correlations from a distinctive doublet at δ_H 2.29 (H-24, J=7.2 Hz) to a ketone at δ_C 212.63 (C-23) and two methyl carbon signals at δ_C 22.57 (C-26), 22.45 (C-27) were observed. In addition, the HMBC correlations from a doublet at δ_H 0.91 (H-21) to δ_C 56.06 (C-17) and δ_C 50.25 (C-22) as well as from δ_H 2.18, 2.42 (H-22) to δ_C 212.63 (C-23) explained the side chain difference between **2** and **3** (Fig. 3). Based on the interpretation of ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC, NOESY, and HR-FAB-MS data, the aglycones of **2** and **3** were completely elucidated as a 5α-cholesta-9(11),24-dien-23-one-3β,6α-diol 3-sulfate and a 5α-cholest-9(11)-en-23-one-3β,6α-diol 3-sulfate, respectively (Figs. 3, 4). The sugar moieties of **2** and **3** were identical based on the analyses of 2D-NMR data (^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC, and NOESY), and GC, and included two β-D-galactose, two β-D-quinovose, and one β-D-fucose (Table 1). The oligosaccharide connected to C-6 was verified by the HMBC (Fig. 3) and NOESY (Fig. 4) cross-peaks, where correlations from δ_H 4.92 (GalI-1′) to δ_C 79.98 (C-6), and between δ_H 4.92 (GalI-1′) and δ_H 3.86 (H-6) were shown. The interglycosidic links from QuiI-1″ to GalI-3′, and from QuiII-1‴ to QuiI-2″ were deduced from the HMBC and NOESY data, where cross-peaks between δ_H 4.96 (QuiI-1″) and δ_C 90.35 (GalI-3′)/δ_H 3.90 (GalI-3′), and between δ_H 4.97 (QuiII-1‴) and δ_C 83.67 (QuiI-2″)/δ_H 3.88 (QuiI-2″), respectively, were observed. The GalII-1⁗ tie to QuiI-4″ was verified by the HMBC correlation from δ_H 4.87 (GalII-1‴) to δ_C 84.64 (QuiI-4″), and the NOE correlation between δ_H 4.87 (GalII-1⁗) and δ_H 3.64 (QuiI-4″). The last connection, Fuc-1⁗→GalII-2⁗, was confirmed by the HMBC and NOESY cross-peaks of δ_H 4.95 (Fuc-1⁗)/δ_C 83.10 (GalII-2⁗), and δ_H 4.95 (Fuc-1⁗)/δ_H 3.95 (GalII-2⁗). As a result, **2** and **3** were elucidated as 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-galactopyranosyl]-5α-cholesta-9(11),24-dien-23-one-3β-yl sodium sulfate (**2**), and 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-galactopyranosyl]-5α-cholest-9(11)-en-23-one-3β-yl sodium sulfate (**3**), respectively.

Compound **4**, a white amorphous powder, displayed a pseudo-molecular ion at m/z 1289.5 $[M+Na]^+$ in the matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)-MS spectrum. The ^{1}H- and ^{13}C-NMR data of **4** revealed a steroidal glycoside. Through the analysis of 2D-NMR data, the aglycone structure of **4** was identified as a 5α-cholest-9(11)-en-23-one-3β,6α,20-triol 3-sulfate.[12—19] Glycosilation at C-6 and other interglycosidic linkages were confirmed by the following HMBC and NOESY correlations: δ_H 4.83 (QuiI-1′)/δ_C 80.86 (C-6) and δ_H 4.83 (QuiI-1′)/δ_H 3.81 (H-6), δ_H 5.05 (Xyl-1″)/δ_C 90.53 (QuiI-3′) and δ_H 5.05 (Xyl-1″)/δ_H 3.83 (QuiI-3′), δ_H 5.34 (QuiII-1‴)/δ_H 4.13 (Xyl-2″), δ_H 5.00 (Gal-1⁗)/δ_C 79.72 (Xyl-4″) and δ_H 5.00 (Gal-1⁗)/δ_H 4.23 (Xyl-4″), δ_H 4.86 (Fuc-1⁗⁗)/δ_C 84.05 (Gal-2⁗) and δ_H 4.86 (Fuc-1⁗⁗)/δ_H 4.49 (Gal-2⁗). The stereochemistry of C-20 was suggested to be 20*S* (α) by comparison of the chemical shift of H-21 (δ_H 1.61) with that in the literature (δ_H 1.56).[24] Considering all the NMR and MS data, compound **4** was identified as thornasteroside A,[12—18] (20*S*)-6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-20-hydroxy-23-one-5α-cholest-9(11)-en-3β-yl sodium sulfate. Even though there are reports on the partial NMR assignment for thornasteroside A, some proton and carbon chemical shifts are quite different from our findings since the previous study was performed after hydrolysis. The chemical shift value of H-6 was assigned to δ_H 5.37,[16] which turned out to be δ_H 3.81 in our study. In addition, the chemical shift values of Gal-2⁗ and Fuc-1⁗⁗ were reported to be δ_C 82.5 and 105.5,[18] respectively, which were assigned to δ_C 84.05 and 107.76, respectively, in our study.

Compound **5** was isolated as a white amorphous powder. The molecular formula was assigned as $C_{62}H_{101}NaO_{33}S$ using the MALDI-TOF-MS data showing an pseudo-molecular ion at m/z 1451.6 $[M+Na]^+$. The difference of 162 mass units in the molecular weight and the appearance of an additional anomeric proton at δ_H 5.09 (1H, d, J=7.9 Hz) implied the addition of one hexosyl moiety in **5** compared to **4**. The structure determination of sugars was accomplished by ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC, and NOE data without hydrolysis. The oligosaccharidic linkage of **5** was almost the same as that of **4** except for the additional GalII, proved by the HMBC correlation from δ_H 5.09 (GalII-1) to δ_C

Table 1. NMR Data of Compound **1** (Pyridine-d_5) and Mixture of **2** and **3** (Pyridine-d_5/D_2O)

	1				Mixture of **2** and **3**	
	δ_C [a]	δ_H, mult. (J in Hz) [b]	HMBC	NOE	δ_C [a]	δ_H, mult. (J in Hz) [b]
1	36.02	1.38, m; 1.62, m			35.79	1.29, m; 1.53, m
2	29.59	1.89, m; 2.81, m			29.03	1.80, m[c]; 2.64, br s
3	77.61	4.91[c]		H-5	78.11	4.77, m
4	30.01	1.72[c]; 3.51, br d (12.7)			30.53	1.72, m; 3.20, br d (10.8)
5	49.41	1.51, m		H-3	48.84	1.45, m[c]
6	80.97	3.82, m		H-1′, 19	79.98	3.86, m[c]
7	41.76	1.24, m; 2.71, m			40.90	1.18, m; 2.64, br s
8	35.60	2.0, m		H-11, 18, 19	35.46	1.96, m
9	145.68	—			145.58	—
10	38.36	—			38.17	—
11	116.48	5.17[d]		H-8, 19	116.41	5.07, br s
12	41.76	1.86, m; 2.01, m			41.65	1.84, m[c]; 1.96, m
13	41.16	—			41.05	—
14	53.75	1.11, m		H-17	53.62	1.07, m
15	25.28	1.01, m; 1.54, m			25.16	1.04, m; 1.66, m
16	28.55	1.14, m; 1.62, m			28.52, 28.58[e]	1.17, m; 1.66, m
17	56.23	1.03, m		H-14	56.36, 56.06	1.04, m
18	11.67	0.56, s	C-12, 13, 14, 17	H-8, 21	11.60, 11.62[e]	0.48, s
19	19.55	0.94, s[c]	C-1, 5, 9, 10	H-6, 8, 11	19.21	0.83, s
20	32.57	2.05, m			33.20, 32.44	2.02, m
21	19.32	0.92, d[c]	C-17, 20, 22	H-18	19.46, 19.51	0.93, d 0.91, d
22	50.63	2.13, m[c]; 2.43, br d (14.8)			51.44 50.25	2,13, m[c]; 2.46, d (14.4) 2.18, m ; 2.42, d (18.0)
23	208.24	—			202.39, 212.63	—
24	30.48	2.08, s	C-22, 23		124.70, 52.40	6.14, s 2.29, d (7.2)
25					155.55, 24.61	— 2.11, m
26					20.63, 22.57	2.13, s 0.87, d (6.0)
27					27.40, 22.45	1.80, s 0.85, d (6.0)
GalI-1′	105.45	4.92, d (8.2)[c]	C-6	H-6, 3′, 5′	103.82	4.92, d (7.2)
2′	73.79	3.97, m	C-1′		73.63	3.89, m
3′	91.69	3.85, m		H-1′, 4′, 1″	90.35	3.90, m
4′	69.75	4.07, m	C-5′	H-3′, 5′	69.18	3.91, m
5′	77.61	3.86, m		H-1′, 4′	76.93	3.86, m
6′	62.35	4.31 dd (12, 5.7); 4.48, d (11.2)			61.81	4.36, m; 4.21, br d (12.0)
QuiI-1″	103.91	4.92, d (8.2)[c]	C-3′	H-3′, 3″, 5″	103.22	4.96, d (6.6)
2″	82.26	4.07, m		H-1‴, 4″	83.67	3.88, m
3″	75.70	4.11, m	C-2″	H-1″, 5″	74.85	4.12, t (9.0)
4″	86.50	3.58, t (8.7)	C-5″	H-2″, 1‴	84.64	3.64, m
5″	71.61	3.85, m		H-1″, 3″	71.58	3.88, m
6″	18.44	1.73, d (5.9)	C-4″, 5″		18.07	1.64, d (6.0)
QuiII-1‴	104.95	5.24, d (6.5)	C-2″	H-2″, 3‴, 5‴	105.71	4.97, d (7.8)
2‴	76.33	4.07, m			75.80	3.91, m
3‴	76.69	4.07, m		H-1‴, 5‴	76.34	4.01, t (9.3)[c]
4‴	75.54	4.07, m		H-6‴	75.39	3.77, t (9.6)
5‴	73.50	3.62, m[c]		H-1‴, 3‴	73.75	3.61, m
6‴	17.90	1.77, d (6.0)	C-4‴, 5‴	H-4‴	18.02	1.76, d (6.0)
GalII-1⁗	102.98	4.96, d (7.7)	C-4″	H-4″, 3⁗, 5⁗	102.13	4.87, d (7.8)
2⁗	84.37	4.07, m	C-1⁗	H-1⁗	83.10	3.95, m
3⁗	78.27	4.26, t (9.0)	C-2⁗	H-1⁗	77.37	4.17, t (8.7)
4⁗	71.30	4.13, t (9.5)[c]			70.70	3.90, m
5⁗	78.21	3.97, m		H-1⁗	77.57	3.90, m
6⁗	62.26	4.21, dd (12.6, 6.2); 4.54, d (10.9)			61.81	4.03, m; 4.40, br d (11.4)
Fuc-1⁗′	107.10	5.06, d (7.7)	C-2⁗	H-2⁗, 3⁗′, 5⁗′	105.93	4.95, d (8.4)
2⁗′	73.87	4.43, t (8.5)	C-1⁗′, 3⁗′		73.13	4.26, t (8.7)
3⁗′	75.14	4.07, m		H-1⁗′, 4⁗′, 5⁗′	74.32	4.01, t (9.3)[c]
4⁗′	72.58	3.99, m		H-3⁗′, 5⁗′	72.10	3.95, m
5⁗′	71.93	3.76, d (6.2)[c]		H-1⁗′, 3⁗′, 4⁗′	71.67	3.68, br d (6.6)
6⁗′	17.19	1.49, d (6.2)[c]	C-4⁗′, 5⁗′		16.83	1.42, d (6.6)

a) Assignment based on HMQC and HMBC NMR data (150 MHz). *b*) Assignment based on COSY, TOCSY, and HMBC NMR data (600 MHz). *c*) Signals partially overlapped. *d*) Signals overlapped with solvent. *e*) Assignments may be interchanged.

83.68 (Fuc-3⁗′), as well as the NOESY (and rotating frame Overhauser enhancement spectroscopy (ROESY)) cross peak between δ_H 5.09 (GalII-1) and δ_H 4.00 (Fuc-3⁗′). The 20*S* (α) configuration was determined by comparison of the chemical shift of H-21 (δ_H 1.57) with that in the literature (δ_H 1.56).[24] Consequently, the structure of **5** was recognized

as versicoside A,[18,19] (20*S*)-6α-*O*-[β-D-galactopyranosyl-(1→3)-β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-20-hydroxy-23-one-5α-cholest-9(11)-en-3β-yl sodium sulfate.

Compound **6** was obtained as a white amorphous powder. The pseudo-molecular ion at *m/z* 1351.4860 $[M+Na]^+$ in the HR-FAB-MS spectrum matched the molecular formula $C_{56}H_{89}NaO_{32}S$. Comparing the NMR data of **6** to those of **1**, the downfield shifted proton signals at δ_H 2.37 (H-21) and 0.61 (H-18), and the downfield shifted carbon signals at δ_C 213.20 (C-20) and 65.10 (C-17) in **6** were different from those in **1**. Furthermore, a secondary methyl group at δ_H 0.92 in **1** was not observed in **6**, suggesting that the aglycone of **6** is the pregnane skeleton. Based on the analysis of ^1H–^1H COSY, TOCSY, HSQC, HMBC, NOESY, and HR-FAB-MS data, the steroidal moiety of **6** was characterized as a 5α-pregn-9(11)-en-20-one-3β,6α-diol 3-sulfate. The type of oligosaccharide moiety and the linkage were the same as in **5**. Accordingly, the structure of **6** was determined to be anasteroside B (**6**),[11] 6α-*O*-[β-D-galactopyranosyl-(1→3)-β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-5α-pregn-9(11)-en-20-one-3β-yl sodium sulfate.

Compound **7**, a white amorphous powder, showed pseudo-molecular ion peaks at *m/z* 1189 $[M+Na]^+$, 1167 $[M+H]^+$, and 1143 $[M-Na]^-$ in the ESI-MS. The overall ^1H- and ^{13}C-NMR spectra were quite similar to those of **6** except for the absence of one anomeric proton at δ_H 5.25 (1H, d, J=7.8 Hz). The lack of one hexosyl unit (*m/z* 162) in the ESI-MS data further supported that one of the hexose units in **7** was detached compared to **6**. A detailed comparison of the 2D-NMR data of **7** with those of **6** suggested that the structure of **7** is 6α-*O*-[β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-5α-pregn-9(11)-en-20-one-3β-yl sodium sulfate.[15,20]

When compounds **1**—**7** were tested for their anti-inflammatory activity in Raw 264.7 cells, compounds **4** and **5** (at 8 μM) inhibited the production of nitric oxide (NO) by up to 41.11%. In case of compounds **1**—**3**, **6** and **7**, a concentration-dependent activity was not observed in our assay due to their cytotoxicity toward the Raw 264.7 cells. Compared to control Raw 264.7 cells treated with lipopolysaccharide, treatment with compound **4** reduced the production of NO by 6.08%, 8.13%, 10.35%, 13.86%, and 19.72% at 0.1, 0.5, 1, 2, and 4 μM, respectively. Compound **5** reduced the production of NO by 5.52%, 8.29%, 12.55%, 14.59%, and 41.11% at 0.5, 1, 2, 4, and 8 μM, respectively. However both compounds exhibited toxicity toward the employed cells at high concentration, suggesting that these steroidal saponins are general toxic constituents of the starfish *A. amurensis*. In association with these results, we evaluated the cytotoxic activity against HL-60 human promelocytic leukemia cells. Compounds **4** and **5** exhibited moderate cytotoxic activity against HL-60 with IC_{50} values ranging from 21.3 to 81.5 μM.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP-370 (Tokyo, Japan) automatic digital polarimeter. UV spectra were obtained in H_2O using a JASCO V-550 UV/vis spectrometer. VLC was performed on Merck silica gel (70—230 mesh). MPLC was carried out with Biotage Isolera^{YM}. Reversed phase C_{18} SNAP Cartridge KP-C18-HS (120 g, Biotage), C_{18} RediSep Rf Flash column (130 g, 40—63 μm, 60 Å, Teledyne), silica gel SNAP Cartridge HP-Sil (25 g, 30 μm, Biotage), and silica gel RediSep Rf Disposable Flash column (12 g, 35—70 μm, 60 Å, Teledyne) were used for MPLC. HPLC separation was performed with a Gilson system with a UV detector and a Luna C_{18} column (250×21.20 mm, 10 μm). High and low resolution FAB-MS data were obtained on a JEOL JMS-700. MALDI-TOF-MS spectra were run on a Bruker Ultraflex III analyzer using DHB (2,5-dihydroxybenzoic acid) as a matrix in reflector mode. The LCQ advantage trap mass spectrometer (Thermo Finnigan, San Jose, CA, U.S.A.) was equipped with an electrospray ionization (ESI) source. TLC was executed on glass plates precoated with silica gel F_{254} (20×20 cm, 200 μm, 60 Å, Merck). The GC column chromatography was performed on a Hewlett Packard GC 6890 with an HP-5 column (cross linked 5% phenyl methyl silicone, 25 m×0.32 mm×0.17 μm) and N_2 as carrier gas (N_2 flow, 3 ml/min; air flow, 450 ml/min).

NMR Spectra ^1H (600 MHz), ^{13}C (150 MHz), and 2D (COSY, TOCSY, HMQC, HSQC, HMBC, NOESY, ROESY) NMR spectra were recorded on a Varian 600 MHz (VNS 600) spectrometer equipped with a 5 mm direct detection PFG probe. All NMR experiments were performed at 294 K, using pyridine-d_5 (**1**, **4**) or pyridine-d_5/D_2O (4 : 1 ratio with **2**, **3**, **5**—**7**) as the solvent. Chemical shifts were given on the δ scale and referenced by pyridine-d_5 as an internal standard (δ_H=7.19, δ_C=123.5). Coupling constants (J) are in Hz. Data processing was carried out with MestReNova v6.0.2 program.

Biological Material The starfish *Asterias amurensis* was collected off the coast of Pohang (36°06′N, 129°29′E), Korea in the spring of 2010. A voucher specimen (YNU00001), identified by Prof. Hyeun Wook Chang, has been deposited in the College of Pharmacy, Yeungnam University. The starfish was kept frozen after collection until the extraction process.

Extraction and Isolation The whole body of *Asterias amurensis* (6.5 kg) was chopped and extracted twice in MeOH (2×12 l) at room temperature for 7 d. The dried MeOH extract (241 g) was suspended in water and partitioned sequentially with *n*-hexane (16.6 g), EtOAc (6.8 g), and *n*-BuOH (34.0 g). A portion of the *n*-BuOH fraction (17.5 g) which exhibited bioactivity such as cytotoxicity and anti-inflammatory effects, was subjected to Si gel VLC (21×17.5 cm), and eluted with a stepwise gradient of CH_2Cl_2 and MeOH [100 : 0, 90 : 10, 80 : 20, 70 : 30, 60 : 40, 50 : 50, 40 : 60, 30 : 70, 20 : 80, 10 : 90, 0 : 100 (each 3 l)] to give 12 fractions (Fr. 1—12). Fr. 7 (4.2 g) was further divided into 6 fractions (Fr. 7-1 to 7-6) using MPLC [C_{18} column (16×4 cm)] with a gradient of MeOH/H_2O (from 50 : 50 to 100 : 0). Fr. 7-2 (63.0 mg) was applied to Si gel (7.5×3 cm) MPLC with isocratic solvent of $CHCl_3$/MeOH/H_2O (60 : 30 : 5), and afforded **1** (12.6 mg) and 6 other subfractions (Fr. 7-2-1 to 7-2-7). Of these, Fr. 7-2-6 was further purified by C_{18} HPLC (250×21.20 mm, 10 μm) with a gradient of MeOH/H_2O (50 : 50→70 : 30) over 60 min to yield **6** (2.5 mg). Fr. 7-4 (81.3 mg) was applied to C_{18} HPLC (250×21.20 mm, 10 μm) with ACN/H_2O (100 : 0→90 : 10) for 80 min, to obtain a mixture of **2** and **3** (9.7 mg). Fr. 7-1 (1688.5 mg) was fractionated by C_{18} MPLC (21×4 cm) using a gradient of MeOH/H_2O (from 10 : 90 to 70 : 30) to yield 7 subfractions (Fr. 7-1-1 to 7-1-7). Among them, Fr. 7-1-5 was further fractionated by Si gel MPLC (7.5×3 cm) with isocratic $CHCl_3$/MeOH/H_2O (60 : 30 : 5) to afford **6** (6.5 mg) and **7** (4.0 mg). Fr. 8 was chromatograghed over C_{18} MPLC (21×4 cm) and eluted with an isocratic MeOH/H_2O (50 : 50) to afford 7 fractions (Fr. 8-1 to 8-7). Compounds **4** (5.8 mg) and **5** (7.2 mg) were isolated from Fr. 8-5 by Si gel MPLC (8×2.2 cm) using a mobile phase of $CHCl_3$/MeOH/H_2O (65 : 35 : 5).

Compound **1**: White amorphous powder; $[\alpha]_D^{25}$ −6.8° (c=0.1, H_2O); ^1H- and ^{13}C-NMR: see Table 1; FAB-MS *m/z*: 1261 $[M+Na]^+$, 1141 $[M+Na-NaSO_4H]^+$, 1083 $[M+Na-NaSO_4H-C_3H_6O$ (aglycone side chain)$]^+$, 995 $[M+Na-NaSO_4H-Fuc]^+$, 833 $[M+Na-NaSO_4H-Fuc-Gal]^+$, 785 [Gal-Qui($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal-Fuc+Na−H]$^+$, 639 [Gal-Qui($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal+Na−H]$^+$, 379 $[M+Na-NaSO_4H-Fuc-Gal^{1\text{-}4}$-(Qui-$^{1\text{-}2}$)Qui-Gal$]^+$. HR-FAB-MS *m/z*: 1261.4910 $[M+Na]^+$ (Calcd for $C_{54}H_{87}Na_2O_{28}S$: 1261.4900).

Compounds **2** and **3**: Pale yellow amorphous powder; $[\alpha]_D^{25}$ −6.8° (c=0.1, H_2O); UV λ_{max} (H_2O) nm (DAD): 200.5, 202.5, 245; ^1H- and ^{13}C-NMR: see Table 1; ESI-MS *m/z*: 1301 $[M+Na]^+$, 1303 $[M+Na]^+$, 1279 $[M+H]^+$, 1281 $[M+H]^+$, 1181 $[M+Na-NaSO_4H]^+$, 1183 $[M+Na-NaSO_4H]^+$, 1133 $[M+H-NaSO_4H-Fuc]^+$, 1135 $[M+H-NaSO_4H-Fuc]^+$, 987 $[M+H-NaSO_4H-Fuc-Qui]^+$, 989 $[M+H-NaSO_4H-Fuc-Qui]^+$. HR-FAB-MS *m/z*: 1301.5204 $[M+Na]^+$ (Calcd for $C_{57}H_{91}Na_2O_{28}S$: 1301.5213), 1303.5361 $[M+Na]^+$ (Calcd for $C_{57}H_{93}Na_2O_{28}S$: 1303.5369).

Compound **4**: White amorphous powder; $[\alpha]_D^{22}$ −4.5° (c=0.3, H_2O); UV

λ_{max} (H_2O) nm (DAD): 203, 250.5, 256.5, 262.5; ^{1}H- and ^{13}C-NMR: see ref. 23); MALDI-TOF-MS *m/z*: 1289 $[M+Na]^+$, 1169 $[M+Na-NaSO_4H]^+$, 1069 $[M+Na-NaSO_4H-C_6H_{12}O$ (aglycone side chain)$]^+$, 1023 $[M+Na-NaSO_4H-Fuc]^+$, 861 $[M+Na-NaSO_4H-Fuc\text{-}Gal]^+$, 755 [Qui-Xyl($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal-Fuc+Na−H]$^+$, 609 [Qui-Xyl($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal+Na−H]$^+$, 437 $[M+Na-NaSO_4H-Fuc\text{-}Gal^{1\text{-}4}$-(Qui-$^{1\text{-}2}$)Xyl-Qui$]^+$.

Compound **5**: White amorphous powder; $[\alpha]_D^{22}$ +11.1° (*c*=0.3, H_2O); UV λ_{max} (H_2O) nm (DAD): 204.5, 250.5, 256.5; ^{1}H- and ^{13}C-NMR: see ref. 23); MALDI-TOF-MS *m/z*: 1451 $[M+Na]^+$, 1331 $[M+Na-NaSO_4H]^+$, 1231 $[M+Na-NaSO_4H-C_6H_{12}O$ (aglycone side chain)$]^+$, 1185 $[M+Na-NaSO_4H-Qui]^+$, 1169 $[M+Na-NaSO_4H-Gal]^+$, 1023 $[M+Na-NaSO_4H-Gal\text{-}Fuc]^+$, 917 [Qui-Xyl($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal-Fuc-Gal+Na−H]$^+$, 755 [Qui-Xyl($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal-Fuc+Na−H]$^+$, 437 $[M+Na-NaSO_4H-Gal\text{-}Fuc\text{-}Gal^{1\text{-}4}$-(Qui-$^{1\text{-}2}$) Xyl-Qui$]^+$.

Compound **6**: White amorphous powder; $[\alpha]_D^{25}$ −33.1° (*c*=0.1, H_2O); UV λ_{max} (H_2O) nm (DAD): 250.0, 255.5, 261.5; ^{1}H- and ^{13}C-NMR: see ref. 23); FAB-MS *m/z*: 1351 $[M+Na]^+$, 1231 $[M+Na-NaSO_4H]^+$, 1085 $[M+Na-NaSO_4H-Fuc]^+$, 923 $[M+Na-NaSO_4H-Fuc\text{-}Gal]^+$, 917 [Qui-Xyl($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal-Fuc-Gal+Na−H]$^+$, 755 [Qui-Xyl($^{2\text{-}1}$-Qui)-$^{4\text{-}1}$Gal-Fuc+Na−H]$^+$, 337 $[M+Na-NaSO_4H-Gal\text{-}Fuc\text{-}Gal^{1\text{-}4}$-(Qui-$^{1\text{-}2}$)Xyl-Qui$]^+$. HR-FAB-MS *m/z*: 1351.4860 $[M+Na]^+$ (Calcd for $C_{54}H_{87}Na_2O_{28}S$: 1351.4853).

Compound **7**: White amorphous powder; $[\alpha]_D^{25}$ −48.4° (*c*=0.1, H_2O); ^{1}H- and ^{13}C-NMR: see ref. 23); ESI-MS *m/z*: 1189 $[M+Na]^+$, 1183 $[M-Na+H+K]^+$, 1167 $[M+H]^+$, 1143 $[M-Na]^-$, 997 $[M-Na-Fuc]^-$, 851 $[M-Na-Fuc-Qui]^-$, 835 $[M-Na-Fuc\text{-}Gal]^-$.

Analysis of Sugar Each saponin **1**—**3** (1 mg) was dissolved in 0.5 ml of 1 N HCl, heated at 90 °C for 2 h, and partitioned with CH_2Cl_2 and H_2O (each 1 ml×3). The aqueous layer was neutralized with Ag_2CO_3 and centrifuged. The dried supernatant was dissolved in 1 ml of anhydrous pyridine and 2 ml of 0.1 M L-cysteine methyl ester hydrochloride in pyridine were added. The mixture was kept at 60 °C for 1 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.5 ml) at 60 °C for 5 min. The obtained mixture was partitioned between hexane and H_2O (each 1 ml×3) and the hexane fraction was analyzed by gas chromatography (GC) [column: HP-5 (25 m×0.32 mm×0.17 mm, HP), injector temperature: 290 °C, column temperature: 160 °C (2 min)→0.5 °C/min↑→170 °C→0.1 °C/min↑→200 °C, carrier gas: N_2]. The retention times of authentic samples were as follows: D-xylose (t_R 23.22 min), L-xylose (26.41 min), D-quinovose (26.49 min), D-fucose (29.28 min), L-fucose (33.64 min), D-galactose (50.53 min), and L-galactose (58.51 min). In the hydrolysate of **1**—**3**, D-quinovose (t_R 26.83—26.97 min), D-fucose (t_R 29.56—29.66 min), and D-galactose (t_R 50.12—50.29 min) were detected.

Cell Culture RAW264.7 cells, a murine macrophage/monocyte cell line, were obtained from the Korean Cell Line Bank (Seoul, Korea) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 100 μM MEM non-essential amino acid solutions. In all experiments, cells were grown to 80—90% confluence and subjected to no more than 20 cell passages.

Measurement of Cell Viability Cell viability was assessed by MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) assay. RAW264.7 cells plated in 96-well plates (5×10^4 cells/well) were treated with ECC for 20 h. Then MTT (5 mg/ml) was added and incubated for 4 h. The culture medium was removed, and the cells were dissolved in 0.04 N HCl/isopropyl alcohol. The optical densities (OD) at 570 nm and 630 nm were measured using a microplate reader.

Measurement of Nitric Oxide RAW264.7 cells (5×10^5 cells) were pre-incubated at 37 °C for 12 h in serum-free medium. Nitric oxide (NO) production was monitored by measuring nitrite levels in the culture media using Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylenediamine dihydrochloride, and 2.5% phosphoric acid). Absorbance was measured at 570 nm after incubation for 10 min.

Cytotoxic Activity The cancer cell lines were maintained in RPMI 1640, which included L-glutamine with 10% FBS and 2% penicillin–streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Viable cells were seeded in growth medium (100 μl) into 96-well microtiter plates (1×10^4 cells/well) and incubated at 37 °C in a 5% CO_2 incubator. The test sample was dissolved in dimethyl sulfoxide (DMSO) and adjusted to final sample concentrations ranging from 5.0 to 150 μM by diluting with growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to <0.1%. After standing for 24 h, 10 ml of the test sample was added to each well. The same volume of DMSO was added to the control wells. Cytotoxicity was measured using a modified MTT assay. On removing medium after 48 h of the test sample treatment, MTT (10 μl) was also added to each well. After 4 h incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 μl). The OD was measured at 570 nm. The IC_{50} value was defined as the concentration of sample that reduced the absorbance by 50% relative to the vehicle-treated control.

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References and Notes

1) Lodge D. M., *Trends Ecol. Evol.*, **8**, 133—137 (1993).
2) Cohen A. N., Carlton J. T., *Sience*, **279**, 555—558 (1998).
3) Ruiz G. M., Rawlings T. K., Dobbs F. C., Drake L. A., Mullady T., Huq A., Colwell R. R., *Nature* (London), **408**, 49—50 (2000).
4) Ross D. J., Johnson C. R., Hewitt C. L., *Biol. Invasions*, **5**, 3—21 (2003).
5) Ross D. J., Johnson C. R., Hewitt C. L., *Mar. Ecol. Prog. Ser.*, **241**, 99—112 (2002).
6) Ross D. J., Johnson C. R., Hewitt C. L., *J. Exp. Mar. Bio. Ecol.*, **288**, 257—278 (2003).
7) Ross D. J., Johnson C. R., Hewitt C. L., *Estuar. Coast. Shelf Sci.*, **67**, 695—707 (2006).
8) D'Auria M. V., Minale L., Riccio R., *Chem. Rev.*, **93**, 1839—1895 (1993).
9) Pettit G. R., Day J. F., Hartwell J. L., Wood H. B., *Natrure* (London), **227**, 962—963 (1970).
10) Fusetani N., Kato Y., Hashimoto K., Komori T., Itakura Y., Kawasaki T., *J. Nat. Prod.*, **47**, 997—1002 (1984).
11) Chludil H. D., Seldes A. M., Maier M. S., *J. Nat. Prod.*, **65**, 153—157 (2002).
12) Bruno I., Minale L., Riccio R., *J. Nat. Prod.*, **52**, 1022—1026 (1989).
13) Kitagawa I., Kobayashi M., *Chem. Pharm. Bull.*, **26**, 1864—1873 (1978).
14) Riccio R., Greco O. S., Minale L., *J. Nat. Prod.*, **48**, 97—101 (1985).
15) Komori T., Matsuo J., Itakura Y., Sakamoto K., Ito Y., Taguchi S., Kawasaki T., *Liebigs Ann. Chem.*, **1983**, 24—36 (1983).
16) Minale L., Riccio R., Greco O. S., Pusset J., Menou J. L., *Comp. Biochem. Physiol.*, **80B**, 113—118 (1985).
17) Maier M. S., Centurión R., Muniain C., Haddad R., Eberlin M. N., *ARKIVOC*, **vii**, 301—309 (2007).
18) Iorizzi M., Minale L., Riccio R., *J. Nat. Prod.*, **56**, 1786—1798 (1993).
19) Findlay J. A., Jaseja M., Burnell D. J., Brisson J. R., *Can. J. Chem.*, **65**, 1384—1391 (1987).
20) Tang H. F., Yi Y. H., Li L., Sun P., Zhou D. Z., Liu B. S., *Chin. Chem. Lett.*, **16**, 619—622 (2005).
21) Findlay J. A., Agarwal V. K., *J. Nat. Prod.*, **46**, 876—880 (1983).
22) Riccio R., D'Auria M. V., Minale L., *J. Org. Chem.*, **51**, 533—536 (1986).
23) Supplemental data can be obtained from the corresponding authors.
24) Tang H. F., Yi Y. H., Li L., Sun P., Zhang S. Q., Zhao Y. P., *Planta Med.*, **71**, 458—463 (2005).