Isotactic Polymethoxydienes from the Philippines Sponge *Myriastra clavosa*

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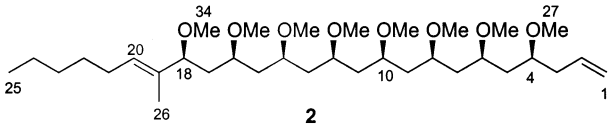
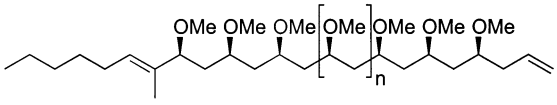
A specimen of the sponge *Myriastra clavosa* from the Philippines contained five new isotactic polymethoxydienes **1**−**5** that form a homologous series. The structures of the isotactic polymethoxydienes, which exhibited moderate cytotoxicity, were elucidated by interpretation of spectroscopic data.

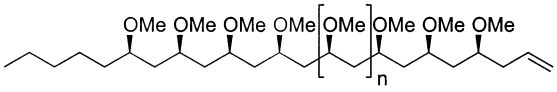
Marine sponges are recognized as a rich source of bioactive metabolites.[1] In our continuing search for cytotoxic agents from marine sponges, we screened many hundreds of sponges from the Philippines in a cell-line panel that was used to assign a priority to crude extracts based on efficacy and selectivity. We had expected the crude extracts of *Myriastra clavosa* to be assigned a high priority in the cell-line panel due to the report by Fu et al.[2] that *M. clavosa* contained the potent cytotoxins clavosines A−C, which are related to the calyculins. During our studies of *M. clavosa*, we have reported the isolation of two dimeric macrolides, clavosolides A and B,[3] but we were unable to isolate or even detect the clavosines. The cytotoxicity of our specimen of *M. clavosa* was traced to two fractions, one of which contained a homologous series of five isotactic polymethoxydienes **1**−**5**, which are the subject of this paper.



2



1 n = 1
3 n = 3
4 n = 4
5 n = 5



6 n = 1
7 n = 2

The sponge *Myriastra clavosa* Ridley 1884 (order Astrophorida, family Ancorinidae) was collected by hand using scuba at a depth of 10−15 m near Boracay Island in the Philippines and was kept frozen until it was extracted with methanol. The ethyl acetate-soluble material from the methanol extract was chromatographed on Sephadex LH-20 and silica gel to obtain five fractions that contained multiple signals in the methoxy region of their ^{1}H NMR spectra. These fractions, which also exhibited moderate cytotoxicity against the HCT-116 human colon tumor cell line, were further purified by HPLC using hexane−EtOAc mixtures as eluants to obtain (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,17*E*)-4,6,8,10,12,14,16-heptamethoxy-17-methyltricosa-1,17-diene (**1**), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,19*E*)-4,6,8,10,12,14,16,18-octamethoxy-19-methylpentacosa-1,19-diene (**2**), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,21*E*)-4,6,8,10,12,14,16,18,20-nonamethoxy-21-methylheptacosa-1,21-diene (**3**), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,23*E*)-4,6,8,10,12,14,16,18,20,22-decamethoxy-23-methylnonacosa-1,23-diene (**4**), and (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,24*S*,25*E*)-4,6,8,10,12,14,16,18,20,22,24-undecamethoxy-25-methyluntriacont-1,25-diene (**5**).

Table 1. ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR Data for 4,6,8,10,12,14,16,18-Octamethoxy-19-methylpentacosa-1,19-diene (**2**)[a]

C #	δ_C	δ_H	mult., *J* (Hz), (# H)	COSY	HMBC
1	117.1	5.09	d, 11	H-2	C-3
		5.11	d, 16	H-2	C-3
2	134.4	5.82	ddt, 16, 11, 6	H-1, H_2-3	
3	37.8	2.29	br q, 6 (2 H)	H-2, H-4	C-1, C-2, C-4, C-5
4	77.4	3.37	m	H-3, H_2-5	
5−17	(see below)				
18	84.4	3.63	br t, 7	H_2-17	C-16, C-17, C-20, C-26, C-34
19	133.4				
20	129.8	5.38	br t, 7	H_3-26, H_2-21	C-18, C-21, C-26
21	27.7	2.02	m (2 H)		
22	29.4	1.34	m (2 H)	H_2-21, H_2-23	C-23
23	31.7	1.30	m (2 H)	H_2-22, H_2-24	
24	22.6	1.25	m (2 H)	H_2-23, H_3-25	C-23
25	14.2	0.87	t, 7 (3 H)	H_2-24	C-23, C-24
26	10.4	1.53	s (3 H)	H-20	C-18, C-19, C-20
27−33	(see below)				
34	55.5	3.16	s (3 H)		C-18

[a] The following signals could not be individually assigned: C#: 5,7,9,11,13,15,17 δ_C 37.8, 37.9, 38.1, 38.3, 38.4, 38.4, 38.5; δ_H 1.57 (m, 7 H), 1.76 (m, 7 H); C#: 6,8,10,12,14,16 δ_C 75.3 (4 C), 75.5 (2 C); δ_H 3.37 (m, 6 H); C#: 27−33 δ_C 56.20, 56.24 (3 C), 56.26, 56.3, 56.4; δ_H 3.29 (s, 3 H), 3.30 (s, 3 H), 3.31 (s, 6 H), 3.33 (s, 6 H), 3.34 (s, 3 H).

(4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,19*E*)-4,6,8,10,12,14,16,18-Octamethoxy-19-methylpentacosa-1,19-diene (**2**), $[\alpha]_D$ +4.4° (*c* 1, $CHCl_3$), was the first of the compounds to be isolated as a viscous oil, which was a very pale green color due to the high chlorophyll content of the extract. The molecular formula, $C_{34}H_{66}O_8$, which was determined from a high-resolution mass measurement on the $[M + Na]^+$ ion at *m*/*z* 625.4635, required 2 unsaturation equivalents. The ^{13}C NMR spectrum (Table 1) contained four signals at δ 117.1 (CH_2), 129.8 (CH), 133.4 (C), and 134.4 (CH), which were assigned to a terminal olefin and a trisubstituted

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olefin, respectively. In the ^{1}H NMR spectrum (Table 1), the terminal olefin gave rise to signals at δ 5.09 (d, 1 H, J = 11 Hz, H-1), 5.11 (d, 1 H, J = 16 Hz, H-1), and 5.82 (ddt, 1 H, J = 16, 11, 6 Hz, H-2). The latter signal was coupled to a methylene signal at δ 2.29 (br q, 2 H, J = 6 Hz, H_2-3), which was in turn coupled to a complex signal centered at δ 3.37 (m, 7 H), which was assigned to H-4, H-6, H-8, H-10, H-12, and H-14. The presence of eight methoxy signals at δ 3.16 (s, 3 H), 3.28 (s, 3 H), 3.30 (s, 3 H), 3.31 (s, 6 H), 3.33 (s, 6 H), and 3.34 (s, 3 H), which show HMBC correlations to a group of carbon signals at δ 75.3 (4C), 75.5 (2C), 77.4, and 84.4, indicated that **2** might be closely related to the known isotactic polymethoxy-1-alkenes previously isolated from the cyanophyte *Tolypothrix conglutinata* var. *chlorata*.[4] The signal at δ 77.4 was assigned to C-4 on the basis of the HMBC correlation to the methylene signal at δ 2.29, while the signals at δ 75.3 and 75.5 were assigned to carbons 6, 8, 10, 12, 14, and 16 on the basis of their HMQC correlations to the complex signal at δ 3.37. The COSY spectrum showed coupling from the signal at δ 3.37 to two mutually coupled signals at δ 1.76 (m, 7 H) and 1.59 (m, 7 H), which are due to magnetically nonequivalent methylene protons which are characteristic of an isotactic arrangement of the methoxy groups.[4−6] The carbon signal at δ 84.4 was assigned to C-18, which is adjacent to the trisubstituted olefin. The HMQC and HMBC spectra show that the attached proton signal is at δ 3.64 (t, 1 H, J = 7 Hz, H-18) and the attached methoxy signal is the one at δ 3.16 (s, 3 H). There are also HMBC correlations from the signal at δ 84.4 to the vinyl methyl signal at δ 1.53 (s, 3 H, δ_C 10.4) and to the olefinic signal at δ 5.38 (br t, 1 H, J = 7 Hz, H-20), which is coupled to a methylene signal at δ 2.06 (m, 2 H, H_2-21, δ_C 27.7). The remaining four carbons comprise a normal alkyl chain, the assignment of which was confirmed by the observation of HMBC correlations from both the signal at δ 2.06 and the methyl signal at δ 0.87 (t, 3 H, J = 7 Hz) to the C-23 signal at δ 31.7. The absolute configuration was assigned as all-*S* by comparison of the optical rotation, $[\alpha]_D$ +4.4°, with that of synthetic (4*S*,6*S*,8*S*,10*S*,12*R*,14*R*,16*R*,18*R*)-4,6,8,10,12,-14,16,18-octamethoxy-1-tricosene (**6**, $[\alpha]_D$ +5.22°) and (4*S*,6*S*,8*S*,10*S*,12*R*,14*R*,16*R*,18*R*,20*R*)-4,6,8,10,12,14,16,18,20-nonamethoxy-1-pentacosene (**7**, $[\alpha]_D$ +4.45°).[5,7]

As was the case for the isotactic polymethoxy-1-alkenes previously isolated from the cyanophyte *Tolypothrix conglutinata* var. *chlorata*,[4] the remaining compounds formed a homologous series differing from each other by −CH_2-CH(OMe)− units (m/z = 58). (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,17*E*)-4,6,8,10,12,14,16-Heptamethoxy-17-methyltricosa-1,17-diene (**1**), the molecular formula of which, $C_{31}H_{60}O_7$, was determined from a high-resolution mass measurement of the $[M + Na]^+$ ion at m/z 567.4247, contained one less C_3H_6O unit than **2**. The only obvious difference in the ^{1}H NMR spectrum of **1** when compared with that of **2** was in the number of methoxyl signals; otherwise the spectra appeared identical. The molecular formulas of the higher homologues, (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,21*E*)-4,6,8,10,12,14,16,18,20-nonamethoxy-21-methylheptacosa-1,21-diene (**3**), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,23*E*)-4,6,8,10,12,14,16,18,20,22-decamethoxy-23-methylnonacosa-1,23-diene (**4**), and (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,24*S*,25*E*)-4,6,8,10,12,14,16,18,20,22,24-undecamethoxy-25-methyluntriacont-1,25-diene (**5**), were determined from high-resolution mass measurements of their $[M + Na]^+$ ions [m/z 683.5067 (**3**); 741.5527 (**4**); 799.5910 (**5**)] as $C_{37}H_{72}O_9$, $C_{40}H_{78}O_{10}$, and $C_{43}H_{84}O_{11}$, respectively. Again, the ^{1}H NMR spectra differed in the expected manner. All of the compounds gave optical rotations of positive sign and have therefore been assigned the all-*S* absolute configuration.

The polymethoxyalkadienes **1**−**5** showed moderate activity against the HCT-116 human colon tumor cell line, with IC_{50}'s of 119, 106, 86, 75, and 92 μM, respectively. There was a second, more active fraction from this sponge that appears to contain a second series of polymethoxyalkenes, but we have been unable to separate these compounds from large quantities of chlorophylls present in the extract of *M. clavosa*. Microscopic examination of the sponge indicates that the chlorophylls arise from cyanobacteria that constitute an appreciable portion of the cellular material. Given the structural similarity to the polymethoxy-1-alkenes from cyanophyte *Tolypothrix conglutinata* var. *chlorata*,[4] it seems reasonable to propose that the polymethoxyalkadienes **1**−**5** are of cyanobacterial origin.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. IR spectra were measured on a Perkin-Elmer 1600 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer, and all 2D experiments were performed on a Varian Inova 300 MHz NMR spectrometer. HRMS data were obtained on a VG ZAB mass spectrometer at the UC Riverside Regional Facility. All solvents were redistilled prior to use.

Animal Material. The sponge *Myriastra clavosa* Ridley 1884 (Collection # NCI-2879) was collected by hand using scuba (−15 m) at Boracay Island, Philippines, in 1998 and was immediately frozen. The sponge was initially identified by Mary Kay Harper, and the identification was confirmed by Dr. John Hooper, Queensland Centre for Biodiversity, Brisbane. Voucher specimens are available on request.

Extraction and Purification. The sponge (350 g wet wt) was extracted with MeOH (6 × 400 mL), and the extracts were concentrated to obtain a dark green gum (ca. 10 g), which was partitioned between EtOAc and H_2O to obtain organic (1.87 g) and aqueous (7.81 g) extracts. Both extracts inhibited the growth of the HCT-116 cell line, with the organic extract being the more active. The organic extract was first chromatographed on Sephadex LH-20 using MeOH as eluant followed by column chromatography on silica gel using a gradient from hexane through EtOAc to MeOH as eluants. Fractions containing strong methoxy signals in their ^{1}H NMR spectra were purified by HPLC on Porisil using mixtures of EtOAc and hexane as eluants to obtain (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,17*E*)-4,6,8,10,12,14,16-heptamethoxy-17-methyltricosa-1,17-diene (**1**, 1.1×10^{-3} % wet wt), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,19*E*)-4,6,8,10,12,14,16,18-octamethoxy-19-methylpentacosa-1,19-diene (**2**, 3.7×10^{-3} % wet wt), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,21*E*)-4,6,8,10,12,14,16,18,20-nonamethoxy-21-methylheptacosa-1,21-diene (**3**, 3.4×10^{-3} % wet wt), (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,23*E*)-4,6,8,10,12,14,16,18,20,22-decamethoxy-23-methylnonacosa-1,23-diene (**4**, 9.1×10^{-3} % wet wt), and (4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,24*S*,25*E*)-4,6,8,10,12,14,16,18,20,22,24-undecamethoxy-25-methyluntriacont-1,25-diene (**5**, 4×10^{-3} % wet wt).

(4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,17*E*)-4,6,8,10,12,14,16-Heptamethoxy-17-methyltricosa-1,17-diene (1): slighly greenish viscous oil; $[\alpha]_D$ +3.2° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, 3 H, J = 7 Hz), 1.26 (m, 2 H), 1.31 (m, 2 H), 1.35 (m, 2 H), 1.55 (s, 3 H); 1.58 (m, 6 H), 1.78 (m, 6 H), 2.04 (m, 2 H), 2.32 (m, 2 H), 3.15 (s, 3 H), 3.29 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 9 H), 3.34 (s, 3 H), 3.37 (m, 6 H), 3.63 (t, 1 H, J = 7 Hz), 5.09 (d, 1 H, J = 11 Hz), 5.11 (d, 1 H, J = 16 Hz), 5.36 (br t, 1 H, J = 6 Hz), 5.78 (m, 1 H); HRFABMS m/z 567.4247 $(M + Na)^+$ (calcd for $C_{31}H_{60}O_7Na$, 567.4236).

(4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,19*E*)-4,6,8,10,12,14,16,18-Octamethoxy-19-methylpentacosa-1,19-diene (2): slighly greenish viscous oil; $[\alpha]_D$ +4.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$,

400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRFABMS obsd *m*/*z* 625.4635 $(M + Na)^+$ (calcd for $C_{34}H_{66}O_8Na$, 625.4655).

(4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,21*E*)-4,6,8,10,12,14,16,18,20-Nonamethoxy-21-methylheptacosa-1,21-diene (3): slighly greenish viscous oil; $[\alpha]_D$ +2.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, 3 H, J = 7 Hz), 1.25 (m, 2 H), 1.30 (m, 2 H), 1.34 (m, 2 H), 1.54 (s, 3 H); 1.57 (m, 8 H), 1.77 (m, 8 H), 2.02 (m, 2 H), 2.30 (m, 2 H), 3.15 (s, 3 H), 3.29 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 15 H), 3.34 (s, 3 H), 3.37 (m, 8 H), 3.63 (t, 1 H, J = 6.5 Hz), 5.08 (d, 1 H, J = 11 Hz), 5.11 (d, 1 H, J = 16 Hz), 5.37 (br t, 1 H, J = 6 Hz), 5.76 (m, 1 H); HRFABMS *m*/*z* 683.5067 $(M + Na)^+$ (calcd for $C_{37}H_{72}O_9Na$, 683.5074).

(4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,23*E*)-4,6,8,10,12,14,16,18,20,22-Decamethoxy-23-methylnonacosa-1,23-diene (4): slighly greenish viscous oil; $[\alpha]_D$ +6.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, 3 H, J = 7 Hz), 1.25 (m, 2 H), 1.30 (m, 2 H), 1.35 (m, 2 H), 1.53 (s, 3 H); 1.56 (m, 9 H), 1.77 (m, 9 H), 2.03 (m, 2 H), 2.29 (m, 2 H), 3.14 (s, 3 H), 3.28 (s, 3 H), 3.29 (s, 3 H), 3.30 (s, 9 H), 3.31 (s, 9 H), 3.34 (s, 3 H), 3.38 (m, 9 H), 3.62 (t, 1 H, J = 6.5 Hz), 5.08 (d, 1 H, J = 11 Hz), 5.11 (d, 1 H, J = 16 Hz), 5.39 (br t, 1 H, J = 6 Hz), 5.81 (m, 1 H); ^{13}C NMR $CDCl_3$, 100 MHz) δ 134.3, 133.4, 129.8, 117.1, 84.4, 77.3, 75.4 (2 C), 75.3 (6 C), 56.4, 56.3, 56.2 (7 C), 55.5, 38.4, 38.3 (3 C), 38.2, 38.1, 38.0, 37.8, 37.7, 31.6, 29.7, 29.4, 27.6, 22.6, 14.2, 10.3; HRFABMS *m*/*z* 741.5527 $(M + Na)^+$ (calcd for $C_{40}H_{78}O_{10}Na$, 741.5492).

(4*S*,6*S*,8*S*,10*S*,12*S*,14*S*,16*S*,18*S*,20*S*,22*S*,24*S*,25*E*)-4,6,8,10,12,14,16,18,20,22,24-Undecamethoxy-25-methyluntriacont-1,25-diene (5): greenish viscous oil; $[\alpha]_D$ +8.0° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, 3 H, J = 7 Hz), 1.27 (m, 2 H), 1.31 (m, 2 H), 1.35 (m, 2 H), 1.53 (s, 3 H); 1.58 (m, 10 H), 1.77 (m, 10 H), 2.04 (m, 2 H), 2.30 (m, 2 H), 3.15 (s, 3 H), 3.28 (s, 3 H), 3.29 (s, 3 H), 3.30 (s, 6 H), 3.31 (s, 15 H), 3.34 (s, 3 H), 3.37 (m, 10 H), 3.63 (t, 1 H, J = 6.5 Hz), 5.08 (d, 1 H, J = 11 Hz), 5.10 (d, 1 H, J = 16 Hz), 5.37 (br t, 1 H, J = 6 Hz), 5.81 (m, 1 H); ^{13}C NMR $CDCl_3$, 100 MHz) δ 134.3, 133.4, 129.8, 117.1, 84.4, 77.3, 75.4 (2 C), 75.3 (7 C), 56.4, 56.3, 56.2 (8 C), 55.5, 38.4, 38.3 (4 C), 38.2, 38.1, 38.0, 37.8, 37.7, 31.6, 29.7, 29.4, 27.6, 22.6, 14.2, 10.3; HRFABMS *m*/*z* 799.5910 $(M + Na)^+$ (calcd for $C_{43}H_{84}O_{11}Na$, 799.5911).

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References and Notes

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(7) Although the absolute configurations at C-12, C-14, C-16, and C-18 are the same in compounds **2** and **6**, the *R*/*S* designation changes due to the relative size of the substituents at C-19.

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