JOC Note

Spirastrellolides C to G: Macrolides Obtained from the Marine Sponge *Spirastrella coccinea*

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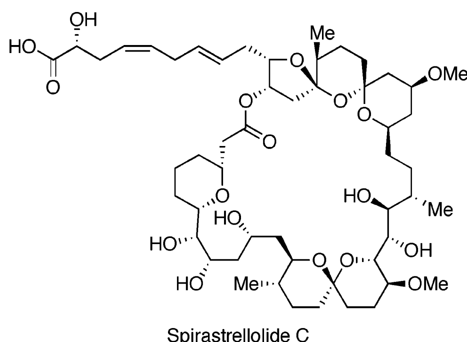
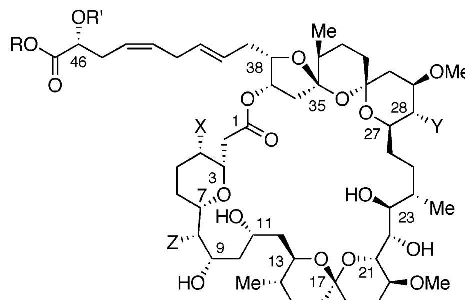


Spirastrellolide C

Five new macrolides, spirastrellolides C (**3**) to G (**7**), have been isolated from extracts of the marine sponge *Spirastrella coccinea* collected in Dominica. Their structures have been elucidated by a combination of spectroscopic analysis and chemical transformations.

The spirastrellolides are a family of biologically active macrolides obtained from extracts of the marine sponge *Spirastrella coccinea* collected in Dominica.[1] Although initially isolated by our group because they showed strong activity and an unusual phenotypic response in a cell-based assay used to discover new natural product antimitotic agents,[1a,2] the spirastrellolides were subsequently found to be potent ($IC_{50} \approx 1$ nM) and selective inhibitors of protein phosphatase 2A.[1b] A number of elegant syntheses of major fragments of spirastrellolide A (**1**)[3] have been reported and they provided initial support for the constitution and partial relative configuration originally assigned from spectroscopic analysis. Recently, the complete absolute configuration of the spirastrellolide macrocyclic core was determined by single-crystal X-ray diffraction analysis of a degradation product of spirastrellolide B (**2**).[4]

As part of our ongoing examination of the *S. coccinea* extract, we have identified the five new spirastrellolides C (**3**) to G (**7**). The new spirastrellolides were isolated from the *S. coccinea* extract as their C-47 methyl esters (**10** to **14**) following the procedure previously reported for the isolation of methylspirastrellolide A (**8**) (Supporting Information).[1] Complete details of the structure elucidation of the methyl esters of **3**−**7** via spectroscopic analysis are presented below.



1 Spirastrellolide A: R=R'=X=Z=H, Y=Cl, $\Delta^{15,16}$
2 Spirastrellolide B: R=R'=X=Y=Z=H
3 Spirastrellolide C: R=R'=X=Y=H, Z=OH
4 Spirastrellolide D: R=R'=Z=H, X=Y=Cl, $\Delta^{15,16}$
5 Spirastrellolide E: R=R'=X=Y=Z=H, $\Delta^{15,16}$
6 Spirastrellolide F: R=R'=X=Z=H, Y=Cl
7 Spirastrellolide G: R=X=Z=H, R'=Me, Y=Cl, $\Delta^{15,16}$
8 R=Me, R'=X=Z=H, Y=Cl, $\Delta^{15,16}$
9 R=Me, R'=X=Y=Z=H
10 R=Me, R'=X=Y=H, Z=OH
11 R=Me, R'=Z=H, X=Y=Cl, $\Delta^{15,16}$
12 R=Me, R'=X=Y=Z=H, $\Delta^{15,16}$
13 R=Me, R'=X=Z=H, Y=Cl
14 R=Me, X=Z=H, R'=Me, Y=Cl, $\Delta^{15,16}$

Methylspirastrellolide C (**10**) gave a $[M + Na]^+$ ion at *m/z* 1033.5721 in the HRESIMS that was consistent with a molecular formula of $C_{53}H_{86}O_{18}$ (calcd for $C_{53}H_{86}O_{18}Na$, 1033.5712). NMR data recorded for methylspirastrellolide C (**10**) showed a strong resemblance to the data previously obtained for the methyl esters **8** and **9** indicating that spirastrellolides A (**1**), B (**2**), and C (**3**) were closely related. The molecular formula of **10** required 11 sites of unsaturation, one less than methylspirastrellolide A (**8**). A low-resolution ESIMS recorded in MeOH

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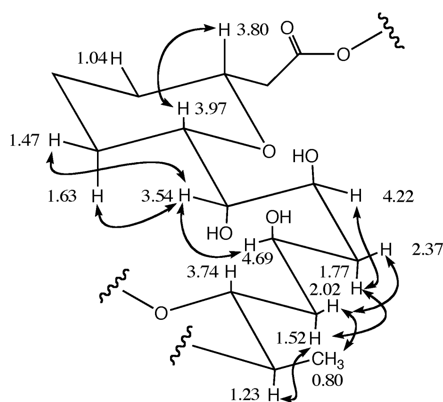


FIGURE 1. ROESY correlations observed for methylspirastrellolide C (**10**).

gave a $[M + Na]^+$ ion at *m/z* 1034 and the corresponding measurement in CD_3OD gave a $[M + Na]^+$ ion at *m/z* 1040 indicating the presence of six exchangeable protons in **10**, one more than in **8**.

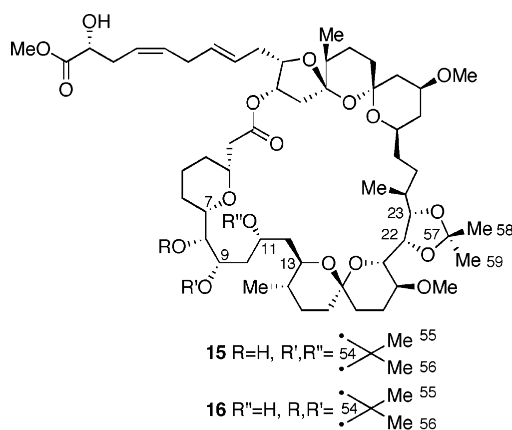
The ^{13}C NMR spectrum (C_6D_6) of **10** showed 52 well-resolved resonances. One broad resonance at δ 35.2 could be assigned to two carbons (C-14 and C-18) on the basis of its relative intensity and HMBC correlations (H-12 and Me-48 to C-14, and H-19 to C-18), thereby accounting for the 53 carbons in the molecular formula obtained from the MS data. Only four olefinic methines were observed in the ^{13}C NMR spectrum of **10**, suggesting that the missing site of unsaturation relative to **8** was due to reduction of one of the double bonds present in **8**.

Analysis of the HSQC, HSQC-TOCSY, COSY, TOCSY, ROESY, and HMBC data recorded for **10** (Supporting Information) showed that methylspirastrellolide C contained a C-27 to C-47 fragment identical in all respects with that found in methylspirastrellolide A (**8**), except that it was missing the chlorine substituent found at C-28 in **8**. Thus, in the COSY spectrum of **10**, the resonance at δ 3.75 assigned to H-29 showed correlations to a pair of geminal methylene proton resonances at δ 1.18 and 1.97, assigned to H-28 and H-28′, which were in turn correlated to a resonance at δ 3.69, assigned to H-27, demonstrating the absence of a substituent at C-28. The 2D NMR data for **10** also confirmed that methylspirastrellolide C contained the C-26 to C-17 fragment found in methylspirastrellolide A and the similarity in ^{13}C and ^1H chemical shift assigments, coupling constants, and observed ROESY correlations for this region of the two molecules indicated that the configurations of the stereogenic centers in this fragment were identical in **8** and **10**.

The absence of ^{13}C resonances that could be assigned to a third olefin in **10** suggested that spirastrellolide C lacked the $\Delta^{15,16}$ double bond found in spirastrellolide A. This was confirmed by the HMBC data for **10**, which showed correlations from a methyl resonance at δ 0.80, assigned to Me-48, to methine carbon resonances at δ 73.8 and 35.2, assigned to C-13 and C-14, respectively, and to a methylene carbon resonance at δ 29.6, assigned to C-15. HSQC correlations were observed from the C-15 resonance (δ 29.6) to proton resonances at δ 1.38 and 1.52. The C-16 carbon resonance at δ 35.6 was identified on the basis of its HSQC-TOCSY correlations to H-15 (δ 1.38), H-15′ (1.52), H-14 (1.23), Me-48 (0.80), and H-13 (3.74); and the H-16 (δ 1.50) and H-16′ (1.40) resonances were assigned on the basis of their HSQC correlations to C-16.

Further 2D NMR analysis showed that **10** contained a linear C-12 to C-9 fragment, a C-3 to C-7 tetrahydropyran fragment, and a C-1 to C-37 macrolide linkage identical with those found in **8**, including the presence of hydroxyl functionalities at C-11 and C-9. The H-9 and H-7 resonances at δ 4.22 and 3.97, respectively, both showed COSY correlations to a deshielded methine resonance at δ 3.54 (H-8), which was correlated in the HSQC spectrum to a carbon resonance at δ 76.6 (C-8), indicating the presence of a hydroxyl functionality at C-8 in methylspirastrellolide C (**10**). H-8 (δ 3.54) was further correlated to an OH proton resonance at δ 3.18 (O**H**-8) in the COSY spectrum. The identification of OH subsitutents at C-8, C-9, C-11, C-22, C-23, and C-46 in methyl ester **10** accounted for the six exchangeable protons observed in the CD_3OD/CH_3OH ESIMS experiments.

Reaction of methyl ester **10** with 2,2-dimethoxypropane and PPTS at rt, followed by HPLC purification, gave bisacetonides **15** and **16**. The geminal methyl carbons (Me-55 and Me-56) in



the C-9/C-11 acetonide **15** had chemical shifts of δ 25.4 and 28.4 demonstrating that the C-9 and C-11 alcohols in **10** were anti as in spirastrellolides A (**1**) and B (**2**).[4] In the C-8/C-9 acetonide **16**, the acetonide methyl resonance at δ 1.44 (Me-55) showed ROESY correlations to both δ 3.83 (H-8, weak) and δ 4.88 (H-9, strong) indicating that H-8 and H-9 were cis. In methylspirastrellolide C (**10**), H-8 appears as a bt with *J* values of ~7.9 Hz with both H-9 and OH-8, which required that H-8 (δ 3.54) and H-9 (δ 4.22) were anti (Figure 1). H-7 (δ 3.97) appears as a bd in **10** with a single large coupling of 11.6 Hz to H-6_{ax} (δ 1.63). The second H-6 resonance at δ 1.47 was assigned to the equatorial proton on the basis of a weak long-range w-coupling observed in the COSY spectrum between H-6 (1.47) and the H-4_{eq} resonance at δ 1.04. The absence of observable coupling between H-7 (δ 3.97) and H-8 (δ 3.54) required a dihedral angle of near 90°. ROESY correlations between δ 3.54 (H-8) and both δ 1.47 (H-6_{eq}, strong NOE) and δ 1.63 (H-6_{ax}, weak NOE) and between H-8 (δ 3.54) and H-11 (δ 4.69) were only consistent with the relative configurations at C-7, C-8, and C-9 drawn in structure **10** (Figure 1). The similarity in ^{13}C and ^1H chemical shift assignments, coupling constants, and observed ROESY correlations for the three molecules **8**, **9**, and **10** indicated that the configurations of the remaining stereogenic centers that are common to the three molecules were identical.

Methylspirastrellolide D (**11**) was isolated as an optically active clear oil that gave a $[M + Na]^+$ ion at *m/z* 1083.4835 in the HRESIMS consistent with a molecular formula of $C_{53}H_{82}O_{17}$-

Cl_2 (calcd for $C_{53}H_{82}O_{17}Cl_2Na$, 1083.4827). The molecular formula of **11** required the 12 sites of unsaturation present in spirastrellolide A (**1**), but the molecular formula of D (**4**) had one more chlorine and one less hydrogen than the molecular formula of **1**. Of the 53 well-resolved resonances observed in the ^{13}C NMR spectrum (C_6D_6) of **11**, all but three (C-4, C-5, and C-6) had chemical shifts comparable to the resonances assigned to the carbons in **8** (Table 2, Supporting Information). Analysis of the COSY, TOCSY, ROESY, HSQC, and HMBC data recorded for **11** showed that methylspirastrellolide D contained a C-8 to C-47 fragment, and a C-1 to C-37 macrolide linkage identical in all respects to those found in methylspirastrellolide A (**8**). Further 2D NMR analysis showed that the C-3 to C-7 tetrahydropyran in **11** contained a chlorine substituent at C-4 (δ 59.8). In the COSY spectrum, the resonance at δ 3.27 assigned to H-4 showed correlations to the H-3 resonance at δ 3.82 and to a pair of geminal methylene proton resonances at δ 1.48 and 1.76, assigned to H-5 and H-5′. HMBC correlations observed between both H-2 (δ 2.17) and H-3 (δ 3.82) and C-4 (δ 59.8) confirmed the placement of the chlorine atom at C-4. The H-4 resonance appeared as a bs and H-3 appeared as a bd with $J = 9.8$ Hz demonstrating that H-4 was equatorial and the chlorine atom was axial. The similarity in ^{13}C and ^{1}H chemical shift assignments, coupling constants, and observed ROESY correlations for **8** and **11** indicated that the configurations of the rest of the stereogenic centers were identical in both molecules.

Methylspirastrellolide E (**12**), which was also isolated as an optically active clear oil, gave a $[M + Na]^+$ ion at *m/z* 1015.5573 in the HRESIMS that was consistent with a molecular formula of $C_{53}H_{84}O_{17}$ (calcd for $C_{53}H_{84}O_{17}Na$, 1015.5606). Analysis of the COSY, HMQC, and HMBC data recorded for methylspirastrellolide E (**12**) (Tables 1 and 2, Supporting Information) showed that it was identical to methylspirastrellolide A (**8**) in all respects except that methylspirastrellolide E (**12**), as with **9** and **10**, was missing the chlorine substituent found at C-28 in **8**. In the COSY spectrum of **12**, the resonance at δ 3.71 assigned to H-29 showed correlations to a pair of geminal methylene proton resonances at δ 1.17 and 1.91, assigned to H-28 and H-28′, which were in turn correlated to a resonance at δ 3.68, assigned to H-27, demonstrating the absence of a substituent at C-28. The similarity in ^{13}C and ^{1}H chemical shift assignments and coupling constants for the three molecules **8**, **9**, and **12** indicated that the configurations of the stereogenic centers that are common to each molecule were identical.

Methyl spirastrellolide F (**13**) gave a $[M + Na]^+$ ion at *m/z* 1051.5421 in the HRESIMS that was consistent with a molecular formula of $C_{53}H_{85}O_{17}Cl$ (calcd for $C_{53}H_{85}O_{17}ClNa$, 1051.5373). The molecular formula of **13** required 11 sites of unsaturation, one less than methylspirastrellolide A (**8**). Only four olefinic methines were observed in the ^{13}C NMR spectrum of **13**, suggesting that the missing site of unsaturation relative to **8** was due to reduction of one of the double bonds present in **8**. Analysis of the COSY, HMQC, and HMBC data recorded for methylspirastrellolide F (**13**) showed that it was identical to methylspirastrellolide A (**8**) except that spirastrellolide F (**6**), as is the case with **9** and **10**, lacked the $\Delta^{15,16}$ double bond. This was confirmed by the HMBC data which showed correlations from a methyl resonance at δ 0.84, assigned to Me-48, to methine carbon resonances at δ 73.7 and 35.0, assigned to C-13 and C-14, respectively, and to a methylene carbon resonance at δ 29.3, assigned to C-15. HMQC correlations were observed from the C-15 resonance (δ 29.3) to proton resonances at δ 1.39 and 1.56. The H-16 and H-16′ proton resonances at δ 1.40 and 1.54 were identified on the basis of their HMBC correlations to C-15 (δ 29.3), and the C-16 carbon resonance at δ 35.5 was assigned on the basis of its HMQC correlations to H-16 and H-16′. The H-15, H-15′, H-16, and H-16′ proton resonances (δ 1.39, 1.56, 1.40, and 1.54) all correlated to the resonance at δ 95.6, assigned to the C-17 ketal carbon.

The ^{1}H NMR spectrum of methylspirastrellolide G (**14**) revealed the presence of an additional singlet methyl resonance at δ 3.20 compared to methylspirastrellolides A−F (**8**−**13**). Methylspirastrellolide G (**14**) gave a $[M + Na]^+$ ion at *m/z* 1063.5409 in the HRESIMS, which was 14 daltons larger than that observed for methylspirastrellolide A (**8**), and was consistent with a molecular formula of $C_{54}H_{85}O_{17}Cl$ (calcd for $C_{54}H_{85}O_{17}ClNa$, 1063.5373). Comparison of the NMR data recorded for **14** with that of **8**, **11**, and **12** (Supporting Information Tables 1 and 2) indicated that the macrocyclic ring of **14** was identical to **8** and that the minor structural difference lay in the side chain. The presence of a C-46 methyl ether in **12** satisfied the data. This was confirmed by the observation of a HMBC correlation between the methyl resonance at δ 3.20 and the methine carbon resonance at δ 80.7, assigned to C-46. The H-46 resonance at δ 3.68 correlated to C-46 in the HMQC data and showed HMBC correlations with both C-44 (δ 124.8) and C-45 (δ 31.1).

The one remaining unresolved structural feature of the spirastrellolides was the absolute configuration at C-46. Treatment of methylspirastrellolide D (**11**) with sodium periodate and a catalytic amount of $RuCl_3$ cleaved the $\Delta^{43,44}$ alkene to give methylmalate containing C-46. Reaction of the methylmalate with MeI and K_2CO_3 in acetone gave dimethylmalate that was shown by chiral GC analysis to be the R enantiomer. Therefore, the C-46 configuration in the spirastrellolides is R.

Methylspirastrellolides C (**10**), D (**11**), and E (**12**) were tested in the cell-based assay for premature mitosis (Supporting Information) and found to have IC_{50} values of 0.4, 0.7, and 0.7 μM, respectively, which is comparable to that of methylspirastrellolide A (**8**) ($IC_{50} = 0.4$ μM).

The spirastrellolides represent a unique family of marine sponge derived macrolides that are potent protein phosphatase 2A inhibitors ($IC_{50} \approx 1$ nM). Their general structure has a 47-carbon linear polyketide chain incorporated into a highly functionalized 38-membered macrolide that has tetrahydropyran, [6,6]-spiroketal, and [5,6,6]-bis-spiroketal substructures embedded in the macrocycle and a side chain terminating in a carboxylic acid. Spirastrellolides C (**3**) to G (**7**) have revealed additional variations in the functionalization of the polyketide backbone. New features include the presence of an additional alcohol at C-8 in spirastrellolide C (**3**), an axial chlorine substituent at C4 in spirastrellolide D (**4**), and a methyl ether in place of an alcohol at C-46 in spirastrellolide G (**7**). The suite of known structural variations in the spirastrellolide family now includes combinations of chlorine or H at C-4, OH or H at C-8, double bond or not at C-15/C-16, chlorine or H at C-28, and OH or OMe at C-46.

Experimental Section

Specimens of *S. coccinea* were collected by hand with use of SCUBA on walls at a depth of 2−5 m off Capucin, Guadeloupe Channel, Dominica. Freshly collected sponge was frozen on site and transported frozen to Vancouver. A voucher sample has been deposited at the Zoological Museum of Amsterdam (ZMA POR. 16778).

Thawed sponge (19 kg) was cut into pieces and extracted with MeOH (3 × 20 L) at rt. The combined MeOH extracts were concentrated in vacuo to give a red gum that was partitioned between EtOAc (5 × 1 L) and H_2O (6.0 L). The EtOAc extract was evaporated to dryness to give 42 g of red oil that was partitioned between hexanes (4 × 300 mL) and 4:1 MeOH/H_2O (1 L). Water was added to adjust the ratio of the MeOH extract to 2:1 MeOH/H_2O (for a total of 1.2 L) and the resulting solution was extracted with CH_2Cl_2 (4 × 200 mL). The CH_2Cl_2 extracts were combined and evaporated to dryness to give 2.2 g of a red solid. To facilitate isolation, the solid was methylated by treatment with CH_2N_2 generated in situ by the addition of 10 mL of 2.0 M trimethylsilyldiazomethane in hexanes to 10 mL of anhydrous MeOH in 40 mL of C_6H_6. After evaporation of the reagents, the sample was fractionated by using Si gel flash chromatography employing a step gradient from 95:5 hexanes/EtOAc to MeOH. A fraction C (364 mg), eluting with EtOAc, and a fraction D (110 mg), eluting with 10% MeOH/EtOAc, were each further fractionated on Sephadex LH-20 with 4:1 MeOH/CH_2Cl_2 as eluent. Reversed-phase HPLC purification eluting with MeOH/H_2O or MeCN/H_2O (complete details are provided in the Supporting Information) gave pure spirastrellolide methyl esters **8−14.** Assigned ^1H and ^{13}C NMR data for the new compounds **10−16** are tabulated in the Supporting Information.

Spirastrellolide C methyl ester 10: isolated as a clear oil; $[\alpha]^{25}_D$ +17.6 (*c* 0.9, CH_2Cl_2); HRESIMS $[M + Na]^+$ *m/z* 1033.5721 (calcd for $C_{53}H_{86}O_{18}Na$, 1033.5712).

Spirastrellolide D methyl ester 11: isolated as a clear oil; $[\alpha]^{25}_D$ +45.9 (c 0.5, MeOH); HRESIMS $[M + Na]^+$ *m/z* 1083.4835 (calcd for $C_{53}H_{82}O_{17}Cl_2Na$, 1083.4827).

Spirastrellolide E methyl ester 12: isolated as a clear oil; $[\alpha]^{25}_D$ +47.4 (*c* 0.5, MeOH); HRESIMS $[M + Na]^+$ *m/z* 1015.5573 (calcd for $C_{53}H_{84}O_{17}Na$, 1015.5606).

Spirastrellolide F methyl ester 13: isolated as a clear oil; $[\alpha]^{25}_D$ +33.8 (*c* 2.30, CH_2Cl_2); HRESIMS $[M + Na]^+$ *m/z* 1051.5421 (calcd for $C_{53}H_{85}O_{17}ClNa$, 1051.5373), negative ion HRESMS $[M + Cl]^-$ *m/z* 1063.5106 (calcd for $C_{53}H_{83}O_{17}Cl_2$, 1063.5164).

Spirastrellolide G methyl ester 14: isolated as a clear oil; $[\alpha]^{25}_D$ +38.3 (*c* 1.46, CH_2Cl_2); HRESIMS $[M + Na]^+$ *m/z* 1063.5409 (calcd for $C_{54}H_{85}O_{17}ClNa$, 1063.5373).

Preparation of the Bisacetonides 15 and 16. Methylspirastrellolide C (**10**) (0.8 mg) was dissolved in CH_2Cl_2 (0.5 mL). To this solution was added dimethoxypropane (0.5 mL) and a catalytic amount of PPTS (∼1 mg). The reaction mixture was stirred for 24 h at rt, then quenched with saturated $NaHCO_3$ and extracted with EtOAc. The EtOAc-soluble material was fractionated with Si gel flash chromatography employing a step gradient from 4:1 hexanes/EtOAc to EtOAc to yield 0.4 mg of a mixture of the bisacetonides **15** and **16**. This mixture was purified by reversed-phase HPLC with MeCN/H_2O as an eluent, to give the bisacetonides **15** and **16** as a clear oils (0.2 and 0.2 mg, respectively).

Bisacetonide 15: isolated as a clear oil; HRESIMS $[M + Na]^+$ *m/z* 1113.6332 (calcd for $C_{59}H_{94}O_{18}Na$, 1113.6338).

Bisacetonide 16: isolated as a clear oil; HRESIMS $[M + Na]^+$ *m/z* 1113.6348 (calcd for $C_{59}H_{94}O_{18}Na$, 1113.6338).

Preparation of Dimethyl Malate from Methylspirastrellolide D (11). To **11** (0.5 mg) dissolved in 1:1:1 CCl_4:CH_3CN:pH 7.2 phosphate buffer (0.9 mL total volume) was added sodium periodate (8.0 mg) and a catalytic amount of $RuCl_3$. After the solution was stirred for 2 h at rt, the organic solvents were evaporated under N_2 and the resulting aqueous suspension was extracted with Et_2O. The aqueous layer was acidified with 100 μL of TFA and then passed through a C_{18} reversed-phase column (0.5 mL). The C_{18} reversed-phase column was washed with H_2O (2 mL) and the entire aqueous eluent was lyophilized and then stirred overnight at rt in acetone (2 mL) containing K_2CO_3 (5.0 mg) and MeI (1 mL) to yield dimethylmalate.

Samples of dimethylmalate were analyzed by using GC/MS with a chiral capillary column (30 m × 250 μm, 0.25 μm film thickness, initial oven temp 50 °C held for 1 min, ramp to 200 °C at 8 deg/min, held for 1 min). Authentic standards of (*R*)- and (*S*)-dimethylmalate eluted with retention times of 14.23 and 14.34 min, respectively, while dimethylmalate from the oxidative cleavage of **11** eluted with a retention time of 14.21 min. EIMS of both standards and the naturally derived dimethylmalate were identical.

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Supporting Information Available: Experimental details, tables of NMR assigments for **10−16**, and NMR spectra for **10−16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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