Brominated Polyacetylenes from the Philippines Sponge *Diplastrella* sp.

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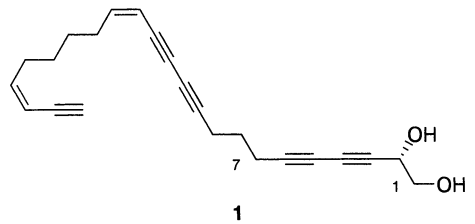
Five novel brominated polyacetylenic diols, diplynes A−E (**2**−**6**), and three sulfated analogues, diplyne A 1-sulfate (**7**), diplyne C 1-sulfate (**8**), and 2-deoxydiplyne D sulfate (**9**), were isolated from the Philippines sponge *Diplastrella* sp. by employing bioassay-guided fractionation using the HIV-1 integrase inhibition assay. The novel metabolites were characterized by interpretation of spectroscopic data.

A review of the literature up to 1996 recorded more than 1600 naturally occurring organobromo compounds,[1] most of which were derived from marine sources. Additional brominated compounds are reported in the marine natural products literature every year.[2] Sponges have yielded a number of brominated fatty acids. In particular, brominated acetylenic fatty acids[3] or polyacetylene sulfates and polyacetylene diols[4] have been isolated from the order Haplosclerida.[5] Siphonodiol (**1**), a polyacetylenic metabolite that contains the diol end group, is the most closely related compound to the diplynes, but it lacks a bromine.[4a] Callyspongin A, the monosulfate of siphonodiol at C-1, and callyspongin B, a monosulfate at C-2, are examples of sulfated polyacetylenes.[4c] Callytriols A−E contain the siphonodiol backbone but have an additional alcohol group.[6] In this paper we describe the first brominated polyacetylenic diols.

As part of our continuing efforts to identify bioactive marine natural products, a collection of marine invertebrates was screened for inhibition in the HIV integrase inhibition assay.[7] In the initial screening of the crude extract of the Philippines sponge *Diplastrella* sp., several fractions of moderate polarity inhibited HIV-1 integrase activity. From these fractions, five novel brominated polyacetylenic diols (**2**-**6**) and three very unstable sulfated derivatives (**7**−**9**) were isolated and their structures elucidated. This is the first report of metabolites from the genus *Diplastrella* (order Hadromerida, family Spirastrellidae).

Results and Discussion

The *Diplastrella* sp. sponge was collected by hand using scuba at a depth of 30 ft off Boracay Island in the Philippines and was kept frozen until it was extracted with methanol. The methanol-soluble material was separated on a Diaion HP-20 column, eluting with increasing amounts of acetone. The 60% aqueous acetone fraction showed HIV integrase inhibition and was separated using flash chromatography on a RP-C_{18} column (methanol/water). Further purification using RP-HPLC using 85% aqueous methanol as eluant resulted in the isolation of diplynes A−E (**2**−**6**). The 40% aqueous acetone fraction was fractionated on a Sephadex LH-20 column using 100% methanol as eluant, followed by RP-HPLC using 85% aqueous methanol as eluant to obtain diplyne A 1-sulfate (**7**), diplyne C 1-sulfate (**8**), and 2-deoxydiplyne D sulfate (**9**).



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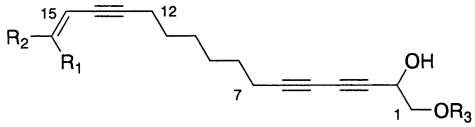
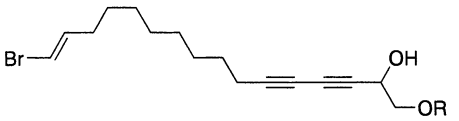
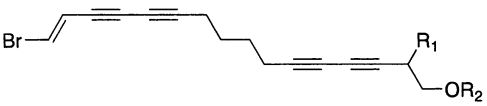


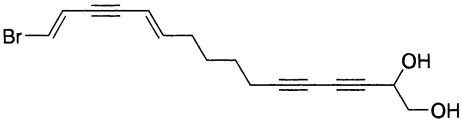
2 R_1= H; R_2 = Br; R_3 = H
3 R_1= Br; R_2 = H; R_3 = H
7 R_1= H; R_2 = Br; R_3 = SO_3H



4 R = H
8 R = SO_3H



5 R_1 = OH; R_2 = H
9 R_1 = H; R_2 = SO_3H



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Diplyne A (**2**), which was isolated as an optically active white powder, showed pseudomolecular $[M + Na]^+$ ions in the ESIMS at *m*/*z* 345 and 347 (1:1), indicating the presence of bromine. The molecular formula, $C_{16}H_{19}O_2Br$, which was deduced by high-resolution mass measurement of the $[M + NH_4]^+$ ion at *m*/*z* 340.0918 (Δ +0.6 mmu), requires seven degrees of unsaturation. Sixteen signals were observed in the ^{13}C NMR spectrum (Table 1), consistent with the molecular formula, six of which were assigned to three acetylenic groups and two to an olefin. Diplyne A

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Table 1. ^{1}H and ^{13}C Data in CD_3OD for Diplyne A and Diplyne A Sulfate

	diplyne A (**2**)		diplyne A sulfate (**7**)	
#	δ_H	δ_C	δ_H	δ_C
1	3.55	67.1	4.02	71.8
	3.58		3.97	
2	4.34	64.5	4.60	62.2
3		75.8		74.6
4		70.7		70.9
5		65.6		65.4
6		81.6		81.9
7	2.30	19.7[a]	2.30	19.7[a]
8	1.52	29.2[b]	1.52	29.2[b]
9	1.41	29.3[b]	1.40	29.3[b]
10	1.41	29.3[b]	1.40	29.3[b]
11	1.52	29.4[b]	1.52	29.4[b]
12	2.30	20.0[a]	2.30	20.0[a]
13		93.6		93.6
14		78.2		78.1
15	6.22	117.7	6.20	117.7
16	6.69	119.0	6.69	119.0

[a] C-7 and C-12 were indistinguishable. [b] C-8, C-9, C-10, and C-11 were indistinguishable.

was therefore acyclic. The UV spectrum contained absorptions at 238 (ϵ 13 300) and 217 nm (ϵ 12 500) due to conjugated unsaturated bonds. The IR spectrum indicated the presence of hydroxyl groups (3310 cm^{-1}), acetylenes (2250, 2210 cm^{-1}), and a vinyl group (1640 cm^{-1}). The ^{1}H NMR spectrum (Table 1) contained three isolated spin systems that are separated by the acetylene groups. Signals at δ 4.34 (1H, dd, J = 6.5, 5 Hz), 3.58 (1H, dd, J = 11, 6.5 Hz), and 3.55 (1H, dd, J = 11, 5 Hz) were assigned to a terminal diol adjacent to an acetylene group. Three signals at 1.41 (4H, m), 1.52 (4H, m), and 2.30 (4H, m) were due to six methylene groups flanked by acetylene groups. Signals at δ 6.22 (1H, dt, J = 14, 2 Hz, C-15) and 6.69 (1H, d, J = 14 Hz, C-16) were assigned to a *trans*-disubstituted double bond adjacent to an acetylene with a terminal bromine atom. The chemical shifts of the H-15 and H-16 signals provided further support for the location of the bromine at the C-16 terminus. The COSY spectrum revealed that the H-15 signal was coupled to the H-12 signal (J = 2 Hz), which is typical of an enyne system. The ^{13}C data for C-1 to C-7 were comparable to those of the C-1 to C-7 region of callytriol A,[6] which led to the placement of acetylenes at C-3, C-5, and C-13. The HSQC and HMBC experiments allowed assignment of the ^{13}C NMR signals, and the HMBC correlations from H-2 to C-1, C-3, C-4, and C-5, from H-7 to C-6, C-5, C-4, and C-3, and from H-12 to C-13 and C-14 confirmed the locations of the diyne and enyne units and established the structure of diplyne A (**2**) as (15*E*)-16-bromohexadeca-15-en-3,5,13-triyne-1,2-diol.

Diplyne B (**3**) was obtained as a white powder. The molecular formula, $C_{16}H_{19}O_2Br$, the same as that of diplyne A (**2**), was determined by high-resolution mass measurement of the $[M + NH_4]^+$ ion at m/z 340.0910 (Δ +0.2 mmu). The ^{1}H and ^{13}C data are nearly identical to diplyne A**,** with the exception of the H-15 signal at δ 6.32 (1H, dt, J = 7.5, 2 Hz) and the H-16 signal at 6.58 (1H, br d, J = 7.5 Hz) that share a coupling constant of 7.5 Hz, indicative of a *cis* olefin. Diplyne B (**3**) is therefore (15*Z*)-16-bromohexadeca-15-en-3,5,13-triyne-1,2-diol.

Diplyne C (**4**), a white powder, has the molecular formula $C_{16}H_{23}O_2Br$, as determined by high-resolution mass measurement of the $[M + NH_4]^+$ ion at m/z 344.1217 (Δ +0.8 mmu). The ^{13}C NMR spectrum contained four carbon signals in the acetylenic region (δ 75.8, 70.7, 65.5, 81.7) and two additional methylene signals (δ 19.8, 29.3, 29.8, 29.8, 30.0, 30.1, 30.3, 33.8), indicating that one of the acetylene groups found in diplyne A had been fully reduced. The ^{1}H spectrum contained signals at δ 6.08 (1H, d, J = 14 Hz, H-16) and 6.13 (1H, dt, J = 14, 7 Hz, H-15) due to the *trans* terminal olefin adjacent to a chain of methylene groups. Diplyne C (**4**) is therefore (15*E*)-16-bromohexadeca-15-en-3,5-diyne-1,2-diol.

Diplyne D (**5**), obtained as a white powder, has the molecular formula of $C_{16}H_{15}O_2Br$, as determined by high-resolution mass measurement of the $[M + NH_4]^+$ ion at m/z 336.0596 (Δ +0.3 mmu). This molecular formula required two degrees of unsaturation more than diplyne A (**2**). The ^{13}C spectrum contained eight acetylenic carbon signals at δ 86.2, 81.6, 77.1, 76.1, 72.1, 70.6, 66.0, and 65.8 and only four methylene carbon signals. The ^{13}C NMR signals at δ 77.1 (C-13), 72.1 (C-14), 122.8 (C-15), and 117.6 (C-16) indicate the additional acetylene must be situated at C-11. In addition, the ^{13}C data for C-1 to C-7 were identical to those of diplyne A, further supporting this assignment. The *trans*-geometry of the olefinic group was defined by the $J_{15,16}$ coupling constant of 14 Hz. Diplyne D (**5**) is therefore (15*E*)-16-bromohexadeca-15-en-3,5,11,13-tetrayne-1,2-diol.

Diplyne E (**6**), a white powder, has a molecular formula of $C_{16}H_{17}O_2Br$, which was determined by high-resolution mass measurement of the $[M + NH_4]^+$ ion at m/z 338.0747 (Δ +0.9 mmu). This molecular formula requires one degree of unsaturation more than diplyne A (**2**). The ^{1}H NMR spectrum contained signals due to an additional double bond at δ 5.60 (1H, dq, J = 16, 1.5 Hz, H-12) and 6.15 (1H, dt, J = 16, 7 Hz, H-11). The large coupling constants between H-11 and H-12 (J = 16 Hz) and between H-15 and H-16 (J = 14 Hz) indicated 11*E*,15*E* geometry. The H-12 signal was also coupled to the H-15 signal ($J_{12,15}$ = 1.5 Hz), which is typical of an ene-yne-ene system. Diplyne E (**6**) is therefore (11*E*,15*E*)-16-bromohexadeca-11,15-dien-3,5, 13-triyne-1,2-diol.

Diplyne A 1-sulfate (**7**) was isolated as an unstable white powder that decomposed both in concentrated solutions and as a powder. The molecular formula, $C_{16}H_{19}O_5SBr$, was determined by high-resolution mass measurement of the $[M - H]^-$ ion at m/z 401.0047 (Δ −1.1 mmu). The IR spectrum showed a characteristic sulfate band at 1220 cm^{-1}. Comparison of ^{1}H and ^{13}C NMR data (Table 1) with those of diplynes A−E revealed that the sulfate was a 1-sulfated derivative of diplyne A (**2**). In the ^{1}H NMR spectrum, H_2-1 and H-2 were at δ 3.97 (H-1), 4.02 (H-1), and 4.60 (H-2) in **7** as opposed to 3.58 (H-1), 3.55 (H-1), and 4.34 (H-2) in **2**. In the ^{13}C NMR spectrum, the C-1 to C-3 signals were at δ 71.8 (C-1), 62.2 (C-2), and 74.6 (C-3) in **7** as opposed to 67.1 (C-1), 64.5 (C-2), and 75.8 (C-3) in **2**. Diplyne A 1-sulfate (**7**) is therefore (15*E*)-16-bromohexadeca-15-en-3,5,13-triyne-1,2-diol 1-sulfate.

Diplyne C sulfate (**8**), an unstable white powder, had the molecular formula $C_{16}H_{23}O_5SBr$, which was determined by high-resolution mass measurement of the $[M - H]^-$ ion at m/z 405.0391 (Δ +2.0 mmu). Comparison of the ^{1}H and ^{13}C NMR spectra with those of diplynes A−E revealed a very close match with those of diplyne C (**4**), except that the signals for H-1, H-2, C-1, C-2, and C-3 were nearly identical to those of diplyne A 1-sulfate (**7**). The compound is therefore diplyne C 1-sulfate (**8**) or (15*E*)-16-bromohexadeca-15-en-3,5-diyne-1,2-diol 1-sulfate.

2-Deoxydiplyne D sulfate (**9**), an unstable powder, had the molecular formula $C_{16}H_{15}O_4SBr$, which was determined by high-resolution mass measurement of the $[M - H]^-$ ion at m/z 380.9796 (Δ 0.0 mmu). Comparison of the ^{1}H and

^{13}C NMR spectra with those of diplynes A−E revealed that the C-5 to C-16 portion of the molecule was the same as that of diplyne D (**5**). In the ^{1}H NMR spectrum, the signal at δ 4.03 (2H, t, J = 7 Hz, H_2-1) was coupled to a methylene signal at 2.64 (2H, t, J = 7 Hz, H_2-2), indicating that **9** was a 2-deoxy derivative of diplyne D (**5**) with a sulfate group at C-1. The ^{13}C NMR signals at δ 65.9 (C-1), 21.1 (C-2), 74.0 (C-3), and 67.3 (C-4) support this assignment. 2-Deoxydiplyne D sulfate (**9**) is therefore (15*E*)-16-bromohexadeca-15-en-3,5,11,13-tetrayne-1-ol 1-sulfate.

Although the compounds were purified on the basis of HIV-1 integrase inhibition, the pure compounds diplynes A−E (**2**−**6**) did not significantly inhibit integrase (IC_{50} >50 μg/mL). The sulfated compounds (**7**−**9**) exhibited mild inhibition of HIV-1 integrase (~30 to 90 μg/mL), which is consistent with the activity observed for sulfated compounds in this assay.[8] However, these compounds degrade rapidly from pure white powders to dark pink to red powders. At the time of the assay, these compounds had partially degraded and it is possible that compounds produced by the degradation process are responsible for the observed activity.

Experimental Section

General Methods. Optical rotations were measured using a Rudolph Autopol III polarimeter at 589 nm. Infared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer, and ultraviolet spectra were recorded using a Varian Cary 50 Bio spectrometer. ^{13}C NMR experiments were recorded on a Varian Gemini 400 MHz spectrometer, and ^{1}H and all two-dimensional NMR experiments were recorded using a Varian Inova 300 MHz spectrometer. High-resolution mass measurements were recorded on a VG 7070 mass spectrometer at the UC Riverside Mass Spectrometry Facility. All solvents were distilled prior to use.

Animal Material. The sponge *Diplastrella* sp. (order Hadromerida, family Spirastrellidae) was collected by hand using scuba at a depth of 30 ft off Boracay Island in the Philippines, in May 1998, and was immediately frozen. The sponge was burnt orange in color, 2−3 mm thick encrusting. The skeleton consists of plumose tracts of tylostyles with a fan of smaller tylostyles protruding through the surface. Diplaster microscleres are concentrated at the base of the fans and are scattered throughout the mesohyl. This sponge is probably a new species, as those already described are from the Mediterranean. Voucher specimens have been deposited in the Queensland Museum (registry # QMG319715) and the Scripps Institution of Oceanography Benthic Invertebrate Collection (# P1182).

Isolation. The sponge (188 g wet wt) was extracted with methanol (2 × 1 L). The crude extract was cyclic loaded onto a Diaion HP-20ss column with increasing amounts of water. Water was washed through the column to remove any excess salts, and the organic material was eluted using increasing amounts of acetone up to 100% acetone. The 60% aqueous acetone fraction showed inhibition of HIV integrase, and a portion was further purified on a silica gel column using 1:1 hexane and ethyl acetate as eluant. The active fractions were combined and separated by reversed-phase HPLC using 85% aqueous methanol as eluant to obtain diplynes A (**2**, 15 mg, 8 × 10^{-3} % wet wt), B (**3**, 2.0 mg, 1 × 10^{-3} % wet wt), C (**4**, 4 mg, 2.1 × 10^{-3} % wet wt), D (**5**, 2.0 mg, 1 × 10^{-3} % wet wt), and E (**6**, 1.5 mg, 8 × 10^{-4} % wet wt). The 40% acetone fraction was chromatographed on a Sephadex LH-20 column using 100% methanol as eluant. Active fractions were combined and separated by reversed-phase HPLC using 85% aqueous methanol as eluant to obtain diplyne A sulfate (**7**, 5 mg, 2.6 × 10^{-3} % wet wt), diplyne C sulfate (**8**, 2.0 mg, 1 × 10^{-3} % wet wt), and 2-deoxydiplyne D sulfate (**9**, 2.0 mg, 1 × 10^{-3} % yield).

Diplyne A (2): white powder; $[\alpha]_D$ −8.7° (*c* 0.33, MeOH); UV (MeOH) 238 (ϵ 13 300), 217 nm (ϵ 12 500); IR (film) 3310, 2250, 2210, 1640, 1460, 1090, 920, 730 cm^{-1}; ESI MS (+ve) *m/z* 345/347 $[M + Na]^+$; HRCIMS $[M + NH_4]^+$ *m/z* 340.0918 (calcd for $C_{16}H_{19}O_2{}^{79}Br$, 340.0912); ^{1}H and ^{13}C NMR (see Table 1).

Diplyne B (3): white powder; UV (MeOH) 246 (ϵ 21 000), 235 (ϵ 21 400), 215 nm (ϵ 40 900); ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.41 (4H, m, H_2-9/H_2-10), 1.52 (4H, m, H_2-8/H_2-11), 2.30 (2H, t, J = 7 Hz, H_2-7), 2.36 (2H, td, J = 7, 2 Hz, H_2-12), 3.54 (1H, dd, J = 11, 5 Hz, H-1), 3.57 (1H, dd, J = 11, 5 Hz, H-1), 4.34 (1H, t, J = 5 Hz, H-2), 6.32 (1H, dt, J = 7.5, 2 Hz, H-15), 6.58 (1H, br d, J = 7.5 Hz, H-16); ^{13}C NMR (100 MHz, MeOH-d_4) δ 117.0 (C-16), 116.7 (C-15), 99.3 (C-13), 81.6 (C-6), 77.8 (C-14), 75.8 (C-3), 70.7 (C-4), 67.1 (C-1), 65.6 (C-5), 64.5 (C-2), 29.4, 29.3, 29.3, 29.2 (C-8 to C-11), 20.0 (C-7 or C-12), 19.7 (C-7 or C-12); ESI MS (+ve) *m/z* 345/347 $[M + Na]^+$; HRCIMS $[M + NH_4]^+$ *m/z* 340.0910 (calcd for $C_{16}H_{19}O_2{}^{79}Br$, 340.0912).

Diplyne C (4): white powder; UV (MeOH) 214 nm (ϵ 37 300); ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.31 (6H, m, H_2-10/H_2-11/H_2-12), 1.39 (4H, m, H_2-9/H_2-13), 1.51 (2H, m, H_2-8), 2.05 (2H, q, J = 7 Hz, H-14), 2.29 (2H, m, J = 6.5 Hz, H-7), 3.50 (1H, dd, J = 11, 6.5 Hz, H-1), 3.52 (1H, dd, J = 11, 5 Hz, H-1), 4.33 (1H, dd, J = 6.5, 5 Hz, H-2), 6.08 (1H, d, J = 14 Hz, H-16), 6.13 (1H, dt, J = 14, 7 Hz, H-15); ^{13}C NMR (100 MHz, MeOH-d_4) δ 139.2 (C-15), 104.9 (C-16), 81.7 (C-6), 77.8 (C-14), 75.8 (C-3), 70.7 (C-4), 67.1 (C-1), 65.5 (C-5), 64.5 (C-2), 33.8 (C-14), 30.3, 30.1, 30.0, 29.8 (2C), 29.3, 29.2, 19.8 (C-7); ESI MS (+ve) *m/z* 349/351 $[M + Na]^+$; HRCIMS $[M + NH_4]^+$ *m/z* 344.1217 (calcd for $C_{16}H_{23}O_2{}^{79}Br$, 344.1225).

Diplyne D (5): white powder; UV (MeOH) 290 (ϵ 13 200), 276 (ϵ 16 700), 260 (ϵ 11 300), 246 (ϵ 6300), 215 nm (ϵ 61 700); ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.63 (m, 4 H, H_2-8/H_2-9), 2.33 (m, 4 H, H_2-7/H_2-10), 3.54 (dd, 1 H, J = 11, 6.5 Hz, H-1), 3.57 (dd, 1 H, J = 11, 6 Hz, H-1), 4.34 (dd, 1 H, J = 6.5, 6 Hz, H-2), 6.32 (dt, 1 H, J = 14, 1 Hz, H-15), 7.00 (d, 1 H, J = 14 Hz, H-16); ^{13}C NMR (100 MHz, MeOH-d_4) δ 122.8 (C-15), 117.6 (C-16), 86.2 (C-11), 81.6 (C-6), 77.1 (C-13), 76.1 (C-3), 72.1 (C-14), 70.6 (C-4), 67.1 (C-1), 66.0 (C-12), 65.8 (C-5), 64.5 (C-2), 28.4 (C-8 or C-9), 28.3 (C-8 or C-9), 19.7 (C-7 or C-10), 19.3 (C-7 or C-10); ESI MS (+ve) *m/z* 341/343 $[M + Na]^+$; HRCIMS $[M + NH_4]^+$ *m/z* 336.0596 (calcd for $C_{16}H_{15}O_2{}^{79}Br$, 336.0599).

Diplyne E (6): white powder; UV (MeOH) 287 (ϵ 3410), 272 (ϵ 4120), 215 nm (ϵ 43 300); ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.52 (4H, m, H_2-8/H_2-9), 2.13 (2H, qd, J = 7, 1.5 Hz, H_2-10), 2.30 (2H, td, J = 6, 1 Hz, H_2-7), 3.54 (1H, dd, J = 11, 7 Hz, H-1), 3.57 (1H, dd, J = 11, 5 Hz, H-1), 4.32 (1H, dd, J = 7, 5 Hz, H-2), 5.60 (1H, dq, J = 16, 1.5 Hz, H-12), 6.15 (1H, dt, J = 16, 7 Hz, H-11), 6.35 (1H, dt, J = 14, 2 Hz, H-15), 6.77 (1H, d, J = 14 Hz, H-16); ^{13}C NMR (100 MHz, MeOH-d_4) δ 146.1 (C-11), 118.7 (C-15 or C-16), 118.4 (C-15 or C-16), 110.6 (C-12), 91.3 (C-13), 85.3 (C-14), 81.4 (C-6), 75.9 (C-3), 70.6 (C-4), 67.1 (C-1), 65.7 (C-5), 64.5 (C-2), 33.5 (C-10), 28.9 (C-8 or C-9), 28.7 (C-8 or C-9), 19.6 (C-7); ESI MS (+ve) *m/z* 343/345 $[M + Na]^+$; HRCIMS $[M + NH_4]^+$ *m/z* 338.0747 (calcd for $C_{16}H_{17}O_2{}^{79}Br$, 338.0755).

Diplyne A sulfate (7): white powder; rapidly decomposes; ESI MS (+ve) *m/z* 447/449 $[M + Na]^+$; HRESIMS $[M - H]^-$ *m/z* 401.0047 (calcd for $C_{16}H_{19}O_5S^{79}Br$, 401.0058); ^{1}H and ^{13}C NMR (see Table 1).

Diplyne C sulfate (8): white powder; rapidly decomposes; UV (MeOH) 240 (ϵ 5000), 215 nm (ϵ 48 400); ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.31 (6H, m, H_2-10/H_2-11/H_2-12), 1.40 (4H, m, H_2-9/H_2-10), 1.52 (4H, m, H_2-8/H_2-11), 2.05 (2H, dd, J = 7, 6.5 Hz, H_2-14), 2.30 (4H, t, J = 7 Hz, H_2-7/H_2-12), 3.95 (1H, dd, J = 10.5, 7 Hz, H-1), 4.02 (dd, 1 H, J = 10.5, 6 Hz, H-1), 4.60 (1H, dd, J = 7, 6 Hz, H-2), 6.12 (1H, d, J = 13.5 Hz, H-16), 6.16 (1H, dt, J = 13.5, 7 Hz, H-15); ^{13}C NMR (100 MHz, MeOH-d_4) δ 139.2 (C-15), 104.9 (C-16), 82.0 (C-6), 74.6 (C-3), 71.8 (C-1), 70.9 (C-4), 65.3 (C-5), 62.2 (C-2), 33.8 (C-14), 30.4, 30.1, 30.0, 29.9, 29.8, 29.3, 19.8 (C-7); ESI MS (+ve) *m/z* 451/453 $[M + Na]^+$; HRESIMS $[M - H]^-$ *m/z* 405.0391 (calcd for $C_{16}H_{23}O_5S^{79}Br$, 405.0371).

2-Deoxydiplyne D sulfate (9): white powder, rapidly decomposes; UV (MeOH) 290 (ϵ 52 400), 274 (ϵ 62 700), 259 (ϵ 42 300), 246 nm (ϵ 22 400); ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.63 (4H, m, H_2-8/H_2-9), 2.29 (2H, t, J = 6 Hz, H_2-7), 2.36 (2H,

t, $J = 6$ Hz, H_2-10), 2.64 (2H, t, $J = 7$ Hz, H_2-2), 4.03 (2H, t, $J = 7$ Hz, H_2-1), 6.33 (1H, d, $J = 14$ Hz, H-15), 7.00 (1H, d, $J = 14$ Hz, H-16); ^{13}C NMR (100 MHz, MeOH-d_4) δ 122.8 (C-16), 117.6 (C-15), 86.2 (C-11), 77.7 (C-6), 77.1 (C-13), 74.0 (C-3), 72.0 (C-14), 67.3 (C-4), 66.5 (C-5, C-12), 65.9 (C-1), 28.5 (C-8 or C-9), 28.3 (C-8 or C-9), 21.1 (C-2), 19.4 (C-7 or C-10), 19.3 (C-7 or C-10); ESI MS (+ve) *m/z* 427/429 $[M + Na]^+$; HRESIMS $[M - H]^-$ *m/z* 380.9796 (calcd for $C_{16}H_{15}O_4S^{79}Br$, 380.9796).

Bioassay. Integrase activity was tested by incubating recombinant integrase, marine extract, or purified compound and DNA substrates together in microtiter plates. Assays were arranged so that the action of integrase connected a biotinylated target DNA to a digoxigenin-modified viral DNA end. Reaction product could thus be assayed by capture on avidin-coated microtiter plates and detected using a colorimetric anti-digoxigenin ELISA.[7]

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Supporting Information Available: Spectra of **2**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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