

Supporting Information for:

Cyclodidemniserinol trisulfate, a sulfated serinolipid from the Palauan ascidian *Didemnum guttatum* that inhibits HIV-1 integrase

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1. Experimental Section (2 pages).
2. ^1H NMR spectrum (500 MHz, DMSO- d_6) of **1**.
3. ^{13}C NMR spectrum (100 MHz, DMSO- d_6) of **1**.
4. COSY spectrum of **1**.
5. HMBC spectrum of **1**.
6. HSQC-TOCSY spectrum of **1**.

Experimental Section

General Methods: The IR and UV spectra were recorded on Perkin-Elmer 1600 and Lambda 3B instruments, respectively. ^1H NMR, COSY, TOCSY, HMQC-TOCSY experiments were recorded on a Varian Inova 300 MHz Spectrometer. The HMBC experiment was acquired on a 500 MHz Varian Gemini spectrometer, and the HETCOR and ^{13}C experiments were performed on a 400 MHz Varian spectrometer equipped with a broad band nano probe tuned to ^{13}C . Chemical shifts are reported in parts per million, referenced to residual solvent peaks, and coupling constants (J), are reported in hertz. High resolution MALDI Fourier transform mass spectra were run on an Ionspec FTMS mass spectrometer at the mass spectrometry facility at the Scripps Research Institute.

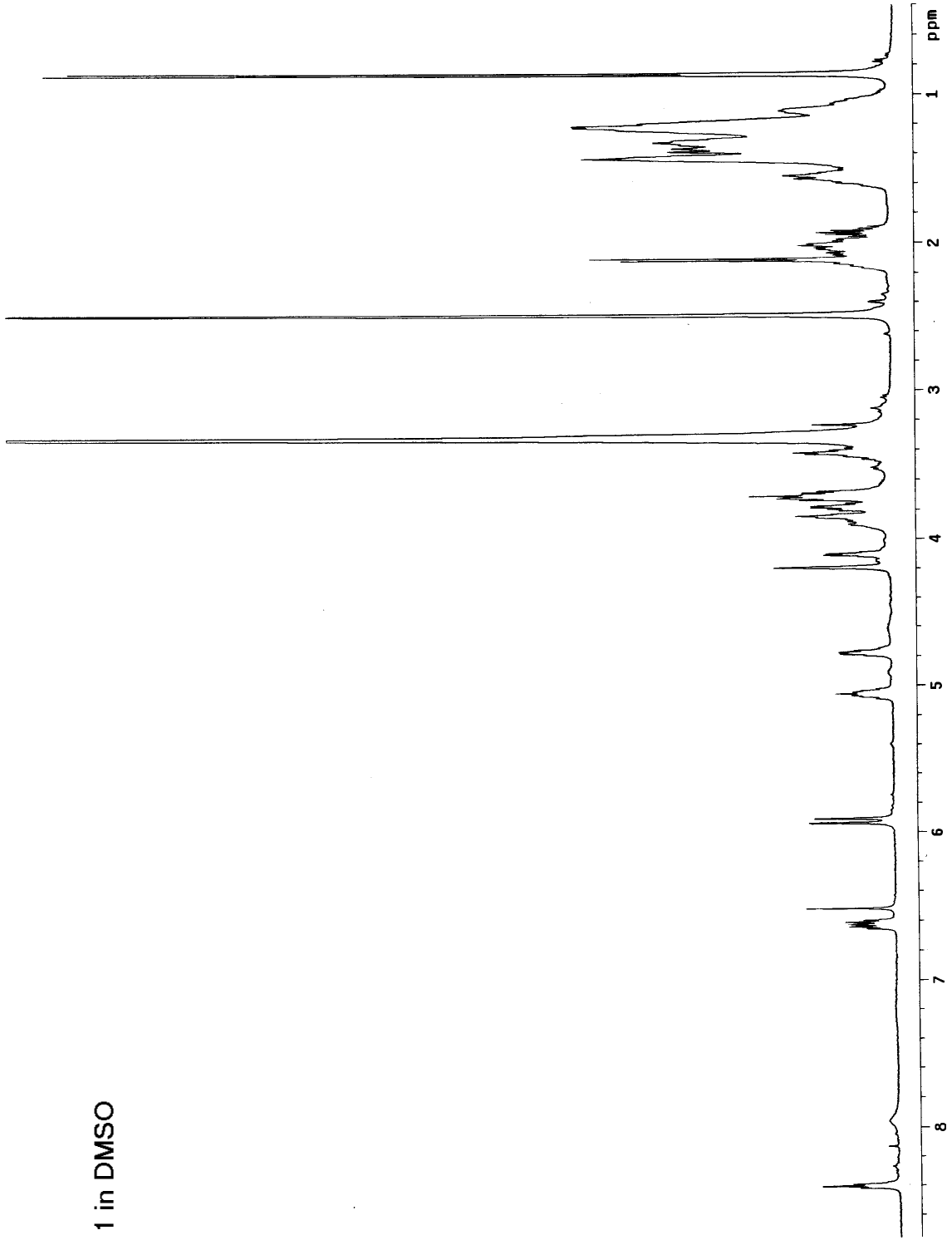
Isolation of cyclodidemniserinol trisulfate (1): The specimen of *Didemnum guttatum* (collection # 96-147) was collected by SCUBA at Ngerchaol Island, Palau and was immediately frozen. The frozen material (400 g wet wt) was extracted with MeOH (4×500 mL), and the extracts were concentrated to obtain a dark green residue (8 g). The residue was partitioned between EtOAc and H_2O , the H_2O -soluble material was lyophilized, and was resuspended in H_2O (10 mL). This solution was loaded onto a C_{18} Sep Pac (12 g), which was subsequently washed with H_2O (150 mL), 90% $\text{H}_2\text{O}/\text{MeOH}$ (100 mL), 75% $\text{H}_2\text{O}/\text{MeOH}$ (100 mL), 50% $\text{H}_2\text{O}/\text{MeOH}$ (100 mL), and 100% MeOH (200 mL). Activity in the HIV integrase assay was traced to the material eluting in 75% $\text{H}_2\text{O}/\text{MeOH}$, and this material was further purified by either C_{18} HPLC or C_{18} flash column chromatography. Final purification by HPLC was achieved on a C_{18} column eluted with a gradient from 90% to 50% $\text{H}_2\text{O}/\text{acetonitrile}$ over 30 minutes to yield cyclodidemniserinol trisulfate (**1**) as a colorless oil (20 mg, 5×10^{-3} % yield): White solid; $[\alpha]_{\text{D}} -26.6^\circ$ (c 0.073, MeOH); UV (MeOH) λ_{max} 210 nm (ϵ 4500); IR (thin film, polystyrene) 2450, 3321, 2068, 1727, 1667, 1625, 1220, 1215, 1119, 1047, 1005 cm^{-1} ; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) see Table 1); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) see Table 1; ESIMS m/z 1039 $[\text{M} + \text{Na}]^+$, 937, 916, 836, 733; HRMS m/z 993.2961 $[\text{M}-\text{Na}]^-$, $\text{C}_{38}\text{H}_{63}\text{N}_2\text{O}_{19}\text{S}_3\text{Na}_2$ requires 933.2983.

Preparation of cyclodidemniserinol (3) for mass spectral analysis:

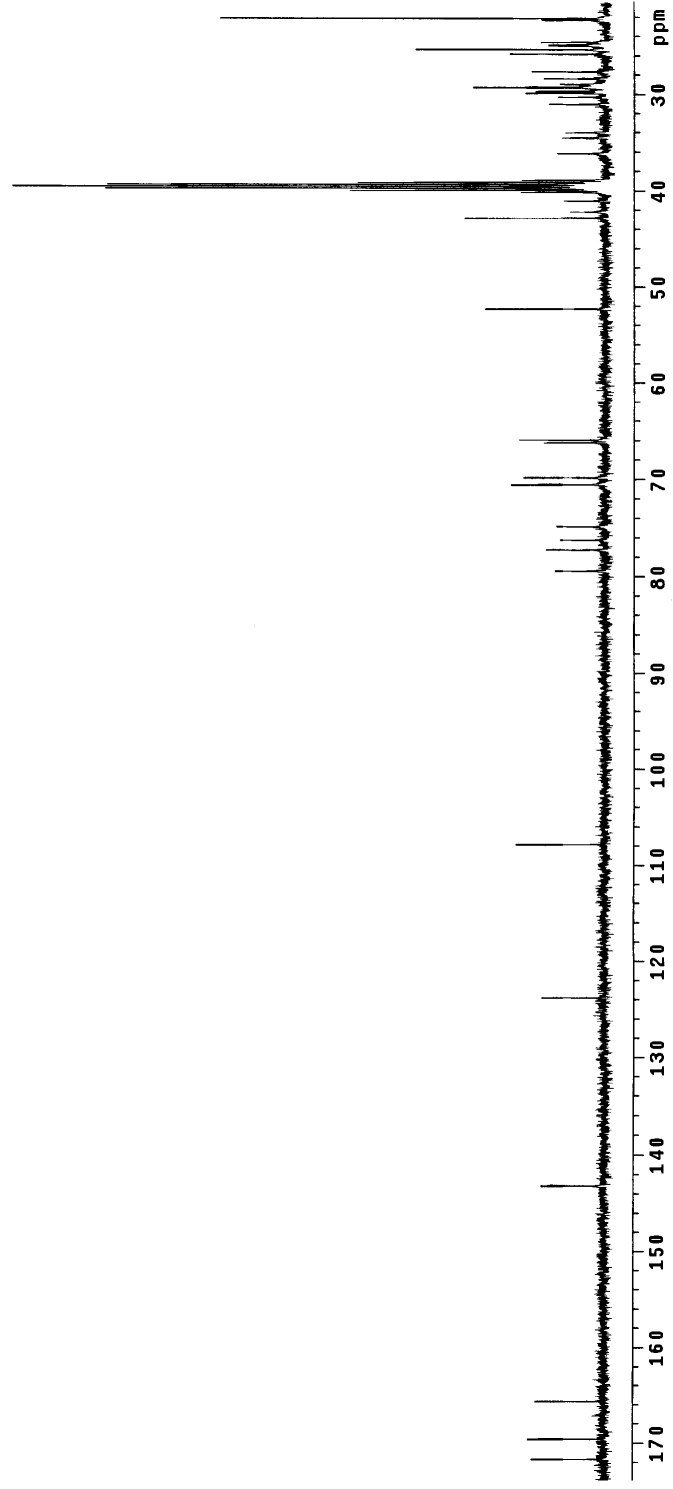
Cyclodidemniserinol trisulfate (**1**, 0.1 mg) was placed in a thick walled 1 ml vial and dissolved in THF (900 μL), H_2O (100 μL), and 1 M H_2SO_4 (5 μL). The vial was tightly capped and heated to 90° for 1 h, then neutralized by adding solid NaCO_3 (20 mg). The supernatant was removed and dried under reduced pressure. The APCI mass spectra of the resulting residue yielded ions at m/z 733 ($\text{M}+\text{Na}^+$), 711 (M^+), and 647 (hydrolysis of isovalerate ester), 376, 334, 203.

Permanganate oxidation of cyclodidemniserinol trisulfate (1): An aqueous solution of cyclodidemniserinol trisulfate (**1**, 0.75 mg in 600 μL) was adjusted to pH 3 with HCl followed by addition of potassium permanganate (3 mg). The reaction mixture was stirred overnight, partitioned between EtOAc (5 mL) and H_2O (5 mL), and the EtOAc soluble material was dried under reduced pressure. The residue was re-dissolved in CH_2Cl_2 (2 mL) and methylated using diazomethane generated using a DMMNG kit (Aldrich Chemical). The resulting derivative was analyzed using GC-MS on an EC-5 column (Alltech) with a temperature gradient from 50 to 150° over 20 minutes. Standards of dimethyl adipate, dimethyl pimelate, and dimethyl suberate were purchased from Arcos Chemical, and a co-injection experiment determined that dimethyl pimelate co-eluted with a major GC-MS peak observed at 6.9 min in the oxidation product of **1**. The EI spectra of the dimethyl pimelate standard and GC-MS peak observed in the reaction product were identical (m/z 157 ($\text{M}-\text{OCH}_3$, 40%), 128 (43 %), 125 (45%), 115 (98%), 74 (100%).

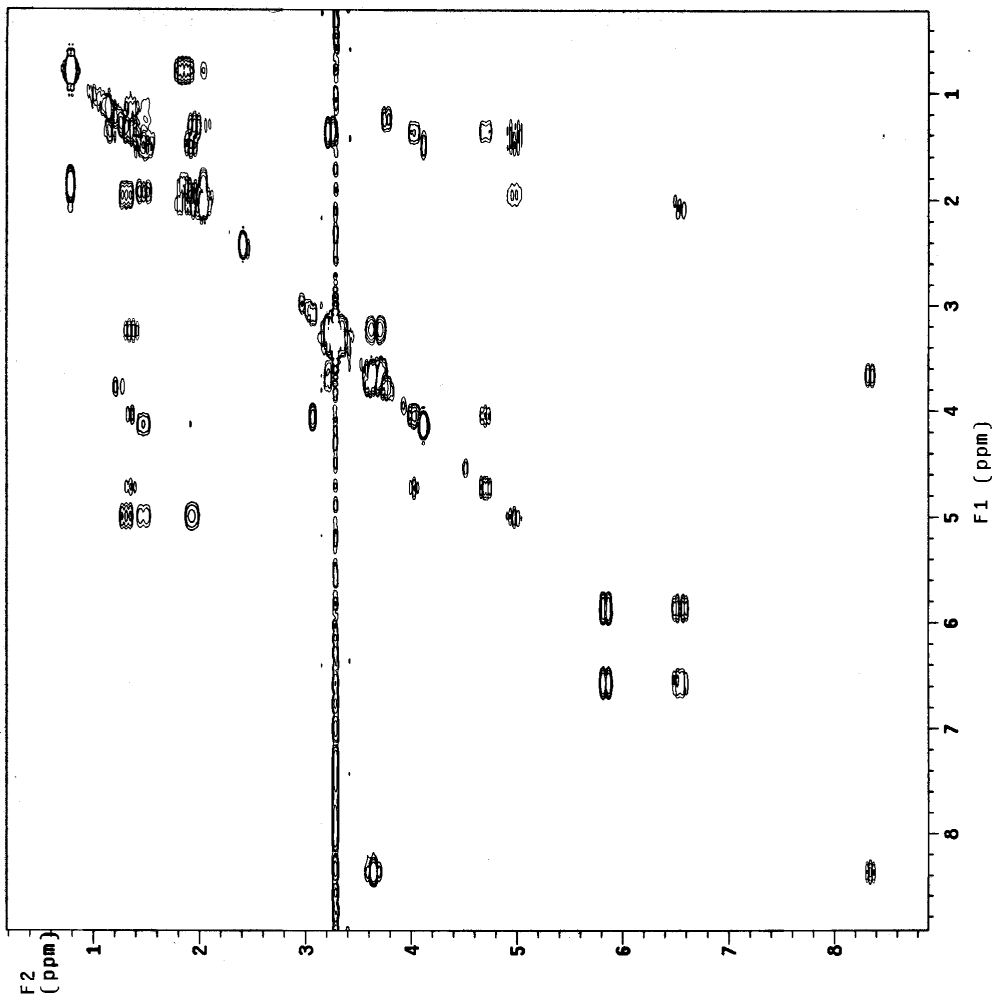
1H of 1 in DMSO



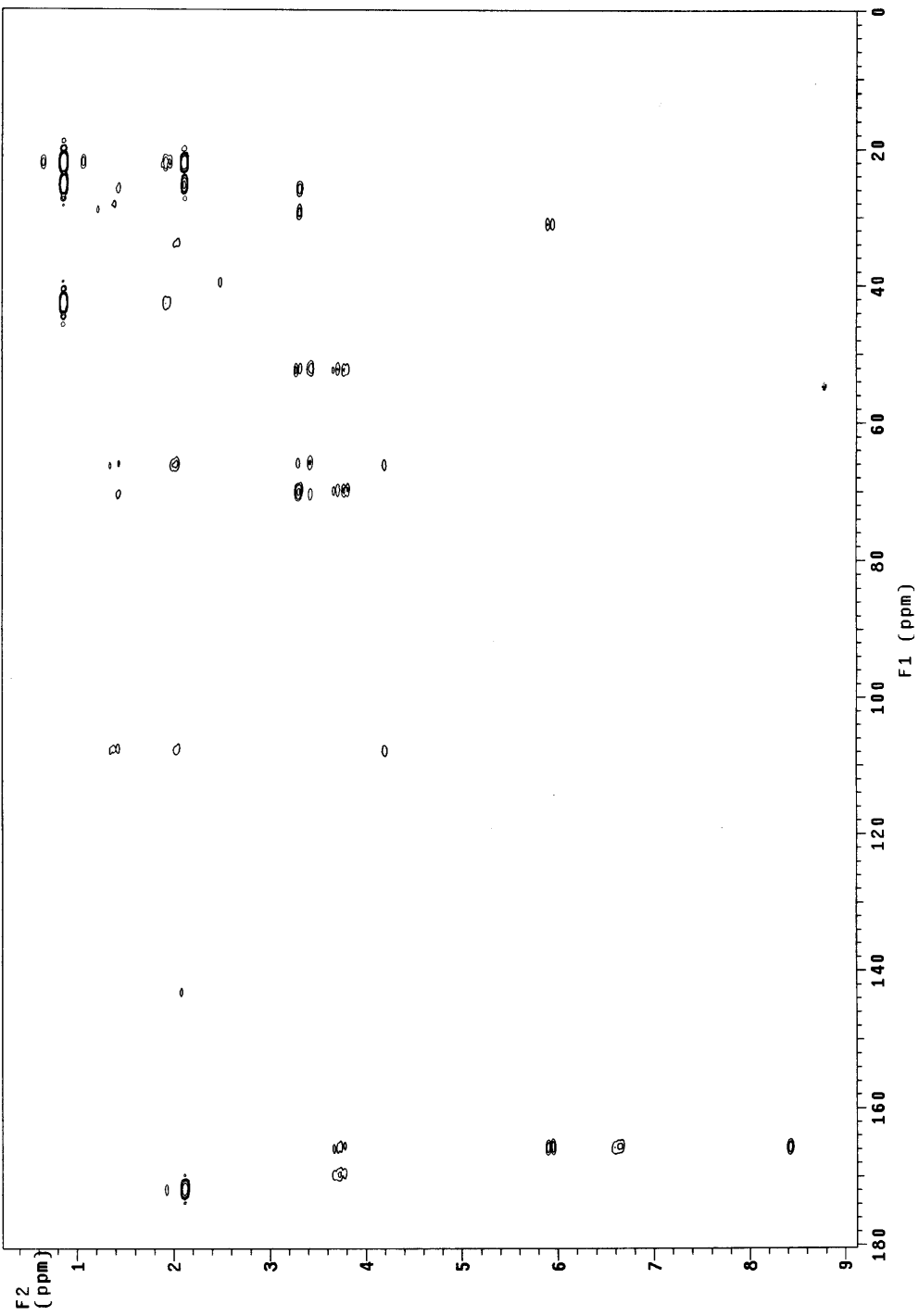
¹³C of 1 in DMSO



COSY of 1 in DMSO



HMBC of 1 in DMSO



HSQC-TOCSY of 1 in DMSO

