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Isolation and structure elucidation of two phosphorylated sterol sulfates, MT1-MMP inhibitors from a marine sponge *Cribrochalina* sp.: revision of the structures of haplosamates A and B☆

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Abstract—Two inhibitors of membrane-type matrix metalloproteinase (MT1-MMP) were isolated from a marine sponge *Cribrochalina* sp. Their structures were elucidated as unusual phosphorylated sterol sulfates by spectroscopic and chemical methods. One of the sterol sulfates was found to be identical to haplosamate A, whose structure was previously proposed to be a steroidal sulfamate ester, which led to structure revisions of both haplosamates A and B. The absolute stereochemistry of haplosamate A was determined by chemical transformation and the modified Mosher's method. The sterol sulfates inhibited MT1-MMP with IC_{50} values of 150 and 160 μg/mL, respectively.

1. Introduction

The membrane-type matrix metalloproteinases (MT-MMPs) are known as key enzymes in tumor metastasis.[2] They activate progelatinase A to the fully matured form which in turn degrades type IV collagen, the major component of the basal membrane which prevents tumor progression. Thus, inhibitors of MT-MMPs are believed to be potential anticancer drugs. In our screening for inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP) in Japanese marine invertebrates,[3] we found significant activity in the methanol extract of a marine sponge *Cribrochalina* sp. collected in western Japan. Bioassay-guided isolation afforded two active compounds whose structures were elucidated as unusual phosphorylated sterol sulfates by spectroscopic and chemical methods. One of these sterol sulfates was found to be identical with haplosamate A which was reported from Philippine haplosclerid sponges and proposed to be a steroidal sulfamate ester.[4] Accordingly, these results led to structure revisions of both haplosamates A and B. This paper describes isolation and structure elucidation of two MT1-MMP inhibitors as well as structure revision of haplosamates A and B.

2. Results and discussion

The frozen sponge was extracted with MeOH, $CHCl_3$/MeOH, and EtOAc. The combined extracts were successively fractionated by solvent partitioning, reverse phase flash chromatography, gel filtration, and ODS HPLC. The active fractions were finally purified by ODS HPLC using a gradient elution system of aq. MeCN containing 250 mM $NaClO_4$ to afford two active compounds **1** and **2** in yields of 2.0 and 0.18×10^{-2}% on the basis of wet sponge, respectively.

The major inhibitor **1** showed a prominent ion peak at *m/z* 653 in the negative mode FAB mass spectrum. It did not take a long time for us to realize that the ^{1}H and ^{13}C NMR spectra were also superimposable on those of haplosamate A (**4**), an HIV integrase inhibitor isolated from two Philippine haplosclerid sponges.[4] Moreover, interpretation of 2D

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Keywords: biologically active compounds; enzyme inhibitors; marine metabolites; steroids and sterols.

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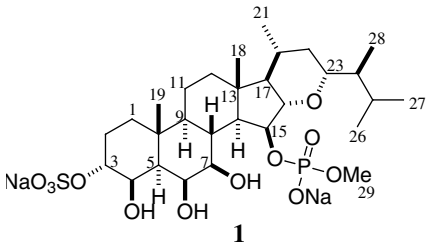

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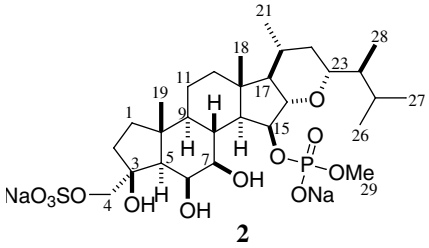

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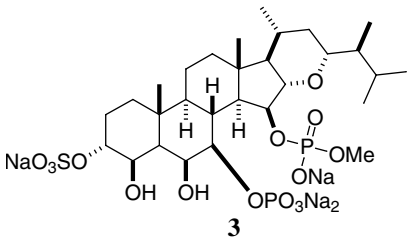

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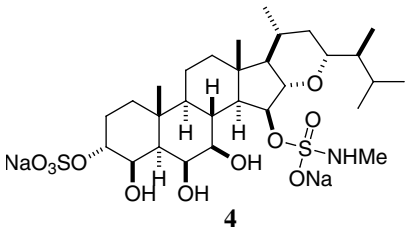

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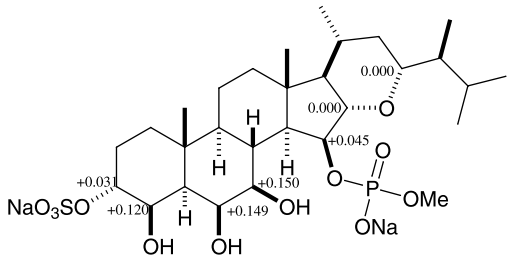
NMR data led to the proposed structure for **4** including the relative stereochemistry, except for the *N*-methylsulfamate group. Then, we became aware of a characteristic *O*-methyl doublet signal coupled to a phosphorus atom in the ^{1}H NMR spectrum. Further inspection of NMR data implied the presence of a methylphosphate functionality; characteristic ^{1}H- and ^{13}C NMR signals coupled to ^{31}P were observed at δ_H 3.61 (1H, d, $^{3}J_{H,P}$=10.4 Hz) and δ_C 53.2 ($^{2}J_{C,P}$=12.9 Hz).[5] This was substantiated by observation of a signal at δ 2.12 in the ^{31}P NMR spectrum.† In fact, the FABMS peaks at *m/z* 653 $(M+H-2Na)^{-}$ matched the elemental composition of $C_{29}H_{50}O_{12}PS$ (*m/z* 653.2766, Δ+0.6 mmu) in the HRFAB mass spectrum. Finally, ^{1}H–^{31}P HMBC[6] cross peaks between the phosphate group and both Me-29 and H-15, supported by couplings observed in the ^{13}C NMR signals of C-14 ($^{3}J_{C,P}$=8.3 Hz) and C-15 ($^{2}J_{C,P}$=7.6 Hz), placed the methylphosphate group at C-15. Thus, the structure of haplosamate A is actually shown as **1**. Finally, direct comparison of haplosamate A with **1** disclosed their identity.

† ^{31}P chemical shift values were significantly dependent on pH of solvents. ^{31}P chemical shift of haplosamate A was shifted to −1.27 ppm upon addition of 1% of TFA.



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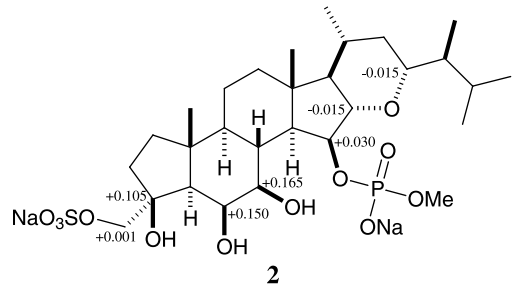

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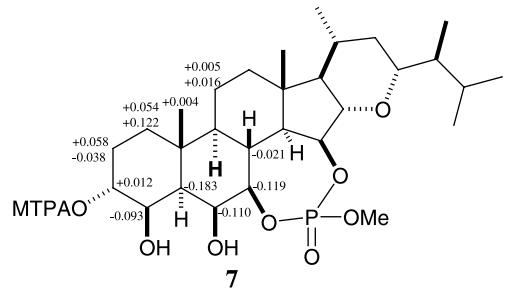
Figure 1. $\Delta\delta$=[δ_C(CD_3OD)−δ_C(CD_3OH)] values obtained for **1** and **2**.

The position of the sulfate group, which had been assigned on the basis of chemical shift arguments, was confirmed by comparing ^{13}C NMR chemical shifts in CD_3OD and CD_3OH. As shown in Fig. 1, C-4, C-6, and C-7 signals experienced chemical shift differences of 0.1 ppm or larger, while other signals including C-3 shifted less than 0.05 ppm. Therefore, the sulfate group was placed on C-3.

To determine the absolute stereochemistry of haplosamate A (**1**), we attempted to apply the modified Mosher's method.[7] Haplosamate A has three secondary hydroxyl groups; two of which are axial, while the rest is equatorial. Therefore, the equatorial hydroxyl group on C-7 was expected to be selectively esterified. In fact, it seemed that esterification at C-7 took place as expected. However,



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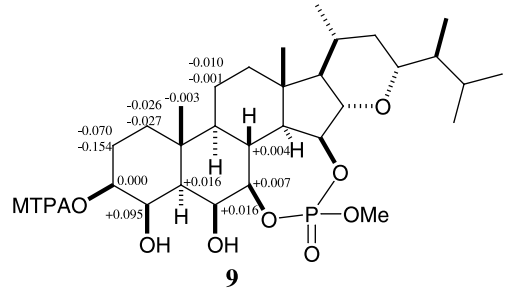

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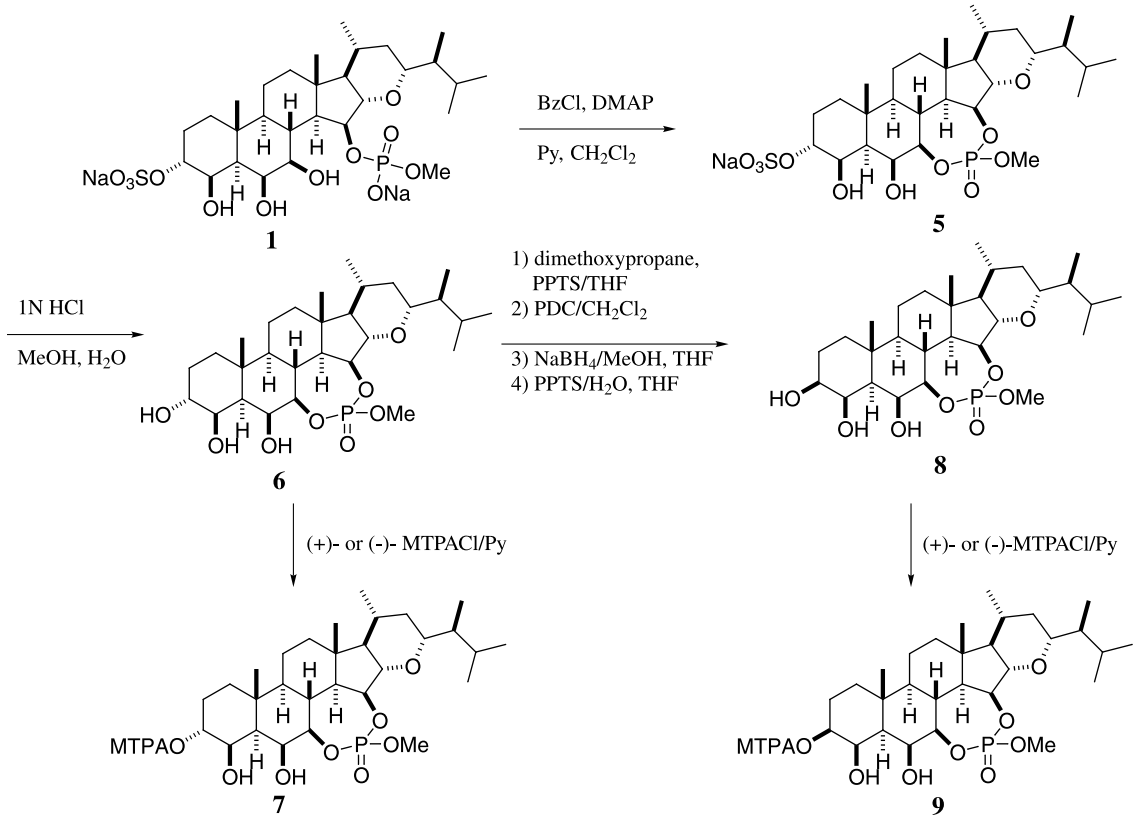
Figure 2. $\Delta\delta$=[$\delta_{S(-)}-\delta_{R(+)}$] values obtained for **7** and **9**.



Scheme 1. Preparation of 3-MTPA esters.

Table 1. ^{1}H, ^{13}C NMR and HMBC data for **2** in CD_3OD

#	δ_H mult. (*J*, Hz)	δ_C	HMBC
1α	1.09 q (10.4)	40.0	C-2, 9, 10, 19
1β	1.54 dd (8.5, 11.5)		C-2, 5, 9, 10
2α	2.12 m	37.9	C-1, 3, 4, 5, 10
2β	1.92 m		C-1, 4
3		81.2	
4	3.88 d (9.6)	73.1	C-2, 4, 5
	3.96 d (9.6)		C-2, 4, 5
5	1.35 d (2.3)	55.3	C-1, 3, 4, 7, 8, 9, 10, 19
6	4.36 dd (1.9, 3.5)	72.5	C-5, 7, 8, 10
7	3.37 dd (9.6, 3.6)	79.8	C-6, 8, 14
8	2.21 q (10.6)	35.6	C-7, 9, 14
9	0.93 m	54.2	C-7, 8, 10, 11, 19
10		45.3	
11α	1.37 m	22.8	
11β	1.64 m		C-9
12α	1.23 dt (3.9, 12.5, 12.5)	41.2	C-13, 18
12β	1.84 dt (15.4, 3.1, 3.1)		C-11
13		43.6	
14	1.40 m	58.9	C-7, 8, 9, 12, 13, 18
15	4.69 dt (2.7, 6.2, 6.2)	81.4	C-13, 16
16	3.83 dd (2.3, 10.0)	92.2	C-15, 20
17	0.73 t (10.4)	62.9	C-12, 13, 16, 18, 20, 21
18	1.01 s	16.9	C-12, 13, 14, 17
19	1.32 s	18.2	C-1, 5, 9, 10
20	1.80 m	34.2	C-17
21	0.98 d	20.8	C-17, 20, 22
22α	0.85 m	40.2	C-20, 21, 23
22β	1.64 m		
23	3.43 m	82.6	C-24
24	1.42 m	45.2	C-23, 25, 26, 27, 28
25	2.08 m	28.3	C-24, 26, 27, 28
26	0.81 d (6.5)	17.6	C-24, 25, 27
27	0.88 d (6.8)	21.8	C-24, 25, 26
28	0.76 d (6.9)	10.6	C-23, 24, 25
29	3.60 d (11.2)	53.2	

undesirable ester exchange with the methyl-phosphate group quickly proceeded to form a cyclic phosphotriester **5**. We decided to take advantage of this transformation; the sulfate ester was expected to be more labile toward acid hydrolysis than the phosphate ester. Haplosamate A (**1**) was treated with benzoyl chloride to afford the cyclic phosphotriester **5**, which was hydrolyzed to yield triol **6**. Although **6** has three hydroxyl groups, among which those on C-4 and C-6 appeared to be less reactive due to steric hinderance of C-19. As expected, reaction of **6** with (+)- or (−)-MTPACl afforded only the monoester **7**. The distribution of $\Delta\delta$ values except for H-2α was consistent with 3*R*-stereochemistry (Fig. 2). Although the overall data indicated the applicability of the MTPA method to this axial alcohol, we decided to confirm this result by preparing the epimeric alcohol. Triol **6** was converted to the 4,6-isopropyridene derivative which was oxidized with PDC to afford 3-ketone. This was reduced with $NaBH_4$ followed by deprotection of the 4,6-isopropyridene group to afford **8** as the major product. Esterification of **8** with (+)- or (−)-MTPACl took place selectively at C-3 to afford monoester **9**. The distribution of $\Delta\delta$ values was solely in agreement with 3*S*-stereochemistry (Fig. 2). Thus, haplosamate A has the stereochemistry of conventional steroids (Scheme 1).

The minor inhibitor **2** had the molecular formula identical with that of haplosamate A (**1**). NMR data indicated that **2** was closely related to **1** except for the ring A structure (Table 1). Interpretation of COSY data disclosed that H-3 and H-4 were missing as evidenced by the lack of further coupling of H-2 and H-5 (Fig. 3). Two new signals were observed instead; an isolated oxygenated methylene (δ_H

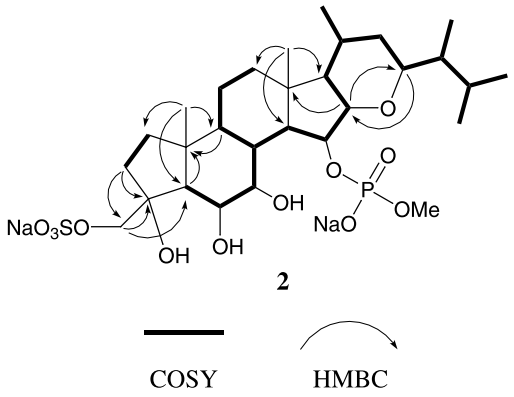


Figure 3. COSY and key HMBC correlations observed for **2**.

3.88, d, J=9.6 Hz, 3.96, d, J=9.6 Hz; δ_C 73.1) and an oxygenated quaternary carbon (δ_C 81.2). HMBC data demonstrated that C-2 and C-5 were attached to the quaternary carbon to which were linked a hydroxymethyl and a hydroxyl group, thus forming the 3-hydroxymethyl-3-hydroxyl nor A-ring (Fig. 3). A deuterium shift experiment in the ^{13}C NMR spectrum implied that C-4 was sulfated (Fig. 1). Other structural features were identical with those of haplosamate A. The relative stereochemistry at C-3 was established on the basis of ROESY[8] data, in which H-5 was correlated with the isolated oxygenated methylene protons, thereby indicating the α-orientation of the hydroxymethyl group and β-orientation of the hydroxyl group on C-3. Thus, the structure of **2** is as shown.

Haplosamate B (**3**), $[\alpha]_D$=−8.0° (c=0.1), had previously been shown to have the same carbon skeleton and substitution pattern as haplosamate A (**1**).[4] The exact mass of the $[M+2H-3Na]^-$ ion in the negative mode HRFAB mass spectrum (m/z 755.2295, Δ mmu+5.2) indicated that the molecular formula was $C_{29}H_{48}O_{15}P_2SNa_3$ and that haplosamate B therefore contained an additional phosphate group. Additional support for the diphosphate structure came from a deuterium-exchanged negative ion ESI mass spectrum that showed a $[M+4D-2H-3Na]^-$ ion at m/z 759, in accord with the predicted number of four exchangeable protons. The presence of two phosphate group was confirmed by the observation of two signals at δ −3.8 and −5.7 in the ^{31}P NMR spectrum. The ^1H NMR spectrum shows proton-phosphorus couplings to H-15 ($^3J_{P,H}$=7.0 Hz) and Me-29 ($^3J_{P,H}$=11.8 Hz). The phosphorus decoupled ^1H NMR spectrum showed the methoxy signal as a singlet at δ 3.60, simplified the H-15 signal at δ 4.85 and sharpened the H-7 signal at δ 3.96, which suggested that the second phosphate group was attached at C-7. The position of the phosphate groups were confirmed by the observation of carbon–phosphorus couplings to C-7 ($^2J_{P,C}$=6 Hz), C-8 ($^3J_{P,C}$=7 Hz), C-14 ($^3J_{P,C}$=10 Hz), C-15 ($^2J_{P,C}$=7 Hz), and C-29 ($^2J_{P,C}$=5 Hz).

To our knowledge, steroids containing both sulfate and phosphate groups have only been reported from the *seaster Tremaster novaecaledoniae*,[9,10] although steroidal sulfates are widely distributed in marine sponges.[11] Compounds **1** and **2** moderately inhibited MT1-MMP with IC_{50} values of 150 and 160 μg/mL, respectively.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 600 MHz for ^1H, 150 MHz for ^{13}C, and 243 MHz for ^{31}P. ^1H and ^{13}C chemical shifts were referenced to the solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD and δ_H 7.24 for $CDCl_3$. ^{31}P chemical shifts were referenced to the peak of external reference of 85% phosphoric acid in H_2O: δ_P 0.00. FAB mass spectra were measured using triethanolamine for negative mode and glycerol for positive mode as matrices. Negative mode HR-FABMS were obtained at resolution of 5000 using PEG sulfate 600 as a marker. Optical rotations were determined in CH_3OH. UV spectra were recorded in CH_3OH.

3.2. Animal material

The sponge samples were collected by hand using SCUBA at depths of 15–20 m off Nihei-jima Island off Kochi (132°33′35′N, 32°45′50″E), frozen immediately, and preserved in a freezer at −20°C until extraction. The sponge was identified as *Cribrochalina* sp. (Demospongiae, Haplosclerida, Petrosiidae) on the basis of the following evidence. Clathrate mass of anastomosed lobes and tubular elevations, with a distinctive ridged-honeycombed surface. Size approx. 14×10×10 cm^3, individual lobes up to 4 cm high and 2.5 cm in diameter, each with a large apical oscule of up to 1 cm wide. Color dark yellow, with greenish tinge. Triangular or square depressions between the ridges are riddled with small apertures. Ectosomal skeleton irregular, with tangential spicules making an irregular halichondroid surface crust carried by strongly developed spicule tracts. Choanosomal skeleton trabecular, with thick flattened often fasciculate spicule tracts making an irregular reticulation. Meshes of skeleton elongate, up to 1200×400 μm. Main spicule tracts 300–600 μm in diameter bounded by spongin, interconnecting spicule tracts up to 150 μm in diameter. Spicules straight abruptly pointed oxeas, 303–378×10–17 μm. The genus identification is tentative. Although this species differs considerably from the type of the genus *Cribrochalina* in growth form and surface characteristics, its skeleton is similar, which is taken as an indication that they are probably related. A voucher fragment is kept in the Zoological Museum of Amsterdam registered under nr. POR 16162.

3.3. MT1-MMP inhibition assay

The recombinant MT1-MMP[12] which is the truncated transmembrane domain and fluorescent substrate MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2 purchased from Peptide Institute, Osaka, were used. Inhibition assay of MT1-MMP was carried out by the modified procedure of Knight et al.[13] Test samples (2 μL) were added to wells of a 96 well microtiter plates, each well containing 100 μL of TNC buffer (50 mM Tris–HCl pH 7.5+150 mM NaCl+10 mM $CaCl_2$+0.02% NaN_3+0.05% Brij-35). Aliquots of 50 μL of enzyme solution (40 ng/mL) were added to the sample solution, and pre-incubated at 37°C for 10 min. After pre-incubation, 50 μL of substrate solution (8 μM) was added to the mixture to begin the reaction. The fluorescence values were measured at an excitation of

328 nm and an emission of 393 nm after incubation at 37°C for 1 h.

3.4. Extraction and isolation

The frozen specimen (390 g) was homogenized and extracted with 50% aqueous MeOH, MeOH, $CHCl_3$/MeOH (1:1), and EtOAc (1.5 L×2, each). The combined extracts were concentrated and partitioned between $CHCl_3$ and H_2O, and the aqueous layer was further extracted with *n*-BuOH. The $CHCl_3$ and *n*-BuOH extracts were separately subjected to the solvent partitioning.[14] They were partitioned between *n*-hexane and MeOH/H_2O (9:1), and the aqueous MeOH fraction was diluted with H_2O to make up 60% MeOH and extracted with $CHCl_3$ to yield six fractions in total. The active $CHCl_3$ and 60% MeOH layers obtained from both extracts were separately fractionated by ODS flash chromatography using stepwise elution of aq MeOH, aq MeCN, and aq *n*-PrOH. Fractions eluted with 80–100% aq MeOH were combined and chromatographed on a Sephadex LH-20 column with 60% aq MeOH to yield three fractions. The first fraction was finally purified by ODS HPLC on COSMOSIL $5C_{18}$ AR-II with 15–60% aq MeCN containing 250 mM $NaClO_4$ to yield the major inhibitor (**1**: 77.8 mg, 2.0×10^{-2}% yield based on wet weight) and the minor inhibitor (**2**: 7.1 mg, 1.8×10^{-3}%).

3.4.1. Major inhibitor (haplosamate A) 1. White powder; $[\alpha]^{24}_D=-6.9°$ (*c* 2.5, MeOH); UV (MeOH) no absorption maximum above 200 nm; HR-FABMS (triethanolamine) *m/z* 653.2784 $(M+H-2Na)^-$ ($C_{29}H_{50}O_{12}PS$: calcd for 653.2760); ^{31}P NMR (CD_3OD) δ_P 2.12.

3.4.2. Minor inhibitor 2. White powder; $[\alpha]^{24}_D=-18°$ (*c* 0.5, MeOH); UV (MeOH) no absorption maximum above 200 nm; HR-FABMS (triethanolamine) *m/z* 653.2766 $(M+H-2Na)^-$ ($C_{29}H_{50}O_{12}PS$: calcd for 653.2760); ^{31}P NMR (CD_3OD) δ_P 2.27.

3.4.3. Preparation of MTPA esters 7 (+) and 7 (−). Haplosamate A (**1**) (50 mg) was dissolved in 3 mL of pyridine containing 2 mg of DMAP. To the mixture was added benzoyl chrolide (30 mg in 3 mL of CH_2Cl_2), and the mixture was stirred at room temperature for 15 min. The reaction mixture was partitioned between H_2O and *n*-BuOH, and the latter phase was dried in vacuo to afford cyclic phosphotriester **5**. This was hydrolyzed in a mixture of 2N HCl and MeOH (4 mL, each) at 80°C for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried in a stream of N_2 gas to yield triol **6**. An aliquot of **6** was dissolved in pyridine and reacted with 10 μL of (+)-MTPACl at room temperature for 30 min. The reaction mixture was diluted with 2 mL of H_2O and extracted with EtOAc. The EtOAc fraction was separated by ODS HPLC (COSMOSIL $5C_{18}$ AR-II; 70% *n*-PrOH) to yield the (+)-MTPA ester **7** (+). (−)-MTPA ester **7** (−) was obtained in the same manner.

MTPA ester **7** (+). ^1H NMR data ($CDCl_3$): δ 7.35–7.40 (5H, m, Ph), 5.177 (1H, d, *J*=2.1 Hz, H3), 4.586 (1H, quint., 3.9, H15), 4.213 (1H, brs, H6), 3.956 (1H, brs, H4), 3.844 (1H, ddd, 20.4, 12.7, 2.3, H7), 3.765 (3H, d, 11.3, H29), 3.720 (1H, dd, 10.2, 3.3, H16), 3.480 (3H, s, OMe), 3.320 (1H, t, 9.3, H23), 2.658 (1H, q, 11.0, H8), 2.149 (1H, t, 14.4, H2β), 1.907 (1H, m, H25), 1.800 (1H, m, H12β), 1.771 (1H, m, H20), 1.740 (1H, m, H2α), 1.615 (1H, d, 13.1, H22β), 1.524 (1H, m, H11α), 1.482 (1H, dd, 11.5, 7.7, H14), 1.420 (1H, m, H11β), 1.419 (1H, m, H24), 1.387 (3H, s, H19), 1.365 (1H, d, 13.1, H1α), 1.173 (1H, dt, 12.6, 12.6, 4.0, H12α), 1.130 (1H, brs, H5), 0.946 (1H, m, H1β), 0.940 (3H, s, H18), 0.931 (3H, d, 6.5, H21), 0.861 (3H, d, 6.9, H27), 0.844 (1H, m, H22α), 0.751 (1H, m, H9), 0.751 (3H, d, 6.5, H26), 0.750 (1H, m, H17), 0.735 (3H, d, 6.9, H28).

MTPA ester **7** (−). ^1H NMR data ($CDCl_3$): δ 7.35–7.40 (5H, m, Ph), 5.189 (1H, d, *J*=2.1 Hz, H3), 4.584 (1H, quint., 3.9, H15), 4.103 (1H, brs, H6), 3.863 (1H, brs, H4), 3.772 (3H, d, 11.3, H29), 3.725 (1H, m, H7), 3.714 (1H, dd, 10.2, 3.3, H16), 3.475 (3H, s, OMe), 3.324 (1H, t, 9.3, H23), 2.637, (1H, q, 11.0, αH8), 2.207 (1H, t, 14.4, H2β), 1.912 (1H, m, H25), 1.799 (1H, m, H12β), 1.764 (1H, m, H20), 1.702 (1H, d, 14.2, H2α), 1.617 (1H, d, 13.5, H22β), 1.529 (1H, m, H11α), 1.450 (1H, m, H14), 1.436 (1H, m, H11β), 1.419 (1H, m, H1α), 1.419 (1H, m, H24), 1.391 (3H, s, H19), 1.168 (1H, dt, 12.6, 12.6, 4.0, H12α), 1.068 (1H, dt, 13.7, 13.7, 3.1, H1β), 0.947 (1H, brs, H5), 0.930 (3H, s, H18), 0.926 (3H, d, 6.5, H21), 0.867 (3H, d, 6.9, H27), 0.837 (1H, m, H22α), 0.771 (3H, d, 6.5, H26), 0.754 (1H, m, H17), 0.740 (1H, m, H9), 0.739 (3H, d, 6.9, H28).

3.4.4. Preparation of 3-*epi*-MTPA ester 9 (+) and 3-*epi*-MTPA ester 9 (−). To the solution of triol **6** in CH_2Cl_2 (7 mL) were added dimethoxypropane (3 mL) and PPTS (5 mg), and the mixture was stirred at room temperature for 4 h. To the reaction mixture was added 50 μL of triethylamine, and the mixture was partitioned between H_2O and EtOAc. The organic phase was dried in vacuo. To the residue dissolved in CH_2Cl_2 (10 mL) were added 400 mg of PDC and 400 mg of celite, and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with 100 mL of diethyl ether and filtered. The filtrate was evaporated to dryness, and to the residue dissolved in 6 mL of MeOH/THF (5:1) was added $NaBH_4$ (130 mg), and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with saturated aqueous solution of NH_4Cl (50 mL) and extracted with EtOAc. The organic extract was dried over anhydrous $MgSO_4$ and evaporated. To the residue dissolved in 50% aq THF (6 mL) was added 3 mg of PPTS, and the mixture was stirred at room temperature overnight. The reaction mixture was briefly evaporated to remove THF, and extracted with EtOAc to afford **8**. Aliquots of **8** were converted to the MTPA esters **9** (+) and **9** (−) as described above.

3-*epi*-MTPA ester **9** (+). ^1H NMR data ($CDCl_3$): δ 7.35–7.40 (5H, m, Ph), 4.855 (1H, dt, *J*=11.9, 3.9, 3.9 Hz, H3), 4.598 (1H, quint., 4.0, H15), 4.336 (1H, brs, H4), 4.304 (1H, brs, H6), 3.930 (1H, ddd, 20.4, 11.5, 2.7, H7), 3.767 (3H, d, 11.4, H29), 3.714 (1H, dd, 10.6, 4.1, H16), 3.470 (3H, s, OMe), 3.324 (1H, ddd, 10.8, 7.3, 1.9), 2.691 (1H, q, 11.1, H8), 2.220 (1H, dq, 12.8, 12.8, 12.8, 3.9, H2β), 1.912 (1H, m, H25), 1.824 (1H, m, H12β), 1.781 (1H, m, H20), 1.772 (1H, m, H2α), 1.687 (1H, dt, 12.7, 3.7, 3.7, H1β), 1.619 (1H, m, H22β), 1.599 (1H, m, H11β), 1.506 (1H, m, H14),

1.474 (1H, m, H11α), 1.455 (3H, s, H19), 1.417 (1H, m, H24), 1.197 (1H, m, H12α), 1.113 (1H, brs, H5), 1.035 (1H, dt, 13.7, 13.7, 3.5, H1α), 0.947 (3H, s, H18), 0.931 (3H, d, 6.3, H21), 0.880 (1H, m, H9), 0.862 (3H, d, 6.5, H27), 0.839 (1H, m, H22α), 0.769 (3H, d, 6.5, H26), 0.767 (1H, m, H17), 0.737 (3H, d, 7.3, H28).

3-*epi*-MTPA ester **9** (−). ^{1}H NMR data ($CDCl_3$): δ 7.35–7.40 (5H, m, Ph), 4.855 (1H, dt, *J*=11.9, 3.9, 3.9 Hz, H3), 4.603 (1H, quint., 4.0, H15), 4.431 (1H, brs, H4), 4.320 (1H, brs, H6), 3.937 (1H, ddd, 20.4, 11.5, 2.7, H7), 3.772 (3H, d, 11.4, H29), 3.728 (1H, dd, 10.6, 4.1, H16), 3.465 (3H, s, OMe), 3.326 (1H, t, 9.4, H23), 2.695 (1H, q, 11.1, H8), 2.150 (1H, dq, 12.8, 12.8, 12.8, 3.9, H2β), 1.912 (1H, m, H25), 1.819 (1H, m, H12β), 1.764 (1H, m, H20), 1.661 (1H, dt, 12.7, 3.7, 3.7, H1β), 1.618 (1H, m, H2α), 1.618 (1H, m, H22β), 1.589 (1H, m, H11β), 1.510 (1H, dd, 11.5, 8.5, H14), 1.473 (1H, m, H11α), 1.452 (3H, s, H19), 1.413 (1H, m, H24), 1.200 (1H, m, H12a), 1.129 (1H, brs, H5), 1.008 (1H, dt, 13.7, 13.7, 3.5, H1α), 0.948 (3H, s, H18), 0.929 (3H, d, 6.3, H21), 0.880 (1H, m, H9), 0.862 (3H, d, 6.5, H27), 0.836 (1H, m, H22α), 0.770 (3H, d, 6.5, H26), 0.738 (3H, d, 7.3, H28).

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