

First Total Synthesis of Symbioramide, a Novel  $\text{Ca}^{2+}$ -ATPaseActivator from *Symbiodinium* sp.

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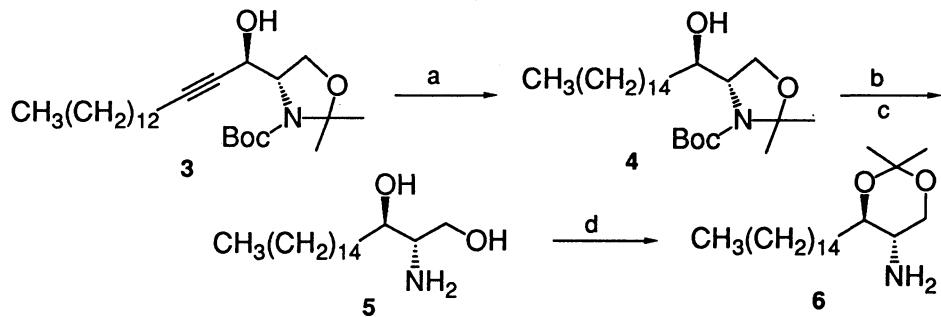
The first total synthesis of symbioramide (**1**) is described and simultaneously established the complete stereostructure of **1** to be (2S,3R,2'R,3'E)-N-(2'-hydroxy-3'-octadecenoyl)-dihydrosphingosine.

Considerable interest has recently been focused on the bioactive ceramides which are the important constituents of sphingolipids distributed widely in biological membranes.<sup>1)</sup>

Symbioramide **1**, a novel ceramide, obtained from the cultured dinoflagellate *Symbiodinium* sp., isolated from the inside of gill cells of the Okinawa bivalve *Fragum* sp., is the first example of SR  $\text{Ca}^{2+}$ -ATPase activator of marine origin and also exhibits antileukemic activity.<sup>2)</sup>

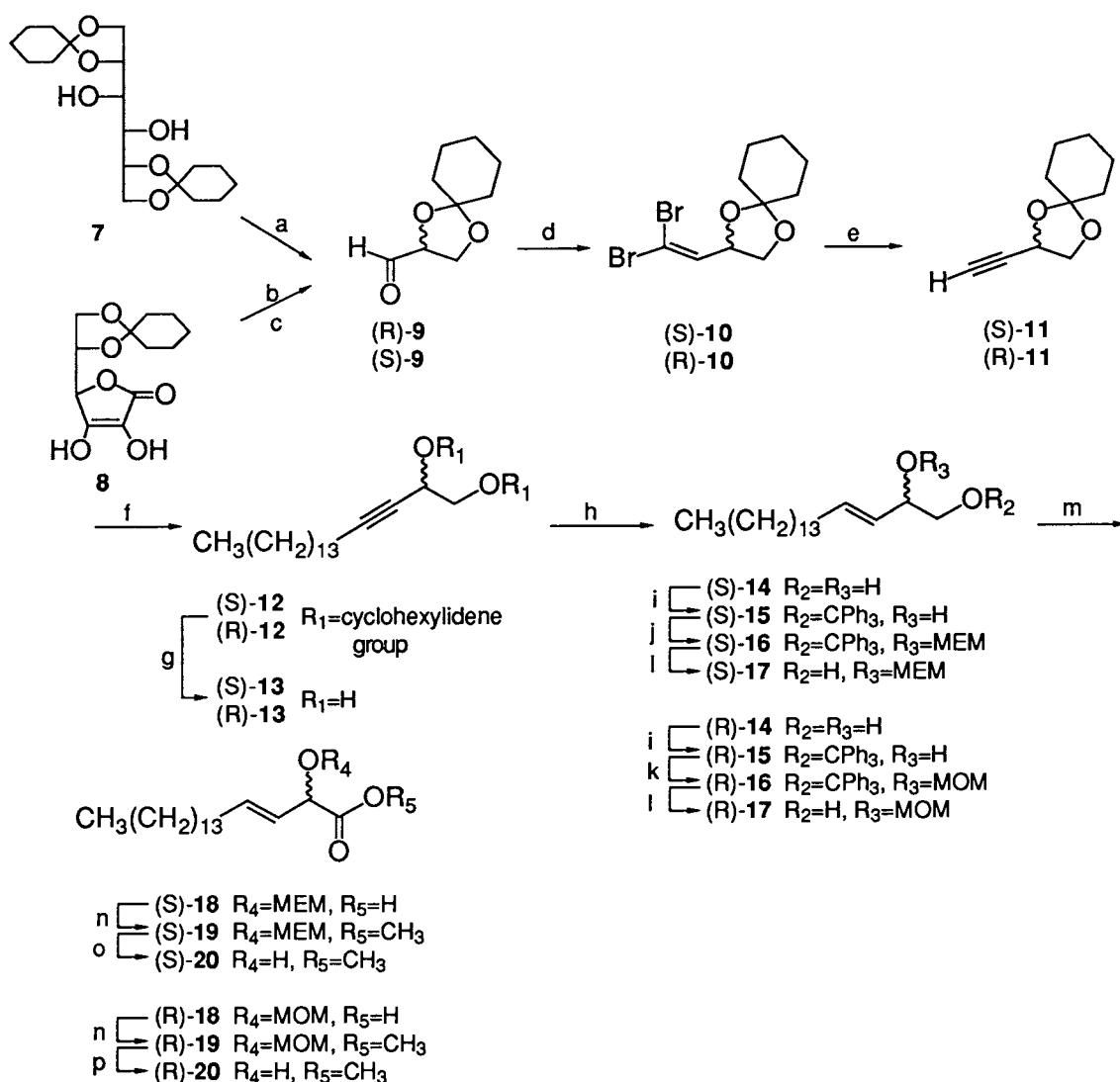
In connection with our synthetic studies on sphingolipids,<sup>3)</sup> this compound attracted our attention both as a synthetic target and as its biological interest. In addition, a total synthesis of **1** would firmly establish the chemical structure of this compound, especially regarding the stereochemistry of the 2'-hydroxyl group. We now wish to report the first total synthesis of **1**.

D-Erythro-dihydrosphingosine **5** was readily prepared by catalytic hydrogenation of **3** ( $[\alpha]_D^{21} -41.3^\circ$  (*c* 2.685,  $\text{CHCl}_3$ )), obtained from L-serine,<sup>4)</sup> followed by deprotection, which without isolation was converted to the acetonide **6** (80%, 4 steps,  $[\alpha]_D^{22} +29.5^\circ$  (*c* 1.178,  $\text{CHCl}_3$ )) (Scheme 1).



Scheme 1. a)  $\text{H}_2$ ,  $\text{PtO}_2$ ,  $\text{AcOEt}$ , room temp, 1 h; b)  $\text{TsOH}$ ,  $\text{MeOH}$ , room temp, 4 h; c)  $d\text{HCl}$ ,  $\text{AcOEt}$ , room temp, 25 min; d)  $\text{CSA}$ ,  $(\text{CH}_3\text{O})_2\text{C}(\text{CH}_3)_2$ , reflux, 1 h.

Our effort was then concentrated on the synthesis of the unusual fatty acid methyl ester (*S*)-**20** and (*R*)-**20**, starting with readily accessible aldehyde (*R*)-**9** and L-ascorbic acid derivative **8**, respectively (Scheme 2). To this goal, the enantiomerically pure aldehyde (*R*)-**9**, obtained by  $\text{NaIO}_4$  oxidation of dicyclohexylidene-D-mannitol **7**,<sup>5)</sup> was converted into the corresponding



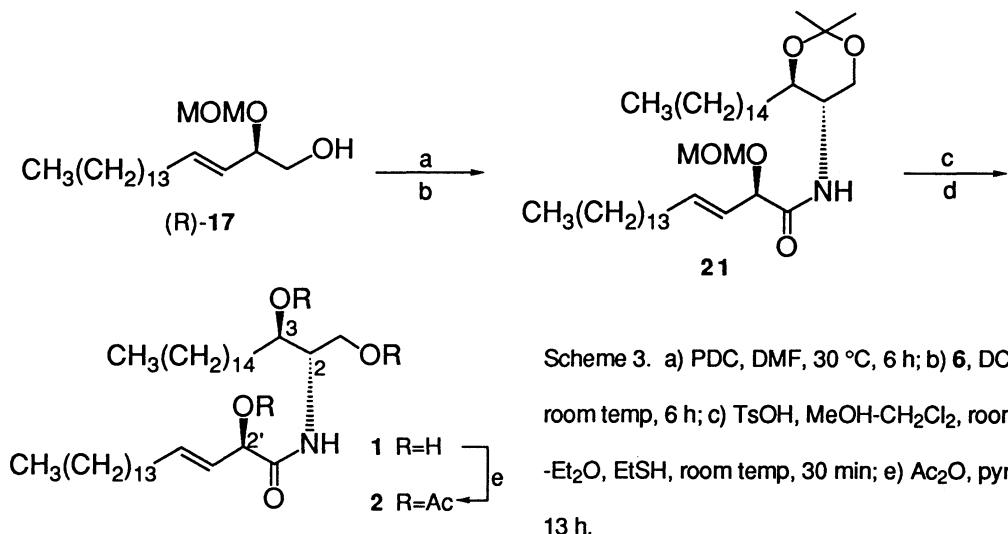
Scheme 2. a)  $\text{NaIO}_4$ ,  $n\text{-Bu}_4\text{NBr}$ ,  $\text{Et}_2\text{O}-\text{H}_2\text{O}$ , room temp, 3 h; b)  $\text{LiAlH}_4$ , THF, reflux, 2 h; c)  $\text{NaIO}_4$ , THF- $\text{H}_2\text{O}$ , 0 °C, 2 h; d)  $\text{Ph}_3\text{P}$ ,  $\text{CBr}_4$ ,  $\text{CH}_2\text{Cl}_2$ , 0 °C, 20 min; e)  $n\text{-BuLi}$ , THF, -78 °C, 1 h, then 0 °C, 1 h; f) (i)  $n\text{-BuLi}$ , THF, -78 °C, then 0 °C, 1 h, (ii)  $\text{CH}_3(\text{CH}_2)_{13}\text{OTs}$ , THF-HMPA, -78 °C, then room temp, 2 h; g)  $d\text{HCl}$ ,  $\text{EtOH}$ , reflux, 4 h; h)  $\text{LiAlH}_4$ ,  $\text{CH}_3\text{O}(\text{CH}_2)_2\text{OCH}_3$ , reflux, 3 h; i)  $\text{Ph}_3\text{CCl}$ , DMAP, pyridine, 100 °C, 2 h; j)  $\text{MEMCl}$ ,  $i\text{-Pr}_2\text{NEt}$ ,  $\text{CH}_2\text{Cl}_2$ , room temp, 18 h; k)  $\text{MOMCl}$ ,  $i\text{-Pr}_2\text{NEt}$ ,  $\text{CH}_2\text{Cl}_2$ , reflux, 30 min; l)  $\text{TsOH}$ ,  $\text{MeOH}-\text{CH}_2\text{Cl}_2$ , room temp, 40 min; m)  $\text{PDC}$ ,  $\text{DMF}$ , 40-50 °C, 3 h; n)  $\text{CH}_3\text{I}$ ,  $i\text{-Pr}_2\text{NEt}$ ,  $\text{CH}_2\text{Cl}_2$ , room temp, 15 h; o)  $\text{ZnBr}_2$ ,  $\text{CH}_2\text{Cl}_2$ , reflux, 8 h; p)  $\text{BF}_3\text{-Et}_2\text{O}$ ,  $\text{EtSH}$ , room temp, 40 min.

olefin (S)-10 ( $[\alpha]_D^{23} -5.23^\circ$  ( $c$  0.968,  $\text{CHCl}_3$ )). Treatment of (S)-10 with  $n\text{-BuLi}$  provided the alkyne (S)-11 ( $[\alpha]_D^{24} +39.3^\circ$  ( $c$  0.890,  $\text{CHCl}_3$ )) which was alkylated to afford (S)-12 (47%, 2 steps,

$[\alpha]_D^{25} +22.7^\circ$  ( $c$  1.020,  $\text{CHCl}_3$ ). Deprotection of (S)-12 gave the diol (S)-13 ( $[\alpha]_D^{24} +11.2^\circ$  ( $c$  0.920,  $\text{CHCl}_3$ )) which was led to (S)-14 (43%, 2 steps,  $[\alpha]_D^{29} +9.05^\circ$  ( $c$  0.398,  $\text{CHCl}_3$ )) by stereoselective  $\text{LiAlH}_4$  reduction.<sup>6</sup> Tritylation of (S)-14 followed by protection of the secondary alcohol gave (S)-16, which on detritylation, afforded (S)-17 (54%, 3 steps,  $[\alpha]_D^{21} +73.2^\circ$  ( $c$  1.242,  $\text{CHCl}_3$ )). Subsequent PDC oxidation of (S)-17 provided the acid (S)-18, which was isolated as its methyl ester (S)-19 (41%, 2 steps,  $[\alpha]_D^{20} +68.0^\circ$  ( $c$  0.500,  $\text{CHCl}_3$ )). Deprotection of (S)-19 gave methyl (2S,3E)-2-hydroxyoctadec-3-enoate (S)-20 (73%,  $[\alpha]_D^{19} +46.4^\circ$  ( $c$  0.278,  $\text{CHCl}_3$ )).

By analogy, the same multi-step sequence starting from cyclohexylidene-L-glyceraldehyde (S)-9, which was obtained by  $\text{LiAlH}_4$  reduction of 8 followed by  $\text{NaIO}_4$  oxidation, afforded the alcohol (R)-17 ( $[\alpha]_D^{15} -73.7^\circ$  ( $c$  0.904,  $\text{CHCl}_3$ )). The latter, on treatment with PDC, followed by methylation and deprotection provided the optically active ester (R)-20 ( $[\alpha]_D^{19} -44.7^\circ$  ( $c$  0.257,  $\text{CHCl}_3$ )) which was identified with the ester obtained from acidic hydrolysis of natural 1 by comparison with the physico-chemical properties (Table 1),<sup>2,7</sup> showing thus the absolute stereochemistry at C-2' position to be (R)-configuration.

Finally, the coupling reaction of (R)-18 with 6 led to the formation of the amide 21. Deprotection of the acetonide and the alcohol function was achieved selectively by treatment with  $\text{TsOH}$ -MeOH followed by  $\text{BF}_3$ -Et<sub>2</sub>O-EtSH to give symbioramide 1 (24% from (R)-17, mp 112-113 °C (benzene/acetone),  $[\alpha]_D^{19} +2.65^\circ$  ( $c$  0.378,  $\text{CHCl}_3$ )). Acetylation of 1 gave the triacetate 2 (97%, mp 75-78 °C) (Scheme 3). The spectral data (IR, NMR, mass) of synthetic 1 and 2 were identical with those of natural product and its triacetate, respectively (Table 1).<sup>2</sup>



Scheme 3. a) PDC, DMF, 30 °C, 6 h; b) 6, DCC, HOBT,  $\text{CH}_2\text{Cl}_2$ , room temp, 6 h; c)  $\text{TsOH}$ ,  $\text{MeOH-CH}_2\text{Cl}_2$ , room temp, 1 h; d)  $\text{BF}_3$ - $\text{Et}_2\text{O}$ , EtSH, room temp, 30 min; e)  $\text{Ac}_2\text{O}$ , pyridine, room temp, 13 h.

In conclusion, the present synthesis unambiguously established the absolute configuration of 1, (2S,3R,2'R,3'E)-N-(2'-hydroxy-3'-octadecenoyl)-dihydrosphingosine.

We thank Prof. J. Kobayashi (Hokkaido Univ.) for a generous gift of the copy of the  $^1\text{H-NMR}$  spectrum of 2 and 20. We also thank members of the Analytical Center of Chiba University for spectral data. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We are also grateful to the Japan Foundation for Optically Active Compounds.

Table 1. Spectral data for 1, 2, and (R)-20

1: IR (KBr):  $\nu$  [cm<sup>-1</sup>] = 3300, 1640, 1530, 1460, and 1060. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.02 (d, J=7.69 Hz, 1H; NH, exch.), 5.90 (dt, J=14.3, 7.0 Hz, 1H; H-4'), 5.56 (dd, J=15.3, 7.3 Hz, 1H; H-3'), 4.53 (dd, J=7.15, 3.58 Hz, 1H; H-2'), 4.03 (dt, J=11.6, 3.58 Hz, 1H; H-1), 3.83-3.76 (m, 3H; H'-1, H-2, and H-3), 3.15 (d, J=3.3 Hz, 1H; OH-2', exch.), 2.64 (br.s, 1H; OH-1, exch.), 2.51 (d, J=6.04 Hz, 1H; OH-3, exch.), 2.08 (q like, 2H; H<sub>2</sub>-5'), 1.55-1.50 (m, 2H; H<sub>2</sub>-4), 1.41-1.36 (m, 2H; H<sub>2</sub>-6'), 1.31-1.26 (m, 48H), 0.88 (t, J=6.88 Hz, 6H; H<sub>3</sub>-18 and H<sub>3</sub>-18'). EIMS:  $m/z$  581 ( $M^+$ , 1.23%), 328 (40.22), 253 (45.87), 43 (100). (Found: C, 74.51; H, 12.29; N, 2.36. C<sub>36</sub>H<sub>71</sub>O<sub>4</sub>N requires C, 74.30; H, 12.30; N, 2.41%)

2: IR (KBr):  $\nu$  [cm<sup>-1</sup>] = 3300, 1730, 1660, 1540, 1460, and 1030. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 6.52 (d, J=8.8 Hz, 1H; NH), 5.90 (dt, J=14.6, 7.0 Hz, 1H; H-4'), 5.52 (dd, J=15.4, 7.15 Hz, 1H; H-3'), 5.49 (d, J=7.15 Hz, 1H; H-2'), 4.90 (dt, J=8.25, 4.95 Hz, 1H; H-3), 4.38-4.33 (m, 1H; H-2), 4.31 (dd, J=11.27, 6.87 Hz, 1H; H-1), 4.04 (dd, J=11.27, 3.29 Hz, 1H; H-1), 2.18 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.08-2.04 (m, 2H; H<sub>2</sub>-5'), 1.62-1.57 (m, 2H; H<sub>2</sub>-4), 1.39-1.35 (m, 2H; H<sub>2</sub>-6'), 1.31-1.25 (m, 48H), 0.88 (t like, 6H; H<sub>3</sub>-18 and H<sub>3</sub>-18'). EIMS:  $m/z$  708 ( $M^+$ , 1.00%), 707 ( $M^+$ -H, 2.29), 648 ( $M^+$ -AcOH, 19.69), 370 (100).

(R)-20: IR (KBr):  $\nu$  [cm<sup>-1</sup>] = 3350, 1760, 1470, and 970. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 5.88 (dt, J=15.4, 6.87 Hz, 1H; H-4), 5.50 (dd, J=15.4, 6.33 Hz, 1H; H-3), 4.61 (t, J=6.05 Hz, 1H; H-2), 3.80 (s, 3H; CO<sub>2</sub>Me), 2.83 (d, J=5.77 Hz, 1H; OH, exch.), 2.06 (q like, 2H; H<sub>2</sub>-5), 1.40-1.37 (m, 2H; H<sub>2</sub>-6), 1.31-1.26 (m, 22H), 0.88 (t, J=6.88 Hz, 3H; H<sub>3</sub>-18). EIMS:  $m/z$  312 ( $M^+$ , 0.86%), 253 ( $M^+$ -CO<sub>2</sub>Me, 100).

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- 7) The low  $[\alpha]_D^{28}$  value ( $[\alpha]_D^{28} -16^\circ$  (c 1, CHCl<sub>3</sub>)) of **20** obtained from **1<sup>2</sup>** may due to the partial epimerization at C-2' or the impurity.

(Received May 21, 1990)