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First Total Synthesis of Symbioramide, a Novel Ca^{2+}-ATPase Activator from *Symbiodinium* sp.

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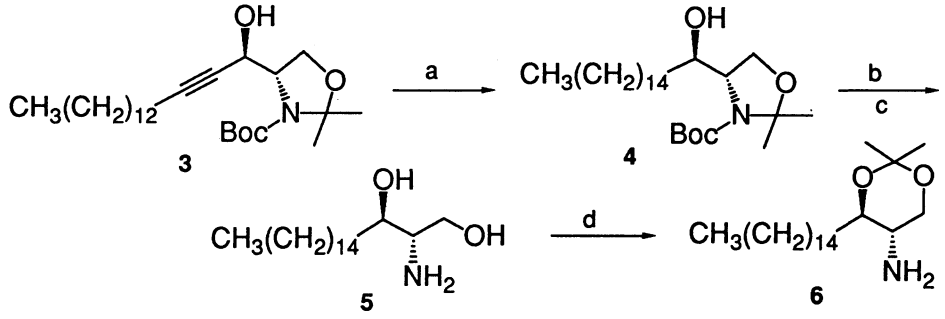
The first total synthesis of symbioramide (**1**) is described and simultaneously established the complete stereostructure of **1** to be (2S,3R,2'R,3'E)-*N*-(2'-hydroxy-3'-octadecenoyl)-dihydrosphingosine.

Considerable interest has recently been focused on the bioactive ceramides which are the important constituents of sphingolipids distributed widely in biological membranes.[1] 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 Ca^{2+}-ATPase activator of marine origin and also exhibits antileukemic activity.[2]

In connection with our synthetic studies on sphingolipids,[3] 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**.

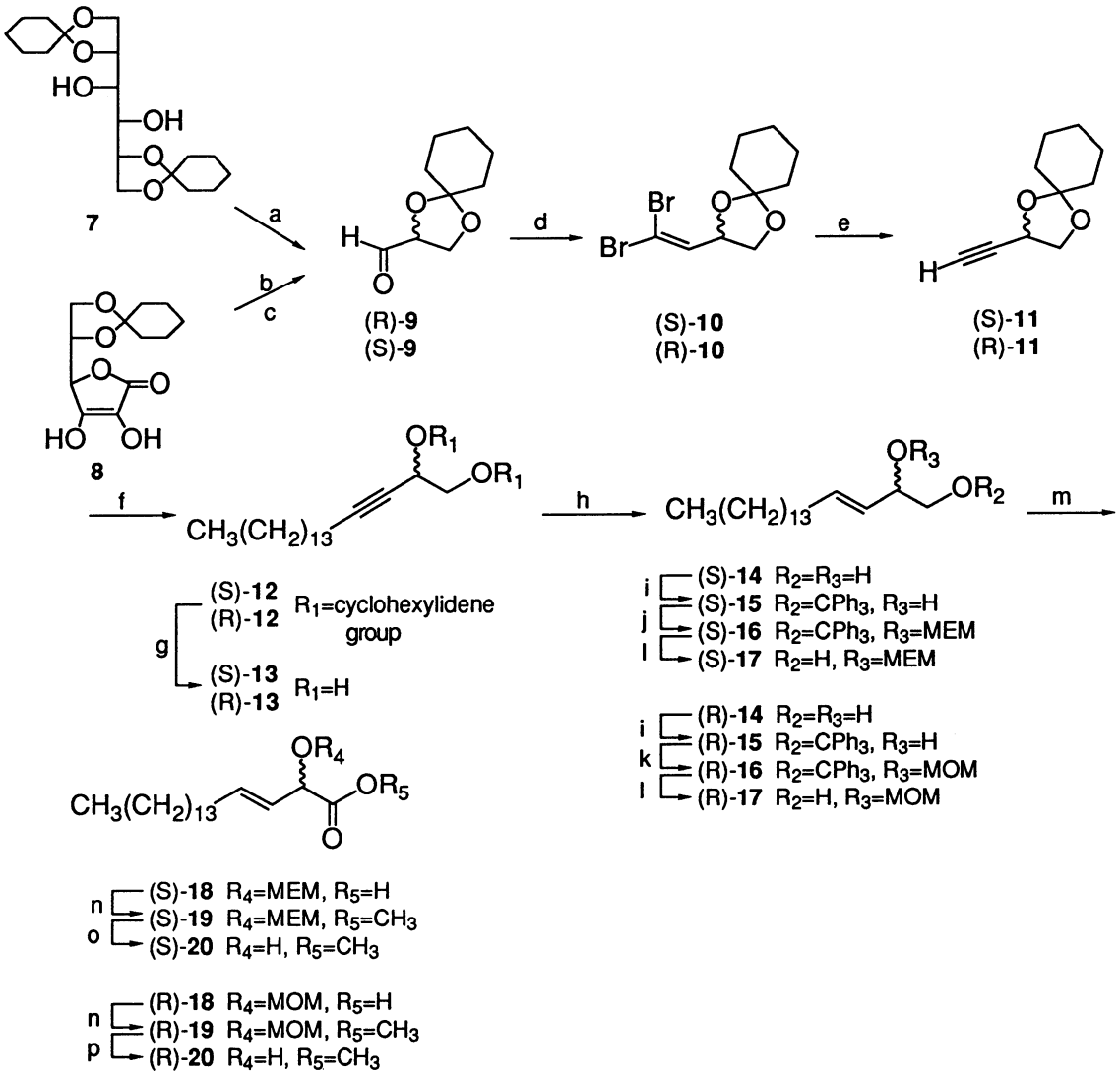
D-*Erythro*-dihydrosphingosine **5** was readily prepared by catalytic hydrogenation of **3** ($[\alpha]_D^{21}$ -41.3° (*c* 2.685, $CHCl_3$)), obtained from L-serine,[4] followed by deprotection, which without isolation was converted to the acetonide **6** (80%, 4 steps, $[\alpha]_D^{22}$ +29.5° (*c* 1.178, $CHCl_3$)) (Scheme 1).



3 4 5 6

Scheme 1. a) H_2, PtO_2, AcOEt, room temp, 1 h; b) TsOH, MeOH, room temp, 4 h; c) *c*HCl, AcOEt, room temp, 25 min; d) CSA, $(CH_3O)_2C(CH_3)_2$, 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 $NaIO_4$ oxidation of dicyclohexylidene-D-mannitol **7**,[5] was converted into the corresponding



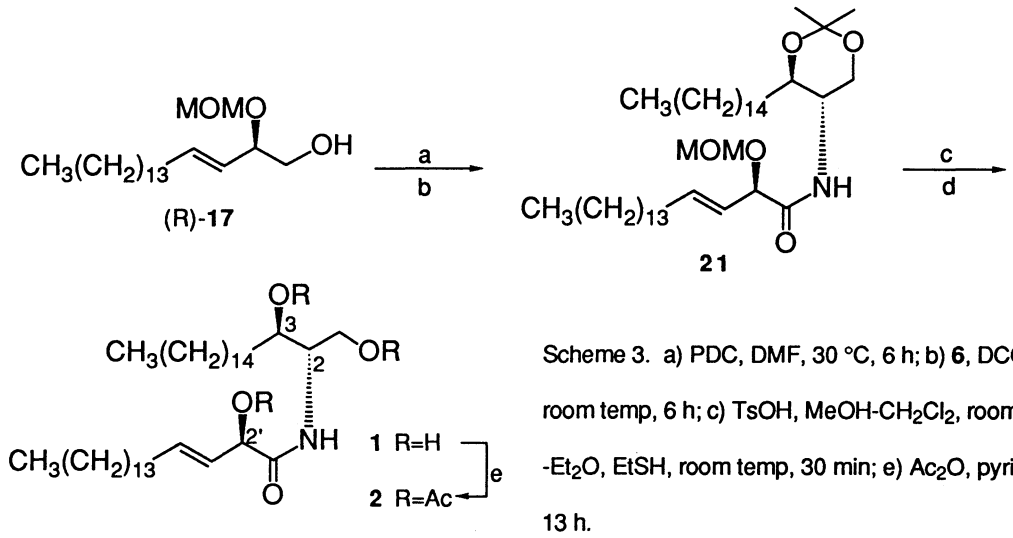
Scheme 2. a) $NaIO_4$, *n*-Bu_4NBr, Et_2O-H_2O, room temp, 3 h; b) $LiAlH_4$, THF, reflux, 2 h; c) $NaIO_4$, THF-H_2O, 0 °C, 2 h; d) Ph_3P, CBr_4, CH_2Cl_2, 0 °C, 20 min; e) *n*-BuLi, THF, -78 °C, 1 h, then 0 °C, 1 h; f) (i) *n*-BuLi, THF, -78 °C, then 0 °C, 1 h, (ii) $CH_3(CH_2)_{13}OTs$, THF-HMPA, -78 °C, then room temp, 2 h; g) *c*HCl, EtOH, reflux, 4 h; h) $LiAlH_4$, $CH_3O(CH_2)_2OCH_3$, refrux, 3 h; i) Ph_3CCl, DMAP, pyridine, 100 °C, 2 h; j) MEMCl, *i*-Pr_2NEt, CH_2Cl_2, room temp, 18 h; k) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, reflux, 30 min; l) TsOH, MeOH-CH_2Cl_2, room temp, 40 min; m) PDC, DMF, 40-50 °C, 3 h; n) CH_3I, *i*-Pr_2NEt, CH_2Cl_2, room temp, 15 h; o) $ZnBr_2$, CH_2Cl_2, reflux, 8 h; p) BF_3-Et_2O, EtSH, room temp, 40 min.

olefin (S)-**10** ($[\alpha]_D^{23}$ -5.23° (*c* 0.968, $CHCl_3$)). Treatment of (S)-**10** with *n*-BuLi provided the alkyne (S)-**11** ($[\alpha]_D^{24}$ +39.3° (*c* 0.890, $CHCl_3$)) which was alkylated to afford (S)-**12** (47%, 2 steps,

$[\alpha]_D^{25}$ +22.7° (*c* 1.020, $CHCl_3$)). Deprotection of (S)-**12** gave the diol (S)-**13** ($[\alpha]_D^{24}$ +11.2° (*c* 0.920, $CHCl_3$)) which was led to (S)-**14** (43%, 2steps, $[\alpha]_D^{29}$ +9.05° (*c* 0.398, $CHCl_3$)) by stereoselective $LiAlH_4$ reduction.[6] 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° (*c* 1.242, $CHCl_3$)). 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° (*c* 0.500, $CHCl_3$)). Deprotection of (S)-**19** gave methyl (2S,3E)-2-hydroxyoctadec-3-enoate (S)-**20** (73%, $[\alpha]_D^{19}$ +46.4° (*c* 0.278, $CHCl_3$)).

By analogy, the same multi-step sequence starting from cyclohexylidene-L-glyceraldehyde (S)-**9**, which was obtained by $LiAlH_4$ reduction of **8** followed by $NaIO_4$ oxidation, afforded the alcohol (R)-**17** ($[\alpha]_D^{15}$ -73.7° (*c* 0.904, $CHCl_3$)). The latter, on treatment with PDC, followed by methylation and deprotection provided the optically active ester (R)-**20** ($[\alpha]_D^{19}$ -44.7° (*c* 0.257, $CHCl_3$)) which was identified with the ester obtained from acidic hydrolysis of natural **1** by comparison with the physico-chemical properties (Table 1),[2,7] 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 TsOH-MeOH followed by BF_3-Et_2O-EtSH to give symbioramide **1** (24% from (R)-**17**, mp 112-113 °C (benzene/acetone), $[\alpha]_D^{19}$ +2.65° (*c* 0.378, $CHCl_3$)). 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).[2]



Scheme 3. a) PDC, DMF, 30 °C, 6 h; b) **6**, DCC, HOBt, CH_2Cl_2, room temp, 6 h; c) TsOH, MeOH-CH_2Cl_2, room temp, 1 h; d) BF_3-Et_2O, EtSH, room temp, 30 min; e) Ac_2O, 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.

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

1: IR (KBr): ν[cm^{-1}]= 3300, 1640, 1530, 1460, and 1060. ^1H NMR (500 MHz, $CDCl_3$): δ= 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_2-5'), 1.55-1.50 (m, 2H; H_2-4), 1.41-1.36 (m, 2H; H_2-6'), 1.31-1.26 (m, 48H), 0.88 (t, J=6.88 Hz, 6H; H_3-18 and H_3-18'). 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. $C_{36}H_{71}O_4N$ requires C, 74.30; H, 12.30; N, 2.41%)

2: IR (KBr): ν[cm^{-1}]= 3300, 1730, 1660, 1540, 1460, and 1030. ^1H NMR (500 MHz, $CDCl_3$): δ= 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_2-5'), 1.62-1.57 (m, 2H; H_2-4), 1.39-1.35 (m, 2H; H_2-6'), 1.31-1.25 (m, 48H), 0.88 (t like, 6H; H_3-18 and H_3-18'). EIMS: m/z 708 (M^+, 1.00%), 707 (M^+-H, 2.29), 648 (M^+-AcOH, 19.69), 370 (100).

(R)-**20**: IR (KBr): ν[cm^{-1}]= 3350, 1760, 1470, and 970. ^1H NMR (500 MHz, $CDCl_3$): δ= 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_2Me), 2.83 (d, J=5.77 Hz, 1H; OH, exch.), 2.06 (q like, 2H; H_2-5), 1.40-1.37 (m, 2H; H_2-6), 1.31-1.26 (m, 22H), 0.88 (t, J=6.88 Hz, 3H; H_3-18). EIMS: m/z 312 (M^+, 0.86%), 253 (M^+-CO_2Me, 100).

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7) The low $[\alpha]_D$ value ($[\alpha]_D^{28}$ -16° (*c* 1, $CHCl_3$)) of **20** obtained from **1**[2] may due to the partial epimerization at C-2' or the impurity.

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