radical **5** which, after recombination with the flavin semiquinone, leads to covalent modification of FAD (Scheme I, route B). Since the reactions of carbon radicals, particularly cyclopropylcarbinyl and homoallylic species, with molecular oxygen are well documented,[14] the inactivation derailment may be envisioned as trapping the acyclic radical **5** with O_2 to form a transient peroxy radical **6** which, upon reduction by one-electron transfer from the active-site-bound flavin semiquinone, gives rise to a peroxy anion **7**. Since the partition ratio of this inactivation is approximately 3, reacting with oxygen instead of coupling with the flavin coenzyme is clearly a more facile process for the ring-opened radical intermediate **5**. As depicted in Scheme II, this reroute is culminated by an intramolecular epoxidation[15] converting **7** via a 1,2-dioxolanylcarbinyl anion **8** to the observed turnover product **9**.[16] The mechanistic insights derived from this study provide highly convincing evidence sustaining our early notion that inactivation of GAD by MCPA-CoA is likely to proceed through a radical mechanism. These results may also be extrapolated to suggest that GAD is capable of mediating one-electron oxidation-reduction.

Acknowledgment. We thank Dr. Vikram Roongta for his assistance with NMR measurements. This work was supported by a National Institutes of Health grant (GM 40541). H.-w.L. also thanks the National Institutes of Health for a Research Career Development Award (GM 00559).

(14) (a) Nakamura, E.; Inubushi, T.; Aoki, S.; Machii, D. *J. Am. Chem. Soc.* **1991**, *113*, 8980. (b) Beckwith, A. L. J. *Tetrahedron* **1981**, *37*, 3073. (c) Porter, N. A. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1980; Vol. IV, p 261. (d) Beckwith, A. L. J.; Ingold, K. U. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980.

(15) (a) Clark, C.; Hermans, P.; Meth-Cohn, O.; Moore, C.; Taljaard, H. C.; van Vuuren, G. *J. Chem. Soc., Chem. Commun.* **1986**, 1378. (b) Rontani, J.-F. *Tetrahedron Lett.* **1991**, 6551. (c) Dowd, P.; Ham, S. W. *J. Am. Chem. Soc.* **1991**, *113*, 9403.

(16) An alternate mechanism is also conceivable involving an intramolecular cyclization of **6** to produce a 1,2-dioxolanylcarbinyl radical (Feldman, K. S.; Simpson, R. E. *J. Am. Chem. Soc.* **1989**, *111*, 4878) and then a one-electron reduction to give the corresponding anion **8**.

Total Synthesis of Halichondrin B and Norhalichondrin B

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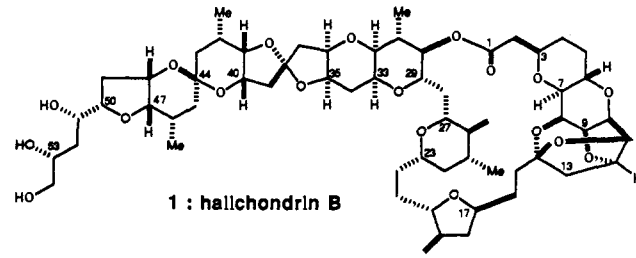
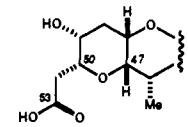
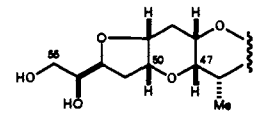
Halichondrins are a class of polyether macrolides isolated originally from the marine sponge *Halichondria okadai* Kadota.[1,2] Halichondrins, especially halichondrin B and homohalichondrin B, exhibit extraordinary in vitro and in vivo antitumor activity. However, the very limited supply of halichondrins from natural sources has prevented further evaluation of their potential clinical application thus far. Their intriguing and challenging structural features, coupled with this fact, encouraged synthetic efforts toward this class of natural products.[3,4] In this paper, we report the first total synthesis of halichondrin B and norhalichondrin B, which has, we believe, potential to meet the demand.



1 : halichondrin B



2 : norhalichondrin B



3 : homohalichondrin B

Scheme I outlines the synthesis of the right half of the halichondrin Bs. We planned to form the C-21-C-22 bond[5] via a Horner-Emmons reaction, followed by conjugate reduction. We were concerned with double-bond isomerization from the C-19 exocyclic to the C-19-C-20 endocyclic position in this process. This transformation was accomplished via the preparation of the aldehyde from the primary alcohol **3**[6,7] by Dess-Martin oxidation,[8] Horner-Emmons reaction under carefully controlled conditions, and the conjugate reduction of the resulting enone by the Stryker reagent,[9] without double-bond isomerization. Hydride reduction of the resulting saturated ketone yielded approximately a 1:1 mixture of the two possible diastereomers. As the stereochemistry of diastereomeric alcohols could not be firmly established at this stage, both diastereomers were transformed separately into the corresponding mesylates and used for the next coupling reaction. However, it is important to note that the two diastereomeric alcohols were readily interconvertible via the Mitsunobu reaction.[10]

(1) (a) Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y.; Hirata, Y. *J. Am. Chem. Soc.* **1985**, *107*, 4796-4798. (b) Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1986**, *58*, 701-710.

(2) A recent publication (Bai, R.; Paull, K. D.; Herald, C. L.; Malspeis, L.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1991**, *266*, 15882-15889) implies that halichondrin B and homohalichondrin B are isolated from *Axinella* sponges.

(3) For the synthetic work from this laboratory, see: (a) Aicher, T. D.; Kishi, Y. *Tetrahedron Lett.* **1987**, *28*, 3463-3466. (b) Aicher, T. D.; Buszek, K. R.; Forsyth, C. J.; Fang, F. G.; Jung, S. H.; Kishi, Y.; Scola, P. M. *Tetrahedron Lett.*, in press. (c) Buszek, K. R.; Forsyth, C. J.; Fank, F. G.; Jung, S. H.; Kishi, Y.; Scola, P. M.; Yoon, S. K. *Tetrahedron Lett.*, in press. (d) Fang, F. G.; Kishi, Y.; Matelich, M. C.; Scola, P. M. *Tetrahedron Lett.*, in press.

(4) For the synthetic work from other laboratories, see: (a) Kim, S.; Salomon, R. G. *Tetrahedron Lett.* **1989**, *30*, 6279-6282. (b) Cooper, A. J.; Salomon, R. G. *Tetrahedron Lett.* **1990**, *31*, 3813-3816. (c) Burke, S. D.; Buchanan, J. L.; Rovin, J. D. *Tetrahedron Lett.* **1991**, *32*, 3961-3964.

(5) The numbering adopted in this paper corresponds to that of halichondrins.

(6) This substance was synthesized from 2-deoxy-L-arabinose diethyl thioacetal 4,5-acetonide (Wong, M. Y. H.; Gray, G. R. *J. Am. Chem. Soc.* **1978**, *100*, 3548) in 47% overall yield in 13 steps: (1) $AcOH/H_2O$/room temperature. (2) TBDPSiCl/imidazole. (3) $I_2/NaHCO_3/H_2O$/acetone. (4) Ac_2O/pyridine/room temperature. (5) $CH_2{=}CHCH_2TMS/BF_3{\cdot}OEt_2/CH_3CN$/0 °C. (6) (a) 9-BBN; (b) H_2O_2. (7) $MMTrCl/Et_3N/CH_2Cl_2$. (8) K_2CO_3/MeOH. (9) Swern oxidation. (10) MeOH/PPTS. (11) Tebbe reagent (Tebbe, F. N.; Parshall, G. W.; Reddy, G. S. *J. Am. Chem. Soc.* **1978**, *100*, 3611-3613. Cannizzo, L. F.; Grubbs, R. H. *J. Org. Chem.* **1985**, *50*, 2386-2397). (12) PvCl/pyridine. (13) TBAF.

(7) Satisfactory spectroscopic data (^1H and ^{13}C NMR, HRMS, MS, IR, UV, $[\alpha]_D$) were obtained for all new compounds reported in this paper.

(8) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155-4156.

(9) (a) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc.* **1988**, *110*, 291-293. (b) Mahoney, W. S.; Stryker, J. M. *J. Am. Chem. Soc.* **1989**, *111*, 8818-8823. (c) We are indebted to Professor Stryker for a sample of this reagent.

(10) For a review on this reaction, see: Mitsunobu, O. *Synthesis* **1981**, 1-28.

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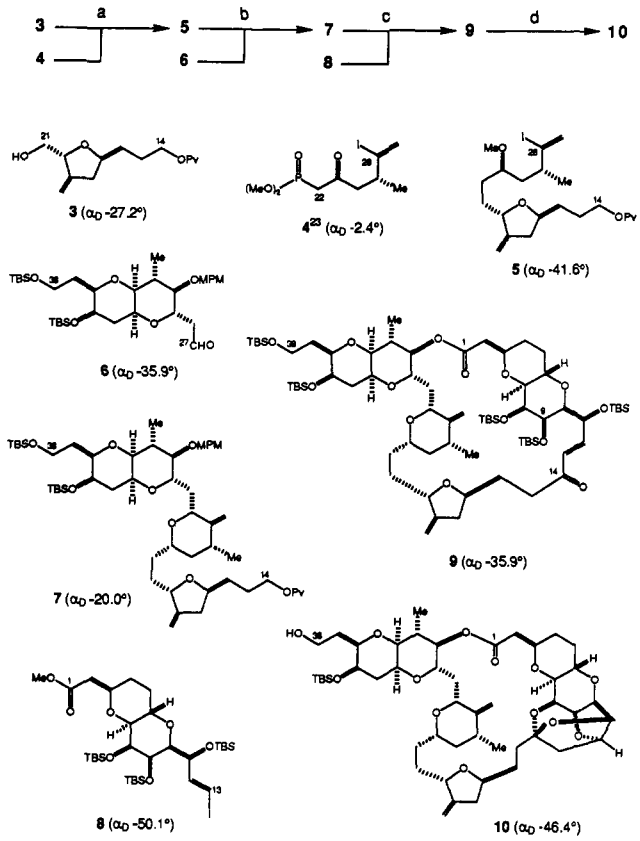
Scheme I[a]

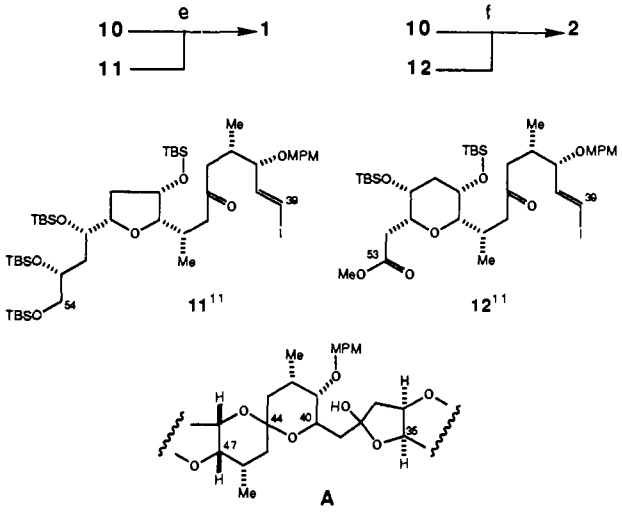


[a] Reagents and reaction conditions: (a) (1) **3**/Dess–Martin reagent/CH_2Cl_2/room temperature. (2) **4** (1.5 equiv)/NaH (1.3 equiv)/THF/0 °C, followed by treatment with the aldehyde at 0 °C. (3) Stryker reagent/wet C_6H_6/room temperature. (4) $NaBH_4$/MeOH/0 °C, followed by silica gel chromatographic separation (EtOAc/hexanes). (5) The more polar alcohol/Ms_2O/Et_3N/0 °C. (b) (1) **5** + **6**/$NiCl_2$ (0.5%)–$CrCl_2$/DMF–THF (1:5)/room temperature. (2) KH/DME/80 °C. (c) (1) **7**/LAH/Et_2O/0 °C. (2) Same as step a1. (3) The aldehyde + **8**/$NiCl_2$ (0.1%)–$CrCl_2$/DMF/room temperature. (4) Same as step a1. (5) DDQ[24]/pH 7.00 phosphate buffer–*t*-BuOH–CH_2Cl_2 (10:1:100)/room temperature. (6) LiOH/H_2O–THF (1:3)/room temperature. (7) Yamaguchi lactonization.[16] (d) (1) TBAF/THF/room temperature. (2) PPTS/CH_2Cl_2/room temperature. (3) *p*-$O_2NPhCOCl$/Py/CH_2Cl_2/room temperature. (4) TBSOTf/Et_3N/CH_2Cl_2/room temperature. (5) K_2CO_3/MeOH/room temperature.

Coupling of segment **5** with segment **6**[11] was accomplished by the Ni(II)/Cr(II)-mediated reaction,[12] to yield approximately a 6:1 mixture of the two possible allylic alcohols, which were immediately subjected to base-induced cyclization to furnish the desired tetrahydropyran **7** in 50–60% overall yield, along with a small amount of the undesired diastereomer. The stereochemistry at the C-23 and C-27 positions was established by NOE experiments.[13] The mesylate **5** was found to be quite labile, presumably due to the participation of the C-20 ether oxygen with the mesylate group,[14] yet **5** survived nicely under the Ni(II)/Cr(II) coupling conditions.

The Ni(II)/Cr(II)-mediated coupling of the C-14 aldehyde derived from **7** with **8**,[15] followed by Dess–Martin oxidation, gave

Scheme II[a]



[a] Reagents and reaction conditions: (e) (1) **10**/Dess–Martin reagent/CH_2Cl_2/room temperature. (2) The aldehyde + **11** (2 equiv)/$NiCl_2$ (0.1%)–$CrCl_2$/DMF/room temperature. (3) Dess–Martin reagent/CH_2Cl_2/room temperature. (4) TBAF/DMF/room temperature. (5) DDQ[24]/pH 7.00 phosphate buffer–*t*-BuOH–CH_2Cl_2 (10:1:100)/room temperature. (6) CSA/CH_2Cl_2/room temperature. (f) (1–6) Same as steps e1–6 with **12** instead of **11**. (7) LiOH/H_2O–THF (1:3)/room temperature.

the expected *trans*-enone in 77% overall yield. After removal of the C-30 (*p*-methoxyphenyl)methyl (MPM) group and hydrolysis of the C-1 methyl ester, this enone was lactonized under Yamaguchi conditions,[16] to afford the lactone enone **9** in 63% overall yield.[17]

The polycyclic ring system around the C-8–C-14 moiety was incorporated cleanly and effectively on treatment of **9** with $(n\text{-Bu})_4NF$ (TBAF) and then *p*-TsOH·Py (PPTS), in 64% yield. The ^1H NMR spectrum showed the product at the TBAF step to be primarily a saturated ketone. The regioselectivity of the Michael reaction was exclusive for the desired five-membered-ring formation, whereas the stereoselectivity was approximately 5–6:1, favoring the desired diastereomer. The undesired Michael adduct, separated from the desired product after PPTS treatment, could be recycled under TBAF conditions. Interestingly, a parallel Michael reaction and subsequent ketalization of the C-1–C-21 segment, which bore no macrolactone ring, also gave the polycyclic product, but the stereoselectivity of the Michael reaction decreased to 2–3:1, still favoring the desired diastereomer. The adjustment of the protecting groups of the polycyclic product furnished the right half, **10**,[18] of the halichondrin B series.

Coupling of the right half aldehyde of halichondrin B, derived from **10**, with the left half, **11**,[11] was effected by Ni(II)/Cr-

(11) For the synthesis and stereochemistry assignment of the corresponding primary alcohol, see refs 3c,d. The aldehyde **6** was obtained by Dess–Martin oxidation of the primary alcohol in 93% yield.

(12) (a) Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644–5646. (b) Takai, K.; Yagashira, M.; Kuroda, T.; Oshima, T.; Uchimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048–6050.

(13) On irradiation of the C-25 proton, 3% and 1.5% NOEs were observed on the C-27 and C-23 protons, respectively.

(14) In wet solvents, this mesylate was readily hydrolyzed to give back the starting alcohol.

(15) The synthesis of this segment was originally studied by using the unnatural antipode.[3a] The natural antipode corresponding to compound **10** in ref 3a was straightforwardly synthesized from D-glucose diacetonide (for transformation of D-glucose diacetonide to L-talofuranoside, see: Brimacombe, J. S.; Mofti, A. M.; Tucker, L. C. N. *J. Chem. Soc. C* **1971**, 2911), and then this intermediate was converted to **8** in 50–55% overall yield in 10 steps: (1) BzCl/Py/room temperature. (2) *p*-TsOH/MeOH/room temperature. (3) TBSOTf/Et_3N/CH_2Cl_2/room temperature. (4) MeONa/MeOH/room temperature. (5) Swern oxidation. (6) IC≡CTMS/$NiCl_2$ (0.01%)–$CrCl_2$/THF/room temperature (stereoselectivity = 8–9:1). (7) $AgNO_3$/H_2O–EtOH (1:4). (8) *n*-Bu_3SnH/AIBN/toluene/80 °C. (9) I_2/CH_2Cl_2/0 °C. (10) TBSOTf/Et_3N/CH_2Cl_2/room temperature.

(16) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.

(17) By using the substrate bearing an acetyl group at the C-11 position, the Ni(II)/Cr(II)-mediated coupling reaction was demonstrated to be effective for this macrocyclic ring formation at the C-13–C-14 position.

(18) Partial ^1H NMR spectral data of **10** (C_6D_6): δ 0.98 (3 H, d, J = 6.5 Hz), 1.09 (3 H, d, J = 7.4 Hz), 4.75 (1 H, d, J = 1.0 Hz), 4.90 (1 H, s), 5.13 (1 H, d, J = 1.8 Hz), 5.28 (1 H, d, J = 1.7 Hz).

(II)-mediated reaction to give, after Dess–Martin oxidation, the expected *trans*-enone in 60% overall yield (Scheme II).[19] The enone was successfully transformed into halichondrin B in three steps. Although this transformation was carried out without isolation of the product(s) at each step, the ^{1}H NMR spectrum indicated the product of the TBAF step to have the partial structure A. Obviously, this process involved deprotection of the C-48 *tert*-butyldimethylsilyl (TBS) group, hemiketal formation between the C-48 hydroxyl group and the C-44 ketone, and Michael addition of the hemiketal hydroxyl group onto the α,β-unsaturated ketone. The 5,5-spiroketal formation was then completed by deprotection of the C-41 MPM group, followed by acid treatment. The C-41 hydroxyl group needed to be protected differently from the others to avoid 5,6-spiroketal formation between the C-41 and C-48 hydroxyl groups and the C-44 ketone. Although this three-step transformation introduced three new chiral centers, its stereoselectivity was very high. The overall yield of the three-step transformation was 50–60%, and the synthetic halichondrin B was confirmed to be identical with natural halichondrin B (**1**)[20] on comparison of spectroscopic (^{1}H NMR, MS, IR, $[\alpha]_D$[21]) and chromatographic data.

The synthesis of norhalichondrin B was carried out in virtually the same way as for halichondrin B except that hydrolysis of the C-53 methyl ester was required as the very last step of the synthesis. The overall yield of the norhalichondrin B synthesis was comparable with that of halichondrin B. On comparison of spectroscopic (^{1}H NMR, MS, IR, $[\alpha]_D$[21]) and chromatographic data, the synthetic norhalichondrin B was proven to be identical with natural norhalichondrin B (**2**).[20,22]

The structure of halichondrin Bs was proposed primarily on the basis of three pieces of evidence: (1) comparison of their spectroscopic data with those of norhalichondrin A, the structure of which was unambiguously established by X-ray analysis; (2) biogenetic considerations of the C-50-and-beyond stereochemistry of halichondrin B; and (3) assumption that the absolute stereochemistry of halichondrin Bs is the same as that of norhalichondrin A, which was deduced from the exciton chirality of its C-12,C-13-bis(*p*-bromobenzoates).[1] The present synthetic work has established unambiguously the relative and absolute stereochemistry of halichondrin B and norhalichondrin B.

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Supplementary Material Available: ^{1}H NMR spectra of the intermediates shown in Schemes I and II and the α,β-unsaturated ketones of halichondrin B and norhalichondrin B (14 pages). Ordering information is given on any current masthead page.

(19) Partial ^{1}H NMR spectral data (C_6D_6) of the enone of the halichondrin B series: δ 4.77 (1 H, s), 4.92 (1 H, s), 5.08 (1 H, s), 5.19 (1 H, s), 6.39 (1 H, d, J = 16.1 Hz), 6.78 (1 H, dd, J = 16.1 and 6.6 Hz). Partial ^{1}H NMR spectral data (C_6D_6) of the enone of the norhalichondrin B series: δ 4.77 (1 H, s), 4.92 (1 H, s), 5.08 (1 H, s), 5.19 (1 H, s), 6.40 (1 H, d, J = 16.1 Hz), 6.79 (1 H, dd, J = 16.1 and 6.7 Hz).

(20) We are indebted to Professors Uemura and Hirata for samples of the naturally occurring halichondrins.

(21) $[\alpha]_D$ of halichondrin B: −51.2° (*c* 0.13 MeOH). $[\alpha]_D$ of norhalichondrin B: −49.0° (*c* 0.08 MeOH).

(22) In a preliminary experiment, we obtained synthetic homohalichondrin B in virtually the same way as halichondrin B.

(23) The keto phosphonate **4** was prepared in 60% overall yield from the known (2*R*,4*S*)-4-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-2-methyl-γ-butyrolactone (Tomooka, K.; Okinaga, T.; Suzuki, K.; Tsuchihashi, G. *Tetrahedron Lett.* **1989**, *30*, 1563–1566) in 10 steps. (1) MeLi/Et_2O. (2) TBSCl/imidazole/CH_2Cl_2/room temperature. (3) $TrisNHNH_2$/THF/cat. HCl. (4) (a) *n*-BuLi/THF; (b) *n*-Bu_3SnCl. (5) I_2/CH_2Cl_2/room temperature. (6) HF·Py/CH_3CN/room temperature. (7) $NaIO_4$/THF–H_2O (2:1)/room temperature. (8) Jones oxidation. (9) $C_6H_5CH_2OH$/DCC. (10) $LiCH_2P(O)(OMe)_2$.

(24) Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885–888, 889–892.