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Synthesis (and Alternative Proof of Configuration) of the Scyphostatin C(1′)–C(20′) Trienoyl Fragment

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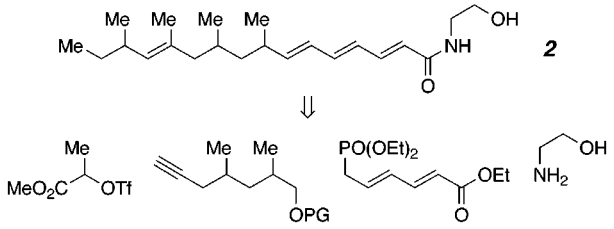
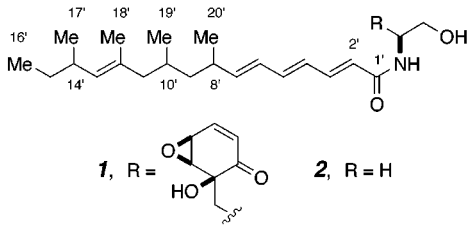
ABSTRACT



Each of four diastereomers of structure 2, corresponding to the lipophilic side chain of scyphostatin (1), were prepared. Careful analysis of their NMR spectral data and comparison with those of the natural product corroborates the recently reported (*Org. Lett.* 2000, *2*, 505) stereochemical assignment. A strategy for the stereoselective synthesis of 2 has been achieved.

Scyphostatin (**1**) was isolated from the mycelial extract of *Trichopeziza mollissima* by Ogita et al. (Sankyo Co., Ltd., Japan) in 1997.[1] It is the most potent (IC_{50} = 1.0 μM)[2] of the few known small molecule inhibitors of membrane-bound neutral sphingomyelinase (N-SMase). N-SMase inhibitors[3] recently have attracted considerable interest since they are believed to be promising candidates for the treatment of inflammation and autoimmune disease.

The structure of scyphostatin (**1**) consists of a lipophilic side chain [C(1′)−C(20′)] and a polar cyclohexenone moiety.



The relative and absolute configuration of the cyclohexenone core was assigned on the basis of NMR and derivatization studies.[1] However, in that initial report the absolute and relative configurations of the three stereocenters at C(8′), C(10′), and C(14′) within the lipid moiety had not yet been addressed. We, therefore, initiated a study aimed at deducing the relative configuration of the C(1′)−C(20′) trienoyl side chain in **1** while simultaneously establishing an efficient synthesis of that subunit. Very recently, the Sankyo group deduced and reported the absolute and relative configuration of the lipophilic moiety, determined by degradation of **1** and chemical correlation to synthetic fragments of unambiguous stereoisomeric composition.[4] In our studies presented here, we have developed a synthesis of the C(1′)−C(20′) side chain and independently determined the relative configuration within that subunit.

Our approach involved the comparison of proton and carbon NMR data from each of the four diastereomeric

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(3) (a) Tanaka, M.; Nara, F.; Yamasato, Y.; Masuda-Inoue, S.; Doi-Yoshioka, H.; Kumakura, S.; Enokita, R.; Ogita, T. *J. Antibiot.* **1999**, *52*, 670−3. (b) Tanaka, M.; Nara, F.; Yamasato, Y.; Ono, Y.; Ogita, T. *J. Antibiot.* **1999**, *52*, 827−30.

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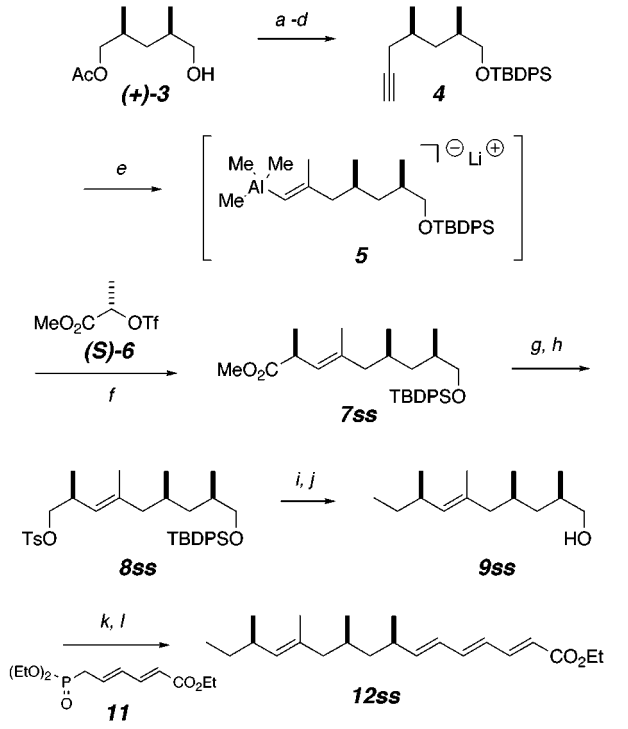
synthetic fragments of constitution **2** with those resonances from analogous protons and carbons in the intact natural product **1**.[5] Anisotropic chemical shift effects within diastereomers often operate over relatively long distances. Hence, we were optimistic that the substituents at the pair of stereogenic centers among C(8′)/C(10′)/C(14′) (1,3-, 1,5-, and 1,7-disposed across an intervening *E*-alkene) in each of the four possible diastereomers of **2** would communicate to a sufficient extent to give rise to distinct sets of NMR data.

The synthesis began (Scheme 1) with the known nonracemic alcohol **3** (92% ee), available in multigram quantities

Scheme 1

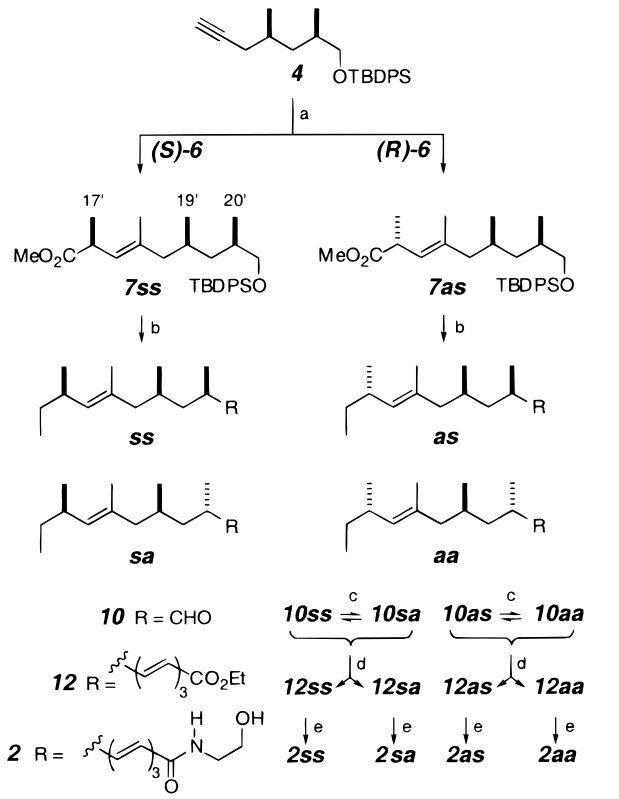


(*a*) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, rt, 99%. (*b*) K_2CO_3, MeOH:H_2O (1:1, v/v), reflux, 88%. (*c*) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 99%. (*d*) TMSC≡CLi, THF/DMSO, −78 °C to rt; then K_2CO_3, MeOH, 67%. (*e*) (i) Me_3Al, Cp_2ZrCl_2, then **4**, CH_2Cl_2, rt; (ii) MeLi, hexanes, −40 °C. (*f*) (*S*)-**6**, hexanes, −40 °C to rt, 51%. (*g*) DIBAL, CH_2Cl_2, −78 °C to 0 °C, 77%. (*h*) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 97%. (*i*) Me_2CuLi, Et_2O, −40 °C to rt. (*j*) TBAF, THF, rt, 72%, two steps. (*k*) TPAP, NMO, CH_2Cl_2, rt. (*l*) $(EtO)_2P(O)CH_2(CH{=}CH)_2CO_2Et$, LDA, −78 to 0 °C, 81%, two steps.

from the porcine pancreatic lipase (PPL) catalyzed resolution of *meso*-2,4-dimethyl-1,5-pentanediol.[6,7] Protection of the alcohol as its TBDPS ether, acetate cleavage, tosylation, displacement with TMSC≡C−Li,[8] and TMS removal gave alkyne **4**. The third stereocenter [C(14′)] was introduced by reaction of the triflate of *S*-methyl lactate [(*S*)-**6**] with the alkenylalanate **5**,[9] which was derived from zirconocene dichloride-promoted addition of trimethylaluminum[10] to alkyne **4**. The resulting *syn,syn*-(*E*)-β,γ-unsaturated ester **7ss** ["ss" = *syn,syn* = Me(17′)/Me(19′)-*syn*,Me(19′)/Me(20′)-*syn*] was reduced (DIBAL) and tosylated to provide **8ss**. Dimethyl cuprate displacement of the tosylate[11] and TBDPS removal gave alcohol **9ss**. TPAP oxidation of **9ss** gave aldehyde **10ss** (not shown) and immediate Horner−Wadsworth−Emmons olefination with phosphonate **11**[12] gave the *E,E,E*-triene[13] ester **12ss**, thereby completing the synthesis of the C(1′)−C(20′) fragment.

Two variations (Scheme 2) to the above theme allowed access to the three additional diastereomers (i.e., "sa", "as", and "aa"). First, coupling **5** with triflate (*R*)-**6** instead of (*S*)-**6** gave the *anti,syn* ester **7as**. Second, each of the *syn,syn*- and

Scheme 2



(*a*) steps *e*−*f* from Scheme 1. (*b*) steps *g*−*k* from Scheme 1. (*c*) KF, florisil, MeOH, rt. (*d*) step *l* from Scheme 1. (*e*) $TBSO(CH_2)_2NH_2$, Me_3Al, CH_2Cl_2, then **12**, rt.

(5) For example, see: (a) Cha, J. K.; Christ, W. J.; Finan, J. M.; Fujioka, H.; Kishi, Y.; Klein, L. L.; Ko, S. S.; Leder, J.; McWhorter, W. W., Jr.; Pfaff, K.-P.; Yonaga, M.; Uemura, D.; Hirata, Y. *J. Am Chem. Soc*. **1982**, *104*, 7369−7371 and preceding papers. (b) Hoye, T. R.; Suhadolnik, J. C. *J. Am. Chem. Soc*. **1987**, *109*, 4402−4403. (c) Gale, J. B.; Yu, J.-G.; Khare, A.; Hu, X. E.; Ho, D. H.; Cassady, J. M. *Tetrahedron Lett*. **1993**, *34*, 5851−4. (d) Oikawa, H.; Matsud, I.; Ichihara, A.; Kohmoto, K. *Tetrahedron Lett*. **1994**, *35*, 1223−6. (e) Boyle, C. D.; Harmange, J.-C.; Kishi, Y. *J. Am. Chem. Soc*. **1994**, *116*, 4995−6. (f) Harmange, J.-C.; Boyle, C. D.; Kishi, Y. *Tetrahedron Lett*. **1994**, *35*, 6819−22. (g) Kishi, Y.; Boyle, C. D. *Tetrahedron Lett*. **1995**, *36*, 5695−8. (h) Kishi, Y.; Boyle, C. D. *Tetrahedron Lett*. **1995**, *36*, 4579−82. (i) Zheng, W.; DeMattei, J. A.; Wu, J. P.; Duan, J. J. W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am Chem. Soc*. **1996**, *118*, 7946−68. (j) Sasaki, M.; Matsumori, N.; Maruyama, T.; Nonomura, T.; Murata, M.; Tachibana, K.; Yasumoto T. *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 1672−5. (k) Nonomura, T.; Sasaki, M.; Matsumori, N.; Murata, M.; Tachibana, K.; Yasumoto T. *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 1675−8. (l) Kishi, Y. *Pure Appl. Chem*. **1998**, *70*, 339−344. (m) Kobayashi, Y.; Lee, J.; Tezuka, K.; Kishi, Y. *Org. Lett*. **1999**, *1*, 2177−80. (n) Lee, J.; Kobayashi, Y.; Tezuka, K.; Kishi, Y. *Org. Lett*. **1999**, *1*, 2181−4.

anti,*syn*-aldehydes **10ss** and **10as** was epimerized with KF and Florisil in methanol to give access to diastereomeric aldehydes **10sa** and **10aa**, respectively. In each case the equilibrium slightly favored ($K_{eq} = \sim 1.5$) the C(8′)−C(10′) *anti* diastereomer (i.e., **10sa** over **10ss** and **10aa** over **10as**). Each of these epimeric mixtures was subjected to coupling with phosphonate **11**. The resulting diastereomeric pairs of trienoates (**12ss**/**12sa** and **12as**/**12aa**) were separable by MPLC on silica gel.

To compare the ^{1}H and ^{13}C NMR spectral data of our synthetic C(1′)−C(20′) fragments with those of the natural scyphostatin (**1**), we chose to convert the ethyl esters **12ss**−**12aa** to the 2-hydroxyethylamides **2ss**−**2aa**. This was accomplished by reacting each of the four diastereomers of **12** with "TBSO$(CH_2)_2$NHAlMe_2",[14] followed by desilylation.

Each of the four diastereomers of **2** gave distinctive ^{1}H and ^{13}C NMR spectra. The proton spectra of **2ss**−**2aa** (as well as of **12ss**−**12aa**) showed small but perceptible differences in many of the chemical shifts and splitting patterns of analogous resonances. The proton chemical shift differences ($\Delta\delta_H$) for H(2′)−H(20′) between scyphostatin (**1**)[15] and each of the diastereomers **2** ($\Delta\delta = \delta_1 - \delta_2$) are plotted in Figure 1 (all data recorded in CD_3OD). Simple visual inspection of these data indicate that the spectrum of the *syn*,*syn* isomer **2ss** most closely matches that of the natural product. To make this a more objective exercise, we also calculated the χ-squared value for the aliphatic protons H(8′)−H(20′) $\{\chi^2 = [\sum_{8'}^{20'}(\delta_1 - \delta_2)^2] \times 10^5\}$ for each of the four sets of ^{1}H NMR data (see insets in the upper right corner of each graph). Again, the best correlation (smallest χ^2) is between **1** and **2ss**. Analogous analysis of the ^{13}C NMR data {graphs not shown; $\chi^2 = [\sum_{8'}^{20'}(\delta_1 - \delta_2)^2] \times 10^2 = 1.3$, 6.2, 166, and 170 for **2ss**, **2as**, **2sa**, and **2aa**, respectively} led to the same conclusion—namely, the relative configuration of the C(1′)−C(20′) fragment is 8′*R**,10′*S**,14′*R**.

Happily, the relative configuration we have deduced is identical to that recently reported by the Sankyo group.[4] Moreover, one of the initial degradation products of scyphostatin (**1**) they prepared was the methyl ester analogue of trienoate **12ss**. Its specific rotation was similar to that of the ethyl ester we synthesized {methyl ester: $[\alpha]_D^{rt} = -2.5$ (*c* 0.50, $CHCl_3$) vs ethyl ester (**12ss**) $[\alpha]_D^{rt} = -2.35$ (*c* 0.34, $CHCl_3$)}, thereby affirming the absolute configuration as well. In conclusion, we have developed both an efficient stereoselective synthesis of the scyphostatin (**1**) lipophilic trienoyl side chain and an alternative proof of its relative and absolute configuration.

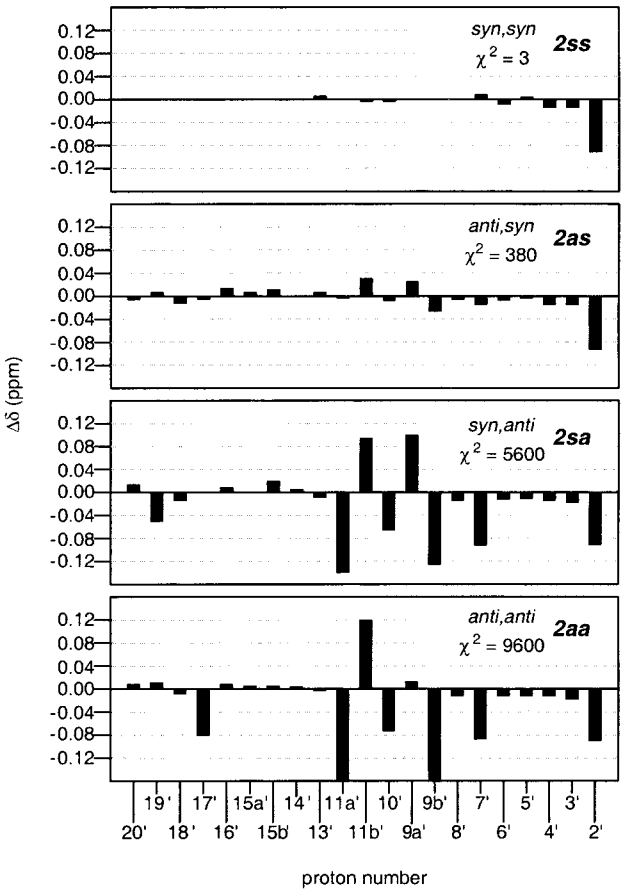


Figure 1. Graphical representation of the differences in ^{1}H NMR chemical shifts between each of the diastereomers of **2** vs scyphostatin (**1**) for ppotons 2′−20′ and the values of χ squared (χ^2, defined in text) for nontrienic protons 8′−20′.

Acknowledgment. This research was supported by the National Institutes of Health (CA-76497). We thank Dr. Masahiro Tanaka, Sankyo Co., Ltd., for providing the ^{1}H and ^{13}C NMR spectra of natural **1**, which were instrumental in our analyses.

Supporting Information Available: Experimental procedures and characterization data for all new compounds. Tables of ^{1}H and ^{13}C NMR spectral data for **2ss**, **2as**, **2sa**, **2aa**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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(13) Analysis of the crude ^{1}H NMR spectrum showed less than ~8% of the (SiO_2 separable) *E*,*E*,*Z*-isomer.

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(15) Original proton and carbon NMR spectra were kindly provided by Dr. M. Tanaka. Since we observed several small but important differences between the spectral data listed in the original report[1] and the values we read from the provided spectra, we have used the latter set for the analyses summarized in Figure 1. The values that we recorded from the provided proton and carbon spectra of scyphostatin can be found in Table C in the Supporting Information.