Natural Products Synthesis

Total Synthesis of (+)-Scyphostatin, a Potent and Specific Inhibitor of Neutral Sphingomyelinase**

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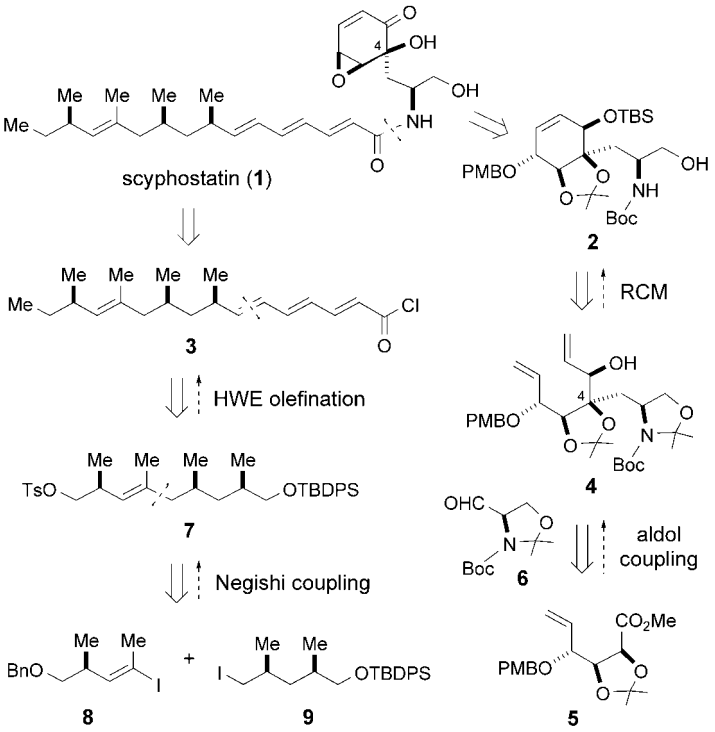
Scyphostatin (**1**, Scheme 1), isolated from a culture broth of *Dasyscyphus mollissimus* SANK-13892 by Ogita and co-workers in 1997,[1] is a powerful and specific inhibitor of neutral sphingomyelinase (N-SMase). It has been reported that **1** inhibits N-SMase and acidic SMase (A-SMase) with IC_{50} values of 1.0 μM and 49.3 μM, respectively.[2] Although a large number of N-SMase inhibitors have been reported to date,[3] **1** is still the most potent inhibitor of the enzyme.[1,2] N-SMase inhibitors regulate the level of ceramide, the hydrolysis product of sphingomyelin, in a wide variety of mammalian cells. Therefore, **1** is expected to be a promising new agent for the treatment of ceramide-mediated pathogenic states such as AIDS,[4] inflammation, and immunological and neurological disorders.[2] Structurally, **1** features a novel, highly oxygenated cyclohexene ring and an aminopropanol side chain linked to a C_{20} unsaturated fatty acid moiety. The significant biological properties coupled with the unique structural features make **1** an exceptionally intriguing and timely target for total synthesis. Although several synthetic approaches, including ours,[5] have appeared recently,[6] no total synthesis has been reported to date. We have already prepared the fully functionalized cyclohexene segment with the *N*,*O*-protected aminopropanol side chain and the correct stereogenic centers;[5b] however, all attempts to remove the *N*,*O*-protecting group (the cyclic carbonate moiety) from the cyclohexene segment met with failure.[5c] Herein we disclose an alternative and more reliable synthetic strategy that led to the completion of the total synthesis of **1**.



Scheme 1. Retrosynthetic plan for scyphostatin (**1**). TBS = *tert*-butyldimethylsilyl, PMB = 4-methoxybenzyl, Boc = *tert*-butoxycarbonyl, Ts = *p*-toluenesulfonyl, TBDPS = *tert*-butyldiphenylsilyl, Bn = benzyl.

The retrosynthetic plan for scyphostatin (**1**) is outlined in Scheme 1. Our preliminary model studies[5a,b] suggested that the labile epoxycyclohexenone moiety should be elaborated at a late stage of the synthesis. We envisaged that the cyclohexene segment **2** would be produced through ring-closing metathesis (RCM) of diene **4**. The RCM substrate **4** would be prepared through aldol coupling of ester **5** with the Garner aldehyde (**6**),[7] which would establish the requisite quaternary stereocenter C4. During the course of our studies, Hoye and Tennakoon reported that the fatty acid side chain (the ethyl ester variant of **3**) can be synthesized from tosylate **7** through Horner–Wadsworth–Emmons (HWE) olefination.[8] Therefore, we planned to employ **7** as a key intermediate in our synthesis and explored an alternative approach to **7**, which features Negishi coupling of vinyl iodide **8** and alkyl iodide **9**.[23]

The synthesis of the cyclohexene segment **2** started from the known compound **10**[9] (Scheme 2), readily prepared from D-arabinose by *O*-benzylglycosylation and acetonide formation. PMB protection of **10** followed by debenzylation and Wittig methylenation provided alcohol **11**, which was subsequently converted into methyl ester **5** through a three-step sequence involving twofold oxidation and methyl esterification. The crucial aldol coupling of **5** with **6** was effected by treatment of **5** with $NaN(SiMe_3)_2$ (1.3 equiv) followed by addition of **6** (1.1 equiv), which led to the desired product **12** as a single diastereomer in 69% yield.[10] The stereochemistry at C4 in **12** was deduced by NOE studies[11] of the deoxygenated compound **13**, which was obtained by applying the procedure reported by Barton and McCombie.[12] The

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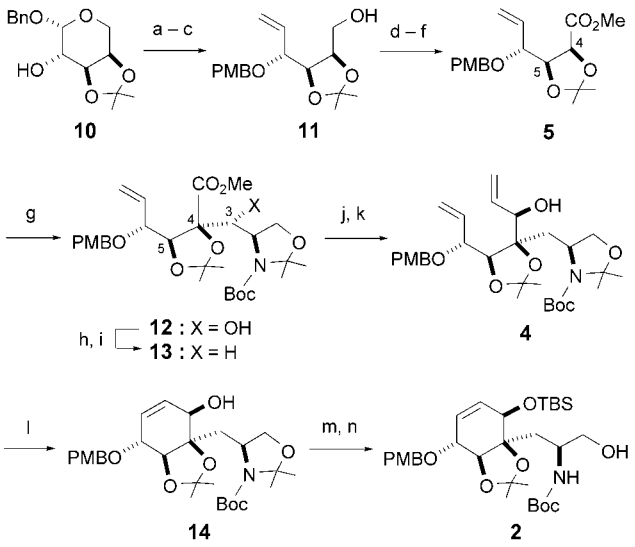
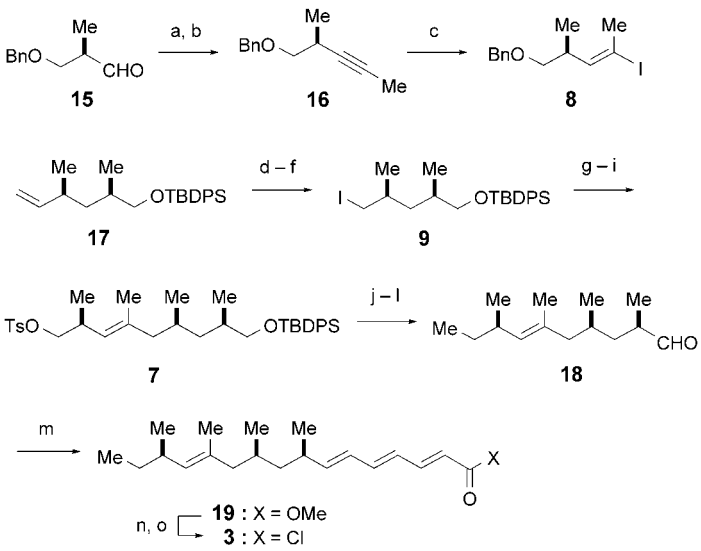


Scheme 2. Synthesis of the cyclohexene segment **2**. a) PMBCl, NaH, DMSO, room temperature, 70%; b) H_2, Raney Ni, EtOH, room temperature, 86%; c) $Ph_3P^+CH_3Br^-$, *t*BuOK, benzene, reflux, 86%; d) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C; *i*Pr_2NEt, −78→0 °C, 95%; e) $NaClO_2$, NaH_2PO_4, DMSO/H_2O, room temperature; f) CH_2N_2, Et_2O/MeOH, 0 °C, 78% (two steps); g) $NaN(SiMe_3)_2$, THF, −78 °C; Garner aldehyde (**6**), −78 °C, 69%; h) $NaN(SiMe_3)_2$, THF, 0 °C; CS_2; MeI, 0 °C→RT; i) *n*Bu_3SnH, AIBN, toluene, reflux, 53% (two steps); j) DIBAL, CH_2Cl_2, −100 °C, 88%; k) vinylmagnesium bromide, THF, 0 °C, 93%; l) $(Cy_3P)_2RuCl_2$(=CHPh) (10 mol%), CH_2Cl_2, reflux, 96%; m) TBSOTf, 2,6-lutidine, CH_2Cl_2, room temperature, 93%; n) PPTS, EtOH, 60 °C, 57%. DMSO = dimethyl sulfoxide, AIBN = 2,2′-azobisisobutyronitrile, DIBAL = diisobutylaluminum hydride, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl, Cy = cyclohexyl, PPTS = pyridinium *p*-toluenesulfonate.



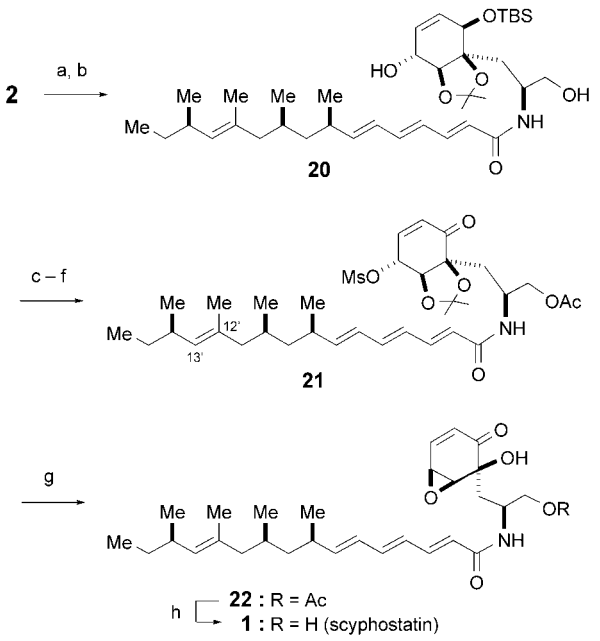
Scheme 3. Synthesis of the fatty acid segment **3**. a) PPh_3, CBr_4, CH_2Cl_2, 0 °C; b) *n*BuLi, THF, −78 °C; MeI, 94%, (two steps); c) Cp_2ZrHCl, benzene, 40 °C; I_2, RT, 63%; d) O_3, CH_2Cl_2/MeOH, −78 °C; $NaBH_4$, room temperature , 84%; e) MsCl, Et_3N, CH_2Cl_2, room temperature; f) NaI, acetone, reflux, 95% (two steps); g) *t*BuLi, $ZnCl_2$, Et_2O, −78→0 °C; **8**, $[Pd(PPh_3)_4]$, THF, 81%; h) lithium 4,4′-di-*tert*-butylbiphenylide, THF, −78 °C, 90%; i) TsCl, Et_3N, DMAP, CH_2Cl_2, RT; j) MeLi, CuI, Et_2O, −40 °C, 97% (two steps); k) TBAF, THF, room temperature, 91%; l) $(nPr)_4NRuO_4$, NMO, CH_2Cl_2, room temperature; m) $(MeO)_2P(O)CH_2CH{=}CH{-}CH{=}CH{-}CO_2Me$, LDA, THF, −78→−30 °C, 46% (two steps); n) KOH (2 M), MeOH/THF, room temperature; o) $(COCl)_2$, DMF, CH_2Cl_2, room temperature. Ms = methanesulfonyl, DMAP = 4-dimethylaminopyridine, TBAF = tetrabutylammonium fluoride, NMO = 4-methylmorpholine *N*-oxide, LDA = lithium diisopropylamide, DMF = *N*,*N*-dimethylformamide.

remarkable stereoselectivity at C4 observed in this coupling reaction seems to be induced by the adjacent C5 stereocenter. Reduction of **13** with DIBAL followed by vinylation of the resulting aldehyde furnished diene **4**. The critical RCM reaction of **4** in the presence of the Grubbs catalyst[13] proceeded smoothly and cleanly to give cyclohexenol **14** in 96% yield. Finally, **14** was converted into **2** by *O*-silylation and selective acetonide cleavage.

The synthesis of the fatty acid segment **3** was next investigated (Scheme 3). Vinyl iodide **8** was prepared from the known aldehyde **15**[14] via alkyne **16** by a Corey–Fuchs reaction[15] and subsequent hydrozirconation/iodination.[16] Alkyl iodide **9**, the coupling partner of **8**, was derived from the known olefin **17**[17] by ozonolysis with reductive workup followed by mesylation and iodination. The key Negishi coupling of **9** and **8** was carried out successfully by employing the modified conditions of Smith et al.[18] to give the desired coupling product (81% yield), which was converted into the Hoye intermediate **7** by debenzylation and tosylation. According to the Hoye protocol,[8] **7** was transformed into aldehyde **18** in a three-step sequence. Aldehyde **18** was then subjected to HWE olefination[19] to yield methyl ester **19**. Spectral data (^1H and ^{13}C NMR, MS) of **19** were in good agreement with those reported.[20] Finally, the desired acid chloride **3** was derived from **19** in two steps.

The projected synthesis was completed as shown in Scheme 4. The Boc and PMB groups of the cyclohexene segment **2** were simultaneously removed with TMSOTf[21] to produce the corresponding amino diol, which was immediately treated with the fatty acid segment **3** to give, after treatment of aqueous AcOH, the desired amide **20** in 73%. Enone **21** was elaborated from **20** by selective acetylation, mesylation, desilylation, and Dess–Martin oxidation. In our previous work,[5a,b] the requisite epoxide moiety was successfully installed by hydrolysis of the acetonide with aqueous CF_3CO_2H, followed by treatment with base. However, upon applying these conditions to **21**, partial isomerization of the C12′-C13′ double bond in the side chain was observed during the acid hydrolysis. Eventually, we overcame this problem by using aqueous CCl_3CO_2H instead of CF_3CO_2H; subsequent treatment with NaOH afforded the desired epoxide **22** without appreciable isomerization of the double bond. The final step was to remove the acetyl group of **22**; deprotection was best brought about by exposure of **22** to lipase PS in phosphate buffer,[22] furnishing (+)-scyphostatin (**1**) in 60% yield, which was identical to natural (+)-**1** in all respects (^1H and ^{13}C NMR, HRMS, and $[\alpha]_D$).[1,2]

In conclusion, we completed the first total synthesis of (+)-scyphostatin (**1**) in an efficient and flexible way. Impor-



Scheme 4. Total synthesis of scyphostatin (**1**). a) TMSOTf, 2,6-lutidine, CH_2Cl_2, room temperature; MeOH; b) **3**, Et_3N, CH_2Cl_2, room temperature; AcOH (aq.), 73% (two steps); c) Ac_2O, pyridine, DMAP, CH_2Cl_2, room temperature, 72%; d) MsCl, Et_3N, CH_2Cl_2, room temperature, 93%; e) TBAF, THF, room temperature; f) Dess–Martin periodinane, CH_2Cl_2, room temperature, 98% (two steps); g) CCl_3CO_2H, CH_2Cl_2/H_2O, reflux; NaOH (2 M), room temperature, 45%; h) lipase PS, pH 7 phosphate buffer/acetone, room temperature, 60%. TMS = trimethylsilyl.

tantly, the synthesis has the potential for producing scyphostatin analogues with a wide variety of fatty acid side chains.

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[22] All attempts at deacetylation of **22** under a various conditions (e.g. K_2CO_3, NaOMe, or KOH in MeOH; aqueous KOH in THF or CH_2Cl_2; DBU or NH_3 in THF) met with failure; presumably, the epoxycyclohexenone moiety present in **22** and/or **1** is sensitive to these basic conditions. Detailed results and discussions will be presented in a full account.

[23] Note added in proof: After submission of this manuscript, we learnt of independent syntheses of the scyphostatin side chain in which similar Negishi-type cross-coupling reactions were employed: a) Z. Tan, E.-i. Negishi, *Angew. Chem.* **2004**, *116*, 2971; *Angew. Chem. Int. Ed.* **2004**, *43*, 2911; b) G. D. McAllister, R. J. K. Taylor, *Tetrahedron Lett.* **2004**, *45*, 2551; we are grateful to Professor Ei-ichi Negishi for kindly providing us with a preprint of his paper prior to publication.

