HETEROCYCLES, Vol. 73, 2007, pp. 263 - 268. © The Japan Institute of Heterocyclic Chemistry
Received, 23rd July, 2007, Accepted, 30th August, 2007, Published online, 4th September, 2007. COM-07-S(U)52

SYNTHESIS OF SCYPHOSTATIN ANALOGS POSSESSING VARIOUS SATURATED FATTY ACID SIDE-CHAINS‡

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Abstract – Scyphostatin analogs **2a–f** possessing various saturated fatty acid side-chains (C_{12}–C_{19}) were efficiently synthesized in enantiomerically pure form starting from the known cyclohexene derivative **3**. These analogs were designed to improve the stability of scyphostatin (**1**), a powerful and specific inhibitor of neutral sphingomyelinase (N-SMase) from a microorganism.

Scyphostatin (**1**, Figure 1), isolated from a culture broth of *Trichopeziza mollissima* SANK 13892 in 1997,[1] has been shown to be a powerful and specific inhibitor of membrane-bound 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 and 49.3 μM, respectively.[1] Remarkably, **1** is the most potent and specific among the many low-molecular weight N-SMase inhibitors, from natural sources[2] or synthesized,[3] known to date.

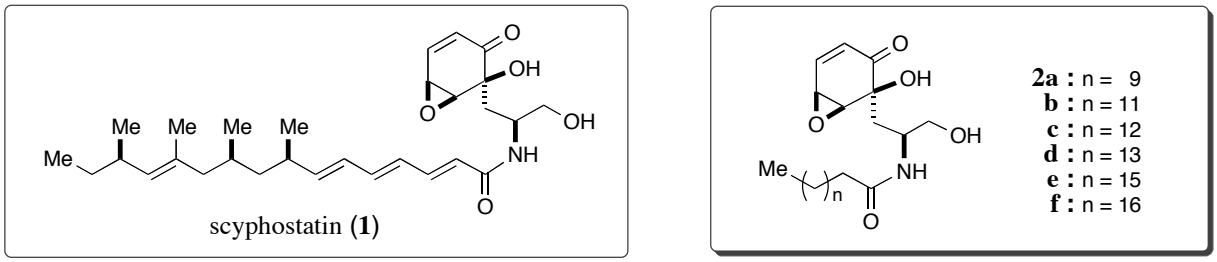


Figure 1. Structures of scyphostatin (**1**) and scyphostatin analogs **2a–f**

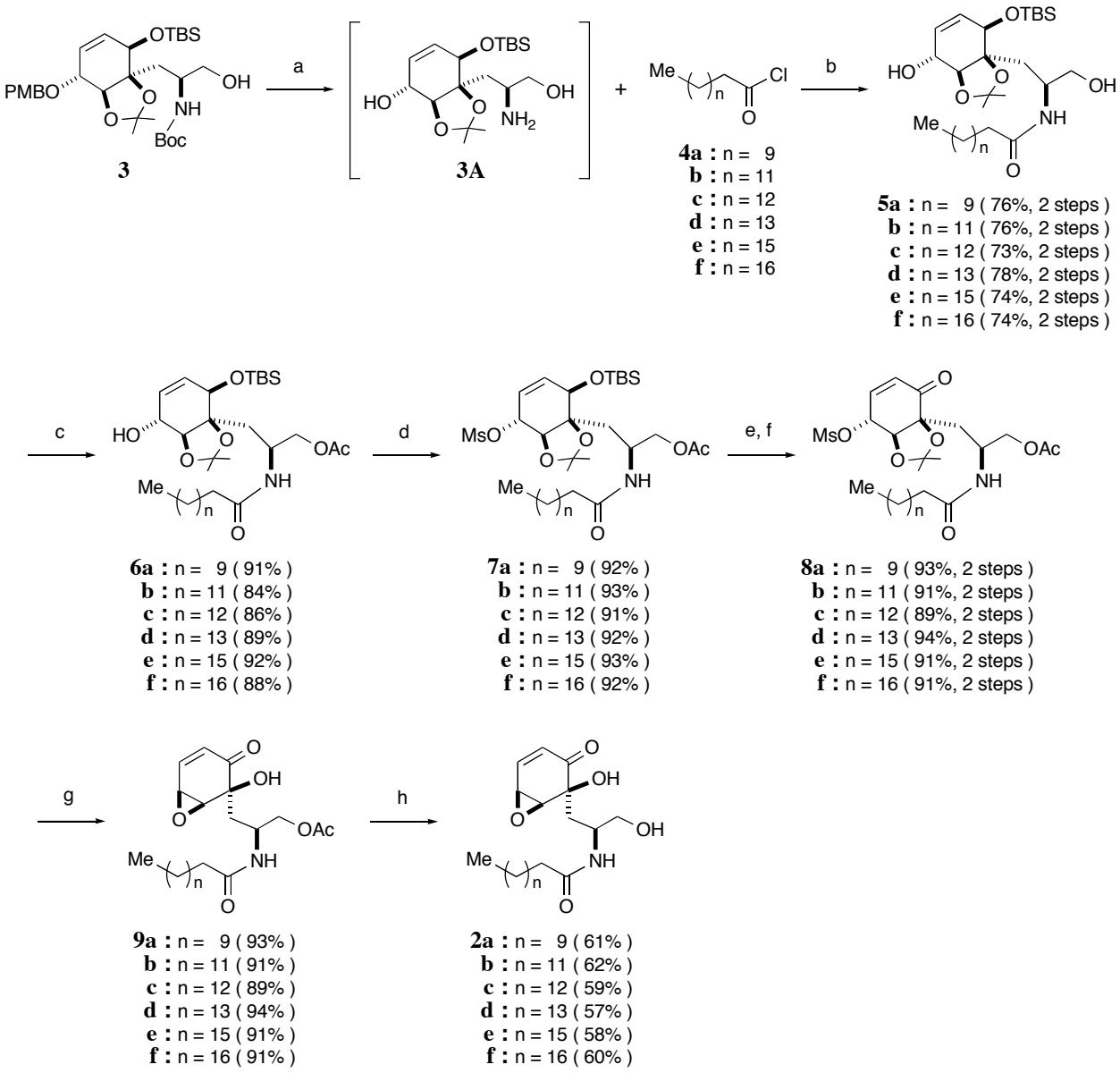
‡This paper is dedicated to the memory of Professor Ivar Ugi of the Technical University of Munich.

N-SMase is an enzyme that specifically cleaves the phosphoester linkage of sphingomyeline (SM) to generate phosphocholine and ceramide. The SM-derived ceramide has been known as an intracellular lipid second messenger in mammalian cell membranes and plays important roles in the cellular signal transmission pathway in particular, as a signal transduction factor in cell differentiation and apoptosis induction.[4] Scyphostatin (**1**), therefore, is recognized as a promising candidate or a potential lead compound for the treatment of ceramide-mediated pathogenic states such as AIDS,[5] inflammation, and immunological and neurological disorders.[1b,c]

Structurally, scyphostatin (**1**) consists of a novel, highly oxygenated cyclohexene ring incorporated with a 20-carbon fatty acid-substituted aminopropanol side-chain. Because of its prominent biological properties and unique structural features, much effort has been devoted in recent years to develop a synthetic route to this natural product.[6] We have already reported our own results about the enantioselective synthesis of the epoxycyclohexenone core.[7] In addition, we have reported an efficient method for the introduction of a fatty acid side-chain at the amino propanol moiety.[8] In 2004, we completed the first total synthesis of (+)-**1**,[9] which facilitated the preparation of scyphostatin analogs in enantiomerically pure form. Recently, Takagi and co-workers disclosed the stereoselective total synthesis of (+)-**1**.[10] Here, we report the synthesis of scyphostatin analogs **2a**–**f** possessing various saturated fatty acid side-chains using a method based on our previously explored strategy.[9]

Scyphostatin (**1**) is very unstable under both acidic and basic conditions.[1b] The conjugated triene moiety of the 20-carbon fatty acid side-chain causes decomposition even under neutral conditions.[1b] Consequently, improving the stability of **1** is highly desirable and worthwhile from the viewpoint of medicinal chemistry and pharmaceuticals. To achieve this, we planned to synthesize six scyphostatin analogs **2a**–**f**, in which the unstable 20-carbon unsaturated fatty acid moiety of **1** is replaced with steady saturated fatty acids such as lauric- (12-carbon), myristic- (14-carbon), *n*-pentadecanoic- (15-carbon), palmitic- (16-carbon), stearic- (18-carbon), and *n*-nonadecanoic- (19-carbon) acids. Although several scyphostatin analogs have been prepared to date,[3a,e,10] this class of analogs has not yet been synthesized owing to the lack of an efficient and reliable synthetic methodology.

As shown in Scheme 1, the synthesis began with the coupling reaction of the highly functionalized cyclohexene derivative **3**,[9] a key intermediate in our total synthesis of (+)-**1**, with commercially available fatty acid chlorides **4a**–**f**. The *tert*-butoxycarbonyl (Boc) and *p*-methoxybenzy (PMB) protecting groups of **3** were removed simultaneously by treatment with trimethylsilyl triflate (TMSOTf)[12] in the presence of 2,6-lutidine to produce the liberated amino alcohol **3A**, whose amino group was acylated immediately with **4a**–**f** in the presence of triethylamine to give the amide coupling products **5a**–**f** in 73–78% yields from **3**. Amides **5a**–**f** were then converted into the corresponding mesylates **7a**–**f** in 78–86% overall yields by selective acetylation (Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 30 min) of the primary hydroxy group



Scheme 1. Synthesis of scyphostatin analogs **2a**–**f**. *Reagents and conditions*: (a) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 3 h; (b) fatty acid chlorides **4a**–**f**, Et_3N, CH_2Cl_2, rt, 1 h; (c) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 30 min; (d) MsCl, Et_3N, CH_2Cl_2, rt, 1 h; (e) TBAF, THF, rt, 40 min; (f) Dess-Martin periodinane, CH_2Cl_2, rt, 30 min; (g) TFA, CH_2Cl_2/ H_2O, reflux, 3 h; then 2M NaOH, rt, 10 min; (h) Lipase PS, PH 7 phosphate buffer/acetone, rt, 12 h.

and subsequent mesylation (MsCl, Et_3N, CH_2Cl_2, rt, 1 h) of the secondary hydroxy group in the resulting acetates **6a**–**f**. Deprotection (TBAF, THF, rt, 40 min) of the *tert*-butyldimethylsilyl (TBS) group in **7a**–**f** followed by Dess–Martin oxidation provided the requisite enones **8a**–**f** in 89–94% yields for the two steps.

The remaining task to complete the synthesis was cleavage of the *O*,*O*-isopropylidene moiety in **8a**–**f** followed by epoxide-ring formation and final deprotection of the acetyl group. To achieve this, hydrolysis of the *O*,*O*-isopropylidene moiety of **8a**–**f** was efficiently carried out by reaction with aqueous trifluoroacetic acid (TFA) in CH_2Cl_2 at reflux for 3 hours to provide liberated vicinal diols. These diols,

without isolation, were then treated with 2M NaOH at ambient temperature for 10 minutes, leading to the formation of the requisite epoxides **9a–f** in 89–94% yields in two steps. The final step was deprotection of the acetyl group in **9a–f**. Initial attempts to achieve this under standard conditions (e.g., aq NaOH in MeOH, THF, CH_2Cl_2; K_2CO_3, NaOMe, NaOH in MeOH; NH_3, DBU in THF) resulted in almost total decomposition of the products, presumably because of the sensitivity of the epoxycyclohexenone moiety to these basic conditions. Therefore, we carried out the delicate acetyl deprotection by employing an enzymatic method which involved exposure of **9a–f** to lipase PS in phosphate buffer–acetone at room temperature for 12 hours. This provided the targeted scyphostatin analogs **2a–f**[13] in 57–62% yields. The synthesized analogues **2a–f** were relatively stable under neutral conditions in a refrigerator.

In conclusion, we synthesized scyphostatin analogs **2a–f** possessing various saturated fatty acid side-chains in enantiomerically pure form starting from the known cyclohexene derivative **3**. This synthetic methodology should be applicable to other important scyphostatin analogs possessing a wide variety of fatty acid side-chains. The biological activities of **2a–f** are under investigation and will be reported in due course.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Area “Creation of Biological Functional Molecules” (No. 17035073), a Grant-in-Aid for Scientific Research (C) (No. 18590013), and the High Technology Research Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

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13. **2a**: $[\alpha]_D^{27}$ +15.9° (*c* 0.05, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (3H, t, *J* = 6.8 Hz), 1.36 (16H, br s), 1.46–1.57 (2H, m), 1.77–1.90 (2H, m), 1.99–2.10 (2H, m), 3.51 (1H, br ddd, *J* = 1.5, 3.9, 3.9 Hz), 3.64 (1H, d, *J* = 3.4 Hz), 3.92 (1H, s), 4.01–4.05 (2H, m), 4.11–4.18 (2H, m), 5.76 (1H, br d, *J* = 7.3 Hz), 6.14 (1H, dd, *J* = 1.5, 10.2 Hz), 7.08 (1H, dd, *J* = 3.9, 10.2 Hz).

2b: $[\alpha]_D^{27}$ +15.0° (*c* 0.04, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, t, *J* = 6.8 Hz), 1.37 (20H, br s), 1.46–1.58 (2H, m), 1.78–1.89 (2H, m), 1.97–2.09 (2H, m), 3.51 (1H, br ddd, *J* = 1.5, 3.9, 3.9 Hz), 3.64 (1H, d, *J* = 3.4 Hz), 3.91 (1H, s), 4.01–4.05 (2H, m), 4.11–4.18 (2H, m), 5.77 (1H, br d, *J* = 7.3 Hz), 6.14 (1H, dd, *J* = 1.5, 10.2 Hz), 7.09 (1H, dd, *J* = 3.9, 10.2 Hz).

2c: $[\alpha]_D^{26}$ +15.9° (*c* 0.05, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, t, *J* = 6.8 Hz), 1.34 (22H, br s), 1.46–1.57 (2H, m), 1.78–1.92 (2H, m), 1.99–2.12 (2H, m), 3.51 (1H, br ddd, *J* = 1.5, 3.9, 3.9 Hz), 3.63 (1H, d, *J* = 3.4 Hz), 3.92 (1H, s), 4.00–4.06 (2H, m), 4.09–4.19 (2H, m), 5.76 (1H, br d, *J* = 7.3 Hz), 6.15 (1H, dd, *J* = 1.5, 10.2 Hz), 7.08 (1H, dd, *J* = 3.9, 10.2 Hz).

2d: $[\alpha]_D^{27}$ +15.0° (*c* 0.04, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, t, *J* = 6.8 Hz), 1.38 (24H, br s), 1.46–1.56 (2H, m), 1.77–1.90 (2H, m), 1.98–2.10 (2H, m), 3.50 (1H, br ddd, *J* = 1.5, 3.9, 3.9 Hz), 3.64 (1H, d, *J* = 3.4 Hz), 3.96 (1H, s), 4.02–4.05 (2H, m), 4.11–4.18 (2H, m), 5.74 (1H, br d, *J* = 7.3 Hz), 6.14 (1H, dd, *J* = 1.5, 10.2 Hz), 7.07 (1H, dd, *J* = 3.9, 10.2 Hz).

2e: $[\alpha]_D^{26}$ +15.9° (*c* 0.05, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (3H, t, *J* = 6.8 Hz), 1.36 (28H, br s), 1.46–1.57 (2H, m), 1.77–1.91 (2H, m), 1.99–2.10 (2H, m), 3.51 (1H, br ddd, *J* = 1.5, 3.9, 3.9 Hz), 3.63 (1H, d, *J* = 3.4 Hz), 3.92 (1H, s, 1H), 4.01–4.05 (2H, m), 4.09–4.19 (2H, m), 5.76 (1H, br d, *J* = 7.3 Hz), 6.12 (1H, dd, *J* = 1.5, 10.2 Hz), 7.08 (1H, dd, *J* = 3.9, 10.2 Hz).

2f: $[\alpha]_D^{27}$ +19.9° (*c* 0.05, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, t, *J* = 6.8 Hz), 1.37 (30H, br s), 1.45–1.57 (2H, m), 1.73–1.93 (2H, m), 1.99–2.13 (2H, m), 3.49 (1H, br ddd, *J* = 1.5, 3.9, 3.9 Hz), 3.64 (1H, d, *J* = 3.4 Hz), 3.90 (1H, s), 4.00–4.08 (2H, m), 4.10–4.22 (2H, m), 5.75 (1H, br d, *J* = 7.3 Hz), 6.14 (1H, dd, *J* = 1.5, 10.2 Hz), 7.08 (1H, dd, *J* = 3.9, 10.2 Hz).