ORGANIC LETTERS

2008
Vol. 10, No. 20
4449−4452

Stereospecific Total Synthesis of Somocystinamide A

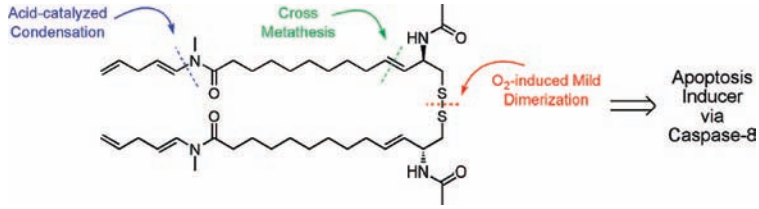
Takashi L. Suyama and William H. Gerwick*

Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, 9500 Gilman Drive, La Jolla, California 92093

wgerwick@ucsd.edu

Received July 24, 2008

ABSTRACT



The first total synthesis of somocystinamide A, a disulfide dimer with extremely labile enamide functional groups, was accomplished in a concise and stereospecific manner. Somocystinamide A is reported to possess exceptionally potent antiangiogenic and tumoricidal activities. The current work should enable further pharmacological investigation of this important natural product.

Marine cyanobacteria are a rich source of biomedically relevant secondary metabolites that are of unique molecular architecture.[1] In line with this theme, somocystinamide A (**1**, Figure 1) was isolated from a mixed assemblage of *Schizothrix* and *Lyngbya* and shown to possess remarkable biological properties.[2,3] Initially, **1** only showed moderate activity against mouse neuroblastoma cells (Neuro-2a).[3] In subsequent studies, however, its IC_{50} against human umbilical vein endothelial cells (HUVECs) was found to be 500 fM.[2] This astonishing in vitro finding was verified in zebra fish wherein antiangiogenetic effects were observed at 80 nM media concentration. Despite this potency, **1** was shown to have no observable adverse effects on zebra fish even at 30 μM. In addition, **1** was shown to trigger apoptosis in tumor cells via caspase-8 activation.[2] This activity profile supports the development of **1**, or analogues thereof, for potential use in cancer treatment.

Biosynthetically, somocystinamide A appears to be assembled through alternating NRPS-PKS elements with a unique termination of a PKS unit via decarboxylation and dehydration to furnish the terminal olefin as seen in curacin A.[4] Methylation of the enamide using *S*-adenosyl methionine, a signature decoration in marine cyano-bacterial natural products,[5] produces the tertiary enamide. Secondary enam-

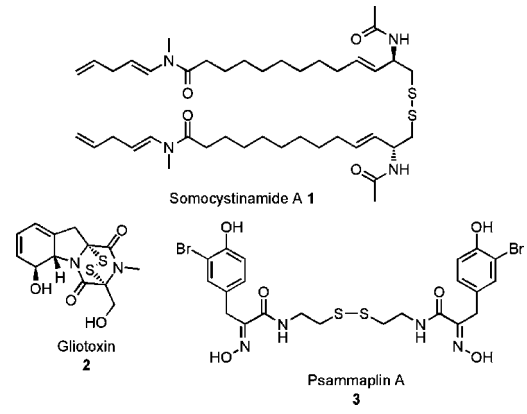


Figure 1. Examples of disulfide-containing natural products.

(1) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. *The Alkaloids*; Cordell, G. A., Ed.; Academic Press; San Diego, CA 2001; Vol. 57, pp 75−184.
(2) Wrasidlo, W.; Mielgo, A.; Torres, V. A.; Barbero, S.; Stoletov, K.; Suyama, T. L.; Klemke, R. L.; Gerwick, W. H.; Carson, D. A.; Stupack, D. G. *Proc. Nat. Acad. Sci. U.S.A.* **2008**, *105*, 2313–2318.
(3) Nogle, L. M.; Gerwick, W. H. *Org. Lett.* **2002**, *4*, 1095–1098.
(4) Moore, B. S. *Nat. Prod. Rep.* **2005**, *22*, 580–593.

10.1021/ol8016947 CCC: $40.75 © 2008 American Chemical Society
Published on Web 09/13/2008

ides have been observed in many natural products, and their preparation has been studied extensively in recent years.[6] Tertiary enamides, however, are encountered very rarely in natural products, and as such, the strategies for their preparation are relatively undeveloped and scarce.[6,7] Furthermore, the presence of the disulfide group in **1** requires great care and consideration during the course of synthesis.[8] For example, synthetic investigation of epidithiapiperazinedione natural products (such as **2**) has met with much difficulty in the installation of the disulfide.[8,9] To date, only one complete total synthesis of a compound of this class has been reported.[10] Another case in point is psammaplin A (**3**); in all three of the published syntheses of **3**, the sulfur atoms were introduced as a disulfide in the final step so as to avoid side reactions.[11]

It was envisioned in the synthesis of **1** that the key carbon−carbon connection at the internal olefin would be made by olefin cross metathesis using a ruthenium catalyst.[12] Accordingly, terminal olefin **6** was prepared from the known aldehyde **5**[13] via a Wittig reaction (Scheme 1).[14] Thiazolidine was chosen as the protecting group for the thiol of **4** because of its relative stability and its tandem protection of the carbamate proton.

Screening of commercially available ruthenium catalysts revealed that the second-generation Hoveyda−Grubbs catalyst (**11**) was optimal (Table 1).[12c] Furthermore, this reaction was optimized for multigram scale by adjusting the concentration and number of equivalents of **7**. Good stereoselectivity was observed in all cases (e.g., trans:cis = 18:1, entry 8). Minimizing the amount of **7** facilitated the purification process because the major side product of the reaction, dimer **12**, closely eluted with the desired product **8** during chromatography. In line with recent reports that some ruthenium catalysts are more functional group-tolerant than initially suspected,[15] alkyl sulfides are apparently very well tolerated by **11**, but not by **9**, suggesting that there may be competition between tricyclohexylphosphine and the sulfide **6** for binding as a ligand on ruthenium.[16]

The methyl ester **8** was hydrolyzed to obtain the carboxylic acid **13** in order to avoid undesired reduction to the primary alcohol in the next step. Afterward, the thiol and the carbamate of **13** were reductively deprotected by sodium in liquid ammonia (Scheme 2).[13a] Reprotection of the carboxylic acid as a methyl ester, deprotection of the amine, and

Scheme 1. Cross-Metathesis to **8**

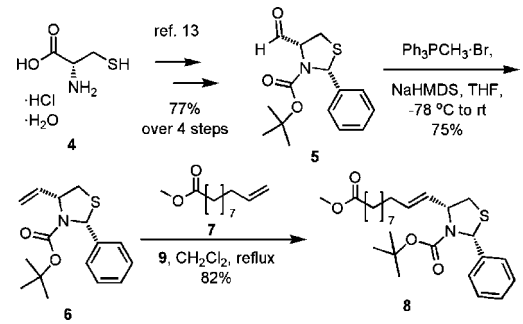
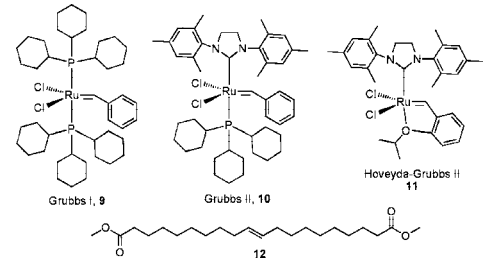


Table 1. Olefin Cross-Metathesis with Various Conditions To Produce **8**

	catalyst (mol %)	conc[a]	ester[b], equiv	yield of **8**[c] (%)
1	**9**, 20	0.04	**7**, 10	0
2	**10**, 20	0.04	**7**, 10	26 (na)
3	**10**, 4	0.04	**7**, 3	23 (57)
4	**11**, 5	0.03	**7**, 3	81 (94)
5	**11**, 5	0.03	**12**, 1.5	53 (55)
6	**11**, 2.5	0.03	**7**, 3	44 (na)
7[d]	**11**, 5	0.04	**7**, 3	73 (83)
8[e]	**11**, 5	0.2	**7**, 2.2	82 (82)

[a] Concentration of **6** (M). [b] Equivalents of **7** or **12** with respect to **6**. [c] Isolated yields. Yields in parentheses are based on recovered starting material. [d] 3.2 g scale (ca. 10 times more than entries 1−6). [e] 5.5 g scale.



(5) Chang, Z; Sitachitta, N.; Rossi, J.; Roberts, M. A.; Flatt, P. M.; Jia, J.; Sherman, D. H.; Gerwick, W. H. *J. Nat. Prod.* **2004**, *67*, 1356–1367.

(6) (a) Tracey, M. R.; Hsung, R. P.; Antoline, J.; Kurtz, K. C.; Shen, L.; Slafer, B. W.; Zhang, Y. *Sci. Synth.* **2005**, *21*, 387–475. (b) Jiang, L.; Job, G. E.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 3667–3669. (c) Coleman, R. S.; Liu, P. *Org. Lett.* **2004**, *6*, 577–580. (d) Dehli, J. R.; Legros, J.; Bolm, C. *Chem. Commun.* **2005**, 973–986. (e) Pan, X.; Cai, Q.; Ma, D. *Org. Lett.* **2004**, *6*, 1809–1812. (f) Han, C.; Shen, R.; Su, S.; Porco, J. A., Jr. *Org. Lett.* **2004**, *6*, 27–30.

(7) (a) Cook, G. R.; Stille, J. R. *Tetrahedron* **1994**, *50*, 4105–4124. (b) Gangadasu, B.; Narender, P.; Kumar, S. B.; Ravinder, M.; Rao, B. A.; Ramesh, C.; Raju, B. C.; Rao, V. J. *Tetrahedron* **2006**, *62*, 8398–8403. (c) Cook, G. R.; Barta, N. S.; Stille, J. R. *J. Org. Chem.* **1992**, *57*, 461–467. (d) Couture, A.; Dubiez, R.; Lablache-Combier, A. *J. Org. Chem.* **1984**, *49*, 714–717. (e) Pattenden, G.; Ashweek, N. J.; Baker-Glenn, C. A. G.; Kempson, J.; Walker, G. M.; Yee, J. G. K. *Org. Biomol. Chem.* **2008**, *6*, 1478–1497. (f) Morales, O.; Seide, W.; Watson, S. E. *Synth. Commun.* **2006**, *36*, 965–973.

(8) Overman, L. E.; Sato, T. *Org. Lett.* **2007**, *9*, 5267–5270.

(9) Middleton, M. D.; Peppers, B. P.; Diver, S. T. *Tetrahedron* **2006**, *62*, 10528–10540.

(10) Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* **1981**, *37*, 2045–2078.

(11) (a) Nicolaou, K. C.; Hughes, R.; Pfefferkorn, J. A.; Barluenga, S.; Roecker, A. J. *Chem.−Eur. J.* **2001**, *7*, 4280–4295. (b) Hoshino, O.; Murakata, M.; Yamada, K. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1561–1562. (c) Coleman, R. S.; Liu, P. *Org. Lett.* **2004**, *6*, 577–580.

(12) (a) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953–956. (b) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490–4527. (c) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168–8179.

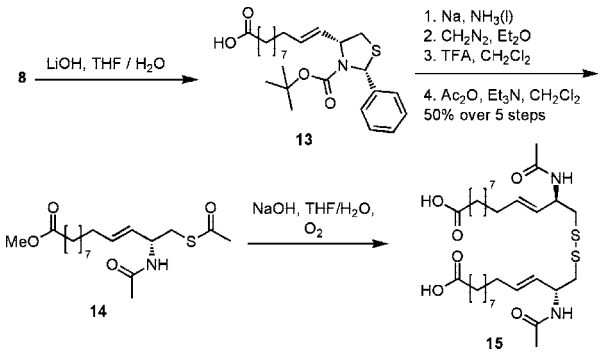
(13) (a) Deroose, F. D.; De Clercq, P. J. *J. Org. Chem.* **1995**, *60*, 321–330. (b) Chavan, S. P.; Chittiboyina, A. G.; Ramakrishna, G.; Tejwani, R. B.; Ravindranathan, T.; Kamat, S. K.; Rai, B.; Sivadasan; L.; Balakrishnan, K.; Ramalingam, S.; Deshpande, V. H. *Tetrahedron* **2005**, *61*, 9273–9280.

(14) Rh-catalyzed methylenation with $TMSCHN_2$ gave poor results. (a) Wittig, G.; Schöllkopf, U. *Ber.* **1954**, *87*, 1318. (b) Lebel, H.; Paquet, V. *J. Am. Chem. Soc.* **2004**, *126*, 320–328.

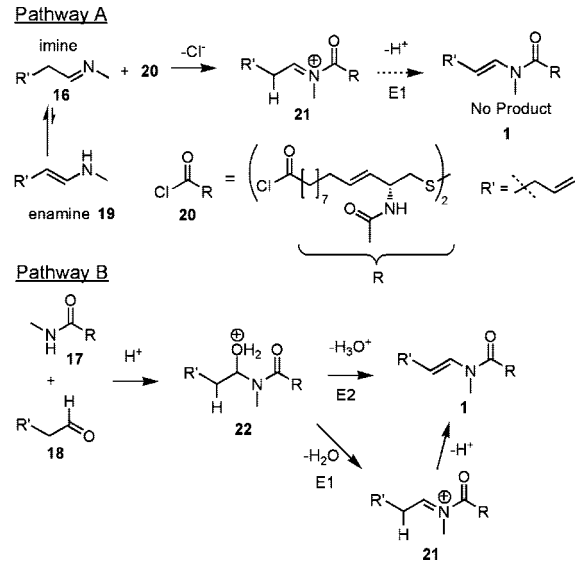
(15) (a) Compain, P. *Adv. Synth. Catal.* **2007**, *349*, 1829–1846. (b) Vernall, A. J.; Abell, A. D. *Aldrichim. Acta* **2003**, *36*, 93–105. (c) Suyama, T. L.; Gerwick, W. H. *Org. Lett.* **2006**, *8*, 4541–4543.

(16) To our knowledge, this is the first example of a successful cross-metathesis of an alkyl sulfide substrate by ruthenium carbene catalysis.

Scheme 2. Synthesis of Disulfide **15** (Somocystinoic Acid)



Scheme 3. Possible Mechanisms for Enamide Formation



acetylation yielded **14** in a good yield. Simultaneous basic hydrolysis of the methyl ester and the thioacetate in the presence of O_2 cleanly caused dimerization to the disulfide in one pot to give **15**. However, attempts to effect the dimerization by conventional means, such as treatment with I_2, did not give good yields.[17]

With **15** (named "somocystinoic acid") in hand, various conditions were investigated to couple the in situ generated imine **16** to the corresponding diacyl chloride **20**, the formation of which was verified by the reaction with methylamine to produce **17**. In most cases, the starting material decomposed while in some cases a trace amount of **1** was observed. This result was curious because there are reports of synthesis of simple enamides via acylation of the corresponding acid chloride with imine.[7,18] It is possible that the putative acyl iminium ion intermediate **21** is intercepted via an intramolecular reaction due to its dimeric nature (Scheme 3).[18] In support of this hypothesis, only tautomer **16** and not **19** was observed by ^{1}H and ^{13}C NMR in CD_2Cl_2.[19]

We then turned our attention to the recently developed Cu-mediated vinylation reaction.[6] It has been reported, although with little experimental evidence, that this approach is inapplicable to acylic secondary amides.[6b,d,f] Therefore, coupling between a simple amide **23** and a commercially available vinyl bromide **24** was investigated, but found ineffective, giving support to these earlier reports.

Observation that the hydrolytic decomposition of **1** to **17** occurs with relatively low activation energy[3] inspired us to carry out the opposite reaction,[20] specifically condensation of the aldehyde **18**[21] with **17**. A Soxhlet extraction apparatus was found to be a convenient vessel for small scale reactions and allowed the use of solvents heavier than water.[22] Observing that the putative intermediate **21** did not yield **1**, we hypothesized that the E1 pathway was not viable (Scheme 3). In support of this hypothesis, use of a more polar solvent, THF, decreased the yield in comparison to 1,2-dichloroethane, a less polar solvent.[23] The best result was obtained when TsOH was used as the catalyst, which gave a 41% yield. Further investigation of this reaction is underway on model systems.

The analytical data (^{1}H and ^{13}C NMR, MS, UV, IR, and optical rotation) for synthetic **1** were essentially identical to those for natural somocystinamide A,[24] thus confirming the originally assigned structure.[3] The bioactivity of the synthetic product **1** was evaluated in the murine Neuro-2a cancer cell line, but its activity was highly variable,[25] possibly due to the unusual solubility properties of **1**, or to factors which we do not currently understand. We also tested synthetic **1** in the brine shrimp toxicity model[26] and observed significantly impaired motility in treated (at 1, 10, and 100 μg/mL) versus control shrimps (DMSO). We also noted a much decreased quanitity of intestinal contents in the treated group. These observations underscore the value of a synthetic supply of somocystinamide A for it is clear that it possesses biological properties not yet understood.

(17) Atmospheric air as oxidant only worked for small scale reactions (ca. 10 mg). Larger scale reactions were run with bubbling O_2 (see the experimental procedures in the Supporting Information). For I_2 examples, see: Bourles, E.; Alves de Sousa, R.; Galardon, E.; Selkti, M.; Tomas, A.; Artaud, I. *Tetrahedron* **2007**, *63*, 2466–2471.

(18) The acetamide, disulfide, or already formed enamide of the other half of the dimeric intermediate may act as a nucleophile to attack the iminium ion. For examples of nucleohilic additions to iminium ions, see: Moonen, K.; Stevens, C. V. *Synthesis* **2005**, *20*, 3603–3612.

(19) In separate studies, reaction of **16** with simple unfunctionalized acid chlorides formed enamides, thus indicating the presence of an acyliminium ion intermediate like **21**. Because **19** was not observed by NMR, an acylenaminium intermediate is not likely involved.

(20) In ref 3, it was found that **1** decomposed after a few days in $CDCl_3$ due to the residual acid and water present in this solvent.

(21) Griffith, G. A.; Percy, J. M.; Pintat, S.; Smith, C. A.; Spencer, N.; Uneyama, E. *Org. Biomol. Chem.* **2005**, *3*, 2701–2712.

(22) Instead of a thimble, glass wool and molecular sieves were used to capture any moisture distilled as an azeotrope.

(23) (a) Saunders, W. H., Jr. *Acc. Chem. Res.* **1976**, *9*, 19–25. (b) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry Part A*, 4th ed.; Kluwer Academic/Pleunum Publishers: New York, 2000; pp 378−383.

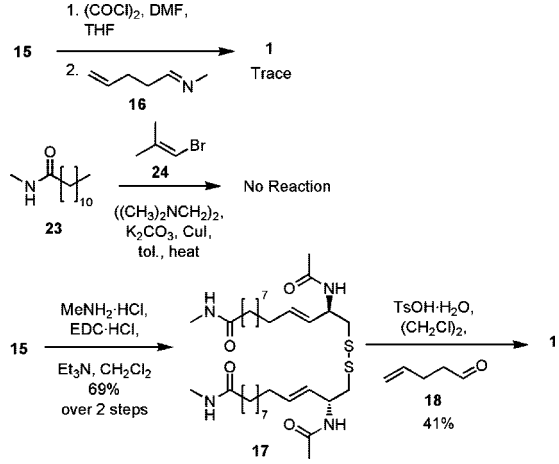
(24) The ^{1}H and ^{13}C were identical between natural and synthetic samples except the shift of C-16 showed a 0.2 ppm downfield shift in the synthetic sample. The UV maximum for synthetic somocystinamide was at 234 nm while natural was reported at 241 nm, both samples in MeOH.

(25) IC_{50} against Neuro-2a cells, synthetic **1** = 0.2 to >10 μM; natural **1** = 1.8 μM (ref 3).

(26) Meyer, B. N.; Ferrigni, N. R.; Putnam, L. B.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31–34.

In conclusion, the first total synthesis of somocystinamide A was achieved despite the presence of two quite challenging functional groups in the molecule (Scheme 4). The current synthesis is fairly robust for all steps but the last two such that >1 g of **15** has been prepared smoothly, and scale-up necessary for in vivo testing of this biomedically exciting compound should now be possible. However, our work demonstrates the need for a more robust reaction to prepare tertiary enamides, a development which would open the door for synthesis of other natural products possessing this functional group, such as the laingolides.[27]

Scheme 4. Synthesis of Somocystinamide A (**1**)



Acknowledgment. T.L.S. thanks D. Carson and the Moores Cancer Center for a student fellowship. Funding is acknowledged from NIH Grant No. NS 053398. We thank J. Wingerd at SIO/UCSD for the Neuro-2a assay and Y. Su at UCSD for the mass spectroscopic analyses. We thank K. Schwartz at Oregon State University for suggesting the use of a Soxhlet extractor, and the J. Bada Laboratory (SIO) for use of liquid NH_3.

Supporting Information Available: Experimental procedures, compound characterization, and ^{1}H and ^{13}C NMR spectra for compounds **6**, **8**, **13**−**15**, **17**, and **1**. Pictures and video of somocystinamide A and control treated brine shrimp. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8016947

(27) (a) Klein, D.; Braekman, J.; Daloze, D.; Hoffman, L.; Demoulin, V. *Tetrahedron Lett.* **1996**, *37*, 7519–7520. (b) Klein, D.; Braekman, J.; Daloze, D.; Hoffman, L.; Castillo, G.; Demoulin, V. *J. Nat. Prod.* **1999**, *62*, 934–936.