Medicinal Chemistry

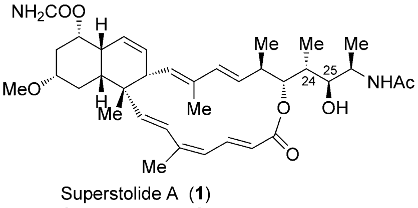
DOI: 10.1002/anie.201209300

Design, Synthesis, and Biological Evaluation of Truncated Superstolide A**

*Lei Chen, Kausar Begam Riaz Ahmed, Peng Huang, and Zhendong Jin**

Dedicated to Professor Philip L. Fuchs on the occasion of his 68th birthday

Marine macrolides are well known for their fascinating molecular structure and potent anticancer activity.[1] Superstolides A (**1**) and B (**2**; Scheme 1), two marine macrolides, were isolated in minute amounts from the deep-water marine sponge *Neosiphonia superstes*.[2] Their absolute structures were determined by using extensive spectroscopic methods. The structural novelty of these two molecules is characterized by a unique 16-membered macrolactone attached to a functionalized *cis*-decalin.



Scheme 1. Anticancer marine macrolide superstolides A and B.

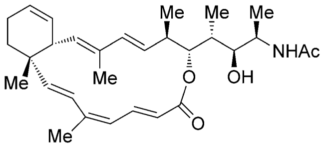
Both superstolides A and B exhibit a potent antiproliferative effect against several tumor cell lines with IC_{50} values ranging from 4.8 to 64 nm.[2] As they have novel and unprecedented structures we can deduce that they might have a unique cellular target(s) and a novel mechanism of action. Unfortunately, the yields of isolation for superstolides A and B are only 0.003 % and 0.0003 %, respectively. In addition, the marine sponge *Neosiphonia superstes* lives at 500–515 meters deep in the ocean off New Caledonia, thus making the collection of a large amount of the marine sponge very difficult and dangerous and its collection has the potential to cause significant damage to the marine habitat. Furthermore, sponges are notorious for being extremely difficult animals to cultivate in controlled systems.[3] The lack of an adequate compound supply severely impedes research toward a thorough understanding of their mechanism of action and hampers the gain of further insight into the clinical potential of this group of fascinating marine macrolides.

These compounds have attracted a great deal of attention from the synthetic organic chemistry community because of their potent anticancer activities coupled with their challenging molecular structures.[4,5] Roush and his co-workers have reported the only completed total synthesis of superstolide A.[5h]

Several years ago, we initiated research directed toward the total synthesis of superstolides A and B.[4] While working on the total synthesis, it became apparent to us that because of the structural complexity of the target molecules, it would be extremely challenging to develop a practical total synthesis that is capable of providing an adequate amount of material for biological investigation, therapeutic evaluation, and possible future clinical trials.

Owing to the lack of a sufficient amount of these natural products and the overwhelming difficulty in the development of a practical total synthesis approach we decided to design a simplified superstolide A analogue that contains the basic pharmacophore and can be easily synthesized in a much shorter reaction sequence. Herein, we report for the first time the design and synthesis of a truncated superstolide A (**3**), in which the *cis*-fused functionalized decalin is simplified to a cyclohexene ring, whereas the 16-membered macrolactone remains intact (Scheme 2).

This design is based on our hypothesis that the 16-membered macrolactone may be the key pharmacophore that interacts with cellular target(s), whereas the *cis*-fused decalin may lock the macrolide into an advantageous conformation. This modification would simplify the synthesis substantially, and at the same time maintain the basic template of the molecule. Such a strategy was considered important in that it could test our hypothesis on the interaction between the



3

Scheme 2. Design of truncated superstolide A.

---

[*] Dr. L. Chen, Prof. Dr. Z. Jin
Division of Medicinal and Natural Products Chemistry
Department of Pharmaceutical Sciences & Experimental Therapeutics
College of Pharmacy, The University of Iowa
Iowa City, IA 52242 (USA)
E-mail: zhendong-jin@uiowa.edu

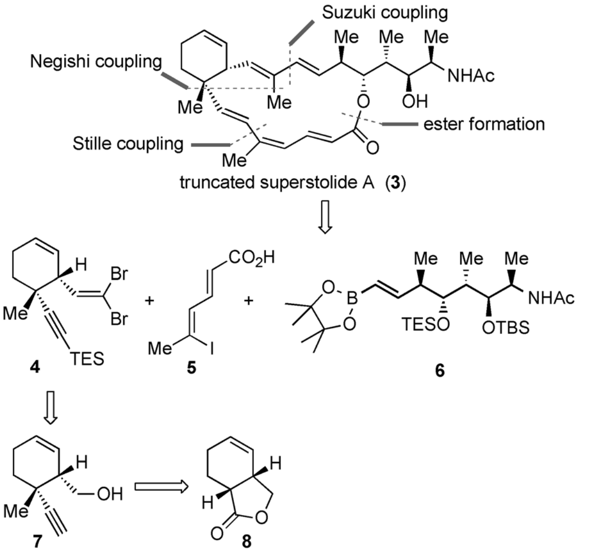
K. B. Riaz Ahmed, Prof. Dr. P. Huang
Department of Molecular Pathology
The University of Texas MD Anderson Cancer Center
Houston, TX 77030 (USA)

[**] This work was made possible by the generous support of the NIH (5R01CA109208).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201209300.

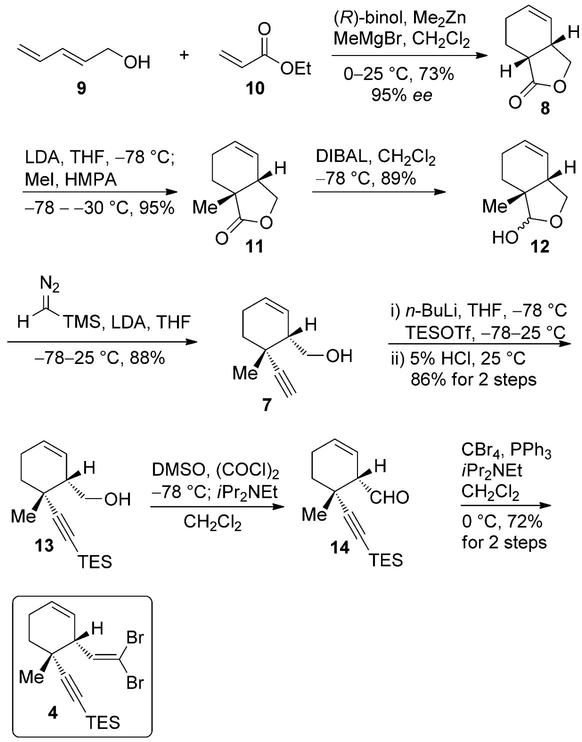
natural product and the receptor and provide important information regarding the structure–activity relationship and pharmacophore identification. In addition, the synthesis of truncated superstolide A (**3**) would also serve as an important model study that would provide some critically important information on the feasibility of three key coupling reactions and an ester formation (or macrolactonization) in our total synthesis strategy especially considering the enormous difficulty involved in the macrolactonization step encountered in the synthesis of Rousch and co-workers.[5i]

Scheme 3 outlines the retrosynthetic analysis of truncated superstolide A (**3**). Sequential disconnections reveal fragments **4**, **5**, and **6** as potential key intermediates, with Suzuki, Negishi, and Stille couplings playing crucial roles in the synthetic strategy.

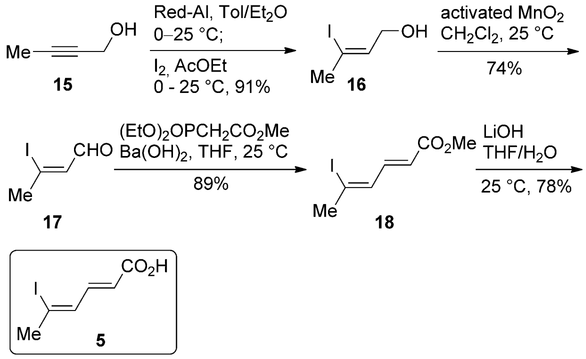


Scheme 3. Retrosynthetic analysis of truncated superstolide A (**3**). TBS = *tert*-butyldimethylsilyl, TES = triethylsilyl.

Our starting material lactone **8** was prepared enantioselectively in 73% yield (95% *ee*) by using an elegant Diels–Alder reaction developed by Ward and Santos (Scheme 4).[6] Lactone **8** was treated with LDA and the the resulting enolate was quenched with MeI to provide compound **11** in 95% yield and with the requisite stereochemistry at the quaternary carbon. Reduction with DIBAL to lactol **12** and subsequent addition of lithiotrimethylsilyldiazomethane gave alkyne **7** in 88% yield.[7] Compound **7** was treated with 2.5 equivalents of *n*BuLi to afford a dianion, which was quenched with 3 equivalents of TESOTf to furnish an intermediate. This intermediate was treated with 5% HCl to chemoselectively cleave the TES ether to provide compound **13** in 86% yield. Homoallylic alcohol **13** was carefully oxidized to its corresponding aldehyde **14**, which was immediately converted into geminal dibromo compound **4** in 72% yield.



Scheme 4. Enantioselective synthesis of fragment **4**. binol = 2,2′-dihydroxy-1,1′-binaphthyl, DIBAL = diisobutylaluminium hydride, DMSO = dimethyl sulfoxide, HMPA = hexamethylphosphorylamide, LDA = lithium diisopropylamide.

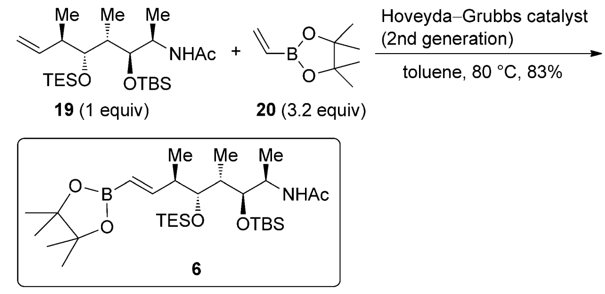


Scheme 5. Synthesis of fragment **5**. Red-Al = sodium bis(2-methoxyethoxy)aluminum hydride.

Compound **5** was synthesized using a modified literature procedure (Scheme 5).[5f] Compound **16**[8] was oxidized to aldehyde **17**, which underwent a Horner–Wadsworth–Emmons olefination to give compound **18** in 89% yield. Hydrolysis of compound **18** furnished carboxylic acid **5** in 78% yield.

The cross-metathesis between olefin **19**[4a] and pinacol vinylboronate **20** proved to be quite challenging (Scheme 6). After much investigation, it was discovered that when the second generation of Grubbs-Hoveyda catalyst was used compound **19** was successfully converted into *trans*-vinylboronate **6** in 83% yield.[9]

The Suzuki coupling between geminal dibromo compound **4** and vinyl boronate **6** provided compound **21** in 70% yield with complete stereoselectivity (Scheme 7).[10] Negishi coupling between vinyl bromide **21** and Me_2Zn gave the



Scheme 6. Synthesis of fragment **6**.

requisite trisubstituted olefin **22** in 86 % yield with complete stereoselectivity.[11] It should be noted that the triethylsilyl group attached to the alkyne moiety of compound **21** was extremely important because it prevented the facile cyclic carbopalladation and subsequent cross-coupling, a major side reaction that competed with the desired Negishi coupling.[12]

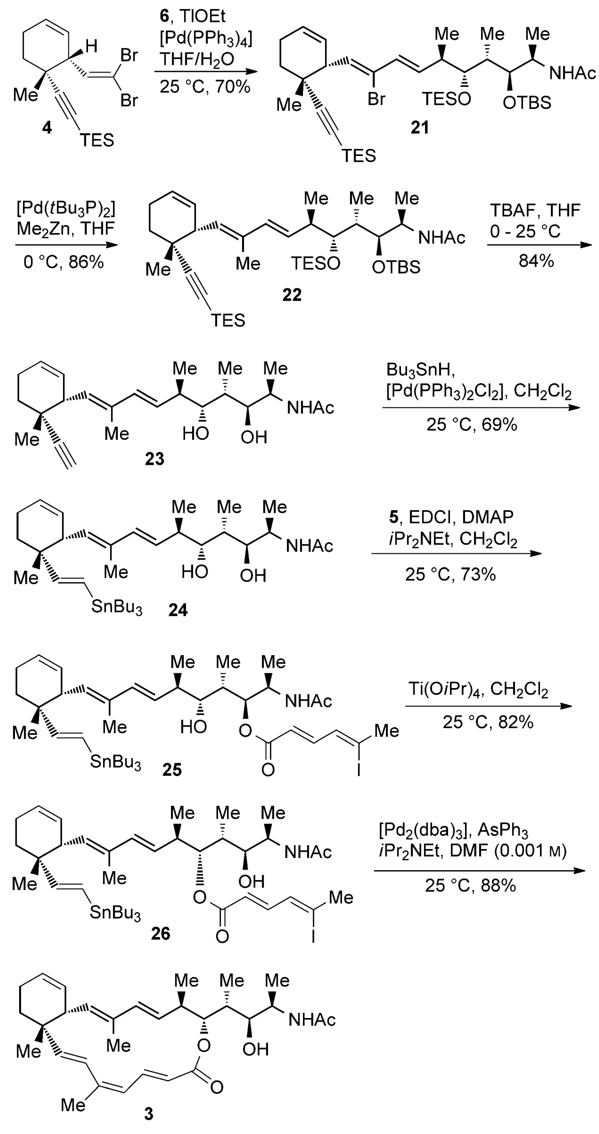
Three silyl protecting groups were removed by TBAF to afford alkyne **23**, which underwent a regio and stereoselective hydrostannylation to furnish vinyl stannane **24** in 69 % yield. A regioselective esterification between alcohol **24** and carboxylic acid **5** provided compound **25**, which isomerized to the desired ester **26** upon treatment with Ti(O*i*Pr)$_4$. This remarkable acyl migration was first reported by Paterson and Mackay.[5f] The formation of the thermodynamically more-stable compound **26** is presumably due to the additional hydrogen bond between the amide group and the neighboring hydroxy group in **26**. Finally, compound **26** underwent a facile intramolecular Stille coupling to give truncated superstolide A (**3**) in 88 % yield.[13]

In summary, the truncated superstolide A (**3**) was successfully synthesized in 15 steps from commercially available starting material **9** in 6.2 % overall yield.[14] It should be noted that all stereogenic carbon centers and double bonds present in the target molecule were constructed stereoselectively.

The antiproliferative effect of compound **3** was evaluated in eight cancer cell lines by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[15] The IC$_{50}$ data are shown in Table 1. We were delighted to discover that compound **3** is about seven times more potent in suppressing tumor cell proliferation than its parent natural product superstolide A in HT-29 cell (the IC$_{50}$ value for superstolide A in HT-29 is 64 nM).[2a] In addition, compound **3** is also potent in suppressing tumor cell proliferation in the other seven tested cell lines with IC$_{50}$ values ranging from 12–77 nM. These results have confirmed our hypothesis that the 16-membered macrolactone is indeed the pharmacophore that interacts with its putative target in the cells, and the modification of the fuctionalized *cis*-decalin to a cyclohexene ring apparently does not affect its potent anticancer activity. This information is highly valuable for the design of other analogues in the future.

Table 1: Antiproliferative effect of compound **3** on various malignant tumor cells (MTT assay).

Entry	Cell Line	IC$_{50}$ [nM]
1	HT29	7.54
2	A375SM	36.52
3	MEL624	53.06
4	SK-MEL-2	63.82
5	H1299	56.75
6	HCT116	52.71
7	Raji	76.73
8	HL60	11.85



Scheme 7. Coupling of the three major fragments and completion of the synthesis. dba = dibenzylideneacetone, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N'*-dimethylformamide, EDCI = 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride, TBAF = tetra-*n*-butylammonium fluoride.

In conclusion, we have designed and synthesized a truncated superstolide A (**3**) that maintains the potent anticancer activity of the original natural product superstolide A. Therefore, we have successfully indirectly solved the supply problem of superstolide A. In addition, our highly convergent and flexible synthetic approach can be easily modified for the synthesis of other designed analogues and bioconjugates in the future. Research in this direction is currently underway and will be reported in due course.

Received: November 20, 2012
Revised: December 27, 2012

Keywords: anticancer agent · asymmetric synthesis · drug design · macrolide · superstolide analogue

---

[1] a) Y. Qi, S. Ma, *ChemMedChem* **2011**, *6*, 399, and references therein; b) I. Bhatnagar, S.-K. Kim, *Mar. Drugs* **2010**, *8*, 2702, and references therein.

[2] a) M. V. D'Auria, C. Debitus, L. G. Paloma, L. Minale, A. Zampella, *J. Am. Chem. Soc.* **1994**, *116*, 6658; b) M. V. D'Auria, C. Debitus, L. G. Paloma, L. Minale, A. Zampella, *J. Nat. Prod.* **1994**, *57*, 1595.

[3] S. Sagar, M. Kaur, K. P. Minneman, *Mar. Drugs* **2010**, *8*, 2619, and references therein.

[4] a) W. Yu, Y. Zhang, Z. Jin, *Org. Lett.* **2001**, *3*, 1447; b) Z. Hua, W. Yu, M. Su, Z. Jin, *Org. Lett.* **2005**, *7*, 1939; c) L. Chen, Z. Hua, G. Li, Z. Jin, *Org. Lett.* **2011**, *13*, 3580.

[5] a) W. R. Roush, J. A. Champoux, B. C. Peterson, *Tetrahedron Lett.* **1996**, *37*, 8989; b) A. Zampella, M. V. D'Auria, *Tetrahedron: Asymmetry* **2001**, *12*, 1543; c) W. R. Roush, L. Hertel, M. J. Schnaderbeck, N. A. Yakelis, *Tetrahedron Lett.* **2002**, *43*, 4885; d) N. A. Yakelis, W. R. Roush, *J. Org. Chem.* **2003**, *68*, 3838; e) J. G. Solsona, P. Romea, F. Urpi, *Org. Lett.* **2003**, *5*, 4681; f) I. Paterson, A. C. Mackay, *Synlett* **2004**, 1359; g) J. A. Marshall, J. J. Mulhearn, *Org. Lett.* **2005**, *7*, 1593; h) M. Tortosa, N. A. Yakelis, W. R. Roush, *J. Am. Chem. Soc.* **2008**, *130*, 2722; i) M. Tortosa, N. A. Yakelis, W. R. Roush, *J. Org. Chem.* **2008**, *73*, 9657.

[6] D. E. Ward, M. S. Santos, *Org. Lett.* **2005**, *7*, 3533.

[7] S. Ohira, K. Okai, T. Moritani, *J. Chem. Soc. Chem. Commun.* **1992**, 721.

[8] Y. Hayashi, M. Shoji, H. Ishikawa, J. Yamaguchi, T. Tamura, H. Imai, Y. Nishigaya, K. Takabe, H. Kakeya, H. Osada, *Angew. Chem.* **2008**, *120*, 6759; *Angew. Chem. Int. Ed.* **2008**, *47*, 6657.

[9] a) C. Morrill, R. H. Grubbs, *J. Org. Chem.* **2003**, *68*, 6031; b) C. Morrill, T. W. Funk, R. H. Grubbs, *Tetrahedron Lett.* **2004**, *45*, 7733.

[10] A. Suzuki, *J. Organomet. Chem.* **1999**, *576*, 147, and references therein.

[11] E.-i. Negishi, Q. Hu, Z. Huang, M. Qian, G. Wang, *Aldrichimica Acta* **2005**, *38*, 71, and references therein.

[12] E.-i. Negishi, Y. Noda, F. Lamaty, E. J. Vawter, *Tetrahedron Lett.* **1990**, *31*, 4393.

[13] a) J. K. Stille, *Angew. Chem.* **1986**, *98*, 504; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508, and references therein; b) G. Pattenden, D. J. Sinclair, *J. Organomet. Chem.* **2002**, *653*, 261, and references therein; c) K. C. Nicolaou, P. G. Bulger, D. Sarlah, *Angew. Chem.* **2005**, *117*, 4516; *Angew. Chem. Int. Ed.* **2005**, *44*, 4442, and references therein.

[14] As 11 steps were needed to prepare compound **19** from (*S*)-(−)-ethyl lactate, the longest linear sequence for the synthesis of compound **3** consists of 19 steps with 3.2% overall yield.

[15] The procedure for cell viability assay and MTT curves are included in the Supporting Information.