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Design, Synthesis and Biological Evaluation of Truncated Superstolide A

Dr. Lei Chen,

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), Fax: (+1) 319-335-8766

Ms. Kausar Begam Riaz Ahmed,

Department of Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030

Prof. Dr. Peng Huang, and

Department of Molecular Pathology, The University of Texas MD Anderson Cancer Center,, Houston, Texas 77030

Prof. Dr. Zhendong 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), Fax: (+1) 319-335-8766

Zhendong Jin: zhendong-jin@uiowa.edu

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

Both superstolides A and B exhibit potent antiproliferative effect against several tumor cell lines with IC₅₀ values ranging from 4.8 to 64 nM.^[2] Their novel and unprecedented structures suggest they might have a unique cellular target(s) and a novel mechanism of action. Unfortunately, the isolation yields for both superstolides A and B are only 0.003% and 0.0003%, respectively. In addition, the marine sponge *Neosiphonia superstes* live at 500-515 meters deep in the ocean off New Caledonia, which makes collecting of a large amount of the marine sponge very difficult and dangerous and 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 adequate compound supply severely impedes research into a thorough understanding of their

Correspondence to: Zhendong Jin, zhendong-jin@uiowa.edu.

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

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

mechanism of action and hampers the gain of further insight into the clinical potential of this group of fascinating marine macrolides.

The potent anticancer activities coupled with their challenging molecular structures have attracted a great deal of attention from the synthetic organic chemistry community.^[4,5] So far the only completed total synthesis of superstolide A was accomplished by Roush and his coworkers.^[5h]

Several years ago, we initiated a program 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.

The lack of a sufficient amount of natural products coupled with the overwhelming difficulty in the development of a practical total synthesis approach entails designing of 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 (Figure 2).

This design is based on our hypothesis that the 16-membered macrolactone may be the key pharmacophore that interacts with cellular target(s) while 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 design was considered important in that it could test our hypothesis on the interaction between the 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 Roush's synthesis.^[5i]

Scheme 1 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.

Our starting material lactone **8** was prepared enantioselectively in 73% yield (95% ee) employing an elegant Diels-Alder reaction developed by Ward (Scheme 2).^[6] Lactone **8** was treated with LDA followed by quenching the resulting enolate with MeI to provide compound **11** in 95% yield with the requisite stereochemistry at the quaternary carbon. DIBAL reduction followed by the addition of lithiotrimethylsilyldiazomethane to lactol **12** gave alkyne **7** in 88% yield.^[7] Compound **7** reacted with 2.5 equivalents of *n*-BuLi to afford a dianion that was quenched with 3 equivalents of TESOTf to furnish an intermediate, which 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 to geminal dibromo compound **4** in 72% yield.

Compound **5** was synthesized using a modified literature procedure (Scheme 3).^[5f] Compound **16**^[8] was oxidized to aldehyde **17**, which underwent 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 4). After much experimentation, it was discovered that employing the second generation of Grubbs-Hoveyda catalyst compound **19** was successfully converted to *trans*-vinylboronate **6** in 83% yield.^[9]

Suzuki coupling between geminal dibromo compound **4** and vinyl boronate **6** provided compound **21** in 70% yield with complete stereoselectivity (Scheme 5).^[10] Negishi coupling between vinyl bromide **21** and Me₂Zn gave the requisite trisubstituted olefin **22** in 86% yield with complete stereoselectivity.^[11] It should be noted that the triethyl silyl group attached to the alkyne moiety of compound **21** was extremely important because it prevented the facile cyclic carbopalladation followed by 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. Regioselective esterification between alcohol **24** and carboxylic acid **5** provided compound **25**, which was isomerized to the desired ester **26** upon treatment with Ti(O-*i*Pr)₄. This remarkable acyl migration was first reported by Paterson.^[5f] The formation of thermodynamically more stable compound **26** is presumably due to the additional hydrogen bond between the amide group and the neighbouring hydroxyl 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 carbons and double bonds present in the target molecule were constructed stereoselectively.

The antiproliferative effect of compound **3** was evaluated in eight cancer cell lines using MTT assay.^[15] The IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 functionalized *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.

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, for the first time 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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14. Because 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.

Figure 1. Anticancer marine macrolide superstolides A and B

Figure 2. Design of truncated superstolide A

Scheme 1. Retrosynthetic analysis of truncated superstolide A (3)

Scheme 2. Enantioselective synthesis of fragment 4

Scheme 3. Synthesis of fragment 5

Scheme 4. Synthesis of fragment **6**

Scheme 5. Coupling three major fragments and completing the synthesis

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