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Enantioselective Synthesis of Both Epimers at C-21 in the Proposed Structure of Cytotoxic Macrolide Callyspongiolide

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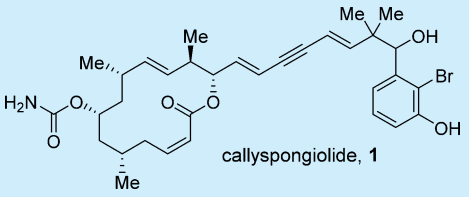
Supporting Information

ABSTRACT: Both epimers at C-21 in the proposed structure of (+)-callyspongiolide have been synthesized in a convergent and enantioselective manner. The 14-membered macrolide with a sensitive C2−C3 *cis*-olefin functionality was installed by a Yamaguchi macrolactonization of hydroxyl alkynoic acid followed by hydrogenation over Lindlar's catalyst. The C5 methyl stereocenter was constructed by a ring-closing olefin metathesis followed by addition of methyl cuprate to an α,β-unsaturated δ-lactone. Other key reactions are chiral Corey−Bakshi−Shibata (CBS) reduction and Sonogashira coupling to conjoin the macrocyclic core and side chain.



Marine sponges of the genus *Callyspongia* have proven to be rich sources of natural products that display a wide spectrum of biological activity.[1−4] Earlier this year, Shin and co-workers isolated callyazepin, a nitrogenous macrocycle displaying moderate cytotoxicity against K562 and A549 cell lines.[5] Proksch and co-workers recently tested a methanolic extract of the marine sponge *Callyspongia* sp., and biological results revealed complete inhibition of the murine lymphoma cell line L5178Y.[6] The structure and relative stereochemistry was determined using NMR and HRMS analysis, yet the sole C21 stereocenter located on the diene-ynic side chain was left unassigned. More interesting was the discovery that against human Jurkat J16 T and Ramos B lymphocytes, callyspongiolide was highly cytotoxic with IC_{50} values of 70 and 60 nM, respectively. With the addition of Q-VD-OPH, apoptosis persisted, suggesting a mode of cell death that does not involve a caspase pathway. Furthermore, increased levels of hypodiploid nuclei were observed at EC_{50} levels of 80 nM for Jurkat J16 T and 50 nM for Ramos B lymphocytes. As part of our continuing interest in the synthesis of bioactive natural products, and taking into consideration the novel structure of this macrolide, we sought to establish a convergent and enantioselective synthesis of callyspongiolide to aid in structural determination, and to expedite further biological studies. Herein, we report our preliminary investigation that has led to a convergent and highly stereoselective synthesis of the proposed structure of both epimers at C-21 of (+)-callyspongiolide.

Our retrosynthetic analysis is shown in Figure 1. We planned a late-stage Sonogashira coupling of vinyl iodide **2** with either enantiomer of enyne **3** to provide access to both epimers of the natural product. The *cis*-macrolactone would be constructed in a similar manner as our reported synthesis of Laulimalide[7−9] by macrolactonization of a hydroxyl alkynoic acid followed by hydrogenation. The *seco* acid **4** could potentially be obtained via the ring-opening of lactone **5**. Olefin **5** would be formed through a Julia−Kocienski olefination[10,11] using an aldehyde obtained from known[12] allyl alcohol **6**, with the sulfone derived from known[13] diol **7**. For the synthesis of enyne **3**, Wittig olefination with the aldehyde derived from olefin **8** would provide **3**. We

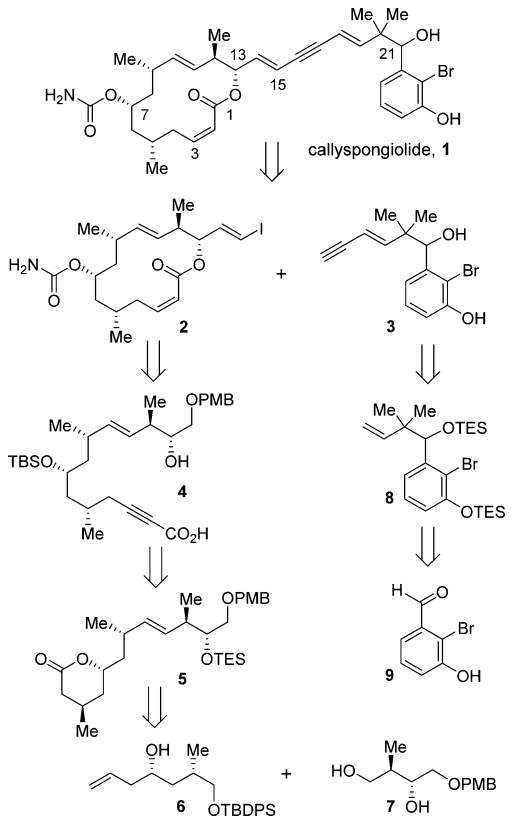


Figure 1. Retrosynthetic analysis of callyspongiolide.

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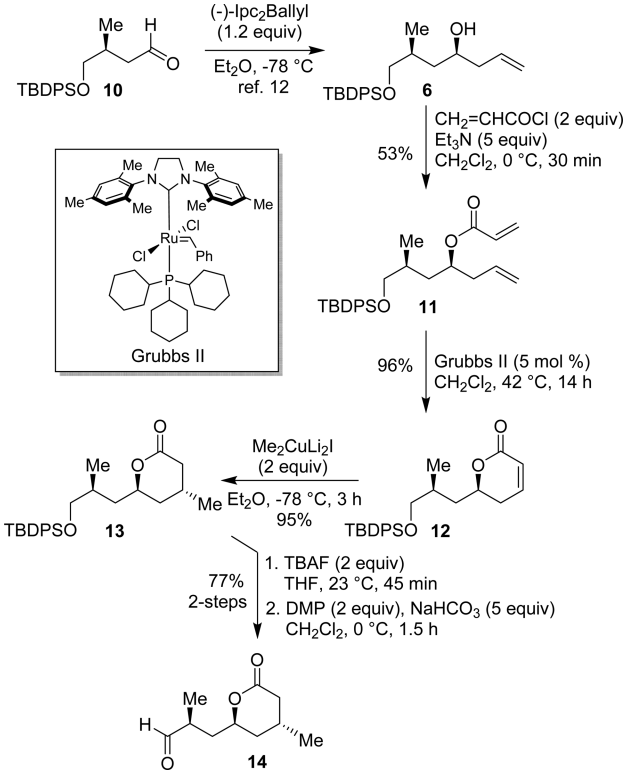
planned a CBS reduction[14,15] of the corresponding ketone derived from **8** to provide access to both enantiomers.

Our synthesis of the macrocyclic core began from the known[12] chiral alcohol **6**. As shown in Scheme 1, treatment of allyl alcohol

Scheme 1. Synthesis of Aldehyde 14

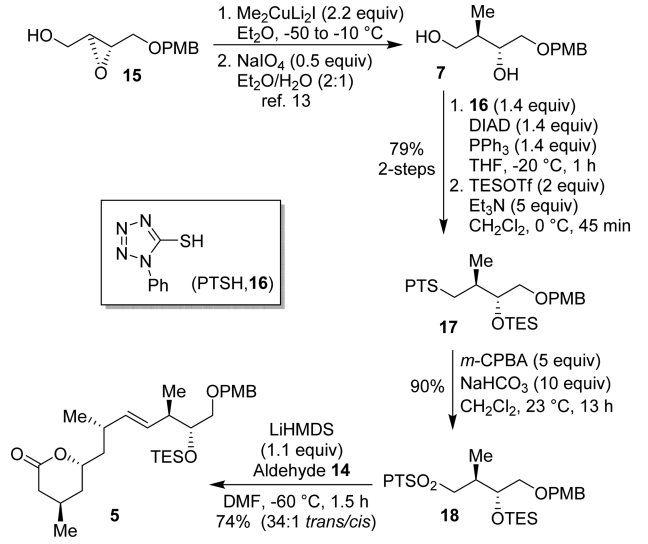


6 with acryloyl chloride and Et_3N gave diene **11**, which was subjected to ring closing metathesis using the Grubbs II catalyst to give *α,β*-unsaturated lactone **12**. Lactone **12** was subjected to 1,4-addition using Me_2CuLi_2I to provide **13** stereoselectively. The methyl group added from the bottom face of the molecule to provide lactone **13** in 95% yield as a single diastereomer by ^1H NMR analysis. Removal of the *tert*-butyldiphenylsilyl (TBDPS) ether with tetrabutylammonium fluoride (TBAF) afforded the corresponding alcohol, which upon oxidation using Dess–Martin periodinane (DMP) in the presence of $NaHCO_3$ furnished aldehyde **14** in 77% yield over two steps.

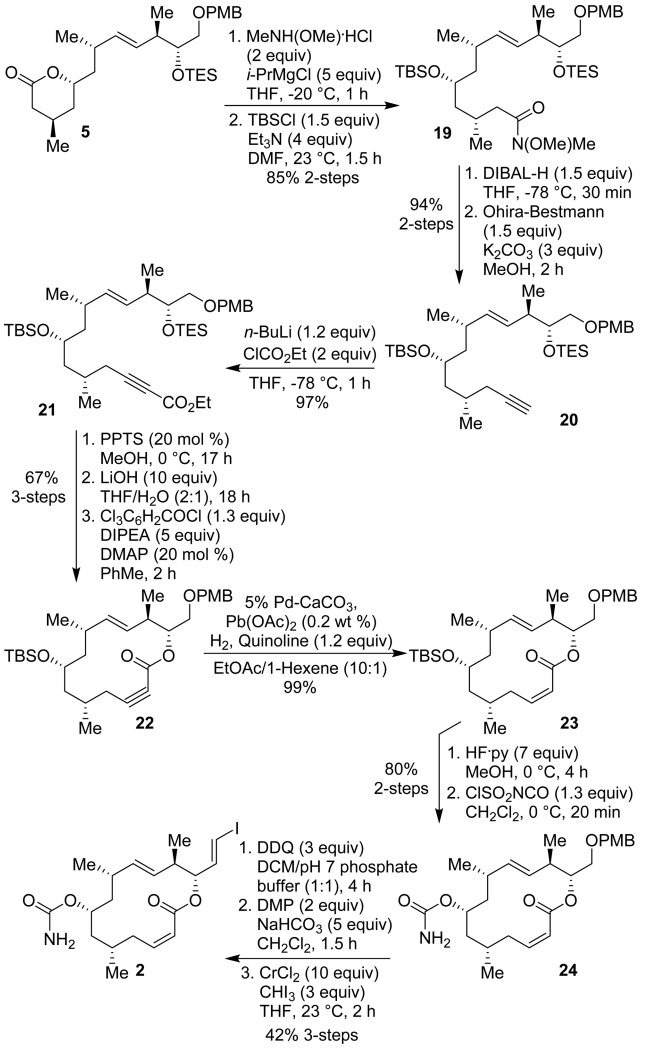
Our elaboration of the C10–C11 *trans*-olefin is shown in Scheme 2. Protected diol **7**[13] was readily prepared from chiral epoxide **15** as described by Crimmins and co-workers.[13] Selective Mitsunobu displacement with 1-phenyl-1*H*-tetrazole-5-thiol (PTSH, **16**) followed by protection of the latent secondary alcohol provided sulfide **17** in 79% yield over two steps. Oxidation using *m*-chloroperbenzoic acid (*m*-CPBA) afforded sulfone **18**. For Julia–Kocienski olefination, sulfone **18** was deprotonated using LiHMDS in DMF at −60 °C. Addition of aldehyde **14** then provided the desired olefin **5** in 74% yield with a *trans*/*cis* ratio of 34:1 as determined by ^1H NMR analysis.

Our synthesis of macrolactone and its conversion to vinyl iodide **2** is shown in Scheme 3. Treatment of lactone **5** with *N,O*-dimethylhydroxylamine hydrochloride and isopropylmagnesium chloride (^{i}PrMgCl) in THF at −20 °C provided the corresponding Weinreb amide. Protection of the resulting secondary alcohol initially proved troublesome, as *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole in either

Scheme 2. Julia–Kocienski Olefination



Scheme 3. Synthesis of Vinyl Iodide 2



DMF or DCM showed low yields as well as silyl migration product. However, switching to Et_3N as base prevented the formation of byproduct, and TBS ether **19** was obtained in 85% yield over two steps. Diisobutylaluminum hydride (DIBAL-H)

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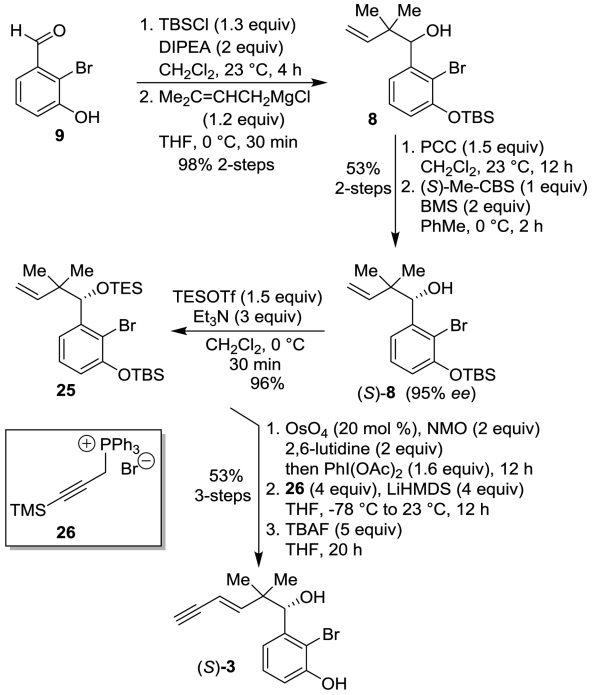
reduction followed by treatment with the Ohira-Bestmann reagent[16] and K_2CO_3 in MeOH gave the homologated alkyne **20**. The terminal acetylene moiety was alkylated by metalation with *n*-BuLi in THF at −78 °C followed by treatment with ethyl chloroformate to give alkynyl ester **21**. Selective removal of the triethylsilyl (TES) ether using pyridinium *p*-toluenesulfonate (PPTS) in MeOH at 0 °C followed by LiOH-mediated hydrolysis provided the corresponding *seco* acid. Yonemitsu's variation[17] for macrolactonization proved very effective, whereby the mixed anhydride is not preformed, but rather the *seco* acid is directly added to the cocktail of reagents (2,4,6-trichlorobenzoyl chloride, DMAP, and DIPEA) to furnish alkynyl lactone **22** in 67% yield over three steps. Reduction of the alkyne moiety proceeded smoothly using Lindlar's catalyst poisoned with quinoline under a hydrogen balloon to provide the *cis*-lactone **23** in 99% yield. Removal of the TBS ether was ineffective using TBAF, even at elevated temperatures. Alternatively, employing HF·pyridine was efficient and afforded the corresponding alcohol. Reaction of the resulting alcohol with chlorosulfonyl isocyanate installed the carbamate moiety of **24** in 80% yield over two steps. Removal of the *p*-methoxybenzyl (PMB) protecting group was accomplished using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) to provide the corresponding alcohol, which was oxidized using DMP and then subjected to Takai olefination[18] to furnish vinyl iodide **2** in 42% yield over three steps.

With the macrocyclic core of the molecule in hand, our attention was directed toward preparation of the side chain. As shown in Scheme 4, commercially available 2-bromo-3-hydroxybenzaldehyde **9** was protected as the TBS ether. Reverse prenylation of the resulting aldehyde using 3-methyl-2-butenylmagnesium chloride furnished alcohol **8** in 98% yield over two steps. The racemic alcohol was oxidized using pyridinium chlorochromate (PCC) in the presence of silica gel in DCM to give the corresponding ketone. The ketone was then subjected to chiral reduction using Corey's (*S*)-Me-CBS catalyst to furnish alcohol (*S*)-**8** in 53% yield over two steps. Chiral HPLC analysis showed that the alcohol was obtained in 95% *ee*.

Scheme 4. Synthesis of Enyne (*S*)-3



Triethylsilyl trifluoromethanesulfonate (TESOTf) was necessary to protect the resulting hindered alcohol **25**, which was obtained in 96% yield. Ozonolysis of the terminal olefin gave a mixture of products, including silyl deprotection. However, Nicolaou's conditions[19] for oxidative cleavage of olefins provided the corresponding aldehyde, which was then subjected to Wittig olefination with commercially available phosphonium bromide **26** to give the corresponding TMS-protected enyne in a *trans*/*cis* ratio of 7:1. Global deprotection of all silyl protecting groups was achieved using TBAF to afford enyne (*S*)-**3** in 53% yield over three steps. In the same manner, (*R*)-**3** was prepared using the same chemistry while employing (*R*)-Me-CBS during the chiral reduction. For determination of absolute configuration, we converted alcohol **8** to its benzoate derivative **27** as shown in Figure 2. X-ray crystallography of the 4-nitrobenzoate derivative confirmed the stereochemical outcome of CBS reduction.[20,21]

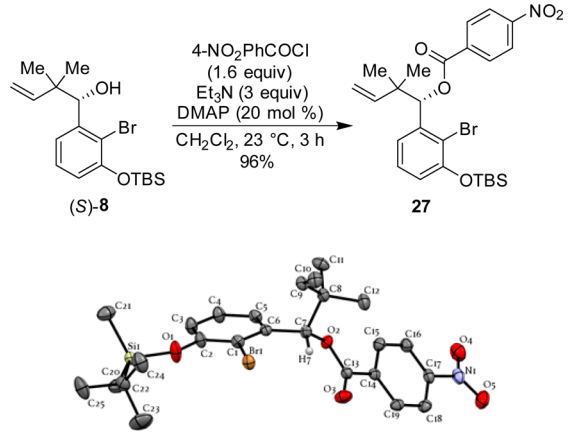


Figure 2. Synthesis and ORTEP drawing of **27**. Gray = carbon; white = hydrogen; orange = bromine; blue = nitrogen; red = oxygen; yellow = silicon.

The synthesis of callyspongiolide is shown in Scheme 5. Sonogashira coupling of vinyl iodide **2** with enyne (*S*)-**3** gave callyspongiolide (*S*)-**1** in 79% yield. Similarly, coupling with the (*R*)-enantiomer furnished callyspongiolide (*R*)-**1** in 82% yield. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of both (*S*)-**1** and (*R*)-**1** epimers are virtually indistinguishable from the spectra reported for the natural product.[6] Further unambiguous assignment was difficult by 800 MHz ^{1}H NMR. Both specific rotations of our HPLC-purified[22] synthetic callyspongiolide epimers, however, showed opposite sign of rotation as well as larger magnitude; synthetic (*S*)-**1**, $[\alpha]_D^{20}$ + 24.5 (*c* 0.1, MeOH); synthetic (*R*)-**1**, $[\alpha]_D^{20}$ + 159 (*c* 0.1, MeOH) compared to that reported for the natural product, $[\alpha]_D^{20}$ − 12.5 (*c* 0.1, MeOH).[6] Based upon these results, we presume that the isolated natural product is the antipode of our synthetic (*S*)-callyspongiolide **1**. Thus, assignment of the absolute structure of natural callyspongiolide requires further synthetic and structural studies.

In summary, we have accomplished the enantioselective synthesis of both epimers at C-21 of the reported structure of callyspongiolide. The specific rotation of both epimers of our synthetic callyspongiolide shows opposite sign to the natural product, and the magnitudes are larger. Our synthesis constructed the 14-membered macrolide with a sensitive C2−C3 *cis*-olefin functionality by Yonemitsu's variation of Yamaguchi

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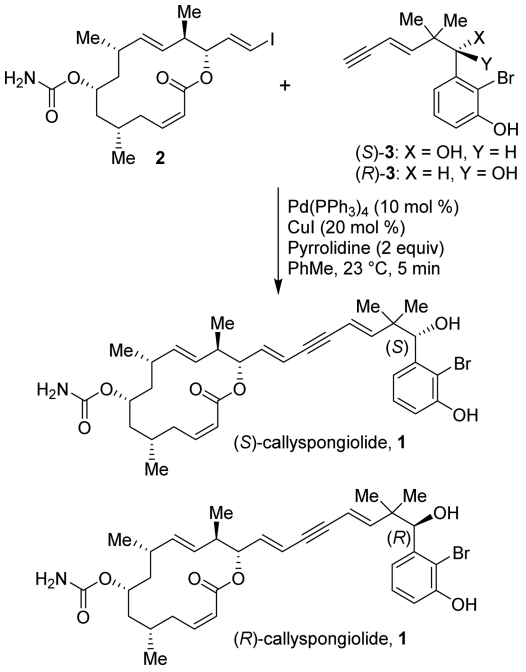
Scheme 5. Sonogashira Coupling and Callyspongiolide Synthesis



macrolactonization followed by hydrogenation over Lindlar's catalyst as the key steps. The C5 methyl stereocenter was constructed by methyl cuprate addition to an α,β-unsaturated δ-lactone. The synthesis also featured a chiral CBS reduction and Sonogashira coupling to conjoin the macrocyclic core and its side chain. The synthesis will provide access to natural callyspongiolide as well as a variety of structural derivatives. Further investigations are in progress.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01523.

Experimental procedures in addition to ^{1}H- and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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(20) X-ray crystallographic structural determination was performed in our X-ray crystallography laboratory by Dr. Matthias Zeller, Department of Chemistry, Purdue University, West Lafayette, IN.

(21) Complete crystallographic data, in CIF format, have been deposited with the Cambridge Crystallographic Data Centre. CCDC 1481162 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(22) HPLC analysis showed the purity of both epimers to be >98%. Conditions: Daicel Chiralpak IA-3, 4.6 × 250 mm, 3 μm, 15% isopropanol/hexane, flow = 1.3 mL/min, T = 25 °C, UV = 254 nm, R_t(*S*)-**1** = 13.6 min, R_t(*R*)-**1** = 12.9 min. Please see Supporting Information for details.

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