Communication

Unconventional fragment usage enables a concise total synthesis of (-)-callyspongiolide

Francesco Manoni, Corentin Rumo, Liubo Li, and Patrick G Harran

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Unconventional fragment usage enables a concise total synthesis of (−)-callyspongiolide

Francesco Manoni, Corentin Rumo, Liubo Li and Patrick G. Harran*

Department of Chemistry and Biochemistry, University of California–Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, United States

Supporting Information Placeholder

ABSTRACT: An asymmetric synthesis of (−)-callyspongiolide is described. The route builds the macrolide domain atypically from a disaccharide and a monoterpene without passing through a *seco*-acid. Chiral iridium catalysis selectively joins fragments. Subsequent degradation of an imbedded butyrolactone *via* perhemiketal fragmentation affords a stereo and regio-defined homoallylic alcohol that is engaged directly in a carbonylative macrolactonization. Further elaboration of the polyunsaturated appendage provides the natural product in a particularly direct and flexible manner.

Polyketide natural products are a large, diverse family of oxygenated hydrocarbons that continue to be valuable in research and medicine. In 2014 Proksch *et al.* described a new member of this group that was named callyspongiolide (**1**, from *Callyspongia* sp., Figure 1).[1] The callyspongiolide macrolactone is reminiscent of those in oscillariolide and the phormidolides,[2] but its phenethylated polyene appendage is unique. The natural product was purified as a cytotoxin. Interestingly, in the cell types examined (Jurkat and Ramos B lymphocytes), its activity was reported to be caspase independent. This implies a non-apoptotic mechanism for cell death. Because apoptotic signaling is suppressed in many cancers, and this often underpins drug resistance in clinical settings,[3] compounds that kill cancer cells by non-apoptotic mechanisms are potentially valuable tools to explore new therapeutic strategies. With this in mind we set out to prepare the molecule. As these studies progressed we benefitted from reports by Ye and Ghosh whose impressive syntheses established absolute stereochemistry of the natural product.[4] These works assembled *seco*-acid precursors to the macrolide using contemporary methods for acyclic diastereocontrol. We had chosen to pursue the problem differently and were exploring alternate means to build the macrolactone. We planned for its relative stereochemical features to be established on medium ring sized intermediates serving as chain synthons,[5] and to circumvent a *seco*-acid precursor. This led to us preparing a polyacetate-derived macrolide from a carbohydrate and a monoterpene.

Callyspongiolide was dissected into two fragments, separating its polyene from the macrocycle at C15 (Figure 1). The C7 oxygenation present on the latter as a urethane was thought accessible *via* ring-opening of the pyran in dioxobicyclic precursor **2**. It was unclear how to control acrylate geometry by this method. However, because the ring system in **2** is found in other bioactive natural products,[6] the risk was offset by opportunities. To construct **2**, the C2-C3 linkage was initially designated the ring-closure bond, such that the *ansa*-bridge might be installed *via* reaction between a pyran derived oxonium ion and an enolized form of the acetate in **3**. This left us to consider how to build the homoallylic acetate segment of **3**, ideally without acylating a C13 alcohol and while installing the *trans* disubstituted alkene with both positional and stereochemical control. It was known that fused bicyclic perhemiketals of type **4** would fragment to macrocyclic homoallylic esters **5** when treated with $FeSO_4$ in the presence of $Cu(OAc)_2$.[7] Both olefin geometry and regiochemistry were controlled – a result rationalized in terms of a transient alkoxy radical fragmenting the Cα-Cβ bond and the incipient carbon radical engaging Cu(II) to form an organometallic that undergoes *syn* co-planar β–hydride elimination.[7] Reasoning by analogy, structure **3** would result from similar fragmentation of butyrolactone-derived perhemiketal **6**. To our knowledge, there was no precedent for the ring system in **6**, providing an occasion to explore new structures while approaching callyspongiolide synthesis in a decidedly unconventional manner.

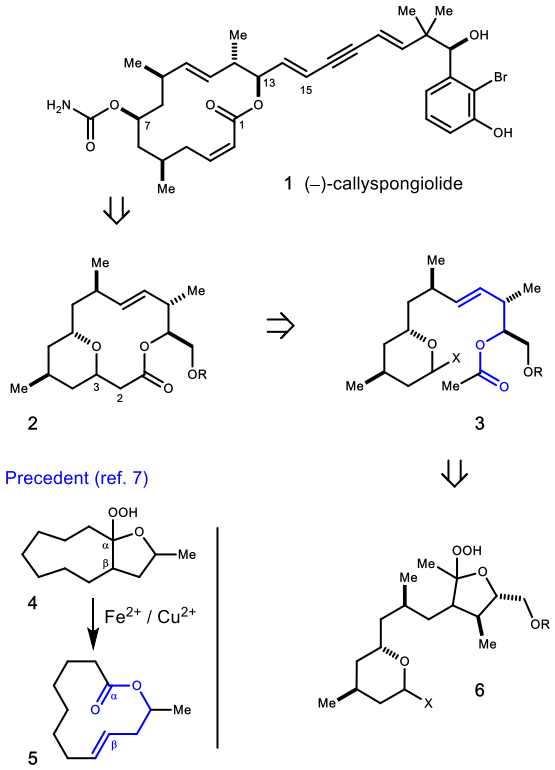
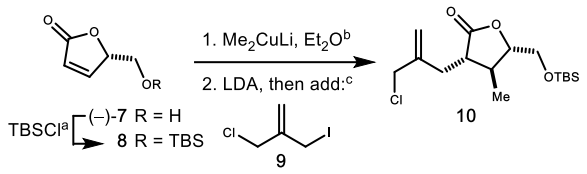


Figure 1. Retrosynthetic analysis of the (−)-callyspongiolide macrolactone.

Optically active butenolide **7** is commercially available and can be prepared on scale from cellulose powder.[8] Silylation of this material, treatment with Me_2CuLi and alkylation of the resultant conjugate addition product with 3-chloro-2-(iodomethyl)-1-propene (**9**)[9] gave allylic

chloride **10**.[10] Both carbon-carbon bond forming steps in the sequence occurred with diastereoselectivity greater than 95:5 (Scheme 1).

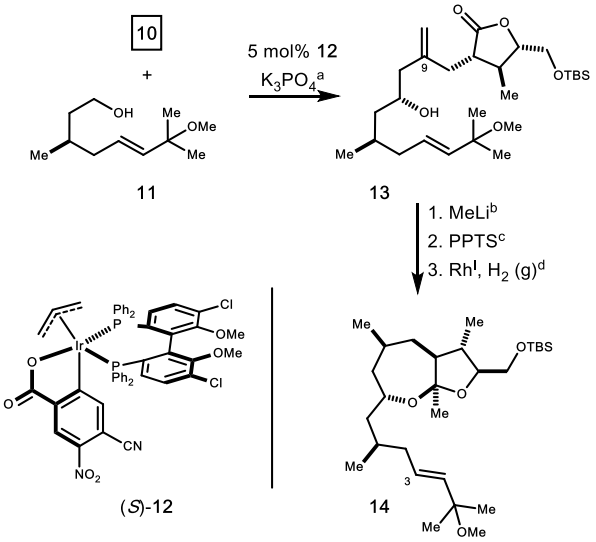
Scheme 1. Elaboration of a cellulose-derived butenolide.



(a) Imidazole (1.5 eq.), TBSCl (1.3 eq.), DCM (0.3 M), 0 °C to RT, 10 h, quant.; (b) CuI (1.1 eq.), MeLi (2.2 eq.), Et_2O (0.36 M), 0 °C, 20 min., then **8**, -20 °C, 2 h, 91%; (c) LDA (1.05 eq.), THF(0.1 M), -78 °C, 1 h, then **9** (1.1 eq.), -78 °C to -40 °C, 2 h, 80%.

We next employed a remarkable method for catalyzed asymmetric allyl transfer to join **10** with citronellol derivative **11**.[11] Warming a stoichiometric mixture of **10** and **11** with 5 mol% of Krische's iridium complex **12**[12] gave a single diastereomer of homoallylic alcohol **13**.[13] While there was room for improving reaction rate and catalyst turnover, the outcome was striking. The reaction forges a carbon-carbon bond between a primary alcohol and an allylic chloride *via* net loss of HCl, and does so with exquisite stereocontrol (*dr* >95:5).[14] With **13** in hand, four of five chiral centers in the target macrocycle had been established. What remained was to saturate the methylidene to set C9 stereochemistry (Scheme 2).

Scheme 2. Iridium catalyzed diastereoselective fragment coupling.

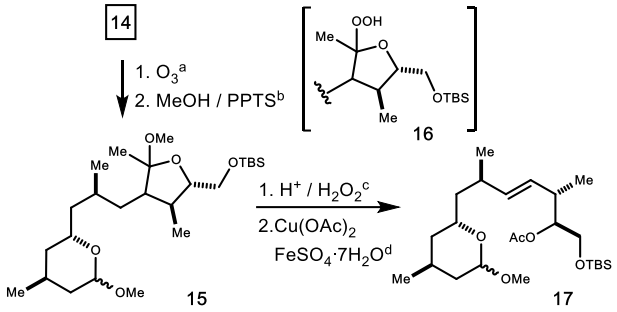


(a) K_3PO_4 (1 eq.), (*S*)-**12** (5 mol%), THF (1 M), 55 °C, 6 days, 62%; (b) MeLi (2.2 eq.), Et_2O (0.1 M), -78 °C to -20 °C, 1 h; (c) PPTS (2 mol%), THF (0.05 M), 50 °C, 2.5 h; (d) $(Ph_3P)_3RhCl$ (2 mol%), H_2 (20 bar), DCM (0.1 M), rt, 3h, 77% from **13**.

While selective hydrogenation of the 1,1-disubstituted alkene (*vs* the 1,2-disubstituted olefin) was achieved readily, controlling facial selectivity in the reduction was not. After much effort, it became clear we needed to impart a facial bias in the substrate by reducing conformational freedom. Since the plan was to convert the lactone in **13** to a perhemiketal of type **6** (Figure 1), we started down this path by adding MeLi to **13**, which provided a corresponding lactol in high yield. Desiccation of that material with dry PPTS then gave an intermediate *cis*-fused dioxaperhydroazulene whose topology allowed for selective hydrogenation using Wilkinson's complex as catalyst to afford a single isomer of bicycle **14** (Scheme 2).

Compound **14** was ozonolyzed to generate a C3 aldehyde. When that molecule was dissolved in MeCN containing catalytic amounts of PPTS, the system smoothly rearranged to linked bis-heterocycle **15**. It was then possible to selectively exchange the furanyl methoxy group with peroxide under mildly acidic conditions (Scheme 3).

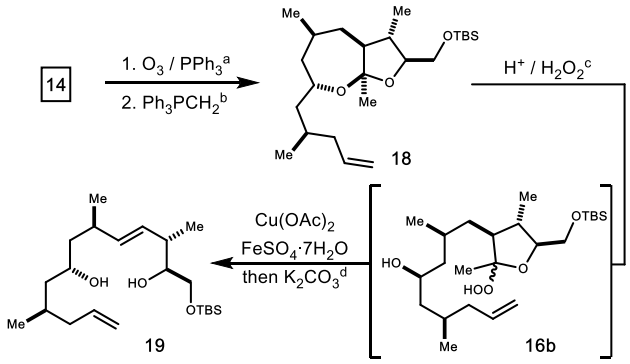
Scheme 3. Equilibrating heterocycles *en route* to a *seco*-acetate *via* perhemiketal fragmentation.



(a) O_3, then PPh_3 (1.1 eq.), DCM (0.05 M), -78 °C, 16 h; (b) PPTS (3 mol%), MeOH (0.05 M), rt, 2 h; (c) H_2O_2 50% v/v (6 eq.), PPTS (10 mol%), MeCN (0.1 M), rt, 30 min.; (d) $Cu(OAc)_2$ (2 eq.), $FeSO_4{\cdot}7H_2O$ (1.2 eq.), MeOH (0.02 M), rt, 20 min., 20% from **14**.

The resultant perhemiketal **16** was dissolved in MeOH and treated with $Cu(OAc)_2$ and $FeSO_4{\cdot}7H_2O$ at room temperature. This caused rapid ring-opening to generate *trans* homoallylic acetate **17** in 20% overall isolated yield from **14**. Consistent with the mechanistic hypothesis described earlier, both Fe(II) and Cu(II) were required, and signals indicative of alkene regioisomers were undetectable in ^1H NMR spectra of crude reaction mixtures.

Scheme 4. Pursuing a modified path to the target macrocycle.

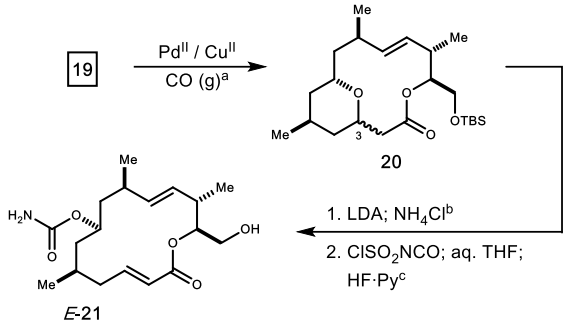


(a) O_3, then PPh_3 (1.1 eq.), DCM (0.05 M), -78 °C, 16 h; (b) CH_3PPh_3Br (1.2 eq.), *n*BuLi (1.2 eq.), THF (0.1 M), -10 °C to rt, 16 h; (c) H_2O_2 50% v/v (6 eq.), PPTS (10 mol%), MeCN (0.1 M), rt, 1 h; (d) $Cu(OAc)_2$ (2 eq.), $FeSO_4{\cdot}7H_2O$ (1.2 eq.), MeOH (0.02 M), rt, 15 min., then K_2CO_3 (10 eq.), rt, 20 h, 21% from **14**.

This result validated a central tenant in our approach and positioned us one bond away from target **2** (Figure 1). The intent was to close the large ring *via* net displacement of the methoxy group with an acetate-derived nucleophile. While we could form silyl ketene acetal derivatives of **17**, as well as various metal enolates, unfortunately none of these species could be coaxed to cyclize in a desired manner. The relative inertness of the methoxy pyran was the problem, yet preparing activated variants *via* additional functional group manipulations was not an attractive solution. Eventually we found an alternative that did not require added steps. Instead of rearranging the ozonolysis product of **14**, it was reacted with Ph_3PCH_2 to afford **18**. That material was treated with aq. H_2O_2 / cat. PPTS in MeCN and the resultant perhemiketal degraded with $FeSO_4{\cdot}7H_2O$ in the presence of $Cu(OAc)_2$. The

homoallylic acetate produced was saponified *in situ* to afford diol **19** in 21% isolated overall yield from **14**. We then used Dai's cascade variant of a Semmelhack cyclization[15] to directly form the target bicycle **20**.[16]

Scheme 5. Sequenced carbonylative macrolactonization and pyran ring-opening.



(a) $Pd(OAc)_2$ (10 mol%), $CuCl_2$ (3 eq.), CO (balloon), 4 Å Sieves, DCE (0.01 M), 40 °C, 24 h, 65%; (b) LDA (1.1 eq.), THF (0.02 M), -78 °C, 2 min., 73%; (c) $ClSO_2NCO$ (1.5 eq.), DCM (0.1 M), rt, 1 h, then add $THF:H_2O$ (4:1), 4 h, rt, then add HF·Py (10 eq.), 0 °C to rt, 2 h, 90% yield over 2 steps.

Slow addition of **19** to a suspension of $CuCl_2$ in dichloroethane containing 10 mol% $Pd(OAc)_2$ at 40 °C under an atmosphere of CO afforded macrolactone **20**. The reaction produced a mixture of C3 epimers (*dr* 6:4), but this was not consequential. Both diastereomers rapidly ring-opened at -78 °C when treated with LDA. Derivatization of the resultant alcohol with $ClSO_2NCO$, diluting the reaction with 20% aq. THF and subsequent addition of HF pyridine complex gave hydroxy urethane **21** in good overall yield (Scheme 5).

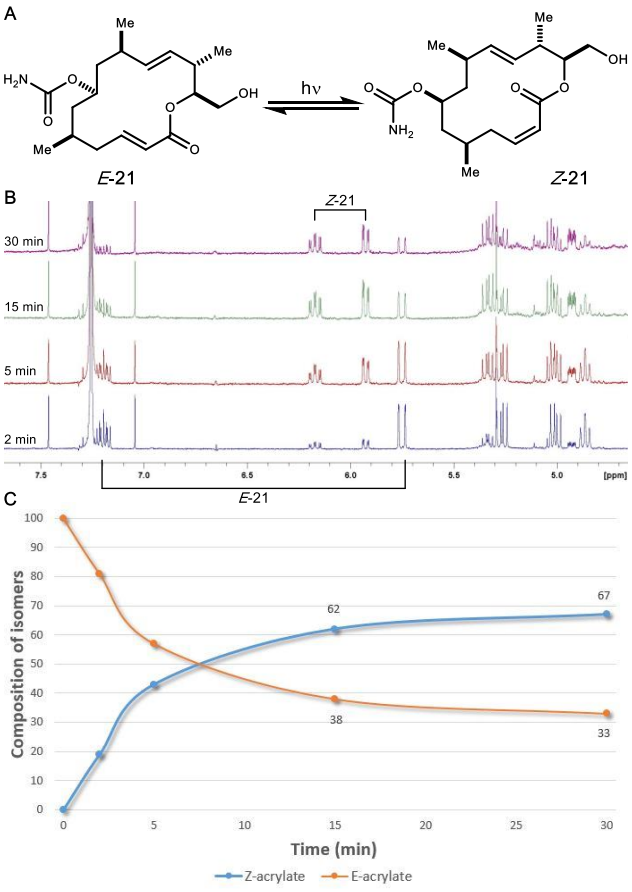
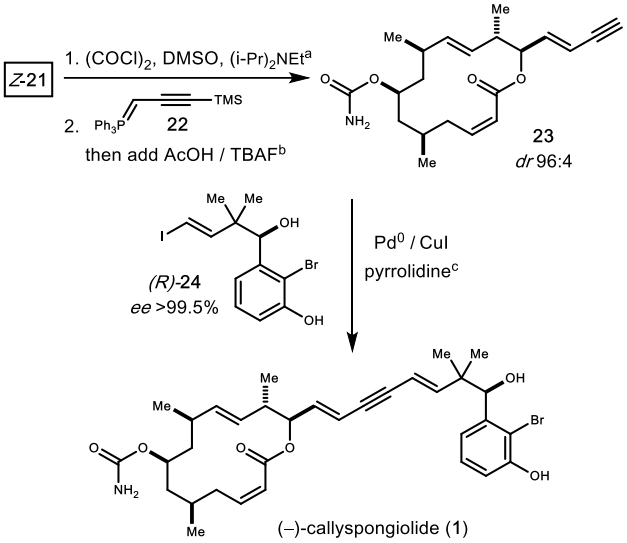


Figure 2. A) Photoisomerization: hν (300 nm, Pyrex, Rayonet), acetone (3 mM), rt, 20 min., 55% isolated *Z*-**21** (90% brsm). B) Partial ^1H NMR spectrum (500 MHz, $CDCl_3$) of aliquots of photolysis reaction. C) Time-course plot of integral ratio for B.

Interestingly, **21** was isolated exclusively as an *E,E* geometric isomer. However, when a dilute acetone solution of that material was irradiated in a Rayonet apparatus (300 nm bulb set) in a Pyrex vessel, a photo equilibrium was established wherein the *Z* acrylate was favored (Figure 2). As depicted in Figure 2, the photolysis was efficient. Performing the reaction in acetone, wherein substrate excitation likely occurred *via* energy transfer from the solvent,[17] prevented secondary photochemistry of the *Z*-enoate that led to deconjugation.[18] This was a major competing process in hexane solvent.

To complete the synthesis, the geometric isomers of **21** were separated by chromatography. This provided pure *Z*-**21** in 55% yield along with recovered *E*-**21** which could be resubjected to the photolysis. *Z*-**21** was oxidized using the Swern protocol. The resulting aldehyde was homologated with trimethylsilyl triphenyl(prop-2-yn-1-ylidene)-λ^5-phosphane (**22**),[19] and the product desilylated *in situ* to afford enyne **23** (Scheme 6).[20] This material was then joined with vinyl iodide (*R*)-**24** (>99.5% *ee*)[4e] *via* Sonogashira coupling[4b] to afford (−)-callyspongiolide (**1**).[21] Spectroscopic data for **1** was identical to that reported in the literature.[1]

Scheme 6. Completion of the synthesis.



(a) $(COCl)_2$ (1.2 eq.), DMSO (1.3 eq.), $(i\text{-}Pr)_2NEt$ (2.4 eq.), DCM (0.05 M), -78 °C 30 min. then rt, 10 min.; (b) $TMSCCCH_2PPh_3Br$ (1.12 eq.), KHMDS (1.1 eq.), THF (0.018 M), -78 °C, 3 h then rt 15 h, then add AcOH (1.1 eq.), TBAF 1 M (1.1 eq.), 0 °C to rt, 1 h, 55% from *Z*-**21**; (c) (*R*)-**24** (1.2 eq.), $Pd(PPh_3)_4$ (10 mol%), CuI (20 mol%), pyrrolidine (2.0 eq.), toluene (0.01 M), rt, 4 h, 82% from **23**.

The longest linear sequence in the above synthesis is 18 steps. It builds the target from three segments while exploiting unique intermediate structures. The design leverages the chiral pool for raw materials and contemporary catalysis to join fragments and form rings. The route makes available callyspongiolide segments, isomers and derivatives on scales useful for driving research into their molecular biology and pharmacology. We have prepared ample amounts (>100 mg) of the natural product [22] and have considerable intermediate supplies for these experiments. In preliminary studies we have confirmed the activity of **1** in Jurkat B lymphoctyes (IC_{50} = 43 nM) and find that its Δ2,3-*E* isomer is comparably potent (IC_{50} = 115 nM). In contrast, neither macrolactone *E*-**21** nor the unsaturated side chain (see SI) possess activity at 3 μM in this cell line. Less than 3% of callyspongiolide toxicity towards Jurkat cells is blocked by pre-treatment with 100 μM Z-VAD-FMK, a pan-caspase inhibitor. Notably, **1** strongly inhibits the growth of mu-

tant *Saccharomyces cerevisiae* lacking the PDR5 drug transporter. In liquid culture, **1** also potently inhibits growth of a yeast ERG3 mutant. This suggests there is a yeast receptor(s) for the compound and opens the possibility of using genome wide mutagenesis to identify it. Experiments to test this exciting prospect are ongoing as are attempts to adapt the synthesis to preparations of biochemical probes and related bioactive natural products.

ASSOCIATED CONTENT

Supporting Information

Supporting Information is available free of charge on the ACS Publications website.
Experimental procedures and spectral data (PDF).

AUTHOR INFORMATION

Corresponding Author

* harran@chem.ucla.edu

Notes

The authors declare no competing financial interests.

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(10) A small amount (ca. 5%) of the iodo variant of **10** is produced during this reaction. Because the iodide interferes with subsequent iridium catalysis (see ref. 13), it was converted to **10** during work-up *via* Finkelstein exchange (LiCl, acetone, rt).
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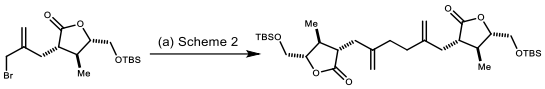
(13) Interestingly, both the bromo and iodo congeners of **10** gave different results. The former was transformed into a mixture (ca. 1:1) of desired product **13** and symmetric dimer **25** (see SI). The latter appeared to poison **12**. Intriguingly **25** was also formed when **10** was contaminated with only minor amounts of its iodo variant (<5%).



(14) Alternatively, **13** could be synthesized from the iodo variant of **10** and the aldehyde derived from **11** using stoichiometric indium metal (THF, rt, 85-90%). However diastereoselectivity was poor (ca. 1:1) and separating the isomers was impractical on scale.
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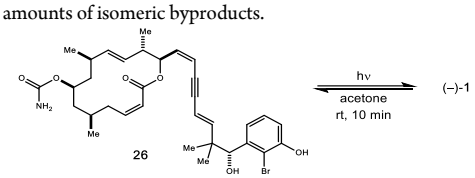
(20) The small amount of *cis* olefin produced in the enyne preparation allowed us to isolate callyspongiolide isomer **26** following Sonogashira coupling of **23** with (*R*)-**24**. Brief photolysis of **26** under conditions identical to those used to interconvert *E*- and *Z*-**21** resulted in roughly a 1:1 mixture of **26** and **1** containing lesser amounts of isomeric byproducts.



(21) Following the protocols reported, the Δ2,3-*E* isomer of (−)-callyspongiolide can be easily accessed from the *E* isomer of **21** (see SI).
(22) Synthetic (−)-callyspongiolide (**1**) was handled following the procedure reported in: Hensgen, M. I.; Stump, B. *Methods Mol. Biol.* **2013**, *1045*, 133-143.

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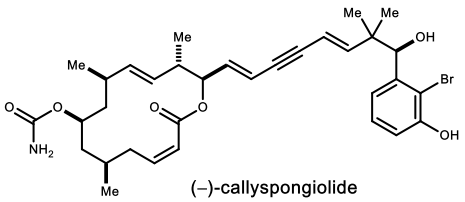

(–)-callyspongiolide