JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Total Synthesis and Stereochemical Assignment of Callyspongiolide

Jingjing Zhou, Bowen Gao, Zhengshuang Xu,* and Tao Ye*

Laboratory of Chemical Genomics, Engineering Laboratory for Chiral Drug Synthesis, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Xili, Nanshan District, Shenzhen 518055, China

Supporting Information

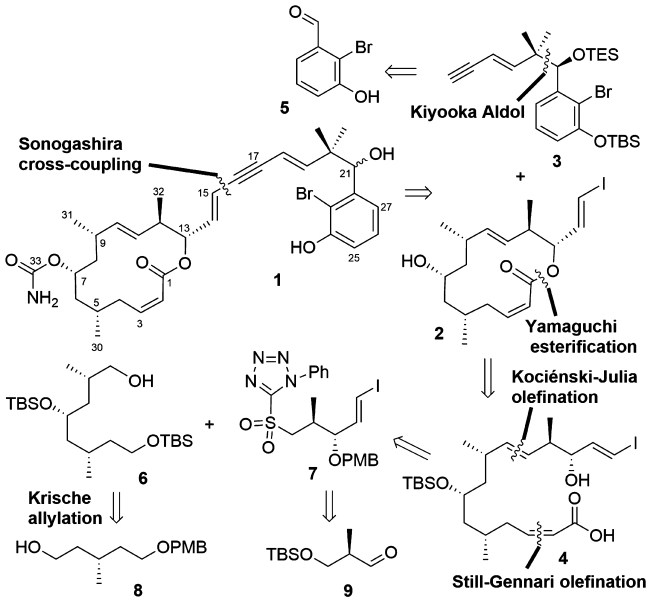
ABSTRACT: Total synthesis of four callyspongiolide stereoisomers led to unambiguous assignment of relative and absolute stereochemistry of the natural product. Key features of the convergent, fully stereocontrolled route include the use of Krische allylation, Kiyooka Aldol reaction, Kociénski–Julia olefination, Still–Gennari olefination, Yamaguchi macrocyclization, and Sonogashira coupling reaction. Biological evaluation of the synthesized compounds against an array of cancer cells revealed that the stereochemistry of the macrolactone core played an important role.

Callyspongiolide (**1**) is an extraordinary marine sponge-derived macrolide, isolated from sponge *Callyspongia* sp., collected in Indonesia.[1] The structure, comprising a conjugated diene-ynic side chain terminating at a brominated benzene ring, appended to the 14-membered macrocyclic lactone ring, was elucidated via a combination of NMR experiments. In addition, six stereogenic centers are present in callyspongiolide, including five in the macrocyclic ring and one in the side chain. The relative stereochemistry of the macrocyclic core was assigned on the basis of coupling constants and advanced 1D NOE and 2D NOESY experiments, but the relative configuration of the side chain with regard to the macrolactone is not known. Furthermore, the absolute stereochemistry of callyspongiolide remains unknown. Callyspongiolide exhibits significant *in vitro* cytotoxicity against human Jurkat J16 T and Ramos B lymphocytes (IC_{50} 70 and 60 nM, respectively). Importantly, callyspongiolide is ~13-fold more active than kahalalide F,[1] which makes callyspongiolide a potential lead compound for the development of new anticancer agents. We have been interested for some time in macrocyclic marine natural products[2] and view their syntheses as a key route to structural assignment, structural modification, and subsequent activity control. Herein, we disclose the total synthesis of callyspongiolide and the resulting assignment of the stereochemical configuration of the natural product.

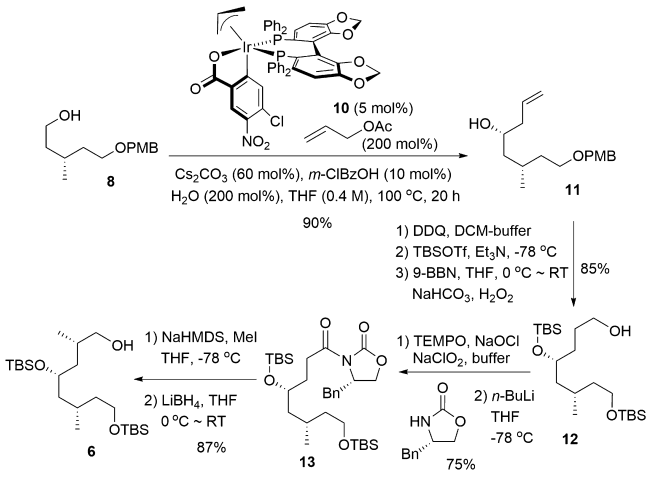
As outlined retrosynthetically in Scheme 1, our synthetic approach relies on assembly of vinyl iodide-containing macrolactone (**2**) and ene-ynic side chain (**3**). The macrolactone (**2**) would be generated by macrolactonization of seco acid **4**, which was planned to arise from a Kociénski–Julia olefination between sulfone **7** and an aldehyde derived from alcohol **6**. Both **6** and **7** could be readily accessed from the known chiral alcohol (**8**) and aldehyde (**9**).

The synthesis of fragment **6** commenced with Krische allylation[3] of the known alcohol **8**.[4] This catalytic reagent-controlled process employs a chiral iridium catalyst to afford homoallylic alcohol **11** in 90% yield and >95% diastereoselectivity (Scheme 2). **11** was converted into primary alcohol **12** in 85% yield by a three-step sequence including the DDQ

Scheme 1. Retrosynthetic Analysis of Callyspongiolide (1)



Scheme 2. Synthesis of Fragment 6



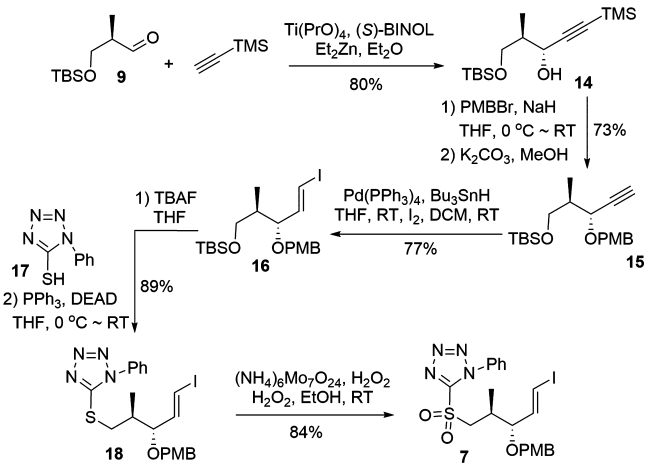
Received: April 6, 2016
Published: May 26, 2016

DOI: 10.1021/jacs.6b03533
J. Am. Chem. Soc. 2016, 138, 6948–6951

oxidative deprotection of the PMB group, followed by protection of the resulting diol as the corresponding TBS ethers, and hydroboration and oxidation of the terminal olefin. Oxidation of the primary hydroxy group of **12** with catalytic TEMPO, NaOCl, and stoichiometric $NaClO_2$ in the MeCN-buffer[5] afforded the desired carboxylic acid, which was then converted to the mixed pivaloyl anhydride and treated with *N*-lithio-(*S*)-4-benzyl-2-oxazolidinone to provide acyl oxazolidinone **13** in 75% yield over three steps. Stereoselective alkylation of imide **13** with iodomethane followed by reductive removal of the chiral auxiliary by using $LiBH_4$ provided primary alcohol **6** in 87% yield over two steps.

With the fragment **6** in hand, we next turned our attention to the synthesis of sulfone **7** (Scheme 3). Thus, treatment of

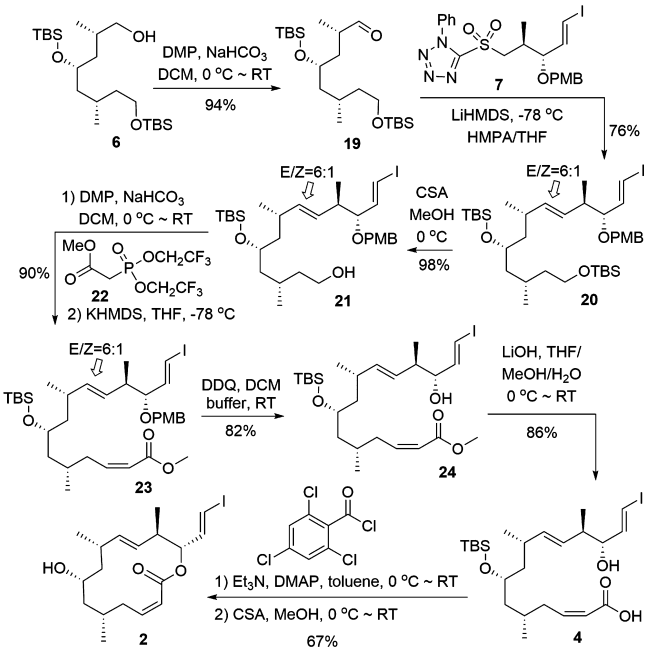
Scheme 3. Synthesis of Fragment 7



aldehyde **9**[6] with (*S*)-BINOL, $Ti(O^iPr)_4$, Et_2Zn, and TMS acetylene provided the corresponding propargylic alcohol (91:9 dr), and the desired anti isomer (**14**) was isolated in 80% yield following chromatographic purification.[7] The propargylic alcohol of **14** was protected as its PMB ether followed by selective desilylation to give rise to **15** in 73% yield. Hydrostannylation of alkyne **15**, followed by iodination of the resulting vinyl stannane, afforded *trans*-vinyl iodide **16** along with its minor regioisomer (<5%), which was removed via silica gel chromatography. The trans-configuration of **16** was confirmed by the coupling constant of the olefinic protons (14.5 Hz). **16** was next transformed into sulfide **18** in 89% yield by a two-step sequence involving removal of the TBS protecting group and Mitsunobu reaction of the resulting primary alcohol with 1-phenyl-1H-tetrazole-5-thiol (**17**). Oxidation of sulfide **18** with ammonium heptamolybdate and H_2O_2 afforded sulfone fragment **7** in 84% yield.[8]

Having both the desired fragments **6** and **7** in hand, we turned to fasten them together to obtain the 14-membered macrocycle **2** (Scheme 4). Thus, oxidation of alcohol **6** with Dess–Martin periodinane provided aldehyde **19** in 94% yield. Kociénski–Julia olefination[8,9] of the resulting aldehyde in THF with lithium anion derived from sulfone **7** proceeded no selectivity, affording the corresponding **20** as a ~1:1 mixture of *E*/*Z* isomers (90% combined yield). To our delight, when the olefination was performed at −78 °C with HMPA as an additive, the *E*/*Z* ratio of alkene **20** was improved to 6:1.[10] As separation of the geometric isomers proved to be difficult, the compounds were carried through the synthetic sequence as a

Scheme 4. Synthesis of Macrolactone 2



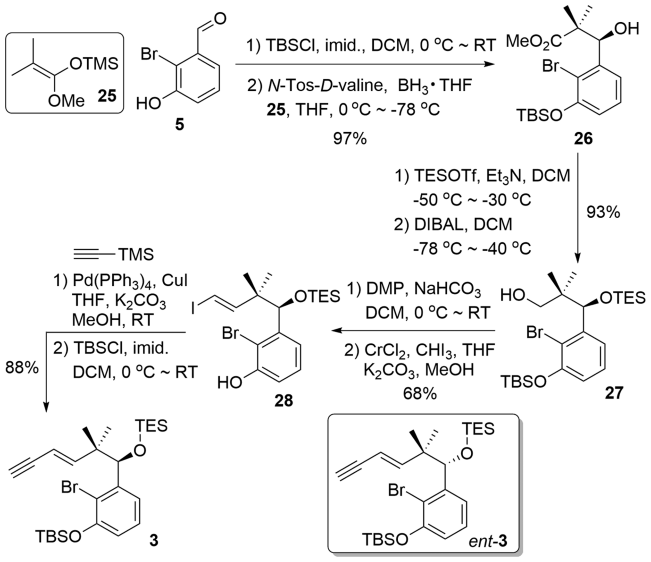
mixture and separated at a later stage of the synthesis. Thus, acid-catalyzed selective desilylation of **20** gave rise to the corresponding alcohol **21** in 98% yield. Oxidation of **21** with Dess–Martin periodinane, followed by immediate reaction with methyl [bis(2,2,2-trifluoroethoxy)phosphoryl]-acetate (**22**) under Still–Gennari conditions,[11] furnished **23** in 90% yield. The geometry of the newly formed olefin was exclusively *Z*. DDQ deprotection of the *p*-methoxybenzyl ether of **23** yielded the corresponding alcohol, and the minor geometric isomer, resulting from the previous Kociénski–Julia olefination, was readily separated by silica gel chromatography. The isomerically pure olefin **24** was subjected to hydrolysis under basic conditions to afford the key seco-acid **4** in 86% yield. Yamaguchi macrolactonization[12] of **4** provided the desired macrocycle, which underwent a facile desilylation to generate the key macrolactone **2** in 67% yield.[13]

The preparation of the ene-ynic fragment **3** began with the protection of the phenolic OH of **5** as its TBS ether, followed by Kiyooka aldol reaction[14] with methyl trimethylsilyl ketene **25**, in the presence of oxazaborolidinone (derived from *N*-Ts-D-valine and BH3·THF), afforded the corresponding (*R*)-*β*-hydroxy ester **26** in 97% yield over two steps (Scheme 5). Silylation of *β*-hydroxy group with triethylsilyl triflate (TESOTf) proceeded smoothly to give the corresponding TES ether and was followed by ester reduction with DIBAL to give primary alcohol **27** in 93% yield over two steps. Oxidation of the primary hydroxy group with Dess–Martin periodinane generated the corresponding aldehyde, which was homologated using the Takai protocol,[15] which proceeded smoothly to give the *E*-vinyl iodide **28** in 68% yield with no observable formation of the *Z* isomer. Sonogashira coupling[16] between vinyl iodide **28** and TMS-acetylene, followed by removal of the TMS group cleanly furnished the corresponding enyne. After protection of the phenolic OH as its TBS ether, **3** was isolated in 88% yield.

With the key intermediates **2** and **3** at hand, their assembly to callyspongiolide was undertaken. Thus, Sonogashira coupling of vinyl iodide **2** with enyne **3** furnished the corresponding conjugated diene-yne in 74% yield. After attachment of the

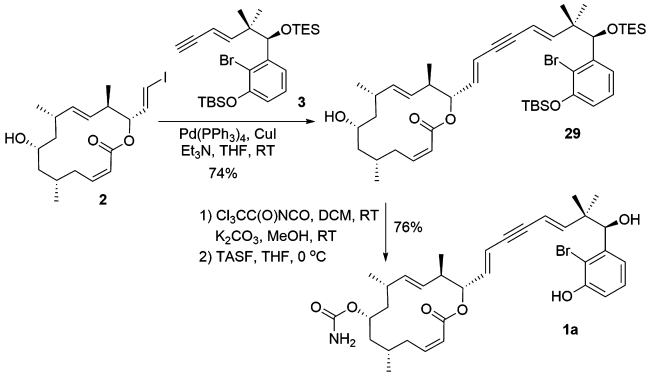
DOI: 10.1021/jacs.6b03533
J. Am. Chem. Soc. 2016, 138, 6948–6951

Scheme 5. Synthesis of Ene-Ynic Fragment 3



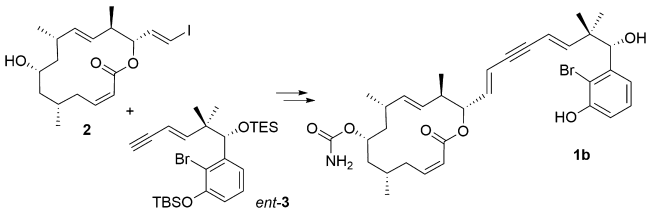
carbamate group[17] onto the free hydroxy group, the resultant product was treated with tris(dimethylamino) sulfur(trimethylsilyl)difluoride in THF to provide **1a** in 76% yield. (Scheme 6).

Scheme 6. Synthesis of Callyspongiolide 1a



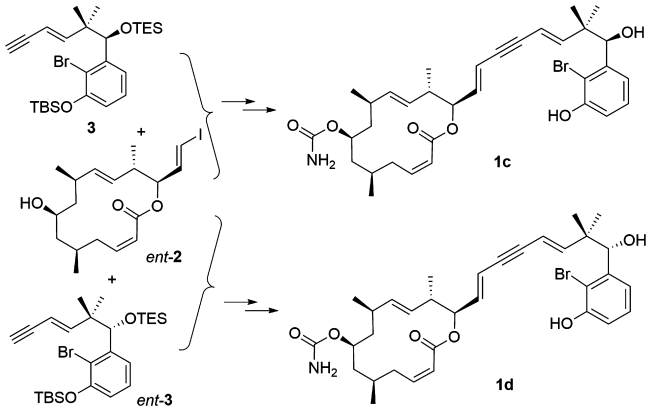
With many building blocks already at hand, the next step was to prepare a batch of *ent*-**3**, and this was readily achieved by following the same synthetic procedure as for **3**, but using the oxazaborolidinone derived from L- instead of D-valine. This proceeded smoothly, and *ent*-**3** was readily incorporated into the synthesis to produce **1b** (Scheme 7). Comparison with an authentic sample of callyspongiolide is not possible as the natural product is no longer available.[18] The NMR spectra of both synthetic materials (**1a**, **1b**) were compared with the data reported for natural callyspongiolide. While both **1a** and **1b** had

Scheme 7. Synthesis of Callyspongiolide 1b



similar ^{1}H and ^{13}C NMR spectra, there were some subtle differences. The chemical shift for the proton on the carbon (C21), attached to the secondary hydroxy substituent, differs slightly in the two epimers. In **1a**, the signal attributable to this proton appears at 4.88 ppm, while the signal for the same proton in **1b** is observed at 4.89 ppm, which is identical to the data reported for the natural product. While the ^{13}C NMR spectra of **1a** and **1b** were similar, a comparison of chemical shifts for all signals indicated that the spectrum of **1b** showed a closer correlation with those of the natural product (maximum $\Delta\delta$ of 0.02 ppm) than that of **1a** (maximum $\Delta\delta$ of 0.04 ppm). The unambiguous assignment of the absolute and relative configuration comes from the rotation values. Indeed, **1b** has a rotation of $[\alpha]_D^{20}$ = +12.0 (*c* 0.1, MeOH), very close in the absolute value and the opposite sign to that reported for the natural sample $[\alpha]_D^{20}$ = −12.5 (*c* 0.1, MeOH), whereas **1a** has a rotation of $[\alpha]_D^{20}$ = +66.0 (*c* 0.1, MeOH). This significant difference suggested that **1b** is the unnatural antipode of callyspongiolide. With ene-ynic side chain **3** and its enentiomer *ent*-**3** in hand, we elected to extend our studies to include the preparation and characterization of the antipodes of **1a** and **1b**. Thus, *ent*-**2** was readily prepared by following a similar synthetic procedure as for **2**, and coupled with enyne **3** or enyne *ent*-**3**, followed by carbamate formation and desilylation provided **1c** and **1d**, respectively. The ^{1}H and ^{13}C NMR spectroscopic data shows, as expected, no difference between **1c**, **1b**, and the natural sample. The optical rotation of **1c** was ($[\alpha]_D^{20}$ = −13.0 (*c* 0.1, MeOH)), which compared well to the data reported under identical conditions for the natural isolate $[\alpha]_D^{20}$ = −12.5 (*c* 0.1, MeOH), thereby confirming that the identity of the absolute stereochemistry of naturally occurring callyspongiolide is **1c** (Scheme 8).

Scheme 8. Synthesis of Callyspongiolide (1c) and Its Epimer 1d



The initial biological evaluation of **1a**−**1d** was performed across a panel of eight cancer cell lines of different histological origins, and cell proliferation was measured by MTS assay. As shown in Table 1, compounds **1a** and **1b**, derived from macrocycle **2**, displayed either no or weak inhibitory activities against the cancer cell lines examined. On the other hand, synthetic callyspongiolide (**1c**) and its epimer **1d** showed potent cytotoxicity in a submicromolar concentration toward most tested cancer cell lines. Importantly, **1c** displayed pronounced inhibitory activity against Jurkat cells, and the IC_{50} value of **1c** was 11.1 nM. The epimer **1d** showed comparable and, in some cases, even more potent anticancer

DOI: 10.1021/jacs.6b03533
J. Am. Chem. Soc. 2016, 138, 6948−6951

Table 1. 50% Antiproliferative Concentrations (IC_{50}, μM) of the Synthetic Samples against Carcinoma Cells

origins	cell line	compounds			
		1a	**1b**	**1c**[a]	**1d**
breast	MCF7	1.42	5.11	0.225	0.0953
brain	SH-SY5Y	2.99	NA[b]	0.467	0.0859
cervix	HeLa	5.40	NA	3.56	1.49
colon	HT-29	5.20	NA	0.260	0.193
colon	RKO	4.56	NA	3.55	1.96
lung	H1299	7.56	NA	0.119	0.572
prostate	PC-3	3.97	8.61	0.276	0.0382
T lymphocyte	Jurkat	1.80	1.72	0.0111	0.205

[a]**1c** is the synthetic sample of callysongiolide. [b]NA = no activity at concentrations of 50 μM.

activities compared to synthetic callyspongiolide (**1c**). For example, **1d** exhibited an IC_{50} value of 38.2 nM against PC-3 cells, 95.3 nM against MCF7 cells, and 85.9 nM against SH-SY5Y cells. These results led us to believe that the stereochemistry of the macrolactone moiety played an important role in the observed inhibitory activity toward the cancer cell lines examined.

In summary, the execution of a highly convergent strategy has led to completion of the first total synthesis of callyspongiolide and three additional stereoisomers. These effects have led to the establishment of the relative stereochemistry as well as the absolute configuration of the natural product. Initial biological assessment of the synthesized compounds against an array of cancer cells revealed that the stereochemistry of the macrolactone core played an important role. Extension of this strategy in the synthesis of various analogues and to establish an SAR profile for callyspongiolide and to identify the targets of its action are currently underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03533.

Experimental details and data (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*yet@pkusz.edu.cn

*xuzs@pkusz.edu.cn

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support provided by NSFC (21572007, 21272011, 21133002), Shenzhen Peacock Plan (KQTD2015-071714043444), SZSTDF (JCYJ20130329175740481, JCYJ20140419131807793, JCYJ20150629144022829, JSGG20140717102922014), GDNSF (2014A030312004),

■ REFERENCES

(1) Pham, C.-D.; Hartmann, R.; Böhler, P.; Stork, B.; Wesselborg, S.; Lin, W.; Lai, D.; Proksch, P. *Org. Lett.* **2014**, *16*, 266.

(2) (a) Liu, J.; Wang, L.; Zhang, J.; Xu, Z.; Ye, T. *Chem. Commun.* **2016**, *52*, 1002. (b) Qu, S.; Chen, Y.; Wang, X.; Chen, S.; Xu, Z.; Ye, T. *Chem. Commun.* **2015**, *51*, 2510. (c) Lei, H.; Yan, J.; Yu, J.; Liu, Y.; Wang, Z.; Xu, Z.; Ye, T. *Angew. Chem., Int. Ed.* **2014**, *53*, 6533. (d) Liu, H.; Liu, Y.; Wang, Z.; Xing, X.; Maguire, A. R.; Luesch, H.; Zhang, H.; Xu, Z.; Ye, T. *Chem. - Eur. J.* **2013**, *19*, 6774. (e) Dai, L.; Chen, B.; Lei, H. H.; Wang, Z.; Liu, Y. Q.; Xu, Z. S.; Ye, T. *Chem. Commun.* **2012**, *48*, 8697.

(3) Hassan, A.; Lu, Y.; Krische, M. *Org. Lett.* **2009**, *11*, 3112.

(4) Fujiwara, K.; Naka, J.; Katagiri, T.; Sato, D.; Kawai, H.; Suzuki, T. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 1173.

(5) (a) Zhao, M.; Li, J.; Mano, E.; Song, Z.; Tschaen, D. M.; Grabowski, E. J. J.; Reider, P. J. *J. Org. Chem.* **1999**, *64*, 2564. (b) De Luca, L.; Giacomelli, G.; Masala, S.; Porcheddu, A. *J. Org. Chem.* **2003**, *68*, 4999.

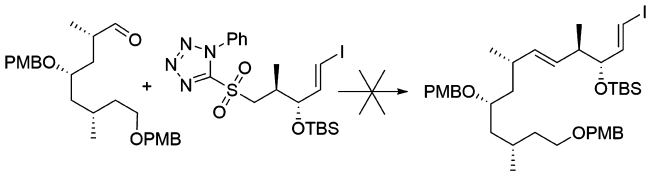
(6) Kirkham, J. E. D.; Lee, V.; Baldwin, J. E. *Chem. Commun.* **2006**, *27*, 2863.

(7) Marshall, J. A.; Bourbeau, M. P. *Org. Lett.* **2003**, *5*, 3197.

(8) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, *1998*, 26.

(9) Blakemore, P. R. *J. Chem. Soc. Perkin Trans. 1* **2002**, 2563.

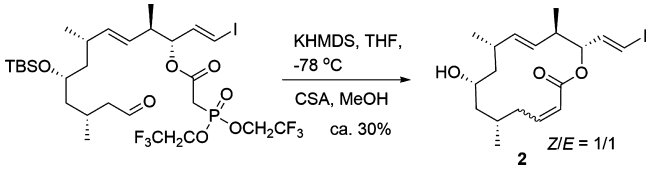
(10) The selection of protective groups on aldehyde (**19**) and sulfone (**7**) proved to be critical for the success of the synthesis of **20**. Interestingly, a similar Kociénski–Julia olefination process with the following aldehyde and sulfone as the condensation partners led to degradation and/or recovery of starting materials under a variety of reaction conditions.



(11) Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.

(12) (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.

(13) An alternative approach for the construction of the macrocycle **2** was also explored. Macrocyclization by an intramolecular Still–Gennari olefination of the *seco*-precursor as shown below gave rise to the corresponding α,β-unsaturated lactone (**2**) as an E/Z mixture of isomers (E/Z = 1:1) in 30% combined yield.



(14) Kiyooka, S.-I.; Kaneko, Y.; Komura, M.; Matsuo, H.; Nakano, M. *J. Org. Chem.* **1991**, *56*, 2276.

(15) Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408.

(16) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.

(17) Kocovsky, P. *Tetrahedron Lett.* **1986**, *27*, 5521.

(18) Proksch, P. Heinrich-Heine University, Düsseldorf, Germany. *Personal communication*, 2015.

DOI: 10.1021/jacs.6b03533
J. Am. Chem. Soc. 2016, 138, 6948–6951