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Design, Synthesis, and Biological Evaluation of EF- and ABEF- Analogues of (+)-Spongistatin 1

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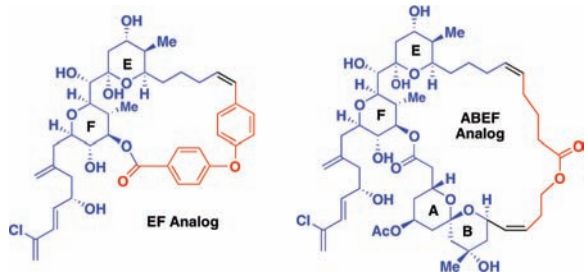
ABSTRACT



The design, synthesis, and biological evaluation of two potential (+)-spongistatin 1 analogues have been achieved. The analogues, incorporating tethers (red) in place of the ABCD and the CD components of the (+)-spongistatin 1 macrolide, were designed such that the conformations of the retained skeleton (blue) would mimic the assigned major solution conformation of the natural product The nanomolar cytotoxicity observed for the ABEF analogue provides strong support for the assigned solution conformation.

Members of the spongistatin family of natural products were independently isolated by three research groups, Pettit[1] (spongistatins), Kitigawa[2] (altohyrtins), and Fusetani[3] (cinachyrolide), from the marine sponges *Spongia*, *Spirastrella*, and *Cinachyra*, respectively. Spongistatin 1 [(+)-**1**, Figure 1], the most abundant congener, has been characterized as an antimitotic agent that

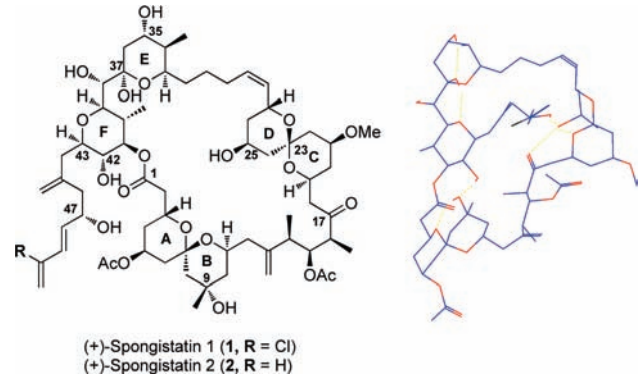


Figure 1. (a) Spongistatins 1 and 2. (b) Calculated hydrogen bonding network of (+)-spongistatin 1.

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inhibits tubulin polymerization by binding in the tubulin vinca alkaloid domain.[4] Importantly, subnanomolar cytotoxicity against several chemoresistant cancer cell lines has been demonstrated, making (+)-spongistatin 1 and congeners thereof important antitumor lead compounds.[5] From a structural perspective, the spongistatins also display significant architectural complexity; common features include the polyether backbone with 24 stereocenters, two spiroketals, a bis-pyran unit embedded within a 42-membered macrolide, and a triene side chain bearing a vinyl chloride. The first total syntheses of (+)-spongistatin 2 by Evans[6] and (+)-spongistatin 1 by Kishi,[7] confirming each structure, have been followed by syntheses from the Smith,[8] Paterson,[9] Crimmins,[10] Heathcock,[11] and Ley[12] laboratories. A variety of synthetic approaches to various fragments of the spongistatins have also been reported,[13] as well as the synthesis of 1 g of (+)-spongistatin 1 (**1**) reported by this laboratory.[14]

The design and synthesis of spongistatin analogues has been impeded by the molecular complexity, with only a few studies reported. Early on, Kishi and co-workers[7] reported that epimerization of the **CD** spiroketal at C(23) affords an analogue possessing cytotoxicity similar to that of (+)-spongistatin 1, while our laboratory reported that side-chain analogues based on the α-D-glucose scaffold maintain modest micromolar activity, albeit most likely via a different mechanism.[15] Subsequently, Paterson et al.[16] disclosed that unsaturation of the **E**-ring, achieved by elimination of water at C(35,36) and formation of a double bond, affords an analogue with increased cytotoxicity relative to (+)-spongistatin 1; a similar observation was made for spongistatin 2 by Heathcock and co-workers,[11] while truncation of the triene side chain by Paterson resulted in a dramatic loss of activity.[16]

More recently, Heathcock reported that spongistatin analogues, including acyclic congeners having only the **E** and **F** rings, as well as cyclic **EF**, **ABEF**, and **ABCD** analogues, where at least one ring had been replaced with a polyethylene linker,[17] do not maintain significant cytotoxicity.

Taken together, the available SAR data suggests that the **E** and **F** rings, as well as the triene side chain, are critical for biological activity. The question thus becomes: How important are the **AB** and **CD** spiroketals? Are these structural units required for potent cyctotoxicity or simply to enforce the bioactive conformation and, in particular, the nearly linear conformation of the western perimeter as observed in the assigned solution conformation.[18]

To achieve answers, we chose a minimalist design strategy, specifically to maintain a macrolide structure, first with excision of the **AB** and **CD** spiroketal units and then with an analogue lacking only the **CD** unit. Two design criteria were foremost: (1) Analogue construction would take advantage of advanced intermediates readily available either from our 1-g synthesis and/or commercially, and (2) we would select tethers that would enforce the western perimeter to maintain the same low energy linear conformation as defined by our solution conformation.[18]

We first targeted macrolide **3**, which maintained the **EF** bis-pyran system but lacked the **AB** and **CD** spiroketals (Figure 2).

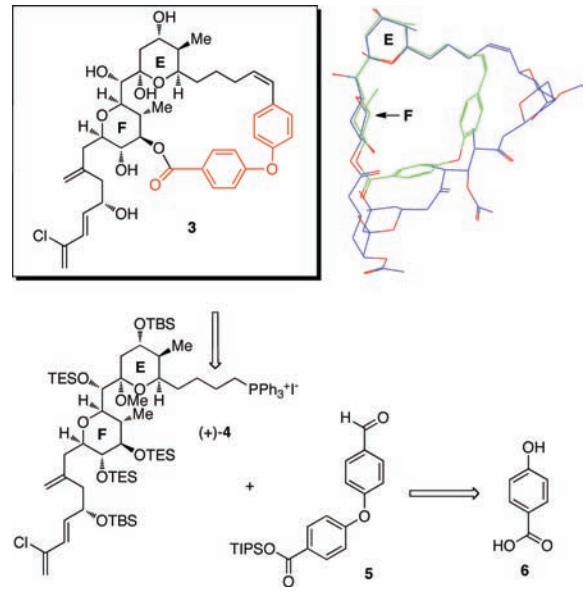

Figure 2. (a) **EF** analogue and (b) overlay of **EF** analogue and predicted solution conformation of (+)-spongistatin 1.

Possible C(1)−C(28) tethers were selected on the basis of length, orientation, and flexibility after in silico screening of known fragment libraries. We then performed Monte Carlo conformational searches (5000 steps) on analogues featuring these linkers. Ana-

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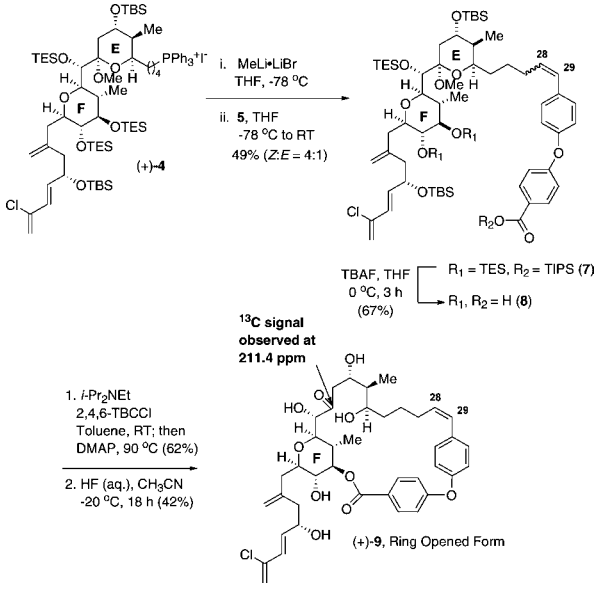
logues preserving the same low energy conformation were subjected to full conformational searches. These studies resulted in selection of the biaryl ether tether (Figure 2). Overlay of the resulting analogue **3** with the twisted or "infinity"-shaped major solution conformation of spongistatin in water revealed excellent overlap along the western perimeter specifically along the **E**, **F**, and **AB** rings.

From the synthetic perspective, the requisite biaryl ether tether was anticipated to be available via a modified Ullmann ether synthesis,[19] employing the TIPS ester of **6** and 4-formylphenyl boronic acid. With aldehyde **5** in hand, analogue **3** would then be constructed using the same endgame strategy as in our gram synthesis of (+)-spongistatin 1, employing **EF** Wittig salt (+)-**4** (Scheme 1).

Scheme 1. Synthesis of **EF** Analogue **3**



Toward this end, deprotonation of (+)-**4** was achieved with MeLi•LiBr followed by union with **5** to lead to a 4:1 mixture of *Z* and *E* olefin isomers which could not be readily separated. The poor selectivity is in contrast to that observed in our (+)-spongistatin 1 synthesis, which produced exclusively the *Z*-olefin isomer. The mixture of olefin isomers was next subjected to 3 equiv of TBAF to remove the TIPS group, as well as the two **F**-ring TES groups; the *seco*-acid olefin isomers (**8**) still could not be separated. Pleasingly, however, macrocyclization occurred only with the *Z* olefin isomer to furnish the desired product in 62% yield. Presumably, the strain associated with incorporating an *E*-olefin prevented macrocyclization. Global deprotection led to an 11:1 mixture of products. However, upon detailed 1- and 2-D NMR analysis, the major product, (+)-**9**, proved to be the ring-opened congener of the desired **EF** analogue **3**, as evidenced by the presence of a ^{13}C NMR signal at 211.4 ppm. Presumably, the ring strain of the tether is sufficient to drive the equilibrium toward the open δ-hydroxy ketone congener of **3**.

We next turned our attention to an analogue lacking only the **CD** spiroketal unit (e.g., **ABEF** analogue **10**, Figure 3). Conformational searches performed on the Heathcock **ABEF** spongistatin 2 analogues indicated that the linkers selected were not capable of preserving the nearly linear western perimeter of the spongistatins. We therefore undertook calculations that eventually led to a range of potential **B** to **E** tethers which were able to maintain the assigned solution conformation of (+)-spongistatin 1 (i.e., linear). Selection of the **B** to **F** tether illustrated in Figure 3 was based both on the ease of synthesis and the ability to engage in hydrogen bonding. That is, the length of the linker, as well as placement and orientation of the ester bond, were optimized to increase the probability of a hydrogen bond between the ester carbonyl and the C(42) hydroxyl group on the **F** ring, which would maintain the desired linear western perimeter conformation. The position of a *Z*-olefin adjacent to the **B** ring was also selected both to rigidify the linker by allylic strain and to simplify the synthesis.

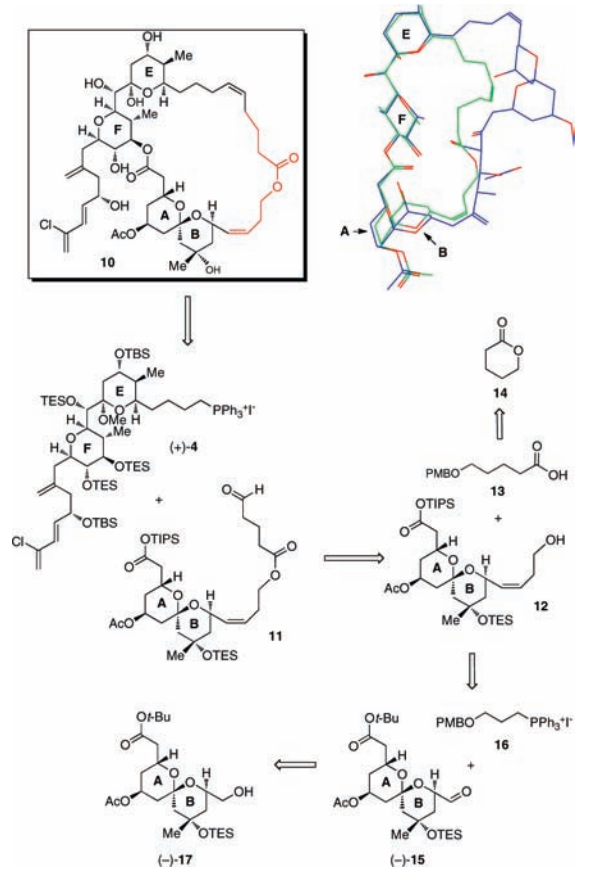


Figure 3. Retrosynthetic Analysis for **ABEF** analogue **10** and overlay with (+)-spongistatin 1.

Construction of the **ABEF** analogue (**10**) was again envisioned to take advantage of **EF** Wittig salt (+)-**4**, now with **AB** aldehyde **11** (Figure 3). The tether would be incorporated

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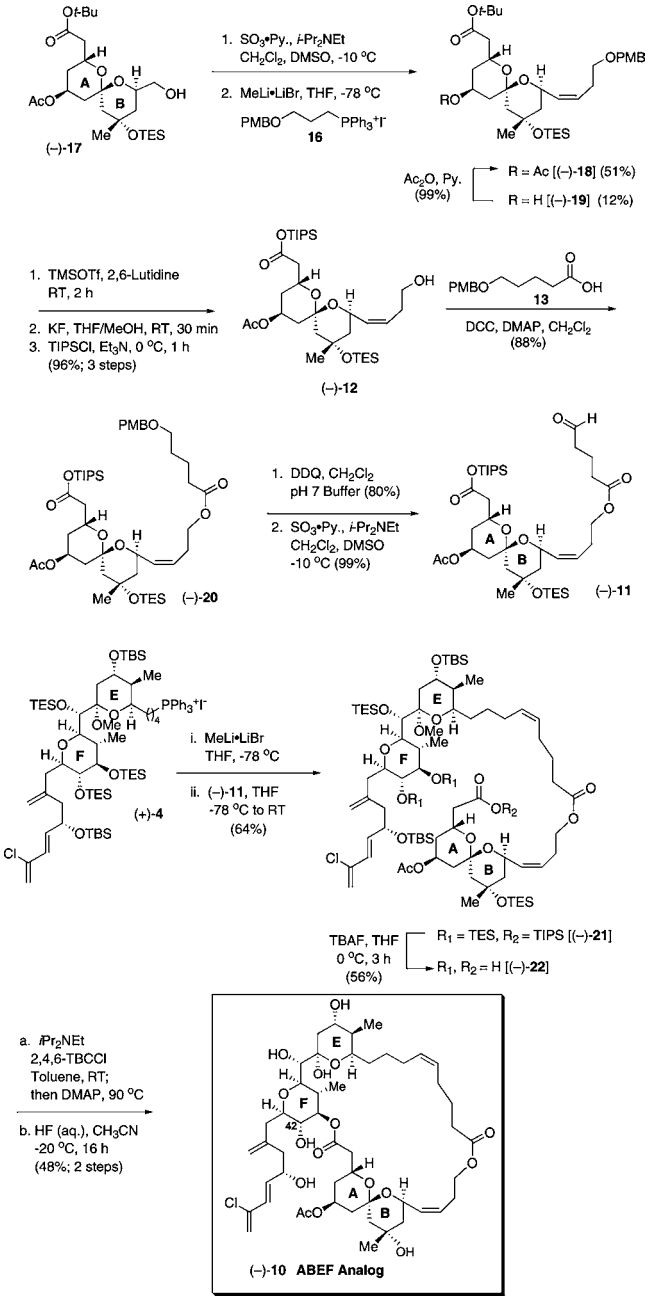
into **11** in two segments; first, a three-carbon unit would be introduced by Wittig olefination of known **AB** aldehyde (−)-**15**,[20] followed by deprotection and esterification of **12** with known acid **13**,[21] available from δ-valerolactone. Toward this end, Parikh−Doering oxidation[22] of alcohol (−)-**17** (Scheme 2), followed by olefination with Wittig salt **16**, produced (−)-

Scheme 2. Synthesis of **ABEF** Analogue (−)-**10**



18 in 51% yield, along with deacylated (−)-**19**, which was reacetylated to furnish an overall yield of 63%.

At this juncture, the *tert*-butyl ester was converted to a TIPS-ester, which was envisioned to be more compatible with our endgame strategy. A three-step sequence was required: (1) treatment with TMSOTf, in the presence of 2,6-lutidine, (2) exposure of the reaction mixture to KF, and (3) conversion to the TIPS-ester by treatment with TIPSCl and Et_3N provided alcohol (−)-**12** in 96% yield. For chain extension, alcohol (−)-**12** was esterified with carboxylic acid **13** (88%), the latter available in one step via hydrolysis of δ-valerolactone in the presence of PMBCl.[21b] Oxidative removal of the PMB ether from (−)-**12**, followed by Parikh−Doering oxidation,[22] then provided (−)-**11**.

With aldehyde (−)-**11** in hand, union with **EF** Wittig salt (+)-**4** furnished the full seco **ABEF** carbon skeleton, possessing exclusively the *Z*-olefin adjacent to the **AB** ring, which upon treatment with 3 equiv of TBAF, Yamaguchi macrocyclization of the derived *seco*-acid (−)-**22**, and global deprotection at low temperature afforded **ABEF** analogue (−)-**10**. The structure, and in particular the integrity of the **E** ring hemiketal, was confirmed by NMR spectroscopy. Importantly, assignment of the solution conformation of analogue (−)-**10**, defined exploiting NMR methods, and deconvolution tactics employed in our solution assignment of (+)-spongistatin 1 are in accordance with the initially calculated conformations.

Biological evaulation revealed that analogue (+)-**9** (Table 1), not surprisingly given the missing **E** ring hemiketal structural component, was inactive against all four cell lines tested. However, analogue (−)-**10**, wherein the **AB** spiroketal is retained but lacks the **CD** spiroketal, displayed nanomolar activities against the tested cancer cell lines. Studies demonstrating that the mode of action of (−)-**10** is similar to that of (+)-spongistatin 1 (**1**) will be published in due course.

Table 1. IC_{50} (nM) Values of Spongistatin 1 and Analogues in the Cell Growth Inhibition Assay with Different Cancer Cell Lines

	MDA-MB-435	HT-29	H522-T1	U937
(+)-spongistatin 1	0.0225	0.058	0.16	0.059
(+)-**9**	>1000	>1000	>1000	>1000
(−)-**10**	82.8	161.2	297.2	60.5

In summary, the design and synthesis of a (+)-spongistatin 1 **ABEF** analogue (−)-**10**, based on the assigned solution conformation of (+)-spongistatin 1 (**1**), possessing nanomolar cell growth inhibitory activity against several human tumor cell lines, has been achieved and as such provides circumstantial evidence for the assigned solution conformation of spongistatin 1 (**1**). Or as often stated by Ralph Hirschmann: "Let the receptor be the judge."

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Supporting Information Available: Spectroscopic and analytical data and selected experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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