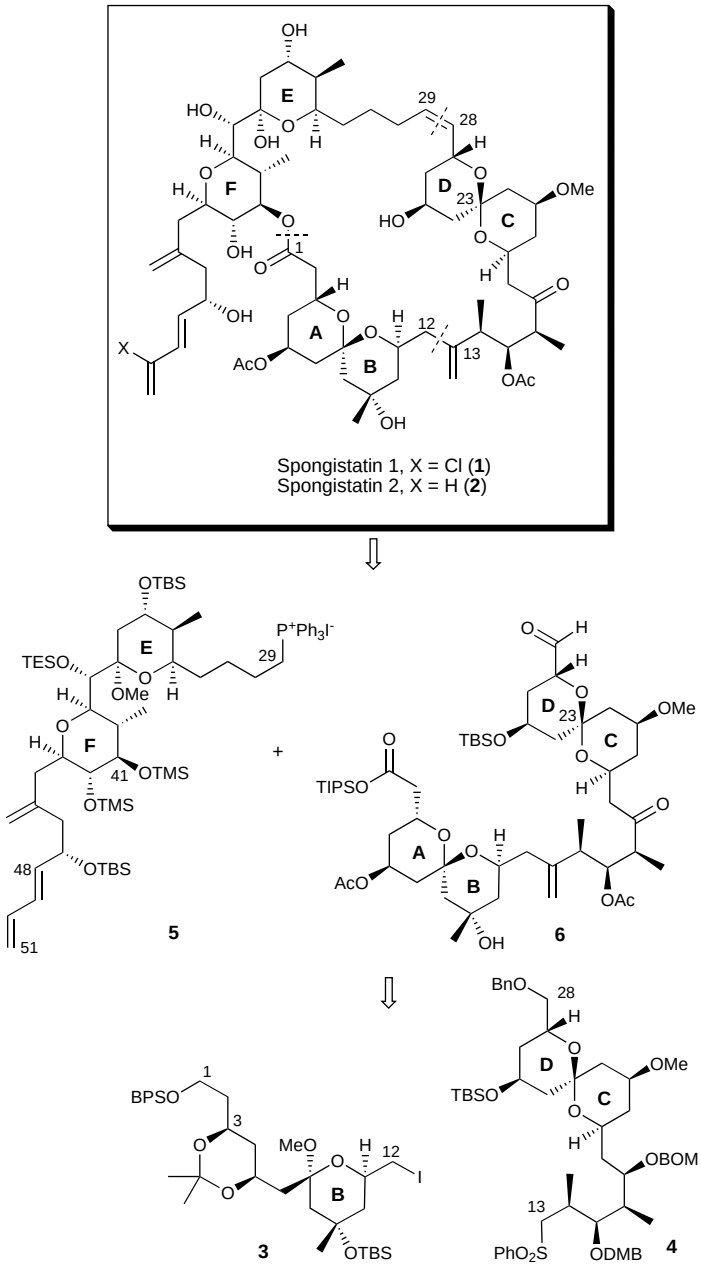
The Spongistatins: Architecturally Complex Natural Products—Part One: A Formal Synthesis of (+)-Spongistatin 1 by Construction of an Advanced ABCD Fragment**

Amos B. Smith III,* Victoria A. Doughty, Qiyan Lin, Linghang Zhuang, Mark D. McBriar, Armen M. Boldi, William H. Moser, Noriaki Murase, Kiyoshi Nakayama, and Masao Sobukawa

The spongipyrans comprise an important family of architecturally unique bis-spiroketal macrolides that display extraordinary (subnanomolar) antitumor activities against a wide variety of human cancer cell lines, including melanoma, lung, colon, and brain.[1] Three research groups (those of Pettit,[1a, 2] Fusetani,[3] and Kitagawa[4]) independently isolated members of the spongipyran family in minute amounts, naming them in turn the spongistatins, cinachyrolide, and the altohyrtins.[5] Whereas the carbon skeleton was common to all three families, the structures differed in the assignment of the relative stereochemical relationships. Kitagawa and coworkers assigned the correct relative and absolute stereochemistries based on extensive Mosher analysis and circular dichroism studies.[4c] These assignments were subsequently confirmed by the total syntheses of (+)-altohyrtin C [spongistatin 2 (**2**)] by Evans et al.[6] and (+)-altohyrtin A [spongistatin 1(**1**)] by Kishi and co-workers.[7] The elegant syntheses at Harvard University demonstrated unequivocally that the altohyrtins and spongistatins were indeed identical.

The spongistatins have attracted wide interest[8] as a result of their architectural complexity, which includes two [6,6] spiroketal moieties,[9] two highly substituted tetrahydropyran rings encased in a 42 membered macrolide framework, 24 stereogenic centers, and a delicate triene side chain. Particularly intriguing is the CD spiroketal possessing only a single anomeric interaction in conjunction with an intramolecular hydrogen bond that stabilizes the axial–equatorial configuration.

To assemble the complex spongipyran skeleton, we envisioned Wittig union of **5** with **6** (Scheme 1), followed by regioselective macrolactonization at C(41), first demonstrated by Evans et al.[6] Advanced intermediate **6** would in turn arise from a sulfone-mediated coupling of subunits **3** and **4** followed by Julia methylenation,[10] elaboration of the AB spiroketal, and oxidation at C(1) and C(28). Herein we report the synthesis of an advanced C(1–28) subunit for the spongistatins. Central to this venture was the development of epimerization conditions to control the C(23) configuration of the CD spiroketal. In conjunction with this effort we achieved a formal synthesis of spongistatin 1 (**1**). In the following communication we describe the synthesis of **5**, fragment assembly, and final elaboration to (+)-spongistatin 2 (**2**).



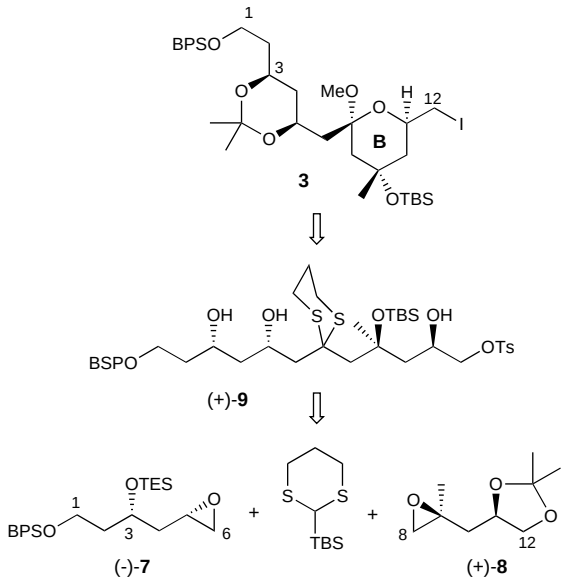
Scheme 1. Retrosynthetic analysis of spongistatin 2 (see ref. [20] for abbreviations).

Continuing with the synthetic analysis, disconnection of methylketal **3** (Scheme 2) revealed the opportunity to exploit a one-pot unsymmetrical bisalkylation of 2-TBS-1,3-dithiane

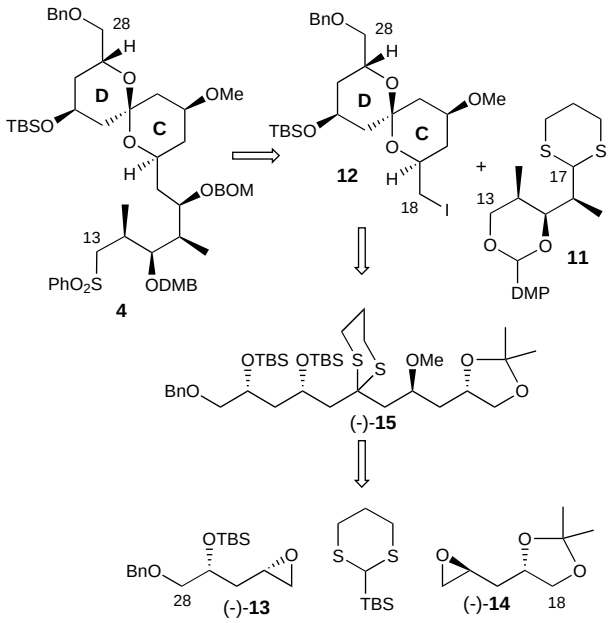
[*] Prof. A. B. Smith III, Dr. V. A. Doughty, Dr. Q. Lin, Dr. L. Zhuang, Dr. M. D. McBriar, A. M. Boldi, Dr. W. H. Moser, Dr. N. Murase, Dr. K. Nakayama, Dr. M. Sobukawa
Department of Chemistry
Laboratory for Research on the Structure of Matter
and Monell Chemical Senses Center, University of Pennsylvania
Philadelphia, PA 19104 (USA)
Fax: (+1) 215-898-5129
E-mail: smithab@sas.upenn.edu

[**] Financial support was provided by the National Institutes of Health (National Cancer Institute) through Grant CA-70329, NIH Postdoctoral Fellowships to A.M.B. and W.H.M., a Japan Society for Promotion of Science Fellowship to N.M., and a Royal Society Fulbright Fellowship to V.A.D. We also thank the Daiichi Pharmaceutical Co., Ltd, and the Tanabe Seiyaku Co., Ltd for financial support. Finally we thank Dr. George T. Furst, Dr. Patrick J. Carroll, Dr. Rakesh Kohli, and Mr John Dykins of the University of Pennsylvania Spectroscopic Service Center for assistance in securing and interpreting high-field NMR spectra, X-ray crystal structures, and mass spectra.

  0044-8249/01/11301-0197 $ 17.50+.50/0



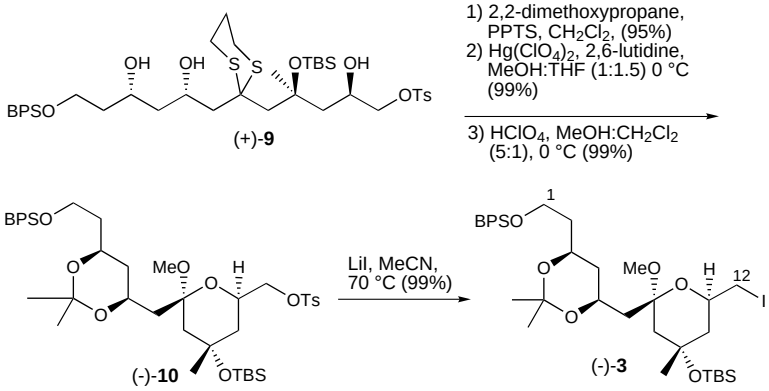
Scheme 2. Retrosynthetic analysis of the AB subunit revealing a one-pot unsymmetrical bis-alkylation disconnection (see ref. [20] for abbreviations).



Scheme 4. Retrosynthesis of the CD spiroketal **4** revealing a one-pot unsymmetrical bis-alkylation disconnection (see ref. [20] for abbreviations).

with Brook rearrangement recently reported by our laboratory.[11] The development of this route and the synthesis of fragments (−)-**7**, (+)-**8**, and (+)-**9** were disclosed earlier.[8b]

Completion of subunit (−)-**3**[12] required four steps beginning with (+)-**9**[8b] (Scheme 3). Protection of the 1,3-diol as the



Scheme 3. Synthesis of the AB subunit (−)-**3** (see ref. [20] for abbreviations).

acetonide, mercury perchlorate mediated removal of the dithiane[13] in the presence of methanol to furnish a mixture of methyl ketals, and acid-catalyzed equilibration led to the thermodynamically more stable ketal (−)-**10** (d.r. > 10:1). Treatment with lithium iodide completed the construction of the AB fragment (−)-**3**; the overall yield was 92% (four steps).

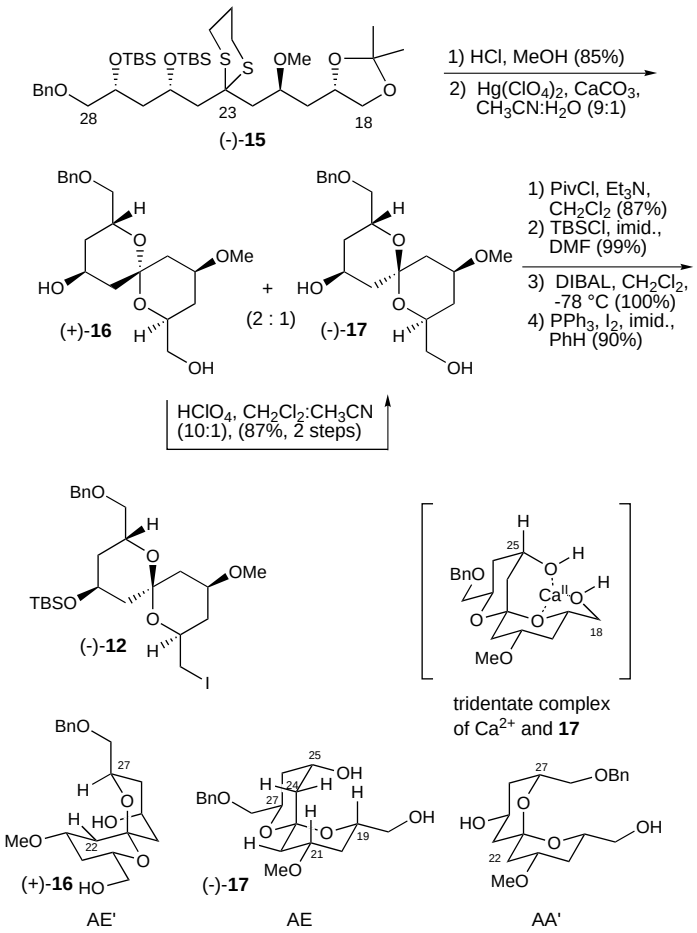
The CD fragment (**4**) was envisioned to arise through the coupling of dithiane **11** with iodide **12** (Scheme 4). Disconnection of **12** at the spiroketal provided another opportunity to employ the one-pot bis-alkylation tactic.[11] Indeed, the coupling of (−)-**13** and (−)-**14** with the lithium anion of 2-TBS-1,3-dithiane followed by methylation furnished (−)-**15** in 68% yield (two steps).[8m]

Assembly of the CD spiroketal required removal of both the TBS and acetonide groups (HCl/MeOH) in (−)-**15** (Scheme 5), followed by treatment with mercury perchlorate buffered with calcium carbonate; the result was a mixture of spiroketals (+)-**16** and (−)-**17** (2:1). After separation, NOE experiments revealed that both spiroketals possessed an "axial–equatorial (AE)" configuration;[14] the "axial–axial (AA′)" congener was not observed. Importantly, treatment of the mixture with perchloric acid prior to purification furnished only the desired spiroketal (−)-**17** (87%, two steps). Subsequent experiments[15] demonstrated that the calcium ion, a remnant of dithiane removal, stabilizes spiroketal (−)-**17**, presumably through coordination with the hydroxyl groups at C(18) and C(25) as well as the C-ring pyran oxygen atom. This observation would prove critical late in the synthesis (see below). Conversion of (−)-**17** into iodide (−)-**12** was then achieved in four steps: pivaloation of the primary hydroxyl group, protection of the secondary hydroxyl group (TBSCl), reductive removal of the pivaloate (DIBAL), and conversion of the primary hydroxyl group into the iodide completed the synthesis of (−)-**12**.

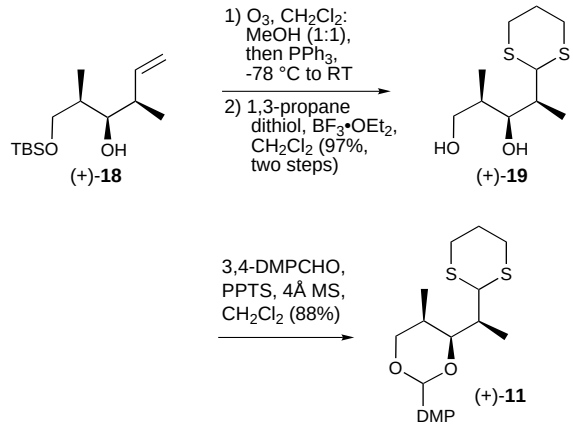
Construction of dithiane (+)-**11** (Scheme 6) required three steps, beginning with (+)-**18**;[8b] ozonolysis, introduction of the dithiane with concomitant loss of the TBS group, and protection of the diol (+)-**19** as the DMP acetal furnished (+)-**11** in 85% yield (three steps).

The coupling of (+)-**11** and (−)-**12** proceeded with high efficiency (95%) to furnish (+)-**20** (Scheme 7). Removal of the dithiane followed by reduction of the resultant ketone ($NaBH_4$) then led to a mixture of alcohols (3.5:1; 97% yield), favoring the C(17) β-epimer **21**. After protection of the

  0044-8249/01/11301-0198 $ 17.50+.50/0



Scheme 5. Synthesis of the CD spiroketal iodide (−)**12** (see ref. [20] for abbreviations).

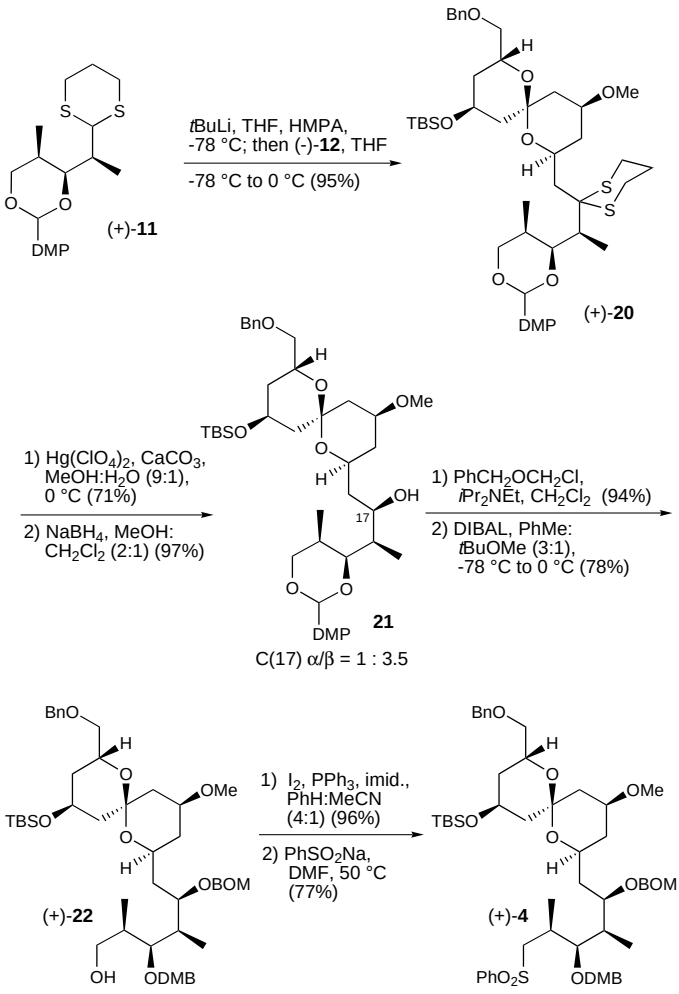


Scheme 6. Synthesis of dithiane (+)-**11** (see ref. [20] for abbreviations).

secondary hydroxyl group (BOMCl), the mixture was separated, and although both epimers were potentially useful, only the major β-epimer was carried forward. Regioselective reduction (DIBAL) of the acetal led to (+)-**22**. The weakly coordinating co-solvent, *tert*-butylmethyl ether, proved essential to minimize reduction of the spiroketal. Conversion of (+)-**22** into the iodide and subsequent introduction of the phenyl sulfone completed the assembly of the CD fragment (+)-**4**.

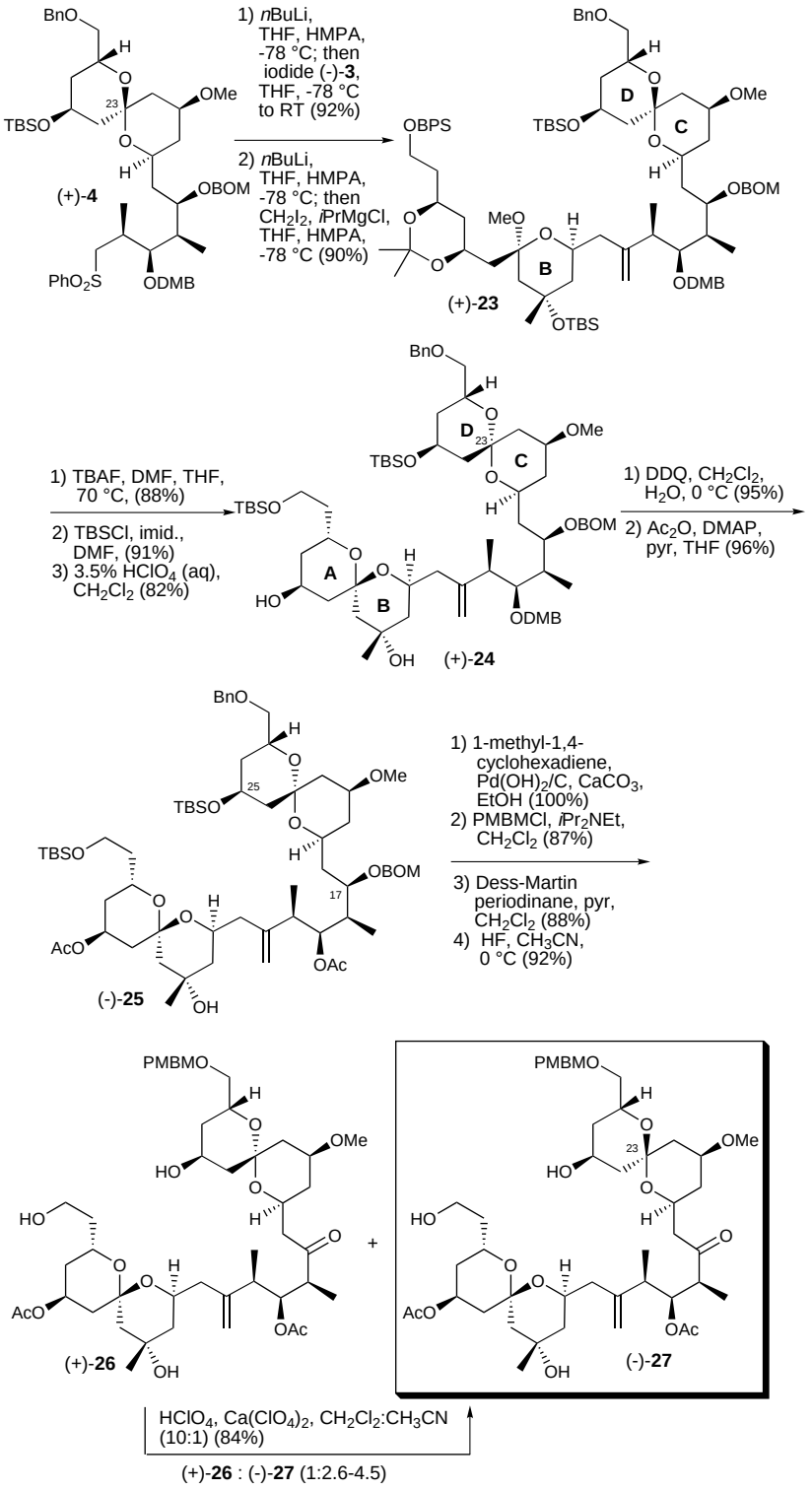
The coupling of (+)-**4** with iodide (−)-**3** also proceeded in excellent yield to furnish an inconsequential mixture of sulfone epimers (Scheme 8). In contrast to a system explored earlier, which possessed a fully elaborated AB spiroketal,[16] Julia methylenation[10] in this case proceeded smoothly to furnish (+)-**23** (90%). Removal of the silicon protecting groups with TBAF, followed in turn by reprotection of both the primary and secondary hydroxyl groups as TBS ethers and hydrolysis of the acetonide with concomitant spiroketalization then furnished spiroketal (+)-**24**. Although not surprising in the retrospect that epimerization of the CD spiroketal had occurred under the acidic conditions, this fact went undetected until the spectroscopic properties of the fully elaborated 23-*epi*-spongistatin was compared with those of an authentic sample of (+)-spongistatin 2 (**1**). Undaunted, we reasoned that it should be possible to correct the CD spiroketal stereochemistry by epimerization (for example, with $HClO_4$/Ca^{2+}) at a late stage in the synthesis.

To confirm rigorously the stereochemistry of (+)-**24**, we prepared (−)-**27**, an advanced intermediate employed in the total synthesis of (+)-spongistatin 1 (**1**) by Kishi and co-workers.[7] Toward this end, oxidative removal of the DMB ether (DDQ), followed by bis-acylation of the triol gave diacetate (−)-**25**. Removal of the C(25) TBS group (HF/CH_3CN) set



Scheme 7. Synthesis of sulfone (+)-**4** (see ref. [20] for abbreviations).

  0044-8249/01/11301-0199 $ 17.50+.50/0



Scheme 8. C(23) Epimerization attempts: Completion of a formal synthesis of (+)-spongistatin 1 (**1**) (see ref. [20] for abbreviations).

the stage for spiroketal correction. Unfortunately, all attempts to effect epimerization at C(23) employing our previously optimized conditions failed. We reasoned that the bulky C(17) BOM group precluded epimerization. Accordingly, the BOM and benzyl groups were removed (transfer hydrogenolysis). Selective protection of the primary hydroxyl group with PMBMCl, oxidation of the secondary hydroxyl group, and treatment with HF/CH_3CN then afforded the desired spiroketal (−)-**27** along with the epimer (+)-**26** (ca. 1:1.3) in 92% yield. Importantly, epimerization of (+)-**26** ($HClO_4/Ca^{2+}$) proceeded consistently with high mass recovery (ca. 80–84%), although the ratio of spiroketals was variable (2.6–4.5:1). In this fashion (−)-**27** could be obtained in approximately 75% yield. Treatment of (+)-**26** with only $HClO_4$ established the critical requirement for a calcium ion; both (+)-**26** and (−)-**27** (6.1:1, 81%) were obtained. The synthesis of (−)-**27**, which was identical to that prepared by Kishi and coworkers, constitutes both a formal total synthesis of (+)-spongistatin 1 (**1**) and confirms our stereochemical assignments.[7, 17]

Continuing with the synthesis of the advanced ABCD fragment (+)-**29** (Scheme 9) for spongistatin 2 (**2**; see accompanying communication), protection of the tertiary hydroxyl group as a TES ether[18] was followed by selective removal of the primary TBS group in the presence of both the secondary TBS and tertiary TES ethers. A two-step oxidation (Dess–Martin periodinane[19] and buffered $NaClO_2$), followed by silylation of the resulting carboxylic acid led to (−)-**28** (67%, five steps). Removal of the benzyl and benzyloxymethyl groups in the presence of the exo-methylene group (transfer hydrogenolysis) then proceeded smoothly. Finally, Dess–Martin oxidation furnished aldehyde (+)-**29**. In the following communication we describe the synthesis of the C(29–51) subunit (**5**), its coupling with (+)-**29**, and final elaboration to afford (+)-spongistatin 2 (**2**).

Received: October 12, 2000 [Z15947]

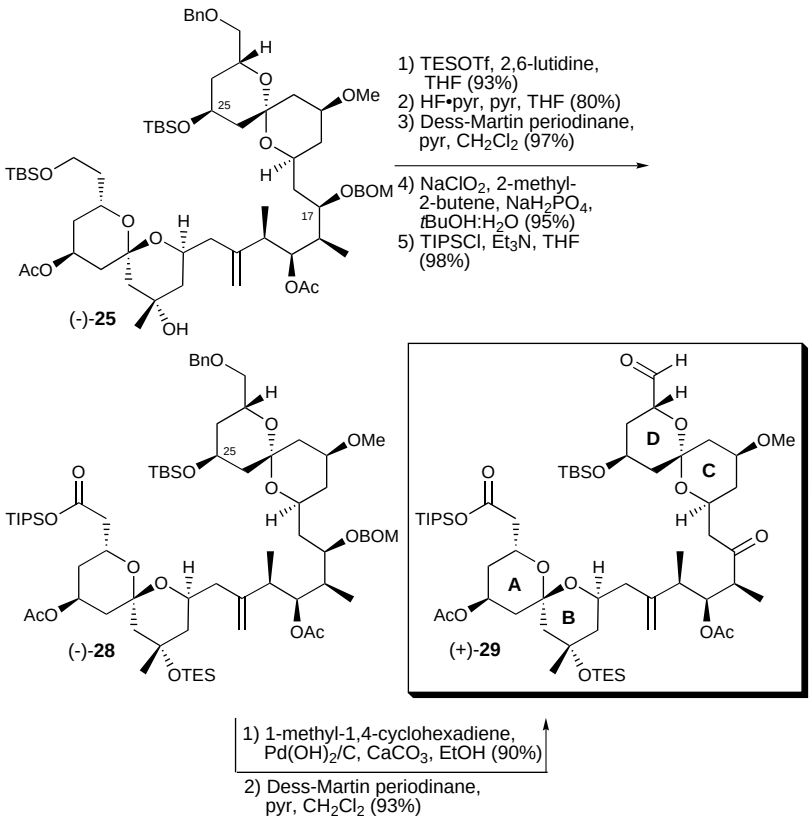
[1] a) G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt, J. N. A. Hooper, *J. Org. Chem.* **1993**, *58*, 1302; b) G. R. Pettit, *Pure Appl. Chem.* **1994**, *66*, 2271; c) R. Bai, G. F. Taylor, Z. A. Cichacz, C. L. Herald, J. A. Kepler, G. R. Pettit, E. Hamel, *Biochemistry* **1995**, *34*, 9714.

[2] a) G. R. Pettit, R. Tan, F. Gao, M. D. Williams, D. L. Doubek, M. R. Boyd, J. M. Schmidt, J.-C. Chapuis, E. Hamel, R. Bai, J. N. A. Hooper, L. P. Tackett, *J. Org. Chem.* **1993**, *58*, 2538; b) G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, *J. Chem. Soc. Chem. Commun.* **1993**, 1166; c) G. R. Pettit, C. L. Herald, Z. A. Cichacz, F. Gao, J. M. Schmidt, M. R. Boyd, N. D. Christie, F. E. Boettner, *J. Chem. Soc. Chem. Commun*. **1993**, 1805; d) G. R. Pettit, C. L. Herald, Z. A. Cichacz, F. Gao, M. R. Boyd, N. D. Christie, J. M. Schmidt, *Nat. Prod. Lett.* **1993**, *3*, 239; e) G. R. Pettit, Z. A. Cichacz, C. L. Herald, F. Gao, M. R. Boyd, J. M. Schmidt, E. Hamel, R. Bai, *J. Chem. Soc. Chem. Commun.* **1994**, 1605.

[3] N. Fusetani, K. Shinoda, S. Matsunaga, *J. Am. Chem. Soc.* **1993**, *115*, 3977.

[4] a) M. Kobayashi, S. Aoki, H. Sakai, K. Kawazoe, N. Kihara, T. Sasaki, I. Kitagawa, *Tetrahedron Lett.* **1993**, *34*, 2795; b) M. Kobayashi, S. Aoki, I. Kitagawa, *Tetrahedron Lett.* **1994**, *35*, 1243; c) M. Kobayashi, S. Aoki, H. Sakai, N. Kihara, T. Sasaki, I. Kitagawa, *Chem. Pharm.*

  0044-8249/01/11301-0200 $ 17.50+.50/0



Scheme 9. Synthesis of (+)-**29** (see ref. [20] for abbreviations).

Bull. **1993**, *41*, 989; d) M. Kobayashi, S. Aoki, K. Gato, I. Kitagawa, *Chem. Pharm. Bull.* **1996**, *44*, 2142.

[5] Since Pettit and co-workers were the first to isolate members of the spongipyran family, we have adopted the spongistatin nomenclature.

[6] a) D. A. Evans, P. J. Coleman, L. C. Dias, *Angew. Chem.* **1997**, *109*, 2951; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2738; b) D. A. Evans, B. W. Trotter, B. Côté, P. J. Coleman, *Angew. Chem.* **1997**, *109*, 2954; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2741; c) D. A. Evans, B. W. Trotter, B. Côté, P. J. Coleman, L. C. Dias, A. N. Tyler, *Angew. Chem.* **1997**, *109*, 2957; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2744; d) D. A. Evans, B. W. Trotter, P. J. Coleman, B. Côté, L. C. Dias, H. A. Rajapakse, A. N. Tyler, *Tetrahedron* **1999**, *55*, 8671.

[7] a) J. Guo, K. J. Duffy, K. L. Stevens, P. I. Dalko, R. M. Roth, M. M. Hayward, Y. Kishi, *Angew. Chem.* **1998**, *110*, 198; *Angew. Chem. Int. Ed.* **1998**, *37*, 187; b) M. M. Hayward, R. M. Roth, K. J. Duffy, P. I. Dalko, K. L. Stevens, J. Guo, Y. Kishi, *Angew. Chem.* **1998**, *110*, 202; *Angew. Chem. Int. Ed.* **1998**, *37*, 192.

[8] For a discussion of synthetic studies towards the spongistatins/altohyrtins/cinachyrolide and their biology, see a) J. Pietruszka, *Angew. Chem.* **1998**, *110*, 2773; *Angew. Chem. Int. Ed.* **1998**, *37*, 2629; for synthetic approaches to the AB spiroketal, see b) A. B. Smith III, Q. Lin, K. Nakayama, A. M. Boldi, C. S. Brook, M. D. McBriar, W. H. Moser, M. Sobukawa, L. Zhuang, *Tetrahedron Lett.* **1997**, *38*, 8675; c) M. M. Claffey, C. H. Heathcock, *J. Org. Chem.* **1996**, *61*, 7646; d) M. M. Claffey, C. J. Hayes, C. H. Heathcock, *J. Org. Chem.* **1999**, *64*, 8267; e) I. Paterson, R. M. Oballa, R. D. Norcross, *Tetrahedron Lett.* **1996**, *37*, 8581; f) I. Paterson, R. M. Oballa, *Tetrahedron Lett.* **1997**, *38*, 8241; g) I. Paterson, D. J. Wallace, R. M. Oballa, *Tetrahedron Lett.* **1998**, *39*, 8545; h) L. A. Paquette, D. Zuev, *Tetrahedron Lett.* **1997**, *38*, 5115; i) D. Zuev, L. A. Paquette, *Org. Lett.* **2000**, *2*, 679; j) M. T. Crimmins, D. G. Washburn, *Tetrahedron Lett.* **1998**, *39*, 7487; k) T. Terauchi, M. Nakata, *Tetrahedron Lett.* **1998**, *39*, 3795; l) A. G. M. Barrett, D. C. Braddock, P. D. de Koning, A. J. P. White, D. J. Williams, *J. Org. Chem.* **2000**, *65*, 375; for synthetic approaches to the CD spiroketal, see m) A. B. Smith III, L. Zhuang, C. S. Brook, Q. Lin, W. H. Moser, R. E. L. Trout, A. M. Boldi, *Tetrahedron Lett.* **1997**, *38*, 8671; n) C. J. Hayes, C. H. Heathcock, *J. Org. Chem.* **1997**, *62*, 2678; o) L. A. Paquette, A. Braun, *Tetrahedron Lett.* **1997**, *38*, 5119; p) I. Paterson, D. J. Wallace, K. R. Gibson, *Tetrahedron Lett.* **1997**, *38*, 8911; q) R. Zemribo, K. T. Mead, *Tetrahedron Lett.* **1998**, *39*, 3895; r) M. T. Crimmins, J. D. Katz, *Org. Lett.* **2000**, *2*, 957; s) T. Terauchi, T. Terauchi, I. Sato, T. Tsukada, N. Kanoh, M. Nakata, *Tetrahedron Lett.* **2000**, *41*, 2649; for synthetic approaches to the E and F rings, see t) A. B. Smith III, L. Zhuang, C. S. Brook, A. M. Boldi, M. D. McBriar, W. H. Moser, N. Murase, K. Nakayama, P. R. Verhoest, Q. Lin, *Tetrahedron Lett.* **1997**, *38*, 8667; u) S. A. Hermitage, S. M. Roberts, D. J. Watson, *Tetrahedron Lett.* **1998**, *39*, 3567; v) P. D. Kary, S. M. Roberts, D. J. Watson, *Tetrahedron: Asymmmetry* **1999**, *10*, 213; w) I. Paterson, L. E. Keown, *Tetrahedron Lett.* **1997**, *38*, 5727; x) G. C. Micalizio, W. R. Roush, *Tetrahedron Lett.* **1999**, *40*, 3351; y) E. Fernandez-Megia, N. Gourlaouen, S. V. Ley, G. J. Rowlands, *Synlett* **1998**, 991; z) S. Lemaire-Audoire, P. Vogel, *Tetrahedron Lett.* **1998**, *39*, 1345; aa) S. Lemaire-Audoire, P. Vogel, *J. Org. Chem.* **2000**, *65*, 3346; ab) R. Dunkel, J. Treu, H. M. R. Hoffmann, *Tetrahedron: Asymmetry* **1999**, *10*, 1539; ac) H. Kim, H. M. R. Hoffmann, *Eur. J. Org. Chem.* **2000**, 2195; ad) R. Dunkel, H. M. R. Hoffmann, *Tetrahedron* **1999**, *55*, 8385; ae) J. C. Anderson, B. P. McDermott, *Tetrahedron Lett.* **1999**, *40*, 7135; af) M. Samadi, C. Munoz-Letelier, S. Poigny, M. Guyot, *Tetrahedron Lett.* **2000**, *41*, 3349; ag) G. A. Wallace, R. W. Scott, C. H. Heathcock, *J. Org. Chem.* **2000**, *65*, 4145; ah) D. A. Evans, B. W. Trotter, B. Côté, *Tetrahedron Lett.* **1998**, *39*, 1709.

[9] For a review of spiroketals, see F. Perron, K. F. Albizati, *Chem. Rev.* **1989**, *89*, 1617.

[10] C. De Lima, M. Julia, J.-N. Verpeaux, *Synlett* **1992**, 133.

[11] A. B. Smith III, A. M. Boldi, *J. Am. Chem. Soc.* **1997**, *119*, 6925.

[12] Satisfactory spectroscopic data (^1H and ^{13}C NMR, LR-MS, HR-MS, $[\alpha]$, and IR) were obtained for all new compounds.

[13] E. Fujita, Y. Nagao, K. Kaneko, *Chem. Pharm. Bull.* **1978**, *26*, 3743.

[14] Nuclear Overhauser experiments (NOE) studies were carried out after selective BPS protection of the primary hydroxyl group (BPSCl, imid., DMF) in both **16** and **17** (80 and 83%, respectively). For the spiroketal derived from **17**, diagnostic NOE observations were seen between H(19a) to H(24e), H(19a) to OH (C25), H(21a) to H(24e), and H(22e) to H(24a). For the spiroketal derived from **16**, a diagnostic NOE was seen between H(22e) and H(27a), which demonstrates that an axial–equatorial rather than an axial–axial configuration exists.

[15] Treatment of a purified mixture of (+)-**16** and (−)-**17** (2:1) with $HClO_4$ afforded a 1:1 mixture of spiroketals. Addition of $Ca(ClO_4)_2$ to the reaction provided a 9:1 mixture favoring the desired spiroketal (−)-**17**.

[16] See ref. [8b]. With the fully formed spiroketal in place, only modest yields for the installation of the exo-methylene group could be obtained.

[17] We thank Prof. Y. Kishi for providing the ^1H NMR spectra of advanced intermediates **26** and **27**.

[18] Initially the corresponding compound with TBS protection on the tertiary hydroxyl group was carried through to the end of the synthesis. However, the TBS group could not be removed.

[19] D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 7277.

[20] Abbreviations used: PG = protecting group; Ac = acetyl; TES = triethylsilyl; TBS = *tert*-butyldimethylsilyl; TMS = trimethylsilyl; BPS = *tert*-butylbiphenylsilyl; TIPS = triisopropylsilyl; DMB = 3,4-dimethoxybenzyl; Bn = benzyl; BOM = benzyloxymethyl; Ts = tosyl = toluene-4-sulfonyl; DMP = dimethoxyphenyl; Piv = pivaloyl = 2,2-dimethylpropanoyl; PPTS = pyridinium *p*-toluenesulfonate; DIBAL = diisobutylaluminum hydride; imid. = imidazole; MS = molecular sieves; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMAP = 4-dimethylaminopyridine; pyr = pyridine; HMPA = hexamethylphosphoramide; DMF = dimethylformamide; PMBM = *para*-methoxybenzyloxymethyl; TBAF = tetrabutylammonium fluoride; THF = tetrahydrofuran.