Total Synthesis of Fostriecin (CI-920)

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Abstract: The first total synthesis of the potent antitumor agent fostriecin (CI-920) is described, confirming the relative and absolute stereochemistry assignments. Fostriecin is a unique phosphate monoester which exhibits weak topoisomerase II inhibition (IC_{50} = 40 μM) and more potent and selective protein phosphatase 2A and 4 (PP2A and PP4) inhibition (IC_{50} = 40−3 nM and 1.5 nM), resulting in mitotic entry checkpoint inhibition. Phase I clinical trials with fostriecin, which were the first to explore the potential of this novel mechanism of action, were halted even before therapeutic concentrations were reached or dose-limiting toxicity established due to problems of drug stability observed during storage of naturally derived material. The synthesis of fostriecin detailed herein is the first stage of efforts that may serve to address these limitations to the clinical examination of this or related promising new antitumor agents.

Fostriecin (**1**, CI-920, Figure 1)[1] is a structurally novel phosphate ester produced by *Streptomyces pulveraceus* that is active in vitro against leukemia (L1210, IC_{50} 0.46 μM), lung cancer, breast cancer, and ovarian cancer, and which exhibits efficacious in vivo antitumor activity.[2] It has been investigated in a Phase I clinical trial at the National Cancer Institute that was halted even before dose-limiting toxicities or therapeutic plasma levels were reached when concerns regarding drug purity and storage stability proved problematic with the naturally occurring material.[3] Although fostriecin inhibits DNA topoisomerase II (IC_{50} = 40 μM)[4] through a novel, non-DNA-strand cleavage mechanism, this activity is weak, and it does not induce G_2 arrest like other topoisomerase II inhibitors. Thus, it is unlikely that this initially suggested target is responsible for the antitumor activity of **1**.[5] Instead, fostriecin has been shown to inhibit the mitotic entry checkpoint through more potent and selective inhibition of protein phosphatases 1 (PP1), 2A (PP2A), and 4 (PP4) (IC_{50} = 45 μM, 1.5 nM, and 3.0 nM, respectively).[6−13] Notable in this regard is the observation that fostriecin

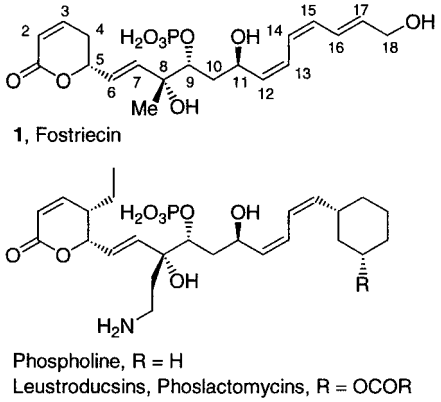

Figure 1.

is the most selective (PP2A/PP4 versus PP1 selectivity = $10^4\times$) protein phosphatase inhibitor known to date. Inhibition of the

(1) Tunac, J. B.; Graham, B. D.; Dobson, W. E. *J. Antibiot.* **1983**, *36*, 1595−1600. Stampwala, S. S.; Bunge, R. H.; Hurley, T. R.; Willmer, N. E.; Brankiewicz, A. J.; Steinman, C. E.; Smitka, T. A.; French, J. C. *J. Antibiot.* **1983**, *36*, 1601−1605.

(2) Reviews: Jackson, R. C.; Fry, D. W.; Boritzki, T. J.; Roberts, B. J.; Hook, K. E.; Leopold, W. R. *Adv. Enzyme Regul.* **1985**, *23*, 193−215. De Jong, R. S.; De Vries, E. G. E.; Mulder, N. H. *Anti-Cancer Drugs* **1997**, *8*, 413−418.

(3) De Jong, R. S.; Mulder, N. H.; Uges, D. R. A.; Sleijfer, D. Th.; Hoppener, F. J. P.; Groen, H. J. M.; Willemse, P. H. B.; Van der Graaf, W. T. A.; De Vries, E. G. E. *Br. J. Cancer* **1999**, *79*, 882−887.

(4) Boritzki, T. J.; Wolfard, T. S.; Besserer, J. A.; Jackson, R. C.; Fry, D. W. *Biochem. Pharmacol.* **1988**, *37*, 4063−4068.

(5) Topoisomerase II inhibitors which induce G_2 arrest are described in the following references. (a) Etoposide: Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560−13566. (b) Doxorubicin: Tewey, K. M.; Rowe, T. C.; Yang, L.; Halligan, B. D.; Liu, L. F. *Science* **1984**, *266*, 466−468. (c) Amsacrine: Nelson, E. M.; Tewey, K. M.; Liu, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1361−1365.

(6) Cheng, A.; Balczon, R.; Zuo, Z.; Koons, J. S.; Walsh, A. H.; Honkanen, R. E. *Cancer Res.* **1998**, *58*, 3611−3619.

(7) Roberge, M.; Tudan, C.; Hung, S. M. F.; Harder, K. W.; Jirik, F. R.; Anderson, H. *Cancer Res.* **1994**, *54*, 6115−6121.

(8) Walsh, A. H.; Cheng, A.; Honkanen, R. E. *FEBS Lett.* **1997**, *416*, 230−234.

(9) Hastie, C. J.; Cohen, P. T. W. *FEBS Lett.* **1998**, *431*, 357−361.

(10) Ho, D. T.; Roberge, M. *Carcinogenesis* **1996**, *17*, 967−972.

(11) Guo, X. W.; Th'ng, J. P. H.; Swank, R. A.; Anderson, H. J.; Tudan, C.; Bradbury, E. M.; Roberge, M. *EMBO J.* **1995**, *14*, 976−85.

(12) For reviews of protein serine/threonine phosphatases, see: Ingrebritsen, T. S.; Cohen, P. *Eur. J. Biochem.* **1983**, *132*, 255−261. Cohen, P. *Annu. Rev. Biochem.* **1989**, *58*, 453−508.

(13) Other inhibitors of protein serine/threonine phosphatases are described in the following references. (a) Okadaic acid: Tachibana, K.; Scheuer, P. J.; Tsukitani, Y.; Kikuchi, Y.; Van Engen, D.; Clardy, J.; Gopichand, Y.; Schmitz, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 2469−2471. (b) Microcystins: Botes, D. P.; Tuinman, A. A.; Wessels, P. L.; Viljoen, C. C.; Kruger, H.; Williams, D. H.; Santikarn, S.; Smith, R. J.; Hammond, S. J. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2311−2318. (c) Calyculin A: Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780−2781. (d) Nodularin: Botes, D. P.; Wessels, P. L.; Kruger, H.; Runnegar, M. T. C.; Santikarn, S.; Smith, R. J.; Barna, J. C. J.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2747−2748. Rinehart, K. L.; Harada, K.; Namikoshi, M.; Chen, C.; Harvis, C. A.; Munro, M. H. G.; Blunt, J. W.; Mulligan, P. E.; Beasley, V. R.; Dahlem, A.; Carmichael, W. *J. Am. Chem. Soc.* **1988**, *110*, 8557−8558. (e) Tautomycin: Cheng, X.-C.; Ubukata, M.; Isono, K. *J. Antibiot.* **1990**, *43*, 809−819. (f) Cantharidine: Li, Y.-M.; Casida, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11867−11870. (g) Motuporin: Valentekovich, R. J.; Schreiber, S. L. *J.Am. Chem. Soc.* **1995**, *117*, 9069−9070. (h) Review: Sheppeck, J. E., II; Gauss, C.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1739−1750.

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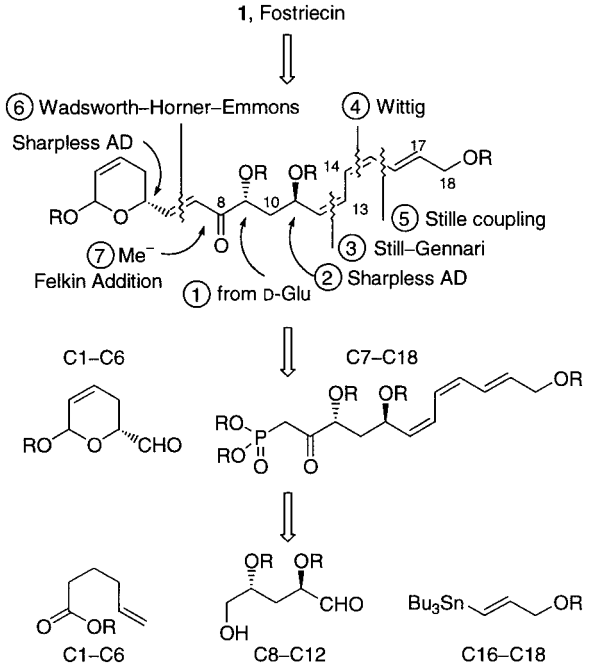
mitotic entry checkpoint and selective protein phosphatase inhibition are novel, clinically unexplored mechanisms worthy of pursuit for introduction of a new class of antitumor agents. Also contributing to its selective antitumor properties, fostriecin was shown to be transported into tumor cells via the reduced folate carrier system.[14] More recently, this selective and potent inhibition of PP2A by fostriecin has also been shown to limit myocardial infarct size and to protect cardiomyocytes during ischemia.[15−17]

At the time the significance of the biological and therapeutic effects of fostriecin was first emerging, only the drug's two-dimensional structure had been established,[18] and its relative and absolute stereochemistry was unknown. In initial efforts to address these limitations to the clinical potential of fostriecin, we first established its relative and absolute stereochemistry.[19,20] This work also confirmed stereochemical assignments[21] for a family of structurally related natural products that include the leustroducsins,[22] phoslactomycins,[23] phosphazomycins,[23] and phospholine (Figure 1).[23] Recently, phoslactomycin F was also found to be a selective, albeit substantially less potent, inhibitor of PP2A ($IC_{50} = 4.7\ \mu M$) versus PP1 ($IC_{50} > 1000\ \mu M$),[24] and these similarities (PP2A ≫ PP1) and distinctions (1000-fold less potent) with fostriecin reveal important structural features contributing to PP2A inhibition. However, the biological properties of these related natural products typically differ from those of fostriecin. In addition to broad-spectrum antifungal activities, the leustroducsins and phoslactomycins also induce production of macrophage colony-stimulating factors by bone marrow stromal cells contributing to the recovery of blood leucocytes.

Herein, we report the first total synthesis of fostriecin that not only serves to confirm the structural and stereochemical assignments (5*R*,8*R*,9*R*,11*R*) of **1** but also possesses the potential of addressing the issues of chemical purity and storage stability limiting clinical trials on the natural product. In this regard, the efforts are complementary to the early and only other synthetic efforts disclosed to date on **1** by Just and O'Connor,[20] which culminated in the preparation of an unnatural 5*R*,8*R*,9*S*,11*R* diastereomer of dephosphorylated fostriecin. In addition, the route detailed herein provides access to structural analogues not accessible by degradation or functionalization of the natural product itself and is sufficiently general as to be adaptable to the leustroducsins, phoslactomycins, and phospholine, for which no synthetic efforts have yet been disclosed. Key elements of the approach include the late-stage deprotection and sequential introduction of the C-terminus lactone and C9 phosphate, a Wadsworth−Horner−Emmons installation of the C6−7 trans double bond, and stepwise assemblage of the sensitive *Z*,*Z*,*E*-triene (Scheme 1). The relative and absolute stereochemistry of the C9 center was set by utilizing an optically active starting material (D-Glu), the C5 and C11 stereocenters were installed via Sharpless AD reactions, and the labile C8 center was installed enlisting an α-$OSiR_3$ enforced Felkin−Anh versus chelation-controlled nucleophilic addition. Throughout the synthesis, the sensitivity of the conjugated *Z*,*Z*,*E*-triene and the three allylic alcohols limited reagent selections and dictated attentive handling during isolation or purification. By design, the tertiary C8 allylic alcohol was introduced late in the synthesis.

Scheme 1



Synthesis of the C1−C6 Subunit of Fostriecin. The precursor to the fostriecin lactone was derived from **2**, which was prepared in four steps from *p*-methoxybenzyl 5-hexenoate enlisting a Sharpless AD reaction as described in our earlier structural assignment work on **1**.[19] Thus, oxidation of **2** to the corresponding selenoxide (H_2O_2) and in situ elimination (85%), Dibal-H reduction of the resulting unsaturated lactone **3**, followed by protection of the lactol provided **4**[25] (90%, two steps), Scheme 2. Deprotection of the TBDPS ether (Bu_4NF, 91%) and oxidation of the resulting alcohol **5**[25] with TPAP/NMO provided the aldehyde **6**[25] (90%). Embodied in this key

(14) Fry, D. W.; Besserer, J. A.; Boritzki, T. J. *Cancer Res.* **1984**, *44*, 3366−3370.

(15) Armstrong, S. C.; Kao, R.; Gao, W.; Shivell, L. C.; Downey, J. M.; Honkanen, R. E.; Ganote, C. E. *J. Mol. Cell. Cardiol.* **1997**, *29*, 3009−3024.

(16) Armstrong, S. C.; Gao, W.; Lane, J. R.; Ganote, C. E. *J. Mol. Cell. Cardiol.* **1998**, *30*, 61−73.

(17) Weinbrenner, C.; Baines, C. P.; Liu, G.-S.; Armstrong, S. C.; Ganote, C. E.; Walsh, A. H.; Honkanen, R. E.; Cohen, M. V.; Downey, J. M. *Circulation* **1998**, *98*, 899−905.

(18) Early work assigned the absolute configuration at C5 as *R*, see: Hokanson, G. C.; French, J. C. *J. Org. Chem.* **1985**, *50*, 462−466.

(19) Boger, D. L.; Hikota, M.; Lewis, B. M. *J. Org. Chem.* **1997**, *62*, 1748−1753.

(20) Just, G.; O'Connor, B. *Tetrahedron Lett.* **1988**, *29*, 753−756.

(21) The absolute stereochemistry of the leustroducsins, phoslactomycins, and phospholine was tentatively assigned on the basis of the chiroptical properties of Mosher esters of the alcohols. Shibata, T.; Kurihara, S.; Yoda, K.; Haruyama, H. *Tetrahedron* **1995**, *51*, 11999−12012.

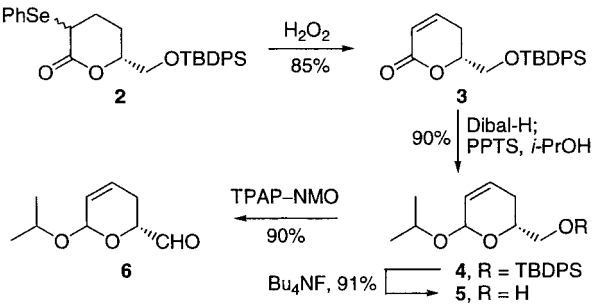
(22) Leustroducsins: Kohama, T.; Enokita, R.; Okazaki, T.; Miyaoka, H.; Torikata, A.; Inukai, M.; Kaneko, I.; Kagasaki, T.; Sakaida, Y.; Satoh, A.; Shiraishi, A. *J. Antibiot.* **1993**, *46*, 1503−1511. Kohama, T.; Nakamura, T.; Kinoshita, T.; Kaneko, I.; Shiraishi, A. *J. Antibiot.* **1993**, *46*, 1512−1519.

(23) Phoslactomycins: Fushimi, S.; Nishikawa, S.; Shimazu, A.; Seto, H. *J. Antibiot.* **1989**, *42*, 1019−1025. Fushimi, S.; Furihata, K.; Seto, H. *J. Antibiot.* **1989**, *42*, 1026−1036. Phosphazomycins: Tomiya, T.; Uramoto, M.; Isono, K. *J. Antibiot.* **1990**, *43*, 118−121. Phospholine: Ozasa, T.; Tanaka, K.; Sasamata, M.; Kaniwa, H.; Shimizu, M.; Matsumoto, H.; Iwanami, M. *J. Antibiot.* **1989**, *42*, 1339−1343. Ozasa, T.; Suzuki, K.; Sasamata, M.; Tanaka, K.; Kobori, M.; Kadota, S.; Nagai, K.; Saito, T.; Watanabe, S.; Iwanami, M. *J. Antibiot.* **1989**, *42*, 1331−1338.

(24) Usui, T.; Marriott, G.; Inagaki, M.; Swarup, G.; Osada, H. *J. Biochem.* (*Tokyo*) **1999**, *125*, 960−965.

(25) The intermediates **4**−**6**, **30**−**33**, and **38** were isolated as a single diastereomer of undefined stereochemistry at the anomeric center. We have assigned this as *trans*-(2*R*,6*R*), in agreement with an independent recent report of **5** and **6**; see: Christmann, M.; Bhatt, U.; Quitschalle, M.; Claus, E.; Kalesse, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4364−4366; Bhatt, U.; Christmann, M.; Quitschille, M.; Claus, E.; Kalesse, M. *J. Org. Chem.* **2001**, *66*, 1885−1893. See also: Murakami, N.; Wang, W.; Aoki, M.; Tsutsui, Y.; Higuchi, K.; Aoki, S.; Kobayashi, M. *Tetrahedron Lett.* **1997**, *38*, 5533−5536.

Scheme 2



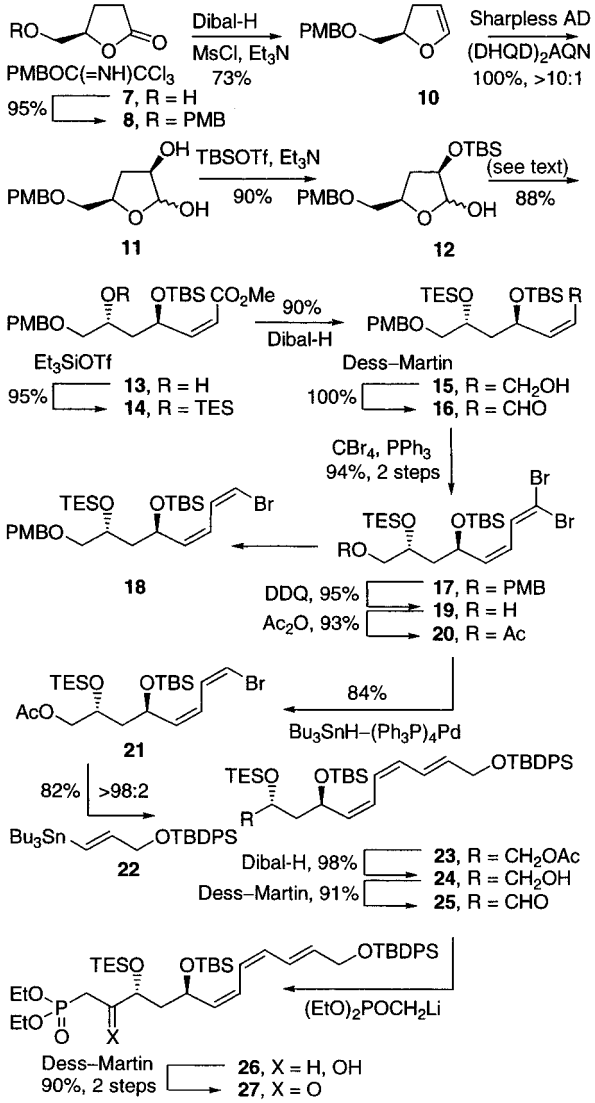
C-terminus subunit of **1** is the C5 stereocenter whose *R* absolute stereochemistry was set through a Sharpless AD reaction.

Synthesis of the C7−C18 Subunit of Fostriecin. The C7−C18 subunit of **1** was prepared by first assembling C8−C12 with introduction of the C9 and C11 stereocenters followed by stepwise introduction of the sensitive triene and finally conversion to the β-ketophosphonate **27** for linkage to C1−C6. The stepwise order to the triene introduction permitted the assessment of the relative stability of the C12−C13 *Z*-alkene, the C12−C15 *Z,Z*-diene, and the C12−C17 *Z,Z,E*-triene and offered the potential of postponing the introduction of any of its sensitive segments to a late stage of the synthesis. PMB protection (95%) and Dibal-H reduction (96%) of optically active lactone **7**, available in two steps from D-glutamic acid,[26] followed by subsequent mesylation and in situ elimination[27] provided the dihydrofuran **10** (73% from **8**), incorporating the C9 stereocenter (Scheme 3). Extensive dimerization of the lactol **9** was observed unless the mesylation was conducted under dilute reaction conditions (ca. 0.02−0.03 M) in the presence of excess Et_3N (15 equiv) at temperatures below −20 °C. Sharpless asymmetric dihydroxylation[28] on **10**, employing $(DHQD)_2AQN$,[29] gave a >10:1 ratio of diol **11** (100%) as an anomeric mixture with clean delivery of the C2 alcohol cis to the C4 hydroxymethyl substituent installing the fostriecin C11 center (Scheme 3).

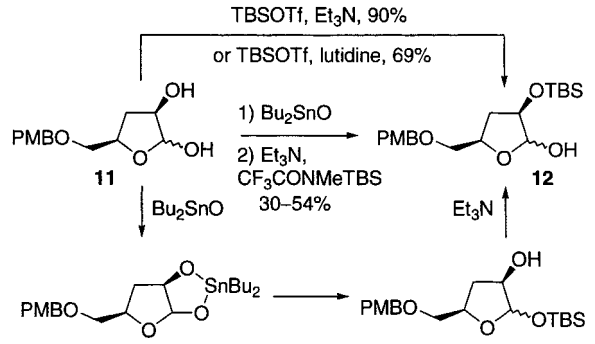
After empirical experimentation, selective protection of the C2 secondary alcohol over the anomeric C1 alcohol was achieved by low-temperature (−78 °C) treatment of **11** with TBSOTf (1.0 equiv, CH_2Cl_2) in the presence of Et_3N (3.0 equiv), providing **12** in superb yield (90%, >10:1 anomeric mixture). Analogous, but less effective, conversions were achieved with TBSOTf−lutidine (1/1.5 equiv, CH_2Cl_2, −78 °C, 1.5 h, 69% based on recovered **11**) or through conversion of diol **11** to the dibutylstannylene (1.1 equiv of Bu_2SnO, CH_3OH, reflux, 1.5 h),[30,31] followed by silylation with $CF_3CONMeTBS/Et_3N$ (3−6 equiv, DMF, 0−5 °C, 3 h, 30−54%). In the latter case, the intermediate silylation of the anomeric alcohol was detected as the predominant product (0.5 h, 0−5 °C) and observed to undergo silyl transfer to the C2 alcohol (3 h, 25 °C), Scheme 4.

Lactol **12** was condensed with the Still−Gennari phosphonate[32] ($(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KHMDS, 18-crown-6, THF, −78 °C) to afford **13** (88%, *Z*:*E* = 29:1), and this step

Scheme 3



Scheme 4



constitutes introduction of the first cis olefin of the sensitive *Z,Z,E*-triene. Notably, **13** cyclized to the corresponding tetrahydrofuran[32] by Michael addition of the liberated alkoxide onto the newly generated *Z* unsaturated ester if excess KHMDS was used as the base, and this could be avoided by careful use of a stoichiometric amount of base. An alternative approach involv-

(26) Herdeis, C. *Synthesis* **1986**, 232−233. Lee, Y. S.; Kang, S. S.; Choi, J. H.; Park, H. *Tetrahedron* **1997**, *53*, 3045−3056.

(27) Takle, A.; Kocienski, P. *Tetrahedron* **1990**, *46*, 4503−4516.

(28) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.

(29) Becker, H.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 448−451.

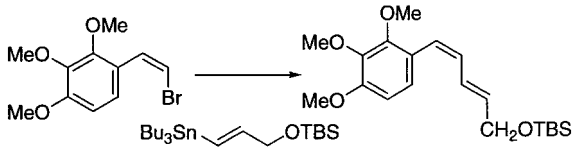
(30) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643−663. De Bernardo, S.; Tengi, J. P.; Sasso, G. J.; Weigele, M. *J. Org. Chem.* **1985**, *50*, 3457−3462.

(31) Nicolaou, K. C.; van Delft, F. L.; Conley, S. R.; Mitchell, H. J.; Jin, Z.; Rodriguez, R. M. *J. Am. Chem. Soc.* **1997**, *119*, 9057−9058.

(32) Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405−4408. For the cyclized tetrahydrofuran: colorless oil, $[\alpha]^{22}_D$ −22 (*c*. 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.26 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 4.52 (1H, d, J = 11.8 Hz), 4.48 (1H, d, J = 11.8 Hz), 4.25 (1H, m), 4.13 (2H, m), 3.80 (3H, s), 3.69 (3H, s), 3.58 (1H, dd, J = 6.6, 9.9 Hz), 3.43 (1H, dd, J = 5.1, 9.9 Hz), 2.50 (1H, dd, J = 5.5, 15.0 Hz), 2.47

ing reaction of lactol **12** with iodomethylenetriphenylphosphorane[33] to provide the corresponding *Z*-iodoalkene was not successful. After protection of **13** with Et_3SiOTf (95%), the resulting ester **14** was reduced with Dibal-H to give alcohol **15** (90%), which was subsequently oxidized with Dess−Martin periodinane[34] to provide aldehyde **16** (100%). Attempts to homologate **16** to the corresponding *Z,Z*-iododiene enlisting iodomethylenetriphenylphosphorane[33] provided the desired iodide[35] (78% from **15**, *Z*:*E* = 4.2:1) but with modest stereoselection. Not only were the two isomers not separable by chromatography, but the product proved labile to isomerization during attempts at purification. Consequently, we employed a two-step procedure enlisting the Corey and Fuchs reaction[36] of aldehyde **16** to first obtain dibromide **17** (94% from **15**), followed by selective reduction of **17** with $Bu_3SnH-Pd(PPh_3)_4$,[37] to cleanly yield the substantially more stable *cis*-vinyl bromide **18**.[35] In contrast to the *Z,Z*-iododiene, **18** proved stable to handling during purification. In initial efforts to elaborate **18** to the requisite *Z,Z,E*-triene unit found in the natural product, the removal of PMB group following Stille coupling with **22** proved problematic. Consequently, the PMB group was exchanged for an acetate by treatment of **17** with DDQ (95%) and acetylation of **19** to provide **20** (93%). Selective reduction of **20** with $Bu_3SnH-Pd(PPh_3)_4$ furnished *cis*-vinyl bromide **21** (84%) with only occasional and trace generation of the over-reduced product (5−10%). Stille coupling[38] of **21** with vinylstannane **22**[39] was found to be low-yielding under typical conditions. Enlisting conditions first established with a model substrate (Scheme 5), the Stille coupling of **21** with **22** conducted in *i*-Pr_2NEt provided the key intermediate, *Z,Z,E*-triene **23**, in excellent yield (82%) with nearly perfect stereochemical intergrity (≥98:2). While **21** and **23** are only marginally stable at room temperature when stored as oils, no decomposition or isomerization was observed over several months when they were stored as solutions in nonpolar solvents at 0−5 °C. Although deacetylation of **23** using standard hydrolytic methods (K_2CO_3/MeOH, LiOH/THF/H_2O) led to acetate removal followed by migration and/or desilylation of the neighboring triethylsilyl group, reduction with Dibal-H gave the desired primary alcohol **24** (98%), which was converted to aldehyde **25** by Dess−Martin oxidation[34] (91%).

Scheme 5



catalyst	co-catalyst/ligand	amine	solvent	product
$Pd_2(dba)_3$	CuI/–	–	NMP	0%
$Pd(CH_3CN)_2Cl_2$	CuI/–	–	NMP	0%
$Pd(PPh_3)_4$	CuI/Fu_3P	Et_3N	NMP	0%
$Pd_2(dba)_3$	CuI/Fu_3P	Et_3N	NMP	9%
$Pd(PhCN)_2Cl_2$	CuI/Fu_3P	Et_3N	NMP	9%
$Pd(CH_3CN)_2Cl_2$	CuI/Fu_3P	Et_3N	NMP	16%
$Pd(CH_3CN)_2Cl_2$	–/Fu_3P	Et_3N	NMP	38%
$Pd(CH_3CN)_2Cl_2$	–/–	Et_3N	NMP	40%
$Pd(CH_3CN)_2Cl_2$	–/–	Et_3N	Et_3N	80%
$Pd(CH_3CN)_2Cl_2$	–/–	pyr	pyr	20%
$Pd(CH_3CN)_2Cl_2$	–/–	*i*-Pr_2NEt	*i*-Pr_2NEt	88%

The remaining conversion of **25** to the ketophosphonate **27** set the stage for a Wadsworth−Horner−Emmons reaction to unite the C1−C6 and C7−C18 subunits. Initial attempts to add the anion derived from diethyl methylphosphonate to aldehyde **25** in THF met with limited success. However, when the reaction was performed in the nonpolar solvent toluene, superb conversions (1:1 mixture of diastereomers) were observed, and the β-ketophosphonate **27** was obtained after Dess−Martin oxidation of **26** (90% for two steps).[40]

Synthesis of Dephosphoryl Fostriecin: Correlation and Confirmation of Structure. Prior to completing the total synthesis of **1**, we first wanted to correlate our synthetic intermediates with those of the natural product to ensure they possessed the C8 natural stereochemistry. This was accomplished by linking the C1−C6 and C7−C18 subunits, the diastereocontrolled introduction of the C8 methyl group, elaboration of the lactone, and correlation with fostriecin derivatives that lack the phosphate. Initial efforts first condensed a β-ketophosphonate related to **27** with the sensitive lactone aldehyde **28**,[19] which provided **29**[41] in modest conversions (10−36%). The best conversions were obtained by using the Roush−Masamune procedure[42] with aldehyde **28** generated in situ by Swern oxidation of the corresponding alcohol as described[19] and appeared to be limited by the sensitivity of the aldehyde to the basic reaction conditions (eq 1). This, coupled with our inability to cleanly add a methyl nucleophile to the C8 carbonyl versus the lactone, led to the use of the lactone precursor **6**. Wadsworth−Horner−Emmons reaction of β-ketophosphonate

(1H, dd, *J* = 7.0, 15.0 Hz), 2.22 (1H, ddd, *J* = 6.2, 7.3, 12.8 Hz), 1.71 (1H, ddd, *J* = 5.9, 11.8 Hz) 0.87 (9H, s), 0.05 (3H, s), 0.04 (3H, s); IR (neat) ν_{max} 2942, 1734, 1515, 1247, 1093, 837 cm^{-1}; MALDIFTMS (DHB) *m/z* 447.2175 (M + Na^+ $C_{22}H_{36}O_6Si$ requires 447.2173).

(33) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 2173-2174.

(34) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287.

(35) Characterization for iodide (4.2:1 *Z,Z*-iododiene versus *E,Z*-iododiene): ^1H NMR (*Z,Z*-iododiene) δ 7.68 (1H, m), 7.19 (2H, m), 6.84 (2H, m), 6.85 (1H, m), 6.25 (1H, t, *J* = 10.9 Hz), 6.08 (1H, d, *J* = 7.6 Hz), 5.70 (1H, t, *J* = 10.1 Hz), 4.97 (1H, m), 4.32 (2H, s), 4.23 (1H, m), 3.36 (2H, m), 3.30 (3H, s), 2.00 (1H, m), 1.82 (1H, m), 1.18 (9H, m), 1.10 (9H, s), 0.08 (6H, m), 0.21 (3H, s), 0.17 (3H, s); ^1H NMR (*E,Z*-iododiene) δ 7.64 (1H, m), 7.21 (2H, m), 6.86 (2H, m), 6.00 (1H, d, *J* = 14.0 Hz), 5.56 (1H, d, *J* = 11.1 Hz), 5.44 (1H, t, *J* = 9.7 Hz), 5.15 (1H, m), 4.37 (2H, s), 4.25 (1H, m), 3.37 (2H, m), 3.31 (3H, s), 2.00 (1H, m), 1.82 (1H, m), 1.18 (9H, m), 1.10 (9H, s), 1.08 (6H, m), 0.22 (3H, s), 0.18 (3H, s); IR (neat) ν_{max} 2936, 2864, 1241, 1092 cm^{-1}; FABHRMS (NBA-CsI) *m/z* 765.1248 (M + Cs^+, $C_{28}H_{49}IO_4Si_2$ requires 765.1269). For **18**: ^1H NMR (C_6D_6, 400 MHz) δ 7.21−7.23 (2H, m), 6.91 (1H, dd, *J* = 7.5, 11.0 Hz), 6.82−6.85 (2H, m), 6.38 (1H, dd, *J* = 10.5, 11.0 Hz), 5.88 (1H, d, *J* = 7.5 Hz), 5.65 (1H, dd, *J* = 9.5, 10.5 Hz), 4.94−4.99 (1H, m), 4.31−4.38 (2H, m), 4.19−4.23 (1H, m), 3.41 (1H, dd, *J* = 6.0, 9.5 Hz), 3.36 (1H, dd, *J* = 4.9, 9.5 Hz), 3.33 (3H, s), 1.97 (1H, ddd, *J* = 4.4, 8.2, 13.8 Hz), 1.73 (1H, ddd, *J* = 4.6, 7.3, 13.8 Hz), 1.10 (9H, t, *J* = 7.9 Hz), 1.02 (9H, s), 0.76 (6H, q, *J* = 7.9 Hz), 0.17 (3H, s); FABHRMS (NBA-CsI) *m/z* 717.1428 (M + Cs^+, $C_{28}H_{49}BrO_4Si_2$ requires 717.1407).

(36) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769−3772.

(37) Uenishi, J.; Kawahama, R.; Yonemitsu, O.; Tsuji, J. *J. Org. Chem.* **1998**, *63*, 8965−8975. Uenishi, J.; Kawahama, R.; Shiga, Y.; Yonemitsu, O.; Tsuji, J. *Tetrahedron Lett.* **1996**, *37*, 6759−6762. Uenishi, J.; Kawahama, R.; Yonemitsu, O.; Tsuji, J. *J. Org. Chem.* **1996**, *61*, 5716−5717.

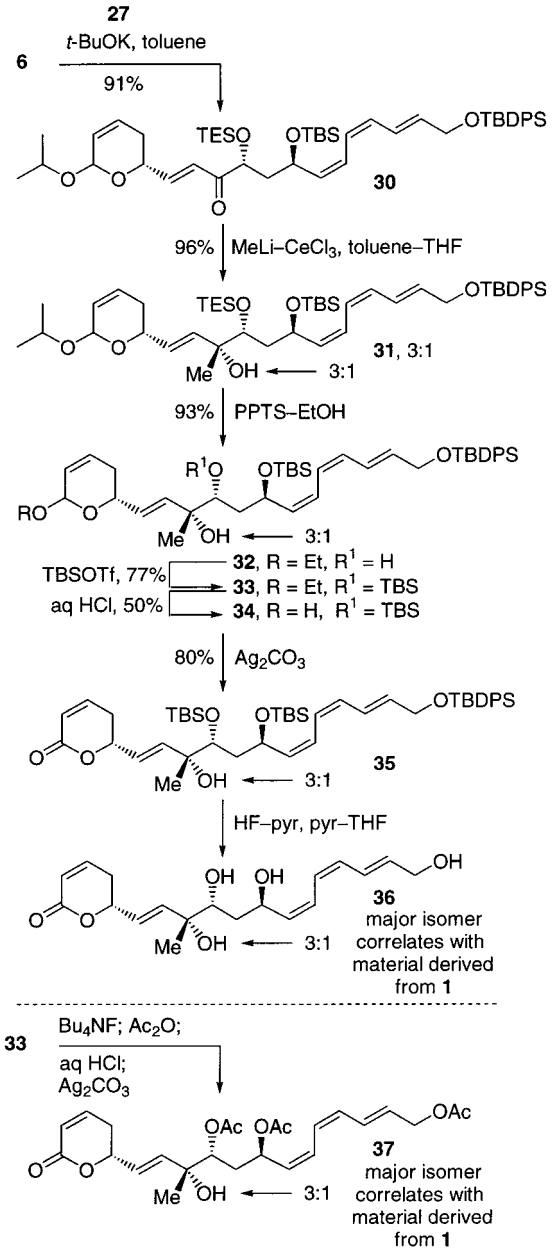
(38) Evans, D. A.; Black, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 4497−4513. Gibbs, R. A.; Krishnan, U. *Tetrahedron Lett.* **1994,** *35*, 2509−2512.

(39) Jung, M. E.; Light, L. A. *Tetrahedron Lett.* **1982**, *23*, 3851−3854. Bansal, R.; Cooper, G. F.; Corey, E. J. *J. Org. Chem.* **1991**, *56*, 1329−1332.

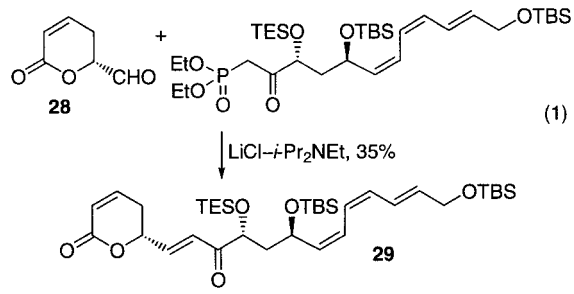
(40) Initial studies conducted with the analogous TBS-protected vinnylstannane related to **22** provided the corresponding C18 OTBS derivatives of **23**−**27**, **30**, and **31**. Selective deprotection of the C9 OTES group was not achieved, leading to the adoption of reagent **22**. Characterization data for these intermediates are provided in the Supporting Information.

(41) Characterization for **29**: ^1H NMR (CD_3CN, 500 MHz) δ 7.02−6.97 (1H, m), 6.92−6.84 (1H, m), 6.80−6.75 (1H, m), 6.60−6.56 (1H, m), 6.19−6.13 (1H, m), 5.97−5.86 (1H, m), 5.82−5.66 (3H, m), 5.59−5.64 (1H, m), 5.23−5.09 (2H, m), 4.70−4.67 (1H, m), 4.13−4.12 (2H, m), 2.28−2.12 (2H, m), 2.08−1.92 (1H, m), 1.68−1.58 (1H, m), 1.13−0.90 (27H, m), 0.80−0.72 (6H, m), 0.24−0.18 (6H, m), 0.09−0.08 (6H, m); FABHRMS (NBA-CsI) *m/z* 825.3367 (M + Cs^+, $C_{37}H_{68}O_6Si_3$ requires 825.3378).

Scheme 6



27 with aldehyde **6** was carried out smoothly in toluene, furnishing the desired trans α,β-unsaturated ketone **30**[25] (91%), Scheme 6.



Direct addition of MeLi to **30** via Felkin−Anh attack was anticipated to provide **31**. However, treatment of **30** with MeLi (THF) gave predominantly the undesired 1,4-adduct[43] (90%, >10:1). Alternative conditions including MeLi/18-crown-6,[44] MeLi/LiCl, and MeTi(^{i}OPr)$_3$[45] either did not substantially improve the selectivity or led to no reaction. Even the combination of MeLi−$CeCl_3$,[46] which gave the most encouraging results, provided a mixture of 1,2-adduct **31** versus 1,4-adduct in THF. Ultimately, we established that when ketone **30** was introduced as a toluene solution into the MeLi/$CeCl_3$ slurry in THF, the 1,2-adduct **31**[25] was formed preferentially (>20:1) in 96% yield as a mixture of two diastereomers (3:1, ^{1}H NMR). Correlation of the major isomer with natural product derivatives as detailed below established its stereochemistry as 8*R* as expected and derived from a Felkin−Anh addition. We briefly explored whether this diastereoselection could be improved by increasing the size of the C9 *O*-silyl protecting group. However, an analogous ratio of diastereomers was obtained with the corresponding C9 OTBS derivative.

Deprotection of the secondary C9 TES ether along with the exchange of the isopropyl acetal to an ethyl acetal was effected by treatment of **31** with PPTS−EtOH (25 °C, 3.5 h, 93%) and was required to permit eventual introduction of the C9 phosphate.[47] However, prior to this completion of the natural product synthesis, correlation of synthetic and naturally derived **36** was carried out to ensure our intermediates bore the correct relative and absolute configurations. Without optimization, reprotection of the secondary alcohol as its OTBS ether **33**[25] (7.2 equiv of TBSOTf, Et_3N, CH_2Cl_2, −78 °C, 4 h, 77%) followed by aqueous acid hydrolysis of the acetal (0.5 N aqueous HCl−acetone 1:4, 0 °C, 1 h, 50%) provided the lactol **34**, which was cleanly oxidized to the lactone **35** (30 equiv of 50% Ag_2CO_3−Celite, benzene, 80 °C, 2 h, 80%). Deprotection of **35** (HF·pyr, pyridine−THF, 25 °C, 4 d) furnished a 3:1 mixture of C8 diastereomers, of which the major diastereomer correlated with the tetraol **36** derived from dephosphorylation of the natural product[18,19] (HPLC: Porasil column, 3.9 mm × 300 mm, 0−50% EtOAc−hexane gradient elution, 1.0 mL/min, R_t = 9.45 min, minor peak at R_t = 8.72 min; NovaPak C18 column, 3.9 mm × 300 mm, 0−50% CH_3CN−H_2O gradient elution, 1.0 mL/min, R_t = 18.43 min, minor peak at R_t = 18.86 min).

In addition, the 9,11,18-triacetate **37** was prepared from naturally derived **36** by acetylation (Ac_2O, pyr, 5 °C, 5 h, 70%)[18] and correlated with the major isomer of a sample prepared from synthetic **33** (i. Bu_4NF, THF, 30 min, 25 °C; ii. Ac_2O, Et_3N, DMAP, CH_2Cl_2, 30 min, 25 °C, 75% for two steps; iii. 0.5 N aqueous HCl−acetone 1:4, 0 °C, 45 min; iv. 50% Ag_2CO_3−Celite, benzene, 80 °C, 1.5 h, 41% for two steps) by HPLC (Chiralcel OD column, 4.6 mm × 250 mm, 10% *i*-PrOH−hexane, 1.0 mL/min, R_t = 48.3 min, minor peak at R_t = 41.3 min) and ^{1}H NMR (C8−CH_3 at δ 1.25, minor isomer C8−CH_3 at δ 1.28).

These studies permitted spectroscopic and HPLC correlation of the synthetic samples with the naturally derived samples of **36** and **37**. However, none of the intermediate C8 diastereomers were chromatographically separable to the extent that they were

(42) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183−2186.

(43) Leonard, J.; Mohialdin, S.; Reed, D.; Ryan, G.; Swain, P. A. *Tetrahedron* **1995**, *51*, 12843−12858.

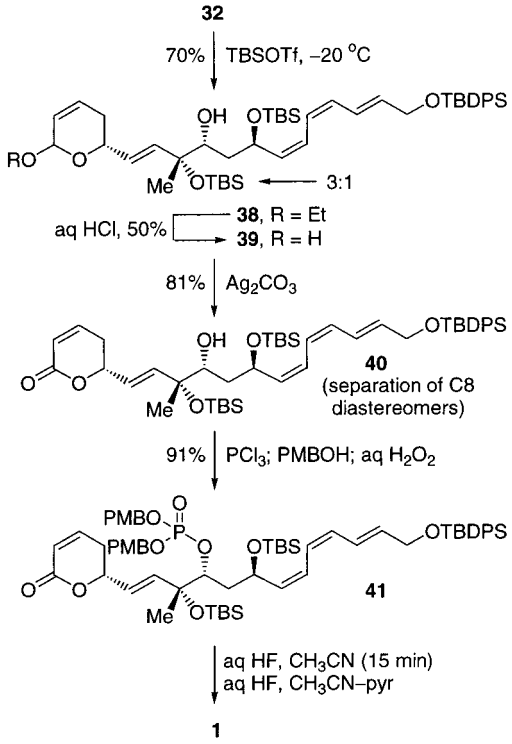
(44) Yamamoto, Y.; Maruyama, K. *J. Am. Chem. Soc.* **1985**, *107*, 6411−6413.

(45) Weidmann, B.; Seebach, D. *Helv. Chim. Acta* **1980**, *63*, 2451−2454.

(46) Imamoto, T. *Pure Appl. Chem.* **1990**, *62*, 747−752.

(47) When this reaction was conducted on the corresponding C18 OTBS derivative, isolation of the C8,C9,C18-triol was observed. Treatment of this triol with TBDPSCl (7 equiv, Et_3N, DMAP, CH_2Cl_2, 1 h, 86%) also provided **32**. Characterization data for C8,C9,C18-triol are provided in the Supporting Information.

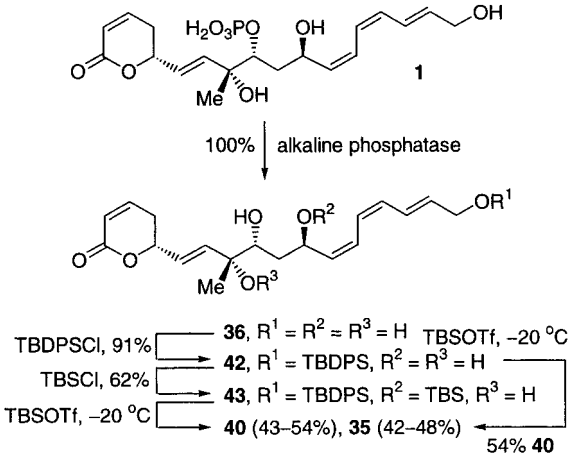
Scheme 7



preparatively useful. Thus, this preliminary correlation was conducted with the 3:1 mixture of C8 diastereomers.

Completion of the Total Synthesis of Fostriecin. In the course of handling **31** and its related C9 OTBS derivative **34**, a base-catalyzed migration of the C9 *O*-silyl group to the adjacent C8 tertiary alcohol was observed. We took advantage of this observation and developed conditions whereby treatment of **32** with TBSOTf (2.0 equiv, 5 equiv of 2,6-lutidine, −20 °C, 45 min) provided predominantly **38**[25] (70%) (Scheme 7). Thus, silylation at −78 °C cleanly provided **33** (77%) as detailed in Scheme 6, but when this reaction was conducted at the higher temperature of −20 °C, a subsequent C9-to-C8 *O*-silyl migration presumably occurred to provide primarily **38**. Hydrolysis to the lactol **39** and oxidation to the lactone cleanly provided **40**, at which point the problematic separation of the C8 diastereomers was straightforward. Thus, only four intermediates in the total synthesis (**31**, **32**, **38**, and **39**) were prepared and characterized as the 3:1 mixture of C8 diastereomers. Synthetic **40** was found to be identical in all respects with a sample of **40** prepared by degradation and functionalization of **1** (^{1}H NMR, ^{13}C NMR, IR, MS, $[\alpha]^{25}_D$). Global silyl ether deprotection of **40** was accomplished by treatment with HF·pyr in pyridine−THF (25 °C, 4 d) with a solid $NaHCO_3$ workup, providing the tetraol **36**, which also proved to be spectroscopically and chromatographically indistinguishable from naturally derived **36**.[18,19] Conversion of **40** to the bis-PMB-protected[48] C9 phosphate upon treatment with PCl_3 (4 equiv, pyridine, 25 °C) followed by PMBOH (9 equiv) and subsequent phosphite oxidation (H_2O_2−H_2O) provided **41** in superb conversion (91%), which upon global silyl ether deprotection (HF; HF−pyridine) provided fostriecin that was identical in all aspects with authentic material. Notably, oxidation of the intermediate phosphite (^{31}P NMR δ 141.0) to the phosphate (^{31}P NMR δ −0.28) was slow and could be monitored by ^{31}P NMR, and the bis-PMB phosphate ester was stable to purification by chromatography. Although not extensively investigated, direct methods of phosphate introduction ($POCl_3$, proton sponge; $Bu_4N{\cdot}H_2PO_4$, Cl_3CCN) were not successful and simply provided recovered starting material. The global deprotection of **41** was carried out in two stages. Prolonged treatment with unbuffered HF (5% H_2O−CH_3CN, 3 d) required for acid-catalyzed PMB ester deprotection led to extensive degradation and olefin isomerization, which precluded the ability to isolate **1** from the reaction mixture. The alternative treatment with HF·pyr (pyr−THF, 4 d) removed the silyl ether protecting groups but failed to cleave the PMB esters cleanly. Thus, we employed a two-stage deprotection, enlisting an initial, brief treatment with HF (5% H_2O−CH_3CN, 15 min) to remove the PMB esters, followed by addition of pyridine (25% pyr−CH_3CN/H_2O) to conduct the slow silyl ether deprotections under buffered conditions. Under these conditions, the C18 and C11 *O*-silyl protecting groups were removed relatively rapidly (1−2 d), whereas the hindered tertiary C8 OTBS required prolonged reactions times.

Scheme 8



Degradation and Functionalization of Natural Fostriecin. Degradation and functionalization of natural fostriecin provided authentic samples of our correlation intermediates (Scheme 8). The tetraol **36** was obtained from **1** by alkaline phosphatase hydrolysis (100%) as previously described.[18,19] Selective protection of the primary alcohol was accomplished by treatment of **1** with TBDPSCl (91%), providing **42** as described.[19] Conversion of **42** to the correlation sample **40** could be accomplished by sequential or concurrent C11 and C8 OTBS ether formation. Thus, treatment of **42** with TBSCl (1.5 equiv, 3.0 equiv of imidazole, DMF, 25 °C, 1 h) cleanly provided **43** (62%) derived from selective C11 OTBS ether formation. Without optimization, treatment of **43** with TBSOTf (3.0 equiv) in the presence of 2,6-lutidine (6.0 equiv) at −20 °C (3 h, CH_2Cl_2) provided **40** (45%), derived from protection of the tertiary C8 versus secondary C9 alcohol, and an equivalent amount of **35** derived from secondary C9 alcohol protection. Like the silylation reaction of **32**, the generation of **40** presumably proceeds by C9 *O*-silyl ether formation and subsequent migration to provide the more stable C8 OTBS ether and is facilitated by conducting the reaction at −20 versus −78 °C. Compound **40** derived from natural fostriecin proved to be identical in all respects (^{1}H NMR, ^{13}C NMR, $[\alpha]^{25}_D$, IR, MS) with synthetic material. The correlation sample **40** could be prepared even more conveniently by direct TBS silylation of **42** (4.0 equiv of TBSOTf, 8.0 equiv of lutidine, CH_2C1_2, −20 °C, 3 h) in higher conversion (54%).

Conclusions

The first total synthesis of fostriecin (CI-920) is disclosed which serves to confirm its relative and absolute stereochemical

(48) Evans, D. A.; Gage, J. R.; Leighton, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 9434−9453.

assignments. Improvements in this approach and its extension to the preparation of key analogues and partial structures of the natural product are in progress in efforts to define the structural features of **1** contributing to the potent and selective protein phosphatase inhibition, and these efforts will be disclosed in due course.

Experimental Section

Full experimental details are provided as Supporting Information.

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Supporting Information Available: Full experimental details of the total synthesis of **1**, details of the coupling defined in Table 1, full experimental details and characterization of the corresponding C18 OTBS route (Supporting Information Scheme 1), the correlation conversions of **32** to **36** and **37**, and the preparations of **43** and **40** from **42** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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