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Total Synthesis, Assignment of the Relative and Absolute Stereochemistry, and Structural Reassignment of Phostriecin (aka Sultriecin)

Christopher P. Burke, **Nadia Haq**, and **Dale L. Boger***
Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Abstract

A total synthesis of phostriecin (**2**) previously known as sultriecin (**1**), its structural reassignment as a phosphate versus sulfate monoester, and the assignment of its relative and absolute stereochemistry are disclosed herein. Key elements of the work, which provided first the originally assigned sulfate monoester **1** and then the reassigned and renamed phosphate monoester **2**, relied on diagnostic ^{1}H NMR spectroscopic properties of the natural product for the assignment of relative and absolute stereochemistry as well as the subsequent structural reassignment, and a convergent asymmetric total synthesis to provide the unequivocal authentic materials. Key steps of the synthetic approach include a Brown allylation for diastereoselective introduction of the C9 stereochemistry, an asymmetric CBS reduction to establish the lactone C5-stereochemistry, diastereoselective oxidative ring expansion of an α-hydroxyfuran to access the pyran lactone precursor, and single-step installation of the sensitive *Z,Z,E*-triene unit through a chelation-controlled cuprate addition with installation of the C11 stereochemistry. The approach allows ready access to analogues that can now be used to probe important structural features required for PP2A inhibition, the mechanism of action defined herein.

Sultriecin (**1**)[1] was identified as an antitumor antibiotic isolated from *Streptomyces roseiscleroticus* No. L827-7 and was an early member of a growing family of related natural products[2] that now include fostriecin (**3**),[3,4,5] cytostatin (**4**),[6] phospholine (**5**, phoslactomycin B),[7] the leustroducsins (**6**),[8] and the phoslactomycins (**7**) (Figure 1).[9] Sultriecin shares several features with other family members including the characteristic electrophilc α,β-unsaturated lactone and hydrophobic *Z,Z,E*-triene capping the ends of an extended structure that contains a central, functionalized 1,3-diol. Unique to **1** and in contrast to other family members that contain phosphate monoesters, sultriecin was assigned as a C9 sulfate ester at the time of its disclosure in 1992.

In efforts on the synthesis and evaluation of members of this class of antitumor agents that have since been shown to act as protein phosphatase 2A (PP2A) inhibitors, we reported total syntheses of **3**[3c] and **4**,[6c] the establishment of their relative and absolute configuration,[4,6] and the preparation of a series of analogues used to define structural features that are key to their potent and unusually selective inhibition of PP2A.[5,6] Based on its functional biological activity and structural similarity to **3** and **4**, we anticipated that **1** would also be a selective PP2A inhibitor, albeit via a sulfate versus phosphate interaction with the enzymatic bimetallic catalytic core. Herein, we report the first total synthesis and stereochemical determination of

boger@scripps.edu.

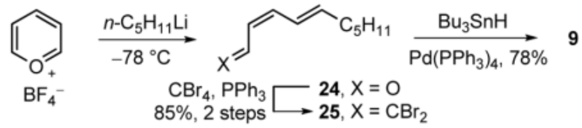
Supporting Information Available: Full experimental details are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

1, an unanticipated and requisite structural reassignment of the natural product as phosphate ester **2** (renamed phostriecin), and the establishment that it does in fact represent an effective inhibitor of PP2A.

The structure of sultriecin was disclosed without a definition of its relative or absolute stereochemistry requiring its assignment prior to initiating synthetic efforts (Figure 1). Earlier, we defined the (5*S*,9*S*,11*S*)-stereochemistry for both fostriecin[4] and cytostatin,[6c] and this assignment was extended to sultriecin. As a result of an intramolecular H-bond between the C9-phosphate and C11-OH of fostriecin and cytostatin, the resulting cyclic structure adopts a twist-boat conformation that gives rise to distinct ^{1}H–^{1}H coupling constants between H11 and H10a (syn) or H10b (anti) (J = 3.7 vs 9.6 Hz)[4] with only the latter capable of being observed with cytostatin (J = 9.4 Hz)[6] and similarly reported for sultriecin (J = 10.2 Hz).[1] Thus, we reasoned that sultriecin, like cytostatin and fostriecin, adopts a rigid H-bonded sulfate conformation exhibiting a H11–H10 coupling constant diagnostic of the 10,11-anti configuration establishing the relative stereochemistry of the C10 methyl substituent and confirming the 9,11-anti configuration. Additionally, the H4–H5 coupling constant reported for sultriecin (J = 2.5 Hz) is diagnostic of the cis-C4/C5 (J = 2–3 Hz) versus trans-C4/C5 (J = 8–9 Hz) substitution on the half chair conformation of the lactone[10] and is identical to that observed with cytostatin (H4–H5 J = 2.7 Hz).[6] Thus, the resulting (4*S*,5*S*,9*S*,10*S*,11*S*)-diastereomer of **1** was targeted for synthesis. A convergent route to sultriecin was designed that not only provides ready access to analogues, but also could be adjusted to allow the preparation of any diastereomer in the event that the initial stereochemical assignment proved incorrect. The approach relies on a late-stage single-step installation of the sensitive *Z*,*Z*,*E*-triene via chelation-controlled addition of the cuprate derived from **9** to aldehyde **8**. In turn, the protected lactol of **8** was envisioned to arise from an oxidative ring expansion of an α-hydroxyfuran, that could be accessed through the coupling of alkyne **10** with 2-furoyl chloride followed by asymmetric (*R*)-CBS ketone reduction and stereoselective alkyne reduction (Figure 1).

Synthesis of alkyne **10** was initiated with protection of methyl (*S*)-3-hydroxy-2-methylpropionate (**11**) as a PMB ether followed by reduction to alcohol **12** (PMBOC(=NH)CCl_3, camphorsulfonic acid, CH_2Cl_2, 25 °C, 12 h; $LiAlH_4$, Et_2O, 0–25 °C, 12 h, 91%, 2 steps) (Scheme 1). After oxidation of **12** to the corresponding aldehyde (DMSO, oxalyl chloride, Et_3N, CH_2Cl_2, −78 to 0 °C, 2 h), asymmetric allylboration (allyldiisopinocampheylborane, −100 °C, 4 h; NaOH, H_2O_2, 25 °C, 16 h, 14:1 dr)[11] gave alcohol **13** that was protected as the ethoxyethyl acetal (**14**) (ethyl vinyl ether, PPTS, CH_2Cl_2, 25 °C, 2 h). The ethoxyethyl acetal (EE), despite the complicating diastereomeric mixture it introduces, was chosen to direct a subsequent chelation-controlled aldehyde addition and represents a uniquely effective protecting group that is capable of selective removal under mild acidic conditions in the presence of the labile triene and sensitive allylic alcohol. Oxidative cleavage of olefin **14** (OsO_4, $NaIO_4$, NMO, THF/H_2O, 25 °C, 18 h, 73% for 4 steps) gave aldehyde **15** that was subjected to Corey–Fuchs homologation (CBr_4, PPh_3, CH_2Cl_2, 0 °C, 10 min, 71%; *n*-BuLi, THF, −78 to 25 °C, 16 h, 93%)[12] to give alkyne **10**. Coupling of **10** with 2-furoyl chloride (Pd$(PPh_3)_2Cl_2$, CuI, Et_3N, 25 °C, 24 h, 85%)[13] provided ketone **17** that was subjected to asymmetric reduction (methyl-(*R*)-CBS-oxazaborolidine,[14] BH_3–Me_2S, THF, −40 °C, 3 h, 12.5:1 dr) setting the C5 stereochemistry and was followed by stereoselective reduction[15] of the alkyne ($LiAlH_4$, THF, 0 to 25 °C, 24 h, 84%, 2 steps) to the trans olefin **18**. The analogous asymmetric CBS reduction of the corresponding α,β-unsaturated ketone with the trans double bond already installed to provide **18** directly was much less diastereoselective (ca. 2.5:1 dr). Intermediate **21** was obtained as a mixture of anomers following oxidative ring expansion of **18** (NBS, $NaHCO_3$/NaOAc, THF/H_2O, 0 °C, 1 h),[16] TBS protection of resultant lactol **19** (TBSCl, $AgNO_3$, pyr, CH_2Cl_2, 25 °C, 15 min),[17] and diastereoselective reduction of ketone **20** ($LiAlH_4$, Et_2O, −60 °C, 2.5 h, 51–64% for 3 steps). The stereochemistry of the resulting

C4 alcohol was necessarily inverted and directly protected as its pivalate ester using the Mitsunobu reaction (DIAD/Ph_3P, pivalic acid, THF, 0 to 25 °C, 75%).[18] Aldehyde **8** was obtained following PMB removal (DDQ, CH_2Cl_2/H_2O, 25 °C, 1 h, 74%) and oxidation of alcohol **23** (DMP, CH_2Cl_2, 25 °C, 1 h, 92%).[19] Following an approach developed by Taylor and adopted in our total synthesis of cytostatin,[20] pyrilium tetrafluoroborate was treated with *n*-pentyllithium (THF, −78 °C, 4 h) giving, after room temperature electrocyclic ring opening of the adduct, the *Z,E*-aldehyde **24** as a single isomer (Equation 1). Aldehyde **24** was converted to **9** via dibromoolefination (CBr_4, PPh_3, Et_3N, CH_2Cl_2, 0 °C, 15 min, 85% for 2 steps)[12] and selective *E*-bromide reduction (Bu_3SnH, $Pd(PPh_3)_4$, Et_2O, 0 °C, 45 min, 78%).[21]



(1)

Incorporation of the sensitive *Z,Z,E*-triene tail commenced with conversion of **9** to the corresponding cuprate (*t*-BuLi, Et_2O, −78 °C, 1 h; CuI–PBu_3, Et_2O, −78 °C, 15 min) followed by slow addition of aldehyde **8** (Et_2O, −78 °C, 1 h, 85%, >5:1 dr), providing **26** derived from chelation-controlled addition to the aldehyde (Scheme 2).[6c,22] Removal of the pivalate ester (DIBAL, CH_2Cl_2, −78 °C, 2 h) followed by silylation of the secondary alcohols of **27** (TBDPSCl, $AgNO_3$, pyr/CH_2Cl_2, 25 °C, 16 h, 80%, 2 steps) afforded **28** that was treated with dilute HCl (THF/H_2O, 25 °C, 12 h, 71%) to simultaneously and selectively remove the EE and TBS protecting groups. The resulting lactol was selectively oxidized to give lactone **29** (Ag_2CO_3–Celite, benzene, 80 °C, 1.5 h, 91%). Sulfate ester introduction (SO_3–pyr, THF, 25 °C, 80%) followed by desilylation (HF–pyr, pyr/THF, 25 °C, 60%) gave **1**, which did not match the spectroscopic (^{1}H NMR, ^{13}C NMR, IR) or physical characteristics (TLC, $[\alpha]_D$, solubility, stability to silica gel) reported for the natural product.

Although several possibilities for this non-correlation with the natural product could be envisioned including the accuracy of our stereochemical assignments as well as spectroscopic perturbations derived from the protonation state or salt form of the sulfate, the only real distinctive difference observed in the ^{1}H NMR of synthetic **1** and the natural product was the chemical shift (CD_3OD, δ 4.82 vs 4.64) and multiplicity (ddd, J = 8.4, 6.0, 1.8 Hz vs dddd, J = 9.6, 7.8, 7.2, 1.8 Hz) of C9-H adjacent to the putative sulfate ester. Diagnostic of what proved to be a required structural reassignment, the C9-H of the natural product exhibited an additional long range coupling ($J_{P\text{-}H9}$ = 7.8 Hz) characteristic of a phosphate (monoisotopic mass = 492.1889) versus sulfate ester (monoisotopic mass = 492.1794).[23] Consequently, phosphate ester **2** was targeted for synthesis (Scheme 3). Alcohol **29** was phosphorylated (*i*-Pr_2NP(OFm)$_2$, tetrazole, CH_2Cl_2/CH_3CN, 25 °C, 1 h; H_2O_2, 15 min, 96%)6 to give **31** that was desilylated (HF–pyr, pyr/THF, 25 °C, 4 d). Removal of the fluorenylmethyl groups in **32** (Et_3N, CH_3CN, 25 °C, 16 h; Dowex Na^+, 63% for 2 steps) unmasked the phosphate giving **2** (phostriecin), that proved identical to the reported properties of **1** as well as a sample[24] of natural "sultriecin" (^{1}H NMR, ^{31}P NMR, $[\alpha]_D$, TLC, HPLC, HRMS), the latter of which displayed a ^{31}P NMR signal like that found with synthetic **2** (δ 3.4, CD_3OD).

Thus, the total syntheses of **1** and **2** led to an unequivocal reassignment of the structural composition and established the relative and absolute stereochemical configuration of the natural product (renamed phostriecin) heretofore known as sultriecin. Key steps include a Brown allylation with controlled introduction of the C9 stereochemistry, a CBS reduction to establish the lactone C5-stereochemistry, diastereoselective oxidative ring expansion of an α-hydroxyfuran to access the pyran lactone precursor, and single-step installation of the sensitive triene unit through a chelation-controlled cuprate addition with installation of the C11 stereochemistry. This approach also allows ready access to analogues that can now be used to probe important structural features required for PP2A inhibition, the mechanism of action defined herein.[25] These and related studies will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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23. It also explains the observation (ref. 6c) that the corresponding sulfate of cytostatin (sulfocytostatin) was found to be inactive against PP2A.
24. Commercially available from Bioaustralis.
25. PP2A inhibition (IC_{50}): 1 (>100 >M), 2 (0.72 μM).

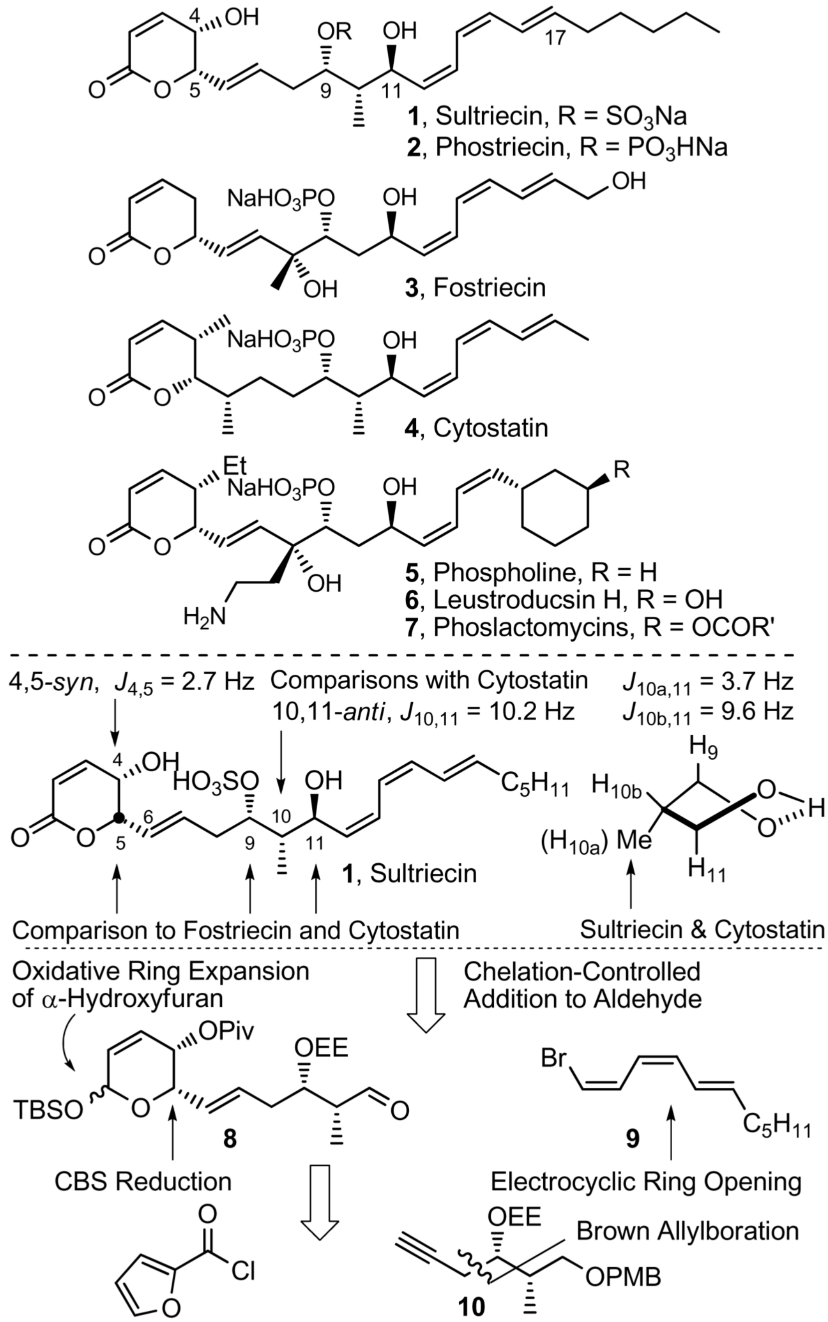
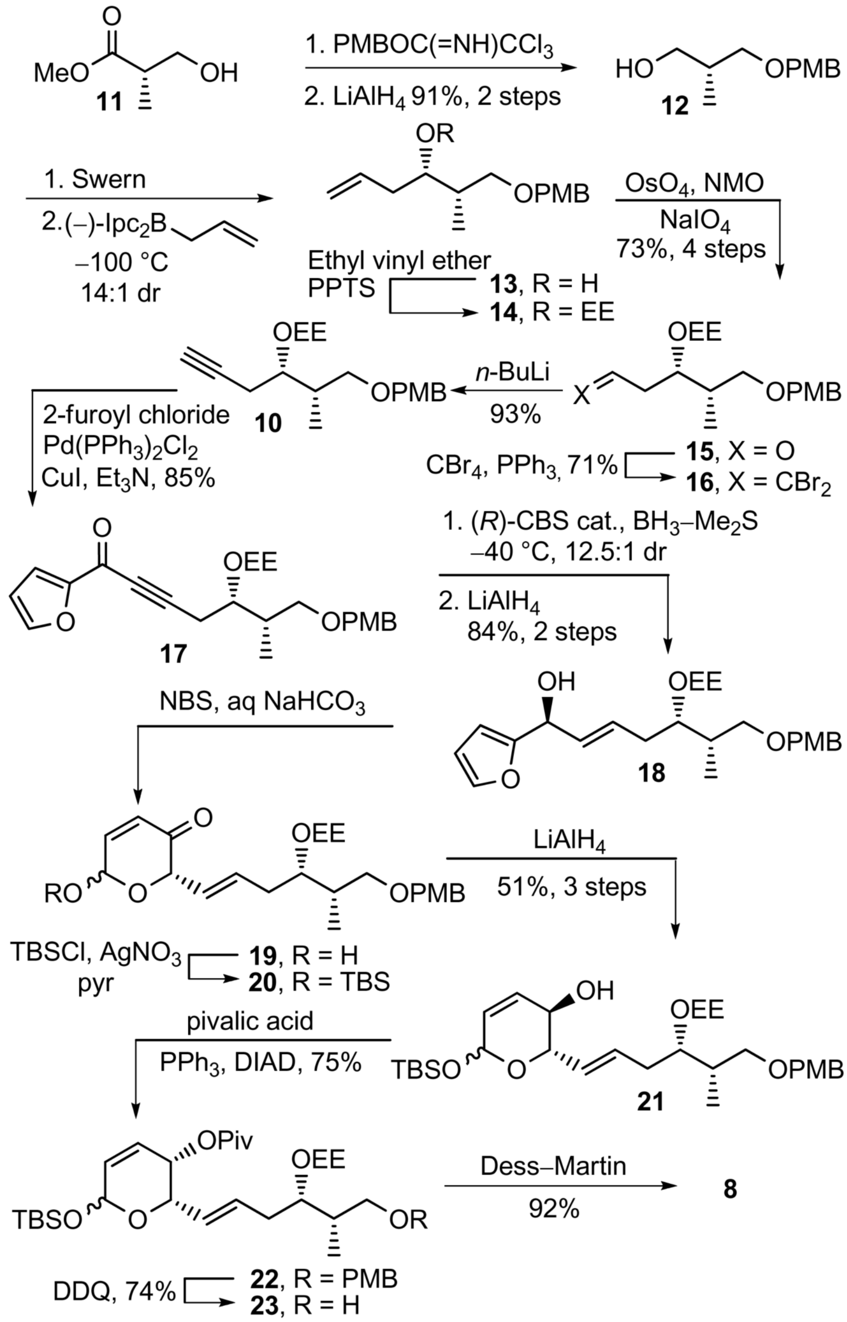


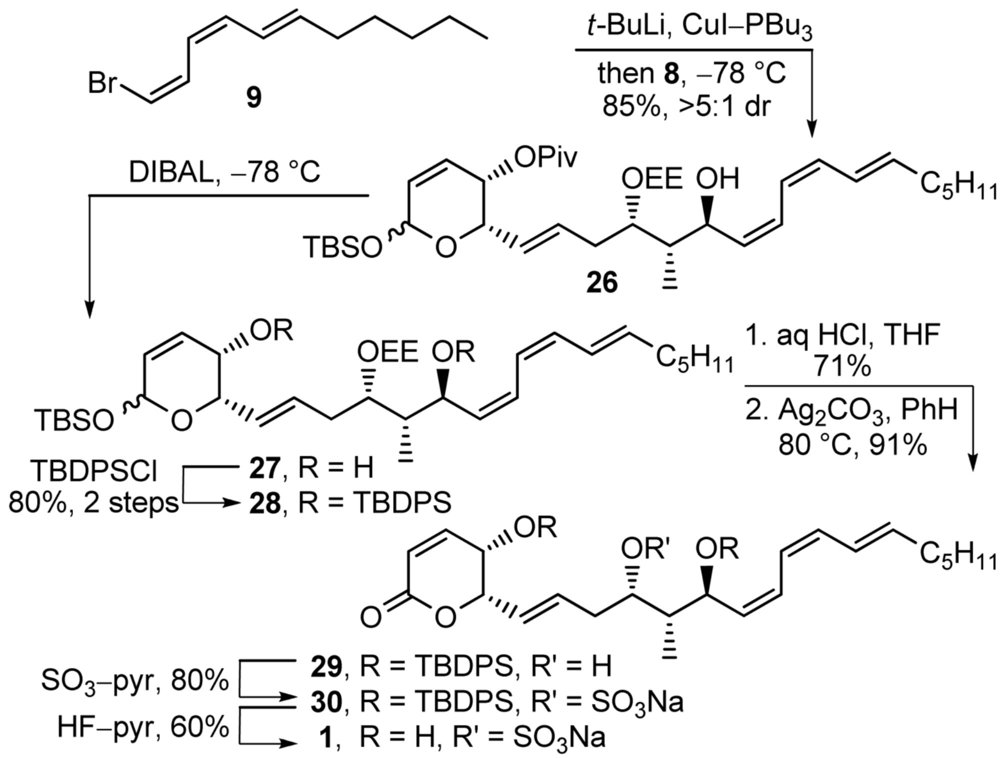
Figure 1.

Top: Natural product structures. Bottom: Assignment of relative and absolute stereochemistry and key retrosynthetic disconnections for sultriecin (**1**).

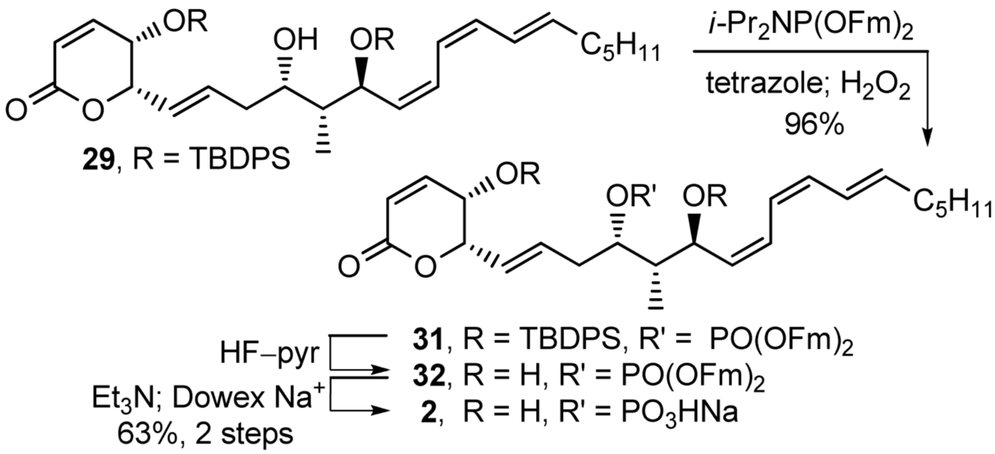


Scheme 1.

Synthesis of **8.**



Scheme 2.
Synthesis of **1** (sultriecin).



Scheme 3.
Synthesis of **2** (phostriecin).