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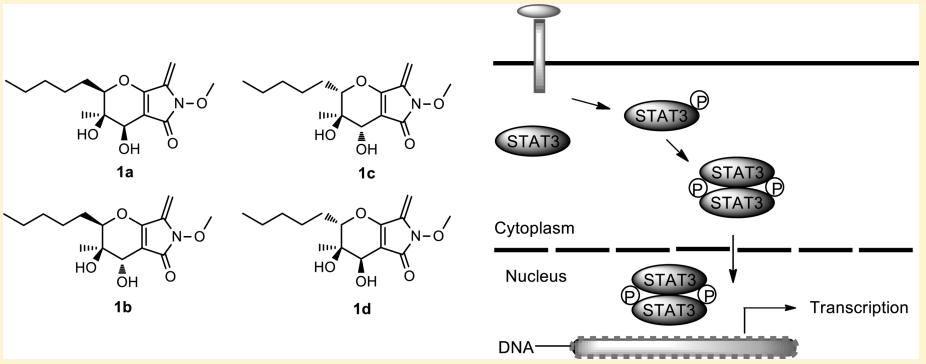
Synthetic and Biological Studies of Phaeosphaerides

Anthoula Chatzimpaloglou,[†] Mikhail Kolosov,[‡] T. Kris Eckols,[‡] David J. Tweardy,[‡] and Vasiliki Sarli*[,†]

[†]Faculty of Chemistry, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece

[‡]Department of Medicine, Section of Infectious Diseases, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, United States

Supporting Information



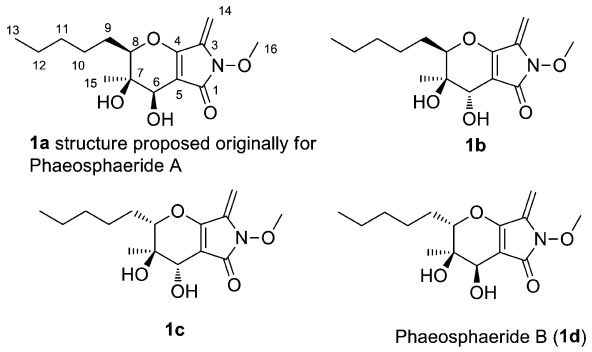
ABSTRACT: The signal transducer and activator of transcription 3 (STAT3) has been validated as a suitable target for cancer therapy. Recent evidence by our group and others has shown that phaeosphaerides act as inhibitors of the STAT3 pathway. An efficient synthetic sequence to phaeosphaeride **1a** has been previously disclosed. In this work, the first total synthesis of (±)-phaeosphaeride B (**1d**) and the unnatural phaeosphaeride **1b** is reported. Additionally, the biological activities of **1a** and **1b** were investigated. (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** inhibited granulocyte colony-stimulating factor (GCSF)-stimulated phosphorylation of STAT1, STAT3, and STAT5 and IL-6-stimulated nuclear translocation of STAT3 alpha. In an SPR-based assay, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** showed minimal ability to inhibit binding of STAT3 to its immobilized phosphotyrosylpeptide ligand (IC_{50} > 100 μM). Thus, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** are likely upstream inhibitors of a kinase in the STAT signaling pathway and do not act through the inhibition of STAT3 dimerization by the blocking of the SH2 binding domain.

INTRODUCTION

The signal transducer and activator of transcription proteins (STATs) play important roles in fundamental cellular processes, including proliferation, development, differentiation, inflammation, and apoptosis.[1] STATs are activated in response to growth factors or cytokines by phosphorylation on specific tyrosyl residues.[2] Phosphorylated STATs interact through their Src homology 2 (SH2) domains, dimerize, translocate into nucleus, and induce transcription on specific target genes.[3] Abnormal activation of STATs, in particular, STAT3, is detected in numerous human cancers. Currently, it is widely recognized that STAT3 is a critical regulator of tumor processes[4] and a suitable target for cancer chemoprevention and therapy.[5] Consequently, several inhibitors of the STAT3 pathway have been designed,[6] and their therapeutic potential is presently evaluated in clinical trials.[7]

Recent evidence by our group and others has shown that phaeosphaerides (Scheme 1) act as inhibitors of the STAT3 pathway.[8,9] Phaeosphaeride A (proposed structure **1a**), a metabolite isolated from the endophytic fungus FA39 (*Phaeosphaeria avenaria*), was identified as a STAT3 inhibitor from a 10000-member library screen consisting of natural product extracts. It was reported to selectively inhibit the STAT3 signaling pathway and suppress cell growth inhibition in U266 multiple myeloma cells with an IC_{50} of 6.7 μM. In 2011, Tamura and co-workers published the first total synthesis of the proposed structure of phaeosphaeride A (**1a**) via a six-

Scheme 1. Phaeosphaerides



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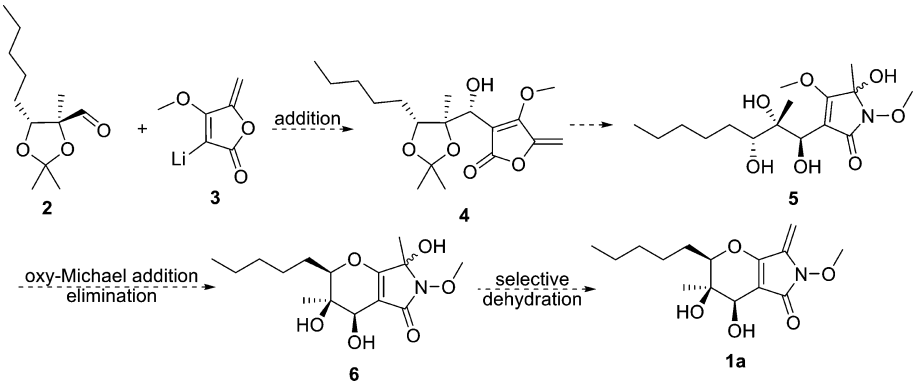
Scheme 2. Previous Synthesis of 1a



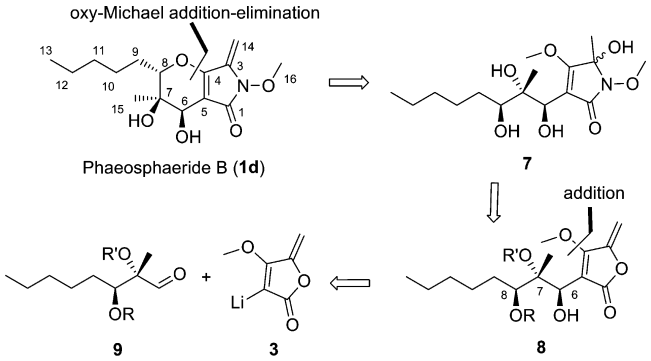
membered ring formation by means of an intramolecular vinyl anion aldol reaction as the key step.[9b] In this study, based on the findings and spectroscopic data, structure **1c** was suggested for the natural phaeosphaeride A. Our group has recently reported an asymmetric synthesis for both enantiomers of **1a** and their biological activities.[9a] We demonstrated that both enantiomers of **1a** decrease STAT3-dependent transcriptional activity and exhibit antiproliferative properties in breast (MDA-MB-231) and pancreatic (PANC-1) cancer cells. As part of these studies, our research included the synthesis of the other phaeosphaeride isomers in order to develop and optimize inhibitors of the STAT3 signaling pathway.

As we recently reported, our strategy for the synthesis of **1a** involved the addition of vinyllithium reagent **3** to the acetonide-protected aldehyde **2**, the introduction of methoxylamino group followed by intramolecular hetero-Michael cyclization, and methanol elimination to form the dihydropyran ring (Scheme 2).[9a] We envisaged that the synthetic sequence employed to **1a** could be used for the preparation of phaeosphaeride B (**1d**) starting from an aldehyde carrying the 2,3-*anti*-diol functionality. Structures **1b** and **1c** could be prepared from **1a** and **1d**, respectively, by inversion of the configuration of the C-6 center by a Mitsunobu reaction or by a two-step oxidation−reduction process. The results of these investigations are reported herein. In addition, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** inhibited GCSF-stimulated phosphorylation of STAT3 and STAT5, IFN-γ-stimulated phosphorylation of STAT1, and IL-6-stimulated nuclear translocation of STAT3 alpha. In an SPR-based assay, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** showed essentially no ability to inhibit binding of STAT3 to its immobilized phosphopeptide ligand (IC_{50} > 1000 μM). Thus, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** do not interact with the SH2 binding domain of STAT3 and are likely upstream inhibitors of a tyrosine kinase in the JAK/STAT pathway.

RESULTS AND DISCUSSION

From the beginning of our studies, we aimed to develop a flexible synthetic route that would give access to phaeosphaeride analogues and enable subsequent structure−activity relationship (SAR) studies. In principle, the synthetic route used to make **1a** should be applicable to phaeosphaeride B, **1d**. Thus, phaeosphaeride B can be envisaged to arise from **7** by an intramolecular oxy-Michael addition followed by methanol elimination (Scheme 3). Formation of **7** was proposed to occur by methoxylamino group introduction and deprotection of tetronate **8**, which in turn can be assembled from building blocks **3** and **9**. Importantly, it was expected that the addition of

Scheme 3. Retrosynthetic Disconnections for Phaeosphaeride B



the α-lithio tetronate reagent **3** to the aldehyde **9** would favor the formation of the *anti*-Felkin-Anh product **8**.

The synthetic approach for the aldehyde **16** was initiated from commercially available hexanal, as illustrated in Scheme 4. Hexanal was subjected to Wittig olefination with the stabilized ylid methyl(triphenylphosphoranylidene) propionate **10** to furnish the (*E*)-α,β-unsaturated ester **11**, as has been previously reported.[2] The ester group was reduced with DIBALH at −78 °C to give allylic alcohol **12**, which was subsequently epoxidized with $NaHCO_3$-buffered *m*CPBA in CH_2C1_2. Epoxy alcohol **13** was then subjected to a $Ti(O\text{-}iPr)_4$-mediated epoxide opening reaction with allyl alcohol to produce an inseparable mixture of the desired 1,2-diol **14a** and a 1,3-diol derivative **14b** in a ratio of approximately 4.2:1 and 79% total yield.[10] Nevertheless, the isomers **14a** and **14b** were oxidized with SO_3·py complex in Et_3N/DMSO, and flash chromatography on silica gel allowed the separation of the aldehyde **15**. In a following step, the tertiary alcohol of **16** was protected as TMS ether by the use of TMSOTf, 2,6-lutidine in CH_2Cl_2 at −78 °C.

Having synthesized the required aldehyde **16**, we next turned our attention to the addition of lithiated tetronate **3** to **16** (Scheme 5). Tetronate derivative **17** was prepared from 4-methoxy-2(5*H*)-furanone in a three-step sequence according to Yoshii et al.[11] Metalation of **17** with LDA in THF at −78 °C as described by Yoshii[12] and addition of the resulting vinyllithium species to aldehyde **16** gave intermediates **18a** and **18b** in 60% yield along with partial recovery of starting materials. In this process, the migration of the TMS group from the tertiary to the newly formed secondary hydroxyl group occurred. That was confirmed by the formation of the single *anti*-product **19** after

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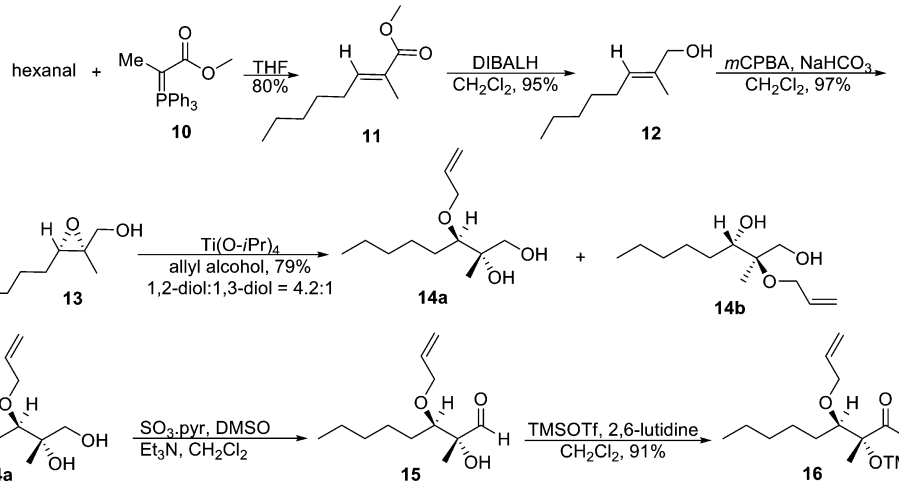
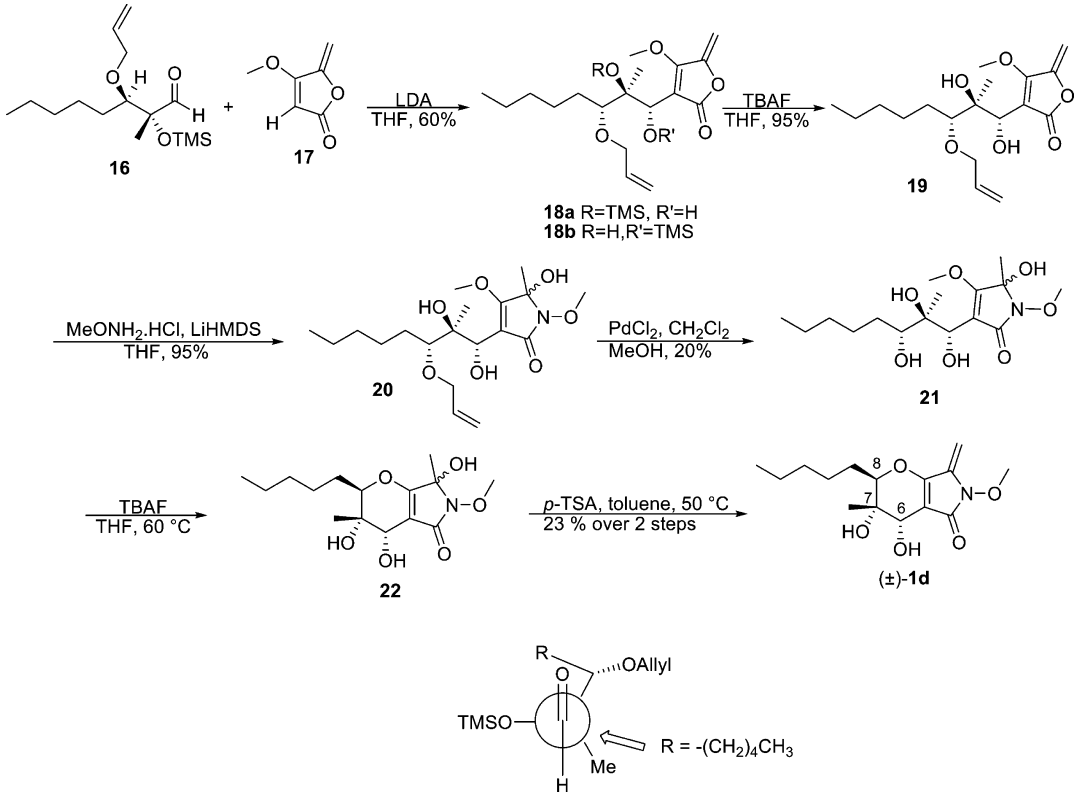
Scheme 4. Synthesis of Aldehyde 16



Scheme 5. Synthesis of (±)-Phaeosphaeride B, 1d



deprotection of the silyl group with TBAF. The stereochemical outcome of the addition reaction can be rationalized by the polar Felkin-Ahn transition state, where the nucleophile approaches the carbonyl group near the methyl substituent as shown below.[13]

The reaction of tetronate **19** with $MeONH_2$·HCl, using LiHMDS as a base in THF at −78 °C, was consecutively employed to provide precursors **20** in 95% yield.[14] The deprotection of the allyl ether in **20** proved to be troublesome. After testing a variety of conditions such as $Pd(PPh_3)_4/K_2CO_3$ in MeOH, PPh_3RhCl/PPh_3 in $EtOH/C_6H_6/H_2O$,[15] $CeCl_3·7H_2O$/NaI in CH_3CN,[16] and $PdCl_2$/AcONa in $AcOH/H_2O$,[17] the removal of the allyl group in **20** with $PdCl_2$ in $MeOH/CH_2Cl_2$ was possible; however, the yield (20%) was far from satisfactory and the reproducibility of the reaction was poor.[18] Nevertheless, **21** was then advanced to the next step. As opposed to the related reaction of **5**, the oxy-Michael addition turned out to be problematic. Even upon prolonged stirring at room temperature, **21** in the presence of TBAF did not cyclize to the dihydropyrans **22**, as was seen with precursors **5**. Heating **21** with 2 equiv of TBAF in THF at 60 °C for 45 min gave **22**, albeit in low yield (23%). Careful control of both reaction temperature and time was critical for **22** formation. In an attempt to optimize the Michael addition/methanol elimination process, the reaction was tested in the presence of varying bases such as Cs_2CO_3/acetonitrile, sodium acetate, triethylamine, DBU, and NaH which failed to give any traces of the desired products. Finally, the resultant dihydropyrans **22** were

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selectively dehydrated to provide (±)-phaeosphaeride B (**1d**) in quantitative yield.

The spectral and analytical data (^{1}H, ^{13}C NMR, MS) of synthetic (±)-**1d** were identical to those of the natural phaeosphaeride B. Two-dimensional NMR was performed to determine the stereochemistry of C-6 of (±)-**1d**. As shown in Figure 1, key NOESY interactions between H-6 with H-15 and H-9, as well as between the two OH protons, indicate that both hydroxyls and H-8 are on the same side of the dihydropyran ring.

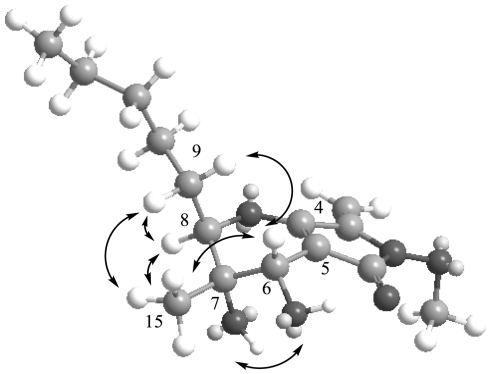


Figure 1. Selected NOESY correlations of (±)-**1d**.

In an attempt to optimize the synthetic route toward phaeosphaeride B and obtain sufficient amount of this material for our studies, an alternative strategy to intermediate **21** was explored. Our approach included a silicon-based protection of the hydroxyl groups in order to overcome the problems associated with deallylation of **20**. We planned to synthesize erythro-diol **26** from the threo-diol **23** that has been used in the synthesis of **1a** (Scheme 6). Compound **23** was converted to cyclic sulfate **24** using thionyl chloride, followed by oxidation of the resulting cyclic sulfite with sodium hypochlorite (94% yield for the two-step conversion).[19] Regioselective ring opening of **24** with sodium acetate (1.1 equiv, *N*,*N*-dimethylformamide, 50 °C) followed by an acidic work up of the monosulfate ester gave acetate **25**. To confirm the position of the acetate group besides NMR studies, **25** was treated with oxidants. It was found that **25** was resistant to oxidation with PCC or Dess Martin in CH_2Cl_2, indicating that the alcohol is bonded to a tertiary carbon atom. The acetate in **25** was then deprotected with K_2CO_3/MeOH to afford compound **26** in 92% yield. The diol **26** was protected as the bis-silyl ether, and the ester group was reduced to the corresponding alcohol by means of DIBALH and back-oxidized to aldehyde **28**.

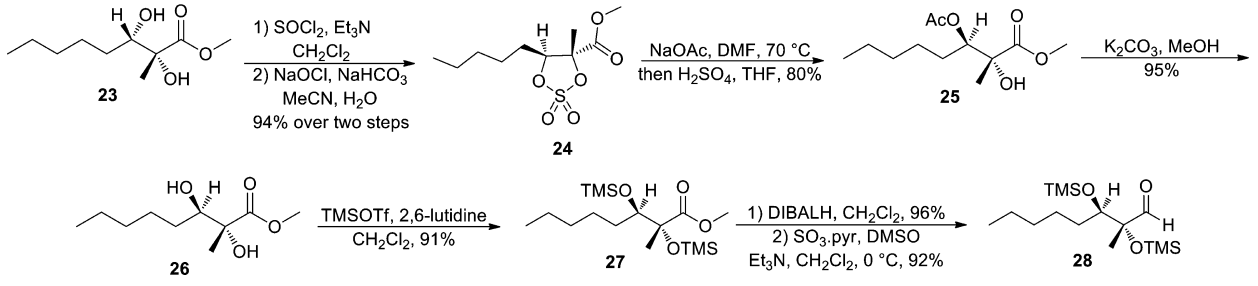
Aldehyde **28** was then reacted with α-lithio tetronate **17** to form **29a** and **29b** in 51% total yield (based on recovered **28**). As expected, migration of the TMS group also occurred from the tertiary to the secondary hydroxy group. Subsequent methoxy amine addition and deprotection of both TMS ethers with TBAF in THF provided **31** in 92% yield. Following the established route, oxy-Michael addition/methanol elimination and selective dehydration yielded (±)-**1d** in 23% yield over two steps (Scheme 7).

The synthesis of the C-6 epimer of the originally proposed structure of phaeosphaeride A (**1a**) was then studied.[9] Our first attempt involved an oxidation/reduction process which was unsuccessful because the unsaturated enamide functionality is very sensitive to both oxidizing and reducing agents. In another effort, **1a** was subjected to Mitsunobu reaction under standard conditions (*p*-nitrobenzoic acid, TPP, DEAD, benzene, 80 °C),[20] which also could not effect this conversion. As an alternative, the conversion of (6*S*,7*S*,8*S*)-**1a** to (6*R*,7*S*,8*S*)-**1b** was performed by a nucleophilic displacement of the mesyl derivative **31** by means of an acetate anion. Mesylation of (6*S*,7*S*,8*S*)-**1a** with $MeSO_2Cl$ and Et_3N in CH_2Cl_2 gave the mesylate **31** as a sole product, which was used in the next step without purification (Scheme 8). Treatment of **31** with $NaNO_2$ in DMF and heating at 70 °C gave the expected (6*R*,7*S*,8*S*)-**1b** in 23% yield. Due to the sensitive nature of the substrate, better yields (80%) were obtained by using NaOAc in dioxane/H_2O and stirring at room temperature overnight.[21]

Neither the spectral data nor the physical data of the diastereomer (6*R*,7*S*,8*S*)-**1b** match with that reported for the natural phaeosphaeride A (Figure 2). The relative configuration of (6*R*,7*S*,8*S*)-**1b** was mainly deduced by analysis of the NOESY spectrum and analogy with phaeosphaeride B and **1a**. In this case, H-8 correlated with H-9 and H-15 correlated with both H-6 and H-8.

The above results imply that the correct stereostructure of natural phaeosphaeride A must be **1c** or its enantiomer. Thus, the inversion of the secondary alcoholic stereocenter of (±)-**1d** to prepare **1c** was attempted. Alcohol (±)-**1d** was treated with mesyl chloride and triethylamine at 0 °C (Scheme 9). After being stirred for 20 min, the starting material disappeared and a new product was observed by TLC. The reaction mixture was then quenched with water and extracted with dichloromethane. The solvent was evaporated, and the residue was treated with sodium acetate in dioxane/water as described above. To our surprise, (±)-**1d** was the only product and it was formed in a clean spot-to-spot reaction with complete retention of configuration. The same result was obtained when the mesylation product was treated with $NaNO_2$ in DMF. We have tried to purify the product observed in the mesylation

Scheme 6. Synthesis of Aldehyde 28



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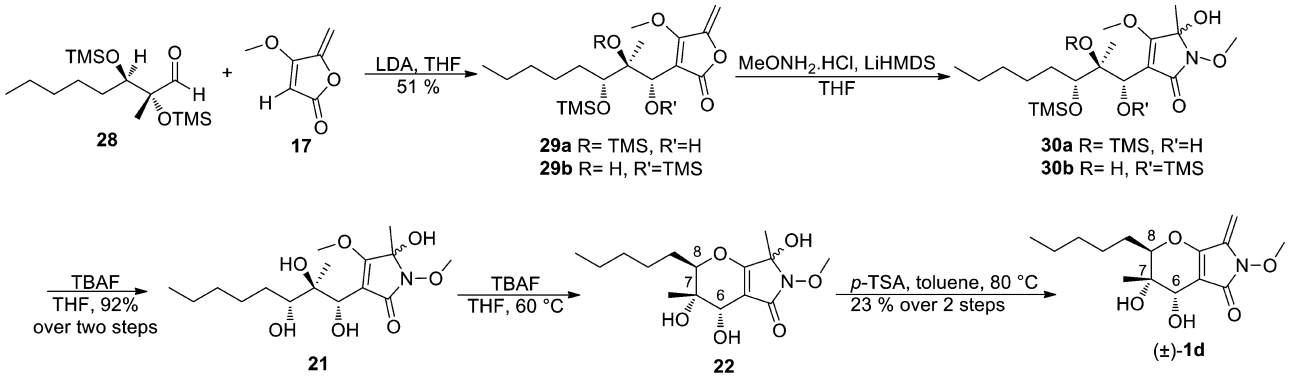
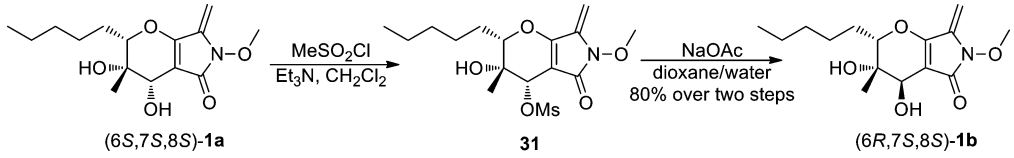
Scheme 7. Synthesis of (±)-1d



Scheme 8. Synthesis of (6*R*,7*S*,8*S*)-1b



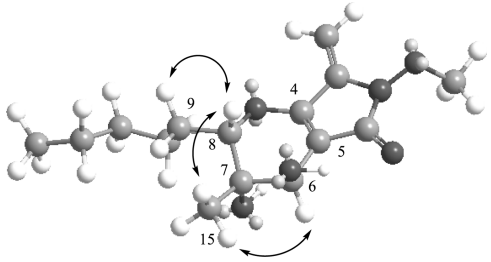


Figure 2. Selected NOESY correlations of (6*R*,7*S*,8*S*)-**1b**.

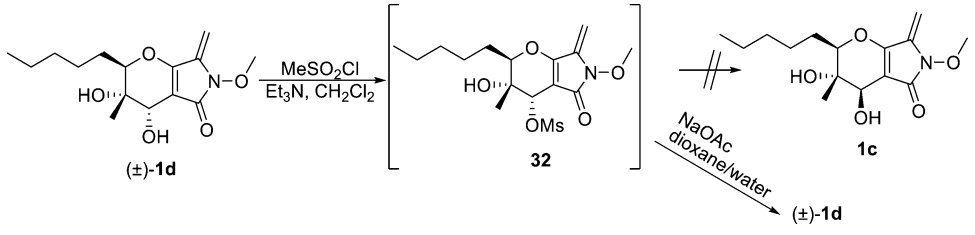
reaction; however, the intermediate was unstable and attempts to isolate and characterize it were unsuccessful.

Biological Evaluation of (6*S*,7*S*,8*S*)-1a and (6R,7S,8S)-1b. We previously reported that both enantiomers of **1a** decrease the STAT3-dependent transcriptional activity and inhibit the malignant cell proliferation in breast (MDA-MB-231) and pancreatic (PANC-1) cancer cells with constitutively active STAT3. In this work, compounds (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** were examined for their effect on STAT phosphorylation. The results demonstrate that (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** inhibited the granulocyte colony-stimulating factor (G-SCF)-stimulated phosphorylation of STAT1, STAT3, and STAT5 (Figure 3) and the IL-6-stimulated nuclear translocation of STAT3 alpha (Figure 4).

Upon cytokine stimulation, STAT1, STAT3, and STAT5 are recruited to cytokine-activated receptor complexes through their SH2 domain binding to pY peptide motifs within these complexes. Once recruited, STATs are activated by phosphorylation on critical tyrosine residues (Tyr702, Tyr705, or Tyr694, respectively). Tyrosine phosphorylation of STATs is mediated by a Janus kinase (JAK), by receptor-associated tyrosine kinases such as PDGF, or by a nonreceptor tyrosine kinase such as Src. STAT tyrosine phosphorylation leads to its release from the receptor complex, followed by its dimerization through reciprocal SH2 domain binding by one partner to the other partner's phosphotyrosyl peptide motif.[22] To determine whether the ability of (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** to inhibit STAT3 activity was due to the interaction of (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** with the pY peptide binding domain within the SH2 binding domain of STAT3, SPR experiments were performed. As illustrated in Figure 5, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** only weakly inhibited STAT3 binding to its pY peptide ligand with IC_{50} values equal to 750 ± 227 and 138 ± 35 μM, respectively. Therefore, the ability of (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** to inhibit STAT3 activity cannot be attributed to blocking pY peptide ligand binding to the STAT3 SH2 domain.

The initial publication of Clardy and co-workers demonstrated that phaeosphaeride A was selective for STAT3, slightly active against STAT1 from U937 cells, and inactive against STAT5 from Nb2 cells in the ELISA-based assay.[8] However, the present work shows that diastereomers (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** are not selective STAT3 inhibitors and have the ability to inhibit cytokine-stimulated phosphorylation of the three STAT proteins. Neither (6*S*,7*S*,8*S*)-**1a** nor (6*R*,7*S*,8*S*)-**1b**, while showing activity in cell-based STAT assays, inhibits

Scheme 9



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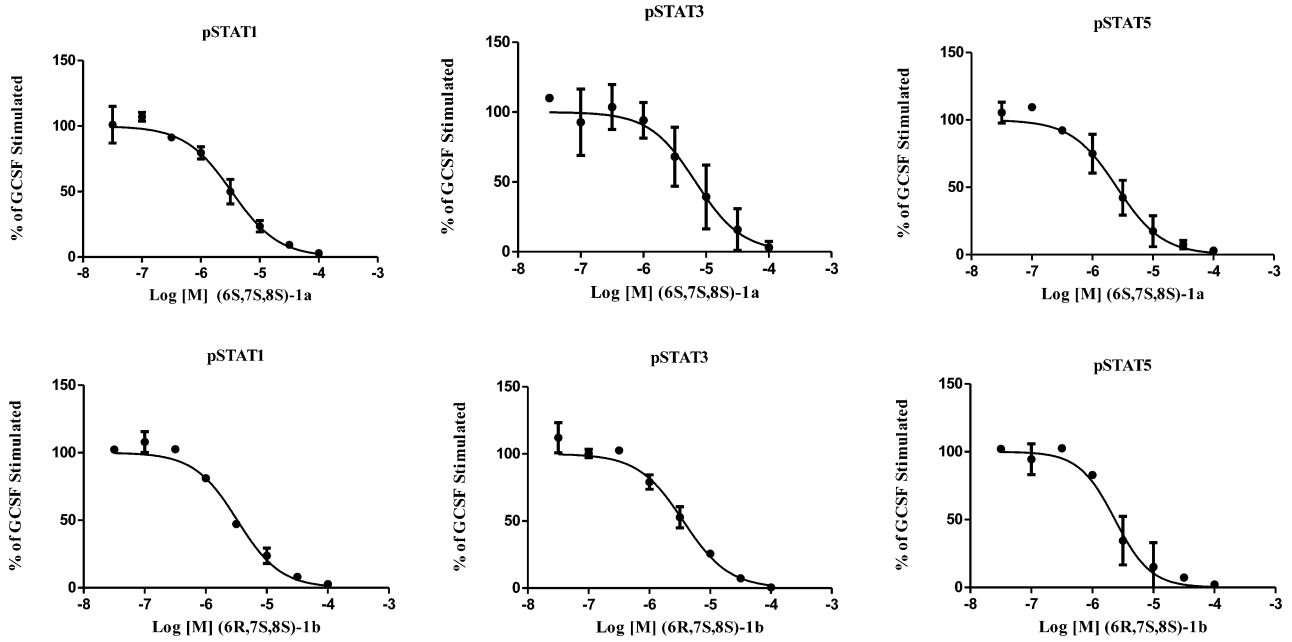

Figure 3. (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** inhibited the GCSF-stimulated phospho-STAT1, phospho-STAT3, and phospho-STAT5 in Kasumi-1 cells. A cell-based assay demonstrated the ability of (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** to inhibit GCSF-stimulated phospho-STAT1, phospho-STAT3, and phospho-STAT5. The IC_{50} values obtained were pSTAT1 at 3.4 ± 0.75 μM, pSTAT3 at 8.5 ± 6.5 μM, and pSTAT5 at 2.5 ± 1.6 μM for (6*S*,7*S*,8*S*)-**1a** and pSTAT1 at 3.0 ± 0.7 μM, pSTAT3 at 1.9 ± 0.8 μM, and pSTAT5 at 1.4 ± 0.9 μM for (6*R*,7*S*,8*S*)-**1b**.

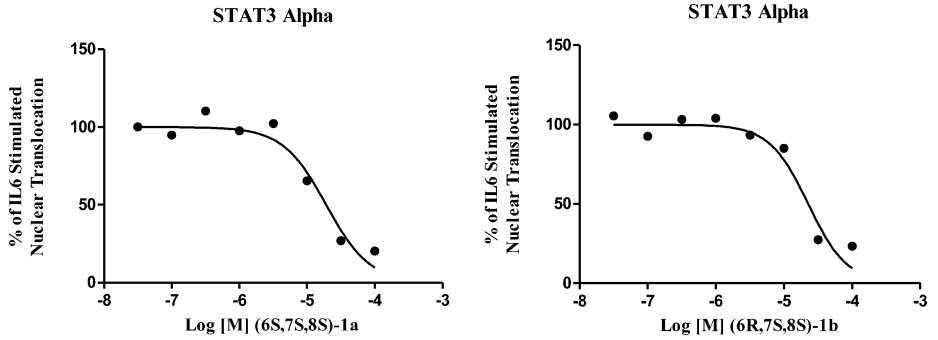

Figure 4. (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** inhibited STAT3 IL-6/sIL-6R-stimulated nuclear translocation in MEF/GFP-STAT3 alpha cells. Two cell-based assay studies examining IL-6/sIL-6R-stimulated nuclear translocation of STAT3 alpha demonstrated the ability of (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** to inhibit nuclear translocation. The IC_{50} values obtained were STAT3 alpha at 23 μM for (6*S*,7*S*,8*S*)-**1a** and 19 μM for (6*R*,7*S*,8*S*)-**1b**.

STAT3 by interacting with the SH2 binding domain and blocking its interaction with the pY peptide. As a conclusion, (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** are likely inhibitors of an upstream kinase in the STAT signaling pathway and do not block STAT3 dimerization by the blocking of the SH2 binding domain.

■ CONCLUSIONS

In summary, the first total synthesis of (±)-phaeosphaeride B has been completed. The C-6 epimer of the proposed structure of phaeosphaeride A, (6*R*,7*S*,8*S*)-**1b**, has also been synthesized. Although our attempts to access **1c** were not successful, based on the current synthesis and the previously reported spectroscopic data for phaeosphaeride A,[8] we propose the structural revision of phaeosphaeride A to compound **1c** or its enantiomer, which is also suggested by Tamura's group.[9b] Additionally, compounds (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** do not interact with the pY peptide binding pocket of the SH2 domain of STAT3 required for STAT3 dimerization, leading to the conclusion that (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** are likely upstream inhibitors of a kinase involved in the STAT signaling pathway.

■ EXPERIMENTAL SECTION

General Experimental Details. All reactions were carried out under an atmosphere of Ar unless otherwise specified. Commercial reagents of high purity were purchased and used without further purification, unless otherwise noted. Reactions were monitored by TLC and using UV light as a visualizing agent and aqueous ceric sulfate/phosphomolybdic acid, ethanolic *p*-anisaldehyde solution, potassium permanganate solution, and heat as developing agents. The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, and tetramethylsilane was used as internal standard. Chemical shifts are indicated in δ values (ppm) from internal reference peaks (TMS ^{1}H 0.00; $CDCl_3$ ^{1}H 7.26, ^{13}C 77.00; DMSO-d_6 ^{1}H 2.50, ^{13}C 39.51). Optical rotations were measured with a sodium lamp and are reported as follows: $[\alpha]_D^{°C}$ (c = g/100 mL, solvent). Melting points (mp) are uncorrected. High-resolution mass spectra (HRMS) were recorded on a mass spectrometer at a 4000 V emitter voltage.

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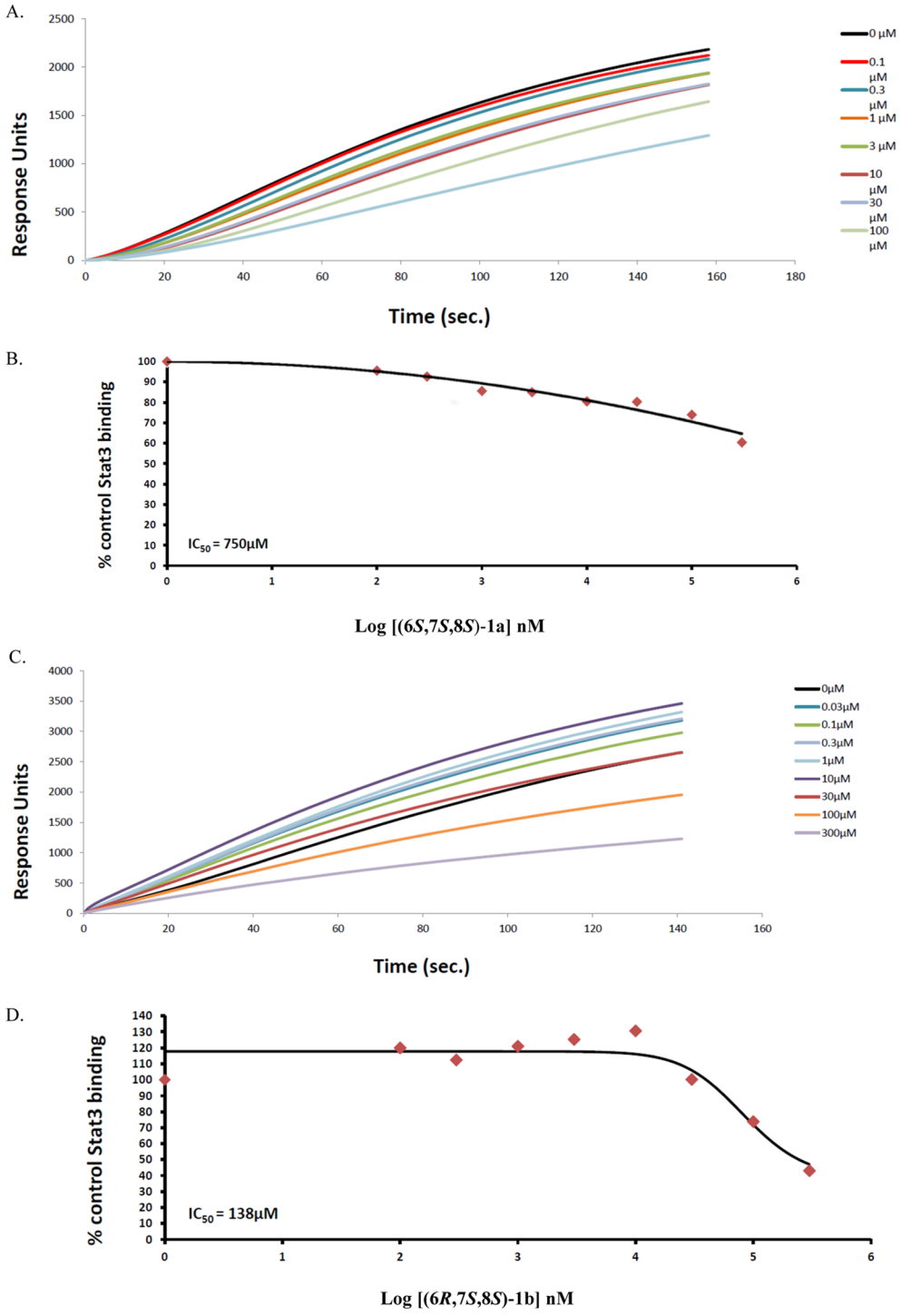

Figure 5. (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-**1b** do not target the phosphopeptide binding pocket within the STAT3 Src homology (SH)2 domain. SPR studies examining the ability of (6*S*,7*S*,8*S*)-**1a** to inhibit STAT3 binding to it phosphotyrosyl (pY) peptide ligand demonstrated an IC_{50} = 750 ± 227 μM in three separate experiments (representative experiment shown), indicating that the (6*S*,7*S*,8*S*)-**1a** does not inhibit STAT3 activation through interfering with STAT3 binding to its pY peptide ligand. Binding of recombinant STAT3 (200 nM) to a sensor chip coated with a phosphododecapeptide (amino acids surrounding Y1068 within EGFR) was measured in real time by SPR in the absence (0 μM) or presence of (6*S*,7*S*,8*S*)-**1a** (0.1 to 300 μM). (A) Response units as a function of time in seconds. (B) (6*S*,7*S*,8*S*)-**1a** equilibrium binding levels obtained, normalized (response obtained in the presence of compound ÷ the response obtained in the absence of compound × 100), and plotted against log [nM] (6*S*,7*S*,8*S*)-**1a** and IC_{50} value calculated. SPR studies examining the ability of (6*R*,7*S*,8*S*)-**1b** to inhibit STAT3 binding to it phosphotyrosyl (pY) peptide ligand demonstrated an IC_{50} 138 ± 35 μM in three separate experiments (representative experiment shown), indicating that the (6*R*,7*S*,8*S*)-**1b** does not inhibit STAT3 activation through interfering with STAT3 binding to its pY peptide ligand. Binding of recombinant STAT3 (200 nM) to a sensor chip coated with a phosphododecapeptide (amino acids surrounding Y1068 within EGFR) was measured in real time by SPR in the absence (0 μM) or presence of (6*S*,7*S*,8*S*)-**1a** (0.1 to 300 μM). (C) Response units as a function of time in seconds. (D) (6*R*,7*S*,8*S*)-**1b** equilibrium binding levels obtained, normalized (response obtained in the presence of compound ÷ the response obtained in the absence of compound × 100), and plotted against log [nM] (6*S*,7*S*,8*S*)-**1a** and IC_{50} value calculated.

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(*E*)-2-Methyloct-2-en-1-ol, 12. To a solution of ester **11** (7 g, 41.14 mmol) in CH_2Cl_2 (200 mL) was added a solution of DIBALH (1 M in CH_2Cl_2, 124 mL, 124 mmol) at −78 °C. After the reaction mixture had been stirred for 2 h at the same temperature, methanol was added. The mixture was allowed to warm to room temperature. Then, saturated aqueous potassium sodium tartrate was added to the solution. The mixture was extracted with CH_2Cl_2; the organic layer was washed with water and brine, dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 5/1) to give 5.5 g of alcohol **12** as colorless oil in 95% yield. **12**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.0 Hz, 3H), 1.27−1.36 (m, 6H), 1.63 (s, 3H), 2.01 (dd, J = 6.0, 7.5 Hz, 2H), 3.96 (s, 2H), 5.39 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 13.9, 22.5, 27.5, 29.1, 31.5, 68.9, 126.5, 134.5; FT-IR 1635, 2856, 2925, 2957, 3431; HRMS (ESI-TOF) m/z for $C_9H_{18}ONa$ $[M + Na]^+$ calcd 165.1255, found 165.1255.

(2-Methyl-3-pentyloxiran-2-yl)methanol, 13. To a solution of the olefin **12** (3.9 g, 27.42 mmol) in 200 mL of CH_2Cl_2 were added $NaHCO_3$ (9.2 g, 109.7 mmol) and *m*CPBA (70%, 10.14 g, 41.13 mmol) at 0 °C. The resulting solution was stirred at room temperature for 8 h. After this time, 10% $Na_2S_2O_3$ and saturated $NaHCO_3$ were added, and stirring was continued at room temperature for an additional 1 h. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with saturated $NaHCO_3$ and brine, then dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 3/1) to provide epoxide **13** in 97% yield (4.2 g). **13**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 6.0 Hz, 3H), 1.28 (s, 3H), 1.31−1.38 (m, 4H), 1.41−1.57 (m, 4H), 2.34 (br, 1H), 3.02 (t, J = 6.0 Hz, 1H), 3.55 (d, J = 12.2 Hz, 1H), 3.67 (d, J = 12.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 14.1, 22.4, 26.0, 28.0, 31.5, 60.3, 60.9, 65.6; FT-IR 1040, 2859, 2927, 2957, 3434; HRMS (ESI-TOF) m/z for $C_9H_{19}O_2$ $[M + H]^+$ calcd 159.1379, found 159.1374.

3-(Allyloxy)-2-methyloctane-1,2-diol, 14a. To a stirred solution of epoxy alcohol **13** (4.69 g, 29.65 mmol) and allyl alcohol (34.44 g, 593 mmol) was added titanium(IV) isopropoxide (12.65 g, 44.50 mmol) under Ar at room temperature. The reaction was stirred at 70 °C for 3 h. After this time, the mixture was allowed to cool to room temperature. The solvent was removed under reduced pressure, and the reaction mixture was diluted with Et_2O. Then, 10% NaOH solution (30 mL) was added, and the mixture was stirred vigorously overnight. Next, the mixture was filtered through a pad of Celite. The filtrate was extracted with ethyl acetate; the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (eluent; hexane/ethyl acetate = 3/1) gave 5.06 g (79% yield) of an inseparable mixture of the desired 1,2-diol and 1,3-diol derivative as a colorless oil (1,2-diol/1,3 diol = 4.2/1). Data of **14a** from the mixture **14a,b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.3 Hz, 3H), 1.08 (s, 3H), 1.30 (m, 6H), 1.51 (m, 2H), 2.79 (br, 2H), 3.31 (d, J = 8.3 Hz, 1H), 3.36 (d, J = 11.2 Hz, 1H), 3.76 (d, J = 11.2 Hz, 1H), 4.14 (m, 2H), 5.15 (m, 1H), 5.27 (m, 1H), 5.90 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 20.7, 22.5, 26.7, 31.0, 32.0, 67.4, 74.0, 74.3, 86.2, 116.7, 134.8; FT-IR 1373, 1448, 2873, 2963, 3411; HRMS (ESI-TOF) m/z for $C_{12}H_{25}O_3$ $[M + H]^+$ calcd 217.1798, found 217.1793.

3-(Allyloxy)-2-hydroxy-2-methyloctanal, 15. Alcohols **14a,b** (2 g, 9.25 mmol) were dissolved in dichloromethane (80 mL). Triethylamine (7.7 mL) was added, and the mixture was cooled to 0 °C. A solution of SO_3−pyridine (5.89 g, 37 mmol) in anhydrous dimethyl sulfoxide (14.8 mL) was added, and the reaction mixture was stirred at 0 °C for 1 h. The mixture was stirred at room temperature overnight and was then extracted with dichloromethane. The organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 10/1) to afford 1.42 g of aldehyde **15**. **15**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.0 Hz, 3H), 1.27 (s, 3H), 1.24 (m, 6H), 1.58 (m, 2H), 3.32 (m, 2H), 4.01 (m, 1H), 4.08 (m, 1H), 5.16 (d, J = 18.0 Hz, 1H), 5.22 (d, J = 9.0 Hz, 1H), 5.85 (m, 1H), 9.67 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 18.7, 22.4, 26.3, 30.7, 31.8, 73.5, 80.0, 84.4, 116.9, 134.4, 203.9; FT-IR 1457, 1728, 2862, 2952, 3450; HRMS (ESI-TOF) m/z for $C_{12}H_{23}O_3$ $[M + H]^+$ calcd 215.1642, found 215.1637.

3-(Allyloxy)-2-methyl-2-((trimethylsilyl)oxy)octanal, 16. To a stirred solution of alcohol **15** (0.98 g, 4.57 mmol) in CH_2Cl_2 (24 mL) was added 2,6-lutidine (1.1 mL, 9.14 mmol), followed by TMSOTf (0.86 mL, 4.79 mmol) at −78 °C. The resultant mixture was stirred at the same temperature for 30 min. After the addition of $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (eluent; hexane/ethyl acetate = 40/1) to afford aldehyde **16** (1.19 g, 91%) as a yellow oil. **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.14 (s, 9H), 0.88 (t, J = 6.0 Hz, 3H), 1.26 (m, 6H), 1.28 (s, 3H), 1.48 (m, 2H), 3.34 (t, J = 6.0 Hz, 1H), 4.05 (m, 2H), 5.13 (m, 1H), 5.24 (m, 1H), 5.84 (m, 1H), 9.60 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 2.2, 13.9, 18.5, 22.5, 26.2, 30.3, 31.9, 73.4, 82.9, 83.8, 116.4, 134.9, 204.1; FT-IR 1088, 1250, 1734, 2857, 2924, 2952; HRMS (ESI-TOF) m/z for $C_{15}H_{30}O_3SiNa$ $[M + Na]^+$ calcd 309.1856, found 309.1859.

3-(3-(Allyloxy)-2-hydroxy-2-methyl-1-(trimethylsilyloxy)-octyl)-4-methoxy-5-methylenefuran-2(5*H*)-one, 18a, and 3-(3-(Allyloxy)-1-hydroxy-2-methyl-2-(trimethylsilyloxy)octyl)-4-methoxy-5-methylenefuran-2(5*H*)-one, 18b. Diisopropylamine (1 mL, 7.26 mmol) was dissolved in THF (6.0 mL) and cooled to −78 °C in a dry ice/acetone bath for 15 min. To this solution was added *n*BuLi (4.55 mL, 1.6 M in hexanes) dropwise over 2 min, and the deprotonation was allowed to proceed for 1 h. Tetronate (832 mg, 6.60 mmol) dissolved in a mixture of THF (6.0 mL) was added dropwise to the LDA solution over 6 min during which the solution turned lemon yellow. The reaction was stirred for exactly 5 min after the addition was complete, during which the color of the reaction darkened to light brown. Aldehyde **16** (630 mg, 2.20 mmol) dissolved in THF (6.0 mL) was added slowly to the lithiated tetronate over 5 min, and the reaction was allowed to proceed for 3 h. The reaction was quenched at −78 °C with a saturated aqueous NH_4Cl solution (20 mL) and allowed to reach room temperature. The mixture was extracted with AcOEt (20 mL), and the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 15/1) to afford 174 mg of A and 305 mg of B (60% total yield) accompanied by recovered starting materials [74 mg of aldehyde (0.26 mmol) and 151 mg of ester (1.2 mmol)]. **18a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.07 (s, 9H), 0.90 (t, J = 6.0 Hz, 3H), 1.26 (m, 6H), 1.30 (s, 3H), 1.66 (m, 2H), 3.35 (d, J = 12.0 Hz, 1H), 4.13 (m, 2H), 4.18 (s, 3H), 4.59 (d, J = 12.0 Hz, 1H), 5.08 (m, 3H), 5.25 (m, 1H), 5.91 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 2.3, 14.0, 18.8, 22.6, 26.6, 30.8, 32.2, 59.8, 69.3, 74.5, 82.5, 85.5, 92.9, 103.0, 115.9, 135.3, 149.6, 162.7, 171.5; FT-IR 1090, 1277, 1378, 1457, 1627, 2850, 2920, 2955, 3467; HRMS (ESI-TOF) m/z for $C_{21}H_{37}O_6Si$ $[M + H]^+$ calcd 413.2354, found 413.2359. **18b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.1 (s, 9H), 0.88 (t, J = 6.7 Hz, 3H), 1.23 (s, 3H), 1.26−1.39 (m, 6H), 1.43−1.69 (m, 2H), 3.16 (m, 1H), 3.92 (m, 1H), 4.08 (m, 1H), 4.21 (s, 3H), 4.86 (s, 1H), 5.03 (m, 3H), 5.19 (m, 1H), 5.85 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.0, 14.0, 21.0, 22.5, 27.6, 30.4, 32.2, 61.0, 69.0, 72.6, 77.8, 85.0, 93.2, 108.4, 115.9, 135.0, 149.7, 163.3, 169.0; FT-IR 1091, 1121, 1264, 1375, 1456, 1635, 2932, 2958, 3446; HRMS (ESI-TOF) m/z for $C_{21}H_{37}O_6Si$ $[M + H]^+$ calcd 413.2354, found 413.2358.

3-(3-(Allyloxy)-1,2-dihydroxy-2-methyloctyl)-4-methoxy-5-methylenefuran-2(5*H*)-one, 19. Compounds **18a** and **18b** (140 mg, 0.34 mmol) were dissolved in THF (3.4 mL) at 0 °C. Then TBAF (0.51 mmol, 1 M solution in THF) was added, and the mixture was stirred at the same temperature for 1 h. After the addition of saturated $NaHCO_3$ solution (2 mL), the reaction mixture was extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 5/1) to afford compound **19** (110 mg, 95%) as a yellow oil. **19**: ^{1}H NMR (300

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MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.26 (s, 3H), 1.29−1.50 (m, 6H), 1.51−1.76 (m, 2H), 2.45 (br, 1H), 3.22 (dd, J = 6.7, 4.3 Hz, 1H), 3.80 (m, 1H), 3.83 (s, 1H), 4.09 (dd, J = 12.4, 5.2 Hz, 1H), 4.20 (s, 3H), 4.87 (d, J = 9.9 Hz, 1H), 5.11 (s, 3H), 5.20 (dd, J = 17.2, 1.4 Hz, 1H), 5.84 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 19.9, 22.5, 27.0, 30.0, 32.2, 60.2, 68.4, 72.3, 77.0, 83.5, 94.0, 105.9, 116.5, 134.6, 149.8, 163.3, 171.8; FT-IR 1119, 1146, 1343, 1372, 1463, 1654, 1738, 2851, 2919, 2955, 3438; HRMS (ESI-TOF) m/z for $C_{18}H_{29}O_6$ $[M + H]^+$ calcd 341.1959, found 341.1969.

3-(3-(Allyloxy)-1,2-dihydroxy-2-methyloctyl)-5-hydroxy-1,4-dimethoxy-5-methyl-1*H*-pyrrol-2(5*H*)-one, 20. A stirred suspension of *O*-Me-hydroxylamine hydrochloride (15 mg, 0.175 mmol) in dry THF (1.6 mL) at −78 °C and under nitrogen atmosphere was treated with a 1 M solution of LiHMDS (1.25 mL, 1.25 mmol). After 10 min, a solution of the ester **19** (40 mg, 0.117 mmol) in a minimum amount of dry THF was added. After being stirred for 2 h at −78 °C, the reaction was quenched with a saturated aqueous solution of NH_4Cl, warmed to room temperature, and extracted with AcOEt. The collected organic layers were dried, filtered, and concentrated under reduced pressure, and the residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 1/1) to give 43 mg of compounds **20** (95%). **20**: FT-IR 1113, 1202, 1348, 1373, 1655, 2862, 2935, 2957, 3204, 3473; HRMS (ESI-TOF) m/z for $C_{19}H_{34}NO_7$ $[M + H]^+$ calcd 388.233, found 388.2336.

5-Hydroxy-1,4-dimethoxy-5-methyl-3-(1,2,3-trihydroxy-2-methyloctyl)-1*H*-pyrrol-2(5*H*)-one, 21. Allylether **20** (40 mg, 0.10 mmol) was dissolved in MeOH/CH_2Cl_2 1/1 (3 mL), and $PdCl_2$ (21 mg, 0.12 mmol) was added. The dark brown suspension was stirred at room temperature for 3 h. The mixture was filtered over a pad of Celite; a saturated NaCl solution (3 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (4 × 10 mL). The organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 1/1) to afford compound **21** (7 mg, 20%) as a yellow oil. **21**: FT-IR 1060, 1116, 1348, 1376, 1460, 1639, 1650, 2857, 2924, 3361; HRMS (ESI-TOF) m/z for $C_{16}H_{30}NO_7$ $[M + H]^+$ calcd 348.2017, found 348.2016.

(±)-Phaeosphaeride B, 1d. Compounds **21** (66 mg, 0.19 mmol) were dissolved in THF (2 mL). Then TBAF (0.38 mmol, 1 M solution in THF) was added, and the mixture was stirred at 60 °C for 45 min. After the addition of saturated $NaHCO_3$ solution (3 mL), the reaction mixture was extracted with AcOEt. The organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The mixture was used to the next step without further purification. Compounds **22** were dissolved in toluene (9 mL). Then *p*-TSA monohydrate (79 mg, 0.42 mmol) was added, and the mixture was stirred at 50 °C for 30 min (control by TLC). The reaction was allowed to cool to rt and treated with 0.2 mL of Et_3N. The mixture was extracted with dichloromethane, and the organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (eluent; hexane/ethyl acetate = 1/1) to afford 13 mg of compound (±)-**1d** in 23% yield over two steps. (±)-**1d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 3H), 1.08 (s, 3H), 1.35 (5H, m), 1.60 (m, 2H), 1.88 (m, 1H), 3.40 (s, 1H), 3.91 (s, 3H), 4.01 (d, J = 10.2 Hz, 1H), 5.03 (d, J = 1.6 Hz, 1H), 5.09 (d, J = 1.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 18.0, 22.5, 26.1, 27.5, 31.6, 64.3, 64.4, 70.0, 82.0, 92.6, 104.0, 136.4, 158.1, 166.3; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89 (m, 6H), 1.24−1.33 (m, 5H), 1.55 (m, 2H), 1.86 (m, 1H), 3.76 (1H overlap with MeO), 3.79 (s, 3H), 3.98 (dd, J = 1.8 Hz, 10.0 Hz, 1H), 5.01 (d, J = 3 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7, 17.9, 21.8, 25.3, 26.7, 30.9, 63.6, 63.8, 69.6, 80.5, 91.4, 105.0, 136.2, 156.9, 165.3; FT-IR 1016, 1447, 1634, 1695, 2920, 3449; HRMS (ESI-TOF) m/z for $C_{15}H_{24}NO_5$ $[M + H]^+$ calcd 298.1649, found 298.1658.

Methyl 4-Methyl-5-pentyl-1,3,2-dioxathiolane-4-carboxylate 2,2-dioxide, 24. To a solution of **23** (1 g, 4.9 mmol) in methylene chloride (12 mL) were added triethylamine (2.5 mL, 17.64 mmol) and thionyl chloride (0.5 mL, 7.35 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min and partitioned between methylene chloride and water. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The cyclic sulfites obtained were oxidized as outlined in the following paragraph directly without purification.

To the remaining organic phase containing cyclic sulfites were added MeCN (50 mL) and solid $NaHCO_3$ (3.92 g, 49 mmol). The mixture was cooled to 0 °C, and bleach (NaOCl solution, ∼5% aqueous solution, 9.8 mmol) was added slowly. The resulting reaction mixture was stirred at room temperature for 8 h. After completion of the reaction, the mixture was cooled to 0 °C and quenched with aqueous Na_2SO_3 solution. Then, the mixture was diluted with ethyl acetate (50 mL). The organic layer was washed with $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography on silica gel (eluent; ethyl acetate/hexanes = 1/4) to afford the cyclic sulfate **24** (1.23 g, 94% over two steps) as a colorless oil. **24**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, J = 6.0 Hz, 3H), 1.34 (m, 4H), 1.40−1.60 (m, 2H), 1.70 (s, 3H), 1.73−1.93 (m, 2H), 3.87 (s, 3H), 5.04 (dd, J = 2.9, 10.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 18.2, 22.2, 25.4, 28.8, 31.0, 53.7, 86.5, 89.1, 168.1; FT-IR 822, 942, 1125, 1209, 1378, 1628, 1743, 2865, 2927, 2954; HRMS (ESI-TOF) m/z for $C_{10}H_{19}O_6S$ $[M + H]^+$ calcd 267.0897, found 267.0896.

Methyl 3-Acetoxy-2-hydroxy-2-methyloctanoate, 25. Sodium acetate (277 mg, 3.38 mmol) was added to a solution of the cyclic sulfate **24** (300 mg, 1.127 mmol) in dimethylformamide (6 mL) at rt. The solution was the heated in an oil bath at 70 °C for 16 h. The heating bath was removed. After being cooled to 23 °C, the product solution was concentrated. The residue was dissolved in tetrahydrofuran (5 mL) at 23 °C, and 20% aqueous sulfuric acid (0.6 mL) was added to the resulting solution. The mixture was stirred at 23 °C for 2 h. The product solution was partitioned between CH_2Cl_2 (30 mL) and water (30 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (eluent; ethyl acetate/hexane = 1/2) to provide **25** (222 mg, 80%) as a colorless oil. **25**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.0 Hz, 3H), 1.27 (m, 6H), 1.36 (s, 3H), 1.37 (m, 1H), 1.67 (m, 1H), 2.11 (s, 3H), 3.25 (s, 1H), 3.80 (s, 3H), 5.13 (dd, J = 2.6, 10.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 20.8, 22.1, 22.3, 25.3, 29.2, 31.4, 53.0, 76.1, 76.4, 170.7, 175.5; FT-IR 1227, 1370, 1632, 1734, 2856, 2954, 3450; HRMS (ESI-TOF) m/z for $C_{12}H_{22}O_5$ $[M + H]^+$ calcd 247.154, found 247.1539.

Methyl 2,3-Dihydroxy-2-methyloctanoate, 26. Potassium carbonate (6 mg, 0.043 mmol) was added to a solution of **25** (105 mg, 0.43 mmol) in methanol (4.2 mL) at rt. The reaction mixture was the heated in an oil bath at 60 °C for 2 h. The product solution was partitioned between saturated aqueous sodium chloride solution (20 mL) and CH_2Cl_2 (30 mL). The layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography on silica gel (eluent; ethyl acetate/hexane = 1/2) to provide **26** (83 mg, 95%) as a colorless oil. **26**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.0 Hz, 3H), 1.26−1.40 (m, 6H), 1.43 (s, 3H), 1.58 (m, 2H), 2.66 (br, 1H), 3.56 (d, J = 9.7, 2.3 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.4, 22.5, 25.9, 31.6, 31.8, 52.7, 76.2, 77.4, 176.0; FT-IR 1063, 1103, 1249, 1383, 1459, 1739, 2847, 2932, 2949, 3452; HRMS (ESI-TOF) m/z for $C_{10}H_{21}O_4$ $[M + H]^+$ calcd 205.1434, found 205.1435.

Methyl 2-Methyl-2,3-bis((trimethylsilyl)oxy)octanoate, 27. To a stirred solution of alcohol **26** (212 mg, 1.04 mmol) in CH_2Cl_2 (5.5 mL) was added 2,6-lutidine (0.7 mL, 6.24 mmol), followed by TMSOTf (0.75 mL, 4.16 mmol) at −78 °C. The resultant mixture was stirred at the same temperature for 10 min and then allowed to warm over 1 h to 0 °C. After the addition of $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash chromatography on silica gel (eluent; hexane/ethyl acetate = 20/1) to afford ester **27** (330 mg, 91%) as a colorless oil. **27**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 9H), 0.10 (s, 9H), 0.89 (t, J =

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6.9 Hz, 3H), 1.21−1.32 (m, 6H), 1.37 (s, 3H), 1.52 (m, 2H), 3.69 (s, 3H), 3.82 (dd, J = 3.0, 8.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.6, 1.7, 14.0, 20.3, 22.6, 26.3, 31.6, 31.9, 51.7, 77.9, 80.4, 174.9; FT-IR 742, 836, 1116, 1258, 1472, 1752, 2945; HRMS (ESI-TOF) m/z for $C_{16}H_{37}O_4Si_2$ $[M + H]^+$ calcd 349.2225, found 349.2223.

2-Methyl-2,3-bis((trimethylsilyl)oxy)octan-1-ol, S1. To a solution of ester **27** (285 mg, 0.819 mmol) in dichloromethane (5 mL) was added a solution of DIBALH (1 M in dichloromethane, 2.0 mL, 2.05 mmol) at −78 °C. After the reaction mixture had been stirred for 1 h at the same temperature, methanol was added. The mixture was allowed to warm to room temperature. Then, saturated aqueous potassium sodium tartrate was added to the solution. The mixture was extracted with dichloromethane, and the organic layer was washed with water and brine, dried over sodium sulfate, and the solvent was evaporated. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 10/1) to give 252 mg of alcohol **S1** as colorless oil in 96% yield. **S1**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.13 (s, 9H), 0.15 (s, 9H), 0.90 (t, J = 6.9 Hz, 3H), 1.11 (s, 3H), 1.24−1.35 (m, 6H), 1.46−1.58 (m, 2H), 2.22 (dd, J = 4.2, 7.7 Hz, 1H), 3.34 (dd, J = 4.2, 11.0 Hz, 1H), 3.55−3.62 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.6, 2.5, 14.0, 19.4, 22.6, 26.8, 31.9, 32.0, 68.4, 76.9, 78.6; FT-IR 836, 1094, 1129, 1249, 1370, 1450, 2860, 2932, 2954, 3461; HRMS (ESI-TOF) m/z for $C_{15}H_{37}O_3Si_2$ $[M + H]^+$ calcd 321.2276, found 321.2276.

2-Methyl-2,3-bis((trimethylsilyl)oxy)octanal, 28. Alcohol **S1** (276 mg, 0.862 mmol) was dissolved in dichloromethane (7 mL). Triethylamine (0.7 mL) was added, and the mixture was cooled to 0 °C. A solution of SO_3−pyridine (550 mg, 3.45 mmol) in anhydrous dimethyl sulfoxide (1.4 mL) was added, and the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 5 h. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography (eluent; pentane/ether = 5/1) to afford 253 mg of aldehyde **28** in 92% yield. **28**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (s, 9H), 0.13 (s, 9H), 0.88 (t, J = 7.0 Hz, 3H), 1.18−1.28 (m, 4H), 1.23 (s, 3H), 1.34−1.48 (m, 4H), 3.65 (dd, J = 2.9, 7.2 Hz, 1H), 9.55 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.6, 2.3, 14.0, 18.2, 22.5, 26.1, 31.7, 77.4, 82.8, 205.1 (one carbon is missing due to overlapping); FT-IR 749, 849, 911, 1249, 1392, 1450, 1641, 1734, 2856, 2918, 2949; HRMS (ESI-TOF) m/z for $C_{15}H_{35}O_3Si_2$ $[M + H]^+$ calcd 319.2119, found 319.2119.

3-(1-Hydroxy-2-methyl-2,3-bis(trimethylsilyloxy)octyl)-4-methoxy-5-methylenefuran-2(5*H*)-one, 29a, and 3-(5-Hydroxy-2,2,5,8,8-pentamethyl-6-pentyl-3,7-dioxa-2,8-disilanonan-4-yl)-4-methoxy-5-methylenefuran-2(5*H*)-one, 29b. Diisopropylamine (0.34 mL, 2.39 mmol) was dissolved in THF (1.5 mL) and cooled to −78 °C in a dry ice/acetone bath for 15 min. To this solution was added *n*BuLi (1.5 mL, 1.6 M in hexanes) dropwise over 2 min, and the deprotonation was allowed to proceed for 1 h. Tetronate (302 mg, 2.39 mmol) dissolved in a mixture of THF (1.8 mL) was added dropwise to the LDA solution over 6 min during which the solution turned lemon yellow. The reaction was stirred for exactly 5 min after the addition was complete, during which the color of the reaction darkened to light brown. Aldehyde **28** (253 mg, 0.795 mmol) dissolved in THF (1.8 mL) was added slowly to the lithiated tetronate over 5 min, and the reaction was allowed to proceed for 3 h. The reaction was quenched at −78 °C with water and allowed to reach room temperature. The mixture was extracted with AcOEt (3 × 10 mL), and the combined organic extracts were dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 10/1) to afford 62 mg of A and 96 mg of B in 51% total yield accompanied by recovered starting materials [30 mg of aldehyde (0.1 mmol) and 51 mg of ester (0.4 mmol)]. **29a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 9H), 0.13 (s, 9H), 0.89 (t, J = 6.8 Hz, 3H), 1.22 (s, 3H), 1.27 (m, 6H), 1.53 (m, 2H), 3.81 (t, J = 4.3 Hz, 1H), 4.19 (s, 3H), 4.66 (d, J = 10.8 Hz, 1H), 4.98 (d, J = 10.8 Hz, 1H), 5.08 (d, J = 2.6 Hz, 1H), 5.11 (d, J = 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.8, 2.4, 14.0, 19.2, 22.6, 26.7, 32.0, 32.3, 59.7, 69.7, 77.4, 82.5, 93.0, 102.9, 149.5, 162.7, 171.6; FT-IR 822, 1040, 1103, 1245, 1281, 1454, 1637, 1748, 2851, 2914, 2945, 3470; HRMS (ESI-TOF) m/z for $C_{21}H_{41}O_6Si_2$ $[M + H]^+$ calcd 445.2436, found 445.2435. (+)-**29b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.09 (s, 9H), 0.10 (s, 9H), 0.89 (t, J = 6.8 Hz, 3H), 1.19 (s, 3H), 1.29 (m, 6H), 1.50 (m, 2H), 2.71 (s, 1H), 3.53 (dd, J = 9.7, 2.5 Hz, 1H), 4.24 (s, 3H), 4.84 (s, 1H), 5.03 (d, J = 2.6 Hz, 1H), 5.05 (d, J = 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −0.02, 0.5, 14.0, 21.3, 22.6, 27.2, 31.9, 32.0, 61.4, 68.4, 77.7, 80.1, 92.7, 108.8, 149.9, 163.0, 169.0; FT-IR 840, 867, 1040, 1249, 1281, 1459, 1619, 1770, 2851, 2918, 2949, 3471; HRMS (ESI-TOF) m/z for $C_{21}H_{41}O_6Si_2$ $[M + H]^+$ calcd 445.2436, found 445.2436.

5-Hydroxy-1,4-dimethoxy-5-methyl-3-(1,2,3-trihydroxy-2-methyloctyl)-1*H*-pyrrol-2(5*H*)-one, 21. A stirred suspension of *O*-Me-hydroxylamine hydrochloride (47 mg, 0.567 mmol) in dry THF (5.1 mL) at −78 °C and under nitrogen atmosphere was treated with a 1 M solution of LiHMDS (3.45 mmol, 3.45 mL). After 10 min, a solution of the mixture **29a** and **29b** (168 mg, 0.378 mmol) in a minimum amount of dry THF was added. After 2 h stirring at −78 °C, the reaction was quenched with a saturated aqueous solution of NH_4Cl, warmed to room temperature, and extracted with AcOEt. The collected organic layers were dried, filtered, and concentrated under reduced pressure. Compounds **30** were used to the next step without further purification. **30**: HRMS (ESI-TOF) m/z for $C_{22}H_{46}NO_7Si_2$ $[M + H]^+$ calcd 492.2807, found 492.2804. Compounds **30** (0.378 mmol) were dissolved in THF (4.5 mL). Then TBAF (1.1 mmol, 1 M solution in THF) was added, and the mixture was stirred at room temperature for 1 h. After the addition of saturated $NaHCO_3$ solution (3 mL), the reaction mixture was extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 10/1) to give 120 mg of alcohols **21** in 92% yield over two steps.

(2*S*,3*R*,4*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[3,2*c*]pyrrol-4-yl methanesulfonate, 31. Compound (6*S*,7*S*,8*S*)-**1a**[9] (40 mg, 0.134 mmol) was dissolved in CH_2Cl_2 (2.8 mL) and cooled to 0 °C. Et_3N (66 μL, 0.469 mmol) was added followed by dropwise addition of methanesulfonyl chloride (26 μL, 0.335 mmol). The mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of saturated $NaHCO_3$ solution, and the mixture was extracted with dichloromethane. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo after filtration. Mesylate **31** was used to the next step without further purification. An analytical sample was obtained by flash chromatography on silica gel eluted with hexane/ethyl acetate = 4/1 as a light yellow oil. **31**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 6.8 Hz, 3H), 1.26 (s, 3H), 1.38 (m, 4H), 1.73 (m, 4H), 3.13 (s, 3H), 3.93 (s, 3H), 4.33 (dd, J = 2.1, 10.2 Hz, 1H), 4.48 (s, 1H), 5.06 (s, 1H), 5.11 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.0, 25.0, 27.5, 29.6, 31.6, 53.2, 64.5, 72.1, 80.7, 92.5, 103.3, 136.2, 157.1, 164.4; FT-IR 998, 1090, 1174, 1270, 1315, 1376, 1446, 1633, 1705, 2856, 2930, 2958, 3452.

(6*R*,7*S*,8*S*)-1b. The crude mesylate was dissolved in dioxane/H_2O (1:1, 0.6 mL) and treated with NaOAc (33 mg, 0.402 mmol) at room temperature for 12 h. The dioxane was evaporated, and the residue was extracted with dichloromethane. After the addition of water (2 mL), the reaction mixture was extracted with CH_2Cl_2. The organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 1/1) to give 32 mg of (6*R*,7*S*,8*S*)-**1b** in 80% yield over two steps. (6*R*,7*S*,8*S*)-**1b**: $[\alpha]_D^{25}$ −13.5 (*c* 2.00, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 6.7 Hz, 3H), 1.28−1.36 (m, 4H), 1.36 (s, 3H), 1.58−1.86 (m, 4H), 2.95 (br, 1H), 3.91 (s, 3H), 4.10 (dd, J = 2.3, 10.2 Hz, 1H), 4.14 (s, 1H), 5.04 (s, 1H), 5.10 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 21.3, 22.5, 25.3, 27.4, 31.6, 64.4, 65.3, 71.3, 81.7, 92.3, 104.1, 136.5, 157.6, 166.3; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (m, 3H), 1.15 (s, 3H), 1.24−1.32 (m, 5H), 1.60 (m, 2H), 1.77 (m, 1H), 3.67 (d, J = 6.2 Hz, 1H), 3.78 (s, 3H), 3.95 (dd, J = 2.1, 10.4 Hz, 1H), 4.70 (s, 1H), 4.97 (s, 2H), 5.35 (d, J = 6.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7, 21.8, 24.6, 26.8, 31.0, 63.5, 63.9, 70.2, 80.4, 90.6, 104.7, 136.9, 157.0, 166.4 (one signal missing due to peak overlapping); FT-

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IR 1020, 1637, 1704, 2929, 3436; HRMS (ESI-TOF) m/z for $C_{15}H_{24}NO_5$ $[M + H]^+$ calcd 298.1649, found 298.1654.

Surface Plasmon Resonance (SPR) Assay. SPR assays were performed as described.[23] Briefly, STAT3 protein was diluted to a concentration of 200 nM in SPR buffer (20 mM Tris buffer, 5% DMSO, pH 7.5) and incubated with (6*S*,7*S*,8*S*)-**1a** or (6*R*,7*S*,8*S*)-**1b** with a concentration range of 0−300 μM at 4 °C for 2 h). Tyrosine phosphorylated and nonphosphorylated biotinylated dodecapeptide based on the STAT3 peptide binding motif within the EGFR at position Y1068 were immobilized onto the surface of two separate channels of a streptavidin chip. Binding of STAT3 to peptides was measured using a biosensor at 25 °C at a flow rate of 10 μL/min for 3 min. The binding curve obtained in the channel containing immobilized phosphorylated peptide was normalized by subtracting the binding measured in the channel containing immobilized nonphosphorylated peptide. IC_{50} values were determined by plotting % maximum response as a function of log concentration of (6*S*,7*S*,8*S*)-**1a** or (6*R*,7*S*,8*S*)-**1b** and fitting the experimental points to a competitive binding model using a four-parameter logistic equation optimized by the Marquardt−Levenberg algorithm (BIAevaluation Software version 4.1).

Assay of Phospho-STAT Inhibition. Kasumi-1 cells (ATCC CRL-2724) were maintained in culture in RPMI1640, 10% FBS, 37 °C, 5% CO_2. The cells were centrifuged and resuspended in RPMI 1640 alone at a density of 2×10^6 cells/mL to serum starved for 1 h. Cells were plated at 100 μL, pretreated with 80 μL of (6*S*,7*S*,8*S*)-**1a** or (6*R*,7*S*,8*S*)-**1b** in DMSO or DMSO (0.7% final) for 1 h, and then stimulated with 20 μL of GCSF (10 ng/mL) for 15 min at 37 °C. The assay was stopped by the addition of 300 μL of ice-cold PBS, and samples were immediately centrifuged at 300*g* for 5 min at 4 °C. Analysis of phospho-STAT inhibition was performed using the Milliplex MAP kit #48-610. Medium was aspirated, and 30 μL of lysis buffer was added. The lysates were diluted 1:1 with assay buffer and plated in each well of a 96-well filter plate preloaded with beads coupled to antibody against the indicated analytes. Plates were incubated overnight at 4 °C with shaking (750 rpm). Bead-bound analytes were measured using biotinylated detection antibody specific for a different epitope and streptavidin−phycoerythrin. Data were collected and analyzed using the Bio-Plex suspension array system. GAPDH-normalized pSTAT1, pSTAT3, and pSTAT5 values from each treatment were corrected for untreated cells, expressed as percentage untreated, and used to determine the IC_{50} using nonlinear regression (log inhibitor vs response), GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

High-Throughput Fluorescence Microscopy (HTFM) Screening Assay. A robust, single-cell, HTFM screening assay was established using GFP-tagged STAT3 alpha, stably expressed (>80% pos) in STAT3−/− MEF cells,[21,24] and MEF/GFP-STAT3 alpha were plated at a density of 1×10^4 cells/well on CC2-coated glass-bottomed 96-well and incubated overnight. Cells were treated with 50 μL (6*S*,7*S*,8*S*)-**1a** or (6*R*,7*S*,8*S*)-**1b** in DMSO or DMSO (0.7% final) at 0.1/0.3/1/3/10/30/100 mM (final) for 1 h and then with 50 μL of IL-6 and sIL-6Ra (150 ng/mL final) for 30 min. The cells were fixed with 4% formaldehyde in PEM buffer (80 mM potassium PIPES, pH 6.8, 5 mM EGTA pH 7.0, 2 mM $MgCl_2$, 30′), counterstained (30′) with DAPI in PEM buffer/0.1% Triton X-100 and then PEM. The cells were imaged by automated microscopy using an image cytometer platform and Cytoshop version 2.1 analysis software (Beckman Coulter). Nuclear localization was measured by using the fraction localized in the nucleus (FLIN).[25] FLIN values were normalized by subtracting the FLIN for unstimulated cells then dividing this difference by the maximum difference (delta, Δ) in FLIN (FLIN in cells stimulated with IL-6/sIL-6R in the absence of compound minus FLIN of unstimulated cells). This ratio was multiplied by 100 to obtain the maximum translocation. The best-fitting curve and IC_{50} value were determined using nonlinear regression (log inhibitor vs response), GraphPad Prism.

■ ASSOCIATED CONTENT

Supporting Information

Characterization data of the described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sarli@chem.auth.gr.

Notes

The authors declare no competing financial interest.

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