

Review

Total Synthesis and Biological Evaluation of Phaeosphaerides

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Abstract: This article reviews studies regarding the total synthesis of phaeosphaerides A and B, nitrogen-containing bicyclic natural products isolated from an endophytic fungus. Numerous synthetic efforts and an X-ray crystal structure analysis of phaeosphaeride A have enabled revision of its originally proposed structure. In addition, a successful protic acid-mediated transformation of phaeosphaeride A to phaeosphaeride B revealed the hypothetical biosynthesis of phaeosphaeride B from phaeosphaeride A. Structure–activity relationship studies of phaeosphaeride derivatives are also discussed.

Keywords: phaeosphaeride A; phaeosphaeride B; total synthesis; structural revision; STAT3; anticancer

1. Introduction

Signal transducer and activator of transcription 3 (STAT3), which belongs to the STAT family of proteins [1], regulates cell proliferation, differentiation, and survival [2]. Non-activated STAT3 is generally localized in the cytoplasm. Once phosphorylated at Tyr705 in the Janus kinase (JAK)/STAT signaling pathway, STAT3 dimerizes, translocates to the nucleus, and binds to a target DNA sequence to induce transcriptional activation [3,4].

Unusual activation of STAT3 is frequently found in various types of tumor cells, leading to apoptosis resistance and tumor cell proliferation via enhanced expression of gene encoding proteins such as Bcl-2, Bcl-xL, and cyclin D1 [5,6]. Therefore, STAT3 has gained considerable interest as a potential target for anticancer therapy. In fact, various STAT3 inhibitors including synthetic small molecules and natural products have been evaluated as prospective anticancer chemotherapeutic agents [7].

In 2006, phaeosphaerides A (proposed structure **1a**) was isolated from the endophytic fungus FA39 (*Phaeosphaeria avenaria*) by Clardy and co-workers as an inhibitor of STAT3-DNA binding (Figure 1) [8]. Phaeosphaeride A possesses a bicyclic structure with three contiguous stereocenters in its dihydropyran ring. Phaeosphaeride A inhibits STAT3 activity with an IC₅₀ of 0.61 mM and also inhibits cell growth in STAT3-dependent U266 multiple myeloma cells with an IC₅₀ of 6.7 μM. Therefore, phaeosphaeride A is expected to be a potential lead compound for anticancer drug candidates. On the other hand, phaeosphaeride B (**1b**), the C-8 stereoisomer of phaeosphaeride A, was reported to have no STAT3 inhibitory activity.

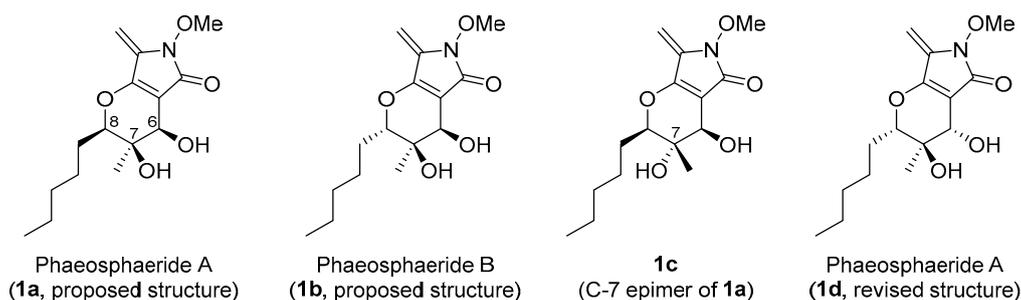


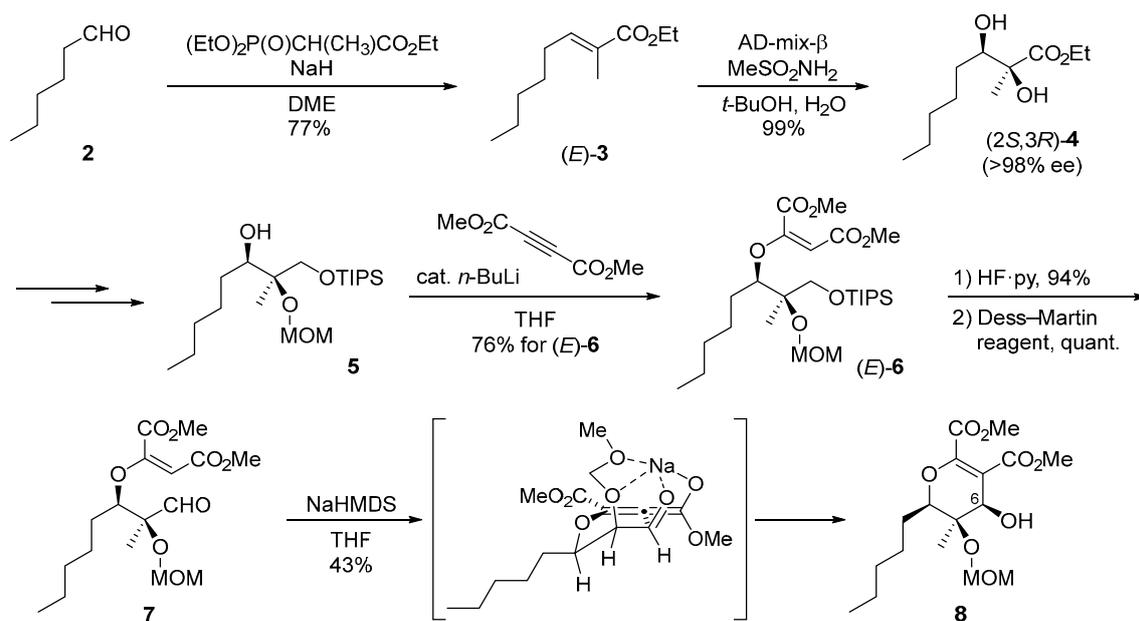
Figure 1. Structures of phaeosphaerides.

The promising biological activity of phaeosphaeride A as well as its simple and unique molecular structure has attracted much attention from the synthetic community. In addition, structure–activity relationship (SAR) studies of STAT3 inhibitory activity should be of significant importance for potential anticancer therapy.

2. Synthetic Approach toward Phaeosphaeride A

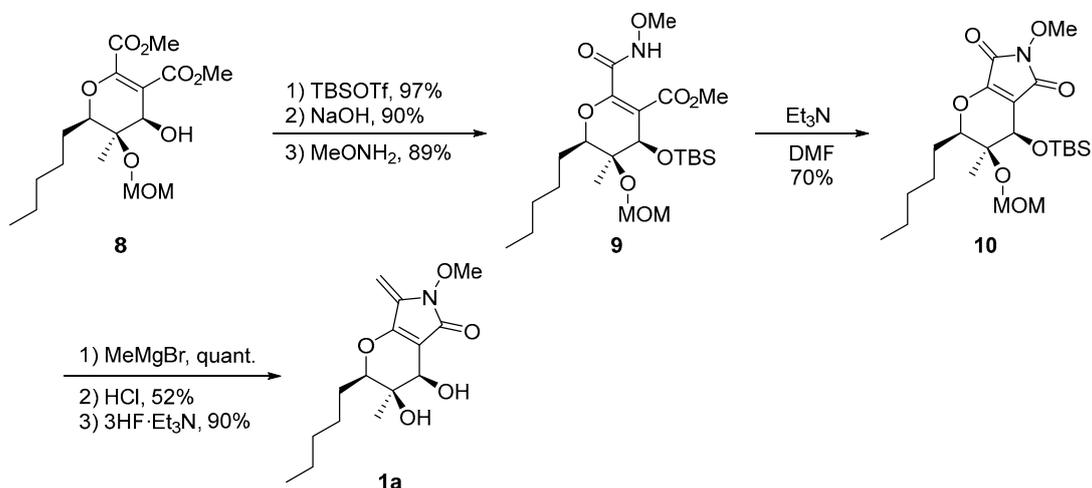
2.1. Synthesis of the Proposed Structure of Phaeosphaeride A

In 2011, our group accomplished the first total synthesis of the proposed structure of phaeosphaeride A (**1a**; Schemes 1 and 2) [9]. In this synthesis, to construct the C-7 and C-8 stereocenters, we used a typical *E*-selective Horner–Wadsworth–Emmons reaction followed by Sharpless asymmetric dihydroxylation using AD-mix- β to obtain the requisite diol (*2S,3R*)-**4** in high yield. In this step, the absolute configuration and the high enantiomeric excess of diol **4** were confirmed by a modified Mosher’s method. After appropriate conversion of diol **4** to secondary alcohol **5**, we found good reaction conditions for an oxy-Michael addition of alcohol **5** to dimethyl acetylenedicarboxylate: Use of a catalytic amount of *n*-BuLi cleanly afforded the desired Michael adduct (*E*)-**6** along with the (*Z*)-isomer in 76% and 18% yields, respectively. The remaining C-6 stereogenic center was installed by a vinyl-anion aldol reaction of aldehyde **7** using sodium bis(trimethylsilyl)amide (NaHMDS), providing the desired dihydropyran derivative **8** via the plausible transition state shown in Scheme 1.



Scheme 1. Synthesis of the dihydropyran intermediate **8**.

The construction of the five-membered ring in phaeosphaeride A was followed by regioselective installation of the exo-methylene group to furnish the proposed structure of phaeosphaeride A (**1a**; Scheme 2). However, the ^1H and ^{13}C NMR spectra of synthetic **1a** did not match those reported for natural phaeosphaeride A. Therefore, this synthesis revealed that the structure of phaeosphaeride A had been incorrectly assigned.



Scheme 2. Tamura's total synthesis of the proposed structure of phaeosphaeride A (**1a**).

For natural phaeosphaeride A, the Clardy group observed nuclear Overhauser effect spectroscopy (NOESY) correlations between H-6 and H-8, and between H-15 and both H-6 and H-8 (Figure 2) [8]. The correlation between H-6 and H-8 clearly indicates a pseudodiaxial relationship between these hydrogens. However, the correlations between H-15 and both H-6 and H-8 do not provide information about the configuration of the C-7 stereocenter. Hence, the correct structure of phaeosphaeride A was presumed to be the C-7 epimer **1c** of the originally proposed structure, or the epimer's enantiomer **1d**.

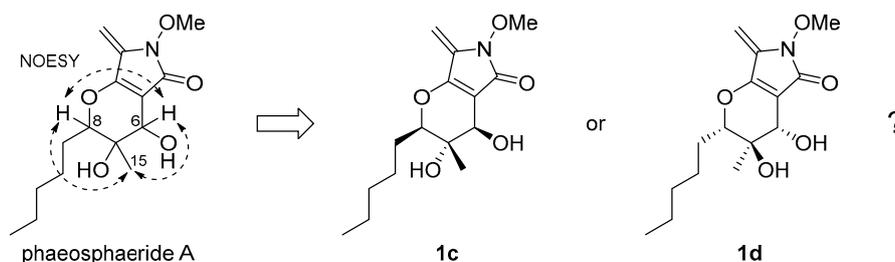
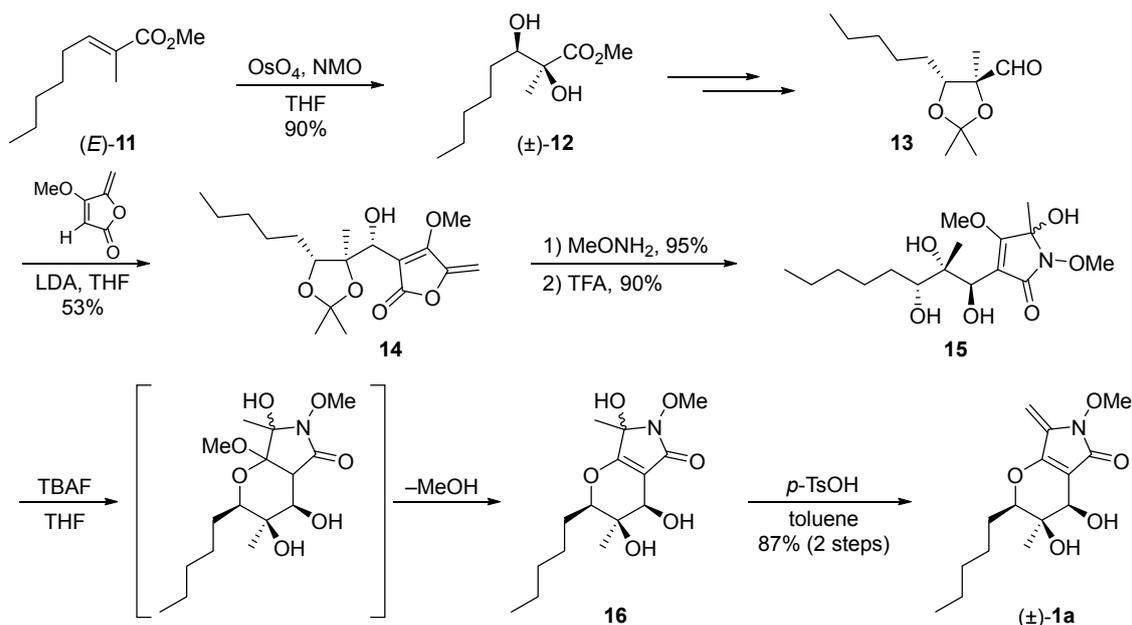


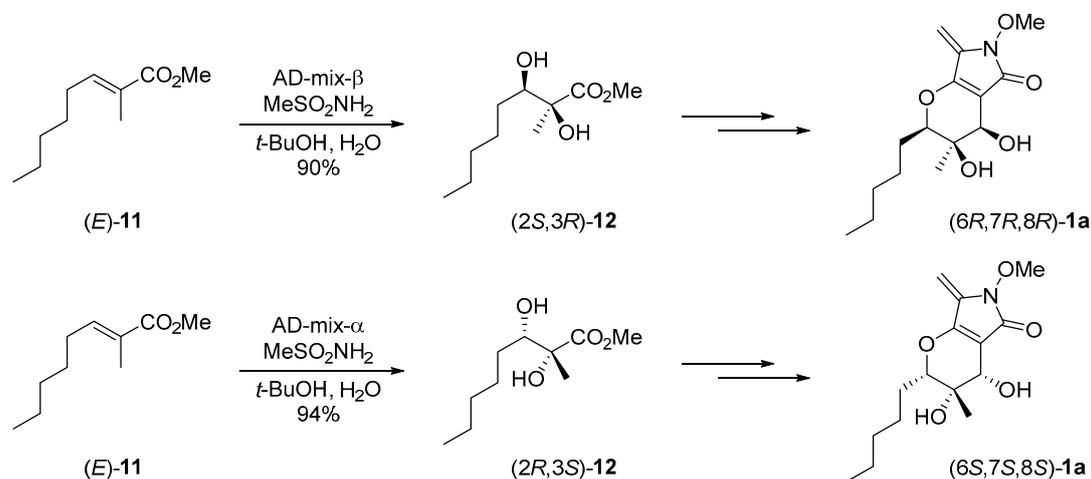
Figure 2. Results of NOESY experiments by Clardy.

In 2012, Sarli's group also succeeded in the total synthesis of the proposed structure of phaeosphaeride A (**1a**) [10]. Their synthesis involved a strategy similar to ours, in which dihydroxylation of unsaturated ester (**E**)-**11** using catalytic OsO₄ and 4-methylmorpholine *N*-oxide (NMO) was used to form the C-7 and C-8 stereocenters. Then, the C-6 center was stereochemically controlled by the anti-selective addition of a vinyl lithium species to aldehyde **13** via the Felkin–Ahn transition state. After establishing the three contiguous stereocenters, sequential oxy-Michael addition/methanol elimination followed by selective dehydration furnished (±)-**1a** (Scheme 3).



Scheme 3. Sarli's total synthesis of (±)-1a.

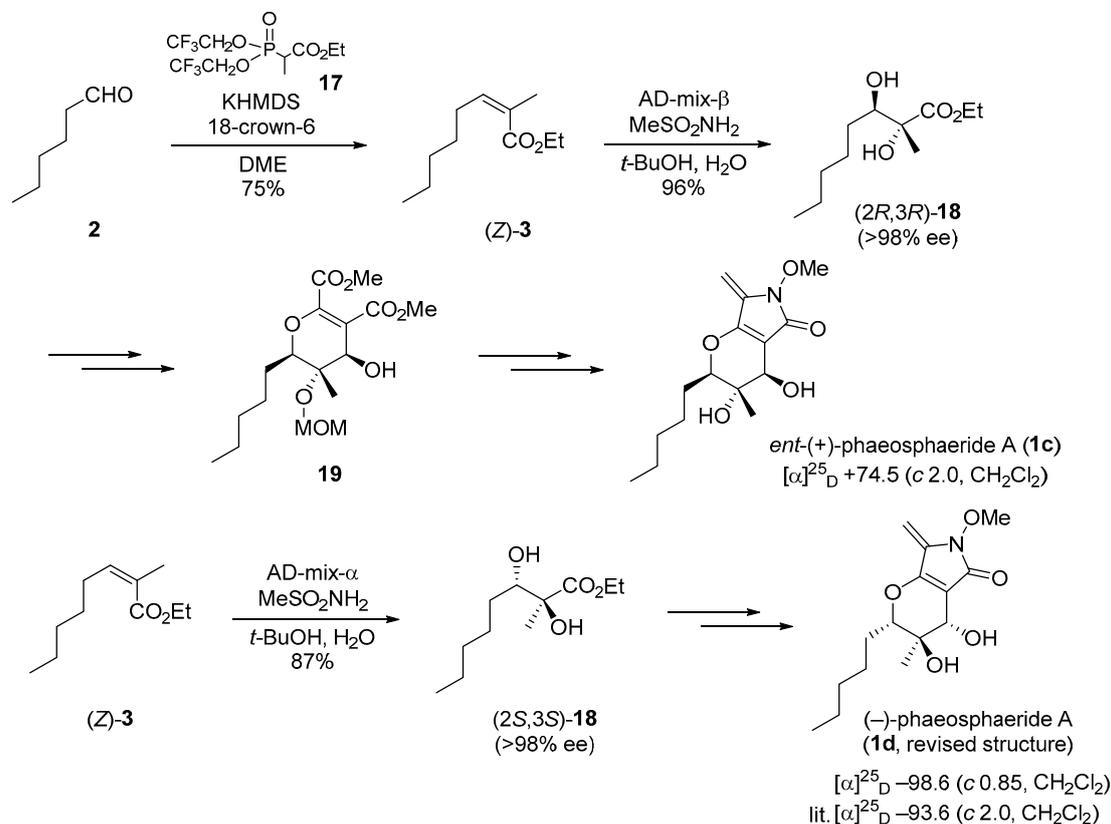
They also synthesized both enantiomers of **1a**, (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a**, by Sharpless asymmetric dihydroxylation of (*E*)-**11** in the first step using AD-mix-β and AD-mix-α, respectively. They obtained the crystal structure of synthetic (6*R*,7*R*,8*R*)-**1a** using synchrotron radiation, which proved their structural assignment of **1a** by NMR. Their studies also pointed to the structural revision of phaeosphaeride A to **1c** or its enantiomer **1d** (Scheme 4).

Scheme 4. Sarli's total synthesis of (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a**.

2.2. Stereochemical Determination of Natural (–)-Phaeosphaeride A

Based on the above achievements by our group and Sarli's group, the C-7 epimer **1c** of the originally proposed structure or the epimer's enantiomer **1d** needed to be synthesized to resolve the issue of the stereochemistry of natural phaeosphaeride A. To access **1c** by a synthetic strategy similar to those shown in Schemes 1 and 2, (*Z*)-α,β-unsaturated ester (*Z*)-**3** was first prepared instead of the (*E*)-ester by Still–Gennari olefination using phosphonate **17**. The ester (*Z*)-**3** was converted into the diol (2*R*,3*R*)-**18** via Sharpless dihydroxylation using AD-mix-β. According to the previous route with slight modification, the intermediate diol (2*R*,3*R*)-**18** was successfully converted via dihydropyran

intermediate **19** into **1c**, the ^1H and ^{13}C NMR spectra of which completely matched the literature data for natural phaeosphaeride A, and the optical rotation of the synthetic compound had the opposite sign to that of the natural product. The correct structure of natural (–)-phaeosphaeride A was thus shown to be the enantiomer **1d** of synthetic **1c** [11]. Then, natural (–)-phaeosphaeride A was synthesized by using AD-mix- α instead of AD-mix- β in the first step (Scheme 5) [12].

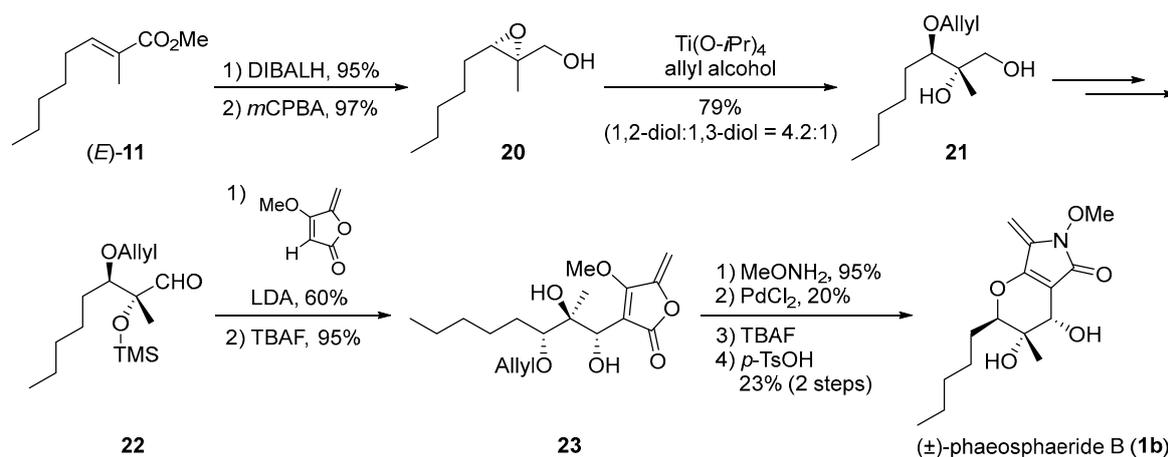


Scheme 5. Kobayashi and Kogen's total synthesis of **1c** and **1d**.

After the reports, Abzianidze et al. reported the crystal structure of natural phaeosphaeride A [13]. This crystal structure clearly supported the results of stereochemical revision by these synthetic approaches.

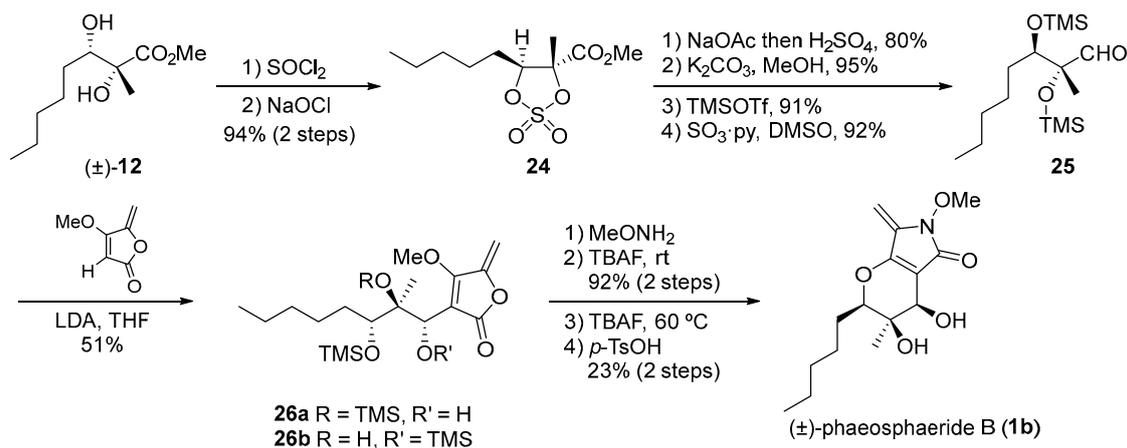
3. Synthetic Approach toward Phaeosphaeride B

The first total synthesis of (±)-phaeosphaeride B (**1b**) was reported by Sarli's group in 2014 [14]. Their synthetic strategy for **1a** (Scheme 3) could be applied to the preparation of (±)-**1b**. In their synthesis, conversion of (*E*)-**11** to an allylic alcohol followed by epoxidation and subsequent regioselective Ti(*i*Pr)₄-mediated epoxide ring-opening of **20** with allyl alcohol enabled introduction of the C-7 and C-8 stereocenters of (±)-**1b**. The key anti-selective nucleophilic addition of a vinyl lithium species to aldehyde **22** delivered the required C-6 stereocenter through the polar Felkin–Ahn transition state. Then, the lactam and dihydropyran rings and exo-methylene group were assembled to complete the total synthesis of (±)-phaeosphaeride B (**1b**) (Scheme 6).



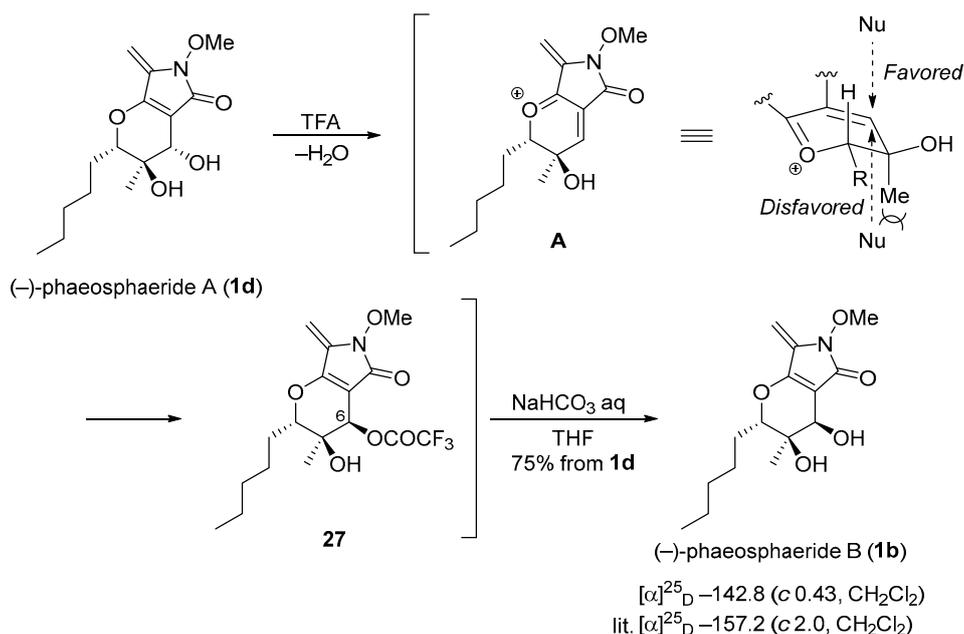
Scheme 6. Sarli's total synthesis of (±)-phaeosphaeride B (1b).

They also developed an improved synthetic scheme for (±)-1b, in which bis-TMS ether aldehyde 25, derived from diol (±)-12 via cyclic sulfate 24, was used in the reaction with α -lithio tetronate to form 26a along with the TMS-migrated product 26b. Subsequent conversion via their established route effectively yielded (±)-1b (Scheme 7).



Scheme 7. Improved synthesis of (±)-1b by Sarli et al.

After the successful total synthesis of (±)-phaeosphaeride B by Sarli, we demonstrated a biomimetic transformation from (−)-phaeosphaeride A to (−)-phaeosphaeride B [12]. We posited that phaeosphaerides A and B would be biosynthetically interconverted under acidic conditions. In testing this hypothesis, treatment of synthetic (−)-phaeosphaeride A (1d) with trifluoroacetic acid (TFA) as a protic acid gave the corresponding trifluoroacetate 27 with stereochemical inversion at C-6 stereocenter via dehydrative formation of the oxonium cation intermediate A. The labile trifluoroacetate 27 was immediately hydrolyzed with aqueous NaHCO₃ in THF to yield (−)-phaeosphaeride B (1b) in a good yield (Scheme 8). In addition, this synthesis confirmed the absolute configuration of natural (−)-phaeosphaeride B as shown in Scheme 8.



Scheme 8. Biomimetic transformation from (–)-phaeosphaeride A to (–)-phaeosphaeride B.

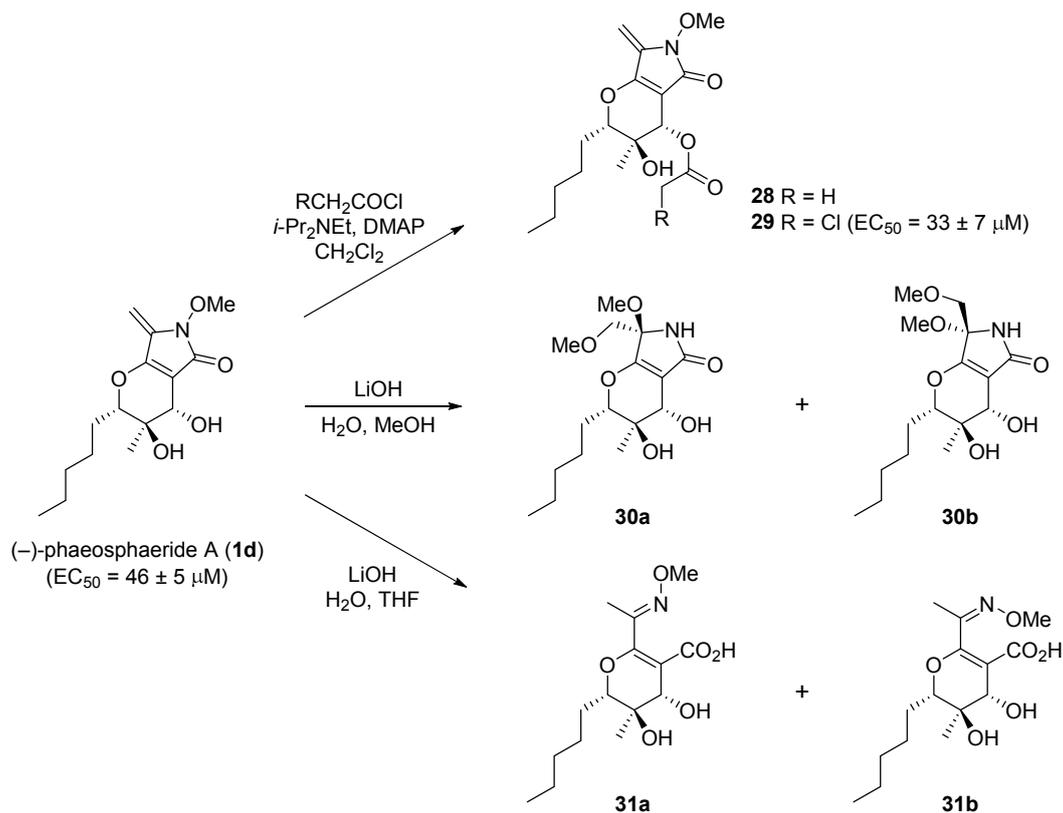
4. Biological Evaluation of Phaeosphaerides and Their Derivatives

Considering their potential as a seed compound for anticancer treatment, Sarli's and Abzianidze's groups evaluated the biological activities of phaeosphaerides and their synthetic derivatives [10,14–17].

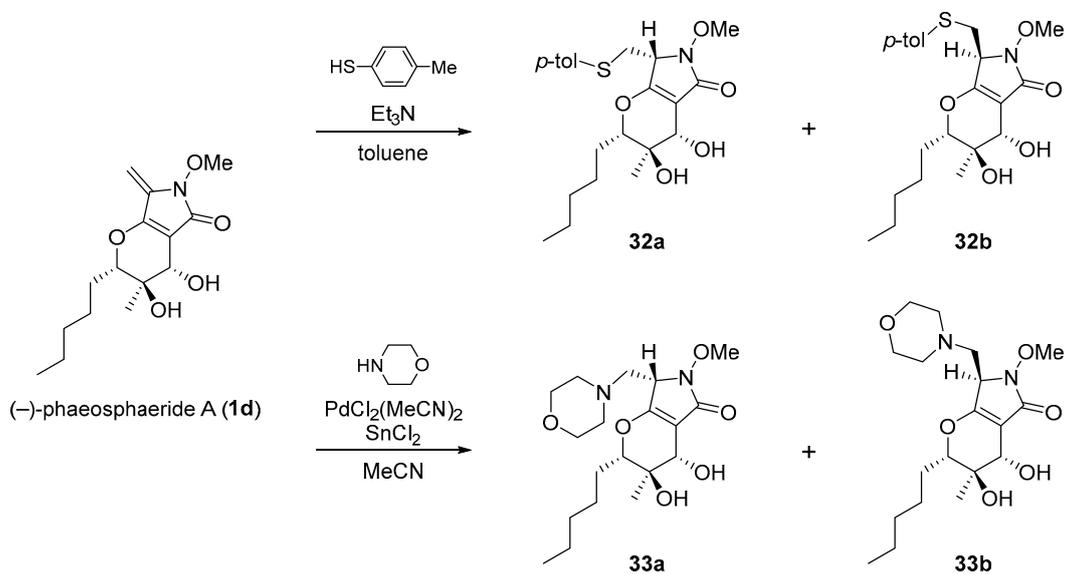
Initially, Sarli and colleagues biologically evaluated the stereoisomers of phaeosphaeride A, (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** [10]. These compounds inhibited STAT3-dependent transcriptional activity in a dose-dependent manner and decreased cell proliferation in breast (MDA-MB-231) and pancreatic (PANC-1) cancer cells in the low micromolar range. After that, they reported that the synthetic (6*S*,7*S*,8*S*)-**1a** and (6*R*,7*S*,8*S*)-phaeosphaeride had only very weak inhibitory activity against binding of STAT3 to its phosphotyrosine peptide ligand, suggesting that phaeosphaerides are upstream inhibitors of a tyrosine kinase in the JAK/STAT pathway [14].

Abzianidze et al. prepared and biologically evaluated the C-6 acyl derivatives **28** and **29**, bis-methanol adducts **30a** and **30b** without the MeO group on the nitrogen, and hydrolyzed products **31a** and **31b** prepared from isolated natural phaeosphaeride A [15]. Compared to natural phaeosphaeride A ($\text{EC}_{50} = 46 \pm 5 \mu\text{M}$), chloroacetyl derivative **29** exhibited more potent cytotoxicity ($\text{EC}_{50} = 33 \pm 7 \mu\text{M}$) against the A549 cancer cell line, while synthetic **30** and **31** had no activity (Scheme 9).

Additionally, they also synthesized 7-(4-methylphenyl)thiomethyl and 7-morpholylmethyl derivatives **32** and **33**, which were less cytotoxic than the parent phaeosphaeride A (Scheme 10) [16]. These results clearly indicated that the exo-methylene and *N*-OMe groups were essential for potent cytotoxicity. Their studies strongly suggest that further SAR studies can provide lead compounds with greater potency for potential use as anticancer chemotherapeutic agents.



Scheme 9. Phaeosphaeride A derivatives **28**, **29**, **30**, and **31** prepared by Abzianidze et al.



Scheme 10. Phaeosphaeride A derivatives **32** and **33** by Abzianidze et al.

5. Conclusions

Phaeosphaerides A and B have attracted considerable attention due not only to their chemical structures but also to their biological activity, and their stereochemical structures have been unambiguously determined through the total synthesis and X-ray crystal structure analysis of phaeosphaeride A. The promising anticancer activity of phaeosphaeride A based on inhibition of STAT3-DNA binding indicates that this natural product is a promising seed compound for anticancer

drug candidates. SAR studies on phaeosphaerides led to the development of more potent compounds such as chloroacetyl derivative **29**, and further SAR studies are awaited for anticancer drug discovery research. In addition, structurally related natural products including paraphaeosphaerides [18,19], phyllostictines [20,21], isoaiqualones [22], benesudon [23], and curvupallides [24] should also attract considerable interest as pharmaceutical targets. Further studies of phaeosphaerides are expected to make valuable contributions to synthetic and medicinal chemistry.

Author Contributions: K.K. and K.T.III prepared the manuscript. K.K. managed the project with assistance from H.K.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Levy, D.E. Physiological significance of STAT proteins: Investigations through gene disruption in vivo. *Cell. Mol. Life Sci.* **1999**, *55*, 1559–1567. [[CrossRef](#)] [[PubMed](#)]
2. Darnell, J.E., Jr. STATs and gene regulation. *Science* **1997**, *277*, 1630–1635. [[CrossRef](#)] [[PubMed](#)]
3. Darnell, J.E., Jr.; Kerr, I.M.; Stark, G.R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **1994**, *264*, 1415–1421. [[CrossRef](#)] [[PubMed](#)]
4. Zhong, Z.; Darnell, J.E., Jr. Stat3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science* **1994**, *264*, 95–98. [[CrossRef](#)] [[PubMed](#)]
5. Bromberg, J.F.; Wrzeszczynska, M.H.; Devgan, G.; Zhao, Y.; Pestell, R.G.; Albanese, C.; Darnell, J.E., Jr. Stat3 as an oncogene. *Cell* **1999**, *98*, 295–303. [[CrossRef](#)]
6. Subramaniam, A.; Shanmugam, M.K.; Perumal, E.; Li, F.; Nachiyappan, A.; Dai, X.; Swamy, S.N.; Ahn, K.S.; Kumar, A.P.; Tan, B.K.; et al. Potential role of signal transducer and activator of transcription (STAT)3 signaling pathway in inflammation, survival, proliferation and invasion of hepatocellular carcinoma. *Biochim. Biophys. Acta Rev. Cancer* **2013**, *1835*, 46–60. [[CrossRef](#)] [[PubMed](#)]
7. Siveen, K.S.; Sikka, S.; Surana, R.; Dai, X.; Zhang, J.; Kumar, A.P.; Tan, B.K.; Sethi, G.; Bishayee, A. Targeting the STAT3 signaling pathway in cancer: Role of synthetic and natural inhibitors. *Biochim. Biophys. Acta Rev. Cancer* **2014**, *1845*, 136–154. [[CrossRef](#)] [[PubMed](#)]
8. Maloney, K.N.; Hao, W.; Xu, J.; Gibbons, J.; Hucul, J.; Roll, D.; Brady, S.F.; Schroeder, F.C.; Clardy, J. Phaeosphaeride A, an Inhibitor of STAT3-dependent signaling isolated from an endophytic fungus. *Org. Lett.* **2006**, *8*, 4067–4070. [[CrossRef](#)] [[PubMed](#)]
9. Kobayashi, K.; Okamoto, I.; Morita, N.; Kiyotani, T.; Tamura, O. Synthesis of the proposed structure of phaeosphaeride A. *Org. Biomol. Chem.* **2011**, *9*, 5825–5832. [[CrossRef](#)] [[PubMed](#)]
10. Chatzimpaloglou, A.; Yavropoulou, M.P.; Rooij, K.E.; Biedermann, R.; Mueller, U.; Kaskel, S.; Sarli, V. Total synthesis and biological activity of the proposed structure of phaeosphaeride A. *J. Org. Chem.* **2012**, *77*, 9659–9667. [[CrossRef](#)] [[PubMed](#)]
11. Kobayashi, K.; Kobayashi, Y.; Nakamura, M.; Tamura, O.; Kogen, H. Establishment of relative and absolute configurations of phaeosphaeride A: Total synthesis of *ent*-phaeosphaeride A. *J. Org. Chem.* **2015**, *80*, 1243–1248. [[CrossRef](#)] [[PubMed](#)]
12. Kobayashi, K.; Kunimura, R.; Tanaka, K., III; Tamura, O.; Kogen, H. Total synthesis of (–)-phaeosphaeride B by a biomimetic conversion from (–)-phaeosphaeride A. *Tetrahedron* **2017**, *73*, 2382–2388. [[CrossRef](#)]
13. Abzianidze, V.V.; Poluektova, E.V.; Bolshakova, K.P.; Panikorovskii, T.L.; Bogachenkov, A.S.; Berestetskiy, A.O. Crystal structure of natural phaeosphaeride A. *Acta Crystallogr.* **2015**, *E71*, o625–o626. [[CrossRef](#)] [[PubMed](#)]
14. Chatzimpaloglou, A.; Kolosov, M.; Eckols, T.K.; Twardy, D.J.; Sarli, V. Synthetic and biological studies of phaeosphaerides. *J. Org. Chem.* **2014**, *79*, 4043–4054. [[CrossRef](#)] [[PubMed](#)]
15. Abzianidze, V.V.; Prokofieva, D.S.; Chisty, L.A.; Bolshakova, K.P.; Berestetskiy, A.O.; Panikorovskii, T.L.; Bogachenkov, A.S.; Holder, A.A. Synthesis of natural phaeosphaeride A derivatives and an in vitro evaluation of their anti-cancer potential. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5566–5569. [[CrossRef](#)] [[PubMed](#)]

16. Abzianidze, V.V.; Bolshakova, K.P.; Prokofieva, D.S.; Berestetskiy, A.O.; Kuznetsova, V.A.; Trishin, Y.G. Synthesis of 7-(4-methylphenyl)thiomethyl and 7-morpholylmethyl derivatives of natural phaeosphaeride A and their cytotoxic activity. *Mendeleev Commun.* **2017**, *27*, 82–84. [[CrossRef](#)]
17. Abzianidze, V.V.; Efimova, K.P.; Poluektova, E.V.; Trishin, Y.G.; Kuznetsov, V.A. Synthesis of natural phaeosphaeride A and semi-natural phaeosphaeride B derivatives. *Mendeleev Commun.* **2017**, *27*, 490–492. [[CrossRef](#)]
18. Li, C.S.; Ding, Y.; Yang, B.J.; Miklossy, G.; Yin, H.Q.; Walker, L.A.; Turkson, J.; Cao, S. A new metabolite with a unique 4-pyranone– γ -lactam–1,4-thiazine moiety from a Hawaiian-plant associated fungus. *Org. Lett.* **2015**, *17*, 3556–3559. [[CrossRef](#)] [[PubMed](#)]
19. Li, C.S.; Sarotti, A.M.; Huang, P.; Dang, U.T.; Hurdle, J.G.; Kondratyuk, T.P.; Pezzuto, J.M.; Turkson, J.; Cao, S. NF- κ B inhibitors, unique γ -pyranol- γ -lactams with sulfide and sulfoxide moieties from Hawaiian plant *Lycopodiella cernua* derived fungus *Paraphaeosphaeria neglecta* FT462. *Sci. Rep.* **2017**, *7*, 10424–10433. [[CrossRef](#)] [[PubMed](#)]
20. Evidente, A.; Cimmino, A.; Andolfi, A.; Vurro, M.; Zonno, M.C.; Cantrell, C.L.; Motta, A. Phyllostictines A–D, oxazatricycloalkenones produced by *Phyllosticta cirsii*, a potential mycoherbicide for *Cirsium arvense* biocontrol. *Tetrahedron* **2008**, *64*, 1612–1619. [[CrossRef](#)]
21. Trenti, F.; Cox, R.J. Structural revision and biosynthesis of the fungal phytotoxins phyllostictines A and B. *J. Nat. Prod.* **2017**, *80*, 1235–1240. [[CrossRef](#)] [[PubMed](#)]
22. Silva, G.H.; Zeraik, M.L.; Oliveira, C.M.; Teles, H.L.; Trevisan, H.C.; Pfenning, L.H.; Nicolli, C.P.; Young, M.C.M.; Mascarenhas, Y.P.; Abreu, L.M.; et al. Lactone derivatives produced by a *Phaeoacremonium* sp., an endophytic fungus from *Senna spectabilis*. *J. Nat. Prod.* **2017**, *80*, 1674–1678. [[CrossRef](#)] [[PubMed](#)]
23. Thines, E.; Arendholz, W.-R.; Anke, H. Bunesudon, a new antibiotic fungal metabolite from cultures of *Mollisia benesuada* (Tul.) Phill. *J. Antibiot.* **1997**, *50*, 13–17. [[CrossRef](#)] [[PubMed](#)]
24. Abraham, W.-R.; Meyer, H.; Abate, D. Curvupallides, a new class of alkaloids from the fungus *Curvularia pallezensis*. *Tetrahedron* **1995**, *51*, 4947–4952. [[CrossRef](#)]



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