Organic LETTERS

Letter

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Total Synthesis and Structural Revision of Chaetoviridins A

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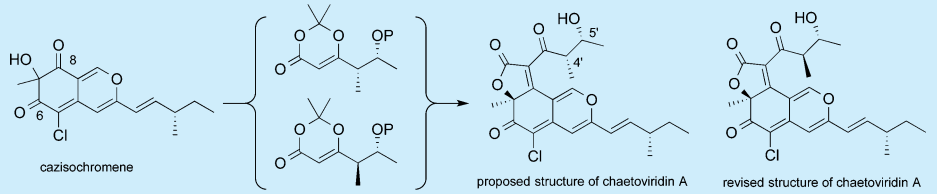
Supporting Information



ABSTRACT: The first synthesis of the proposed structures of chaetoviridins A **1**−**4** has been achieved in 10 steps by controlling the *syn*- or *anti*-aldol side chain. The angular lactone has been regioselectively introduced by condensation of a chiral dioxin-4-one to cazisochromene. Comparison of the NMR and circular dichroism data of the synthesized and reported natural products led to the complete reassignment and renaming of the chaetoviridins.

Azaphilones are bioactive secondary metabolites isolated from various fungi; they present an extremely large structural diversity as well as wide biological activity spectrum.[1] Azaphilones are characterized by an oxabicyclic scaffold that bears an oxygenated quaternary center at the C-7 position (Figure 1). This 7-hydroxyl can be of (*R*) or (*S*) absolute

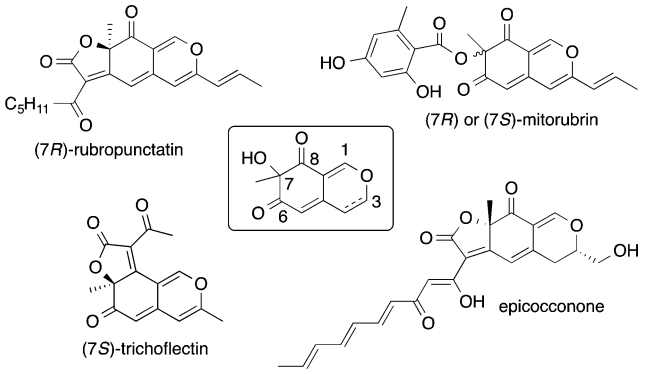


Figure 1. General structure of azaphilones.

configuration such as in (−) or (+) mitorubrin, respectively,[2a] with (7*S*)-isomers being the most common among the azaphilones. However, the 7-hydroxyl can be part of an angular or linear furanone ring such as in trichoflectin[2b] or rubropunctatin,[2c] respectively. The pyranoquinone cycle can also be reduced to a dihydropyranic ring such as in epicocconone.[2d] In addition to that, many reduced or rearranged skeletons exist, enhancing the structural diversity and biological activity spectrum of this family.

Azaphilones and more particularly chaetoviridins A are in the limelight of numerous researches in particular regarding the identification of gene cluster responsible for their biosynthesis and genome mining approaches providing new access to diversity in natural products.[3] Chaetoviridins A have been reported to have wide biological activities, such as inhibitors of caspase 3 and cholesteryl ester transfer protein (CETP), and have antimalarial, antimycobacterial, antifungal, and cytotoxic activity.[1] However, structural variations associated with a panel of biological activities make it difficult to draw any sort of structure−activity relationship.

Chaetoviridins A,[4−9] isolated from diverse *Chaetomium* species, have an angular lactone structure, a branched pentenyl side chain at position 3, and a chlorine atom at position 5 (Figure 2). On the lactone ring can be found a *syn* or *anti* aldol side chain such as in chaetoviridin A **1**[4,5,7,9] and 4′- or 5′-epimers **2** and **3**.[7] (7*R*)- or (7*S*)-Epimers can also be found naturally such as in 5′-

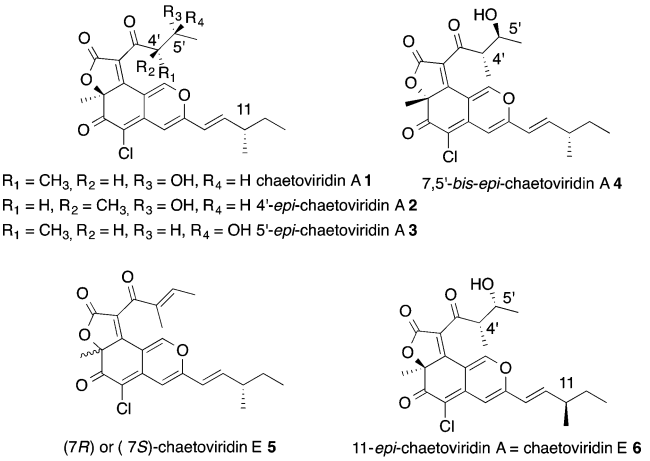


Figure 2. Proposed structures of chaetoviridin A and epimers.

Received: July 5, 2017

DOI: 10.1021/acs.orglett.7b02053
Org. Lett. XXXX, XXX, XXX−XXX

epi-chaetoviridin A **3**[7] (7*S*) or in 7,5′-*bis*-*epi*-chaetoviridin A **4**[8] (7*R*) as well as in dehydrated (7*R*)- or (7*S*)-chaetoviridin E **5**.[5,8] Another epimer of chaetoviridin A has been described, being the C-11 epimer **6**; this compound was also named chaetoviridin E.[6] As a consequence, two "chaetoviridin E" exist with different structures (**5**, **6**).

The isolation and structural elucidation of chaetoviridin A **1** was achieved by Natori in 1990, who established the configuration of the aldol as *syn*, based on NMR studies.[4] This configuration was adopted in subsequent studies, in particular for the structural determination of other epimers.[5,7,9] However, without this being ever mentioned, Collado and Pupo reported in 2011 X-ray data of **1** showing an *anti* (4′*R*,5′*R*) aldol chain.[7] This absolute configuration was, however, already assigned to the 4′-*epi*-chaetoviridin A **2**, which exhibited a ^{1}H and ^{13}C NMR spectrum different from that of **1**. Accordingly, following suspected structural misassignment(s), we undertook the total synthesis of chaetoviridin A and its epimers in order to unambiguously attribute their structure. Besides, while ester-functionalized azaphilones have already been synthesized,[1] no linear or angular lactone-bearing natural azaphilone has ever been synthesized.[10−24]

The retrosynthetic analysis of chaetoviridin A **1** with *syn* aldol side chain highlights two main fragments: the bicyclic oxygenated fragment **14**, a recently identified intermediate in the biosynthesis of azaphilones, named cazisochromene,[2b,c] and a chiral dioxin-4-one **21** bearing the *syn* aldol chain (Figure 3). Due to the possibility of either 7*R* or 7*S* configuration in chaetoviridin A and to remove any structural ambiguity, synthesis of both C7-epimers was undertaken.

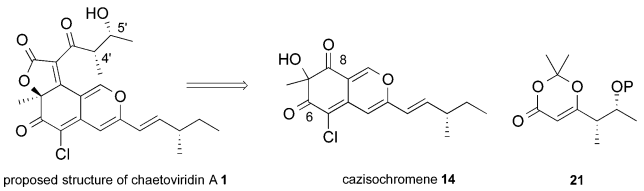
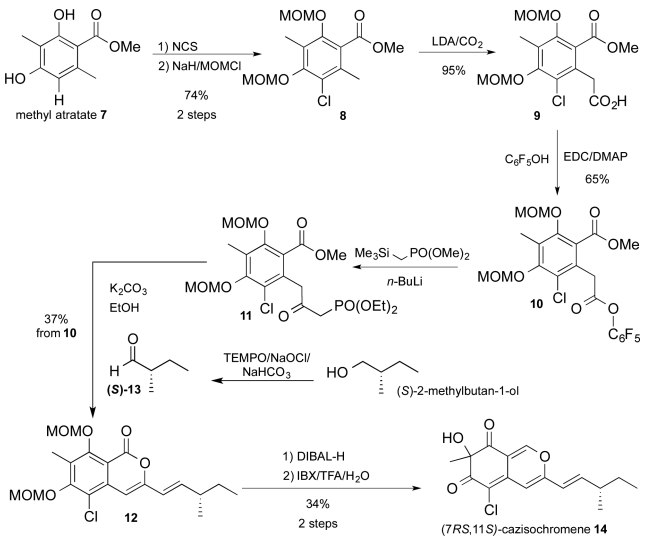


Figure 3. Retrosynthetic analysis.

The synthesis of the key intermediate cazisochromene **14** started with chlorination of methyl atratate **7** before protecting the phenol groups as methoxymethyl ethers (Scheme 1). Benzylic deprotonation of **8** followed by addition of CO_2 yielded the carboxylic acid **9**, which was activated as pentafluorophenol ester **10**. Addition of the lithiated anion of trimethylsilyl methyl phosphonate onto **10** gave the *β*-ketoester **11** following Cossy's procedure.[27a] It should be noted at this stage that use of other carboxylic acid derivatives (acid chlorides, Weinreb amides, acylbenzotriazoles, etc.) and methylphosphonate as the nucleophile did not yield the desired *β*-ketoester **11** with acceptable yields.[27b−d] Then treating **11** with freshly prepared chiral aldehyde **13** in the presence of potassium carbonate performed the Horner−Wadsworth−Emmons (HWE) reaction and lactonization to **12**, installing the pentenyl side chain with 37% yield for the last two steps and without epimerization (Figure S1). Lactone **12** was reduced to the lactol by 1 equiv of DIBAL-H and oxidatively dearomatized in the presence of IBX, TFA, and water[25,26] to yield cazisochromene **14** as a ∼1:1 mixture of inseparable C7-epimers but with controlled 11*S* absolute configuration.

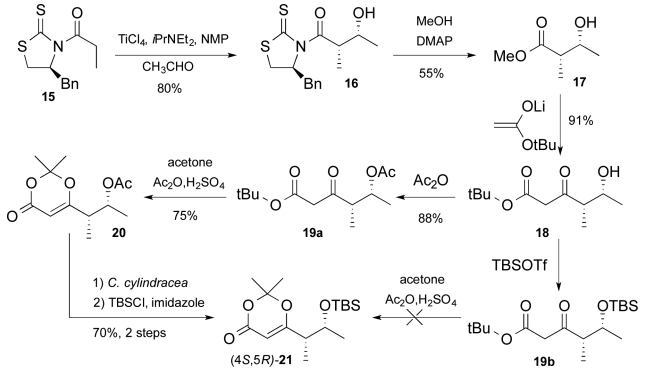
The second fragment, identified as dioxinone **21**, was prepared via a diastereoselective titanium-based aldolization, installing the

Scheme 1. Synthesis of Cazisochromene 14



chiral *syn* aldol moiety of the natural product. *N*-Propionyl (*S*)-benzyl thiazolidin-2-thione **15** reacted under standard conditions[28] with acetaldehyde to give the *syn* Evans aldol **16** in 80% yield. The chiral auxiliary was smoothly removed by methanolysis to give the corresponding methyl ester **17** in a moderate 55% yield. Claisen condensation of the lithium enolate of *t*-BuOAc with methyl ester **17** gave the *β*-ketoester **18** (91% yield), and protection of the secondary hydroxyl as an acetate gave the *β*-ketoester **19a** in 88% yield. The formation of the dioxinone skeleton **20** was performed in 75% yield by adding H_2SO_4 to a mixture of **19a**, Ac_2O, and acetone.[29] To avoid *β*-elimination of acetate **20** occurring under standard deacetylating conditions (MeOH in the presence of K_2CO_3 or other bases), enzymatic hydrolysis with *Candida cylindracea*[30] was performed, giving clean deacetylation of the dioxinone **20**, albeit with a slow reaction rate. The so-obtained free hydroxyl group was then reprotected as a TBS-ether giving (4*S*,5*R*)-dioxinone **21** with 70% yield for the last two steps (Scheme 2).

Scheme 2. Synthesis of Dioxinone (4*S*,5*R*)-21



With the two key intermediates **14** and **21** in hand, we undertook their condensation according to our previous studies.[25,26] Dioxinone **21** and cazisochromene **14** were heated in toluene for 30 min before adding Et_3N. Under these conditions, the intermediate *β*-ketoester **22** was formed and gratifyingly condensed regioselectively at position 8, installing the angular lactone of the natural product. It is worth noting that opposite regioselectivity was observed when preparing analogues

DOI: 10.1021/acs.orglett.7b02053
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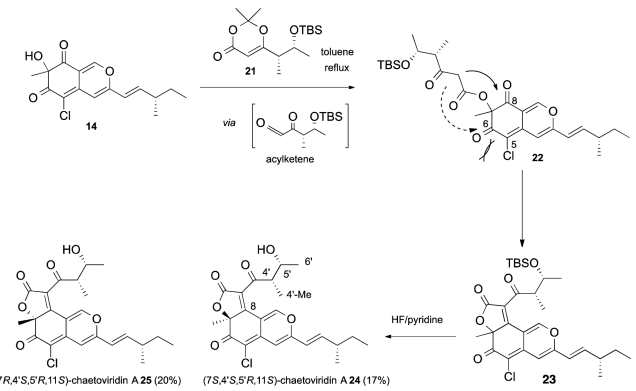
of the nonchlorinated dihydropyranic azaphilone epicocconone, thus demonstrating the importance of the chlorine atom in the regio-outcome of the process.[26] The silylated crude product **23** was directly treated by HF/pyridine to give the (7*S*,4′*S*,5′*R*)-chaetoviridin A **24** with 17% yield and its 7-epimer (7*R*,4′*S*,5′*R*)-chaetoviridin A **25** with 20% yield over two steps (Scheme 3).

Scheme 3. Synthesis of (7*S*,4′*S*,5′*R*,11*S*)-Chaetoviridin A 24 and (7*R*,4′*S*,5′*R*,11*S*)-Chaetoviridin A 25



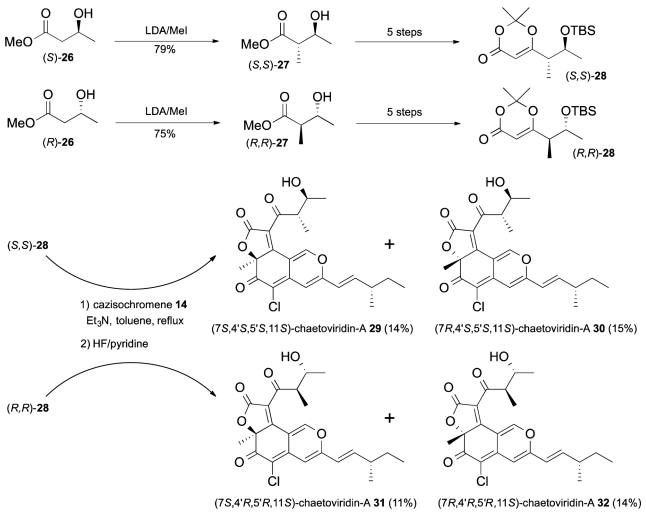
Significantly, no trace of retro-aldolization, *β*-elimination, or epimerization products was observed, and so despite the basic conditions. Note that for simplification of the discussion throughout the Letter, the 11*S* stereochemical information will not be specifically mentioned, unless necessary, as it is common to all the chaetoviridins prepared here.

At this stage, the UV CD spectra of **24** and **25** were recorded and a negative Cotton effect ($\Delta\varepsilon_{371}$ −24.3) allowed assigning the (7*S*) configuration for **24**, whereas **25** showed a positive Cotton effect ($\Delta\varepsilon_{364}$ +19.7), establishing the (7*R*) configuration (Figure S2).[8]

Surprisingly, by comparing the ^{1}H and ^{13}C NMR spectra of the synthetic (7*S*,4′*S*,5′*R*)-chaetoviridin A **24** with literature data of chaetoviridin A **1**, important differences were observed in particular at δ_H 4.30 vs 3.89 ppm ($H_{5'}$) and 1.06 vs 1.18 ppm (4′-CH_3) and at δ_C 9.9 vs 13.5 ppm (4′-CH_3), 19.4 vs 21.4 ppm (C6′), 67.4 vs 70.9 ppm (C5′), and 165.3 vs 162.7 ppm (C8).[4,5,7] However, it turned out that ^{1}H and ^{13}C NMR data of **24** were identical to those reported by Borges et al. for the 4′-*epi*-chaetoviridin **2**, which has been described bearing an *anti*-aldol side chain.[7] In our synthesis, the *syn*-stereocontrolled construction of the aldol moiety unambiguously fixes the *syn* relationship between $H_{4'}$ and $H_{5'}$ in **24**. Accordingly, the reported 4′-*epi*-chaetoviridin A **2**, described with an *anti* aldol side chain, has to be corrected to *syn* such as in (7*S*,4′*S*,5′*R*)-chaetoviridin A **24**.

To further confirm the misassignment of the aldol side chain of the chaetoviridins A, we undertook the preparation of chaetoviridin A epimers bearing *anti* aldol moieties. To this end, we synthesized the two enantiomers of the dioxinones **28** bearing the *anti* aldol side chain (Scheme 4). Their synthesis started with the *anti*-methylation of both enantiomers of methyl 3-hydroxybutanoate **26** to give the *anti* aldols **27**.[31] Then, each enantiomer was individually converted to the (*R*,*R*)- or (*S*,*S*)-dioxinone **28** in five steps, following the procedure developed for the conversion of **17** to the *syn* dioxinone **21** (Scheme 2). Each *anti*-dioxinone was then reacted with cazisochromene **14** under thermal basic conditions, and the crude mixtures were desilylated with HF/pyridine to yield the chaetoviridins with *anti* aldol side

Scheme 4. Synthesis of *anti* Chaetoviridins A 29−32



chains. Starting from (*S*,*S*)-dioxinone **28**, (7*S*,4′*S*,5′*S*)-chaetoviridin A **29** and (7*R*,4′*S*,5′*S*)-chaetoviridin A **30** were obtained with 14 and 15% yield, respectively. The other set of *anti* chaetoviridins A was prepared starting from (*R*,*R*)-dioxinone **28**; accordingly, (7*S*,4′*R*,5′*R*)-chaetoviridin A **31** and (7*R*,4′*R*,5′*R*)-chaetoviridin A **32** were obtained with 11 and 14% yield, respectively. For the four compounds **29**−**32**, the absolute configuration at C-7 was attributable, thanks to the circular dichroism, as for **24** and **25** (Figure S2).

Analysis of the NMR data of **31** revealed, as expected, that they perfectly matched the reported data of natural chaetoviridin A **1**; therefore confirming that the natural product has structure **31**. This also confirms that the *anti* structure of reported natural 4′-*epi*-chaetoviridin **2** has to be corrected to *syn* such as in **24**.

In addition, NMR data of (7*S*,4′*S*,5′*S*)-chaetoviridin A **29** perfectly match the reported data for the natural product 5′-*epi*-chaetoviridin A **3**, validating the *anti* structure of the natural product; however, natural compound **3** should be renamed 4′,5′-*bis*-*epi*-chaetoviridin A.

Concerning the reported 7′,5′-*bis*-*epi*-chaetoviridin A **4**, our NMR data were found to be identical to (7*R*,4′*S*,5′*S*)-chaetoviridin A **30**; this is in accord with the reported structure[8] and further confirms the presence of the unusual epimeric (7*R*) center in this azaphilone; however natural compound **4** should be renamed as 7,4′,5′-*tris*-*epi*-chaetoviridin A.

For the 11-epimer of chaetoviridin A,[6] named chaetoviridin E **6**, we propose to revise its name to 11-*epi*-chaetoviridin A and its structure to the *anti* aldol side chain and (7*S*,4′*R*,5′*R*,*11R*) absolute configuration. Consequently, only one chaetoviridin E will remain, possessing the *β*-eliminated side-chain such as in **5**.

In conclusion, we have synthesized the natural product chaetoviridin A along with three related natural epimers in ten steps by preparing their biogenetic precursor cazisochromene. The synthesis of this fragment involved two key steps, the lactonisation/HWE one-pot process to build the 3-vinyl-isocoumarine core, and an oxidative dearomatization to obtain the pyranoquinone scaffold. The condensation of a functionalized chiral acylketene to cazisochromene allowed the tricyclic skeleton of chaetoviridins A to be built regioselectively. Detailed analysis of NMR and circular dichroism data allowed us to unambiguously revise the structure of all the chaetoviridins A. Of note, easy separation of C7-epimers at the last step of the

DOI: 10.1021/acs.orglett.7b02053
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synthesis offers a divergent access to natural and/or unnatural chaetoviridins A.

Accordingly, these results will now allow clear identification of these metabolites when studying their biosynthesis and the gene clusters of their producing fungi. Quantification of the production of these metabolites is also made possible by providing standards.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02053.

Experimental procedures, characterization data, and copies of the ^{1}H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the Fondation pour le Développement de la Chimie des Substances Naturelles et ses Applications (Académie des Sciences) for a grant to M.M. This work was partially supported by INSA Rouen, Rouen University, CNRS, EFRD, Labex SynOrg (ANR-11-LABX-0029), and Région Normandie. We are grateful to Pr. Watanabe (University of Shizuoka, Japan) for pointing out data discrepancy on chaetoviridin A. We are grateful to Dr. Laure Guilhaudis (Rouen University) for her help in acquisition of CD spectra, to Emilie Petit (INSA de Rouen) for chiral HPLC analyses, to Patricia Martel (University of Rouen) for IR analyses, and to Albert Marcual (CNRS) for HRMS analyses. We thank Dr. Hao Gao (Jinan University, China) for kindly providing us with NMR spectra of 7,4′,5′-*tris-epi*-chaetoviridin A (formerly 7,5′-*bis-epi*-chaetoviridin A). We also thank Pr. Peter Karuso (Macquarie University) for reviewing this manuscript.

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DOI: 10.1021/acs.orglett.7b02053
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