COMMUNICATION

Flow Chemistry-Enabled Divergent and Enantioselective Total Syntheses of Massarinolin A, Purpurolides B, D, E, 2,3-Deoxypurpurolide C, and Structural Revision of Massarinolin A

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In memory of Professor Ei-ichi Negishi

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Abstract: Massarinolin A and purpurolides are bioactive bergamotane sesquiterpenes condensed with a variety of synthetically challenging ring systems: a bicyclo[3.1.1]heptane, an oxasipro[3.4]octane, and a dioxaspiro[4.4]nonane (oxaspirolactone). Herein, we report the first enantioselective total syntheses of massarinolin A, purpurolides B, D, E, and 2,3-deoxypurpurolide C. Our synthesis and computational analysis also led to a structural revision of massarinolin A. The divergent approach features an enantioselective organocatalyzed Diels-Alder reaction to install the first stereogenic center in high ee, a scalable flow photochemical Wolff rearrangement to build the key bicyclo[3.1.1]heptane, a furan oxidative cyclization to form the oxaspirolactone, a late-stage allylic C-H oxidation, and a Myers' NBSH-promoted sigmatropic elimination to install the *exo* methylene group of massarinolin A.

Massarinolin A (**1a**, Figure1), a bergamotane sesquiterpene, was isolated by Gloer and co-workers in 1999 from the liquid cultures of the aquatic fungus *Massarina tunicata*.[1] Its structure was determined primarily by analysis of its NMR data and the absolute configuration was not confirmed. As a sesquiterpene with only 15 carbon atoms, massarinolin A features a remarkably complex ring system. A strained bicyclo[3.1.1]heptane, an oxaspiro[3.4]octane with an all-carbon quaternary center, and an acid-labile dioxaspiro[4.4]nonane (oxaspirolactone) are packed in its structure. Together with massarinolin A, simpler analogs massarinolins B and C without the two spirocyclic ring systems were isolated as well. Prior to the isolation of massarinolin A, only expansolides A and B (**5a**, **5b**), a pair of C4 epimers, share the same tetracyclic ring system.[2] Later on, closely related molecules such as purpurolides B, D, and E (**2**, **3**, and **4**),[3] expansolides C and D (**6a**, **6b**),[4] and decipienolides A and B (**7a**, **7b**)[5] were isolated and characterized. Purpurolides C and F (**8**, **9**) and eutypellacytosporins A-D[6] (**10a**, **10b**, **11a**, **11b**) with higher oxidation state at C14 were isolated as well. For the eutypellacytosporins, the bergamotane sesquiterpene core is connected with cytosporin D via an acetal bond. Additionally, spiroaminal derivatives sporulaminals A and B (**12a**, **12b**) were discovered.[7] Interestingly, for expansolides, decipienolides, eutypellacytosporins, and sporulaminals, which lack the C5 hydroxyl group, a pair of C4 epimers was isolated and the epimers interconvert to each other easily. However, for massarinolin A and purpurolides B-F containing the C5 hydroxyl group, only one epimer was isolated, indicating that the C5 hydroxyl group and its stereochemistry help control the stereoselectivity of the C4 spirocenter. Notably, while for massarinolin A, the C5 hydroxyl group and the C1 carboxylate are *cis* according to the central THF ring, for purpurolides B-F, these two groups are *trans* to each other, which renders suspicion about one of their structural assignment. Since purpurolides B and C's structures were unambiguously confirmed by X-ray analysis, we speculated that the originally proposed massarinolin A (**1a**) structure might be incorrect. Thus, we first computed the ^{1}H and ^{13}C NMR of **1a** and its C4 epimer **1b** with Gaussian 16 and found that **1b** has better match with the reported NMR data of massarinolin A (see Supporting Information).

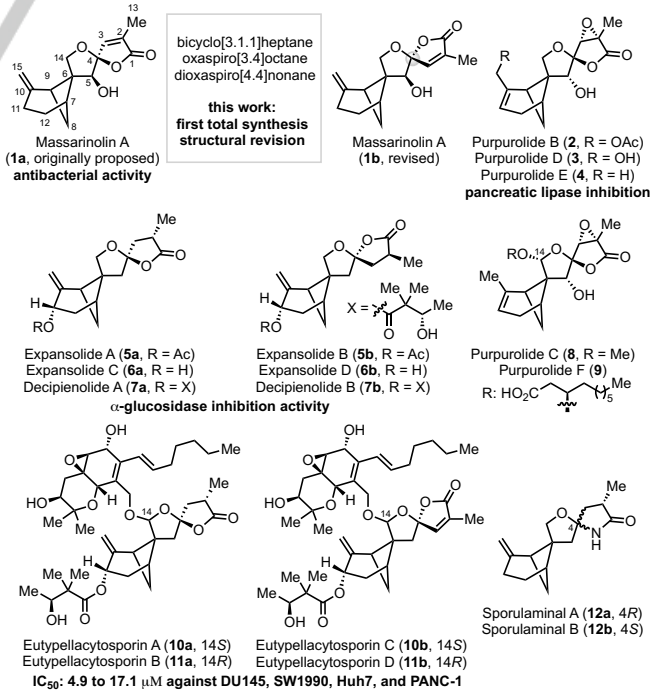


Figure 1. Structures and activity of selected bergamotane sesquiterpenes.

The unique structures of these bergamotane sesquiterpenes are accompanied with intriguing biological activities. Massarinolin A demonstrated activity against Gram-positive bacteria including *Bacillus subtilis* and *Staphylococcus aureus*. Purpurolides D, E, and F showed significant inhibitory activity against pancreatic lipase, an important anti-obesity drug target. Expansolides C and D were active against α-glucosidase, a target for type 2 diabetes drug discovery. The eutypellacytosporins exhibited low μM IC_{50} against a panel of cancer cell lines. These bergamotane natural products were isolated in low yield and quantity, which are not sufficient for further biological evaluations. Thus, a total synthesis is needed for material supply and analog/probe preparation.

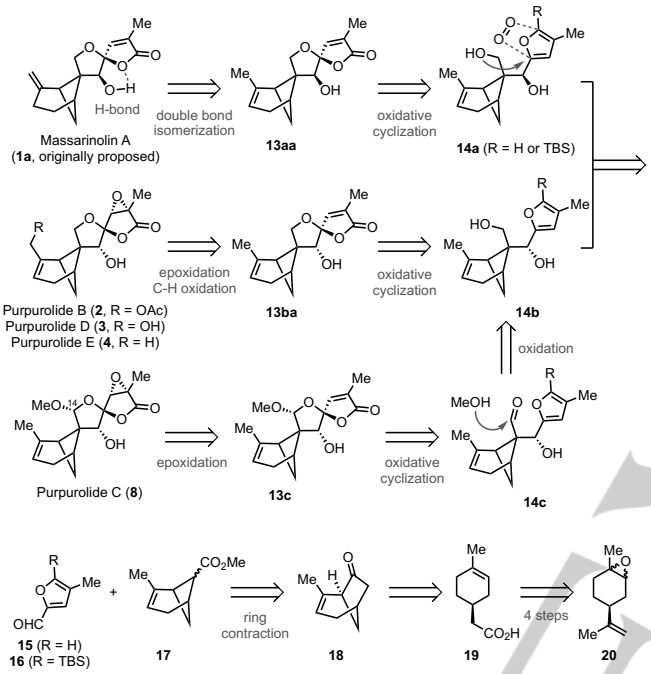


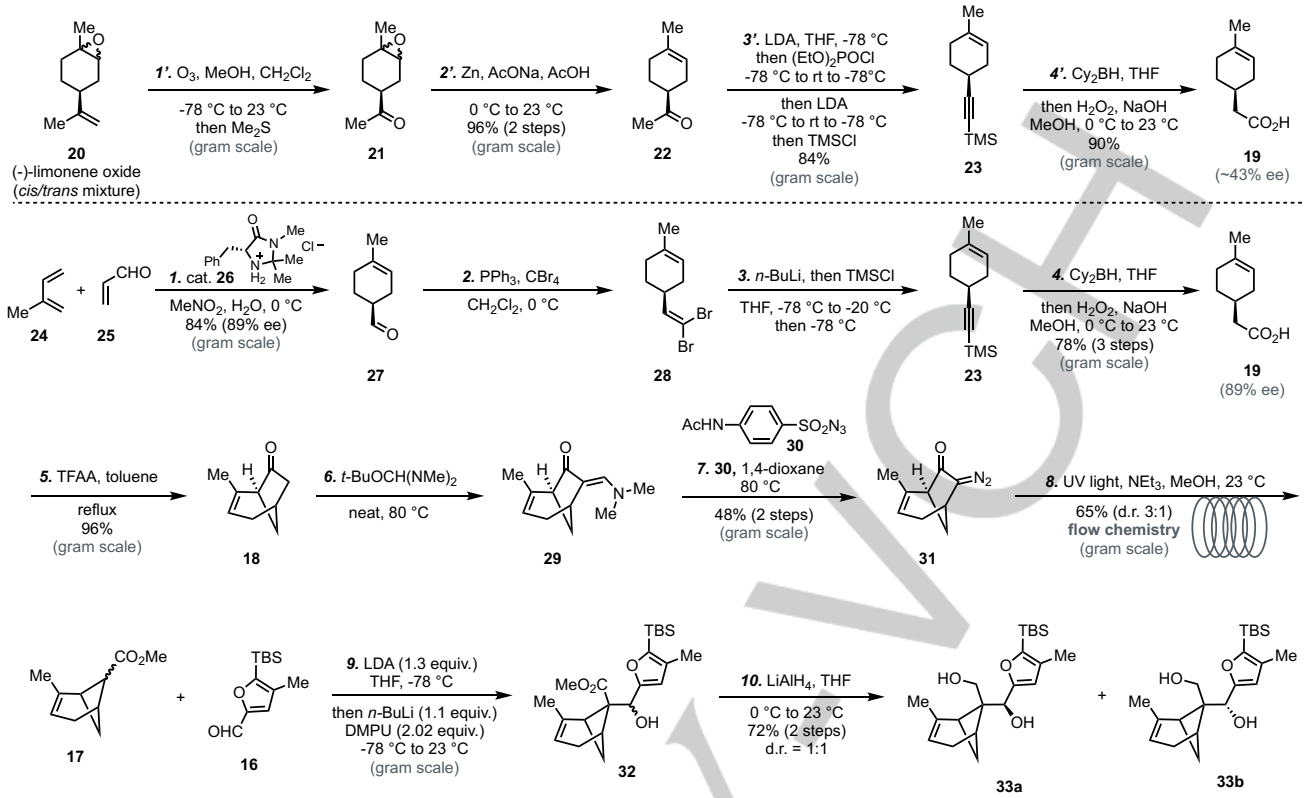
Figure 2. Retrosynthetic analysis.

Our synthetic and biological interest in natural products which feature an oxaspirolactone moiety[8] and have the potential to covalently modify cellular proteins[9] led us to massarinolin A and its closely related analogs (Figure 1). Prior to our study, there was no reported total synthesis of these tetracyclic bergamotane sesquiterpenes. We decided to develop a divergent approach to synthesize these intriguing sesquiterpenes with the aim to fully establish their chemical structures and provide material for comprehensive biological profiling and understand their mode of actions. Herein, we report the first and enantioselective total syntheses of massarinolin A (**1a** and **1b**), purpurolides B (**2**), D (**3**), E (**4**) and 2,3-deoxypurpurolide C (**13c**). Our total synthesis led to a structural revision of massarinolin A (**1b**) and establishment of the absolute configuration of massarinolin A and purpurolides B, D and E.

Retrosynthetically (Figure 2), we proposed that massarinolin A (**1a**) could be derived from **13aa** via an energetically disfavored isomerization of an endocyclic olefin to an *exo*-methylene. **13aa** could be prepared from **14a** via a furan oxidative cyclization.[10] Purpurolide B/D/E could be prepared via oxidation (epoxidation and allylic C-H oxidation) of **13ba**, which could be obtained from a furan oxidative cyclization of **14b**. **14a** and **14b** differ at the secondary alcohol stereochemistry of C5. We intentionally planned a divergent approach to prepare both of them via a non-stereoselective aldol reaction between ester **17** and aldehyde **15** or **16** followed by ester reduction. We were hoping that the aldol reaction would be able to construct the challenging all-carbon quaternary center. Both **15**[11] and **16**[12] are known compounds and can be prepared readily in large scale. For **17**, we envisioned a ring contraction from **18** to build its challenging bicyclo[3.1.1]heptane ring system. **18** could be prepared from **19** via an intramolecular acylation. The latter is a known compound derived from (–)-limonene oxide **20**.[13] Meanwhile, we were interested in accessing purpurolide C (**8**) and others with an acetal at C14. A direct C-H oxidation of purpurolide E (**4**) to purpurolide C (**8**) is appealing, but challenging due to the highly strained bicyclo[3.1.1]heptane ring system, which can undergo facile rearrangement or fragmentation once a cation or radical is generated at C14. Thus, an alternative plan was to oxidize **14b** to aldehyde **14c** followed by a tandem acetal formation-oxidative cyclization to provide **13c**, which could then be epoxidized to purpurolide C. Notably, such furan oxidative cyclization with an aldehyde was unprecedented, rendering uncertainty of this step.

We started by preparing **19** from (–)-limonene oxide **20** via the reported four-step sequence,[13] namely, ozonolytic cleavage of the double bond (**20**→**21**, Scheme 1), reduction of the epoxide to an olefin (**21**→**22**), a one-pot protocol to convert the methyl ketone to a TMS-alkyne (**22**→**23**), and Brown hydroboration-oxidation to convert the alkyne to a carboxylic acid (**23**→**19**). This four-step sequence worked smoothly with high yield and scalability. However, **19** was produced with only about 43% ee. We believe that partial racemization happened in the alkyne formation step, which requires highly basic conditions. We tried to optimize the reaction conditions by tuning the reaction temperatures and the amounts of LDA, but were not successful in preventing the stereochemistry erosion. Therefore, we decided to develop a new enantioselective approach to prepare **19**. An organocatalyzed Diels-Alder reaction between **24** and **25** with the chiral amine catalyst (**26**) developed by MacMillan et al. was used to prepare aldehyde **27** (84% yield and 89% ee).[14] Corey-Fuchs homologation then converted **27** to **23**. The latter was oxidized to **19** via the Brown hydroboration-oxidation. In this new route, **19** was produced in 89% ee and 66% overall yield from **24** and **25**. Each step can be conducted at gram scale.

With a reliable approach to prepare **19**, we started to synthesize bicyclic ketone **18** for the proposed ring contraction. **19** was treated with trifluoroacetic anhydride to form a mixed anhydride, which under the same thermal conditions was converted to **18** via an intramolecular acylation. We decided to explore the Wolff rearrangement for the proposed ring contraction.[15] Thus, α-diazoketone **31** was needed. After some investigations, **18** was converted to vinylogous amide **29** by heating with neat Bredereck's reagent. Heating **29** with 4-acetamidobenzenesulfonyl azide (*p*-ABSA, **30**) in 1,4-dioxane at 80 °C afforded α-diazoketone **31** in 48% yield over two steps.[16] We then explored the photochemical Wolff rearrangement to convert **31** to **17**. Irradiation of **31** in MeOH with triethylamine as base provided **17** as a 3:1 mixture of diastereomers at small scale, but the batch reaction was very difficult to scale up to at least gram scale because it would require a large quartz reaction flask and big photoreactor. Flow chemistry offers many advantages over batch chemistry especially when scaleup is considered,[17] but its application in complex natural product total synthesis is still quite rare.[18] We decided to explore the possibility of using flow

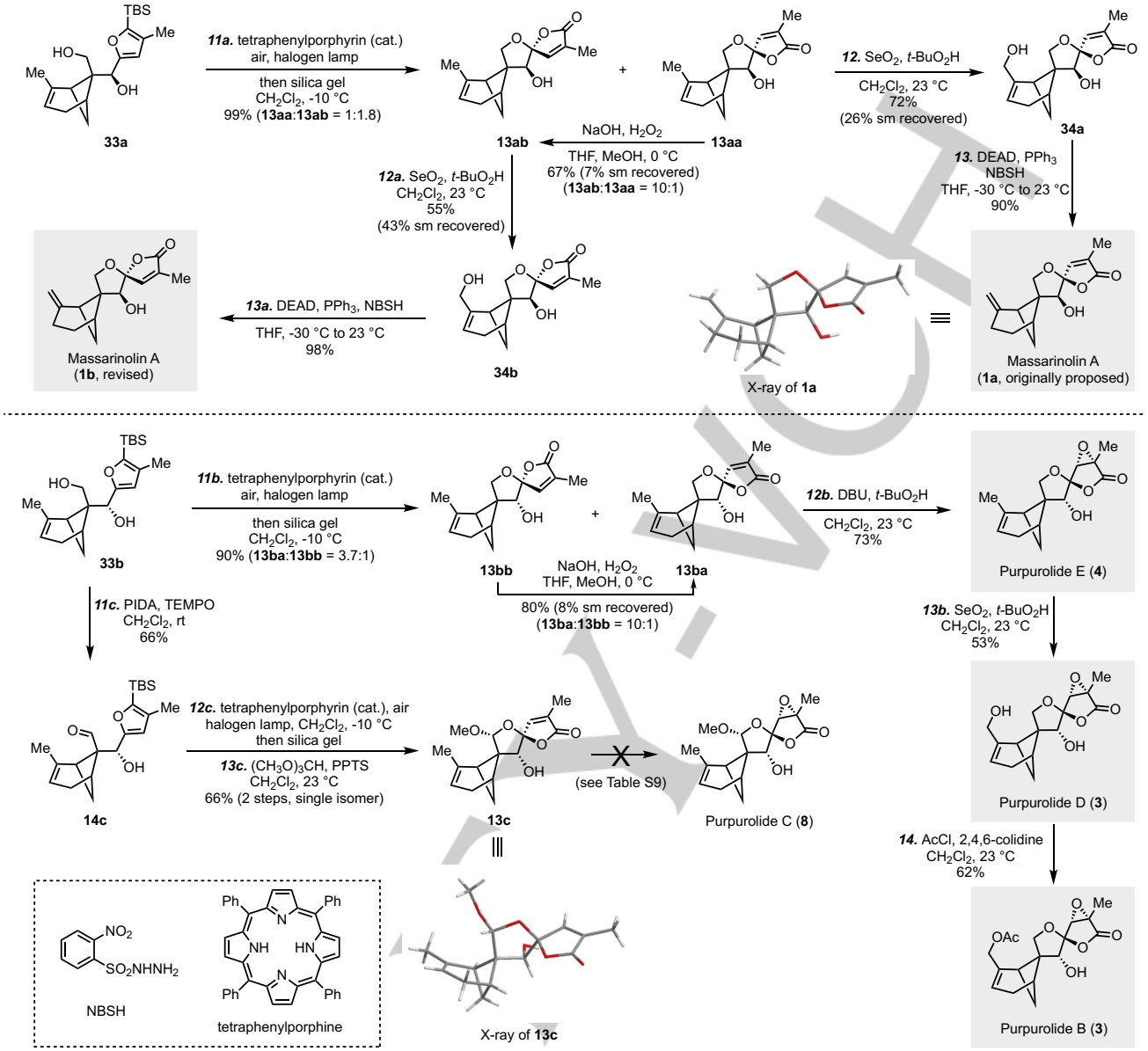


Scheme 1. Synthesis of key intermediates **33a** and **33b**.

photochemical Wolff rearrangement[19] to produce key intermediate **17** in large scale. Fortunately, we were able to transfer the batch chemistry result to flow chemistry at a much larger scale to produce **17** in 65% yield. With **17** in hand, we started to investigate the aldol reaction, which turned out to be nontrivial at all because a sterically congested all-carbon quaternary center is formed in this step. Using furfural as a model aldehyde, we explored the conditions of employing LDA, LDA with HMPA, LiHMDS, NaHMDS, KHDMS, NaH, KH, or KH with 18-crown-6 as base to generate the corresponding enolate, but none of them gave any aldol product. Eventually, we learned that the aldol product could be formed with a combination of LDA followed by *n*-BuLi and DMPU. After further optimizations with aldehyde **16**[12] as the electrophile we were able to realize the aldol reaction by first treating **17** with 1.3 equiv. of LDA to form the corresponding lithium enolate followed by adding 1.1 equiv. of *n*-BuLi to quench the newly formed diisopropylamine and 2.02 equiv. of DMPU (*N,N'*-dimethylpropyleneurea) to break the Li enolate aggregates. The resulting aldol products (d.r. 1:1) were further reduced by $LiAlH_4$ to give a separable 1:1 mixture of **33a** and **33b** in 72% yield (two steps) for the proposed divergent synthesis.

We next started to advance **33a** to massarinolin A (**1a**). Photochemical oxidative furan cyclization with tetraphenylporphine as catalyst in presence of oxygen followed by treating the reaction mixture with silica gel successfully formed the oxaspirolactone moiety and delivered a separable mixture of **13ab** and **13aa** (d.r. = 1:1.8) in 99% yield slightly favoring **13ab** with an opposite stereochemistry of **1a** at the newly formed spirocenter. Notably, the TBS group on the furan ring has a dramatic impact on the oxidative cyclization. The substrate lacking the TBS group was much less effective. We further learned that **13aa** could be converted to **13ab** in 67% yield (d.r. = 10:1) with a combination of NaOH and H_2O_2. These results indicate that **13ab** is thermodynamically more stable than **13aa**, while the C5 hydroxyl group and the C1 carbonyl oxygen of **13aa** can potentially form a hydrogen bond. The fact that **13aa** is less polar than **13ab** is presumably due to this hydrogen bond effect. The epimerization results also led us to further suspect the proposed structure (**1a**) of massarinolin A. Nevertheless, we decided to move forward with **13aa**. At this stage, the endocyclic olefin needed to be isomerized to an *exo* cyclic olefin, an energetically disfavored process. After failing to photochemically isomerize this double bond, we proceeded with a two-step sequence. First, allylic C-H oxidation converted **13aa** to **34a**. Next, the Myers' NBSH-promoted sigmatropic elimination[20] converted **34a** to the proposed structure of massarinolin A (**1a**) in 90% yield. The structure of synthetic **1a** was unambiguously confirmed by X-ray analysis (CCDC 2073790).[21] However, the ^1H and ^{13}C NMR spectra and optical rotation ($[\alpha]^{25.5}_D = -124.6°$) of the synthetic sample don't matched with the ones of the natural sample, which confirms our earlier suspicion about its structural misassignment. We then advanced epimer **13ab** to **1b** via the two-step allylic C-H oxidation and Myers' sigmatropic elimination. The ^1H and ^{13}C NMR spectra and optical rotation ($[\alpha]^{24.7}_D = +29.0°$) of **1b** match well with the ones of the natural sample. Thus, we revised the structure of massarinolin A from **1a** to **1b**.

We then continued to synthesize the purpurolides from **33b**. Photochemical oxidative furan cyclization converted **33b** to a 3.7:1 mixture of **13ba** and **13bb** in 90% yield favoring **13ba**. The minor epimer **13bb** could be further transformed to **13ba** in 80% yield (d.r. = 10:1) via the $NaOH/H_2O_2$-promoted epimerization. In both epimeric pairs (**13aa**/**13ab** and **13ba**/**13bb**), the thermodynamically more stable product is the one where the C5 hydroxyl group and the C1 carboxylate have a *trans* relationship. Epoxidation of **13ba** with *t*-BuOOH and DBU completed the first total synthesis of purpurolide E (**4**), which further underwent allylic



Scheme 2. Total synthesis of massarinolin A, purpurolide B, D, E, 2,3-deoxypurpurolide C, and structural revision of massarinolin A

C-H oxidation with SeO_2 and *t*-BuOOH to afford purpurolide D (**3**). Selective acetylation of the primary alcohol of purpurolide D gave purpurolide B (**2**). The spectroscopic data of synthetic purpurolides B (**2**), D (**3**) and E (**4**) match well with the reported ones of the natural samples.

We next started to prepare purpurolide C with a higher oxidation state at C14. In this case, aldehyde **14c** was prepared from **33b** in 66% yield via a PIDA/TEMPO-mediated oxidation. We first tried to form the C14 acetal in one step by using MeOH as solvent or co-solvent, but observed only the starting material decomposition. Thus, a 2-step procedure was developed. The photochemical oxidative cyclization first led to a hemiacetal intermediate in high yield and diastereoselectivity. The hemiacetal was then treated with trimethyl orthoformate and PPTS to deliver **13c** (2,3-deoxypurpurolide C) in 66% yield, the structure of which was unambiguously confirmed by X-ray analysis (CCDC 2089353). Notably, this conversion is nontrivial at all. The conditions directly involving MeOH failed to afford the desired product, highlighting the structural fragility of **13c**. Unlike the facile transformation of **13ba** to purpurolide E, the epoxidation of **13c** was surprisingly difficult. Various conditions were investigated, but were fruitless (see Table S9 in the Supporting Information). Our attempts to directly oxidize purpurolide E to C were not successful as well. While this branch of synthesis only led to 2,3-deoxypurpurolide C thus far, it provides an avenue to other C14 acetal-containing molecules such as the eutypellacytosporins.

In summary, we developed a divergent and enantioselective approach to complete the first total syntheses of several structurally challenging and unique bergamotane sesquiterpenes including massarinolin A, purpurolides B, D, E, and 2,3-deoxypurpurolide C in 12 to 14 steps. Our synthesis and computational analysis also led to a structural revision of massarinolin A and established its absolute configuration. This synthesis was enabled by a scalable flow photochemical Wolff rearrangement and features an enantioselective organocatalyzed Diels-Alder reaction, a furan oxidative cyclization, a late-stage allylic C-H oxidation, and a Myers' NBSH-promoted sigmatropic elimination. Additionally, a novel furan oxidative cyclization

involving an aldehyde was developed, which could be useful in making other natural products including the anticancer eutypellacytosporins. Comprehensive biological evaluations of the bergamotane sesquiterpenes we prepared are undergoing and will be reported in due course.

Acknowledgements

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Keywords: total synthesis • flow chemistry • massarinolin A • purpurolide • photochemistry

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[21] CCDC 2073790 and CCDC 2089353 contain the supplementary crystallographic data for **1a** and **13c** reported in this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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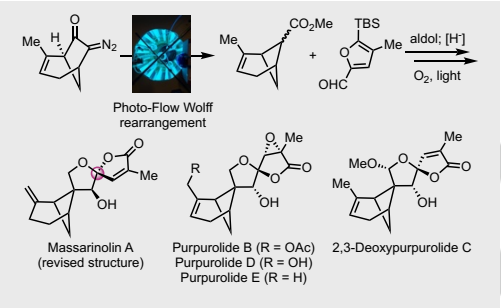
COMMUNICATION

The first total syntheses of complex bergamotane sesquiterpenes massarinolin A, purpurolides B, D, E and 2,3-deoxypurpurolide C were achieved with key steps involving an enantioselective organocatalyzed Diels-Alder reaction, a scalable flow photochemical Wolff rearrangement, a furan oxidative cyclization, a late-stage allylic C-H oxidation and sigmatropic elimination. Our synthesis also led to a structural revision of massarinolin A.



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