Angewandte Chemie
International Edition

Natural Product Synthesis **Hot Paper**

DOI: 10.1002/anie.201407726

Enantioselective Divergent Syntheses of Several Polyhalogenated *Plocamium* Monoterpenes and Evaluation of Their Selectivity for Solid Tumors**

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***Abstract:** The family of polyhalogenated monoterpenes from Plocamium counts over a hundred known members. Using glyceraldehyde acetonide as a chiral-pool precursor, an enantioselective and divergent strategy was developed that provides a blueprint for the synthesis of many of the small yet complex acyclic members of this family. The broad applicability of this approach is demonstrated with the short, eight-step synthesis of four natural products and three analogues. These syntheses are the first of any members of the acyclic polyhalogenated Plocamium monoterpenes and permitted the evaluation of their selectivity against a range of tumor cell lines.*

In 1973, Faulkner reported the discovery of (3*R*,4*S*,7*S*)-3,7-dimethyl-1,8,8-tribromo-3,4,7-trichloro-(1*E*,5*E*)-octadiene (**1**, Figure 1) from the digestive glands of the sea hare *Aplysia californica* as the first in a series of polyhalogenated monoterpenes.[1] Shortly thereafter, it became clear that these types of secondary metabolites were the products of red algae from the genus *Plocamium*, a major food source for the sea hares.[2] In the time since, over a hundred highly halogenated monoterpenes, both cyclic and acyclic, have been isolated from many different *Plocamium* species and from various locations.[3]

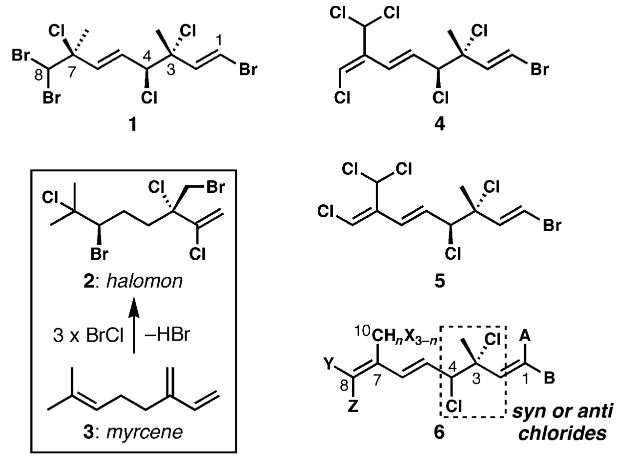

Figure 1. Representative acyclic polyhalogenated monoterpenes from *Plocamium* and the related natural product halomon with its biogenesis from myrcene. A, B, X, Y = Cl, Br, or H.

The family of acyclic polyhalogenated monoterpenes gained some renown in the 1990s with the discovery that halomon (**2**) had a unique profile of cytotoxicity when tested against the NCI 60-cell line. It demonstrated particular selectivity for cell lines that are typically resistant to chemotherapy (renal, brain, colon, and non-small-cell lung cancer), with less efficacy towards leukemia and melanoma lines.[4,5] Isolated from the red alga *Portieria hornemannii*, this compound was the subject of preclinical development at the NCI. The biogenesis of halomon from myrcene (**3**) is easily explained by sequential bromochlorination of each alkene followed by a single elimination of HBr (**3**→**2**). This hypothesis was used as an inspiration for the exceptional three-step synthesis of racemic halomon by Hirama and coworkers.[6] The biogenesis of many of the *Plocamium* monoterpenes (including **1**) is not so easily explained,[3] and a straightforward laboratory conversion of an acyclic monoterpene feedstock into these targets is not likely.

Syntheses of the acyclic polyhalogenated *Plocamium* monoterpenes have not yet been described,[7,8] in spite of their reported anticancer and antimalarial activities.[9,10] Previously unpublished work from two of our laboratories established that isomeric compounds **4** and **5**[2] are selectively cytotoxic to several solid tumor cell lines,[11] as defined by the

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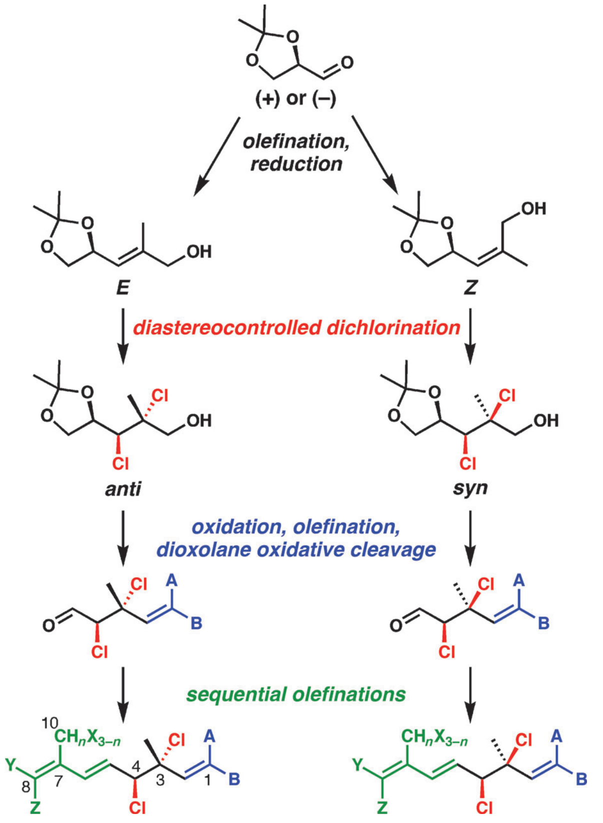
[**] This work was supported by the NIH (R01 GM086483 to C.D.V., F31 CA174176 to C.V.V., and R01 CA100851 to W.H.G. and F.A.V.) and by the UC Cancer Research Coordinating Committee (to C.D.V.). We thank Dr. John Greaves for support with mass spectrometry and Dr. Joseph Ziller and Jordan Corbey for assistance with X-ray crystallography.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201407726.

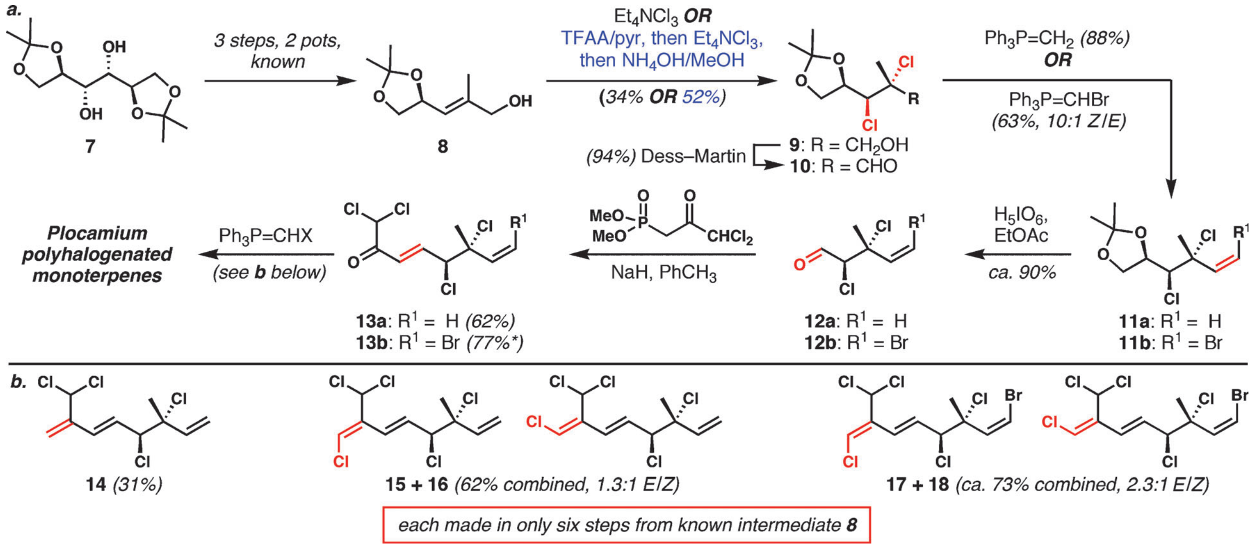


Scheme 1. Divergent synthesis plan for the *Plocamium* monoterpenes. Colored regions are diversifiable. A, B, X, Y = Cl, Br, or H.

disk diffusion assay pioneered by Valeriote and co-workers.[12] Our goal in this work was to generate many more compounds of this series (the natural variability is shown by general structure **6**). We targeted a significant array of natural and unnatural analogues for further testing of activity and selectivity, with the eventual goal of performing mechanism-of-action and pharmacological studies on the most promising candidates. Herein, we describe a simple strategy for the enantioselective and divergent synthesis of many of these natural products from glyceraldehyde acetonide, with proof-of-principle execution resulting in access to four different natural products and three analogues.

In considering a synthesis of compounds of type **4** and **5**, we recognized multiple challenges in spite of the small molecular size of the targets. First, the vicinal secondary and tertiary chloride-bearing centers at the C3 and C4 positions, which are present in both the *syn* and *anti* diastereomeric forms within this family of natural products,[2,3,10] are both allylic and might cause undesired reactivity. Second, the extensive halogenation on either side of the "central" C5–C6 alkene appears to necessitate a convergent approach; however, the method chosen to unite two precursor fragments must be tolerant of the potentially reactive halogen atoms. Third, as we wanted to access tens or hundreds of milligrams of many different natural (and unnatural) polyhalogenated *Plocamium* monoterpenes for biological evaluation, we placed a premium on the development of a short and scalable, yet divergent sequence that could effectively provide access to dozens of different targets. The culmination of our work to address these challenges is the flexible approach shown in Scheme 1, which permits access to both enantiomeric series, both diastereomeric forms of the C3/C4 vicinal dichloride, and a wide range of halogen substitutions at the C1, C8, and C10 positions. It features the non-obvious use of glyceraldehyde acetonide as the source of the C4 and C5 carbon atoms, as a formal "chiral glyoxal" equivalent that permits the stereocontrolled introduction of the C3 and C4 chlorine-bearing stereogenic centers, and as a linchpin for the entire synthesis.

Mannitol diacetonide (**7**; Scheme 2a) has previously been converted into allylic alcohol **8** by oxidative cleavage to

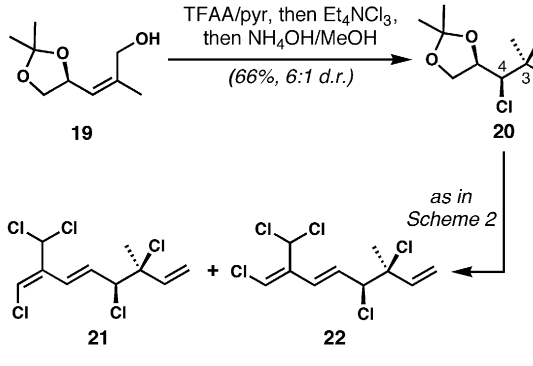


Scheme 2. a) General sequence for the synthesis of polyhalogenated monoterpenes. *: Olefination product **13b** is contaminated by about 10% of the elimination product (conjugated trienone). b) Examples of five completed targets, with yields for the final olefination steps. Geometrical isomers **15**/**16** and **17**/**18** were completely separated by HPLC. TFAA = trifluoroacetic anhydride.

glyceraldehyde acetonide, stereoselective olefination, and ester reduction.[13] Dichlorination of the free allylic alcohol **8**, although highly diastereoselective, was plagued with competitive oxidation to an enal[14] that resisted chlorination; this undesired reactivity could not be prevented by tuning of the reaction conditions. Therefore, a one-pot sequence of in situ trifluoroacetylation, dichlorination, and deacylation was developed, which afforded higher overall yields and high selectivity (> 10:1 d.r.). It is likely that the minimization of 1,3-allylic ($A^{1,3}$) strain[15] in substrate **8** and its trifluoroacetylated derivative is important, and although the outcome mirrors the studies of Chamberlin and Hehre on the iodofunctionalization of allylic alcohols,[16,17] the mechanism underlying stereocontrol is not completely understood. X-ray crystallographic analysis on a mesylated derivative of alcohol **9**[18] enabled the unequivocal assignment of the absolute and relative configuration for compounds produced by this synthetic route.

Oxidation of alcohol **9** to aldehyde **10** was followed by methylenation or bromomethylenation to afford products **11a** or **11b**, respectively. Direct oxidative cleavage of the dioxolane produced sensitive aldehydes **12a**/**12b** in high yield. Olefination of these products was plagued by β-elimination under many conditions examined; however, the use of NaH in toluene with the Horner–Wadsworth–Emmons reagent shown provided enones **13a**/**13b** in good yields with high levels of stereocontrol. Finally, methylenation or chloromethylenation provided targets **14**–**18** in the yields shown in Scheme 2b. The chloromethylenation products were obtained as mixtures at the newly formed alkenes, but they could be separated easily by preparative HPLC.[19] Compounds **14**–**16** and **18** are known natural products,[2,3,10] but **17** has not yet been reported.

The synthesis shown in Scheme 2 is highly divergent and will provide access to many more naturally occurring polyhalogenated natural products from *Plocamium* as well as unnatural analogues. However, there remains another important aspect of our design: The *Z*-isomer of allylic alcohol **8** is also readily available (see **19**, Scheme 3),[20–22] and we have shown that it also undergoes stereocontrolled dichlorination to procure the *syn*-configured C3/C4 dichloride **20**. The relative configuration of product **20** was established by X-ray crystallographic analysis of the corresponding aldehyde obtained by oxidation.[23] Dichloroalcohol **20** was converted into **21** and **22** by similar reactions to those shown in Scheme 2.[23,24] The final products thus produced are in the unnatural enantiomeric series because we used the same enantiomer of glyceraldehyde acetonide as we did for **14**–**18**. Clearly, natural products in both diastereomeric series, as well as in both enantiomeric forms, are available by this route, because glyceraldehyde acetonide is readily available in both mirror-image forms. Each of the target molecules (**14**–**18**, **21**, and **22**) was obtained in only six steps from allylic alcohols **8** or **19** (eight steps from inexpensive commercially available mannitol derivative **7**). Although some of the steps proceeded in only moderate yields, and the chemistry is "classical", these small but complex molecules have never been synthesized before, and the sequence is direct enough to procure plenty of material for biological evaluation.[25]



Scheme 3. Synthesis of 3,4-*syn* *Plocamium* monoterpenes **21** and **22** through diastereocontrolled dichlorination of *Z*-allylic alcohol **19**. See the Supporting Information for details.

Stereocontrolled access to the chloride-bearing stereogenic centers at the C3 and C4 positions of the *Plocamium* monoterpenes would not be easily accomplished by other existing methods. The two best currently available methods to obtain vicinal dichlorides in enantioenriched form—the asymmetric dichlorination of allylic alcohols put forth by the Nicolaou group[26] and the stereospecific deoxydichlorination of epoxides developed by the Yoshimitsu group[27]—do not appear to tolerate the formation of tertiary chlorides, such as that at the C3 position. Moreover, the ready availability of substrate **8** and the utility of the dioxolane for more than just the control of diastereoselectivity in the dichlorination (see below) is particularly attractive.

Although the synthesis of a ten-carbon target by four classical olefination reactions might seem far from ideal, this approach permits maximum divergence both with respect to constitution and configuration (Scheme 1) and suffers from few non-productive operations. The biggest liability, at this stage of development, is the lack of stereocontrol in the final olefination step; this apparent problem is mitigated by the fact that many of these natural products are found in both C7–C8 isomeric forms,[2,3] and that the isomers are readily separable. Furthermore, in the context of compounds **4** and **5** at least, these isomers are known to readily equilibrate upon exposure to minimally acidic medium.[19] Overall, the strategic use of glyceraldehyde acetonide as a chiral glyoxal equivalent is significant; it not only plays the role of a chiral auxiliary to control the absolute configuration of the C3/C4 dichloride, it also serves as a truly effective linchpin for our short synthesis by virtue of the one-step conversion of the dioxolane into an aldehyde. At current count, we have made four naturally occurring polyhalogenated *Plocamium* monoterpenes, one that is likely to be an as yet undiscovered natural product, and two unnatural enantiomers, thereby showcasing the generality of our approach.

Compounds **14**–**16** were subjected to the disk diffusion assay, which provides an indication of the selectivity of compounds for human solid-tumor cell lines compared with the CCRF–CEM human leukemia cell line. These data were compared with the data obtained for halomon (**2**) and compound **4** using the same assay (Table 1).[11,28] All five compounds exhibited selectivity for solid tumors, with some

Table 1: Selectivity of halomon (**2**), compound **4**, and synthetic compounds **14**–**16** for different solid-tumor cell lines[a] with respect to the CCRF–CEM leukemia cell lines according to the disk diffusion assay,[b] and their activity measured as the IC_{50} value against HCT-116 cells. A "⋆" indicates selectivity for the indicated cell line.

Compound	H116	H125	MCF-7	LNCaP	OVC-5	U251N	MDA	PANC-1	HepG2	IC_{50} [$\mu g\,mL^{-1}$]
halomon (**2**)	⋆	⋆		⋆	⋆	⋆	⋆		⋆	0.37[c]
4		⋆		⋆	⋆	⋆	⋆		⋆	1.3
14	⋆	⋆		⋆	⋆	⋆				15
15	⋆	⋆	⋆	⋆	⋆	⋆	⋆		⋆	1.3
16	⋆	⋆	⋆	⋆	⋆	⋆				3.6

[a] H116: HCT-116 human colon carcinoma; H125: human non-small-cell lung carcinoma; MCF-7: human breast adenocarcinoma; LNCaP: androgen-sensitive human prostate adenocarcinoma; OVC-5: OVCAR-5 human ovary carcinoma; U251N: human glioblastoma; MDA: MDA-MB-231 human breast carcinoma; PANC-1: human pancreatic carcinoma; HepG2: human hepatocellular carcinoma. [b] The disk-diffusion soft-agar colony formation assay is used to identify compounds that are selective for solid-tumor lines. The observation of a substantial difference in the zone of growth inhibition between solid-tumor cell lines and leukemia cell lines indicates solid-tumor selectivity. See the Supporting Information for raw data. [c] Insufficient amounts of halomon were available to determine its IC_{50} value against HCT-116 cells in our laboratories; this value is taken from Ref. [4].

differences in the selectivity profiles. None showed selectivity for PANC-1, whereas all were selective for LNCaP, OVCAR-5, and U251N tumor cells with respect to the leukemia cell line. Of the three synthetic compounds evaluated, **15** proved to be the most potent, with an IC_{50} value of $1.3\ \mu g\,mL^{-1}$ against the HCT-116 colon carcinoma cell line, and it demonstrated similar activity to previously isolated compound **4**. This level of activity, coupled with the good selectivity for solid tumors, is sufficient to warrant further studies, including the development of a detailed SAR profile for this class of compounds and the synthesis of more bioavailable analogues. Our versatile synthesis is ideal to fuel these studies.

Received: July 29, 2014
Revised: August 20, 2014
Published online: September 12, 2014

Keywords: antitumor agents · chlorination · olefination · stereocontrol · total synthesis

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[22] We generated compound **19** through a reasonably *Z*-selective (6:1) Horner–Wadsworth–Emmons olefination, which is much more economical than the corresponding modified Still–Gennari reaction. See the Supporting Information for details.

[23] Please see the Supporting Information for details.

[24] The enantiomer of **21** has not yet been reported, but that of **22** is known (see Ref. [2]). On the basis of the ready equilibration of the "left-hand" trichloropropenyl moieties found in **15**–**18**, **21**, and **22**, and the fact that in many cases both geometrical isomers are isolated from the same source, it is reasonable to assume that both **17** and *ent*-**21** are natural products that have not yet been described in the literature.

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