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Highly Selective Synthesis of Halomon, Plocamenone, and Isoplocamenone

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Supporting Information

ABSTRACT: Over 160 chiral vicinal bromochlorinated natural products have been identified; however, a lack of synthetic methods for the selective incorporation of halogens into organic molecules has hindered their synthesis. Here we disclose the first total synthesis and structural confirmation of isoplocamenone and plocamenone, as well as the first selective and scalable synthesis of the preclinical anticancer natural product halomon. The synthesis of these inter-halogenated compounds has been enabled by our recently developed chemo-, regio-, and enantioselective dihalogenation reaction.

Over the course of the past few decades, a plethora of halogenated natural products have been isolated, primarily from marine sources.[1a−c] Roughly 240 contain chiral vicinal dihalide motifs, with the majority (ca. 70%) being bromochlorinated terpenes.[1d] Many of these inter-halogenated compounds are reported to possess promising biological activity, including unique cytotoxicity profiles. Additionally, such molecules pose exquisite challenges to selective synthesis and structural characterization. This is highlighted by the lack of strategies that provide stereocontrolled access to relevant halogenated motifs and by the repeated misassignment of such natural products.

The most-studied molecule of this class, halomon (**1**, Figure 1), was originally isolated in 1975 from the red algae *Portieria hornemannii*.[2a] Full structural data as well as relative and absolute stereochemical assignments were presented 17 years later by the National Cancer Institute (NCI) upon its reisolation and crystallization. In this study, **1** produced "one of the most extreme cases of differential cytotoxicity"[2b] in the NCI-60 human tumor cell line screen. Preliminary *in vivo* studies were so encouraging that the compound was selected by the NCI for preclinical drug development, but it was dropped *explicitly due to lack of sufficient material*.[3a] Despite intense interest in **1** from the scientific community,[3] efforts to isolate sufficient material from natural sources have failed,[3a,h] likely due to local and seasonal variations in terpene content.[3g,h] Although no comprehensive study on the mechanism of action has been reported, insight is gained by the discovery that halomon inhibits DNA methyltransferase-1,[4] an important target for cancer research and therapy.[5] Surprisingly, on the basis of computational analysis of its cytotoxicity profile, researchers have concluded that the activity of **1** is unlikely to stem from it acting as a nonspecific alkylating agent.[3b,6] Experimental investigations into the validity of this statement have not been conducted.

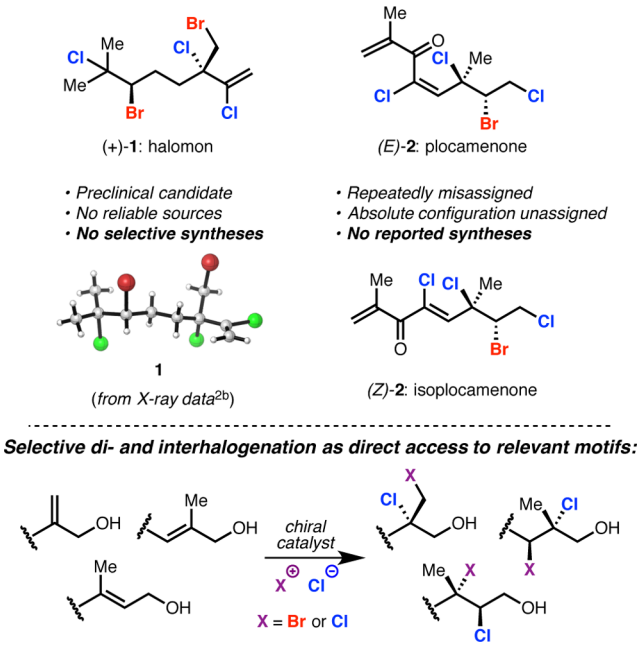

Figure 1. Structures of halomon, plocamenone, and isoplocamenone and our approach to such motifs.

Two nonselective total syntheses of halomon have been reported by Mioskowsky and Hirama.[7] Both efforts afforded halomon as mixtures of all possible diastereo- and enantiomers and rely on silica gel chromatography and two consecutive semipreparative HPLC separations with achiral followed by chiral columns to afford pure (+)-**1** in undisclosed amounts. Hirama reported a discrepancy in optical rotation between synthetic and natural material, leading them to posit the originally isolated halomon may have been impure. While this could call into question the original NCI-60 screen, subsequent NCI-60 screens with halomon from different sources have revealed similar cytotoxicity profiles.[7g−i]

In addition to providing material for biological evaluation, total synthesis is an invaluable tool for the structural assignment of natural produts.[8] Noncrystalline linear polyhalogenated terpenes are notoriously difficult to characterize, as illustrated by numerous NMR studies relying on comparison to synthetic halogenated model systems for structure elucidation.[2a,9] For example, the cytotoxic agent plocamenone (*E*)-**2** has been repeatedly misassigned in the literature,[9b,10] with the closest

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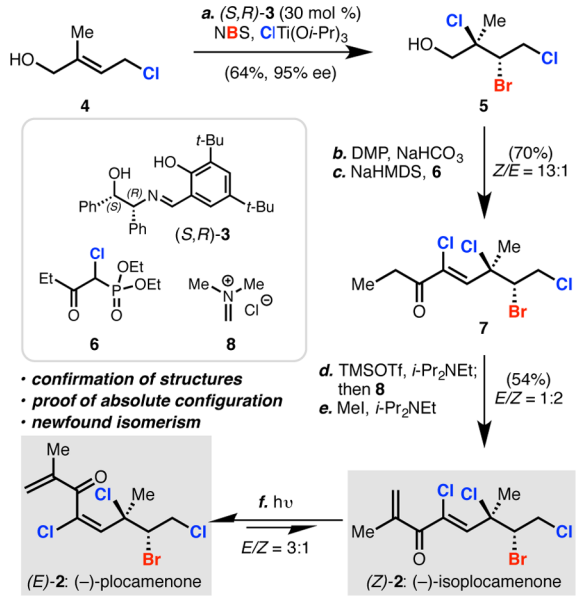
proposed structure being the (*Z*)-isomer.[10b] More recently, Urban isolated and characterized both double-bond isomers from *Plocamium angustum* and assigned the previously isolated compound, plocamenone, as the (*E*)-isomer ((*E*)-**2**). The minor isomer, termed isoplocamenone ((*Z*)-**2**) was reportedly prone to decomposition.[11]

A recent advance made in this laboratory allows for the chemo-, regio-, and enantioselective bromochlorination, dibromination, and dichlorination of allylic alcohols (Figure 1, bottom).[12] Here, this method is used strategically to achieve the first total syntheses, structural confirmation, and determination of the natural enantiomer of isoplocamenone and plocamenone. Further, we report the first total synthesis of (+)-halomon with essentially complete stereocontrol.

Selective Syntheses of (−)-Plocamenone and (−)-Isoplocamenone. We began our syntheses of plocamenone and isoplocamenone with (*S,R*)-Schiff base **3**-catalyzed bromochlorination[12b] of known allylic alcohol **4** (Scheme 1), which

Scheme 1. Synthesis of (−)-Plocamenone and (−)-Isoplocamenone[a]



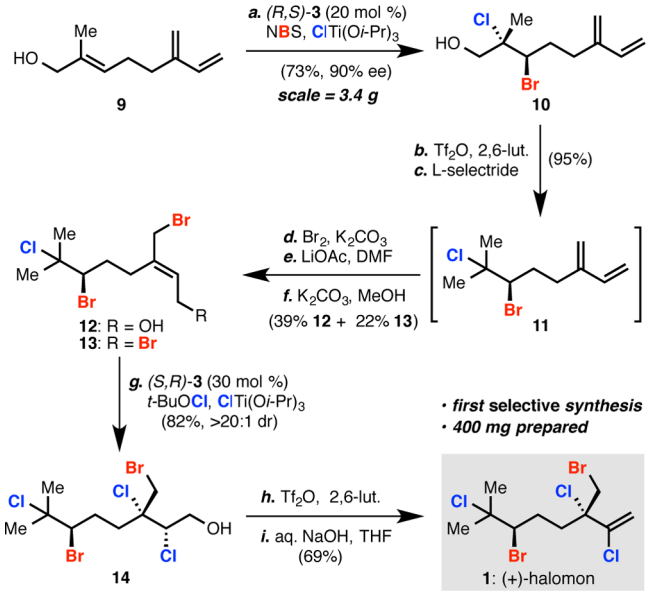
[a]Reagents and conditions: (a) NBS (1.05 equiv), ClTi(O*i*-Pr)$_3$ (1.10 equiv), 20 mol% (*S,R*)-**3**, hexanes, −20 °C, 64%; (b) Dess−Martin periodinane (1.5 equiv), $NaHCO_3$ (10.0 equiv), CH_2Cl_2, rt; (c) diethyl (1-chloro-2-oxobutyl)phosphonate (**6**) (2.0 equiv), sodium hexamethyldisilazide (1.4 equiv), THF, −78 to 0 °C, 70% from **5**; (d) trimethylsilyl trifluoromethanesulfonate (2.0 equiv), *N,N*-diisopropylethylamine (3.0 equiv), CH_2Cl_2, −78 °C to rt; **8** (5.0 equiv), −78 °C to rt; (e) iodomethane (5.0 equiv), *N,N*-diisopropylethylamine (3.6 equiv), THF, rt, 54% from **7**; (f) floodlamp, Et_2O, 5 days, rt.

can be synthesized in one step from commercially available isoprene oxide.[13] Absolute configuration was assigned through X-ray crystallographic analysis (see Supporting Information). The resulting alcohol **5** was then oxidized with Dess−Martin periodinane and subjected to Horner−Wadsworth−Emmons olefination with **6**[14] to yield ketone **7** in a 13:1 ratio of double-bond isomers (see Supporting Information for NOE structural assignment). Treatment with Eschenmoser's chloride salt (**8**) and subsequent activation with iodomethane and elimination[15] yielded a separable 1:2 mixture of plocamenone ((*E*)-**2**) and isoplocamenone ((*Z*)-**2**).[16a] The use of Eschenmoser's iodide salt produced a significantly messier reaction mixture but showed no signs of double-bond isomerization. This leads us to surmise that conjugate addition of chloride followed by C−C bond rotation and elimination may be responsible for this isomerization of the trisubstituted olefin.

In its most recent isolation and structural report,[11] isoplocamenone was reported to be unstable, but no mention of decomposition products was made. In light of the apparent isomerization during the α-methylenation, we suspected that isoplocamenone could possibly convert into its double-bond isomer plocamenone. Indeed, when irradiated with a flood lamp for 5 days, a pure sample of isoplocamenone was found to isomerize to a 3:1 mixture of plocamenone/isoplocamenone.[16b] These syntheses have also established the absolute configuration of the natural products as the shown enantiomers.[17]

Selective Synthesis of (+)-Halomon. Our highly selective route to halomon starts with known allylic alcohol **9**,[18] which was bromochlorinated according to reported conditions[12b] with (*R,S*)-Schiff base **3** to provide **10** in 90% ee on multigram scale (Scheme 2). It was found that the resulting bromochloro

Scheme 2. Synthesis of (+)-Halomon[a]



[a]Reagents and conditions: (a) NBS (1.05 equiv), ClTi(O*i*-Pr)$_3$ (1.10 equiv), 20 mol% (*R,S*)-**3**, hexanes, −20 °C, 73%; (b) trifluoromethanesulfonic anhydride (1.2 equiv), 2,6-lutidine (1.5 equiv), CH_2Cl_2, −78 °C; (c) L-Selectride (2.2 equiv), THF, −78 °C, crude 95% from **10**; (d) Br_2 (1.0 equiv), K_2CO_3 (0.1 equiv) hexanes, −78 °C; (e) lithium acetate (1 equiv), DMF, 0 °C; (f) K_2CO_3 (1 equiv), methanol, 0 °C to rt, 39% **12** + 22% **13**; (g) *t*-BuOCl (1.5 equiv), ClTi(O*i*-Pr)$_3$ (1.10 equiv), 30 mol% (*S,R*)-**3**, hexanes, −20 °C, 82%; (h) trifluoromethanesulfonic anhydride (1.2 equiv), 2,6-lutidine (1.5 equiv), CH_2Cl_2, −78 °C; (i) sodium hydroxide, H_2O, THF, 0 °C to rt, 69% from **14**.

alcohol could be deoxygenated in nearly quantitative yield via activation as the trifluoromethanesulfonate ester followed by treatment with L-Selectride.[19] The resulting bromochlorinated myrcene derivative **11** was taken on without purification and subjected to a formal 1,4-bromohydration sequence,[20] which occurs via dibromide **13** in moderate overall yield but excellent selectivity for the *trans* double-bond isomer **12** which is readily isolable in pure form. Several aspects of this sequence are of note. The initial 1,4-dibromination to **13** is highly selective for

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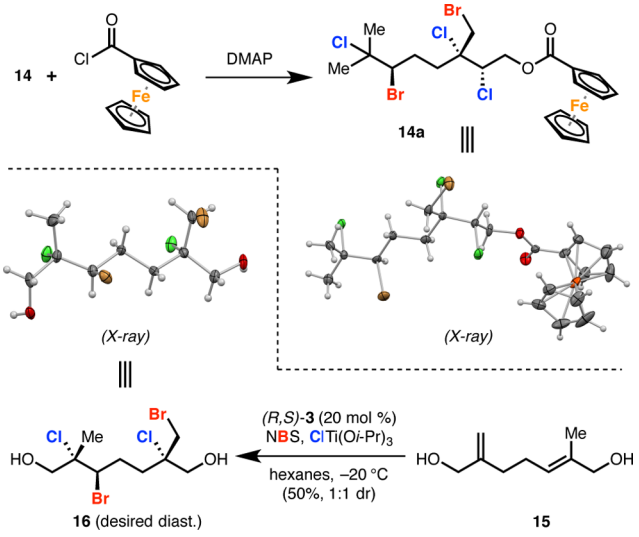
the *E*-isomer, presumably due to dibromination occurring on the *s-trans* conformational isomer of **11**. Bromide displacement with acetate occurs with high selectivity for the less-hindered terminal allylic site, but at high conversion subsequent displacement of the second allylic bromide occurs, prompting us to stop this reaction prior to full conversion. In our hands, direct bromoacetylation of **11** with acetyl hypobromite gave mixtures of 1,4- and 1,2-bromoacetylated products.

Bromohydrin **12** was then subjected to dichlorination conditions[12b] with (*S,R*)-Schiff base **3** to give alcohol **14** in >20:1 diastereomeric ratio. *In the absence of chiral ligand, an inseperable 1:1 mixture of dichloride diastereomers was obtained.* Other dichlorination conditions such as Et_4NCl_3 also resulted in no diastereoselectivity. At this point, the absolute and relative configuration was confirmed by X-ray crystal structure analysis of ferrocenecarboxylate derivative **14a** (Scheme 3, top).

Scheme 3. Stereochemical Confirmation of 14 and a First-Generation Approach to Halomon



We have identified this derivatization as being particularly valuable in cases where common benzoate and sulfonate derivatives fail to induce adequate crystallinity. Alcohol **14** was then converted to the primary trifluoromethanesulfonate ester and selectively eliminated with aqueous hydroxide in the presence of five alkyl halides to afford (+)-halomon (**1**). The route thus developed has allowed for the synthesis of 400 mg of pure (+)-**1**. In initial biological activity investigations (K562 human leukemia), we have repeatedly measured 50% growth inhibitory concentrations of 1.3−2.3 μM, closely matching the originally reported 1.2 μM.[2b] We also have used this chemistry to synthesize unnatural enantiomer (−)-**1**, demonstrating the power of the developed dihalogenation reaction in this context.[21] The stage is now set for the synthesis of other unnatural stereoisomers as well as analogues for structure−activity relationship and target-identification studies.[22]

Our first strategy toward a selective synthesis of halomon involved attempted double bromochlorination of bis-allylic alcohol **15** (Scheme 3, bottom). Likely due to poor solubility of the substrate in the reaction solvent, the diastereoselectivity of the reaction was found to be poor. Whereas a 20 mol% ligand loading afforded a 1:1 mixture of diastereomers, the best result was obtained with 100 mol%, giving a meager ratio of 3:1 favoring the desired diastereomer **16** (confirmed via X-ray crystallographic analysis, Scheme 3). The identity of the minor diastereomer was not unambiguously determined.

The chemo-, enantio-, and regioselective di- and interhalogenation developed in this laboratory has proven to be an invaluable tool for the synthesis of stereocomplex polyhalogenated natural products. It has enabled the first total synthesis of isoplocamenone and plocamenone, as well as the first selective and scalable total synthesis of halomon. The syntheses have made possible the structural confirmation of isoplocamenone and plocamenone, the determination of their natural enantiomers, and the finding of an interesting photoisomerization between the two compounds. In addition, the synthesis reported here proved to be a reliable source of pure (+)-halomon, allowing a total of more than 400 mg to have been synthesized to date. This material will be used in future studies toward answering important questions pertinent to the mechanism of action of halomon.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b08398.

Experimental procedures, characterizations, and spectral data (PDF)
X-ray crystallographic data for **14a** (CIF)
X-ray crystallographic data for **16** (CIF)
X-ray crystallographic data for **S5** (CIF)

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Notes

The authors declare no competing financial interest.

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(16) (a) Conducting the α-methylenation in the dark yielded the same 1:2 mixture of double-bond isomers. (b) Irradiation for 12 h produced a 2:1 plocamenone/isoplocamenone ratio.

(17) Natural (*E*)-**2**: $[\alpha]_D = -14.3°$ (c = 0.8) from ref 10a. Synthetic (*E*)-**2**: $[\alpha]_D = -15.6°$ (c = 0.5, $CHCl_3$).

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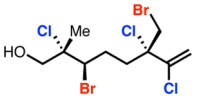
(21) Both enantiomers of synthetic halomon were analyzed by reverse-phase chiral HPLC according to conditions reported by Hirama (ref 7b) and found to be of >99% ee (see Supporting Information).

(22) For example, silyl protection of **10** has allowed for the synthesis of the following derivatizable analogue:



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