Halogenated Secondary Metabolites

Samantha Green

"Halogen": derived from the Greek word meaning "salt-producing"[1]

[1]Natural Abundance of Halides/mg kg^{-1}

Halide	Oceans	Sedimentary Rock	Fungi	Wood Pulp	Plants
F^-	1.4	270-740			
Cl^-	19,000	10-320		70-2100	200-10,000
Br^-	65	1.6-3	100		
I^-	0.05	0.3			

Each year, the oceans and volcanoes emit around 210 MT of chlorine equivalents into the atmosphere. HCl, NaCl[2]

Water: [1,3]
Ocean: nearly 2% Cl^- by weight; Great Salt Lake: 9% Cl^- by weight
The enzymes in algae are able to more easily oxidize bromide, resulting in a higher occurrence of brominated secondary metabolites despite the higher concentration of chloride in the ocean.

Terrestrial Environment: [1]
Halogens found in sedements, soils, plants, fungi, lichen, volcanoes, biomass combustion, bacteria, insects, and higher organisms.

Peatlands:[1]
Wetlands with a thick water-logged organic soil layer composed of dead or decaying plant material and comprise 2% of the earth's surface.
Major reservoir of organically bound iodine.
91% of bromine found in peat is organically bound.

Not surprisingly, many halogenated secondary metabolites originate from the ocean. These compounds have shown antimicrobial, antifeedant, antihelmintic, and cytotoxic activities and are increasingly being investigated as clinical targets

[1]Gribble, G.W. *Progress in the Chemistry of Organic Natural Products,* 2010, **91**, 613.
[2]Leveque, C. *Ecology: From Ecosystem to Biosphere*, 2003. 398.
[3]The Chemistry of Halogens. http://chemed.chem.purdue.edu/

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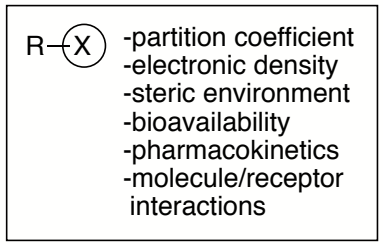
The Role of Halogen Bonding

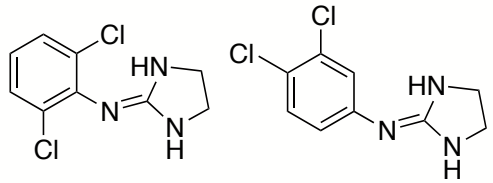
Samantha Green

1820s: Marks the emergence of halogenated drug candidates[4]

Halides are used in place of hydrogen atoms in drug targets to make the molecules more lipophilic and hydrophobic i.e. they are better able to permeate lipid membranes and enter cells. As a negative result, they often accumulate in lipid (adipose) tissue.[5]



Chlonidine: antihypertensive[5]



ED_{20} = 0.01 mg/kg ED_{20} = 3.0 mg/kg

Molecule efficacy can be largely influenced by orientation of substituents

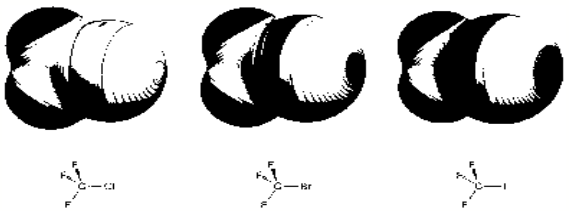
Sterics: Halogens responsible for preventing rotation and positioning the rings in a perpendicular orientation

Halogen Bonding: "Occurs when there is evidence of a net attractive interaction between an electrophilic region associated with a halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity"[7]

The halogen acts as lewis acid while the Y is electron donating: R–X---Y

Advantages of Halogen bonding > hydrogen bonding

1) Halogen bonds are directional due to their narrowly confined σ–hole (region of positive electrostatic potential) on the outersurface



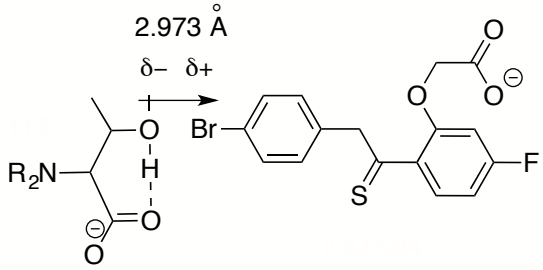
σ-hole on hydrogen atoms is largely dispersed among the surface

2) Tunibility: strength of halogen bond: I > Br > Cl. Additionally strengthened when attached to an electron withdrawing moiety. Allows for single atom mutation.

3) Halogen bonds=hydrophobic. Hydrogen bonds=hydrophilic

4) The sheer size of halogens can alter the light-emitting properties of halogenated dyes by promoting singlet-to-triplet intercrossing.

"Specificity Pocket"



Aldose Reductase: enzyme responsbible for the reduction of glucose to sorbitol. Long believed to be responsible for diabetic complications in various organs. Several aldose reductase inhibitors have been developed and withdrawn from the market due to toxicity effects resulting from off target inhibition of Aldehyde Reductase.

Solution: Halogen bonding.

Upon IDD 594 binding, Aldose Reductase undergoes a conformational change, creating a "specificity pocket." In this pocket, IDD 594 forms a halogen bond with Thr 113 at an unusually short bond length, contributing to the bonding specificity to aldose reductase. Aldehyde reductase contains a tyrosine moiety in place of the threonine and the increased steric bulky discourages non-specific binding.[8]

[4]Meyer, H.P, *Chemistry Today*, **2011**, *29* (4) [5]http://shodhganga.inflibnet.ac.in/bitstream/10603/2389/9/09_chapter%201.pdf
[6] Lu, Y. et al. *J. Med. Chem.* ***2009****, 52*, 2854-2862. [7] Priimagi, A. et al. *Acc. Chem. Res.* **2013**, *46* (11) 2686–2695
[8] Howard, E.I. et al. *Proteins: Structure, Function, and Bioinformatics* **2004**, *55*, 792– 804

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Halogenated Drug Targets

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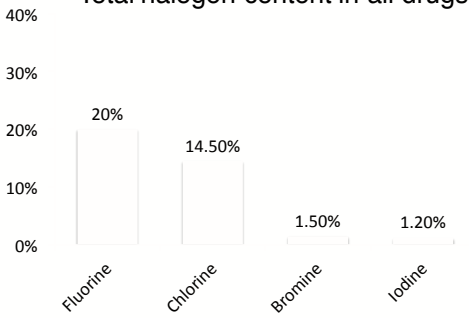
There are discrepencies in the composition of halogenated drug candidates.

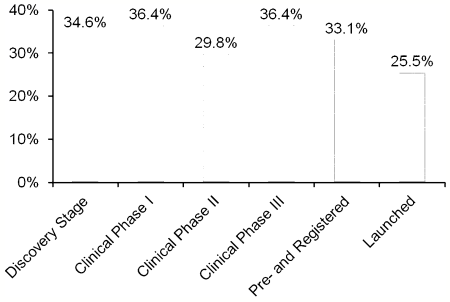
Total halogen content in all drugs

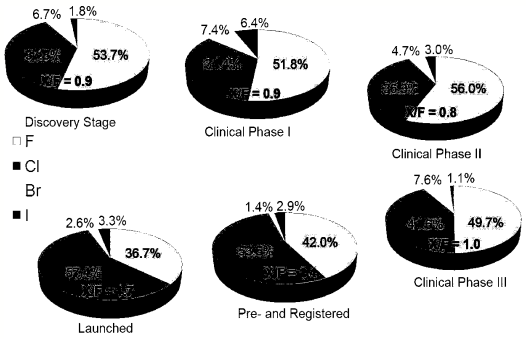


Percentages include drugs containing multiple halogens. Source: Novartis, 2013[9]

Halogenated drug composition specifically at Thomson Reuters Pharma; however, these statistics match with other literature sources. Percentages include drugs containing multiple halogens.[10]

Thomson Reuters Pharma

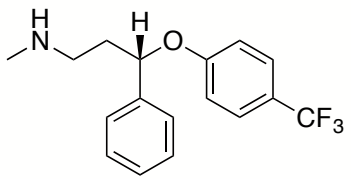




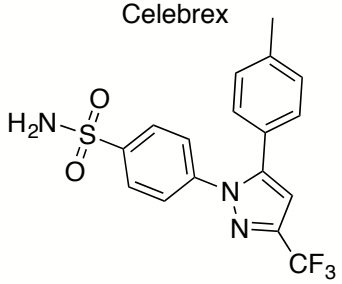
"The molecules in discovery stage represent the current opinions and interests in drug discovery, and the drug molecules in clinical phases represent the opinions and interests in drug discovery about a decade ago, while the launched phase collects the successful drugs over the past one hundred years."[10]

Fluorine: commonly found as a trifluoromethyl substituent. Used in place of a chlorine atom or a methyl group at risk of metabolic oxidation. Also used to facilitate access to the CNS and transmission through the blood brain barrier.[5]

Fluoxetine (Prozac)

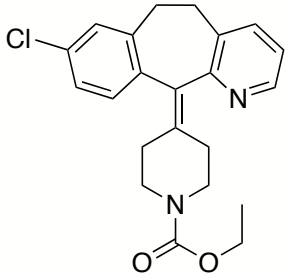


Celebrex

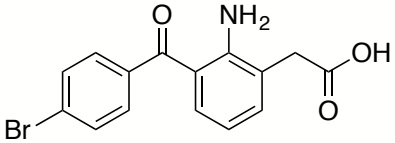


Chlorine: provides increased lipophilicity, a source of hydrogen bonding, and a metabolic obstruction[5]

Loratadine (Claritin)

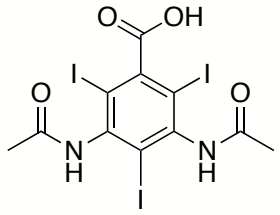


Bromfenac



Bromine: less commonly used and mainly seen as a bromoaryl. Bromine can more easily generate alkylating intermediates than chlorine or fluorine, leading to toxicity issues (example: bromfenac, withdrawn from market)[5].

Iodine: Used by the body to produce thyroid hormones. KI is a treatment for deficiecies in hormone production. Organoiodide compounds are used as X-ray radiocontrast agents.[5]



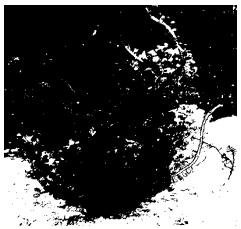
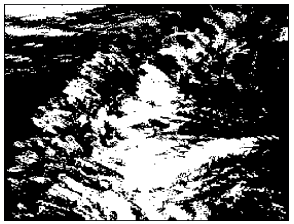
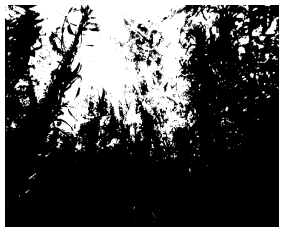
Diatrizoic acid

[9] Vulpetti, A. "Protein interactions with fluorine and other halogens" Novartis, 2013
[10] Xu, Z. et al. *J. Chem. Inf. Model*, **2014**, *54*, 69-78

Marine Organisms

Samantha Green

Percentage of chlorinated or brominated secondary metabolites[11]

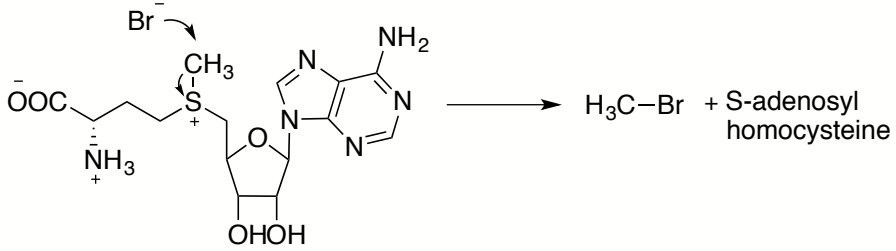
Red Algae	Green Algae	Brown Algae
		
90%	7%	1%
Over 800 chlorinated and/or brominated forms of terpenes		Brown algae utilizes iodine instead.

The formation of organohalogenated compounds is thought to have developed in prokaryotes in response to the generation of reactive oxygen species (ROS). Similarly in algae, the halogonated compounds eliminate ROS while also playing a role in defense and structure maitenance.[11]

Biosynthesis of methyl bromide/iodide

Iodide and bromide are actively taken up from seawater and nucleophilicily attack the CH_3S site of S-adenosylmethionine (SAM) halide/thiol methyltransferase. Limited to monohalogenation.

Methylbromide formation

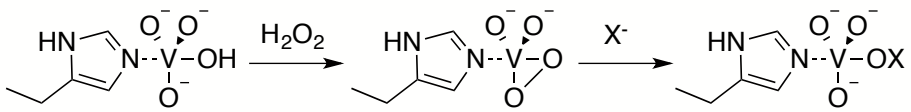


Two classes of enzymes involved in halogenation:[12]

1) highly substrate-specific halogenases requiring dioxygen for enzymatic activity. Co-substrates: Flavin ($FADH_2$-dependent halogenases) or R-ketoglutarate (non-heme Fe^{II}/R-ketoglutarate/O_2-dependent halogenases).

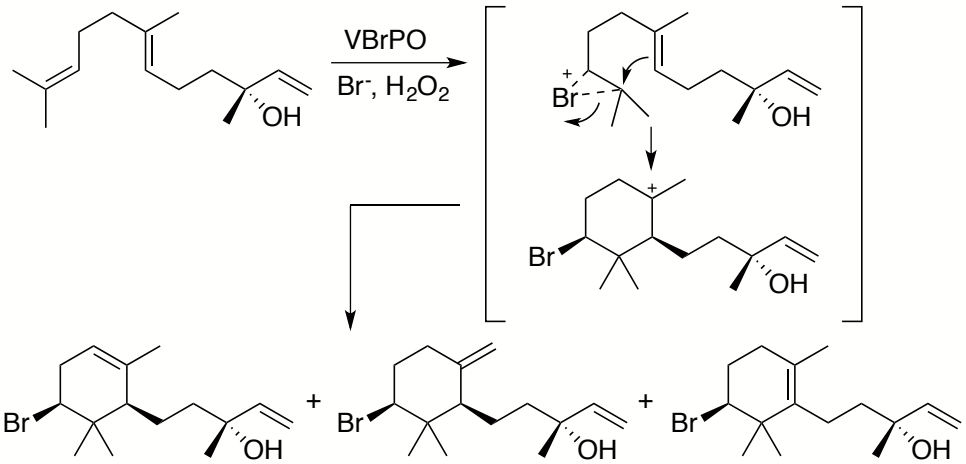
2) less specific haloperoxidases (HPO) utilizing hydrogen peroxide (i.e. vanadium or heme hydroperoxidases).

In the 1980s, haloperoxidases were isolated from algae and the researchers were surprised to find stoichiometric quantities of vanadium rather than heme.



Bound hypohalite (–OX) intermediates react as "X^+" equivalents

Formation of α–, β–, and γ–Snyderol from Sesquiterpene Nerolidol



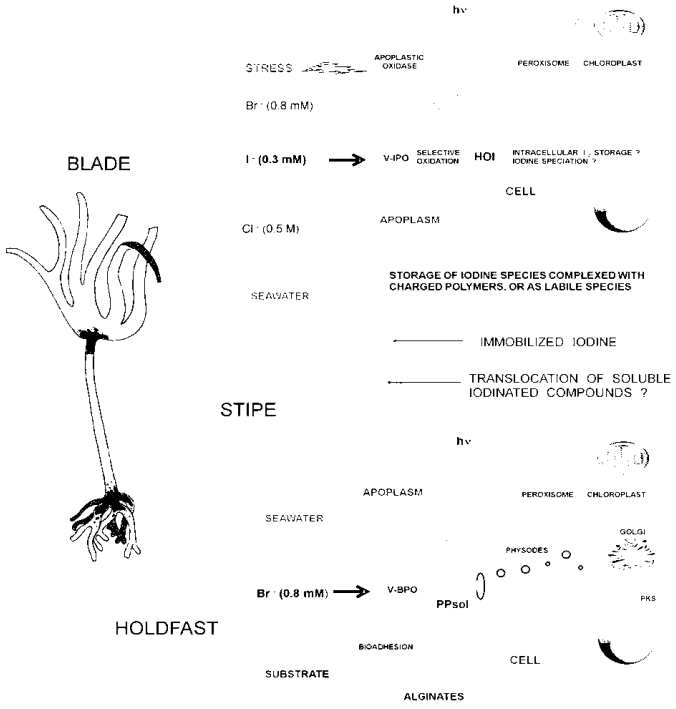
[11] Barre, S.L. et al. *Mar. Drugs* **2010**, *8*, 988-1010.
[12] Walsh, C.T. et al. *Chem. Rev.*, **2006**, *106*(8), 3364-3378.

Brown Algae

Samantha Green

Vanadium dependent hydroperoxidases are named based on the most electronegative element they can oxidize. These enzymes are highly substrate non-specific.

The oxidation potential of the halides is pH-dependent, generally requiring a higher pH to oxidize the more electronegative halides.[11]



Brown algae VHPOs have a common ancestor to the VHPOs of red algae; however, as first observed in *Laminaria digitata*, the brown algae developed novel biochemical function of iodine oxidation.

VIPOs are upregulated after an oxidative burst to restore iodine levels while VBrPOs are specifically activated and play a role in oxygen detoxification

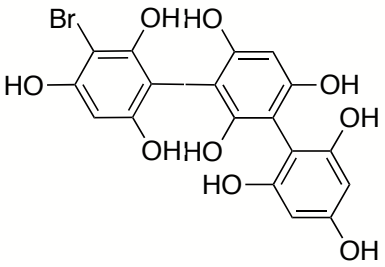
Laminaria digitata: approximately 1% iodide by dry weight. The kelp contains a 30,000 fold accumulation of iodide compared to seawater.

Iodide is thought to be stored *via* chelation with apoplastic macromolecules. This ensures abundant and readily acessible reduced iodine. However, the mechanism is not yet confirmed.

These iodine and bromine containing secondary metabolites primarily play a role in oxygen detoxicification (quenching excess H_2O_2 and ROS) and bioadhesion; but also act in an antibacterial role on the kelp exterior.

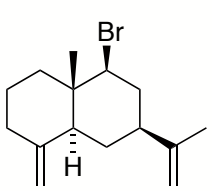
Secondary Metabolites from Brown Algae:

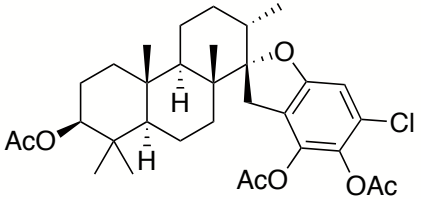
Phlorotannins and Phloroglucinol



Biosynthesis with hydroxylation at the meta position are derived from the acetate-malonate pathway (additionally a PKS-type enzyme complex (?)). The triketide formed after cyclization undergoes tautomerization to give phenol. VHPOs shown to crosslink the monomers *in vitro*. Sulfated polyphenols are common in brown algae; but sulfur and halogen-substituted phenols have yet to be reported.

Halogenated Terpenes





Presense of terpenes is far more rare in brown algae than red, most likely due to red algae's ability to diversify their defense strategy.

Secondary metabolites from brown algae have not yet found a clinical use.

"The fact that some of the newly found halogenated compounds show minor or no activity at all against a specific target does not exclude the possibility of other hidden unidentified active biological effects."[13]

[13] Cabrita, M.T. et al. *Mar. Drugs* **2010**, *8*, 2301-2317

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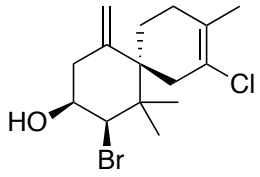
Red Algae[13] and Bromoindoles[14]

Samantha Green

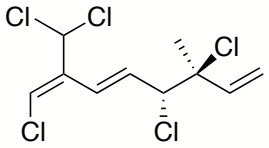
Red Algae are the largest source of halogenated secondary metabolites. Specifically, *Laurencia* (shown right) is considered one of the most prolific. Diterpenes, sesquiterpenes, triterpenes, and C15-acetogenins are the main natural products and have been shown to have antimicrobial, antifeedant, antihelmintic, and cytotoxic properties[13]

Various Isolated Compounds:

Elatol:Potent antibacterial

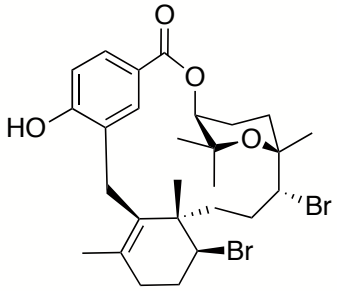


chamigrene subclass of sesquiterpenes



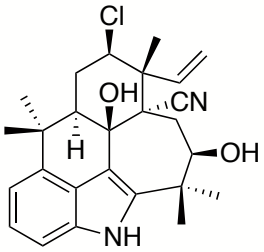
isolated from *Plocamium cornutum*.
IC_{50} = 16 μM against Plasmodium falciparum (most deadly human malaria parasite)

Isolated from *Callophycus serratus*



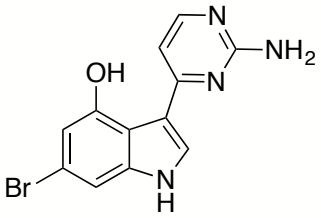
Bromphycolide active against *S. aureus* and cytotoxic but showed little cell line selectvity

Ambiguine M

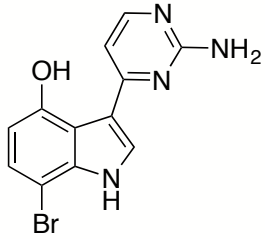


Isolated from cyanobacteria. Potent activity against *Mycobacterium tuberculosis*.

Meridianin B

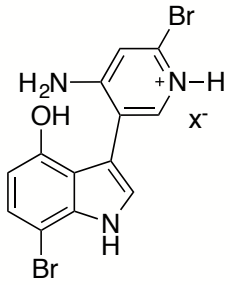


Meridianin E



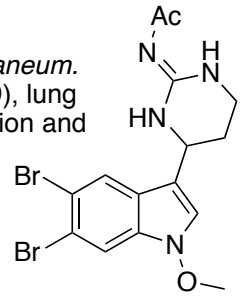
The two most potent of the family. Activity: inhibition of various protein kinases (protein phosphorylation) and cytotoxicity LMM3 (murine mammalian adenocarcinoma cell line) IC_{50} of 11.4μM and 11.1 μM

Psammopemmin B



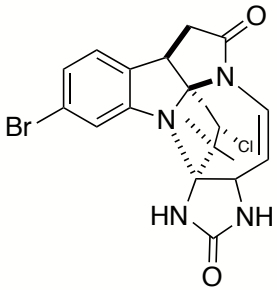
Isolated as the amine salt from Antarctic marine sponge *Psammopemma sp.* Suspected to have pharmacological activity due to the 4-hydroxyindole but the isolated quantity was too little for bio testing. Still awaiting structure confirmation via total synthesis .

Aplicyanin F



Isolated from the Antarctic tunicate *Aplidium cyaneum.* Cytotoxic against cancer cell lines: colon (HT-29), lung (A-549), and breast (MDA-MB-231). The 5 position and the acetyl group on the imine nitrogen important for activity.

Securamine G



Isolated from *bryozoans S. securifrons*.
Not aparent that there's biological activity from this scaffold. In different variants of this scaffold, they exist in equilibrium with securine dependent on solvent (DMSO-d_6 vs $CDCl_3$).

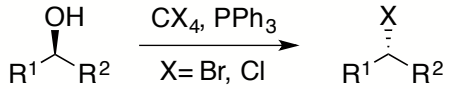
[14] Pauletti, P.M. et al. *Mar. Drugs* **2010**, *8*, 1526-1549

Methods of Halogen Insertion in Synthesis[15]

Samantha Green

****This is by no means a complete list of available methods****

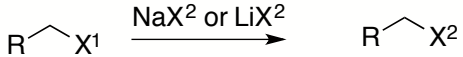
Appel Reaction



primary or secondary alcohols
inversion of stereochemistry

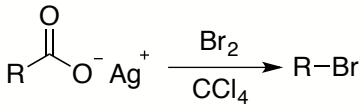
Tertiary Alcohols: Strong acid (HCl, HI, HBr)
Primary/Secondary Alcohols: various methods of S_n2: $SOCl_2$, $SOBr_2$, PBr_3, PCl_3, PCl_5, PBr_5, $MsCl/EtN_3$ (can directly replace with chlorides in some substrates) or TsCl/pyridine and then nucleophilic substitution with halide.

Finkelstein Reaction (alcohol or halide displacement)



Inversion of stereochemistry
X^1= F, Cl, Br, I, OMs, OTs
Constrained by solubility of the sodium or lithium halide

Hunsdiecker Reaction

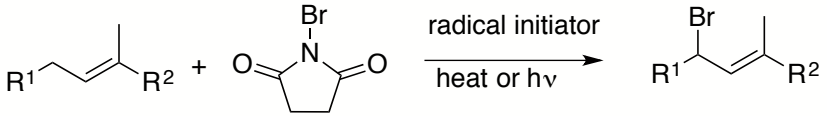


Radical mechansim for the formation of alkyl or aryl halides

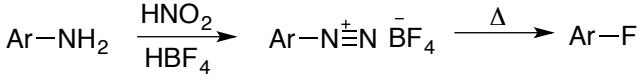
Modifications:

1) Thallium(I)-carboxylates instead of silver salts
2) Cristol-Firth modification- excess red HgO and one equivalent of halgen
3) Suarez modification- treatment of acid with hypervalent iodine in CCl_4
4) Kochi modification- $Pb(OAc)_4$ with iodine or lithium halde
5) Barton modification- thermal of photolytic decomposition of thiohydroxamate esters in halogen donor solvents
6) AIBN can decarboxylate aromatic acids

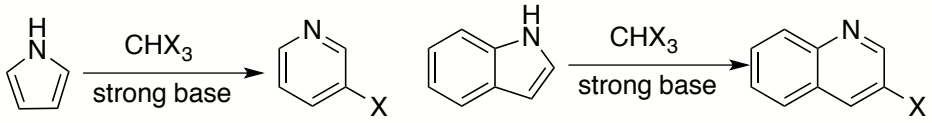
Wohl-Ziegler Bromination



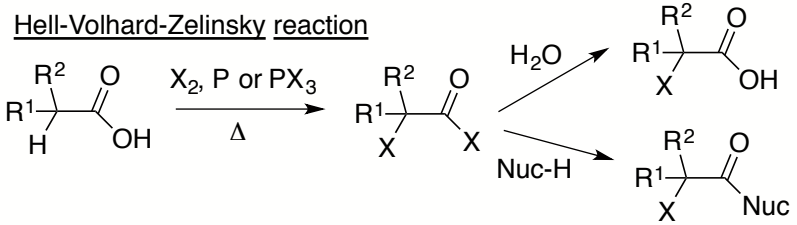
Balz-Schiemann Reaction



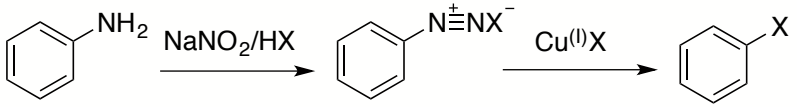
Ciamician-Dennstedt Rearrangement



Hell-Volhard-Zelinsky reaction



Sandmeyer Reaction



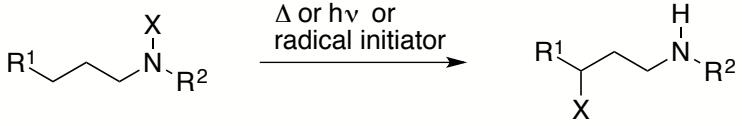
[15]Czako B., Kurti, L. *Strategic Applications of Named Reactions in Organic Synthesis*.

Methods of Halogen Insertion in Synthesis

Samantha Green

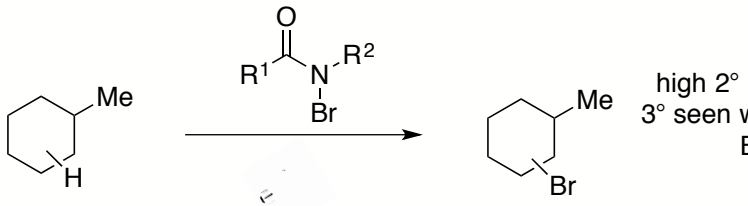
C-H aliphatic Bromination

Hofmann-Loffler-Freytag Reaction

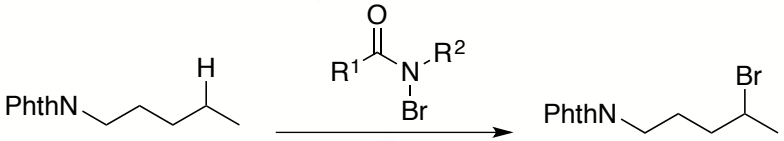


Nitrogen-centered radica to do site-selective, intramolecular C-H functionalizations

Erik Alexanian, JACS 2014[16]

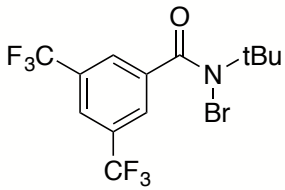


high 2° selectivity
3° seen with NBS or Br_2



1 equiv 1 equiv

-Reactions run with a 100 W household lightbulb.
-Run under Ar; however, little decrease in yield when run under air
-no dihalogenation observed (electronic deactivation of bromoalkane pdt)

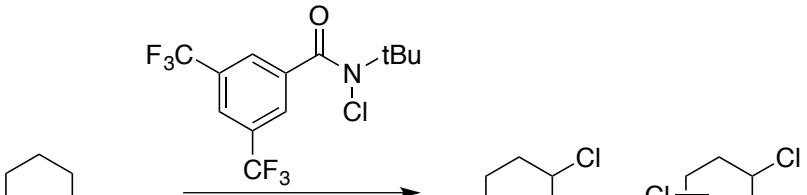


provided the highest yields (75%, 98.5% selective for 2°>3° bromination.Favors tertiary bromination with adamantane. In other cases though, highest selectivity for methelene yet reported.

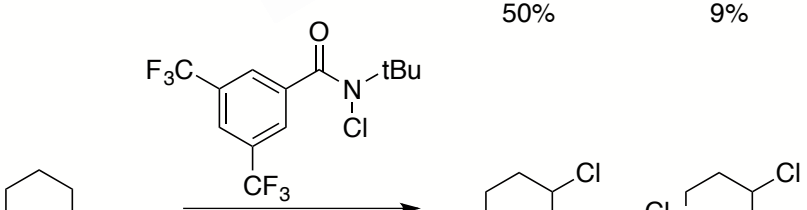
C-H aliphatic Chlorination

Vanderwal and Alexanian, JACS 2015[17]

Chlorine free radicals are far more promiscuous than bromine. Bromine is often site-selective for the weakest C-H bond while chlorine undergoes polyhalogenation and poor site selectivity.



50% 9%



96.9% 3.1%

total conversion: 71.6%

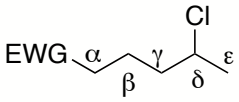
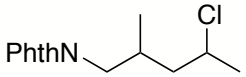
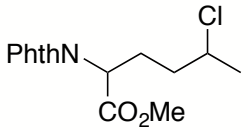
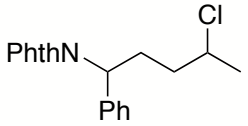
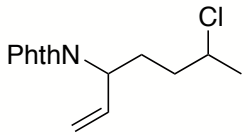
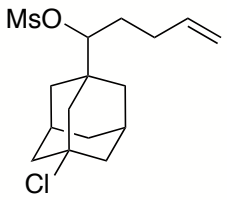
Substrate	% selectivity of chlorination α	β	γ	δ	ε	total yield (%)
	EWG—CH_2—	CH_2—	CH_2—	CH_2—	CH_2	
1. PhthN	-	-	4.9	81.2	13.9	80
2. NC	-	-	15.0	65.9	19.1	79
3. Cl	9.2	5.7	15.3	56.9	12.9	74
4.			23.9	65.5	7.5	70

[16]Alexanian, E.J. et al. *J. Am. Chem. Soc.* **2014**, *136*, 14389-14392
[17]Alexanian, E.J. and Vanderwal, C.D. et al. *J. Am. Chem. Soc.* **2016**, *138*, 696-702

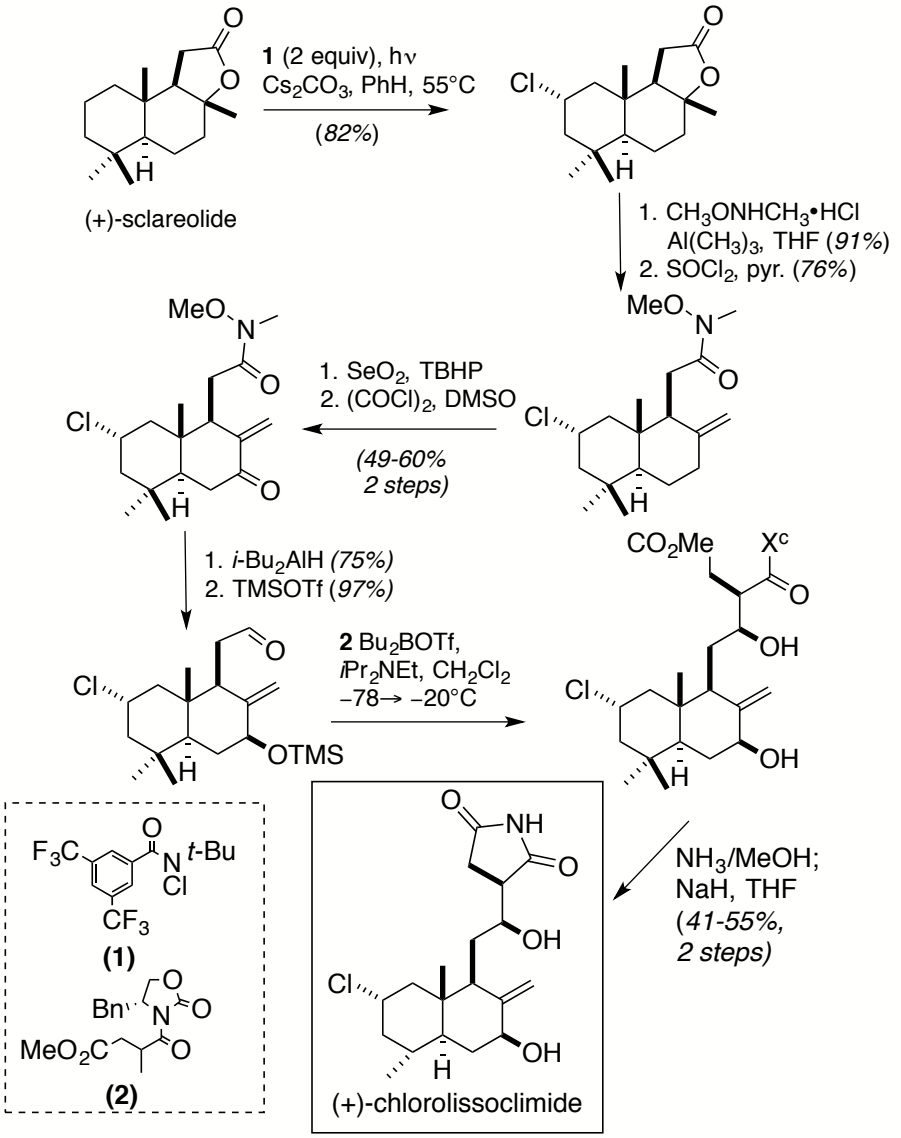
Methods of Halogen Insertion in Synthesis

Samantha Green

Vanderwal and Alexanian, JACS 2015 (cont.)

entry	major chlorination product	% select.	Total yield (%)	sites of minor chlorniation (% selectively)
				
1.		63.6	69	β=2.4; γ=7.5; ε=26.5
2.		77.5	66	ε=26.5
3.		67.9	81	γ=14.9; ε=17.2
4.		74.0	78	α=6.5; ε=18.9
5.		100	65	

Application in synthesis of (+)-Chlorolissoclimide

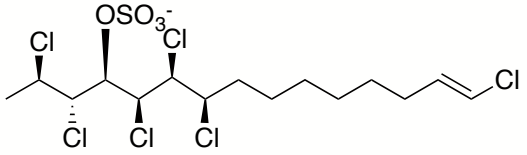


Shenvi Lab Group Meeting
2/1/16

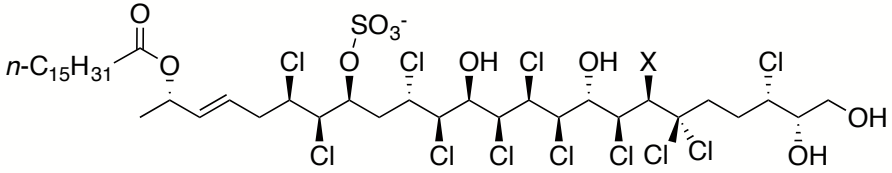
Chlorosulfolipids

Samantha Green

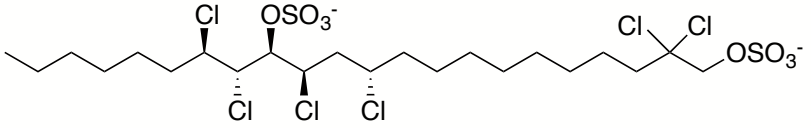
Molecule significance:
1) allow for confirmation of stereochemistry
2) One has been found to be a protein kinase inhibitor (no given IC data)
3) Synthetic challenge
4) "electronically distinct isosteres of polyketide natural products." polyketides are pharmaceutically relevant so maybe these are too...?



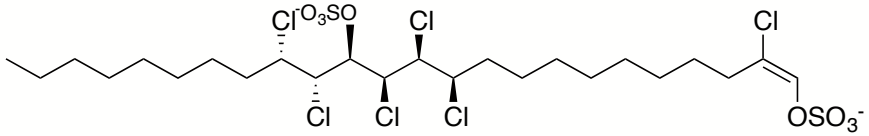
1 mytilipin A (hexachlorosulfolipid)



2: X = H **mytilipin B**
3: X = OH **mytilipin C**



4: (±)- danicalipin A

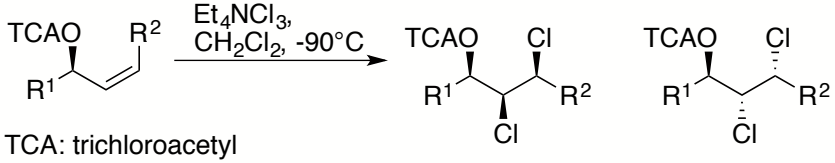


5: malhamensilipin A

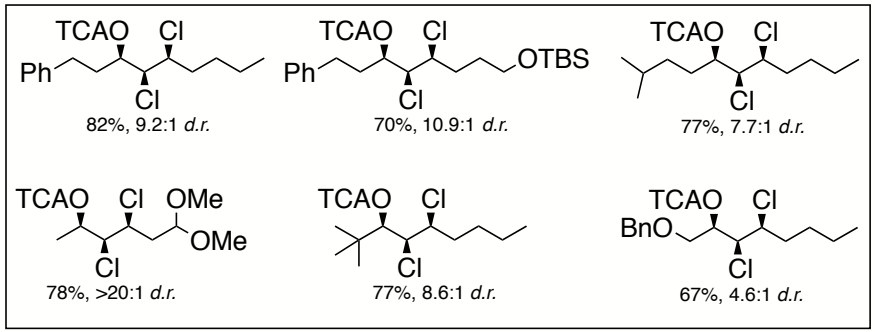
Studies into the biosynthesis reveal that the hydrocarbon chain is constructed *via* the normal fatty acid biosynthetic pathway and subsequently functionalized with the polar substituents. The biosynthesis of the chlorination is unknown. The chlorides reside on unactivated carbons, so it is unlikely to be electrophilic sources of halogen. Less chlorinated lipids were resubjected to culture media containing chlorine sources and were further chlorinated.[18]

Methodologies

Vanderwal, JACS 2008[19] Anti-dichlorination using Mioskowski reagent

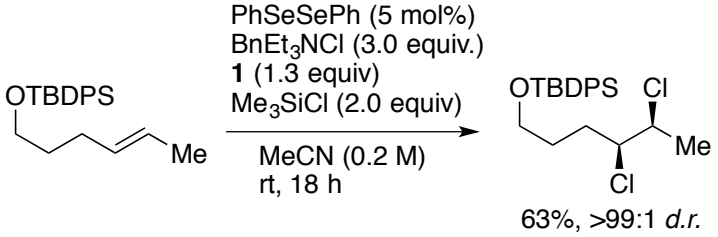


TCA: trichloroacetyl

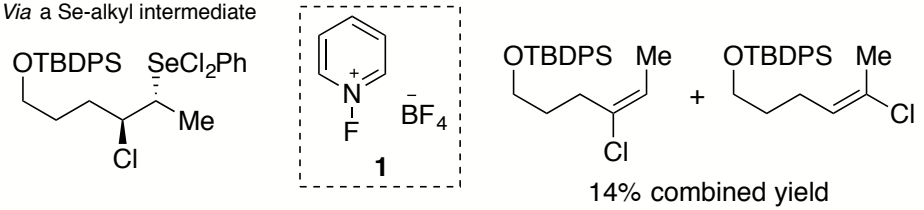


Yields refer to isolated yield. Diastereometric ratios determined by integration using ^{1}H NMR

Denmark, Nature Chemistry 2015[20] Syn-dichlorination



Via a Se-alkyl intermediate



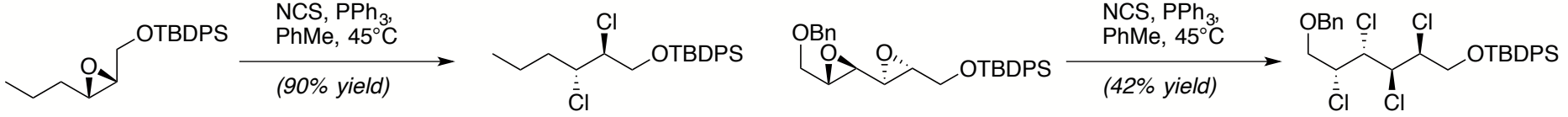
14% combined yield

[18] Bedke, D.K. and Vanderwal, C.D. *Nat. Prod. Rep.*, **2011**, *28,* 15-25.
[19] Vanderwal C.D. et al. *J. Am. Chem. Soc.,* **2008**, *130*, 12514-12518 [20]Denmark, S.E. et al. *Nat. Chem.*, **2015**, *7*, 146-152.

Chlorosulfolipids

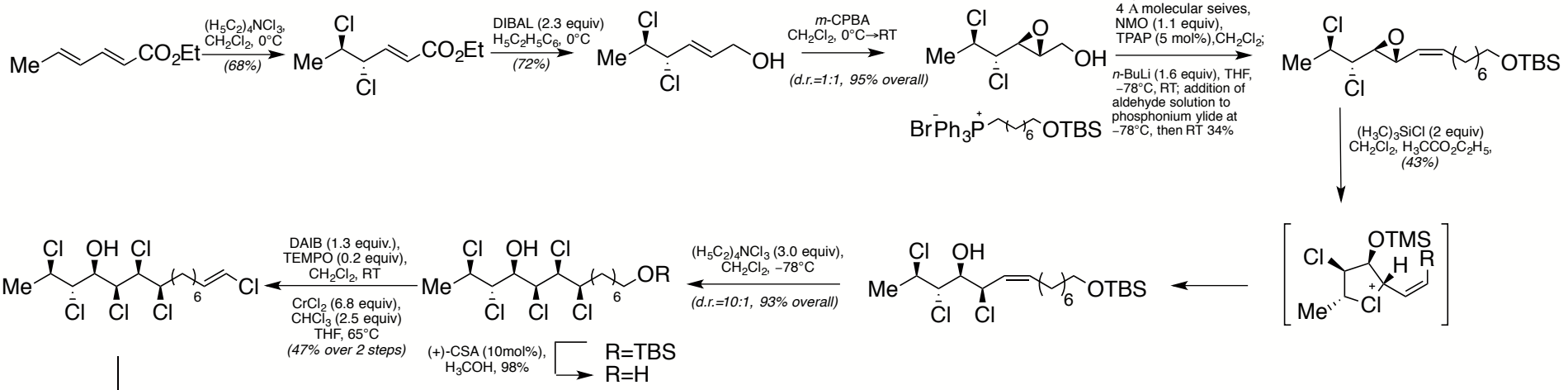
Samantha Green

Yoshimitsu, JOC 2009[21] Deoxydichlorination of epoxides



Total Synthesis of mytilipin A (hexachlorosulfolipid)

Carriera, Nature 2009[22]



(±)-hexachlorosulfolipid

TPAP=tetra-*n*-propylammonium perruthenate(VII)

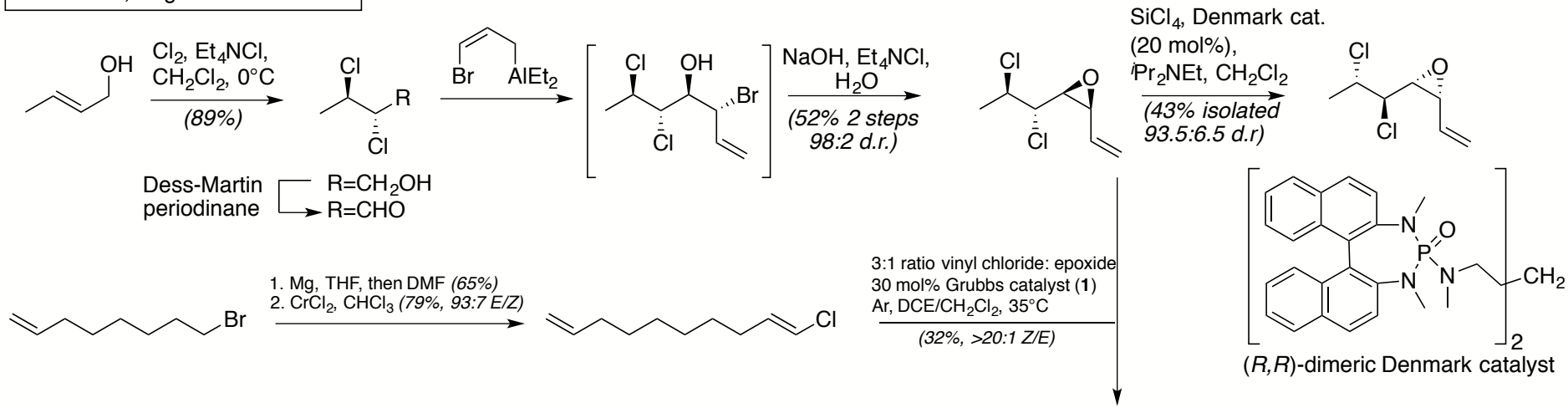
Comments: Initial route used the *cis* epoxide in place of *trans*. During the first route, they assumed that chlorohydrin diastereomer after epoxide opening had a *syn* relative configuration and proceeded to the end. When the natural product NMR did not match the isolated NMR, they reasses the configuration, realizing the epoxide opening gave *anti*-selectivity. The *trans* epoxide gave similarly *anti*-configuration and they were able to proceed with this new route. The *anti*-configuration is thought to occur due to anchimeric participation of one of the chlorides at C2 and C3 during the ring opening.

[21] Yoshimitsu, T. et al. *J. Org. Chem.*, **2009**, *74*, 696-702
[22] Carreira, E.M. et al. *Nature*, **2009**, *457*, 573-576

Chlorosulfolipids

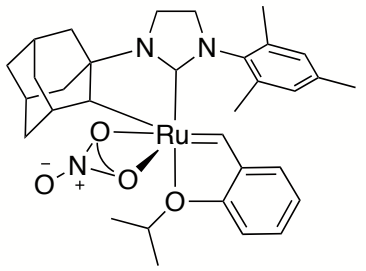
Samantha Green

Vanderwal, Angew. Chem. 2013[23]



(+)-hexachlorosulfolipid

Grubbs catalyst



1

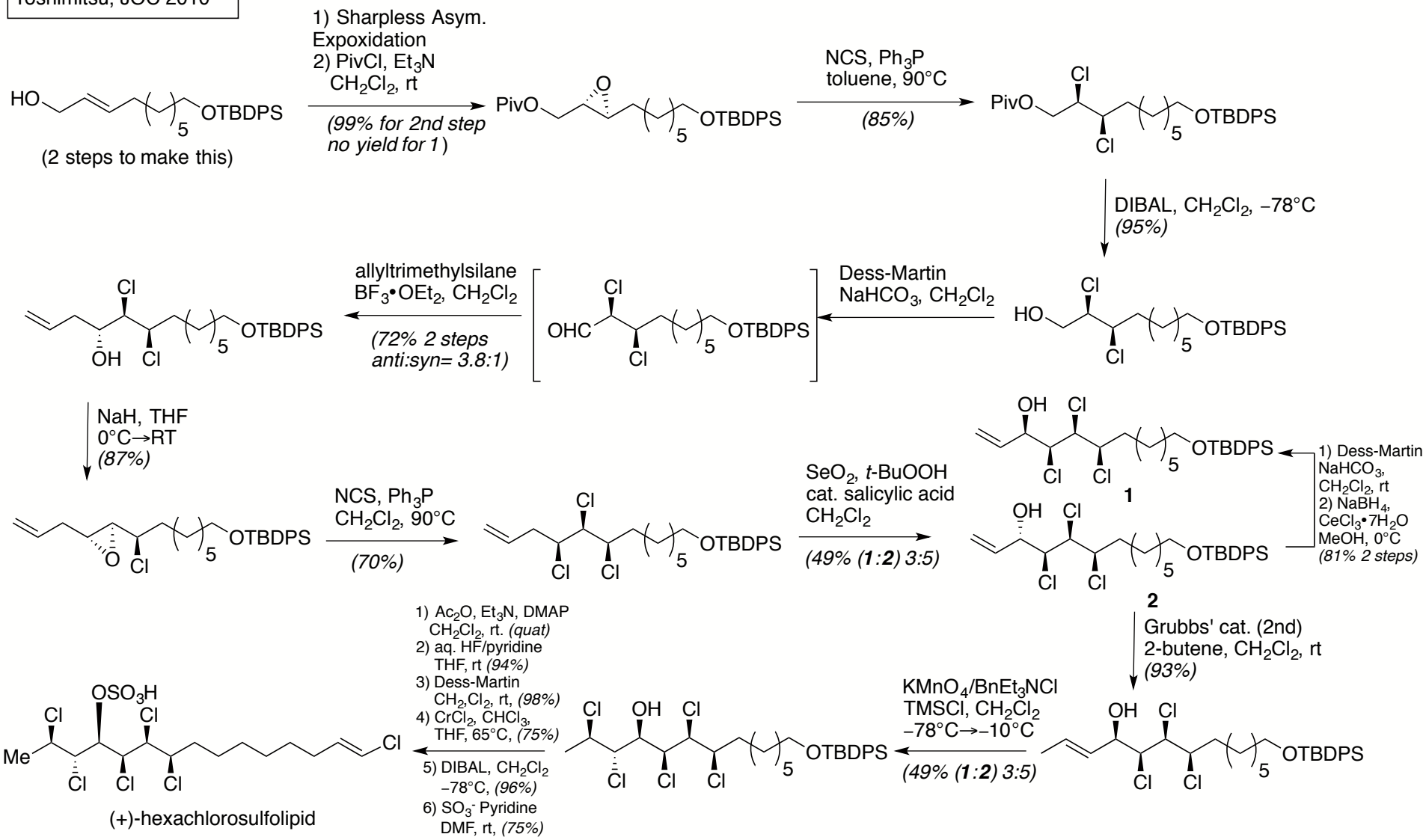
Comments: longest linear is 7 steps, overall 9% yield, prepared over 100 mg. Enantioselective synthesis is 8 steps longest linear ((+)-mytilipin A after kinetic resolution).

[23] Vanderwal, C. D. et al. *Angew. Chem. Int. Ed.*, **2013**, *52*, 10052-10055

Chlorosulfolipids

Samantha Green

Yoshimitsu, JOC 2010[24]



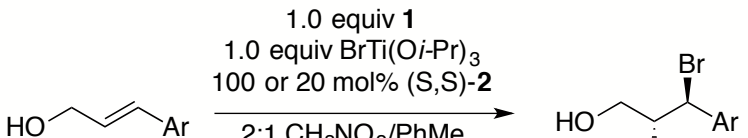
[24] Yoshimitsu, T. et al., *J. Org. Chem.*, **2010**, *75*, 5425-5437.

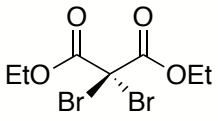
Dihalogenation of Alkenes

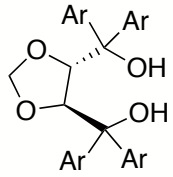
Samantha Green

Burns, JACS 2013[25] Dibromination

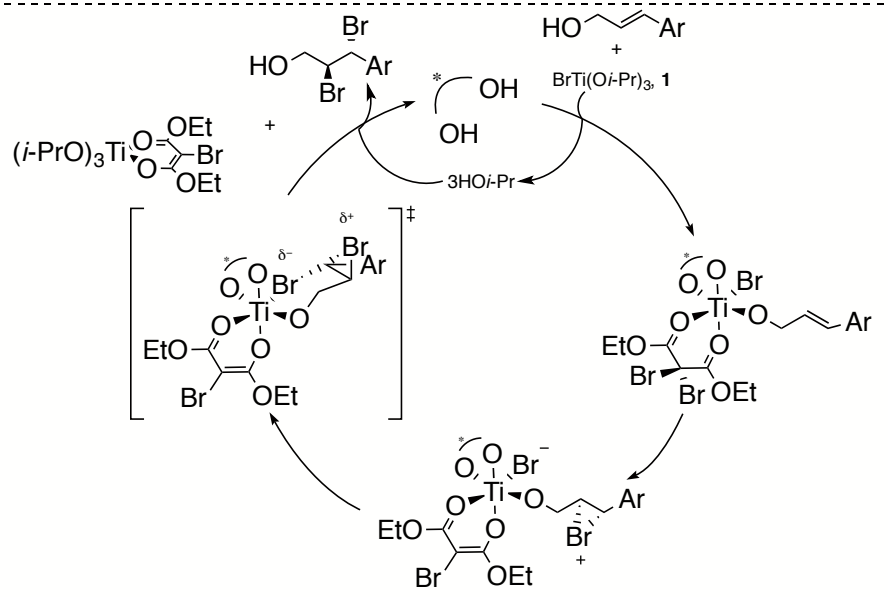
Problems with dibromination:
1) racemization of chiral bromonium ions in the presense of alkenes (Denmark)
2) Regioselectivity during bromide addition for stereocontrol of the product.




1

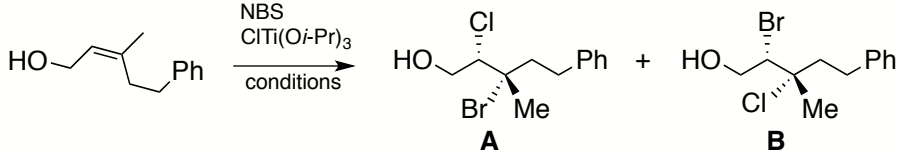

2

Ar= 2-naphthyl

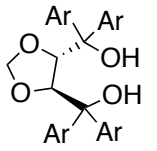


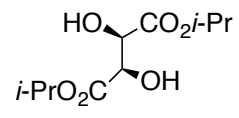
Burns, JACS 2015[26] Dihalogenation

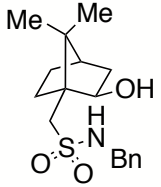
First general method for the enantio- and regioselective addition of two different halogen atoms across alkene

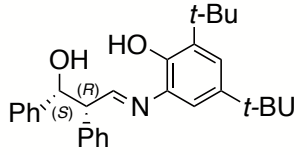


Entry	conditions	yield A + B	A:B	ee A,B (%)
1	Pyr•HCl instead of $ClTi(O\textit{i}\text{-}Pr)_3$ CH_2Cl_2, r.t.	80	1:4	–
2	CH_2Cl_2, r.t.	85	1:2	–
3	50 mol %, **1**, CH_2Cl_2, r.t.	88	1:2	6,8
4	50 mol %, **2**, CH_2Cl_2, r.t.	73	1:2	12,6
5	50 mol %, **3**, CH_2Cl_2, r.t.	89	1:1	17,0
6	50 mol %, **4**, CH_2Cl_2, r.t.	67	1:1	63,6
7	50 mol %, **4**, hexanes, r.t.	70	8:1	94,52
8	50 mol %, **4**, hexanes, -20°C	80	>20:1	98,–
9	10 mol %, **4**, hexanes, –20°C	88	>20:1	94,–


Ar= 2-naphthyl
1


2


3


4

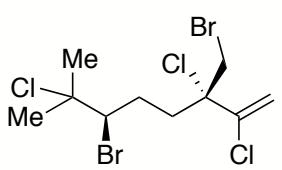
[25]Burns, N.Z., et al. *J. Am. Chem. Soc.,* **2013**, *135*, 12960-12963
[26]Burns, N.Z., et al. *J. Am. Chem. Soc.,* **2015**, 137, 3795-3798

Plocamium Monoterpenes

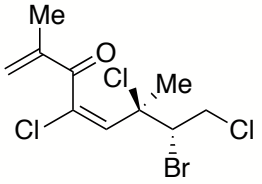
Samantha Green

Vanderwal, Angew 2014[27]

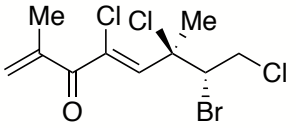
Plocamium, a species of red algae, is a prolific source of halogenated monoterpenes, both cyclic and acyclic. Halomon shows cyctoxicity against chemotherapy-resistant cell lines (renal, brain, colon, non-small-cell lung). Overall, the family seems promising for their cyctoxic potential.



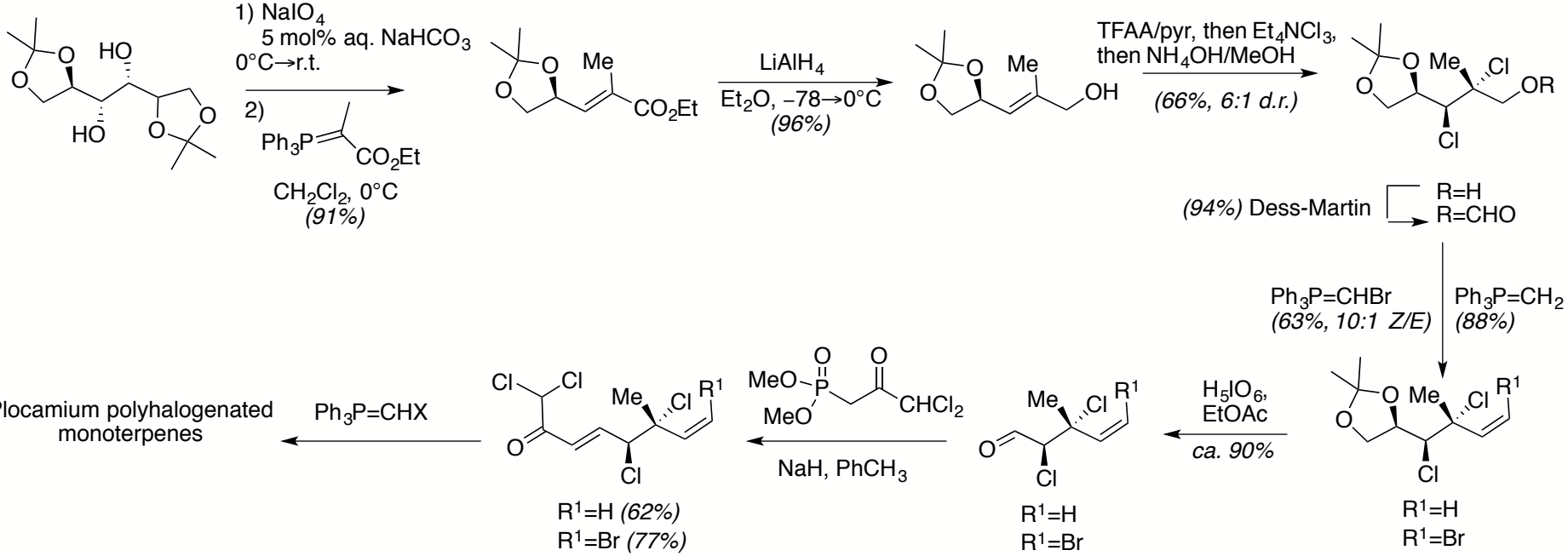
(+)-halomon

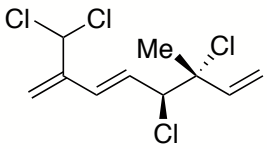


(*E*)-plocamenone

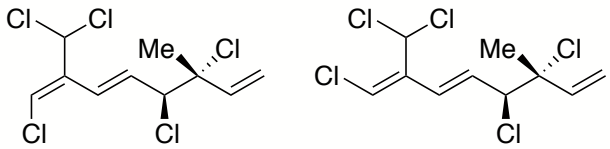


(*Z*)-isoplocamenone

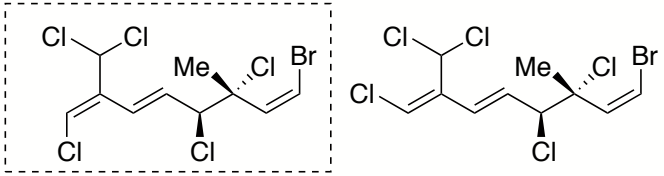




31%



62% combined, 1.3:1 E/Z



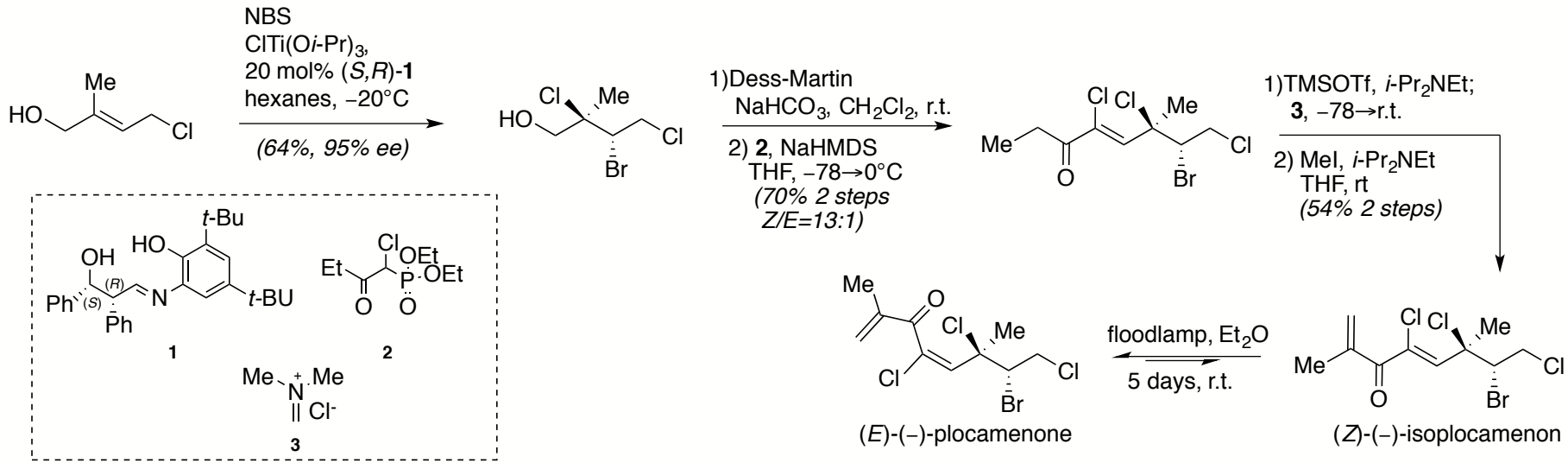
ca. 73% combined, 2.3:1 E/Z

[27] Vanderwal, C. D., et al. *Angew. Chem. Int. Ed.,* **2014**, *53*, 12205-12209

Plocamium Monoterpenes

Samantha Green

Burns, JACS 2015[28] (−)-Plocamenone and (−)-Isoplocamenone



[28] Burns, N.Z. et al., *J. Am. Chem. Soc.,* **2015**, *137*, 12784-12787