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Synthesis of the cryptophycins

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1. Introduction

For many years, the Chemistry Department at the University of Hawaii housed an interdisciplinary research program involving the collection and cultivation of cyanophytes (blue-green algae), and the screening of their extracts for anti-cancer activity. During the 1980s, my colleagues in the Chemistry Department, Richard Moore and Gregory Patterson, examined several hundreds of culture extracts in a bioassay-guided search for new leads for cancer chemotherapeutic agents.[1] The blue-green algae suggested themselves as a promising group of organisms for scrutiny in a search for novel cytotoxins, since there had been numerous reports of the adverse effects of algal blooms on human and animal health. The Moore–Patterson team discovered a large number of new cytotoxic compounds.[2–4] Many of these had unique and bizarre structures, and many were potent and selective cytotoxins. The most promising of all of these was isolated in 1991 after more than 1000 cyanophytes had been screened. The crude lipophilic extract from *Nostoc* sp. GSV 224 was found to be cytotoxic against the KB cell line (a human nasopharyngeal carcinoma) at 0.24 ng/mL, and against the LoVo cell line (a human colorectal adenocarcinoma) at 6 ng/mL.[5] Moreover, the crude extract showed tumor-selective cytotoxicity. The major cytotoxin which was isolated through bioassay-guided fractionation of the crude cyanobacterial extracts was cryptophycin 1 (cryptophycin A), a cyclic depsipeptide. Cryptophycin 1 co-occurs with a number of structurally related cryptophycins. After the Hawaii team had elucidated the gross structure of cryptophycin 1, it was found that a group at Merck had isolated the same compound some years earlier from the extracts of a related cyanophyte, *Nostoc* sp. ATCC 53789.[6,7] The Merck team published neither the

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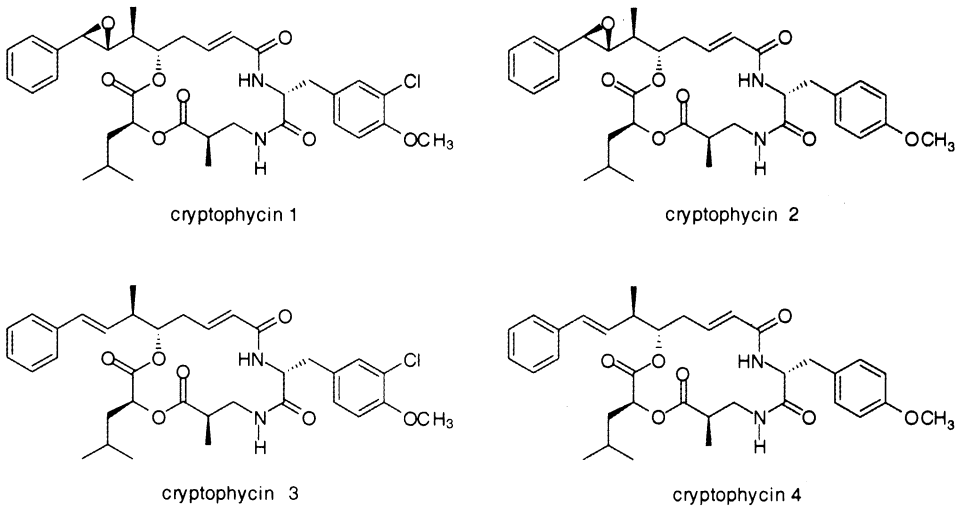


Figure 1. Some common cryptophycins.

relative nor the absolute stereochemistry in their patent. It is surprising that they apparently failed to discover the extraordinary selective cytotoxicity of the natural product, although they did note strong anti-fungal activity against *Cryptococcus* (Fig. 1).

2. The first Hawaii synthesis

The Hawaii group was the first to publish the relative and absolute stereochemistry for the cryptophycins in 1994.[8] Our first synthesis, in 1995, corrected the structures of cryptophycins 1 and 3. The modular nature of the depsipeptide structure renders the synthetic approach much simpler than it would otherwise be. For the purposes of the retrosynthetic analysis, cryptophycin 1 can be disconnected into four units. Unit A, the only polyketide-derived molecular fragment, provides the greatest synthetic challenge. Unit B is *O*-methyl-D-chlorotyrosine, whereas units C and D correspond to (*R*)-3-amino-2-methylpropanoic acid and (*S*)-2-hydroxy-4-methylvaleric (L-leucic) acid, respectively. There are a number of alternatives for macrocyclization, all of which appear to be viable and attractive. One of the most appealing is to form the unit B to unit C amide bond during the key step. Such an approach takes advantage of the small steric requirement of the primary amino group of unit C (Fig. 2).

The epoxide function of unit A poses a problem. The high reactivity of this styrene oxide suggests that it would be most prudent to introduce it as late in the synthetic sequence as possible, preferably in the very last step. We had assumed

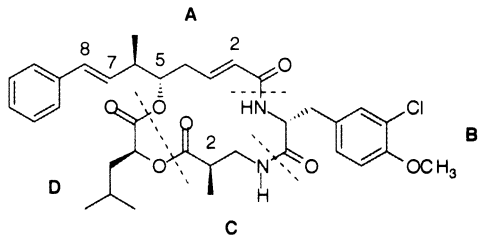
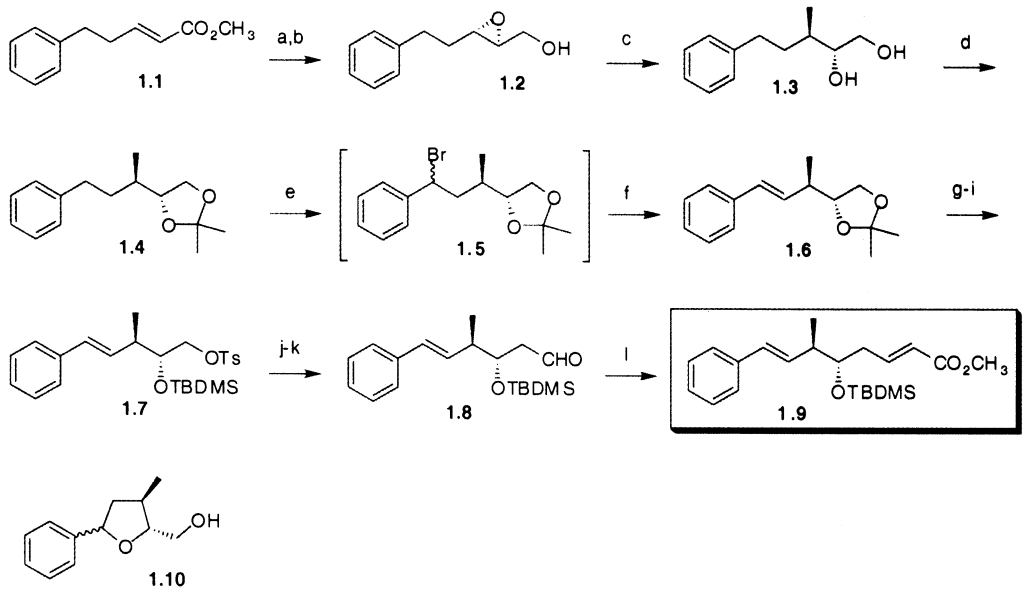


Figure 2. Retrosynthetic disconnection showing units A, B, C and D.

that in a macrocyclic cryptophycin intermediate one diastereoface of the styrene double bond would be shielded by the bulk of the molecule, leaving the other face open for a selective reaction with electrophilic epoxidizing reagents. As will be seen, this assumption was wrong.

Scheme 1 summarizes the first successful approach to unit A. Enoate **1.1** was prepared in 86% yield from commercially available dihydrocinnamaldehyde and trimethylphosphonoacetate in the presence of tetramethylguanidine (TMG) in THF. Reduction of the ester function in **1.1** with DIBAL-H led to the anticipated allylic alcohol in 90% isolated yield. Sharpless asymmetric epoxidation, using L-(+)-diethyl tartrate as the chiral inducer, led to epoxy alcohol **1.2** (94% yield; >95% ee). Regio- and stereospecific epoxide ring-opening with trimethylaluminum led to the desired 1,2-diol **1.3** in 95% yield. None of the 1,3-diol that would have resulted from the alternative mode of attack by the trimethylaluminum could be detected in the reaction mixture. Protection of the diol as acetonide **1.4** (97% yield) was straightforward, setting the stage for the introduction of the benzylic *E* double bond. Benzylic bromination of **1.4** with NBS in carbon tetrachloride led to unstable bromide **1.5** which was immediately dehydrobrominated by exposure to DBU at 70°C. This provided **1.6** in 80% overall yield for the two steps. Although this process worked well on small scale, upon scaleup we found that the yield of **1.6** was eroded by the formation of diastereomeric tetrahydrofuryl alcohols **1.10** as undesired reaction byproducts. The appearance of **1.10** is most easily rationalized as arising from acetonide hydrolysis by adventitious HBr which is formed during the radical halogenation reaction, followed by intramolecular displacement of bromide to generate more HBr. Consistent with this mechanistic hypothesis, slow addition of 1 equiv. of 2,2-dimethoxypropane to the radical halogenation reaction mixture as an acid scavenger effectively suppressed the formation of **1.10**. Similar reactivity of the benzylic carbon atom of unit A is a recurring motif in cryptophycin chemistry.

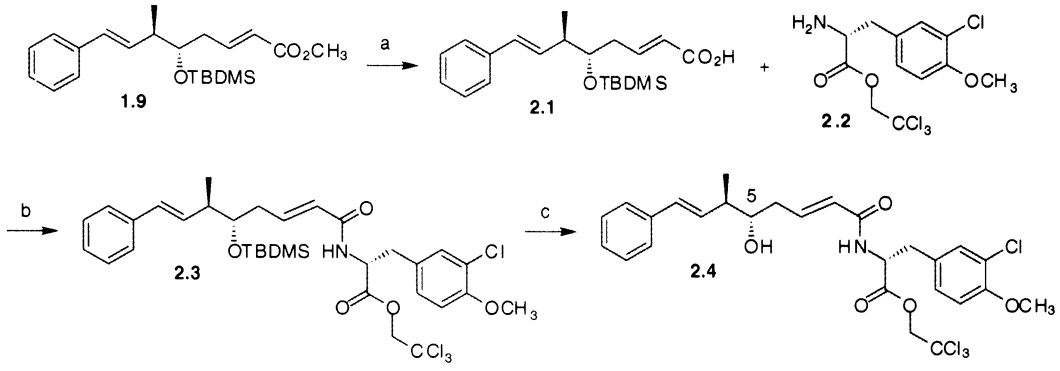
Hydrolysis of the acetonide group in **1.6** with aqueous methanolic HCl led to a diol (93% yield) which was selectively monotosylated at the primary position according to



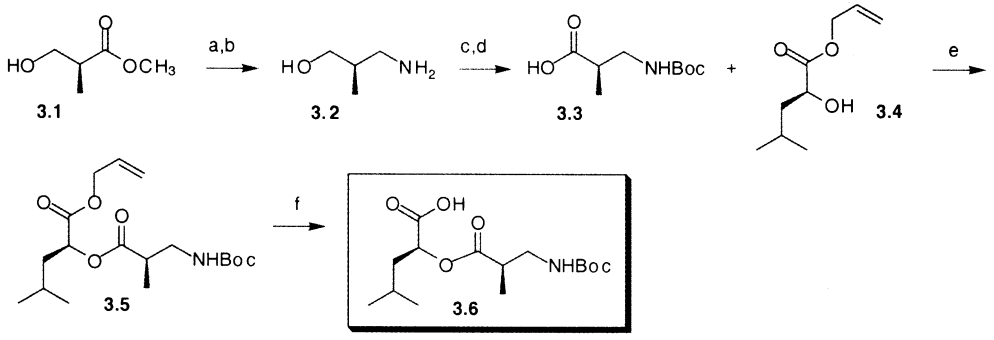
Scheme 1. (a) DIBAL-H, THF, −78 to 25°C, 90%; (b) L-(+)-DET, Ti(O-*i*Pr)$_4$, *t*-BuOOH, CH_2Cl_2, −20°C, 94%; (c) $AlMe_3$, hexane/CH_2Cl_2, 0–25°C, 95%; (d) $(MeO)_2CMe_2$, PPTS, CH_2Cl_2, 25°C, 97%; (e) NBS, CCl_4, $(MeO)_2CMe_2$, $h\nu$, 25°C; (f) DBU, 70°C, 80% (two steps); (g) 1% aqueous HCl/MeOH, 25°C, 93%; (h) $Bu_2Sn(OMe)_2$, PhMe, Dean-Stark; TsCl, Et_3N, 0–25°C, 82%; (i) $TBSOSO_2CF_3$, Et_3N, CH_2Cl_2, 25°C, 98%; (j) KCN, DMSO, 60°C, 92%; (k) DIBAL-H, CH_2Cl_2, −78 to 25°C, 95%; (l) $(MeO)_2POCH_2CO_2Me$, TMG, THF, −78 to 25°C, 83%.

Ley's procedure.[9] Exposure of the diol to dibutyldimethoxystannane in refluxing toluene with azeotropic removal of methanol led to a dibutylstannylene ketal which was directly converted to the primary tosylate in 82% overall yield from the diol by treatment with tosyl chloride and triethylamine. Protection of the secondary alcohol as the TBS ether led to **1.7** in 98% yield. The remaining carbon atoms of unit A were introduced as follows. Displacement of the tosylate by cyanide in DMSO (92% yield) was followed by reduction with DIBAL-H in dichloromethane to give aldehyde **1.8** in 95% yield. Horner–Emmons homologation with trimethylphosphonoacetate in the presence of TMG led to key intermediate **1.9** in 83% yield. This first synthesis of unit A is fairly long, however, the overall yield from dihydrocinnamaldehyde is high at 29%. Moreover, none of the steps makes use of exotic reagents or extreme conditions, therefore this is an eminently *practical* synthesis of unit A. Considerations of practicality informed our synthetic plan, because it was obvious to us from the outset that the cryptophycins were destined for the clinic, and also that it would not be possible to use *Nostoc* in culture for the production of material. The reasons for this last point may not be obvious, and therefore deserve some comment. *Nostoc* is an obligate phototroph, so large-scale fermentation in a closed system, as required by cGMP standards, presents an insurmountable engineering problem. Also, it is often the case that the natural product is not the optimal clinical candidate. For these reasons total synthesis provides the only means to secure material for the clinic.

Scheme 2 shows the assembly of unit A+unit B. Methyl ester **1.9** was hydrolyzed to carboxylic acid **2.1** with lithium hydroxide in acetone (95% yield). Protected unit B **2.2** was prepared from commercially available D-tyrosine in five steps. Chlorination with sulfuryl chloride in glacial acetic acid[10] was followed by protection of the nitrogen atom as the Boc derivative with di-*tert*-butyldicarbonate and triethylamine (94% yield). Methylation of this material in acetone took place in the presence of potassium carbonate



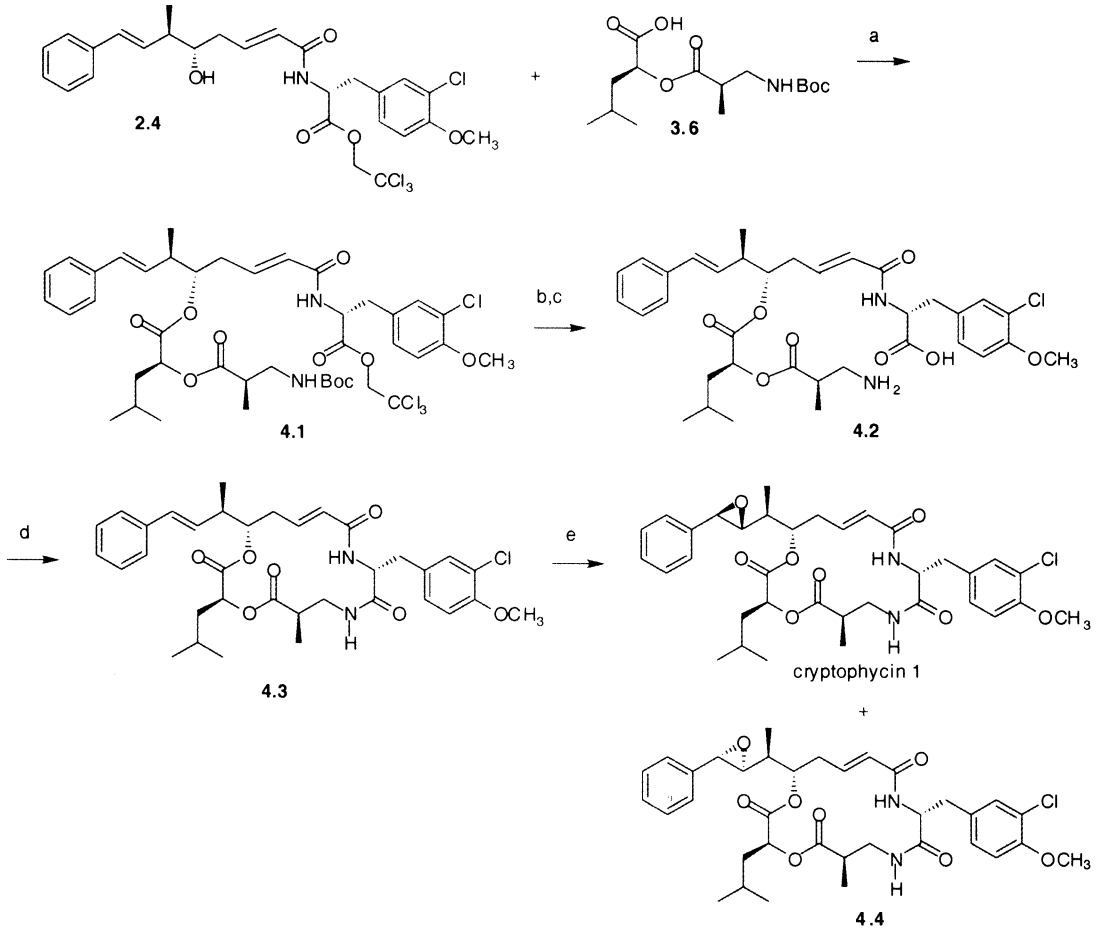
Scheme 2. (a) LiOH, acetone, 25°C, 95%; (b) FDPP, DIEA, DMF, 25°C, 73%; (c) MeCN, 50% aqueous HF (95/5), 25°C, 90%.



Scheme 3. (a) NH_3, MeOH, 50°C, sealed tube, 7 days, 66%; (b) BH_3–THF, THF, 0°C to reflux, 77%; (c) $(Boc)_2O$, Et_3N, MeOH, 25°C, 100%; (d) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O, 25°C, 74%; (e) DMAP, DCC, CH_2Cl_2, 0–25°C, 92%; (f) THF, morpholine, $Pd(PPh_3)_4$, 25°C, 100%.

and dimethyl sulfate at reflux for 4 h to produce the methyl ester of *N*-(*tert*-butoxycarbonyl)-3-(3-chloro-4-methoxyphenyl)-D-alanine in 84% yield. Hydrolysis back to the carboxylic acid took place with aqueous NaOH in dioxane in 86% yield. Esterification of the free acid with 2,2,2-trichloroethanol in the presence of DCC and pyridine gave the Troc ester in 65% yield after recrystallization of the crude product from hexanes. Exposure of this material to trifluoroacetic acid at room temperature led to quantitative cleavage of the Boc group, leading to the trifluoroacetate salt of **2.2**. The trifluoroacetate was converted to **2.2** in situ, during the condensation with **2.1**. Mixing a solution of **2.1** in anhydrous DMF with pentafluorophenyl diphenylphosphinate (FDPP), the trifluoroacetate salt of **2.2** and diisopropylethylamine (DIEA) at room temperature led to amide **2.3** in 73% yield.[11] Cleavage of the silyl ether protecting group with aqueous HF in acetonitrile produced **2.4** in 90% yield.

The Hawaii synthesis follows a highly convergent strategy whereby **2.4** was joined to unit C+D fragment **3.6** (Scheme 3). Commercially available (*S*)-(+)-3-hydroxy-2-



Scheme 4. (a) DMAP, DCC, CH_2Cl_2, 0–25°C, 94%; (b) Zn, THF, AcOH, sonicate, 25°C; (c) TFA (neat), 25°C, 89% (two steps); (d) FDPP, DIEA, DMF, 25°C, 64%; (e) *m*-CPBA, CH_2Cl_2, 0°C, ca. 2/1 cryptophycin 1/**4.4**.

methylpropanoate **3.1** was first converted to the amide by treatment with ammonia in methanol at 50°C in a sealed tube. The amide was isolated in 66% yield, along with unreacted **3.1** that was separated from the product and was recycled. Reduction of the amide function with borane in THF led to amine **3.2** in 77% yield. Protection of the amino group as the Boc derivative (100% yield), followed by oxidation of the primary alcohol with ruthenium tetroxide (74% yield) led to carboxylic acid **3.3**. Commercially available L-leucic acid was converted to allyl ester **3.4** in 93% yield under phase-transfer conditions, by stirring a two-phase mixture of the acid and allyl bromide in dichloromethane with aqueous sodium bicarbonate containing tetra-*n*-butylammonium chloride. The coupling of **3.3** with **3.4** was accomplished with DCC and DMAP in dichloromethane to produce **3.5** in 92% yield. Cleavage of the allyl ester was carried out under neutral conditions, by exposure of **3.5** to dry morpholine and catalytic $Pd(PPh_3)_4$ in THF.[12] Unit C+D acid **3.6** was isolated in quantitative yield.

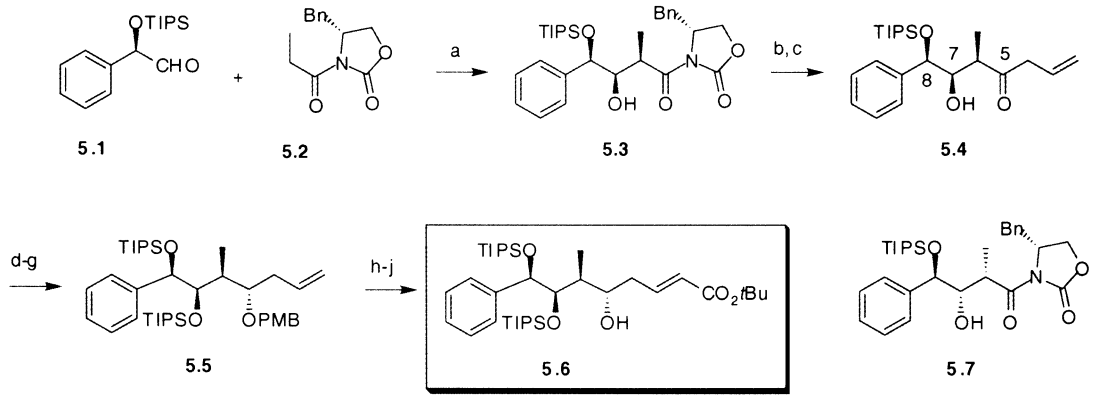
The joining together of the two large fragments and the macrolactamization are summarized in Scheme 4. The ester linkage between **2.4** and **3.6** was installed through the use of DCC/DMAP to produce *seco* compound **4.1** in 94% yield. Sequential removal of the Troc and Boc protecting groups was accomplished in 89% yield following treatment with zinc and acetic acid, followed by neat trifluoroacetic acid. Macrolactamization of **4.2** with FDPP in the presence of DIEA in DMF gave **4.3** (cryptophycin 3) in 64% yield. Epoxidation of **4.3** with *m*-CPBA at 0°C led to a ca. 2/1 mixture of cryptophycin 1 and the corresponding (7*S*,8*S*)-*trans*-epoxide **4.4**. As will be seen from the discussion of the subsequent cryptophycin syntheses, this ratio is essentially invariant regardless of the oxidant or the conditions that are chosen. A more vexing problem is that the separation of cryptophycin 1 from **4.4** can only be accomplished by HPLC, which greatly complicates the preparative task. Several solutions to the stereochemical problem posed by the epoxide have been developed, both by our group and by others. These will be discussed in some detail in what follows.

The first Hawaii synthesis has been discussed in considerable detail because the syntheses that followed invariably borrowed from it to varying degrees.

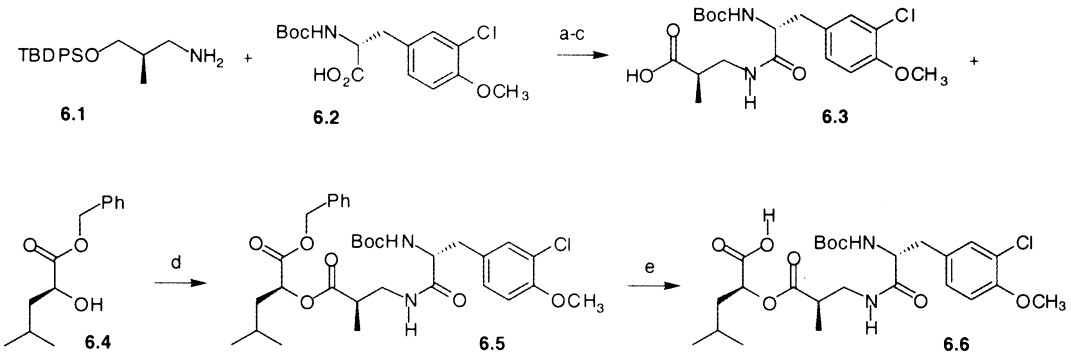
3. The Leahy–Gardinier synthesis

The first synthesis to address the stereochemical problem posed by the epoxide is due to Leahy and Gardinier.[13] A diol was used as a convenient synthetic equivalent of the epoxide. The stereochemistry at carbon atoms C7 and C8 was established from the outset thereby avoiding any ambiguity regarding the epoxide stereochemistry. Leahy and Gardinier's highly successful synthesis of unit A is summarized in Scheme 5. Two asymmetric fragments were coupled in the first step, (*R*)-mandelaldehyde derivative **5.1** and the boron enolate derived from Evans chiral imide **5.2**.[14] This led to a single crystalline product, **5.3**, in 84% yield. Three of the four stereocenters were thus established in the first step. Trimethylaluminum-mediated transamidation of **5.3** gave the corresponding Weinreb amide in 93% yield. Following treatment with allylmagnesium bromide, allyl ketone **5.4** was isolated (92% yield). The stereochemistry at C5 was controlled by means of the Evans–Hoveyda tandem aldol–Tishchenko reaction: exposure of **5.4** to acetaldehyde and samarium iodide reduced the C5 ketone carbonyl group to the α alcohol, and at the same time converted the C7 hydroxyl to the corresponding acetate (96% yield).[15] Protection of the C5 hydroxyl group as the *p*-methoxybenzyl (PMB) ether (70% yield) was followed by treatment with DIBAL-H to cleave the C7 acetate reductively (90% yield). Finally, protection as the TIPS derivative gave **5.5** in 93% yield. The conversion of **5.5** to unit A **5.6** was accomplished by oxidative cleavage of the terminal alkene to the aldehyde (91% yield), followed by Horner–Emmons homologation using the Masamune–Roush conditions (90% yield) and oxidative cleavage of the PMB protecting group with DDQ (95% yield). This represents the first highly efficient synthesis of unit A with control of C7 and C8 stereochemistry. There is one limitation of this route, namely that the Evans aldol reaction is sensitive to scale. On larger scale, increasing quantities of the undesired isomer **5.7** are generated, therefore this reaction was limited to a 2 g scale.

Leahy and Gardinier follow a rather different strategy for the assembly of units B, C, and D. Protected aminoalcohol **6.1** (Scheme 6) was prepared from the reduction of the known amide.[16] The amine was then coupled with unit B acid **6.2**, which was prepared according to the first Hawaii synthesis, in 97% yield. Fluorodesilylation with TBAF



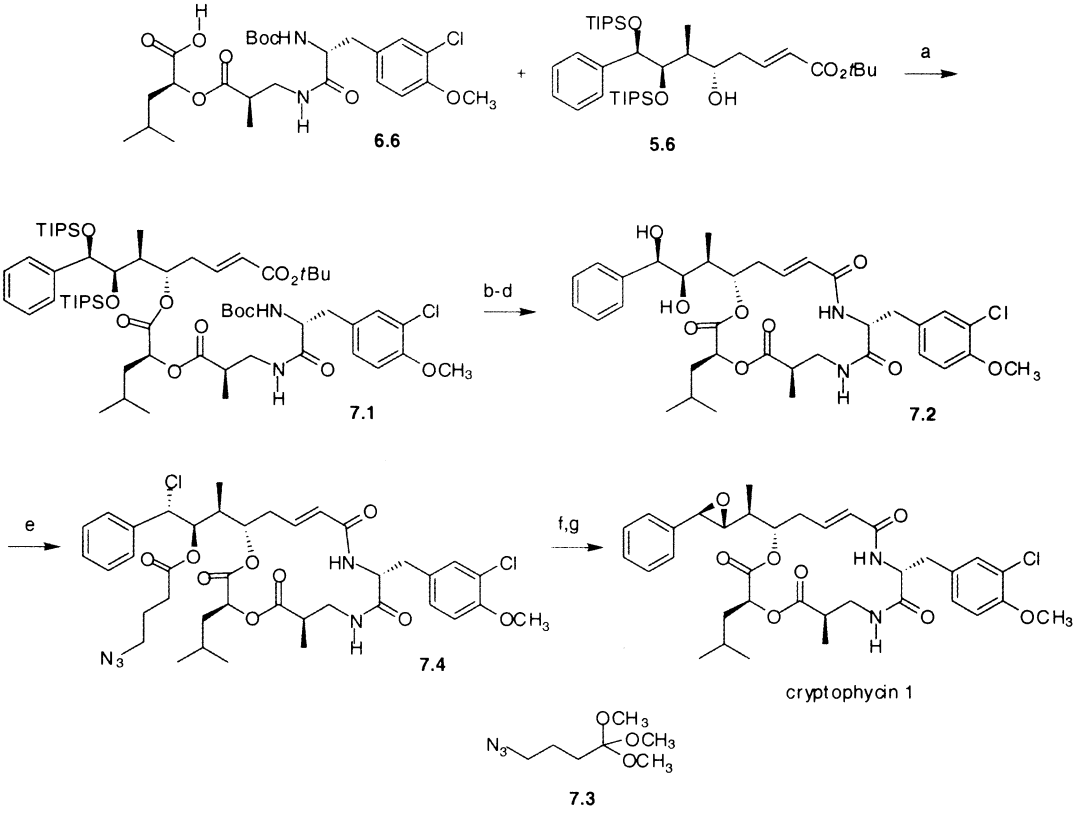
Scheme 5. (a) Bu_2BOTf, DIEA, 84%; (b) Me_3Al, MeONHMe·HCl, 93%; (c) $CH_2{=}CHCH_2MgBr$, 92%; (d) MeCHO, SmI_2, 96%; (e) $PMBOC(NH)CCl_3$, cat. TfOH, 70%; (f) DIBAL-H, 90%; (g) TIPSOTf, DIEA, 93%; (h) OsO_4, NMO; $NaIO_4$, 91%; (i) $(EtO)_2POCH_2CO_2tBu$, DBU, LiCl, 90%; (j) DDQ, 95%.



Scheme 6. (a) EDC, HOBT, 97%; (b) TBAF, 96%; (c) $RuCl_3$, $NaIO_4$, 83%; (d) DCC, 95%; (e) Ra/Ni, H_2, 84%.

(96% yield) was followed by oxidation of the primary alcohol function with ruthenium tetroxide (83% yield) to give unit B+C compound **6.3**. Oxidation of alcohol to acid, rather than coupling **6.2** with a β-alanine ester, is one of several noteworthy features of this total synthesis. Coupling of **6.3** with the benzyl ester of L-leucic acid **6.4** took place with DCC to give **6.5** in 95% yield. Finally, reductive cleavage of the benzyl ester with Raney nickel led to unit B+C+D compound **6.6** in 84% yield.

The final steps of the Leahy–Gardinier synthesis are summarized in Scheme 7. Coupling of unit A compound **5.6** with unit B+C+D **6.6** under Yamaguchi conditions produced **7.1** in 91% yield.[17] Cleavage of the Boc and *tert*-butyl ester protecting groups took place in a single operation, by exposure of **7.1** to HCl in ethyl acetate. The silyl ether protecting groups were inert under these conditions. Macrolactamization was carried out with *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-bis(pentamethylene)uronium hexafluorophosphate to give the macrocycle in 76% overall yield for the two steps.[18] Cleavage of the two silyl protecting groups with TBAF gave diol **7.2** in 95% yield. The final problem in the synthesis was to convert the *syn*-diol to the β-epoxide. Although Sharpless had published an excellent method for the conversion of vicinal diols to epoxides,[19] the strongly basic conditions of the procedure are incompatible with the macrocycle. In particular, the unit C to unit D ester link is readily cleaved in the presence of base. This is, in fact, one of the pathways for the deactivation of the cryptophycins in vivo. As a consequence, Leahy and Gardinier developed a very ingenious modification of the Sharpless procedure. 4-Azido-1,1,1-trimethoxybutane **7.3**, prepared in



Scheme 7. (a) DIPEA, 2,4,6-trichlorobenzoyl chloride, 91%; (b) HCl, EtOAc; (c) *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-bis(pentamethylene)uronium hexafluorophosphate, 76% (two steps); (d) TBAF, 95%; (e) **7.3**, TMS–Cl, 63%; (f) PPh_3, H_2O, 63%; (g) K_2CO_3, acetone, 98%.

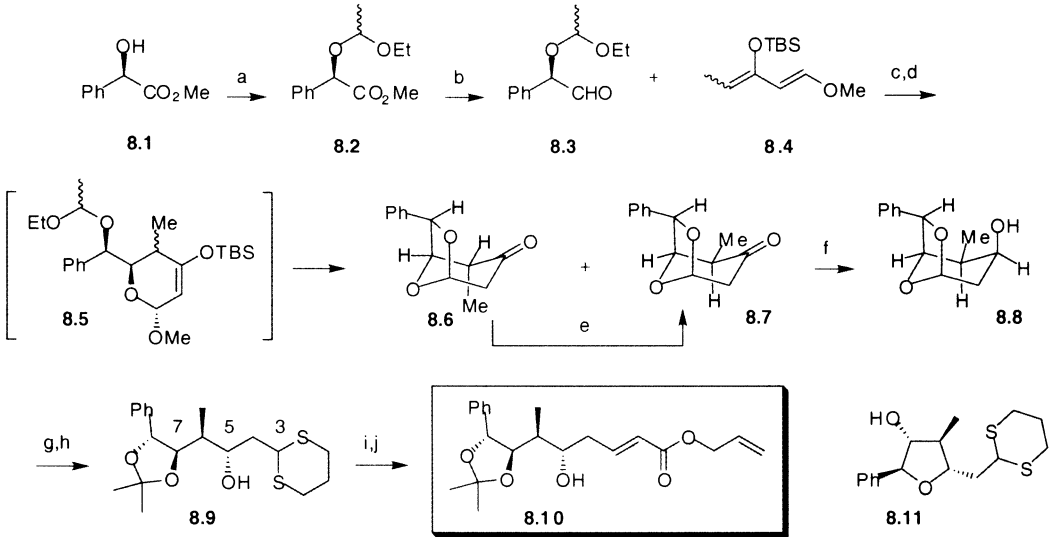
two steps from commercially available 4-chlorobutyronitrile, was allowed to react with diol **7.2** in the presence of trimethylchlorosilane according to Sharpless' conditions. This led stereospecifically to the anticipated azidobutyrate **7.4** with inversion of configuration at C7 (63% yield). Exposure of **7.4** to triphenylphosphine and water led to a Staudinger reaction with generation of a primary amino group that underwent an intramolecular lactamization reaction to produce the C7–C8 chlorohydrin. Brief exposure of the chlorohydrin to potassium carbonate in acetone led to cryptophycin 1.

The Leahy–Gardinier synthesis is exceptionally clever, and is noteworthy for solving several of the problems that are posed by the structure in unique and ingenious ways. The use of the Evans aldol reaction with mandelaldehyde derivative **5.1**, the Tishchenko reduction of the C5 keto group and the design of azidoester **7.3** so as to overcome the limitations of the conventional Sharpless procedure distinguish this work.

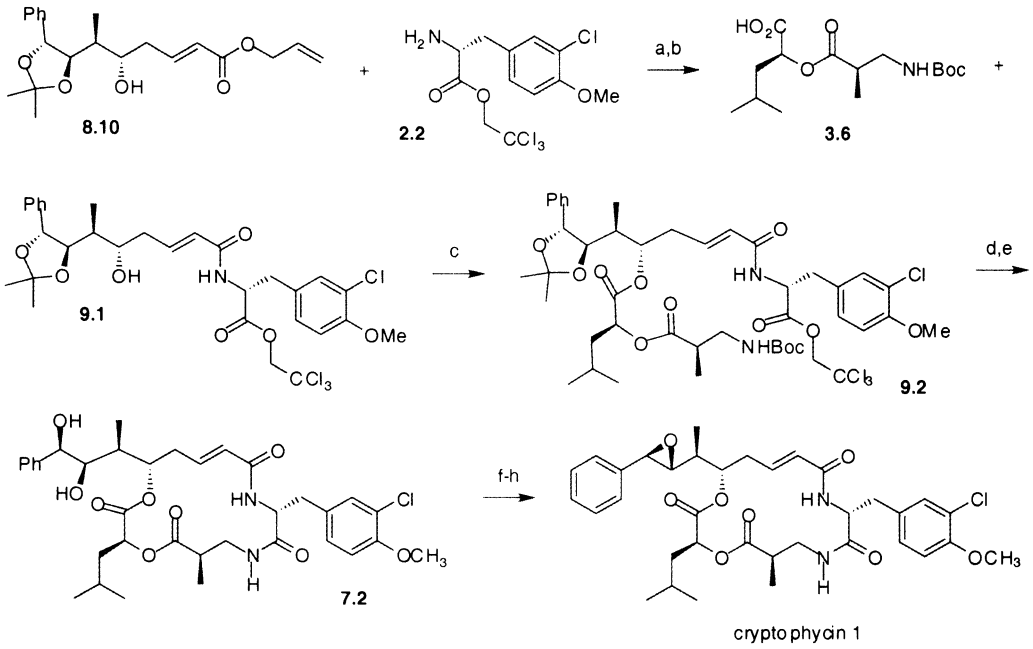
4. The Tius–Li synthesis

(*R*)-Mandelic acid is an attractive starting material for unit A. It occurred to us that it should be possible to assemble unit A using mandelic acid as the *sole* source of asymmetry. Scheme 8 summarizes our successful unit A synthesis.[20] (*R*)-Methyl mandelate **8.1** was converted to *O*-ethoxyethyl derivative **8.2**. Although the presence of an asymmetric center on the protecting group complicates the interpretation of the NMR spectra, we found that this choice of oxygen protecting group was critical for the success of the subsequent reactions in the scheme. Reduction of the ester function in **8.2** with DIBAL-H gave protected mandelaldehyde **8.3**, which was combined with diene **8.4** in THF in a magnesium bromide-catalyzed hetero-Diels–Alder reaction.[21] Exposure of the crude Diels–Alder product **8.5** to trifluoroacetic acid, followed by workup, gave a 10/1 mixture of products **8.6** and **8.7**. Although this cycloaddition process is likely to be mechanistically distinct from the conventional concerted Diels–Alder process, the stereochemistry of the products can be rationalized as resulting from an *exo* transition state in a chelation controlled process.[22] Acid catalyzed cleavage of the ethoxyethyl protecting group was followed by an intramolecular Michael addition of the hydroxyl to give bicyclic products **8.6** and **8.7**. Stirring a solution of this product mixture in acetonitrile over KF/alumina[23] for 48 h epimerized **8.6**, leading to a 1/4 mixture of axial methyl **8.6** to equatorial methyl **8.7** products in 48% overall yield from **8.1**. It is noteworthy that diene **8.4** was used as a 9/1 mixture of (*E*,*Z*) to (*Z*,*Z*) geometrical isomers. The stereochemistry of the diene is reflected in the ratio of Diels–Alder products, but since one can epimerize the product mixture, one need not separate the isomers of the diene.

Reduction of the 1/4 mixture of **8.6** and **8.7** with L-selectride at −78°C in THF gave alcohol **8.8** as a single (axial) isomer in 93% yield, based on recovered starting material. The recovered ketone was determined to be a ca. 6–7/1 mixture of **8.6** and **8.7**. It therefore appears that reduction of axial isomer **8.6** by L-selectride is very slow, and since the reaction medium is basic, some epimerization of **8.6** to **8.7** can take place during the course of the reduction. Intermediate **8.8** incorporates all four asymmetric carbon atoms of unit A. No adjustment of the oxidation state of C3 is necessary, since it is present in **8.9** as a ketal. Careful exposure of **8.8** to boron trifluoride etherate and 1,3-propanedithiol in dichloromethane at 0°C converted the ketal function to the dithioketal in 85% yield. Protection of the *syn* C7–C8 diol as the acetonide gave **8.9** in 93% yield. It was necessary to exercise care during the formation of the dithioketal. If the temperature was allowed to rise much above 0°C, or if the reaction was not quenched promptly following the consumption of **8.8**, tetrahydrofuran **8.11** was isolated in yields up to 30%. This compound is analogous to **1.10**, and its formation provides another example of the reactive nature of the benzylic carbon atom in unit A. Treatment of dithioketal **8.9** with iodomethane and calcium carbonate in aqueous acetonitrile led to the expected β-hydroxyaldehyde.



Scheme 8. (a) ethyl vinyl ether, PPTS; (b) DIBAL-H, ether, −78°C; (c) $MgBr_2$, THF, 36–42°C; (d) TFA, THF, **8.6/8.7**=10/1; (e) KF, alumina, MeCN, **8.6/8.7**=1/4, 48% of **8.7** overall from **8.1**; (f) L-selectride, THF, −78°C, 93%; (g) $BF_3{\cdot}Et_2O$, CH_2Cl_2, $HS(CH_2)_3SH$, 0°C, 85%; (h) $Me_2C(OMe)_2$, TsOH, acetone, 93%; (i) MeI, $CaCO_3$, MeCN, H_2O, 70°C; (j) $LiClO_4$, DIPEA, MeCN, $(EtO)_2POCH_2CO_2CH_2CH{=}CH_2$, 68% from **8.9**.



Scheme 9. (a) $Pd(PPh_3)_4$, morpholine, 100%; (b) Et_3N, EDCI, CH_2Cl_2, 0°C to rt, 81%; (c) DCC, DMAP, CH_2Cl_2, 81%; (d) TFA, CH_2Cl_2, 0°C; (e) 2-hydroxypyridine, PhMe, rt, 62% from **9.2**; (f) $(MeO)_3CH$, PPTS, CH_2Cl_2, rt; (g) AcBr, CH_2Cl_2, rt; (h) $KHCO_3$, DME/EtOH/MeOH (6/4/1), 40°C, 70% from **7.2**.

Horner–Emmons reaction with allyldiethylphosphonoacetate led to unit A compound **8.10** in 68% overall yield for the two steps from **8.9**. It is noteworthy that the C5 hydroxyl group was not protected at any time during this synthesis.

The allyl ester in **8.10** was cleaved in quantitative yield in the presence of catalytic palladium(0) and morpholine (Scheme 9).[12] Coupling with unit B compound **2.2** took place in the presence of EDCI and triethylamine to produce **9.1** in 81% yield. Esterification of the C5 hydroxyl group with unit C+D **3.6** took place in the presence of DCC and DMAP in 81% yield to give *seco* compound **9.2**. Simultaneous removal of the Boc and acetonide protecting groups took place with trifluoroacetic acid in dichloromethane at 0°C. The elegant conditions that were developed at Eli Lilly Co. were applied to the macrolactamization step.[18] Simple treatment in toluene at room temperature with 2-hydroxypyridine led to **7.2**, the same intermediate from Leahy's synthesis, in 62% overall yield for the two steps from **9.2**. The trichloroethyl protecting group in unit B serves to activate the ester during the macrolactamization, so it serves two complementary roles during the synthesis. The Lilly conditions for the macrolactamization do not require exotic or expensive peptide coupling reagents, and are well suited for large-scale work.

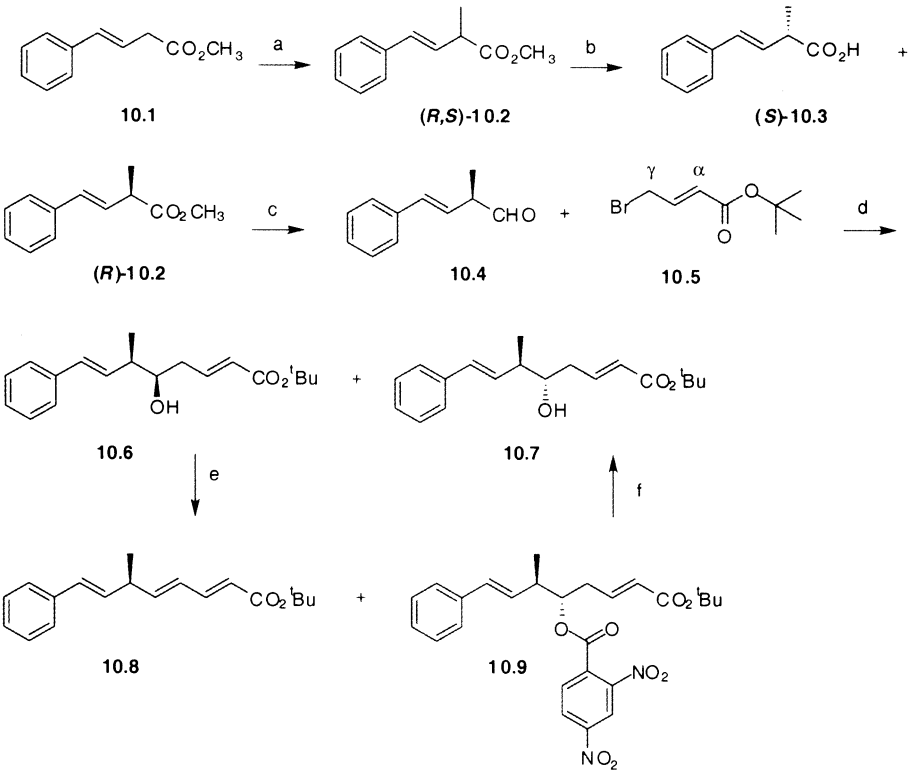
Since one of our goals for this work was to develop as simple a cryptophycin synthesis as possible, we decided not to make use of azido orthoformate **7.3** for the critical diol to epoxide transformation. We were mindful of the lability of the unit C–unit D ester linkage toward base, so we used a modification of the Sharpless procedure. Treatment of **7.2** with trimethyl orthoformate and PPTS, followed by acetyl bromide, led to a sensitive bromohydrin formate that was not purified. Exposure of this intermediate product to powdered potassium bicarbonate in a 6/4/1 mixture of DME/ethanol/methanol at 40°C for 24 h led to cryptophycin 1 in 70% overall yield from **7.2**. The conditions for the conversion of bromohydrin formate to epoxide were developed through a painstaking optimization process. The small amount of methanol in the reaction mixture was necessary to partially dissolve the bicarbonate. However, larger proportions of methanol led to methanolysis of the unit C–unit D ester.

In summary, this cryptophycin synthesis is both efficient (6.8% overall yield) and stereospecific. (*R*)-Methyl mandelate serves as the sole source of asymmetry for unit A. The key feature of this synthesis is the stereoconvergent hetero-Diels–Alder reaction that is used to assemble unit A. In Section 5, each of the other approaches to unit A that have been described to date is discussed.

5. Syntheses of unit A

5.1. The Sih chemoenzymatic synthesis

The Sih synthesis of unit A relies upon an enzymatic hydrolysis of a racemic ester for the key step (Scheme 10).[24] (*E*)-Methyl styryl acetate **10.1** was first treated with LDA, followed by dimethyl sulfate to produce (±)-**10.2** in 92% yield. Exposure of the racemic ester to *Candida rugosa* lipase from Sigma Chemical Co. at room temperature in a phosphate buffer led to carboxylic acid (*S*)-**10.3** in 45% yield and methyl ester (*R*)-**10.2** in 48% yield. Both products were isolated in >96% ee, therefore the kinetic resolution was highly successful. Separation of methyl ester (*R*)-**10.2** from the carboxylic acid was followed by reduction with DIBAL-H to give aldehyde **10.4** in 95% yield. The introduction of the remaining four carbon atoms of unit A was

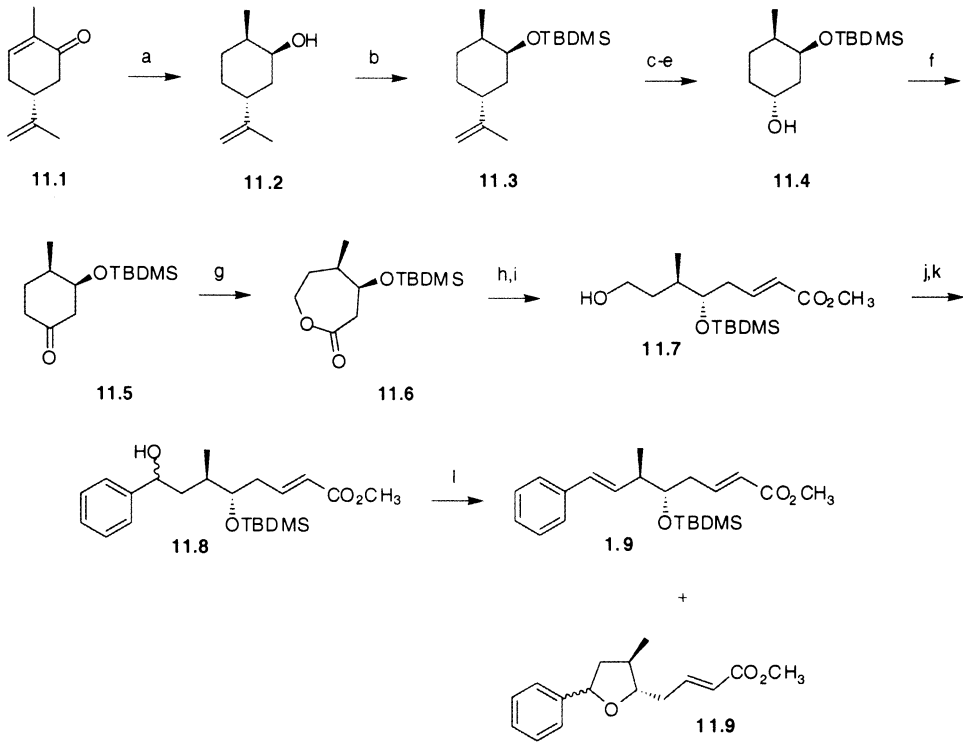


Scheme 10. (a) LDA, Me_2SO_4, THF, 92%; (b) 2-propanol-treated *Candida rugosa* lipase, 0.2 M phosphate buffer, rt, (*S*)-**10.3** 45% (ee>96%), (*R*)-**10.2** 48% (ee>96%); (c) DIBAL-H, Et_2O, −70°C, 95%; (d) Zn–Pb, Et_2O/PhH (1/1), **10.6** 22% (ee>96%), **10.7** 18% (ee>96%), α-isomers 25%; (e) 2,4-dinitrobenzoic acid, DEAD, PPh_3, **10.8** 15%, **10.9** 59%; (f) K_2CO_3, MeOH, 30 min, 92%.

accomplished by means of a Reformatsky reaction with *tert*-butyl 4-bromocrotonate **10.5**. This is not a straightforward reaction, since there are a number of pathways that are available to **10.5**. In fact, in spite of the considerable efforts of the Sih group to optimize the Reformatsky reaction, the process was not selective, and all possible products were formed: the undesired *syn* γ-adduct **10.6** was isolated in 22% yield along with the desired *anti*-γ-adduct **10.7** (18% yield). The α-adducts were also formed in 25% yield. Since the major product had the wrong stereochemistry it was necessary to convert **10.6** to **10.7** through a Mitsunobu inversion. Exposure of **10.6** to 2,4-dinitrobenzoic acid, diethylazodicarboxylate (DEAD) and triphenylphosphine led in 59% yield to dinitrobenzoate **10.9** with the correct C5 stereochemistry, along with 15% of triene **10.8**. The presence of the enoate function renders the protons at C4 relatively acidic, consequently the Mitsunobu process in this instance was accompanied by unusually large amounts of elimination product **10.8**. Nitrobenzoate **10.9** was hydrolyzed to **10.7** with methanolic potassium carbonate in 92% yield. The overall yield of **10.7** from **10.1** was 12.6%. Although Sih's synthesis of unit A provides an illustration of the synthetic utility of an enzyme-mediated kinetic resolution, the yield is inferior to that of the first Hawaii synthesis (29% for unit A). Also, the need to separate and recycle **10.6** to **10.7** diminishes the preparative utility of the Sih approach. Nevertheless, Sih notes that the Hawaii synthesis '…is relatively lengthy and is not easily adaptable for the synthesis of cryptophycins in gram quantities.' Sih's statement should be interpreted in light of the following comments by Moher, Moore and co-workers: 'As part of our initial efforts to generate supplies of **2** and synthetic intermediates to fuel clinical development and SAR, respectively, we adopted the Moore–Tius synthesis of **2**, making necessary adjustments for *multi-kilo* operations.'[25] (Compound **2** is cryptophycin 52; see Scheme 23 for the structure of cryptophycin 52.)

5.2. The Lilly chemoenzymatic synthesis

Chemists at Eli Lilly Company have described a successful chemoenzymatic approach to unit A (Scheme 11).[26] Commercially available (*R*)-carvone **11.1** was bioreduced with *Trigonopsis variabilis* (ATCC 10679) using glucose as a carbon source to produce saturated alcohol **11.2** in 50–62% isolated yield without the need for chromatography. The product was isolated as a single diastereomer (de>98%, as judged by GC). The first step of this synthesis serves to establish the stereochemistry at C5 and C6 of unit A. Protection of the secondary hydroxyl group as the TBS derivative (87% yield) was followed by ozonolysis and Criegee rearrangement to produce alcohol **11.4** after hydrolytic cleavage in 73% overall yield from **11.3**.[27,28] Oxidation to ketone **11.5** took place in a two-phase system with aqueous sodium hypochlorite and dichloromethane in the presence of catalytic TEMPO, to give cyclohexanone **11.5**.[29] At this stage it was necessary to perform the C–C bond cleavage that converts cyclic intermediate **11.5** to a linear product that can be elaborated to unit A. Since both α carbon atoms in **11.5** are unsubstituted, the only control element that can be used to influence the regiochemistry of the cleavage is the β-silyloxy group. The electron-withdrawing effect of the β-silyloxy substituent was expected to favor rearrangement to **11.6** during a Baeyer–Villiger



Scheme 11. (a) *Trigonopsis variabilis*, pH 7 buffer, glucose, 50–62%; (b) TBDMSCl, DBU, DMF, 87%; (c) O_3, MeOH, CH_2Cl_2; (d) Ac_2O, DMAP; (e) NaOH, MeOH, 73% (three steps); (f) TEMPO (cat.), NaOCl, CH_2Cl_2, 87%; (g) CF_3CO_3H, CH_2Cl_2, TFA (1/4), −15°C, 2 h, 81%; (h) DIBAL-H, PhMe, −78°C; (i) $(MeO)_2POCH_2CO_2Me$, TMG, THF, 66% (two steps); (j) TEMPO (cat.), NaOCl, CH_2Cl_2; (k) PhMgBr, −78°C, 67% (two steps); (l) 3 equiv. Ms_2O, 9 equiv. Et_3N, DMAP, CH_2Cl_2, 42 h, 53%.

oxidation.[30] The optimized conditions involved the use of 30% aqueous hydrogen peroxide as the oxidant (1.6 equiv. oxidant) with sufficient trifluoroacetic anhydride to form peroxytrifluoroacetic acid and to consume all the water, leading to a solvent mixture of dichloromethane and trifluoroacetic acid (4/1). At −15°C under these conditions lactone **11.6** was formed in a 98/2 ratio with its regioisomer in a combined yield of 83% (81% yield of **11.6**). The authors note that consistently better results for the Baeyer–Villiger were obtained from the reactions that used 30% hydrogen peroxide and trifluoroacetic anhydride, than from hydrogen peroxide urea complex and trifluoroacetic anhydride. They postulate that conditions that favor the protonation of the β-silyloxy group, thereby amplifying its inductive electron-withdrawing effect, favor the production of **11.6**.

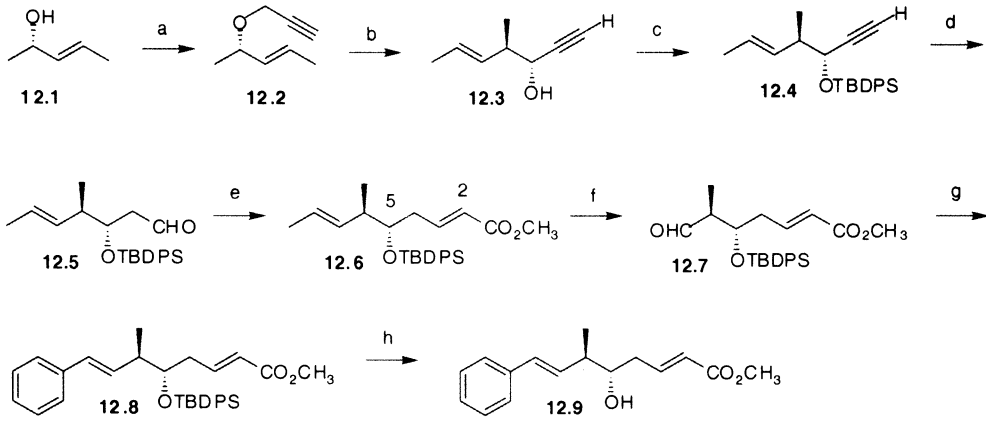
The conversion of **11.6** to unit A follows a conventional strategy. Reduction of lactone to lactol with DIBAL-H was followed by a Horner–Emmons reaction with trimethylphosphonoacetate to give **11.7** in 66% yield for the two steps. Oxidation of the primary hydroxyl group in **11.7** to the aldehyde, followed by addition of phenylmagnesium bromide gave diastereomeric benzylic alcohols **11.8** in 67% overall yield for the two steps from **11.7**. The route was designed to allow introduction of the phenyl group toward the end of the unit A synthesis. In this way, a series of cryptophycin phenyl ring analogs can be prepared from a common precursor. The elimination of water from benzylic alcohol **11.8** proved to be surprisingly difficult. The optimized conditions involved treatment of **11.8** with 3 equiv. of methanesulfonic anhydride and 9 equiv. triethylamine in the presence of catalytic DMAP in dichloromethane. This led to styrene **1.9**, the intermediate from the first Hawaii synthesis, in 53% yield (97.3% ee). The desired **1.9** was formed in a 4/1 ratio with tetrahydrofuran **11.9**, the product of intramolecular displacement of the mesylate by the oxygen atom at C5. During the course of this study it was found that *para* substitution on the phenyl ring by electron-donating substituents (e.g. methyl) *increases* the rate of the desired elimination reaction relative to tetrahydrofuran formation, perhaps by rendering the intermediate cation more stable, and hence longer lived, thereby allowing the elimination process to compete with the cyclization. Alternatively, stabilization of the transition state for the elimination process would account for the observation.

The use of methanesulfonic anhydride for the elimination also deserves comment. When methanesulfonyl chloride was used instead of the anhydride for the elimination of water from **11.8**, the benzylic chloride was isolated in 25% yield. The chloride did not undergo elimination under the reaction conditions.

The Lilly chemoenzymatic synthesis of unit A is interesting because it relies upon a non-obvious (to this writer) strategy. The next unit A synthesis to be discussed makes use of a classical organic reaction in the key step.

5.3. Synthesis of unit A via a [2,3]-Wittig rearrangement

The comprehensive and far-ranging treatment of the anionic [2,3]-Wittig rearrangement by Nakai and Mikami[31] strongly suggested that the critical C5–C6 stereochemistry within

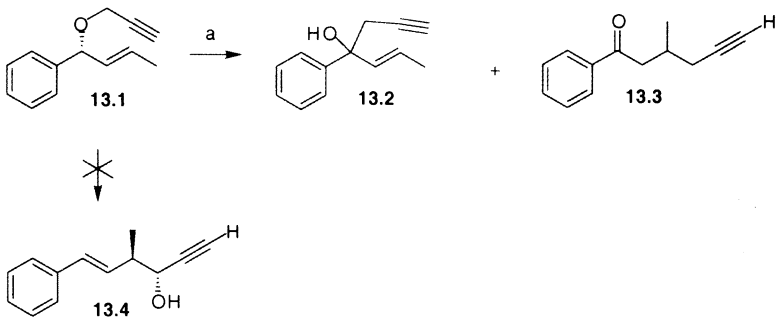


Scheme 12. (a) propargyl bromide, 50% aqueous NaOH, *n*-Bu_4NHSO_4 (cat.), 86%; (b) *n*-BuLi, THF, −90°C to rt, 71%; (c) TBDPSCl, DMF, imidazole, 92%; (d) 2-methyl-2-butene, BH_3·THF, 0°C; H_2O_2, aqueous KH_2PO_4/K_2HPO_4 (2.2 M), 0°C, 83%; (e) $(MeO)_2POCH_2CO_2Me$, TMG, THF, −78°C, 92%; (f) O_3, CH_2Cl_2, pyridine, Sudan 7B, −78°C; Zn, HOAc, 85%; (g) $PhCH{=}PPh_3$, THF, −78°C to rt; PhSH, VAZO 88, PhMe, reflux, 82%; (h) aqueous HF, MeCN, rt, 93%.

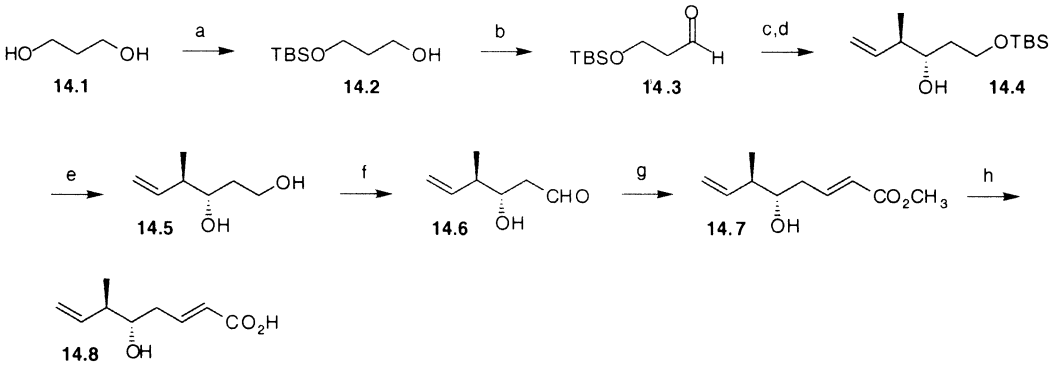
unit A would be accessible through the rearrangement of a suitably substituted, non-racemic allylpropargyl ether.[32] The optically enriched starting material for the synthesis was prepared by incubating commercially available racemic (*E*)-3-penten-2-ol with porcine pancreatic lipase (PPL) and trifluoroethyl laurate in anhydrous ether for 80 h.[33] This resulted in a kinetic resolution in which the slow-reacting (*S*) enantiomer **12.1** (Scheme 12) could be distilled from the reaction mixture in 40% yield. This material was >95% ee, as judged by analysis of the ^{1}H NMR spectrum of the Mosher ester.[34] Exposure of **12.1** to propargyl bromide and strong base under phase transfer reaction conditions led to allyl propargyl ether **12.2** in 86% isolated yield. The conditions for the subsequent anionic [2,3]-Wittig rearrangement were developed with care. An excess of *n*-butyllithium in hexanes was transferred to the reaction flask that was cooled to −90°C. Evaporation of most of the hexanes under vacuum produced a residue to which was slowly added a solution of **12.2** in anhydrous THF. The reaction mixture was allowed to warm from −90°C to room temperature overnight, and was quenched with ammonium chloride and worked up to give a 9/1 mixture of diastereomers in 71% yield (the major diastereomer **12.3**, (3*R*,4*R*)-4-methylhept-5-(*E*)-en-1-yn-3-ol, is shown in Scheme 12). The solvent exchange was found to be critical to the success of the Wittig rearrangement. The diastereomers were separated by column chromatography on silica gel to produce **12.3** in >95% ee. This intermediate requires elaboration at both ends of the molecule for its eventual conversion to unit A. Protection of the C5 alcohol as the TBS ether took place in 92% yield to give silyl ether **12.4**. Selective hydroboration of the terminal alkyne in the presence of the 1,2-disubstituted alkene with disamylborane (or catecholborane), followed by oxidation of the intermediate dialkylvinyl boron species, led to aldehyde **12.5** in 83% yield. Horner–Emmons homologation of the aldehyde to α,β-unsaturated ester **12.6** took place in 92% yield.

It was now necessary to differentiate between the electron-poor and electron-rich alkene groups of **12.6**. Ozonolysis of **12.6** at −78°C in dichloromethane containing pyridine, and monitoring the disappearance of the red color of a small amount of Sudan 7B which was added as an indicator made it possible to terminate the ozonolysis before significant cleavage of the C2–C3 double bond had taken place.[35] Reductive workup with zinc powder and acetic acid led to aldehyde **12.7** in 85% yield.

Wittig reaction with benzylidene triphenylphosphorane led to an *E*/*Z* mixture (ca. 4/1) of styrenes that were isomerized to the more stable *E* geometrical isomer **12.8** by refluxing the mixture in toluene in the presence of thiophenol and 1,1′-azobis(cyclohexanecarbonitrile) (VAZO 88). The overall yield of **12.8** for the two steps from **12.7** was 82%. Cleavage of the TBDPS protecting group from **12.7** took place with aqueous HF in acetonitrile, to give unit A compound **12.9** in 93% yield.



Scheme 13. (a) *n*-BuLi, PhLi or *t*-BuLi, **13.2/13.3** ca. 1/2.5.

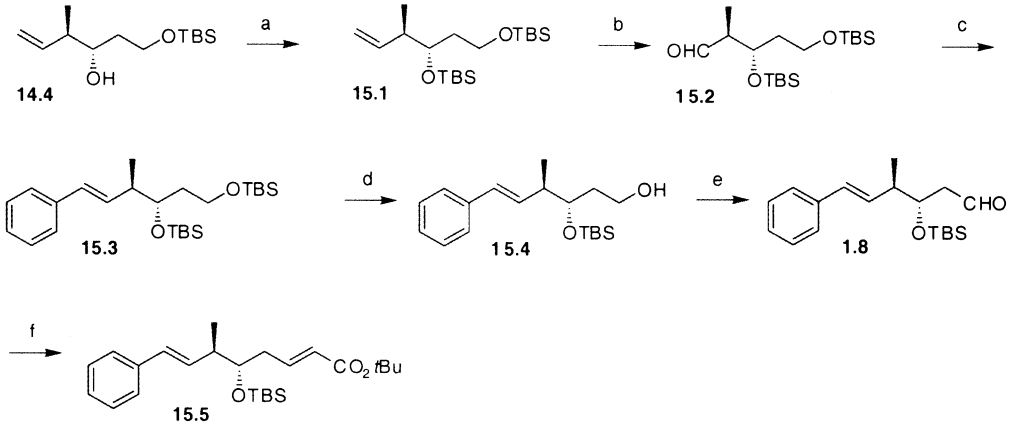


Scheme 14. (a) NaH, TBSCl, THF, 0°C, 95%; (b) TEMPO (cat.), NaOCl, $NaHCO_3$, CH_2Cl_2, (pH 9.0–9.2), 91%; (c) (*E*)-crotyldiisopinocampheylborane, Et_2O, −78°C; (d) H_2O_2, NaOAc, 67% from **14.3**; (e) TBAF, 100%; (f) TEMPO (cat.), NaOCl, $NaHCO_3$, CH_2Cl_2, (pH 9.0–9.2), 100%; (g) $(MeO)_2POCH_2CO_2Me$, base; (h) KOH, 85% overall from **14.4** (four steps).

This synthesis of unit A, like the Lilly chemoenzymatic synthesis, follows a 'phenyl last' strategy. While this is well-suited for the preparation of diverse phenyl ring analogs of unit A from a common precursor, in the present case one might wonder whether a more efficient synthesis of **12.9** might have been possible by incorporating the phenyl group at the beginning of the sequence. In fact, this strategy was examined (Scheme 13). Racemic propargyl ether **13.1** was prepared in a straightforward way from phenyl propenyl ketone in two steps. In this case, all attempts to perform the anionic [2,3]-Wittig rearrangement failed. Exposure of **13.1** either to *n*-butyllithium, phenyllithium or *tert*-butyllithium led to a mixture of tertiary alcohol **13.2** and phenone **13.3**. None of the desired [2,3]-Wittig product **13.4** was obtained. Tertiary alcohol **13.2** is apparently formed by means of a [1,2]-Wittig rearrangement of a benzyl carbanion, whereas rearrangement of the same carbanion through a vinylogous process can rationalize the occurrence of phenone **13.3**. These results underscore the idiosyncratic nature of the anionic [2,3]-Wittig rearrangement. The sensitivity of this process to solvent, base, and substitution pattern add to the challenge of incorporating it into synthetic planning.

5.4. Asymmetric crotylboration

The Brown asymmetric crotylboration reaction provides an excellent means for controlling relative and absolute stereochemistry at C5 and C6 of unit A.[36–38] Michael Martinelli and coworkers in the Chemical Process Group at Eli Lilly Company have described a very brief synthesis of unit A based on this strategy.[39] The starting point for the synthesis is 1,3-propanediol **14.1** (Scheme 14) which was selectively *mono* protected to give TBS ether **14.2** in 95% yield. Oxidation of the free hydroxyl group in **14.2** with sodium hypochlorite in the presence of catalytic TEMPO led to aldehyde **14.3** in 91% yield.[29] The enantiomerically pure Brown asymmetric crotylboron reagent was prepared in situ from (−)-α-pinene of 81% ee, according to the published protocols.[36–38] Addition of the crotylboron reagent to **14.3** at −78°C, followed by oxidation with hydrogen peroxide in the presence of sodium acetate, led to homoallylic alcohol **14.4** in 67% yield and ≥99% ee and de. Quantitative removal of the silyl ether protecting group with fluoride gave diol **14.5**. Oxidation of this material with hypochlorite and TEMPO, as described previously, produced β-hydroxyaldehyde **14.6** in quantitative yield. Homologation via the phosphonate led to methyl ester **14.7** which was hydrolyzed with base to produce carboxylic acid **14.8** in 85% overall yield for the four steps from **14.4**. The phenyl ring of cryptophycin 1 was introduced by means of a Heck reaction with iodobenzene at the end of a synthetic sequence that led to cryptophycin 52, a synthetic analog of the natural product. Whereas the overall yield of **14.8** was excellent, the Heck process on the macrocyclic intermediate proceeded in only 31% yield, underscoring



Scheme 15. (a) TBSCl, imidazole, DMF, rt, 93%; (b) O_3, −78°C; Me_2S, warm to rt, 62%; (c) $PhCH_2PO(OEt)_2$, *n*-BuLi, THF, −78°C to rt, 79%; (d) HF–pyridine, THF, 99%; (e) Dess–Martin, CH_2Cl_2, 98%; (f) Ph_3PCHCO_2tBu, CH_2Cl_2, 85%.

once again the difficulties that are encountered when attempting to functionalize C7 or C8 once the macrocycle is in place. The goal of this synthesis was to prepare an advanced intermediate that could be converted to a series of phenyl ring analogs, and in this it was highly successful.

Georg[40] and White[41,42] and their respective co-workers have applied a similar strategy to the synthesis of unit A. White and co-workers have prepared **14.4** in the same way as the Lilly group, however, their synthesis diverges thereafter (Scheme 15). Protection of the free hydroxyl group in **14.4** as the TBS ether led to *bis*-TBS ether **15.1** in 93% yield. Ozonolytic cleavage of the terminal olefin in **15.1** gave aldehyde **15.2** (62% yield). The styryl group was introduced through the Horner–Emmons reaction of the aldehyde with diethylbenzylphosphonate.[43] The *E* isomer **15.3** was the exclusive reaction product, isolated in 79% yield. The benzyl Horner–Emmons reagent is therefore to be preferred over the corresponding phosphorane (see **12.7→12.8**, Scheme 12) which requires an additional isomerization step. Selective removal of the primary silyl ether in **15.3** took place in nearly quantitative yield with HF–pyridine complex. The Dess–Martin periodinane oxidized alcohol to aldehyde in 98% yield to give **1.8**, the intermediate from the first Hawaii synthesis. Homologation of **1.8** with (*tert*-butoxycarbonylmethylene)triphenylphosphorane[44] in dichloromethane led to protected unit A compound **15.5** in 85% yield.

The White synthesis is noteworthy for several reasons. The overall yield is high: 20% from **14.3**. Also, the use of diethylbenzylphosphonate defines a useful alternative to the conventional Wittig reagent for installing the styrene function. In addition to the enantioselective crotylboration approach, White has also disclosed a related strategy, based on a diastereoselective allylation of a chiral, non-racemic aldehyde. The allylstannation approach will be discussed in Section 5.5.

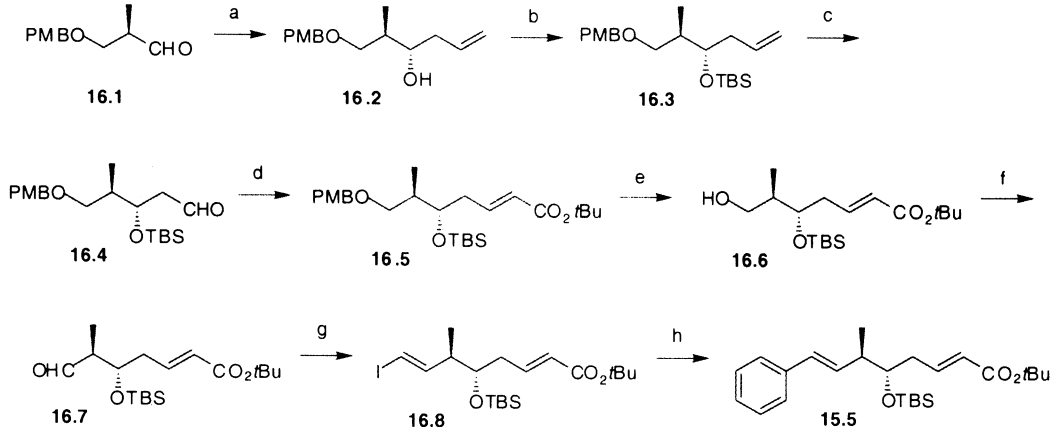
5.5. Diastereoselective allylstannation

The allylstannation synthesis of unit A by White and co-workers makes use of (*R*) aldehyde **16.1** (Scheme 16).[41] This compound was converted to a 11/1 mixture of diastereomeric homoallylic alcohols **16.2** according to Keck's conditions.[45] The ratio of diastereoisomers was improved to 20/1 by conducting the allylstannation at −100°C, according to Linderman's conditions, rather than at −78°C.[46] The major isomer **16.2** was isolated in 76% yield. Protection of the hydroxyl group as the TBS ether led to **16.3** which was oxidatively cleaved to produce aldehyde **16.4** in 76% yield. Homologation with (*tert*-butoxycarbonylmethylene)-triphenylphosphorane,[44] as in White's previously discussed synthesis of unit A, led to **16.5**. Introduction of the styryl portion of unit A was accomplished through aldehyde **16.7**, which was prepared in the conventional way from **16.5**. The Takai reaction of aldehyde **16.7** with iodoform led to (*E*)-vinyl iodide **16.8** in 92% yield.[47] This was followed by a Stille reaction with phenyltrimethylstannane to give unit A compound **15.5** in 67% yield.[48]

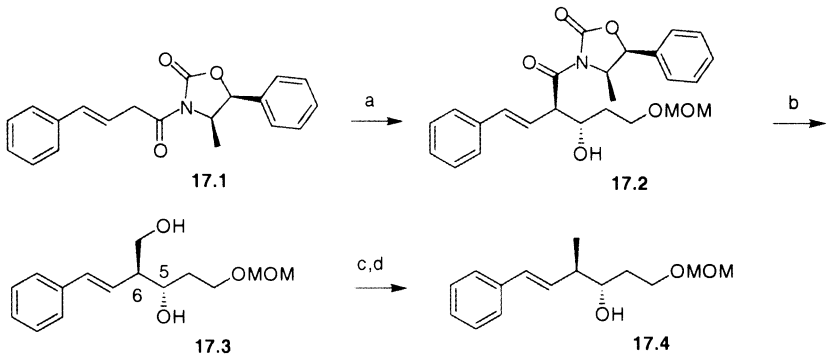
Although the allylation approach to unit A was successful and efficient (14% overall yield from **16.1**) the modest diastereoselectivity of the key step, and the attendant need to separate the minor diastereoisomer from the mixture, diminished the appeal of this route, and prompted White and coworkers to develop the synthesis of Scheme 15.

5.6. A chiral auxiliary approach

A conceptually distinct strategy for the synthesis of unit A was developed by Kobayashi and co-workers in the context of their improved synthesis of arenastatin A.[49] The arenastatins were isolated from the Okinawan sponge *Dysidea arenaria*, and they are structurally similar to the cryptophycins.[50,51] Unit B of the arenastatins is D-*O*-methyltyrosine, which is easily prepared from the commercially available unnatural amino acid, whereas unit C of the arenastatins is achiral β-alanine. For these reasons arenastatin synthesis is less challenging than cryptophycin synthesis. Although the arenastatins have good in vitro anti-tumor activity, they are essentially inactive in vivo. This is almost certainly in large part a consequence of the much greater propensity for hydrolytic cleavage of the unit C–unit D ester link in the arenastatins relative to the cryptophycins. As a result of the attenuated activity of the arenastatins, whatever interest their synthesis has generated has been related to their utility as simpler cryptophycin analogs.



Scheme 16. (a) $Bu_3SnCH_2CH{=}CH_2$, $SnCl_4$, CH_2Cl_2, −100°C, 76% (20/1); (b) TBSCl, DMF, imidazole, 91%; (c) cat. OsO_4, $NaIO_4$, aqueous THF, 76%; (d) $Ph_3P{=}CHCO_2tBu$, CH_2Cl_2, 95%; (e) DDQ, CH_2Cl_2, H_2O, 0°C, 92%; (f) Dess–Martin, CH_2Cl_2, 92%; (g) CHI_3, $CrCl_2$, THF, 0°C, 92%; (h) $PhSnMe_3$, $PdCl_2(MeCN)_2$, DMF, 67%.



Scheme 17. (a) Bu_2BOTf, Et_3N, CH_2Cl_2; $MeOCH_2O(CH_2)_2CHO$, 95% (de>99%); (b) Bu_3B, AcOH, $LiBH_4$, THF, 80%; (c) TsCl, pyr; (d) $LiBEt_3H$, THF, 88% (two steps).

The synthesis of Kobayashi and co-workers is summarized in Scheme 17. The diastereoselective aldol reaction through the dibutylboryl enolate of **17.1** with β-(methoxy)methoxypropanal led to **17.2** in 95% yield (de>99%). Reduction of imide to primary alcohol **17.3** took place in 80% yield with lithium borohydride in the presence of dibutylacetoxyborane. Selective tosylation of the primary hydroxyl group in **17.3**, followed by reduction with lithium triethylborohydride led to **17.4** in 88% yield for the two steps. This compound was not homologated to unit A, because Kobayashi and co-workers used a strategy that does not proceed through a discreet unit A compound. Although this unit A synthesis is highly stereoselective, the need to adjust the oxidation state of the group at C6 adds to the number of steps.

A closely related synthesis of unit A has been described by Ghosh and Bischoff, the main point of difference between this work and the route that is summarized in Scheme 17 being the use of (+)-(1*R*,2*S*)-1-(*N*-tosylamino)-2-indanol as the chiral auxiliary group.[52]

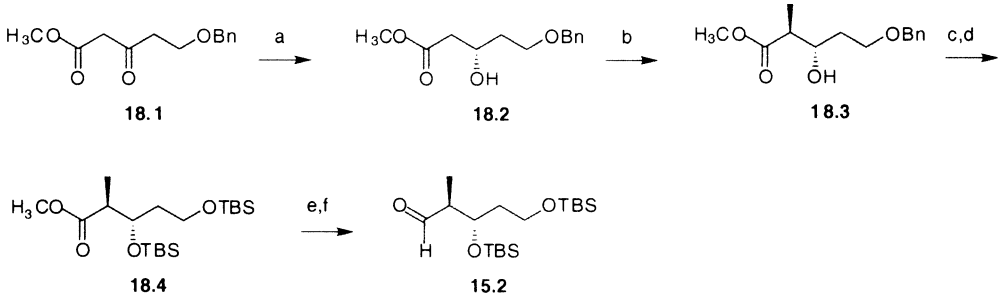
5.7. Noyori hydrogenation—Fráter alkylation

Georg and co-workers[40] have made clever use of the Noyori asymmetric hydrogenation in order to control the absolute stereochemistry at C5 of unit A (Scheme 18).[53] Methyl 5-benzyloxy-3-oxopentanoate **18.1** was hydrogenated at 50 psi and 50°C for 5 h in methanol in the presence of (*S*)-BINAP/$RuBr_2$ to give the (*R*) alcohol **18.2** in 97% yield and 97% ee, as determined by chiral HPLC analysis. Exposure of **18.2** to LDA in THF, followed by a solution of iodomethane in HMPA, the conditions for the Fráter alkylation, led to *anti*-product in 74% yield.[54] The diastereomeric excess of the product was determined to be 92% by integration of the signals for the C3 methine proton in the ^1H NMR spectrum of the reaction mixture. Hydrogenolytic cleavage of the benzyl ether protecting group, followed by protection of the primary alcohol as the TBS derivative, led to **18.4** in 93% yield for the two steps. The reduction of the ester group in **18.4** with DIBAL-H in THF led to the anticipated primary alcohol in 90% yield. This was followed by oxidation with TPAP/NMO[55] to produce aldehyde **15.2** in 88% yield. This is the same intermediate that White uses, and from this point on, Georg's synthesis parallels White's.

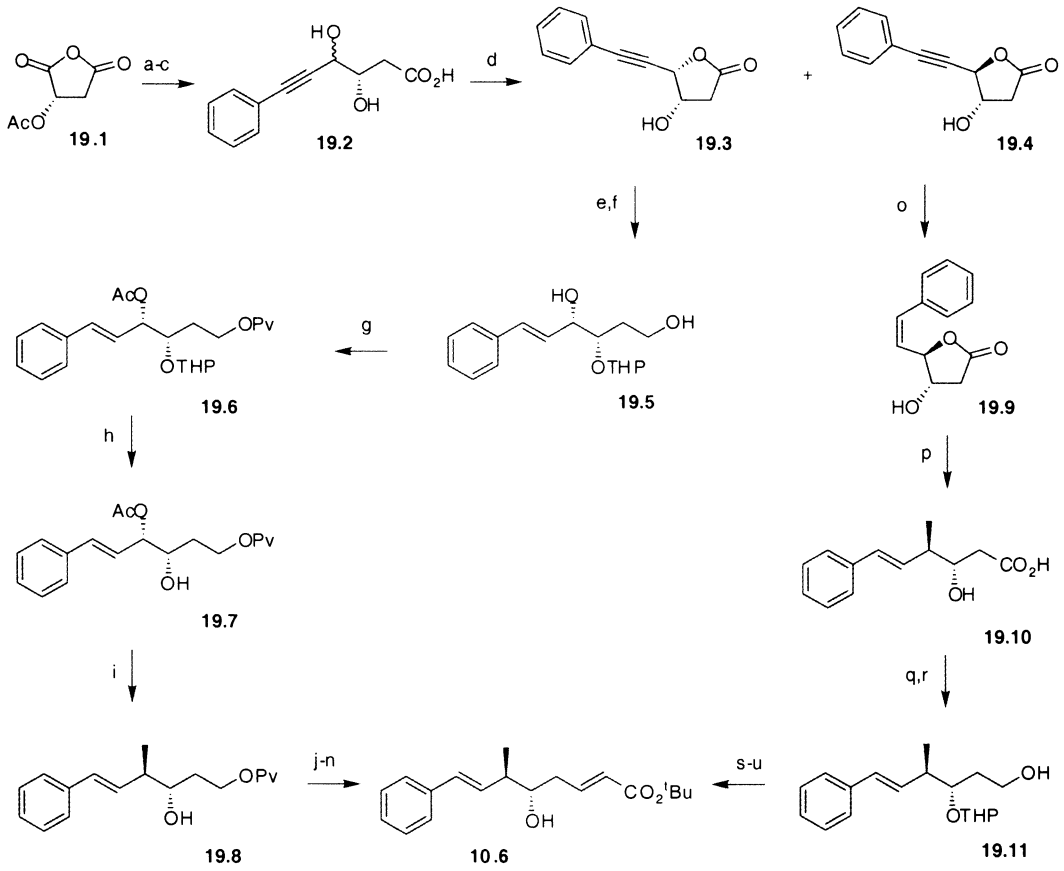
Since the development of catalytic methods for performing diastereo- and enantioselective aldol reactions, as well as ones that make use of chiral auxiliaries, the Fráter alkylation has not seen much use. Georg's synthesis provides a nice illustration of this method's enduring utility in synthesis.

5.8. (*S*)-(−)-2-Acetoxysuccinic anhydride as a starting material for unit A

A very unconventional unit A synthesis has been described by Lavalée and co-workers at BioChem Therapeutics, Inc. (Scheme 19).[56] The sole source of asymmetry for unit A was (*S*)-2-acetoxysuccinic anhydride **19.1**. Regioselective ring opening at C1 of **19.1** with lithium phenylacetylide was followed by non-stereoselective reduction of the intermediate ynone by sodium borohydride. Base-mediated hydrolysis of the acetate led to a 1/1 mixture of diastereomeric diols **19.2** in 73% yield. Exposure of this mixture to tosic acid in benzene at 50°C led to diastereomeric lactones **19.3** (43% yield) and **19.4** (45% yield). The overall yield of



Scheme 18. (a) H_2, (*S*)-BINAP-$RuBr_2$, 97% (97% ee); (b) LDA, HMPA, MeI, THF, 74% (92% de); (c) H_2, Pd/C, THF; (d) TBSCl, DMF, imidazole, 93% (two steps); (e) DIBAL-H, THF, −78 to −10°C, 90%; (f) TPAP, NMO, CH_2Cl_2/MeCN (10/1), 4 Å sieves, 88%.



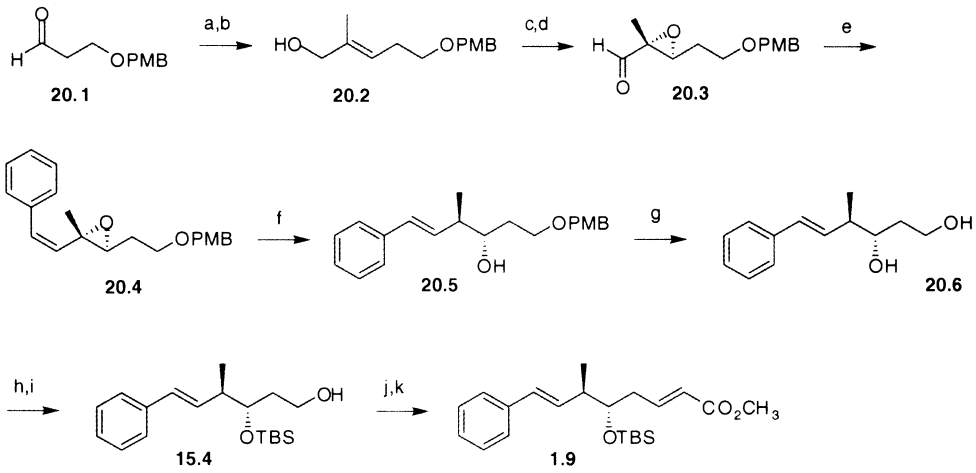
Scheme 19. (a) Li–phenylacetylide, THF, −78°C; (b) $NaBH_4$, EtOH, −78°C; (c) NaOH, 0°C, 73% (three steps); (d) TsOH, PhH, 50°C, 63% overall from phenylacetylene; 43% for **19.3**, 45% for **19.4**; (e) DHP, TsOH, THF, rt, 92%; (f) LAH, Et_2O, rt, 100%; (g) PivCl, pyr, DMAP, 0°C to rt; Ac_2O, 59%; (h) HOAc, H_2O, rt, 3 h, 100%; (i) Me_2CuLi, Et_2O, 0°C, 34%; (j) DHP, TsOH, THF, rt, 98%; (k) LAH, THF, 0°C, 97%; (l) DMSO, TEA, $(COCl)_2$, CH_2Cl_2, −78 to 0°C; (m) $Ph_3P{=}CHCO_2tBu$, CH_2Cl_2, 73% (two steps); (n) HOAc, H_2O, 40°C, 80%; (o) H_2, Lindlar, quinoline, EtOAc, MeOH, 0°C, 85%; (p) MeLi, CuBr·Me_2S, Et_2O, −35°C; (q) DHP, TsOH, THF, rt; (r) LAH, 0°C; (s) DMSO, TEA, $(COCl)_2$, −78 to 0°C; (t) $Ph_3P{=}CHCO_2tBu$, CH_2Cl_2; (u) HOAc, H_2O, 40°C, 23% overall from **19.9**.

lactones from phenylacetylene was 63%. It is somewhat unusual that the homochiral building block was used in excess, whereas phenylacetylene was the limiting species.

Each of the diastereomeric lactones **19.3** and **19.4** was independently converted to the same unit A fragment. The route that proceeds from **19.3** will be discussed first. Protection of the free hydroxyl group in **19.3** as the tetrahydropyranyloxy ether (92% yield) was followed by quantitative reduction of the butyrolactone with lithium aluminum hydride to produce diol **19.5**. Sequential pivaloylation of the primary hydroxyl group, followed by acetylation of the secondary hydroxyl in situ, led to fully protected triol **19.6** in 59% yield. Selective hydrolysis of the THP protecting group with aqueous acetic acid took place in quantitative yield to give **19.7**. Exposure of this allylic acetate to lithium dimethyl cuprate in ether at 0°C led to **19.8** in 34% yield. Unfortunately, the key cuprate step was not regioselective, and led to a 1/1 mixture of S_N2 (desired) and S_N2' (undesired) products. The cuprate reaction of the fully protected intermediate **19.6** failed to provide a pathway to **19.8**, leading instead to a mixture of conjugated dienes from a reduction/elimination process. This made it necessary to deprotect the hydroxyl group at C5 (unit A numbering) only to reprotect it following the cuprate step (vide infra). This all contributes to the length of the synthesis.

Conversion of **19.8** to unit A compound **10.6**, the same one that Sih subsequently reported in 1996,[24] took place in five steps. The C5 alcohol was protected once again as the THP derivative (98% yield), and the pivaloate was cleaved reductively with lithium aluminum hydride (97% yield). Swern oxidation of the primary alcohol to the aldehyde, followed by exposure to (*tert*-butoxycarbonylmethylene)triphenylphosphorane[44] led to the tetrahydropyranyloxy *tert*-butyl ester in 73% yield for the two steps. Hydrolytic cleavage of the THP protecting group with aqueous acetic acid led to unit A compound **10.6** in 80% yield.

A slightly shorter synthesis of **10.6** is possible from **19.4**. Semihydrogenation of the alkyne group over Lindlar's catalyst led to *Z* styryl butyrolactone **19.9** in 85% yield. Methyl cuprate addition to **19.9** took place with retention of stereochemistry at the reacting carbon atom, but complete conversion to the *E* geometry of the styrene was observed in product **19.10**. Protection of the C5 hydroxyl group as the THP ether, followed by reduction of the carboxylic acid to the primary alcohol with lithium aluminum hydride led to **19.11**. This material was elaborated to **10.6** through the same three steps (Swern oxidation; Wittig homologation; hydrolysis) as has been described for the synthesis that proceeds through intermediate **19.8**. The overall yield of **10.6** from **19.9** was 23%.



Scheme 20. (a) $Ph_3P{=}CMeCO_2Et$, THF, 0°C, >74%; (b) DIBAL-H, Et_2O, 0°C, 97%; (c) $Ti(OiPr)_4$, (+)-DET, *t*-BuOOH, CH_2Cl_2, −25°C, 4 Å sieves, 95%; (d) DMSO, TEA, $(COCl)_2$, CH_2Cl_2, −78°C, 1.5 h; Et_3N, rt, 1.5 h, 80%; (e) $PhCH{=}PPh_3$, −100°C, THF, 10 h, rt, 67%; (f) $Pd_2dba_3{\cdot}CHCl_3$, nBu_3P, HCO_2H, TEA, 1,4-dioxane, rt, 10 h, 91%; (g) $AlCl_3$, CH_2Cl_2, rt, 30 min, 75%; (h) TBSCl, DMAP, imidazole, DMF, rt, 8 h, 94%; (i) HOAc, H_2O, THF (1/1/2), 72 h, rt, 85%; (j) DMSO, TEA, $(COCl)_2$, CH_2Cl_2, −78°C, 1 h; Et_3N, rt, 1 h; (k) $(MeO)_2POCH_2CO_2Me$, NaH, THF, rt, 4 h, 72% (two steps).

Some total syntheses are noteworthy because of their brevity and/or their efficiency, whereas others are distinguished by a non-obvious strategy, or by their use of unusual reagents or reaction conditions. The Lavalée synthesis falls in the second category. The use of non-racemic acetoxysuccinic anhydride for unit A synthesis is unique to this research group. One suspects that there are other syntheses of unit A waiting to be discovered which make use of this chiral pool starting material. Finally, both pathways to unit A which have been summarized in Scheme 19 could probably have been made a bit shorter by forgoing the protection–deprotection of the C5 hydroxyl (Scheme 19, protection steps (j) and (q), and deprotection steps (n) and (u)). To wit, selective oxidation of the primary hydroxyl group in diol **14.5** (Scheme 14) by the hypochlorite/TEMPO reagent proceeds in good yield, as does the subsequent Horner–Emmons reaction that leads to **14.7**. These results suggest that protection at C5 in **19.8** and **19.10** may not have been necessary.

5.9. Vinyl epoxide reduction

Like the original Hawaii work, the unit A synthesis of Furuyama and Shimizu makes use of the Sharpless asymmetric epoxidation for control of the absolute stereochemistry.[57] The *para*-methoxybenzyl derivative **20.1** of 3-hydroxypropanal (Scheme 20) was treated first with [1-(ethoxycarbonyl)ethyl]triphenylphosphorane in THF at 0°C (>74% yield), followed by reduction of the ester function with DIBAL-H in ether to give primary alcohol **20.2** (97% yield). This material was converted to the non-racemic epoxide under the Sharpless conditions, using (+)-diethyl tartrate as the chiral inducer (95% yield). Swern oxidation to epoxyaldehyde **20.3** took place in 80% yield. Exposure of **20.3** to benzylidenetriphenylphosphorane at −100°C, followed by warming to room temperature led to *Z* styryl compound **20.4** in 67% yield. The key step in the sequence is the palladium mediated reduction–isomerization step that converts **20.4** into **20.5**, and which sets the relative and absolute stereochemistry at C5 and C6 (unit A numbering). The conversion to **20.6** took place in 91% yield upon exposure to catalytic Pd(0), tri-*n*-butylphosphine and triethylammonium formate. The stereochemistry requires some comment. The process is initiated by the (conventional) epoxide ring opening with formation of a π-allyl palladium species. Interconversion between this π-allyl intermediate and a σ-bonded palladium species is reversible, allowing for rotation about C7–C8 to take place. Relief of non-bonded interactions between the phenyl and methyl groups favors one of the two rotamers of the σ-bonded intermediate. Conversion to another π-allyl species, followed by the reductive elimination of palladium, completes the catalytic cycle (Fig. 3). This is very clever.

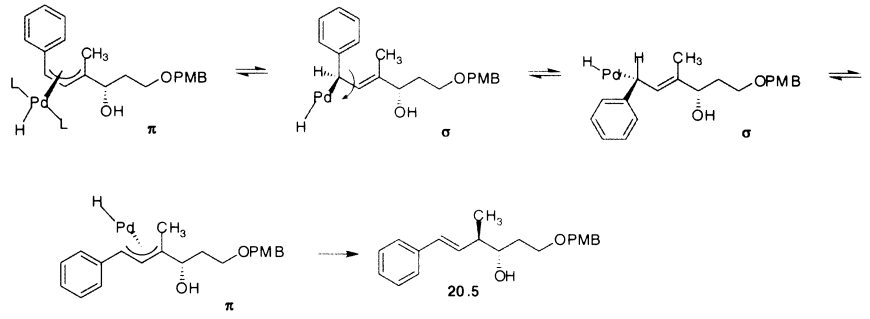
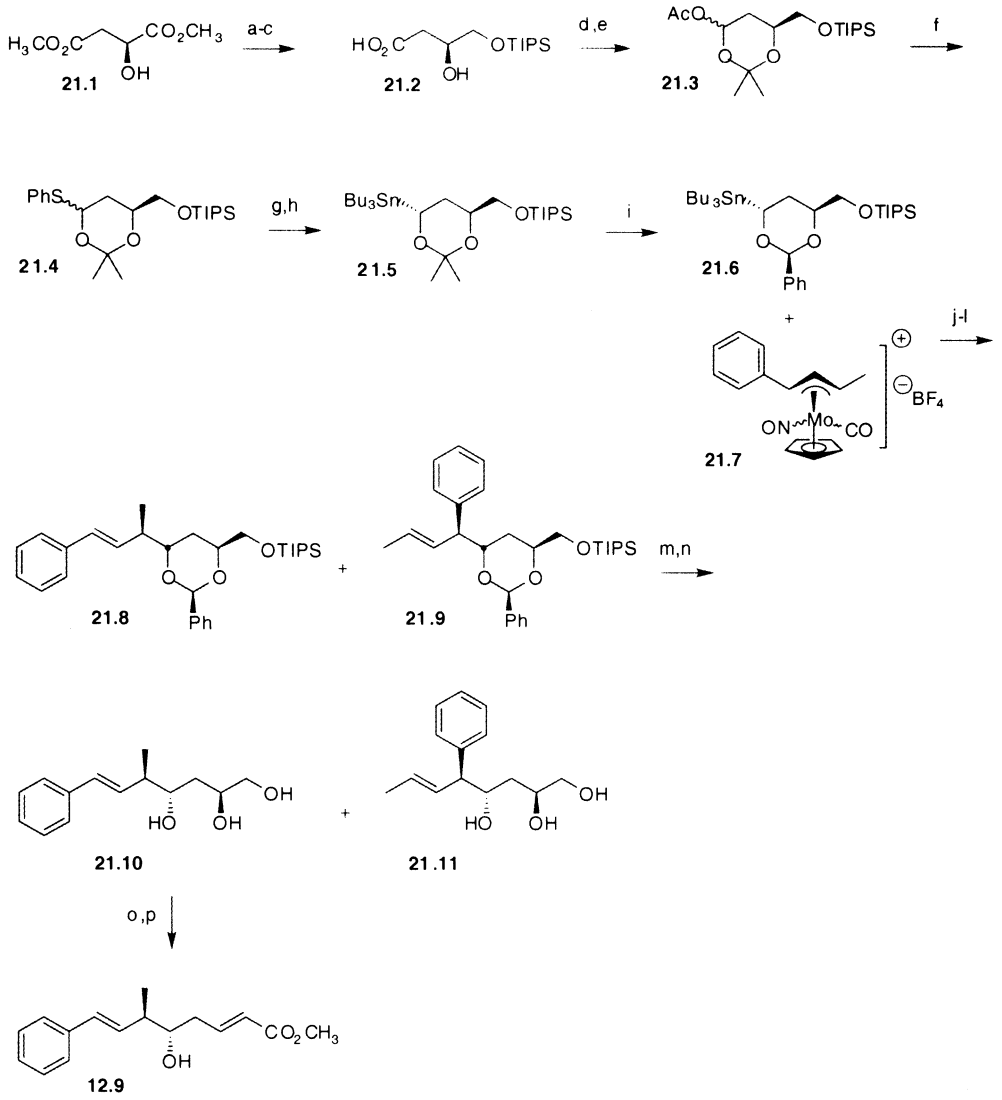


Figure 3. The proposed mechanism for the conversion of **20.4** to **20.5**.



Scheme 21. (a) $BH_3{\cdot}Me_2S$, THF, rt; $NaBH_4$, THF, rt, 89%; (b) TIPSCl, imidazole, DMF, −30°C, 71%; (c) K_2CO_3, MeOH, heat, 70%; (d) $H_2C{=}CMeOMe$, PPTS, CH_2Cl_2, 84%; (e) DIBAL-H, CH_2Cl_2, −78°C; pyr, DMAP, Ac_2O, 84%; (f) PhSH, $ZnCl_2$, CH_2Cl_2, −30°C, 87%; (g) LDBB, Li, THF, −78°C; (h) Bu_3SnCl, THF, −78°C, 71% from **21.4**; (i) $PhCH(OMe)_2$, TsOH, CH_2Cl_2, rt, 89%; (j) *n*BuLi, THF, −78°C; (k) $CuBr{\cdot}Me_2S$, −78°C; add **21.7**; (l) O_2, $CHCl_3$, rt, 71% from **21.6**; **21.8**/**21.9**=1.2/1; (m) TsOH, MeOH, rt, 63%; (n) chromatographic separation; (o) $NaIO_4$, MeOH, H_2O (3/1), rt; (p) $(MeO)_2POCH_2CO_2Me$, TMG, THF, −78°C to rt, 83% (two steps), (*E*/*Z*≥25/1).

From **20.6**, the synthesis follows familiar ground. Protection of both hydroxyl groups as the TBS ethers (94% yield), followed by selective hydrolysis of the primary TBS group with aqueous acetic acid (85% yield) led to **15.4**, the same intermediate that White reported. Swern oxidation of **15.4** to the aldehyde, followed by Horner–Emmons homologation, led to **1.9**, the unit A compound of the first Hawaii synthesis, in 72% yield for the two steps from **15.4**. For the reason discussed in the preceding section, it was probably not necessary to protect the C5 alcohol.

5.10. Planar chiral molybdenum cationic complex

One of the more unusual syntheses of unit A is due to Kocienski and co-workers.[58] It makes use of methyl (*S*)-malate as the starting material, however, the stereogenic center in the malate is used as a control element for a diastereoselective alkylation step of a planar chiral cationic molybdenum complex, and does not appear in unit A. The synthesis is outlined in Scheme 21.

Selective reduction of dimethyl malate **21.1** according to Moriwake's procedure was followed by protection of the primary hydroxyl group as the TIPS derivative and saponification of the C4 carboxylate.[59] This led to β-hydroxyacid **21.2** in 53% overall yield from **21.1**. This compound was converted to a dioxanone (84% yield) that was reduced with DIBAL-H and immediately acetylated according to Rychnovsky's conditions (84% yield) to produce a diastereomeric mixture of *O*-acetyl acetals **21.3**.[60] Transacetalization with benzenethiol was catalyzed by zinc chloride to give a 9/1 mixture of diastereomeric thioacetals **21.4** in 87% yield. Reductive lithiation with 4,4′-di-*tert*-butylbiphenyl (LDBB) and lithium led to an intermediate α-alkoxylithium species which was intercepted with tri-*n*-butyltin chloride to produce axial tributylstannane **21.6** in 71% yield, epimerization having taken place at the stage of

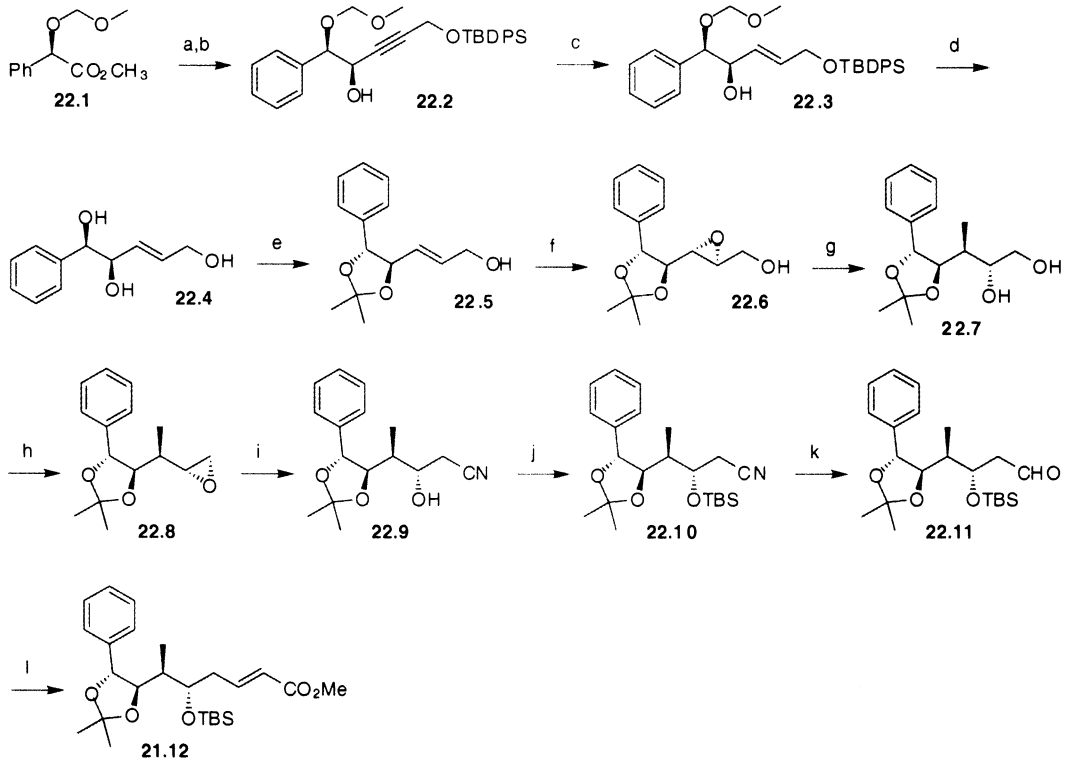
the lithium species.[61] Transacetalization of the acetonide with benzaldehyde dimethyl acetal in the presence of catalytic tosic acid led to benzylidene acetal **21.6** as a chromatographically separable 7/1 mixture of diastereomers at the acetal carbon atom in 89% yield.

The key steps in the synthesis of unit A by Kocienski and co-workers took place through a series of transmetalations of **21.6**. Exposure to *n*-butyllithium at −78°C led to the lithio species, which was treated with cuprous bromide to produce the axial copper species. To this was added cationic molybdenum complex **21.7**. The axially chiral molybdenum complex was prepared as a diastereomeric mixture from (*S*)-4-phenyl-(*E*)-3-buten-2-ol.[62,63] Oxidative decomplexation of the molybdenum led to a 1.2/1 mixture of products **21.8** and **21.9** in 71% yield. The organocopper species that was derived from **21.6** evidently did not discriminate between the methyl- and phenyl-substituted termini of complex **21.7**.[64] Unfortunately, **21.8** and **21.9** were not separable by chromatography, so they were carried through to the next step as a mixture. Exposure of this mixture to tosic acid in methanol at room temperature led to a mixture of triol **21.10** and **21.11** in 63% yield. These were separated, and **21.10** was treated sequentially with sodium periodate to generate the C3 (unit A numbering) aldehyde. The aldehyde was elaborated to unit A compound **12.9** in 83% yield for the two steps from **21.10**. Note that the stereogenic center that was derived from dimethyl malate is lost during the oxidative cleavage step.

One of the uses of natural products total synthesis is to define the limits and to test the scope of applicability of new methodology. The unit A synthesis by Kocienski and co-workers should be judged in this light. The lack of regioselectivity in the key step diminishes the preparative utility of this route. However, had preparative utility been the overriding concern, the desired C5–C6 stereochemistry could have been secured in one step, through stereospecific methylation of the commercially available enantiomer of **21.1** according to Seebach's published procedure.[65,66]

5.11. Diastereoselective addition to (*R*)-mandelaldehyde

A synthesis of unit A that controls the stereochemistry at C7 and C8 is due to Larchevêque and co-workers.[67] The starting material was the methoxymethyl ether **22.1** of (*R*)-methyl mandelate (Scheme 22). Reduction of the ester function with DIBAL-H gave the protected mandelaldehyde which underwent chelation-controlled addition of the bromomagnesium derivative of protected propargyl alcohol to give **22.2** in 87% overall yield for the two steps from **22.1**. Careful reduction of the propargyl alcohol with RedAl® in ether at −25°C led to *E* allylic alcohol **22.3** in 72% yield. The first strategy for introduction of the C6 methyl group failed. Epoxidation of **22.3** under the Sharpless conditions failed to take place, whereas vanadyl acetoacetate-catalyzed epoxidation with *tert*-butyl hydroperoxide led to the *anti*-epoxide diastereomer with very low diastereomeric excess. Moreover, epoxide ring-opening by lithium dimethylcuprate was difficult, consequently the synthetic strategy was modified.



Scheme 22. (a) DIBAL-H, Et_2O/pentane (1/1), −78°C; (b) $BrMgCCCH_2OTBDPS$, Et_2O, 87% (two steps); (c) Red-Al, Et_2O, −25°C, 72%; (d) 2 M HCl, MeOH, 40°C, 2 d, 92%; (e) $CuSO_4$ (anh), TsOH, acetone, rt, 2 d, 91%; (f) $Ti(OiPr)_4$, (+)-DIPT, −20°C, 4 Å sieves, CH_2Cl_2, *t*BuOOH, 4 d, 76% (95/5); (g) hexane/CH_2Cl_2, (5/1), Me_3Al, −10°C, 1 h; rt, 20 h, 44+18% **22.6**; (h) TMSCl, $MeC(OMe)_3$, CH_2Cl_2, 0°C, 1H, MeOH, K_2CO_3, 2 h, 65%; (i) MeCN (anh), $LiClO_4$, KCN, 70°C, 6 h, 70%; (j) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0°C, 92%; (k) PhMe, DIBAL-H, −78°C, 58%; (l) $(MeO)_2POCH_2CO_2Me$, TMG, THF, −78°C to rt, 78%.

Global deprotection of **22.3** by heating to 40°C in methanol with 2 M hydrochloric acid for two days led to triol **22.4** in 92% yield. The *syn* diol portion of the molecule was protected as acetonide **22.5** (91% yield). Sharpless asymmetric epoxidation, using (+)-diisopropyl tartrate as the chiral inducer, led to a 95/5 mixture of diastereomeric epoxy alcohols from which **22.6** was isolated in 76% yield. Introduction of the C6 methyl group was accomplished by exposing **22.6** to trimethylaluminum over a prolonged period. After 20 h at room temperature, **22.7** was isolated in 44% yield, along with 18% of the starting material **22.6**. The low reactivity of **22.6** is most likely due to competition for the Lewis acidic reagent by the ketal oxygen atoms. Although Larchevêque and co-workers do not provide an explanation for the low material balance for the epoxide ring-opening reaction, it seems likely that in the presence of trimethylaluminum some cyclization of **22.7** to a tetrahydrofuran byproduct takes place. This fits the reactivity pattern for the benzylic carbon atom of unit A that has been described by others (see **1.10**, **8.10** and **11.9**).

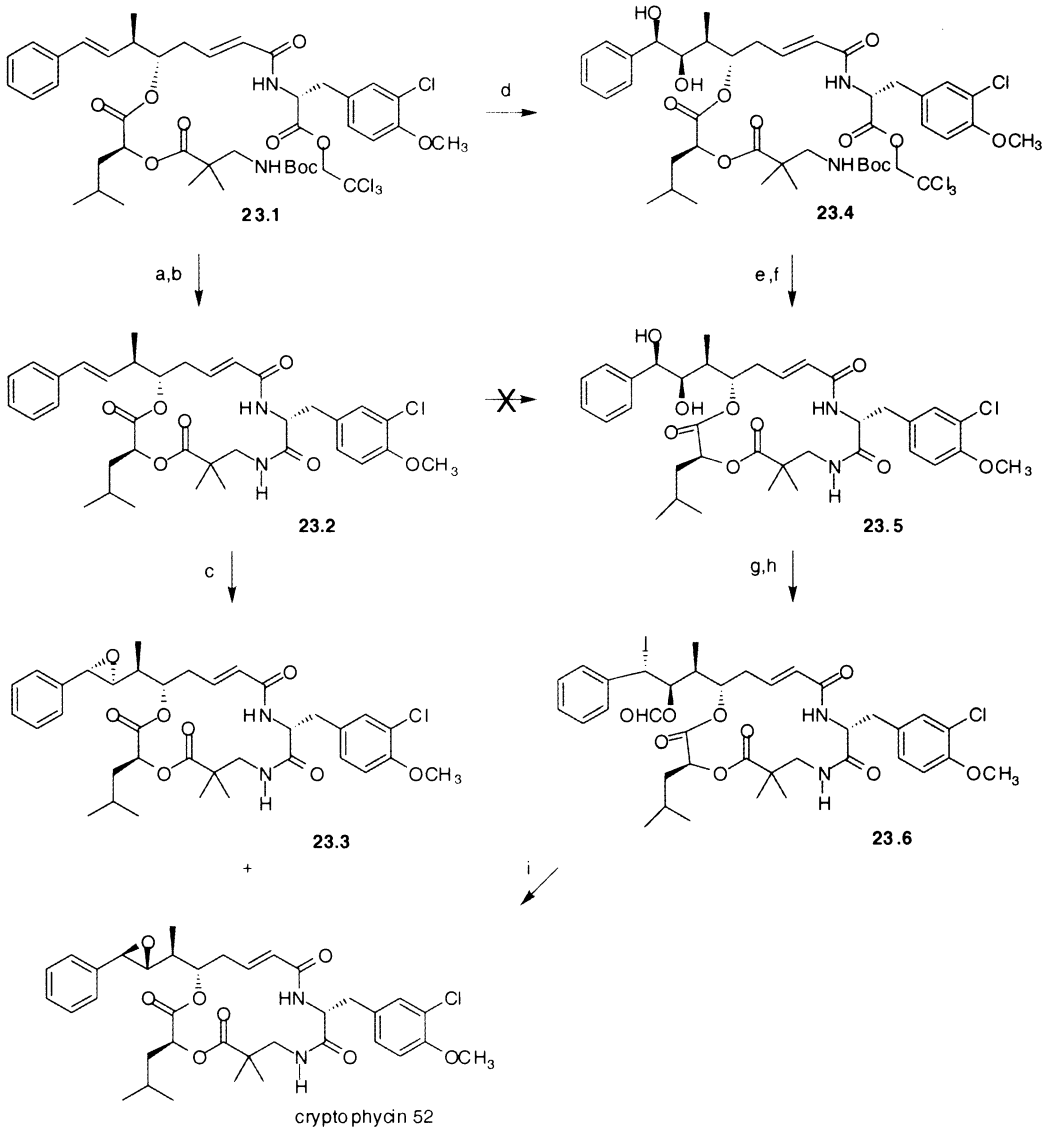
Diol **22.7** was converted to epoxide **22.8** through Sharpless' conditions in 65% yield.[19] One-carbon homologation of **22.8** with potassium cyanide in the presence of lithium perchlorate led to nitrile **22.9** (70% yield). Protection of the free C5 hydroxyl group as the TBS ether (92% yield), followed by reduction with DIBAL-H in toluene (58% yield) led to aldehyde **22.11**. Immediate reaction with trimethylphosphonoacetate with TMG in THF produced unit A compound **22.12** in 78% yield. The overall yield of the sequence of reactions leading from **22.1** to **22.12** was 3.3%, the largest loss of material having taken place at the point of introduction of the C6 methyl group. Although this synthesis has the advantage of being able to control C7–C8 stereochemistry from the outset, the low overall yield renders it an unattractive choice for preparative scale work. An alternative approach to the control of C7–C8 stereochemistry is given in Section 6.

6. Dihydroxylation of *seco*-cryptophycin 52

A solution to the stereochemical problem posed by the epoxide in unit A was solved in the context of the total synthesis of cryptophycin 52, a hydrolytically stable analog of the natural product (Scheme 23).[25] The only difference between cryptophycins 1 and 52 is in unit C. In cryptophycin 52, unit C bears an additional methyl group on the α carbon atom. The small increase in steric hindrance engendered by the quaternary carbon atom of the modified unit C diminishes the rate of hydrolytic cleavage of the unit C–unit D ester link. Fray[18] has shown that brief exposure of *seco*-cryptophycin 52 **23.1** (Scheme 23) to neat trifluoroacetic acid at room temperature selectively removes the Boc protecting group. Macrocyclization is mediated by 2-hydroxypyridine, yielding **23.2** in 80% yield for the two steps from **23.1**. Epoxidation of **23.2** with *m*-CPBA gave at best a 2/1 mixture of cryptophycin 52 along with the α-epoxy diastereomer **23.3**. Following separation of the two diastereoisomers by HPLC, cryptophycin 52 could be isolated in 51% overall yield from **23.1**. The need for a separation by HPLC in the last step, and the loss of material from the epoxidation are major shortcomings. An obvious solution to the problem is to oxidize **23.2** to the *syn*-β-diol, and then to convert diol to epoxide. Unfortunately, all efforts to convert styrene **23.2** to diol **23.5** failed. Osmium tetroxide, catalytic or stoichiometric, gave roughly equimolar mixtures of the four diastereomers resulting from indiscriminate attack at each of the two carbon–carbon double bonds in **23.2**. Ruthenium-catalyzed dihydroxylation also failed, and led to destruction of **23.2** with the formation of aldehydes. The results from the Sharpless AD which were obtained with the dihydroquinidine based phthalazine ligand $(DHQD)_2PHAL$ were disappointing because of the insolubility of **23.2** in the reaction solvent, aqueous *tert*-butanol.[68]

The sum of these negative results suggested that the styryl carbon–carbon double bond in *cyclic* compound **23.2** would be very difficult to functionalize, and that it was unlikely that this could be done either regio- or stereoselectively. The obvious way to circumvent this problem would be to perform the oxidation on a *ring-open* compound, such as **23.1**. Scheme 23 summarizes the successful route that is based on this premise. The Sharpless AD of **23.1** with $(DHQD)_2PHAL$ in the presence of catalytic $K_2OsO_2(OH)_2$ led to the desired *syn* diol **23.4** in 61% yield. The conditions for the AD had to be optimized carefully, and differ a little from the conventional conditions. Even with **23.1**, the reaction is slow and 1–2 mol% osmium must be used, rather than the 0.2 mol% osmium that suffices for many alkenes. One must use a correspondingly larger amount of the chiral ligand, at least 2 mol%. Decreasing the concentration of osmium and/or the ligand results in slower kinetics and an inferior yield of **23.4**, because of exposure of starting material and product to the basic reaction conditions over a prolonged period of time. Buffering the reaction mixture, adding the base slowly, or adding more than 1 equiv. of methanesulfonamide as an accelerant failed to improve the yield.

The conversion of *seco* diol **23.4** to cryptophycin 52 was accomplished in excellent yield, once the optimal reaction conditions were defined. Acidolysis of the Boc protecting group with trifluoroacetic acid in dichloromethane took place during 1.5 h at 0°C. It is noteworthy that no side reactions involving a benzylic carbocation took place during the treatment with acid. Macrolactamization was accomplished with 2-hydroxypyridine to give **23.5** in 82% yield for the two steps from **23.4**. Diol to epoxide conversion took place through a modified Sharpless procedure.[69] Trimethyl orthoformate in the presence of PPTS converted the diol to the cyclic orthoformate, which upon exposure to iodotrimethylsilane at 0°C and reductive workup furnished iodoformate **23.6** in 93% overall yield from diol **23.5**. Potassium carbonate in THF/methanol at 0°C gave cryptophycin 52 in 98% yield. The diol to epoxide conversion was also accomplished through the use of acetyl bromide in place of iodotrimethylsilane. This led to a bromohydrin formate, which was converted in high yield to cryptophycin 52. Trimethyl orthoformate was used in all successful cases, rather than trimethyl orthoacetate: if one uses orthoacetate, and performs the next step with chlorotrimethylsilane, the product is a chlorohydrin *acetate*, hydrolytic cleavage of which with base requires more vigorous conditions than does cleavage of the formate. Inevitably, some base



Scheme 23. (a) TFA (neat), rt, 15 min, 0.5 M NaOH; (b) 2-hydroxypyridine (2.0 equiv.), PhMe, 0.02 M, 20°C, 80% from **23.1**; (c) *m*-CPBA, CH_2Cl_2, **23.3**/cryptophycin 52=1/2, 51% of cryptophycin 52 overall from **23.1**; (d) $K_2OsO_2(OH)_2$, $(DHQD)_2PHAL$, K_2CO_3, $K_3Fe(CN)_6$, $MeSO_2NH_2$, *t*BuOH/H_2O (1/1), rt, 20.5 h, 61%; (e) TFA, CH_2Cl_2, 0°C, 1.5 h; aqueous K_2CO_3; (f) 2-hydroxypyridine (2.0 equiv.), MeCN/PhMe (1/1), 40°C, 21 h, 82% (two steps); (g) PPTS, CH_2Cl_2, $(MeO)_3CH$, rt; aqueous $NaHCO_3$; (h) CH_2Cl_2, 0°C, TMSI, 45 min; 10% aqueous $Na_2S_2O_3$, 93% (two steps) (i) THF/MeOH (1/1), K_2CO_3, 0°C, 6 h, 98%.

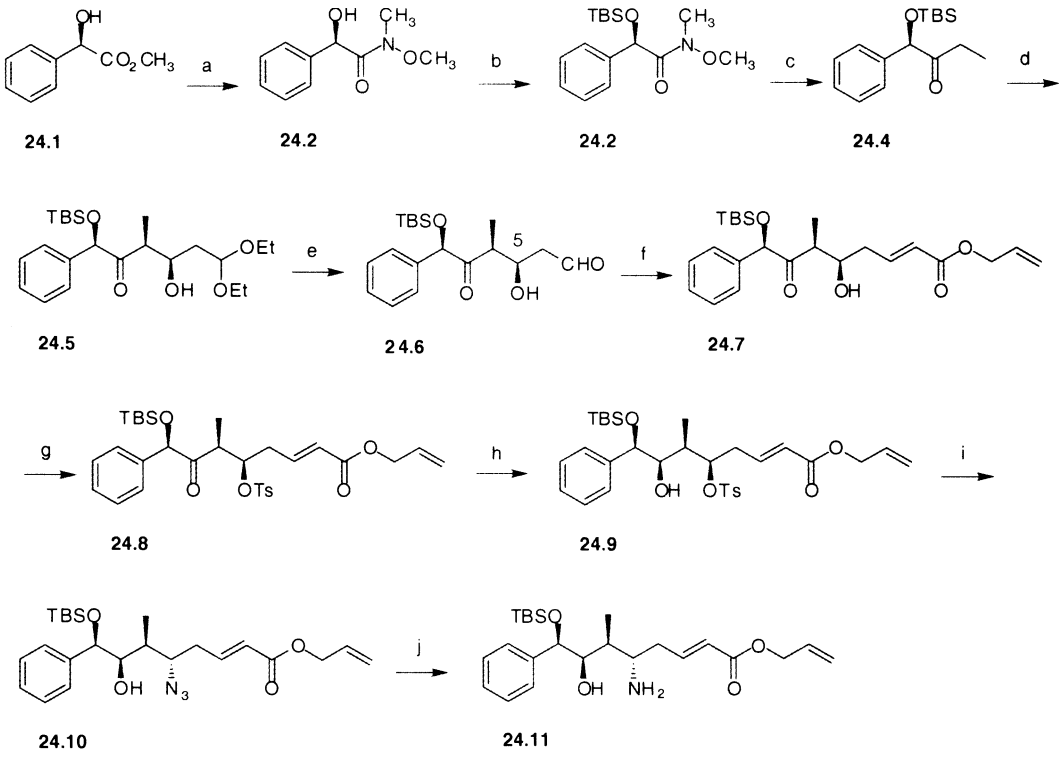
catalyzed ring-opening of the macrocycle also takes place under these conditions, hence the preference for the orthoformate.

The protocols that are summarized in Scheme 23 underscore an unanticipated feature of cryptophycin reactivity. The styrene function is held in close proximity to unit D in the cyclic compounds, and is not easily accessible to electrophilic species. Molecular modeling confirms this. Section 7 serves to illustrate a consequence of the conformational rigidity of the cyclic cryptophycins in a different context.

7. 1-Aza-cryptophycin 1, an unstable cryptophycin

The importance of the cryptophycins as potential cancer chemotherapeutic agents and the propensity for in vivo deactivation through hydrolytic cleavage of the ester bonds prompted the design and synthesis of analogs that would retain the high activity of the natural product while being resistant to this decomposition pathway. Cryptophycin 52 accomplished this goal by introducing steric hindrance into unit C, thereby stabilizing the unit C–unit D link. An alternative strategy for stabilizing the cryptophycins against hydrolytic cleavage is by substituting amide links for ester links. In this section, the synthesis by the Hawaii group and aspects of the reactivity of 1-*aza*-cryptophycin 1 is be discussed.[70]

Scheme 24 summarizes the synthesis of **24.11**, the *aza* analog of unit A. This synthesis controls the epoxide stereochemistry from the outset, by using (*R*)-methyl mandelate **24.1** as the starting material and as the sole source of asymmetry for unit A. Conversion of methyl ester **24.1** to Weinreb amide **24.2** was mediated by trimethylaluminum



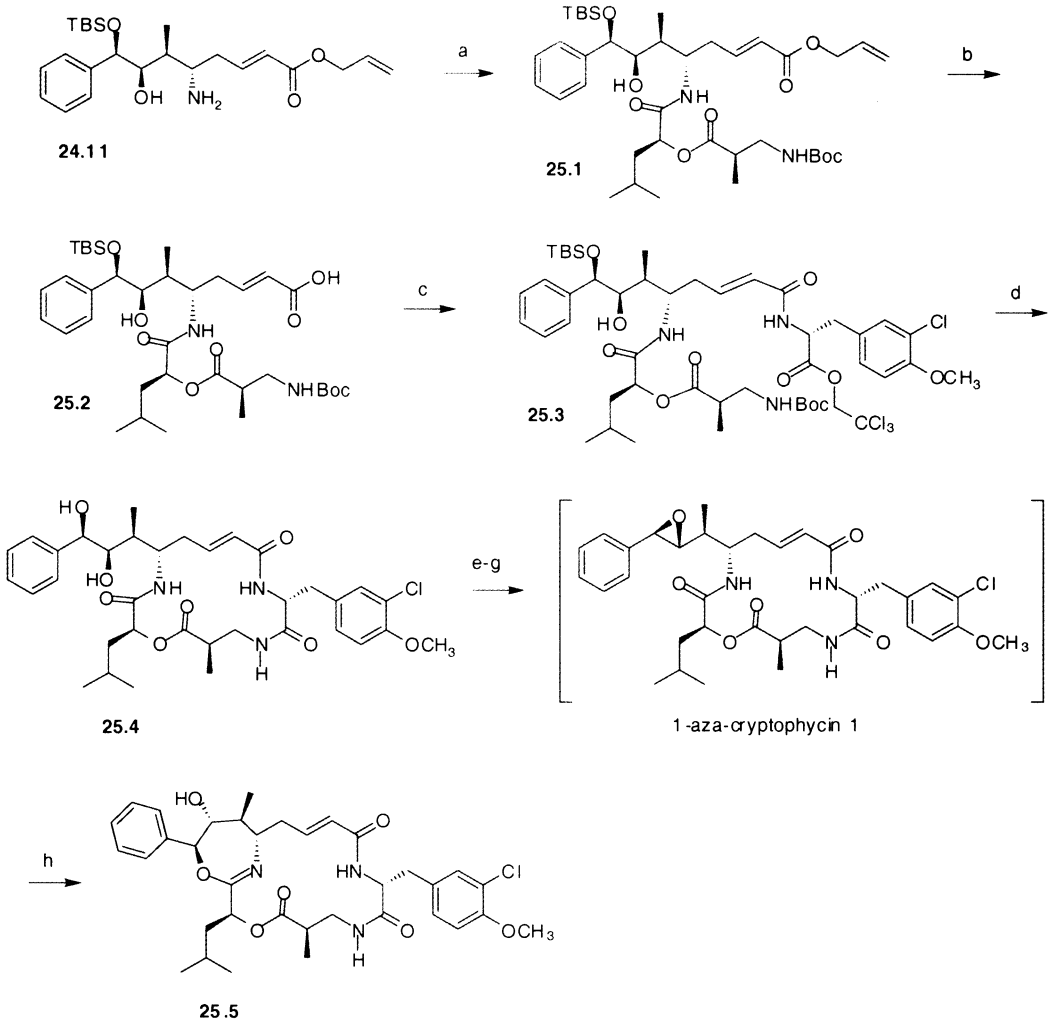
Scheme 24. (a) HNMe(OMe)·HCl, Me_3Al, PhH, 0–5°C, 2 h; add **24.1**, rt, 12 h, 93%; (b) TBSOTf, Et_3N, CH_2Cl_2, 0°C, 3 h, 93%; (c) EtMgBr, THF, 5 h, 94%; (d) Bu_2BOTf, DIPEA, CH_2Cl_2, −78°C, 15 min; 0°C, 2 h; add 3,3-diethoxypropanal, −78°C, 1 h, 0°C, 2 h, MeOH, H_2O_2, 0°C, 2 h, 65%; (e) Cl_3CCO_2H, CH_2Cl_2/H_2O (20/1), rt, 1 h, 87%; (f) $(EtO)_2POCH_2CO_2CH_2CH{=}CH_2$, LiCl, DIPEA, MeCN, rt, ca. 4 h, 63%; (g) TsCl, KOH (s), THF, 0°C to rt, 86%; (h) $NaBH_4$, $CeCl_3·7H_2O$, EtOH, 0°C, 96%; (i) tetramethylguanidinium azide, $MeNO_2$, 70–80°C, 5 h, 69%; (j) PPh_3, THF, H_2O, 50–60°C, 12 h.

(93% yield). Protection of the hydroxyl group in **24.2** as the TBS ether (93% yield), followed by exposure of the product to ethylmagnesium bromide led to ethyl ketone **24.4** (94% yield). Condensation of the di-*n*-butylboryl enolate[71,72] derived from **24.4** with 3,3-diethoxypropanal,[73,74] followed by oxidative workup, led exclusively to *syn* aldol **24.5** in 65% yield following purification by flash column chromatography. Condensation of **24.4** with 3,3-diethoxypropanal through the lithium enolate, generated with LDA, led to a ca. 10/1 mixture of *syn* and *anti*-aldol products. Acid hydrolysis of the diethyl acetal function proceeded to give aldehyde **24.6** in 87% yield. Elimination of the C5 (unit A numbering) hydroxyl, which is β to both the ketone and aldehyde functions, did not take place to any appreciable extent. Horner–Emmons homologation of **24.6** under Masamune–Roush conditions gave allyl ester **24.7** in 63% yield. There are two remaining tasks that must be performed on this intermediate to transform it to unit A. First, the C5 nitrogen atom must be introduced with inversion of stereochemistry. Second, stereoselective reduction of the C7 keto group to either α or β alcohol can lead to the desired α epoxide. Introduction of the nitrogen atom was done classically: conversion of alcohol to tosylate **24.8** (86% yield) was followed by Luche reduction of the ketone with 20/1 stereoselectivity (96% yield).[75] Exposure of alcohol **24.9** to tetramethylguanidinium azide (69% yield) led to **24.10**. Reduction of azide **24.10** to primary amine **24.11** took place with triphenylphosphine in aqueous THF.

Assembly of the units of 1-*aza*-cryptophycin 1 is shown in Scheme 25. The condensation of **24.11** with unit C–unit D fragment **3.6** was mediated by FDPP to give **25.1** in 74% overall yield from **24.10**. Palladium catalyzed cleavage of the allyl ester produced carboxylic acid **25.2** in 91% yield. Carboxylate **25.2** was coupled with protected unit B **2.2** to give protected *seco* compound **25.3** in 69% yield. Exposure of this material to trifluoroacetic acid at 0°C, followed by evaporation to dryness and resuspension in toluene containing 2-hydroxypyridine, led to **25.4** in 50% overall yield. Treatment with trifluoroacetic acid cleaved both Boc and TBS protecting groups, however, reactions involving the intermediacy of a benzylic carbocation did not take place to any appreciable extent. The trichloroethyl ester group in **25.3** served two functions, as before: it masked the carboxy group in unit B during the coupling step, and it activated the same carbonyl group for the macrolactamization.

Conversion of the *syn* diol unit in **25.4** to the epoxide was accomplished through the orthoformate, which was treated with acetyl bromide to produce the anticipated formyloxy bromide.[25] Exposure of this compound to potassium bicarbonate led to an approximately equimolar mixture of two compounds, one of which was the desired epoxide, and another was identified as **25.5**. The conversion of 1-*aza*-1-cryptophycin 1 to **25.5** took place spontaneously, even upon storage at −5°C. Chromatographic separation on basic alumina, or reverse phase HPLC produced only mixtures of the two compounds. Although a pure sample of 1-*aza*-1-cryptophycin could not be obtained, its presence could be inferred from the characteristic signals at 3.0 (H7) and 3.7 (H8) ppm in the ^1H NMR spectrum of the mixture.

The rearrangement of 1-*aza*-cryptophycin 1 to **25.5** was certainly not anticipated, however, the mechanism probably



Scheme 25. (a) **3.6**, DMF, FDPP, DIPEA, rt, 5 min, add **24.11**, rt, 4 h, 74% from **24.10**; (b) $Pd(PPh_3)_4$, THF, morpholine, rt, 4 h, 91%; (c) FDPP, DMF, add **2.2**, Et_3N, rt, 5 h, 69%; (d) TFA (neat), 0°C to rt, 1 h; evaporate to dryness; 2-hydroxypyridine, PhMe, rt, 20 h, 50%; (e) PPTS, $(MeO)_3CH$, CH_2Cl_2, rt, 2 h; (f) MeCOBr, CH_2Cl_2, 6 h, rt; (g) aqueous $NaHCO_3$; DME, MeOH, EtOH, $KHCO_3$, 40°C, 15 h; (h) chromatography on silica gel, 62% from **25.4**.

follows the pathway indicated in Scheme 25. Activation of the epoxide is followed by nucleophilic ring opening by the amide carbonyl oxygen atom of unit D, leading to the seven-membered ring imino ether **25.5**. Release of ring strain of the epoxide presumably provides sufficient thermodynamic driving force. No such rearrangement takes place with cryptophycin 1. For example, treatment of cryptophycin 1 with ferric chloride leads to the C7 ketone. It therefore appears that the enforced proximity of the unit D amide carbonyl oxygen atom to the benzylic carbon atom in 1-*aza*-cryptophycin 1, as well as the greater electron density of the amide oxygen atom relative to the ester oxygen atom of cryptophycin 1, are responsible for the marked difference in reactivity of these two cryptophycins. This unanticipated reactivity precludes 1-*aza*-cryptophycin 1 from consideration as a clinical candidate.

8. Conclusion

The discovery of the cryptophycins and the recognition of their exceptional potency as anti-microtubule agents has led to the relatively large number of independent efforts directed at partial or total syntheses that are the subject of this report. The molecular complexity of the cryptophycins is modest by contemporary standards, therefore it seems likely that most of the research activity has been driven by a realization of the potential that these compounds have as a new class of chemotherapeutic agents against solid tumors. Although the intellectual challenges of devising and executing a laboratory scale synthesis of the cryptophycins are also modest, the same cannot be said for the design of a synthesis efficient enough to supply clinical trials. Such a route must be able to produce multi-kilogram quantities of material, and clearly this is at or near the upper limit of what is currently feasible in pharmaceutical development. That the art and practice of organic synthesis has reached maturity is reflected in the diversity of methods and strategies that have been brought to bear on unit A. These span the spectrum from classical aldol and Diels–Alder methods to chemoenzymatic approaches as well as methods based on carbon–carbon bond forming reactions by means of transition metal complexes. Given the clinical potential of this group of natural products, it seems likely that new and perhaps better cryptophycin syntheses will continue to be developed for some time to come.

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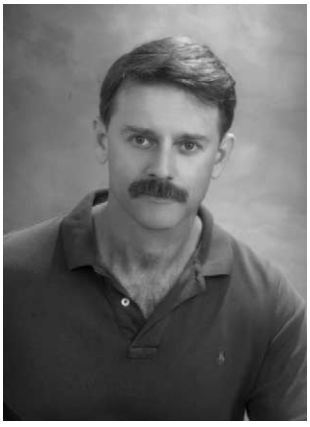
References

1. Moore, R. E.; Corbett, T. H.; Patterson, G. M. L.; Valeriote, F. A. *Curr. Pharm. Des.* **1996**, *2*, 317. A review of cryptophycin synthesis and anticancer activity has been recently published: Eggen, M.; Georg, G. *Med. Res. Revs.* **2002**, *22*, 85.
2. Tolytoxin: Carmeli, S.; Moore, R. E.; Patterson, G. M. L. *J. Nat. Prod.* **1990**, *53*, 1533.
3. Tantazoles: Carmeli, S.; Moore, R. E.; Patterson, G. M. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1990**, *112*, 8195.
4. Ambiguines: Smitka, T. A.; Bonjouklian, R.; Doolin, L.; Jones, N. D.; Deeter, J. B.; Prinsep, M. R.; Yoshida, W.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1992**, *57*, 857.
5. Trimurtulu, G.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. *J. Am. Chem. Soc.* **1994**, *116*, 4729.
6. Schwartz, R. E.; Hirsch, C. F.; Sesin, D. F.; Flor, J. E.; Chartrain, M.; Fromtling, R. E.; Harris, G. H.; Salvatore, M. J.; Liesch, J. M.; Yudin, K. *J. Ind. Microbiol.* **1990**, *5*, 113.
7. Hirsch, C. F.; Liesch, J. M.; Salvatore, M. J.; Schwartz, R. E.; Sesin, D. F. US Patent 4,946,835, issued August 7, 1990.
8. Barrow, R.; Hemscheidt, T.; Paik, S.; Liang, J.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479.
9. Boons, G.-J.; Castle, G. H.; Clase, J. A.; Grice, P.; Ley, S. V.; Pinel, C. *Synlett* **1993**, 913.
10. Zeynek, R.; Hoppe-Seyler's, Z. *Physiol. Chem.* **1926**, *144*, 247.
11. Dudash, Jr., J.; Jiang, J.; Mayer, S. C.; Joullié, M. M. *Synth. Commun.* **1993**, *23*, 349.
12. Jefferey, P. D.; McCombie, S. W. *J. Org. Chem.* **1982**, *47*, 587.
13. Gardinier, K. M.; Leahy, J. W. *J. Org. Chem.* **1997**, *62*, 7098.
14. Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 7001.
15. Evans, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1990**, *112*, 6447.
16. Hioki, H.; Okuda, M.; Miyagi, W.; Ito, S. *Tetrahedron Lett.* **1993**, *34*, 6131.
17. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn* **1979**, *52*, 1989.
18. The macrolactamization is easily carried out with cheap 2-hydroxypyridine: Fray, A. H. *Tetrahedron: Asymmetry* **1998**, *9*, 2777.
19. Kolb, H. C.; Sharpless, K. B. *Tetrahedron* **1992**, *48*, 10515.
20. Li, L.-H.; Tius, M. A. *Org. Lett*. in press.
21. Danishefsky, S. J. *Chemtracts* **1989**, 273.
22. Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F.; Maring, C. J.; Springer, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1256.
23. Tius, M. A.; Busch-Petersen, J. *Synlett* **1997**, 531.
24. Salamonczyk, G. M.; Han, K.; Guo, Z.-W.; Sih, C. J. *J. Org. Chem.* **1996**, *61*, 6893.
25. Liang, J.; Moher, E. D.; Moore, R. E.; Hoard, D. W. *J. Org. Chem.* **2000**, *65*, 3143.
26. Varie, D. L.; Brennan, J.; Briggs, B.; Cronin, J. S.; Hay, D. A.; Rieck, J. A.; Zmijewski, M. J. *Tetrahedron Lett.* **1998**, *39*, 8405.
27. Schreiber, S. L.; Liew, W.-F. *Tetrahedron Lett.* **1983**, *24*, 2363.
28. Swarts, H. J.; Verstegen-Hacksma, A. A.; Jansen, B. J. M.; deGroot, A. *Tetrahedron* **1994**, *43*, 251.
29. Anelli, P.; Montanari, F.; Quici, S. *Org. Synth.* **1990**, *69*, 212.
30. Krow, G. R. *Org. React.* **1993**, *43*, 251.
31. Nakai, T.; Mikami, K. *Org. React.* **1994**, *46*, 105.
32. Liang, J.; Hoard, D. W.; Khau, V. V.; Martinelli, M. J.; Moher, E. D.; Moore, R. E.; Tius, M. A. *J. Org. Chem.* **1999**, *64*, 1459.
33. Morgan, B.; Oelschlager, A. C.; Stokes, T. M. *J. Org. Chem.* **1992**, *57*, 3231.
34. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
35. Veysoglu, Y.; Mitscher, L. A.; Swayze, J. K. *Synthesis* **1980**, 807.
36. Brown, H. C.; Bhat, K. S.; Randad, R. S. *J. Org. Chem.* **1989**, *54*, 1570.
37. Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919.
38. Brown, H. C.; Joshi, N. N. *J. Org. Chem.* **1988**, *53*, 4059.
39. Dhokte, U. P.; Khau, V. V.; Hutchison, D. R.; Martinelli, M. J. *Tetrahedron Lett.* **1998**, *39*, 8771.
40. Eggen, M.; Mossman, C. J.; Buck, S. B.; Nair, S. K.; Bhat, L.; Ali, S. M.; Reiff, E. A.; Boge, T. C.; Georg, G. I. *J. Org. Chem.* **2000**, *65*, 7792.
41. White, J. D.; Hong, J.; Robarge, L. A. *J. Org. Chem.* **1999**, *64*, 6206.
42. White, J. D.; Hong, J.; Robarge, L. A. *Tetrahedron Lett.* **1998**, *39*, 8779.
43. Johns, A.; Murphy, J. A.; Sherburn, M. S. *Tetrahedron* **1989**, *45*, 7835.
44. Chuang, C.-P.; Hart, D. J. *J. Org. Chem.* **1983**, *48*, 1782.
45. Keck, G. E.; Park, M.; Krishnamurthy, D. *J. Org. Chem.* **1993**, *58*, 3787.
46. Linderman, R. J.; Cusack, K. P.; Taber, M. R. *Tetrahedron Lett.* **1996**, *37*, 6649.
47. Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408.
48. Stille, J. K.; Groh, B. L. *J. Am. Chem. Soc.* **1987**, *109*, 813.
49. Kobayashi, M.; Wang, W.; Ohyabu, N.; Kurosu, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1995**, *43*, 1598.
50. Kobayashi, M.; Kurosu, M.; Ohyabu, N.; Wang, W.; Fujii, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2196.
51. Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 7969.
52. Ghosh, A. K.; Bischoff, A. *Org. Lett.* **2000**, *2*, 1573.
53. Noyori, R. *Asymmetric Catalysis in Organic Synthesis*; Wiley: New York, 1994.
54. Fráter, G. *Helv. Chim. Acta* **1979**, *62*, 2825.
55. Ley, S. V.; Norman, J.; Griffith, W. P. *Synthesis* **1994**, 639.
56. Rej, R.; Nguyen, D.; Go, B.; Fortin, S.; Lavalée, J.-F. *J. Org. Chem.* **1996**, *61*, 6289.
57. Furuyama, M.; Shimizu, I. *Tetrahedron: Asymmetry* **1998**, *9*, 1351.

58. Christopher, J. A.; Kocienski, P. J.; Kuhl, A.; Bell, R. *Synlett* **2000**, 463.
59. Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T.; Nomizu, S.; Moriwake, T. *Chem. Lett.* **1984**, 1389.
60. Dahanukar, V. H.; Rychnovsky, S. D. *J. Org. Chem.* **1996**, *61*, 8317.
61. Rychnovsky, S. D.; Buckmelter, A. J.; Dahanukar, V. H.; Skalitzky, D. J. *J. Org. Chem.* **1999**, *64*, 6849.
62. Tate, D. P.; Knipple, W. R.; Augl, J. M. *Inorg. Chem.* **1962**, *1*, 433.
63. Faller, J. W.; Linebarrier, D. *Organometallics* **1988**, *7*, 1670.
64. Faller, J. W.; Lambert, C.; Mazzieri, M. R. *J. Organomet. Chem.* **1990**, *383*, 161.
65. Seebach, D.; Wasmuth, D. *Helv. Chim. Acta* **1980**, *63*, 197.
66. Aebi, J. D.; Sutter, M. A.; Wasmuth, D.; Seebach, D. *Annalen* **1983**, 2115.
67. Pousset, C.; Haddad, M.; Larchevêque, M. *Tetrahedron* **2001**, *57*, 7163.
68. Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
69. Chang, H.-T.; Sharpless, K. B. *J. Org. Chem.* **1996**, *61*, 6456.
70. Barrow, R.; Moore, R. E.; Li, L.-H.; Tius, M. A. *Tetrahedron* **2000**, *56*, 3339.
71. Masamune, S.; Choy, W.; Kerdesky, F. A. J.; Imperiali, B. *J. Am. Chem. Soc.* **1981**, *103*, 1566.
72. Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099.
73. Makin, S. M.; Raifel'd, Y. E.; Limanova, O. V.; Shavrygina, O. A.; Kosheleva, L. M. *J. Org. Chem. USSR* **1979**, 1665.
74. Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.* **1992**, *114*, 9836.
75. Gemal, A. L.; Luche, J.-L. *J. Am. Chem. Soc.* **1981**, *103*, 5454.

Biographical sketch



Marc Tius was born in 1953 in Izmir, on the Aegean coast of Turkey. He moved to Greece when he was 5 years old. He attended Elementary school for six years in Kavala, a town in Eastern Macedonia, and Gymnasium for another six years in Thessaloniki, a larger city in northern Greece. In 1971 he enrolled as an undergraduate at Dartmouth College in Hanover, New Hampshire, where he majored in Mathematics and Chemistry. His first research experience was in Professor Gordon Gribble's labs. In 1975 he moved south from Hanover to Cambridge and started graduate studies at Harvard, where he joined Professor E. J. Corey's group. For his thesis he completed the synthesis of aphidicolin, working with Larry Blaszczak first, and then with Jagabandhu Das. After a brief postdoc in the Corey group, he moved to Hawaii in August 1980, where he has been ever since. He currently has a joint appointment in the Chemistry Department of the University of Hawaii, and at the Cancer Research Center of Hawaii.