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Award Number: DAMD17-98-1-8200

TITLE: Total Synthesis of Cryptophycin A and Analogs

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REPORT DATE: April 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2000	Annual Summary (16 Mar 99 - 15 Mar 00)

4. TITLE AND SUBTITLE
Total Synthesis of Cryptophycin A and Analogues

5. FUNDING NUMBERS
DAMD17-98-1-8200

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Cryptophycins are antimitotic metabolites isolated from an algae. By interacting with tubulin, Cryptophycin A induces apoptosis. Preliminary *in vivo* studies showed that the cryptophycins are active against breast tumors. To date, structure-activity relationship studies regarding the C3 and C10 side chains have not been fully probed.

The synthesis of the epi-C3 cryptophycin analogues has been completed. Scale-up of the synthesis is underway so full characterization and the biological testing can be completed. The total synthesis of the C10 analogue containing the C3' chloro group, derived from the substitution of the D-3-chlorophenylalanine for the D-tyrosine moiety, is nearly complete. *In vitro* biological testing will be performed to determine biological activity in selected breast cancer cell lines and in a tubulin assay.

The studies outlined in this research summary will provide insight into how cryptophycin and tubulin interact. Information obtained about the binding site and where cryptophycin binds to tubulin will help reveal how the potent cytotoxic effects are exerted in cancerous cells. This may inturn help scientists better understand tubulin's structure so that more potent anticancer agents can be prepared in the future.

14. SUBJECT TERMS
Cryptophycin A, synthesis, analogues, tubulin

15. NUMBER OF PAGES
27

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Suzanne Buck 4-11-2000

PI - Signature Date

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Introduction

Cryptophycins are antimitotic, cyanobacterial metabolites isolated from a blue-green algae. By interacting with tubulin, Cryptophycin A (Figure 1) induces apoptosis.[1-3] Preliminary *in vivo* studies showed that the cryptophycins are active against breast, pancreatic and colon tumors.[2,4] Cryptophycin-52 (Figure 1), the C6 *gem*-dimethyl analogue of Cryptophycin A, is currently in clinical trials.[5] To date, structure-activity relationship studies regarding the C3 and C10 side chains have not been fully probed. Although analogues were isolated containing varied side chains at C3, no isolation or synthesis of the C3 epimer has been reported. Inversion of the C3 stereochemistry will provide information regarding the orientation of the hydrophobic binding site at C3. Regarding the C10 side chain, the necessity of the aromatic oxygen has not been fully studied. Analogues containing C3'-chloro and C3', C5'-dichloro phenylalanine moieties are being prepared. The synthesis of analogues with modifications on the C10 side chain, and at C3 are being carried out. *In vitro* biological testing will be performed to determine biological activity in selected breast cancer cell lines and in a tubulin assay.

The studies outlined in this research summary will provide insight into how cryptophycin and tubulin interact. Information obtained about the binding site and where cryptophycin binds to tubulin will help reveal how the potent cytotoxic effects are exerted in cancerous cells. This may inturn help scientists better understand tubulin's structure so that more potent anticancer agents can be prepared in the future.

Body

I. Retrosynthesis of Cryptophycin Analogues

Arenastatin A is synthesized from two key fragments: the "northern half" **1.1**, and the "southern half" **1.2** (see Scheme 1 for retrosynthetic analysis). The northern half is obtained through asymmetric synthesis, while the southern half is formed through the coupling of three easily accessed building blocks: 1) L-leucic acid (**1.3**), 2) β-alanine (**1.4**), and 3) D-tyrosine (**1.5**). The leucic acid and the β-alanine, which are incorportated into the synthesis of the southern half, are commercially available. The tyrosine moiety is easily derived in a few steps from D-tyrosine.

Synthesis of Northern Half 1.1

As covered in last year's report, the original asymmetric synthesis of **1.1**, developed in the Georg laboratory, was completed using two key reactions, a Noyori asymmetric hydrogenation[6] followed by a Frater reaction,[7,8] to set the two adjacent stereocenters (Scheme 2).[9] As in many total syntheses, the need for more starting material is always present. Therefore, a second route more amenable to scale-up, was developed which uses the *B*-allyldiisopinocampheyl borane developed by H. C. Brown, to set both stereocenters of **1.1** in a single step (Scheme 3).[10] Starting from 1,3-propanediol (**3.1**), the northern half **1.1** is obtained in nine steps in 17% overall yield. This is in contrast to the Noyori/Frater route in which the overall yield for the twelve step synthesis, starting from diketo ester **2.1**, was 4%. During the development of the crotylboration route, another similar route was also investigated. The final crotylboration route, the alternative crotylboration route and other routes to **1.1** are discussed.

A. Original Noyori/Frater Route to Northern Half 1.1

In the Noyori/Frater route, the dianion of methyl acetoacetate (**2.1**) is alkylated with benzyl chloromethyl ether to provide benzyl ester **2.2** (Scheme 2). This first step was easily completed on a 10 g scale in 85% yield. Using the Noyori asymmetric hydrogenation,[6] 8 g of ester **2.2** was reduced, giving the *S* stereochemistry (63% yield, 97% ee) at C3 of compound **2.3**. The Frater procedure[7,8] allowed stereoselective introduction of the methyl group of **2.4** (71% yield, 95% de). The benzyl group of **2.4** was easily cleaved via hydrogenation, yielding the diol in 99%, which was diprotected as a *tert*-butyldimethylsilyl (TBS) ether **2.5** (88% yield). Because the diisobutylaluminum hydride (DIBAL-H) reduction of **2.5** provided a mixture of the desired aldehyde and overreduced alcohol, reduction to the alcohol (accomplished in 90% yield) and reoxidation to form **2.6** (80% yield) was employed to obtain the necessary synthon for the first Horner-Emmons-Wadsworth reaction. The olefination successfully formed **2.7** in 63%. Selective mono-desilylated was achieved in 65% using a solution of acetic acid, water, and tetrahydrofuran (1.0: 1.0: 2.5), and the resulting primary alcohol was cleanly oxidized with tetrapropylammonium perruthenate (TPAP) to provide the second Horner-Emmons-Wadsworth synthon, **2.8** in 80%. *t*-Butyl ester **2.9** was obtained from **2.8** in 76%,[10] and the TBS ether was selectively cleaved using tetrabutylammonium fluoride (TBAF) to form **1.1** in 78%.[11]

B. Crotyl Boration Route to Northern Half 1.1

Synthesis of **1.1** via the crotyl boration route begins with the protection of 1,3-propanediol (**3.1**) with *p*-methoxybenzyl chloride (64% on 10g scale, Scheme 3). The monoprotected alcohol was cleanly oxidized to aldehyde **3.2** in 96% yield using sodium hypochlorite as the oxidant in the presence of catalytic 2,2,6,6-tetramethyl-1-piperdinyloxy, free radical (TEMPO) and potassium bromide. Aldehyde **3.2** was reacted with *B*-allyldiisopinocampheyl borane **3.3** to provide **3.4** (76%, 91% de, 16 g scale). Use of the crotyl boration reaction is key to obtaining **1.1** in fewer overall steps because it sets two stereocenters in one step as compared to the Noyori/Frater route. An added benefit is the ease with which this reaction is scaled up. The protection of alcohol **3.4**, followed by the oxidative cleavage of the *p*-methoxybenzyl (PMB) group using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) affords the primary alcohol. This alcohol was then oxidized to

provide aldehyde **3.5** in 68% yield, three steps. Aldehyde **3.5** was subjected to a Horner-Emmons-Wadsworth reaction (81%) similar to the olefination described previously in the Noyori/Frater route in Scheme 2. After completing the construction of α,β-unsaturated *t*-buty ester **3.6**, the aryl ring was installed using a Heck coupling (84%) to afford **2.9**. The TBS ether was selectively cleaved to form **1.1** using the same procedure outlined in scheme 2.[11]

C. Alternative Crotyl Boration Route to Northern Half 1.1

During the development of the crotylboration route (Scheme 3), an alternative approach (Scheme 4) was pursued in which I intended to construct the two asymmetric centers after the α,β-unsaturated ester was established. This route was pursued in the hopes of simplifying the purification necessary after the crotylboration reaction. The one drawback to the crotylboration route was the fact that the borane byproduct travelled closely to the desired product during chromatography. This made the separation of the product from the byproduct tedious, and it was therefore envisioned that substituting another substrate might allow for easier separation of the product from the byproduct.

Starting with *tert*-butyldiphenylsilyl (TBDPS) protection of 1,3-propane diol (**3.1**) in 89%, the resulting alcohol **4.1a** was oxidized to aldehyde **4.2a** using TPAP and 4-morpholine-*N*-oxide (NMO) (86%, Scheme 4). This aldehyde was then subjected to a Horner-Emmons-Wadsworth reaction to yield α,β-unsaturated methylester **4.3a** in 69%. The protecting group was then cleaved using hydrofluoric acid in pyridine (**4.4a**, 86%). The oxidation of alcohol **4.4a** to provide synthon **4.5a** for the key crotylboration reaction proved troublesome. Initial attempts to oxidize the alcohol to **4.5a** using TPAP or swern conditions failed.

During the development of the published crotylboration procedure it was determined that the *tert*-butylester protection of the acid moiety of the backbone was optimal. Therefore, the *tert*-butylester was synthesized in a manner similar to the methyl ester (Scheme 4). Unfortunately the TBDPS protecting group of **4.3b** could not be removed without cleaving the *t*-butyl ester, providing **4.4b**. Therefore the route was again modified, substituting the *tert*-butyldimethylsilyl (TBS) ether for the TBDPS-ether. Scheme 4 outlines this synthetic route.

The TBS protecting group on **4.3c** was removed using hydrofluoric acid in pyridine provided alcohol **4.4c** (91%). Oxidation procedures to obtain **4.5b** from **4.4c** using varied reagents such as TEMPO, Dess-Martin periodinane, and 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX) were unsucessful.

Interestingly, on small scale aldehyde **5.1** was obtained under the Dess-Martin reaction conditions, where the double bond had migrated into conjugation with the aldehyde (Scheme 5). The possibility of the crotyl boration being attempted on **5.1** was considered. If the crotyl boration worked, there was hope that the double bond would be easily isomerized into conjugation with the ester. Unfortunatley, due to the high instability of **5.1** on scale up, this route was no longer pursued.

D. Alternative route to Desired Intermediate Aldehyde

Seeing that a fewer step synthesis of aldehyde **4.5** would allow for the most convergent synthesis of backbone **1.1**, we pursued other routes. In the literature, a procedure was found in which 3-propenal (**6.4**) was formed and the double bond migration had been avoided (Scheme 6). Allylbromide (**6.1**) was reacted with tin and glyoxal (**6.2**) under sonication, to form diol **6.3**.[12] Clean oxidation of the diol to 3-propenal (**6.4**) was achieved using sodium periodate.[13]

Following the procedure, we chose as our substrate methyl 4-bromocrotonate (**7.1**, Scheme 7). Unfortunately, only trace amounts of product **7.2** was formed. Upon reviewing the protocol, it was determined that the methyl 4-bromocrotonate (**7.1**) decomposed when reacted with the tin under sonication.

One last protocol was found for obtaining aldehyde **4.5a**[14] utilizing α-pyrone (**8.1**, Scheme 8) which undergoes a Norrish type I cleavage when irradiated with 300 nm light in a photoreactor. In the presence of dry methanol, aldehyde **4.5a** is produced. Unfortunately, scale up and purification of the product were not easily completed, so the sequence was no longer pursued.

E. Summary of the Synthesis of the Northern Half

Because a more concise route to **1.1** was not identified, the initial crotyl boration route (Scheme 3) will be used to produce the necessary quantities of **1.1** required for future studies.

II. C3 Analogues of Cryptophycin A-SAR Study of Inversion of Stereochemistry at C3

Moore *et al.* isolated cryptophycin analogues from *Nostoc* sp. GSV 224[15] with varying C3 groups. The analogues containing substitutions such as an isopropyl group, an *n*-propyl group or *sec*-butyl group all had greatly diminished cytotoxicity. Therefore, the data does not support alterating the C3 isobutyl group in order to obtain a more potent compound. Currently in the Georg group, we hypothesize that the binding site of the cryptophycins contains a hydrophobic pocket where the C3 isobutyl group interacts favorably. Of all of the analogues isolated by Moore and coworkers, none contained the inverted C3 stereocenter, and to date no synthesis of such compounds have been published. I have proposed that by inverting the stereochemistry at C3, analogues **A** and **B** in Figure 2, information regarding the orientation of the hydrophobic binding site will be gained.

The analogues of interest, **A** - **D** in Figure 2, all lack the C6 methyl group, which is not necessary for *in vitro* activity, but is necessary for *in vivo* activity where it slows hydrolysis of the ester bond. Since all analogues will be screened for biological activity in an *in vitro* tubulin assay, and *in vitro* cell line assays the analogues lacking the substitution at C6 have been selected for synthesis and testing.

A. Synthesis of the Epi-C3 Fragment

The synthesis of the C3 epimer of Arenastatin A is accomplished by substituting D-leucic acid (**9.2**) for L-leucic acid (**1.3**). Although L-leucic acid is commercially available, D-leucic acid is not. Therefore, a diazotization procedure[16] was used to gain access to D-leucic acid (**9.2**) in one step from D-leucine (**9.1**) which is commerically available (Scheme 9).

B. Synthesis of the Southern Half of Epi-C3 Analogues

D-Leucic acid was protected as its allyl ester **9.3**[17] and coupled[1] to *N*-Boc-protected β-alanine, to afford compound **9.4** (Scheme 9). Intermediate **9.4** was deprotected using trifluoroacetic acid[18] and subsequently joined to the desired tyrosine moiety,[18] affording protected southern half **9.5** of the Arenastatin analogues. The allyl group was cleaved using tetrakis(triphenylphosphine)-palladium(0) and pyrrolidine to reveal acid **9.6**.[19]

C. Completion of Epi-C3 Analogues

The facile joining of **9.6** and **1.1** was completed via a Yamaguchi coupling. Acid **9.6** was activated using the Yamaguchi reagent, 2,4,6-trichlorobenzoyl chloride, and coupled to **1.1** (Scheme 10).[16] The *N*-Boc group and the *tert*-butyl ester were simultaneously cleaved from intermediate **10.1** using trifluoroacetic acid.[20] The crude trifluoroacetic acid salt was joined to the acid moiety using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent,[20] thus closing the macrolide and forming **10.2**. Epoxidation using *m*-chloroperbenzoic acid (*m*-CPBA) yielded mixtures of the α and β epoxides. Syntheses of **A** and **B** was confirmed by high-resolution mass spectrometry (HRMS), but not enough material was obtained to fully characterize the compounds and report accurate yields. Resynthesis of **A** and **B** is currently underway on larger scale.

III. C10 Analogues of Cryptophycin A-SAR Study of the C10 Side Chain

The SAR studies done by Moore *et al.* have shown that removal of the C10 aryl C3' chlorine or the C4'-*O*-methyl group causes loss of *in vivo* activity[15] (see Figure 1 for numbering system). Because removal of the chlorine or the methyl group decreases the lipophilicity of Cryptophycin A, this implies that lipophilicity may be very important in the activity of the molecule.

A steric component may also be important as revealed by other isolated cryptophycin analogues. Aryl C3',5'-dichloro, C4'-*O*-methyl substituted and aryl C3',5'-dichloro, C4'-hydroxy compounds also had decreased activity *in vitro*.[15] This could be due to the increased steric bulk introduced by the second chlorine on the aryl ring. In order to more thoroughly test the hypothesis that the the steric bulk introduced by the second chlorine is not tolerated by the binding site, the C10 mono and dichlorophenylalanine analogues, **C** and **D** respectively, will be synthesized (Figure 2). If the hypothesis is correct, then the monochlorinated compound should have better activity than the dichloro analogue.

Synthesis of the C10 Analogues

The C10 analogues will be accessed by substituting 3-chloro and 3,5-dichlorophenylalanine for the D-tyrosine moiety. D-3-Chlorophenylalanine is commericially available but the D-3,5-dichlorophenylalanine is not. Therefore, synthesis of the 3,5-dichloro compound via dichlorination of D-tyrosine, followed by standard deoxygenation of the aryl ring was pursued.

The straightforward deoxygenation procedure in development may be used to obtain either the D- or L-chlorophenylalanine analogues that are not readily available. This deoxygenation is currently being optimized and studied to determine its feasiblity as a route to enantiomerically pure 3-chloro and 3,5-dichlorophenylalanines.

A. Synthesis of Chlorophenylalanines

Synthesis of the dichlorinated analogue began with the chlorination of D-tyrosine methyl ester hydrochloride (**11.1**), using two equivlents of sulfuryl chloride to afford the dichlorinated product (Scheme 11). The chlorinated compound was *N*-Boc protected yielding **11.2**. Since last reported, no further deoxygenations have been attempted. New procedures are being reviewed, and will be attempted in the future to optimize the modest 28% yield of dichlorinated product **11.4**. Further studies will examine the altering the conditions and/or reagent equivalents to increase the yield. Methyl ester **11.4** will be hydrolyzed using 1 N sodium hydroxide, forming dichlorinated acid **11.5**.

B. Completion of the C10 Analogues

As with the C3 analogues, the synthesis of the C10 analogues begins with L-leucic acid. The chlorophenylalanine side chains will be substituted for the tyrosine moieties to complete analogues **C** and **D**.

L-Leucic acid (**1.3**) was protected as its allyl ester and then coupled to Boc-β-alanine to afford **12.1** (Scheme 12). After deprotection, **12.1** will be joined with 3-chlorophenylalanine or 3,5-dichlorophenylalanine. Following removal of the allyl protecting group of **12.2**, the southern half will be coupled to **1.1** to provide **12.3**. The *N*-Boc group and the *tert*-butyl ester will be cleaved[20] and HBTU will be used to close the macrolide. Epoxidation using *m*-CPBA will provide mixtures of the α and β epoxides of analogues **C** and **D**.

Key Research Accomplishments

- The synthesis of the arenastatin epi-C3 analogues has been completed. Scale-up of the synthesis is underway so full characterization and biological testing can be completed.
- The total synthesis of the C10 analogue containing the C3' chloro group, derived from the substitution of the D-3-chlorophenylalanine for the D-tyrosine moiety, is nearly complete. Biological results should be forthcoming.

Reportable Outcomes

DEGREE: M.S., Medicinal Chemistry, August 1999, Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas.

ORAL PRESENTATION: "Studies Towards the Total Synthesis of Cryptophycin A and Arenastatin A Analogues," July 9, 1999, National Institutes of Health Symposium given by NIH predoctoral trainees, Lawrence, Kansas.

FUNDING: CA70369 (P.I. G.I. Georg and Co.-P.I. R. H. Himes) 06/01/99-5/31/02
National Institutes of Health, National Cancer Institute
Pharmacophore/Interactions with Tubulin of Cryptophycin
The major goal of this project is to prepare cryptophycin affinity labels to charachterize the cryptophycin binding site.

POSTER: Buck, Suzanne B.; Georg, Gunda I,. "Studies Towards the Total Synthesis of Cryptophycin A and Analogues." April 16-18, 1999, Presented at the 37th MIKI Medicinal Chemistry Meeting, Lawrence, Kansas.

LECTURE: "Studies Towards the Synthesis of Cryptophycin A and Arenastatin A Analogues." October, 29, 1998, Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas.

POSTER: Hanna, Suzanne B.; Eggen, MariJean; Mossman, Craig J.; Reiff, Emily A.; Boge, Thomas C.; Georg, Gunda I., "Studies Towards the Total Synthesis of Cryptophycin A and Analogues." May 22-24, 1998, Presented at the 36th MIKI Medicinal Chemistry Meeting, Chicago, Illinois.

Conclusions

There are five major areas that I have planned to addressed in my research project. (1) Cyclizing the cryptophycin macrocycle, utilizing a novel lactonization technique involving a highly activated β-lactam. (2) Synthesizing the arenastatin analogues with the inverted C3 center, using D-leucic acid instead of the biologically common L-leucic acid. (3) Modifiying the β-lactam to introduce both the ethyl and *gem*-dimethyl groups at C6, and to invert the stereochemistry at C6. (4) Modifying the aryl moiety in the side chain of C16. (5) Modifying the aryl ring at C10 by substituting various phenylalanine and tyrosine derivatives during the synthesis.

The first area of my research project, cyclizing the macrolide via a novel lactonization technique, has been completed by other researchers in the Georg group. They determined that the route is feasible, but it takes more synthetic steps to obtain the analogues. Therefore, the focus of my research has shifted towards completing the syntheses of the previously described C3 and C10 analogues for biological testing.

The second area of my project, synthesis of the arenastatin analogues **A** and **B**, containing the inverted C3 center, has been completed. Currently the synthesis is being scaled up to produce enough material for full characterization of each compound and for *in vitro* biological testing.

While reviewing the recent literature regarding C6 analogues of cryptophycin that have been synthesize, it came to my attention that the third area of my project, synthesis of *gem*-dimethyl and *gem*-diethyl analogues and inverted C6 analogues of Cryptophycin A, is no longer pertinent. In a study published by Varie *et al.* they tested the effects of dialkyl substitution at C6 of Cryptophycin A, in human leukemia cell lines.[21] Following the premise that the *gem*-dimethyl group of Cryptophycin-52 slowed rate of ester hydrolysis *in vivo*, they synthesized analogues to test the theory that increased stabilty of the ester moiety would lead to more potent cytotoxic analogues. After preparing C6 analogues where R = R_1 = ethyl (*gem*-diethyl), propyl, $(CH_2)_4$, and $(CH_2)_5$, and spiropropyl $(CH_2)_2$, no increased potency of the new analogues was observed. The most active compound was the spirocyclopropyl compound which was 10 fold less active than Cryptophycin-52.

With this research in mind, the synthesis of analogues with inversion of the C6 methyl group, is no longer warranted since this analogue is expected to be as succeptible to inactivation by ester hydrolysis as Cryptophycin A. It could be argued that the enzymatic cleavage is expected to be affected by the chirality of the molecule, and that inversion of the stereochemistry at C6 might reduce hydrolysis of this bond. This theory could be tested by synthesizing the C6 epimer of cryptophycin to determine the selectiviy of the esterase involved with the hydrolysis, but at this juncture, the synthesis of analogues to probe the interaction of tubulin and cryptophycn are more pertinent.

The fourth area of my project, modification of the C16 aryl moiety has yet to be initiated. Studies will be performed on the cryptophycin family will revolve around the synthesis of analogues for use in biological assays to determine where cryptophycin interacts with tubulin. Flourescent labels will be introduced at the C10 side chain using the phenolic oxygen as a linker. Also, photoaffinity probes at C16 are currenty being completed by another member of the Georg group. Therefore, radioactive analogues of the photolabelled compounds will be prepared for biological testing. The introduction of the radiolabel and flourescent groups will be accomplished by demethylation of Cryptophycin A's C10 side chain using boron tribromide to form the reactive phenol. The phenol will then be converted to the tritiated methoxy compound or coupled to a flourescent moiety.

The fifth area of my research, synthesis of C10 analogues substituting 3-chloro and 3,5-dichloro phenylalanine for the D-tyrosine moiety, is in progress. Currently, ways to optimize the deoxygenation step are being pursued.

In the future, further development of the SAR of cryptophycin via semisynthesis will be pursued. The cyclopropyl bioisostere of the epoxide of Cryptophycin A will be pursued. Reduction of the epoxide to a double bond, followed by Simmons-Smith cyclopropanation, should afford the desired analogue. If this procedure proves not to be selective for the styrene site, then

total synthesis of the desired analogue will be completed via cyclopropanation of intermediate **3.4** in the backbone synthesis (Scheme 3).

The studies outlined in this research summary will provide insight into how cryptophycin and tubulin interact. Information obtained about the binding site and where cryptophycin binds to tubulin will help reveal how the potent cytotoxic effects of are exerted in cancerous cells. This may inturn help scientists better understand tubulin's structure so that more potent compounds anticancer agents can be prepared in the future.

References

(1) 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*, 4729-4737.

(2) Smith, C. D.; Zhang, X.; Mooberry, S. L.; Patterson, G. M. L.; Moore, R. E. *Cancer Res.* **1994**, *54*, 3779-3784.

(3) Subbaraju, G. V.; Golakoti, T.; Patterson, G. M. L.; Moore, R. E. *J. Nat. Prod.* **1997**, *60*, 302-305.

(4) Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479-2490.

(5) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Husebo, T. L.; Jensen, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1995**, *117*, 12030-12049.

(6) Kobayashi, M.; Wang, W.; Ohyabu, N.; Kurosu, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1995**, *43*, 1598-1600.

(7) Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 7969-7972.

(8) Kobayashi, M.; Kurosu, M.; Wang, W.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2394-2396.

(9) Kobayashi, M.; Kurosu, M.; Ohyabu, N.; Wang, W.; Fujii, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2196-2198.

(10) Salamonczyk, G. M.; Han, K.; Guo, Z.-W.; Sih, C. J. *J. Org. Chem.* **1996**, *61*, 6893-6900.

(11) Moore, R. E. *(private communication with Gunda I. Georg).*

(12) Patel, V. F.; Andis, S. L.; Kennedy, J. H.; Ray, J. E.; Schultz, R. M. *J. Med. Chem.* **1999**, *42*, 2588-2603.

(13) Panda, D.; DeLuca, K.; Williams, D.; Jordan, M. A.; Wilson, L. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9313-9318.

(14) Panda, D.; Himes, R. H.; Moore, R. E.; Wilson, L.; Jordan, M. A. *Biochemistry* **1997**, *36*, 12948-12953.

(15) Kerksiek, K.; Mejillano, M. R.; Schwartz, R. E.; Georg, G. I.; Himes, R. H. *FEBS Letters* **1995**, *377*, 59-61.

(16) Smith, C. D.; Zhang, X. *J. Biol. Chem.* **1996**, *271*, 6192-6198.

(17) de Muys, J.-M.; Rej, R.; Nguyen, D.; Go, B.; Fortin, S.; Lavallee, J.-F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1111-1116.

(18) White, J. D.; Hong, J.; Robarge, L. A. *J. Org. Chem.* **1999**, *64*, 6206-6216.

(19) Gardinier, K. M.; Leahy, J. W. *J. Org. Chem.* **1997**, *62*, 7098-7099.

(20) Dhokte, U. P.; Khau, V. V.; Hutchison, D. R.; Martinelli, M. J. *Tetrahedron Lett.* **1998**, *39*, 8771-8774.

(21) 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*, 1459-1463.

(22) White, J. D.; Hong, J.; Robarge, L. A. *Tetrahedron Lett.* **1998**, *39*, 8779-8782.

(23) Norman, B. H.; Hemscheidt, T.; Schultz, R. M.; Andis, S. L. *J. Org. Chem.* **1998**, *63*, 5288-5294.

(24) Georg, G. I.; Ali, S. M.; Stella, V. J.; Waugh, W. N.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1959-1962.

(25) Varie, D. L.; Shih, C.; Hay, D. A.; Andis, S. L.; Corbett, T. H.; Gossett, L. S.; Janisse, S. K.; Martinelli, M. J.; Moher, E. D.; Schultz, R. M.; Toth, J. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 369-374.

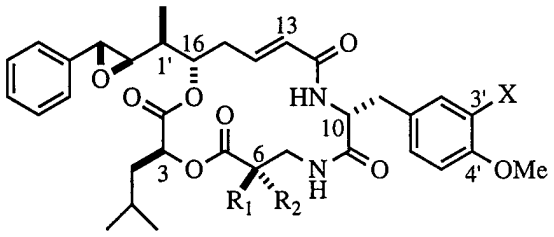
(26) Fray, A. H. *Tetrahedron: Asymmetry* **1998**, *9*, 2777-2781.

(27) Shih, C.; Gossett, L. S.; Gruber, J. M.; Grossman, C. S.; Andis, S. L.; Schultz, R. M.; Worzalla, J. F.; Corbett, T. H.; Metz, J. T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 69-74.

(28) Rej, R.; Nguyen, D.; Go, B.; Fortin, S.; Lavallee, J.-F. *J. Org. Chem.* **1996**, *61*, 6289-6295.
(29) Genet, J. P.; Ratovelomanana-Vidal, V.; Can de Andrade, M. C.; Pfister, X.; Guerreiro, P.; Lenoir, J. Y. *Tetrahedron Lett.* **1995**, *36*, 4801-4804.
(30) Frater, G. *Helv. Chim. Acta* **1979**, *62*, 2825-2832.
(31) Georg, G. I.; Kant, J.; Gill, H. J. *J. Am. Chem. Soc.* **1987**, *109*, 1129-1135.
(32) Ali, S. M.; Georg, G. I. *Tetrahedron Lett.* **1997**, *38*, 1703-1706.
(33) Eggen, M.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3177-3180.
(34) Parlow, J. J.; Vazquez, M. L.; Flynn, D. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2391-2394.
(35) Einhorn, C.; Luche, J.-L. *J. Organomet. Chem.* **1987**, *322*, 177-183.
(36) Crimmins, M. T.; Wells, A. J.; Kirincich, S. J.; Choy, A. *Synth. Commun.* **1998**, *28*, 3675-3679.
(37) Pirkle, W. H.; McKendry, L. H. *J. Am. Chem. Soc.* **1969**, *91*, 1179-1186.
(38) Mori, K. *Tetrahedron* **1976**, *32*, 1101-1106.
(39) Friedrich-Bochnitschek, S.; Waldmann, H.; Kunz, H. *J. Org. Chem.* **1989**, *54*, 751-756.
(40) Deziel, R. *Tetrahedron Lett.* **1987**, *28*, 4371-4372.

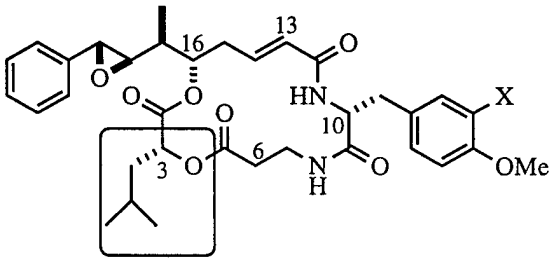
Addena A:

Figure 1. Structures of Cryptophycin A, Arenastatin A and Cryptophycin-52.

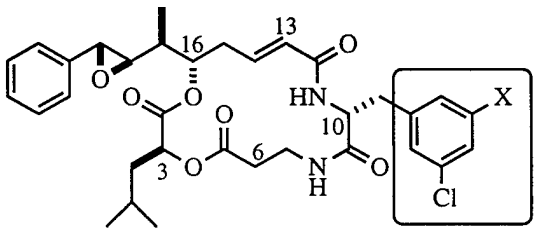


Cryptophycin A, X = Cl; R_1 = Me; R_2 = H
Arenastatin A, X = H; R_1 = R_2 = H
Cryptophycin-52, X = Cl; R_1 = R_2 = Me

Figure 2. Structures of analogues to be completed.



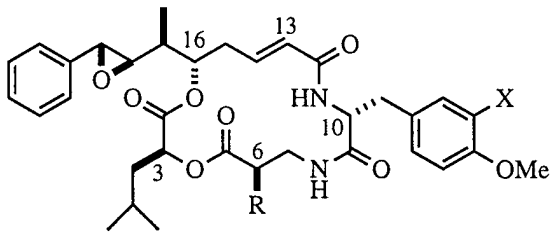
A, X = H
B, X = Cl



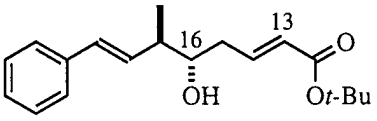
C, X = H
D, X = Cl

Addena B:

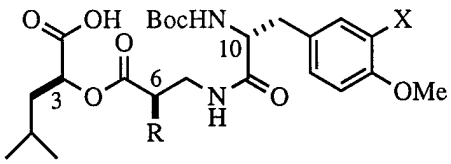
Scheme 1



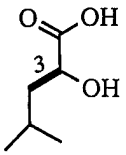
Cryptophycin A, R = Me; X = Cl
Arenastatin A, R = X = H



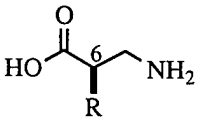
"Northern Half" **1.1**



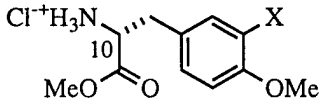
"Southern Half" **1.2**



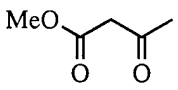
L-Leucic Acid (**1.3**)



β-Alanine (**1.4**)



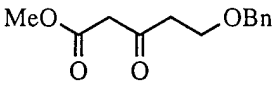
Tyrosine Derivative **1.5**



2.1

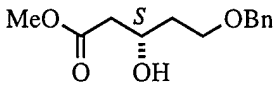
NaH, THF, 0 °C, 30 min
n-BuLi, -30 °C, 30 min

benzyl chloromethyl ether, -30 °C, 1 hr, (85%)



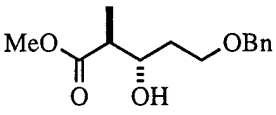
2.2

Noyori Ru-binap catalyst, H_2, 55 psi, CH_3OH, 50 °C, 16 hr, (63%)



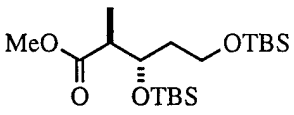
2.3, (97% ee)

1. LDA (2 equiv), THF, -78 °C, then to -20 °C, 90 min
2. MeI, HMPA, THF, -78 °C, then -20 °C, 19 hr, (71%)



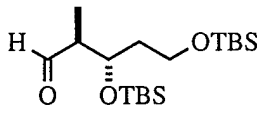
2.4, (95% de)

1. H_2, 52 psi, Pd-OH/C, THF, RT, 5 hr, (99%)
2. TBSCl, imidazole, THF, 0 °C, then RT, 18 hr, (88%)



2.5

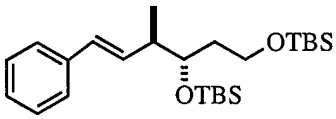
1. DIBAL-H, THF, -78 °C to -10 °C, 3hr, (90%)
2. TPAP, NMO, CH_3CN, CH_2Cl_2, 4 Å MS, RT, 1 hr, (80%)



2.6

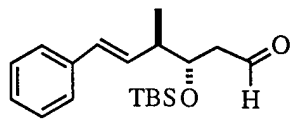
$PhCH_2PO(OEt)_2$, *n*-BuLi, THF, -78 °C, 1 hr

then, **1.6**, THF, -78 °C, 2 hr, RT, 16 hr, (63%)



2.7

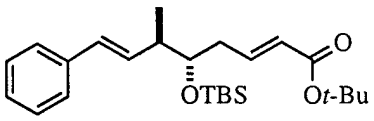
1. AcOH/THF/H_2O, RT, 3 d, (65%)
2. TPAP, NMO, CH_3CN, CH_2Cl_2, 4 Å MS, RT, 1 hr, (80%)



2.8

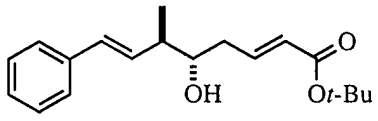
t-$BuO_2CCH_2PO(OEt)_2$, DBU, LiCl, CH_3CN,

RT, 30 min, (76%)

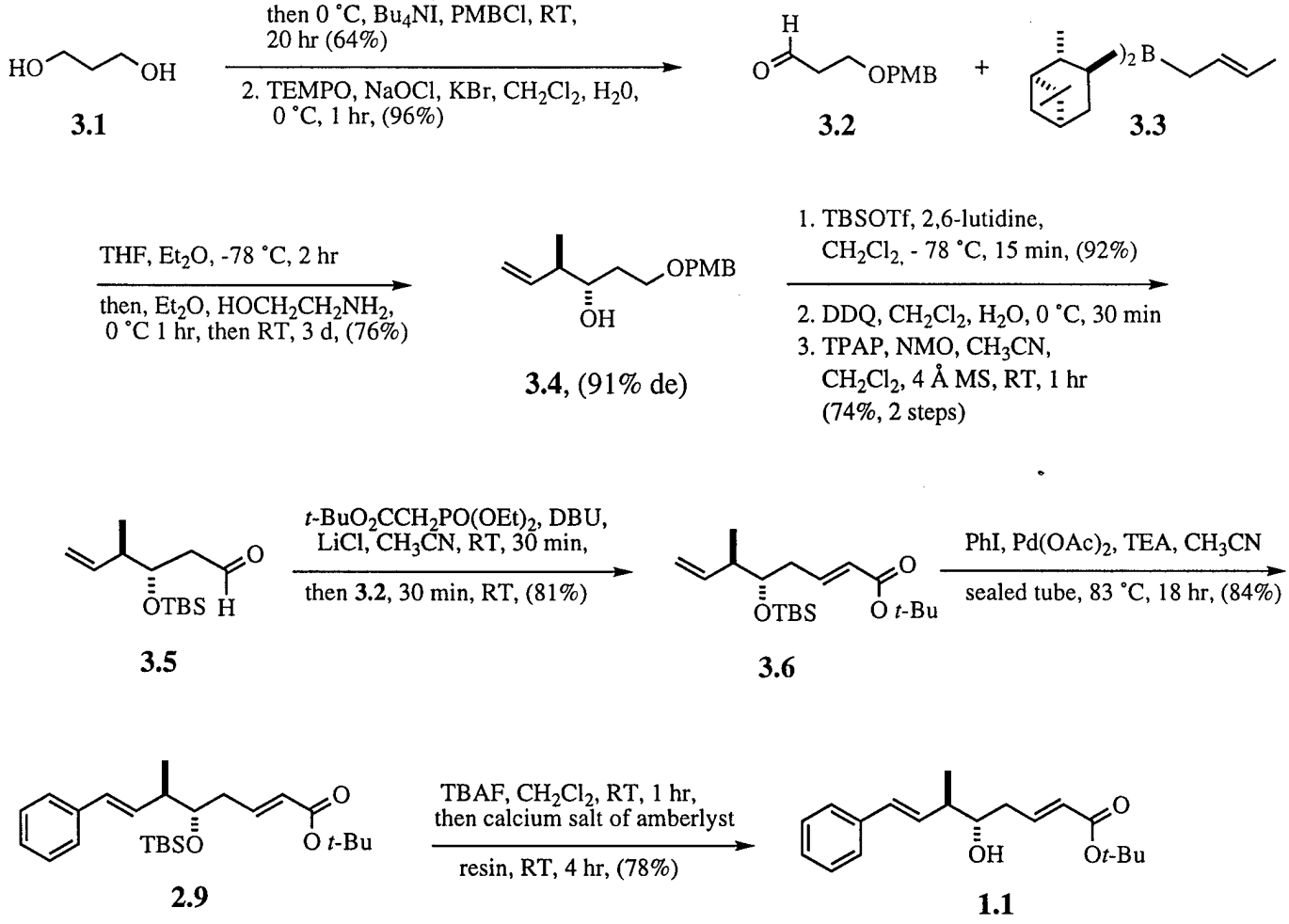


2.9

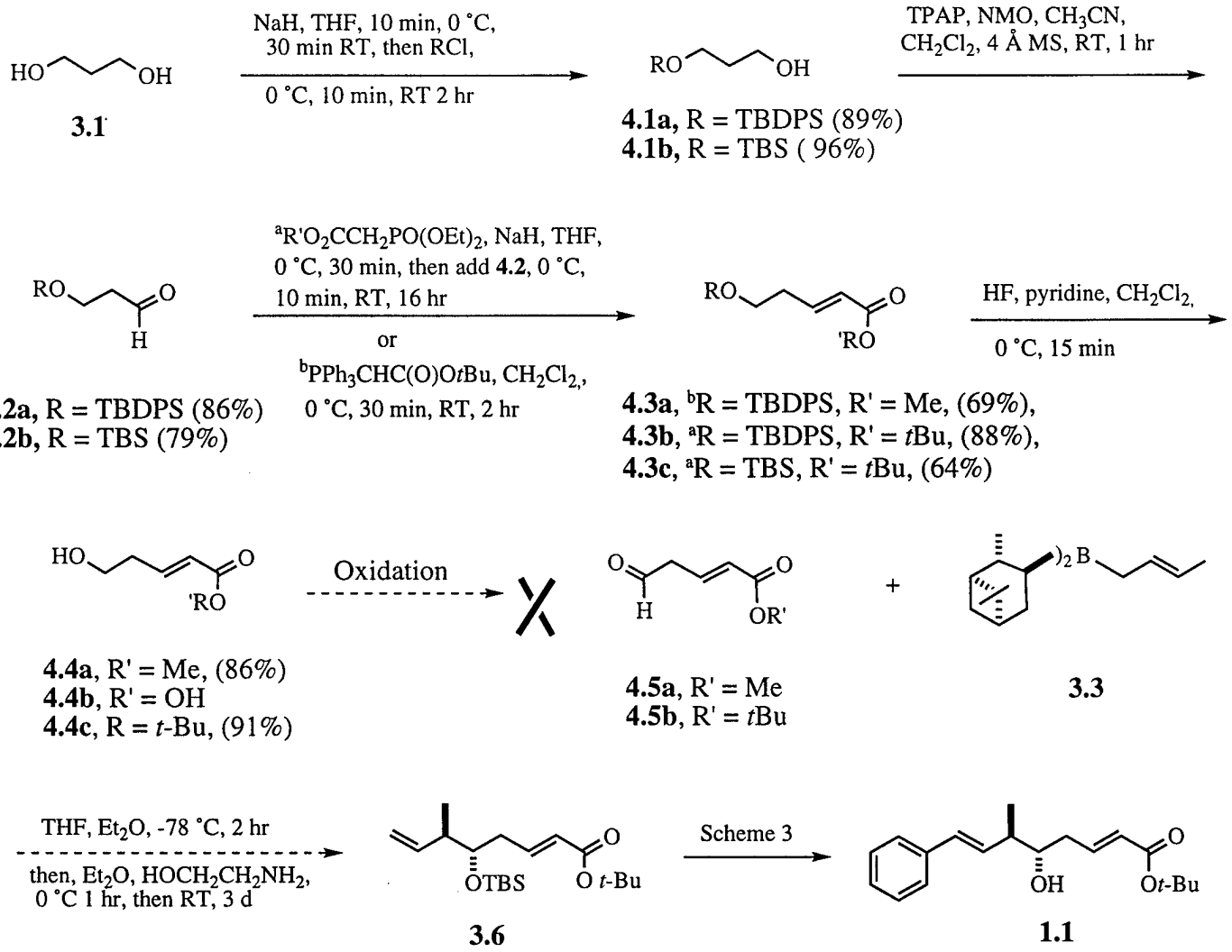
TBAF, CH_2Cl_2, RT, 1 hr, then calcium salt of amberlyst resin, RT, 4 hr, (78%)



1.1


1. NaH, THF, 0 °C, then RT 1 hr,
then 0 °C, Bu4NI, PMBCl, RT,
20 hr (64%)
2. TEMPO, NaOCl, KBr, CH2Cl2, H20,
0 °C, 1 hr, (96%)
HO
OH
3.1
H
O
OPMB
3.2
)2 B
3.3
THF, Et2O, -78 °C, 2 hr
then, Et2O, HOCH2CH2NH2,
0 °C 1 hr, then RT, 3 d, (76%)
OPMB
OH
3.4, (91% de)
1. TBSOTf, 2,6-lutidine,
CH2Cl2, - 78 °C, 15 min, (92%)
2. DDQ, CH2Cl2, H2O, 0 °C, 30 min
3. TPAP, NMO, CH3CN,
CH2Cl2, 4 Å MS, RT, 1 hr
(74%, 2 steps)
O
OTBS H
3.5
t-BuO2CCH2PO(OEt)2, DBU,
LiCl, CH3CN, RT, 30 min,
then 3.2, 30 min, RT, (81%)
O
OTBS
O t-Bu
3.6
PhI, Pd(OAc)2, TEA, CH3CN
sealed tube, 83 °C, 18 hr, (84%)
O
TBSO
O t-Bu
2.9
TBAF, CH2Cl2, RT, 1 hr,
then calcium salt of amberlyst
resin, RT, 4 hr, (78%)
O
OH
Ot-Bu
1.1

Scheme 4



3.1

4.1a, R = TBDPS (89%)
4.1b, R = TBS (96%)

4.2a, R = TBDPS (86%)
4.2b, R = TBS (79%)

4.3a, [b]R = TBDPS, R' = Me, (69%),
4.3b, [a]R = TBDPS, R' = *t*Bu, (88%),
4.3c, [a]R = TBS, R' = *t*Bu, (64%)

4.4a, R' = Me, (86%)
4.4b, R' = OH
4.4c, R = *t*-Bu, (91%)

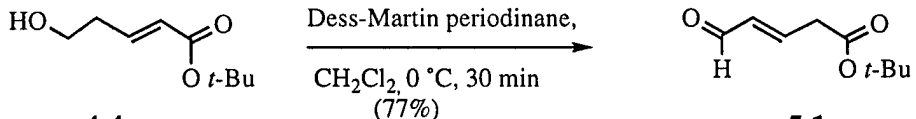
4.5a, R' = Me
4.5b, R' = *t*Bu

3.3

3.6

1.1

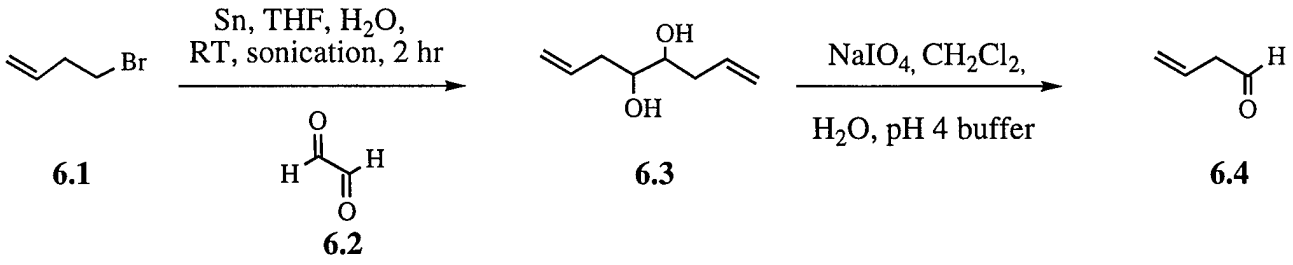
Scheme 5



4.4c

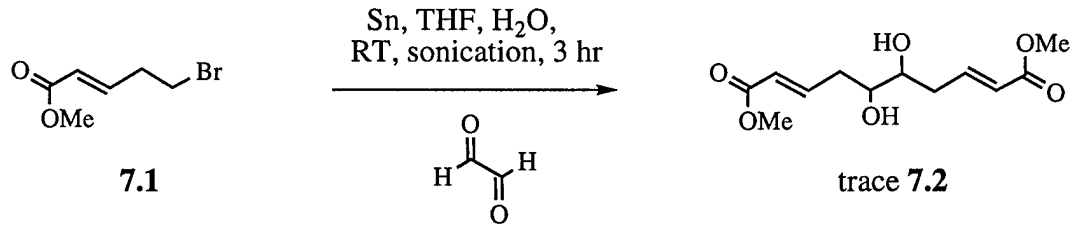
5.1

Scheme 6

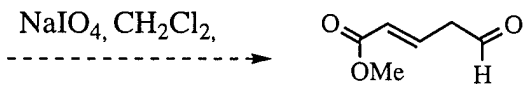


6.1 **6.2** **6.3** **6.4**

Scheme 7

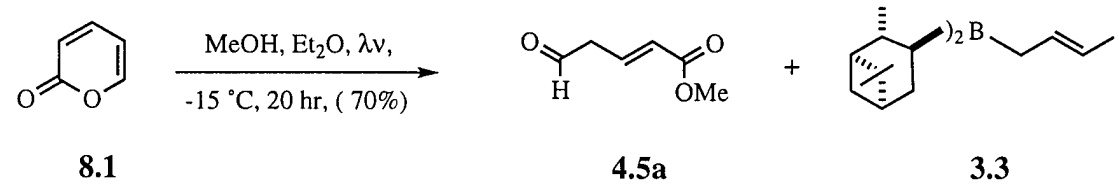


7.1 **6.2** trace **7.2**

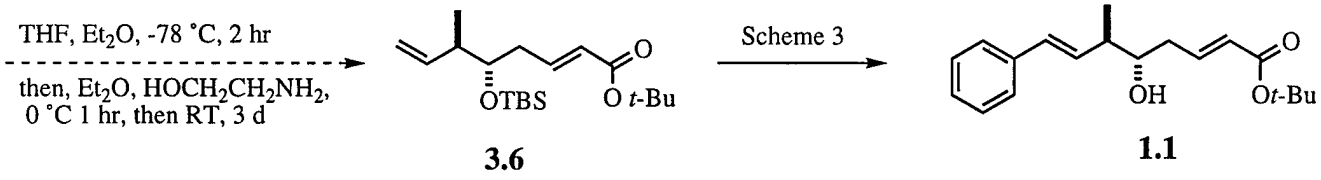


7.3

Scheme 8

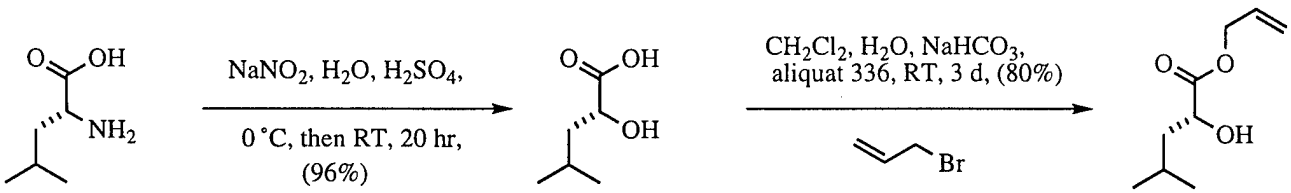


8.1 **4.5a** **3.3**



3.6 **1.1**

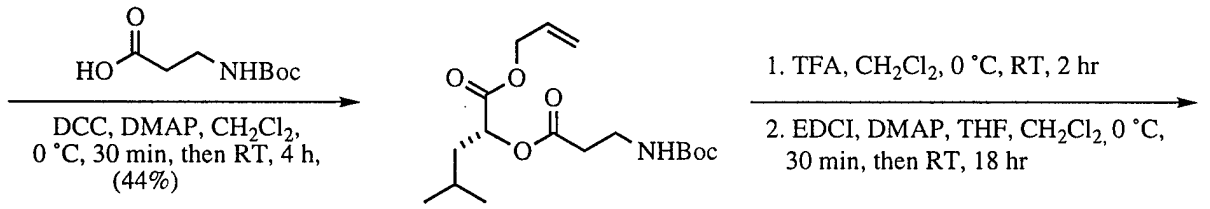
Scheme 9



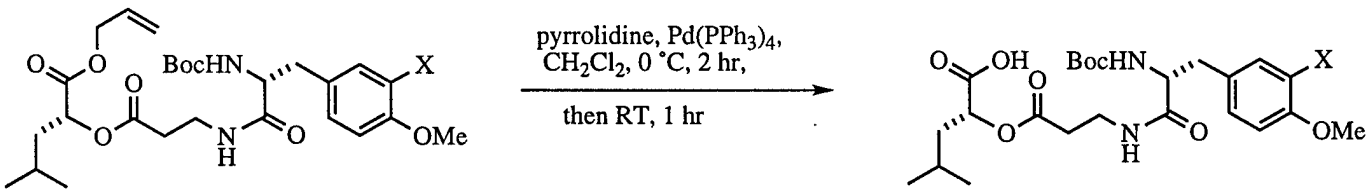
D-leucine
9.1

D-leucic acid
9.2

9.3



9.4

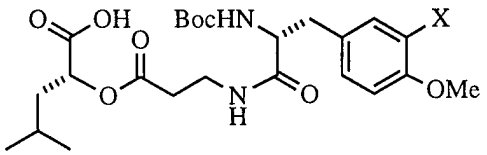


protected southern half

9.5a, X = H, 80%
9.5b, X = Cl, 92%

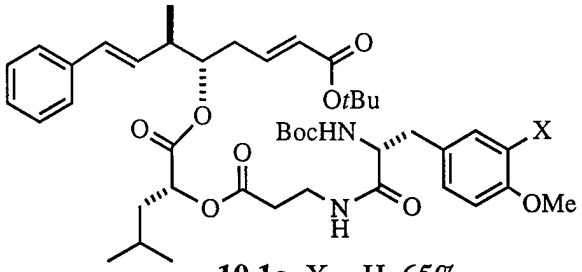
9.6a, X = H, 87%
9.6b, X=Cl, 67%

Scheme 10



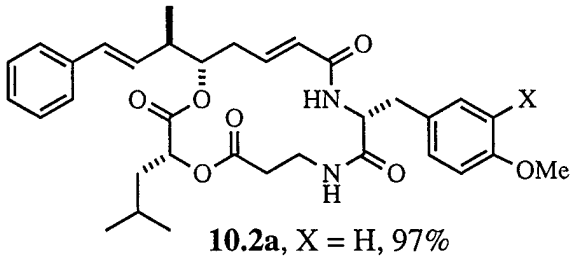
9.6a, X = H
9.6b, X = Cl

DIEA, DMAP, Yamaguchi Reagent,
THF, RT, 1 hr, then **1.1**, RT, 14 hr



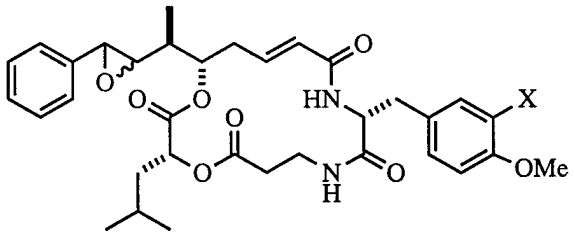
10.1a, X = H, 65%
10.1b, X = Cl, 99%

1. TFA, CH_2Cl_2, 0 °C, then RT, 2 hr
2. HBTU, DIEA, CH_3CN, RT, 3.5 hr



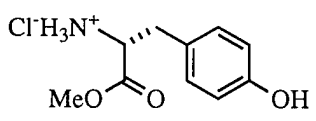
10.2a, X = H, 97%
10.2b, X = Cl, 27% unoptimized

m-CPBA, CH_2Cl_2, RT, 21 hr



mixture of α and β epoxides

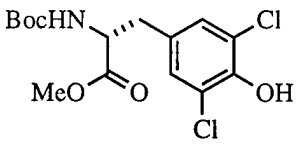
A, X = H, 47%, unoptimized
B, X = Cl, trace product isolated



D-tyrosine methyl ester hydrochloride (**11.1**)

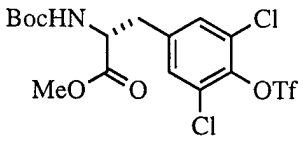
1. SO_2Cl_2, AcOH, Δ, 60 °C, 10 min, (90%)

2. Boc_2O, TEA, CH_2Cl_2 0 °C, 5 min, RT, 2 hr, (74%)



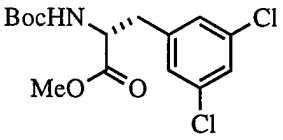
11.2a

Tf_2O, 2,6-lutidine, CH_2Cl_2, 0 °C, 30 min, (100%)



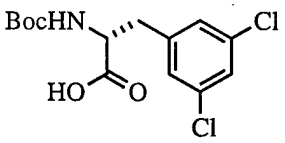
11.3

$Pd(OAc)_2$, DPPF, TEA, HCO_2H, DMF, 60 °C, 16 hr, (28%, unoptimized)



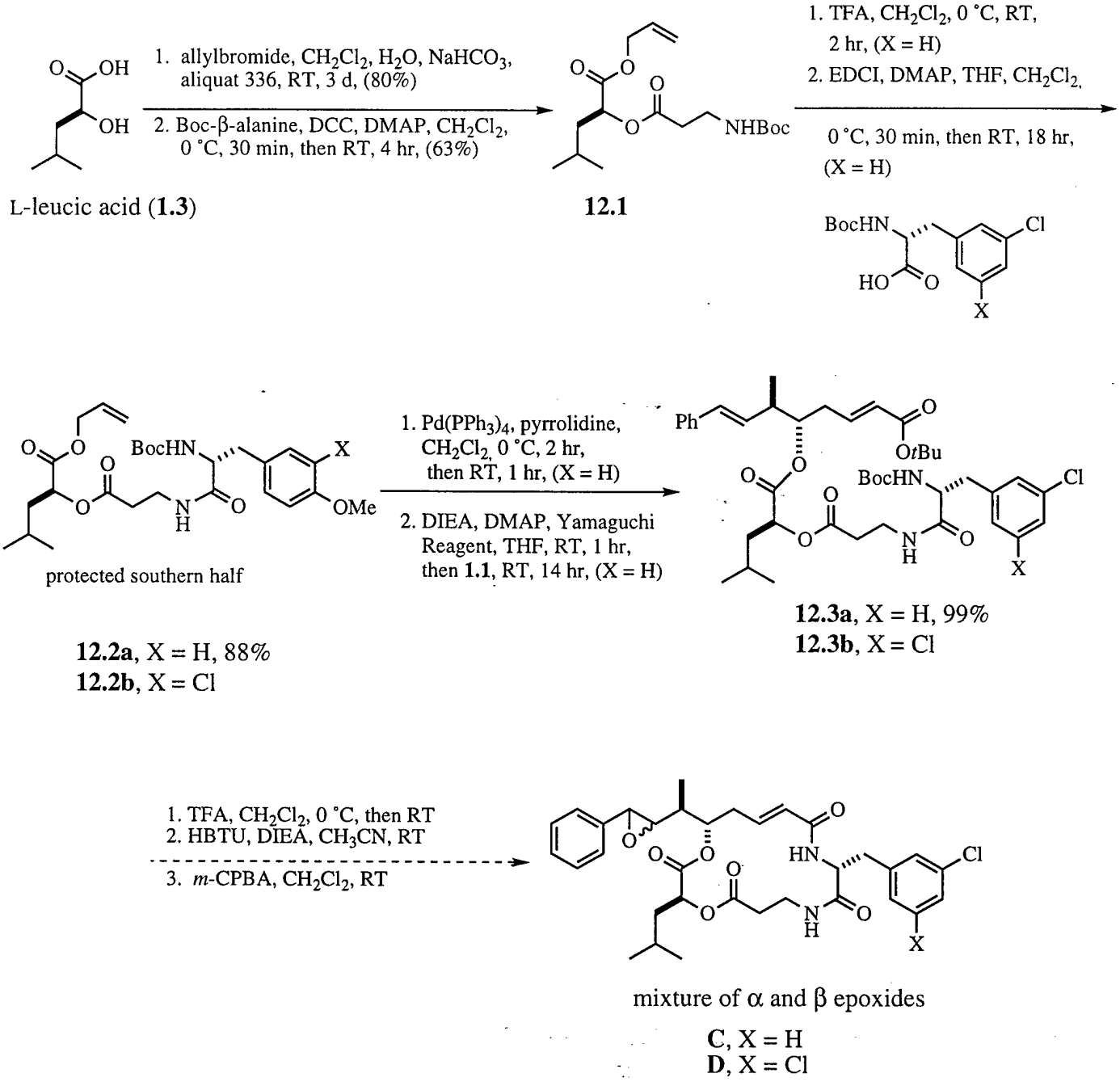
11.4

1N NaOH, dioxane



11.5

Scheme 12



L-leucic acid (**1.3**)

12.1

protected southern half

12.2a, X = H, 88%
12.2b, X = Cl

12.3a, X = H, 99%
12.3b, X = Cl

mixture of α and β epoxides

C, X = H
D, X = Cl

Addena C:
Acronyms

AcOH - acetic acid
Boc - *tert*-butoxycarbonyl
Boc_2O - di-*tert*-butyl dicarbonate
CH_3CN - acetonitrile
DBU - 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC - 1,3-dicyclohexylcarbodiimide
DDQ - 2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H - diisobutylaluminum hydride
DIEA - diisopropyl ethylamine
DMAP - 4-dimethylamino pyridine
DMF - dimethylforamide
DPPF - 1,1'-bis(diphenylphosphino)ferrocene
EDCI - 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
HBTU - *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate
HF - hydrogen flouride
HMPA - hexamethylphophoramide
HRMS - high-resolution mass spectrometry
IBX -1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide
LDA - lithium diisopropyl amide
LiOH - lithium hydroxide
m-CPBA - *m*-chloroperbenzoic acid
MDR - multidrug resistance
MS - molecular sieves
NMO - 4-morpholine-*N*-oxide
Pd(OAc) - palladium(II)acetate
Pd(PPh3)4 - tetrakis(triphenylphosphine)-palladium(0)
PMB - *p*-methoxybenzyl
PMBCl - *p*-methoxybenzylchloride
RT - room termperature
SAR - structure-activity relationship
Sn - tin
TBAF - tetrabutylammonium fluoride
TBDPS - *tert*-butyldiphenylsilylether
TBS - *tert*-butyldimethylsilylether
TBSOTf - *tert*-butyldimethylsilyl trifluoromethanesulfonate
TBSCl - *tert*-butyldimethylsilyl chloride
TEA - triethylamine
TEMPO - (2,2,6,6-tetramethyl-1-piperdinyloxy, free radical
TF_2O - triflic anhydride
TFA - trifluoroacetic acid
THF - tetrahydrofuran
TPAP - tetrapropylammonium perruthenate
Yamaguchi reagent - 2,4,6-trichlorobenzoyl chloride