DISSERTATION

A CONCISE TOTAL SYNTHESIS OF THE TMC-95A AND TMC-95B PROTEASOME INHIBITORS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY BRIAN KEITH ALBRECHT ENTITLED A CONCISE TOTAL SYNTHESIS OF THE TMC-95A AND TMC-95B PROTEASOME INHIBITORS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

A CONCISE TOTAL SYNTHESIS OF THE

TMC-95A AND TMC-95B PROTEASOME INHIBITORS

A concise total synthesis of the TMC-95A/B proteasome inhibitors is presented. The synthesis features the use of an L-serine derived *E*-selective modified Julia olefination reaction that ultimately controls the stereochemical outcome of the highly oxidized tryptophan fragment. A diastereoselective dihydroxylation, a Suzuki coupling, macrocyclization and *cis*-propenyl amide formation were also employed.

In the process of the total synthesis, a suitable intermediate was converted to a late stage intermediate in the Danishefsky total synthesis, effectively completing a formal synthesis.

The limited use of protecting groups allowed for an efficient route that is amenable to the preparation of a variety of analogs due to its convergency.

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smile, especially during the trials and tribulations of graduate school. My accomplishments would be inconsequential without you to share them with.

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List of Abbreviations

Ac_2O	acetic anhydride
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyldicarbonate
BnBr	benzyl bromide
Bu_2BOTf	dibutylboron trifluoromethanesulfonate
^{i}BuOCOCl	isobutylchloroformate
BzCl	benzoyl chloride
Cbz	benzyloxycarbonyl
CbzCl	benzylchloroformate
DEAD	diethyl azodicarboxylate
Dess-Martin Periodinane	triacetoxy *o*-iodoxybenzoic acid
$(DHQ)_2PHAL$	hydroquinine 1,4-phthalazinediyl diether
$(DHQD)_2PHAL$	hydroquinidine 1,4-phthalazinediyl diether
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DMAP	4-(dimethylamino)-pyridine
DMDO	dimethyldioxirane
DME	dimethoxyethane
DMF	dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1 *H*)-pyrimidinone
DMS	dimethylsulfide

EDCI	ethyl-dimethylaminopropylcarbodiimide hydrochloride
Et_3N	triethylamine
EtOAc	ethyl acetate
HOAc	acetic acid
HOAt	1-hydroxyazabenzotriazole
HOBt	1-hydroxybenzotriazole
IBX	*o*-iodoxybenzoic acid
Jones reagent	CrO_3 and aq. H_2SO_4
KHMDS	potassium bis(trimethylsilyl)amide
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
*m*CPBA	*m*-chloroperbenzoic acid
MeCN	acetonitrile
MOMCl	chloromethyl methyl ether
MsCl	methanesulfonyl chloride
NaHMDS	sodium bis(trimethylsilyl)amide
nOe	nuclear Overhauser effect
NMM	*N*-methylmorpholine
NMO	4-methyl morpholine *N*-oxide
NMP	1-methyl-2-pyrrolidinone
Pd/C	palladium on carbon
$PdCl_2(dppf)\cdot CH_2Cl_2$	dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium
$Pd_2(dba)_3\cdot CHCl_3$	tris(dibenzylideneaceone)dipalladium

PhH	benzene
PhMe	toluene
PPTS	pyridinium *p*-toluenesulfonate
$^{i}Pr_2NEt$	diisopropylethylamine
TBAF	tetrabutyl ammonium fluoride
TBSCl	*tert*-butyldimethylsilyl chloride
TBSOTf	*tert*-butyl dimethylsilyl trifluoromethansulfonate
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy, free radical
TESOTf	triethylsilyl trifluoromethanesulfonate
TFA	trifluoroacetic acid
TfOH	trifluoromethane sulfonic acid
THF	tetrahydrofuran
TIPSCl	triisopropylsilyl chloride
TsCl	*p*-toluenesulfonyl chloride
p-TsOH	*p*-toluenesulfonic acid

Chapter 1

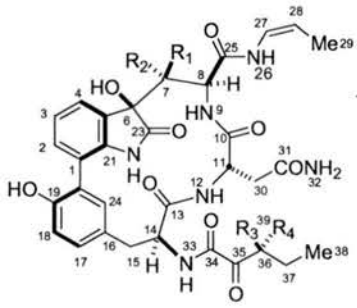
TMC-95; Novel Proteasome Inhibitors

1.1 Introduction

The ubiquitin-proteasome pathway is an ATP-dependent process discovered more than 20 years ago and is the major proteolytic mechanism in the cytosol and nucleus of all eukaryotic cells.[1] Initial studies focused on understanding the importance of this pathway in the regulation of cellular processes originated with biological studies in extracts of mammalian cells and genetic studies in yeasts.[2] It was not until the availability of cell permeable proteasome inhibitors that the physiological roles of the proteasome were understood. These findings have shown that the proteasome catalyzes the degradation of the majority of mammalian proteins, both short- and long-lived.[3] Proteasomal degradation of a large variety of cellular proteins is vital to many of the intracellular processes such as cell cycle progression, apoptosis, inflammation, immune surveillance, selective removal of misfolded or damaged proteins, and the regulation of metabolic pathways.[1] Therefore, specific proteasome inhibitors are of great interest not only as a tool for understanding the ubiquitin-proteasome pathway but also as potential drug candidates.

In early 2000, Kohno and co-workers reported the isolation of novel cyclic tripeptides TMC-95A-D (**1-4**) (Figure 1).[4] TMC-95A-D are potent proteasome inhibitors

Figure 1. Structures of TMC-95A-D



TMC-95	R_1	R_2	R_3	R_4
A (**1**)	H	OH	Me	H
B (**2**)	H	OH	H	Me
C (**3**)	OH	H	Me	H
D (**4**)	OH	H	H	Me

isolated from the fermentation broth of *Apiospora montagnei Sacc. TC 1093,* derived from soil samples. These natural products are unique cyclic peptides containing L-tyrosine, L-asparagine, a highly oxidized L-tryptophan derivative, *(Z)*-1-propenylamine, and 3-methyl-2-oxopentanoic acid subunits. It has been shown that these compounds are biologically active against the chymotrypsin-like (ChT-L), trypsin-like (T-L), and peptidylglutamyl-peptide hydrolyzing activities (PGPH) of the 20S proteasome (Table 1.1).[4]

Table 1.1. Inhibitory activities of TMC-95A-D and ALLN against the 20S proteasome.

Compound	In the presence or absence of 0.02% SDS[a]	IC_{50} (μM)		
		ChT-L	T-L	PGPH
TMC-95A	+SDS	0.0054	0.20	0.060
	-SDS	0.012	1.5	6.7
TMC-95B	+SDS	0.0087	0.49	0.060
TMC-95C	+SDS	0.36	14	8.7
TMC-95D	+SDS	0.27	9.3	3.3
ALLN[b]	+SDS	6.6	6.0	21

[a] Known to activate the 20S proteasome. [b] *N*-acetyl-Leu-Leu-nLeu-CHO, a known proteasome inhibitor.

Since the isolation of TMC-95A-D, it has been determined that TMC-95A shows non-covalent and reversible inhibition of the proteasome, an action not previously observed

with other inhibitors.[5] The great interest emerging in the field of proteasome inhibition, the considerable biological activity, and the distinctive structures of the TMC-95 class of natural products have provided motivation for many research groups to undertake total synthesis programs towards these compounds. Additionally, it is desirable to develop a synthesis that would be readily adaptable for the preparation of analogs.

1.2 Synthetic Efforts toward TMC-95A/B

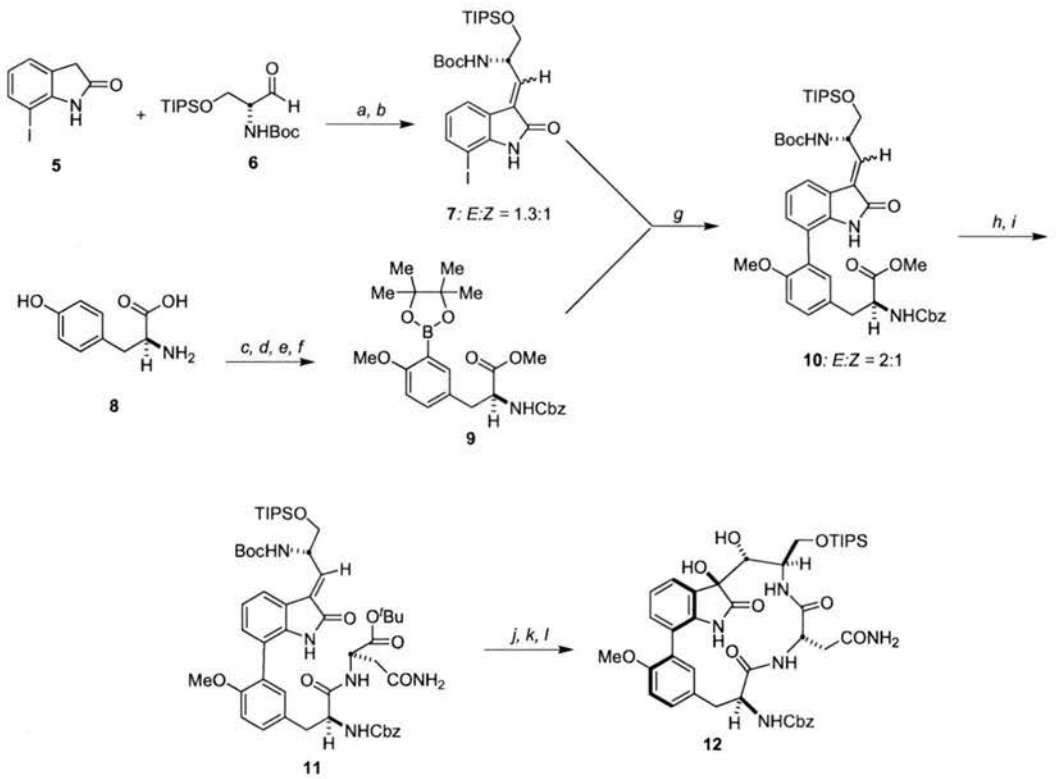
Since the isolation of TMC-95A-D there have been numerous synthetic efforts aimed at the total synthesis of TMC-95A/B. The major hurdles in a total synthesis of TMC-95 that must be faced are the asymmetric preparation of the highly oxidized tryptophan moiety, the biaryl linkage, the macrocyclic core, and the *cis*-enamide. Efforts in each of the above areas have culminated in the total syntheses completed by the Williams[6], Danishefsky[7], and Hirama-Inoue[8] research groups. In addition to the total syntheses reported by these groups, the Ma[9] and Feldman[10] groups have reported efforts toward a total synthesis.

1.2.1a. Danishefsky's Synthesis of TMC-95A/B Macrocyclic Core.

The first total synthesis of TMC-95A/B was reported by Danishefsky and co-workers in 2002.[7] Prior to the completion of the total synthesis, they reported a synthesis of the macrocyclic core of TMC-95A/B. Their synthesis focused on the key biaryl formation via the Suzuki protocol[11] and macrolactamization at the C10-N9 amide bond linkage. The initial stages of the Danishefsky synthesis began with the preparation of the highly oxidized tryptophan fragment. In a pivotal step of their synthesis, Danishefsky found that condensation of the D-serine-derived aldehyde **6** (prepared in two steps from *N*-Boc-D-serine-*O*-methyl ester) with 7-iodooxindole **5** (prepared in four steps from 2-

iodoaniline via the Sandmeyer isatin synthesis[12] and subsequent hydrazine reduction[13] of the ketone) yielded oxindolene **7** (Scheme 1). It was found that a two-step protocol first using lithium diisopropyl amide (LDA) followed by methanesulfonyl chloride in the

Scheme 1.[a] **Danishefsky's synthesis of the TMC-95A/B macrocyclic core**



[a] (a) LDA, THF, -78°C; (b) Et_3N, MsCl, CH_2Cl_2, -60° to -30°C, 76% (2 steps); (c) 1. MeOH, $SOCl_2$; 2. Cbz-Cl, K_2CO_3, H_2O, acetone, 96% (2 steps); (d) LiOH, Me_2SO_4, 86%; (e) I_2, Ag_2SO_4, MeOH, 93%; (f) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, DMSO, 80°C, 95%; (g) $PdCl_2(dppf)$, K_2CO_3, DME, 80°C, 72%; (h) LiOH, THF, H_2O, 0°C; (i) H-Asn-*O*-^{t}Bu, EDCI, HOAt, THF, 70% (2 steps); (j) OsO_4, NMO, $(DHQ)_2PHAL$, ^{t}BuOH, H_2O, 81% of a diastereomeric mixture (34% of desired); (k) TFA, CH_2Cl_2; (l) EDCI, HOAt, $^i Pr_2NEt$, CH_2Cl_2/DMF, 4 mM, 55% (2 steps).

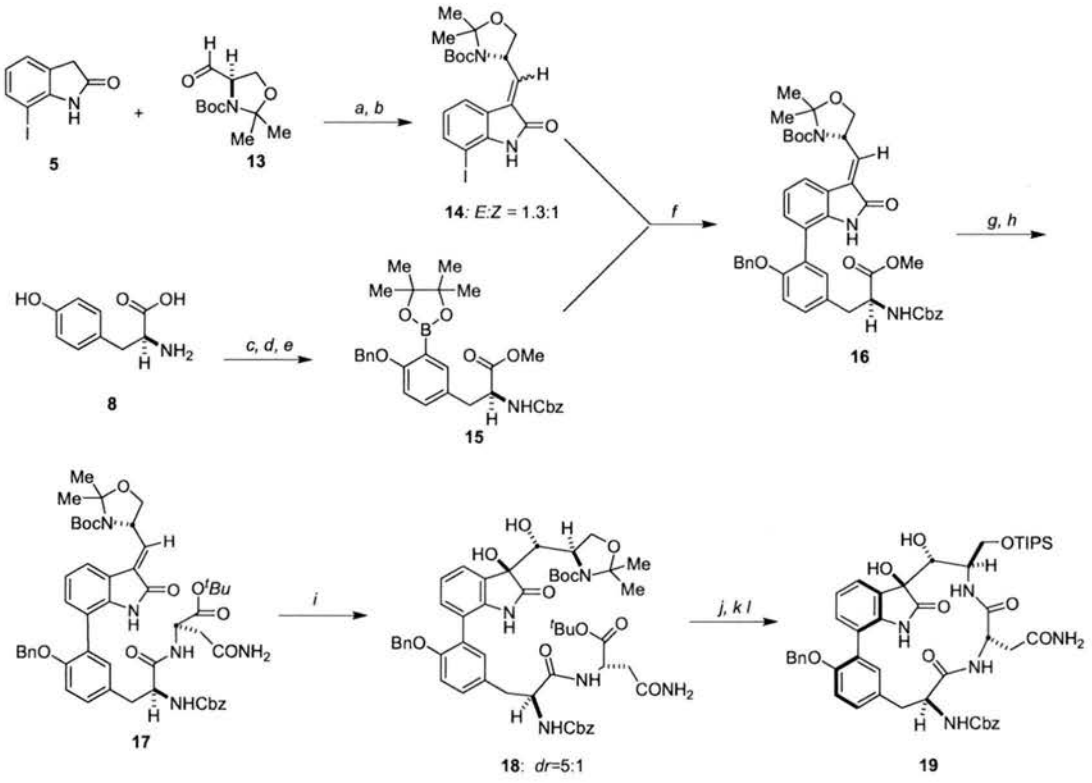
presence of triethylamine gave sufficient yields without the racemization of the serine derived stereocenter. Previous efforts using a one-step protocol catalyzed by piperidine furnished the desired compound with racemization. Using this approach they found that at best they were able to achieve a 1.3:1 *E:Z* ratio of the resulting double bond geometry.

Treatment of the mixture of geometric isomers with boronic ester **9** (prepared in four steps from L-tyrosine utilizing the Miyaura protocol[14]) provided biaryl-coupled product **10**. It was found under these reaction conditions no matter what ratio of double bond isomers was present going into the reaction, a 2:1 *E:Z* mixture was always obtained afterward. At this point, they were able to separate the two isomers, and convert the undesired *Z*-isomer to the desired *E*-isomer with catalytic I_2. Saponification of the tyrosine methyl ester and amide bond formation with L-asparagine-*O*-^{t}Bu-ester provided pseudotripeptide **11**. Incorporation of the C6-C7 diol was accomplished using Sharpless' asymmetric dihydroxylation[15] conditions yielding the diol in a 1:1.4 ratio of desired to undesired stereochemistry. Separation of the two isomers and treatment with TFA allowed for removal of both the Boc carbamate and the *tert*-butyl ester giving the requisite amino acid necessary for macrocyclization. The macrocyclic core of TMC-95 was furnished upon treatment of the amino acid with EDCI and HOAt at high dilution. Although Danishefsky was the first to prepare the macrocyclic core of TMC-95A/B, he does note that the above synthesis contains serious issues regarding the selectivity of the double bond geometry and the stereochemical outcome of the dihydroxylation that must be overcome in order to complete a total synthesis.

1.2.1b. Danishefsky's Total Synthesis of TMC-95A/B

After the initial report on the synthesis of the macrocyclic core of TMC-95A/B, Danishefky's research group reported the first total synthesis of TMC-95A/B. As they stated in their initial report, there still remained significant synthetic issues that must be overcome in order to complete a total synthesis. Some of the issues that still remained in heir synthesis were the incorporation of the 3-methyl-2-oxo-ketoamide at N33, oxidation.

Scheme 2.[a] **Danishefky's total synthesis of TMC-95A/B: Part 1**



[a] (a) LDA, THF, -78°C; (b) MsCl, Et_3N, CH_2Cl_2, 81%; (c) 1. MeOH, $SOCl_2$; 2. Cbz-Cl, K_2CO_3, H_2O, acetone; 3. BnBr, Cs_2CO_3, acetone, Δ, 88% (3 steps); (d) I_2, Ag_2SO_4, MeOH, 99%; (e) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMSO, 80°C, 91%; (f) $PdCl_2$(dppf), K_2CO_3, DME, 80°C, 75%; (g) LiOH, THF, H_2O, 0°C; (h) H-Asn-*O*-^{t}Bu, EDCI, HOAt, THF, 85% (2 steps); (i) OsO_4, NMO, $(DHQD)_2PHAL$, ^{t}BuOH, H_2O, 88% (73% of desired isomer); (j) PPTS, MeOH, Δ; (k) TIPSCl, imidazole, DMAP, CH_2Cl_2, 88%; (l) 1. TFA, CH_2Cl_2; 2. EDCI, HOAt, $^i Pr_2NEt$, CH_2Cl_2/DMF, 2 mM, 52% (2 steps).

of C25 to its carboxyl oxidation state, the facial selectivity in the oxidation of C6 and C7 and the preparation of the *cis*-propenylamide at N26. In the revised synthesis of the highly oxidized tryptophan fragment, Danishefsky's group performed a condensation reaction similar to that which the Ma group had previously reported (see Section 1.2.3). Treatment of 7-iodooxindole **5** with the D-serine-derived Garner aldehyde **13**[16] yielded oxindolene **14** in a 1.3:1 *E:Z* ratio (Scheme 2). Suzuki coupling of aryl iodide **14** with

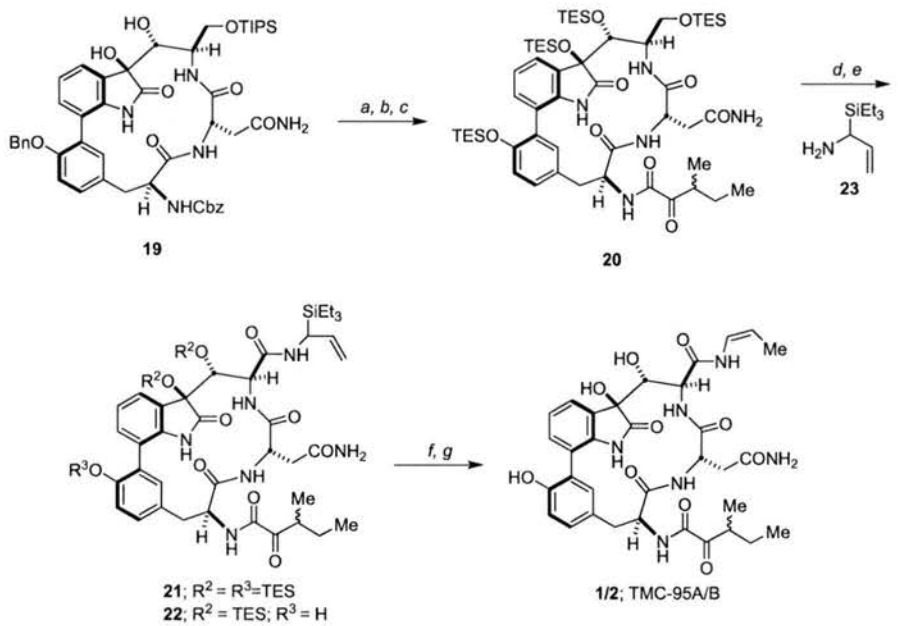
the boronic ester **15** (prepared in 5 steps from L-tyrosine) afforded the corresponding biaryl **16**

Upon the completion of the biaryl portion of TMC-95A/B, the next focus was on the completion of the macrocyclic core. Hydrolysis of methyl ester **16** followed by amide bond formation with asparagine-*tert*-butyl ester afforded pseudo tripeptide **17**. As in the previous synthesis of the macrocyclic core, Sharpless asymmetric dihydroxylation afforded the C6-C7 diol in a modest 5:1 diastereomeric ratio (favored to disfavored). They found that in order to obtain satisfactory facial selectivity in the dihydroxylation of the C6-C7 double bond it was necessary to have the allylic oxazolidine ring system present. Over the next few steps in Danishefsky's total synthesis, several protecting group manipulations were necessary in order to complete TMC-95A/B. First, removal of the *N,O*-acetonide liberated the free primary alcohol which was reprotected as a TIPS ether. The requisite amino acid for macrocyclization was prepared by treatment of the Boc-carbamate and the *tert*-butyl ester with TFA. Macrocyclization of this amino acid was mediated by EDCI and HOAt under high dilution to give macrocycle **19** in 52% yield over the two steps.

With macrocycle **19** in hand, what remained for the Danishefsky group was the oxidation of C25 and the incorporation of both the ketoamide side chain and the *cis*-propenyl amide. Macrocycle **19** was treated under Pd-mediated hydrogenolysis to remove the phenolic benzyl ether and the Cbz-carbamate in a single manipulation (Scheme 3). Selective amide bond formation at N33 with (±)-3-methyl-2-oxo-pentanoic acid afforded the corresponding amide. Danishefsky stated that incorporation of an

optically active pentanoic acid derivative would be futile due to the propensity for epimerization of the C36 methyl group. In any event, the resulting ketoamide was treated

Scheme 3.[a] Danishefky's total synthesis of TMC-95A/B: Part 2

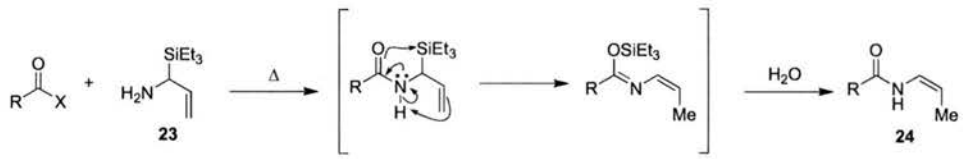


[a] (a) 1. Pd/C, H_2, EtOH; 2. (±)-3-methyl-2-oxo-pentanoic acid, EDCI, HOAt, CH_2Cl_2, DMF, 85% (2 steps); (b) HF/pyridine; (c) 1. TESOTf, 2,6-lutidine, CH_2Cl_2; 2. $NaHCO_3$; 3. citric acid, EtOAc, H_2O, 73% (4 steps); (d) Jones reagent, acetone, 0°C; (e) **23**, EDCI, HOAt, CH_2Cl_2, DMF, 45% (2 steps); (f) *o*-xylene, 140°C, 2 days; (g) HF/pyridine, THF, pyridine; then Me_3SiOMe, 49% (2 steps).

with TBAF to remove the primary TIPS ether. The resulting tetraol was then globally protected with TES groups to afford the protected macrocycle **20**. In a key step, Danishefsky found that treatment of TES-protected macrocycle **20** with Jones reagent[17] allowed for sequential deprotection of the primary silyl ether followed by oxidation of the alcohol to the acid. It must also be noted that this sequence deprotected some of the phenolic silyl group. This four-component mixture was carried throughout the remainder of the synthesis without separation.

In addition to the total synthesis, Danishefsky's group developed a new method for the preparation of *cis*-propenyl amides. They found that when amine **23** was condensed to form a variety of amides that when heated underwent a silyl rearrangement to form *cis*-propenyl amides of type **24** (Scheme 4). It was this method

Scheme 4. Generation of *cis*-propenyl amides.



that Danishefsky used to complete the total synthesis. Treatment of the resultant carboxylic acid from the oxidation of **20** with amine **23** followed by heating in *o*-xylene at 140°C for 2 days furnished the corresponding *cis*-propenyl amide. The final step in Danishefsky's total synthesis was the deprotection of the remaining silyl ethers to afford TMC-95A/B as a mixture of C36 diastereomers which could be separated.

In summary, Danishefsky's total synthesis features the preparation of the highly oxidized tryptophan moiety from a condensation reaction of 7-iodooxindole and the Garner aldehyde, Suzuki coupling for the incorporation of the biaryl bond and a modestly selective dihydroxylation. Most notable in Danishefsky's total synthesis is the new methodology developed for the preparation of *cis*-propenyl amides. Overall Danishefky's synthesis was accomplished in 28 total steps with 23 steps in the longest linear sequence.

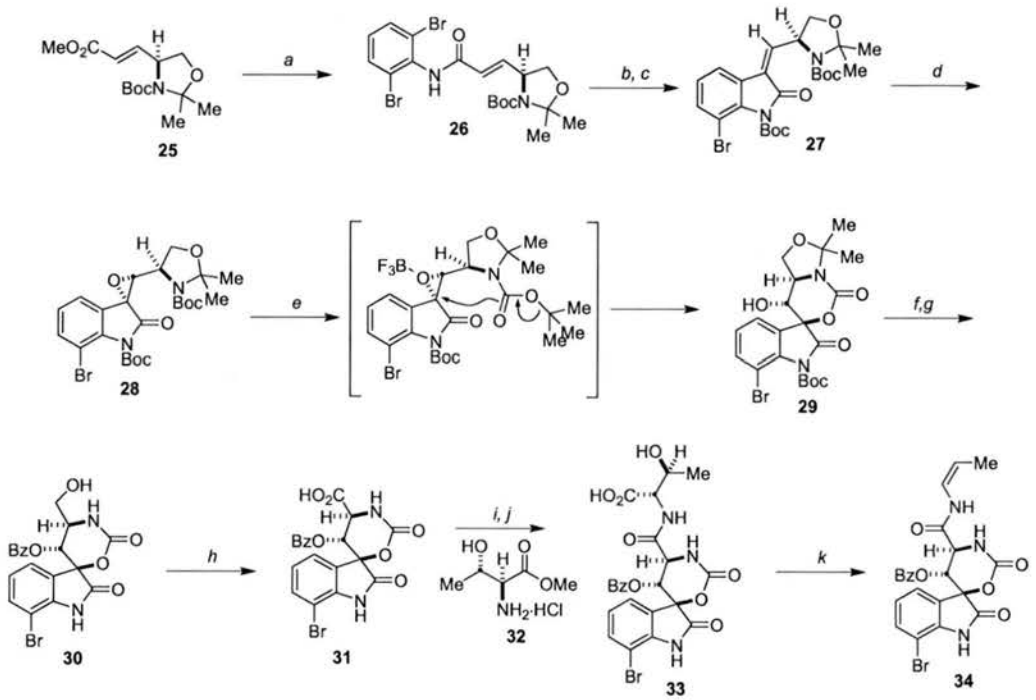
1.2.2a. Hirama and Inoue's Synthesis of the Northern Fragment of TMC-95A

In their initial communication, Hirama, Inoue and co-workers reported the synthesis of the highly oxidized tryptophan fragment. Their synthesis showcased a stereocontrolled synthesis of the highly oxidized tryptophan residue via a *Z*-selective

Heck[18] coupling, a diastereoselective epoxidation to incorporate the C6-C7 diol and a 1,3-elimination reaction of an L-*allo*-threonine derivative to prepare the *cis*-propenyl amide.

Amidation of the α,β-unsaturated methyl ester **25** (derived from the Garner aldehyde in one step) with 2,6-dibromoaniline afforded α,β-unsaturated amide **26** (Scheme 5). Intramolecular Heck reaction produced only the *E*-isomer of **27**.

Scheme 5.[a] The Hirama/Inoue synthesis of the northern fragment.



[a] (a) 2,6-dibromoaniline, Me_3Al, PhMe, 0°C→RT, 59%; (b) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 88%; (c) $Pd_2(dba)_3{\cdot}CHCl_3$, Et_3N, THF-NMP, 86%; (d) DMDO, CH_2Cl_2; (e) $BF_3{\cdot}Et_2O$, CH_2Cl_2, -78°C→0°C, 87% (2 steps); (f) BzCl, Et_3N, DMAP, CH_2Cl_2, 83%; (g) TfOH, CF_3CH_2OH, 0°C→rt, 64%; (h) CrO_3, H_5IO_6, wet MeCN, 0°C; (i) **32**, EDCI, HOBt, NMM, DMF, 0°C→rt, 60%(2 steps); (j) LiOH, THF, H_2O, 0°C; (k) DEAD, PPh_3, THF, -78°C→RT, 40% (2 steps).

Diastereoselective epoxidation of **27** with DMDO, followed by intramolecular opening of the epoxide provided spiro-oxazolidinone **29** which possessed the desired

stereochemistry at both C6 and C7. Esterification of the C7 secondary alcohol with benzoyl chloride followed by triflic acid-mediated removal of the Boc group and acetonide to produced primary alcohol **30**. Oxidation of the primary alcohol to the carboxylic acid, followed by coupling to L-*allo*-threonine methyl ester produced the desired dipeptide. The subsequent step involved conversion of the L-*allo*-threonine residue to the *cis*-propenyl amide via a decarboxylation-elimination method previously described by the Vederas[19] group. Hydrolysis of the L-*allo*-threonine methyl ester followed by treatment of the resulting β-hydroxycarboxylic acid with Mitsunobu conditions[20] produced the desired *cis*-propenyl amide **34** as a single isomer. Their preparation of **34** was achieved in 13 linear steps.

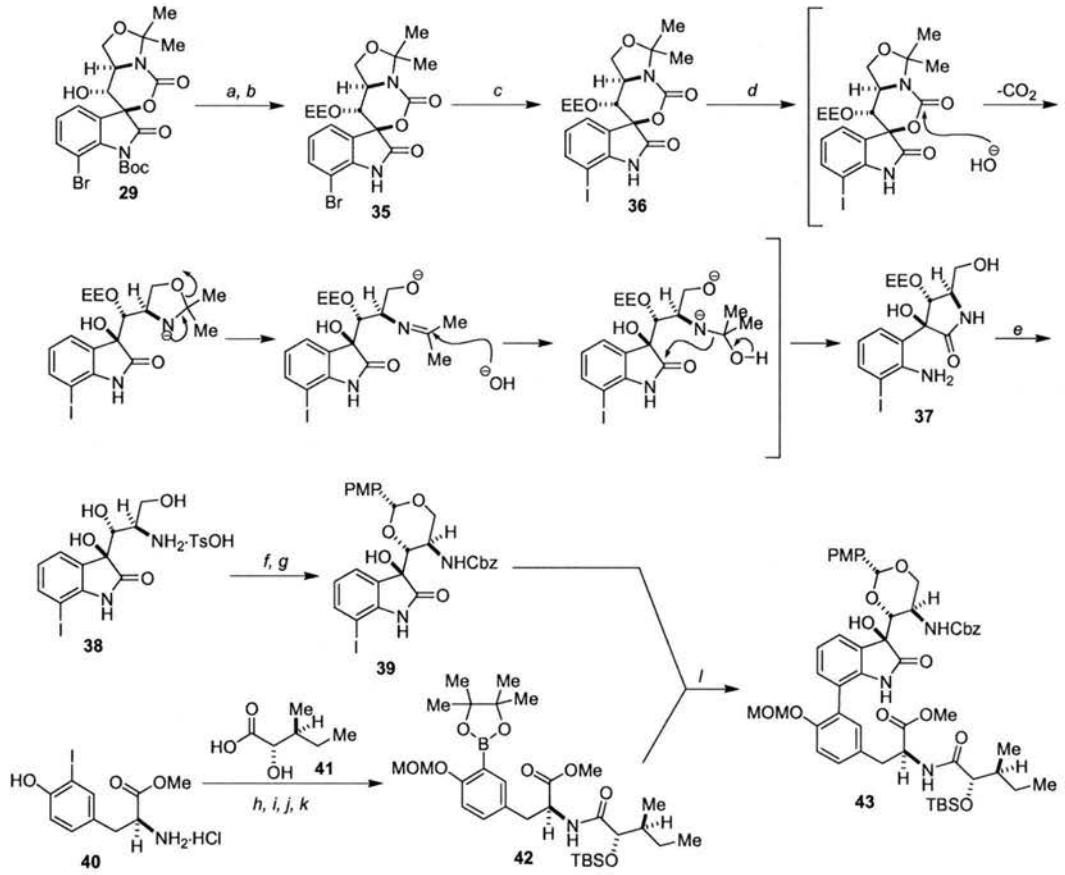
1.2.2b. Hirama and Inoue's Total Synthesis of TMC-95A

The efforts reported by Hirama, Inoue and co-workers culminated in the first total synthesis of TMC-95A with absolute control of all stereochemical entities. Where Danishefsky had shown that the C36 α and β stereoisomers convert very rapidly under a variety of conditions, Hirama and Inoue have reported their method for circumventing this problem and ultimately preparing only TMC-95A. Initially they aimed to utilize enamide **34** as a starting point for the completion of the total synthesis. Unfortunately, elaboration of this intermediate was unsuccessful. They were, however, able to utilize many of their previously reported key transformations in their total synthesis.

Although they were unable to transform enamide **34** to TMC-95A, they were able to elaborate on spiro-oxazolidinone **29.** Removal of the Boc group followed by treatment with ethyl vinyl ether afforded the C7 ethoxy ethyl ether **35** (Scheme 6). Presumably the

aryl bromide in **35** was not an efficient precursor to a palladium-catalyzed biaryl coupling reaction. Therefore it was necessary to convert the aryl bromide to the aryl iodide **36**

Scheme 6[a]. Hirama, Inoue's total synthesis of TMC-95A: Part 1



[a] (a) $Mg(ClO_4)_2$, MeCN, 50°C; (b) ethyl vinyl ether, PPTS, THF, 35°C, 94% (2 steps); (c) ^{n}BuLi, ICH_2CH_2I, THF, -60°C→rt, 86%; (d) ^{t}BuOK (15 eq.), H_2O (10 eq.), Et_2O, RT, 74%; (e) *p*-TSOH, H_2O, MeOH, RT; (f) CbzCl, Et_3N, DMF, 0°C→rt, 55% (3 steps); (g) *p*-MeOPhCH(OMe)$_2$, *p*-TsOH, THF, 84%; (h) **41** (2 eq.), EDCI, HOBt, NMM, DMF, 0°C, 56%; (i) MOMCl, K_2CO_3, acetone, 85%; (j) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0°C, 88%; (k) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMSO, 80°C, 71%; (l) $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O (4:1), 95°C, 84%.

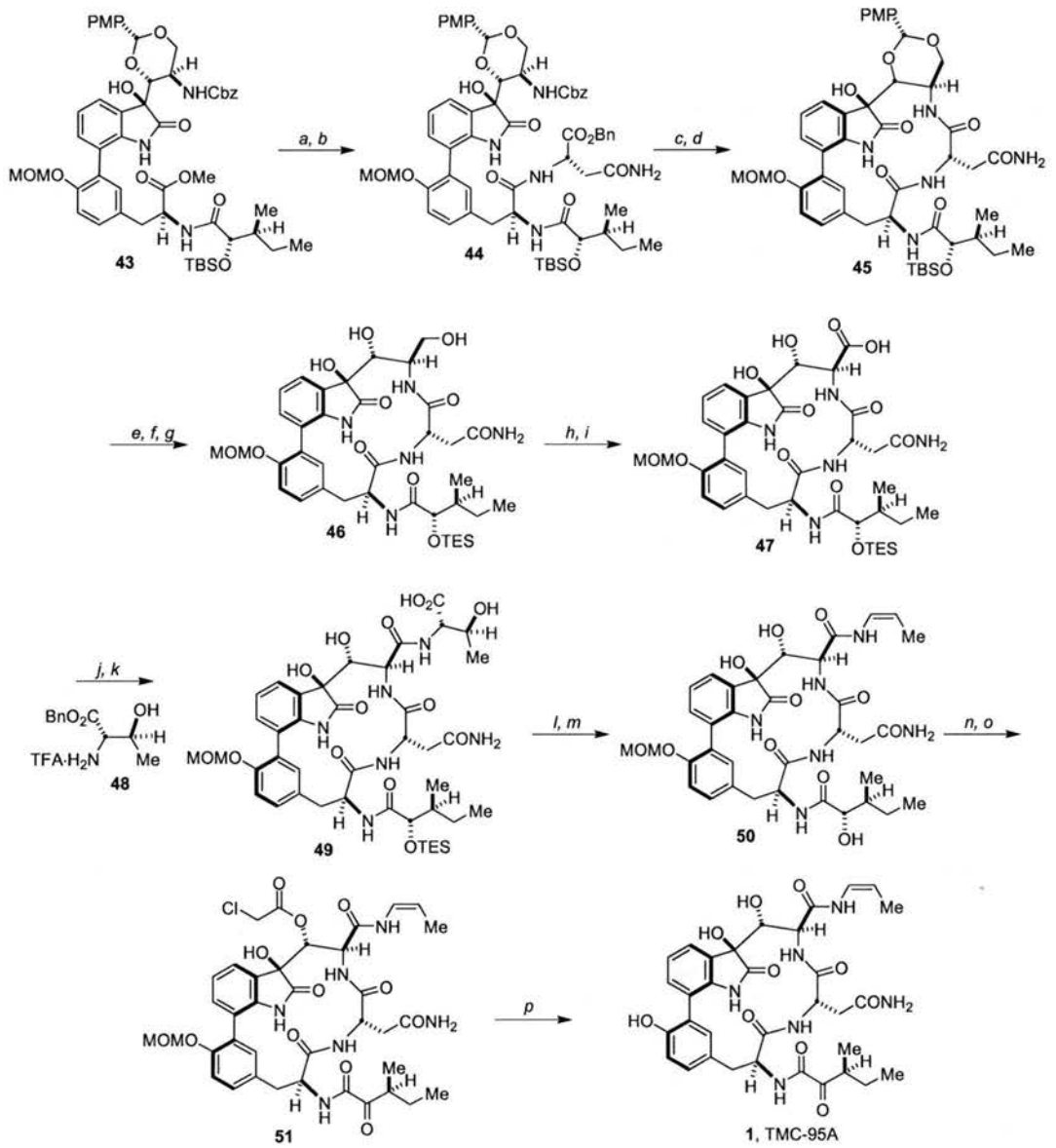
through halogen metal exchange and iodination with diiodoethane. Next it was necessary to remove the cyclic urethane in **36**. They found that the urethane was extremely resistant to a variety of conditions. Ultimately it was found that treatment of **36** with

Gassman's "anhydrous hydroxide" conditions[21] provided aniline **37** in 74% yield. It was proposed that **37** was produced by first loss of carbon dioxide upon addition of hydroxide to the carbamate, followed by ketimine formation, loss of acetone, and oxindole acyl migration to the more nucleophilic primary amine, affording aniline **37**. Although not the desired product, they were able to convert **37** to the suitably protected oxindole **39** over 3 steps.

Simultaneously, the Hirama/Inoue group prepared the necessary precursor for the critical biaryl-forming reaction. Coupling of 3-iodo-L-tyrosine methyl ester hydrochloride **40** with α-hydroxycarboxylic acid **41**, followed by several protecting group manipulations resulted in the desired dipeptide aryl iodide. Conversion of the aryl iodide to its boronic ester afforded biaryl-coupling precursor **42**. Aryl iodide **39** and aryl boronic ester **42** underwent facile Suzuki coupling to provide biaryl **43**.

Incorporation of the asparagine residue was accomplished via coupling of L-asparagine benzyl ester to the saponification product of methyl ester **43** yielding the pseudotripeptide **44** (Scheme 7). Hydrogenolysis of the benzyl ester and Cbz group liberated the requisite macrocyclization precursor. Subjection of this amino acid to EDCI and HOBt in DMF at 0°C gave an impressive 77% yield of the cyclized product **45**. Initial studies toward the completion of TMC-95A had shown that late stage removal of the C35 TBS-ether proved to be problematic. Therefore, it was decided to remove the TBS group from **45** and replace it with the more labile TES-ether, allowing for a more facile removal of this protecting group at a later stage. Next, the PMP benzilidene acetal was removed with $Zn(OTf)_2$, EtSH and $NaHCO_3$ allowing for subsequent oxidation.

Scheme 7[a]. Hirama, Inoue's total synthesis of TMC-95A: Part 2



[a] (a) LiOH, THF/H_2O (1:1), 0°C; (b) H-Asn-OBn·TFA, EDCI, HOBt, DMF, 0°C, 75% (2 steps) (c) $Pd(OH)_2/C$, H_2, THF/H_2O (1:1); (d) EDCI, HOAt, DMF, 0°C, 77% (2 steps); (e) TBAF, 4 Å MS, THF, 86%; (f) TESCl, imidazole, DMF, 85%; (g) $Zn(OTf)_2$, EtSH, $NaHCO_3$, CH_2Cl_2, 100%; (h) SO_3·pyridine, Et_3N, DMSO/CH_2Cl_2 (1:3), rt; (i) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, $^tBuOH/H_2O$ (5:1), rt; (j) **48**, EDCI, HOBt, DMF, 0°C, 67% (3 steps); (k) $Pd(OH)_2/C$, H_2, THF/H_2O (1:2); (l) DEAD, PPh_3, 4 Å MS, 0°C→rt, 59% (2 steps); (m) HF·pyridine, THF, 79%; (n) Dess-Martin periodinane, CH_2Cl_2, 80%; (o) $(ClCH_2CO)_2O$, pyridine, CH_2Cl_2, 0°C; (p) aq. 1M HCl/THF (3:1), RT; then sat. $NaHCO_3$, 64% (2 steps).

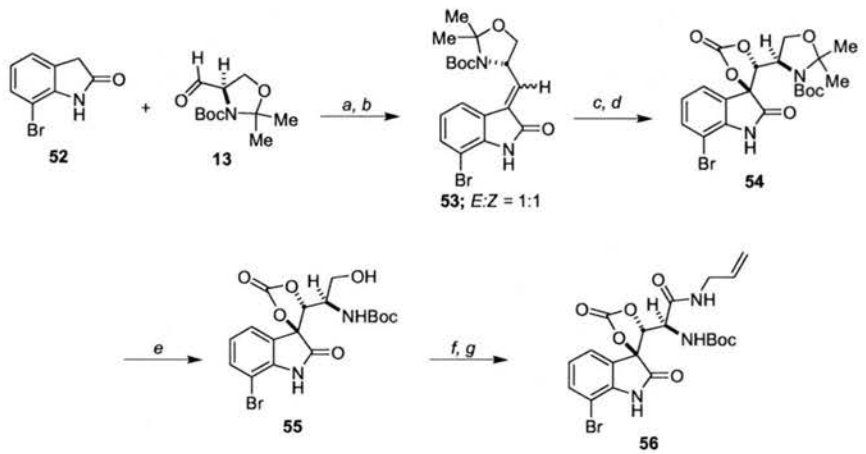
Oxidation of the primary alcohol to the corresponding carboxylic acid was accomplished in a two-step protocol using SO_3·pyridine, DMSO and Et_3N followed by sodium chlorite oxidation of the resulting aldehyde to afford carboxylic acid **47**. In a similar manner to what Hirama/Inoue had previously reported, carboxylic acid **47** was coupled to L-*allo*-threonine residue **48**. Palladium-catalyzed hydrogenolysis, treatment with Mitsunobu conditions and removal of the C35 TES-ether afforded compound **50.** Oxidation of the resultant C35 secondary alcohol under Dess-Martin periodinane[22] conditions, and protection of the C7 secondary alcohol as its chloroacetyl ester provided **51**. With the key C35 ketone intact it was necessary to complete the total synthesis without the epimerization of the C36 methyl group. It was found that the protection of the C7 secondary alcohol was necessary in order to complete this process. If the C7 alcohol was left unprotected, they were unable to deprotect the phenolic MOM-ether without decomposition. Fortunately, treatment of the bis-protected compound **51** with hydrochloric acid followed by sodium bicarbonate afforded TMC-95A in 64% yield over the last two steps. Overall the Hirama/Inoue total synthesis of TMC-95A proceeds in 40 total steps and 35 linear steps.

1.2.3. Ma's Synthesis of the Highly Oxidized Tryptophan Fragment.

The first reported synthetic efforts towards the TMC-95 class of proteasome inhibitors came from the Ma group. In their initial report, they demonstrated an approach to the highly oxidized tryptophan moiety. First, treatment of 7-bromoisatin (prepared via the Sandmeyer procedure) with hydrazine afforded 7-bromooxindole **52**. After a survey of experimental conditions the stereochemical integrity of the product was retained when 7-bromooxindole was treated with ^{n}BuLi followed by the Garner aldehyde **13** to furnish

the aldol product (Scheme 8). In order to obtain the desired alkene, the diastereomeric mixture was treated with methanesulfonyl chloride and triethylamine yielding

Scheme 8[a]. Ma's synthesis of the highly oxidized tryptophan fragment.



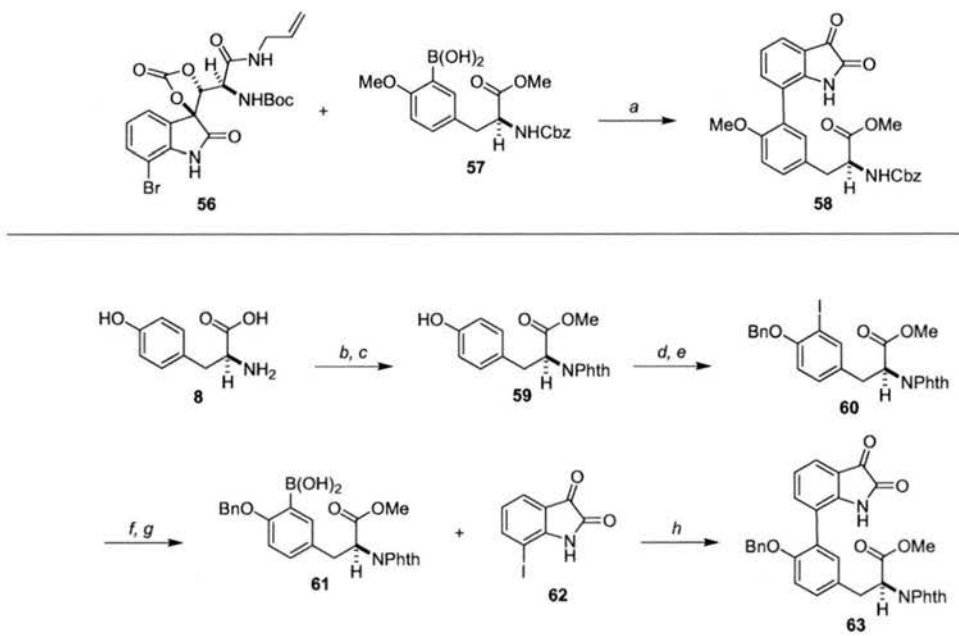
[a] (a) ^{n}BuLi, THF, -78°C; (b) MsCl, Et_3N, CH_2Cl_2, -60°C, 76%; (c) OsO_4, pyridine, THF, 0°C, 79%; (d) CDI, 91%; (e) TMSCl, $^{n}Bu_4NI$; (f) Jones oxidation; (g) allylamine, DCC, HOBt, 65% (3 steps).

oxindolene **53** as a ~1:1 mixture of *E:Z* isomers. This method was later used in the total synthesis of TMC-95A/B by the Danishefsky group. After separation of the two geometric isomers and isomerization of the *Z*-isomer to the *E*-isomer catalyzed by iodine, the *E*-isomer was treated with stoichiometric OsO_4 in pyridine and THF to yield the desired diol. Protection of the diol as its cyclic carbonate with carbonyl diimidazole provided **54**. Removal of the *N,O*-acetonide afforded primary alcohol **55**. Jones oxidation and coupling to allylamine gave the fully oxidized tryptophan fragment **56**.

In a second report, the Ma research group attempted to realize a biaryl coupling with previously synthesized fully oxidized tryptophan fragment **56**. They found that treatment of aryl bromide **56** with a tyrosine-derived boronic acid **57** afforded only the undesired coupled product **58** in 19% yield (Scheme 9). In light of these results they

decided to pursue the Suzuki coupling of a simplified substrate. Preparation of the tyrosine-derived boronic acid **61** began with the protection of L-tyrosine **8** as its phthalate methyl ester **59**. Benzylation of the phenol and *ortho*-iodination provided the corresponding aryl iodide **60**. Palladium-catalyzed formation of the boronic ester was followed by hydrolysis to the boronic acid **61**. Finally, ligandless palladium-catalyzed Suzuki coupling of boronic acid **61** and 7-iodoisatin **62** with $Pd(OAc)_2$ and KF in methanol at 20°C for 3 days yielded coupled product **63** in 64% yield. The Ma group was able to prepare a highly oxidized tryptophan fragment **56** in 7 steps and also utilized a ligandless palladium-catalyzed Suzuki coupling to form the biaryl portion.

Scheme 9[a]. Ma's synthesis of the biaryl moiety of TMC-95.



[a] (a) $Pd(OAc)_2$, Na_2CO_3, 19%; (b) *N*-carboethoxypthalimide; (c) HCl, MeOH, 76% (2 steps); (d) BnBr, K_2CO_3; (e) I_2, AgOTFA, 85% (2 steps); (f) pinacolborane, $PdCl_2(dppf)$, Et_3N; (g) 1. diethanolamine; 2. 1 M HCl, 67% (3 steps); (h) $Pd(OAc)_2$, KF, MeOH, 20°C, 3 days, 64%.

1.3 Biological Studies of TMC-95 Analogs

1.3.1 TMC-95A-20S Proteasome Co-Crystal Structure

Shortly after the isolation of the TMC-95 proteasome inhibitors, there was a flurry of reports focusing on the biological activity of these molecules. The preparation of synthetic analogs that followed were based on the structural work done by Groll et. al.[5] Groll and co-workers were able to co-crystallize TMC-95A and the 20S proteasome derived from yeast. The TMC-95A:20S proteasome complex revealed that TMC-95A was a non-covalent proteasome inhibitor and did not modify any of the nucleophilic threonine residues necessary for hydrolytic activity (Figure 1.3.1). From the co-crystal structure, they were able to determine which portions of TMC-95A were important for binding to the proteasome (Figure 1.3.2). It was observed that the *cis*-propenyl amide interacted specifically with the S1 pocket of the proteasome and the asparagine side chain interacted with the S3 pocket. Also, the rigidity of the macrocycle was essential for binding due to entropic considerations. Therefore, simplified TMC-95A analogs

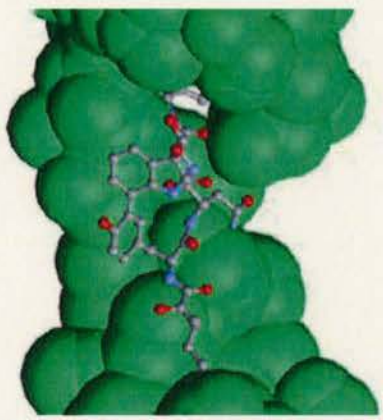


Figure 1.3.1. Magnification of the TMC-95A:20S proteasome crystal structure adapted from the Protein Data Bank. One should note the absence of any covalent bonds between TMC-95A (gray backbone) and the 20S proteasome (green).

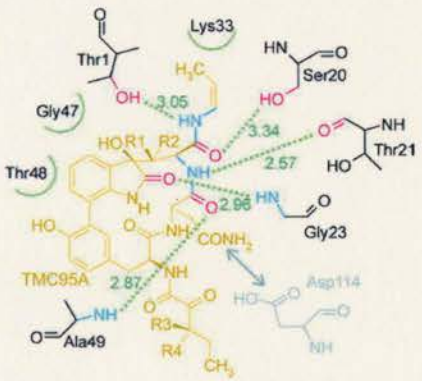


Figure 1.3.2. Hydrogen bonding and hydrophobic interactions between TMC-95A and the 20S proteasome necessary for efficient binding. (taken from ref. 5)

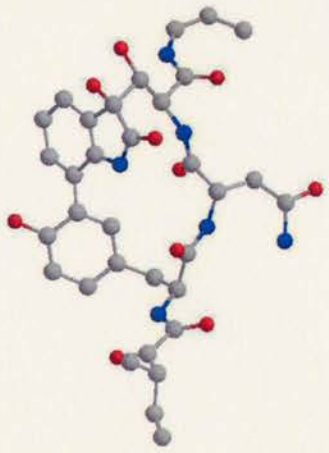


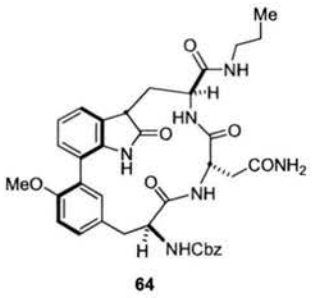
Figure 1.3.3. Crystal structure of TMC-95A extracted from the TMC-95A:20S proteasome crystal structure complex.

could be prepared that still retain biological activity if these essential binding motifs were still present.

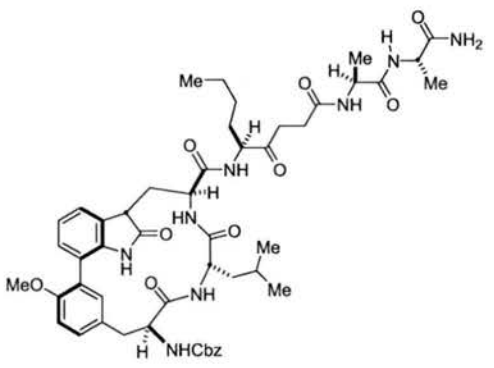
1.3.2 Moroder Analogs

Shortly after the crystal structure of TMC-95A and the 20S proteasome complex was reported, Moroder et. al. reported the first active synthetic analog of TMC-95.[23] Based on results from the co-crystal structure, the Moroder group hypothesized that perhaps they could prepare simplified TMC-95 analogs by modification of the *cis*-propenyl amide and ketoamide moieties. They were able to show that this hypothesis was valid through the preparation of macrocyclic analog **64** (Figure 1.3.3). Although not as potent against ChT-L as TMC-95A, analog **64** maintained the same order of magnitude potency against T-L and PGHP compared with TMC-95A. With the above information in hand, Moroder and co-workers prepared analog **65** hoping to increase the potency against ChT-L through an increased binding affinity with the S1 pocket.[24] As proposed, analog **65** did indeed increase in potency against ChT-L while retaining its T-L and PGHP activity.

Figure 1.3.3. Moroder et. al. analogs.



64



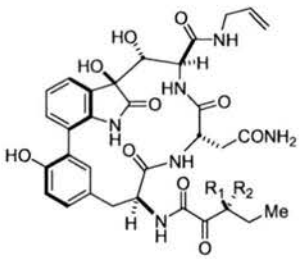
65

1.3.2 Danishefsky Analogs

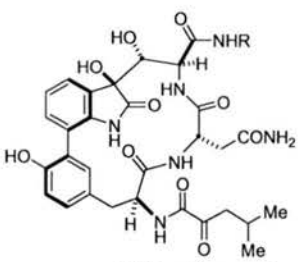
After the completion of their total synthesis, the Danishefsky group utilized their synthetic route to prepare a variety of simplified TMC-95 analogs (Figure 1.3.4).[25] In all of their synthetic analogs, Danishefsky decided to leave the macrocyclic backbone unchanged, especially the oxidation at C6 and C7. Similar to what the Moroder group had done, the Danishefsky group decided to modify the portions of TMC-95A that interact with the S1 and S3 pockets of the proteasome. The analogs that were prepared by the Danishefsky group have four different variations: (1) substitution of the *cis*-propenyl amide by either an allyl amide or propyl amide (compounds **66**, **67**, **68**, **69**, **70**, **71**, and **72**); (2) oxidation state of the C25 carbon reduced to an alcohol (compounds **73**, **74**, and **75**); (3) substitution of the asparagine amide side chain by a nitrile (compounds **73** and **74**); (4) simplification of the ketoamide side chain by preparing analogs without a stereogenic center (compounds **68**, **69**, **70**, **71**, **72**, **73**, **74**, and **75**). They determined that the ketoamide side chain has little effect on proteasome inhibition and can be simplified in order to avoid mixtures of diastereomers that are associated with the natural products.

It also appears that the *cis*-propenyl amide can be replaced with an allyl amide without significant loss of activity, but that the more flexible propyl amide caused a dramatic decrease in all three activities. They also found that analogs containing the C25 alcohol oxidation state have almost no activity. They hypothesized that this may be due to the lack of hydrogen bonding and hydrophobic contacts with the proteasome.

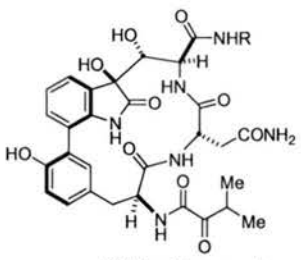
Figure 1.3.4. Danishefsky analogs.



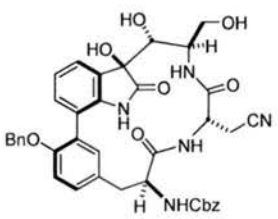
66; R_1= Me; R_2=H
67; R_1= H; R_2=Me



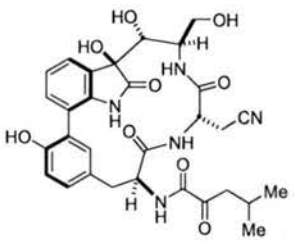
68; R = *(Z)*-propenyl
69; R = allyl



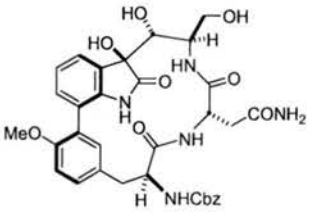
70; R = *(Z)*-propenyl
71; R = allyl
72; R = *n*-proply



73



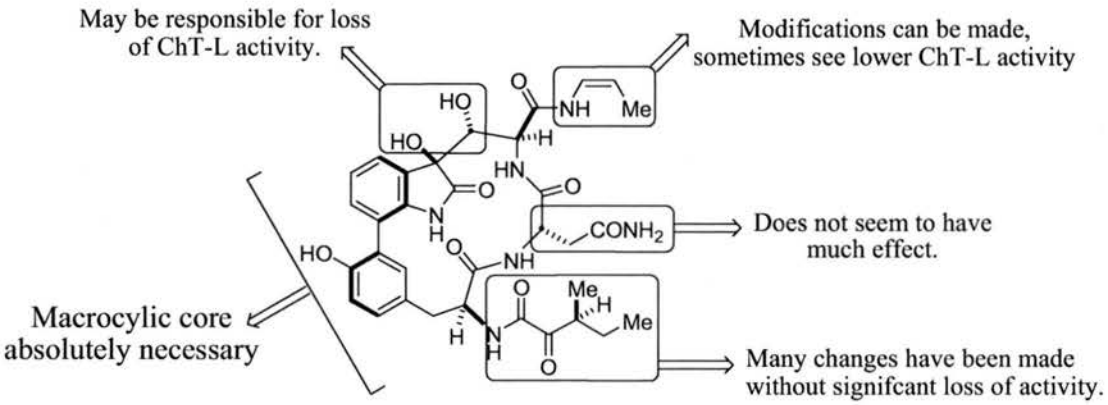
74



75

Unfortunately the analog activity data from each research group is reported using different measurements. Therefore, only through an indirect comparison to the natural products is one able to compare the different analogs. At this stage of the molecule's existence it might be more noteworthy to comment on the flexibility of the manipulations that can be made while still retaining active compounds. Below is a quick summary of which parts of TMC-95A can be modified while still retaining activity (Figure 1.3.3).

Figure 1.3.3. Where modifications to TMC-95A can exist.



1.4 Conclusion

In only a short period of time since their isolation, the TMC-95 proteasome inhibitors have garnered tremendous amounts of synthetic attention. Presumably this is due to the novel structures and the potent inhibitory activity. In addition to the work done in the total synthesis arena, these compounds have received considerable attention as a starting point for the preparation of synthetic analogs. Also, the results described by a variety of groups show the power of chemical synthesis to identify simpler analogs while still retaining inhibitory activity.[23-25]

Chapter 2

Total Synthesis of TMC-95A/B: Initial research

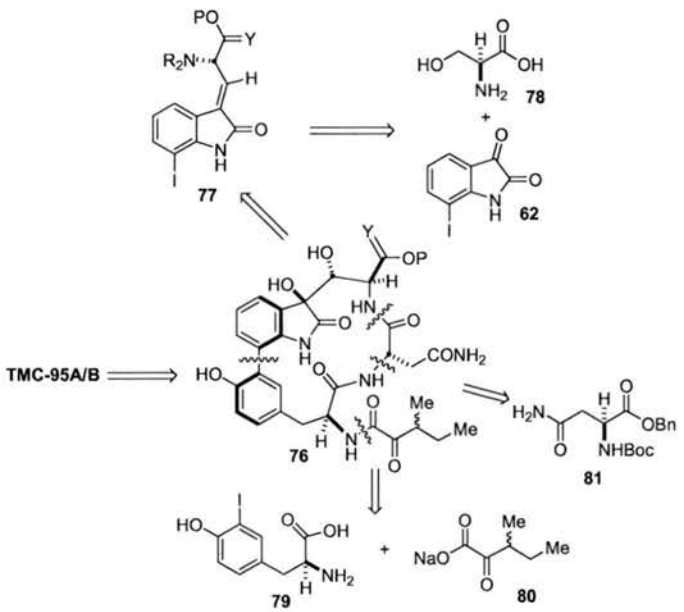
2.1 Retrosynthetic Analysis of TMC-95A/B

In choosing TMC-95A/B as a total synthesis target and the project on which my graduate education would be based, it was felt that this class of molecules would invoke a vast array of synthetic chemistry. Our goal was to prepare TMC-95A/B in a convergent and stereoselective manner. Initially TMC-95A/B were chosen as synthetic targets over TMC-95C/D for two reasons; (1) TMC-95A/B have more potent activity; and (2) it was felt incorporation of the C6, C7 *anti*-diol relationship would lend itself nicely to our proposed synthetic route.

Retrosynthetically, it was felt that TMC-95A/B could eventually be derived from a macrocyclic core such as **76** (Figure 2.1). In order to complete this macrocyclic core, it was necessary to first prepare the highly oxidized tryptophan moiety (northern half), a tyrosine derivative (southern half), and an asparagine residue. Through a biaryl bond-forming reaction and two amide bond couplings the macrocycle **76** would be complete. As simple as this concept appeared, we understood the complexity that stood in front of us.

It was reasoned that the highly oxidized tryptophan fragment could arise from an oxindolene derivative of type **77** via oxidation of the C6-C7 double bond to the fully oxidized tryptophan fragment. At the initial stage, we were uncertain which oxidation state of C25 would allow for the completion of TMC-95A/B, therefore it has generically

Figure 2.1 Retrosynthetic analysis of TMC-95A/B



been assigned as Y. It was also felt that the oxindolene derivative **77** would eventually evolve from L-serine **78** and 7-iodoisatin **62.**

Upon preparation of the highly oxidized tryptophan derivative, the biaryl portion could be installed through either a Stille[26] or Suzuki coupling of a derivative of commercially available 3-iodo-L-tyrosine **79**. Incorporation of the ketoamide side chain from commercially available (±)-3-methyl-2-oxo-pentanoic acid sodium salt **80** followed by installation of the asparagine residue from a protected L-asparagine derivative **81**. Therefore, through a convergent synthesis we would be able to prepare macrocycle **76**.

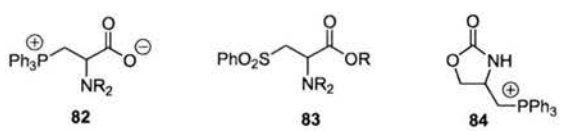
Our initial efforts towards TMC-95A/B were mainly focused on the preparation of the macrocycle described above. First, it was necessary to deal with the oxidation state of C25. Our initial plan was to have the correct oxidation state in place before the preparation of the macrocycle. However we knew that this strategy might prove problematic because β-γ unsaturated amino acid derivatives of type **77** are known to

undergo facile epimerization[27] in the carboxyl oxidation state, therefore we devised another strategy utilizing the alcohol oxidation state. If the latter case proved to be the viable route, then we would have to deal with a selective oxidation of C25. In either event, our initial attempts were to prepare substrates containing both the C25 carboxyl oxidation state and the alcohol oxidation state.

2.2 Initial Attempts Toward the Highly Oxidized Tryptophan Fragment

Since our research group is heavily involved in the asymmetric synthesis of non-proteinogenic amino acids, the highly oxidized tryptophan fragment was the most intriguing component of TMC-95A/B for us. Our synthetic plan for the preparation of this component was to oxidize an oxindolene of type **77**. In the process of preparing oxindolene **77**, we were hoping to develop a general method for the preparation of β,γ-unsaturated amino acids and/or amino alcohols. A survey of the literature shows that there are a few methods for the preparation of β,γ-unsaturated amino acids/alcohols from nucleophilic serine-derived synthons. These synthons have been developed in the labs of Itaya[28] (general structure **82**), Sasaki[29] (**83**), and Sibi[30] (**84**) (Figure 2.2) and all have their advantages and limitations. Although we felt that these synthons could perhaps accomplish the task at hand, we wanted to explore novel, and perhaps more general methodology in this arena.

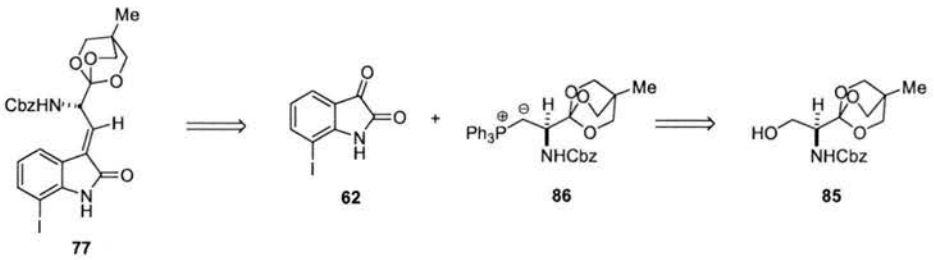
Figure 2.2 Serine-derived synthons for preparing β,γ-unsaturated amino acids



2.2.1. Phosphorus Ylide Approach.

Our initial plan for the preparation of an oxindolene derivative **77** was to incorporate the double bond functionality through a Wittig olefination between 7-iodoisatin **62** and a phosphorus ylide derived from serine. Although Itaya and Sibi have developed similar chemistry, we hoped to develop a serine-derived phosphorus ylide that retained the carboxyl oxidation state in a protected form. Therefore, our initial focus was to utilize the L-serine-derived OBO-ester **85** previously reported by Lajoie and co-workers[31] (Figure 2.3). It was felt that treatment of phosphonium salt **86** with the strong base necessary for ylide formation would not racemize the corresponding α-center. Also, with the OBO-ester intact, we would not have to be concerned with racemization of the resulting β,γ-unsaturated amino acid derivative.

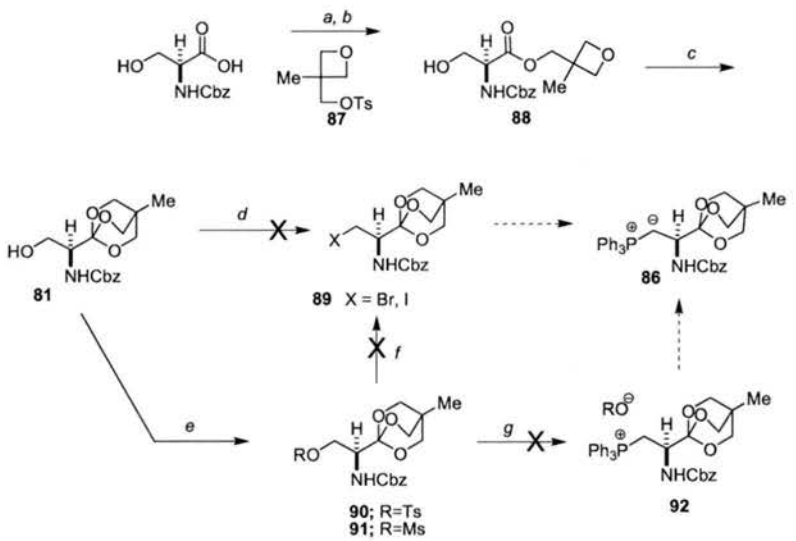
Figure 2.3 Retrosynthesis of oxindolene 77 utilizing an OBO-ester derived serine



Lajoie and co-workers have shown that treatment of the *N*-Cbz-L-serine cesium salt with oxetane tosylate **87** yields the corresponding oxetane ester **88** (Scheme 10). $BF_3 \cdot Et_2O$ rearrangement of oxetane ester **88** afforded OBO-ester **85**. With this substrate in hand, several attempts were made to convert it to the corresponding phosphonium ylide **86**. Treatment of **85** under a variety of conditions did not directly convert the primary alcohol into the corresponding halide **89** necessary for triphenyl phosphine displacement. It seemed that perhaps we must first convert the primary alcohol into a

suitable leaving group, then prepare the necessary primary halide. Formation of both the tosylate **90** and the mesylate **91** were successful. However, attempts to convert those substrates to the corresponding halides proved futile. Also, direct conversion of either

Scheme 10.[a] Attempts at preparing phosphorus ylide 82.



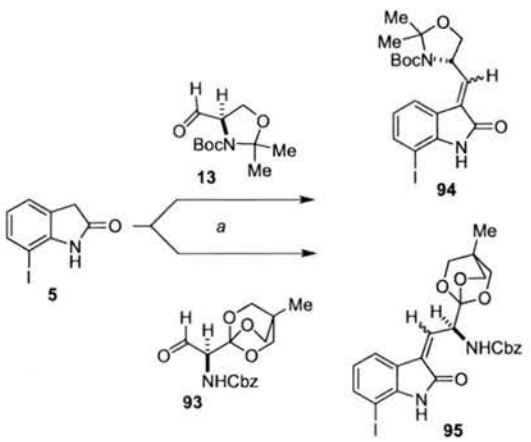
[a] (a) Cs_2CO_3, THF, H_2O; (b) **83**, DMF, NaI, rt, 85% (2 steps); (c) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0°C, 93%; (d) PPh_3, I_2, imidazole, MeCN; or, PPh_3, Br_2, MeCN; or, PPh_3Br_2, MeCN, Δ; or, PPh_3, CBr_4, CH_2Cl_2; (e) TsCl, pyridine or MsCl, Et_3N, CH_2Cl_2; (f) NaI or NaBr, DMF (all combinations tried) (g) PPh_3, PhMe, Δ.

tosylate **90** or mesylate **91** to phosphonium salt **92** was unsuccessful. Because of these difficulties we decided to abandon this route.

2.2.2. Condensation Route.

Our next plan was to utilize similar starting materials for the preparation of oxindolene **77** but reverse the oxidation states of the two coupling partners. One could envision using an aldol-type condensation between an oxindole and a serine-derived aldehyde. Therefore we prepared 7-iodooxindole **5**, the Garner aldehyde **13**, and the L-serine derived OBO-ester aldehyde **93**. Knoevenagel condensation of 7-iodooxindole **5**

Scheme 11.[a] Knoevenagel condensation.



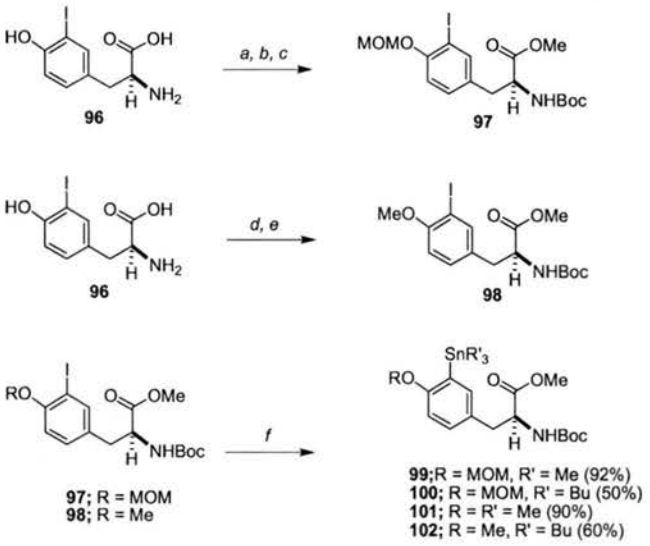
[a] (a) cat. piperidine, MeOH, Δ, yield not determined.

with the Garner aldehyde **13** and OBO-ester aldehyde **93** provided the desired unsaturation (**94** and **95**, respectively) necessary for the preparation of the highly oxidized tryptophan moiety. These findings were of great significance to our research program at the time. Unfortunately, at the time we were conducting the above research, the Ma research group reported similar findings in the literature. This being the case, in order to both delineate our work from Ma's and to overcome the limitations present in their results we decided to abandon the Knoevenagel approach towards the highly oxidized tryptophan fragment.

2.3. Stille Coupling to Form Biaryl.

While working on the northern half of TMC-95A/B, efforts were also being put toward developing an efficient method for incorporation of the biaryl linkage. With some initial results in hand, we decided to fully pursue the Stille coupling for the formation of the biaryl portion in order to present our work on this aspect of the project preventing any further setbacks. Protection of commercially available 3-iodo- L-tyrosine **96** as the fully protected synthons **97** and **98** was accomplished without incident (Scheme 12).

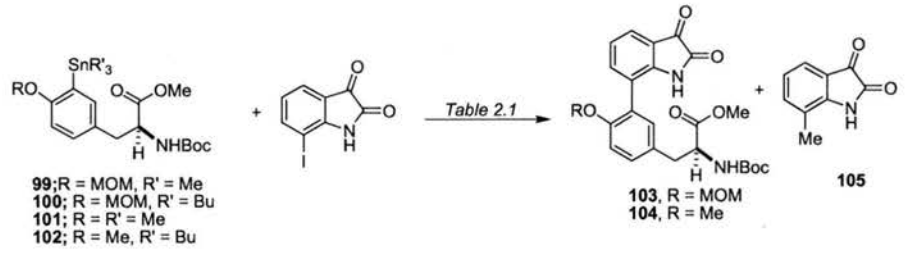
Scheme 12.[a] Tyrosine subunit synthesis.



[a] (a) $SOCl_2$, MeOH, -45°C→rt, 48h; (b) Boc_2O, CH_2Cl_2, sat. aq. $NaHCO_3$, 0°C→rt, 14h; (c) MOMCl, $^iPr_2NEt$, CH_2Cl_2, 0°C, 4h, 95% (3 steps); (d) Boc_2O, aq. NaOH, dioxane, 0°C→rt, 5h; (e) MeI, acetone, Δ (quant., 2 steps); (f) $(Me_3Sn)_2$ or $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, PhMe, Δ, see above for yields.

With aryl iodides **97** and **98** in hand, we felt that the palladium catalyzed Stille coupling reaction for the coupling of sp^2-sp^2carbon centers was most suitable for our system. Treatment of aryl iodides **97** and **98** with a hexaalkyl ditin species in the presence of catalytic palladium(0) afforded the aryl stannanes **99**, **100**, **101**, **and 102** in modest to excellent yields depending on the alkyl substituents on the tin center. Although all the reactions proceeded in a facile manner, the diminished yields in the tributyltin derivatives were due to the tedious manipulations necessary to remove tributyltin iodide (a byproduct in the reaction) from the product.

With the aryl stannanes in hand, we required proof of concept that these would undergo cross coupling reactions with an electrophilic isatinyl iodide **62**. Initially, we felt that the trimethyl stannanes would be an ideal coupling partner for 7- iodoisatin.



99;R = MOM, R' = Me
100; R = MOM, R' = Bu
101; R = R' = Me
102; R = Me, R' = Bu

103, R = MOM
104, R = Me

105

Table 2.1. Results from Stille coupling model study.

Entry	R	R'	Conditions	Product, Ratio	Yield
1	MOM	Me	$Pd(PPh_3)_2Cl_2$[a], DMF, LiCl, 100°, 20 h	**103**:**105**, 1:1	< 5%
2	MOM	Me	$Pd(dppf)Cl_2$[a], DMF, LiCl, 100°, 5.5 h	**103**:**105**, 1:1	< 5%
3	MOM	Me	$Pd(PPh_3)_2Cl_2$[a], THF, CuI, reflux, 24 h	no reaction	-
4	MOM	Me	$Pd(dppf)Cl_2$[a], dioxane, CuI, reflux, 6 h	**103**	< 2%
5	MOM	Me	$Pd(PhCN)_2Cl_2$[b], DMF, $AsPPh_3$, CuI, 100°, 1h	**103**:**105**, 1:1	18%
6	Me	Me	$Pd(dppf)Cl_2$[b], DMF, CuI, dppf, 100°, 10 h	**104**:**105**, 1:1	6%
7	Me	Bu	$Pd(dppf)Cl_2$[b], DMF, CuI, 100°, 2.5 h	**104**	15%
8	Me	Bu	$Pd(PhCN)_2Cl_2$[b], DMF, $AsPPh_3$, CuI, 3 h	**14**	20%
9	Me	Bu	$Pd(dppf)Cl_2$[a], MeCN, CuBr, reflux, 7.5 h	**104**	80%[d]
10	Me	Bu	$Pd(dppf)Cl_2$[a], PhMe, CuI, reflux, 24 h	no reaction	-
11	MOM	Bu	$Pd(dppf)Cl_2$[c], MeCN, CuBr, reflux, 24 h	**103**	68%
12	MOM	Bu	$Pd(PPh_3)_2Cl_2$[c], MeCN, CuBr, reflux, 48 h	**103**	57%

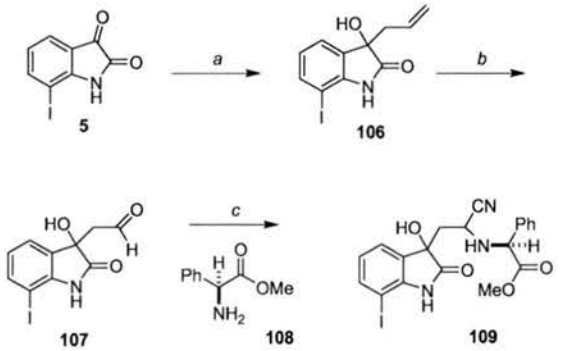
[a] 5 mol % catalyst, [b] 10 mol % catalyst, [c] 7 mol % catalyst, [d] Based on recovered starting

Unfortunately, all attempts to cross-couple both trimethyl tin derivatives **99** and **101** with 7-iodoisatin afforded the coupled products **103** or **104** in low yields, accompanied by methyl group transfer to the isatin derivative (compound **105**, Table 2.1). It was found to be imperative to employ the tributyl stannane species as the coupling partner in order to obtain satisfactory yields of biaryls **103** and **104**. Although 7-iodoisatin was a simplified system compared to what our synthetic plans projected, we felt that it laid the groundwork for our synthetic endeavor and this work was communicated in early 2001.[32] With this study complete we refocused our efforts on the initial goal of preparing the highly oxidized tryptophan fragment.

2.4.1. Strecker Amino Acid Synthesis

After the Stille coupling model study, our efforts turned towards the Strecker amino acid synthesis[33] to complete the tryptophan fragment. Treatment of 7-iodoisatin with allyl magnesium bromide afforded the corresponding homo-allylic alcohol **106** in 85% yield (Scheme 12). Our plan was to eliminate the 3° alcohol at a later stage to afford an oxindolene derivative. Initial studies to eliminate the alcohol at this stage proved futile and it was felt that it was best for the Strecker synthesis to eliminate the 3° hydroxyl group at a later stage. Oxidative cleavage of the double bond of **106** with ozone afforded aldehyde **107**. Treatment of **107** under Strecker conditions with TMSCN and L-phenyl glycine methyl ester **108** afforded amino nitrile **109**. Initially we were excited about these results, but eventually it was decided to abandon this route for a variety of reasons. First, amino nitrile **109** was isolated as a mixture of diastereomers at C6 and C25. Therefore, in order to achieve an efficient and stereoselective synthesis, we needed to determine a method to afford the desired stereochemistry at C25. Secondly, all attempts at eliminating the 3° alcohol at this stage or any subsequent stages were

Scheme 13.[a] Strecker amino acid synthesis.



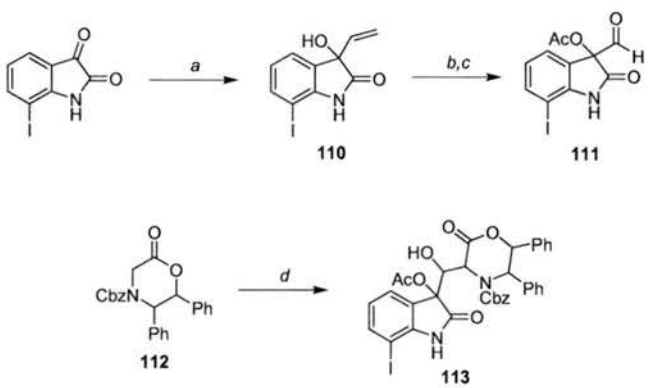
[a] (a) 2.2 eq. allyl magnesium bromide, THF, -78°C, 77%; (b) O_3, MeOH, CH_2Cl_2, -82°C; then Me_2S, -82°C→rt, 14 h, 73%; (c) **98**, TMSCN, EtOH, Δ, 45%.

unsuccessful. Therefore this route did not lend itself to the preparation of an oxindolene, and at the same time it lacked the oxidation at C7 necessary for the natural products. Finally, the sequence and yields described above were a best-case scenario for the Strecker reaction. Typically the yields in the ozonolysis step ranged from 40-70% and in the Strecker reaction between 20 and 45%.

2.4.2. Chiral Glycine Template Aldol Reaction.

Based on the initial results described in Scheme 13 and results previously reported in our group, we attempted to utilize the chiral oxazinone template developed by Williams and co-workers for the preparation of α-amino acids.[34] Treatment of

Scheme 14.[a] Chiral template design towards highly oxidized tryptophan moiety.



[a] (a) 2.2 eq. vinyl magnesium bromide, THF, -78°C, 77%; (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2; (c) O_3, CH_2Cl_2, -78°C, then DMS, -78°C→rt, 14h; (d) Bu_2BOTf, Et_3N, CH_2Cl_2, -78°C, then **111** →0°C, ~10% (3 steps).

7-iodoisatin with vinyl magnesium bromide afforded allylic alcohol **110** (Scheme 14). Acetate protection of the 3° alcohol and oxidative cleavage of the terminal alkene afforded aldehyde **111**. Initial studies had shown that we were able to treat the boron enolate of **112** with aldehyde **111** yielding the resultant coupled product **113** in a poor (~10%) yield over the three steps. Although the yield was significantly lower than desired, we felt that further experimentation would provide optimal reaction conditions.

In order for this chemistry to be relevant in an asymmetric total synthesis, the above sequence needed to be rendered stereoselective. It is known that the boron enolate of **112** gives excellent stereoselectivity and modest diastereoselectivity for the resulting 2° alcohol in the aldol reaction. Therefore, we must determine conditions to render the initial vinyl addition to isatin asymmetric. It was decided to apply chemistry developed by Oppolzer and co-workers for the enantioselective addition of alkenyl zinc bromides to aldehydes.[35] Treatment of 7-iodoisatin (along with the MOM-protected amide derivative) with a variety of chiral alkenyl zinc complexes afforded the desired allylic alcohol **110** in low yields with insufficient poor selectivity (Table 2.2). The majority of asymmetric alkyl zinc nucleophilic additions to carbonyls have been demonstrated on aldehydes. Therefore, the low yields and poor selectivity in our system may be a result of decreased reactivity of the isatin ketone. In any event, these results were deemed to be unsuitable for the goals of our total synthesis project.

Table 2.2. Asymmetric vinyl additions.

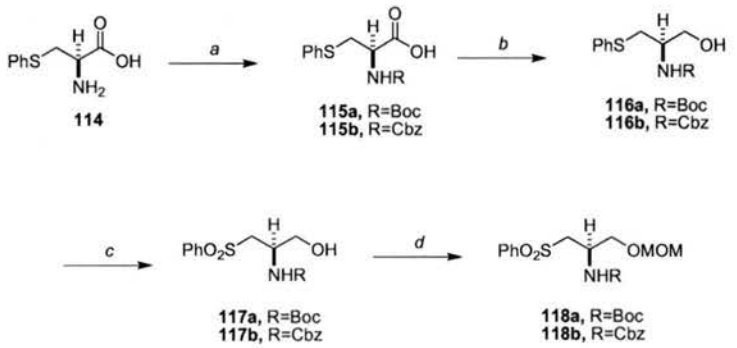
Entry	R_1	Ligand	Solvent	Temp (°C)	Yield (%)	% ee
1	H	*(1S, 2R)* R_2=Me, R_3=H	Et_2O	0	33	18
2	H	*(1S, 2R)* R_2=Me, R_3=Me	Et_2O	-78	40	61
3	H	*(1S, 2R)* R_2=Me, R_3=Me	PhMe	-78	19	0
4	H	*(1S, 2R)* R_2=Me, R_3=Me	Et_2O	-78 to 0	42	22
5	H	*(1S, 2R)* R_2=Me, R_3=Me	CH_2Cl_2	-78	25	0
6	MOM	*(1S, 2R)* R_2=Me, R_3=Me	1:1 Et_2O:THF	-78	53	40
7	MOM	*(1S, 2R)* R_2=Me, R_3=Me	Et_2O	-45	50	40
8	H	*(1S, 2R)* R_2=Ph, R_3=Me	Et_2O	-78	NR	-
9	MOM	*(1S, 2R)* R_2=Ph, R_3=Me	Et_2O	-78	NR	-

2.4.3. Julia Olefination.

After numerous unsuccessful attempts at the preparation of the highly oxidized tryptophan fragment, our focus was turned to the utilization of a Julia olefination[36] reaction for the synthesis of β,γ-unsaturated amino acids. It had been shown by Sasaki and co-workers[5] that L-cysteine-derived precursors could be utilized in the preparation of β,γ-unsaturated amino acids (Although briefly discussed earlier in this chapter for background purposes, it was not until this stage of the project that we decided to investigate this type of chemistry). Protection of *S*-phenyl-L-cysteine **114** afforded both the Boc and Cbz carbamates **115a/b** (Scheme 15). It was decided to prepare both protected forms of the cysteine derivative in order to determine the superiority of either substrate in the Julia olefination. Formation of the mixed carbonic anhydride of **115a/b** followed by subsequent reduction with $NaBH_4$ yielded the primary alcohols **116**. Oxidation of the thioethers was accomplished with *m*-CPBA. The oxidation routinely provided a mixture of the sulfone **117a/b** and sulfoxide. It was possible to separate the two at this stage and resubject the sulfoxide to the oxidation conditions to yield the desired sulfone. Finally, protection of the primary alcohols as their MOM-ether afforded the Julia olefination precursors **118a/b**.

Upon preparation of the Julia olefination precursors, we focused our efforts on coupling sulfones **118** with 7-iodoisatin. Treatment of a mixture of sulfones **118** and 7-iodoisatin with NaHMDS provided the corresponding β-hydroxy sulfones **119** (Scheme 16). Although it is known that subjection of β-hydroxy sulfones to Na/Hg amalgam provides the alkene substrate, we also prepared the four acylated products **120**.

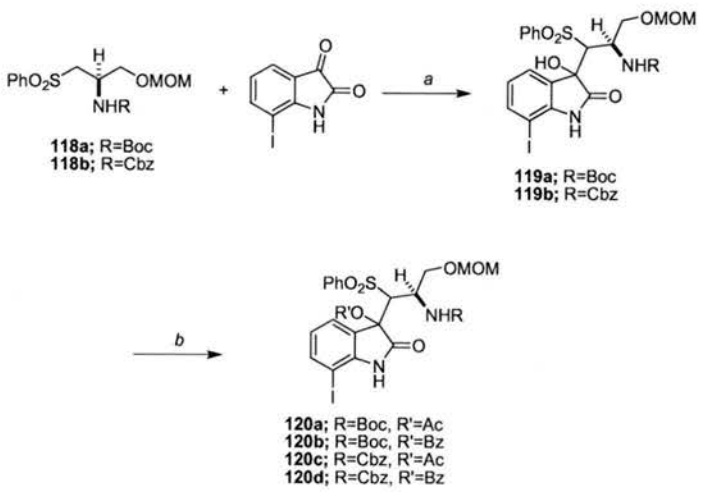
Scheme 15.[a] Preparation of Julia olefination precursors



[a] (a) Boc_2O or CbzCl, aq. NaOH, dioxane, 0°C→rt, ~quantitative for both substrates; (b) ^{i}BuOCOCl, Et_3N, CH_2Cl_2, 0°C; then $NaBH_4$, THF, H_2O (~10:1), 0°C→rt, R=Boc; 44%, R=Cbz; 88%; (c) *m*CPBA, $NaHCO_3$, CH_2Cl_2, R=Boc; 77%, R=Cbz; 92%; (e) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, 0°C (quantitative for both substrates).

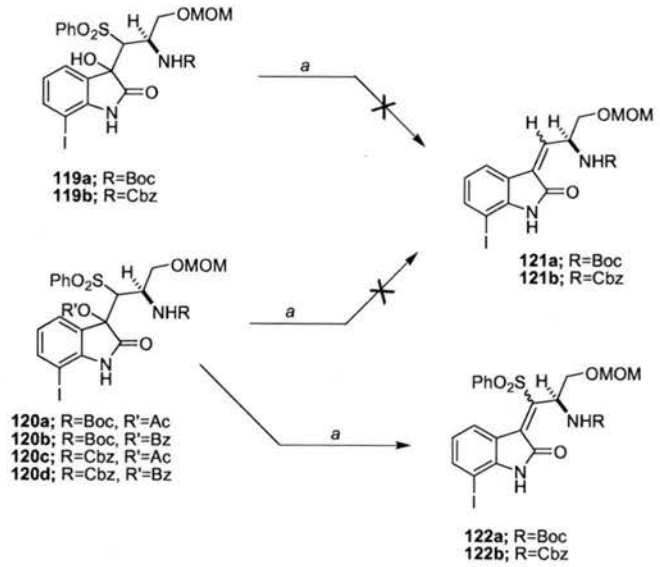
We hoped that β-hydroxy sulfones **119**, when treated with Na/Hg amalgam, would produce the desired alkenes (Scheme 17). Unfortunately, after exhaustive experimentation we were unable to determine any reaction conditions that would provide the desired alkene **121**. Therefore we turned to the reductive elimination of the acylated substrates **120** with Na/Hg amalgam. Again, subjection of these substrates to the above conditions did not provide any of the desired alkene. These reaction conditions did eliminate the 3° acyl groups providing the vinyl sulfones **122** in an undetermined yield. Attempts at utilizing vinyl sulfone **122** to produce alkenes **121** also did not yield any of the desired products. We also felt that this line of chemistry was neither elegant nor useful and thus it was abandoned.

Scheme 16.[a] Coupling of sulfones to 7-iodoisatin.



[a] (a) 6 eq. NaHMDS, THF, -78°C; R=Boc; 70%, R=Cbz; 90%; (b) Ac2O or BzCl, DMAP, Et_3N, CH_2Cl_2, quant. for all substrates.

Scheme 17.[a] Elimination attempts.

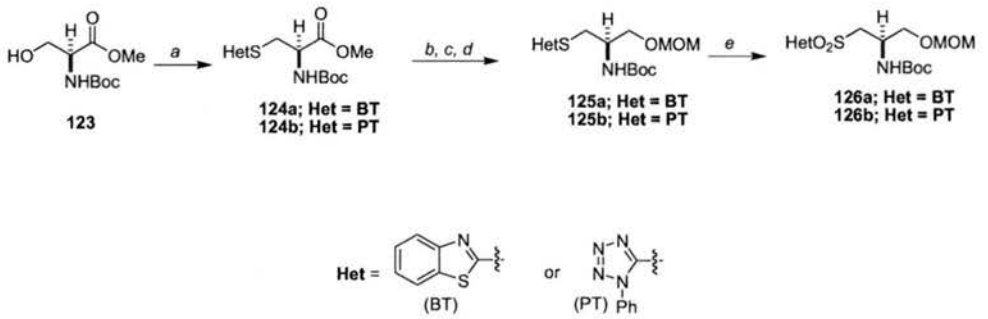


[a] (a) 5% Na/Hg amalgam, Na_2HPO_4, THF, MeOH (5:1).

2.4.4. Modified Julia Olefination.

A survey of the literature had shown that the majority of recent publications utilizing Julia olefinations employed heteroaromatic sulfones as one-half of the coupling partner. Julia[37] and later Kocieński[38] developed one-pot heteroaromatic-sulfone modified Julia olefinations that eliminated the need for Na/Hg amalgam in the alkene-forming step. It was felt that this modified Julia olefination might be suitable for our system because the alkene-forming reaction was proving to be the problematic step. Treatment of serine derivative **123** under Mitsunobu conditions with either 2-mercaptobenzathiazole (BTSH)

Scheme 18.[a] Preparation of modified Julia olefination precursors.

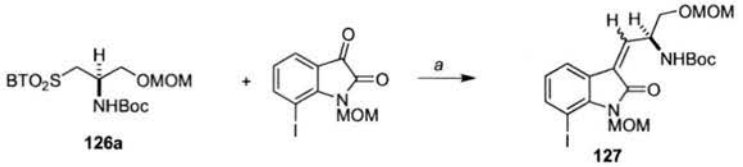


[a] (a) HetSH, DIAD, PPh_3, THF, rt, ~89% yield both substrates; (b) DIBAL, THF, -78°C, 14h; (c) $NaBH_4$, THF, 0°C; (d) MOMCl, $^iPr_2NEt$, CH_2Cl_2, 85 % (2 steps) (e) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 93%.

or 1-phenyl-1*H*-tetrazole-5-thiol (PTSH), DIAD, and PPh_3 furnished *S*-heteroaromatic cysteine derivatives **124** (Scheme 18). Reduction of the methyl ester was accomplished through a two-step protocol utilizing DIBAL followed by $NaBH_4$ yielding the corresponding primary alcohol, which was then protected as its MOM-ether affording **125**. Oxidation of the thioether **125** with *m*CPBA produced sulfones **126**. Treatment of **126a** (BT-sulfone) and *N*-MOM-7-iodoisatin with NaHMDS provided the desired alkene in an undetermined yield and selectivity (Scheme 19). For the first time, we had been

able to prepare the desired alkene starting with L-serine and 7-iodoisatin. Since our initial attempt at this reaction proceeded with the desired outcome we did not pursue the PT-sulfone any further in this approach. Although we saw that the reaction produced the

Scheme 19.[a] Initial modified Julia olefination.

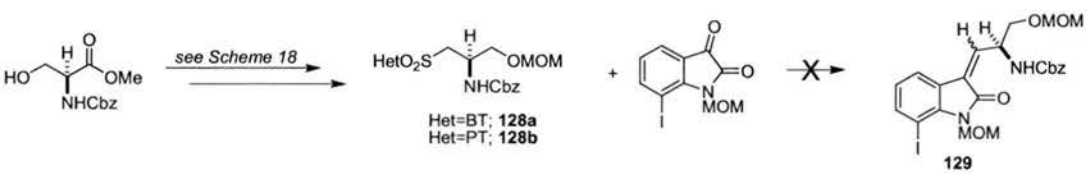


[a] (a) excess NaHMDS, THF, -78°C (yield not determined).

desired outcome, these initial studies were used only as proof of concept. Our ideal goal was to begin with *N*-Cbz-L-serine methyl ester because this protecting group scheme was most compatible with our synthetic plan. Therefore, our next focus was to prepare a Cbz-sulfone similar to **125**.

Starting with *N*-Cbz-L-serine methyl ester we were readily able to produce sulfone **128** in a sequence and with yields similar to those outlined in Scheme 18 (Scheme 20). With both the BT and PT sulfones in hand, we attempted to perform the modified Julia olefination as seen previously. Much to our surprise, by altering the nitrogen protecting group the

Scheme 20. *N*-Cbz protected sulfone.

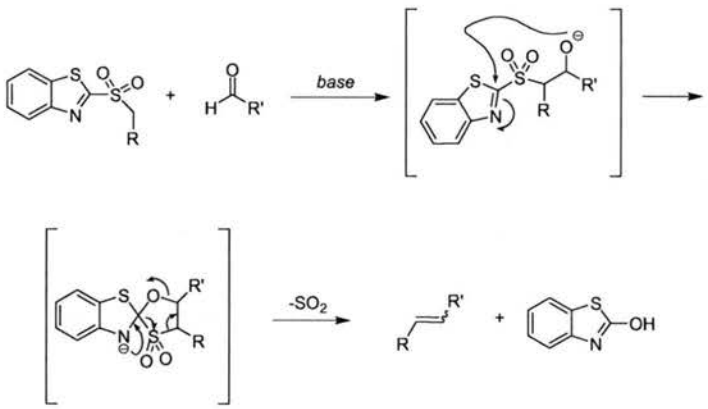


reaction ceased to yield any of the desired product. Confused by these results, numerous reaction conditions (combinations of base, solvent, temperature) were attempted, yet the desired product was never obtained. Since an excess of base was necessary (due to the

relatively low pKa of the carbamate proton) in the modified Julia olefination, we knew that the carbamate was deprotonated under the reaction conditions that produced desired product as seen in Scheme 19. Perhaps with the less sterically hindered Cbz-carbamate (versus the deprotonated Boc-carbamate) the deprotonated carbamate was acting as a reactant in this sequence.

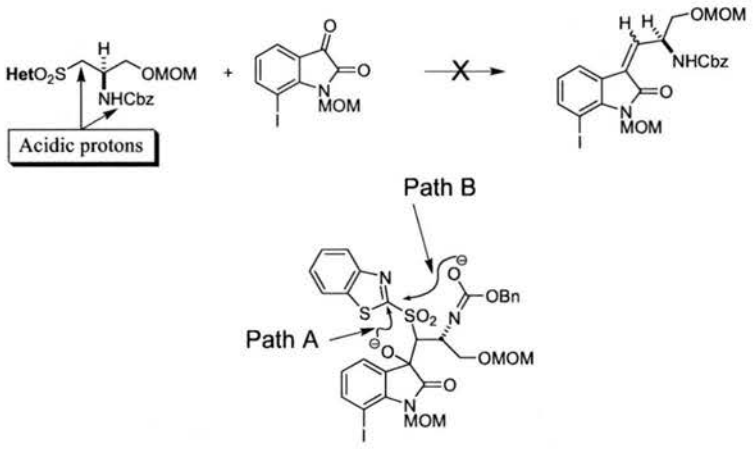
Julia and Kocieński have shown that the mechanism of the modified Julia olefination is most likely as shown in Scheme 21. Utilizing this mechanism to understand our situation, we knew that sulfones **128** contained two acidic protons and thus the deprotonated carbamate may be accountable for our problems (Scheme 22). Assuming that the initial dianion adds to the isatin ketone, intermediate **130** should be formed. At this stage, there are two competing pathways; pathway A would provide the

Scheme 21. Mechanism of the modified Julia olefination.



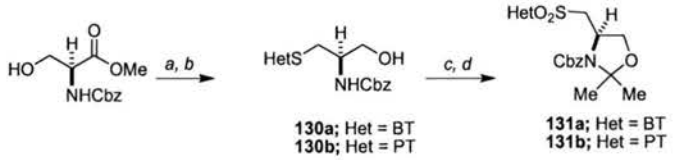
desired product and pathway B would give an undesired product and possibly decomposition. Although we did not have any concrete evidence for pathway B inhibiting our desired outcome, it was felt that if we could prepare a substrate that would not allow pathway B to operate that we would be able to get the approach to work utilizing the Cbz-carbamate.

Scheme 22. Mechanistic insight to the modified Julia olefination.



To eliminate the possibility for path B to operate we decided to manipulate the protecting groups in sulfone **128**. Similar to what was carried out before, *N*-Cbz-L-serine methyl ester was treated with the BTSH and PTSH heteroaromatic thiols under Mitsunobu conditions to afford the *S*-heteroaromatic cysteine derivatives

Scheme 23.[a] Second-generation Julia olefination precursor.



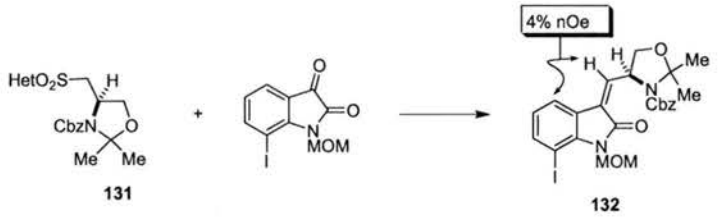
[a] (a) HetSH, DIAD, PPh_3, THF, rt, ~89% yield both substrates; (b) $CaCl_2$, $NaBH_4$, THF, 0°C then **130,** 95%; (c) 2,2-dimethoxypropane, *p*-TsOH, CH_2Cl_2, rt; (d) $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, H_2O_2, EtOH, (77% two steps).

(Scheme 23). Reduction of the methyl ester was accomplished with $Ca(BH_4)_2$ in THF, yielding alcohol **130** in excellent yield. Blocking the carbamate nitrogen and the primary alcohol in a single step was accomplished by forming the *N,O*-acetonide with dimethoxypropane and *p*-toluenesulfonic acid, and finally oxidation[39] of the thioether to sulfones **131** afforded the second-generation Julia olefination precursors. With these substrates we found that a molybdenum/hydrogen peroxide mixture gave far more

reproducible results than *m*-CPBA and yielded only the sulfones (no sulfoxide was isolated). It should be noted that both the BT and PT-sulfones were prepared in the same manner and in similar yields. The above four-step sequence can be carried out without any purification until the isolation of the final sulfones. With the PT-sulfone, column chromatography is necessary, but the BT-sulfone can be selectively crystallized from the crude mixture in absolute ethanol.

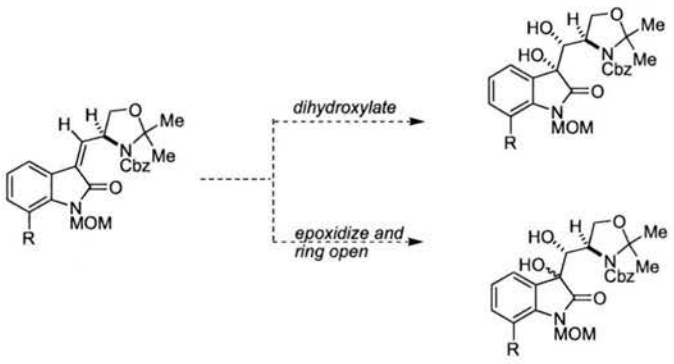
Upon completion of the second-generation sulfones we attempted to couple the sulfones to an isatin derivative. Initially it was felt that the *N*-MOM-protected isatin

Scheme 24. First successful Julia olefination with Cbz sulfones.



would be a better substrate rather than the free amide. Our first successful modified Julia olefination with the Cbz-sulfones afforded the undesired *Z*-isomer **132** (Scheme 24). All combinations of sulfone, solvent (THF, DMF, DMPU, PhMe), base (NaHMDS, LiHMDS, KHMDS, LDA) and temperature afforded only the *Z*-isomer. Although we were able to get the desired coupling reaction to proceed, this result was undesired due to the isolation of the unwanted geometric isomer. It is known[9] that the dihydroxylation of the *E*-isomer will give the desired stereochemistry for TMC-95A/B whereas the *Z*-isomer will produce the undesired diol stereochemistry that does not correspond to any of the natural products (Scheme 25). Also, if we first formed the epoxide followed by a subsequent acid-catalyzed opening to produce the diol, there was the likelihood of forming stereo-random products (compound **134**). Unfortunately at this time we had not

Scheme 25. Inherent problems with the *Z*-alkene.



determined conditions to afford the desired *E*-alkene. Therefore we accepted the fact that we will have to utilize the *Z*-alkene in our synthesis.

A concern that we had with the sequence outlined in Scheme 24 was the eventual deprotection of the *N*-MOM oxindole portion. The typical procedure for the removal of MOM-protecting groups from oxindoles utilizes a two step-protocol.[40] In an effort to reduce the number of chemical steps necessary for protections and deprotections, we attempted the modified Julia olefination on the unprotected 7-iodoisatin. Realizing that the amide proton of 7-iodoisatin would be removed under the basic reaction conditions, we utilized a large excess of base, also realizing that we may encounter similar mechanistic problems as discussed earlier. Our first study was to treat a mixture of 7-iodoisatin and BT-sulfone **131a** in THF at -78°C with an excess of NaHMDS. Initial inspection of the reaction mixture revealed that two new compounds were produced. Upon further analysis, it was found that the reaction of unprotected 7-iodoisatin and BT-sulfone **131a** produced a 1:1 mixture of *E:Z* alkene isomers **133** as determined by nOe experiments (Table 2.3). This was the first time in which we had been able to prepare the desired *E*-alkene. To this date we are unsure of the reason why the selectivity is affected

by the *N*-MOM protecting group. Perhaps it is due to a steric interaction between the MOM-group and the sulfone after the initial addition to the ketone that does not allow for elimination to occur to yield the *E*-isomer. In any event, we were excited about these results and we set out to evaluate other reaction conditions hoping for an increase in *E*-selectivity.

Through exhaustive experimentation we eventually determined reaction conditions that would allow for increased *E*-selectivity. Initially we wanted to determine which sulfone gave the best selectivity. Olefination of 7-iodoisatin with either of the sulfones **131** under the initial reaction conditions (THF, NaHMDS, -78°C) provided the desired product in a 1:1 *E:Z* mixture (Table 2.3; Entries 1 and 3). All attempts at this reaction in solvents such as DME and toluene gave low conversion and were not pursued further. At the same time, Lui and Jacobsen had shown that by utilizing DMF and DMPU as co-solvents and LiHMDS as a base in a modified Julia olefination for the total synthesis of ambruticin, they were able to achieve high *E*-selectivity in their reaction.[41] This being the case, we attempted to utilize these conditions for our modified Julia olefination. Our comparison for this system was carried out with both the BT-sulfone and the PT-sulfone. It was found that under the same conditions the BT-sulfone gave better *E*-selectivity (Table 2.3, Entries 2 and 4). Our efforts were then focused on optimizing the olefination with **131a**. We noticed that the more thermodynamically stable *E*-isomer can be preferentially prepared in greater selectivity by increasing the reaction temperature from -45°C to 0°C (Table 2.3, Entries 4, 5, and 6). Increasing the

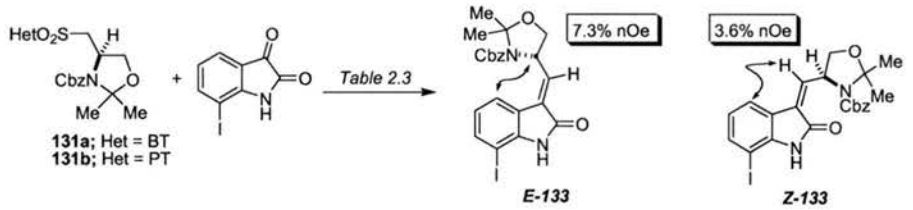


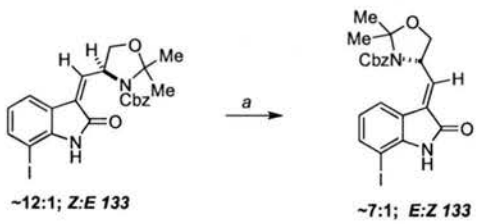
Table 2.3. Optimization of the modified Julia olefination.

Entry	Het	Conditions[a]	E/Z ratio[b]
1	PT	THF, NaHMDS, -78°C	1:1
2	PT	DMF, DMPU, LiHMDS, -45° C	2:1
3	BT	THF, NaHMDS, -78° C	1:1
4	BT	DMF, DMPU, LiHMDS, -45° C	2.5:1
5	BT	DMF, DMPU, LiHMDS, -45° C	3:1
6	BT	DMF, DMPU, LiHMDS, 0° C	5:1
7	BT	DMF, DMPU, LiHMDS, rt	1.5:1[c]

[a] In all cases yields were at least 79% (see note C). [b] *E/Z* ratios were determined by ^{1}H analysis of crude product mixtures. [c] Yield under these conditions was ~40%.

reaction temperatures above 0°C gave lower selectivity, presumably because of decomposition at these higher temperatures (Table 2.3, Entry 7). Ultimately, the ideal reaction conditions were found to be treatment of BT-sulfone **131a** and 7-iodoisatin with LiHMDS in DMF:DMPU (1:1) at 0°C to give a 5:1 *E:Z* ratio of oxindolene **21** (Table 2.3, Entry 6). It is also possible to separate the two isomers by column chromatography and isomerize the undesired *Z*-isomer to the *E*-isomer under conditions reported by the Danishefsky group in their total synthesis (Scheme 26).

Scheme 26.[a] Isomerization of *Z*-133 to *E*-133.



[a] (a) I_2, PhH, Δ, ~75%.

2.5. Conclusion

Finally, we were able to achieve our immediate goal of the project by completing the preparation of oxindolene **133** in an efficient manner and good selectivity. After surveying a variety of different types of chemistry including an aldol-type reaction, the Strecker reaction, asymmetric zinc additions, and Julia olefinations it has been determined that the modified Julia olefination proved superior in our system. Although we prepared oxindolene **131,** we realized later that this was only a small hurdle in our work towards a concise total synthesis of TMC-95A/B.

Chapter 3

Concise Total Synthesis of TMC-95A/B

3.1 Introduction.

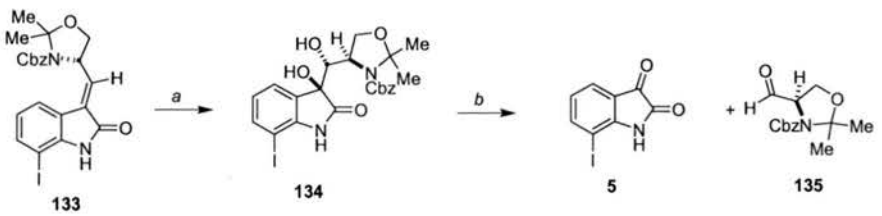
Completion of TMC-95A/B in an efficient manner can conceivably be accomplished via numerous synthetic routes. At this stage of our research program the Danishefsky group had already completed and reported their synthetic strategy to TMC-95A/B. This gave us a benchmark which we hoped to improve upon. As impressive as the Danishefsky and Hirama/Inoue syntheses are, there are still some limitations, such as the selectivity in the double bond geometry, the poor facial selectivity during the dihydroxylation and the overall number of steps. Our synthetic plan would allow for a much more efficient and concise total synthesis if it reached fruition.

3.2 Initial Synthetic Route.

3.2.1 Early Oxidation of the C6-C7 alkene.

In an effort to realize the total synthesis of TMC-95A/B in the most convergent manner possible it was felt that an ideal route would be to oxidize the C6-C7 alkene of *E*-oxindolene **133** at an early stage. Treatment of *E*-oxindolene **133** with stoichiometric OsO_4 in water and pyridine afforded the corresponding diol **134** as a single diastereomer in 90% yield (Scheme 27). All efforts at making the oxidation catalytic in OsO_4 with a stoichiometric reoxidant proved futile. It is believed that this is due to the inherent stability of the osmate ester, not allowing for decomplexation and reoxidation of the osmium center.[42]

Scheme 27.[a] Initial oxidation.



[a] (a) OsO_4 (0.1M in H_2O), pyridine, H_2O, 0°C; then sat. aq. $NaHSO_3$, THF, MeOH, 90%.; (b) DMSO-d_6, 120°C.

Unfortunately, we found that the thermal stability of diol **134** was extremely low. Attempts at an elevated-temperature ^1H NMR in DMSO-d_6 (120°C) gave the decomposition products 7-iodoisatin and aldehyde **135**. Our hypothesis is that diol **134** undergoes a retro-aldol reaction followed by rapid oxidation of the resulting hydroxyoxindole to isatin. In any event, these results made us reluctant to advance diol **134** any further in the synthesis, especially since we plan to utilize a Stille coupling to form the biaryl, which requires elevated temperatures. Therefore, we planned to install the C6-C7 diol after assembly of the biaryl portion.

3.2.2. Stille Coupling.

As seen earlier the Stille coupling is an efficient method for the preparation of a simplified TMC-95 biaryl system. It was our plan to extrapolate this chemistry to the more advanced coupling partner, oxindolene aryl iodide **133**. With both the trimethyl stannane **99** and the tributyl stannane **100** in hand, we subjected both substrates to oxindolene aryl iodide **133** under the previously developed conditions. Much to our surprise, these reaction conditions did not provide the corresponding biaryl **135** in any significant amount (Table 3.1, Entries 1 and 2). Despite extensive experimentation, we

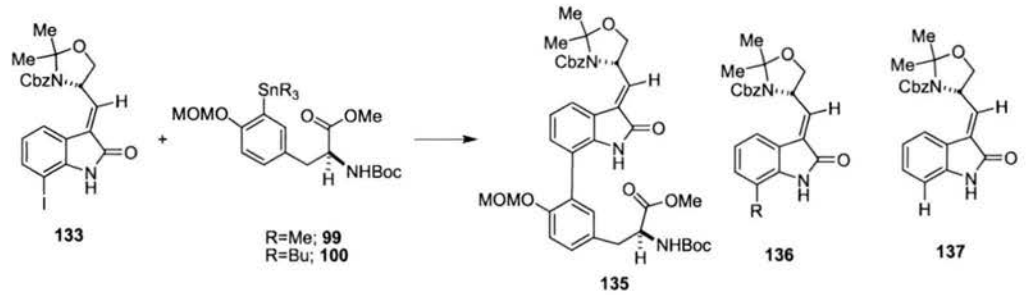


Table 3.1. Attempted Stille coupling of advanced intermediates.

Entry	R	conditions	Results[a] (**135**:**136**:**137**)
1	Me	MeCN, $PdCl_2$ (dppf), CuBr, Δ	1:2:0
2	Bu	MeCN, $PdCl_2$ (dppf), CuBr, 80°	1:3:0
3	Me	NMP, $PdCl_2$ (dppf), CuBr, 60°	1:4:0
4	Bu	DMF, $PdCl_2$ (dppf), CuBr, 80°	Trace:1:2
5	Bu	NMP, $PdCl_2$ (dppf), CuBr, 60°	Trace:1:2
6	Bu	NMP, Pd_2 (dba)3 CuBr, 60°	Trace:2:1

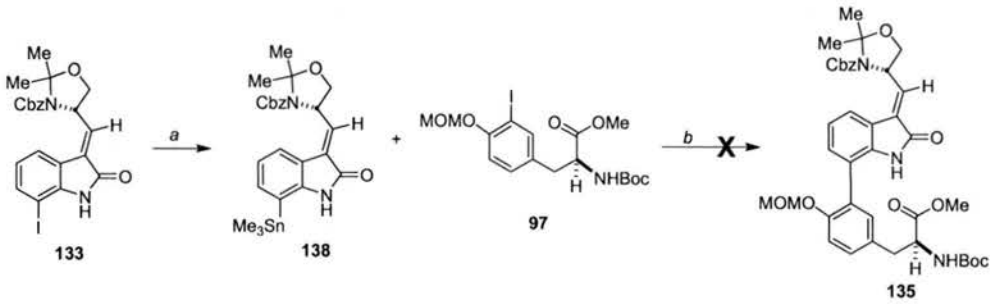
[a] Combined yields were less than 40%.

found that numerous combinations of Pd-catalyst and ligand gave unsatisfactory yields of the biaryl product **135**. The best isolated yield of coupled product **135** was ~20%, and it was routinely accompanied by side-products resulting from alkyl group transfer from the stannane (**136**) and reductive removal of the iodine atom (**137**).

Our main rationale for these results was that there are likely to be significant electronic differences between 7-iodoisatin and aryl iodide **133**. Therefore we thought that by reversing the polarity of the coupling partners the desired biaryl might be produced. Treatment of aryl iodide **133** with hexamethylditin catalyzed by palladium (0) afforded the corresponding aryl stannane **138** (Scheme 28) . Unfortunately the analogous reaction with hexabutylditin did not afford any of the desired aryl stannane. Treatment of aryl stannane **138** with tyrosine aryl iodide **97** under palladium catalyzed cross-coupling conditions again did not afford any of the desired biaryl **135**. Frustrated by these results,

we decided to abandon the Stille coupling reaction as a means for preparing the more complex TMC-95 biaryl.

Scheme 28.[a] Reversed polarity Stille coupling.



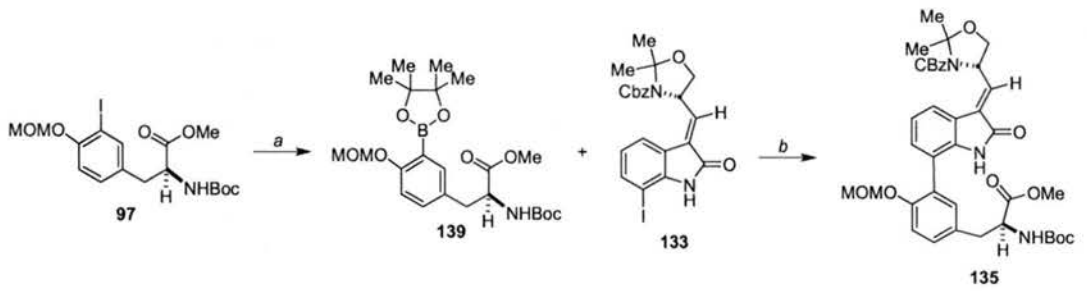
[a] (a) hexamethylditin, $Pd(PPh_3)_4$, PhMe, Δ, 92%.; (b) $PdCl_2(dppf)$, CuBr, MeCN, Δ.

3.3 Suzuki Coupling and Asparagine Incorporation.

Since the Stille coupling did not afford the desired biaryl coupled product in the more complex system, the next logical choice was to test the feasibility of the Suzuki coupling to provide the TMC-95 biaryl system. Conversion of aryl iodide **97** to the boronic ester **139** was accomplished via the Miyaura protocol (Scheme 29). The resulting boronate ester was used crude due to decomposition observed upon silica gel purification. Treatment of crude boronic ester **139** with aryl iodide **133** and K_2CO_3 in refluxing aqueous DME catalyzed by $Pd(dppf)Cl_2$ smoothly installed the biaryl linkage yielding **135** in 90% yield with no alkene isomerization.

With biaryl **135** finally in hand, we once again faced the issue of which synthetic route we should choose, and due to the lability of the C6-C7 diol that we had seen earlier, it was decided to postpone the oxidation until a later stage. Incorporation of the asparagine residue was readily accomplished by first saponifying methyl ester **135** (Scheme 30). Amide bond formation between carboxylic acid **140** and readily available asparagine

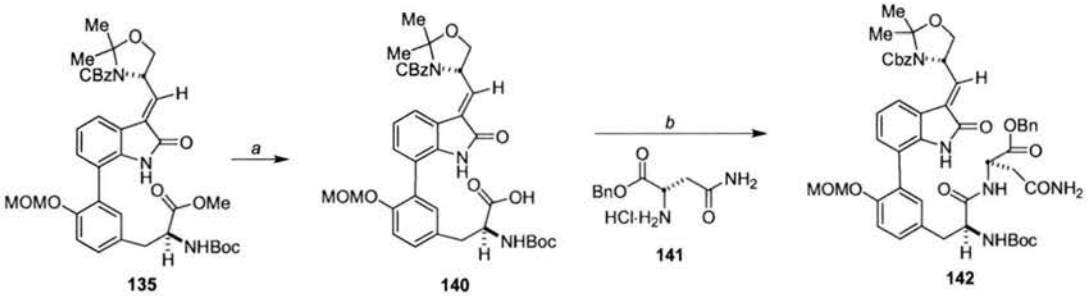
Scheme 29.[a] Suzuki coupling.



[a] (a) bispinacolatodiboron, $PdCl_2$(dppf), KOAc, DMSO, 80°C, 4h; (b) $PdCl_2$(dppf), K_2CO_3, DME, H_2O (~8:1), Δ, 2h, 90% (based on **133**).

benzyl ester hydrochloride[43] **141** mediated by EDCI and HOAt afforded pseudotripeptide **142** in 98% yield over the two steps. With the incorporation of the asparagine residue the carbon framework necessary for the macrocyclic core of TMC-95A/B was complete.

Scheme 30.[a] Asparagine incorporation.



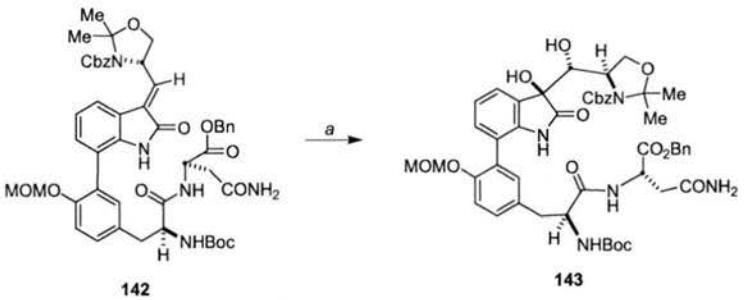
[a] (a) LiOH, THF, H_2O (1:1), 0°C, 1h; (b) EDCI, HOAt, $^iPr_2NEt$, CH_2Cl_2, 0°C, 2h, 98% (2 steps).

3.4 Diastereoselective Dihydroxylation.

Upon completion of the linear pseudopeptide necessary for the macrocyclic framework of TMC-95A/B, it was felt that this was an ideal point in which to install the C6-C7 diol. We knew from previous work that a stoichiometric OsO_4 oxidation of a similar C6-C7 alkene afforded the corresponding diol with complete facial selectivity.

Therefore we felt that alkene **142** was an ideal substrate since it retains identical chemical features near the reaction center. Not surprisingly, treatment of **142** under the same conditions described earlier afforded the corresponding diol **143** as a single diastereomer in 87% yield with the reaction occurring from the opposite face of the allylic carbamate (Scheme 31). Again, all attempts at rendering this reaction catalytic in osmium proved futile and it was felt that it was more important to sacrifice OsO_4 for a completely diastereoselective reaction. (Note that Danishefsky[9b] had shown that a catalytic asymmetric oxidation of a similar alkene afforded a 5:1 mixture of diol diastereomers.)

Scheme 31.[a] Diastereoselective dihydroxylation.



142 143

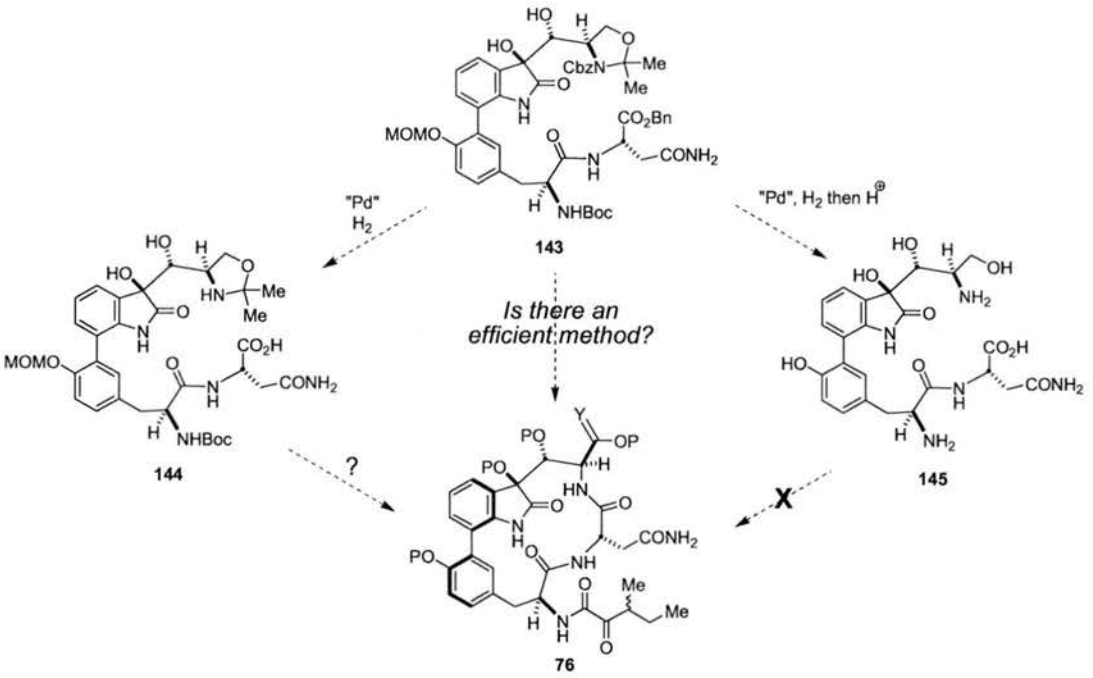
[a] (a) OsO_4 (0.1M in H_2O), pyridine, 0°C, 1h; then sat. aq. $NaHSO_3$, THF, MeOH, 87%.

3.5 Ketoamide Formation and Macrocyclization.

With the C6-C7 oxidation complete, our synthetic plan was at a point of uncertainty. Our next main goal was to prepare a macrocycle of type **76**. Through careful planning we had been able to utilize five different protecting groups that can be removed with only two chemical manipulations. Although this has its advantages, at this stage it had serious limitations. In order to form a macrocyclic compound it would be necessary to first remove the benzyl ester and Cbz-carbamate to afford the seco-acid for macrocyclization. Palladium-catalyzed hydrogenolysis should afford amino acid **144** in a facile manner (Scheme 32). The problem that we faced was whether or not the

secondary neopentyl-type amine would cyclize. If attempts were made at cleavage of the *N,O*- acetonide, there was also a risk of removing the *N*-Boc carbamates, resulting in two different amine functionalities (**145**) that could form amide bonds. Therefore we

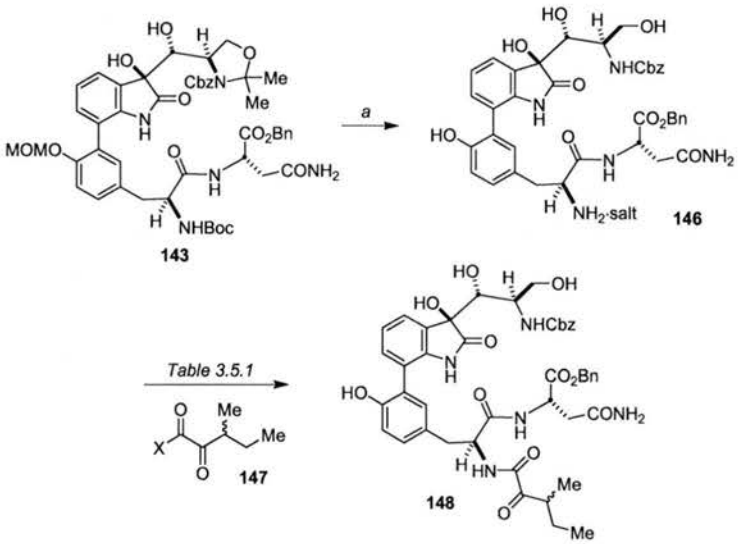
Scheme 32. Protecting group issues.



were not fully confident that there was an efficient route to form a macrocyclic compound from diol **143**.

Since it was realized that we had a variety of protecting group issues to face we evaluated our synthetic options. We could either return to previous intermediates and manipulate the protecting groups or we could attempt the higher risk proposition of continuing forward with limited protecting groups. At a critical stage in the synthesis, it was decided to remove all of the acid-labile protecting groups, liberating the C14 amine, the C25 primary alcohol, and the C19 phenol. Although we realized that the four free alcohols may prove to be problematic in both the incorporation of the ketoamide and the

macrocyclization, it was felt that in order to have an efficient synthesis we must take this risk. It was found that treatment of diol **143** with aqueous acid (either TFA or HCl) provided the corresponding amine salt **146**. Next we surveyed a variety of conditions for incorporation of the ketoamide with both salt forms (Table 3.5.1). Subjection of the amine salt **146** to a (±)-3-methyl-2-oxo-pentanoic acid-derived coupling partner **147** afforded the corresponding ketoamide **148** in a range of yields. It was decided to utilize



[a] (a) TFA:H_2O (1:1), rt, 4h or conc. HCl, MeOH, H_2O, rt, 4h.

Table 3.5.1. Ketoamide incorporation.

Entry	Salt	X	Conditions	Yield of **148** (%)from **143**
1	TFA	Cl	sat. $NaHCO_3$, CH_2Cl_2, 0°C	<10
2	TFA	Cl	CH_2Cl_2, DMF, Et_3N	17
3	HCl	ONa	EDCI, HOAt, DMF, CH_2Cl_2	24
4	HCl	ONa	EDCI, HOAt, DMF, CH_2Cl_2	70
5	TFA	ONa	EDCI, HOAt, CH_2Cl_2, DMF, 0°C	54
6	TFA	ONa	EDCI, HOAt, THF, 0°C	98

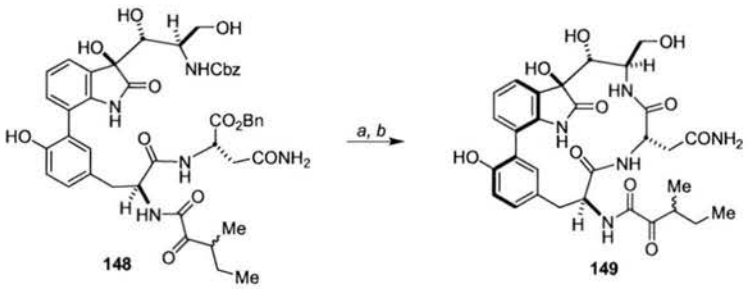
the racemic (±)-3-methyl-2-oxo-pentanoic acid derivative **147** due to the fact that it would eventually provide TMC-95A and B, and also because Danishefsky[9] stated that a single isomer of the ketoamide rapidly equilibrates under a variety of conditions.

Initially we attempted to couple the TFA-salt **147** with the corresponding acid chloride of the ketoacid. In both cases we saw low conversion to ketoamide **148** (Table 5.3.1, Entries 1 and 2). In the first entry we believe this is due to the water solubility of both the starting material and product. Since the sodium salt of **147** is commercially available, we attempted to directly couple it to amine **146.** Ultimately we found that the TFA amine salt **146** could be coupled to (±)-3-methyl-2-oxo-pentanoic acid sodium salt mediated by EDCI and HOAt to afford the corresponding amide **26** in ~98% yield. The high yield in this reaction was promising for the macrocyclization step in that there was little coupling to any of the free alcohols, so protecting groups might not have to be employed.

In light of the previous results, we attempted to utilize **148** as a macrocyclic precursor. We had planned the synthesis so that we could employ a palladium-catalyzed hydrogenation as a mild deprotection step on the complex substrate. Typically, these reactions are facile and high yielding. Unfortunately that was not the case in early studies. Utilizing 10% palladium on activated charcoal in an alcoholic solvent under an atmosphere of hydrogen typically gave low yields of the resulting seco-amino acid. It was hypothesized that the activated charcoal was sequestering either the starting material or the product. This led us to investigate palladium black as the catalyst system. Attempts at the hydrogenation with ~10 mol % of palladium black led to low conversion to the amino acid. Due to the nature of these compounds, chromatographic separation of the starting material and products was not a viable option. Finally, it was found that treatment of **148** with 400 mol % of palladium black under an atmosphere of hydrogen in MeOH completely converted it to the amino acid (Scheme 33). At this pivotal point in the synthesis, the resulting amino acid was treated with EDCI and HOAt at high dilution

to yield the desired unprotected macrocycle **149** in 49% yield for the two steps. Crude ^{1}H NMR analysis showed that besides macrocycle **149** there were no other macrocyclic compounds related to lactone formation or biaryl atropisomers.[44]

Scheme 33.[a] Macrocyclization

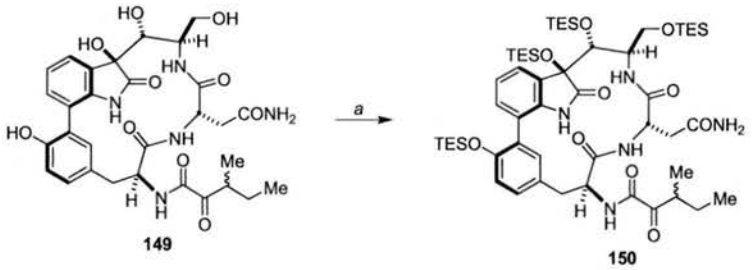


[a] (a) Pd black, H_2, MeOH, 14h; (b) EDCI, HOAt, CH_2Cl_2, DMF, 1:1 (1 mM), rt, 24h, 49% (2 steps).

3.6 Formal Synthesis

Macrocycle **149** is only a single synthetic manipulation from a key late-stage intermediate in the Lin and Danishefsky total synthesis. The structure of **149** was secured by intersecting the Danishefsky late-stage intermediate. Treatment of macrocyclic tetraol **149** with an excess of TESOTf in the presence of 2,6-lutidine to afford the fully protected TES-macrocycle **150** in approximately 40% yield (Scheme 34). The lower yield is due to the fact that the excess TESOTf necessary for complete conversion appears to form the silyl enol ether of the ketoamide as well as Si-N bonds with some of the amides. In an effort to hydrolyze the unwanted silicon bonds there was also some partial hydrolysis of the phenolic silyl ether and/or the 3° silyl ether. In any event, TES-macrocycle **150** was prepared and the ^{1}H spectral characteristics of this substance exactly matched those of the ^{1}H NMR spectrum provided to us by Professor Danishefsky.[45] This work was communicated in early 2003.[46]

Scheme 34.[a] Intersection of Danishefsky intermediate.

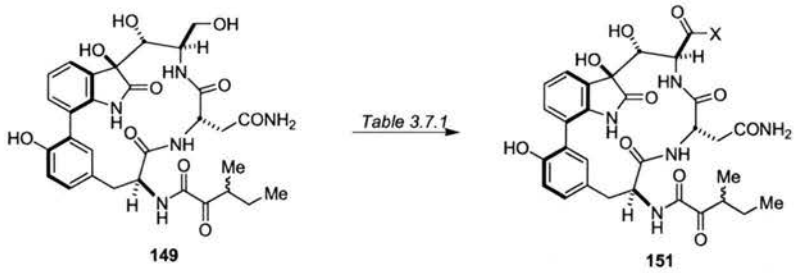


[a] (a) TESOTf, 2,6-lutidine, CH_2Cl_2, DMF, 0°C→rt, 14h; then sat. aq. $NaHCO_3$ followed by citric acid, ~40%.

3.7 Selective Oxidation.

Although we were able to secure the structure of macrocycle **149** by intersecting a late-stage intermediate in the Danishefsky synthesis, it was felt that an efficient synthesis of TMC-95A/B could be accomplished via direct elaboration of macrocycle **149**. In order for this to happen, it was necessary to selectively oxidize the C25 primary alcohol in the presence of the C7 secondary alcohol and, even more problematically, avoid the oxidative cleavage of the C6-C7 diol. Initially it was felt that we would be able to perform a selective oxidation of the primary alcohol directly to the necessary carboxylic acid utilizing a platinum-catalyzed dehydrogenation reaction.[47] Unfortunately it was found that no reaction occurred or, if a base additive was used, complete decomposition occurred (Table 3.7., Entries 1 and 2).

Since the platinum-catalyzed dehydrogenation failed to yield the desired product, we searched the literature for other methods that would selectively oxidize a primary alcohol directly to the carboxylic acid while not altering the oxidation state of secondary alcohols. TEMPO oxidation systems are known to preferentially oxidize primary



149 151

Table 3.7. Selective primary oxidation.

Entry	Conditions	X	Results
1	PtO_2, O_2, acetone, H_2O, Δ	OH	Recovered SM
2	PtO_2, O_2, $NaHCO_3$ acetone, H_2O, Δ	OH	Decomposition
3	TEMPO, $NaClO_2$, cat. NaOCl, phosphate buffer, 50°C	OH	Decomposition
4	excess TEMPO	H	Recovered SM
5	$(COCl)_2$, $^{i}Pr_2NEt$, DMSO	H	~30 %
6	1. SO_3·pyridine, DMSO, $^{i}Pr_2NEt$, CH_2Cl_2; 2. $NaClO_2$, $^{t}BuOH$, H_2O, NaH_2PO_4, 2-methyl-2-butene	OH	~80%
7	IBX, DMSO, acetone	H	~10%,
8	Dess-Martin Periodinane, DMSO	H	Decomposition

alcohols to aldehydes in the presence of a secondary alcohol.[48] A research group at Merck has shown that this methodology can be extended to perform a second oxidation on the initially formed aldehyde using $NaClO_2$ in the presence of catalytic amounts of sodium hypochlorite.[49] Subjection of our substrate to TEMPO lead only to recovered starting material and, when forcing conditions such as elevated temperatures were utilized, decomposition occurred (Table 3.7, Entry 3).

Since it appeared that the direct oxidation of the primary alcohol to the carboxylic acid appeared unattainable, we decided to attempt a two-step protocol by first forming the aldehyde. After testing a variety of conditions (Swern oxidation,[50] SO_3·pyridine, Dess-Martin periodinane,[51] IBX[52], and other TEMPO systems) we finally determined exact reaction conditions for treatment of macrocycle **5** with SO_3·pyridine in DMSO and

CH_2Cl_2 affording the desired aldehyde as a complex inseparable mixture of aldehyde, C6-, and C7-lactol isomers. It was possible, however, to treat this mixture with $NaClO_2$, NaH_2PO_4, and 2-methyl-2-butene to produce the desired carboxylic acid **151** (X=OH). With the carboxylic acid in place, the only synthetic challenge left was the preparation of the *cis*-propenyl amide to provide TMC-95A/B.

3.8 Installation of the *cis*-Propenyl Amide.

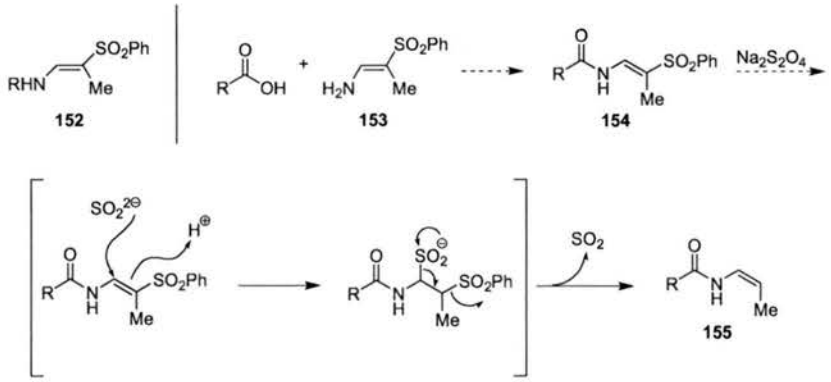
Recently the preparation of enamides has received considerable attention due to the fact that they are present in many biologically active natural products.[53] That said, it was not clear whether or not the dense functionality in TMC-95A/B will withstand the vast array of enamide chemistry described in the literature. Therefore a careful survey of the literature was essential in choosing a method by which to install the *cis*-propenyl side chain.

3.8.1. New Methodology for the Preparation of Enamides.

3.8.1.1 Stabilized Enamine Sulfones

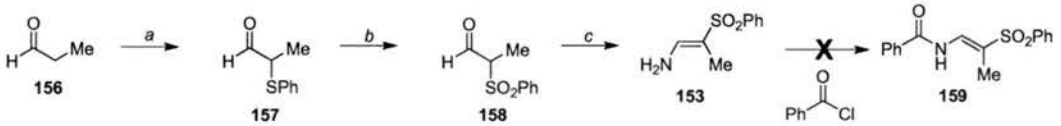
The presence of the *cis*-propenyl amide in the complex structures of TMC-95A/B prompted us to investigate new, mild, synthetic methods for the preparation of this functionality. Alper and co-workers[54] have shown that enamine sulfones of type **152** are relatively stable (Scheme 35). We envisioned coupling the hypothetical primary enamine **153** to an activated carboxyl group, affording enamide sulfone **154**. Julia had studied the mechanism of the stereospecific reduction of vinylic sulfones with sodium dithionite affording the alkene with retention of double-bond geometry.[55] Therefore our proposal was to reduce enamide sulfone **154** with sodium dithionite affording the *cis*-propenyl amide **155** as a single isomer.

Scheme 35. Proposed enamide preparation utilizing stabilized enamines.



To determine the feasibility of this sequence, we first prepared enamine sulfone **153**. Although Alper described the preparation of a variety of secondary enamine sulfones through a hydroformylation of vinyl sulfones catalyzed by rhodium complexes, we decided to prepare this substrate through more traditional methods. Treatment of propionaldehyde **156** with Br_2 followed by thiophenol affords thioether **157**[56] (Scheme 36). Oxidation of thioether **157** with *m*CPBA afforded sulfone **158**. Condensation of **158** with ammonia (saturated solution in CH_2Cl_2) in the presence of alumina afforded enamine sulfone **153** as a single isomer. All attempts to couple **153** to simple electrophiles were unsuccessful. Hence, we were never able to determine if the key reduction of the vinyl sulfone would afford the desired *cis*-propenyl amide **155**.

Scheme 36.[a] Enamide sulfone as enamide precursor.

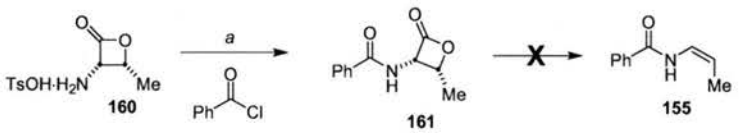


[a] (a) 1. Br_2, dioxane, Et_2O; 2. PhSH, $^iPr_2NEt$, CH_2Cl_2, ~25% (2 steps); (b) *m*CPBA, CH_2Cl_2, NaH_2PO_4, ~quant.; (c) $NH_{3(g)}$, CH_2Cl_2, RT, sealed tube, yield not determined.

3.8.1.2. β-lactones as Precursors to Enamides.

It is known that upon heating β-lactones, a [2+2] retrocycloaddition occurs forming an alkene and carbon dioxide.[57] It was hypothesized that upon heating β-lactone amide **161** it would liberate carbon dioxide affording the desired *cis*-propenyl amide (Scheme 37). Amide bond formation between known L-threonine-derived β-lactone **160**[58] and benzoyl chloride afforded β-lactone amide **161**. All attempts to cyclorevert this substrate led to complete decomposition. At this point it was decided to rely on literature-precedented enamide formations for the completion of our total synthesis.

Scheme 37.[a] β-lactone as a precursor to enamides.



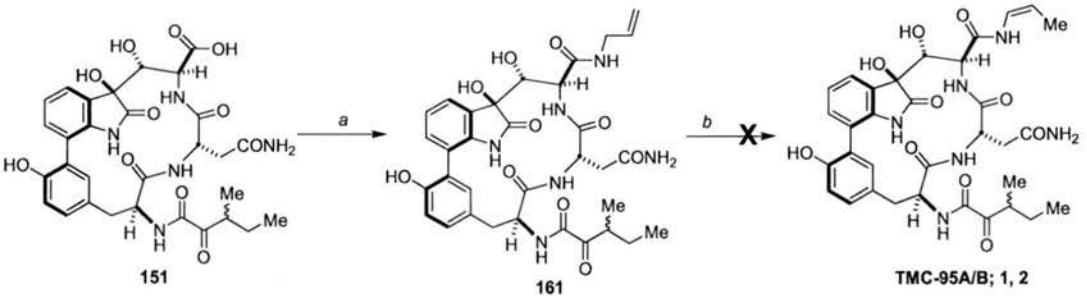
[a] (a) $^{i}Pr_2NEt$, CH_2Cl_2, 0°C, ~85%.

3.8.2. Isomerization of an Allyl Amide.

Stille and co-workers developed a method for transition-metal mediated conversion of allyl amides to enamides where the *cis*-configuration predominates.[59] This reaction manifold was chosen as an initial study because the preparation of the necessary precursor was trivial. Carboxylic acid **151** was readily coupled to allyl amine in the presence of EDCI and HOAt to afford the corresponding allyl amide **162** (Scheme 35). Stille and co-workers have shown that the isomerization takes place with a variety of catalysts. A rhodium hydride catalyst was chosen because it is specific for terminal olefins and tolerates a wide variety of functionality.[60] Subjection of allyl amide **162** to the isomerization conditions led to decomposition of the starting material. We realized that the potential for problems in this reaction existed. The solvent (PhMe) reported by

Stille was not compatible for our substrate and these reactions were typically carried out at elevated temperatures. In spite of these foreseen problems the possibility of the desired outcome outweighed the drawbacks.

Scheme 38[a]. Attempted isomerization of an allyl amide.



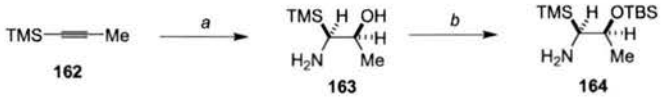
[a] (a) allylamine, EDCI, HOAt, DMF, CH_2Cl_2, 0°C→rt, 64% (from **149**).; (b) $RhHCO(PPh_3)_4$, DMF, 100°C.

3.8.3. Peterson Olefination to Enamides.

Next we looked at methodology developed by Fürstner and co-workers utilizing the Peterson olefination manifold as an efficient method for the preparation of both *E* and *Z* enamides.[61] Fürstner has shown that the treatment of β-hydroxy alkyl silanes with a strong base smoothly affords the expected enamides. Although we would attempt this exact chemistry on our system, our experience has shown that the TMC-95A/B macrocyclic core is not always stable to base. Also, due to the numerous acidic protons in the molecule this reaction would necessitate a large excess of base. Therefore, we strove to prepare a masked alkoxide that could be generated under mild conditions facilitating the desired Peterson olefination. A survey of the literature showed that this mode of reactivity has also been utilized for the preparation of another enamide-containing natural product, crocacin D.[62]

Preparation of the requisite silyl amino alcohols began with hydroboration-protonation of TMS-propyne **162**[63] (Scheme 39). Epoxidation, ring opening with NaN_3, and reduction of the azide afforded amine **163** which was to be deployed in the base-promoted Peterson olefination. Protection of the alcohol as its TBS-ether afforded amine **164** which could be used in a fluoride-promoted Peterson olefination.

Scheme 39.[a] Preparation of silyl amino alcohols.

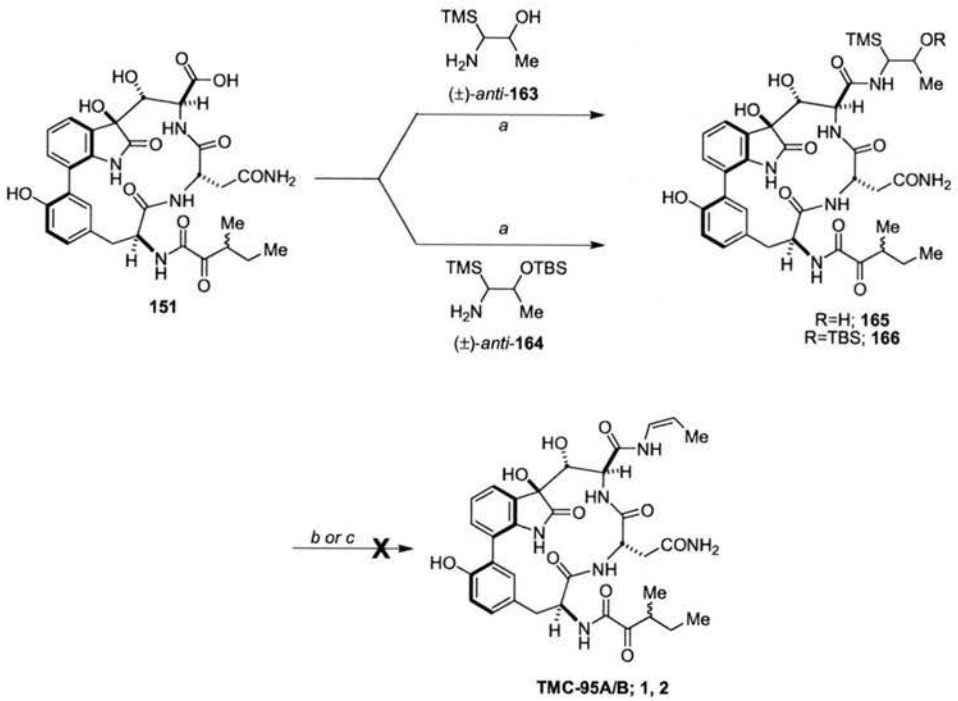


162 **163** **164**

[a] (a) 1. BH_3·DMS, cyclohexene, 0°C, 3h; then **162**, 1h; then EtOH, HOAc, 1h; then H_2O_2; due to the volatility of this intermediate it is difficult to isolate without solvent. Therefore a yield is not listed.; 2. *m*CPBA, CH_2Cl_2, 89%; 3. NaN_3, NH_4Cl, MeOH, H_2O, 66%; 4. LAH, THF, rt, 3h, quantitative.; (b) TBSCl, imidazole, CH_2Cl_2, 0°C→rt, 14 h, 75%.

Coupling of amines **163** and **164** proceeded smoothly to provide amides **165** and **166.** As expected, treatment of amide **165** under strongly basic conditions (such as KHMDS, or KO^{t}Bu) led to decomposition, presumably through a retro-aldol type reaction of the C6-C7 diol as discussed earlier (Scheme 40). We were much more hopeful that the fluoride-mediated Peterson olefination would provide TMC-95A/B, since this reaction had worked in our hands on simple substrates.[64] Subjection of amide **166** under the conditions (CsF, DMF), initially tested in our model system, resulted in only recovered starting material. Amide **166** was then subjected to a variety of fluoride sources in hopes of producing TMC-95A/B. Under all the conditions tested (TBAF, DMF, THF; KF, 18-crown-6, THF, MeCN; HF·pyridine, pyridine, THF) there was no reaction. Upon extended reaction times the starting material began to decompose or there

Scheme 40.[a] Peterson manifold to TMC-95A/B



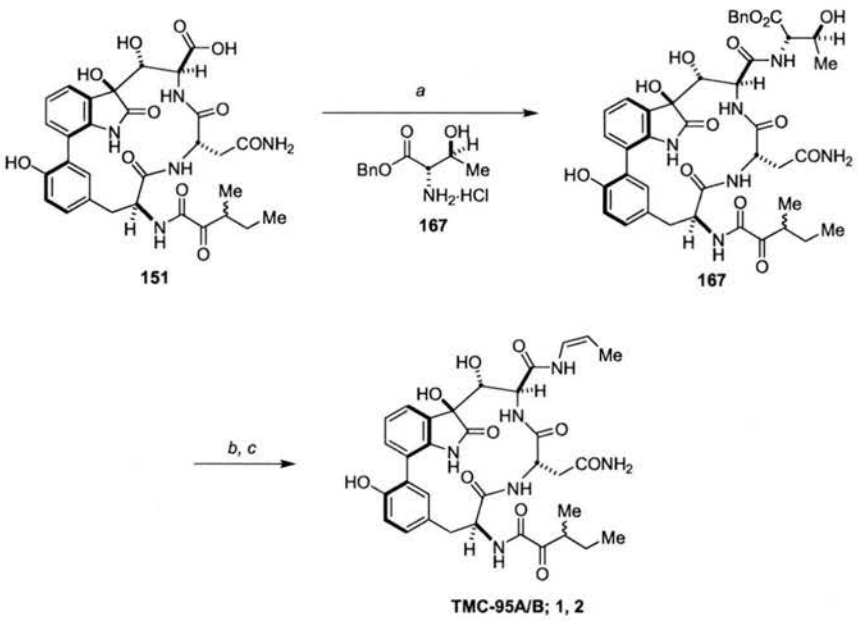
[a] (a) EDCI, HOAt, CH_2Cl_2, DMF, 0°C→RT, 18h (yields ranged from ~35-55% from **149**); (b) KHMDS, DMF, THF, 0°C or KO^{t}Bu, THF, 0°C; (c) fluoride source.

was deprotection of the TBS-ether affording amide **165** (this typically occurred when TBAF was used as a fluoride source).

3.8.4. Mitsunobu Decarboxylation and the Completion of TMC-95A/B

Based on the aforementioned setbacks, it was decided to abandon the above methods. It was felt that the enamide preparation utilized by the Hirama/Inoue group for their total synthesis of TMC-95A would be sufficient for our system. Treatment of carboxylic acid **151** with L-*allo*-threonine-benzyl ester hydrochloride salt **167**[65] mediated by EDCI and HOAt afforded amide **168** (Scheme 41). Hydrogenolysis of **168** with palladium black under an atmosphere of hydrogen produced the resultant carboxylic acid. Subjection of this material to Mitsunobu conditions afforded TMC-95A/B as a 1:1 mixture which could be separated by RP-HPLC. The synthetic TMC-95A and TMC-95B

Scheme 41.[a] Completion of TMC-95A/B



[a] (a) **167**, EDCI, HOAt, $^{i}Pr_2NEt$, DMF, CH_2Cl_2 (1:1), 0°C, 18-24h, 46% (from **149**); (b) Pd black, H_2 (1 atm), MeOH, 2h, RT; (c) DIAD, PPh_3, DMF, THF, 0°→RT; then H_2O, 70% (2 steps).

material identically matched the natural samples[66] in regards to ^{1}H NMR, ^{13}C NMR, optical rotation, TLC, high-resolution mass spectrometry and RP-HPLC.

3.9. Conclusion.

In conclusion, a concise and efficient total synthesis of TMC-95A/B has been accomplished. The synthesis was completed in 22 total steps with 18 in the longest linear sequence (as compared to Danishefsky: 28 total, 23 longest linear sequence; and Hirama/Inoue: 40 total, 35 longest linear sequence). It should be noted that currently this is the shortest overall synthesis and the only synthesis to be derived from L-serine instead of D-serine. Our synthesis features an *E*-selective modified Julia olefination to form the key oxindolene. It has been found that this transformation is also a viable route to other protected β-γ unsaturated amino alcohols.[67] Also, this synthesis is amenable to

the preparation of a variety of analogs due to its convergency and the fact that minimal protecting groups are employed.

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[62] *Total synthesis of (+)-crocacin D.* Chakraborty, T. K.; Laxman, P. *Tetrahedron Lett.* **2002**, *43*, 2645-2648.

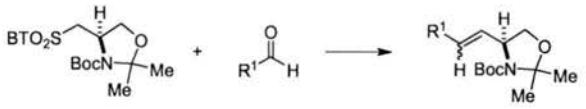
[63] *The Stereoselective Conversion of Vinylsilanes to γ-Substituted Allylsilanes Via Higher-Order Cuprates.* Soderquist, J. A.; Santiago, B. *Tetrahedron Lett.* **1989**, *30*, 5693-5696, and references therin.

[64] The following reaction cleanly provided the desired enamide.

[65] *Total synthesis of the Ramoplanin A2 and Ramoplanose Aglycon.* Jiang, W. L.; Wanner, J.; Lee, R. J.; Bounaud, P. Y.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5288-5290.

[66] We would like to gratefully thank Dr. Jun Kohno, Tanabe Seiyaku Co., Ltd., for generously providing authentic samples of TMC-95A and TMC-95B which were invaluable for comparison.

[67] A brief study has shown that this chemistry also works with aliphatic and aromatic aldehydes:



R = Ph, *p*-OMePh, Pr

Chapter 4. Experimental Section

General Procedures.

Unless otherwise noted, materials were obtained from commercial sources and utilized without purification. All reactions requiring anhydrous conditions were performed under a postive pressure of argon using flame-dried glassware that was cooled under dry argon. Tetrahydrofuran, dimethylformamide and toluene were degassed with argon and passed through a solvent purification system (J.C Meyer of Glass Contour) containing alumina or molecular seives. Dichloromethane was distilled from CaH_2 prior to use.

Column chromatography was performed on Merck silica gel Kieselgel 60 (230-400 mesh).

Mass spectra were obtained on Fisons VG Autospec.

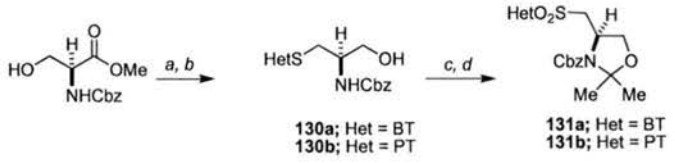
HPLC data were obtained on a Waters 600 HPLC.

^1H NMR, ^{13}C NMR and nOe experiments were recorded on a Varian 300 or 400 MHz spectrometer. Chemical shifts (δ) were given in ppm and were recorded relative to the residual solvent peak unless otherwise noted. ^1H NMR were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When a signal was deemed "broad" it was noted as such.

IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer.

Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

4.1 Experimental procedures for compounds leading to the total synthesis.



130a; Het = BT
130b; Het = PT

131a; Het = BT
131b; Het = PT

General Procedure for the preparation of sulfones 131. A mixture of *N*-Cbz-serine methyl ester (4.0 g, 15.8 mmol), HetSH (31.6 mmol), and PPh_3 (6.28 g, 23.7 mmol) were taken up in dry THF (160 mL) under argon. To the resulting mixture was added DIAD (5.90 mL, 28.44 mmol) at room temperature and allowed to stir for 15 minutes. The reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was removed and the aqueous layer was extracted with diethyl ether (3 x 200 mL). The combined organics were washed with water (1 x 200 mL) and brine (1 x 200 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered through ~ 40 mL of silica gel and concentrated under reduced pressure. The crude mixture was used without further purification.

To a mixture of $CaCl_2$ (8.8 g, 79 mmol) in THF (400 mL) at 0°C was added $NaBH_4$ (6.04 g, 158.0 mmol) and allowed to stir for 10 min. A solution of the crude product from previous, THF (200 mL) and water (40 mL) were added via addition funnel over 20 min at 0°C. The resulting mixture was allowed to warm to room temperature and stir for 24 h. The reaction was quenched with water and acidified to pH~7 with 1 M HCl. The resulting mixture was extracted with Et_2O (3 x 200 mL), and the combined organics were washed with water (1 x 200 mL), brine (1 x 40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to an oil.

The resulting crude oil was taken up in 600 mL of CH_2Cl_2 to which was added

p-toluene sulfonic acid monohydrate (536 mg, 2.78 mmol) and 2,2-dimethoxypropane (60 mL). The resulting mixtue was allowed to stir for 5 h at which time the reaction mixture was diluted with CH_2Cl_2 (200 mL). The resulting mixture was washed with saturated aq. $NaHCO_3$ (1 x 200 mL), water (1 x 200 mL), 0.1 M HCl (1 x 200 mL), and brine (1 x 200 mL). The organic layer was dried over dried over anhydrous Na_2SO_4, filtered and concentrated to an oil.

The crude oil was taken up in EtOH (600 mL). To the ethanol solution was added a mixture of $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$ (1.84 g, 1.49 mmol) and H_2O_2 (30% in H_2O, 14.24 mL, 139 mmol) and allowed to stir at room temperature for 12 h. The resulting mixture was diluted with water and extracted with CH_2Cl_2 (3 x 200 mL). The combined organics were washed with water (1 x 100 mL), brine (1 x 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated.

NOTE: In practice, the above reactions were carried out without purification until isolation of the final sulfones **131**. Some intermediates were isolated for analytical purposes only, see below.

Alcohol 130a (Het=BT). Purified via flash chromatography (silica gel, 2:1 → 1:1 hexanes:EtOAc) to afford alcohol **130a** as a colorless oil which solidified upon standing. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 3.50 (dd, *J*=14.3, 4.4 Hz, 1H), 3.62 (m, 2H), 3.87 (dd, *J*=12.2, 2.6 Hz, 1H), 3.97 (m, 1H), 5.12 (s, 2H), 5.77 (d, *J*=7.9 Hz, 1H), 7.37-7.29 (m, 6H), 7.42 (dt, *J*=7.3, 1.5 Hz, 1H), 7.73 (d, *J*=8.5 Hz, 1H), 7.80 (d, *J*= 8.8 Hz, 1H). ^{13}C (75 MHz, $CDCl_3$, 273K) δ: 33.2, 53.3, 61.3, 67.1, 121.0,, 121.3, 124.9, 126.5, 128.1, 128.3, 128.6, 135.0, 136.4, 151.9, 155.9, 169.0. HRMS(FABH+) Calcd: 375.0837 (*m/z*). Found: 375.0837 (*m/z*).

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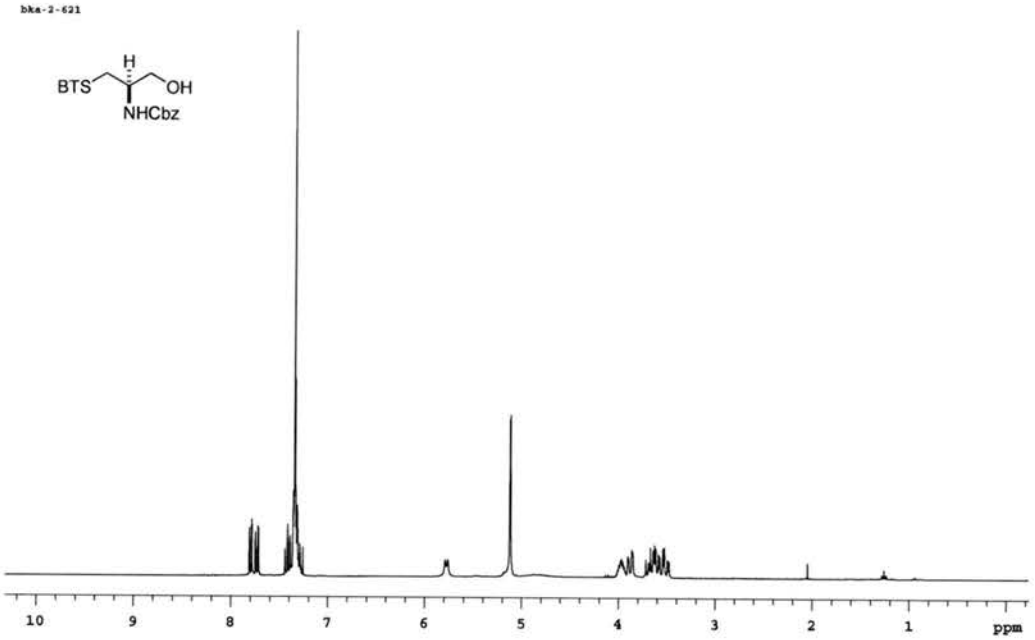
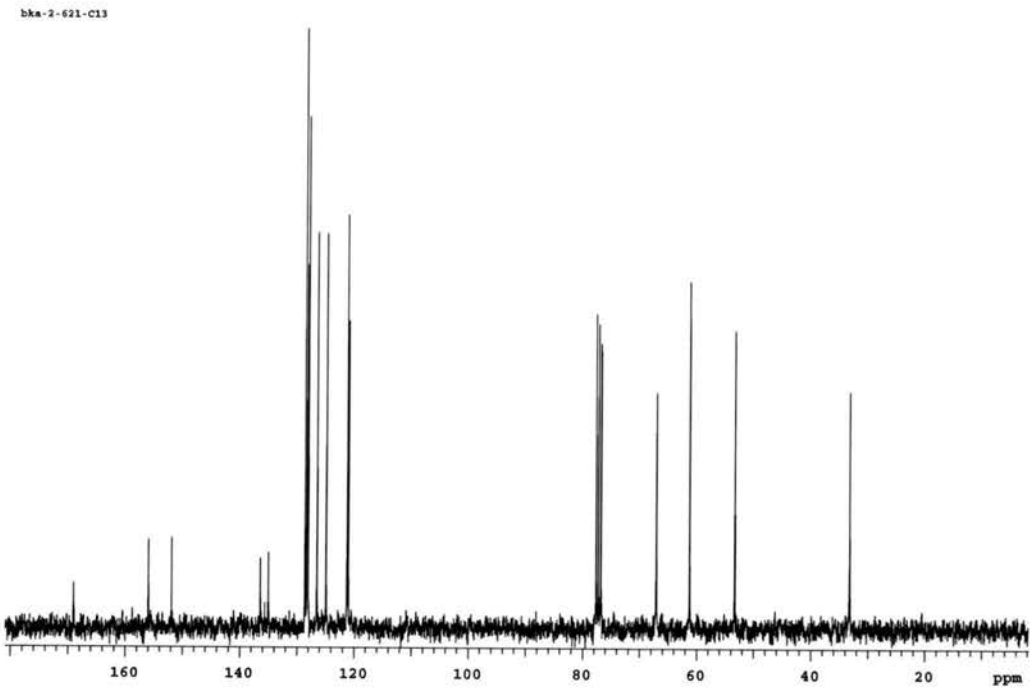
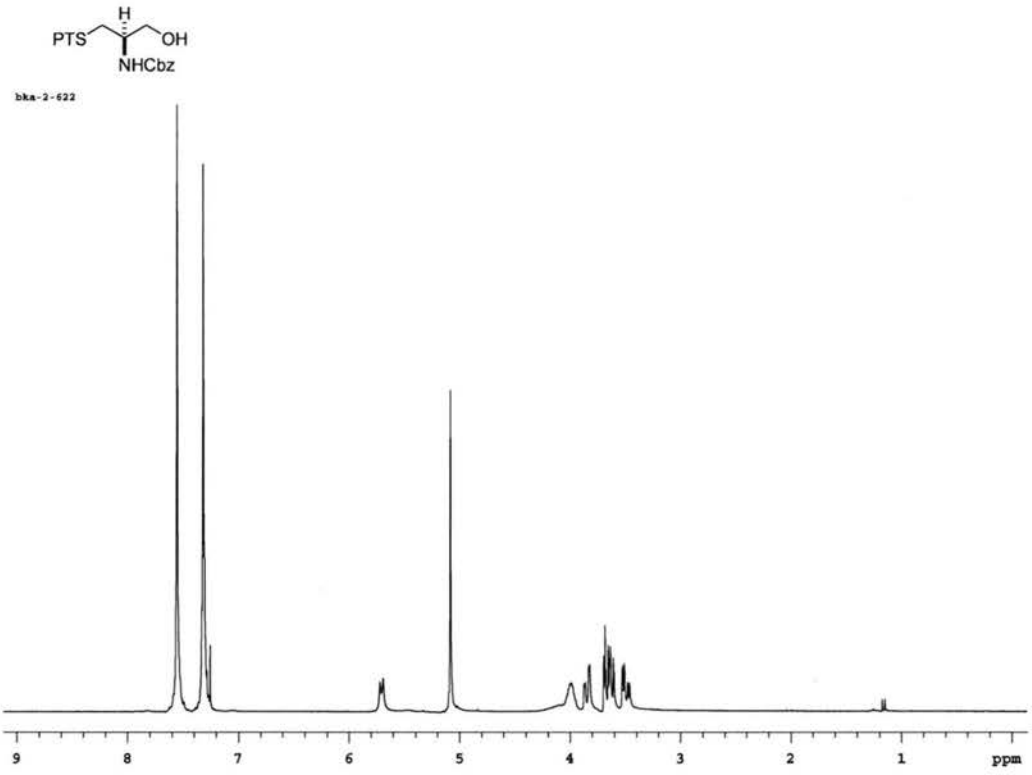


Filename:bka-2-621-C13



Alcohol 130b (Het=PT). Purified via flash chromatography (silica gel, 2:1 → 1:1 hexanes:EtOAc) to afford alcohol **130b** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 3.49 (dd, *J*= 14.4, 5.4 Hz, 1H), 3.65 (m, 2H), 3.85 (dd, *J*=12.2, 2.4 Hz, 1H), 4.00 (m, 1H), 4.10 (br s, 1H), 5.08 (s, 2H), 5.71 (br d, *J*=8.0 Hz, 1H), 7.32 (br s, 5H), 7.56 (br s, 5H). ^{13}C (75 MHz, $CDCl_3$, 273K) δ 33.6, 53.2, 61.5, 67.1, 124.0, 128.1, 128.3, 128.6, 130.0, 130.6, 133.3, 136.2, 155.3, 156.0.

Filename: bka-2-622



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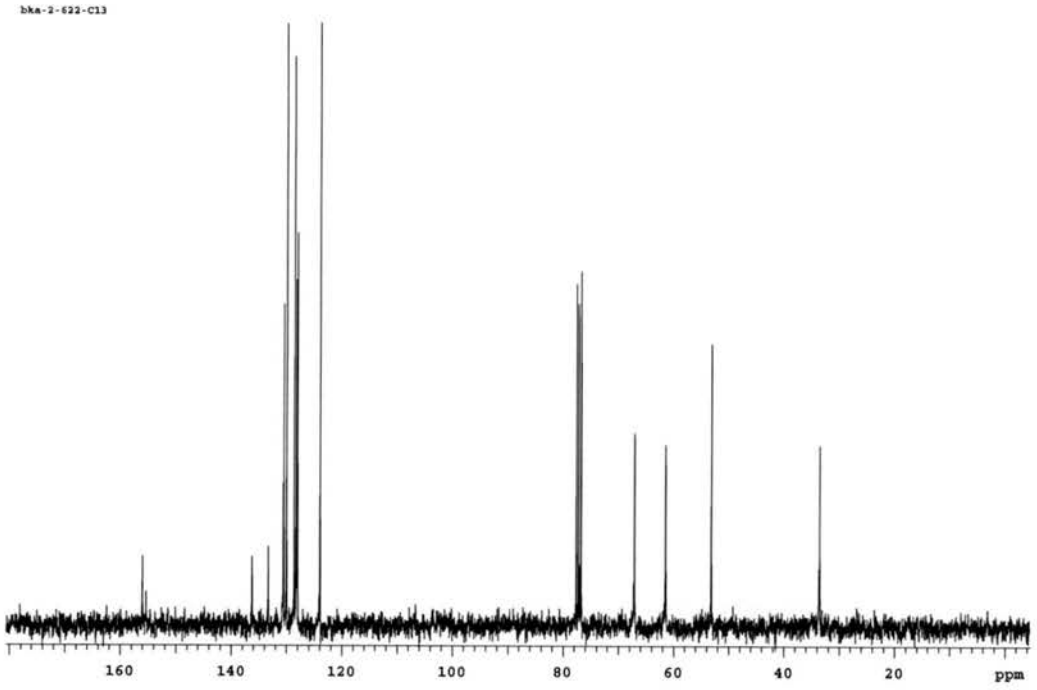
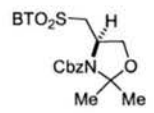
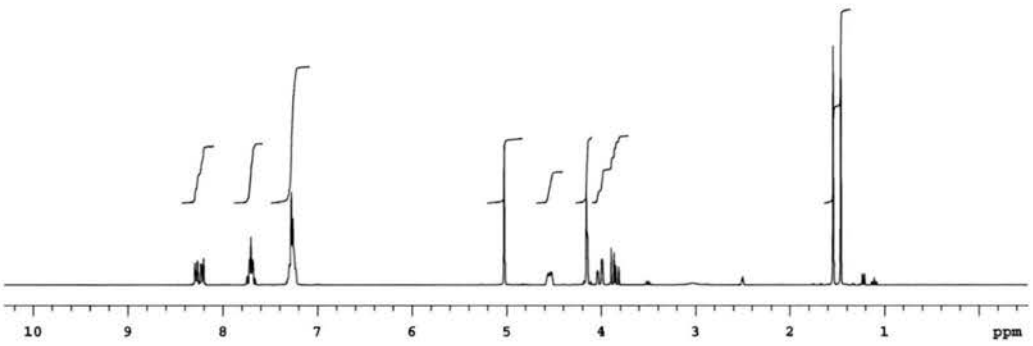


Sulfone 131a (Het=BT). Crude isolate is an off white solid. The resulting solid was recrystallized from absolute EtOH to afford sulfone **131** (65 %, three steps) as a shiny white solid. Mp = 118-121°C (uncorrected). $[\alpha]_D^{25}$ = + 16.4 (*c* 0.86, $CHCl_3$); ^1H NMR (300 MHz, dmso-d_6, 393 K, mixture of non-coalescing rotamers) δ 1.45 (s, 3H), 1.53 (s, 3H), 3.86 (1/2 ABX, dd, *J*= 14.3, 9.7 Hz, 1H), 4.01 (1/2 ABX, dd, *J*= 14.3, 2.0 Hz, 1H), 4.16-4.13 (m, 2H), 4.57-4.49 (m, 1H), 5.01 (s, 2H), 7.40-7.20 (m, 5H), 7.80-7.65 (m, 2H), 8.32-8.19 (m, 2H).; ^{13}C (100 MHz, DMSO-d_6, 273 K, mixture of rotamers, major listed) δ 22.7, 26.2, 51.5, 55.8, 65.9, 66.4, 93.5, 123.6, 124.9, 127.3, 127.8, 128.1, 128.2, 128.3, 136.2, 136.4, 150.7, 152.2, 165.7; IR ($CHCl_3$ film): 2985, 1709, 1406, 1350, 1330, 1138, 1092, 762; HRMS (FABH+) calcd for $C_{21}H_{23}N_2O_5S_2$ (*m/z*) 447.1048; found (*m/z*) 447.1047.

Filename:bka-3-976

STANDARD 1H OBSERVE





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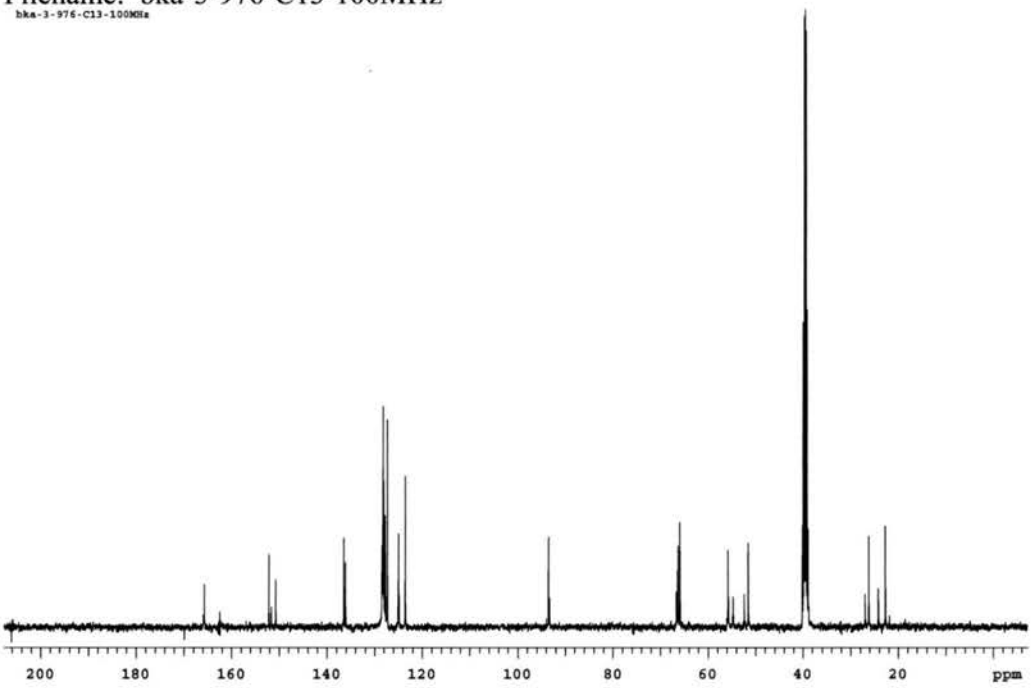

Sulfone 131b (Het=PT). Purified via flash chromatography (silica gel, 2:1 hexanes:EtOAc). ^{1}H NMR (300 MHz, dmso-d_6, 393 K) δ 1.46 (s, 3H), 1.53 (s, 3H), 4.15-4.00 (m, 4H), 4.58-4.49 (m, 1H), 5.18 (1/2 ABq, J=12.6 Hz, 1H), 5.08 (1/2 ABq, J=12.6 Hz, 1H), 7.27-7.40 (m, 5H), 7.60-7.74 (m, 5H). ^{13}C (750 MHz, dmso-d_6, 273 K, mixture of rotamers) δ 22.8, 24.09, 24.10, 26.3, 27.0, 51.0, 52.2, 57.1, 57.3, 66.2, 66.3, 66.5, 66.8, 93.5, 93.6, 126.4, 126.44, 127.5, 127.8, 127.9, 128.05, 128.07, 128.1, 128.4, 128.45, 128.47, 128.51, 129.4, 131.6, 132.9, 136.1,136.3, 150.82, 150.83, 151.8, 153.40, 155.43, 171.3. IR (CH_2Cl_2 film) 1708, 1497, 1350, 1147. HRMS (FABH+) Calcd: 458.1498 (*m/z*); Found: 458.1485 (*m/z*).

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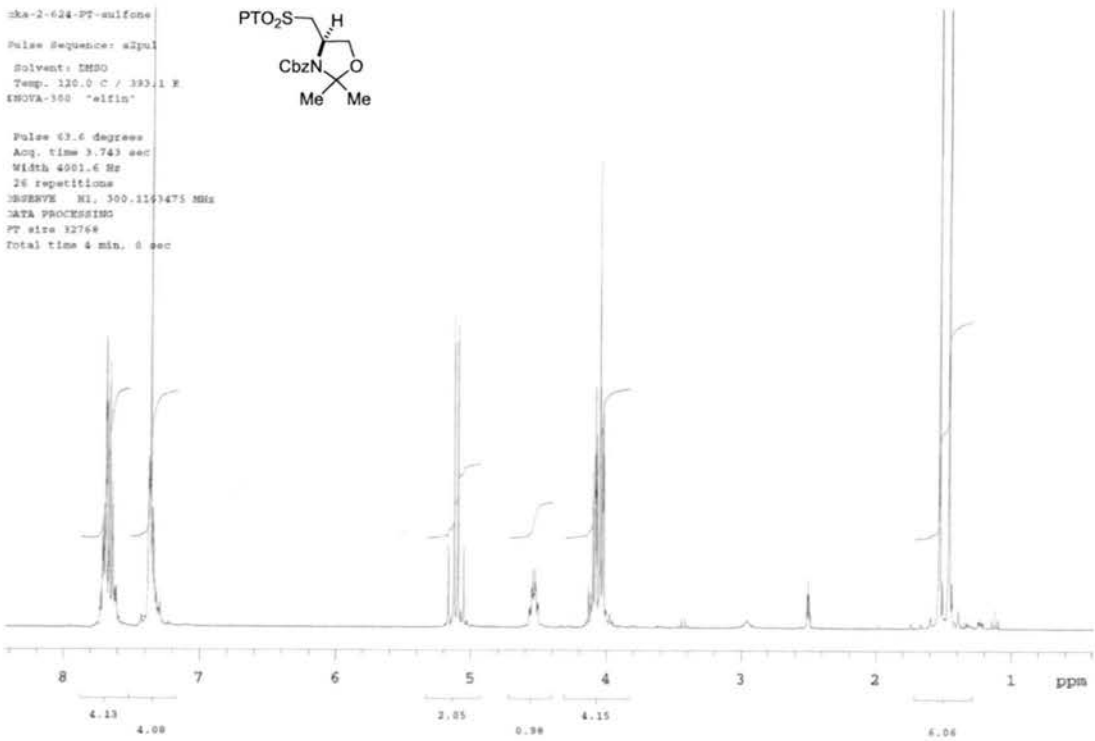

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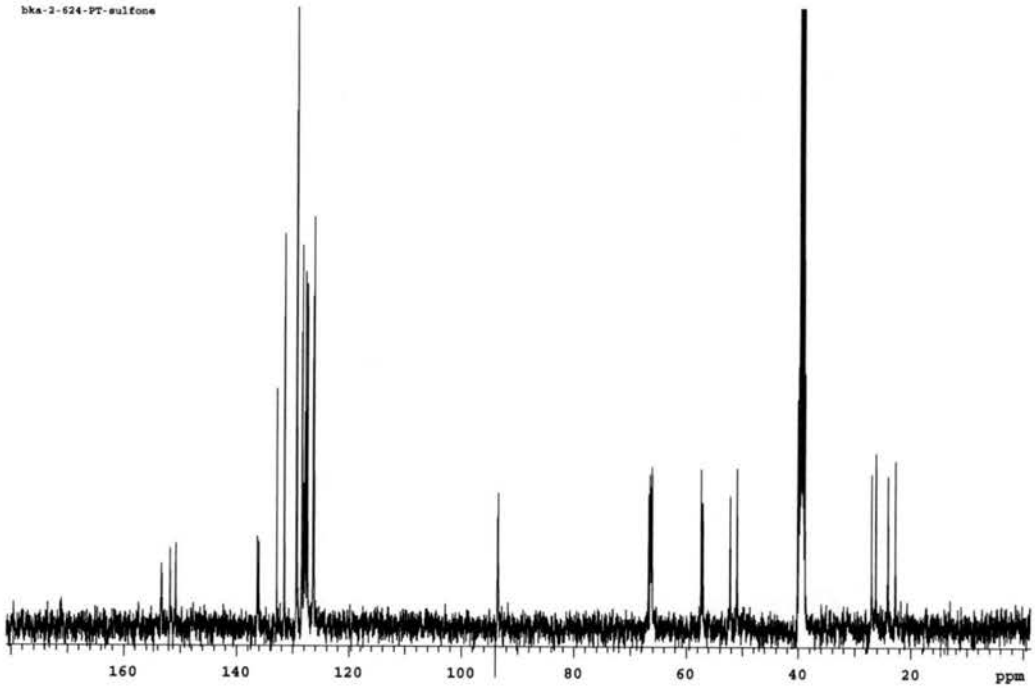

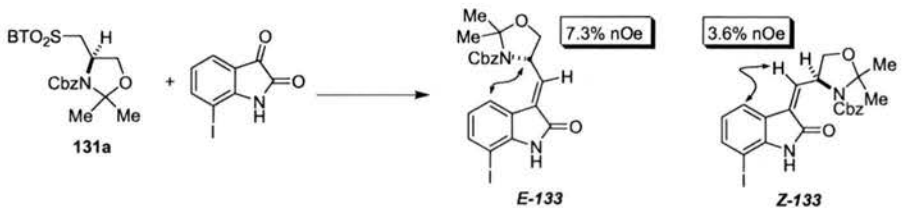

Note: In an effort to simplify the separation of the *E* and *Z* isomers of above, the reaction was routinely performed on the scale described below. In order to obtain sufficient material for the total synthesis, iterations of below were performed and the products pooled.

Oxindolene 133. To solution of sulfone **131a** (1.29 mg, 2.88 mmol), 7-iodoisatin (660 mg, 2.4 mmol), DMF (50 mL) and DMPU (50 mL) at 0°C under argon was added LiHMDS (1.0 M, 7.2 mL, 7.2 mmol) and allowed to stir at 0°C for 20 min. The reaction was quenched with 3% aq. NH_4Cl (~100 mL). The resulting mixture was extracted with Et_2O:THF (10:1) (4 x 40 mL). The combined organics were washed with 10% aq. NaOH (2 x 20 mL), water (1 x 40 mL) and brine (1 x 40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford a crude mixture of 5:1 *E:Z* geometric isomers which on purification by flash chromatography (3:1:1 Hexanes, EtOAc, $CHCl_3$) furnished the *Z*-isomer (242 mg, 13%) and the *E*-isomer (1.2 g, 66%) both as yellow solids.

***E*-isomer 133**: $[\alpha]_D^{25} = -10.5$ (*c* 1.56, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6, 383 K) δ 1.56 (s, 3H), 1.65 (s, 3H), 3.87 (dd, *J*=9.2, 3.0 Hz, 1H), 4.38 (dd, *J*=9.2, 6.8 Hz, 1H), 4.96 (1/2 ABq, *J*=12.6 Hz, 1H), 5.07 (1/2 ABq, *J*=12.6 Hz, 1H), 5.34 (ddd, *J*=9.6, 6.8, 3.0 Hz, 1H), 6.66 (d, *J*=9.6 Hz, 1H), 6.73 (dd, *J*=7.9, 7.7 Hz, 1 H), 7.25-7.15 (m, 5H), 7.56

(overlapping d, *J*=7.9 Hz, 2H), 9.99 (br s, 1H); ^{13}C (100 MHz, dmso-d_6, 273 K, mixture of rotamers, major rotamer listed) δ 23.5, 26.3, 54.4, 66.0, 67.7, 75.4, 94.2, 121.9, 123.3, 124.0, 127.2, 127.6, 128.0, 128.7, 136.1, 138.4, 139.8, 145.2, 151.6, 167.6; IR ($CHCl_3$ film): 3205, 1703, 1676, 1606, 1469, 1347; HRMS (FABH+) calcd for $C_{22}H_{22}N_2O_4I$ (*m/z*) 505.0624; found (*m/z*) 505.0624.

***Z*-isomer 133.** ^1H NMR (300 MHz, dmso-d_6, 393 K) δ 1.55 (s, 3H), 1.67 (s, 3H), 3.82 (dd, *J*=9.0, 3.7 Hz, 1H), 4.31 (dd, *J*=9.0, 6.8 Hz, 1H), 4.99 (1/2 ABq, *J*=12.7 Hz, 1H), 5.13 (1/2 ABq, *J*=12.7 Hz, 1H), 5.86 (ddd, *J*=8.2, 6.8, 3.7 Hz, 1H), 6.77 (overlap dd, *J*=7.7, 7.7 Hz, 1 H),6.93 (d, *J*=8.2 Hz, 1H), 7.25-7.15 (m, 5H), 7.56 (overlap dd, *J*=7.7, 7.7 Hz, 2H), 9.99 (br s, 1H). ^{13}C (75 MHz, dmso-d_6, 273 K, mixture of rotamers, major rotamer listed) δ 23.6, 26.1, 53.7, 65.7, 68.3, 74.8, 94.2, 120.2, 123.2, 123.5, 126.9, 127.5, 128.0, 136.4, 138.0,142.6, 144.0, 151.6, 167.5. IR ($CHCl_3$ film): 3205, 1703, 1676, 1606, 1469, 1347. HRMS (FABH+) calcd for $C_{22}H_{22}N_2O_4I$ (*m/z*) 505.0624; found (*m/z*) 505.0624.

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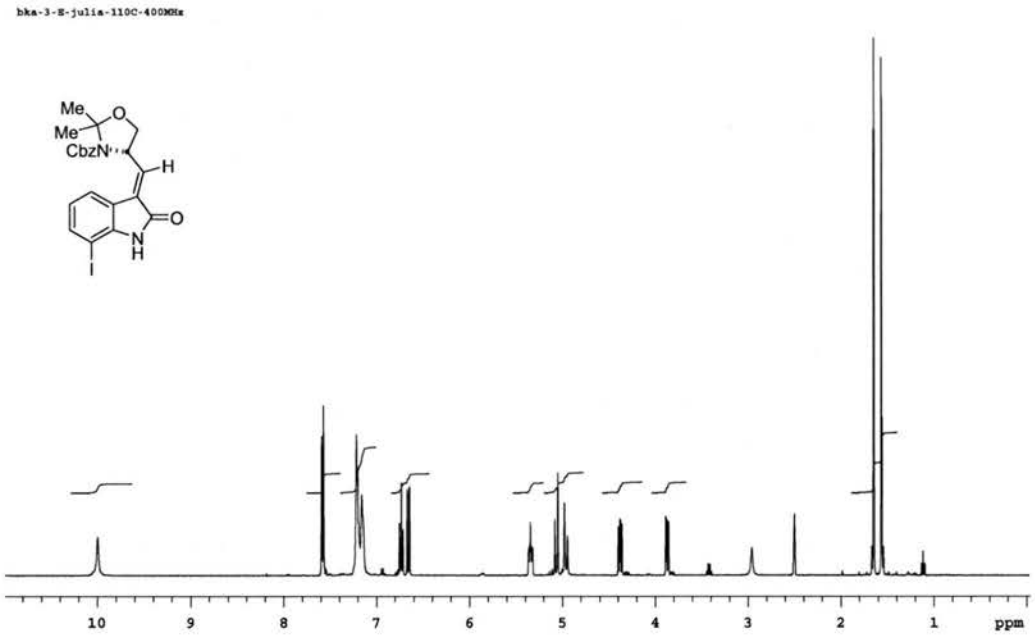

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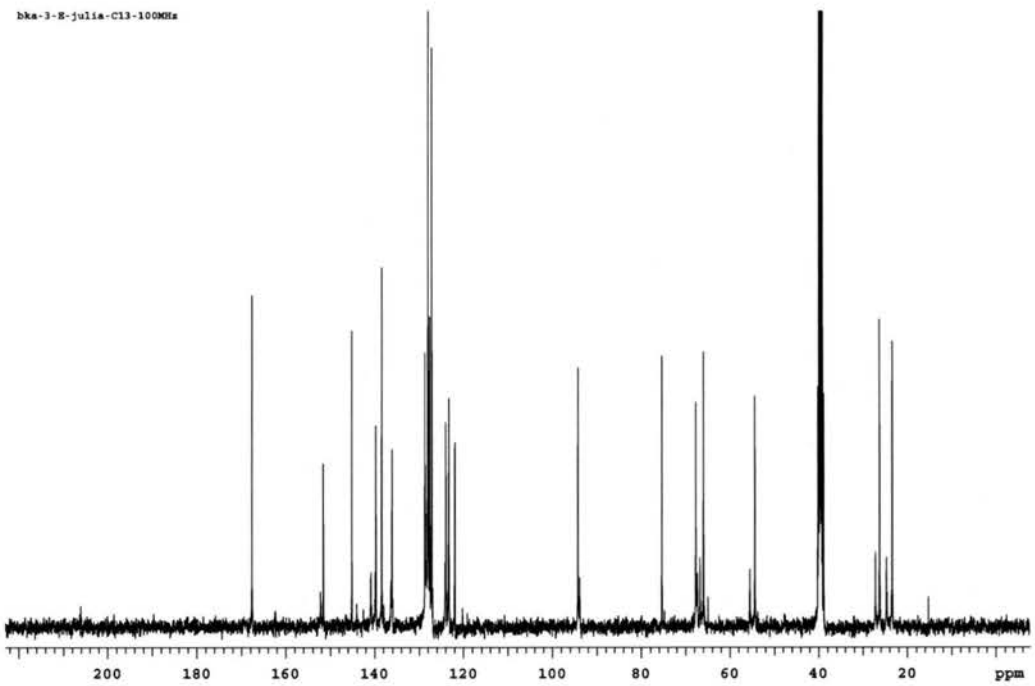

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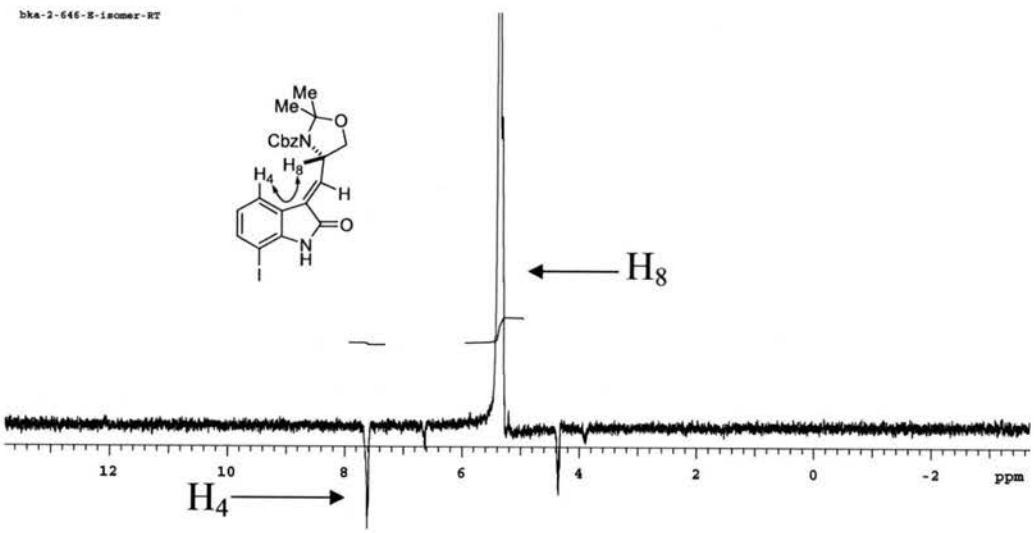

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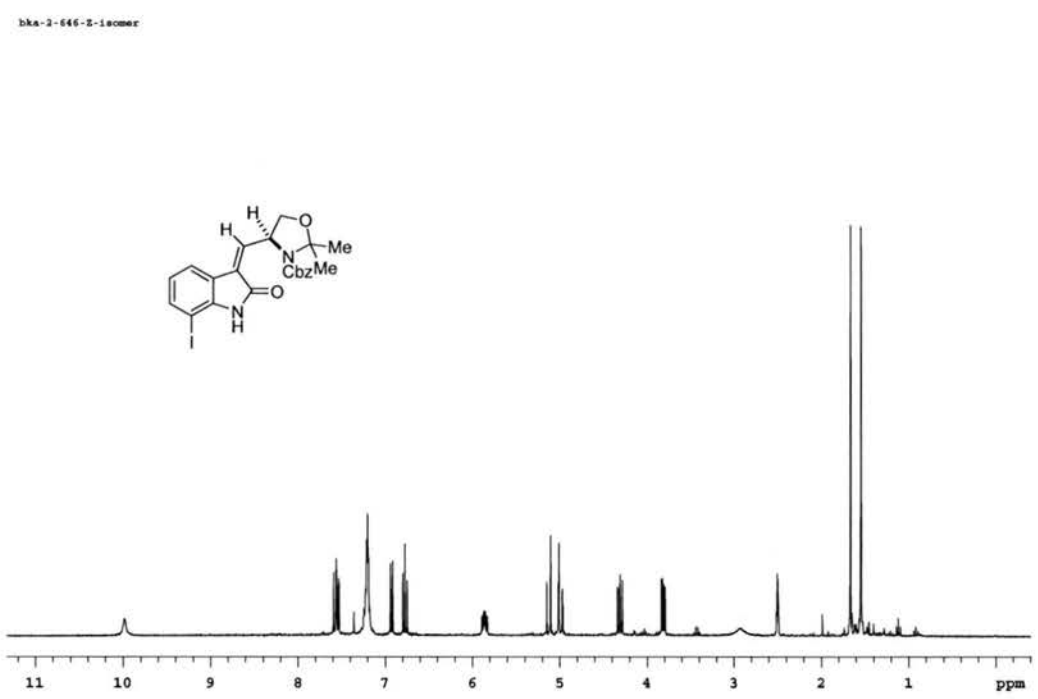

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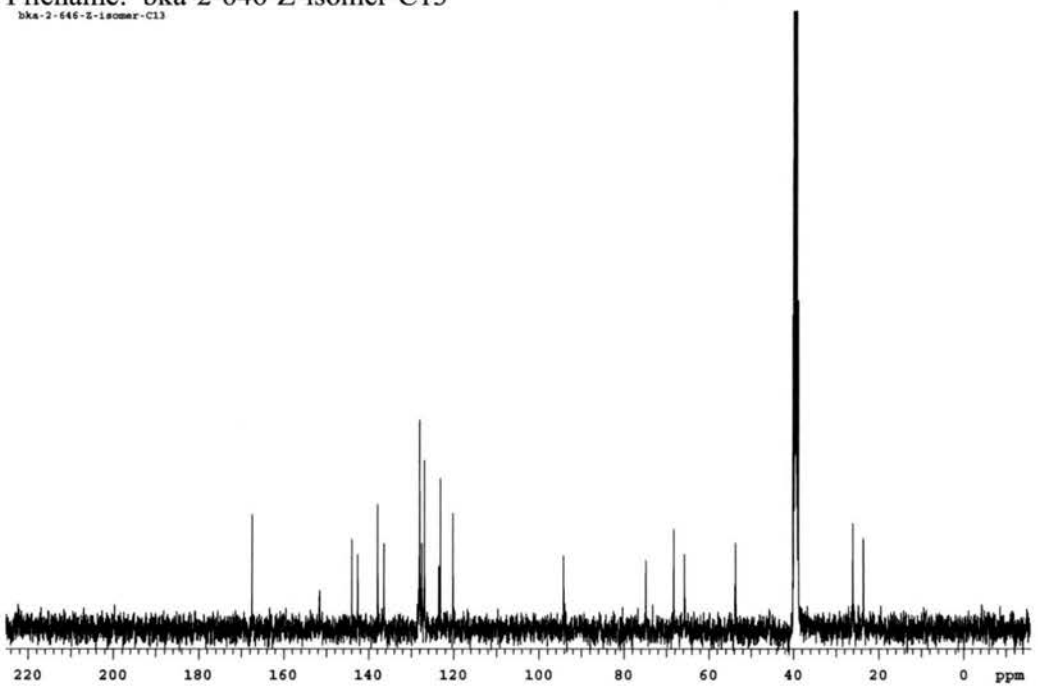

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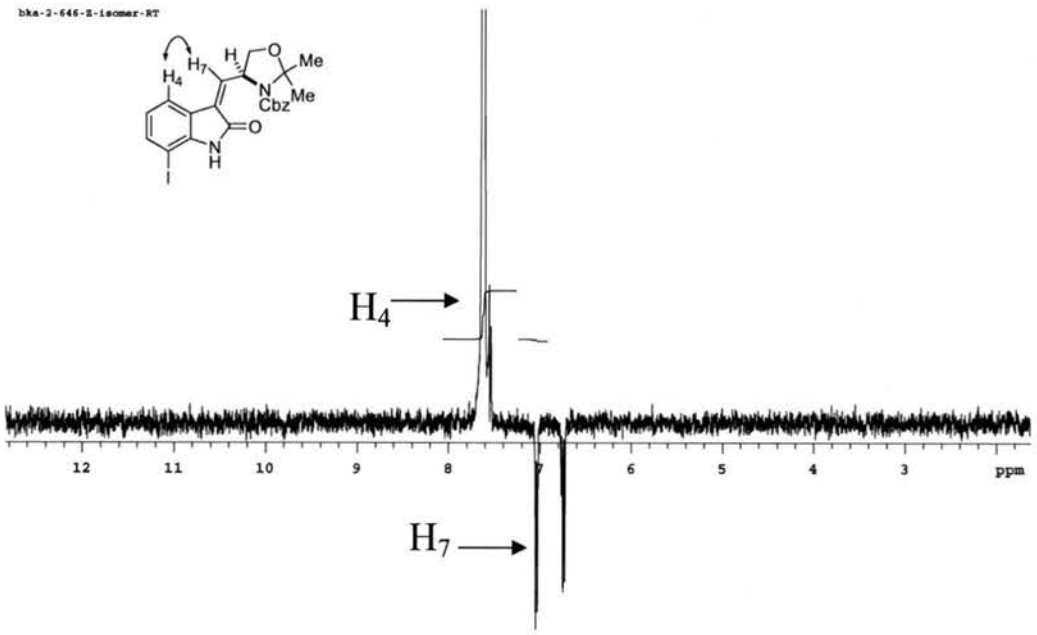
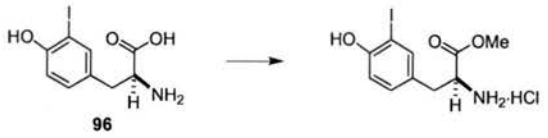
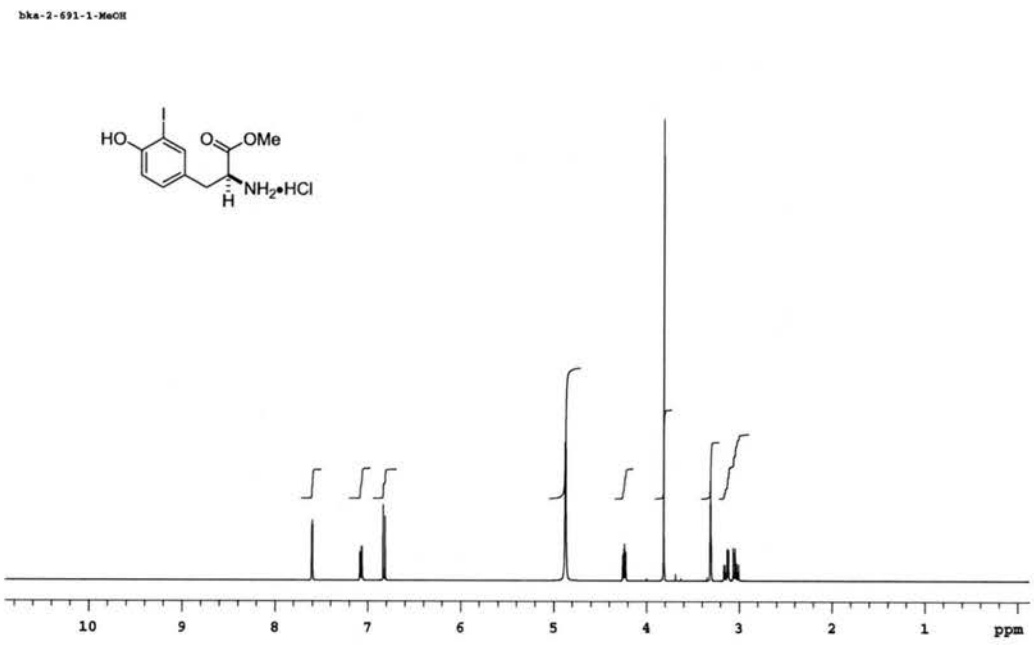




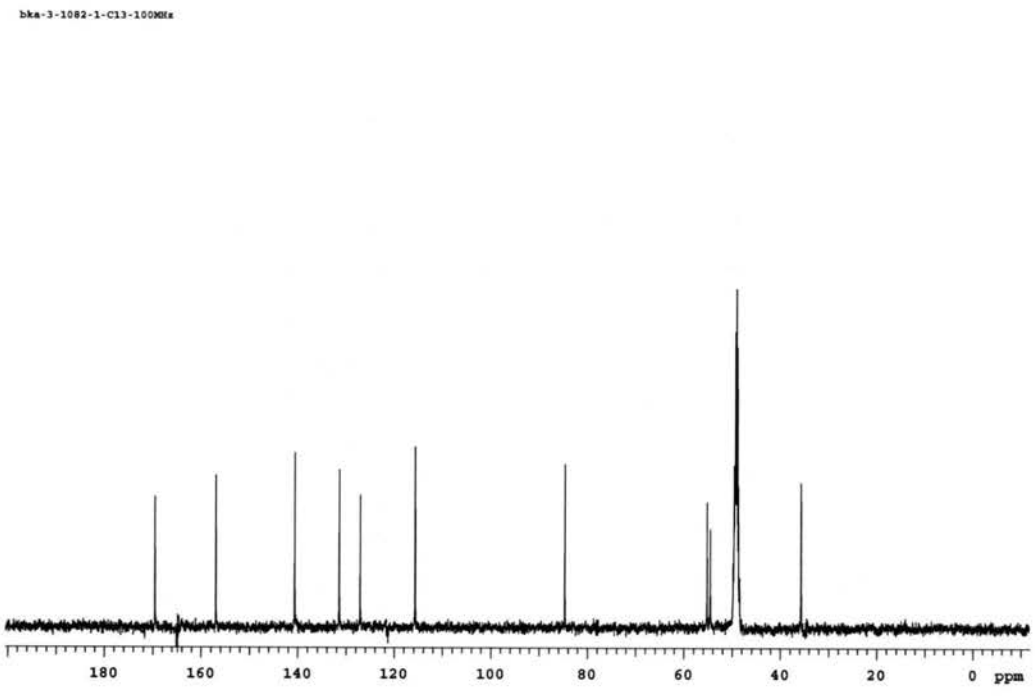
96

3-iodotyrosine-OMe-ester hydrochloride. To a solution of 3-iodotyrosine (2.0 g, 6.52 mmol) in methanol (80 mL) at room temperature was added $SOCl_2$ (8.4 mL) and allowed to stir for 18 h. The resulting mixture was concentrated under reduced pressure to afford 3-iodotyrosine-OMe-ester hydrochloride (2.34 g, 100%) as a white solid.$[\alpha]_D^{25}$ = + 9.3 (*c* 0.56, CH_3OH); ^1H NMR (400 MHz, CD_3OD, 273 K) δ 3.03 (dd, *J*=14.5, 7.5 Hz, 1H), 3.14 (dd, *J*=14.5, 6.1 Hz, 1H), 3.82 (s, 3H), 4.24 (dd, *J*=7.5, 6.1 Hz, 1H), 6.83 (d, *J*=8.3 Hz, 1H), 7.08 (dd, *J*=8.3, 2.2 Hz, 1H), 7.60 (d, *J*=2.2 Hz, 1H); ^{13}C (100 MHz, CD_3OD, 273 K) δ: 35.7, 54.6, 55.2, 84.7, 115.7, 127.1, 131.5, 140.7, 157.0, 169.5; IR (MeOH film): 3132, 2952, 1740, 1505, 1416, 1290, 1244; HRMS (FABH+) calcd for $C_{10}H_{13}N_1O_3I_1$ (*m/z*) 321.9940; found (*m/z*) 321.9946.

Filename: bka-2-691-MeOH

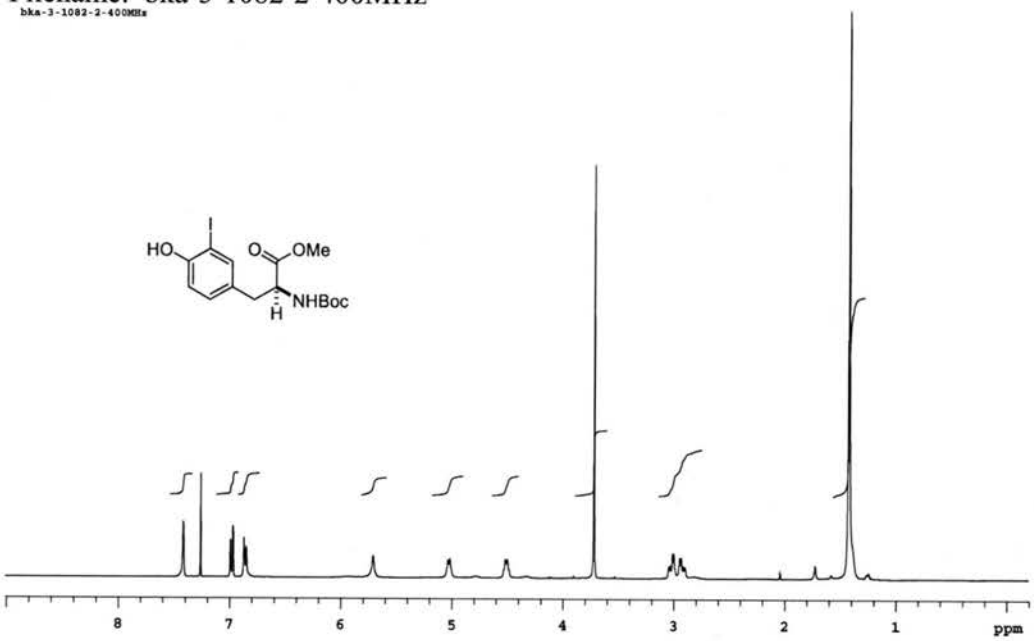


Filename: bka-3-1082-1-C13-100MHz

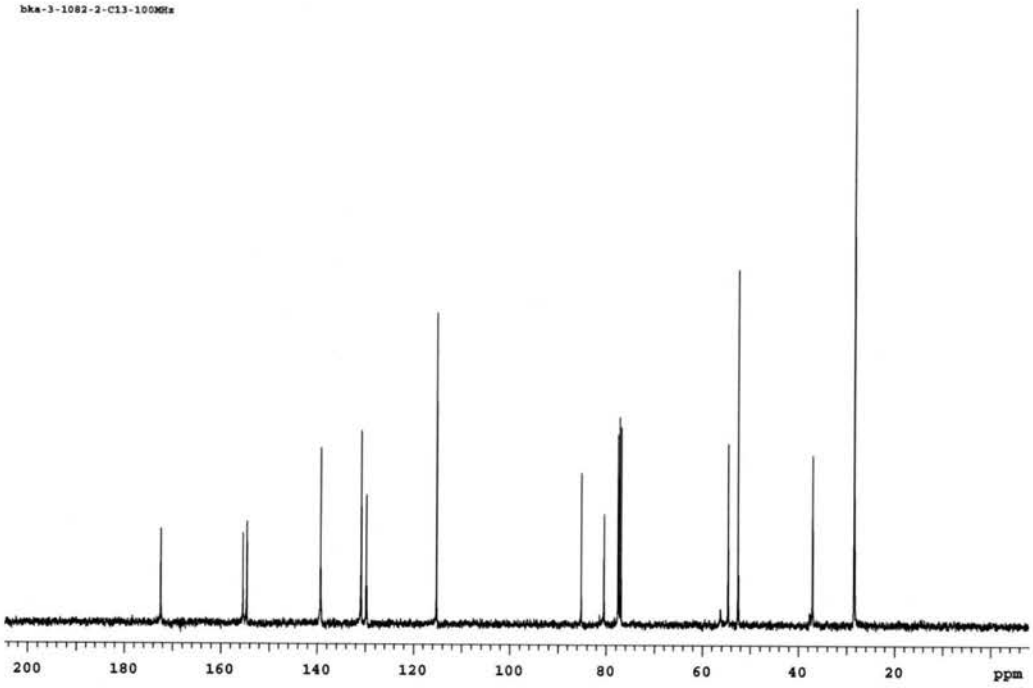


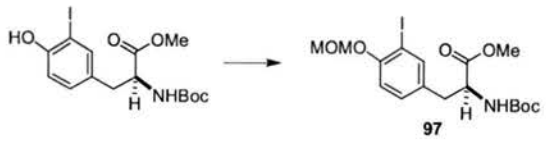
***N*-Boc-3-iodotyrosine methyl ester. Note: Althought the product of this reaction is used crude see below for data.** To a biphasic mixture of 3-iodotyrosine-OMe-ester hydrochloride (2.34 g, 6.52 mmol), CH_2Cl_2 (100 mL) and sat. aq. $NaHCO_3$ (60 mL) at 0° C was added Boc_2O (1.47 g, 6.52 mmol). The reaction was allowed to warm to room temperature and stir for 24 h. The organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were washed with 1 M HCl (1 x 10 mL), sat. aq. $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to a colorless oil which was used without further purification. $[\alpha]_D^{25} = +43.5$ (*c* 0.69, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD, 273 K) δ 1.43 (s, 9H), 2.92 (dd, *J*=13.8, 5.8 Hz, 1H), 3.02 (dd, *J*=13.8, 5.8 Hz, 1H), 3.72 (s, 3H), 4.51 (overlap dd, *J*=5.8, 5.8 Hz, 1H), 5.02 (brd, *J*=7.7 Hz, 1H), 5.71 (brs, 1H) 6.86 (d, *J*=8.3 Hz, 1H), 6.98 (dd, *J*=8.3, 1.7 Hz, 1H), 7.43 (br s, 1H); ^{13}C (100 MHz, CD_3OD, 273 K) δ: 28.4, 37.1, 55.6, 54.6, 80.5, 85.2, 115.2, 129.8, 130.8, 139.3, 154.6, 155.4, 172.4. IR (MeOH film): 3362, 2977, 1740, 1684, 1505, 1366, 1163; HRMS (FABH+) calcd for $C_{15}H_{21}N_1O_5I_1$ (*m/z*) 422.0464; found (*m/z*) 422.0465.

Filename: bka-3-1082-2-400MHz



Filename: bka-3-1082-2-C13-100MHz



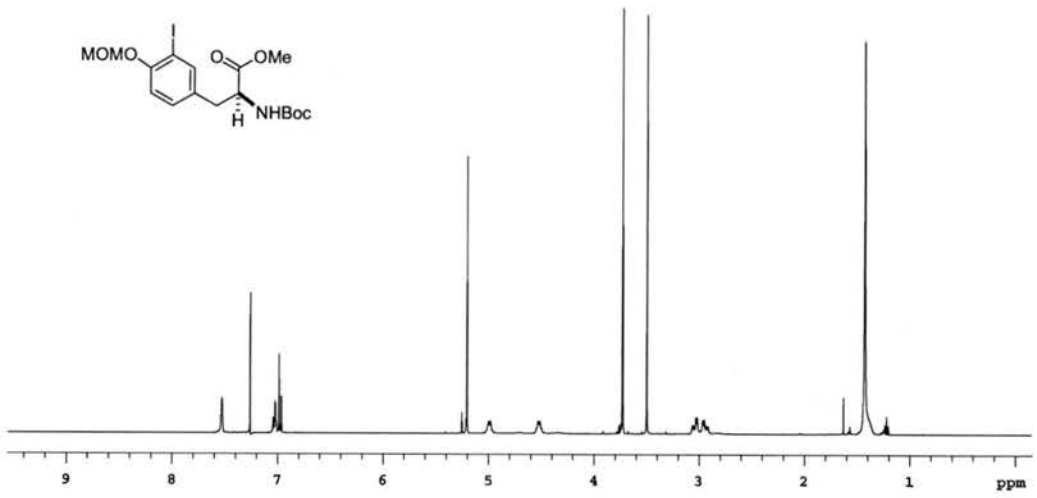


97

The resulting oil was taken up in anhydrous CH_2Cl_2 (35 mL) under argon and cooled to 0° C. To the resulting solution was added $^{i}Pr_2NEt$ (1.6 mL, 9.2 mmol) followed by MOMCl (0.78 mL, 9.2 mmol). The reaction was allowed to stir for 1.5 h then diluted with CH_2Cl_2 (100 mL). The organic layer was washed with 1 M HCl (20 mL), water (1 x 20 mL), sat. aq. $NaHCO_3$ (1 x 40 mL) and brine (1 x 40 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford tyrosine derivative **97** (2.98 g, 98%, two steps) which formed a white solid upon standing. $[\alpha]_D^{25}$ = +41.62 (*c* 1.48, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 273 K) δ: 1.44 (s, 9H), 2.94 (dd, *J*=13.9, 5.9 Hz, 1H), 3.04 (dd, *J*=13.9, 5.5 Hz, 1H), 3.51 (s, 3H), 4.52 (m, 1H), 5.00 (br d, *J*=7.7 Hz, 1H), 5.21 (s, 2H), 6.97 (d, *J*=8.4 Hz, 1H), 7.03 (dd, *J*=8.4, 1.8 Hz, 1H), 7.53 (d, *J*=1.8 Hz, 1H); ^{13}C (100 MHz, $CDCl_3$, 273 K) δ 28.4, 36.9, 52.4, 54.5, 56.6, 80.0, 87.2, 95.0, 114.8, 130.4, 131.5, 140.3, 155.1, 155.2, 172.1; IR ($CHCl_3$ film): 3364, 2975, 2932, 1744, 1714, 1489, 1366, 1242, 1162, 991; HRMS (FABH+) calcd for $C_{17}H_{25}N_1O_6I_1$ (*m/z*) 466.0727; found (*m/z*) 466.0718.

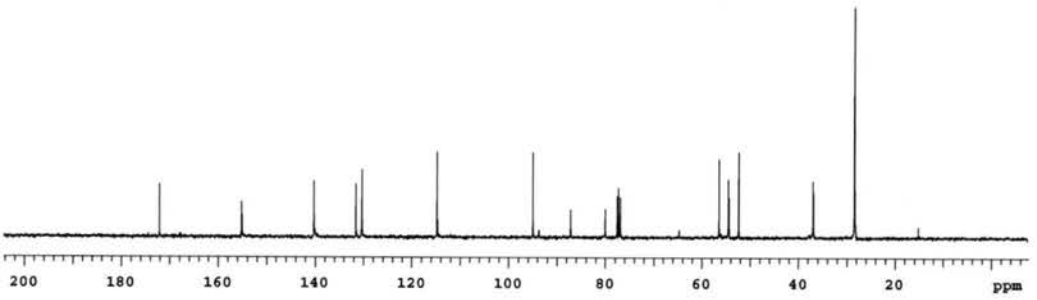
Filename: bka-2-692

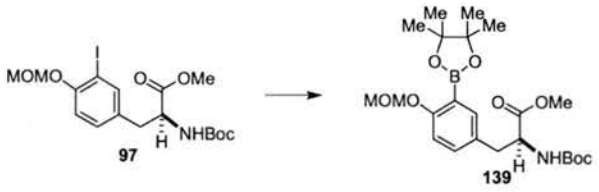
bka--2-692-400MHz



Filename: bka-2-692-C13-100MHz

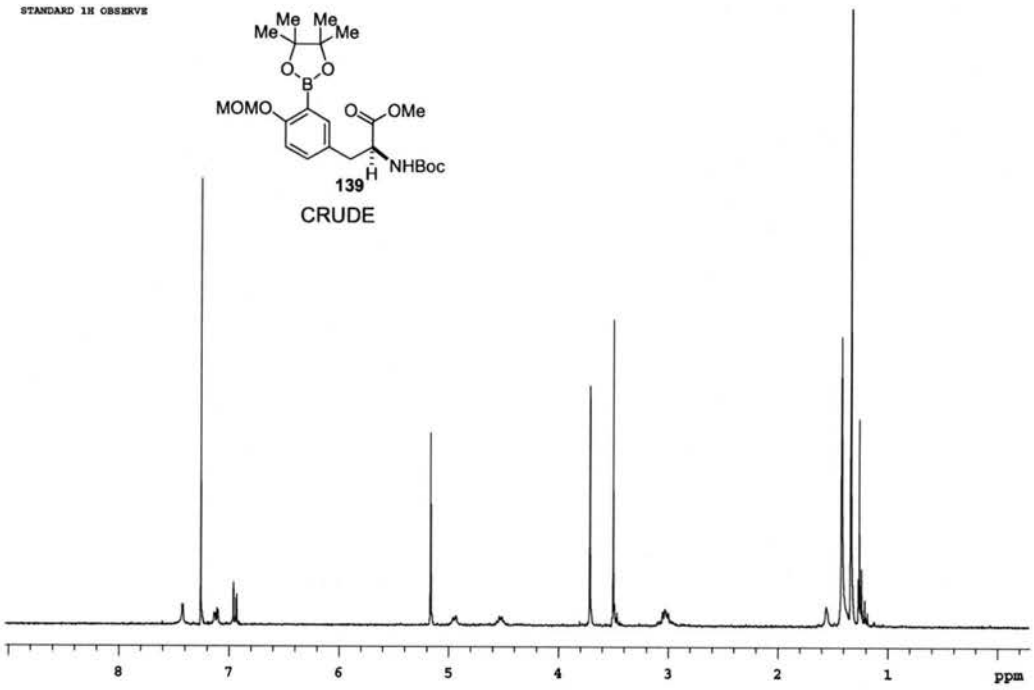
bka-2-692-C13-100MHz

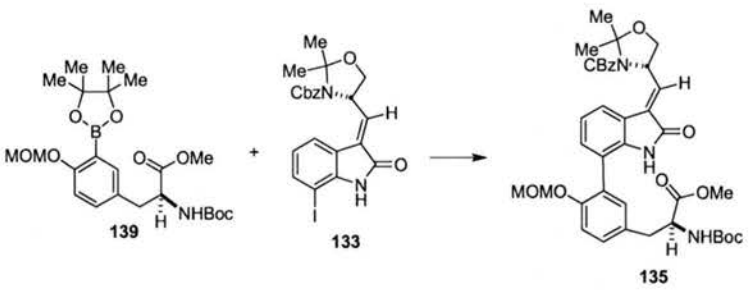




Boronic ester 139. Tyrosine derivative **97** (1.5 g, 3.29 mmol) bispinacolatodiborn (918 mg, 3.62 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (188 mg, 0.23 mmol) and KOAc (969 mg, 9.87 mmol) were taken up in DMSO (30 mL) and heated to 80° C for 4 h. The reaction mixture was allowed to cool to room temperature and poured onto water (60 mL). The resulting mixture was extracted with Et_2O (3 x 30 mL) and the combined organics were washed with water (1 x 10 mL) and brine (1 x 10 mL), dried over anhydrous Na_2SO_4, filtered through Celite® and concentrated under reduced pressure to afford tyrosine boronic ester **139** which was used without any further purification. ^1H NMR (300 MHz, $CDCl_3$, 273 K) δ: 1.34 (s, 12H), 1.42 (s, 9H), 3.00 (dd, *J*=13.9, 7.7 Hz, 1H), 3.09 (dd, *J*=13.9, 5.5 Hz, 1H), 3.50 (s, 3H), 3.71 (s, 3H), 4.53 (m, 1H), 4.92 (br d, *J*=7.2 Hz, 1H), 5.17 (s, 2H), 6.95 (d, *J*=8.4 Hz, 1H), 7.12 (dd, *J*=8.4, 2.2 Hz, 1H), 7.42 (d, *J*=2.2 Hz).

Filename: bka-2-685-1

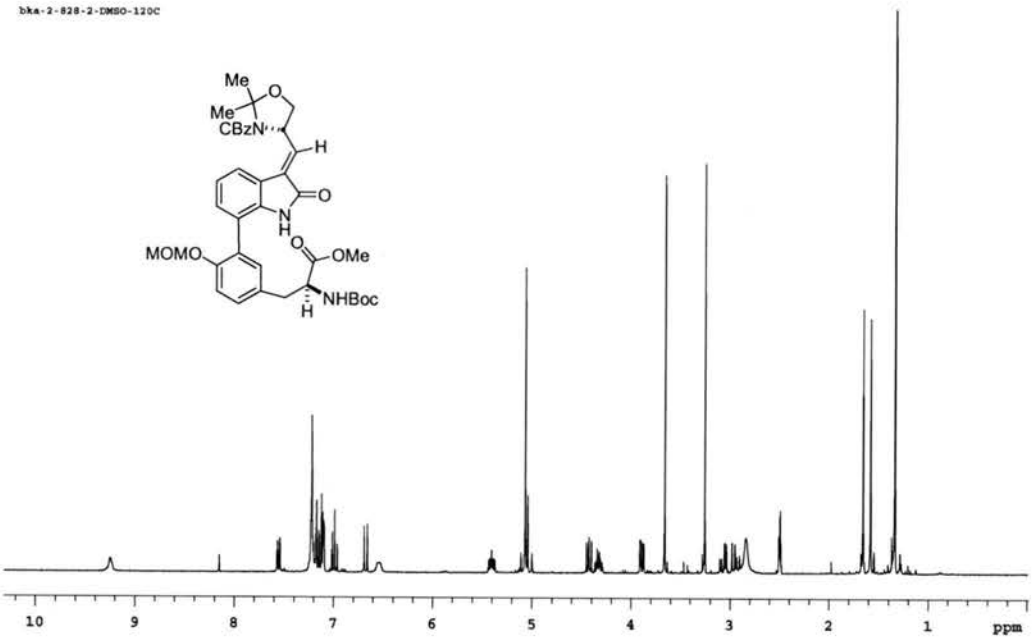




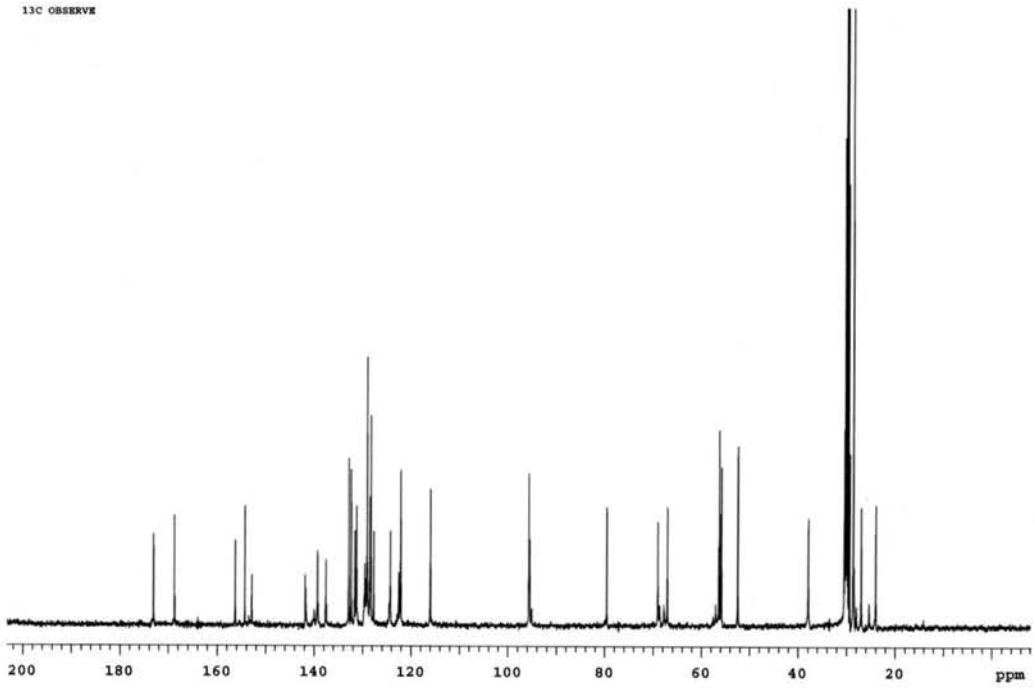
Biaryl 135. Tyrosine borinic ester **139** oxindolene aryl iodide **133**, (1.38 mg, 2.74 mmol), $PdCl_2(dppf) \cdot CH_2Cl_2$ (179 mg, 0.22 mmol) and K_2CO_3 (1.14 g, 8.22 mmol) were taken up in DME (50 mL) and doubly distilled H_2O (7 ml) and brought to reflux for 2 h. The reaction mixture was concentrated then diluted with EtOAc (20 mL) and poured onto brine. The organic layer was removed and the aqueous layer extracted with EtOAc (2 x 40 mL). The combined organics were washed with H_2O (1 x 10 mL), brine (1x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purified via flash chromatography (silica gel, 1:1:1 hexanes:EtOAc:CH_2Cl_2) to afford biaryl **135** (1.76 g, 90%, based on alkene **133**) as a yellow amorphous solid: $[\alpha]_D^{25} = +5.54°$ (*c*1.1, $CHCl_3$); ^{1} H NMR (300 MHz, DMSO-d_6, 393 K) δ: 1.34 (s, 9H), 1.58 (s, 3H), 1.66 (s, 3H), 2.94 (dd, J = 14.1, 8.6 Hz, 1H), 3.07 (dd, J = 14.1, 5.4 Hz, 1H), 3.26 (s, 3H), 3.66 (s, 3H), 3.89 (dd, J = 9.1, 3.2 Hz, 1H), 4.30 (ddd, J = 14.1, 8.6, 5.4 Hz, 1H), 4.43 (dd, J = 9.1, 6.7 Hz, 1H), 5.02 (1/2 ABq, J = 12.5 Hz, 1H), 5.07 (s, 2H), 5.09 (1/2 ABq, J = 12.5 Hz, 1H), 5.40 (ddd, J = 9.5, 6.7, 3.2 Hz, 1H), 6.54, (br s, 1H), 6.67 (d, J = 9.2 Hz, 1H), 6.99 (t, J = 7.7 Hz, 1H), 7.08-7.25 (m, 9H), 7.55 (d, J = 7.5 Hz, 1 H), 9.25 (br s, 1H); ^{13}C NMR (100 MHz, acetone-d_6, major rotamer) 24.0, 26.9, 28.6, 37.9, 52.4, 55.8, 56.0, 56.4, 67.0, 69.0, 79.6, 95.7, 116.0, 122.07, 122.13, 122.5, 124.3, 127.7, 128.3, 128.5, 129.1, 129.4, 129.6, 131.3, 131.6, 132.3, 132.9, 137.5, 139.3, 141.8, 152.9, 154.3, 156.3, 168.8,

173.1; IR ($CHCl_3$ film) 3283, 2980, 1708, 1366, 1206, 1059; HRMS (FABH+) calcd for $C_{39}H_{46}N_3O_{10}$ (*m/z*) 716.3183; found (*m/z*) 716.3161.

Filename: bka-2-828-2-DMSO-120C

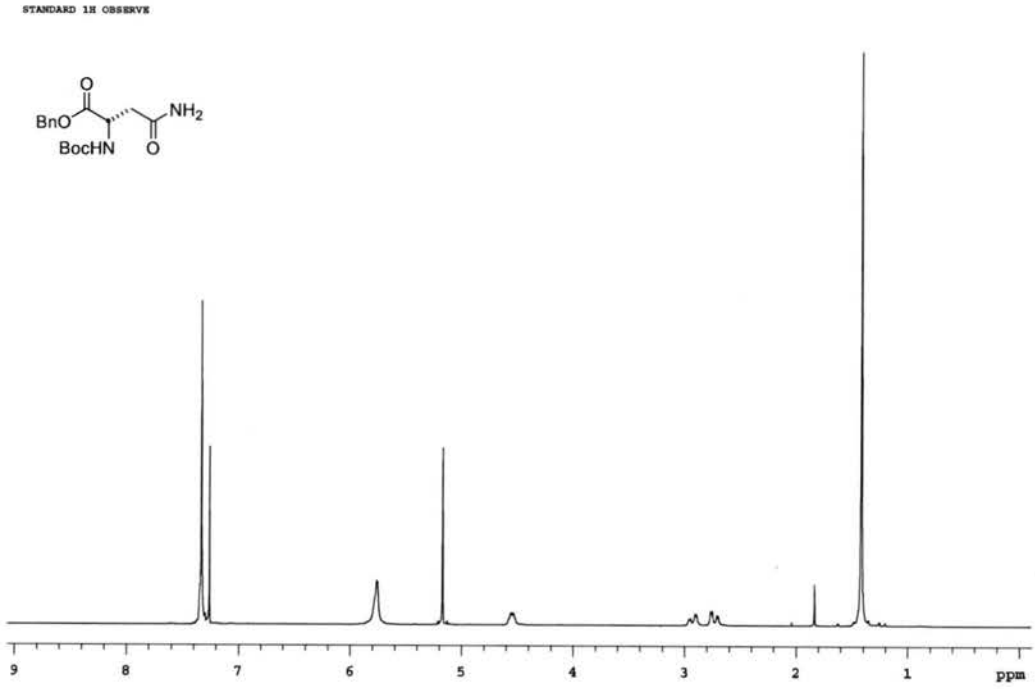


Filename: bka-3-1083-2-C13

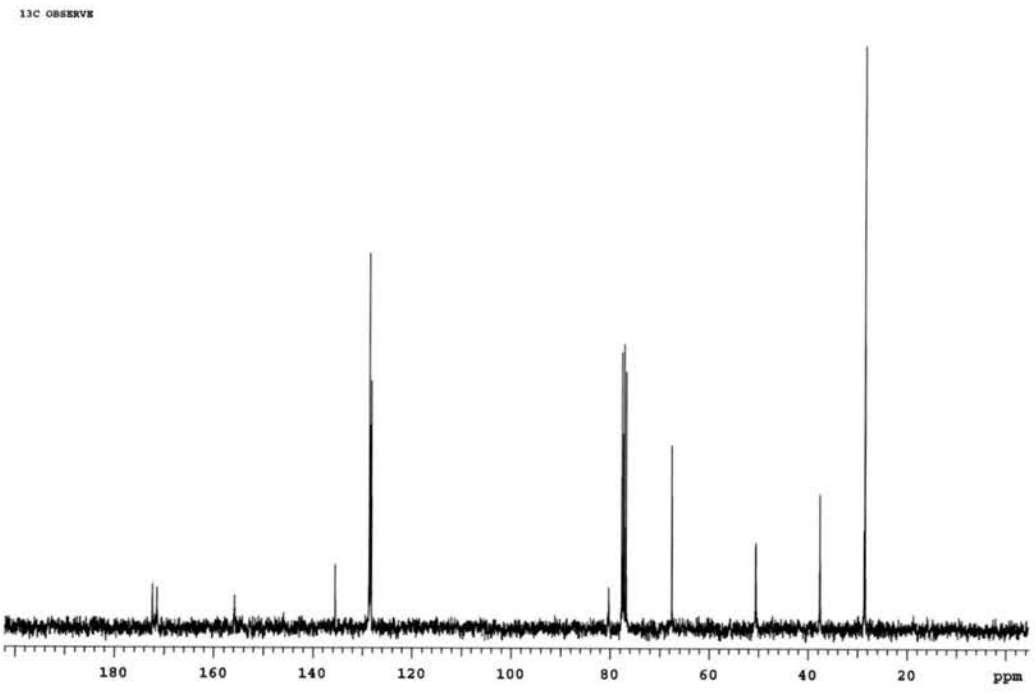


α-*N*-Boc-L-asparagine benzyl ester.[43] α-*N*-Boc-L-asparagine (3.0 g, 12.9 mmol) was dissolved in 50 mL H_2O and 50 mL THF. The solution was titrated to pH=~7 (note: the asparagine derivative undergoes cyclization to the succinimide at pH>7) with 20% aq. Cs_2CO_3 (pH paper). The volatiles were removed followed by lyopholization for ~18 h. The residual cesium salt was reconstituted in dry DMF (100 mL) and benzyl bromide was added (1.52 mL, 12.8 mmol). The resulting mixture was allowed to stir at room temperature for 12 h. Removed DMF in vacuo followed by the addition of a large volume of H_2O (~200 mL). Extracted aqueous layer with Et_2O (3 x 50 mL), washed with water (1 x 20 mL), aq. LiBr (1 x 30 mL) and brine (1 x 30 mL). Concentrated under reduced pressure to an oil and crystallized from EtOAc:hexanes (~1:5) to afford α-*N*-Boc-L-asparagine benzyl ester as a white solid (3.7 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$, 273 K) δ: 1.43 (s, 9H), 2.73 (dd, *J*=16.1, 4.0 Hz, 1H), 2.93 (dd, *J*=16.1, 4.0 Hz, 1H), 4.55 (m, 1H), 5.17 (s, 2H), 5.76 (br s, 3H), 7.37 (s, 5H); ^{13}C (75 MHz, $CDCl_3$) δ: 28.6, 37.6, 50.6, 67.6, 80.3, 128.3, 128.4, 128.6, 135.5, 155.8, 171.4, 172.3. HRMS (FABH+) calcd for $C_{16}H_{23}N_2O_5$ (*m/z*) 323.1607; found (*m/z*) 323.1605.

Filename: bka-1-384



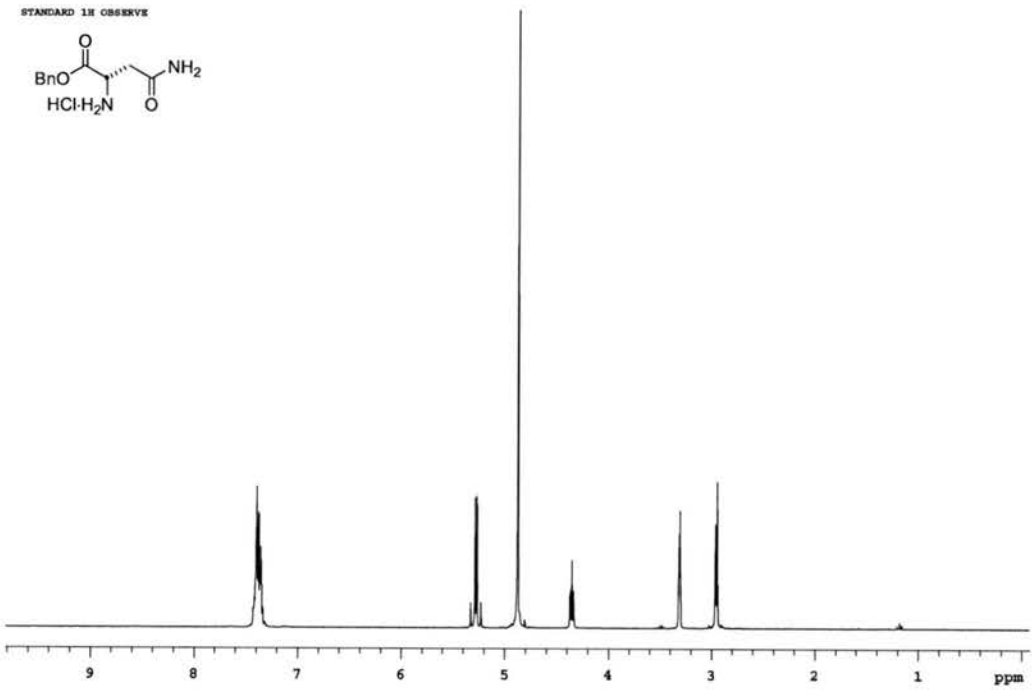
Filename: bka-1-384-13C



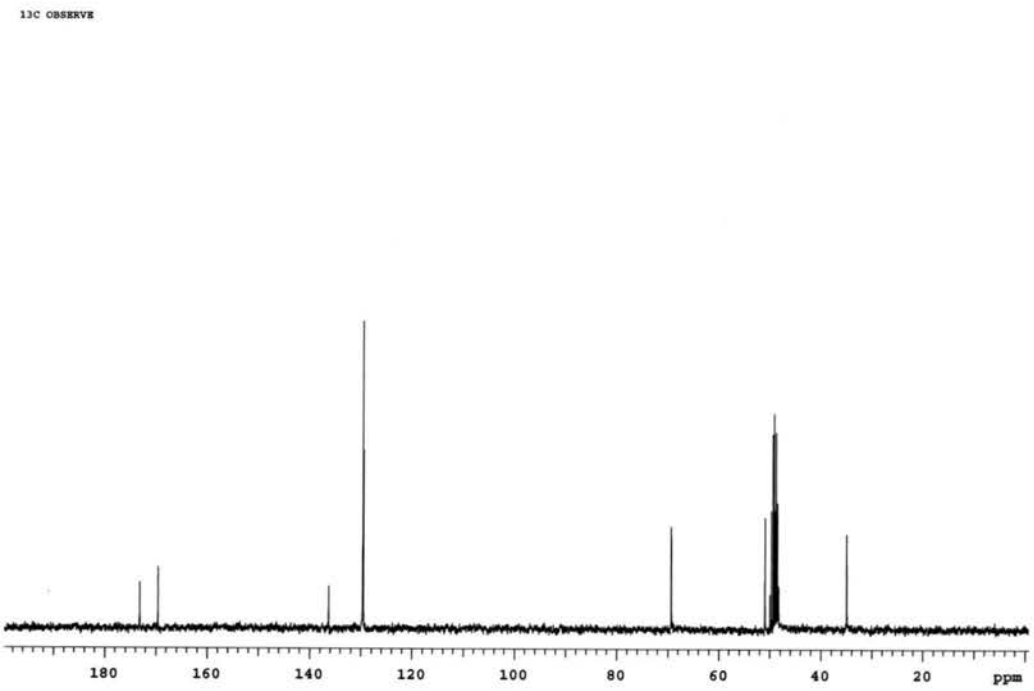
141

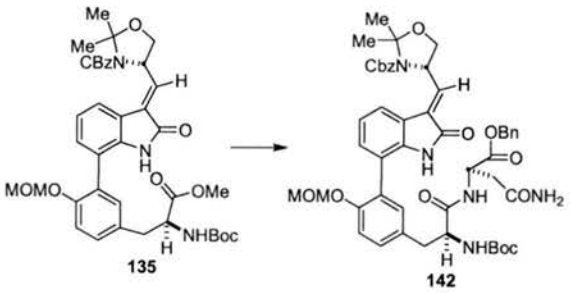
L-asparagine benzyl ester hydrochloride.[43] α-*N*-Boc-L-asparagine benzyl ester (1.0 g, 3.01 mmol) was dissolved in 5 mL TFA. Allowed to stir for 30 min then removed volatiles under reduced pressure. Added a 2 mL of HCl in MeOH (2 mL AcCl in 10 mL MeOH) and allowed to stir for 5 min. Removed volatiles under reduced pressure and repeated. The viscous oil was taken up in minimal amount anhydrous dioxane (distilled from benzophenone sodium ketyl) and added ~25 mL anhydrous Et_2O. Allowed to stir for 1 h and a white precipitate formed. Decanted off liquids and washed with 2 mL dry Et_2O to afford L-asparagine benzyl ester hydrochloride (620 mg, 100%) as a white solid. ^1H NMR (300 MHz, CD_3OD , 273 K) δ: 2.95 (d, *J*=5.5 Hz, 2H), 4.35 (t, *J*=5.5 Hz, 1H), 5.24 (1/2 ABq, *J*=12.1 Hz, 1H), 5.30 (1/2 ABq, *J*=12.1 Hz, 1H), 7.40 (m, 5H) ^{13}C (75 MHz, $CDCl_3$) δ: 34.9, 50.6, 129.5, 129.6, 136.3, 169.6, 173.1. HRMS (FABH+) calcd for $C_{11}H_{15}N_2O_3$ (*m/z*) 223.1083; found (*m/z*) 223.1079.

Filename: bka-1-387



Filename: bka-1-387-C13



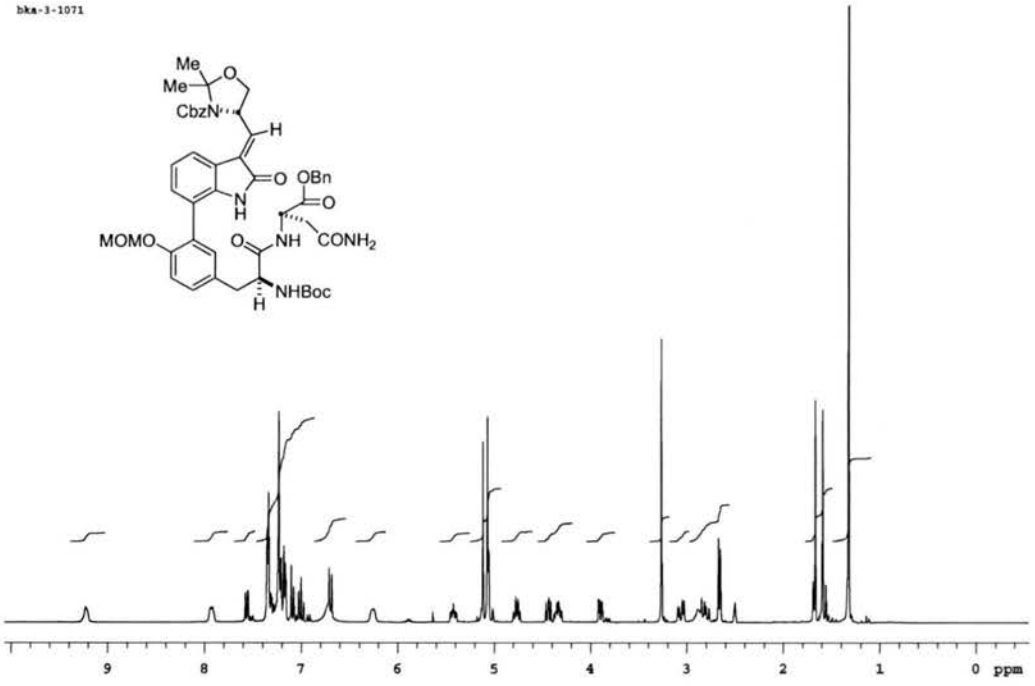


Pseudotripeptide 142. To a solution of biaryl **135** (1.6 g, 2.34 mmol), THF (20 mL), H_2O (20 mL) at 0° C was added $LiOH{\cdot}H_2O$ (246 mg, 5.89 mmol) and allowed to stir for 1 h. The resulting mixture is diluted with water and extracted with Et_2O (3 x 5 mL). The aqueous portion is acidified to pH = 4 with conc. HCl, which is then extracted with EtOAc (3 x 10 mL). The combined organics are washed with water (1 x 10 mL), brine (1 x 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting carboxylic acid is used without further purification.

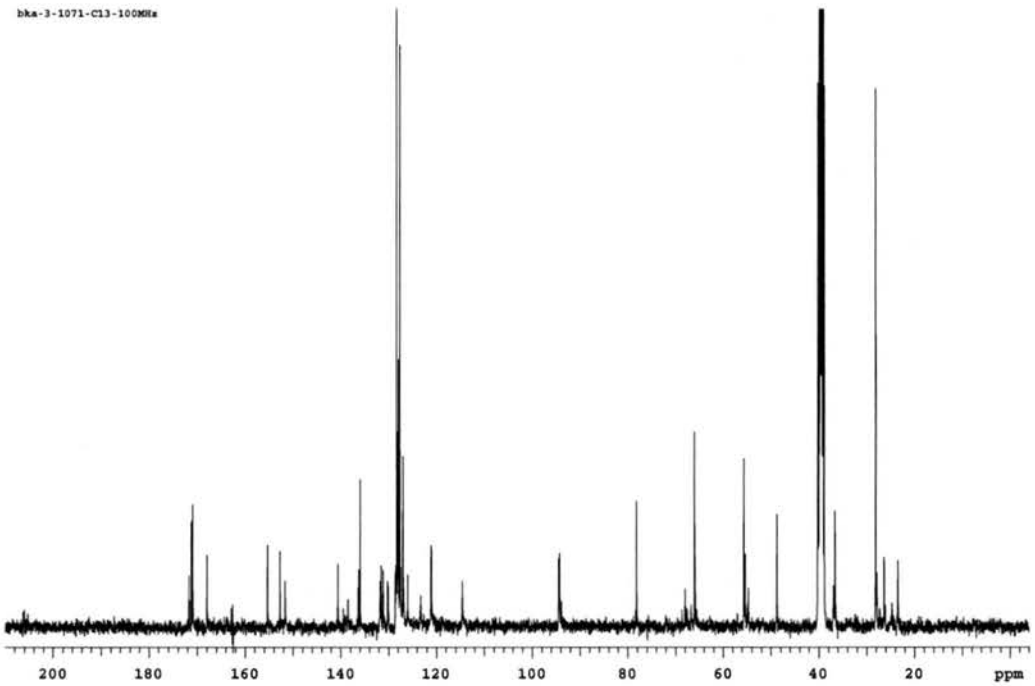
The resulting carboxylic acid, EDCI (240 mg, 1.23 mmol) and HOAt (175 mg, 1.29 mmol) are taken up in dry CH_2Cl_2 (100 mL) at 0° C and allowed to stir for 10 min. To the resulting mixture is added asparagine benzyl ester hydrochloride (363 mg, 1.4 mmol) and $^{i}Pr_2NEt$ (244 μL, 1.4 mmol) and allowed to stir at 0° C for 2 h. The reaction mixture is diluted with CH_2Cl_2 (50 mL) and washed sequentially with sat. $NaHCO_3$ (20 mL), 1 M HCl (20 mL), water (20 mL) and brine (20 mL). The organics are dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purified via flash chromatography (silica gel, 2%→5% MeOH in CH_2Cl_2) to afford **142** (1.04 g, 98%) as a yellow amorphous solid: $[\alpha]_D^{25}$ = + 18.72° (*c* 1.945, $CHCl_3$); ^{1} H NMR (300 MHz, DMSO-d_6, 393 K, mixture of rotamers, major listed) δ: 1.32 (s, 9H), 1.59 (s, 3H), 1.66 (s, 3H), 2.63-2.68 (m, 2H), 2.80 (dd, *J* = 14.1, 9.0 Hz, 1H), 3.05 (dd, *J* = 14.1, 4.9 Hz, 1H), 3.26 (s, 3H), 3.89 (dd, *J* = 9.0, 3.2 Hz, 1H), 4.32 (ddd, *J* = 13.4, 8.8, 5.1 Hz, 1H),

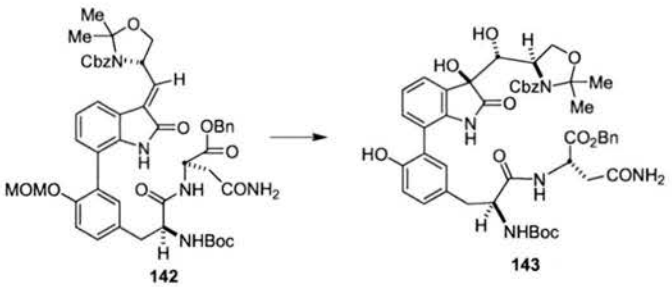
4.43 (dd, J = 9.0, 6.5 Hz, 1H), 4.76 (dd, J = 13.9, 6.1 Hz, 1H), 5.03-5.15 (m, 6H), 5.41 (ddd, J = 9.5, 6.5, 3.2 Hz, 1H), 6.25 (br d, J = 7.9 Hz, 1H), 6.69 (d, J = 9.2 Hz, 1H), 6.72 (br s, 2H), 7.00 (t, J = 7.7 Hz, 1H), 7.05-7.40 (m, 14H), 7.56 (d, J = 7.5 Hz, 1 H), 7.91 (br d, J = 7.8 Hz, 1H), 9.20 (br s, 1H),; ^{13}C NMR (100 MHz, DMSO-d_6, major rotamer) δ: 23.4, 26.3, 28.1, 36.6, 36.9, 48.7, 54.7, 55.3, 55.6, 65.8, 66.0, 68.0, 78.1, 94.2, 94.4, 114.5, 121.1, 123.3, 125.9, 126.8, 127.0, 127.6, 127.9, 128.1, 128.3, 128.5, 130.1, 131.1, 131.5, 131.8, 136.0, 136.3, 138.5, 140.6, 151.6, 152.7, 155.3, 162.5, 170.0, 170.9, 171.2, 171.7; IR ($CHCl_3$ film) 3315, 2979, 1707, 1613, 1501, 1405, 1348, 1203, 1162; HRMS (FABH+) calcd for $C_{49}H_{56}N_5O_{12}$ (*m/z*) 906.3925; found (*m/z*) 906.3934.

Filename: bka-3-1071



Filename: bka-3-1071-C13-100MHz

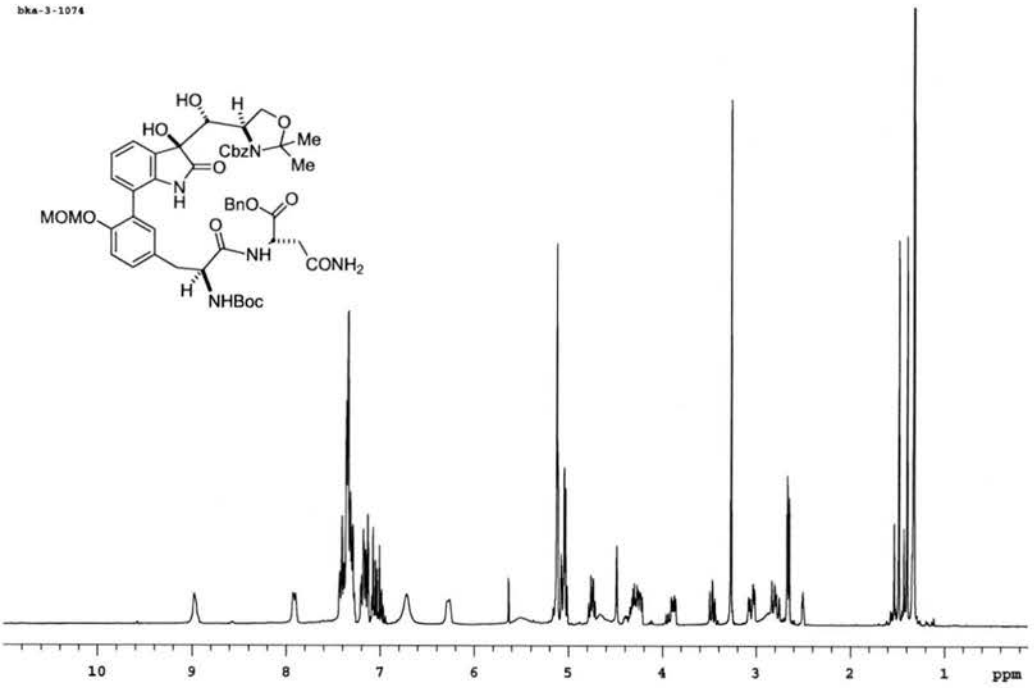




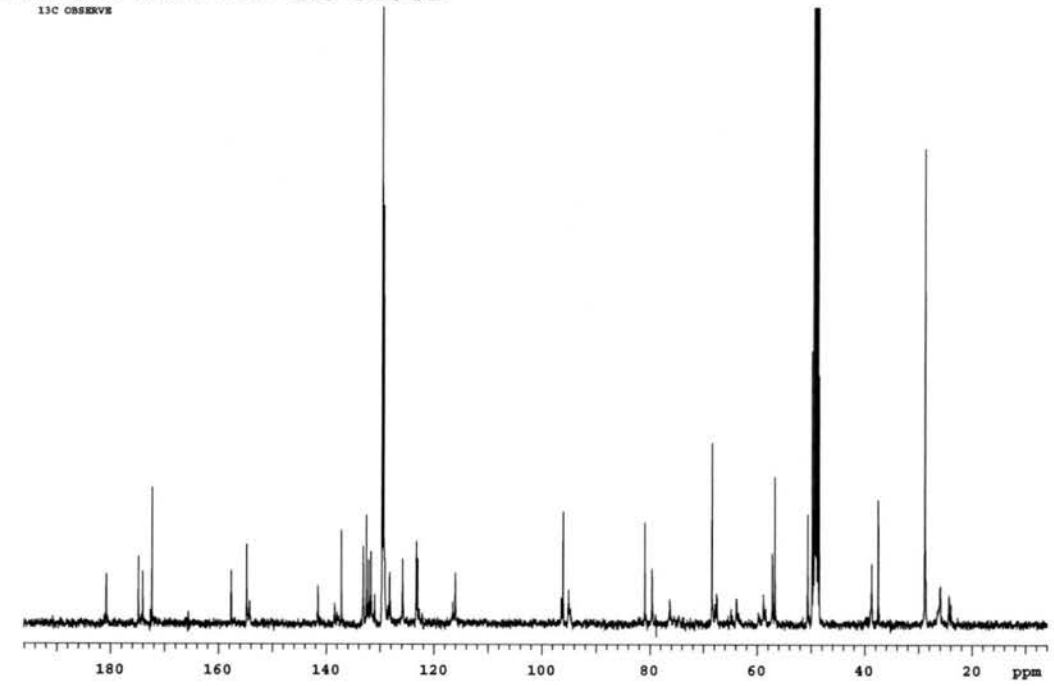
Diol 143. To a solution of alkene **142** (438 mg, 0.48 mmol) in pyridine (10 mL) at 0° C was added OsO_4 (0.1 M H_2O, 5 mL, 0.5 mmol) and allowed to stir for 1h. The reaction mixture was diluted with THF (5 mL) and MeOH (5 mL) and sat. aq. $NaHSO_3$ (10 mL) was added. The resulting mixture was vigorously stirred at 0° C for 2 h. The mixture was diluted with water and the aqueous layer was extracted with CH_2Cl_2 (3 x 30 mL). The combined organics were washed with water (1 x 10 mL), brine (1 x 20 mL) dried

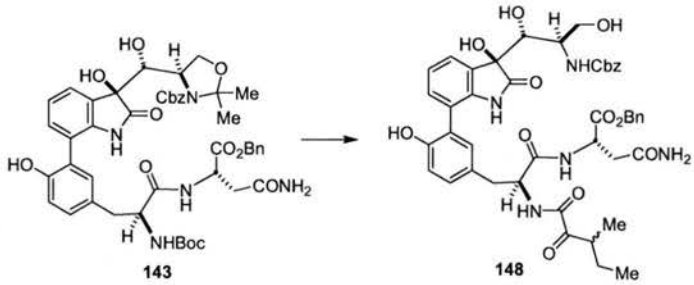
over Na_2SO_4, filtered, and concentrated under reduced pressure. Purified via flash chromatography (silica gel, 5 % MeOH in CH_2Cl_2) to afford **143** (392 mg, 87%) as a white film: $[\alpha]_D^{25}$ = + 58.74° (*c*1.11, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6, 393 K, mixture of rotamers that begins spontaneous decomposition upon elevated temperatures, major listed) δ: 0.88 (s, 9H), 0.94 (s, 3H), 1.03 (s, 3H), 2.21 (d, *J*=6.2 Hz, 2H), 2.34 (dd, *J*=13.9, 9.2 Hz, 1H), 2.60 (dd, *J*=13.9, 4.7 Hz, 1H), 2.82 (s, 3H), 3.02 (dd, *J*=8.4, 7.8 Hz, 1H), 3.52-3.40 (m, 1H), 3.90-3.75 (m, 2H), 4.04 (s, 1H), 4.30 (dd, *J*=13.9, 6.2 Hz, 1H), 4.68-4.56 (m, 6H), 5.05 (br s, 1H), 5.82 (br s, 1H), 6.27 (br s, 2H), 7.00-6.50 (m, 16H), 7.46 (br d, *J*=7.5 Hz, 1H), 8.53 (br s, 1H); ^{13}C (100 MHz, CD_3OD, 273 K) δ 24.0, 24.4, 26.0, 26.1, 28.8, 37.5, 38.7, 50.6, 56.7, 57.2, 67.5, 67.7, 68.4, 76.2, 79.6, 80.9, 95.0, 96.1, 96.4, 116.0, 116.6, 123.1, 123.3, 125.8, 128.1, 128.3, 129.4, 129.7, 131.0, 131.7, 132.2, 132.5, 133.1, 137.2, 138.5, 141.6, 154.2, 154.8, 157.7, 172.3, 174.0, 174.8, 180.8,; IR ($CHCl_3$ film) 3350, 2925, 1719, 1672, 1500, 1411, 1351, 1168; HRMS (FABH+) calcd for $C_{49}H_{58}N_5O_{14}$ (*m/z*) 940.3980; found (*m/z*) 940.3993.

Filename: bka-3-1074



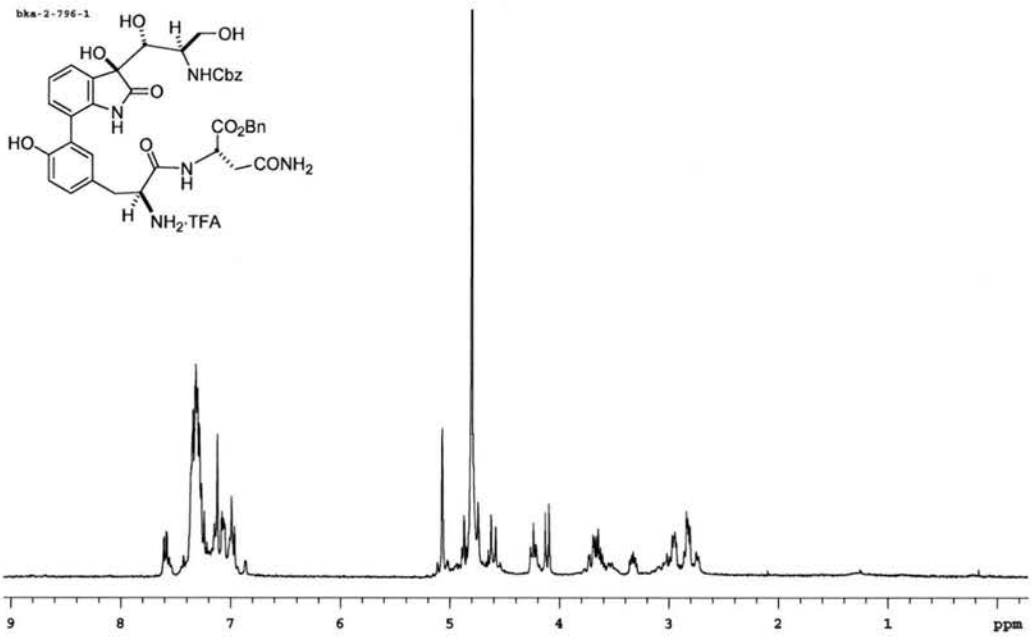
Filename: bka-3-1074-C13-CD3OD





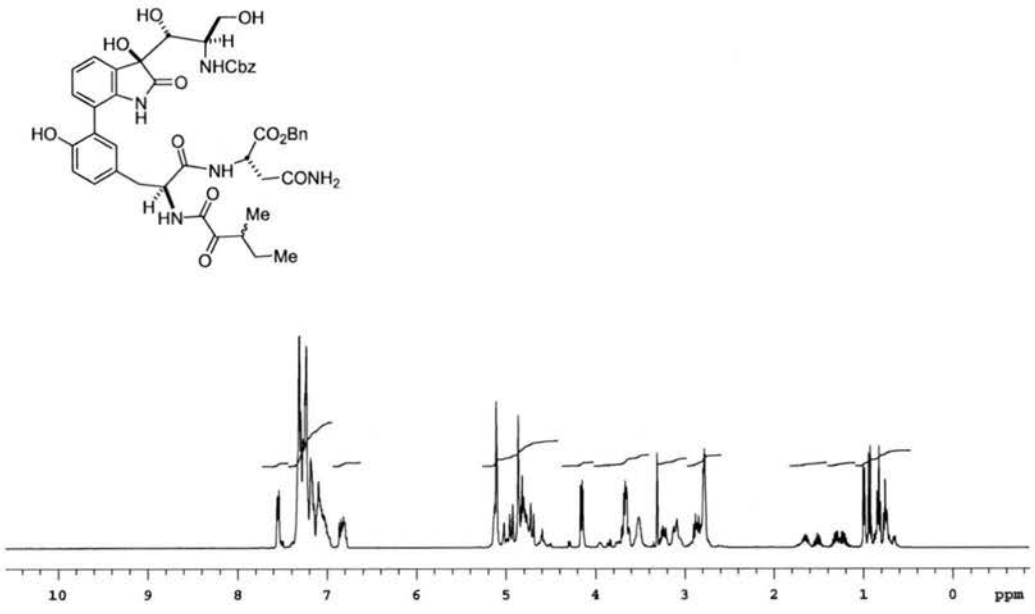
Ketoamide 148. Diol **143** (313 mg, 0.33 mmol) was taken up in 5 mL TFA and 5 mL water and allowed to stir for 4 h. The reaction mixture was concentrated under reduced pressure and azeotroped with toluene. The TFA·amine salt was used without further purification. ^{1}H NMR is convoluted at room temperature due to urethane rotamers on the NMR timescale. Attempts at elevated temperature necessary for coalescence lead to complete decomposition of the product. See attached room temperature ^{1}H NMR (300 MHz, D_2O, 273K). HRMS (FABH+) calcd for $C_{39}H_{42}N_5O_{11}$ (*m/z*) 756.2881; found (*m/z*) 756.2894.

Filename: bka-2-796-1

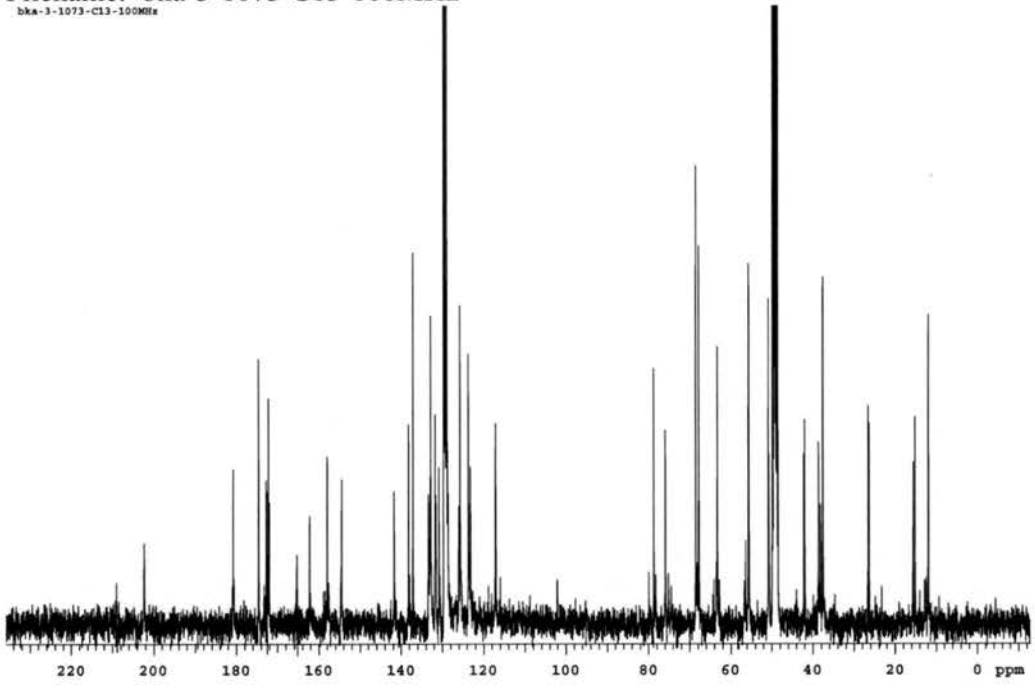


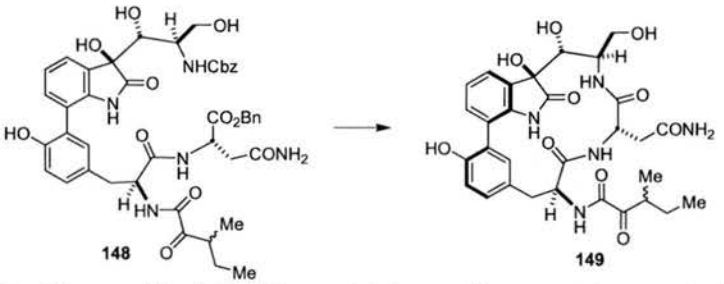
To the resulting film was added 3-methyl-2-oxo-pentanoic acid sodium salt (56 mg, 0.36 mmol), EDCI (77 mg, 0.4 mmol) and HOAt (54 mg, 0.4 mmol) which was taken up in THF (25 mL) at 0° C and allowed to stir for 1 h. The reaction mixture was concentrated under reduced pressure and immediately subjected to flash chromatography (silica gel, 10% MeOH in CH_2Cl_2) to afford **148** (286 mg, ~ 99%) as a film and a mixture of diastereomers. ^{1}H NMR is convoluted at room temperature due to urethane rotamers on the NMR timescale. Attempts at elevated temperature necessary for coalescence lead to complete decomposition of the product. See attached room temperature ^{1}H NMR (400 MHz, CD_3OD, 273K). ^{13}C NMR (100MHz, CD_3OD, 273K, mixture of rotamers and C36 diastereomers) δ: 11.8, 12.0, 15.1, 15.6, 26.3, 26.5, 37.5, 38.1, 38.6, 41.9, 42.2, 50.8, 55.6, 63.2, 67.8, 68.5, 75.9, 78.7, 117.2, 123.2, 123.7, 125.7, 126.0, 128.7, 128.9, 129.2, 129.3, 129.4, 129.6, 129.7, 130.8, 131.7, 132.8, 133.3, 133.4, 137.1, 138.2, 141.7, 154.4, 158.0, 162.2, 162.3, 165.4, 172.0, 172.3, 172.8, 174.6, 174.7, 180.9, 202.4. IR (MeOH film): 3334, 2961, 1721, 1673, 1513, 1452, 1415. HRMS (FABH+) calcd for $C_{45}H_{50}N_5O_{13}$ (*m/z*) 868.3405; found (*m/z*) 868.3414.

Filename: bka-3-1073-400MHz



Filename: bka-3-1073-C13-100MHz

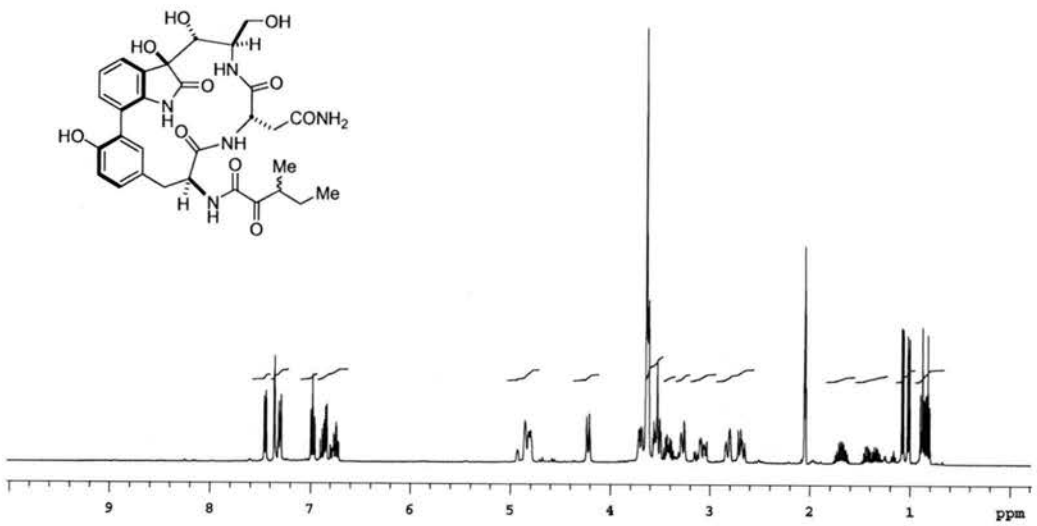




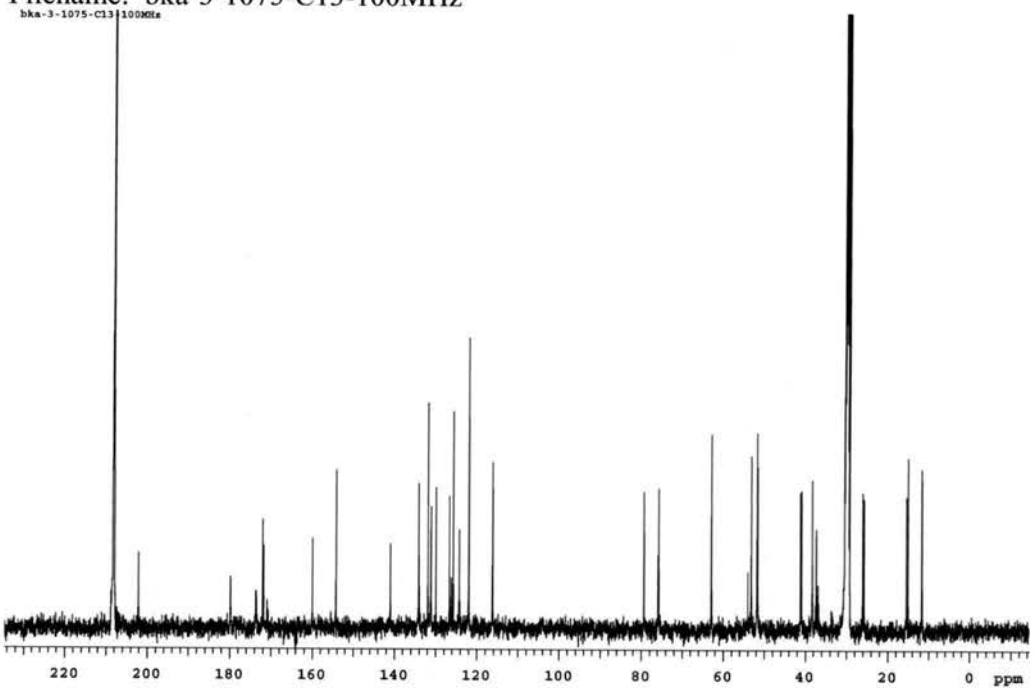
Macrocycle 149. Ketoamide **148** (96 mg, 0.11 mmol) was taken up in MeOH (5 mL). The solution was purged with Ar for 10 min at which time Pd black (75 mg, 0.7 mmol) was added. Hydrogen gas was bubbled through the resulting heterogeneous mixture for 20 min then the mixture was allowed to stir under a balloon of H_2 for 14 h. The mixture was then purged again with Ar for 10 min and filtered through a PTFE Acrodisc® (0.45 μM) filter and concentrated under reduced pressure. The resulting film was taken up in a minimal amout of MeOH followed by the addition of about 100X the volume of dry PhMe which was then concentrated under reduced pressure. Treated one more time with the same volume of PhMe and concentrated. The resulting white film, EDCI (173 mg, 0.88 mmol) and HOAt (120 mg, 0.88 mmol) were taken up in anhydrous CH_2Cl_2 (65 mL) and anhydrous DMF (65 mL) and allowed to stir at room temperature for 24 h. The reaction was concentrated under reduced pressure to a yellow oil. The resulting oil was first run through a small silica gel column (25% MeOH in CH_2Cl_2) and the fractions containing macrocycle (Rf = ~.1 and stains red with vanillin) were concentrated. This crude mixture was taken up in water (~5 mL) and ran through a Waters Sep-Pak C18 cartridge. Rinsed with H_2O (3 x 5mL) and collected the desired compound by eluting with MeOH (4 x 5mL). The resulting mixture was subjected to flash chromatography (silica gel, 25% MeOH in CH_2Cl_2) to afford macrocycle **149** (33 mg, 49%). ^{1}H NMR (400 MHz, acetone-d_6 and D_2O, 273 K) δ: 0.82 (t, J=7.5 Hz, diastereomeric H38), 0.88 (t,

J=7.5 Hz, diastereomeric H38), 1.02 (d, *J*=7.0 Hz, diastereomeric H39), 1.08 (d, *J*=7.0 Hz, diastereomeric H39), 1.24-1.42 (m, 1H, diastereomeric H37), 1.60-1.80 (m, 1H, diastereomeric H37), 2.68 (dd, *J*=15.0, 9.6 Hz, 1H), 2.82 (dd, *J*=15.0, 3.4 Hz, 1H), 3.04-3.16 (m, 1H), 3.28 (d, *J*=11.9 Hz, 1H), 3.41 (m, 1H, diastereomeric H36), 3.58 (m, 2H), 3.71 (overlap with HOD, m, 1H), 4.22 (d, *J*=10.2 Hz, 1H), 4.79-4.92 (m, 2H), 6.76 (m, 1H), 6.87 (m, 1H), 6.98 (t, *J*=7.7 Hz, 1H), 7.31 (dd, *J*=7.9, 3.2 Hz, 1H), 7.36 (d, *J*=1.9 Hz, 1H), 7.45 (d, *J*=7.5 Hz, 1H); ^{13}C NMR (100MHz, acetone-d_6 and D_2O , 273K, mixture C36 diastereomers) δ: 11.6, 11.7, 15.1, 15.4, 25.8, 26.1, 37.3, 37.4, 38.3, 40.9, 41.2, 51.6, 51.7, 53.2, 62.9, 75.8, 79.3, 116.2, 122.0, 124.2, 125.7, 126.3, 126.6, 129.9, 131.1, 131.9, 134.2, 141.0, 154.2, 154.3, 160.0, 171.8, 172.1, 173.6, 179.8, 202.2. IR (MeOH film): 3388, 2961, 1713, 1667, 1573, 1555, 1514. HRMS (FABH+) calcd for $C_{30}H_{36}N_5O_{10}$ (*m/z*) 626.2462; found (*m/z*) 626.2439.

Filename: bka-3-1075-400MHz

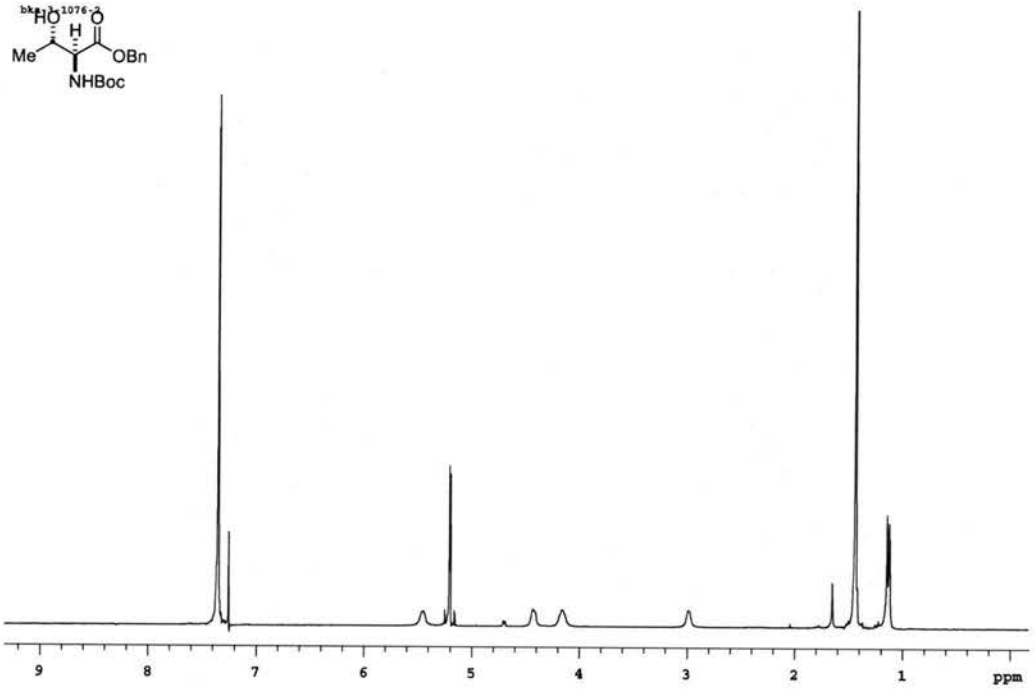


Filename: bka-3-1075-C13-100MHz



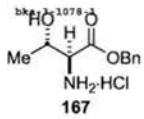
***N*-Boc-L-*allo*-threonine benzyle ester.** To L-allo-threonine (91 mg, 0.76 mmol) in THF (1 mL) and sat. aq. Na_2CO_3 (1 mL) at room temperature was added Boc_2O (175 mg, 0.8 mmol) and allowed to stir overnight. The reaction was acidified to pH ~4 and extracted with EtOAc (3 x 10 mL). Washed combined organics with water (1 x 5 mL) and brine (1 x 5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was taken up in dry DMF (1 mL) and cooled to 0°C under Ar. To the solution was added $NaHCO_3$ (64mg, 0.76 mmol) and benzyl bromide (95 μL, 0.78 mmol) and allowed to stir for 18h. Concentrated reaction mixture under reduced pressure then diluted with H_2O (~10 mL). Extracted with EtOAc (3 x 5 mL) and the combined organics were washed with H_2O, (1 x 5 mL), aq. LiBr (1 x 5 mL) and brine (1 x 5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Purified via flash chromatography (silica gel, 2:1 hexanes:EtOAc→1:1 hexanes:EtOAc) to afford *N*-Boc-L-*allo*-threonine benzyle ester (204 mg, 87%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 1.13 (d, *J*=6.2 Hz, 3H), 1.41 (s, 9H), 2.99 (br s, 1H), 4.16 (br m, 1H), 4.25 (br m, 1H), 5.18 (1/2 ABq, *J*=12.3 Hz, 1H), 5.23 (1/2 ABq, *J*=12.3 Hz, 1H), 5.45 (br d, *J*=5.9 Hz, 1H), 7.36 (m, 5H).

Filename: bka-3-1076-2

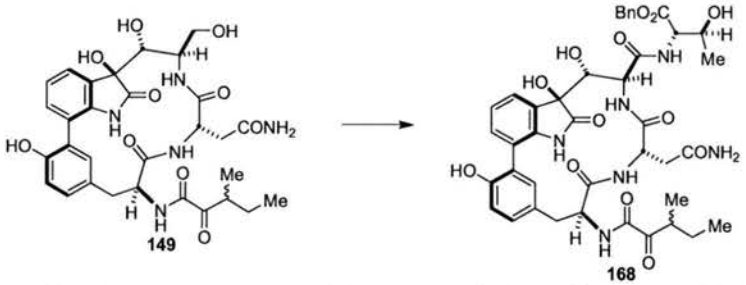


L-*allo*-threonine benzyl ester hydrochloride. *N*-Boc-L-*allo*-threonine benzyle ester (35 mg, 0.113 mmol) was dissolved in 1 mL TFA. Allowed to stir for 30 min then removed volatiles under reduced pressure. Added a 1 mL of HCl in MeOH (2 mL AcCl in 10 mL MeOH) and allowed to stir for 5 min. Removed volatiles under reduced pressure and repeated. To the viscous oil was added anhydrous Et_2O (5 mL) and allowed to stir for 1 h as a white precipitate formed. Decanted off liquids and washed with 2 mL dry Et_2O to afford L-*allo*-threonine benzyl ester hydrochloride (22 mg, 100%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6 , 273 K) δ: 1.15 (d, J=6.2 Hz, 3H), 4.08 (m, 2H), 5.25 (s, 2H), 5.64 (d, J=4.4 Hz, 1H), 7.40 (m, 5H), 8.38 (br s, 3H).

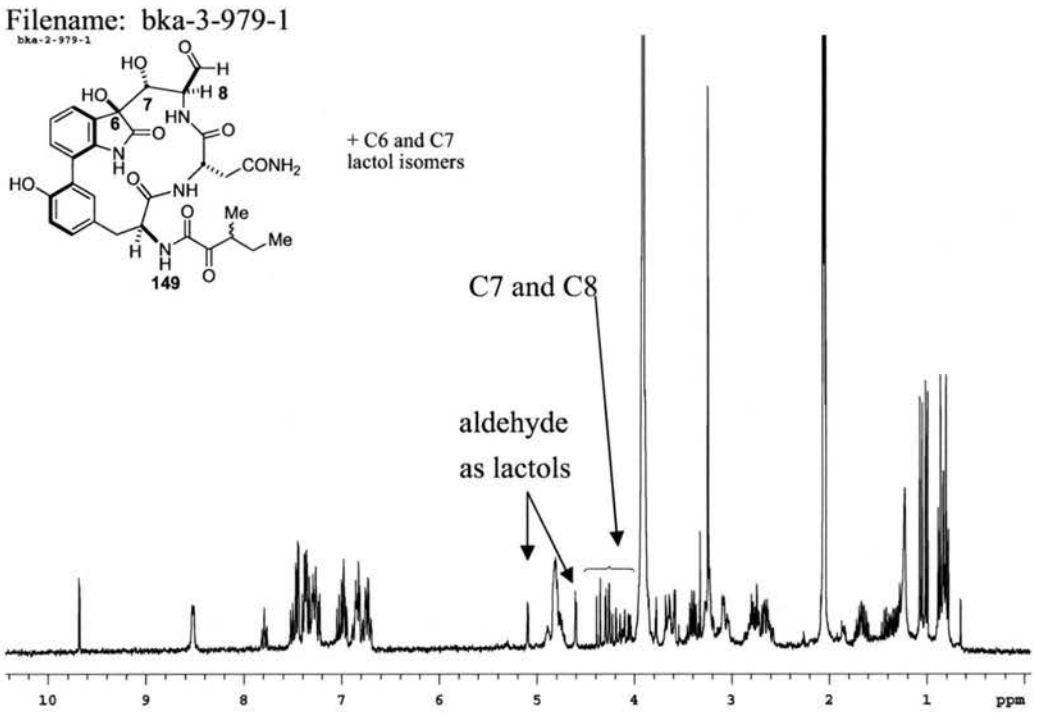
Filename: bka-3-1078-1



167



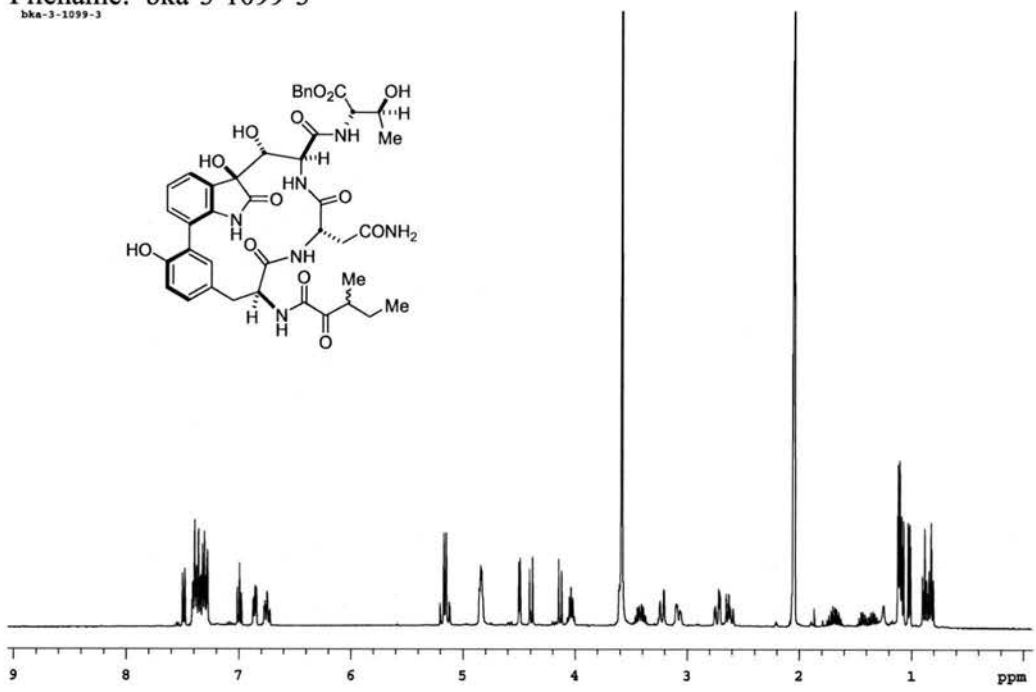
Threonine benzyl ester macrocycle 168. To a solution of macrocycle **149** (10.2 mg, 0.016 mmol), $^{i}Pr_2NEt$ (14 μL, 0.081 mmol), DMSO (0.5 mL) and dry CH_2Cl_2 (1.5 mL) at room temperature was added SO_3·pyridine complex (13.2 mg, 0.081 mmol) and allowed to stir for 20 min. Quenched with 2 drops of 1M HCl and removed volatiles under reduced pressure. Reconstituted in H_2O (5 mL) and ran through a Waters Sep-Pak C18 cartridge. Rinsed with H_2O (3 x 5mL) and collected the desired compound by eluting with MeOH (4 x 5mL). The resulting mixture was flushed through a plug of silica gel (25% MeOH in CH_2Cl_2) to afford the corresponding aldehyde as a mixture of lactol isomers and was used without any further purification (see below for crude ^1H NMR; acetoned_6-D_2O, 300MHz).



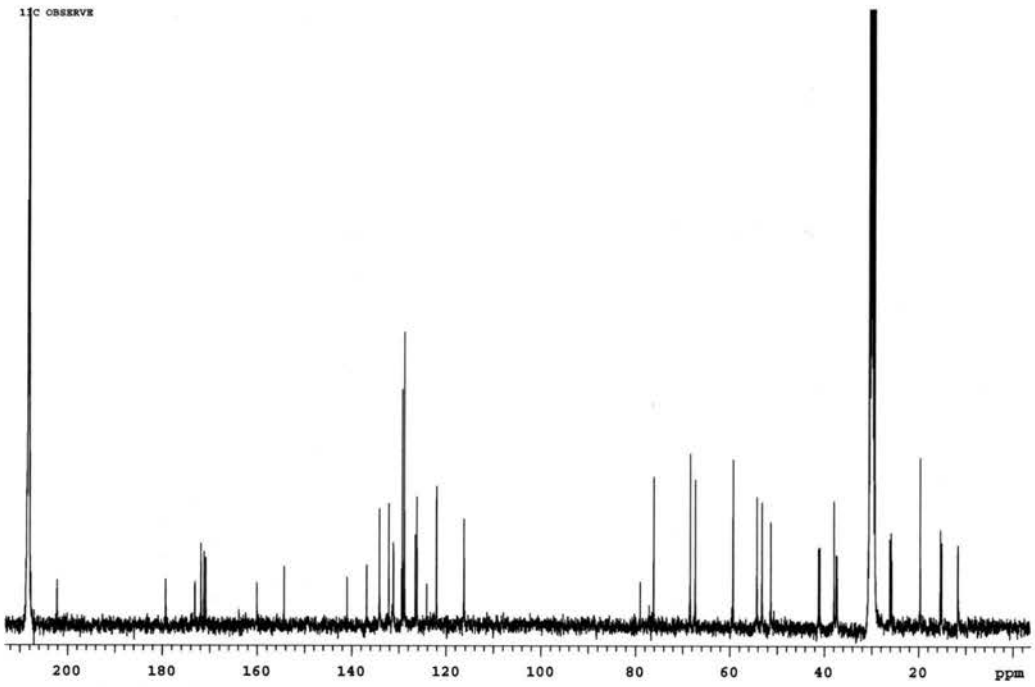
The resulting crude thin film (from SO_3·pyridine reaction) was taken up in H_2O (0.5 mL), ^{t}BuOH (0.5 mL) and 2-methyl-2-butene was added. To the vigorously stirred mixture was added a solution of $NaClO_2$ (5.6 mg, 0.049 mmol, 80%) and NaH_2PO_4 (6.8 mg, 0.049 mmol) in H_2O (0.5 mL) and allowed to stir for 5 h. The reaction was quenched with 2 drops sat. aq. $NaHSO_3$ then 2 drops 1M HCl. The volatiles were removed in vacuo and the mixture was reconstituted in H_2O (0.5 mL) and ran through a Waters Sep-Pak C18 cartridge. Rinsed with H_2O (3 x 5mL) and collected the desired compound by eluting with MeOH (4 x 5mL) and concentrated. The resulting film was taken up in a minimal amount of MeOH followed by anhydrous PhMe and concentrated to a white film. To this white film was added EDCI (3.2 mg, 0.016 mmol), HOAt (2.3 mg, 0.016 mmol) and L-*allo*-threonine benzyl ester hydrochloride (3.6 mg, 0.015 mmol). The resulting mixture was taken up in dry DMF (0.5 mL) and dry CH_2Cl_2 (0.5 mL) at

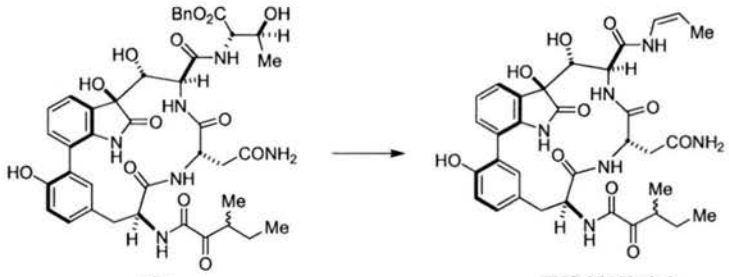
0°C followed by the addition of $^{i}Pr_2NEt$ (2.5 μL, 0.015 mmol) and allowed to stir at ~0°C for 18 h. Concentrated under reduced pressure and pufified by PTLC (25% MeOH in CH_2Cl_2) to afford coupled product **168** (5.6 mg, 46% three steps). ^{1}H NMR (400 MHz, acetone-d_6 and D_2O, 273 K) δ: 0.82 (t, *J*=7.5 Hz, diastereomeric H38), 0.88 (t, *J*=7.5 Hz, diastereomeric H38), 1.02 (d, *J*=7.0 Hz, diastereomeric H39), 1.08 (d, *J*=7.0 Hz, diastereomeric H39), 1.11 (d, *J*=6.4 Hz, 3H), 1.25-1.44 (m, diastereomeric H37) 1.68 (m, diasteromeric H37), 2.62 (dd, *J*=15.7, 9.2 Hz, 1H), 2.65 (dd, *J*=15.7, 4.1 Hz, 1H), 3.08 (m, 1H), 3.22 (dd, *J*=13.8, 2.3 Hz, 1H), 3.42 (m, diasteromeric H36), 4.04 (dq, *J*=6.4, 5.1 Hz, 1H), 4.13 (d, *J*=10.7 Hz, 1H), 4.39 (d, *J*=10.7 Hz, 1H), 4.50 (d, *J*=5.1 Hz, 1H), 4.84 (m, 2H), 5.14 (1/2ABq, *J*=12.6 Hz, 1H), 5.19 (1/2ABq, *J*=12.6 Hz, 1H), 6.75 (m, 1H), 6.86 (dd, *J*=8.1, 3.8 Hz), 7.00 (dd, *J*=7.7, 7.5 Hz, 1H), 7.26-7.42 (m, 7H), 7.49 (dd, *J*=7.5, 1.3 Hz, 1H); ^{13}C NMR (100 MHz, acetone-d_6 and D_2O) δ: 11.6, 11.7, 15.115.4, 19.6, 25.8, 26.1, 37.4, 37.9, 41.0, 41.2, 51.3, 53.2, 54.3, 59.3, 67.3, 68.4, 76.1, 79.0, 116.2, 122.1, 124.2, 126.2, 126.6, 128.8, 128.9, 129.3, 129.4, 131.2, 132.1, 134.1, 136.8, 141.0, 154.3, 160.0, 170.8, 171.2, 171.8, 171.9, 173.1, 179.2, 202.1; IR (MeOH film): 3335, 2963, 2923, 2849, 1722, 1665, 1517; HRMS (FABH+) calcd for $C_{41}H_{47}N_6O_{13}$ (*m/z*) 831.3201; found (*m/z*) 831.3204.

Filename: bka-3-1099-3



Filename: bka-3-1099-3-C13





168 TMC-95A/B; 1, 2

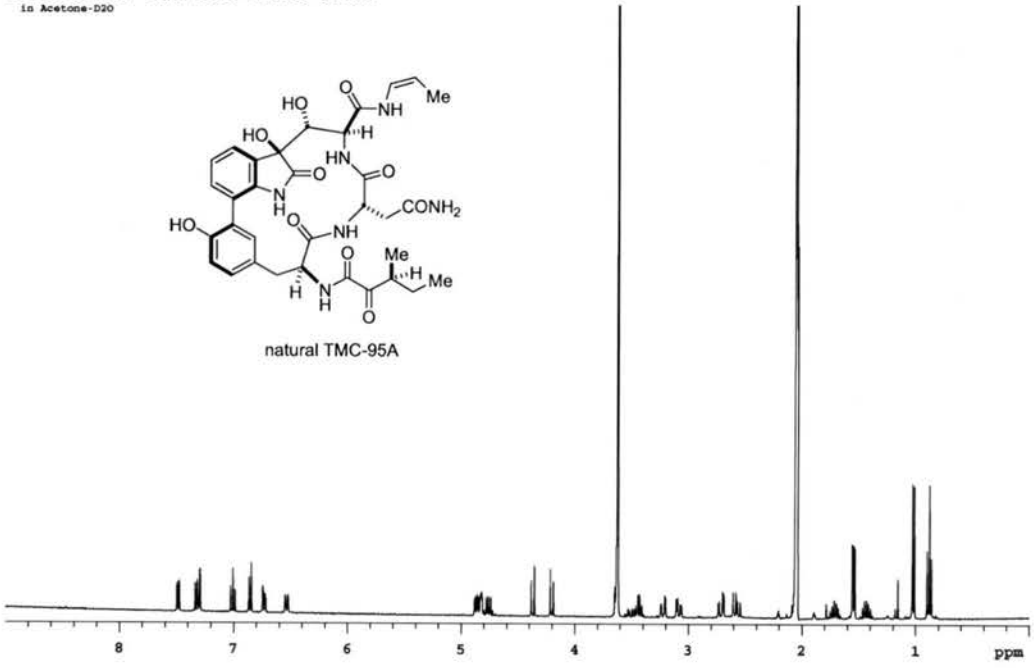
A solution of macrocylic threonine derivative **168** (4 mg, 0.0048 mmol) in MeOH (2 mL) was purged with argon for 10 min. To this solution was added Pd black (~2 mg) then bubbled H_2 through the solution for 20 min. Put under an atmosphere of H_2 for an additional 2 h. Purged reaction mixture with argon then filtered through a PTFE Acrodisc® (0.45 μM) filter and concentrated. Reconstituted in a minimal amount of MeOH and added a large excess of dry PhMe and concentrated to a white film. The resulting white film and PPh_3 (~3 mg) were taken up in dry DMF (0.3 mL) and dry THF (0.5 mL) under argon at 0°C. Treated the solution with DIAD (~6 μL) and allowed to warm to room temperature while stirring for 20 min. Quenched reaction with water and concentrated to a yellow oil. Initial purification by PTLC (25% MeOH in CH_2Cl_2) afforded TMC-95A and B as a 1:1 mixture of diastereomers (2.3 mg, 70%). TMC-95A and B were separated by RP-HPLC (Waters Symmetry C18 column, 4.6 mm x 250 mm, 20% MeCN in H_2O at 1.5 mL/min, RT=41.49 min. (TMC-95B) and 46.6 min. (TMC-95A). The synthetic compounds was identical to the natural samples by ^{1}H NMR, HPLC, TLC and HRMS (FABH+) calcd for $C_{33}H_{39}N_6O_{10}$ (*m/z*) 679.2728; found (*m/z*) 679.2727.

TMC-95A: ^{1}H NMR (400 MHz, acetone-d_6 and D_2O, 273 K) δ: 0.87 (t, *J*=7.5 Hz, 3H), 1.01 (d, *J*=6.8 Hz, 3H), 1.43 (m, 1H), 1.54 (dd, *J*=7.2, 1.7 Hz, 3H), 1.71 (m, 1H), 2.57 (dd, *J*=15.3, 9.8 Hz, 1H), 2.71 (dd, *J*=15.3, 4.3 Hz, 1H), 3.08 (dd, *J*=14.3, 4.8 Hz, 1H),

3.22 (dd, *J*=14.3, 2.3 Hz, 1H), 3.43 (m, 1H), 4.20 (d, *J*=10.5 Hz, 1H), 4.36 (d, *J*=10.5 Hz, 1H), 4.74-4.88 (m, 3H), 6.52 (dd, *J*=8.9, 1.7 Hz, 1H), 6.73 (dd, *J*=8.3, 2.2 Hz, 1H), 6.85 (d, *J*=8.3 Hz, 1H), 7.00 (t, *J*=7.7 Hz, 1H), 7.28 (d, *J*=2.2 Hz, 1H), 7.32 (dd, *J*=7.7, 1.1 Hz, 1H), 7.48 (dd, *J*=7.7, 1.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, 278K): see below. $[\alpha]_D^{25}$ = +93, synthetic (*c* 0.015, MeOH); lit: +102 (*c* 0.54, MeOH).

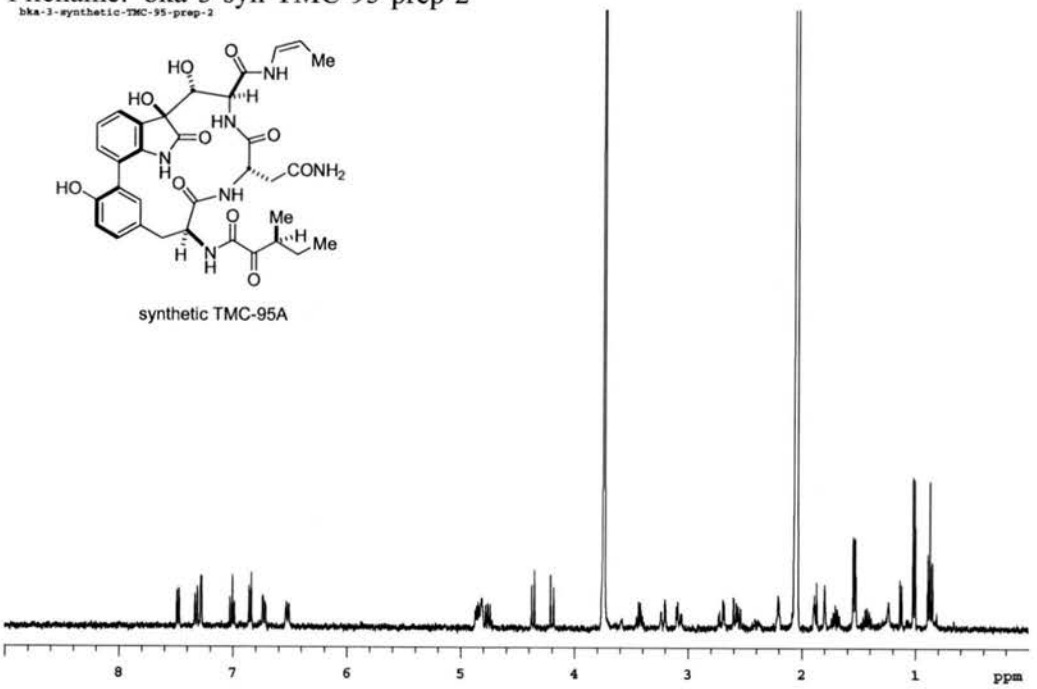
TMC-95B: ^{1}H NMR (400 MHz, acetone-d_6 and D_2O, 273 K) δ: 0.82 (t, *J*=7.5 Hz, 3H), 1.08 (d, *J*=7.0 Hz, 3H), 1.32 (m, 1H), 1.54 (dd, *J*=7.0, 1.7 Hz, 3H), 1.66 (m, 1H), 2.57 (dd, *J*=15.5, 9.6 Hz, 1H), 2.71 (dd, *J*=15.5, 4.5 Hz, 1H), 3.08 (dd, *J*=13.8, 5.1 Hz, 1H), 3.22 (dd, *J*=13.8, 2.3 Hz, 1H), 3.40 (m, 1H), 4.20 (d, *J*=10.6 Hz, 1H), 4.37 (d, *J*=10.6 Hz, 1H), 4.72-4.88 (m, 3H), 6.53 (dd, *J*=8.9, 1.7 Hz, 1H), 6.76 (dd, *J*=8.3, 2.1 Hz, 1H), 6.86 (d, *J*=8.1 Hz, 1H), 7.01 (t, *J*=7.7 Hz, 1H), 7.29 (d, *J*=2.1 Hz, 1H), 7.32 (dd, *J*=7.7, 1.1 Hz, 1H), 7.48 (dd, *J*=7.7, 1.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, 278K): see below. $[\alpha]_D^{25}$ = +73, synthetic (*c* 0.015, MeOH); lit: +74 (*c* 0.47, MeOH).

Filename: bka-nat-TMC-95A



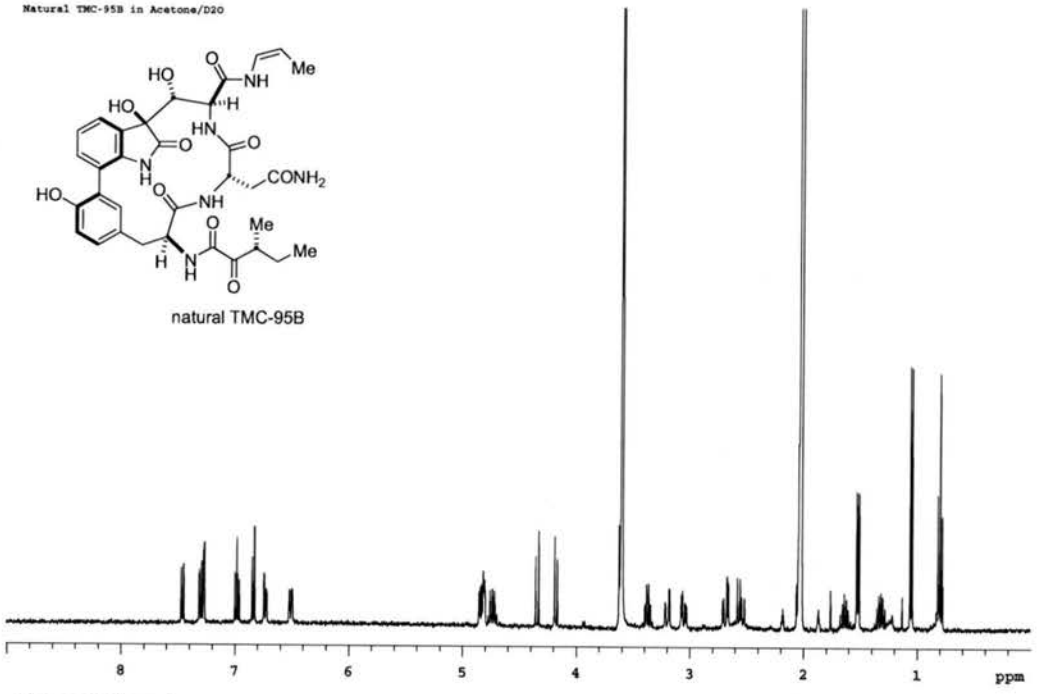
natural TMC-95A

Filename: bka-3-syn-TMC-95-prep-2

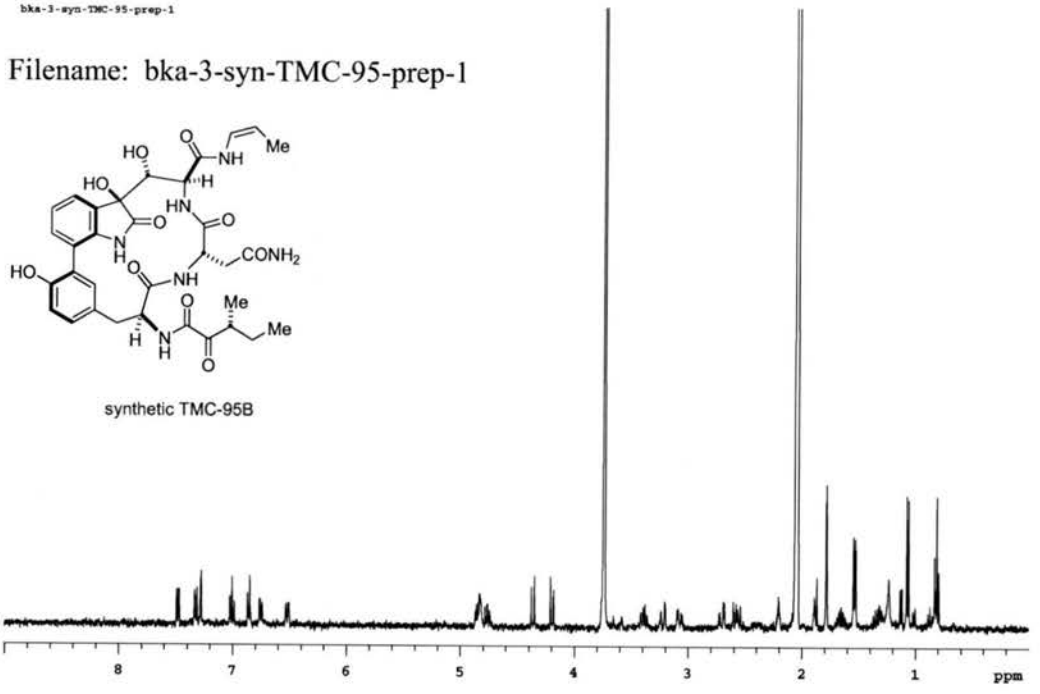


synthetic TMC-95A

Filename: bka-nat-TMC-95B

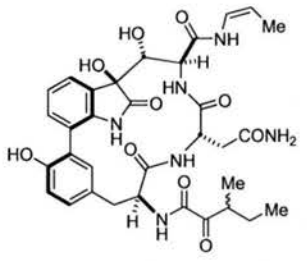


natural TMC-95B



Filename: bka-3-syn-TMC-95-prep-1

synthetic TMC-95B



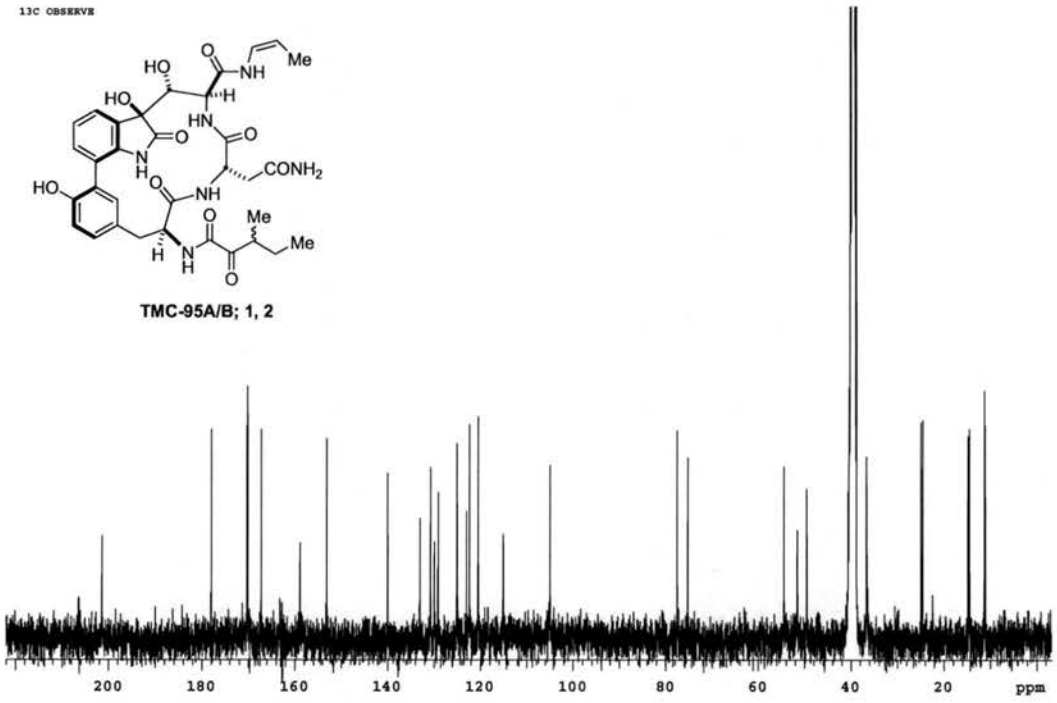
TMC-95A/B; 1, 2

Table 1. Comparison of ^{13}C NMR-Data between Synthetic TMC-95A/B and Natural TMC-95A/B.

Carbon #	Literature Reported ^{13}C Chemical Shifts (100 MHz, DMSO-d_6, 298K)		Synthetic TMC-95A/B ^{13}C Chemical Shifts (100 MHz, DMSO-d_6, 298K)
	TMC-95A	TMC-95B	1:1 Mixture TMC-95A/B
1	120.4	120.4	120.5
2	130.7	130.7	130.7
3	120.4	120.4	120.5
4	124.9	124.9	125.0
5	129.1	129.1	129.1
6	77.5	77.5	77.5
7	75.2	75.2	75.2
8	54.4	54.4	54.5
10	170.3	170.3	170.3
11	49.5	49.5	49.6
13	170.1	170.1	170.1
14	51.6	51.5	51.5, 51.6
15	36.5	36.6	36.5, 36.6
16	125.0	125.0	125.1
17	129.8	129.8	129.8

18	115.0	115.1	115.0, 115.1
19	153.1	153.1	153.2
20	122.9	122.9	123.0
21	139.9	139.9	139.9
23	177.9	177.9	178.0
24	132.9	133.0	133.0
25	167.2	167.2	167.2
27	122.3	122.3	122.4
28	105.0	105.0	105.0
29	11.2	11.2	11.2
30	36.6	36.6	36.6
31	170.1	170.1	170.2
34	158.8	158.9	158.9, 159.0
35	201.3	201.3	201.3, 201.4
36	39.5	40.0	overlap with DMSO-d_6 signal
37	24.8	24.5	24.6, 24.9
38	11.0	11.1	11.0, 11.2
39	14.5	14.7	14.5, 14.8

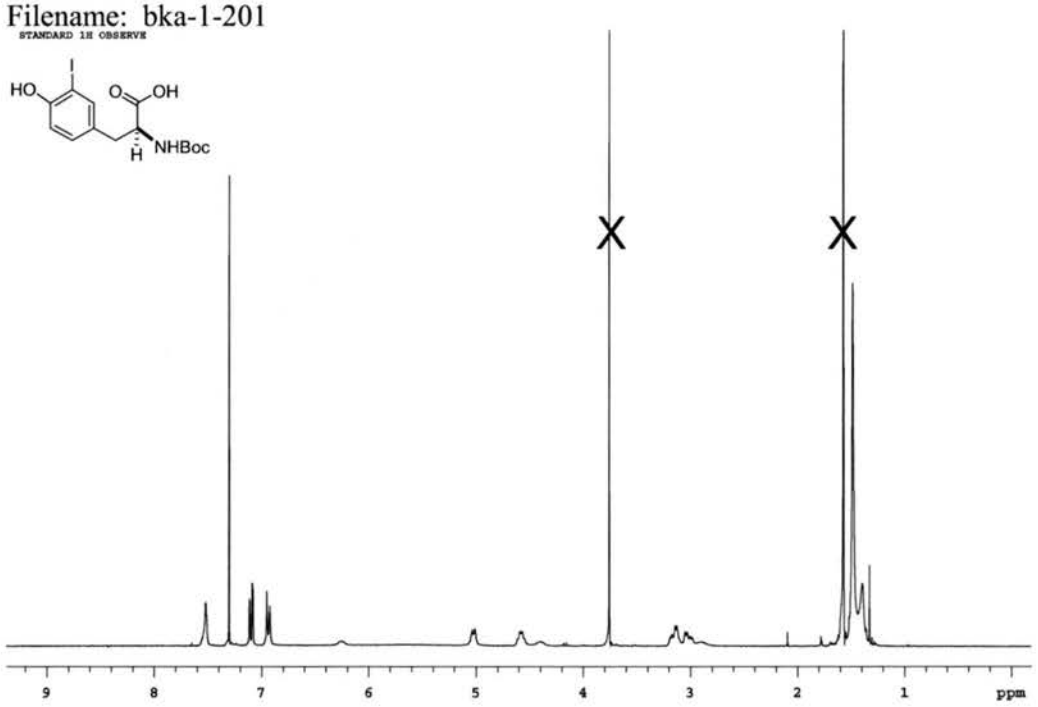
Filename: bka-3-1107-13C-DMSO



Experimental Section 2.

***N*-Boc-3-iodo-L-tyrosine**. To a mixture of 3-iodo-L-tyrosine (1.03 g, 3.35 mmol), dioxane (10 mL) and 2.5 M aq. NaOH (10 mL) at 0°C was added Boc_2O (0.80 g, 3.3 mmol) and allowed to stir for 4 h. Acidified to pH~3 with 1 M HCl and extracted with EtOAc (3 x 50 mL). Washed combined organics with H_2O (1 x 20 mL) and brine (1 x 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to afford *N*-Boc-3-iodo-L-tyrosine (1.37 g, 100%). ^1H NMR (300 MHz, $CDCl_3$, 273K) δ 1.45 (s, 9H), 2.98 (dd, *J*=13.9, 5.9 Hz, 1H), 3.13 (dd, *J*=13.9, 5.1 Hz, 1H), 4.55 (m, 1H), 4.99 (br d, *J*=7.3 Hz, 1H)), 6.91 (d, *J*=8.4 Hz, 1H), 7.06 (dd, *J*=8.4, ~2.0 Hz, 1H), 7.49 (br s, 1H). HRMS (FABH+) calcd for $C_{14}H_{19}N_1O_5I_1$ (*m/z*) 408.0308; found (*m/z*) 408.0316.

Filename: bka-1-201

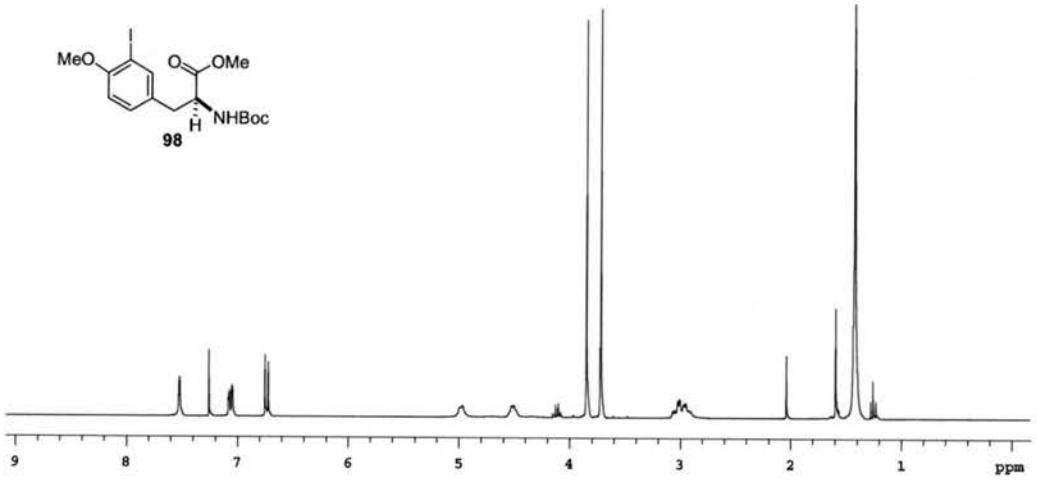


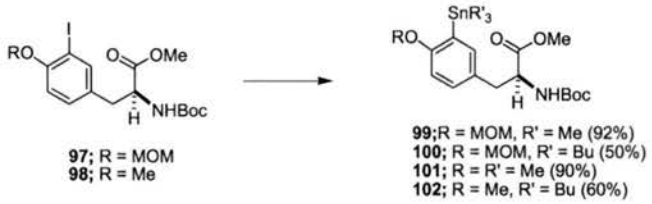
98

3-iodo-L-tyrosine derivative 98. To a solution of *N*-Boc-3-iodo-L-tyrosine (1.36 g, 3.34 mmol) and acetone (30 mL) was added K_2CO_3 and MeI (0.62 mL, 10 mmol) and brought to reflux for 12 h. Concentrated under reduced pressure and reconstituted in EtOAc (100 mL). Washed organic layer with sat. aq. $NaHCO_3$ (1 x 10 mL), 0.5 M HCl (1 x 10 mL) and brine (1 x 20 mL). Dried over anhydrous Na_2SO_4, filtered and concentrated to provide tyrosine derivative **98** (1.45 g, 100%) as a colorless oil that solidified upon standing. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 1.43 (s, 9H), 2.94 (dd, *J*=13.9, 5.9 Hz, 1H), 3.04 (dd, *J*=13.9, 5.5 Hz, 1H), 3.72 (s, 3H), 3.85 (s, 3H), 4.51 (ddd, *J*=7.3, 5.9, 5.5 Hz, 1H), 4.98 (br d, *J*=7.3 Hz, 1H), 6.74 (d, *J*=8.4 Hz, 1H), 7.06 (dd, *J*=8.4, 2.2 Hz, 1H), 7.52 (br s, 1H).

Filename:bka-1-118A

STANDARD 1H OBSERVE





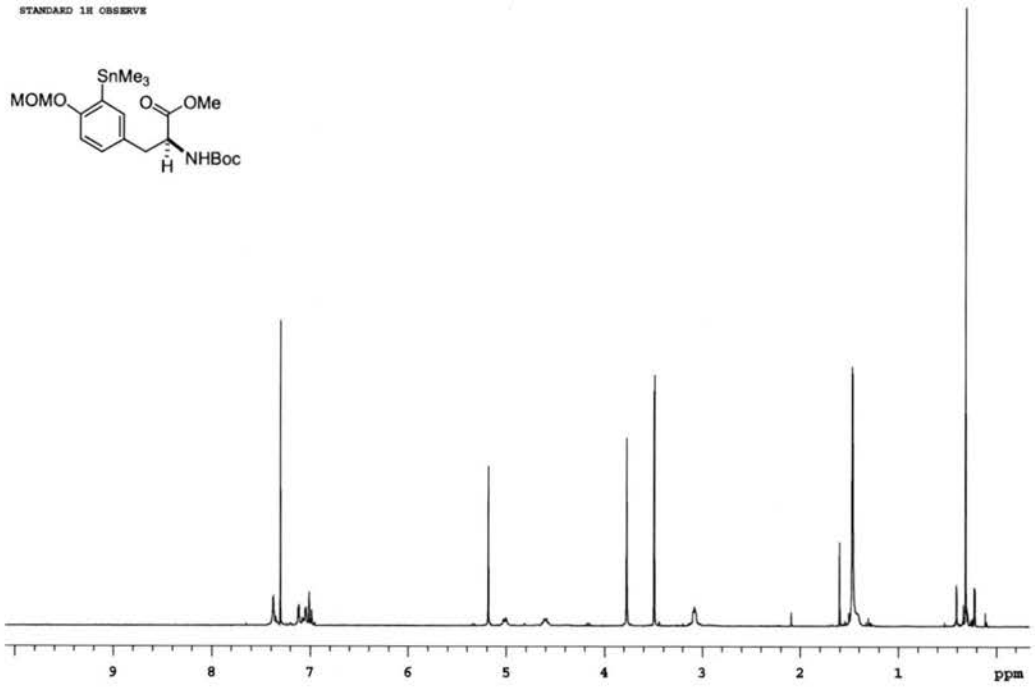
97; R = MOM
98; R = Me

99;R = MOM, R' = Me (92%)
100; R = MOM, R' = Bu (50%)
101; R = R' = Me (90%)
102; R = Me, R' = Bu (60%)

Representative procedure for converting tyrosine aryl idodides to aryl stannanes. To a flask of tyrosine aryl iodide (0.49 mmol), $Pd(PPh_3)_4$, and dry PhMe under argon fixed with a condenser was added $(R'_3Sn)_2$ (0.664 mmol) and allowed to reflux until complete consumption of the starting material (TLC, 2:1 hexanes:EtOAc, typically Pd black crashes out of solution). Filtered reaction mixture through Celite® and concentrated under reduced pressure. Took up mixture in EtOAc and washed organics with 0.5 M HCl (1 x 10 mL), H_2O (1 x 10 mL), brine (1 x 10 mL) and dried over Na_2SO_4. Filtered, concentrated and purified via flash chromatography (silica gel, 2:1 hexanes:EtOAc). For the tributyl stannane derivatives, after removing EtOAc under pressure, the crude mixture as taken up in MeCN (30 mL) and washed with hexanes (3 x 10 mL) to remove excess tributyltin iodide then concentrated under reduced pressure and purified as described above. See above for yields.

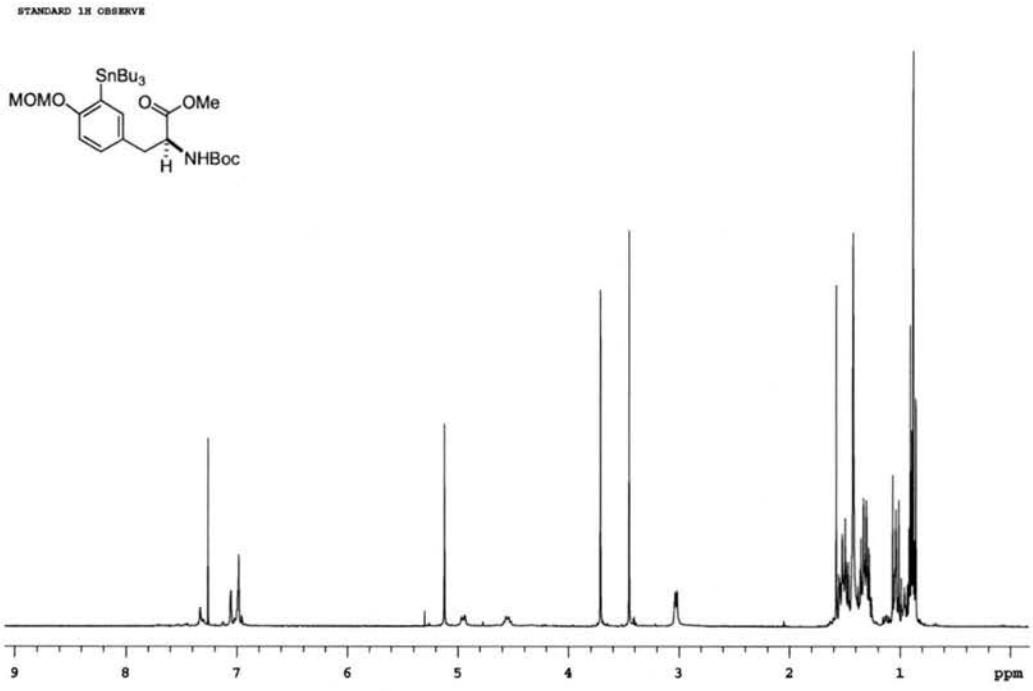
Stannane 99. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 0.32 (s (with satellites), 9H), 1.47 (s, 9H), 3.08 (m, 2H), 3.49 (s, 3H), 3.77 (s, 3H), 4.60 (dd, *J*=13.6, 5.1 Hz, 1H), 5.02 (br d, *J*=8.4 Hz, 1H), 5.18 (s, 2H), 7.01 (m, 1H), 7.12 (m, 1H), 7.37 (d, *J*=1.4 Hz, 1H).

Filename: bka-1-271



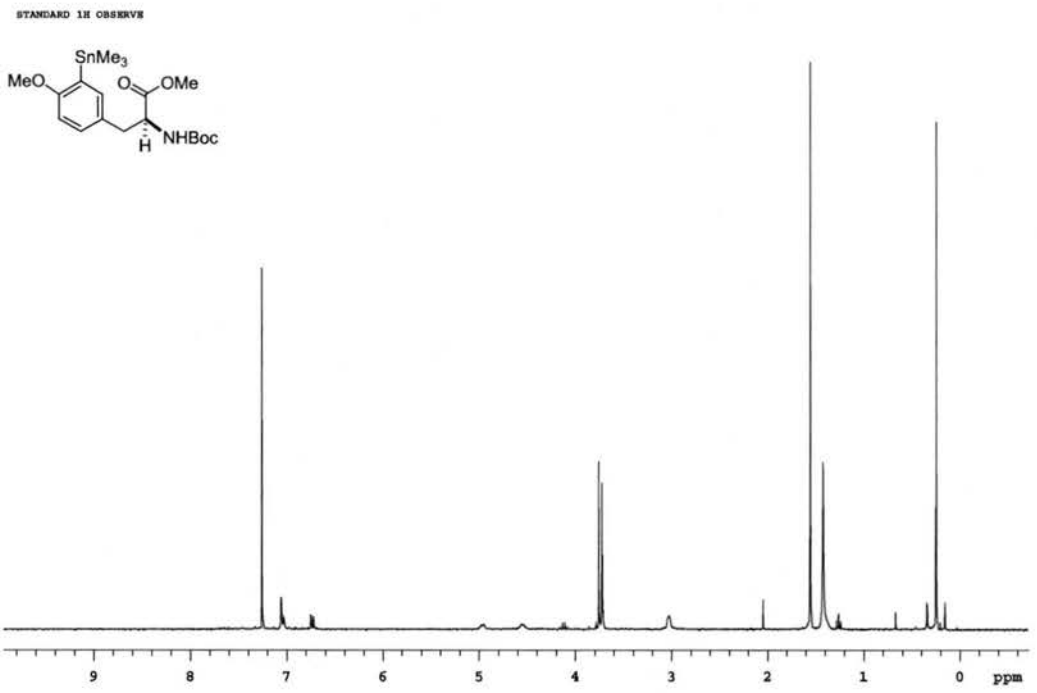
Stannane 100. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 0.89 (m, 9H), 1.05 (m, 6H), 1.20-1.60 (m, 15H), 3.03 (d, *J*=5.9 Hz, 2H), 3.45 (s, 3H), 3.71 (s, 3H), 4.55 (dd, *J*=8.0, 5.5 Hz, 1H), 4.95 (br d, *J*=8.0 Hz, 1H), 5.12 (s, 2H), 7.02 (m, 2H), 7.33 (d, *J*=1.5 Hz, 1H).

Filename: bka-1-472-1



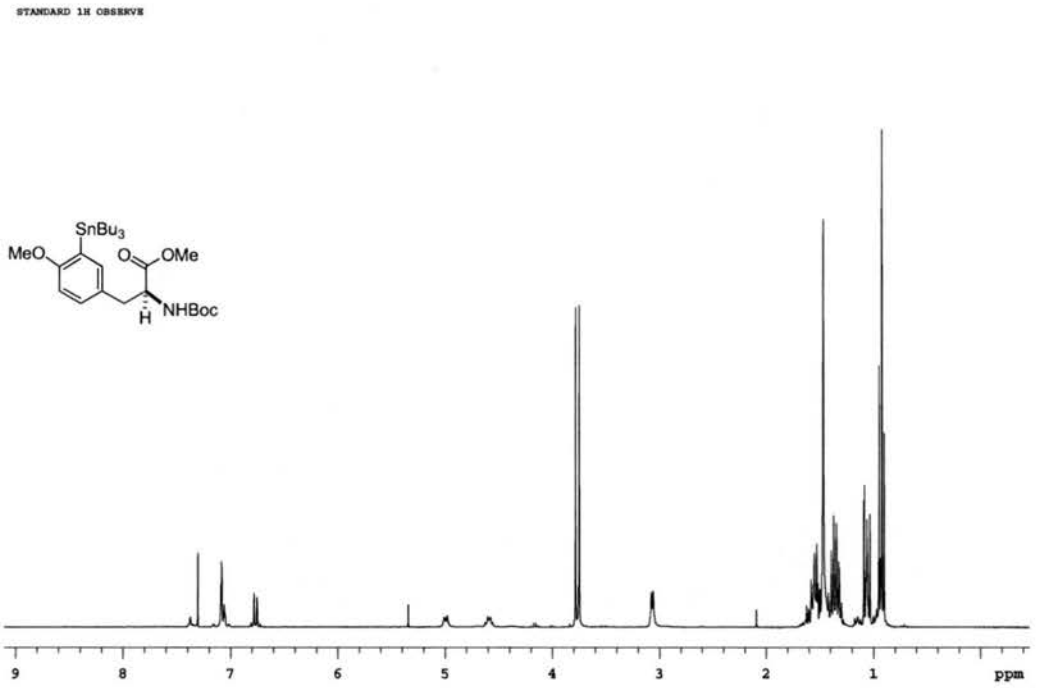
Stannane 101. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 0.25 (s (with satellites), 9H), 1.42 (s, 9H), 3.03 (br m, 2H), 4.55 (m, 1H), 4.97 (br d, *J*=9.5 Hz, 1H), 6.74 (d, *J*=8.8 Hz, 1H), 7.05 (m, 1H).

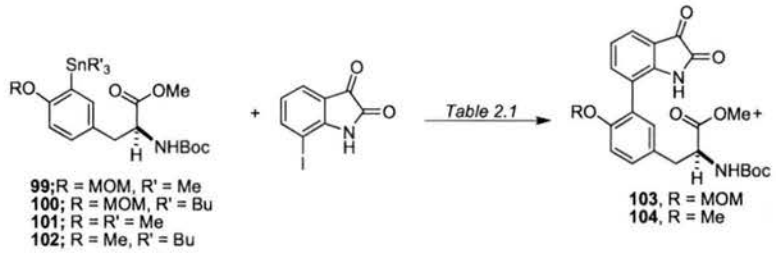
Filename: bka-1-123



Stannane 102. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 0.89 (t, *J*=7.3 Hz, 9H), 1.03 (t, *J*=8.1 Hz, 6H), 1.32 (m, 6H), 1.44 (s, 9H), 1.51 (m, 6H), 3.03 (d, *J*=5.5 Hz, 2H), 3.72 (s, 3H), 3.75 (s, 3H), 4.56 (dd, *J*=8.1, 5.5 Hz, 1H), 4.96 (br d, *J*=8.1 Hz, 1H), 6.73 (m, 1H), 7.03 (m, 2H).

Filename: bka-1-283

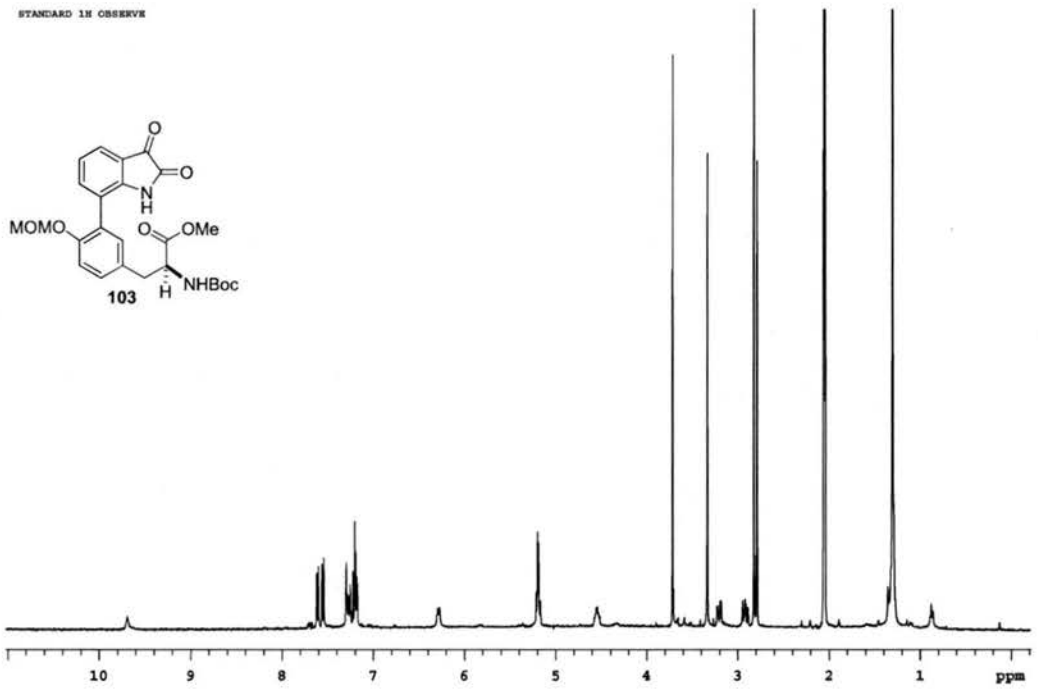




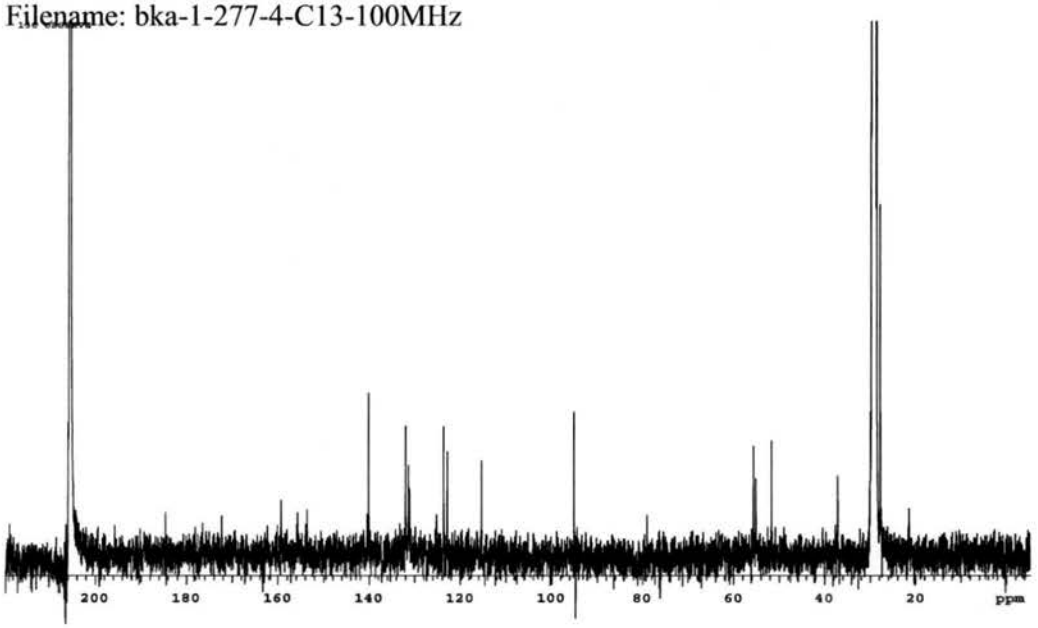
General procedure for Stille coupling coupling of 7-iodoisatin and tyrosine arylstannanes yielding biaryls 103 and 104 (See Table 2.1 for equivalents). To a flame dried flask fixed with a reflux condenser under argon was added 7-iodoisatin (1 eq), arylstannane (1.1 eq), Pd catalyst, and CuX, which was then taken up in dry MeCN and refluxed for the appropriate time (see Table 2.1 for times). The resulting hot mixture was filtered through celite and washed with additional MeCN. The resulting solution was washed with hexanes (3 x 5 mL) to remove Bu_3SnI. The MeCN phase was concentrated to dryness under vacuum and then taken up in EtOAc (20 mL). The organic layer was washed consecutively with aq. KF (2 x 5 mL), aq. $NaHCO_3$ (2 x 5 mL), water (1 x 5 mL), brine (1 x 5 mL), dried over $MgSO_4$, filtered and concentrated. Purified via flash column chromatography (2:1 hexanes:EtOAc) to afford coupled product as a yellow film.

Biaryl 103: ^{1}H NMR (400 MHz, acetone-d_6, 273 K) δ: 1.30 (s, 9H), 2.92 (dd, *J*=13.7, 9.9 Hz, 1H), 3.21 (dd, *J*=13.7, 4.8 Hz, 1H), 3.34 (s, 2H), 3.72 (s, 3H), 4.55 (m, 1H), 5.18 (overlapping 1/2 ABq, *J*=11.6 Hz, 1H), 5.20 (overlapping 1/2 ABq, *J*=11.6 Hz, 1H), 6.28 (br d, *J*=8.5 Hz, 1H), 7.17-7.29 (m, 4H), 7.55 (d, *J*=7.5 Hz, 1H), 7.61 (d, *J*=7.7 Hz, 1H), 9.69 (br s, 1H). ^{13}C NMR (100 MHz, acetone-d_6, 273 K) δ: 21.4, 27.8, 37.2, 51.7, 55.1, 55.7, 76.1, 78.9, 95.1, 115.3, 122.8, 123.6, 125.2, 131.3, 131.9, 140.1, 153.6, 155.6, 159.3, 172.3, 184.5. HRMS (FABH+) calcd for $C_{25}H_{29}N_2O_8$ (*m/z*) 485.1924; found (*m/z*) 485.1917.

Filename: bka-1-277-4

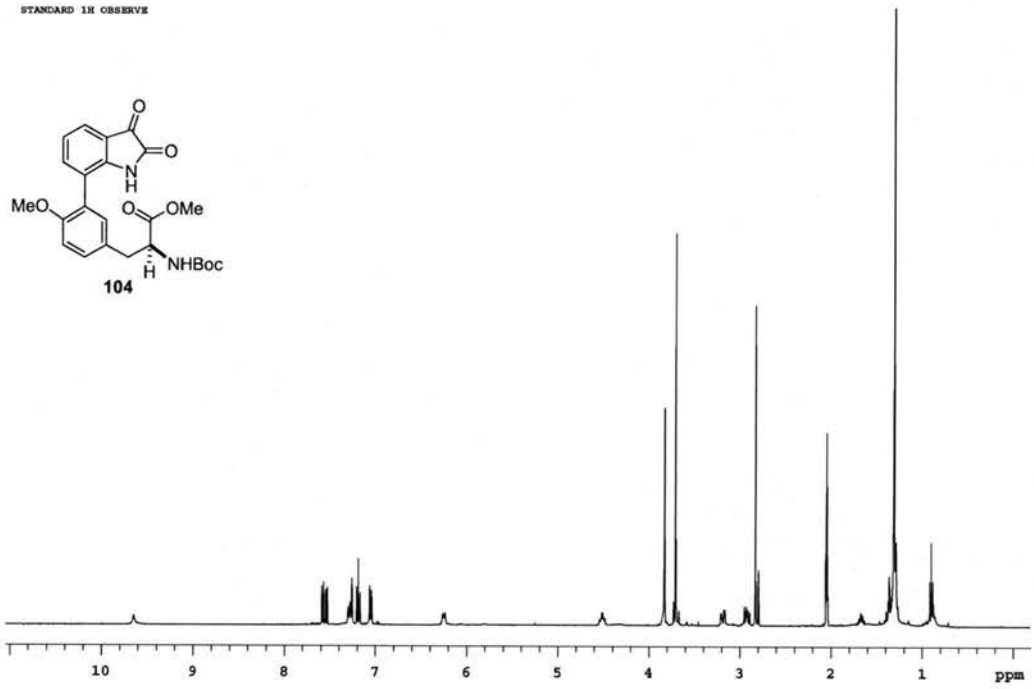


Filename: bka-1-277-4-C13-100MHz

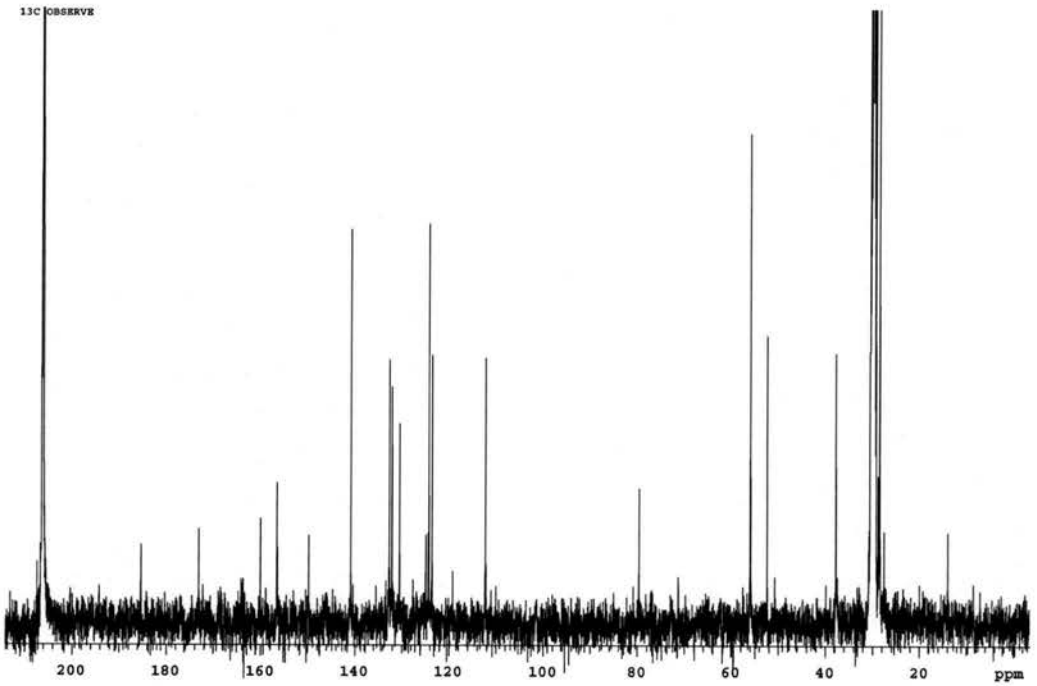


Biaryl 104: ^{1}H NMR (400 MHz, acetone-d_6, 273 K) δ: 1.31 (s, 9H), 2.92 (dd, *J*=13.8, 9.5 Hz, 1H), 3.19 (dd, *J*=13.8, 4.9 Hz, 1H), 3.71 (s, 3H), 3.83 (s, 3H), 4.52 (ddd, *J*=9.5, 8.3, 4.9 Hz, 1H), 6.25 (br d, *J*=8.3 Hz, 1H), 7.05 (d, *J*=8.3 Hz, 1H), 7.19 (dd, *J*=7.7, 7.5 Hz, 1H), 7.27-7.30 (m, 2H), 7.54 (dd, *J*=8.7, 1.1 Hz, 1H), 7.58 (dd, *J*=7.7, 1.3 Hz, 1H), 9.65 (br s, 1H). ^{13}C NMR (100 MHz, acetone-d_6, 273 K) δ: 14.0, 28.6, 28.8, 37.8, 52.4, 56.0, 79.7, 112.3, 123.6, 1124.3, 124.5, 124.9, 130.5, 132.1, 132.7, 140.9, 149.8, 156.6, 160.0, 173.1, 185.3. HRMS (FABH+) calcd for $C_{24}H_{27}N_2O_7$ (*m/z*) 455.1818; found (*m/z*) 455.1805.

Filename: bka-1-290-4



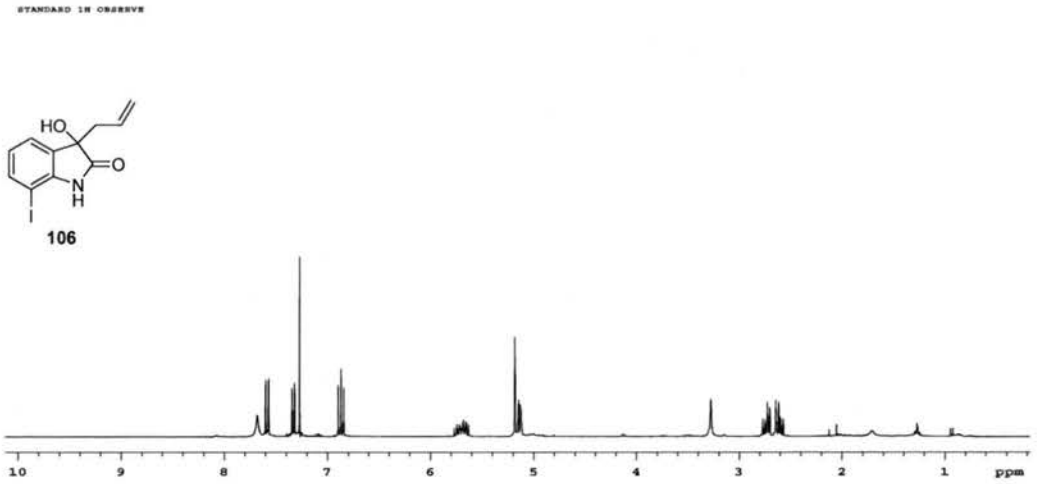
Filename:bka-1-290-C13-100MHz

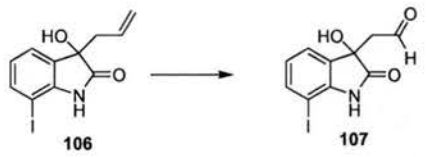


106

homo-allylic alcohol 106. To a solution of 7-iodoisatin (415 mg, 1.52 mmol) in anhydrous THF (15 mL) at –78° C under Ar was added allyl magnesium bromide (3.10 mL, 1.0 M ether, 3.07 mmol). The resulting mixture was allowed to stir for 5 min. then quenched with 2 mL 1 M HCl. The mixture was extracted with EtOAc (3 x 10 mL), washed 0.5 M HCl (2 mL), brine (2 mL), dried anhydrous Na_2SO_4, filtered, concentrated under vacuum. Purified via flash column chromatography (1:1 hexanes:EtOAc) to yield a yellow oil which solidified upon standing (77% yield). ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 2.60 (1/2 ABX, *J*=13.5, 8.4 Hz, 1H), 2.73 (1/2 ABX, *J*=13.5, 6.3 Hz, 1H), 3.27 (br s, 1H), 5.13 (m, 2H), 5.57 (m, 1H), 6.86 (dd, *J* =8.1, 7.2 Hz, IH), 7.32 (d, *J* = 7.2 Hz, 1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.67 (br s, 1H). HRMS (FABH+) calcd for $C_{11}H_{11}N_1O_2I$ (*m/z*) 315.9835; found (*m/z*) 315.9833.

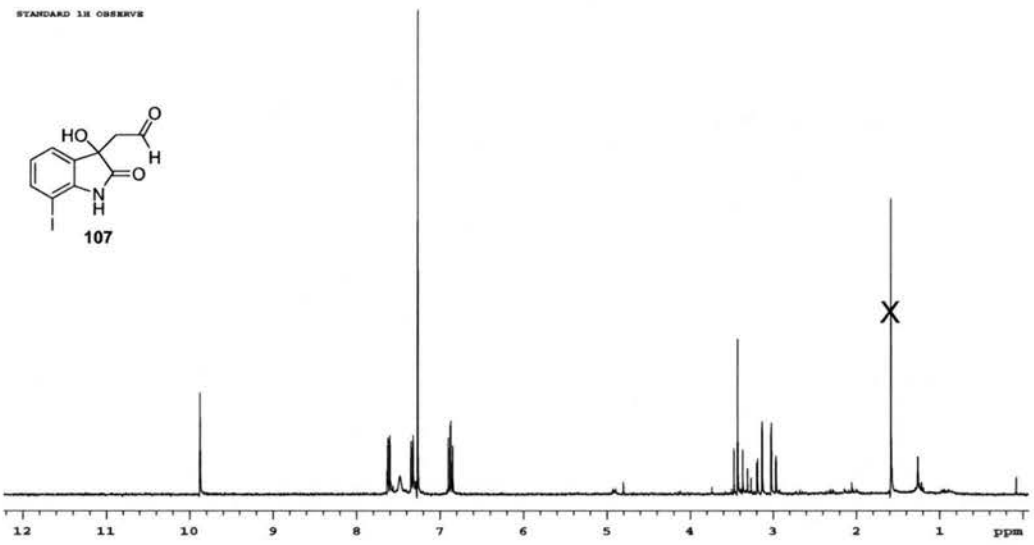
Filename: bka-1-351-1

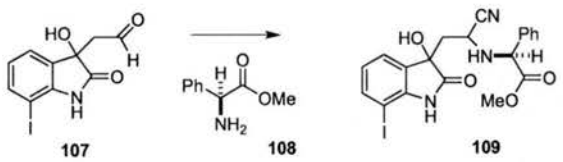




aldehyde 107. Ozone was bubbled though a solution of homoallylic alchol (38 mg, 0.12 mmol), methanol (1 mL) and CH_2Cl_2 (1 mL) until a blue color persisted. Argon was bubbled through the resulting mixture until the blue color diminished at which time 0.5 mL DMS was added and allowed to stir for 12 h. The resulting mixture was concentrated to a yellow oil (28 mg, 73%). Due to the reactivity of the aldehyde it was used without purification and decomposed upon standing. Crude ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 2.99 (1/2 ABX, *J*=16.8, 1.5 Hz, 1H), 3.16 (1/2 ABX, *J*=16.8, 1.5 Hz, 1H), 3.40 (m, 1H), 6.87 (dd, *J*=8.1, 7.5 Hz, 1H), 7.34 (d, 7.5 Hz, 1H), 7.48 (br s, 1H), 7.62 (d, *J*=8.1 Hz, 1H), 9.88 (t, *J*=1.5 Hz, 1H). HRMS (FABH+) calcd for $C_{10}H_9N_1O_3I$ (*m/z*) 317.9627; found (*m/z*) 317.9632.

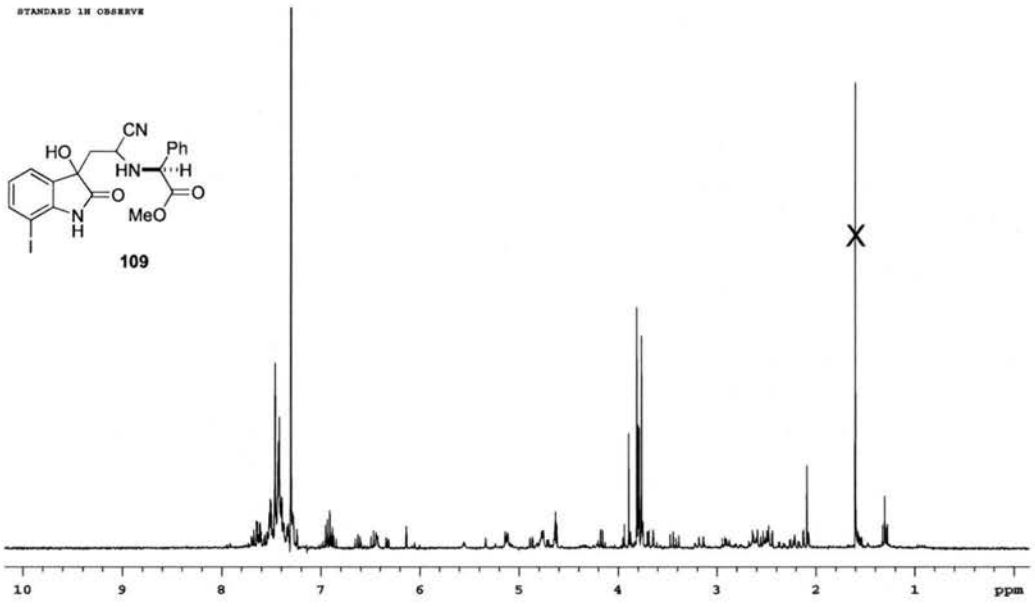
Filename: bka-1-355





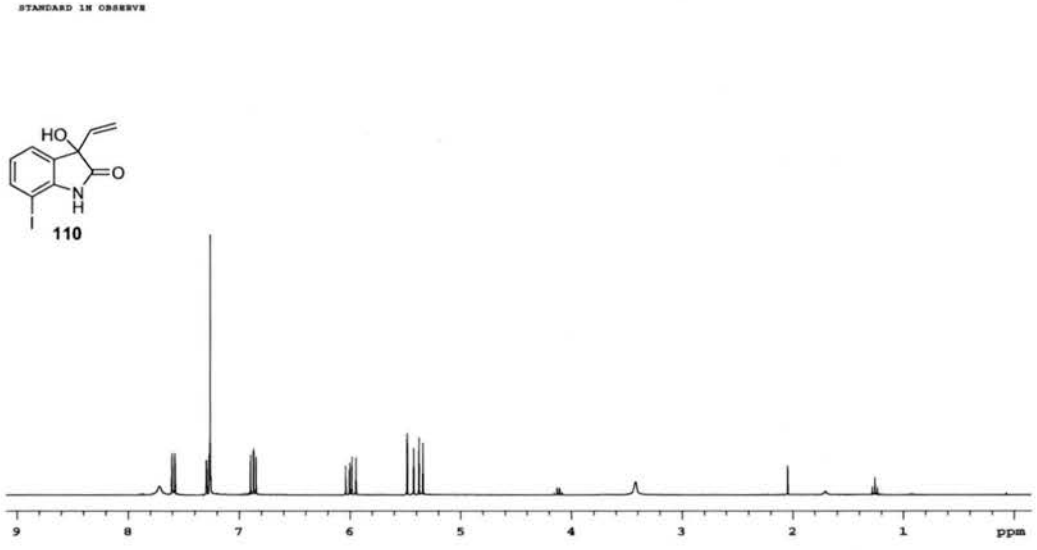
Amino nitrile 109. To a solution of aldehyde **107** (17 mg, 0.054 mmol) and phenyl glycine methyl ester **108** (9.7 mg, 0.059 mmol) in absolute EtOH (5 mL) was added TMSCN (8.8 μL, 0.065 mmol) and the mixture was brought to reflux for 4.5 h. Quenched with sat. aq. $NaHCO_3$ (0.5 mL) and concentrated. Reconstituted in EtOAc (30 mL) and washed with H_2O (1 x 5 mL) and brine (1 x 5 mL). Dried organics over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purified via flash chromatography to afford amino nitrile **109** (12 mg, 45%) as a mixture of inseparable diasteromers. ^1H NMR (300 MHz, $CDCl_3$, 273K) See spectrum of diastereomeric mixture.

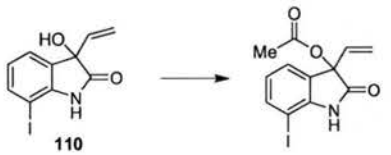
Filename: bka-1-370-A



110

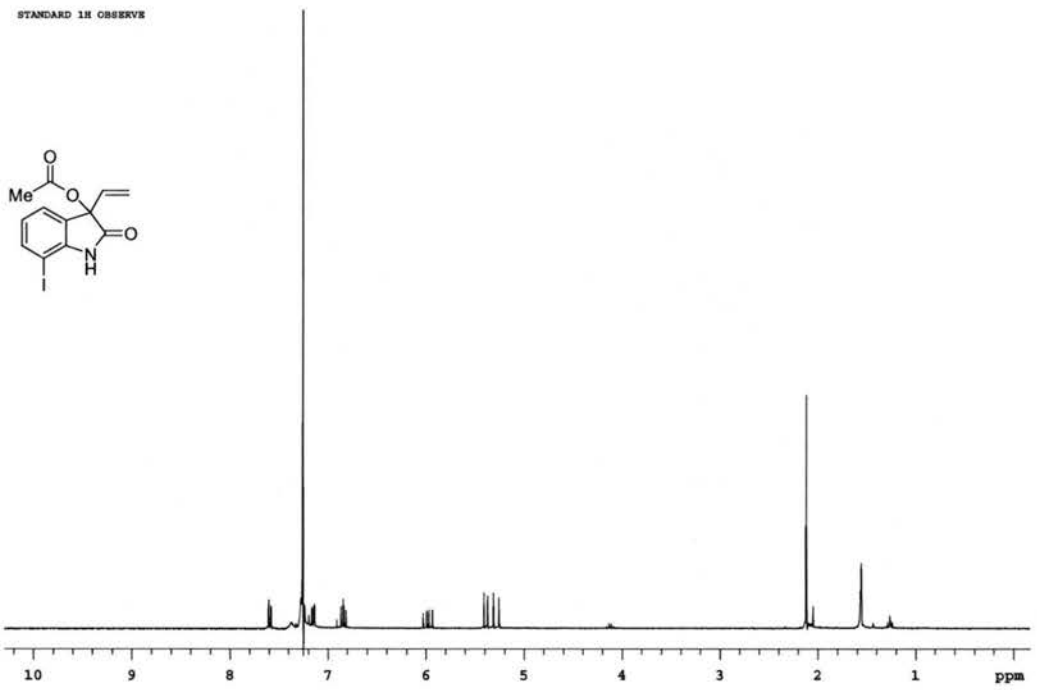
Allylic alcohol 110. To a solution of 7-iodoisatin (200 mg, 0.732 mmol) in anhydrous THF (7 mL) at –78° C under argon was added vinyl magnesium bromide (1.61 mL, 1.0 M THF, 1.61 mmol). The resulting mixture was allowed to stir for 5 min. then quenched with 1 M HCl (2 mL). The mixture was extracted with EtOAc (3 x 10 mL), washed 0.5 M HCl (2 mL), brine (2 mL), dried anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purified via flash column chromatography (1:1 hexanes:EtOAc) to afford allylic alcohol **110** as a yellow oil (170 mg, 77% yield). ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 3.40 (br s, 1H), 5.36 (d, *J*=10.6 Hz, 1H), 5.45 (d, *J*=17.3 Hz, 1H), 5.99 (dd, *J* = 17.3, 10.6 Hz, 1H), 6.87 (dd, *J*=8.1, 7.2 Hz, 1H), 7.28 (dd, *J*=7.2, 1.2 Hz, 1H), 7.59 (dd, *J*=8.1, 1.2 Hz, 1H), 7.72 (br s, 1H).

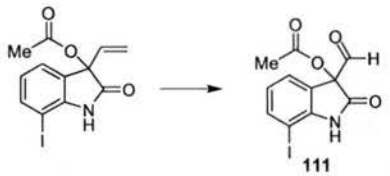




To a solution of alcohol (145 mg, 0.484 mmol) in dry CH_2Cl_2 (5 mL) at RT under Ar was added DMAP (6 mg, 0.048 mmol), $^{i}Pr_2NEt$ (94 μL, 0.532 mmol), Ac_2O (55 μL, 0.581 mmol) and allowed to stir for 1 h. Concentrated resulting mixture in vacuo, took up in EtOAc (30 mL), washed 0.5 M HCl (2 mL), H_2O (2 mL), sat. $NaHCO_3$ (2 mL), brine (2 mL), dried Na_2SO_4, filtered, concentrated in vacuo. Recrystallized from EtOAc to yield a white solid (138 mg, 83 %). ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 2.10 (s, 3H), 5.28 (d, *J*=17.3 Hz, 1H), 5.39 (d, *J*=10.6 Hz, 1H), 5.99 (dd, *J* = 17.3, 10.6 Hz, 1H), 6.82 (dd, *J*=8.1, 7.2 Hz, 1H), 7.18 (d, *J* = 7.2 Hz, 1H), 7.28 (br s, 1H), 7.60 (d, *J*=8.1 Hz, 1H).

Filename: bka-1-386



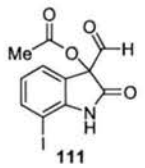


111

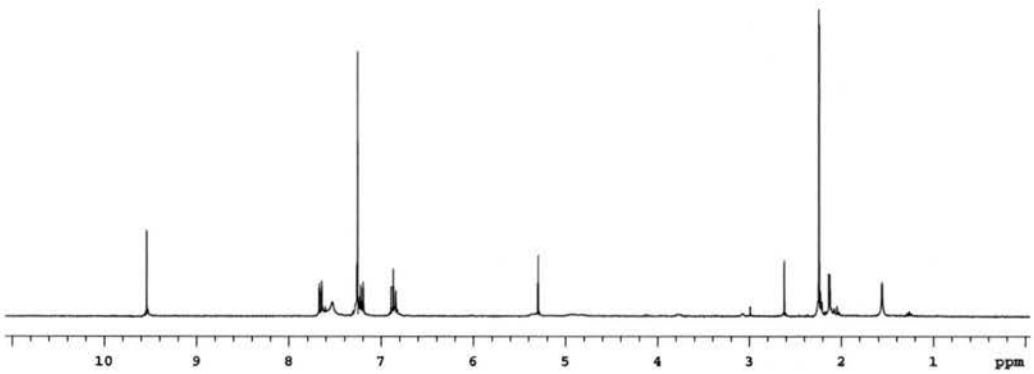
Aldehyde 111. Ozone was bubbled though a solution of allylic alchol (90 mg, 0.26 mmol) and CH_2Cl_2 (1 mL) at –78° C until a blue color persisted. Argon was bubbled through the resulting mixture until the blue color diminished at which time 0.5 mL DMS was added and allowed to stir for 12 h. The resulting mixture was concentrated to a colorless oil. Due to the reactivity of the aldehyde it was used without purification. Crude ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 2.43 (s, 3H), 6.87 (dd, *J*=7.8, 7.5 Hz, 1H), 7.21 (d, *J*=7.5 Hz, 1H), 7.52 (br s, 1H), 7.66 (d, *J*=7.8 Hz, 1H), 9.55 (s, 1H).

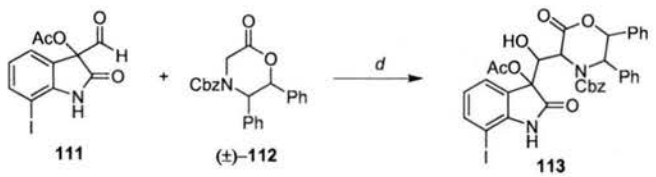
Filename: bka-1-389-2

STANDARD 1H OBSERVE

Crude 

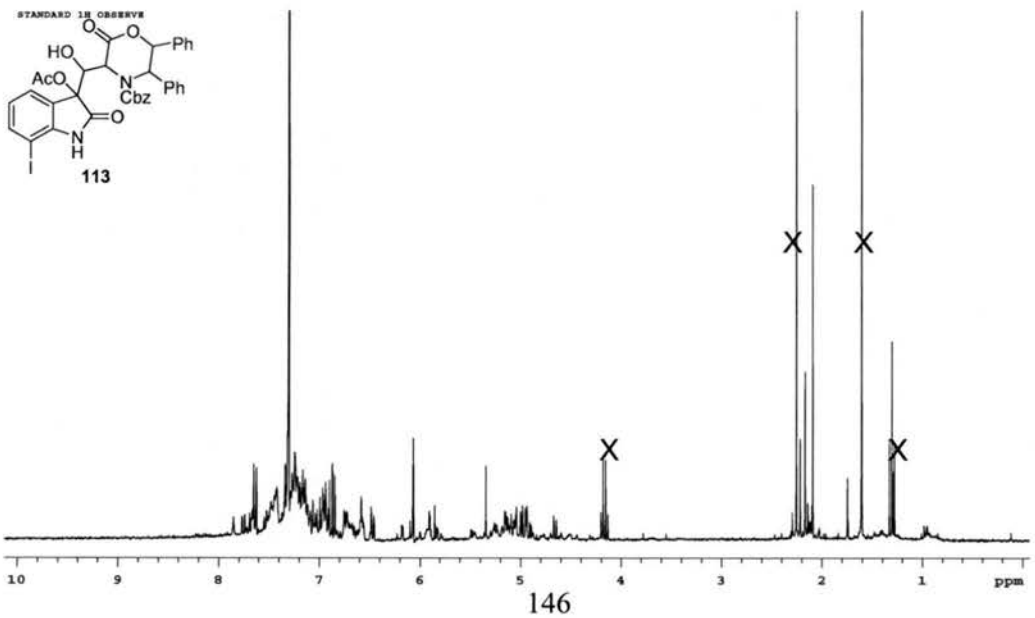
111





Aldol product 113. To a solution of lactone (101.04 mg, 0.26 mmol) in dry CH_2Cl_2 (3 mL) at –5°C under Ar was added Bu_2BOTf (0.39 mL, 1.0 M in CH_2Cl_2, 0.39 mmol) then Et_3N (80 μL, 0.572 mmol) and allowed to stir for about 20 min. A solution of aldehyde (90 mg, 0.26 mmol) in dry CH_2Cl_2 (4 mL) at –78° C was added via canula to the lactone mixture precooled to –78° C. The resulting mixture was allowed to stir at –78° C for 1h then added 0.025 M $KHPO_4$ buffer (pH=7) and allowed to stir for 15 min, then diluted with H_2O, extracted with CH_2Cl_2 (3 x 3 mL), washed brine (3 mL), dried Na_2SO_4, filtered, concentrated. Purified via flash column chromatography (silica gel, 1:1 hexanes: EtOAc). Inseparable complex mixture of diastereomers and rotamers see crude ^1H NMR (300 MHz, $CDCl_3$, 273K) below. HRMS (FABH+) calcd for $C_{35}H_{30}N_2O_8I$ (*m/z*) 733.1047; found (*m/z*) 733.1056.

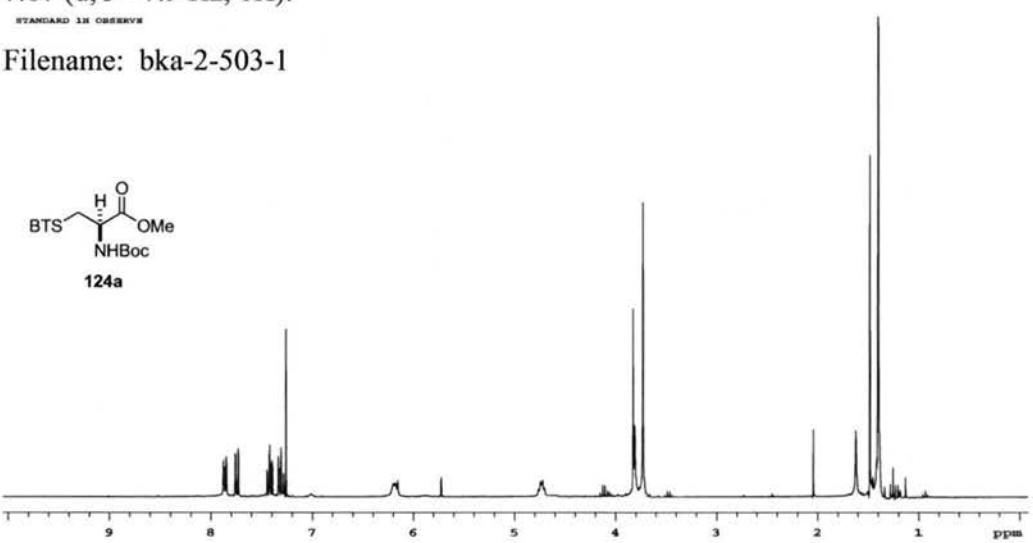
Filename: bka-1-390



123 124a

BT-cysteine derivative 124a. A mixture of *N*-Boc-serine methyl ester (1.7 g, 7.9 mmol), BTSH (2.6 g, 15.8 mmol), and PPh_3 (3.14 g, 11.8 mmol) were taken up in dry THF (80 mL) under argon. To the resulting mixture was added DIAD (2.95 mL, 14.2 mmol) at room temperature and allowed to stir for 15 minutes. The reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was removed and the aqueous layer was extracted with diethyl ether (3 x 200 mL). The combined organics were washed with water (1 x 200 mL) and brine (1 x 200 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered through ~ 40 mL of silica gel and concentrated under reduced pressure. Purified via flash chromatography (silica gel, 2:1 hexanes, EtOAc) to afford BT-cysteine derivative **124a** (2.6 g, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) 1.40 (s, 9H), 3.72 (s, 3H), 3.81 (m, 2H), 4.73 (m, 1 H), 6.20 (br d, *J*=7.4 Hz, 1 H), 7.30 (ddd, *J*=7.3, 1.3, 0.5 Hz, 1 H), 7.42 (dt, *J*=7.3, 1.3 Hz, 1 H), 7.74 (ddd, *J*=7.9, 1.3, 0.5 Hz, 1H), 7.87 (d, *J*= 7.9 Hz, 1H).

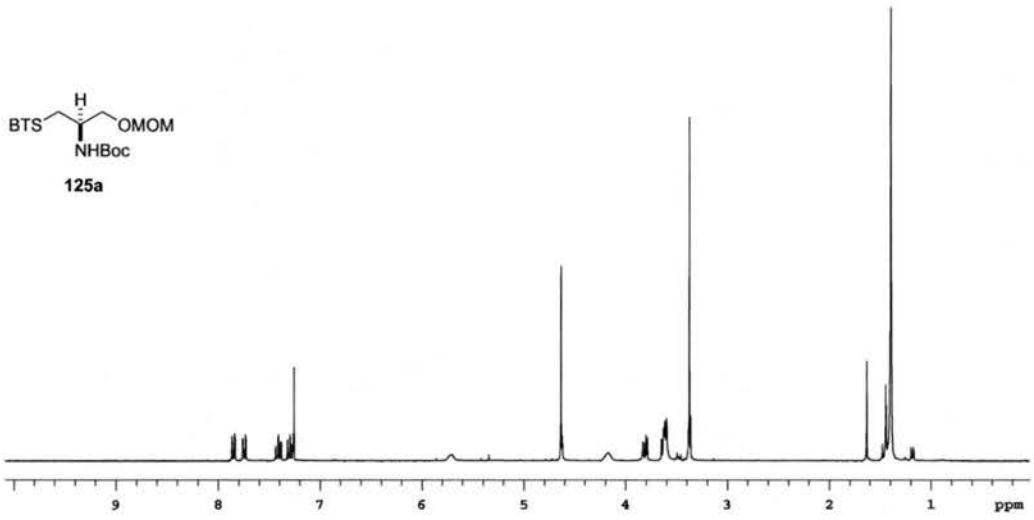
Filename: bka-2-503-1



124a

MOM-ether 125. To BT-cysteine derivative **124a** (199 mg, 0.54 mmol) in dry THF (7 mL) under Ar at –78°C was added DIBAL (1.0 M in PhMe, 0.65 mL, 0.65 mmol) and allowed to stir at –78°C for 14 hr. Quenched with 10 mL absolute EtOH and allowed to stir until evolution of H_2 ceased. Concentrated to an oil, took up in 10 mL absolute EtOH, cooled to 0°C and added $NaBH_4$ (103 mg, 2.7 mmol) and allowed to stir for 7 hr. Quenched with 1 M HCl (10 mL), extracted EtOAc (3 x 10 mL), washed brine (10 mL), dried Na_2SO_4, concentrated to an oil. Used without further purification. To the resulting alcohol (156 mg, 0.46 mmol), $^{i}Pr_2NEt$ (0.12 mL, 0.68 mmol) in dry CH_2Cl_2 (10 mL) was added MOM-Cl (58 μL, 0.68 mmol) and allowed to reflux overnight. Concentrated to an oil, took up in EtOAc (30 mL), washed 0.5 M HCl, H_2O, brine, dried Na_2SO_4, filtered, concentrated to an oil. Purified via flash chromatography (silica gel, 2:1 hexanes:EtOAc) to yield **MOM-ether 125** (176 mg, 85 %, two steps) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 1.40 (s, 9H), 3.37 (s, 3H), 3.55-3.64 (m, 2H), 3.62 (dd, *J*=9.8, 5.7 Hz, 1H), 3.82 (dd, *J*=9.8, 3.8 Hz, 1H), 4.18 (m, 1H), 4.63 (s, 2H), 5.70 (br d, 1H), 7.30 (ddd, *J*=7.9, 7.3, 1.2 Hz, 1H), 7.41 (ddd, *J*= 8.1, 7.3, 1.5 Hz, 1H), 7.75 (ddd, *J*=7.9, 1.5, 0.6 Hz, 1H), 7.86 (ddd, *J*= 8.1, 1.2, 0.6 Hz, 1H).

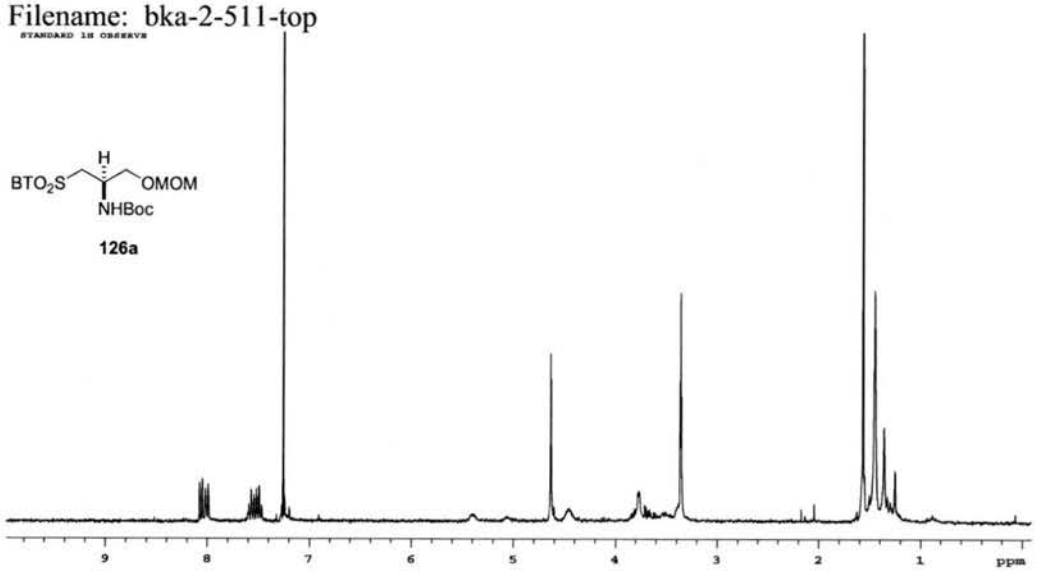
Filename: bka-2-508

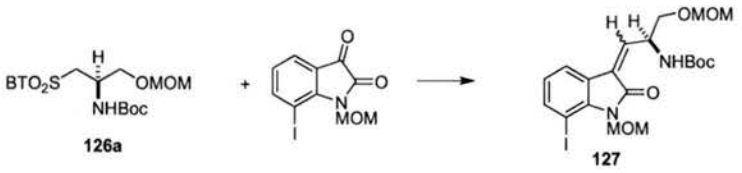


125a **126a**

Sulfone 126a. To a heterogeneous mixture of thioether **125a** (50 mg, 0.13 mmol), $NaHCO_3$ (65.5 mg, 0.78 mmol) and CH_2Cl_2 (5 mL) at room temperature was added *m*CPBA (86 mg, 0.33 mmol, 65%) and allowed to stir overnight. Diluted reaction mixture with CH_2Cl_2 (10 mL) and sequentially washed the organic layer with H_2O (1 x 5 mL), sat. aq. $NaHCO_3$ (1 x 5 mL), sat. aq. $Na_2S_2O_3$ (3 x 5 mL) and brine (1 x 5 mL). Dried organics over anhydrous Na_2SO_4, filtered and concentrated. Purified via flash chromatography (silica gel, 1:1 hexanes:EtOAc) to afford sulfone **126a** (50 mg, 93%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$, 273K) δ 1.45 (s, 9H), 3.36 (s, 3H), 3.51-3.88 (m, 4H), 4.45 (m, 1H), 4.63 (s, 2H), 5.40 (m, 1H), 7.47-7.59 (m, 2H), 8.00 (d, *J*=8.1 Hz, 1H), 8.06 (d, *J*=8.1 Hz, 1H). HRMS (FABH+) calcd for $C_{17}H_{25}N_2O_6S_2$ (*m/z*) 417.1154; found (*m/z*) 417.1159.

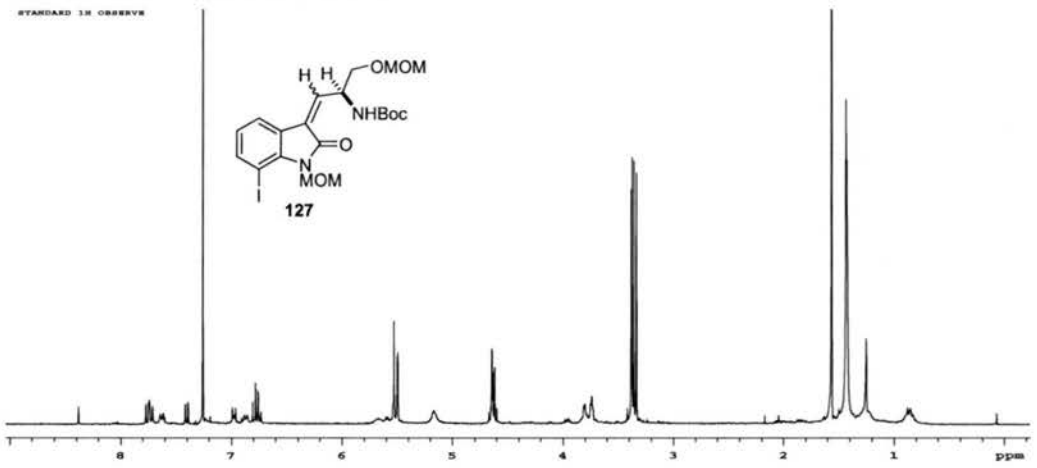
Filename: bka-2-511-top

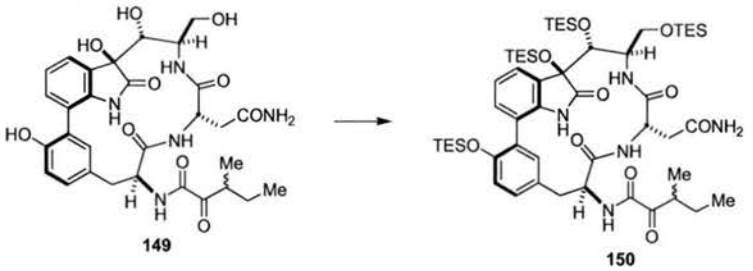




Oxindolene 127. To a solution of sulfone **126a** (21 mg, 0.054 mmol) and *N*-MOM-7-iodoisatin (19 mg, 0.061 mmol) in dry THF (1 mL) at -78°C under argon was added NaHMDS (1.0 M THF, 0.11 mL, 0.11 mmol). Allowed the reaction mixture to stir for about 15 min. then quenched with sat. aq. NH_4Cl. Extracted aqueous with EtOAc (3 x 5 mL) and washed combined organics with water (1 x 5 mL) and brine (1 x 5 mL). Dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purified via PTLC (silica gel, 4:1 hexanes:EtOAc, 2 x) to afford oxindolene **127** as a ~1:1 mixture of *E*:*Z* isomers (yield not determined). ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 1.43 (s, 9H), 3.34-3.38 (4 s, 2 geometric isomers, 6H), 3.78 (m, 2H), 4.64 (m, 2H), 5.17 (br s, 0.5 H), 5.50 and 5.53 (2 s, 2 geometric isomers, 2H), 5.66 (br s, 0.5 H), 6.77 (m, 1H), 6.87 (d, *J*=6.6 Hz, 0.5 H), 6.98 (m, 1H), 7.40 (dd, *J*=7.5, 1.2 Hz, 1H), 7.63 (d, *J*=9 Hz, 0.5 H), 7.74 (dd, *J*=9.9, 7.8 Hz, 1H). HRMS (FABH+) calcd for $C_{20}H_{28}N_2O_6I$ (*m/z*) 519.0992; found (*m/z*) 519.0974.

Filename: bka-2-512-middle



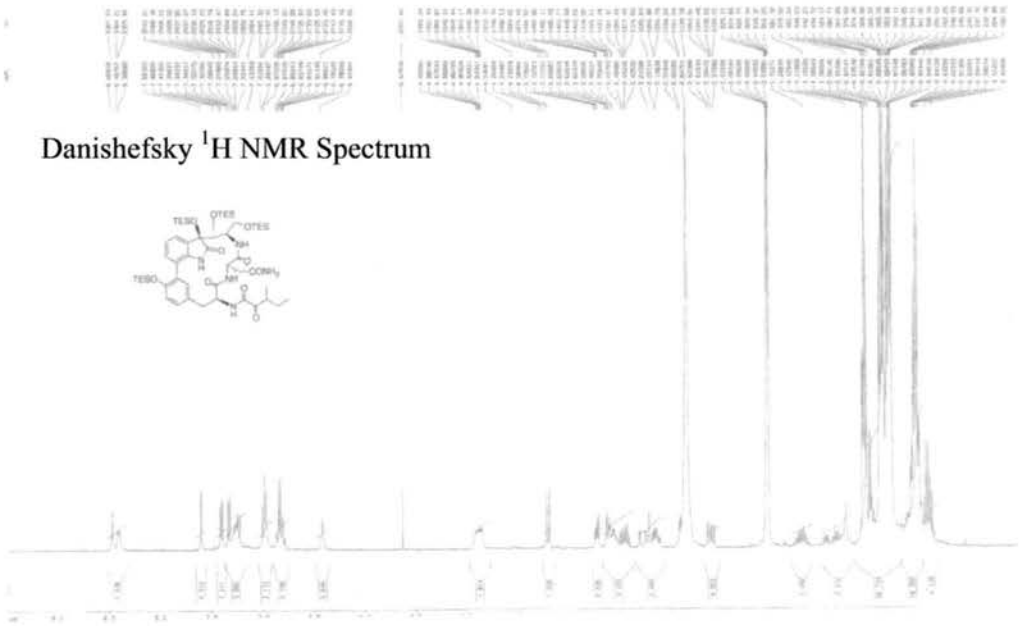


149 150

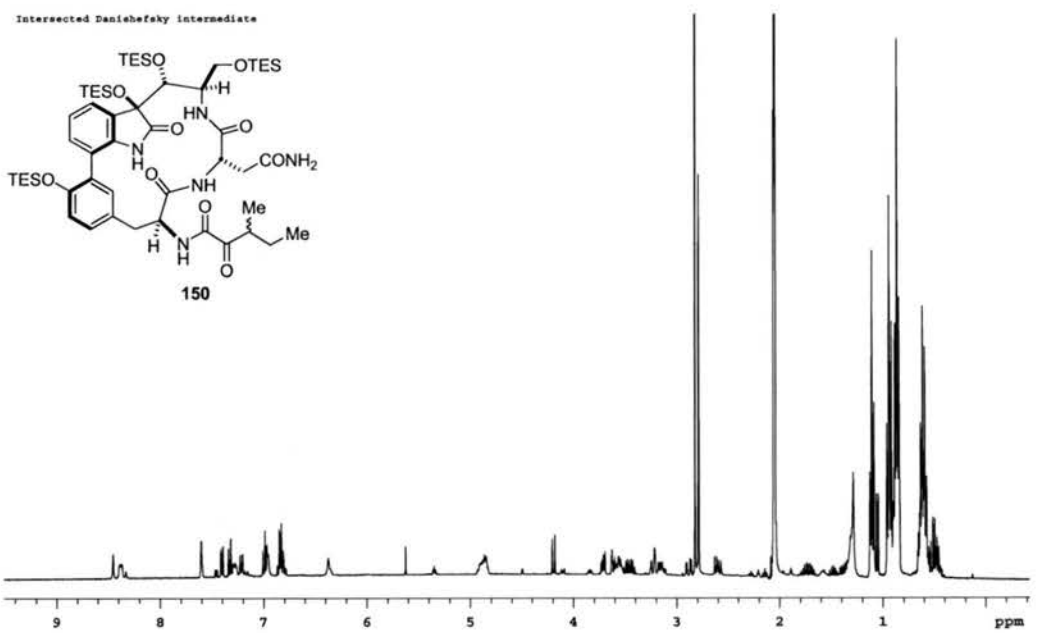
TES-protected macrocycle 150. To macrocycle **149** (3.2 mg, 0.0051 mmol) in dry DMF (0.5 mL) and CH_2Cl_2 (0.5 mL) at 0°C under argon was added 2,6-lutidine (100 μL) followed by TESOTf (200 μL). Allowed the reaction to slowly warm to room temperature and stir for ~18 h. Diluted reaction mixture with MeOH (2 mL) and THF (2 mL) and added enough $NaHCO_3$ to saturate the solution and stirred for 1 h. Concentrated under reduced pressure and reconstituted in EtOAc/H_2O (1:1, 5 mL). Separated organics and extracted aqueous with EtOAc (3 x 3 mL) and washed combined organics with H_2O (1 x 5 mL). The combined organics were placed in a flask along with citric acid (~200 mg) and allowed to stir for 1h. Washed organic layer with H_2O (3 x 5 mL), 9% aq. Na_2CO_3 (1 x 5 mL) and brine (1 x 5 mL). Dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purified via PTLC (silica gel, 25% MeOH in CH_2Cl_2) to afford TES-protected macrocycle (~2 mg, 40%) as a thin oil. The ^1H spectral characteristics of this substance exactly matched those of the ^1H NMR spectrum provided to us by Professor Danishefsky (see below). ^1H NMR (400 MHz, acetone-d_6, 273 K) δ: 0.50-1.14 (m, 66 H), 1.30-1.52 (m, 1H), 1.49 (m, 1H), 1.74 (m, 1H), 2.60 (dd, *J*=15.8, 9.2 Hz, 1H), 2.88 (dd, *J*=15.8, 5.1 Hz, 1H), 3.16 (m, 1H), 3.23 (m, 1H), 3.46 (m, 1H), 3.52-3.64 (m, 1H), 3.72 (dd, *J*=9.8, 5.1 Hz, 1H), 4.20 (d, *J*=10.2 Hz, 1H), 4.87 (m, 2H), 6.38 (br s, 1H), 6.83 (m, 2H), 6.80 (m, 2H), 7.22 (d, *J*=9.6 Hz, 1H), 7.28 (m, 1H), 7.33 (d,

J=7.9 Hz, 1H), 7.40 (d, *J*=7.2 Hz, 1H), 7.61 (s, 1H), 8.38 (m, 1H), 8.46 (br s, 1H). HRMS (FABH+) calcd for $C_{54}H_{92}N_5O_{10}Si_4$ (*m/z*) 1082.5921; found (*m/z*) 1082.5944.

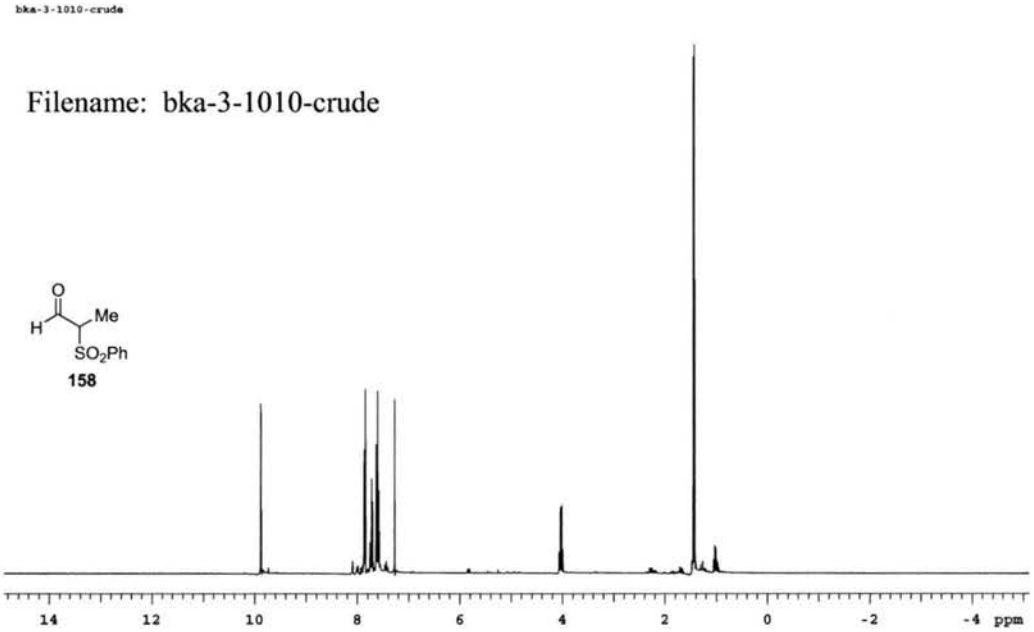
Filename: bka-3-868-3

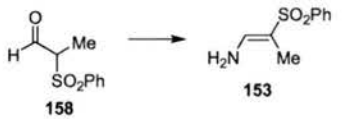


Danishefsky ^{1}H NMR Spectrum



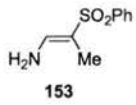
Sulfone 158. To thioether **157**[56] (112 mg, 0.67 mmol) and NaH_2PO_4 (420 mg, 2.96 mmol) in CH_2Cl_2 (10 mL) at room temperature was added *m*CPBA (270 mg, 1.48 mmol, ~95%). The mixture was allowed to stir for 1 h then quenched with sat. aq. $Na_2S_2O_3$ (5 mL) and allowed the resulting mixture to stir for 10 min. Separated the two layers and extracted the aqueous layer with CH_2Cl_2 (3 x 10 mL). Washed combined organics with sat. aq. $NaHCO_3$ (1 x 10 mL) and brine (1 x 10 mL). Dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford sufone **158** (133 mg, 100%) as a colorless oil that solidified upon standing. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 1.44 (d, *J*=7.0 Hz, 1H), 4.03 (dq, *J*=7.0, 1.5 Hz, 1H), 7.60 (m, 2H), 7.21 (m, 1H), 7.85 (m, 2H), 9.88 (d, *J*=1.5 Hz, 1H). IR ($CHCl_3$ film): 3457, 3063, 1731, 1445, 1306, 1148, 1076, 697.

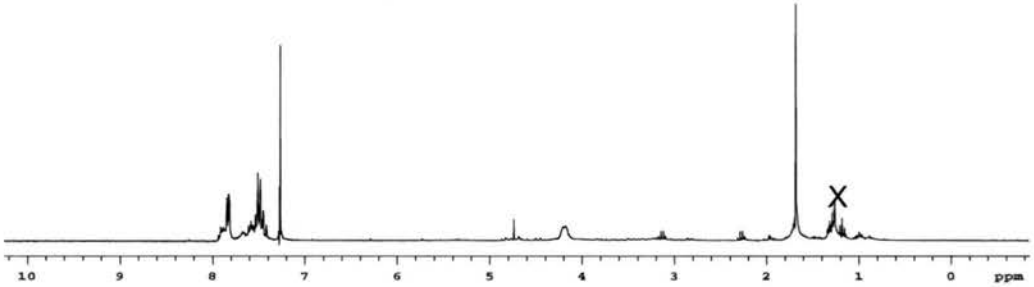


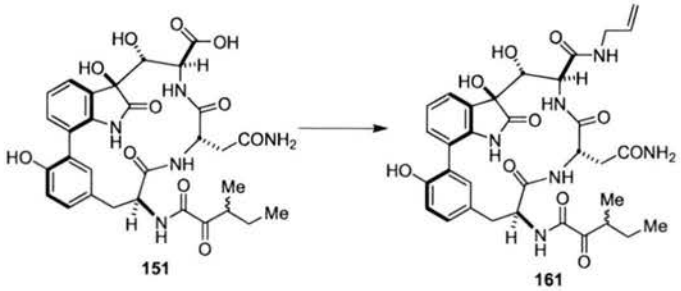


Enamine 153. A mixture of sulfone aldehyde **158** (24 mg, 0.12 mmol) and alumina (123 mg, 1.2 mmol) in CH_2Cl_2 was cooled to -78° C in a sealed tube. Anhydrous $NH_{3(g)}$ was bubbled through the mixture for about 5 min. at which time the tube was sealed and allowed to warm to room temperature while stirring for 14 h. The mixture was then cooled to -78°C and the screw cap was removed. The mixture was slowly allowed to warm to room temperature allowing the $NH_{3(g)}$ to escape. The alumina was removed by filtration through Celite ® and the resultant mixture was concentrated to afford enamine **153** (yield not determined) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 1.68 (s, 3H), 4.22 (br d, *J*=8.7 Hz, 2H), 7.50 (m, 4H), 7.83 (m, 2H). IR ($CHCl_3$ film): 3368, 2923, 1651, 1446, 1723, 1132, 1072. HRMS (FABH+) calcd for $C_9H_{12}N_1O_2S_1$ (*m/z*) 198.0589; found (*m/z*) 198.0587.

Filename: bka-3-101102-crude

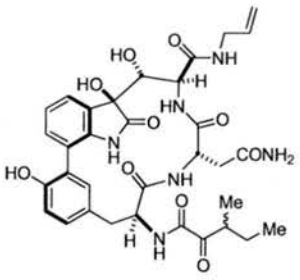






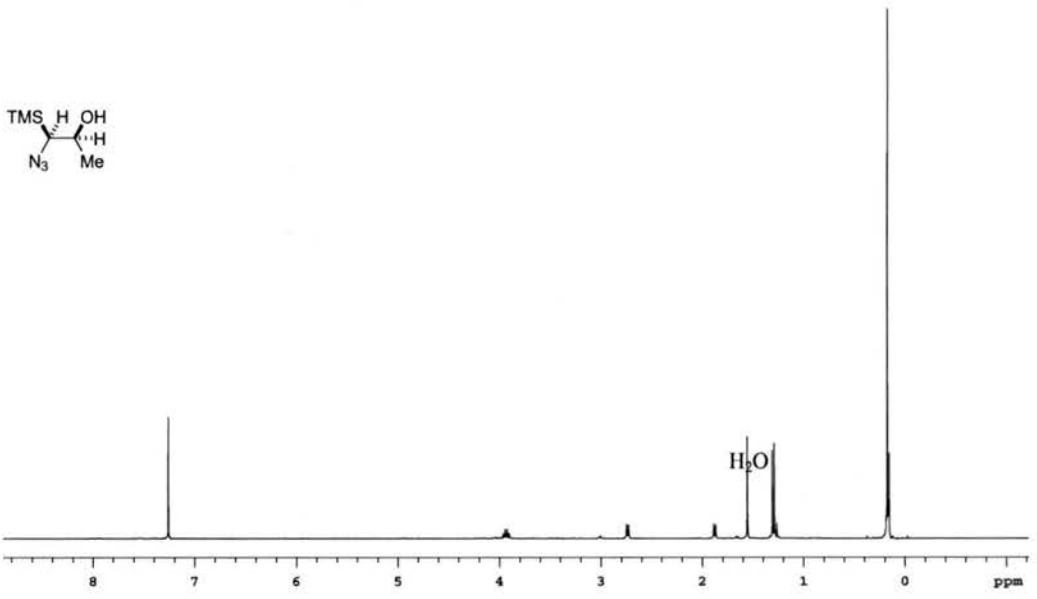
Allyl amide 161. Crude macrocyclic acid (from the oxidation of 3.1 mg, 0.00496 mmol of alcohol **149**), EDCI (1.5 mg, 0.0074 mmol), HOAt (1 mg, 0.0074 mmol) were taken up in dry DMF (0.5 mL) and dry CH_2Cl_2 (0.5 mL) and cooled to 0°C under argon. To the resulting solution was added allylamine (0.6 μL, 0.0074 mmol) and allowed to stir for 3 h. Concentrated to a yellow oil under reduced pressure and purified via PTLC (silica gel, 25% MeOH in CH_2Cl_2) to afford allyl amide **161** (2 mg, 64% from **149**) as a thin film. ^{1}H NMR (300 MHz, acetone-d_6 and D_2O , 273K) δ: 0.80 (t, *J*=7.7, diastereomeric H38), 0.86 (t, *J*=7.7, diastereomeric H38), 1.00 (d, *J*=7.0, diastereomeric H39), 1.06 (d, *J*=7.0, diastereomeric H39), 1.25-1.45 (m, diastereomeric H37) 1.68 (m, diasteromeric H37), 2.57 (dd, *J*=15.4, 9.5 Hz, 1H), 2.69 (dd, *J*=15.4, 4.8 Hz, 1H), 3.07 (m, 1H), 3.18 (m, 1H), 3.40 (m, diasteromeric H36), 3.73 (d, *J*=5.1 Hz, 2H), 4.02 (d, *J*=10.6 Hz, 1H), 4.34 (d, *J*=10.6 Hz, 1H), 4.81 (m, 2H), 4.98 (dd, *J*=10.3, 1.5 Hz, 1H), 5.12 (dd, *J*=17.2, 1.5 Hz, 1H), 5.75 (ddd, *J*=17.2, 10.3, 5.1 Hz, 1H), 6.73 (dt, *J*=8.1, 2.2 Hz, 1H), 6.86 (m, 1H), 6.99 (t, *J*=7.7 Hz, 1H), 7.25 (d, *J*=2.2 Hz, 1H), 7.30 (dd, *J*=7.7, 1.1 Hz, 1H), 7.46 (dd, *J*=7.3, 1.1 Hz, 1H).

Filename: bka-3-992-1



Azido alcohol. To a solution of epoxide[63] (572 mg, 4.4 mmol) and NH_4Cl (518 mg, 9.68 mmol) in MeOH:H_2O (4:1, 50 mL) at room temperature was added NaN_3 (1.14 g, 17.6 mmol) and allowed to stir for 16 h. Quenched reaction with sat. aq. NH_4Cl (10 mL). Removed volatiles under reduced pressure and extracted aqueous layer with Et_2O (3 x 30 mL). The combined organics were washed with H_2O (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purified via flash chromatography (silica gel, 4:1 hexanes:EtOAc) to afford the corresponding azido alcohol (503 mg, 66%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$, 273K) δ: 0.17 (s, 9H), 1.30 (d, *J*=6.2 Hz, 3H), 1.88 (d, *J*=5.9 Hz, 1H), 2.74 (d, *J*=5.9 Hz, 1H), 3.94 (ddq, *J*=6.2, 5.9, 5.9 Hz, 1H).

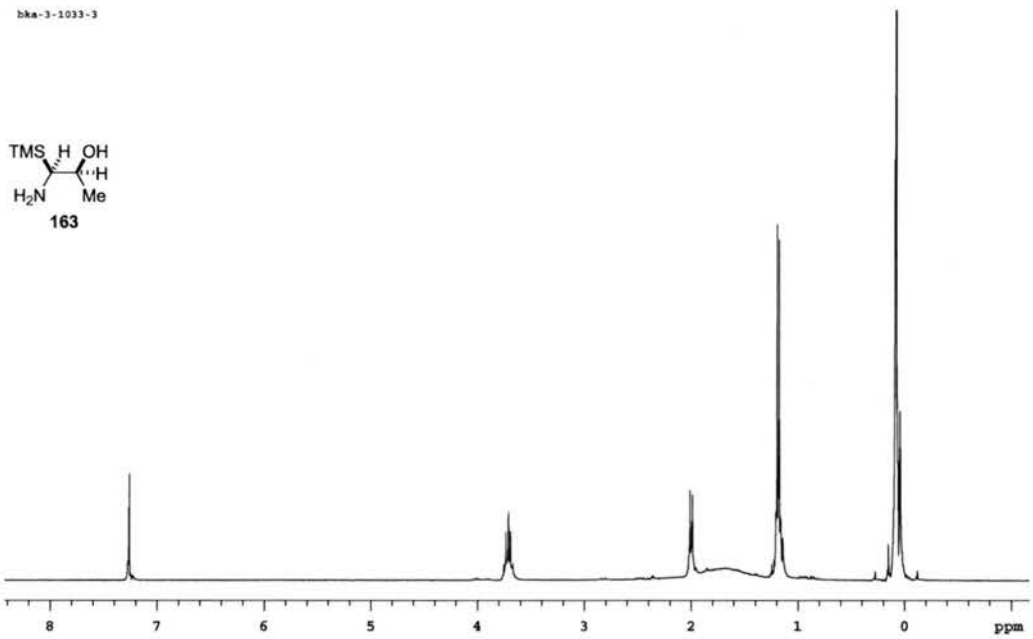
Filename: bka-3-1019-2-pure-1



163

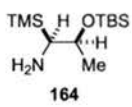
Amino alcohol 163. To lithium aluminum hydride (220 mg, 5.8 mmol) in dry THF (30 mL) at room temperature was added a solution of azide (503 mg, 2.9 mmol) in dry THF (5 mL) slowly over 10 min. The resulting mixture was allowed to stir at room temperature for 3h. Slowly added the following sequentially: H_2O (0.22 mL), aq. NaOH (15%, 0.22 mL) and H_2O (0.66 mL). The mixture was allowed to stir until no gray solid persisted (~1 h). Filtered mixture through Celite® and washed white cake with THF (5 mL). Removed volatiles in vacuo to afford amino alcohol **163** (427 mg, 100%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ: 0.08 (s, 9H), 1.18 (d, *J*=6.0 Hz, 3H), 1.42-1.84 (br s, 3H), 2.00 (d, *J*=6.3 Hz, 1H), 3.71 (dq, *J*=6.3, 6.0 Hz, 1H).

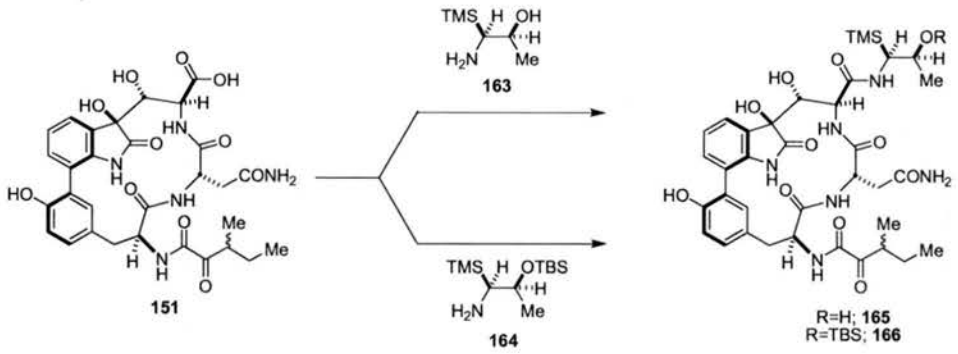
Filename: bka-3-1033-1



Amine 164. To a solution of amine **163** (100 mg, 0.68 mmol), imidazole (139 mg, 2.04 mmol) in dry CH_2Cl_2 (1 mL) under argon at 0°C was added TBSCl (104 mg, 0.68 mmol). The mixture was allowed to warm to room temperature while stirring for 17 h. Removed volatiles in vacuo then reconstituted in EtOAc (50 mL). Washed organic layer with 1 M NaOH (4 x 5 mL) and brine (1 x 10 mL). Dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purified via flash chromatography (silica gel, 3:1 hexane:Et_2O→3:1 Et_2O:hexanes→100% Et_2O) to afford TBS protected amino alcohol **164** (133 mg, 75%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$, 273K) δ: 0.07 (m, 15 H), 0.89 (s, 9H), 1.15 (d, *J*=6.3 Hz, 3H), 1.42 (br, s, 2H), 2.07 (d, *J*=6.9 Hz, 1H), 3.82 (dq, *J*=6.9, 6.3 Hz, 1H).

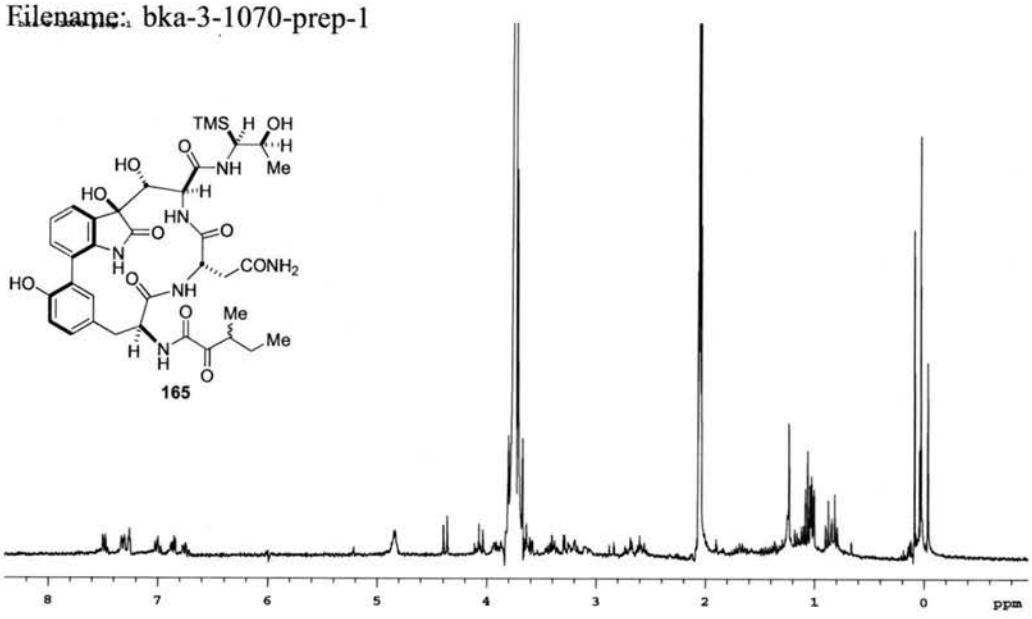
Filename: bka-3-1048-1





General procedure for the preparation of amides 165 and 166. Crude Carboxylic acid **151** (1 eq), amine **163** or **164** (1.5 eq), EDCI (2 eq) and HOAt (2 eq) were taken up in dry DMF (0.5 mL) and dry CH_2Cl_2 (0.5 mL) at 0°C under argon. The mixtures were allowed to stir at ~4°C (cold room) under argon for 22 h. Removed volatiles under reduced pressure. Reconstituted in reconstituted in H_2O (5 mL) and ran through a Waters Sep-Pak C18 cartridge. Rinsed with H_2O (3 x 5mL) and collected the desired compound by eluting with MeOH (4 x 5mL) and concentrating. Purified via PTLC (silica gel, 15% MeOH in CH_2Cl_2) to afford amids **165** and **166** as thin films (was never able to get 100% purity. Usually accompanied by amines **164** and **165**. Yields ranges from ~30-35 % from alcohol **149**). See below for ^{1}H NMR (300 MHz, acetone-d_6 and D_2O , 273K).

Filename: bka-3-1070-prep-1



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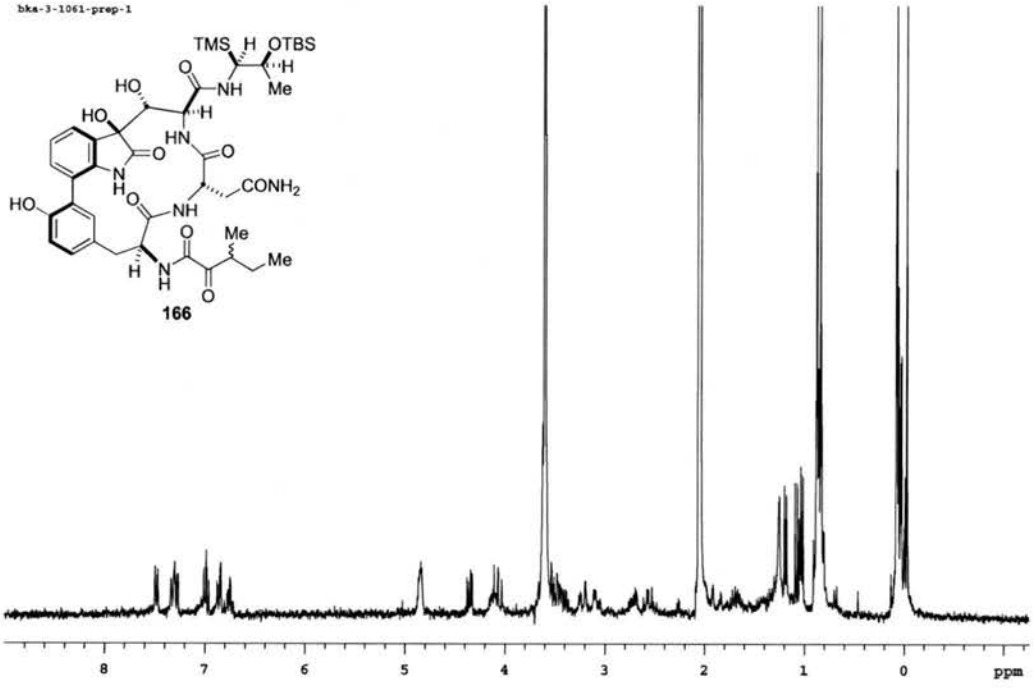

Appendix 1. Publications

Pergamon

Tetrahedron Letters 42 (2001) 2755–2757

TETRAHEDRON LETTERS

Entry into the bi-aryl moiety of the TMC-95 proteasome inhibitors via the Stille protocol

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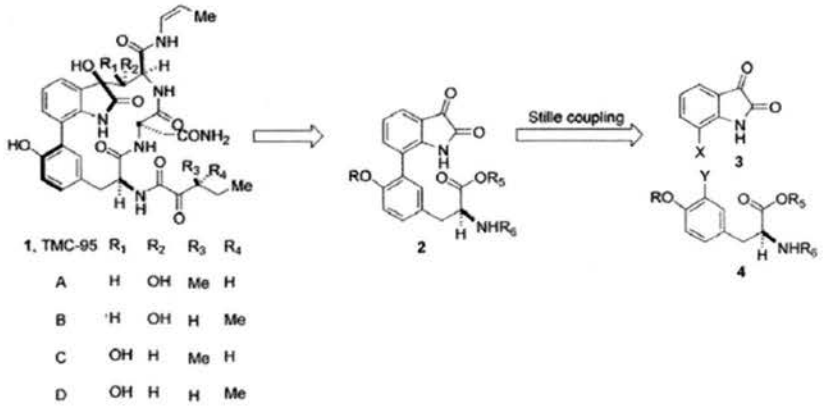
Abstract—The synthesis of the bi-aryl moiety of the TMC-95 natural products has been achieved via a palladium-catalyzed Stille cross-coupling reaction of an aryl stannane tyrosine derivative and 7-iodoisatin.

TMC-95 A–D are potent proteasome inhibitors isolated from the fermentation broth of *Apiosopora montagnei* Sacc. TC 1093 by Kohno and co-workers.[1] These natural products are unique cyclic peptides containing L-tyrosine, L-asparagine, a highly oxidized L-tryptophan-derived oxindole, (*Z*)-1-propenylamine, and 3-methyl-2-oxopentanoic acid units. It has been shown that these compounds are biologically active against chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptide hydrolysing proteases.[1] Recently, proteasome inhibitors have received considerable attention due to the critical role they play in intracellular processes such as cell progression, antigen presentation, and cytokine-stimulated signal transduction.[2] The great interest emerging in the field of proteasome inhibition, the considerable biological activity, and the distinctive structures of the TMC-95s have provided motivation to contemplate a total synthesis of these compounds that would be readily adaptable to preparing analogs.

When contemplating the synthesis of the TMC-95s, it was envisioned that the biaryl moiety could best be constructed via a palladium-catalyzed cross-coupling reaction.[3] When considering coupling partners, we envi-



1, TMC-95	R_1	R_2	R_3	R_4
A	H	OH	Me	H
B	H	OH	H	Me
C	OH	H	Me	H
D	OH	H	H	Me

Figure 1.

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PII: S0040-4039(01)00299-4

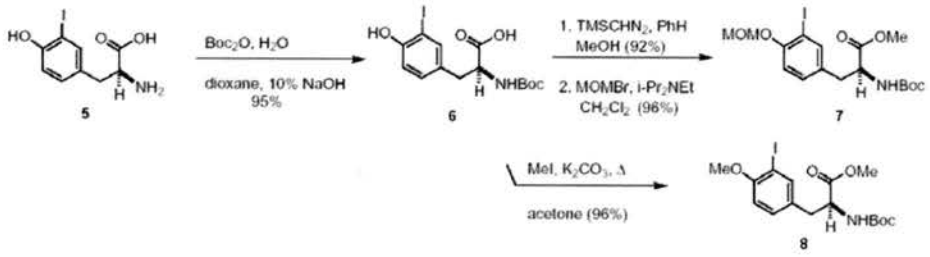
sioned coupling two readily available, chemically manipulative fragments such as 7-iodoisatin **3** (X = I) and a 3-stannyltyrosine derivative **4** (Y = SnR'_3, Fig. 1). It is conceivable that any type of biaryl coupling[4] method could lead to the biaryl portion, but in our hands the Stille coupling protocol proved to be both compatible with other functionality and high yielding. Herein we report the entrance into the biaryl moiety of the TMC-95 natural products via a palladium-catalyzed Stille cross-coupling reaction.[5]

7-Iodoisatin was prepared from 2-iodoaniline via the Sandmeyer procedure.[6] In order to obtain the appropriate aryl stannane necessary for the Stille coupling, manipulations were made to commercially available 3-iodotyrosine **5**. The free amino acid was first protected to give the *N*-Boc amino acid **6**. Next, we decided to explore several protecting group alternatives to determine the effect of the blocking groups on the coupling yields. First, compound **6** was subjected to $TMSCHN_2$, followed by bromomethyl methyl ether and *i*-Pr_2NEt to furnish fully protected MOM-ether-3-iodotyrosine-O-Me ester **7**. Secondly, compound **6** was treated with 2.2 equiv. of MeI and 1.5 equiv. of K_2CO_3 in refluxing acetone to give the *O*-methyl ether of 3-iodotyrosine-O-Me ester **8** (Scheme 1).

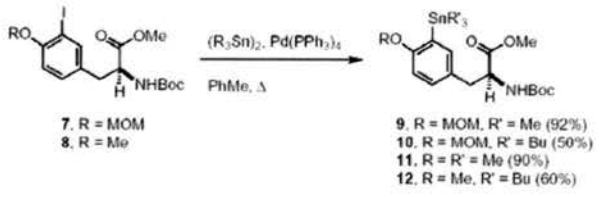
The aryl iodides were converted to the corresponding aryl stannane by treatment with either hexamethylditin or hexabutylditin and $Pd(PPh_3)_4$ in refluxing toluene (Scheme 2).[7]

With both coupling partners in hand, we focused our attention on optimising the Stille coupling conditions. Stille coupling of 7-iodoisatin and aryl stannane (Scheme 3) was attempted under several conditions, as summarized in Table 1.

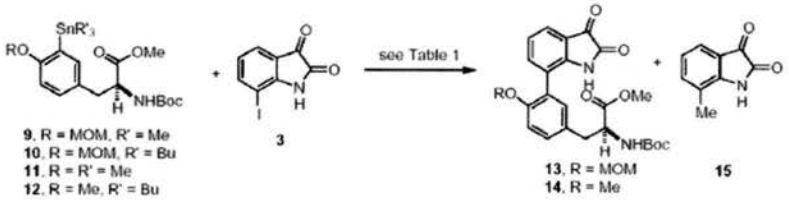
We had initially envisioned using the aryl trimethylstannane derivatives due to the fact that access to these compounds was accompanied by very high yields. In the event, coupling experiments demonstrated that there was competitive methyl group transfer to 7-iodoisatin, especially in polar solvents such as DMF (Table 1, entries 1, 2, 5, and 6). It has been shown that aryl stannanes with *ortho*-substituents have significant



Scheme 1.



Scheme 2.



Scheme 3.

Table 1. Reaction conditions for the Stille coupling outlined in Scheme 3

Entry	R	R′	Conditions	Product, ratio	Yield (%)
1	MOM	Me	$Pd(PPh_3)_2Cl_2$,[a] DMF, LiCl, 100°, 20 h	**13:15**, 1:1	<5
2	MOM	Me	$Pd(dppf)Cl_2$,[a] DMF, LiCl, 100°, 5.5 h	**13:15**, 1:1	<5
3	MOM	Me	$Pd(PPh_3)_2Cl_2$,[a] THF, CuI, reflux, 24 h	No reaction	–
4	MOM	Me	$Pd(dppf)Cl_2$,[a] dioxane, CuI, reflux, 6 h	13	<2
5	MOM	Me	$Pd(PhCN)_2Cl_2$,[b] DMF, $AsPh_3$, CuI, 100°, 1 h	**13:15**, 1:1	18
6	Me	Me	$Pd(dppf)Cl_2$,[b] DMF, CuI, dppf, 100°, 10 h	**14:15**, 1:1	6
7	Me	Bu	$Pd(dppf)Cl_2$,[b] DMF, CuI, 100°, 2.5 h	14	15
8	Me	Bu	$Pd(PhCN)_2Cl_2$,[b] DMF, $AsPh_3$, CuI, 3 h	14	20
9	Me	Bu	$Pd(dppf)Cl_2$,[a] MeCN, CuBr, reflux, 7.5 h	14	80[d]
10	Me	Bu	$Pd(dppf)Cl_2$,[a] PhMe, CuI, reflux, 24 h	No reaction	
11	MOM	Bu	$Pd(dppf)Cl_2$,[c] MeCN, CuBr, reflux, 24 h	13	68
12	MOM	Bu	$Pd(PPh_3)_2Cl_2$,[c] MeCN, CuBr, reflux, 48 h	13	57

[a] 5 mol% catalyst.
[b] 10 mol% catalyst.
[c] 7 mol% catalyst.
[d] Based on recovered starting material, 60% otherwise.

methyl group transfer, with phenyl transfer being only five times faster than methyl transfer.[5] These results made us turn our attention to preparing the tributylstannanes as potential coupling partners. We observed that, although the yields for incorporation of the tributylstannane are lower, the coupling reactions proceed much more smoothly, with no butyl transfer observed. It was also seen that solvents played an important role in the coupling with, MeCN being better than DMF, whereas THF, dioxane, and toluene displayed virtually no reaction at all. As expected, we were unable to observe any significant difference between the two phenolic protecting groups used.

In summary, we have found that a palladium-catalyzed Stille cross-coupling reaction between a tri-*n*-butylstannane derivative of tyrosine and 7-iodoisatin is an efficient method for the synthesis of the biaryl moiety of the TMC-95 class of natural products. Studies to apply this method for the construction of the TMC-95 proteasome inhibitors and select analogs is currently in progress in these laboratories.

Acknowledgements

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1. (a) Khono, J.; Koguchi, Y.; Nishio, M.; Najao, K.; Juroda, M.; Shimizu, R.; Ohmuki, T.; Komatsubara, S. *J. Org. Chem.* **2000**, *65*, 990; (b) Koguchi, Y.; Khono, J.; Nishio, M.; Takahashi, K.; Okuda, T.; Ohmuki, T.; Komatsubara, S. *J. Antibiot.* **2000**, *53*, 105. For recent work on TMC-95, see: Ma, D.; Wu, Q. *Tetrahedron Lett.* **2000**, *41*, 9089.
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ORGANIC LETTERS

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A Concise Formal Total Synthesis of TMC-95A/B Proteasome Inhibitors

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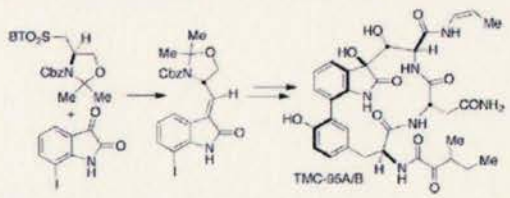
ABSTRACT



A formal total synthesis of proteasome inhibitors TMC-95A/B is described. The synthesis features a stereoselective modified Julia olefination and a diastereoselective dihydroxylation to construct the highly oxidized tryptophan residue.

TMC-95 A–D (**1**–**4**, Figure 1) are potent proteasome inhibitors isolated from the fermentation broth of *Apiospora montagnei* Sacc. TC 1093, derived from soil samples.[1] These natural products are unique cyclic peptides containing L-tyrosine, L-asparagine, a highly oxidized L-tryptophan, (*Z*)-1-propenylamine, and 3-methyl-2-oxopentanoic acid units. It has been demonstrated that these compounds are biologically active against chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptide hydrolyzing proteases.[1b] Proteasome inhibitors have received considerable attention recently due to the role they play in intracellular processes such as cell progression, antigen presentation, and cytokine-stimulated signal transduction. In addition, proteasome inhibitors are proving to be valuable tools for probing the function of the proteasome in cells.[2]

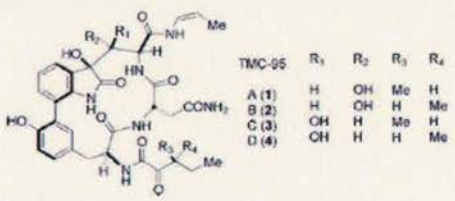


Figure 1. Structures of TMC-95 A–D.

The great interest emerging in the field of proteasome inhibition, the considerable biological activity, and the distinctive structures of the TMC-95 class of natural products have provided motivation to contemplate a total synthesis of these compounds. Recently, it has been determined that TMC-95A displays noncovalent and reversible inhibition of the proteasome, a mode of action not observed until recently with other inhibitors.[3] With this in mind, our goal was to establish a concise and convergent total synthesis that would be ammenable to the preparation of a variety of analogues that could exploit TMC-95s mode of action. Immediately following the publication of the structures of these novel

(1) (a) Khono, J.; Koguchi, Y.; Nishio, M.; Najao, K.; Juroda, M.; Shimizu, R.; Ohnuki, T.; Komatsubara, S. *J. Org. Chem.* **2000**, *65*, 990. (b) Koguchi, Y.; Khono, J.; Nishio, M.; Takahashi, K.; Okuda, T.; Ohnuki, T.; Komatsubara, S. *J. Antibiot.* **2000**, *53*, 105.

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10.1021/ol0272545 CCC: $25.00
Published on Web 12/21/2002

cyclic peptide natural products, significant synthetic activity in this field commenced.[4]

In this paper, we describe a stereocontrolled approach to the core macrocycle of TMC-95A/B. Although the pioneering work of Danishefsky, Hirama, and Ma proved to be an invaluable resource in our synthesis, we felt that the number of synthetic steps[4c] and the lack of stereocontrol in the construction of the oxidized tryptophan moiety had to be addressed.[4a,b,c,f] Our approach is concise and provides a stereocontrolled route to the dihydroxylated oxindole fragment. Also, we have been able to intercept a late-stage intermediate in the Danishefsky synthesis and this report thus constitutes a formal total synthesis of TMC-95A/B.

When contemplating the total synthesis of TMC-95A/B, we felt that these natural products could ultimately be prepared from a macrocylic peptide such as **5** (Figure 2).

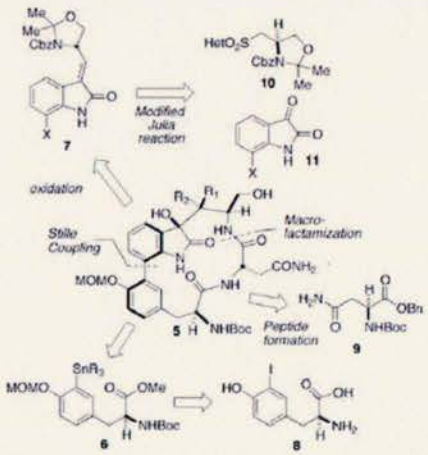
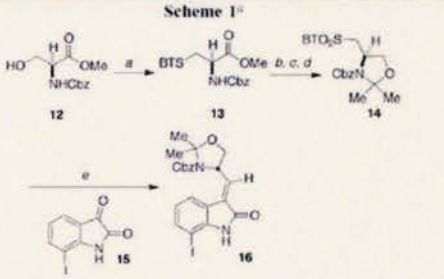


Figure 2. Retrosynthetic Analysis.

Stille coupling[5] of aryl stannane **6** with tryptophan moiety **7** followed by oxidation to the diol, peptide formation with asparagine derivative **9**, deprotection, and macrolactamization was anticipated to furnish the requisite macrocycle **5**. We envisioned that aryl stannane **6** would be derived from commercially available 3-iodotyrosine **8**. Modified Julia[6] olefination of a heteroaromatic sulfone **10** and readily available 7-substituted isatin[7] **11** would in turn furnish oxindolene **7**.

Synthesis of the highly oxidized tryptophan moiety began with treatment of readily available *N*-Cbz-serine methyl ester **12** under Mitsunobu[8] conditions with 2-mercaptobenzathiazole (BTSH), DIAD, and PPh_3 to furnish *S*-heteroaromatic cysteine derivative **13** (Scheme 1). Completion of the

Scheme 1[a]



[a] Reaction conditions: (a) BTSH, DIAD, PPh_3, THF, rt, 89%; (b) $CaCl_2$, $NaBH_4$, THF, 0 °C, and then **13**, 95%; (c) 2,2-dimethoxypropane, *p*-TsOH, CH_2Cl_2, rt; (d) $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, H_2O_2, EtOH, 77%, two steps; (e) LiHMDS, DMF, DMPU, 0 °C, 79%, *E*:*Z* = 5:1.

modified Julia coupling partner was accomplished by (1) reduction of the methyl ester with $Ca(BH_4)_2$, (2) blocking of the carbamate nitrogen and the primary alcohol as the acetonide with DMP and *p*-toluenesulfonic acid, and (3) oxidation[9] of the thioether to sulfone **14**.

Next, our efforts were focused on optimizing the modified Julia coupling reaction between sulfone **14** and 7-iodoisatin **15**. It was determined that conditions similar to those reported by Jacobsen[10] and co-workers gave the best selectivity in the modified Julia olefination, furnishing the desired oxindolene **16** in 79% yield. By increasing the reaction temperature to 0 °C, we were able to increase the selectivity to 5:1 (*E*:*Z*), yielding the thermodynamically favored product.

With alkene **16** and aryl stannane **6**[4d] in hand, attempts were made at constructing the biaryl moiety of the TMC-95 proteasome inhibitors under the Stille conditions developed earlier in our laboratory.[4d] Despite extensive experimentation, we found that numerous combinations of Pd-catalyst and ligand gave unsatisfactory yields of biaryl product **18**

(4) For synthetic efforts on TMC-95s, see: (a) Lin, S.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 512. (b) Lin, S.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 1967. (c) Inoue, M.; Furuyama, H.; Sakazaki, H.; Hirama, M. *Org. Lett.* **2001**, *3*, 2863. (d) Albrecht, B. K.; Williams, R. M. *Tetrahedron Lett.* **2001**, *42*, 2755. (e) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2001**, *42*, 5279. (f) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2000**, *41*, 9089. (g) Karatjas, A. G.; Feldman, K. S. *Abstracts of Papers*, 223rd National Meeting of the American Chemical Society, Orlando, FL, April 7−11, 2002; American Chemical Society: Washington, DC; ORGN-400. (h) Albrecht, B. K.; Williams, R. M. *Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States, August 18−22*, **2002**, ORGN-819.

(5) (a) Farina, V.; Krishnamurthy, V.; Scott, W. J. **1997**, *50*, 1−652. (b) Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508.

(6) (a) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, 28. (b) Baudin, J. B.; Hareau, G.; Julia, S. A.; Ruel, O. *Tetrahedron Lett.* **1991**, *32*, 1175. (c) Julia, M.; Paris, J.-M. *Tetrahedron Lett.* **1973**, *14*, 4833.

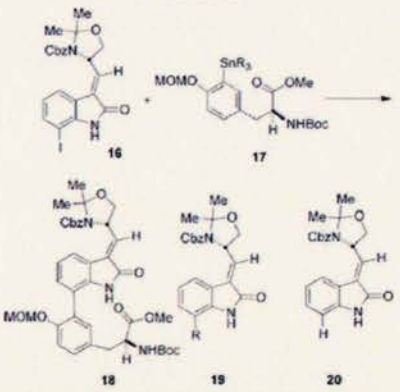
(7) (a) Sandmeyer, T. *Helv. Chim. Acta* **1919**, *2*, 234. (b) Marvel, C. S.; Hiers, G. S. *Organic Syntheses*; Wiley: New York, 1941; Collect. Vol. I, p 327. (c) Lisowski, V.; Robba, M.; Rault, S. *J. Org. Chem.* **2000**, *65*, 4193.

(8) Mitsunobu, O. *Synthesis* **1981**, 1.

(9) Schultz, H. S.; Freyermuth, H. B.; Buc, S. R. *J. Org. Chem.* **1963**, *28*, 1140.

(10) Liu, P.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2001**, *123*, 10772.

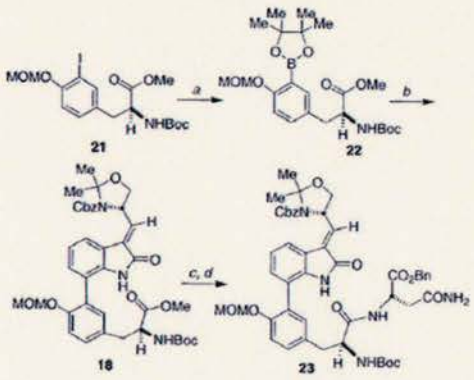
Scheme 2



(Scheme 2). The best isolated yield of coupled product **18** was ~20%, which was routinely accompanied by side-products resulting from alkyl group transfer from the stannane (**19**) and reductive removal of the iodine atom (**20**). Due to the fact that the Stille coupling gave undesired side products and insufficient yields, we decided that the Suzuki[4a,b,11] coupling was the next logical choice for constructing the biaryl bond.

Treatment of tyrosine derivative **21**[4d] with bis(pinacolato)-diboron, $Pd(dppf)Cl_2$, and KOAc in DMSO via the Miyaura protocol[12] gave boronic ester **22** (Scheme 3). Treatment of boronic ester **22** under Suzuki conditions with aryl iodide **16** and K_2CO_3 in refluxing aqueous DME catalyzed by $Pd(dppf)Cl_2$ gave the desired biaryl product **18** in 90% yield.

Scheme 3[a]

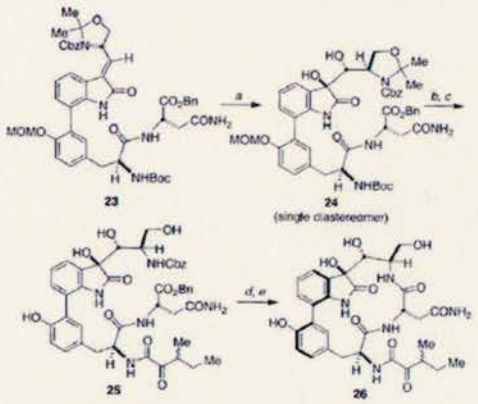


[a] Reaction conditions: (a) bis(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, DMSO, 80 °C, 4 h, (80%); (b) **16**, K_2CO_3, $Pd(dppf)Cl_2$, aqueous DME, 90%; (c) LiOH, THF, H_2O, 0 °C; (d) H_2N—Asn—OBn, HOAt, EDCI, NMM, CH_2Cl_2, 0 °C, 4 h, 98%, two steps.

Incorporation of the asparagine residue was readily accomplished first via saponification of methyl ester **18**. The resulting carboxylic acid was coupled to NH_2—Asn—OBn ester[13] mediated by HOAt and EDCI in CH_2Cl_2 to give the pseudotripeptide **23** (98% yield from **18**).

Treatment of alkene **23** with OsO_4 in aqueous pyridine afforded diol **24** as a single diastereomer in 87% yield (Scheme 4). Treatment of this substance with a 1:1 mixture

Scheme 4[a]



[a] Reaction conditions: (a) OsO_4, py., H_2O, 0 °C, then $NaHSO_3$, THF, MeOH, (87%); (b) TFA, H_2O, 1:1; (c) 3-methyl-2-oxopentanoic acid, HOAt, EDCI, THF, 98%, two steps; (d) Pd black, H_2, EtOH; (e) EDCI, HOAt, CH_2Cl_2, DMF (1:1), 1 μM, 49%, two steps.

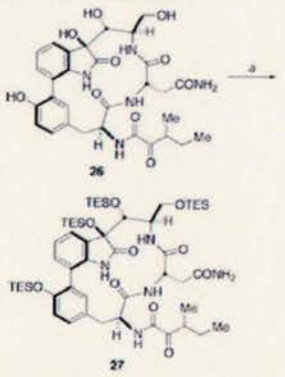
of trifluoroacetic acid—water resulted in the liberation of the acid-labile protecting groups. Coupling of the resultant free amine salt with *d,l*-3-methyl-2-oxo-pentanoic acid gave ketoamide **25** as a mixture of inseparable diastereomers. Since it is known that the ketoamide residue is labile to epimerization,[4] no attempt was made to effect the coupling of either (*R*)- or (*S*)-3-methyl-2-oxo-pentanoic acid because the same diastereomeric mixture would result.

Hydrogenolysis of the benzyloxy carbamate and the benzyl ester residues of **25** produced the requisite amino acid substrate for macrocyclization. Subjecting this substance to EDCI and HOAt afforded the TMC-95 macrocyclic core structure **26** in 49% overall yield from **25**. It was previously known[4a,b] that macrocyclization would only provide the desired atropisomer; therefore, this was of no synthetic concern. The structure of this substance was secured by conversion into a late-stage intermediate reported by Lin and Danishefsky.[4a]

The completion of the formal synthesis was accomplished by treatment of macrocyclic tetraol **26** with TESOTf and

(11) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
(12) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508.
(13) Yoshimura, S.; Miki, M.; Ikemura, H.; Amoto, S.; Shunooishi, Y.; Takeda, T.; Takeda, Y.; Miwatani, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 125.

Scheme 5[a]



[a] Reaction conditions: (a) TESOTf, 2,6-lutidine, CH_2Cl_2, DMF, from 0 °C to rt, 12 h, ~40%.

2,6-lutidine to afford **27** in approximately 40% isolated yield (Scheme 5). The ^{1}H NMR spectral characteristics of this substance exactly matched those of the ^{1}H NMR spectrum kindly provided to us by Professor Danishefsky (see Supporting Information).[2a]

In summary, we have effectively applied a stereoselective modified Julia olefination reaction, followed by a diastereoselective dihydroxylation and macrocyclization with limited use of protecting group chemistry as key transformations in a concise formal total synthesis of the TMC-95 A/B proteasome inhibitors. It is felt that an efficient total synthesis of TMC-95A/B can be accomplished via elaboration of the unprotected macrocycle **26**. These efforts along with studies focused on preparing novel TMC-95 analogues are currently under investigation in our laboratories.

Acknowledgment. This material is based upon work supported by the National Science Foundation under Grant 0202827 and the National Institutes of Health. We are also grateful to Boehringer-Ingelheim Pharmaceuticals for partial support of this work. Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631. We are indebted to Dr. Jun Kolmo, Tanabe Seiyaku Co., for providing authentic samples of TMC-95A/B that were valuable for spectral comparison. We would also like to thank Prof. Samuel J. Danishefsky (Columbia University, Sloan–Kettering) for providing a ^{1}H NMR spectrum of compound **27**.

Supporting Information Available: Complete spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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A concise, total synthesis of the TMC-95A/B proteasome inhibitors

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A concise, total synthesis of the proteasome inhibitors TMC-95A/B has been accomplished. The synthesis features the use of an L-serine-derived *E*-selective modified Julia olefination reaction to ultimately control the stereochemical outcome of the highly oxidized tryptophan fragment. Additionally, the limited use of protecting groups at a late stage of the total synthesis allowed for its completion in an efficient manner.

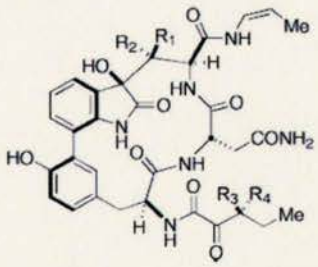
Julia olefination | Suzuki biaryl coupling | Stille coupling

The ubiquitin-proteasome pathway is an ATP-dependent pathway discovered >20 years ago and is the major proteolytic pathway in the cytosol and nucleus of all eukaryotic cells (ref. 1 and references within). Initial studies focused on understanding the importance of this pathway in the regulation of cellular processes and benefited from biological studies in extracts of mammalian cells and genetic studies in yeasts (2). It was not until the development or isolation of cell-permeable proteasome inhibitors that the physiological roles of the proteasome were understood. These findings have shown that the proteasome catalyzes the degradation of the majority of mammalian proteins, both short- and long-lived (3, 4). The proteasomal degradation of a large variety of cellular proteins is vital to many of the intracellular processes such as cell-cycle progression, apoptosis, inflammation, immune surveillance, selective removal of misfolded or damaged proteins, and the regulation of metabolic pathways (ref. 1 and references within). Therefore, specific proteasome inhibitors are of great interest not only for use as a tool for understanding the ubiquitin-proteasome pathway but also as potential drug candidates.

In early 2000, Kohno and coworkers (5) reported the isolation of novel cyclic tripeptides TMC-95A–D (**1–4**) (Fig. 1). TMC-95A–D are potent proteasome inhibitors isolated from the fermentation broth of *Apiospora montagnei Sacc.* TC 1093, derived from soil samples. These natural products are unique cyclic peptides containing L-tyrosine, L-asparagine, a highly oxidized L-tryptophan, (*Z*)-1-propenylamide, and 3-methyl-2-oxopentanoic acid subunits. It has been shown that these compounds are biologically active against the chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptide-hydrolyzing activities of the 20S proteasome. Recently, it was determined that TMC-95A displays noncovalent and reversible inhibition of the proteasome, a mode of action not observed with other inhibitors until recently (6).

The great interest emerging in the field of proteasome inhibition, the considerable biological activity, and the distinctive structures of the TMC-95 class of natural products have provided motivation to contemplate a total synthesis of these compounds that would be readily adaptable to preparing biologically active analogs (7–11). Indeed, immediately after the publication of the structures of these novel cyclic peptide natural products, significant synthetic activity in this field commenced (12, 13, †), resulting in total syntheses being reported by Lin and Danishefsky (14, 15) and Inoue *et al.* (16, 17).

Recently we reported a formal total synthesis of TMC-95A/B by intersecting a late-stage intermediate in the Danishefsky total synthesis (18). In that communication we reported the synthesis



TMC-95	R_1	R_2	R_3	R_4
A (**1**)	H	OH	Me	H
B (**2**)	H	OH	H	Me
C (**3**)	OH	H	Me	H
D (**4**)	OH	H	H	Me

Fig. 1. Structures of TMC-95A–D.

of the unprotected macrocyclic intermediate **5** (Fig. 2), and since that time, we have endeavored to elaborate this substance to TMC-95A/B in an efficient manner. Herein we report the realization of that goal along with a full account of our research program in this arena.

Materials and Methods

General Procedures. Unless otherwise noted, materials were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon by using flame-dried glassware that was cooled under dry argon. Tetrahydrofuran (THF), dimethylformamide (DMF), and toluene were degassed with argon and passed through a solvent-purification system (J. C. Meyer, Glass Contour, Laguna Beach, CA) containing alumina or molecular sieves. Dichloromethane was distilled from CaH_2 before use. Column chromatography was performed on Merck silica gel Kieselgel 60 (230–400 mesh). Mass spectra were obtained on Fisons VG Autospec. HPLC data were obtained on a Waters 600 high-pressure liquid chromatograph. ^{1}H NMR, ^{13}C NMR, and nuclear Overhauser effect (NOE) experiments were recorded on a Varian 300- or 400-MHz spectrometer. Chemical shifts (δ) were given in parts per million and recorded relative to the residual solvent peak unless otherwise noted. ^{1}H NMR were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant (in hertz), and number of protons. When a signal was deemed "broad," it was noted as such. IR spectra were recorded on a Nicolet Avatar 320 Fourier transform IR spectrometer. Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: THF, tetrahydrofuran; DMF, dimethylformamide; NOE, nuclear Overhauser effect; BT, benzothiazole; PT, phenyl tetrazole; LiHMDS, lithium bis(trimethylsilyl)amide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; RT, room temperature; EDCI, ethyl-dimethylaminopropyl carbodiimide hydrochloride; HOAt, 1-hydroxyazabenzotriazole.

*To whom correspondence should be addressed. E-mail: rmw@chem.colostate.edu.

†Karatjas, A. G. & Feldman, K. S., Abstracts of Papers, 223rd American Chemical Society National Meeting, April 7–11, 2002, Orlando, FL, ORGN-400.

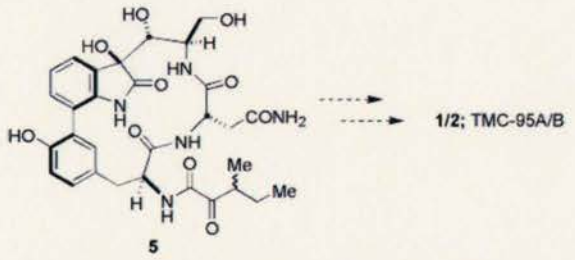


Fig. 2. Strategy to convert tetraol 5 into TMC-95A/B.

Complete experimental procedures and spectroscopic and analytical data including NMR spectra can be found in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

Results and Discussion

Synthetic Plan. Retrosynthetically, we reasoned that macrocycle **5** could be elaborated to TMC-95A/B (Fig. 3) and was derived from the building blocks **6–11**. The highly oxidized tryptophan moiety was envisioned to be installed via a protected oxindolene of type **6**, which could ultimately come from L-serine (**7**) and a 7-substituted isatin **8**. Stille coupling (19) was planned to form the biaryl bond linkage between the oxidized tryptophan and the bottom-half tyrosine portion, which in turn could come from commercially available 3-iodotyrosine (**9**) and 3-methyl-2-oxopentanoic acid sodium salt (**10**). Incorporation of an asparagine residue (**11**) and macrolactamization at the C10–N9 amide bond thus would afford macrocycle **5**.

Total Synthesis of TMC-95A/B. The synthesis of TMC-95A/B began with the preparation of the highly oxidized tryptophan fragment. Initially, it was determined that treatment of 7-iodooxindole **12** [readily prepared from 7-iodoisatin via hydrazine reduction (20)] with either the Garner aldehyde **13** (21, 22) or the L-serine-derived OBO-ester aldehyde **14** (23) under condensation conditions produced oxindolenes **15** and **16** (Scheme 1). Although these results proved to be very promising, Ma and Wu (13) and later Lin and Danishefsky (14, 15) reported a very similar

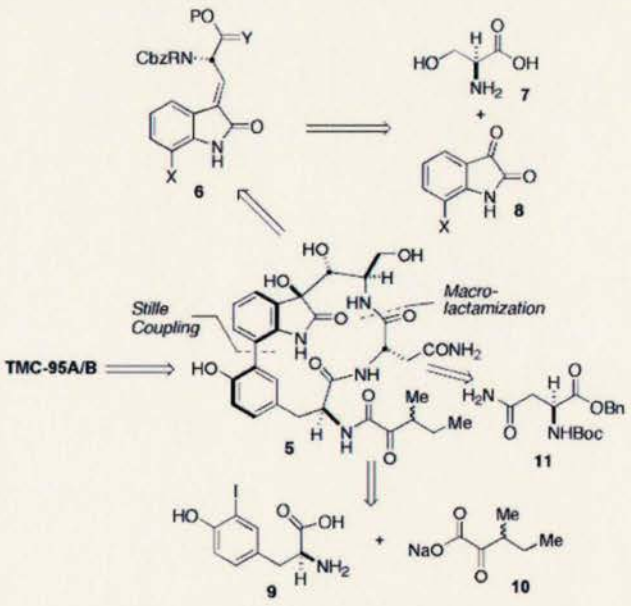
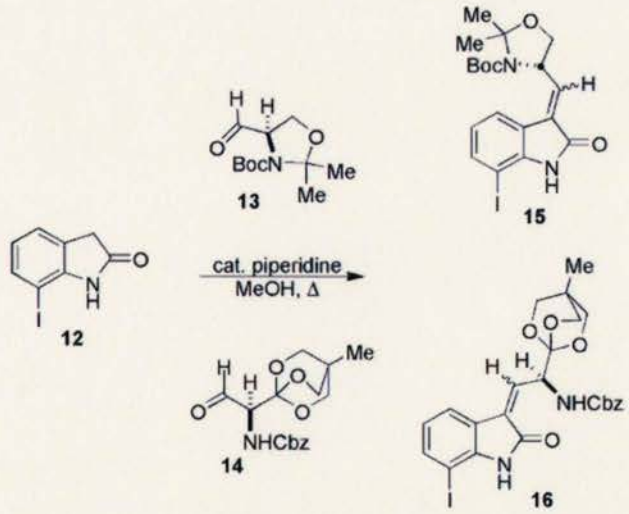


Fig. 3. Retrosynthetic analysis of TMC-95A/B.

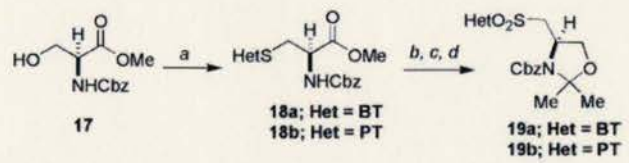


Scheme 1. Initial studies toward the highly oxidized tryptophan fragment.

transformation in their syntheses of the highly oxidized tryptophan fragment. It was therefore decided that an alternate and more efficient route to the highly oxidized tryptophan fragment should be developed.

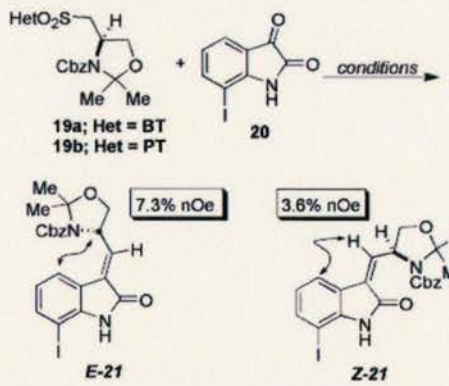
Ultimately, it was found that a modified Julia olefination (24–26) proved to be an effective method to furnish an oxindolene derivative **6**. The synthesis of the highly oxidized tryptophan fragment began with treatment of readily available *N*-benzyloxycarbonyl-L-serine methyl ester (**17**) under Mitsunobu (27) conditions with either 2-mercaptobenzothiazole or 1-phenyl-1*H*-tetrazole-5-thiol, diisopropyl azodicarboxylate, and PPh_3 to furnish *S*-heteroaromatic cysteine derivatives **18** (Scheme 2). Completion of the modified Julia sulfone coupling partners was accomplished by (*i*) reduction of the methyl ester with $Ca(BH_4)_2$, (*ii*) blocking of the carbamate nitrogen and the primary alcohol as the acetonide with 2,2-dimethoxypropane and *p*-toluenesulfonic acid, and (*iii*) oxidation (28) of the thioether to the sulfone **19**. It should be noted that both the benzothiazole (BT) and phenyl tetrazole (PT) sulfone were prepared in like manner and similar yields.

With sulfones **19** in hand, we set out to determine the optimal reaction conditions necessary to couple sulfones **19** with readily available 7-iodoisatin (29–31) **20** that would give both a high-yielding and highly diastereoselective process (Table 1). It was found that conditions similar to those reported by Liu and Jacobsen (32) gave the best selectivity in the modified Julia olefination, furnishing the desired oxindolene **21**. Under the same reaction conditions, we saw that the BT sulfone gave superior selectivity over that of the corresponding phenyltetrazole derivative (Table 1, entry 2 vs. 4). We also noticed that the more thermodynamically stable *E*-isomer can preferentially



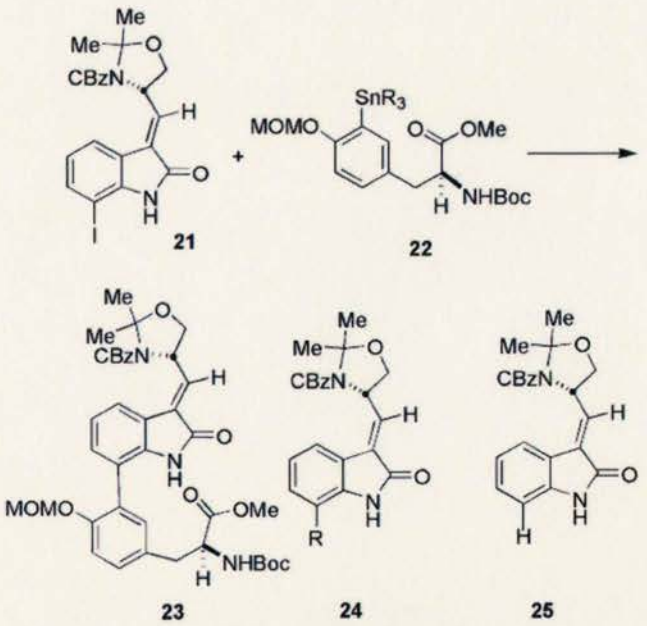
Scheme 2. Preparation of the modified Julia sulfone. Reaction conditions: a, 2-mercaptobenzothiazole, diisopropyl azodicarboxylate, PPh_3, THF, room temperature (RT) (89%); b, $CaCl_2$, $NaBH_4$, THF, 0°C to RT (95%); c, 2,2-dimethoxypropane, *p*-toluenesulfonic acid, CH_2Cl_2, RT; d, $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, H_2O_2, EtOH (77%, two steps).

Table 1. Modified Julia olefination



Entry	Heterocycle (Het)	Conditions*	E/Z ratio†
1	PT	THF, NaHMDS, −78°C	1:1
2	PT	DMF, DMPU, LiHMDS, −45°C	2:1
3	BT	THF, NaHMDS, −78°C	1:1
4	BT	DMF, DMPU, LiHMDS, −78°C	2.5:1
5	BT	DMF, DMPU, LiHMDS, −45°C	3:1
6	BT	DMF, DMPU, LiHMDS, 0°C	5:1

NaHMDS, sodium bis(trimethylsilyl)amide.
*In all cases yields were at least 79%.
†E/Z ratios were determined by ^{1}H analysis of crude product mixtures.



Scheme 3. Attempted Stille coupling.

be prepared with greater selectivity by increasing the reaction temperature. Ultimately the optimized reaction conditions were found to involve treating the BT sulfone **19a** and 7-iodoisatin with lithium bis(trimethylsilyl)amide (LiHMDS) in DMF/1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) (1:1) at 0°C, affording a 5:1 *E*/*Z* ratio of oxindolene **21** (Table 1, entry 6). It was also possible to separate the two isomers and then isomerize the undesired *Z*-isomer to the *E*-isomer under conditions reported by the Danishefsky group (14, 15).

With oxindolene **21** in hand, we considered numerous synthetic strategies that could be used to complete the total synthesis. Of those contemplated, two disconnections involving either C6–C7 oxidation to the diol or biaryl formation were seriously considered. Because our laboratory had previously developed a Stille coupling protocol for the preparation of a simplified TMC-95 biaryl (33) and studies have shown that the C6–C7 diol is somewhat labile (34, ‡), it was decided to form the biaryl bond before installation of the C6–C7 diol.

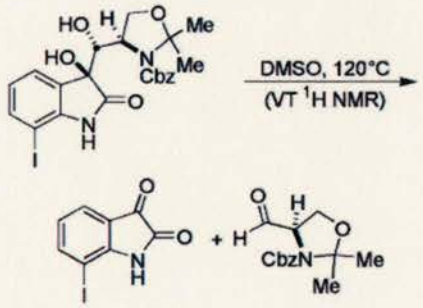
With aryl iodide **21** and aryl stannane **22** (33) in hand, attempts were made at constructing the biaryl moiety of the TMC-95 proteasome inhibitors under the Stille conditions discussed earlier. Despite extensive experimentation, we found that numerous combinations of Pd-catalyst and ligand gave unsatisfactory yields of the biaryl product **23** (Scheme 3). The best isolated yield of coupled product **23** was ≈20%, which was routinely accompanied by side products resulting from alkyl group transfer from the stannane (**24**) and reductive removal of the iodine atom (**25**). Because of the fact that the Stille coupling gave undesired side products and insufficient yields, we decided that the Suzuki (35) coupling protocol was the next logical choice for constructing the biaryl bond.

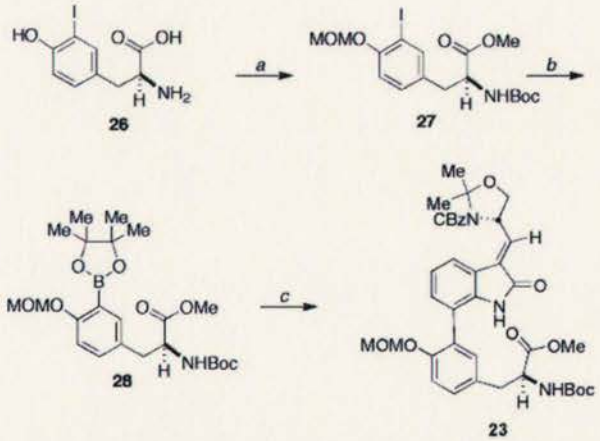
Preparation of the requisite boronic ester necessary for the Suzuki coupling began with the protection of commercially available 3-iodo-L-tyrosine **26**. Subjection of 3-iodo-L-tyrosine **26** to (*i*) thionyl chloride in methanol, (*ii*) di-*tert*-butyldicarbonate, and (*iii*) chloromethyl methyl ether and diisopropylethylamine afforded the fully protected tyrosine derivative **27** in near quantitative yield (Scheme 4). Conversion of the aryl iodide in **27** to the boronic ester **28** was accomplished via the Miyaura protocol (36). Treatment of boronic ester **28** under Suzuki conditions with aryl iodide **21** and K_2CO_3 in refluxing aqueous dimethoxyethane catalyzed by dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium smoothly installed the biaryl linkage yielding **23** in 90% yield.

Saponification of the methyl ester in **23** allowed for amide bond formation between the resulting carboxylic acid and L-asparagine benzyl ester mediated by ethyl-dimethylaminopropyl carbodiimide hydrochloride (EDCI) and 1-hydroxyazabenzotriazole (HOAt) to yield pseudotripeptide **29** in 98% yield over the two steps (Scheme 5). Pseudotripeptide **29** constitutes the complete carbon framework for the macrocyclic core. It is significant to note that the judicious choice of protecting groups has allowed for complete removal of all protecting groups in two simple transformations. With pseudotripeptide **29** in hand, we found that this was the ideal juncture in the synthesis for the oxidation to the C6–C7 diol. Subjection of **29** to OsO_4 in pyridine at 0°C allowed for the oxidation of the C6–C7 double bond with complete facial selectivity opposite to the allylic carbamate yielding diol **30** in 87% yield as a single diastereomer with the correct relative configuration.

At this stage, we decided to remove all the acid labile protecting groups, liberating the C14 amine, the C25 primary

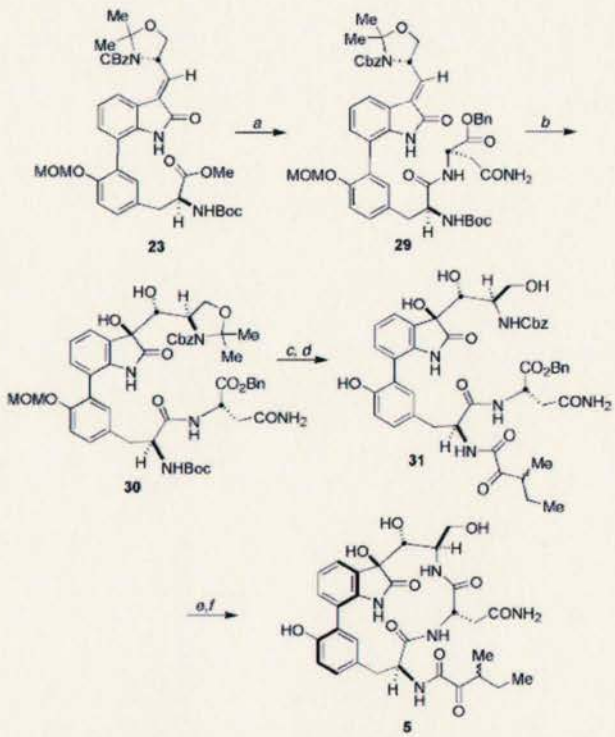
‡^{1}H NMR at 393 K revealed the following retro-aldol cleavage:



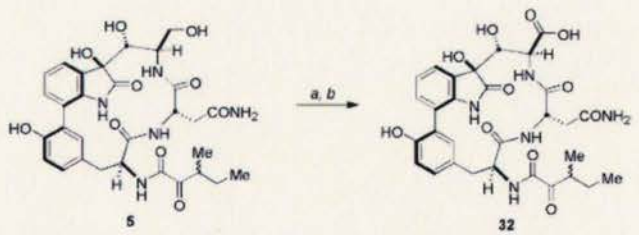


Scheme 4. Suzuki biaryl formation. Reaction conditions: a1, $SOCl_2$, MeOH, RT, 18 h; a2, di-*tert*-butyldicarbonate, saturated $NaHCO_3$, CH_2Cl_2, 0°C → RT, ≈12 h; a3, chloromethyl methyl ether, diisopropylethylamine, CH_2Cl_2, 0°C, 3 h, 95% (three steps); b, bis(pinacolato)diboron, KOAc, dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium, DMSO, 80°C, 4 h, 80–89%; c, **21**, K_2CO_3, dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium, aqueous dimethoxyethane, Δ 90%.

alcohol, and the C19 phenol with trifluoroacetic acid/H_2O (1:1) (Scheme 5). Although we realized that the four free alcohols may prove to be problematic in both the incorporation of the ketoamide and the macrocyclization, the potential payoff in



Scheme 5. Preparation of the macrocyclic core. Reaction conditions: a1, LiOH, THF, H_2O, 0°C; a2, H_2N-Asn-OBn, HOAt, EDCI, diisopropylethylamine, CH_2Cl_2, 0°C, 4 h (98%, two steps); b, OsO_4, pyridine, 0°C, 1 h, and then saturated $NaHSO_3$, 87%; c, trifluoroacetic acid/H_2O (1:1), RT, 4 h; d, 3-methyl-2-oxopentanoic acid sodium salt, HOAt, EDCI, THF, 0°C (98%, two steps); e, Pd black, H_2, MeOH, RT, 6 h; f, EDCI, HOAt, CH_2Cl_2, DMF (1:1), RT, 1 mM (49%, two steps).



Scheme 6. Selective oxidation of C25. Reaction conditions: a, SO_3-pyridine, DMSO, CH_2Cl_2 (3:1), RT, 15 min; b, $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^tBuOH, H_2O, RT, 5 h.

terms of efficiency motivated us to explore this approach. The resulting trifluoroacetic acid-amine salt was coupled to *d,l*-3-methyl-2-oxo-pentanoic acid sodium salt mediated by EDCI and HOAt, affording the corresponding amide **31** in ≈98% yield over the two steps. The high yield in this reaction proved promising for the macrocyclization step, because there is no observable competing acylation of any of the free alcohols, and promised to obviate the need for protecting groups.

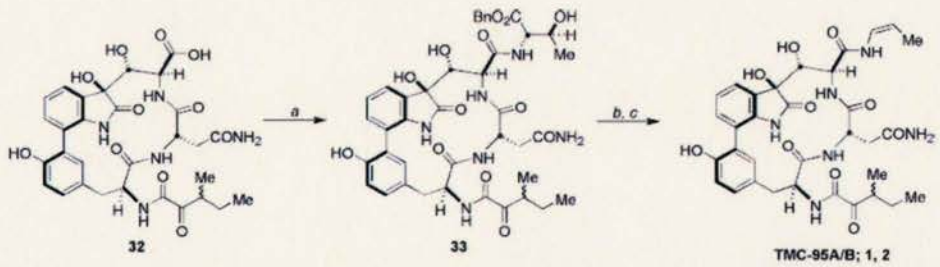
Hydrogenolysis of both the benzyl ester and the *N*-benzyloxy carbamate with palladium black afforded the requisite amino acid necessary for macrocyclization. The resulting amino acid was treated with EDCI and HOAt to yield the key unprotected macrocycle **5**. Crude ^1H NMR analysis showed that besides macrocycle **5**, there were no other macrocyclic compounds related to lactone formation or biaryl atropisomers.[§]

As stated earlier, although we were able to secure the structure of macrocycle **5** by intersecting a late-stage intermediate in the Danishefsky synthesis, it was felt that an efficient synthesis of TMC-95A/B could be accomplished via direct elaboration of macrocycle **5**. To realize this objective, selective oxidation of the C25 primary alcohol in the presence of the C7 secondary alcohol would need to be achieved. In addition and, even more problematic, the oxidative cleavage of the C6–C7 diol loomed as a potential pitfall. Initially, a selective oxidation of the primary alcohol directly to the necessary carboxylic acid using a platinum-catalyzed dehydrogenation reaction (37) was examined. Unfortunately, it was found that no reaction occurred or, if base was added, complete decomposition occurred.

Other direct oxidation methods of the primary alcohol to the carboxylic acid were considered, including the 2,2,6,6-tetramethyl-1-piperidinyloxy (free radical)/$NaClO_2$/NaOCl combination; unfortunately, no desired carboxylic acid product was observed (38). Finally, after exhaustive experimentation [Swern oxidation (39), SO_3·pyridine, Dess–Martin periodinane (40), *o*-iodoxybenzoic acid (41, 42), and other 2,2,6,6-tetramethyl-1-piperidinyloxy systems (43)], it was found that a two-step protocol proved successful in obtaining the desired carboxylic acid. Thus, treating macrocycle **5** with SO_3-pyridine in DMSO-CH_2Cl_2 afforded the desired aldehyde as an inseparable complex mixture of aldehyde, plus C6 and C7 lactol isomers (Scheme 6). Fortunately, subjection of this mixture to $NaClO_2$ and NaH_2PO_4 in the presence of 2-methyl-2-butene to produce desired carboxylic acid **32**.

With carboxylic acid **32** in hand, all that remained was the incorporation of the *cis*-propenyl amide. There were several avenues that we chose to investigate for this transformation. Initially, we decided to test the method developed by Stille and Becker (44), which involved a transition-metal-mediated process wherein allyl amides are converted to the corresponding enamides in which the cis-configuration predominates. Coupling of

[§]Lin and Danishefsky (14, 15) showed that the atropisomeric outcome follows the C6 stereochemistry.



Scheme 7. Completion of TMC-95A/B. Reaction conditions: a, L-*allo*-threonine-benzyl ester hydrochloride, EDCI, HOAt, diisopropylethylamine, CH_2Cl_2, DMF, 0°C, 16 h, 49% (from **5**); b, Pd black, MeOH, H_2 [1 atm (1 atm = 101.3 kPa)], RT; c, diisopropyl azodicarboxylate, PPh_3, DMF, THF, RT, 70% (two steps).

allyl amine with the carboxylic **32** readily provided the corresponding allyl amide. Unfortunately, all attempts to isomerize this product proved futile.

Next, the Peterson olefination method developed by the Fürstner laboratory (ref. 45 and references therein) that utilizes hydroxyalkyl silanes for the preparation of enamides was evaluated. The requisite strong base used in this Peterson olefination protocol was anticipated to be too harsh for the sensitive functionality present in the TMC-95 macrocyclic core. Therefore, we examined the liberation of a masked alkoxide under mild conditions as a means to trigger the desired olefination. The literature revealed that fluoride-based deprotection of a *tert*-butyldimethylsilyl ether unleashes an alkoxide species reactive enough to suffer facile Peterson olefination to yield an enamide as reported in the synthesis of crocacin D (46). Although we were able to conduct this reaction on a very simplified substrate, we were unable to produce TMC-95A/B after subjection of the corresponding hydroxyalkyl silyl amide with a variety of fluoride sources.

Based on the aforementioned setbacks, we evaluated the enamide preparation developed by Pansare and Vederas (47) and used by Inoue *et al.* (16, 17) in their synthesis of TMC-95A. Treatment of carboxylic acid **32** with L-*allo*-threonine-benzyl ester hydrochloride salt (48) mediated by EDCI and HOAt afforded the corresponding amide **33** in 49% overall yield from **5** (Scheme 7). Hydrogenolysis of the benzyl ester in **33** with palladium black under an atmosphere of hydrogen produced the resultant carboxylic acid. Subjection of this material to Mitsunobu conditions afforded TMC-95A/B in 70% yield for the two steps. The individual diastereomers TMC-95A and TMC-95B were separated by HPLC to collect analytical data on each. The synthetic samples of TMC-95A and TMC-95B and the natural materials proved identical by ^{1}H NMR, ^{13}C NMR, mobility on TLC, mobility on HPLC, optical rotation, and high-resolution mass spectrometry.

Conclusions

A concise and efficient total synthesis of TMC-95A/B has been accomplished. The synthesis was completed in 22 total steps with only 18 steps in the longest linear sequence. It should be noted that this is a very short and efficient total synthesis of these natural substances and is an approach to commence with L-serine instead of D-serine. Our synthesis features an *E*-selective modified Julia olefination to form the key oxindolene. It has been found that this transformation is also a viable route to other β,γ-unsaturated protected amino alcohols. The synthesis recorded here constitutes an efficient strategy that is amenable to the preparation of a variety of analogs due to being highly convergent and requiring minimal protecting group manipulations.

We are indebted to Dr. Jun Kohno (Tanabe Seiyaku Co., Toda-shi, Saitama, Japan) for providing authentic samples of TMC-95A/B, which were valuable for spectral comparison. This material is based on work supported by National Science Foundation Grant 0202827 and the National Institutes of Health. We are also grateful to Boehringer Ingelheim Pharmaceuticals for partial support of this work. Mass spectra were obtained on instruments supported by National Institutes of Health Shared Instrumentation Grant GM49631.

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Appendix 2. Research Proposal

Independent Research Proposal: A Rhodium-Catalyzed C-H insertion of α-Ketoamides and its Application to the Total Synthesis of Salinosporamide A.

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Department of Chemistry

Colorado State University

Recently Du Bois and co-workers have developed a stereospecific C-H oxidation with carbamates and sulfamates to form five- and six-membered heterocyclic compounds, respectively (Scheme 1).[1] Du Bois proposes that the carbamates and sulfamates react through a rhodium-stabilized nitrene-type intermediate. Although this methodology has significant synthetic power[2] it also incurs inherent limitations,[3] specifically when attempted on primary amides. The purpose of this proposal is to show how rhodium nitrene chemistry can be adapted to modified primary amides and how this synthetic methodology can be applied to the total synthesis of salinosporamide A (**1**) and, potentially the related natural products omuralide (**2**) and lactacystin (**3**) (Figure 1).

Scheme 1. Du Bois and co-workers Rh catalyzed C-H activation chemistry.

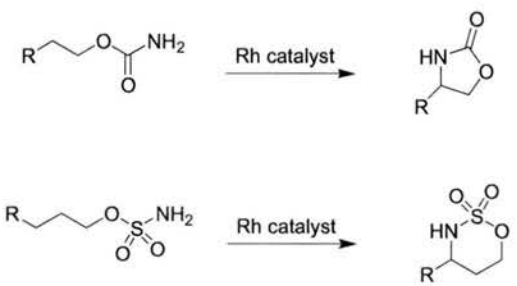
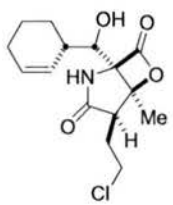
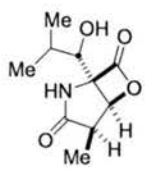


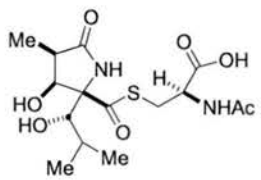
Figure 1. Structures of salinosporamide A (**1**), omuralide (**2**), lactacystin (**3**).



salinosporamide A, **1**



omuralide, **2**

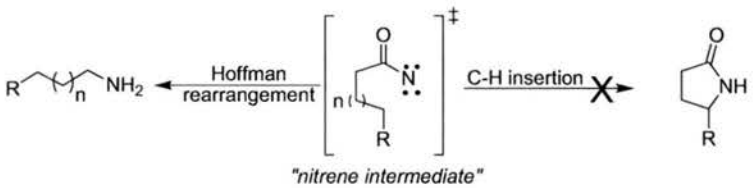


lactacystin, **3**

A significant drawback to the rhodium nitrene chemistry is that it cannot be applied to primary amides. This is presumably due to the fact that the intermediate "nitrene" undergoes faster Hoffman rearrangement as compared to C-H activation (Scheme 2). Therefore, in order to make the C-H insertion reaction of primary amides

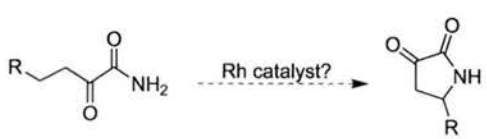
proceed as desired one must determine how to slow down nitrene rearrangement in order to favor C-H

Scheme 2. C-H insertion versus Hoffman rearrangement.



insertion. When substituted benzamides are treated under Hoffman rearrangement conditions, electron withdrawing groups on the aromatic ring retard the rate of the reaction as compared to electron donating groups.[4] Therefore, in order to promote C-H insertion of nitrenes derived from primary amides one would have to substitute the α-position of the amide with a "non-transferable" group. Based on the aforementioned observations it is my hypothesis that α-ketoamides, when subjected to rhodium-catalyzed C-H insertion conditions, will produce α-ketolactams (Scheme 3).

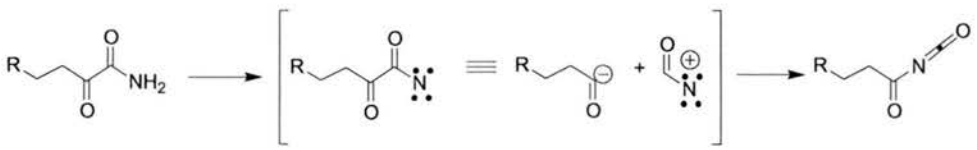
Scheme 3. Proposed formation of α-ketolactams through a rhodium C-H insertion reaction.



In order to evaluate the probability of this reaction manifold, one must compare it to known entities. It is known that nitrenes readily insert into a variety of C-H bonds. Therefore, this should not be the problematic step in this reaction manifold.[1,2] The problem that needs to be addressed is that of the migratory aptitude of the neighboring group to the nitrene. As stated earlier, the migratory propensity depends on the electronic effects of the rearranging group, according to which electron-rich carbon-carbon bonds

rearrange faster than electron-poor carbon-carbon bonds. Although it is known that the Hoffman rearrangement is a concerted reaction having no discrete charge separation one could consider the nitrene to be the electrophilic partner and the migrating carbon group to be the nucleophilic partner. If this analogy were to be used for the α-ketoamide class of reactants one would have to consider an "acyl anion" as the nucleophilic migratory partner (Scheme 4).

Scheme 4. Hoffman rearrangement of α-ketoamides broken into nucleophilic and electrophilic partners.

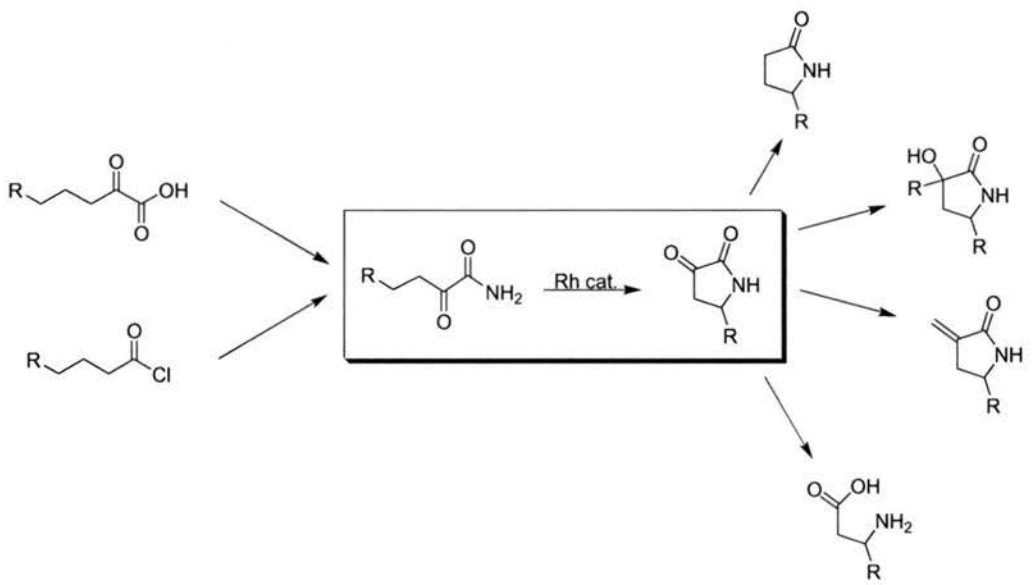


It is proposed that, since acyl anions do not exist, "*per se,*"[5] and are only known in masked forms (Umpulong chemistry), C-H activation of this class of nitrenes should be far more facile than the corresponding Hoffman rearrangement.

In order for this type of chemistry to be useful, the starting materials must be readily accessible and the products must be synthetically useful. α -Ketoamides can be prepared in a number of ways[6] and perhaps the most useful routes to them evolve from pyruvic acids or acid chlorides (Scheme 5). The α-ketolactam derived from the proposed rhodium-catalyzed C-H insertion can be synthetically converted to a variety of substrates[7] including the saturated alkane, secondary or tertiary alcohols, alkenes and amino acids along with other conversions typical for ketones (Scheme 5).

The second purpose of this proposal is to show how the above chemistry can be applied to the total synthesis of salinosporamide A (**1**) and, potentially, the related natural products omuralide (**2**) and lactacystin (**3**)[8] (Figure 1). Recently Fenical and co-workers

Scheme 5. Preparation of α-ketoamides and uses of α-ketolactams.

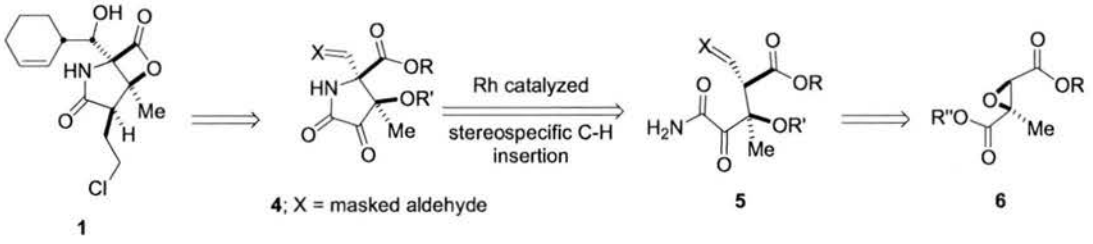


have reported the isolation of the highly cytotoxic proteasome inhibitor salinosporamide A from the novel marine bacterium *Salinospora.*[9] Salinosporamide A shows potent cytotoxicity to a variety of cancer cell lines, including HCT-116 human colon carcinoma cells; the NCI's 60-cell-line panel; non-small lung cancer cells; CNS cancer cells; melanoma and breast cancer. Salinosporamide A inhibited proteasomal activity by more than 35 times that of the known proteasome inhibitor omuralide, the current standard for this class of molecules. Along with its potent biological activity, salinosporamide A has a very intriguing molecular structure containing a fused γ-lactam-β-lactone ring system, five contiguous stereocenters, a cyclohexene ring system and a pendant chloroethyl group. It is for the above stated reasons that it is felt salinosporamide A is an interesting target for total synthesis that would allow for the use of the rhodium-catalyzed C-H insertion chemistry of α -ketoamides described above.

Retrosynthetically, salinosporamide A (**1**) could come from α-ketolactam derivative (**4**) by incorporation of both the chloroethyl side chain and the cyclohexene

side chain. The α-ketolactam derivative (**4**) could be prepared from the primary α-ketoamide (**5**) through a stereospecific rhodium-catalyzed C-H insertion reaction. In turn α-ketoamide (**5**) could be prepared from optically active epoxide (**6**) (Scheme 6).

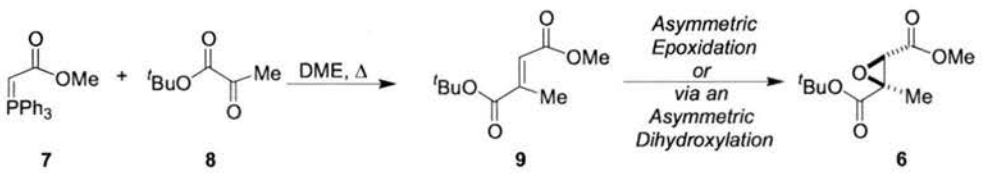
Scheme 6. Retrosynthetic analysis of salinosporamide A.



It is proposed that epoxide **6** can be readily prepared in optically active form starting with readily available starting materials. Wittig olefination of commercially available stabilized ylide **7** with readily available pyruvic acid *tert*-butyl ester[10] **8** would give the corresponding α-ß-unsaturated ester **9** with orthogonal protecting groups on each ester (Scheme 7). A pivotal step in the synthesis is the preparation of optically active α-ß-epoxy ester **6** which can be secured either through an asymmetric epoxidation or *via* an asymmetric dihydroxylation. Recently Shi and co-workers have shown the asymmetric epoxidation of tri-substituted α-ß-unsaturated esters with a chiral dioxirane.[11] Although substrates similar to **9** were not treated under the reaction conditions, it is felt that this is a logical starting point for the synthesis of **6**. In the event the asymmetric epoxidation does not give the desired results, there are methods for which similar compounds have been prepared asymmetrically through a Sharpless asymmetric dihydroxylation.[12]

With epoxide **6** in hand, two of the five stereocenters of salinosporamide A have been set. In order for the incorporation of the cyclohexene side chain at a later stage of the

Scheme 7. Preparation of the key asymmetric epoxide.

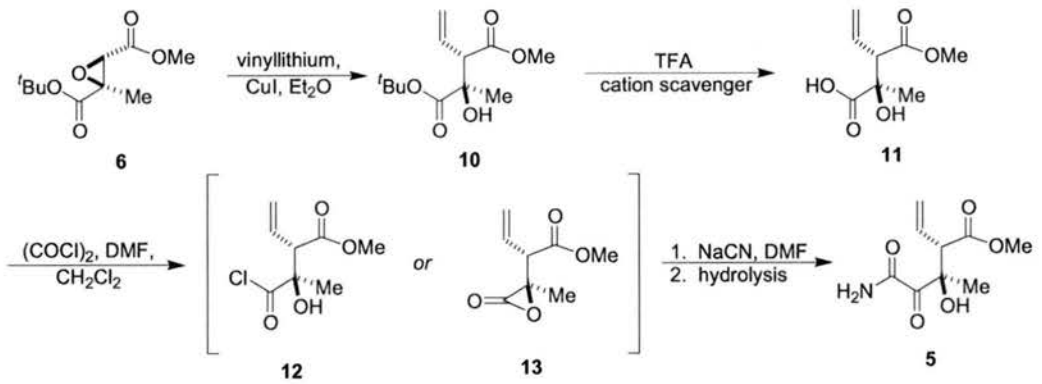


7 8 9 6

synthesis, a regioselective epoxide ring opening must occur. It is known[13] that when α-ß-epoxy esters are treated with organocuprates derived from organolithiums and a Cu(I)salt a regioselective ring opening occurs at the more sterically accessible carbon (Scheme 8). Therefore, treatment of epoxide **6** with vinyl lithium and CuI should afford alkene **10**. With alkene **10** in hand, selective deprotection of the *tert*-butyl ester with anhydrous TFA and a cation scavenger should afford carboxylic acid **11**. Treatment of carboxylic acid **11** with oxalyl chloride and catalytic DMF should afford the corresponding acid chloride **12**. Although not probable, there is a possibility of the 3° alcohol closing on the acid chloride **12** to form the α-lactone **13**. In either event, both the acid chloride **12** or the α-lactone **13** should prove as useful intermediates for the next transformation. Treatment of acid chloride **12** or α-lactone **13** with NaCN followed by hydrolysis will afford the key ketoamide **14**. Another potential problem during this reaction sequence is the potential hydrolysis of the methyl ester. Although the conditions necessary for the hydrolysis of the nitrile to the amide are typically somewhat mild[6a] it is possible that the methyl ester would also be hydrolyzed. If this is the case, a simple solution would be to regenerate the methyl ester using diazomethane.

Upon the preparation of α-ketoamide **5**, the proposed synthesis is at a point to test the feasibility of the above rhodium-catalyzed C-H insertion of α-ketoamides. A potential problem with α-ketoamide **5** is the fact that there is a free 3° alcohol which may

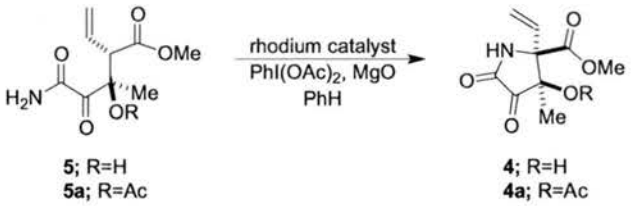
Scheme 8. Preparation of requisite ketoamide for Rh catalyzed C-H insertion.



interfere with the desired insertion reaction. If the 3° alcohol proves problematic, it can be protected as its corresponding acetate **5a**, thus allowing for a deprotection concurrently with the methyl ester prior to β-lactone formation.

Du Bois and co-workers have shown that the rhodium-catalyzed C-H insertion of both carbamates and sulfamates is highly dependent on substrate and rhodium catalyst.[1,2] Therefore, for this proposal we will not suggest a specific rhodium catalyst necessary for this reaction and do realize that extensive experimentation would be necessary to afford the corresponding α-ketolactam **4** (Scheme 9).

Scheme 9. Formation of α-ketolactams via rhodium catalyzed C-H insertion reaction.

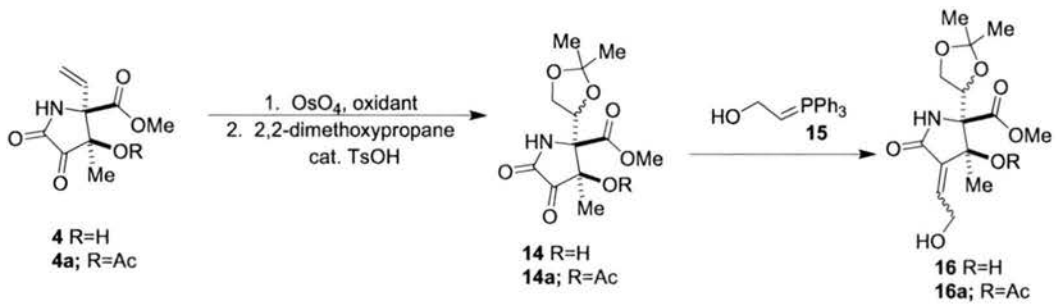


It is known that the C-H insertion chemistry is a stereospecific reaction manifold and the newly formed C-N bond retains the stereochemistry of the original C-H bond. Therefore, since α-ketoamide **5** was prepared as a single isomer this should prove to be an efficient method for the asymmetric preparation of two adjacent tetrasubstituted carbons. α-

Ketolactam **4** provides the necessary functionality that allows for the facile and expedient incorporation of the chloroethyl side chain of salinosporamide A.

Incorporation of the chloroethyl and cyclohexene side chains of salinosporamide A will require synthetic manipulations to both the terminal alkene and the ketone. Conversion of the ketone into the chloroethyl side chain will first require the transformation of the pendant terminal alkene in lactam **4**. Treatment of alkene **4** with catalytic OsO_4, and a stoichiometric oxidant such as NMO or trimethylamine *N*-oxide followed by 2,2-dimethoxypropane and a catalytic acid such as *p*-toluenesulfonic acid should produce acetonide **14** (Scheme 10). With the masked alkene in place, the ketone can now be converted to the chloroethyl side chain. Wittig olefination of the ketone with the ylide from commercially available (2-hydroxyethyl)triphenylphosphonium chloride **15** and a strong base should yield the α-β-unsaturated amide **16**.

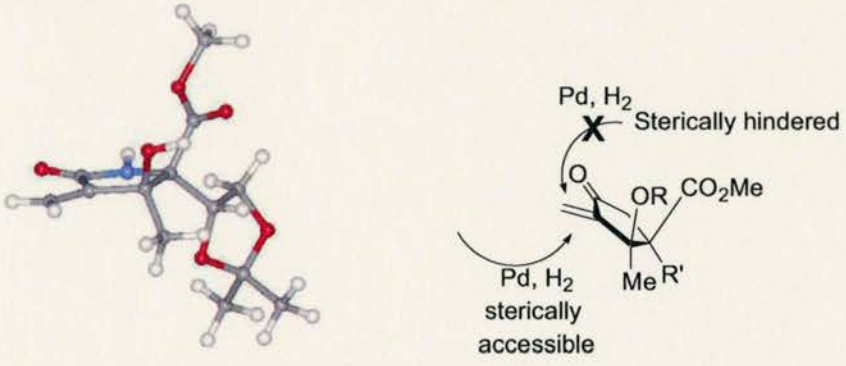
Scheme 10.



With α-β-unsaturated amide **16** in hand the synthesis is at a stage where the third contiguous stereocenter can be set. At this point in the synthesis only experimentation can tell which substrate would be an ideal substrate for the incorporation of this side chain. It is envisioned that the double bond will be reduced under transition-metal mediated hydrogenation conditions. PM3 energy minimization calculations show that there may be some facial selectivity for hydrogenation from the opposite side of both the

alcohol and the carbomethoxy group (Figure 2). It is felt that these calculations do not definitively show the desired stereochemical outcome from this hydrogenation. Initial attempts at this transformation will be conducted without the presence of a chiral ligand with the hope of achieving the desired product, but it is also understood that only modest stereoinduction may be achieved, if any at all. Therefore, the transition metal-catalyzed hydrogenation may have to be accomplished in the presence of a chiral ligand. Currently

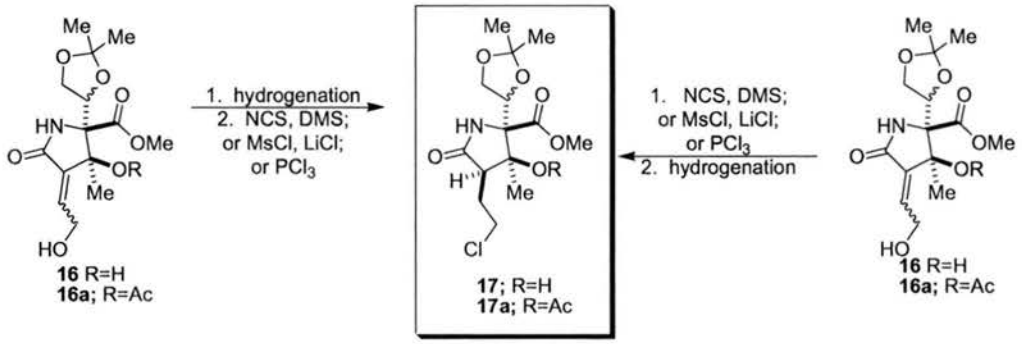
Figure 2. Semi-empirical PM3 energy minimization of a simplified alkene **16**.



there are a variety of asymmetric hydrogenation catalysts that are available for α-β-unsaturated carbonyl systems.[14] There are two possible routes that could provide the chloroethyl side chain. First would be the hydrogenation of the double bond followed by conversion of the primary alcohol to the primary chloride. The second option would be the reverse sequence. Either sequence of reaction conditions should yield the same product. Therefore, hydrogenation of α-β-unsaturated amide **16** followed by conversion of the primary alcohol to the primary chloride will provide the chloroethyl compound **17** (Scheme 11). There are numerous ways of converting the primary alcohol to the primary chloride including NCS and dimethylsulfide; PCl_3; and methanesulfonyl chloride and LiCl.[15] This sequence can also be reversed to yield chloroethyl compound **17**. As stated

earlier, it would be ideal if the 3° alcohol did not have to be protected during the rhodium catalyzed C-H activation.

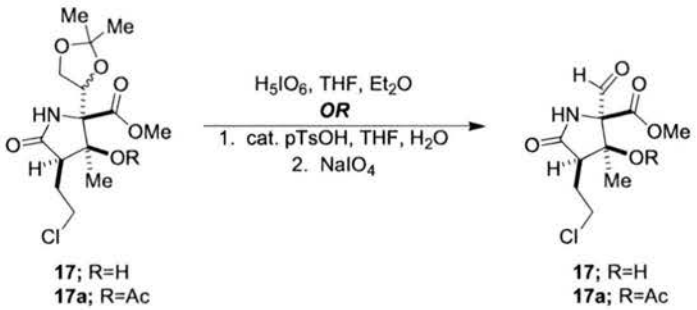
Scheme 11. Completing the chloroethyl side chain.



With the unprotected alcohol the above sequence would have competition between a 1° and a 3° alcohol. Although this may prove problematic it is not seen as a serious issue due to the fact that under the reaction conditions the 1° alcohol should react at a much faster rate than the 3° alcohol.

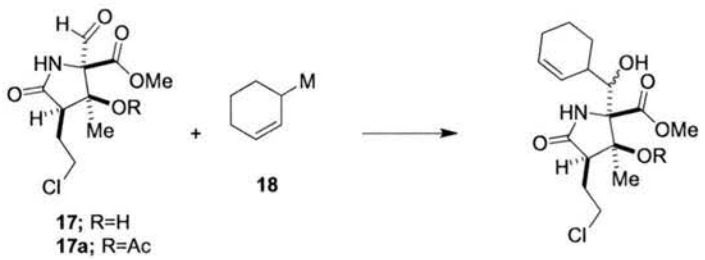
With the completion of the chloroethyl side chain, the next focal point will be the installation of the cyclohexene side chain. The prerequisite aldehyde can readily be prepared by treating the acetonide **17** with H_5IO_6[16] in a single step (Scheme 12). If this reaction proves to be problematic, a two-step protocol using catalytic acid followed by sodium periodate should yield the aldehyde.

Scheme 12. Oxidative cleave of cyclic acetonide.



It is proposed that incorporation of the cyclohexene side chain will be accomplished *via* treatment of aldehyde **17** with cyclohexene allyl organometallic species of type **18** (Scheme 13). Although it is known that when a *Z*-allyl organometallic (which is definitively incorporated into this cyclohexene ring system) is reacted with an aldehyde high *syn* diastereoselectivity is achieved between the newly formed stereocenters. However, it is also known that poor facial diastereoselectivity is achieved when using an achiral allyl organometallic and a chiral aldehyde.[17] In one sense this is to our advantage

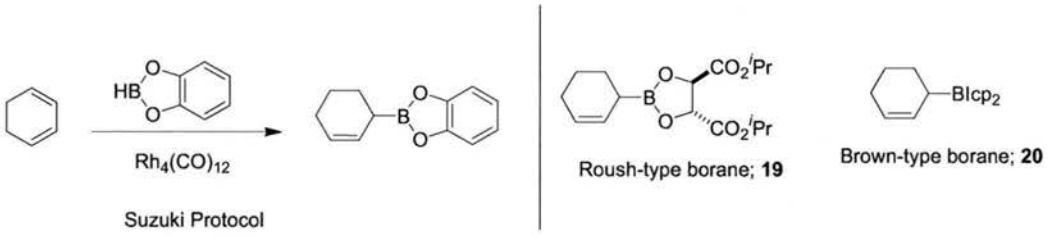
Scheme 13. General reaction for the incorporation of cyclohexene side chain.



since in salinosporamide A the connectivity between these two stereocenters is *syn,* but at the same time, in order to achieve the desired facial selectivity and ultimate stereochemical outcome, we must invoke a doubly diastereoselective reaction by treating aldehyde **17** with a chiral allyl organometallic.

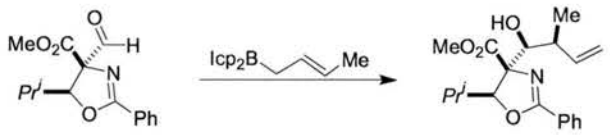
Suzuki and co-workers have shown a convenient approach to the preparation of racemic cyclohexenal allyl boronates (Scheme 14)[18]. It is felt that the chemistry developed by Suzuki and co-workers can be used to prepare Roush-type[19] allylic borane **19** and Brown-type[20] allylic borane **20**. Recent work has described the use of Roush-type boranes to produce the desired relative stereochemistry when treating chiral aldehydes under these reaction conditions.[21] Smith and co-workers have also used a Brown allylation in their total synthesis

Scheme 14. Cyclohexene allylic borane.



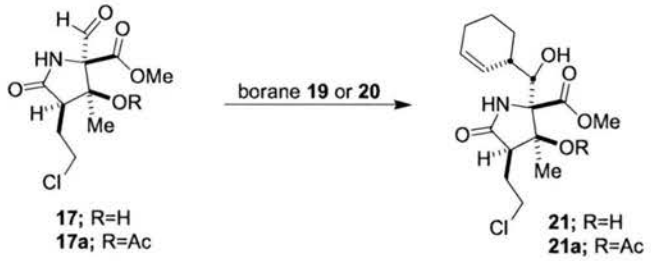
of lactacystin.[22] Although the Smith synthesis of lactacystin does not use this chemistry to install a similar type of side chain, the substitution pattern near the aldehyde is very similar to the substitution pattern necessary for this synthesis (Scheme 15). With the above precedents in hand, it is felt that treatment of aldehyde **17** with either the

Scheme 15. Smith's allylation reaction.



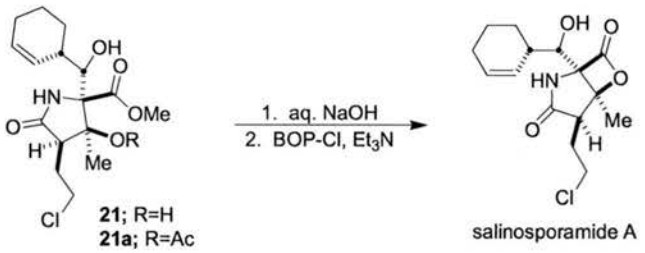
Roush-type borane **19** or the Brown-type borane **20** should yield the corresponding homoallylic alcohol **21** (Scheme 16).

Scheme 16. Asymmetric installation of cyclohexene side chain.



After the installation of the cyclohexene side chain, the only remaining synthetic efforts involve the preparation of the fused γ-lactam-β-lactone ring system. Following the protocol set by Corey and co-workers[23], treatment of either **21** or **21a** with aqueous NaOH followed by BOP-Cl and Et_3N should provide salinosporamide A (Scheme 17).

Scheme 17. Completion of salinosporamide A.



In conclusion, it has been proposed that a novel rhodium-catalyzed C-H insertion reaction involving a nitrene-type intermediate of an α-ketoamide can be used to prepare a complex natural product such as salinosporamide A in a concise and asymmetric manner. Through minor substituent modifications, it can be seen how the above synthesis of salinosporamide A may be evolved to prepare both omuralide (**2**) and lactacystin (**3**). Likewise, the above synthetic scheme should lend itself to the preparation of a variety of analogs because the basic starting materials are derived from pyruvates or glyoxylates, Wittig reagents, and organometallic reagents.

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