DISSERTATION

ASYMMETRIC TOTAL SYNTHESES OF (+)- AND (-)-SPIROTRYPROSTATINS A AND B

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

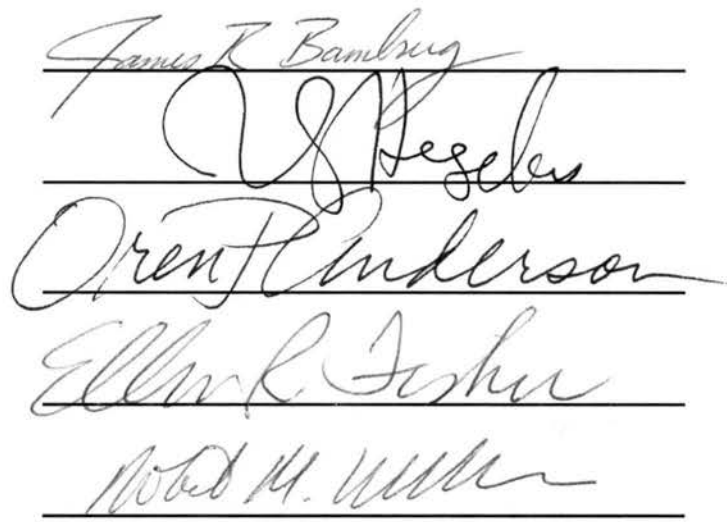
Spring 2003

COLORADO STATE UNIVERSITY

April 12, 2002

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY PAUL RICHARD SEBAHAR ENTITLED ASYMMETRIC TOTAL SYNTHESES OF (+)- AND (-)-SPIROTRYPROSTATINS A AND B BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work



Advisor

Department Head

ABSTRACT OF DISSERTATION

ASYMMETRIC TOTAL SYNTHESES OF (+)- AND (-)-SPIROTRYPROSTATINS A AND B

The first published total synthesis of (+)- and (-)-spirotryprostatin B is presented. The synthesis features an asymmetric azomethine ylide [1,3]-dipolar cycloaddition reaction. Additionally, a Barton-modified Hunsdiecker reaction was demonstrated as means of affecting an oxidative decarboxylation. Intermediates along the synthesis were studied for their biological activity as G2/M phase cell cycle inhibitors and microtubule assembly inhibitors.

The asymmetric azomethine ylide [1,3]-dipolar cycloaddition was also studied in greater detail. Varying the aldehyde component of the reaction resulted in the formation three different cycloadducts. Theoretical calculations for the reaction were compared with observed results.

Attempts to synthesize (-)-spirotryprostatin A are also presented. Two different strategies based on the synthesis of (+)- and (-)-spirotryprostatin B were explored.

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Spring 2003

ACKNOWLEDGEMENTS

I would like to thank Professor Robert M. Williams for providing the resources and the stimulating research environment that enabled me to develop as a synthetic chemist. The freedom given to explore such an exciting project and the autonomy granted in conducting my research was invaluable to my career as a graduate student. Additionally, I would like to thank Bob for the friendliness that he and his wife Jill extended to both my wife and I outside of the laboratory.

Thanks to the members of the Williams Group, past and present, for the various roles they filled while I was at Colorado State University. The expertise, advice, and laboratory antics of the Williams group were instrumental in being able to complete a total synthesis of spirotryprostatin B. Thank you the friends I had at Colorado State University. The camping, fishing, football games and parties made the time spent in graduate school much more enjoyable.

Finally, I would like to thank my family. I am indebted to my parents, Charles Joseph Sebahar and Karen Lou Sebahar, for their love and support. It is without question that I would not have been able to complete either my BS in chemistry or my PhD in organic chemistry without their guidance. My brothers, Chuck, Dave, Steve and Ron, have proved to be sources of inspiration through the success of both their families and their careers. Finally, I would like to thank my wife Holly Lynn (Norling) Sebahar for her continued love and support. My accomplishments would be inconsequential without her by my side.

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Chapter 1

Spirotryprostatins A and B

1.1 Introduction

Elucidating the regulatory machinery of the cell cycle is crucial to understanding how defects in the regulatory mechanism of the cell result in uncontrolled growth and differentiation, such as cancer.[1] Small-molecule natural products are proving invaluable in contemporary studies of cellular probes through their ability to specifically bind target proteins that modulate signal transduction cascades. Numerous examples exist in which the biological function of a particular cellular factor have been investigated through the use of such compounds.[2] Therefore, the development of new and specific inhibitors of signal transduction cascade pathways will continue to be extremely important in the understanding of the regulatory mechanism of the cell cycle.

Recently, powerful bioassays have been developed to specifically identify new natural products that inhibit the progression of the cell cycle at distinct phases. Using temperature-sensitive mammalian tsFT210 cells and rat normal fibroblast 3Y1 cells, Osada, et al. have exploited these screening technologies to identify a wide array of interesting natural products from the fermentation broth of the fungus *Aspergillus fumigatus* and other microbial sources.[3]

Included in the families of fungal metabolites identified in this manner are the fumitremorgins,[4] the tryprostatins,[5] the cyclotryprostatins[6] and the spirotryprostatins[7] (**1** and **2**, Figure 1). The primary target of tryprostatin A and cyclotryprostatins A and B are microtubules, which induce M-phase specific inhibition and microtubule disassembly.[8]

These substances have attracted considerable synthetic attention and individual total syntheses of the tryprostatins[9] and some of the fumitremorgins[10] have been reported.

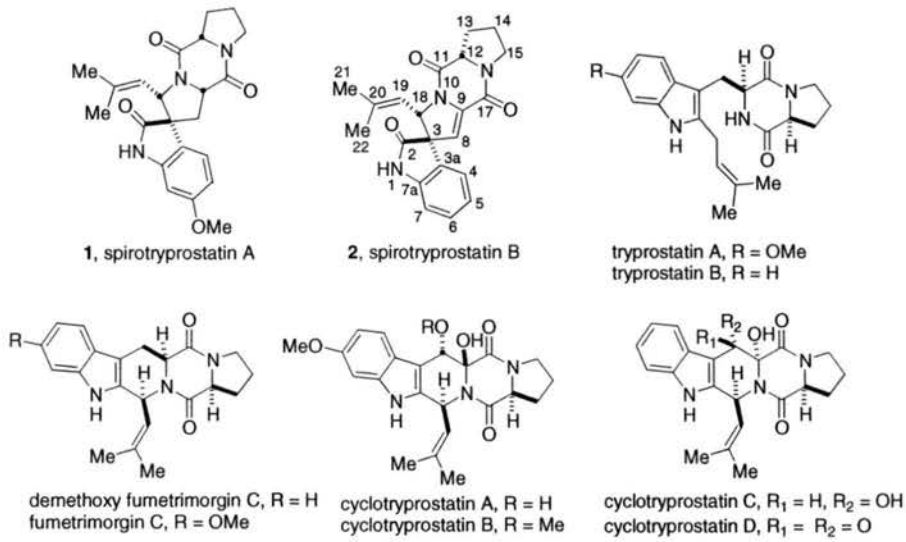


Figure 1

The structurally more interesting and complex members of this family of compounds are the spirotryprostatins, which display the weakest biological activity as cell cycle inhibitors. Isolated in 1996, from *Aspergillus fumigatus*, spirotryprostatin A (**1**) and spirotryprostatin B (**2**) were shown to completely inhibit the progression of cells at concentrations greater than 253 μM and 34.4 μM, respectively. The detailed mechanism of action by which these substances inhibit microtubule assembly is presently not known and studies to discover the target of these natural products have been hampered by the small quantities of these substances that can be conveniently isolated from the producing organism. Despite their relatively modest biological activity relative to other members of this family, the spirotryprostatins have nonetheless garnered the most attention due to their intriguing molecular structures. The spirotryprostatins are characterized by a unique spirooxindole substituted *cis*-prolyl-prolyl-diketopiperazine that is prenylated at C-18.

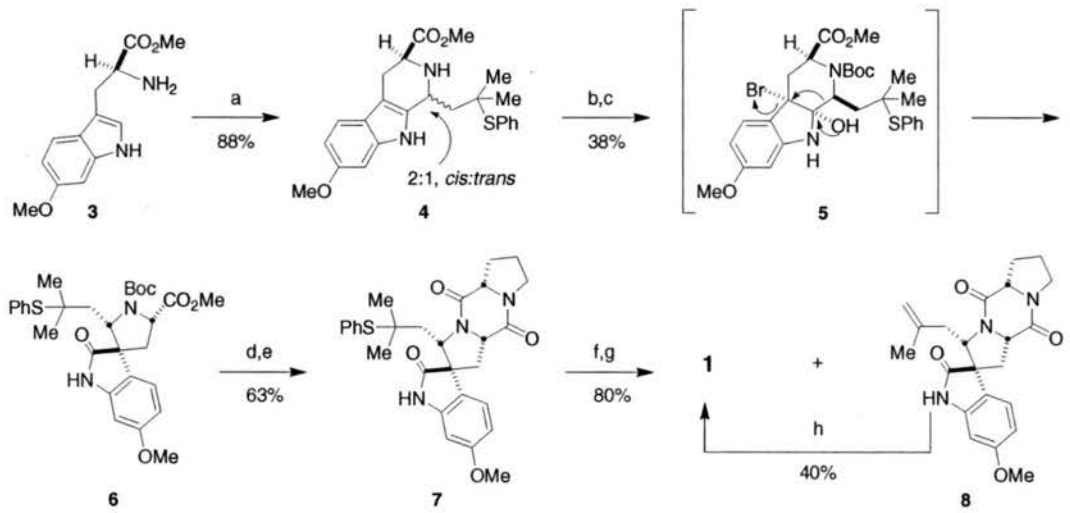
Spirotryprostatin A (**1**) differs from spirotryprostatin B (**2**) in that it is saturated at C-8 and C-9 and is substituted at C-6 by a methoxy group, whereas spirotryprostatin B is absent of functionality on the aromatic ring and contains the characteristic eneamide moiety.

1.2 Total Syntheses of Spirotryprostatin A and B

Since isolation of the spirotryprostatins in 1996, numerous groups have embarked on research programs directed at the synthesis of spirotryprostatins A and B. Various research groups have focused their efforts on the development of synthetic methodology towards the spirooxindole pyrrolidine core of the natural products. Recent approaches include a [5+2]-cycloaddition of enantiomerically pure η^3-pyridinylmolybdenum complexes,[11] directed radical cyclizations,[12] ring expansion of cyclopropanes by aldimines[13] and iodide ion induced rearrangement of [(*N*-aziridinomethylthio)methylene]-oxindoles.[14] In addition to the asymmetric generation of the spirooxindole quaternary carbon, a total synthesis of the spirotryprostatins would require installation of the prenyl side-chain, formation of three or four stereogenic centers and generation of the eneamide moiety. These issues have been addressed by various strategies and have culiminated in the total synthesis of spirotryprostatin B (**2**) by the groups of Williams,[15] Danishefsky,[16] Ganesan,[17] Overman[18] and Fuji.[19] The Danishefsky research group has also reported the total synthesis of (-)-spirotryprostatin A (**1**).[20]

1.2.1 Danishefsky's Synthesis of Spirotryprostatin A

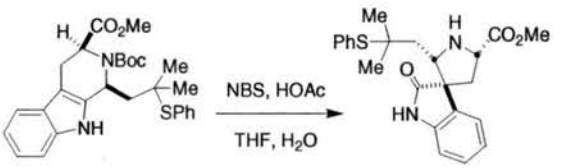
The first total synthesis of (-)-spirotryprostatin A was reported by Danishefsky *et al.* in 1998.[20] The group used a biomimetic strategy that revolved around a Pictet-Spengler reaction of an appropriately substituted tryptophan derivative and oxidation of the resulting β-carboline to form the key spirooxindole pyrrolidine amino acid (Scheme 1). The synthesis began with the enantioselective generation of 6-methoxy tryptophan methyl ester **3** and formation of the requisite 3-methyl-3-phenylsufanyl butyraldehyde.[21] Subjecting these two reagents to the action of CF_3CO_2H in CH_2Cl_2 and 4Å Molecular Sieves according to the protocol of Cook[22] afforded a 2:1 mixture of *cis/trans*-tetrahydro-β-carboline diastereomers **4**. Isolation of the desired *cis*-isomer and protection of the free amine as the *t*-butyl carbamate afforded a suitable precursor for the oxindole spiro-ring-forming contraction sequence. Bromination at C-3 of the indole occurred *anti* to the alkyl side-chain and the resulting imine was trapped with water to generate intermediate **5**. The alcohol then collapsed to form spirooxindole **6** in 46% yield. Deprotection of the Boc group followed by coupling with trichloroethyl chloroformate (Troc) protected L-proline acid chloride and Zn^0 assisted removal of the carbamate protecting group effected cyclization and resulted in a 63% yield of the diketopiperazine **7**. Installation of the prenyl side-chain was accomplished by oxidation of the thio-ether with sodium periodate and heating the resulting sulfoxide in toluene. The reaction sequence provided 80% of a separable mixture of spirotryprostatin A (**1**) and olefin isomer **8** in a 2.6:1 ratio. The undesired isomer **8** was then converted to the natural product by isomerization with rhodium trichloride, improving the overall yield to 12%.



Scheme 1. Danishefsky's synthesis of spirotryprostatin A (**1**). (a) 3-Methy-3-phenylsulfanyl butyraldehyde, 4Å sieves, TFA, CH_2Cl_2; (b) $(Boc)_2O$, Et_3N, CH_2Cl_2; (c) NBS, HOAc, H_2O, THF; (d) TFA, CH_2Cl_2; (e) Troc-L-pro-Cl, Et_3N, DMAP, CH_2Cl_2; (f) Et_3N, CH_2Cl_2; Zn^o, MeOH, THF, NH_4Cl; (g) $NaIO_4$, H_2O, MeOH; toluene, Δ; (h) $RhCl_3 \cdot 3H_2O$, EtOH, Δ.

1.2.2 Danishefsky's Synthesis of Spirotryprostatin B

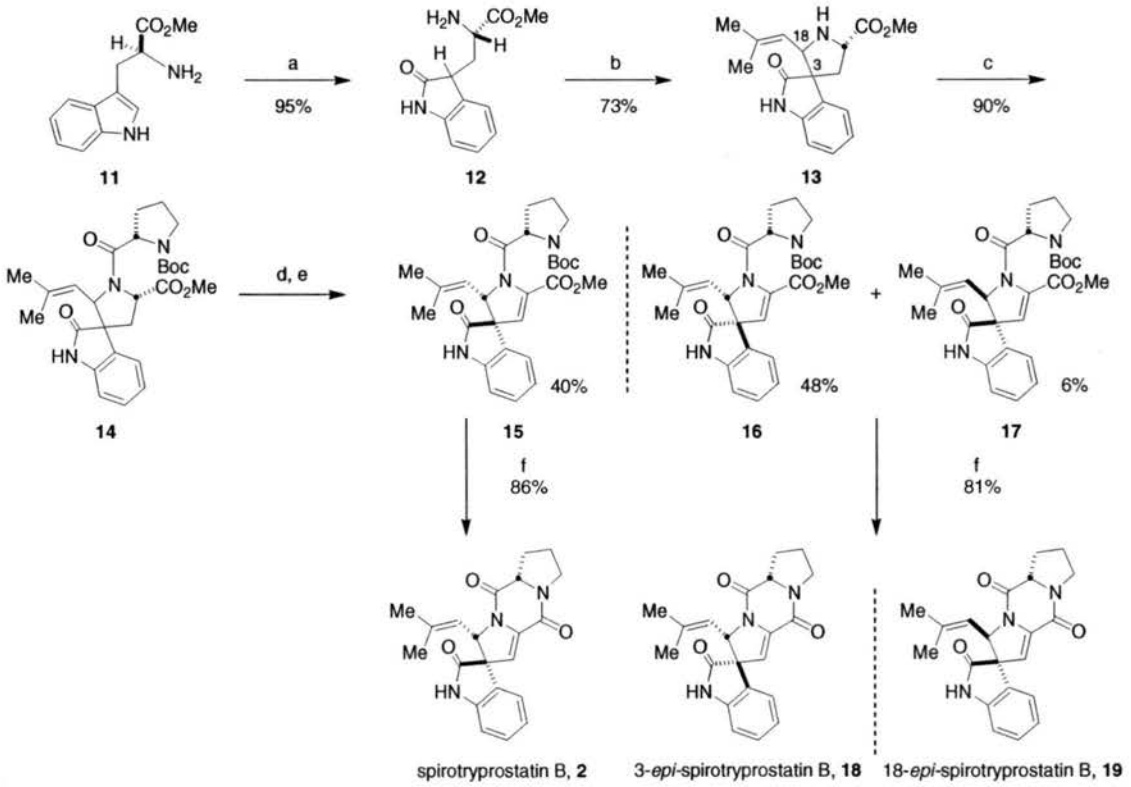
Concurrent with the efforts of Williams, Ganesan and Overman, Danishefsky reported a total synthesis of spirotryprostatin B in 2000.[16] The strategy developed for spirotryprostatin A was explored as a potential means of accessing spirotryprostatin B as well. However, this approach proved unsuccessful, as specificity issues were encountered that lengthened the synthesis and relied heavily on protecting groups. Attempts to apply the Pinnacol-type *spiro*-rearrangment used in the synthesis of spirotryprostatin A to the 6-demethoxy congener **9**, led to the C-3 *epi*-amino acid methyl ester **10** (Scheme 2). Investigations reported by Danishefsky also indicated that installation of the eneamide functionality could not be accomplished later in the synthesis by the planned selenation-elimination protocol as no selectivity was achieved in the deprotonation of the C-9 versus the C-12 proton. As a result, an alternate route to spirotryprostatin B was explored.



9 10

Scheme 2. Pinnacol-type spiro-rearrangement of tetrahydro-β-carboline **9**.

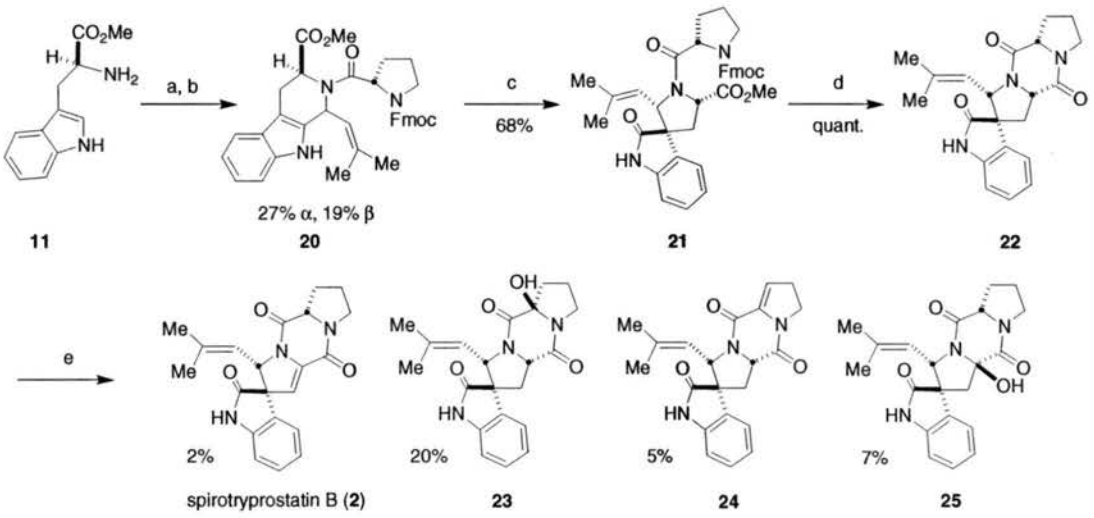
Danishefsky et al. were eventually able to complete a total synthesis of (-)-spirotryprostatin B via the route shown in Scheme 3. L-Tryptophan methyl ester **11** was converted to the oxindole **12** by Kornblum oxidation in 95% yield. Intramolecular Mannich reaction of **12** with senicaldehyde afforded amino acid methyl ester **13** (73%) as an inseparable mixture of four diastereomers at C-3 and C-18. The resulting mixture was coupled to Boc-L-proline with BOPCl as the activating agent and resulted in dipeptides **14**. Installation of the eneamide was accomplished by selenation, oxidation and elimination to form three products **15**, **16** and **17** in 40%, 48% and 6% yields, respectively. Precursor **15** was isolated and converted to spirotryprostatin B (**2**) by TFA-assisted removal of the Boc group and triethylamine-induced cyclization. The other two diastereomers (**16** and **17**) were subjected as a mixture to the deprotection conditions and produced the corresponding 3-*epi* and 18-*epi* spirotryprostatins (**18** and **19**). Thus, Danishefsky's synthesis of spirotryprostatin B (**2**) was accomplished in 5 steps and 5% overall yield.



Scheme 3. Danishefsky's synthesis of spirotryprostatin B (**2**). (a) DMSO, 12N HCl, HOAc; (b) senicaldehyde, Et_3N, 3Å sieves, pyridine; (c) BOPCl, Et_3N, Boc-L-proline; (d) LiHMDS, THF, 0°C; PhSeCl, THF, 0°C; (e) DMDO, THF, 0°C; (f) TFA, CH_2Cl_2; Et_3N, CH_2Cl_2.

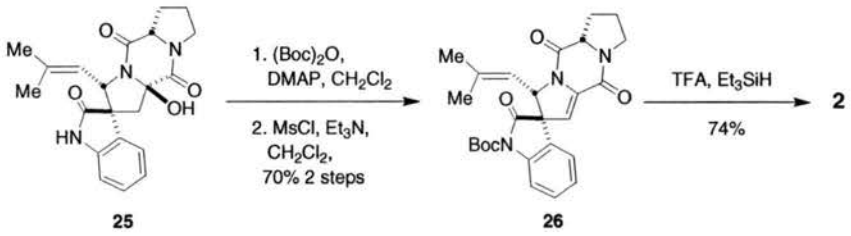
1.2.3 Ganesan's Synthesis of Spirotryprostatin B

Ganesan reported a synthesis of spirotryprostatin B in 2000,[17] using an approach similar to that deploted in Danishefsky's synthesis of spirotryprostatin A. Starting with tryptophan methyl ester **11**, Pictet-Spengler reaction with senicaldehyde afforded the tetrahydro-β-carboline **20** as two diastereomers (α:β) in 46% overall yield (Scheme 4). The desired dipeptide (**20β**) was isolated as the minor component after acylation with Fmoc-L-proline acid chloride and separation from the *trans*-isomer. Oxidation and *spiro*-rearrangement was then effected by the addition of one equivalent of N-bromosuccinimide in acetic acid, THF and water.



Scheme 4. Ganesan's Synthesis of Spirotryprostatin B. (a) $CH(OMe)_3$, senicaldehyde; (b) Fmoc-L-proline acid chloride, pyridine, CH_2Cl_2; (c) NBS, HOAc, H_2O, THF; (d) piperdine, CH_2Cl_2; (e) LDA, THF, -78°C; PhSeCl.

Timing of the *spiro*-rearrangement proved to be important as attempts to effect the rearrangement earlier or later in the synthesis resulted in the formation of the C-3 epimer. This required the chemoselective oxidation of the indole without disturbing the isopropylidene group. By the careful control of reagent amounts, Ganesan et al. were able to selectively oxidize the indole without disturbing the isopropylidene group. This circumvented masking of the prenyl side-chain as the thioether (**6**) which Danishefsky utilized in the synthesis of spirotryprostatin A. Acylation of the free amine with Fmoc-L-proline acid chloride, followed by base-induced cyclization generated diketopiperazine **22**. Installation of the eneamide was required for completion of the total synthesis. However as Danishefsky and coworkers had alluded to in their explorations of spirotryprostatin B, there was no way to distinguish the central spirooxindole-substituted pyrrolidine ring over the proline portion of the molecule. As a result, subjecting **22** to standard oxidation conditions led to the isolation of four products (**2**, **23**, **24** and **25**) as



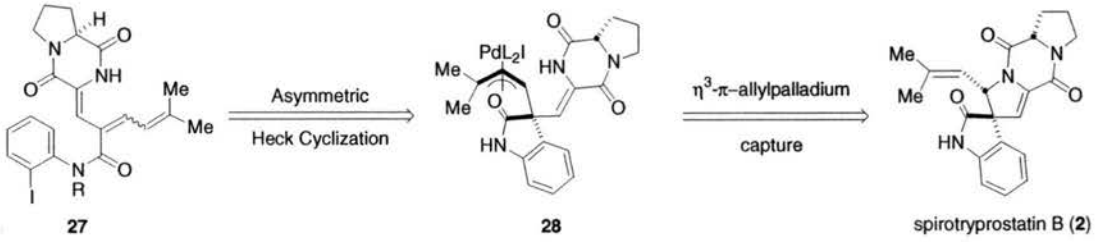
Scheme 5. Conversion of 25 into spirotryprostatin B (**2**).

well as 24% of unreacted starting material. The total synthesis of spirotryprostatin B (**2**) was completed (albeit in 2% yield). The overall yield was improved slightly by the three-step conversion of the minor side-product **25** into the natural product (Scheme 5). Protection of the oxindole nitrogen with Boc anhydride, mesylation of the tertiary alcohol and concominant elimination resulted in the formation of **26** in 70% yield for the two steps. Removal of the Boc group with the aid of trifluoroacetic acid and triethylsilane afforded the natural product. Thus, the synthesis of spirotryprostatin B (**2**) was accomplished in <1% over five steps.

1.2.4 Overman's Synthesis of Spirotryprostatin B

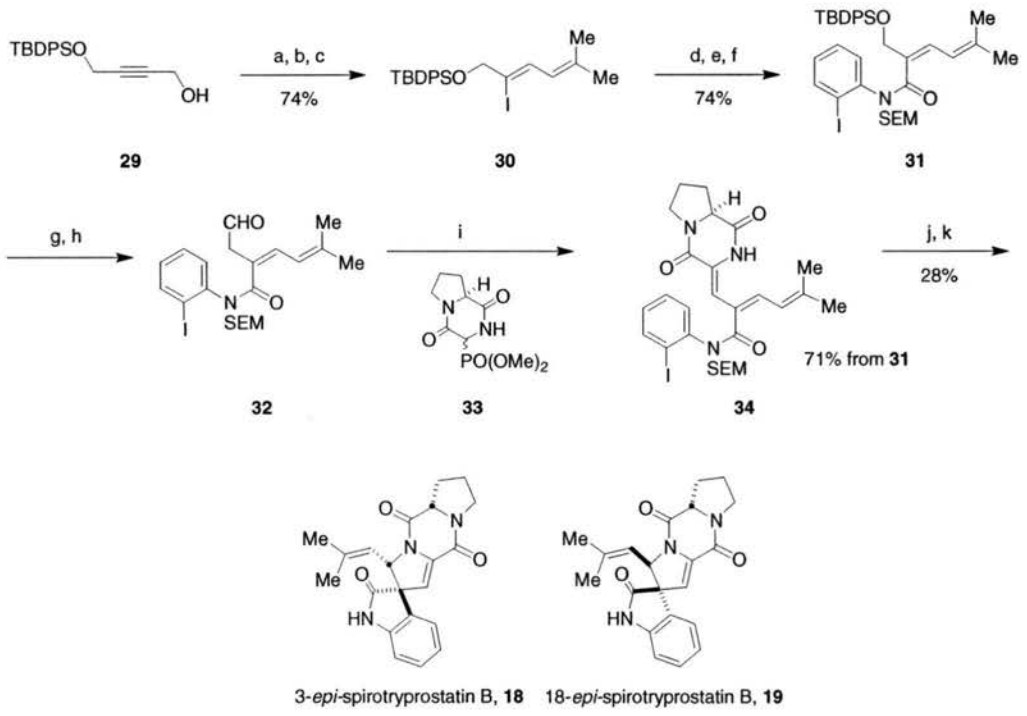
Utilizing an entirely different strategy, Overman and Rosen reported a synthesis of spirotryprostatin B in 2000.[18] The approach centered on the formation of the spirooxindole and the prenyl side-chain through an asymmetric Heck reaction followed by trapping of the η^3-π-allyl intermediate (Scheme 6). Oxidative addition of palladium into the aryl iodide bond of **27**, followed by migratory insertion would generate 28. Attack by the diketopiperazine amide nitrogen on the π-allylpalladium intermediate **28** would afford the natural product. This strategy would require the use of a chiral ligand to

induce asymmetry and would be dependent on the configuration of the triene, but would set two contiguous stereogenic centers in one step.



Scheme 6. Asymmetric Heck-η^3-allylpalladium approach to spirotryprostatin B (**2**).

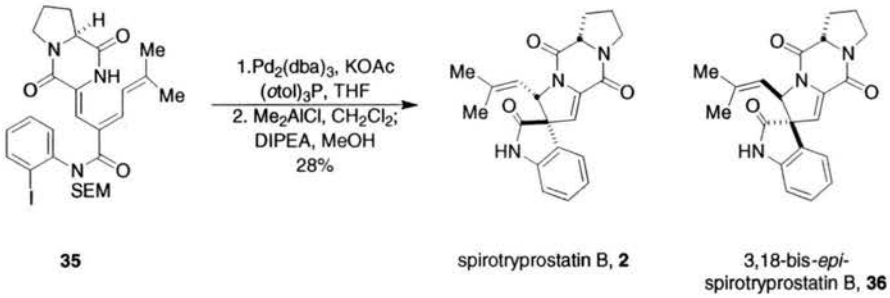
Synthesis of the requisite Heck precursor began with the protected propargyl alcohol **29**, which was converted in high yield (74% over three steps) into dienyl iodide **30** (Scheme 7). Carbonylation of **30**, coupling with 2-iodoaniline and protection of the resulting amide with SEM-Cl generated **31** in 74% overall yield. Fluoride anion-assisted removal of the silyl protecting group and Swern oxidation afforded aldehyde **32**. Olefination of **32** with the diketopiperazine phosphonate (**33**) derived from glycine and L-proline, generated the key intermediate **34** (71% yield from **31**). Palladium/(*S*)-BINAP-mediated cyclization of **34** proceeded as planned but did not produce the natural product. Rather two diastereomers were isolated and determined to be 3-*epi* and 18-*epi* spirotryprostatins B (**18** and **19**) in a 6:1 ratio and 28% yield. Similarly, (*R*)-BINAP gave the opposite isomers with similar selectivity. However, changing the configuration of the triene from *E* to *Z* would generate the natural product and the *bis*-3,18-*epi* isomer.



Scheme 7. Overman's synthesis of C-3 and C18-*epi*-spirotryprostatin B (**2**). (a) Red-Al, I_2; (b) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2; (c) *i*PrPPh$_3$I, *n*-BuLi (d) [Pd(dppf)]Cl_2, CO, MeOH; (e) 2-iodoaniline, Me_2AlCl; (f) SEMCl, NaH, DMF; (g) *n*-Bu_4NF, THF; (h) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2; (i) **33**, *t*-BuOK, [18]-crown-6, THF, -78°C; (j) $Pd(dba)_2 \cdot CHCl_3$, (*S*)-BINAP, pentamethylpiperdine, DMA; (k) Me_2AlCl, THF; DIPEA, MeOH, 100°C.

Preparation of substrate **35**, which ultimately led to the natural product, was accomplished in a similar fashion to the synthesis of the *Z*-isomer **34**. Application of the earlier developed cyclization protocol surprisingly led to the isolation of a compound that corresponded to 3-*epi*-spirotryprostatin B (**18**). Control experiments indicated that the triene underwent isomerization to the more stable *E*-configuration. Changing the ligand from (*S*)-BINAP to tri-*o*-tolylphosphine and the base from pentamethylpiperdine to potassium acetate (Scheme 8) suppressed the isomerization but removed the source of chirality (Scheme 8) . As a result, cyclization of **35** and removal of the SEM protecting group resulted in the formation of the natural product **2** along with the bis-*epi*

diastereomer **34** as a 1:1 mixture. Thus the Overman group completed the total synthesis of spirotryprostatin B (**2**) in 15% from a synthetically available precursor.

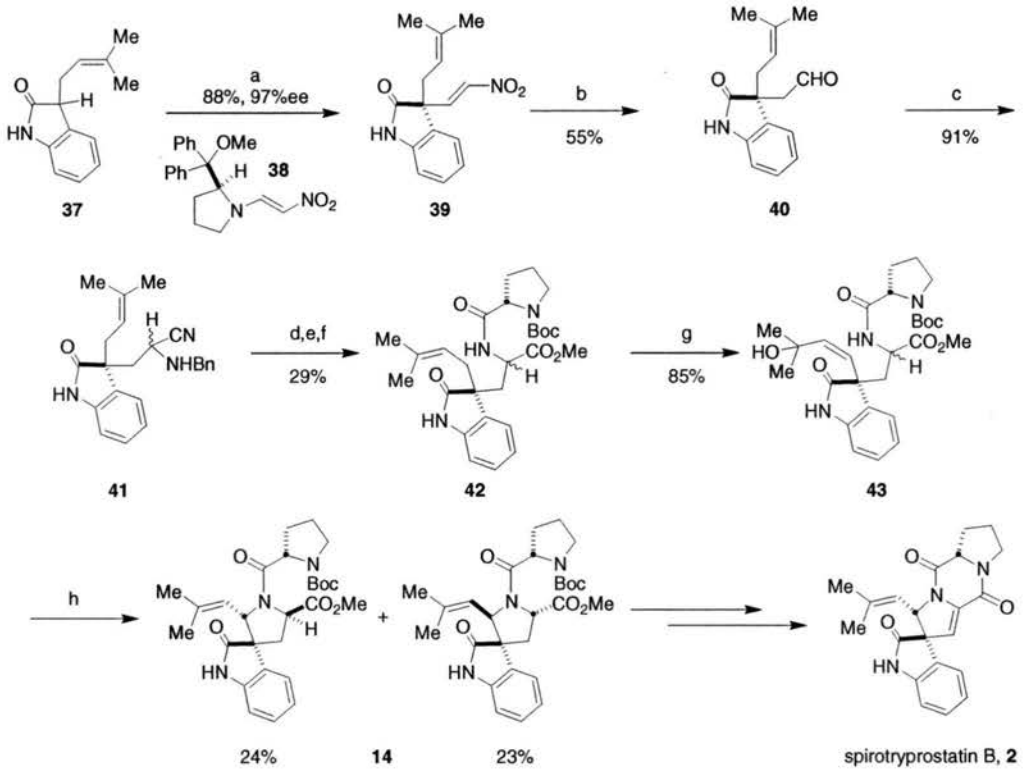


35 spirotryprostatin B, **2** 3,18-bis-*epi*-spirotryprostatin B, **36**

Scheme 8. Completion of the total synthesis of spirotryprostatin B (**2**).

1.2.4 Fuji's Formal Synthesis of Spirotryprostatin B

The most recent synthesis of spirotryprostatin B has been accomplished by Fuji and coworkers.[19] The strategy, which resulted in a formal synthesis, relied on installation of the C-3 quaternary spirooxindole at the outset of the synthesis and advancement of the key intermediate through a Strecker reaction and coupling with L-proline (Scheme 9). The synthesis began with the asymmetric alkylation of racemic oxindole **37**. Utilizing methodology developed within the Fuji group, formation of the lithium enolate of **37** and addition of nitroeneamine **38** generated the dialkylated product **39** in 88% yield and 97% ee.[23] Reduction of the nitro group and hydrolysis of the resulting eneamine afforded (*S*)-aldehyde **40** in 55% yield. Strecker reaction with TMSCN proceeded accordingly but resulted in a 1:1 mixture of diastereomers (**41**). Although the newly formed stereogenic center would eventually be eliminated to form the characteristic eneamide of spirotryprostatin B, the lack of selectivity affected the planned cyclization.



Scheme 9. Fuji's synthesis of spirotryprostatin B (**2**). (a) THF, *n*-BuLi, -30°C, **38**; (b) $TiCl_3$, NH_4OAc, MeOH, H_2O; (c) $BnNH_2$, CH_2Cl_2; TMSCN; (d) CBzCl, Et_3N, CH_2Cl_2; (e) K_2CO_3, MeOH; 1M HCl; (f) Pd black, 5% AcOH/MeOH; *N*-Boc-L-proline, EDCI, CH_2Cl_2; (g) *m*CPBA, CH_2Cl_2; PhSeSePh, $NaBH_4$, MeOH, Δ; 30% aq. H_2O_2, THF 0°C; (h) TsOH, CH_3CN, Δ.

Protection of amine **41** as the benzyl carbamate, hydrolysis of the nitrile, hydrogenolysis of the benzyl carbamate and benzyl group and coupling with Boc-L-proline generated **42** in 28% yield for the three steps. Transformation of the prenyl side-chain into allylic alcohol **43** was required prior to cyclization and formation of the core pyrrolidine ring. This was accomplished by epoxidation of the olefin with *m*CPBA, ring opening, oxidation and thermal elimination of the resulting selenoxide. Treatment of **43** with *p*-toluenesulfonic acid in acetonitrile afforded the dipeptides **14** as a 1:1 mixture of diastereomers in 47% yield. The same intermediates (**14**) were reported by Danishefsky

and therefore represented a formal total synthesis of (-)-spirotryprostatin B (**2**) Thus, Fuji and coworkers accessed the natural product in <1% over ten steps.

Four distinctly different strategies were utilized in the total syntheses of spirotryprostatins A and B. The seminal synthesis was completed by the Danishefsky group in their approach to spirotryprostatin A. The strategy resulted in a 10% yield over eight steps and was highlighted by the stereospecific spirorearrangement of *cis*-substituted tetrahydro-β-carboline **4** into an oxindole **6** (Scheme 1). This required the enantioselective synthesis of the methoxy-tryptophan derivative **3** and the tricyclic indole precursor **4**. Although, moderate selectivity was achieved in the formation of the tetrahydro-β-carboline **4**, the subsequent steps overcame any shortcomings resulting from the modest stereochemical control. The scope of the approach proved to be the weakest aspect of the synthesis as attempts to apply the strategy to the syntheses of spirotryprostatin B were unsuccessful. Still, the synthesis served as a basis for the syntheses that followed.

Ganesan and coworkers also used a spiro-rearrangment protocol in the synthesis of spirotryprostatin B but were limited by the stage at which the reaction could be applied. This forced formation of the oxindole **21** early in the synthesis and complicated attempts to install the eneamide moiety. Differentiation of the nearly identical α-protons (**22**) was unsuccessful and an extremely low yield of spirotryprostatin B was obtained (Scheme 4). The synthesis seemed to validate Danishefsky's decision to abandon this strategy.

Danishefsky et al. utilized a more direct route towards spirotryprostatin B that relied on an intramolecular Mannich reaction (Scheme 2). The synthesis was extremely

concise (five steps) but lacked stereocontrol. Arguably, a very practical synthesis (~500 mg of product was obtained from 5 g of starting material), the loss of 72% of the original mass to diastereomeric side-products limits the overall value of the approach.

Overman utilized a stereoselective Pd-catalyzed Heck insertion and trapping of the resulting η^3-allylpalladium intermediate to access spirotryprostatin B (Scheme 7). The strategy set two contiguous stereocenters in one step with good selectivity but in low yield (28%). Unfortunately, the requisite *Z*-triene precursor **35** was unstable to the conditions developed for the asymmetric system and no selectivity was observed. However, optimization increased the yield for the mixture of diastereomers to 72% (36% for (-)-spirotryprostatin B). Although the synthesis was somewhat lengthy as compared to both the Danishefsky and Ganesan approaches, it was convergent, straightforward and the highest yielding (15%).

Fuji's approach to spirotryprostatin B revolved around the enantioselective installation of the spirooxindole using an asymmetric nitroolefination (Scheme 9). This method efficiently generated oxindole **39** in high enantiomeric excess. Unfortunately the subsequent transformations suffered from a lack of stereocontrol. The Strecker reaction used to incorporate the necessary amino acid functionality resulted in a 1:1 mixture of diastereomers (**41**) that were carried on through five steps in the synthesis. The undefined stereogenic center combined with the poor yield (24%) observed for cyclization of the core pyrrolidine ring made this an inefficient strategy.

1.3 Biological Studies of Spirotryprostatin Analogs

As a result of the aforementioned synthetic efforts, a number of analogs were generated and screened for biological activity. Osada and coworkers have reported the cell cycle inhibition of mouse tsFT210 cell lines at the G2/M phase for spirotryprostatin A **1** (IC_{50} = 197.5 μM) and spirotryprostatin B **2** (IC_{50} = 14.0 μM). In general, it appears that the spirooxindole alkaloids are in most cases, less active than other members of the tryptophan-proline derived natural products. However, some analogs showed encouraging results towards human breast cancer cell lines

Danishefsky and coworkers selected various intermediates from their approach to spirotryprostatin A and tested them for biological activity. In addition to the natural product **1**, demethoxyspirotryprostatin A (**44**), compounds **45**, **46**, **47** and **48** were assayed against MCF-7 and MDA MB-468 human breast cancer cell lines (Figure 2). Spirotryprostatin A, demethoxyspirotryprostatin A (**44**) and isospirotryprostatin (**48**) did not show significant activity against MCF-7 cells and high micromolar concentrations (>100 μM) were required for the inhibition of MDA MB-468 cell lines.

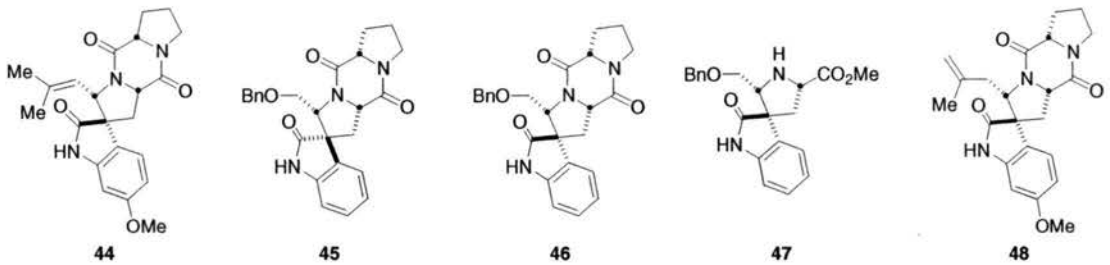


Figure 2. Intermediates tested for MCF-7 and MDA MB-468 human breast cancer cells.

However, compounds **45-47** were markedly more active against both cell lines. Against the MCF-7 cells, compounds **45-47** had IC_{50}'s in the 40-100 μM range, whereas

the same compounds inhibited anchorage dependent tumor growth of MDA MB-468 cells at concentrations of 20-25 nM. Although further studies focused on understanding the structure-activity relationship of these compounds, this data illustrated the potential of spirooxindole pyrrolidines as chemotherapeutic agents.

Intermediates from the syntheses of Ganesan and Overman were submitted to Osada et al. for testing as cell cycle and microtuble inhibitors. Compound **22** and the saturated prenyl congener from the Ganesan approach, (Scheme 1.4) did not inhibit cell progression ($IC_{50} > 500$ μM) nor did they disrupt microtubule assembly. In addition, the analogs were submitted to the National Cancer Institute's (NCI) 60-cell line in vitro antitumor assay but showed little activity as IC_{50} values for the NCI's assays were >100 μM. The two late stage intermediates from the Overman synthesis, 3-*epi* and 18-*epi*-spirotryprostatin B (Scheme 7, **18** and **19**) closely resemble the natural product and therefore were expected to have similar activity. However, the concentrations at which 50% cell cycle inhibition was achieved were markedly increased from the natural product for both analogs, 125 μM versus 14 μM respectively. Compounds **18** and **19** also showed 15% and 8% inhibition of microtubule assembly at a concentration of 250 μM, however a comparison with spirotryprostatin B cannot be made as data has not been reported for the natural product.

The spirotryprostatins have garnered a lot of synthetic attention, however the enthusiasm about these natural products has been tempered due to their modest biological activity. The structurally simpler tryprostatins and fumitremorgins seem to be at least as active and in some case more active than the spirooxindole-containing natural products. The compounds generated by Danishefsky et al. however, did show promise and could

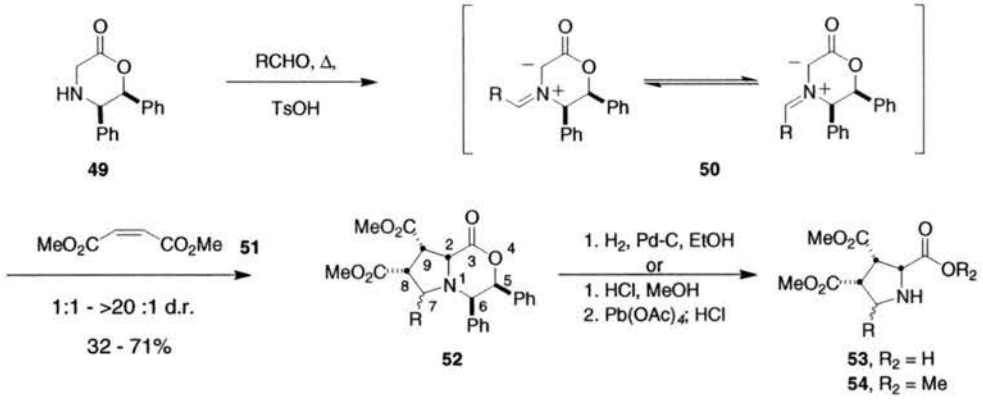
serve as leads for potential chemotherapeutic agents. The source of activity in the spirotryprostatins is still under investigation and future studies will help elucidate the structure-activity relationships of these natural products.

Chapter 2

Asymmetric, Stereocontrolled Total Synthesis and Biological Studies of (+)- and (-)-Spirotryprostatin B

2.1 Initial Synthetic Route to Spirotryprostatin B

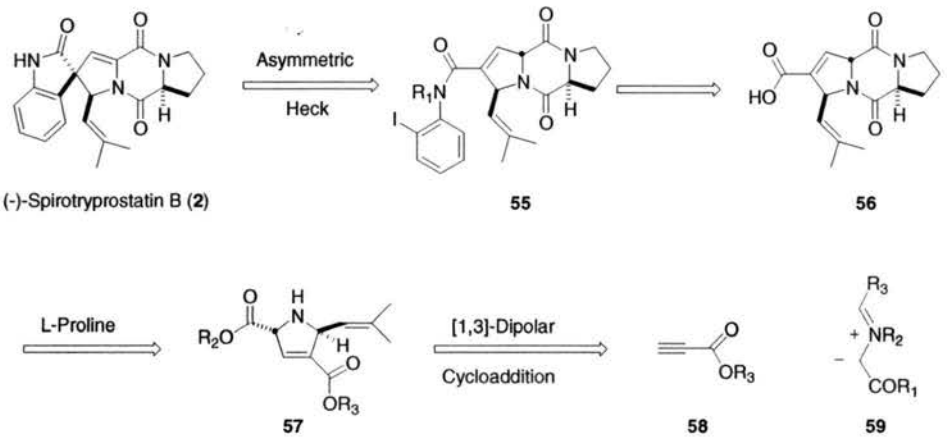
At the outset, the focus was on devising an efficient and stereocontrolled method to construct the core *spiro*-oxindole-containing pyrrolidine ring as the backbone to the synthetic strategy. The [1,3]-dipolar cycloaddition reaction ([1,3]-DPC) has proven to be a versatile and powerful method in the synthesis of natural products that possess the pyrrolidine ring system.[24] It was therefore rationalized that an asymmetric [1,3]-DPC could provide an efficient method for construction of the central pyrrolidine ring. The Williams group had previously established methodology for the asymmetric [1,3]-DPC of azomethines ylides derived from (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-ones[25] (Scheme 10).



Scheme 10. Asymmetric [1,3]-dipolar cycloaddition of chiral azomethine ylides.

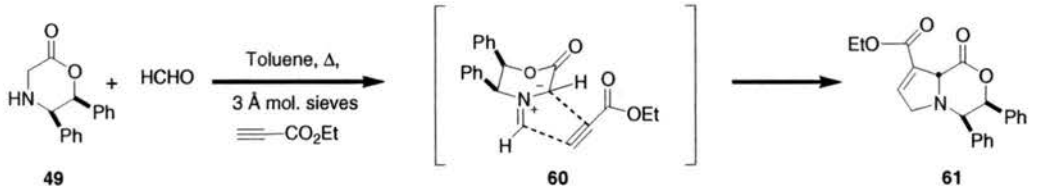
Addition of both aliphatic and aromatic aldehydes to morpholinone **49** under acidic conditions generated a mixture of *E* and *Z* ylides **50** that underwent reaction with dimethyl maleate (**51**) to provide the bicyclic cycloadducts **52** in 32-71% yield. Formation of the corresponding amino acids (**53**) or methyl esters (**54**) was accomplished by hydrogenolysis or ring-opening and either oxidative or reductive cleavage of the chiral auxiliary. In all cases the [1,3]-DPC proceeded with excellent *endo* selectivity resulting in the stereochemistry depicted. The diastereoselectivity at the C-7 position ranged from 1:1 to > 20:1 and depended on the nature of the aldehyde constituent.

The initial strategy toward (-)-spirotryprostatin B revolved around installation of the eneamide functionality and the spirooxindole stereogenic center at the last step in the synthesis through an asymmetric Heck reaction. Heck precursor **55** would result from amidation of carboxylic acid **56** with 2-iodoaniline. Diketopiperazine **56** could result from coupling of amino acid **57** with L-proline and concominant cyclization. The highly functionalized pyrrolidine (**57**) would be generated by a [1,3]-DPC of chiral azomethine ylide **59** and propiolate ester **58**.



Scheme 11. Initial retrosynthetic analysis of (-)-spirotryprostatin B **2**.

Preliminary efforts were directed at the generation of pyrrolidine **57**. Addition of paraformaldehyde to (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** and ethyl propiolate in toluene at reflux afforded cycloadduct **61** (Scheme 12).



49 60 61

Scheme 12. Cycloaddition of azomethine ylide derived from paraformaldehyde.

As expected, approach of the alkyne occurred *anti* to the bulky phenyl groups and resulted in the formation of cycloadduct **61**. However, the regiochemistry was opposite to that which was desired as the carboethoxy group was bound at the C-9 rather than the C-8 position. Single crystal X-ray diffraction indeed confirmed the structure of **61** (Figure 3). This regiochemical result represented a fatal flaw in the planned synthetic route.

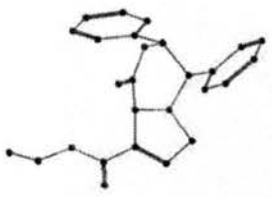
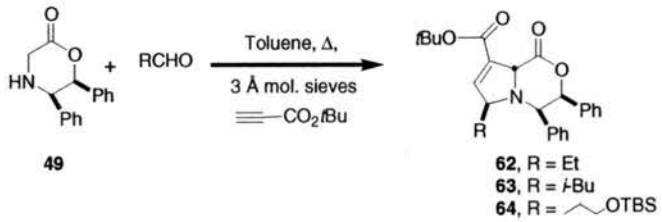


Figure 3. Single crystal X-ray of bicyclic cycloadduct **61**.

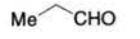
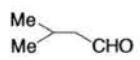
Focus shifted towards reversing the regiochemical outcome of the reaction. It seemed plausible that substituting a bulky aldehyde for paraformaldehyde might induce formation of the opposite regiochemistry (Scheme 13). *t*-Butyl propiolate was substituted



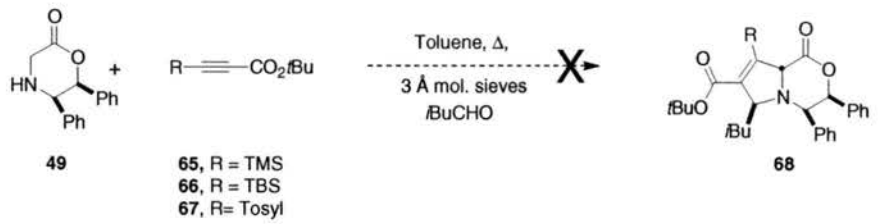
Scheme 13. Cycloaddition of azomethine ylide derived from various aldehydes.

for ethyl propiolate as a means of differentiating the ester from the lactone. Varying the aldehyde constituent did not alter the regiochemistry of the cycloadducts (Table 1) but provided some valuable data. The cycloaddition with propionaldehyde (entry 2) resulted in 7:1 ratio of diastereomers, the major product being the C-7 β-isomer. Initially, only small amounts of the product (**62**) was isolated. It was suspected that the aldehyde was not stable to the acidic conditions. Exclusion of TsOH from the reaction and addition of 3Å Molecular Sieves to remove the water generated in the reaction increased the yield to 23%. Application of these new conditions to the azomethine ylide derived from isovaleraldehyde (entry 3) resulted in only the production of the β-isomer. Although varying the components in the reaction did not solve the regiochemical problem, it suggested that the stereogenic center at C-7 could be controlled with the judicious choice of the aldehyde.

Table 1. [1,3]-Dipolar Cycloaddition of Azomethine Ylides Derived from Various Aldehydes and *t*-Butyl Propiolate

Entry	RCHO	Conditions	Product	Yield(%)	Diast. Ratio
1		TsOH, Tol. Δ	**61**	62	-
2		Tol. mol. sieves	**62**	23	7:1
3		Tol. Δ, mol. sieves	**63**	76	>20:1
4		Tol. Δ, mol. sieves	**64**	70	1:1

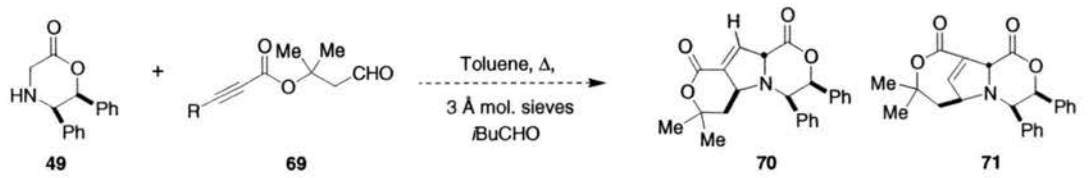
To address the regiochemical issue, three different approaches were explored. The first strategy involved a di-substituted, differentiated alkyne dipolarophile. Cycloadditions with alkynes **65-67** were expected to result in a reversal of the previously observed regiochemistry and afford cycloadducts **68** (Scheme 14). However, application of the standard [1,3]-DPC conditions did not yield the desired product. Cycloaddition with alkyne **65** did afforded compound **63**, which was a consequence of protio-desilylation followed by [1,3]-DPC to yield cycloadduct **63**. This was supported by the fact that when alkyne **66** (R = TBS), was reacted with the azomethine ylide derived from morpholinone **49** and isovaleraldehyde, only starting materials were observed. Similarly, the tosyl derivative **67** did not result in the formation of any cycloadduct. However, the desired outcome of reversing the regiochemistry was not realized and no further experiments were conducted.



49 **65**, R = TMS; **66**, R = TBS; **67**, R= Tosyl **68**

Scheme 14. Attempted [1,3]-DPC with non-symmetrical alkyne dipolarophiles.

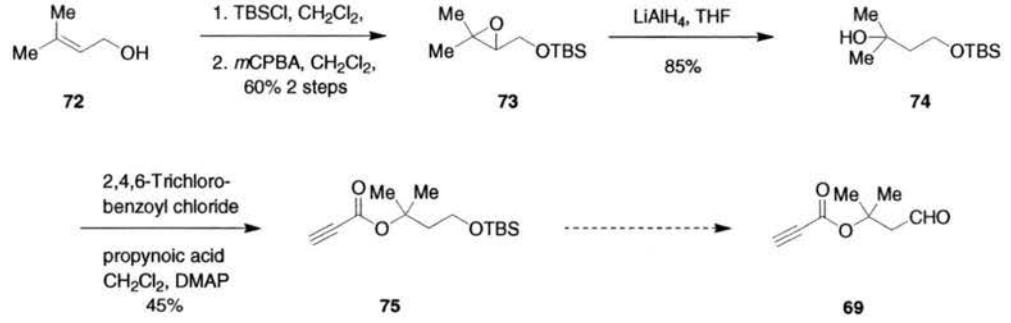
Another stategy that was explored involved tethering the alkyne directly to the aldehyde in the form of **69**. Addition of **69** to morphilinone **49**, would generated and azomethine ylide and undergo an intramolecular azomethine ylide [1,3]-DPC (Scheme 15). While the reaction could potentially result in two products (**70** and **71**), it was expected that cycloadduct **70** with the thermodynamically more stable 6-5-6 tricyclic ring system would be formed preferentially to product **71**.



49 69 70 71

Scheme 15. Intramolecular [1,3]-DPC Approach.

The ultimately unsuccessful synthesis of the requisite aldehyde (**69**) began with commercially available alcohol **72** (Scheme 16). Protection as the TBS-ether and epoxidation afforded **73** in 60% yield for the two steps. Reduction with lithium aluminum hydride resulted in tertiary alcohol **74** in 85% yield. Esterification of propynoic acid and alcohol **74** with DCC/DMAP or EDCI protocols failed to afford the desired product (**75**). Eventually it was discovered that formation of the mixed anhydride with propynoic acid and 2,4,6-trichlorobenzoyl chloride followed by the addition of alcohol **74** resulted in a 45% yield of **75**. However, attempts to deprotect the silyl-ether using acidic, basic and fluoride anion-assisted conditions all resulted in the decomposition of the starting material.

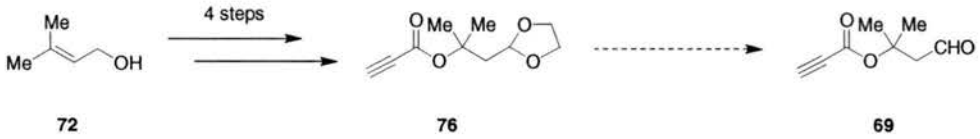


72 73 74 75 69

Scheme 16. Attempted synthesis of aldehyde **69**.

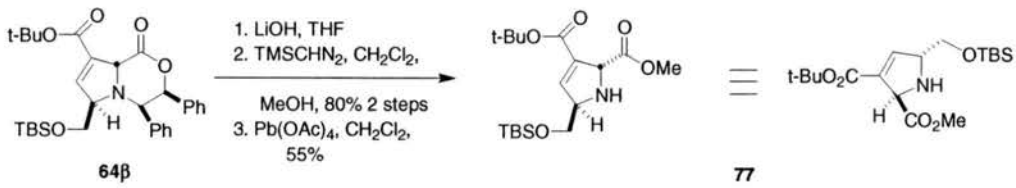
Attempts to obviate the deprotection/oxidation sequence that were necessary to access **69** were not realized. Acetal **76** was synthesized in a similar fashion to silyl-ether **75**, but also failed to afford aldehyde **69** (Scheme 17). Again, conditions that were

expected to release the aldehyde always resulted in decomposition of the starting material. As a result, the strategy was abandoned.



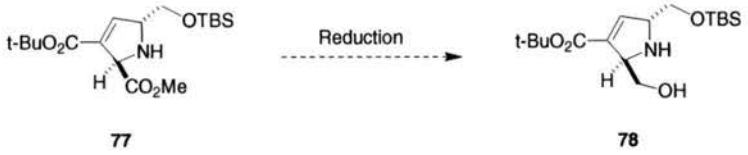
Scheme 17. Attempted synthesis of aldehyde **69**.

In parallel with the previously described strategies, another approach that utilized cycloadduct **64** (Table 1, entry 4) was explored. Although the reaction generated a 1:1 mixture of diastereomers, the β-isomer represented a compound with the desired stereo- and regiochemistry (Scheme 18). Hydrolysis of **64β** followed by esterification and lead(IV)-mediated cleavage of the resulting amino alcohol generated **77**. This intermediate has the correct regiochemistry and stereochemistry at both positions α to the amino group but would require oxidation of the silyl ether and reduction of the ester.



Scheme 18. Conversion of cycloadduct **64β** into amino acid methyl ester **77**.

The strategy required reduction of the ester functionality before manipulation of the silyl ether (Scheme 19). Conditions were explored for the reduction of **77** to alcohol **78**, however various hydride sources led to either decomposition or recovery of the starting material, Table 2. Also, **77** proved to be unstable for any extended period of time as auto-oxidation occurred and resulted in pyrrole formation.



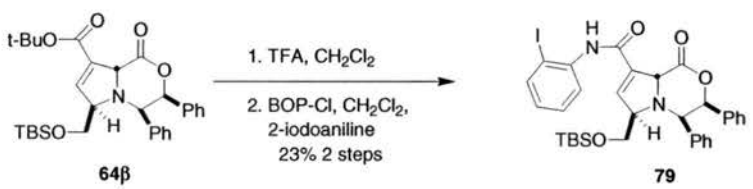
77 78

Scheme 19. Attempted reduction of amino acid methyl ester **77**.

Table 2. Conditions Explored for the Reduction of Ester 77

Entry	Conditions	Temp.(°C)	Time (h)	Result
1	BH_3·THF	0	4	Decomp.
2	DIBAL, THF	-78	4	SM
3	DIBAL, THF	-78 - 0	24	SM
4	$LiAlH_4$, THF	-78	1	Decomp.
5	$LiBH_4$, MeOH	25	1	Decomp.

It was at this stage that the potential of this strategy came into question. The failure to access amino alcohol **78**, the numerous steps required for elaboration to the natural product and the tendency of dehydroproline derivatives to undergo auto-oxidation made the planned synthetic route impractical. One last set of experiments confirmed the decision to abandon this approach (Scheme 20). Amide **79** was synthesized, by deprotection of the t-butyl ester and BOP-Cl mediated coupling with 2-iodoaniline, to test if it would serve as a suitable precursor for the asymmetric Heck reaction. Unfortunately, amide **79** decomposed before any investigations into the Heck reaction were attempted and efforts to develop a new strategy began in earnest.

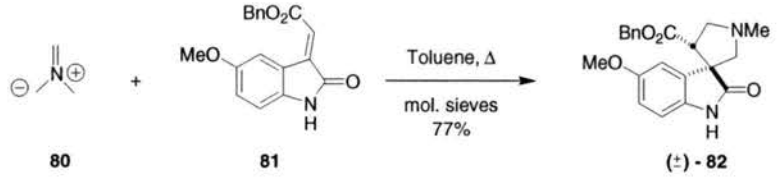


64β 79

Scheme 20. Amidation of cycloadduct **64β**.

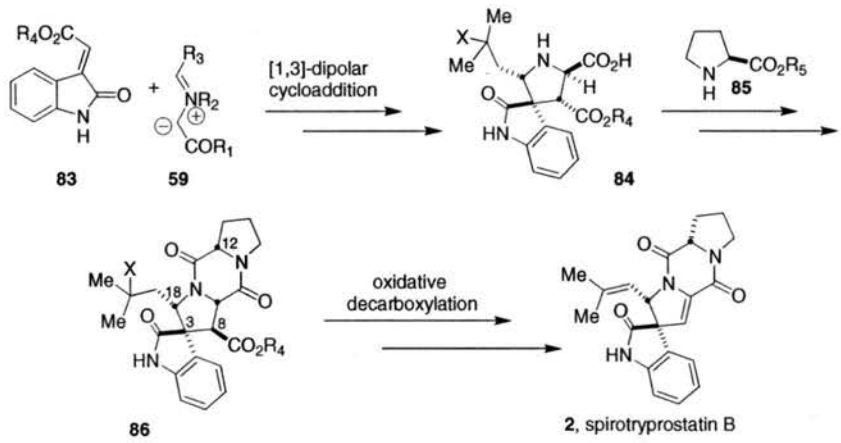
2.2 Revised Synthetic Route to Spirotryprostatin B

It was expected that an azomethine ylide derived from morpholinone **49** would still serve as an efficient template for the formation of the core pyrrolidine and a new route was devised. The strategy was based on the synthesis of (-)-horsfiline by Palmisano and coworkers in which they reacted the symmetrical ylide **80**, derived from sarcosine and paraformaldehyde, with benzyl oxindolylidene acetate (**81**) and generated the racemic spirooxindole **82** (Scheme 21).[26]



Scheme 21. [1,3]-Dipolar cycloaddition with oxindolylidene acetate **81**.

It was envisioned that an asymmetric [1,3]-DPC between a chiral azomethine ylide of the general type **59** and an oxindolylidene acetate (**83**) could, in both a relative and absolute sense, generate the desired *spiro*-amino acid **84** (Scheme 22). If successful, the reaction would generate two of the three necessary stereogenic centers in the natural product. Coupling with a suitable proline derivative (**85**) followed by cyclization would yield the diketopiperazine **86**. Having accessed the core framework of pentacyclic substance **86**, completion of the synthesis would mandate a judiciously timed oxidative decarboxylation and installation of the isoprene-derived unsaturation *via* elaboration of the pentacyclic substance **86**.



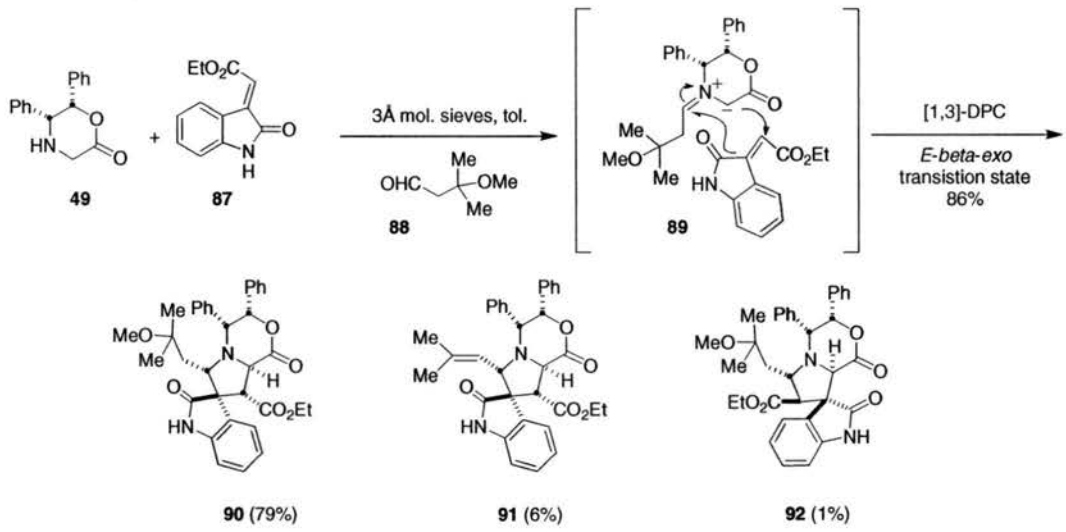
Scheme 22. General synthetic plan for the synthesis of **2**.

Numerous methods exist for the construction of *spiro*-oxindole systems related to that present in **1** and **2**.[27] The literature contains conflicting evidence as to the regio- and diastereochemical outcome of such reactions. Azomethine ylides derived from Williams' diphenyloxazinone-based glycine template and related chiral glycine-based azomethine ylide equivalents,[28] reveal that the regio- and stereochemistry of the resulting cycloadducts are dependent upon both the nature of the aldehyde and the dipolarophile. Although reactions with simple symmetrical alkenyl dipolarophiles (i.e. dimethylmaleate **51**, Scheme 10) usually proceed with a high degree of *endo*-selectivity, there are few studies that address the regiochemical aspects of asymmetrically substituted dipolarophiles. It was therefore difficult to predict if the amide or the ester moiety of the oxindolylidene acetate **83** would dominate in directing the facial approach of the dipole. In this particular instance, there are thus eight possible diastereomeric transition state structures, only one of which culminates in the desired spirotryprostatin stereostructure. With respect to the relative stereochemistry of the prenyl side-chain, the reaction was expected to be diastereoselective for the desired isomer since earlier studies suggested that bulky aliphatic aldehydes preferentially form the *E*-ylide. Assuming that the *E*-ylide

geometry would dominate in the present case, four possible diastereomers could be reasonably expected to result from the planned cycloaddition.

2.2.1 Synthesis of a Spirooxindole Amino Acid

As shown (Scheme 23) reaction of the azomethine ylide derived from oxazinone **49** and aldehyde **88**, prepared by Swern oxidation of the commercially available alcohol, with ethyl oxindolylidene acetate **87**[29] in the presence of Molecular Sieves, resulted in the formation of two cycloadducts **90** and **91** in a 1:2 ratio and 86% combined yield. The initial set of reaction conditions indeed afforded the desired cycloadduct as evidenced by ^{1}H NMR, ^{13}C NMR and nOe experiments. Additionally, a small amount of a third product **92** (1%) was produced and confirmed to be the regio- and stereoisomer opposite that of the desired cycloadduct. The relative and absolute stereochemistry of the desired cycloadduct **90** was further secured through single-crystal X-ray analysis (Figure 4).



Scheme 23. Asymmetric [1,3]-dipolar cycloaddition reaction.

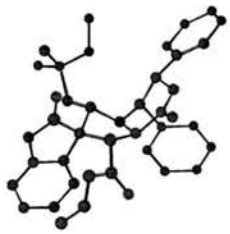


Figure 4. Single crystal X-ray of cycloadduct **90**.

The observed products suggested that approach of the dipolarophile to the azomethine ylide occurred with the carboethoxy group being positioned opposite to the bulky phenyl groups in an *exo*-fashion. The reaction must have therefore proceeded *via* an *E-beta-exo* transition state (**89**) and constructed the entire prenylated tryptophyl moiety of spirotryprostatin B in a single, simple operation (Figure 5). *E-beta-exo* refers to the preferential formation of the *E*-azomethine ylide and approach of the dipolarophile *anti* or *beta* to the phenyl groups with the carboethoxy acting in a *exo* fashion. However, the yield was far from ideal since **90** was isolated as a 1:2 mixture along with **91**, which results from the elimination of methanol from the desired cycloadduct. Therefore, additional effort was directed towards shifting the ratio of cycloadducts towards compound **90**. To minimize formation of the undesired cycloadduct, the reaction was performed at 60 °C instead of at reflux, and the yield of **90** was improved to 82% with only 6% of **91** being formed.

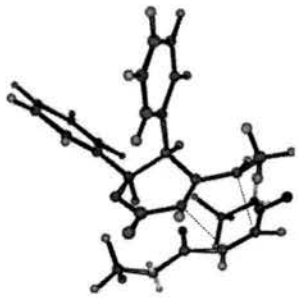


Figure 5. Minimized *E-beta-exo* transition state **89**.[30]

The possible loss of methanol from the alkene progenitor had not been foreseen, since these conditions had heretofore proven to be very mild and tolerant of a range of aldehydes. It was not clear whether the elimination was occurring during the reaction or after formation of the cycloadduct. Re-subjecting **90** to toluene at reflux in the presence of Molecular Sieves did not afford any of the eliminated cycloadduct **91** suggesting that the loss of methanol occurred from a different pathway. To complicate matters further, efforts to grow a suitable crystal of **90** for analysis also resulted in the elucidation of another cycloadduct **93** (Figure 6). To account for **93** and the other spirooxindoles (**90-92**) that resulted from the reaction mixture, a mechanism was proposed (Scheme 24).

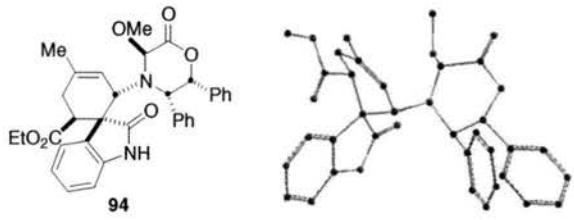
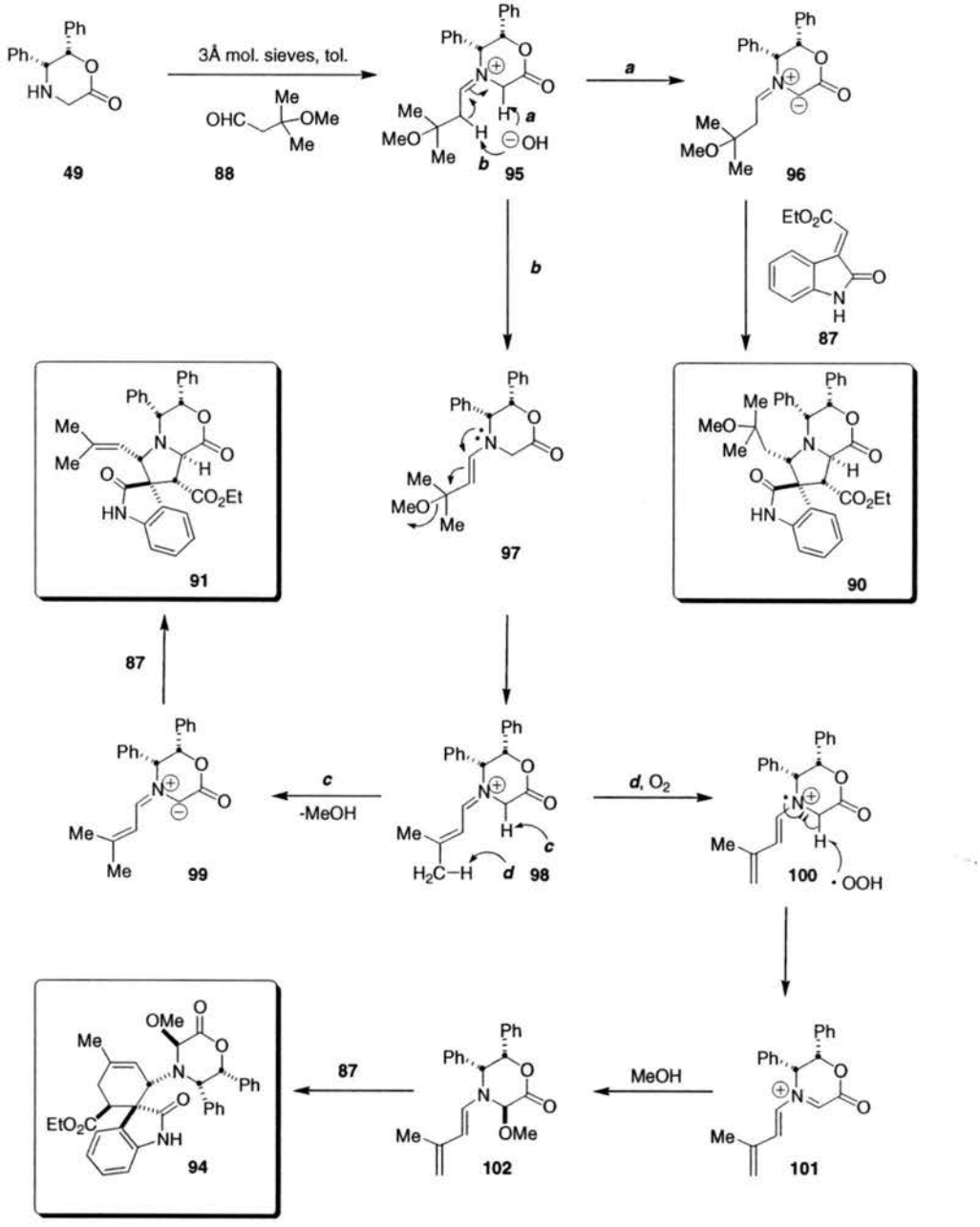


Figure 6. Structure and single crystal X-ray of cycloadduct **94**.

Addition of aldehyde **88** to oxazinone **49** should initially generate the salt **95** which could then be deprotonated α- to the lactone carbonyl (path *a*) or β- to the nitrogen atom (path *b*) to give the ylide **96** or the eneamine **97**, respectively (Scheme 18). Dipole **96** could condense with ethyl oxindolylidene acetate (**87**) to yield the desired cycloadduct **90**. If eneamine **97** was formed then, under the thermal conditions of the reaction, nitrogen-assisted extrusion of methoxide could furnish the thermodynamically more stable (relative to **96**) conjugated iminium ion species **98**. Deprotonation α- to the carbonyl (path *c*) could then generate the azomethine ylide **99** and suffer [1,3]-DPC to yield **91**. Alternatively, **98** could undergo auto-oxidation (path *d*) to yield radical-cation **100**. Further oxidation could result in the formation of iminium species **101** which could

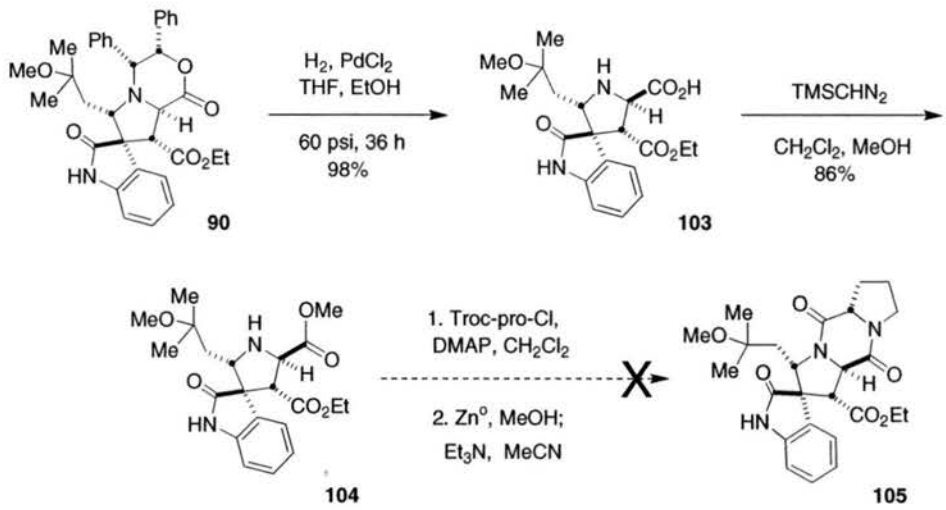
be trapped by methanol with approach of the nucleophile facially opposite the bulky phenyl substituents to give **102**. Diene **102** could then react with ethyl oxindolylidene acetate **87** via a 4+2 cycloaddition to yield side-product **94**.



Scheme 24. Mechanism proposed for the formation of **90**, **91** and **94**.

2.2.2 Elaboration to the Diketopiperazine

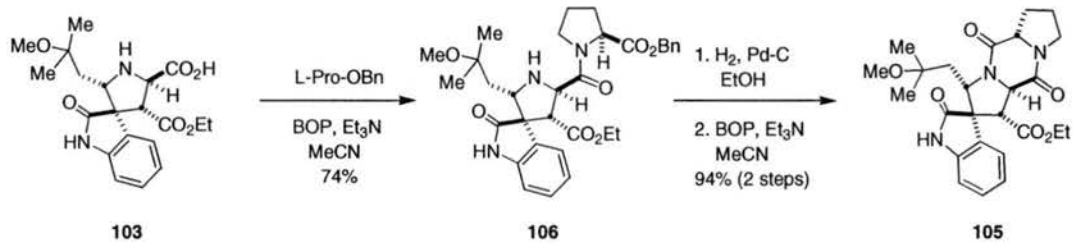
With the key *spiro*-tetracyclic intermediate **90** in hand, focus shifted to construction of the diketopiperazine (Scheme 25). Reductive cleavage of the chiral auxiliary afforded carboxylic acid **103**, which was esterified with $TMSCHN_2$ to yield the corresponding methyl ester **104** in 86% yield. Attempts to acylate the nitrogen of the pyrrolidine ring of **104** failed under a number of conditions. Only trace amounts of the desired product **105** were ever obtained as the reaction was complicated by acylation of the oxindole nitrogen. The decreased nucleophilicity of the pyrrolidine nitrogen can be attributed to the surrounding steric bulk. The *anti*- configuration of the ester and isopropylidene groups α- to the amine effectively blocking each face of the nitrogen from acylation.



Scheme 25. Elaboration of cycloadduct **90** and attempted acylation.

This initially discouraging result eventually became an asset, since it was realized that the steric hindrance about the nitrogen might allow for direct coupling on the free, zwitterionic amino acid without concomitant self-condensation. Thus, amino acid **103** was taken on crude from the preceding hydrogenation and directly coupled with L-proline benzyl ester and benzotriazol-1-yloxy-tris(dimethylamino)phosphonium

hexafluorophosphate (BOP) as the activating agent to give the dipeptide in 74% yield for the two steps (Scheme 26). Reduction of the benzyl ester followed by BOP-mediated cyclization afforded the desired diketopiperazine in excellent yield. The stage was now set for sequential installation of the two olefinic moieties.



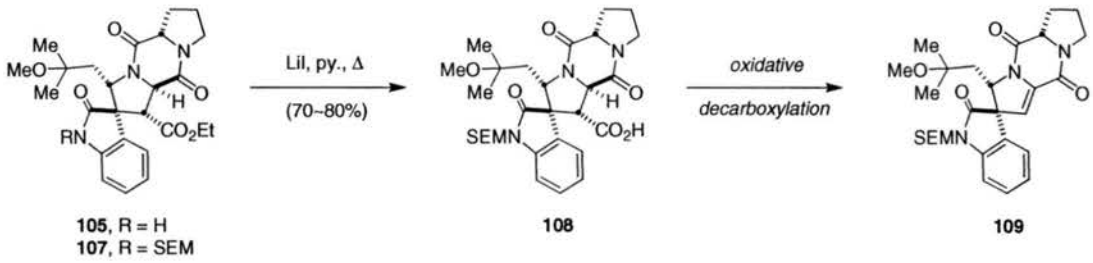
103 106 105

Scheme 26. Formation of diketopiperazine **105**.

2.2.3 Oxidative Decarboxylation

Several strategies were examined for the installation of the eneamide functionality and the prenyl side-chain. The initial plan was to first form the C-8/C-9 unsaturation and subsequently secure the C-19/C-20 olefin since the planned oxidative decarboxylation would involve an alkyl radical that might react with a proximal olefinic group. However, it was recognized that for an undesired intramolecular radical cyclization process to occur, it must proceed *via* a stereoelectronically disfavored *5-endo-trig* cyclization.[31] With these considerations in mind, attempts to effect a radical-based oxidative decarboxylation were pursued. Saponification of the ethyl ester **105** was attempted using LiOH in THF/MeOH/H_2O, but failed to afford the desired carboxylic acid. After some exploration, it was found that LiI in pyridine[32] at reflux furnished the desired carboxylic acid **105** (Scheme 27). However, attempts to promote oxidative decarboxylation either through the use of $Pb(OAc)_4$[33] or iodosobenzene diacetate[34] with carboxylic acid **105**

were unsuccessful, apparently due to the lability of the oxindole amide. If this indeed were the case, then protection of the oxindole nitrogen would prevent decomposition. Thus, **105** was converted to the corresponding SEM derivative **107**. Cleavage of the ethyl ester with LiI in pyridine at reflux furnished the corresponding carboxylic acid **108** which was subjected to Kochi-type conditions ($Pb(OAc)_4$, $Cu(OAc)_2$) generating the eneamide **109** in poor yields (10-25%).

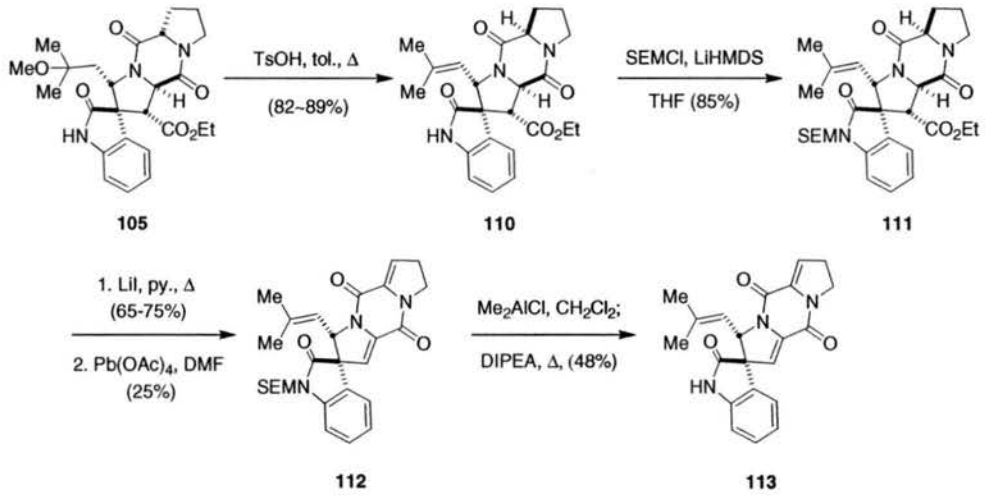


Scheme 27. Lead (IV) mediated formation of eneamide **109**.

Unfortunately, all attempts to install the C-19/C-20 unsaturation with **109** as a substrate were uniformly unsuccessful under a range of acidic elimination conditions. Although the eneamide proved to be stable to both mildly basic and acidic conditions, more vigorous conditions resulted in decomposition. These results suggested that the isopropylidene group needed to be in place prior to installation of the C-8/C-9 unsaturation. To this end, diketopiperazine **105** was subjected to treatment with TsOH in toluene at reflux, resulting in the formation of the desired olefin **110** in good yield with only trace amounts of the isomeric disubstituted olefin present (Scheme 28). As before, the SEM group was used to protect the oxindole nitrogen (**111**).

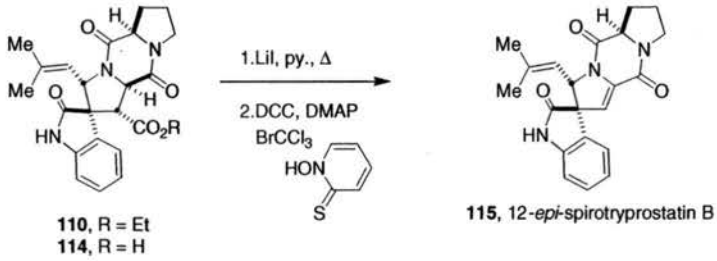
Subjecting the carboxylic acid resulting from saponification of ethyl ester **111** to a classical Kochi-type oxidative decarboxylation protocol produced the over-oxidized triene **112**. Attempts to obviate oxidation of the proline residue under a wide range of

Kochi-type conditions were unsuccessful. Deprotection of triene **112** using dimethylaluminum chloride provided an intriguing analog of spirotryprostatin B (**113**).[18]



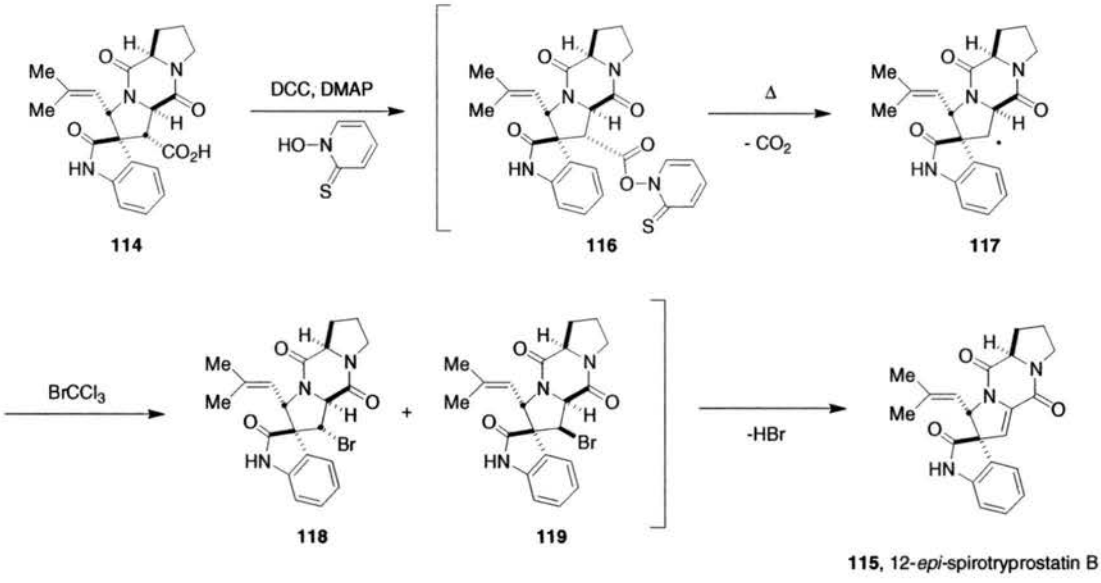
Scheme 28. Formation of undesired tri-olefinic analog **113**.

Focus turned to the examination of a Barton-modified Hunsdiecker reaction as a possible solution to the oxidative decarboxylation problem.[35] This reaction has found utility in the generation of alkyl halides. However, the application of this method for the formation of α,β-unsaturated amino acid derivatives has not been reported. The ethyl ester **110** was converted to the carboxylic acid **114** as above with lithium iodide in hot pyridine (Scheme 29). Treatment of **114** with DCC, DMAP and *N*-hydroxypyridine-2-thione yielded a product **115** whose ^{1}H NMR spectroscopic signatures closely resembled those of the natural product with the exception of slight variations in the chemical shifts of several resonances.



Scheme 30. Barton-modified Hunsdiecker reaction of **114**.

The Barton-modified Hunsdiecker protocol converted carboxylic acid **114** to olefin **115** (Scheme 31). The reaction mechanism involves radical decarboxylation of the *N*-hydroxypyridine-2-thione ester **116**, into a secondary alkyl radical (**117**) that was quenched by the solvent, $BrCCl_3$, into the corresponding alkyl bromides (**118** and **119**). Thermal elimination of HBr from **119** resulted in the formation of 12-*epi*-spirotryprostatin B (**115**).



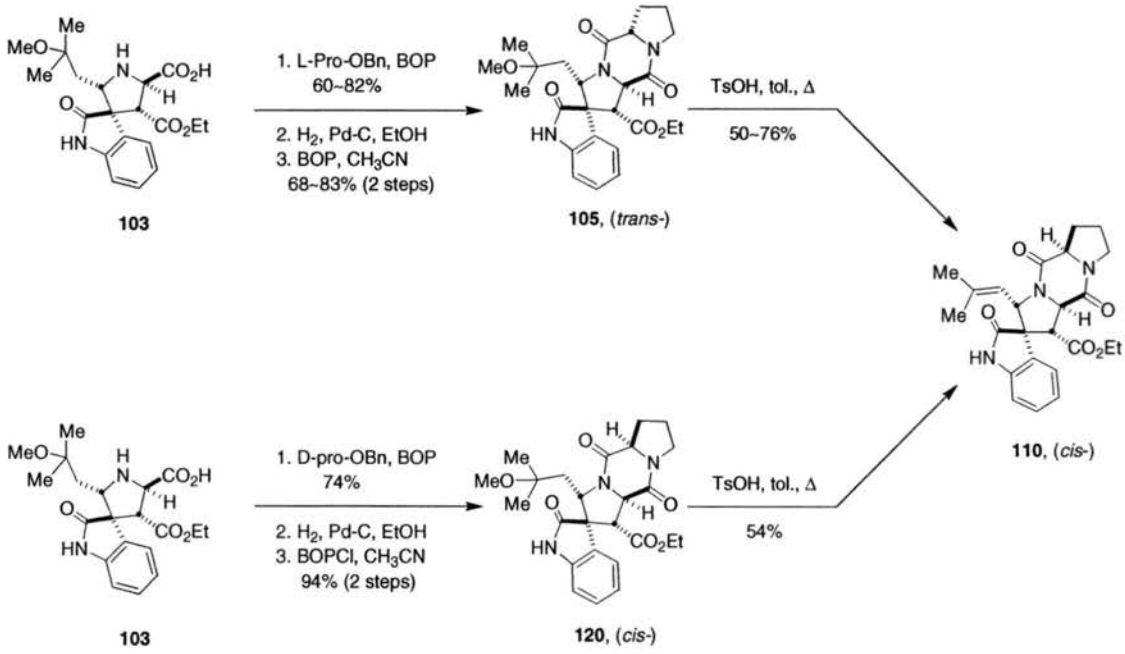
Scheme 31. Mechanism of the Barton-modified Hunsdiecker reaction.

The overall yield for this process was far from exceptional (34 - 43%) and it was possible that the formation and difference in the relative rates of elimination of the two

diastereomeric bromides might have contributed to the recovery of only moderate amounts of the desired product. It was suspected that only the bromide that was positioned *trans*-antiperiplanar to the α-hydrogen, suffered facile elimination to give 12-*epi*-spirotryprostatin B (**115**).

A comparison of the ^{1}H NMR data for the natural spirotryprostatin B and product **115** revealed discrepancies that suggested an epimerization had occurred. The absolute stereochemistry of the L-proline residue was not in doubt in the initial stages of the synthesis and both the relative and absolute stereochemistry of the spirooxindole moiety had been secured by X-ray crystallographic analysis of **90**. Thus, it was suspected that an epimerization in the proline ring had occurred, either at the stage of the elimination of methanol from **105** or during the ethyl ester cleavage, to ultimately give 12-*epi*-spirotryprostain B **115**.

To decipher at what stage the suspected epimerization reaction had occurred, the complementary D-proline-derived *cis*-diketopiperazine **120**, was constructed as shown in Scheme 32. This was accomplished in a similar fashion to that utilized for the formation of the *trans*-diketopiperazine **105**. Thus, coupling of amino acid **103** with D-proline benzyl ester (74%) followed by hydrogenation of the benzyl ester and cyclization (94% over two steps) afforded **120**.



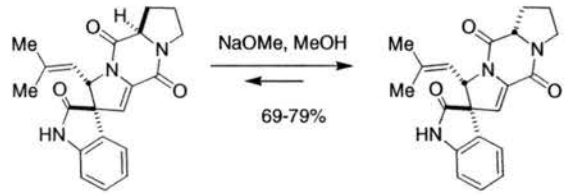
Scheme 32. Thermodynamic instability of *trans*-diketopiperazine **105.**

If the dehydration step resulted in the loss of stereochemical integrity of **105**, then subjecting the two substrates (**105** and **120**) separately to the elimination conditions would yield the same product. This indeed proved to be the case as the pentacyclic product **110** was formed exclusively from either substrate when treated with TsOH in hot toluene. It is well known that *cis*-diketopiperazines are thermodynamically more stable than the corresponding *trans*-isomers for cyclic anhydrides of proline.[36] In contrast, syntheses of the [6,6,5]-ring system of the fumetrimorgins (Figure 1) have exhibited a preference for the *trans*-configuration.[37]

2.2.4 Completion of the Total Synthesis of Spirotryprostatin B

With the stereochemical issues clarified, focus returned to the task of converting 12-*epi*-spirotryprostatin B (**115**) into the natural stereoisomer (Scheme 33). Addition of NaOMe in MeOH at 0°C yielded an equilibrium mixture of spirotryprostatin B (**2**) and

12-*epi*-spirotryprostatin B (**115**) in a 2:1 ratio. These diastereomers were easily separated by chromatography and the recovered **115** could be re-subjected to the epimerization protocol giving **2** in 62% overall yield for the two cycles. The synthetic and natural specimens of (-)-spirotryprostatin B displayed identical spectroscopic data including optical rotation. In like fashion, (+)-*ent*-spirotryprostatin B was synthesized starting with the opposite antipode of **49**.[17]



115, 12-*epi*-spirotryprostatin B **2**, spirotryprostatin B

Scheme 33. Thermodynamic epimerization of **115** to **2**.

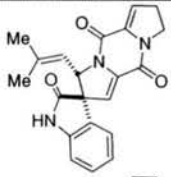
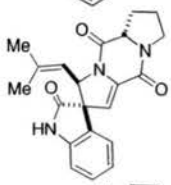
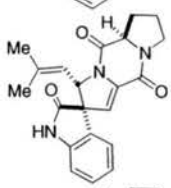
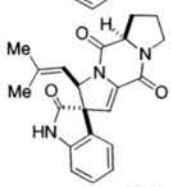
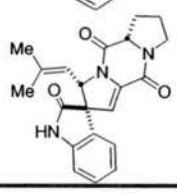
2.3 Biological Testing

The effects of compounds **90**, **103-106**, **110**, **113-115**, their enantiomers, and *ent*-spirotryprostatin B on cell cycle control and microtuble assembly were examined by Osada and coworkers. Given the moderate activities of the title compounds (IC_{50} = 14.0 μM for spirotryprostatin B), it was not surprising to find that all of the spirotryprostatin analogs prepared in this study that were tested had no effect on *in vitro* microtubule assembly and had little or no effect on *in vitro* cell cycle inhibition. Three compounds (**115**, *ent*-**115**, and *ent*-**2**) did however, provide some intriguing results.

12-*epi*-Spirotryprostatin B (**115**) caused partial accumulation of cells at the G_2/M phase at concentrations of 125 μM but was toxic to 3Y1 and tsFT210 cells at 250 μM or higher concentrations (Table 3). The enantiomer of **115** was however, neither toxic to the

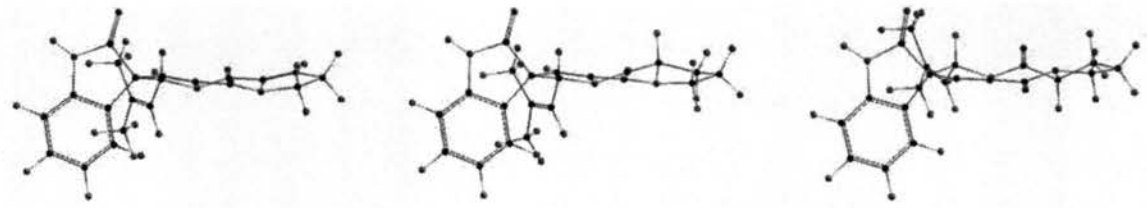
cells nor showed any activity for cell cycle proliferation and microtuble assembly. Similar results were seen in the testing of spirotryprostatin B (**2**) and *ent*-**2**. Spirotryprostatin B has been reported to inhibit tubulin polymerization and to be cytotoxic to mammalian cells[6] whereas *ent*-**2** had no effect on *in vitro* microtubule assembly or *in vitro* cell cycle inhibition but was toxic to 3Y1 and tsFT210 cells at 31.3 μM and 15.6 μM concentrations, respectively. These data suggest that the molecular target of *ent*-spirotryprostatin B is different from that of the natural enantiomer.

Table 3. Biological Activity of Selected Analogs for G_2/M Phase Inhibition.

Compound #	Structure	G_2/M Phase Accumulation	tsFT210 Cells IC_{50}	3Y1 Cells IC_{50}
113		None	Inactive	Inactive
Ent-**115**		None	Inactive	Inactive
115		Partial @ 125 μM	> 250 μM	> 250 μM
Ent-**2**		None	15.6 μM	31.3 μM
2		Accumulation at the G_2/M Phase	14 μM	Not Reported

It is also interesting to note that the slight variation in structure caused by going from *epi*-spirotryprostatin B (**115**) to the dehydro-derivative **113** and eventually to spirotryprostatin B (**2**) resulted in a dramatic change in the activity. A comparison of the minimized structures for **113**, **115** and the natural product (**2**) reveal only subtle differences (Figure 7). The configuration (or lack thereof) of the α-proton does not seem to induce any dramatic changes in the confirmation of the molecules as the diketopiperazine is relatively planar in all cases. There is not any obvious structure-activity relationship between the three molecules. It was suspected that 12-*epi*-spirotryprostatin B (**115**) might be less active than the natural product (**2**) due to the difference in stereochemistry of the proline stereogenic center, however it seemed logical that if indeed this was true then dehydrospirotryprostatin B **113**, which more closely resembled the natural product (**2**), would show some intermediate activity. This was not the case however, the discrepancy could be a result of the instability of **113**. After any prolonged time, the compound began to turn from an off white amorphous solid to a yellowish, sticky solid. This seems likely, as there are two Michael-acceptors in the **113** as compared to just one for both 12-*epi*-spirotryprostatin B and spirotryprostatin B.

Aside from the absolute configuration of the proline stereogenic center, only the position of the prenyl side-chain in the natural product represents any differences in the three structures. In the active cell-cycle inhibitor spirotryprostatin B (**2**) the olefin is "higher" relative to the diketopiperazine than in both the moderately active 12-*epi*-spirotryprostatin B **113** and the inactive dehydro-congener **115**. The significance of this observation is questionable, yet there are reports that suggest that unsaturation in this scaffold is an important factor in the cell cycle inhibition activity of these molecules.[8]



113 **115** **2**

Figure 7. Comparison of minimized conformations for 113, 115 and 2.

2.4 Conclusion

The syntheses of both antipodes of spirotryprostatin B (**2**) have been achieved utilizing a diastereoselective, asymmetric [1,3]-dipolar cycloaddition reaction as the key step. This strategy sets four contiguous stereogenic centers and generates the core spirooxindole pyrrolidine in one step. In addition, a tertiary methyl ether was demonstrated to serve as a suitable progenitor of the prenyl group, providing an alternative method for the introduction of the isopropylidene functionality. Elaboration to the *trans*-diketopiperazine **105** was accomplished by coupling and cyclization with L-proline benzyl ester which was shown to be thermodynamically unstable. Subjecting the *trans*-diketopiperazine system to acidic dehydration conditions resulted in epimerization and the preferential formation of the diketopiperazine with the *cis*-configuration. Various methods for the installation of the characteristic eneamide functionality via oxidative decarboxylation were explored. The Barton-modified Hunsdiecker reaction was eventually found to provide the desired effect and afforded 12-*epi*-spirotyrpostatin B (**115**). Epimerization under basic conditions generated the natural product and completed the asymmetric total synthesis of (+)- and (-)-spirotryprostatin B (**2**).

Intermediates along the route were assayed for their activity as cell cycle and microtubule assembly inhibitors. The analogs tested did not show any improved activity over that of the natural products **1** and **2**. However, these studies did suggest that the *ent*-spirotryprostatin B and the 12-*epi* isomer are not acting via the same mechanism as the natural product.

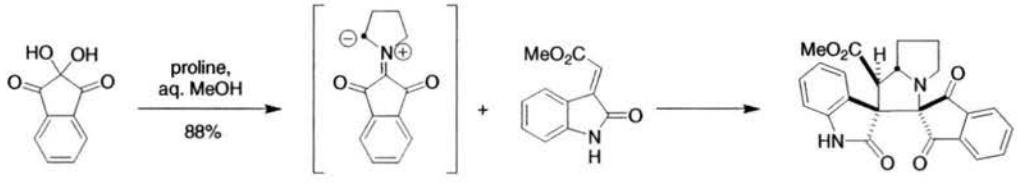
Chapter 3

Asymmetric Azomethine Ylide [1,3]-Dipolar Cycloaddition with Ethyl Oxindolylidene Acetate

3.1 Introduction

The [1,3]-dipolar cycloaddition reaction ([1,3]-DPC) is a versatile and powerful method for the synthesis of natural products that possess the pyrrolidine ring system.[24] The reaction generates highly substituted proline derivatives in one simple step. The transformation has found application in the construction of spiroxindole pyrrolidine containing natural products as evident by the total syntheses of horsfiline[26,27] and spirotryprostatin B.[15]

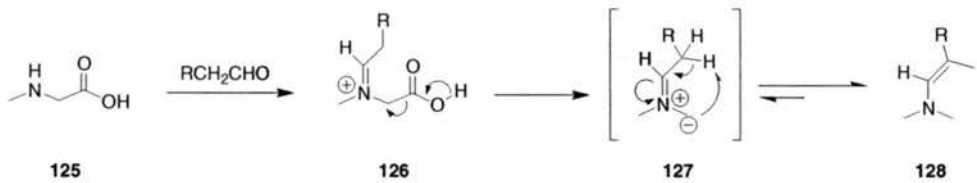
Grigg and coworkers were the first to demonstrate the utility of the [1,3]-DPC reaction of azomethine ylides and oxindolylidene acetates (Scheme 27). Addition of L-proline to ninhydrin (**121**) generated the azomethine ylide **122**, upon decarboxylation, which reacted with methyl oxindolylidene acetate **123** to afford racemic spirooxindole pyrrolidine **124**. Since this initial publication, numerous groups have exploited this decarboxylative approach for the generation of azomethine ylides and the synthesis of highly functionalized pyrrolidines.[4]



121 **122** **123** (±) **124**

Scheme 34. Cycloaddition of an azomethine ylide with oxindolylidene acetate **123**.

The synthesis of the core of spirotryprostatin B represented the first time a chiral azomethine ylide had been utilized in the synthesis of spirooxindole pyrrolidines. The reaction involves a stabilized azomethine ylide, which offer an advantage to non-stabilized ylides in that decarboxylation is not required. This allows utilization of the carboxy group in a chiral template such as **49**. Secondly, it expands the scope of substrates that can be used for ylide formation. With the decarboxylative approach, azomethine ylides derived from aldehydes are prone to eneamine formation (Scheme 35). Addition of an aldehyde with α-protons, such as sarcosine (**125**), generates an iminium ion **126** which decarboxylates to give [1,3]-dipole **127**. The azomethine ylide can then undergo tautomerization to the more thermodynamically stable eneamine **128**. Stabilized azomethine ylides are less prone to eneamine formation as a manifestation of resonance of the carbanion into the carboxy group.



Scheme 35. Eneamine formation with non-stabilized azomethine ylides.

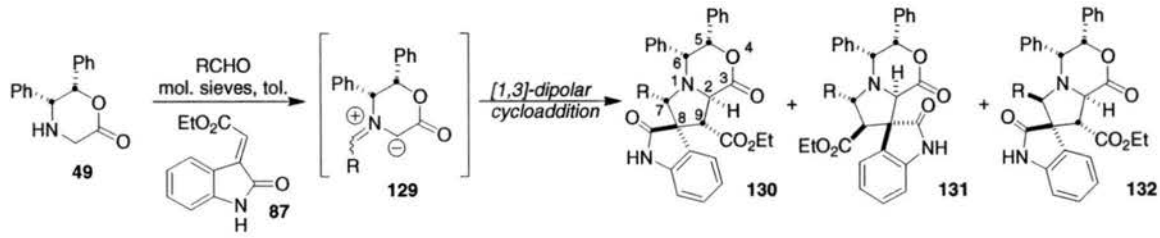
In the synthesis of spirotryprostatin B, the use of the bulky aldehyde 3-methoxy-3-methylbutanal **88** resulted in preferential formation of the *E*-ylide and high diastereoselectivities at the resulting stereogenic center. It was suspected that less sterically demanding aldehydes would result in lower selectivities and possibly products with different regiochemistry. The [1,3]-DPC reaction of azomethine ylides derived from various aldehydes with ethyl oxindolylidene acetate **87** were investigated. Conversion of

the resulting cycloadducts to the corresponding amino acid methyl esters was also accomplished.

3.2 Azomethine Ylides Derived from Various Aldehydes

The amine source for this investigation was (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** (Scheme 36). Addition of an aldehyde to morpholinone **49** generates the azomethine ylide (**129**). In the presence of the ethyl oxindolylidene acetate (**87**), a [1,3]-DPC reaction occurs stereoselectively to furnish spirooxindole pyrrolidines. For the five aldehydes tested in this study, three products were observed (**130**, **131** and **132**). The specific examples, reaction temperature, yields and diastereomeric ratio of **130** to **132** are recorded in Table 4.

The regio- and stereochemistry of the resulting cycloadducts were dependent on the nature of the aldehyde constituents. Bulky aldehydes favored the formation of the *E*-ylides and resulted in the preferential formation of cycloadducts **130**, (entries f-h). Isobutyraldehyde was expected to follow this trend, however the reaction produced three products **130**, **131** and **132** and resulted in an 8.6:1 diastereomeric ratio of **130**:**132**. For the less branched systems high diastereoselectivity resulted (>20:1), however only moderate *exo*-selectivity with respect to the carboethoxy group (*endo* for the oxindole carbonyl) was observed resulting in mixtures of **130** and **131** (entries a-c). The azomethine ylide generated from paraformaldehyde yielded three products, **130**, **131**, and ***endo*-130**. Product ***endo*-130**, which was a result of approach of the ester in an *endo*-fashion, was isolated in 9%.



Scheme 36. [1,3]-Dipolar cycloaddition reaction with various aldehyde constituents.

Table 4. Spirooxindole Pyrrolidine Cycloadducts 130, 131 and 132

entry	aldehyde	temperature	yield (% **130**)	yield (% **131**)	yield (% **132**)	diast. ratio (**130**:**132**)
a	Paraformaldehyde[a]	reflux	28	11	0	-
b	benzyloxy-acetaldehyde	reflux	44	14	0	>20:1
c	benzyloxy-acetaldehyde	60°C	54	8	0	>20:1
d	isobutyraldehyde	reflux	43	11	5	8.6:1
e	isobutyraldehyde	60°C	74	6	Trace	>20:1
f	isovaleraldehyde	reflux	84	1	0	>20:1
g	isovaleraldehyde	60°C	86	0	0	>20:1
h	*p*-anisaldehyde	reflux	60	0	0	>20:1

a) An additional product was also isolated in 9% yield that proved to be the regiochemically identical cycloadduct to **131**, but as a result of approach of the dipolarophile in an *endo* approach.

Reaction temperature also seemed to affect the regiochemistry and stereochemistry of the reaction. In the case of isobutyraldehyde, moderate regioselectivity and diastereoselectivity was obtained when the reaction was performed under refluxing toluene conditions. When the temperature of the system was lowered to 60°C, the ratio of cycloadducts **130** and **131** was increased from ~4:1 to ~12:1 and the diastereomeric ratio of products **130** and **132** improved to greater than 20:1 (entries d-e). In contrast,

cycloaddition of the ylide derived from *p*-anisaldehyde required refluxing conditions for the reaction to occur (entry h). Presumably, the electron donating effect of the methoxy group attenuates the electrophilicity of the aldehyde by the incoming nucleophile and requires elevated temperature for the formation of the azomethine ylide.

The regiochemistry of the cycloadducts was easily determined by the multiplicity of the ^{1}H NMR signal for the C2 hydrogen; a doublet was observed in the case of cycloadducts **130** and **132** whereas as singlet resulted for cycloadduct **131**. The relative and absolute stereochemistry was determined by difference nOe ^{1}H NMR spectroscopy and correlation to the known stereocenters (C5 and C6) of the starting material (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (Figure 8).

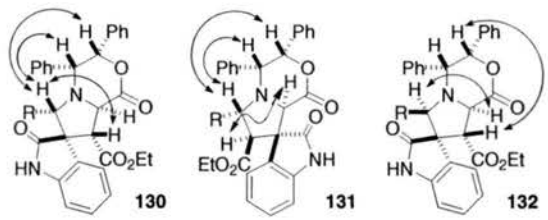


Figure 8. Observed nOe enhancements for cycloadducts **130**, **131** and **132**.

In conjunction with Professor Gyooson Park of Kookmin University in Korea, the relative energies for four of the possible eight transition state energies have been calculated. The transition state energies are for the [1,3]-DPC reaction of the azomethine ylide derived from acetaldehyde with methyl oxindolylidene acetate (Figure 9). This system was chosen as it was the simplest system that closely resembled the real system and would require the least amount of CPU times. Preliminary AM1 calculations suggested that only the *E*-ylide was about 1.0 Kcal more stable than the corresponding *Z*-azomethine ylide. Although the data does not match exactly with the experimental

results, the calculations did predict that transition state **133**, which corresponds to product **130**, would be the most stable. Transition states **134-136** were calculated to be between 3.0 and 4.4 Kcal higher than **133** and the corresponding products would not be expected to be isolated in significant quantities. Experimentally cycloadduct **131**, which corresponds to transition state structure **134**, was produced in 1-14%. One explanation for this discrepancy is that the calculations were based on room temperature, whereas the reactions were performed at 60°C or at 110°C (refluxing toluene).

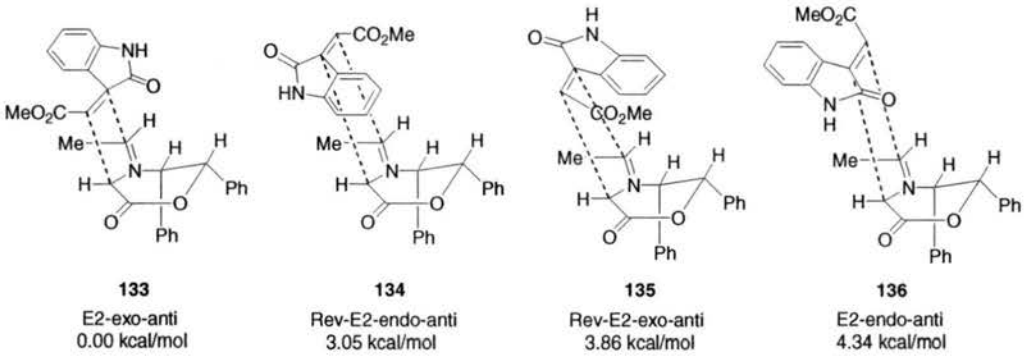
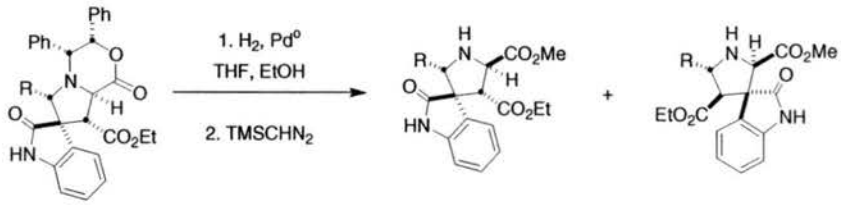


Figure 9. AM1 calculation of four possible transition state energies.

Conversion of the tetra-cyclic products into the corresponding spirooxindole-substituted proline derivatives could be accomplished by catalytic hydrogenation (Scheme 37). For characterization purposes, the amino acids were converted to the corresponding methyl esters. Hydrogenolysis of the chiral auxiliary was accomplished in most cases with palladium chloride at room temperature and elevated pressures (70 psi of hydrogen, Table 3). However, *p*-anisaldehyde derivative **130h** proved resistant to these conditions and only partial reduction was observed, (entry 5). Elevated temperatures resulted in a complex mixture of products. Pearlman's catalyst, which has been shown to selectively reduce the benzylic C-N bond of an unsubstituted aromatic in the presence of

a *p*-methoxy derivative,[38] failed to dramatically improve formation of the desired product. The major product proved to be the partially reduced compound. Addition of 1N hydrochloric acid to the reaction mixture[39] resulted in the complete removal of bibenzyl (entry 6). However, small amounts of epimerization at the α-position and cleavage of the pyrrolidine C-N bond were observed along with 59% of the desired product. It is noteworthy to mention that any attempt to remove the chiral auxiliary via an oxidative protocol, such as $Pb(OAc)_4$ or $NaIO_4$ resulted in decomposition of the starting material. The oxindole moiety presumably interferes with the oxidizing agents.



Scheme 37. Hydrogenolysis and esterification of cycloadducts **130** and **131**.

Table 5. Conversion of dipolar cycloadducts into amino acid methyl esters 137 and 138

entry	substrate	method	yield (%)
1	**130**a	H_2, $PdCl_2$	93
2	**131**a	H_2, $PdCl_2$	73
3	**130**f	H_2, $PdCl_2$	89
4	**130**h	H_2, $PdCl_2$	5
5	**130**h	H_2, $Pd(OH)_2$	25
6	**130**h	H_2, Pd-C, 1N HCl	59

3.3 Conclusion

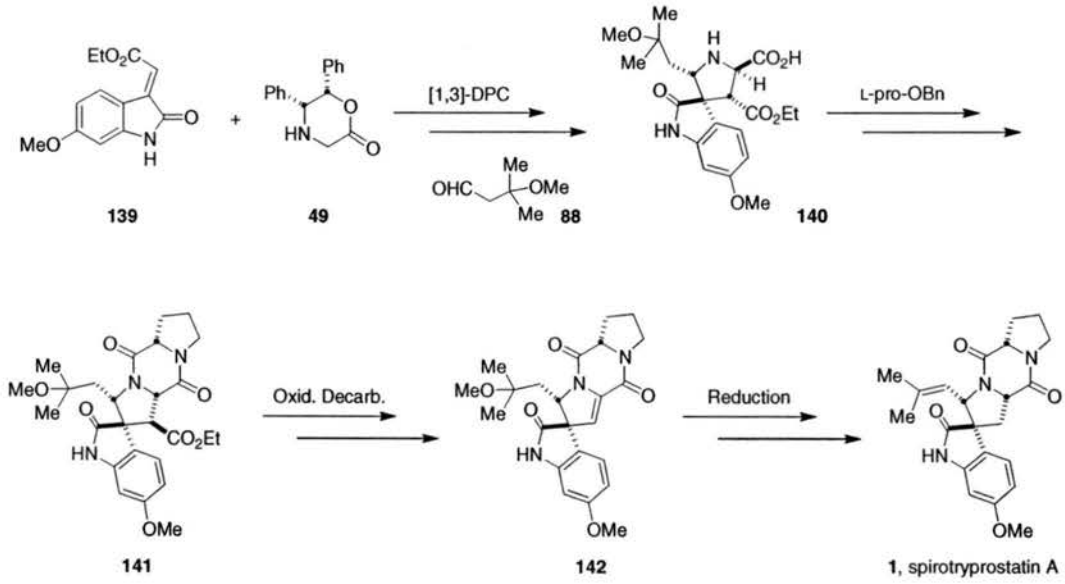
In summary, the asymmetric synthesis of spirooxindole substituted pyrrolidines via the [1,3]-dipolar cycloaddition of azomethine ylides derived from a chiral, non-racemic glycinate and ethyl oxindolylidene acetate are described. The reaction is, in most cases, *exo*-selective for the carboethoxy group of the dipolarophile and sets three or four contiguous stereocenters including the quaternary carbon of a spirooxindole. Two regioisomers were isolated in good to excellent diastereoselectivity for four out of five of the reactions. Computational experiments on a simplified version of this system were done and roughly matched the results observed experimentally. Selected cycloadducts were also converted to the corresponding amino acid methyl ester and highlighted the [1,3]-DPC reaction of asymmetric azomethine ylides with oxindolylidene acetate as an efficient method for the synthesis of spirooxindole-substituted proline derivatives.

Chapter 4

Progress Towards the Synthesis of Spirotryprostatin A

4.1 Initial Synthetic Route to Spirotryprostatin A

The strategy developed for (+)- and (-)-spirotryprostatin B also seemed applicable to the total synthesis of spirotryprostatin A (Scheme 38). The approach would have to account for the substitution of the aromatic ring and the formation of the fourth stereogenic center. [1,3]-DPC reaction with methoxy-substituted oxindolylidene acetate **139** would yield spirooxindole pyrrolidine amino acid **140** upon reductive cleavage of the chiral auxiliary. Coupling of **140** to L-proline benzyl ester and concomitant cyclization would afford diketopiperazine **141**. Hydrolysis of the ester followed by a Barton-modified Hunsdiecker reaction would result in the formation of eneamide **142**.

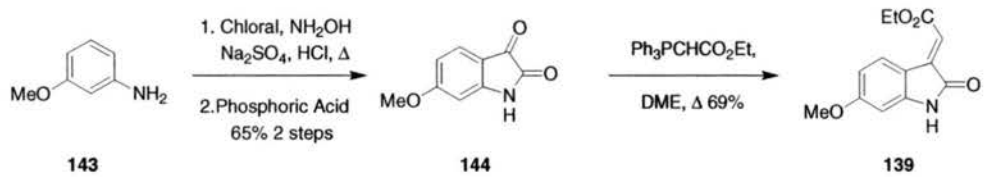


Scheme 38. Initial strategy to spirotryprostatin A (**1**).

Palladium-catalyzed reduction of the olefin would occur opposite the isopropylidene group and the aromatic ring to form the *cis*-diketopiperazine. Acid-catalyzed elimination would then afford spirotryprostatin A (**1**).

4.1.1 Sandmeyer Synthesis of 6-Methoxyisatin

In the synthesis of spirotryprostatin B, ethyl oxindolylidene acetate was synthesized by Wittig reaction of commercially available 1H-indole-2,3-dione (isatin) and the requisite stabilized ylide. For spirotryprostatin A, 6-Methoxyisatin (**144**) was not commercially available and necessitated preparation. The Sandmeyer reaction provided an efficient method for the synthesis of **144** (Scheme 39).[40] Addition of 2,2,2-trichloroethane-1,1-diol (chloral) to *m*-anisidine (**143**) in the presence of hydroxylamine and acid, followed by polyphosphoric acid induced cyclization, generated **144** in 69% yield. Wittig reaction with (carbethoxymethylene)triphenyl phosphorane then afforded ethyl 6-methoxyoxindolylidene acetate **139**. Crystallization of the product mixture afforded only the *E*-isomer.



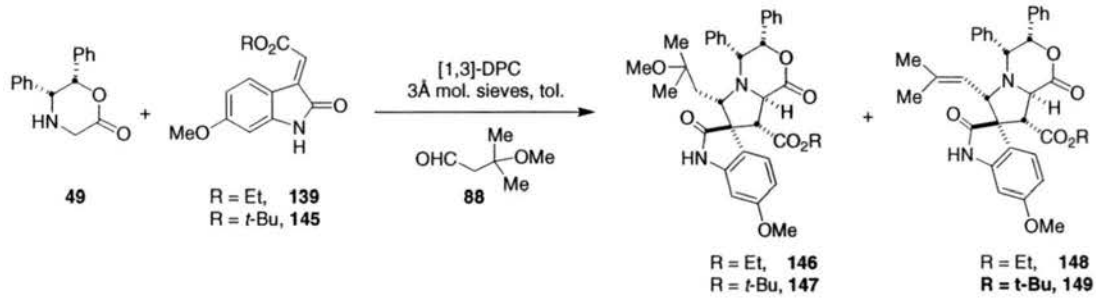
143 **144** **139**

Scheme 39. Synthesis of starting material **139** by Sandmeyer and Wittig reactions.

4.1.2 Cycloaddition and Elaboration to the Diketopiperazine

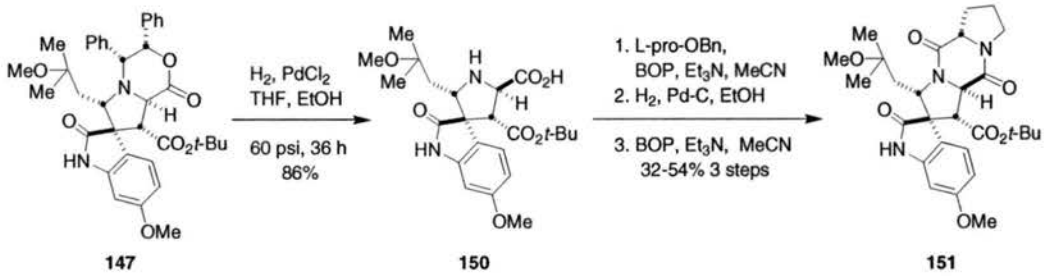
Addition of dipolarophile **139** to the azomethine ylide derived from morpholinone **49** and aldehyde **88** yielded cycloadducts **146** and **148** (Scheme 40). The reaction

proceeded in only modest yields (50-70%) and afforded the two products as a 1:1 mixture. It was suspected that the yield and selectivity were a result of the insolubility of oxindolylidene acetate **139** in toluene at room temperature. This allowed the competing pathway that results in olefin formation to prevail (Scheme 24). It was theorized that an increase in the number of aliphatic carbons on the dipolarophile would aid in the solubility. Therefore, *t*-butyl analog **145** was synthesized which proved to be readily soluble in toluene at room temperature. Subjecting **145** to the standard [1,3]-DPC reaction conditions resulted in an improved yield (60-70%) and an increase in the ratio (2:1) of desired cycloadduct **147** to eliminated cycloadduct **149**.



Scheme 40. [1,3]-DPC with 6-methoxy-ethyl oxindolylidene acetates **139** and **145**.

Construction of the diketopiperazine began with palladium-catalyzed hydrogenolysis of cycloadduct **146** (Scheme 41). The resulting amino acid **150** was coupled without purification to L-proline benzyl esters with BOP as the activating agent. Reduction of the resulting dipeptide-benyzl ester, followed by intramolecular cyclization afforded diketopiperazine **151** in identical yields (32-54% over three steps). This is in contrast to the 69% yield observed for the same steps in the spirotryprostatin B synthesis (Scheme 26). It is not currently understood why substitution of the aromatic ring or exchange of the ethyl ester for a *t*-butyl ester caused such a decrease in the overall yield.

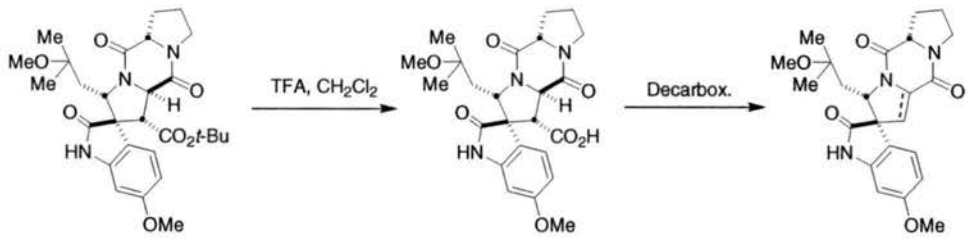


147 150 151

Scheme 41. Elaboration to diketopiperazine **151**.

4.1.3 Attempts at Oxidative Decarboxylation

Completion of the synthesis of spirotryprostatin A required hydrolysis of the ester functionality and Barton-modified Hunsdiecker reaction to afford eneamide **153** (Scheme 42). The *t*-butyl ester **151** was hydrolyzed using trifluoroacetic acid in yields ranging from 42-58%. However, subjecting the resulting carboxylic acid **152** to oxidative decarboxylation conditions failed to provide eneamide **153** (Table 6, entry 1). Kochi-type conditions ($Pb(OAc)_4$ and thermal or photolytic clevage of a benzophenone oxime ester were also unsuccessful (entries 2-3).



151 152 153

Scheme 42. Attempted decarboxylation of **152**.

Table 6. Attempted Decarboxylation Conditions

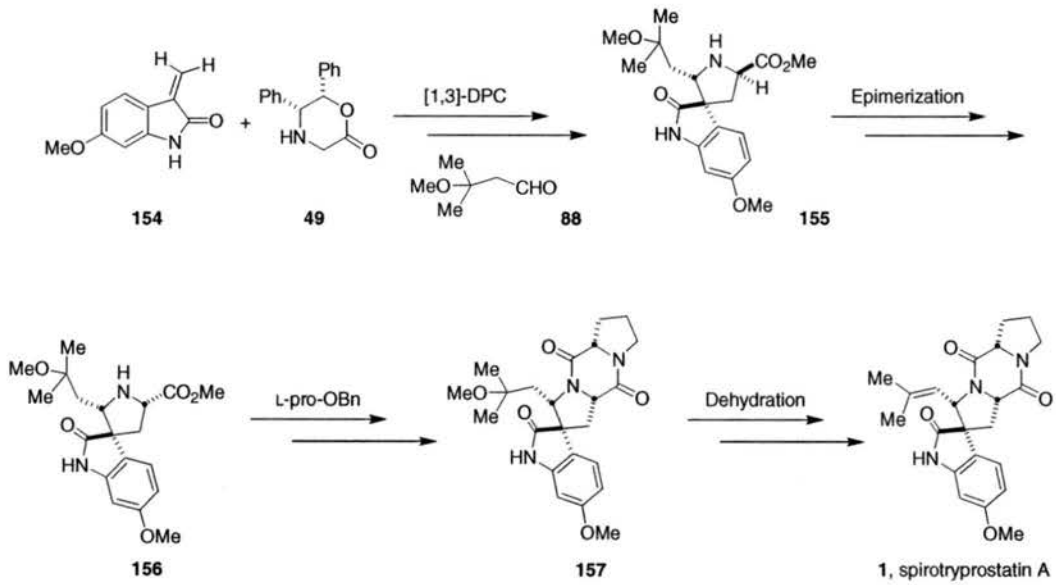
entry	initiation	conditions
1	hν or Δ	DCC/DMAP, $BrCCl_3$ thiohydroxyamic acid
2	Δ	$Pb(OAc)_4$, $Cu(OAc)_2$ DMF,
3	hν or Δ	DCC/DMAP, $BrCCl_3$ benzophenone oxime
4	hν or Δ	DCC/DMAP, *t*-BuSH thiohydroxyamic acid
5	hν or Δ	DCC/DMAP, *t*-BuSH benzophenone oxime
6	hν or Δ	DCC/DMAP, Bu_3SnH benzophenone oxime

Reductive decarboxylation conditions also resulted in decomposition of the starting material (entries 4-6). Attempts to affect either the oxidative or the reductive decarboxylation at an earlier stage in the synthesis were met with similar results. One potential explanation for the cause of failure is that the oxindole could undergo a retro-Mannich reaction and then the resulting charged intermediate could undergo decomposition.[41] Nonetheless, the complications in the elimination of the carboxy group and the low yields observed for the previous steps warranted exploration of a new strategy.

4.1.2 Revised Synthetic Route to Spirotryprostatin A

A new approach, one that avoided the problematic decarboxylation step, was devised (Scheme 43). Elimination of the carboxy group from the dipolarophile at the outset would alleviate the problems associated with its removal. The strategy therefore, revolved around formation and [1,3]-DPC reaction of 6-methoxy-3-methylene-1,3-dihydro-indol-2-one **154**. The synthesis of **154** would not be trivial in lieu of its reported

instability.[42] If successful, cycloadduct **155** would exist with the correct configuration at two of the stereogenic centers and without the carboxy group. However, an epimerization of the α-position would need to occur before coupling to L-proline benzyl ester and elaboration to the DKP **157**. Formation of the amino acid with the correct stereochemistry would need to occur at this stage, as the spirotryprostatin B synthesis had shown the thermodynamic instability of *trans*-DKP resulted in the epimerization of the prolyl-stereogenic center. Elimination of the tertiary methyl ether would afford the natural product (**1**).

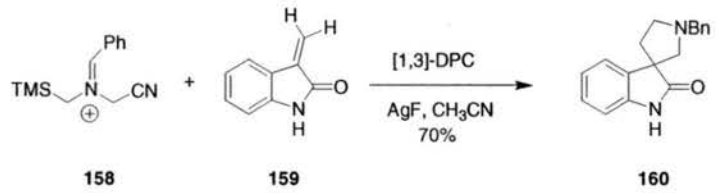


Scheme 43. Revised synthetic route to spirotryprostatin A (**1**).

4.2.1 Cycloaddition with methylene dihydroindolinone

Recently, Liebeskind et al. reported the generation and use of the demethoxy-derivative of **154** as a dienophile in a [5+2]-cycloaddition, suggesting that **154** would perhaps react under [1,3]-DPC reaction conditions.[11] Indeed, Horvath and coworkers recently showed that the azomethine ylides generated from silylaminonitriles (**158**) and

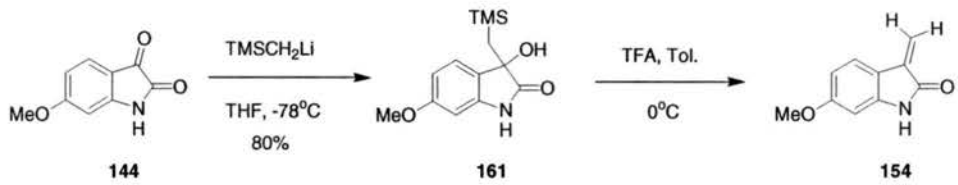
3-methyleneindolin-2-one **159** reacted to give **160** in 70% yield (Scheme 37).[4i] However, the dipolarophile was generated by flash vacuum pyrolysis and did not seem compatible with the synthesis of the methoxy-substituted derivative and a different approach was sought.



158 159 160

Scheme 44. [1,3]-DPC reaction of 159.

The Peterson olefination, which has proved to be an efficient method for the generation of terminal olefins, provided a potential route to methylene indolinone **154**. Addition of trimethylsilylmethyl lithium to 6-Methoxyisatin **144** afforded tertiary alcohol **161** in 80% yield (Scheme 38). After some exploration it was found that addition of 1 eq. of trifluoroacetic acid promoted the generation of methylene indolin-2-one **154**. However, the compound was not isolable due to polymer formation upon concentration.

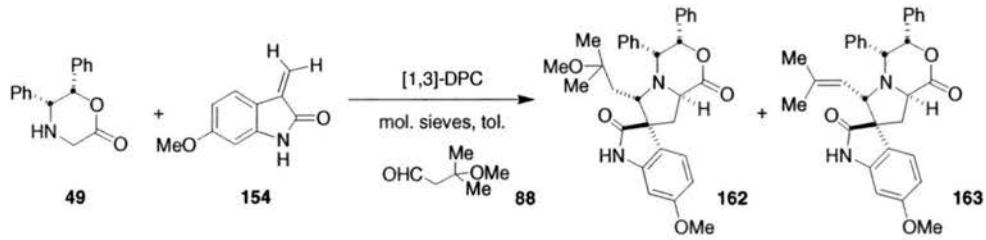


144 161 154

Scheme 45. Formation of methylene indolin-2-one (**154**) via a Peterson olefination.

Neutralization of the acid and reaction of the crude product to the standard [1,3]-DPC reaction conditions afforded two cycloadducts (**162** and **163**) in 45-80% yield (Scheme 46). Unfortunately, the observed ratio of **162** to **163** was 1:10 favoring the eliminated product **163**. Changing the solvent (benzene, ethyl acetate), temperature (0-60°C) and

concentrations (0.01-0.1M) did not improve the yield of the desired cycloadduct **162**. nOe Experiments confirmed the stereochemical assignment of the prenyl side-chain, however the stereochemistry of the quaternary stereogenic center has not been unambiguously determined. NMR studies were inconclusive and attempts to form a crystal suitable for X-ray analysis have been unsuccessful.

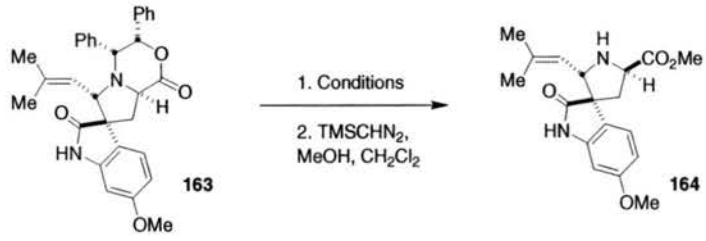


Scheme 46. [1,3]-DPC reaction with methylene indolinone **154**.

4.2.1 Attempts to Remove the Chiral Auxiliary

Unable to obtain useful amounts of **162**, focus shifted towards elaboration of **163** to the amino acid. Incorporation of the prenyl group at such an early stage presented a difficult challenge. Removal of the chiral auxiliary was required without affecting the isopropylidene group (Scheme 47). Various conditions were explored but failed to yield the desired amino acid methyl ester **164** (Table 7). Birch conditions using Li^{o}, Na^{o} or K^{o} all resulted in decomposition of the starting material. Oxidative cleavage methods were also unsuccessful. Saponification with LiOH or esterification with acidic methanol of **163** followed by the addition of $Pb(OAc)_4$ or $NaIO_4$ to the resulting 1,2-amino alcohols did not afford any of the desired product (entries 4-5). If the oxindole of the intermediate methyl ester was protected as the lactam ether, decomposition of the starting material still

resulted (entry 6). It appears that cleavage of the chiral auxiliary from the spirooxindole pyrrolidine cycloadduct is best accomplished by palladium-catalyzed hydrogenolysis.



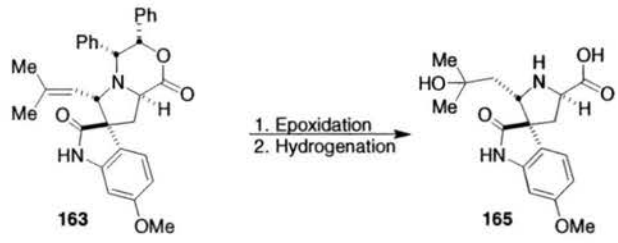
Scheme 47. Attempted removal of the chiral auxiliary of **163**.

Table 7. Conditions Explored for Removal of the Chiral Auxiliary

entry	conditions	result
1	Li^{o}, NH_3	Decomp.
2	Na^{o}, NH_3	Decomp.
3	K^{o}, NH_3	Decomp.
4	LiOH; $NaIO_4$	SM and Decomp.
5	HCl, MeOH; $Pb(OAc)_4$	Decomp.
6	HCl, MeOH; $BF_4 \bullet OEt_3$; $Pb(OAc)_4$	Decomp.

Another potential method for removal of the chiral auxiliary was to mask the olefin in **163** as an epoxide and subject it to palladium-catalyzed hydrogenolysis (Scheme 48). If the intermediate epoxide was reduced along with the chiral auxiliary, then amino acid **165** would be produced. However, standards conditions (*m*-CPBA) failed to afford more than a 15% yield of the epoxide. Alternative oxidation sources (*per*-acetic acid and trifluoro *per*-acetic acid) resulted in similar yields whereas dimethyl dioxirane resulted in complete decomposition of the starting material. It is possible that the steric bulk around the olefin hinders epoxide formation and oxidation of the oxindole nitrogen may compete. The inability to remove the chiral auxiliary or modify **163** in such a way to

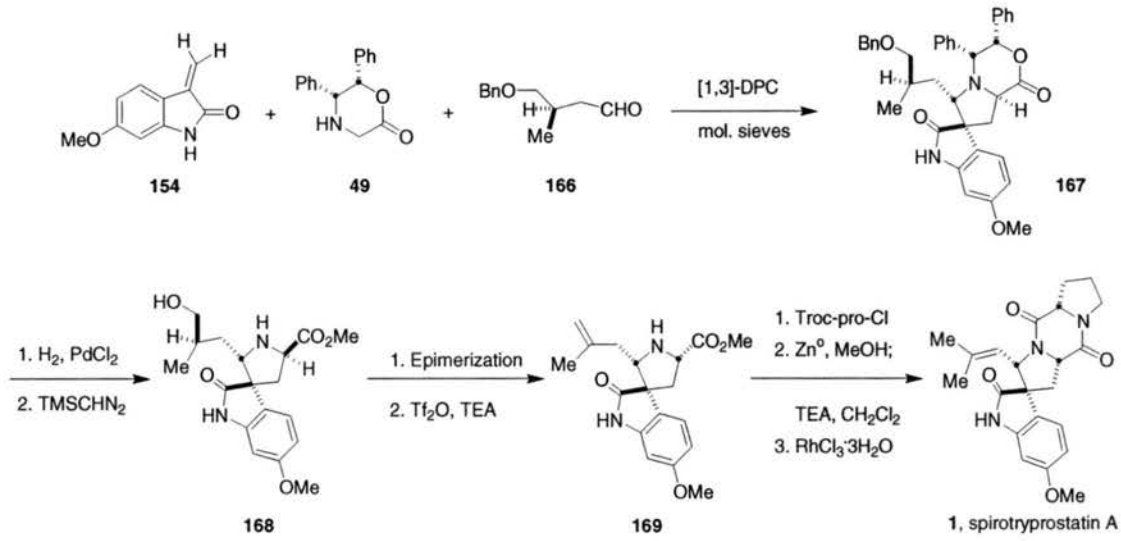
allow for hydrogenolysis suggests that the present route will not afford the natural product.



Scheme 48. Epoxidation of olefin **163**.

4.3 Modified Aldehyde Approach

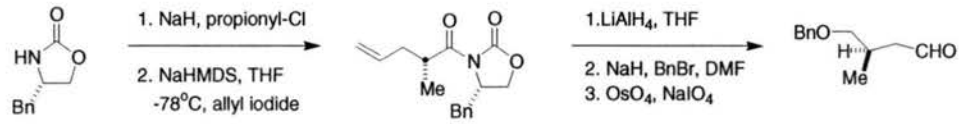
6-Methoxy-3-methylene indolinone (**154**) still seemed to have potential as a precursor to the core spirooxindole pyrrolidine ring and ultimately spirotryprostatin A. If olefin formation could be prevented in the [1,3]-DPC reaction then the resulting cycloadduct could be subjected to hydrogenolysis conditions. Based on this principle a third strategy was devised (Scheme 49). Aldehyde **166** would serve as a prenyl side-chain progenitor that was incapable of olefin formation. [1,3]-DPC reaction would afford **167**, which would be elaborated to amino acid methyl ester **168** by hydrogenolysis of the chiral auxiliary and esterification. Epimerization followed by elimination of the tertiary methyl ether would provide **169**. Coupling and cyclization with L-proline would afford spirotryprostatin A (**1**). This strategy would represent a formal synthesis, as the last three steps would mimic Danishefsky and coworkers synthesis of spirotryprostatin A.



154 49 166 167 168 169 1, spirotryprostatin A

Scheme 49. Modified aldehyde **166** approach to spirotryprostatin A (**1**).

Synthesis of the optically active aldehyde **166** was accomplished by an asymmetric aldol reaction (Scheme 50).[43] Acylation of Evans' oxazolidinone (**170**) followed by diastereoselective alkylation with allyl iodide afforded imide **171** in 80% yield. Reduction of imide **171** gave the free oxazolidinone **170** and the primary alcohol, which was protected as the benzyl ether. The resulting ether was converted to aldehyde **166** by oxidation of the terminal olefin with osmium tetraoxide and sodium periodate mediated oxidative cleavage of the intermediate diol in 60% yield over the three steps.



170 171 166

Scheme 50. Synthesis of aldehyde **166**.

Preliminary studies suggest that [1,3]-DPC reaction with aldehyde **166** occurred to yield cycloadduct **167**. However, attempts to characterize and confirm the stereochemistry by NMR have been hampered by an equal amount of another

diastereomer. Further exploration of this strategy is still required.

4.4 Conclusion

Progress towards the synthesis of spirotryprostatin A (**1**) has been described. The initial strategy was based on the approach to the dehydro-demethoxy congener spirotryprostatin B (**2**). The Sandmeyer reaction of *m*-anisidine provided an efficient means to access 6-Methoxyisatin. Conversion to the 6-methoxy-oxindolylidene acetate was accomplished by Wittig reaction with a stabilized ylide. Elaboration to the diketopiperazine succeeded, however the oxidative decarboxylation protocol failed on substrate **152**. Numerous conditions were explored, yet the desired eneamide was never obtained and the initial strategy was abandoned.

Another approach, based on 3-methylene indolinone **154**, was explored as a means of avoiding the oxidative decarboxylation step. A Peterson olefination proved to be a mild method for the formation of the terminal olefin. However, [1,3]-DPC reaction of **154** resulted in the formation of the undesired cycloadduct **163**. Attempts to remove the chiral auxiliary from this substrate proved fruitless under a variety of approaches. The strategy still holds promise if the ratio of cycloadduct **162** to **163** could be improved. Additionally, [1,3]-DPC reaction of aldehyde **166** could provide a third alternative strategy to the synthesis of spirotryprostatin A.

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Experimental Section

5.1 General Procedures

Unless otherwise noted, materials were obtained from commercially available sources and used without purification. Toluene was freshly distilled from CaH_2. Diethyl ether and THF were freshly distilled from sodium benzophenone ketyl. 3Å molecular sieves were activated by heating for three minutes at the highest setting in a microwave followed by cooling under argon.

All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware (120°C) that was cooled in a dessicator, unless stated otherwise.

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

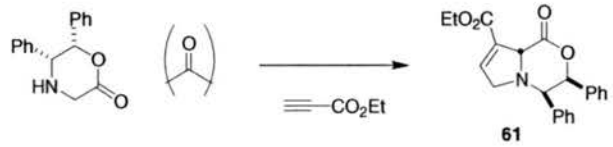
Mass spectra were obtained on Fisons VG Autospec.

^1H NMR, ^{13}C NMR, HSQC and nOe experiments were recorded on a Varian 300 or 400 MHz spectrometer. Spectra were recorded in $CDCl_3$ and chemical shifts (δ) were given in ppm and were relative to $CHCl_3$. Proton ^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 appropiate, the multiplicity of a signal is denoted as "br" to indicate the signal was broad.

IR spectra were recorded on a Perkin-elmer 1600 series FT-IR spectrometer.

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

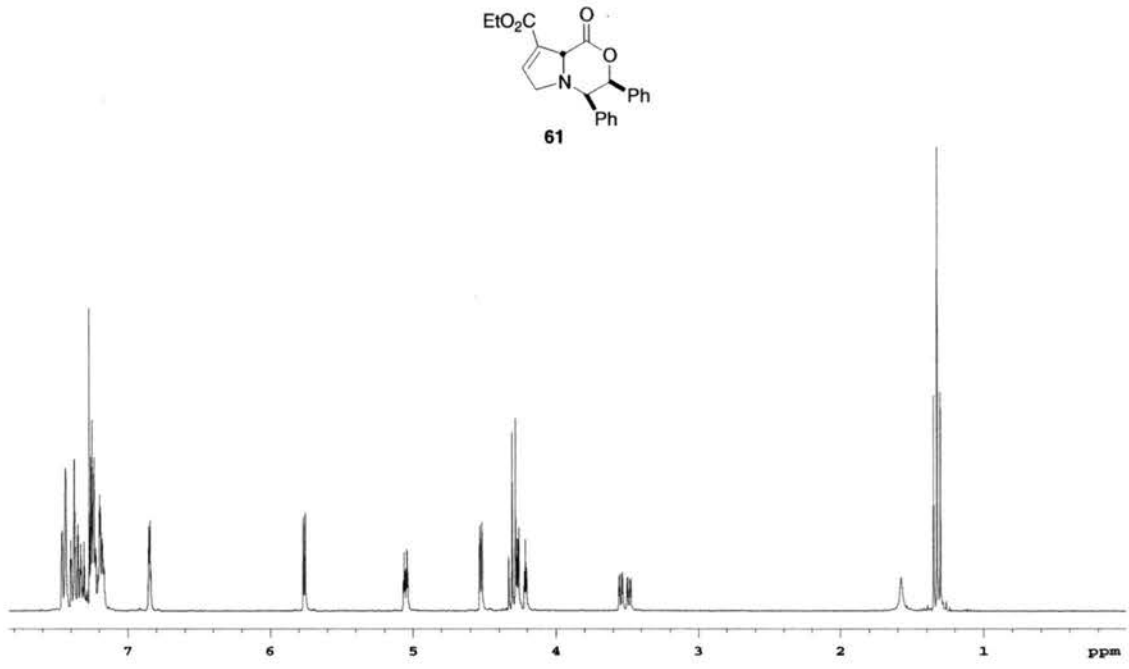
5.1 Experimental Procedures



61

1-Oxo-3,4-diphenyl-3,4,6,8a-tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazine-8-carboxylic acid ethyl ester 61.

To a flame dried 50 mL round bottom flask with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (125 mg, 0.49 mmol), paraformaldehyde (75 mg, 2.47 mmol), *p*-toluene sulphonic acid (37 mg, 0.20 mmol) and 2.5 g of activated 3Å molecular sieves. An oven-dried condenser was attached and the system was flushed with argon. Freshly distilled toluene (250 mL) was added followed by ethyl propiolate (0.10 mL, 0.98 mmol). The reaction was then heated to reflux and kept at that temperature for 12 h at which time the heating mantle was turned off. The reaction was allowed to cool to room temperature and filtered through celite to remove the sieves. Concentration afforded clear oil which was chromatographed (SiO_2, 2:1 hexanes:EtOAC → 1:1 hexanes:EtOAc) to afford 0.11 g of 59 (62%) as a white solid. **61**: $[\alpha]_D^{25}$ = -137.6 (c = 0.46, $CHCl_3$). m.p. = 117-118 °C (recryst. MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 1.32 (t, J = 6.9, 3H), 3.48 (ddd, J = 2.1 Hz, J = 6.3 Hz, J = 17.1 Hz, 1H), 4.23 (dt, J = 2.1 Hz, J = 17.1 Hz, 1H), 4.29 (q, J = 6.9 Hz, 2H), 4.52 (d, J = 3.9 Hz, 1H), 5.05 (dt, J = 2.1 Hz, J = 6.3 Hz, 1H), 5.76 (d, J = 3.9 Hz, 1H), 6.85 (dd, J = 2.1 Hz, J = 3.9 Hz 1H), 7.16 - 7.46 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 14.5, 61.2, 62.8, 65.5, 68.6, 83.1, 128.1, 128.2, 128.3, 128.4, 128.6, 128.9, 132.2, 134.3, 136.6, 139.3, 162.9, 169.4. IR (NaCl/neat) 1748, 1718.



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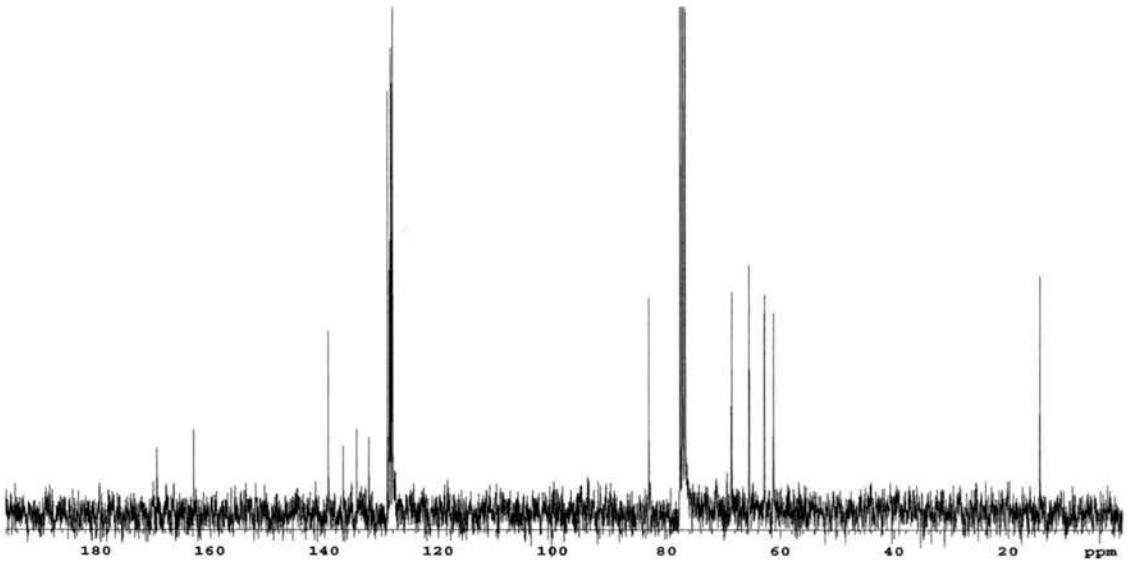
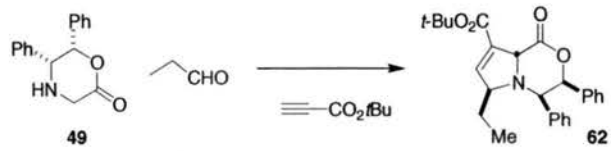


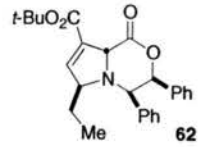
^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS1-225-C13



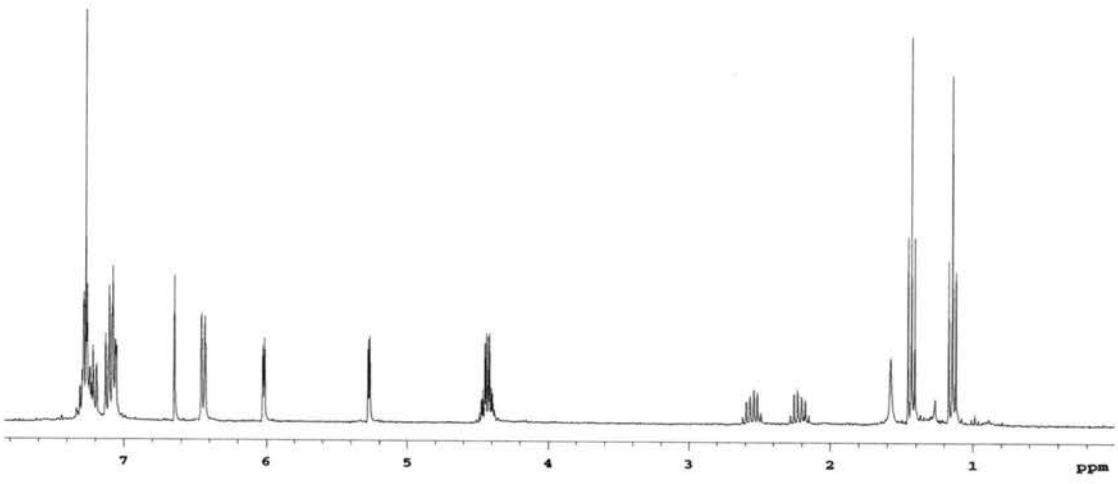
6-Ethyl-1-oxo-3,4-diphenyl-3,4,6,8a-tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazine-8-carboxylic acid tert-butyl ester 62.

To a flame dried 50 mL round bottom flask with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (125 mg, 0.49 mmol), propionaldehyde (178 mL, 2.47 mmol) and 2.5 g of activated 3Å molecular sieves. An oven-dried condenser was attached and the system was flushed with argon. Freshly distilled toluene (250 mL) was added followed by ethyl propiolate (0.10 mL, 0.98 mmol). The reaction was then stirred at room temperature for 12 h. The reaction was filtered through celite, concentrated and chromatographed (SiO_2, 3:1 hexanes:EtOAC → 2:1 hexanes:EtOAc) to afford 0. g of 59 (23%) as an off-white solid.

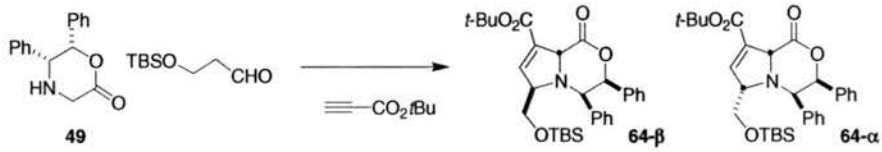
62: $[\alpha]_D^{25}$ = 52.5 (*c* 0.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 1.14 (t, J = 7.8, 3H), 1.43 (t, J = 7.8, 3H), 2.22 (q, J = 7.8 Hz, 1H), 2.56 (q, J = 7.8 Hz, 1H), 4.38 – 4.48 (m, 1H), 5.27 (d, J = 3.3 Hz, 1H), 6.02 (d, J = 3.3 Hz, 1H), 6.44 (d, J = 7.2 Hz, 2H), 6.65 (s, 1H), 7.05 - 7.30 (m, 8H). IR (NaCl/neat) 1742, 1738. HRMS (FAB+) calcd for $C_{26}H_{29}O_4N$ (*m/z*) , found (*m/z*).



62



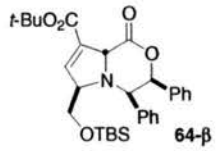
^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS1-44-1H



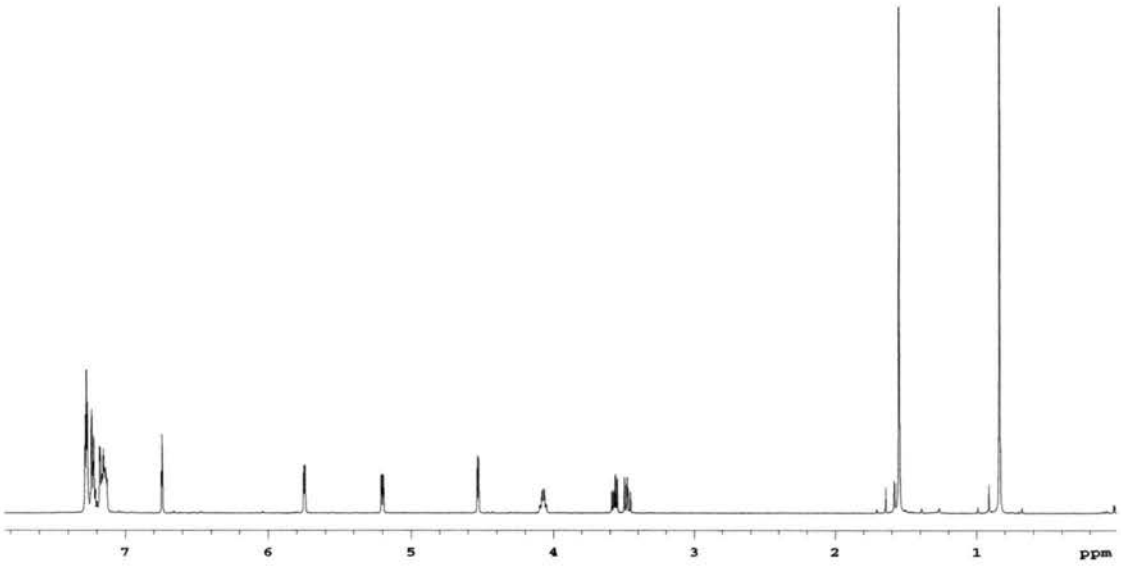
6-Benzyloxymethyl-1-oxo-3,4-diphenyl-3,4,6,8a-tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazine-8-carboxylic acid tert-butyl ester 64.

To a flame dried 50 mL round bottom flask with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (125 mg, 0.49 mmol), 3-(tert-Butyl-dimethyl-silanyloxy)-propionaldehyde (103 mg, 0.59 mmol) and 2.5 g of activated 3Å molecular sieves. An oven-dried condenser was attached and the system was flushed with argon. Freshly distilled toluene (250 mL) was added followed by *t*-butyl propiolate (0.10 mL, 0.98 mmol). The reaction was then stirred at room temperature for 12 h. The reaction was filtered through celite, concentrated and chromatographed (SiO_2, 3:1 hexanes:EtOAC → 2:1 hexanes:EtOAc) to afford 88 mg of **64-β** (35%) **64-α** (35%) as off-white solids.

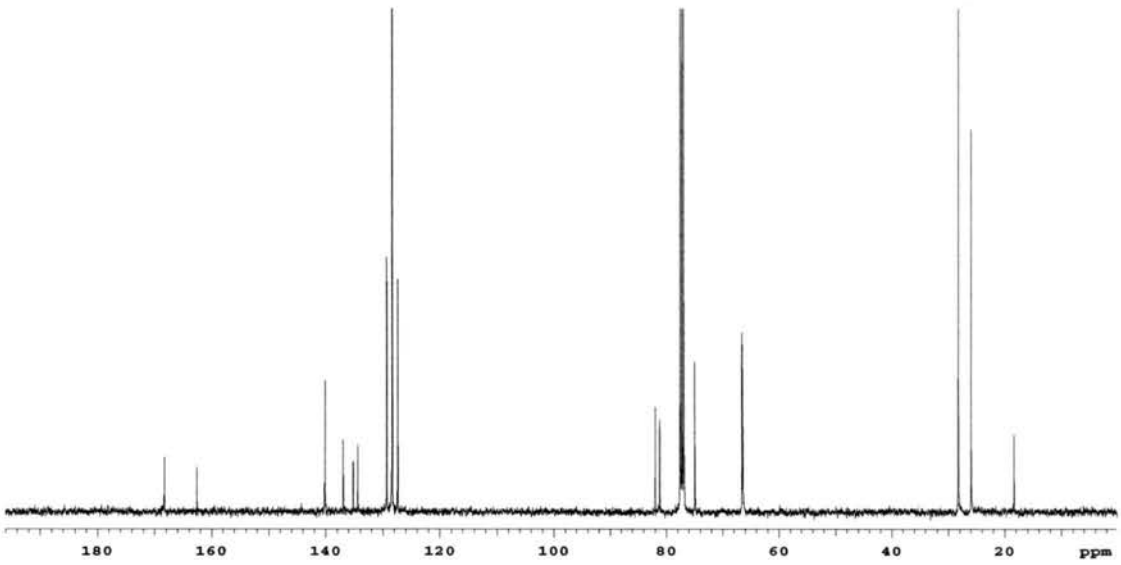
64-β: $[\alpha]_D^{25}$ = -33.8 (c = 1.27, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.80 (s, 9H), 1.51 (s, 9H), 3.44 (dd, J = 7.6 Hz, J = 9.6 Hz, 1H), 3.53 (dd, J = 7.6 Hz, J = 9.6 Hz, 1H), 4.01-4.06 (m, 1H), 4.49 (d, J = 3.6 Hz, 1H), 5.16 (dd, J = 2.0 Hz, J = 4.6 Hz, 1H), 5.71 (d, J = 3.6 Hz, 1H), 6.70 (t, J = 2.0 Hz,1H), 7.09 - 7.25 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -5.3, 18.4, 26.0, 28.2, 66.4, 66.5, 74.9, 81.1, 81.9, 127.3, 128.3, 129.3, 134.4, 135.2, 136.9, 140.1, 162.5, 168.3. IR (NaCl/neat) 1748, 1719. HRMS (FAB+) calcd for $C_{31}H_{42}O_5NSi$ (*m/z*) 536.2832, found 536.2820 (*m/z*).



64-β

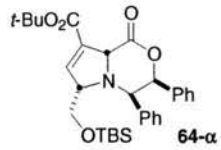


^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS1-265-2H

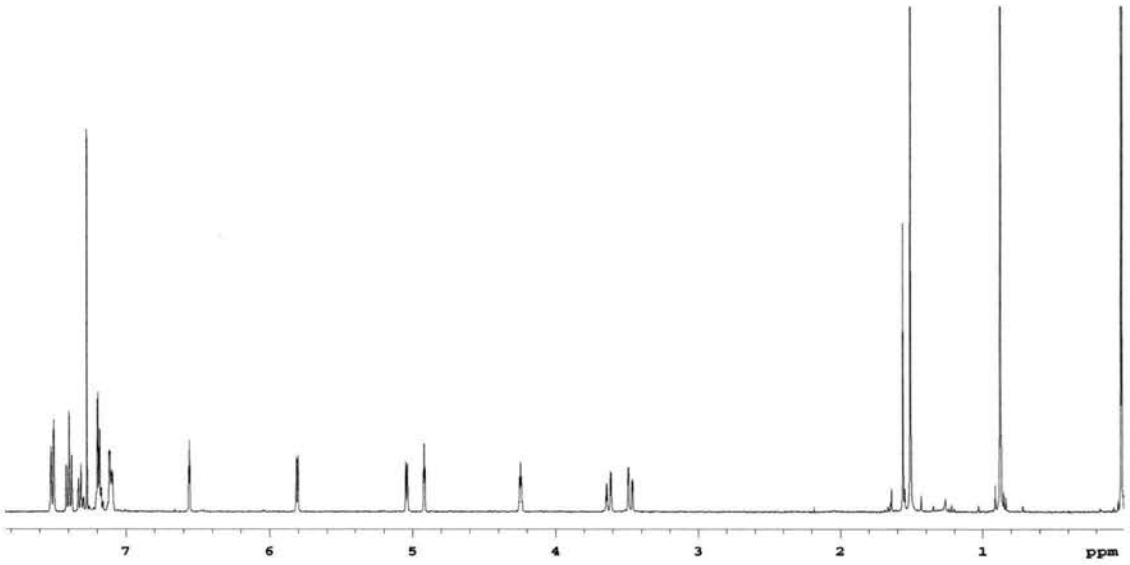


^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS1-265-2HC13

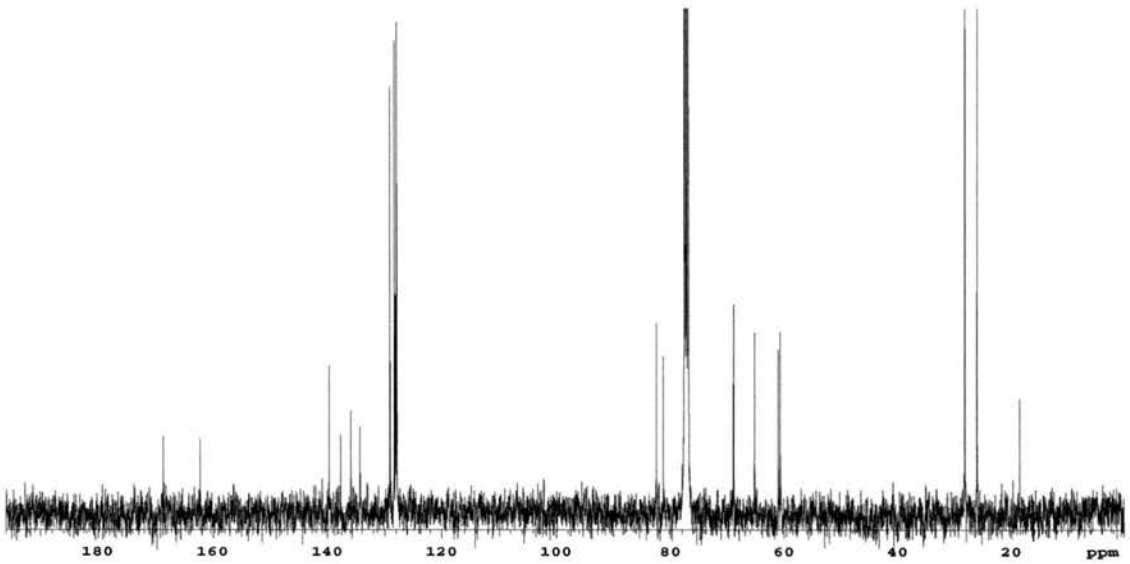
64-α: $[\alpha]_D^{25}$ = 131.5 (c = 0.33, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.84 (s, 9H), 1.47 (s, 9H), 3.44 (dd, J = 2.0 Hz, J = 11.6 Hz, 1H), 3.59 (dd, J = 2.8 Hz, J = 11.6 Hz, 1H), 4.21 (t, J = 2.4 Hz, 1H), 4.88 (t, J = 2.0 Hz, 1H), 5.01 (d, J = 4.8 Hz, 1H), 5.77 (d, J = 4.8 Hz, 1H), 6.52 (t, J = 2.4 Hz,1H), 7.05 - 7.48 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -5.3, 18.6, 26.0, 28.2, 60.6, 60.9, 65.1, 688, 81.2, 82.4, 127.8, 127.9, 128.0, 128.1, 128.3, 129.0, 134.6, 135.9, 137.7, 139.7, 162.2, 168.6. IR (NaCl/neat) 1759, 1719. HRMS (FAB+) calcd for $C_{31}H_{42}O_5NSi$ (*m/z*) 536.2832, found 536.2820 (*m/z*).



64-α



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS1-265-1H



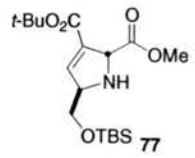
^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS1-265-1HC13

6-Benzyloxymethyl-1-oxo-3,4-diphenyl-3,4,6,8a-tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazine-8-carboxylic acid tert-butyl ester 77.

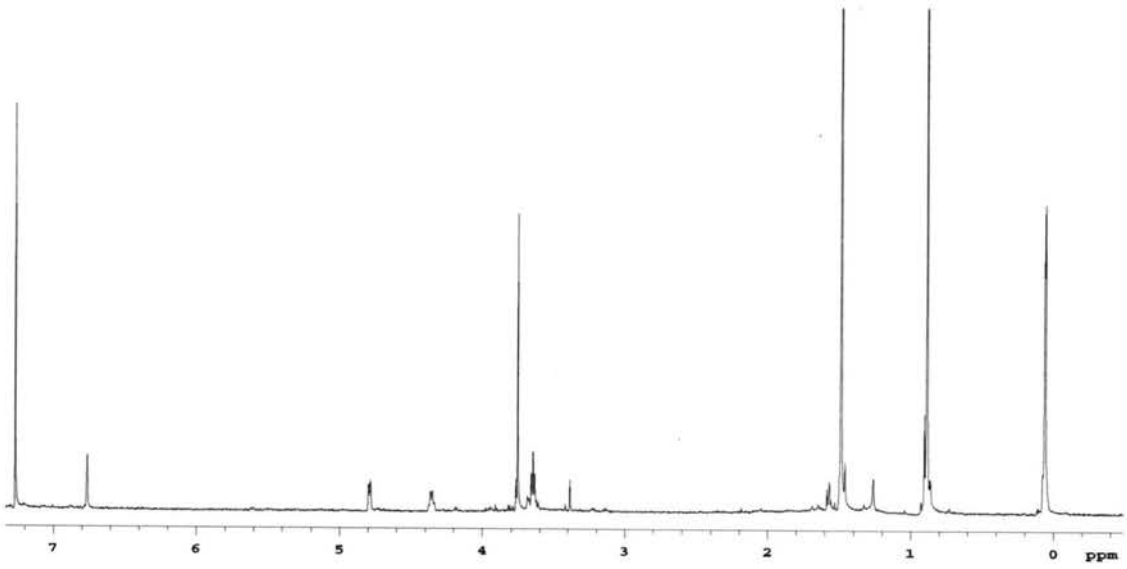
To 25 mL round bottom flask with stir bar was added **64-β** (0.25 mg, 0.047 mmol) dissolved in 1 mL of THF. MeOH (1 mL) and water (1 mL) were added followed by LiOH (11 mg, 0.50 mmol). The reaction was stirred at room temperature for 14 hours and then acidified to pH 3-4 with 1N HCl. The mixture was extracted with EtOAc (3 x 5 mL), the organic layers combined, dried over Na_2SO_4, filtered and evaporated. The oily residue was taken up in 2 mL of a 1:1 mixture of MeOH:CH_2Cl_2 and $TMSCHN_2$ (2.0 M sol. in hexanes) was added until a yellow color persisted. The reaction was stirred for 15 min. and evaporated to leave an oily residue. The crude reaction mixture was taken up in CH_2Cl_2 (2 mL) and MeOH (1 mL) and cooled to 0°C. To the resulting solution was added $Pb(OAc)_4$ (0.25 mg, 0.056 mmol) and stirred for 10 min and then 10 drops of 1N HCl was added. The reaction was allowed to warm to room temperature and stirred another 45 min. EtOAC was added (5 mL), the aq. layer was separated and extracted with EtOAC (3 x 5 mL). The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oily residue was purified by PTLC (2 x ½ 250 μm plates) with 3:1 hexanes: EtOAC as the eluent to afford 8 mg (46%) of **77** as a clear oil.

77: $[\alpha]_D^{25}$ = 76.3 (c = 0.11, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.60 (s, 6H), 0.88 (s, 9H), 1.49 (s, 9H), 3.65 (t, J = 5.2 Hz, 2H), 3.75 (s, 3H), 4.35 (d, J = 6.0 Hz, 1H), 4.79 (d, J = 4.4 Hz, 1H), 6.76 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -0.58, 18.4,

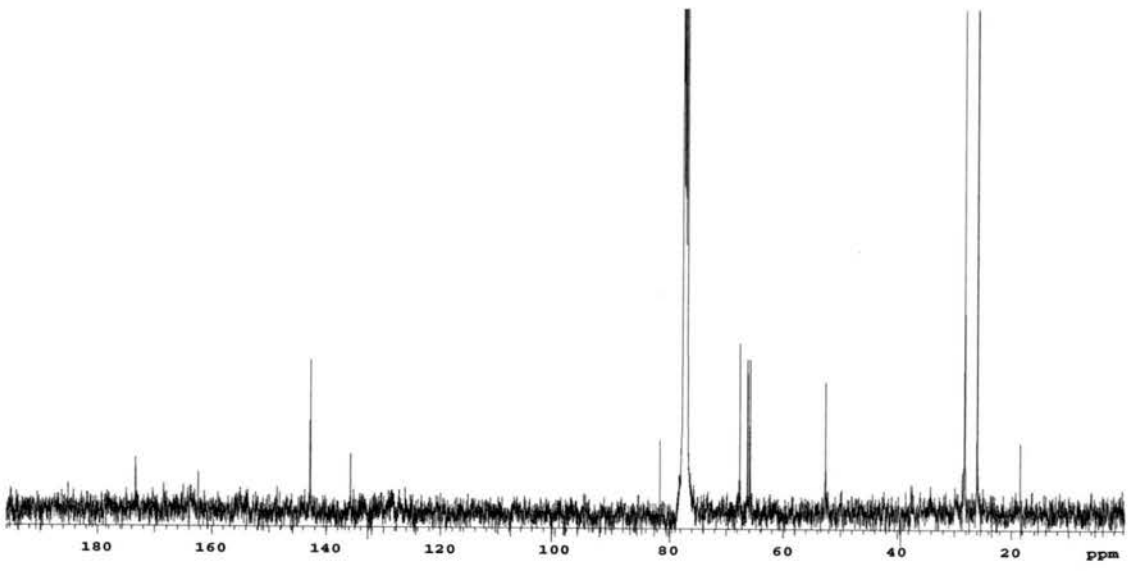
26.0, 28.2, 52.5, 65.8, 66.2, 67.6, 81.5, 135.8, 142.8, 135.8, 142.8, 162.3, 173.3. IR (NaCl/neat) 1754, 1716. HRMS (FAB+) calcd for $C_{18}H_{34}O_5NSi$ (*m/z*) 372.2206, found 372.2208 (*m/z*).



77



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS1-190-1H

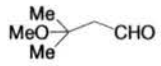


^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS1-190-C13

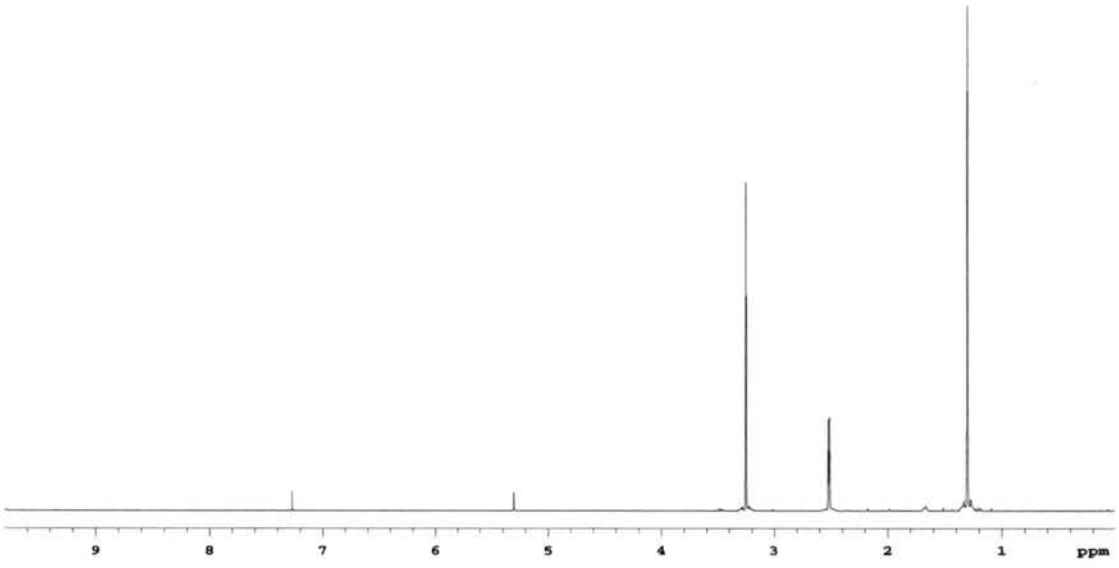
88

3-Methyl-3-methoxybutanal 88.

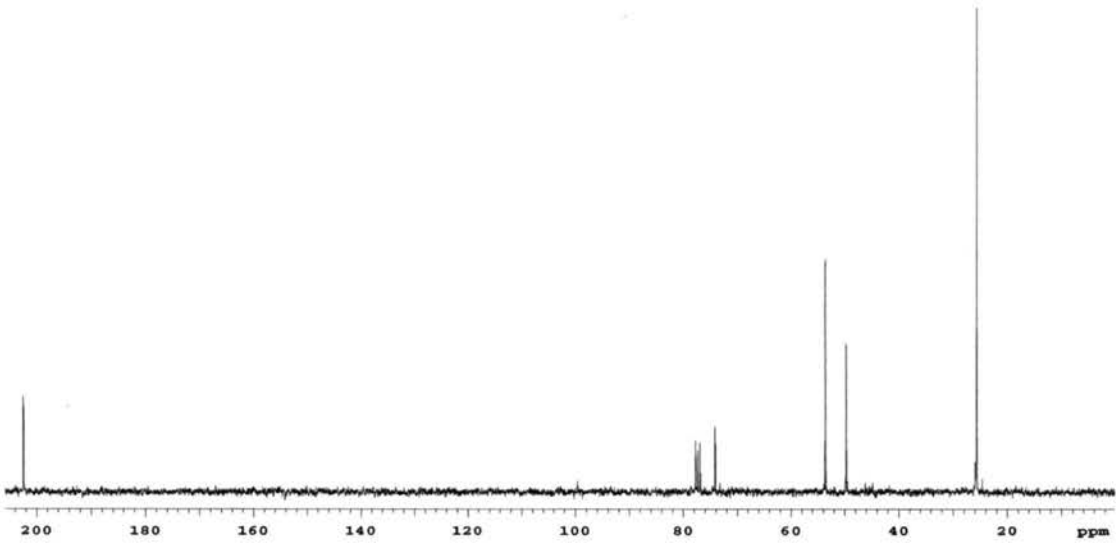
To an oven-dried 2000 mL three-neck round-bottom flask with stir bar was added DMSO (15.8 mL, 22.3 mmol) in 50 mL of CH_2Cl_2. The solution was cooled to -78°C under argon and oxalyl chloride (10 mL, 112 mmol) in 250 mL of CH_2Cl_2 was added dropwise over 15 min. 3-Methyl-3-methoxybutan-1-ol (12.0 g, 100 mmol) along with pyridine (16.5 mL, 200 mmol) in 100 mL of CH_2Cl_2 was added dropwise over 15 min. The reaction was stirred 15 min. more at -78°C and then TEA (75 mL, 0.5 mol) in 75 mL of CH_2Cl_2 was added over 15 min. with vigourous stirring. The solution was kept at 78°C for 15 min and then warmed to 4°C and stirred for another 15 min. 1N HCl was used to acidify to pH ~4 and the layers separated. The aqueous layers were extracted 3 x 50 mL and the organic layers combined, dried over Na_2SO_4, filtered and evaporated. The crude product could be obtained by column chromatography with 2:1 CH_2Cl_2:Et_2O as the eluent to yield 11.5 g (97%) of a yellow oil. The product was further purified by distillation under reduced pressure (aspirator) to yield 10.5 g of **88** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 1.31 (s, 6H), 2.53 (d, J = 3.3 Hz, 2H), 3.26, (s, 3H), 9.84 (t, J = 3.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 18.6, 42.7, 46.6, 67.1, 195.4. IR (NaCl/neat) 2974, 2937, 2828, 1732. LRMS (EI+) calcd for $C_6H_{13}O_2$ (*m/z*) 117.1, found (*m/z*) 117.1.



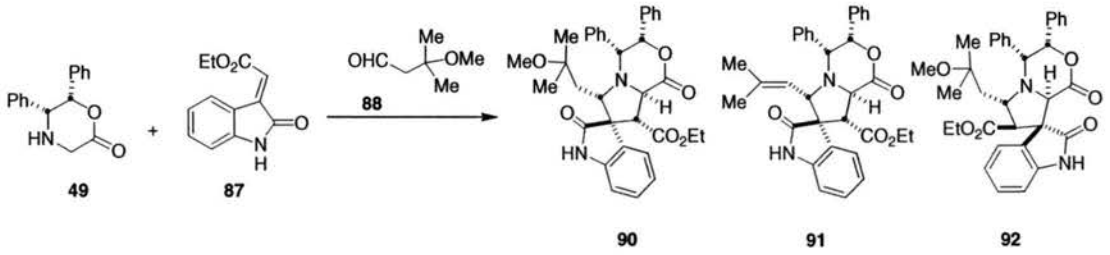
88



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS1-291-1H



^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS1-291-C13



Spiro[3H-indole-3,7'(6'H)-[1H]pyrrolo[2,1-c][1,4]oxazine]-8'-carboxylic acid, 1,2,3',4',8',8'a-hexahydro-6'-(2-methoxy-2-methylpropyl)-1',2-dioxo-3',4'-diphenyl-, ethyl ester, (3S,3'S,4'R,6'S,8'R,8'aR) 90.

Spiro[3H-indole-3,7'(6'H)-[1H]pyrrolo[2,1-c][1,4]oxazine]-8'-carboxylic acid, 1,2,3',4',8',8'a-hexahydro-6'-(2-methyl-2-prop-ene-yl)-1',2-dioxo-3',4'-diphenyl-, ethyl ester, (3S,3'S,4'R,6'S,8'R,8'aR) 91.

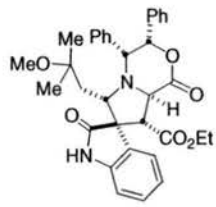
Spiro[3H-indole-3,7'(6'H)-[1H]pyrrolo[2,1-c][1,4]oxazine]-8'-carboxylic acid, 1,2,3',4',8',8'a-hexahydro-6'-(2-methoxy-2-methylpropyl)-1',2-dioxo-3',4'-diphenyl-, ethyl ester, (3S,3'S,4'R,6'S,8'R,8'aR) 92.

To a 500 mL round-bottom flask with stir bar was added (5R,6S)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (5.0 g, 19.8 mmol), ethyl oxindolylidene acetate (**87**) (6.4 g, 29.6 mmol) and 5.0 g of activated 3Å molecular sieves. An oven-dried condenser was attached and the system was flushed with argon. Freshly distilled toluene (250 mL) was added followed by 3-methyl-3-methoxybutanal **88** (2.75 g, 23.7 mmol). The reaction was then heated to 60°C and kept at that temperature for 1 h at which time the heating mantle was turned off. The reaction was allowed to cool to room temperature and filtered through celite to remove the sieves. Concentration afforded an orange solid which was chromatographed (SiO_2, 4:1 hexanes:EtOAC $\rightarrow$ 1:1

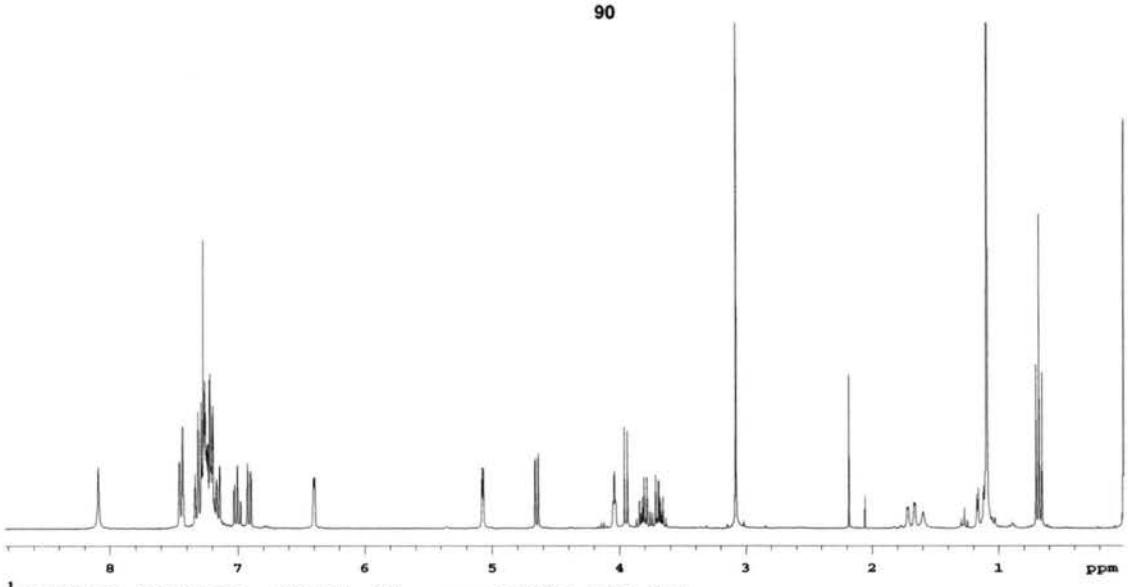
hexanes:EtOAc) to afford 9.2 g of **90** (82%) and 0.70 g of **91** (6.3%) and 0.12 g of **92** (1.1%) as white solids.

90: $[\alpha]_D^{25}$ = -88.8 (c = 1.0, CH_2Cl_2). m.p. = 225-227 °C (recryst. EtOH). ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.68 (t, J = 6.9, 3H), 1.09 (s, 6 H), 1.14 (dd, J = 3.6 Hz, J = 16.2 Hz, 2H), 1.70 (d, J = 3.3 Hz, J = 15.9 Hz, 2H), 3.08 (s, 3H), 3.63 - 3.85 (m, 2H), 3.95 (d, J = 7.5 Hz, 1H), 4.04 (t, J = 3.3 Hz, 1H), 4.65 (d, J = 7.5 Hz, 1H), 5.07 (d, J = 3.3 Hz, 1H), 6.40 (d, J = 3.3 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 7.00 (dt, J = 0.9 Hz, J = 7.5 Hz, 1H), 7.15 (d, J = 7.5 Hz, 1H), 7.18 - 7.33 (m, 10H), 7.44 (d, J = 7.5 Hz, 1H), 8.09 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 23.6, 26.9, 45.4, 50.6, 53.3, 56.0, 57.1, 57.5, 61.5, 65.5, 74.5, 77.1, 110.8, 124.0, 126.2, 127.2, 128.3, 128.4, 128.5, 129.4, 129.5, 130.2, 130.4, 137.5, 138.3, 142.3, 170.1, 173.0, 178.9. IR (NaCl/neat) 3308, 1734, 1618.

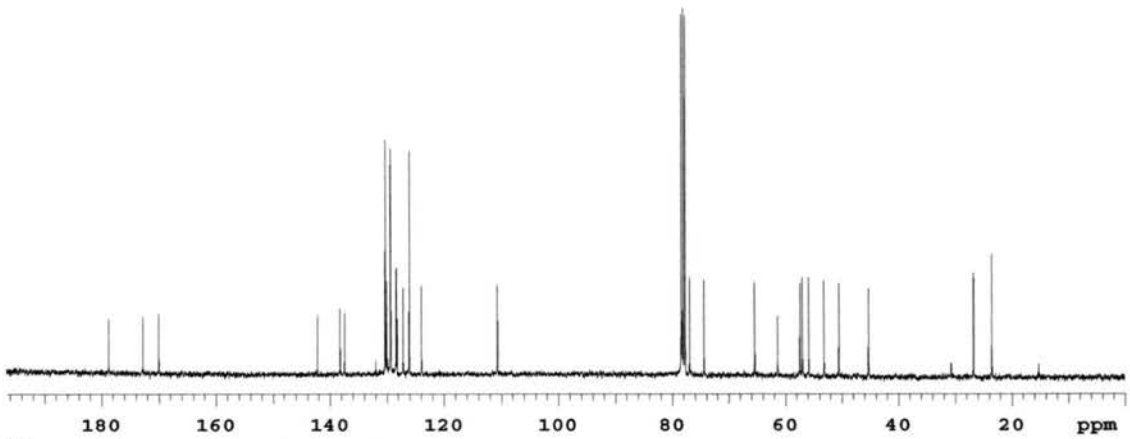
***ent*-90**: $[\alpha]_D^{25}$ = 91.7 (c = 1.0, CH_2Cl_2).



90

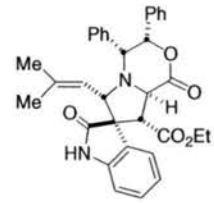


^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS1-292-1H

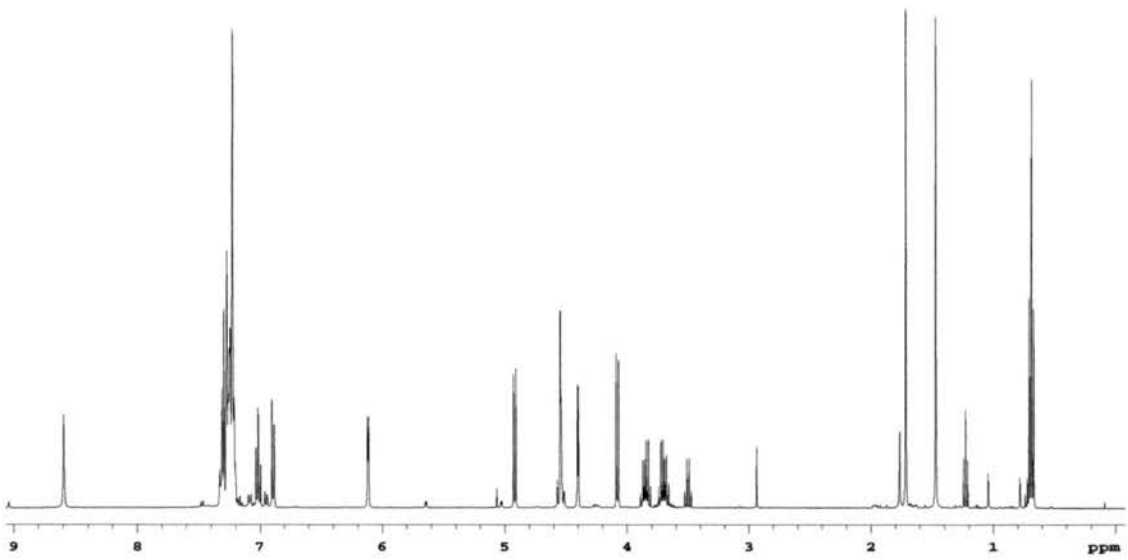


^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS1-292-C13

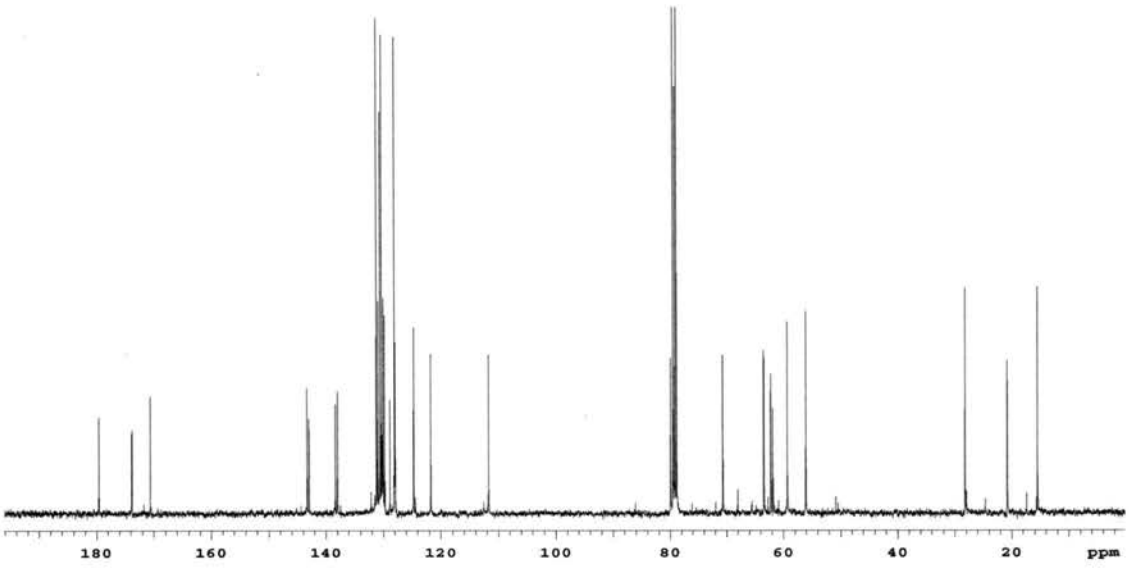
91: $[\alpha]_D^{25} = 52.8$ (c = 0.95, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 1.11 (t, J = 6.8, 3H), 1.16 (s, 3 H), 1.19 (s, 3 H), 1.80 - 1.94 (m, 2H), 3.18 (s, 3H), 3.41 (d, J = 6.0 Hz, 1H), 4.00 - 4.14 (m, 2H), 4.53 (m, 1H), 4.69 (d, J = 7.6 Hz, 1H), 5.0 (s, 1H), 6.41 (d, J = 2.8 Hz, 1H), 6.84 (d, J = 7.6 Hz, 2H), 7.01 - 7.35 (m, 12H), 7.62 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: 13.5, 18.8, 26.2, 54.1, 57.3, 59.8, 60.2, 61.4, 68.7, 78.0, 109.8, 119.8, 122.7, 125.9, 126.1, 126.9, 127.8, 128.1, 128.3, 128.6, 129.0, 129.2, 136.0, 136.4, 141.1, 141.4, 168.6, 171.8, 177.6. IR (NaCl/neat) 3305, 1730, 1618.



91

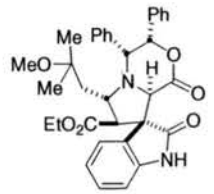


^1H NMR, 400 MHz, $CDCl_3$, filename: PRS1-292-elim1H

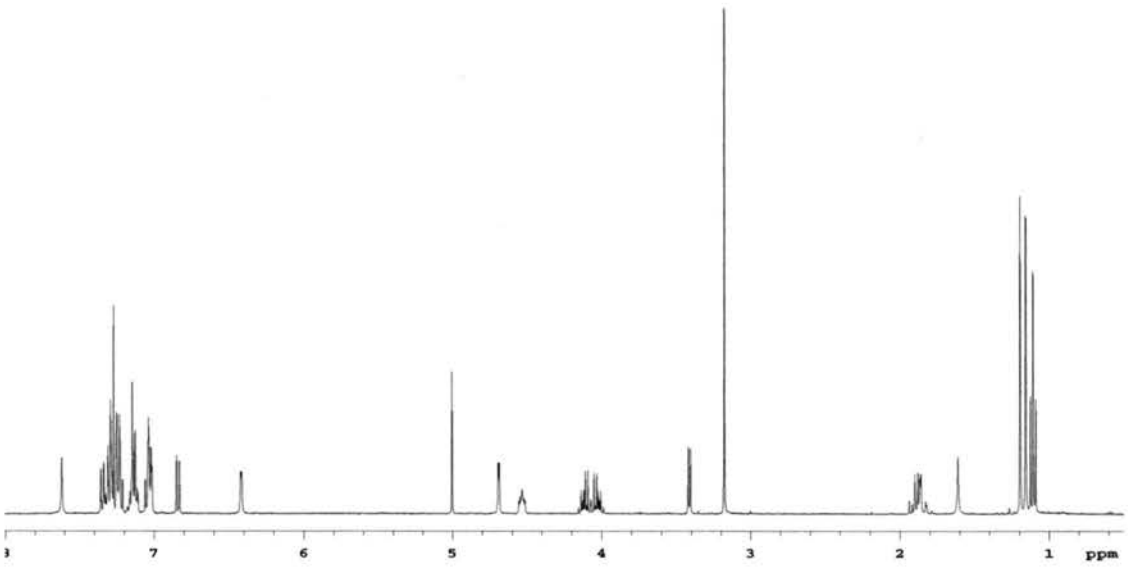


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS1-292-elimC13

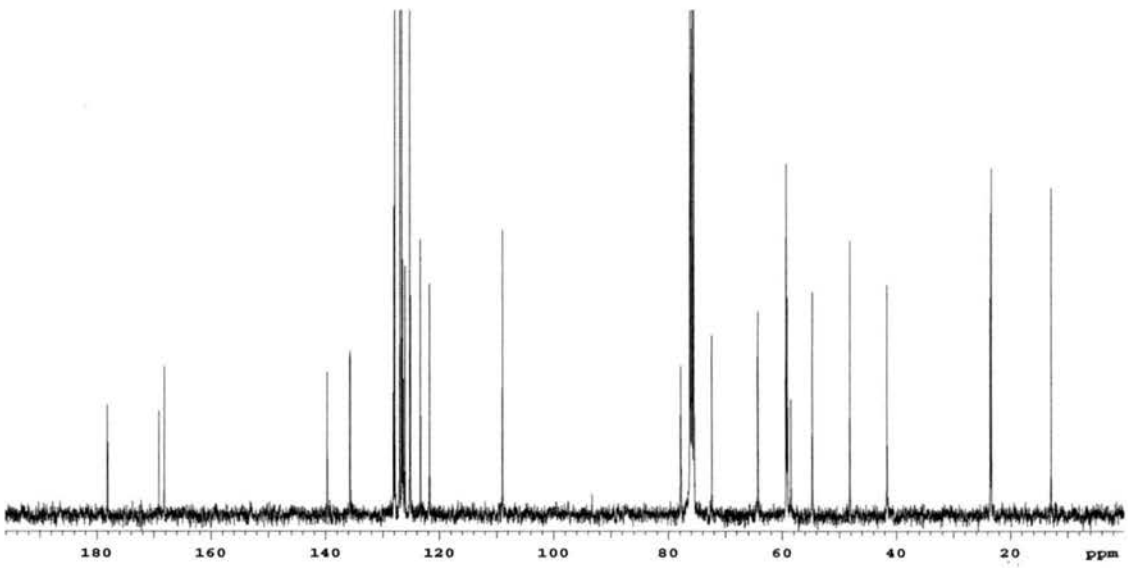
92: $[\alpha]_D^{25}$ = 118.1 (c = 1.05, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.64 (t, J = 6.8, 3H), 1.42 (s, 3 H), 1.67 (s, 3 H), 3.46 - 3.68 (m, 1H), 3.78 - 3.83 (m, 1H), 4.04 (d, J = 7.6 Hz, 1H), 4.36 (d, J = 3.6 Hz, 1H), 4.50 (t, J = 7.6 Hz, 1H), 4.51 (s, 1H), 4.87 (d, J = 7.6 Hz, 1H), 5.0 (s, 1H), 6.08 (d, J = 3.6 Hz, 2H), 6.97, (t, J = 6.8 Hz, 1H), 6.84, (d, J = 6.8 Hz, 1H), 7.16 - 7.28 (m, 12H), 7.62 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: 14.2, 24.7, 24.8, 43.0, 49.5, 56.1, 59.8, 60.4, 60.5, 60.7, 65.6, 73.7, 79.2, 110.3, 123.1, 124.7, 126.5, 127.4, 127.7, 127.8, 128.0, 128.3, 129.2, 129.4, 137.0, 137.1, 141.1, 169.5, 170.4, 179.4. IR (NaCl/neat) 3288, 1718, 1621.



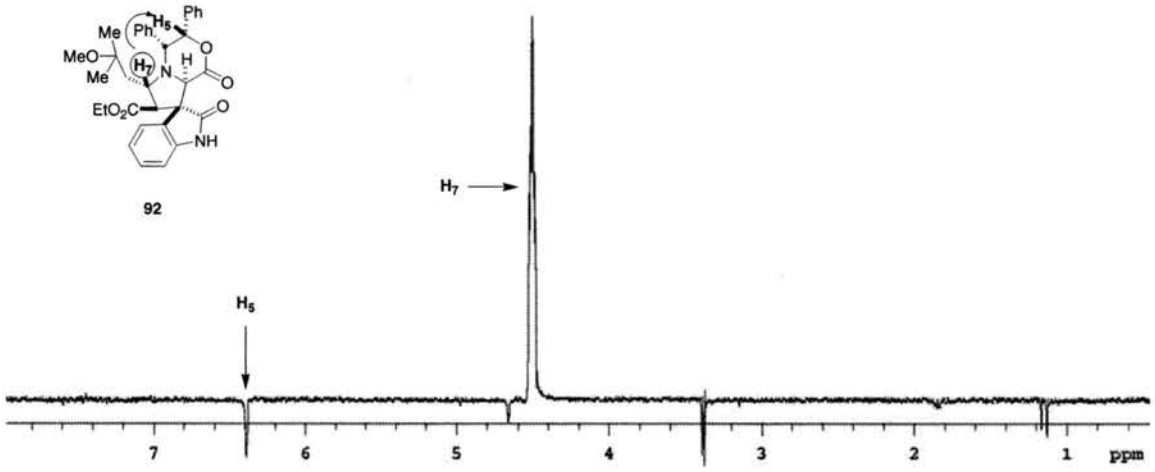
92



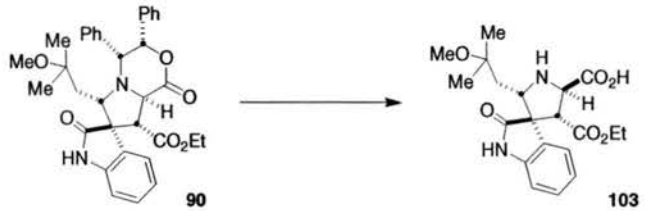
^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS1-292-rr1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS1-292-rrC13

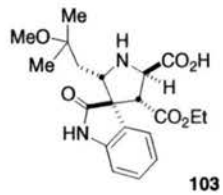


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS1-292-rr

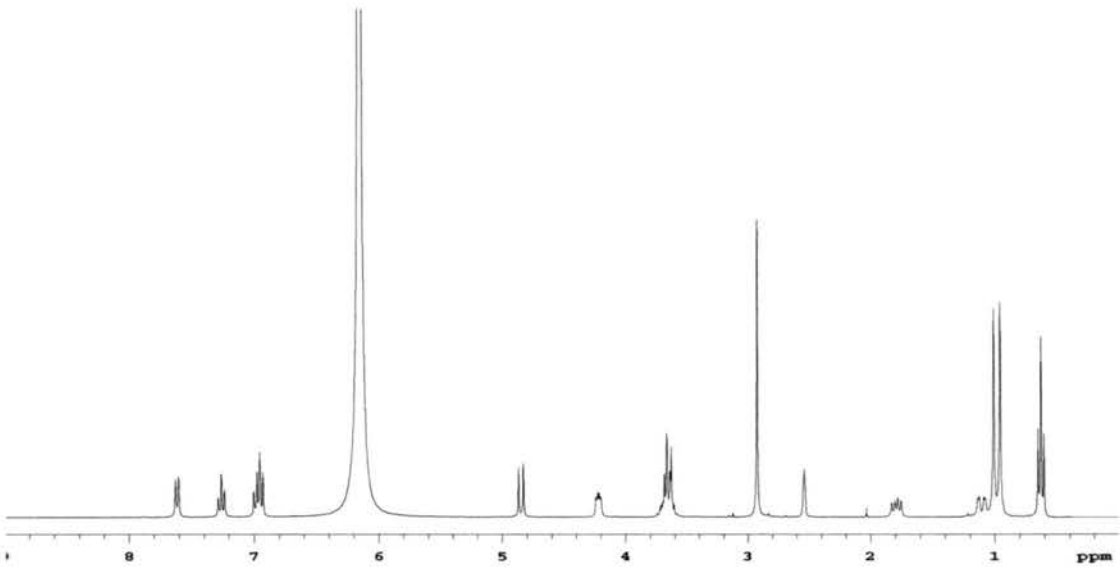


Spiro[3H-indole-3,3'-pyrrolidine]-4',5'-dicarboxylic acid, 1,2-dihydro-2'-(2-methoxy-2-methylpropyl)-2-oxo-, 4'-ethyl ester, monohydrochloride, (2'S,3S,4'R,5'R) 103.

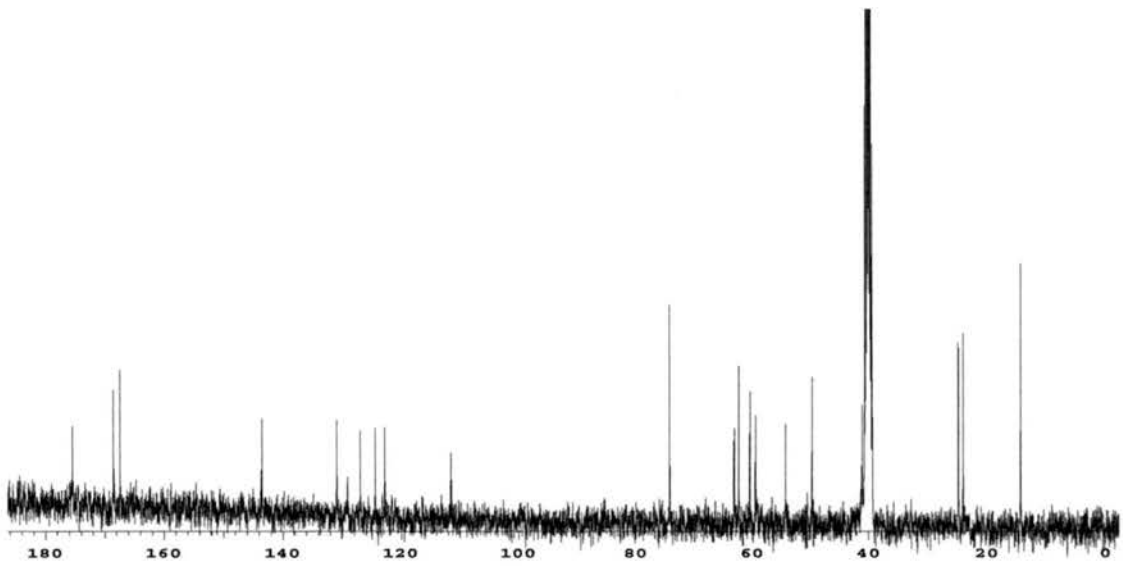
Recrystallized cycloadduct **90** (5.0 g, 8.8 mmol) was added to a sealable pressure tube and dissolved in 200 mL of 1:1 THF:EtOH. The solvent was purged with argon for 5 min and $PdCl_2$ (1.55 g, 8.80 mmol) was added. The tube was sealed and flushed with H_2 before finally pressurizing to 70 PSI. The reaction was stirred for 36 h and then filtered through celite to remove the palladium catalyst. Concentration afforded a viscous oil which was triturated with 1 x 25 mL Et_2O, 1 x 25 mL EtOAc, and 1 x 25 mL Et_2O to give 3.75 g (quant. yield) of **103** as a white solid upon drying under high vaccum. $[\alpha]_D^{25}$ = -14.0 (c = 1.0, MeOH). ^{1}H NMR (300 MHz, DMSO) δ HOD: 0.64 (t, J = 6.9, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.14 (dd, J = 3.6 Hz, J = 14.7 Hz, 1H), 1.80 (dd, J = 8.4 Hz, J = 15.0 Hz, 2H), 2.93 (s, 3H), 3.61 - 3.73 (m, 3H), 4.22 (dd, J = 4.2 Hz, J = 8.1 Hz, 1H), 4.85 (d, J = 11.4 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H), 7.00 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 7.62 (d, J = 7.5 Hz, 1H), 11.1 (br s, 1H). ^{13}C NMR (75 MHz, DMSO) δ HOD: 15.0, 24.8, 25.6, 41.9, 50.5, 55.0, 60.2, 61.1, 63.1, 63.9, 75.0, 112.3, 120.2, 123.6, 124.6, 125.2, 125.7, 127.7, 129.9, 130.9, 131.7, 144.3, 168.2, 169.3, 176.3. IR (NaCl/neat) 3444, 3098, 3058, 2977, 1746, 1771, 1634. HRMS (FAB+) calcd. for $C_{20}H_{27}O_6N_2$ (*m/z*) 391.1869, found (*m/z*) 391.1866. ***ent*- 103**: $[\alpha]_D^{25}$ = 10.0 (c = 1.0, MeOH).



103

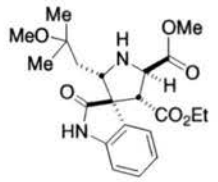


^{1}H NMR, 300 MHz, DMSO, filename: PRS1-317-1H

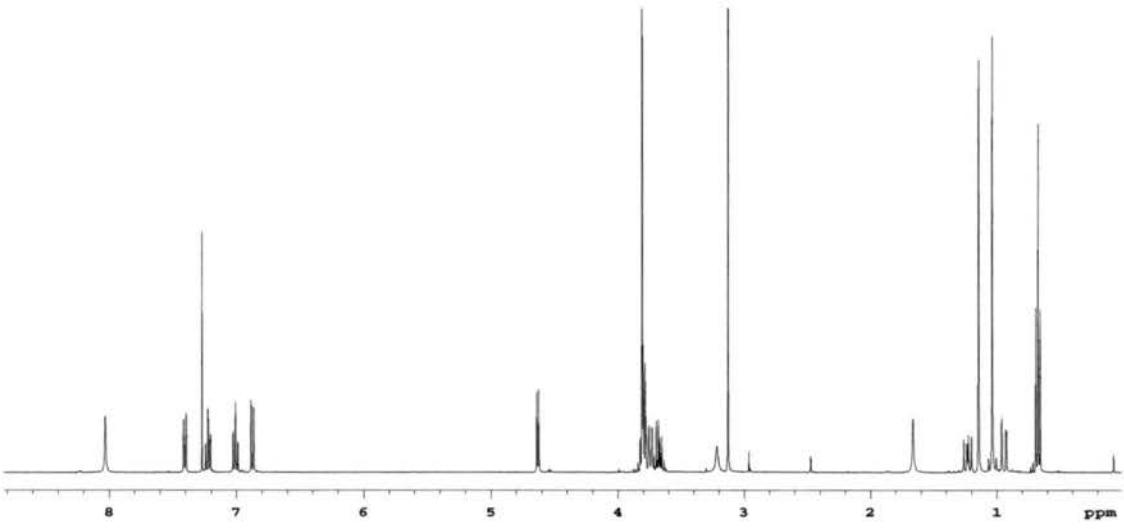


^{13}C NMR, 75 MHz, DMSO, filename: PRS1-317-C13

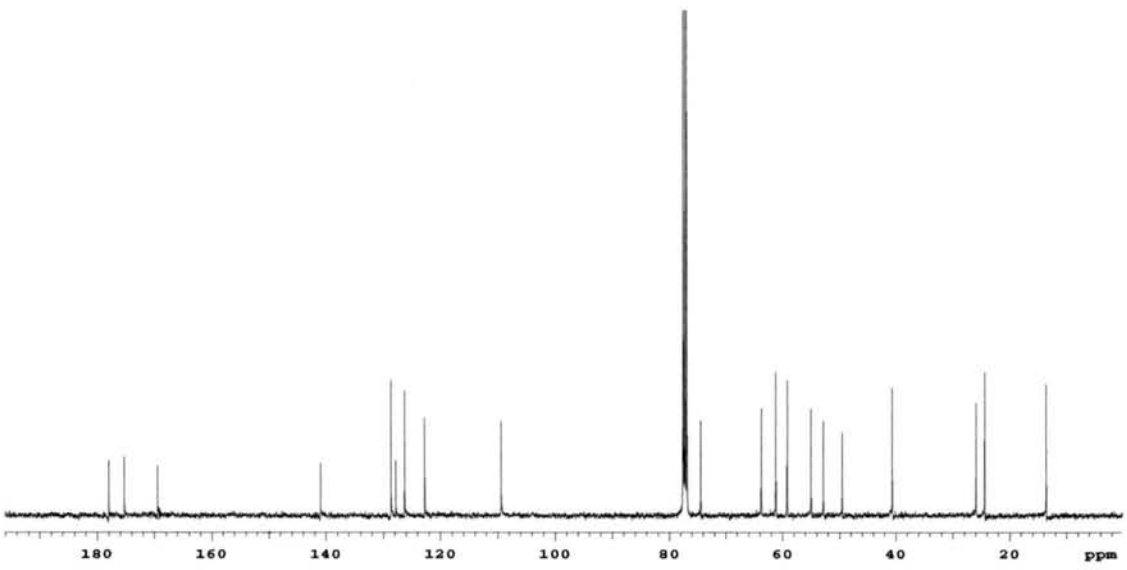
Spiro[3H-indole-3,3'-pyrrolidine]-4',5'-dicarboxylic acid, 1,2-dihydro-2'-(2-methoxy-2-methylpropyl)-2-oxo-, 4'-ethyl ester, 5'-methyl ester, (2'S,3S,4'R,5'R) 104. Recrystallized cycloadduct **90** (0.50 g, 0.88 mmol) was added to a sealable pressure tube and dissolved in 10 mL of 1:1 THF:EtOH. The solvent was purged with argon for 5 min and $PdCl_2$ (155 mg, 0.88 mmol) was added. The tube was sealed and flushed with H_2 before finally pressurizing to 70 PSI. The reaction was stirred for 36 h and then filtered through celite to remove the palladium catalyst. Concentration afforded a viscous oil which was taken up 5 mL of 1:1 CH_2Cl_2:MeOH. $TMSCHN_2$ (~1.0 mL of a 2.0 M solution in hexanes) was added until a yellow color persisted. The reaction was stirred 5 min. and then concentrated under reduced pressure. Column Chromatography with 1:1 hexanes:EtOAc affored 325 mg (91%) of **104** as a white amorphous solid. $[\alpha]_D^{25}$ = -27.3 (c = 0.97, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.63 (t, J = 6.8, 3H), 0.90 (dd, J = 1.6 Hz, J = 14.4 Hz, 1 H), 0.99 (s, 3H), 1.10 (s, 3H), 1.19 (dd, J = 9.6 Hz, J = 14.4 Hz, 1H), 3.08 (s, 3H), 3.17 (br s, 1H), 3.58 - 3.66 (m, 1H), 3.70 (d, J = 8.8 Hz, 1H), 3.72 - 3.80 (m, 2H) 3.76 (s, 3H), 4.58 (d, J = 8.8 Hz, 1H), 6.82 (d, J = 7.6 Hz, 1H), 6.96 (dt, J = 0.8 Hz, J = 7.6 Hz, 1H), 7.18 (dt, J = 0.8 Hz, J = 7.6 Hz, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.98 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: 1.53, 24.4, 25.8, 40.6, 49.4, 52.8, 54.9, 59.1, 61.0, 61.1, 63.7, 74.4, 109.4, 122.7, 126.2, 127.8, 128.6, 140.9, 169.4, 175.2, 178.0. IR (NaCl/neat) 3244, 1734. HRMS (FAB+) calcd for $C_{21}H_{29}O_6N_2$ (*m/z*) 405.2025, found (*m/z*) 405.2024.



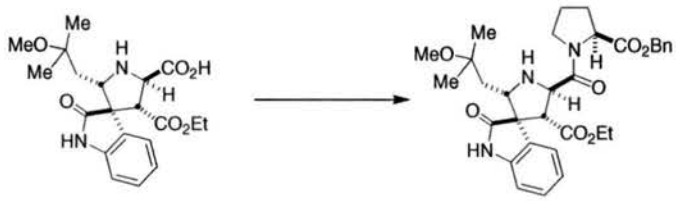
104



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS1-299-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS1-317-C13

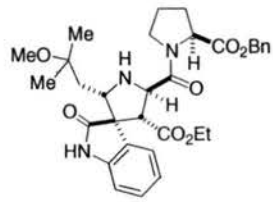


103 106

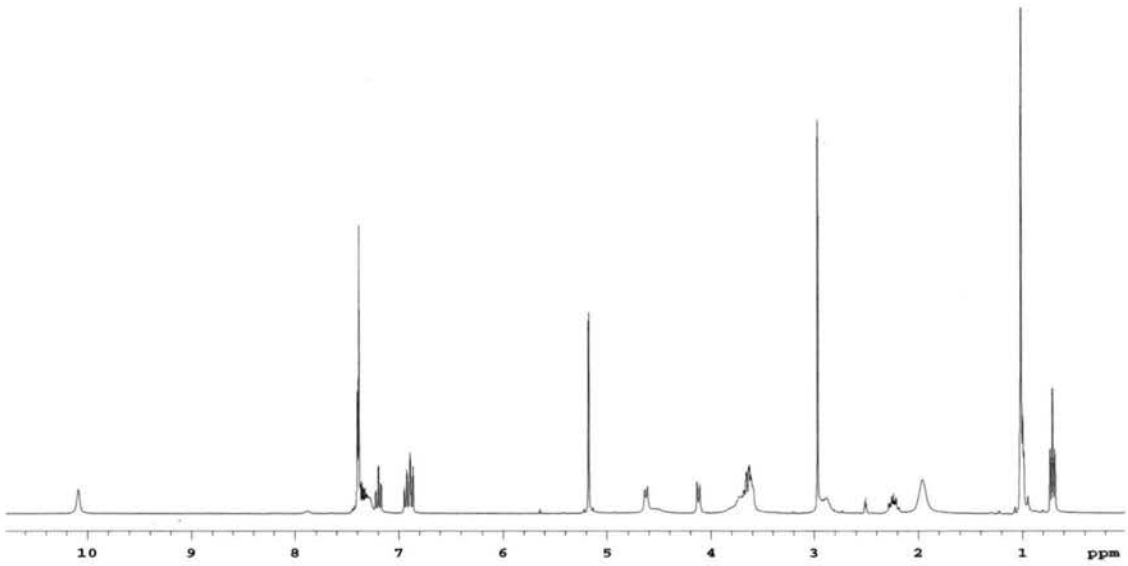
Spiro[3H-indole-3,3'-pyrrolidine]-4'-carboxylic acid, 1,2-dihydro-2'-(2-methoxy-2-methylpropyl)-2-oxo-5'-[[(2S)-2-[(phenylmethoxy)carbonyl]-1-pyrrolidinyl] carbonyl]-, ethyl ester, (2'S,3S,4'R,5'R) 106.

To a 200 mL round-bottom flask that contained amino acid **103** (3.75 g, 8.8 mmol) and was placed under high vacuum for 24 h was added BOP (4.25 g, 9.7 mmol) and L-proline benzyl ester hydrochloride (2.35 g, 9.7 mmol). The flask was flushed with argon, 100 mL of CH_3CN was added and the reaction mixture cooled to 0°C. With stirring, triethylamine (2.70 mL, 19.3 mmol) was added dropwise and the solution allowed to warm to room temperature and stir for 8 h. The solvent was then evaporated, replaced with 100 mL of EtOAc, washed with 2 x 15 mL 1N HCl, 1 x 15 mL H_2O, 2 x 15 mL 5% $NaHCO_3$, 1 x 10 mL sat. brine sol., dried over Na_2SO_4, filtered and evaporated to yield 5.0 g of a brown foam **106** which was taken on crude. An analytical sample of **106** was generated by column chromatography with 1:1 hexanes:EtOAc: $[\alpha]_D^{25}$ = -75.3 (c = 1.0, CH_2Cl_2). ^1H NMR (300 MHz, 120 °C, DMSO) δ DMSO: 0.60 (t, J = 7.2, 3H), 0.88 (d, J = 3.9 Hz, 2H), 0.90 (s, 6 H), 1.84 (br s, 1H), 2.05 - 2.16 (m, 1H), 2.75 (br s, 2H), 2.85 (s, 3H), 3.47 - 3.66 (m, 3H), 4.00 (d, J = 7.2 Hz, 1H), 4.51 (d, J = 7.5 Hz, 1H), 5.06 (s, 1H), 6.77 (t, J = 7.5 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H), 7.08 (dt, J = 1.2 Hz, J = 7.5 Hz, 1H), 7.15 - 7.28 (m, 6H), 9.97 (br s, 1H). ^{13}C NMR (75 MHz, DMSO) δ DMSO: 13.8, 25.6, 25.7, 47.4, 48.7, 49.0, 55.9, 59.9, 60.2, 60.6, 60.8, 62.7, 64.5, 66.6, 74.2, 109.9, 121.5, 122.2, 1222.6, 125.5, 128.2, 128.3, 128.4, 128.5, 129.0, 143.4, 170.2, 171.2, 172.3,

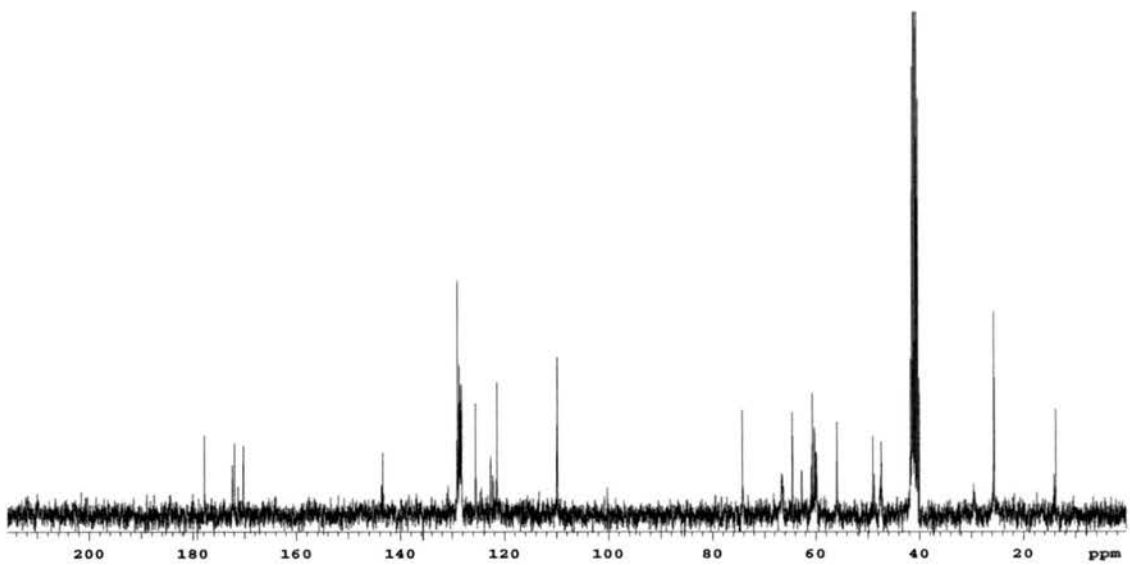
177.8. IR (NaCl/neat) 3239, 1731, 1725, 1645, 1618. HRMS (FAB+) calcd for $C_{32}H_{40}O_7N_3$ (*m/z*) 578.2866, found (*m/z*) 578.2862. ***ent*-106:** $[\alpha]_D^{25}$ = 81.5 (c = 1.0, CH_2Cl_2).



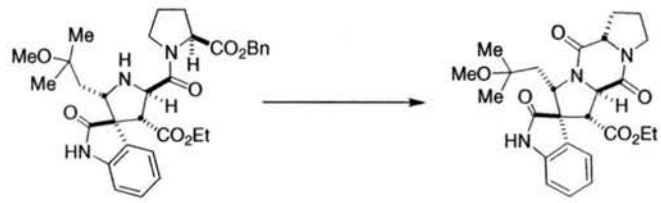
106



^{1}H NMR, 300 MHz, DMSO, filename: PRS2-602-1H



^{13}C NMR, 750 MHz, DMSO, filename: PRS2-602-C13H

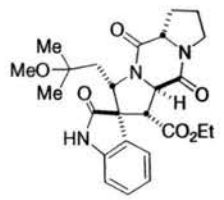


106 105

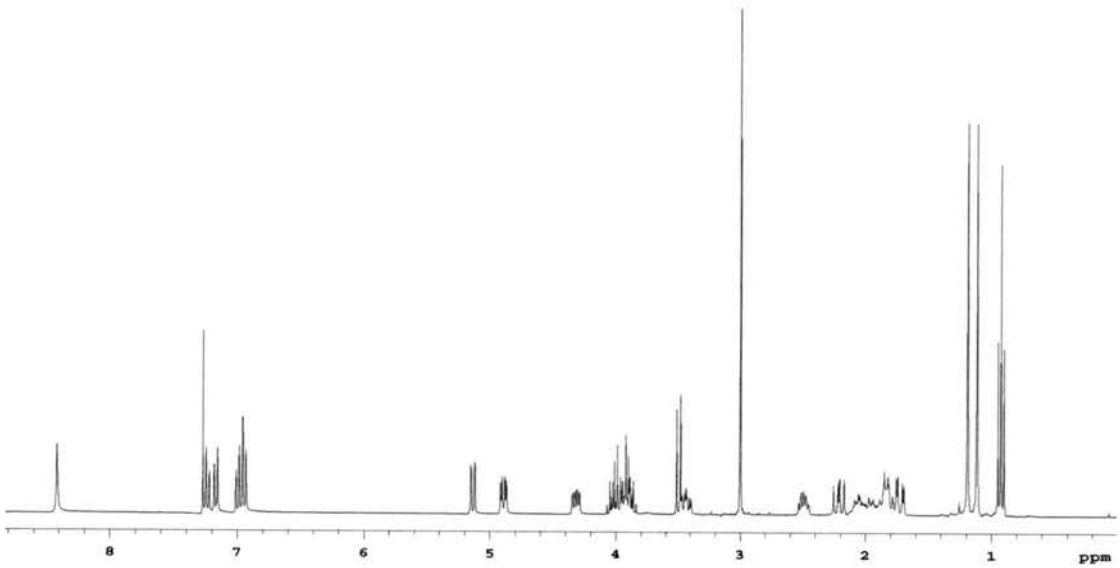
Spiro[1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3H),3'-[3H]indole]-1-carboxylic acid, 1',2',5a,6,7,8,10,10a-octahydro-3-(2-methoxy-2-methylpropyl)-2',5,10-trioxo-, ethyl ester, (1R,2S,3S,5aS,10aR) 105.

To a 100 mL round-bottom flask that contained **106** (5.0 g, 8.7 mmol) was added a stir bar and 20 mL of EtOH. Argon was bubbled through for 5 min. and 10% Pd/C (0.5 g) was added. The system was flushed with H_2 and a balloon of H_2 was attached. The solution was stirred vigorously for 1.5 h and then filtered through Celite, evaporated and placed on high vacuum overnight. To the crude mixture was added a stir bar, BOP (3.83 g, 8.6 mmol) and 80 mL of CH_3CN. Triethylamine (1.2 mL, 8.6 mmol) was added dropwise and the reaction was allowed to stir for 8 h at which time the solvent was evaporated. Purification *via* column chromatography with 75:20:5 CH_2Cl_2:EtOAc:IPA afforded 2.75 g (68%) of **105** as a white solid. $[\alpha]_D^{25}$ = -92.0 (c = 1.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.92 (t, J = 7.2, 3H) 1.11 (s, 3H), 1.19 (s, 3H), 1.72 (dd, J = 4.2 Hz, J = 14.4 Hz, 1H), 1.75 - 2.08 (m, 3H), 2.21 (dd, J = 10.5 Hz, J = 14.4 Hz, 1H), 2.49 (h, J = 6.0, 1H), 3.0 (s, 3H), 3.42 (ddd, J = 3.9 Hz, J = 7.5 Hz, J = 9.9 Hz, 1H), 3.49 (d, J = 9.3 Hz, 1H), 4.67 (d, J = 9.9 Hz, 1H), 3.84 - 4.07 (m, 3H), 4.31 (dd, J = 5.4 Hz, J = 9.9 Hz, 1H), 4.89 (dd, J = 3.9 Hz, J = 10.5 Hz, 1H), 5.13 (dd, J = 1.2 Hz, J = 9.6 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H), 6.99 (d, J = 7.5 Hz, 1H), 7.17 (d, J = 7.5 Hz, 1H), 7.25 (dt, J = 1.9 Hz, J = 7.5 Hz, 1H), 8.42 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 12.9, 20.7, 23.1, 23.7, 29.1, 38.4, 43.8, 47.9, 53.3, 56.1, 59.3, 59.6, 60.1, 60.6, 73.5, 109.6, 121.1,

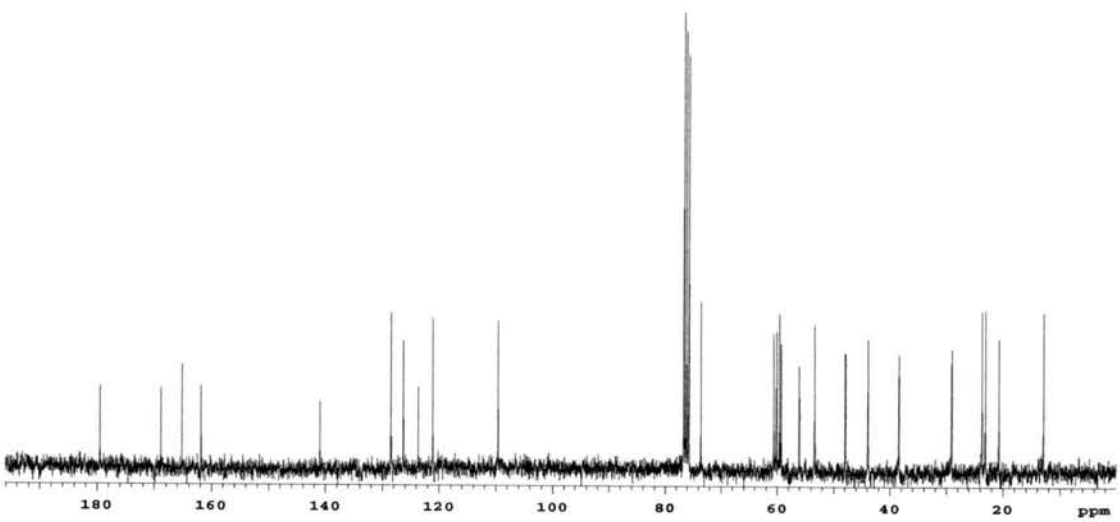
123.6, 126.3, 128.5, 141.0, 161.8, 165.2, 168.8, 179.5. IR (NaCl/neat) 3244, 1763, 1667, 1665. HRMS (FAB+) calcd for $C_{25}H_{32}O_6N_3$ (*m/z*) 470.2291, found (*m/z*) 470.2280. ***ent*-105:** $[\alpha]_D^{25} = 95.8$ (c = 1.2, CH_2Cl_2).



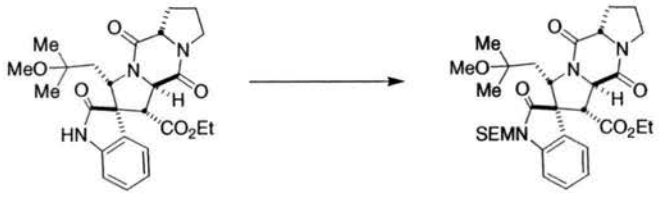
105



^{1}H NMR, 300 MHz, DMSO, filename: PRS2-602-1H



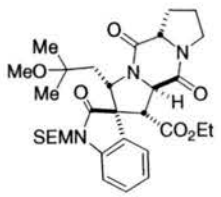
^{1}H NMR, 300 MHz, DMSO, filename: PRS2-602-1H



_N_-SEM-spiro[1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3H),3'-[3H]indole]-1-carboxylic acid, 1',2',5a,6,7,8,10,10a-octahydro-3-(2-methoxy-2-methylpropyl)-2',5,10-trioxo-, ethyl ester, (1R,2S,3S,5aS,10aR) 107:

To a flame-dried 10 mL round-bottom flask with stir bar was added diketopiperazine **105** (65 mg, 0.14 mmol). The system was flushed with Ar, THF added and cooled to –78°C. KHMDS (0.33 mL of a 0.5 M sol., 0.16 mmol) was added and stirred for 15 min. SEMCl (0.03 mL, 0.16 mmol) was added dropwise and the reaction allowed to warm to room temperature and stirred for 8 h. Sat. NH_4Cl was added and the reaction mixture poured into 10 mL EtOAc. The aq. layer was extracted 3 x 5 mL with EtOAC, the organic layers combine, dried over Na_2SO_4, filtered, evaporated and chromatographed with 75:20:5 CH_2Cl_2:EtOAc:IPA to yield 60 mg (72%) of **107** as a white solid. $[\alpha]_D^{25}$ = -72.4 (c = 0.74, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: -0.04 (s, 9H), 0.8 (t, J = 6.8, 6H), 1.12 (s, 3H), 1.18 (s, 3H), 1.70 (dd, J = 4.0 Hz, J = 14.4 Hz, 1H), 1.81 (dt, J = 3.2 Hz, J = 11.6 Hz, 1H), 1.88 - 2.01 (m, 1H), 2.02 - 2.12 (m, 1H), 2.21 (dd, J = 10.8 Hz, J = 14.4 Hz, 1H), 2.50 (quint, J = 6.0 Hz,1H), 3.01 (s, 3H), 3.40 - 3.48 (m, 1H), 3.47 (d, J = 9.2 Hz, 1H), 3.54 (t, J = 8.8 Hz, 2H), 3.83 - 3.90 (m, 1H), 3.92 - 3.96 (m, 1H), 3.97 - 4.04 (m, 1H), 4.32 (dd , J = 5.6 Hz, J = 11.6 Hz, 1H), 4.85 (dd , J = 4.0 Hz, J = 10.8 Hz, 1H), 5.05 (1/2 Abq, J = 11.2 Hz, 1H), 5.13, (dd, J = 1.2 Hz, J = 9.2 Hz, 1H), 5.20 (1/2 Abq, J = 11.2 Hz, 1H), 7.06 - 7.09 (m, 2H), 7.22 (d, J = 7.6 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -2.09, 13.0, 17.1, 20.9,

23.2, 23.8, 29.2, 38.8, 44.0, 48.2, 54.2, 56.2, 59.7, 60.2, 60.8, 65.6, 69.0, 73.7, 109.5, 121.9, 123.0, 126.4, 128.7, 142.1, 161.9, 164.9, 168.8, 178.6. IR (NaCl/neat) 2971, 1724, 1668. HRMS (FAB+) calcd for $C_{31}H_{46}O_7N_3Si_1$ (*m/z*) 600.3105, found (*m/z*) 600.3109.



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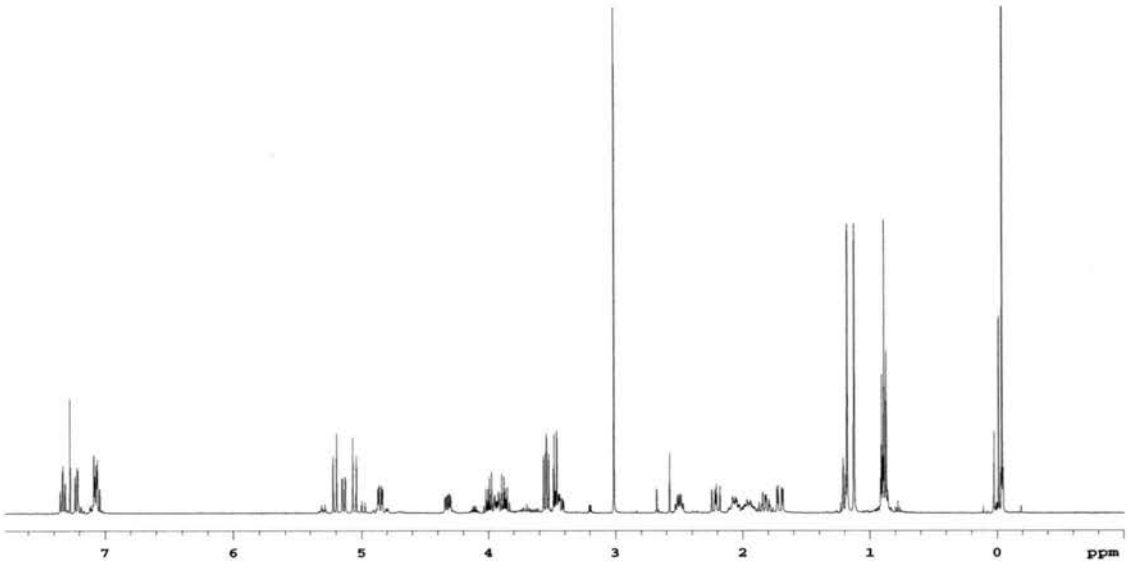
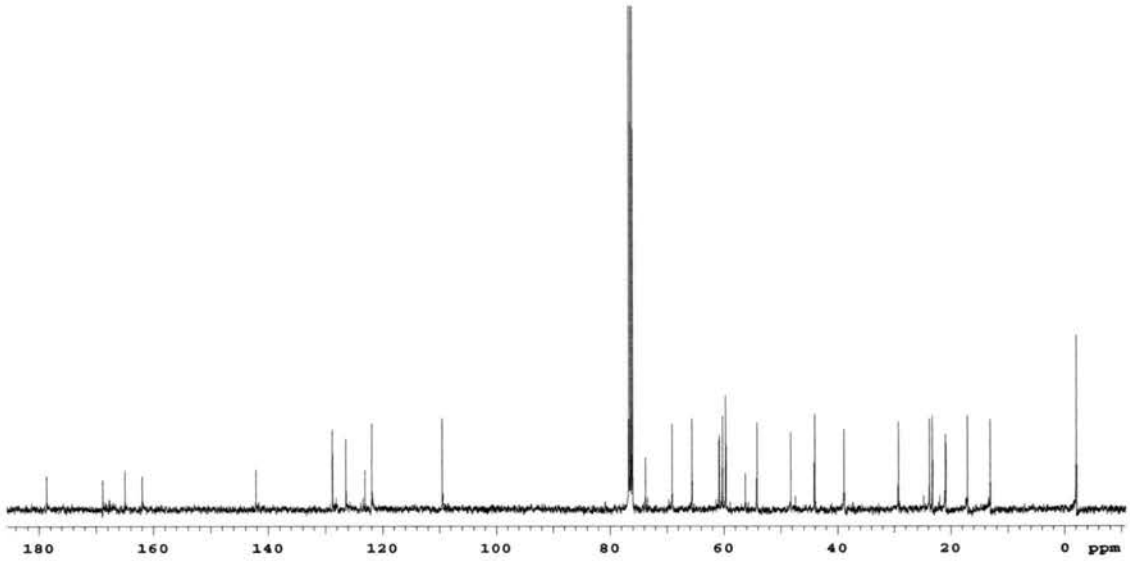
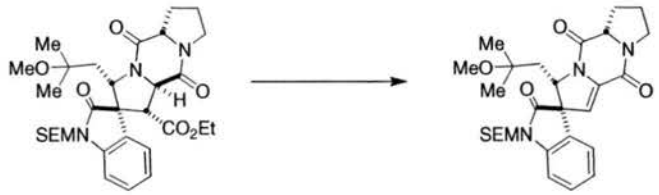


^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-561-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-561-C13

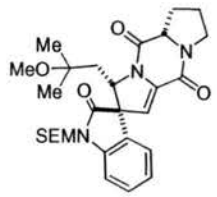


107 109

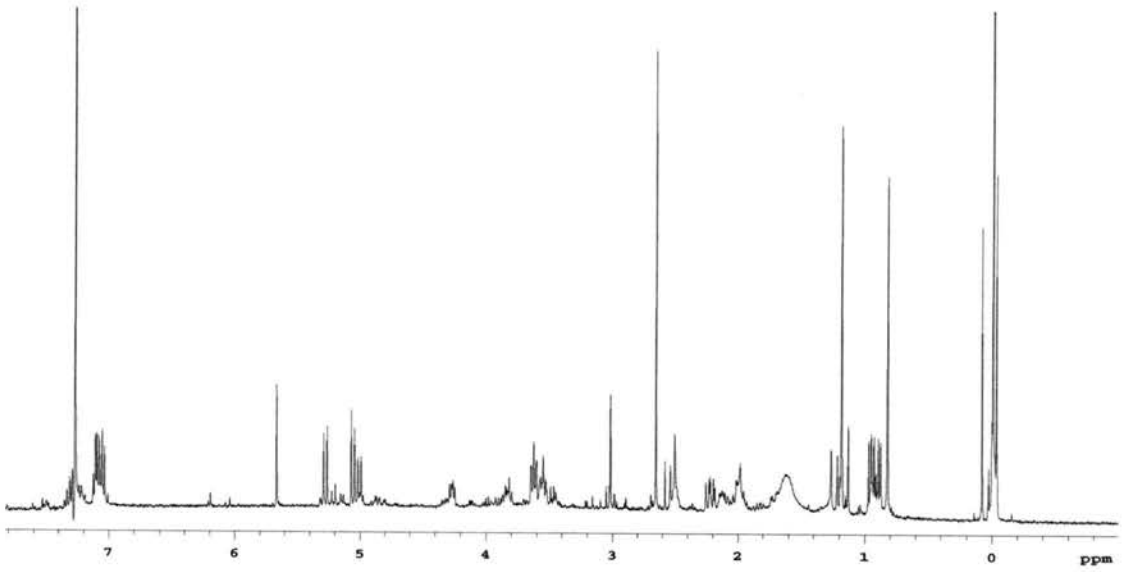
Eneamide 109.

To a flame-dried 10 mL round-bottom flask with stir bar was added SEM protected diketopiperazine **107** (70 mg, 0.08 mmol) and LiI (110 mg, 0.80 mmol). An oven dried condensor was attached and the system was flushed with argon, freshly distilled pyridine (5 mL) was added and the system heated to reflux for 48 hrs. The solvent was evaporated and replaced with 10 mL of EtOAc, extracted with 5 x 2 mL 5% $NaHCO_3$ and the aqueous layers combined. The solution was then saturated with NaCl, acidified to pH 4 with 1N HCl and extracted with 5 x 5 mL EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to yield a white solid, which was used without further purification. To the flask which contained the crude carboxylic acid was added $Cu(OAc)_2$ (1 mg, 0.006 mmol) and an oven dried condensor was attached. The system was flushed with Ar and distilled DMF (1 mL) was added. The reaction was wrapped in tin foil and stirred for 15 min. at which time $Pb(OAc)_4$ (55 mg, 0.12 mmol) was added. The mixture was stirred (still in the dark) for 15 min. more and then heated to reflux for 1.5 h. Evaporation of the solvent and purification via column chromatography with 75:20:5 CH_2Cl_2:EtOAc:IPA to yielded 5 mg (11%) of **109** as a clear oil. $[\alpha]_D^{25}$ = -6.25 (c = 0.16, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: -0.04 (s, 9H), 0.79 (s, 3H), 0.91 (t, J = 7.6, 3H), 1.15 (s, 3H), 1.22 (d, J = 8.4 Hz, 1H), 1.92 - 2.00 (m, 2H), 2.10 - 2.1 (m, 1H), 2.18 (dd, J = 10.8 Hz, J = 13.6 Hz, 1H), 2.40 - 2.50 (m, 1H), 2.62 (s, 3H), 3.40 - 3.48 (m, 1H), 3.49 - 3.54 (m, 1H), 3.60 (t, J = 8.8 Hz, Hz, 1H), 4.21 -

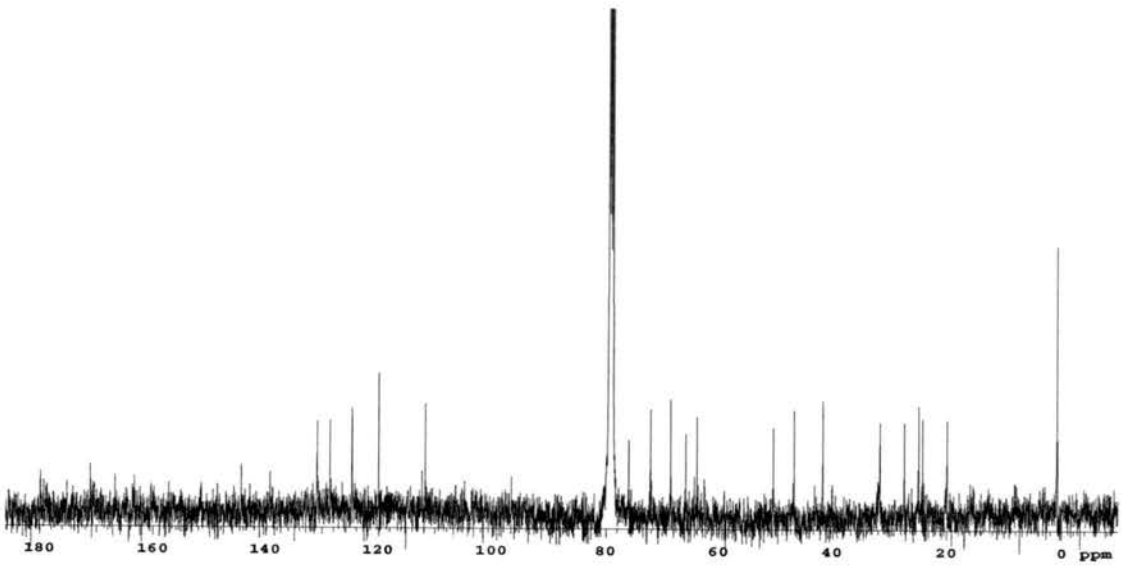
4.25 (m, 1H), 3.78 - 3.81 (m, 1H), 4.97 (d, J = 10.0 Hz, 1H), 5.02 (1/2 ABq, J = 10.8 Hz, 1H), 5.24 (1/2 ABq, J=10.8 Hz, 1H), 5.63 (s, 1H), 6.99 - 7.09 (m, H), 7.2 (d, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -1.23, 18.0, 22.2, 22.9, 25.4, 29.7, 39.7, 44.7, 48.4, 61.9, 63.8, 66.5, 70.1, 70.6, 109.7, 110.3, 118.0, 122.7, 126.6, 128.8, 137.2, 142.3, 161.3, 164.6, 169.0 . IR (NaCl/neat) 2952, 1727, 1683, 1650. HRMS (FAB+) calcd for $C_{28}H_{40}O_5N_3Si_1$ (*m/z*) 526.2737, found (*m/z*) 527.2727.



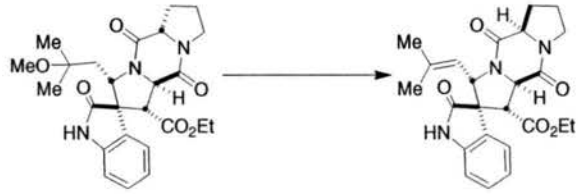
109



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-447-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-447-C13

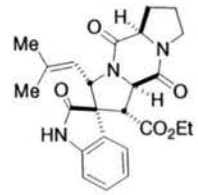


105 110

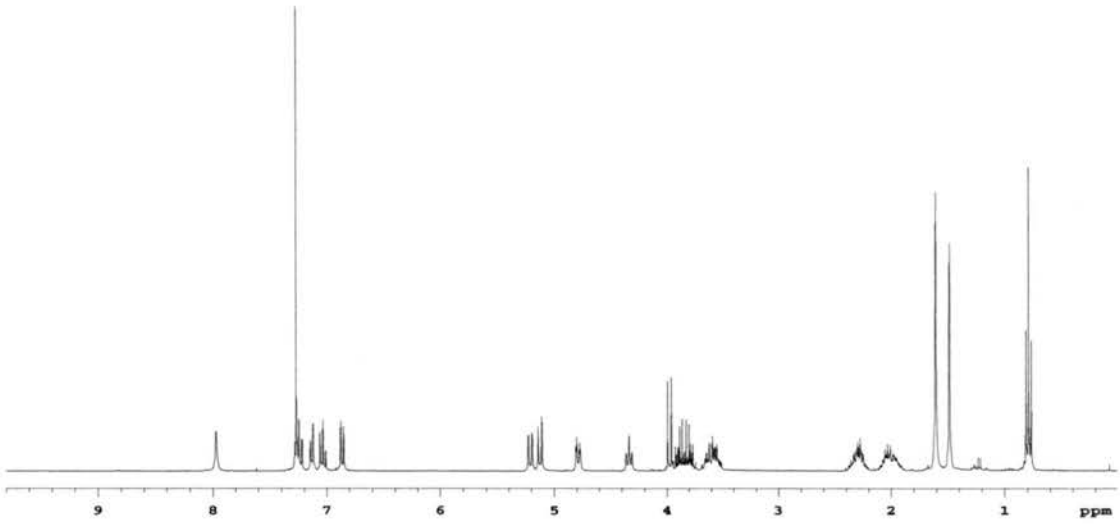
Spiro[1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3H),3'-[3H]indole]-1-carboxylic acid, 1',2',5a,6,7,8,10,10a-octahydro-3-(2-methyl-1-propenyl)-2',5,10-trioxo-, ethyl ester, (1R,2S,3S,5aR,10aR 110.

To a flame-dried 250 mL round-bottom flask with stir bar was added diketopiperazine **105** (2.70 g, 5.75 mmol), 4Å molecular sieves (5.0 g) and TsOH (1.0 g, 5.75 mmol). An oven-dried condensor was attached, the system was flushed with argon, freshly distilled toluene (200 mL) was added and the system heated to reflux temperature for 8 h. The solvent was evaporated and replaced with 100 mL of EtOAc, washed with 2 x 15 mL 5% $NaHCO_3$, 1 x 10 mL sat. brine sol., dried over Na_2SO_4, filtered, evaporated and chromatographed with 75:20:5 CH_2Cl_2:EtOAc:IPA to yield 1.75 g (70%) of **110** as a white amorphous solid. $[\alpha]_D^{25} = 78.5$ (c = 1.0, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.79 (t, J = 7.5, 3H) 1.48 (d, J = 1.5 Hz, 3H), 1.61 (d, J = 1.5 Hz, 3H), 1.90 - 2.10 (m, 2H), 2.20 - 2.40 (m, 2H), 3.50 - 3.70 (m, 2H), 3.74 - 3.92 (m, 2H), 3.97 (d, J = 10.2 Hz, 1H), 4.33 (t, J = 7.5 Hz, 1H), 4.78 (dt, J = 1.5 Hz, J = 9.6 Hz, 1H), 5.12 (d, J = 9.6 Hz, 1H), 5.21 (d, J = 10.2 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 7.03 (dt, J = 1.9 Hz, J = 7.5 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 7.24 (dt, J = 1.9 Hz, J = 7.5 Hz, 1H), 7.97 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 7.27, 11.9, 17.4, 19.4, 20.8, 39.3, 45.2, 52.8, 54.3, 54.9, 55.3, 57.2, 103.8, 113.1, 116.0, 119.2, 119.3, 122.8, 130.8, 134.8, 158.5, 160.3, 161.5, 171.0. IR (NaCl/neat) 3219, 1723, 1663, 1648. HRMS (FAB+) calcd for

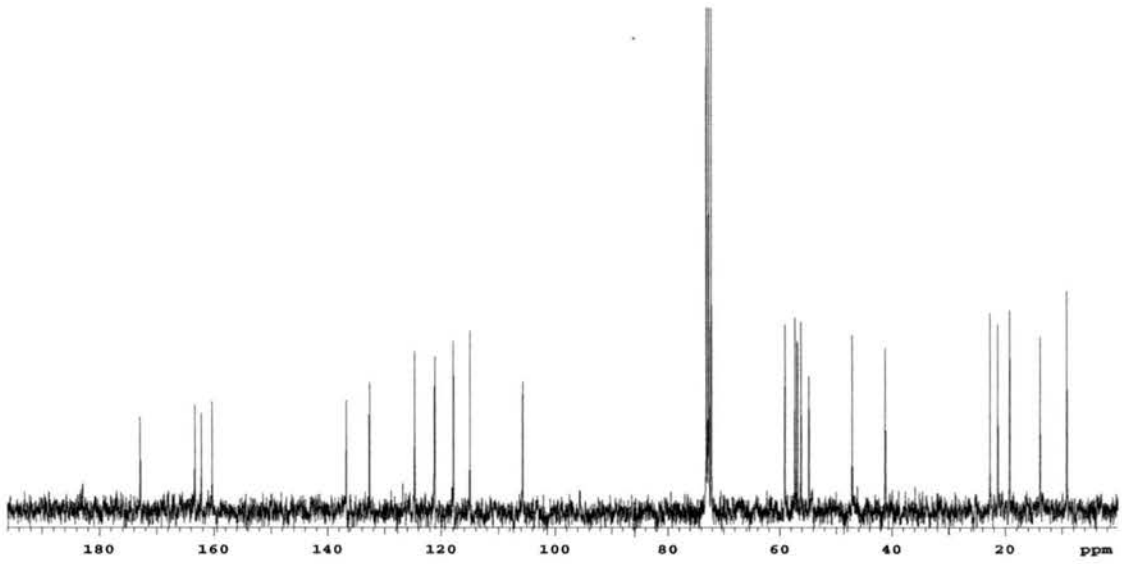
$C_{24}H_{28}O_5N_3$ (*m/z*) 438.2029, found (*m/z*) 438.2017. ***ent*-110:** $[\alpha]_D^{25}$ = -74.0 (c = 1.0, CH_2Cl_2).



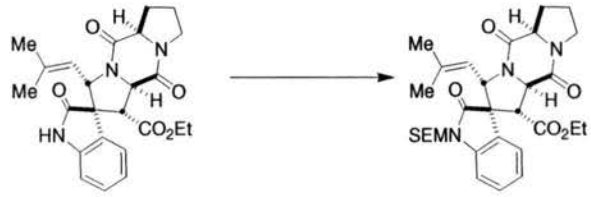
110



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-583-1H



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-583-1H

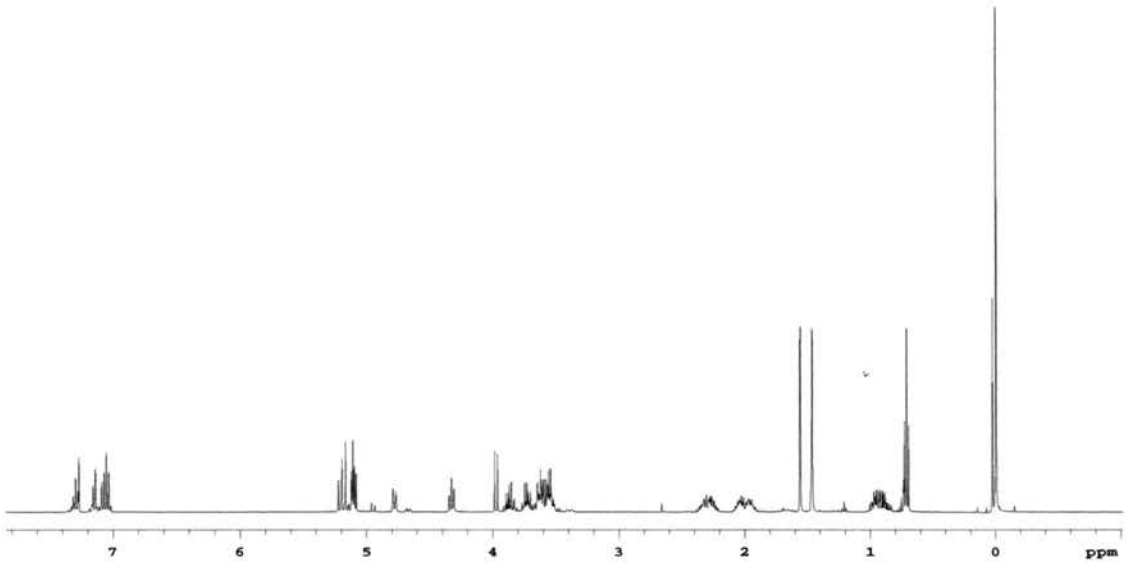


***N*-SEM-spiro[1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3H),3'-[3H]indole]-1-carboxylic acid, 1',2',5a,6,7,8,10,10a-octahydro-3-(2-methyl-1-propenyl)-2',5,10-trioxo-, ethyl ester, (1R,2S,3S,5aR,10aR 111.**

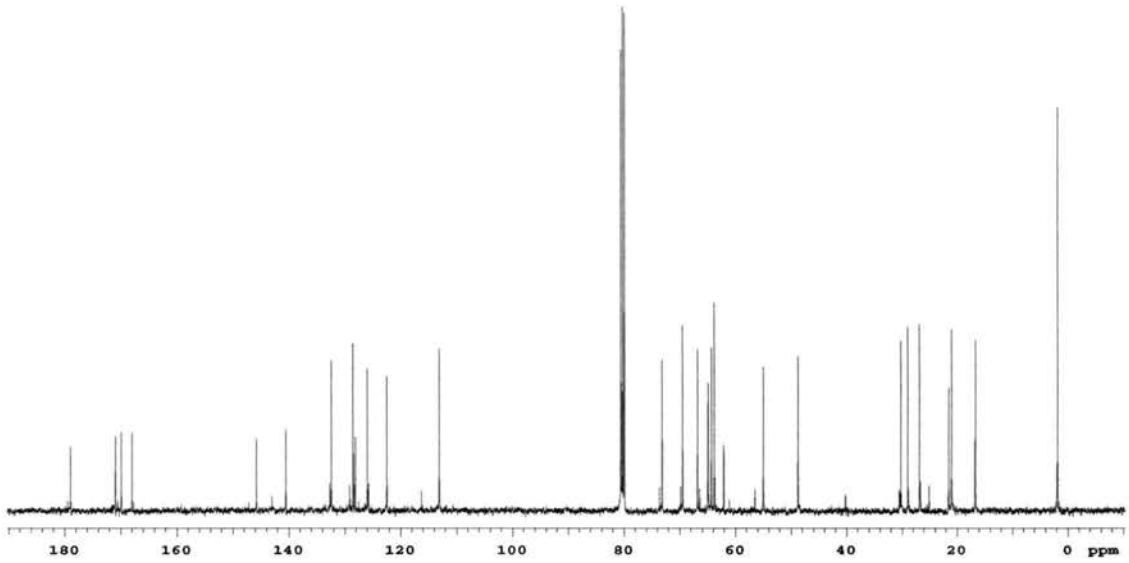
To a flame-dried 10 mL round-bottom flask with stir bar was added **110** (65 mg, 0.15 mmol). The system was flushed with Ar, THF added and cooled to –78°C. KHMDS (0.35 mL of a 0.5 M sol., 0.18 mmol) was added and stirred for 15 min. SEMCl (0.035 mL, 0.18 mmol) was added dropwise and the reaction allowed to warm to room temperature and stirred for 8 h. Sat. NH_4Cl was added and the reaction mixture poured into 10 mL EtOAc. The aq. layer was extracted 3 x 5 mL with EtOAC, the organic layers combine, dried over Na_2SO_4, filtered, evaporated and chromatographed with 75:20:5 CH_2Cl_2:EtOAc:IPA to yield 70 mg (84%) of the white solid **111**. $[\alpha]_D^{25}$ = -63.5 (c = 0.97, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: -0.04 (s, 9H), 0.71 (t, J = 7.2, 3H), 0.86 - 1.00 (m, 2H), (1.46 (s, 3H), 1.55 (s, 3H), 1.95 – 2.02 (m, 2H), 2.24 - 2.40 (m, 2H), 3.52 - 3.65 (m, 3H), 3.68 - 3.76 (m, H), 3.82 - 3.90 (m, 1H), 3.97 (d, J = 10.0 Hz, 1H), 4.32 (t, J = 8.0 Hz, 1H), 4.78 (d, J = 14.8 Hz, 1H), 5.09 (1/2 ABq, J = 11.2 Hz, 1H), 5.10 (d, J = 10.0 Hz, 1H), 5.20 (1/2 ABq, J = 11.2 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -1.16, 13.7, 17.9, 18.5, 23.8, 25.9, 27.2, 45.7, 51.9, 59.0, 60.7 ,61.3, 61.8, 63.7, 66.4, 70.1, 110.0, 119.4, 122.9, 125.1, 125.5, 129.4, 137.5, 142.8, 165.0, 166.8, 167.9,

175.9. IR (NaCl/neat) 1728, 1678. HRMS (FAB+) calcd for $C_{30}H_{42}O_6N_3Si_1$ (*m/z*) 568.2843, found (*m/z*) 568.2827.

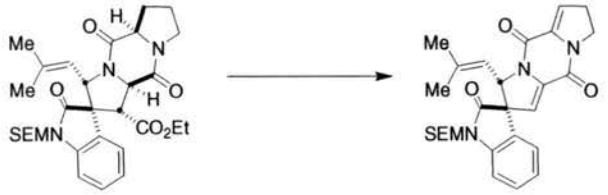
111



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-507-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-507-C13

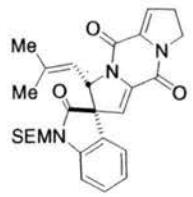


111 **112**

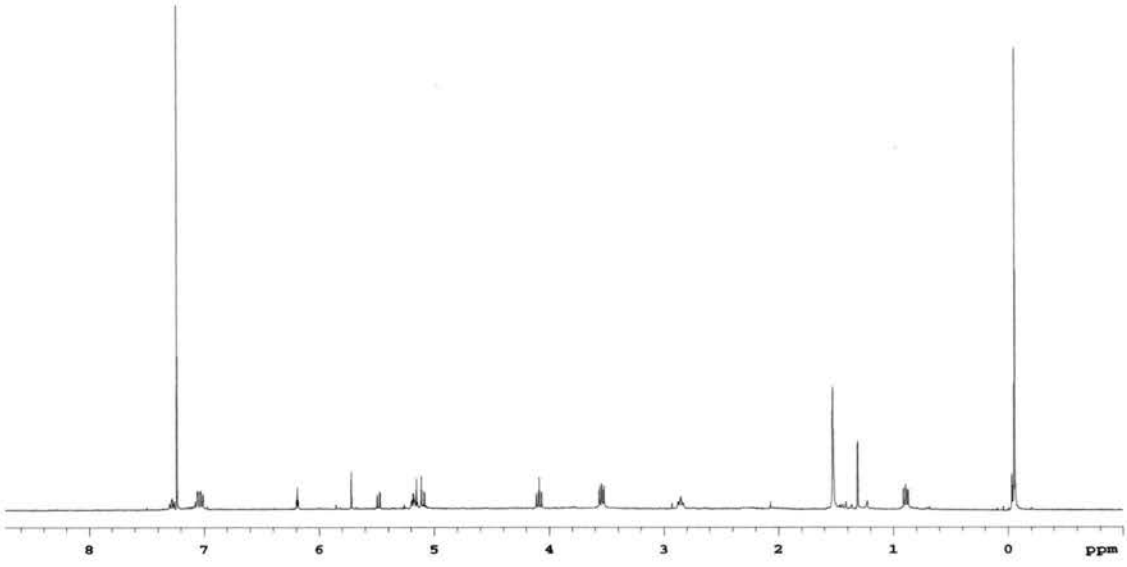
***N*-SEM Triene 112.**

To a flame-dried 10 mL round-bottom flask with stir bar was added **111** (70 mg, 0.12 mmol) and LiI (165 mg, 1.2 mmol). An oven dried condensor was attached and the system was flushed with argon, freshly distilled pyridine (5 mL) was added and the system heated to reflux for 48 hrs. The solvent was evaporated and replaced with 10 mL of EtOAc, extracted with 5 x 2 mL 5% $NaHCO_3$ and the aqueous layers combined. The solution was then saturated with NaCl, acidified to pH 4 with 1N HCl and extracted with 5 x 5 mL EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to yield 45 mg (68%) a white solid, which was used without further purification. To the flask which contained the crude carboxylic acid was added $Cu(OAc)_2$ (1.5 mg, 0.008 mmol) and an oven dried condensor was attached. The system was flushed with Ar and distilled DMF (1 mL) was added. The reaction was wrapped in tin foil and stirred for 15 min. at which time $Pb(OAc)_4$ (55 mg, 0.12 mmol) was added. The mixture was stirred (still in the dark) for 15 min. more and then heated to reflux for 1.5 h. Evaporation of the solvent and purification via column chromatography with 75:20:5 CH_2Cl_2:EtOAc:IPA to yielded 8 mg (20%) of **112** as a clear oil. $[\alpha]_D^{25}$ = -60.0 (c = 0.05, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: -0.02 (s, 9H), 0.93 (t, J = 7.6, 3H), (1.34 (s, 3H), 1.56 (s, 3H), 2.89 (dt, J = 2.4 Hz, J = 8.0 Hz, 2H), 3.57 (t, J = 7.6 Hz, 2H), 4.12 (t, J = 8.8 Hz, 2H), 5.13 (d, J = 10.8 Hz, 1H), 5.21 (t, J = 11.2 Hz, 2H), 5.20 (d, J = 8.0 Hz, 1H), 5.75 (s, 1H), 6.22 (t, J = 3.2 Hz, 1H), 7.04 - 7.11 (m, 3H), 7.31 (t, J = 7.6 Hz,

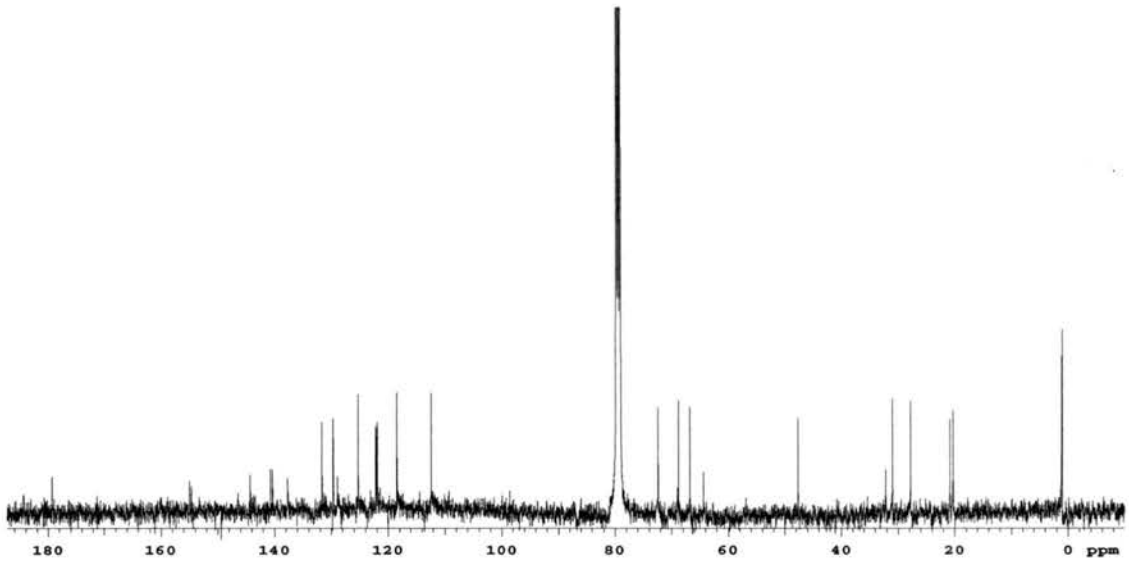
1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -1.2, 18.0, 18.5, 25.4, 28.7, 29.9, 45.4, 62.1, 64.5, 66.6, 70.2, 110.1, 116.2, 119.7, 119.9, 123.0, 126.7, 127.4, 129.4, 135.5, 138.1, 138.5, 142.0, 152.2, 152.7, 177.0. IR (NaCl/neat) 1727, 1683. HRMS (FAB+) calcd for $C_{27}H_{33}O_4N_3Si_1$ (*m/z*) 491.2240, found (*m/z*) 491.2226.



112



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-509-1H

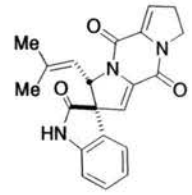


^{1}H NMR, 100 MHz, $CDCl_3$, filename: PRS2-509-C13

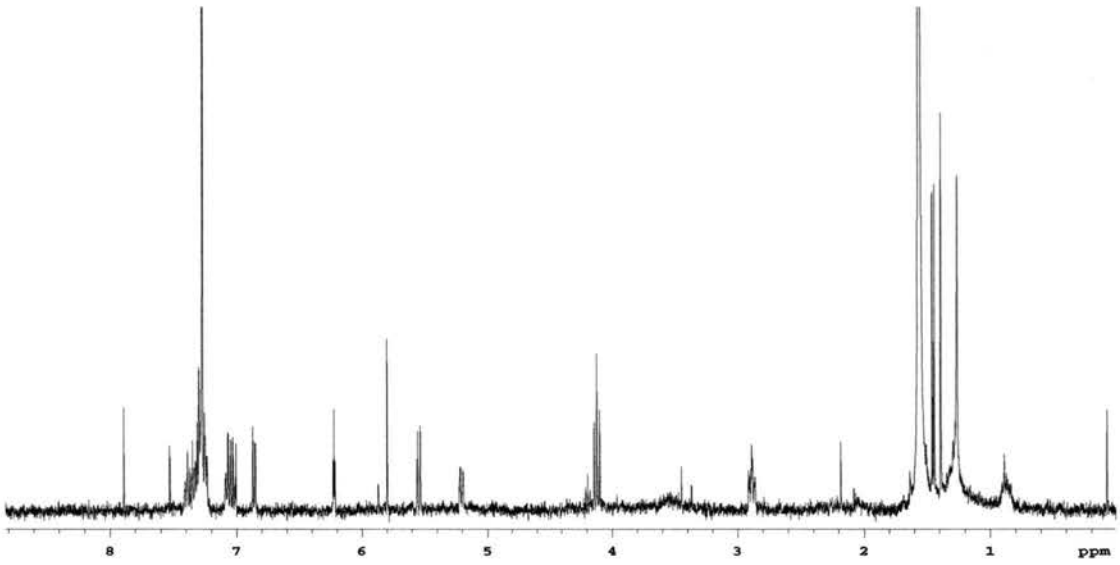
112 113

Triene 113.

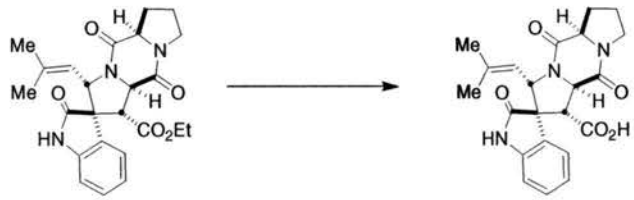
To a flame-dried 10 mL round-bottom flask with stir bar was added **112** (8 mg, 0.017 mmol) dissolved in CH_2Cl_2 (2 mL) and cooled to –78°C. A 1.0M hexane solution of Me_2AlCl (0.086 mL, 0.086 mmol) was added dropwise under Ar. The mixture was warmed to room temperature and stirred for 15 min. The solution was cooled to 0°C and poured into a sat. Na/K tartrate solution (2 ml) also at 0°C. The mixture was allowed to warm to room temperature and stirred vigorously for 1 h. The aq. layer was then extracted 3 x 5 mL with EtOAc, the organic layers combined, dried over Na_2SO_4, filtered and evaporated. Purification was accomplished by PTLC (1/2 of a 250μ plate) with 75:20:5 CH_2Cl_2:EtOAc:IPA as the eluent to yield 3 mg (48%) of **113** as a clear oil. ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 1.22 (s, 3H), 1.35 (s, 3H), 2.85 (dt, J = 3.2 Hz, J = 8.0 Hz, 2H), 4.09 (t, J = 8.8 Hz, 2H), 5.17 (d, J = 8.8 Hz, 1H), 5.51 (d, J = 8.8 Hz, 1H), 5.75 (s, 1H), 6.19 (t, J = 3.2 Hz, 1H), 6.82 (d, J = 7.6 Hz, 1H), 6.97 - 7.05 (m, 2H), 7.37 (t, J = 7.6 Hz, 1H), 7.85 (br s, 1H). IR (NaCl/neat) 1763, 1667. HRMS (FAB+) calcd for $C_{21}H_{20}O_3N_3$ (*m/z*) 362.1504, found (*m/z*) 362.1484.



113



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-696-1H

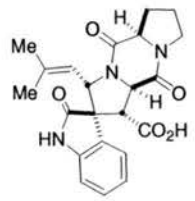


110 114

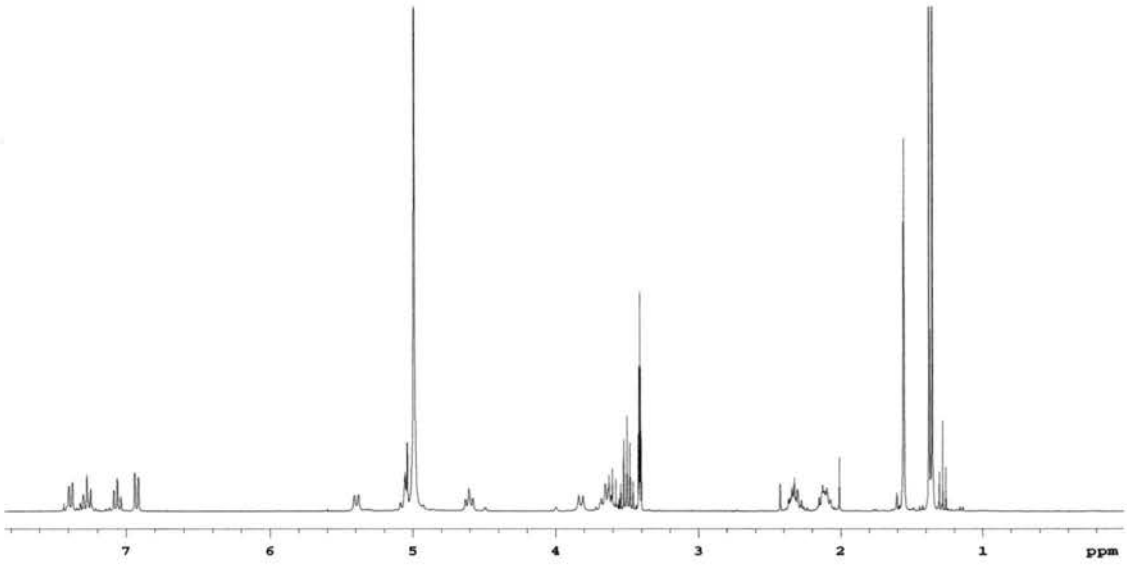
Spiro[1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3H),3'-[3H]indole]-1-carboxylic acid, 1',2',5a,6,7,8,10,10a-octahydro-3-(2-methyl-1-propenyl)-2',5,10-trioxo-, (1R,2S,3S,5aR,10aR) 114.

To a flame dried 100 mL round-bottom flask with stir bar was added diketopiperazine **110** (0.87 g, 2.0 mmol) and LiI (2.66 g, 20.0 mmol). An oven dried condensor was attached and the system was flushed with argon, freshly distilled pyridine (50 mL) was added and the system heated to reflux for 48 hrs. The solvent was evaporated and replaced with 50 mL of EtOAc, extracted with 5 x 10 mL 5% $NaHCO_3$ and the aqueous layers combined. The organic layer was dried over Na_2SO_4, filtered, evaporated and purified via column chromatography with 75:20:5 CH_2Cl_2:EtOAc:IPA to recover 80 mg of unreacted starting material **110**. The aqueous phase was saturated with NaCl, acidified to pH 4 with 1N HCl and extracted with 5 x 10 mL EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to yield 0.58 g (71%) of **114** as white amorphous solid. $[\alpha]_D^{25}$ = 73.0 (c = 0.8, MeOH). ^1H NMR (300 MHz, CD_3OD) δ MeOH: 1.27 (s, 3H), 1.29 (s, 3H), 1.98 - 2.04 (m, 2H), 2.18 - 2.30 (m, 2H), 3.50 - 3.70 (m, 2H), 3.37 - 3.46 (m, 1H), 3.51 - 3.58 (m, 2H), 3.74 (d, J = 10.2 Hz, 1H), 4.52 (t, J = 7.5 Hz, 1H), 4.96 (d, J = 5.1 Hz), 5.32 (d, J = 9.3 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.98 (dt, J = 1.9 Hz, J = 7.5 Hz, 1H), 7.19 (dt, J = 1.9 Hz, J = 7.5 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ MeOH: 12.5, 18.7, 19.9, 22.3, 40.7, 47.0,

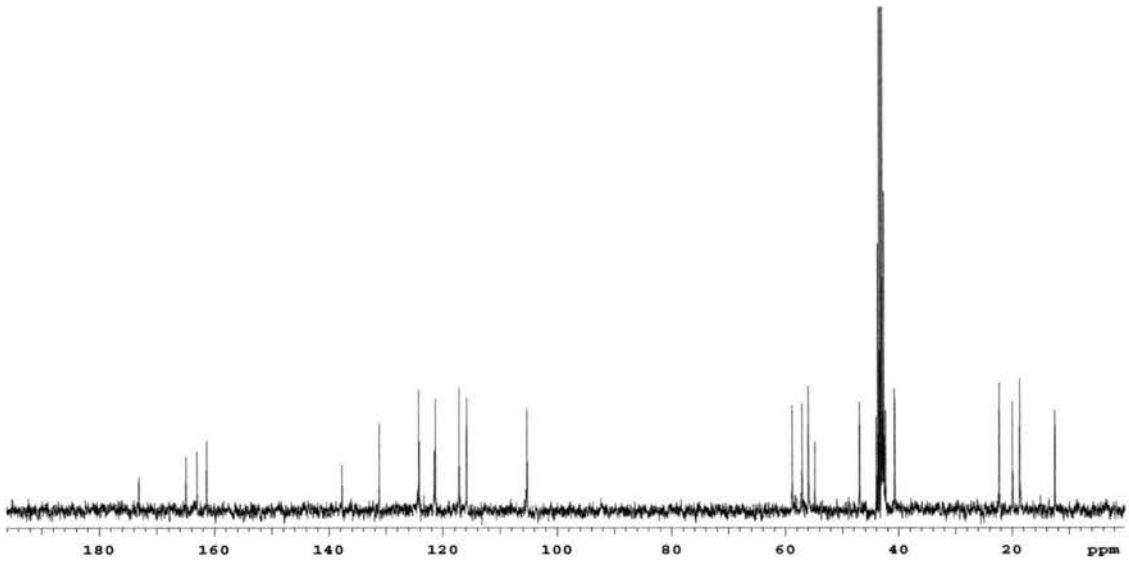
54.8, 55.9, 57.1, 58.8, 105.2, 115.8, 117.1, 121.3, 121.5, 124.2, 131.1, 137.7, 161.3, 163.0, 164.9, 173.1. IR (NaCl/neat 3248, 1731, 1678, 1668. HRMS (FAB+) calcd for $C_{22}H_{24}O_5N_3$ (*m/z*) 410.1716, found (*m/z*) 410.1698. ***ent*-114:** $[\alpha]_D^{25}$ = -75.0 (c = 1.0, MeOH).



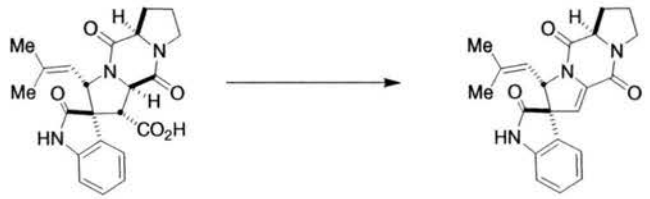
114



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-607-2H



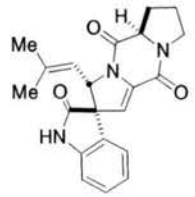
^{1}H NMR, 75 MHz, $CDCl_3$, filename: PRS2-607-C13



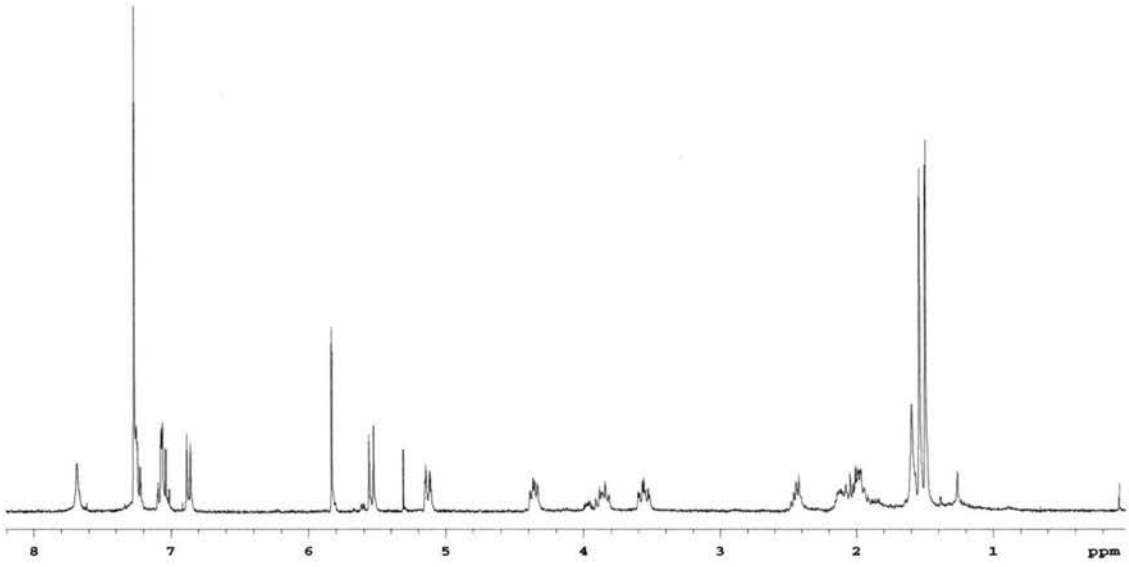
92 93

Spiro[3H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(10H),3'-[3H]indole]-2',5,10(1'H)-trione, 5a,6,7,8-tetrahydro-3-(2-methyl-1-propenyl)-, (2S,3S,5aR) (12-*epi*-Spirotyprostatin B) 115. To a flame-dried 100 mL round-bottom flask with stir bar was added carboxylic acid **114** (0.290 g, 0.26 mmol), DCC (0.22 g, 1.06 mmol), DMAP (0.13 g, 1.06 mmol) and 2-mercaptopyridine *N*-oxide (0.112, 0.88 mmol). An oven-dried condenser was attached and the system was flushed with argon and wrapped in tin foil. Freshly distilled $BrCCl_3$ (25 mL) was added and the system was heated to 60°C for 1 h. The foil was then removed and the reaction heated to reflux for 1.5 h. The solvent was evaporated and the resulting oil was purified by chromatography (silica gel, eluted with 75:20:5 CH_2Cl_2:EtOAc:IPA) to yield 0.095 g (37%) of **115**. $[\alpha]_D^{25}$ = 41.3 (c = 0.8, CH_2Cl_2). ^1H NMR (300 MHz $CDCl_3$) δ $CHCl_3$: 1.50 (d, J = 1.5 Hz, 3H), 1.54 (d, J = 1.5 Hz, 3H), 1.90 - 2.16 (m, 3H), 2.18 - 2.30 (m, 2H), 3.40 - 3.48 (m, 1H), 3.52 - 3.60 (m, 1H), 3.81 - 3.92 (m, 2H), 4.36 (dd, J = 6.9 Hz, J = 10.5 Hz, 1H), 5.13 (dt, J = 1.5 Hz, J = 8.1 Hz, 1H), 5.54 (d, J = 9.3), 5.83 (s,1H), 6.87 (d, J = 7.5 Hz, 1H), 7.01 - 7.09 (m, 2H), 7.19 (dt, J = 1.9 Hz, J = 7.5 Hz, 1H), 7.22 - 7.27 (m, 1H) 7.69 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: 18.6, 22.2, 25.7, 29.3, 45.3, 62.0, 62.1, 64.8, 110.1, 115.8, 119.3, 121.8, 122.8, 127.2, 128.6, 129.3, 155.7, 162.5, 178.2. IR (NaCl/neat) 3196, 1724, 1676, 1639. HRMS (FAB+) calcd for $C_{21}H_{21}O_3N_3$ (*m/z*) 364. 1661, found (*m/z*) 364.1658.

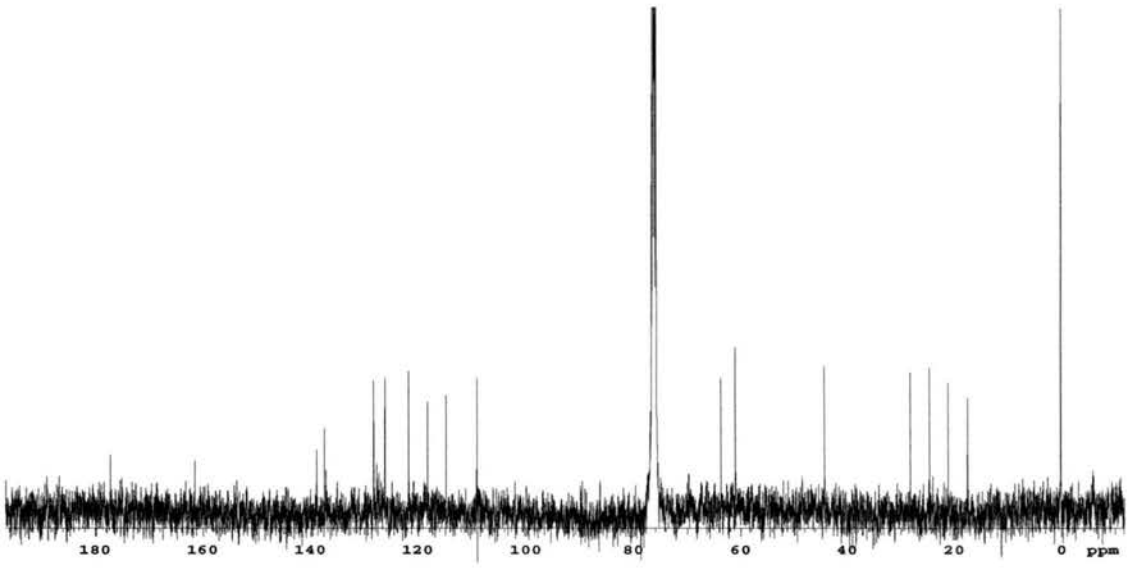
12-*epi*-*ent*-Spirotyprostatin B: $[\alpha]_D^{25}$ =- 42.5 (c = 0.8,CH_2Cl_2).



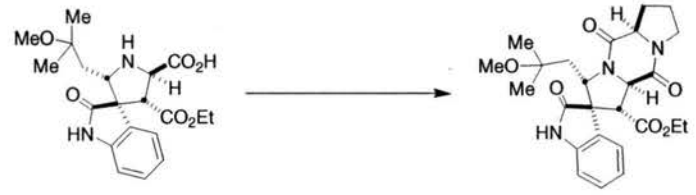
115



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-618-1H



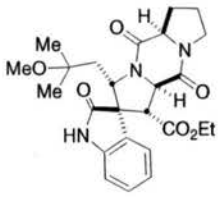
^{1}H NMR, 75 MHz, $CDCl_3$, filename: PRS2-618-C13



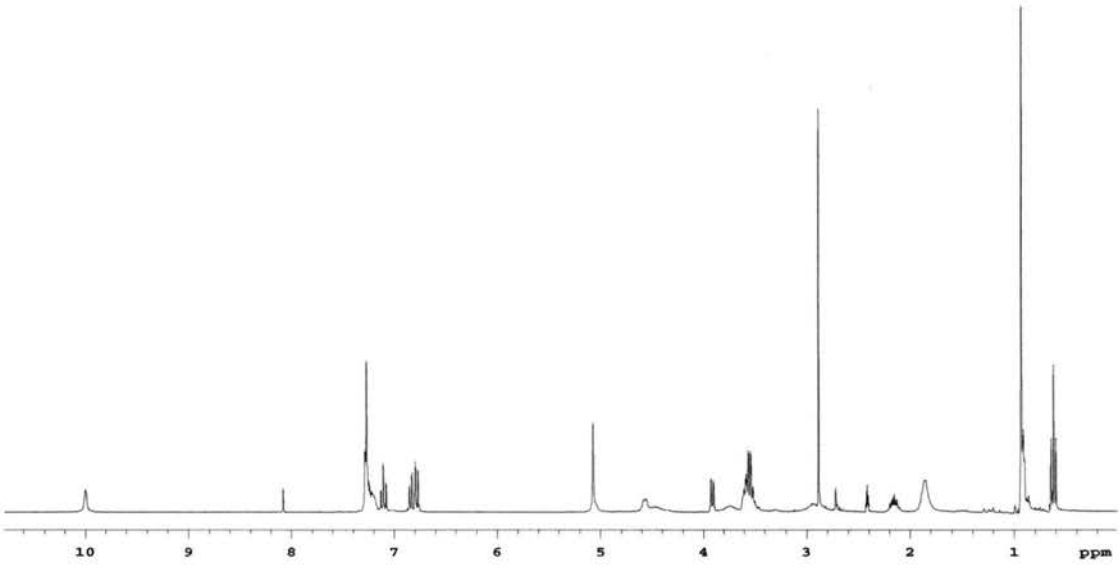
114 120

Spiro[1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3H),3'-[3H]indole]-1-carboxylic acid, 5a,6,7,8,10,10a-hexahydro-3-(2-methoxy-2-methylpropyl)-2',5,10-trioxo-, ethyl ester, (1R,2S,3S,5aR,10aR) 120.

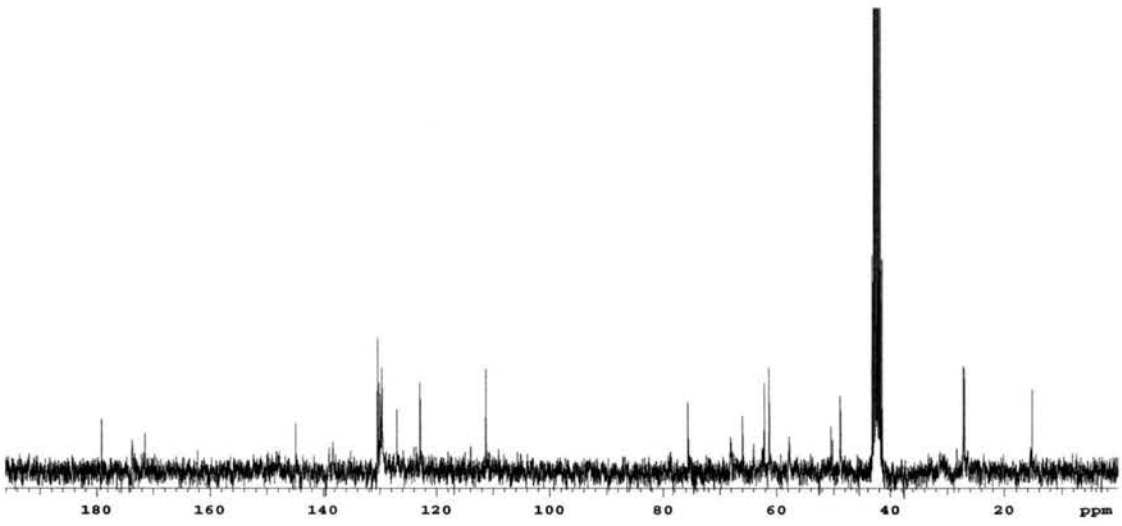
Compound **120** was generated in an identical fashion to diketopiperazine **105** yet afforded a higher yield (94%). $[\alpha]_D^{25}$ =81.7 (c = 1.0, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.85 (s, 3H), 0.87 (t, J = 7.2, 3H), 1.24, (s, 3H), 1.77 (dd, J = 9.9 Hz, J = 14.1 Hz, 1H), 1.90 - 2.11 (m, 2H), 2.25 - 2.33 (m, 2H), 2.52 (d, J = 13.8 Hz, 1H), 2.79 (s, 3H), 3.56 - 3.65 (m, 2H), 3.73 - 3.81 (m, 3H), 4.31 (t, J = 7.5 Hz, 1H), 4.67 (d, J = 9.9 Hz, 1H), 5.09 (d, J = 9.9 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 7.5 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 8.26 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 7.27, 15.8, 17.0, 19.2, 21.0, 33.5, 39.1, 41.3, 48.1, 52.7, 54.1, 54.7, 55.0, 55.5, 67.3, 103.2, 115.2, 119.8, 120.2, 122.3, 135.3, 158.0, 159.7, 161.0, 171.5. IR (NaCl/neat) 3268, 1729, 1671, 1669. HRMS (FAB+) calcd for $C_{25}H_{32}O_6N_3$ (*m/z*) 470.2291, found (*m/z*) 470.2296. ***ent*-Diketopiperazine 120**: $[\alpha]_D^{25}$ = -81.2 (c = 1.0, CH_2Cl_2)



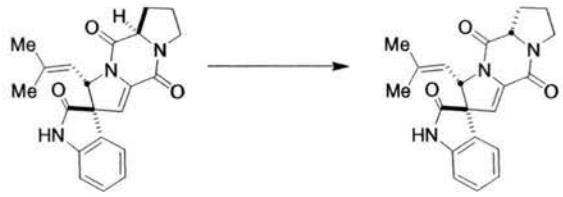
120



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-593-2H



^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS2-593-C13

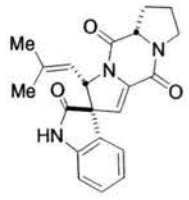


115 2

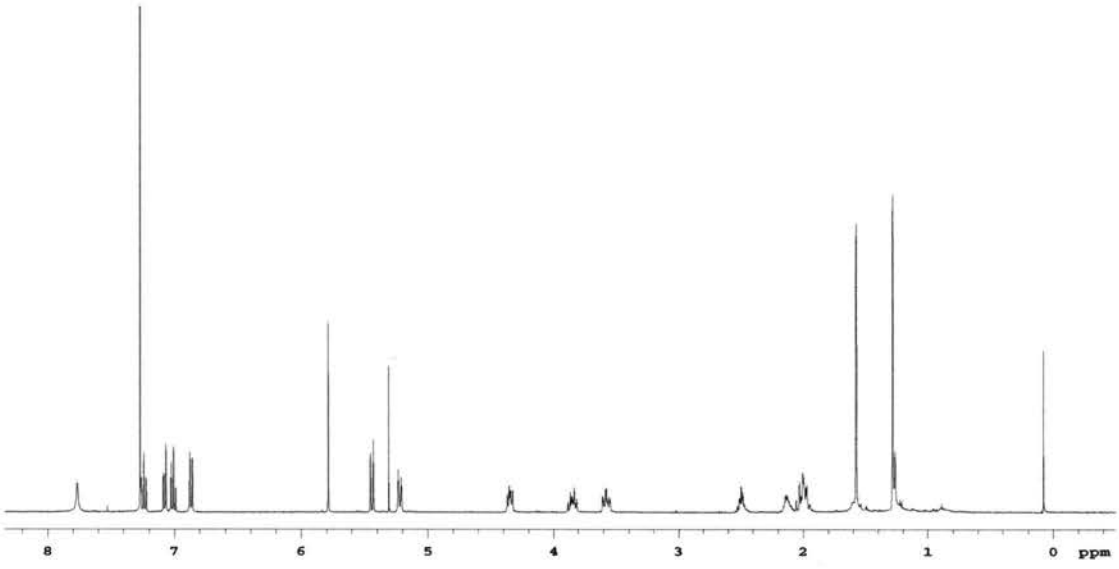
Spirotyprostatin B (2).

To a flame-dried 10 mL round-bottom flask with stir bar was added 12-*epi*-spirotyprostatin B (**115**) (0.95 g, 0.26 mmol), MeOH (2 mL) was added and the system cooled to 0°C. A 1M solution of NaOMe in MeOH (0.26 mL) was added dropwise and the mixture was stirred at 0 °C for 2 h at which time 5 mL of saturated aqueous NH_4Cl was added along with 5 mL of EtOAc. The aqueous layer was extracted with EtOAc (3 x 5 mL) and the organic layers combined, dried over Na_2SO_4, filtered, evaporated and purifed by chromatography (silica gel, eluted with 75:20:5 CH_2Cl_2:EtOAc:IPA) to yield 0.044 g (46%) of **2** as an off-white amorphous solid and 0.28 g (30%) of **115**. $[\alpha]_D^{25}$ = -151.1 (c = 0.45, $CHCl_3$). ^1H NMR (400 MHz $CDCl_3$) δ $CHCl_3$: 1.28 (d, J = 0.9 Hz, 3H), 1.57 (d, J = 0.9 Hz, 3H), 1.94 - 2.05 (m, 2H), 2.08 - 2.16 (m, 1H), 2.46 - 2.53 (m, 1H), 3.58 (ddd, J = 2.9 Hz, J = 9.3 Hz, J = 12.2 Hz, 1H), 3.84 (dt, J = 8.3 Hz, J = 12.2 Hz, 1H), 4.35 (dd, J = 6.1 Hz, J = 10.5 Hz, 1H), 5.22 (dt, J = 1.2 Hz, J = 8.8 Hz, 1H), 5.h4 (d, J = 8.8), 5.79 (s,1H), 6.89 (d, J = 7.6 Hz, 1H), 6.99 (dt, J = 1.0 Hz, J = 7.6 Hz, 2H), 7.06 (dt, J = 1.0 Hz, J = 7.6 Hz, 1H), 7.23 (dt, J = 1.0 Hz, J = 7.6 Hz, 1H) 7.77 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 18.4, 22.3, 25.5, 29.5, 45.0, 61.8, 61.9, 64.3, 110.0, 116.4, 120.7, 122.5, 127.4, 128.1, 129.3, 138.4, 138.5, 140.6, 155.2, 162.7, 178.1. IR

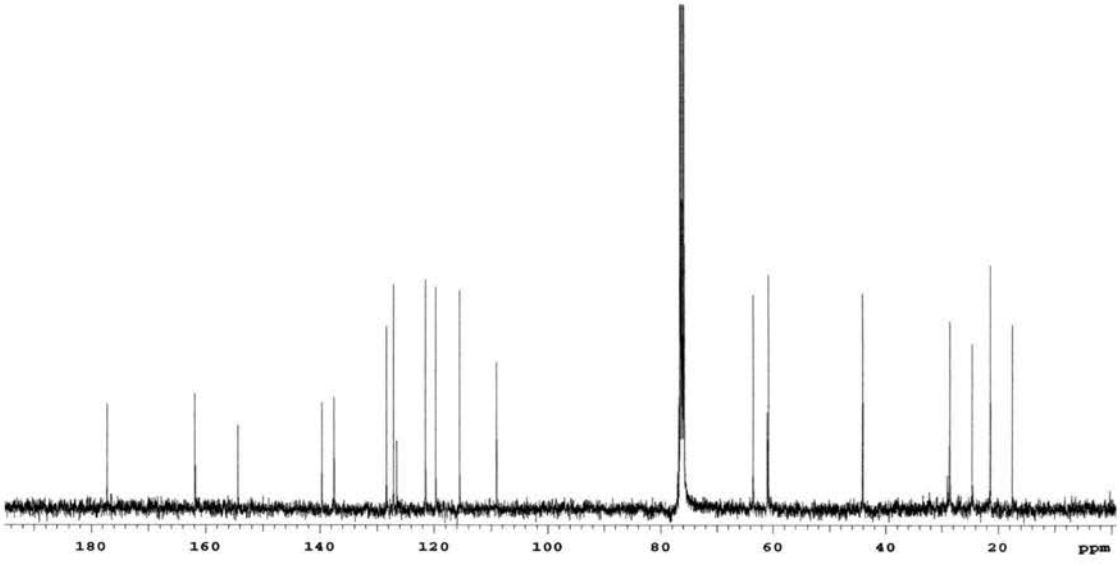
(NaCl/neat) 3235, 1718, 1677, 1690. HRMS (EI) calcd for $C_{21}H_{21}O_3N_3$ (*m/z*) 363.1583, found (*m/z*) 363.1584. ***ent*-Spirotryprostatin B:** $[\alpha]_D^{25} = 155.1$ (c = 0.33 ,CH_2Cl_2).



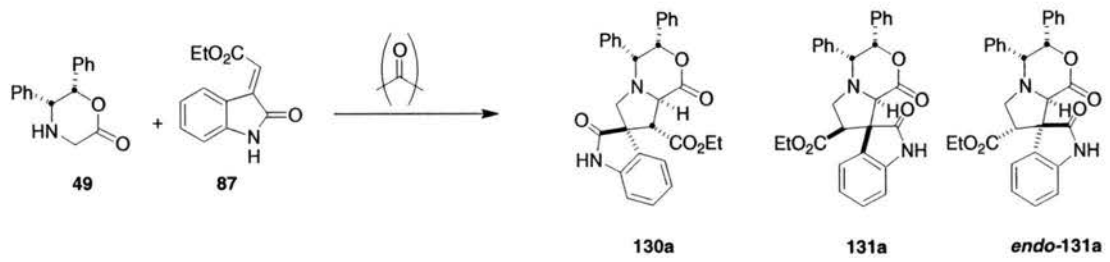
2



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-622-1H



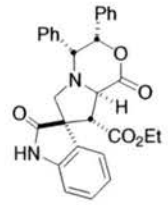
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-622-C13



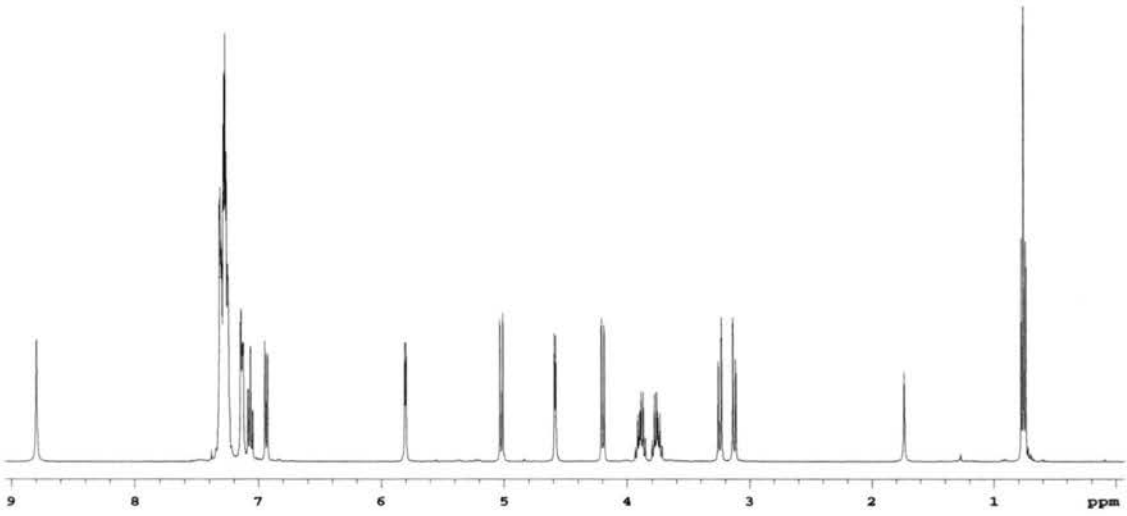
Cycloaddition of azomethine ylide derived from paraformaldehyde.

To a flame dried 25 ml round bottom with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** (253 mg 1.0 mmol), ethyl oxindolyl acetate **87** (325 mg, 1.5 mmol), paraformaldehyde (360 mg, 10.0 mmol) and 0.50 g of activated 3A molecular sieves. An oven-dried condensor was attached and the system was flushed with Ar. Freshly distilled toluene (10 ml) was added and the system was heated to reflux under Ar and kept at that temperature for two hours. The reaction mixture was allowed to cool to room temperature, filtered through celite to remove the mol. sieves and purified by flash chromatography using 2:1 hexane/EtOAc as the eluent to obtain 135 mg of **130a** (28%), 53 mg of **131a** (11%), and 43 mg of ***endo*-131a** (9%) as white amorphous solids **130a:** $[\alpha]_D^{25}$ =-32.0 (*c* 0.78, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.72 (t, J=6.8 Hz, 3H) 3.08 (d, J=9.6 Hz, 1H), 3.20 (d, J=9.6 Hz, 1H), 3.75-3.69 (m, 1H), 3.87-3.81 (m, 1H), 4.15 (d, J=8.8 Hz, 1H), 4.54 (d, J=4.0 Hz, 1H), 4.98 (d, J=8.8 Hz, 1H), 5.75 (d, J=4.0 Hz, 1H), 7.09 (dd, J=1.6 Hz, J = 6.8 Hz, 1H), 7.27–7.21 (m, 10H), 8.74 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 54.3, 55.5, 60.7, 61.4, 63.7, 67.5, 84.4, 110.1, 123.2, 124.6, 127.9, 128.4, 128.7, 128.8, 129.1, 129.8, 134.4, 136.1, 140.8, 168.7, 171.4, 178.2. IR (NaCl/neat) 1735, 1618; HRMS (FAB+) Calcd for $C_{29}H_{27}N_2O_5$ (*m/z*) 483.1920, found

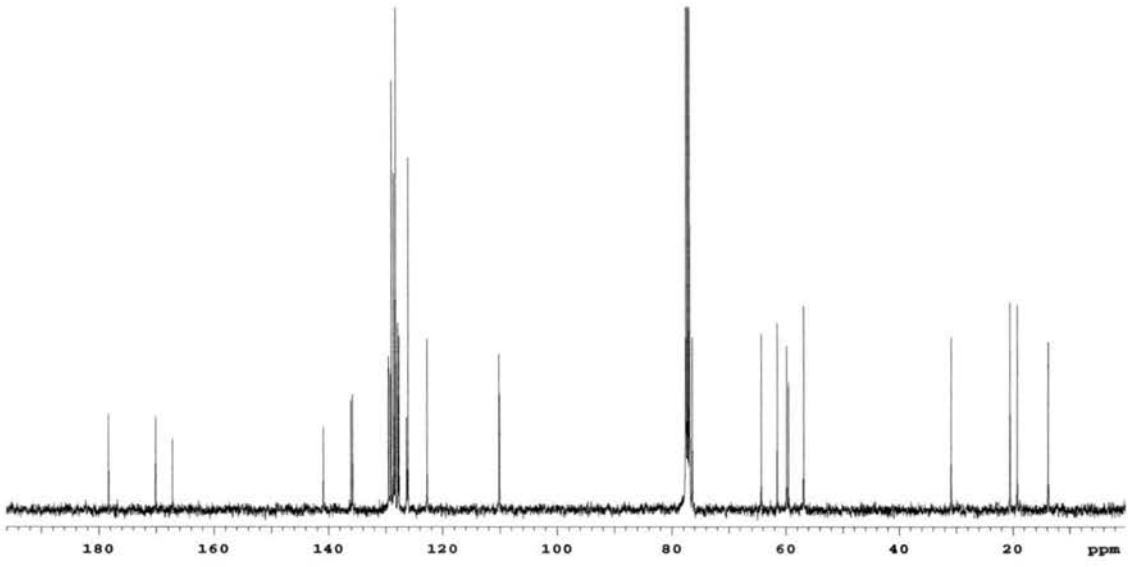
(*m/z*) 483.1917; NOE data: irradiation of H_6 enhanced H_7-α: (3.45%); irradiation of H_7-α enhanced H_9 (3.61%).



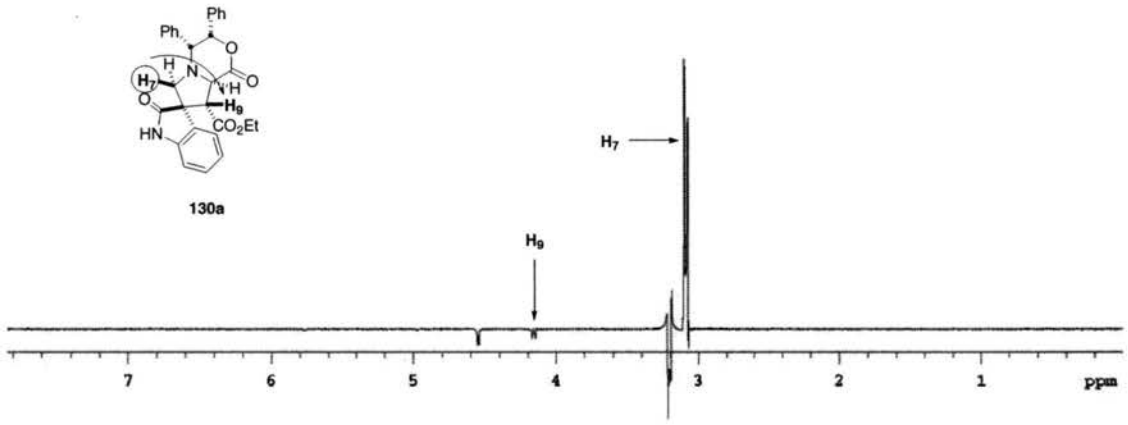
130a



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-865-3H

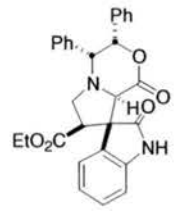


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-865-3HC13

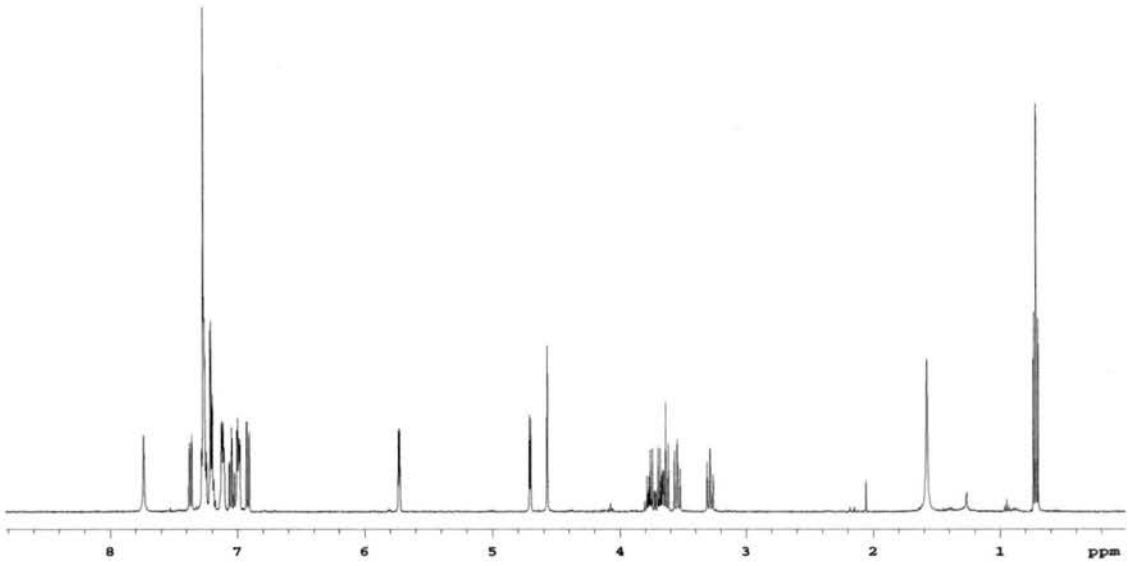


nOe, 400 MHz, $CDCl_3$, filename: PRS2-865-3Hnoe

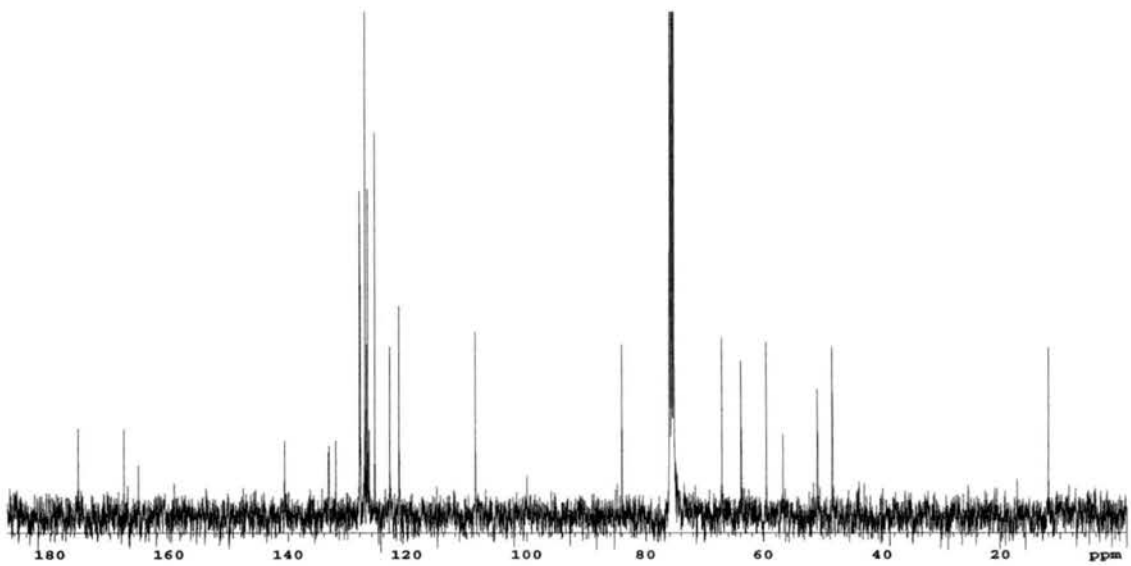
131a: $[\alpha]_D^{25}$ =-111.0 (*c* 1.09, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (br s, 1H), 7.23–7.13 (m, 9H), 7.02 (t, J=7.6 Hz, 1H), 6.98 (d, J=6.8 5.52 (d, J=4.0 Hz, 1H), 5.02 (d, J=4.0 Hz, 1H), 4.77 (s, 1H), 3.70-3.60 (m, 2H), 3.56 (dd, J=6.4 Hz, J=10.8 Hz, 1H), 3.46 (t, J=10.8 Hz, 1H), 3.32 (dd, J=6.4 Hz, J=10.8 Hz, 1H), 0.68 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.7, 169.8, 167.9, 141.5, 135.4, 134.7, 130.3, 129.4, 129.3, 128.6, 128.5, 128.4, 127.6, 123.7, 123.0, 110.2, 86.2, 72.9, 62.6, 61.6, 60.9, 54.1, 51.5, 13.6. IR (NaCl/neat) 3307, 1726, 1620; HRMS (FAB+) calcd for $C_{29}H_{27}N_2O_5$ (*m/z*) 483.1920, found (*m/z*) 483.1911.



131a

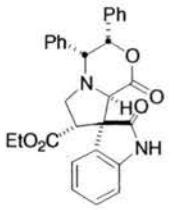


^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-865-1H

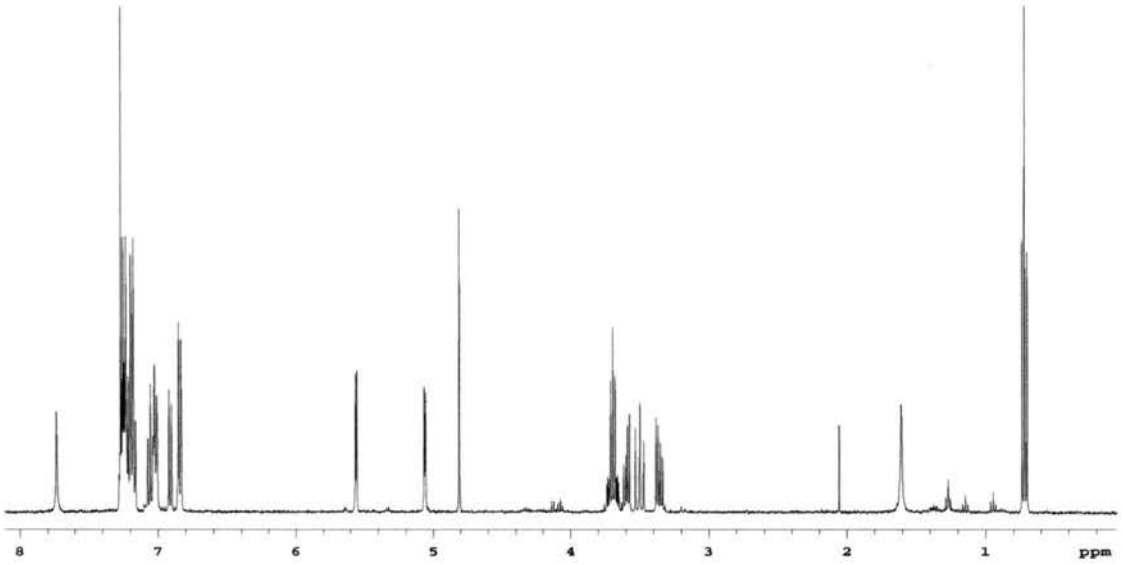


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-865-C13

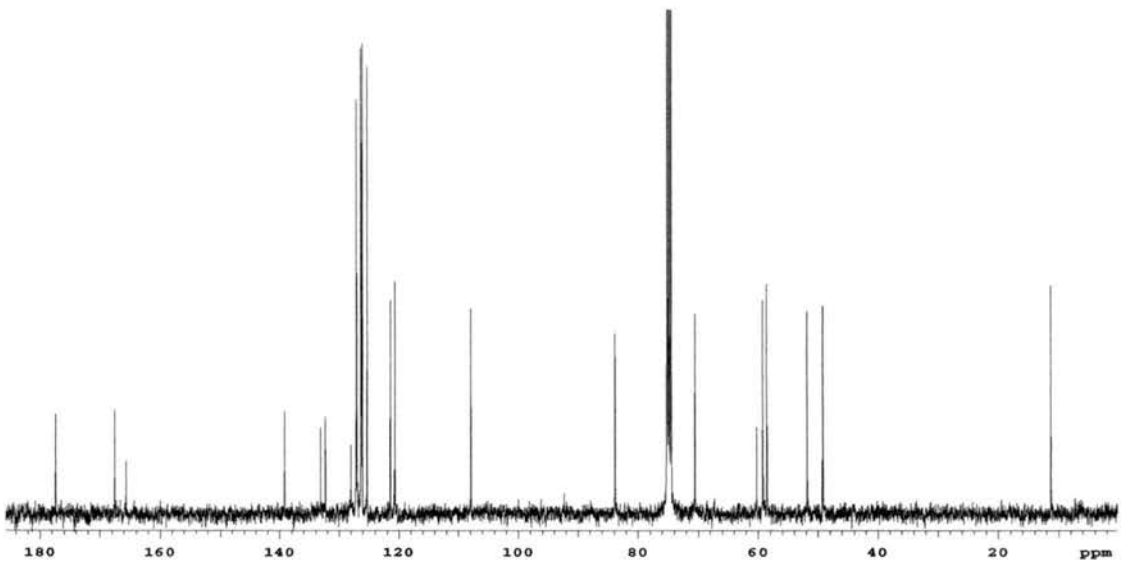
***endo*-131a:** $[\alpha]_D^{25}$ =-123.0 (*c* 0.43, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (br s, 1H), 7.33 (d, J=7.2 Hz, 1H), 7.23–6.95 (m,12H), 6.88 (d, J=7.2 Hz, 1H), 5.69 (d, J=4.0 Hz, 1H), 4.67 (d, J=4.0 Hz, 1H), 4.54 (s, 1H), 3.75-3.60 (m, 2H), 3.60 (t, J=8.4 Hz, 1H), 3.51 (t, J=9.2 Hz, 1H), 3.25 (t, J=9.2 Hz, 1H), 0.68 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 169.2, 166.7, 142.2, 134.7, 133.5, 129.5, 129.4, 128.6, 128.4, 128.1, 127.9, 127.0, 124.4, 122.8, 110.1, 85.6, 68.7, 65.4, 61.1, 58.3, 52.5, 50.0, 13.5; IR (NaCl/neat) 3313, 1731; 1619; HRMS (FAB+) Calcd for $C_{29}H_{27}N_2O_5$ (*m/z*) 483.1920, found (*m/z*) 483.1904; NOE data: irradiation of H_2 enhanced H_8 (1.54%).



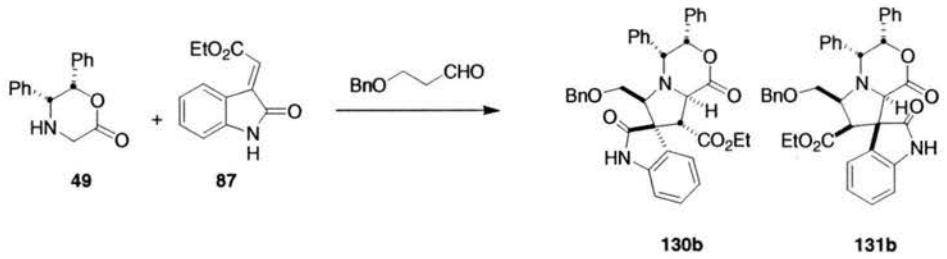
***endo*-131a**



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-865-2H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-865-2HC13



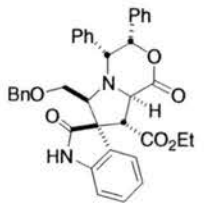
Cycloaddition of azomethine ylide derived from benzyloxy-acetaldehyde.

Method A (Reflux): To a flame dried 25 ml round bottom with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** (253 mg 1.0 mmol), ethyl oxindolyl acetate **87** (325 mg, 1.5 mmol) and 0.50 g of activated 3A molecular sieves. An oven-dried condensor was attached and the system was flushed with Ar. Freshly distilled toluene (10 ml) followed by benzyloxy-acetaldehyde (180 mg, 1.2 mmol). The system was heated to reflux under Ar and kept at that temperature for two hours. The system was allowed to cool to room temperature, filtered through celite to remove the sieves and purified by flash chromatography using 3:1 hexanes/EtOAc as the eluent to afford 265 mg of **130b** (44%) and 85 mg of **131b** (14%) as white amorphous solids. Analytical samples were prepared by HPLC using 3:1 hexanes/EtOAc as the eluent.

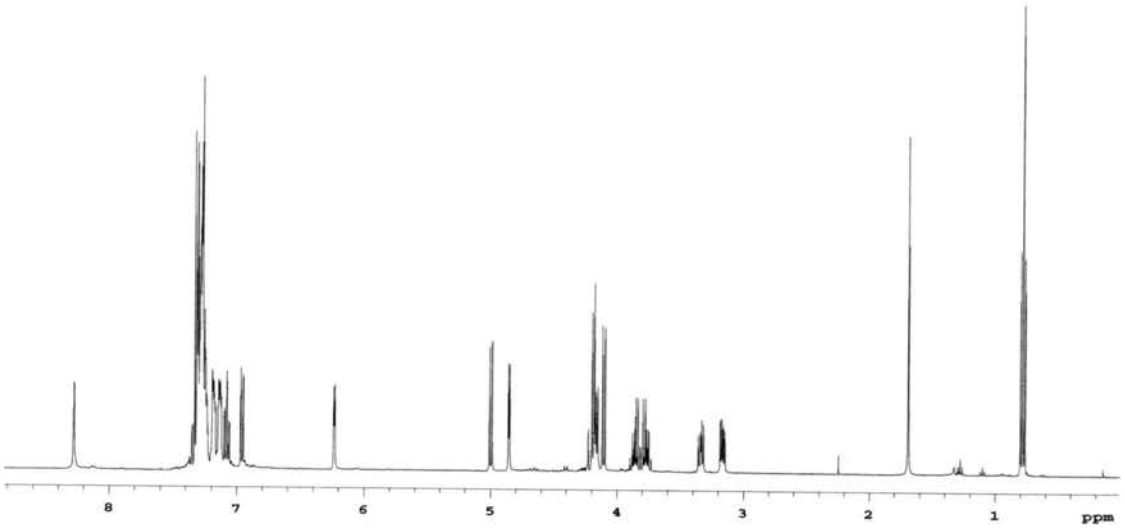
Method B (60°C): To a flame dried 25 ml round bottom with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** (253 mg 1.0 mmol), ethyl oxindolyl acetate **87** (325 mg, 1.5 mmol) and 0.50 g of activated 3A molecular sieves. The system was flushed with Ar. Freshly distilled toluene (10 ml) was added followed by benzyloxy-acetaldehyde (180 mg, 1.2 mmol). The system was warmed to 60°C under Ar, as measured by a thermocouple, and kept at that temperature for two hours. The reaction was allowed to cool to room temperature, filtered through celite and purified by flash chromatography using 3:1 hexanes/EtOAc as the eluent to afford 325 mg of **130b** (54%)

and 50 mg of **131b** (8%) as white amorphous solids. Analytical samples were prepared by HPLC using 3:1 hexanes/EtOAc as the eluent.

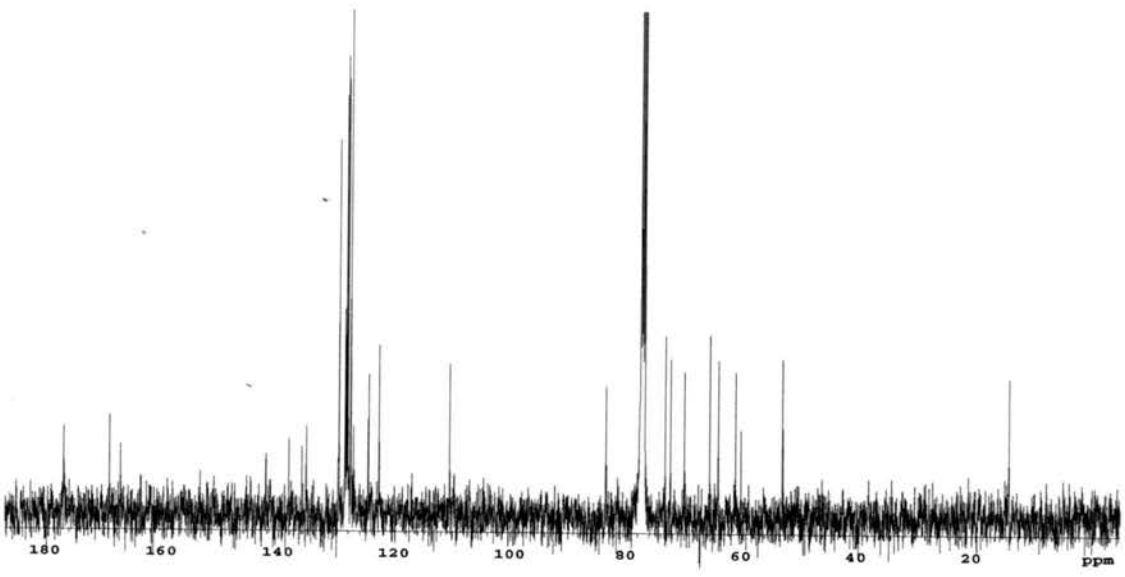
130b: $[\alpha]_D^{25}$ =-32.5 (*c* 0.56, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (br s, 1H), 7.24-6.95 (m, 18H), 6.85 (d, J=7.6 Hz, 1H), 4.89 (d, J=8.0 Hz, 1H), 4.75 (d, J=3.2 Hz, 1H), 4.12-4.05 (m, 3H), 4.00 (d, J=8.0 Hz, 1H), 3.77-3.73 (m, 1H), 3.73-3.65 (m, 1H), 3.23 (dd, J=6.0 Hz, 9.6 Hz, 1H), 3.06 (dd, J=4.2 Hz, 9.6 Hz, 1H), 0.68 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 171.4, 168.2, 141.4, 137.4, 136.4, 135.8, 129.3, 129.1, 128.5, 128.4, 128.0, 127.9, 127.8, 126.2, 126.1, 122.8, 109.9, 78.2, 73.7, 70.9, 69.9, 62.2, 61.5, 58.6, 58.2, 54.5, 13.6; IR (NaCl/neat) 3269, 1732, 1618; HRMS (FAB+) Calcd for $C_{37}H_{35}N_2O_6$ (*m/z*) 603.2495, found (*m/z*) 603.2477; NOE data: irradiation of H_6 enhanced H_7 (3.07%).



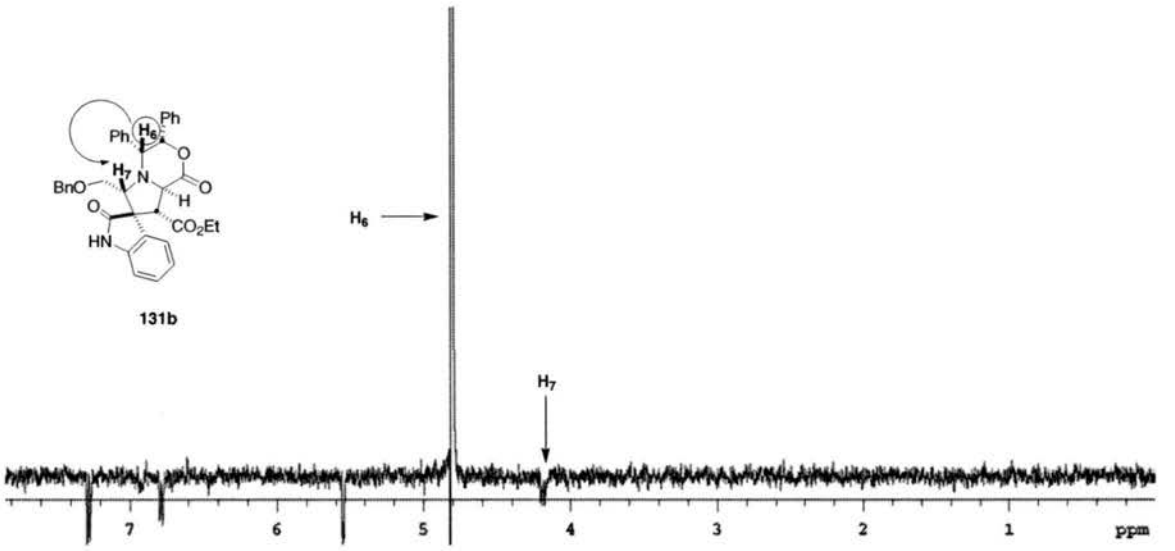
130b



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-866-2H

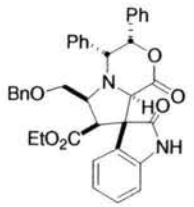


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-866-2HC13

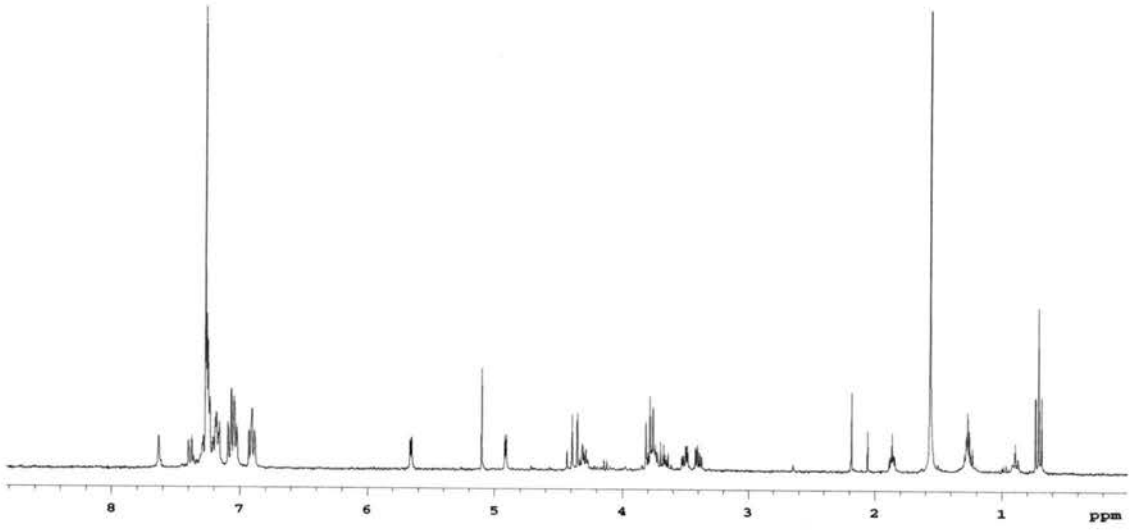


nOe, 400 MHz, $CDCl_3$, filename: PRS2-866-2HC13noe

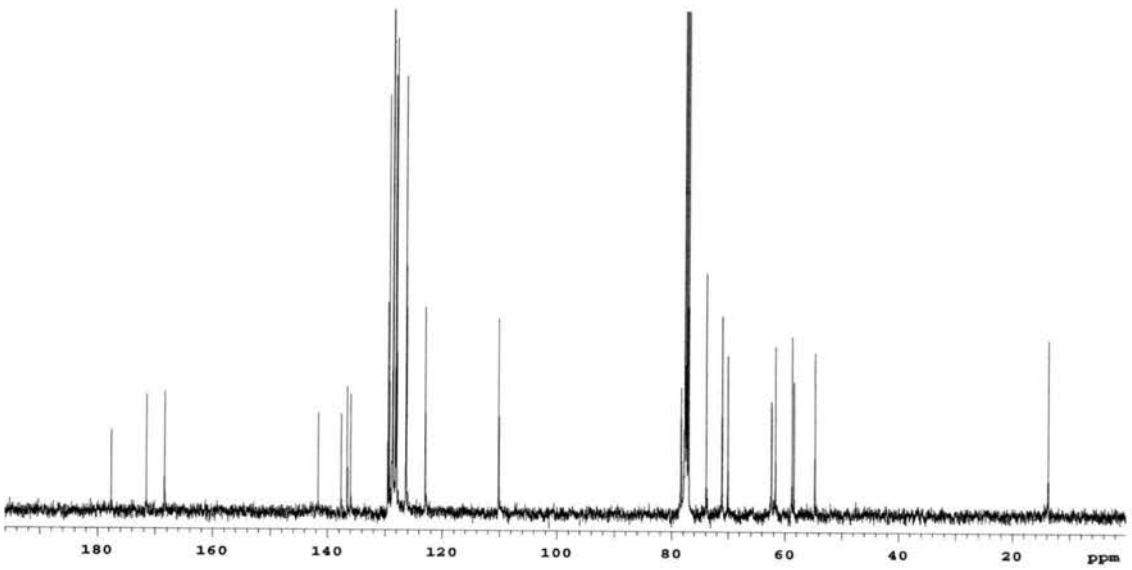
131b: $[\alpha]_D^{25}$ =-161.8 (*c* 0.22, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (br s, 1H), 7.34 (d, J=7.2 Hz, 1H), 7.22-7.12 (m, 12H) 7.04-6.98 (m,5.06 (s, 1H), 4.87 (d, J=3.6 Hz, 1H), 4.37 (1/2ABq, J=12.0 Hz, 1H), 4.29 (1/2ABq, J=12.0 Hz, 1H), 4.30-4.24 (m, 1H), 3.65-3.60 (m, 1H), 3.46 (dd, J=4.0 Hz, 9.6 Hz, 1H), 3.36 (dd, J=4.8 Hz, 9.6 Hz, 1H), 0.66 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 169.0, 167.0, 142.2, 138.2, 136.0, 135.3, 129.7, 129.6, 128.6, 128.5, 128.2, 128.1, 127.6, 127.1, 124.6, 122.7, 110.4, 83.5, 73.3, 72.3, 69.9, 65.5, 64.1, 61.1, 60.2, 52.9, 13.5; HRMS (FAB+) Calcd. for $C_{37}H_{35}N_2O_6$ (*m/z*) 603.2495, found (*m/z*) 603.2483.



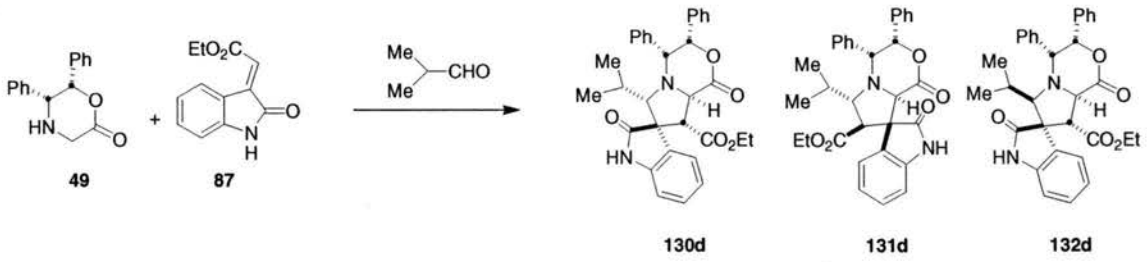
131b



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-866-1H



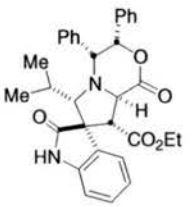
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-866-C13



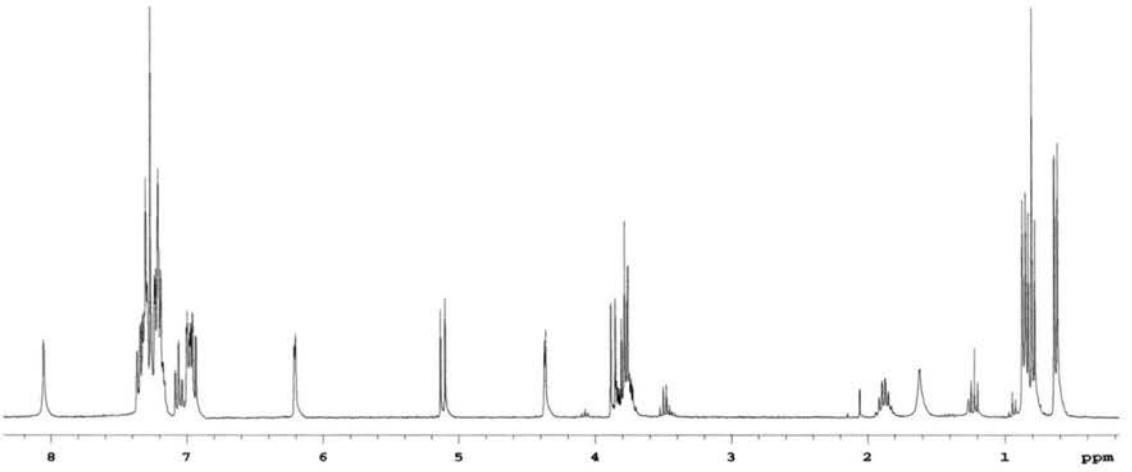
Cycloaddition of azomethine ylide derived from isobutyraldehyde.

The reaction was performed in an identical fashion to the cycloaddition of benzyloxy-acetaldehyde. Isobutyraldehyde (86 mg, 1.2 mmol) was used as received from Aldrich. **Method A:** 225 mg of **130d** (43%), 73 mg of **131d** (11%) and 25 mg of **132d** (5%) were obtained as white amorphous solids. **Method B:** 387 mg of **130d** (74%), 30 mg of **131d** (6%) and trace amounts of **132d** (<1%) were obtained as white amorphous solids:

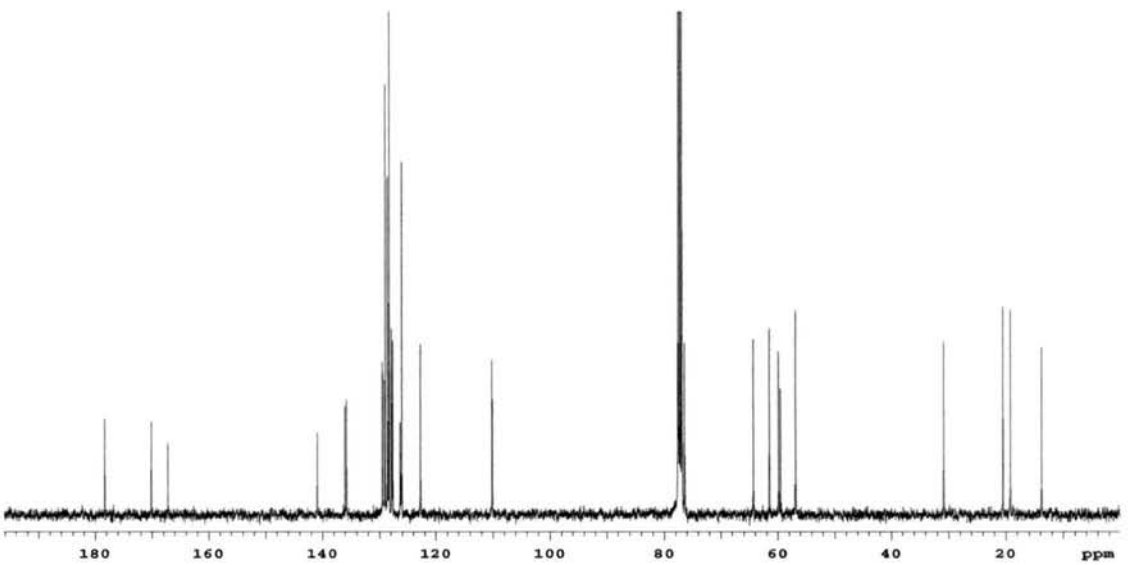
130d: $[\alpha]_D^{25}$ =-58.8 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (br s, 1H), 7.31–7.16 (m,10 H), 7.08–6.91 (m, 4H), 6.19 (d, J=4.0 Hz, 1H), 5.12 (d, J=13.2 Hz, 1H), 4.36 (d, J=4.0 Hz, 1H), 3.86 (d, J=13.2 Hz, 1H), 3.85-3.73 (m, 3H), 1.88 (sept, J=9.2 Hz, 1H), 0.86 (d, J=9.2 Hz, 3H), 0.81 (t, J=9.6 Hz, 3H), 0.63 (d, J=9.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 170.1, 167.2, 140.9, 136.0, 135.7, 129.5, 129.0, 128.5, 128.2, 127.8, 127.6, 126.3, 126.1, 122.7, 110.1, 77.5, 76.4, 64.3, 61.5, 59.8, 59.5, 56.8, 30.8, 20.5, 19.2, 13.7;. IR (NaCl/neat) 3288, 1729, 1618; HRMS (FAB+) Calcd. for $C_{32}H_{33}N_2O_5$ (*m/z*) 525.2389, found (*m/z*) 525.2390. NOE data: irradiation of H_9 enhanced H_5 (2.62%).



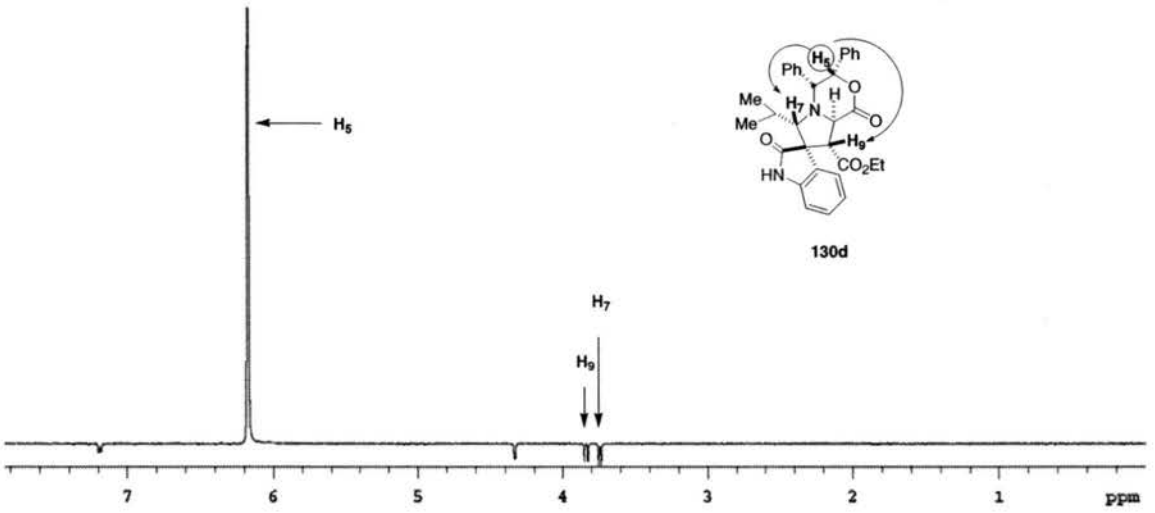
130d



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-864-3H



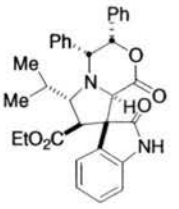
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-864-3HC13



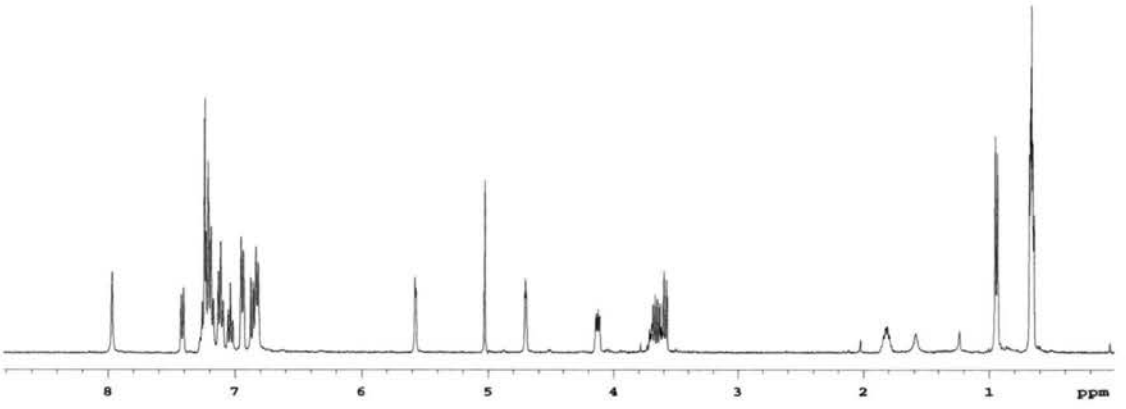
130d

nOe, 400 MHz, $CDCl_3$, filename: PRS2-864-3Hnoe

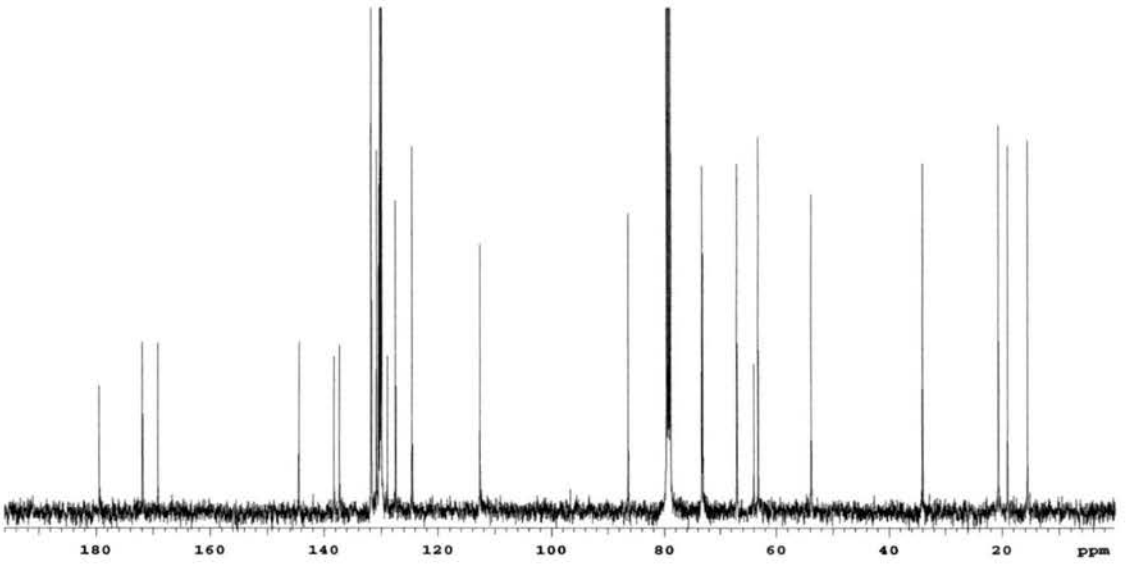
131d: $[\alpha]_D^{25}$ =-20.7 (*c* 0.9, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 7.87 (br s,1H), 7.43 (d, J=10.0 Hz, 1H), 7.37–7.03 (m,9H), 6.97-6.83 (m,4H), 5.59 (d, J=4.8 Hz,1H), 5.03 (s, 1H), 4.71 (d, J=4.8 Hz,1H), 4.14 (dd, J=6.0, J=12.0 Hz,1H), 3.74-3.63 (m,2H), 3.60 (d, J=12.0 Hz, 1H), 1.86-1.80 (m, 1H), 0.96 (d, J=9.2 Hz, 3H), 0.68 (t, J=6.4 Hz,6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 169.8, 167.1, 142.4, 136.2, 135.2, 129.7, 129.6, 128.7, 128.3, 128.1, 128.0, 127.8, 126.8, 125.4, 122.5, 110.6, 84.4, 71.2, 71.1, 64.9, 61.9, 61.1, 51.7, 32.0, 18.6, 17.0, 13.5;. IR (NaCl/neat) 3300, 1727, 1618; HRMS (FAB+) Calcd. for $C_{32}H_{33}N_2O_5$ (*m/z*) 525.2389, found (*m/z*) 525.2378; NOE data: irradiation of H_5 enhanced H_7 (4.16%).



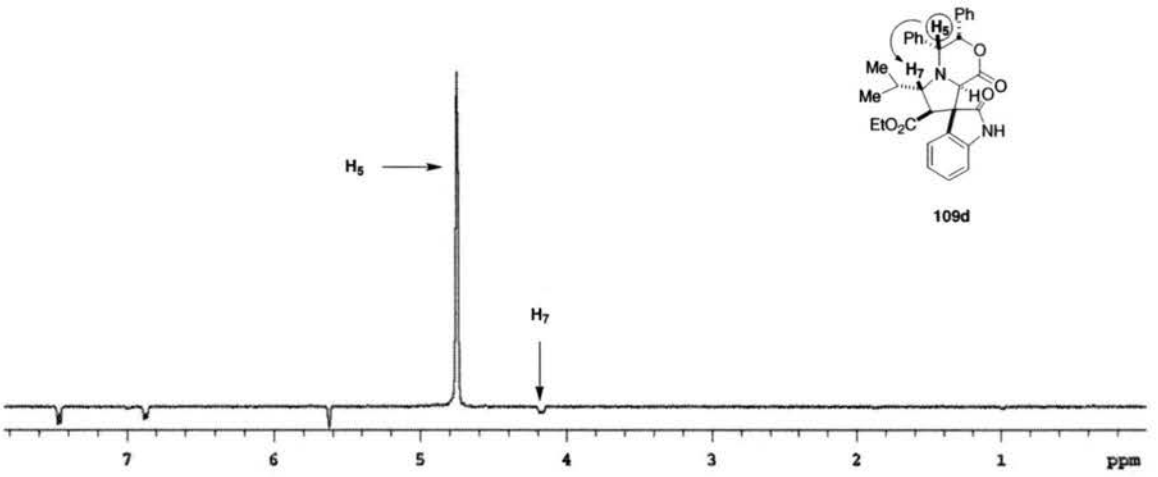
131d



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-864-1H#2

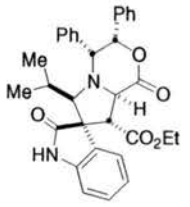


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-864-1H#2C13

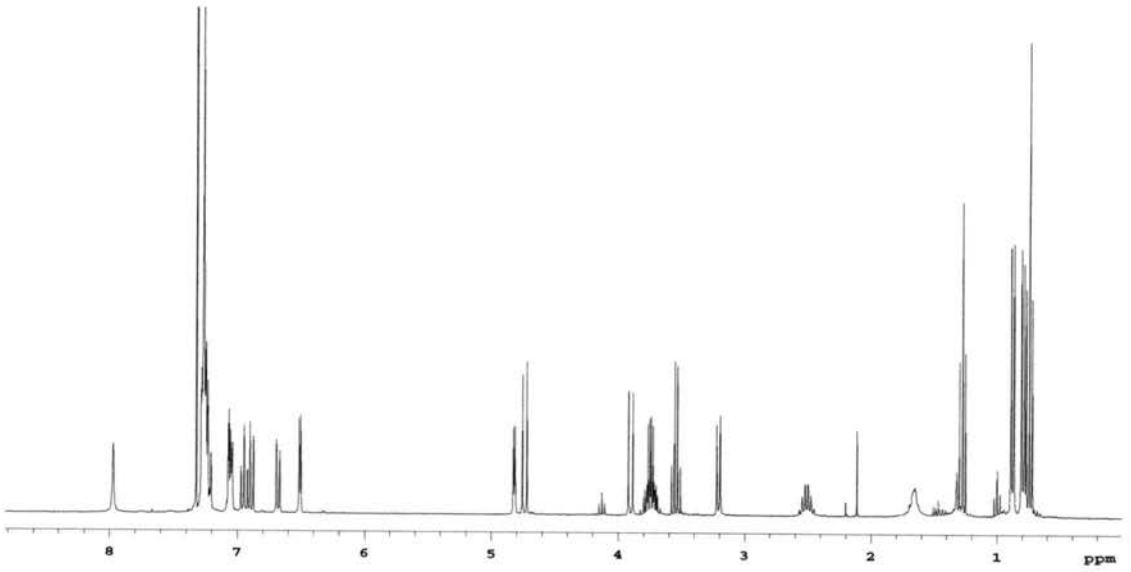


nOe, 400 MHz, $CDCl_3$, filename: PRS2-864-1H#2noe

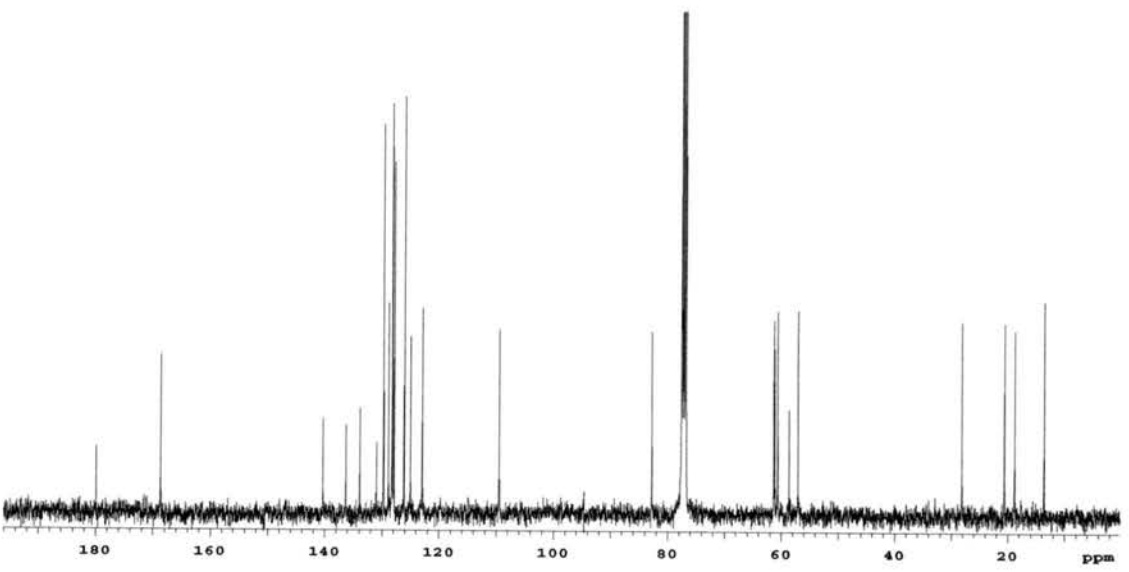
132d: $[\alpha]_D^{25}$ =-24.0 (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (br s, 1H), 7.32–7.21 (m, 10H), 7.06–7.04 (m, 2H), 6.94 (t, J=10.4 Hz, 1H), 6.89 (d, J=10.0 Hz, 1H), 6.68 (d, J=10.0 Hz, 1H), 6.50 (d, J=5.2 Hz, 1H), 4.81 (d, J=5.2 Hz, 1H), 4.74 (d, J=14.0 Hz, 1H), 3.90 (d, J=14.0 Hz, 1H), 3.80-3.69 (m, 2H), 3.21 (d, J=10.4 Hz, 1H), 2.51 (sept, J=9.2 Hz, 1H), 0.88 (d, J=10.4 Hz, 3H), 0.79 (d, J=10.4 Hz, 3H), 0.74 (t, J=9.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 168.6, 140.3, 140.9, 136.2, 133.9, 130.9, 129.7, 128.8, 128.2, 128.1, 127.8, 126.1, 125.0, 122.9, 109.7, 82.7, 77.4, 61.3, 61.1, 60.6, 58.6, 57.0, 28.1, 20.6, 18.9, 13.6;. IR (NaCl/neat) 3296, 1734, 1715, 1618; HRMS (FAB+) Calcd. for $C_{32}H_{33}N_2O_5$ (*m/z*) 525.2389, found (*m/z*) 525.2386; NOE data: irradiation of H_5 enhanced H_9 (6.88%); irradiation of H_7 enhanced H_2 (4.30%).



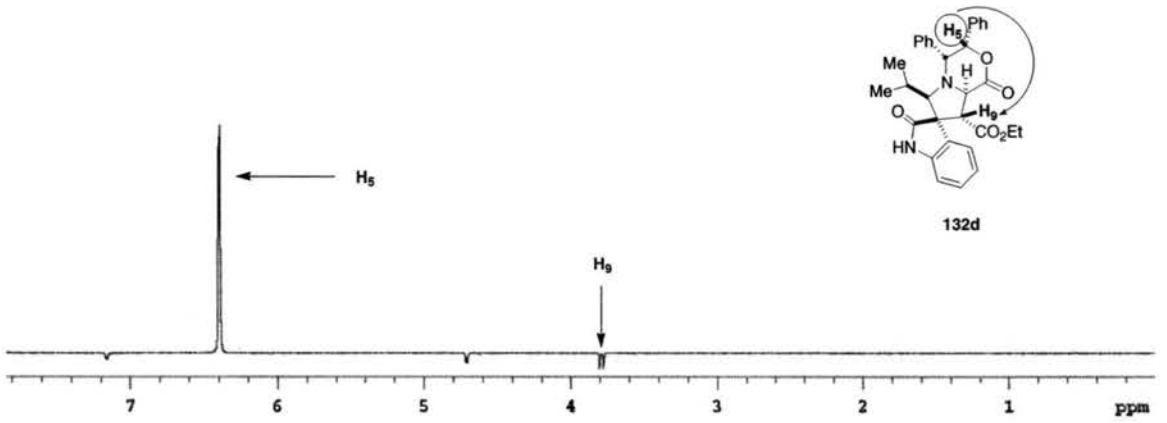
132d



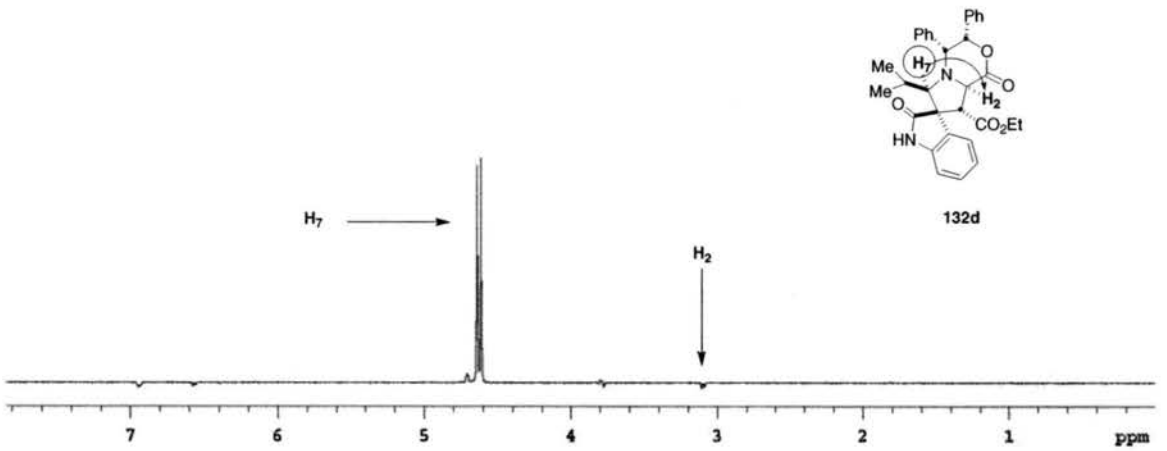
^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-864-1H



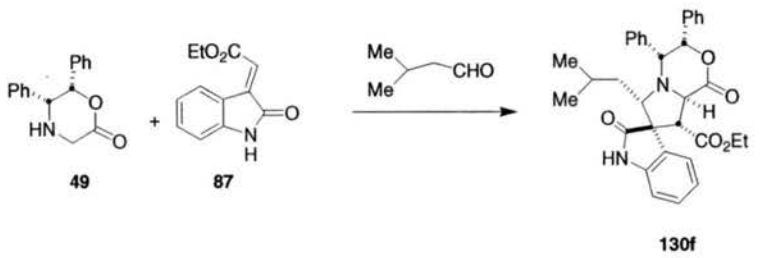
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-864-C13



nOe, 400 MHz, $CDCl_3$, filename: PRS2-864-1Hnoe1



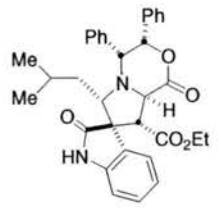
nOe, 400 MHz, $CDCl_3$, filename: PRS2-864-1Hnoe2



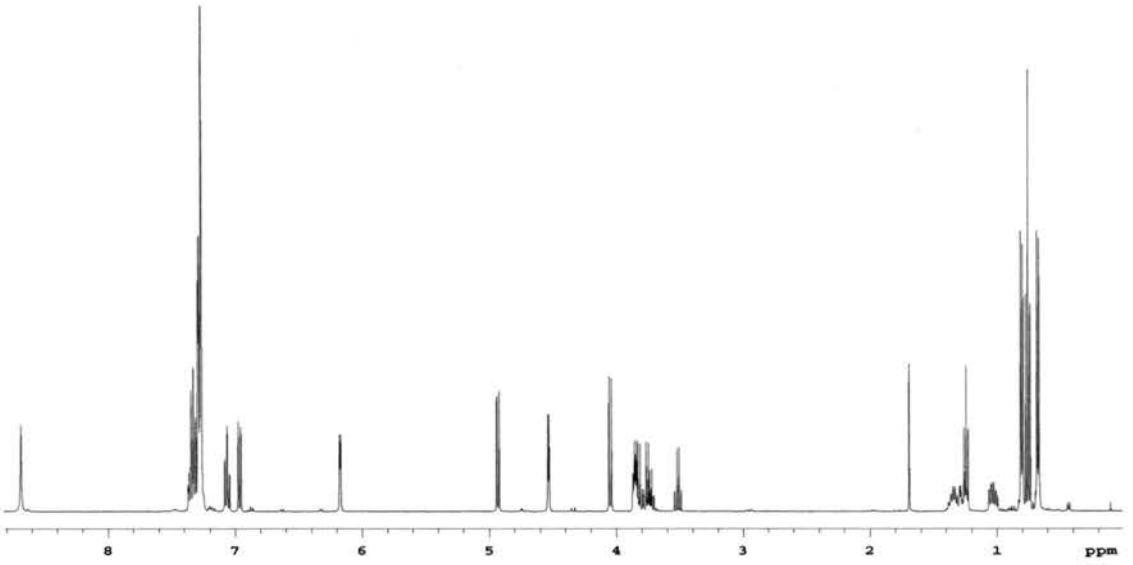
Cycloaddition of azomethine ylide derived from isovaleraldehyde.

The reaction was performed in an identical fashion to the cycloaddition of benzyloxy-acetaldehyde. Isovaleraldehyde (103 mg, 1.2 mmol) was used as received from Aldrich. **Method A:** 452 mg of **130f** (84%) was obtained as white amorphous solids and a trace amount of **131f** (~1%) was observed in the ^{1}H NMR spectra but not isolated. **Method B:** 463 mg of **130f** (86%) was obtained as a white amorphous solid.

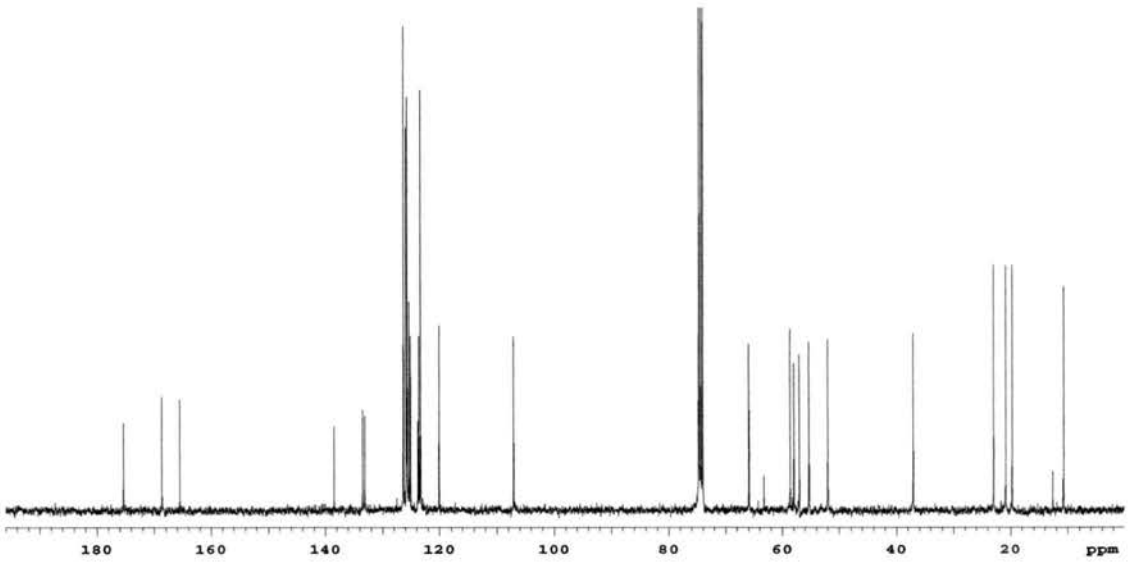
130f: $[\alpha]_D^{25}$ =62.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (br s, 1H), 7.31–7.20 (m, 12H), 7.00 (t, J=7.6 Hz, 1H), 6.90 (d, J=7.6 Hz, 1H), 6.11 (d, J=3.2 Hz, 1H), 6.50 (d, J=5.2 Hz, 1H), 4.87 (d, J=8.0 Hz, 1H), 4.48 (d, J=3.2 Hz, 1H), 3.99 (d, J=8.0 Hz, 1H), 3.81-3.64 (m, 3H), 1.34-1.17 (m, 2H), 1.00-0.93 (m, 3H), 0.74 (d, J=6.4 Hz, 3H), 0.70 (d, J=7.2 Hz, 3H), 0.62 (t, J=6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 171.5, 168.4, 141.4, 136.3, 136.0, 129.2, 129.1, 128.6, 128.5, 128.2, 128.0, 126.6, 126.5, 126.2, 122.9, 109.9, 77.6, 68.8, 61.5, 60.8, 59.8, 58.2, 54.8, 39.9, 25.8, 23.7, 22.6, 13.6;. IR (NaCl/neat) 3284, 1732, 1618; HRMS (FAB+) Calcd. for $C_{33}H_{35}N_2O_5$ (*m/z*) 539.2546, found (*m/z*) 539.2544; NOE data: irradiation of H_5 enhanced H_9 (2.17%).



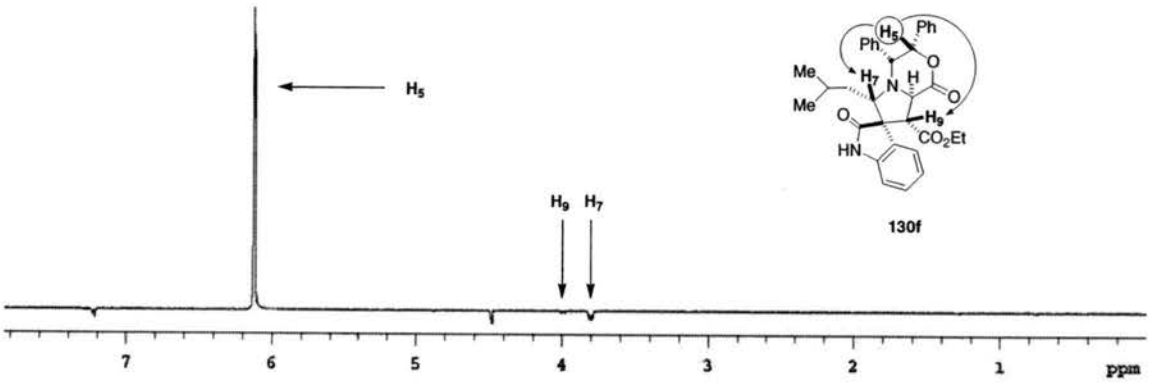
130f



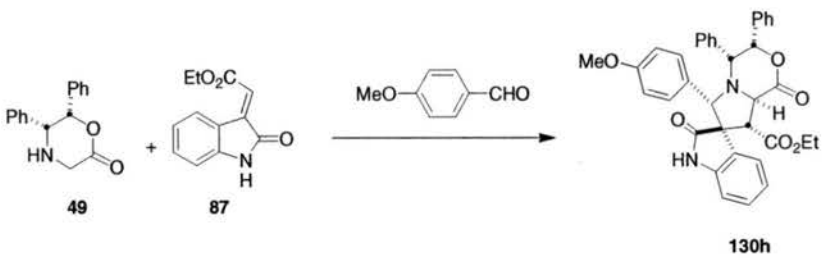
^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-503-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-503-C13

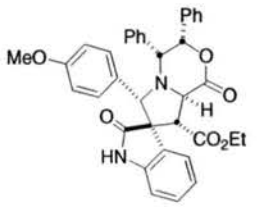


nOe, 400 MHz, $CDCl_3$, filename: PRS2-503-noe

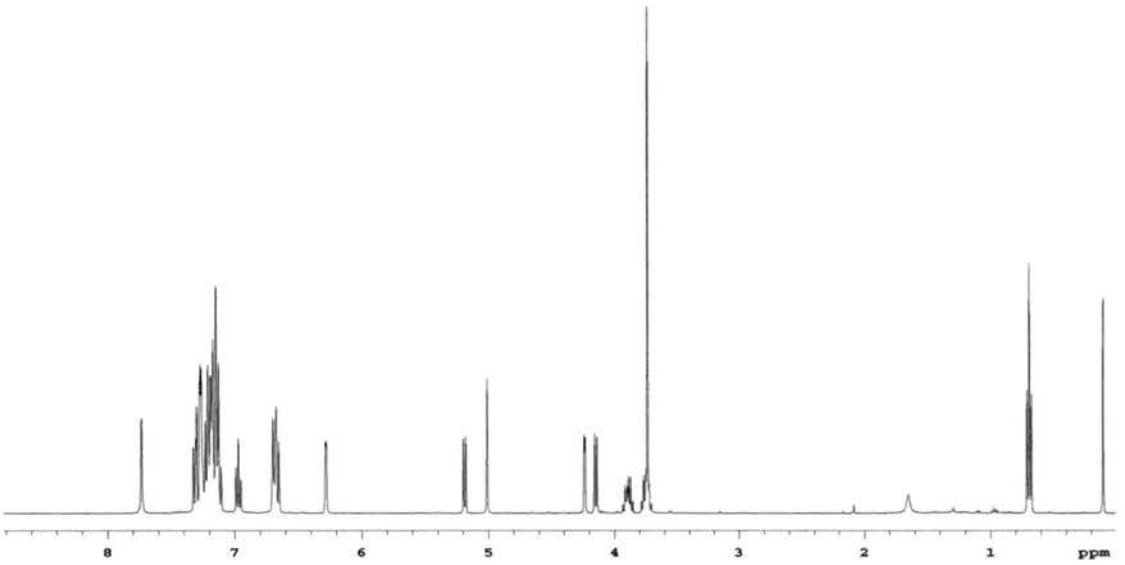


Cycloaddition of azomethine ylide derived from *p*-anisaldehyde.

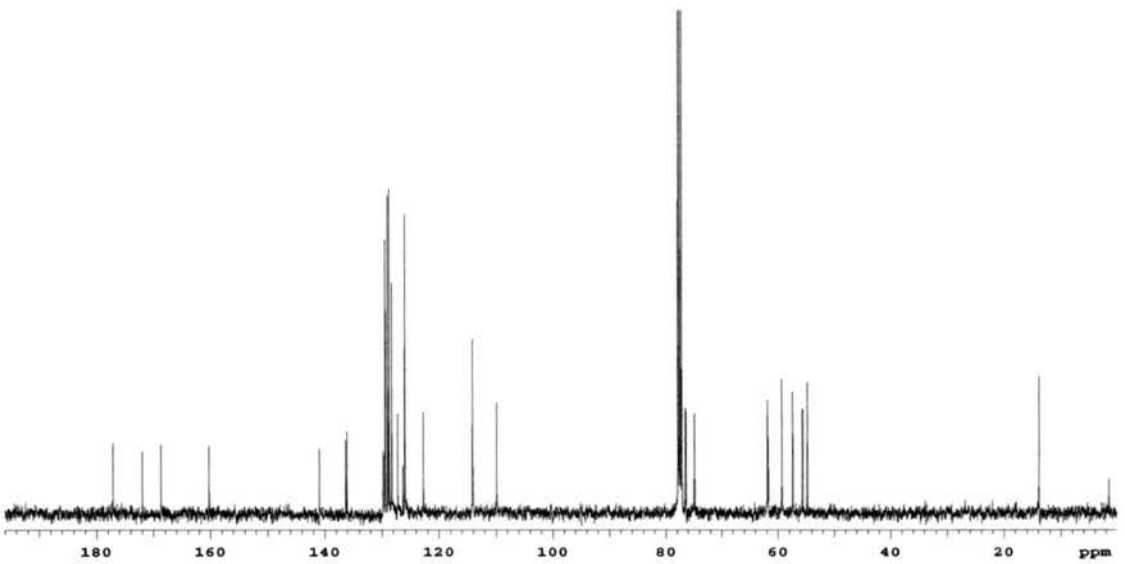
The reaction was performed in an identical fashion to the cycloaddition of benzyloxy-acetaldehyde. *p*-Anisaldehyde (163 mg, 1.2 mmol) was used as received from Aldrich. **Method A:** 353 mg of **130h** (60%) was obtained as white amorphous solid. **130h:** $[\alpha]_D^{25}$ =80.8 (*c* 0.47, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (br s, 1H), 7.26–7.04 (m, 15H), 6.91 (t, J=7.6 Hz, 1H), 6.61 (d, J=7.6 Hz, 2H), 6.22 (d, J=3.2 Hz, 1H), 5.12 (d, J=8.0 Hz, 1H), 4.95 (s, 1H), 4.17 (d, J=3.2 Hz, 1H), 4.09 (d, J=8.0 Hz, 1H), 3.87-3.79 (m, 1H), 3.72-3.64 (m, 4H), 0.63 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 171.6, 168.4, 140.6, 136.0, 135.8, 129.4, 129.1, 129.0, 128.6, 128.4, 127.9, 126.8, 126.0, 125.7, 125.6, 122.4, 113.8, 109.6, 76.2, 74.6, 61.5, 61.4, 59.0, 57.1, 55.3, 54.4, 13.5. IR (NaCl/neat) 3296, 1728, 1612. HRMS (FAB+) Calcd. for $C_{36}H_{33}N_2O_6$ (*m/z*) 589.2338, found (*m/z*) 589.2327. NOE data: irradiation of H_7 enhanced H_5 (10.2%) and H_9 (4.38%)



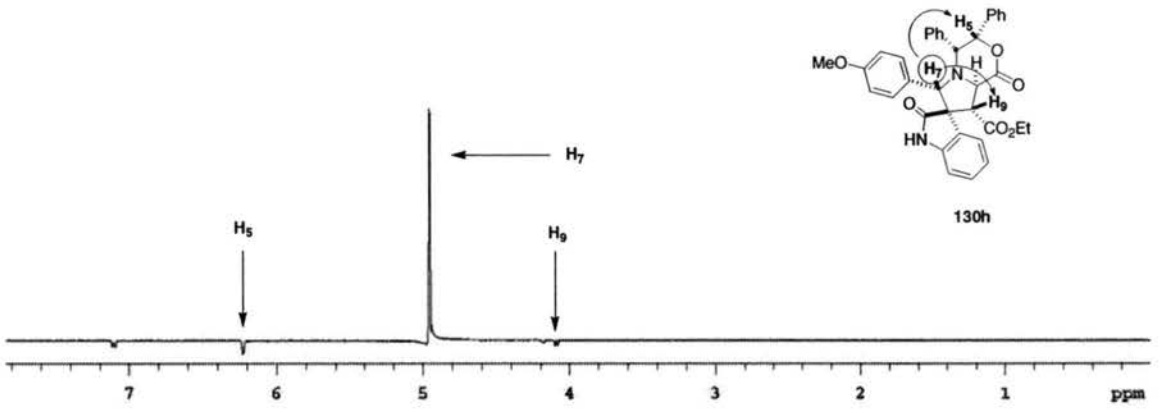
130h



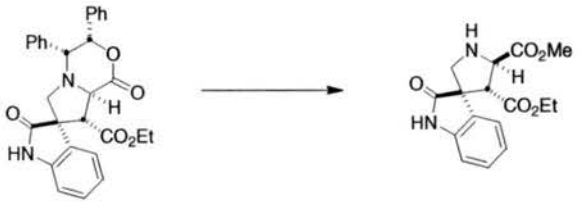
^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-538-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-538-C13



nOe, 400 MHz, $CDCl_3$, filename: PRS2-538-noe

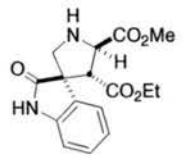


130a 137a

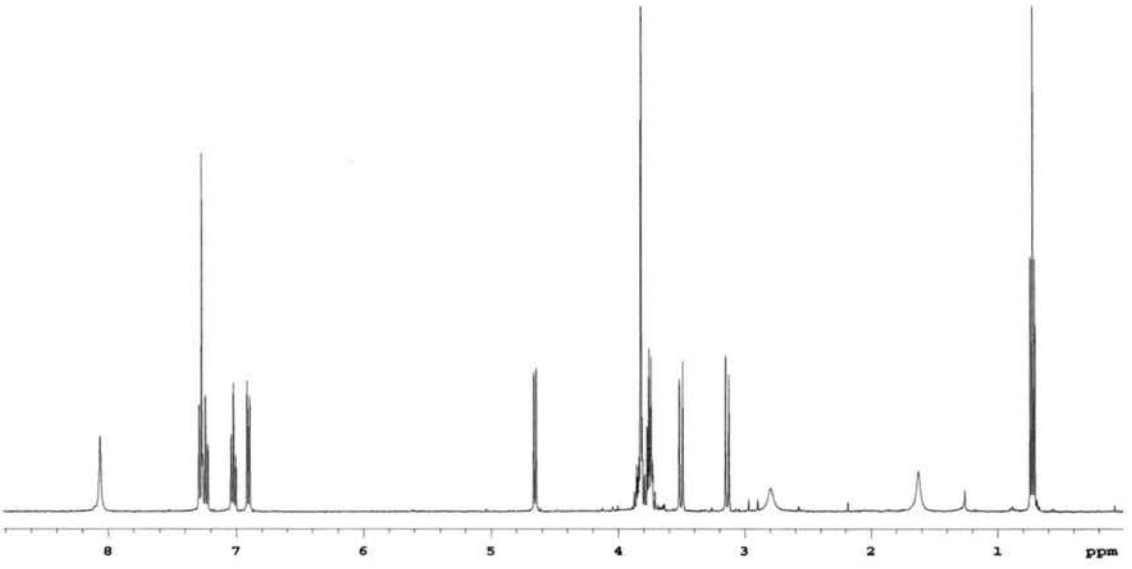
Amino acid methyl ester 137a.

Cycloadduct **137a** (50 mg, 0.1 mmol) was taken up in THF:MeOH 1:1 (2 mL) and transferred to a pressurizable tube. Argon was bubbled through for 5 min. and $PdCl_2$ (17 mg, 0.1 mmol) added. The system was sealed and hydrogenated (65-75 Psi) for 36 h at room temperature. The heterogeneous solution was filtered through celite and evaporated under reduced pressure. The resulting oil was taken up in CH_2Cl_2:MeOH 1:1 (2 mL), a stir bar added and $TMSCHN_2$, available from Aldrich as a 2.0 M solution in hexanes, was added until a yellow color persisted. The reaction was stirred for 15 min. and then evaporated under reduced pressure. Purification by flash chromatography using 2:1 hexanes/EtOAc as the eluent yielded 30 mg (93%)of **137a** as a white amorphous solid.

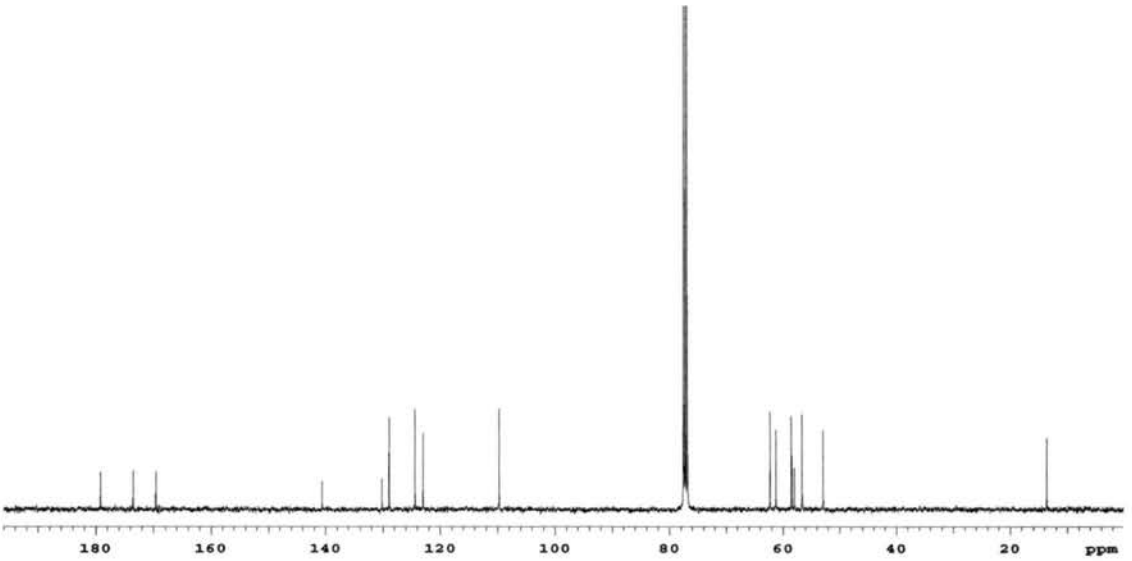
137a: $[\alpha]_D^{25}$ =-23.0 (*c* 0.40, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 7.24 (d, J=7.6 Hz, 1H), 7.20 (t, J=7.6 Hz, 1H), 6.98 (t, J=7.6 Hz, 1H), 6.87 (d, J=7.6 Hz, 1H), 4.62 (d, J=7.6 Hz, 1H), 3.82-3.73 (m, 1H), 3.79 (s, 3H), 3.73-3.68 (m, 1H), 3.47 (1/2ABq, J=10.8 Hz, 1H), 3.10 (1/2ABq, J=10.8 Hz, 1H), 2.76 (br s, 1H), 0.69 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.1, 173.5, 169.6, 140.6, 130.1, 128.9, 124.4, 123.0, 109.7, 62.3, 61.2, 58.5, 58.0, 56.6, 52.9, 13.6; IR (NaCl/neat) 3303, 1732, 1618; HRMS (FAB+) Calcd. for $C_{36}H_{33}N_2O_6$ (*m/z*) 319.1294, found (*m/z*) 319.1286.



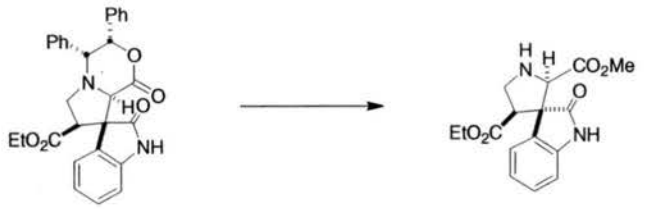
137a



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-906-2H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-906-2HC13

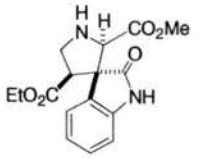


131a 138a

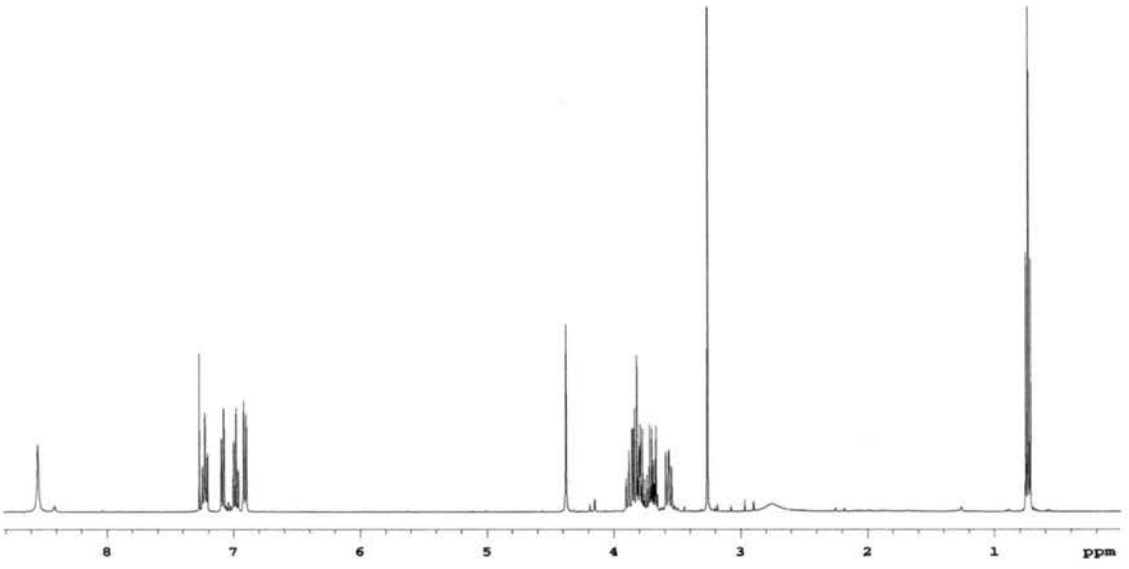
Amino acid methyl ester 138a.

Cycloadduct **131a** (50 mg, 0.1 mmol) was taken up in THF:MeOH 1:1 (2 mL) and transferred to a pressurizable tube. Argon was bubbled through for 5 min. and $PdCl_2$ (17 mg, 0.1 mmol) added. The system was sealed and hydrogenated (65-75 Psi) for 36 h at room temperature. The heterogeneous solution was filtered through celite and evaporated under reduced pressure. The resulting oil was taken up in CH_2Cl_2:MeOH 1:1 (2 mL), a stir bar added and $TMSCHN_2$, available from Aldrich as a 2.0 M solution in hexanes, was added until a yellow color persisted. The reaction was stirred for 15 min. and then evaporated under reduced pressure. Purification by flash chromatography using 2:1 hexanes/EtOAc as the eluent, yielded 24 mg (73%) of **138a** as a white amorphous solid.

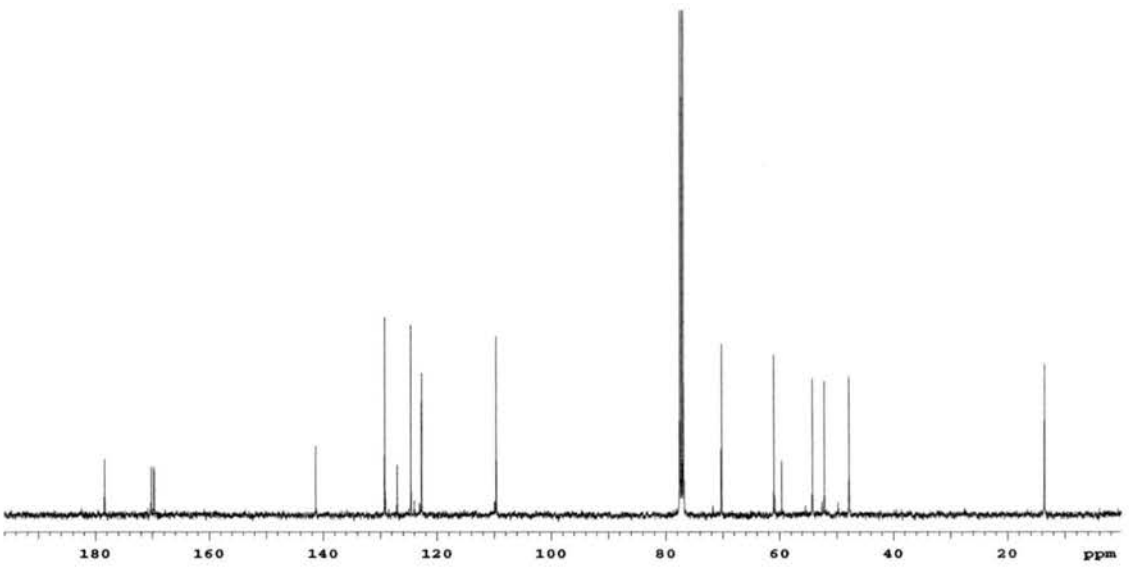
138a: $[\alpha]_D^{25}$ =-61.1 (*c* 0.61, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.51 (br s, 1H), 7.19 (dt, J=0.8 Hz, 7.6 Hz, 1H), 7.05 (d, J=7.6 Hz, 1H), 6.94 (dt, J=0.8 Hz, 7.6 Hz, 1H), 6.87 (d, J=7.6 Hz, 1H), 4.34 (s, 1H), 3.87-3.63 (m, 4H), 3.53 (dd, J=8.4 Hz, 10.8 Hz, 1H), 3.23 (s, 3H), 2.76 (br s, 1H), 0.70 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4, 170.2, 169.7, 141.3, 129.2, 127.0, 124.6, 122.7, 109.6, 70.2, 61.0, 59.6, 54.2, 52.1, 47.8, 13.6; IR (NaCl/neat) 3326, 1730, 1615; HRMS (FAB+) Calcd. for $C_{36}H_{33}N_2O_6$ (*m/z*) 319.1294, found (*m/z*) 319.1289.



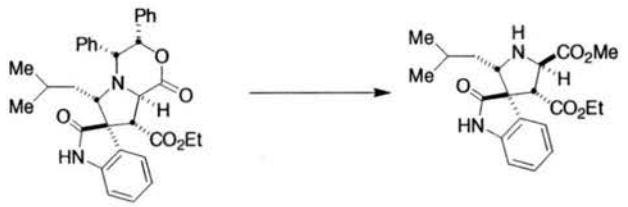
138a



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-906-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-906-C13

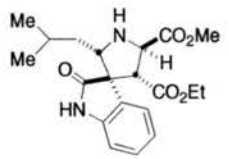


130f 137f

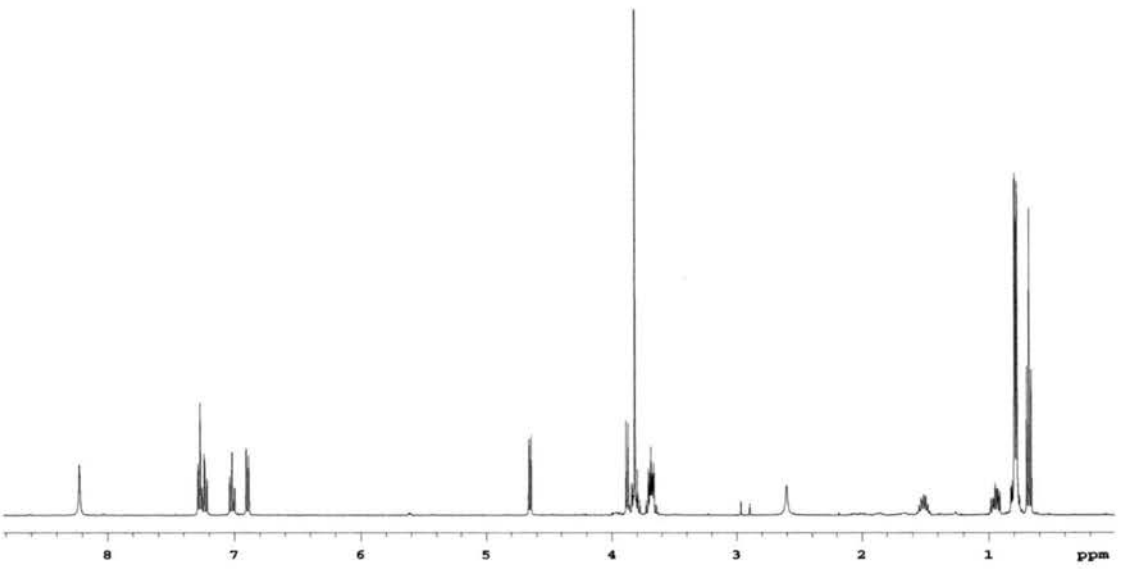
Amino acid methyl ester 137f.

Cycloadduct **130f** (50 mg, 0.09 mmol) was taken up in THF:MeOH 1:1 (2 mL) and transferred to a pressurizable tube. Argon was bubbled through for 5 min. and $PdCl_2$ (16 mg, 0.09 mmol) added. The system was sealed and hydrogenated (65-75 Psi) for 36 h at room temperature. The heterogeneous solution was filtered through celite and evaporated under reduced pressure. The resulting oil was taken up in CH_2Cl_2:MeOH 1:1 (2 mL), a stir bar added and $TMSCHN_2$, available from Aldrich as a 2.0 M solution in hexanes, was added until a yellow color persisted. The reaction was stirred for 15 min. and then evaporated under reduced pressure. Purification by flash chromatography using 2:1 hexanes/EtOAc as the eluent, yielded 31 mg (89%)of **137f** as a white amorphous solid.

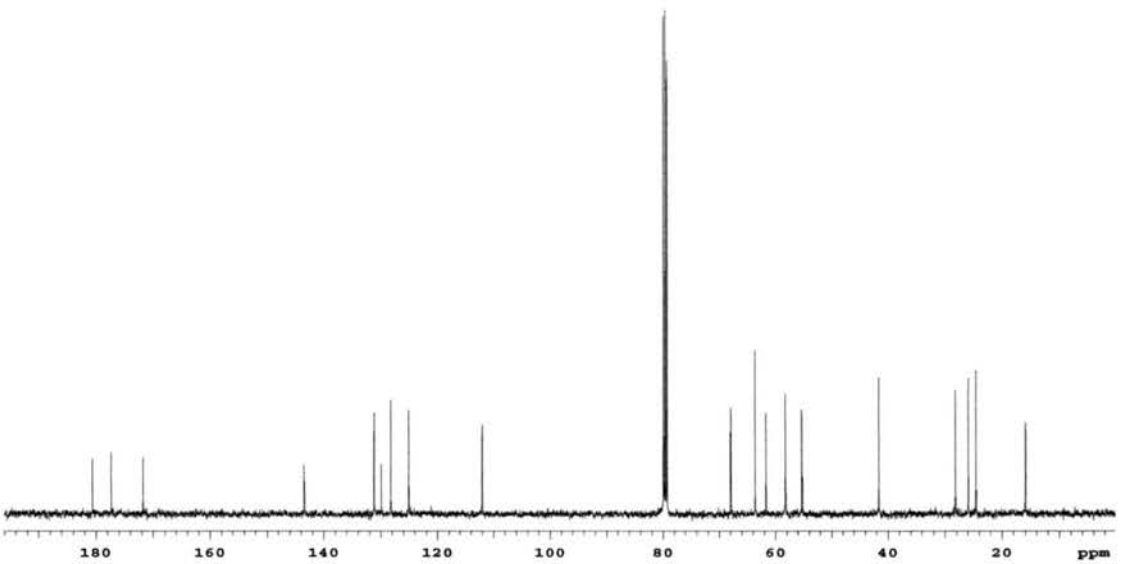
137f $[\alpha]_D^{25}$ =24.8 (*c* 0.64, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (br s, 1H), 7.24 (d, J=7.6 Hz, 1H), 7.20 (dt, J=1.2 Hz, 7.6 Hz, 1H), 6.98 (dt, J=1.2 Hz, 7.6 Hz, 1H), 6.86 (d, J=7.6 Hz, 1H), 4.61 (d, J=6.8 Hz, 1H), 3.84 (d, J=6.8 Hz, 1H), -3.80-3.75 (m, 1H), 3.78 (s, 3H), 3.68-3.60 (m, 2H), 2.59 (br s, 1H), 1.50-1.45 (m, 1H), 0.95-0.87 (m, 1H), 0.79-0.72 (m, 1H), 0.76 (d, J=6.8 Hz, 6H), 0.65 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 174.9, 169.4, 141.1, 128.7, 127.5, 125.8, 122.7, 109.6, 65.5, 61.2, 59.3, 55.8, 52.9, 39.2, 25.8, 23.5, 22.2, 13.5; IR (NaCl/neat) 3325, 1728, 1617; HRMS (FAB+) Calcd. for $C_{36}H_{33}N_2O_6$ (*m/z*) 375.1920, found (*m/z*) 375.1922.



137f



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-907-1H



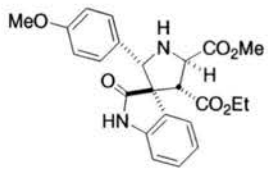
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-907-C13

130j 137j

Amino acid methyl ester 137j.

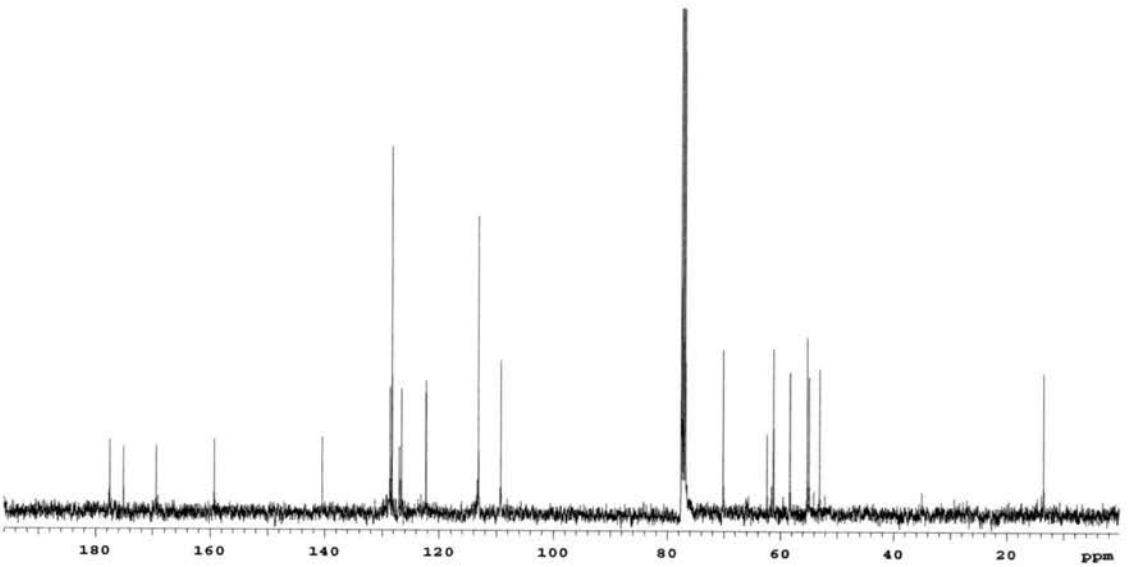
Cycloadduct **130j** (50 mg, 0.08 mmol) was taken up in 10% HCl:MeOH 1:1 (2 mL) and transferred to a pressurizable tube. Argon was bubbled through for 5 min. and 10% Pd-C (2 mg) was added. The system was sealed, pressurized to 40 psi and placed in an oil bath maintained at 40°C. The reaction was stirred for 36 h, filtered through celite and evaporated under reduced pressure. The resulting oil was taken up in CH_2Cl_2:MeOH 1:1 (2 mL), a stir bar added and $TMSCHN_2$, available from Aldrich as a 2.0 M solution in hexanes, was added until a yellow color persisted. The reaction was stirred for 15 min. and then evaporated under reduced pressure. Purification by flash chromatography using 75:20:5 CH_2Cl_2:EtOAc:IPA as the eluent, yielded 20 mg (59%) of **137j** as a white amorphous solid.

137j: $[\alpha]_D^{25}$ = 30.8 (*c* 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (br s, 1H), 7.43 (d, J=7.2 Hz, 1H), 7.04 (d, J=8.8 Hz, 1H), 6.99 (t, J=7.2 Hz, 1H), 6.53 (d, J=8.8 Hz, 3H), 4.77 (d, J=6.8 Hz, 3H), 4.73 (s, 1H), 3.96 (d, J=6.8 Hz, 3H), 3.82-3.75 (m, 1H), 3.80 (m, 3H), 3.69-3.59 (m, 1H), 3.62 (m, 3H), 2.79 (br s, 1H), 0.69 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 175.1, 169.3, 159.3, 140.4, 128.6, 128.3, 126.9, 126.5, 122.2, 113.1, 109.2, 70.1, 62.4, 61.2, 58.2, 55.2, 54.8, 52.9, 13.5; IR (NaCl/neat) 3265, 1735, 1713, 1618; HRMS (FAB+) Calcd. for $C_{36}H_{33}N_2O_6$ (*m/z*) 425.1713, found (*m/z*) 425.1706.



137j

^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-908-1H



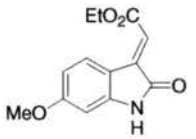
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-908-C13

144 139

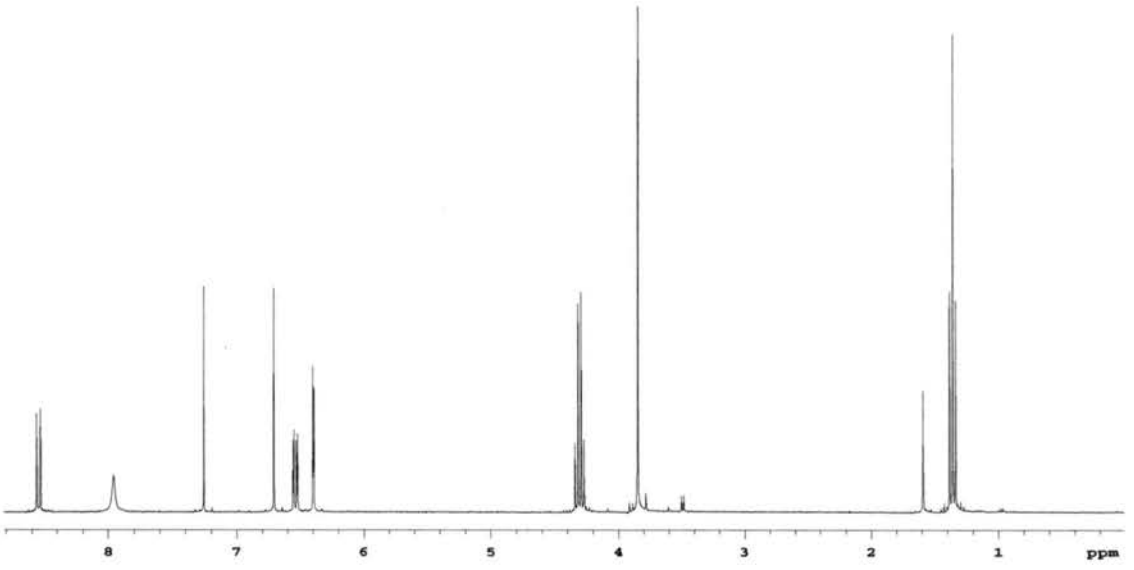
(6-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidene)-acetic acid ethyl ester 139.

To a 25 ml oven dried round bottom flask with stir bar was added 6-methoxy isatin **144** (0.50 g, 2.8 mmol) and carboethoxy triphenylphospylidene (1.1 g, 3.1 mmol). An oven-dried condensor was attached and the system flushed with Ar. DME (30 ml) was added via syringe and the system heated to reflux with stirring. Heating continued for 14 hours followed by filtering through a pad of celite. The solution was then evaporated to dryness and recrystallized from EtOH to yield 0.70 g (69%) of 6-methoxy carboethoxy oxindolylidene acetate **139** as an orange solid. The mother liquor, evaporated to dryness and recyrstallized a second time.

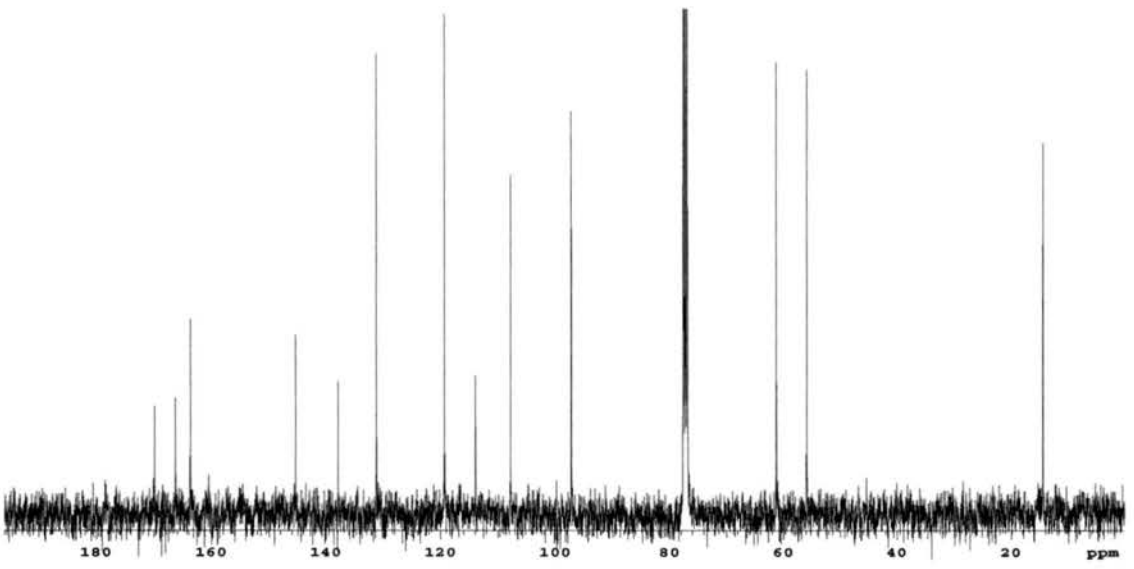
139: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (t, J = 7.5 Hz, 3H), 3.85 (s, 3H), 4.30 (q, J = 7.5 Hz, 2H), 6.40 (d, J = 2.1 Hz, 1H), 6.54 (dd, J = 2.1 Hz, J = 8.7 Hz, 1H), 6.71 (s, 1H), 7.96 (br s, 1H), 8.54 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 55.8, 61.1, 97.2, 107.7, 113.8, 119.3, 131.2, 137.9, 145.3, 164.6, 166.3, 169.9. IR (NaCl/neat) 3170, 1709, 1615. HRMS (FAB+) Calcd. for $C_{13}H_{13}N_1O_4$ (*m/z*) 247.0844, found (*m/z*) 247.0844.



139



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-641-1H



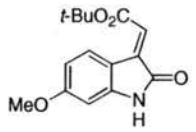
^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS2-641-C13

144 145

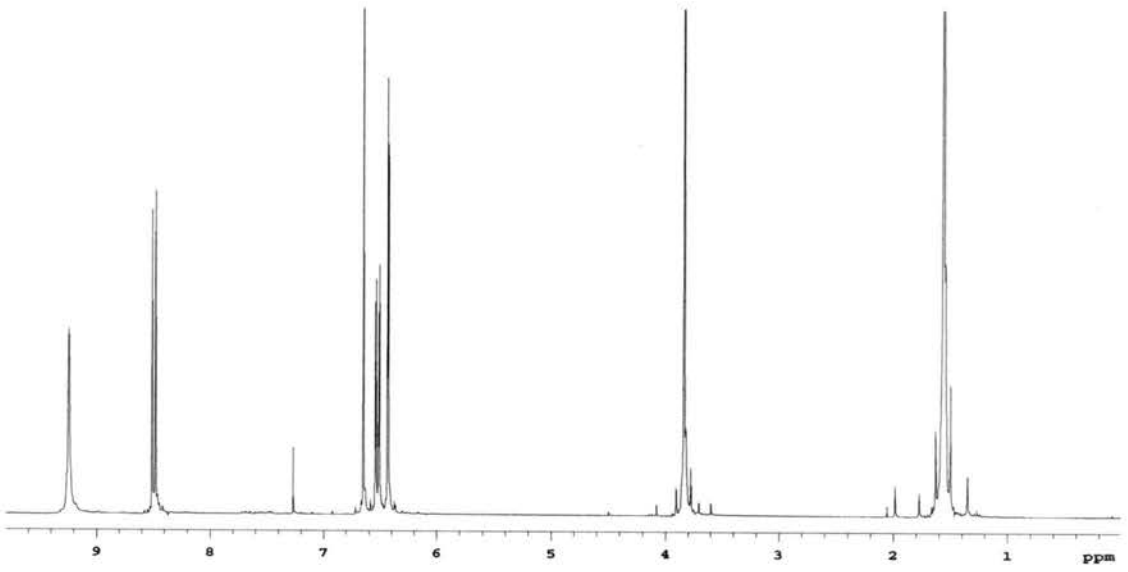
(6-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidene)-acetic acid *tert*-butyl ester 145.

To a 25 ml oven dried round bottom flask with stir bar was added 6-methoxy isatin **144** (0.50 g, 2.8 mmol) and carbo-*tert*-butyloxy triphenylphospylidene (1.15 g, 3.1 mmol). An oven-dried condensor was attached and the system flushed with Ar. DME (30 ml) was added via syringe and the system heated to reflux with stirring. Heating continued for 14 hours followed by filtering through a pad of celite. The solution was then evaporated to dryness and recrystallized from MeOH to yield 0.42 g (54%) of 6-methoxy carbo-*tert*-butoxy oxindolylidene acetate **145** as an orange solid. The mother liquor, evaporated to dryness and recyrstallized a second time.

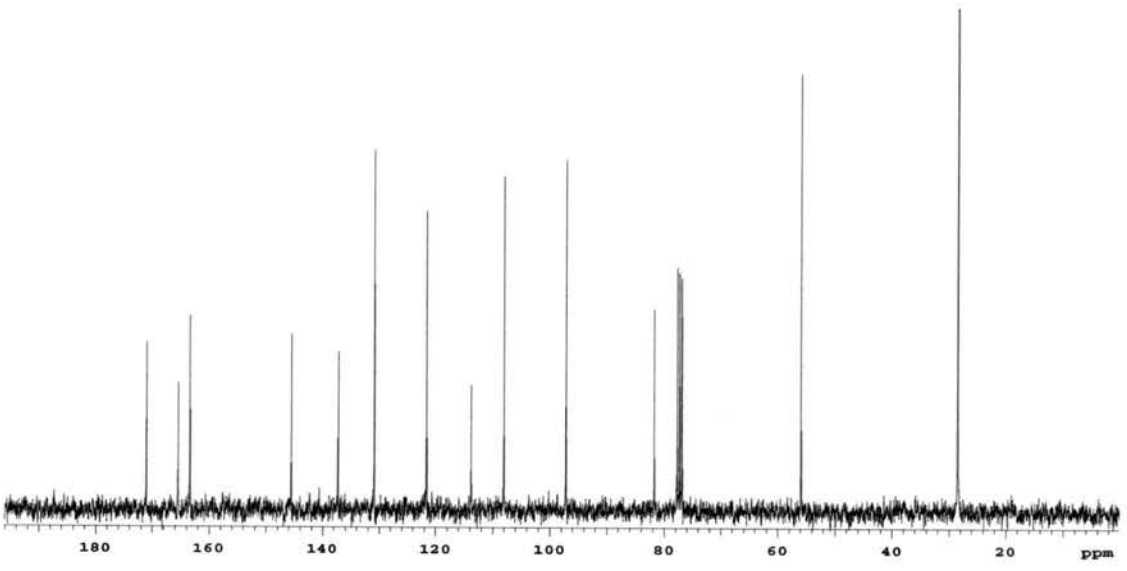
145: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 (s, 9H), 3.84 (s, 3H), 4.30 (q, J = 7.5 Hz, 2H), 6.43 (d, J = 2.1 Hz, 1H), 6.53 (dd, J = 2.1 Hz, J = 8.7 Hz, 1H), 6.65 (s, 1H), 8.50 (d, J = 8.7 Hz, 1H), 9.25 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 55.9, 81.7, 97.1, 108.0, 113.7, 121.6, 130.9, 137.3, 145.5, 163.4, 165.5, 171.0. IR (NaCl/neat) 3219, 1726, 1700. HRMS (FAB+) Calcd. for $C_{15}H_{17}N_1O_4$ (*m/z*) 275.1157, found (*m/z*) 275.1156.



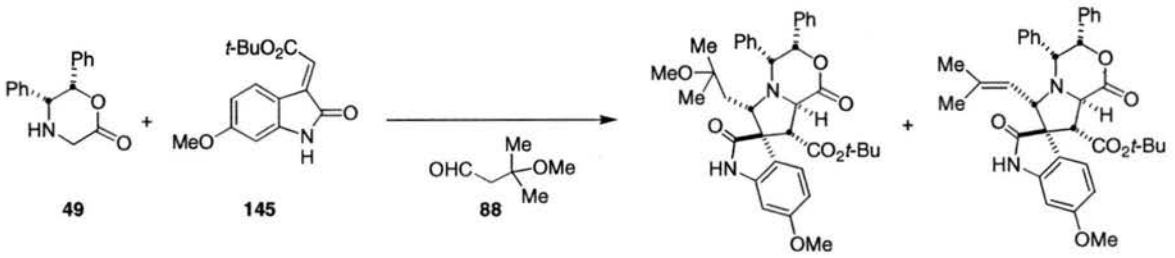
145



^{1}H NMR, 300 MHz, $CDCl_3$, filename: PRS2-727-1H



^{13}C NMR, 75 MHz, $CDCl_3$, filename: PRS2-727-C13



49 145 88 147 149

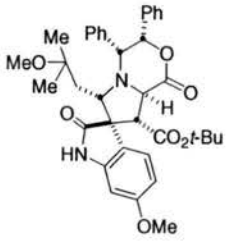
Cycloadduct 147.

Cycloadduct 149.

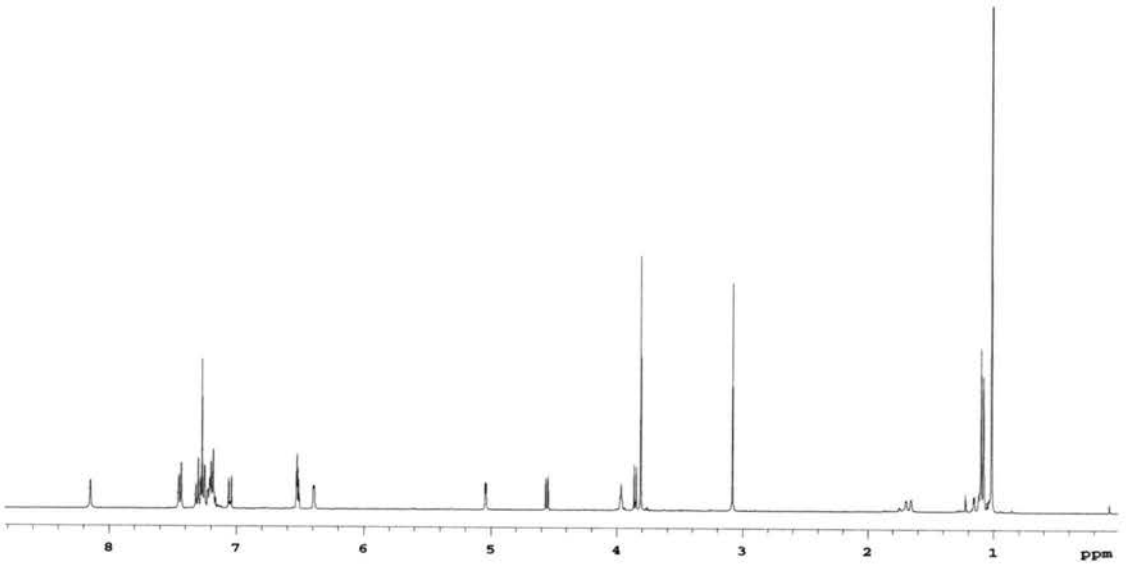
To a flame dried 100 ml round bottom flask with stir bar was added 6-methoxy carbotertbutyloxy oxindolylidene acetate **145** (0.60 g, 2.2 mmol), (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** (0.40 g, 1.5 mmol) and 2.5 g of 3 Å mol.sieves. An oven-dried condensor was attached and the system flushed with Ar. Distilled toluene (25 ml) was added via syringe followed by the addition of 3-methoxy-3-methyl butanal **88** (0.22 g, 1.8 mmol) via syringe. The reaction mixture was kept at room temperature for 14 hours while stirring. The reaction was then filtered through a pad of celite with toluene as the eluent and the resulting solution was evaporated under reduced pressure. Column chromatography with 3:1 hex:EtOAc furnished 0.44 g (45%) of cycloadduct **147** and 0.25 g (25%) of cycloadduct **149** as white solids.

147: $[\alpha]_D^{25}$ =86.3 (*c* 0.63, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (s, 9H), 1.03 (s, 3H), 1.05 (s, 3H), 1.14 (dd, J = 1.6 Hz, J = 16.4 Hz, 1H), 1.63 (dd, J = 1.6 Hz, J = 16.4 Hz, 1H), 3.03 (s, 3H), 3.76 (s, 3H), 3.80 (d, J = 7.2 Hz, 1H), 3.92 (d, J = 1.6 Hz, 1H), 4.50 (d, J = 7.2 Hz, 1H), 4.99 (d, J = 3.2 Hz, 1H), 6.34 (d, J = 3.2 Hz, 1H), 6.45 – 6.49 (m, 2H), 7.00 (d, J = 8.8 Hz, 1H), 7.12- 7.38 (m, 8H), 7.39 (d, J = 8.8 Hz, 1H), 8.10 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ.22.6, 26.0, 27.5, 44.3, 49.7, 55.2, 55.7, 56.0, 56.2, 60.0, 64.8, 73.5, 75.9, 82.0, 97.1, 107.2, 119.9, 125.1, 127.3, 127.4, 128.4, 128.5, 129.5,

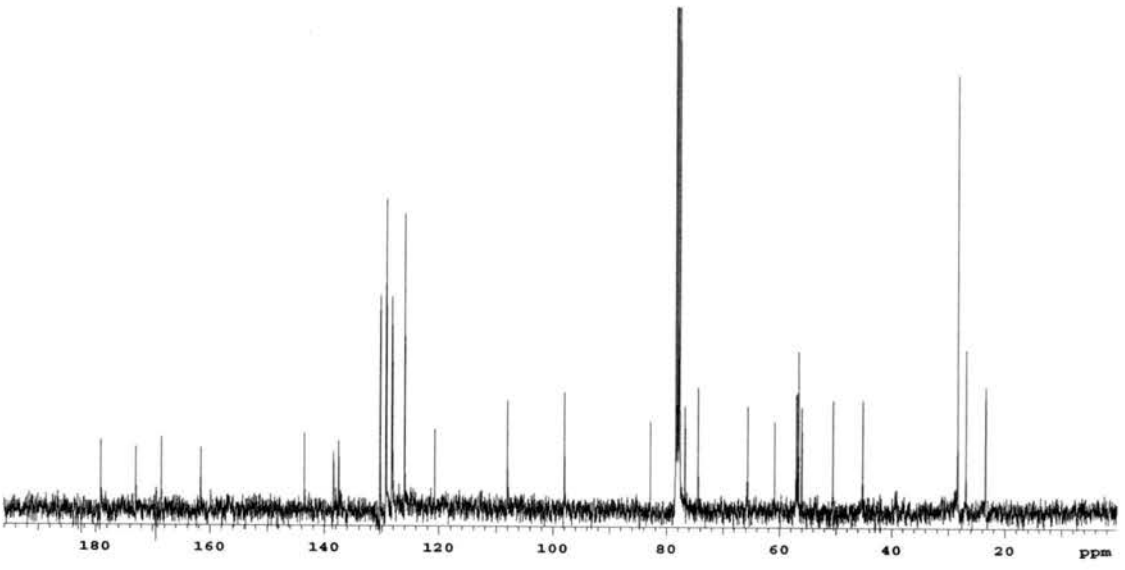
136.7, 137.5, 142.6, 160.8, 167.7, 172.1, 178.2. IR (NaCl/neat) 1733, 1628. HRMS (FAB+) Calcd. for $C_{37}H_{43}N_2O_7$ (*m/z*) 627.3070, found (*m/z*) 627.3074. NOE data: irradiation of H_7 enhanced H_5 (1.12%) and H_9 (2.21%).



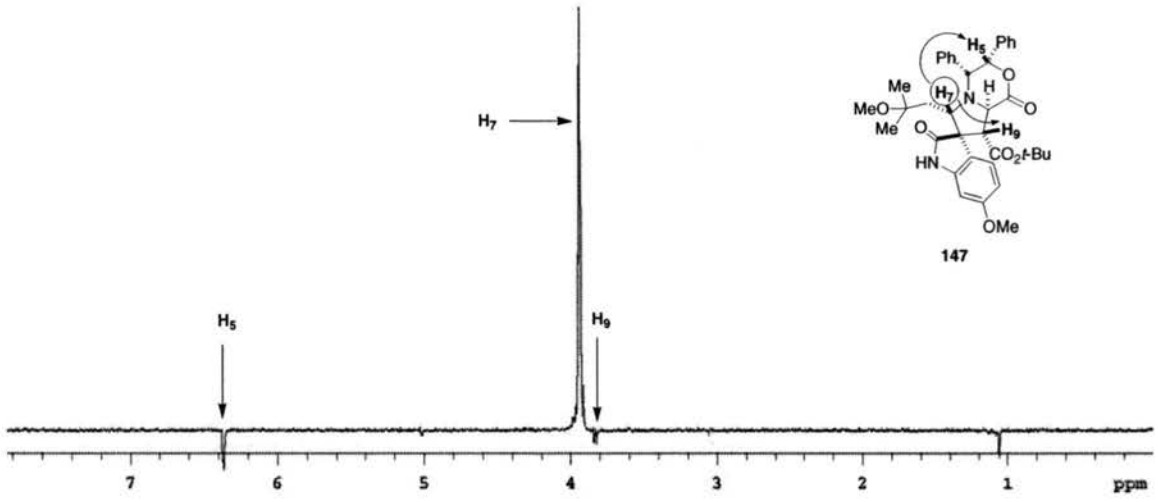
147



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-729-1H

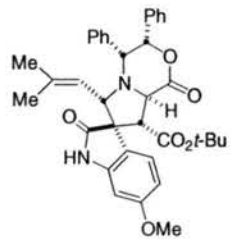


^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-729-C13

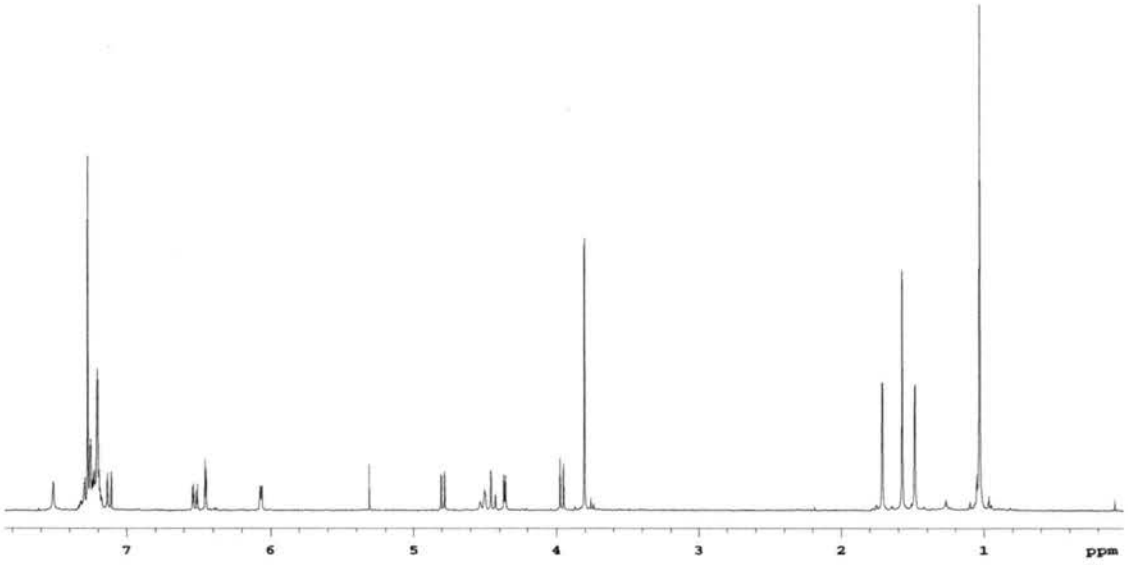


nOe, 400 MHz, $CDCl_3$, filename: PRS2-729-noe

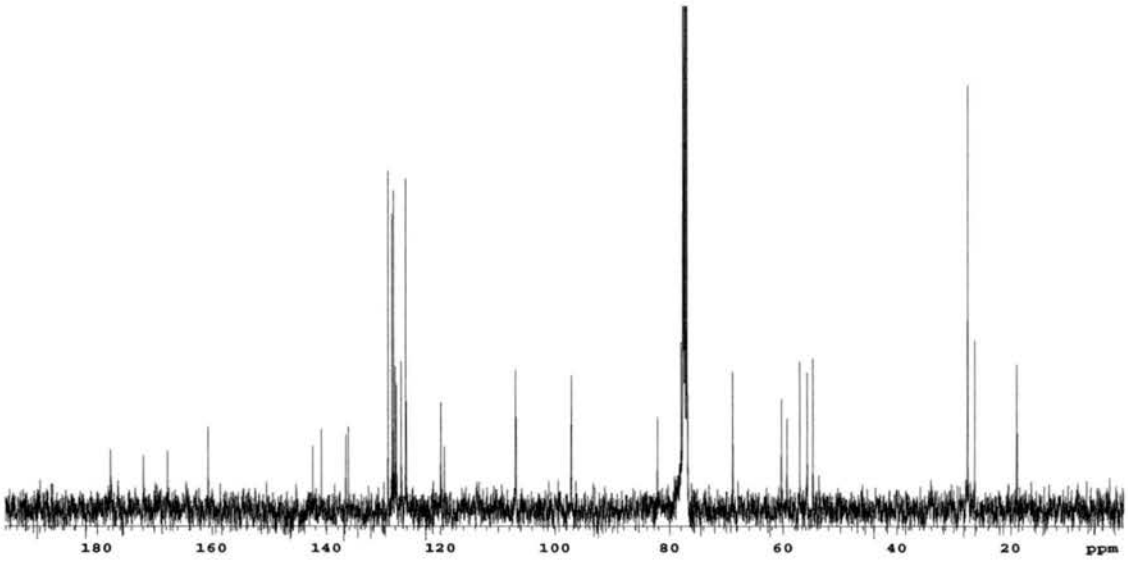
149: $[\alpha]_D^{25}$ =-12.7 (*c* 0.29, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.99 (s, 9H), 1.44 (s, 3H), 1.67 (s, 3H), 3.76 (s, 3H), 3.93 (d, J = 7.2 Hz, 1H), 4.33 (d, J = 3.2 Hz, 1H), 4.41 (d, J = 9.2 Hz, 1H), 4.48 (d, J = 9..2 Hz, 1H), 4.76 (d, J = 7.2 Hz, 1H), 6.03 (d, J = 3.2 Hz, 1H), 6.42 (d, J = 2.0 Hz, 1H), 6.49 (dd, J = 2.0 Hz, J = 8.2Hz, 1H), 7.08 (d, J = 7.2 Hz, 1H), 7.15 – 7.24 (m, 10H), 7.52 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ.18.9, 26.3, 27.5, 54.7, 55.7, 57.0, 59.3, 60.2, 68.8, 77.9, 82.1, 97.1, 106.8, 119.3, 119.9, 126.1, 126.9, 127.7, 128.0, 128.3, 128.6, 129.3, 136.2, 136.6, 140.8, 142.4, 160.6, 167.7, 171.9, 177.6. IR (NaCl/neat) 1730, 1632 cm^{-1}. HRMS (FAB+) Calcd. for $C_{36}H_{39}N_2O_6$ (*m/z*) 595.2808, found (*m/z*) 595.2804. NOE data: irradiation of H_9 enhanced H_7 (2.02%) and H_6 (1.31%).



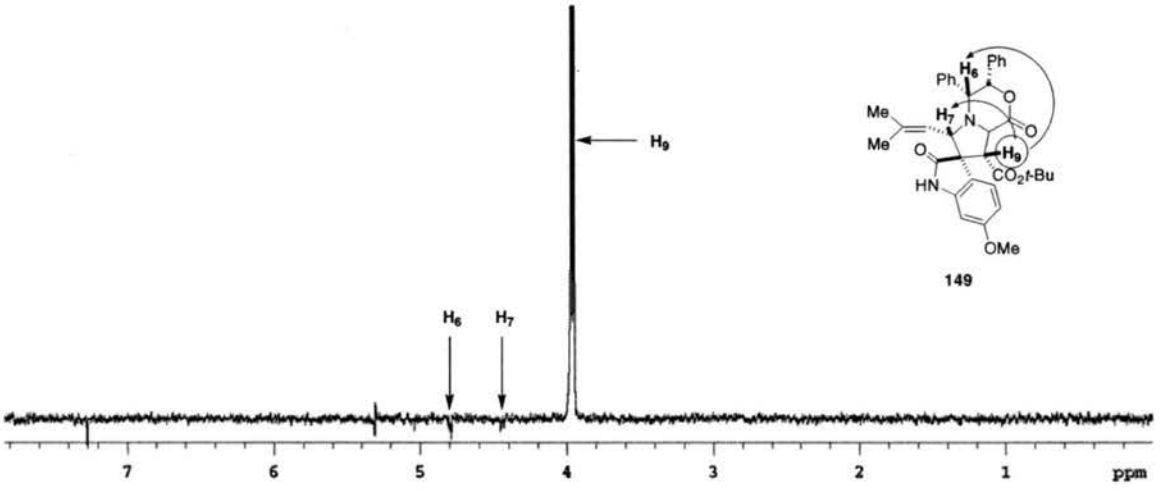
149



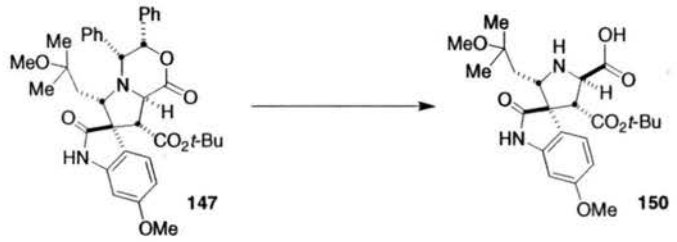
^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-729-elim-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-729-elim-C13



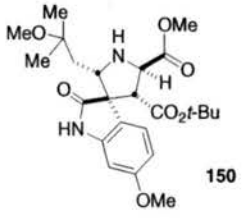
nOe, 400 MHz, $CDCl_3$, filename: PRS2-729-elim-noe



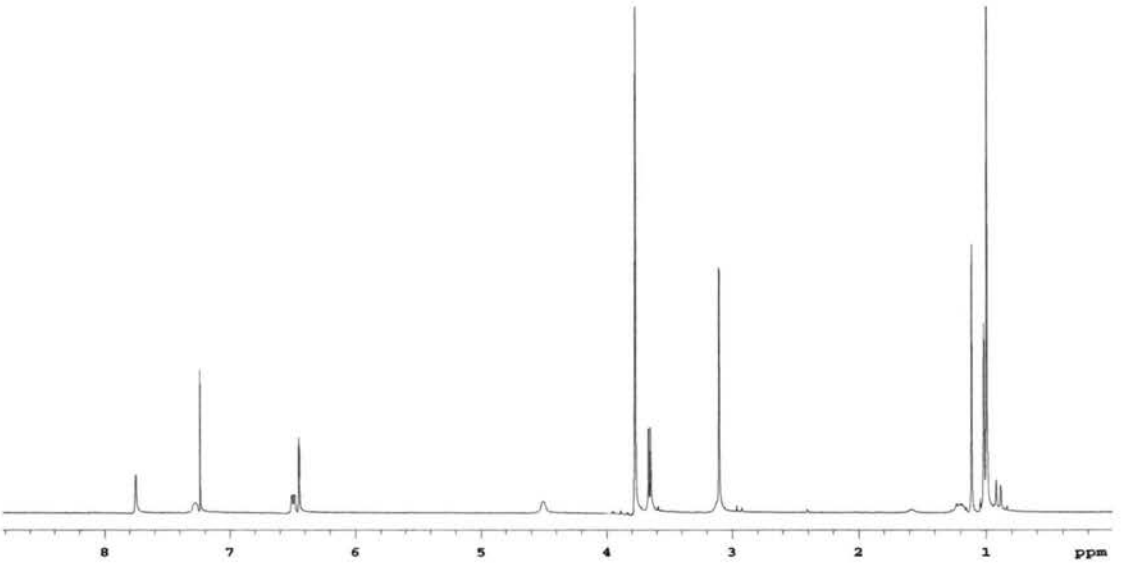
6-Methoxy-2'-(2-methoxy-2-methyl-propyl)-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-4',5'-dicarboxylic acid 4'-tert-butyl ester 150. Cycloadduct **147** (0.50 g, 0.80 mmol) was added to a sealable pressure tube and dissolved in 10 mL of 1:1 THF:EtOH. The solvent was purged with argon for 5 min and $PdCl_2$ (140 mg, 0.80 mmol) was added. The tube was sealed and flushed with H_2 before finally pressurizing to 70 PSI. The reaction was stirred for 36 h and then filtered through celite to remove the palladium catalyst. Concentration afforded a viscous oil which was triturated with 1 x 5 mL of freshly distilled Et_2O to afford the crude amino acid as a white solid. The crude product was used without purification. For characterization purposes a small amount of the amino acid was converted to the methyl ester. The carboxylic acid was dissolved in 5 mL of 1:1 CH_2Cl_2:MeOH. $TMSCHN_2$ (~0.2 mL of a 2.0 M solution in hexanes) was added until a yellow color persisted. The reaction was stirred 5 min. and then concentrated under reduced pressure. PTLC (1 x ½ 250 μm plate) with 1:1 hexanes:EtOAc afforded the methyl ester of **150** as a white amorphous solid.

150: $[\alpha]_D^{25}$ = -17.2 (*c* 0.64, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.89 (dd, J = 13.6 Hz, 1 H), 0.98 (s, 9H), 1.00 (s, 3H), 1.10 (s, 3H), 1.17 – 1.21 (m, 1H), 3.09 (s, 4H), 3.64 (d, J = 6.4 Hz, 2H), 3.76 (s, 6H), 4.49 (br s, 1H), 6.43 (d, J = 2.0 Hz, 1H), 6.48 (d, J = 8.4 Hz, 1H), 7.25 (s, 1H), 7.78 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: 21.8, 24.0, 24.9, 28.0, 30.1, 39.8, 44.8, 48.9, 55.7, 57.0, 60.4, 60.5, 61.6, 74.5, 82.6, 97.4,

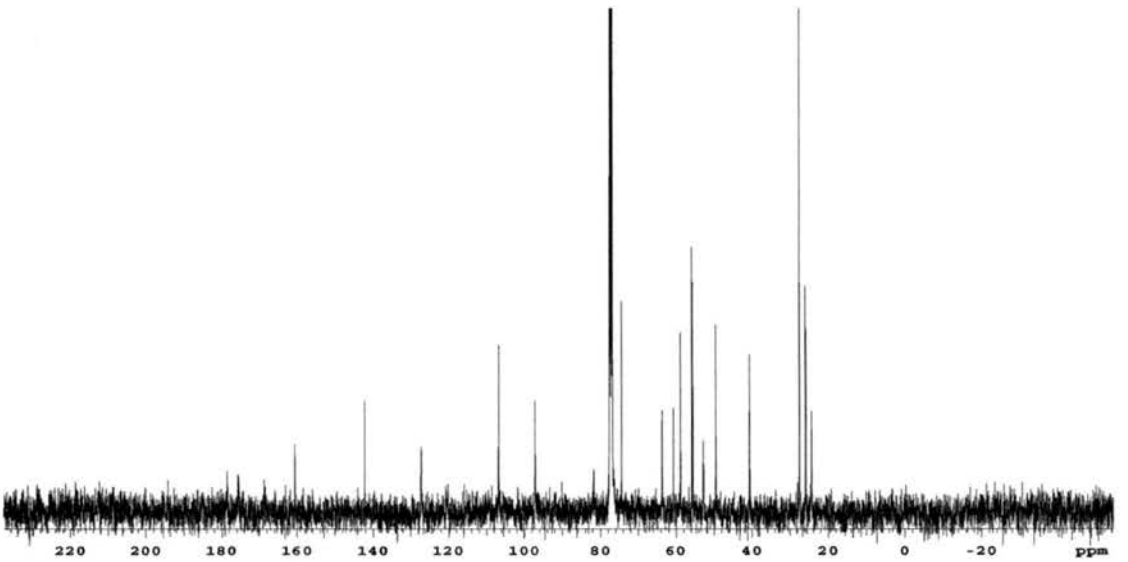
106.9, 116.6, 129.2, 143.1, 160.9, 162.9, 166.2, 168.7, 181.1. IR (NaCl/neat) 1724, 1662. HRMS (FAB+) calcd for $C_{28}H_{38}O_7N_3$ (*m/z*) 528.2710, found (*m/z*) 528.2714.



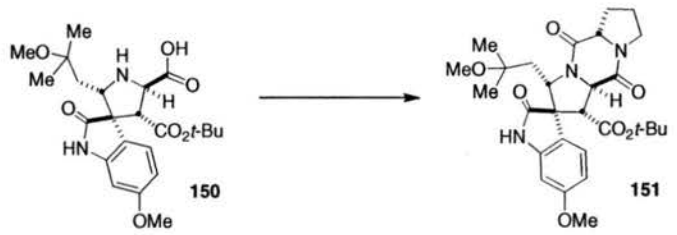
150



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-840-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-840-C13

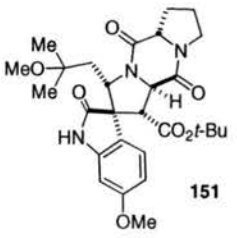


Diketopiperazine 151.

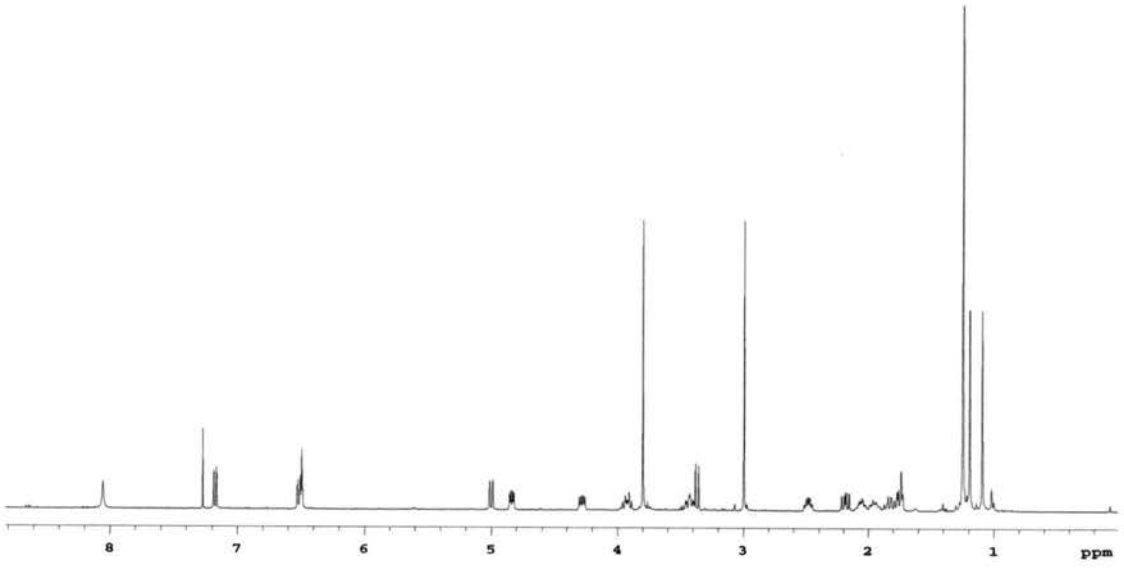
To a 50 mL round-bottom flask that contained the crude amino acid **150** (0.30 g, 0.62 mmol) and was placed under high vacuum for 24 h was added BOP (0.30 g, 0.68 mmol) and L-proline benzyl ester hydrochloride (0.16 g, 0.68 mmol). The flask was flushed with argon, 15 mL of CH_3CN was added and the reaction mixture cooled to 0°C. With stirring, triethylamine (0.19 mL, 1.3 mmol) was added dropwise, the solution allowed to warm to room temperature and stirred for 8 h. The solvent was then evaporated, replaced with 10 mL of EtOAc, washed with 2 x 2.5 mL 1N HCl, 1 x 2.5 mL H_2O, 2 x 2.5 mL 5% $NaHCO_3$, 1 x 1 mL sat. brine sol., dried over Na_2SO_4, filtered and evaporated to yield the crude dipeptide as a brown foam which was taken on crude. To the foam was added a stir bar and 10 mL of EtOH. Argon was bubbled through for 5 min. and 10% Pd/C (0.04 g) was added. The system was flushed with H_2 and a balloon of H_2 was attached. The solution was stirred vigorously for 1.5 h and then filtered through Celite, evaporated and placed on high vacuum overnight. To the crude mixture was added a stir bar, BOP (0.27 g, 0.62 mmol) and 5 mL of CH_3CN. Triethylamine (0.086 mL, 0.62 mmol) was added dropwise and the reaction was allowed to stir for 8 h at which time the solvent was evaporated. Purification *via* column chromatography with 75:20:5 CH_2Cl_2:EtOAc:IPA afforded 127 mg (39%) of **151** as a white solid.

151: $[\alpha]_D^{25}$ = -57.3 (*c* 1.1, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 1.05 (s, 3H), 1.15 (s, 3H), 1.21 (s, 9H), 1.70 (dd, J = 4.0 Hz, J = 18.4 Hz, 2H), 1.78 (quint., J = 8.4 Hz,

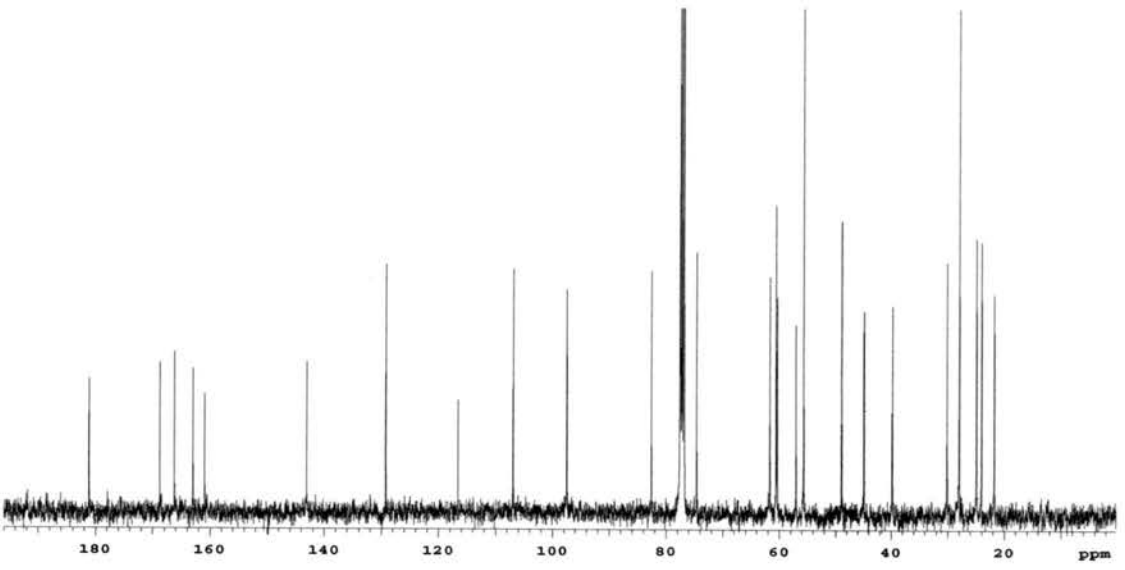
1H), 1.85 – 1.96 (m, 1H), 1.96 - 2.08 (m, 1H), 2.15 (dd, J = 9.6 Hz, J = 14.0 Hz, 1H), 2.49 (quint, J = 6.0, 1H), 2.95 (s, 3H), 3.43 (d, J = 9.6 Hz, 1H), 3.39 (ddd, J = 3.6 Hz, J = 10.0 Hz, J = 13.6 Hz, 1H), 3.76 (s, 3H), 3.89 (dt, J = 8.0 Hz, J = 12.4 Hz, 1H), 4.24 (dd, J = 6.0 Hz, J = 11.6 Hz, 1H), 4.80 (dd, J = 4.4 Hz, J = 9.6 Hz, 1H), 4.96 (d, J = 10.0 Hz, 1H), 6.45 (d, J = 2.0 Hz, 1H), 6.47 (dd, J = 2.0 Hz, J = 8.4 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 8.01 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ $CHCl_3$: 12.9, 20.7, 23.1, 23.7, 29.1, 38.4, 43.8, 47.9, 53.3, 56.1, 59.3, 59.6, 60.1, 60.6, 73.5, 109.6, 121.1, 123.6, 126.3, 128.5, 141.0, 161.8, 165.2, 168.8, 179.5. IR (NaCl/neat) 3244, 1763, 1667, 1665. HRMS (FAB+) calcd for $C_{25}H_{32}O_6N_3$ (*m/z*) 470.2291, found (*m/z*) 470.2280.



151



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-573-1H



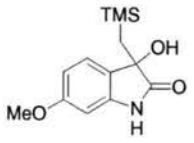
^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-573-C13

144 161

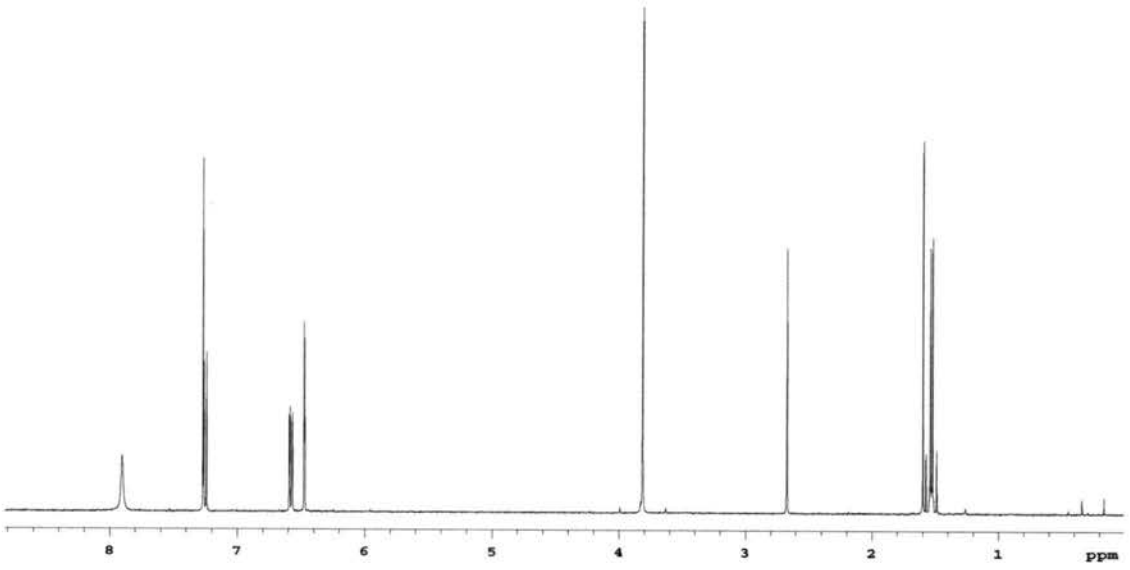
3-Hydroxy-6-methoxy-3-trimethylsilanylmethyl-1,3-dihydro-indol-2-one 161.

To a flame dried 250 mL round bottom flask with stir bar was added 6-methoxy isatin **144** (2.00 g, 11.3 mmol). The system was flushed with Ar and sealed with a rubber septum. THF (100 mL) was added and cooled to -78^{O}C. A solution of trimethylsilyl methyl lithium (1.25 mL, 1.0 M) was added dropwise over 15 min. The reaction was stirred for 1 hour, still at -78^{O}C. Saturated aq. NH_4Cl was added until a pH of 7-8 was obtained. 50 mL of EtOAc was added and the layers were separated. The aqueous layer was extracted 3 x 50 mL with EtOAc, the organic layers combined, washed with 1 x 10 mL of brine, dried over Na_2SO_4, filtered and evaporated to yield a yellowish solid. To the resulting solid, CH_2Cl_2 (100 mL) was added, heated briefly to reflux and allowed to stand for 1 h. The white precipitate was filtered and washed with CH_2Cl_2 (10 mL) to afford 2.40 g (80%) **161** as an off-white solid.

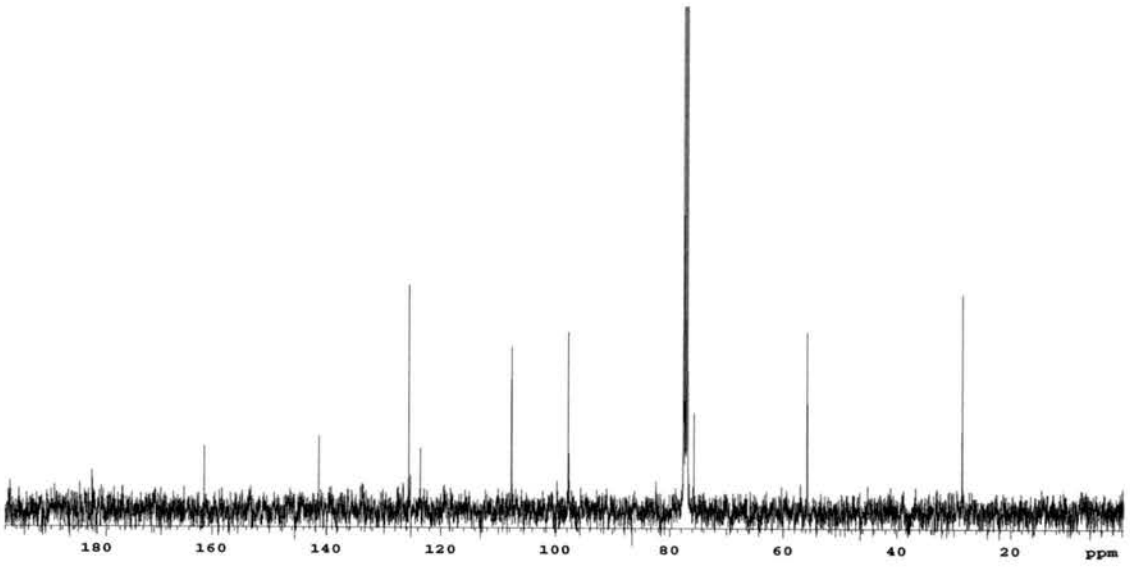
161: ^{1}H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: -0.20 (s, 9H), 1.53 (ABq, J = 7.2 Hz, 2H), 2.67 (s, 1H), 3.81 (s, 3H), 6.47 (d, J = 2.0 Hz, 1H), 6.58 (dd, J = 2.0 Hz, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 7.90 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ $CHCl_3$: -0.87, 28.5, 55.7, 75.7, 97.7, 107.6, 123.4, 125.6, 141.3, 161.4, 180.5. IR (NaCl/neat) 3388, 1713, 1633. HRMS (FAB+) calcd for $C_{13}H_{19}O_3NSi_1$ (*m/z*) 265.1134, found (*m/z*) 265.1132.



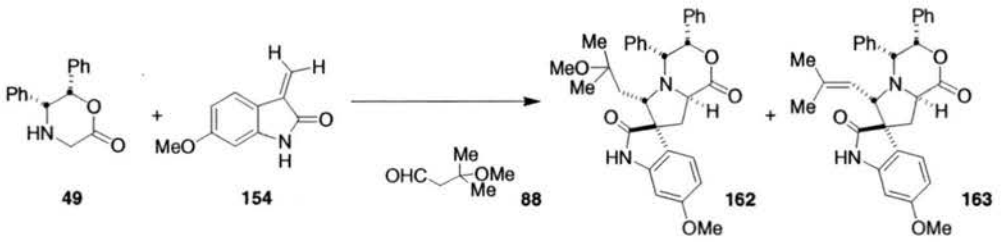
161



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-880-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-880-C13



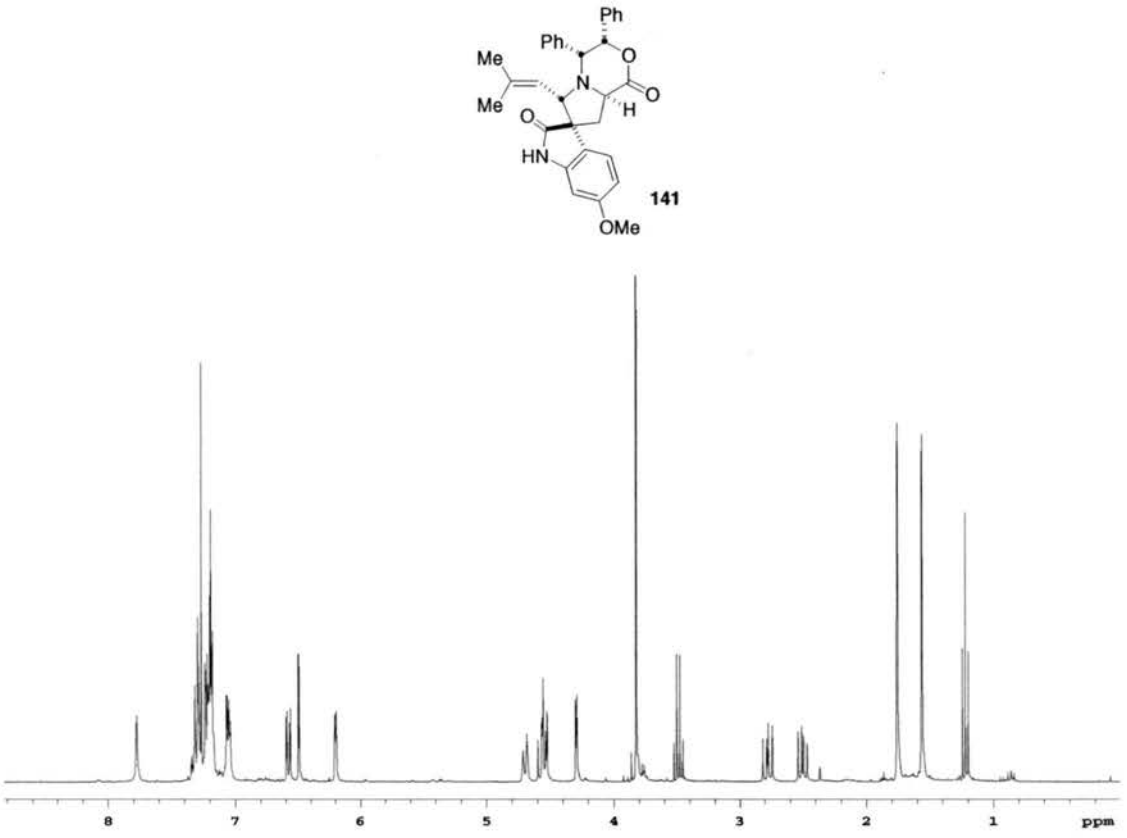
Cycloadduct 162.

Cycloadduct 163.

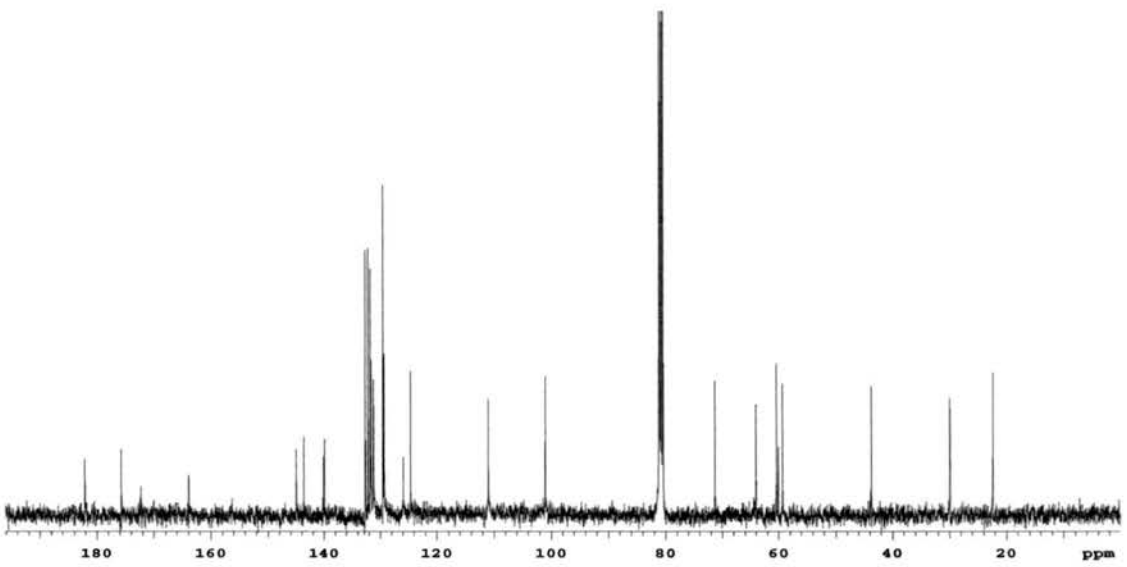
To a flame dried 25 mL round bottom flask with a stir bar was added 3-hydroxy-6-methoxy-3-trimethylsilylmethyl-1,3-dihydro-indol-2-one **154** (100 mg, 0.38 mmol). The system was flushed with Ar and sealed with a rubber septum. Freshly distilled toluene was added (5 mL) and the reaction mixture cooled to 0°C. Trifluoroacetic acid (50 μL, 0.65 mmol) was added dropwise and the reaction was allowed to stir for 15 min. Triethylamine (90 μL, 0.65 mmol) was added dropwise and stirred for 5 min. at 0°C. The organic layer was then extracted with 3x 2 mL water followed by 1 x 2 mL brine, dried over Na_2SO_4, and filtered into an oven dried flask with a stir bar. To the crude reaction mixture was added activated 3Å molecular sieves, (5R,6S)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one **49** (60 mg, 0.24 mmol) and 3-methoxy-3-methylbutanal **88** (33 mg, 0.28 mmol). The reaction mixture was stirred for 6 h at room temperature, the solution was decanted from the molecular sieves and concentrated under reduced pressure. The resulting oil was purified by column chromatography with 2:1 hexanes:EtOAc as the eluent to provide 90 mg (77%) of **162** and 11 mg (8.8%) of **163** as a white foams.

163: $[\alpha]_D^{25}$ =-60.1 (*c* 1.39, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ = 1.56 (d, J= 1.6 Hz, 3H), 1.76 (d, J= 1.6 Hz, 3H), 2.38 (dd, J=8.4 Hz, 12.6 Hz, 1H), 2.78 (dd, J= 9.6 Hz, 12.6 Hz, 1H), 3.82 (s, 3H), 4.29 (d, J= 4.4 Hz, 1H), 4.54 (d, J= 9.0 Hz, 1H), 4.56 (dd, J= 8.4

Hz, 9.6 Hz, 1H), 4.70 (dt, J= 1.6 Hz, 12.8 Hz, 1H), 6.20 (d, J= 4.0 Hz, 1H), 6.50 (d, J= 3.2 Hz, 1H), 6.58 (dd, J= 3.2 Hz, 11.2 Hz, 1H), 7.04-7.07 (m, 2H), 7.18–7.32 (m, 11H), 7.76 (br s, 1H),. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.8, 26.4, 40.1, 55.7, 56.4, 56.8, 60.4, 67.7, 97.4, 107.4, 121.0, 122.3, 125.7, 125.9, 127.6, 128.1, 128.2, 128.6, 129.1, 136.3, 136.5, 140.1, 141.4, 160.3, 168.7, 172.1, 178.5.IR (NaCl/neat) 3261, 1724, 1630. HRMS (FAB+) calcd for $C_{31}H_{31}N_2O_4$ (*m/z*) 495.2284: Found (*m/z*) 495.2267.



^{1}H NMR, 400 MHz, $CDCl_3$, filename: PRS2-882-1H



^{13}C NMR, 100 MHz, $CDCl_3$, filename: PRS2-882-C13

Appendix 1

X-ray Crystal Structure Data for Compound 61

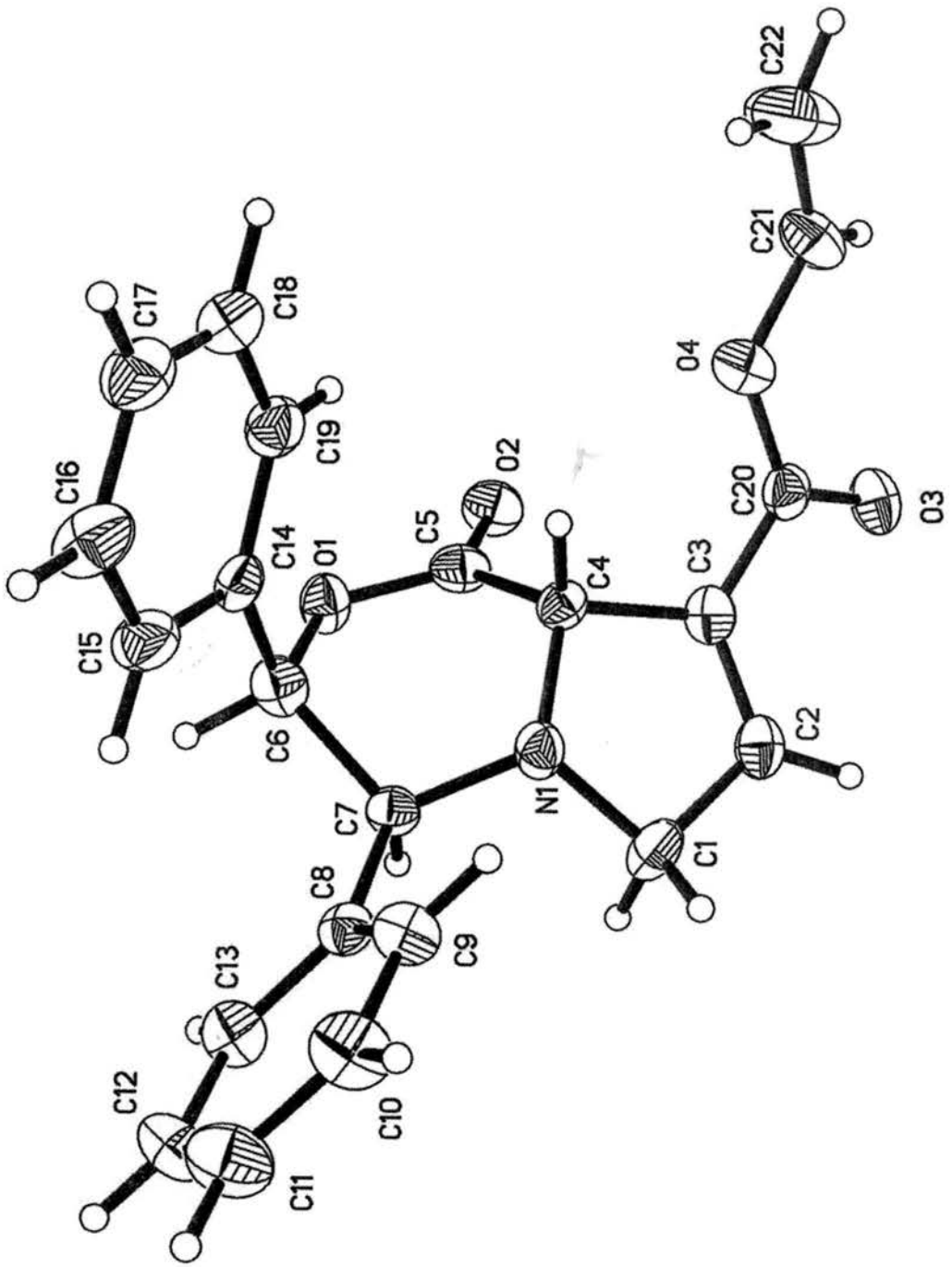

C22
C21
O4
C20
O3
C3
C4
C5
O2
O1
C14
C16
C17
C18
C19
C15
C6
C7
N1
C2
C1
C8
C9
C13
C12
C10
C11

Table 1. Crystal data and structure refinement for **61**.

Identification code	rw5 Sebahar/williams
Empirical formula	$C_{22}H_{20}NO_4$
Formula weight	362.39
Temperature	163(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 8.8975(7) Å alpha = 90° b = 11.5548(9) Å beta = 90° c = 18.953(2) Å gamma = 90°
Volume, Z	1948.5(3) $Å^3$, 4
Density (calculated)	1.235 Mg/m3
Absorption coefficient	0.085 mm^{-1}
F(OOO)	764
Crystal size	0.40 x 0.20 x 0.08 mm
θ range for data collection	2.06 to 28.31°
Limiting indices	$-11 \leq h \leq 8, -15 \leq k \leq 14, -24 \leq 1 \leq 25$
Reflections collected	13038
Independent reflections	4675 (R_{int} = 0.0990)
Absorption correction	SADABS (G. Sheldrick, private comm.)
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4671 / 0 / 244
Goodness-of-fit on F^2	0.993
Final R indices [I>2σ(I)]	Rl = 0.0712, wR2 = 0.1266
R indices (all data)	Rl = 0.1685, wR2 = 0.1726
Absolute structure parameter	unable to determine accurately
Largest diff. peak and hole	0.407 and -0.265 $eÅ^{-3}$

Table2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3]for **59**. U(eq) is defined as one third of the trace of the orthogonalized tensor.

	X	Y	Z	U(eq)
N (1)	-907 (4)	4913 (3)	27 (2)	25 (1)
O (1)	773 (3)	3315 (2)	890 (2)	28 (1)
O (2)	-1319 (3)	2360 (2)	1083 (1)	32 (1)
O (3)	-5378 (3)	2945 (3)	621 (2)	34 (1)
O (4)	-4138 (3)	3898 (3)	1488 (2)	37 (1)
C (1)	-1837 (5)	4724 (4)	-603 (2)	32 (1)
C (2)	-3248 (5)	4211 (3)	-315 (2)	28 (1)
C (3)	-3125 (5)	3985 (3)	362 (2)	26 (1)
C (4)	-1583 (4)	4296 (3)	627 (2)	22 (1)
C (5)	-727 (5)	3234 (4)	875 (2)	27 (1)
C (6)	1475 (5)	4370 (3)	616 (2)	28 (1)
C (7)	691 (4)	4686 (3)	-84 (2)	22 1)
C (8)	1469 (4)	5680 (3)	-462 (2)	23 (1)
C (9)	933 (5)	6804 (3)	-433 (2)	30 (1)
C (10)	1718 (5)	7689 (4)	-753 (2)	37 (1)
C (10)	3051 (5)	7468 (4)	-1102 (2)	42 (1)
C (12)	3599 (5)	6355 (4)	-1134 (2)	37 (1)
C (13)	2804 (5)	5458 (4)	-819 (2)	32 (1)
C (14)	1590 (4)	5285 (4)	1186 (2)	26 (1)
C (15)	2577 (5)	6211 (4)	1083 (2)	35 (1)
C (16)	2752 (5)	7059 (4)	1593 (2)	39 (1)
C (17)	1971 (5)	6992 (4)	2224 (2)	38 (1)
C (18)	1027 (5)	6065 (4)	2343 (2)	36(1)
C (19)	854 (5)	5217 (4)	1827 (2)	30(1)
C (20)	-4330 (5)	3534 (3)	819 (2)	27 (1)
C (21)	-5266 (5)	3549 (5)	2000 (2)	45 (1)
C (22)	-5164 (7)	4385 (5)	2597 (3)	62 (2)

Table 3. Bond lengths [Å] and angles [°] for 59.

N (1)–C (7)	1.461(5)	N (1)–C (1)	1.470(5)
N (1)–C (4)	1.470(5)	O (1)–C (5)	1.338(5)
O (1)–C (6)	1.465(5)	O (2)–C (5)	1.205(5)
O (3)–C (20)	1.214(5)	O (4)–C (20)	1.346(5)
O (4)–C (21)	1.454(5)	C (1)–C (2)	1.492(6)
C (2)–C (3)	1.315(5)	C (3)–C (20)	1.474(6)
C (3)–C (4)	1.504(5)	C (4)–C (5)	1.519(5)
C (6)–C (14)	1.514(6)	C (6)–C (7)	1.542(5)
C (7)–C (8)	1.520(5)	C (8)–C (9)	1.385(6)
C (8)–C (13)	1.391(6)	C (9)–C (10)	1.379(6)
C (10)–C (11)	1.382(6)	C (11)–C (12)	1.377(6)
C (12)–C (13)	1.389(6)	C (14)–C (19)	1.384(6)
C (14)–C (15)	1.399(6)	C (15)–C (16)	1.384(6)
C (16)–C (17)	1.385(6)	C (17)–C (18)	1.379(6)
C (18)–C (19)	1.392(6)	C (21)–C (22)	1.492(7)
C(7)–N(1)–C(1)	113.8(3)	C(7)–N(1)–C(4)	115.0(3)
C(1)–N(1)–C(4)	109.1(3)	C(5)–O(1)–C(6)	118.4(3)
C(20)–O(4)–C(21)	117.0(3)	N(1)–C(1)–C(2)	103.6(3)
C(3)– C(2)–C(1)	111.5(4)	C(2)–C(3)–C(20)	125.7(4)
C(2)– C(3)–C(4)	110.8(4)	C(20)–C(3)–C(4)	123.5(4)
N(1)– C(4)–C(3)	103.4(3)	N(1)–C(4)–C(5)	115.2(3)
C(3)– C(4)–C(5)	111.6(3)	O(2)–C(5)–C(1)	119.2(4)
O(2)– C(5)–C(4)	124.0(4)	O(1)–C(5)–C(4)	116.7(4)
O(1)– C(6)–C(14)	110.9(3)	O(1)–C(6)–C(7)	108.0(3)
C(14)– C(6)–C(7)	118.6(3)	N(1)–C(7)–C(8)	112.0(3)
N(1)– C(7)–C(6)	111.0(3)	C(8)–C(7)–C(6)	112.2(3)
C(9)– C(8)–C(13)	119.0(4)	C(9)–C(8)–C(7)	122.3(4)
C(13)– C(8)–C(7)	118.6(4)	C(10)–C(9)–C(8)	120.3(4)
C(9)– C(10)–C(11)	120.6(4)	C(12)–C(11)–C(10)	119.9(4)
C(11)– C(12)–C(13)	119.8(4)	C(12)–C(13)–C(8)	120.5(4)
C(19)– C(14)–C(15)	117.6(4)	C(19)–C(14)–C(6)	123.7(4)
C(15)– C(14)–C(6)	118.514)	C(16)–C(15)–C(14)	121.0(4)
C(15)– C(16)–C(17)	120.4(4)	C(18)–C(17)–C(16)	119.3(4)
C(17)– C(18)–C(19)	120.0(4)	C(14)–C(19)–C(18)	121.6(4)
O(3)– C(20)–O(4)	124.4(4)	O(3)–C(20)–C(3)	125.1(4)
O(4)– C(20)–C(3)	110.5(4)	O(4)–C(21)–C(22)	106.5(4)

Table 4. Anisotropic displacement parameters [$Å^2 \times 10^3$] for **59**. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [(ha^*)^2U_{11} + \ldots 2hka^*b^*U_{12}]$

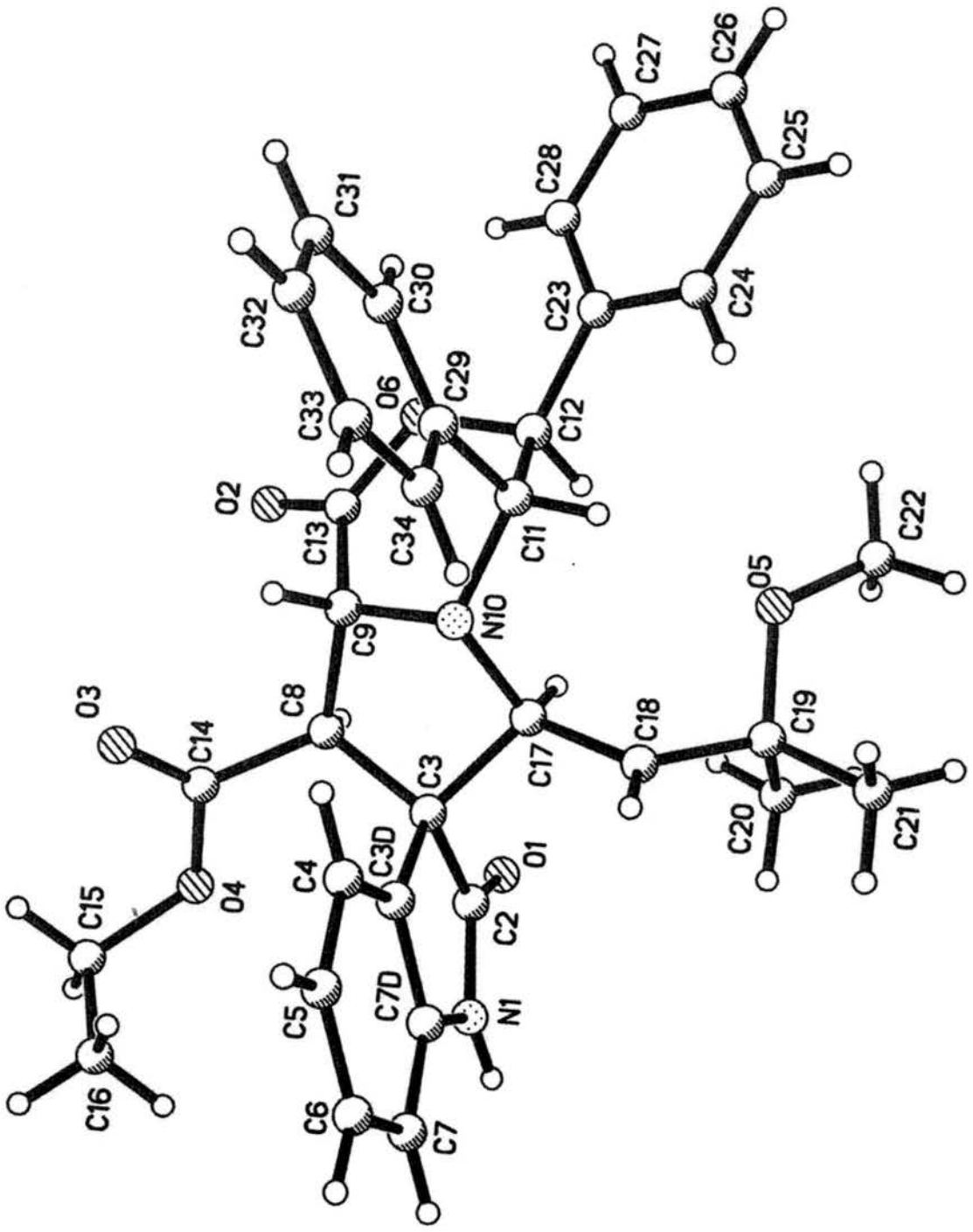
	U11	U22	U33	U23	U13	U12
N(l)	21(2)	26(2)	29(2)	3(2)	-2(2)	-4(2)
O(l)	24(2)	24(2)	34(2)	6(1)	-2(l)	0(l)
O(2)	37(2)	25(2)	33(2)	6(1)	4 (1)	-3(1)
O(3)	22(2)	35(2)	45(2)	-2(2)	-l(l)	-4(l)
O(4)	34(2)	45(2)	31(2)	-3(2)	6(1)	2(2)
C(l)	34(3)	35(3)	29(2)	6(2)	- l0(2)	-3 (2)
C(2)	23(2)	27(2)	34(2)	-3(2)	-4(2)	-1(2)
C(3)	24(2)	22(2)	31(2)	-1(2)	-2(2)	-1(2)
C(4)	25(2)	21(2)	21(2)	-1(2)	-2(2)	l(2)
C(5)	35(3)	24(2)	22(2)	-2(2)	4(2)	2(2)
C(6)	28(2)	23(2)	35(2)	6(2)	2(2)	-I(2)
C(7)	24(2)	20(2)	22(2)	1(2)	l(2)	-2(2)
C(8)	26(2)	23(2)	21(2)	-3(2)	-2(2)	-4(2)
C(9)	33(2)	25(2)	31(2)	-2(2)	4(2)	-1(2)
C(l0)	45(3)	19(2)	46(3)	3(2)	5(2)	-3(2)
C(11)	49(3)	31(3)	46(3)	2(2)	8(3)	-10(2)
C(l2)	31(3)	39(3)	41(3)	-4(2)	12(2)	-4(2)
C(l3)	33(3)	29(2)	36(2)	0(2)	l(2)	0(2)
C(l4)	22(2)	34(2)	23(2)	2(2)	-6(2)	5(2)
C(l5)	37(3)	42(3)	26(2)	1(2)	-4(2)	-12(2)
C(l6)	53(3)	32(3)	32(3)	3(2)	-9(2)	-12(2)
C(17)	42(3)	37(3)	34(3)	-7(2)	-6(2)	0(2)
C(18)	35(3)	46(3)	27(2)	-1(2)	-4(2)	1(2)
C(19)	30(2)	31(2)	28(2)	5(2)	-3(2)	-3(2)
C(20)	25(2)	27(2)	29(2)	1(2)	-3(2)	7(2)
C(21)	30(3)	66(4)	39(3)	6(3)	14(2)	-8(3)
C(22)	77(4)	70(4)	38(3)	0(3)	21(3)	12(3)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 59.

	X	Y	Z	U(eq)
H(1A)	-1339(5)	4185(4)	-936(2)	39
H(1B)	-2043(5)	5463(4)	-849(2)	39
H(2A)	-4125(5)	4066(3)	-587(2)	34
H(4A)	-1687(4)	4846(3)	1031(2)	27
H(6A)	2530(5)	4156(3)	490(2)	34
H(7A)	765(4)	3994(3)	-398(2)	27
H(9A)	20(5)	6967(3)	-193(2)	35
H(10A)	1340(5)	8458(4)	-733(2)	44
H(11A)	3589(5)	8083(4)	-1318(2)	51
H(12A)	4518(5)	6200(4)	-1371(2)	44
H(13A)	3175(5)	4689(4)	-848(2)	39
H(15A)	3137(5)	6260(4)	657(2)	42
H(16A)	3412(5)	7691(4)	1509(2)	47
H(17A)	2085(5)	7578(4)	2571(2)	45
H(18A)	495(5)	6006(4)	2776(2)	43
H(19A)	215(5)	4575(4)	1919(2)	36
H(21A)	-5938(5)	2912(5)	1962(2)	54
H(22C)	-4448(7)	4995(5)	2583(3)	74
H(22A)	-5814(7)	4309(5)	2992(3)	74

Appendix 2

X-ray Crystal Structure Data for Compound 90


C26
C27
C25
C28
C31
C24
C23
C30
C32
C29
O6
C33
C12
O2
C34
C11
C13
C22
O5
C9
N10
O3
C8
C18
C19
C14
C17
C3
C20
C21
C4
C3D
O1
O4
C15
C2
C5
C7D
N1
C6
C7
C16

Crystal data and structure refinement for **90**.

Identification code	rwccdl3 (Sebahar/Williams)
Empirical formula	$C_{35}H_{38.50}N_2O_{6.50}$
Formula weight	591.18
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 12.4236(2) Å alpha = 90°
	b =20.5339(2) Å beta = 113.1830(10)°
	c = 14.1279(2) Å gamma = 90°
Volume, Z	3313.07(8) $Å^3$, 4
Density (calculated)	1.185 Mg/m^3
Absorption coefficient	0.082 mm^{-1}
F(000)	1258
Crystal size	0.33 x 0.36 x 0.40 mm
θ range for data collection	1.57 to 28.16°
Limiting indices	$-16 \le h \le 8, -26 \le k \le 26, -18 \le 1 \le 18$
Reflections collected	21584
Independent reflections	14556 (R_{int} = 0.0203)
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	14556 / 1 / 785
Goodness-of-fit on F^2	1.092
Final R indices [1>2σ(I)]	Rl = 0.0721, wR2 = 0.1707
R indices (all data)	Rl = 0.1045, wR2 = 0.1968
Extinction coefficient	0.0027(7)
Largest diff. peak and hole	0.933 and -0.402 $eÅ^{-3}$
Color of Crystal	Colorless

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for **90**. U (eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
N (1)	855 (3)	1118 (2)	-1878 (3)	64 (1)
N (10)	-116 (3)	3289 (1)	-2145 (2)	44 (1)
O (1)	384 (3)	1463 (1)	-529 (2)	70 (1)
O (2)	1662 (3)	3806 (2)	510 (2)	65 (1)
O (3)	3330 (3)	3109 (2)	-989 (4)	101 (1)
O (4)	3213 (3)	2090 (2)	-431 (3)	86 (1)
O (5)	-3057 (3)	2822 (2)	-2768 (3)	87 (1)
O (6)	-116 (2)	4125 (1)	-489 (2)	53 (1)
C (2)	631 (4)	1571 (2)	-1269 (3)	54 (1)
C (3D)	1047 (3)	2089 (2)	-2610 (3)	49 (1)
C (3)	694 (3)	2257 (2)	-1720 (3)	46 (1)
C (4)	1283 (4)	2478 (2)	-3307 (3)	58 (1)
C (5)	1576 (5)	2183 (3)	-4063 (4)	75 (1)
C (6)	1640 (5)	1511 (3)	-4109 (4)	82 (1)
C (7D)	1107 (4)	1410 (2)	-2674 (3)	54 (1)
C (7)	1399 (4)	1106 (2)	-3421 (4)	71 (1)
C (8)	1525 (3)	2731 (2)	-896 (3)	47 (1)
C (9)	968 (3)	3409 (2)	-1230 (3)	44 (1)
C (11)	-970 (3)	3826 (2)	-2340 (3)	45 (1)
C (12)	-1210 (3)	3957 (2)	-1368 (3)	49 (1)
C (13)	859 (3)	3777 (2)	-319 (3)	48 (1)
C (14)	2793 (4)	2687 (2)	-781 (3)	68 (1)
C (15)	4376 (6)	1928 (4)	-369 (7)	129 (3)
C (16)	4332 (8)	1603 (7)	-1252 (9)	193 (5)
C (17)	-497 (3)	2615 (2)	-2047 (2)	44 (1)
C (18)	-1473 (3)	2366 (2)	-3051 (3)	54 (1)
C (19)	-2667 (4)	2211 (2)	-3025 (3)	62 (1)
C (20)	-2586 (5)	1687 (3)	-2230 (5)	95 (2)
C (21)	-3502 (5)	1992 (4)	-4103 (5)	110 (2)
C (22)	-4104 (7)	2809 (4)	-2563 (10)	183 (5)
C (23)	-2114 (3)	4478 (2)	-1453 (3)	54 (1)
C (24)	-3134 (4)	4508 (3)	-2348 (4)	80 (1)
C (25)	-4020 (4)	4950 (3)	-2429 (5)	90 (2)
C (26)	-3902 (5)	5362 (2)	-1629 (5)	87 (2)
C (27)	-2882 (5)	5349 (2)	-747 (5)	81 (1)
C (28)	-1986 (4)	4910 (2)	-666 (3)	66 (1)
C (29)	-563 (3)	4403 (2)	-2814 (3)	46 (1)
C (30)	-350 (4)	5026 (2)	-2392 (3)	60 (1)
C (31)	-7 (4)	5524 (2)	-2890 (4)	72 (1)
C (32)	132 (4)	5406 (2)	-3800 (4)	74 (1)
C (33)	-68 (4)	4789 (3)	-4223 (4)	75 (1)
C (34)	-398 (4)	4286 (2)	-3726 (3)	60 (1)
N (1B)	-1356 (3)	2210 (2)	1323 (3)	65 (1)
N (10B)	1428 (3)	3650 (1)	3321 (2)	46 (1)
O (1B)	-511 (3)	2757 (1)	368 (2)	66 (1)

O (2B)	646 (3)	5108 (1)	1677 (2)	69 (1)
O (3B)	-1695 (3)	4625 (2)	2143 (3)	90 (1)
O (4B)	-2422 (3)	3772 (2)	1085 (3)	74 (l)
O (5B)	3473 (3)	2538 (l)	3065 (2)	70 (l)
O (6B)	2393 (2)	4696 (l)	2521 (2)	56 (l)
C (2B)	-698 (3)	2682 (2)	1150 (3)	51 (l)
C (3B)	-199 (3)	3116 (2)	2127 (3)	44 (l)
C (3C)	-829 (3)	2840 (2)	2778 (3)	50 (l)
C (4B)	-833 (4)	3041 (2)	3717 (3)	63 (l)
C (5B)	-1507 (5)	2684 (3)	4119 (4)	80 (l)
C (6B)	-2138 (5)	2152 (3)	3617 (5)	90 (2)
C (7C)	-1477 (4)	2301 (2)	2265 (3)	59 (1)
C (7B)	-2141 (5)	1945 (2)	2684 (5)	82 (l)
C (8B)	-373 (3)	3853 (2)	1870 (3)	46 (l)
C (9B)	682 (31	4186 (2)	2722 (3)	48 (1)
C (11B)	2664 (3)	3848 (2)	3855 (3)	49 (1)
C (12B)	3125 (3)	4149 (2)	3098 (3)	50 (l)
C (13B)	1241 (3)	4688 (2)	2253 (3)	51 (l)
C (14B)	-1553 (4)	4128 (2)	1745 (3)	58 (l)
C (15B)	-3629 (5)	3976 (3)	861 (6)	104 (2)
C (16B)	-4057 (7)	3684 (6)	1560 (9)	168 (4)
C (17B)	1151 (3)	3064 (2)	2637 (3)	44 (l)
C (18B)	1672 (3)	2435 (2)	3245 (3)	49 (1)
C (19B)	2562 (4)	2062 (2)	2950 (3)	55 (l)
C (20B)	1990 (5)	1801 (3)	1842 (3)	78 (1)
C (21B)	3072 (5)	1494 (2)	3707 (4)	76 (l)
C (22B)	4370 (6)	2350 (4)	2736 (6)	117 (2)
C (23B)	4382 (3)	4394 (2)	3567 (3)	57 (l)
C (24B)	5258 (4)	3997 (2)	4244 (4)	80 (1)
C (25B)	6408 (5)	4224 (3)	4674 (5)	98 (21
C (26B)	6710 (5)	4830 (4)	4461 (6)	129 (3)
C (27B)	5866 (6)	5219 (4)	3801 (8)	151 (4)
C (28B)	4703 (5)	5006 (3)	3348 (6)	112 (2)
C (29B)	2791 (3)	4251 (2)	4806 (3)	54 (l)
C (30B)	2772 (4)	4927 (2)	4829 (4)	77 (l)
C (31B)	2824 (5)	5245 (3)	5729 (5)	95 (2)
C (32B)	2921 (5)	4916 (4)	6582 (5)	97 (2)
C (33B)	2972 (5)	4238 (4)	6591 (4)	96 (2)
C (34B)	2899 (4)	3914 (3)	5696 (3)	78 (l)
O (7)	-1397 (7)	984 (3)	187 (4)	169 (3)
C (35)	-2207 (10)	474 (4)	-176 (7)	154 (3)
C (36)	-2987 (9)	309 (8)	453 (8)	211 (6)

Table 3. Bond lengths [Å] and angles [°l for **90**.

C (4) - C (3D) - C (7D)	119.8 (3)	C (4) - C (3D) - C (3)	131.7 (3)
C (7D) -C (3D) -C (3)	108.5 (3)	C (3D) -C (3) -C (17)	114.3 (3)
C (3D) - C (3) - C (8)	115.9 (3)	C (17) - C (3) - C (8)	101.6 (3)
C (3D) - C (3) - C (2)	102.1 (3)	C (17) - C (3) - C (2)	110.7 (3)
C (8) - C (3) - C (2)	112.6 (3)	C (3D) - C (4) - C (5)	119.0 (4)
C (6) - C (5) - C (4)	120.4 (4)	C (5) - C (6) - C (7)	121.8 (4)
C (7) - C (7D) - C (3D)	122.1 (4)	C (7) - C (7D) -N (l)	128.3 (4)
C (3D) - C (7D) - N (1)	109.6 (3)	C (7D) - C (7) - C (6)	116.9 (4)
C (14) - C (8) - C (9)	114.0 (3)	C (14) - C (8) - C (3)	113.4 (3)
C (9) - C (8) - C (3)	104.5 (3)	N (10) - C (9) - C (8)	105.1 (3)
N (10) - C (9) - C (13)	117.6 (3)	C (8) - C (9) - C (13)	111.3 (3)
N (10) - C (ll) - C (12)	110.0 (3)	N (10) - C (11) - C (29)	108.6 (3)
C (l2) - C (ll) - C (29)	117.7 (3)	O (6) - C (12) - C (23)	108.0 (3)
O (6) - C (12) - C (ll)	110.7 (3)	C (23) - C (12) - C (11)	116.7 (3)
O (2) - C (13) - O (6)	119.4 (3)	O (2) - C (13) - C (9)	121.6 (3)
O (6) - C (13) - C (9)	118.7 (3)	O (3) - C (14) - O (4)	125.1 (4)
O (3) - C (14) - C (8)	125.7 (4)	O (4) - C (14) - C (8)	109.2 (4)
C (16) - C (15) -O (4)	111.5 (7)	N (10) - C (17) - C (18)	112.7 (3)
N (10) - C (17) - C (3)	99.6 (3)	C (l8) - C (17) - C (3)	115.4 (3)
C (19) - C (18) - C (17)	117.6 (3)	0 (5) - C (19) - C (20)	111.0 (4)
O (5) - C (19) - C (18)	104.5 (3)	C (20) - C (19) - C (18)	112.2 (4)
O (5) - C (19) - C (21)	110.0 (4)	C (20) - C (19) - C (21)	110.2 (4)
C (l8) - C (19) - C (21)	108.7 (4)	C (28) - C (23) - C (24)	118.5 (4)
C (28) - C (23) - C (12)	122.7 (4)	C (24) - C (23) - C (12)	118.7 (4)
C (23) - C (24) - C (25)	120.5 (5)	C (26) - C (25) - C (24)	120.6 (5)
C (25) - C (26) - C (27)	119.5 (5)	C (26) - C (27) - C (28)	120.1 (5)
C (23) - C (28) - C (27)	120.7 (5)	C (30) - C (29) - C (34)	118.6 (3)
C (30) - C (29) - C (11)	124.0 (3)	C (34) - C (29) - C (11)	117.3 (3)
C (29) - C (30) - C (31)	120.1 (4)	C (32) - C (31) - C (30)	120.8 (4)
C (33) - C (32) - C (31)	119.8 (4)	C (32) - C (33) - C (34)	119.9 (4)
C (33) - C (34) - C (29)	120.8 (4)	C (2B) - N (lB) - C (7C)	111.8 (3)
C (9B) - N (10B) - C(11B)	112.4 (3)	C (9B) - N (10B) - C (17B)	107.3 (3)
C (11B) - N (10B) - C (17B)	118.7 (3)	C (14B) - 0 (4B) - C (15B)	118.2 (4)
C (22B) - 0 (5B) - C (19B)	117.2 (4)	C (13B) - 0 (6B) - C (12B)	120.6 (3)
O (lB) - C (2B) - N (lB)	126.6 (3)	O (lB) - C (2B) - C (3B)	125.4 (3)
N (lB) - C (2B) - C (3B)	108.1 (3)	C (3C) - C (3B) - C (17B)	114.3 (3)
C (3C) - C (3B) - C (8B)	116.4 (3)	C (17B) - C (3B) - C (8B)	101.6 (3)
C (3C) - C (3B) - C (2B)	101.7 (3)	C (17B) - C (3B) - C (2B)	110.8 (3)
C (8B) - C (3B) - C (2B)	112.4 (3)	C (7C) - C (3C) - C (4B)	120.9 (4)
C (7C) - C (3C) - C (3B)	108.0 (3)	C (4B) - C (3C) - C (3B)	131.1 (3)
C (5B) - C (4B) - C (3C)	117.5 (4)	C (6B) - C (5B) - C (4B)	121.6 (5)
C (5B) - C (6B) - C (7B)	121.7 (5)	C (3C) - C (7C) - C (7B)	120.9 (4)
C (3C) - C (7C) - N (lB)	110.0 (3)	C (7B) - C (7C) -N (1B)	129.1 (4)
C (6B) - C (7B) - C (7C)	117.5 (5)	C (l4B) - C (8B) - C (9B)	114.2 (3)
C (14B) - C (8B) - C (3B)	115.5 (3)	C (9B) - C (8B) - C (3B)	104.6 (3)
N (10B) - C (9B) - C (13B)	117.5 (3)	N (10B) - C (9B) - C (8B)	105.4 (3)
C (13B) - C (9B) - C (8B)	110.5 (3)	N (10B) - C (11B) - C (l2B)	110.8 (3)
N (10B) - C (11B) - C (29B)	108.9 (3)	C (12B) - C (11B) - C (29B)	117.2 (3)
O (6B) - C (12B) - C (23B)	107.1 (3)	O (6B) - C (12B) - C (11B)	112.1 (3)
C (23B) - C (l2B) - C (11B)	115.6 (3)	O (2B) - C (13B) -0 (6B)	118.9 (3)
O (2B) - C (13B) - C (9B)	120.5 (3)	O (6B) - C (l3B) - C (9B)	120.5 (3)
O (3B) - C (14B) - O (4B)	124.1 (4)	O (3B) - C (14B) - C (8B)	124.9 (4)
O (4B) - C (14B) - C (8B)	110.9 (3)	C (l6B) - C (15B) - O (4B)	111.0 (6)

N (10B) - C (17B) - C (18B)	111.9 (3)	N (10B) - C (17B) - C (3B)	100.2 (3)
C (l8B) - C (17B) - C (3B)	117.1 (3)	C (19B) - C (18B) - C (17B)	117.2 (3)
O (5B) - C (19B) - C (18B)	104.2 (3)	O (5B) - C (19B) - C (20B)	112.0 (4)
C (18B) -C (19B) -C (20B)	110.9 (3)	O (5B) - C (19B) - C (21B)	110.0 (4)
C (18B) - C (19B) - C (2lB)	109.5 (3)	C (20B) - C (19B) - C (21B)	110.1 (4)
C (28B) - C (23B) - C (24B)	117.8 (4)	C (28B) - C (23B) - C (12B)	122.5 (4)
C (24B) - C (23B) - C (12B)	119.7 (4)	C (25B) - C (24B) - C (23B)	119.5 (5)
C (26B) - C (25B) - C (24B)	122.1 (5)	C (27B) - C (26B) - C (25B)	118.9 (6)
C (26B) - C (27B) - C (28B)	120.6 (6)	C (23B) - C (28B) - C (27B)	121.0 (6)
C (34B) - C (29B) - C (30B)	118.1 (4)	C (34B) - C (29B) - C (11B)	117.6 (4)
C (30B) - C (29B) - C (11B)	124.2 (4)	C (29B) - C (30B) - C (31B)	119.3 (5)
C (32B) - C (31B) - C (30B)	122.1 (6)	C (31B) - C (32B) - C (33B)	119.9 (5)
C (32B) - C (33B) - C (34B)	118.7 (6)	C (29B) - C (34B) - C (33B)	121.9 (5)
O (7) - C (35) - C (36)	117.6 (8)		

Table 4. Anisotropic displacement parameters [$Å^2$ x 10^3] for **90**.
The anisotropic displacement factor exponent takes the form:
$-2\pi^2$ [(ha*)$2U_{11}$ + . . . + 2hka*b*U_{12}]

	U11	U22	U33	U23	U13	U12
N (1)	99 (3)	33 (2)	61 (2)	-4 (l)	35 (2)	2 (2)
N (10)	57 (2)	37 (l)	38 (1)	3 (l)	18 (1)	-1 (1)
O (1)	113 (3)	52 (2)	52 (2)	7 (1)	39 (2)	-8 (2)
O (2)	71 (21	63 (2)	47 (l)	-9 (1)	9 (1)	-1 (1)
O (3)	65 (2)	78 (2)	161 (4)	12 (2)	46 (2)	-6 (2)
O (4)	78 (2)	67 (2)	106 (3)	7 (2)	30 (2)	20 (21
O (5)	72 (2)	64 (2)	134 (3)	-9 (2)	51 (2)	-11 (2)
O (6)	67 (2)	44 (l)	44 (l)	-4 (l)	19 (1)	0 (1)
C (2)	74 (3)	41 (2)	4412)	3 (2)	20 (21	-5 (2)
C (3D)	58 (2)	46 (2)	44 (2)	l (2)	22 (2)	3 (2)
C (3)	63 (2)	38 (2)	40 (2)	l (l)	22 (2)	0 (2)
C (4)	79 (3)	50 (2)	56 (2)	3 (2)	37 (2)	-1 (2)
C (5)	99 (3)	79 (3)	64 (3)	0 (2)	51 (3)	-4 (3)
C (6)	99 (4)	92 (4)	72 (3)	-18 (3)	54 (3)	-7 (3)
C (7D)	68 (2)	49 (2)	48 (2)	-2 (2)	27 (2)	-1 (2)
C (7)	92 (3)	57 (3)	70 (3)	-13 (2)	40 (3)	5 (2)
C (8)	59 (2)	38 (2)	40 (2)	0 (1)	17 (2)	l (2)
C (9)	52 (2)	36 (2)	44 (2)	3 (1)	18 (2)	-6 (1)
C (11)	50 (2)	39 (2)	44 (2)	3 (l)	16 (2)	-2 (2)
C (12)	55 (2)	40 (2)	51 (2)	0 (2)	21 (2)	-3 (2)
C (13)	60 (2)	35 (2)	47 (2)	-2 (2)	18 (2)	-3 (2)
C (14)	62 (3)	62 (3)	68 (3)	-5 (2)	13 (2)	10 (2)
C (15)	78 (4)	112 (5)	182 (8)	-3 (5)	35 (5)	40 (4)
C (16)	116 (7)	297 (16)	194 (10)	-37 (11)	91 (7)	39 (8)
C (17)	59 (2)	40 (2)	39 (2)	-3 (l)	24 (2)	-6 (2)
C (18)	62 (2)	57 (2)	43 (2)	-9 (2)	20 (2)	-12 (2)
C (19)	64 (2)	50 (2)	68 (2)	-8 (2)	21 (2)	-17 (2)
C (20)	99 (4)	82 (3)	110 (4)	8 (3)	48 (3)	-32 (3)
C (21)	84 (4)	139 (6)	90 (4)	-23 (4)	16 (3)	-42 (4)
C (22)	118 (6)	120 (6)	372 (16)	-33 (8)	162 (9)	-13 (5)
C (23)	58 (2)	43 (2)	69 (2)	3 (2)	33 (2)	-4 (2)
C (24)	59 (3)	88 (3)	86 (3)	-15 (3)	21 (2)	5 (2)
C (25)	60 (3)	85 (3)	116 (4)	-10 (3)	23 (3)	8 (3)
C (26)	67 (3)	64 (3)	142 (5)	7 (3)	53 (3)	14 (2)
C (27)	96 (4)	55 (3)	109 (4)	7 (3)	60 (3)	14 (2)
C (28)	8l (3)	55 (2)	67 (2)	7 (2)	36 (2)	5 (2)
C (29)	46 (2)	44 (2)	44 (2)	10 (2)	13 (2)	2 (1)
C (30)	66 (2)	47 (2)	72 (2)	4 (2)	34 (2)	0 (2)
C (31)	76 (3)	42 (2)	107 (4)	11 (2)	47 (3)	3 (2)
C (32)	65 (3)	69 (3)	91 (3)	40 (3)	33 (2)	3 (2)
C (33)	71 (3)	92 (4)	64 (3)	19 (2)	30 (2)	-4 (2)
C (34)	65 (2)	60 (2)	53 (2)	11 (2)	23 (2)	-2 (2)
N (1B)	85 (2)	45 (2)	61 (2)	-10 (2)	25 (2)	-15 (2)
N (10B)	55 (2)	39 (1)	42 (1)	-3 (l)	18 (l)	-1 (1)
O (1B)	89 (2)	64 (2)	43 (l)	-7 (l)	26 (1)	0 (2)
O (2B)	69 (2)	44 (1)	90 (2)	15 (l)	28 (2)	9 (1)
O (3B)	73 (2)	66 (2)	137 (3)	-26 (2)	47 (2)	8 (2)
O (4B)	56 (2)	62 (2)	93 (2)	2 (2)	17 (2)	0 (1)
O (5B)	73 (2)	114 (6)	93 (2)	-3 (2)	52 (2)	1 (l)

O (6B)	53 (2)	49 (1)	61 (l)	8 (1)	19 (1)	-5 (l)
C (2B)	67 (2)	39 (2)	45 (2)	-1 (2)	21 (2)	2 (2)
C (3B)	54 (2)	38 (2)	43 (2)	-2 (1)	22 (2)	l (1)
C (3C)	61 (2)	42 (2)	49 (2)	8 (2)	23 (2)	2 (2)
C (4B)	82 (3)	58 (21	59 (2)	2 (2)	40 (2)	2 (2)
C (5B)	101 (4)	85 (3)	75 (3)	8 (3)	57 (3)	2 (3)
C (6B)	103 (4)	87 (4)	105 (4)	24 (3)	66 (4)	-3 (3)
C (7C)	70 (2)	43 (2)	64 (2)	6 (2)	27 (2)	-1 (2)
C (7B)	91 (3)	59 (3)	103 (4)	6 (3)	47 (3)	-19 (2)
C (8B)	54 (2)	40 (2)	47 (2)	1 (2)	24 (2)	4 (2)
C (9B)	54 (2)	45 (2)	50 (2)	-3 (2)	27 (2)	2 (2)
C (11B)	53 (2)	46 (2)	49 (2)	0 (2)	20 (2)	2 (2)
C (12B)	53 (2)	47 (2)	48 (2)	l (2)	17 (2)	3 (2)
C (13B)	60 (21	35 (2)	57 (2)	-2 (2)	23 (2)	-2 (2)
C (14B)	56 (2)	44 (2)	71 (2)	3 (2)	21 (2)	l (2)
C (15B)	61 (3)	100 (4)	142 (5)	22 (4)	31 (3)	-1 (3)
C (16B)	100 (5)	197 (10)	223 (10)	47 (9)	80 (6)	22 (6)
C (17B)	56 (2)	38 (2)	42 (2)	l (1)	24 (2)	2 (2)
C (18B)	60 (2)	42 (2)	47 (2)	0 (2)	25 (2)	l (2)
C (19B)	66 (2)	48 (2)	53 (2)	2 (2)	26 (2)	10 (2)
C (20B)	95 (3)	78 (3)	60 (2)	-15 (2)	31 (2)	19 (3)
C (21B)	84 (3)	66 (3)	78 (3)	16 (2)	32 (3)	24 (2)
C (22B)	102 (4)	120 (5)	165 (6)	-13 (5)	91 (5)	5 (4)
C (23B)	55 (2)	55 (2)	61 (2)	-1 (2)	23 (2)	-1 (2)
C (24B)	63 (3)	72 (3)	92 (3)	14 (3)	18 (2)	-3 (2)
C (25B)	61 (3)	109 (4)	109 (4)	27 (4)	16 (3)	8 (3)
C (26B)	55 (3)	127 (6)	164 (7)	34 (5)	2 (4)	-19 (3)
C (27B)	79 (4)	96 (5)	237 (9)	41 (6)	19 (5)	-27 (4)
C (28B)	65 (3)	83 (4)	163 (6)	44 (4)	18 (3)	-12 (3)
C (29B)	50 (2)	63 (21	48 (2)	-7 (2)	18 (2)	-3 (2)
C (30B)	90 (3)	63 (3)	69 (3)	-14 (2)	23 (2)	7 (2)
C (31B)	98 (4)	87 (4)	92 (4)	-38 (3)	29 (3)	9 (3)
C (32B)	67 (3)	151 (6)	76 (3)	-59 (4)	30 (3)	-21 (3)
C (33B)	92 (4)	142 (6)	57 (3)	-20 (3)	35 (3)	-34 (4)
C (34B)	87 (3)	88 (3)	59 (3)	-13 (2)	27 (2)	-24 (3)
O (7)	257 (7)	129 (4)	94 (3)	8 (3)	39 (4)	-95 (5)
O (35)	218 (9)	61 (2)	131 (6)	-12 (5)	70 (7)	3 (7)
O (36)	166 (9)	342 (18)	144 (8)	75 (10)	82 (7)	-2 (10)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for **90**.

H (1A)	843 (3)	705 (2)	-1788 (3)	76
H (4A)	1247 (4)	2929 (2)	-3272 (3)	70
H (5A)	1729 (5)	2438 (3)	-4539 (4)	90
H (6A)	1850 (5)	1324 (3)	-4612 (4)	98
H (7A)	1432 (4)	655 (2)	-3460 (4)	85
H (8A)	1495 (3)	2624 (2)	-231 (3)	56
H (9A)	1494 (3)	3662 (2)	-1454 (3)	53
H (11A)	-1707 (3)	3671 (2)	-2869 (3)	54
H (12A)	-1496 (3)	3549 (2)	-1193 (3)	58
H (15A)	4831 (6)	2324 (4)	-281 (7)	155
H (15B)	4767 (6)	1654 (4)	228 (7)	155
H (16A)	5112 (8)	1502 (7)	-1186 (9)	290
H (16B)	3960 (8)	1877 (7)	-1842 (9)	290
H (16C)	3891 (8)	1208 (7)	-1335 (9)	290
H (17A)	-770 (3)	2597 (2)	-1485 (2)	53
H (18A)	-1340 (3)	2311 (2)	-3649 (3)	65
H (20A)	-3351 (5)	1606 (3)	-2240 (5)	143
H (20B)	-2075 (5)	1834 (3)	-1557 (5)	143
H (20C)	-2280 (5)	1293 (3)	-2394 (5)	143
H (21A)	-3545 (5)	2324 (4)	-4595 (5)	165
H (21B)	-4267 (5)	1919 (4)	-4107 (5)	165
H (21C)	-3216 (5)	1595 (4)	-4280 (5)	165
H (22A)	-4277 (7)	3240 (4)	-2403 (10)	275
H (22B)	-3988 (7)	2526 (4)	-1990 (10)	275
H (22C)	-4745 (7)	2651 (4)	-3160 (10)	275
H (24A)	-3224 (4)	4231 (3)	-2895 (4)	96
H (25A)	-4695 (4)	4965 (3)	-3030 (5)	109
H (26A)	-4504 (5)	5648 (2)	-1680 (5)	105
H (27A)	-2790 (5)	5633 (2)	-208 (5)	97
H (28A)	-1298 (4)	4909 (2)	-75 (3)	79
H (30A)	-435 (4)	5111 (2)	-1779 (3)	72
H (31A)	129 (4)	5940 (2)	-2606 (4)	86
H (32A)	359 (4)	5741 (2)	-4125 (4)	89
H (33A)	18 (4)	4708 (3)	-4837 (4)	90
H (34A)	-511 (4)	3869 (2)	-4004 (3)	72
H (1BA)	-1663 (3)	1892 (2)	908 (3)	78
H (4BA)	-402 (4)	3401 (2)	4063 (3)	75
H (5BA)	-1529 (5)	2810 (3)	4743 (4)	96
H (6BA)	-2575 (5)	1924 (3)	3911 (5)	108
H (7BA)	-2570 (5)	1582 (2)	2348 (5)	98
H (8BA)	-286 (3)	3919 (2)	1216 (3)	55
H (9BA)	386 (3)	4420 (2)	3175 (3)	57
H (11B)	3112 (3)	3447 (2)	4111 (3)	59
H (12B)	3088 (3)	3811 (2)	2596 (3)	60
H (15C)	-3659 (5)	4446 (3)	912 (6)	125
H (15D)	-4127 (5)	3852 (3)	162 (6)	125
H (16D)	-4847 (7)	3825 (6)	1398 (9)	252
H (16E)	-3572 (7)	3812 (6)	2250 (9)	252
H (16F)	-4040 (7)	3219 (6)	1502 (9)	252

H (17B)	1470 (7)	3127 (2)	2110 (3)	53
H (18B)	2046 (3)	2548 (2)	3969 (3)	59
H (18C)	1030 (3)	2143 (2)	3168 (3)	59
H (20D)	2562 (5)	1569 (3)	1674 (3)	116
H (20E)	1691 (5)	2159 (3)	1374 (3)	116
H (20F)	1359 (5)	1513 (3)	1786 (3)	116
H (21D)	3628 (5)	1260 (2)	3521 (4)	114
H (21E)	2451 (5)	1207 (2)	3679 (4)	114
H (21F)	3454 (5)	1663 (2)	4393 (4)	114
H (22D)	4916 (6)	2702 (4)	2850 (6)	175
H (22E)	4030 (6)	2246 (4)	2016 (6)	175
H (22F)	4772 (6)	1976 (4)	3120 (6)	175
H (24B)	5077 (4)	3584 (2)	4407 (4)	96
H (25B)	6988 (5)	3955 (3)	5121 (5)	118
H (26B)	7482 (5)	4973 (4)	4763 (6)	154
H (27B)	6062 (6)	5632 (4)	3647 (8)	181
H (28B)	4136 (5)	5279 (3)	2895 (6)	134
H (30B)	2727 (4)	5168 (2)	4256 (4)	92
H (31B)	2789 (5)	5698 (3)	5734 (5)	114
H (32B)	2954 (5)	5140 (4)	7165 (5)	117
H (33B)	3053 (5)	4006 (4)	7180 (4)	115
H (34B)	2924 (4)	3462 (3)	5695 (3)	94
H (7B)	-1045 (7)	1028 (3)	-193 (4)	254
H (35A)	-1781 (10)	84 (4)	-203 (7)	185
H (35B)	-2730 (10)	577 (4)	-877 (7)	185
H (36A)	-3495 (9)	-50 (8)	133 (8)	316
H (36B)	-3449 (9)	682 (8)	460 (8)	316
H (36C)	-2489 (9)	194 (8)	1147 (8)	316

Appendix 3

X-ray Crystal Structure Data for Compound 94

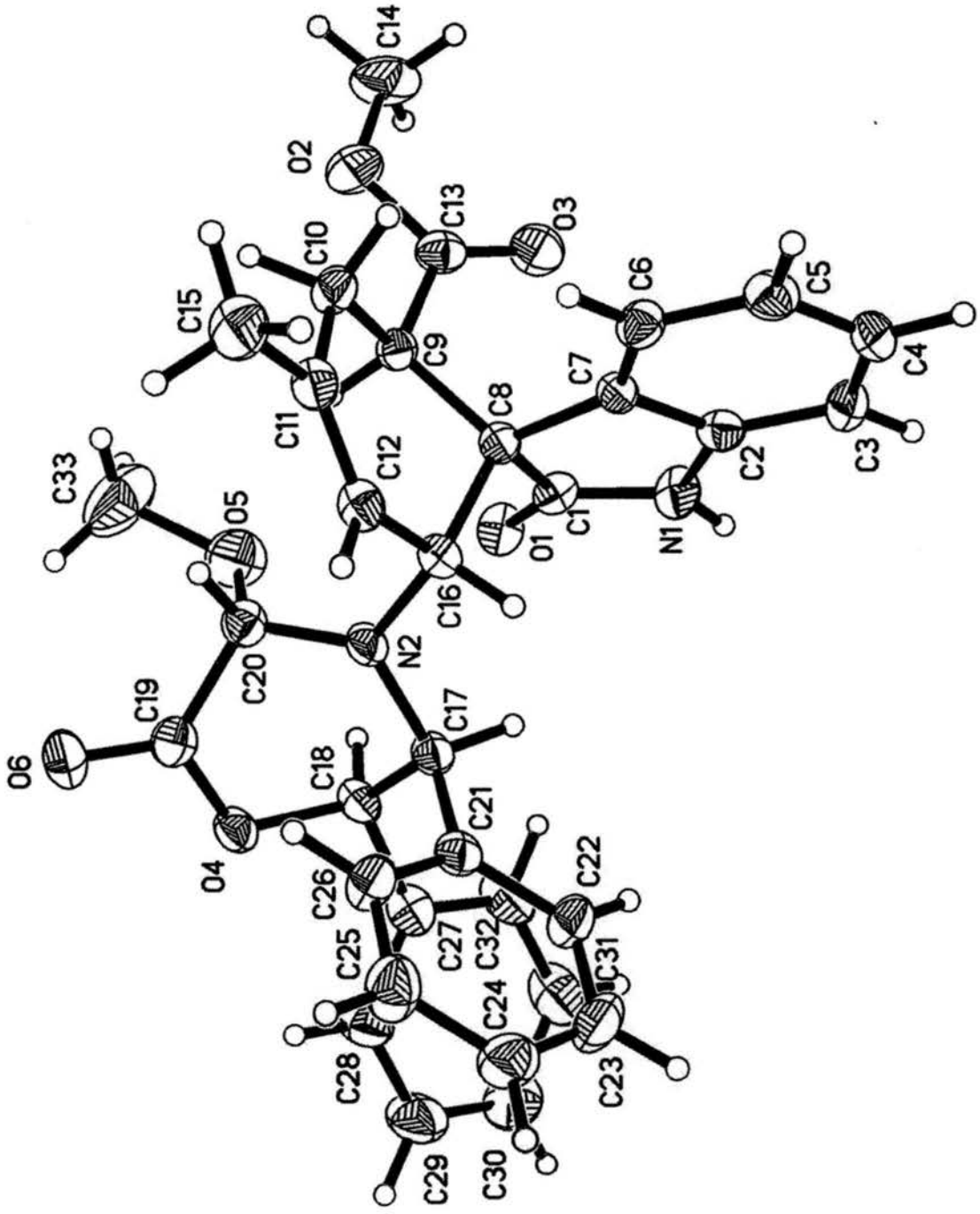

C14
O2
C13
O3
C10
C15
C6
C5
C9
C4
C7
C11
C8
C2
C12
C3
C33
O5
O1
C1
N1
C16
N2
C20
C19
C17
O6
C18
C21
O4
C22
C26
C27
C32
C25
C31
C24
C28
C23
C29
C30

Table 1. Crystal data and structure refinement for **94**.

Identification code	rw11m	
Empirical formula	C33 H32 N2 O6	
Formula weight	552.61	
Temperature	167(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.3761(7) Å	α= 108.640(2)°.
	b = 12.3319(9) Å	β= 97.220(2)°.
	c = 13.1524(10) Å	γ = 97.298(2)°.
Volume	1406.76(18) $Å^3$	
Z	2	
Density (calculated)	1.305 Mg/m^3	
Absorption coefficient	0.090 mm^{-1}	
F(000)	584	
Crystal size	0.08 x 0.20 x 0.42 mm^3	
Theta range for data collection	1.66 to 28.36°.	
Index ranges	-10<=h<=12, -15<=k<=16, -15<=l<=17	
Reflections collected	9233	
Independent reflections	6374 [R(int) = 0.0502]	
Completeness to theta = 28.36°	90.4 %	
Absorption correction	SADABS	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6374 / 0 / 371	
Goodness-of-fit on F^2	0.975	
Final R indices [I>2sigma(I)]	R1 = 0.0766, wR2 = 0.1702	
R indices (all data)	R1 = 0.1640, wR2 = 0.2116	
Extinction coefficient	0.004(2)	
Largest diff. peak and hole	0.514 and -0.510 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for rw11m. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	6923(2)	-38(2)	5575(2)	28(1)
O(2)	8929(3)	-3119(2)	4138(2)	39(1)
O(3)	6686(3)	-2671(2)	3967(2)	37(1)
O(4)	9754(2)	2411(2)	8796(2)	27(1)
O(5)	10068(3)	433(2)	6791(2)	46(1)
O(6)	11619(3)	1633(2)	9252(2)	41(1)
N(1)	4738(3)	-1164(2)	5494(2)	28(1)
N(2)	8206(3)	130(2)	7832(2)	24(1)
C(1)	6215(4)	-871(3)	5730(3)	25(1)
C(2)	4263(4)	-2221(3)	5649(3)	24(1)
C(3)	2849(4)	-2813(3)	5433(3)	29(1)
C(4)	2651(4)	-3855(3)	5658(3)	31(1)
C(5)	3813(4)	-4248(3)	6077(3)	29(1)
C(6)	5218(4)	-3641(3)	6291(3)	26(1)
C(7)	5468(3)	-2610(3)	6068(3)	20(1)
C(8)	6830(3)	-1703(3)	6253(3)	20(1)
C(9)	8140(3)	-2139(3)	5762(3)	22(1)
C(10)	8751(4)	-2990(3)	6266(3)	28(1)
C(11)	8744(4)	-2675(3)	7467(3)	26(1)
C(12)	8150(3)	-1802(3)	8006(3)	25(1)
C(13)	7798(4)	-2666(3)	4532(3)	27(1)
C(14)	8741(5)	-3609(3)	2962(3)	48(1)
C(15)	9403(4)	-3467(3)	8000(3)	38(1)
C(16)	7357(4)	-1051(3)	7513(3)	22(1)
C(17)	7393(4)	1093(3)	8132(3)	25(1)
C(18)	8351(4)	2177(3)	8083(3)	26(1)
C(19)	10453(4)	1499(3)	8687(3)	27(1)
C(20)	9709(4)	356(3)	7823(3)	26(1)
C(21)	6908(4)	1275(3)	9229(3)	25(1)
C(22)	5562(4)	1601(3)	9380(3)	34(1)
C(23)	5134(4)	1815(3)	10383(4)	41(1)

C(24)	6043(4)	1729(3)	11251(3)	39(1)
C(25)	7351(4)	1378(3)	11103(3)	40(1)
C(26)	7775(4)	1134(3)	10087(3)	32(1)
C(27)	7675(4)	3253(3)	8423(3)	30(1)
C(28)	8110(4)	4109(3)	9448(3)	37(1)
C(29)	7425(5)	5064(3)	9748(4)	48(1)
C(30)	6295(5)	5182(3)	9039(4)	50(1)
C(31)	5857(5)	4356(3)	8030(4)	49(1)
C(32)	6549(4)	3392(3)	7709(3)	41(1)
C(33)	11556(5)	256(4)	6625(5)	68(2)

Table 3. Bond lengths [Å] and angles [°] for **94**.

O(1)-C(1)	1.238(4)	C(8)-C(16)	1.574(4)
O(2)-C(13)	1.344(4)	C(9)-C(13)	1.512(5)
O(2)-C(14)	1.447(4)	C(9)-C(10)	1.540(4)
O(3)-C(13)	1.200(4)	C(10)-C(11)	1.501(5)
O(4)-C(19)	1.351(4)	C(11)-C(12)	1.317(5)
O(4)-C(18)	1.454(4)	C(11)-C(15)	1.520(4)
O(5)-C(20)	1.468(4)	C(12)-C(16)	1.507(4)
O(5)-C(33)	1.472(5)	C(17)-C(21)	1.524(5)
O(6)-C(19)	1.202(4)	C(17)-C(18)	1.537(4)
N(1)-C(1)	1.355(4)	C(18)-C(27)	1.506(5)
N(1)-C(2)	1.409(4)	C(19)-C(20)	1.514(5)
N(2)-C(20)	1.403(4)	C(21)-C(26)	1.376(5)
N(2)-C(17)	1.467(4)	C(21)-C(22)	1.390(5)
N(2)-C(16)	1.471(4)	C(22)-C(23)	1.381(5)
C(1)-C(8)	1.537(4)	C(23)-C(24)	1.378(5)
C(2)-C(3)	1.378(4)	C(24)-C(25)	1.367(5)
C(2)-C(7)	1.401(4)	C(25)-C(26)	1.393(5)
C(3)-C(4)	1.403(5)	C(27)-C(32)	1.389(5)
C(4)-C(5)	1.371(5)	C(27)-C(28)	1.393(5)
C(5)-C(6)	1.377(5)	C(28)-C(29)	1.383(5)
C(6)-C(7)	1.391(4)	C(29)-C(30)	1.376(6)
C(7)-C(8)	1.526(4)	C(30)-C(31)	1.363(6)
C(8)-C(9)	1.536(4)	C(31)-C(32)	1.398(5)

C(13)-O(2)-C(14)	115.6(3)	C(3)-C(2)-C(7)	123.4(3)
C(19)-O(4)-C(18)	116.1(2)	C(3)-C(2)-N(1)	127.2(3)
C(20)-O(5)-C(33)	114.5(3)	C(7)-C(2)-N(1)	109.5(3)
C(1)-N(1)-C(2)	111.1(3)	C(2)-C(3)-C(4)	116.5(3)
C(20)-N(2)-C(17)	120.2(3)	C(5)-C(4)-C(3)	121.1(3)
C(20)-N(2)-C(16)	123.1(3)	C(4)-C(5)-C(6)	121.5(3)
C(17)-N(2)-C(16)	116.6(2)	C(5)-C(6)-C(7)	119.4(3)
O(1)-C(1)-N(1)	124.4(3)	C(6)-C(7)-C(2)	118.1(3)
O(1)-C(1)-C(8)	126.6(3)	C(6)-C(7)-C(8)	133.3(3)
N(1)-C(1)-C(8)	109.0(3)	C(2)-C(7)-C(8)	108.4(3)

C(7)-C(8)-C(9)	116.9(3)	O(4)-C(18)-C(17)	109.9(3)
C(7)-C(8)-C(1)	101.2(2)	C(27)-C(18)-C(17)	113.2(3)
C(9)-C(8)-C(1)	111.4(3)	O(6)-C(19)-O(4)	119.5(3)
C(7)-C(8)-C(16)	109.4(2)	O(6)-C(19)-C(20)	123.9(3)
C(9)-C(8)-C(16)	108.2(3)	O(4)-C(19)-C(20)	116.6(3)
C(1)-C(8)-C(16)	109.6(2)	N(2)-C(20)-O(5)	114.4(3)
C(13)-C(9)-C(8)	112.6(3)	N(2)-C(20)-C(19)	111.8(3)
C(13)-C(9)-C(10)	110.9(3)	O(5)-C(20)-C(19)	105.5(3)
C(8)-C(9)-C(10)	111.9(3)	C(26)-C(21)-C(22)	118.6(3)
C(11)-C(10)-C(9)	114.0(3)	C(26)-C(21)-C(17)	121.7(3)
C(12)-C(11)-C(10)	122.1(3)	C(22)-C(21)-C(17)	119.7(3)
C(12)-C(11)-C(15)	122.5(3)	C(23)-C(22)-C(21)	120.3(4)
C(10)-C(11)-C(15)	115.3(3)	C(24)-C(23)-C(22)	120.5(4)
C(11)-C(12)-C(16)	125.5(3)	C(25)-C(24)-C(23)	119.5(4)
O(3)-C(13)-O(2)	123.6(3)	C(24)-C(25)-C(26)	120.2(4)
O(3)-C(13)-C(9)	125.5(3)	C(21)-C(26)-C(25)	120.7(3)
O(2)-C(13)-C(9)	110.9(3)	C(32)-C(27)-C(28)	118.1(3)
N(2)-C(16)-C(12)	111.5(3)	C(32)-C(27)-C(18)	119.4(3)
N(2)-C(16)-C(8)	116.0(2)	C(28)-C(27)-C(18)	122.4(3)
C(12)-C(16)-C(8)	109.9(3)	C(29)-C(28)-C(27)	120.8(4)
N(2)-C(17)-C(21)	112.6(3)	C(30)-C(29)-C(28)	120.5(4)
N(2)-C(17)-C(18)	107.4(3)	C(31)-C(30)-C(29)	119.6(4)
C(21)-C(17)-C(18)	113.3(3)	C(30)-C(31)-C(32)	120.7(4)
O(4)-C(18)-C(27)	107.8(3)	C(27)-C(32)-C(31)	120.3(4)

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for rw11m. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$ for **94**.

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	29(1)	28(1)	36(2)	20(1)	7(1)	5(1)
O(2)	40(2)	44(2)	35(2)	8(1)	18(1)	12(1)
O(3)	40(2)	42(2)	27(1)	9(1)	4(1)	8(1)
O(4)	25(1)	21(1)	30(1)	6(1)	0(1)	-1(1)
O(5)	45(2)	46(2)	47(2)	13(1)	16(1)	3(1)
O(6)	27(2)	40(2)	47(2)	6(1)	-6(1)	4(1)
N(1)	26(2)	27(2)	36(2)	19(1)	1(1)	10(1)
N(2)	22(2)	17(1)	30(2)	6(1)	0(1)	1(1)
C(1)	26(2)	24(2)	26(2)	9(1)	7(2)	8(2)
C(2)	28(2)	23(2)	21(2)	6(1)	4(1)	7(2)
C(3)	20(2)	31(2)	37(2)	10(2)	3(2)	7(2)
C(4)	23(2)	22(2)	42(2)	6(2)	6(2)	-2(2)
C(5)	32(2)	21(2)	35(2)	11(2)	9(2)	4(2)
C(6)	25(2)	24(2)	30(2)	11(2)	6(2)	8(2)
C(7)	20(2)	20(2)	20(2)	6(1)	3(1)	2(1)
C(8)	21(2)	18(2)	22(2)	8(1)	2(1)	4(1)
C(9)	20(2)	21(2)	24(2)	8(1)	6(1)	2(1)
C(10)	24(2)	27(2)	33(2)	11(2)	4(2)	9(2)
C(11)	24(2)	23(2)	33(2)	13(2)	1(2)	3(2)
C(12)	26(2)	24(2)	27(2)	12(2)	-1(2)	4(2)
C(13)	33(2)	22(2)	28(2)	9(2)	9(2)	3(2)
C(14)	63(3)	46(2)	34(2)	3(2)	28(2)	8(2)
C(15)	38(2)	33(2)	46(3)	19(2)	-2(2)	11(2)
C(16)	23(2)	21(2)	21(2)	6(1)	2(1)	1(1)
C(17)	24(2)	17(2)	32(2)	6(1)	-2(2)	4(1)
C(18)	26(2)	21(2)	26(2)	7(1)	-4(2)	1(2)
C(19)	25(2)	29(2)	29(2)	13(2)	7(2)	4(2)
C(20)	24(2)	25(2)	29(2)	10(2)	5(2)	3(2)
C(21)	26(2)	17(2)	30(2)	6(1)	3(2)	2(1)
C(22)	26(2)	34(2)	43(2)	11(2)	6(2)	12(2)
C(23)	27(2)	40(2)	57(3)	13(2)	16(2)	12(2)

C(24)	38(2)	40(2)	35(2)	8(2)	15(2)	3(2)
C(25)	33(2)	55(3)	36(2)	20(2)	5(2)	9(2)
C(26)	27(2)	39(2)	33(2)	15(2)	8(2)	11(2)
C(27)	33(2)	25(2)	33(2)	15(2)	0(2)	3(2)
C(28)	46(2)	28(2)	32(2)	7(2)	-2(2)	7(2)
C(29)	56(3)	28(2)	50(3)	2(2)	0(2)	10(2)
C(30)	49(3)	23(2)	77(3)	15(2)	11(2)	13(2)
C(31)	49(3)	32(2)	59(3)	17(2)	-15(2)	9(2)
C(32)	46(3)	27(2)	42(2)	10(2)	-12(2)	4(2)
C(33)	51(3)	61(3)	107(5)	35(3)	44(3)	20(3)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **94**.

	x	y	z	U(eq)
H(1A)	4154	-748	5273	33
H(3A)	2050	-2531	5147	35
H(4A)	1696	-4295	5519	37
H(5A)	3646	-4956	6222	35
H(6A)	6008	-3923	6588	31
H(9A)	8931	-1445	5949	26
H(10A)	8165	-3777	5885	33
H(10B)	9766	-3021	6140	33
H(12A)	8228	-1633	8770	30
H(14A)	9618	-3915	2756	73
H(14B)	8586	-3004	2647	73
H(14C)	7893	-4241	2688	73
H(15A)	9784	-4056	7459	57
H(15B)	8650	-3851	8286	57
H(15C)	10201	-3005	8598	57
H(16A)	6452	-968	7840	26
H(17A)	6495	897	7564	30
H(18A)	8521	2023	7318	31
H(20A)	10157	-283	7975	31
H(22A)	4934	1676	8790	41
H(23A)	4206	2024	10474	49
H(24A)	5764	1912	11948	46
H(25A)	7972	1299	11695	48
H(26A)	8671	868	9986	38
H(28A)	8886	4037	9947	44
H(29A)	7737	5642	10449	58
H(30A)	5822	5836	9250	60
H(31A)	5074	4435	7541	58
H(32A)	6247	2831	6998	49
H(33A)	11708	321	5922	102
H(33B)	11682	-519	6631	102
H(33C)	12268	848	7212	102

Appendix 4

Publications

The Asymmetric Total Synthesis of (+)- and (−)-Spirotryprostatin B

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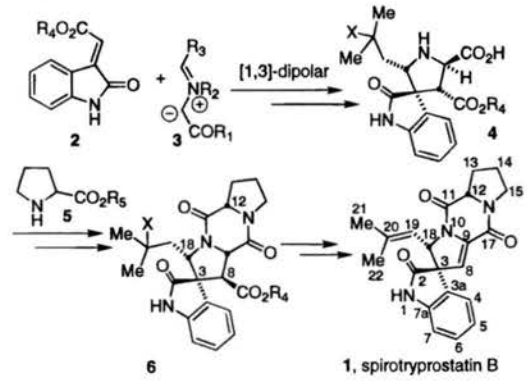
The spirotryprostatins,[1] tryprostatins,[2] and cyclotryprostatins[3] represent a promising class of antimitotic arrest agents. Isolated from the fermentation broth of *Aspergillus fumigatus*, spirotryprostatin B has been shown to completely inhibit the G2/M progression of mammalian tsFT210 cells at concentrations over 12.5 μg/mL. While the cyclotryprostatins and tryprostatins have been shown to act by affecting microtubule assembly,[4] much less is known about the spirotryprostatins due to the limited availability of these compounds. Fermentation of 400 L of culture medium yielded 1 mg of spirotryprostatin A and 11 mg of spirotryprostatin B (**1**), respectively.

Although numerous naturally occurring prenylated alkaloids derived from proline and tryptophan are known,[5] the unique spirooxindole ring system distinguishes the spirotryprostatins. This unique structural array along with the limited quantities and the interesting biological activity render the spirotryprostatins attractive synthetic targets. Recently, the total synthesis of spirotryprostatin A was completed using the classical halohydrin to oxindole spiro-ring-forming contraction sequence.[6] We have directed our research interests toward the total synthesis of the more biologically active congener, spirotryprostatin B, using an entirely new strategy to access this type of amino acid substructure.

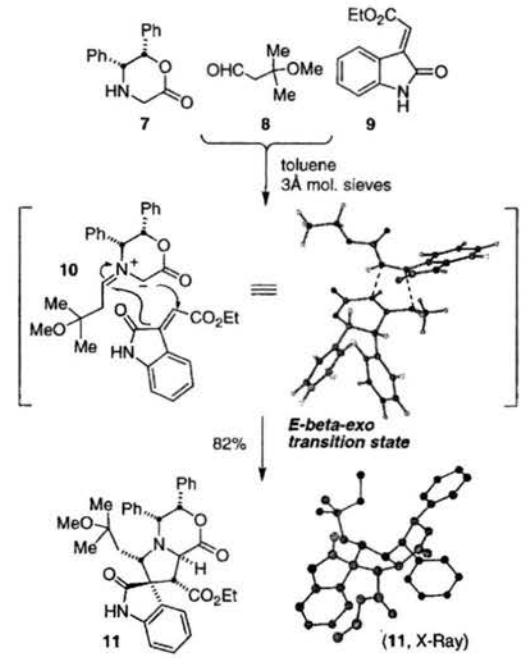
In contemplating the synthesis of spirotryprostatin B, it was envisioned that the core pyrrolidine ring could be formed through an asymmetric [1,3]-dipolar cycloaddition reaction, (Scheme 1).[7] Reaction of a chiral azomethine ylide of the type **3** with an oxindolylideneacetate **2** could set four contiguous stereogenic centers. Of these, the quaternary spirooxindolyl center at C3 and the adjacent C18 center would have to be controlled in a relative and absolute sense culminating in amino acid **4**. Standard peptide coupling protocol with protected proline **5** followed by cyclization would generate the diketopiperazine **6**. Completion of the synthesis mandates unmasking of the prenyl side-chain followed by oxidative decarboxylation. We record here the successful execution of this strategy.

We have previously reported that the addition of an aldehyde to 5,6-diphenylmorpholin-2-one (**7**) generates a mixture of the corresponding *E*- and *Z*-azomethine ylides with a preference for the *E*-ylide in the cases of sterically demanding aldehydes.[8] Dipolar cycloadditions of ylides generated from this system proceed with a high degree of endo selectivity to give substituted pyrrolidines. On the basis of this premise that a bulky isoprene aldehyde progenitor would favor the *E*-ylide geometry, the relative stereochemistry at the isoprene-bearing carbon (C18) of spirotryprostatin seemed attainable. However, it was more difficult to predict the regio- and stereochemical course at the C3 and consequently the C8 positions. Previous reports with azomethine ylides and oxindolylideneacetate dipolarophiles related to **2** suggested that the undesired regiochemistry may result from this type of cycloaddition.[9] The reaction of oxazinone[8] **7** with aldehyde **8**[10] and oxindole **9**[11] in toluene at room temperature in the presence of 3 Å mol sieves, however, afforded cycloadduct **11** in 82% yield.

Scheme 1



Scheme 2



(1) (a) Cui, C. B.; Kakeya, H.; Osada, H. *J. Antibiot.* **1996**, *49*, 832−835. (b) Cui, C. B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, *51*, 12651−12666.

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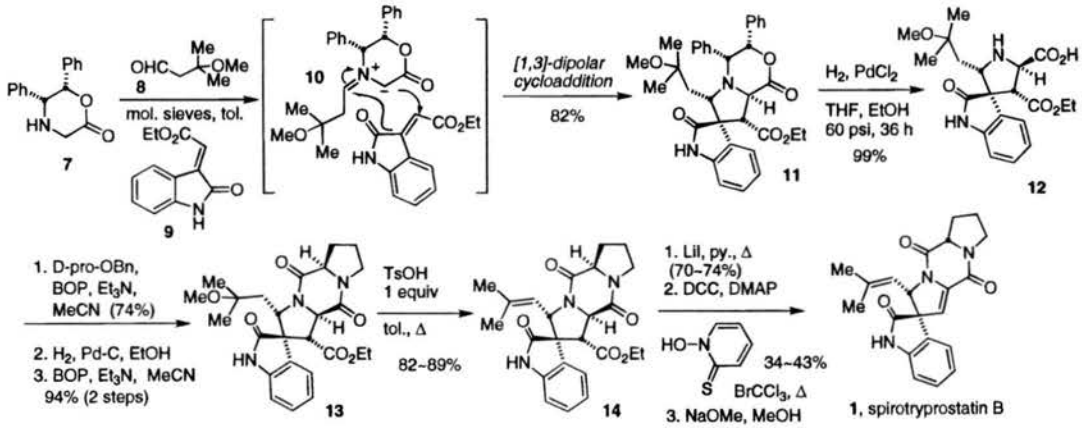
10.1021/ja001133n CCC: $19.00
Published on Web 05/23/2000

Scheme 3



The relative and absolute stereochemistry of this substance was firmly secured through single-crystal X-ray analysis (see Supporting Information). The dipolar cycloaddition reaction of azomethine ylide **10** therefore must proceed via the *E-β-exo* transition state (shown in Scheme 2).[12] This reaction, which sets four contiguous stereogenic centers, constructs the entire prenylated tryptophyl moiety of spirotryprostatin B in a single, simple operation.

With this key intermediate in hand, the synthesis of spirotryprostatin B required coupling of the spriooxindole amino acid with proline, and installation of the two olefinic units (Scheme 3). Thus, reductive cleavage of bibenzyl from oxazinone **11** proceeded in essentially quantitative yield affording the amino acid **12**. Coupling with D-proline benzyl ester (BOP reagent, MeCN, triethylamine, 74%) furnished the requisite dipeptide.[13] It is interesting to note that the steric bulk of the environment around the amino group of **12** obviated the need for a protecting group during the peptide coupling procedure and the free amino acid **12** was directly and effectively used in the reaction. Deprotection of the benzyl ester under standard conditions followed by BOP-mediated cyclization generated the diketopiperazine **13** in 94% yield over 2 steps.

Several strategies were examined for the installation of the two olefinic units that had to be judiciously sequenced with the planned oxidative decarboxylation. Ultimately, it was found that installation of the isoprenyl unsaturation could be accomplished by subjecting **13** to dehydrating conditions in the presence of TsOH in refluxing toluene yielding **14** in 82−89% yield *without* the production of double bond isomers. It should be noted that hydrolysis of the ethyl ester of **14** under standard conditions (LiOH in THF/MeOH/H_2O) failed to give any of the desired product. We found that the use of LiI in refluxing pyridine produced the desired carboxylic acid in 74% yield.[14] The final oxidative decarboxylation proved problematic under a range of Kochi-type conditions ($Pb(OAc)_4$[15] or iodosobenzene diacetate[16]). After extensive experimentation, we found that a modified Hunsdiecker reaction using conditions developed by Barton et al.[17] gave 12-*epi*-spirotryprostatin B in 34−43% yield. This substance was then epimerized with NaOMe in MeOH to give a 2:1 ratio of **1** to 12-*epi*-spirotryprostatin B that were easily separable by silica gel chromatography. The synthetic (−)-spirotryprostatin B (**1**) spectra were identical with the ^{1}H NMR, ^{13}C NMR, IR, and HR-EI-MS spectra of the natural product kindly provided by Dr. Hiroyuki Osada.[1] With use of the antipode of **7**, (+)-*ent*-spirotryprostatin B was prepared in like manner (see data in Supporting Information).

In summary, the application of a stereochemically distinct asymmetric 1,3 dipolar cycloaddition provides access to both antipodes of spirotryprostatin B in an efficient nine-step sequence. This approach appears well-suited to preparing the simpler congener spirotryprostatin A, and several analogues that may prove useful in defining the antimitotic properties of this class of unique spirooxindole alkaloids and studies toward those objectives are in progress in these laboratories.[18]

Acknowledgment. Financial support is gratefully acknowledged from the National Science Foundation (CHE9731947). Dr. Hiroyuki Osada of the Riken Institute, Japan, is acknowledged for providing detailed spectra of natural spirotryprostatin B.

Supporting Information Available: Complete spectroscopic data for all new compounds including details of the X-ray structure determination for compound **11** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA001133N

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(10) Aldehyde **8** is obtained from inexpensive, commercially available 3-methoxy-3-methyl-1-butanol (Aldrich Chemical Co.) by Swern oxidation in 89% yield.

(11) The unsaturated oxindole **10** is readily prepared from isatin (Aldrich Chemical Co.) by condensation with $(Ph)_3PCHCO_2Et$ in refluxing diglyme in 84% yield.

(12) "*β*" refers to the approach of the dipolarophile from the top face as drawn in Scheme 2.

(13) The yield for the conversion of **12** to the L-proline isomer corresponding to **13** was 52% and this diminished yield appears to reflect the thermodynamic instability of forming the corresponding *trans*-diketopiperazine.

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(18) Since submission of our paper, we have learned that Profs. Overman and Danishefsky have also independently achieved the synthesis of spirotryprostatin B; we thank both Profs. Danishefsky and Overman for making us aware of their work prior to publication.

Pergamon

Tetrahedron 58 (2002) 6311–6322

TETRAHEDRON

Asymmetric, stereocontrolled total synthesis of (+) and (−)-spirotryprostatin B via a diastereoselective azomethine ylide [1,3]-dipolar cycloaddition reaction

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Received 21 March 2002; accepted 22 April 2002

The authors wish to dedicate this paper to the myriad of impressive accomplishments of Professor Yoshito Kishi of Harvard University and in recognition of his receiving the Tetrahedron Prize

Abstract—The asymmetric, stereocontrolled total syntheses of (+) and (−)-spirotryprostatin B (**2**) are described. Formation of the core pyrrolidine ring was accomplished via a diastereoselective asymmetric [1,3]-dipolar cycloaddition reaction. Addition of 3-methoxy-3-methylbutanal to (5*R*,6*S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one generated an azomethine ylide that reacted with ethyl oxindolylidene acetate to furnish the desired cycloadduct (**11**) that possessed the correct relative and absolute stereochemistry of natural spirotryprostatin B. The key dipolar cycloaddition reaction sets four contiguous stereogenic centers. Reductive cleavage of the oxazinone generated the spiro-oxindole pyrrolidine (**19**) that was coupled to D-proline benzyl ester and cyclized to the pentacyclic diketopiperazine **22**. A Barton-modified Hunsdiecker protocol effected oxidative decarboxylation to yield 12-*epi*-spirotryprostatin B (**30**). Thermodynamic epimerization of the D-proline stereogenic center with sodium methoxide yielded spirotryprostatin B as the major product. The antipode of the natural product, ent-spirotryprostatin B, was prepared from (5*S*,6*R*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one. Several synthetic intermediates and spirotryprostatin analogs were tested for their activity as G2/M phase cell cycle inhibitors and microtuble assembly against 3Y1 and tsFT210 mammalian cells.

1. Introduction

Elucidating the regulatory machinery of the cell cycle is crucial to understanding how defects in the regulatory mechanism of the cell result in uncontrolled growth and differentiation, such as cancer.[1] Small-molecule natural products are proving invaluable in contemporary studies of cellular probes through their ability to specifically bind target proteins that modulate signal transduction cascades. Numerous examples exist in which the biological function of a particular cellular factor have been investigated through the use such compounds.[2] Therefore, the development of new and specific inhibitors of signal transduction cascade pathways will continue to be extremely important in the understanding of the regulatory mechanism of the cell cycle.

Recently, powerful bioassays have been developed to specifically identify new natural products that inhibit the progression of the cell cycle at distinct phases. Using temperature-sensitive mammalian tsFT210 cells and rat normal fibroblast 3Y1 cells, Osada et al., have exploited these screening technologies to identify a wide array of interesting natural products from the fermentation broth of the fungus *Aspergillus fumigatus* and other microbial sources.[3]

Included in the families of fungal metabolites identified in this manner are the fumitremorgins,[4] the tryprostatins,[5] the cyclotryprostatins[4] and the spirotryprostatins (**1** and **2**, Fig. 1).[6] The primary target of tryprostatin A and cyclotryprostatins A and B are microtubules which induce M-phase specific inhibition and microtubule disassembly.[7] This family of prenylated, cyclo-L-Trp-L-Pro-derived polycyclic alkaloids has received considerable attention recently due to their unique biological activities and interesting chemical structures. These substances have therefore attracted considerable synthetic attention and individual total syntheses of each representative class have been reported.[8–10]

The tryprostatin family of secondary metabolites are the consequence of several modes of isoprenylation of the tryptophan moiety of the simple cyclic dipeptide progenitor cyclo-L-Trp-L-Pro.[11] The structurally most interesting and complex members of this family are the spirotryprostatins A and B which, curiously display among the weakest

Keywords: cycloaddition reaction; azomethine; aldehydes.
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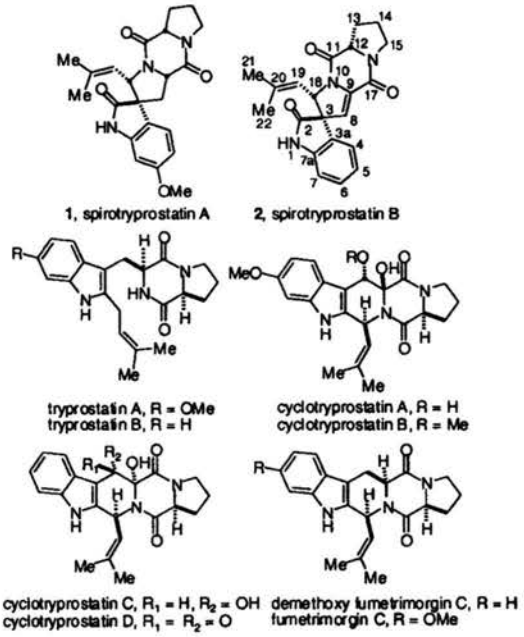
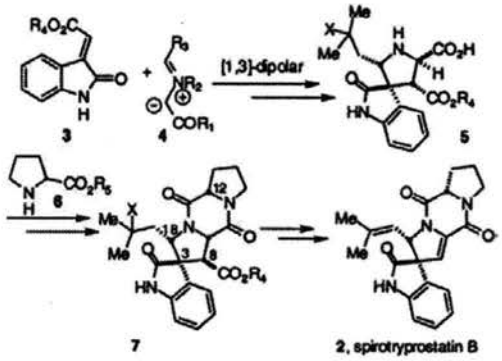


Figure 1. Structures of the spirotryprostatins, tryprostatins, cyclotryprostatins and fumitremorgins.

biological activity of this family of cell cycle inhibitors. Isolated in 1996, from *A. fumigatus*, spirotryprostatin A (**1**) and spirotryprostatin B (**2**) were shown to completely inhibit the progression of cells at concentrations greater than 253 and 34.4 μM, respectively. Despite their relatively modest biological activity relative to other members of this family, the spirotryprostatins have nonetheless garnered the most attention due to their intriguing molecular structures. The detailed mechanism of action by which these substances inhibit microtubule assembly is presently not known and studies to discover the target of these natural products have been hampered by the small quantities of these substances that can be conveniently isolated from the producing organism. Herein, we present a full account of our efforts towards the total synthesis of both antipodes of spirotryprostatin B and analogs.[12]

2. Results and discussion

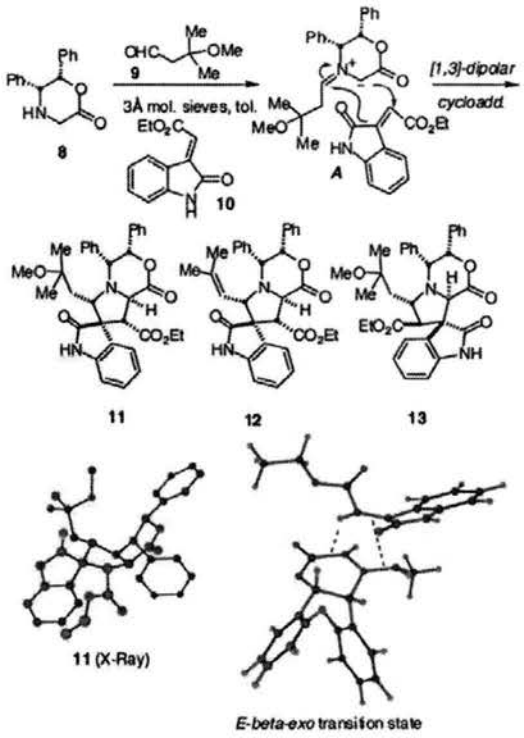
At the outset, we focused on devising an efficient and stereocontrolled method to construct the core spirooxindole-containing pyrrolidine ring as the backbone to our synthetic strategy as shown Scheme 1. It was envisioned that an asymmetric [1,3]-dipolar cycloaddition between a chiral azomethine ylide of the general type **4** and an oxindolylidene acetate (**3**) could, in both a relative and absolute sense, generate the desired spiro-amino acid **5**. If successful, the reaction would generate two of the three necessary stereogenic centers contained in the natural product. Coupling with a suitable proline derivative (**6**) followed by cyclization would yield the diketopiperazine **7**.



Scheme 1. General synthetic plan for the synthesis of **2**.

With the construction of the desired framework represented as in the pentacyclic substance **7**, completion of the synthesis would mandate judiciously timed oxidative decarboxylation and installation of the isoprene-derived unsaturation via elaboration of the pentacyclic substance **7**.

The utility of [1,3]-dipolar cycloadditions is a well-established synthetic method for the formation of variously substituted pyrrolidine rings.[13] Numerous methods exist, including reaction of azomethine ylides with oxindolylidene acetate dipolarophiles, for the construction of spirooxindole systems related to that present in **1** and **2**.[14] However, the literature contains conflicting evidence as to the regio- and diastereochemical outcome of such reactions.



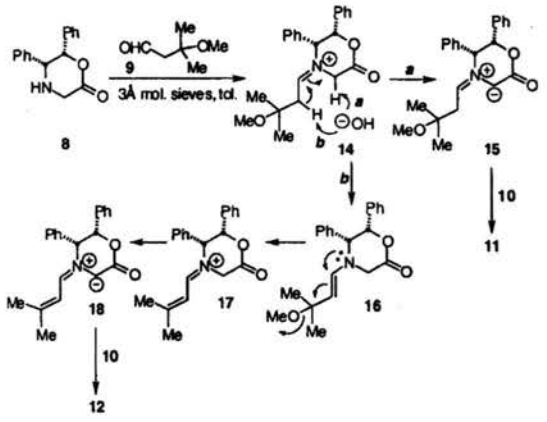
Scheme 2.

Azomethine ylides derived from our diphenyl oxazinone-based glycine template,[15] and related chiral glycine-based azomethine ylide equivalents,[16] reveal that the regio- and stereochemistry of the resulting cycloadducts are dependent upon both the nature of the aldehyde and the dipolarophile. While simple symmetrical alkenyl dipolarophiles (i.e. dimethylmaleate) usually proceed with a high degree of *endo*-selectivity, there are few studies that address the regiochemical aspects of asymmetrically substituted dipolarophiles. It was therefore difficult to predict if the amide or the ester moiety of the oxindolylidene acetate (**3**) would dominate in directing the facial approach of the dipole. In this particular instance, there are thus eight possible diastereomeric transition state structures, and only one of which culminates in the desired spirotryprostatin stereostructure.

With respect to the relative stereochemistry of the prenyl side-chain, the reaction was expected to be diastereoselective in the desired sense since, earlier studies in our laboratories suggested that bulky aliphatic aldehydes preferentially form the *E*-ylide.[15] Assuming that the *E*-ylide geometry would dominate in the present case, four possible diastereomers could be reasonably expected to result from the planned cycloaddition. As shown in Scheme 2, reaction of the azomethine ylide derived from oxazinone **8**[17] and aldehyde **9**[18] with ethyl oxindolylidene acetate **10**[19] in the presence of molecular sieves in hot toluene, resulted in the formation of two cycloadducts **11** and **12** in a 1:2 ratio and 86% combined yield. We were pleased to observe that this initial set of reaction conditions indeed afforded the desired cycloadduct as evidenced by ^1H, ^{13}C NMR and nOe experiments.

The relative and absolute stereochemistry of the desired cycloadduct **11** was further secured through single-crystal X-ray analysis as depicted in Scheme 2. This result suggested that approach of the dipolarophile to the azomethine ylide occurs with the carboethoxy group being positioned opposite to the bulky phenyl groups in an *exo*-fashion. The reaction must therefore proceed via an *E*-beta-*exo* transition state[20] and constructs the entire prenylated tryptophyl moiety of spirotryprostatin B in a single, simple operation. However, the yield was far from ideal since **11** was isolated as a 1:2 mixture along with **12**, which results from the elimination of methanol from the desired cycloadduct. Additionally, a small amount of a third product **13** was produced and confirmed to be the reversed regio- and stereoisomer of the desired cycloadduct. Therefore, additional effort was directed at shifting the ratio of cycloadducts towards compound **11**.

We had not foreseen the possible loss of methanol from the aldehyde progenitor as these conditions had heretofore proven to be very mild and tolerated a wide range of aromatic and aliphatic aldehydes.[21] It was not clear whether the elimination was occurring during the reaction or after formation of the cycloadduct. Re-subjecting **11** to refluxing toluene in the presence of molecular sieves did not afford any of the eliminated cycloadduct **12** suggesting that a distinct azomethine ylide was being formed in situ and a proposed mechanism for the formation of **12** is illustrated in Scheme 3.

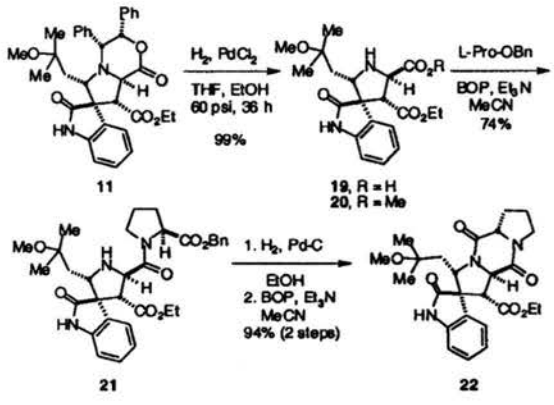


Scheme 3. Mechanism proposed for partitioning of **14** to cycloadducts **11** and **12**.

Addition of the aldehyde **9** to oxazinone **8** should initially generate the salt **14** which can then be deprotonated α- to the lactone carbonyl or β- to the nitrogen atom to give the ylide **15** or the eneamine **16**, respectively. Dipole **15** can then condense with ethyl oxindolylidene acetate (**10**) to generate the desired cycloadduct **11**. If eneamine **16** is formed, then under the thermal conditions of the reaction, nitrogen-assisted extrusion of methoxide furnishes the thermodynamically more stable (relative **14**) conjugated iminium ion species **17**.

Deprotonation α- to the carbonyl would then generate the azomethine ylide **18** that can suffer cycloaddition to yield **12**. To minimize formation of the undesired cycloadduct, the reaction was performed at 60°C instead of at reflux temperature, improving the yield of **11** to 82% with only 6% of **12** being formed.

With the key spiro-tetracyclic intermediate (**11**) in hand, focus shifted to construction of the diketopiperazine as detailed in Scheme 4. Reductive cleavage of the chiral auxiliary afforded acid **19** which was esterified with $TMSCHN_2$ to yield the corresponding methyl ester **20** in 86%. Attempts to acylate the nitrogen of the pyrrolidine ring



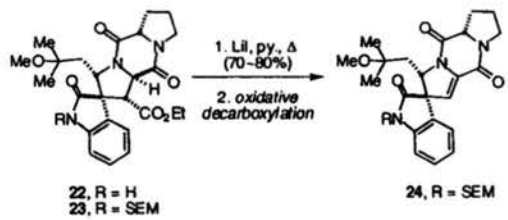
Scheme 4.

of **20** failed under a number of conditions. Only trace amounts of product were ever obtained and were complicated by acylation of the oxindole nitrogen. The decreased nucleophilicity of the pyrrolidine nitrogen can be attributed to the surrounding steric bulk. The ester and propylidene groups which are α- to the amine, are in an *anti*-configuration effectively blocking each face of the nitrogen from electrophilic attack.

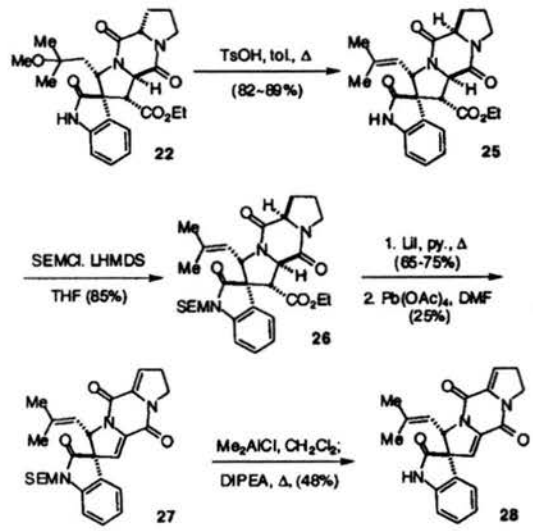
This initially discouraging result was eventually turned into an asset since, it was soon realized that the steric hindrance about the nitrogen might allow for direct coupling on the free, zwitterionic amino acid without concomitant self-condensation with the active ester. Thus, amino acid **19** was taken on crude from the preceeding hydrogenation step and directly coupled with L-proline benzyl ester with BOP[22] as the activating agent to give the dipeptide **21** in 74% for the two steps. Reduction of the benzyl ester followed by BOP-mediated cyclization afforded the desired diketopiperazine (DKP) **22** in excellent yield. The stage was now set for sequential installation of the two olefinic moieties.

Several strategies were examined for the installation of the enamide functionality and the prenyl side-chain. The initial plan was to first form the C-8/C-9 unsaturation and subsequently secure the C-19/C-20 olefinic group since the planned oxidative decarboxylation would involve an alkyl radical that might react with a proximal prenyl group. However, it was recognized that if an undesired intramolecular radical cyclization process were to occur, it would have to occur via a stereoelectronically disfavored 5-*endo-trig* cyclization.[23] With these considerations in mind attempts to effect a radical-based oxidative decarboxylation were pursued. Saponification of the ethyl ester of **22** was attempted using LiOH in THF/MeOH/H_2O, but failed to give any of the desired carboxylic acid. After some exploration, it was found that LiI in refluxing pyridine[24] furnished the desired carboxylic acid (Scheme 5). However, attempts to affect the oxidative decarboxylation either through the use of $Pb(OAc)_4$[25] or iodosobenzene diacetate[26] were unsuccessful, apparently due to the lability of the oxindole 2° amide. The oxindole nitrogen atom of **22** was protected as the corresponding SEM derivative **23**. Cleavage of the ethyl ester with LiI in refluxing pyridine furnished the corresponding acid that was subjected to Kochi-type conditions generating the eneamide **24**, albeit only in poor yields (10–25%).

Unfortunately, all attempts to install the C-19/C-20 unsaturation with **24** as a substrate were uniformly unsuccessful under a range of acidic elimination conditions. While the enamide proved to be stable to both mildly basic



Scheme 5.

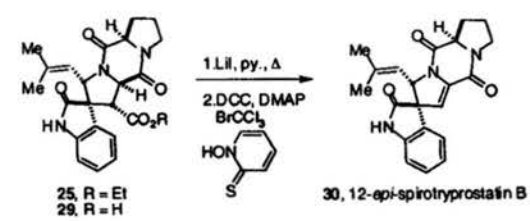


Scheme 6.

and acidic conditions, more vigorous conditions resulted in decomposition. These results suggested that the isopropylidene group needed to be in place prior to installation of the C-8/C-9 unsaturation. To this end, diketopiperazine **22** was subjected to treatment with TsOH in refluxing toluene resulting in the formation of the desired olefin **25** in good yield with only trace amounts of the isomeric disubstituted olefin present (Scheme 6). As before, the SEM group was used to protect the oxindole nitrogen (**26**).

Subjecting the carboxylic acid, resulting from saponification of the ethyl ester **26**, to a classical Kochi-type oxidative decarboxylation protocol produced the over-oxidized triene **27**. Despite extensive effort, we were unable to obviate oxidation of the proline residue under a wide range of Kochi-type conditions. Triene **27** provided an intriguing analog of spirotryprostatin B once the protecting group was removed. The free oxindole **28** was thus obtained using dimethyl aluminum chloride followed by heating in diisopropylethyl amine.[27]

We next turned to examining a Barton-modified Hunsdiecker reaction as a possible solution to the oxidative decarboxylation problem.[28] This reaction has found utility in a number of applications for the generation of alkyl halides, however the application of this method for the formation of α,β-unsaturated amino acid derivatives has seldom been reported. The ethyl ester was converted to the acid as above with lithium iodide in hot pyridine yielding



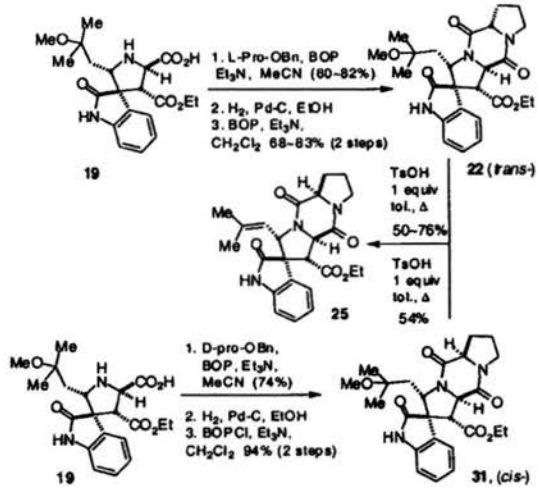
Scheme 7.

carboxylic acid **29**, (Scheme 7). Treatment of **29** with DCC, DMAP and *N*-hydroxy pyridine-2-thione yielded a product **30** whose ^{1}H NMR spectroscopic signatures closely resembled those of the natural product with the exception of some slight variations in the chemical shifts of several resonances.

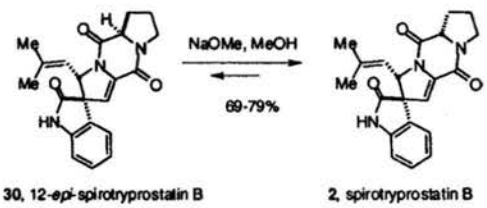
The Barton-modified Hunsdiecker protocol converts the carboxylic acid, via radical decarboxylation of the *N*-hydroxy pyridine-2-thione ester, into a secondary alkyl radical that is quenched by the solvent, $BrCCl_3$, into the corresponding alkyl bromides, which under thermal conditions, eliminate HBr to form the olefin. The overall yield for this process was far from exceptional (34–43%) and, it is possible that the formation and relative rates of elimination of the two diastereomeric bromides, might have contributed to the recovery of only moderate amounts of the desired product. We suspect that only the bromide that is positioned *trans*, antiperiplanar to the α-hydrogen, suffers facile elimination to give 12-*epi*-spirotryprostatin B. Our excitement that the reaction had occurred as planned was tempered by the discrepancies observed in the ^{1}H NMR data between the natural spirotryprostatin B and product **30**.

The absolute stereochemistry of the L-proline residue was not in doubt in the initial stages of the synthesis and both the relative and absolute stereochemistry of the spiro-oxindole moiety had been secured by X-ray crystallographic analysis of **11**. Thus, we suspected that an epimerization had occurred in the proline ring either at the stage of the elimination of methanol from **22**, or during the ethyl ester cleavage to ultimately give 12-*epi*-spirotryprostain B **30**.

To decipher at what stage the suspected epimerization reaction was occurring, the complementary D-proline-derived *cis*-diketopiperazine **31**, was constructed as shown in Scheme 8. This was accomplished in a similar fashion to that utilized for the formation of the *trans*-diketopiperazine **22**. Thus, coupling of amino acid **19** with D-proline benzyl ester (74%) followed by hydrogenation of the benzyl ester and cyclization (94% over two steps) afforded **31**.



Scheme 8.



Scheme 9. Thermodynamic epimerization of **32** to **2**.

If the dehydration step was the culprit in the loss of stereochemical integrity of **22**, then subjecting the two substrates (**22** and **31**) separately to the elimination conditions would yield the same product. This indeed proved to be the case wherein it was observed that the pentacyclic product **25** was formed exclusively from either substrate when treated with TsOH in hot toluene. It is well-known that *cis*-diketopiperazines are thermodynamically more stable than the corresponding *trans*-isomers for cyclic anhydrides of proline.[29] In contrast, reported syntheses of the fumetrimorgins have demonstrated the ability of substrates with the 6-6-5-ring system to undergo epimerization from the *cis*-configuration to the *trans*-configuration.[30]

With the stereochemical issues clarified, we returned to the task of converting 12-*epi*-spirotryprostatin B (**30**) into the natural stereoisomer as shown in Scheme 9. Addition of NaOMe in MeOH at 0°C to **30** yielded an equilibrium mixture of spirotryprostatin B (2): 12-*epi*-spirotryprostatin B (**30**) in a 2:1 ratio. These diastereomers were easily separated by chromatography and the recovered **30** could be re-subjected to the epimerization protocol giving **2** in 62% overall yield for the two cycles. The synthetic and natural specimens of (−)-spirotryprostatin B displayed identical spectroscopic data including optical rotation. In like fashion, (+)-*ent*-spirotryprostatin B was synthesized starting with the opposite antipode of **8**.[17]

3. Biological activity

The effects of compounds **11**, **19**, **20**, **21**, **22**, **25**, **28**, **29**, **30**, their enantiomers, and *ent*-spirotryprostatin B on cell cycle control and microtuble assembly were examined. Given the moderate activities of the title compounds (IC_{50}=14.0 μM for spirotryprostatin B), it was not surprising to find that all of the spirotryprostatin analogs prepared in this study that were tested had no effect on in vitro microtubule assembly and had little or no effect on in vitro cell cycle inhibition. Three compounds (**30**, *ent*-**30**, and *ent*-**2**) did however, provide some intriguing results.

12-*epi*-Spirotryprostatin B (**30**) was shown to cause partial accumulation of cells at the G_2/M phase at concentrations of 125 μM but were toxic to 3Y1 and tsFT210 cells at 250 μM or higher concentrations. The enantiomer of **30** was however, neither toxic to the cells nor showed any activity for cell cycle proliferation and microtuble assembly. Similar results were seen in the testing of spirotryprostatin (**2**) and *ent*-**2**. Spirotryprostatin B has been reported to inhibit tubulin polymerization and to be cytotoxic to mammalian cells[6] whereas *ent*-**2** had no effect on in vitro microtubule

assembly or in vitro cell cycle inhibition but was toxic to 3Y1 and tsFT210 cells at 31.3 and 15.6 μM concentrations, respectively. These data suggest that the molecular target of *ent*-spirotryprostatin B is different from that of the natural product. Further studies aimed at elucidating the cellular target of these substances and the molecular mechanism by which they arrest the cell cycle are currently under investigation in these laboratories.

4. Conclusion

In summary, the synthesis of both antipodes of spirotryprostatin B (**2**) has been achieved utilizing a diastereoselective, asymmetric [1,3]-dipolar cycloaddition reaction as the key step. This strategy, which sets four contiguous stereogenic centers in one step, also appears to be adaptable to the synthesis of spirotryprostatin A and efforts are underway in this regard. In addition, a tertiary methyl ether was demonstrated to serve as a suitable progenitor of the prenyl group providing an alternative method for the introduction of the isopropylidene functionality. Moreover, the inherent thermodynamic stability of diketopiperazines with the 5-6-5 ring system have been shown to preferentially favor the *cis*-configuration. Application of the methodology developed in this work is being applied to the synthesis of other potentially biologically useful members of the spirotryprostatin structural class.

5. Experimental

5.1. Cell culture and proliferation assay

Rat normal fibroblast 3Y1 cells[31] were grown in Dulbecco's modified MEM culture medium supplemented with 10% fetal calf serum under a humidified atmosphere containing 5% CO_2. Exponentially growing 3Y1 cells were treated with the test compounds for 24 h. The distribution of DNA content was determined by flow cytometry and relative cell numbers (cell number at 24 h per initial cell number at 0 h×100) were counted. MTT assay is a colorimetric assay using 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide. The cell viability was determined by this assay with minor modifications.[32]

5.2. Preparation of microtubule and turbidity assay (in vitro microtubule assembly assay)

Calf brain microtubule protein was prepared by two cycles of assembly-disassembly[33] and stored at −80°C in Mes buffer (100 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), 1 mM EGTA and 0.5 mM $MgCl_2$) at pH 6.8. Protein concentrations were determined by using the Dc Protein Assay (BioRad, Hercules, CA). Microtubule assembly was monitored by the turbidity assay as described previously.[34,35]

5.2.1. 3-Methyl-3-methoxybutanal (9). To an oven-dried 2000 mL three-neck round-bottom flask with stir bar was added DMSO (15.8 mL, 22.3 mmol) in 50 mL of CH_2Cl_2. The solution was cooled to −78°C under argon and oxalyl chloride (10 mL, 112 mmol) in 250 mL of CH_2Cl_2 was added dropwise over 15 min. 3-Methyl-3-methoxybutan-1-ol (12.0 g, 100 mmol) along with pyridine (16.5 mL, 200 mmol) in 100 mL of CH_2Cl_2 was added dropwise over 15 min. The reaction was stirred 15 min. more at −78°C and then Et_3N (75 mL, 0.5 mol) in 75 mL of CH_2Cl_2 was added over 15 min with vigourous stirring. The solution was kept at 78°C for 15 min and then warmed to 4°C and stirred for another 15 min. 1N HCl was used to acidify to pH −4 and the layers separated. The aqueous layers were extracted with three 50 mL portions of CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The crude product could be obtained by column chromatography with 2:1 CH_2Cl_2/Et_2O as the eluent to yield 11.5 g (97%). The product was further purified by distillation to remove any impurities. For **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 1.31 (s, 6H), 2.53 (d, J=3.3 Hz, 2H), 3.26, (s, 3H), 9.84 (t, J=3.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ $CDCl_3$: 18.6, 42.7, 46.6, 67.1, 195.4; IR (NaCl/neat) 2974, 2937, 2828, 1732 cm^{-1}; LRMS (EI+) calcd for $C_6H_{13}O_2$ (*m/z*) 117.1, found (*m/z*) 117.1.

5.3. Cycloaddition

To a 500 mL round-bottom flask with stir bar was added (5*R*,6*S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (5.0 g, 19.8 mmol), ethyl oxindolylidene acetate (**10**) (6.4 g, 29.6 mmol) and 5.0 g of activated 3 Å molecular sieves. An oven-dried condenser was attached and the system was flushed with argon. Freshly distilled toluene (250 mL) was added followed by 3-methyl-3-methoxybutanal (**9**, 2.75 g, 23.7 mmol). The reaction was then heated to 60°C and kept at that temperature for 1 h at which time the heating mantle was turned off. The reaction was allowed to cool to room temperature and filtered through Celite to remove the sieves. Concentration afforded an orange solid which was chromatographed (SiO_2, 4:1 hexanes/EtOAC→1:1 hexanes/EtOAc) to afford 9.2 g of **11** (82%) and 0.70 g of **12** (6.3%) and 0.12 g of **13** (1.1%). Analytical samples of **11** were generated by recrystallization from EtOH.

5.3.1. Spiro[3*H*-indole-3,7′ (6′*H*)-[1*H*]pyrrolo[2,1-*c*][1,4]oxazine]-8′-carboxylic acid, 1,2,3′,4′,8′,8′a-hexahydro-6′-(2-methoxy-2-methylpropyl)-1′,2-dioxo-3′,4′-diphenyl, ethyl ester, (3*S*,3′*S*,4′*R*,6′*S*,8′*R*,8′a*R*) (11). $[\alpha]_D^{25}=-88.8$ (*c* 1.0, CH_2Cl_2); melting point: 225–227°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.68 (t, J=6.9 Hz, 3H), 1.09 (s, 6H), 1.14 (dd, J=3.6, 16.2 Hz, 2H), 1.70 (d, J=3.3, 15.9 Hz, 2H), 3.08 (s, 3H), 3.63–3.85 (m, 2H), 3.95 (d, J=7.5 Hz, 1H), 4.04 (t, J=3.3 Hz, 1H), 4.65 (d, J=7.5 Hz, 1H), 5.07 (d, J=3.3 Hz, 1H), 6.40 (d, J=3.3 Hz, 1H), 6.91 (d, J=7.5 Hz, 1H), 7.00 (dt, J=0.9, 7.5 Hz, 1H), 7.15 (d, J=7.5 Hz, 1H), 7.18–7.33 (m, 10H), 7.44 (d, J=7.5 Hz, 1H), 8.09 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ $CDCl_3$: 23.6, 26.9, 45.4, 50.6, 53.3, 56.0, 57.1, 57.5, 61.5, 65.5, 74.5, 77.1, 110.8, 124.0, 126.2, 127.2, 128.3, 128.4, 128.5, 129.4, 129.5, 130.2, 130.4, 137.5, 138.3, 142.3, 170.1, 173.0, 178.9; IR (NaCl/neat) 3308, 1734, 1618 cm^{-1}.

ent-**11**: $[\alpha]_D^{25}=91.7$ (*c* 1.0, CH_2Cl_2).

5.3.2. Spiro[3*H*-indole-3,7′ (6′*H*)-[1*H*]pyrrolo[2,1-*c*][1,4]oxazine]-8′-carboxylic acid, 1,2,3′,4′,8′,8′a-hexahydro-6′-(2-methyl-2-prop-ene-yl)-1′,2-dioxo-3′,4′-

diphenyl-, ethyl ester, (3*S*,3′*S*,4′*R*,6′*S*,8′*R*,8′a*R*) (12). $[\alpha]_D^{25}=52.8$ (*c* 0.95, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 1.11 (t, $J=6.8$ Hz, 3H), 1.16 (s, 3H), 1.19 (s, 3H), 1.80–1.94 (m, 2H), 3.18 (s, 3H), 3.41 (d, $J=6.0$ Hz, 1H), 4.00–4.14 (m, 2H), 4.53 (m, 1H), 4.69 (d, $J=7.6$ Hz, 1H), 5.0 (s, 1H), 6.41 (d, $J=2.8$ Hz, 1H), 6.84 (d, $J=7.6$ Hz, 2H), 7.01–7.35 (m, 12H), 7.62 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: 13.5, 18.8, 26.2, 54.1, 57.3, 59.8, 60.2, 61.4, 68.7, 78.0, 109.8, 119.8, 122.7, 125.9, 126.1, 126.9, 127.8, 128.1, 128.3, 128.6, 129.0, 129.2, 136.0, 136.4, 141.1, 141.4, 168.6, 171.8, 177.6; IR (NaCl/neat) 3305, 1730, 1618 cm^{-1}; HRMS (FAB+) calcd for $C_{23}H_{33}O_5N_2$ (*m/z*) 537.2389, found (*m/z*) 537.2383.

5.3.3. Spiro[3*H*-indole-3,7′ (6′*H*)-[1*H*]pyrrolo[2,1-*c*][1,4]oxazine]-8′-carboxylic acid, 1,2,3′,4′,8′,8′a-hexahydro-6′-(2-methoxy-2-methylpropyl)-1′,2-dioxo-3′,4′-diphenyl-, ethyl ester, (3*S*,3′*S*,4′*R*,6′*S*,8′*R*,8′a*R*) (13). $[\alpha]_D^{25}=118.1$ (*c* 1.05, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.64 (t, $J=6.8$ Hz, 3H), 1.42 (s, 3H), 1.67 (s, 3H), 3.46–3.68 (m, 1H), 3.78–3.83 (m, 1H), 4.04 (d, $J=7.6$ Hz, 1H), 4.36 (d, $J=3.6$ Hz, 1H), 4.50 (t, $J=7.6$ Hz, 1H), 4.51 (s, 1H), 4.87 (d, $J=7.6$ Hz, 1H), 5.0 (s, 1H), 6.08 (d, $J=3.6$ Hz, 2H), 6.97, (t, $J=6.8$ Hz, 1H), 6.84, (d, $J=6.8$ Hz, 1H), 7.16–7.28 (m, 12H), 7.62 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: 14.2, 24.7, 24.8, 43.0, 49.5, 56.1, 59.8, 60.4, 60.5, 60.7, 65.6, 73.7, 79.2, 110.3, 123.1, 124.7, 126.5, 127.4, 127.7, 127.8, 128.0, 128.3, 129.2, 129.4, 137.0, 137.1, 141.1, 169.5, 170.4, 179.4; IR (NaCl/neat) 3288, 1718, 1621 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{36}O_6N_2$ (*m/z*) 569.2651, found (*m/z*) 569.2640.

5.3.4. Spiro[3*H*-indole-3,3′-pyrrolidine]-4′,5′-dicarboxylic acid, 1,2-dihydro-2′-(2-methoxy-2-methylpropyl)-2-oxo-, 4′-ethyl ester, monohydrochloride, (2′*S*,3*S*,4′*R*,5′*R*) (19). Recrystallized cycloadduct **11** (5.0 g, 8.8 mmol) was added to a sealable pressure tube and dissolved in 200 mL of 1:1 THF/EtOH. The solvent was purged with argon for 5 min and $PdCl_2$ (1.55 g, 8.80 mmol) was added. The tube was sealed and flushed with H_2 before finally pressurizing to 70 Psi. The reaction was stirred for 36 h and then filtered through Celite to remove the palladium catalyst. Concentration afforded a viscous oil which was triturated with 1×25 mL Et_2O, 1×25 mL EtOAc, and 1×25 mL Et_2O to give 3.75 g (quant. yield) of a white solid **19** upon drying under high vaccum. $[\alpha]_D^{25}=-14.0$ (*c* 1.0, MeOH); ^1H NMR (300 MHz, DMSO d_6) δ HOD: 0.64 (t, $J=6.9$ Hz, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.14 (dd, $J=3.6$, 14.7 Hz, 1H), 1.80 (dd, $J=8.4$, 15.0 Hz, 2H), 2.93 (s, 3H), 3.61–3.73 (m, 3H), 4.22 (dd, $J=4.2$, 8.1 Hz, 1H), 4.85 (d, $J=11.4$ Hz, 1H), 6.96 (t, $J=7.5$ Hz, 1H), 7.00 (d, $J=7.5$ Hz, 1H), 7.27 (d, $J=7.5$ Hz, 1H), 7.62 (d, $J=7.5$ Hz, 1H), 11.1 (br s, 1H); ^{13}C NMR (75 MHz, DMSO d_6) δ HOD: 15.0, 24.8, 25.6, 41.9, 50.5, 55.0, 60.2, 61.1, 63.1, 63.9, 75.0, 112.3, 120.2, 123.6, 124.6, 125.2, 125.7, 127.7, 129.9, 130.9, 131.7, 144.3, 168.2, 169.3, 176.3; IR (NaCl/neat) 3444, 3098, 3058, 2977, 1746, 1771, 1634, 1568 cm^{-1}; HRMS (FAB+) calcd for $C_{20}H_{27}O_6N_2$ (*m/z*) 391.1869, found (*m/z*) 391.1866.

ent-Amino acid **19**: $[\alpha]_D^{25}=10.0$ (*c* 1.0, MeOH).

5.3.5. Spiro[3*H*-indole-3,3′-pyrrolidine]-4′,5′-dicarboxylic acid, 1,2-dihydro-2′-(2-methoxy-2-methylpropyl)-2-oxo-, 4′-ethyl ester, 5′-methyl ester, (2′*S*,3*S*,4′*R*,5′*R*) (20). Recrystallized cycloadduct **11** (0.50 g, 0.88 mmol) was added to a sealable pressure tube and dissolved in 10 mL of 1:1 THF/EtOH. The solvent was purged with argon for 5 min and $PdCl_2$ (155 mg, 0.88 mmol) was added. The tube was sealed and flushed with H_2 before finally pressurizing to 70 Psi. The reaction was stirred for 36 h and then filtered through Celite to remove the palladium catalyst. Concentration afforded a viscous oil which was taken up 5 mL of 1:1 CH_2Cl_2/MeOH. $TMSCHN_2$ (−1.0 mL of a 2.0 M solution in hexanes) was added until a yellow color persisted. The reaction was stirred 5 min. and then concentrated under reduced pressure. Column Chromatography with 1:1 hexanes/EtOAc affored 325 mg (91%) of white solid **20**. $[\alpha]^{25}=-27.3$ (*c* 0.97, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.63 (t, $J=6.8$ Hz, 3H), 0.90 (dd, $J=1.6$, 14.4 Hz, 1H), 0.99 (s, 3H), 1.10 (s, 3H), 1.19 (dd, $J=9.6$, 14.4 Hz, 1H), 3.08 (s, 3H), 3.17 (br s, 1H), 3.58–3.66 (m, 1H), 3.70 (d, $J=8.8$ Hz, 1H), 3.72–3.80 (m, 2H), 3.76 (s, 3H), 4.58 (d, $J=8.8$ Hz, 1H), 6.82 (d, $J=7.6$ Hz, 1H), 6.96 (dt, $J=0.8$, 7.6 Hz, 1H), 7.18 (dt, $J=0.8$, 7.6 Hz, 1H), 7.36 (d, $J=7.6$ Hz, 1H), 7.98 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: 1.53, 24.4, 25.8, 40.6, 49.4, 52.8, 54.9, 59.1, 61.0, 61.1, 63.7, 74.4, 109.4, 122.7, 126.2, 127.8, 128.6, 140.9, 169.4, 175.2, 178.0; IR (NaCl/neat) 3244, 1734 cm^{-1}; HRMS (FAB+) calcd for $C_{21}H_{29}O_6N_2$ (*m/z*) 405.2025, found (*m/z*) 405.2024.

5.3.6. Spiro[3*H*-indole-3,3′-pyrrolidine]-4′-carboxylic acid, 1,2-dihydro-2′-(2-methoxy-2-methylpropyl)-2-oxo-5′-[[(2*S*)-2-[(phenylmethoxy)carbonyl]-1-pyrrolidinyl]-carbonyl]-, ethyl ester, (2′*S*,3*S*,4′*R*,5′*R*) 21. To a 200 mL round-bottom flask that contained amino acid **19** (3.75 g, 8.8 mmol) and was placed under high vacuum for 24 h was added BOP[22] (4.25 g, 9.7 mmol) and L-proline benzyl ester hydrochloride (2.35 g, 9.7 mmol). The flask was flushed with argon, 100 mL of CH_3CN was added and the reaction mixture cooled to 0°C. With stirring, triethylamine (2.70 mL, 19.3 mmol) was added dropwise and the solution allowed to warm to room temperature and stir for 8 h. The solvent was then evaporated, replaced with 100 mL of EtOAc, washed with 2×15 mL, 1N HCl, 1×15 mL H_2O, 2×15 mL 5% $NaHCO_3$, 1×10 mL sat. brine sol., dried over Na_2SO_4, filtered and evaporated to yield 5.0 g of a brown foam **21** which was taken on crude. An analytical sample of **21** was generated by column chromatography with 1:1 hexanes/EtOAc: $[\alpha]_D^{25}=-75.3$ (*c* 1.0, CH_2Cl_2); ^1H NMR (300 MHz, 120°C, DMSO) δ DMSO: 0.60 (t, $J=7.2$ Hz, 3H), 0.88 (d, $J=3.9$ Hz, 2H), 0.90 (s, 6H), 1.84 (br s, 1H), 2.05–2.16 (m, 1H), 2.75 (br s, 2H), 2.85 (s, 3H), 3.47–3.66 (m, 3H), 4.00 (d, $J=7.2$ Hz, 1H), 4.51 (d, $J=7.5$ Hz, 1H), 5.06 (s, 1H), 6.77 (t, $J=7.5$ Hz, 1H), 6.81 (d, $J=7.5$ Hz, 1H), 7.08 (dt, $J=1.2$, 7.5 Hz, 1H), 7.15–7.28 (m, 6H), 9.97 (br s, 1H); ^{13}C NMR (75 MHz, DMSO d_6) δ DMSO d_6: 13.8, 25.6, 25.7, 47.4, 48.7, 49.0, 55.9, 59.9, 60.2, 60.6, 60.8, 62.7, 64.5, 66.6, 74.2, 109.9, 121.5, 122.2, 1222.6, 125.5, 128.2, 128.3, 128.4, 128.5, 129.0, 143.4, 170.2, 171.2, 172.3, 177.8; IR (NaCl/neat) 3239, 1731, 1725, 1645, 1618 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{40}O_7N_3$ (*m/z*) 578.2866, found (*m/z*) 578.2862.

ent-**21**: $[\alpha]_D^{25}=81.5$ (*c* 1.0, CH_2Cl_2).

5.3.7. Spiro[1*H*,5*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-2(3*H*),3′-[3*H*]indole]-1-carboxylic acid, 1′,2′,5a,6,7,8,10,10a-octahydro-3-(2-methoxy-2-methylpropyl)-2′,5,10-trioxo-, ethyl ester, (1*R*,2*S*,3*S*,5a*S*,10a*R*) 22. To a 100 mL round-bottom flask that contained **21** (5.0 g, 8.7 mmol) was added a stir bar and 20 mL of EtOH. Argon was bubbled through for 5 min. and 10% Pd/C (0.5 g) was added. The system was flushed with H_2 and a balloon of H_2 was attached. The solution was stirred vigorously for 1.5 h and then filtered through Celite, evaporated and placed on high vacuum overnight. To the crude mixture was added a stir bar, BOP[22] (3.83 g, 8.6 mmol) and 80 mL of CH_3CN. Triethylamine (1.2 mL, 8.6 mmol) was added dropwise and the reaction was allowed to stir for 8 h at which time the solvent was evaporated. Purification via column chromatography with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH afforded 2.75 g (68%) of **22** as a white solid. For **22**: $[\alpha]_D^{25}=-92.0$ (*c* 1.0, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.92 (t, $J=7.2$ Hz, 3H), 1.11 (s, 3H), 1.19 (s, 3H), 1.72 (dd, $J=4.2$, 14.4 Hz, 1H), 1.75–2.08 (m, 3H), 2.21 (dd, $J=10.5$, 14.4 Hz, 1H), 2.49 (h, $J=6.0$ Hz, 1H), 3.0 (s, 3H), 3.42 (ddd, $J=3.9$, 7.5, 9.9 Hz, 1H), 3.49 (d, $J=9.3$ Hz, 1H), 4.67 (d, $J=9.9$ Hz, 1H), 3.84–4.07 (m, 3H), 4.31 (dd, $J=5.4$, 9.9 Hz, 1H), 4.89 (dd, $J=3.9$, 10.5 Hz, 1H), 5.13 (dd, $J=1.2$, 9.6 Hz, 1H), 6.96 (t, $J=7.5$ Hz, 1H), 6.99 (d, $J=7.5$ Hz, 1H), 7.17 (d, $J=7.5$ Hz, 1H), 7.25 (dt, $J=1.9$, 7.5 Hz, 1H), 8.42 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ $CDCl_3$: 12.9, 20.7, 23.1, 23.7, 29.1, 38.4, 43.8, 47.9, 53.3, 56.1, 59.3, 59.6, 60.1, 60.6, 73.5, 109.6, 121.1, 123.6, 126.3, 128.5, 141.0, 161.8, 165.2, 168.8, 179.5; IR (NaCl/neat) 3244, 1763, 1667, 1665 cm^{-1}; HRMS (FAB+) calcd for $C_{25}H_{32}O_6N_3$ (*m/z*) 470.2291, found (*m/z*) 470.2280.

ent-**22**: $[\alpha]_D^{25}=95.8$ (*c* 1.2, CH_2Cl_2).

5.3.8. *N*-SEM diketopiperazine 23. To a flame-dried 10 mL round-bottom flask with stir bar was added diketopiperazine **22** (65 mg, 0.14 mmol). The system was flushed with Ar, THF added and cooled to −78°C. KHMDS (0.33 mL of a 0.5 M sol., 0.16 mmol) was added and stirred for 15 min. SEMCl (0.03 mL, 0.16 mmol) was added dropwise and the reaction allowed to warm to room temperature and stirred for 8 h. Sat. NH_4Cl was added and the reaction mixture poured into 10 mL EtOAc. The aq. layer was extracted 3×5 mL with EtOAc, the organic layers combine, dried over Na_2SO_4, filtered, evaporated and chromatographed with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH to yield 60 mg (72%) of the white solid **23**: $[\alpha]_D^{25}=-2.4$ (*c* 0.74, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: −0.04 (s, 9H), 0.8 (t, $J=6.8$ Hz, 6H), 1.12 (s, 3H), 1.18 (s, 3H), 1.70 (dd, $J=4.0$, 14.4 Hz, 1H), 1.81 (dt, $J=3.2$, 11.6 Hz, 1H), 1.88–2.01 (m, 1H), 2.02–2.12 (m, 1H), 2.21 (dd, $J=10.8$, 14.4 Hz, 1H), 2.50 (quint, $J=6.0$ Hz, 1H), 3.01 (s, 3H), 3.40–3.48 (m, 1H), 3.47 (d, $J=9.2$ Hz, 1H), 3.54 (t, $J=8.8$ Hz, 2H), 3.83–3.90 (m, 1H), 3.92–3.96 (m, 1H), 3.97–4.04 (m, 1H), 4.32 (dd, $J=5.6$, 11.6 Hz, 1H), 4.85 (dd, $J=4.0$, 10.8 Hz, 1H), 5.05 (1/2 Abq, $J=11.2$ Hz, 1H), 5.13, (dd, $J=1.2$, 9.2 Hz, 1H), 5.20 (1/2 Abq, $J=11.2$ Hz, 1H), 7.06–7.09 (m, 2H), 7.22 (d, $J=7.6$ Hz, 1H), 7.33 (t, $J=7.6$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: −2.09, 13.0, 17.1, 20.9, 23.2, 23.8, 29.2, 38.8, 44.0, 48.2, 54.2, 56.2, 59.7, 60.2, 60.8, 65.6, 69.0, 73.7, 109.5, 121.9, 123.0, 126.4, 128.7, 142.1, 161.9, 164.9, 168.8, 178.6; IR (NaCl/neat) 2971, 1724, 1668 cm^{-1}; HRMS (FAB+) calcd for $C_{31}H_{46}O_7N_3Si_1$ (*m/z*) 600.3105, found (*m/z*) 600.3109.

5.3.9. Eneamide 24. To a flame-dried 10 mL round-bottom flask with stir bar was added SEM protected diketopiperazine **23** (70 mg, 0.08 mmol) and LiI (110 mg, 0.80 mmol). An oven dried condensor was attached and the system was flushed with argon, freshly distilled pyridine (5 mL) was added and the system heated to reflux for 48 h. The solvent was evaporated and replaced with 10 mL of EtOAc, extracted with 5×2 mL 5% $NaHCO_3$ and the aqueous layers combined. The solution was then saturated with NaCl, acidified to pH 4 with 1N HCl and extracted with 5×5 mL EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to yield a white solid, which was used without further purification. To the flask which contained the crude carboxylic acid was added $Cu(OAc)_2$ (1 mg, 0.006 mmol) and an oven dried condensor was attached. The system was flushed with Ar and distilled DMF (1 mL) was added. The reaction was wrapped in tin foil and stirred for 15 min. at which time $Pb(OAc)_4$ (55 mg, 0.12 mmol) was added. The mixture was stirred (still in the dark) for 15 min more and then heated to reflux for 1.5 h. Evaporation of the solvent and purification via column chromatography with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH to yielded 5 mg (11%) of a clear oil. For **24**: $[\alpha]^{25}=-6.2$ (*c* 0.16, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: −0.04 (s, 9H), 0.79 (s, 3H), 0.91 (t, $J=7.6$ Hz, 3H), 1.15 (s, 3H), 1.22 (d, $J=8.4$ Hz, 1H), 1.92–2.00 (m, 2H), 2.10–2.1 (m, 1H), 2.18 (dd, $J=10.8$, 13.6 Hz, 1H), 2.40–2.50 (m, 1H), 2.62 (s, 3H), 3.40–3.48 (m, 1H), 3.49–3.54 (m, 1H), 3.60 (t, $J=8.8$ Hz, 1H), 4.21–4.25 (m, 1H), 3.78–3.81 (m, 1H), 4.97 (d, $J=10.0$ Hz, 1H), 5.02 (1/2 ABq, $J=10.8$ Hz, 1H), 5.24 (1/2 ABq, $J=10.8$ Hz, 1H), 5.63 (s, 1H), 6.99–7.09 (m, H), 7.2 (d, $J=7.6$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: −1.23, 18.0, 22.2, 22.9, 25.4, 29.7, 39.7, 44.7, 48.4, 61.9, 63.8, 66.5, 70.1, 70.6, 109.7, 110.3, 118.0, 122.7, 126.6, 128.8, 137.2, 142.3, 161.3, 164.6, 169.0; IR (NaCl/neat) 2952, 1727, 1683, 1650 cm^{-1}; HRMS (FAB+) calcd for $C_{28}H_{40}O_5N_3Si_1$ (*m/z*) 526.2737, found (*m/z*) 527.2727.

5.3.10. Spiro[1*H*,5*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-2(3*H*),3′-[3*H*]indole]-1-carboxylic acid, 1′,2′,5a,6,7,8,10,10a-octahydro-3-(2-methyl-1-propenyl)-2′,5,10-trioxo-, ethyl ester, (1*R*,2*S*,3*S*,5a*R*,10a*R*-25). To a flame-dried 250 mL round-bottom flask with stir bar was added diketopiperazine **22** (2.70 g, 5.75 mmol), 4 Å molecular sieves (5.0 g) and TsOH (1.0 g, 5.75 mmol). An oven-dried condensor was attached, the system was flushed with argon, freshly distilled toluene (200 mL) was added and the system heated to reflux temperature for 8 h. The solvent was evaporated and replaced with 100 mL of EtOAc, washed with 2×15 mL 5% $NaHCO_3$, 1×10 mL sat. brine sol., dried over Na_2SO_4, filtered, evaporated and chromatographed with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH to yield 1.75 g (70%) of **25**: $[\alpha]_D^{25}=78.5$ (*c* 1.0, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.79 (t, $J=7.5$ Hz, 3H) 1.48 (d, $J=1.5$ Hz, 3H), 1.61 (d, $J=1.5$ Hz, 3H), 1.90–2.10 (m,

2H), 2.20–2.40 (m, 2H), 3.50–3.70 (m, 2H), 3.74–3.92 (m, 2H), 3.97 (d, J=10.2 Hz, 1H), 4.33 (t, J=7.5 Hz, 1H), 4.78 (dt, J=1.5, 9.6 Hz, 1H), 5.12 (d, J=9.6 Hz, 1H), 5.21 (d, J=10.2 Hz, 1H), 6.86 (d, J=7.5 Hz, 1H), 7.03 (dt, J=1.9, 7.5 Hz, 1H), 7.13 (d, J=7.5 Hz, 1H), 7.24 (dt, J=1.9, 7.5 Hz, 1H), 7.97 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ $CDCl_3$: 7.27, 11.9, 17.4, 19.4, 20.8, 39.3, 45.2, 52.8, 54.3, 54.9, 55.3, 57.2, 103.8, 113.1, 116.0, 119.2, 119.3, 122.8, 130.8, 134.8, 158.5, 160.3, 161.5, 171.0; IR (NaCl/neat) 3219, 1723, 1663, 1648 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{28}O_5N_3$ (m/z) 438.2029, found (m/z) 438.2017.

ent-**25**: $[\alpha]_D^{25}=-74.0$ (c 1.0, CH_2Cl_2).

5.3.11. *N*-SEM diketopiperazine 26. To a flame-dried 10 mL round-bottom flask with stir bar was added **25** (65 mg, 0.15 mmol). The system was flushed with Ar, THF added and cooled to −78°C. KHMDS (0.35 mL of a 0.5 M sol., 0.18 mmol) was added and stirred for 15 min. SEMCl (0.035 mL, 0.18 mmol) was added dropwise and the reaction allowed to warm to room temperature and stirred for 8 h. Sat. NH_4Cl was added and the reaction mixture poured into 10 mL EtOAc. The aq. layer was extracted 3×5 mL with EtOAC, the organic layers combine, dried over Na_2SO_4, filtered, evaporated and chromatographed with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH to yield 70 mg (84%) of the white solid **26**: $[\alpha]_D^{25}=-63.5$ (c 0.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: −0.04 (s, 9H), 0.71 (t, J=7.2 Hz, 3H), 0.86–1.00 (m, 2H), (1.46 (s, 3H), 1.55 (s, 3H), 1.95 – 2.02 (m, 2H), 2.24–2.40 (m, 2H), 3.52–3.65 (m, 3H), 3.68–3.76 (m, H), 3.82–3.90 (m, 1H), 3.97 (d, J=10.0 Hz, 1H), 4.32 (t, J=8.0 Hz, 1H), 4.78 (d, J=14.8 Hz, 1H), 5.09 (1/2 ABq, J=11.2 Hz, 1H), 5.10 (d, J=10.0 Hz, 1H), 5.20 (1/2 ABq, J=11.2 Hz, 1H), 7.04 (d, J=8.0 Hz, 1H), 7.07 (t, J=8.0 Hz, 1H), 7.15 (d, J=8.0 Hz, 1H), 7.30 (t, J=8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: −1.16, 13.7, 17.9, 18.5, 23.8, 25.9, 27.2, 45.7, 51.9, 59.0, 60.7, 61.3, 61.8, 63.7, 66.4, 70.1, 110.0, 119.4, 122.9, 125.1, 125.5, 129.4, 137.5, 142.8, 165.0, 166.8, 167.9, 175.9; IR (NaCl/neat) 1728, 1678 cm^{-1}; HRMS (FAB+) calcd for $C_{30}H_{42}O_6N_3Si_1$ (m/z) 568.2843, found (m/z) 568.2827.

5.3.12. *N*-SEM triene 27. To a flame-dried 10 mL round-bottom flask with stir bar was added **26** (70 mg, 0.12 mmol) and LiI (165 mg, 1.2 mmol). An oven dried condensor was attached and the system was flushed with argon, freshly distilled pyridine (5 mL) was added and the system heated to reflux for 48 h. The solvent was evaporated and replaced with 10 mL of EtOAc, extracted with 5×2 mL 5% $NaHCO_3$ and the aqueous layers combined. The solution was then saturated with NaCl, acidified to pH 4 with 1N HCl and extracted with 5×5 mL EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to yield 45 mg (68%) a white solid, which was used without further purification. To the flask which contained the crude carboxylic acid was added $Cu(OAc)_2$ (1.5 mg, 0.008 mmol) and an oven dried condensor was attached. The system was flushed with Ar and distilled DMF (1 mL) was added. The reaction was wrapped in tin foil and stirred for 15 min. at which time $Pb(OAc)_4$ (55 mg, 0.12 mmol) was added. The mixture was stirred (still in the dark) for 15 min. more and then heated to reflux for 1.5 h. Evaporation of the solvent and purification via column chromatography with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH to yielded 8 mg (20%) of **26** as a clear oil. For **26**: $[\alpha]^{25}=-60.0$ (c 0.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: −0.02 (s, 9H), 0.93 (t, J=7.6 Hz, 3H), (1.34 (s, 3H), 1.56 (s, 3H), 2.89 (dt, J=2.4, 8.0 Hz, 2H), 3.57 (t, J=7.6 Hz, 2H), 4.12 (t, J=8.8 Hz, 2H), 5.13 (d, J=10.8 Hz, 1H), 5.21 (t, J=11.2 Hz, 2H), 5.20 (d, J=8.0 Hz, 1H), 5.75 (s, 1H), 6.22 (t, J=3.2 Hz, 1H), 7.04–7.11 (m, 3H), 7.31 (t, J=7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: −1.2, 18.0, 18.5, 25.4, 28.7, 29.9, 45.4, 62.1, 64.5, 66.6, 70.2, 110.1, 116.2, 119.7, 119.9, 123.0, 126.7, 127.4, 129.4, 135.5, 138.1, 138.5, 142.0, 152.2, 152.7, 177.0; IR (NaCl/neat) 1727, 1683 cm^{-1}; HRMS (FAB+) calcd for $C_{27}H_{33}O_4N_3Si_1$ (m/z) 491.2240, found (m/z) 491.2226.

5.3.13. Triene 28. To a flame-dried 10 mL round-bottom flask with stir bar was added **27** (8 mg, 0.017 mmol) dissolved in CH_2Cl_2 (2 mL) and cooled to −78°C. A 1.0 M hexane solution of Me_2AlCl (0.086 mL, 0.086 mmol) was added dropwise under Ar. The mixture was warmed to room temperature and stirred for 15 min. The solution was cooled to 0°C and poured into a sat. Na/K tartrate solution (2 mL) also at 0°C. The mixture was allowed to warm to room temperature and stirred vigorously for 1 h. The aq. layer was then extracted 3×5 mL with EtOAc, the organic layers combined, dried over Na_2SO_4, filtered and evaporated. Purification was accomplished by PTLC (1/2 of a 250 μm plate) with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH as the eluent to yield 3 mg (48%) of **28** as a clear oil. For **28**: ^{1}H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 1.22 (s, 3H), 1.35 (s, 3H), 2.85 (dt, J=3.2, 8.0 Hz, 2H), 4.09 (t, J=8.8 Hz, 2H), 5.17 (d, J=8.8 Hz, 1H), 5.51 (d, J=8.8 Hz, 1H), 5.75 (s, 1H), 6.19 (t, J=3.2 Hz, 1H), 6.82 (d, J=7.6 Hz, 1H), 6.97–7.05 (m, 2H), 7.37 (t, J=7.6 Hz, 1H), 7.85 (br s, 1H); IR (NaCl/neat) 1763, 1667 cm^{-1}; HRMS (FAB+) calcd for $C_{21}H_{20}O_3N_3$ (m/z) 362.1504, found (m/z) 362.1484.

5.3.14. Spiro[1*H*,5*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-2(3*H*),3′-[3*H*]indole]-1-carboxylic acid, 1′,2′,5a,6,7,8,10,10a-octahydro-3-(2-methyl-1-propenyl)-2′,5,10-trioxo-, (1*R*,2*S*,3*S*,5a*R*,10a*R*) 29. To a flame dried 100 mL round-bottom flask with stir bar was added olefin **25** (0.87 g, 2.0 mmol) and LiI (2.66 g, 20.0 mmol). An oven-dried condensor was attached and the system was flushed with argon, freshly distilled pyridine (50 mL) was added and the system heated to reflux for 48 h. The solvent was evaporated and replaced with 50 mL of EtOAc, extracted with 5×10 mL 5% $NaHCO_3$ and the aqueous layers combined. The solution was then saturated with NaCl, acidified to pH 4 with 1N HCl and extracted with 5×10 mL EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to yield 0.58 g (71%) of **29**. The organic layer from the first extraction was dried over Na_2SO_4, filtered, evaporated and purified via column chromatography with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH to recover 80 mg of unreacted starting material **25**. For **29**: $[\alpha]_D^{25}=73.0$ (c 0.8, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ MeOH: 1.27 (s, 3H), 1.29 (s, 3H), 1.98–2.04 (m, 2H), 2.18–2.30 (m, 2H), 3.50–3.70 (m, 2H), 3.37–3.46 (m, 1H), 3.51–3.58 (m, 2H), 3.74 (d, J=10.2 Hz, 1H), 4.52 (t, J=7.5 Hz, 1H), 4.96 (d, J=5.1 Hz), 5.32 (d, J=9.3 Hz, 1H), 6.84 (d, J=7.5 Hz, 1H), 6.98 (dt, J=1.9, 7.5 Hz, 1H),

7.19 (dt, J=1.9, 7.5 Hz, 1H), 7.24 (d, J=7.5 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ CD_3OD: 12.5, 18.7, 19.9, 22.3, 40.7, 47.0, 54.8, 55.9, 57.1, 58.8, 105.2, 115.8, 117.1, 121.3, 121.5, 124.2, 131.1, 137.7, 161.3, 163.0, 164.9, 173.1; IR (NaCl/neat 3248, 1731, 1678, 1668 cm^{-1}; HRMS (FAB+) calcd for $C_{22}H_{24}O_5N_3$ (m/z) 410.1716, found (m/z) 410.1698.

ent-**29**: $[\alpha]_D^{25}=-75.0$ (c 1.0, MeOH).

5.3.15. Spiro[1*H*,5*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-2(3*H*),3′-[3*H*]indole]-1-carboxylic acid, 5a,6,7,8,10,10a-hexahydro-3-(2-methoxy-2-methylpropyl)-2′,5,10-trioxo-, ethyl ester, (1*R*,2*S*,3*S*,5a*R*,10a*R*-31). Compound **31** was generated in an identical fashion to diketopiperazine **22** yet afforded a higher yield (94%). For **31**: $[\alpha]_D^{25}=81.7$ (c 1.0, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.85 (s, 3H), 0.87 (t, J=7.2 Hz, 3H), 1.24, (s, 3H), 1.77 (dd, J=9.9, 14.1 Hz, 1H), 1.90–2.11 (m, 2H), 2.25–2.33 (m, 2H), 2.52 (d, J=13.8 Hz, 1H), 2.79 (s, 3H), 3.56–3.65 (m, 2H), 3.73–3.81 (m, 3H), 4.31 (t, J=7.5 Hz, 1H), 4.67 (d, J=9.9 Hz, 1H), 5.09 (d, J=9.9 Hz, 1H), 6.86 (d, J=7.5 Hz, 1H), 7.01 (t, J=7.5 Hz, 1H), 7.09 (d, J=7.5 Hz, 1H), 7.23 (t, J=7.5 Hz, 1H), 8.26 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ $CDCl_3$: 7.27, 15.8, 17.0, 19.2, 21.0, 33.5, 39.1, 41.3, 48.1, 52.7, 54.1, 54.7, 55.0, 55.5, 67.3, 103.2, 115.2, 119.8, 120.2, 122.3, 135.3, 158.0, 159.7, 161.0, 171.5; IR (NaCl/neat) 3268, 1729, 1671, 1669 cm^{-1}; HRMS (FAB+) calcd for $C_{25}H_{32}O_6N_3$ (m/z) 470.2291, found (m/z) 470.2296.

ent-Diketopiperazine **31**: $[\alpha]_D^{25}=-81.2$ (c 1.0, CH_2Cl_2).

5.3.16. Spiro[3*H*,5*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-2(10*H*),3′-[3*H*]indole]-2′,5,10(1′*H*)-trione, 5a,6,7,8-tetrahydro-3-(2-methyl-1-propenyl)-, (2*S*,3*S*,5a*R*) (12-*epi*-spirotyprostatin B) (30). To a flame-dried 100 mL round-bottom flask with stir bar was added carboxylic acid **31** (0.29 g, 0.26 mmol), DCC (0.22 g, 1.06 mmol), DMAP (0.13 g, 1.06 mmol) and 2-mercaptopyridine *N*-oxide (0.112 g, 0.88 mmol). An oven-dried condenser was attached and the system was flushed with argon and wrapped in tin foil. Freshly distilled $BrCCl_3$ (25 mL) was added and the system was heated to 60°C for 1 h. The foil was then removed and the reaction heated to reflux for 1.5 h. The solvent was evaporated and the resulting oil was purified by chromatography (silica gel, eluted with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH) to yield 0.095 g (37%) of **32**. $[\alpha]_D^{25}=41.3$ (c 0.8, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 1.50 (d, J=1.5 Hz, 3H), 1.54 (d, J=1.5 Hz, 3H), 1.90–2.16 (m, 3H), 2.18–2.30 (m, 2H), 3.40–3.48 (m, 1H), 3.52–3.60 (m, 1H), 3.81–3.92 (m, 2H), 4.36 (dd, J=6.9, 10.5 Hz, 1H), 5.13 (dt, J=1.5, 8.1 Hz, 1H), 5.54 (d, J=9.3 Hz), 5.83 (s, 1H), 6.87 (d, J=7.5 Hz, 1H), 7.01–7.09 (m, 2H), 7.19 (dt, J=1.9, 7.5 Hz, 1H), 7.22–7.27 (m, 1H) 7.69 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ $CDCl_3$: 18.6, 22.2, 25.7, 29.3, 45.3, 62.0, 62.1, 64.8, 110.1, 115.8, 119.3, 121.8, 122.8, 127.2, 128.6, 129.3, 155.7, 162.5, 178.2; IR (NaCl/neat) 3196, 1724, 1676, 1639 cm^{-1}; HRMS (FAB+) calcd for $C_{21}H_{21}O_3N_3$ (m/z) 364.1661, found (m/z) 364.1658.

epi-ent-Spirotyprostatin B: $[\alpha]_D^{25}=-42.5$ (c 0.8, CH_2Cl_2).

5.3.17. Spirotyprostatin B (2). To a flame-dried 10 mL round-bottom flask with stir bar was added 12-*epi*-spirotyprostatin B (**32**) (0.95 g, 0.26 mmol), MeOH (2 mL) was added and the system cooled to 0°C. A 1 M solution of NaOMe in MeOH (0.26 mL) was added dropwise and the mixture was stirred at 0°C for 2 h at which time 5 mL of saturated aqueous NH_4Cl was added along with 5 mL of EtOAc. The aqueous layer was extracted with EtOAc (3×5 mL) and the organic layers combined, dried over Na_2SO_4, filtered, evaporated and purifed by chromatography (silica gel, eluted with 75:20:5 CH_2Cl_2/EtOAc/*i*-PrOH) to yield 0.044 g (46%) of **2** and 0.28 g (30%) of **32**. For **2**: $[\alpha]_D^{25}=-151.1$ (c 0.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 1.28 (d, J=0.9 Hz, 3H), 1.57 (d, J=0.9 Hz, 3H), 1.94–2.05 (m, 2H), 2.08–2.16 (m, 1H), 2.46–2.53 (m, 1H), 3.58 (ddd, J=2.9, 9.3, 12.2 Hz, 1H), 3.84 (dt, J=8.3, 12.2 Hz, 1H), 4.35 (dd, J=6.1, 10.5 Hz, 1H), 5.22 (dt, J=1.2, 8.8 Hz, 1H), 5.4 (d, J=8.8), 5.79 (s, 1H), 6.89 (d, J=7.6 Hz, 1H), 6.99 (dt, J=1.0, 7.6 Hz, 2H), 7.06 (dt, J=1.0, 7.6 Hz, 1H), 7.23 (dt, J=1.0, 7.6 Hz, 1H) 7.77 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ $CDCl_3$: 18.4, 22.3, 25.5, 29.5, 45.0, 61.8, 61.9, 64.3, 110.0, 116.4, 120.7, 122.5, 127.4, 128.1, 129.3, 138.4, 138.5, 140.6, 155.2, 162.7, 178.1; IR (NaCl/neat) 3235, 1718, 1677, 1690 cm^{-1}; HRMS (EI) calcd for $C_{21}H_{21}O_3N_3$ (m/z) 363.1583, found (m/z) 363.1584.

ent-Spirotryprostatin B: $[\alpha]_D^{25}=155.1$ (c 0.33, CH_2Cl_2).

Acknowledgments

We thank the National Science Foundation (Grant No. CHE 9731947) for financial support. The authors are also grateful to Professor Raymond L. Funk of Pennsylvania State University with helpful discussions concerning the mechanism of formation of compound **12**. The authors also thank Professor Larry E. Overman, of the University of California, Irvine, and Professor Samuel J. Danishefsky of the Sloan-Kettering Institute for communicating their results on spirotryprostatin B prior to publication. RMW wishes to acknowledge Professor Kishi's passion for natural products chemistry as this has provided the inspiration for this work.[36]

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18. Aldehyde **9** is obtained from inexpensive, commercially available 3-methoxy-3-methyl-1-butanol (Aldrich Chemical Co.) by Swern oxidation in 89% yield (see Section 5).
19. The unsaturated oxindolylidene acetate **10** is readily prepared from isatin (Aldrich Chemical Co.) by condensation with $(Ph)_3PCHCO_2Et$ (Aldrich Chemical Co.) in refluxing diglyme in 84% yield (see Ref. 14c).
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HETEROCYCLES, Vol. 58, 2002, pp. 563-575, Received, 15th April, 2002

THE SYNTHESIS OF SPIROOXINDOLE PYRROLIDINES *VIA* AN ASYMMETRIC AZOMETHINE YLIDE [1,3]-DIPOLAR CYCLOADDITION REACTION

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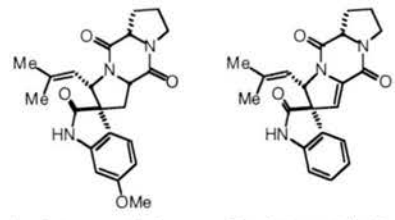
Abstract—The asymmetric [1,3] dipolar cycloaddition reactions of azomethine ylides derived from 5,6-diphenylmorpholin-2-one with various aldehydes and ethyl oxindolylideneacetate are described. Addition of an aldehyde to the morpholin-2-one, under essentially neutral conditions, results in the preferential formation of the *E*-ylide which then reacts with the dipolarophile to yield spirooxindole pyrrolidine derivatives in moderate to excellent regio- and diastereoselectivities. The resulting cycloadducts were easily separated by column chromatography and converted to the corresponding amino acid methyl esters through catalytic hydrogenolysis.

INTRODUCTION

The [1,3] dipolar cycloaddition reactions of azomethine ylides with alkene dipolarophiles have proven invaluable for the construction of highly substituted pyrrolidine derivatives.[1] Chiral pyrrolidines constitute the main structural element of a number of alkaloid natural products, including the microtubule inhibitors spirotryprostatins A and B (Figure 1).[2] The development of a general, stereoselective version of the reaction, either through the use of a chiral auxiliary attached to the dipolarophile or through the use of a chiral azomethine ylide is therefore an important synthetic objective.

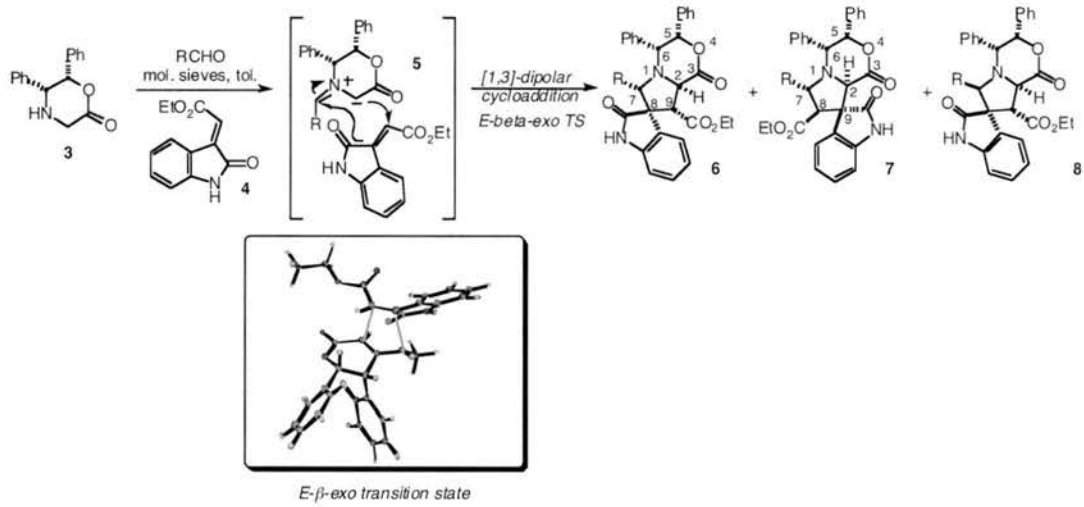


1, spirotryprostatin A **2**, spirotryprostatin B

Figure 1

Dedicated to Professor A. I. Meyers on the occasion of his 70th birthday.

Azomethine ylides derived from chiral, non-racemic glycinates have been shown to serve as effective templates for the synthesis of highly substituted pyrrolidines.[3] While a number of groups have explored the reaction of achiral azomethine ylides with oxindolylidene acetates,[4] to our knowledge there are no examples reported in the literature of [1,3] dipolar cycloaddition reactions between azomethine ylides derived from 5,6-diphenylmorpholin-2-one and oxindolylideneacetates. In our total synthesis of (-)-spirotryprostatin B, we demonstrated the viability of this approach for the formation of spirooxindole pyrrolidine derivatives.[5] In a continuation of this work, the effects of various aldehydes on the regio- and diastereoselectivity of this system and conversion of the cycloadducts to the corresponding amino acid methyl esters has been explored (Scheme 1).



Scheme 1

RESULTS AND DISCUSSION

The starting material for this investigation, (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (**3**), was conveniently prepared[6] from the commercially available *N-t*-BOC derivative.[7] Addition of an aldehyde to the amino lactone (**3**) in the presence 3Å molecular sieves in toluene generates an *E*/*Z* mixture of azomethine ylide (**5**). We have previously reported that in the case of sterically demanding aldehydes, the *E*-ylide was preferentially favored and that dipolar cycloadditions of ylides generated from this system proceeded with a high degree of *endo*-selectivity to give substituted pyrrolidines.[6] In the present system, the [1,3] dipole reacts with ethyl oxindolylidene acetate (**4**) *via* an *E-β-exo* transition state to yield spirooxindole cycloadducts (**6**) (Scheme 1). *E-β-exo* refers to the preferential formation of the *E*-azomethine ylide and approach of the dipolarophile *anti*- or *β*- to the phenyl groups with the carboethoxy

acting in an *exo*-fashion (inset, Scheme 1). Two other products were also isolated, **7** and **8**, and were the consequence of approach of the dipolarophile in an *endo*-fashion and *via* cycloaddition with the *Z*-ylide, respectively. The specific examples, reaction temperature, yields and diastereomeric ratios for **6**:**8** are recorded in Table 1.

Table 1. Spirooxindole Pyrrolidine Cycloadducts (**6**, **7** and **8**).

Entry	Aldehyde	R	Temp	Yield (% **6**)	Yield (% **7**)	Yield (% **8**)	Diast. ratio (**6**:**8**)
a	paraformaldehyde[a]	H	reflux	28	11	0	-
b	benzyloxy-acetaldehyde	$BzOCH_2$	reflux	44	14	0	>20:1
c	benzyloxy-acetaldehyde	$BzOCH_2$	60°C	54	8	0	>20:1
d	isobutyraldehyde	*i*-Pr	reflux	43	11	5	8.6:1
e	isobutyraldehyde	*i*-Pr	60°C	74	6	trace	>20:1
f	isovaleraldehyde	*i*-Bu	reflux	84	1	0	>20:1
g	isovaleraldehyde	*i*-Bu	60°C	86	0	0	>20:1
h	3-methoxy-3-methylbutanal[b]	$(Me)_2(OMe)CCH_2$	reflux	29	0	0	>20:1
i	3-methoxy-3-methylbutanal[c]	$(Me)_2(OMe)CCH_2$	60°C	82	1	0	>20:1
j	*p*-anisaldehyde[d]	*p*-MeOPh	reflux	60	0	0	>20:1

a) In addition, 9% of another compound was isolated, which was the result of addition of the dipolarophile in a regiochemically similar fashion to **7**, but with the carboethoxy group adding via an *exo*-approach. b) A second product (59%) was obtained as a result of elimination of the tertiary alcohol to afford the trisubstituted olefin derivative. c) The trisubstituted olefin was also obtained at 60°C for **6g**, although in reduced yield (6%). d) The reaction required prolonged heating (>24 h) to obtain the reported yield, whereas most reaction were complete between 2 and 8 h.

The regio- and stereochemistry of the resulting cycloadducts was dependent on the nature of the aldehyde constituents. Bulky aldehydes favored the formation of *E*-ylides and therefore cycloadducts (**6**), (Table 1, Entries f-j). *Ab initio* calculations on this system as well as a similar system[8] are in agreement with this observation. Isobutyraldehyde was expected to follow this trend, however the reaction produced three products, (**6d**, **7d** and **8d**) and resulted in an 8.6:1 diastereomeric ratio of **6d**:**8d**. For the less branched

systems, high diastereoselectivity resulted (>20:1), however only moderate *exo*-selectivity with respect to the carboethoxy group (*endo* for the oxindole) was observed, (Table 1, Entries a-c). The ylide generated from paraformaldehyde yielded three products, one of which was the result of the ester reacting in an *endo*-fashion, (**7a**). The more sterically demanding aldehydes resulted in high *exo*-selectivity as well as high diastereoselectivity (Table 1, Entries f-j).

Reaction temperature also seemed to affect the regiochemistry and stereochemistry of the resulting products. In the case of isobutyraldehyde moderate regioselectivity and diastereoselectivity was observed when the reaction was performed under refluxing toluene conditions. When the temperature of the system was lowered to 60 °C, the ratio of cycloadducts (**6**) and (**7**) was increased from ~4:1 to ~12:1 and the diastereomeric ratio of products (**6**) and (**8**) improved to greater than 20:1 (Table 1, Entries d and e). On the contrary, cycloaddition of the ylide derived from *p*-anisaldehyde (Entry j), required refluxing conditions for the reaction to occur. Presumably, the electron-donating effect of the methoxy group deters attack of the aldehyde by the incoming nucleophile and requires elevated temperatures for the formation of the azomethine ylide.

The regiochemistry of the cycloadducts were easily determined by the mutiplicity of the C2 hydrogen; a doublet was observed in the case of cycloadducts (**6**) and (**8**) whereas as singlet is exhibited for cycloadduct (**7**). The relative and absolute stereochemistry was determined by difference nOe ^{1}H NMR spectroscopy and correlation to the known stereogenic centers (C5 and C6) of the starting material (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (Figure 2). The structure of Entry **6i** was confirmed by single crystal X-Ray analysis as reported in the earlier account of the total synthesis of spirotryprostatin B.

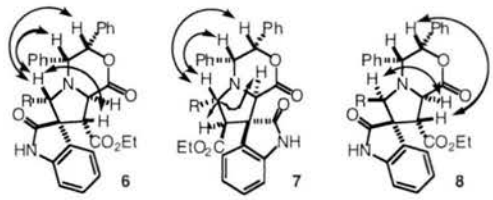
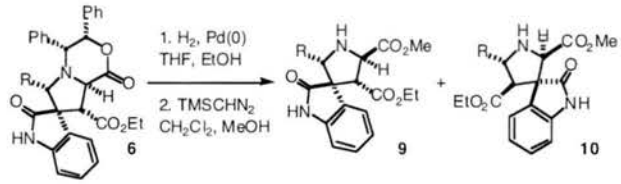


Figure 2. Observed nOe enhancements for cycloadducts (**6**, **7** and **8**).

Conversion of the tetracyclic products into the corresponding spirooxindole-substituted proline methyl ester derivatives was accomplished by catalytic hydrogenation (Scheme 2). For characterization purposes, the amino acids were converted to the corresponding methyl esters. Hydrogenolysis of the chiral auxiliary was accomplished in most cases by the use of palladium chloride under 70 psi of hydrogen for 36 hs (Table 2). However, *p*-anisaldehyde derivative (**6j**) proved resistant to these conditions and only partial

reduction was observed, (Table 2, Entry 5). Elevated temperatures and pressures resulted in a complex mixture of products. Pearlman's catalyst, which has been shown to selectively reduce the benzylic C-N bond of an unsubstituted aromatic in the presence of a *p*-methoxy derivative,[9] failed to dramatically improve formation of the desired product. The bulk of the reaction proved again, to be under-reduction. A search for alternative sources of Pd(0) yielded conditions[10] for the complete removal of the bibenzyl moiety (Table 2, Entry 7). Small amounts of epimerization at the α-position and cleavage of the pyrrolidine C-N bond were observed along with 59% of the desired product for the two steps. It is noteworthy to mention that any attempt to remove the chiral auxiliary *via* an oxidative protocol, such as $Pb(OAc)_4$ or $NaIO_4$ resulted in decomposition of the starting material. The electron-rich oxindole moiety presumably reacts with the oxidizing agents examined.



Scheme 2

Table 2. Conversion of dipolar cycloadducts (**6**) into amino acids methyl esters (**9**) and (**10**).

Entry	Substrate	Method	Yield (% **9** and **10**)
1	**6a**	H_2, $PdCl_2$	93
2	**7a**	H_2, $PdCl_2$	73
3	**6f**	H_2, $PdCl_2$	89
4	**6h**	H_2, $PdCl_2$	85
5	**6j**	H_2, $PdCl_2$	5
6	**6j**	H_2, $Pd(OH)_2$	25
7	**6j**	H_2, Pd-C, 1N HCl	59

CONCLUSION

In summary, the asymmetric syntheses of spirooxindole-substituted pyrrolidines *via* diastereoselective [1,3] dipolar cycloaddition of azomethine ylides derived from a chiral, non-racemic glycinate and ethyl oxindolylidene are described. The reaction is highly *exo*-selective for the carboethoxy group of the dipolarophile and sets three or four contiguous stereogenic centers including the quaternary carbon of a spirooxindole. In most cases two regioisomers were detected and were isolated with good to excellent diastereoselectivity. This methodology should find uslefu applications for the synthesis of

spirooxindole-substituted natural products and their derivatives.

ACKNOWLEDGMENT

Financial support is gratefully acknowledged from the National Science Foundation (CHE0202827).

EXPERIMENTAL

All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware (120°C) that was cooled in a dessicator, unless stated otherwise. Toluene was freshly distilled from CaH_2. THF was freshly distilled from sodium benzophenone ketyl. 3Å molecular sieves were activated by heating for three minutes at the highest setting in a microwave followed by cooling under argon. Column chromatography was performed on Merck silica gel Kiesel 60 (230-400 mesh). ^1H NMR, ^{13}C NMR, HSQC and nOe experiments were recorded on a Varian 400 MHz spectrometer. Spectra were recorded in $CDCl_3$ and chemical shifts (δ) were given in ppm and were relative to $CHCl_3$. MS were obtained on Fisons VG Autospec. IR spectra were recorded on a Perkin-elmer 1600 series FT-IR spectrophotometer. Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

General procedure for the [1,3] dipolar cycloaddition of oxindolyl acetates with azomethine ylides derived from (5*R*,6*S*)-5,6-diphenylmorpholin-2-one:

Method A (Reflux): To a flame dried 25 mL round bottom with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (253 mg 1.0 mmol), ethyl oxindolyl acetate (325 mg, 1.5 mmol) and 0.50 g of activated 3Å molecular sieves. An oven-dried condensor was attached and the system was flushed with Ar. Freshly distilled toluene (10 mL) followed by the aldehyde (1.2 mmol). The system was heated to reflux under Ar and kept at that temperature for two hs. The system was allowed to cool to rt, filtered through celite to remove the sieves and purified by flash chromatography using hexane/EtOAc as the eluents. Analytical samples were prepared by HPLC.

Method B (60°C): To a flame dried 25 mL round bottom with stir bar was added (*5R,6S*)-2,3,5,6-tetrahydro-5,6-diphenyl-1,4-oxazin-2-one (253 mg 1.0 mmol), ethyl oxindolylacetate (325 mg, 1.5 mmol) and 0.50 g of activated 3Å molecular sieves. The system was flushed with Ar. Freshly distilled toluene (10 mL) was added followed by the aldehyde (1.2 mmol). The system was warmed to 60°C under Ar, as measured by a thermocouple, and kept at that temperature for 2 h. The reaction was allowed to cool to rt, filtered through celite to remove the sieves and purified by flash chromatography using hexane/EtOAc as the eluents. Analytical samples were prepared by HPLC.

Cycloaddition of azomethine ylide derived from paraformaldehyde. Method B: From 360 mg of paraformaldehyde (10.0 mmol) was obtained 135 mg of **6a** (28%), 53 mg of **7a** (11%), and 43 mg of ***7a-exo*** (9%) as white amorphous solids after refluxing for 10 h and purification by flash chromatography on silica gel (2:1 hexanes:ethyl acetate). For **6a:** $[\alpha]_D^{25}$ = -32.0° (*c* 0.78, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.74 (br s, 1H), 7.27–7.21 (m, 10H), 7.09 (dd, J=1.6 Hz, 6.8 5.75 (d, J=4.0 Hz, 1H), 4.98 (d, J=8.8 Hz, 1H), 4.54 (d, J=4.0 Hz, 1H), 4.15 (d, J=8.8 Hz, 1H), 3.87-3.81 (m, 1H), 3.75-3.69 (m, 1H), 3.20 (d, J=9.6 Hz, 1H), 3.08 (d, J=9.6 Hz, 1H), 0.72 (t, J=6.8 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.2, 171.4, 168.7, 140.8, 136.1, 134.4, 129.8, 129.1, 128.8, 128.7, 128.4, 127.9, 124.6, 123.2, 110.1, 84.4, 67.5, 63.7, 61.4, 60.7, 55.5, 54.3, 13.6;. **IR** (neat) 3302, 1735, 1618; **HRMS** (FAB+) Calcd for $C_{29}H_{27}N_2O_5$ (*m/z*) 483.1920, found 483.1917; **NOE** data: irradiation of H_6 enhanced H_7-α: (3.45%); irradiation of H_7-α enhanced H_9 (3.61%). For **7a:** $[\alpha]_D^{25}$ = - 111.0° (*c* 1.09, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.70 (br s, 1H), 7.23–7.13 (m, 9H), 7.02 (t, J = 7.6 Hz, 1H), 7.04-6.97 (m, 2H), 6.88 (d, J = 7.6 Hz, 1H), 6.81 (d, J = 7.6 Hz, 2H), 5.52 (d, J = 4.0 Hz, 1H), 5.02 (d, J = 4.0 Hz, 1H), 4.77 (s, 1H), 3.70-3.60 (m, 2H), 3.56 (dd, J = 6.4 Hz, J = 10.8 Hz, 1H), 3.46 (t, J = 10.8 Hz, 1H), 3.32 (dd, J = 6.4 Hz, J = 10.8 Hz, 1H), 0.68 (t, J=6.8 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.7, 169.8, 167.9, 141.5, 135.4, 134.7, 130.3, 129.4, 129.3, 128.6, 128.5, 128.4, 127.6, 123.7, 123.0, 110.2, 86.2, 72.9, 62.6, 61.6, 60.9, 54.1, 51.5, 13.6. **IR** (neat) 3307, 1726, 1620 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{29}H_{27}N_2O_5$ (*m/z*) 483.1920, found 483.1911. For **7a–*exo*:** $[\alpha]_D^{25}$ = -123.0° (*c* 0.43, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.70 (br s, 1H), 7.33 (d, J=7.2 Hz, 1H), 7.23–6.95 (m,12H), 6.88 (d, J=7.2 Hz, 1H), 5.69 (d, J=4.0 Hz, 1H), 4.67 (d, J=4.0 Hz, 1H), 4.54 (s, 1H), 3.75-3.60 (m, 2H), 3.60 (t, J=8.4 Hz, 1H), 3.51 (t, J=9.2 Hz, 1H), 3.25 (t, J=9.2 Hz, 1H), 0.68 (t, J=7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 176.9, 169.2, 166.7, 142.2, 134.7, 133.5, 129.5, 129.4, 128.6, 128.4, 128.1, 127.9, 127.0, 124.4, 122.8, 110.1, 85.6, 68.7, 65.4, 61.1, 58.3, 52.5, 50.0, 13.5; **IR** (neat) 3313, 1731, 1619 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{29}H_{27}N_2O_5$ (*m/z*) 483.1920, found 483.1904; **NOE** data: irradiation of H_2 enhanced H_8 (1.54%).

Cycloaddition of azomethine ylide derived from benzyloxyacetaldehyde. Benzyloxyacetaldehyde (180 mg, 1.2 mmol) was prepared according to literature procedure.[11] **Method A:** 265 mg of **6b** (44%) and 85 mg of **7b** (14%) were obtained as white amorphous solids. **Method B:** 325 mg of **6b** (54%) and 50 mg of **7b** (8%) were obtained as white amorphous solids. For **6b:** $[\alpha]_D^{25}$ = -32.5° (*c* 0.56, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.17 (br s, 1H), 7.24-6.95 (m, 18H), 6.85 (d, J=7.6 Hz, 1H), 4.89 (d, J=8.0 Hz, 1H), 4.75 (d, J=3.2 Hz, 1H), 4.12-4.05 (m, 3H), 4.00 (d, J=8.0 Hz, 1H), 3.77-3.73 (m, 1H), 3.73-3.65 (m, 1H), 3.23 (dd, J = 6.0 Hz, J = 9.6 Hz, 1H), 3.06 (dd, J=4.2 Hz, J = 9.6 Hz, 1H), 0.68 (t, J=7.2 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 171.4, 168.2, 141.4, 137.4, 136.4, 135.8, 129.3, 129.1, 128.5, 128.4, 128.0, 127.9, 127.8, 126.2, 126.1, 122.8, 109.9, 78.2, 73.7, 70.9, 69.9, 62.2, 61.5, 58.6, 58.2, 54.5, 13.6; **IR** (neat) 3269, 1732, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{37}H_{35}N_2O_6$ (*m/z*) 603.2495, found 603.2477; **NOE** data: irradiation of H_6 enhanced H_7 (3.07%). For **7b:** $[\alpha]_D^{25}$ = -161.8° (c 0.22, $CHCl_3$); **^{1}H NMR** (400 MHz, CDCl3) δ 7.69 (br s, 1H), 7.34 (d, J=7.2 Hz, 1H), 7.22-7.12 (m, 12H) 7.04-6.98 (m, 4H), 6.86 (t, J = 7.2 Hz, 2H), 5.61 (d, J = 3.6 Hz, 1H), 5.06 (s, 1H), 4.87 (d, J = 3.6 Hz, 1H), 4.37 (1/2ABq, J = 12.0 Hz, 1H), 4.29 (1/2ABq, J = 12.0 Hz, 1H), 4.30-4.24 (m, 1H), 3.65-3.60 (m, 1H), 3.46 (dd, J = 4.0 Hz, J = 9.6 Hz, 1H), 3.36 (dd, J = 4.8 Hz, J = 9.6 Hz, 1H), 0.66 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, CDCl3) δ 177.0, 169.0, 167.0, 142.2, 138.2, 136.0, 135.3, 129.7, 129.6, 128.6, 128.5, 128.2, 128.1, 127.6, 127.1, 124.6, 122.7, 110.4, 83.5, 73.3, 72.3, 69.9, 65.5, 64.1, 61.1, 60.2, 52.9, 13.5; **IR** (neat) 3269, 1732, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{37}H_{35}N_2O_6$ (m/z) 603.2495, found 603.2483.

Cycloaddition of azomethine ylide derived from isobutyraldehyde. Isobutyraldehyde (86 mg, 1.2 mmol) was used as received from Aldrich. **Method A:** 225 mg of **6c** (43%), 73 mg of **7d** (11%) and 25 mg of **8d** (5%) were obtained as white amorphous solids. Method B: 387 mg of **6d** (74%), 30 mg of **7d** (6%) and a trace amount of **8d** (<1%) were obtained as white amorphous solids. For **6d:** $[\alpha]_D^{25}$ = -58.8° (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.77 (br s, 1H), 7.31–7.16 (m, 10 H), 7.08–6.91 (m, 4H), 6.19 (d, J = 4.0 Hz, 1H), 5.12 (d, J = 13.2 Hz, 1H), 4.36 (d, J = 4.0 Hz, 1H), 3.86 (d, J = 13.2 Hz, 1H), 3.85-3.73 (m, 3H), 1.88 (sept, J = 9.2 Hz, 1H), 0.86 (d, J = 9.2 Hz, 3H), 0.81 (t, J = 9.6 Hz, 3H), 0.63 (d, J=9.2 Hz, 3H); **^{13}C NMR** (100 MHz, CDCl3) δ 178.3, 170.1, 167.2, 140.9, 136.0, 135.7, 129.5, 129.0, 128.5, 128.2, 127.8, 127.6, 126.3, 126.1, 122.7, 110.1, 77.5, 76.4, 64.3, 61.5, 59.8, 59.5, 56.8, 30.8, 20.5, 19.2, 13.7;. **IR** (neat) 3288, 1729, 1618 cm^{-1}; HRMS (FAB+) Calcd for $C_{32}H_{33}N_2O_5$ (m/z) 525.2389, found 525.2390. NOE data: irradiation of H9 enhanced H5 (2.62%). For 7d: $[\alpha]_D^{25}$ = -20.7° (c 0.9, $CHCl_3$); **^{1}H NMR** (400 MHz, CDCl3) δ 7.87 (br s,1H), 7.43 (d, J = 10.0 Hz, 1H), 7.37–7.03 (m, 9H), 6.97-6.83 (m, 4H), 5.59 (d, J = 4.8 Hz, 1H), 5.03 (s, 1H), 4.71 (d, J = 4.8 Hz, 1H), 4.14 (dd, J = 6.0, J = 12.0 Hz, 1H), 3.74-3.63 (m, 2H), 3.60 (d, J = 12.0 Hz, 1H), 1.86-1.80 (m, 1H), 0.96 (d, J = 9.2 Hz, 3H), 0.68 (t, J = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.3, 169.8, 167.1, 142.4, 136.2, 135.2, 129.7, 129.6, 128.7, 128.3, 128.1, 128.0, 127.8, 126.8, 125.4, 122.5, 110.6, 84.4, 71.2, 71.1, 64.9, 61.9, 61.1, 51.7, 32.0, 18.6, 17.0, 13.5;. **IR** (neat) 3300, 1727, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{32}H_{33}N_2O_5$ (*m/z*) 525.2389, found 525.2378; **NOE** data: irradiation of H_5 enhanced H_7 (4.16%). For **8d:** $[\alpha]_D^{25}$ = -24.0° (*c* 0.5, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.98 (br s, 1H), 7.32–7.21 (m, 10H), 7.06–7.04 (m, 2H), 6.94 (t, J = 10.4 Hz, 1H), 6.89 (d, J = 10.0 Hz, 1H), 6.68 (d, J = 10.0 Hz, 1H), 6.50 (d, J = 5.2 Hz,

1H), 4.81 (d, J = 5.2 Hz, 1H), 4.74 (d, J = 14.0 Hz, 1H), 3.90 (d, J = 14.0 Hz, 1H), 3.80-3.69 (m, 2H), 3.21 (d, J = 10.4 Hz, 1H), 2.51 (sept, J = 9.2 Hz, 1H), 0.88 (d, J = 10.4 Hz, 3H), 0.79 (d, J = 10.4 Hz, 3H), 0.74 (t, J = 9.6 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.8, 168.6, 140.3, 140.9, 136.2, 133.9, 130.9, 129.7, 128.8, 128.2, 128.1, 127.8, 126.1, 125.0, 122.9, 109.7, 82.7, 77.4, 61.3, 61.1, 60.6, 58.6, 57.0, 28.1, 20.6, 18.9, 13.6;. **IR** (neat) 3296, 1734, 1715, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{32}H_{33}N_2O_5$ (*m/z*) 525.2389, found 525.2386; **NOE** data: irradiation of H_5 enhanced H_9 (6.88%); irradiation of H_7 enhanced H_2 (4.30%).

Cycloaddition of azomethine ylide derived from isovaleraldehyde. Isovaleraldehyde (103 mg, 1.2 mmol) was used as received from Aldrich. **Method A:** 452 mg of **6f** (84%) was obtained as white amorphous solids and a trace amount of **7f** (~1%) was observed in the ^{1}H NMR spectra but not isolated. **Method B:** 463 mg of **7f** (86%) was obtained as a white amorphous solid. For **7f:** $[\alpha]_D^{25}$ = +62.7° (*c* 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.63 (br s, 1H), 7.31–7.20 (m, 12H), 7.00 (t, J = 7.6 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 6.11 (d, J = 3.2 Hz, 1H), 6.50 (d, J = 5.2 Hz, 1H), 4.87 (d, J = 8.0 Hz, 1H), 4.48 (d, J = 3.2 Hz, 1H), 3.99 (d, J = 8.0 Hz, 1H), 3.81-3.64 (m, 3H), 1.34-1.17 (m, 2H), 1.00-0.93 (m, 3H), 0.74 (d, J = 6.4 Hz, 3H), 0.70 (d, J = 7.2 Hz, 3H), 0.62 (t, J = 6.4 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.2, 171.5, 168.4, 141.4, 136.3, 136.0, 129.2, 129.1, 128.6, 128.5, 128.2, 128.0, 126.6, 126.5, 126.2, 122.9, 109.9, 77.6, 68.8, 61.5, 60.8, 59.8, 58.2, 54.8, 39.9, 25.8, 23.7, 22.6, 13.6;. **IR** (neat) 3284, 1732, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{33}H_{35}N_2O_5$ (*m/z*) 539.2546, found 539.2544; **NOE** data: irradiation of H_5 enhanced H_9 (2.17%).

Cycloaddition of azomethine ylide derived from 3-methyl-3-methoxybutanal. 3-Methyl-3-methoxybutanal (116 mg, 1.2 mmol) was prepared by Swern oxidation of 3-methyl-3-methoxybutan-ol, which is commercially available from Aldrich. **Method A:** 165 mg of **6h** (29%) and 335 mg of **6h-*elim*** (59%) were obtained as white amorphous solids. **Method B:** 465 mg of **6h** (82%), 34 mg of **6h-*elim*** (6%) and 5 mg of **7h** (1%) were obtained as white amorphous solids. For **6h:** $[\alpha]_D^{25}$ = -14.0° (c 1.0, CH_2Cl_2); melting point: 225-227 °C; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.09 (br s, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.18-7.33 (m, 10H), 7.15 (d, J = 7.5 Hz, 1H), 7.00 (dt, J = 0.9 Hz, 7.5Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.40 (d, J=3.3 Hz, 1H), 5.07 (d, J = 3.3 Hz, 1H), 4.65 (d, J = 7.5 Hz, 1H), 4.04 (t, J = 3.3 Hz, 1H), 3.95 (d, J = 7.5 Hz, 1H), 3.63-3.85 (m, 2H), 3.08 (s, 3H), 1.70 (d, J = 3.3 Hz, J = 15.9 Hz, 2H), 1.14 (dd, J = 3.6 Hz, J = 16.2 Hz, 2H), 1.09 (s, 6 H), 0.68 (t, J = 6.9, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) δ $CHCl_3$: 178.9, 173.0, 170.1, 142.3, 138.3, 137.5, 130.4, 130.2, 129.5, 129.4, 128.5, 128.4, 128.3, 127.2, 126.2, 124.0, 110.8, 77.1, 74.5, 65.5, 61.5, 57.5, 57.1, 56.0, 53.3, 50.6, 45.4, 26.9, 23.6; **IR** (NaCl/neat) 3308, 1734, 1618 cm^{-1}

An X-Ray crystal structural analysis for this compound has been previously reported.[5] For **6h-*elim*: [α]** $^{25}_{D}$ = +52.8° (c 0.95, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 (br s, 1H), 7.01-7.35 (m, 12H), 6.84 (d, J = 7.6 Hz, 2H), 6.41 (d, J = 2.8 Hz, 1H), 5.0 (s, 1H), 4.69 (d, J = 7.6 Hz, 1H), 4.53 (m, 1H), 4.00-4.14 (m, 2H), 3.41 (d, J = 6.0 Hz, 1H), 3.18 (s, 3H), 1.80-1.94 (m, 2H), 1.19 (s, 3 H), 1.16 (s, 3 H), 1.11 (t, J = 6.8 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.6, 171.8, 168.6, 141.4, 136.4, 136.0, 129.2, 129.0, 128.6, 128.3, 128.1, 127.8, 126.9, 126.1, 125.9, 122.7, 119.8, 109.8, 78.0, 68.7, 61.4, 60.2, 59.8, 57.3, 54.1, 26.2, 18.8, 13.5; **IR** (NaCl/neat) 3305, 1730, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{33}H_{33}N_2O_5$ (*m/z*) 537.2389, found 537.2383. For **7h: $[\alpha]_D^{25}$** = +118.1° (c 1.05, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 (br s, 1H), 7.16-7.28 (m, 12H), 6.97, (t, J = 6.8 Hz, 1H), 6.84 (d, J = 6.8 Hz, 1H), 6.08 (d, J = 3.6 Hz, 2H), 5.0 (s, 1H), 4.87 (d, J = 7.6 Hz, 1H), 4.51 (s, 1H), 4.50 (t, J = 7.6 Hz, 1H), 4.36 (d, J = 3.6 Hz, 1H), 4.04 (d, J = 7.6 Hz, 1H), 3.78-3.83 (m, 1H), 3.46-3.68 (m, 1H), 1.67 (s, 3H), 1.42 (s, 3H), 0.64 (t, J = 6.8, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ $CHCl_3$: 179.4, 170.4, 169.5, 141.1, 137.1, 137.0, 129.4, 129.2, 128.3, 128.0, 127.8, 127.7, 127.4, 126.5, 124.7, 123.1, 110.3, 79.2, 73.7, 65.6, 60.7, 60.5, 60.4, 59.8, 56.1, 49.5, 43.0, 24.8, 24.7, 14.2; **IR** (NaCl/neat) 3288, 1718, 1621 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{33}H_{37}N_2O_6$ (*m/z*) 569.2652, found 569.2640.

Cycloaddition of azomethine ylide derived from *p*-anisaldehyde. *p*-Anisaldehyde (163 mg, 1.2 mmol) was used as received from Aldrich. **Method A:** 353 mg of **6j** (60%) was obtained as white amorphous solid. For **6j: $[\alpha]_D^{25}$** = +80.8° (*c* 0.47, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.67 (br s, 1H), 7.26–7.04 (m, 15H), 6.91 (t, J = 7.6 Hz, 1H), 6.61 (d, J = 7.6 Hz, 2H), 6.22 (d, J = 3.2 Hz, 1H), 5.12 (d, J = 8.0 Hz, 1H), 4.95 (s, 1H), 4.17 (d, J = 3.2 Hz, 1H), 4.09 (d, J = 8.0 Hz, 1H), 3.87-3.79 (m, 1H), 3.72-3.64 (m, 4H), 0.63 (m, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 176.8, 171.6, 168.4, 140.6, 136.0, 135.8, 129.4, 129.1, 129.0, 128.6, 128.4, 127.9, 126.8, 126.0, 125.7, 125.6, 122.4, 113.8, 109.6, 76.2, 74.6, 61.5, 61.4, 59.0, 57.1, 55.3, 54.4, 13.5; **IR** (neat) 3296, 1728, 1612 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{36}H_{33}N_2O_6$ (*m/z*) 589.2338, found 589.2327; **NOE** data: irradiation of H_7 enhanced H_5 (10.2%) and H_9 (4.38%)

General procedure for the reduction of spirooxindole pyrrolidine derivatives to the corresponding amino acid methyl esters: The cycloadducts (0.1 mmol) were taken up in THF:MeOH 1:1 (2 mL) and transferred to a pressurizable tube. Argon was bubbled through for 5 min and $PdCl_2$ (18 mg, 0.1 mmol) added. The system was sealed and hydrogenated (65-75 Psi) for 36 h at rt. The heterogeneous solution was filtered through celite and evaporated under reduced pressure. The resulting oil was taken up in CH_2Cl_2:MeOH 1:1 (2 mL), a stir bar added and $TMSCHN_2$, available from Aldrich as a 2.0 M solution in hexanes, was added until a yellow color persisted. The reaction was stirred for 15 min and then

evaporated under reduced pressure. Purification by flash chromatography using hexanes/EtOAc as the eluents yielded white amorphous solids. Analytical samples were prepared by PTLC.

Amino acid methyl ester (9a): Prepared by hydrogenation of cycloadduct (**6a**) (50 mg, 1.0 mmol). Column chromatography with 2:1 hexanes:EtOAc yielded 30 mg (93%) of **9a** as a white amorphous solid. For **9a:** $[\alpha]_D^{25}$ = -23.0° (*c* 0.40, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 7.24 (d, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 6.98 (t, J = 7.6 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 4.62 (d, J = 7.6 Hz, 1H), 3.82-3.73 (m, 1H), 3.79 (s, 3H), 3.73-3.68 (m, 1H), 3.47 (1/2ABq, J = 10.8 Hz, 1H), 3.10 (1/2ABq, J = 10.8 Hz, 1H), 2.76 (br s, 1H), 0.69 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.1, 173.5, 169.6, 140.6, 130.1, 128.9, 124.4, 123.0, 109.7, 62.3, 61.2, 58.5, 58.0, 56.6, 52.9, 13.6; **IR** (neat) 3303, 1732, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{16}H_{19}N_2O_5$ (*m/z*) 319.1294, found 319.1286.

Amino acid methyl ester (10a): Prepared by hydrogenation of cycloadduct (**7a**) (50 mg, 1.0 mmol). Column chromatography with 2:1 hexanes:EtOAc yielded 24 mg (73%) of **10a** as a white amorphous solid. For **10a:** $[\alpha]_D^{25}$ = -61.1° (*c* 0.61, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.51 (br s, 1H), 7.19 (dt, J = 0.8 Hz, J = 7.6 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 6.94 (dt, J = 0.8 Hz, J = 7.6 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 4.34 (s, 1H), 3.87-3.63 (m, 4H), 3.53 (dd, J = 8.4 Hz, J = 10.8 Hz, 1H), 3.23 (s, 3H), 2.76 (br s, 1H), 0.70 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.4, 170.2, 169.7, 141.3, 129.2, 127.0, 124.6, 122.7, 109.6, 70.2, 61.0, 59.6, 54.2, 52.1, 47.8, 13.6; **IR** (neat) 3326, 1730, 1615 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{16}H_{19}N_2O_5$ (*m/z*) 319.1294, found 319.1289.

Amino acid methyl ester (9f): Prepared by hydrogenation of cycloadduct (**6f**) (50 mg, 0.9 mmol). Column chromatography with 2:1 hexanes:EtOAc yielded 31 mg (89%) of **9f** as a white amorphous solid. For **9f:** $[\alpha]_D^{25}$ = +24.8° (*c* 0.64, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.18 (br s, 1H), 7.24 (d, J = 7.6 Hz, 1H), 7.20 (dt, J = 1.2 Hz, J = 7.6 Hz, 1H), 6.98 (dt, J = 1.2 Hz, J = 7.6 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 4.61 (d, J = 6.8 Hz, 1H), 3.84 (d, J = 6.8 Hz, 1H), 3.80-3.75 (m, 1H), 3.78 (s, 3H), 3.68-3.60 (m, 2H), 2.59 (br s, 1H), 1.50-1.45 (m, 1H), 0.95-0.87 (m, 1H), 0.79-0.72 (m, 1H), 0.76 (d, J = 6.8 Hz, 6H), 0.65 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.2, 174.9, 169.4, 141.1, 128.7, 127.5, 125.8, 122.7, 109.6, 65.5, 61.2, 59.3, 55.8, 52.9, 39.2, 25.8, 23.5, 22.2, 13.5; **IR** (neat) 3325, 1728, 1617 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{20}H_{27}N_2O_5$ (*m/z*) 375.1920, found 375.1922.

Amino acid methyl ester (9h): Prepared by hydrogenation of cycloadduct (**6h**) (50 mg, 1.0 mmol). Column chromatography with 2:1 hexanes:EtOAc yielded 30 mg (93%) of **9h** as a white amorphous

solid. For **9h:** $[\alpha]_D^{25}$ = -27.3° (c 0.97, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.98 (br s, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.18 (dt, J= 0.8 Hz, J = 7.6 Hz, 1H), 6.96 (dt, J = 0.8 Hz, J = 7.6 Hz, 1H), 6.82 (d, J = 7.6 Hz, 1H), 4.58 (d, J = 8.8 Hz, 1H), 3.80-3.72 (m, 1H), 3.76 (s, 3H), 3.70 (d, J = 8.8 Hz, 1H), 3.66-3.58 (m, 1H), 3.17 (br s, 1H), 3.08 (s, 3H), 1.19 (dd, J = 9.6 Hz, 14.4 Hz, 1H), 1.10 (s, 3H), 0.99 (s, 3H), 0.90 (dd, J = 1.6 Hz, J = 14.4 Hz, 1 H), 0.63 (t, J = 6.8 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.0, 175.2, 169.4, 140.9, 128.6, 127.8, 126.2, 122.7, 109.4, 74.4, 63.7, 61.1, 61.0, 59.1, 54.9, 52.8, 49.4, 40.6, 25.8, 24.4, 13.5; **IR** (neat) 3244, 1734, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{21}H_{29}N_2O_6$ (*m/z*) 405.2025, found 405.2024.

Amino acid methyl ester 9j: Prepared by hydrogenation of cycloadduct **6j** (50 mg, 1.0 mmol). Column chromatography with 2:1 hexanes:EtOAc yielded 30 mg (93%)of **9j** as a white amorphous solid. For **9j:** $[\alpha]_D^{25}$ = +30.8° (*c* 0.65, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.51 (br s, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H), 6.99 (t, J = 7.2 Hz, 1H), 6.53 (d, J = 8.8 Hz, 3H), 4.77 (d, J = 6.8 Hz, 3H), 4.73 (s, 1H), 3.96 (d, J = 6.8 Hz, 3H), 3.82-3.75 (m, 1H), 3.80 (m, 3H), 3.69-3.59 (m, 1H), 3.62 (m, 3H), 2.79 (br s, 1H), 0.69 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.4, 175.1, 169.3, 159.3, 140.4, 128.6, 128.3, 126.9, 126.5, 122.2, 113.1, 109.2, 70.1, 62.4, 61.2, 58.2, 55.2, 54.8, 52.9, 13.5; **IR** (neat) 3265, 1735, 1713, 1618 cm^{-1}; **HRMS** (FAB+) Calcd for $C_{23}H_{25}N_2O_6$ (*m/z*) 425.1713, found 425.1706.

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Appendix 5

Research Proposal

Asymmetric Azomethine Ylide [1,3]-Dipolar Cycloaddition with Ethyl Oxindolylidene Acetate

Introduction

Recently, Ishii and coworkers isolated a novel Ras-farnesyltransferase inhibitor designated TAN-1813 from the culture broth of the *Phoma* sp. FL-41510 fungus strain.[1] The importance of small binding proteins such as Ras proteins in regulating cell proliferation and differentiation has been well documented.[2] While the IC_{50} value of 47 μg/ml for the inhibition of rat brain farnesyltransferase was only moderate, TAN-1813 (Figure 1) represents a possible lead as an anti-cancer compound.

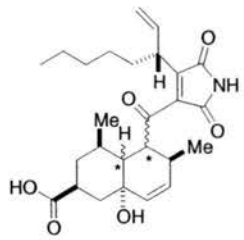
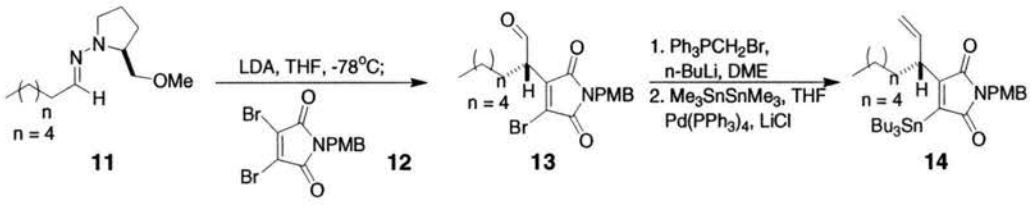


Figure 1. Structure of TAN-1813. * Denotes unknown stereochemistry.

While the potential therapeutic uses alone warrant synthetic investigation, the novel structure adds to the need for a route to TAN-1813. The seven stereocenters, highly functionalized [4.4.0] bicyclic ring system and the di-substituted maleimide make it an intriguing compound. In addition, the absolute and relative configuration is unknown as two of the seven stereocenters have not been defined. This information combined with the interesting structure and potential applications as a chemotherapeutic substantiate the need for a total synthesis TAN-1813.

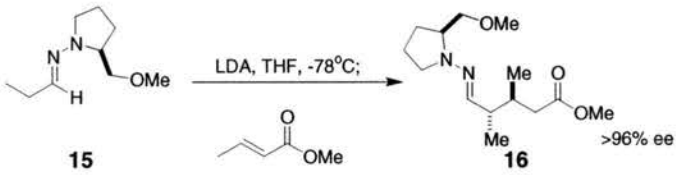
In contemplating the total synthesis of TAN-1813 a number of considerations had to be taken into account. First, the strategy must allow for the stereoselective generation of all four possible diastereomers. Interestingly, it is not known why NMR studies have not been able to determine the relative configuration of all stereocenters. A synthesis will allow for a number of intermediates that should provide both the absolute and relative stereochemistry. Secondly, installation of the maleimide group will need to occur late in the synthesis due to the limitations imposed by the electrophilicity of the maleimide. Additionally, introduction of the maleimide last would help determine if it is necessary for the biological activity of TAN 1813. Synthesis and biological evaluation of each piece would help elucidate which portion of the molecule is important for the natural products biological activity. Lastly, an overall convergent approach would be the most efficient and would allow for a number of different analogues to be generated. With these considerations in mind, a novel retrosynthetic analysis for the synthesis of TAN-1813 **1** (Scheme 1). The key transformations to this approach involve formation of the maleimide precursor **3** through an asymmetric conjugate addition and formation of the core bicyclic ring **7** through an asymmetric Ene cyclization.

The natural product will result from a Stille reaction between acid chloride **2** and maleimide **3**. The maleimide **3** will be generated from conjugate addition of a chiral hydrazone **4** to dibromomaleimide **5**. The core bicyclic acid chloride **2** serves as the backbone of TAN 1813 and will be formed from the ester **6** after chemoselective epoxidation and reduction. Formation of compound **6** will in turn, be generated by two different routes. The first strategy involves displacement of the alcohol by cyanide, hydrolysis and methyl ester formation. The second strategy will involve enone formation



Scheme 2. Synthesis of Stille precursor 14.

The addition/elimination will be expected to proceed with nearly complete enantioselectivity as Enders and Redenbach have shown that the SAMP hydrazones can be added to Michael acceptors with high stereoselectivities.[4] Hydrazone **15** was added to methyl-2-butenoate to yield **16** in quantitative yield and in greater than 96% enantioselectivity (Scheme 3). The choice of commercially available dibromomaleimide as the Michael acceptor in the reaction was based on its use in a number of syntheses,[5] and will be expected to react as predicted. This protocol will however, require protection of the maleimide nitrogen due to its acidity. The Mitsunobu reaction with para-methoxybenzyl alcohol has proven a reliable method for such alkylations.[6]



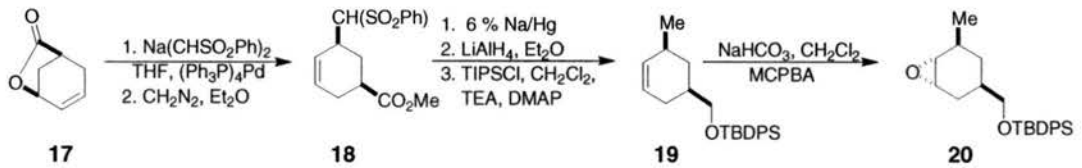
Scheme 3. Asymmetric conjugate addition using SAMP hydrazone.

Installation of the terminal olefin may require more extensive studies as the integrity of the newly formed stereocenter may not withstand standard methods for the formation of the exo-methylene, such as a Wittig reaction. The use of non-basic titanium-based methylenating reagents such as Petasis' reagent, Cp_2TiMe_2,[7] or Nozaki's reagent, $CH_2I_2/Zn/TiCl_4$,[8] will provide an alternative to the classical methods. Upon generation of

the terminal olefin, conversion of the bromide to the vinyl tin will commence. Palladium-catalyzed stannylations[9] have proven tolerant of various functionalities and will be expected to yield Stille precursor **14**.

Synthesis of Intermediates 9 and 10

Synthon **9** will be generated starting with known lactone **17** (Scheme 4).[10] Trost and coworkers have shown that palladium-catalyzed allylic alkylation of **17** with the sodium salt of bis(benzenesulfonyl)methane proceeds stereospecifically to yield **18** upon esterification with diazomethane.[11] Additionally, they proved that the reduction of the sulfonyl groups could be accomplished with 6% sodium-mercury amalgam. Further reduction of the ester to the primary alcohol will be accomplished by lithium aluminum hydride. Protection of the resulting alcohol as the tert-butyldiphenylsilyl ether **19** will provide a robust protecting group as it will not be removed until the late stages of the synthesis.[12] Lastly, epoxidation with meta-chloroperbenzoic acid will furnish the desired compound **20**.

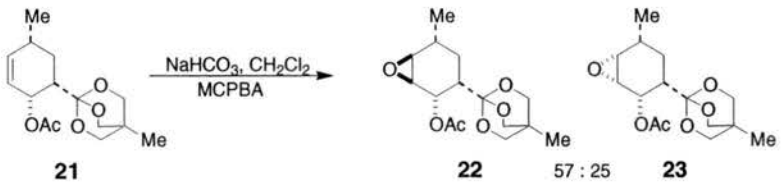


17 **18** **19** **20**

Scheme 4. Synthesis of Epoxide **20**.

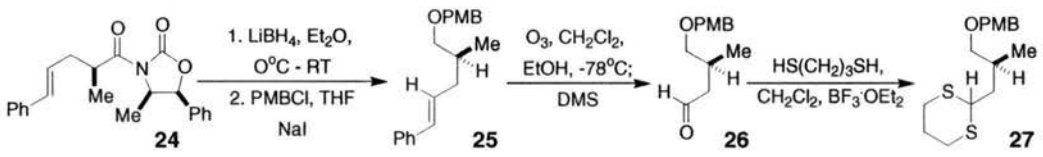
Based on the work of Barrett et al., the epoxidation is expected to occur opposite the two alkyl groups. They showed that oxirane formation of a similar substrate **21**, proceeded stereoselectively away from the two alkyl groups to give a ~2:1 mixture of separable diastereomers (**22** and **23**) (Scheme 5).[13] The similarities between substrates **20**

and **21** suggest that the *syn* configuration and *meta* orientation of the methyl group and alkoxy group in cyclohexene derivative **20** will provide steric hindrance so as to favor approach of the electrophilic oxygen source opposite the side-chains. While recent studies provide alternative methods that may improve the diastereomeric ratio above that of the Barrett protocol, efforts will not focus heavily on improving the ratio as the proposed strategy will provide copious amounts of the desired product.



Scheme 5. Diastereoselective epoxidation.

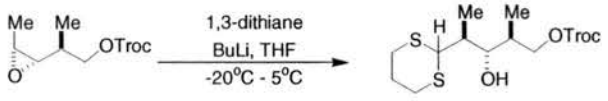
The straightforward synthesis of synthon **10** will begin with known oxazolidinone **24** (Scheme 6).[14] Reduction with lithium borohydride followed by protection of the resulting alcohol as the *p*-methoxybenzyl ether according to a literature protocol will generate **25**.[15] Ozonolysis followed by reductive work-up with dimethyl sulfide will result in the formation of aldehyde **26**. As this strategy mirrors the reference cited, except for the choice of the protecting group for the primary alcohol, the sequence will proceed as shown. Addition of propane dithiol in the presence of boron trifluoride etherate will then convert the aldehyde to yield cyclic dithiane **27**.[16]



Scheme 6. Synthesis of Dithiane **27**.

Coupling and Elaboration to Acid Chloride 2

With the requisite compounds in hand, elaboration to the desired acid chloride **2** will begin with the addition of **27** to the epoxide **20**. Oishi and coworkers have shown that lithiodithianes add with complete regioselectively to disubstituted oxiranes with a directing allylic methyl group (Scheme 7).[17] Approach of the bulky lithiodithiane occurred from the top face and away from the branched methyl group to yield only alcohol **29**.

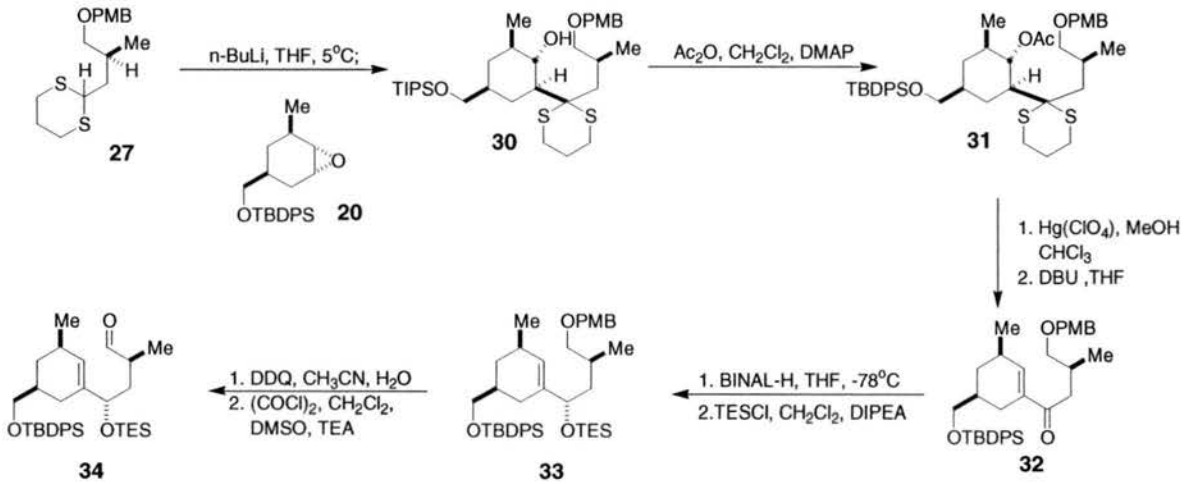


28 **29**

Scheme 7. Nucleophilic opening of oxirane 28 by dithiane.

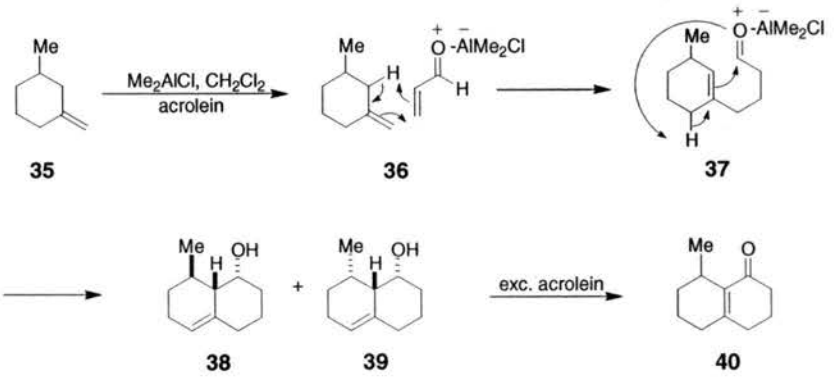
While the cited example is acyclic, the same rational will be used in the cyclic case, suggesting that addition of **27** to **20** will yield **30** exclusively (Scheme 8).[18] The methyl group is expected to create steric bulk above the bridgehead carbon of the epoxide and direct the nucleophile to the opposite terminus. Acetylation with acetic anhydride will provide **31**. Standard mercury perchlorate mediated conversion of dithiane **31** to the ketone will be used followed by base induced elimination of the acetate to give enone **32**.[19] At this stage it will be necessary to asymmetrically reduce the enone to the resulting allylic alcohol, which will be later utilized to direct an epoxidation. Noyori's binapthylaluminum hydride has proven to be a quite general method for the asymmetric reduction of enones to the corresponding allylic alcohol (Scheme 11).[20] While the catalyst should direct the hydride source opposite the alkyl groups, it is plausible that only moderate diastereoselectivity will be obtained, in which case the triisopropyl group

could be easily removed and used to direct the reduction from the top face to give the stereochemistry shown in **33**. Protection of the resulting alcohol as the triethylsilyl ether (TES) will provide an orthogonal group that can be selectively cleaved in the presence triisopropylsilyl ether. Some exploration in choice of protecting group maybe required as the TES group may prove to labile. Deprotection of the para-methoxybenzyl ether with dichlorodicyano quinone[21] followed by Swern oxidation[22] will generate the aldehyde **34**.



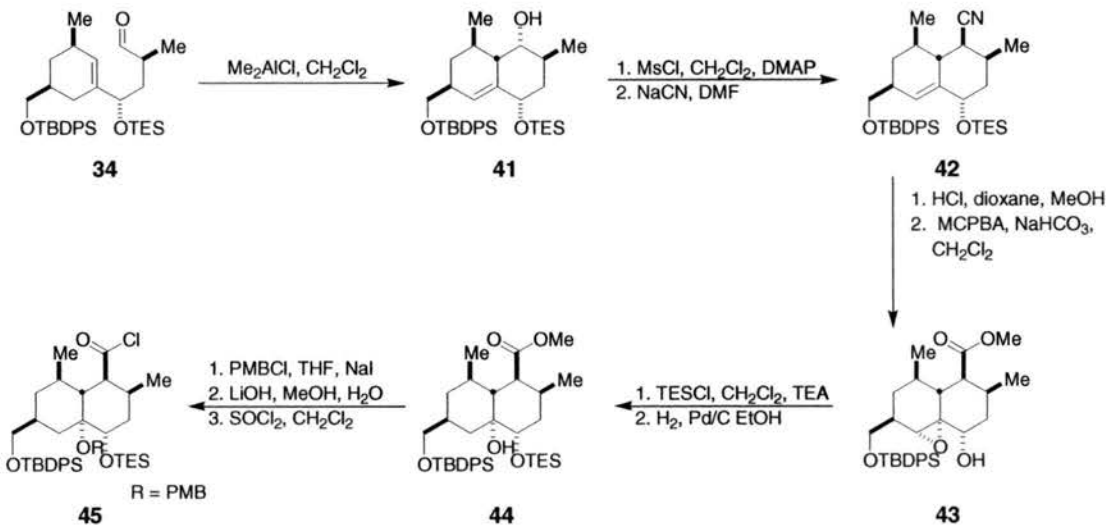
Scheme 8. Synthesis of Ene Precursor 27.

With the key precursor **34** in hand, the Ene cyclization will be attempted. Snider and Goldman have shown that reaction of acrolein with racemic methyl-enecyclohexane **35** generates an equal mixture of diastereomers **38** and **39** through the mechanism shown (Scheme 9).[23] Dimethyl aluminum chloride reacts with acrolein to form the "ate" complex **36**, which undergoes an intermolecular Ene reaction to generate **37**. This then reacts intramolecularly in a second Ene reaction to give only the two diastereomers **38** and **39**. If excess acrolein is used then only the product **40** is observed, a result of an Oppenaur oxidation.



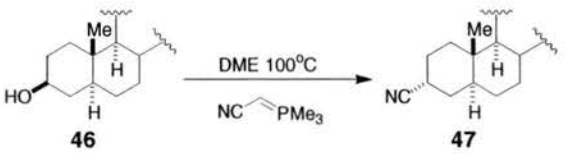
Scheme 9. Mechanism of Sequential Ene Reactions.

In the case of precursor **34**, the first Ene reaction has been eliminated and the starting material is optically active. This reaction will therefore proceed to give alcohol **41**, stereoselectively (Scheme 10). At this point, the strategy will diverge so as to be able to account for the synthesis of all four possible diastereomers. Formation of the mesylate and displacement with potassium cyanide in dimethyl sulfoxide will yield the nitrile **41**.[24] The reaction will be expected to yield the desired product however, mesylates are prone to eliminate lowering yields and complicating the reaction.



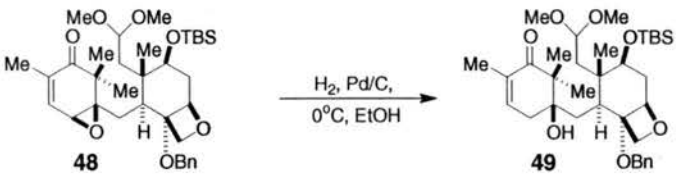
Scheme 10. Synthesis of Acid Chloride **45**.

Nucleophilic displacement of cyclohexanol **34** therefore could result in significant formations of the olefin. Alternatively, Tsunoda and co-workers have developed a one-pot cyanation of secondary alcohols based on the Mitsunobu reaction (Scheme 11).[25] Addition of the cyano-Wittig reagent, shown below, to cyclic alcohol **46** reacted smoothly to give cyanide **47** with only small amounts of the olefin detected. Application of this method will provide a complimentary method for cyanation of the alcohol **42**.



Scheme 11. One Pot Cyanation.

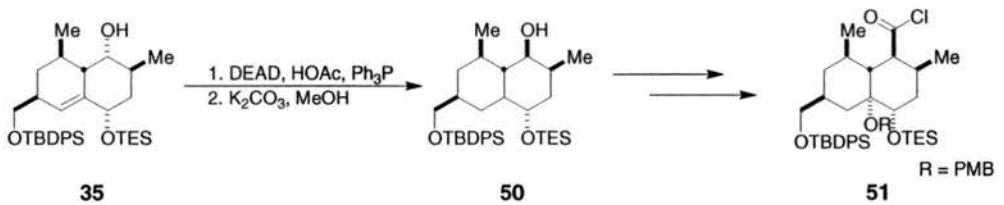
Conversion of **42** to the ester will be accomplished by acidic methanol.[26] The TBDPS group will be stable to these conditions, however literature suggests that the triethylsilyl ether will not survive, thereby decreasing the need for a deprotection step.[27] Judicious choice of reaction conditions will be required and will be reflected in the choice of reagents from this point forward as more vigorous methods might affect an epimerization of the newly formed stereocenter. Directed epoxidation will follow and yield **43**. Due to the neighboring alcohol, this reaction is expected to proceed stereospecifically with meta-chloroperbenzoic acid as the oxidant,[28] however a number of vanadium reagents would provide the desired selectivity as well.[29] Reprotection of the alcohol as the triethylsilyl ether followed by reduction of the epoxide with palladium on carbon will yield alcohol **44**. Danishefsky and coworkers have shown in there total synthesis of Taxol® that trisubstituted epoxides are opened chemoselectively in the presence of an enones (Scheme 12).[30]



Scheme 12. Palladium Catalyzed Reduction of Epoxide **48**.

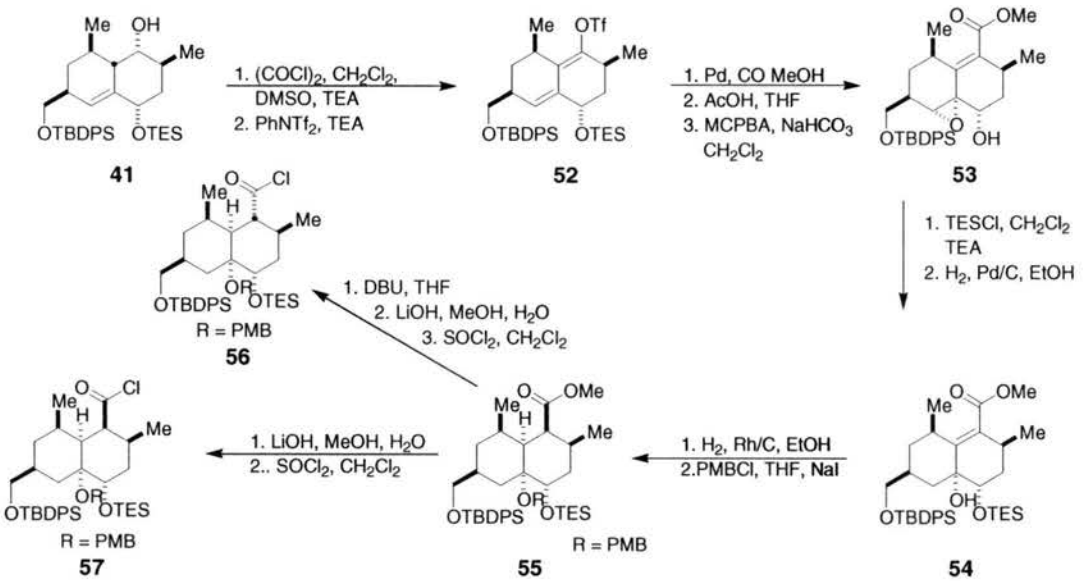
This will provide an excellent method for the introduction of the tertiary alcohol. Protection with para-methoxybenzyl chloride followed by saponification of the ester with lithium hydroxide and acid chloride formation will generate the second coupling partner **45**, for the Stille reaction (Scheme 10). Although this synthesis will be somewhat long, it allows for the straightforward formation of both diastereomers at the α position of acid chloride **45**.

The Mitsunobu reaction[31] will be used to convert alcohol **41** into its diastereomer **50** (Scheme 13). Again, the potential for olefin formation exists but will be expected to furnish the cyanide rather than elimination product with Tsunoda's work serving as a back-up approach.[26] Addition of diethyl azodicarboxylate to alcohol **41** in the presence of triphenylphosphine and acetic acid will give the acetate, which upon subjecting to potassium carbonate in methanol will generate, alcohol **50**. This compound can then be elaborated to the diastereomeric acid chloride **51** as before (Scheme 10).



Scheme 13. Formation of the Second Diastereomer **51**.

The other two possible diastereomers will be formed by taking in account the ease of which the product from the Ene cyclization was oxidized and isomerized to **40**, Scheme 9. Similarly, Swern oxidation of **41** and concominant isomerization will yield the intermediate enone, which will be converted to the triflate **52** (Scheme 14).[32] Carbonylation[33] will yield the methyl ester followed by cleavage of the TES group and chemoselective, asymmetric epoxidation will give **53**. Conjugated diene **52** could potentially be too electron deficient, however the δ, γ olefin of enones have been differentiated in a number of systems and will react as expected.[34] Palladium catalyzed reduction of the epoxide will then afford tertiary alcohol **54**.

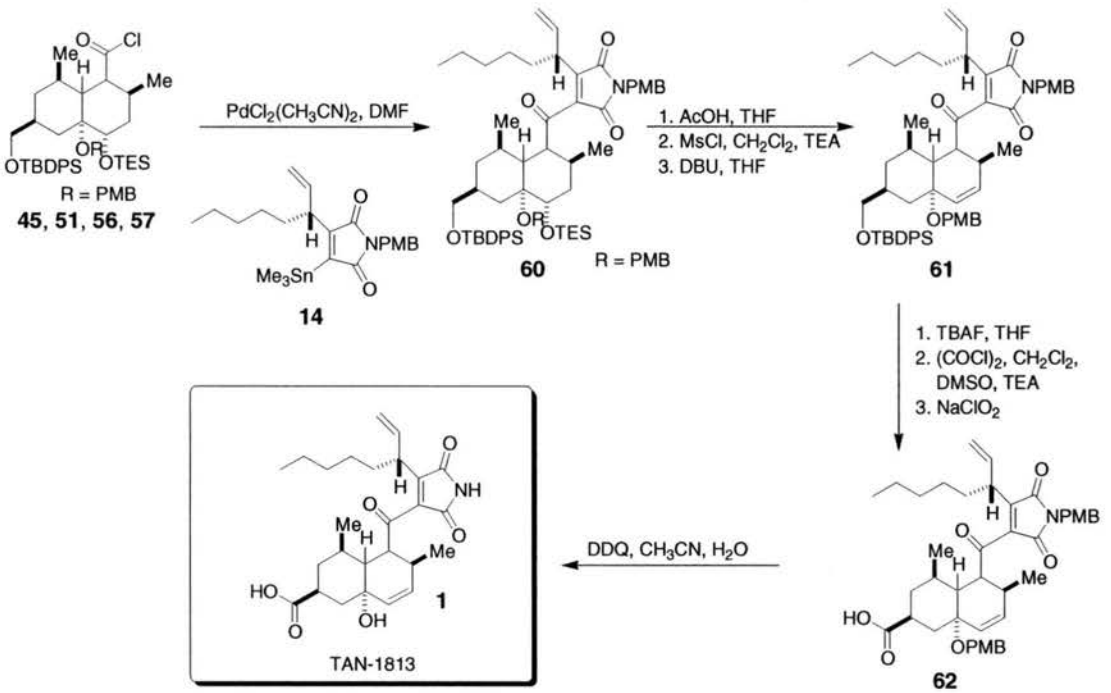


Scheme 14. Synthesis of Other Diastereomers.

An asymmetric 1,4-reduction of the enone will efficiently set two contiguous stereocenter with addition of hydrogen occurring from the same face as the tertiary alcohol. A similar approach has been recorded by Mori and was accomplished in high yield (Scheme 15).[35] The enone was reduced with approach of hydrogen directed by the

Completion of TAN 1813

With vinyl stannane and all four diastereomers of the acid chloride in hand, coupling and functional group transformations will be all that is required to complete the total synthesis (Scheme 16). The Stille reaction of acid chlorides **45**, **51**, **56**, and **57**, with stannane **14**, will result in the formation of ketones **60**.[37] Again, selective hydrolysis of the triethylsilyl ether will yield the secondary alcohol. Mesylation followed by base catalyzed elimination will generate the internal olefin of **61**. Deprotection of the tert-butyldiphenylsilyl ether with tetrabutylammonium fluoride[38] will finally release the primary alcohol. Oxidation to the carboxylic acid will be accomplished by a two-step protocol. First, Swern oxidation[22] will form the aldehyde and then sodium chlorite[39] will further oxidize the compound to give **62**. Deprotection of both the tertiary ether and the maleimide with dichlorodicyanoquinone[21] will complete the synthesis of TAN 1813.



Scheme 16. Completion of TAN-1813.

In summary, the asymmetric total synthesis of TAN 1813 and three diastereomers has been proposed. The strategy utilizes an intramolecular Ene cyclization as the key step and will disclose the absolute structure of the natural product. The synthesis will also be highly convergent and provide a number of analogues that will help elucidate the biological activity of these compounds.

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