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A Modular Tandem Michael Or Aza Michael Approach to Obtain Indoline Alkaloid-Like Policyclic Derivatives

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A MODULAR TANDEM MICHAEL OR AZA MICHAEL APPROACH TO OBTAIN INDOLINE ALKALOID-LIKE POLYCYCLIC DERIVATIVES

by

Jean-Louis Brochu
B.Sc. (honours), University of Ottawa, 2004

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In Partial Fulfillment of the Requirements for the
M.Sc. Degree in the
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Candidate | Supervisor

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LIST OF ABBREVIATIONS

2D	2 dimensional
Alloc	Allyloxycarbonyl
br s	Broad singlet
i-Bu	*iso*-Butyl
t-Bu	*tert*-butyl
C	Carbon
^{0}C	Degree Celsius
COSY	^{1}H-^{1}H NMR correlation spectroscopy
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublets
ddd	Doublet doublet of doublets
dddd	Doublet doublet doublet of doublets
ddt	Doublet doublet of triplets
DEPT	Distortionless Enhancement by Polarization Transfer
DIC	*N*,*N*-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	*N*,*N*-dimethylforamide
DMSO	Dimethylsulfoxide
ee	Enantiomeric excess
eq.	Equivalent
ES+	Positive electrospray
Fmoc	9-fluorenylmethoxycarbonyl
g	Gram(s)
H	Hydrogen, proton
HATU	*O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyl-uronium hexafluophosphate
HBTU	*O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyl-uronium hexafluophosphate
HOBT	1-Hydroxybenzotriazole
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography mass spectroscopy
Hr(s)	Hour(s)
HSQC	Heteronuclear Single-Quantum Coherence
Hz	Hertz
i-Bu	iso-butyl
J	Coupling constant
LDA	Lithium diisopropylamide
LiHMDS	Lithium bis(trimethylsilyl)-amide
LRMS	Low resolution mass spectroscopy
m	Multiplet

M	Molar
Me	Methyl
MEM	Methoxyethoxymethoxy
mg	Milligram(s)
mL	Milliliter(s)
mmol	Millimole(s)
mp	Melting point
Ms	Mass spectroscopy
mw	Molecular weight
N	Normal
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
Ph	Phenyl
ppm	Parts Per Million
PREP LC	Preparative liquid chromatography
Py	Pyridine
PyBrop	Bromotripyrrolidinophosphonium hexafluorophosphate
q	Quartet
RT	Room Temperature
s	Singlet
SAR	Structure activity relationship
S_N2	Type 2 nucleophilic substitution
SPS	Solid phase synthesis
t	Triplet
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate
Teoc	Trimethylsilylethoxycarbonyl
TESOTf	Triethylsilyl trifluoromethanesulfonate
THF	Tetrahydofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TOCSY	Total correlation spectroscopy
TS	Transition state
µL	Microlitre(s)
uv	Ultraviolet

ABSTRACT

The use of small molecule chemical probes is highly attractive in dissecting complex biological processes (i.e. multiple protein-protein interactions and protein complexes-derived signaling networks) because of the probe's ability to induce subtle, and generally reversible, changes in protein dynamics.

With the goal of developing new synthesis methods leading to high-throughput generation of natural product-like Indoline derivatives having different architectures, this thesis will highlight a modular, tandem reaction approach in which a key reaction is the use of a tandem Michael or aza-Michael reaction to obtain indoline alkaloid-like polycyclic architectures. An interesting feature is that the choice of an amino acid moiety in the side chain allowed the formation of different fused ring systems. The solution phase synthesis method was then developed on solid phase with an objective of generating few analogs in a high-throughput manner.

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À mon fils, Miika Thomas Brochu

CHAPTER 1: INTRODUCTION

1.1 Human Genome and Protein-Protein Interactions

The Human Genome Project was brought to completion officially April 2003, with Nature's complete genetic blueprint of a human being having over 30 000 genes counting for 100 000 to 450 000 different proteins[1a, 1b]. Currently, there is a need to apply this acquired knowledge leading to a better understanding of cellular processes at the molecular level[1a]. The post-genomic age has brought challenges to the biomedical research community in developing a better understanding of protein-protein interactions-based signaling networks[2]. It is becoming clear with all the recent advances that signaling networks relate to cell functioning in normal cells as well as dysfunctional cellular processes[3]. Unfortunately, these signaling networks involve multiple dynamic and highly complex protein-protein interactions, thus severely limiting our ability to exploit this knowledge in generating new therapeutic approaches by modulating these interactions in a controlled and reversible manner[4]. This effort must be undertaken by the entire scientific community to benefit from the high-throughput technologies in accessing and evaluating new compounds in order to identify the functions of these newly discovered genes[5, 6].

1.2 Genetic and Chemical Genetic Approach

The genetic approach, also known as classical genetics, is the most common, yet indirect, way of determining the cellular functions of a protein[7]. In traditional genetics, there are two approaches; forward and reverse genetics, **table 1**. In forward genetics, biologists induce specific mutations in a known genome and the effects are interpreted to explain the function of the gene. In reverse genetics, a mutation is induced to an already branded gene to identify the effects of the resulting phenotype[5]. These irreversible mutations and genetic screens are much more feasible on simple organisms such as yeast or small insects due to their rate of reproduction. Unfortunately, these approaches are

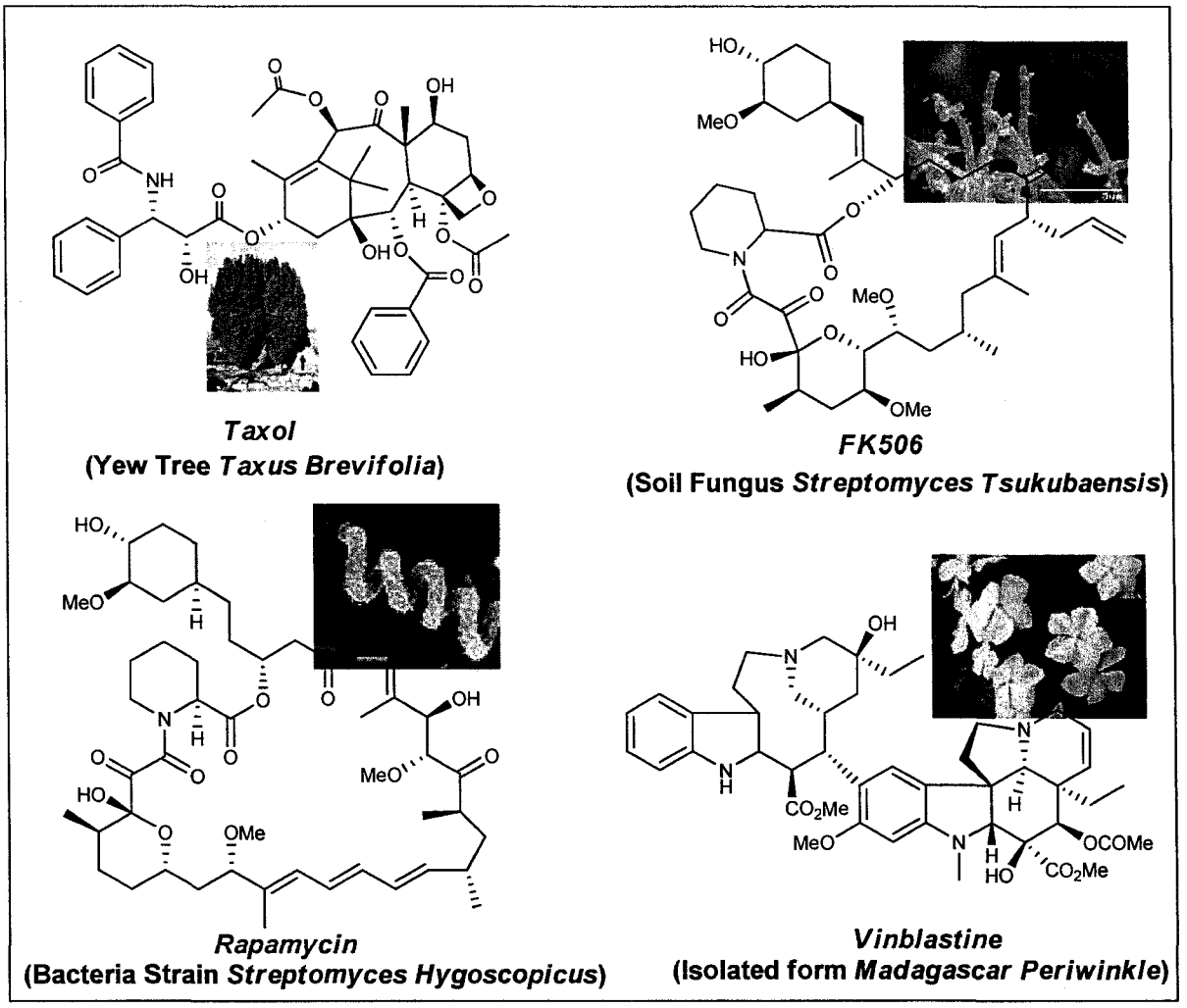
difficult to accomplish and time consuming on more complex organisms[8]. The need for immediate knowledge in the understanding of protein functions will lead to new therapeutics for improving human life, thus employing the chemical genetic approach to identify genes that regulate biological processes is becoming increasingly more attractive to the scientific community. Chemical genetics employs the use of small molecules as probes to serve as highly specific modulators (i.e. activators or inhibitors) of proteins and protein-protein interactions. It is used to identify which proteins regulate different biological processes and to further understand, in molecular detail, how proteins perform their biological functions enabling the kinetic analysis of the *in vivo* consequences of these changes[6ab, 8]. This chemical genetic approach is restrictive and easily introduced to cells given that the ligand can be added or removed at any time[8]. This modern field brings together cell biologist and synthetic organic chemist. The idea of using small molecular probes originates from Nature. In most cases, natural products, where known modulators of protein-protein interactions by interacting with enzymes and proteins, are chiral and highly complex in Nature and possesses several stereogenic centers as well as diverse functional groups resulting into a three dimensionally architectured molecule[9]. These natural products could serve as good starting points for the design of new enantioenriched natural product-like compounds. Nature presents different scaffolds (structural motifs), in which can provide synthetic interest, for instance; polycyclic amino alkaloids, indolines, hydroxyquinolines, benzofurans, benzopyrans and many more. The origins of these chemical genetic ideas came about in the eighteenth century where researchers suggested that in plant extracts, in which animals are affected, each contain a single active ingredient that acts on a distinct part of the animal, subsequently morphine was the first active compounds isolated from opium[8, 10].

Table 1: Comparing Classical Genetic and Chemical-Genetic Approaches[8]

Forward Genetics (from phenotype to gene/protein)		**Reverse Genetics (from gene/protein to phenotype)**	
Classical genetic approach	***Chemical-genetic approach***	***Classical genetic approach***	***Chemical-genetic approach***
Random mutagenesis (for example, irradiating cells)	Add library of small molecules to cells or animals	Mutate single gene of interest in cells or animals (for example, a knockout mouse)	Add a library of small molecules to a purified protein of interest
Select mutants with the phenotype of interest	Select small molecules that produce the phenotype of interest	Generate cells or animals with mutant gene	Add the molecules that bind to the protein of interest to cells or animals
Identify mutated gene	Identify the protein to which the small molecules bind	Look at phenotype	Look at phenotype

The first era of chemical genetics synthesized sets of molecules with never before seen properties. The outcome of these synthetic trials showed poor hits during bio-assays, it was determined that these new molecules poorly occupied populated regions of multidimensional, chemical descriptor space[11a]. The approach for designing new molecular probes is to generate similar compounds that interact with enzyme specificity, like natural products (**Figure 1**).

Figure 1: Natural products as Chemical Probes[12]



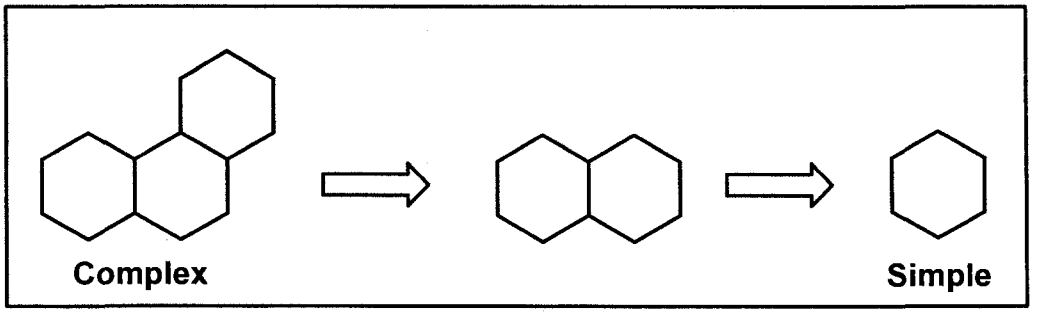
Many old and new synthetic techniques can be used to achieve our goal. Many different retrosynthetic pathways can be employed starting with only one compound, which gives alternate routes if you hit an unexpected brick wall.

1.3 Target-Oriented Synthesis (TOS)

Target-oriented synthesis (TOS) has been a very successful and broadly used technique in drug discovery. This synthetic procedure involves preselected protein targets, where the retrosynthetic analyses of these key scaffolds are used for the design of small molecules (probes)[7]. In TOS, the retrosynthetic analysis begins with a known and structurally complex target where the research is based

on the discovery of simplified precursors to initiate the synthesis, **Figure 2**[7]. TOS targets are classically natural products or analogues of these products which serve as potential lead candidates for developing drugs[7]. The major problem is that we know very little about protein functions. At present, only 0.1% of human proteins are now drug targets[13]. Many unknown protein interactions and functionalities are left unidentified applying this synthetic technique.

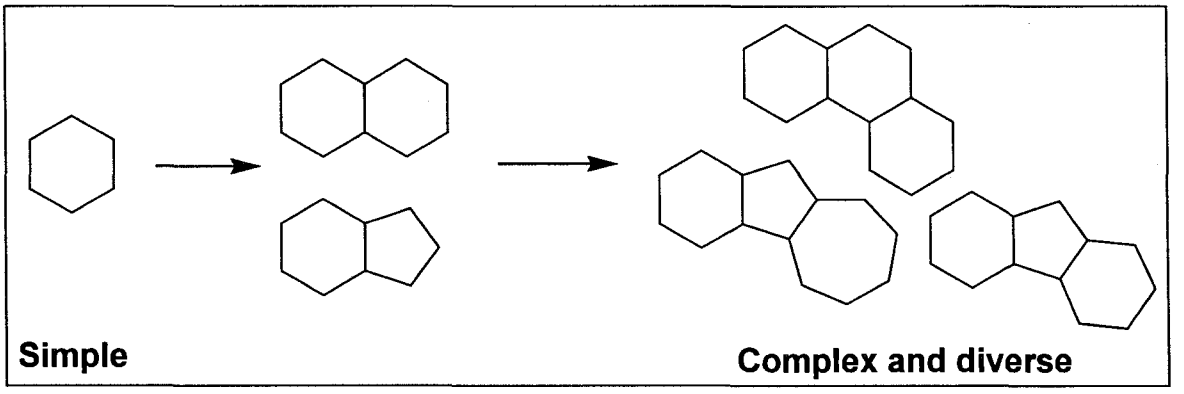
Figure 2: Retrosynthetic analysis in Target-Oriented Synthesis[7]



1.4 Diversity-Oriented Synthesis (DOS)

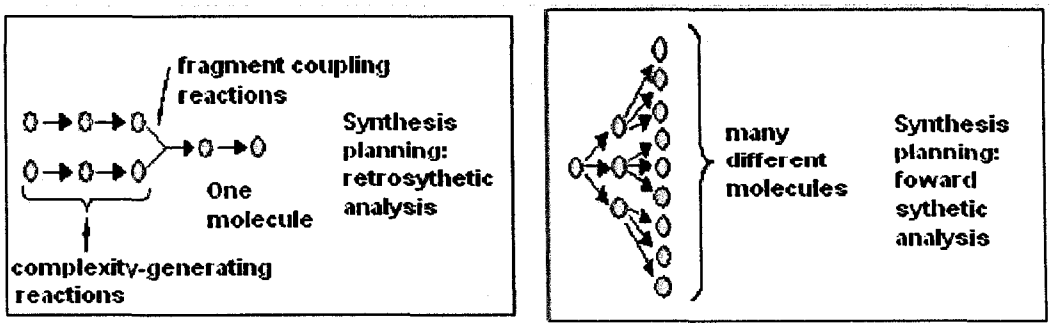
In Diversity-Oriented Synthesis (DOS), the focus is not on a specific known target but on the discovery of new ones. One of the goals of diversity-oriented synthesis is to efficiently synthesize a collection of small molecules or natural product-like compounds capable of perturbing any disease-related biological pathway[11a]. This can eventually lead to the identification of therapeutic protein targets capable of being modulated by small molecules. The focus is mainly on the chemical reactions where the diversity is a key feature. This is achieved by adding and replacing different building blocks and changing the stereochemistry of different natural product-like scaffolds, **Figure 3**.

Figure 3: The complexity and diversity of Diversity-Oriented Synthesis targets



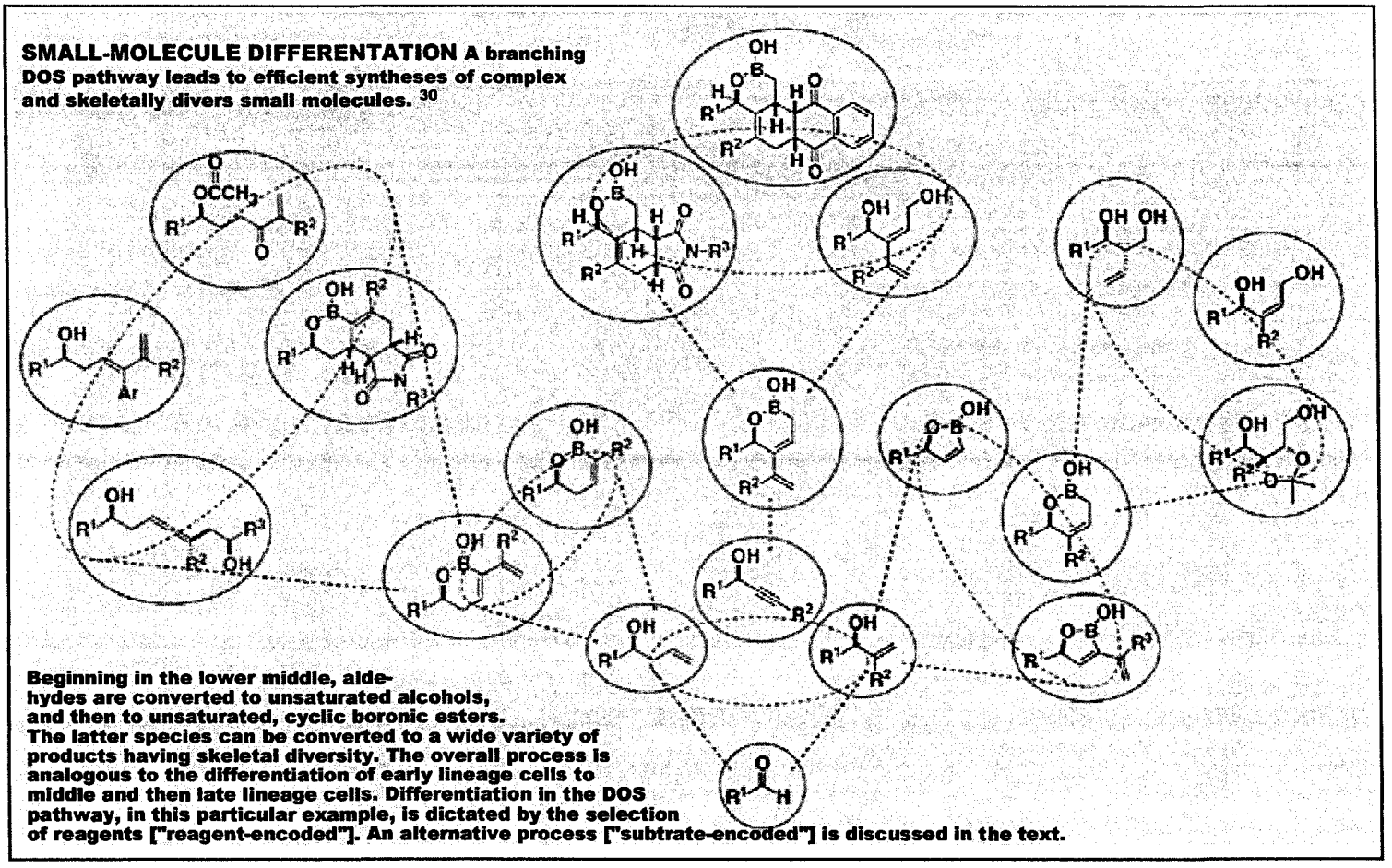
By looking at **Figure 4**, diversity-oriented synthesis has a branching motif and can produce skeletally diverse products compared to TOS, which demonstrates a bottleneck motif where more work is done per product. An example of small-molecule differentiation in DOS research is presented in **Figure 5.1**. With these advancements in combinatorial chemistry, it is now possible to generate a reasonable sized library of structurally diverse compounds. It is important to prepare complex molecular skeletons with many diversity sites in addition to being able to load the compound on solid support to maximize the outcome. DOS was capable to utilize technical developments in combinatorial synthesis, which is frequently applied in TOS[11a]. Combinatorial chemistry starts with a small molecule probe, a lead drug, and the synthesis is aimed at highly populating a region of the chemical space occupied by that lead target, in order words, to make analogues[11a].

Figure 4: Comparing TOS and DOS synthetic techniques



TOS **DOS**

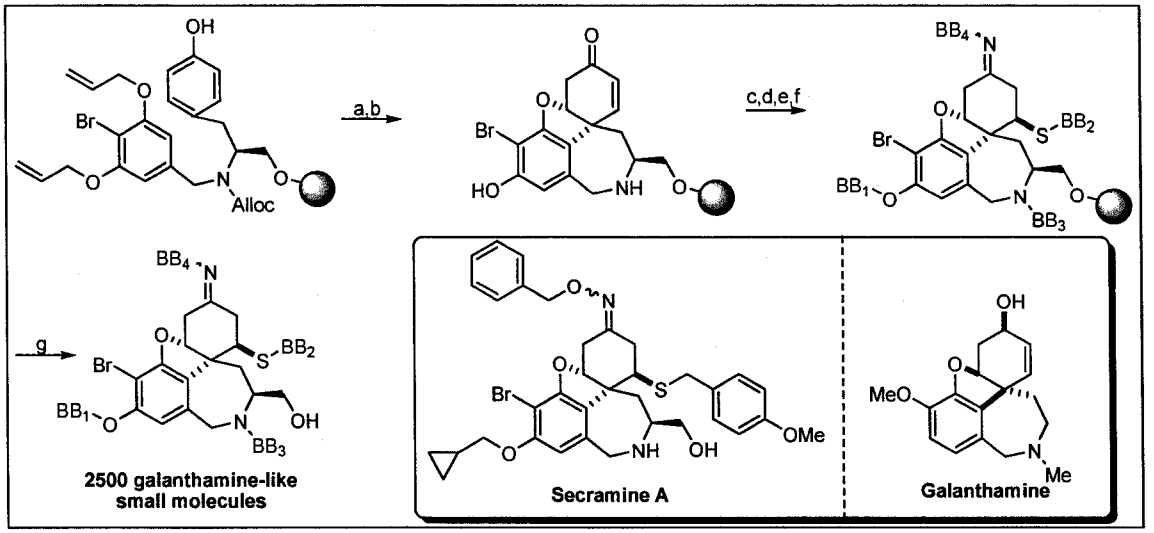
Figure 5.1: An example of DOS from Schreiber and co-workers group[11a]



Many exciting DOS success stories are arising in the scientific community. At the department of chemistry, Harvard University, Henry Pelish and co-workers were inspired by the usefulness of small molecules to study protein traffic, **Figure 5.2**. Through DOS-oriented high-throughput synthesis and phenotypic screening, they have discovered secramine A, a small molecule that inhibits protein traffic out of the Golgi apparatus[11b]. This new small molecule was found to inhibit activation of the Rho GTPase Cdc42, a protein implicated in trafficking, by a mechanism dependent upon the guanine dissociation inhibitor RhoGDI[11b]. RhoGDI binds Cdc42 and antagonizes its own membrane association, nucleotide exchange and effector binding[11b]. In vitro, secramine inhibits Cdc42 binding to membranes, GTP and effectors in a RhoGDI-dependent manner[11b]. In cells, secramine mimics the effects of dominant-negative Cdc42 expression on protein export from the Golgi and on Golgi polarization in migrating cells[11b]. RhoGDI-

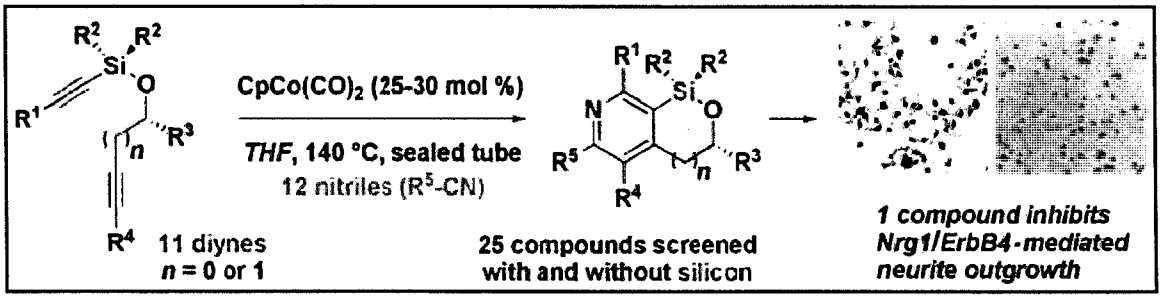
dependent Cdc42 inhibition by secramine illustrates a new way to inhibit Rho GTPases with small molecules and provides a new means to study Cdc42, RhoGDI and the cellular processes they mediate[11b]. The Harvard team synthesized a library of complex, natural product–like small molecules resembling galanthamine. Galanthamine is a bioactive natural product drug used for the treatment of mild to moderate Alzheimer's disease and various memory impairments. It is an alkaloid that is obtained synthetically or from the bulbs and flowers of the Caucasian snowdrop (Voronov's snowdrop), *Galanthus woronowii* (Amaryllidaceae) and related genera.

Figure 5.2: The discovery of Secramine A through DOS methods[11b]



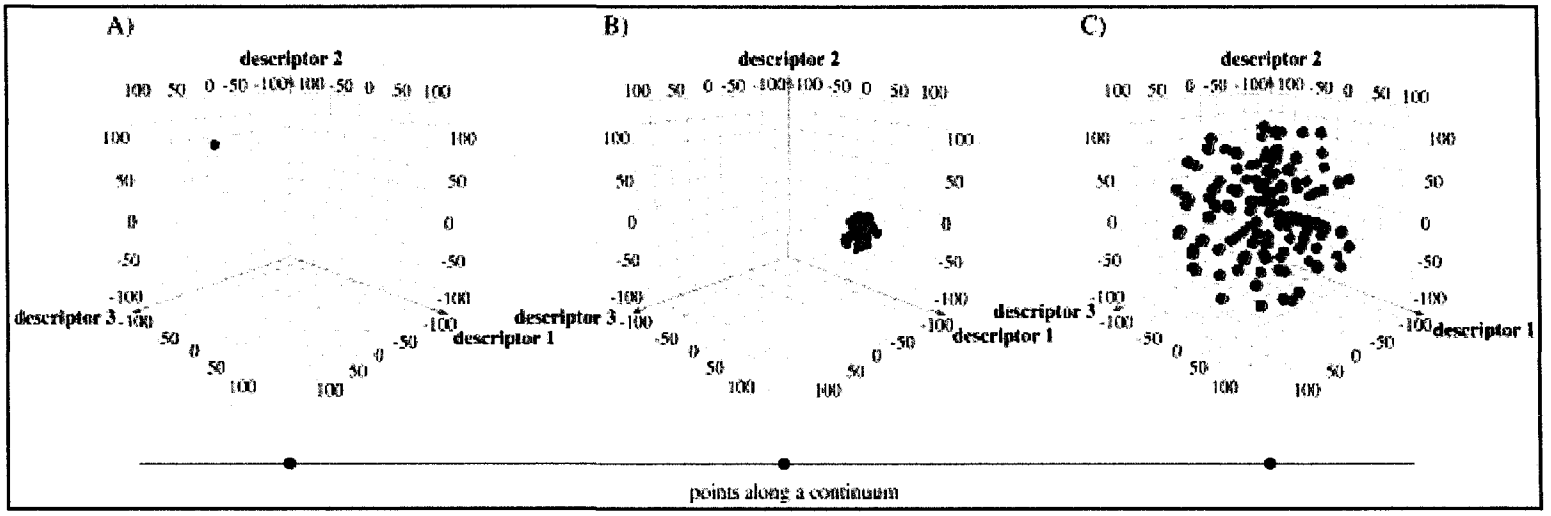
The Schreiber team recognized the importance of exploring intricate pyridines in small-molecule screening, **Figure 5.3**. They have developed a previously unexplored [2+2+2]-cycloaddition of silyl-tethered diynes with nitriles[11c]. The tether grants high regioselectivity, while the solvent (THF) permits the catalytic CpCo(CO)2 to be used without exogenous irradiation[11c]. One of the resulting bicyclic and monocyclic (desilylated) pyridines was identified as an inhibitor of neuregulin-induced neurite outgrowth (EC50 = 0.30μM) in a screen that probes a pathway likely to be involved in breast cancers and schizophrenia[11c].

Figure 5.3: The discovery of a neuregulin-induced neurite outgrowth inhibitor through DOS methods[11c]



Diversity-oriented synthesis is aimed at reaching the objective of broadly populating the unexplored, natural product chemical space that is currently not occupied by conventional combinatorial chemistry[14]. The combinatorial chemistry program in DOS utilizes stereo- and enantio-selective organic synthesis reactions and is designed to provide small molecules that are rich in stereochemically-defined polyfunctional groups and are conformationally diverse, natural product-like skeletons. On the contrary, with few exceptions, classical combinatorial chemistry efforts have led to simple compounds lacking 3-dimensional architectures. These simple compounds may not populate the chemical space that is occupied by bioactive natural products, and perhaps, are likely to be less attractive as useful chemical probes in exploring them as modulators of protein-proteins interactions in dissecting dynamic signaling networks. With DOS, a synthetic platform is already established where re-synthesis at any stage is achievable and the second generation architecturally complex compounds could be easily obtained. **Figure 6** compares (A) TOS, (B) combinatorial chemistry and (C) DOS as a three-dimensional plot representing the chemical product or compilation of products resulting from a single synthesis pathway. The axis plots a calculable or measurable property of a small molecule (molecular weight, solubility). In TOS, a single target structure is envisioned. In medicinal or combinatorial chemistry, a group of analogues with known or expected properties is synthesized. In DOS, the graph (chemical space) is broadly populated with an assortment of products having unknown properties.

Figure 6: Comparing TOS, Combinatorial Chemistry and DOS with their relative product outcome[14]

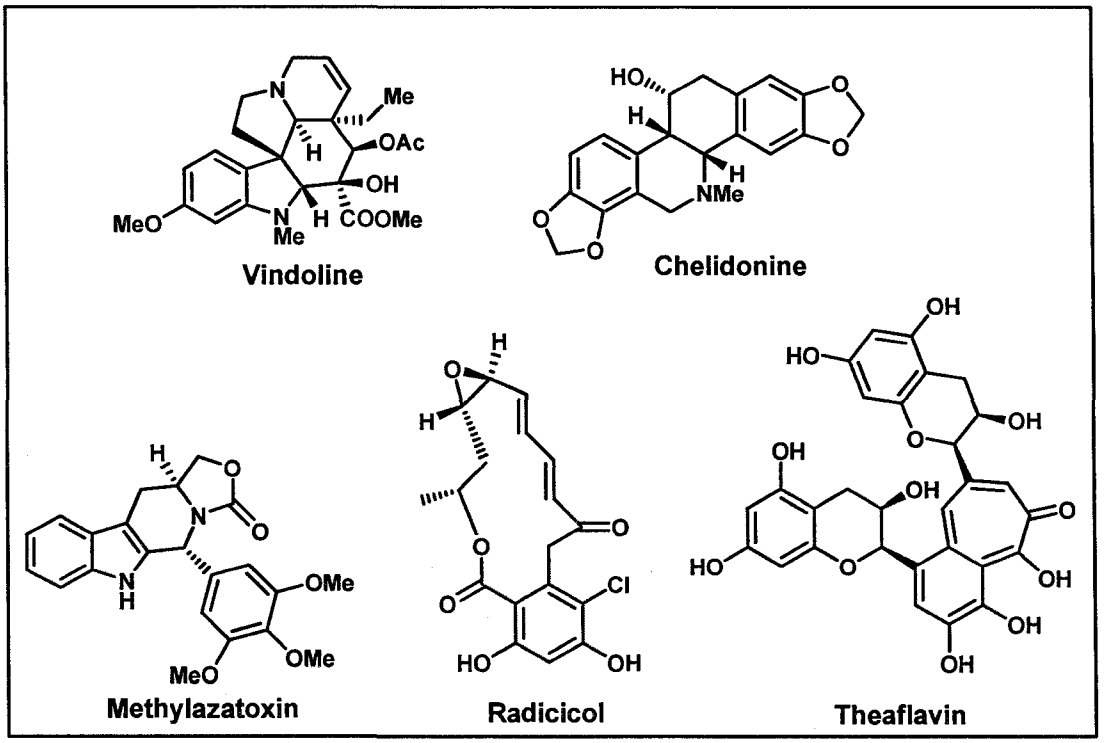


1.5 Approach to Natural Product-inspired, Diversity-Oriented Synthesis

1.5.1 Interest in Alkaloid and Flavonoid Scaffolds

Alkaloids and flavonoids are natural products generally found in plants. Several of their derivatives are known to have a diverse range of biological properties. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and flavonoids contain oxygenated functional groups and these structures also contain aromatic moieties which are ideal for protein binding. Abundant quantities of these natural products are known to interact with protein surfaces that are involved in protein-protein interactions.

Figure 7: Bioactive Alkaloid and Flavonoid Natural Products

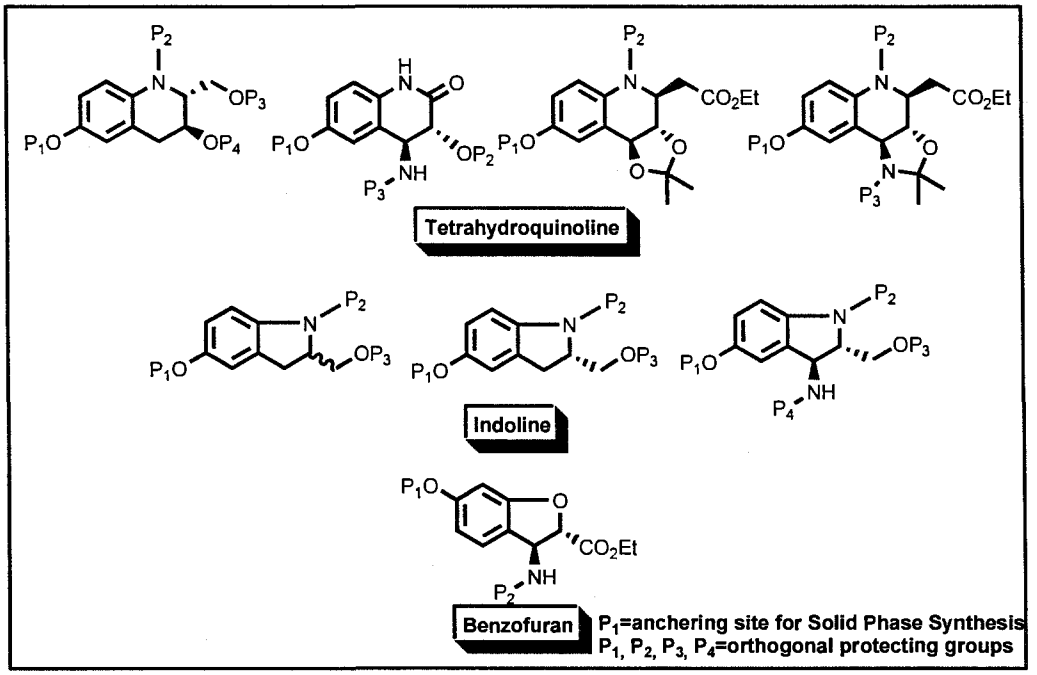


Some of the bioactive alkaloids and flavonoids are shown in **Figures 7**. Belonging to the family of *Vinca alkaloids*, vindoline contains indoline moieties and acts as an antimitotic agent as it interferes and inhibits the microtubule assembly formation[15]. Chelidonine is also an antimitotic alkaloid natural product and it contains a tetrahydroisoquinoline moiety. Most flavonoids are rich in oxygenated functional groups and contain polyphenolic moieties. These two features are ideal for protein binding and several of these natural products are known to interfere with protein surfaces that are involved in protein-protein interactions. Radicicol is a flavonoid-based macrolide derivative and is known to interact with the heat shock protein 90 (Hsp90)[16]. This protein is a molecular chaperone and plays an important role in stabilizing and correctly folding several oncogenic proteins. Finding the inhibitors of this protein could be very useful in developing novel anti-tumor agents as its inhibition could affect several unwanted signaling pathways involving Hsp90. The benzopyran-based flavonoid natural product theaflavin has been shown to interfere with the protein-protein interactions involving the Bcl-2 protein family and related proteins[17]. The ability

to modulate these networks in a highly selective manner provides a mean of obtaining a better understanding of these signaling pathways and their specific roles in apoptosis[18]. These are just a very few examples of alkaloid and flavonoid natural products which demonstrates the importance of these specific scaffolds that interfere with protein surfaces involved in protein-protein interactions, further validating the need for charting the chemical space that is currently occupied by these bioactive natural products.

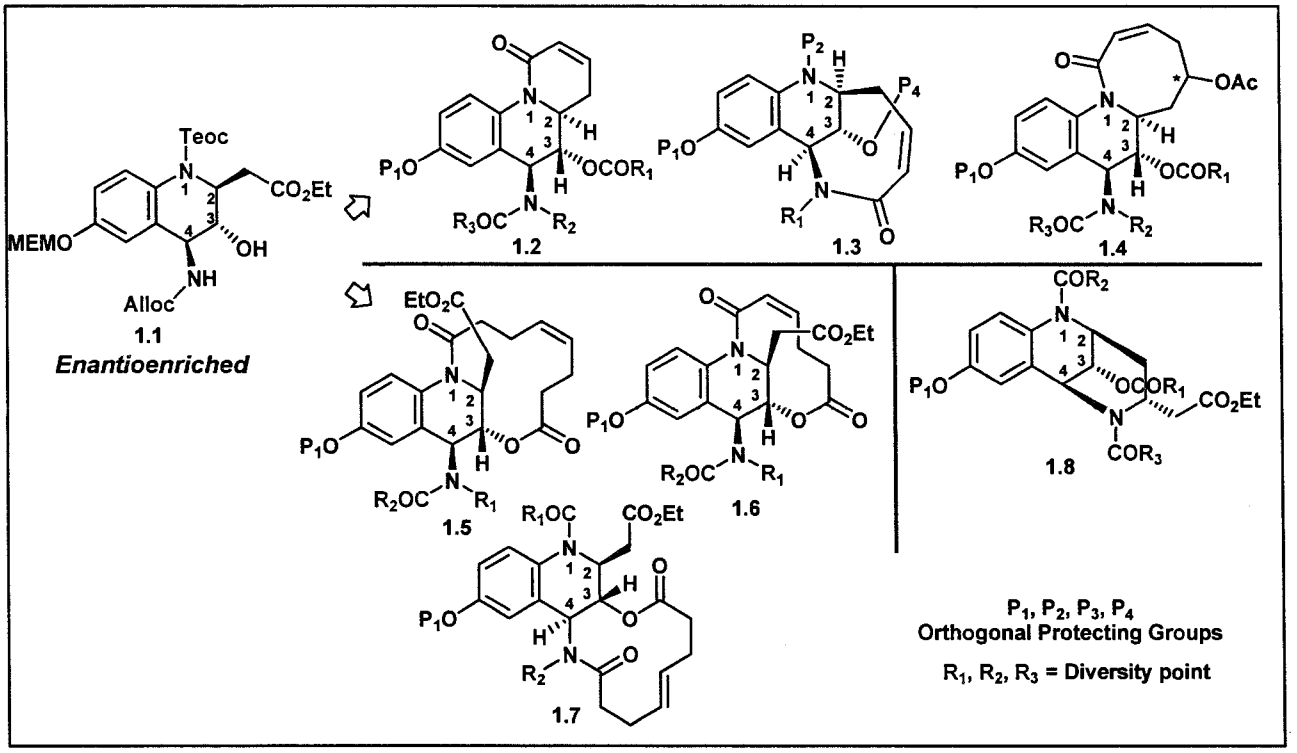
The primary research interest in the Arya group is to utilize modern organic chemistry and to develop novel approaches to obtain complex, polycyclic, natural product-like compounds having diverse 3D skeletal and stereochemical architectures. The Arya group is developing the natural product-inspired DOS program in three scaffolds of interests; tetrahydroquinolines, benzofurans and indolines, **Figure 8**. Once the synthesis methodology is accomplished in solution phase, manual solid phase is then developed which then allows generating the libraries in a high-throughput manner. The Arya group libraries, in collaboration with the Broad Institute of Harvard University and MIT, are also utilized in printing small molecule microarrays. The group's hypothesis is to explore the unexplored chemical territory using diversity-oriented synthesis as a method to obtain alkaloid and flavonoid natural product-like compounds which are likely to provide for useful small-molecule modulators of protein-protein interactions and dissectors of signalling networks because these compounds are anticipated to occupy the chemical space currently being championed by bioactive alkaloid and flavonoid natural products.

Figure 8: The Arya group's chemistry teams



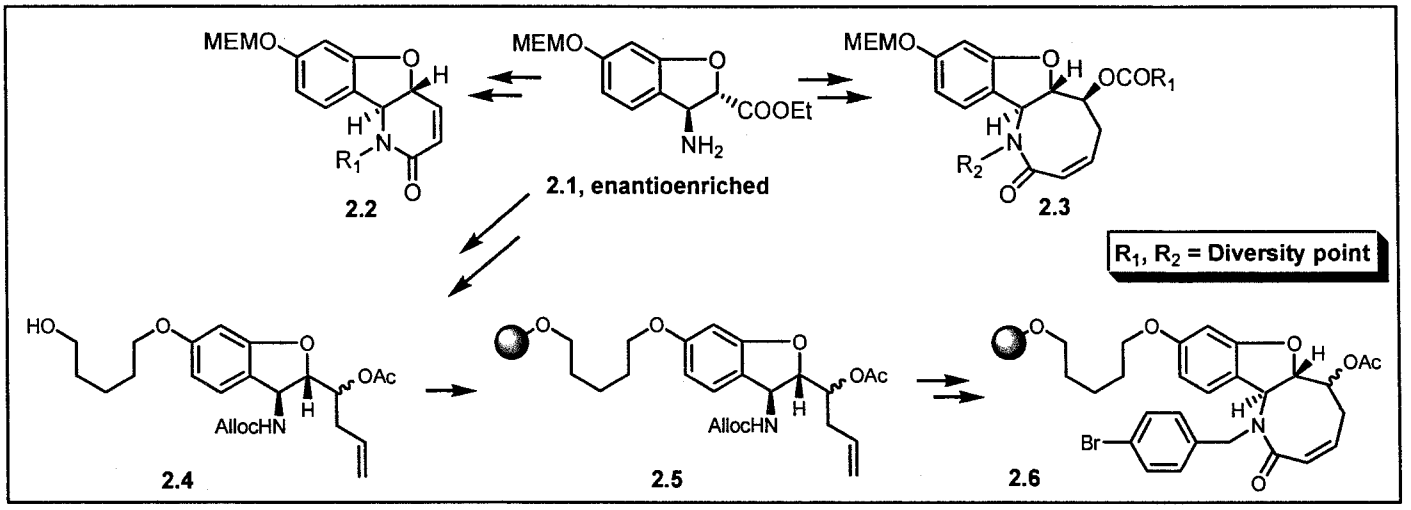
Arya and co-workers[19] developed a highly practical, enantioselective synthesis of functionalized, tetrahydroaminoquinoline scaffold, **1.1 (Scheme 1)**. There are several key features on this scaffold such as; the presence of an δ-amino acid moiety, β-amino acid moiety, γ-hydroxy carboxyl ester functionality, and a phenolic hydroxyl that could be exploited as an immobilization site in the planning of a solid phase synthesis. For example, scaffold, **1.1** can be used as a starting point for the synthesis of compounds; **1.3**, **1.4**, **1.5**, **1.6** and **1.7** using a ring closing metathesis strategy. Starting compound **1.1** is prepared in 12 steps, 9.8% overall yield[19]. Compound **1.6** is highly unique as it contains a bridged, ten-membered ring unsaturated lactam moiety. Compound **1.5** also has a bridged twelve-membered ring with a cis-olefin. Compound **1.8** was prepared from **1.1** in five steps; the final step was achieved through a stereoselective *in situ* aza-Michael reaction. Several of these compounds were synthesized on solid support and led to the creation of several small molecule libraries. This tetrahydroaminoquinoline-based scaffold is highly versatile in mapping the chemical space leading to a wide variety of very different polycyclic architectures.

Scheme 1: Examples of the Arya group approach to obtain different tetrahydroquinoline-based tricyclic compounds



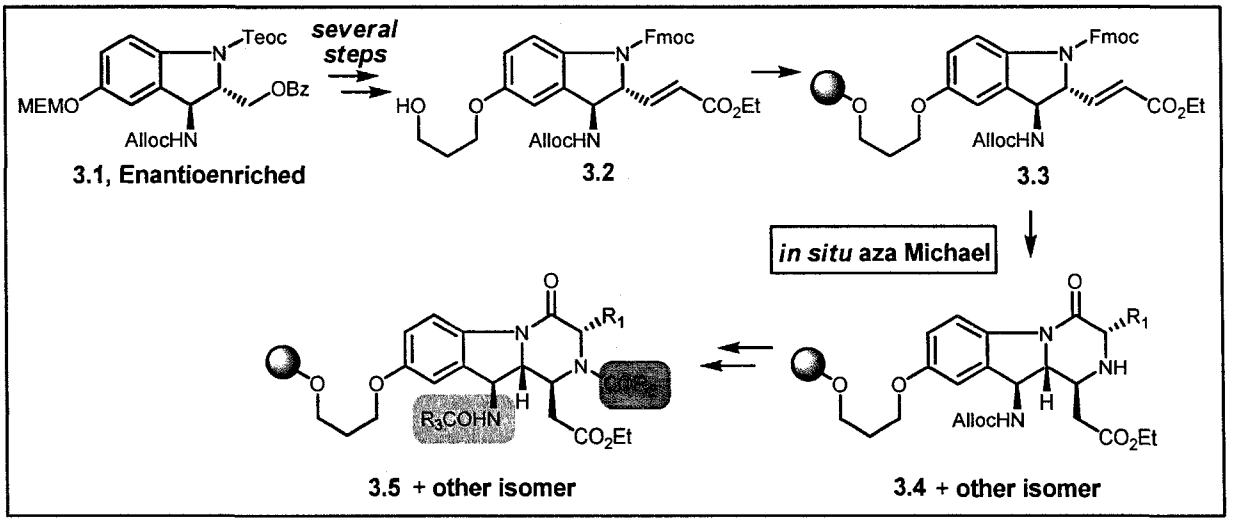
Arya and co-workers[20] have also developed a solution and solid phase approach to obtain benzofuran-based small molecules having two different tricyclic architectures, six and eight membered unsaturated lactams ring, **2.2** and **2.3** (**Scheme 2**). Starting compound **2.1** is prepared in 6 steps, 30% overall yield[20]. With a 5 carbon spacer, compound **2.4** was loaded onto the solid support and the manual solid phase synthesis of **2.6** was successful. The library generation of this scaffold is currently in progress.

Scheme 2: Examples of the Arya group approach to obtain different benzofuran-based tricyclic compounds



In the first generation of Indoline-based polycyclic derivatives, Arya and co-workers[21] developed a practical, enantio-controlled synthesis of an aminoindoline scaffold, **3.1 (Scheme 3)**. Having many striking features, this scaffold includes the presence of four orthogonally protected functional groups, making it highly versatile for producing different polycyclic architectures. The phenolic hydroxyl group serves as a future anchoring site for the solid phase synthesis and further library generation. The aminoindoline scaffold, **3.1** is converted into **3.2** as a starting material in developing the solid phase synthesis projects. The Broad institute alkylsilyl linker-based polystyrene macrobeads are used to immobilize compound **3.2** onto the solid support, **3.3**. Using the Broad institute loading protocol generally the yields are very high, in the 75 to 95% range. The key reaction in this methodology was the formation of the additional ring by *in situ* stereo-controlled, aza-Michael reaction to yield compound **3.4** as the major diastereoisomer following the *N*-Fmoc removal and the amino acid coupling. Using the three diversity sites, as shown in compound **3.5**, a 90-membered library was obtained by employing the IRORI split-and-mix technology[21].

Scheme 3: The first generation of the Arya group approach to obtain Indoline polycyclic derivatives



1.5.2 High-Throughput Organic Synthesis

The outcome of Diversity-Oriented Synthesis is optimized on solid support, utilizing split and pool technique championed by Schreiber and co-workers[11a]. This procedure consists of splitting plastic beads (high capacity macro beads) in different containers and adding different building blocks to each division. Then the beads are pooled together and split again in different containers (X-Kans) to then add different building blocks in each division to react at different diversity sites. The great advantage of this technique is to as have many different molecules with the minimal amount of reactions performed. An example of this technology is the IRORY split-and-mix method. Continuing this process creates an enormous collection of small molecules ready for bio-assays.

Solid phase synthesis has been practiced for almost 50 years, before the birth of combinatorial chemistry[22]. Professor Bruce Merrifield of Rockefeller University invented solid phase synthesis (SPS) in 1959: notebook entry 5/26/59 — "A New Approach to the Continuous, Stepwise Synthesis of Peptides". Bruce Merrifield was searching for the right support, linker, amino protecting group and cleavage procedure in peptide synthesis. He acquired a simple polymer, polystyrene cross-linked with divinylbenzene (DVB) which was used as ion-

exchange resins for column chromatography. The DVB polystyrene was further functionalized by Friedel-craft kylation to create linkage sites. Referees critiqued the first publication, suggesting that this SPS should be shunned as it violated the basic principles of synthetic organic chemistry[22]. The official SPS protocol was later published in 1963. Bruce Merrifield won the Nobel Prize for chemistry on October 17, 1984.

The Arya group uses the Broad institute's alkylsilyl linker-based polystyrene macrobeads, **Figure 9**[23]. The Alkylsilyl macrobead is initially inactive, subsequent to swelling in a BIORAD column; the resin is treated with a solution of trifluoromethanesulfonate and becomes orange. The activated beads are treated with 2,6-lutidine followed by the compound to be loaded. A 3 or 5 carbon hydroxyl spacer is coupled to a phenolic hydroxyl serving as the anchoring site.

Figure 9: The Broad institute loading protocol on Alkylsilyl Macrobeads[23]

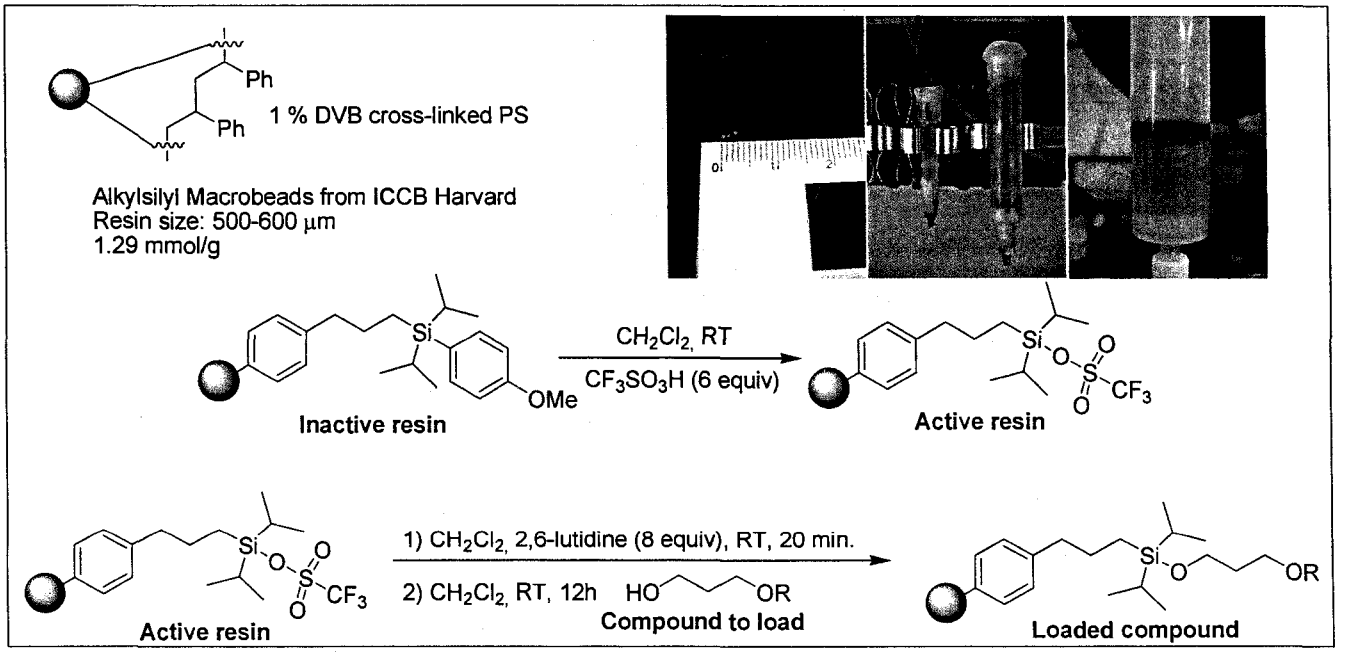
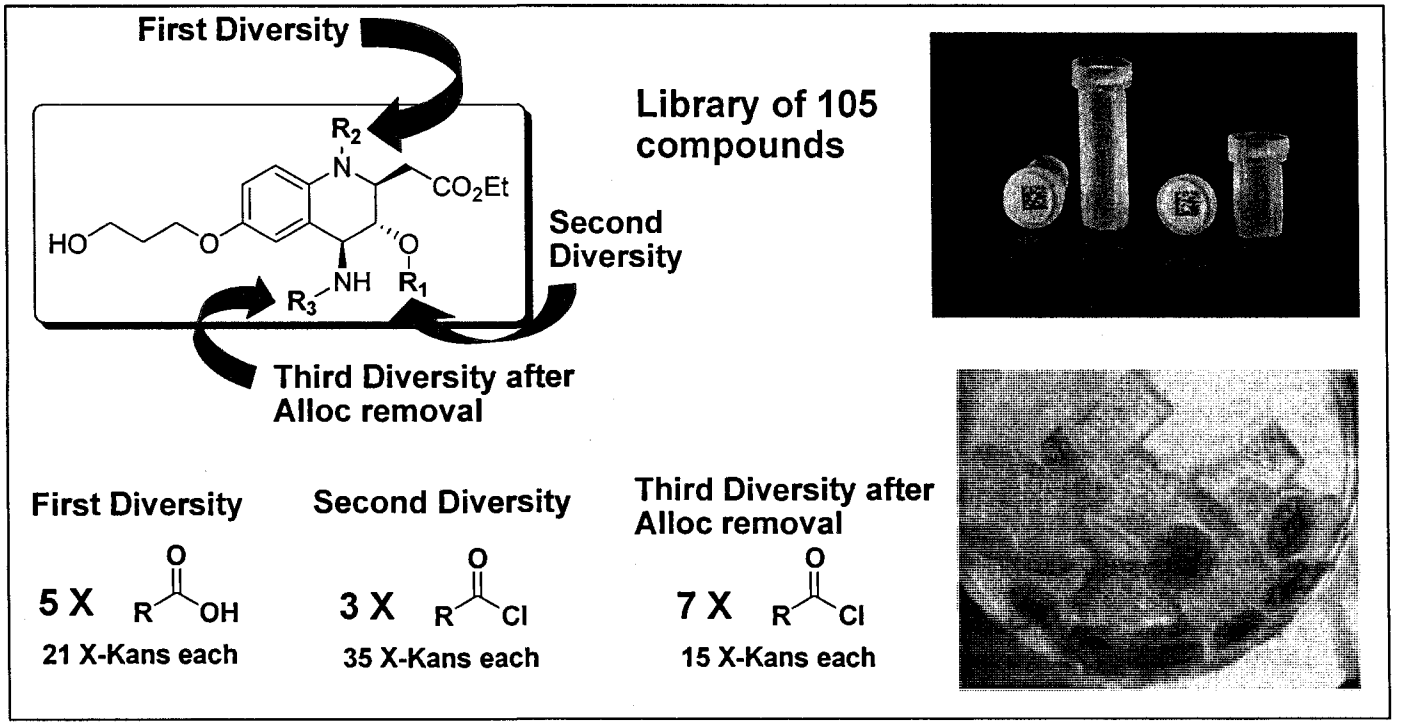


Figure 10 demonstrates how to generate a library of 105 compounds using the IRORI split-and-mix-type technology. The starting compound has 3

diversity sites; R_1 a free hydroxyl, R_2 Fmoc protected secondary amine and R_3 Alloc protected primary amine. Subsequent to loading, the resin is partitioned in a minimum of 105 X-Kans micro-reactors labeled with a 2D bar code, and then 105 compounds (5x3x7) can be generated in 16 reactions. All the compounds were obtained after cleavage from the macrobeads and no purification are performed. After four reaction steps and cleavage with HF pyridine, the purity of all the compounds was more than 95% (checked by HPLC/MS). Starting from 1.6 g of loaded resin, the 105 compounds were synthesized in a range of 9 to 13 mg.

Figure 10: Generation of a 105-membered library using IRORI® technology[23]

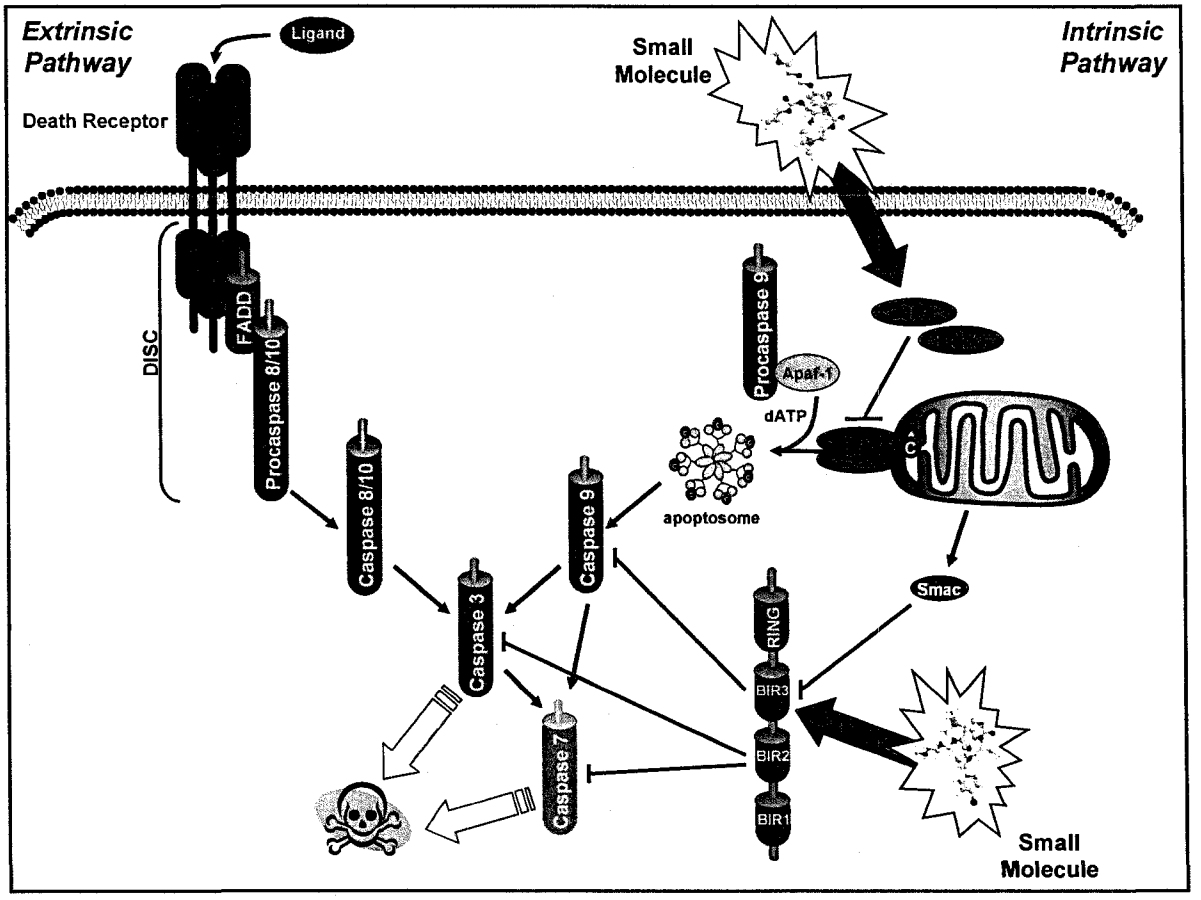


1.5.3 Interest in Apoptosis

Human cells in normal tissue within homeostasis are created and destroyed at the same rate. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult[24]. If the cell death rate is higher than cell creation, diseases such as neuro-degenerations, infertility and immuno-deficiencies can

occur. If new cells are created at a higher rate than cell death, such disorders can produce cancers. There are 2 different types of cell death; necrosis, acute cellular injury or lysosomes-assisted death and apoptosis, regulated cell death. A simplified apoptotic pathway is illustrated in **Figure 11**.

Figure 11: The Apoptotic pathway

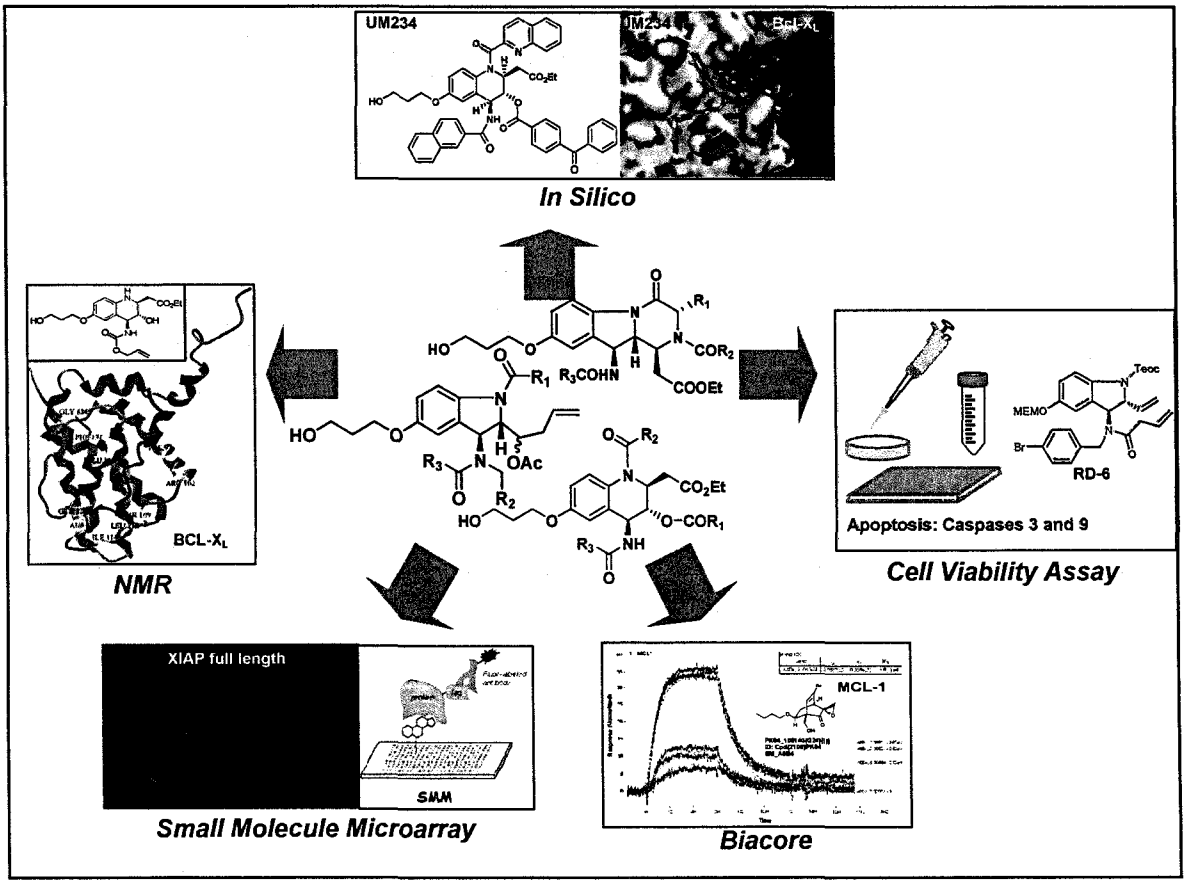


Proteins in the Bcl family are central regulators of programmed cell death in which its members inhibit apoptosis[24]. Bcl-2 and Bcl-X_L are such proteins and are found to be over-expressed as a protein-protein complex in many cancers and are supplement to tumor initiation, progression and resistance to therapy[24]. Inhibition of this protein-protein interaction by a small molecule can prove to be a useful therapeutic and to induce apoptosis in cancer cells. Another route to cell death in the apoptotic pathway is through binding of the Smac protein with the BIR3 domain. IAPs, Inhibitor-of-Apoptosis-Proteins, are also regulators of programmed cell death in which inhibit apoptosis and are found to be over-

expressed in cancer cells. IAPs bind to the Smac protein, preventing the complexation of Smac and BIR3, thus inhibiting apoptosis. A small molecule can also be found useful in binding with IAPs leading to the promotion of apoptosis. An abundance of research has been done on these protein-protein interactions, Bcl-2 - Bcl-X_L and IAPs – Smac, important structural information such as X-Ray, *In silico* studies, NMR and SAR by NMR has already been reported and should aid in the design of small molecules[24]. In collaboration with Professor Robert Korneluk laboratory, CHEO's Apoptosis Research Center, the Arya group has identified interesting small molecules which interfere with the Apoptotic pathways.

1.5.4 DOS Chemistry Validation Tools

Figure 12: DOS Chemistry Validation Tools

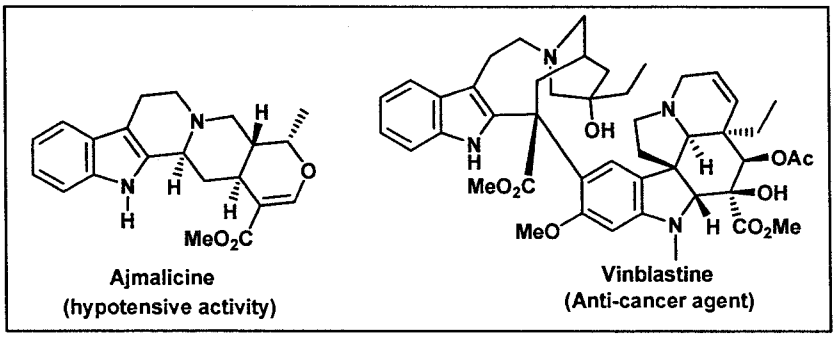


Upon the creation of a library of compounds comes the importance of collaborations. Many validation tools are at hand of the Arya group, **Figure 12**. In the chemical biology of Apoptosis project, the compounds libraries are screen through protein NMR, *in silico* studies, cell viability assays as well as through small molecules microarrays. In the Structure/Function Characterization of Kinase Signaling Networks project, Biocore analysis is done with our libraries in which surface plasma resonance is collected through the interested protein which is bound on a gold sheet. These tools help the group identify and modify good hits, build strategies in designing new scaffolds and validate how small molecule probe can help us further understand protein-protein interactions.

1.6 Project Goal

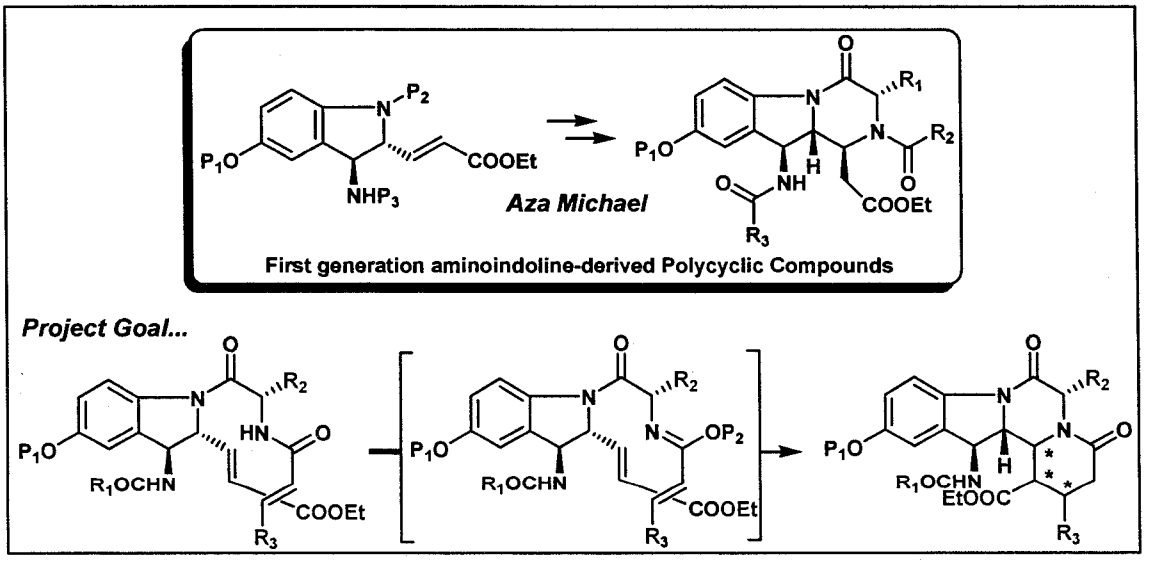
The goal of this project is founded in producing indoline-based polycyclic derivatives. There are several natural products known in Nature that possess the indole and indoline scaffolds in which their derivatives demonstrate a wide range of biological activities [25]. These natural product-like cores are excellent starting points to apply the tools of diversity-oriented syntheses. In **Figure 13**, two indole alkaloids-based natural products are presented. Ajmalicine is found in the *Rauwolfia serpentine* plant, a small shrub mostly found in Asia, and is used as an antihypertensive.[26] Vinblastine is found in *Catharanthus roseus*, in which Ajmalicine can also be extracted.[26] This natural product is used in cancer chemotherapy and it is one of the most effective anti-cancer agents available.

Figure 13: Indole alkaloid-based natural products[26]



The target derivatives of this project are part of a new class of Indoline polycyclic alkaloid-like compounds having an acid functionality. Initially, the chemistry is developed by a model study on a simple indoline core then pursued on the enantio-enriched indoline core. Once the solution phase chemistry has been developed, a manual solid phase synthesis will complete this methodology study. As mentioned, this methodology thesis is divided in three parts; the model study, the enantio-enriched study and the manual solid phase synthesis.

Scheme 4: Tetracyclic indoline derivatives as target molecules

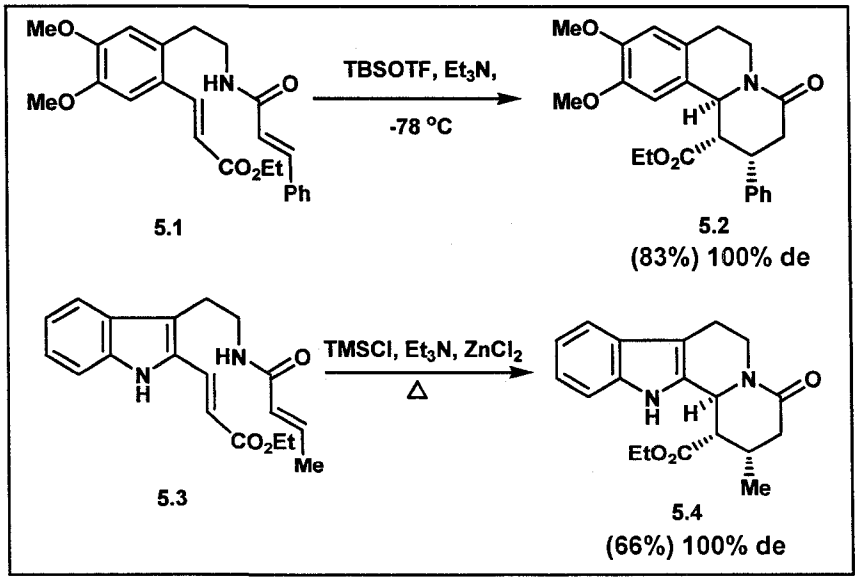


The project goal is to develop a second generation methodology to obtain polycyclic indoline derivatives. As seen in **Scheme 4**, the first generation of the indoline project featured a tricyclic system and the creation of a new stereogenic

center. This system was fully developed in solution and solid phase and having three diversity points, a 90-membered library has been generated. The next step was to generate a tetracyclic system. The coupling of a peptidic side-chain to the indoline core followed by a tandem aza-Michael cyclization can potentially generate our tetracyclic compound having three diversity points and three new stereogenic centers. The enantio-enriched indoline core first requires a 14 step synthesis. To initially validate this methodology, a model study was envisioned.

To lead the model study, we found an interesting literature by Ihara and Fukumoto, on the synthesis of polycyclic natural products employing highly diastereoselective intramolecular double Michael reactions[27]. **Scheme 5** demonstrates the Lewis acid-catalyzed double Michael reaction. **5.1** and **5.3** were each converted to the benzo[α]quinolizidine **5.2** and the indolo[2,3-α]quinolizidine **5.4** respectively as single isomers. It was found that treatment of the amide esters with bases such as LDA, LiHMDS or NaH only led to mono-Michael products.

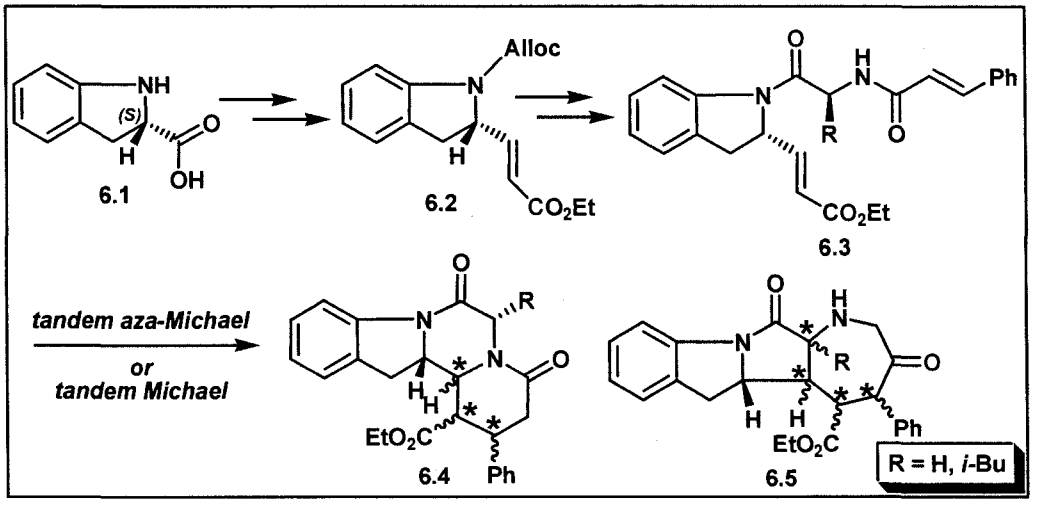
Scheme 5: Literature example to lead model study



Starting from commercially available (*S*)-(−)-Indoline-2-carboxylic acid, **6.1** (**Scheme 6**) can be converted to **6.2** through carboxylic reduction, amine protection, oxidation to the aldehyde and Wittig reaction. Upon the coupling of a peptidic side-chain, **6.4** or **6.5** can potentially be synthesized through tandem aza-Michael or Michael addition respectfully. The aza-Michael mechanistic route was firstly proposed but as we applied different triflates as the Lewis acids, the tandem Michael product appeared. This late discovery can prove very useful in a DOS project as well as in a library generation. In one step, we can produce 3 to 4 chiral centers depending on the Lewis acid used; **6.4**, **6.5**.

The peptidic side-chain is very interesting for inducing diversity to the compound since many amino acids can be used (Glycine, leucine, alanine, phenylalanine, etc.) as well as different acryloyls and cinnamoyls.

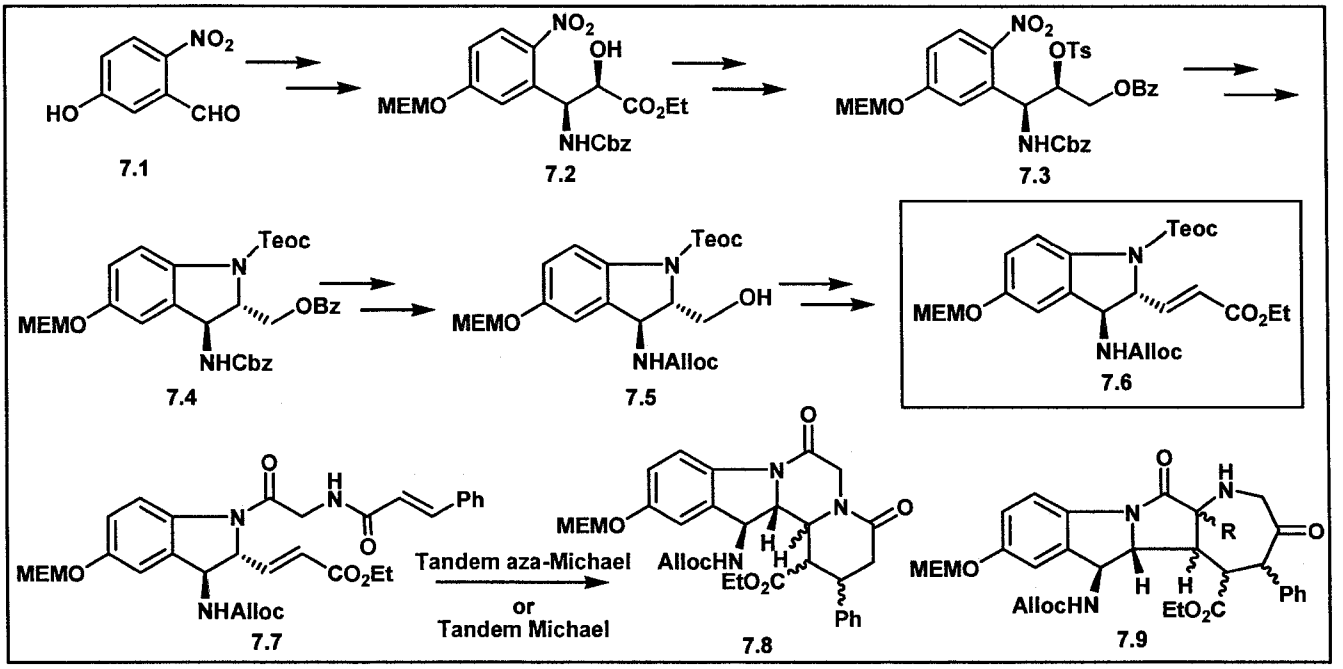
Scheme 6: Model system approach to tetracyclic compound



Upon completion of the model study, its application to an enantio-enriched scaffold will be made. The starting compound **7.6** (**Scheme 7**) is prepared through a previously published, 14 step synthesis starting with 5-hydroxy-2-nitro-benzaldehyde, **7.1**[21]. This synthetic route features a Sharpless asymmetric aminohydroxylation reaction to give compound **7.2** in 79% yield (>92% ee). The indoline core **7.4** is cyclized through mild basic conditions upon selective nitro-reduction. The overall sequence is very clean and the desired aminoindoline **7.6**

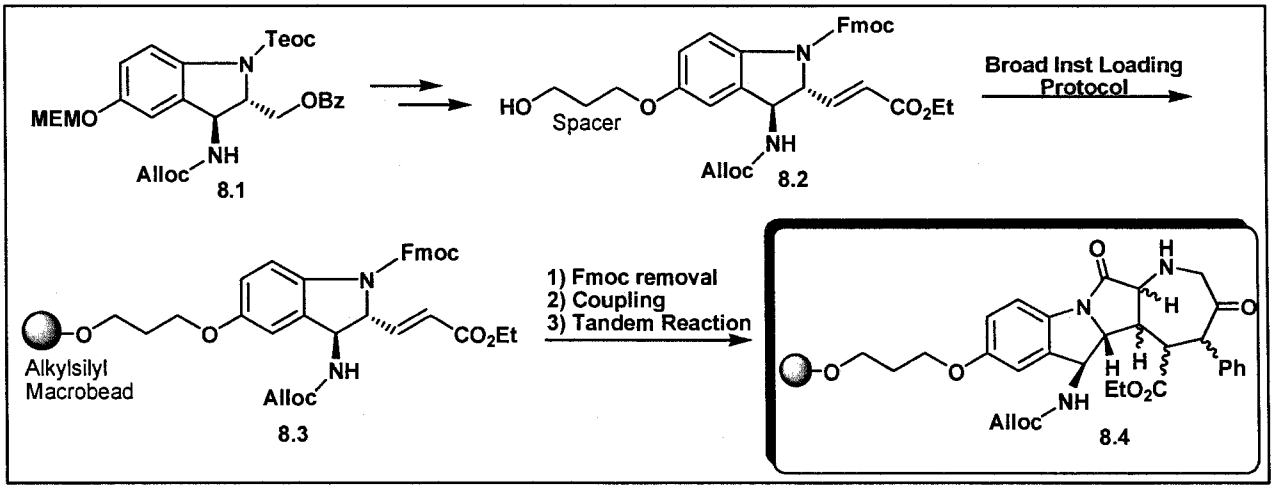
could be obtained in large quantities (5–10 g) in a short period. With a practical method in hand for this scaffold, the next step is to couple our peptidic side-chains and to investigate the tandem aza-Michael/Michael additions, **Scheme 7**.

Scheme 7: Application of model study to enantio-enriched scaffold



To complete this methodology study, a manual solid phase synthesis (**Scheme 8**) is to be attempted on the enantio-enriched indoline and attain tetracyclic indoline derivatives for potential library generation. Compound **8.1** will serve as a start point for loading the indoline core onto alkylsilyl macrobeads. Following extensive loading experience in the lab, a three carbon spacer is suggested prior loading. The removal of the OMEM group will provide the immobilization site.

Scheme 8: Manual solid phase synthesis of enantio-enriched system

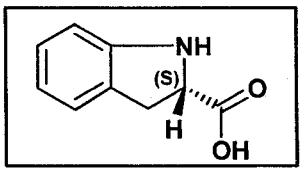


CHAPTER 2: Model Study

2.1 Introduction

The goal of this model study was to demonstrate the coupling of the peptide side chains to the Indoline moiety and then to attempt the following tandem Michael additions. The success of this model study will serve as a benchmark upon its application to the enantio-enriched Indoline core and then again with the solid phase synthesis. As a good starting compound, we came up with an optically active indoline-carboxylic acid, **Figure 14**. This starting material will allow us to reach the indoline core equipped with a Michael acceptor in a few steps.

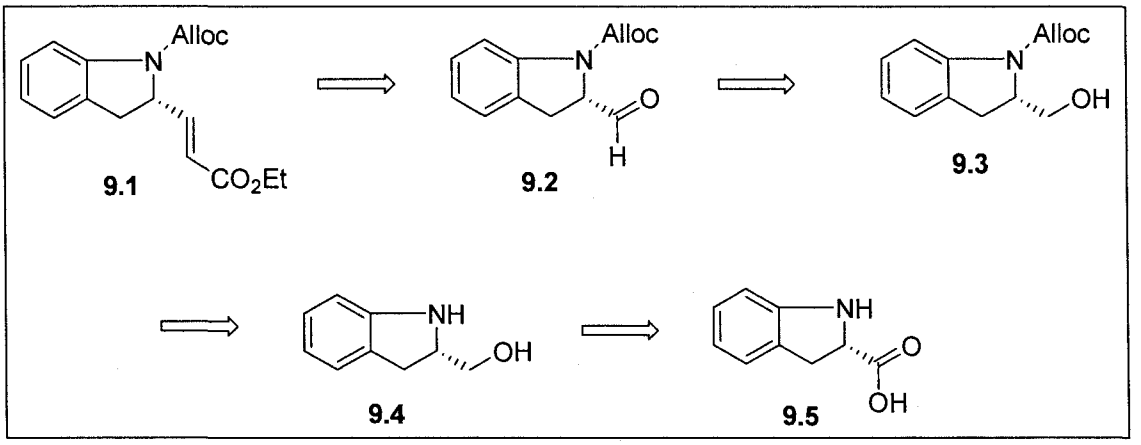
Figure 14: (*S*)-(−)-Indoline-2-carboxylic acid as a starting point for the Model Study



2.2 *Retro-Synthetic Analysis*

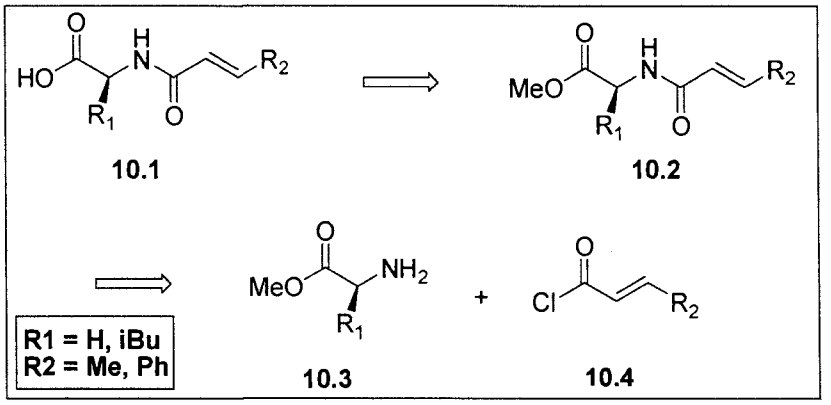
The indoline core **9.1** is our first target. This indoline scaffold has a protected secondary amine as well as a chiral Michael acceptor with a *trans* ethyl ester functionality. This electro-deficient derivative (**9.1**) can be prepared through a Wittig reaction starting with aldehyde **9.2**. Aldehyde **9.2** can be prepared by an oxidation of alcohol **9.3**. At this point the secondary amine of **9.4** is protected as the *N*-Alloc group. Lastly, our starting material **9.5** is to be reduced to yield primary alcohol **9.4**.

Scheme 9: Retro-Synthetic Analysis of the indoline core



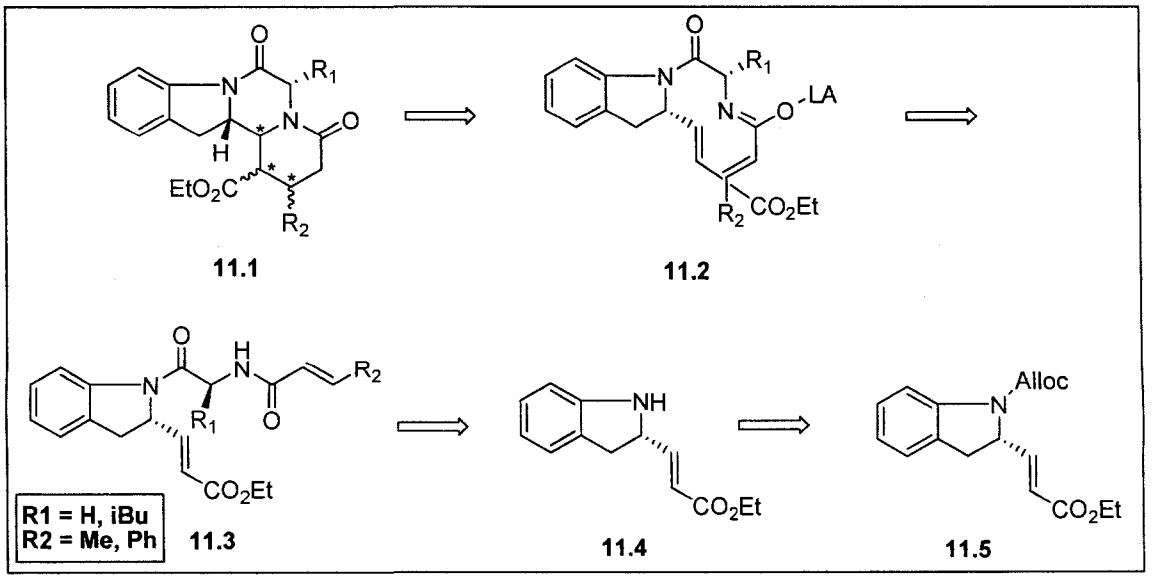
Compound **9.1** will serve as an initial point for introducing diversity to the indoline scaffold. **Scheme 10** demonstrates the retro-synthetic analysis for the peptide side-chains as the second Michael acceptor site. At this point, we can introduce 2 diversity points. The first diversity is defined by the choice of L-amino esters and the second diversity by the choice of acid chlorides which has an olefin. These building blocks can be easily condensed and further hydrolyzed to yield the peptide side chains **10.1**. Initially, we are interested in the glycine amino-ester with the cinnamoyl and crotyl adducts as the coupling of these peptides to the indoline core can be more feasible due to potential steric hindrance.

Scheme 10: Retro-Synthetic Analysis of the peptide side chains



Having these peptide side-chains in-hand, we can now concentrate on the coupling of these adducts to the indoline core and attempt the tandem aza-Michael cyclization, **Scheme 11**. Tetracyclic compound **11.1** is believed to go through the aza-enol **11.2** starting with the coupled precursor **11.3**. The presence of a Lewis acid is envisioned to induce the aza-enol formation under basic conditions. This tandem aza-Michael reaction would generate three new chiral centers. The removal of the *N*-Alloc protecting group **11.5** is needed to obtain amine **11.4** to then attempt the coupling of these peptides with the help of a coupling agent.

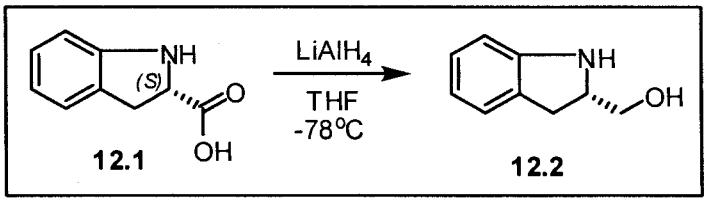
Scheme 11: Retro-Synthetic Analysis of the tetracyclic formation



2.3 *Synthesis of the indoline core*

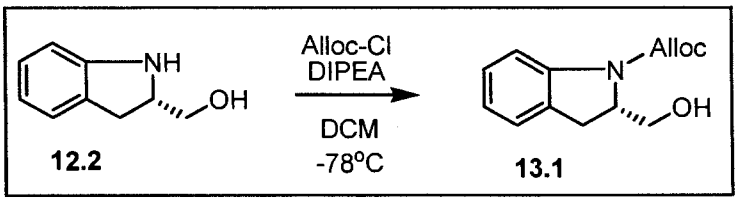
The first step was a simple reduction of the indoline carboxylic acid. This was achieved by using lithium aluminum hydride which reduced the acid into a primary alcohol (**Scheme 12**). The product **12.2** was obtained in high yields (84%) and was characterized by NMR and MS analyses. Its formation was confirmed by the appearance of [δ 3.78 ppm (dd, J = 11.0, 3.8 Hz, 1H), 3.64 ppm (dd, J = 11.0, 6.3 Hz, 1H)] in the ^{1}H NMR spectrum representing the two protons of the newly formed CH_2.

Scheme 12: Reduction of the (S)-indoline-2-carboxylic acid



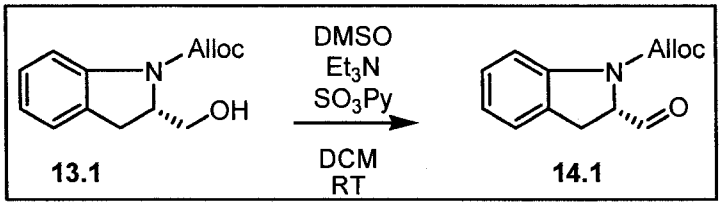
The second step involved the protection of the secondary amine of the indoline core using allyl chloroformate (**Scheme 13**). The reaction had to be performed at low temperature to avoid protection of the primary alcohol. Only one equivalent of Alloc-Cl was required and the use of DIPEA was needed to neutralize the formation of HCl in this nucleophilic attack. Compound **13.1** was isolated in 99% yield. After purification, the ^{1}H NMR confirmed the formation of the product. The olefinic protons appeared as two doublets at 5.42 ppm (J = 17.2 Hz) and 5.32 ppm (J = 10.4 Hz) and as a multiplet between 6.09 and 6.00 ppm integrating for one proton each.

Scheme 13: Protection of the indoline secondary amine



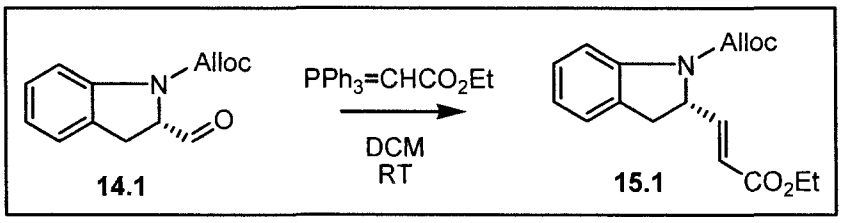
The next step was the oxidation (Parikh-Doering) of the alcohol to form an aldehyde. The experimental conditions for oxidation found in the total synthesis of bafilomycin A_1 by Hanessian et *al.* were used to generate aldehyde **14.1** from the alcohol (**Scheme 14**)[28]. The DMSO and SO_3·Py form a charged dimethylthiopyridine complex where the nucleophilic alcohol attacks the charged sulfur eliminating the pyridine. Triethylamine is used to activate the methyl so the aldehyde can be formed. The reaction was quenched by introducing a saturated solution of ammonium chloride. The product was isolated in 84% yield. After purification, the product was characterized by NMR and MS analyses. The formation of the aldehyde functionality was confirmed by the appearance of a singlet at 9.69 ppm, integrating for an aldehyde proton in the ^{1}H NMR spectrum.

Scheme 14: Alcohol oxidation to aldehyde



The next step was the Wittig reaction where a stabilized ylide was added to the aldehyde. On a TLC, two (*cis* & *trans*) product isomers can be clearly seen. By flash chromatography, separation of the products was possible. A 98:2 ratio of *trans* to *cis* was obtained, with the *E* conformation of the new Michael acceptor as the major product. The formation of the major isomer is explained by the presence and hindrance of the triphenylphosphine. The product was isolated in 90% yield and characterized by NMR and MS analyses. ^{1}H NMR confirmed the formation of the Michael-type adduct with the appearance of new olefinic protons at 6.90 ppm (dd, J = 15.6, 6.4 Hz, 1H), 5.91 ppm (d, J = 15.6 Hz, 1H) and the presence of the ethyl esters at 4.17 ppm (q, J = 7.1 Hz, 2H) and 1.27 ppm (t, J = 7.1 Hz, 3H).

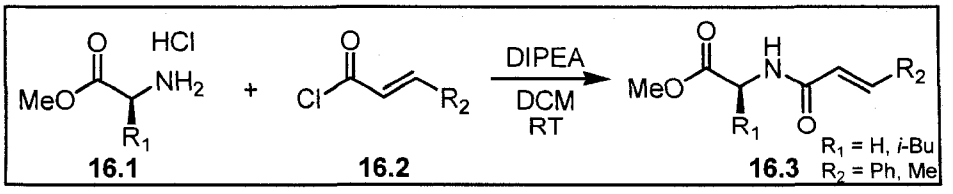
Scheme 15: Michael acceptor induced by Wittig reaction



2.4 Synthesis of the tetracyclic ring system

Before attempting the tandem Michael addition we need to prepare and couple the peptide side-chains. As a model study, ester **16.1** was first used as glycine and afterward, leucine (**Scheme 16**), but different amino acids can be used to generate the first diversity in a future library. The reaction involved a simple nucleophilic attack (S_N2 type) where the chlorine atom of the cinnamoyl-chloride or crotonyl-chloride, acts as a good leaving group. Again, many different acid chlorides could have been used. The phenyl and methyl group of this particular example represents the second diversity site. Aromatic groups are useful in providing hydrophilicity in a small molecule as well as in exhibiting membrane penetration properties. As for this reaction, DIPEA was used to neutralize the salt and to further activate the primary amine for the attack of the carbonyl. After purification, the product was characterized by NMR and MS analyses. Presence of a large singlet in the 3.7-3.8ppm allocates the methyl esters and the company of olefinic protons is also present.

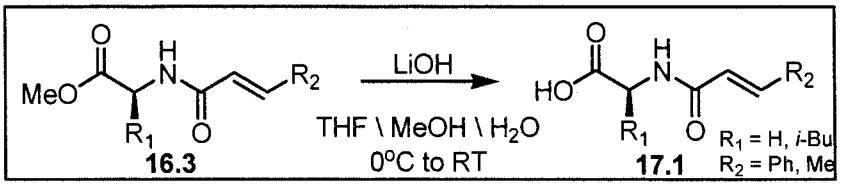
Scheme 16: Preparation of the side-chains peptide methyl esters



A hydrolysis reaction completes the formation of the desired side-chains to be coupled with the indoline core (**Scheme 17**). This is a well known reaction in which an ester undergoes a saponification to yield a carboxylic acid. Once

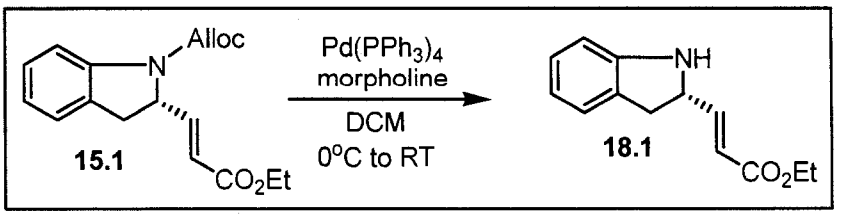
completed, the reaction mixture is neutralized. Due to the acid's hydrophilicity, the organic workup was very difficult and therefore not applied. Flash chromatography was also very difficult even when using 20% methanol in dichloromethane. These acids were then simply to be used as crude acids in the coupling reactions. The products were characterized by NMR and MS analyses. The ^{1}H NMR spectrum illustrated the disappearance of the methyl esters in the 3.7- 3.8 ppm regions.

Scheme 17: Preparation of the side-chains peptide acids



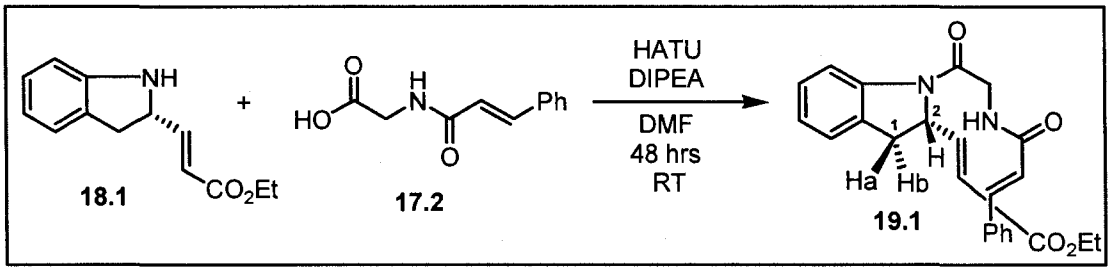
Deprotection of the secondary amine was the next step in the synthesis, **Scheme 18**. Liberating the amine will allow the addition of the peptide acid side-chains. The reaction was achieved using a palladium catalysis that binds to the π system of the allyloxycarbonyl group and then further releases CO_2. Morpholine is required to trap the remaining allyl group off the palladium complex and regenerate the catalyst. The product was obtained in 90% yield and was confirmed by the disappearance of the allylic protons of the alloc group in the ^{1}H NMR spectrum. It was also confirmed by the appearance of the N-H broad singlet at 3.90 ppm.

Scheme 18: Palladium-catalyzed Alloc deprotection



The next step was vital for the future of this project. The glycine-cinnamoyl acid (**17.1**) was first to be attempted in the coupling with the indoline scaffold, **Scheme 19**. The coupling of the side-chain was tried with several different coupling reagents; DIC/HOBT, HATU, HBTU and through the conversion of the side-chain into the acid chloride with $SOCl_2$. The best conditions were found to be with the use of HATU in DMF (to increase the solubility of the polar glycine-cinnamoyl). The coupling was monitored by TLC. The new observed spots were the activated ester and the coupling product **19.1**. The reaction was allowed to stir for 2 days. After purification, the product was characterized by NMR and MS analyses.

Scheme 19: Coupling of the glycine-cinnamoyl side-chain to the indoline core

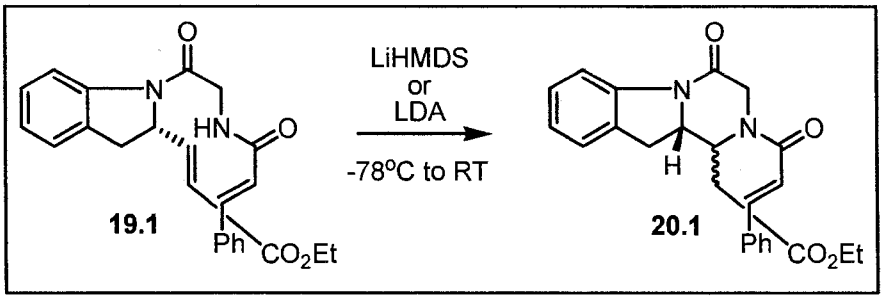


2D-NMR became very useful in the elucidation of this compound. The orientation of the protons on C1 is determined with the support of the COSY spectrum in which the doublet of doublet at 3.65 ppm and the doublet at 2.98 ppm represent H1a and H1b respectively. In the COSY spectrum, H2 can only see one of the two protons on C1, which explains that H1b is at a 90° angle to H2 in respects to the Karplus plot (J = 0 Hz). The product is also confirmed with the presence of 2 sets of olefinic protons, the first, which is adjacent and seen by H2 in the COSY spectrum and the other with the presence of 2 doublets at 6.52 and 5.92ppm having a common 15.7Hz coupling constant.

The final step in the synthesis was first attempted using the literature conditions reported by Ihara and Fukumoto[27d)e)]. The conditions stated the use

of LiHMDS or LDA as the base. The reaction led to the formation of a mixture of tricylic compounds, **Scheme 20**. The NMR clearly indicates the disappearance of 2 olefinic protons and the remaining olefinic protons adjacent to the phenyl group. Many condition variations were attempted. I tried increasing the temperature, adding more equivalents of base, using different solvents but the outcome remained the same.

Scheme 20: First attempt of the tetracyclic ring formation

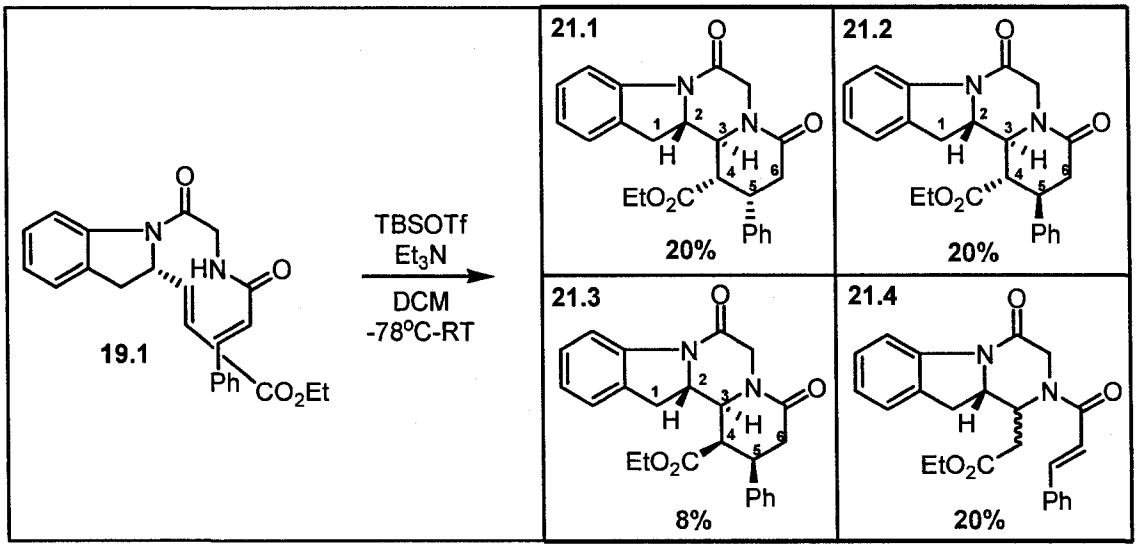


The first cyclization (i.e. aza-Micheal) is known in the Arya group, through previous work, to be quick and easy in basic conditions[21]. The second Michael addition seems to need a driving force for the formation of the enolate. In more recent literatures by Ihara and Fukumoto[27], the use of an excess of TBSOTf as a Lewis acid was mentioned, in the presence of Et_3N. The oxaphillic silyl-group would reduce the activation energy of these Michael additions and generate the enolate.

We applied the new reaction conditions but allowed the reaction temperature to warm to room temperature for complete consumption of the starting material. This reaction yielded 5 new compounds, **Scheme 21**. We isolated 3 different tetracyclic compounds and the racemic mixture of tricyclic compounds seen in **Scheme 20**. Tetracycles **21.1**, **21.2** and **21.3** were isolated by PREP HPLC in 20%, 20% and 8% respectfully with a total reaction yield of 68%. The formation of 3 new chiral centers has been achieved with these new compounds. As we are applying this project through a DOS program, these results can be very efficient. If generating a library of compounds while applying

this chemistry, we can be screening the mixture of compounds at once. Upon positive results, we can separate and individually screen yet again to determine the specific hit.

Scheme 21: Lewis acid mediated tandem aza-Michael cyclization with the glycine-cinnamoyl derived indoline



Upon isolation of these tetracyclic compounds, 2D NMR became indispensable in identifying these structures. The first clue in determining the formation of the tetracycles is the disappearance of both pairs of olefinic protons in the proton NMR. The second clue is the proton chain at C2 through C5 with a CH_2 (C1 and C6) at each end identifiable with the COSY spectrum. In **Figure 15**, we can see an example this proton chain in the COSY spectrum of compound **21.1**. The red line represents the correlation between CH protons on C2 through C5. The green line represents the correlation between CH_2 protons and their adjacent CH. The proton at C2 was identified and referenced upon previous NMRs as the C5 and C6 protons were recognized by the adjacent carbonyl with the help of the HMBC spectrum. All tetracycles were fully characterized using ^{1}H and ^{13}C NMR and COSY, HSQC, HMBC 2D-NMR. Relative stereochemistry has been confirmed with the help of NOESY 2D-NMR which is all summarized in **Figure 16**.

Figure 15: COSY spectrum of 21.1 illustrating an example of the proton chain of C1 to C6 of the formation of the tetracyclic compounds

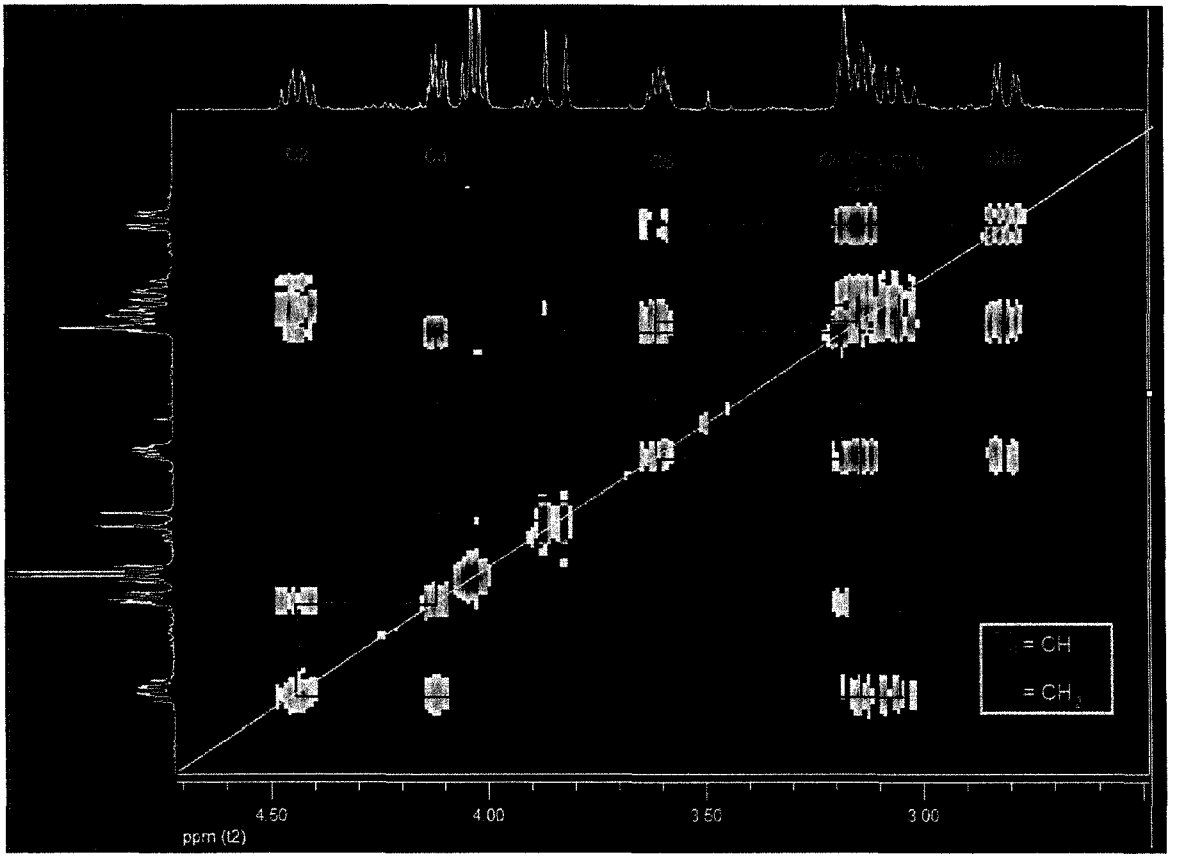
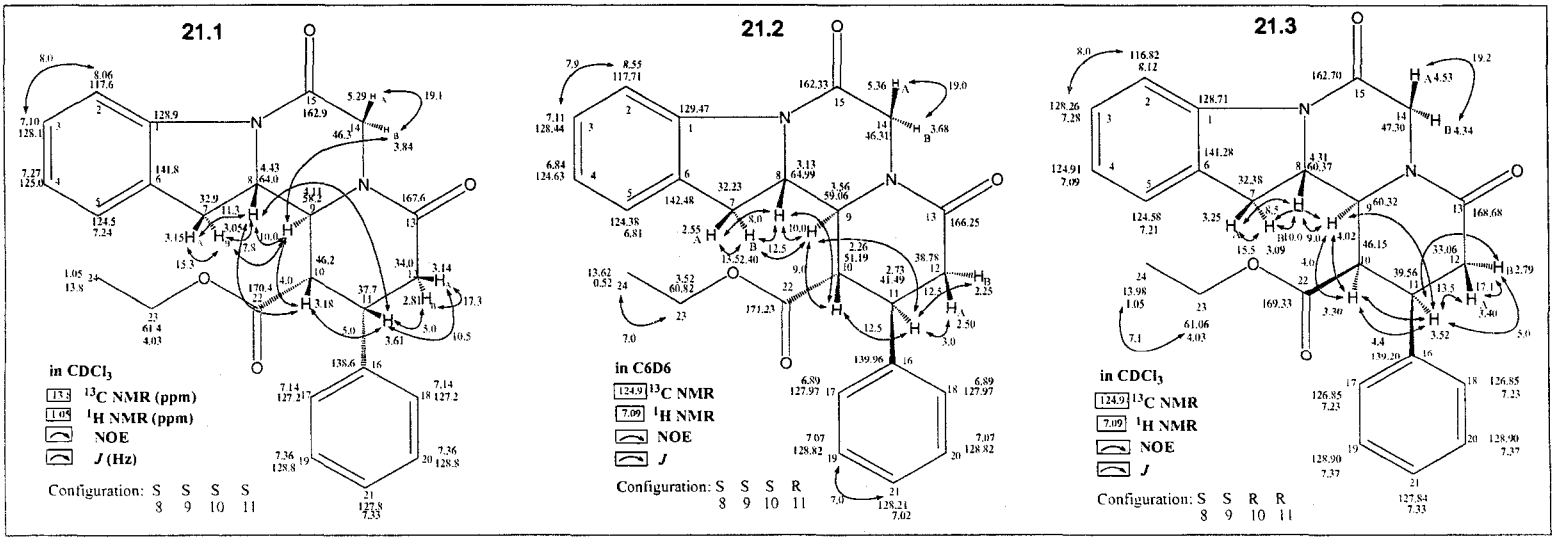
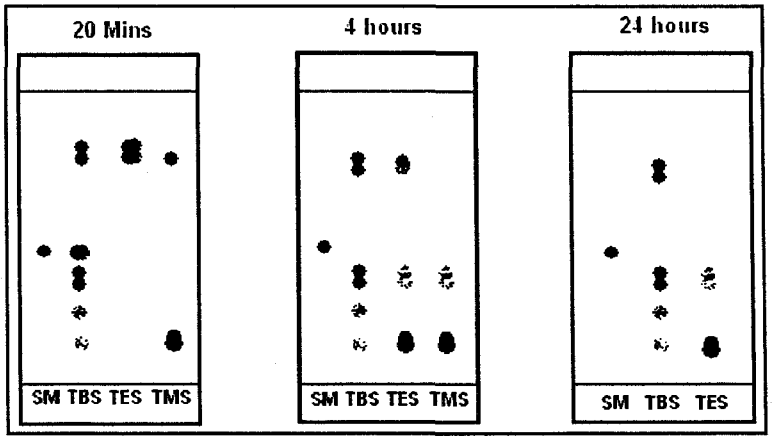


Figure 16: Full characterization of tetracyclic compounds 21



At this point we wanted to optimize the tandem aza-Michael reaction from a stereoselectivity point-of-view. We started a Lewis acid study in which we wanted to vary the size of these silyl groups by replacing the TBSOTf reagent with TESOTf and again with TMSOTf which will be considered hereon as reaction conditions A, B and C respectively. Three reactions were started side-by-side and monitored by TLC. **Figure 17** represents the TLC plates of this Lewis acid study. The first TLC was taken after 20 minutes. At this point, reaction A was as seen before in **Scheme 21**, with the starting material as the major spot. Reaction B had no more starting material and a large spot at the top of the plate comparing to the racemic mixture of tricyclic compounds previously found. Reaction C also had no more starting material but the TLC illustrated a dominant new spot in the polar region of the plate with a minor spot in the tricyclic compound region. A second TLC analysis took place at the 4 hour mark. Reaction A illustrated full consumption of the starting material with the same products as before. Reaction B interestingly illustrated as well the same new spot in the polar region of the plate with a minor and reduced spot in the tricyclic compound region. It is appearing as the tricyclic compound is being transformed into this new product which is not corresponding to the previously found tetracyclic compounds. Reaction C now illustrates one major spot with the disappearance of the minor product in the tricyclic compound region and was, at

Figure 17: Representation of the TLC plate of the Lewis acid study



TLC Plate: (SM) starting material, (TBS) reaction with TBSOtf; (TES) reaction with TESOtf; (TMS) reaction with TMSOtf; Solvent system (3:1, EtOAc:Hexane)

this point, worked-up and purified. Again, in reaction C, the tricyclic compound spot seems to be converted into this new product. The last TLC analysis was taken at the 24 hour mark. Reaction A had no noticeable changes consequently was worked-up and purified. Reaction B illustrated as reaction C carried out, the tricyclic compound spot disappeared and all that remained was again the new polar compound spot. The reaction was worked-up and then subjected to purification. Reaction B and C had identical results with each 90% yields. This new compound's mass corresponded to the wanted product, but in this step, mass confirmation is not a good method to verify the product as the starting material, tricyclic and tetracyclic compounds all have the same molecular weight.

The NMR analysis illustrated that both sets of olefinic protons had disappeared, indicating we may have a new tetracyclic compound. At first we thought it was the forth possible diastereoisomer, having the phenyl and ester *anti* to each other in a (*R*, *S*) orientation. This compound was later rejected as the possible product since the HSQC spectrum demonstrated the presence of an NH functionality in the compound in which a broad singlet was not correlating to any carbon peak and in addition, the previously seen C1 to C6 proton chain was not observed in the COSY spectrum. With the help of numerous 2-D NMR experiments, the product was identified as a tetracyclic compound having a 6x5x5x7 ring system (**22.1**). The initial enolate was not formed upon the aza-intermediate but through an enolate adjacent to the indoline nitrogen. The second cyclization would follow the same mechanistic path as seen in the previous reaction conditions. The newly formation of this 6x5x5x7 ring system would generate 4 chiral centers in this one step. This compound was isolated as a single compound in 90% yield, **Scheme 22**. This discovery was very fascinating; with the choice of Lewis acid, we can potentially control the size of the ring system generated by the tandem aza-Michael or tandem Michael addition.

Scheme 22: Lewis acid study on the tandem aza-Michael cyclization

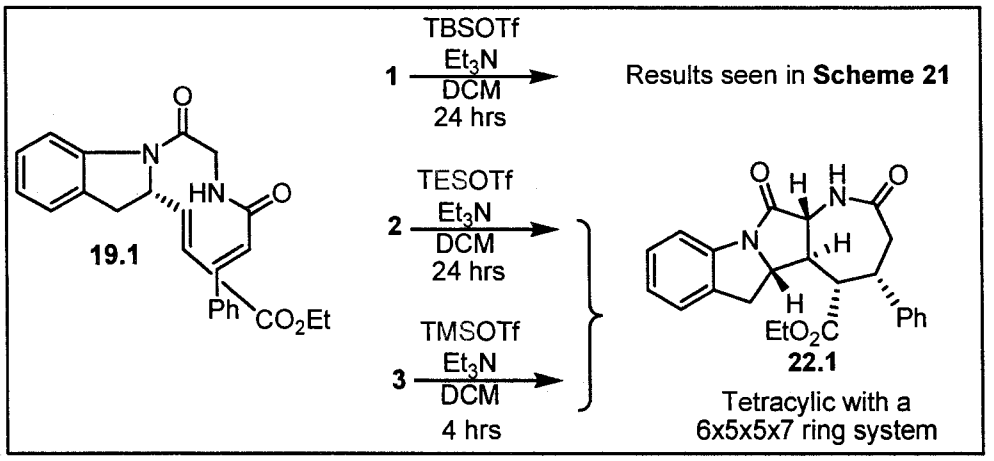
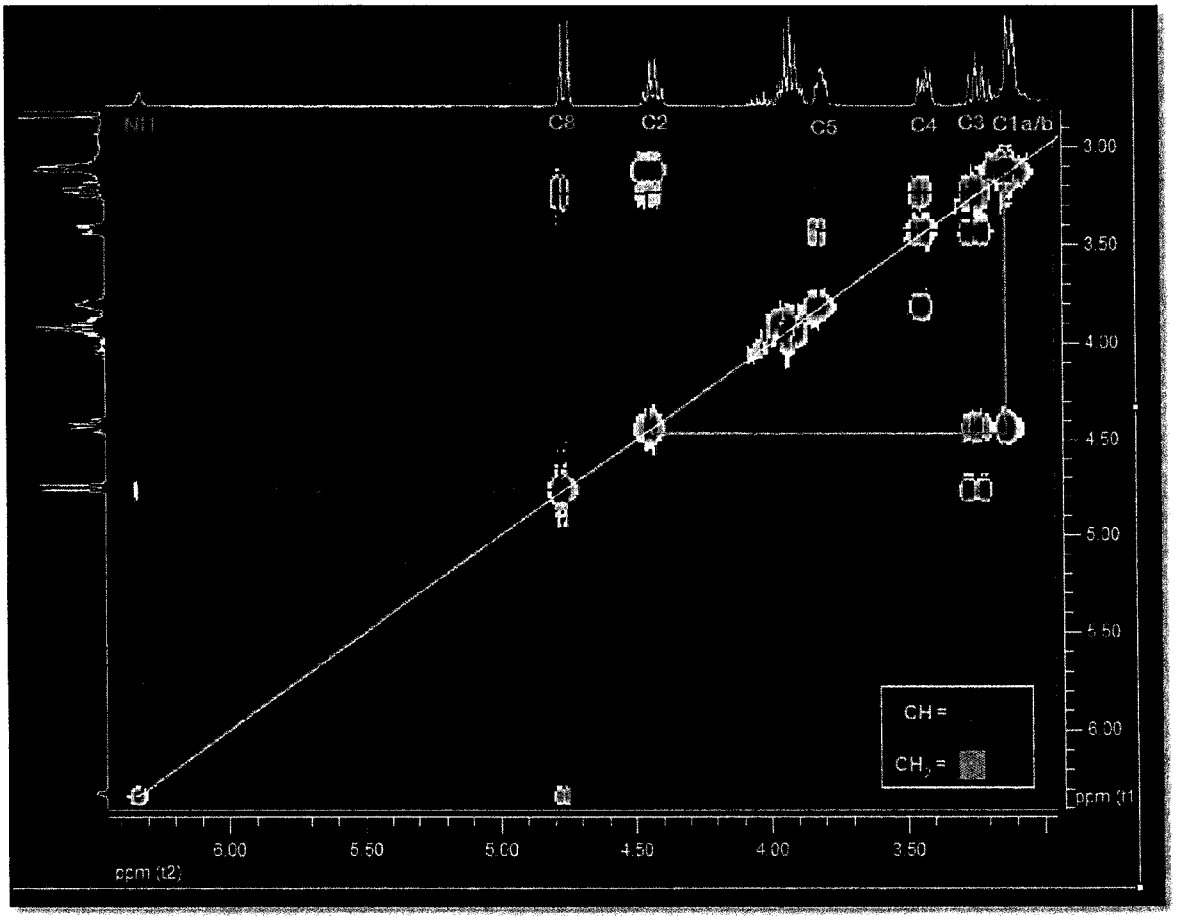


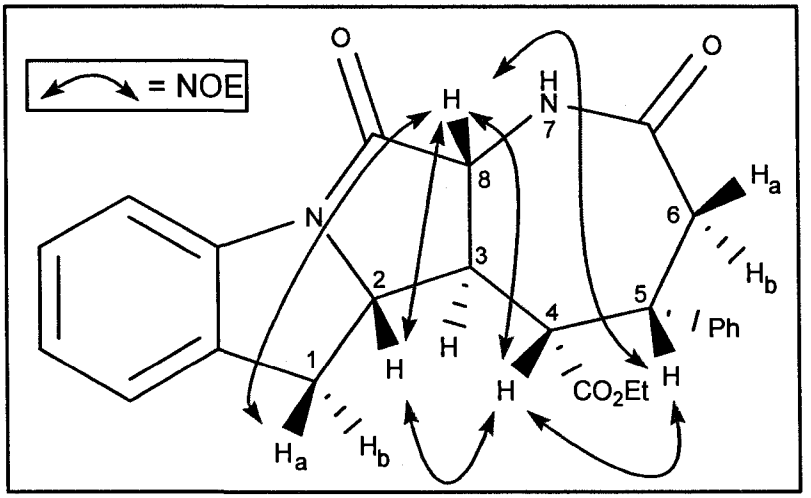
Figure 18: COSY spectrum of tetracyclic compound 22.1



In **Figure 18**, we can clearly see the correlation between protons starting with the NH which associates with H8 and H8 to H3. H3 correlates with C2 and

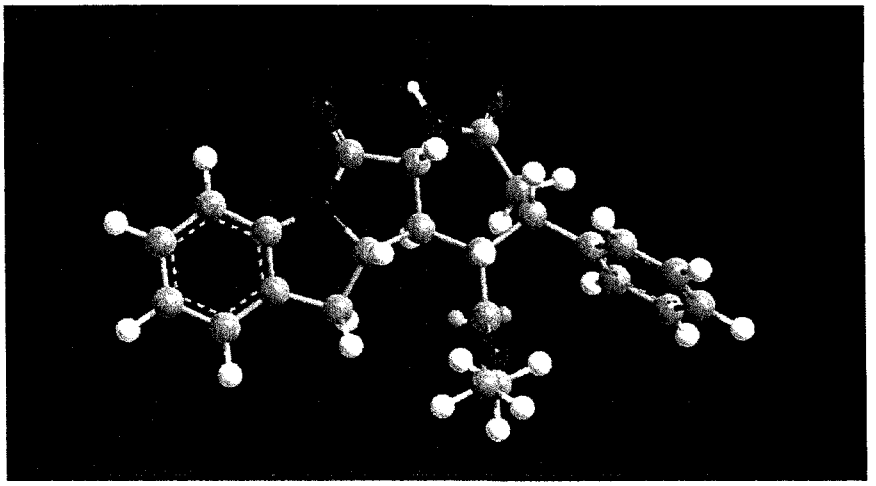
C4, indicating the split between the 5 and 7 ring formation. The stereochemistry was confirmed with the help of 2D NOESY experiments. **Figure 19** highlights the NOE effect between neighboring protons. To solve the stereochemistry, we must begin with H2, as it is the only known orientation and so, our point of reference. In the NOESY spectrum, H2 has 2 important 1-3 correlations with H8 and H4. H8 and H5 also have an important long distant correlation, indicating that H2, H4, H5, H8 are all on the same face of the molecule. In ^{1}H NMR, H3 is seen as a doublet doublet of doublets where H2 and H3 share a coupling constant of 8.8Hz; H3-H4 as well as H3H8 also share a coupling constant of 11.3Hz. These coupling constants help us position H3 as being *trans* to H2, H4 and H8. On top of all these analyses, a DEPT 135 experiments also illustrated the presence of 3 CH_2s in which would associate to C1, C6 and the ethyl ester.

Figure 19: NOE correlation of tetracyclic compound 22.1

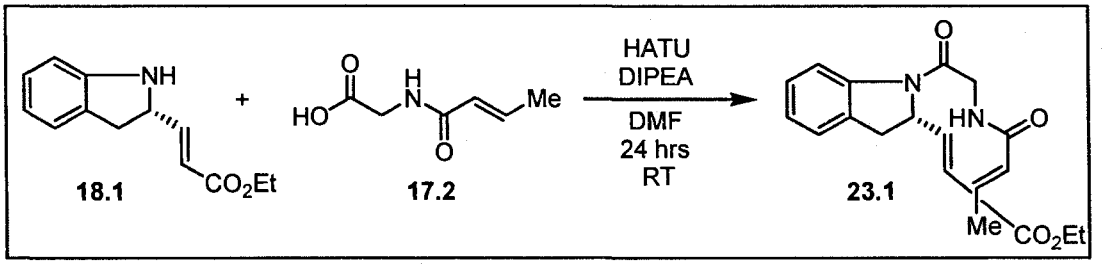


Later, we were able to grow crystals of compound **22.1**. It took a lengthy amount of time to grow good enough quality crystals for the crystallographer to start working. At first, crystals were very fine needles and too small to analyze. In a solvent system consisting of chloroform and heptane, the compound was able to crystallize throughout slow evaporation at room temperature. The stereochemistry was re-confirmed as we acquired the x-ray structure of **22.1** (**Figure 20**).

Figure 20: X-ray structure of tetracyclic compound 22.1

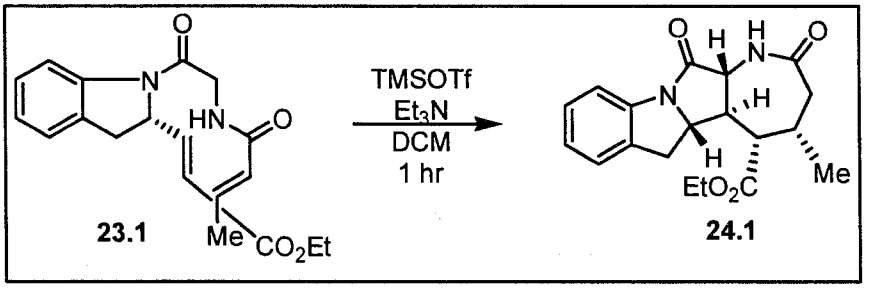


Scheme 23: Coupling of the glycine-crotyl side-chain to the indoline core



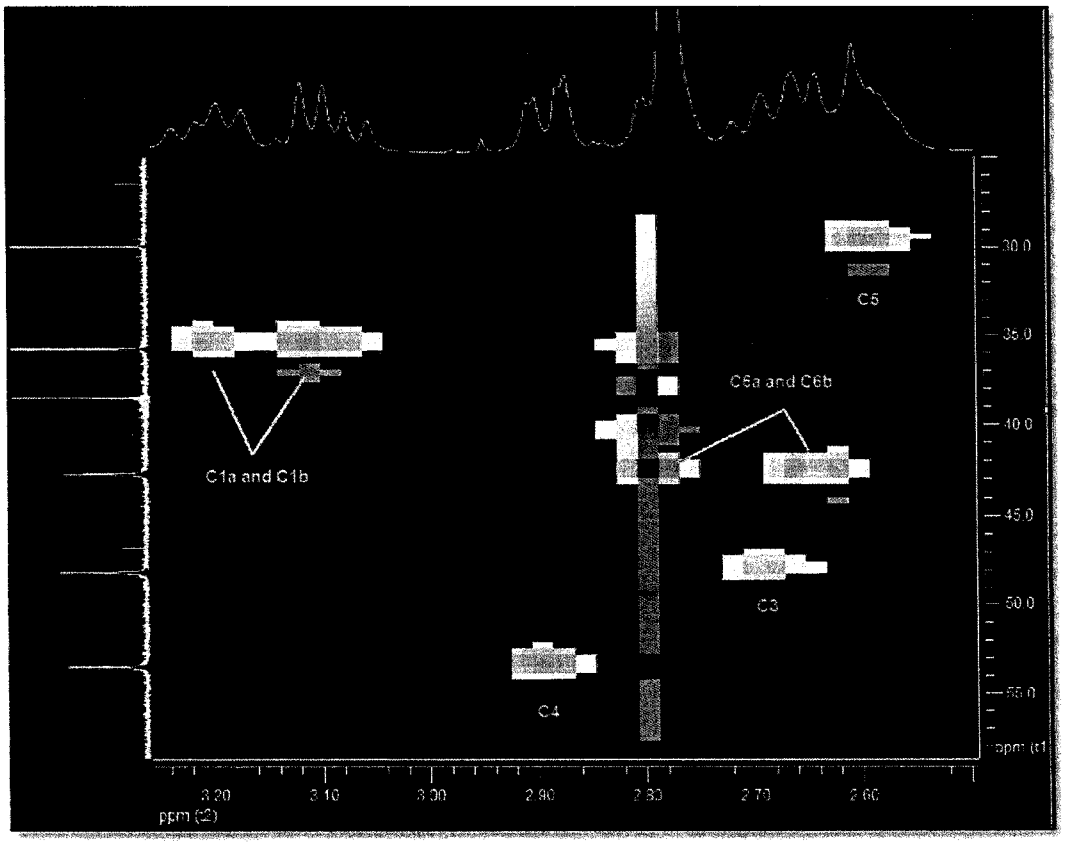
The next step in this model study was to validate this methodology with a second case study, to couple the glycine-crotyl side-chain to the indoline core and try this reaction again, **Scheme 23**. This coupling was completed in similar yields in half the time. NMR analysis clearly illustrated the presence of both sets of olefinic protons and a very large doublet at 1.88ppm representing the methyl group. Upon isolation, we submitted this compound to the TMSOTf-mediated tandem Michael conditions. We were pleased to observe very similar results.

Scheme 24: Lewis acid mediated tandem Michael cyclization with the glycine-crotyl derived indoline



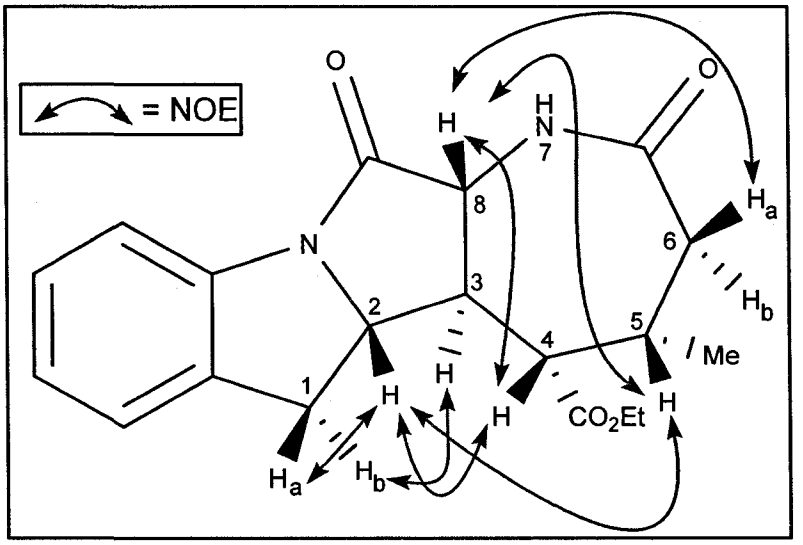
This reaction resulted in the isolation of again, a single product in 80% yield, **Scheme 24**. Multiple NMR experiments were once more vital to the elucidation of this compound. 1D ^{1}H-NMR displayed the disappearance of both sets of allylic protons as well as the HSQC indicated a broad singlet not correlating to a carbon peak. NMR analysis was a little more challenging as the splitting wasn't as good as compound **22.1**'s spectra.

Figure 21: HSQC spectrum enlargement of tetracyclic compound 24.1



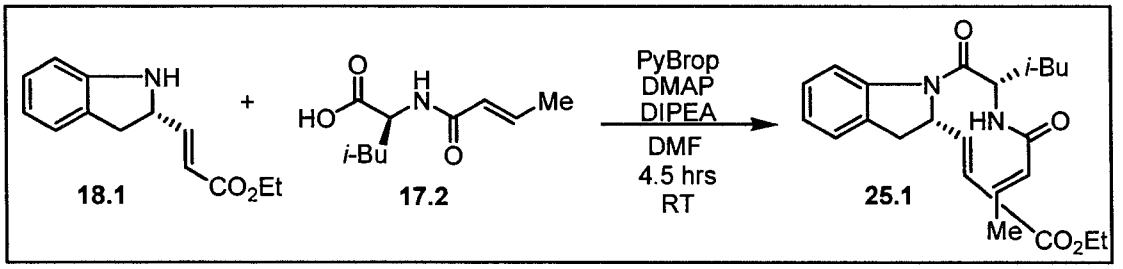
As seen in **Figure 21**, the HSQC was very useful in identifying CH_2s; both C1 and C6 proton shifts correlated with their associated carbon. Proton-proton correlation was then easier to associate with the help of the COSY spectrum. Once again, NOESY experiments were utilized for stereochemistry assignments. NOE correlation is summarized in **Figure 22**, omitting most 1-2 correlations as relationships can prove misleading. H2 and H8 have too similar chemical shift to be able to display proper correlation. As a difference from the NOESY spectrum of compound **22.1**, a correlation between H1b and H3 as well as H1a and H2 can be seen. We weren't able to grow crystal for this compound following numerous attempts.

Figure 22: NOE correlation of tetracyclic compound 24.1



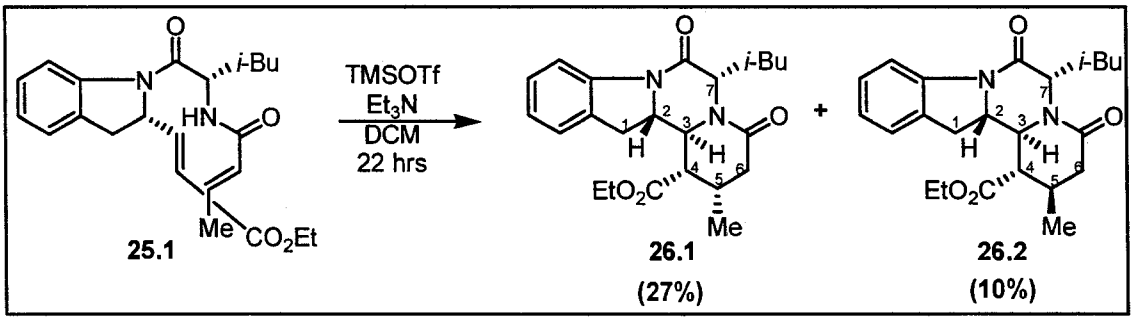
As a final case study, we wanted to try and add an extra diversity in the scaffold by changing the amino acid in the side-chain. Glycine was replaced with leucine, in which an *iso*-butyl group is added to the side-chain. This would add a bulky group to the molecule. The coupling of this side-chain was very difficult and not possible with HATU; the added steric bulk is hindering the condensation of the 2 subunits. At this time we once again started multiple coupling attempts with various reagents. Coupling was found successful with the use of PyBrop and DMAP at the reduced yield of 52% (**Scheme 25**).

Scheme 25: Coupling of the leucine-crotyl side-chain to the indoline core



The coupling was confirmed by NMR analysis. Several highlights of the ^{1}H-NMR are the presence of both sets of allylic protons, the presence of 2 large doublets integrating for 3 protons and one large triplet integrating for 2 protons (*i*-Bu) as well as a large doublet integrating for 3 protons (Me), all in the aliphatic area. Upon isolation, we submitted this compound **25.1** to the TMSOTf-mediated tandem Michael conditions, **Scheme 26**.

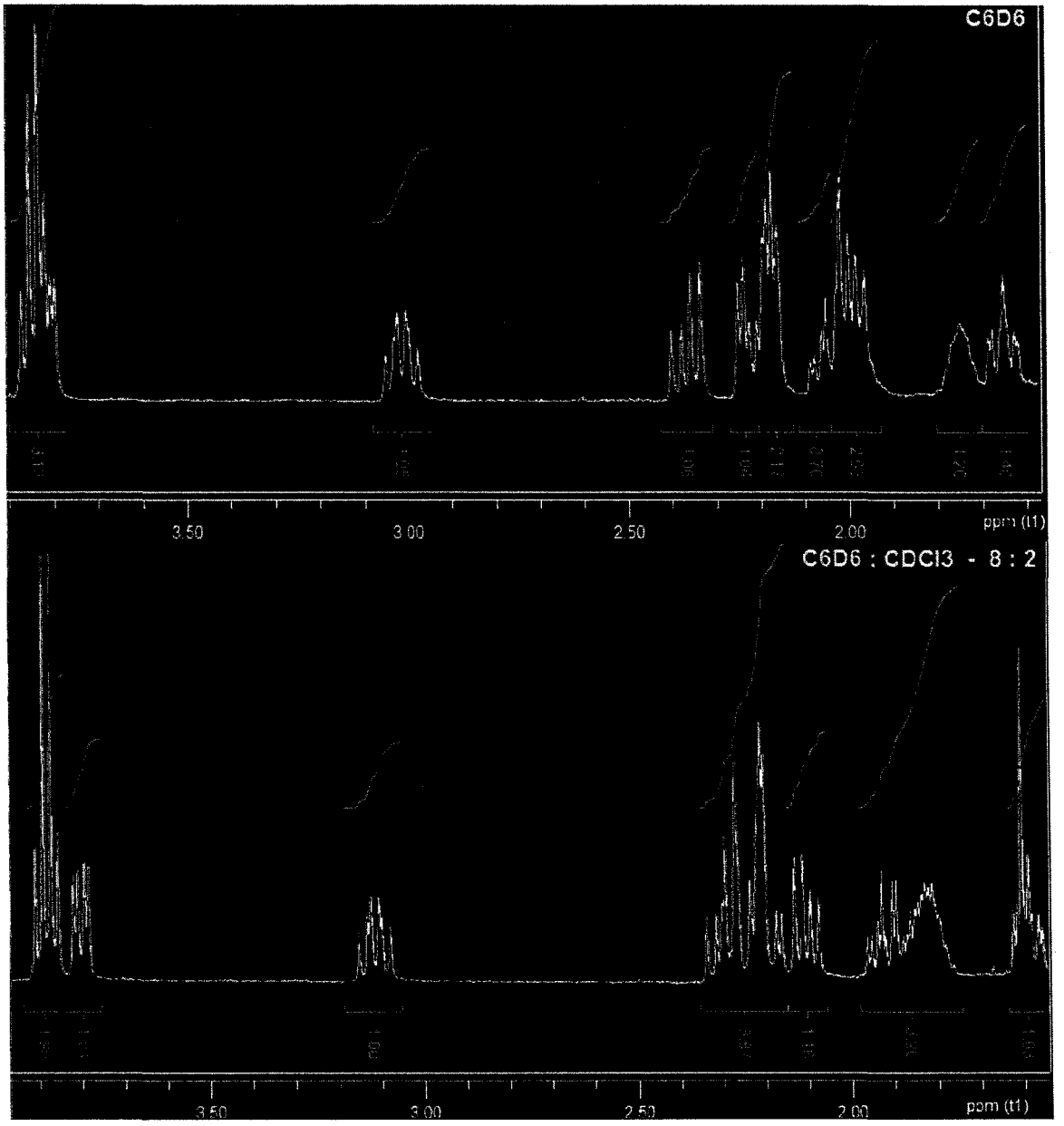
Scheme 26: Lewis acid mediated tandem aza-Michael cyclization with the leucine-crotyl derived indoline



This reaction was slower than previous TMSOTf-mediated cyclizations; the starting material was fully consumed after 22 hours of stirring at room temperature, according to TLC. The TLC indicated 2 new spots, very close to each other, one in which was more dominant. Upon purification, the 2 new compounds were isolated in 27% and 10% yield. Following the NMR analysis, two compounds were indeed tetracycles but to our surprise, these tetracycles were in a 6x5x6x6 ring system. Interestingly, by introducing the *iso*-butyl group on the side-chain, we are again generating the 6x5x6x6 ring formation. NMR

studies highlight the disappearance of both sets of olefinic protons and we can notice the C1 to C6 proton chain in the COSY spectrum, previously seen in **Figure 15**. 2-D NMR including NOESY experiments were very helpful to fully characterize these compounds with their relative stereochemistry, seen in **Scheme 26**.

Figure 23: Deuterated solvent study for optimal characterization of 26.1



NMR experiments were conducted in deuterated benzene but some vital proton peaks were superimposed with other peaks. At this time we added 20% $CDCl_3$ and noticed better splitting between the H3 doublet of doublet and the ester quartet, **Figure 23**, in the 3.8-3.9ppm region. The 1 to 2ppm region of the spectrum was also different; some superimposed peaks were free and *vice versa*. 2-D experiments were done again in this new solvent ratio and proved very useful in the NOESY spectrum and the stereochemistry determination.

With the goal of explaining the mechanistic aspects of the tandem aza-Micheal and tandem Michael cyclizations, transition states have been proposed with the help of a molecular model. **Figure 24** illustrates the proposed transition states for the aza-Michael cyclization producing a 6x5x6x6 ring system. In the first cyclization, transition states **A** and **B** are to be favored over transition states **C** and **D** as the protons identified in red can have a favoring *trans* (TS **A** and **B**) or disfavoring *cis* (TS **C** and **D**) orientation. Moreover, transition states **C** and **D** have the Michael acceptor in a pseudo-axial position in which would not be favored due to hindrance to the indoline bicyclic skeleton. Product **B** should be more stable than product **A** since the enol in product **A** has a disfavoring *Z* configuration compared to product **B** who has a more stable enol having an *E* configuration. Moreover, intermediates can potentially have secondary interactions between the Lewis acid and neighboring amide (TS **A/C**) or ester (TS **B/D**).

Having the favored transition state **B**, transition states **E**, **F**, **G** and **H** have been proposed again with the explicit use of a molecular model. In the second cyclization, transition states **E** and **F** are to be favored over transition states **G** and **H** as the protons identified in red can have a favoring *trans* (TS **E** and **F**) or disfavoring *cis* (TS **G** and **H**) orientation. In the disfavoring boat-shaped transition state **E**, both olefins have a favoring *trans* orientation although in the favoring chair-shaped transition state **F**, both olefins have a disfavoring *cis* orientation. In any case, both products through transition **E** and **F** have been isolated in identical 20% yields. As for transition states **G** and **H**; transition state **G** has disfavoring boat-shaped as well as having both olefins have a disfavoring

cis orientation. No product has been isolated with this stereochemistry. Although in the favoring chair-shaped transition state **H**, both olefins have also a favoring *trans* orientation and this product was isolated in 8% yield.

Figure 24: Proposed transition states of the tandem aza-Michael cyclizations with glycine-based side-chain, generating a 6x5x6x6 ring system (TBSOTf)

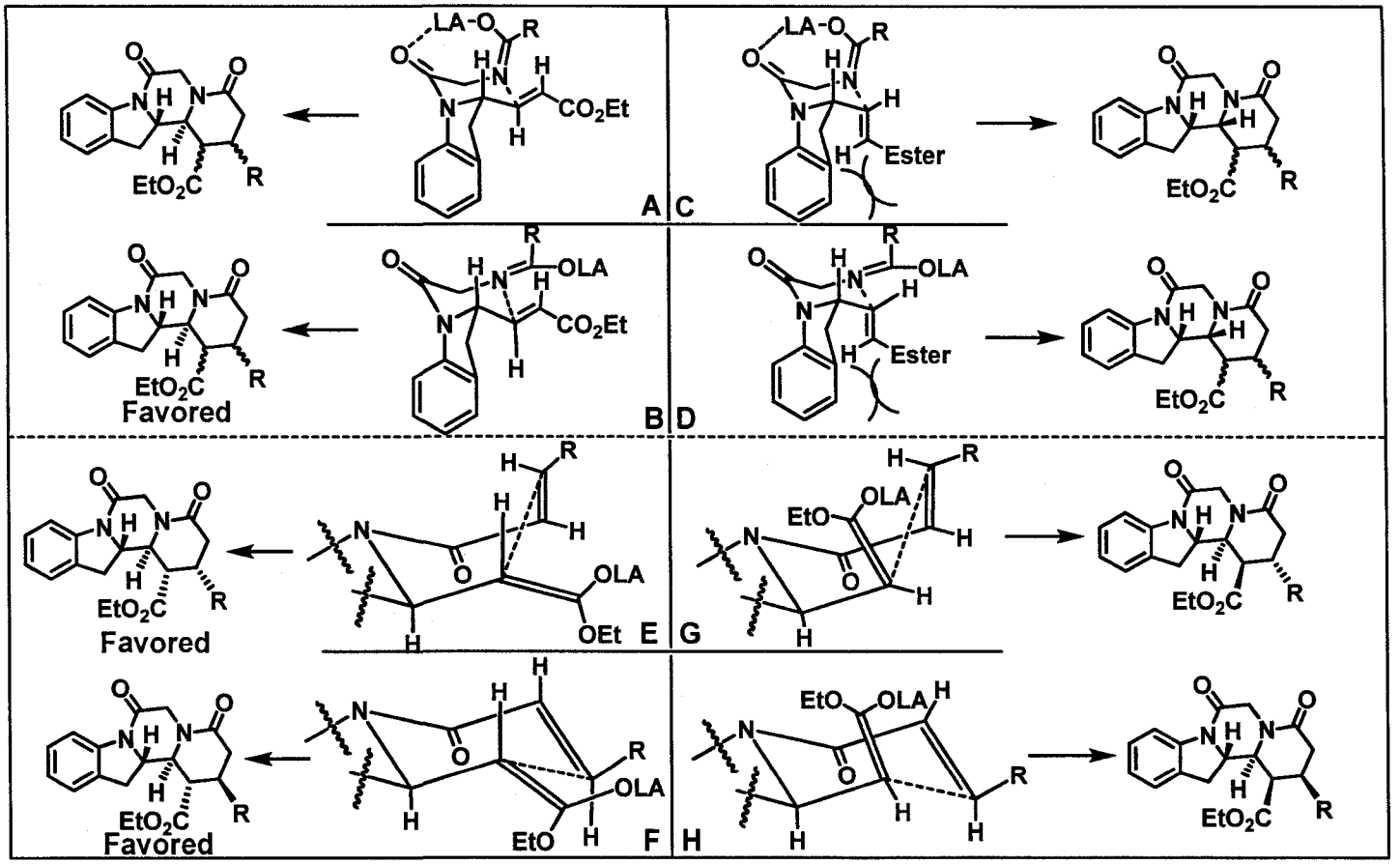
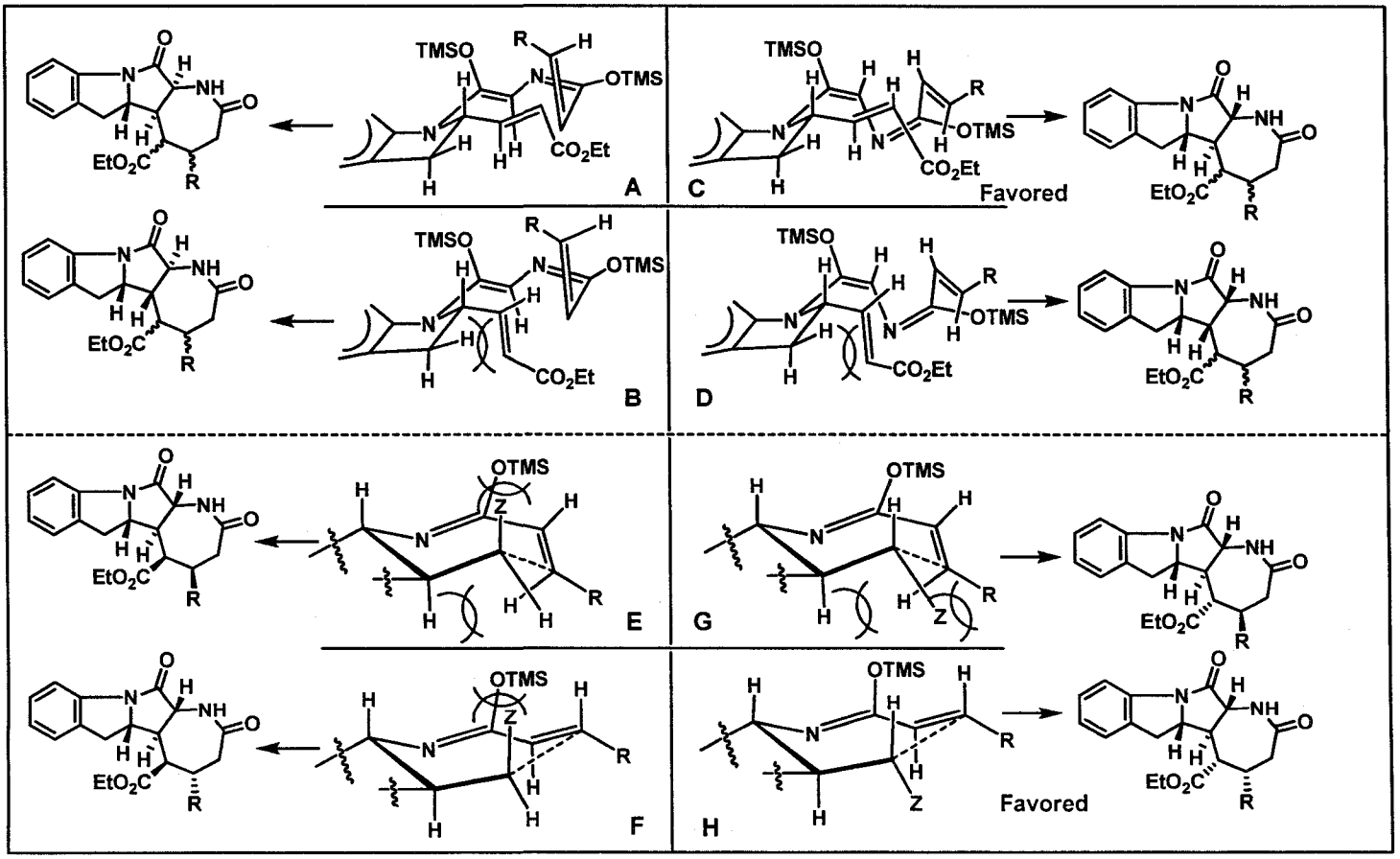


Figure 25 illustrates the proposed transition states for the Michael cyclization producing a 6x5x5x7 ring system. To explain the transfused system obtained in the first cyclization, four transition states could again be drawn with the help of a molecular model; **A**, **B**, **C** and **D**. On structure **A** and **B**, the first enol adopts a *Z* configuration which is less stable than the *E* configuration shown in structures **C** and **D**. Moreover, structures **B** and **D** have a disfavoring interaction between the electro-deficient alkene and the equatorial proton from

the indoline core allowing us to postulate the privileged structure **C** to lead to the right stereochemistry observed in the X-ray of the final compound.

As for the second cyclization, transitions states **E** and **F** showed a strong disfavored interaction from the ester group (Z) in a less stable axial position with the Lewis acid complex. Structure **E** and **G** also showed disfavored interaction between one axial proton and the proton from the Michael acceptor. Finally, a strong disfavoring interaction between the ester group (Z) and the R group in the transition state **G** helped to indicate that structure **H** was the most favorable transition stated as observed in the X-ray of the final compound.

Figure 25: Proposed transition states of the tandem Michael cyclizations with glycine-based side-chain, generating a 6x5x5x7 ring system (TMSOTf)



The tandem aza-Michael cyclization with its application to the leucine-crotyl side-chain and TMSOTf as the Lewis acid would follow the proposed transition states in **Figure 24**. Having a bulky *iso*-butyl group adjacent to the indoline amide would strongly disfavor the formation of a 5-membered ring. As for the second cyclization, the boat-shaped transition state E having both olefins a more-favoring *trans* orientation (27%) would demonstrate to be more stable over the chair-shaped transition state F having both olefins have a disfavoring *cis* orientation (10%).

2.5 Conclusion

At this point, we have conducted three case studies, in which we coupled 3 different side-chains; two having a glycine amino acid and each with either a methyl or phenyl functionality and one with a functionalized amino acid (leucine) with the methyl group at the end.

This model study has proven to be successful towards the initial goal of being able to couple such side-chains to an indoline core and further undergo a double cyclization to form a tetracyclic molecule. At this point, we can now apply the knowledge of the model study to the enantio-enriched indoline scaffold.

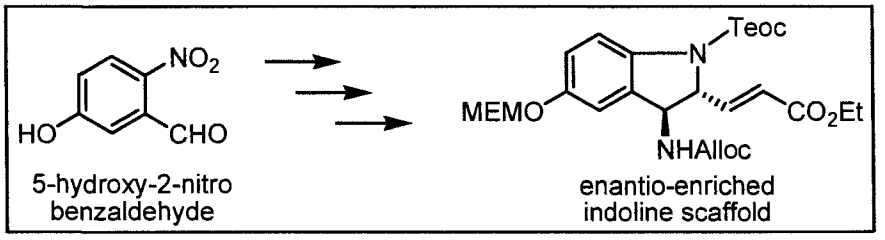
This model study has many variations and optimizations to be studied to have an in-depth and complete understanding of this tandem Michael cyclization but we concentrated our efforts of this project with its application to the enantio-enriched indoline scaffold and further on solid support.

CHAPTER 3: Enantio-enriched System Study

3.1 *Introduction*

This project turns a new chapter in which the model study is to be tested on the enantio-enriched indoline scaffold. This scaffold is specifically designed to maximize chirality and to possess multiple diversity sites. This scaffold is equipped with a hydroxyl group as an anchoring site for immobilization on solid support. **Figure 26** illustrates 5-hydroxy-2-nitrobenzaldehyde as the starting material for the synthesis of an enantio-enriched indoline scaffold. This starting compound has the necessary functionalities for the formation the indoline rings and a hydroxyl anchoring site for future solid phase synthesis projects. The synthetic route to this enhanced indoline core was previously developed by the Arya group[21].

Figure 26: 5-hydroxy-2-nitrobenzaldehyde as the starting material for the synthesis of the enantio-enriched indoline scaffold

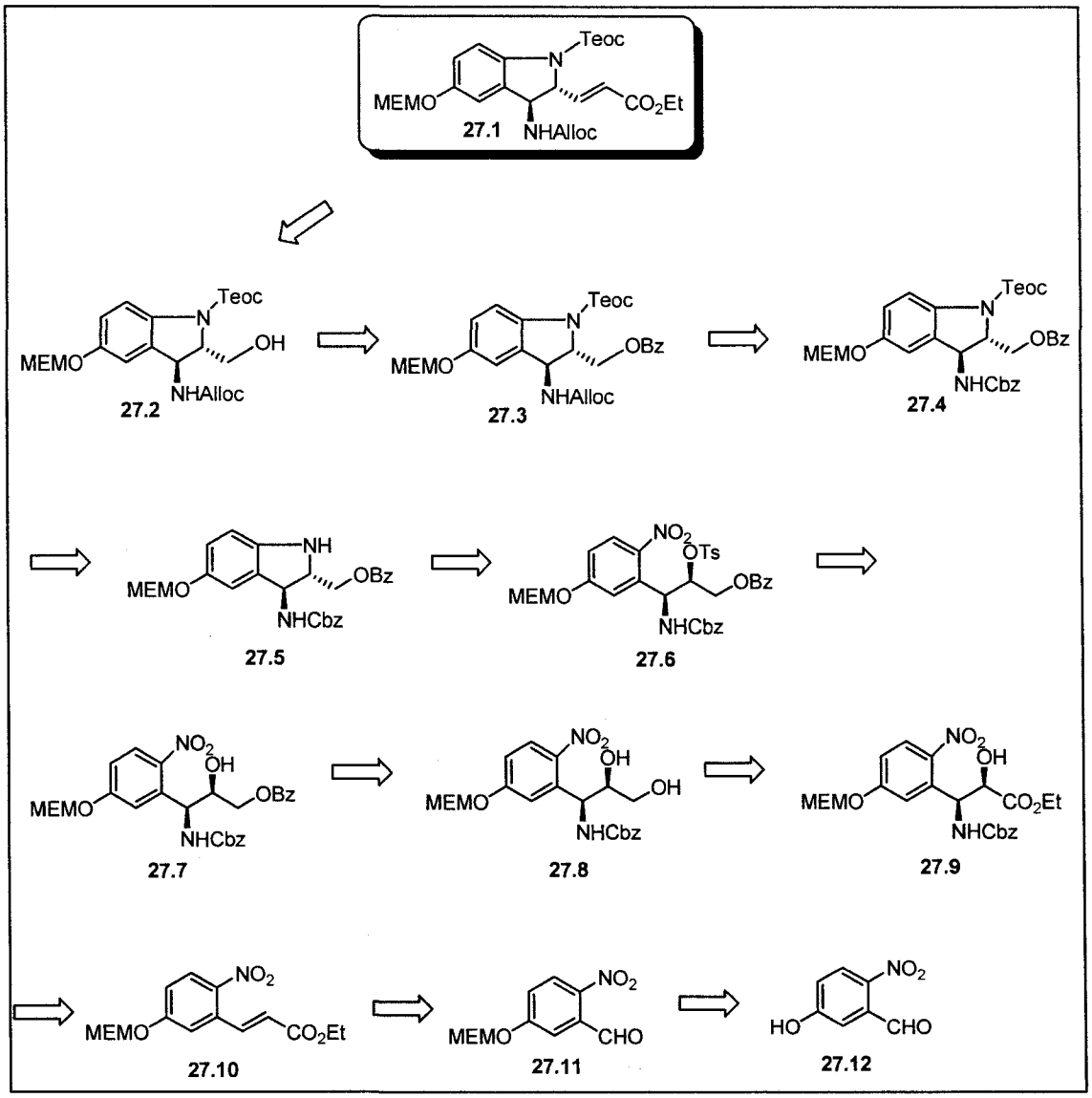


3.2 *Retro-Synthetic Analysis*

Our primary target is the enantio-enriched indoline core **27.1**. This indoline scaffold has a MEM-protected benzylic hydroxyl and a *N*-alloc-protected chiral primary amine. The indoline secondary amine is protected with a Teoc group and there is the needed chiral Michael acceptor with an ethyl ester functionality. The first disconnection, **Scheme 27**, is the Michael acceptor which

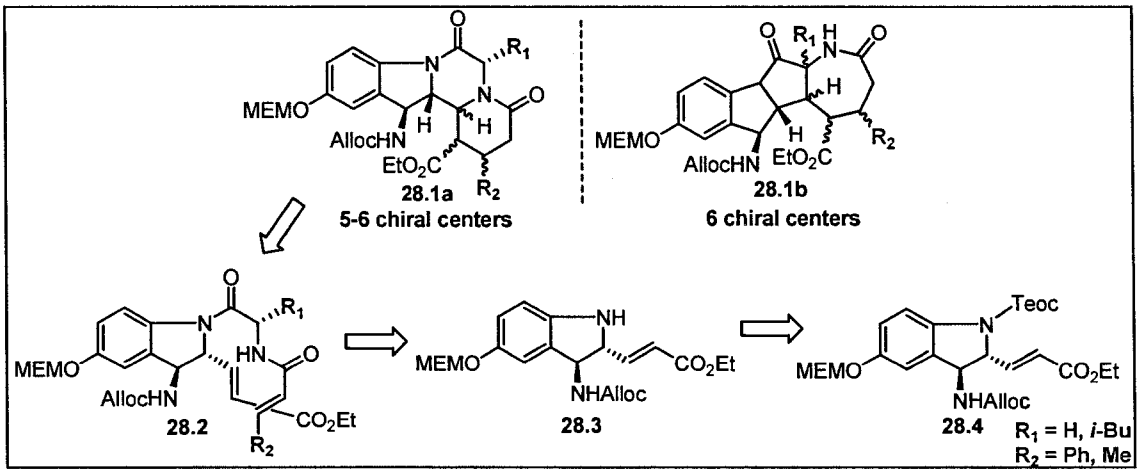
can be achieved through Witting conditions from the oxidized alcohol **27.2.** This alcohol was protected with a benzyl group, **27.3**. Changing the protecting group of the chiral primary amine was necessary since hydrogenation is needed to remove this benzyl-carbamate and further in the synthesis, there will be olefin functionalities which are sensitive to hydrogenation. The benzyl-carbamate on **27.4** needs to be removed through hydrogenation and the free amine was protected as *N*-Alloc protecting group. The indoline secondary amine **27.5** is protected with a Teoc group. The next disconnection was the formation of the indoline through cyclization of the tosylated alcohol **27.6 .** The reduction of the nitro group is needed prior to the cyclization. The diol **27.8** can be prepared by reducing ester **27.9**. This ester is the product of a Sharpless catalytic-asymmetric aminohydroxylation from olefin **27.10**. This step has been well used in the group and known to produce in about 80% yield (>92%ee)[21]. Olefin **27.10** can be prepared through Wittig conditions with aldehyde **27.11**. Our commercially available starting material is first to be protected with a MEM group for future solid phase loading.

Scheme 27: Retro-synthetic analysis of the Arya enantio-enriched indoline scaffold



Scheme 28 demonstrates the retro-synthetic analysis for the tandem Michael cyclization study. Upon Teoc removal of compound **28.4**, we couple the side-chain to the enantio-enriched indoline scaffold, **28.3**. The final step will be to apply the TBSOTf and TMSOTf-mediated double Michael conditions to scaffold **28.2**. Once completed, this methodology will be applied on solid support.

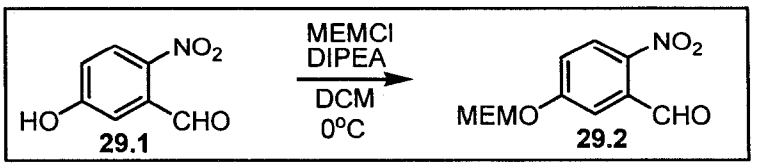
Scheme 28: Retro-Synthetic Analysis of the tetracyclic formation on the Arya enantio-enriched indoline scaffold



3.3 Synthesis of the indoline core

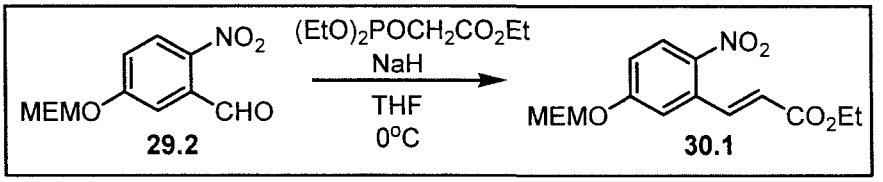
We began with 25g of the 5-hydroxy-2-nitrobenzaldehyde to yield a good amount of indoline core to complete this study. We also need enough compound to branch-off this scale-up synthesis to generate loading material for the solid-phase synthesis. The first step of the scale-up to prepare the advanced indoline starting material was the MEM-protection of phenolic hydroxyl. This reaction is completed in the presence of DIPEA at low temperature giving quantitative yields of **29.2**, **Scheme 29**. Upon purification, NMR experiments were conducted to confirm the product. NMR analysis highlighted the presence of the 4 large MEM peaks; 1 large methoxy singlet and 3 methylene peaks corresponding to the added methoxy-ethoxy-methoxy group.

Scheme 29: MEM protection on phenolic hydroxyl



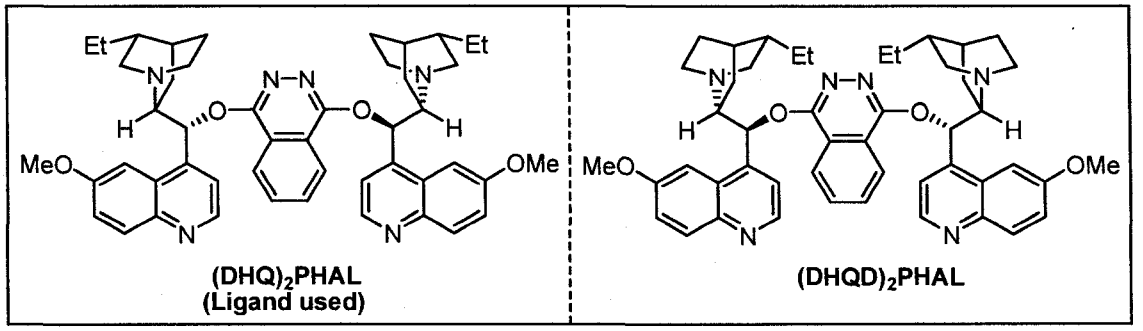
The second step was a carbon-chain extension to yield olefin **30.1**. This high yielding Horner-Wittig reaction was confirmed by NMR in which the addition of olefinic doublets and the ethyl ester's triplet and quartet.

Scheme 30: Carbon chain extension by Horner-Wittig reaction



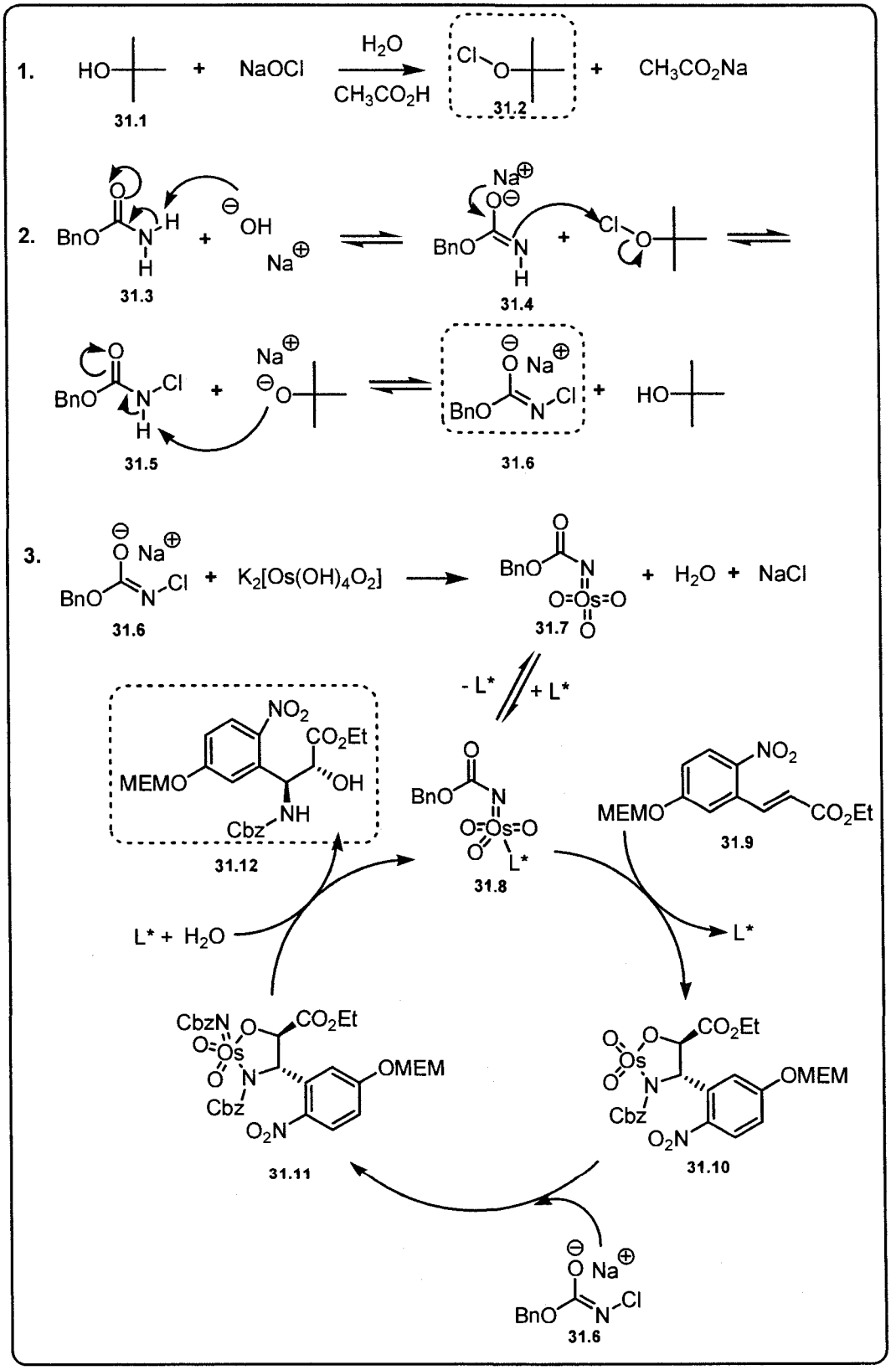
The following step was the Sharpless catalytic asymmetric amino-hydroxylation. This reaction proves to be an excellent way to introduce chirality from an olefin. This asymmetric amino-hydroxylation and similar asymmetric dihydroxylation reactions were developed by K. Barry Sharpless.[7,29] The selective chirality is driven by the use of modified cinchona alkaloids as chiral ligands; dihydroquinine $(DHQ)_2PHAL$ and dihydroquinidine $(DHQD)_2PHAL$ seen in **Figure 27** [7,29].

Figure 27: Chiral ligands used in Sharpless' amino-hydroxylation



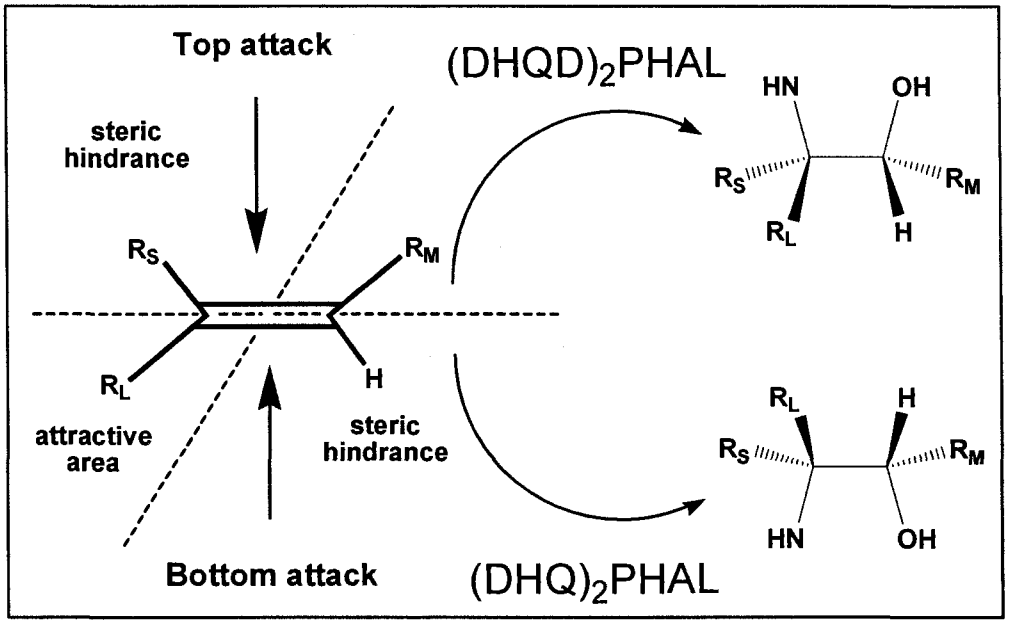
The reaction mechanism is summarized in **Scheme 31**. Prior to the reaction, the *tert*-butyl hypochlorite **31.2** is freshly prepared (1). The benzyl carbamate forms an aza-enolate in the basic media and further captures the chloride from the *tert*-butyl hypochlorite to form the chloro-benzamide **31.5**; this intermediate is again deprotonated to form the Sodium-chlorobenzimidate **31.6**, (2).

Scheme 31: Catalytic cycle to Sharpless' amino-hydroxylation



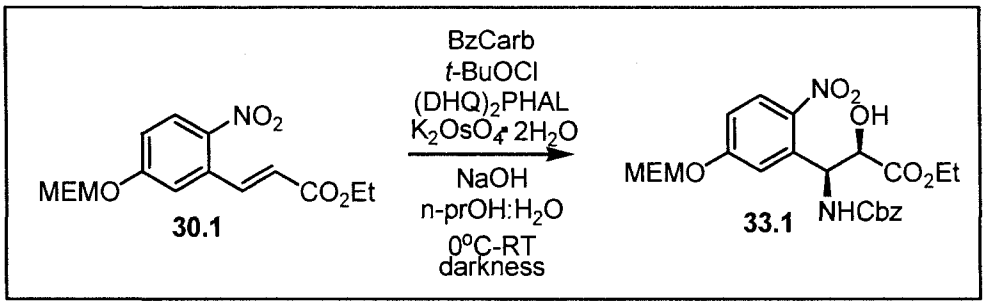
The activated benzylcarbamate will coordinate with the osmium catalyst in addition to the chiral ligand to form intermediate **31.8** as it enters the catalytic cycle, (3). The Olefin **31.9** coordinates with the complex through *syn* addition. **Scheme 32** illustrates the predictions of the enantiofacial selectivity of the reaction where Rs and H represent the smallest substituents, R_M for moderate substituents and R_L as the attractive area to lodge flat aromatic substituents. [7] Following these constraints, we can predict which face of the olefin will be attacked using the proper chiral ligand.

Scheme 32: Olefin face selectivity with choice of chiral ligand



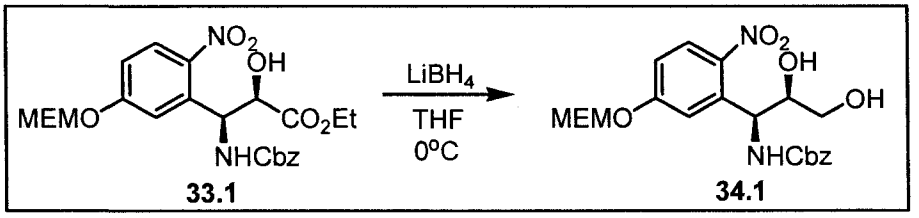
This reaction is often used in the lab and it was found to be optimal at a maximum of 10g of the olefin, **Scheme 33**. Reagents in these reaction conditions are very sensitive to light and needs to be conducted in complete obscurity. Upon product isolation through flash chromatography, NMR analysis indicated the disappearance of the olefinic protons and the emergence of benzylic protons representing the benzyl carbamate. The more polar (on TLC) less dominant isomer wasn't isolated.

Scheme 33: The Sharpless catalytic asymmetric amino-hydroxylation



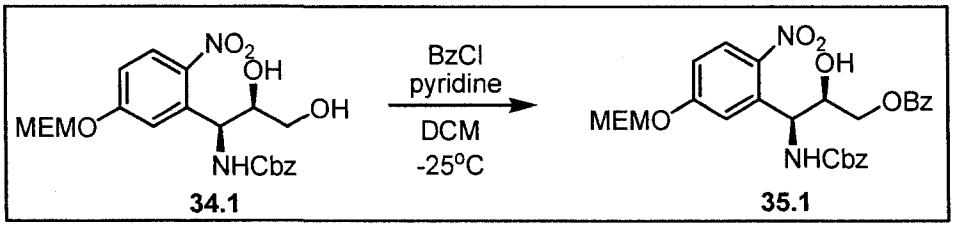
The following step exhibited the reduction of the ethyl ester with the goal of generating a diol, **Scheme 34**. Numerous ethyl acetate extracts during the work-up were necessary due to the polarity of the compound since the diol has a mild affinity to water. NMR experiments illustrated the supportive disappearance of the ethyl ester's triplet and quartet.

Scheme 34: Reduction of ester to construct diol



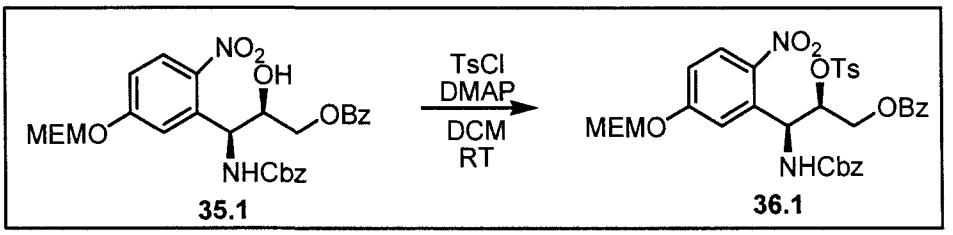
The next step was the monobenzoylation of the primary alcohol **34.1**. This reaction had to be done at low temperature following the slow addition of benzoyl chloride, via a syringe pump, to restrain to the benzoylation to the primary alcohol. Minor dibenzoylation product was observed by TLC and MS. Upon purification, NMR experiments confirmed the formation of compound **35.1**. Some NMR highlights demonstrated the appearance of new benzylic protons in the aromatic region of the ^{1}H spectrum as well as the appearance of a carbonyl carbon at 167.4ppm in the ^{13}C spectrum.

Scheme 35: Monobenzoylation of primary alcohol



The secondary hydroxyl group of **35.1** was subjected to tosylation giving the OTs derivative **36.1**. This tosyl group will act as a good leaving group upon the upcoming indoline cyclization, **Scheme 36**. This tosylation was carried out on a 34g scale and completed in 24 hours with a 95% yield. Upon purification, ^{1}H NMR experiments highlighted the tosyl's methyl as a large singlet at 2.30ppm integrating for 3 protons in addition to a pair of new aromatic signals.

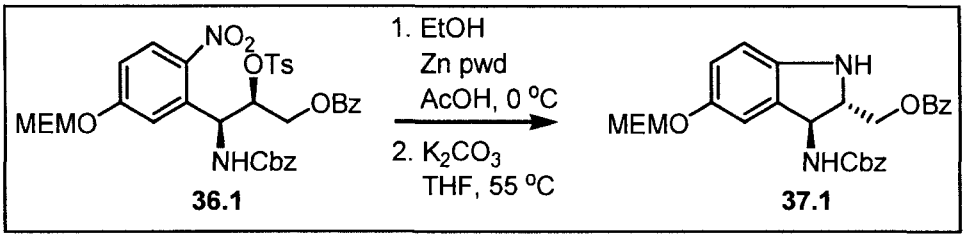
Scheme 36: Secondary alcohol tosylation



At this point in the scale-up, we have 41g of compound **36.1**. The next combined steps included the reduction of the nitro group by hydrogenation producing an amino group following the indoline ring closing in basic conditions. The common conditions previously used in the lab for the hydrogenation was the use of Lindlar's catalysts (5% Pd on $CaCO_3$) but the yield was found to significantly decrease if we exceeded 2g of starting material. It would take over 20 reactions since I had 41g of compound. Other group members had also investigated a large scale reduction of the nitro group by means of zinc powder and acetic acid in ethanol, **Scheme 37**. Using these conditions, I submitted two 20g reactions. The reactions were monitored by MS and TLC. Upon completion, the amino intermediate was immediately submitted to basic media to complete

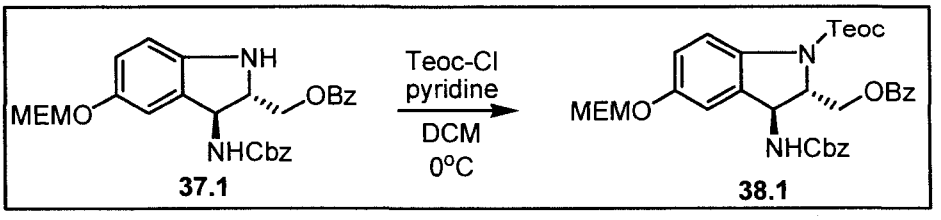
the cyclization. Upon isolation, the product was confirmed with the help of MS and ^{1}H NMR experiments, in which highlighted the disappearance of the tosyl peaks and the appearance of the NH broad singlet at 5.35ppm. This reaction had a combined yield of 78% for both reactions with a significant drop in molecular weight.

Scheme 37: Cyclization generating the indoline scaffold



The next step was to protect the newly formed secondary amine with a Teoc group. It is important when choosing the type of protecting groups that each adduct needs different conditions to be removed. This allows the chemist to have complete control of his molecule and be able to remove a single protecting group or undergo chemical reactions without affecting other groups on the scaffold. Most protection and deprotection reactions are usually straight forward and high yielding. Teoc-Cl is first made with 2-(trimethylsilyl)ethanol and triphosgene; the protection is done *in-situ*, **Scheme 38**. Much attention is needed in handling triphosgene as it is very toxic. Upon isolation, ^{1}H NMR highlighted the disappearance the NH broad singlet and the appearance of the very large singlet at 0.06ppm and its relative carbon peak at -1.1ppm in the ^{13}C NMR, representing the trimethylsilyl group.

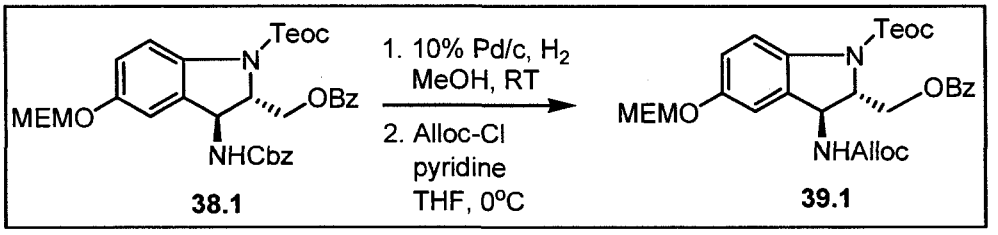
Scheme 38: Teoc protection on indoline core



The benzyl-carbamate on **38.1** is to be removed and replaced with the Alloc protecting group, **Scheme 39**. This change of protecting group is necessary since Pd/c-catalyzed hydrogenation is needed to remove this benzyl-carbamate and further in the synthesis, we will be inserting an olefin by Wittig methodology and this functionality will be vulnerable to hydrogenation. This said, the benzylcarbamate was removed with 10% Pd/c and upon full conversion confirmation by TLC, the amino-intermediate was submitted to immediate Alloc protection. Following purification, ^{1}H NMR analysis displayed the disappearance of the benzylcarbamate aromatic proton peaks and the rising of the Alloc allylic peaks.

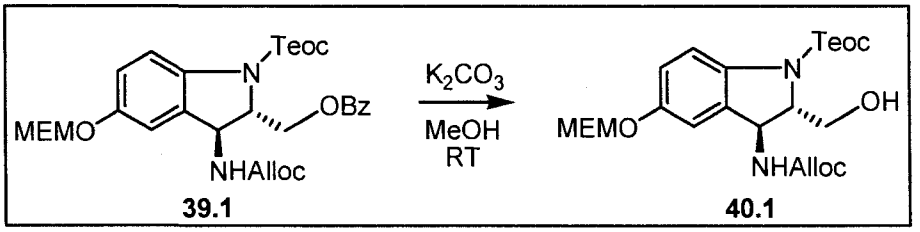
At this point of the synthesis of the scale-up, we can branch-off the sequence to convert this indoline scaffold into material capable to be loaded on solid support. This secondary sequence will be discussed in chapter 5. This said, a significant portion of compound **39.1** was kept aside for the solid phase project.

Scheme 39: Protecting group exchange



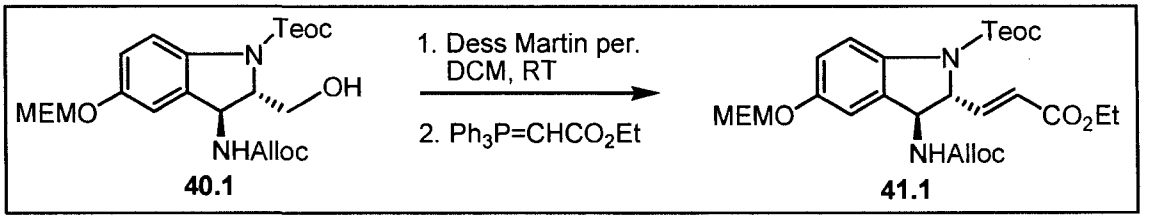
To complete the indole core, we need to insert the Michael acceptor on the eastern part of the scaffold. This said, the primary alcohol needs to be deprotected, **Scheme 40**. In presence of K_2CO_3 in methanol, alcohol **40.1** is formed within 2 hours, in quantitative yields. The disappearance of the benzyl's aromatic peaks in the ^{1}H NMR confirmed the conversion.

Scheme 40: Primary alcohol deprotection



The next step employed the use of the Dess Martin periodinane to oxidize the primary alcohol to the needed α-amino aldehyde. Full conversion was confirmed by TLC and HPLC/MS after 1 hour of stirring and then the Wittig triphospherane was added *in situ* to complete the formation of the Michael acceptor, **Scheme 41**. Upon isolation, NMR experiments were conducted; some highlights illustrated the appearance of the ethyl ester's quartet and triplet at 4.18 and 1.28ppm respectfully as well as the newly added olefinic peaks of the Michael acceptor.

Scheme 41: Dess Martin oxidation followed by a Wittig reaction



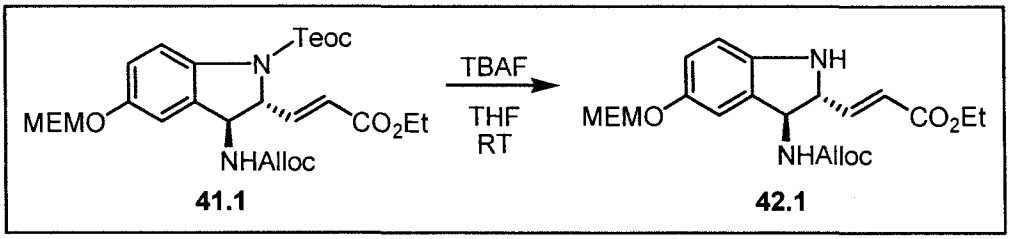
This concludes the synthesis of the starting material needed to conduct the tandem Michael cyclization study on the enantio-enriched indoline scaffold.

3.4 Synthesis of the tetracyclic ring system

Before coupling our peptide side-chains, we need to remove the Teoc protecting group which is easily done in the presence of a fluoride reagent, **Scheme 42**. Upon isolation, ^1H NMR highlighted the reappearance the NH broad singlet and the disappearance of the very large singlet at 0.06ppm and its

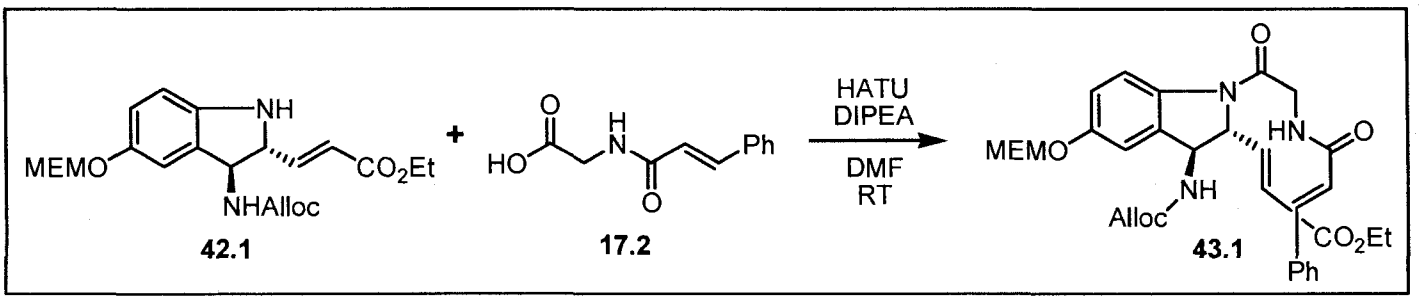
relative carbon peak at -1.1ppm in the ^{13}C NMR, representing the trimethylsilyl group.

Scheme 42: Teoc deproctection



Coupling of the glycine cinnamic acid was similar to the model study's results with a slight reduction in yield (60% instead of 80%) with an increase in reaction time (72 hours instead of 48 hours), **Scheme 43**. Previous NMR analysis completed with the model study was very useful in elucidating these new compounds with, regardless; a full 2D-NMR analysis was conducted. NMR highlights included the usual appearance of cinnamoyl olefins and aromatic protons. 2D NMR is again required in analyzing the data as the MEM and Alloc protecting group tremendously populate the spectrum.

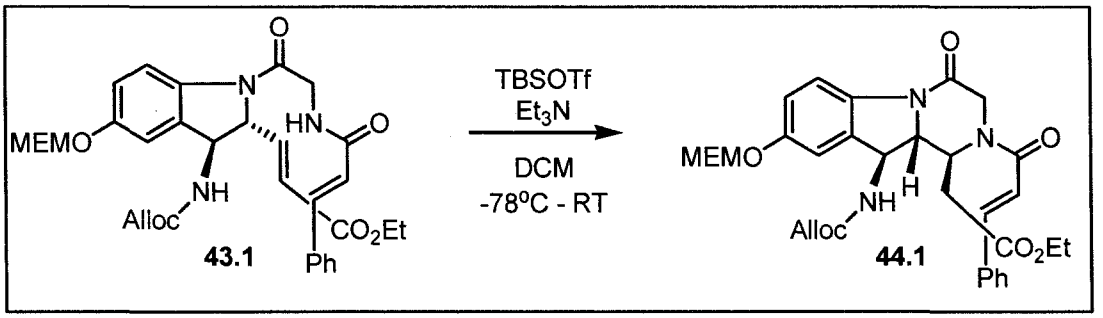
Scheme 43: Coupling of the glycine-cinnamoyl side-chain to the enantio-enriched indoline core



Upon isolation of the coupled glycine-cinnamoyl-indoline scaffold, the final step was to attempt cyclization. We wanted to take the same approach as in the model study without the use of only strong bases like LDA, seen in **Scheme 20**, as it only led to a mixture of tricyclic compounds. We were only interested in

using the Lewis-acid catalyzed conditions. This said, the first conditions applied were the use of TBSOTf as the Lewis acid (**Scheme 44**). To summarize, these conditions applied in the model study led to a mixture of tetracyclic and tricyclic compounds. This reaction led to the formation of tricyclic compound **44.1** as a single diastereoisomer in 76% yield. Further attempts to complete the final cyclization have failed. While we did not get the tetracyclic product, yielding one single product in good yield was promising.

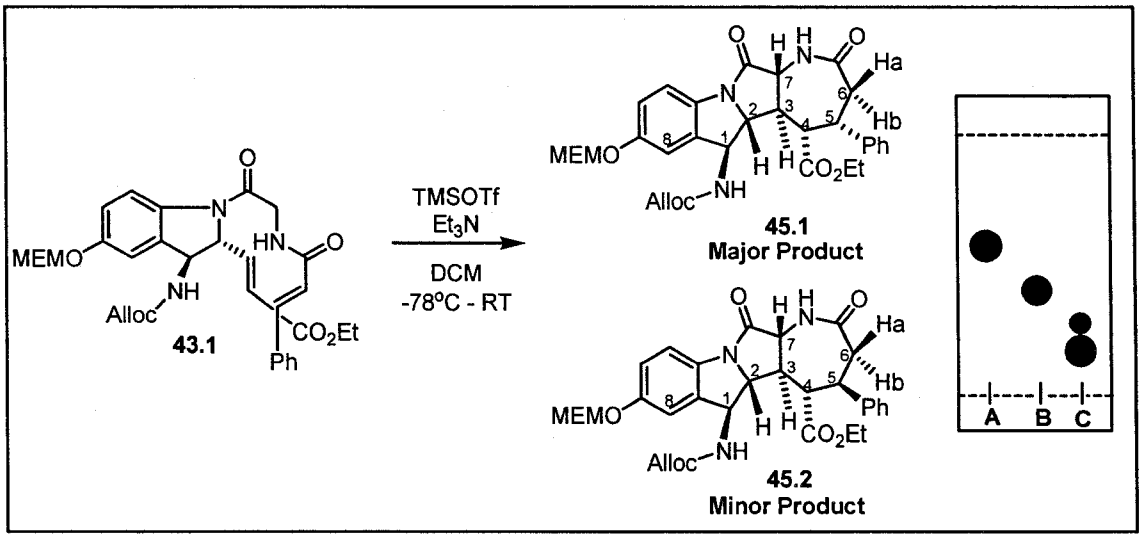
Scheme 44: Lewis acid mediated tandem Michael cyclization with the glycine-cinnamoyl-derived enantio-enriched indoline using TBSOTf



The next conditions to be attempted were the use of TMSOTf as the Lewis acid. Keep in mind that these conditions applied in the model study led to the formation of a tetracyclic compounds having a 6x5x5x7 ring system with high yield.

As seen in the TLC plate of **Scheme 45**, the starting material was fully consumed using these conditions and led to the formation of 2 new compounds with the same molecular weight and did not correspond with the tricyclic compound **44.1**. The 2 new compounds were isolated as a major and minor product in 55% and 12% yield.

Scheme 45: Lewis acid mediated tandem Michael cyclization with the glycine-cinnamoyl-derived enantio-enriched indoline using TMSOTf



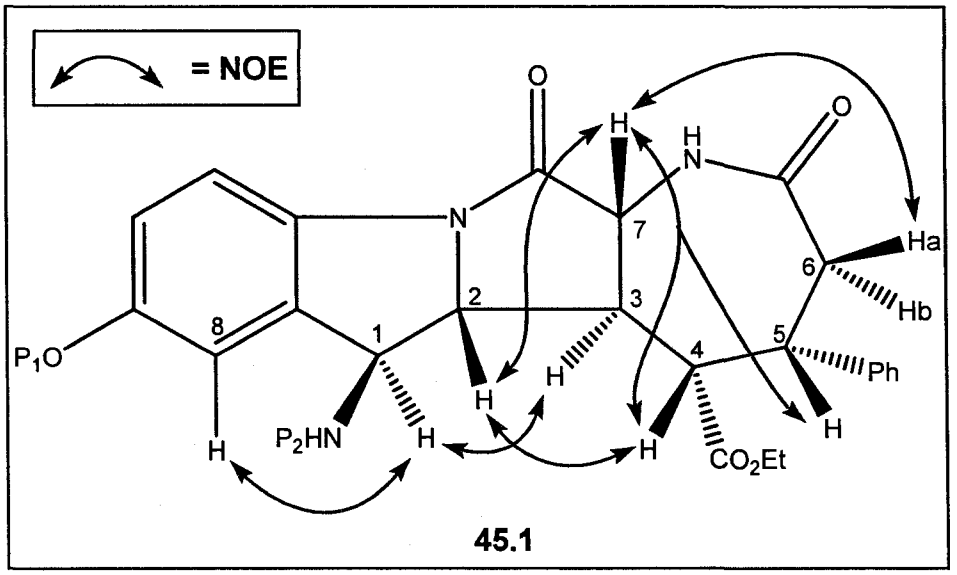
TLC Plate: (A) starting material, **44.1**; (B) Tricylic compound **44.2**; (C) Crude product; Solvent system (3:1, EtOAc:Hexane)

In the early stages of the NMR analysis, the spectra for these 2 new products were difficult to analyze; many signals were superimposed and were very broad. The Alloc's protons were essentially in the way and so I decided to remove and replace the protecting group with a benzyl group to facilitate characterization, **Scheme 46**. NMR experiments were repeated at a higher temperature, 58°C. This elevated temperature sharpened-up the spectra and having analyzed products **46.1** and **46.2** first, characterization was more feasible. 2D NMR was very essential for the elucidations of these compounds.

The NMR analysis of the product **45.1** started by observing the disappearance of the olefinic protons of **43.1**. The 5x7 ring system was first thought since there was the presence of only 1 aliphatic CH_2 signal on the HSQC (H6). H6a was confirmed as well by correlating with the adjacent carbonyl in the HMBC spectrum. The HSQC only illustrated one free NH signal; the other was hidden in a multiplet. The free NH signal (^1H), confirmed in the HSQC by having no correlation with any carbons, had a weak correlation in the COSY spectrum with a broad doublet integrating for 1 proton. This proton could only be H1 or H7. In previous NOESY analysis, H1 would always correlate with the aromatic singlet

H8 in which this wasn't the case with this signal. H8 correlated with a multiplet in which H1 was really hiding amongst other signals (OMEM and Alloc). Having H1, H6 and H7 characterized, the COSY NMR portrayed the rest of the correlating protons (H2, H3, H4 and H5). The relative stereochemistry was determined by 2-D NOESY analysis, **Figure 28**. The stereochemistry at H1 and H2 is predetermined and therefore are key references in determining the NOE correlation. There is an important 1-3 correlation between H1 and H3 which indicates there on the same face of the molecule. H2 has 1-3 correlation with H4 and H7 indicating their presence on the same face. Now determined to be on the top face of the molecule, H7 has a long distance 1-4 correlation with H6a and H5 as well as 1-3 correlation with H4. This NOE study suggests that both the ethyl ester and phenyl groups are oriented towards the bottom face of the molecule in a *cis* orientation.

Figure 28: NOE correlation of compound 45.1

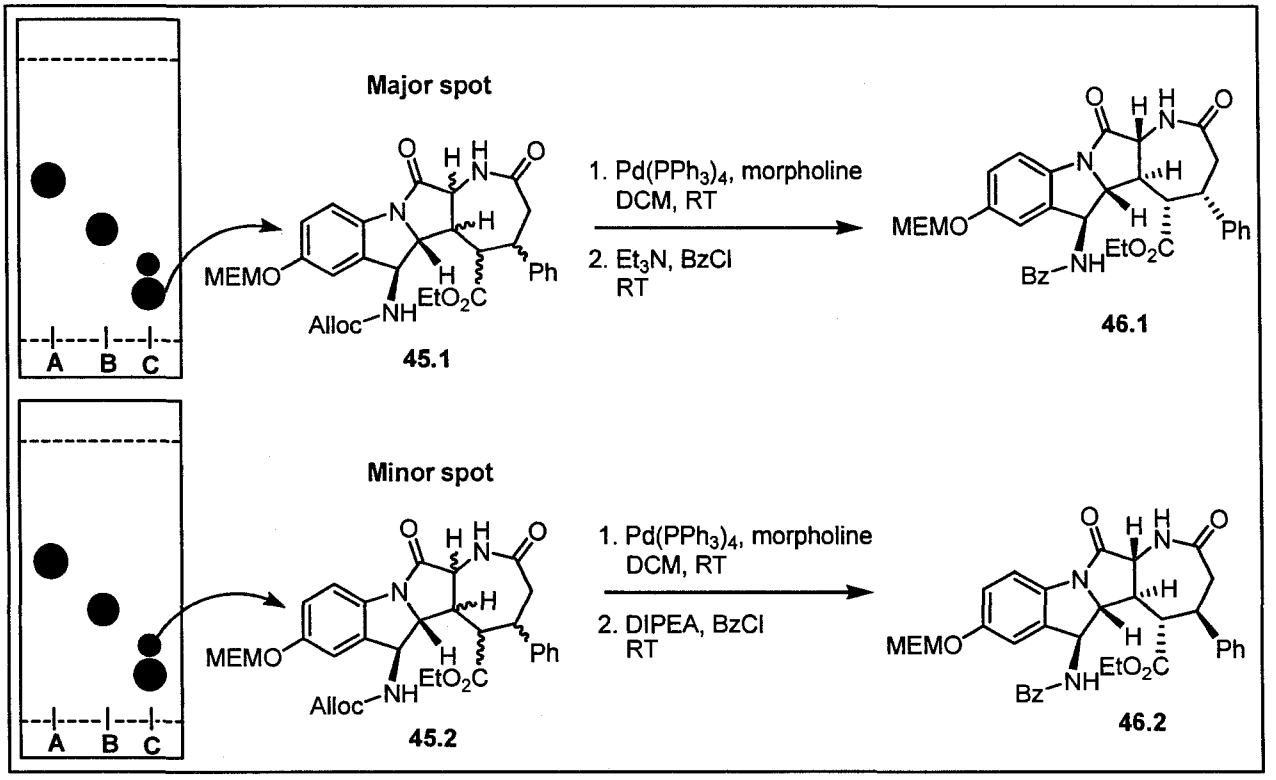


45.1

As previously mentioned, the *N*-alloc protecting group of compounds **45.1** and **45.2** was removed and immediately submitted to benzoylation with the goal of having a much cleaner NMR spectrum, especially in the 4 to 6ppm region, **Scheme 46**. At this time, the relative stereochemistry of these compounds was not assigned. Compound **45.1** was first converted to compound **46.1**. The alloc

deprotection was completed in 60 minutes and the benzoylation was carried out *in situ*.

Scheme 46: Alloc deprotection followed by benzoylation in goal of facilitating NMR analysis

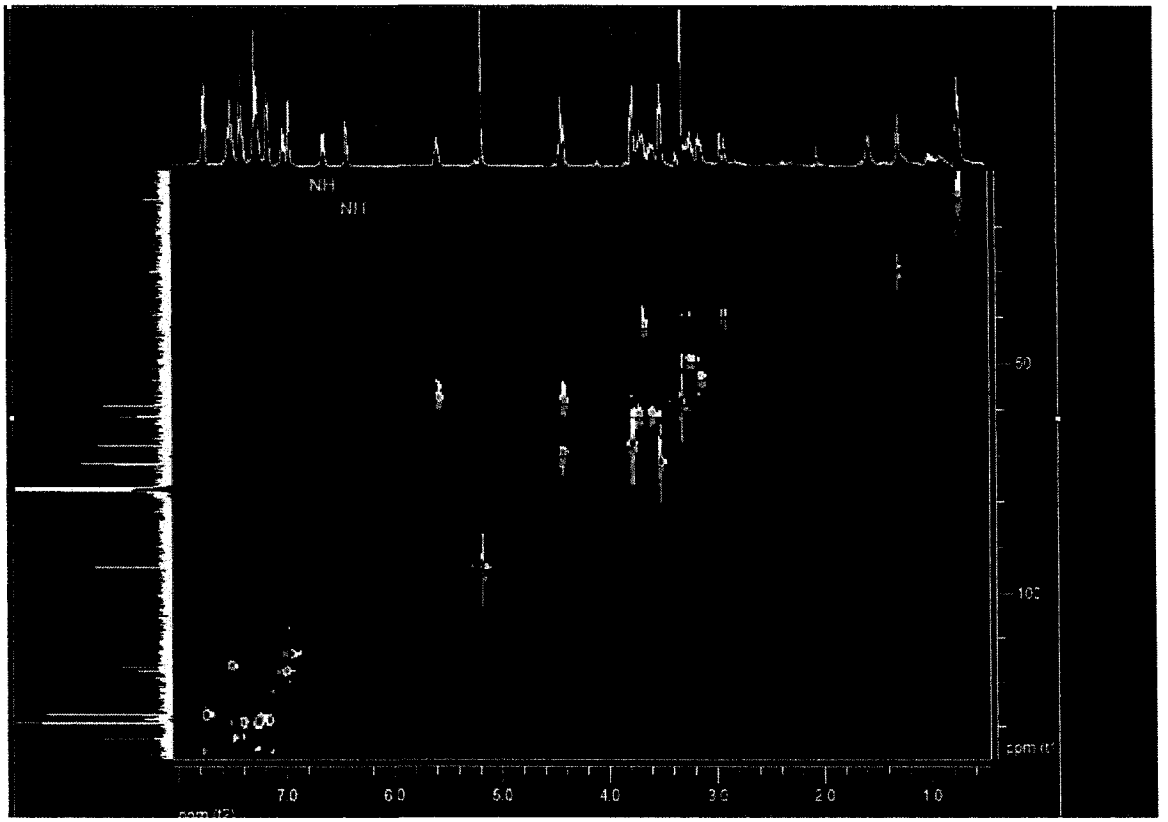


TLC Plate: (A) starting material, **44.1**; (B) Tricylic compound **44.2**; (C) Crude product; Solvent system (3:1, EtOAc:Hexane)

Upon isolation of product **46.1**, NMR experiments were conducted. The olefinic protons disappeared as new aromatic signals surfaced. The ^{13}C DEPT135 spectrum identified 5 CH_2 signals; 3 CH_2 in the MEM group, one CH_2 for the ethyl ester and one more in the 7-membered ring. This analysis tells us that we have a 5 and 7-membered ring system. If the product molecule had a 6x6 ring system, we would see a sixth CH_2 signal, one in both 6-membered rings. As a second clue in identifying the 5x7 ring system, the HSQC spectrum was very clear in identifying the presence of the 2 NH signals; broad proton signal not correlating with a carbon signal, **Figure 29**. From those 2 signals, the proton-proton correlation in the COSY spectrum was clear in identifying the all the

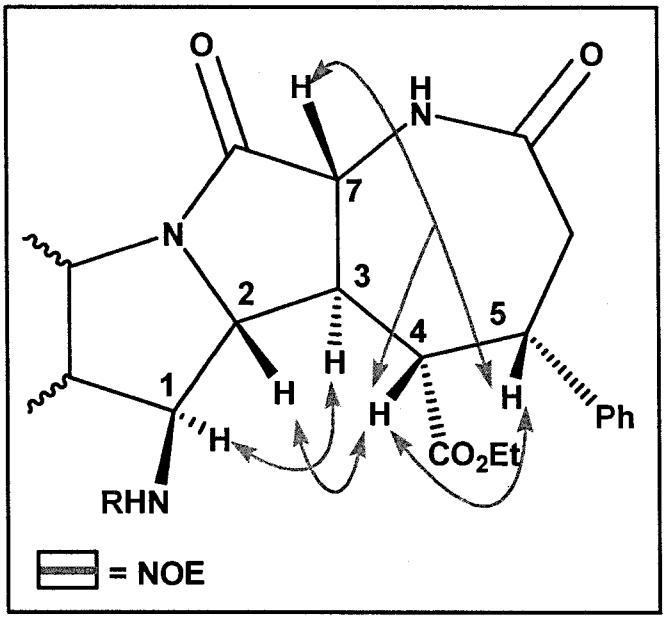
proton of the molecule. In general, the NMR spectra were much cleaner and easier to analyze than compound **45.1**.

Figure 29: HSQC spectrum identifying the presence of 2 NH proton signals

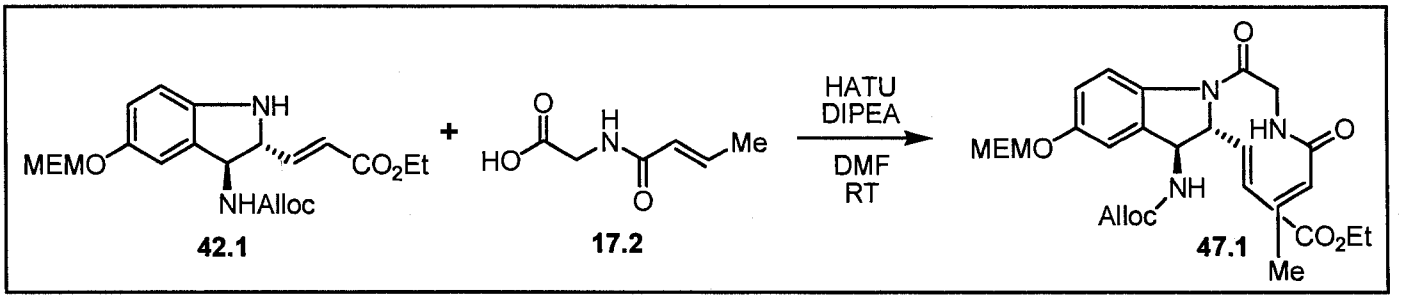


The relative stereochemistry was assigned with the help of the 2D NOESY spectrum which is summarized in **Figure 30**. H1 and H3 have a positive NOE correlation indicating their orientation towards the bottom face of the molecule. H2 and H7 are superimposed on the proton spectrum but they still can be told apart as they correlate with all other top-face protons of the molecule, H4 and H5.

Figure 30: NOE correlation of compound 46.1

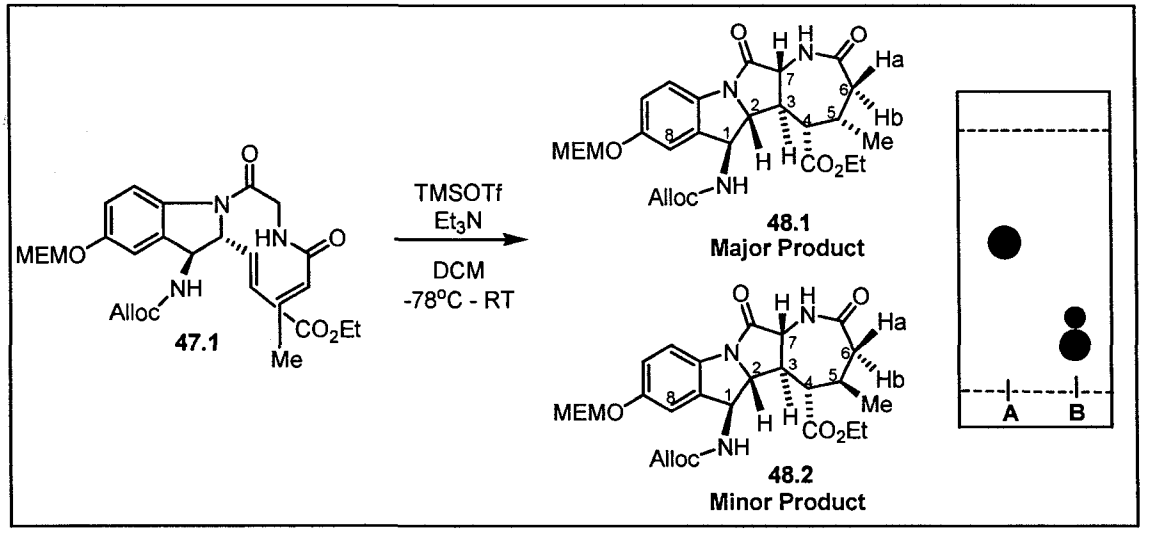


Scheme 47: Coupling of the glycine-crotyl side-chain to the enantio-enriched indoline core



The next case study investigated the reproducibility of these results with the glycine-crotyl side-chain. First, the side-chain **17.2** is to be coupled to the enantio-enriched indoline core **42.1**. This coupling was completed within 24 hours and in a high 92% yield. Upon isolation, the product was fully assigned by NMR experiments; new olefinic signals and a large doublet of doublets at 1.87ppm integrating for 3 protons are among the NMR highlights.

Scheme 48: Lewis acid mediated tandem Michael cyclization with the glycine-crotyl-derived enantio-enriched indoline using TMSOTf

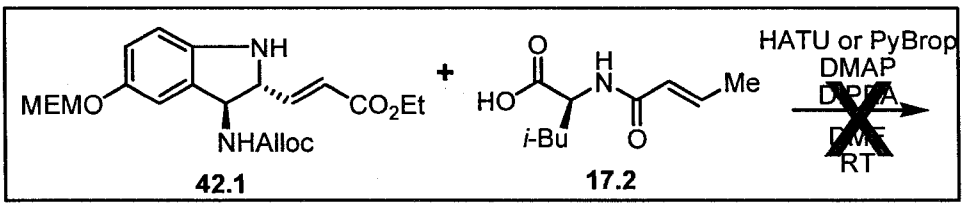


TLC Plate: (A) starting material, **47.1**; (B) Crude product; Solvent system (3:1, EtOAc:Hexane)

Submitting the coupled product **47.1** to our tandem Michael cyclization conditions gave very similar results compared to the phenyl derivative, **Scheme 48**. The TLC of the crude product illustrated full conversion into 2 new products. Upon isolation, the product yield was 50% and 24% for the major and minor products respectively. Both products also shared the same and predicted molecular weight, according to mass spectra. The NMR experiments were very difficult to assign as previously seen with the *N*-alloc group in the molecule. The alloc protons hide important proton signals and many signals were grouped into multiplets. These 2 compounds were purified using Preparative HPLC; however, very little compound was retrieved. NMR experiments were still possible, ^{1}H, COSY, NOESY, HSQC and a faint ^{13}C. We did not have enough product to undergo the *N*-Alloc removal and subsequent benzoylation. The COSY spectrum was difficult to extract information since the spectrum has many multiplets; multiplets correlating with other multiplets. NMR experiments were repeated in deuterated benzene instead of chloroform but the spectrum was worst. Some positive analysis illustrated a pair of NH signals in the HSQC of the major product; two broad signal not correlating with a carbon. This can lead us

to believe that the product has a 6x5x5x7 ring system. With the help other colleagues, the compounds were characterized as **48.1** and **48.2**.

Scheme 49: Coupling of the leucine-crotyl side-chain to the enantio-enriched indoline core



The final case study was to couple a leucine-crotyl side-chain to the enantio-enriched indoline core and attempt the cyclization. Unfortunately, this coupling was not achieved, **Scheme 49**. Two reactions using different coupling agents were tried, HATU and PyBrop. Only the starting material was recovered. These were the only condition attempted due to time constraints.

Figure 31: Proposed transition states of the tandem Michael cyclizations on the enantio-enriched indoline scaffold, generating a 6x5x5x7 ring system (TMSOTf)

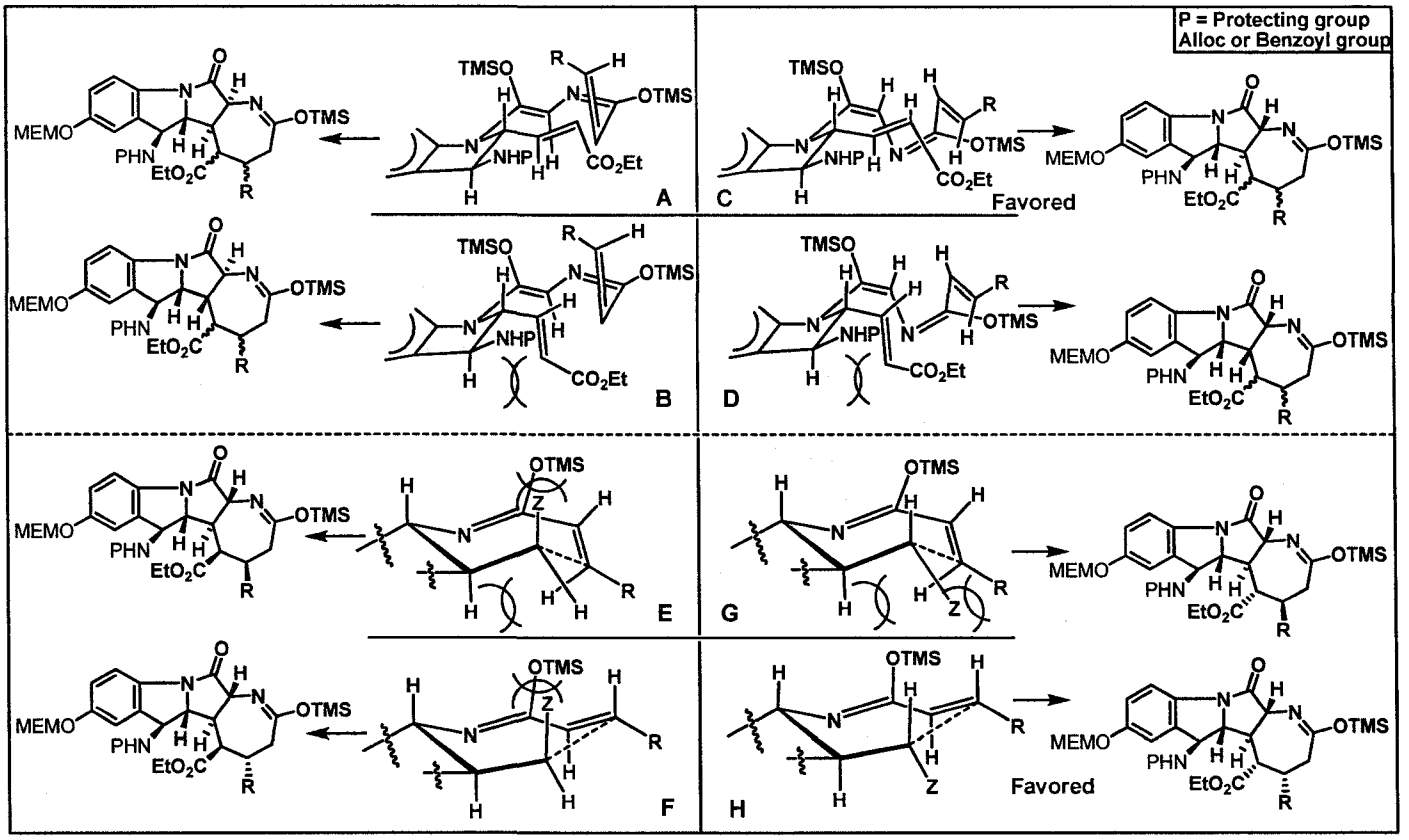


Figure 31 illustrates the proposed transition states for the Michael cyclization producing a 6x5x5x7 ring system on the enantio-enriched system. The reaction mechanism is believed to follow the same path and transition states as proposed in **Figure 25**. To once again explain the transfused system obtained in the first cyclization, four transition states could be drawn with the help of a molecular model; **A**, **B**, **C** and **D**. On structure **A** and **B**, the first enol adopts a *Z* configuration which is less stable than the *E* configuration shown in structures **C** and **D**. Moreover, structures **B** and **D** now have an even more disfavoring interaction between the electro-deficient alkene and the equatorial proton from the indoline core; allowing us to postulate the privileged structure **C** to lead to the right stereochemistry observed in the final compound.

As for the second cyclization, transitions states **E** and **F** showed a strong disfavored interaction from the ester group (Z) in a less stable axial position with the Lewis acid complex. Structure **E** and **G** also showed disfavored interaction between one axial proton and the proton from the Michael acceptor. Finally, a strong disfavoring interaction between the ester group (Z) and the R group in the transition state **G** helped to indicate that structure **H** was the most favorable transition stated as observed in the final compounds.

3.5 Conclusion

In this chapter, we have accomplished 2 case studies, in which we coupled 2 different side chains; both having a glycine amino acid and each with a methyl or a phenyl functionality. Upon the coupling of the glycine-cinnamoyl side-chain to the new indoline core, its application to the TBSOTf-mediated tandem Michael conditions led to the formation of a tricyclic product as one single diastereoisomer in 76% yield in a 6x5x6 ring system. When we changed the Lewis acid from TBSOTf to TMSOTf we observed the formation of a dominant and minor product having a 6x5x5x7 ring system as a tetracyclic molecule. The results were reflecting those found in the model study with the addition of a new minor product. Similar observations were found with both crotyl and cinnamoyl derivatives.

NMR analyses were much more difficult and unfortunately we were not able to crop any form of crystals good enough for X-ray analysis.

This enantio-enriched system study has shown moderate to great success towards the initial goal of being able to couple such side-chains to an indoline core and further undergo a double cyclization to form a tetracyclic molecule. At this point, we can now apply the knowledge of these 2 case studies onto solid support and see if the generation of library is feasible applying this methodology.

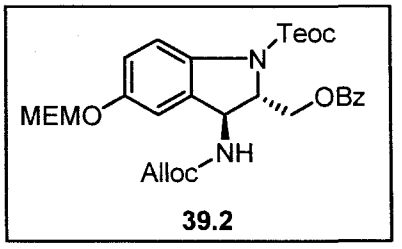
Unfortunately the case study with our functionalized amino acid (leucine) with the methyl/crotyl group was not completed. We were unable to couple this side-chain to the enantio-enriched indoline scaffold, more coupling conditions are to be attempted in the future of this project.

CHAPTER 4: Manual Solid-Phase Synthesis

4.1 Introduction

This project has now reached its last chapter. At this point, a model study of tandem Michael addition has been completed on 3 cases and 2 cases were applied towards the enantio-enriched indoline scaffold. Only the coupling of glycine-derived side-chains were completed on the enantio-enriched scaffold therefore we restricted the nature of the amino acid of the side-chains to remain of the glycine species for the time being. This methodology is now to be implemented onto the solid support and repeat the 2 case studies on the enantio-enriched indoline scaffold. Upon successful loading of the indoline core, the glycine-cinnamoyl and glycine-crotyl side-chains are to be coupled to the anchored compound. A 3-carbon spacer is to be inserted on the MEM-protected hydroxyl before loading the indoline core onto solid support. The synthetic route towards the loading process of the enantio-enhanced Indoline core has been previously completed and published in the Arya group[21]. As mentioned previously, compound **39.2** will serve as the starting material for the manual solid-phase synthesis, (see **Figure 32**).

Figure 32: Starting material for the manual solid-phase synthesis

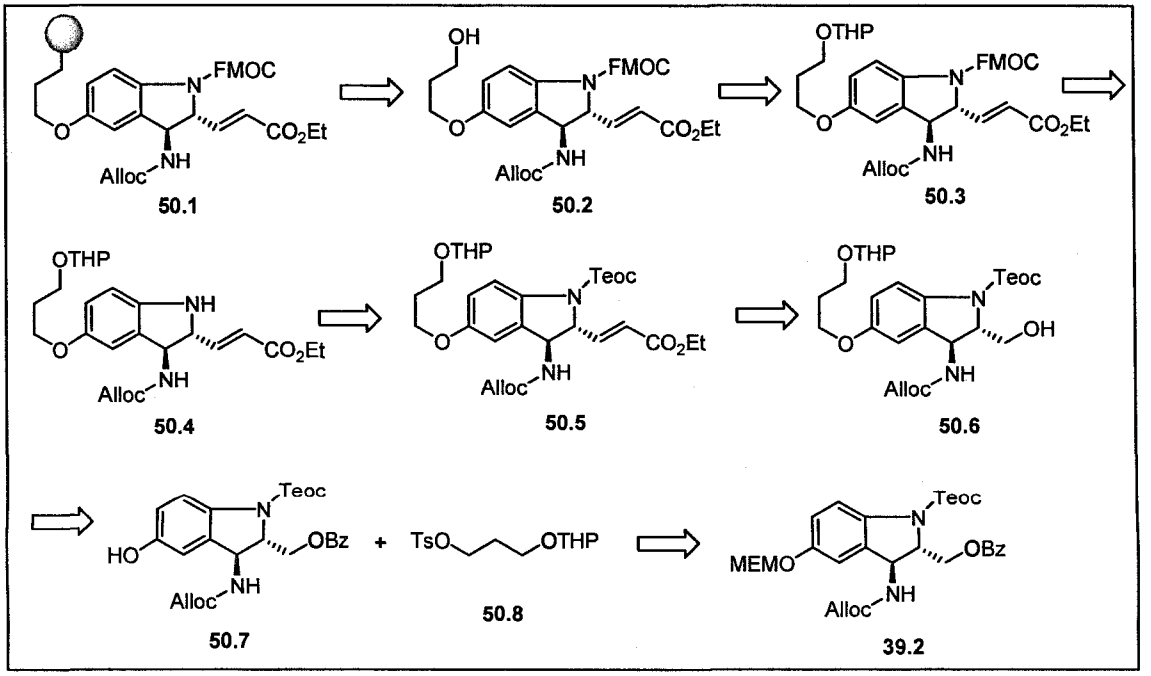


39.2

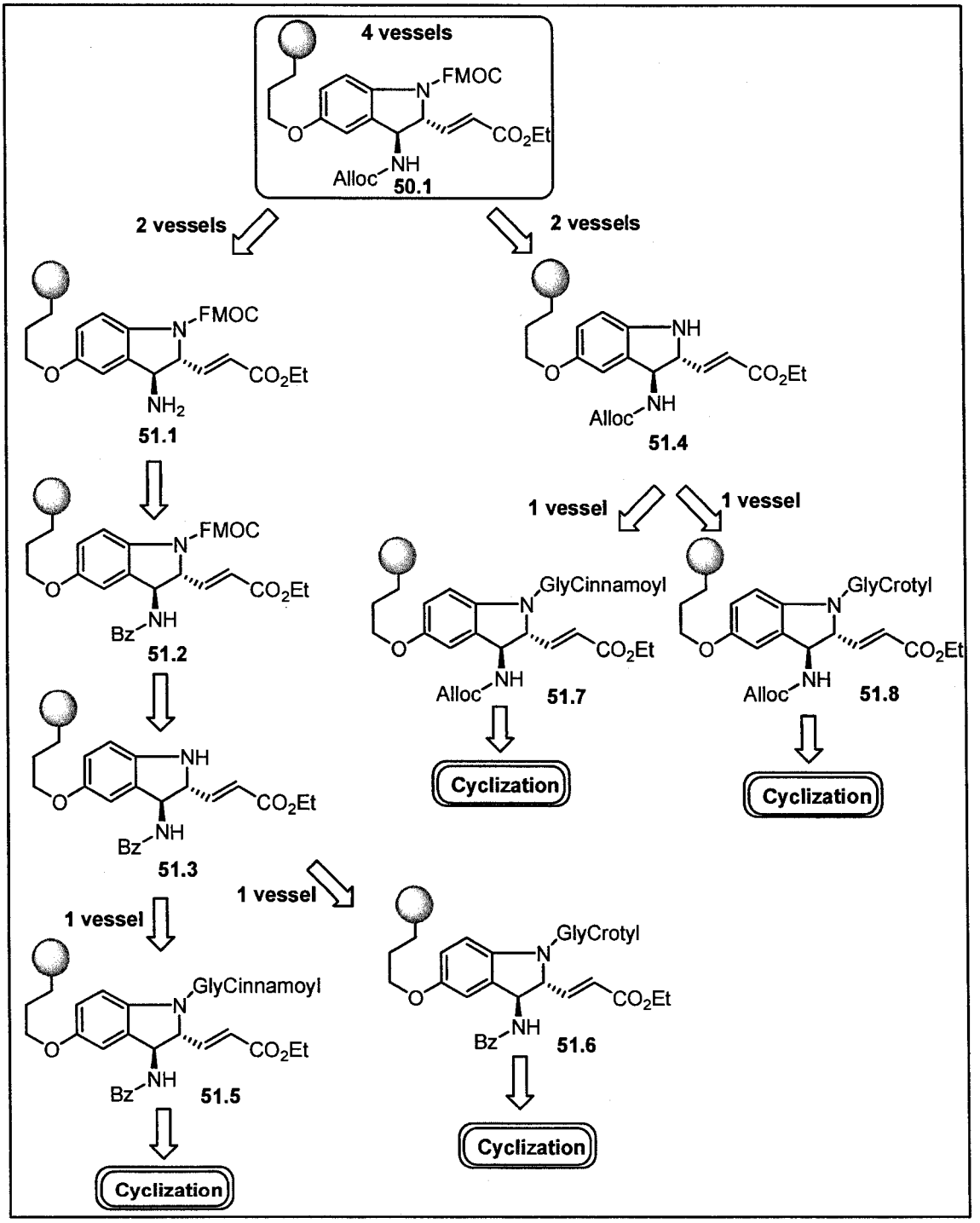
4.2 *Retro-synthetic Analysis*

Scheme 50 illustrates the retro-synthetic analysis of the indoline core prior to its loading onto solid support, **50.1**. The loading protocol and alkylsilyl-linker-based macrobeads are provided by the Broad Institute of Harvard and MIT[23]. The first disconnection off the resin gives alcohol **50.2**. This hydroxyl was deprotected from its THP counterpart **50.3** in converting the indoline scaffold into the loadable material is the removal of alcohol **50.2.** The loading percentage was found to be optimal when the indoline secondary amine is protected with Fmoc. A change of protection groups is necessary since the Teoc group is sensitive to cleaving reagents (HF), **50.3→50.5**. The next disconnection was the synthesis of the Michael acceptor **50.5** through Dess-Martin oxidation and following Wittig reaction from alcohol **50.6.** The next disconnection is the insertion of a THP-protected 3-carbon spacer **50.8** from hydroxyl **50.7**, deprotected from its MEM precursor **39.2**.

Scheme 50: Retro-Synthetic analysis of the indoline core prior to loading on solid support



Scheme 51: Synthetic analysis of the Manual Solid-Phase Synthesis



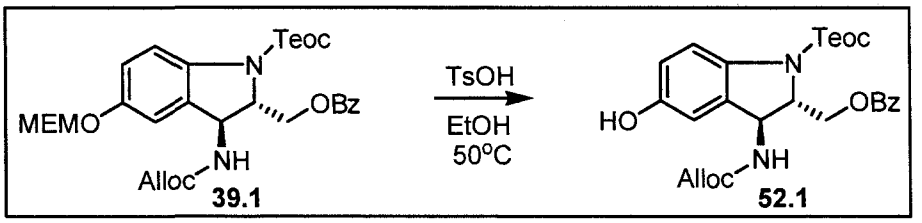
In the above **Scheme 51**, is the overall manual solid-phase synthesis plan. Resin **50.9** is to be split in 4 BIORAD vessels with the goal of achieving 4 different structures. As previously attempted in the previous chapter and for the

sake of applying the DOS chemistry to the synthesis, 2 vessels are to be submitted to Alloc removal **51.1** and subsequent benzoylation **51.2**. These 2 vessels are submitted to Fmoc-removal, **51.3**, and then each vessel will be coupled a different side-chain; glycine cinnamic acid **51.5** and the glycine crotyl acid **51.6**. The 2 original vessels are to be immediately submitted to Fmoc removal, keeping the Alloc group; and as well, each vessel will be coupled a different side-chain; glycine cinnamic acid **51.7** and the glycine crotyl acid **51.8**. Each of the vessels will then be submitted to the tandem cyclization conditions using TMSOTf as the Lewis acid.

4.3 Solid-Phase Synthesis of the indoline core

As mentioned above, the first step is the deprotection of the MEM group. Like most hydroxyl protecting groups, the presence of an acid in a protic solvent will free the alcohol, **Scheme 52**. This reaction is completed in quantitative yields and NMR highlights consists of the noticeable disappearance of the large methoxy singlet and 3 methylene peaks.

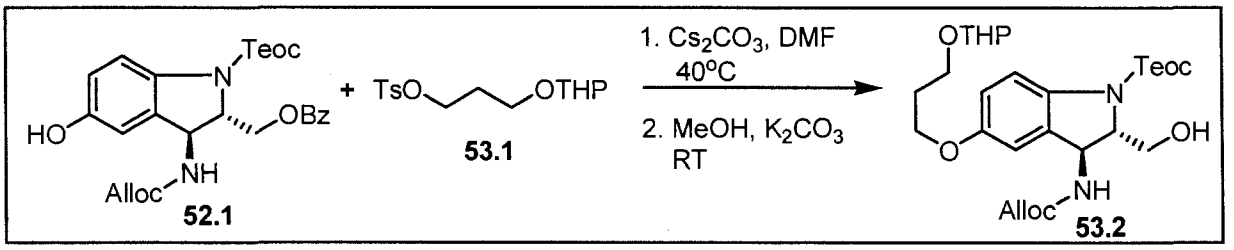
Scheme 52: MEM deprotection on the anchoring site



The following step was the insertion of the 3-carbon spacer, **Scheme 53**. This spacer is a propane-1,3-diol in which one hydroxyl is THP-protected and the other is tosylated, therefore cesium carbonate is adequate for this addition [30]. The intermediate was directly used in the next step consisting of the primary alcohol deprotection, **53.2**. Upon isolation, NMR experiments were conducted; some highlights illustrated the disappearance in aromatic signals and the

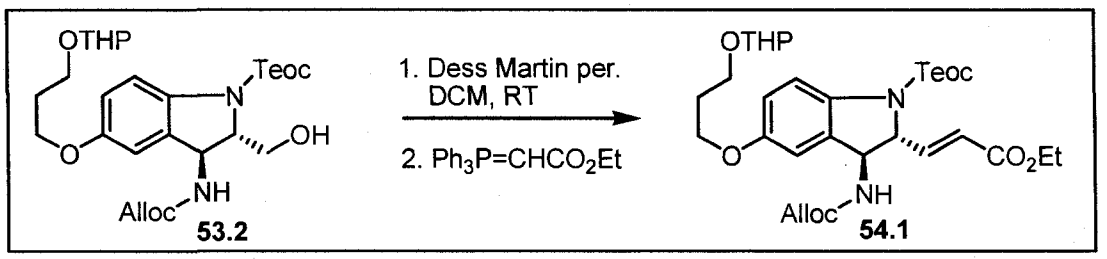
appearance of the aliphatic protons corresponding to the 3 methylenes in the spacer as well as for the THP group.

Scheme 53: 3-carbon spacer insertion followed with the primary alcohol deprotection



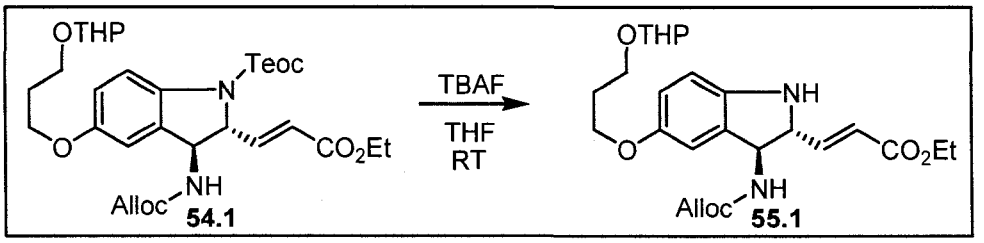
The next step employed the use of the Dess Martin periodinane to oxidize the primary alcohol to the needed α-amino aldehyde. After 1 hour of stirring and full conversion confirmed by TLC, the Wittig triphospherane is added *in situ* to complete the formation of the Michael acceptor, **Scheme 54**. Upon isolation, NMR experiments were conducted; some highlights illustrated the appearance of the ethyl ester's quartet and triplet at 4.17 and 1.27ppm respectfully as well as the newly added olefinic peaks of the Michael acceptor.

Scheme 54: Dess Martin oxidation followed by Wittig reaction



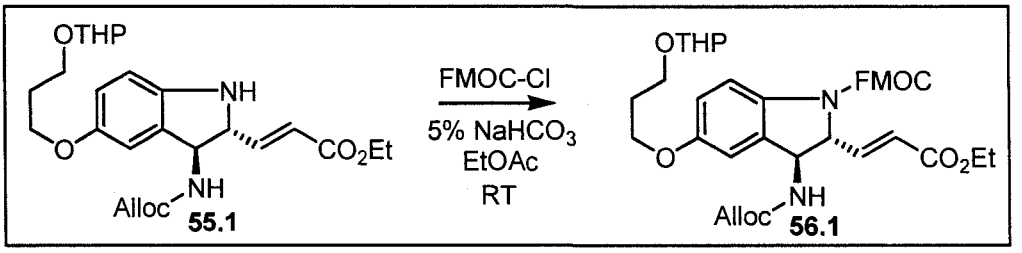
The use of the Fmoc instead of the Teoc group as the protecting group was found to give a better loading percentage; therefore the Teoc group was replaced with the Fmoc group. The Teoc protecting group is removed in presence of fluoride, **Scheme 55**. This reaction has been previously carried-out in the earlier chapter, **Scheme 42**.

Scheme 55: Change of protecting group; Teoc deprotection



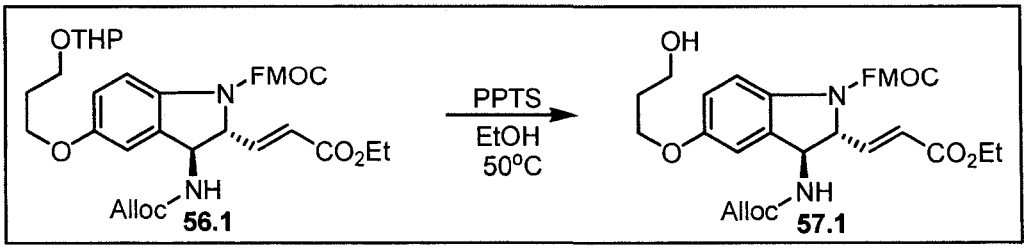
The following step was the Fmoc protection, summarized in **Scheme 56**. This reaction was quantitative and the product was easily purified. NMR analysis illustrated the disappearance of the large singlet near the 0 ppm to the Teoc group and the relative appearance of the aromatic peaks of the newly added Fmoc group.

Scheme 56: Change of protecting group; Fmoc protection



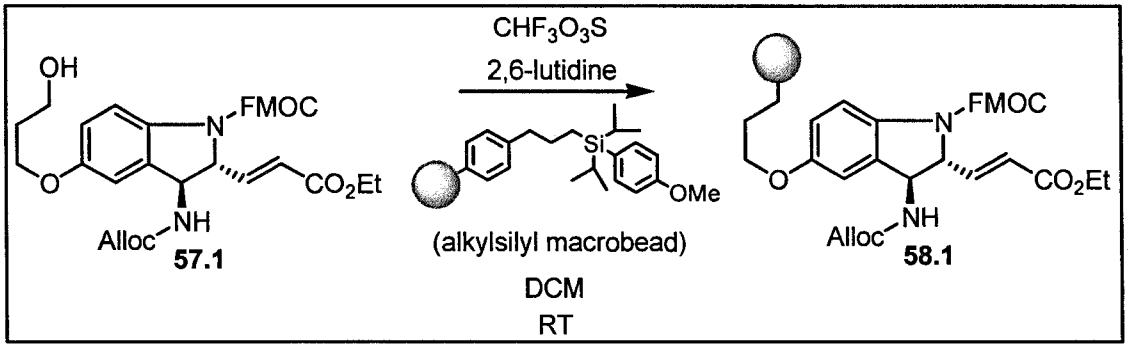
There is one last deprotection step before loading of the indoline core onto the solid support. THP deprotection, summarized in **Scheme 57**, is easily done in the presence of a mild acid in protic solvent. NMR analysis clearly illustrated the disappearance of the tetrahydropyran protons. Upon isolation of the compound, the scaffold is ready to be loaded onto the resin.

Scheme 57: THP removal



To end with the solution phase of this project, we move on to the loading step, **Scheme 58**. There is a short loading summary back in **Figure 9**, in the introduction. The loading protocol is very sensitive to moisture; we often have to delay loading if the lab atmosphere is too humid. The trifluoromethanesulfonate is always used fresh and diluted to a 0.45M concentration in DCM. The loading protocol indicates that 2 equivalents of the loading compound are necessary. We can still recover the starting material after the loading. Consecutive loadings are always necessary, particularly for library generation.

Scheme 58: Loading of the Arya enantio-enriched indoline core onto solid support

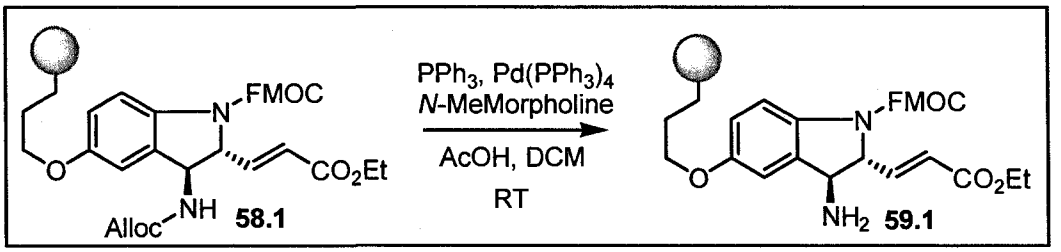


The loading procedure is a lengthy process. The resin is difficult to weight since the beads are sensitive to static. The use of a static gun and not wearing latex gloves can ease the weighing process. Next, the resin needs to be swelled in DCM for 30 minutes before it can be activated by the trifluoromethanesulfonate (6 equivalences). Upon activation of the resin, the beads become orange; an excess of lutidine (8.0 equivalents) is added followed by the starting material. The macrobeads used have a 0.99 mmol/g loading capacity; this will help us calculate the loading percentage, essential for yield calculation upon future solid-phase reactions. After lengthy and numerous washings, the beads are dried under high vacuum. We utilized 514mg of starting material to load for completing this manual solid-phase project. The rest of the starting compound would be used for the future synthesis of a small compound library.

The loading percentage was calculated (644mg of resin, 95% loading) and the purity was verified (96%) via the cleavage of 3 beads and the use of an HPLC/MS. The loaded resin is usually stored in a dessicator or under vacuum.

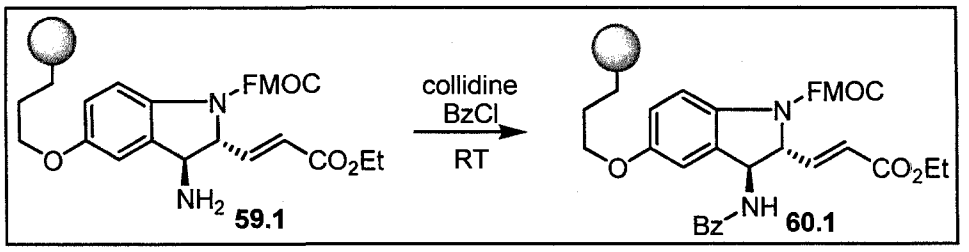
As previously mentioned in the solid-phase retro-synthetic analysis, **Scheme 51**, four reaction vessels of 100mg of resin each are to be done in parallel. The first step was to remove the *N*-alloc group on the south end of the scaffold, **Scheme 59**. As pointed out earlier, the reagents used in solid-phase reactions are often used in excess as well as reaction time with the goal of maximizing yield to complete conversion. This said, an excess of palladium catalyst is used along with an excess ligand. The reaction was stopped after 18 hours; 3 beads were cleaved and purity and full conversion was determined via the HPLC/MS analysis.

Scheme 59: Alloc deprotection on solid support



The benzoylation on the primary amine was conducted, **Scheme 60**. This reaction takes only 8 hours for full conversion. Upon washing and the cleavage of 3 beads, purity and full conversion was determined via HPLC/MS analysis.

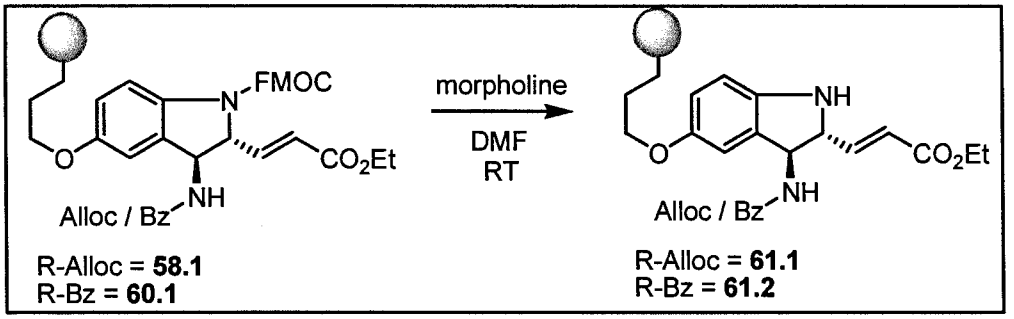
Scheme 60: Benzoylation on solid support



4.4 Solid-Phase Synthesis of the tetracyclic ring system

At this point, we are moving forward with 4 parallel reactions with 2 vessels with resin **58.1** and 2 vessels with resin **60.1**. In this next step, we are removing the Fmoc protecting group to free the secondary amine, **Scheme 61**. This deprotection step is easily accomplished in 30 minutes with the presence of excess morpholine. Upon washing and the cleavage of 3 beads, purity (>95%) and full conversion was determined via HPLC/MS analysis.

Scheme 61: Fmoc removal on solid support

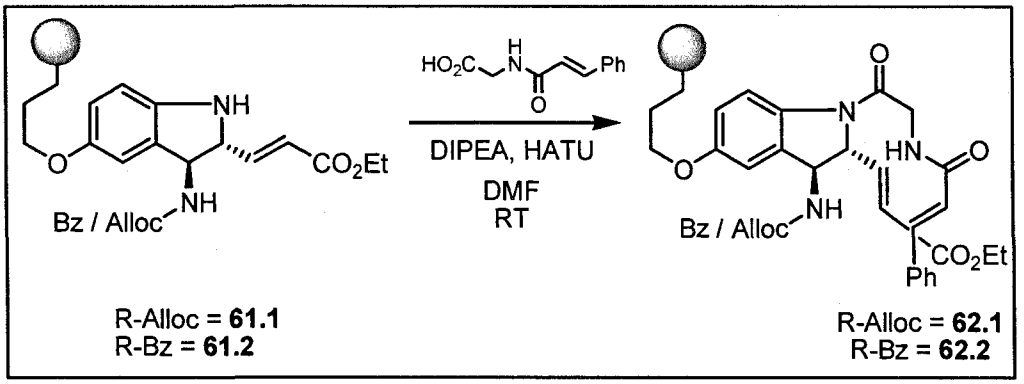


R-Alloc = **58.1**
R-Bz = **60.1**

R-Alloc = **61.1**
R-Bz = **61.2**

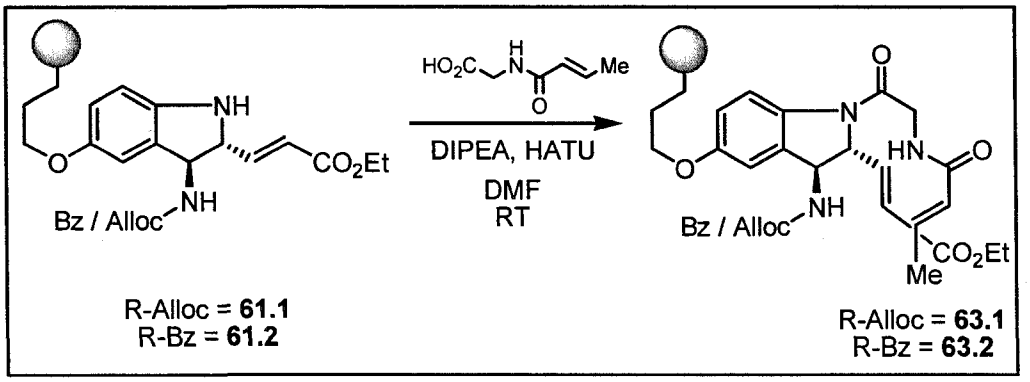
At this point, 2 vessels will be put aside as the 2 remaining vessels, resin **61.1** and **61.2**, will be submitted to our glycine-cinnamoyl side-chain coupling, illustrated in **Scheme 62**. Upon washing and the cleavage of 3 beads, purity and full conversion was determined via HPLC/MS analysis. Occasionally, re-submissions to the coupling conditions were required to maximize conversion as the starting material was still present.

Scheme 62: Coupling of the glycine-cinnamoyl side-chain to the enantio-enriched indoline core onto the solid support



With the other 2 vessels of resin **61.1** and **61.2**, we submitted these to our glycine-crotyl side-chain coupling, illustrated in **Scheme 63**. Upon washing and the cleavage of 3 beads, purity and full conversion was determined via HPLC/MS analysis. At some occasions, the starting material was still present on the resin; therefore was again re-submitted to the coupling conditions to maximize conversion.

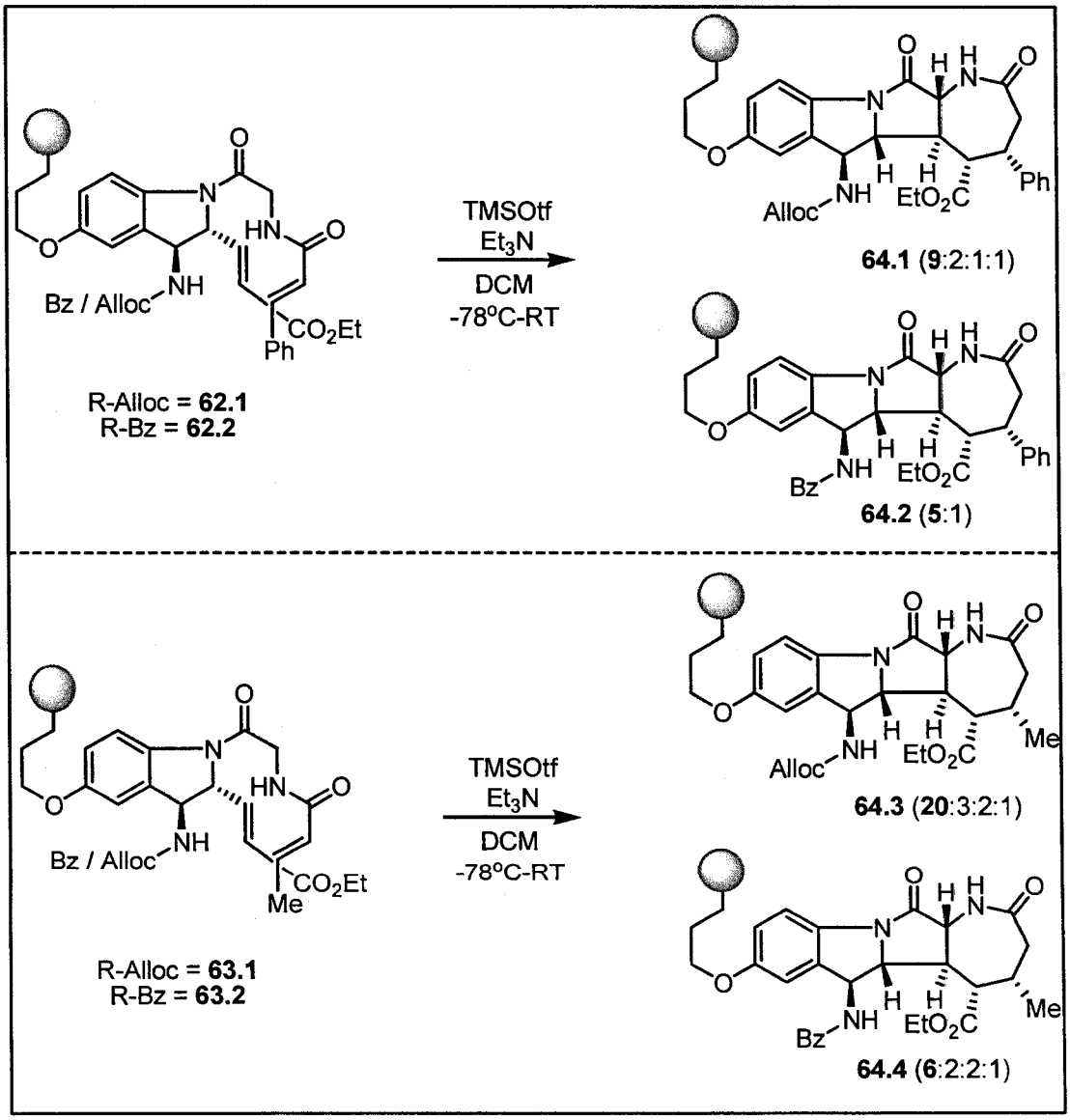
Scheme 63: Coupling of the glycine-crotyl side-chain to the enantio-enriched indoline core onto the solid support



We now have 4 different indoline cores which are coupled with an amino peptidic side-chain. The last and final step for these scaffolds is the Lewis acid catalyzed tandem Michael cyclization. These 4 reactions are illustrated in **Scheme 64**. After completing the resin washings and the compound cleavage,

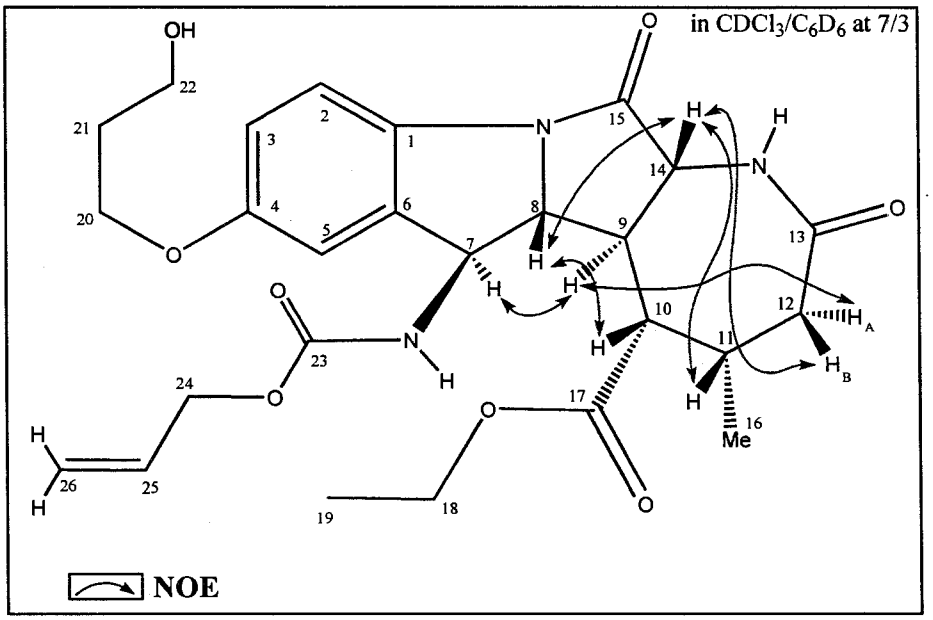
all products were analyzed via HPLC/MS. The biggest issue in using HPLC/MS for analysis is that all molecular weights (M+1) are identical. The molecule ion can represent the starting material, the tricyclic products or the tetracyclic products. Retention times are important to take note. For all four reactions, the starting material was fully consumed. In **Scheme 64**, the product ratio represents all compounds with the product ions mass. In each case, a major product is found.

Scheme 64: Lewis acid mediated tandem Michael cyclization with the enantio-enriched indoline onto the solid support



Clean NMR experiments were difficult to accomplish due to the scale of the reaction. Compound **64.3** was the only product fully assigned by NMR therefore all stereochemistry associated to compounds **64.1**, **64.2** and **64.4** are predicted by the results in the formation of **64.3**. The NMR elucidation of compound **64.3** was difficult as most signals were broad and coupling constances hidden. 2D NMR was again essential to assign this product. Many ^1H NMR spectra were performed in a multitude of different combinations of deuterated chloroform and benzene in goal of getting the best splitting for a high quality NOESY spectrum. **Figure 33** illustrates the NOE correlation of product **64.3**, performed in a 7:3 ratio of deuterated chloroform and benzene respectfully. H_7 and H_8 were perfect references for the complete assignment of **64.3**.

Figure 33: NOE Correlation of tetracyclic compound 64.3

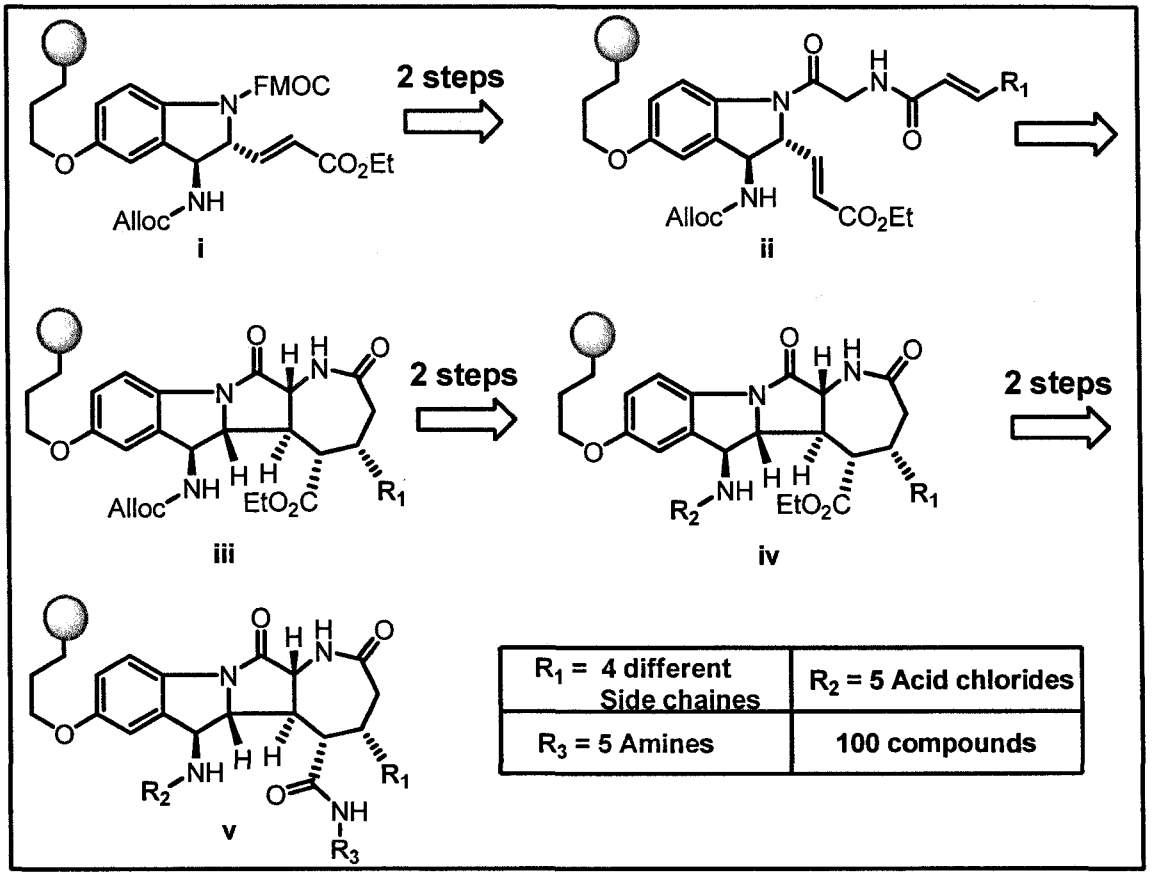


4.5 Library Generation Plan

The goal of this project was to develop a methodology in generating indoline polycyclic derivatives in a diversity oriented synthesis. This methodology is to be applied for the generation of a library. The library generation will be attempted in the near future. **Scheme 65** illustrates an envisioned library generation plan. As for the first diversity, following an Fmoc removal, the resin is

split in 4 reactions using 4 glycine-based side-chains which are to be coupled to the indoline core (**i**) and undergo the tandem Michael cyclization (**iii**). As for the second diversity, all the resin is pooled together for removing the *N*-alloc protecting group to be again split into 5 reactions using 5 acid chlorides to be coupled to the indoline core (iii).

Scheme 65: Envisioned Library Generation Plan



As for the third diversity, all the resin is again pooled together for the hydrolysis of the ethyl ester and split again to couple 5 different amines with the newly formed acid. Upon cleavage off the resin, this sums up to a 100 compound library, or 120 if you prepared extra resin to have the acid functionality after the second diversity. The purity of each compound can be verified by HPLC/MS. If this methodology proves to be successful, many different libraries can be generated in the future. Cristal structures (X-ray) of the target protein can

prove to be very useful in designing a library and choosing appropriate acid chlorides, amines, aldehydes or acids.

4.6 Conclusions and Future work

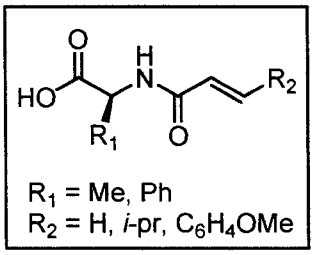
In conclusion, many objectives have been met and goals achieved. In the beginning of this project, a model study on a simplified indoline scaffold has been completed in which a synthetic peptide side-chain having an olefin functionality was successfully coupled to a simple indoline core. Upon the successful coupling of 3 different side-chains, a reaction condition has been developed in order to undergo a Lewis-acid catalyzed tandem aza-Micheal or tandem Michael cyclization producing tetracyclic compounds in moderate to very good yields. We were able to synthesize these tetracyclic compounds having 6x5x6x6 or 6x5x5x7 ring systems depending on the selection of the Lewis acid used. Moreover, explicit 1D and 2D NMR experiments and X-ray analysis helped us in the characterization of these compounds. Applying this methodology to the enantio-enriched indoline scaffold was also successful. Unfortunately, we were not able to successfully coupled the Leucine-crotyl side chain to the bulkier indoline core although both glycine-cinnamoyl and glycine crotyl side-chains were accomplished. Upon submission to the tandem Michael cyclization conditions, our target tetracyclic products have been achieved. NMR studies were much more challenging however characterization was completed. Upon successful loading of the enantio-enriched indoline onto solid support, these experiments have been repeated on the resin with similar yields.

At this stage, the next is to ahead with a library generation however this library should be kept small; not bigger than seen in **Scheme 65**. Alas we have only scratched the surface in fully understanding the full potential of this methodology. Our original objective was to develop a second generation methodology to synthesize polycyclic indoline derivatives to further be applied in the generation of a library of compounds via solid phase synthesis. At this point,

we have developed a new methodology nevertheless many areas have yet to be entirely explored and understood. Some future work has to be done in order to invest in the application of this methodology in the generation of larger libraries of >100 to 1000 members.

To start with the model study, more different side-chains need to be attempted applying different amino acids and different terminal functionalities. **Figure 34** illustrated potential artificial peptides to be used as side-chains to couple onto the indoline scaffold. Alanine (Me) and phenylalanine (Ph) can prove to be very useful in discovering the bulkiness limits upon the formation of the 6x5x6x6 or 6x5x5x7 ring system of the tetracyclic product during the tandem cyclization. The size of the terminal functionality has yet to show disruption of the formation of the product however having different sizes and functionalities will expand our knowledge of the reaction mechanistic.

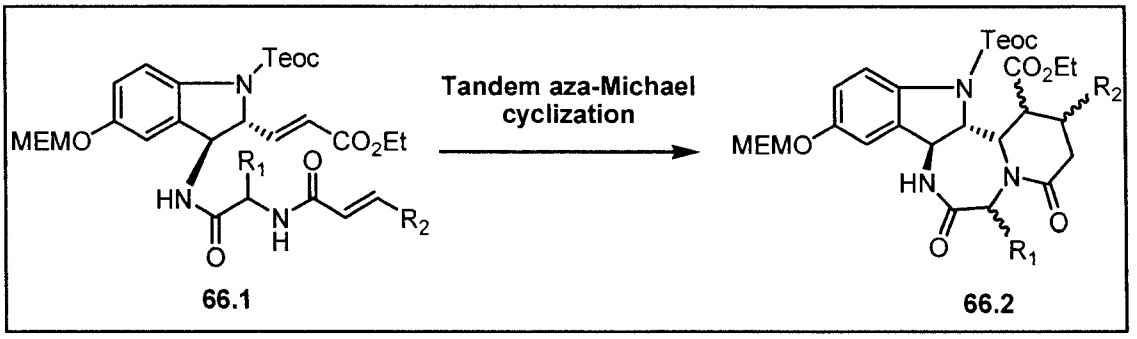
Figure 34: Potential artificial peptides to be used as side-chains to couple onto the indoline scaffold



The Lewis acid study conducted was not fully sufficient. We only explored the bulkiness of triflate Lewis acids. A broader study should be done with different types of Lewis acids like ZnCl, $AlCl_3$, BF_3 and many others. This study can potentially discover a better control over the formation of the tetracyclic ring systems. These essays should be then transferred to the enantio-enriched indoline scaffold and further onto solid support.

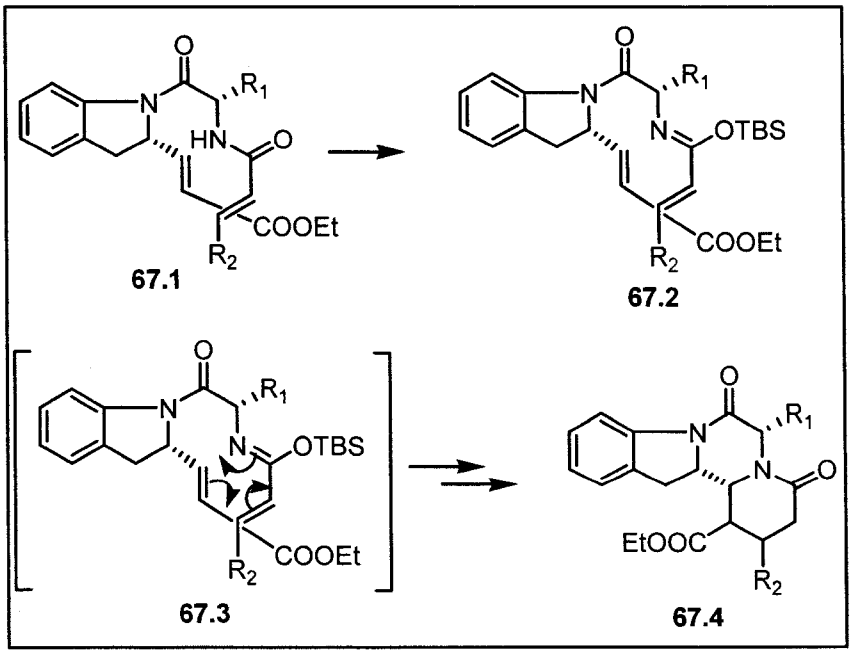
To further explore this methodology, we can employ the use of β-amino acids in the side chains and a well as coupling the side chains on the bottom of the indoline scaffold and attempt the tandem Michael cyclization conditions.

Scheme 66: Potential exploration of the Tandem aza-Michael cyclization



A growing interest has come up in attempting to trap the enol of the couples indoline scaffold and attempt Diels-Alder conditions to form our tetracyclic targets, **Scheme 67**.

Scheme 67: Potential exploration under Diels-Alder conditions



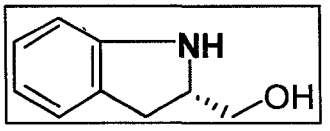
CHAPTER 5: Experimental Procedure

5.1 General methods

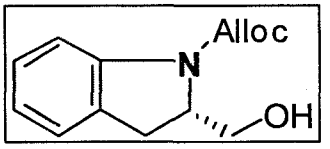
The Starting materials, reagents and chemicals were obtained from commercial suppliers and used without purification, mostly through Sigma-Aldrich. THF, CH_2Cl_2, and DMF were passed through activated alumina columns to remove impurities prior to use. Unless otherwise stated, all non-aqueous reactions were performed under an atmosphere of dry nitrogen or argon in oven-dried glassware. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents and products. Reactions were monitored by thin layer chromatography (TLC) using Merck 60 F254 0.25 mm silica gel plates. The TLC spots were viewed under ultraviolet light and by heating the TLC plate after treatment with a solution of ammonium molybdate in 10% aqueous H_2SO_4. Conventional flash column chromatography, using Silicycle Ultra Pure Silica Gel (230-400 mesh), was performed to purify all compounds. Automated flash chromatography was performed of a Biotage system equipped with a Flash collector and Horizon detector and recorder. Removal of organic solvents was performed by roto-evaporation on a Büchi R-114 Rotovapor using a Buchi B-178 vacuum system. Trace solvents were removed on a high vacuum pump. Vacuum removal of solvents for the linker cleavage reactions (solid phase) was accomplished using Genevac HT-4 Atlas Evaporator. All NMR experiments were recorded on an AC-Bruker instrument (400 MHz). Unless otherwise noted, proton and carbon chemical shifts are reported in parts per million using deuterated $CDCl_3$, as an internal standard at 7.26 and 77.0 ppm, respectively. Other solvents like deuterated benzene (C_6D_6), deuterated dimethylsulfoxide (C_2D_6OS) or deuterated acetone [$(CD_3)_2CO$], were used. The multiplicity, coupling constants (*J* in Hz), and number of protons were indicated in parentheses after each chemical shift. Analysis by mass spectrometry was performed on a VG Quattro I (Micromass) mass spectrometer equipped with a pneumatically assisted electrospray ionization source operating in positive mode. The enantiomeric excess (ee%) was determined by chiral HPLC using a Hewlett-

Packard (Agilent) 1090 LC equipped with a diode array detector and Chiracel-OD column. The HPLC spectra were recorded on an Agilent 1100 Series HPLC system. The HPLC/MS spectra were recorded on a Waters 2795 separation module (LC), equipped with a ZQ 2000 ES+ MS and uv absorption is standardized at 254nm. The PREP HPLC purifications were performed and recorded on a Gilson apparatus equipped with automatic injection and fraction collection.

5.2 Syntheses for Model Study System

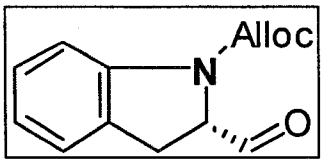


Compound 12.2: To a solution of (S)–indoline-2-carboxylic acid (2.00 g, 12.19 mmol) in THF (50 mL) at -78°C, was added $LiAlH_4$ (1M solution in hexane, 24.38 mL, 24.38 mmol). The reaction mixture was stirred at -78°C under N_2 for 3 hours. The reaction was quenched with water, and then extracted with dichloromethane. The combined organic layers were washed with brine then dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane: ethyl acetate (3:2 v/v) gave 1.53 g (84%) of product as a light brown solid; MS (ES+) *m/z* (M+1) 150.1; HRMS (FAB) *m/z* (M^+) calcd 149.08 for $C_9H_{11}NO$, obsd 149.09; mp = 65.5 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 6.7 Hz, 1H), 7.07 (dd, *J* = 7.6, 7.6 Hz, 1H), 6.79 (dd, *J* = 7.5, 7.4 Hz, 1H), 6.73 (d, *J* = 7.8 Hz, 1H), 4.15-4.08 (m, 1H), 3.78 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.64 (dd, *J* = 11.0, 6.3 Hz, 1H), 3.36 (br s, 2H), 3.15 (dd, *J* = 15.8, 9.2 Hz, 1H), 2.89 (dd, *J* = 15.8, 7.8 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.0, 129.6, 127.9, 125.3, 120.3, 111.10, 65.4, 60.9, 32.3 ppm.

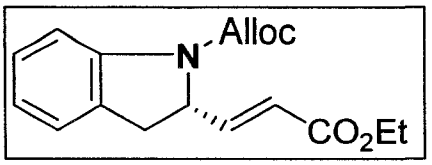


Compound 13.1: To a solution of the alcohol (0.74 g, 4.90 mmol) in dichloromethane (69 mL) at -78°C, was added DIPEA (0.86 g, 1.15 mL, 4.90 mmol) followed by the addition of allylchloroformate (0.59 g, 0.52 mL, 4.90 mmol). The reaction

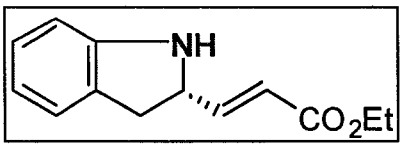
mixture was stirred at -78°C under N_2 for 2 hours. The reaction was quenched by adding saturated ammonium chloride solution (25 mL), and then extracted with dichloromethane and ethyl acetate. The combined organic layers were washed with brine and dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane : ethyl acetate (3:1 v/v) gave 1.14 g (99%) of product as a light yellow oil; MS (ES+) *m/z* (M+1) 234.0; HRMS (FAB) *m/z* (M^+) calcd 233.11 for $C_{13}H_{15}NO_3$, obsd 233.113; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (br s, 1H), 7.22-7.18 (m, 2H), 7.01 (dd, *J* = 7.4, 7.3 Hz, 1H), 6.09-6.00 (m, 1H), 5.42 (d, *J* = 17.2 Hz, 1H), 5.32 (d, *J* = 10.4 Hz, 1H), 4.79 (d, *J* = 4.2 Hz, 2H), 4.66 (br s, 1H), 3.81 (dd, *J* = 11.1, 5.9 Hz, 1H), 3.75 (dd, *J* = 11.2, 5.0 Hz, 1H), 3.38 (dd, *J* = 16.4, 10.1 Hz, 1H), 2.93 (br s, 1H), 2.54 (br s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 142.1, 132.7, 130.5, 127.9, 125.2, 123.5, 118.8, 116.1, 67.0, 65.8, 61.5, 31.7 ppm.



Compound 14.1: To a solution of the indoline alcohol (3.80g, 16.31 mmol) in dichloromethane (178 mL), was added DMSO (45 mL) followed by Et_3N (11.4mL, 81.55 mmol) then SO_3·Py (10.38g, 65.24 mmol) in that specific order. The reaction mixture was stirred at room temperature under N_2 for 3 hours. The reaction was quenched by adding saturated ammonium chloride solution, and then extracted with dichloromethane and ethyl acetate. The combined organic layers were washed with brine and dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane : ethyl acetate (3:1 v/v) gave 3.18 g (84%) of product as a light yellow oil; MS (ES+) *m/z* = 232.0 (M+1); HRMS (FAB) *m/z* (M^+) calcd 232.10 for $C_{13}H_{13}NO_3$, obsd 232.0985; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.96 (br s, 1H), 7.28-7.26 (m, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.02 (dd, *J* = 7.4, 7.3, 1H), 5.96 (br s, 1H), 5.44-5.30 (m, 2H), 4.87-4.75 (m, 3H), 3.46-3.44 (m, 1H), 3.22 (dd, *J* = 16.6, 4.4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.2, 153.0, 142.5, 132.4, 130.5, 128.6, 125.1, 123.8, 119.1, 115.5, 66.9, 66.3, 30.3 ppm.

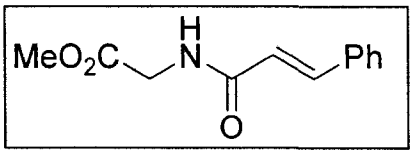


Compound 15.1: To a solution of the indoline aldehyde (3.17g, 13.71 mmol) in dichloromethane (102 mL), was added the Wittig reagent, (carbethoxymethylene) triphenylphosphorane (5.75g, 16.51 mmol). The reaction mixture was stirred at RT, under N_2 for 2:20 hours. The reaction was quenched by adding brine, and then extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane: ethyl acetate (9:1 v/v) gave 3.84 g, (95%) of the *trans* product and 0.089 g of the *cis* product. The trans product was a yellow oil; MS (ES+) *m/z* = 302.2 (M+1); HRMS (FAB) *m/z* (M^+) calcd 301.13 for $C_{17}H_{19}NO_4$, obsd 301.14; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (br s, 1H), 7.23 (dd, *J* = 7.8, 7.5 Hz, 1H), 7.16 (d, *J* = 7.4 Hz, 1H), 7.00 (dd, *J* = 7.5, 7.4 Hz, 1H), 6.90 (dd, *J* = 15.6, 6.4 1H), 5.97 (br s, 1H), 5.91 (d, *J* = 15.6 Hz, 1H), 5.36 (d, *J* = 17.0 Hz 1H), 5.27 (d, *J* = 10.3 Hz, 1H), 5.12 (br s, 1H), 4.73 (br s, 2H), 4.17 (q, *J* = 7.1 Hz, 2 H), 3.50 (dd, *J* = 16.1, 10.4 Hz, 1H), 2.88 (dd, *J* = 16.2, 2.0 Hz, 1H), 1.27 (t, *J* = 7.1, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 153.0, 146.4, 142.5, 132.7, 129.2, 128.3, 125.4, 123.7, 121.5, 118.7, 115.8, 66.7, 60.9, 59.8, 34.6, 14.6 ppm.

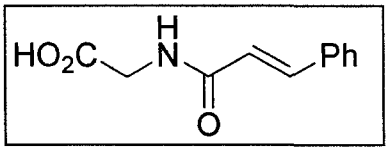


Compound 18.1: To a solution of the alloc indoline core (1.03g, 3.40 mmol) in dichloromethane (71 mL) at 0°C, was added $Pd(Ph_3)_4$ (0.39g, 0.34 mmol) followed by morpholine (0.62 mL, 7.14 mmol). The reaction mixture was stirred from 0°C to room temperature under N_2 for 4 hours. The reaction solution was evaporated under vacuum pressure then purified by flash chromatography. Elution with hexane: ethyl acetate (9:1 v/v) gave 0.67 (90%) of product as a yellow oil; MS (ES+) *m/z* = 218.1 (M+1); HRMS (FAB) *m/z* (M^+) calcd 211.11 for $C_{13}H_{15}NO_2$, obsd 217.1116; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12-7.03 (m, 3H), 6.76 (dd, *J* = 7.4, 7.4Hz, 1H), 6.67 (d, *J* = 7.8, Hz, 1H), 6.05 (d, *J* = 15.6 Hz, 1H), 4.54-4.48 (m, 1H), 4.23 (q, *J* = 7.2 Hz, 2H), 3.90 (br s, 1H), 3.31 (dd, *J* = 15.5, 9.2 Hz, 1H), 2.88 (dd, *J* = 15.5, 7.8 Hz, 1H), 1.32 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (100

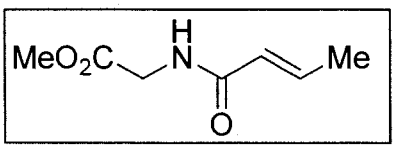
MHz, $CDCl_3$) δ 166.9, 150.5, 149.2, 134.9, 128.1, 125.1, 121.5, 119.6, 109.9, 60.9, 60.6, 36.5, 14.7 ppm



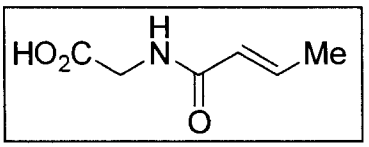
Compound 16.3: To a solution of Gly-OMe HCl (1.31g, 10.36 mmol) in dichloromethane (57 mL), was added DIPEA (3.51 mL, 20.72 mmol). The reaction mixture was stirred under N_2 for 15 minutes at room temperature. To this reaction mixture, was added cinnamoyl-chloride (2.07g, 12.43 mmol). The reaction mixture was stirred under N_2 for 1:25 hours at RT. The reaction was extracted with dichloromethane. The combined organic layers were washed with brine then dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with 3:10 ethyl acetate: hexane gave 1.75 g (77%) of product as a yellow oil; MS (ES+) *m/z* = 220.1 (M+1); HRMS (FAB) *m/z* (MH^+) calcd 220.10 for $C_{12}H_{14}NO_3$, obsd 220.1303; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.68 (d, *J* = 15.6 Hz, 1H), 7.55-7.52 (m, 2H), 7.38-7.41 (m, 3H), 6.49 (d, *J* = 15.6 Hz, 1H), 6.19 (br s, 1H), 4.22 (d, *J* = 5.1 Hz, 2H), 3.82 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 166.2, 142.3, 135.0, 130.3, 129.3, 128.3, 120.1, 52.9, 41.2 ppm.



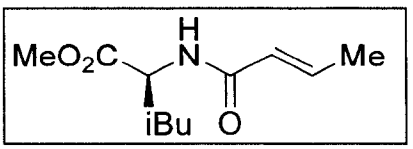
Compound 17.1: To a solution of the peptide ester (0.10 g, 0.47 mmol) in 8:2:1, THF:water:MeOH (10 ml) at 0°C, was added LiOH (0.039g, 0.93 mmol). The reaction mixture was stirred at 0°C to room temperature under N_2 for 3 hours. The reaction was neutralized with Amberlite® H^+ resin to pH 6 then evaporated by vacuum pressure to give 0.10 g (99%) of product as a white solid; MS (ES+) *m/z* = 206.1 (M+1); HRMS (FAB) *m/z* (MH^+) calcd 206.08 for $C_{11}H_{12}NO_3$, obsd 206.087; ^1H NMR (400 MHz, Acetone d_6) δ 7.63-7.57 (m, 3H), 7.44-7.37 (m, 3H), 6.82 (d, *J* = 15.7 Hz, 1H), 4.11 (d, *J* = 5.0 Hz, 2H), 2.83 (br s, 1H) ppm; ^{13}C NMR (100 MHz, Acetone d_6) δ 172.4, 167.3, 141.9, 137.1, 131.3, 130.7, 129.5, 123.2, 42.6 ppm.



Compound 16.3: To a solution of Gly-OMe HCl (4.01g, 31.62 mmol) in dichloromethane (150 mL), was added DIPEA (11.00 mL, 63.24 mmol). The reaction mixture was stirred under N_2 for 15 minutes at room temperature. To this reaction mixture, was added crotonyl chloride 90% (4.07ml, 42.06 mmol). The reaction mixture was stirred under N_2 for 2 hours at RT. The reaction was extracted with dichloromethane. The combined organic layers were washed with brine then dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with 3:10 ethyl acetate: hexane gave 4.60g (93%) of product as a yellow oil; MS (ES+) *m/z* = 158.0 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (dq, *J* = 15.3, 7.0 Hz, 1H), 6.22 (br s, 1H), 5.86 (dq, *J* = 15.3, 1.8 Hz, 1H), 4.08 (d, *J* = 5.3 Hz, 2H), 3.74 (s, 3H), 1.84 (dd, *J* = 6.8, 1.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 166.0, 140.9, 124.2, 52.3, 41.1, 17.7 ppm.

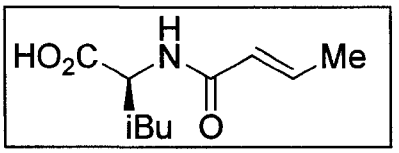


Compound 17.1: To a solution of the peptide ester (2.11g, 13.41 mmol) in 8:2:1, THF:water:MeOH (125ml) at 0°C, was added LiOH (1.15g, 26.82 mmol). The reaction mixture was stirred at 0°C to room temperature under N_2 for 3 hours. The reaction was neutralized with Amberlite® H^+ resin to pH 6 then evaporated by vacuum pressure to give 1.78g (90%) of product as an off white solid; MS (ES+) *m/z* = 144.0 (M+1); ^{1}H NMR (400 MHz, DMSO) δ 12.54 (br s, 1H), 8.21(t, J = 5.5 Hz, 1H), 6.64 (dq, *J* = 15.3, 7.0 Hz, 1H), 5.97 (dq, *J* = 15.3, 1.8 Hz, 1H), 3.80 (d, *J* = 5.7 Hz, 2H), 1.80 (dd, *J* = 7.0, 1.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 171.3, 165.1, 138.3, 125.3, 40.5, 17.3 ppm.

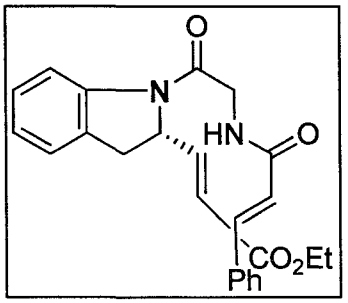


Compound 16.3: To a solution of Leu-OMe HCl (4.02g, 22.01 mmol) in DCM (400 mL), was added DIPEA (7.67 mL, 44.03 mmol). The reaction mixture was stirred under N_2 for 15 minutes at room temperature. To this reaction mixture, was added crotonyl chloride 90% (2.84 ml, 26.42 mmol). The reaction mixture was stirred under N_2 for 2 hours at RT. The reaction was extracted with DCM. The combined organic layers were washed with brine then dried over

magnesium sulfate, filtered and purified by flash chromatography. Elution with 1:5 ethyl acetate:hexane gave 4.08g (87%) of product as a clear oil; MS (ES+) *m/z* = 214.2 (M+1); ^{1}H NMR (400 MHz, C_6D_6) δ 6.99 (dq, *J* = 15.1, 7.0 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 5.75 (dq, *J* = 15.3, 1.5 Hz, 1H), 4.99 (ddd, *J* = 9.3, 8.3, 5.0 Hz, 1H), 3.31 (s, 3H), 1.76-1.51 (m, 3H), 1.48 (dd, *J* = 6.8, 1.5 Hz, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.85 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 165.6, 140.0, 125.5, 51.7, 50.9, 41.8, 25.1, 23.0, 17.5 ppm.



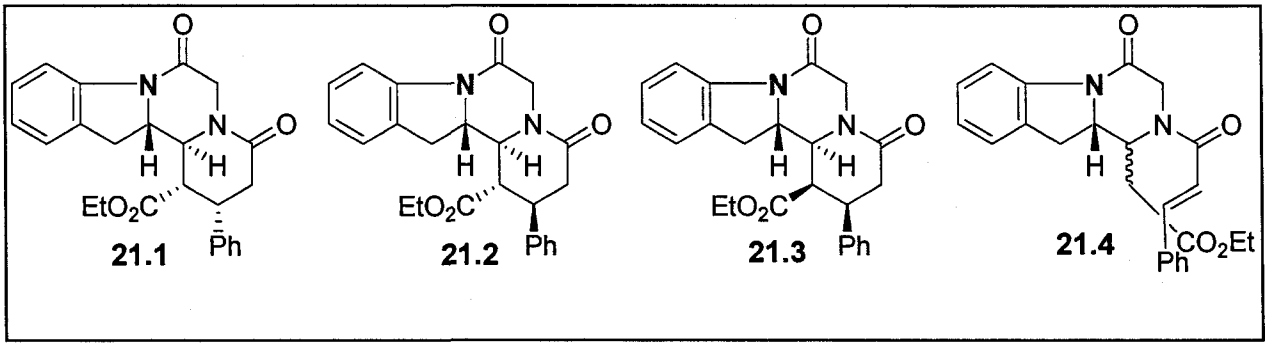
Compound 17.1: To a solution of the peptide ester (4.00g, 18.76 mmol) in 8:2:1, THF:water:MeOH (82ml) at 0°C, was added LiOH (1.57g, 37.51 mmol). The reaction mixture was stirred at 0°C to room temperature under N_2 for 3 hours. The reaction was neutralized with Amberlite® H^+ resin to pH 6 then evaporated by vacuum pressure to give 3.84g(quantitative yield) of the crude product as a very viscous clear oil solid; MS (ES+) *m/z* = 200.3 (M+1); ^{1}H NMR (400 MHz, DMSO) δ 7.63 (d, *J* = 7.8 Hz, 1H), 6.55 (dq, *J* = 15.3, 7.0 Hz, 1H), 5.99 (d, *J* = 15.3 Hz, 1H), 4.13-4.06 (m, 1H), 1.77 (d, *J* = 6.8 Hz, 3H), 1.63-1.35 (m, 3H), 0.84 (t, *J* = 6.3 Hz, 6H); ^{13}C NMR (100 MHz, DMSO) δ 175.4, 165.0, 137.9, 127.9, 127.3, 52.8, 42.7, 25.4, 24.1, 22.8, 17.3 ppm.



Compound 19.1: To a solution of the glycine-cinnamoyl acid (2.50g, 12.21 mmol) in DMF (106 mL), was added HATU (4.64g, 12.21 mmol) followed by the addition of DIPEA (2.13mL, 12.21 mmol) and then the deprotected indoline core (0.53g, 2.44 mmol). The reaction mixture was stirred at room temperature under N_2 for 48 hours. The reaction mixture was condensed and washed with ammonium chloride then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane: ethyl acetate (5:2 v/v) gave 0.79g (81%) of product as a light orange solid; MS (ES+) *m/z* = 405.1 (M+1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 15.7 Hz, 1H), 7.55-7.49 (m,

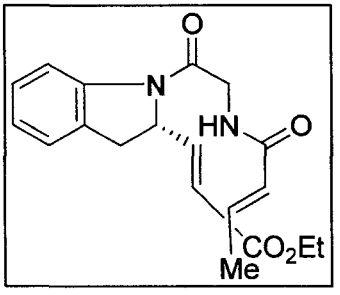
2H), 7.43-7.33 (m, 3H), 7.32-7.24 (m, 1H), 7.21 (d, *J* = 7.3 Hz, 1H), 7.11 (t, *J* = 7.3 Hz, 1H), 6.92 (dd, *J* = 15.7, 5.9 Hz, 1H), 6.75 (br s, 1H), 6.52 (d, *J* = 15.7 Hz, 1H), 5.92 (d, *J* = 15.7 Hz, 1H), 5.14-5.06 (m, 1H), 4.47 (dd, *J* = 17.9, 4.4 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.07 (dd, *J* = 17.9, 3.2 Hz, 1H), 3.63 (dd, *J* = 15.9, 10.0 Hz, 1H), 2.95 (d, *J* = 15.9 Hz, 1H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.2, 165.9, 145.0, 142.0, 135.1, 130.3, 129.5, 129.3, 129.2, 128.5, 128.3, 125.54, 125.52, 122.2, 120.4, 117.9, 61.4, 59.4, 43.2, 35.8, 14.6 ppm.

Compounds 21.1, 21.2, 21.3 and 21.4.



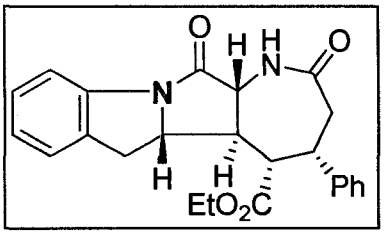
To a solution of the Glycine-cinnamoyl coupled indoline moiety (0.20g, 0.49 mmol) in DCM (10 mL) at -78°C, was added Et_3N (0.076ml, 0.54 mmol) followed by the addition of TBSOTf (0.23ml, 0.99 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 24 hours. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (9:1 v/v) gave 25.6mg (20%), 25.7mg (20%), 10.3mg (8%) and 25.6g (20%) of products **21.1**, **21.2**, **21.3** and **21.4**, respectively as an off white solid; MS (ES+) *m/z* = 405.1 (M+1); ^1H NMR (compound **21.1**): (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.0 Hz, 1H), 7.39-7.22 (m, 5H), 7.16-7.08 (m, 3H), 5.29 (d, *J* = 18.8 Hz, 1H), 4.48-4.39 (m, 1H), 4.11 (dd, *J* = 10.3, 4.0 Hz, 1H), 4.03 (q, *J* = 7.0 Hz, 2H), 3.84 (d, *J* = 18.8 Hz, 1H), 3.64-3.57 (m, 1H), 3.21-3.10 (m, 3H), 3.05 (dd, *J* = 15.1, 11.3 Hz, 1H), 2.81 (dd, *J* = 17.3, 5.0 Hz, 1H), 1.05 (t,

J = 7.0 Hz, 3H) ppm; ^{13}C NMR (compound **21.1**): (100 MHz, $CDCl_3$) δ 170.4, 167.6, 162.9, 141.8, 138.6, 128.9, 128.8, 128.1, 127.8, 127.2, 125.0, 124.5, 117.6, 64.0, 61.4, 58.2, 46.3, 46.2, 37.7, 34.0, 32.9, 13.8 ppm. ^1H NMR (compound **21.2**): (400 MHz, C_6D_6) δ 8.55 (d, J = 8.0 Hz, 1H), 7.19-6.98 (m, 4H), 6.92-6.78 (m, 4H), 5.36 (d, J = 19.1 Hz, 1H), 3.68 (d, J = 19.1 Hz, 1H), 3.61-3.43 (m, 3H), 3.56 (dd, J = 10.0 Hz, J = 9.0 Hz, 1H), 3.60-3.53 (m, 2H), 3.13 (ddd, J = 12.5 Hz, J = 10.0 Hz, J = 8.0 Hz, 1H), 2.73 (td, J = 12.5 Hz, J = 3.0 Hz, 1H), 2.56 (dd, J = 15.1, 8.0 Hz, 1H), 2.50 (dd, J = 16.1, 3.0 Hz, 1H), 2.40 (dd, J = 15.1, J = 12.5 Hz, 1H), 2.32-2.21 (m, 2H), 0.52 (t, J = 7.0 Hz, 3H) ppm; ^{13}C NMR (compound **21.2**): (100 MHz, C_6D_6) δ 171.2, 166.2, 162.3, 142.4, 139.9, 129.4, 128.7, 128.1, 127.9, 124.6, 124.3, 117.6, 64.9, 60.7, 59.0, 51.1, 46.2, 41.4, 38.7, 32.2, 13.5 ppm. ^1H NMR (compound **21.3**): (400 MHz, $CDCl_3$) δ 8.12 (d, J = 8.0 Hz, 1H), 7.40-7.19 (m, 7H), 7.09 (t, J = 7.4 Hz, 1H), 4.53 (d, J = 19.2 Hz, 1H), 4.34 (d, J = 19.2 Hz, 1H), 4.35-4.26 (m, 1H), 4.07-3.98 (m, 3H), 3.55-3.47 (m, 1H), 3.39 (dd, J = 17.1, 13.6 Hz, 1H), 3.30 (dd, J = 4.0, 4.0 Hz, 1H), 3.25 (dd, J = 15.6, 8.5 Hz, 1H), 3.07 (dd, J = 15.5, 10.1 Hz, 1H), 2.79 (dd, J = 17.1, 5.2 Hz, 1H), 1.05 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (compound **21.3**): (100 MHz, $CDCl_3$) δ 169.3, 168.7, 162.7, 141.3, 139.0, 128.9, 128.7, 128.3, 127.8, 126.9, 124.9, 124.6, 116.8, 61.1, 60.4, 60.3, 47.3, 46.1, 39.6, 33.1, 32.4, 14.0 ppm.

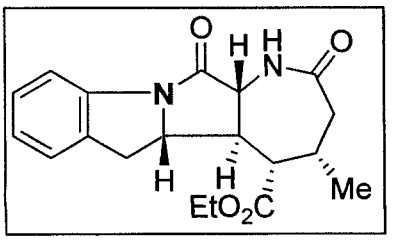


Compound 23.1: To a solution of the glycine-crotyl acid (0.54g, 3.7421 mmol) in DMF (160 mL), was added HATU (1.42g, 3.74 mmol) followed by the addition of DIPEA (0.65ml, 3.74 mmol) and then the deprotected indoline core **18.1** (0.16g, 0.75 mmol). The reaction mixture was stirred at room temperature under N_2 for 24 hours. The reaction mixture was condensed and washed with ammonium chloride then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (5:2 v/v) gave 0.21g (83%) of product as a light yellow oil; MS (ES+) m/z = 343.1 (M+1); ^1H NMR: (400 MHz, $CDCl_3$) δ 8.15 (d, J = 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.20 (d, J = 7.2 Hz, 1H), 7.10 (t, J = 7.3

Hz, 1H), 6.96-6.85 (m, 2H), 6.74 (br s, 1H), 5.94 (d, J = 15.1 Hz, 1H), 5.90 (d, J = 15.1 Hz, 1H), 5.09 (br t, 1H), 4.40 (d, J = 17.8 Hz, 1H), 4.12 (q, J = 7.0 Hz, 2H), 4.00 (d, J = 17.8 Hz, 1H), 3.61 (dd, J = 15.8, 10.0 Hz, 1H), 2.93 (d, J = 16.0 Hz, 1H), 1.88 (d, J = 6.8 Hz, 3H), 1.25 (t, J = 7.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 165.9, 165.4, 144.6, 141.5, 140.5, 128.7, 128.0, 125.0, 124.9, 124.4, 121.7, 117.4, 60.3, 58.9, 42.5, 35.3, 17.7, 14.1 ppm.

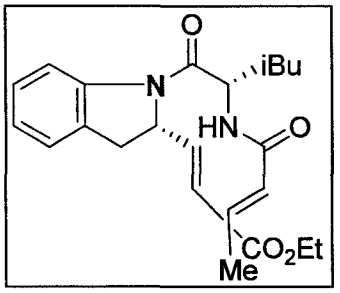


Compound 22.1: To a solution of the Glycine-cinnamoyl coupled indoline moiety (0.11g, 0.25 mmol) in DCM (5 mL) at -78°C, was added Et_3N (0.140 ml, 0.99mmol) followed by the addition of TMSOTf (0.18 ml, 0.99 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 4 hours. The reaction mixture was quenched with brine then extracted with DCM and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (4:1 v/v) gave 0.095g (91%) of product as white needles. Stereochemistry was confirmed by crystal X-ray structure; MS (ES+) m/z = 405.1 (M+1); ^{1}H NMR: (400 MHz, Acetone D_6) δ 7.48 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 7.3 Hz, 2H), 7.30-7.20 (m, 5H), 7.06 (t, J = 7.5 Hz, 1H), 6.32 (br s, 1H), 4.78 (d, J = 10.8 Hz, 1H), 4.43 (q, J = 8.8 Hz, 1H), 3.98-3.87 (m, 2H) 3.84-3.77 (m, 1H), 3.43 (dd, J = 11.5, 5.5 Hz, 1H), 3.24 (ddd, J = 11.2, 11.1, 8.8 Hz, 1H), 3.18-2.94 (m, 4H), 1.04 (t, J = 7.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, Acetone D_6) δ 173.7, 173.4, 169.2, 142.2, 140.8, 136.5, 130.9, 130.2, 129.2, 129.0, 127.4, 126.5, 116.7, 66.2, 62.2, 60.8, 55.2, 52.1, 44.3, 42.6, 37.2, 15.3 ppm; carbon displacements for 62.2, 42.6 and 37.2 are CH_2 carbons, determined by DEPT 135 analysis.



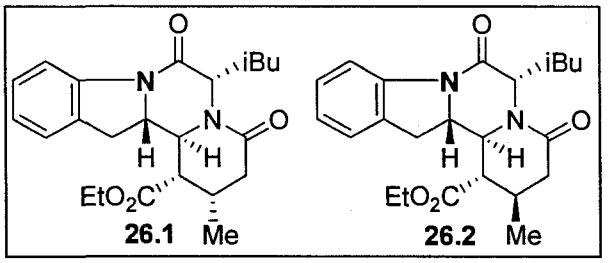
Compound 24.1: To a solution of the Glycine-crotyl coupled indoline moiety (0.077g, 0.23 mmol) in DCM (5 mL) at -78°C, was added Et_3N (0.16 ml,

1.17mmol) followed by the addition of TMSOTf (0.22 ml, 1.17 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 1 hour. The reaction mixture was quenched with brine then extracted with DCM and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (4:1 v/v) gave 0.062g (80%) of product as an off white solid; MS (ES+) *m/z* = 343.1 (M+1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.9 Hz, 1H), 7.20 (t, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.3 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.54 (br s, 1H), 4.28 (dd, *J* = 10.5, 3.0 Hz, 1H), 4.24-4.15 (m, 3H) 3.21 (dd, *J* = 16.3, 9.3 Hz, 1H), 3.09 (dd, *J* = 16.3, 8.8 Hz, 1H), 2.89 (dd, *J* = 11.3, 3.0 Hz, 1H), 2.82-2.53 (m, 4H), 1.30 (t, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 172.0, 166.2, 137.9, 134.0, 127.7, 125.2, 125.1, 115.1, 63.8, 61.0, 59.8, 53.5, 48.1, 42.7, 35.8, 30.0, 14.2, 13.5 ppm.



Compound 25.1: To a solution of the leucine-crotyl acid (0.92g, 4.60 mmol) in DMF (25 mL), was added PyBrop® (2.15g, 4.60 mmol) followed by the addition of DMAP (0.56g, 4.60 mmol), DIPEA (1.00ml, 5.74 mmol) and then the deprotected indoline core (0.20g, 0.92 mmol). The reaction mixture was stirred at room temperature under N_2 for 4.5 hours. The reaction mixture was condensed and washed with ammonium chloride then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (5:2 v/v) gave 0.19g (52%) of product as a clear viscous oil; MS (ES+) *m/z* = 399.4 (M+1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 8.3 Hz, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.89 (dd, *J* = 15.8, 5.5 Hz, 1H), 6.80 (dq, *J* = 15.1, 7.0 Hz, 1H), 6.20 (d, *J* = 8.5 Hz, 1H), 5.86 (d, *J* = 15.8 Hz, 1H), 5.81 (d, *J* = 15.3 Hz, 1H), 5.08 (dd, *J* = 9.8, 5.5 Hz, 1H), 4.91 (ddd, *J* = 9.2, 9.1, 3.8 Hz, 1H), 4.16-4.05 (m, 2H), 3.59 (dd, *J* = 15.8, 9.8 Hz, 1H), 2.95 (d, *J* = 15.8 Hz, 1H) 1.83 (d, *J* = 6.8 Hz, 3H), 1.77-1.60 (m, 2H), 1.58-1.50 (m, 1H) 1.23 (t, *J* = 7.0 Hz, 3H) 1.04

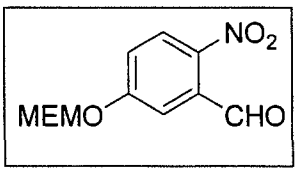
(d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 165.5, 164.9, 144.3, 141.7, 140.3, 129.1, 128.0, 125.0, 124.9, 124.8, 122.0, 117.8, 60.7, 59.4, 49.6, 44.0, 35.1, 24.8, 23.5, 22.1, 17.7, 14.1 ppm.



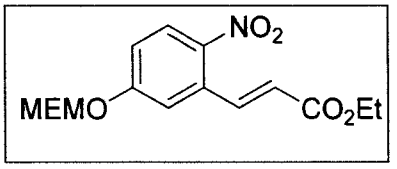
Compounds 26.1 & 26.2: To a solution of the Leucine-crotyl coupled indoline moiety (0.18g, 0.46 mmol) in DCM (5 mL) at -78°C, was added Et_3N (0.51 ml, 3.65 mmol) followed by the addition of TMSOTf (0.66 ml, 3.65 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 22 hours. The reaction mixture was quenched with brine then extracted with DCM and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (9:1 v/v) gave 0.048g (27%) and 0.018g (10%) of products **26.1** and **26.2** respectively as off white solids; MS (ES+) *m/z* = 399.4 (M+1); ^{1}H NMR (compound **26.1**): (400 MHz, C_6D_6) δ 8.55 (d, J = 8.0 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.90 (t, J = 7.5 Hz, 1H), 6.85 (d, J = 7.5 Hz, 1H), 5.84 (dd, J = 10.0, 3.8 Hz, 1H), 3.85 (q, J = 7.0 Hz, 2H), 3.82 (dd, J = 10.5, 4.0 Hz, 1H), 3.12 (ddd, J = 11.0, 10.8, 8.0 Hz, 1H), 2.37 (dd, J = 17.1, 8.8 Hz, 1H), 2.23 (dd, J = 17.1, 5.0 Hz, 1H), 2.23-2.15 (m, 2H), 2.07 (dd, J = 9.8, 3.8 Hz, 1H), 2.10-1.94 (m, 3H), 1.80-1.71 (m, 1H), 1.70-1.61 (m, 1H), 1.23 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H) 0.89 (t, J = 7.0 Hz, 3H), 0.81 (d, J = 7.0 Hz, 3H) ppm; ^{13}C NMR (compound **26.1**) (100 MHz, C_6D_6) δ 170.7, 166.5, 166.1, 143.3, 129.6, 128.1, 124.4, 124.3, 118.1, 64.2, 61.0, 54.1, 53.6, 45.1, 41.9, 37.6, 32.3, 27.0, 25.2, 23.7, 22.2, 17.2, 14.2 ppm. ^{1}H NMR (compound **26.2**): (400 MHz, C_6D_6) δ 8.54 (d, J = 8.0 Hz, 1H), 7.13-7.06 (m, 1H), 6.90-6.82 (m, 2H), 5.82 (dd, J = 9.5, 4.8 Hz, 1H), 3.94 (dd, J = 9.5, 8.8 Hz, 1H), 3.84 (q, J = 7.0 Hz, 2H), 3.05-2.95 (m, 1H), 2.50 (dd, J = 14.7, 7.4 Hz, 1H), 2.38-2.28 (m, 2H), 2.07 (ddd, J = 13.8, 8.5, 5.0 Hz, 1H), 1.96-1.78 (m, 2H), 1.75-1.58 (m, 3H), 1.15 (d, J = 6.5 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H), 0.88 (t, J = 7.0 Hz, 3H), 0.61 (d J = 6.3 Hz, 3H) ppm; ^{13}C NMR (compound **26.2**) (100 MHz, C_6D_6) δ 172.4, 167.0, 166.3, 143.0, 129.5,

128.1, 124.44, 124.38, 117.9, 66.0, 61.1, 55.7, 53.0, 51.2, 42.0, 39.7, 32.3, 30.1, 25.4, 23.5, 22.5, 18.6, 14.1 ppm.

5.3 Syntheses for Enantio-Enriched System

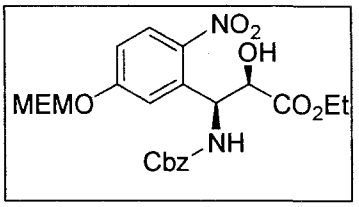


Compound 29.2: DIPEA (52.2 mL, 299.78 mmol) and MEM chloride (25 g, 201.09 mmol) were added to a solution of 5-hydroxy-2-nitro-benzaldehyde (25 g, 151.39 mmol) in DCM (500 mL) at 0 °C. The mixture was warmed to room temperature and stirred for another 4 hours. The reaction was quenched with saturated ammonium chloride solution and extracted with DCM. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:3 ethyl acetate/hexanes) to give the pure product (38.63 g, 99%) as a pale yellow oil; MS (ES+) *m/z* (M+1) 256.0; ^{1}H NMR (400MHz, $CDCl_3$) δ 10.46 (s, 1H), 8.16 (d, *J* = 9.0 Hz, 1H), 7.48 (s, 1H), 7.34 (d, *J* = 8.9 Hz, 1H), 5.40 (s, 2H), 3.84 (d, *J* = 3.0 Hz, 2H), 3.56 (d, *J* = 2.9 Hz, 2H), 3.37 (s, 3H) ppm; ^{13}C NMR (100MHz, $CDCl_3$) δ 188.7, 162.1, 143.3, 134.7, 127.5, 120.2, 116.7, 93.9, 71.8, 68.9, 59.4 ppm.

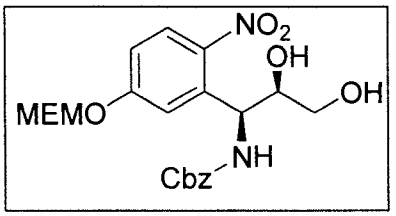


Compound 30.1: To a suspension of 60% NaH (9.00 g, 224.81 mmol) in THF (750mL) was added triethyl phosphono acetate (35.99 mL, 179.84 mmol) at 0°C. The reaction mixture was stirred for 30 min at 0 °C. To the reaction mixture was added the above aldehyde (38.25 g, 149.87 mmol) at 0 °C. The reaction mixture was stirred for 6 h at 0 °C and quenched with water. The mixture was extracted with EtOAc, and then the combined organic layers were washed with water and brine then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (1:1 ethyl acetate/hexanes) to give the pure product (46.15 g, 95%) as a yellow oil; MS (ES+) *m/z* 326.1 (M+1); ^{1}H NMR

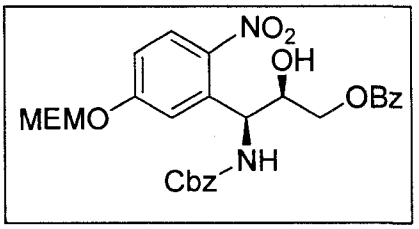
(400MHz, $CDCl_3$) δ 8.18 (d, *J* = 15.7 Hz, 1H), 8.11 (d, *J* = 9.0 Hz, 1H), 7.21 (d, *J* = 2.6 Hz, 1H), 7.16 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.32 (d, *J* = 15.7 Hz, 1H), 5.37 (s, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.84 (t, *J* = 4.6 Hz, 2H), 3.38(t, *J* = 4.6 Hz, 2H), 3.38 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (100MHz, $CDCl_3$) δ 165.5, 161.5, 142.3, 141.1, 133.9, 127.8, 123.7, 117.4, 116.5, 93.8, 71.8, 68.6, 61.2, 59.4, 14.6 ppm.



Compound 33.1: A solution of Benzyl carbamate (11.07 g, 109.87 mmol) in *n*-propyl alcohol (100 mL) is slowly added to a freshly prepared solution of NaOH (2.88 g, 108.10 mmol) in water (167 mL), then stirred for 10 minutes at 0 °C. As of this moment, the reagent where added in absolute darkness. To this reaction mixture, was added freshly prepared *tert*butyl hypochlorite (8.27 mL, 108.10 mmol) followed by a solution of the ligand $(DHQ)_2PHAL$ (920 mg, 1.77 mmol, 5 mol%) in *n*-propyl alcohol (33 mL). The reaction mixture was allowed to warm to room temperature for 15 minutes. A solution of the above olefin (7.69 g, 35.44 mmol) in *n*-propyl alcohol (15 mL) was added. The reaction mixture was cooled again to 0 °C, then potassium osmate dihydrate (348 mg, 1.42 mmol, 4 mmol%) was added. The reaction mixture was stirred for 4 hours in the dark as the dark green solution becomes dark yellow. After TLC analysis confirmed the absence of starting material, the reaction mixture is condensed and extracted with ethyl acetate. The combined organic extracts were washed with water and brine and dried over Na_2SO_4. Purification by column chromatography (1:1 ethyl acetate/hexanes) afforded the product (9.98 g, 79%) as yellow oil; MS (ES+) *m/z* 493.3 (M+1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 9.1 Hz, 1H), 7.38-7.30 (m, 5H), 7.19 (d, *J* = 2.4 Hz, 1H), 7.11 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.09 (d, *J* = 8.9 Hz, 1H), 5.88 (d, *J* = 8.9 Hz, 1H), 5.33 (s, 2H), 5.11 (d, *J* = 12.1 Hz, 1H), 5.03 (d, *J* = 11.9 Hz, 1H), 4.66(s, 1H), 4.31 (q, *J* = 7.2 Hz, 2H), 3.82 (t, *J* = 4.6 Hz, 2H), 3.54 (t, *J* = 4.7 Hz, 2H) 3.36 (s, 3H), 3.31 (s, 1H), 1.28 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 161.6, 155.7, 142.1, 138.5, 136.5, 128.9, 128.6, 128.5,128.1, 117.4, 115.4, 93.7, 72.4, 71.8, 68.4, 67.5, 63.2, 59.4, 53.3, 14.4 ppm.

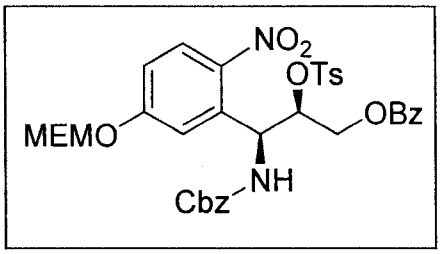


Compound 34.1: To a solution of the above aminohydroxylester (7.97 g, 16.19 mmol) in dry THF (190 mL) was added a solution of 2 M $LiBH_4$ in THF (8.18 mL,16.35 mmol) under N_2 at 0°C. The solution was allowed to warm to room temperature, and then stirred overnight. The reaction was quenched with saturated ammonium chloride solution and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (3:1 ethyl acetate/hexanes) to give the product (6.47 g, 89%) as a yellowish oil; MS (ES+) *m/z* 451.2 (M+1); ^{1}H NMR (400MHz, $CDCl_3$) δ 8.04 (d, *J* = 9.0 Hz, 1H), 7.42-7.20 (m, 5H), 7.12-6.85 (m, 2H), 6.42 (d, *J* = 8.1 Hz, 1H), 5.53 (d, *J* = 5.6 Hz, 1H), 5.32-5.18 (m, 2H), 5.05 (s, 2H), 4.01 (s, 1H), 3.85-3.60 (m, 5H), 3.54 (m, 2H) 3.27 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 156.5, 142.1, 140.2, 136.6, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 127.6, 116.5, 116.0, 115.5, 93.6, 73.8, 71.8, 68.2, 67.5, 64.7, 59.2, 52.5 ppm.

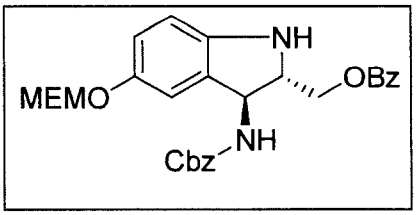


Compound 35.1: To a solution of the above diol (16.0589 g, 35.6706 mmol) in dry DCM (483 mL) was added dry pyridine (3.17 mL, 39.24 mmol). The solution was cooled to –25 ^{0}C. A solution of benzoyl chloride (3.93 mL, 33.89 mmol) in 10 ml of DCM was added to the solution via syringe pump (0.2 mL/min). The solution was stirred at –25 ^{0}C for 2 hours. The reaction was quenched with methanol, extracted with DCM and washed with 1% HCl, brine, and a saturated $NaHCO_3$ solution. The organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 ethyl acetate/hexanes) to give the product (17.41 g, 88%) as a yellow oil; MS (ES+) *m/z* 555.3.2 (M+1); ^{1}H NMR (400MHz, CDCl3) δ 8.11 (m, 3H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.40-7.20 (m, 7H), 7.06 (dd, *J* = 9.0, 2.1 Hz, 1H), 6.17 (d, *J* = 8.3 Hz, 1H), 5.78 (d, *J* = 8.3 Hz, 1H), 5.36

(d, J = 7.1 Hz, 1H), 5.31 (d, J = 7.1 Hz, 1H), 5.06 (s, 2H), 4.61 (dd, J = 11.4, 6.8 Hz, 1H), 4.51 (dd, J = 11.4, 4.8 Hz, 1H), 4.38 (m, 1 H), 3.80 (m, 2H), 3.56 (m, 1H), 3.49 (m, 1H), 3.28 (s, 3H) ppm; ^{13}C NMR (100 MHz, CDCl3) δ 167.4, 161.3, 156.2, 142.0, 139.9, 136.5, 133.7, 130.2, 130.0, 129.0, 128.9, 128.6, 128.5, 128.3, 116.6, 115.6, 93.4, 71.8, 71.7, 68.0, 67.6, 66.9, 59.2, 52.5 ppm.

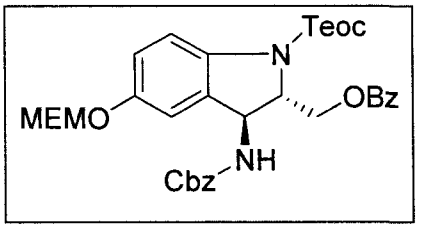


Compound 36.1: To a solution of the above secondary alcohol (33.54 g, 60.48 mmol) in dry DCM (610 mL) were added TsCl (17.30 g, 90.72 mmol) and DMAP (14.78 g, 120.96 mmol). The solution was stirred at room temperature for 24 hours. The solution was then washed with brine, extracted with DCM. The organic layers were dried over sodium sulfate and concentrated under reduced pressured. The crude product was purified by column chromatography (1:1 ethyl acetate/hexanes) to give the product (40.84 g, 95%) as a yellow oil; MS (ES+) *m/z* 709.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 9.1 Hz, 1H), 8.02 (d, J = 7.6 Hz, 2H), 7.59-7.51 (m, 3H), 7.43 (t, J = 7.6 Hz, 2H,), 7.35 (m, 5H), 7.11-7.04 (m, 4H), 6.02 (d, J = 9.2 Hz, 1H), 5.96 (d, J = 9.2 Hz, 1H), 5.35-5.28 (m, 3H), 5.07 (s, 2H), 4.68 (dd, J = 11.8, 5.9 Hz, 1H), 4.52 (dd, J = 11.8, 6.2 Hz, 1H), 3.83 (dd, J = 9.1, 4.4 Hz, 2H), 3.55 (dd, J = 9.1, 4.4 Hz, 2H), 3.36 (s, 3H), 2.30 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 162.0, 155.8, 145.4, 141.7, 137.6, 136.2, 133.7, 132.9, 130.3, 130.2, 129.6, 128.8, 128.7, 128.6, 127.9, 93.8, 80.5, 71.8, 68.6, 67.9, 63.3, 59.4, 52.6, 22.0 ppm.



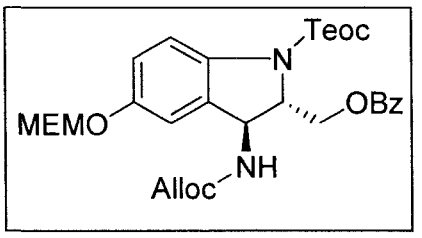
Compound 37.1: To a solution of the above nitro/tosyl compound 2x(20.42 g, 28.83 mmol) in anhydrous ethanol 2x(250 mL) at 0°C was added Zinc powder 2x(19.23 g, 288.30 mmol) followed by 2x16.36 mL of Glacial acetic acid. The reaction mixture was then stirred overnight under argon atmosphere. The mixture was filtered through celite and the organic solution was concentrated under

reduced pressure to then dissolve the amine in dry THF 2x(600 mL). Anhydrous potassium carbonate 2x(7.97 g, 57.66 mmol) was added to the solution and the reaction mixture was stirred at 55 °C for 24 hours. The reaction mixture was washed with brine and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 ethyl acetate/hexanes) to give the cyclized indoline product (22.72 g, 78%) as a yellow oil; MS (ES+) *m/z* 507.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.44-7.28 (m, 7H), 6.99 (s, 1H), 6.86 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.57 (d, *J* = 8.4 Hz, 1H), 5.35 (bs, 1H), 5.21-5.11 (m, 5H), 4.62 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.40 (dd, *J* = 11.0, 7.4 Hz, 1H), 4.10-3.90 (m, 2H), 3.83 (t, *J* = 4.5 Hz, 2H), 3.57 (t, *J* = 4.6 Hz, 2H), 3.38 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 156.3, 151.7, 145.4, 136.7, 133.5, 130.3, 130.1, 129.0, 128.8, 128.7, 128.6, 119.0, 114.4, 111.4, 95.1, 72.0, 67.9, 67.4, 67.1, 66.7, 59.4, 56.9 ppm.

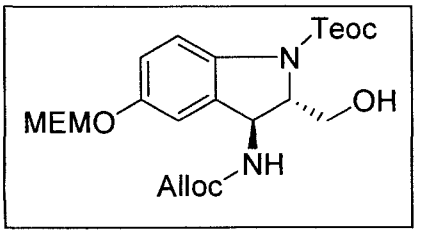


Compound 38.1: To a solution of 2-(trimethylsilyl)ethanol (12.80 mL, 89.75 mmol) and bis(trichloromethyl)-carbonate (11.55 g, 31.41 mmol) in dry DCM (250 mL) cooled to 0°C, was added pyridine (7.26 mL, 89.75 mmol) dropwise. The solution was stirred at 0°C for 2 hours. Then a solution of the indoline secondary amine above (22.72 g, 44.87 mmol) and pyridine (10.89 mL, 134.62 mmol) in dry DCM (350 mL) was added dropwise to the solution of 2-trimethylsilylethoxycarbonyl chloride (Teoc-Cl). The reaction was continued for 1 hour at 0°C. The reaction mixture was washed with 1% HCl solution, water, and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the protected indoline compound (20.32 g, 70%) as a yellow oil; MS (ES+) *m/z* 651.4 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (bs, 1H), 7.70 (d, *J* = 7.4 Hz, 2H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.38-7.29 (m, 7H), 7.10 (s, 1H), 7.04 (dd, *J* = 8.8, 2.0 Hz, 1H), 5.21-5.10 (m, 6H), 4.65-4.55 (m,

3H), 4.29 (m, 2H), 3.82 (t, J = 4.5 Hz, 2H), 3.55 (t, J = 4.6 Hz, 2H), 3.36 (s, 3H), 1.09 (m, 2H), 0.06 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 155.8, 154.1, 136.5, 134.0, 130.0, 129.0, 128.7, 128.3, 118.9, 116.8, 114.1, 94.7, 72.0, 68.0, 67.5, 66.5, 65.1, 64.8, 59.4, 18.2, -1.1 ppm.

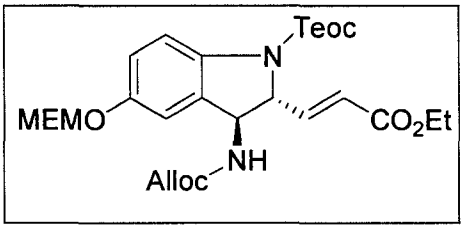


Compound 39.1: To a solution of the above protected indoline core (20.32 g, 31.24 mmol) in methanol (517 mL) was added the Palladium catalyst (4.07 g, 10% palladium over activated carbon). The reaction mixture was then subjected to hydrogenation under atmospheric pressure for 3 hours. The reaction mixture was filtered through celite and the organic solution was concentrated under reduced pressure. Then the intermediate was dissolved in dry THF (394 mL) and cooled at 0°C. Alloc chloride (4.14 mL, 39.05 mmol) and pyridine (3.79 mL, 46.86 mmol) were then added to the solution. The mixture was stirred at 0°C for 1 hour. The reaction mixture was diluted with ethyl acetate, and washed with 1% HCl solution and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the Teoc/Alloc protected indoline core (15.24 g, 81%) as a light yellow oil; MS (ES+) *m/z* 601.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (bs, 1H), 7.68 (d, J = 7.7 Hz, 2H), 7.49 (t, J = 7.3 Hz, 1H), 7.33-7.28 (m, 2H), 7.09 (d, J = 2.2 Hz, 1H), 7.03 (dd, J = 8.8, 2.0 Hz, 1H), 5.92 (m, 1H), 5.34-5.05 (m, 5H), 4.62-4.57 (m, 5H), 4.29 (bs, 2H), 3.83 (t, J = 4.6 Hz, 2H), 3.54 (t, J = 4.6 Hz, 2H), 3.37 (s, 3H), 1.09 (bs, 2H), 0.05 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 155.6, 154.1, 133.3, 132.9, 130.0, 128.6, 118.9, 188.4, 116.8, 114.1, 94.7, 72.0, 68.0, 66.5, 66.3, 65.1, 64.8, 59.4, 18.2, -1.1 ppm.



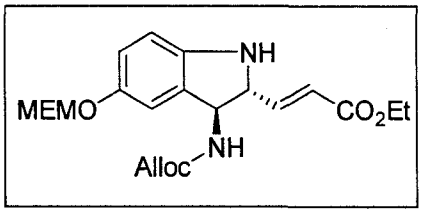
Compound 40.1: To a solution of the above compound (15.24 g, 25.39 mmol) in methanol (642 mL) was added anhydrous potassium

carbonate (3.51 g, 25.39 mmol). The mixture was stirred at room temperature for 2 hours under argon atmosphere. The reaction was then neutralized with Amberlite H+ resin to pH = 7. The mixture was filtered and concentrated under reduced pressure. The crude product was diluted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 ethyl acetate/hexanes) to give the primary alcohol (11.97 g, 95%) as a yellow oil; MS (ES+) *m/z* 497.4 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (bs, 1H), 7.06 (d, *J* = 2.4 Hz, 1H), 7.03 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.94 (m, 1H), 5.34 (d, *J* = 17.2 Hz, 1H), 5.27-5.23 (m, 3H), 4.99 (d, *J* = 5.6 Hz, 1H), 4.63 (d, *J* = 4.6 Hz, 1H), 4.38-4.30 (m, 2H), 3.98 (bs, 1H), 3.83 (t, *J* = 4.6 Hz, 2H), 3.70 (bs, 1H), 3.58 (t, *J* = 4.6 Hz, 2H), 3.39 (s, 3H), 3.13 (bs, 1H), 1.13 (t, *J* = 8.6 Hz, 2H), 0.08 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 154.1, 132.7, 118.9, 118.7, 117.0, 113.9, 94.5, 72.0, 69.9, 68.0, 66.6, 64.8, 63.8, 59.4, 55.2, 18.4, -1.1 ppm.

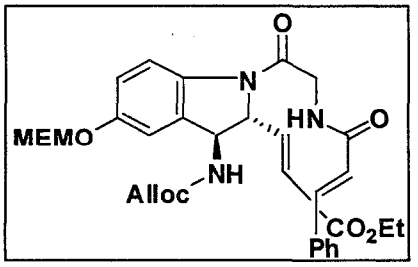


Compound 41.1: The Dess-Martin periodinane (4.40 g, 10.07 mmol) was added to a solution of the above primary alcohol (2.50 g, 5.04 mmol) in dry DCM (130 mL). The resulting suspension was stirred at room temperature under argon atmosphere and TLC showed complete conversion to the aldehyde after 1 hour. Then (carbethoxymethylene)triphosphorane (4.62 g, 12.59 mmol) was added to the reaction mixture, then stirred for an additional hour. The reaction mixture was washed with a saturated sodium bicarbonate solution and brine sequentially. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (2:3 ethyl acetate/hexanes) to give the final compound (2.02 g, 71%) as a yellow oil; MS (ES+) *m/z* 565.4 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (bs, 1H), 7.07-7.05 (m, 2H), 6.95 (dd, *J* = 15.6, 5.7 Hz, 1H), 5.98-5.89 (m, 2H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.25 (d, *J* = 15.3 Hz, 1H), 5.23 (s, 2H), 5.06 (d, *J* = 6.7 Hz, 1H), 4.92 (bs, 1H), 4.84 (d, *J* = 6.7 Hz, 1H), 4.62 (bs, 2H),

4.30 (bs, 2H), 4.18 (q, J = 7.1 Hz, 2H), 3.83 (t, J = 4.6 Hz, 2H), 3.58 (t, J = 4.6 Hz, 2H), 3.39 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H), 1.07 (bs, 2H), 0.07 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 155.6, 154.2, 144.2, 132.8, 122.6, 119.3, 118.5, 117.0, 114.4, 94.5, 72.0, 68.3, 68.1, 66.4, 64.8, 61.0, 59.4, 57.2, 18.2, 14.6, -1.1 ppm.

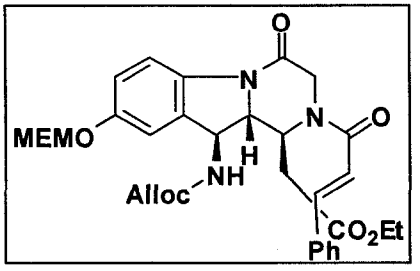


Compound 42.1: A TBAF solution (1 M, 1.28 mL, 1.28 mmol) was added to the solution of the above Teoc protected compound (0.36 g, 0.64 mmol) in dry THF (15 mL). The solution was stirred at room temperature for 2 hours under argon atmosphere. The solution was then washed with a saturated NH_4Cl solution, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (2:3 ethyl acetate/hexanes) to give the secondary amine (233.1 mg, 87%)as a dark yellow oil; MS (ES+) *m/z* 421.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (dd, J = 15.6, 6.7 Hz, 1H), 6.92 (d, J = 2.1 Hz, 1H), 6.85 (dd, J = 2.1, 8.5 Hz, 1H), 6.56 (d, J = 8.5 Hz, 1H), 6.05 (d, J = 15.6 Hz, 1H), 5.92 (m, 1H), 5.39 (d, J = 8.3 Hz, 1H), 5.31 (d, J = 15.6 Hz, 1H), 5.22 (d, J = 10.7 Hz, 1H), 5.14 (s, 2H), 4.98 (t, J = 6.8 Hz, 1H), 4.59 (d, J = 5.3 Hz, 1H), 4.20-4.14 (m, 3H), 3.81 (t, J = 4.6 Hz, 2 H), 3.56 (t, J = 4.6 Hz, 2H), 3.37 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 156.0, 151.7, 147.0, 145.1, 133.0, 128.3, 122.6, 119.0, 118.3, 114.5, 111.3, 95.1, 72.0, 68.5, 67.9, 66.2, 60.9, 59.5, 59.4, 14.6 ppm.



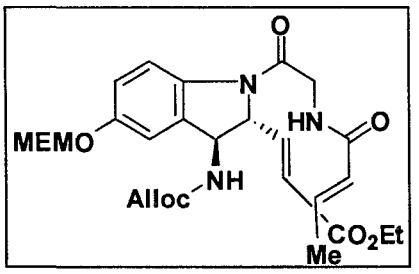
Compound 43.1: To a stirred solution of glycine cinnamic acid (255 mg, 1.24 mmol) in dry DMF (10 mL) was added HATU (473 mg, 1.24 mmol) followed by DIPEA (217 µL, 1.24 mmol). To the reaction mixture was added the above secondary amine (105 mg, 0.25 mmol) and the mixture was stirred 72 hours under argon atmosphere. The solution was condensed and dissolved in Ethyl acetate, then washed with a saturated NH_4Cl solution, dried over sodium sulfate and

concentrated under reduced pressure. The crude product was purified by column chromatography (1:5 ethyl acetate/hexanes) to give the coupled product (90.2 mg, 60%) as a dark yellow mousse; MS (ES+) *m/z* 608.4 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 9.3 Hz, 1H), 7.63 (d, *J* = 15.8 Hz, 1H), 7.54-7.49 (m, 2H), 7.41-7.34 (m, 3H), 7.13-7.06 (m, 2H), 6.97 (dd, *J* = 15.5, 4.5 Hz, 1H), 6.68 (br s, 1H), 6.51 (d, *J* = 15.6 Hz, 1H), 5.98 (d, *J* = 15.8 Hz, 1H), 5.98-5.87 (m, 1H), 5.38-5.20 (m, 5H), 4.96 (br d, *J* = 4.8, 0.0 Hz, 1H), 4.86 (br d, *J* = 5.8, 0.0 Hz, 1H), 4.67-4.58 (m, 2H), 4.45 (dd, *J* = 17.8, 4.3 Hz, 1H), 4.16 (q, *J* = 7.0 Hz, 2H), 3.98 (d, *J* = 17.8 Hz, 1H), 3.84-3.80 (m, 2H), 3.58-3.54 (m, 2H), 3.37 (s, 3 H), 1.26 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.9, 165.2, 164.3, 155.1, 142.4, 141.7, 136.7, 134.8, 134.6, 132.2, 129.8, 128.8, 127.9, 123.3, 119.9, 118.8, 118.7, 114.1, 113.7, 93.9, 71.5, 67.7, 67.5, 66.2, 60.9, 59.0, 57.8, 42.5, 14.1 ppm.



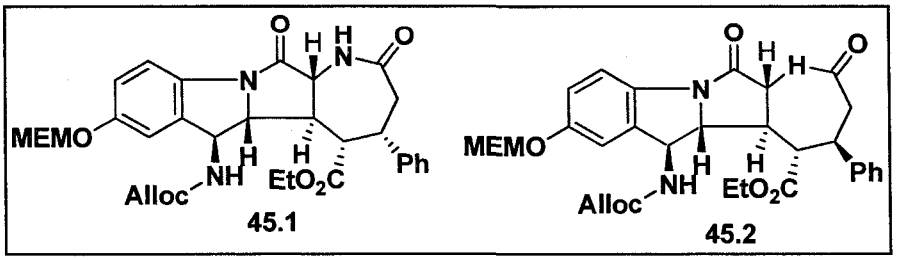
Compound 44.1: To a solution of the Glycine-cinnamoyl coupled indoline moiety (0.94g, 0.15 mmol) in DCM (4 mL) at -78°C, was added Et_3N (0.22ml, 1.55 mmol) followed by the addition of TBSOTf (0.36ml, 1.55 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 4 hours. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and purified by flash chromatography. Elution with hexane:ethyl acetate (4:1 v/v) gave 55.0mg (58%) of the tricyclic product as a beige mousse; MS (ES+) *m/z* = 608.4 (M+1); ^{1}H NMR: (400 MHz, C_6D_6) δ 7.84 (d, *J* = 15.8 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.19-7.11 (m, 2H), 7.00-6.54 (m, 3H), 6.89 (br s, 1H), 6.63 (d, *J* = 15.6 Hz, 1H), 5.81-5.69 (m, 1H), 5.43 (t, *J* = 9.9 Hz, 1H), 5.17 (t, *J* = 6.8 Hz, 1H), 5.11 (d, *J* = 7.0 Hz, 1H), 5.01 (d, *J* = 10.5 Hz, 1H), 4.84-4.75 (m, 2H), 4.46 (d, *J* = 4.5 Hz, 2H), 4.05-3.95 (m, 2H), 3.68 (dd, *J* = 5.8, 3.8 Hz, 2H), 3.59 (dd, *J* = 8.5, 7.0 Hz, 1H), 3.32 (dd, *J* = 5.3, 4.3 Hz, 2H), 3.10 (s, 3H), 2.87 (dd, *J* = 16.3, 4.0 Hz, 1H), 2.81-2.61 (m, 2H), 1.03 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ

171.6, 169.8, 166.4, 155.9, 155.5, 141.7, 135.4, 133.4, 129.5, 128.8, 128.4, 128.2, 128.0, 121.1, 117.4, 117.3, 115.9, 114.2, 94.4, 72.0, 70.0, 68.2, 65.8, 60.7, 58.7, 58.6, 58.4, 47.3, 34.0, 14.3 ppm.



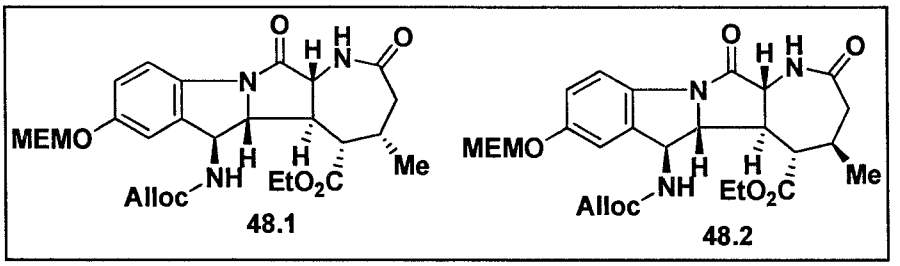
Compound 47.1: To a stirred solution of glycine crotyl acid (170 mg, 1.19 mmol) in dry DMF (10 mL) was added HATU (452 mg, 1.19 mmol) followed by DIPEA (207 µL, 1.19 mmol). To the reaction mixture was added the above secondary amine (103 mg, 0.25 mmol) and the mixture was stirred 24 hours under argon atmosphere. The solution was condensed and dissolved in Ethyl acetate, then washed with a saturated NH_4Cl solution, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:4 ethyl acetate/hexanes) to give the coupled product (124 mg, 92%) as a dark yellow mousse; MS (ES+) *m/z* 546.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 9.5 Hz, 1H), 7.08 (d, *J* = 2.5 Hz, 1H), 7.06 (br s, 1H), 6.94 (dd, *J* = 15.8, 5.8 Hz, 1H), 6.85 (dq, *J* = 15.1, 6.8 Hz, 1H), 6.46 (br s, 1H), 5.95 (d, *J* = 15.8 Hz, 1H), 5.89 (dq, *J* = 15.3, 1.8 Hz, 1H), 5.34-5.24 (m, 3H), 5,22 (s, 2H), 4.92 (d, *J* = 5.0 Hz, 1H), 4.84 (d, *J* = 6.0 Hz, 1H), 4.70-4.57 (m, 2H), 4.37 (dd, *J* = 18.1, 4.8 Hz, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.89 (br d, *J* = 18.1 Hz, 1 H), 3.83-3.79 (m, 2H), 3.58-3.53 (m, 2H), 3.37 (s, 3H), 1.87 (dd, *J* = 6.8, 1.3 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 166.0, 165.9, 165.2, 155.3, 155.0, 142.4, 140.8, 136.8, 132.2, 124.3, 123.2, 118.8, 118.6, 118.4, 113.7, 93.9, 71.5, 67.7, 66.2, 64.3, 60.4, 59.0, 57.8, 42.3, 17.7, 14.1 ppm.

Compounds 45.1 and 45.2.



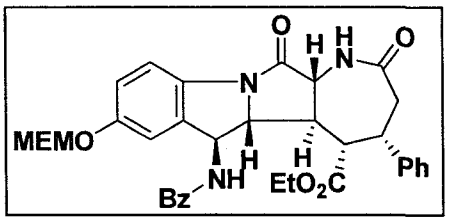
To a stirred solution of glycine cinnamoyl coupled indoline (81 mg, 0.13 mmol) in dry DCM (10 mL) at -78°C was added Et_3N (93 µL, 0.66 mmol) followed by TMSOTf (120 µL, 0.66 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 24 hours under argon atmosphere. The solution was washed with a saturated brine solution, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:4 ethyl acetate/hexanes) and by PREP HPLC to give 2 diastereoisomers of the cyclized product (**45.1**: 44mg, 55% and **45.2**: 10 mg, 12%) as a light yellow mousse; MS (ES+) *m/z* 608.4 (M+1); ^{1}H NMR (Compound **45.1**): (400 MHz, C_6D_6) δ 7.70 (broad d, *J* = 8.3 Hz, 1H), 7.22 (s, 1H), 7.18-6.99 (m, H_{Ph}), 6.88 (broad s, 1H), 6.01 (broad s, 1H), 5.85-5.70 (broad m, 1H), 5.16 (d, *J* = 17.3 Hz, 1H), 5.08-4.87 (broad m, 4H), 4.64-4.41 (broad m, 2H), 3.97 (broad s, 1H), 3.84-3.66 (broad m, 3H), 3.63-3.55 (m, 2H), 3.36-3.22 (broad m, 3H), 3.08 (s, 3H), 2.85-2.65 (broad m, 3H), 1.43-1.10 (broad m, 3H); ^{13}C NMR (Compound **45.1**): (100 MHz, C_6D_6) δ 172.4, 171.4, 166.1, 155.9, 137.3, 133.0, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9, 127.8, 117.4, 117.30, 117.25, 116.1, 113.6, 94.1, 71.9, 68.1, 65.6, 60.9, 58.6, 32.3, 30,2, 30.1, 29.8, 23.1, 14.4; ^{1}H NMR (Compound **45.2**): (400 MHz, $CDCl_3$ at 58°C) δ 7.50 (d, *J* = 8.5 Hz, 1H), 7.32-7.22 (m, H_{Ph}), 7.20-7.15 (m, 2H), 7.00 (dd, *J* = 8.5 Hz, *J* = 2.0 Hz, 1H), 6.93 (d, *J* = 2.0 Hz, 1H), 6.40 (broad s, 1H), 5.91-5.77 (m, 1H), 5.24 (d, *J* = 17.6 Hz, 1H), 5.21-5.12 (m, 4H), 4.97 (broad m, 1H), 4.58-4.45 (m, 2H), 4.42 (dd, *J* = 10.5 Hz, *J* = 1.3 Hz, 1H), 4.27-4.19 (m, 1H), 3.94-3.84 (m, 2H), 3.81-3.76 (m, 2H), 3.73-3.67 (m, 1H), 3.56-3.51 (m, 2H), 3.36 (s, 3H), 3.22 (t, *J* = 10.8 Hz, 1H), 3.18 (d, *J* = 8.5 Hz, 1H), 3.13 (dd, *J* = 11.0 Hz, *J* = 5.5 Hz, 1H), 2.96 (broad d, *J* = 16.3 Hz, 1H), 1.00 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (Compound **45.2**): (100 MHz, C_6D_6) δ 173.4, 172.8, 169.4, 166.5, 156.1, 155.4, 150.0, 136.3, 133.9, 132.7, 132.3, 128.9, 127.1, 118.3, 118.1, 116.5, 112.8, 93.8, 71.5, 70.6, 67.7, 66.4, 60.9, 59.0, 55.9, 48.2, 38.6, 29.9, 29.7, 14.1.

Compounds 48.1 and 48.2.

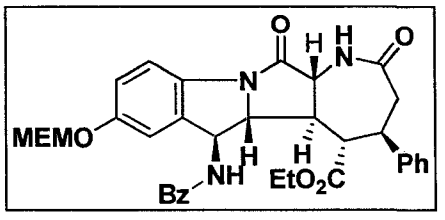


To a stirred solution of glycine crotyl coupled indoline (108 mg, 0.20 mmol) in dry DCM (10 mL) at -78°C was added Et_3N (165 µL, 1.20 mmol) followed by TMSOTf (213 µL, 1.20 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 4 hours under argon atmosphere. The solution was washed with a saturated brine solution, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:5 ethyl acetate/hexanes) and by preparative HPLC to give 2 diastereoisomers of the cyclized product (54 mg, 50% and 24 mg, 22%) as a light yellow mousse; MS (ES+) *m/z* 546.3 (M+1); ^{1}H NMR (Compound **48.1**): (400 MHz, $CDCl_3$) δ 7.42 (broad d, *J* = 8.3 Hz, 1H), 7.02-6.86 (broad m, 2H), 6.42 (very broad s, 1H), 5.95-5.78 (broad m, 1H), 5.41 (broad s, 1H), 5.35-5.01 (broad m, 4H), 5.27 (d, *J* = 16.8 Hz, 1H), 4.52 (broad s, 2H), 4.33-4.06 (broad m, 4H), 3.79 (broad s, 2H), 3.57-3.50 (m, 2H), 3.36 (s, 3H), 2.96-2.44 (broad m, 5H), 1.27 (t, *J* = 7.0 Hz, 3H), 1.26 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR (Compound **48.1**): (100 MHz, $CDCl_3$) δ 174.2, 173.6, 173.1, 166.3, 155.8, 136.2, 132.9, 132.4, 129.2, 126.7, 118.4, 117.3, 116.3, 113.1, 94.3, 71.9, 68.1, 66.2, 61.7, 59.4, 53.4, 50.7, 47.8, 43.0, 30.4, 14.6, 13.8; ^{1}H NMR (Compound **48.2**): (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.5 Hz, 1H), 7.05-7.00 (m, 2H), 6.18 (broad s, 1H), 5.96 (ddt, *J* = 17.1 Hz, *J* = 10.8 Hz, *J* = 5.8 Hz, 1H), 5.35 (dd, *J* = 17.1 Hz, *J* = 1.3 Hz, 1H), 5.27 (d, *J* = 10.8 Hz, 1H), 5.23 (s, 2H), 5.28-5.20 (m, 1H), 5.15 (dd, *J* = 11.0 Hz, *J* = 2.8 Hz, 1H), 4.68-4.63 (m 2H), 4.24-4,13 (m, 3H), 3.83-3.78 (m, 2H), 3.58-3.53 (m, 2H), 3.50 (s, 3H), 3.30 (dd, *J* = 7.0 Hz, *J* = 4.0 Hz, 1H), 3.01 (d, *J* = 14.6 Hz, 1H), 2.63 (td, *J* = 10.8 Hz, *J* = 4.3 Hz, 1H), 2.54 (t, *J* = 7.0 Hz, 1H), 2.42 (dd, *J* = 14.6 Hz, *J* = 7.0 Hz, 1H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.26-1.22 (m, 3H); ^{13}C NMR (Compound **48.2**): (100 MHz, $CDCl_3$) δ 173.7, 173.5, 167.2, 164.3, 155.9, 155.4, 133.7,

133.0, 132.3, 118.3, 118.2, 116.6, 112.8, 93.8, 71.5, 67.7, 66.3, 60.8, 59.1, 55.9, 50.9, 47.8, 46.0, 39.4, 29.7, 17.7, 14.2.



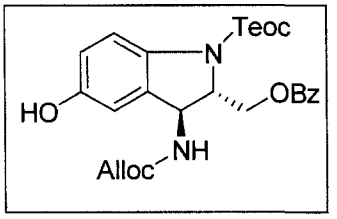
Compound 46.1. To a stirred solution of the cyclized glycine cinnamoyl functionalized indoline tetracycle (27 mg, 0.045 mmol) in dry DCM (2 mL) was added morpholine (8 µL, 0.090 mmol) followed by $Pd(0)(PPh_3)_4$ (5.2 mg, 0.0045 mmol). The reaction mixture was stirred for 30 minutes under argon atmosphere. To the reaction mixture was directly added Et_3N (64 µL, 0.45 mmol) followed by Benzoyl chloride (32 µL, 0.27 mmol). The rection mixture was stirred for an additional hour. The reaction mixture was washed with a saturated brine solution, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the benzoylated compound (18 mg, 67%) as a light yellow mousse; MS (ES+) m/z 628.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$ at 58°C) δ 7.73 (d, J = 7.3 Hz, 2H), 7.50 (d, J = 8.8 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.38 (t, J = 7.5 Hz, 2H), 7.29-7.20 (m, 3H), 7.14 (d, J = 6.8 Hz, 2H), 6.99 (d, J = 8.5 Hz, 1H), 6.94 (s, 1H), 6.62 (d, J = 8.0 Hz, 1H), 6.41 (s, 1H), 5.57 (t, J = 7.5 Hz, 1H), 5.16 (s, 2H), 4.45-4.37 (m, 2H), 3.75 (t, J = 4.5 Hz, 2H), 3.71-3.62 (m, 2H), 3.61-3.52 (m, 1H), 3.49 (t, J = 4.5 Hz, 2H), 3.30 (s, 3H), 3.27-3.16 (m, 2H), 3.12 (dd, J = 10.8, 5.8 Hz, 1 H), 2.91 (d, J = 16.3 Hz, 1H), 0.72 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171,6, 166.6, 165.9, 155.5, 141.9, 138.8, 135.7, 134.7, 133.4, 132.1, 128.75, 128.70, 128.1, 127.8, 126.9, 117.0, 116.1, 112.8, 93.8, 71.5, 69.3, 67.6, 61.3, 59.3, 59.0, 58.6, 58.2, 53.5, 49.1, 42.1, 40.0, 13.4 ppm.



Compound 46.2. To a stirred solution of the cyclized glycine cinnamoyl functionalized indoline tetracycle (14 mg, 0.023 mmol) in dry DCM (1 mL) was added morpholine (2 µL, 0.023 mmol) followed by $Pd(0)(PPh_3)_4$ (2.6 mg, 0.0023 mmol). The reaction mixture was stirred for 20 minutes under argon atmosphere. To the reaction mixture was

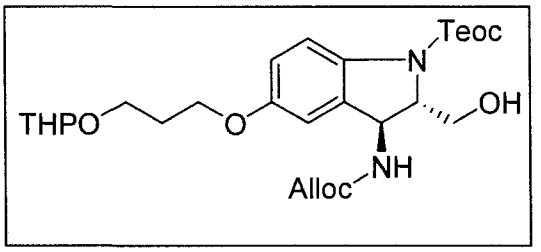
directly added Et_3N (20 µL, 0.139 mmol) followed by Benzoyl chloride (8 µL, 0.070 mmol). The rection mixture was stirred for an additional hour. The reaction mixture was washed with a saturated brine solution, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the benzoylated compound (12 mg, 67%) as a light yellow oil; MS (ES+) m/z 628.5 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 7.5 Hz, 2H), 7.59-7.43 (m, 7H), 7.36 (t, J = 7.5 Hz, 2H), 7.06 (d, J = 7.3 Hz, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 6.56 (broad d, J = 7.8 Hz, 1H), 6.34 (s, 1H), 5.65 (t, J = 7.5 Hz, 1H), 5.27-5.20 (m, 2H), 5.11 (d, J = 10.5 Hz, 1H), 4.29-4.18 (broad m, 1H), 4.08-3.92 (broad m, 2H), 3.88 (s, 1H), 3.82-3.77 (m, 2H), 3.73 (t, J = 7.8 Hz, 1H), 3.57-3.50 (m, 2H), 3.34 (s, 3H), 3.16 (dd, J = 16.6, 8.8 Hz, 1H), 3.00 (d, J = 16.6 Hz, 1H), 2.50 (dt, J = 10.5, 7.5 Hz, 1H), 1.29 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 167,9, 155.5, 150.0, 136.2, 133.9, 133.4, 132.3, 129.8, 128.9 (2C), 128.83, 128.79 (2C), 128.6, 128.5, 127.21 (2C), 127.18, 127,0 (2C), 126.6, 126.3, 118,0, 116.7, 113.0, 93.9, 71.5, 70.8, 67.7, 60.8, 59.0, 57.3, 55.8, 48.3, 34.3, 30.0, 29.7, 13.9 ppm.

5.4 Syntheses for Manual Solid Phase

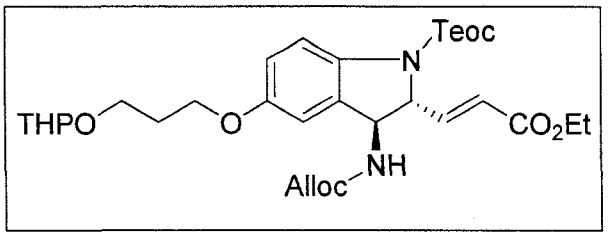


Compound 52.1: To a solution of the MEM protected compound (6.18 g, 10.30 mmol) in anhydrous Ethanol (250 mL) was added *p*-TSA (2.20 g, 11.33 mmol). The solution was stirred at 50°C for 24 hours. The solution was then diluted with ethyl acetate and washed with a saturated sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (2:3 ethyl acetate/hexanes) to give the benzilic alcohol (5.07 g, 96%) as a very viscous light yellow oil; MS (ES+) *m/z* 513.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.5 Hz, 2H), 7.47 (t, *J* = 7.1 Hz, 1H), 7.31 (dd, *J* = 8.6, 7.5 Hz, 2H), 6.86 (s, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 5.90 (m, 1H), 5.50 (d, *J* = 6.7 Hz, 1H), 5.31 (d, *J* = 17.1 Hz, 1H),

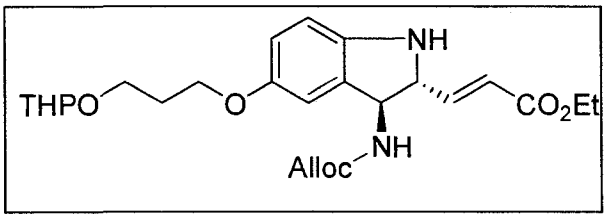
5.22 (d, J = 10.4 Hz, 1H), 5.06 (d, J = 7.2 Hz, 1H), 4.64-4.55 (m, 4H), 4.49 (d, J = 8.4 Hz, 1H), 4.26 (bs, 2H), 1.07 (bs, 2H), 0.04 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 156.0, 153.5, 153.1, 133.4, 132.8, 130.0, 129.9, 128.7, 118.5, 117.0, 112.8, 66.4, 66.3, 65.1, 64.9, 55.0, 18.2, -1.1 ppm.



Compound 53.2: To a solution of the above benzilic alcohol (5.07 g, 9.40 mmol) in dry DMF (120 mL), was added 3-(tetrahydro-*2H*pyran- 2-yloxy)- propyl-4-methyl-benzenesulfonate[30] (3.73 g, 11.88 mmol), and cesium carbonate (8.07 g, 24.74 mmol). The reaction mixture was stirred at 40°C for 12 hours under argon atmosphere. The organic solvent was removed and the reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes). The intermediate was directly used in the next step. To a solution of this intermediate in methanol (250 mL) was added potassium carbonate (1.27 g, 9.17 mmol). The reaction mixture was stirred at room temperature for 2 hours under argon atmosphere. The solution was diluted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (2:3 ethyl acetate/hexanes) to give the primary alcohol (4.30 g, 85%) as a very viscous light yellow oil; MS (ES+) *m/z* 551.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (bs, 1H), 6.91-6.63 (m, 2H), 5.92 (m, 1H), 5.35-5.13 (m, 3H), 4.98 (bs, 1H), 4.60 (m, 3H), 4.31 (m, 3H), 4.10-3.47 (m, 8H), 2.74 (bs, 1H), 2.04 (m, 2H), 1.86-1.48 (m, 6H), 0.94 (t, J = 7.4 Hz, 2H), 0.07 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 156.0, 155.7, 132.8, 118.6, 116.9, 116.8, 116.7, 111.8, 99.3, 66.8, 66.5, 66.0, 64.4, 64.3, 62.7, 60.5, 55.2, 31.1, 30.1, 25.8, 20.0, 18.3, -1.1 ppm.

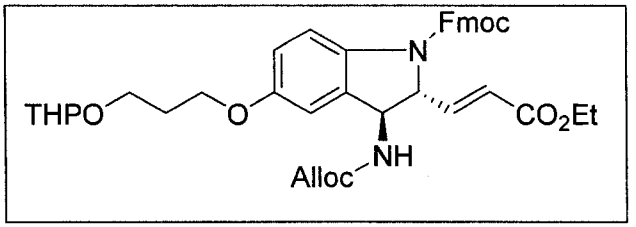


Compound 54.1: The Dess-Martin periodinane (6.83 g, 15.63 mmol) was added to a solution of the above primary alcohol (4.30 g, 7.81 mmol) in dry DCM (200 mL). The resulting suspension was stirred at room temperature under argon atmosphere and TLC showed complete conversion to the aldehyde after 5 hour. Then (carbethoxymethylene)triphosphorane (7.16 g, 19.53 mmol) was added to the reaction mixture, then stirred for an additional 2 hours. The reaction mixture was washed with a saturated sodium bicarbonate solution and brine sequentially. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:3 ethyl acetate/hexanes) to give the final product (3.86 g, 80%) as a very viscous light yellow oil; MS (ES+) *m/z* 619.5 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (bs, 1H), 6.97-6.88 (m, 3H), 5.97-5.89 (m, 2H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.19 (d, *J* = 10.3 Hz, 1H), 5.12 (d, *J* = 6.7 Hz, 1H), 4.90 (bs, 1H), 4.84 (d, *J* = 7.1 Hz, 1H), 4.61 (m, 3H), 4.29 (bs, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.05 (t, *J* = 6.2 Hz, 2H), 3.95 –3.83 (m, 2H), 3.60-3.49 (m, 2H), 2.06 (t, *J* = 6.2 Hz, 2H), 1.83-1.47 (m, 6H), 1.27 (t, *J* = 7.2 Hz, 3H), 0.06 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 156.1, 155.6, 144.3, 132.8, 122.5, 118.5, 117.2, 117.1, 117.0, 112.2, 99.4, 68.2, 66.4, 66.0, 64.7, 64.3, 62.7, 60.9, 57.4, 31.1, 30.1, 25.8, 20.0, 18.2, 14.6, -1.1 ppm.

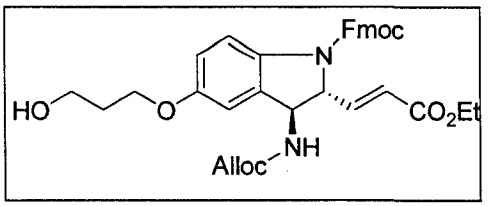


Compound 55.1: A 1M TBAF solution (12.48 mL, 12.48 mmol) was added to a solution of the above Teoc protected indoline (3.86 g, 6.24 mmol) in dry THF (150 mL). The reaction mixture was stirred at room temperature for 1 hour under argon atmosphere. The organic solution was washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the

secondary amine (2.95 g, >99%)as a very viscous light yellow oil; MS (ES+) *m/z* 475.4 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (dd, *J* = 15.6, 6.7 Hz, 1H), 6.77 (s, 1H), 6.70 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.56 (d, *J* = 8.4 Hz, 1H), 6.04 (d, *J* = 15.6 Hz, 1H), 5.92 (m, 1H), 5.48 (d, *J* = 8.4 Hz, 1H), 5.32 (d, *J* = 17.2 Hz, 1H), 5.21 (d, *J* = 10.4 Hz, 1H), 4.97 (t, *J* = 7.0 Hz, 1H), 4.58 (m, 3H), 4.16 (q, *J* = 7.1 Hz, 3H), 3.97 (t, *J* = 6.3 Hz, 2H), 3.92-3.78 (m, 3H), 3.57-3.45 (m, 2H), 2.01 (t, *J* = 6.3 Hz, 2H), 1.85-1.43 (m, 6H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 156.0, 146.0, 133.0, 129.1, 123.2, 118.4, 117.0, 112.8, 112.6, 99.4, 68.4, 66.9, 66.3, 64.5, 62.8, 61.0, 59.6, 31.1, 30.1, 25.8, 20.0, 14.6 ppm.

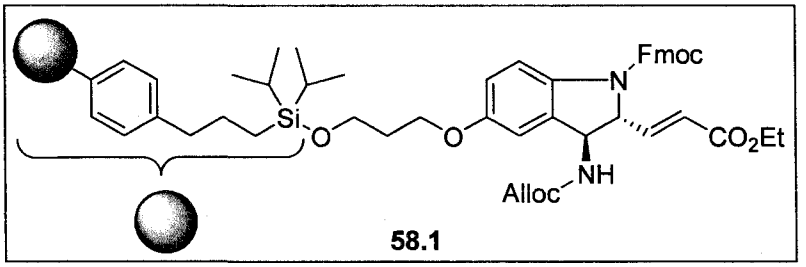


Compound 56.1: To a solution of the above secondary amine (2.95 g, 6.22 mmol) in ethyl acetate (150 mL) was added a 5% sodium bicarbonate solution (20 mL) followed by Fmoc-chloride (3.32 g, 14.44 mmol). The reaction mixture was stirred at room temperature for 3 hours under argon atmosphere. The organic phase was washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the Fmoc protected amine (3.77 g, 87%) as a very viscous light yellow oil; MS (ES+) *m/z* 697.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 3H), 7.57 (d, *J* = 6.1 Hz, 2H), 7.41 (t, *J* = 7.3 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 6.95-6.50 (m, 3H), 5.96 (m, 1H), 5.79 (m, 1H), 5.43-5.22 (m, 2H), 5.03 (m, 1H), 4.92-4.50 (m, 7H), 4.28 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.03 (m, 2H), 3.94- 3.82(m, 2H), 3.61-3.48 (m, 2H), 2.06 (t, *J* = 6.3 Hz, 2H), 1.90-1.48 (m, 6H), 1.29 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 155.6, 144.0, 141.9, 132.8, 128.2, 127.6, 125.3, 125.1, 122.6, 120.5, 118.6, 117.2, 117.0, 112.1, 99.4, 68.3, 67.8, 66.4, 66.0, 64.3, 62.8, 60.9, 57.6, 47.6, 31.1, 30.1, 25.8, 20.0, 14.6 ppm.



Compound 57.1: PPTS (0.74 g, 2.87 mmol) was added to a solution of the above THP protected spacer/indoline (2.00 g, 2.87 mmol) in anhydrous ethanol (160 mL). The reaction mixture was stirred at 55°C for 8 hours under argon atmosphere. The reaction mixture was then diluted with ethyl acetate, washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 ethyl acetate/hexanes) to give the final product (1.01 g, 98%)as a very viscous light yellow oil; MS (ES+) *m/z* 613.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 3H), 7.57 (d, *J* = 6.2 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 2H), 6.97-6.50 (m, 3H), 5.96 (m, 1H), 5.78 (m, 1H), 5.44- 5.18 (m, 2H), 4.95-4.50 (m, 6H), 4.28 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.07 (m, 2H), 3.86 (t, *J* = 6.0 Hz, 2H), 2.03 (m, 2H), 1.78 (s, 1H), 1.29 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 156.0, 155.6, 152.9, 144.1, 141.9, 132.8, 128.2, 127.6, 125.3, 125.1, 122.6, 120.5, 120.4, 118.6, 117.2, 117.0, 112.1, 68.3, 67.8, 66.6, 66.4, 61.0, 60.7, 57.5, 47.6, 32.4, 14.6 ppm.

The Broad Institute Loading Procedure on the Indoline core

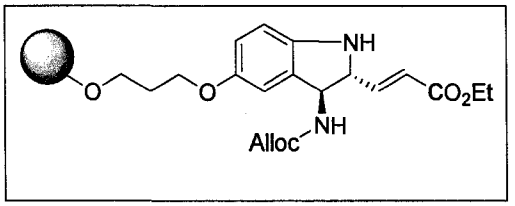


58.1

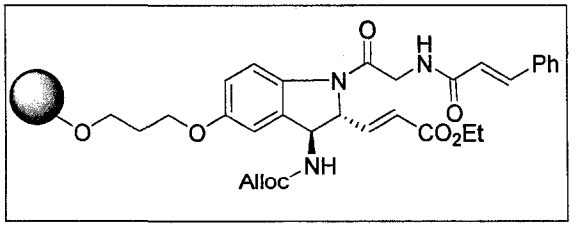
Loading: 3-[Diisopropyl-(*p*-methoxyphenyl) silyl] propyl functionalized resin (420.4 mg, 0.4162 mmol) was swollen in dry DCM (2.5 mL) under argon for 30 minutes in a BIORAD tude. The solvent was then drained to add, by syringe, a solution of trifluoromethanesulfonate acid in dry DCM (4%, 5.61 mL). The resin was then gently agitated for 30 minutes under argon. The acidic solution was drained and the activated resin was treated with 2,6-lutidine (364 µL, 3.36 mmol)

for 15 minutes followed by the addition of a solution of the compound to be loaded (515 mg, 0.841 mmol) in dry DCM (1 mL). The resin was gently shaken overnight. The resin was washed with DMF (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 643.75 mg of the loaded resin (95%).

Cleavage: The loaded resin (3 beads) in an Eppendorf tube was swelled in THF (0.5 mL) for 10 min, and treated with HF-pyridine solution (15 µL). The reaction tube was shaken for 45 minutes then quenched with methoxytrimethylsilane (100 µL) and shaken for another 10 minutes. The solution was concentrated and submitted to MS and HPLC/MS analysis; MS & HPLC/MS (ES+) *m/z* 613.4 (M+1); HPLC/MS purity >96%.

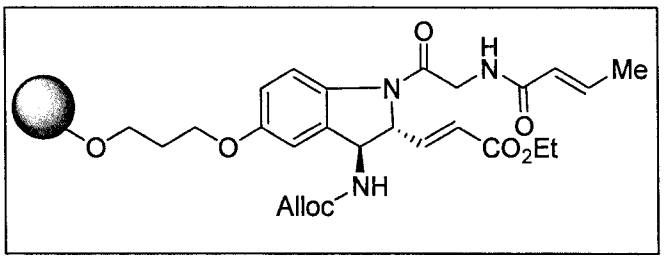


Compound 61.1: The Fmoc protected resin (40.0 mg, 0.03960 mmol) was swelled in DMF (2.5 mL) for 30 minutes. Morpholine (1.0 mL) was added the reaction mixture and shaken for 30 minutes. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 32.7 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 391.2 (M+1); HPLC/MS purity >95%.

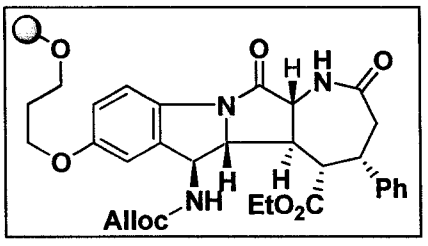


Compound 62.1: To a stirred solution of glycine cinnamic acid (26.0 mg, 0.1208 mmol) in dry DMF (2.5 mL) was added HATU (46.0 mg, 0.1208 mmol) followed by DIPEA (21.0 µL, 0.1208 mmol). The reaction mixture was added to the above secondary amine resin (24.4 mg, 0.02416 mmol) which was swelled in DMF (2.5 mL) for 30 minutes. The reaction mixture was shaken for 48 hours. The reaction mixture was then drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a

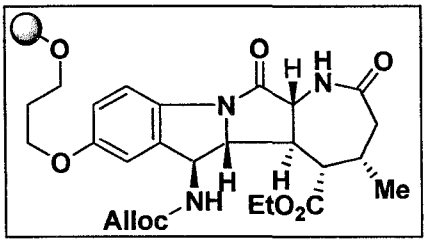
period of 90 minutes. The resin was then dried under vacuum overnight to give 26.4 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 578.3 (M+1); HPLC/MS purity >90%.



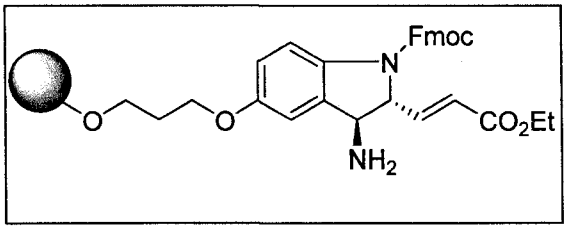
Compound 63.1: To a stirred solution of glycine crotyl acid (30.0 mg, 0.2093 mmol) in dry DMF (2.5 mL) was added HATU (79.6 mg, 0.2093 mmol) followed by DIPEA (36.5 µL, 0.2093 mmol). The reaction mixture was added to the above secondary amine resin (35.2 mg, 0.04288 mmol) which was swelled in DMF (2.5 mL) for 30 minutes. The reaction mixture was shaken for 48 hours. The reaction mixture was then drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 34.5 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 516.2 (M+1); HPLC/MS purity >94%.



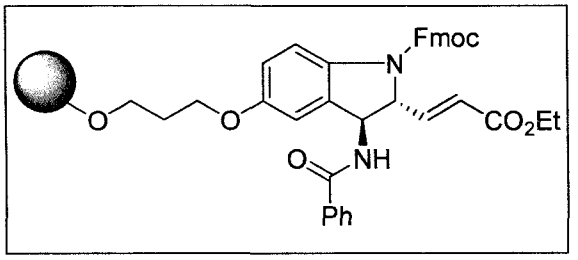
Compound 64.1: The glycine cinnamoyl coupled indoline resin (94.0 mg, 0.099 mmol) was swelled in DCM (2.5 mL) for 30 minutes. Triethylamine (55.5 µL, 0.3960 mmol) and TMSOTf (73.9 µL, 0.3960 mmol) were added to the swelled resin and then shaken for 20 hours. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 93.8 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 578.3 (M+1); HPLC/MS purity >80%.



Compound 64.3: The glycine crotyl coupled indoline resin (100.8 mg, 0.100 mmol) was swelled in DCM (2.5 mL) for 30 minutes. Triethylamine (56.0 µL, 0.400 mmol) and TMSOTf (74.6 µL, 0.400 mmol) were added to the swelled resin and then shaken for 20 hours. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 100.0 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 516.2 (M+1); HPLC/MS purity >95%.

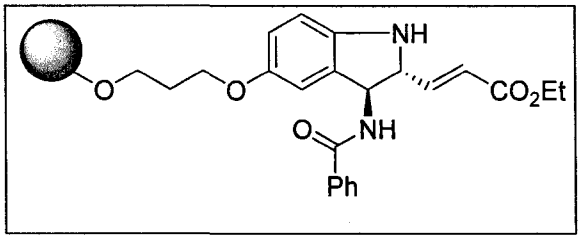


Compound 59.1: The Fmoc protected resin (27.0 mg, 0.02673 mmol) was swelled in DCM (2.5 mL) for 30 minutes. The mixture was drained and of a solution of DCM (5 mL), N-methylmorpholine (0.32 mL) and acetic acid (0.66 mL), 2.5 mL was added the swelled resin. To this mixture was added the ligand PPh_3 (90.3 mg, 0.3408 mmol) and the catalyst tetrakis $Pd_{(0)}(PPh_3)_4$ (83.0 mg, 0.07110 mmol). The reaction mixture was shaken for 18 hours. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 24.5 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 529.2 (M+1).

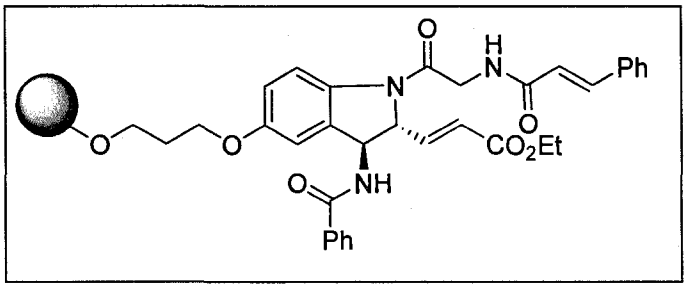


Compound 60.1: The above primary amine resin (24.5 mg, 0.02673 mmol) was swelled in DCM (2.5 mL) for 30 minutes. Collidine (0.032 µL, 0.2426 mmol) and Benzoyl chloride (0.014

μL, 0.1213 mmol) were added the reaction mixture and shaken for 8 hours. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 24.5 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 633.3 (M+1); HPLC/MS purity >73%.

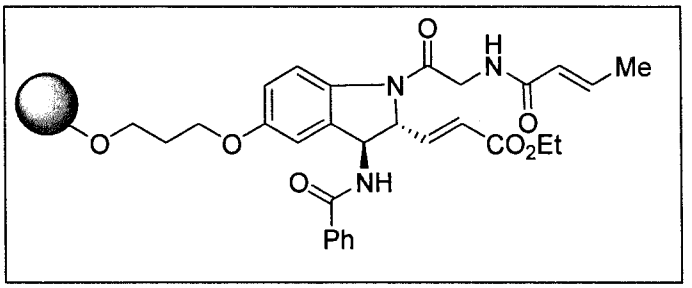


Compound 61.2: The Fmoc protected resin (24.5 mg, 0.02673 mmol) was swelled in DMF (2.5 mL) for 30 minutes. Morpholine (1.0 mL) was added the reaction mixture and shaken for 30 minutes. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 18.0 mg of the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 411.2 (M+1).

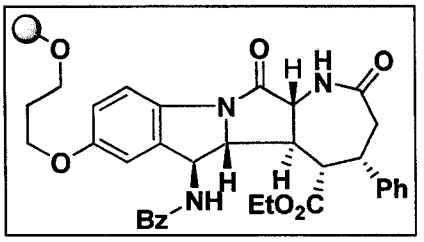


Compound 62.2: To a stirred solution of glycine cinnamic acid (19.0 mg, 0.08663 mmol) in dry DMF (2.5 mL) was added HATU (33.0 mg, 0.08663 mmol) followed by DIPEA (15.0 μL, 0.08663 mmol). The reaction mixture was added to the above secondary amine resin (18.0 mg, 0.02673 mmol) which was swelled in DMF (2.5 mL) for 30 minutes. The reaction mixture was shaken for 48 hours. The reaction mixture was then drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give 18.6 mg of the resulting resin. A sample of the dried resin (3 beads) was

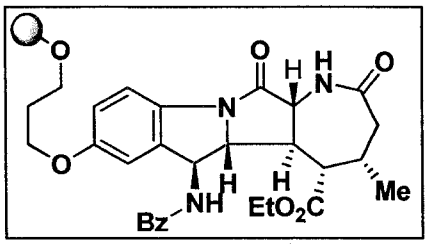
cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 598.3 (M+1).



Compound 63.2: To a stirred solution of glycine crotyl acid (79.0 mg, 0.5445 mmol) in dry DMF (2.5 mL) was added HATU (207.0 mg, 0.5445 mmol) followed by DIPEA (95.0 µL, 0.5445 mmol). The reaction mixture was added to the above secondary amine resin (0.1089 mmol) which was swelled in DMF (2.5 mL) for 30 minutes. The reaction mixture was shaken for 48 hours. The reaction mixture was then drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give the resulting resin. A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 536.2 (M+1).



Compound 64.2: The glycine cinnamoyl coupled indoline resin (0.1089 mmol) was swelled in DCM (2.5 mL) for 30 minutes. Triethylamine (77.0 µL, 0.5445 mmol) and TMSOTf (102 µL, 0.5445 mmol) were added to the swelled resin and then shaken for 20 hours. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give the resulting resin (102.1 mg). A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 598.3 (M+1).



Compound 64.4: The glycine crotyl coupled indoline resin (0.1089 mmol) was swelled in DCM (2.5 mL) for 30 minutes. Triethylamine (77.0 µL, 0.5445 mmol) and TMSOTf (102 µL, 0.5445 mmol) were added to the swelled resin and then shaken for 20 hours. The reaction mixture was drained and washed with DCM (3 x), THF (3 x), and DCM (3 x) over a period of 90 minutes. The resin was then dried under vacuum overnight to give the resulting resin (103.5 mg). A sample of the dried resin (3 beads) was cleaved and the resulting compound was analyzed by MS & HPLC/MS; MS (ES+) *m/z* 536.2 (M+1).

Claims to Original Research

1. The synthesis of tricyclic and tetracyclic indoline-based derivatives using Lewis-acid mediated tandem Michael and tandem aza-Michael methodology.

2. The development of a synthetic tandem Michael and tandem aza-Michael approach to obtain polycyclic derivatives on a simple and enantio-enriched indoline scaffold as well as the development and its application on solid support.

3. Publication:

   Brochu, J; Prakesch, M; Enright, G; Leek, D; Arya, P. *J. Comb. Chem.* **2008**, *10*, 405-420.

4. Poster Presentation:

   (a) "A modular tandem aza Michael or aza Diels-Alder approach to obtain indoline alkaloid-like polycyclic derivatives" Brochu, J; Prakesch, M; Leek, D; Arya, P. at the 16th Quebec/Ontario Minisynposium in Synthetic and BioOrganic chemistry, Nov 11-13, 2005, McGill University, Saint Adèle, QC, Canada.

   (b) "A modular tandem aza Michael or aza Diels-Alder approach to obtain indoline alkaloid-like polycyclic derivatives" Brochu, J; Prakesch, M; Poondra, R; Leek, D; Arya, P. at the 17th Quebec/Ontario Minisynposium in Synthetic and BioOrganic chemistry, Nov 3-5, 2006, University of Western Ontario, London, ON, Canada.

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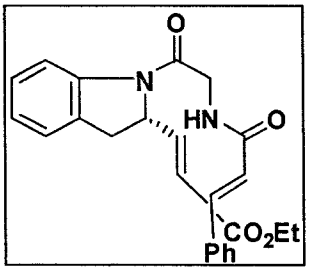
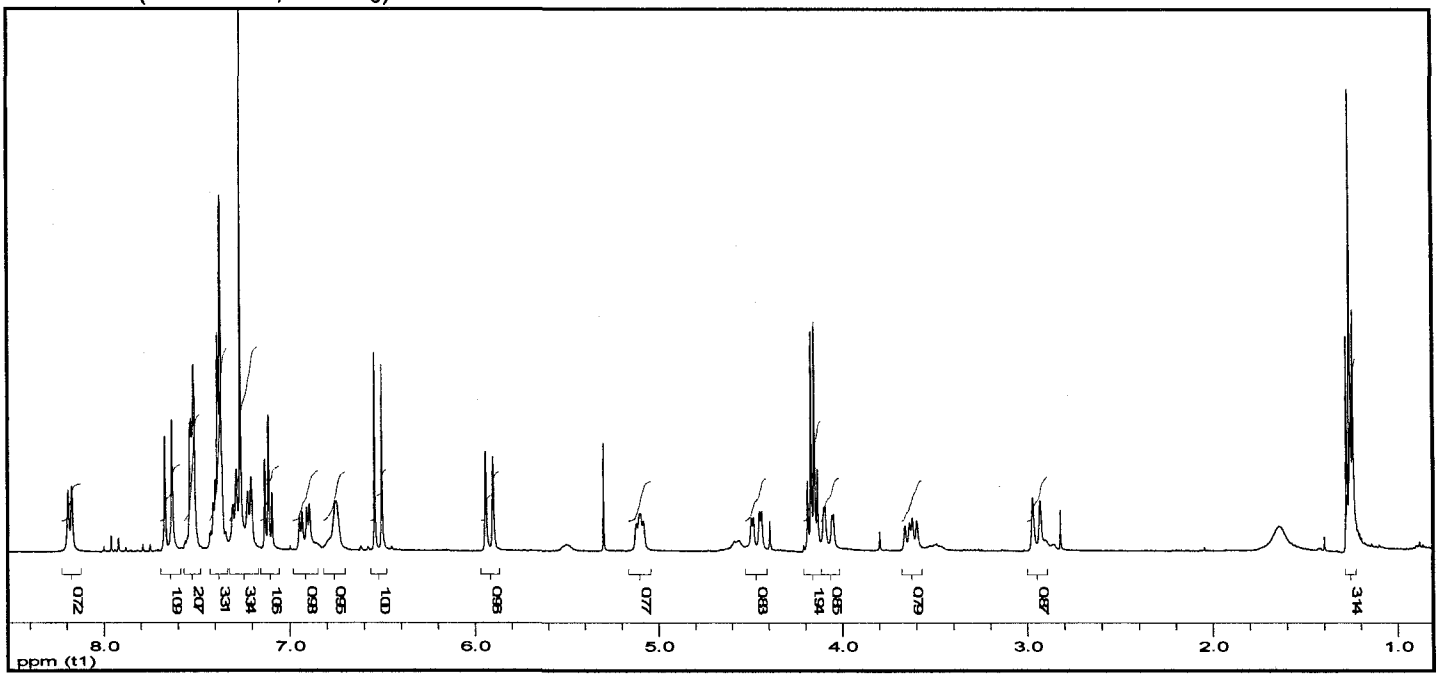
Appendix

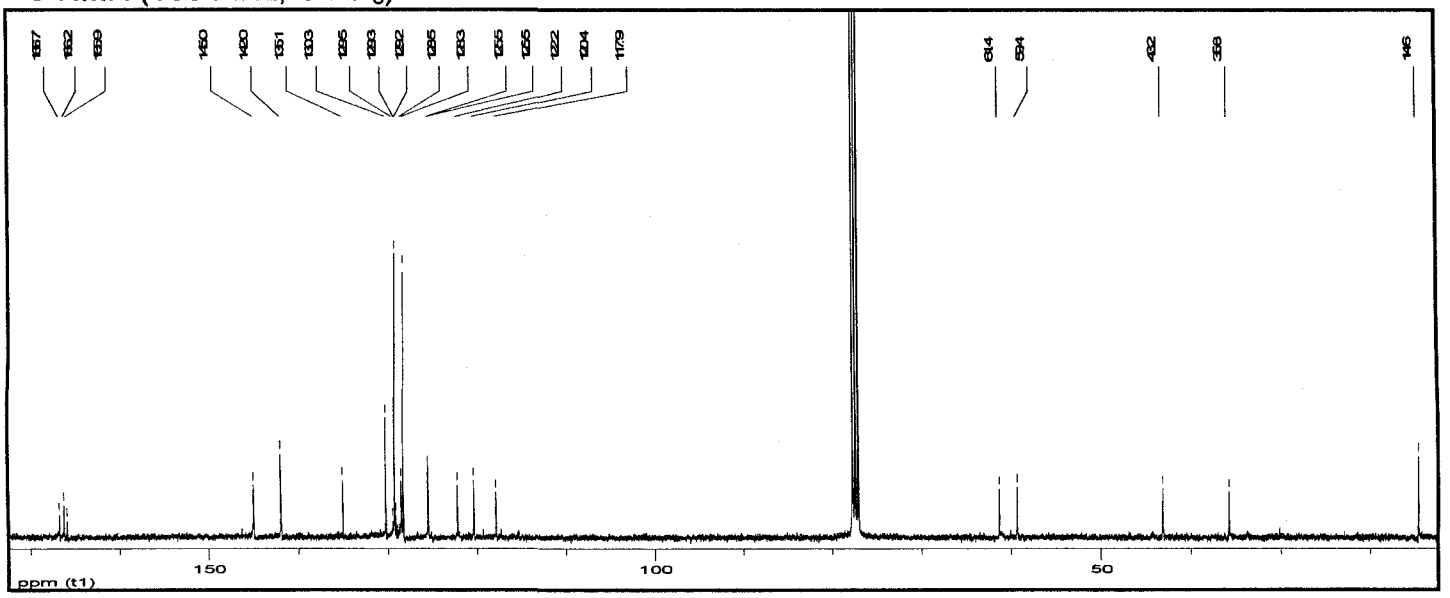
Compound 19.1:



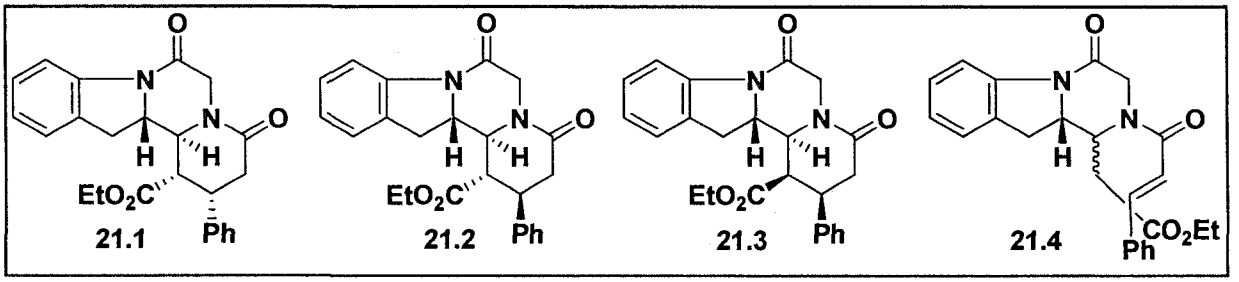
^{1}H NMR: (400 MHz, $CDCl_3$)



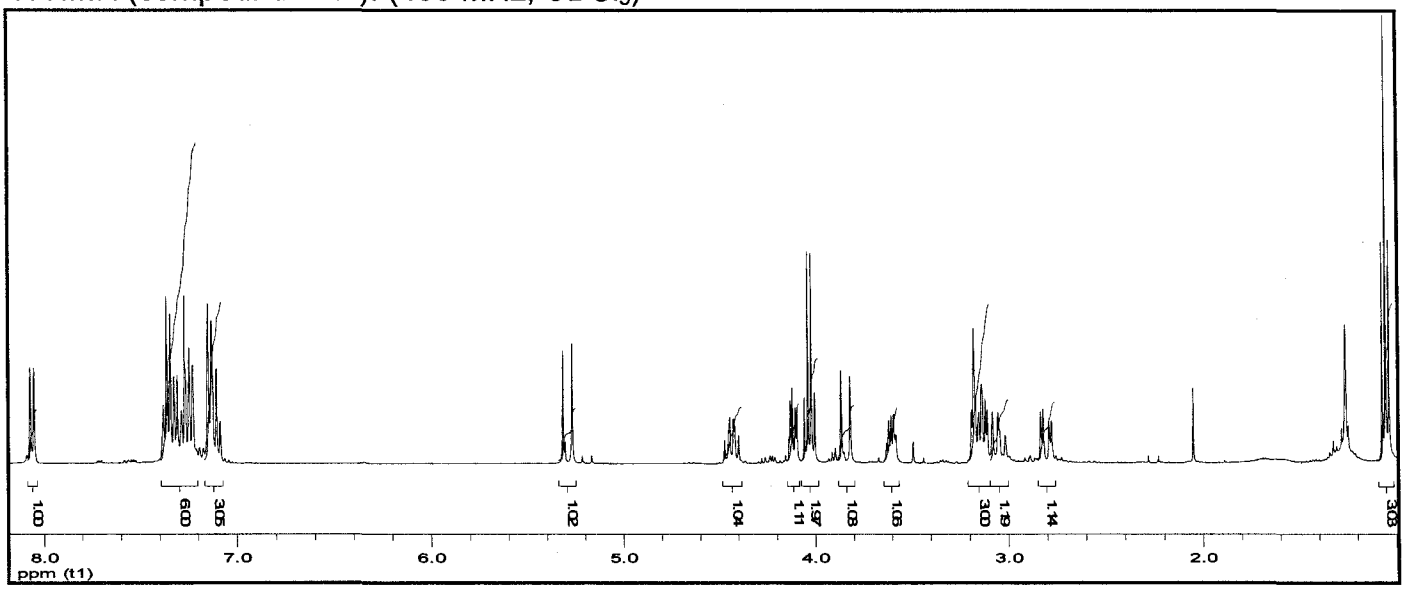
^{13}C NMR (100 MHz, $CDCl_3$)



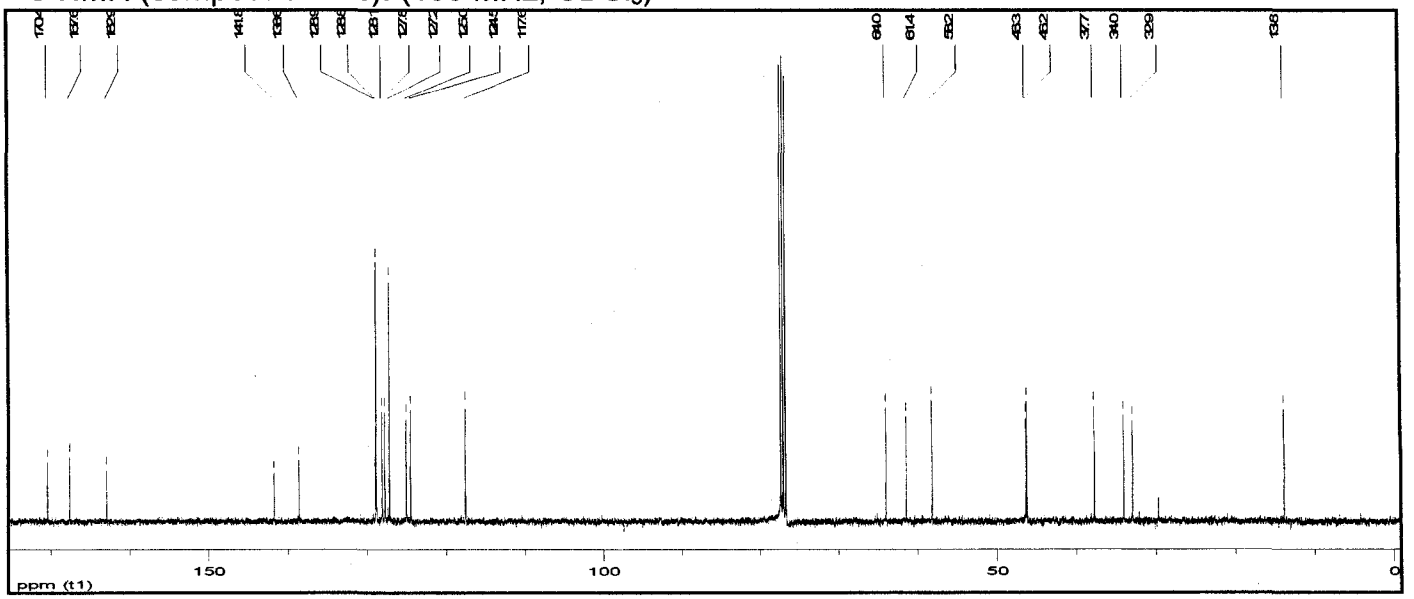
Compounds 21.1, 21.2, 21.3 and 21.4:

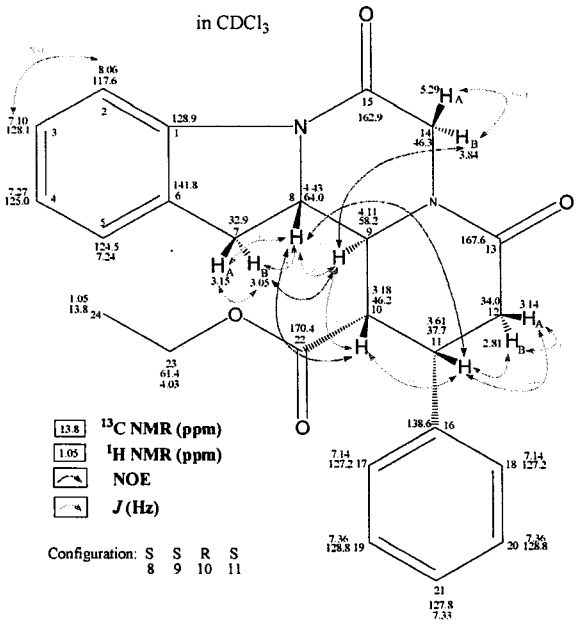


^{1}H NMR (compound **21.1**): (400 MHz, $CDCl_3$)

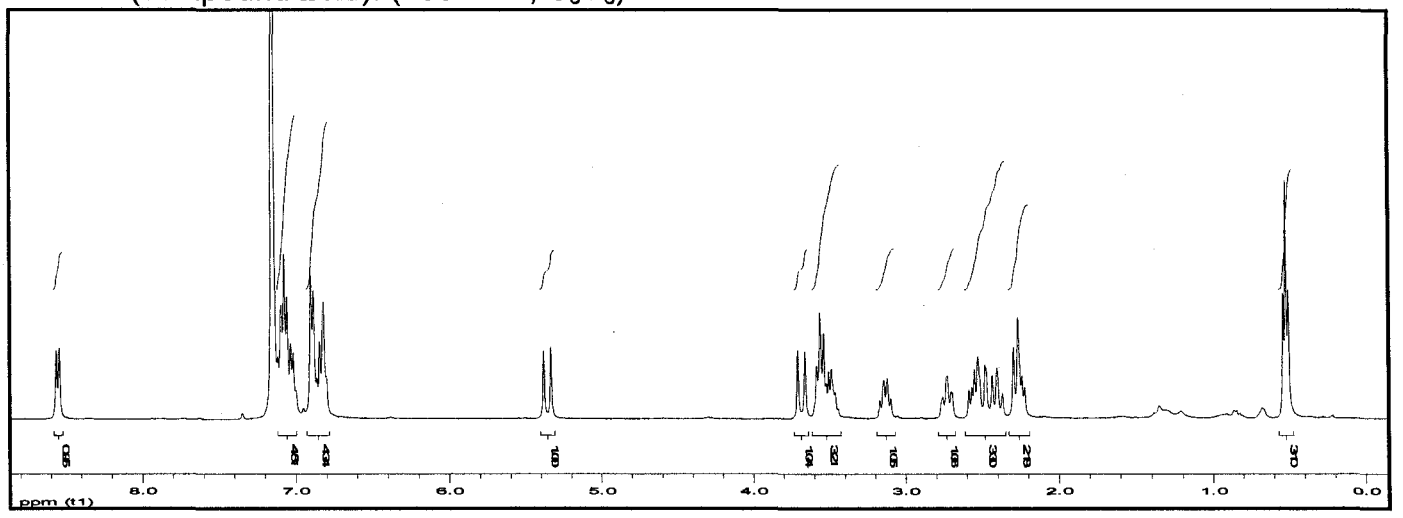


^{13}C NMR (compound **21.1**): (100 MHz, $CDCl_3$)

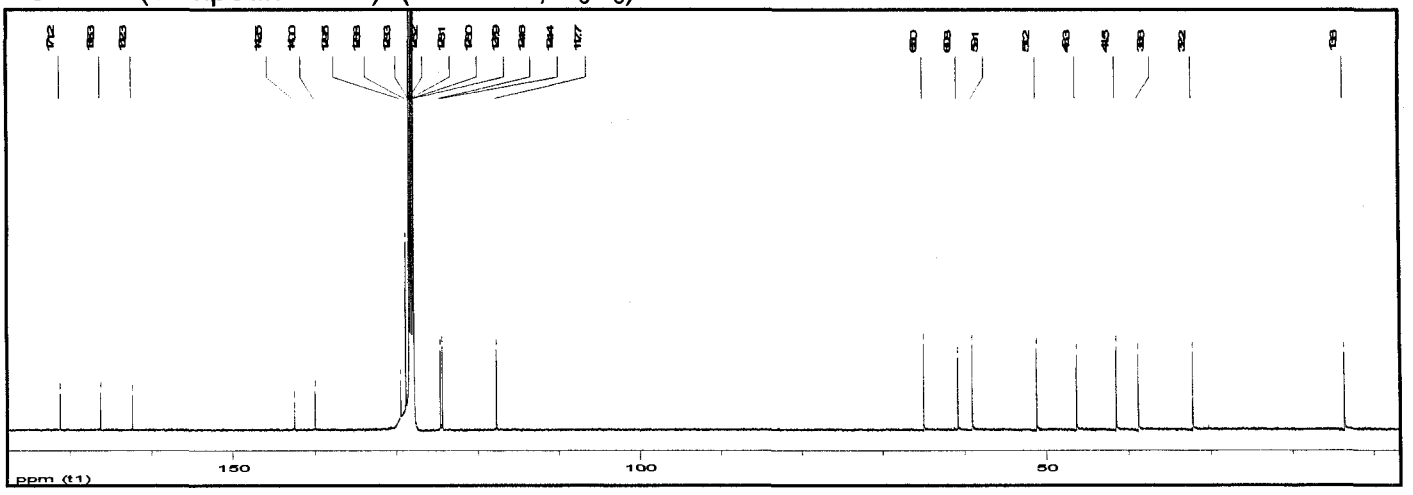


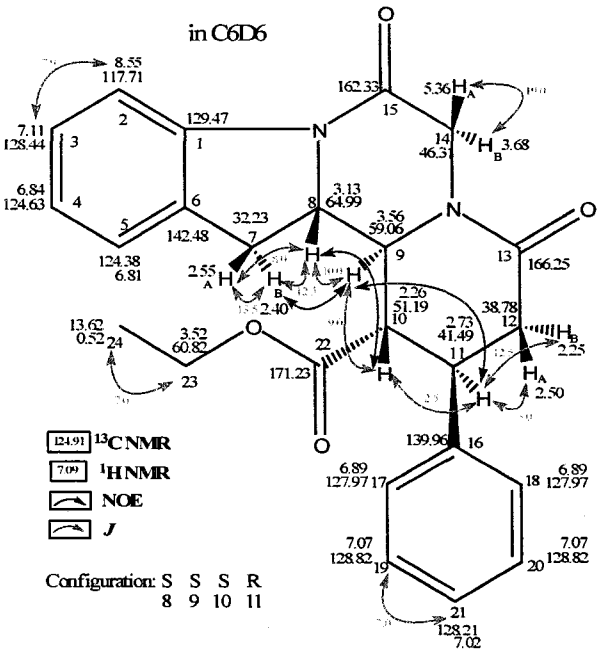


^{1}H NMR (compound **21.2**): (400 MHz, C_6D_6)

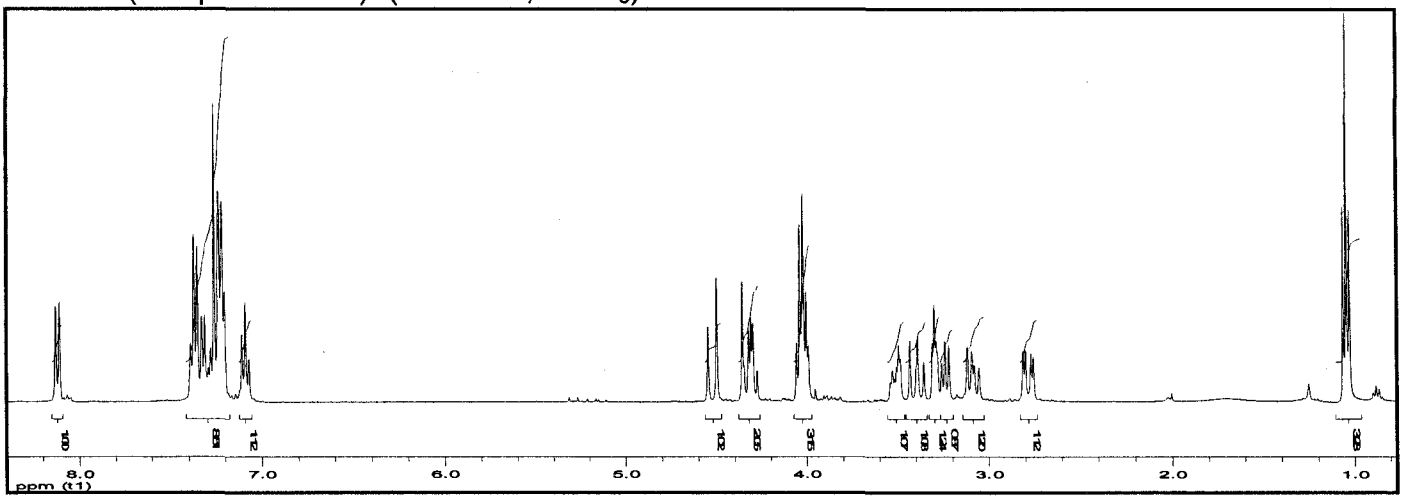


^{13}C NMR (compound **21.2**): (100 MHz, C_6D_6)

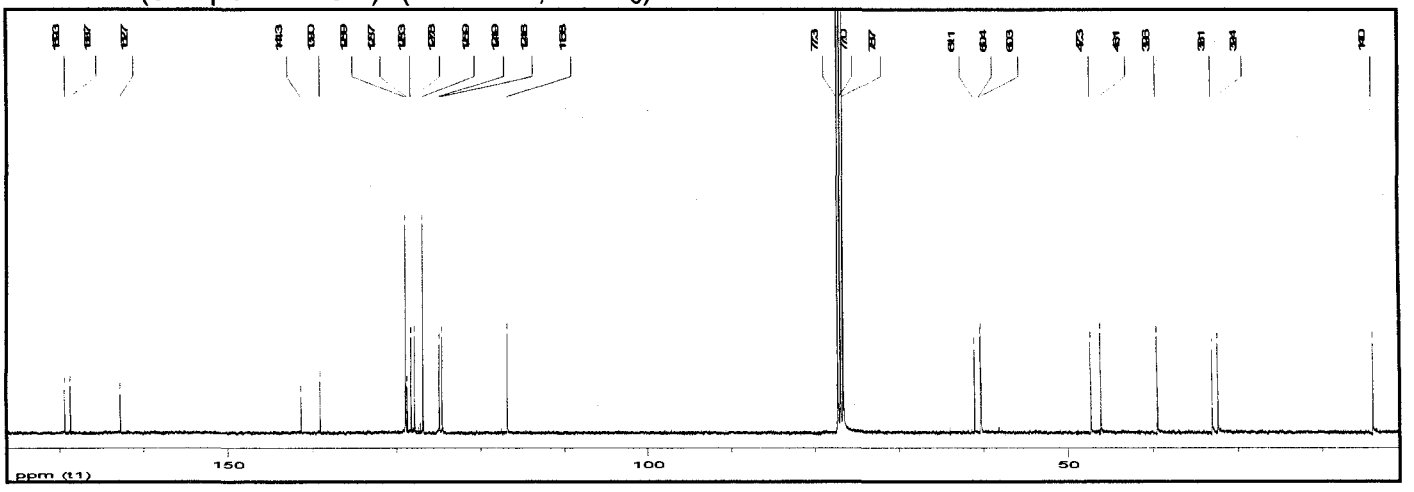


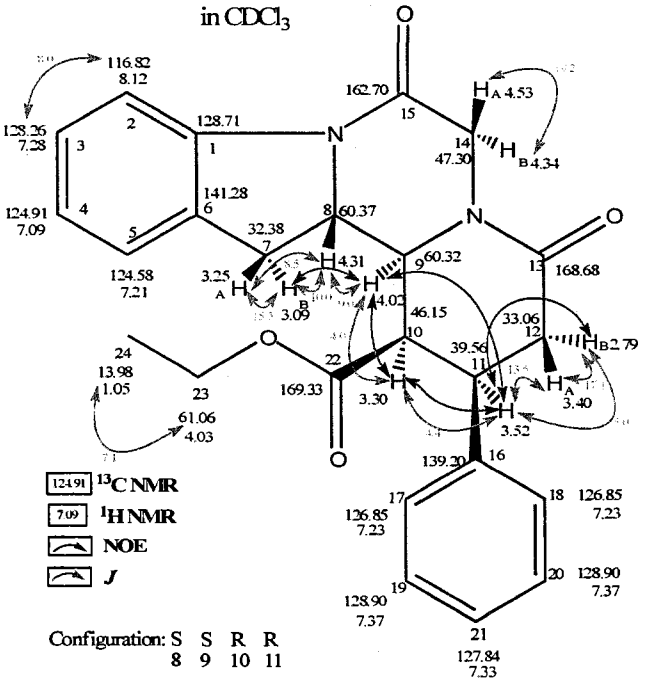


^{1}H NMR (compound **21.3**): (400 MHz, $CDCl_3$)

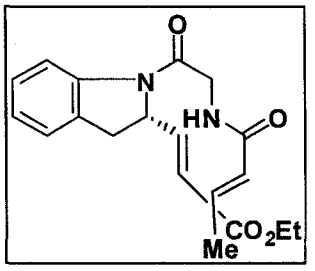


^{13}C NMR (compound **21.3**): (100 MHz, $CDCl_3$)

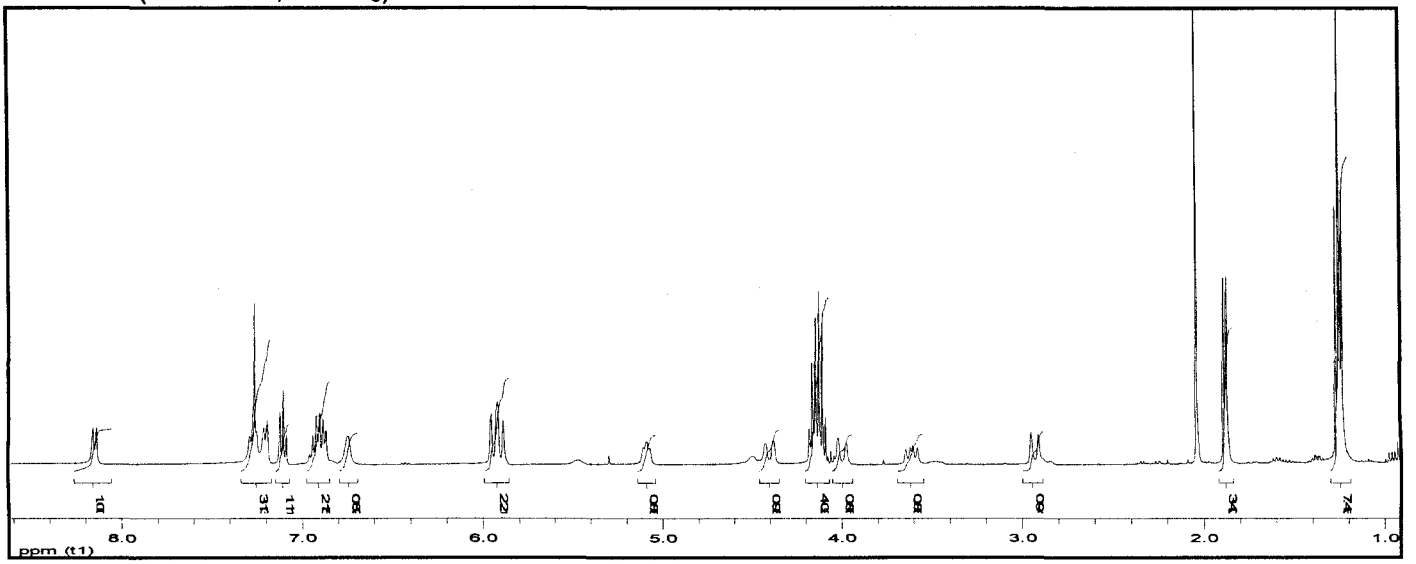




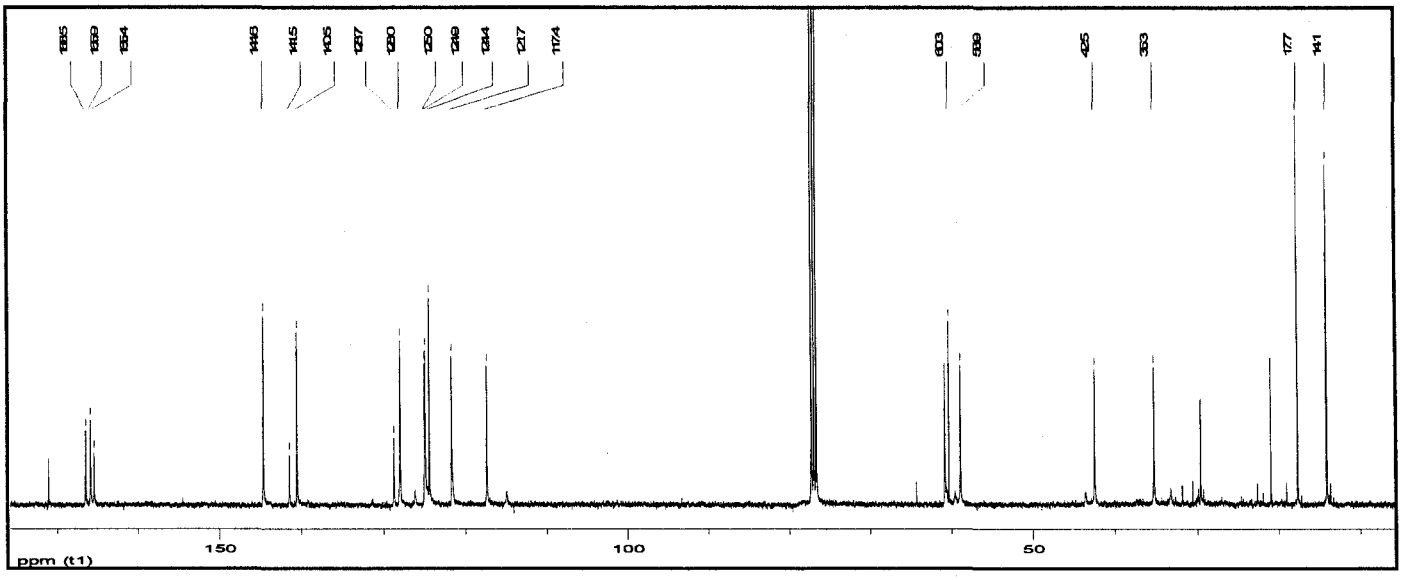
Compound 23.1:



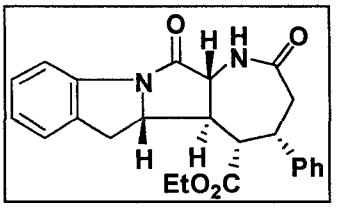
^{1}H NMR: (400 MHz, $CDCl_3$)



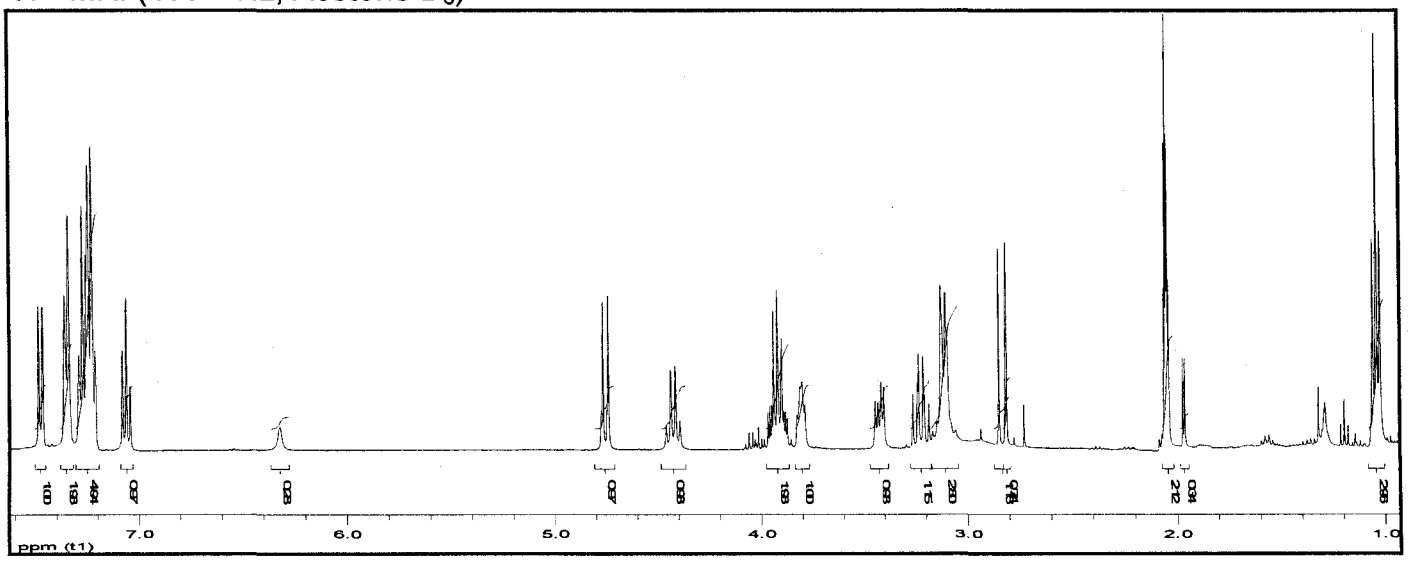
^{13}C NMR (100 MHz, $CDCl_3$)



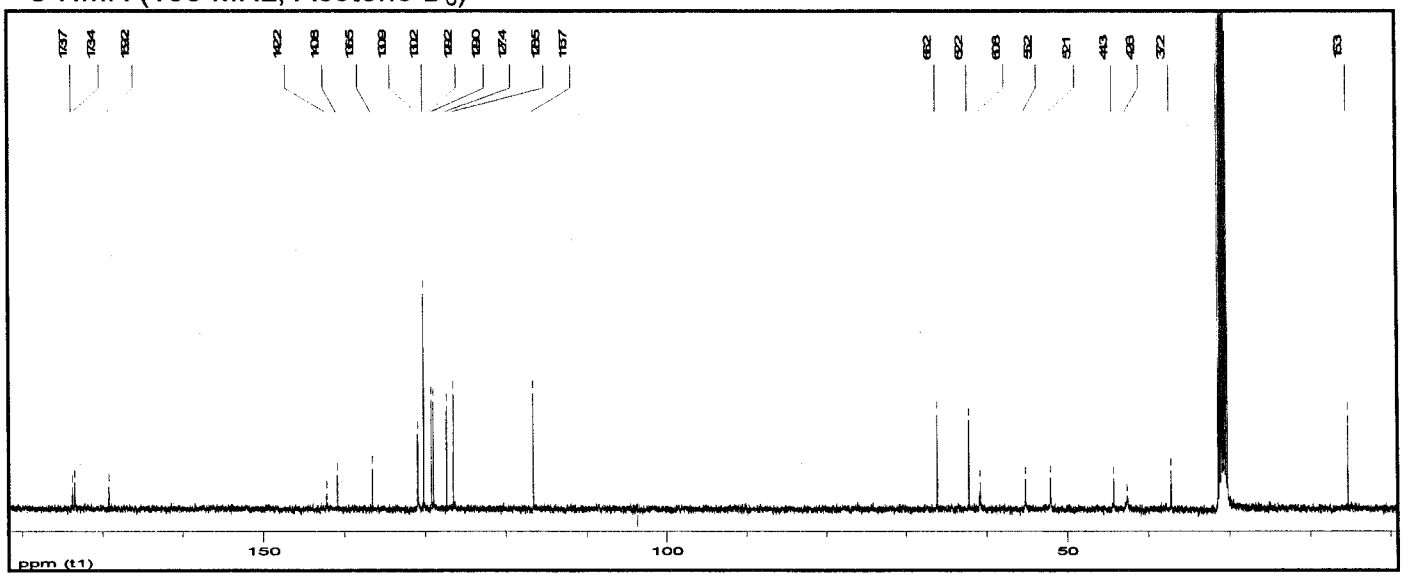
Compound 22.1:



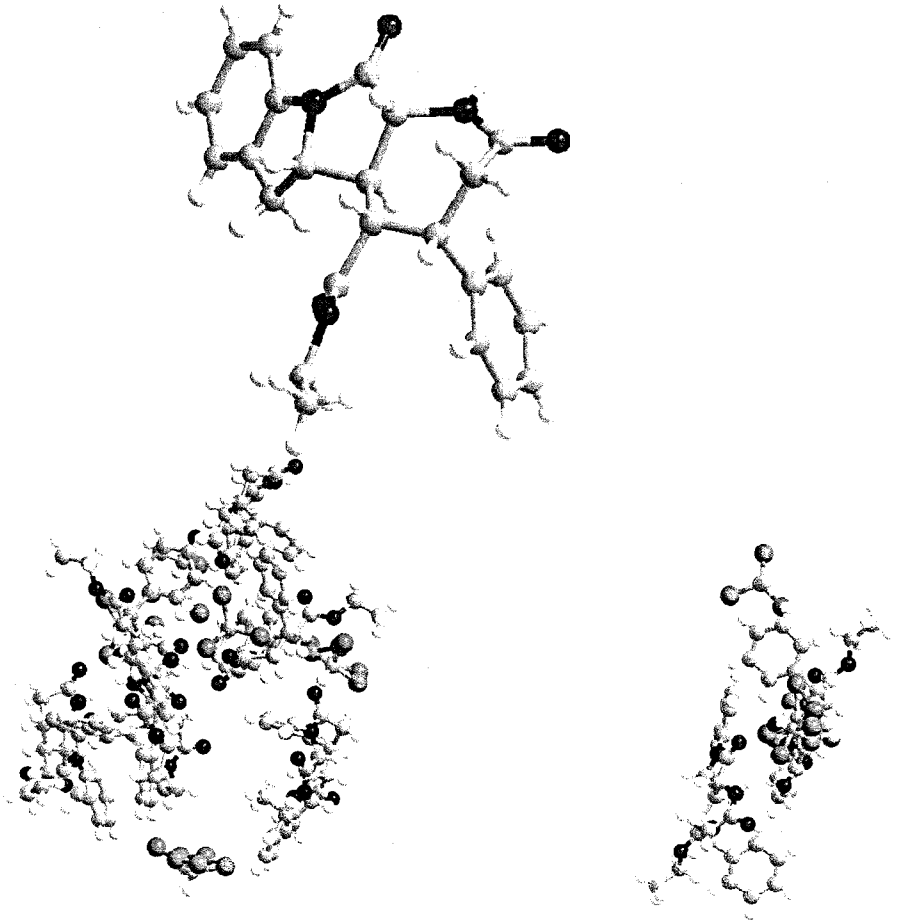
^{1}H NMR: (400 MHz, Acetone D_6)



^{13}C NMR (100 MHz, Acetone D_6)

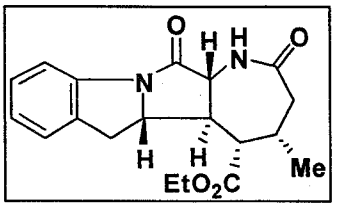


X-ray structure of the compound **22.1** and organization of compound **22.1** in the crystalline box.

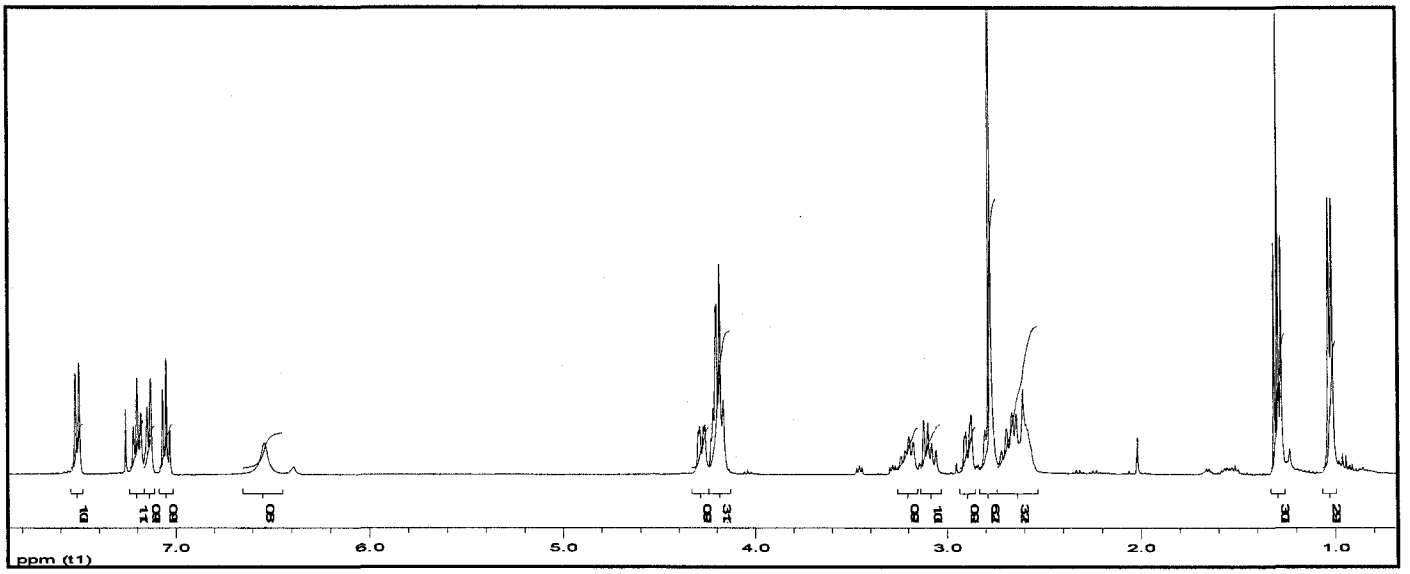


See detailed data at the end of the experimental section.

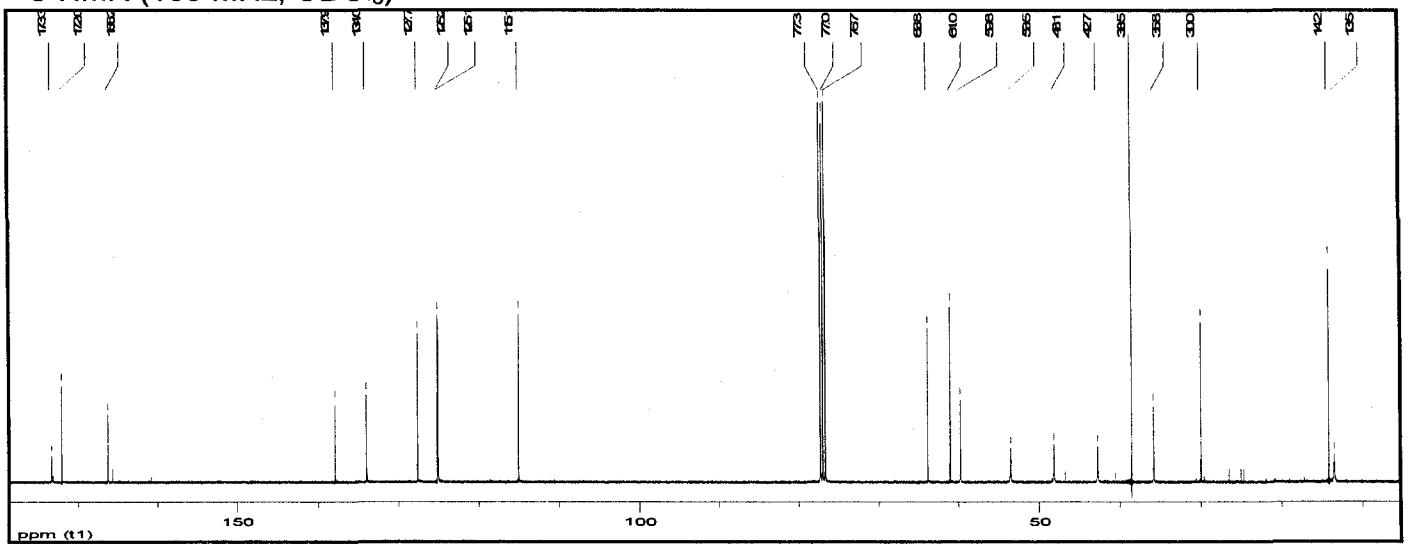
Compound 24.1:



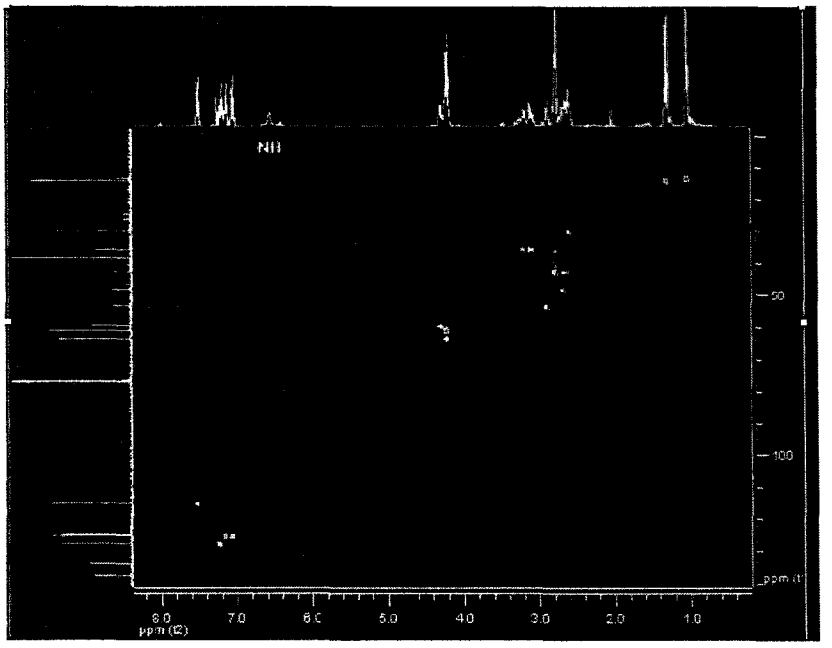
^{1}H NMR: (400 MHz, $CDCl_3$)



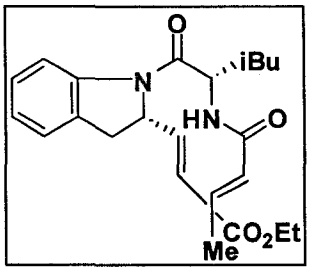
^{13}C NMR (100 MHz, $CDCl_3$)



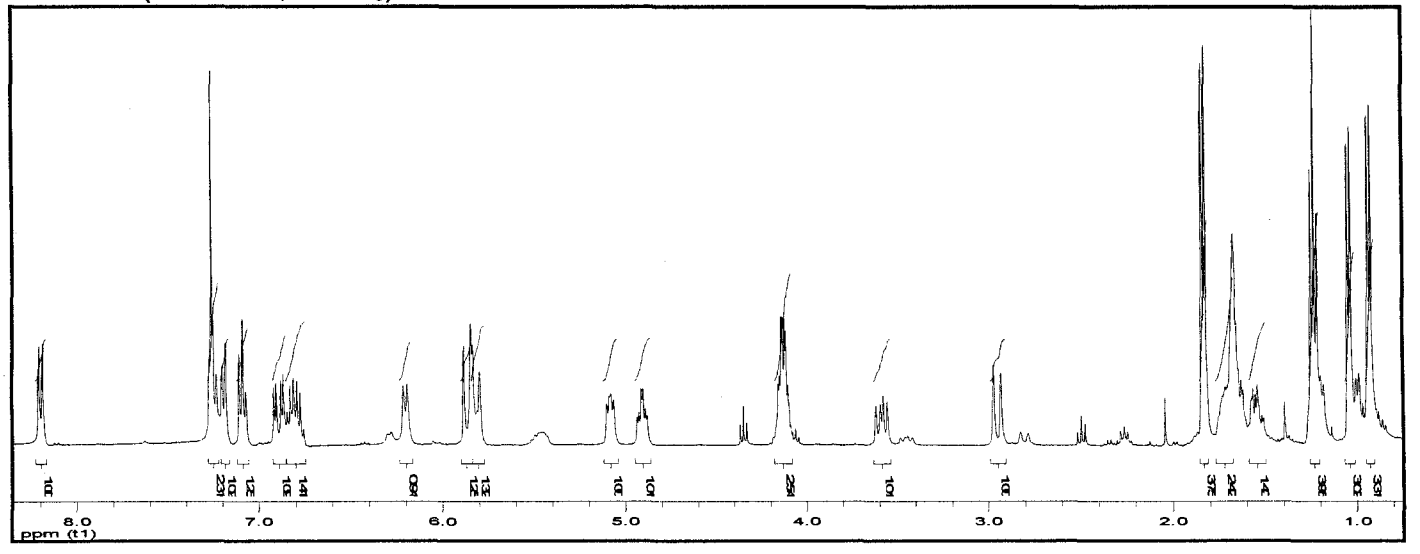
HSQC experiment showing the free NH within the 7-membered ring (no crosspeak for NH)



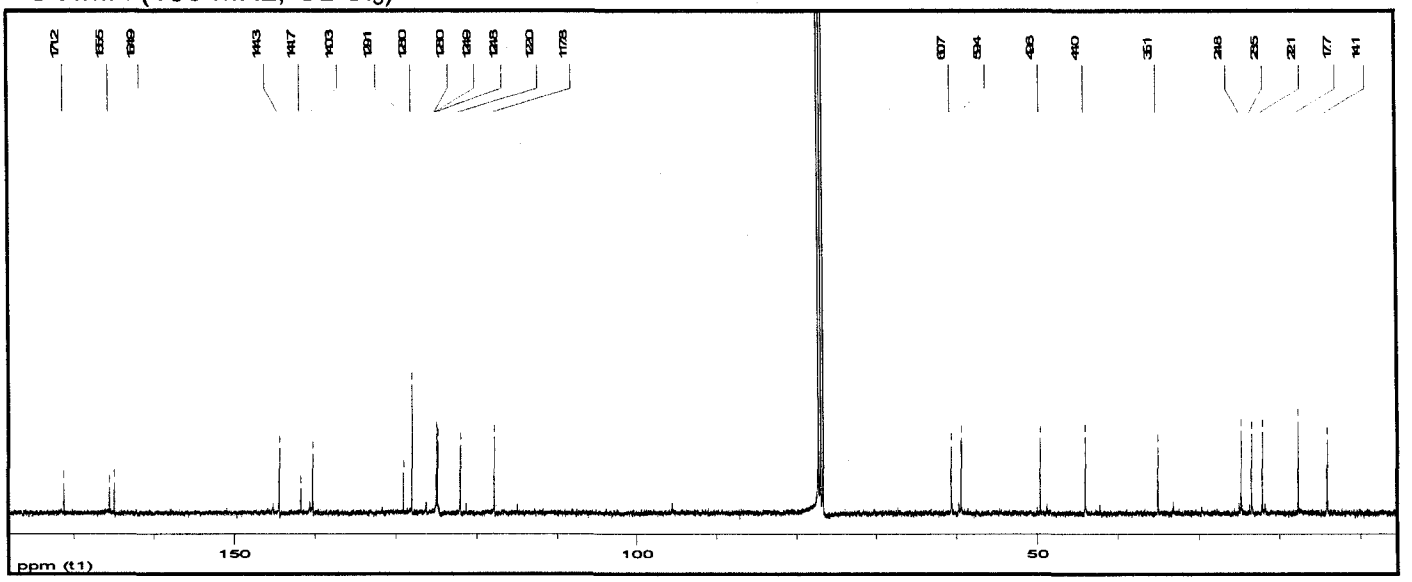
Compound 25.1:



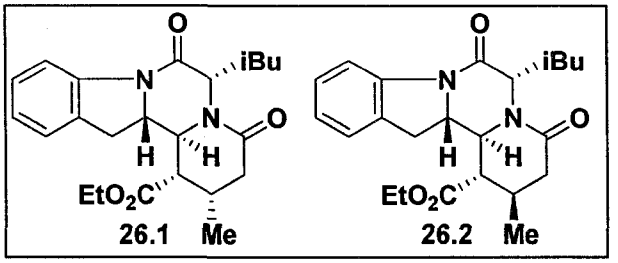
^{1}H NMR: (400 MHz, $CDCl_3$)



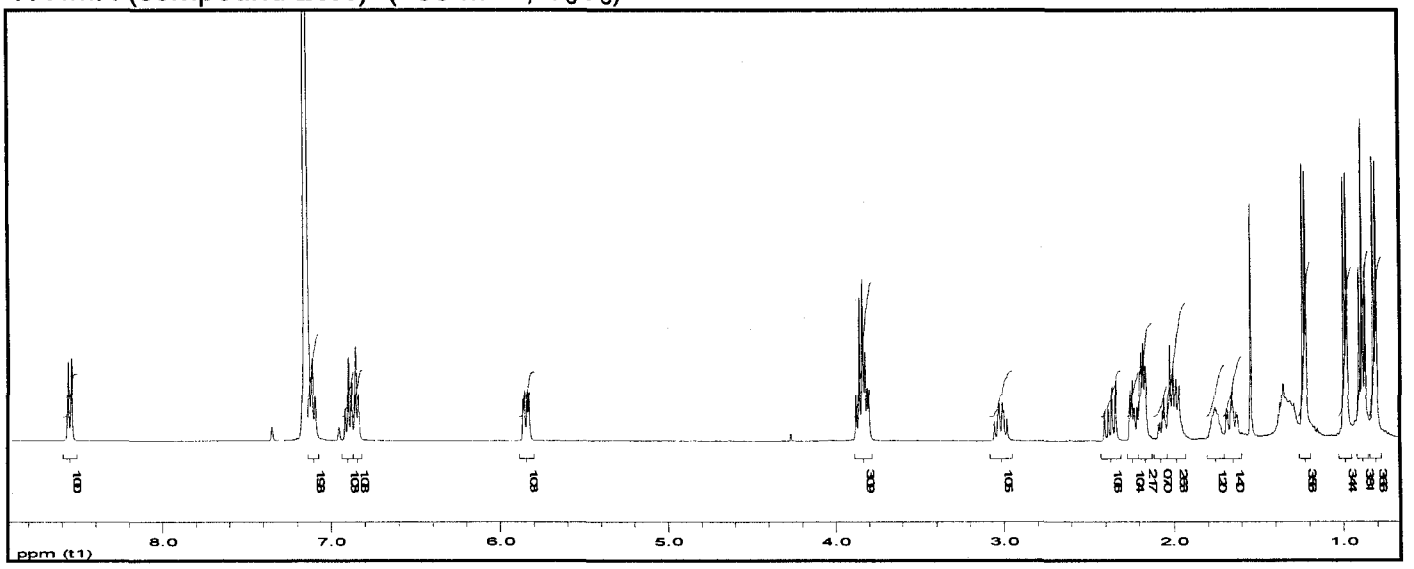
^{13}C NMR (100 MHz, $CDCl_3$)



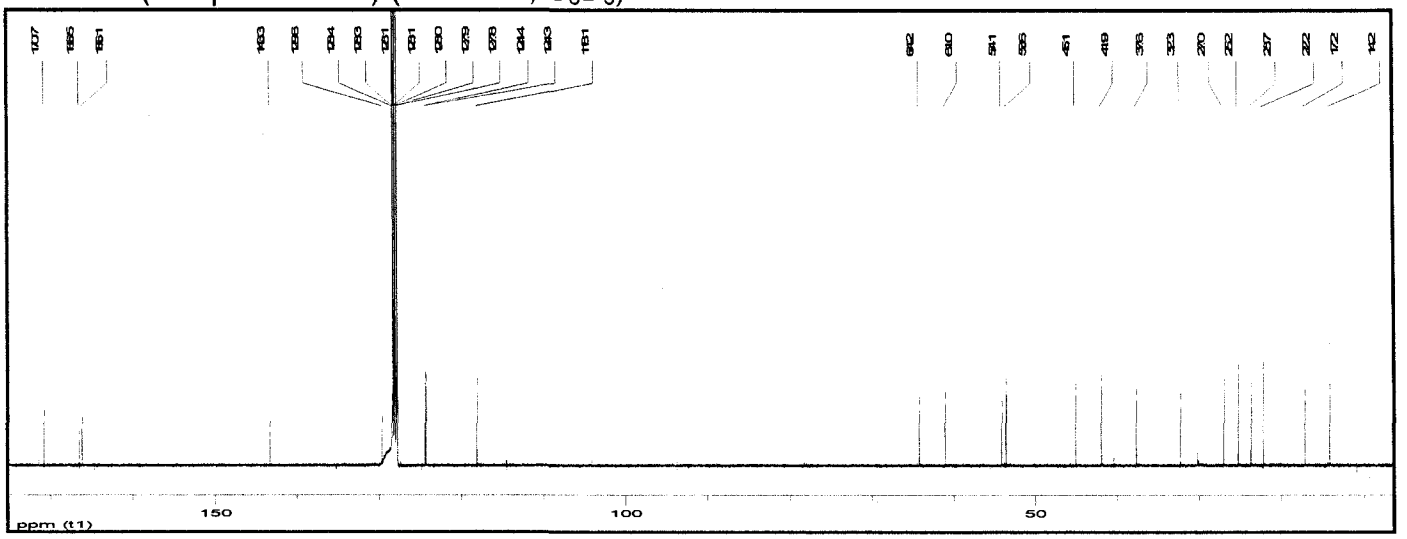
Compounds 26.1 and 26.2:



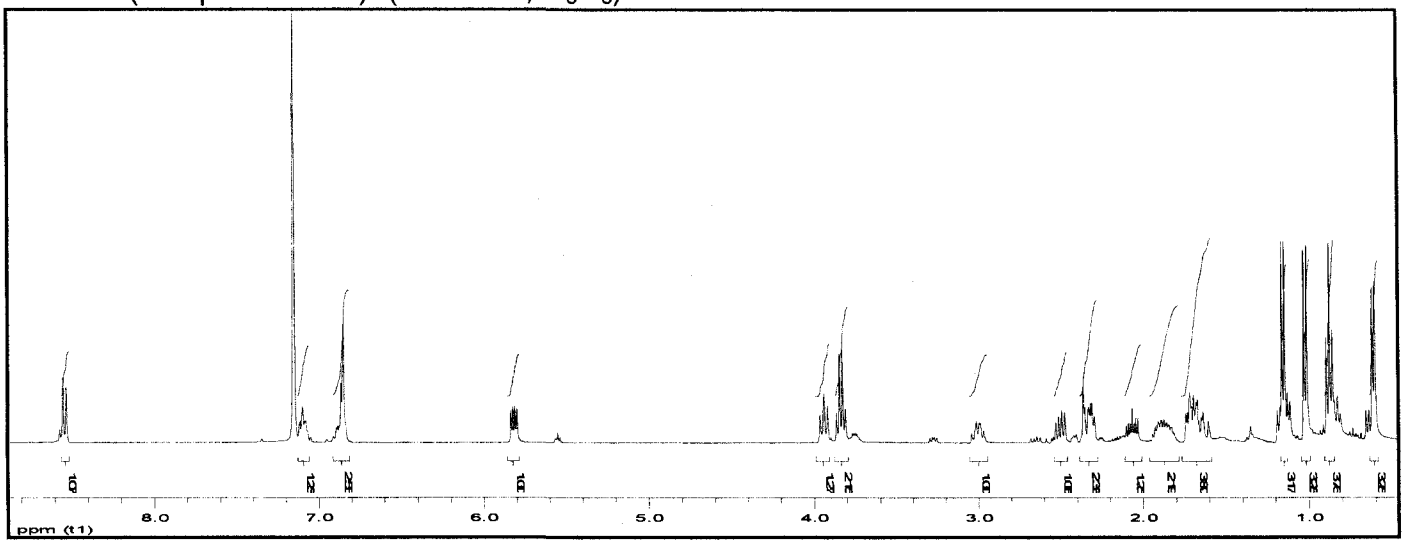
^{1}H NMR (compound **26.1**): (400 MHz, C_6D_6)



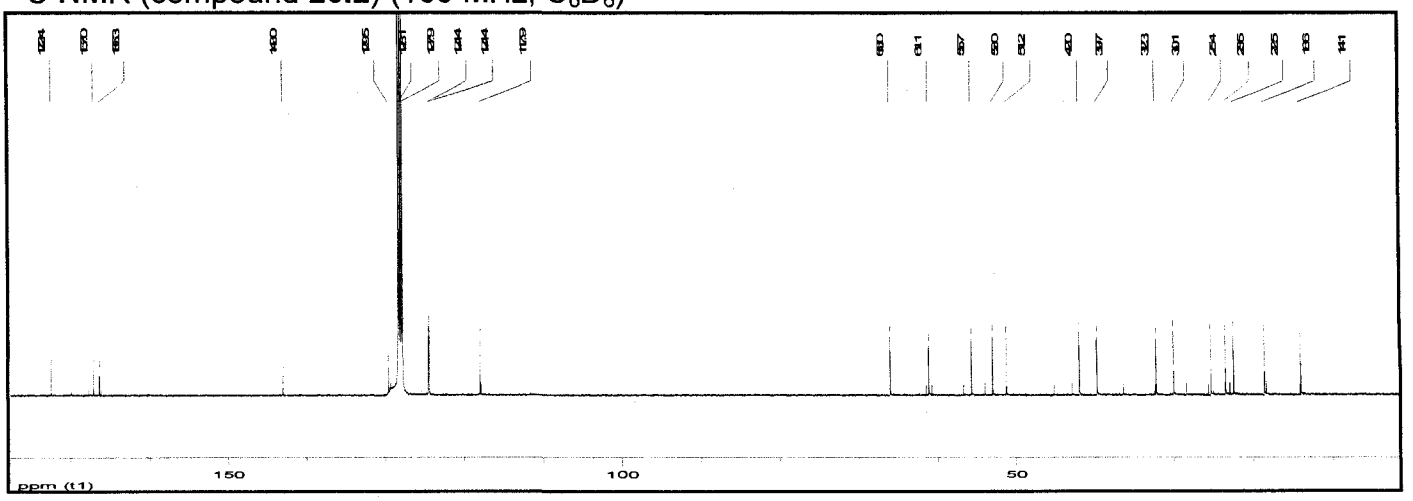
^{13}C NMR (compound **26.1**) (100 MHz, C_6D_6)



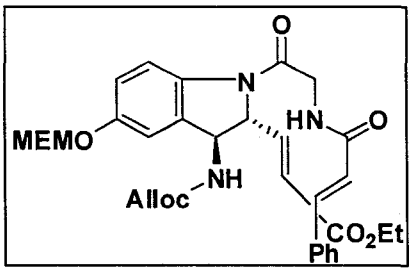
^{1}H NMR (compound **26.2**): (400 MHz, C_6D_6)



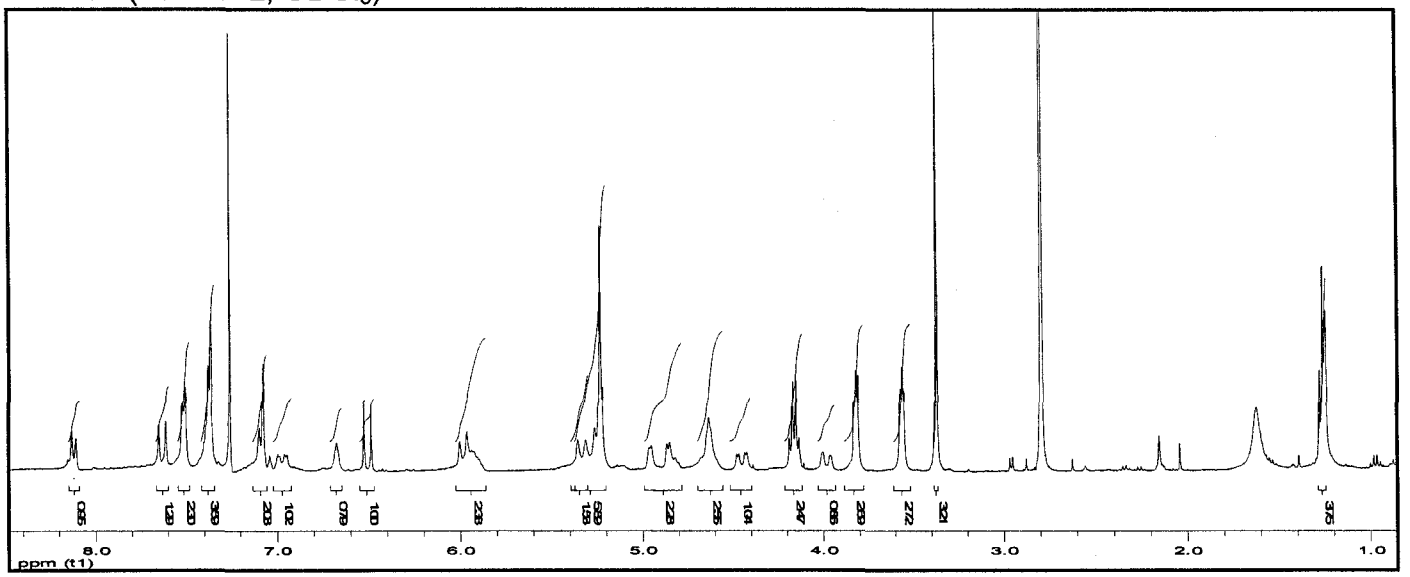
^{13}C NMR (compound **26.2**) (100 MHz, C_6D_6)



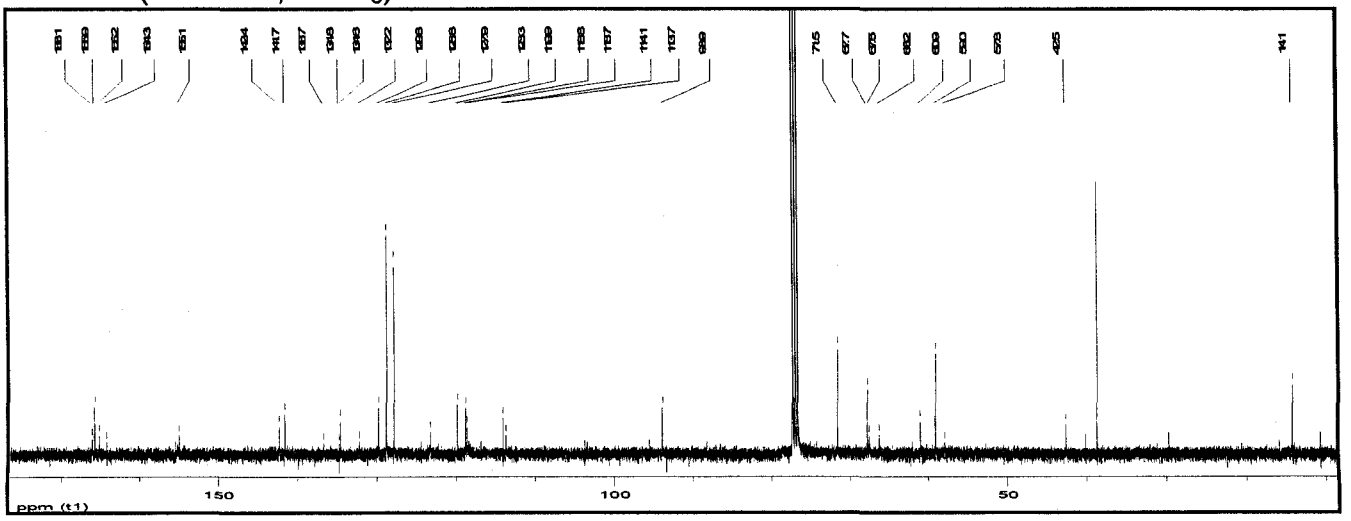
Compound 43.1:



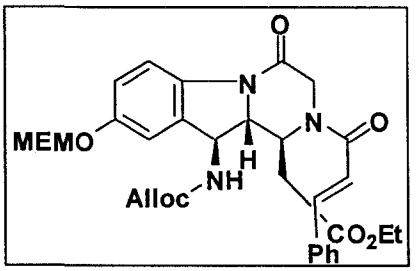
^{1}H NMR (400 MHz, $CDCl_3$)



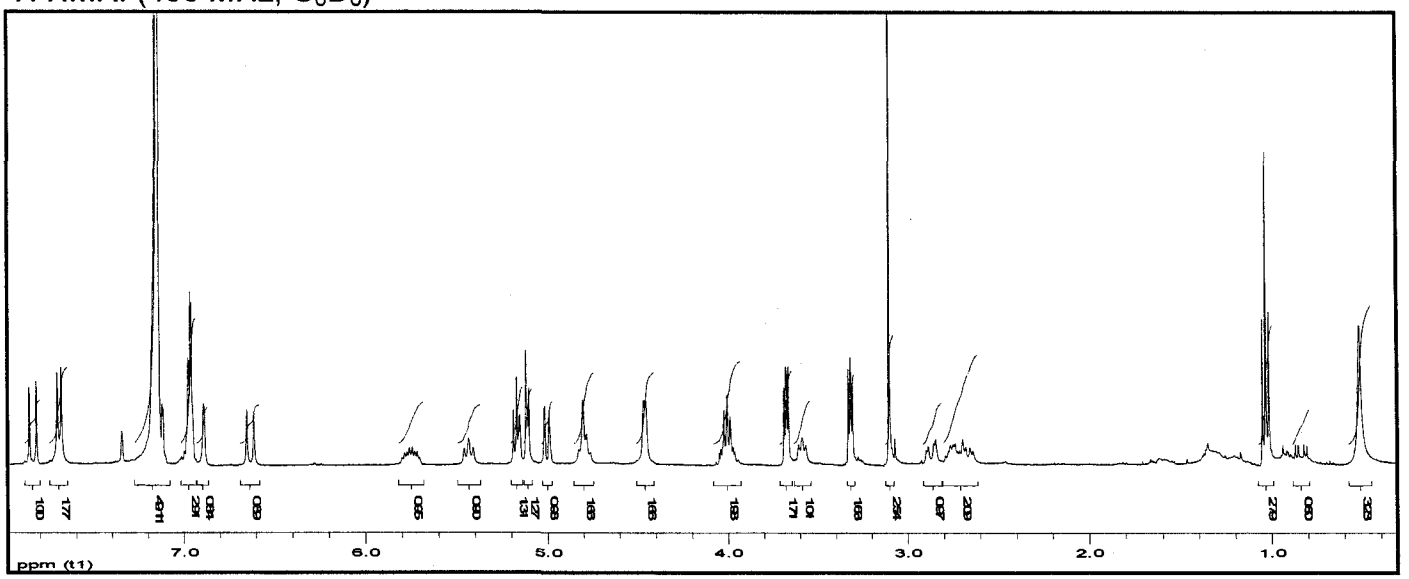
^{13}C NMR (100 MHz, $CDCl_3$)



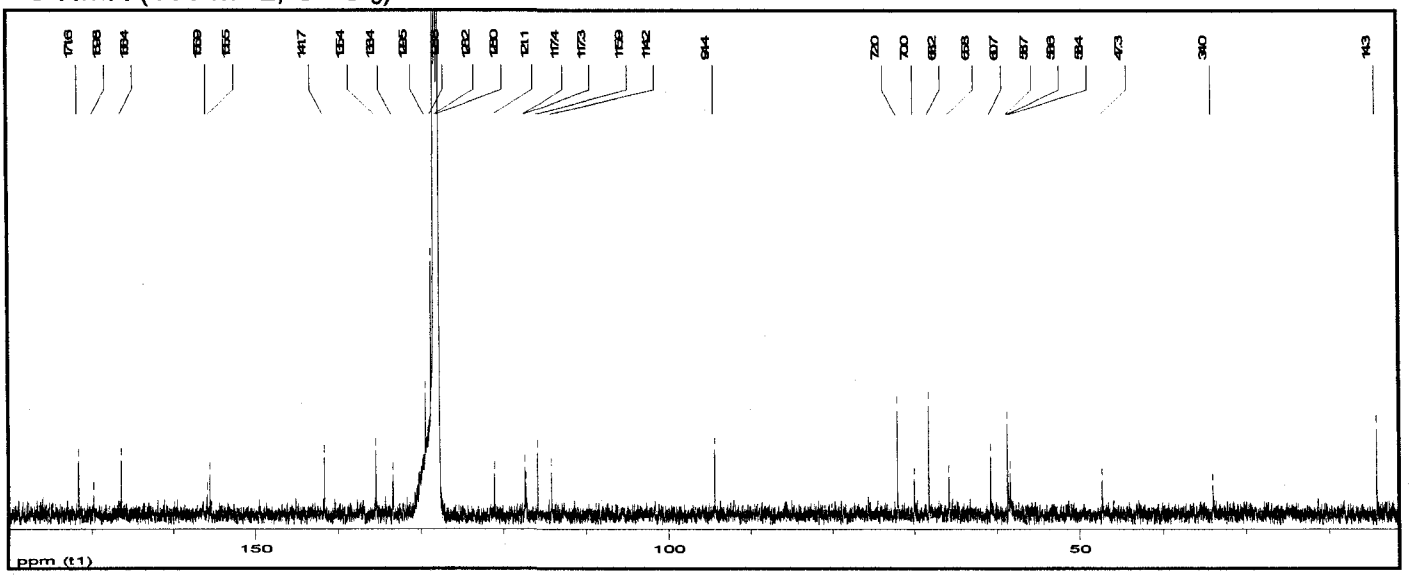
Compound 44.1:



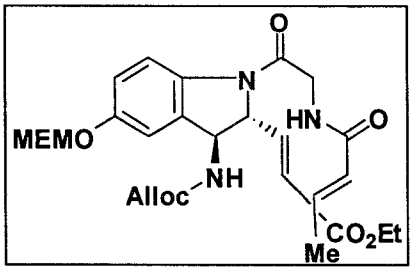
^{1}H NMR: (400 MHz, C_6D_6)



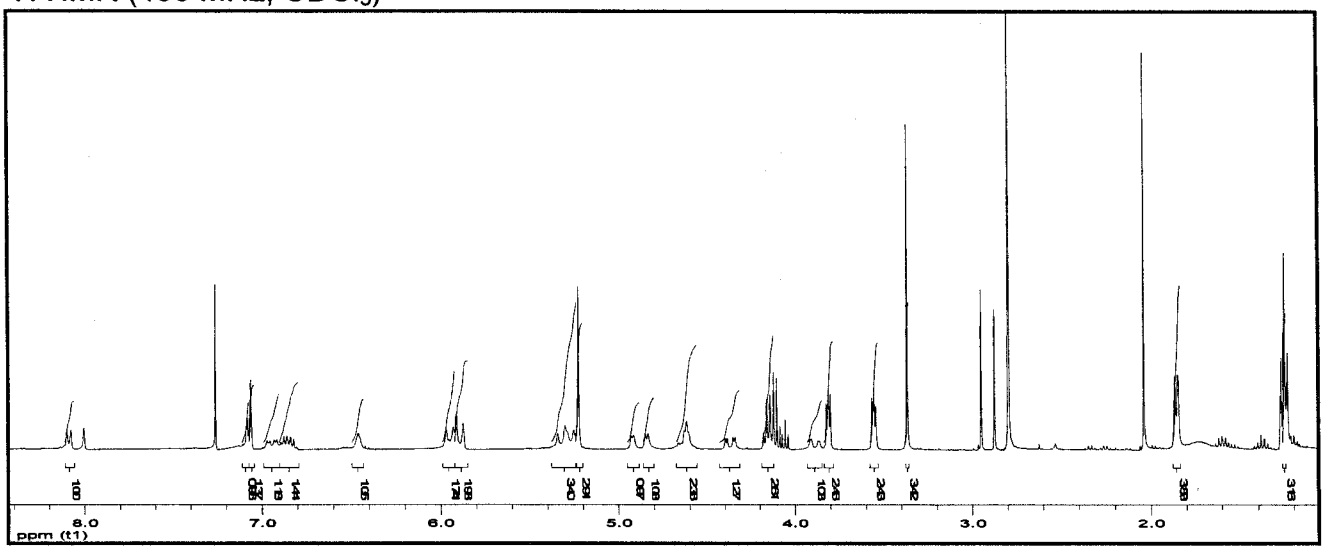
^{13}C NMR (100 MHz, $CDCl_3$)



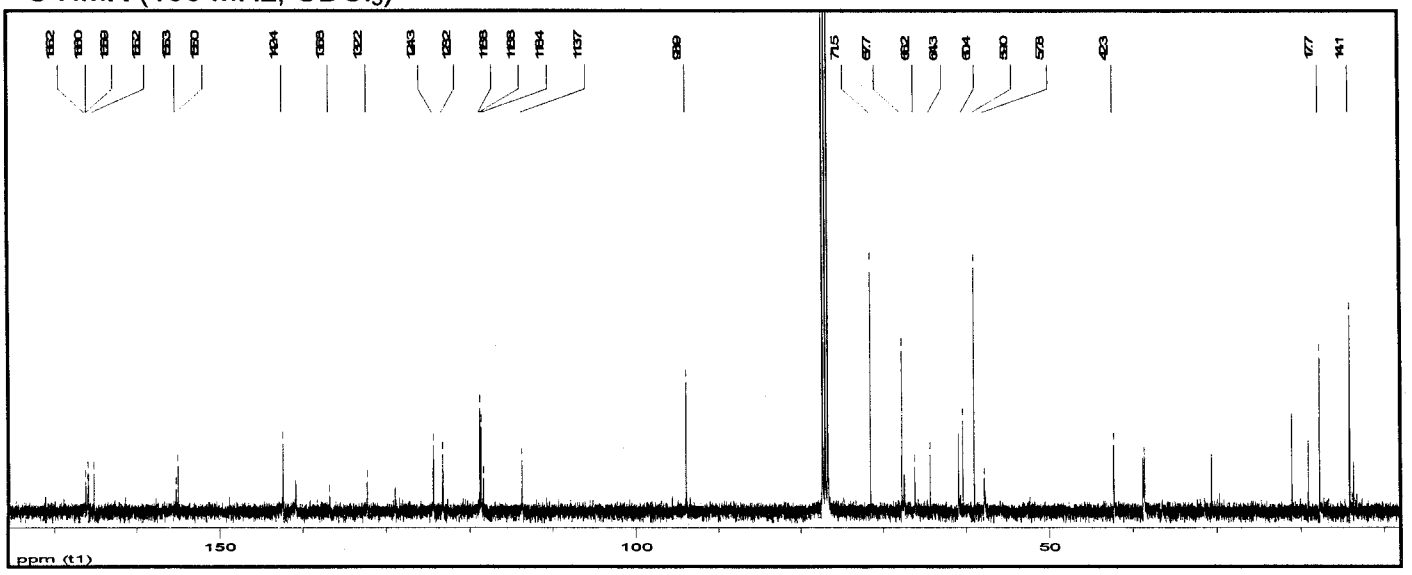
Compound 47.1:



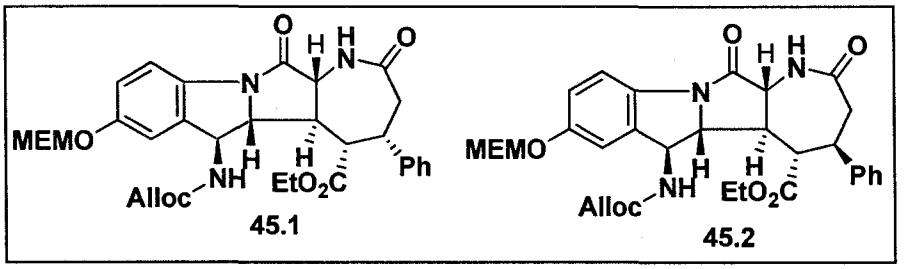
^{1}H NMR (400 MHz, $CDCl_3$)



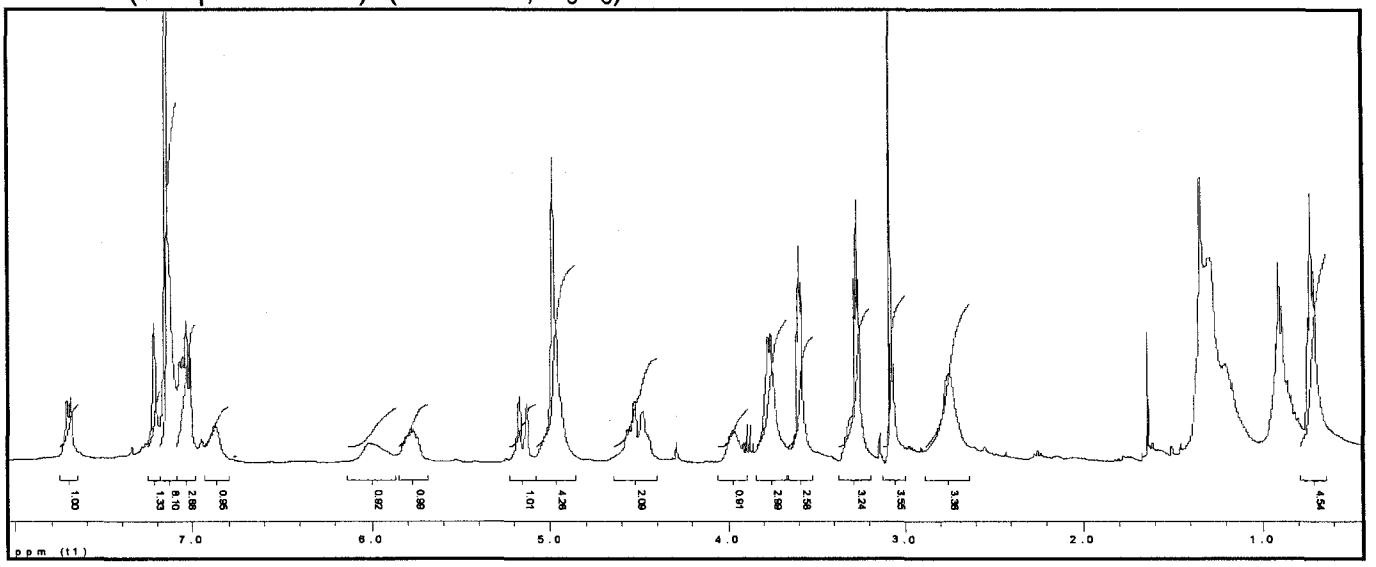
^{13}C NMR (100 MHz, $CDCl_3$)



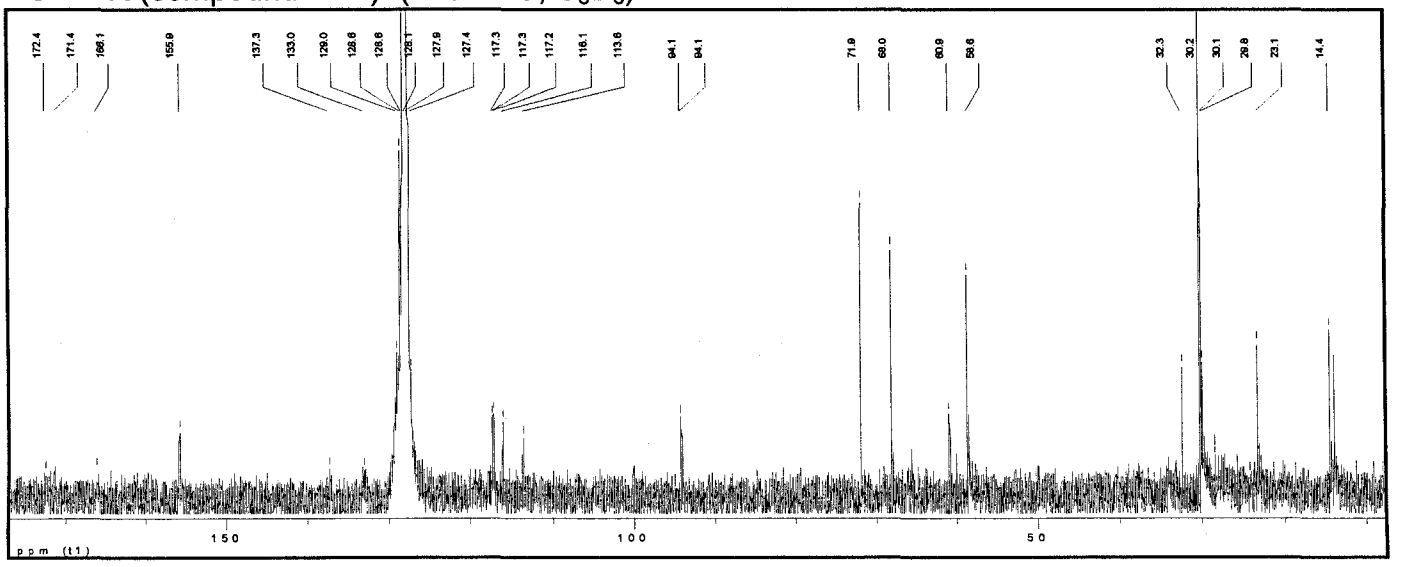
Compounds 45.1 and 45.2:



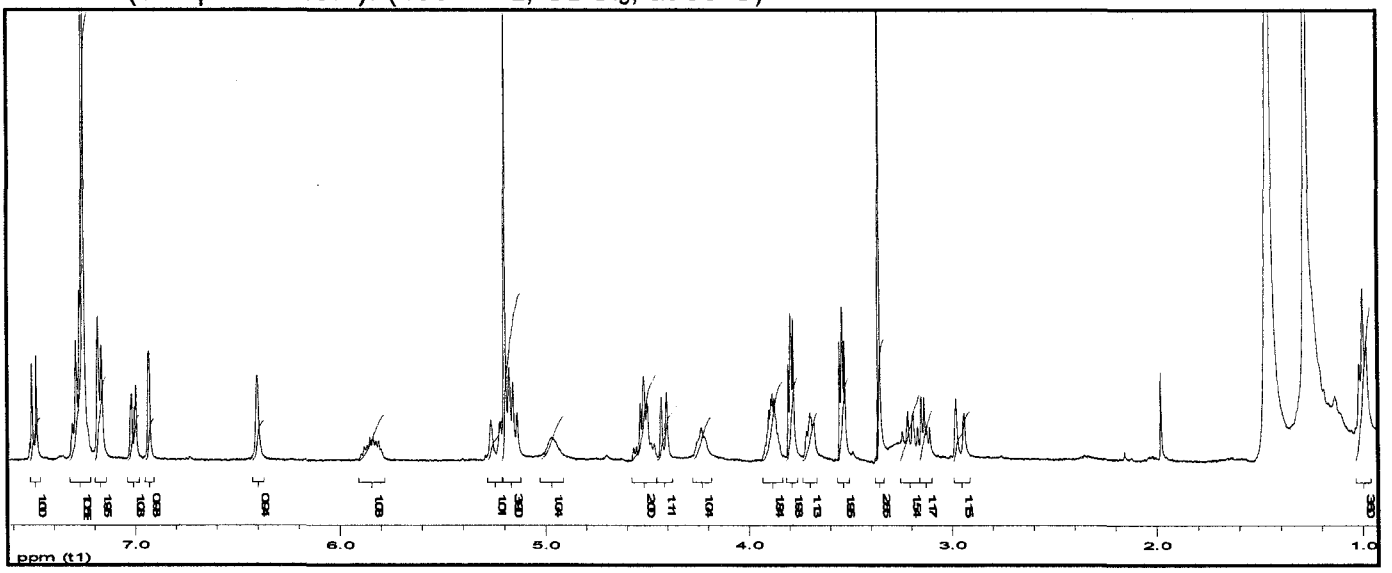
^{1}H NMR (compound **45.1**): (400 MHz, C_6D_6)



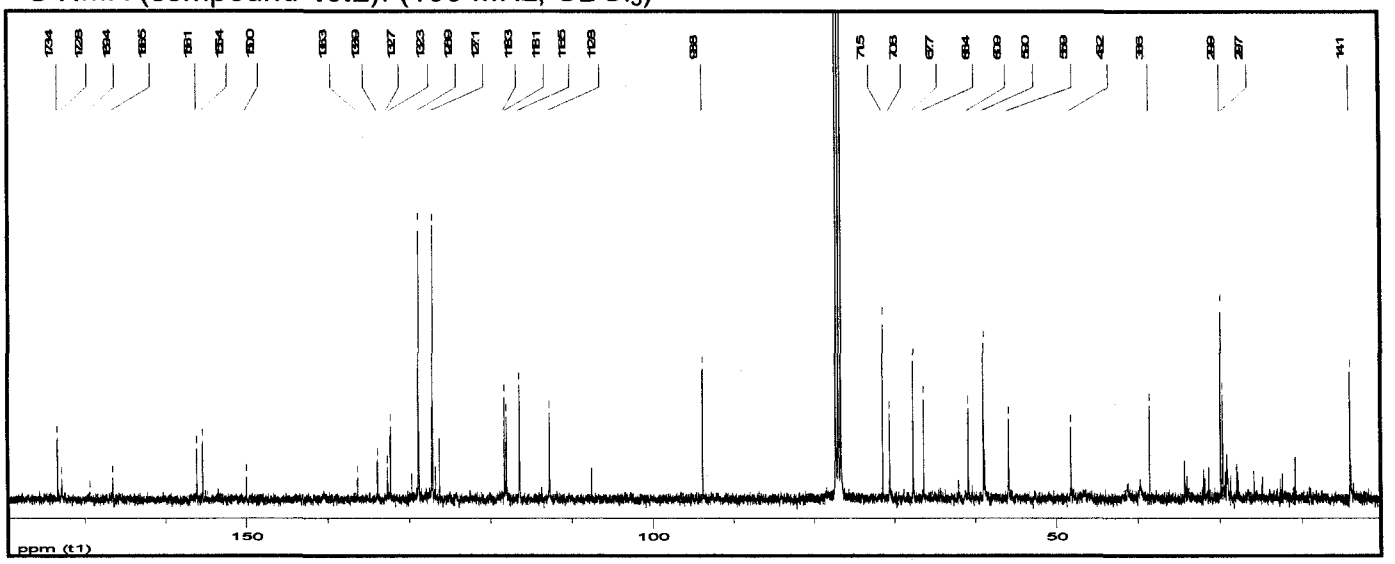
^{13}C NMR (compound **45.1**): (100 MHz, C_6D_6)



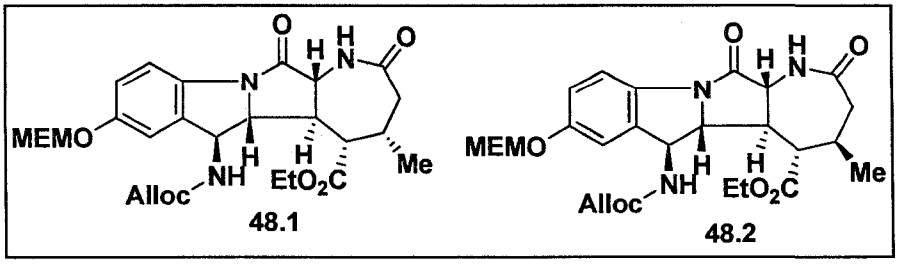
^{1}H NMR (compound **45.2**): (400 MHz, $CDCl_3$, at 58°C)



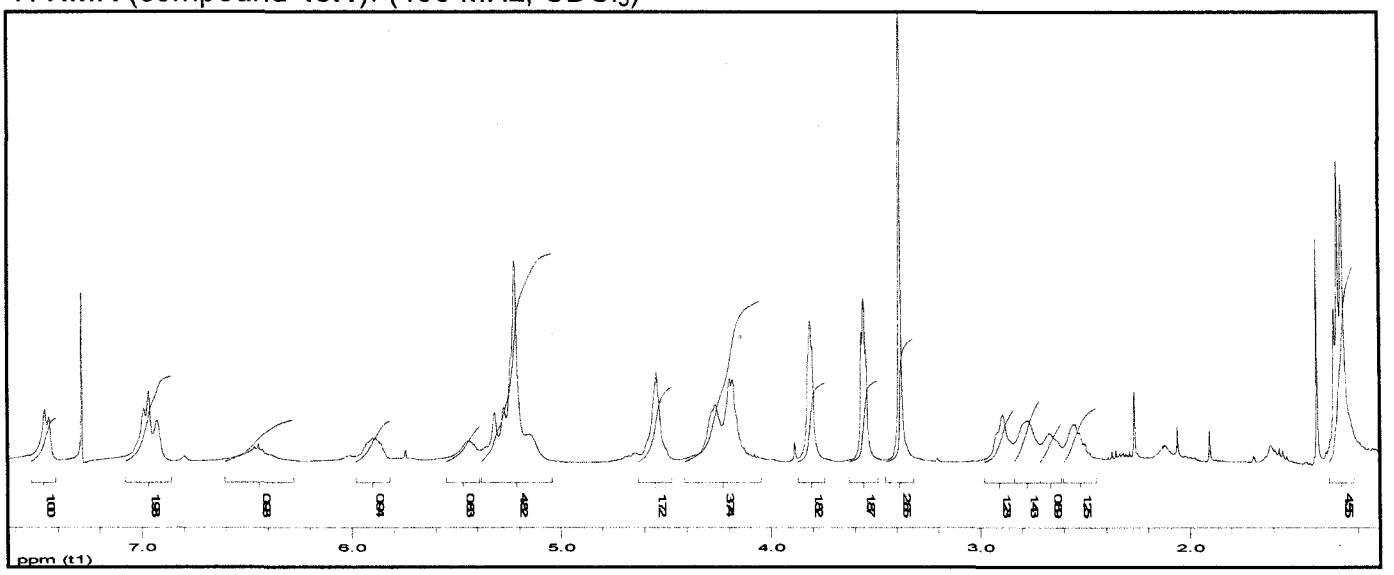
^{13}C NMR (compound **45.2**): (100 MHz, $CDCl_3$)



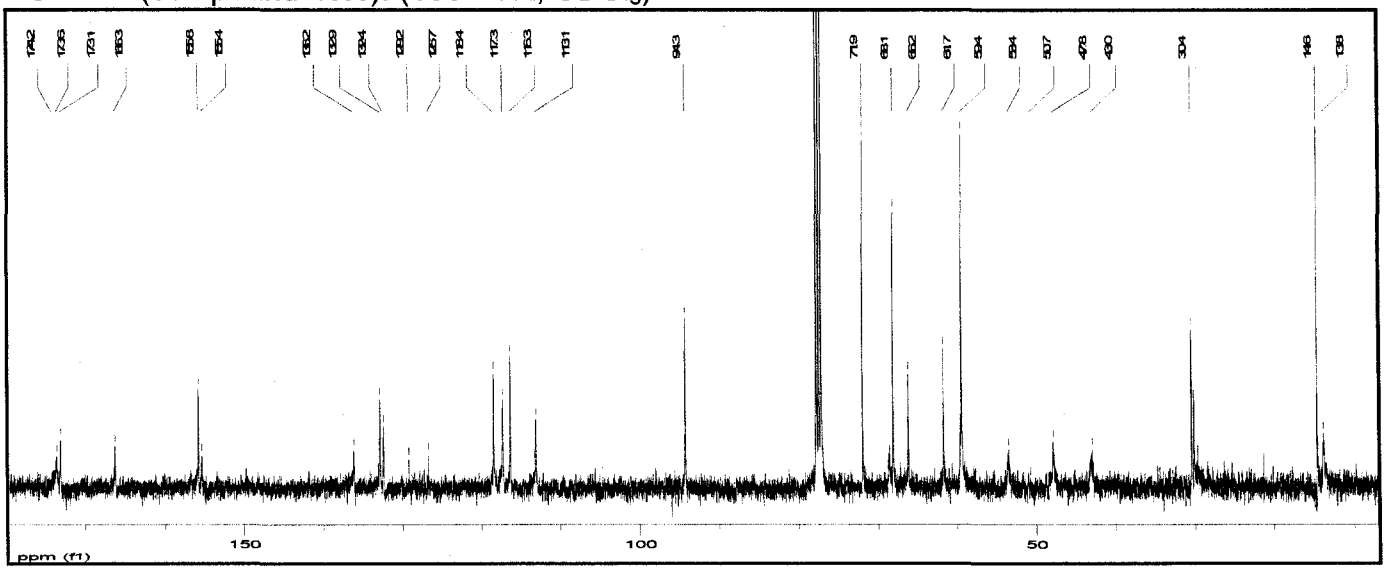
Compounds 48.1 and 48.2:



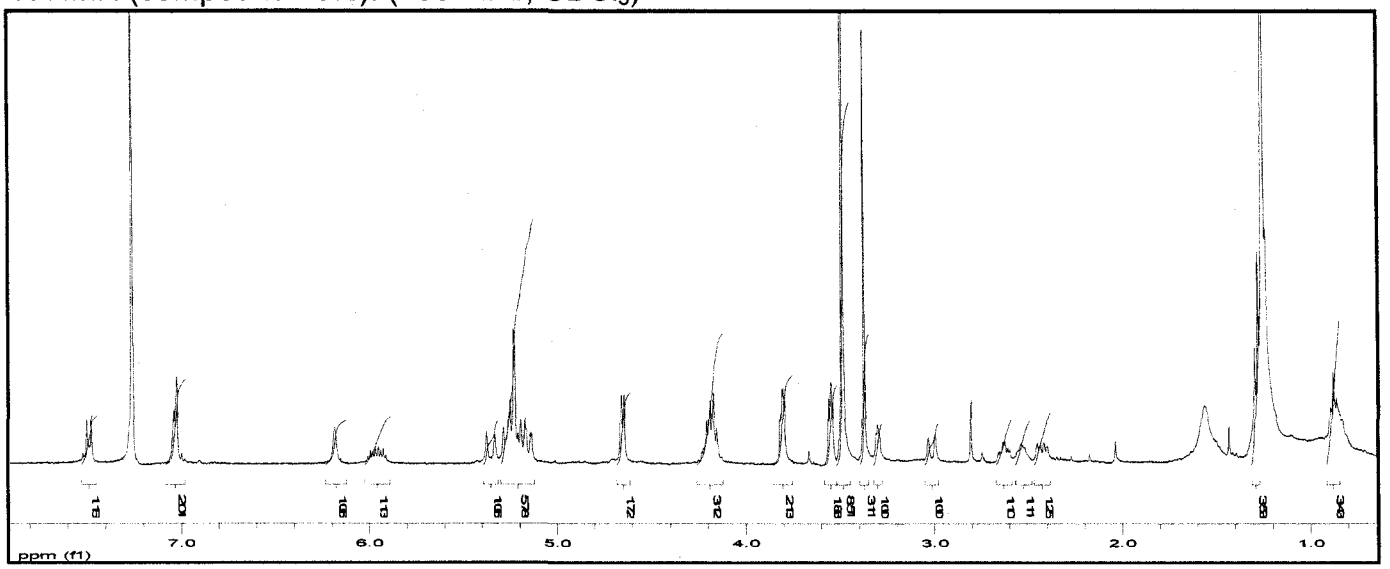
^{1}H NMR (compound **48.1**): (400 MHz, $CDCl_3$)



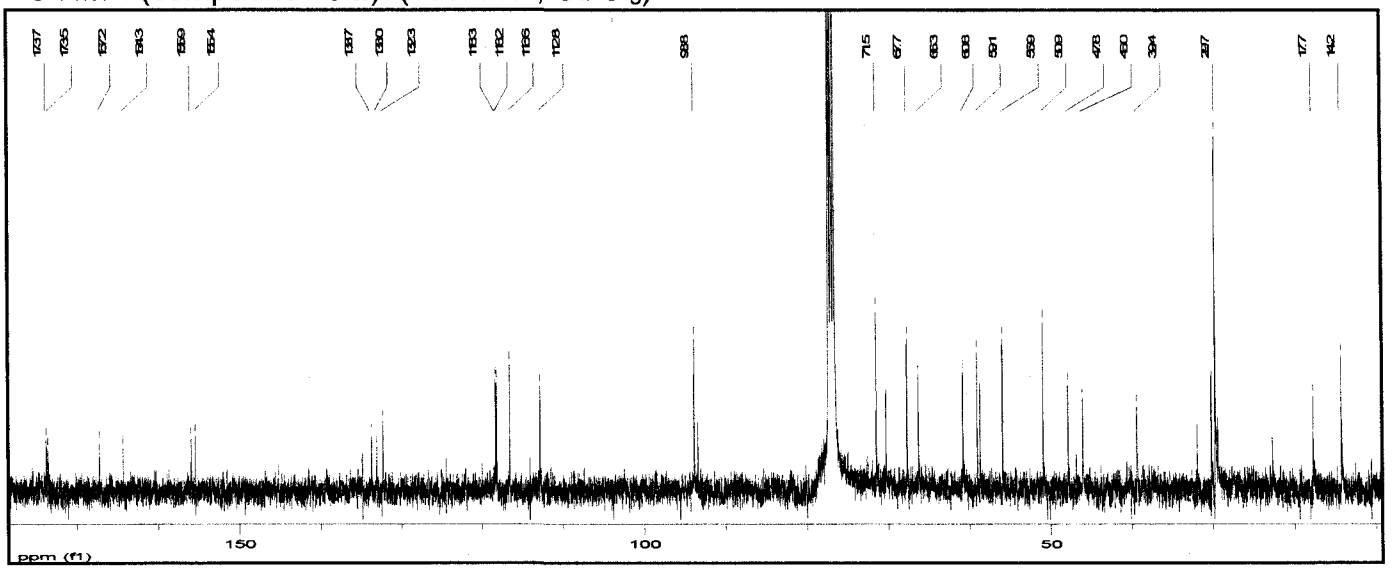
^{13}C NMR (compound **48.1**): (100 MHz, $CDCl_3$)



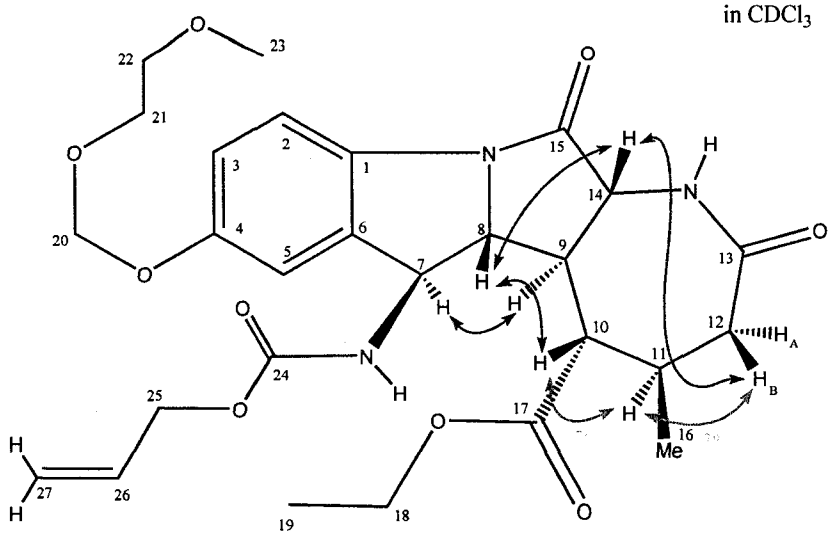
^{1}H NMR (compound **48.2**): (400 MHz, $CDCl_3$)



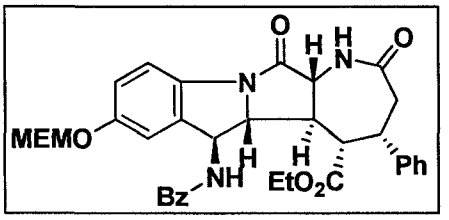
^{13}C NMR (compound **48.2**): (100 MHz, $CDCl_3$)



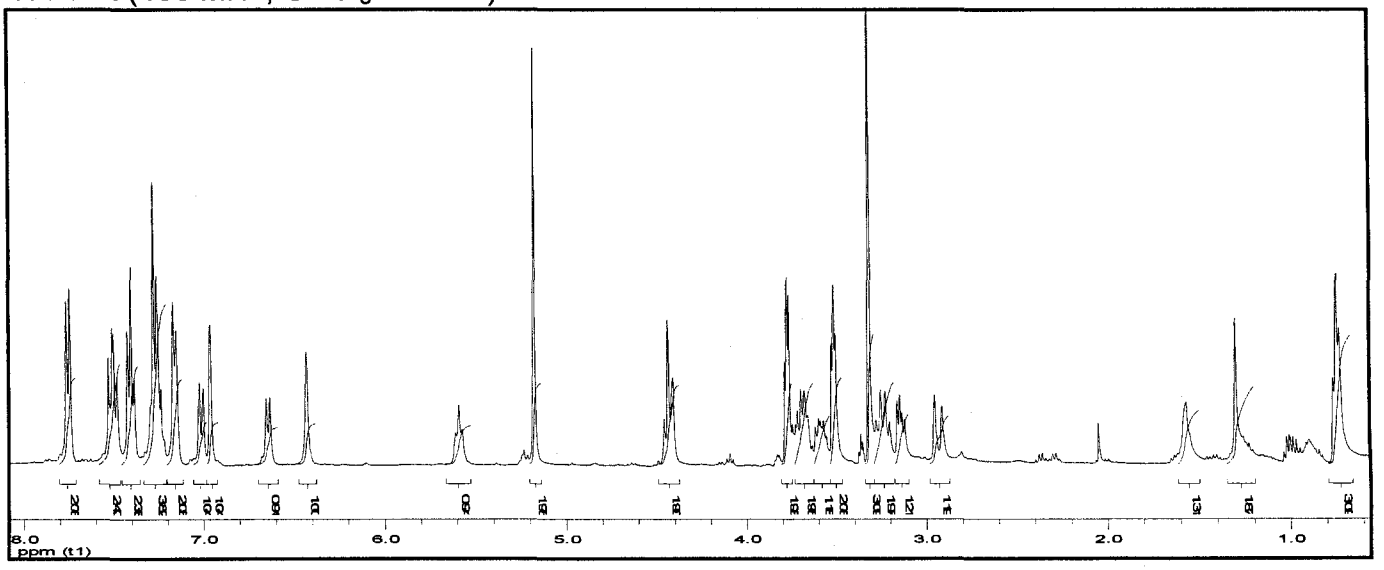
in $CDCl_3$



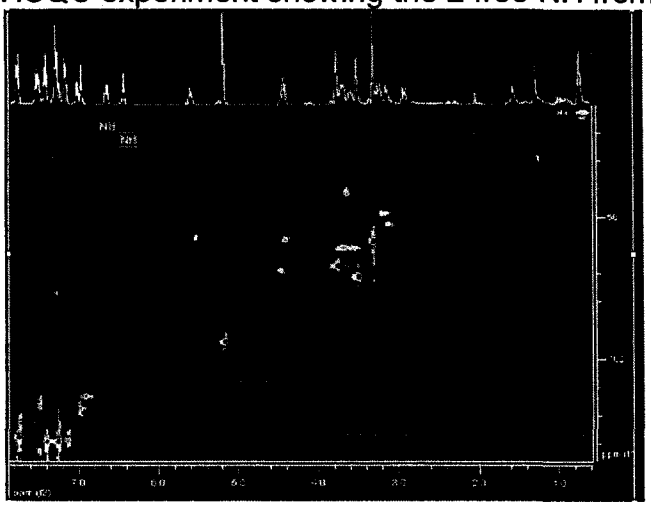
Compound 46.1:

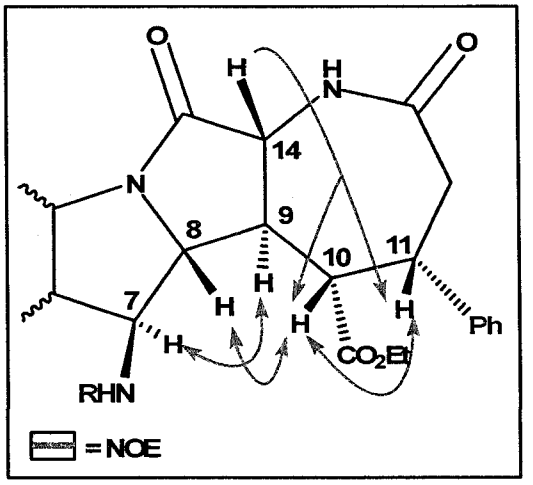


^{1}H NMR (400 MHz, $CDCl_3$ at 58°C)

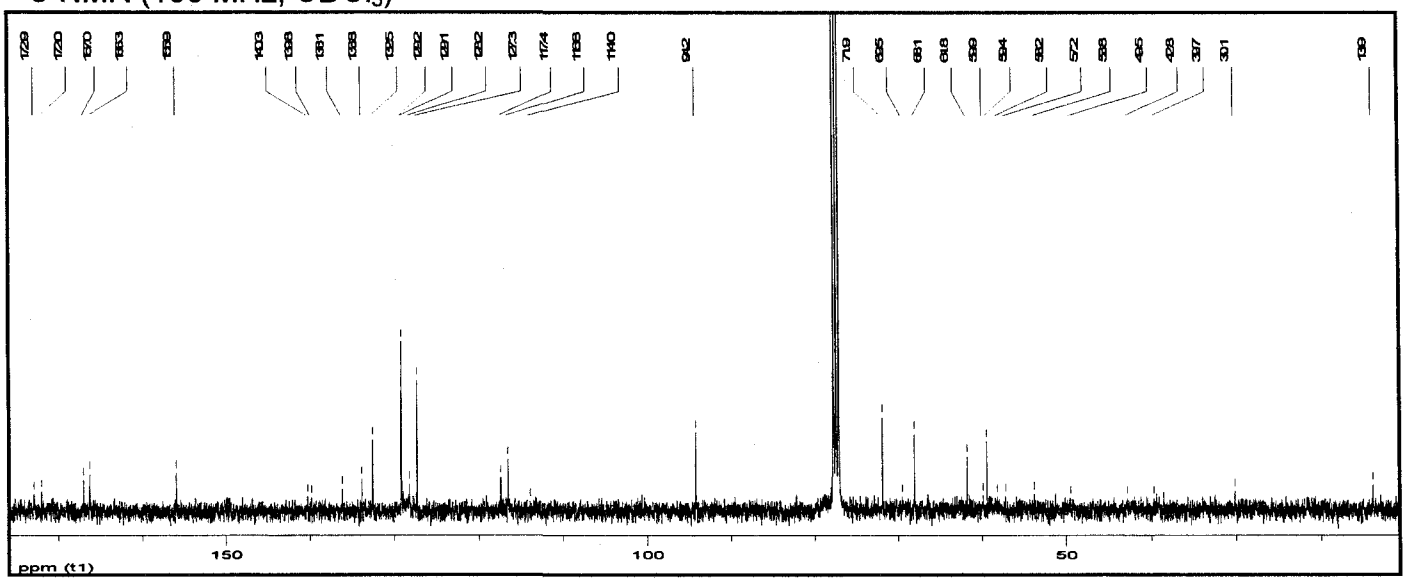


HSQC experiment showing the 2 free NH from the compound **46.1**.

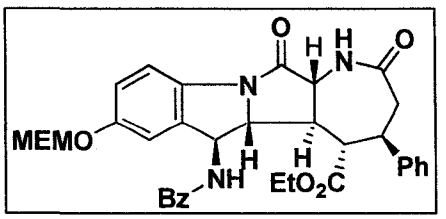




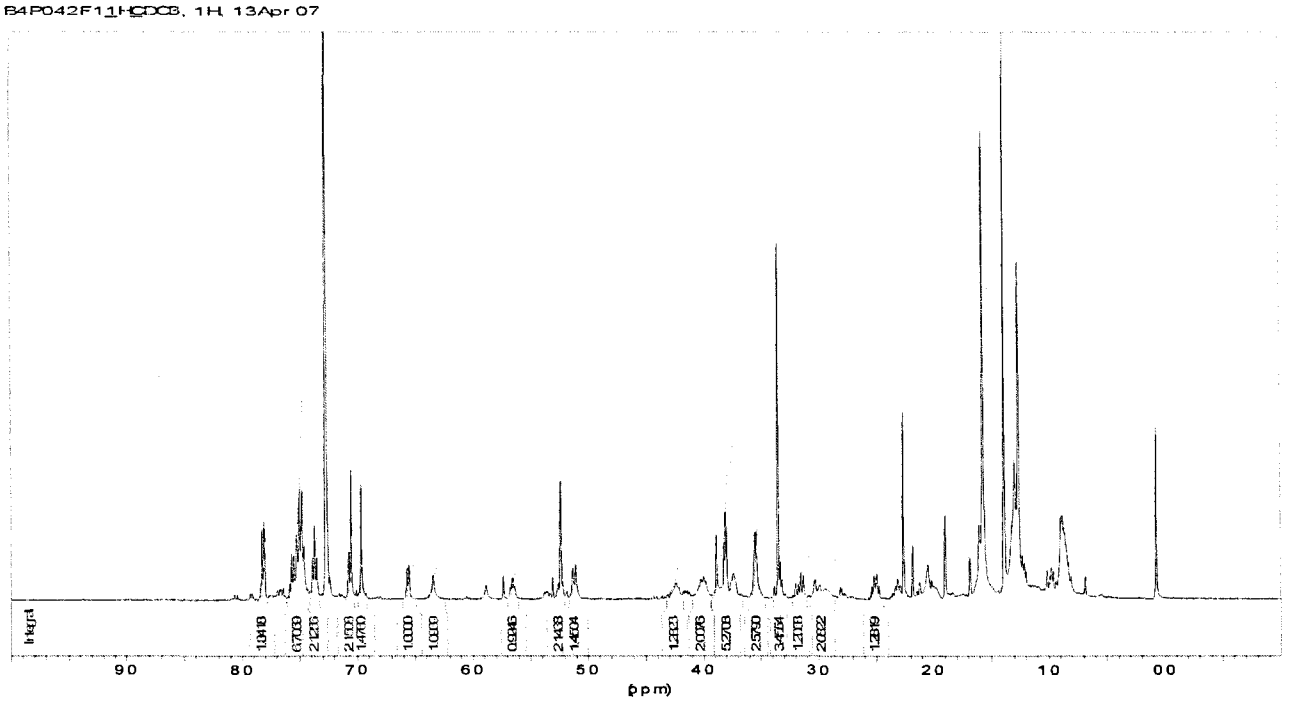
^{13}C NMR (100 MHz, $CDCl_3$)



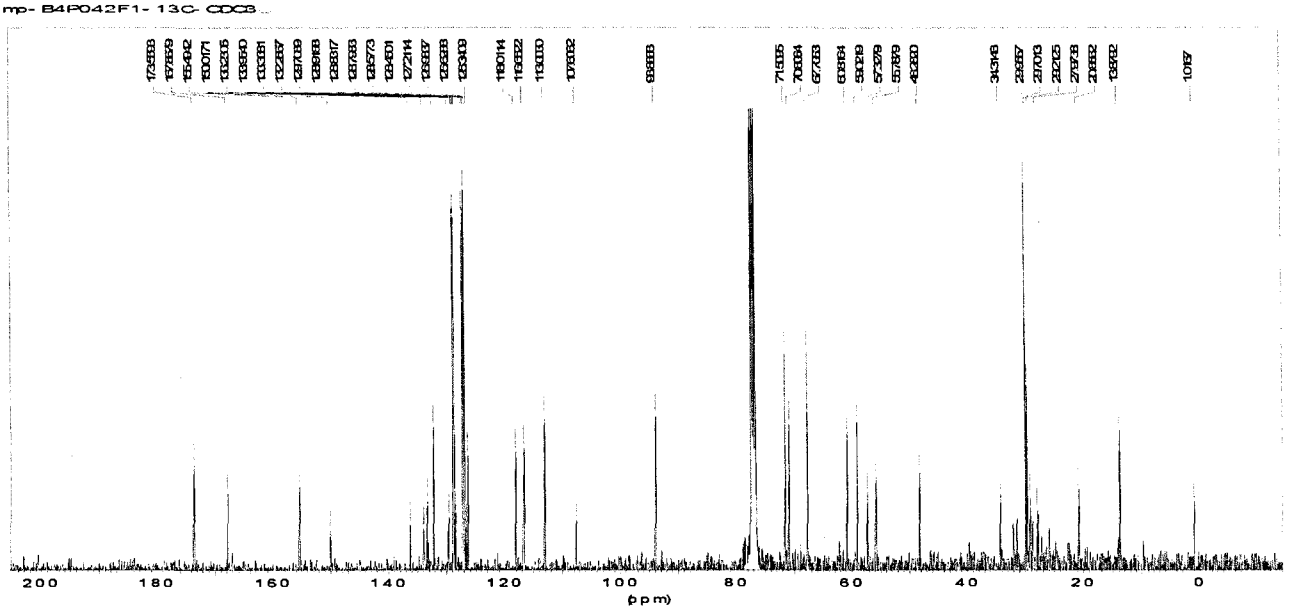
Compound 46.2:



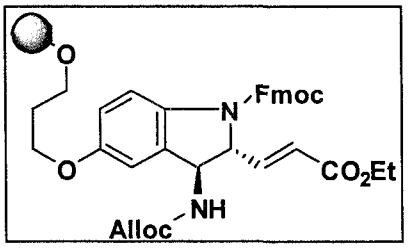
^{1}H NMR (400 MHz, $CDCl_3$)

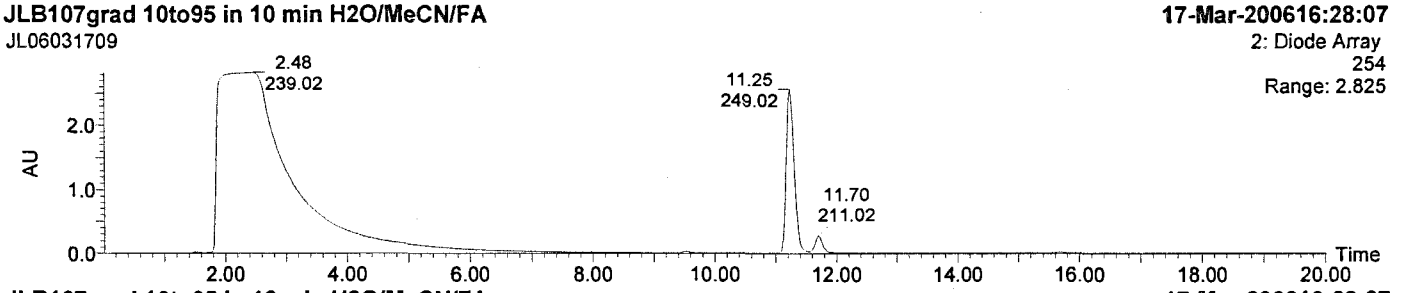


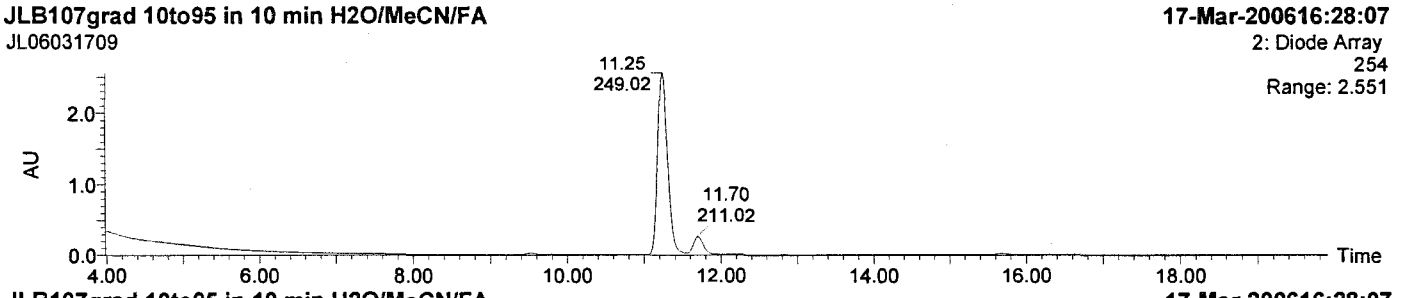
^{13}C NMR (100 MHz, $CDCl_3$)

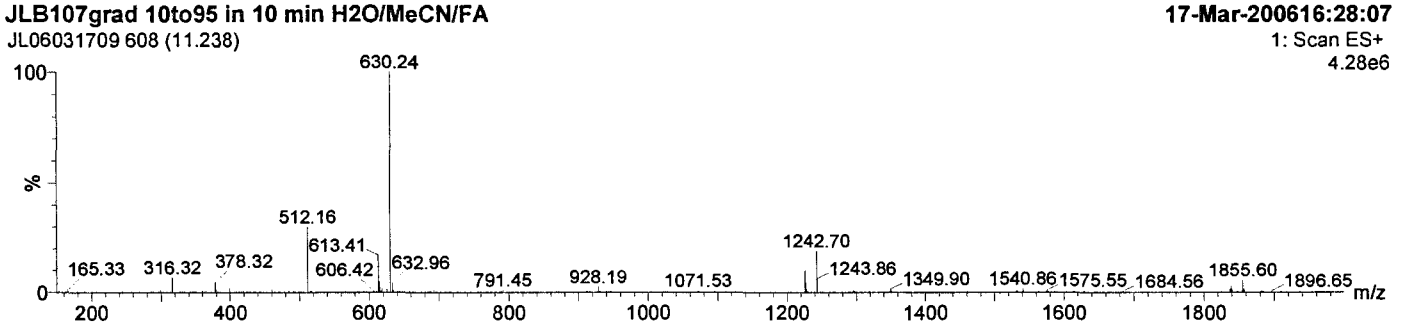


Compound 58.1:

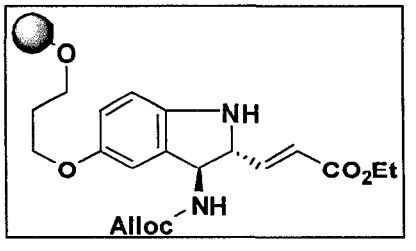


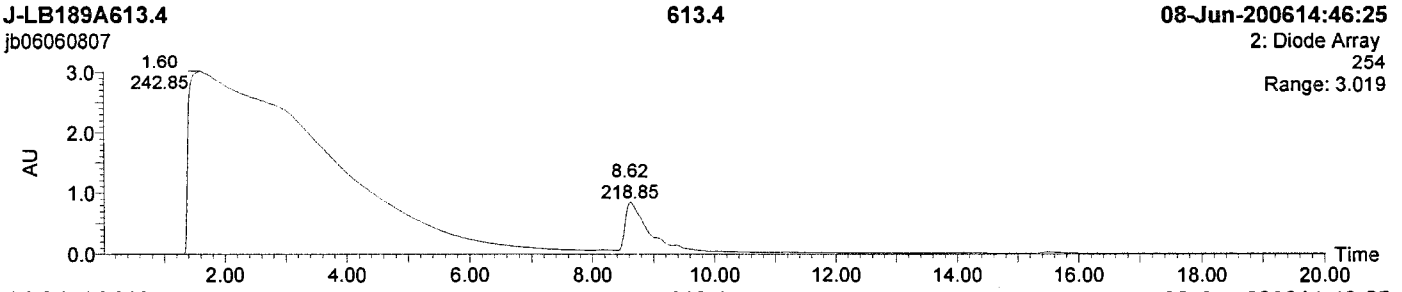


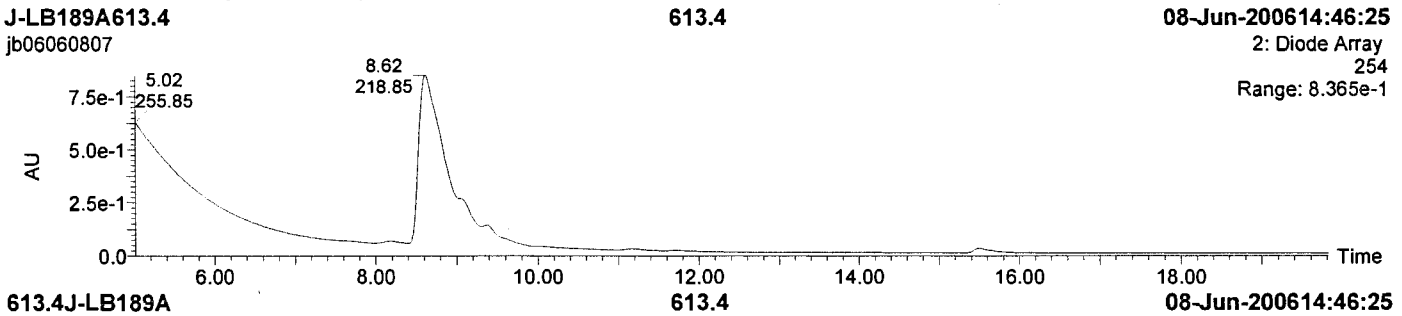


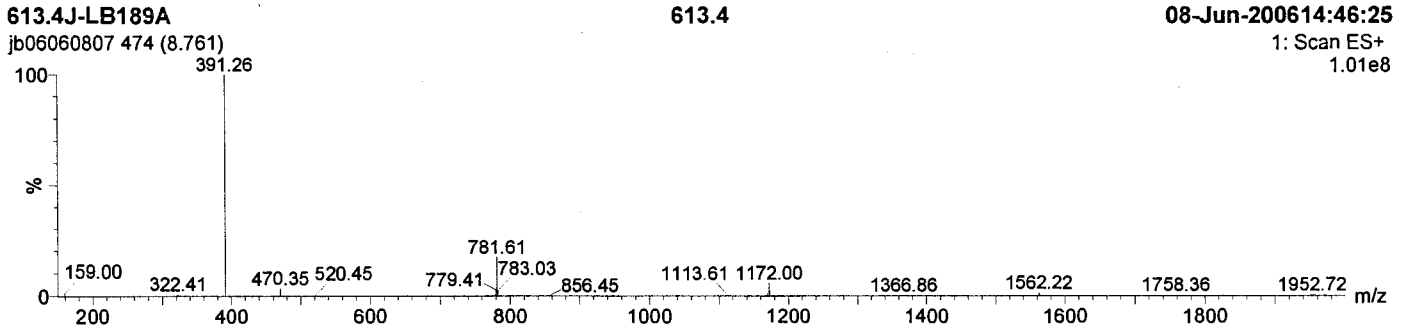


Compound 61.1:

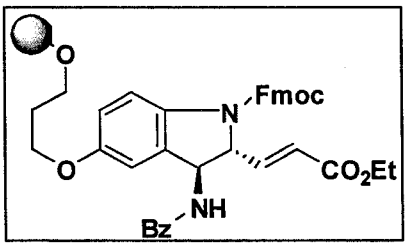


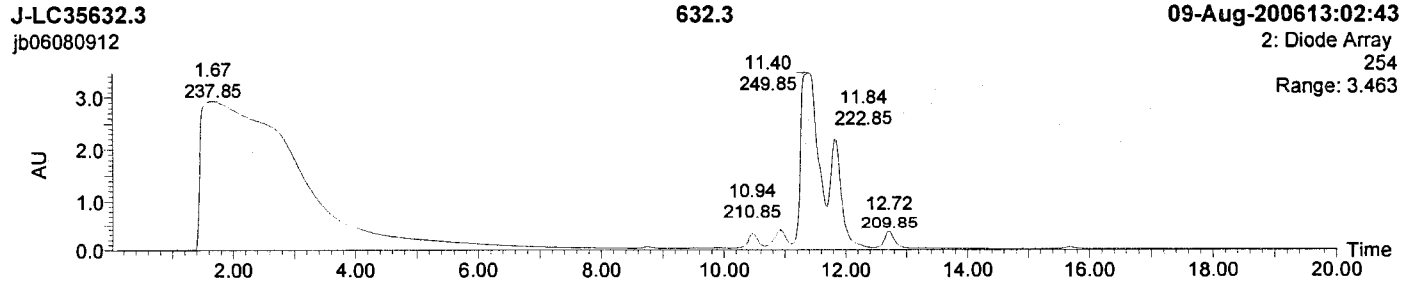


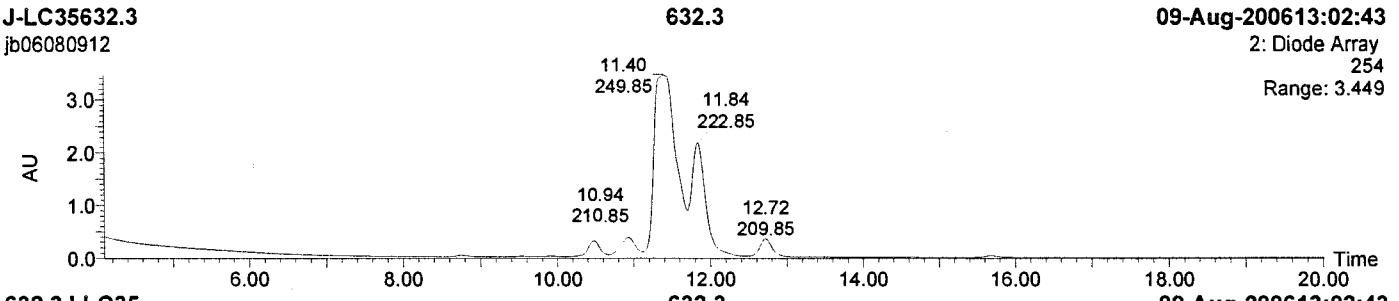


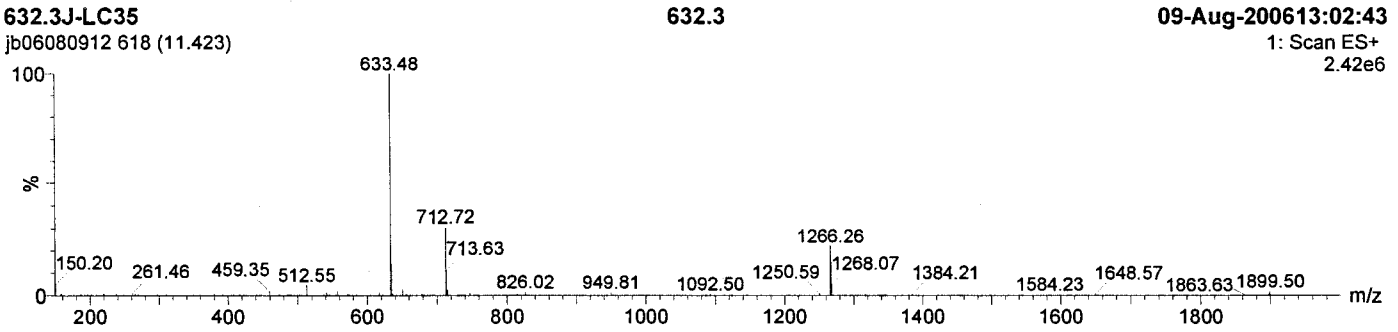


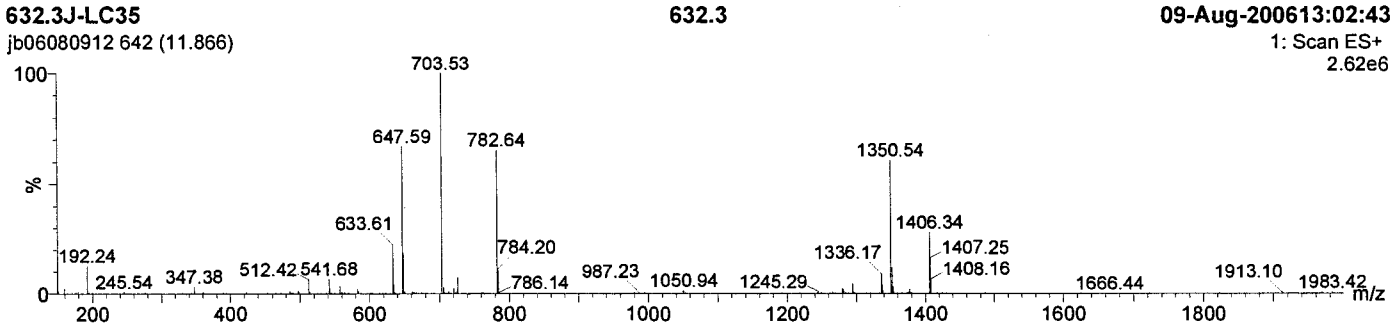
Compound 60.1:



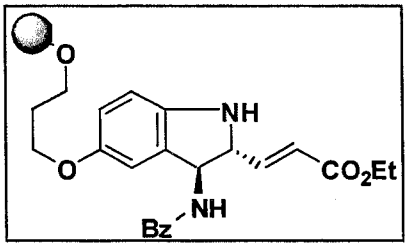


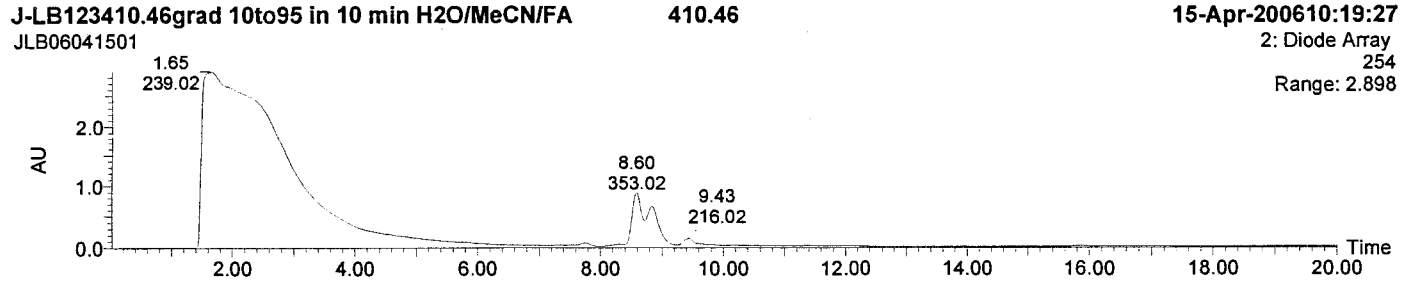


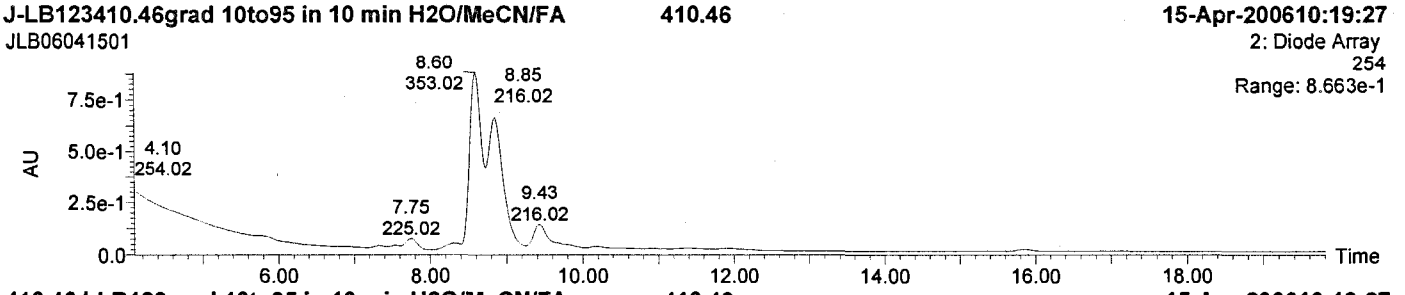


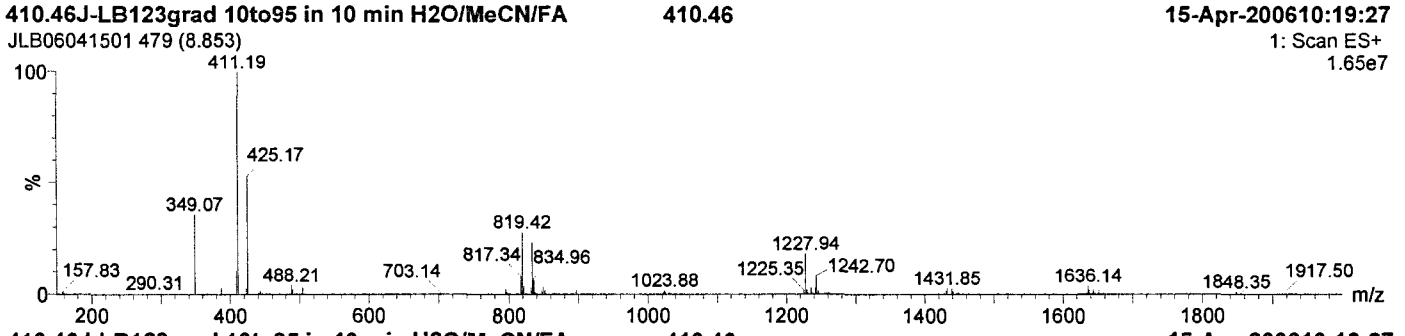


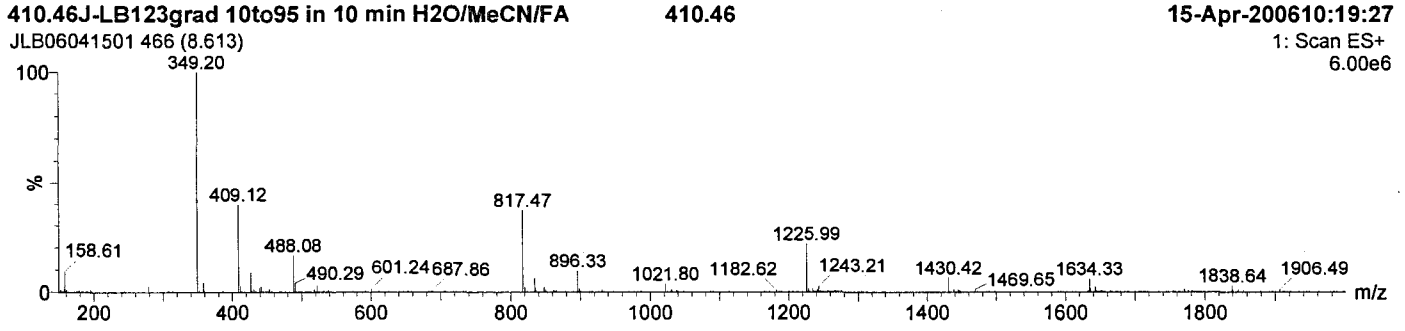
Compound 61.2:



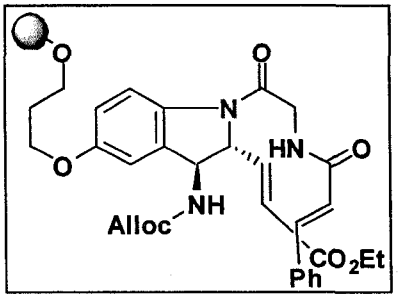


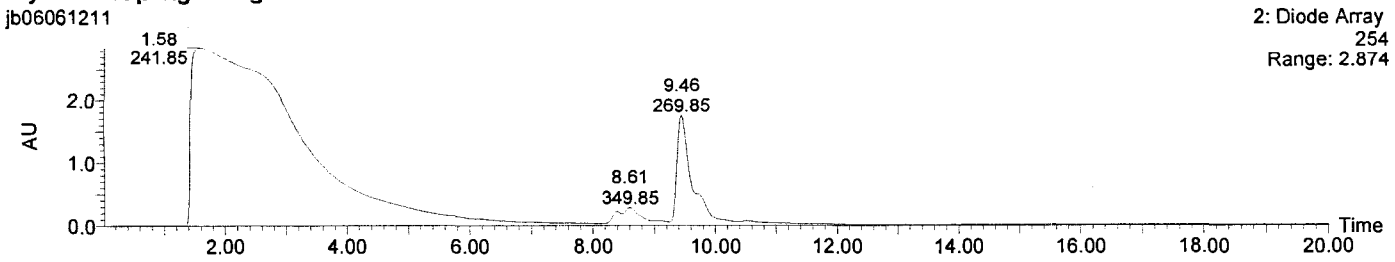


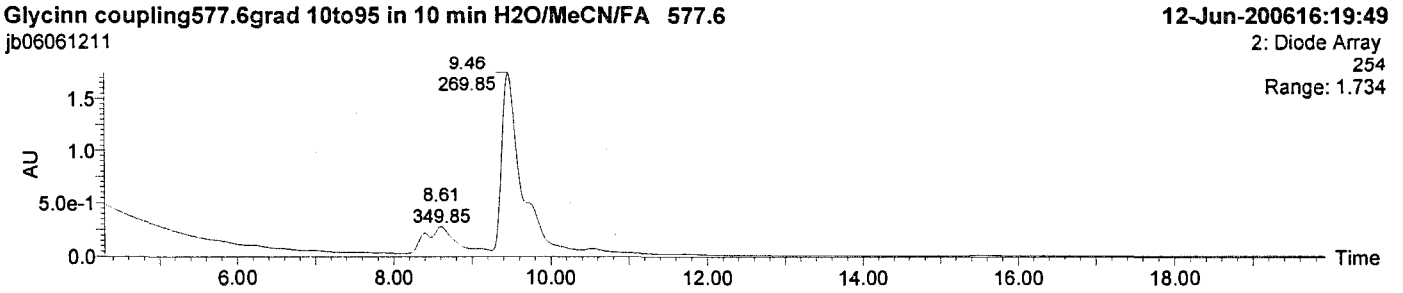


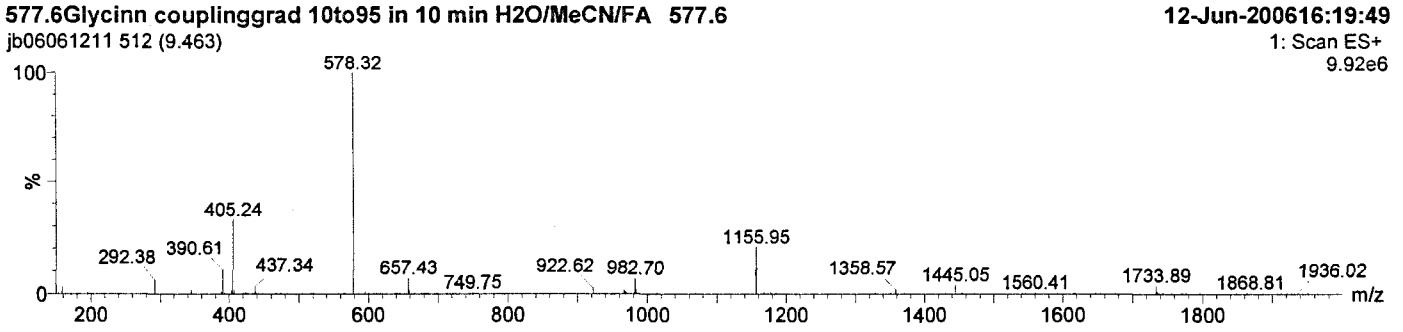


Compound 62.1:

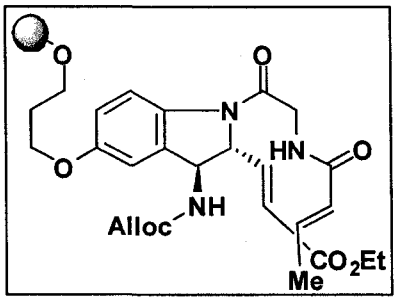


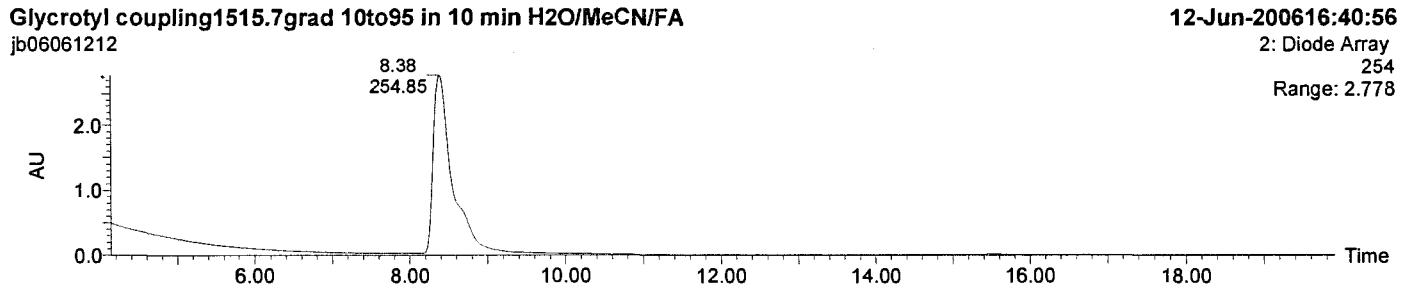


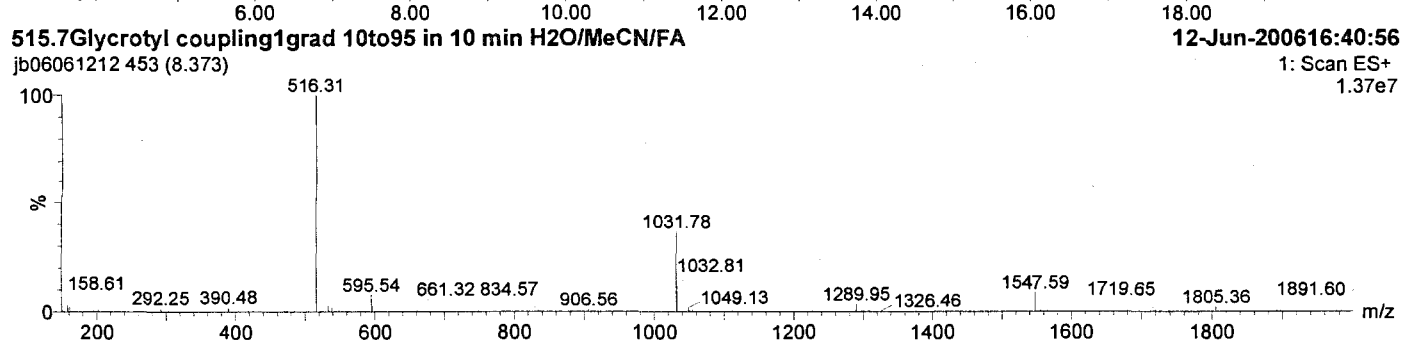




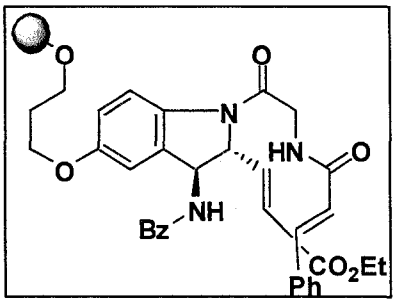
Compound 63.1:

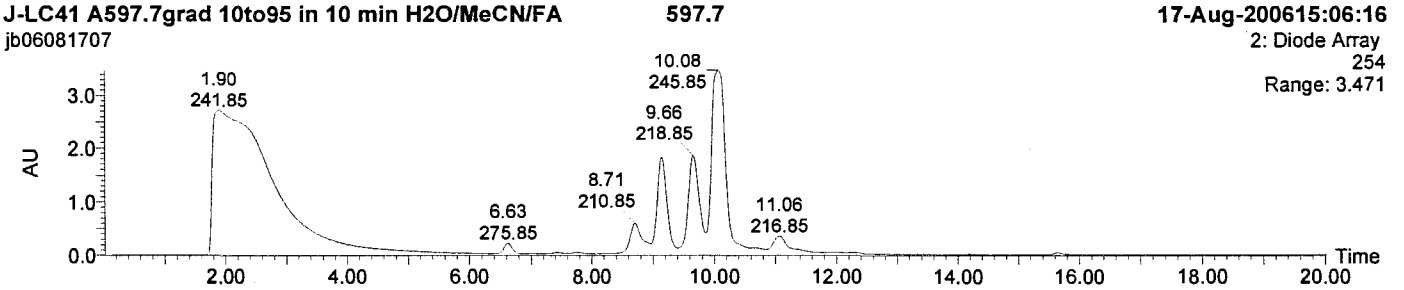


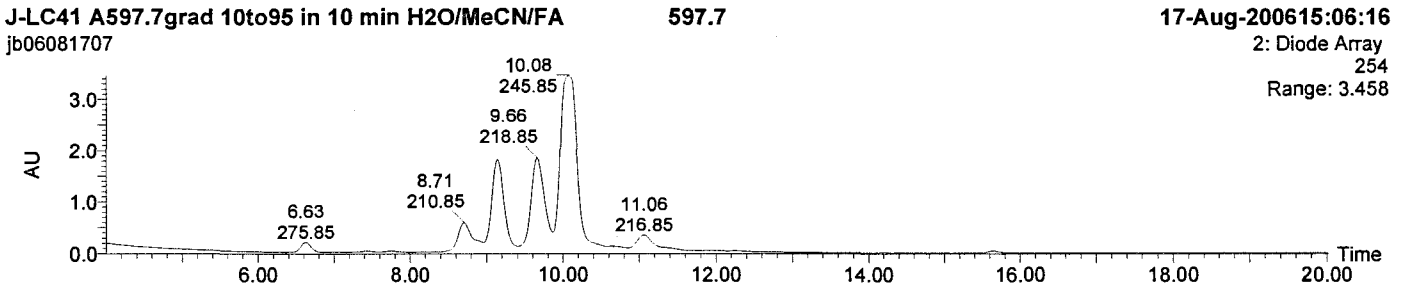


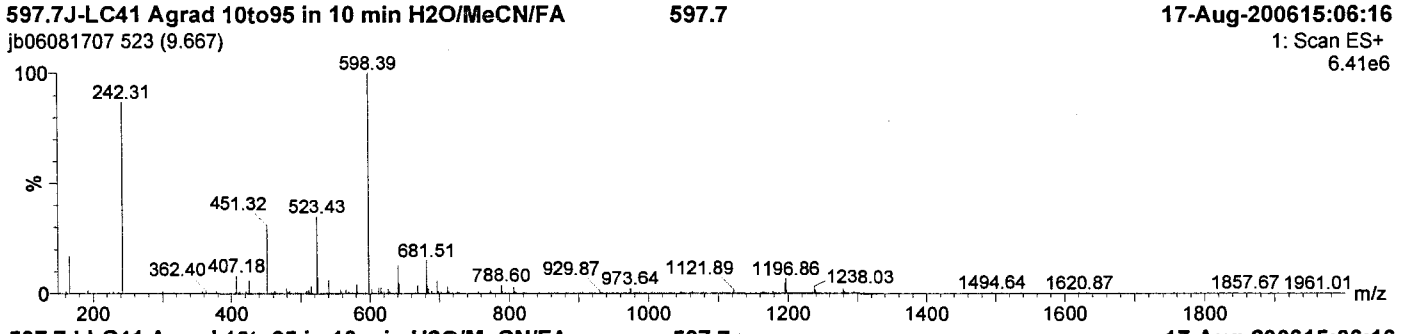


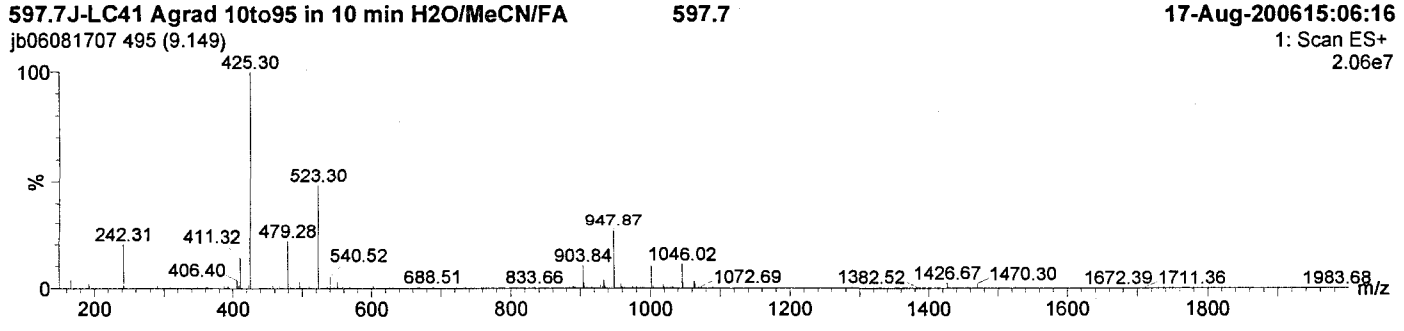
Compound 62.2:

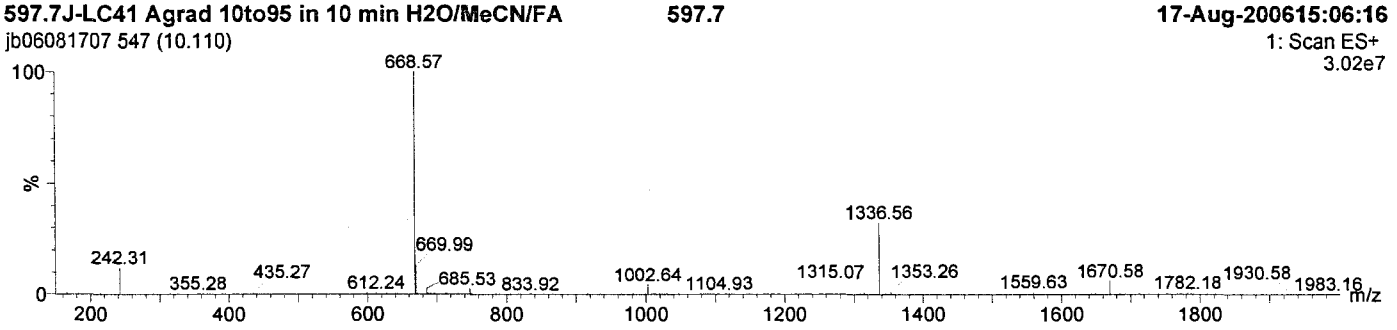




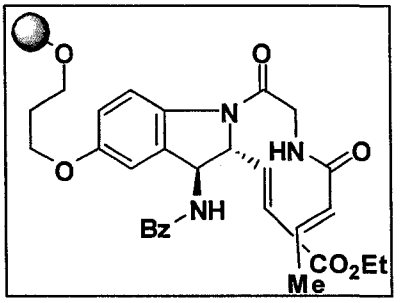


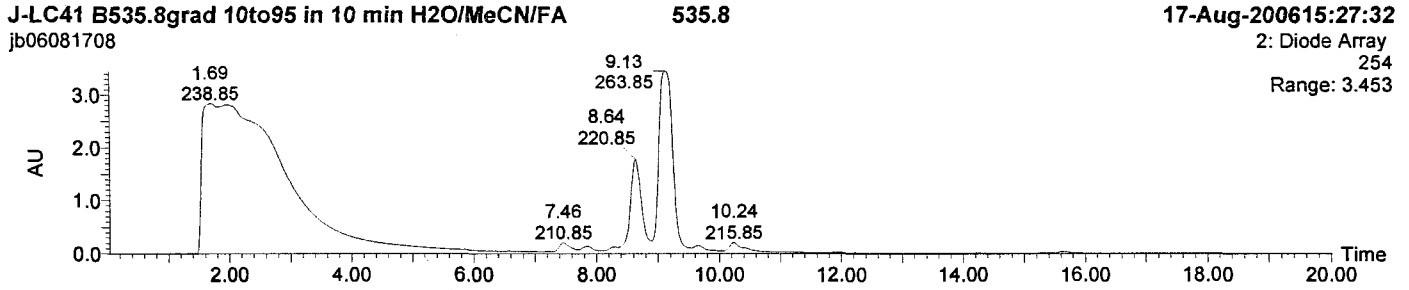


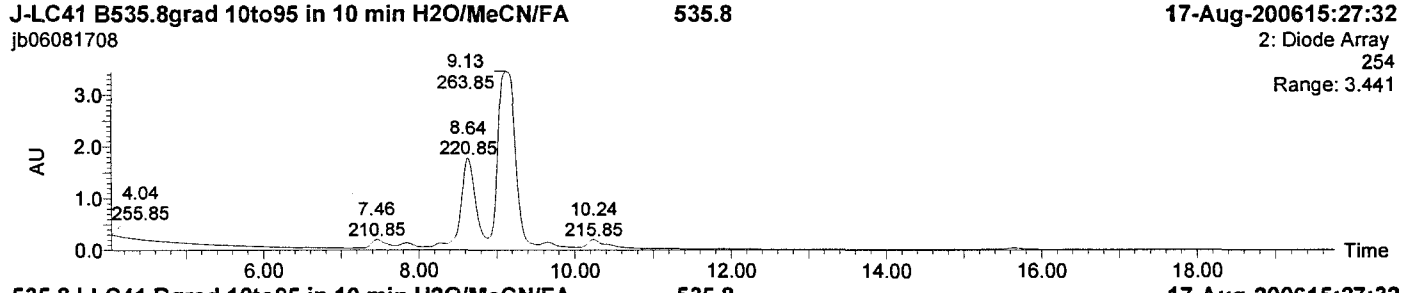


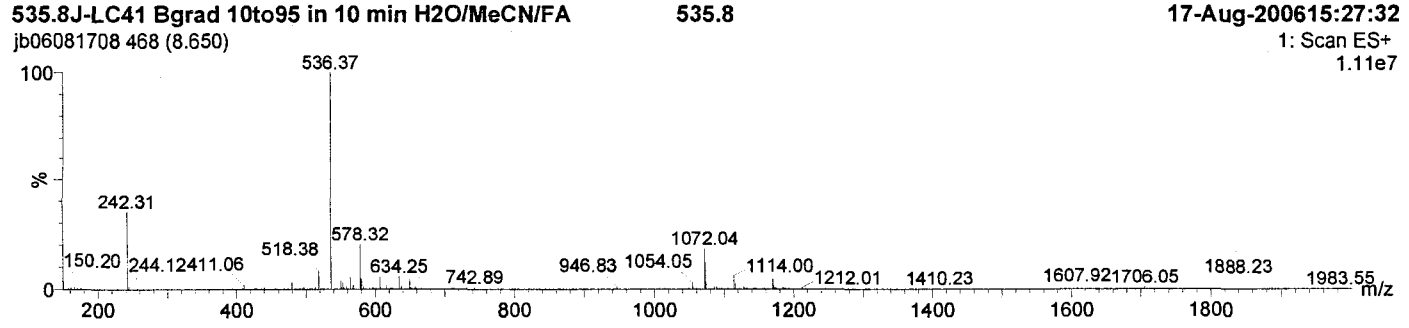


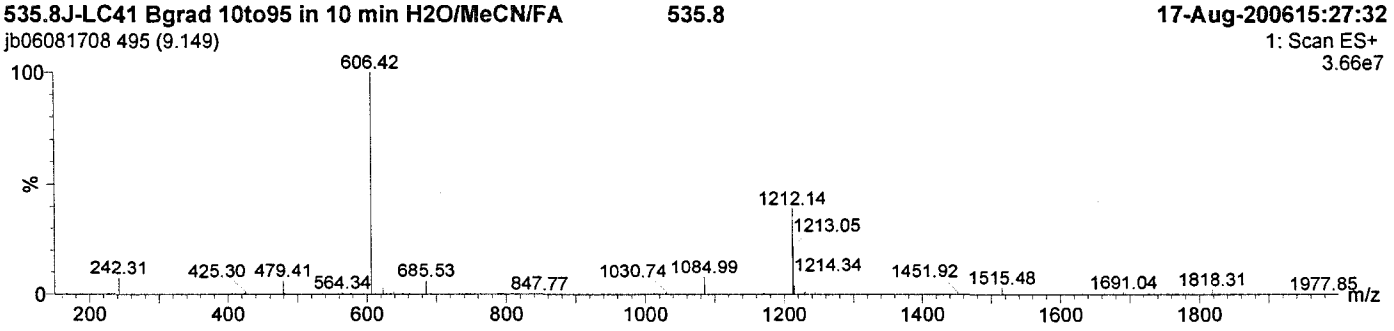
Compound 63.2:



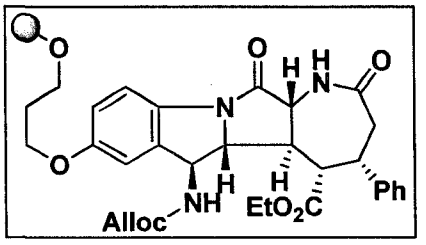


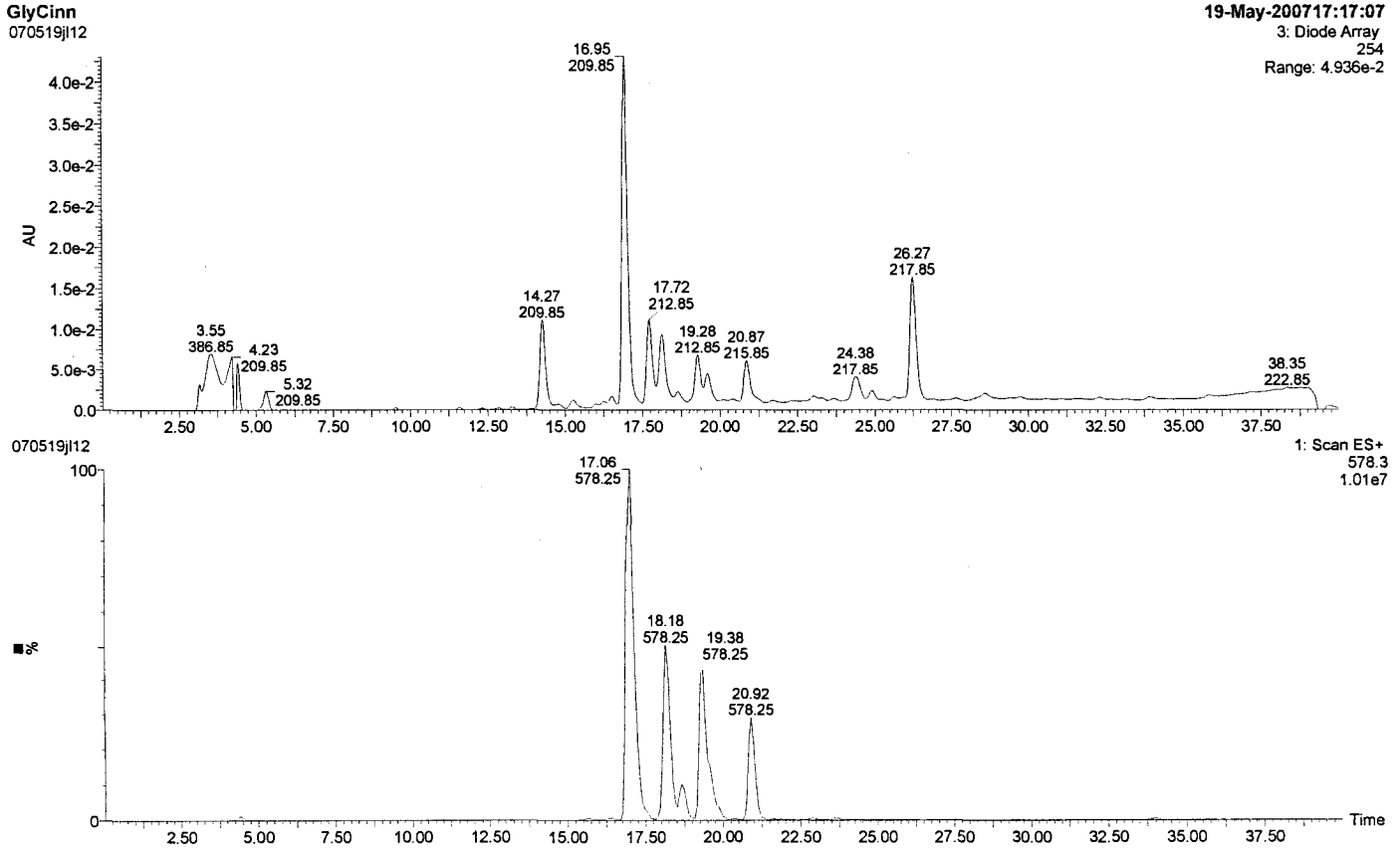


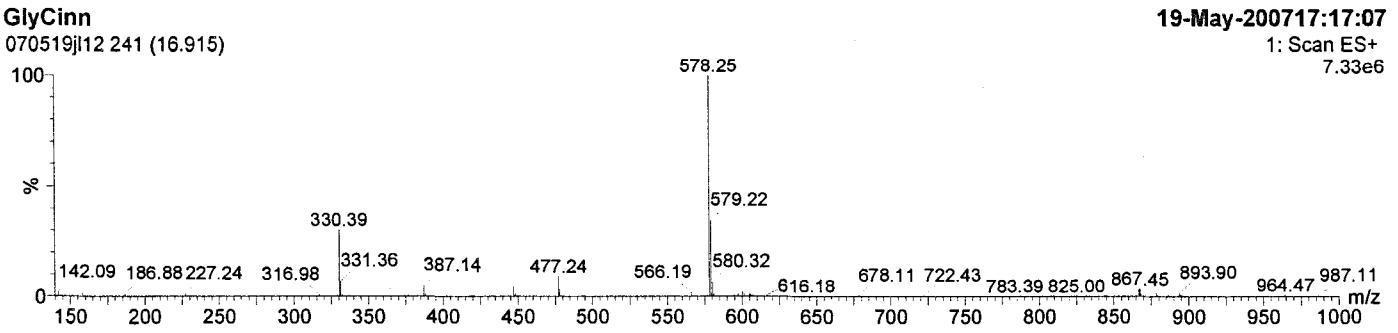




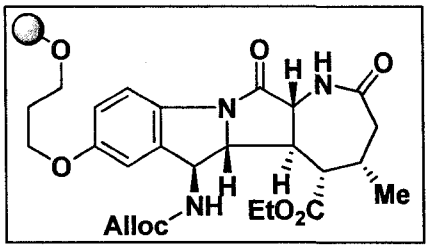
Compounds 64.1:

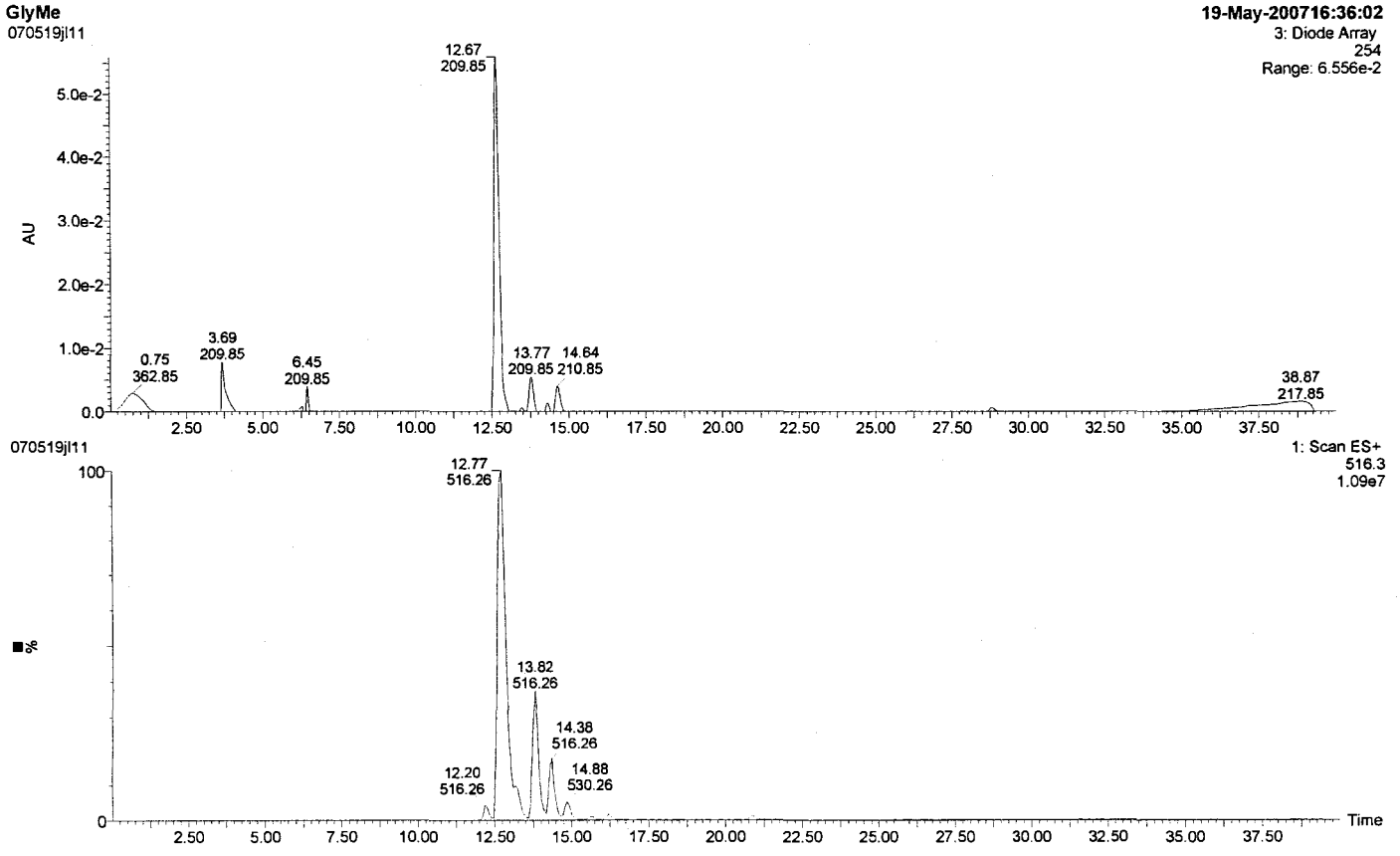


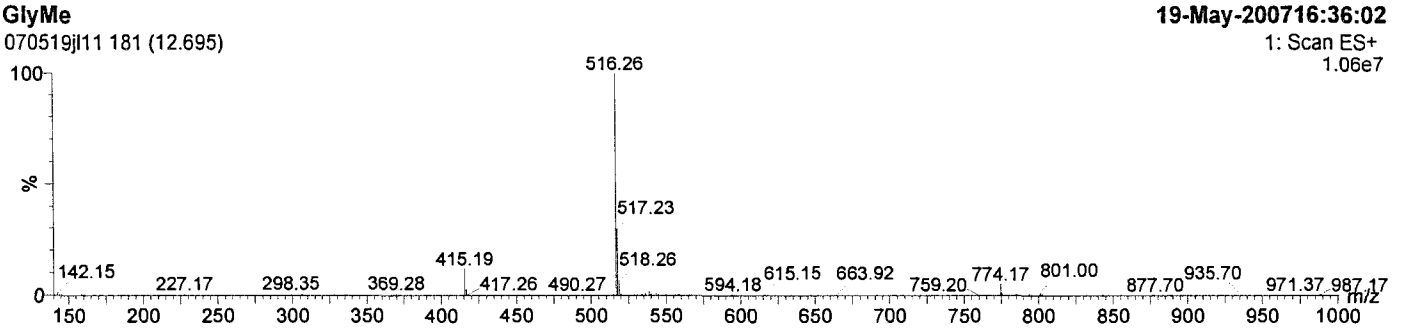




Compounds 64.3:

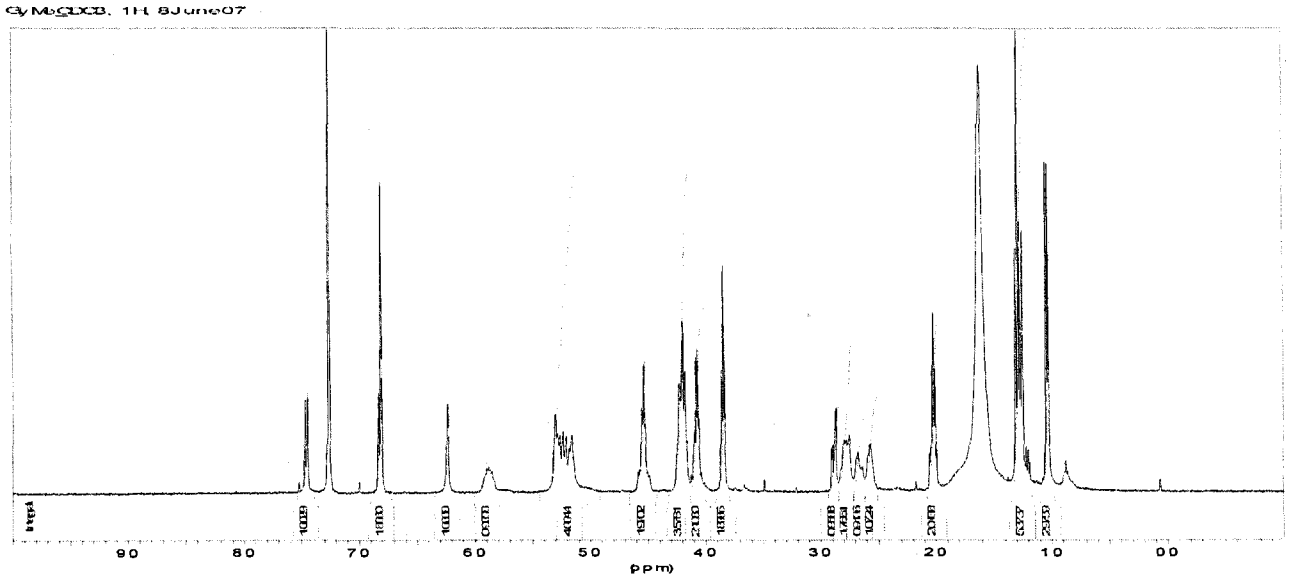




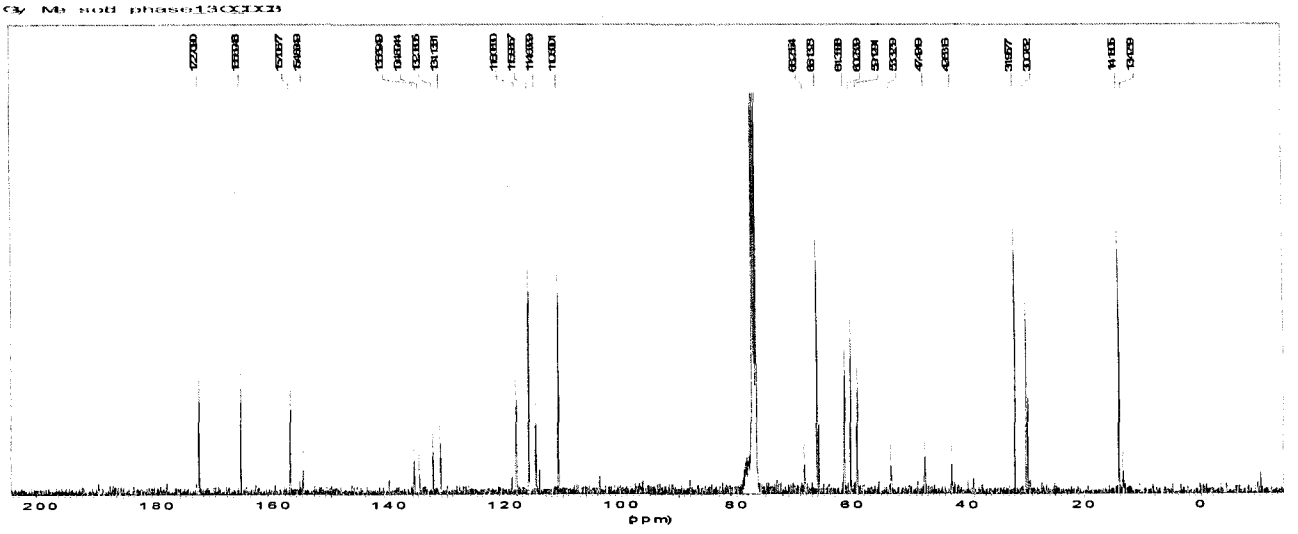


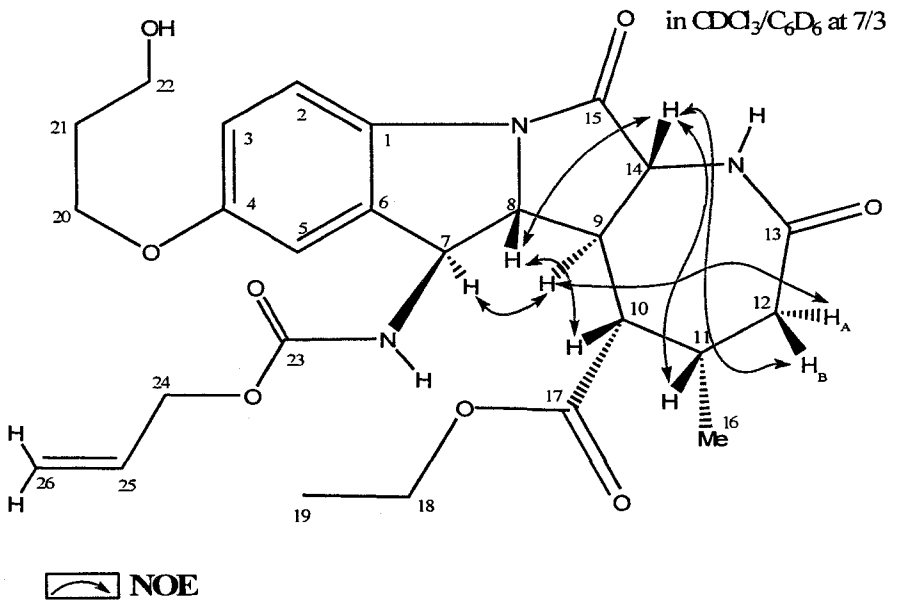
Analysis of the compound **64.3**:

^{1}H NMR (400 MHz, $CDCl_3$)

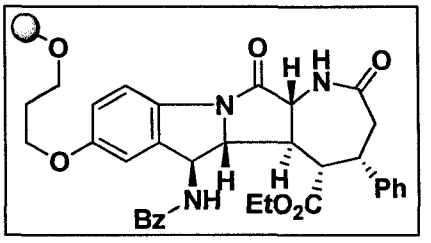


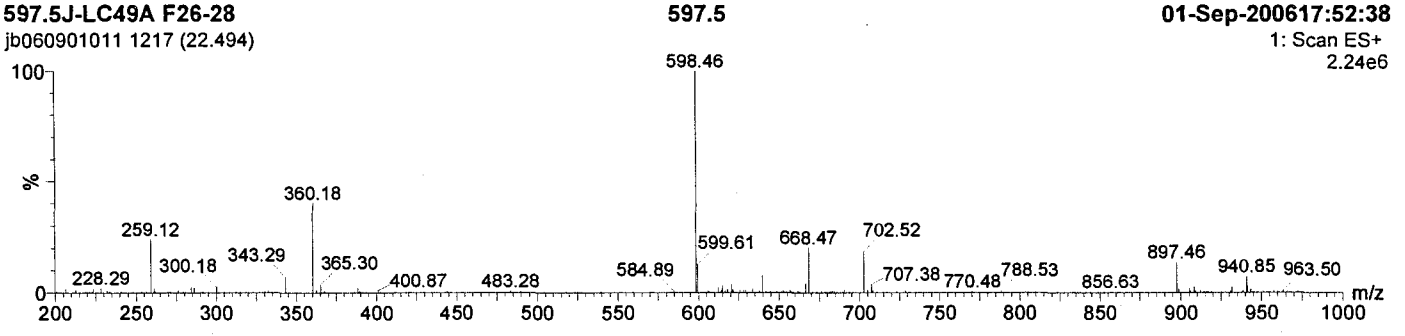
^{13}C NMR (100 MHz, $CDCl_3$)



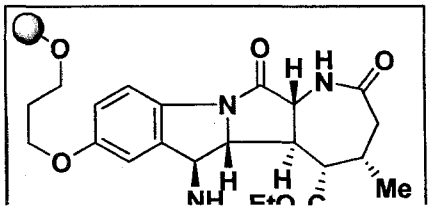


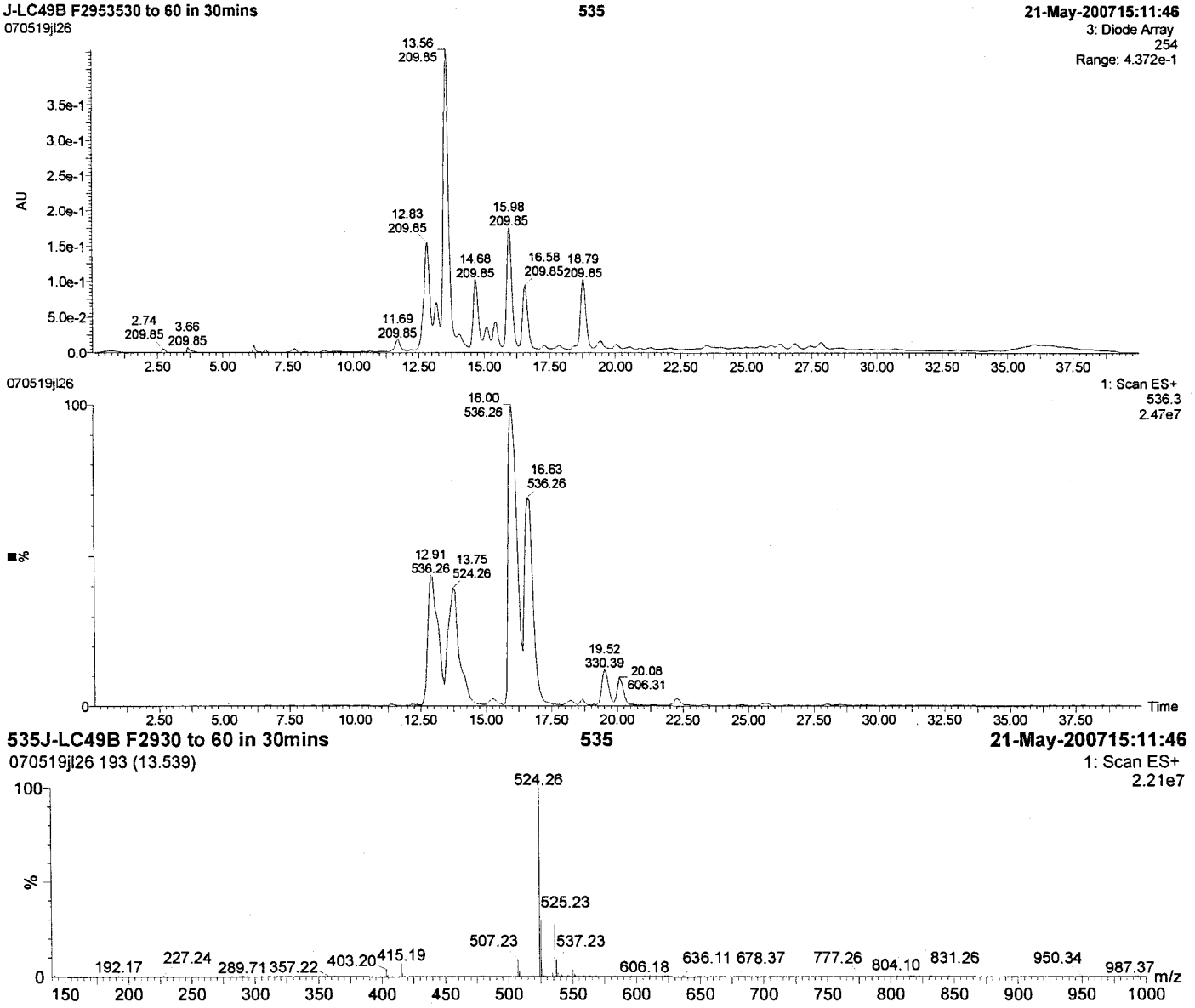
Compounds 64.2:





Compound 64.4:





Data on the X-ray structure of compound 22.1:

Table 1. Crystal data and structure refinement for sadf.

Identification code	sadf
Empirical formula	C99.24 H99.24 Cl9.71 N8 O16
Formula weight	2004.26
Temperature	398(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 17.1257(13) Å □= 90°.
	b = 29.451(2) Å □= 90°.
	c = 39.372(3) Å □ = 90°.
Volume	19858(3) $Å^3$
Z	8
Density (calculated)	1.341 Mg/m^3
Absorption coefficient	0.341 mm^{-1}
F(000)	8350
Crystal size	0.50 x 0.40 x 0.10 mm^3
Theta range for data collection	1.03 to 20.75°.
Index ranges	-17<=h<=16, -29<=k<=29, -39<=l<=39
Reflections collected	118886
Independent reflections	20487 [R(int) = 0.0585]
Completeness to theta = 20.75°	99.8 %
Absorption correction	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	20487 / 1680 / 2095
Goodness-of-fit on F^2	1.026
Final R indices [I>2sigma(I)]	R1 = 0.0891, wR2 = 0.2320
R indices (all data)	R1 = 0.1251, wR2 = 0.2713
Absolute structure parameter	0.05(10)
Extinction coefficient	0.00069(10)
Largest diff. peak and hole	0.727 and -0.456 $e.Å^{-3}$

Publication:

Reagent-Based, Modular, Tandem Michael Approach for Obtaining Different Indoline Alkaloid-Inspired Polycyclic Architectures

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Received December 14, 2007

A modular, reagent-based approach to obtain different indoline alkaloid-inspired, tetracyclic architectures is developed. With the use of TBSOTf as a Lewis acid, we report here a tandem Michael-based approach that led to the synthesis of a diastereomeric mixture of tetracyclic derivatives with two additional six-membered rings. By simply changing the Lewis acid to TMSOTf, we were able to obtain a different tetracyclic compound having additional functionalized 5- and 7-membered rings with complete stereocontrol.

With the rapid rise in utilization of small molecule probes in understanding biological functions, the need for accessing natural products, natural product analogs and natural product-inspired small molecules has also grown.[1] In particular, the development of modular approaches on solid phase is attractive because they allow the possibility of generating different architectures in a high-throughput manner.[2]

Herein, we report a modular, reagent-based approach for developing the synthesis of enantio-enriched, alkaloid-inspired, polycyclic derivatives. The key reaction in our approach is the tandem Michael/Aza−Michael reaction that was reported in the literature.[3] In particular, we were interested in developing this methodology on the indoline/aminoindoline scaffolds because of our interest in having a wide access to different indoline alkaloid natural product-inspired polycyclic architectures.[4] In addition to developing this modular approach in solution, we were also interested in solid-phase synthesis leading to high-throughput generation of several derivatives.

In a model study, the commercially available (*S*)-indoline-2-carboxylic acid **1** (Scheme 1) yielded compound **2** in four steps. Following *N*-Alloc removal, coupling of modified amino acid **3** was then carried out giving compound **4** as the starting material to explore the tandem Michael reaction. The first reaction conditions, TBSOTf/NEt_3 at a low temperature, produced a mixture of tetracyclic derivatives (48%, see products **5a**, **5b**, and **5c**) and the tricyclic compounds (20%, **5d**). All the products were assigned by 2D NMR studies. To increase the yield of the tetracyclic compounds in this reaction, other Lewis acids, TESOTf and TMSOTf, were then used.

With the use of TMSOTf, we were pleased to observe the complete transformation, yielding only one tetracyclic compound. Another surprise came from the structural assignment that revealed a totally different architecture (Scheme 2, compounds **7** and **8**). In addition to the NMR studies, the X-ray structure of the unique compound **7** finally confirmed the stereochemistry (Figure 3). Compound **7** crystallizes as a solvate from chloroform in the orthorhombic space group $P2_12_12_1$ with a = 17.1257 (13) Å, b = 29.451(2) Å, and c = 39.372(3) Å (Table 1). There are eight molecules of **7** in the asymmetric unit. All exhibit the same stereochemistry. Complete X-ray data for **7** can be found in the Supporting Information. With the use of TMSOTf, the reaction was very fast and the complete disappearance of the starting material took place in a less than 20 min yielding compound **7**. This reaction was repeated with the other starting material, **6**, and as observed previously, only a single tetracyclic compound **8** was obtained with a complete stereocontrol.

To explain the stereochemical outcome of this reaction, four transition states could be postulated as shown in Figure 1 (**A**, **B**, **C** and **D**). In structures **A** and **B**, the first enol adopts the Z configuration, which is less stable than the E configuration shown in structures **C** and **D**. Moreover, in the **B** and **D** structures, the disfavored interaction between an electron-deficient alkene and the equatorial proton from the indoline core allows us to postulate that it is the privileged structure **C** that leads to the correct stereochemistry. The generation of next two stereocenters could be explained by consideration of the four privileged conformations of the precursor of the 7-membered ring (Figure 1). Transition states **E** and **F** (Figure 1) indicate a strong disfavored interaction from the ester group in the less stable axial position with the Lewis Acid complex. Finally, a strong disfavored interaction between the side chain and an electron-deficient olefin in transition state **G** led us to propose structure **H** as the favored one leading to the observed stereochemistry.

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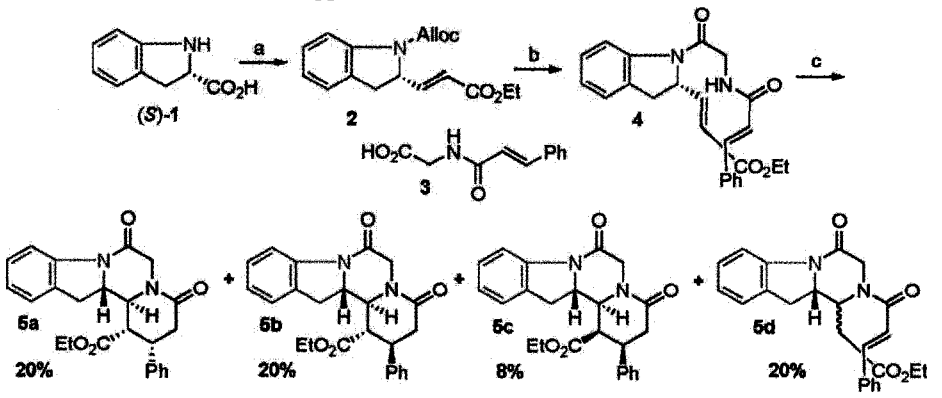
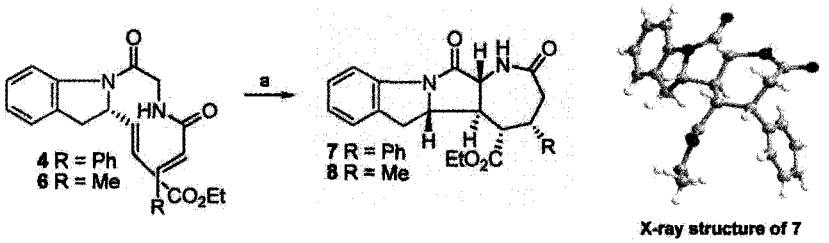
Scheme 1. TBSOTf-Mediated Tandem Michael Approach[a]



[a] Reagents and conditions: (a) (i) $LiAlH_4$, 84% (ii) Alloc-Cl, DIPEA, 99% (iii) SO_3–pyridine, DMSO, Et_3N, 84% (iv) $Ph_3C{=}CHCO_2Et$, 95%; (b) (i) $Pd(Ph_3)_4$, morpholine, 90% (ii) **3**, HATU, DIPEA, 83%; (c) TBSOTf, Et_3N, 68%.

Scheme 2. TMSOTf-Mediated Tandem Michael Approach[a]



[a] Reagents and conditions: (a) TESOTf or TMSOTf, Et_3N (for **7**, 91% and **8**, 80%).

Table 1. Crystal Data and Structure Refinement for the Compound **7**

identification code	sadf
empirical formula	$C_{99.24}H_{99.24}Cl_{9.71}N_8O_{16}$
fw	2004.26
temp	398(2) K
wavelength	0.71073 Å
cryst syst	orthorhombic
space group	$P2_12_12_1$
unit cell dimensions	
a	17.1257(13) Å
b	29.451(2) Å
c	39.372(3) Å
α	90°
β	90°
γ	90°
vol	19858(3) Å^3
Z	8
density (calcd)	1.341 Mg m^{-3}
abs coeff	0.341 mm^{-1}
F(000)	8350
cryst size	0.50 × 0.40 × 0.10 mm^3
θ range for data collection	1.03–20.75°
index ranges	$-17 \le h \le 16$
	$-29 \le k \le 29$
	$-39 \le l \le 39$
reflns collected	118 886
independent reflns	20 487 [R_{int} = 0.0585]
completeness to θ = 20.75°	99.8%
abs correction;	semiempirical from equivalents
refinement method	full-matrix least-squares on F^2
data/restraints/params	20 487/1680/2095
GOF on F^2	1.026
final R indices [$I > 2\sigma(I)$]	R1 = 0.0891, wR2 = 0.2320
R indices (all data)	R1 = 0.1251, wR2 = 0.2713
absolute structure parameter	0.05(10)
extinction coeff	0.00069(10)
largest diff. peak and hole	0.727 and −0.456 e Å^{-3}

The next series of experiments was then tried with a starting material in which the Gly-moiety in the side chain (**9**, Scheme 3) was replaced by another amino acids. The cyclization of compound **10** led to a mixture of **11a** and **11b** in a ratio of 3:1. It appears that the bulky isobutyl group induces the cyclization to occur through the pathway shown in Scheme 1. After this, we then decided to work with the systems that were derived from aminoindoline derivatives **13** and **15** (Scheme 4), which could be easily obtained from the starting material **12**. When TBSOTf was used as the Lewis acid, there was no sign of the cyclization reaction. This may be attributed to steric effects because this study only led to the tricyclic **14** with the predictable stereochemistry (see the Supporting Information). When the Lewis acid used was changed to TMSOTf, two isomers of tetracyclic compounds **16a/17a** and **16b/17b** were formed in a 2:1 ratio with the same architecture observed with the model study. At this stage it was difficult to assign the stereochemistry because the *N*-Alloc group usually results in broad signals in NMR. To solve this problem, the *N*-Alloc group was replaced with the *N*-benzoyl group and this replacement gave sharp NMR signals.

After the successful development of the tandem reaction-based method in solution, the method was then applied to the solid phase. To our knowledge, there are no examples in the literature that describe the application of this approach on the solid phase leading to library generation. For this study, compound **19** (Scheme 5) was prepared with the three-carbon spacer and the appropriate protecting groups to be compatible for the solid-phase synthesis. The loading of compound **19** was near quantitative, giving compound **20** anchored onto the solid support. After a few steps on the solid phase support, compounds **21**–**24** were obtained. Compound **21** (Scheme 5) was subjected to cyclization to

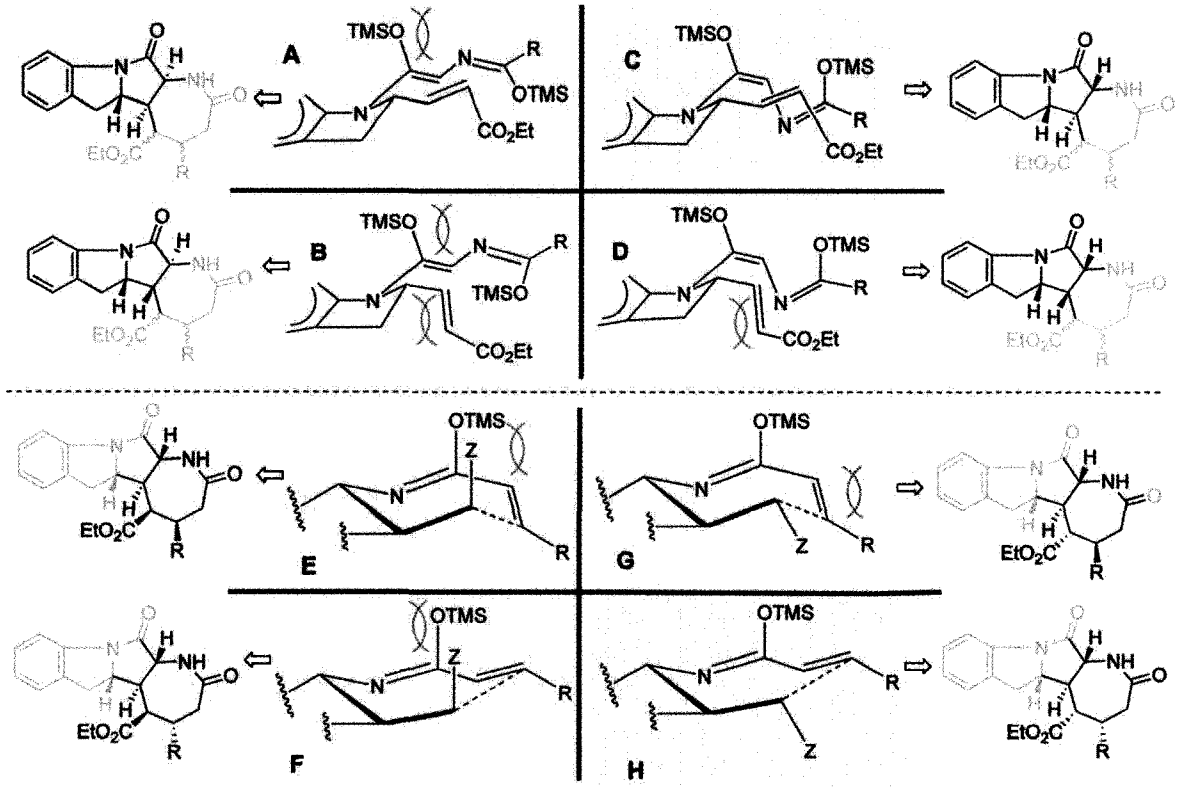


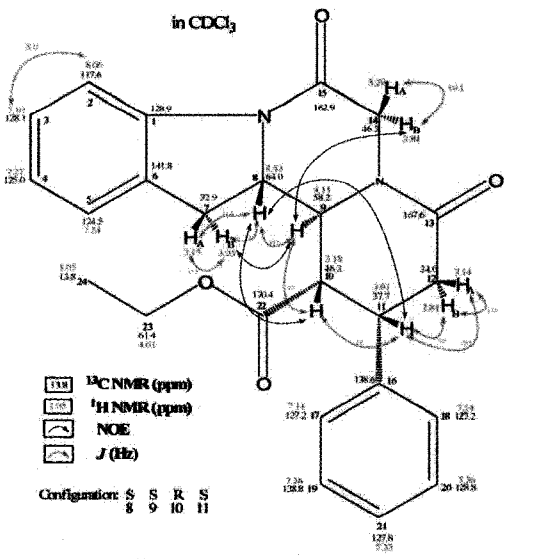
Figure 1. Proposed transition states to explain the observed trans-fused system and the seven-membered ring stereochemistry.

give a mixture of four isomers in an excellent yield (98%) with a ratio of 7.4:1.2:0.3:1.1. The major isomer **25a** was separated by preparative HPLC, and the stereochemistry was thoroughly assigned using 2D NMR. Compounds **22**, **23**, and **24**, upon cyclization reaction, gave the corresponding desired tetracyclic products **26**, **27**, and **28** in moderate to good yields (60%, 45%, and 72%) as diastereomeric mixtures (**26** = 6.7:1.6:0.9:0.8, **27** = 2.2:7.8, and **28** = 2:5:1.9:1.1). For **26** and **27**, the major isomers were separated by preparative HPLC, but their stereochemistry could not be assigned because of an overlap of signals in NMR even in various solvents. Further work, using this solid-phase methodology, is ongoing in library generation, and applications of these compounds will be reported in the near future.

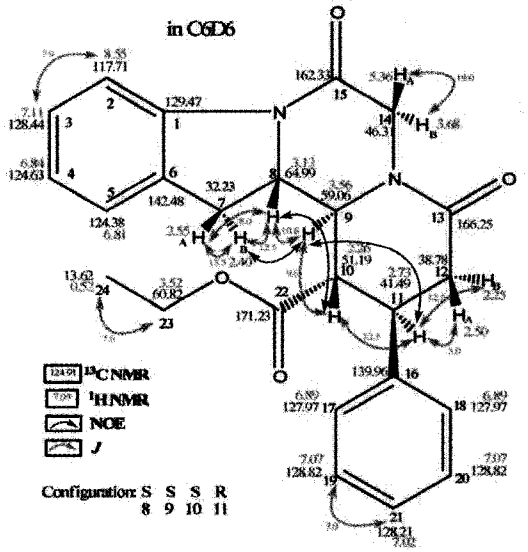
Experimental Section

All reactions were carried out in flame-dried glassware under an atmosphere of nitrogen with magnetic stirring. Thin-layer chromatography (TLC) was done on EMD (art. no. 5715-7) precoated silica gel 60 F_{254} glass plates (layer thickness 0.25 mm). Visualization was achieved with a UV lamp (254 nm) or by staining with vanillin, $KMnO_4$ solution, ammonium molybdate/ceric sulfate solution. Flash column chromatography was performed using silica gel 60 (40−63 μm, Silicycle) or Biotage Horizon Flash Chromatography System. Solvents were purified as follows: trace amounts of water and oxygen from THF, DMF, and dichloromethane were removed using columns containing activated alumina and copper under N_2. Triethylamine, pyridine, ethyl ether and toluene were obtained from commercial suppliers (EMD and Aldrich) and used without further purification. NMR spectra were recorded on a Bruker DRX 400 MHz spectrometer. All chemical shifts are reported in parts per million (δ). ^{1}H NMR (400 MHz) spectra were recorded at room temperature in $CDCl_3$, C_6D_6, or DMSO-d_6 solutions and referenced to residual $CHCl_3$ (7.27 ppm) or C_6H_6 (7.16 ppm) or DMSO (2.50 ppm). Fully decoupled ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$, C_6D_6, or DMSO-d_6 solutions. The center peaks of $CDCl_3$ (77.0 ppm), C_6D_6 (128.7 ppm), and DMSO-d_6 (39.43 ppm) were used as the internal reference. Mass spectra were carried out on a VG Quattro I (Micromass) mass spectrometer equipped with a pneumatically assisted electrospray ionization source, operating in positive mode. HPLC were performed using a Hewlett-Packard (Agilent) 1100 Series equipped with a diode array detector and a NovaPack C18 (3.9 × 300 mm) column. The enantiomeric excess was determined by chiral HPLC, using a Hewlett-Packard (Agilent) 1090 Series II Liquid Chromatograph equipped with a diode array detector and a CHIRACEL-OD column. HPLC/MS were performed using Waters equipment: Waters micromass ZQ ESCI Multi-Mode ionization, Waters 996 Photodiode Array Detector (254 nm), and Waters 2795 Separation Module with Phenomenex Spherisorb 3 ODS-2 column.

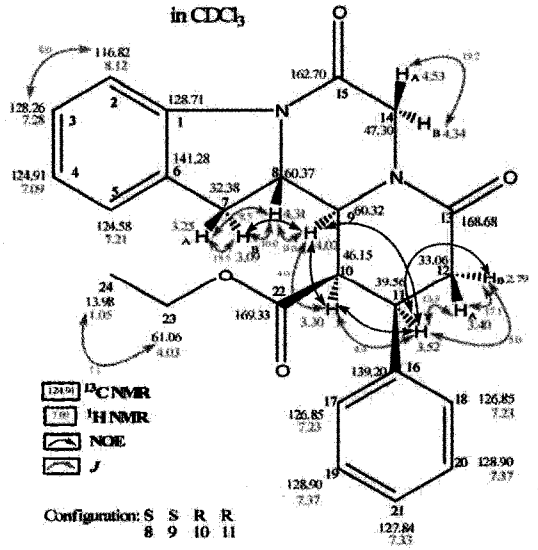
Small-scale solid-phase reactions (1−10 mg resin) were performed in 2 mL fritted polypropylene Bio-Spin chromatography columns. Medium-scale solid-phase reactions (10−200 mg) were performed in 10 mL polypropylene PD-10 columns. Agitation of solid-phase reaction mixtures was performed using a Barnstead-Thermolyne Labquake shaker. The linker cleavage reactions (<20 mg of beads) were carried out in 1.5 mL eppendorf tubes. Vacuum removal of solvents for the linker cleavage reactions was accomplished using Genevac HT-4 Atlas Evaporator. In cases where the products



Compound 5a



Compound 5b



Compound 5c

Figure 2. Diagram showing the ^{13}C NMR, ^{1}H NMR, NOE, and J values corresponding to the structures of compounds **5a**–**5c**.

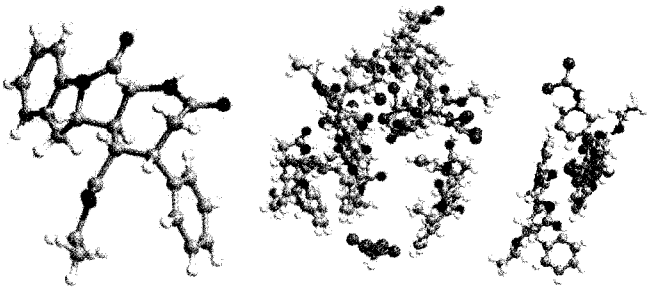


Figure 3. X-ray structure of compound **7** and the organization of compound **7** in the crystalline box.

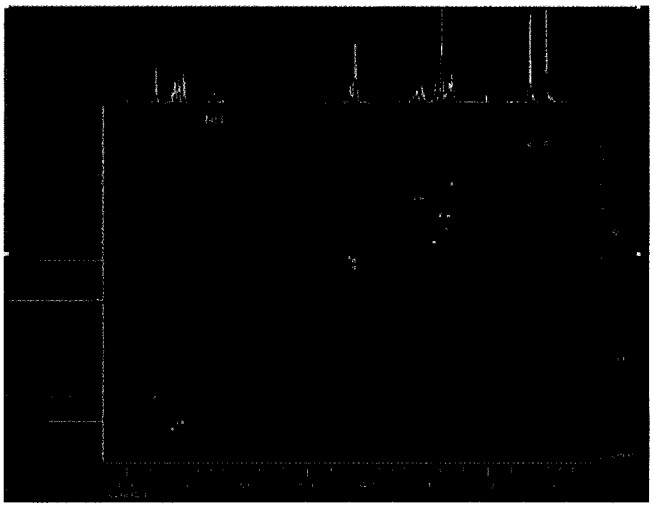
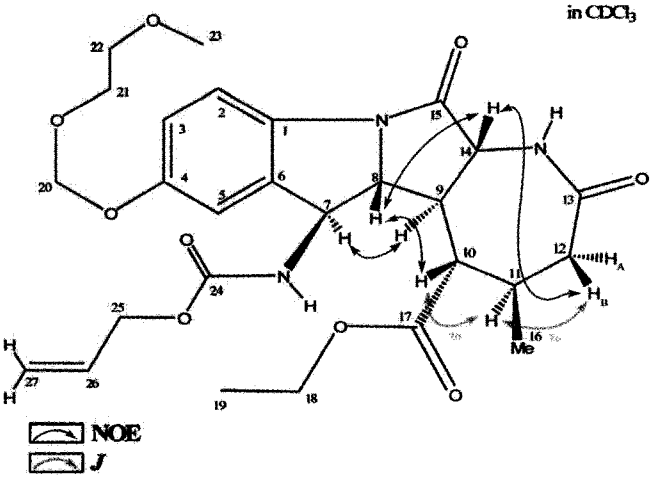


Figure 4. HSQC experiment showing the free NH within the 7-membered ring (no cross peak for −NH proton)

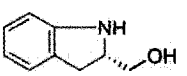


Compound 17b

Figure 5. NOE and J values for the structure of compound **17b**.

from the solid phase were characterized by NMR, the reactions were carried out on a 15–20 mg resin scale.

Compound 1a, (*S*)-Indolin-2-ylmethanol.



To a solution of (*S*)-indoline-2-carboxylic acid (2.00 g, 12.19 mmol) in THF (50 mL) at −78 °C, was added $LiAlH_4$ (1 M solution in hexane, 24.38 mL, 24.38 mmol). The reaction mixture was stirred at −78 °C under N_2 for 3 h. The reaction

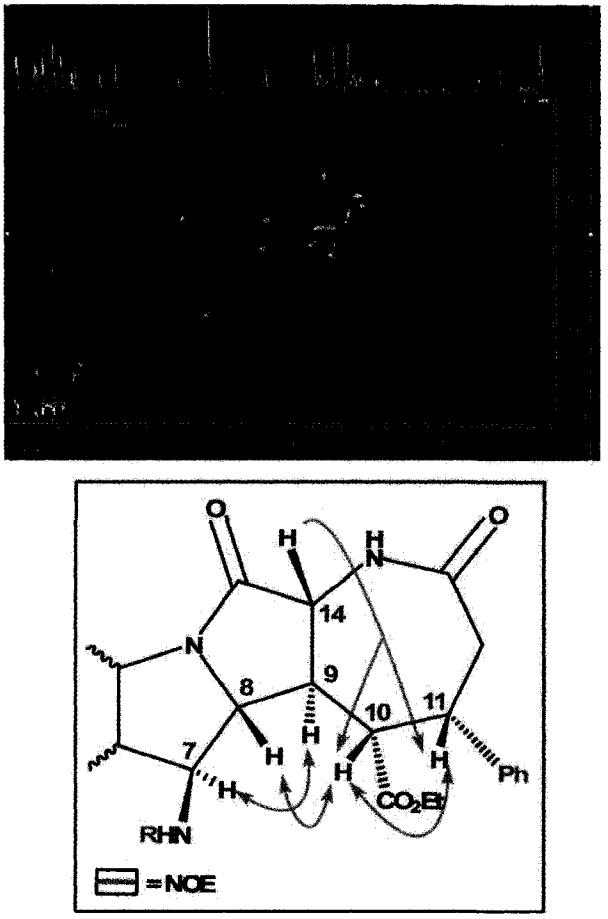
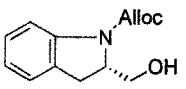


Figure 6. HSQC experiment showing the two free NH moieties from compound **18b**.

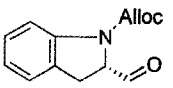
mixture was quenched with water and then extracted with dichloromethane. The combined organic layers were washed with brine then dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (3:2 v/v) gave 1.53 g (84%) of product as a light brown solid: MS (ES+) *m/z* (M + 1) 150.1; HRMS (FAB) *m/z* (M^+) calcd 149.08 for $C_9H_{11}NO$, obsd 149.09; mp = 65.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, J = 6.7 Hz, 1H), 7.07 (dd, J = 7.6, 7.6 Hz, 1H), 6.79 (dd, J = 7.5, 7.4 Hz, 1H), 6.73 (d, J = 7.8 Hz, 1H), 4.15−4.08 (m, 1H), 3.78 (dd, J = 11.0, 3.8 Hz, 1H), 3.64 (dd, J = 11.0, 6.3 Hz, 1H), 3.36 (br s, 2H), 3.15 (dd, J = 15.8, 9.2 Hz, 1H), 2.89 (dd, J = 15.8, 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.0, 129.6, 127.9, 125.3, 120.3, 111.10, 65.4, 60.9, 32.3.

Compound 1b, (*S*)-Allyl 2-(hydroxymethyl)indoline-1-carboxylate.



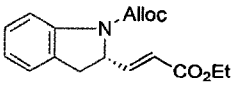
To a solution of the alcohol **2** (0.74 g, 4.90 mmol) in dichloromethane (69 mL) at −78 °C was added DIPEA (0.86 g, 1.15 mL, 4.90 mmol), followed by the addition of allylchloroformate (0.59 g, 0.52 mL, 4.90 mmol). The reaction mixture was stirred at −78 °C under N_2 for 2 h. The reaction mixture was quenched by addition of saturated ammonium chloride solution (25 mL), and then it was extracted with dichloromethane and ethyl acetate. The combined organic layers were washed with brine and dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (3:1 v/v) gave 1.14 g (99%) of product as a light yellow oil: MS (ES+) *m/z* (M + 1) 234.0; HRMS (FAB) *m/z* (M^+) calcd 233.11 for $C_{13}H_{15}NO_3$, obsd 233.113; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (br s, 1H), 7.22−7.18 (m, 2H), 7.01 (dd, J = 7.4, 7.3 Hz, 1H), 6.09−6.00 (m, 1H), 5.42 (d, J = 17.2 Hz, 1H), 5.32 (d, J = 10.4 Hz, 1H), 4.79 (d, J = 4.2 Hz, 2H), 4.66 (br s, 1H), 3.81 (dd, J = 11.1, 5.9 Hz, 1H), 3.75 (dd, J = 11.2, 5.0 Hz, 1H), 3.38 (dd, J = 16.4, 10.1 Hz, 1H), 2.93 (br s, 1H), 2.54 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 142.1, 132.7, 130.5, 127.9, 125.2, 123.5, 118.8, 116.1, 67.0, 65.8, 61.5, 31.7.

Compound 1c, (*S*)-Allyl 2-formylindoline-1-carboxylate.



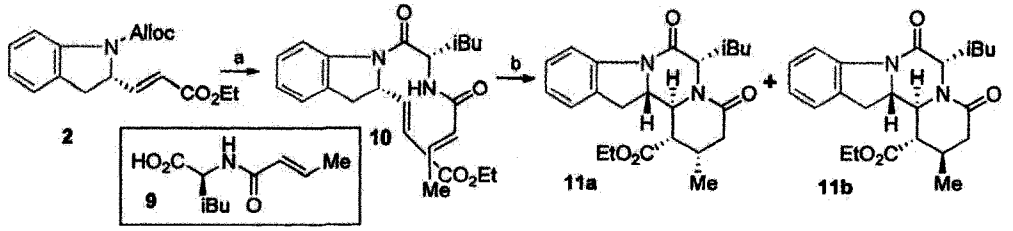
To a solution of the indoline alcohol **3** (3.80 g, 16.31 mmol) in dichloromethane (178 mL) was added DMSO (45 mL), followed by Et_3N (11.4 mL, 81.55 mmol) and then $SO_3 \cdot Py$ (10.38 g, 65.24 mmol), in that specific order. The reaction mixture was stirred at room temperature under N_2 for 3 h. The reaction mixture was quenched by addition of a saturated ammonium chloride solution, and then it was extracted with dichloromethane and ethyl acetate. The combined organic layers were washed with brine and dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (3:1 v/v) gave 3.18 g (84%) of product as a light yellow oil: MS (ES+) *m/z* (M + 1) 232.0; HRMS (FAB) *m/z* (M^+) calcd 232.10 for $C_{13}H_{13}NO_3$, obsd 232.0985; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.96 (br s, 1H), 7.28−7.26 (m, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.02 (dd, J = 7.4, 7.3, 1H), 5.96 (br s, 1H), 5.44−5.30 (m, 2H), 4.87−4.75 (m, 3H), 3.46−3.44 (m, 1H), 3.22 (dd, J = 16.6, 4.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.2, 153.0, 142.5, 132.4, 130.5, 128.6, 125.1, 123.8, 119.1, 115.5, 66.9, 66.3, 30.3.

Compound 2, (*S,E*)-Allyl 2-(3-ethoxy-3-oxoprop-1-enyl)indoline-1-carboxylate.



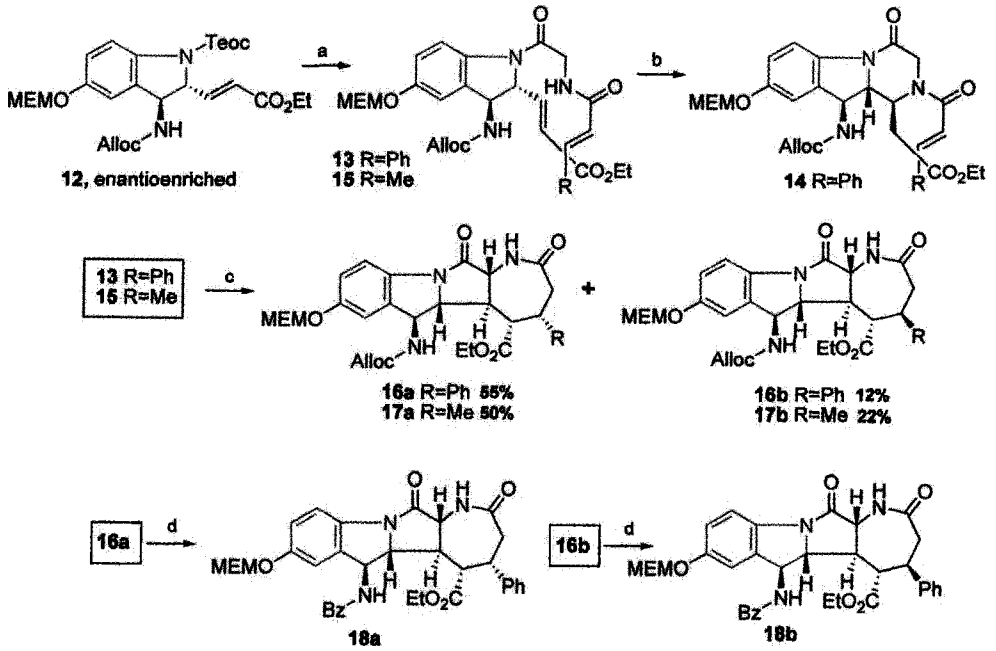
To a solution of the indoline aldehyde **4** (3.17 g, 13.71 mmol) in dichloromethane (102 mL) was added the Wittig reagent, (carbethoxymethylene) triphenylphosphorane (5.75 g, 16.51 mmol). The reaction mixture was stirred at RT, under N_2 for 2 h 20 min. The reaction mixture was quenched by adding brine, and then it was extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (9:1 v/v) gave 3.84 g (95%) of the trans product and 0.089 g of the cis product. The trans product **2** was a yellow oil: MS (ES+) *m/z* (M + 1) 302.2; HRMS

Scheme 3. Tandem Michael Reaction with the Substituted Amino Acid Derivative[a]



[a] Reagents and conditions: (a) (i) $Pd(Ph_3)_4$, morpholine, 90% (ii) **9**, PyBroP, DMAP, DIPEA, 52%; (b) TMSOTf, Et_3N, 37%.

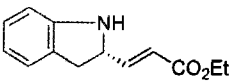
Scheme 4. Tandem Michael Reaction with an Aminoindoline Scaffold[a]



[a] Reagents and conditions: (a) (i) TBAF, 87% (ii) **3**, HATU, DIPEA, 58%; (b) TBSOTf, Et_3N, 76%; (c) TMSOTf, Et_3N, 67−72%; (d) (i) $Pd(Ph_3)_4$, morpholine (ii) BzCl, Et_3N, 67−70% for 2 steps.

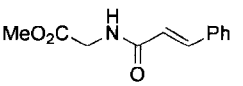
(FAB) *m/z* (M^+) calcd 301.13 for $C_{17}H_{19}NO_4$, obsd 301.14; ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 (br s, 1H), 7.23 (dd, J = 7.8, 7.5 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.00 (dd, J = 7.5, 7.4 Hz, 1H), 6.90 (dd, J = 15.6, 6.4 1H), 5.97 (br s, 1H), 5.91 (d, J = 15.6 Hz, 1H), 5.36 (d, J = 17.0 Hz 1H), 5.27 (d, J = 10.3 Hz, 1H), 5.12 (br s, 1H), 4.73 (br s, 2H), 4.17 (q, J = 7.1 Hz, 2 H), 3.50 (dd, J = 16.1, 10.4 Hz, 1H), 2.88 (dd, J = 16.2, 2.0 Hz, 1H), 1.27 (t, J = 7.1, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 153.0, 146.4, 142.5, 132.7, 129.2, 128.3, 125.4, 123.7, 121.5, 118.7, 115.8, 66.7, 60.9, 59.8, 34.6, 14.6.

Compound 2a, (*S*,*E*)-Ethyl 3-(indolin-2-yl)acrylate.



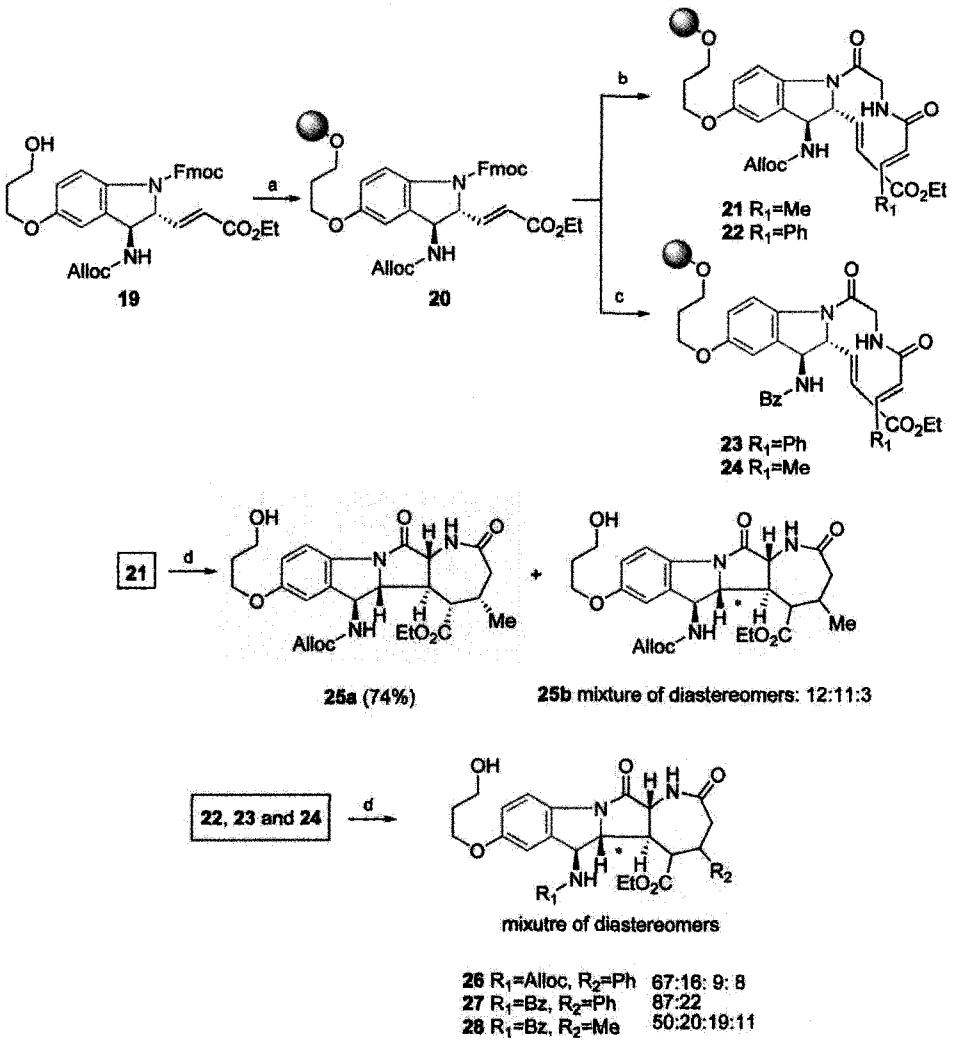
To a solution of the alloc indoline core **2** (1.03 g, 3.40 mmol) in dichloromethane (71 mL) at 0 °C was added $Pd(Ph_3)_4$ (0.39 g, 0.34 mmol), followed by morpholine (0.62 mL, 7.14 mmol). The reaction mixture was stirred from 0 °C to room temperature under N_2 for 4 h. The reaction solution was evaporated under vacuum pressure then purified by flash chromatography. Elution with hexane/ethyl acetate (9:1 v/v) gave 0.67 (90%) of product as a yellow oil: MS (ES+) *m/z* (M + 1) 218.1; HRMS (FAB) *m/z* (M^+) calcd 211.11 for $C_{13}H_{15}NO_2$, obsd 217.1116; ^1H NMR (400 MHz, $CDCl_3$) δ 7.12−7.03 (m, 3H), 6.76 (dd, J = 7.4, 7.4 Hz, 1H), 6.67 (d, J = 7.8, Hz, 1H), 6.05 (d, J = 15.6 Hz, 1H), 4.54−4.48 (m, 1H), 4.23 (q, J = 7.2 Hz, 2H), 3.90 (br s, 1H), 3.31 (dd, J = 15.5, 9.2 Hz, 1H), 2.88 (dd, J = 15.5, 7.8 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 150.5, 149.2, 134.9, 128.1, 125.1, 121.5, 119.6, 109.9, 60.9, 60.6, 36.5, 14.7.

Compound 3a, Methyl 2-cinnamamidoacetate.



To a solution of Gly−OMe HCl (1.31 g, 10.36 mmol) in dichloromethane (57 mL) was added DIPEA (3.51 mL, 20.72 mmol). The reaction mixture was stirred under N_2 for 15 min at room temperature. To this reaction mixture was added cinnamoyl-chloride (2.07 g, 12.43 mmol). The reaction mixture was stirred under N_2 for 1:25 h at RT. The reaction mixture was extracted with dichloromethane. The combined organic layers were washed with brine then dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with 3:10 ethyl acetate/hexane gave 1.75 g

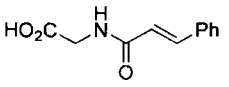
Scheme 5. Manual Solid-Phase Synthesis[a]



[a] Reagents and conditions: (a) alkylsilyl macrobeads (loading 0.99 mmol/g, 500−560μm), 95%; (b) (i) morpholine (ii) glycine acids, HATU, DIPEA; (c) (i) $Pd(PPh_3)_4$, PPh_3, 4-NMM, CH_3CO_2H, (ii) BzCl, 2,4,6-collidine, (iii) repeat b; (d) (i) TMSOTf, Et_3N (ii) HF-pyridine.

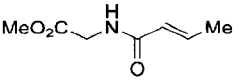
(77%) of product as a yellow oil: MS (ES+) *m/z* (M + 1) 220.1; HRMS (FAB) *m/z* (MH^+) calcd 220.10 for $C_{12}H_{14}NO_3$, obsd 220.1303; ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 15.6 Hz, 1H), 7.55−7.52 (m, 2H), 7.38−7.41 (m, 3H), 6.49 (d, J = 15.6 Hz, 1H), 6.19 (br s, 1H), 4.22 (d, J = 5.1 Hz, 2H), 3.82 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 166.2, 142.3, 135.0, 130.3, 129.3, 128.3, 120.1, 52.9, 41.2.

Compound 3, 2-Cinnamamidoacetic Acid.



To a solution of the peptide ester (0.10 g, 0.47 mmol) in 8:2:1 THF/water/MeOH (10 mL) at 0 °C was added LiOH (0.039 g, 0.93 mmol). The reaction mixture was stirred at 0 °C to room temperature under N_2 for 3 h. The reaction mixture was neutralized with Amberlite H^+ resin to pH 6 and then evaporated by vacuum pressure to give 0.10 g (99%) of product as a white solid: MS (ES+) *m/z* (M + 1) 206.1; HRMS (FAB) *m/z* (MH^+) calcd 206.08 for $C_{11}H_{12}NO_3$, obsd 206.087; ^1H NMR (400 MHz, acetone-d_6) δ 7.63−7.57 (m, 3H), 7.44−7.37 (m, 3H), 6.82 (d, J = 15.7 Hz, 1H), 4.11 (d, J = 5.0 Hz, 2H), 2.83 (br s, 1H); ^{13}C NMR (100 MHz, acetone-d_6) δ 172.4, 167.3, 141.9, 137.1, 131.3, 130.7, 129.5, 123.2, 42.6.

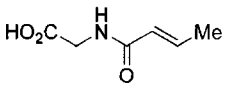
Compound 3b, (*E*)-Methyl 2-but-2-enamidoacetate.



To a solution of Gly-OMe HCl (4.01 g, 31.62 mmol) in dichloromethane (150 mL) was added DIPEA (11.00 mL, 63.24 mmol). The reaction mixture was stirred under N_2 for 15 min at room temperature. To this reaction mixture was added crotonyl chloride 90% (4.07 mL, 42.06 mmol). The reaction mixture was stirred under N_2 for 2 h at RT. The reaction was extracted with dichloromethane. The combined organic layers were washed with brine, then dried over magnesium sulfate, filtered, and purified by flash chroma-

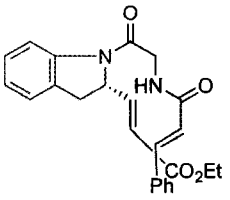
tography. Elution with 3:10 ethyl acetate/hexane gave 4.60 g (93%) of product as a yellow oil: MS (ES+) *m/z* (M + 1) 158.0; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (dq, J = 15.3, 7.0 Hz, 1H), 6.22 (br s, 1H), 5.86 (dq, J = 15.3, 1.8 Hz, 1H), 4.08 (d, J = 5.3 Hz, 2H), 3.74 (s, 3H), 1.84 (dd, J = 6.8, 1.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 166.0, 140.9, 124.2, 52.3, 41.1, 17.7.

Compound 3c, (*E*)-2-But-2-enamidoacetic Acid.



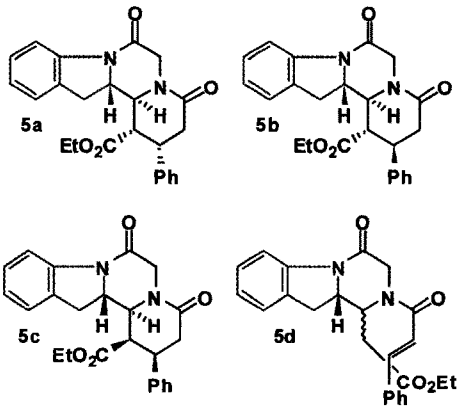
To a solution of the peptide ester (2.11 g, 13.41 mmol) in 8:2:1 THF/water/MeOH (125 mL) at 0 °C was added LiOH (1.15 g, 26.82 mmol). The reaction mixture was stirred at 0 °C to room temperature under N_2 for 3 h. The reaction was neutralized with Amberlite H^+ resin to pH 6 and then evaporated by vacuum pressure to give 1.78 g (90%) of product as an off-white solid: MS (ES+) *m/z* (M + 1) 144.0; ^{1}H NMR (400 MHz, DMSO) δ 12.54 (br s, 1H), 8.21(t, J = 5.5 Hz, 1H), 6.64 (dq, J = 15.3, 7.0 Hz, 1H), 5.97 (dq, J = 15.3, 1.8 Hz, 1H), 3.80 (d, J = 5.7 Hz, 2H), 1.80 (dd, J = 7.0, 1.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 171.3, 165.1, 138.3, 125.3, 40.5, 17.3.

Compound 4, (*E*)-Ethyl 3-((*S*)-1-(2-cinnamamidoacetyl)-indolin-2-yl)acrylate.



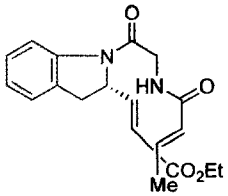
To a solution of the glycine-cinnamoyl acid (2.50 g, 12.21 mmol) in DMF (106 mL) was added HATU (4.64 g, 12.21 mmol), followed by the addition of DIPEA (2.13 mL, 12.21 mmol) and then the deprotected indoline core **2a** (0.53 g, 2.44 mmol). The reaction mixture was stirred at room temperature under N_2 for 48 h. The reaction mixture was condensed and washed with ammonium chloride then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (5:2 v/v) gave 0.79 g (81%) of product as a light orange solid: MS (ES+) *m/z* (M + 1) 405.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 15.7 Hz, 1H), 7.55−7.49 (m, 2H), 7.43−7.33 (m, 3H), 7.32−7.24 (m, 1H), 7.21 (d, J = 7.3 Hz, 1H), 7.11 (t, J = 7.3 Hz, 1H), 6.92 (dd, J = 15.7, 5.9 Hz, 1H), 6.75 (br s, 1H), 6.52 (d, J = 15.7 Hz, 1H), 5.92 (d, J = 15.7 Hz, 1H), 5.14−5.06 (m, 1H), 4.47 (dd, J = 17.9, 4.4 Hz, 1H), 4.16 (q, J = 7.1 Hz, 2H), 4.07 (dd, J = 17.9, 3.2 Hz, 1H), 3.63 (dd, J = 15.9, 10.0 Hz, 1H), 2.95 (d, J = 15.9 Hz, 1H), 1.26 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.2, 165.9, 145.0, 142.0, 135.1, 130.3, 129.5, 129.3, 129.2, 128.5, 128.3, 125.54, 125.52, 122.2, 120.4, 117.9, 61.4, 59.4, 43.2, 35.8, 14.6.

Compounds 5a−5d.



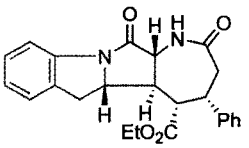
To a solution of the glycine−cinnamoyl-coupled indoline moiety (0.20 g, 0.49 mmol) in DCM (10 mL) at −78 °C was added Et_3N (0.076 mL, 0.54 mmol), followed by the addition of TBSOTf (0.23 mL, 0.99 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under an argon atmosphere for 24 h. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ and then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (9:1 v/v) gave 25.6 (20%), 25.7 (20%), 10.3 (8%), and 25.6 mg (20%) of products **5a−5d**, respectively, as an off-white solid: MS (ES+) *m/z* (M + 1) 405.1; ^{1}H NMR (compound **5a**; 400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.0 Hz, 1H), 7.39−7.22 (m, 5H), 7.16−7.08 (m, 3H), 5.29 (d, J = 18.8 Hz, 1H), 4.48−4.39 (m, 1H), 4.11 (dd, J = 10.3, 4.0 Hz, 1H), 4.03 (q, J = 7.0 Hz, 2H), 3.84 (d, J = 18.8 Hz, 1H), 3.64−3.57 (m, 1H), 3.21−3.10 (m, 3H), 3.05 (dd, J = 15.1, 11.3 Hz, 1H), 2.81 (dd, J = 17.3, 5.0 Hz, 1H), 1.05 (t, J = 7.0 Hz, 3H); ^{13}C NMR (compound **5a**; 100 MHz, $CDCl_3$) δ 170.4, 167.6, 162.9, 141.8, 138.6, 128.9, 128.8, 128.1, 127.8, 127.2, 125.0, 124.5, 117.6, 64.0, 61.4, 58.2, 46.3, 46.2, 37.7, 34.0, 32.9, 13.8; ^{1}H NMR (compound **5b**; 400 MHz, C_6D_6) δ 8.55 (d, J = 8.0 Hz, 1H), 7.19−6.98 (m, 4H), 6.92−6.78 (m, 4H), 5.36 (d, J = 19.1 Hz, 1H), 3.68 (d, J = 19.1 Hz, 1H), 3.61−3.43 (m, 3H), 3.56 (dd, J = 10.0 Hz, J = 9.0 Hz, 1H), 3.60−3.53 (m, 2H), 3.13 (ddd, J = 12.5 Hz, J = 10.0 Hz, J = 8.0 Hz, 1H), 2.73 (td, J = 12.5 Hz, J = 3.0 Hz, 1H), 2.56 (dd, J = 15.1, 8.0 Hz, 1H), 2.50 (dd, J = 16.1, 3.0 Hz, 1H), 2.40 (dd, J = 15.1, J = 12.5 Hz, 1H), 2.32−2.21 (m, 2H), 0.52 (t, J = 7.0 Hz, 3H); ^{13}C NMR (compound **5b**; 100 MHz, C_6D_6) δ 171.2, 166.2, 162.3, 142.4, 139.9, 129.4, 128.7, 128.1, 127.9, 124.6, 124.3, 117.6, 64.9, 60.7, 59.0, 51.1, 46.2, 41.4, 38.7, 32.2, 13.5; ^{1}H NMR (compound **5c**; 400 MHz, $CDCl_3$) δ 8.12 (d, J = 8.0 Hz, 1H), 7.40−7.19 (m, 7H), 7.09 (t, J = 7.4 Hz, 1H), 4.53 (d, J = 19.2 Hz, 1H), 4.34 (d, J = 19.2 Hz, 1H), 4.35−4.26 (m, 1H), 4.07−3.98 (m, 3H), 3.55−3.47 (m, 1H), 3.39 (dd, J = 17.1, 13.6 Hz, 1H), 3.30 (dd, J = 4.0, 4.0 Hz, 1H), 3.25 (dd, J = 15.6, 8.5 Hz, 1H), 3.07 (dd, J = 15.5, 10.1 Hz, 1H), 2.79 (dd, J = 17.1, 5.2 Hz, 1H), 1.05 (t, J = 7.1 Hz, 3H); ^{13}C NMR (compound **5c**; 100 MHz, $CDCl_3$) δ 169.3, 168.7, 162.7, 141.3, 139.0, 128.9, 128.7, 128.3, 127.8, 126.9, 124.9, 124.6, 116.8, 61.1, 60.4, 60.3, 47.3, 46.1, 39.6, 33.1, 32.4, 14.0.

Compound 6, (*E*)-Ethyl 3-((*S*)-1-(2-(*E*)-but-2-enamidoacetyl)indolin-2-yl)acrylate.



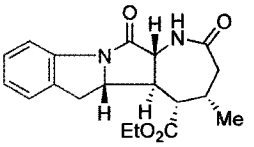
To a solution of the glycine-crotyl acid (0.54 g, 3.7421 mmol) in DMF (160 mL) was added HATU (1.42 g, 3.74 mmol), followed by the addition of DIPEA (0.65 mL, 3.74 mmol) and then the deprotected indoline core **2a** (0.16 g, 0.75 mmol). The reaction mixture was stirred at room temperature under N_2 for 24 h. The reaction mixture was condensed, washed with ammonium chloride, and then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (5:2 v/v) gave 0.21 g (83%) of product as a light yellow oil: MS (ES+) *m/z* (M + 1) 343.1; ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.15 (d, J = 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.20 (d, J = 7.2 Hz, 1H), 7.10 (t, J = 7.3 Hz, 1H), 6.96−6.85 (m, 2H), 6.74 (br s, 1H), 5.94 (d, J = 15.1 Hz, 1H), 5.90 (d, J = 15.1 Hz, 1H), 5.09 (br t, 1H), 4.40 (d, J = 17.8 Hz, 1H), 4.12 (q, J = 7.0 Hz, 2H), 4.00 (d, J = 17.8 Hz, 1H), 3.61 (dd, J = 15.8, 10.0 Hz, 1H), 2.93 (d, J = 16.0 Hz, 1H), 1.88 (d, J = 6.8 Hz, 3H), 1.25 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 165.9, 165.4, 144.6, 141.5, 140.5, 128.7, 128.0, 125.0, 124.9, 124.4, 121.7, 117.4, 60.3, 58.9, 42.5, 35.3, 17.7, 14.1.

Compound 7.



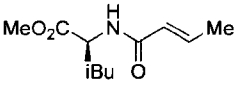
To a solution of the glycine−cinnamoyl-coupled indoline moiety **4** (0.11 g, 0.25 mmol) in DCM (5 mL) at −78 °C was added Et_3N (0.140 mL, 0.99mmol), followed by the addition of TMSOTf (0.18 mL, 0.99 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under an argon atmosphere for 4 h. The reaction mixture was quenched with brine and then extracted with DCM and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (4:1 v/v) gave 0.095 g (91%) of product as white needles. Stereochemistry was confirmed by crystal X-ray structure: MS (ES+) *m/z* (M + 1) 405.1; ^{1}H NMR: (400 MHz, acetone-d_6) δ 7.48 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 7.3 Hz, 2H), 7.30−7.20 (m, 5H), 7.06 (t, J = 7.5 Hz, 1H), 6.32 (br s, 1H), 4.78 (d, J = 10.8 Hz, 1H), 4.43 (q, J = 8.8 Hz, 1H), 3.98−3.87 (m, 2H) 3.84−3.77 (m, 1H), 3.43 (dd, J = 11.5, 5.5 Hz, 1H), 3.24 (ddd, J = 11.2, 11.1, 8.8 Hz, 1H), 3.18−2.94 (m, 4H), 1.04 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ 173.7, 173.4, 169.2, 142.2, 140.8, 136.5, 130.9, 130.2, 129.2, 129.0, 127.4, 126.5, 116.7, 66.2, 62.2, 60.8, 55.2, 52.1, 44.3, 42.6, 37.2, 15.3; carbon displacements for 62.2, 42.6, and 37.2 are CH_2 carbons, determined by DEPT 135 analysis.

Compound 8.



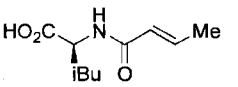
To a solution of the glycine−crotyl-coupled indoline moiety 6 (0.077 g, 0.23 mmol) in DCM (5 mL) at −78 °C was added Et_3N (0.16 mL, 1.17 mmol), followed by the addition of TMSOTf (0.22 mL, 1.17 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 1 h. The reaction mixture was quenched with brine then extracted with DCM and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (4:1 v/v) gave 0.062 g (80%) of product as an off-white solid; MS (ES+) *m/z* (M + 1) 343.1; ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.51 (d, J = 7.9 Hz, 1H), 7.20 (t, J = 7.8 Hz, 1H), 7.14 (d, J = 7.3 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 6.54 (br s, 1H), 4.28 (dd, J = 10.5, 3.0 Hz, 1H), 4.24−4.15 (m, 3H) 3.21 (dd, J = 16.3, 9.3 Hz, 1H), 3.09 (dd, J = 16.3, 8.8 Hz, 1H), 2.89 (dd, J = 11.3, 3.0 Hz, 1H), 2.82−2.53 (m, 4H), 1.30 (t, J = 7.0 Hz, 3H), 1.03 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 172.0, 166.2, 137.9, 134.0, 127.7, 125.2, 125.1, 115.1, 63.8, 61.0, 59.8, 53.5, 48.1, 42.7, 35.8, 30.0, 14.2, 13.5.

Compound 9a, (*S,E*)-Methyl 2-but-2-enamido-4-methylpentanoate.



To a solution of Leu-OMe HCl (4.02 g, 22.01 mmol) in DCM (400 mL) was added DIPEA (7.67 mL, 44.03 mmol). The reaction mixture was stirred under N_2 for 15 min at room temperature. To this reaction mixture was added crotonyl chloride 90% (2.84 mL, 26.42 mmol). The reaction mixture was stirred under N_2 for 2 h at RT. The reaction mixture was extracted with DCM. The combined organic layers were washed with brine, then dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with 1:5 ethyl acetate/hexane gave 4.08 g (87%) of product as a clear oil: MS (ES+) *m/z* (M + 1) 214.2; ^{1}H NMR (400 MHz, C_6D_6) δ 6.99 (dq, J = 15.1, 7.0 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 5.75 (dq, J = 15.3, 1.5 Hz, 1H), 4.99 (ddd, J = 9.3, 8.3, 5.0 Hz, 1H), 3.31 (s, 3H), 1.76−1.51 (m, 3H), 1.48 (dd, J = 6.8, 1.5 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 165.6, 140.0, 125.5, 51.7, 50.9, 41.8, 25.1, 23.0, 17.5.

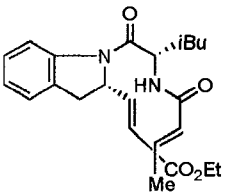
Compound 9, (*S,E*)-2-But-2-enamido-4-methylpentanoic Acid.



To a solution of the peptide ester (4.00 g, 18.76 mmol) in

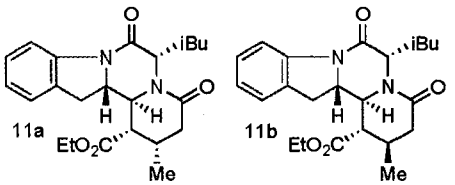
8:2:1 THF/water/MeOH (82 mL) at 0 °C, was added LiOH (1.57 g, 37.51 mmol). The reaction mixture was stirred at 0 °C to room temperature under N_2 for 3 h. The reaction was neutralized with Amberlite H^+ resin to pH 6 and then evaporated by vacuum pressure to give 3.84 g(quantitative yield) of the crude product as a very viscous clear oil solid: MS (ES+) *m/z* (M + 1) 200.3; ^{1}H NMR (400 MHz, DMSO) δ 7.63 (d, J = 7.8 Hz, 1H), 6.55 (dq, J = 15.3, 7.0 Hz, 1H), 5.99 (d, J = 15.3 Hz, 1H), 4.13−4.06 (m, 1H), 1.77 (d, J = 6.8 Hz, 3H), 1.63−1.35 (m, 3H), 0.84 (t, J = 6.3 Hz, 6H); ^{13}C NMR (100 MHz, DMSO) δ 175.4, 165.0, 137.9, 127.9, 127.3, 52.8, 42.7, 25.4, 24.1, 22.8, 17.3.

Compound 10, (*E*)-Ethyl 3-((*S*)-1-((*S*)-2-((*E*)-but-2-enamido)-4-methylpentanoyl)indolin-2-yl)acrylate.



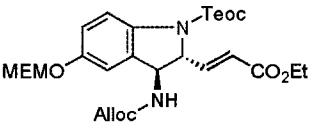
To a solution of the leucine−crotyl acid (0.92 g, 4.60 mmol) in DMF (25 mL) was added PyBrop (2.15 g, 4.60 mmol), followed by the addition of DMAP (0.56 g, 4.60 mmol), DIPEA (1.00 mL, 5.74 mmol), and then the deprotected indoline core **2a** (0.20 g, 0.92 mmol). The reaction mixture was stirred at room temperature under N_2 for 4.5 h. The reaction mixture was condensed, washed with ammonium chloride, and then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (5:2 v/v) gave 0.19 g (52%) of product as a clear viscous oil: MS (ES+) *m/z* (M + 1) 399.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 8.0 Hz, 1H), 7.25 (t, J = 8.3 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 6.89 (dd, J = 15.8, 5.5 Hz, 1H), 6.80 (dq, J = 15.1, 7.0 Hz, 1H), 6.20 (d, J = 8.5 Hz, 1H), 5.86 (d, J = 15.8 Hz, 1H), 5.81 (d, J = 15.3 Hz, 1H), 5.08 (dd, J = 9.8, 5.5 Hz, 1H), 4.91 (ddd, J = 9.2, 9.1, 3.8 Hz, 1H), 4.16−4.05 (m, 2H), 3.59 (dd, J = 15.8, 9.8 Hz, 1H), 2.95 (d, J = 15.8 Hz, 1H) 1.83 (d, J = 6.8 Hz, 3H), 1.77−1.60 (m, 2H), 1.58−1.50 (m, 1H) 1.23 (t, J = 7.0 Hz, 3H) 1.04 (d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 165.5, 164.9, 144.3, 141.7, 140.3, 129.1, 128.0, 125.0, 124.9, 124.8, 122.0, 117.8, 60.7, 59.4, 49.6, 44.0, 35.1, 24.8, 23.5, 22.1, 17.7, 14.1.

Compounds 11a and 11b.



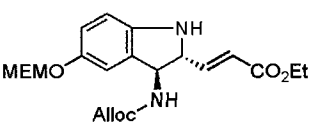
To a solution of the leucine−crotyl-coupled indoline moiety **10** (0.18 g, 0.46 mmol) in DCM (5 mL) at −78 °C was added Et_3N (0.51 mL, 3.65 mmol), followed by the addition of TMSOTf (0.66 mL, 3.65 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under argon atmosphere for 22 h. The reaction mixture was quenched with brine and then extracted with DCM and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (9:1 v/v) gave 0.048 (27%) and 0.018 g (10%) of products **11a** and **11b**, respectively, as off-white solids: MS (ES+) *m/z* (M + 1) 399.4; ^{1}H NMR (compound **11a**; 400 MHz, C_6D_6) δ 8.55 (d, J = 8.0 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.90 (t, J = 7.5 Hz, 1H), 6.85 (d, J = 7.5 Hz, 1H), 5.84 (dd, J = 10.0, 3.8 Hz, 1H), 3.85 (q, J = 7.0 Hz, 2H), 3.82 (dd, J = 10.5, 4.0 Hz, 1H), 3.12 (ddd, J = 11.0, 10.8, 8.0 Hz, 1H), 2.37 (dd, J = 17.1, 8.8 Hz, 1H), 2.23 (dd, J = 17.1, 5.0 Hz, 1H), 2.23−2.15 (m, 2H), 2.07 (dd, J = 9.8, 3.8 Hz, 1H), 2.10−1.94 (m, 3H), 1.80−1.71 (m, 1H), 1.70−1.61 (m, 1H), 1.23 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H) 0.89 (t, J = 7.0 Hz, 3H), 0.81 (d, J = 7.0 Hz, 3H); ^{13}C NMR (compound **11a**; 100 MHz, C_6D_6) δ 170.7, 166.5, 166.1, 143.3, 129.6, 128.1, 124.4, 124.3, 118.1, 64.2, 61.0, 54.1, 53.6, 45.1, 41.9, 37.6, 32.3, 27.0, 25.2, 23.7, 22.2, 17.2, 14.2 ppm; ^{1}H NMR (compound **11b**; 400 MHz, C_6D_6) δ 8.54 (d, J = 8.0 Hz, 1H), 7.13−7.06 (m, 1H), 6.90−6.82 (m, 2H), 5.82 (dd, J = 9.5, 4.8 Hz, 1H), 3.94 (dd, J = 9.5, 8.8 Hz, 1H), 3.84 (q, J = 7.0 Hz, 2H), 3.05−2.95 (m, 1H), 2.50 (dd, J = 14.7, 7.4 Hz, 1H), 2.38−2.28 (m, 2H), 2.07 (ddd, J = 13.8, 8.5, 5.0 Hz, 1H), 1.96−1.78 (m, 2H), 1.75−1.58 (m, 3H), 1.15 (d, J = 6.5 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H), 0.88 (t, J = 7.0 Hz, 3H), 0.61 (d J = 6.3 Hz, 3H); ^{13}C NMR (compound **11b**; 100 MHz, C_6D_6) δ 172.4, 167.0, 166.3, 143.0, 129.5, 128.1, 124.44, 124.38, 117.9, 66.0, 61.1, 55.7, 53.0, 51.2, 42.0, 39.7, 32.3, 30.1, 25.4, 23.5, 22.5, 18.6, 14.1.

Compound 12, (2*R*,3*S*)-2-(Trimethylsilyl)ethyl 3-(allyloxycarbonylamino)-2-((E)-3-ethoxy-3-oxoprop-1-enyl)-5-((2-methoxyethoxy)methoxy)indoline-1-carboxylate.



The synthetic procedure was already described:[4] yellow oil; MS (ES+) *m/z* (M + 1) 565.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (bs, 1H), 7.07−7.05 (m, 2H), 6.95 (dd, J = 15.6, 5.7 Hz, 1H), 5.98−5.89 (m, 2H), 5.33 (d, J = 17.2 Hz, 1H), 5.25 (d, J = 15.3 Hz, 1H), 5.23 (s, 2H), 5.06 (d, J = 6.7 Hz, 1H), 4.92 (bs, 1H), 4.84 (d, J = 6.7 Hz, 1H), 4.62 (bs, 2H), 4.30 (bs, 2H), 4.18 (q, J = 7.1 Hz, 2H), 3.83 (t, J = 4.6 Hz, 2H), 3.58 (t, J = 4.6 Hz, 2H), 3.39 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H), 1.07 (bs, 2H), 0.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 155.6, 154.2, 144.2, 132.8, 122.6, 119.3, 118.5, 117.0, 114.4, 94.5, 72.0, 68.3, 68.1, 66.4, 64.8, 61.0, 59.4, 57.2, 18.2, 14.6, −1.1.

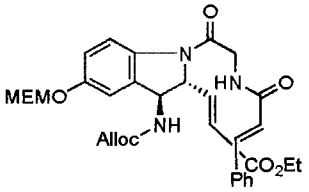
Compound 12a, (*E*)-Ethyl-3-((2*R*,3*S*)-3-(allyloxycarbonylamino)-5-((2-methoxyethoxy)methoxy)indolin-2-yl)acrylate.



The synthetic procedure was already described:[4] dark yellow oil; MS (ES+) *m/z* (M + 1) 421.3; ^{1}H NMR (400 MHz, $CDCl_3$)

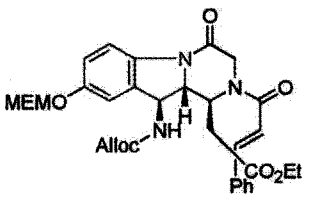
δ 6.98 (dd, J = 15.6, 6.7 Hz, 1H), 6.92 (d, J = 2.1 Hz, 1H), 6.85 (dd, J = 2.1, 8.5 Hz, 1H), 6.56 (d, J = 8.5 Hz, 1H), 6.05 (d, J = 15.6 Hz, 1H), 5.92 (m, 1H), 5.39 (d, J = 8.3 Hz, 1H), 5.31 (d, J = 15.6 Hz, 1H), 5.22 (d, J = 10.7 Hz, 1H), 5.14 (s, 2H), 4.98 (t, J = 6.8 Hz, 1H), 4.59 (d, J = 5.3 Hz, 1H), 4.20−4.14 (m, 3H), 3.81 (t, J = 4.6 Hz, 2 H), 3.56 (t, J = 4.6 Hz, 2H), 3.37 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 156.0, 151.7, 147.0, 145.1, 133.0, 128.3, 122.6, 119.0, 118.3, 114.5, 111.3, 95.1, 72.0, 68.5, 67.9, 66.2, 60.9, 59.5, 59.4, 14.6.

Compound 13, (*E*)-Ethyl-3-((2*R*,3*S*)-3-(allyloxycarbonylamino)-1-(2-cinnamamidoacetyl)-5-((2-methoxyethoxy)methoxy)indolin-2-yl)acrylate.



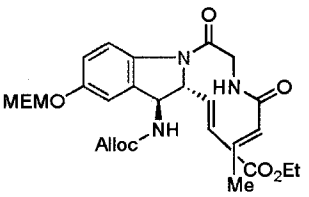
To a stirred solution of glycine cinnamic acid (255 mg, 1.24 mmol) in dry DMF (10 mL) was added HATU (473 mg, 1.24 mmol), followed by DIPEA (217 μL, 1.24 mmol). To the reaction mixture was added the above secondary amine (105 mg, 0.25 mmol), and the mixture was stirred for 72 h under argon atmosphere. The solution was condensed and dissolved in ethyl acetate, then washed with a saturated NH_4Cl solution, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:5 ethyl acetate/hexanes) to give the coupled product (90.2 mg, 60%) as a dark yellow mousse: MS (ES+) m/z (M + 1) 608.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 9.3 Hz, 1H), 7.63 (d, J = 15.8 Hz, 1H), 7.54−7.49 (m, 2H), 7.41−7.34 (m, 3H), 7.13−7.06 (m, 2H), 6.97 (dd, J = 15.5, 4.5 Hz, 1H), 6.68 (br s, 1H), 6.51 (d, J = 15.6 Hz, 1H), 5.98 (d, J = 15.8 Hz, 1H), 5.98−5.87 (m, 1H), 5.38−5.20 (m, 5H), 4.96 (br d, J = 4.8, 0.0 Hz, 1H), 4.86 (br d, J = 5.8, 0.0 Hz, 1H), 4.67−4.58 (m, 2H), 4.45 (dd, J = 17.8, 4.3 Hz, 1H), 4.16 (q, J = 7.0 Hz, 2H), 3.98 (d, J = 17.8 Hz, 1H), 3.84−3.80 (m, 2H), 3.58−3.54 (m, 2H), 3.37 (s, 3 H), 1.26 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.9, 165.2, 164.3, 155.1, 142.4, 141.7, 136.7, 134.8, 134.6, 132.2, 129.8, 128.8, 127.9, 123.3, 119.9, 118.8, 118.7, 114.1, 113.7, 93.9, 71.5, 67.7, 67.5, 66.2, 60.9, 59.0, 57.8, 42.5, 14.1.

Compound 14, Ethyl-2-((1*S*,10*S*,10a*S*)-10-(allyloxycarbonylamino)-2-cinnamoyl-8-((2-methoxyethoxy)methoxy)-4-oxo-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indol-1-yl)acetate.



To a solution of the glycine−cinnamoyl-coupled indoline moiety (0.94 g, 0.15 mmol) in DCM (4 mL) at −78 °C was added Et_3N (0.22 mL, 1.55 mmol), followed by the addition of TBSOTf (0.36 mL, 1.55 mmol). The reaction mixture was allowed to warm to room temperature as it was stirred under an argon atmosphere for 4 h. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ and then extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and purified by flash chromatography. Elution with hexane/ethyl acetate (4:1 v/v) gave 55.0 mg (58%) of the tricyclic product as a beige mousse: MS (ES+) m/z (M + 1) 608.4; ^{1}H NMR (400 MHz, C_6D_6) δ 7.84 (d, J = 15.8 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.19−7.11 (m, 2H), 7.00−6.54 (m, 3H), 6.89 (br s, 1H), 6.63 (d, J = 15.6 Hz, 1H), 5.81−5.69 (m, 1H), 5.43 (t, J = 9.9 Hz, 1H), 5.17 (t, J = 6.8 Hz, 1H), 5.11 (d, J = 7.0 Hz, 1H), 5.01 (d, J = 10.5 Hz, 1H), 4.84−4.75 (m, 2H), 4.46 (d, J = 4.5 Hz, 2H), 4.05−3.95 (m, 2H), 3.68 (dd, J = 5.8, 3.8 Hz, 2H), 3.59 (dd, J = 8.5, 7.0 Hz, 1H), 3.32 (dd, J = 5.3, 4.3 Hz, 2H), 3.10 (s, 3H), 2.87 (dd, J = 16.3, 4.0 Hz, 1H), 2.81−2.61 (m, 2H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 169.8, 166.4, 155.9, 155.5, 141.7, 135.4, 133.4, 129.5, 128.8, 128.4, 128.2, 128.0, 121.1, 117.4, 117.3, 115.9, 114.2, 94.4, 72.0, 70.0, 68.2, 65.8, 60.7, 58.7, 58.6, 58.4, 47.3, 34.0, 14.3.

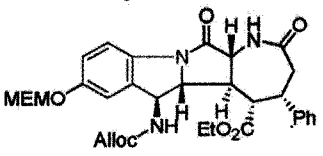
Compound 15, (*E*)-Ethyl-3-((2*R*,3*S*)-3-(allyloxycarbonylamino)-1-(2-(*E*)-but-2-enamidoacetyl)-5-((2-methoxyethoxy)methoxy)indolin-2-yl)acrylate.



To a stirred solution of glycine crotyl acid (170 mg, 1.19 mmol) in dry DMF (10 mL) was added HATU (452 mg, 1.19 mmol), followed by DIPEA (207 μL, 1.19 mmol). To the reaction mixture was added the above secondary amine (103 mg, 0.25 mmol), and the mixture was stirred for 24 h under argon atmosphere. The solution was condensed and dissolved in ethyl acetate, then washed with a saturated NH_4Cl solution, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:4 ethyl acetate/hexanes) to give the coupled product (124 mg, 92%) as a dark yellow mousse: MS (ES+) m/z (M + 1) 546.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 9.5 Hz, 1H), 7.08 (d, J = 2.5 Hz, 1H), 7.06 (br s, 1H), 6.94 (dd, J = 15.8, 5.8 Hz, 1H), 6.85 (dq, J = 15.1, 6.8 Hz, 1H), 6.46 (br s, 1H), 5.95 (d, J = 15.8 Hz, 1H), 5.89 (dq, J = 15.3, 1.8 Hz, 1H), 5.34−5.24 (m, 3H), 5,22 (s, 2H), 4.92 (d, J = 5.0 Hz, 1H), 4.84 (d, J = 6.0 Hz, 1H), 4.70−4.57 (m, 2H), 4.37 (dd, J = 18.1, 4.8 Hz, 1H), 4.15 (q, J = 7.0 Hz, 2H), 3.89 (br d, J = 18.1 Hz, 1 H), 3.83−3.79 (m, 2H), 3.58−3.53 (m, 2H), 3.37 (s, 3H), 1.87 (dd, J = 6.8, 1.3 Hz, 3H), 1.25 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 166.0, 165.9, 165.2, 155.3, 155.0, 142.4, 140.8, 136.8, 132.2, 124.3, 123.2, 118.8, 118.6, 118.4, 113.7, 93.9, 71.5, 67.7, 66.2, 64.3, 60.4, 59.0, 57.8, 42.3, 17.7, 14.1.

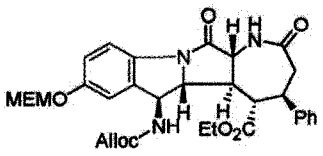
Compounds 16a and 16b.

major



16a

minor

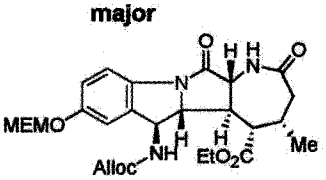


16b

To a stirred solution of glycine−cinnamoyl-coupled indoline (81 mg, 0.13 mmol) in dry DCM (10 mL) at −78 °C was added Et_3N (93 μL, 0.66 mmol), followed by TMSOTf (120 μL, 0.66 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 24 h under an argon atmosphere. The solution was washed with a saturated brine solution, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:4 ethyl acetate/hexanes) and by PREP HPLC to give two diastereoisomers of the cyclized product (**16a** 44 mg, 55%; **16b** 10 mg, 12%) as a light yellow mousse: MS (ES+) *m/z* (M + 1) 608.4; ^{1}H NMR (compound **16a**; 400 MHz, C_6D_6) δ 7.70 (broad d, *J* = 8.3 Hz, 1H), 7.22 (s, 1H), 7.18−6.99 (m, H_{Ph}), 6.88 (broad s, 1H), 6.01 (broad s, 1H), 5.85−5.70 (broad m, 1H), 5.16 (d, *J* = 17.3 Hz, 1H), 5.08−4.87 (broad m, 4H), 4.64−4.41 (broad m, 2H), 3.97 (broad s, 1H), 3.84−3.66 (broad m, 3H), 3.63−3.55 (m, 2H), 3.36−3.22 (broad m, 3H), 3.08 (s, 3H), 2.85−2.65 (broad m, 3H), 1.43−1.10 (broad m, 3H); ^{13}C NMR (compound **16a**; 100 MHz, C_6D_6) δ 172.4, 171.4, 166.1, 155.9, 137.3, 133.0, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9, 127.8, 117.4, 117.30, 117.25, 116.1, 113.6, 94.1, 71.9, 68.1, 65.6, 60.9, 58.6, 32.3, 30,2, 30.1, 29.8, 23.1, 14.4; ^{1}H NMR (compound **16b**; 400 MHz, $CDCl_3$ at 58 °C) δ 7.50 (d, *J* = 8.5 Hz, 1H), 7.32−7.22 (m, H_{Ph}), 7.20−7.15 (m, 2H), 7.00 (dd, *J* = 8.5 Hz, *J* = 2.0 Hz, 1H), 6.93 (d, *J* = 2.0 Hz, 1H), 6.40 (broad s, 1H), 5.91−5.77 (m, 1H), 5.24 (d, *J* = 17.6 Hz, 1H), 5.21−5.12 (m, 4H), 4.97 (broad m, 1H), 4.58−4.45 (m, 2H), 4.42 (dd, *J* = 10.5 Hz, *J* = 1.3 Hz, 1H), 4.27−4.19 (m, 1H), 3.94−3.84 (m, 2H), 3.81−3.76 (m, 2H), 3.73−3.67 (m, 1H), 3.56−3.51 (m, 2H), 3.36 (s, 3H), 3.22 (t, *J* = 10.8 Hz, 1H), 3.18 (d, *J* = 8.5 Hz, 1H), 3.13 (dd, *J* = 11.0 Hz, *J* = 5.5 Hz, 1H), 2.96 (broad d, *J* = 16.3 Hz, 1H), 1.00 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (compound **16b**; 100 MHz, C_6D_6) δ 173.4, 172.8, 169.4, 166.5, 156.1, 155.4, 150.0, 136.3, 133.9, 132.7, 132.3, 128.9, 127.1, 118.3, 118.1, 116.5, 112.8, 93.8, 71.5, 70.6, 67.7, 66.4, 60.9, 59.0, 55.9, 48.2, 38.6, 29.9, 29.7, 14.1.

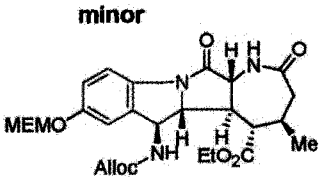
Compounds 17a and 17b.

major



17a

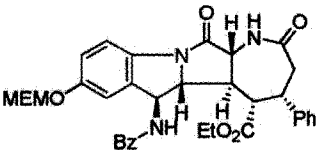
minor



17b

To a stirred solution of glycine−crotyl-coupled indoline (108 mg, 0.20 mmol) in dry DCM (10 mL) at −78 °C was added Et_3N (165 μL, 1.20 mmol), followed by TMSOTf (213 μL, 1.20 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 4 h under an argon atmosphere. The solution was washed with a saturated brine solution, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:5 ethyl acetate/hexanes) and by preparative HPLC to give two diastereoisomers of the cyclized product (54 mg, 50% and 24 mg, 22%) as a light yellow mousse: MS (ES+) *m/z* (M + 1) 546.3; ^{1}H NMR (compound **17a**; 400 MHz, $CDCl_3$) δ 7.42 (broad d, *J* = 8.3 Hz, 1H), 7.02−6.86 (broad m, 2H), 6.42 (very broad s, 1H), 5.95−5.78 (broad m, 1H), 5.41 (broad s, 1H), 5.35−5.01 (broad m, 4H), 5.27 (d, *J* = 16.8 Hz, 1H), 4.52 (broad s, 2H), 4.33−4.06 (broad m, 4H), 3.79 (broad s, 2H), 3.57−3.50 (m, 2H), 3.36 (s, 3H), 2.96−2.44 (broad m, 5H), 1.27 (t, *J* = 7.0 Hz, 3H), 1.26 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR (compound **17a**; 100 MHz, $CDCl_3$) δ 174.2, 173.6, 173.1, 166.3, 155.8, 136.2, 132.9, 132.4, 129.2, 126.7, 118.4, 117.3, 116.3, 113.1, 94.3, 71.9, 68.1, 66.2, 61.7, 59.4, 53.4, 50.7, 47.8, 43.0, 30.4, 14.6, 13.8; ^{1}H NMR (compound **17b**; 400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.5 Hz, 1H), 7.05−7.00 (m, 2H), 6.18 (broad s, 1H), 5.96 (ddt, *J* = 17.1 Hz, *J* = 10.8 Hz, *J* = 5.8 Hz, 1H), 5.35 (dd, *J* = 17.1 Hz, *J* = 1.3 Hz, 1H), 5.27 (d, *J* = 10.8 Hz, 1H), 5.23 (s, 2H), 5.28−5.20 (m, 1H), 5.15 (dd, *J* = 11.0 Hz, *J* = 2.8 Hz, 1H), 4.68−4.63 (m 2H), 4.24−4,13 (m, 3H), 3.83−3.78 (m, 2H), 3.58−3.53 (m, 2H), 3.50 (s, 3H), 3.30 (dd, *J* = 7.0 Hz, *J* = 4.0 Hz, 1H), 3.01 (d, *J* = 14.6 Hz, 1H), 2.63 (td, *J* = 10.8 Hz, *J* = 4.3 Hz, 1H), 2.54 (t, *J* = 7.0 Hz, 1H), 2.42 (dd, *J* = 14.6 Hz, *J* = 7.0 Hz, 1H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.26−1.22 (m, 3H); ^{13}C NMR (compound **17b**; 100 MHz, $CDCl_3$) δ 173.7, 173.5, 167.2, 164.3, 155.9, 155.4, 133.7, 133.0, 132.3, 118.3, 118.2, 116.6, 112.8, 93.8, 71.5, 67.7, 66.3, 60.8, 59.1, 55.9, 50.9, 47.8, 46.0, 39.4, 29.7, 17.7, 14.2.

Compound 18a.

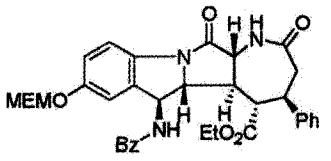


18a

To a stirred solution of the cyclized glycine cinnamoyl-functionalized indoline tetracycle (27 mg, 0.045 mmol) in dry DCM (2 mL) was added morpholine (8 μL, 0.090 mmol), followed by $Pd(0)(PPh_3)_4$ (5.2 mg, 0.0045 mmol). The reaction mixture was stirred for 30 min under argon atmosphere. To the reaction mixture was directly added Et_3N (64 μL, 0.45 mmol), followed by benzoyl chloride (32 μL, 0.27 mmol). The reaction mixture was stirred for an additional hour. The reaction mixture was washed with a saturated brine solution, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the benzoylated compound (18 mg, 67%) as a light yellow mousse: MS (ES+) *m/z* (M + 1) 628.3; ^{1}H NMR (400 MHz, $CDCl_3$ at 58 °C) δ 7.73 (d, *J* = 7.3 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.29−7.20 (m, 3H), 7.14 (d, *J* = 6.8 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 1H), 6.94 (s, 1H), 6.62 (d,

J = 8.0 Hz, 1H), 6.41 (s, 1H), 5.57 (t, J = 7.5 Hz, 1H), 5.16 (s, 2H), 4.45−4.37 (m, 2H), 3.75 (t, J = 4.5 Hz, 2H), 3.71−3.62 (m, 2H), 3.61−3.52 (m, 1H), 3.49 (t, J = 4.5 Hz, 2H), 3.30 (s, 3H), 3.27−3.16 (m, 2H), 3.12 (dd, J = 10.8, 5.8 Hz, 1 H), 2.91 (d, J = 16.3 Hz, 1H), 0.72 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171,6, 166.6, 165.9, 155.5, 141.9, 138.8, 135.7, 134.7, 133.4, 132.1, 128.75, 128.70, 128.1, 127.8, 126.9, 117.0, 116.1, 112.8, 93.8, 71.5, 69.3, 67.6, 61.3, 59.3, 59.0, 58.6, 58.2, 53.5, 49.1, 42.1, 40.0, 13.4.

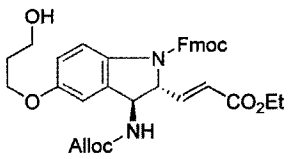
Compound 18b.



18b

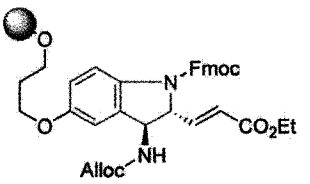
To a stirred solution of the cyclized glycine cinnamoyl-functionalized indoline tetracycle (14 mg, 0.023 mmol) in dry DCM (1 mL) was added morpholine (2 μL, 0.023 mmol), followed by Pd(0)$(PPh_3)_4$ (2.6 mg, 0.0023 mmol). The reaction mixture was stirred for 20 min under argon atmosphere. To the reaction mixture was directly added Et_3N (20 μL, 0.139 mmol), followed by benzoyl chloride (8 μL, 0.070 mmol). The reaction mixture was stirred for an additional hour. The reaction mixture was washed with a saturated brine solution, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 ethyl acetate/hexanes) to give the benzoylated compound (12 mg, 67%) as a light yellow oil: MS (ES+) m/z (M + 1) 628.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 7.5 Hz, 2H), 7.59−7.43 (m, 7H), 7.36 (t, J = 7.5 Hz, 2H), 7.06 (d, J = 7.3 Hz, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 6.56 (broad d, J = 7.8 Hz, 1H), 6.34 (s, 1H), 5.65 (t, J = 7.5 Hz, 1H), 5.27−5.20 (m, 2H), 5.11 (d, J = 10.5 Hz, 1H), 4.29−4.18 (broad m, 1H), 4.08−3.92 (broad m, 2H), 3.88 (s, 1H), 3.82−3.77 (m, 2H), 3.73 (t, J = 7.8 Hz, 1H), 3.57−3.50 (m, 2H), 3.34 (s, 3H), 3.16 (dd, J = 16.6, 8.8 Hz, 1H), 3.00 (d, J = 16.6 Hz, 1H), 2.50 (dt, J = 10.5, 7.5 Hz, 1H), 1.29 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 167,9, 155.5, 150.0, 136.2, 133.9, 133.4, 132.3, 129.8, 128.9 (2C), 128.83, 128.79 (2C), 128.6, 128.5, 127.21 (2C), 127.18, 127,0 (2C), 126.6, 126.3, 118,0, 116.7, 113.0, 93.9, 71.5, 70.8, 67.7, 60.8, 59.0, 57.3, 55.8, 48.3, 34.3, 30.0, 29.7, 13.9.

Compound 19, (2*R*,3*S*)-(9*H*-Fluoren-9-yl)methyl-3-(allyloxycarbonylamino)-2-((*E*)-3-ethoxy-3-oxoprop-1-enyl)-5-(3-hydroxypropoxy)indoline-1-carboxylate.



The synthetic procedure was already described:[4] viscous light yellow oil; MS (ES+) m/z (M + 1) 613.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 3H), 7.57 (d, J = 6.2 Hz, 2H), 7.42 (t, J = 7.3 Hz, 2H), 7.30 (t, J = 7.3 Hz, 2H), 6.97−6.50 (m, 3H), 5.96 (m, 1H), 5.78 (m, 1H), 5.44− 5.18 (m, 2H), 4.95−4.50 (m, 6H), 4.28 (m, 1H), 4.18 (q, J = 7.1 Hz, 2H), 4.07 (m, 2H), 3.86 (t, J = 6.0 Hz, 2H), 2.03 (m, 2H), 1.78 (s, 1H), 1.29 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 156.0, 155.6, 152.9, 144.1, 141.9, 132.8, 128.2, 127.6, 125.3, 125.1, 122.6, 120.5, 120.4, 118.6, 117.2, 117.0, 112.1, 68.3, 67.8, 66.6, 66.4, 61.0, 60.7, 57.5, 47.6, 32.4, 14.6.

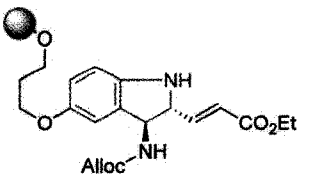
Compound 20, (2*R*,3*S*)-(9*H*-fluoren-9-yl)methyl-3-(allyloxycarbonylamino)-2-((*E*)-3-ethoxy-3-oxoprop-1-enyl)-5-(3-hydroxypropoxy)indoline-1-carboxylate.



The synthetic procedure was already described.[4] 3-[Diisopropyl-(*p*-methoxyphenyl) silyl] propyl-functionalized resin (420.4 mg, 0.4162 mmol) was swollen in dry DCM (2.5 mL) under argon for 30 min in a BIORAD tude. The solvent was then drained to add, by syringe, a solution of trifluoromethanesulfonate acid in dry DCM (4%, 5.61 mL). The resin was then gently agitated for 30 min under argon. The acidic solution was drained, and the activated resin was treated with 2,6-lutidine (364 μL, 3.36 mmol) for 15 min, followed by the addition of a solution of the compound to be loaded (515 mg, 0.841 mmol) in dry DCM (1 mL). The resin was gently shaken overnight. The resin was washed with DMF (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 598.4 mg of the loaded resin (>95%).

Cleavage. The loaded resin (three beads) in an Eppendorf tube was swelled in THF (0.5 mL) for 10 min and treated with HF−pyridine solution (15 μL). The reaction tube was shaken for 45 min; then the reaction was quenched with methoxytrimethylsilane (100 μL), and the tube was shaken for another 10 min. The solution was concentrated and submitted to MS and HPLC/MS analysis: MS and HPLC/MS (ES+) m/z (M + 1) 613.4; HPLC/MS purity >96%.

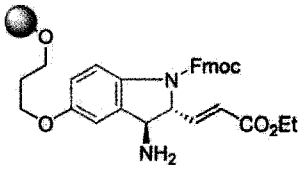
Compound 20a, (*E*)-Ethyl-3-((2R,3S)-3-(allyloxycarbonylamino)-5-(3-hydroxypropoxy) indolin-2-yl)acrylate.



The synthetic procedure was already described.[4] The Fmoc-protected resin (40.0 mg, 0.03960 mmol) was swelled in DMF (2.5 mL) for 30 min. Morpholine (1.0 mL) was added to the reaction mixture, and it was shaken for 30 min. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 32.7 mg of the resulting resin. A sample of the dried resin (three

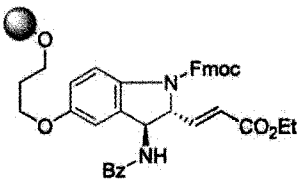
beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 391.2; HPLC/MS purity >95%.

Compound 20b, (2*R*,3*S*)-(9*H*-Fluoren-9-yl)methyl-3-amino-2-((*E*)-3-ethoxy-3-oxoprop-1-enyl)-5-(3-hydroxypropoxy)indoline-1-carboxylate.



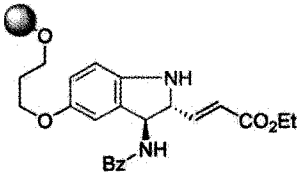
The Fmoc-protected resin (27.0 mg, 0.02673 mmol) was swelled in DCM (2.5 mL) for 30 min. The mixture was drained, and 2.5 mL of a solution of DCM (5 mL), *N*-methylmorpholine (0.32 mL), and acetic acid (0.66 mL) was added the swelled resin. To this mixture was added the ligand PPh_3 (90.3 mg, 0.3408 mmol) and the catalyst $Pd(0)(PPh_3)_4$ (83.0 mg, 0.07110 mmol). The reaction mixture was shaken for 18 h. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 24.5 mg of the resulting resin.

Compound 20c, (2*R*,3*S*)-(9*H*-Fluoren-9-yl)methyl-3-benzamido-2-((E)-3-ethoxy-3-oxoprop-1-enyl)-5-(3-hydroxypropoxy)indoline-1-carboxylate.



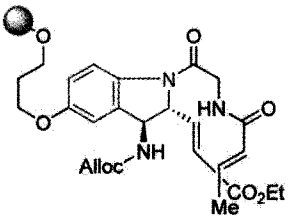
The above primary amine resin (24.5 mg, 0.02673 mmol) was swelled in DCM (2.5 mL) for 30 min. Collidine (0.032 μL, 0.2426 mmol) and benzoyl chloride (0.014 μL, 0.1213 mmol) were added to the reaction mixture, and it was shaken for 8 h. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 24.5 mg of the resulting resin. A sample of the dried resin (three beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 633.3; HPLC/MS purity >73%.

Compound 20d, (*E*)-Ethyl-3-((2*R*,3*S*)-3-benzamido-5-(3-hydroxypropoxy)indolin-2-yl)acrylate.



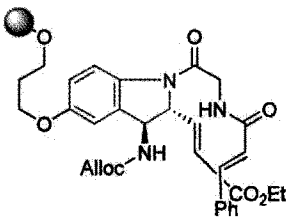
The Fmoc-protected resin (24.5 mg, 0.02673 mmol) was swelled in DMF (2.5 mL) for 30 min. Morpholine (1.0 mL) was added, and the reaction mixture was shaken for 30 min. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 18.0 mg of the resulting resin. A sample of the dried resin (three beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 411.2.

Compound 21, (*E*)-Ethyl-3-((2*R*,3*S*)-3-(allyloxycarbonylamino)-1-(2-(*E*)-but-2-enamidoacetyl)-5-(3-hydroxypropoxy)indolin-2-yl)acrylate.



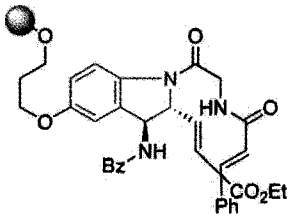
To a stirred solution of glycine crotyl acid (30.0 mg, 0.2093 mmol) in dry DMF (2.5 mL) was added HATU (79.6 mg, 0.2093 mmol), followed by DIPEA (36.5 μL, 0.2093 mmol). The reaction mixture was added to the above secondary amine resin (35.2 mg, 0.04288 mmol), which was swelled in DMF (2.5 mL) for 30 min. The reaction mixture was shaken for 48 h. The reaction mixture was then drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 34.5 mg of the resulting resin. A sample of the dried resin (three beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 516.2; HPLC/MS purity >94%.

Compound 22, (*E*)-Ethyl-3-((2*R*,3*S*)-3-(allyloxycarbonylamino)-1-(2-cinnamamidoacetyl)-5-(3-hydroxypropoxy)indolin-2-yl)acrylate.



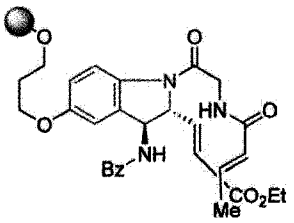
To a stirred solution of glycine cinnamic acid (26.0 mg, 0.1208 mmol) in dry DMF (2.5 mL) was added HATU (46.0 mg, 0.1208 mmol), followed by DIPEA (21.0 μL, 0.1208 mmol). The reaction mixture was added to the above secondary amine resin (24.4 mg, 0.02416 mmol), which was swelled in DMF (2.5 mL) for 30 min. The reaction mixture was shaken for 48 h. The reaction mixture was then drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 26.4 mg of the resulting resin. A sample of the dried resin (three beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 578.3; HPLC/MS purity >90%.

Compound 23, (*E*)-Ethyl-3-((2*R*,3*S*)-3-benzamido-1-(2-cinnamamidoacetyl)-5-(3-hydroxypropoxy) indolin-2-yl)acrylate.



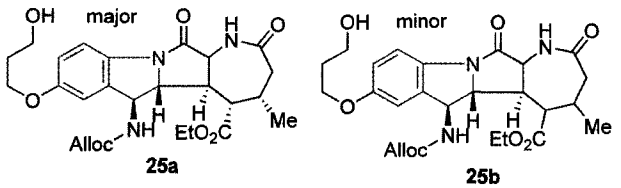
To a stirred solution of glycine cinnamic acid (19.0 mg, 0.08663 mmol) in dry DMF (2.5 mL) was added HATU (33.0 mg, 0.08663 mmol), followed by DIPEA (15.0 μL, 0.08663 mmol). The reaction mixture was added to the above secondary amine resin (18.0 mg, 0.02673 mmol), which was swelled in DMF (2.5 mL) for 30 min. The reaction mixture was shaken for 48 h. The reaction mixture was then drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 18.6 mg of the resulting resin. A sample of the dried resin (three beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 598.3.

Compound 24, (*E*)-Ethyl-3-((2*R*,3*S*)-3-benzamido-1-(2-(*E*)-but-2-enamidoacetyl)-5-(3-hydroxy propoxy)indolin-2-yl)acrylate.



To a stirred solution of glycine crotyl acid (79.0 mg, 0.5445 mmol) in dry DMF (2.5 mL) was added HATU (207.0 mg, 0.5445 mmol), followed by DIPEA (95.0 μL, 0.5445 mmol). The reaction mixture was added to the above secondary amine resin (0.1089 mmol), which was swelled in DMF (2.5 mL) for 30 min. The reaction mixture was shaken for 48 h. The reaction mixture was then drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give the resulting resin. A sample of the dried resin (three beads) was cleaved, and the resulting compound was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 536.2.

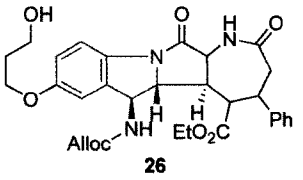
Compounds 25a and 25b.



25a **25b**

The glycine−crotyl-coupled indoline resin **21** (34 mg, 0.033 mmol) was swelled in DCM (2.5 mL) for 30 min. Triethylamine (19.0 μL, 0.132 mmol) and TMSOTf (25 μL, 0.132 mmol) were added to the swelled resin, and then the mixture was shaken for 20 h. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 30 mg of the resulting resin. All of the dried resin was cleaved, and the resulting crude compound (15 mg) was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 516.2; HPLC/MS purity >98% showing 4 isomers 74%, 12%, 3%, and 11%. Analysis of the major product **25a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.3 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 6.81 (s, 1H), 6.24 (broad s, 1H), 5.95−5.81 (m, 1H), 5.30 (d, *J* = 8.0 Hz, 1H), 5.29 (d, *J* = 15.8 Hz, 1H), 5.22 (d, *J* = 10.5 Hz, 1H), 5.16 (t, *J* = 8.0 Hz, 1H), 4.61−4.48 (m, 2H), 4.28−4.14 (m, 4H), 4.13−4.03 (m, 2H), 3.85 (t, *J* = 6.0 Hz, 2H), 2.89 (dd, *J* = 11.3, 3.8 Hz, 1H), 2.84−2.72 (m, 2H), 2.71−2.68 (m, 1H), 2.61−2.51 (m, 1H), 2.02 (quint, *J* = 6.0 Hz, 2H), 1.29 (t, *J* = 7.0 Hz, 3H), 1.04 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 165.6, 157.1, 154.9, 135.7, 134.8, 132.4, 131.1, 118.1, 115.9, 114.7, 110.8, 68.3, 66.1, 65.8, 61.4, 60.2, 59.18, 59.13, 53.3, 47.5, 42.8, 32.0, 30.1, 14.2, 13.4 (See the Supporting Information).

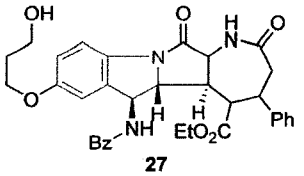
Compound 26 (Mixture of Diastereomers).



26

The glycine−cinnamoyl-coupled indoline resin **22** (26 mg, 0.026 mmol) was swelled in DCM (2.5 mL) for 30 min. Triethylamine (15 μL, 0.104 mmol) and TMSOTf (20 μL, 0.104 mmol) were added to the swelled resin, and then the mixture was shaken for 20 h. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give 25 mg of the resulting resin. All of the dried resin was cleaved, and the resulting crude compound (16 mg) was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 578.3; HPLC/MS purity >60% showing 4 isomers: 67%, 16%, 9%, and 8%. The major compound could be isolated after purification by preparative HPLC (6 mg, 60%): MS (ES+) *m/z* (M + 1) 578.5; HPLC/MS purity >95%. Analysis of the major product of **26**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.5 Hz, 1H), 7.40−7.22 (m, 3H), 7.17 (d, *J* = 6.5 Hz, 2H), 6.83 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H), 6.78 (d, *J* = 2.0 Hz, 1H), 6.50 (broad s, 1H), 5.93−5.79 (m, 1H), 5.43−5.07 (m, 4H), 4.59−4.45 (m, 2H), 4.41 (d, *J* = 10.5 Hz, 1H), 4.25 (broad s, 1H), 4.13−4.01 (m, 2H), 3.93−3.79 (m, 3H), 3.76−3.59 (m, 5H), 3.23−3.08 (m, 2H), 2.95 (broad d, *J* = 15.8 Hz, 1H), 2.07−1.97 (m, 4H), 1.39−1.17 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 164.8, 156.5, 154.3, 142.2, 138.6, 135.1, 131.6, 130.3, 128.03 (2C), 128.02 (2C), 127.3, 127.1, 126.8, 117.4, 115.3, 114.1, 110.2, 69.8, 65.3, 65.2, 60.6, 59.4, 58.0, 31.2, 29.0, 21.9, 13.0 (see the Supporting Information).

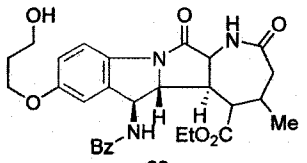
Compound 27 (Mixture of Diastereomers).



27

The glycine−cinnamoyl-coupled indoline resin **23** (18 mg, 0.020 mmol) was swelled in DCM (2.5 mL) for 30 min. Triethylamine (12 μL, 0.080 mmol) and TMSOTf (15 μL, 0.080 mmol) were added to the swelled resin, and then the mixtuer was shaken for 20 h. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give the resulting resin (17 mg). All the dried resin was cleaved, and the resulting crude compound (20 mg) was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 598.5; HPLC/MS purity >45% showing only 2 isomers 22% and 78%.The major compound could be isolated after purification by preparative HPLC (5 mg): MS (ES+) *m/z* (M + 1) 598.1; HPLC/MS purity >99%. Analysis of the major product of **27**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 7.8 Hz, 1H), 7.60−7.42 (m, 6H), 7.37 (d, J = 7.8 Hz, 2H), 6.95−6.87 (m, 2H), 6.62−6.46 (m, 1H), 5.67 (broad s, 1H), 5.11 (broad d, J = 10.8 Hz, 1H), 4.53 (t, J = 6.3 Hz, 1H), 4.23 (broad s, 1H), 4.17−3.93 (m, 5H), 3.92−3.81 (m, 2H), 3.78−3.64 (m, 2H), 3.17 (dd, J = 16.6 Hz, 8.8 Hz, 1H), 3.02 (broad d, J = 16.6 Hz, 1H), 2.99−2.86 (m, 1H), 2.28−2.15 (m, 2H), 1.96−1.73 (m, 1H), 1.34−1.19 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 168.9, 167.8, 157.2, 133.3, 132.3, 128.9 (2C), 128.8 (2C), 128.7, 128.5, 127.2 (2C), 127.0 (2C), 126.93, 126.90, 116.6, 115.4, 114.1, 111.3, 70.68, 70.65, 66.2, 60.8, 60.0, 55.8, 48.2, 43.1, 31.9, 29.7, 13.9 (see the Supporting Information).

Compound 28 (Mixture of Diastereomers).



28

The glycine−crotyl-coupled indoline resin **24** (75 mg, 0.073 mmol) was swelled in DCM (2.5 mL) for 30 min. Triethylamine (41.0 μL, 0.292 mmol) and TMSOTf (55 μL, 0.292 mmol) were added to the swelled resin, and then the mixture was shaken for 20 h. The reaction mixture was drained and washed with DCM (3×), THF (3×), and DCM (3×) over a period of 90 min. The resin was then dried under vacuum overnight to give the resulting resin (70 mg). All the dried resin was cleaved, and the resulting crude compound (60 mg) was analyzed by MS and HPLC/MS: MS (ES+) *m/z* (M + 1) 536.2; HPLC/MS purity >72% showing 4 isomers 20%, 50%, 19%, and 11% (see the Supporting Information).

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Supporting Information Available. Additional figures showing NMR, HPLC, and MS data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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