# SYNTHETIC APPROACHES TOWARD AZABICYCLIC AND AZASPIRO-BICYCLIC SCAFFOLDS RELEVANT TO PEPTIDE CONFORMATION CONTROL

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

SWAPNA BHAGWANTH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

RODNEY L. JOHNSON, Advisor
December, 2009

## Acknowledgment

*"Pigmaei gigantum humeris impositi plusquam ipsi gigantes vident."*
translated in English to:
*"If I have seen further it is only by standing on the shoulders of giants."*
– Sir Isaac Newton in a letter dated 15th February 1676 to Robert Hooke [original quote credited to Bernard of Chartres (d. after 1124 A.D.)]

In what I consider to be the most important section of my dissertation, I shall earnestly attempt, to express my gratitude to all those who have provided me with the wherewithal, to achieve this five-year goal. My sincere hope is that, all of these special people will continue to support me in all my endeavors, for years to come. The following is in no particular order, and I hope no one has been left out.

This work is, in main part, due to the steadfast direction, faith, and generous encouragement of my graduate research advisor, Prof. Rodney L. Johnson. His trust in me and my work was, and is, unfailing. He never replied in the negative to any of my requests, no matter how outrageous they were, as long as I justified them to the best of my abilities. His regular suggestions have kept me on the scientific "straight and narrow". I believed all along that the road to a doctoral degree was as much about the intellectual pursuits, as it was about building character. In that regard, I am a transformed individual because of Prof. Johnson. I will forever endeavor to reach *his* ideal sense of organization and scientific discipline. My attempts to complete this dissertation in a timely manner would have been utterly futile, had it not been for this mentoring.

Of course, none of this would have been possible, even unimaginable, without the very first scientific mentor in my life, my father, Dr. M. R. Ramnath Bhagwanth. My father completed his doctoral research in synthetic chemistry working with Prof. K. Venkataraman (of the Baker-Venkataraman transformation) over forty years ago. If I have never thought of pursuing, anything other than organic synthesis in my life, it is solely because I wished to emulate my father. I would listen bright-eyed to his *exploits* as a chemist, and was forever enchanted by the *mystical* powers of Organic Chemistry. I followed his footsteps and received my baccalaureate from the same institute where he had studied several years before. Even today, he never fails to amaze me with his mastery of organic synthesis, both theoretical and experimental. His skills as a process

chemist are legendary. It is indeed *fortuitous* that I inherited his keen intellect and analytical mind, and perhaps more importantly, his sense of discipline, professional ethics and conscience. With all confidence, I can say that I will never meet anyone who is so whole-heartedly and passionately devoted to their work. To my father, I do certainly hope you are proud of me today.

My development as an organic chemist would have been impossible, without the constant presence of Dr. Ashish P. Vartak during the formative first three years of my Ph.D. I have never met a more consummate experimentalist in synthetic organic chemistry than Ashish. Organic chemistry is his first language and no one else speaks it more fluently. His analytical bent is unsurpassed and he is genuinely *scary* brilliant. He immediately understood the connection between $\alpha$-vinylproline derivatives and ring closing metathesis and *implored* me to pursue that project. In this dissertation, I am honored to have answers to some questions posed by him (in his Ph.D. thesis). My sincere hope is that keen students of organic chemistry are trained under him for years to come. A willing student cannot find a more seasoned mentor than Ashish.

I have been truly blessed to have more than one mentor during the course of my Ph.D. studies. In fact, I have been fortunate to have had (and continue to have) three. Dr. Keith R. Hornberger was my manager, during my stint as a research intern, at Glaxo SmithKline (GSK, RTP) over the summers of 2007 and 2008. Keith has since then gone on from being *boss* to *mentor* and finally to a close friend (almost like older brother to me). Few people in this world can match his spectacular analytical prowess, his buoyant and infectious energy, and fewer still possess his “smarts”. Soon after working with Keith, I regained confidence in my scientific abilities, learned how to function successfully within a deadline, and improved my professional communication skills. Keith provided instant feed-back, which was essential to my development as a scientific professional. In addition, he is a consummate synthetic and medicinal chemist, who is truly committed to oncology drug discovery. I genuinely hope more advanced students of synthetic chemistry the opportunity to work with him. I also appreciate Keith for taking the time off from his hectic work schedule, and flying from New York to Minneapolis on November 5$^{th}$, 2009, to attend my Ph.D. defense seminar. My experience at GSK with Keith and the rest of the Oncology Medicinal Chemistry department was extremely positive, and I hope to find myself in such a productive and fulfilling professional atmosphere in the future.

This would also be a good time to thank Dr. Kirk L. Stevens, who also interviewed me at GSK for an internship position. In addition to being a brilliant medicinal chemist, Kirk is by far one of the best managers I have met. His communication and networking skills and the respect with which he treats everyone around him are admirable. In addition, Kirk is the most positive person I have ever met; his attitude is enviable. I can only *hope* to reach those ideals sometime in my professional life. I have Kirk to thank for, for the next phase of my professional life. He directly recommended me for the Research Associate position at Boston University, a position I will assume on November 16$^{th}$, 2009, after this dissertation is completed.

A colleague once told me that a Ph.D. was a marathon and not a sprint. It is very easy to lose focus and confidence when things aren't working out as planned in the laboratory. In that regard, Dr. Bhooma Raghavan has been my rock; and my punching bag! Bhooma was the first person I worked with in the Johnson laboratory; it was very kind of her to acknowledge me in her *J. Org. Chem.* paper and Ph.D. thesis. The door to Bhooma's laboratory/office was always open so I could bounce-off ideas for my projects, air out pet-peeves and complaints, and seek advice on important decision-making. Bhooma is a genuinely caring individual, and only wants the best for everyone around her. She is a fantastic team-player and a strong group motivator. Thanks for providing me bonafide home-cooked South Indian meals whenever I was ill (which was a lot). There's a cupboard in my kitchen filled with her dishes and containers, which I will hopefully return before I leave from here. Bhooma also opened her home to me when I was recuperating from surgery in early January 2009. Those were the 3 best days of the last five years. She took care of me as only family can. Bhooma is the best friend I made during my Ph.D. and I sincerely hope we stay that way for years and years to come. I only wish the best possible things for her family and herself.

I sincerely appreciate the mentoring by Prof. Patrick E. Hanna. Thank you for scanning my written proposal and my Ph.D. dissertation with a fine-toothed comb. Every graduate student in medicinal chemistry should have the opportunity to learn from him; for not only is he one of the best medicinal chemists, but his professional ethics are outstanding. Thank you for serving as a dissertation committee member. I also sincerely appreciate feedback from Prof. Robert A. Fecik. Robert offered worldly and practical advice when it came time for me to apply for post-doctoral positions. Thank you for serving as the Chairperson of my dissertation committee, and for including me in student

recruitment activities. I also would like to thank Prof. Craig J. Forsyth for serving on my preliminary examination committee and to Prof. T. Andrew Taton for taking over as the external member. I also truly appreciate Dr. Taton for teaching my first (and one of my favorite) courses (CHEM 8411) I took as a graduate student. In addition, a special shout-out to Prof. Gunda I. Georg; she went over and above her duties, in mentoring me while I was applying for post-doctoral positions in late 2008. Her commitment to graduate education is unparalleled. Dr. Georg, I truly appreciate the time and effort you took to read my research summary (three drafts no less!!). I also appreciate the time and effort taken by Dr. Alex G. Waterson in reviewing my post-doctoral application dossier. He also went the extra mile and recommended me to several organic chemists within the Meyers network. I would like to thank our collaborator, Dr. Ram K. Mishra, and his colleagues at McMaster University, for conducting the pharmacological evaluation of the PLG $D_2$ receptor modulators. I would also like to thank Dr. Beverly Ostrowski, for putting up with me as the NMR research assistant for two years, and training me on the Varian instruments. I would also like to thank Dr Victor G. Young and Lindsay Hinkle for procuring X-ray crystallographic structures of some of my compounds.

Weaver Densford Hall (WDH) is one of the *tallest* buildings at the University of Minnesota. On a clear day, you can see it from far, far away from any direction. I looked forward to going up to the $8^{th}$ floor every day that I worked for five years. Much of that was because of the co-inhabitants of lab. 8-167. In addition to Bhooma and Ashish, a few special folks made my life worthwhile. Thank you, Dr. Abigail L. Fisher, for being the coolest person to hang out with! In addition, I truly appreciate your efforts in proof-reading two chapters of this thesis. My colleague, Dr. Cory S. Kending was a kindred spirit; except when he was rock-climbing or training for marathons! His work ethic and organizational skills are outstanding. Cory is a true-blue midwestern gentleman. I truly hope he is "*living the dream*". Also, I appreciate Dr. Xiaochun Dong's patience with teaching me the best way to conduct HRMS data collection. I wish him all the best in his new career at Fudan University in Shanghai, China.

I truly appreciate members of my *surrogate* research group, the Fecik group members. Erick Leggans will go a long way; not merely as an organic chemist, but as a team leader, motivator, mentor and teacher; Dr. Ranganathan Balasubramanian for being an excellent sport; Dr. Amber Onorato for being the quintessential extrovert, for *ripping apart* the first draft of my research summary (the closest friends are brutally

honest indeed) and for buying obscenely priced Vikings tickets so I would have company to watch my first and perhaps last game at the Mall of America field (Sunday Oct. 18th 2009, Vikings vs. Ravens, Go Vikings!!!); and Dr. Dennis Brown for coming to my rescue and taking over my apartment lease once I left Minneapolis.

To Sharon Baker, my best wishes and I hope we can travel to India and Tibet together sometime. All the nefarious printing activities paid off! Thank you for also proof-reading two chapters of my dissertation. You might just be the nicest person on Earth. Thank you, Joyce Reha, for being the person truly in the driver's seat at the department of Medicinal Chemistry. Is there anything you cannot do? I appreciate you for accompanying me to Tiffany's to get my engagement ring and for setting up my initial accommodation at Boston with Tom and Majella. *I am still shaking my head in disbelief.*

Life at WDH was bearable mainly due to the strong, life-long bonds Jolanna Norton and Sadiya Addo shared with me. It was hard when they left, but I always knew that they believed in me and everything that I could achieve in Minnesota.

To Aaron Teitelbaum, thank you for being a good friend, and an even better chauffeur; and boy am I glad you decided not to dabble in synthetic chemistry! Thanks to Katie Pietsch for helping me with the business cards and for being an all-around good egg. I also appreciate my room-mate of three years, Nitya Jacob, who is a very caring person. I wish her the best in all walks of life.

I would like to extend my heart-felt gratitude and also wish my best to the following from my time at UMN: Dr. Earl W. Dunham, Dr. Shana J. Sturla, Dr. Swati S. More, Sally Kessler (for proof-reading two chapters of my thesis), and Rajiv Taribagil and the following from my time @ RTP, NC: Dr. Gregory M. Schaaf, George M. Adjabeng, Ronda Davis-Ward, James Salovich, Hamilton Dickson, Dr. Samarjit Patnaik, Dr. Khalida Shamim, Stephon C. Smith and John Catalano, and from back home in India: Mahesh Subramaniam (brother-in-law extraordinaire), Akriti and Dhruv (niece and nephew respectively, just for being adorable and the last people I think about every night) and Chandrasekhar Sridhar (my best friend and brother-from-another-mother).

Finally, none of this would have been imaginable, let alone possible without the incessant support of the following special members of my family. My mom, Leela Bhagwanth, was always there with her patient listening ear, especially when I became really ill in 2006. For three years, when the doctors could not pin down my condition and when I was ready to give up, it was mainly because of my mother's unfailing words of

encouragement that I held it together here. Even till today, I can depend on her for practically anything. Another important person in my life has been my older sister, Shreya Bhagwanth. Shreya has a very strong personality, and I have always wanted to emulate her confidence and her no-holds-barred approach to anything she sets her mind to. She has kept me pragmatic and grounded all my life, and the last five years were no different. I truly would be lost without her. Finally, my fiancé, Dr. Siddharthya K. Mujumdar has been my rock of Gibraltar over the last few years. We started off as quiz partners in UDCT, Bombay back in 2002. No one would have imagined that we would find ourselves at University of Minnesota (he in 2003, and me in 2004), pursuing our respective Ph.D. degrees, nine thousand miles away from home. Siddharthya, had to be my dad, mom, and sis put together, to get me through this past five years. We have gone through some tough, grueling times together, and *I don't think there is anything we cannot achieve together*. I am truly blessed to have someone who loves and cares for me as much as he does. And now, after spending two years apart, I am ecstatic to be moving closer to him.

Guys, I hope you are proud of my accomplishments.

*For Amma and Appa,*

*Who brought me into this world and gave me more than I could have asked for.*

## Abstract

A ring closing metathesis (RCM) strategy was explored toward synthesis of a conformationally constrained Type VIa *β*-turn mimic of the tripeptide dopaminergic receptor modulator, Pro-Leu-Gly-$NH_2$ (PLG). It was anticipated that this strategy would result in a generalized route to synthesize the indolizidinone scaffold-based Type VIa *β*-turn mimic of any Xaa-Pro dipeptide. Although this strategy was unyielding in the desired result, the study highlighted the recalcitrant reactivity of sterically hindered amino acid residues.

The interaction between the *N*-terminal tetrapeptide, Ala-Val-Pro-Ile (AVPI) of the pro-apoptotic protein, second mitochondrial activator of caspases (Smac), and the binding site on the X-linked inhibitor of apoptosis protein (XIAP) has been previously validated for its chemotherapeutic potential. We, designed and synthesized, conformationally-constrained diastereoisomeric AVPI analogues, containing the 5.5-fused bicyclic thiazolidine scaffold. Although the synthesis of this scaffold presented several challenges; the base- and acid-lability of the thermodynamically unstable diastereomer as well as difficulties in the vital amidation step notwithstanding; synthesis of one diastereomer was successfully completed.

A diastereomeric pair of 5.6.5-azaspirobicyclic lactams, synthesized to mimic the biological activity of PLG and possessing opposite stereochemistry at the C-8a' position, was recently found to have *opposing* dopamine $D_2$ receptor modulatory activities. Our goal was to identify the structural elements in this scaffold that were responsible for this opposite activity. We therefore, utilized molecular modeling on existing PLG modulators containing this scaffold and designed more 5.6.5-azaspirobicyclic lactams containing: (1) different stereochemistry at the C-3' position and (2) *various* substitutions at the C-2' position. Following the established paradigm toward synthesis of the target molecules

only led to inconvenient epimerization/racemization issues. However, with a seemingly simple change in the order of events provided the desired target molecules. In addition, our initial hypothesis was verified by pharmacological testing of the synthesized molecules. Eventually it is anticipated that use of both positive and negative modulators will serve to understand the overall structural and biochemical mechanism of PLG-mediated $D_2$ receptor modulation.

## Table of Contents

## List of Tables

## List of Figures

## List of Schemes

## Abbreviations

| | |
|---|---|
| $[\alpha]_D$ | Specific rotation |
| π-Allyl Pd | π-Allyl palladium chloride dimer |
| 6-OHDA | 6-Hydroxydopamine |
| ADTN | 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene |
| Ala | Alanine |
| anhyd. | Anhydrous |
| AMPT | DL-Amphetamine |
| Approx. | Approximately |
| BAIB | *bis*-Acetoxy iodobenzene |
| Bn | Benzyl |
| BnBr | Benzyl bromide |
| $BnNH_2$ | Benzylamine |
| Boc | *tert*-Butyloxycarbonyl |
| $Boc_2O$ | Di-*tert*-butyloxy dicarbonate |
| BOP-Cl | *bis*-(2-Oxo-3-oxazolidinyl)phosphonic chloride |
| nBuLi | *n*-Butyllithium |
| α-C | Alpha carbon (with respect to a functional group) |
| $CaH_2$ | Calcium hydride |
| *c*AMP | cyclic Adenosine 3',5'-monophosphate |
| Cbz | Benzyloxycarbonyl |
| CbzCl | Benzyl chloroformate |
| CD | Circular Dichroism |
| $CDCl_3$ | Deuterated chloroform |
| $CD_3OD$ | Deuterated methanol |
| $CH_2Cl_2$ | Dichloromethane |
| $CH_3CN$ | Acetonitrile |
| $CH_3I$ | Iodomethane |
| $CH_2N_2$ | Diazomethane |
| CNS | Central Nervous System |
| conc. | Concentrated |
| $C_q$ | Quaternary carbon |
| $Cs_2CO_3$ | Cesium carbonate |
| COSY | Correlation Spectroscopy |
| gCOSY | gradient Correlation Spectroscopy |
| $CrO_3$ | Chromium trioxide [Cr(VI)] |
| D- | Dextrorotatory |
| DABCO | 1,4-Diazabicyclo[2.2.2]octane |
| (*S*,*S*)-DACH | *N*,*N*'-bis(2-Diphenylphosphino-1-naphthoyl)-(1*S*,2*S*)-1,2-diaminocyclohexane |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| DCC | 1,3-Dicyclohexylcarbodiimide |
| dec. | Decomposed |
| $D_2$ | Dopamine $D_2$ receptor |
| DEPT-135 | Distortionless Enhancement by Polarization Transfer (flip angle 135°) |
| DIBAL-H | Diisobutylamluminium hydride |
| DIPEA | Diisopropylethyl amine (Hünig's base) |

| | |
|---|---|
| DMF | *N,N*-Dimethylformamide |
| DMSO-$d_6$ | Deuterated dimethyl sulfoxide |
| DMAP | 4-Dimethylaminopyridine |
| DYKAT | Dynamic Kinetic Asymmetric Transformation |
| EDC | 1-[3-(Dimethylamino)propyl)]-3-ethylcarbodiimide |
| EPS | Extrapyramidal side effects |
| Equiv. | Equivalents |
| EtOH | Ethanol |
| $Et_3N$ | Triethylamine |
| $Et_2O$ | Diethyl ether |
| EtOAc | Ethyl acetate |
| ESI | Electrospray Ionization |
| Fmoc | 9-Fluorenylmethyloxycarbonyl |
| GDP | Guanosine 5'-diphosphate |
| Gly | Glycine |
| Gpp(NH)p | 5'-Guanylyimidodiphosphate |
| GTP | Guanosine 5'-triphosphate |
| $H_2O$ | Water |
| HCl | Hydrochloric Acid |
| $H_2SO_4$ | Sulfuric Acid |
| HATU | *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate |
| HMBC | Heteronuclear Multiple Bond Correlation |
| HMDS | Hexamethyldisilzane |
| HMPA | Hexamethylphosphoramide |
| HMQC | Heteronuclear Multiple Quantum Coherence |
| gHMQC | gradient Heteronuclear Multiple Quantum Coherence |
| HOAt | 1-Hydroxy-7-azabenzotriazole |
| HOBt | 1-Hydroxybenzotriazole |
| HPLC | High Pressure Liquid Chromatography |
| HRMS | High Resolution Mass Spectrometry |
| IBCF | Isobutylchloroformate |
| Ile | Isoleucine |
| IR | Infared spectroscopy |
| $K^\circ$ | Potassium (metal) |
| $K_2CO_3$ | Potassium carbonate |
| KHMDS | Potassium hexamethyldisilazide |
| KO*t*Bu | Potassium *tert*-butoxide |
| L- | Levorotatory |
| LDA | Lithium Diisopropylamide |
| Leu | Leucine |
| LHMDS | Lithim hexamethyldisilazide |
| $Li^\circ$ | Lithium (metal) |
| LiCl | Lithium chloride |
| LiOH | Lithium hydroxide |
| lit. | Literature reference |
| LiO*t*Bu | Lithium *tert*-butoxide |
| LRMS | Low Resolution Mass Spectrometry |
| $Li/NH_3$ | Lithium/ammonia |

| | |
|---|---|
| MeI | Iodomethane |
| MeOH | Methanol |
| MIF-1 | Melanocyte Inhibiting Factor-1 |
| mp | Melting Point |
| mmol | Millimoles |
| mL | Milliliter |
| MPTP | 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| MsCl | Methanesulfonyl chloride |
| MSH | Melanocyte-Stimulating Hormone |
| Mukaiyama's Reagent | 2-Chloro-1-methylpyridinium Iodide |
| $NaBH_4$ | Sodium borohydride |
| NaClO | Sodium hypochlorite/bleach |
| $NaClO_2$ | Sodium chlorite |
| NaH | Sodium hydride-60% Mineral oil dispersion |
| $NaHCO_3$ | Sodium bicarbonate |
| $NaIO_4$ | Sodium periodate |
| NaOH | Sodium hydroxide |
| $Na_2SO_3$ | Sodium sulfite |
| $NH_2NH_2 \bullet H_2O$ | Hydrazine hydrate |
| $NH_3$ | Ammonia |
| NMO | *N*-Methylmorpholine-*N*-oxide |
| NMM | *N*-Methylmorpholine |
| NMR | Nuclear Magnetic Resonance |
| NOESY | Nuclear Overhauser Effect Spectroscopy |
| NPA | *N*-Propylnorapomorphine |
| $OsO_4$ | Osmium tetroxide |
| PD | Parkinson's Disease |
| $Pd^{\circ}$ | Elemental Palladium |
| Pd/C | Palladium on carbon |
| PLG | L-Prolyl-L-leucyl-glycinamide |
| Pro | Proline |
| psi | Pound per square inch |
| PyBrOP | Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate) |
| Ref. | Reference |
| $R_f$ | Ratio to Front |
| r.t. | Room temperature |
| SAR | Structure Activity Relationships |
| sat. | Saturated |
| $SiO_2$ | Silica gel |
| $SOCl_2$ | Thionyl chloride |
| TEMPO | 2,2,6,6-Tetramethylpiperidine-1-oxyl |
| TD | Tardive dyskinesia |
| THF | Tetrahydrofuran |
| TFA | Trifluoroacetic acid |
| TLC | Thin Layer Chromatography |
| $t_R$ | Retention Time |
| Tr | Trityl/Triphenylmethyl |
| TrCl | Trityl Chloride |
| *p*-TsOH | *para*-Toluenesulfonic acid |

| | |
|---|---|
| Val | Valine |
| Xaa | Any α-amino acid |
| $Zr(O\mathit{t}Bu)_4$ | Zirconium (IV) *tert*-butoxide |

# Chapter 1

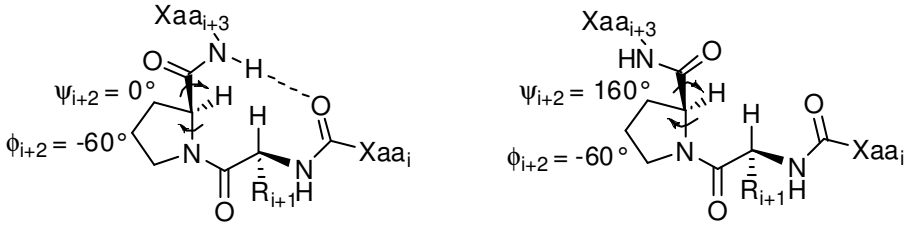
## *Rationale for Developing a Different Synthetic Strategy toward Indolizidinone-Based Type VI β-Turn Mimics*

### 1.1. Introduction

All 20 naturally occurring common α-amino acids, except L-proline (Pro), are primary amines. The latter is a cyclic imino acid containing a secondary amine which imparts unique conformational characteristics to Pro. For the *N*-terminal amides of primary amino acids, the difference in free energy between the *trans*- and *cis* amide bond conformers is significant ($\Delta G° = 2.6$ kcal/mol), the *trans* isomer being the more stable one mainly due to steric preference.[1] For *N*-terminal amides of proline, the free energy difference is lowered to a value of 0.5 kcal/mol.[1] This leads to an increased potential for the *cis* conformer being present in significant proportions compared to the *trans* form, even in biological conditions.[2] In fact, *cis*-proline is the second-most conserved residue encountered biologically in proteins, the first being half-cystine.[3]

The type VI *β*-turn is a conformational feature of certain biologically relevant peptides. The observation pertinent to this discussion is that a type VI *β*-turn incorporates a *cis*-proline in its i+2 position. *It is important to emphasize that the presence of L-proline in the i+2 position does not connote the presence of the type VI β-turn conformation.*[4]

The type VI *β*-turn family consists of two sub-types: type VIa and type VIb (Figure 1.1). A rotation of the ψ(i+2) torsion angle by 160° prevents an intramolecular hydrogen bond between the i-C=*O* and i+3-N*H* in the type VIb *β*-turn. The *ideal* torsion angles of the backbone i+1 and i+2 residues of a type VI *β*-turn are shown in Table 1.1.[5]




Type VIa *β*-turn  Type VIb *β*-turn

**Figure 1.1.** Type VI *β*-turn sub-types

**Table 1.1.** Defining torsional angles of i+1 and i+2 residues of type VI *β*-turns

| Turn type | φ(i+1) | ψ(i+1) | φ(i+2) | ψ(i+2) |
|---|---|---|---|---|
| *VIa* | –60° | 120° | –90° | 0° |
| *VIb* | –135° | 135° | –75° | 160° |
| *II* | –60° | 120° | 80° | 0° |

### 1.2. Type VI *β*-turn and the tripepide L-Pro-L-Leu-Gly-$NH_2$ (PLG)

The solid state conformation of the positive allosteric dopamine modulator, PLG, was determined to be a type II *β*-turn.[6] Previous attempts at understanding the conformation of this tripeptide in solution gave widely ranging results.[7–10] For instance, NMR experiments indicated that it was the *protonated* form of the peptide that was partially capable of adopting a more ordered structure (such as a type II *β*-turn) in solution. Conversely, the unprotonated form was unable to adopt any particular conformation namely because of significant aggregation at concentrations used in the NMR analyses.

The positional propensity of Pro at each residue positions in *β*-turns[11] is shown in Table 1.2. This indicates that Pro is almost never found in the i+2 and i+3 position of a

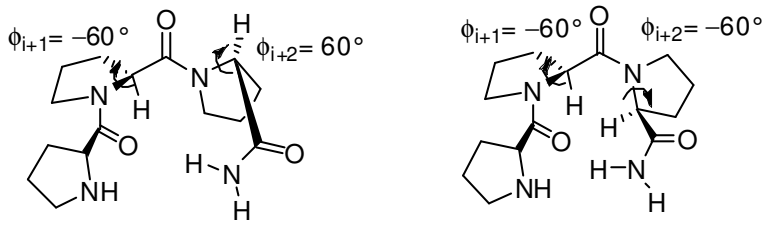
**Table 1.2.** Positional propensity of L-proline

| | **Turn** | **i** | **i+1** | **i+2** | **i+3** |
|---|---|---|---|---|---|
| Pro | *generic* | 1.48 | 2.45 | 0.63 | 0.96 |
| Pro | *type II* | 1.99 | 4.91 | 0.00 | 0.00 |

type II *β*-turn and that it has a 2.5-fold propensity to be found in the i+1 position compared to the i position. Although position potentials are not available for the type VI *β*-turn, it should be noted that based on literature precedent, out of 18 examples where Pro can be found to stabilize a type VIa *β*-turn, Pro occupies the i+1 position in 3 examples and the i+2 position in 15 examples.

While investigating the correlation between the conformation and bioactivity of peptidomimetics based on PLG, there was a need for constraining the tripeptide, L-Pro-L-Pro-L-Pro-$NH_2$, in a type VI *β*-turn conformation. It was hoped, that the triprolines would lead to a more flexible peptidomimetic scaffold, as compared to the spirobicyclic PLG mimetics that had been designed, synthesized and tested biologically. On the basis of data in Table 1.1., it is obvious that the all-L-triproline would be incapable of maintaining a type II *β*-turn conformation. Yet, through pharmacological testing of the triprolines, it was evident that the homochiral triproline peptide was far superior in its activity than the corresponding analogues that were conformationally capable of mimicking the type II *β*-turn.

Since the presence of Pro in the i+2 increases the possibility of a *cis*-amide bond, it also introduces the possibility of a type VI *β*-turn conformation as the bio-active conformation. Since the original hypothesis was that the bio-active conformation of PLG is a type II *β*-turn, a keen observer would realize the similarity between the two turns described by the triprolines (Figure 1.2.).



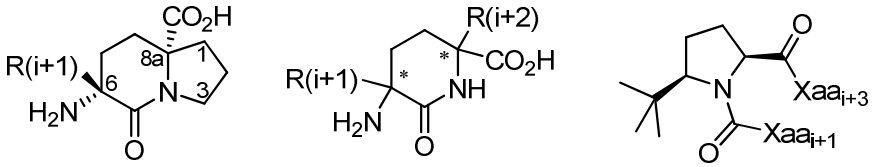

L-Pro-L-Pro-D-Pro-$NH_2$
Type II $\beta$-turn conformation

L-Pro-L-Pro-L-Pro-$NH_2$
Type VI $\beta$-turn conformation

**Figure 1.2.** Comparison between triprolines in type II and type VI conformations

### 1.3. Rationale for selection of the indolizidinone-based type VI *β*-turn mimic

Most literature reports directed at developing small molecule mimics of the type VI *β*-turn, concentrate on *cis*-Pro amide mimicry. Some examples are shown in Figure 1.3., and include 6-amino-8a-carboxyindolizidinone **1.1**,[12] *cis*-(rac)-3-amino-6-carboxypiperidinone **1.2**[13] and 5-*tert*-butylproline **1.3**[14]. Although the *cis*–Pro amide form is inherently unstable, the type VI *β*-turn containing *cis*-Pro is found in biologically relevant locations and therefore there has been a steady growth in developing mimics of this conformation.




**1.1** **1.2** **1.3**

**Figure 1.3.** Examples of type VI $\beta$-turn mimics

Of the existing type VI *β*-turn mimics in the literature, the indolizidinone mimic developed concurrently albeit independently by the Gramberg[12a] and Germanas[12b] groups was selected. The bicyclic lactam exhibits minimal deviation from the parent peptide being constrained. As shown in Figure 1.4., the lactam is developed by bridging the α-carbons of the i+1 and i+2 residues with an ethylene linker. The structure of the indolizidinone Gly-Pro mimic (where $R_{i+1}$ = H) was determined by X-ray crystallography.[12b] The main features were: (1) the pseudo-chair (almost cyclohexene-like) conformation of the 6-membered lactam ring with the amino terminus in a pseudo-equatorial position and (2) the *twisted* amide, where the amide bond was twisted out of planarity (ω= –14.6°) due to the strained bridgehead position of the lactam nitrogen. It is also important to note in this discussion that the indolizidinone is *cis*-substituted at the C-6 amino and C-8a carboxy terminal positions.

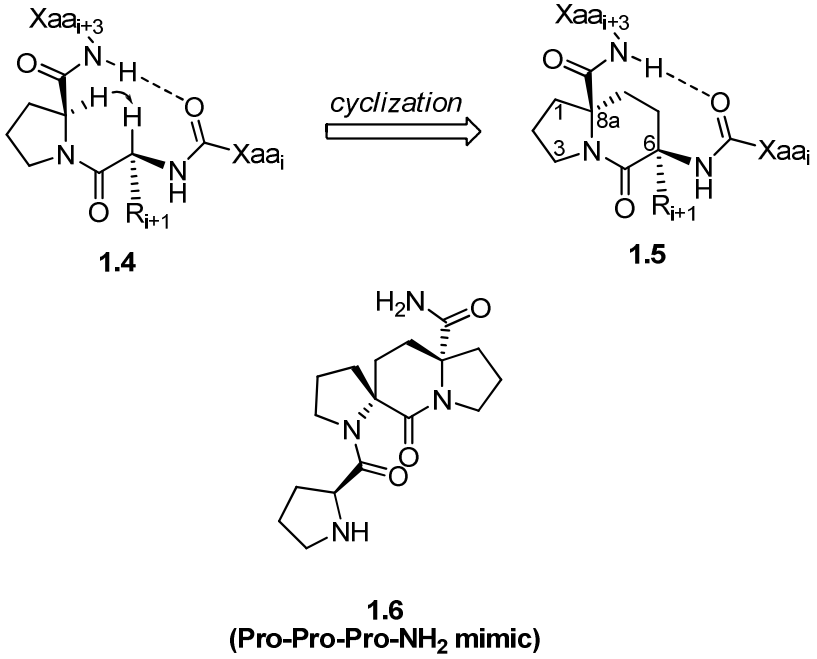
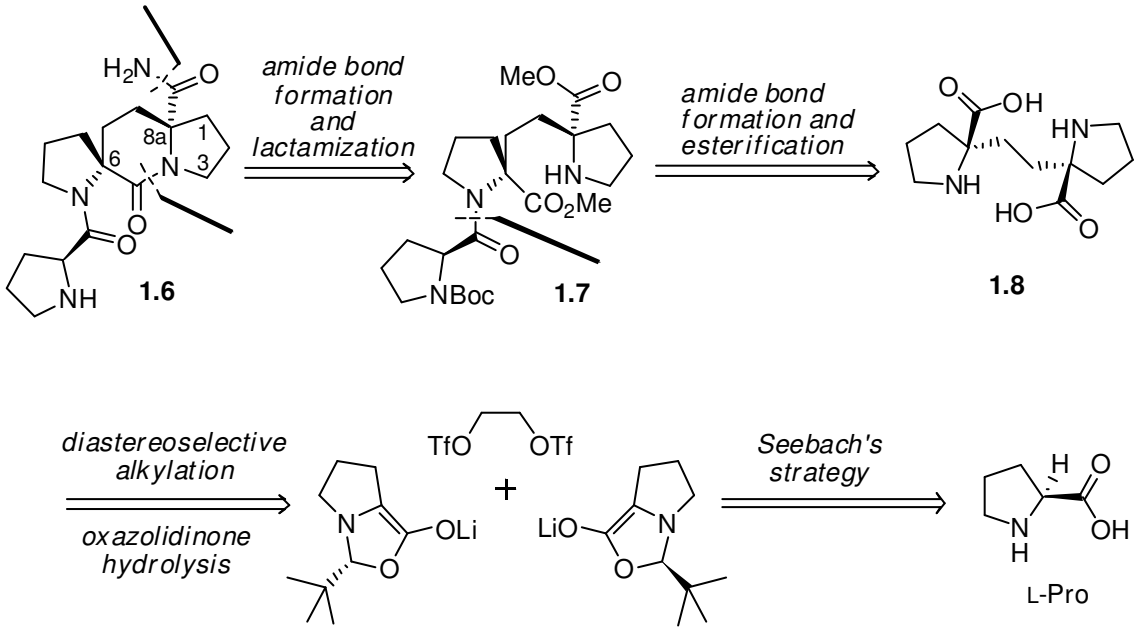



**Figure 1.4.** Indolizidinone-based type VI *β*-turn mimic

### 1.4. Previous approach toward synthesis of indolizidinone mimic 6

Realizing immediately that the L-Pro-L-Pro-L-Pro-$NH_2$ indolizidinone mimic had identical i+1 and i+2 Pro residues, Dr. Ashish P. Vartak, a previous graduate student in the Johnson laboratory, explored a concerted approach toward its synthesis.[15] His strategy hinged in the simultaneous generation of the desired stereochemistry about both C-6 and C-8a of the ultimate indolizidinone in one step (Scheme 1.1). This was accomplished by alkyating Seebach's oxazolidinone with ethylene glycol bis-triflate as the electrophile. This strategy was successful toward the synthesis of the desired indolizidinone mimic.[16] Although an elegant concerted strategy, this approach was limited toward synthesis of the type VI *β*-turn mimic where **i+1 = i+2 = Pro**.

**Scheme 1.1.** Retrosynthetic strategy for previous synthesis of **1.6**



## 1.5. Ring Closing Metathesis (RCM)-approach toward synthesis of type VI *β*-turn mimics

The RCM-approach toward synthesis of indolizidinone type VI *β*-turn mimics where **i+1 ≠ i+2** was inspired in part by investigations conducted by the Gmeiner group in 2001[17] and in part by a comment made by Dr. Ashish P. Vartak in his doctoral dissertation.[18] In the former report, the rational design and synthesis of lactam-bridged type VI *β*-turn inducing peptide mimics using RCM was delineated. According to their Molecular Dynamics (MD) calculations, the *trans*-substituted 8-membered bicyclic lactam containing the internal olefin was the most optimal type VI *β*-turn mimic capable of sustaining a stable turn conformation (Figure 1.5). The existence of an intra-molecular hydrogen bond was partially confirmed by variable temperature NMR (VT-NMR) spectroscopy. As shown in the scheme below, this approach hinged on the enantioselective synthesis of α-allylproline and α-allylglycine derivatives. In an independent study by Derrer *et al.*,[19] the same 8-membered lactam was found to be capable of type VIb conformational mimicry.

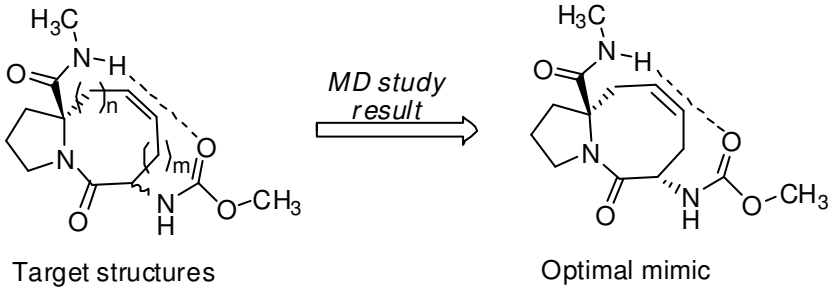


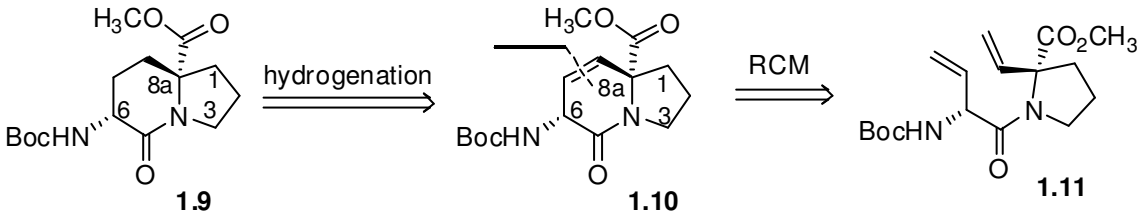

**Figure 1.5.** Structure optimization by the Gmeiner group varying m,n from 0–1

These two studies differ from the detailed conformational analyses conducted by Germanas[12b] in the support of the 6-membered indolizidinone mimic. Our main aim

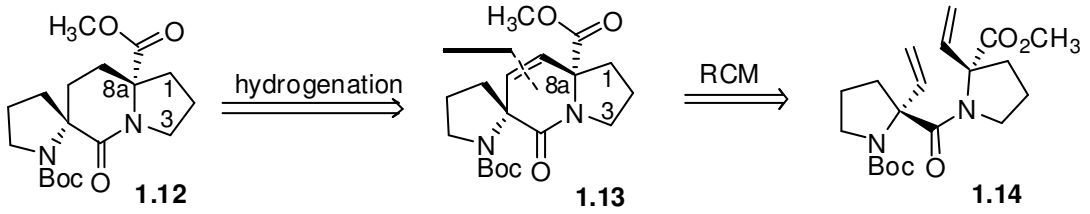
therefore was to develop an RCM-based strategy toward synthesis of a generic indolizidinone-based type VI *β*-turn mimic (*of the tripeptide Pro-Xaa-Pro*).

To test the feasibility of this synthetic route, two target molecules were proposed. In addition to the Pro-Pro indolizidinone scaffold **1.12**, the sterically less-hindered Gly-Pro scaffold **1.9** was also selected. Retrosynthetically, the target molecules can be obtained by hydrogenation of the didehydro intermediates **1.10** and **1.13** (Scheme 1.2). It was hoped that these intermediates would be obtained by an RCM reaction on the corresponding Gly-Pro and Pro-Pro dipeptides.

**Scheme 1.2.** Retrosynthetic analysis involving RCM




Gly-Pro indolizidinone mimic




Pro-Pro indolizidinone mimic

In order to synthesize **1.11** and **1.14**, the strategy requires the ready availability of enantiopure α-vinyl amino acids. As will be discussed in the following chapter, the synthesis of the α-vinylproline and α-vinylglycine residues proved to be an extremely non-trivial task.

# CHAPTER 2

*Attempts Toward the Construction of the Indolizidinone Nucleus of Type VIa β-turn mimics of the type Pro-Xaa-Pro Using Ring Closing Metathesis (RCM)*

## 2.1. Introduction

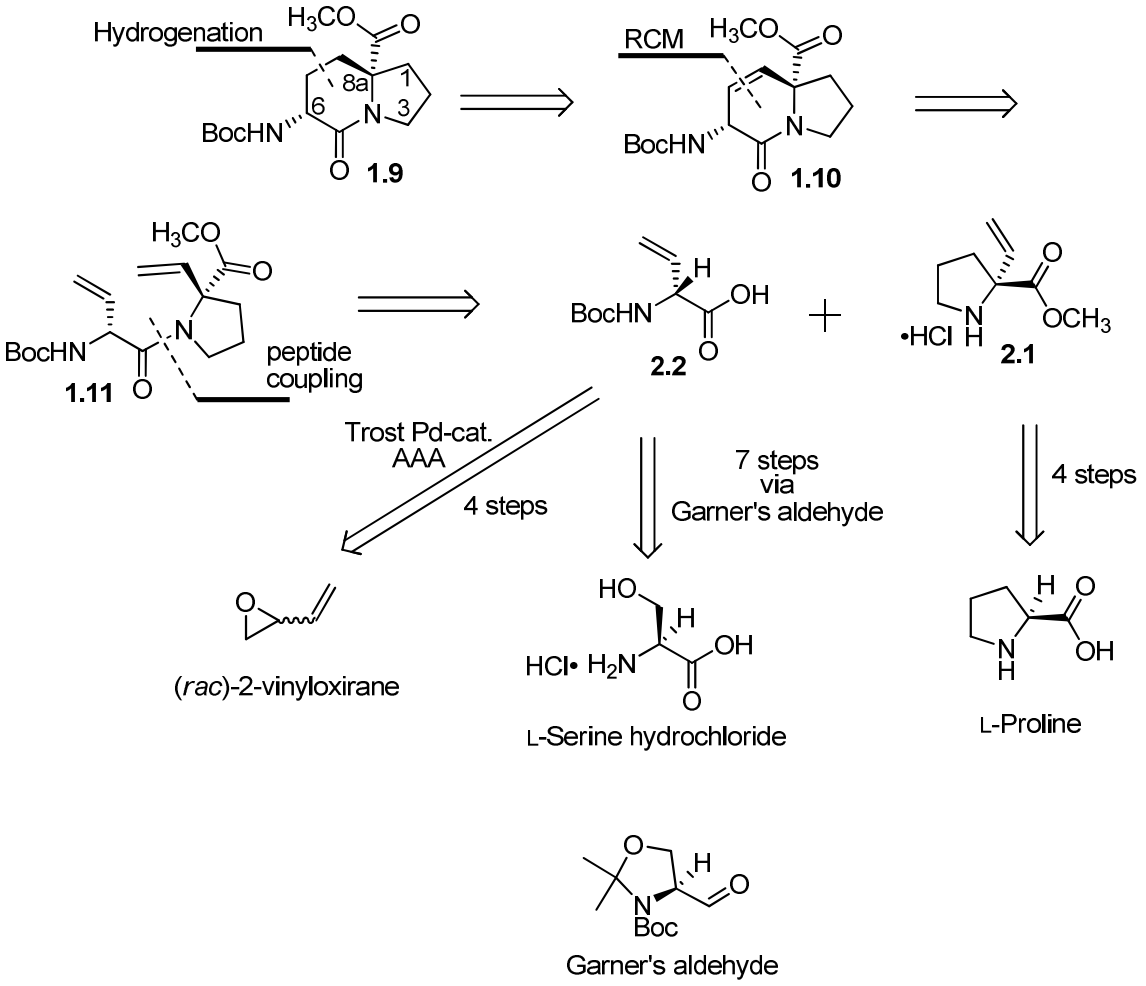
The attempted synthesis of the two target indolizidinone mimics, **1.9** and **1.12** via the RCM strategy will be discussed in the following order:

1. Retrosynthetic analyses of indolizidinones **1.9** and **1.12**
2. Synthesis of key intermediates, α-vinyl and α-allyl amino acids in an optically pure form.
3. Synthesis of α-vinylglycine–α-vinylproline (vinylGly-vinylPro) and α-vinylproline–α-vinylproline (vinylPro-vinylPro) dipeptides, **1.11** and **1.14**
4. Synthesis of dipeptides with homologated side chains (α-allylglycine–α-allyl proline (allylGly-allylPro), α-allylproline–α-allylproline (allylPro-allylPro), α-allyl proline–α-vinylproline (allylPro-vinylPro)
5. Ring Closing Metathesis reaction attempts

Retrosynthetically, the indolizidinone **1.9** can be reduced to two α-vinyl amino acid subunits, **2.1** and **2.2** (Scheme 2.1). The first challenge therefore involved the stereoselective syntheses of two unnatural amino acids, (*R*)-α-vinylglycine and (*R*)-α-vinylproline. For the α-vinylproline residue, **2.1**, we anticipated applying Seebach's "self regeneration of stereocenters" strategy utilizing an electrophile that could *indirectly* introduce the vinyl substitution at the α-carbon (α-C) of proline. Initial retrosynthesis for **2.2** indicated that L-serine could be a viable starting material. Garner's aldehyde would serve as an important intermediate. Another proposed route involved use of the Trost Pd-catalyzed asymmetric allylic alkylation strategy and hinged on the possible one-pot

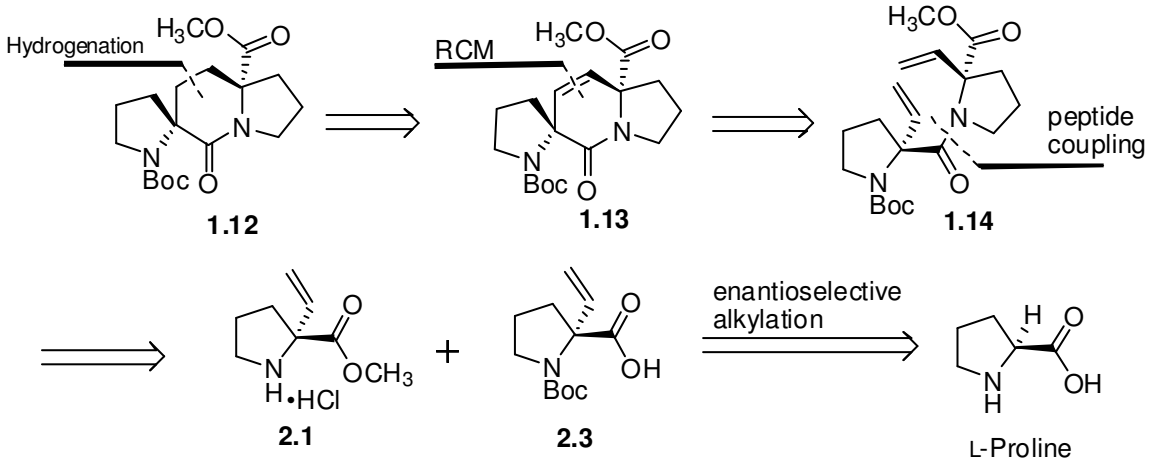
oxidation of 2-amino-2-vinylalcohols into α-vinyl aminoacids. It was also anticipated that in addition to the RCM reaction, the coupling reaction between the two amino acids would be challenging and would require extensive optimization.

**Scheme 2.1.** Retrosynthetic analysis for **1.9**




The retrosynthetic analysis (Scheme 2.2) for indolizidinone **1.12** was similar to that of **1.9.** The key intermediates **2.1** and **2.3** can both be derived from L-proline.

**Scheme 2.2.** Retrosynthetic analysis for **1.12**




**1.12** **1.13** **1.14** **2.1** **2.3**

## 2.2. α-Vinylglycine: Challenges associated with synthesis and manipulation

α-Vinylglycine was first isolated from mushrooms in 1974 in partially racemic form with high proportion of the D- (or (*R*)-form).[20] Following initial efforts at the synthesis of the racemic form,[21] a few reports detailing syntheses of the optically pure α-amino acid have steadily appeared in the literature since 1980.[20, 22, 23] It is evident from these studies, that synthetic manipulation involving α-vinylglycine is plagued by two stability issues (Figure 2.1).

Under *basic* conditions (aqueous or organic), vinylglycine under goes (1) racemization/epimerization at the α-center or (2) isomerization of the terminal olefin to form the internal α,β-unsaturated amino acid. The latter process can also occur under high temperature conditions (*vide infra*). Since these processes can also occur in the *N*- and *C*-protected forms, acid-mediated deprotections have to be employed to remove the carbamate and ester protecting groups. In light of this, some literature accounts utilize the Cbz protecting group as opposed to the more ubiquitously encountered Boc group.[24] Orthogonality can be maintained since two-step deprotection can be conveniently achieved under acidic conditions to remove the methyl ester (OMe) and Cbz groups.

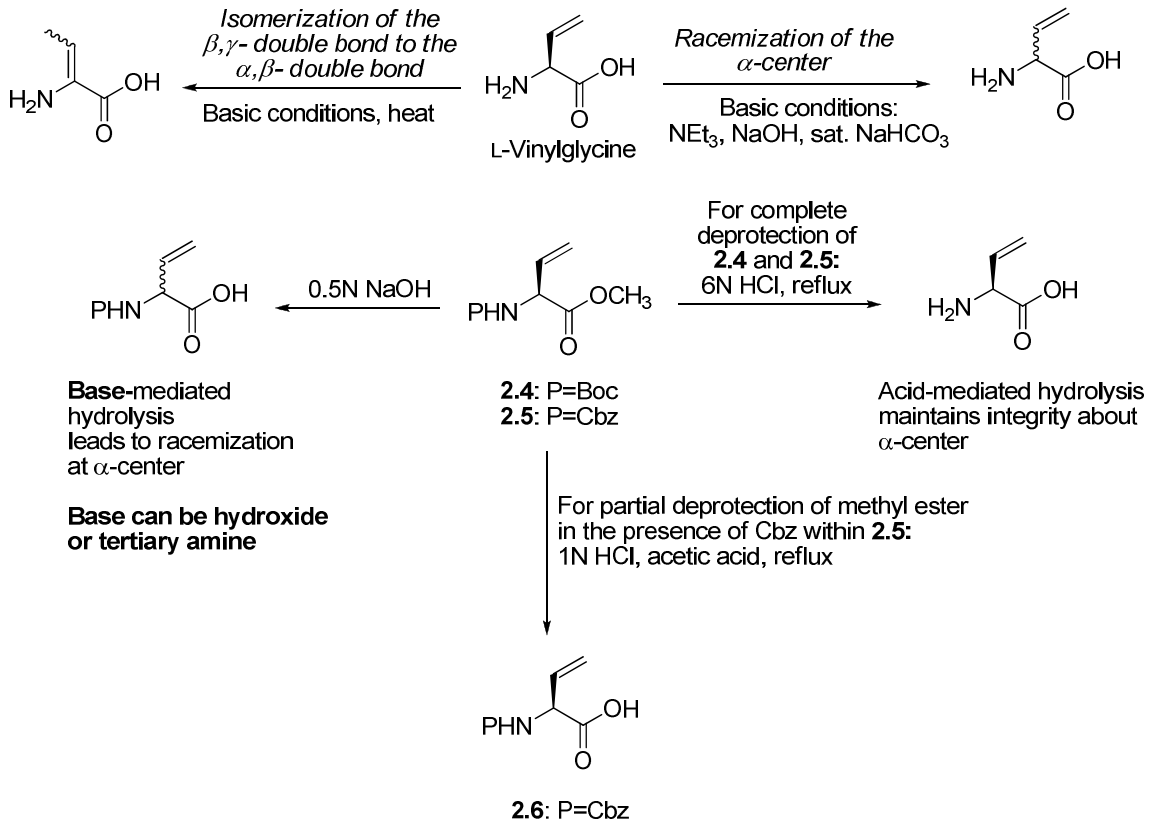
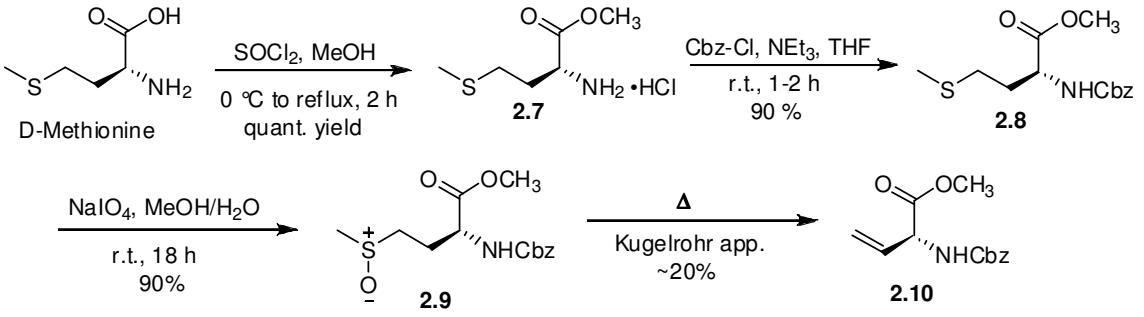



**Figure 2.1.** Instability of α-Vinylglycine and its derivatives

### 2.3.a. Synthesis of optically pure (*R*)-α-vinylglycine

As per the original report by the Rapoport group,[20] our first attempt (Scheme 2.3) at α-vinylglycine synthesis started with D-methionine, which, following suitable carbamate and ester protection, was subjected to S-oxidation by sodium periodate ($NaIO_4$). The resulting sulfoxide **2.9** was subjected to thermal *syn*-elimination using a Kugelrohr distillation apparatus resulting in Cbz-(*R*)-α-vinylglycine-OMe **2.10**. Unfortunately, in my hands, the Kugelrohr distillation resulted in a complex mixture with only 20% yield of **2.10**. The rest of the mixture was composed of unreacted starting material and the α,β-isomerized olefin (*vide supra*).

**Scheme 2.3.** Rapoport approach starting with methionine

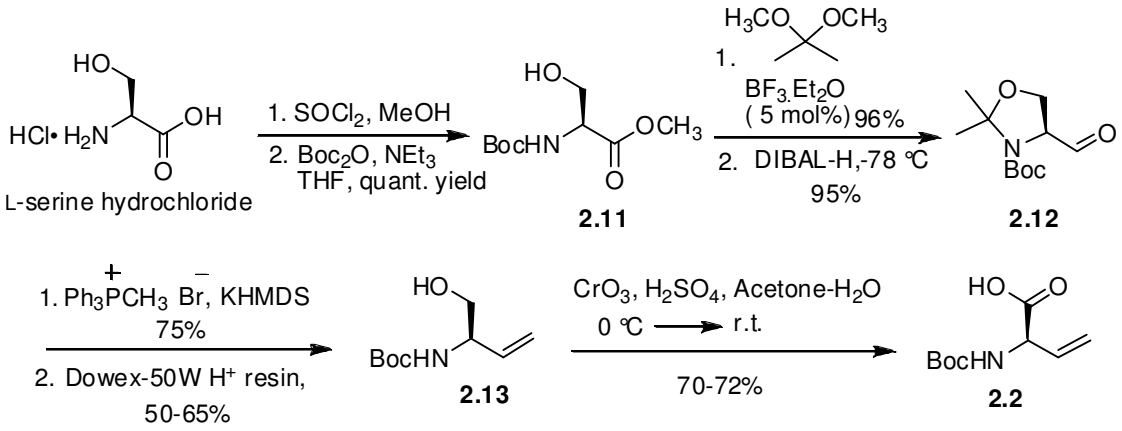



The second approach was dependent on the possibility of converting α-vinyl amino alcohols to the corresponding acid via either a one-pot two step oxidation protocol,[25,26] such as Jones oxidation[26] or alternately possibly in discrete steps; first oxidation to the aldehyde followed by conversion to the acid.

The synthesis is depicted in Scheme 2.4., and, it was based on a route developed by the Taylor group.[27] It required 7 steps toward the synthesis of *N*-protected-α-vinylglycine **2.2**. Commercially available hydrochloride salt of L-serine was first protected to form the Boc-L-serine methyl ester **2.11** in quantitative yield. This was converted to Garner's aldehyde **2.12** in two steps by first reacting with 2,2-dimethoxypropane (DMP) in the presence of catalytic Lewis acid and then reducing the ester to the aldehyde using diisobutyl aluminum hydride (DIBAL-H). Wittig methylenation followed by acidic oxazolidine hydrolysis gave rise to the crucial intermediate **2.13**. Several one-pot oxidations were attempted with Jones oxidation giving the most reproducible yields. Catalytic 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO)-based oxidations failed to form the desired product in good yields. Despite the highly acidic reaction conditions (3 N $H_2SO_4$) found in Jones oxidation, the integrity of the Boc-

protecting group[23,28] was maintained and Boc-(*R*)-α-vinylglycine **2.2** obtained in a crude form as a yellow-colored oil. Although this procedure was reproducible and highly

**Scheme 2.4.** Synthesis of Boc-(*R*)-α-vinylglycine *via* Garner's aldehyde




Scalable, it was significantly labor-intensive. Purification via flash chromatography was necessary after each step and large-scale reactions were required due to the number of steps in the synthetic plan.

### 2.3.b. Trost's Pd-catalyzed Asymmetric Allylic Alkylation (AAA) strategy

To obviate the laborious route, a different strategy was adopted toward the synthesis of the crucial *N*-protected-α-vinylglycinol intermediate **2.13**. In 2000, the Trost group reported the enantioselective synthesis of vinylglycinol using Pd-mediated catalysis.[29] The methodology developed was a *Dy*namic *K*inetic *A*symmetric *T*ransformation (DYKAT) of *racemic* monoepoxides. Butadiene monoepoxides (or vinyl oxiranes) are commercially available and are therefore readily obtained substrates for this palladium-catalyzed reaction. The DYKAT reaction of butadiene monoepoxide is hypothesized to proceed via a mechanism indicated in Figure 2.2. The first step is an

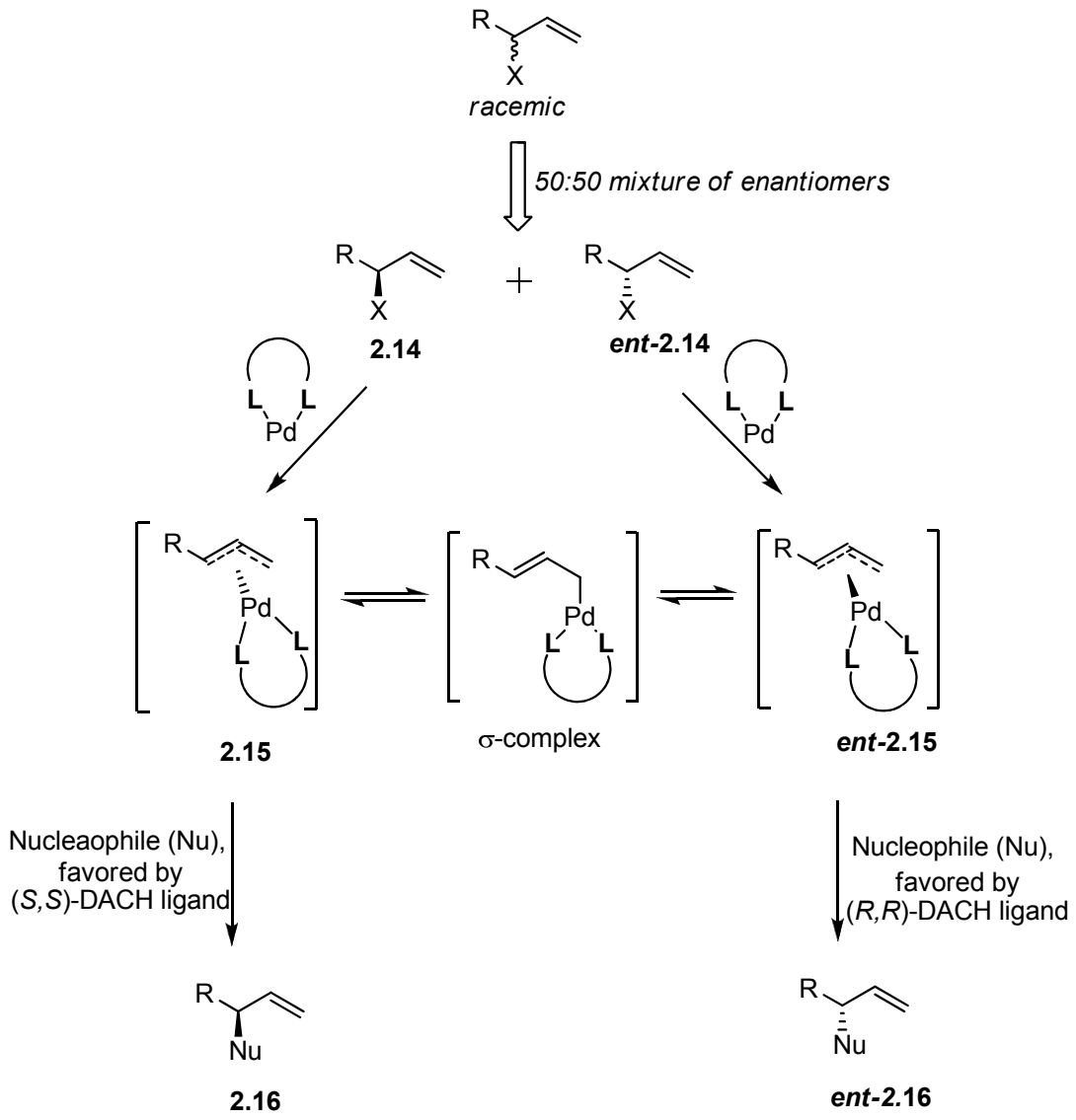



**Figure 2.2.** Origin of enantiodiscrimination in Pd-catalyzed DYKAT of epoxides

$S_N1$-type loss of the enantiotopic *good leaving group*, which in the case of racemic 2-vinyloxirane, leads to epoxide opening. This is followed by formation of a π-allyl palladium species, where Pd is tightly bound by the enantiopure Trost standard ligand; the DACH ligand (derived from 1,2-*dia*mino*cyclo*h*exane*). For (*rac*)-2-vinyloxirane, both enantiomers undergo these two steps at the same rate. Eventually, the two π-allyl

palladium species, **2.15** and ***ent*-2.15** rapidly interconvert via a dynamic equilibrium process, which occurs via the formation of an intermediate σ-complex. *Enantiodiscrimination occurs at the next step, wherein there is a rate differential in the reaction between the incoming nucleophile* (Nu) *on either **2.15** or **ent-2.15**, depending on the chirality of the ligand.* As per the Trost mnemonic, the naphthyl (*S,S*)-DACH ligand preferentially gives rise to **2.16,** while the (*R,R*)-DACH ligand gives rise to ***ent*-2.16.** A recent report details new mechanistic insights of the generic Pd-catalyzed AAA reaction.[30]

This particular subset of the Trost reaction is also regioselective. As shown in Figure 2.3., it was hypothesized that the use of (*rac*)-2-vinyloxiranes would be

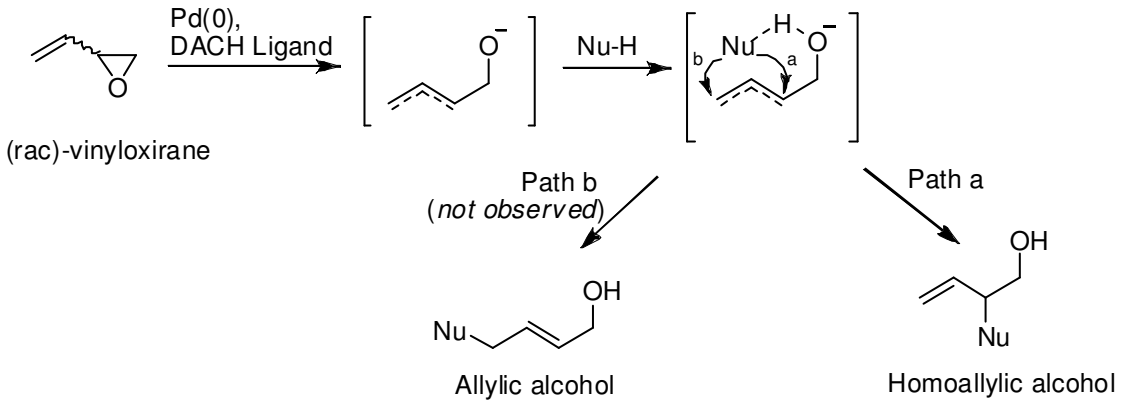


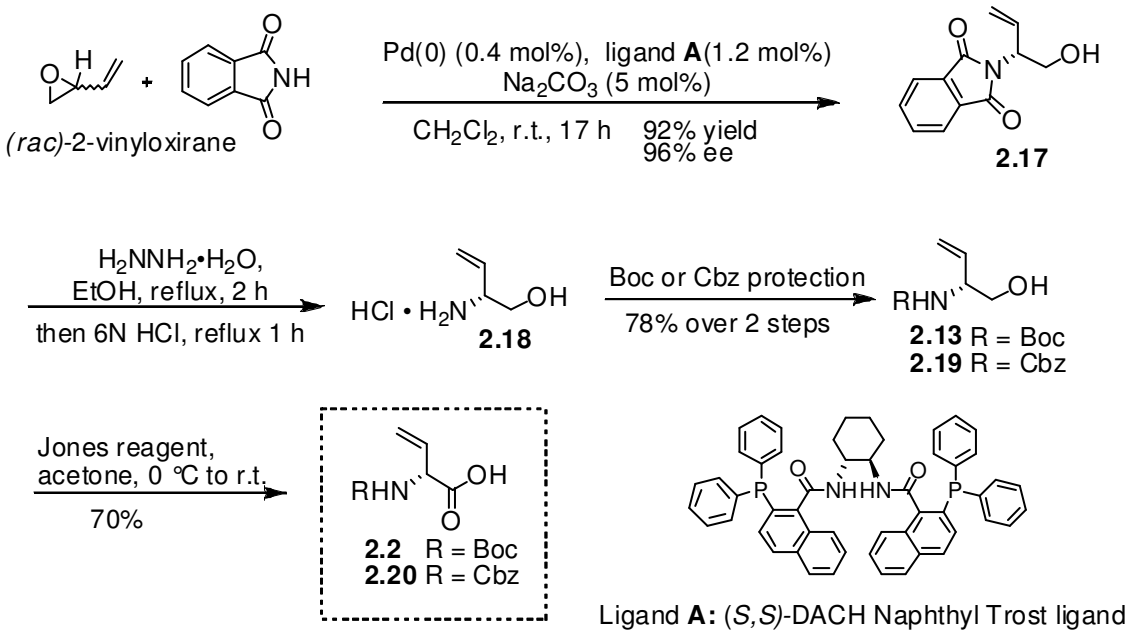

**Figure 2.3.** Origin of regioselectivity in the Trost DYKAT reaction of epoxides

advantageous for DYKAT, since the incoming nucleophile (Nu) would be capable of co-ordinating with the oxygen of the newly opened epoxide, and that this co-ordination would lead to regioselective attack of the Nu on the adjacent allylic carbon (*path a*) as opposed to the distal allylic position (*path b*). By correctly "matching" the substrate and

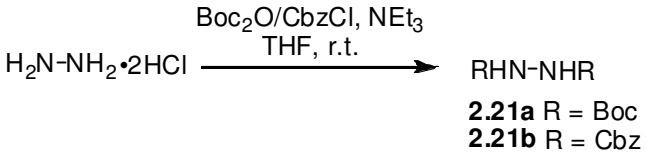
ligand it is therefore possible to obtain an enantiopure substituted homoallylic alcohol product.

For the synthesis of (*R*)-α-vinylglycinol, (*rac*)-2-vinyloxirane was reacted with a slight excess of commercially available phthalimide in the presence of catalytic amounts of π-allyl Pd dimer and the (*S,S*)-DACH Trost ligand (Scheme 2.5.). A catalytic amount (5 mol%) of base such as $Na_2CO_3$ deprotonates the weakly basic phthalimide N*H* (pka ~ 8) to render it nucleophilic for the allylic attack. The alkoxide anion (pka ~ 17–18) formed by epoxide ring opening can function as a base as the reaction continues. Methylene chloride ($CH_2Cl_2$) appears to be the most suitable solvent for this reaction which takes place at room temperature and requires approximately 12–17 h for completion. The phthalimido vinylglycinol **2.17** was re-crystallized from diethyl ether ($Et_2O$) to increase the enantiopurity (96% ee ➔ 98% ee). Hydrazinolysis and 6 N hydrochloric acid (HCl) refluxing treatment gave the de-protected (*R*)-α-vinylglycinol hydrochloride salt **2.18** which was not further purified. When greater than 5 g of the phthalimido compound was subjected to hydrazinolysis, the subsequent 6 N HCl reflux conditions produced large amounts of hydrazine as by-product, and, separation from desired amino alcohol product was not achieved until the next step, wherein the free amine was protected as a carbamate (Boc or Cbz). This was delineated since the yield of the desired Boc- or Cbz-protected compound **2.13** or **2.19** was surprisingly low and another more non-polar by-product was isolated in almost 0.5–1:1 ratio by weight. It was determined by NMR that this by-product was the corresponding bis-N-Boc/Cbz hydrazine **2.21a** or **2.21b**. In the same reaction context, attempts using *tert*-butylcarbamate (Boc-$NH_2$) as the nucleophile failed under various basic conditions (see Scheme 2.5.). Perhaps, catalytic amount of a strong base, such as NaH, is not sufficient for initial deprotonation of the carbamate (pka

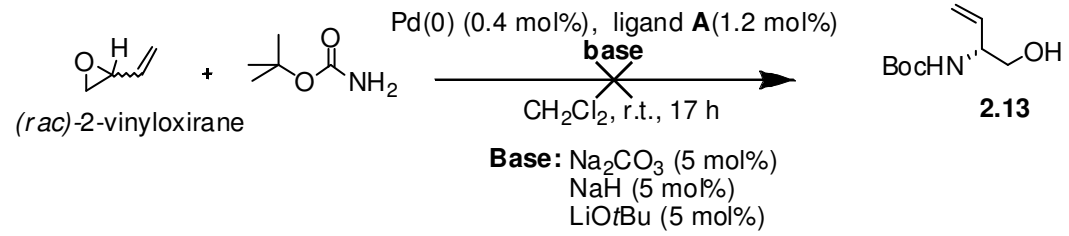
**Scheme 2.5.** (*R*)-α-Vinylglycine using Trost Pd-AAA




Unwanted side reaction on large scale:




Failed attempts:




~ 26). The use of an imide-type Boc derivative, where the N*H* has lower pka, has been recently shown to give the product in two steps.[31]

The Boc- or Cbz-protected alcohol was oxidized to a satisfactory extent using the Jones oxidation protocol[23] to the corresponding Boc- or Cbz-(*R*)-α-vinylglycine **2.2** or

**2.20**. Unfortunately, on a large scale, both alcohols were isolated by flash chromatography containing a UV-active impurity that co-eluted with the desired products respectively. The impurities were probably to the extent of 5% since they were not discernable by NMR, but were definitely subjected to oxidation to more polar by-products in the Jones oxidation. Once again, the crude material obtained after Jones oxidation was pure by NMR and used without further purification.

### 2.4. Synthesis of (*R*)-α-vinylproline

The title compound was synthesized by a method developed by the Gmeiner group.[32] The key step in the diastereoselective synthesis, is the Claisen condensation of methyl formate with the *non-racemic* enolate, generated from the trichloromethyl oxazolidinone **2.22** of L-proline. The use of this oxazolidinone was first reported by the Germanas group[33] as a more stable alternative to using the pivaloyl oxazolidinone of proline developed by Dieter Seebach.[34] The predictable stereochemical outcome of enolate additions of Seebach oxazolidinones is termed as "*S*elf *R*egeneration of *S*tereocenters (*SRS*). That is, additions of electrophiles at the α-position of proline occur with the same stereochemical sense as the original proline (Figure 2.4).

SRS has its origins in the diastereospecific formation of a single oxazolidinone diastereomer **2.22** shown in the figure. Its formation is driven by a thermodynamic equilibrium of the ring-closed and ring-opened form of the condensed iminium carboxylate between proline and chloral hydrate (or pivalaldehyde). The two main events responsible for the stereospecific outcome of this reaction are (1) propensity of 5.5-fused rings to be *cis*–fused and (2) the propensity of the bulky trichloromethyl (or *tert*-butyl) group for occupying the equatorial position to minimize 1,3-diaxial interactions.

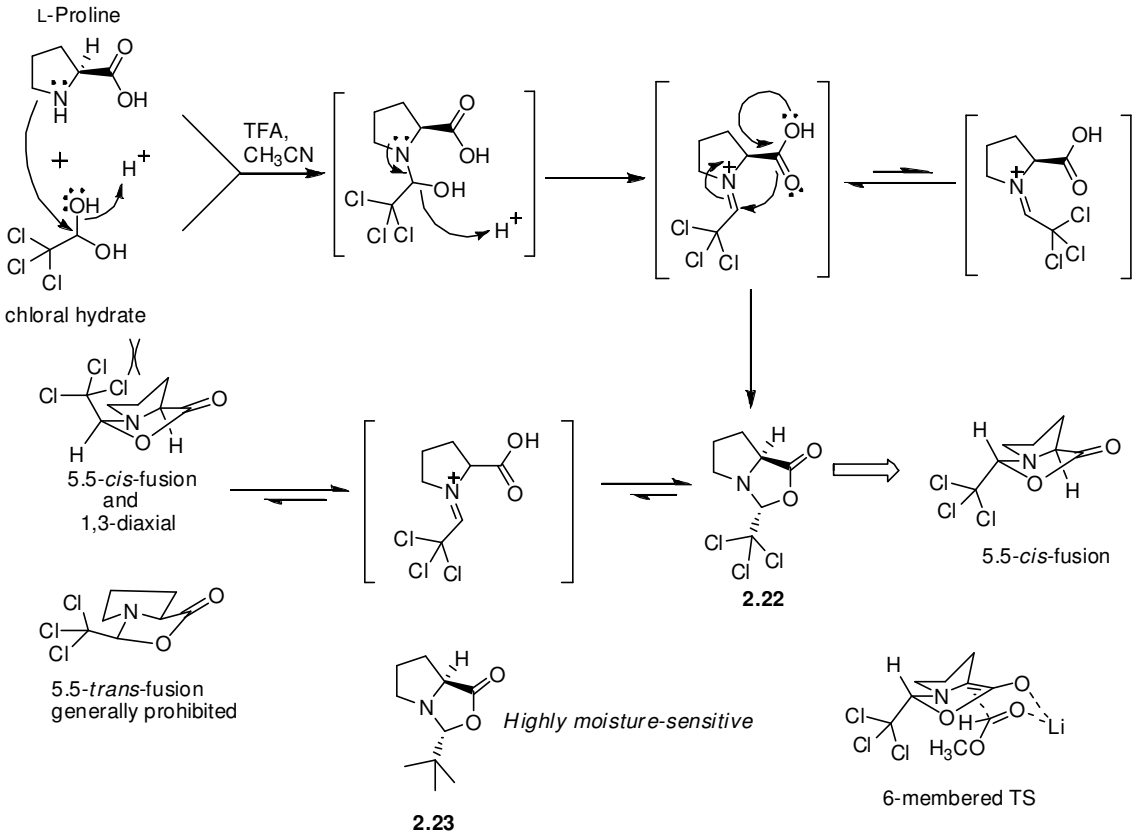
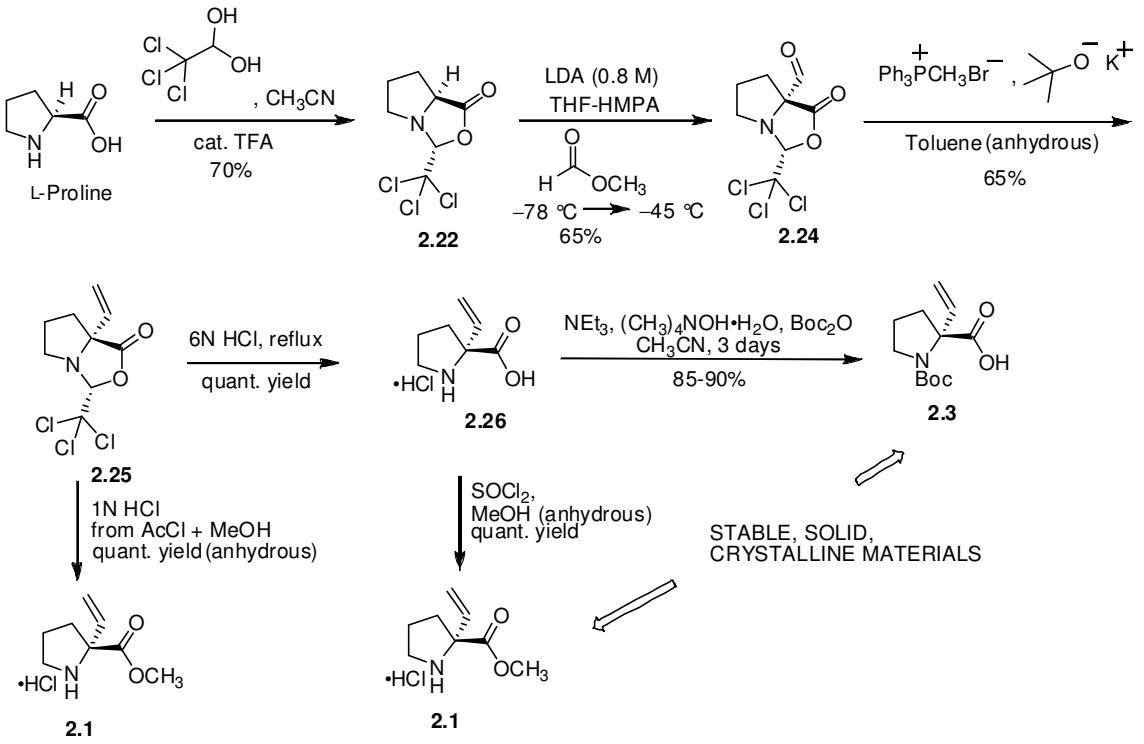



**Figure 2.4.** Thermodynamic basis for diastereoselective oxazolidinone formation

In addition, the enolates derived from such oxazolidinones are excellent "*carbonylophiles*" since the preferential O-Li enolate can easily form a six-membered transition state, leading to carbonyl addition products easily. The trichloromethyl derivative **2.22** is a stable crystalline solid and can be stored indefinitely at room temperature in a tightly sealed container. This is opposed to the lability of the pivaloyl oxazolidinone **2.23** which is *highly* moisture-unstable and has to be used immediately toward the enolate formation. However, there is a marked trade-off between the relative stability of the oxazolidinones and the corresponding enolate addition yields and diastereoselectivities.

Toward the synthesis of α-vinylproline (Scheme 2.6), L-proline was condensed with commercially available chloral hydrate using a protocol developed by Dr. Ashish Vartak in our laboratory. The use of chloral hydrate obviates the use of anhydrous

**Scheme 2.6.** Synthesis of (*R*)-α-vinylproline




chloral, which is difficult to purify and maintain in the anhydrous state, as well as avoids the use of a Dean-Stark apparatus while giving similar yields. The oxazolidinone **2.22** was crystallized in 60–70% yields using EtOAc/absolute EtOH. Claisen condensation of the lithium enolate derived from the oxazolidinone with methyl formate at –40 °C gave rise to the corresponding α-formyl oxazolidinone **2.24** in 65% yield. The yield was improved marginally by adding a co-solvent such as HMPA in stoichiometric quantities. One carbon Wittig methylenation using a potassium base such as potassium *tert*-butoxide (KO*t*Bu) gave the α-vinyloxazolidinone **2.25** in reproducible yields. All three

steps are amenable to scale-up. The Claisen condensation and Wittig reactions gave closer to 70% yield on a 15–20 g scale. The Wittig reaction requires special mention since it was subject to extensive optimization studies (Table 2.1). As reported in the

**Table 2.1.** Optimization of the Wittig reaction

| Olefination conditions | Yield |
|---|---|
| $Ph_3P\text{-}CH_3^+\ Br^-$ , KO$^t$Bu (1.7 equiv.),toluene | 65–67% |
| $Ph_3P\text{-}CH_3^+\ Br^-$ , KO$^t$Bu (1.2 equiv.),toluene | 50–55% |
| $Ph_3P\text{-}CH_3^+\ Br^-$ , KO$^t$Bu, 18-crown-6, toluene | 50% |
| $Ph_3P\text{-}CH_3^+\ Br^-$ , KO$^t$Bu, DME | No product |
| $Ph_3P\text{-}CH_3^+\ Br^-$ , *n*-BuLi (2.5M in hexanes), THF | 100% **2.22** |
| Tebbe reagent (0.5M in toluene), Toluene, pyridine | <20% |
| Tebbe reagent (0.5M in toluene), THF, pyridine | <20%, dimethylenation |

literature,[32] use of a lithium base led to complete reversion of the α-formyl oxazolidinone substrate **2.24** to the un-alkylated oxazolidinone **2.22** via a putative *retro-claisen* reaction. Toluene was the solvent of choice; use of a polar solvent such as dimethoxyethane (DME) gave no conversion to product. Use of the Tebbe reagent[35, 36] as the methylenating agent gave poor to no conversion to desired product. When tetrahydrofuran (THF) was used as solvent (and as an intrinsic Lewis base capable of activating the Tebbe reagent to its more active form), the dimethylenated product, where the lactone carbonyl was also methylenated, was observed. As a deuterated chloroform ($CDCl_3$) solution, this molecule decomposed at room temperature within a day after the $^1$H NMR was obtained, perhaps due to an unstable strained *enol ether* type structure.

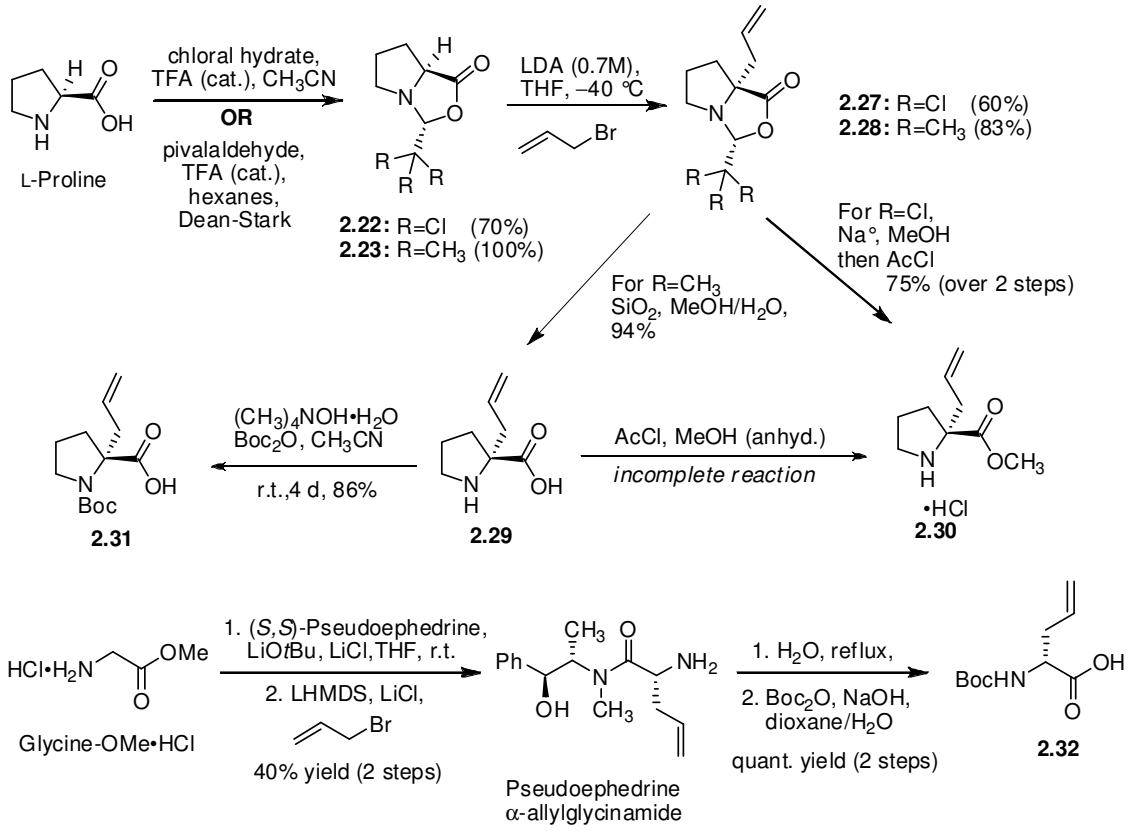
The α-vinyl oxazolidinone was either hydrolyzed under aqueous acidic conditions to form the amino acid hydrochloride **2.26** or anhydrous acidic conditions, wherein it was converted to the methyl ester hydrochloride **2.1** via a one-pot two step reaction. The α-

vinylproline•HCl could also be converted to the methyl ester **2.1** using thionyl chloride or acetyl chloride and anhydrous methanol (MeOH). The discrete two step method gave higher yields and compound of higher purity, since the side products of the oxazolidinone hydrolysis could be easily separated from the desired product at the end of the 6 N HCl hydrolysis. Also, **2.26** serves as an intermediate for synthesis of the Boc-protected amino acid as well. Boc-protection of **2.26** was conducted as per an optimized methodology developed previously in our laboratory for *N*-Boc protection of sterically hindered proline derivatives.[37] Nearly quantitative yield of **2.3** was obtained using the basic phase transfer reagent tetramethyl ammonium hydroxide (TMAH) and di-*tert*-butyl dicarbonate ($Boc_2O$) in anhydrous acetonitrile ($CH_3CN$) for 4 days. Both the α-vinylproline derivatives are stable, crystalline solids.

### 2.5. Synthesis of (*R*)-α-allylproline and (*R*)-α-allylglycine

In order to test the effect of steric hindrance on the peptide coupling and RCM reactions, derivatives of the one-carbon higher homologues, (*R*)-α-allylproline and (*R*)-α-allylglycine were also synthesized. α-Allylproline was also synthesized using Seebach's SRS strategy (Scheme 2.7). Large-scale syntheses using both pivalaldehyde[38] and chloral hydrate to form the oxazolidinone chiral auxiliaries **2.22** and **2.23** were successfully conducted. Either oxazolidinone was then subjected to enolate generation (using 0.7–1.0 M LDA in THF) and subsequent alkylation using commercially available allyl bromide. The yields typically ranged from 60–80%, with the trichloromethyl analogue giving lower yields. The alkylated oxazolidinone was subjected to an aqueous extraction work-up and generally used without further purification. Silica gel-mediated hydrolysis[38] of the alkylated pivaloyl oxazolidinone **2.28** gave rise to (*R*)-α-allylproline

**Scheme 2.7.** Synthesis of (*R*)-α-allylproline, (*R*)-α-allylglycine




**2.29** which was then converted to the methyl ester **2.30** in refluxing acetyl chloride in methanol (AcCl/MeOH). This reaction was usually incomplete even after 48 h reflux.[39] The alternate procedure involved subjecting the alkylated trichloro oxazolidinone **2.27** to sodium methoxide-mediated lactone opening and methyl esterification in the same reaction via the *N*-formyl intermediate.[33, 39] The methyl ester formation reaction was instructive in indicating the opposing labile natures of the two oxazolidinones. The pivaloyl oxazolidinone is susceptible to even *mildly acidic* hydrolysis, while the trichloromethyl analogue, which is stable on $SiO_2$ gel, is more susceptible to *nucleophilic/basic* hydrolysis. The trichloromethyl analogue is not completely hydrolyzed by $SiO_2$ gel in MeOH/$H_2O$ even after prolonged reflux. Only refluxing in 6 N HCl for 4–5 h

completely hydrolyzes the oxazolidinone; on the other hand, the NaOMe/MeOH reaction is complete within 30 min. at room temperature and forms the *N*-formyl derivative indicating the base lability of the *N*,*O*-acetal methine proton. α-Allylproline **2.29** was also converted to the Boc-protected derivative **2.31**. Boc-α-(*R*)-allylglycine **2.32** was synthesized as per the Myers' strategy utilizing (*S,S*)-pseudoephedrine as a chiral auxiliary. The details are discussed elsewhere in this dissertation (Chapter 4, Scheme 4.3).

**2.6.a. Synthesis of the peptide precursors for the RCM reaction**

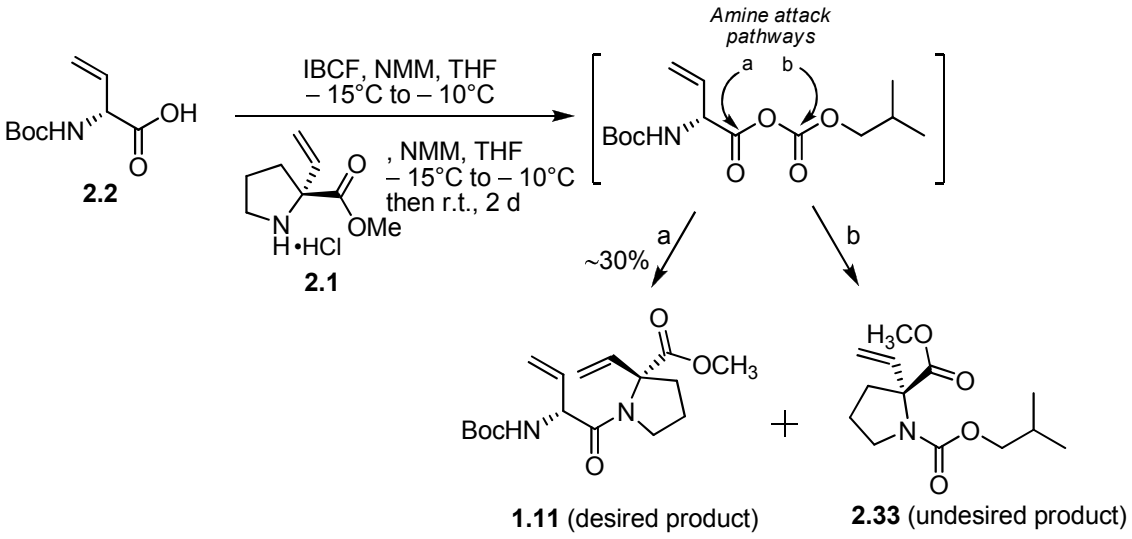
There are two major hurdles to be overcome while synthesizing dipeptides such as **1.11** and **1.14**. Firstly, α-vinylglycine as discussed before (*vide supra*) is unstable in the presence of tertiary bases for prolonged time periods. Secondly, *steric hindrance* is extremely pertinent to the discussion involving synthetic manipulations of α-alkylated proline residues. In fact, there is *no literature precedent* for amide bond formation between orthogonally protected, sterically encumbered α-alkylated proline residues as in **1.14**. Even recent studies[40] that attempt to improve the peptide coupling efficiencies of sterically hindered amino acids report cases where *only* one coupling partner is highly sterically hindered (e.g. α-center being a quaternary center). It was therefore hoped that the use of several peptide coupling conditions for synthesis of the dipeptide RCM precursors and the use of α-allylamino acids in the dipeptide synthesis would be instructive in understanding the steric demands of the α-alkylated proline and glycine residues. Based on literature precedent for coupling reactions involving α-vinylglycine,[41, 42] and α-allylproline,[39, 43, 44] four highly reactive peptide coupling agents/conditions were selected: (1) 7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium

hexafluorophosphate (HATU), (2) bis-(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl), (3) mixed anhydride method using isobutyl chloroformate (IBCF), and (4) acid fluoride method using Deoxo-Fluor®.[45]

### 2.6.b. Synthesis of dipeptide precursors 1.11 and 1.14 for RCM

The two target dipeptides were composed of an *N*-protected α-vinylglycine or α-vinylproline and α-vinylproline methyl ester. The mixed anhydride method employing isobutyl chloroformate (IBCF) gave the optimal result for coupling Boc-α-vinylglycine and α-vinylproline methyl ester (Scheme 2.8).

**Scheme 2.8.** Synthesis of Boc-(*R*)-vinylGly-(*R*)-vinylPro-OMe



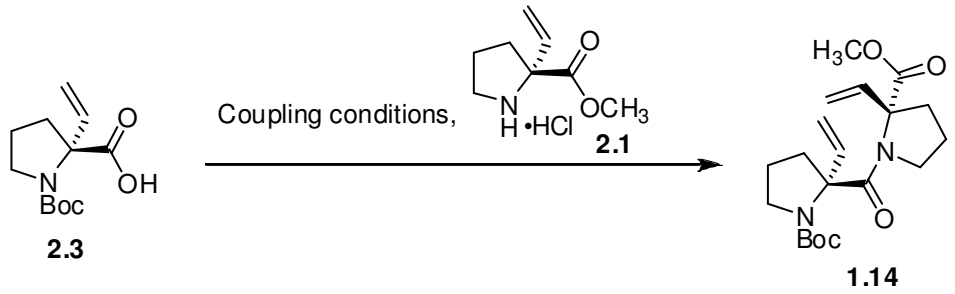

Although an optimized 30% yield was obtained, 1.5-fold quantity of a side product was isolated and characterized. This product, **2.33**, arises from the attack of the amine on the *wrongly activated* carbonyl of the mixed anhydride. Formation of this urethane has been reported in the literature in the context of the secondary amines of proline and sterically encumbered proline residues as the attacking nucleophiles.[46, 47]

This issue will be addressed again in the next sections. The reaction was not reproducible with respect to the yields of **1.11**. In addition, the product was unstable even within the NMR tube as a $CDCl_3$ solution giving rise to seemingly impure samples as per $^1H$ NMR.

Coupling between the orthogonally protected α-vinylproline residues was extremely challenging perhaps due to the steric environment about the quaternary α-carbon (Scheme 2.9). There is no literature precedent for amide bond formation between two α-alkylated proline residues. The most optimal conditions led to 17% yield of **1.14** and involved use of BOP-Cl as the stoichiometric coupling agent. This coupling agent is known to promote amide bond formations of *N*–alkylated amino acids.[48]

Slightly lower yields were also obtained with bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP). The acid fluoride technique using Deoxo-fluor and 2-chloro-1-methylpyridinium iodide (CMPI/Mukaiyama's reagent) gave no conversion. The coupling reaction of Boc-α-vinylproline in the presence of HATU resulted in the recovery of the active HOAt ester in over 50% yield which was extremely stable to aminolysis. No amide product was obtained even when the ester was subjected to amine attack under refluxing basic conditions. It should be noted that this HOAt ester was not stable for prolonged periods of time at room temperature.

**Scheme 2.9./Table 2.2.** Synthesis of Boc-(*R*)-vinylPro-(*R*)-vinylPro-OMe




**2.3** **2.1** **1.14**

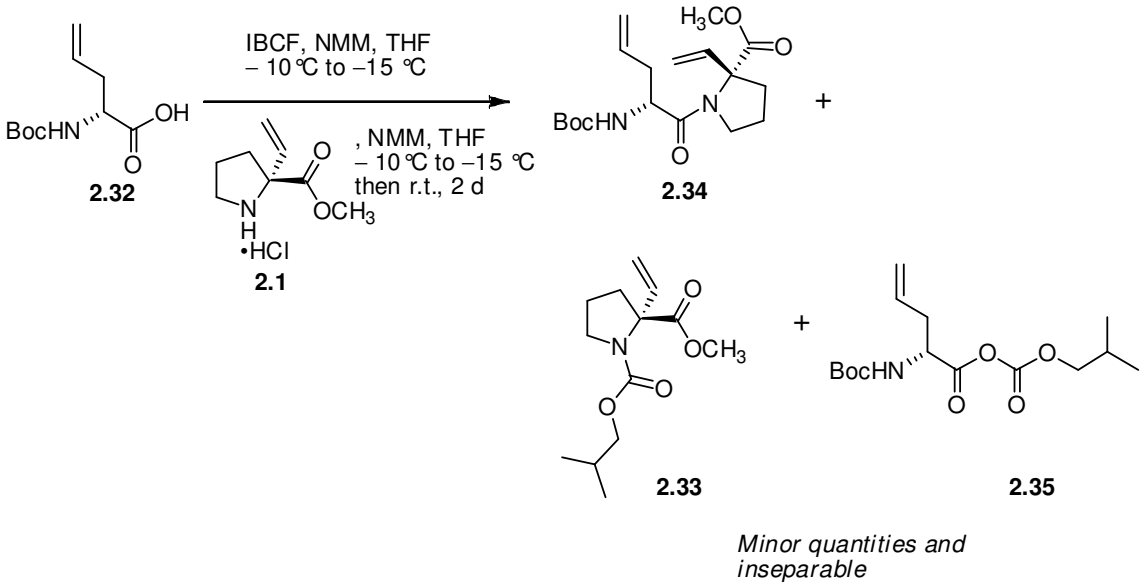
| Coupling conditions | Yield |
|---|---|
| BOP-Cl, DIPEA, $CH_2Cl_2$ | 17% |
| PyBroP, DIPEA, $CH_2Cl_2$ | 13% |
| Deoxo-fluor, DIPEA, $CH_2Cl_2$ | No product |
| HATU, NMM/DIPEA, DMF | HOAt-ester recovered |
| CMPI (Mukaiyama's reagent), $NEt_3$, $CH_2Cl_2$ | No product |

### 2.6.c. Synthesis of one-carbon higher homologue dipeptides: Precursors to the 7- and 8-membered RCM products

Similar to the reaction with α-vinylglycine, the mixed anhydride method gave the best yields for dipeptide formation between Boc-(*R*)-α-allylglycine and α-vinylproline methyl ester (Scheme 2.10.).

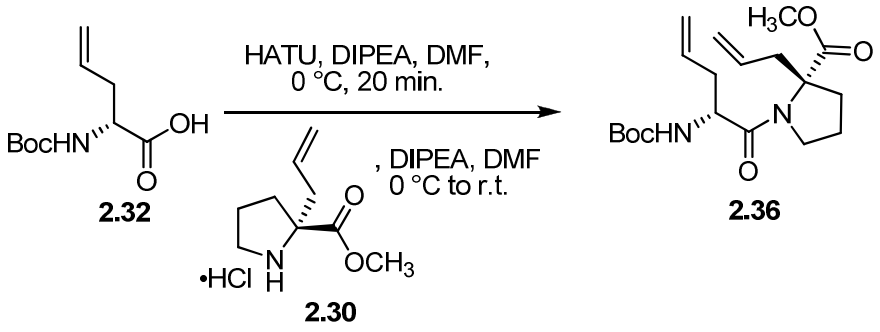
Once again, a small quantity of a mixture of **2.33** and **2.35** were obtained, indicating that nucleophilic attack of the sterically hindered amine on the active carboxyl carbonyl was inefficient. As per mass balance, it should be noted that active ester formation on either α-alkylglycine was relatively easy.

**Scheme 2.10.** Synthesis of Boc-(*R*)-allylGly-(*R*)-vinylPro-OMe



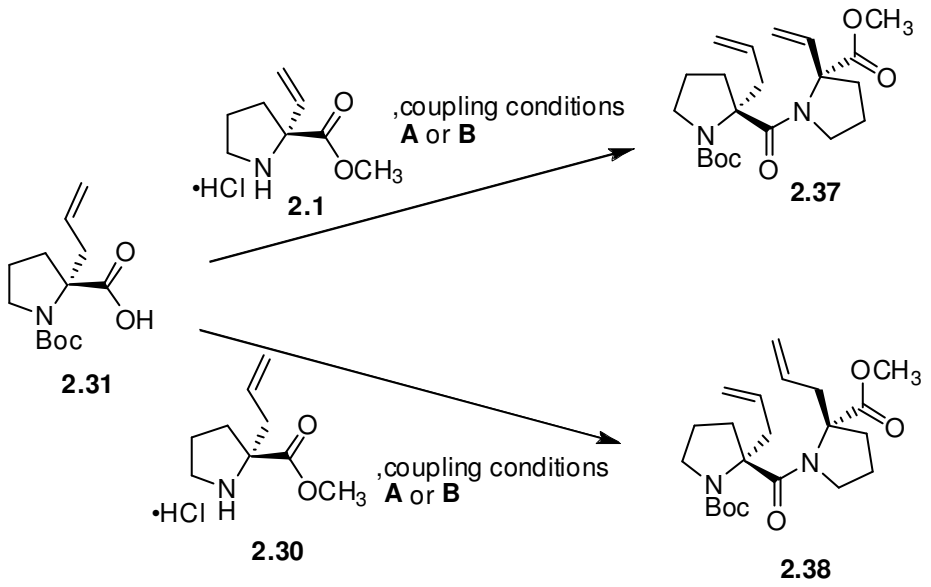

To avoid formation of the unproductive adduct **2.33**, coupling between **2.32** and α-allylproline methyl ester•HCl **2.30** was conducted in the presence of HATU (Scheme 2.11). The desired product was isolated in good yields and purification by flash chromatography was simplified.

**Scheme 2.11.** Synthesis of Boc-(*R*)-allylGly-(*R*)-allylPro-OMe



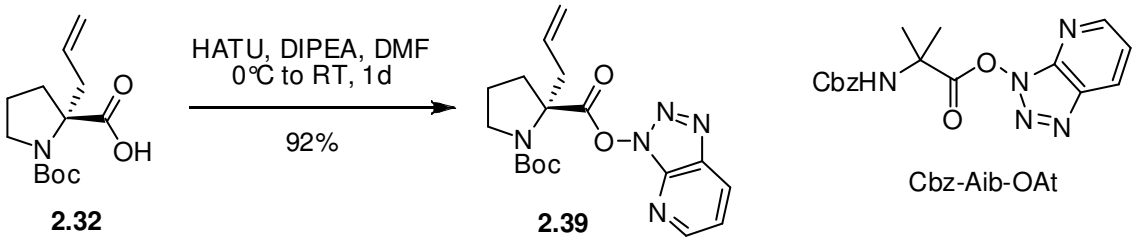
Both HATU (coupling condition **A**) and BOP-Cl (coupling condition **B**) were used to effect the formation of the dipeptides between Boc-α-allylproline and α-vinyl and α-allylproline methyl esters (Scheme 2.12). These two coupling agents are highly reactive and result in clean reaction mixtures. Between the two reaction conditions, BOP-Cl (**B**) gave higher yields. The reaction of **2.31** with α-allylproline methyl ester hydrochloride **2.30** gave higher yields of the desired dipeptide using either **A** or **B** than the reaction with **2.1.** This was presumably demonstrative of the slight relief from steric crowding about the α-quaternary carbon (α-$C_q$) of the attacking nucleophilic amine due to the presence of a more rotatable methylene group. The coupling reaction in the presence of HATU resulted in the isolation of relatively large quantities of the HOAt active ester. This active ester is formed almost quantitatively and is stable indefinitely when stored below 0°C as was demonstrated when the reaction was repeated in the absence of added

**Scheme 2.12.** Syntheses of Boc-(*R*)-allylPro-(*R*)-vin/allylPro-OMe



nucleophile (**A** without **2.1** or **2.30**, Scheme 2.13). The HOAt ester **2.39** is a colorless oil and slowly decomposes to 7-azabenzotriazole (HOAt) and the free acid when stored at ambient temperature. This decomposition appears to be faster in solution than in the solid state. One attempt to crystallize the ester from a mixture of EtOAc/$Et_2O$/1–3 drops of MeOH over 1 day at room temperature resulted in decomposition and crystallization of HOAt as a new polymorph. HOAt esters of most α-amino acids are highly reactive and not stable enough for capture under ambient conditions. However, there is literature

**Scheme 2.13.** Model synthesis of Boc-(*R*)-α-allylPro-OAt

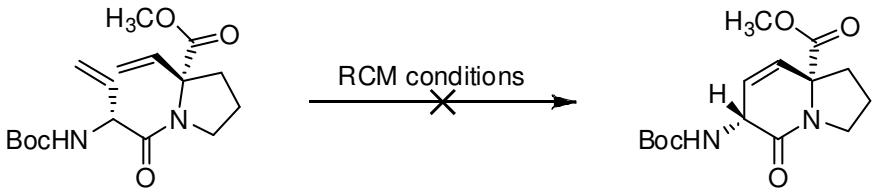



precedent for the isolation of HOAt esters of *sterically hindered* α-alkylated amino acids, such as, benzyloxycarbonyl protected α-aminoisobutyric acid (Cbz-Aib-OH).[49, 50] There is also a debate about the connectivity of the ester in such stable adducts, since *O* ➔ *N* acyl transfer is known and would result in a more stable amide linkage capable of resisting hydrolysis.

### 2.7. Attempts at Ring Closing Metathesis (RCM)

With approximately 30 mg of the Boc-(*R*)-vinylGly-(*R*)-vinylPro-OMe **1.11**, in hand, I proceeded to conduct the RCM reaction. The reactions (Scheme 2.14) were

**Scheme 2.14.** RCM reaction toward synthesis of indolizidinone **1.10**




**1.11** **1.10**

conducted at moderate dilutions (1–3 mM). The solvents were freshly distilled and de-gassed by Ar sparge for 1 hour before the reaction and the reaction was conducted under inert gas. Toluene was distilled from sodium/benzophenone which further ensured the absence of dissolved oxygen. Even with increased catalytic loading and reaction times, the desired product was not obtained. At a higher temperature, after prolonged reaction times, a minor appearance of the product was detected by ESI-MS (Table 2.3., entry 3).

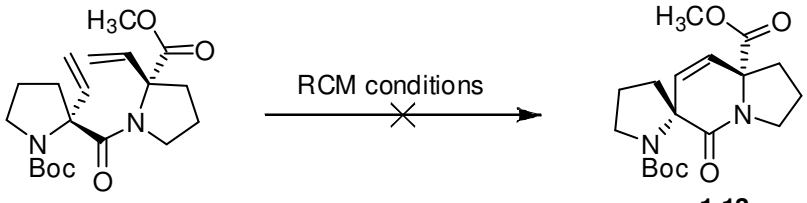
**Table 2.3.** RCM reaction toward synthesis of indolizidinone **1.10**

| Entry | RCM catalyst, equiv. | Solvent | T (°C) | time (h) | Yield (%) |
|---|---|---|---|---|---|
| 1 | Grubbs' gen. I, 10–20 mol% | $CH_2Cl_2$ | 40 | 24 | – |
| 2 | Grubbs' gen. II, 10–20 mol% | $CH_2Cl_2$ | 40 | 24 | – |
| 3 | Grubbs' gen. II, 60 mol% | PhMe | 80 | 48–72 | trace |

The RCM metathesis toward synthesis of **1.13** was also attempted (Scheme 2.15). In addition to similar reaction conditions for **1.10**, microwave-assisted (μ-wave) synthesis was also attempted. No product was observed under micro-wave conditions.[51–53] The reaction failed to give desired product even after *refluxing for 1 week!* The reactions were conducted under Ar sparge since this has been found to be beneficial in RCM reactions to form tetrasubstituted olefins.[52] The pre-catalyst was

replaced up to 10 mol% twice a day leading to stoichiometric amounts by the end of a week, since it has been shown that the Grubbs' catalysts suffer marked decomposition at high temperatures after prolonged reaction time.[54]

**Scheme 2.15.** RCM reaction toward synthesis of indolizidinone **1.13**




**1.14** **1.13**

**Table 2.4** RCM reaction toward synthesis of indolizidinone **1.13**

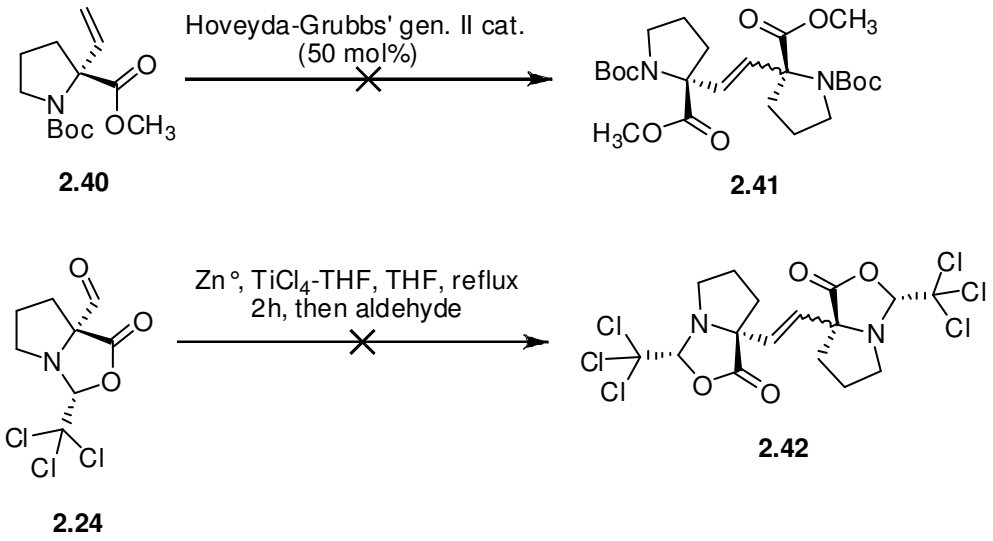
| **Entry** | **RCM catalyst, equiv.** | **Solvent/ additive** | **T (°C)** | **time (h)** | **Yield (%)** |
|---|---|---|---|---|---|
| 1 | Grubbs' gen. II, ~100 mol% | PhMe | 80 | 168 | – |
| 2 | Grubbs' gen. II, ~50 mol% | PhMe | 150, μwave | 0.5–1 | – |
| 3 | Grubbs' gen. II, ~50 mol% | PhMe/ $Ti(OiPr)_4$ | 80 | 48–72 | – |

A cross-metathesis attempt and a McMurry coupling[55] also resulted in no product formation (Scheme 2.16).

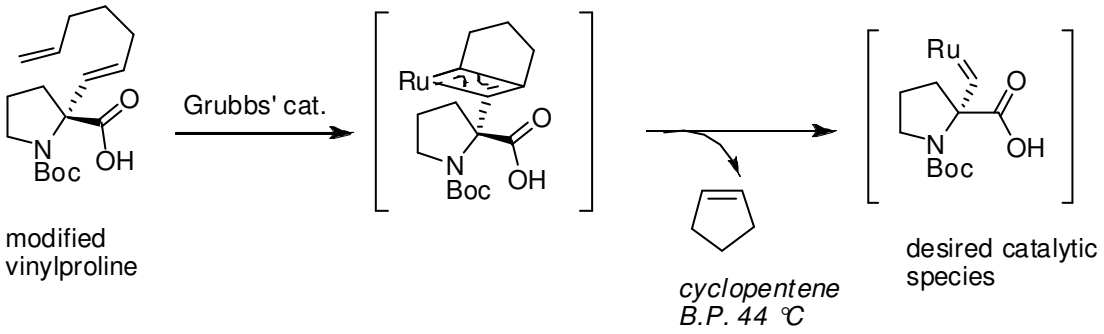
Since 2004, Relay RCM (RRCM) has emerged as an important strategy for overcoming low-yielding RCM reactions, especially in the case of problematic RCM reactions involving sterically hindered olefins.[56, 57] In such cases, it is presumed that the formation of the initial catalytic species is slow due to the steric hindrance. As shown in Scheme 2.17, taking advantage of the facile nature of RCM to form a 5-membered ring and the entropic benefit of forming a volatile side product, it would be expected that the

pre-catalytic Ru species would be capable of loading onto the sterically hindered olefin. Unfortunately, efforts to synthesize the requisite substrate for RRCM failed. In the hope

**Scheme 2.16.** Alternate routes attempted



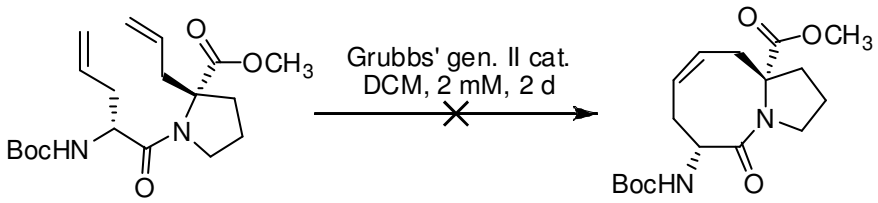

**Scheme 2.17.** Relay RCM rationale




that the formation of the 8-membered ring might be more facile, RCM reaction of dipeptide **2.36** to **2.43** was attempted (Scheme 2.18). No product formation was detected. It is important to note that the dipeptide containing the opposite stereochemistry at the α-position of glycine (*S*) has been successfully converted to the 8-

membered lactam by RCM.[58] In such a dipeptide within the *cis*-amide conformer, the two olefins have a *syn* arrangement relative to each other.

**Scheme 2.18.** Attempts toward synthesis of the 8-membered lactam by RCM




**2.36** **2.43**

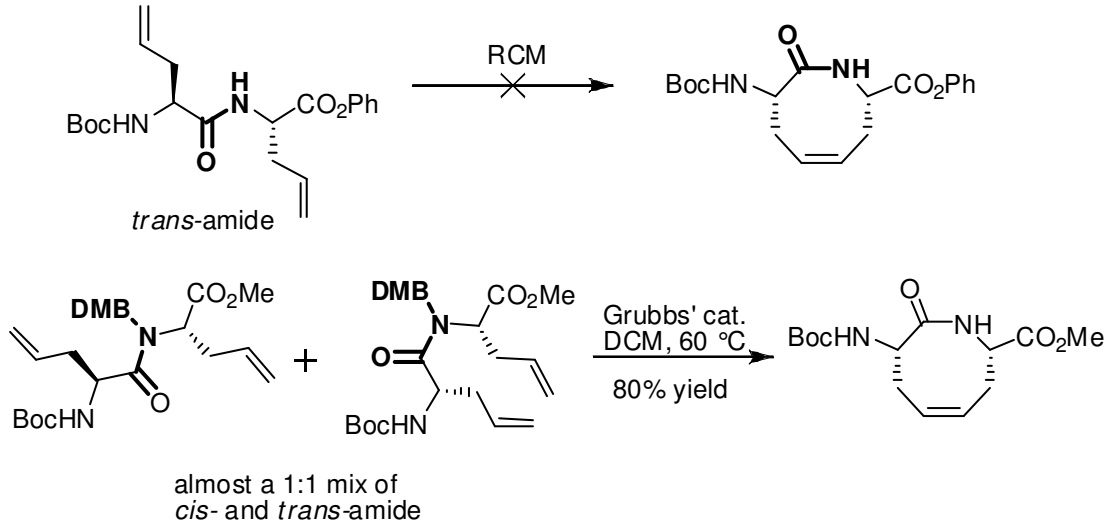
### 2.8. Possible explanation for failure of RCM of dipeptide substrates

The first major hurdle in the RCM reaction of the above dipeptides was the steric interference. The RCM reaction between two olefins, each of which are vinylic and stem out of quaternary carbons is currently unreported in the literature. If one extrapolates Robert Grubbs' classification of the reactivity of olefins to cross metathesis (CM)[59], to include ring closing metathesis, these vinyl olefins would fall under Type IV olefins; that is the olefins are "spectators" in the metathesis reaction. However; unlike in the case of CM, where *low reactivity* can be a key to product *selectivity* by correct substrate-substrate matching; since *both* the olefins participating in the RCM are sterically hindered and type IV, there is no product formation. From the reaction in the presence of a Lewis acid, it is also obvious that the dipeptides containing the olefins do not inhibit the pre-catalyst activity. It might be hypothesized that even at high pre-catalyst loadings, the active catalytic species is perhaps unable to load onto either olefin to form the necessary catalytic species in the first place. It was unfortunate then that the Relay RCM (RRCM)

strategy[56] was not possible due to the failure in synthesizing the substrate for such a reaction.

The second hurdle to be overcome is the conformational energetics of the dipeptides. In attempting to form the lactam via RCM, a larger proportion of the amide bond conformers need to exist in the *cis* form as opposed to the thermodynamically stable *trans*. This issue has recent literature precedent.[60] In trying to synthesize the 8-membered carbocylic peptide mimic of a cyclic disulfide, Creighton *et al.* observed that the dipeptide, Boc-(*S*)-allylgly-(*S*)-allyl-OPh, was not amenable toward RCM (Scheme 2.19). On blocking the NH of the amide bond by "transient alkylation" using the 2,6-dimethoxybenzyl (DMB) protecting group, a more significant proportion of the *cis* rotamer resulted as evidenced by (1) an almost 3:2 ratio of the rotameric forms by $^1$H NMR (in $CDCl_3$) and (2) a successful RCM reaction that proceeded in 80% yield.

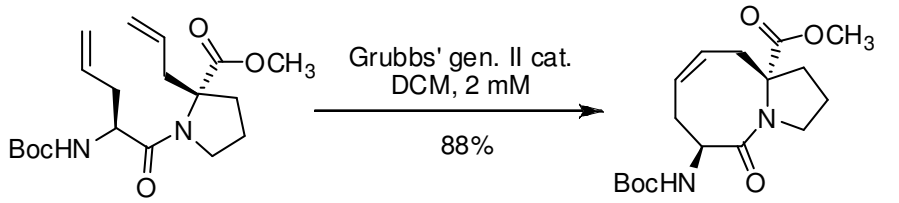
**Scheme 2.19.** *cis*-conformer and ease of RCM




In case of *N*-terminal amides of proline, there is a natural tendency for higher *cis*-rotamer proportions. This being the case for **1.11** and **1.14**, one would expect the RCM reaction to be *relatively facile* within the context of sterically hindered olefins. However,

this also places the two reacting olefins in a *trans* relationship to each other; which can still lead to problems in 6–8 membered ring formations. For example, although synthesis of the 8-membered analogue **2.43** (Scheme 2.18) was unsuccessful, synthesis of the corresponding *syn* substituted 8-membered lactam by RCM has been reported in the literature (Scheme 2.20).[58]

**Scheme 2.20.** Literature precedent for RCM to form 8-membered mimic




*syn*-allyl groups

*trans*-substitued lactam

In addition, even in the *trans* amide rotamer, the olefins are completely directed away from each other (Figure 2.5). Given that the correlation between conformational predisposition of the starting material toward ring closure and facility of the RCM reaction being used to form the ring is a contentious issue,[61, 62] it is perhaps prudent to hypothesize that the unreactivity of the olefins toward the RCM pre-catalysts is responsible for the failure in ring formation.

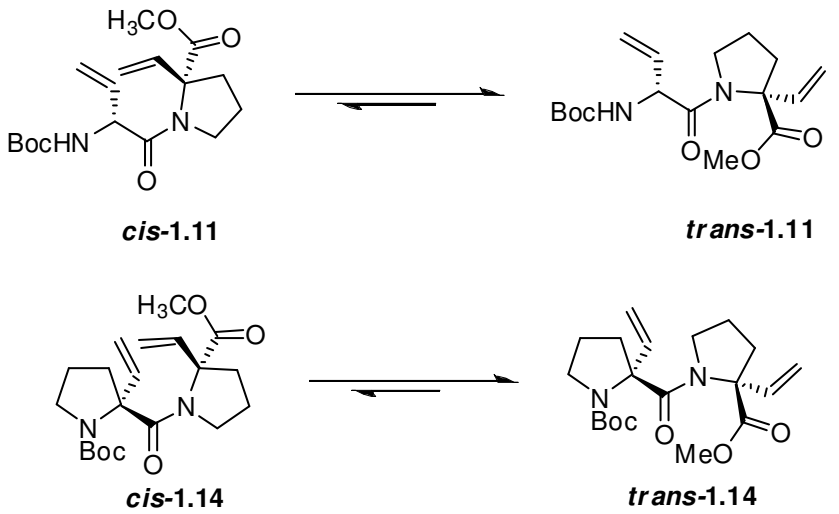
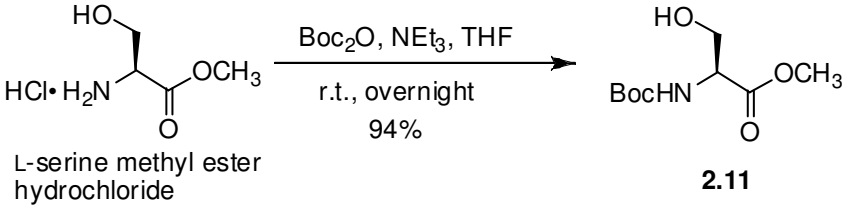



**Figure 2.5.** Dipeptide rotamers

**2.9.** Experimental section:

***N*-(*tert*-Butyloxycarbonyl) serine Methyl Ester (2.11)**




A suspension of L-serine methyl ester hydrochloride (5 g, 32.1 mmol, 1 equiv.) in THF (80 mL) was cooled in a salt-ice bath. Triethylamine (9.0 mL, 64.3 mmol, 2 equiv.) was added drop-wise to the suspension until a thick white precipitate was produced indicating triethylammonium chloride formation (Note 1). A solution of di-*tert*-butyl dicarbonate (10.5 g, 48.2 mmol, 1.5 equiv.) in THF (30 mL) was added to this thick

suspension and stirring was continued overnight at room temperature. After 15 h, the reaction was filtered to remove the salt and filtrate was concentrated to give the crude product which was purified by flash chromatography (gradient elution: 15–60% EtOAc in hexanes) to give the pure product **2.11** as a colorless oil weighing 6.6 g (94% yield).

TLC $R_f$ 0.41 (EtOAc/hexanes, 1:1) [lit.[27a] 0.38, same solvent system]

$[\alpha]_D$ –19.2 (*c* 5.0, $CH_3OH$) [lit.[27c] –18.9 (*c* 5.0, $CH_3OH$)]

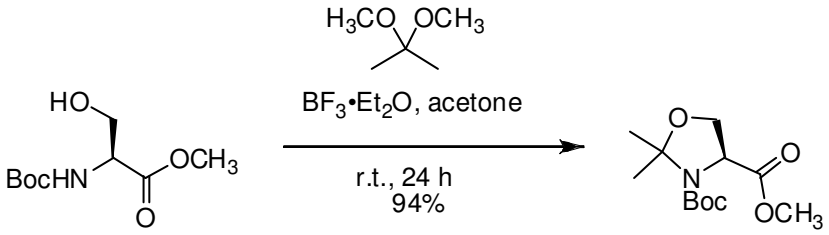
$^1H$ (300 MHz, $CDCl_3$) δ 5.55 (d, 1H, *J* = 6.8 Hz, N*H*–(C=O)–O*t*Bu), 4.35 (m, 1H, α-C*H*), 3.96–3.82 (m, 2H, β-C$H_2$), 3.75 (s, 3H, OC$H_3$), 2.97 (br. s, 1H, O*H*), 1.42 (s, 9H, NH–(C=O)–OC(C$H_3$)$_3$)

$^{13}C$ (75 MHz, $CDCl_3$) δ 170.0 ((*C*=O)–$OCH_3$), 154.6 (NH–(*C*=O)–O*t*Bu), 80.3 (NH–(C=O)–O*C*$(CH_3)_3$), 63.7 (α-*C*H), 56.2 (β-*C*$H_2$), 53.1 (O*C*$H_3$), 29.2 (NH–(C=O)–OC(*C*$H_3$)$_3$)

Notes:

1. THF is a better solvent than DCM since the triethylammonium chloride by-product is sparingly soluble in THF and can therefore be simply filtered off. The salt has reasonable solubility in DCM and this complicates the work-up protocol.

**4(*S*)-*N*-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(methoxycarbonyl) oxazolidine**




**2.11**

Boc-protected-L-serine methyl ester **2.11** (6.6 g, 30 mmol, 1 equiv.) was dissolved in 100 mL anhydrous acetone (Note 1). 2,2-Dimethoxypropane (30 mL, 244. 8

mmol, 8 equiv.) was added to this solution under Ar and the solution was stirred at room temperature. Boron trifluoride etherate (0.2 mL, 1.5 mmol, 5 mol%) was added to this solution which immediately changed from a colorless to a bright yellow solution. The solution was stirred for an additional 24 h at which point the solution turned pale, amber colored. Acetone was removed by rotary evaporation and the residue taken up in $Et_2O$. This solution was washed with a saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$ , filtered and concentrated to give a crude yellow oil which was purified by flash chromatography using EtOAc and hexanes to give 7.3 g of pure product (93.5% yield)

TLC $R_f$ 0.68 (EtOAc/hexanes, 1:1) [lit.[27a] 0.78, same solvent system]

$[\alpha]_D$ –58.3 (*c* 1.35, $CHCl_3$) [lit.[27b, 27c] –57 (*c* 1.3, $CHCl_3$); –54 (*c* 1.3, $CHCl_3$)]

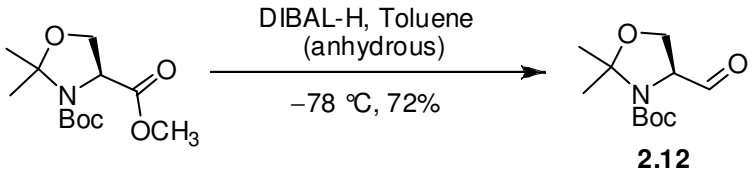
$^1H$ (300 MHz, $CDCl_3$, rotamers about the NH–(C=O)–O*t*Bu bond in 2–4:1 ratio) δ 4.36 (dd, 0.4H, *J* = 6.3, 2.4 Hz, α-C*H*), 4.26 (dd, 0.5H, *J* = 6.9, 3.0 Hz, α-C*H*), 4.06–3.88 (m, 2H, β-C$H_2$), 3.63 (app. d, 3H, O-C$H_3$), 1.52 (app.d, 3H, 2-C$H_3$), 1.38 (app.d, 3H, 2-C$H_3$), 1.29 (s, 9H, C(C$H_3$)$_3$)

$^{13}C$ (300 MHz, $CDCl_3$, rotamers about the NH–(C=O)–O*t*Bu bond in approx. 1:1 ratio) δ 171.4 and 170.9 (*C*(=O)$OCH_3$), 151.8 and 150.9 (NH–(*C*=O)–O*t*Bu), 94.8 and 94.2 (*C*($CH_3$)$_2$), 80.7 and 80.1 (NH–(C=O)–O*C*($CH_3$)$_3$), 66.2 and 65.9 (α-*C*H), 59.2 and 59.1 (β-*C*$H_2$), 52.3 and 52.2 (O*C*$H_3$), 28.3 and 28.2 (NH–(C=O)–OC(*C*$H_3$)$_3$), 26.0 and 25.1 (C(*C*$H_3$)$_2$), 24.9 and 24.4 (C(*C*$H_3$)$_2$)

Notes:

1. Acetone was rendered anhydrous by stirring over Drierite (25 g/l) for 3 h followed by distilling the mixture.

**4(*S*)-*N*-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-formyl-oxazolidine (2.12)**




**2.12**

The oxazolidine ester (6 g, 23.1 mmol, 1 equiv.) was dissolved in 45 mL anhydrous toluene (Note 1) and transferred to an oven-dried 3-neck flask equipped with a stir bar and thermometer. The solution was cooled to –78 °C using dry-ice/acetone. Diisobutyl aluminium hydride or DIBAL-H (26.2 mL, 39.3 mmol, 1.7 equiv.) was added dropwise (Note 2) to ensure that the internal temperature of the reaction did not increase above –70 °C. Once addition was complete, the reaction mixture was stirred for 2 h at which point the reaction was judged to be complete by TLC. Methanol (10 mL) was added dropwise to quench the reaction while ensuring that the reaction temperature did not increase above –65 °C and excessive foaming was avoided. The resulting white colored emulsion was poured into 150 mL HCl (previously cooled to 0 °C) over a 5 min period while the mixture was vigorously stirred. The aqueous layer was extracted thrice with 150 mL portions of EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ , filtered and concentrated to give a crude oil which was purified by flash chromatography (isocratic elution: 20% EtOAc in hexanes) to give 3.8 g of the purified product **2.12** (72% yield).

TLC $R_f$ 0.37 (EtOAc/hexanes, 1:4) [lit.[27a] 0.33, same solvent system]

$[\alpha]_D$ –88.6 (*c* 1.7, $CHCl_3$) [lit.[27c] –89 (*c* 1.0, $CHCl_3$)]

$^1H$ (300 MHz, $CDCl_3$, rotamers about the NH–(C=O)–O*t*Bu bond in various ratios) δ 9.51 (app.d, 1H, *H*–C=O), 4.82–4.26 (m, 0.5H, α-C*H*), 4.16–4.11 (m, 0.5H, α-C*H*), 4.06–3.88

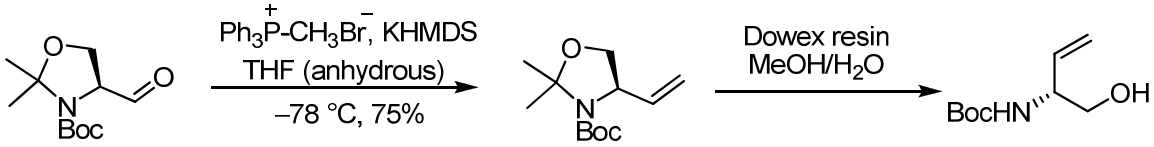
(m, 2H, β-C$H_2$),1.56 (app.d, 3H, C(C$H_3$)$_2$), 1.47 (app.d, 6H, C(C$H_3$)$_2$ and NH–(C=O)–OC(C$H_3$)$_3$),1.36 (s, 6H, NH–(C=O)–OC(C$H_3$)$_3$)

$^{13}$C (75 MHz, $CDCl_3$, rotamers about the NH–(C=O)–O*t*Bu bond in an approx. 1:1 ratio) δ 199.2 (H–*C*=O), 152.5 and 151.2 (NH–(*C*=O)–O*t*Bu), 95.0 and 94.3 (*C*(CH$_3$)$_2$), 81.3 and 80.1 (NH–(C=O)–O*C*(CH$_3$)$_3$), 64.7 (α-*C*H), 63.9 and 63.5 (β-*C*H$_2$), 28.4 (NH–(C=O)–OC(*C*H$_3$)$_3$), 26.8 and 25.8 (C(*C*H$_3$)$_2$), 24.8 and 23.9 (C(*C*H$_3$)$_2$)

Notes:

1. Toluene was rendered anhydrous by distilling over sodium sand.
2. DIBAL-H was purchased as a 1.5 M solution in toluene and used as received.

**4(*S*)-*N*-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-vinyl oxazolidine**




**2.12** **2.13**

Methyltriphenylphosphonium bromide (4.9 g, 13.1 mmol, 3 equiv.) was suspended in 57 mL anhydrous THF (Note 1). A solution of potassium hexamethyldisilazide in toluene (26.2 mL, 0.5 M solution, 3 equiv.) was added dropwise at room temperature. The suspension turned a bright yellow color immediately. This suspension was stirred under Ar for 1 h at room temperature after which it was cooled to –78 °C. A solution of Garner's aldehyde (1 g, 4.36 mmol, 1 equiv.) in 20 mL THF was then added dropwise to the bright yellow suspension of the Wittig reagent. Once addition was complete, the final color of the suspension was bright orange. The cooling bath was removed and the reaction was warmed to room temperature where it was stirred for 3 h.

Once reaction was complete as determined by TLC, the unreacted base was quenched using MeOH. An off-white to tan colored precipitate resulted and the solvent was removed *in vacuo.* The residue was partitioned between $Et_2O$ and a 1:1 mix of water and saturated sodium potassium tartrate. The layers were separated and the aqueous layer was washed thrice with $Et_2O$. The ethereal layer was dried over $Na_2SO_4$, filtered and concentrated to give a pale yellow-colored oil. Flash chromatography (isocratic elution: 6:1 hexanes/ethyl acetate) gave 0.75 g pure product as a colorless oil (75% yield).

TLC $R_f$ 0.66 (EtOAc/hexanes, 1:4)

$[\alpha]_D$ +17.4 (*c* 1.1, $CHCl_3$) [lit.[27] +15.6 (*c* 2.0, $CHCl_3$)]

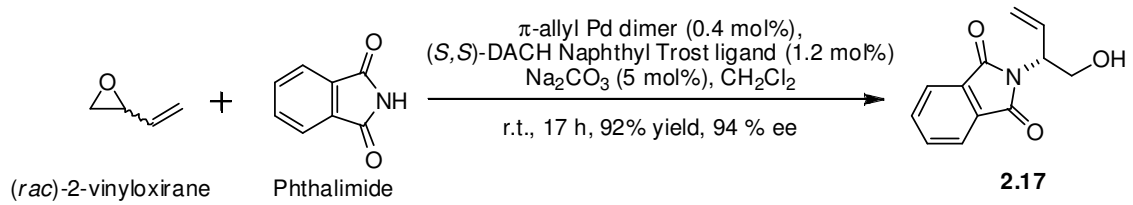
$^1H$ (300 MHz, $CDCl_3$) δ 5.79–5.66 (m, 1H, C*H*=$CH_2$), 5.18–5.04 (m, 2H, CH=C*H*$_2$), 4.36–4.14 (m, 1H, α–C*H*), 3.99–3.93 (m, 1H, β-C*H*$_2$), 3.68–3.64 (m, 1H, β-C*H*$_2$), 1.52 (s, 3H, C(C*H*$_3$)$_2$), 1.43–1.35 (m, 12H, C(C*H*$_3$)$_2$ and NH–(C=O)–OC(C*H*$_3$)$_3$)

$^{13}C$ (75 MHz, $CDCl_3$, rotameric peaks observed about NH–(C=O)–O*t*Bu bond in an approx. 2:1 ratio) δ 151.8 (NH–(*C*=O)–O*t*Bu), 137.3 and 136.7 (*C*H=$CH_2$), 115.7 (CH=*C*H$_2$), 93.9 and 93.5 (*C*(CH$_3$)$_2$), 80.1 and 79.5 (NH–(C=O)–O*C*(CH$_3$)$_3$), 68.1 (α-*C*H), 59.7 (β-*C*H$_2$), 28.5 (NH–(C=O)–OC(*C*H$_3$)$_3$), 27.3 and 26.6 (C(*C*H$_3$)$_2$), 24.9 and 23.8 (C(*C*H$_3$)$_2$)

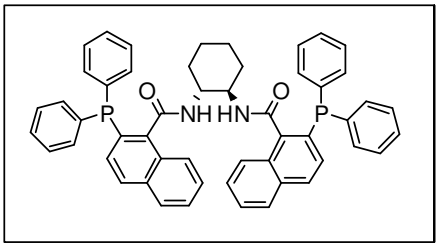
<u>Notes:</u>

1. THF was rendered anhydrous by distilling from sodium-benzophenone under Ar.

**_N_-Phthaloyl (_R_)-2-amino-2-vinylethanol (2.17)**




(*rac*)-2-vinyloxirane Phthalimide **2.17**



Ligand **A**:(*S,S*)-DACH Naphthyl Trost ligand

A mixture of π-allyl palladium dimer (7.3 mg, 0.02 mmol, 0.4 mol%), Trost ligand **A** (47.5 mg, 0.06 mmol, 1.2 mol%), sodium carbonate (26.5 mg, 0.25 mmol, 5 mol%) and phthalimide (809 mg, 5.5 mmol, 1.1 equiv.) was purged under Ar in a flask for 30 minutes. DCM (40 mL, Note 1) was then added to this mixture. The suspension was stirred under Ar for 10 minutes after which (*rac*)-2-vinyloxirane (0.4 mL, 5 mmol, 1 equiv.) was added. The yellow-colored cloudy solution was stirred for 17 h at room temperature under Ar at which point TLC indicated reaction to be complete. DCM was removed by rotary evaporation and the yellow residue directly subjected to flash chromatography (gradient elution: 10% to 75% EtOAc in hexanes). The pure product eluted around 50% gradient and was obtained as a white solid that could be further crystallized from diethyl ether to give 1 g solid **2.17** (92% yield).

mp: 64–66 °C [lit.[29] 62 °C]

TLC $R_f$ 0.55 (EtOAc/hexanes, 3:1)

$[\alpha]_D$ = + 76.1 (*c* 1.0, $CH_2Cl_2$) [lit.[29], for the enantiomer, –72.2 (*c* 2.02, $CH_2Cl_2$)]

$^1$H (300 MHz, $CDCl_3$) δ 7.84–7.81 (m, 2H, Ar-C*H*), 7.74–7.69 (m, 2H, Ar-C*H*), 6.14 (ddd, $J_1$ = 17.4 Hz, $J_2$ = 9.9 Hz, $J_3$ = 6.9 Hz, 1H, C*H*=$CH_2$), 5.29–5.23 (m, 2H, CH=C$H_2$), 4.95–

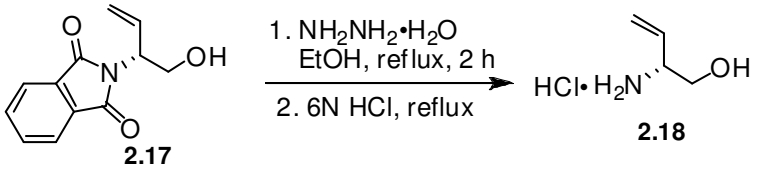
4.86 (m, 1H, 1-C*H*), 4.18–4.09 (m, 1H, 2-C$H_2$), 3.98–3.91 (m, 1H, 2-C$H_2$), 2.85–2.77 (app. m, 1H, O*H*)

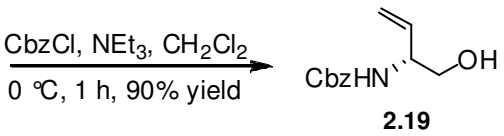
$^{13}$C (75 MHz, $CDCl_3$) δ 168.7 (C=O), 134.3 (Ar-*C*H), 132.1 (Ar-$C_q$), 131.9 (*C*H=$CH_2$), 123.6 (Ar-*C*H), 118.9 (CH=*C*$H_2$), 63.0 (*C*$H_2$–OH), 56.0 (N–*C*H–$CH_2$)

Notes:

1. DCM was rendered anhydrous by distilling from $CaH_2$.

***N*-Benzyloxycarbonyl-2-amino-2(*R*)-vinyl ethanol (2.19)**




The phthalimido protected compound **2.17** (5.3 g, 24.2 mmol, 1 equiv.) was dissolved in 150 mL absolute EtOH. Hydrazine hydrate (1.3 mL, 26.6 mmol, 1.1 equiv.) was added to the solution which was then refluxed for 2 h. A thick white precipate formed during the course of the 2 h. The suspension was cooled to room temperature. 6N HCl (350–400 mL) was then added to the precipitate which was then once again refluxed for 2 h. After cooling to room temperature, the volume was reduced to one half (250–300 mL) and the suspension then cooled to 0 °C. The cooled solution was filtered and the filtrate further concentrated to give **2.18** as a white solid weighing 3 g (75% yield, Note 1).

mp: 83–84 °C

$[\alpha]_D$ = –8.6 (*c* 1.0, $CH_3OH$) [lit.[21] $[\alpha]_D$ for enantiomer = + 10 (*c* 0.53, $CH_3OH$)]

$^1H$ (400 MHz, DMSO-$d_6$) δ 8.33 (br. s, 3H, N*H*$_3$), 5.84 (ddd, 1H, $J_1$ = 17.4 Hz, $J_2$ = 10.7 Hz, $J_3$ = 6.7 Hz, C*H*=$CH_2$), 5.41–5.29 (m, 2H, CH=C*H*$_2$), 3.70–3.62 (m, 1H, α-C*H*), 3.61–3.49 (m, 2H, C*H*$_2$OH)

$^{13}C$ (100 MHz, DMSO-$d_6$) δ 132.5 (*C*H=$CH_2$), 119.7 (CH=*C*$H_2$), 61.5 (*C*$H_2$OH), 54.5 (α-*C*H)

A suspension of the hydrochloride salt **2.18** (1 g, 8.1 mmol, 1 equiv.) in 30 mL anhydrous DCM (Note 2) was cooled in an ice-salt bath. Triethylamine (3.4 mL, 24. 3 mmol, 3 equiv.) was added dropwise to this suspension. Benzyl chloroformate (1.4 mL, 9.7 mmol, 1.2 equiv.) was added to the resulting mildly cloudy suspension (Note 3). The reaction was stirred for 1 h at 0 °C after which the suspension was diluted to 60 mL and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions (60 mL each). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to give a pale-yellow colored oil which was subjected to flash chromatography twice (gradient elution:5% to 30% EtOAc in hexanes) to give the pure product **2.19** as a white solid weighing 1.4 g (80% yield).

mp: 55–57 °C

TLC $R_f$ 0.47 (EtOAc/hexanes, 3:1)

$[\alpha]_D$ = +33.1 (*c* 1.1, $CHCl_3$) [lit.[29] $[\alpha]_D$ for enantiomer –32.2 (*c* 1.46, $CHCl_3$)]

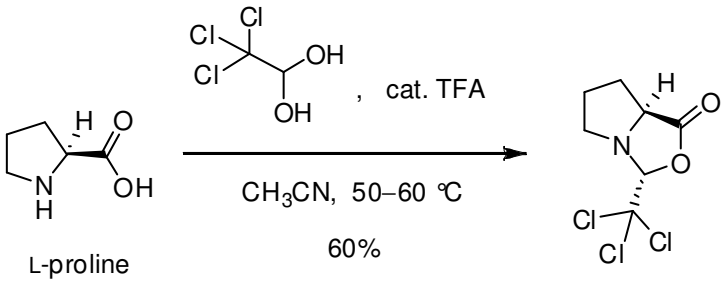
$^1H$ (400 MHz, $CDCl_3$) δ 7.29–7.22 (m, 5H, Ar-C*H*), 5.73 (ddd, $J_1$ = 16.2 Hz, $J_2$ = 10.5 Hz, $J_3$ = 5.3 Hz, 1H, C*H*=$CH_2$), 5.22–5.14 (m, 2H, CH=C*H*$_2$), 5.03 (s, 2H, C*H*$_2$Ph), 4.27 (m, 1H, α-C*H*), 3.67–3.52 (m, 2H, C*H*$_2$OH), 2.31 (br. s, 1H, O*H*)

$^{13}C$ (100 MHz, $CDCl_3$, gHMQC and DEPT-135 assignment) δ 156.5 (NH(*C*=O)–O), 136.4 (Ar-*C*–$CH_2$), 135.2 (*C*H=$CH_2$), 128.6, 128.3, 128.2 (Ar-*C*H), 116.9 (CH=*C*$H_2$), 66.9 (*C*$H_2$Ph), 64.9 (*C*$H_2$OH), 55.0 (α-*C*H)

Notes:

1. When the free vinylglycinol was subjected to Boc-protection, reasonable amount of a solid by-product was isolated. This was analyzed to be the bis-Boc hydrazine derivative. No doubt that hydrazine•2HCl was isolated along with the desired product after hydrazinolysis. This also explains the appearance of "extra" proton integration in the $^1$H NMR of the intermediate.
2. DCM was rendered anhydrous by distilling from $CaH_2$.
3. DCM can be replaced by THF to ensure that the side product, triethylammonium chloride, is insoluble and can be filtered off.

**(*2R*,*5S*)-1-Aza-2-trichloromethyl-3-oxabicyclo[3.3.0]octane-4-one (2.22)**




**2.22**

To a well-stirred suspension of L-proline (7.5 g, 65.2 mmol) and chloral hydrate (12.9 g, 78.2 mmol, 1.2 equiv.) in acetonitrile (100 mL), was added 1–2 drops of trifluoroacetic acid (Note 1). The suspension was rapidly warmed to 45 °C and then gradually to 60 °C. A pale yellow colored solution resulted within an hour of stirring at this temperature. The same conditions were maintained for another 2 h following which the solvent was removed using a rotary evaporator. The yellow solid was taken up in 80–100 mL ethyl acetate and the undissolved solid was filtered off. Slow removal of ethyl acetate *in vacuo* resulted in a crystalline solid residue. The solid was re-crystallized from

hot ethyl acetate and the crystals were filtered and washed with cold ethanol. Further 3–4 crops of crystals of **2.22** gave an overall yield of 9.5 g (60% based on L-proline) whose spectral properties were consistent with those reported in literature. The crystals were dried well and stored under Ar.

mp 108–110 °C [lit.[33a] 105–108 °C; lit.[43] 107–109 °C; lit.[33b] 108 °C]

TLC $R_f$ 0.7 (Hexanes/Ethyl acetate, 1:1)

$[\alpha]_D$ +35.2 (*c* 1.1, $C_6H_6$) [lit.[33b] +33 (*c* 2.0, $C_6H_6$)]

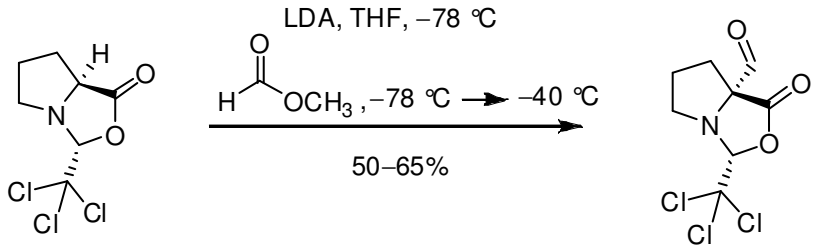
$^1$H NMR (300 MHz, $CDCl_3$) δ 5.16 (s, 1H , N–C*H*–O), 4.13 (dd, $J_1$ = 8.7 Hz, $J_2$ = 4.8 Hz, 1H, α-C*H*), 3.43 (ddd, $J_1$ = 10.8 Hz, $J_2$ = 7.8 Hz, $J_3$ = 5.7 Hz, 1H, δ-C*H*), 3.13 (ddd, $J_1$ = 11.4 Hz, $J_2$ = 6.3 Hz, $J_3$ = 6.3 Hz, 1H, δ-C*H*), 2.27–2.06 (m, 2H, γ-C*H*), 1.98–1.88 (m, 1H, β-C*H*), 1.81–1.68 (m, 1H, β-C*H*)

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 175.2 (O=*C*–O), 103.5 (N–*C*H–O), 100.4 ($Cl_3$–*C*), 62.3(α-*C*), 57.8(δ-*C*), 29.9 (β-*C*), 25.3 (γ-*C*)

Notes:

1. Use of greater than 7 mol% TFA with respect to L-proline results in low yields.

**(*2R*, *5R*)-1-Aza-5-formyl-2-trichloromethyl-3-oxabicyclo[3.3.0]octan-4-one (2.24)**




**2.22** **2.24**

A solution of LDA (0.8 M , 82.2 mL , 58.3 mmol), freshly prepared from diisopropylamine (9.3 mL, 66.1 mmol) and *n*-butyllithium (23.3 mL, 58.3 mmol) in THF (49.5 mL), was added drop-wise to a solution of the chloral-derived oxazolidinone **2.22** (9.5 g, 38.9 mmol) in THF (47.5 mL) that was previously cooled to –78 °C. The deep-red colored enolate solution was stirred at –78 °C for 30 min, after which anhydrous methyl formate (19.2 mL, 310.8 mmol) was added over 5 min. The solution was stirred at –78 °C for 10 min and then warmed to –40 °C where it was then maintained for 1 h. The reaction was then quenched with sat. $NH_4Cl$ solution and the solvent removed *in vacuo.* The residue was dissolved in $Et_2O$ (150 mL) and the solution was washed sequentially with 10% citric acid solution (2 x 100 mL), saturated $NaHCO_3$ (2 x 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered (the second time using a 0.45 µm PTFE filter disc), evaporated and purified by flash chromatography (gradient elution: 15 – 35% EtOAc in hexanes) to furnish 5.2 g (50% yield, Note 1) of alkylated product **2.24** as white crystals.

mp 86–87 °C [lit.[32] 89 °C]

TLC $R_f$ 0.6 (Hexanes/EtOAc, 1:1) [lit.[32] $R_f$ 0.4 (Hexanes/EtOAc, 1:1)]

$[\alpha]^{25}_D$ : +31.8 (*c* 2.0, $CHCl_3$) [lit.[32] +29.5 (*c* 2.0, $CHCl_3$)]

$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment) δ 9.6 (s, 1H, O=C–*H*), 5.18 (s, 1H, N–C*H*–O), 3.53 (ddd, $J_1$ = 8.1 Hz , $J_2$ = 6.6 Hz, $J_3$ = 6.6 Hz ,1H, δ-C*H*), 3.33 (ddd, $J_1$ = 11.7

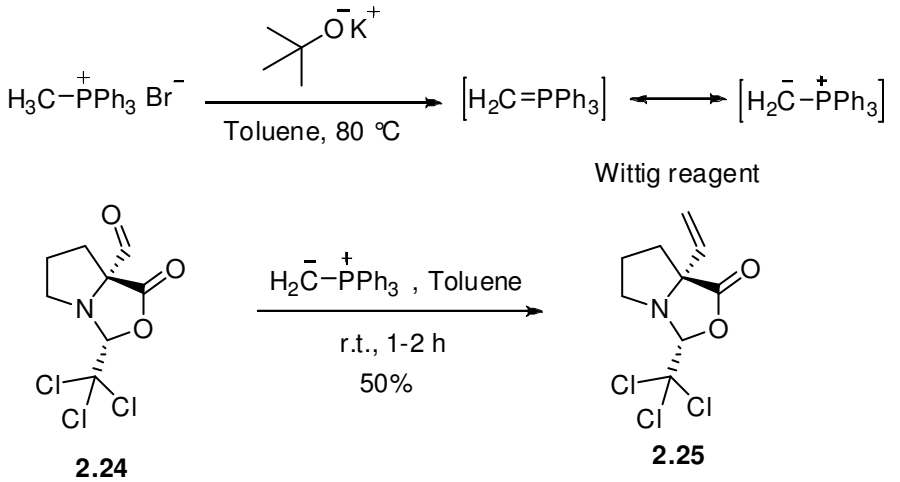
Hz, $J_2$ = 6.3 Hz, $J_3$ = 6.3 Hz, 1H, δ-C*H*), 2.41–2.21 (m, 2H, β-C$H_2$), 2.03–1.80 (m, 2H, γ-C$H_2$)

$^{13}$C NMR (75 MHz, $CDCl_3$, gHMQC assignment) δ 193.2 (O=*C*–H), 168.9 (O=*C*–O) 102.2 (N–*C*H–O), 99.7($Cl_3$–*C*), 78.0 (α-*C*), 58.9 (δ-*C*), 33.8 (β-*C*), 25.4 (γ-*C*)

Notes:

1. When the reaction scale was doubled (to 20 g), the yield obtained increased to 64%.

**(*2R*,*5R*)-1-Aza-2-trichloromethyl-5-vinyl-3-oxabicyclo[3.3.0]octan-4-one (2.25)**




Methyltriphenylphosphonium bromide (16.7 g, 46.9 mmol, 1.7 equiv.) and potassium *tert*-butoxide (5.2 g, 46.9 mmol, 1.7 equiv.) were suspended in anhydrous toluene (Note 1). The suspension, which immediately took on a canary-yellow color, was heated for 2 h at 80°C (Note 2). At the end of 2 h, this reaction mixture was cooled to room temperature. A solution of the 2-formyl proline derivative **2.24** (7.5 g, 27.6 mmol, 1 equiv.), in anhydrous toluene (75 mL), was added drop wise into the mixture containing the Wittig reagent. The suspension turned light brown in color (with a tan color

precipitate) once the addition was complete. The reaction was quenched after 2 hours when TLC displayed no further change in product distribution. Toluene was removed on a rotary evaporator and the residue was diluted with 300 mL diethyl ether. This suspension was then stored overnight at –20 °C to ensure complete precipitation of the triphenylphosphine oxide side product. The precipitate was then filtered off the next day. The slightly turbid solution was further diluted with ether and filtered using a 0.45 µm PTFE filter disc. The clear solution was evaporated to give an orange–yellow oil which was further purified by flash chromatography (gradient elution: 5–15% EtOAc in hexanes) to give the product **2.25** as a colorless oil (Note 3) weighing 3.5 g (50% yield).

TLC $R_f$ 0.50 (hexanes/EtOAc, 4:1) [lit.[32] $R_f$ 0.27 (hexanes/EtOAc, 4:1)]

$[\alpha]_D$ +48.3 [lit.[32] +47.2 (*c* 0.25, $CHCl_3$)]

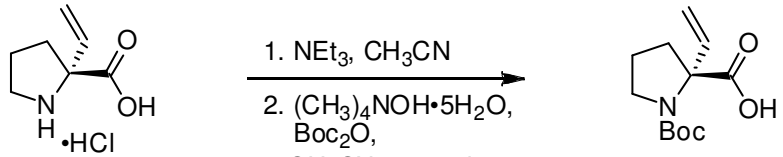
$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment) δ 6.02 (dd, $J_1$ = 16.8 Hz, $J_2$ = 10.2 Hz, 1H, –C*H*=$CH_2$), 5.52 (dd, $J_1$ = 16.8 Hz, $J_2$ = 1.2 Hz, 1H, CH=C$H_{trans}$H), 5.23 (dd, $J_1$ = 10.2 Hz, $J_2$ = 0.9 Hz, 1H, –CH=C$H_{cis}$H), 5.09 (s, 1H, N–C*H*–O), 3.46 (ddd, $J_1$ = 11.1 Hz , $J_2$ = 6.3 Hz, $J_3$ = 6.3 Hz ,1H, δ-C*H*), 3.20 (ddd, $J_1$ = 11.1 Hz, $J_2$ = 6.6 Hz, $J_3$ = 6.6 Hz, 1H, δ-C*H*), 2.23–2.14 (m, 2H, β-C*H*), 2.08–1.79 (m, 2H, γ-C*H*)

$^{13}$C NMR (75 MHz, $CDCl_3$, gHMQC assignment) δ 174.0 (O=*C*–O), 135.7 (–*C*H=$CH_2$), 116.1 (–CH=*C*$H_2$), 102.6 (N–*C*H–O), 100.6 ($Cl_3$–*C*), 73.6 (α-*C*), 58.4 (δ-*C*), 38.4 (β-*C*), 25.0 (γ-*C*)

<u>Notes:</u>

1. Toluene was rendered anhydrous by distilling from sodium metal.
2. Temperature was maintained at 70–80 °C. Do not reflux reaction mixture.
3. A white solid was obtained when the reaction was conducted on a scale less than 1.0 g.

**(2*R*)-*N*-*tert*-Butyloxycarbonyl-2-vinylpyrrolidine-2-carboxylic acid (2.3)**




**2.26** **2.3**

2-Vinylproline hydrochloride **2.26** (1 g, 5.6 mmol, 1 equiv.) was suspended in 50 mL anhydrous acetonitrile (Note 1). The resulting solution was cooled in an ice-/salt bath to which triethylamine (1 mL, 6.8 mmol, 1.2 equiv.) was added drop-wise. The solution was stirred for 10 minutes at room temperature after which tetramethyl ammonium hydroxide pentahydrate (1.1 g, 5.6 mmol, 1 equiv.) was added. This was followed by the addition of di-*tert*-butyldicarbonate (1.84 g, 8.5 mmol, 1.5 equiv.). The cloudy reaction mixture was stirred under Ar at room temperature for 3 d. A further 0.5 equiv. of $Boc_2O$ (0.6 g, 2.8 mmol) was added and the reaction was allowed to proceed for another day. At the end of 4 d, the solvent was removed *in vacuo* to give a white solid residue which was partitioned between diethyl ether and 1N NaOH (100 mL each). Unreacted $Boc_2O$ was removed by washing with ether. The aqueous layer was acidified to pH 2 using 4N HCl followed by extraction of the liberated acid into ethyl acetate (3 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated to give the Boc-protected amino acid **2.3** as a crystalline white solid weighing 1.2 g (85% yield).

m.p. 134–136 °C [lit.[32] 134 °C]

TLC $R_f$ 0.52 (Isopropanol/$NH_4OH$, 4:1) [lit.[32] $R_f$ 0.49 ($CH_2Cl_2$/MeOH, 9:1)]

$[\alpha]_D$ –57.1 (*c* 0.35, $CHCl_3$) [lit.[32] –60.3 (*c* 0.4, $CHCl_3$)]

$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment, rotamers observed in a 2:1 ratio) δ 6.28 (dd, $J_1$ = 17.4 Hz, $J_2$ = 10.5 Hz, 0.4H, –C*H*=$CH_2$), 6.02 (dd, $J_1$ = 17.1 Hz, $J_2$ = 10.5 Hz, 0.6H, –C*H*=$CH_2$), 5.27–5.03 (m, 2H, –CH=C$H_2$), 3.69–3.48 (m, 2H, δ-C*H*), 2.69–2.60

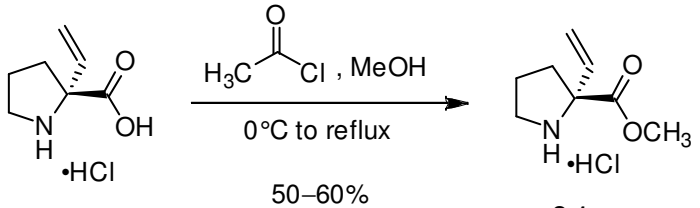
(m, 0.6H, β-C*H*), 2.35–2.25 (m, 0.4H, β-C*H*), 2.08–1.79 (m, 3H, β-C*H* and γ-C*H*), 1.48 and 1.36 (s, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (75 MHz, $CDCl_3$, gHMQC assignment, rotamers observed about the Boc group in a 2:1 ratio) δ 178.8 and 175.2 (O=*C*–OH), 155.8 and 153.5 (NH–(*C*=O)–O–), 136.6 and 136.5 (–*C*H=$CH_2$), 114.8 and 113.3 (–CH=*C*$H_2$), 81.6 and 80.7 (*C*(C$H_3$)$_3$), 71.0 and 69.4 (α-*C*), 48.8 and 48.0 (δ-*C*), 39.5 and 38.1 (β-*C*), 28.6 and 28.3 (C(*C*$H_3$)$_3$), 22.9 and 22.1 (γ-*C*)

Notes:

1. Acetonitrile was rendered anhydrous by distilling from $CaH_2$.

**Methyl (*R*)-2-Vinylpyrrolidine-2-carboxylate (2.1)**




**2.26** **2.1**

2-Vinylproline hydrochloride **2.26** (1.2 g, 6.5 mmol, 1 equiv.) was dissolved in 60 mL anhydrous MeOH (Note 1). The solution was cooled to –5 °C using an ice/salt bath. Acetyl chloride (9.3 mL, 130.6 mmol, 20 equiv.) was added drop-wise over 20 min using an addition funnel. Once the addition was complete, the clear solution was stirred at this temperature for 5 min followed by first warming to r.t. and finally refluxing for 7 h. The solution was cooled back to r.t. and stirred overnight. At the end of this time, TLC indicated complete consumption of the starting material (Note 2). The solution was concentrated to give a brown-colored oil to which 20 mL portions (4 x) of xylenes were added and removed *in vacuo.* This treatment ensured complete removal of solvent and

excess HCl. The oil solidified to give a crystalline yellow-brown solid weighing 1.3 g (quantitative yield) on the high-vac pump. Re-crystallization attempts from a mixture of iPrOH/$CHCl_3$/hexanes failed to yield crystals. $^1H$ and $^{13}C$ NMR of the solid obtained indicated greater than 95% purity and the material was used without further purification.

TLC $R_f$ 0.73 (iPrOH/$NH_4OH$, 4:1)

$[\alpha]_D$ –65.9 (*c* 1.2, $CH_3OH$)

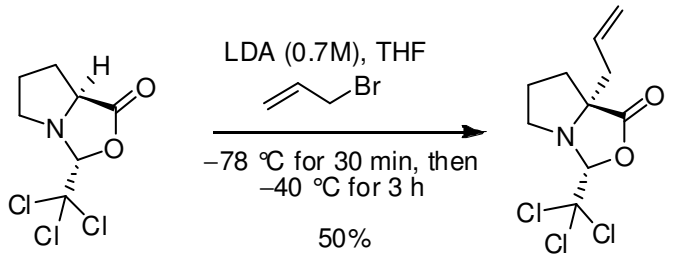
$^1H$ NMR (400 MHz, $CDCl_3$) δ 10.32 and 9.77 (br. s, 1H, N$H_2$), 6.15 (dd, $J_1$ = 17.4 Hz, $J_2$ = 10.8 Hz, 1H, C$H$=$CH_2$), 5.84 (br. s, 1H, N$H_2$), 5.64–5.41 (m, 2H, CH=C$H_2$), 3.76 (s, 3H, OC$H_3$), 3.57–3.42 (m, 2H, δ-C$H_2$), 2.50–2.38 (m, 1H, β-C$H_2$), 2.26–2.15 (m, 1H, β-C$H_2$), 2.08–1.87 (m, 2H, γ-C$H_2$)

$^{13}C$ NMR (100 MHz, $CDCl_3$, HMQC and DEPT assignment, rotamers observed) δ 170.1 and 168.8 (*C*=O), 131.4 and 131.1 (*C*H=$CH_2$), 121.3 and 120.9 (CH=*C*$H_2$), 73.3 and 73.0 (α-*C*), 53.9 (O*C*$H_3$), 44.8 and 44.6 (δ-*C*), 33.6 (β-*C*), 22.1 and 21.9 (γ-*C*)

Notes:

1. MeOH was rendered anhydrous by reacting with 1.5 g/100 mL Mg metal which forms Mg methoxide *in situ* followed by distillation.
2. Literature procedure calls for one pot hydrolysis and methyl ester formation.[32] This reaction takes place at r.t. and requires 7 days.

**(*2R*,*5R*)-5-Allyl-1-aza-2-trichloromethyl-3-oxabicyclo[3.3.0]octan-4-one (2.27)**




**2.22** **2.27**

A solution of LDA (0.8 M , 72 mL, 54.9 mmol), freshly prepared from diisopropylamine (8.4 mL, 59.9 mmol, 1.2 equiv.) and *n*-butyllithium (22 mL, 54.9 mmol, 1.1 equiv.) in THF (50 mL), was added drop-wise to a solution of the chloral-derived oxazolidinone **2.22** (12.2 g, 49.9 mmol) in THF (100 mL) that was previously cooled to –78 °C. The deep-red colored enolate solution was stirred at –78 °C for 30 min, after which anhydrous allyl bromide (8.6 mL, 99.8 mmol, 2.0 equiv.) was added over 5 min. The solution was stirred at –78 °C for 10 min and then warmed to –40 °C where it was then maintained for 3 h. The reaction was then quenched with sat. $NH_4Cl$ solution and the solvent removed *in vacuo.* The residue was dissolved in $Et_2O$ (150 mL) and the solution was washed sequentially with 10% citric acid solution (2 x 100 mL), saturated $NaHCO_3$ (2 x 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered (the second time using a 0.45 µm PTFE filter disc), evaporated and purified by flash chromatography (gradient elution: 15 – 35% EtOAc in hexanes) to furnish 5.2 g (50% yield, Note 1) of alkylated product **2.27** as a very viscous oil (mostly oil at r.t.; mp reported as 20–24 °C in leading reference).

TLC $R_f$ 0.52 (Hexanes/EtOAc, 3:1)

$[\alpha]_D$ +42.4 (*c* 1.0, $CHCl_3$) [lit.[33a] $[\alpha]_D$ +44.6 (*c* 2.0, $CHCl_3$)]

$^1$H NMR (400 MHz, $CDCl_3$) δ 5.87 (dddd, 1H, $J_1$ = 16.8 Hz, $J_2$ = 10.8 Hz, $J_3$ = $J_4$ = 6.4 Hz, C*H*=$CH_2$), 5.20–5.16 (m, 2H, CH=C$H_2$), 4.98 (s, 1H, N-C*H*-O), 3.24–3.11 (m, 2H, δ-

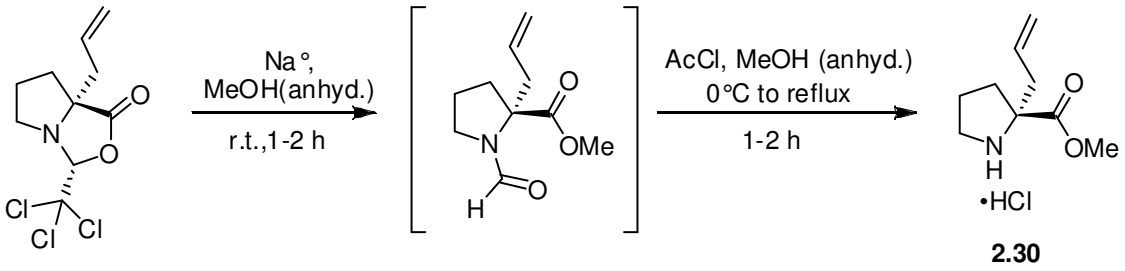
C$H_2$), 2.65–2.51 (m, 2H, C$H_2$CH=CH$_2$), 2.17–2.11 (m, 1H, β-C$H_2$), 2.05–1.97 (m, 1H, β-C$H_2$), 1.93–1.85 (m, 1H, γ-C$H_2$), 1.69–1.56 (m ,1H, γ-C$H_2$)

$^{13}$C NMR (100 MHz, $CDCl_3$) δ 176.6 (*C*=O), 132.0 (*C*H=CH$_2$), 120.2 (CH=*C*H$_2$), 102.4 (N-*C*-O), 100.4 (*C*Cl$_3$), 58.5 (δ-*C*), 41.6 (*C*H$_2$CH=CH$_2$), 35.3 (β-*C*), 25.3 (γ-*C*)

Notes:

1. The crude material before flash chromatography can be used directly without any further purification. The yield before flash chromatography was approx. 60%.

**Methyl (*R*)-2-(2'-Propenyl)pyrrolidine-2-carboxylate•hydrochloride (2.30)**




**2.27** **2.30**

71% over both steps

A solution of the oxazolidinone **2.27** (4.0 g, 14.1 mmol, 1.0 equiv.) was prepared in anhydrous MeOH (50 mL) (Note 1). Metallic sodium (196 mg, 8.5 mmol, 0.6 equiv.) was cut into small pieces and added to the solution at room temperature. A mild exotherm was detected (35–37 °C) at the completion of addition. The clear and colorless solution was stirred at r.t. under Ar for 1 h. Complete consumption of the starting material ($R_f$ 0.52 in hexanes/EtOAc- 3:1) was determined by TLC examination. The solution was pale-yellow colored at the end of the reaction. The solution was cooled to 0 °C and freshly distilled acetyl chloride was added dropwise. The resulting off-white thick

suspension was warmed to r.t. and then refluxed for 1–2 h until the intermediate *N*-formyl derivative ($R_f$ 0.1–0.2 in hexanes/EtOAc-3:1) was completely consumed as determined by TLC. The volatiles were removed by rotary evaporation and further azeotroping with benzene. The yellow oil obtained was purified by flash chromatography (gradient elution: 0–10% MeOH/DCM) to give a pale-yellow oil which crystallized under reduced vacuum to the pure product **2.30** as an off-white solid weighing 2.0 g (71% yield over 2 steps).

TLC $R_f$ 0.76 (iPrOH/$NH_4OH$, 4:1); $R_f$ 0.36 ($CH_2Cl_2$/MeOH, 9:1)

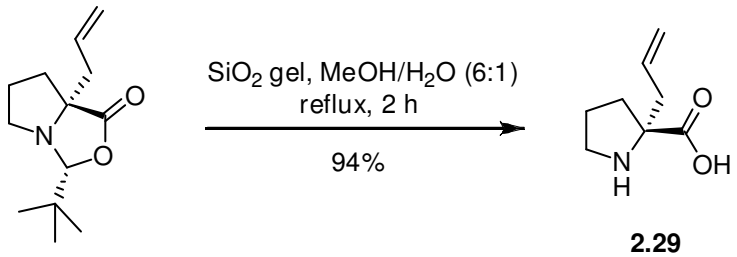
$^1$H NMR (400 MHz, $CDCl_3$) δ 10.10 (br.s, 2H, N*H*$_2$), 5.85 (m, 1H, C*H*=CH$_2$), 5.34–5.20 (m, 2H, CH=C*H*$_2$), 3.83 (s, 3H, $OCH_3$), 3.63–3.49 (m, 2H, δ-C*H*$_2$), 3.07–3.02 (m, 1H, C*H*$_2$-CH=CH$_2$), 2.48–2.40 (m, 1H, β-C*H*$_2$), 2.16–2.7 (m, 2H, β-C*H*$_2$, γ-C*H*$_2$), 1.94–.88 (m, 1H, γ-C*H*$_2$)

$^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.2 (*C*=O), 130.3 (*C*H=CH$_2$), 121.6 (CH=*C*H$_2$), 72.7 (α-*C*), 53.8 (O*C*H$_3$), 45.5 (δ-*C*), 39.3 (*C*H$_2$CH=CH$_2$), 34.6 (β-*C*), 22.4 (γ-*C*)

Notes:

1. MeOH was rendered anhydrous by distilling from Mg metal.

**(*R*)-2-(2'-Propenyl)proline (2.29)**




**2.28** **2.29**

The alkylated oxazolidinone **2.28** (5 g, 22.4 mmol, 1.0 equiv.) was dissolved in 6:1 MeOH/$H_2O$ (100 mL). Silica gel (5 g) was added to the solution and the slurry was heated at reflux for 2 h at which point TLC indicated that the starting material had been consumed. The reaction mixture was cooled to r.t. and the residue was triturated and suspended in 20:1 $CH_2Cl_2$/MeOH. The suspension was filtered and the filtrate concentrated to give an off-white solid. The solid was triturated with hot hexanes/$Et_2O$ (2 x 50 mL portions) and the suspension filtered to yield the product **2.29** as a fluffy, amorphous white solid weighing 3.2 g (94% yield). All physical and spectral properties of this solid were consistent with values reported in the literature. No further purification was necessary.

mp >250 °C (dec.)

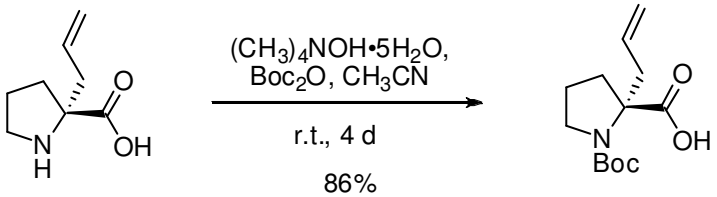
TLC $R_f$ 0.4 (iPrOH/$NH_4OH$,4:1) [lit.[63] $R_f$ 0.4 (same solvent system)]

$[\alpha]_D$ –48.6 (*c* 0.99, $H_2O$) [lit.[63] $[\alpha]_D$ –48.6 (*c* 1.46, $H_2O$)]

$^1$H NMR (400 MHz, $D_2O$, gCOSY assignment) δ 5.79–5.69 (m, 1H, C*H*$_2$=$CH_2$), 5.31–5.24 (m, 2H, CH=C*H*$_2$), 3.47–3.33 (m, 2H, δ-C*H*$_2$), 2.88 (dd, $J_1$ = 14.8 Hz, $J_2$ = 6.4 Hz, 1H, C*H*$_2$CH=$CH_2$), 2.54 (dd, $J_1$ = 14.8 Hz, $J_2$ = 8.1 Hz, 1H, C*H*$_2$CH=$CH_2$), 2.43–2.35 (m, 1H, γ-C*H*$_2$), 2.09–1.92 (m, 3H, β-C*H*$_2$, γ-C*H*$_2$)

$^{13}$C NMR (100 MHz, $D_2O$, gHMQC assignment) δ 175.7 (*C*=O), 130.9 (*C*H=$CH_2$), 120.4 (CH=*C*H$_2$), 74.1 (α-*C*), 45.7 (δ-*C*), 39.4 (*C*H$_2$CH=$CH_2$), 34.7 (γ-*C*), 22.8 (β-*C*)

**(*R*)-*N-tert*-Butyloxycarbonyl-2-(2'-propenyl)pyrrolidine Carboxylic Acid (2.31)**




**2.29** **2.31**

2-Allylproline **2.29** (1 g, 6.4 mmol, 1 equiv.) was suspended in 64 mL anhydrous acetonitrile (Note 1). Tetramethyl ammonium hydroxide pentahydrate (1.2 g, 6.4 mmol, 1 equiv.) was then added to the suspension. A clear solution resulted in approx. 20 minutes indicating salt formation. This was followed by the addition of $Boc_2O$ (2.1 g, 9.6 mmol, 1.5 equiv.). The cloudy reaction mixture was stirred under Ar at room temperature for 3 d. A further 0.5 equiv. of $Boc_2O$ (0.7 g, 3.2 mmol) was added and the reaction was allowed to proceed for another day. At the end of 4 d, the solvent was removed *in vacuo* to give a white solid residue which was partitioned between diethyl ether and 1 N NaOH (100 mL each). Unreacted $Boc_2O$ was removed by washing with ether. The aqueous layer was acidified to pH 2 using 4 N HCl followed by extraction of the liberated acid into ethyl acetate (3 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated to give the product **2.31** as a white solid weighing 1.4 g (86% yield). The solid was re-crystallized from /$Et_2O$/hexanes to give thick, long needle-shaped crystals.

mp 121–122 °C [lit.[64] mp 118–119 °C]

TLC $R_f$ 0.45 (iPrOH/$NH_4OH$, 4:1)

$[\alpha]_D$ +3.1 (*c* 1.0, $CHCl_3$); +72.1 (*c* 0.52, MeOH) [lit.[64] $[\alpha]_D$ +72.5 (*c* 1.2, MeOH)]

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.78–5.60 (m, 1H, C*H*=$CH_2$), 5.16–5.09 (m, 2H, CH=C*H*$_2$), 3.72–3.66 (m, 0.5H, δ-C*H*$_2$), 3.55–3.49 ( m, 0.5H, δ-C*H*$_2$), 3.39–3.25 (m, 1H, δ-C*H*$_2$), 2.99–2.88 (m, 1H, C*H*$_2$CH=$CH_2$), 2.67–2.53 (m, 1H, C*H*$_2$CH=$CH_2$),

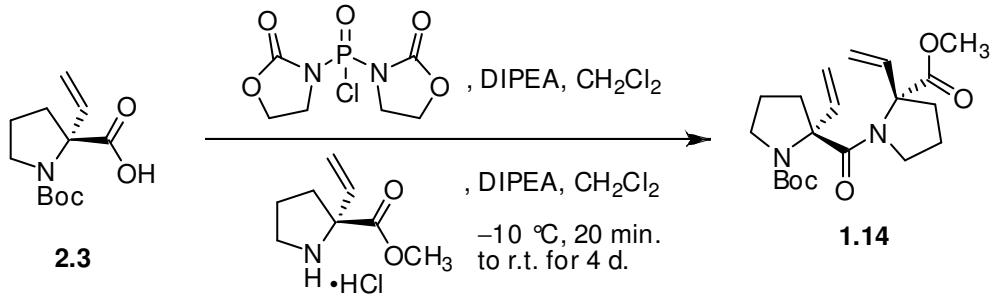
2.41 (br.s, 0.5H, γ-C$H_2$), 2.15–2.11 (m, 1H, β-C$H_2$), 2.02–1.75 (m, 2.5H, β-C$H_2$, γ-C$H_2$), 1.45 and 1.41 (s, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and DEPT-135 assignment, rotamers observed about the carbamate bond in a 2–4:1 ratio) δ 180.8 and 177.0 ((*C*=O)-OH), 156.1 and 153.7 (NH–(*C*=O)), 133.2 and 132.2 (*C*H=$CH_2$), 119.9 and 119.3 (CH=*C*$H_2$), 81.4 and 80.7 (*C*(C$H_3$)$_3$), 69.1 and 67.0 (α-*C*), 49.2 and 48.6 (δ-*C*), 39.3 and 38.1 (*C*$H_2$CH=$CH_2$), 37.2 and 35.0 (β-*C*), 28.5 and 28.4 (C(*C*$H_3$)$_3$), 22.8 and 22.7 (γ-*C*)

Notes:

1. $CH_3CN$ was rendered anhydrous by distilling from $CaH_2$.

**(2*R*)-Methyl 1-((2'*R*)-1'-(*N''*-(*tert*-Butyloxycarbonyl)-2'-vinylpyrrolidine)carbonyl)-2-vinylpyrrolidine-2-carboxylate (1.14)**




15–17%

Boc-α-vinylproline **2.3** (940 mg, 3.9 mmol, 1.5 equiv.) was dissolved in 30 mL anhydrous DCM (Note 1). The solution was cooled to –10 °C using an ice/salt bath. This was followed by the sequential addition of α-vinylproline methyl ester hydrochloride **2.1** (491 mg, 2.6 mmol, 1.0 equiv.) and BOP-Cl (993 mg, 3.9 mmol, 1.5 equiv.). The mild suspension was stirred for 10 min. after which DIPEA (0.9 mL, 10.4 mmol, 4.0 equiv.) was added in one portion. The yellow-colored solution was stirred at –10 °C for another

10 min and then warmed to r.t. where it was stirred for 4 d under Ar. After 4 d, the reaction was diluted to 100 mL and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was subjected to flash chromatography (gradient elution: 0–70% EtOAc in hexanes) to give the desired product **1.14** as a clear oil weighing 146 mg (15% yield, Note 2).

TLC $R_f$ 0.48 (EtOAc/hexanes, 3:1)

$[\alpha]_D$ +35.3 (*c* 2.13, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment), δ 6.49 (dd, $J_{trans}$ = 17.4 Hz, $J_{cis}$ = 10.6 Hz, 1H, C*H*=$CH_2$), 6.33 (dd, $J_{trans}$ = 17.4 Hz, $J_{cis}$ = 10.7 Hz, 1H, C*H*=$CH_2$), 5.12–4.89 (m, 4H, 2 x CH=C*H*$_2$), 3.72–3.69 (ovlp. m, 2H, vinylPro δ-C*H*$_2$), 3.69 (s, 3H, OC*H*$_3$), 3.52–3.39 (m, 2H, vinylPro δ-C*H*$_2$), 2.17–1.83 (m, 8H, vinylPro β-C*H*$_2$, vinylPro γ-C*H*$_2$), 1.39 (s, 9H, C(C*H*$_3$)$_3$)

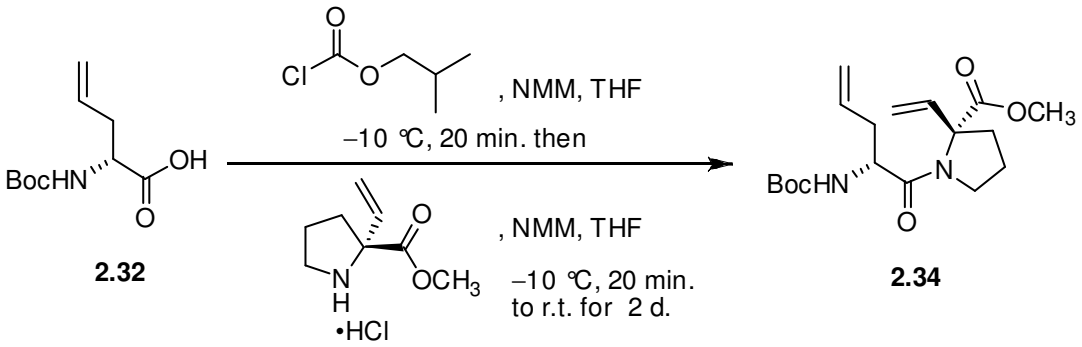
$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment, minor rotameric peak appearance for some peaks indicated by *) 172.3* and 172.2 (amide *C*=O), 170.0 (ester *C*=O), 154.3 and 153.8* (carbamate *C*=O), 139.0 and 138.8*, 137.5 and 137.4* (*C*H=$CH_2$), 113.5, 113.0 (CH=*C*H$_2$), 80.75 and 79.4* (*C*(CH$_3$)$_3$), 71.5, 70.1 (vinylPro α-*C*), 52.4 (O*C*H$_3$), 49.0* and 48.1, 47.9 (vinylPro δ-*C*), 37.6, 35.8* and 35.7 (vinylPro β-*C*), 28.4* and 28.1 (C(*C*H$_3$)$_3$), 24.4, 21.2 (vinylPro γ-*C*)

ESI HRMS *m/z* 401.2042 $[M+Na]^+$ $C_{20}H_{30}N_2O_5$+Na requires 401.2047

Notes:

1. DCM was rendered anhydrous by distilling from $CaH_2$
2. The most optimal yield of this reaction is 17% on a smaller scale. The yield can be improved by ensuring the purity of the starting material, specifically the amine hydrochloride salt.

**(2*R*)-Methyl 1-((4'*R*)-2'-(*tert*-Butyloxycarbonylamino)pent-4'-enoyl)-2-vinylpyrrolidine-2-carboxylate (2.34)**




Boc-α-allylglycine **2.32** (215 mg, 1.0 mmol, 1 equiv.) was dissolved in 3–4 mL anhydrous THF (Note 1) and the solution was cooled to –10 °C in an ice-salt bath. NMM (0.1 mL, 1.0 mmol, 1equiv.) was added drop-wise to this solution followed by very slow drop-wise addition of isobutylchloroformate (0.13 mL, 1.0 mmol, 1 equiv.). A white suspension was obtained (indicative of NMM•HCl formation). This suspension was stirred at –10 °C for 20 min. following which a suspension of α-vinylproline methyl ester **2.1** in THF [formed by neutralization of the corresponding HCl salt (210.6 mg, 1.1 mmol, 1.1 equiv.) in 4 mL THF using NMM (0.12 mL, 1.1 mmol, 1.1 equiv.)] was added in two portions to the mixed anhydride. After stirring at –10 °C for another 20 min., the suspension was warmed to R.T. where it was stirred for 2 d. At this point, TLC indicated complete consumption of the limiting reagent. NMM•HCl was removed by filtration and the volatiles removed by rotary evaporation. The residue was taken up in EtOAc (30 mL) and the solution washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The pale yellow-colored crude material was purified by flash chromatography (gradient elution: 0–30 % EtOAc in hexanes) to give the desired product **2.34** as a clear oil weighing 150 mg (45% yield).

TLC $R_f$ 0.59 (EtOAc/hexanes, 2:1)

$[\alpha]_D$ +56.3 (*c* 0.86, $CDCl_3$)

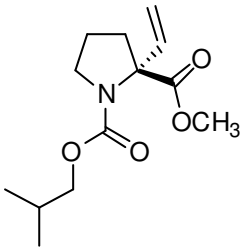
$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 6.35 (dd, $J_1$ = 17.5 Hz, $J_2$ = 10.9 Hz, 1H, vinylic group C*H*=$CH_2$), 5.76 (dddd, or app. ddt., $J_1$ = 17.2 Hz, $J_2$ = 10.2 Hz, $J_3$ = $J_4$ = 7.1 Hz, 1H, allylic group C*H*=$CH_2$), 5.24 (br. d, *J* = 8.6 Hz, 1H, N*H*), 5.16–5.09 (m, 3H, allylic group CH=C$H_2$, vinylic group CH=C$H_{cis}$H), 4.97 (app. d, *J* = 17.3 Hz, 1H, vinylic group CH=C$H_{trans}$H), 4.54 (ddd or app. dt, $J_1$ = 8.6 Hz, $J_2$ = $J_3$ = 6.3 Hz, 1H, Gly α-C*H*), 3.90–3.81 (m, 1H, Pro δ-C$H_2$), 3.73–3.68 (ovlp. m, 1H, Pro δ-C$H_2$), 3.72 (s, 3H, OC$H_3$), 2.57–2.50 (m, 1H, allylic C$H_2$), 2.39–2.32 (m, 1H, allylic C$H_2$), 2.23–2.15 (m, 1H, Pro β-C$H_2$), 2.08–1.92 (m, 3H, β-C$H_2$, γ-C$H_2$), 1.43 (s, 9H, NH(C=O)C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 172.6 (amide (*C*=O)), 169.7 (ester (*C*=O)), 155.4 (NH(*C*=O)C($CH_3$)$_3$), 135.7 (vinylic group *C*H=$CH_2$), 132.8 (allylic group *C*H=$CH_2$), 118.9 (allylic group CH=*C*$H_2$), 113.5 (vinylic group CH=*C*$H_2$), 79.7 (Boc *C*($CH_3$)$_3$), 70.2 (Pro α-*C*), 52.7 (ester O*C*$H_3$), 51.6 (Gly α-*C*), 48.4 (Pro δ-*C*), 37.4 (Gly β-*C*), 37.1 (Pro β-*C*), 28.5 (Boc C(*C*$H_3$)$_3$), 23.6 (Pro γ-*C*)

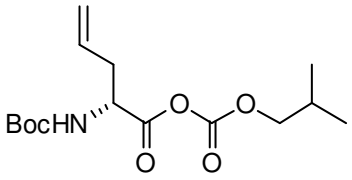
ESI HRMS *m/z* 375.1894 $[M+Na]^+$ ($C_{18}H_{28}N_2O_5$ + Na)$^+$ requires 375.1890

<u>Notes:</u>

1. THF was rendered anhydrous by distilling from sodium-benzophenone
2. A solid weighing 50 mg was also recovered as being an inseparable mixture of two molecules having higher $R_f$ values than the desired product. By NMR, the solid appears to be a mixture of the following molecules.

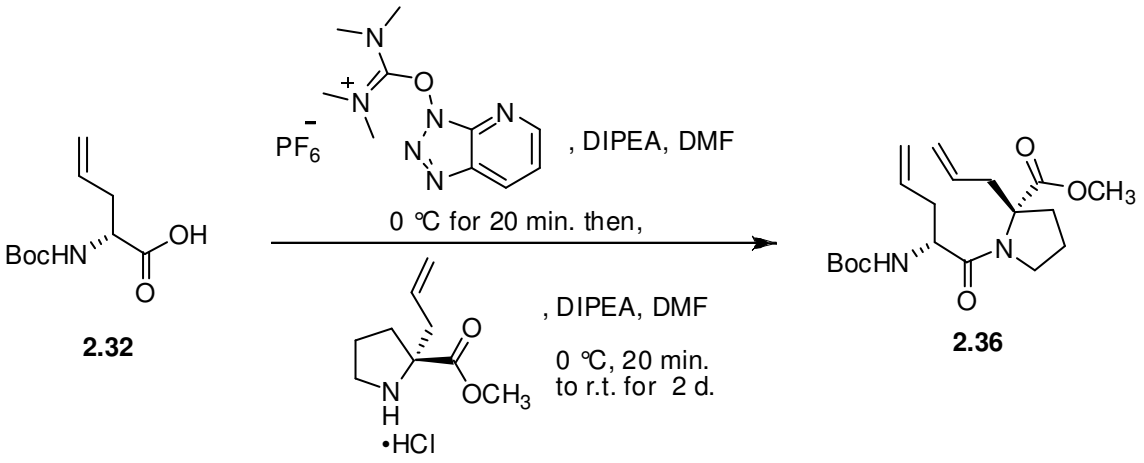


**2.33**
Amine attacking *wrong* carbonyl of mixed anhydride



**2.35**
Active mixed anhydride

**(2*R*)-Methyl 2-Allyl-1-((4'*R*)-2'-(*tert*-butyloxycarbonylamino)pent-4'-enoyl)pyrrolidine-2-carboxylate (2.36)**




Boc-α-Allylglycine **2.32** (215 mg, 1.0 mmol, 1 equiv.) was dissolved in 2 mL anhydrous DMF (Note 1) and the solution was cooled to 0 °C. DIPEA (0.17 mL, 1.0 mmol, 1 equiv.) was added to the solution followed by HATU (380 mg, 1.0 mmol, 1 equiv.) at which point the solution turned a fluorescent yellow (Note 2). This solution was stirred for 20 min. after which a solution of α-allylproline methyl ester **2.30** in DMF [formed by neutralization of the corresponding HCl salt (226 mg, 1.1 mmol, 1.1 equiv.) in 2 mL using DIPEA (0.2 mL, 1.1 mmol, 1.1 equiv.)] was added to the activated ester. The

resulting yellow-orange colored solution was stirred for 2 d, during which the color of the solution darkened. DMF was removed *in vacuo* and the residue was dissolved in EtOAc (30 mL) and the solution was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude material was subjected to purification by flash chromatography (gradient elution: 0–40 % EtOAc in hexanes) to give the desired product **2.36** as a colorless oil weighing 110 mg (30 % yield).

TLC $R_f$ 0.62 (EtOAc/hexanes, 2:1)

$[\alpha]_D$ +44.2 (*c* 1.39, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.77–5.59 (m, 2H, 2 x C*H*=$CH_2$), 5.26 (br. d, *J* = 8.2 Hz, 1H, N*H*), 5.15–5.05 (m, 4H, 2 x CH=C$H_2$), 4.52–4.44 (m, 1H, Gly α-C*H*), 3.92–3.82 (m, 1H, Pro δ-C$H_2$), 3.66 (s, 3H, OC$H_3$), 3.50–3.41 (m, 1H, Pro δ-C$H_2$), 3.10–3.00 (m, 1H, Pro allylic C$H_2$), 2.66–2.56 (m, 1H, Pro allylic C$H_2$), 2.53–2.41 (m,1H, Gly allylic C$H_2$), 2.35–2.22 (m, 1H, Gly allylic C$H_2$), 2.14–1.85 (m, 4H, β-C$H_2$, γ-C$H_2$), 1.39 (s, 9H, NH(C=O)C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 173.9 (amide (*C*=O)), 170.2 (ester (*C*=O)), 155.4 (NH(*C*=O)C($CH_3$)$_3$), 133.1 and 132.8 (allylic group *C*H=$CH_2$), 119.4 and 118.7 (allylic group CH=*C*$H_2$), 79.6 (Boc *C*($CH_3$)$_3$), 68.5 (Pro α-*C*), 52.4 (ester O*C*$H_3$), 51.8 (Gly α-*C*), 48.9 (Pro δ-*C*), 37.7 (Pro allylic-*C*), 37.3 (Gly β-*C*), 34.9 (Pro β-*C*), 28.4 (Boc C(*C*$H_3$)$_3$), 23.9 (Pro γ-*C*)

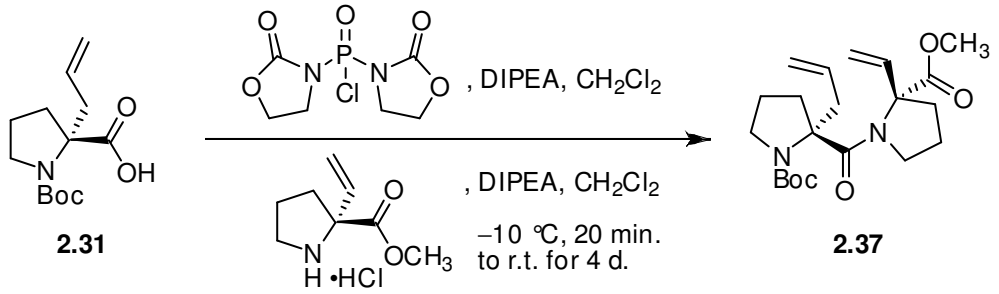
ESI HRMS *m/z* 389.2070 $[M+Na]^+$ $C_{19}H_{30}N_2O_5$ + Na requires 389.2047

Notes:

1. DMF purchased from Fisher was stored over 4 Å molecular sieves and used as it is.

2. The color of the active ester formation varies from pale yellow to fluorescent canary yellow with concentration and scale.

**(2*R*)-Methyl 1-((2'*R*)-2'-Allyl-2'-(*N''*-(*tert*-butyloxycarbonyl)-2'-pyrrolidine)carbonyl)-2-vinylpyrrolidine-2-carboxylate (2.37)**




**2.31** **2.1** 17% **2.37**

Boc-α-allylproline **2.31** (200 mg, 0.8 mmol, 1 equiv.) was dissolved in anhydrous DCM (Note 1). The solution was cooled to –10 °C using an ice/salt bath. This was followed by the sequential addition of α-vinylproline methyl ester hydrochloride **2.1** (230 mg, 1.2 mmol, 1.5 equiv.), BOP-Cl (306 mg, 1.2 mmol, 1.5 equiv.). The mild suspension was stirred for 10 min. after which DIPEA (0.5 mL, 2.8 mmol, 3.5 equiv.) was added in one portion. The yellow-colored solution was stirred at –10 °C for another 10 min and then warmed to r.t. at which it was stirred for 4 d under Ar. After 4 d, the reaction was diluted to 30 mL and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was subjected to flash chromatography (gradient elution: 0–40% EtOAc in hexanes) to give the desired product **2.37** as a clear oil weighing 65 mg (17% yield).

TLC $R_f$ 0.52 (EtOAc/hexanes, 3:1)

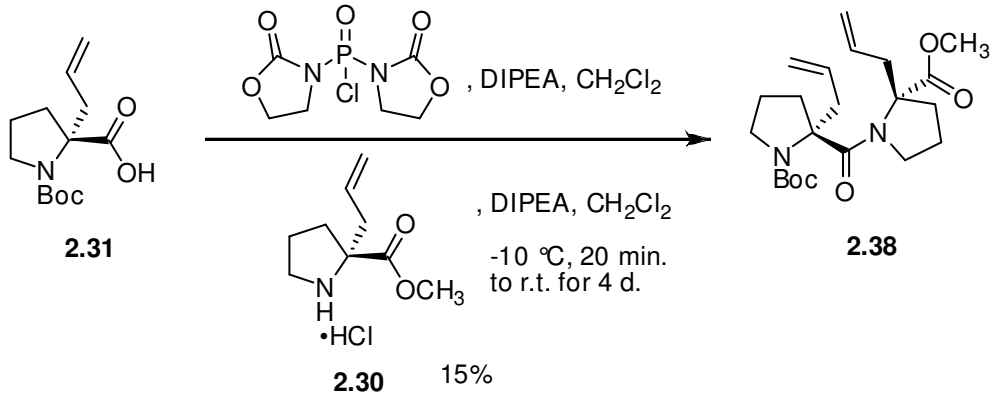
$[\alpha]_D$ + 41.2 (*c* 3.0, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$) δ 6.45–6.28 (m, 1H, vinylic C*H*=$CH_2$), 5.80–5.66 (m, 1H, allylic C*H*=$CH_2$), 5.12–4.89 (m, 4H, allylic and vinylic CH=C*H*$_2$), 3.72 (s, 3H, OC*H*$_3$), 3.64–3.42 (m, 4H, 2 x Pro δ-C*H*$_2$), 3.10–2.92 (m, 1H, allylic C*H*$_2$), 2.74–2.65 (m ,1H, allylic C*H*$_2$), 2.20–2.00 (m, 4H, β-C*H*$_2$), 2.00–1.75 (m, 4H, γ-C*H*$_2$), 1.48 and 1,44 (s, 9H, C(C*H*$_3$))

$^{13}$C NMR (100 MHz, $CDCl_3$, rotamers observed about the carbamate bond in a 7:1 ratio) δ 173.2 and 173.1 (amide *C*=O), 171.9 and 171.7 (ester *C*=O), 153.9 and 152.9 (carbamate *C*=O), 136.7 and 136.6 (vinylic *C*H=$CH_2$), 134.7 and 134.3 (allylic *C*H=$CH_2$), 118.7 and 118.3 (allylic CH=*C*H$_2$), 112.5 and 112.3 (vinylic CH=*C*H$_2$), 80.9 and 79.3 (*C*(CH$_3$)$_3$), 71.8 and 71.6 (vinylPro α-*C*), 68.9 and 68.4 (allylPro α-*C*), 52.5 and 52.3 (O*C*H$_3$), 48.5, 48.2, 48.0 (Pro δ-*C*), 40.9 and 39.6 (allylic *C*), 37.7, 35.7, 35.5 (Pro β-*C*), 28.5 and 28.2 (C(*C*H$_3$)$_3$), 24.5, 22.8, 22.1 (Pro γ-*C*)

ESI HRMS *m/z* 415.2212 [M+Na]$^+$ $C_{22}H_{34}N_2O_5$+Na requires 415.2203

**(*R*)-Methyl 2-Allyl-1-((*R*)-2'-allyl-2'-(*N''*-(*tert*-butyloxycarbonyl)-2''-pyrrolidine)carbonyl)pyrrolidine-2-carboxylate (2.38)**




Boc-α-allylproline **2.31** (200 mg, 0.8 mmol, 1 equiv.) was dissolved in anhydrous DCM (Note 1). The solution was cooled to –10 °C using an ice/salt bath. This was

followed by the sequential addition of α-allylproline methyl ester hydrochloride **2.30** (247 mg, 1.2 mmol, 1.5 equiv.), BOP-Cl (306 mg, 1.2 mmol, 1.5 equiv.). The mild suspension was stirred for 10 min. after which DIPEA (0.5 mL, 2.8 mmol, 3.5 equiv.) was added in one portion. The yellow-colored solution was stirred at –10 °C for another 10 min and then warmed to r.t. at which it was stirred for 4 d under Ar. After 4 d, the reaction was diluted to 30 mL and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was subjected to flash chromatography (gradient elution: 0–40% EtOAc in hexanes) to give the desired product **2.38** as a clear oil weighing 60 mg (15% yield).

TLC $R_f$ 0.59 (EtOAc/hexanes, 2:1)

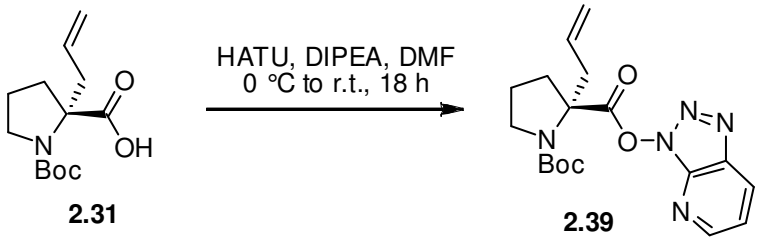
$[\alpha]_D$ +14.4 (*c* 1.0, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$) δ 5.80–5.62 (m, 2H, 2 x C*H*=$CH_2$), 5.18–5.00 (m, 4H, 2 x CH=C*H*$_2$), 3.70 (s, 3H, $OCH_3$), 3.68–3.37 (m, 4H, 2 x δ-C*H*$_2$), 3.20–2.95 (m, 2H, allylic C*H*$_2$), 2.73–2.54 (m, 2H, allylic C*H*$_2$), 2.16–1.70 (m, 8H, 2 x β-C*H*$_2$, 2 x γ-C*H*$_2$), 1.47 and 1.45 (s, 9H, C(C*H*$_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.3 (amide *C*=O), 172.1 (ester *C*=O), 154.1 (NH-(*C*=O)-C($CH_3$)$_3$), 134.4, 133.8 (allylic *C*H=$CH_2$), 119.0, 118.6 (allylic CH=*C*H$_2$), 80.8 (*C*($CH_3$)$_3$), 70.1, 68.7 (Pro α-*C*), 52.4 (O*C*H$_3$), 48.5, 48.4 (Pro δ-*C*), 40.8, 37.8 (allylic *C*H$_2$), 37.5, 34.3 (Pro β-*C*), 28.5, 28.3 (C(*C*H$_3$)$_3$), 25.0, 22.1 (Pro γ-*C*H$_2$)

ESI HRMS *m/z* 429.2364 [M+Na]$^+$ $C_{22}H_{34}N_2O_5$+Na requires 429.2360

**7''-Aza-benzotriazolyl (2*R*)-*N-tert*-Butyloxycarbonyl-2-(2'-propenyl)pyrrolidine carboxylate (2.39)**




**2.31** **2.39**

A solution of Boc-α-(*R*)-allylproline **2.31** (383 mg, 1.5 mmol, 1 equiv.) in 4 mL anhydrous DMF (Note 1) was cooled to 0°C. DIPEA (0.7 mL, 3.7 mmol, 2.5 equiv.) was added drop-wise followed by HATU (570 mg, 1.5 mmol, 1 equiv.). The pale yellow-colored solution was stirred under Ar for 18 h while the solution was warmed to ambient temperature. The solvent and other volatiles were removed by rotary evaporation (water bath < 50 °C). The residue was dissolved in EtOAc (50 mL) and washed sequentially with 10% citric acid and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The yellow residue was purified by flash chromatography (gradient elution: 0–50 % EtOAc/hexanes). The product **2.39** was obtained as a clear, colorless oil weighing 514 mg (92% yield). The compound was stored under Ar at –20 °C. It appears to hydrolyze under ambient conditions over a 4–5 day period.

TLC $R_f$ 0.62 (EtOAc/hexanes, 3:1)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY, rotamers observed about the carbamate bond in a 3:1 ratio) δ 8.61–8.60 (m, 1H, 6''-C*H*), 8.33–8.28 (m, 1H, 4''-C*H*), 7.36–7.29 (m, 1H, 5''-C*H*), 5.77–5.67 (m, 1H, C*H*=$CH_2$), 5.17–5.11 (m, 2H, CH=C$H_2$), 3.77–3.74 (m, 0.7H, Pro δ-C$H_2$), 3.69–3.63 (m, 0.3H, Pro δ-C$H_2$), 3.47–3.37 (m, 1H, Pro δ-C$H_2$), 3.25 (dd, $J_1$ = 14 Hz, $J_2$ = 6.7 Hz, 0.3H, C$H_2$CH=$CH_2$), 3.07 (dd, $J_1$ = 14.1 Hz, $J_2$ = 6.6 Hz, 0.7H, C$H_2$CH=$CH_2$), 2.73–2.53 (m, 2H, C$H_2$CH=$CH_2$, Pro β-C$H_2$), 2.39–2.26 (m, 1H, Pro β-

C*H*$_2$), 2.16–2.05 (m, 1H, Pro γ-C*H*$_2$), 1.97–1.89 (m, 1H, Pro γ-C*H*$_2$), 1.50 and 1.45 (s, 9H, C(C*H*$_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$. gHMQC, rotamers observed about the carbamate bond in a 3:1 ratio for most peaks) δ 170.6 and 170.3 (ester *C*=O), 153.9 and 153.0 (carbamate *C*=O), 151.7 and 151.6 (6"-*C*), 140.7 and 140.6 (aromatic $C_q$), 134.9 (aromatic $C_q$), 132.0 and 131.6 (*C*H=$CH_2$), 129.4 and 129.3 (4"-*C*), 120.9 and 120.7 (5"-*C*), 120.5 and 120.1 (CH=*C*$H_2$), 82.2 and 80.8 (*C*($CH_3$)$_3$), 67.0 and 66.3 (Pro α-*C*), 48.5 and 48.4 (Pro δ-*C*), 39.6 and 38.4 (*C*$H_2$CH=$CH_2$), 37.6 and 35.9 (Pro β-*C*), 28.5 and 28.1 (C(*C*$H_3$)$_3$), 23.7 and 22.9 (Pro γ-*C*)

ESI HRMS *m/z* 396.1649 $[M+Na]^+$ $C_{18}H_{23}N_5O_4$+Na requires 396.1642

Notes:

1. DMF was stored over 4Å molecular sieves and used without further treatment.

# Chapter 3

*Rationale for the Design of Constrained Peptidomimetics Targeting the X-Linked Inhibitor of Apoptosis Protein (XIAP) for Cancer Chemotherapy*

## 3.1. Introduction to apoptosis

*Apoptosis* is the term used to describe the process of "programmed cellular death", a physiologically natural process that occurs in an evolutionarily conserved manner in all multi-cellular organisms.[65] Derived from the Greek language, apoptosis translates to the "dropping off" of "petals or leaves from plants or trees". Prof. James Cormack of the department of Greek language at the University of Aberdeen is credited as first using the term to describe the process.[66] Apoptosis is characterized by biochemical signal transduction processes that lead among others to the following distinct cellular morphological changes: (1) blebbing of the plasma membrane, (2) shrinkage of the cell, (3) cell membrane changes such as loss of cell asymmetry and attachment, (4) chromatin hypercondensation, (5) chromosomal DNA cleavage/fragmentation, and (6) packaging of cellular contents into membrane-bound vesicles. It is important to note that apoptosis differs from necrosis since premature tissue death involved in the latter is usually a result of external causes such as infection, toxins or trauma and therefore is detrimental to the organism.

Although now well established as an essential part of maintaining homeostasis, the delicate balance between cell proliferation and cell death, and survival of multi-cellular organisms; the significance of apoptosis in cell growth and differentiation was elucidated only in the 1990's.[66–70] In humans, it has been delineated that dysfunction in apoptosis kinetics can lead to a number of disease conditions. Excessive up-regulation of apoptosis, leading to uncontrolled cellular death is implicated in the development of many auto-immune disease conditions and in neurodegenerative conditions such as

Alzheimer's disease. The converse scenario, the abnormal down-regulation of apoptosis, has been strongly implicated in development of several human cancers, mainly caused by uncontrolled cellular proliferation.[71–74]

### 3.2.a Mechanism of apoptosis

Apoptosis is brought about by a series of signal transduction events facilitated by a family of cysteine proteases termed as *caspases*, which proteolytically cleave their substrate after a conserved aspartate residue on the target protein/peptide sequence.[75] As shown in Figure 3.1., depending on the source of the death stimuli, the apoptotic response is mediated either by (1) the *intrinsic* pathway, initiated by the mitochondria or (2) the *extrinsic* pathway, initiated by activation of cell-surface receptors called the "death receptors". In the first pathway, intrinsic death stimuli[66] such as activation of tumor suppressor proteins (e.g. p53), oncogenes (e.g. c-Myc) or DNA damage lead to the release of proteins such as cytochrome *c* from the mitochondria which in turn activates caspase-9, an initiator caspase, in the context of the apoptosome complex. Formation of this complex triggers a downstream cascade of caspase activation, subsequently causing cell death. In the extrinsic pathway, specific extracellular ligands, such as FasL, bind to their cell-surface death receptors causing them to oligomerize resulting in the formation of the death-inducing signaling complex (DISC). This process eventually leads to the activation of caspase-8 by autocatalytic cleavage of procaspase-8. In a process analogous to that in the intrinsic pathway, this activates the further downstream caspases and leads to cell death. The two pathways merge at the stage involving mature caspase-3. Generally, dysfunction/inactivation of the intrinsic apoptotic pathway is regarded as a hallmark of cancer.[76] Reining in control over regulation of

apoptosis therefore has important ramifications in the chemotherapy of several malignant diseases.

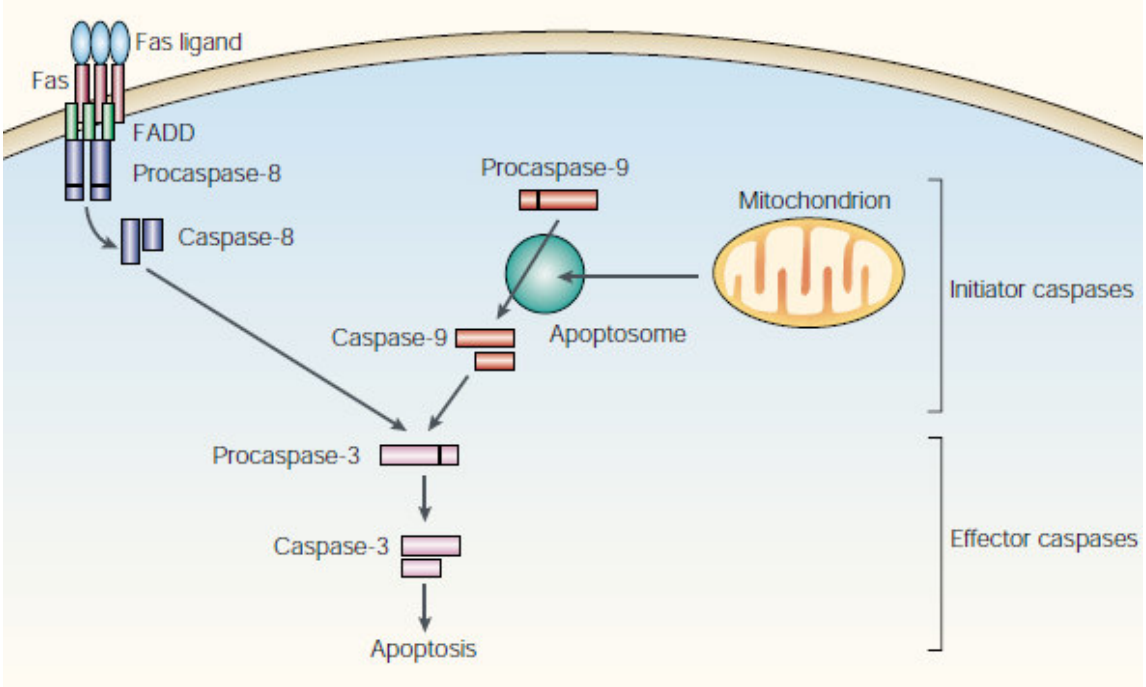



**Figure. 3.1.**[81] Overall signal transduction processes in apoptosis
Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Molecular Cellular Biology] (Holcik, M.; Korneluk, R. G. XIAP, The Guardian Angel. *Nature Rev. Mol. Cell. Biol.* **2001,** *2*, 550–556), copyright (2001)

### 3.2.b Regulation of apoptosis: Structural and biochemical basis of XIAP-BIR3 domain and caspase 9 interaction

Although caspases are subject to transcriptional regulation and post-translational modifications,[77] a direct and unique natural mechanism avoids inadvertent destruction of healthy cells caused by caspase activation. The enzymatic activity of both the initiator (caspases 8, 9 etc.) and effector (caspases 3, 7 etc.) caspases is inhibited by the

Inhibitor of Apoptosis Protein (IAP) protein family.[78–80] Since the IAPs directly bind to and inhibit the caspases, there are capable of inhibiting both apoptotic pathways.[81]

In humans, there are eight sub-types of IAPs. X-linked inhibitor of apoptosis protein (XIAP), is the most well-characterized member of the IAPs. Being a multi-modular protein, XIAP contains several baculoviral inhibitor of apoptosis repeat (BIR) domains, each of which has a distinct function. As depicted in Figure 3.2., the third domain (BIR3) potently binds and inhibits the initiator caspase-9, while the linker region between the BIR2 and BIR2 domains inhibits effector caspases 3 and 7 with lesser affinity. As per *in vitro* kinetic studies, XIAP is the most potent caspase inhibitor in the IAP family and has a $K_i$ = 0.2–0.8 nM.[78]

Based on X-ray crystal structure of caspase-9 bound to XIAP[82] as well as mutational analysis,[83] three residues on XIAP-BIR3 domain are indispensible to caspase-9 inhibition: Trp 310, Glu 314 and His 343. The first two residues line a conserved groove on the XIAP binding site which interacts with the *N*-terminal residue of caspase-9 (Ala). His 343 on the other hand, lies in the opposite side of the BIR3 domain. A His→Ala mutation did not affect binding but abrogated caspase-9 inhibition. This leads to the conclusion that binding is only a necessary but not sufficient event for caspase inactivation. It was later determined that XIAP inhibits caspase-9 by preventing its conversion from a monomeric to a homodimeric form which was required for its enzymatic activity.[82]

It is important to note the following point at this time. Although it has been demonstrated, that small molecule inhibitors of XIAP bind it with higher affinity when different portions of the inhibitor concurrently interact with both the BIR2 and BIR3 domains, most of the following discussion will concentrate on the BIR3 domain since that appears necessary and sufficient to inhibit XIAP activity.

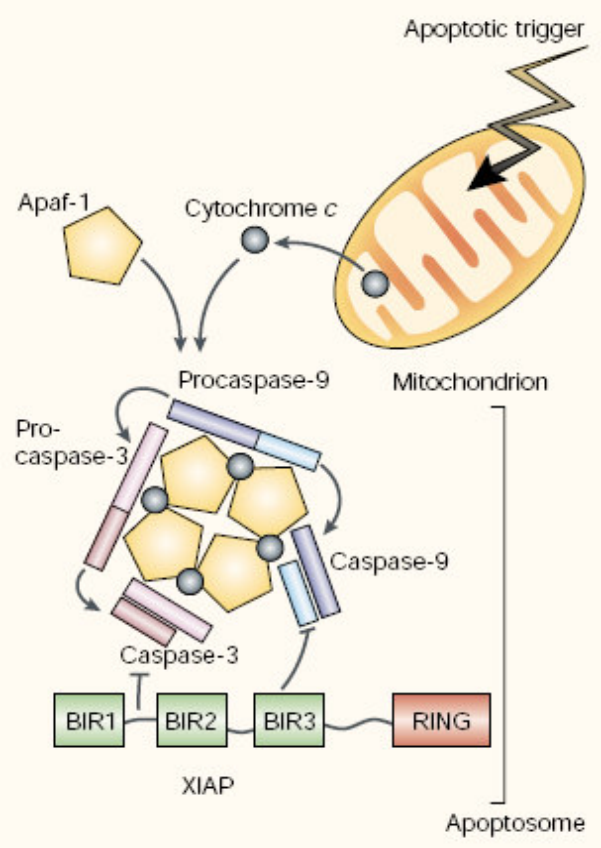



**Figure 3.2.**[81] Inhibition of caspases by XIAP BIR domains within the apoptosome
Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Molecular Cellular Biology] (Holcik, M.; Korneluk, R. G. XIAP, The Guardian Angel. *Nature Rev. Mol. Cell. Biol.* **2001,** *2*, 550–556), copyright (2001)

### 3.3. Validating XIAP as a potential therapeutic target

Based on the following experimental evidence, XIAP has been validated as a target for developing cancer chemotherapeutic agents with a novel mode of action.[84]

1. *In vitro* studies using cultured cells indicate over-expression of XIAP in human cancer cell lines and tumor tissues.
2. The same studies also indicate that this over-expression of XIAP in tumor cells confers resistance to combination drug chemotherapy.

3. Knocking down XIAP in several malignant cell lines using small interfering ribonucleotides (siRNA) and antisense nucleotides restores the sensitivity of the cell lines toward chemotherapy.
4. XIAP knock-outs do not appear toxic to normal cells as evidenced within the XIAP knockout mouse.
5. Inhibition of XIAP potentiates caspase activation leading to apoptosis.

### 3.4. Endogenous XIAP inhibitors as a proof of concept for development of therapeutic agents

During apoptosis, a family of pro-apoptotic proteins antagonizes the XIAP-mediated inhibition of the caspases.[85] The second mitochondria-derived activator of caspases or direct IAP-binding protein with low pI (Smac or DIABLO) is a prominent member of this family and it negatively regulates the caspase-inhibiting activity of the IAPs.[86, 87] That is, they promote apoptosis by inhibiting the inhibitors of apoptosis (Figure 3.3).

Full length Smac protein contains 239 amino acids, with the *N*-terminal 55 residues coding for the mitochondria-targeting sequence that is proteolyzed upon import. Mature Smac therefore has 184 amino acids with a very important *N*-terminal tetrapeptide, Ala-Val-Pro-Ile (AVPI) which serves as the IAP tetrapeptide binding motif similar to the *N*-terminal tetrapeptide, ATPF, in caspase-9.[66] Mature Smac is released from the mitochondria along with cytochrome *c* within the intrinsic apoptotic pathway. Based on high resolution structural evidence provided by NMR[88] and X-ray analyses,[89,90] AVPI, has been shown to interact with the same conserved surface groove in the XIAP-BIR3 domain to which caspase-9 binds to; and therefore competes[91] for the *identical* binding pocket as caspase-9. These studies conclude that the competition between

Smac and caspase-9 for the same binding site on XIAP releases bound caspase-9 and promotes apoptosis.

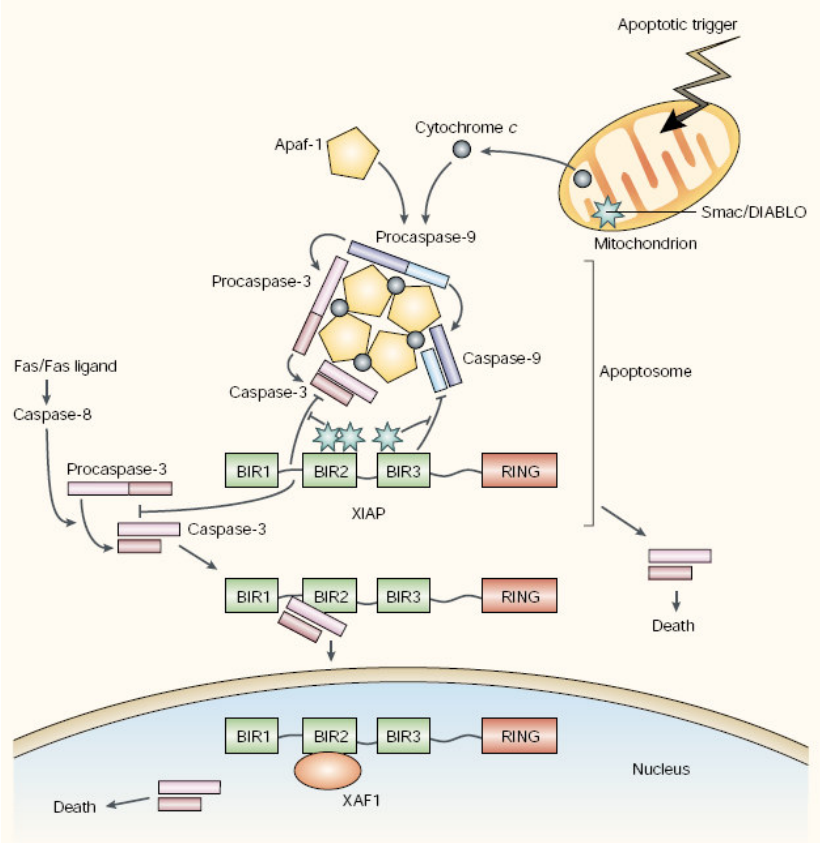



**Figure 3.3**.[81] Inhibition of caspases and promotion of apoptosis by Smac
Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Molecular Cellular Biology] (Holcik, M.; Korneluk, R. G. XIAP, The Guardian Angel. *Nature Rev. Mol. Cell. Biol.* **2001,** *2*, 550–556), copyright (2001)

It has been shown that short peptide sequences conserving the *N*-terminal AVPI tetrapeptide are capable of binding to and inhibiting XIAP in a competitive manner with respect to Smac and caspase-9 in both *in vitro* and *in vivo* studies.[92–94] Although these short peptides were highly active *in vitro*, the diminution of *in vivo* activity was surprising. It was hypothesized that cell-permeability was a major hurdle. When tethered to carrier

peptides these relatively cell-permeable Smac-based mimics increased the sensitivity of tumor cells *in vitro*[92] and *in vivo*[93] to the anti-tumor activity of the Apo-2L/TNF-related apoptosis-inducing ligand (TRAIL) and reduced the resistance of these cells to chemotherapeutic agents such as paclitaxel, etoposide, doxorubicin, and cisplatin. When injected directly into the tumor in conjunction with cisplatin, one of these peptides was capable of regressing tumor growth *in vivo* in H460 non small-cell lung cancer xenografts with little toxicity to mice.[94]

### 3.5. Binding conformation of Smac

It is important to note that developing inhibitors of XIAP based on Smac essentially involves disrupting protein-protein interaction, which unlike typical enzyme-small molecule substrate interaction, can span large areas that can be flat lacking specific binding sites. It is therefore a formidable task to develop *small-molecule* inhibitors of XIAP. It is indeed fortuitous that peptides resembling the short *N*-terminal tetrapeptide of Smac, AVPI, alone can bind to the Smac/caspase-9 shallow binding site. As shown in Figure 3.4,[91] the tetrapeptide, AVPI binds in a conserved surface groove within the BIR3 domain of XIAP whilst making a network of hydrogen bonding interactions and a series of Van der Waals contacts. The first residue, Ala, binds to a conserved pocket formed in part by Trp 310 and Leu 307 in XIAP. The free $NH_2$ forms a pair of charge-stabilized hydrogen bonds with Glu 314. Ala is essential and contributes the largest to the specific recognition of Smac to XIAP; mutation to another amino acid completely abrogates this affinity.[90] The remaining three residues; Val, Pro, and Ile are all hydrophobic and interact extensively with hydrophobic residues lining the BIR3 domain groove (Trp 323, Tyr 324, Gly 306). These residues can tolerate variation but for

optimal binding affinity, Pro is preferred in the third position and a bulky hydrophobic residue in the fourth position.

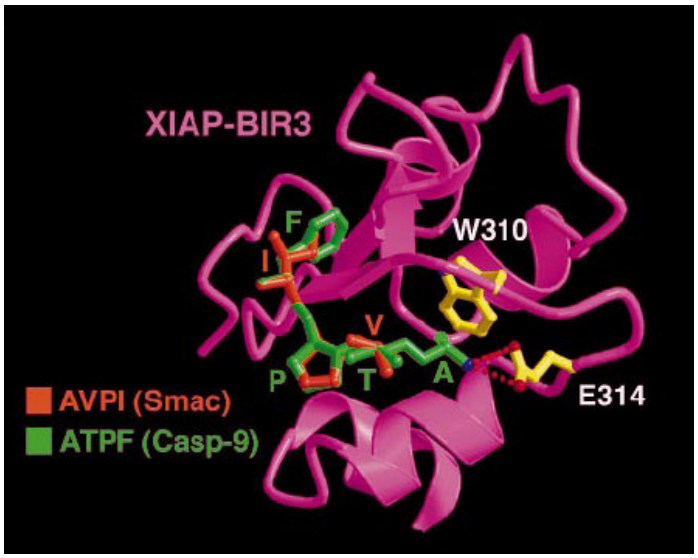



**Figure 3.4.**[91] X-ray structure overlaps of Smac and caspase-9 with XIAP BIR3
Reprinted by permission from Macmillan Publishers Ltd: [Nature] (Srinivasula, S. M.; Hegde, R.; Saleh, A.; Datta, P.; Shiozaki, E.; Chai, J.; Lee, R.-A.; Robbins, P. D.; Fernandes-Alnemri, T.; Shi, Y.; Alnemri, E.S. A Conserved XIAP Interaction Motif in Caspase-9 and Smac/Diablo Regulates Caspase Activity and Apoptosis. *Nature* **2001,** 410, 112–116.), copyright (2001)

### 3.6. SAR of the Smac-based tetrapeptide, AVPI

A graphical representation of detailed structure-activity relationship (SAR) studies that have been conducted on the tetrapeptide is shown in Figure 3.5.[95, 96] The parent tetrapeptide, AVPI (**3.1**), binds to XIAP BIR3 domain with a $K_d$ value of 480 nM. The following changes either increase of decrease this affinity of the modified peptide for its receptor site. The absolute requirement of Ala containing a free amine at the *N*-terminal paralleled the mutation studies that were conducted previously.[88, 90] Val can be replaced by other amino acid side chains; in fact Kipp *et al.* recently determined that arginine (Arg) can make optimal interactions with the binding pocket.[96] The pyrrolidine side chain

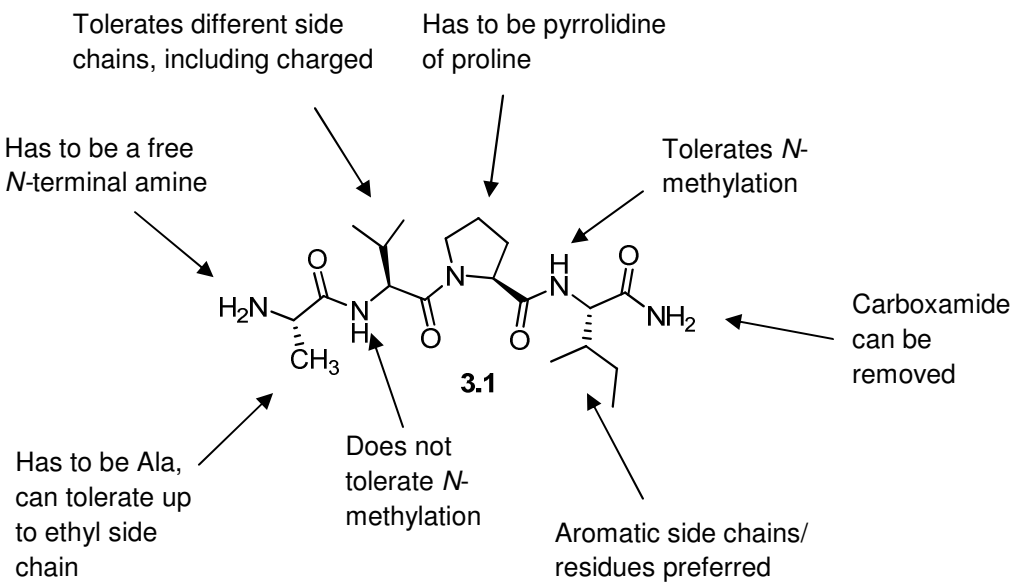


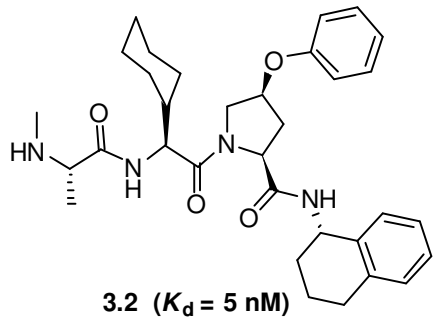

**Figure 3.5**. SAR studies conducted on the minimal tetrapeptide requirements

of Pro is essential in maintaining the extended conformation (β-strand) of the tetrapeptide in the active site. Due to a high number of aromatic hydrophobic residues lining the BIR3 domain groove, Ile in the natural substrate can be readily substituted with bulky, hydrophobic residues to achieve increased affinity. Additionally, *N*-alkylation of the Ala-Val amide is not tolerated while the Pro-Ile amide can be substituted/alkylated.

### 3.7. Recent developments in small molecule Smac mimetics

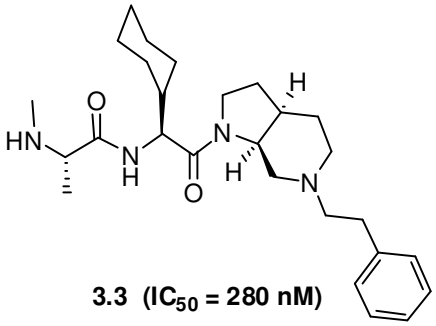
Over the last five years, there has been a steady disclosure of Smac-based small molecule inhibitors of XIAP in the scientific literature.[95,97] Abbott Laboratories were the first to disclose potent Smac-based peptides (Figure 3.6., **3.2**) resulting from extensive modification of the parent tetrapeptide.[98] In terms of absolute requirement for biological activity, the results of this study were consistent with that of McLenden's SAR analysis,

discussed in the previous section.[84, 96] In addition to Abbott, both Novartis[99] and Genentech[100] have developed conformationally constrained peptidomimetics, **3.3** and **3.5** respectively based on Smac that are potent both *in vitro* and *in vivo*.



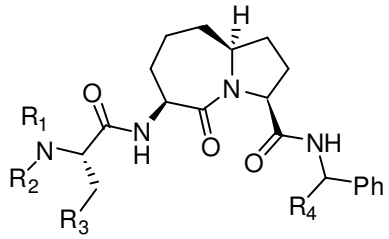
**3.2 ($K_d$ = 5 nM)**

Abbott Laboratories



**3.3 ($IC_{50}$ = 280 nM)**

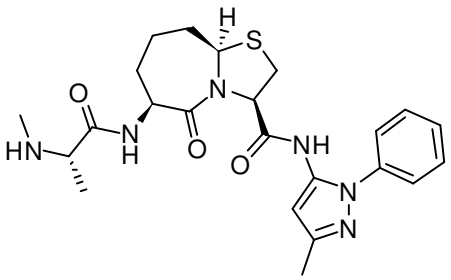
Novartis



**3.4 a-d**

**a: $R_1$ =$R_2$ = $R_3$ =$R_4$ = H ($K_i$ = 150 nM)**
**b: $R_1$ =$R_2$ = H ;$R_3$ = $CH_3$; $R_4$ = Ph ($K_i$ = 25 nM)**
**c: $R_1$ = H; $R_2$ = Me ;$R_3$ = Me; $R_4$ = Ph ($K_i$ = 61 nM)**
**c: $R_1$ =$R_2$ = Me ;$R_3$ = Me; $R_4$ = Ph ($K_i$ = 14 μM)**

Shaomeng Wang Lab.,
Univ. of Michigan



**3.5 ($K_i$ = 270 nM)**

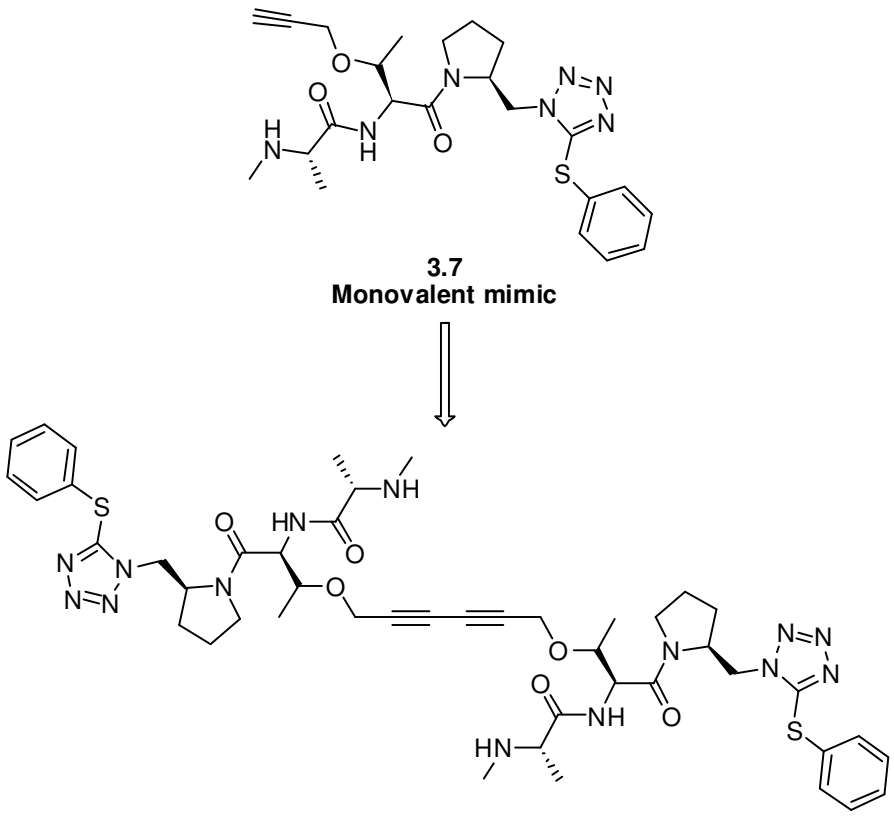
Genentech

**Figure 3.6.** Representative Smac mimetics and XIAP inhibitors

Shaomeng Wang and his research group at the University of Michigan are at the forefront of developing Smac-based constrained XIAP inhibitors.[101–103] They were the first to report the design, synthesis and biological evaluation of the conformationally rigid azabicyclo[5.3.0]alkanones (Figure 3.6., **3.4**) as Smac mimetics.[102] These bicyclics were

developed by rational design aided by competent computational analyses based on existing Smac/XIAP crystal structures. Consistent with existing SAR, molecules containing the free *N*-terminal amine, **3.4a** and **3.4b**, were highly potent; however they were virtually inactive in *in vivo* assays.[103] With the hypothesis that the free amine was detrimental to cell permeability and metabolic stability of the mimics, the corresponding *N*-alkylated molecules, **3.4c** and **3.4d** were synthesized and evaluated. Although the binding affinity was either the same or slightly lowered, the cellular activity of these molecules was approximately 500 times higher than that of the unalkylated amines.[101]

It has been recently demonstrated that using Smac mimic dimers (Figure 3.7)



**3.7**
**Monovalent mimic**

**3.8**
**Bivalent mimic ($K_i$ = 0.7 nM)**

**Figure 3.7**. Representative bivalent Smac mimetic and XIAP inhibitor

capable of simultaneously binding to both the BIR2 and BIR3 domains of XIAP results in inhibitors with higher affinity and biological activity.[104, 105] These molecules are termed as the bivalent Smac mimetics. One of the most potent is shown in Figure 3.7.[104]

### 3.8. Rationale for developing Smac mimetics based on the bicyclic thiazolidine scaffold (BTD)

In keeping with the main research aim in our laboratory, we hoped to design and synthesize small-molecule mimics of AVPI that were capable of adopting the bioactive conformation of the Smac tetrapeptide at the XIAP-BIR3 domain binding site. Several conformationally constrained molecules were designed, out of which the bicycle thiazolidines (BTD, shown in Figure 3.8), were the most constrained since both $\psi_2$ and $\varphi_3$ torsion angles were simultaneously restricted.

The diastereoisomeric BTDs are a versatile peptidomimetic scaffold. We have previously utilized them in our laboratory as moderately constrained external type II *β*-turn mimics of the dopamine modulator, Pro-Leu-Gly-$NH_2$ (PLG).[106, 107] In order to maintain the extended conformation of AVPI as observed within the X-ray crystal

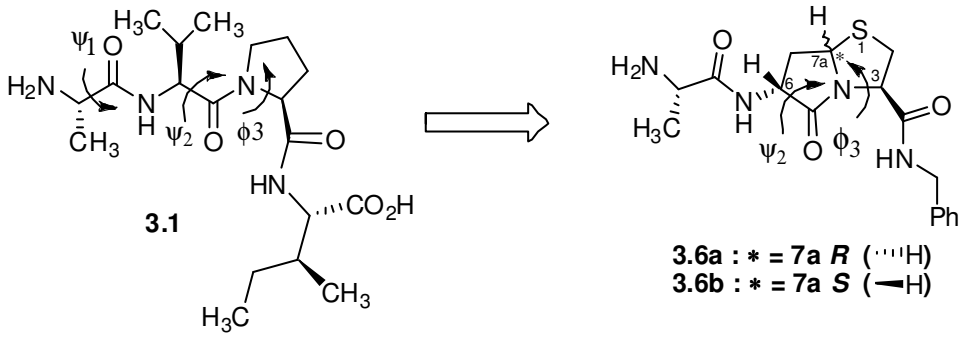



**Figure 3.8** Constraining AVPI into the BTD peptidomimetic

structure of the Smac/XIAP complex, the stereochemistry about the C-3 and the C-6 is maintained in a *trans* manner as opposed to the *cis* relative configuration held in order to mimic the *β*-turn conformation. The constrained molecules also incorporate the requisite functionalities to hopefully maintain potent binding affinity at the XIAP-BIR3 domain site. Alanine is maintained as per SAR requirements. The lactam ring partially occupies the space occupied by the isopropyl (iPr) side chain of Val. The thiazolidine ring has two functions; the thiazolidine ring can mimic to a certain extent the pyrrolidine ring of Pro (pseudoproline)[108] and it is anticipated that the d-orbital of the sulfur atom can engage in fruitful d-π interactions with the π-cloud of the numerous aromatic residues lining the BIR3 active site. As suggested by the previous SAR studies, the carboxyl terminal can be avoided and the Ile side chain is replaced by an aromatic hydrophobic residue.

The BTDs appear structurally similar to the azabicyclo[x.y.0]alkanes designed by the Wang group with the exception of opposite relative stereochemistry at the terminal extension points of the bicyclic ring system. It is anticipated that analogous to the non-sulfur containing bicyclics, the diastereomer with *R* stereochemistry at the bridgehead (C-7a) position will be more bioactive than the diastereomer with the *S* stereochemistry at C-7a. The latter can possibly act as a negative control.

# CHAPTER 4

*Synthesis of the conformationally constrained bicyclic thiazolidine (BTD) AVPI mimics for XIAP inhibiton*

## 4.1. Introduction

This chapter will encompass the discussion regarding the challenges involved in the synthetic manipulations of the bicyclic thiazolidine (BTD) scaffold and is divided into the following sub-sections:

1. Retrosynthetic analysis for the diastereomeric target molecules
2. Various synthetic attempts toward synthesis of the key intermediate, (*R*)-α-allylglycine.
3. Physico-chemical properties of the two diastereomers following lactamization
4. Reactions toward synthesis of the major diastereomer **4.1a**
5. Reactions toward synthesis of the minor diastereomer **4.1b**

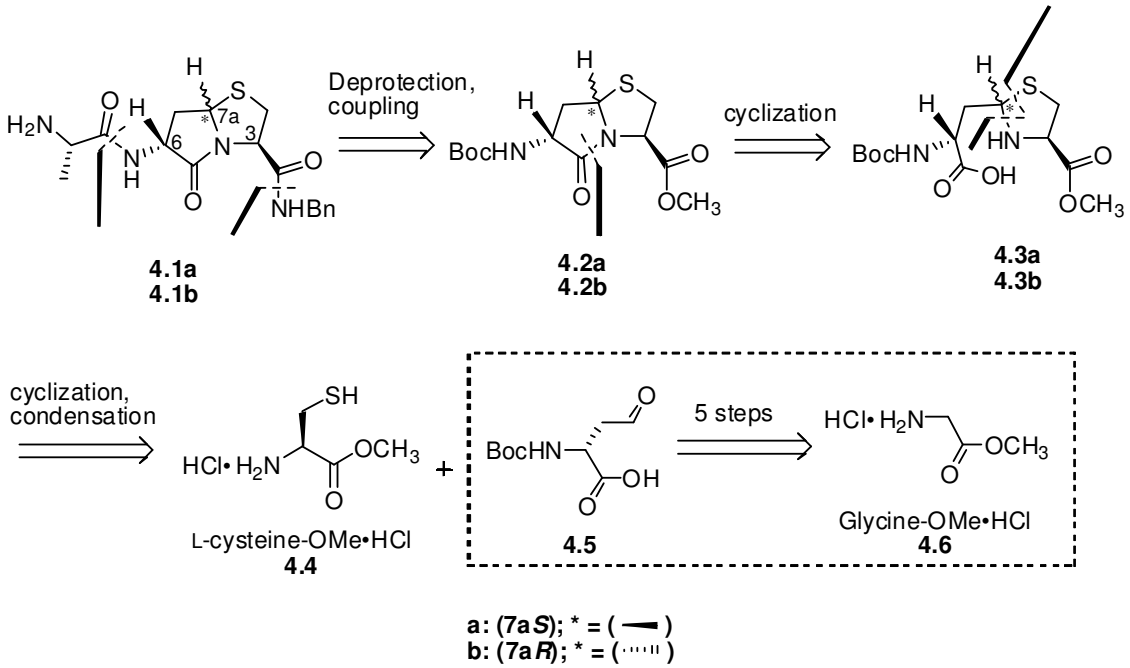
## 4.2. Retrosynthetic analysis

The bicyclic thiazolidine, **4.1a**, derived from L-cysteine methyl ester was first synthesized by Baldwin *et al.*[109] The molecule reported in the publication was derived from the L-aspartic acid semi-aldehyde derivative and therefore had opposite stereochemistry (6*S*) at the C-6 position compared to our desired final products (*Note: The original atom numbering scheme by Baldwin did not number the sulfur atom and therefore C-6 was actually C-5*). We had previously reported synthesis of the BTD with the (3*S*,6*R*,7a*R*) stereochemistry as a result of thermodynamic bias.[110] However, in the present scenario, in order to obtain both epimers at C-7a, a modified kinetic methodology developed first by Mukaiyama *et al.* toward the synthesis of β-lactams[111]

and used previously in our laboratory toward the synthesis of (5,5)- and (6,5)-bicyclic thiazolidines,[110, 112] was used to effect the lactamization reaction.

The initial retrosynthetic disconnections (Scheme 4.1) are about the linear amide bonds, which can be constructed using solution-phase peptide coupling conditions. This strips down the molecules to the main BTD scaffold. It is anticipated that the central

**Scheme 4.1**. Retrosynthetic analysis of target molecules



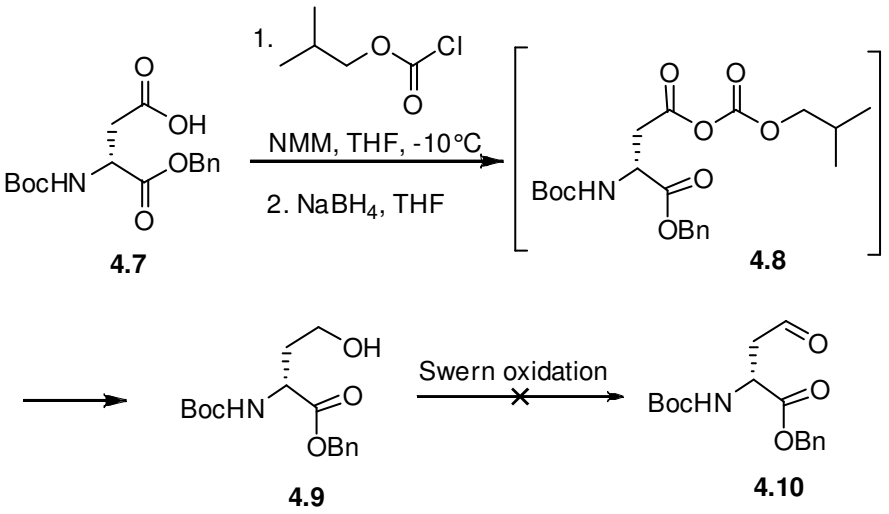

pyrrolo-thiazolidine core of **4.2a** and **4.2b** can be derived from a condensation-lactamization sequence on Boc-(*R*)-aspartic acid semi aldehyde **4.5** derived from (*R*)-α-allylglycine and an ester of commercially available L-cysteine•HCl. Although the condensation that generates the *N,S*-thioaminal center is nonstereoselective, there is precedent for the product BTD diastereomers **4.2a** and **4.2b** being separable by chromatography.[112] Enantiopure (*R*)-α-allylglycine can be obtained from various approaches; one such method is the Myers' diastereoselective alkylation of (*S,S*)-

pseudoephedrine glycinamide, which itself can be obtained from cheap commercially available starting materials, glycine methyl ester•HCl and (*S*,*S*)-(+)-pseudoephedrine.[113–116]

### 4.3. Synthesis of (*R*)-α-allylglycine and (*R*)-α-aspartic semi aldehyde

The initial route toward the synthesis of the semi aldehyde derivative involved manipulating the oxidation state of the side chain of commercially available *N*-Boc-(*R*)-aspartic acid α-benzyl ester (**4.7**, Scheme 4.2.).

**Scheme 4.2.** Attempts at synthesis of (*R*)-aspartic acid semi aldehyde

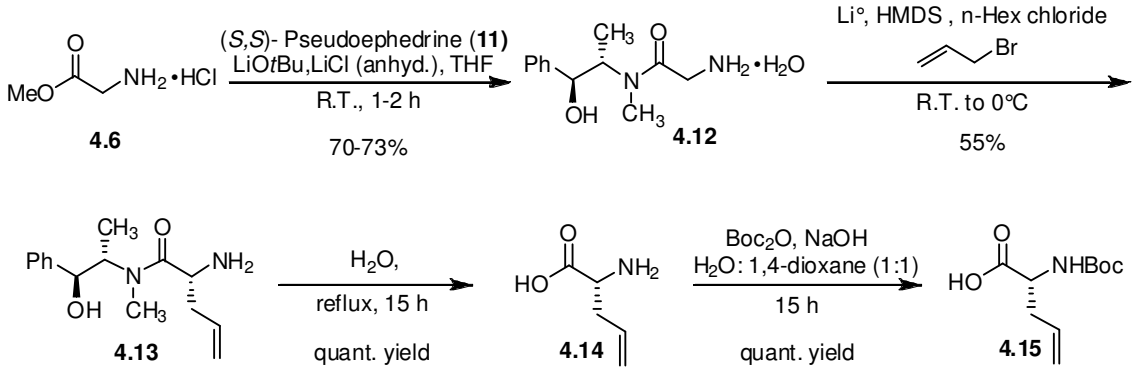



Sodium borohydride reduction of the side chain active ester **4.8** derived from reaction of the carboxylic acid with isobutyl chloroformate (IBCF) gave rise to the corresponding side chain alcohol **4.9** in 65% yield.[117] Swern oxidation to the corresponding aldehyde **4.10** failed in my hands. This route was therefore abandoned in favor of the following route.

The successful route (Scheme 4.3.) toward the synthesis of the semi aldehyde **4.5** hinges on the potential of converting the terminal olefin of an appropriately protected (*R*)-α-allylglycine derivative to the corresponding aldehyde by oxidative cleavage. The methodology, which was first reported in 1978 by Larcheveque *et al.*[118] and further developed by Myers *et al.*,[114] involves diastereoselective alkylation of the dianion enolate derived from ephedrine/pseudoephedrine glycinamide. The glycinamide **4.12** is derived by coupling glycine methyl ester hydrochloride **4.6** with (*S*,*S*)-(+)-pseudoephedrine **4.11**, where the latter acts as a chiral auxiliary. Although pseudoephedrine is used in stoichiometric amounts, the process has the following advantages: (1) Both enantiomeric forms of pseudoephedrine are cheaply available in bulk quantities. (2) The reaction is highly diastereoselective (>19:1 d.e. for the reaction with reactive electrophiles such as allyl bromide). (3) Reaction can be performed without need for *N*-protection for the glycyl unit. (4) Presence of the pseudoephedrine moiety confers high degree of crystallinity to the amides and therefore the latter are easily purified. (5) There is no attenuation of stereochemical integrity during the auxiliary hydrolysis step. (6) The chiral auxiliary is 100% recoverable and can be re-used.[116]

As shown in Scheme 4.3., glycine was first coupled to (*S,S*)-pseudoephedrine in the presence of approximately 1.8 equivalents of lithium *tert*-butoxide (LiO*t*Bu) and catalytic amounts of anhydrous lithium chloride (LiCl) in tetrahydrofuran (THF) as solvent at ambient temperature. One equivalent of the base neutralizes the hydrochloride salt, which liberates the free amine and simultaneously forms an equivalent of LiCl *in situ.* The remaining LiO*t*Bu deprotonates the benzylic alcohol in pseudoephedrine to form the lithium alkoxide. The reaction is presumed to proceed by transesterification of

**Scheme 4.3.** Myers' methodology for (*R*)-α-allylglycine




glycine methyl ester with this alcohol followed by an *O* ➔ *N* acyl transfer to form the desired product.[113, 119] Since lithium methoxide (LiOMe) is constantly generated during the acylation, less than one equivalent of base is sufficient at the beginning of the reaction after accounting for hydrochloride salt neutralization. LiCl is known to play an important role in improving yields of this acyl transfer reaction by reducing nucleophilicity of the glycine and the glycinamide free amino groups and thereby reducing the rates of two main competitive processes: (1) glycine oligomerization and (2) overglycylation of the product.[113] Also, addition of LiCl in reactions conducted in THF has been shown to improve and maintain the solubility of various amino acid intermediates and peptides.[120] Although LiCl is generated *in situ*, it was found that adding LiCl externally increased the yields of this reaction up to 75%. The glycinamide is highly hygroscopic and is therefore isolated as its crystalline hydrate. The subsequent enolization and alkylation was performed using lithium hexamethyldisilazide (LHMDS) as the base and in the presence of LiCl both of which were generated *in situ.* This was achieved by first reacting lithium metal (Sigma Aldrich, lithium wire, Li°) with hexamethyldisilazane (HMDS) and *n*-hexylchloride at room temperature for 12–15 hours and then conducting enolization of

**4.12**. The alkylation was complete within 1–2 h at 0 °C and the alkylated product **4.13** was re-crystallized from toluene/ether in approximately 55% yield.

Myers *et al.* offer an interesting explanation for the observed high diastereoselectivities for this reaction (Figure 4.1).[114] The chiral auxiliary itself is devoid

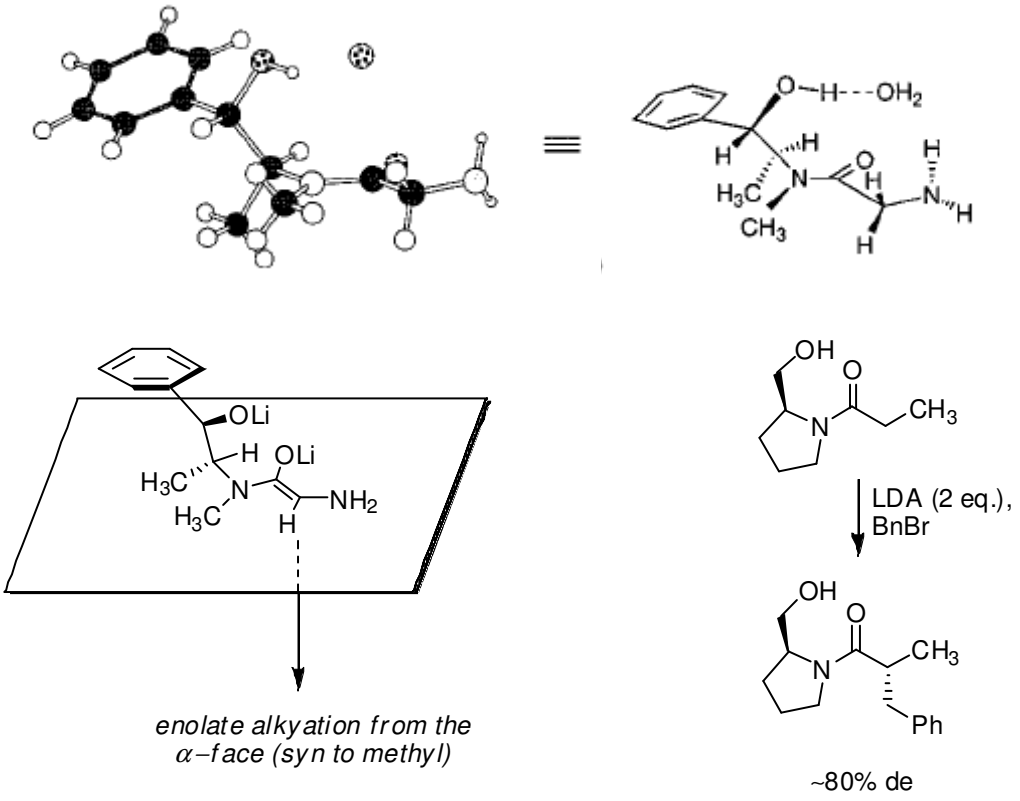


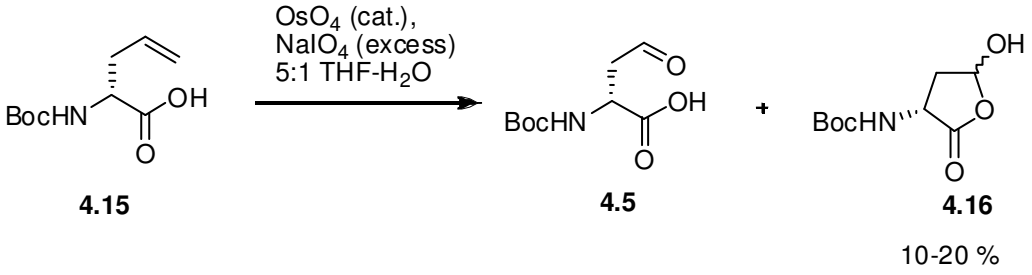

**Figure 4.1.** *Top:*[114] X-ray structure of pseudoephedrine glycinamide•$H_2O$.(Reprinted with permission from Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. Highly Practical Methodology for the Synthesis of D- and L-α-Amino Acids, N-Protected α-Amino Acids, and N-Methyl- α-Amino Acids. *J. Am. Chem. Soc.* **1997,** *119*, 656–673. Copyright 1997 American Chemical Society) *Bottom left:* Model to explain diastereoselectivity of alkylation
*Bottom right:* Diastereoselectivity with prolinol amides[123]

of any 3-dimensional facial or organizational features commonly observed in other chiral auxiliaries that are encountered in the literature. Based on the single molecule X-ray structure of pseudoephedrine glycinamide•$H_2O$ and based on existing allylic strain ($A_{1,3}$)

arguments;[121] it is predicted that the thermodynamic *Z*-enolate adopts an extended conformation as shown in the figure. It can then be seen that the secondary Li-alkoxide and the solvent molecules coordinated to the Li cation together block the β-face.[122] The alkylation therefore preferentially occurs from the α-face of the enolate. This also compares well with existing literature precedent for the alkylation distereoselectivities of other tertiary amide enolates such as those derived from prolinol amides[123] (Figure 4.1.*bottom right*).

(*R*)-α-Allylglycine **4.14** was synthesized from the glycinamide following hydrolysis under reflux. The chiral auxiliary was recovered in almost quantitative yield and can be re-used for another cycle. The alkylated glycine derivative was formed in an enantiopure fashion (no epimerization was detectable during the hydrolysis. *N*-Boc protection gave the requisite Boc-(*R*)-α-allylglycine (**4.15**) in quantitative yield. The olefin was then oxidatively cleaved using the Lemieux-Johnson protocol to give the corresponding aldehyde (Scheme 4.4).[124] The reaction was conducted in the presence of 5 mol%

**Scheme 4.4.** Oxidative cleavage of the olefin




osmium tetroxide ($OsO_4$, as a 2.5 w/w% suspension in *tert*-butanol) and excess sodium periodate ($NaIO_4$), usually in a 5:1 mixture of THF:$H_2O$. The crude product was directly used in the next reaction without further purification.

The biggest challenge involved the purification of the formed aldehyde **4.5**. The general work-up protocol that is followed for oxidations/oxidative cleavage reactions catalyzed by $OsO_4$ requires a sodium bisulfite/ sodium sulfite wash to reductively quench the excess oxidizing species. However, in this case such washes were not possible, since (1) sodium bisulfite could react with the aldehyde to form a bisulfite adduct and (2) an aqueous solution of sodium sulfite is alkaline (pH ~ 9.0). This would have lead to loss of product due to solubilization of the free carboxylic acid as the sodium salt in the aqueous layer. Therefore multiple filtration operations were conducted to ensure the complete removal of any insoluble osmium or sodium iodate impurities prior to removal of THF *in vacuo.* Additionally, since the reaction was conducted in the presence of the free carboxylic acid, the desired product was isolated as a 4–5:1 mixture with the cyclized γ- hydroxy lactone **4.16** in the minority.[112] This was concluded by a complex $^{1}H$ and $^{13}C$ NMR which indicated the presence of the γ-hydroxy carbon at approximately 90–100 ppm. The product was immediately subjected to the next reaction as soon as it was synthesized, although the lactone has also been shown to participate in lactamization.[112]

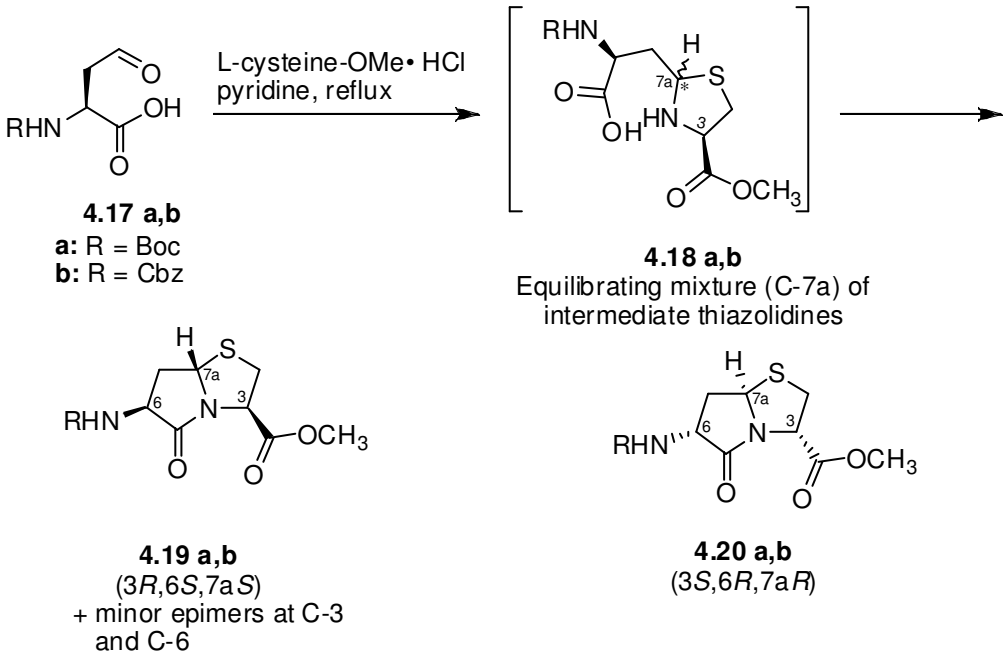
### 4.4. Synthesis of the bicyclic thiazolidine scaffold

As reported first by Baldwin *et al.*[109] and later by the Johnson group,[110] the 5,5-bicyclic thiazolidine (BTD) scaffold can be derived from the cyclization and lactamization reaction between a modified cysteine derivative **4.4** and the *N*-protected aspartic acid semi-aldehyde derivative **4.17a** and **4.17b** (**a** or **b** refers to the nature of the *N*-protecting group) as shown in Scheme 4.5.

Under the original conditions, the two substrates were first condensed to form the intermediate equilibrating mixture of thiazolidines, which would then be captured into the

product after lactamization. In both reports, the lactamization step was conducted under thermal conditions or “thermodynamic control”, which led to the preferential formation of a single epimer, **4.19a** and **4.19b** about the bridgehead carbon (C-7a, 7a*S*). Steric control was a possible rationale for this preference.[109] That is, in case of the reaction with the L-cysteine methyl ester hydrochloride **4.4**, the intermediate thiazolidine **4.18a** and **4.18b** containing the (3*R*, 7a*S*) stereochemistry or the 3,7a-*trans* relationship, was better arranged for lactam formation than the diastereomer with the 3,7a-*cis* relationship.

**Scheme 4.5.** Representative BTD formation

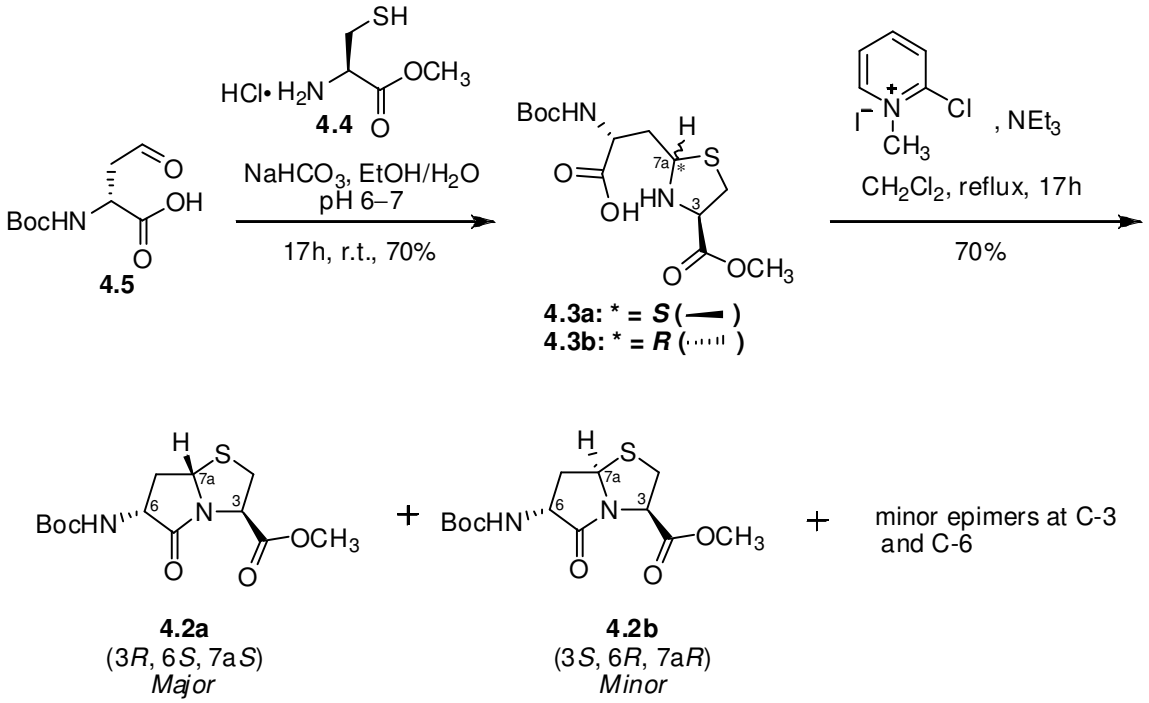



This selectivity was observed even when the reaction was conducted with D-cysteine methyl ester hydrochloride. The corresponding (3*S*,7a*R*) product **4.20a** and **4.20b** was obtained.

In order to obtain both C-7a bridgehead epimers, **4.2a** and **4.2b**, we had to alter the lactamization conditions. It has been hypothesized that if the rate of the subsequent lactamization was greater than the rate of C-7a epimer equilibration within the

diastereomeric intermediate thiazolidines, then *both* bridgehead diastereomers could be theoretically obtained.[109] As reported previously by our laboratory in the synthesis of BTDs, Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide or CMPI) is an ideal acid activating agent for this purpose. The corresponding active ester is highly reactive and under DCM reflux (40 °C), results in lactamization with formation of both C-7a epimers. First, the condensation (Scheme 4.6.) was conducted by stirring L-cysteine

**Scheme 4.6.** Condensation and lactamization




methyl ester hydrochloride **4.4** and the aldehyde **4.5** in a 1:1 EtOH:$H_2O$ mixture with the addition of solid sodium bicarbonate ($NaHCO_3$). The pH of the reaction medium was adjusted to 6–7 and the reaction stirred overnight at ambient temperature. The diastereomeric mixture of thiazolidines **4.3a** and **4.3b** was isolated as a white foam. The crude product of condensation was subjected to lactamization in the presence of equimolar amounts of CMPI and excess $NEt_3$. The reaction was complete within 15–17 h

at reflux in DCM. The crude material was a complex mixture and required purification at least twice by flash chromatography to obtain the pure diastereomers **4.2a** and **4.2b**. Under the "kinetic control" of this reaction condition, the diastereomers are obtained in a 2-3:1 ratio of the 7a*S*:7a*R* epimers. Very minor amounts of other diastereomers were also detected by $^1$H NMR of other chromatographic fractions (higher $R_f$ value). Formation of ketenes from active esters derived from reaction between carboxylic acids (where α-C ≠ quaternary carbon) and CMPI in the presence of $NEt_3$ is known.[125] It is possible that the minor amounts of the C-6 epimers are produced due to the epimerization at this carbon caused by a ketene formation-lactamization-proton quench sequence. The major diastereomer was determined to be the (3*R* 6*R*,7a*S*)-BTD as per X-ray crystal structure analysis (Figure 4.2). Nuclear Overhauser effects were inconclusive at this stage in the synthesis of the bicyclic diastereomers; this is mainly caused by significant chemical shift overlaps of hydrogen atoms at the C-3, C-7a and N*H*Boc positions in $^1$H NMR in $CDCl_3$ and MeOH-$d_4$.

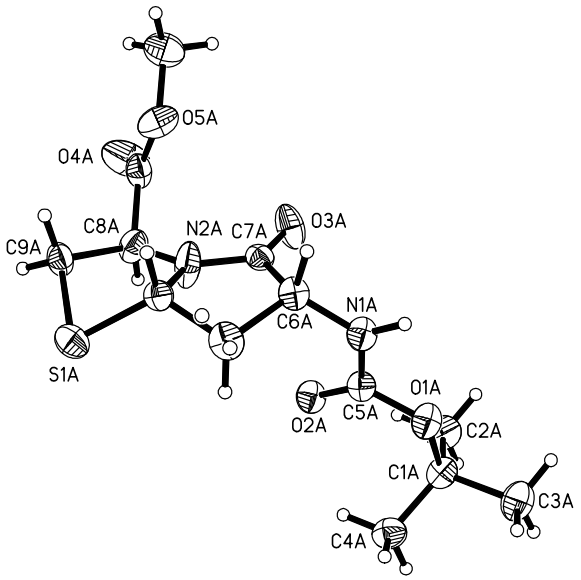
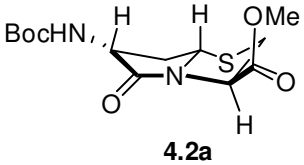



**Figure 4.2.** X-ray crystal structure of major BTD diastereomer **4.2a**

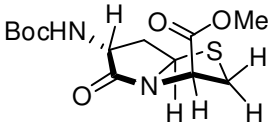
### 4.5. Stability and reactivity of the bicyclic thiazolidine diastereomers

There is an inherent bias in the stability between the two bridgehead (C-7a) epimers. Based on crystallographic data and the fact that [3.3.0] ring systems are preferentially *cis*-fused; the following conformations for the two diastereomers can be envisioned (Figure 4.3).




**4.2a**

*equatorial- and exocyclic-like positioning of the methoxycarbonyl*




**4.2b**

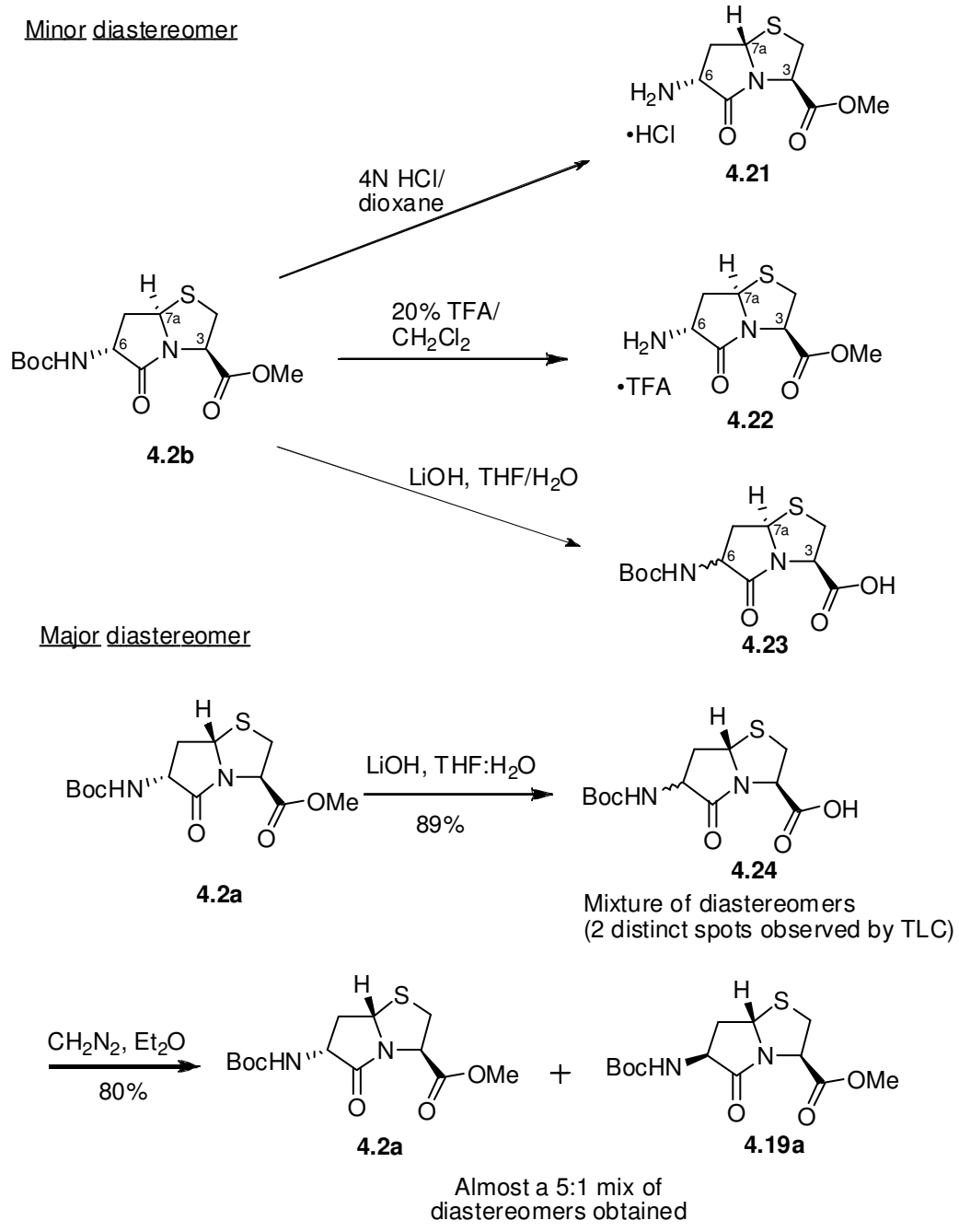
*axial- and endocyclic-like positioning of the methoxycarbonyl*

**Figure 4.3.** Possible conformations of the two BTD diastereomers

Under very strong Brønsted acid conditions (e.g. 4 N HCl/dioxane; pka ~ –9), the minor diastereomer **4.2b** was unstable and racemized almost entirely at the C-7a position to give the corresponding Boc-deprotected derivative **4.21** of the major diastereomer **4.2a** (Scheme 4.7). This racemization perhaps proceeds via an *N*-acyliminium intermediate, leading to opening of the thiazolidine and therefore re-equilibration to the thermodynamically more stable diastereomer **4.21**. The rate of this process was slowed down when a weaker Brønsted acid, such as trifluoroacetic acid (TFA; pka ~ –1) was used for Boc-deprotection to give **4.22**. It is important to note that this racemization process occurs regardless of the nature of the terminal substitution and order of events; that is it occurs in the absence and presence of the eventual benzylamide and alanine sub-units. Needless to say, Boc-deprotection of the major diastereomer **4.2a** in the presence of either Brønsted acid gives rise to the product with desired C-7a stereochemistry without racemization/epimerization.

Under ester hydrolysis conditions, both diastereomers led to moderate amounts of epimerized product. In case of the major diastereomer, the epimer was determined to be present to an extent of 17% as per $^{1}$H NMR. Based on previous reports,[110] it was hypothesized that the epimerized side product was the C-6 epimer. This was confirmed experimentally by re-converting the carboxylic acid mixture **4.24** to the corresponding methyl ester and separating the diastereomers **4.2a** and **4.19a** and conducting NOE analysis on the minor product **4.19a**. The extent of epimerization in case of the minor BTD diastereomer **4.2b** was higher and more complex. The situation was perhaps complicated by the fact that the methyl ester was in a sterically crowded milieu being in almost an axial position (*vide supra*). The hydrolysis then was perhaps plagued by 1,3-diaxial interactions and **4.2b** possibly underwent epimerization at C-3 and C-6.

**Scheme 4.7.** Chemical instabilities of the BTD diastereomers

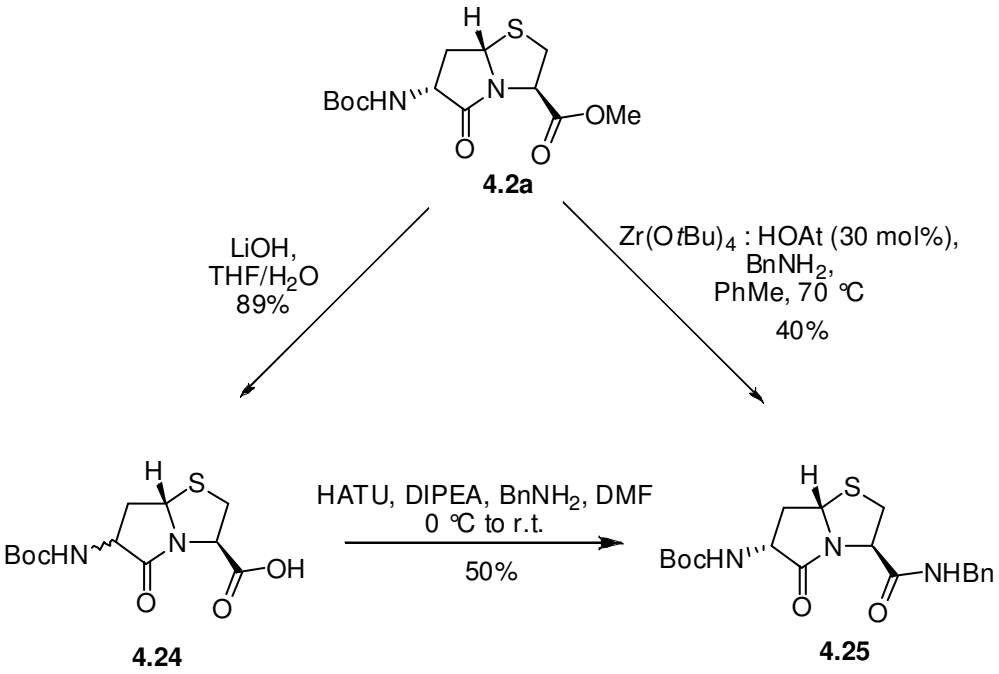



### 4.6.a. Further reactions of the major diastereomer: *Amidation*

In the case of the major BTD diastereomer, two routes were explored toward synthesis of the *C*-terminal benzylamide (Scheme 4.8). Initially, methyl ester hydrolysis

of **4.2a** to **4.24**, followed by coupling with benzylamine to form **4.25** (as hydrochloride salt) in the presence of HATU and DIPEA was attempted. Although this method resulted in the formation of the desired benzylamide product **4.25**, it was plagued by base-mediated epimerization, as discussed before.

**Scheme 4.8.** Amidation of major BTD diastereomer

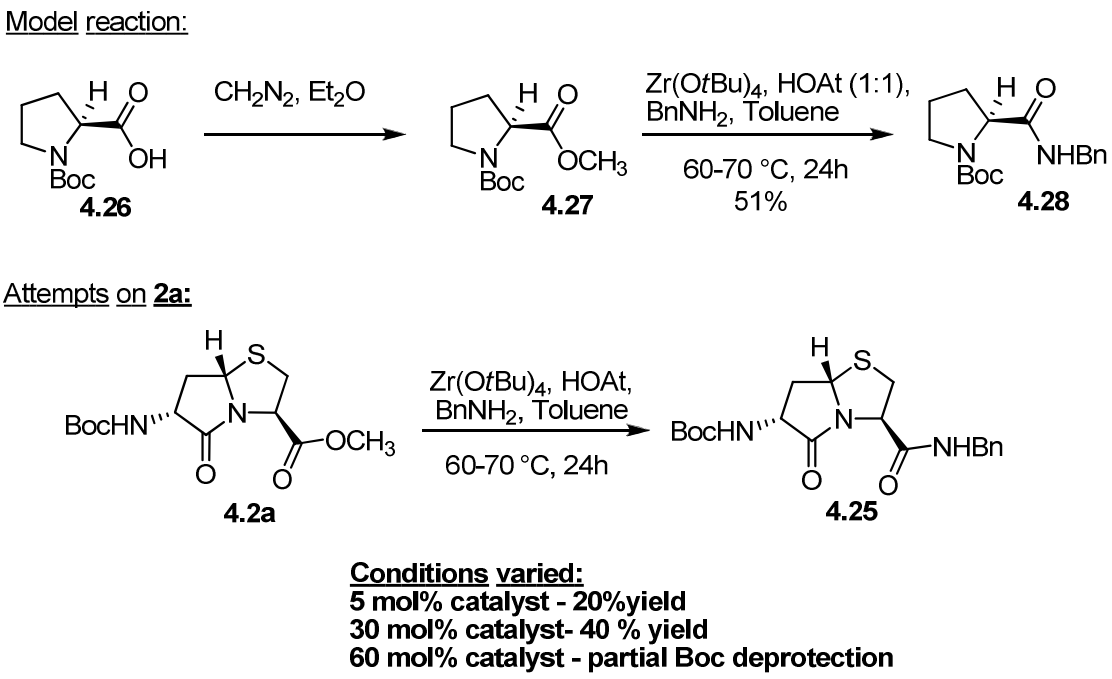



To circumvent this, an alternative route was attempted. Dr. Ravindranadh Somu, a former post-doctoral research associate in our laboratory, pointed me in the direction of a publication by the Porco group detailing direct conversion of esters to amides.[126] Although direct amidation of esters (particularly methyl and ethyl esters) promoted by *stoichiometric* amounts of metal-based mediators and Lewis acid-amine adducts is known,[127] there is scant literature precedent for conducting this process in a *catalytic* fashion. In addition, we also hoped to conduct this reaction under mild conditions of

temperature and avoid any strong base-mediated reactions (*vide supra*). As detailed in this paper, amidation of methyl esters mediated by catalytic group (IV) metal alkoxide s (e.g. Zr(*Ot*Bu)$_4$) in the presence of activators such as 1-hydroxy-azabenzotriazole (HOAt) resulted in high yields (76–99%) of the desired amide without significant racemization. Another encouraging aspect of this account was the complex nature of substrates successfully amenable to amide formation.

As shown in Scheme 4.9., we first conducted the amidation on a model system using the standard conditions reported in the paper for *N*-Boc alanine methyl ester. We

**Scheme 4.9.** Zr(O*t*Bu)$_4$ and HOAt- mediated amidation




hoped that use of *N*-Boc proline methyl ester **4.27** would best mimic the structural rigidity of the BTD diastereomer. With the use of 10 mol% each of zirconium tetra *tert*-butoxide

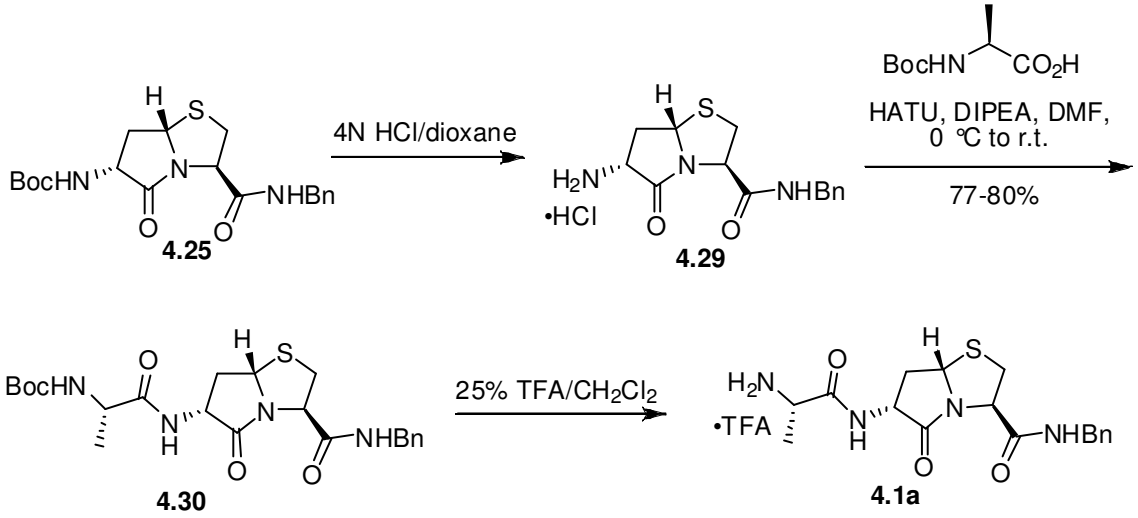
($Zr(OtBu)_4$) and HOAt in the presence of excess benzylamine in toluene, the benzylamide **4.28** was obtained in a 51% yield after 24h. Although this did not represent stellar yields (vis à vis those reported in the paper), we nevertheless decided to proceed with the reaction on the BTD methyl ester using the same conditions.

After much experimentation, it was determined that 30 mol% alkoxide and HOAt in toluene at 70 °C for one day was optimal for conversion to the amide **4.25**. Higher equivalents led to competitive Boc-deprotection, perhaps enhanced by the Lewis acid properties of the metal alkoxide.

### 4.6.b. Further reactions of the major diastereomer: *Coupling with Alanine*

For the major diastereomer, Boc-deprotection (Scheme 4.10) using 4N HCl in 1,4-dioxane proceededly smoothly and the corresponding ammonium hydrochloride **4.29**

**Scheme 4.10.** Major diastereomer synthesis




was neutralized using Hunig's base (DIPEA) and then coupled to *N*-Boc alanine using HATU as the coupling reagent. The product **4.30** was obtained as a colorless oil/foam in

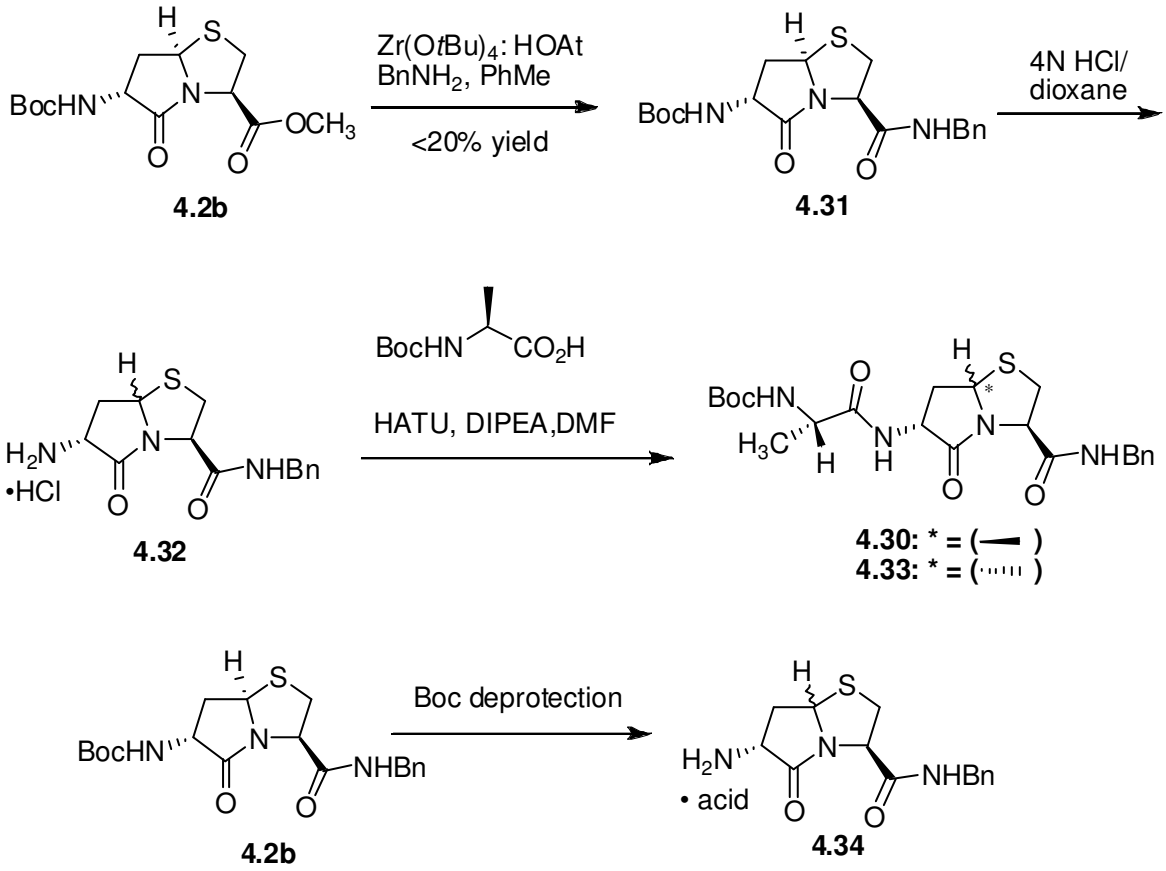
almost 80% yield. It is highly stable even at ambient temperature for extended time periods (~ 2 years). The final molecule was obtained by Boc-protection of the precursor using trifluoroacetic acid in quantitative yield.

### 4.7. Further reactions of the minor diastereomer

One of the main reasons for exploring the catalytic amidation route was due to the chemical instability of the minor diastereomer **4.2b**.The minor diastereomer was not amenable to the LiOH-mediated hydrolysis/benzylamine coupling route, largely due to the large extent of epimerization at the base hydrolysis stage and to the poor yield in the subsequent coupling reaction. As alluded to before (Figure 4.1, *vide supra*), it is hypothesized that the almost axial orientation of the methoxycarbonyl group in the minor diastereomer is responsible for the poor yields.

Unfortunately, the zirconium-mediated amidation of the methyl ester of the minor epimer was also very inefficient resulting in less than 20% product formation (**4.31**). Further, the acid-mediated racemization about the C-7a position was discovered only after the Boc-deprotection/Boc-alanine-OH coupling sequence led to the formation of **4.30** almost exclusively instead of **4.33**. Although, it was eventually determined that this competitive process could be avoided in the presence of TFA, the handleability of the minor diastereomer had severely diminished. Coupled with the issues of chemical instability and minute quantities of material, the attempt toward the synthesis of the final minor diastereomer was therefore abandoned (see experimental section for more details).

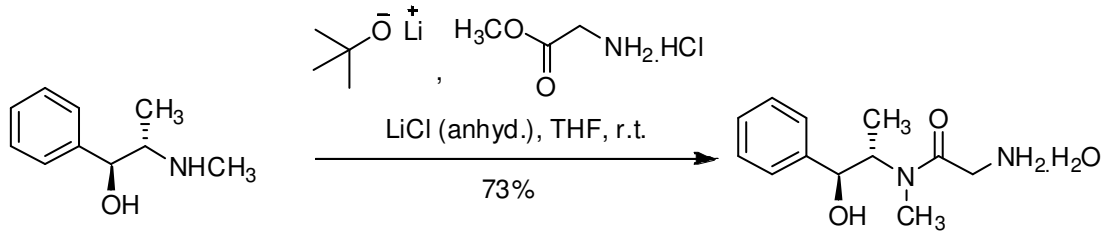
**Scheme 4.11.** Minor diastereomer synthesis




**1**. $BF_3.Et_2O$ - epimerization of bridgehead
**2.** Ceric ammonium nitrate (CAN) - Incomplete deprotection and epimerization
**3.** CAN (with pH buffer) - Incomplete deprotection
**4.** 25% TFA in $CH_2Cl_2$ (16 equiv.) - Complete deprotection, epimerization undetected.

**4.8.** Experimental section:

**(*S*,*S*)-(+)-Pseudoephedrine Glycinamide Monohydrate (4.12)**




(*S*, *S*)-(+)-Pseudoephedrine

**4.12**

(*S*,*S*)-Pseudoephedrine (13.7 g, 83 mmol, previously re-crystallized from benzene) and glycine methyl ester hydrochlodride **4.6** (13.6 g, 108 mmol, 1.3 equiv.) were added to a 250 mL flask containing anhydrous THF (110 mL) under Ar. The suspension was stirred vigorously for 15 min. LiCl (Note 1) was also added to this suspension. LiO*t*Bu (12 g, 150 mmol, 1.8 equiv.) was added in one portion under a stream of Ar (Note 2). The reaction was stirred at ambient temperature for a 1 h. The reaction was quenched by addition of saturated $NH_4Cl$ solution and THF was removed *in vacuo*. The aqueous mixture was transferred into a 500 mL separatory funnel and extracted with a 10:1 $CH_2Cl_2$ /isopropyl alcohol solution (3 x 200 ml). The organic extracts were pooled together and dried over anhydrous $K_2CO_3$. Filtration and solvent removal furnished a pale yellow-colored oil, which was dissolved in a minimum quantity of hot toluene. Approximately 2 mL $H_2O$ was added and the flask was cooled gradually on a tepid water bath. Crystals started to appear almost as soon as the solution cooled to room temperature. Further re-crystallization of the first crop from hot toluene gave 14.5 g of pure product **4.12** (73% yield).

mp 87–89 °C [lit.[114] 84–86 °C]

TLC $R_f$ 0.25 ($CH_2Cl_2$ /MeOH /$NEt_3$, 90:5:5) [lit.[114] $R_f$ 0.18, same system]

$[\alpha]_D$ + 98.3 (*c* 1.2, MeOH) [lit.[114] +101.2 (*c* 1.2, MeOH)]

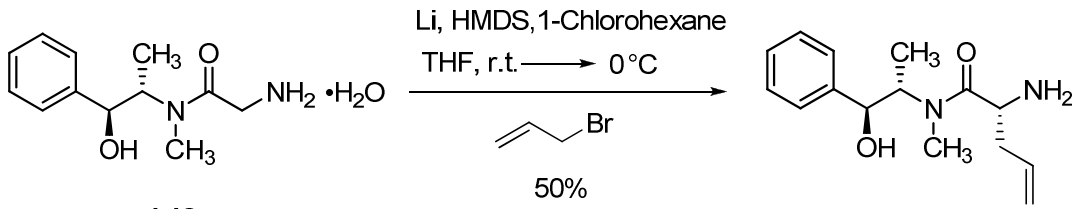
$^{1}$H NMR (300 MHz, $CDCl_3$, rotamers present in a 1:1 ratio) δ 7.38–7.25 (m, 5H, aromatic C*H*), 4.62–4.49 (m, 1.5H, C*H*$CH_3$ and C*H*OH), 3.84–3.78 (m, 0.5H, C*H*$CH_3$), 3.72 (d, 0.5H, *J*= 15.6 Hz, C*H*$_2$$NH_2$), 3.38 (app. d, 1.5H, *J* = 15 Hz, C*H*$_2$$NH_2$), 2.93 and 2.77 (2 s, 3H, NC*H*$_3$), 2.52 (br, 5H, CHO*H*, $CH_2$N*H*$_2$, *H*$_2$O), 1.01 and 0.94 (2 d, 3H, *J* = 6.9 Hz, CHC*H*$_3$).

$^{13}$C NMR (75MHz, $CDCl_3$, 2 rotamers present in a 1.5:1 ratio) δ 174.3 and 173.6 (*C*=O), 142.2 and 141.9, 128.86, 128.7, 128.5, 128.4, 128.1, 127.1, 127.0, 126.8 (aromatic *C*H), 76.2 and 75.3 (*C*HOH), 57.8 (*C*H$CH_3$), 44.0 and 43.7 (*C*$H_2$$NH_2$), 30.5 and 27.3 (N*C*$H_3$), 15.8 and 14.7 (CH*C*$H_3$).

Notes:

1. LiCl was rendered anhydrous by flame-drying thrice before use and cooled under Ar.
2. LiO*t*Bu was prepared fresh in the laboratory using a procedure delineated below.

**(*S*,*S*)-(+)-Pseudoephedrine α-allylglycinamide (4.13)**




**4.12** **4.13**

Li metal (2.0 g, 290 mmol, 6.8 equiv.) was freed from oil by rinsing in hexanes, cut into small pieces and introduced into an oven-dried flask containing anhydrous THF (170 mL) under a stream of Ar under stirring. HMDS (33.4 mL, 160 mmol, 3.7 equiv.) and 1-chlorohexane (20.6 mL, 150 mmol, 3.6 equiv.) were sequentially added to the

reaction mixture. The flask was then immersed in a 20 °C water bath. A cloudy precipitate, presumed to be that of LiCl, started to form. The reaction mixture started to warm gradually. Ice was added to the water bath in order to maintain the reaction temperature below 30 °C at all times. Once the initial exothermicity was controlled, the water bath was removed and stirring was continued at room temperature overnight. The next day, the dark, amber-colored suspension showed no traces of Li metal. (*S,S*)-(+)-pseudoephedrine glycinamide monohydrate **4.12** (10 g, 42 mmol, 1 equiv.) was added in one portion after the reaction mixture had been cooled to 0 °C. The reaction mixture was stirred vigorously for 2.5 h to allow the bright orange-colored enolate suspension to form. Allyl bromide (3.7 mL, 44 mmol, 1.05 equiv.) was added dropwise over 5–6 min so as to maintain the reaction temperature below 10 °C. The reaction mixture was stirred at 0 °C for 1 h, after which the reaction was quenched by the addition of 100 mL $H_2O$.THF was removed by rotary evaporator and the remaining aqueous mixture was adjusted to pH 0 using 6 N HCl. The pH adjustment was done carefully ensuring that the temperature did not rise above 10 °C. The aqueous mixture was then extracted with EtOAc. The organic layer was further washed with 100 mL portions of 3 N and 1 N HCl. The three aqueous layers were combined and cooled in an ice-salt bath. The solution was then basified to pH 14 with 50% NaOH solution, again ensuring the temperature did not rise above 20 °C. Extraction of the basic solution with $CH_2Cl_2$ (3 x 200 mL) was followed by drying over anhydrous $K_2CO_3$. Filtration of the salt followed by concentration of the organic solution furnished the crude product as a yellow-colored oil. The oil was dissolved in toluene and the resulting solution was evaporated *in vacuo* to furnish a yellow-colored solid, which was crystallized from hot toluene-ether. The product crystals weighed 5.5 g (50% yield). The product **4.13** was diastereomerically pure as determined by spectroscopic studies. mp 73–74 °C [lit.[114] 79–83 °C]

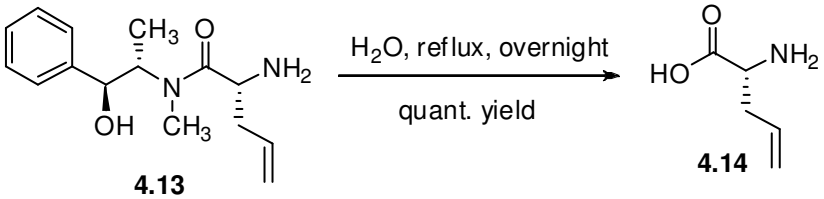
TLC $R_f$ 0.4 ($CH_2Cl_2$/MeOH /$NEt_3$, 90:5:5)

$[\alpha]_D$ +89.1 (*c* 1.1, $CHCl_3$) [lit.[114] +86.4 (*c* 1.1, $CHCl_3$)]

$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment, rotamers present in a 3:1 ratio) δ 7.33–7.26 (m, 5H, aromatic C*H*), 5.85–5.62 (m, 1H, C*H*=$CH_2$), 5.19–5.05 (m, 2H, CH=C$H_2$), 4.59–4.44 (m, 2H, C*H*$CH_3$ and C*H*OH), 3.71–3.61 (m, 2H, α-C*H*), 2.93 and 2.85 (2 s, 3H, NC$H_3$), 2.36–2.04 (m, 2H, C$H_2$CH=$CH_2$), 1.04 and 0.91 (2 d, 3H, *J* = 6.6 Hz, CHC$H_3$).

$^{13}$C NMR (75MHz, $CDCl_3$, gHMQC assignment, rotamers present in a 5:1 ratio) δ 176.6 and 174.9 (*C*=O), 142.4 and 141.8 (aromatic *C*H), 134.6 and 133.6 (*C*H=$CH_2$), 128.5, 128.1, 127.5, 126.7, 126.3 (aromatic *C*H), 118.0 and 117.9 (CH=*C*$H_2$), 75.7 and 75.0 (*C*HOH), 58.1 and 57.7 (*C*H$CH_3$), 51.4 and 51.2 (α-*C*H), 39.9 and 39.7 (β-*C*$H_2$), 31.9 and 27.0 (N*C*$H_3$), 15.6 and 14.5 (CH*C*$H_3$)

**(*R*)-α-Allylglycine (4.14)**




**4.13** **4.14**

(*S*,*S*)-(+)-Pseudoephedrine-(*R*)-α-allylglycinamide **4.13** (3 g, 11 mmol) was suspended in water (60 mL) and refluxed overnight. The next day, the pale yellow-colored solution was cooled to room temperature and approx. 5 mL concentrated $NH_4OH$ solution was added to this solution. The resulting basic solution was extracted with $CH_2Cl_2$ (3 x 100 mL). The 3 organic extracts (Note 1) were separately and

sequentially extracted with 50 mL aqueous solution containing 1 mL concentrated $NH_4OH$. The aqueous extracts were combined and the water evaporated to give a pale yellow solid. The solid was triturated with absolute ethanol under heat and filtered to yield the product **4.14** as a pure, white solid weighing 1.3 g (100% yield).

mp > 280 °C (dec.)

TLC $R_f$ 0.4 ($CH_2Cl_2$/ MeOH /$NEt_3$, 90:5:5)

$[\alpha]_D$ +37.3 (*c* 0.75, $H_2O$) [lit.[114] +37.5 (*c* 4.09, $H_2O$)]

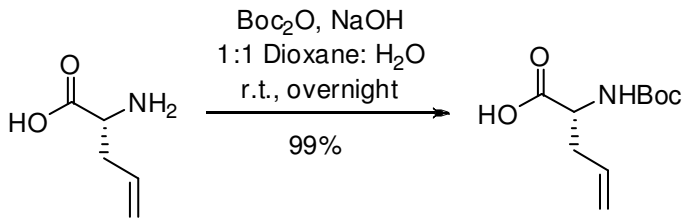
$^1$H NMR (300 MHz, $CDCl_3$) δ 5.68–5.52 (m, 1H, C*H*=$CH_2$), 5.13–5.07 (m, 2H, CH=C*H*$_2$), 3.65–3.61 (m, 1H, α-C*H*), 2.52–2.39 (m, 2H, C*H*$_2$–CH=$CH_2$)

$^{13}$C NMR (75MHz, $CDCl_3$) δ 174.1 (*C*=O), 131.3 (*C*H=$CH_2$), 120.6 (CH=*C*H$_2$), 54.1 (α-*C*H), 35.1 (*C*H$_2$–CH=$CH_2$)

Notes:

1. The chiral auxiliary (*S*,*S*)-(+)-pseudoephedrine was recovered by combining the $CH_2Cl_2$ layers and removing the solvent *in vacuo*. In various attempts, 83–95% of the chiral auxiliary has been recovered.

**(*R*)-*N*-(*tert*-Butyloxycarbonyl)-α-allylglycine (4.15)**




**4.14** **4.15**

(*R*)-Allylglycine **4.14** (1.0 g, 8.7 mmol, 1 equiv.) was dissolved in a solution of NaOH (350 mg, 8.7 mmol, 1 equiv.) in water (12.5 mL). To this was added a solution of $Boc_2O$ (2.9 g, 13.1 mmol, 1.5 equiv.) in 1,4-dioxane(12.5 mL). The reaction mixture was stirred at room temperature overnight. The next day, 1,4-dioxane was removed on the rotary evaporator and the aqueous solution was extracted with EtOAc (50 mL). The aqueous layer was then acidified to pH 2–3 using solid citric acid. The acidic mixture was then extracted with $CH_2Cl_2$ (80 mL). The organic layer was dried over $MgSO_4$. Filtration, followed by removal of the solvent *in vacuo* furnished 1.86 g of the Boc-protected amino acid **4.15** (99.4% yield) as a colorless oil.

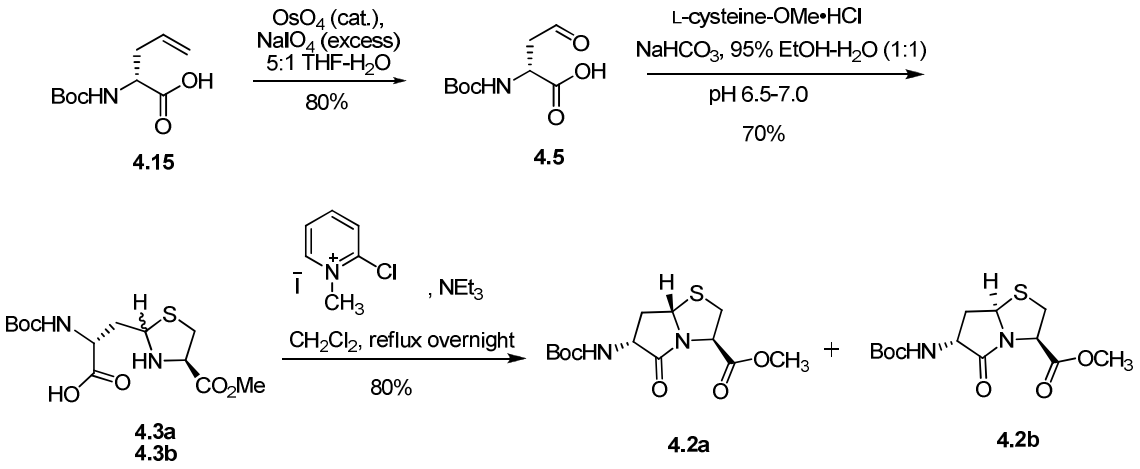
TLC $R_f$ 0.6 (iPrOH/$NH_4OH$, 4:1)

$[\alpha]_D$ –8.4 (*c* 2.13, $CH_3OH$) [lit.[116] +8.6 to +11.4 (*c* 1.4, $CH_3OH$) for enantiomer]

$^1$H NMR (300 MHz, $CDCl_3$) δ 10.8 (br. s, 1H, (C=O)-O*H*), 6.42 (app. d, 0.3H, N*H*), 5.79–5.65 (m, 1H, C*H*=$CH_2$), 5.19–5.07 (m, 2.6H, CH=C*H*$_2$, N*H*), 4.39 (AB quartet, 0.63H, α-C*H*), 4.17 (AB quartet, 0.33H, α-C*H*), 2.64–2.46 (m, 2H, C*H*$_2$CH=$CH_2$), 1.43 (s, 9H, C(C*H*$_3$)$_3$)

$^{13}$C NMR (75MHz, $CDCl_3$) δ 176.8 and 176.5 (acid *C*=O), 156.8 and 155.6 (carbamate *C*=O), 132.3 (*C*H=$CH_2$), 119.6 (CH=*C*$H_2$), 81.9 and 80.6 (*C*(C$H_3$)$_3$), 54.6 and 53.0 (α-*C*), 37.0 and 36.8 (*C*$H_2$CH=$CH_2$), 28.6 (C(*C*$H_3$)$_3$)

**(3*R*,6*R*,7a*S*)-Methyl 6-(*tert*-Butyloxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (4.2a) and (3*R*,6*R*,7a*R*)-Methyl 6-(*tert*-Butyloxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (4.2b)**




Boc-(*R*)-α-allylglycine **4.15** (3.3 g, 15.3 mmol) was dissolved in a mixture of 5:1 THF-$H_2O$ under Ar. $OsO_4$ (200 mg, 9 mL of a 2.5% w/v solution, 0.8 mmol) was added all a once. A brownish-black precipitate started to form. $NaIO_4$ (13.1 g, 61.4 mmol) was added in portions to this suspension. When the addition was completed within 30 min, a thick white precipitate of reduced sodium iodate remained. This suspension was stirred vigorously under Ar overnight. Next morning, the white precipitate was filtered off and THF removed *in vacuo.* The pH of the aqueous residue was adjusted to 2–3 and the product was extracted into EtOAc (3 x) (Note 1). The organic layer was dried using $MgSO_4$ and the solvent removed in the rotary evaporator to obtain colorless oil, which changed to white foam and finally solidified under vacuum. The crude product **4.5** weighed 2.67 g (80% yield). It was directly used in the subsequent reaction without purification.

To a chilled (ice/water/salt) solution of Boc-D-aspartic acid semialdehyde **4.5** (2.67 g, 12.3 mmol) in 1:1 95% EtOH–$H_2O$ was added one equivalent of sodium bicarbonate (1.0 g, 12.3 mmol). L-Cysteine methyl ester hydrochloride (2.1 g, 12.3 mmol) was then added as a solid in one portion. The pH of the resulting solution was adjusted to 6.5–7.0 using sat. $NaHCO_3$ solution. This solution was stirred overnight at room temperature under Ar. The next morning EtOH was removed on the rotary evaporator and the aqueous solution was acidified to pH 4–5. This solution/emulsion was extracted using a mixture of $Et_2O$ and EtOAc (3:1) three to four times (Note 2). The organic layer was dried using $MgSO_4$ and concentrated *in vacuo* to give a colorless oil that turned into a white foam under vacuum. The crude product containing the diastereomeric mixture of **4.3a** and **4.3b** weighed 2.9 g (70% yield).

To a solution of the diastereomeric thiazolidine carboxylic acid mixture **4.3 a-b** (3.1 g, 9.3 mmol, 1 equiv.) in anhydrous dichloromethane, was added one equivalent of triethylamine (2.86 mL, 20.4 mmol) followed by addition of slight excess of Mukaiyama's reagent (2.85 g, 11.2 mmol). The solution was refluxed overnight. The next morning the solution was washed sequentially with 10% citric acid, saturated $NaHCO_3$ and brine solution. The organic layer was dried over $MgSO_4$ and concentrated to give an amber-colored oil. The crude oil was then chromatographed using hexanes: EtOAc to give the two separate diastereomers, **4.2a** and **4.2b** in a 1.5–3:1 ratio (2.74 g total, 80% yield) respectively. The major diastereomer, **4.2a** was an easily crystallizable solid, while the minor diastereomer solidified under reduced pressure.

Diastereomer **4.2a:**

mp 143–144 °C

TLC $R_f$ 0.45 (EtOAc/hexanes, 1:1)

$[\alpha]_D$ –187.8 (*c* 0.55, $CHCl_3$)

$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment) 5.25 (app. t, 1H, *J* = 5.7 Hz, 7a-C*H*), 5.11 (br. s, 1H, N*H*), 4.31–4.24 (m, 2H, 3-C*H*, 6-C*H*), 3.79 (s, 3H, OC$H_3$), 3.55 (dd, 1H, $J_1$ = 12.3 Hz, $J_2$ = 7.5 Hz, 2-C$H_{\text{trans to 3-CH}}$), 3.40 (dd, 1H, $J_1$ = 12.3 Hz, $J_2$ = 3.6 Hz, 2-C$H_{\text{cis to 3-CH}}$), 3.18 (ddd, $J_1$ = 13.2 Hz, $J_2$ = $J_3$ = 7.2 Hz, 1H, 7-C$H_{\text{syn to bridgehead}}$), 1.99 (ddd, $J_1$ = 12 Hz, $J_2$ = 10.8 Hz, $J_3$ = 7.5 Hz, 1H, 7-C$H_{\text{trans to bridgehead}}$), 1.44 (s, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 171.6 (lactam *C*=O), 169.6 (ester *C*=O), 155.5 (carbamate *C*=O), 80.5 (*C*($CH_3$)$_3$), 62.0 (7a-*C*), 57.9 (3-*C*), 54.6 (6-*C*), 53.3 (O*C*$H_3$), 39.3 (7-*C*), 35.4 (2-*C*), 28.6 (C(*C*$H_3$)$_3$)

ESI HRMS *m/z* 339.0987 $[M+Na]^+$ $(C_{13}H_{20}N_2O_5S + Na)^+$ requires 339.0985

Diastereomer **4.2b**

TLC $R_f$ 0.38 (EtOAc/hexanes, 1:1)

$[\alpha]_D$ +9.3 (*c* 0.59, $CHCl_3$)

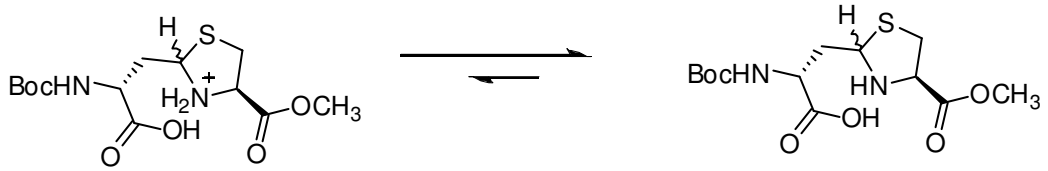
$^1$H NMR (300 MHz, $CDCl_3$) 5.16–5.08 (m, 3H, 3-C*H*, 7a-C*H*, N*H*), 4.62 (app. q, 1H, 6-C*H*), 3.77 (s, 3H, OC$H_3$), 3.41–3.29 (m, 2H, 2-C$H_2$), 2.61 (ddd, $J_1$ = 13.8 Hz, $J_2$ = 9.6 Hz, $J_3$ = 5.1Hz, 1H, 7-C$H_{\text{syn to bridgehead}}$), 2.45 (ddd, $J_1$ = 13.8 Hz, $J_2$ = 7.2 Hz, $J_3$ = 5.1 Hz, 1H, 7-C$H_{\text{trans to bridgehead}}$), 1.43 (s, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 171.8 (lactam *C*=O), 168.6 (ester *C*=O), 155.4 (carbamate *C*=O), 80.6 (*C*($CH_3$)$_3$), 65.9 (7a-*C*), 58.1 (3-*C*), 55.1 (6-*C*), 52.9 (O*C*$H_3$), 38.6 (7-*C*), 31.9 (2-*C*), 28.4 (C(*C*$H_3$)$_3$)

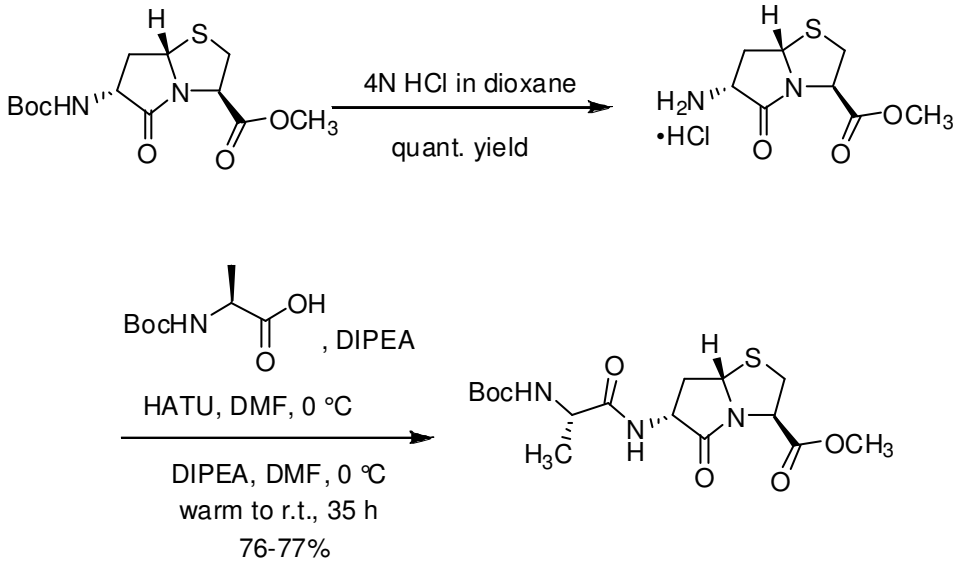
ESI HRMS *m/z* 339.0961 $[M+Na]^+$ $(C_{13}H_{20}N_2O_5S + Na)^+$ requires 339.0985

Notes*:*

1. The general work-up protocol that is followed for oxidations/oxidative cleavage reactions catalyzed by $OsO_4$ requires a sodium bisulfite/ sodium sulfite wash to reduce any unreacted oxidizing species. However, in this case such washes are *NOT* to be used since 1.) sodium bisulfite can react with the aldehyde to form a bisulfite adduct and 2.) an aqueous solution of sodium sulfite is alkaline (pH ~ 9.0). This will lead to loss of product due to solubilization of the acid as the sodium salt in the aqueous layer. Multiple filtrations can be done to ensure the complete removal of any insoluble osmium or sodium iodate impurities prior to removal of THF *in vacuo.*
2. The original work-up for this reaction involved acidifying the aqueous layer to pH 6.0 followed by repetitive extractions into EtOAc. However, application of this protocol only led to partial recovery of the product amino acid. Further acidification of the aqueous layer to pH 3–5 was found to be more advantageous. At this pH, the product amino acid is almost completely soluble in a largely diethyl ether wash. The removal of the product from the aqueous layer also facilitates the shifting of the following thermodynamic equilibrium to the right and leads to almost complete recovery of the amino acid.



**(3*R*,6*R*,7a*S*)-Methyl 6-[2'-(*tert*-Butoxycarbonyl)amino-1'-oxopropane]amino-5-oxo-(6*H*)-pyrrolo[2,1-*b*]thiazole-3-carboxylate**

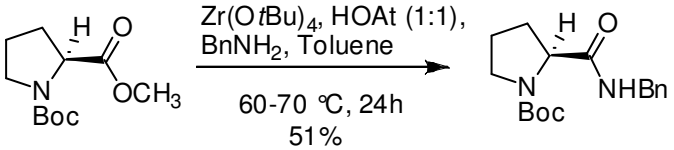



The Boc-protected bicyclic thiazolidine (125.1 mg, 0.4 mmol) was deprotected by stirring 4 N HCl in dioxane (3 mL) under Ar for 1 hour and then removing the excess HCl and dioxane in the rotary evaporator. A DMF solution of Boc-alanine-OH (75.1 mg, 0.4 mmol) was first stirred under Ar with diisopropylethylamine (0.1 mL, 0.6 mmol, 1.5 equiv.) and HATU (148.3 mg, 0.4 mmol, 1 equiv) at 0 °C. After 20 minutes at this temperature, a solution of the white hydrochloride salt (100 mg, 0.4 mmol) and diisopropylethylamine (0.1 mL, 0.6 mmol, 1.5 equiv.) in DMF was added to the activated ester. The solution was stirred under Ar at room temperature for about 16 hours. DMF was removed on the high vacuum rotary evaporator and the residue dissolved in $CH_2Cl_2$. The organic layer was washed sequentially with 10% citric acid solution, saturated $NaHCO_3$ solution, brine and then dried over $MgSO_4$. After concentrating the organic layer, the residue was subjected to flash chromatography using a gradient solvent system (EtOAc: hexanes 1:1 increasing to 3:1) to produce the product as a white solid weighing 110 mg (72% yield)

$^{1}$H NMR (300 MHz, $CDCl_3$) δ 7.1 (br. s, 1H, N*H*), 5.17–5.09 (m, 3H, N*H*Boc, 3-C*H*, 7a-*H*), 4.83 (ddd, 1H, $J_1$ = 7.2 Hz, $J_2$ = 8.4 Hz, $J_3$ = 10.2 Hz, 6-C*H*), 4.22 (br. m, 1H, alanine α-C*H*), 3.75 (s, 3H, OC$H_3$), 3.40–3.29 (m, 2H, $J_1$ = 4.2 Hz, $J_2$ = 11.4 Hz, $J_3$ = 13.5 Hz, 2-C*H*), 3.16 (ddd, 1H, $J_1$ = 6.3 Hz, $J_2$ = 8.4 Hz, $J_3$ = 12.6 Hz, 7-C*H*), 1.99 (ddd, 1H, $J_1$ = 7.2 Hz, $J_2$ = 10.5 Hz, $J_3$ = 12.6 Hz, 7-C*H*), 1.42 (s, 9H, C(C$H_3$)$_3$), 1.34 (d, 3H, $J$ = 7.2 Hz, alanine C$H_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 173.4 (HN-*C*=O), 171.4 (*C*=O), 169.6 (HN-*C*=O), 155.7 (O-*C*=O), 80.4 ((C$H_3$)$_3$*C*), 62.0 (cysteine α-*C*H), 58.0 (=N-*C*H-S-), 53.4 (lactam α-*C*H ), 53.3 (O*C*$H_3$), 50.1 (alanine α-*C*H), 38.1 (*C*$H_2$), 35.4 (*C*$H_2$S), 28.7 ((*C*$H_3$)$_3$C), 18.7 (alanine β-$CH_3$)

**Benzyl (*S*)-1-(*tert*–Butyloxycarbonyl)pyrrolidine-2-carboxamide (4.28)**




**4.27** **4.28**

Boc-proline methyl ester **4.27** (210 mg, 0.9 mmol, 1 equiv.) and benzylamine (0.1 mL, 1.1 mmol, 1.2 equiv.) were dissolved in 1 mL anhydrous toluene (Note 1). This was followed by the sequential addition of 7-aza-1-hydroxybenzotriazole (HOAt, 13 mg, 0.09 mmol, 10 mol%) and zirconium tetra *tert*-butoxide (Zr(O*t*Bu)$_4$, 0.04 mL, 0.09 mmol, 10 mol%). The initial turbidity caused by HOAt addition disappeared once Zr(O*t*Bu)$_4$ was added. The pale yellow-colored solution was stirred under Ar in a sealed tube at 70 °C for 1d. The reaction was quenched after this time period by sequential addition of 2 mL MeOH and 2 mL $CH_2Cl_2$ which led to the formation of a suspension. The reaction

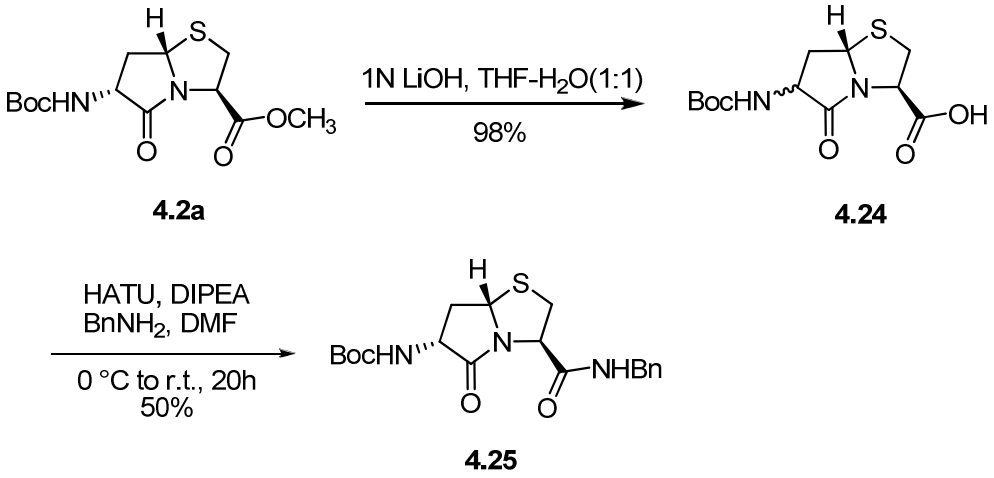
mixture was passed through a $SiO_2$ gel plug (4 cm x 1.5 cm) and washed well with a 1:1 mixture of $CH_2Cl_2$. The eluted solution was filtered once more through a 0.45 μm filter disc and concentrated. $^1H$ NMR analysis of the crude material indicated presence of the desired product in majority. Further purification by flash chromatography (gradient elution: 50–75% EtOAc/hexanes) led to the isolation of the pure product **4.28** as a white solid weighing 138 mg (51% yield) with identical spectral properties as reported in the literature.

mp 126–127 °C

TLC $R_f$ 0.47 (EtOAc/hexanes, 3:1)

$[\alpha]_D$ –84.4 (*c* 0.54, $CHCl_3$)

**(3*R*,6*R*,7a*S*)-Benzyl 6-[*N*-(*tert*-Butoxycarbonyl)amino]-5-oxo-(6*H*)-pyrrolo[2,1-*b*]thiazole-3-carboxamide (4.25)**




**4.2a** **4.24** **4.25**

A solution of the ester **4.2a** (100 mg, 0.32 mmol, 1 equiv.) and lithium hydroxide (23 mg, 0.96 mmol, 3 equiv.) was stirred in a 2 mL volume of a 1:1 mixture of THF and water for 18 h at room temperature. The organic solvent was removed on a rotary

evaporator and the pH of the aqueous solution was adjusted to 2–3 with 1N HCl. The aqueous layer was washed thrice with ethyl acetate. The organic layer was dried with $MgSO_4$ and concentrated to give a nearly white solid weighing 95 mg (98% yield). As per $^1$H NMR analysis, the carboxylic acid obtained was a mixture of diastereomers in a 5–9:1 ratio, with the major diastereomer corresponding to the desired product. Based on preliminary 1-D NOE analysis and based on precedent, the minor diastereomer was the carboxylic acid where the 6-C had epimerized to the opposite stereochemistry (Note 1).

A solution of the diastereomeric mixture of carboxylic acids (95 mg, 0.32 mmol, 1 equiv.) in 2 mL anhydrous DMF (Note 2) was cooled to 0 °C. This was followed by the sequential addition of DIPEA (0.1 mL, 0.48 mmol, 1.5 equiv.) and HATU (122 mg, 0.32 mmol, 1 equiv.). The mixture was stirred at this temperature under Ar for 20 min. This was followed by addition of a mixture of benzylamine (0.1 mL, 0.96 mmol, 3 equiv.) and DIPEA (0.2 mL, 0.96 mmol, 3 equiv.) drop-wise over 10 minutes. The yellow-colored solution was stirred under Ar for 20 h. The volatiles were removed by rotary evaporation. The residue was dissolved in EtOAc (30 mL) and the solution was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to give a yellow oil which was purified by flash chromatography (gradient elution: 0–60% EtOAc/hexanes) to give the pure product weighing 63 mg (50% yield).

TLC $R_f$ 0.51 (EtOAc/hexanes, 3:1)

$[\alpha]_D$ –163.8 (*c* 0.5, $CHCl_3$)

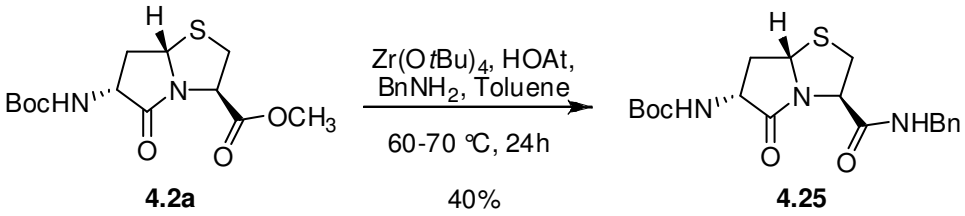
$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment) δ 7.33–7.23 (m, 6H, Ar-C*H*, N*H*Bn), 5.18 (d, 1H, *J* = 7.2 Hz, N*H*Boc), 4.98 (dd, AB pattern, 1H, $J_1$ = 7.2 Hz, $J_2$ = 6.0 Hz, 7a-C*H*), 4.83 (app. t, 1H, *J* = 6.6 Hz, 3-C*H*), 4.67 (app. q, AB pattern, 1H, *J* = 7.8Hz, 6-C*H*), 4.43–4.35 (m, 2H, C$H_2$Bn), 3.74 (dd, 1H, $J_{1(geminal)}$ = 11.7 Hz, $J_2$ = 5.7 Hz, 2-C$H_2$), 3.28

(dd, 1H, $J_{1(geminal)}$ = 11.7 Hz, $J_2$ = 7.5 Hz, 2-C$H_2$) , 3.11–3.02 (m, 1H, 7-C$H_2$), 2.06–1.94 (m, 1H, 7-C$H_2$), 1.45 (C(C$H_3$)$_3$)

$^{13}$C NMR (75 MHz, $CDCl_3$, gHMQC assignment) δ 173.3 (lactam *C*=O), 167.9 (amide *C*=O), 155.3 (carbamate *C*=O), 137.7 (Ar-*C*), 128.8, 127.6, (Ar-C*H*), 80.5 (*C*(CH$_3$)$_3$), 62.5 (7a-*C*), 59.6 (3-*C*), 54.6 (6-*C*), 43.9 (*C*H$_2$Bn), 37.9 (7-*C*), 34.8 (2-*C*), 28.5 (C(*C*H$_3$)$_3$)

ESI HRMS *m/z* 414.1454 $[M+Na]^+$ $(C_{19}H_{25}N_3O_4S+Na)^+$ requires 414.1458

Alternate route[18] toward **4.25**




**4.2a** 40% **4.25**

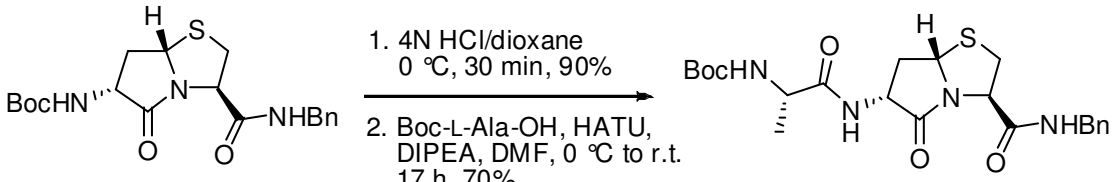
The bicyclic thiazolidine methyl ester **4.2a** (158 mg, 0.5 mmol, 1 equiv.) and benzylamine (0.0.7 mL, 0.6 mmol, 1.2 equiv.) were dissolved in 1 mL anhydrous toluene (Note 3). This was followed by the sequential addition of 7-aza-1-hydroxybenzotriazole (HOAt, 7 mg, 0.05 mmol, 10 mol%) and zirconium tetra *tert*-butoxide (Zr(O*t*Bu)$_4$, 0.0.02 mL, 0.0.05 mmol, 10 mol%). The initial turbidity caused by HOAt addition disappeared once Zr(O*t*Bu)$_4$ was added. The pale yellow-colored solution was stirred under Ar in a sealed tube at 70 °C for 1 d. Since the reaction was incomplete as determined by TLC analysis, another 20 mol% each of HOAt and Zr(O*t*Bu)$_4$ was added to the reaction mixture, which was stirred for another day. The reaction was quenched after this time period by sequential addition of 2 mL MeOH and 2 mL $CH_2Cl_2$ which led to the formation of a suspension. The reaction mixture was passed through a $SiO_2$ gel plug (4 cm x 1.5 cm) and washed well with a 1:1 mixture of $CH_2Cl_2$ and MeOH. The eluted solution was

filtered once more through a 0.45 μm filter disc and concentrated. Further purification by flash chromatography (gradient elution: 20–75% EtOAc/hexanes) led to the isolation of the pure product as a colorless oil weighing 78 mg (40% yield). The physical and spectral properties of this material were identical to those of **4.25** obtained via the ester hydrolysis/benzylamine coupling sequence.

Notes:

1. On a previous attempt, the diastereomeric mixture after ester hydrolysis was subjected to re-esterification with diazomethane to give the epimeric mixture of methyl esters which was easily separable by flash chromatography. The minor diastereomer **4.19a** had a higher $R_f$ value than the major diastereomer.
   $^1$H NMR (300 MHz, $CDCl_3$) δ 5.16 (d, 1H, 3-*C*), 5.05 (dd, 1H, 7a-C*H*), 5.00 (br. s, .5H, N*H*Boc), 4.40 (dd, 1H, 6-*C*), 3.51–3.35 (m, dd or AB pattern, 2H, 2-C$H_2$), 2.74–2.67 (m, 1H, 7-C$H_2$), 2.47–2.37 (m, 1H, 7-C$H_2$), 1.45 (C(C$H_3$)$_3$)
2. DMF was stored over 4 Å molecular sieves after the bottle was first opened and was used without further purification
3. Toluene was rigorously rendered anhydrous by stirring and distilling from sodium sand.

**(3*R*,6*R*,7a*S*)-Benzyl 6-[2'-(*tert*-Butoxycarbonyl)amino-1'-oxopropane]amino-5-oxo-(6*H*)-pyrrolo[2,1-*b*]thiazole-3-carboxamide (4.30)**




**4.25** **4.30**

A solution of 4 N HCl in dioxane (2 mL) was added to the Boc-protected bicyclic thiazolidine benzylamide **4.25** (39 mg, 0.1 mmol, 1 equiv.). The solution was stirred for 30 min. at 0 °C. The reaction mixture was warmed to ambient temperature and the solvent and excess HCl were removed *in vacuo*. The hydrochloride salt **4.29** was obtained as a white solid weighing 32 mg (90% yield) after it has been dried under high vacuum.

Boc-L-alanine (17 mg, 0.09, 1 equiv.) was dissolved in anhydrous DMF (1 mL) and the solution cooled in an ice/salt bath. HATU (34 mg, 0.09 mmol, 1 equiv.) and DIPEA (0.02 mL, 0.14 mmol, 1.5 equiv.) were sequentially added to the DMF solution which was then stirred for 20 min. under Ar. The hydrochloride salt **4.29** (32 mg, 0.09 mmol, 1 equiv.) was separately neutralized in 1 mL DMF using DIPEA (0.02 mL, 0.09 mmol, 1 equiv.). The latter solution was then added to the activated ester. The color of the solution turned from a pale yellow to a bright yellow-color. The solution was stirred under Ar for 17h at room temperature. The next day, DMF was removed *in vacuo* and the mixture directly subjected to purification by flash chromatography (gradient elution: 60–85% EtOAc/hexanes). The pure product **4.30** was obtained as a colorless oil and weighed 30 mg (70% yield).

TLC $R_f$ 0.31 (EtOAc/hexanes, 3:1)

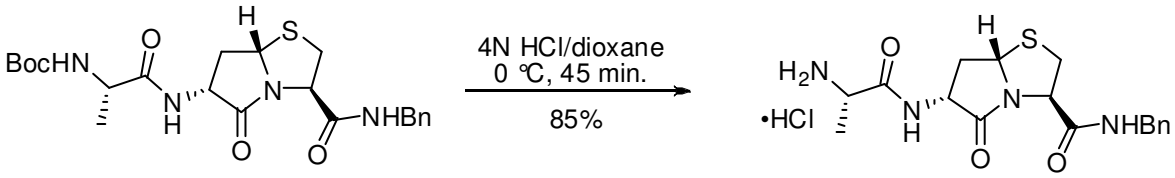
$[\alpha]_D$ –147.6 (*c* 1.45, $CH_3OH$)

$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment) δ 7.31–7.22 (m, 5H, Ar-C*H*), 5.09 (app. t, 1H, *J* = 6.3 Hz, 7a-C*H*), 4.97 (dd, AB pattern, 1H, $J_1$ = 6.9 Hz, $J_2$ = 4.5 Hz, 6-C*H*), 4.89 (m, 1H, 3-C*H*), 4.40 (m, AB pattern, 2H, C$H_2$Bn ), 4.09 (app. q, 1H, *J* = 5.7 Hz, Ala α-C*H*), 3.49–3.34 (m, 2H, 2-C$H_2$), 2.94 (ddd, 1H, $J_1$ = 15.3 Hz, $J_2$ = 8.7 Hz, $J_3$ = 6.9 Hz, 7-C$H_2$), 2.15–2.05 (m, 1H, 7-C$H_2$), 1.44 (C(C$H_3$)$_3$), 1.31 (d, 3H, $J_1$ = 7.2 Hz, Ala-C$H_3$)

$^{13}$C NMR (75 MHz, $CDCl_3$, gHMQC assignment) δ 174.6 (amide *C*=O), 173.0 (lactam *C*=O), 169.9 (amide *C*=O), 156.3 (carbamate *C*=O), 138.4 (Ar-*C*), 128.3, 127.1, (Ar-C*H*), 79.5 (*C*$(CH_3)_3$), 62.0 (7a-*C*), 60.2 (3-*C*), 53.3 (6-*C*), 50.5 (Ala α-*C*), 43.1 (*C*$H_2$Bn), 36.8 (7-*C*), 34.9 (2-*C*), 27.6 (C(*C*$H_3)_3$), 17.4 (Ala *C*$H_3$)

ESI HRMS *m/z* 485.1814 $[M+Na]^+$ $(C_{22}H_{30}N_4O_5S+Na)^+$ requires 485.1829

**(3*R*,6*R*,7a*S*)-Benzyl 6-[(2'*S*)-Amino-1'-oxopropane]amino-5-oxo-(6*H*)-pyrrolo[2,1-*b*]thiazole-3-carboxamide Hydrochloride salt (4.1a)**




**4.30** **4.1a**

A solution of 4N HCl in dioxane (3 mL) was added to the Boc-protected bicyclic thiazolidine benzylamide **4.30** (73 mg, 0.16 mmol, 1 equiv.). The solution was stirred for 45 min. at 0 °C. The reaction mixture was warmed to ambient temperature and the solvent and excess HCl were removed *in vacuo*. The hydrochloride salt **4.1a** was obtained as a white solid weighing 32 mg (90% yield) after it has been dried under high vacuum.

TLC $R_f$ 0.31 (EtOAc/hexanes, 3:1)

$[\alpha]_D$ –147.6 (*c* 1.45, $CH_3OH$)

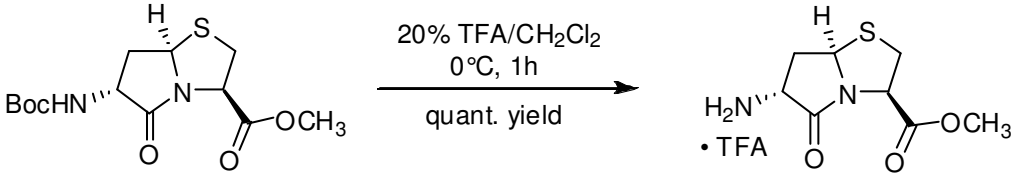
$^1$H NMR (300 MHz, $CDCl_3$, gCOSY assignment) δ 7.31–7.22 (m, 5H, Ar-C*H*), 5.09 (app. t, 1H, *J* = 6.3 Hz, 7a-C*H*), 4.97 (dd, AB pattern, 1H, $J_1$ = 6.9 Hz, $J_2$ = 4.5 Hz, 6-C*H*), 4.89 (m, 1H, 3-C*H*), 4.40 (m, AB pattern, 2H, C$H_2$Bn ), 4.09 (app. q, 1H, *J* = 5.7 Hz, Ala α-

C*H*), 3.49–3.34 (m, 2H, 2-C$H_2$), 2.94 (ddd, 1H, $J_1$ = 15.3 Hz, $J_2$ = 8.7 Hz, $J_3$ = 6.9 Hz, 7-C$H_2$), 2.15–2.05 (m, 1H, 7-C$H_2$), 1.44 (C(C$H_3$)$_3$), 1.31 (d, 3H, $J_1$ = 7.2 Hz, Ala-C$H_3$)

$^{13}$C NMR (75 MHz, $CDCl_3$, gHMQC assignment) δ 174.6 (amide *C*=O), 173.0 (lactam *C*=O), 169.9 (amide *C*=O), 156.3 (carbamate *C*=O), 138.4 (Ar-*C*), 128.3, 127.1, (Ar-C*H*), 79.5 (*C*(CH$_3$)$_3$), 62.0 (7a-*C*), 60.2 (3-*C*), 53.3 (6-*C*), 50.5 (Ala α-*C*), 43.1 (*C*H$_2$Bn), 36.8 (7-*C*), 34.9 (2-*C*), 27.6 (C(*C*H$_3$)$_3$), 17.4 (Ala *C*H$_3$)

ESI HRMS *m/z* 485.1814 $[M+Na]^+$ $(C_{22}H_{30}N_4O_5S+Na)^+$ requires 485.1829

**(3*R*,6*R*,7a*R*)-Methyl 6-Amino-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate• Trifluoroacetic acid (4.22)**




**4.2b** **4.22**

The bicyclic thiazolidine minor diastereomer **4.2b** was dissolved in a solution of 20–25% trifluoroacetic acid in anhydrous DCM. The solution was cooled to 0 °C and the reaction stirred in a closed flask until complete consumption was determined by TLC analysis. This was typically between 45–60 min. After this period of time, the volatiles were removed *in vacuo*. Excess TFA was removed by azeotroping it from DCM (2x) and xylenes (2x) and the pale yellow-colored residue **4.22** was dried for 1 d under high vacuum. Usually quantitative yield was obtained.

$^1$H NMR (300 MHz, $CD_3OD$, assignments based in *J* values) δ 5.30 (dd, 1H, $J_1$ = 6.9 Hz, $J_2$ = 5.4 Hz, 7a-C*H*), 4.38 (dd, 1H, $J_1$ = 8.1 Hz, $J_2$ = 3.0 Hz, 3-C*H*), 4.22 (dd, 1H, $J_1$ = 9.6 Hz, $J_2$ = 4.5 Hz, 6-C*H*), 3.76 (s, 3H, OC$H_3$), 3.70 (dd, 1H, $J_{1(geminal)}$ = 12.3 Hz, $J_2$ = 7.5 Hz,

2-C$H_{\text{trans to 3-C}H}$), 3.49 (dd, 1H, $J_{1(\text{geminal})}$ = 12.3 Hz, $J_2$ = 3.0 Hz, 2-C$H_{\text{cis to 3-C}H}$), 2.71 (ddd, 1H, $J_{1(\text{geminal})}$ = 14.7 Hz, $J_2$ = 9.6 Hz, $J_3$ = 5.4 Hz, 7-C$H_{\text{cis to 7a-C}H}$), 2.54 (ddd, 1H, $J_{1(\text{geminal})}$ = 14.7 Hz, $J_2$ = 6.9 Hz, $J_3$ = 4.5 Hz, 7-C$H_{\text{trans to 7a-C}H}$)

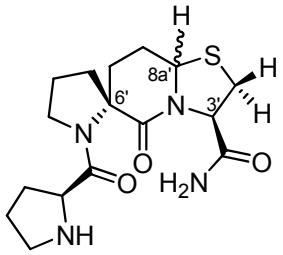
$^{13}$C NMR (75 MHz, $CD_3OD$) δ 169.7, 169.2 (*C*=O), 66.7 (7a-*C*), 58.7 (3-*C*), 54.5 (6-*C*), 53.3 (O*C*$H_3$), 39.5 (7-*C*), 29.6 (2-*C*)

Chapter 5

*Design and Synthesis of Peptidomimetic Negative Allosteric Modulators of the Dopamine $D_2$ Receptor Based on the Endogenous Tripeptide, Pro-Leu-Gly-$NH_2$ (PLG)*

## 5.1. Background and Significance

The endogenous tripeptide neurotransmitter, Pro-Leu-Gly-$NH_2$ (PLG) has unique neuropharmacological activity. PLG modulates dopaminergic neurotransmission within the central nervous system (CNS) in an allosteric manner.[128,129] Until recently, PLG and its peptidomimetic analogues were shown to be positive allosteric modulators of the dopamine $D_2$ and $D_4$ receptors.[130–132] We have demonstrated that a diastereomeric pair of 5.6.5-spiro bicyclic lactam polyproline II helix mimics (Figure 5.1.) that differ in stereochemistry about a single center (C-8a') have, in fact, *opposite* dopamine $D_2$ modulatory activities; that is, **5.1a** is a positive modulator, while **5.1b** reduces the affinity of traditional $D_2$ agonists at their orthosteric site, and thereby is a *negative* $D_2$ receptor modulator.[133]




**5.1a**: 8'a = *R* ( ⋯ )
**5.1b**: 8'a = *S* ( ▬ )

**Figure 5.1.** C-8a' epimeric pair of modulators with *opposite* activities

We believe that an arsenal of conformationally constrained analogues of PLG that are capable of producing either a positive or a negative modulatory response will aid

in our overall goal of understanding the structural and biochemical mechanism of the allosteric modulation of the $D_2$ receptor by PLG.

### 5.2. Clinical requirement for developing $D_2$ allosteric modulators

The dopamine receptors are part of the G-protein-coupled receptor (GPCR) superfamily of integral membrane-bound receptors within the mammalian system.[134] The dopaminergic receptors are classified into: (1) presynaptic $D_1$-like or (2) post-synaptic $D_2$-like, depending upon the stimulation or inhibition of the dopamine-dependent intracellular adenylyl cyclase activity.[135] In relevance to this discussion, the presence of the $D_2$ receptors in the substantia nigra, striatum and the limbic cortex directly implicates the involvement of the $D_2$ subtype in normal motor control and cognitive functioning.

Impaired receptor pharmacology resulting from either the destruction of receptors or via changes in receptor sensitivity toward the natural ligand, dopamine, has been implicated in neurological disorders such as Parkinson's disease (PD), schizophrenia, Gilles de la Tourette syndrome and tardive dyskinesia, among others.[136, 137] Parkinson's disease is in most part characterized by dysfunctional motor neuron activity, caused mainly by destruction of the substantia nigra region of the CNS, which in turn is essential for the proper functioning of the basal ganglia. Since normal motor control is achieved by an equilibrium between the dopaminergic and cholinergic response, the destruction of dopaminergic neurons leads to unchecked excitatory activity characterized by tremors, muscular rigidity, bradykinesia and gait disturbances.[138, 139]

The most widely used treatment for PD is L-3,4-dihydroxy phenylalanine (L-DOPA), which is the precursor to endogenous dopamine. Clinically administered L-DOPA is converted to dopamine within the dopaminergic neurons (via the action of DOPA decarboxylase) and attempts to restore dopamine levels in the CNS. The

prolonged clinical use of L-DOPA is not without drawbacks. These include: (1) poor bioavailability of L-DOPA within the neurons, since majority of the drug is metabolized within the peripheral system and (2) prolonged therapy is ineffective, since with disease progression, motor complications, including dyskinesia (involuntary movements) become increasingly evident.[140, 141] To counteract the former, a peripheral DOPA decarboxylase inhibitor (e.g. carbidopa) is administered in conjunction with L-DOPA .

A second line of therapy involves the use of dopamine receptor agonists. The use of agonists, however, is only moderately effective and is accompanied by a battery of side effects that can include: somnolence, hallucinations, nausea, dizziness etc. These agonists are also non-selective toward any particular dopamine receptor subtype. It has also been observed that dopamine receptor sensitivity undergoes marked change during the course of agonist administration. For example, deficiency of dopaminergic stimulation initially leads to a rebound over-expression of $D_1$ and $D_2$ dopamine receptors, which causes clinical super-sensitivity to dopamine or its agonists in the early stages. Although this can be reversed by L-DOPA administration, prolonged use of such drugs can lead to desensitization of the receptors and renders the therapy ineffective.[141]

### 5.3. Concept of allosteric modulation

Based on the above observations, it is imperative to develop therapeutic agents with a mechanism characterized by greater control over receptor selectivity and sensitivity. In this context, we believe that *allosteric modulation* is a novel approach of fine-tuning dopamine receptor response to the action of direct-acting agonists or antagonists.[142] As shown in Figure 5.2., allosteric modulators bind to a secondary site (allosteric site) that is topographically distinct from the orthosteric site on the relevant GPCR[142–144] causing a conformational change in the receptor. Allosteric occupation

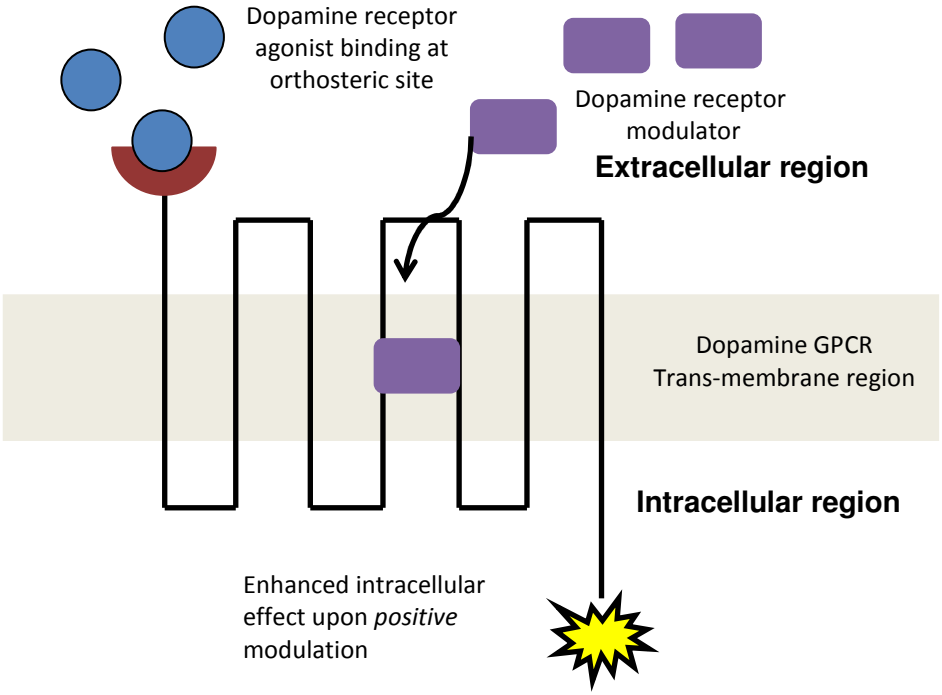


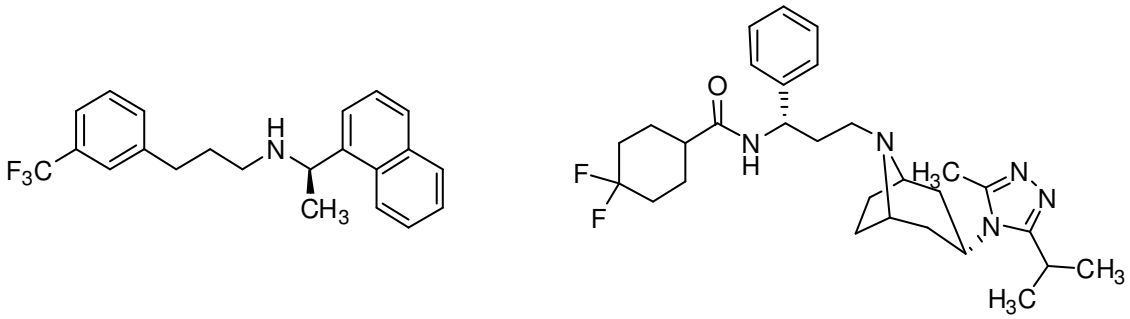

**Figure 5.2.** Allosteric modulation of GPCRs

can therefore lead to modification of the interactive properties of the receptor (e.g., GPCR) with respect to orthosteric ligands as well as the intracellular environment in either a positive or a negative manner.

Typically, allosteric modulators can effect the following pharmacological events: (1) affinity modulation, in which the resulting conformational change impacts the affinity of the receptor for the orthosteric ligand (e.g. the rate of association or dissociation or both of an agonist are affected) and/or (2) efficacy modulation, in which the conformational change causes a change in the intensity of the intracellular signaling process. Allosteric modulation has several advantages over conventional drug-receptor targeting. First, in the case of affinity modulation, the allosteric modulator is latent in the absence of the orthosteric ligand and exerts its effects *only* following the binding of the

ligand to its orthosteric site. Such molecules therefore have the capacity to maintain the temporal and spatial aspects of endogenous signaling. Second, since affinity modulation only effects a conformational change, the activity of the modulator (in the positive or negative direction) is limited by the cooperativity factor, which imposes a threshold on the magnitude of the effect. Therefore, a high degree of control can be imposed on the desired pharmacological effect.[144] Finally, there is a possibility of higher receptor sub-type selectivity in the presence of allosteric modulators resulting from the fact that: (1) compared to highly conserved orthosteric binding domains within GPCR subfamilies, there is less sequence homology among the various allosteric binding sites within the same sub-families[145] and (2) the cooperativity factor can be highly specific for a particular GPCR sub-type.[146]

For some classes of GPCRs, (e.g. muscarinic receptors, metabotropic glutamate receptors-mGluRs) only the location of the allosteric site has been determined based on mutagenesis experiments.[142] Currently, there are two marketed drugs that work via GPCR allosteric modulation, Cinacalcet and Maraviroc (Figure 5.3.). Although there has




Cincalcet
(Calcium sensing receptor
Positive allosteric modulator)

Maraviroc
(Chemokine CCR5
Negative allosteric modulator)

**Figure 5.3.** Marketed allosteric modulators

been an increased fervor in exploring alternative allosteric strategies targeting GPCRs, the overall biochemical mechanism of allosteric modulation remains elusive.

### 5.4. Pharmacological effects of PLG mediated via the $D_2$ receptor

PLG (Figure 5.4.) was first isolated from brain tissue and determined to be the *C*-terminal metabolite of the pituitary peptide hormone, oxytocin. Due to its regulatory effect on melanocyte stimulating hormone (MSH), PLG was initially identified as melanostatin or MSH-release inhibitory factor (MIF-1).[147] Further studies have demonstrated that its main neuropharmacological activity involves positive modulation of the dopamine receptors.[128]

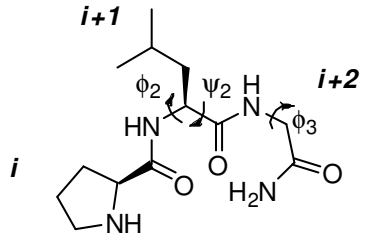



**Figure 5.4.** Structure of PLG

This modulation is specific for the dopamine receptors and PLG does not interact with adrenergic,[129] serotonergic,[148] or GABA-ergic receptors.[149] The effects of positive modulation are also specific within the dopaminergic receptor subtype; studies carried out in cell lines transfected with human dopamine receptor subtypes have shown that PLG enhances agonist binding to the $D_2$ and $D_4$ subtypes, whereas the $D_1$ and $D_3$ subtypes are unaffected.[150] Additionally, it has been demonstrated that PLG does not directly affect the synthesis, uptake or metabolism of dopamine itself.[151] Finally, it is

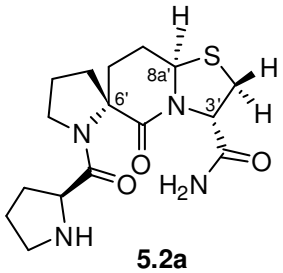
significant that PLG has been shown to modulate the $D_2$ receptors in the presence of agonists, but not antagonists.[129]

### 5.5. Probing the bioactive conformation of PLG

The overall research goal in our laboratory has been to elucidate the bioactive conformation of biologically relevant peptides such as PLG. In this regard, we are specifically interested in understanding the conformation adopted by PLG during its interaction with the allosteric site on the dopamine $D_2$ receptor. Since there is always an entropic cost of maintaining a ligand at its active site in a specific orientation, with minimal deviation about freely rotatable bonds; we believe that the use of conformationally constrained analogues of PLG will lead to: (1) determination of the bioactive conformation of PLG at its active site and (2) the design of more potent PLG analogues with higher binding affinity to the receptor site.

The main approach taken in our research is the systematic restriction about the rotatable bonds; this involves constraining the back-bone torsion angles within the tripeptide sequence of PLG. Since original solid-state[152] and solution-state studies[153] (X-ray crystallographic and NMR studies, respectively) had indicated that PLG might exist in a type II *β*-turn conformation, our initial efforts were directed toward designing analogues of PLG where the $\varphi_2$, $\psi_2$, and $\varphi_3$ torsion angles were held as close as possible to those found in an ideal type II *β*-turn. As shown in Figure 5.5., **5.2a** which possesses the highly rigid 5.6.5 spiro bicyclic lactam type II *β*-turn mimic, was found to be significantly more potent than PLG in both the $^3$[H]spiroperidol/*N*-propylnorapomorphine (NPA) competition binding assay and the 6-hydroxydopamine (6-OHDA)-lesioned model of PD. This conformationally constrained analogue of PLG also increased, to a more significant degree than PLG, the ratio of dopamine receptors in the

high-affinity state. The results with this highly constrained PLG peptidomimetic provided strong support for the hypothesis that the bioactive conformation of PLG is a type II *β*-turn.[132]




**5.2a**

**Figure 5.5.** Most active type II *β*-turn mimic of PLG

The following *in vitro* and *in vivo* pharmacological studies and their results indicate that modulation of the $D_2$-like receptors by PLG and its active peptidomimetic analogues (e.g., **5.2a** among others) is affected by increasing the sensitivity of the receptor toward dopaminergic orthosteric agonists.

1. In *in vitro* radio-ligand binding assay, PLG selectively enhances the binding affinity of dopamine receptor agonists, such as: 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN),[154, 155] apomorphine,[156–158] and *N*-propylnorapomorphine (NPA)[129, 159] to the dopamine receptors.
2. PLG inhibits dopamine-dependent adenylyl cyclase activity in rat striatal membranes.[160] As discussed before, this is indicative of binding to the $D_2$-like receptor sub-type.
3. It has been shown that the dopamine $D_2$ receptors exist in two states: (1) $D_H$ , the high affinity state and (2) $D_L$, the low affinity state. Only the high affinity state is coupled to the inhibitory guanine nucleotide regulatory protein ($G_i$), the main G-protein in the context of the GPCR that forms the link between ligand binding and

adenylyl cyclase activity. Agonists and antagonists of the $D_2$ receptor favor the two states in an orthogonal manner. PLG enhances agonist-stimulated (e.g. NPA) GTPase activity in rat striatal membranes,[160] an observation that is consistent with the hypothesis that GTP hydrolysis is involved in maintaining high-affinity agonist binding to the $D_2$ receptor by PLG.

4. *In vivo* studies have demonstrated that PLG potentiates the effects of L-DOPA[161] and apomorphine.[162]
5. Lesions within the nigrostriatal region of rat brain caused by 6-hydroxydopamine administration results in decreased $D_2$ receptor density, which temporarily causes rebound receptor supersensitivity and leads to increased rotational behavior within the rat upon agonist administration. PLG and its peptidomimetics enhance both amphetamine and apomorphine-dependent rotational behavior in 6-hydroxydopamine lesioned rats.[163–165]
6. Oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the toxic 1-methyl-4-phenylpyridinium cation ($MPP^+$) leads to destruction of dopamine-producing neurons in the substantia nigra and is frequently used as a rat PD model. PLG and its analogues have been found to be partially neuroprotective against this destruction.[166, 167]

As was discussed previously (Chapter 1 p. 3–4), the original triproline scaffold (L-Pro-L-Pro-D-Pro-$NH_2$ (**5.3b**) and L-Pro-L-Pro-L-Pro-$NH_2$ (**5.3a**)) was designed to replace the highly constrained spiro bicyclic analogue with a more flexible back-bone, where only the φ torsion angles of the i+1 and i+2 residues were constrained by the natural pyrrolidine ring of proline (Pro).[168] Since a type II *β*-turn cannot be adopted when the i+2 position is held by L-Pro as in **5.3a**, this analogue was originally designed as a negative

control. However, L-Pro-L-Pro-L-Pro-$NH_2$ was pharmacologically effective within the *in vitro* and *in vivo* studies, while the triproline capable of adopting the type II *β*-turn, L-Pro-L-Pro-D-Pro-$NH_2$ was less active.[165, 168] a result that was inconsistent with the basic hypothesis that the bioactive conformation of PLG was a type II *β*-turn.

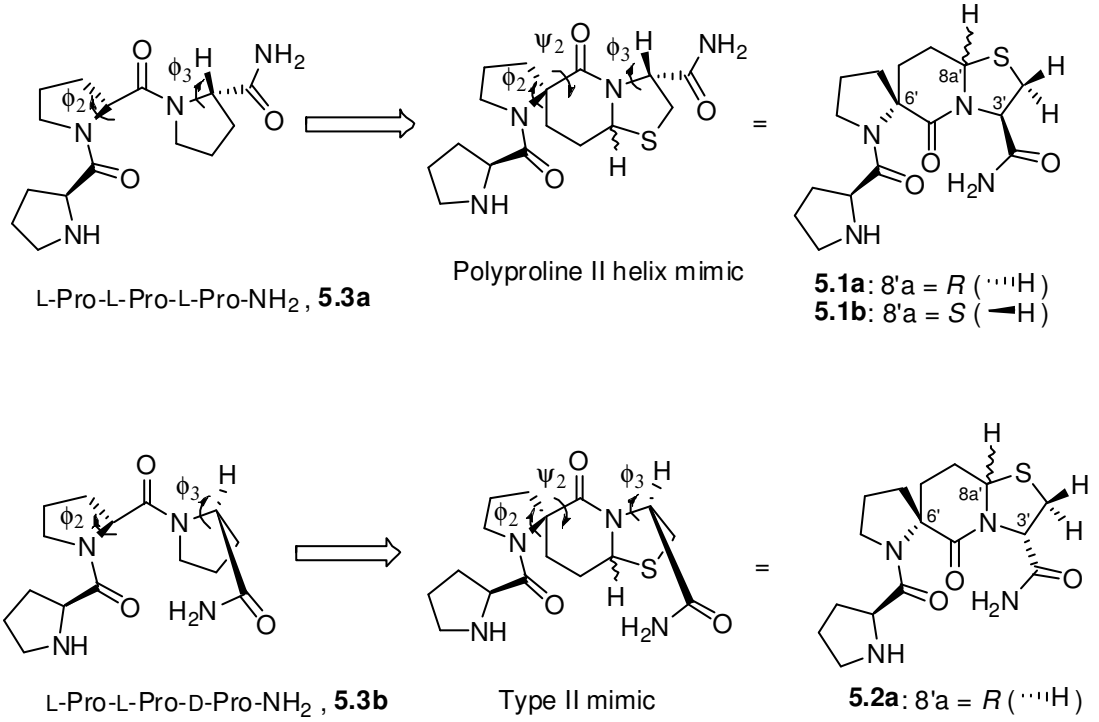



**Figure 5.6.** Type II *β*-turn and polyproline II helix mimics of PLG

In order to reconcile this anomaly we hypothesized that the all L-triproline PLG analogue **5.3a** adopted a different conformation that placed the important pharmacophores in the same orientation as done by highly active type II *β*-turn mimicking analogues of PLG, such as **5.2a**. Therefore, we decided to explore the possibility of two seemingly different secondary structures. In addition to the possibility of a type VIa *β*-turn (as discussed in Chapter 1), in an all-*trans* amide bond situation, a

non-turn secondary structure that could also be envisioned for L-Pro-L-Pro-L-Pro-$NH_2$. This is the polyproline II helix conformation.

On the basis of torsion angle comparison of ideal type II *β*-turn and polyproline II helix as well as computational overlays of energy minimized triproline analogue, *N*-acetyl-L-Pro-L-Pro-$NH_2$ (Ac-Pro-Pro-$NH_2$) and the 5.6.5-spiro bicyclic lactam **5.2a**, we concluded that inverting the stereochemistry about the C-3' center of the rigid type II *β*-turn mimic (which uses a derivative of L-cysteine instead of D-cysteine) would result in a polyproline II helix mimic. Both bridgehead (C-8a') epimers were synthesized to test the hypothesis (Figure 5.6., **5.1a** and **5.1b**).

Compounds **5.1a** and **5.1b** were subjected to pharmacological evaluation similar to **5.2a**.[133] Analogue **1a** behaved in a manner similar to PLG and previously tested PLG analogues (e.g. **5.2a**) wherein both enhanced the binding of a tritiated $D_2$ agonist (e.g. [$^3$H]-NPA) to isolated dopamine $D_2$ receptors and they exhibited bell-shaped dose response curves. Interestingly, the C-8a' epimer of **5.1a**, the spiro bicyclic compound **5.1b**, *decreased* the binding of [$^3$H]-NPA to isolated dopamine $D_2$ receptors in a characteristic bell-shaped dose-response curve. It is important to understand that all three PLG analogues were tested for their ability to bind to the *same allosteric* site on the $D_2$ receptor by a competitive radioligand binding assay.[133]

### 5.6. Rationale for negative modulatory activity: Structural perturbations in the molecule due to differences in C-8a' stereochemistries

Molecular modeling studies were conducted on the pharmacologically tested molecules in the hope of understanding the *opposing* modulatory activities of **5.1a** and **5.1b**. Computational overlay (Figure 5.7.A) indicated that majority of the energy-minimized 5.6.5-spiro bicyclic scaffold of **5.2a** matched with the minimized structure of **5.1a**, except for the carboxamide region. This was expected if one compares the back-

bone torsion angles for an ideal type II $\beta$-turn and polyproline II helix ($\psi_3$ = 0 ° vs. 155 ° respectively). A second overlay of the Ac-Pro-Pro-$NH_2$ polyproline and **5.2a** confirms this opposing hinging of the primary carboxamide (Figure 5.7.B). The most important overlay was that between **5.1a** and two different energy-minimized conformations of **5.1b**, since any deviation from matched conformation could hypothetically be responsible for the opposing pharmacological activity observed for **5.1b**. As Figure 5.7.C indicates, while most of the two molecules align with each other, the main difference is in the directionality of the C-2' methylene (circled in the figure).

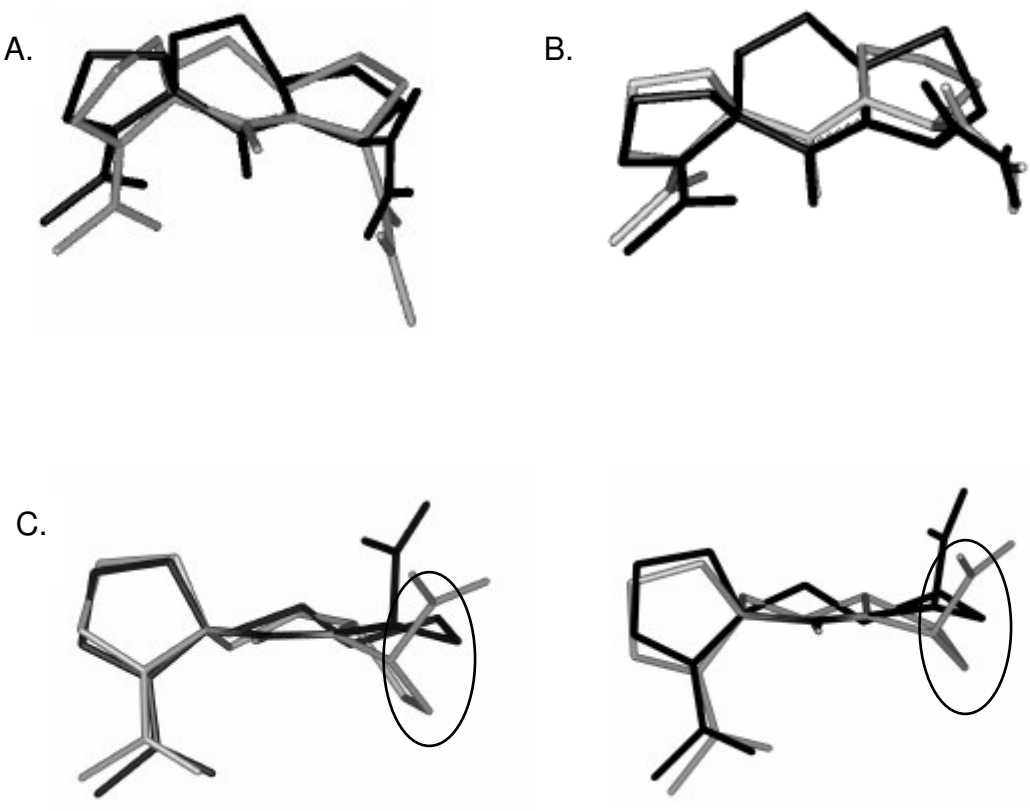
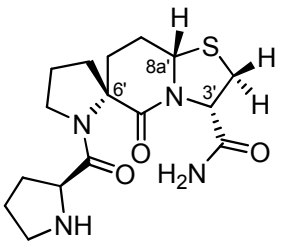



**Figure 5.7.** Overlays of (A) 5.6.5-spirobicyclic type II $\beta$-turn mimic **5.2a** (gray) and polyproline II helix mimic **5.1a** (black), (B) Ac-Pro-Pro-$NH_2$ (gray) and polyproline II helix

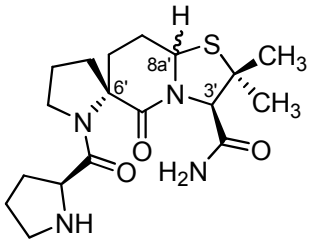
mimic **5.1a** (black), (C) Polyproline II helix mimic-positive modulator **5.1a** (black) and two conformations of polyproline II helix mimic-negative modulator **5.1b** (gray)

### 5.7. Molecular design to test the hypothesis for negative modulatory activity of 5.6.5-spirobicyclic PLG analogue

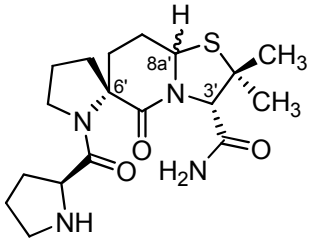
On the basis of molecular modeling studies discussed above, we postulate that the negative modulatory effect of **5.1b** is brought about by positioning the C-2' methylene group into a different area of space within the allosteric binding site on the dopamine $D_2$ receptor. To test this hypothesis, the following series of molecules will be synthesized and tested (Figure 5.8).



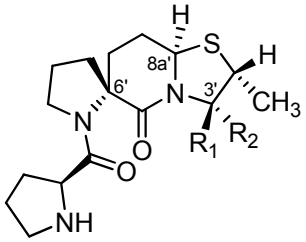
**5.2b**



**5.4a: 8a' = *R* (⋯)**
**5.4b: 8a' = *S* (▬)**



**5.5a: 8a' = *R* (⋯)**
**5.5b: 8a' = *S* (▬)**



**5.6a: $R_1$ = H; $R_2$ = $CONH_2$**
**5.6b: $R_1$ = $CONH_2$; $R_2$ = H**

**Figure 5.8.** Proposed dopamine $D_2$ receptor modulators based on PLG

1. *Convert positive modulator to a negative modulator*: Molecular modeling and pharmacological precedent of the 5.6.5-spiro bicyclic polyproline II helix mimics, **5.1a** and **5.1b**, suggest that by inverting the stereochemistry about the C-8a' (bridgehead carbon) of **5.2a**, the corresponding diastereomer, **5.2b**, would be capable of adopting a conformation identical to that found within the established negative modulator, **5.1b**.
2. *Enhance the negative modulatory activity of **5.1b** via C-2' substitution*: We anticipate that by a double substitution of the C-2' methylene of the existing negative modulator **5.1b** with methyl groups, we might be able to enhance this opposing activity by further perturbing the projection of this region of the molecule into the active site on the $D_2$ receptor. Although the promising target here would be **5.4b**, on the basis of the divergent synthetic route and eventual results from testing **5.1b**, we also decided to simultaneously attempt the synthesis of **5.4a** as well as **5.5a** and **5.5b**.
3. *Substitutions of the C-2' methylene of the positive modulators*: We anticipate that placing a methyl substitution on the C-2' carbon of the positive modulators **5.1a** and **5.2a**, will lead to the formation of molecules **5.6a** and **5.6b**, capable of negative modulatory action. We hope that this methyl group will project in the same space that the C-2' methylene occupies within the existing negative modulator **5.1b**.

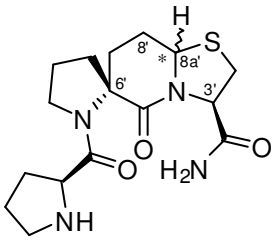
Chapter 6

*Synthesis and pharmacological evaluation of the constrained 5.6.5-spiro bicyclic lactams capable of negatively modulating the dopaminergic response via the PLG allosteric site*

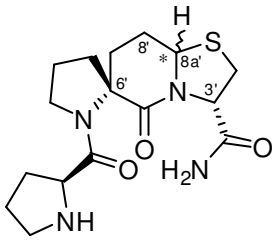
## 6.1. Introduction

This chapter will be divided into the following parts:

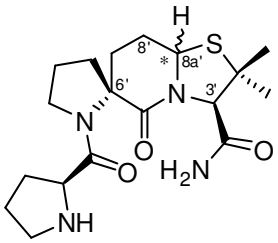
1) Synthesis of negative modulators
   a) Representative retrosynthetic analysis (**5.2b**)
   b) Synthesis of *N*-Boc-α-(propan-3'-al)proline, **6.4**
   c) Synthesis of the second negative modulator, **5.2b**
   d) Synthesis of potential modulators, **5.4b** and **5.5a**
   e) Stereochemical outcome of the condensation/lactamization sequence



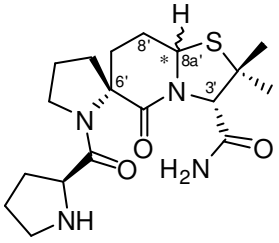
**5.1a: * = *R*** (dashed)
**5.1b: * = *S*** (wedge)



**5.2a: * = *R*** (dashed)
**5.2b: * = *S*** (wedge)



**5.4a: * = *R*** (dashed)
**5.4b: * = *S*** (wedge)



**5.5a: * = *R*** (dashed)
**5.5b: * = *S*** (wedge)

**Figure 6.1.** 5.6.5-Spiro bicyclic lactams based on PLG

f) Challenges in ammonolysis and/or $NH_3$ amidation reaction

g) Advantages of altering order of reactions

2) Pharmacological evaluation

a) Negative modulatory activity

3) Future studies

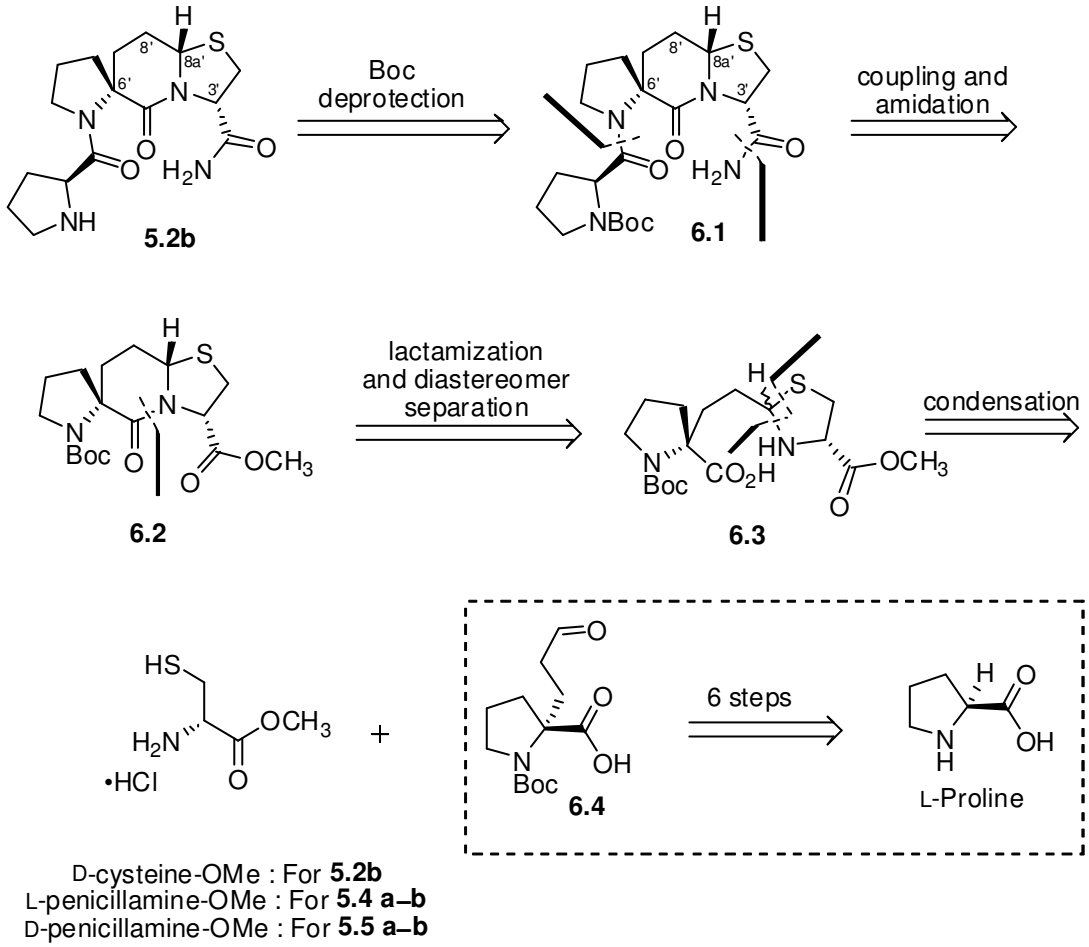
a) Pharmacolgical testing of **5.4 a–b** and **5.5 a–b**.

b) Completion of *β*-methylcysteine synthesis, **5.6 a–b**

### 6.2. Retrosynthesis

A representative disconnection analysis (for **5.2b**) is shown in Scheme 6.1. The core scaffold of **5.2b**, is the 5.6.5-spiro bicyclic lactam, **6.2**, which can be potentially accessed through lactamization of the diastereomeric thiazolidine adduct mixture **6.3** that is formed from the condensation between an ester of D-cysteine with the prolyl aldehyde derivative **6.4**. The stereochemical configuration about the $\alpha$-carbon of the aldehyde derivative can be created by once again applying Seebach's "self-regeneration of stereocenters" principle starting with L-proline. For synthesis of the other target molecules, the D-cysteine derivative can be substituted by D- and L-penicillamine derivatives. Unprotected penicillamine is commercially available in both enantiopure forms.

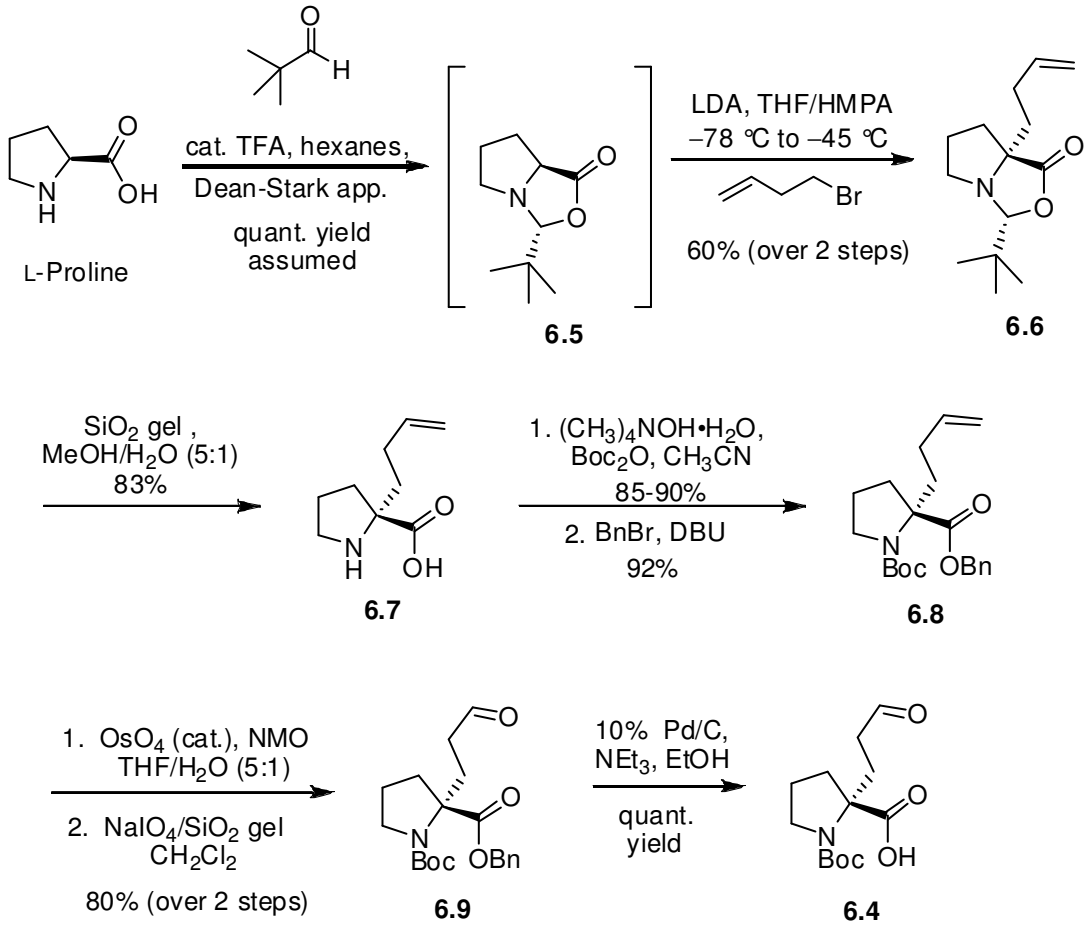
**Scheme 6.1.** Representative retrosynthetic analysis




### 6.3. Synthesis of (*R*)-*N*-Boc-2-(propan-3'-al)pyrrolidine-2-carboxylate

L-Proline was used as the starting material in the synthesis of the common aldehyde intermediate **6.4** (Scheme 6.2.); enantioselective α-alkylation of proline was achieved by applying Seebach's "Self-regeneration of Stereocenters" strategy once again.[169] Majority of this scheme was first developed by Dr. Ehab Khalil[170] and subsequently improved by Dr. Bhooma Raghavan,[171] both past graduate students in our laboratory. In this method, L-proline was first condensed with pivalaldehyde to form the *tert*-butyloxazolidinone **6.5** (also see Chapter 2).The procedure for oxazolidinone

**Scheme 6.2.** Synthesis of the common aldehyde intermediate




formation and the rationale for the diastereospecificity have been discussed in detail in Chapter 2. As discussed before this oxazolidinone is very moisture-labile and was therefore immediately subjected to the subsequent enolization and alkylation using 4-bromo-1-butene. In order to improve the efficiency of alkylation, the nucleophilicity of the enolate was increased by conducting the reaction in the presence of one equivalent of a co-solvent such as hexamethylphosphoramide (HMPA). Unfortunately, it had been previously determined that replacing HMPA with a less-toxic additive such as 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (or *N,N*-dimethylpropylene urea, DMPU)[172] was not advantageous as it did not improve reaction yields as compared to

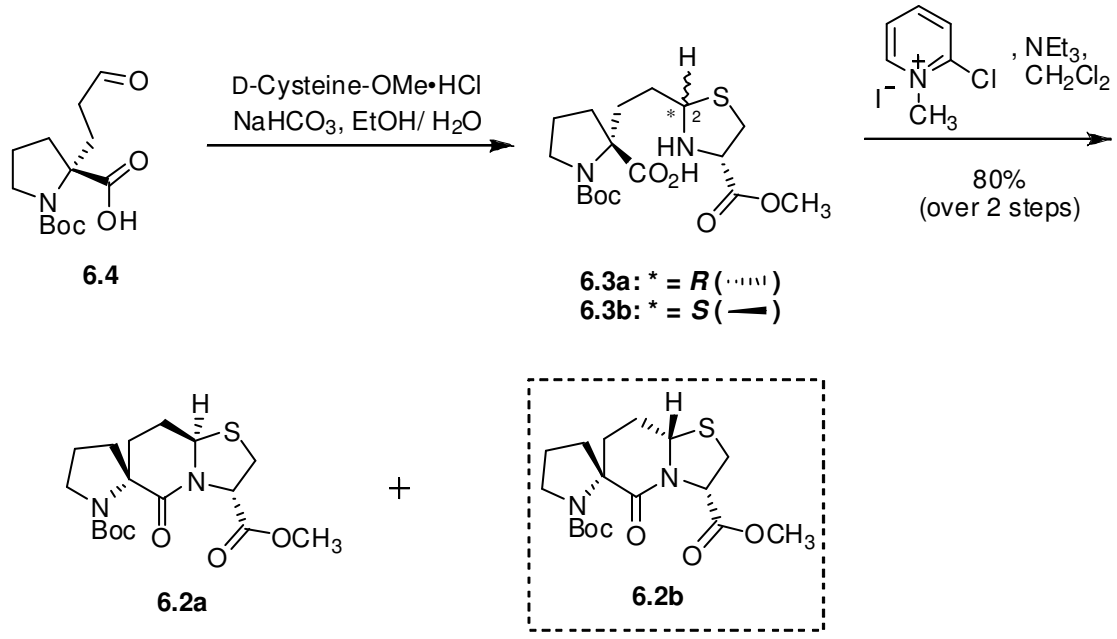
background yields when the reaction was conducted in the absence of any co-solvents. The enolate formation is conducted at –78 °C, while the 6-hour diastereospecific alkylation is conducted at a slightly higher temperature of –45 °C. Although the oxazolidinone is labile in the presence of silica gel (due to the latter's acidic nature), the product can be purified by flash chromatography while eluting with a mixture of hexanes and ethyl acetate. The α-butenyl oxazolidinone **6.6** is stable under ambient conditions for a few days; slow hydrolysis becomes evident by increased presence of a white precipitate in the colorless-yellow oil. It should be noted that in contrast to the crystalline nature of α-allyloxazolidinone at temperatures < 25 °C, the higher homologue is a colorless oil of very light viscosity even at –20 °C.

Silica gel-mediated hydrolysis[173] of the oxazolidinone **6.6** gave rise to the corresponding α-butenylproline **6.7** which can be re-crystallized to give the pure α-imino acid. Both the *N*- and *C*-terminals of the proline derivative were then protected in that order to give rise to the *N*-Boc-α-butenylproline benzyl ester **6.8**. Oxidative cleavage of the olefin to the corresponding aldehyde was then conducted using the two-step protocol. Catalytic osmium tetroxide ($OsO_4$) and *N*-methyl morpholine *N*-oxide (NMO) were first used to form the vicinal diol, followed by silica gel-supported sodium periodate ($NaIO_4/SiO_2$ gel)-mediated diol cleavage to aldehyde **6.9**.[174] This two step sequence usually results in higher yields than the direct Lemieux-Johnson one-pot transformation that uses excess $NaIO_4$. The purified aldehyde **6.9** was then stored at 4 °C where it was stable for extended periods of time (several months). The subsequent hydrogenolytic removal of the benzyl ester to form **6.4** was conducted just before the ensuing condensation to form the diastereomeric spirobicyclic thiazolidines. This prevents formation of the internal lactone, which is rather unproductive when used in the subsequent step.

## 6.4. Synthesis of the negative modulator 5.2b: The diastereoisomeric 5.6.5-spiro bicyclic thiazolidines derived from D-cysteine

The *N*-Boc α-alkylated proline derivative **6.4** was subjected to a tandem condensation/lactamization sequence with the hydrochloride salt of D-cysteine methyl ester. As shown in Scheme 6.4, the condensation was conducted at slightly acidic to neutral pH (6.5–7.0) in an aqueous ethanolic solution. The intermediate equilibrating

**Scheme 6.3.** Synthesis of diastereomeric 5.6.5-spirobicylic thiazolidines




diastereomeric thiazolidine mixture containing **6.3a** and **6.3b** was obtained as a white-colored foam, which was immediately subjected to lactamization using 2-chloro-1-methylpyridinium iodide (CMPI) as the activating agent. As encountered in Chapter 4, CMPI facilitates the formation of an active ester at the free carboxylic acid center. The ester is then capable of undergoing lactamization at a faster rate than the diastereomer

interconversion rate, leading to the trapping of both diastereomeric 5.6.5-spiro bicyclic thiazolidines **6.2a** and **6.2b** in a 1:1 ratio.

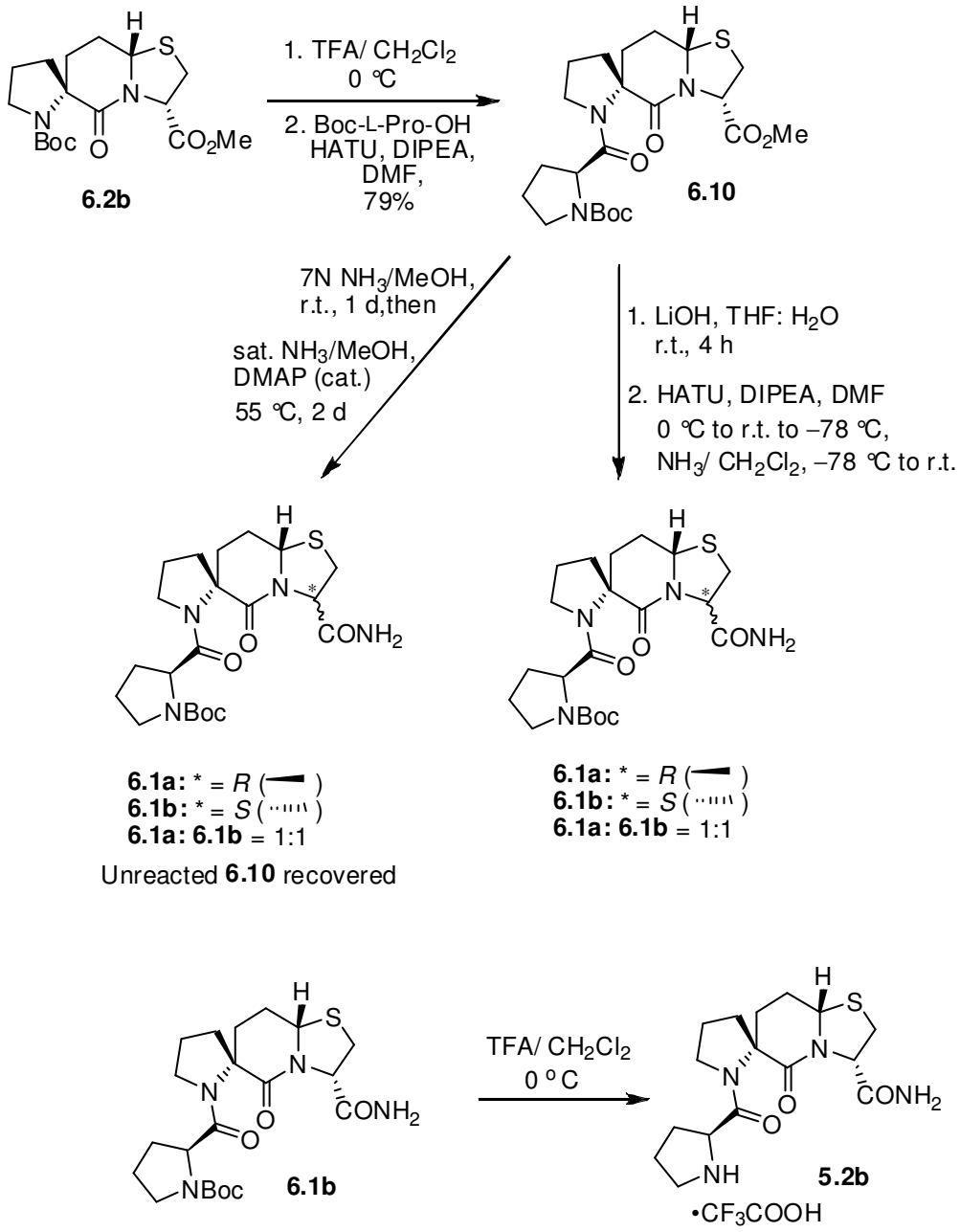
The diastereomers **6.2a** and **6.2b** were easily separated by flash chromatography and based on precedent (since **6.2a** has been synthesized before in our laboratory) the relative stereochemistry assigned by differences in the $^{1}$H NMR chemical shifts and 1-D Nuclear Overhauser Effect Spectroscopy (NOESY).

### 6.5. Synthesis of the negative modulator 5.2b: Challenges with the ammonolysis/amidation reactions

The synthesis of **5.2b** was achieved from **6.2b** as outlined in Scheme 6.4. Treatment of **6.2b** with trifluoroacetic acid to remove the *N*-Boc group gave the corresponding trifluoroacetate salt, which was coupled to Boc-L-Pro-OH using *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent. This provided **6.10** in a 79% yield. This represents a substantial improvement in yield and ease of the reaction as compared to previously developed conditions in our laboratory.[170, 171]

Initially, ammonolysis of **6.10** was attempted with a saturated solution of ammonia in dichloromethane/methanol first at room temperature then followed by heating to 55 °C for 2 days (in the presence of 10 mol% DMAP on the third day). These conditions only resulted in *partial conversion* of **6.10** to a 1:1 mixture of carboxamides **6.1a** and **6.1b.** This difficulty in ammonolysis was analogous to that observed with the diastereomer derived from L-cysteine-OMe•HCl, also an isomer where the bridgehead hydrogen and carboxamide group are in a *trans* relationship.[171,175] We therefore hydrolyzed the methyl ester and then coupled the resulting acid to ammonia using HATU

**Scheme 6.4.** Final steps toward synthesis of **5.2b**




as the coupling reagent. Although complete conversion was obtained, a 1:1 mixture of **6.1a** and **6.1b** was obtained once again, indicating epimerization at the C-3' position. It was determined by further experimentation that this competing reaction occurred during the ester hydrolysis step. Attempting ester hydrolysis under cold conditions (–5 °C to 0

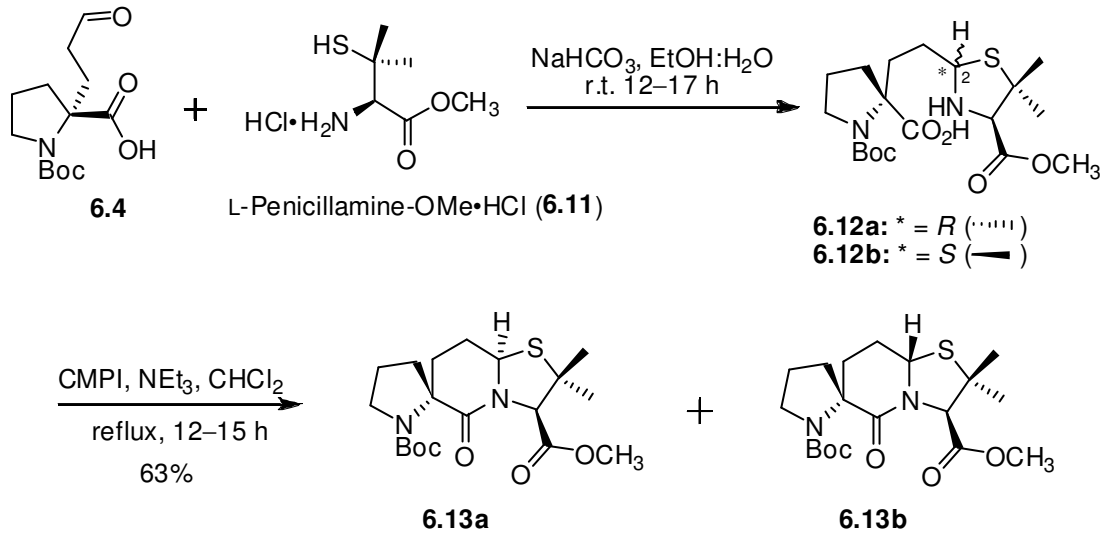
°C) only served to reduce the rate of the main hydrolysis reaction. Diastereoisomers **6.1b** and **6.1b** were therefore simply chromatographically separated. Removal of the *N*-Boc protecting group from **6.1b** with TFA/$CH_2Cl_2$ provided the desired PLG peptidomimetic **5.2b** as its trifluoroacetate salt, the purity of which was confirmed by HPLC analysis. The results of pharmacological testing of **5.2b** will be discussed in a later section.

### 6.6. Synthesis of dopaminergic modulators derived from L- and D-penicillamines

A similar sequence of condensation/lactamization was used to synthesize the potential dopaminergic modulators **5.4a** and **5.4b** derived from L-penicillamine and **5.5a** and **5.5b** derived from D-penicillamine. *N*-Boc-α-butenylproline **6.4** was condensed separately with the hydrochloride salts of both L- and D-penicillamine methyl esters **6.11** and **6.14** respectively, to give the corresponding pairs of 5.6.5-spiro bicyclic thiazolidines in 57% and 63% yields respectively (Schemes 6.5 and 6.6).

An important observation is the ratio of the diastereomers obtained for each reaction set. For the condensation/lactamization sequence with the L-penicillamine derivative, a 3:1 ratio of the two C-8a' epimers **6.13a** and **6.13b** was obtained with the major diastereromer **6.13a** having the C-8a' α-hydrogen (bridgehead proton down) configuration; while for the reaction sequence with D-penicillamine-OMe•HCl, a 1:1 ratio of the diastereomers **6.16a** and **6.16b** was obtained. This directly mirrors the diastereoselectivities observed previously with D- and L-cysteine-OMe•HCl respectively.[170, 171]

**Scheme 6.5.** Synthesis of the 5.6.5-spiro bicyclic lactams derived from L-penicillamine-OMe



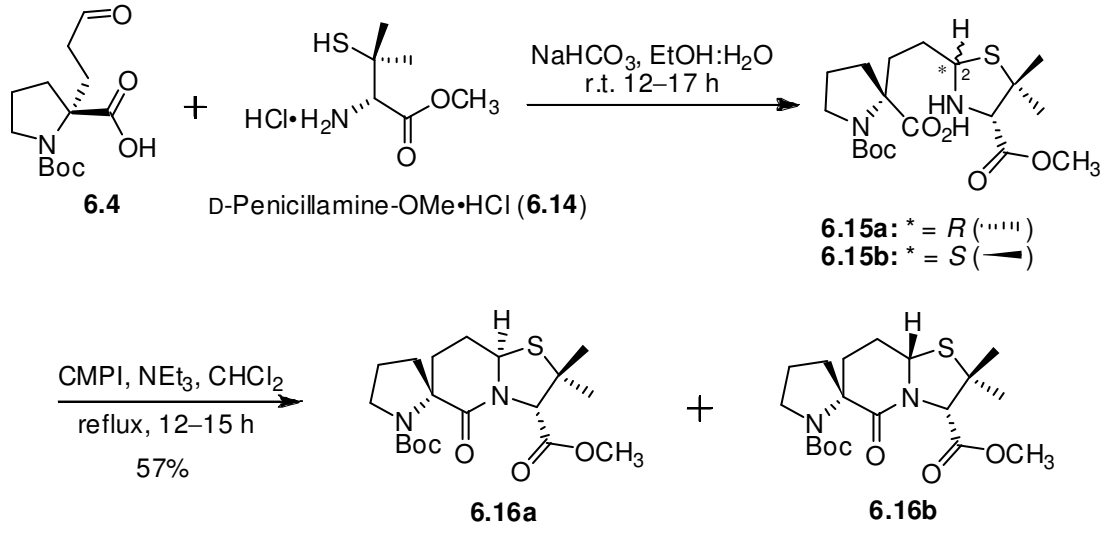

**6.4** L-Penicillamine-OMe•HCl (**6.11**)

**6.12a:** * = *R* (dashed) **6.12b:** * = *S* (wedge)

**6.13a** **6.13b**

**6.3a : 6.3b = 3:1**

**Scheme 6.6.** Synthesis of the 5.6.5-spiro bicyclic lactams derived from L-penicillamine-OMe



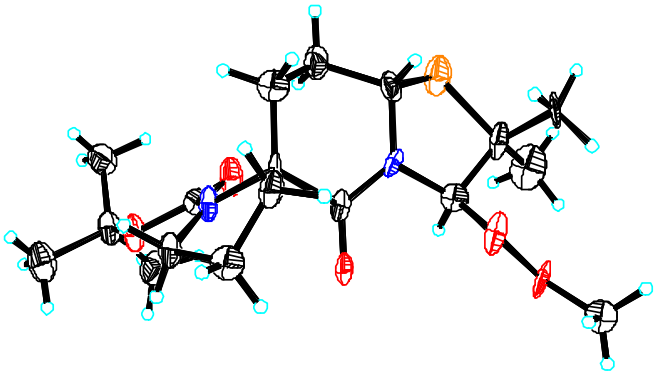

**6.4** D-Penicillamine-OMe•HCl (**6.14**)

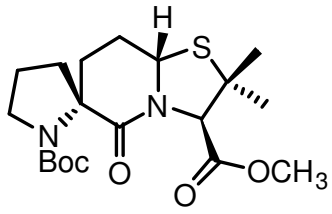
**6.15a:** * = *R* (dashed) **6.15b:** * = *S* (wedge)

**6.16a** **6.16b**

**6.16a : 6.16b = 1:1**

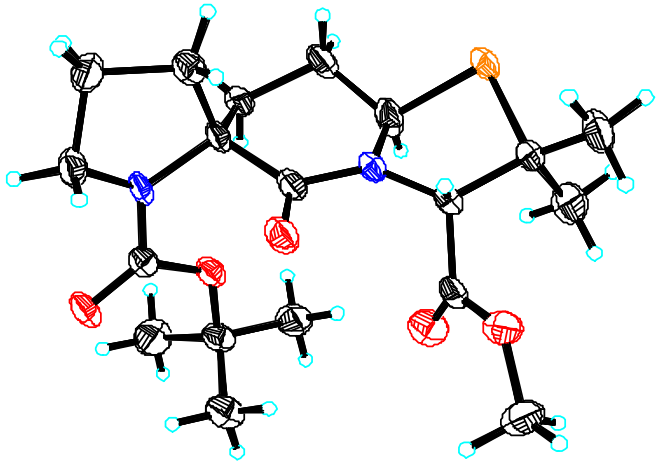
The absolute and relative stereochemical configuration about the bridgehead carbons (C-8a') for each diastereomer was established by 1-D NOESY (see experimental section) as well as by single molecule X-ray structure analysis (Figure 6.2.a., 6.2.b., and 6.2.c.) for three of the four diastereomers.

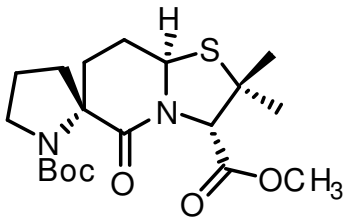





**6.13b**

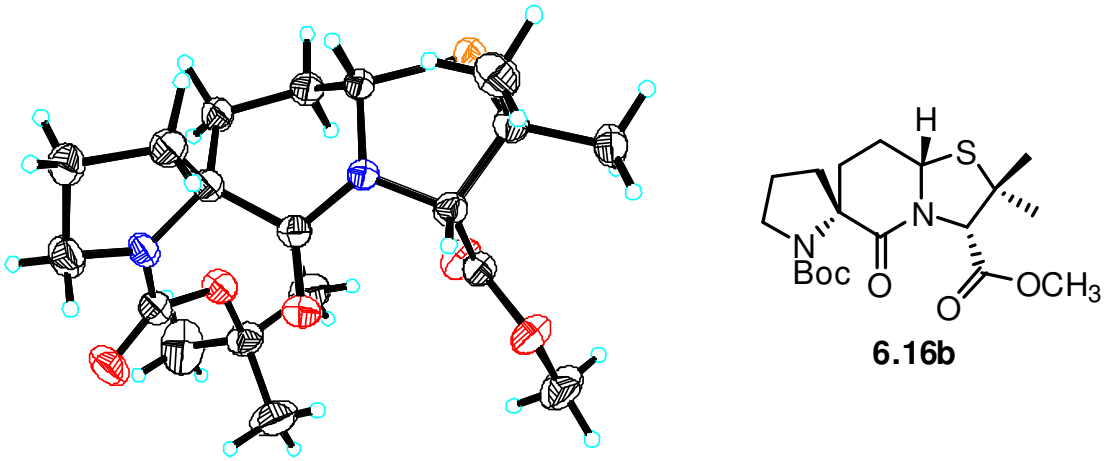
**Figure 6.2.a** X-ray crystal structure of **6.13b**






**6.16a**

**Figure 6.2.b** X-ray crystal structure of **6.16a**




**6.16b**

**Figure 6.2.c** X-ray crystal structure of **6.16b** (hydrogens removed for clarity)

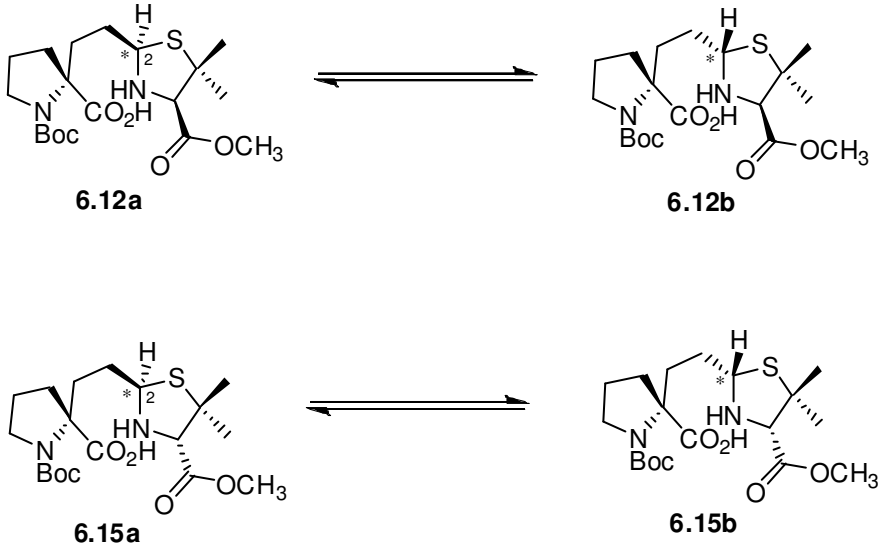
### 6.7. Rationale for diastereomeric ratios in condensation/lactamization: Correlation between C-3' and C-8a' stereochemistries

The advantage of the existing condensation/lactamization sequence is the stereodivergent nature; both possible stereoisomers can be obtained in the same reaction. However, it is imperative to rationalize the observed stereoselectivity, especially in case of the formation of unequal quantities of **6.13a** and **6.13b**; when the C-3' has the (*R*) configuration. The stereochemical outcome can be assumed to be dependent on the following factors:

1. Ratio of the intermediate thiazolidines formed; that is the equilibrium constant of the thermodynamic interconversion between the two C-2 (thiazolidine nomenclature) epimers.
2. 6.5-fused bicyclic and 5.6.5-spiro bicyclic *cis*- and *trans*-ring fusion propensity.
3. Steric interaction experienced by the attacking amine nucleophile of the thiazolidine on the activated ester with respect to the left hand hemisphere (positioning of the pyrrolidinyl ring).

4. Steric influence of the methoxycarbonyl moiety of the thiazolidine ring on the attacking amine nucleophile; that is influence of the configuration of the soon-to-be C-3' center on lactam formation.

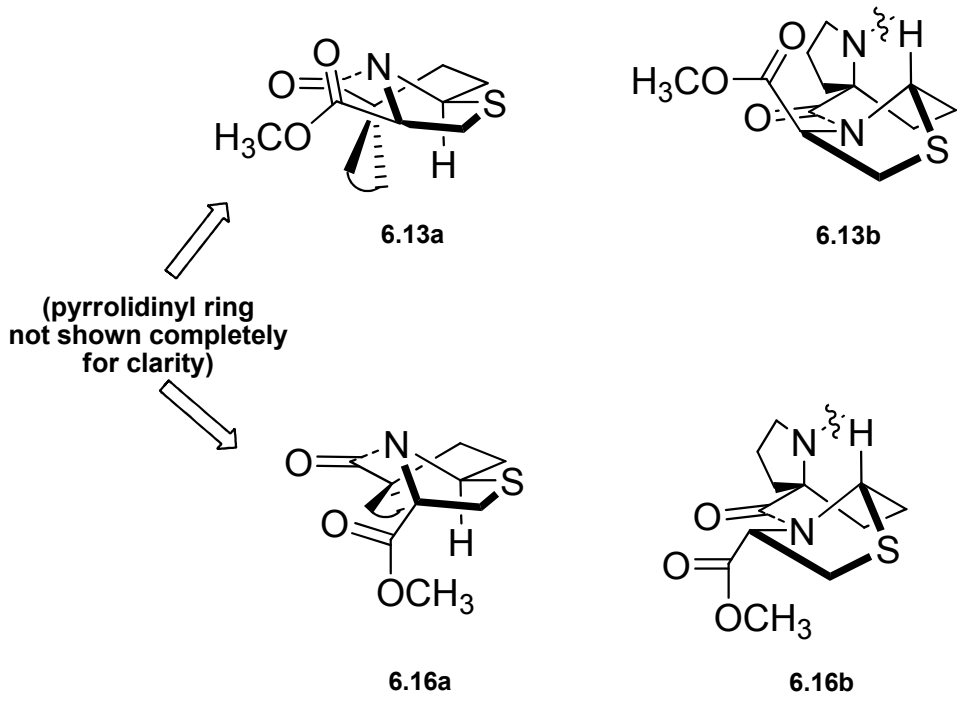
Based on $^1$H NMR of the crude intermediate thiazolidine mixture, in case of either (*R*)- or (*S*)-configuration at the carbon containing the methoxycarbonyl group, there appears to be no bias in the epimeric equilibrium at the thio-aminal position (C-2 within the thiazolidine). As long as the thiazolidine contains a secondary amine *NH*, either the *cis*-2,4- or the *trans*-2,4-substituted thiazolidine appears to be equally stable (Scheme 6.7.). Compared to 5.5-bicyclic lactams encountered in Chapters 3 and 4 of this thesis




**Scheme 6.7.** Equilibrium between diastereomeric pair: No bias

and their propensity to be *cis*-fused (with respect to the directionality of the bridgehead nitrogen lone pair and C-8a' hydrogen), the 5.6.5-ring systems appear to be *trans*-fused as per Figures 6.2. Although the lactam nitrogen is $sp^2$-hybridized, it is a bridgehead nitrogen and is perhaps slightly twisted in order to maintain good orbital overlap.

Therefore, it can be hypothesized that in the event of lactamization, the Burgi-Dunitz trajectory of the amine NH is highly influenced by diaxial interactions (as shown in Figure 6.3., C-2' *gem*-dimethyl group and C-6' pyrrolidinyl ring removed for clarity). If this is indeed true, then steric hindrance caused by the pseudo-equatorial methoxycarbonyl in case of the (*S*)-configuration (D-Cys-OMe or D-Pen-OMe) as in the formation of **6.16a** and **6.16b** is *far less* than the diaxial interaction contributions by the pseudo-axial methoxycarbonyl in case of the (*R*)-configuration (L-Cys-OMe or L-Pen-OMe) as in the




*Lactam bond is dotted to indicate possible attacking trajectory of the intermediate.* N*H* and O-*active ester not shown for clarity.Pyrrolidinyl ring indicated by a loop in 2 out of 4 images for clarity*

**Figure 6.3.** Depiction of possible stable conformations of the four diastereomeric products of lactamization

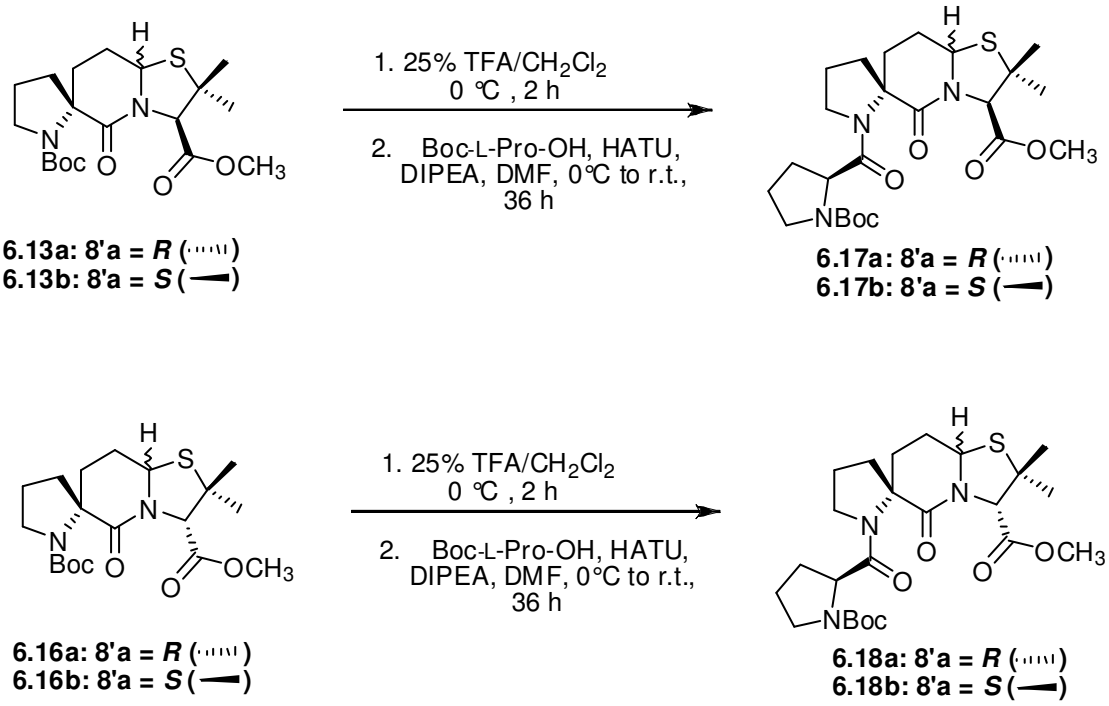
formation of **6.13a** and **6.13b**. In case of L-Pen-OMe, although the intermediate thiazolidines are rapidly interconverting at the C-2 position, there is significant diaxial

interaction when the intermediate thiazolidine ring is *trans*-2,4-fused (**6.12b→6.13b**), in comparison to the *cis*-2,4-fused (**6.12a→6.13a**) during the lactamization process. This perhaps translates to the 3:1 ratio of the respective epimers (**6.13a**:**6.13b**) observed experimentally. Although this argument can also be applied to the case of the 5.6.5-spirobicyclic lactams derived from D-Cys-OMe/D-Pen-OMe, since the methoxycarbonyl group is relatively distant in the pseudoequatorial position for **6.15a** and **6.15b**, it perhaps does not interfere as much during the lactamization resulting in a lack of stereoselectivity and a 1:1 ratio of the two C-8a' epimers. The pseudo-equatorial position of the ester will also come to play an important role in the observed difficulty in nucleophilic reactions at that group and epimerization issues further on in the synthetic scheme.

### 6.8. Synthesis of two potential modulators 5.4b and 5.5a: Challenges with the ammonolysis/amidation reactions

As with the cysteine derivative, all four diastereomers derived from the penicillamines were separately *N*-deprotected in the presence of TFA in anhydrous $CH_2Cl_2$ and subsequently coupled to *N*-Boc proline in the presence of HATU as the activating agent to the give the two corresponding diastereomersic sets **6.17a**/**6.17b** and **6.18a**/**6.18b** (Scheme 6.8). As per our design rationale, since **6.17b** would potentially lead to the formation of a negative modulator, we proceeded to subject it to further reactions first. Unfortunately, the next ammonolysis step for converting the methyl ester of **6.17b** to the carboxamide even under forcing conditions resulted in complete recovery

**Scheme 6.8** Boc-Pro-OH coupled diastereomers




**6.13a: 8'a = *R*** (dashed)
**6.13b: 8'a = *S*** (wedge)

**6.17a: 8'a = *R*** (dashed)
**6.17b: 8'a = *S*** (wedge)

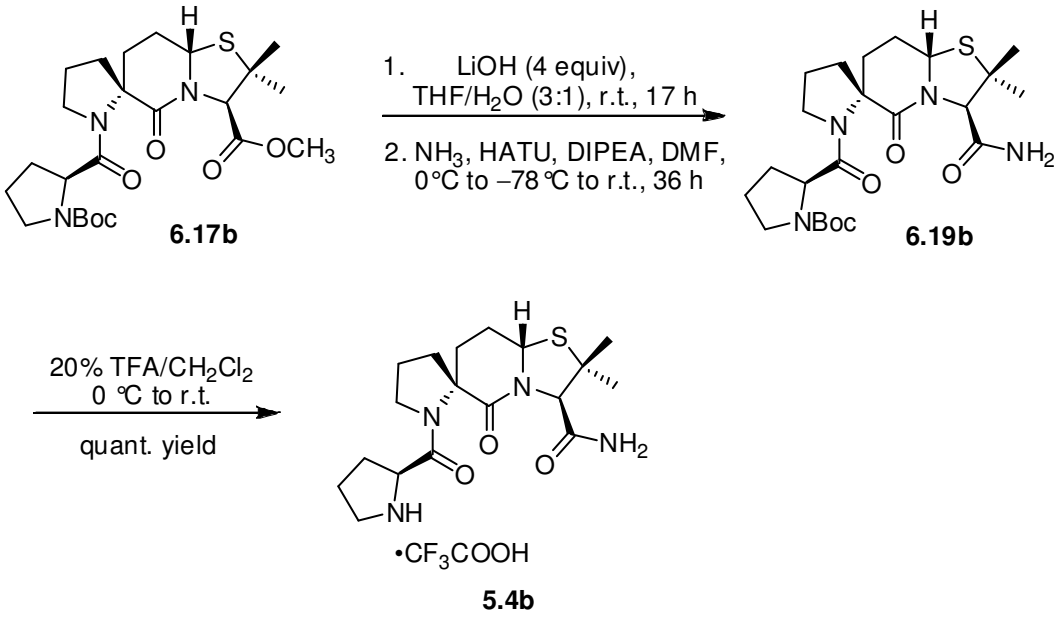
**6.16a: 8'a = *R*** (dashed)
**6.16b: 8'a = *S*** (wedge)

**6.18a: 8'a = *R*** (dashed)
**6.18b: 8'a = *S*** (wedge)

of the starting material. Since previous experience with the L-cysteine-OMe derivative in our laboratory indicated that this was not an issue,[171, 175] it was hypothesized that the added steric bulk of the 2'-*gem*-dimethyl group hindered the nucleophilic attack on the methoxy carbonyl. We therefore decided to use the ester hydrolysis/ammonia coupling protocol that had been successfully used before.

Ester hydrolysis of **6.17b** proceeded smoothly under standard conditions without any epimerization (Scheme 6.9). The subsequent coupling with ammonia resulted in a 20–25% yield of the carboxamide **6.19b**. Despite the poor yield, the final *N*-deprotection was conducted using TFA/$CH_2Cl_2$ to give the ammonium trifluoroacetate salt **5.4b** in quantitative yield. On repeated trituration with a hot dichloromethane/ethyl ether mixture, the product precipitated as a white crystalline solid.

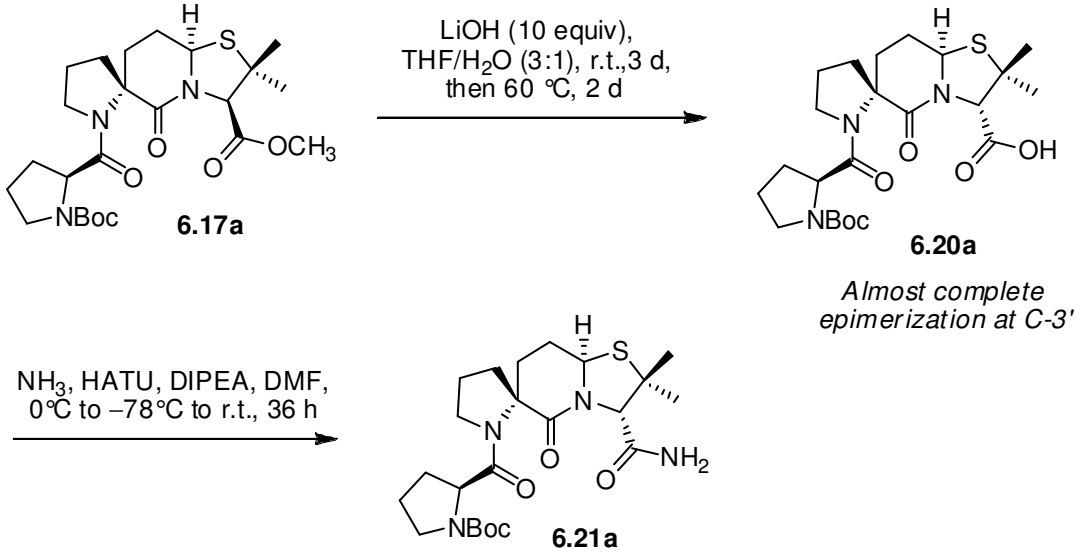
**Scheme 6.9** Synthesis of potentially negative modulator **5.4b**




**6.17b** **6.19b** **5.4b**

Unfortunately, as shown in Scheme 6.10, the C-8a' epimer **6.17a** was recalcitrant toward ester hydrolysis under standard conditions and required almost 10 equivalents

**Scheme 6.10** Epimerization during ester hydrolysis of **6.17a**




**6.17a** **6.20a**

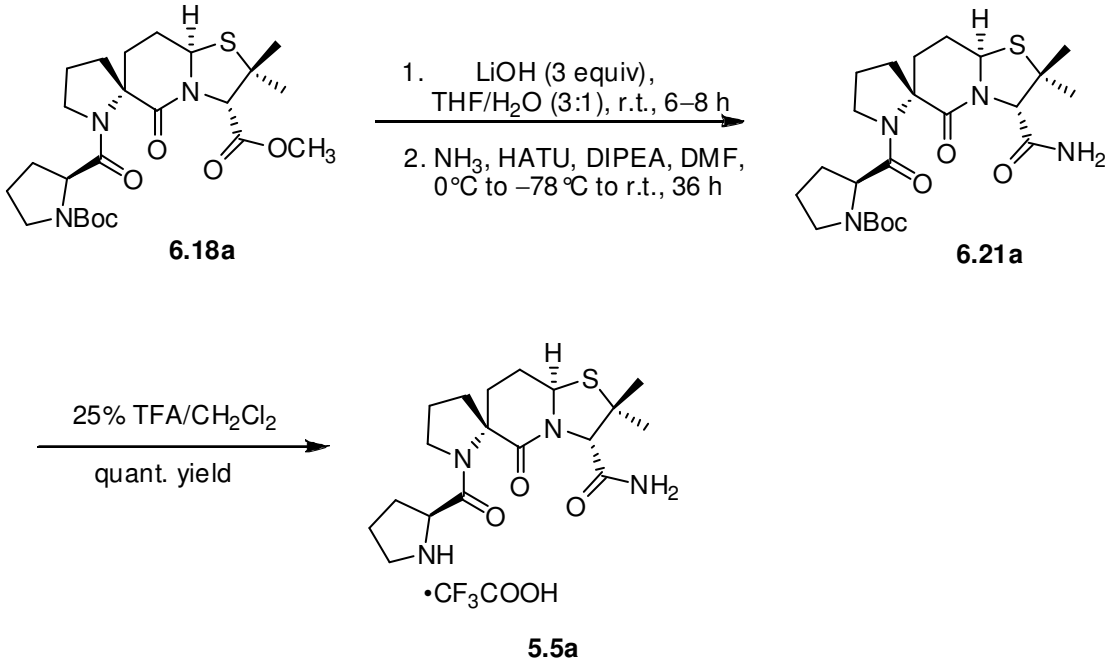
*Almost complete epimerization at C-3'*

**6.21a**

LiOH, refluxing conditions and prolonged reaction times for complete conversion. Under these forced conditions, almost complete epimerization at the C-3' position (as determined by NOE analysis) was observed. Although epimerization under ester hydrolysis had not been observed for the previously synthesized des-dimethyl derivative, there is precedent for C-3' epimerization under forced ammonolysis conditions.[171,175] It is hypothesized that the pseudo-axial positioning of the methoxycarbonyl in **6.17a** (*vide supra*) in addition to the C-2'-*gem*-dimethyl group hinders ester hydrolysis and reinforces the epimerization under the harsh conditions. The carboxylic acid obtained was further subjected to amidation via coupling with ammonia using HATU and spectral properties of the carboxamide **6.21a** were thoroughly analyzed.

Next, the diastereomer **6.18a** was subjected to similar reactions (Scheme 6.11). This would potentially lead to a control compound within the pharmacological studies

**Scheme 6.11.** Synthesis of **5.5a**

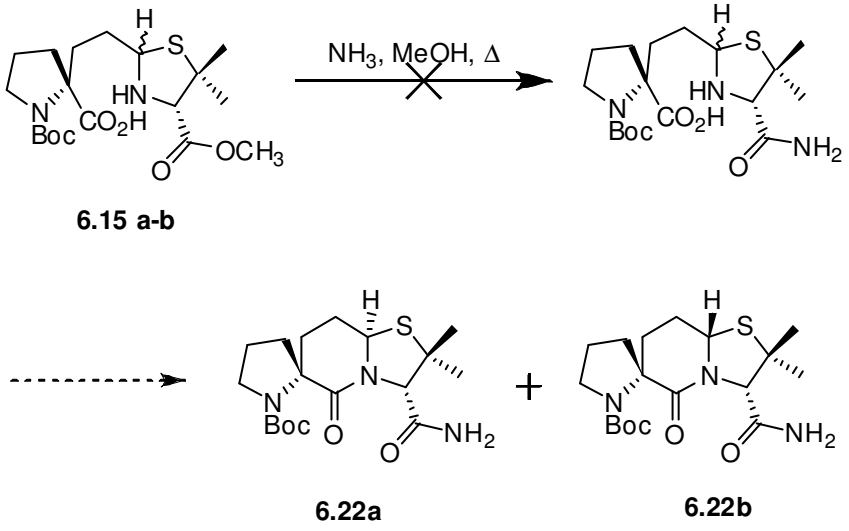


and structural analysis would assist us in confirming the stereochemistry of the previous product **6.21a**. The ester hydrolysis was unsurprisingly facile; the carboxylic acid formation being complete in 6–8 hours using 3 equivalents of LiOH *without epimerization*.[170] The carboxylic acid was then coupled to ammonia to form the carboxamide **6.21a** in good yields with identical spectral properties as of the carboxamide obtained in Scheme 6.10. *N*-Boc deprotection using TFA/$CH_2Cl_2$ gave the corresponding TFA salt **5.5a** as a crystalline white solid.

### 6.9. Alternate strategy to synthesize 5.6.5-spiro bicyclic lactams: Reaction sequence switch and results

Since the conversion of the ester to the primary carboxamide was plagued with epimerization issues and low conversions, we decided to change the sequence of reactions as per the original retrosynthetic scheme; *the carboxamide formation would be conducted earlier within the synthetic scheme.* As shown in Scheme 6.12., the first

**Scheme 6.12** Ammonolysis attempt on intermediate thiazolidine mixture **6.15 a–b**




**6.15 a-b**

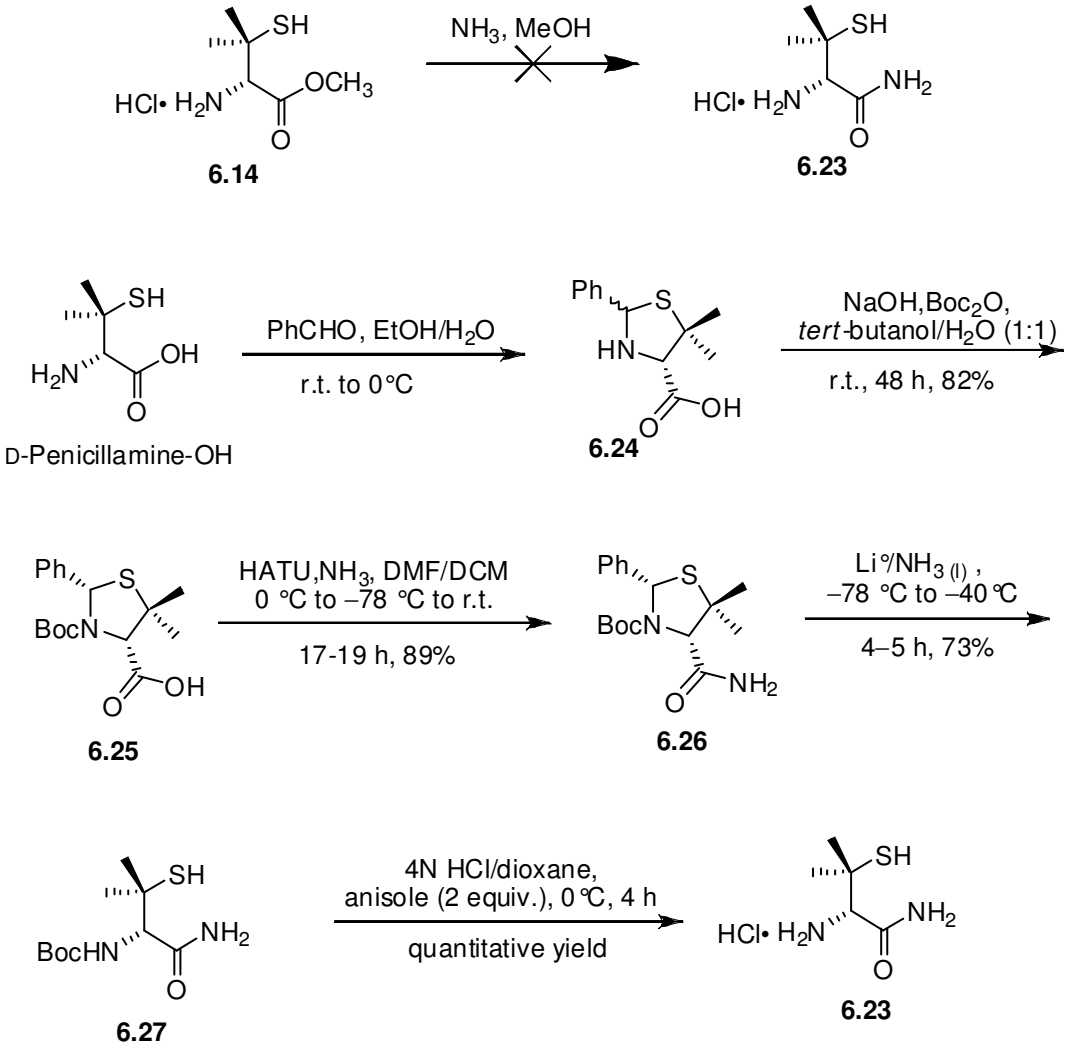
**6.22a** **6.22b**

attempt involved ammonolysis of the intermediate diastereomeric thiazolidine mixture **6.15a**/**6.15b**, followed by subjecting the carboxamide derivative to lactam formation. Unfortunately, the ammonolysis reaction itself was unsuccessful even under forced reaction conditions.

It was then decided that we would utilize the carboxamide derivative of penicillamine (D- or L-penicillamide•HCl) in the condensation/lactamization sequence. The reactions were first attempted with D-penicillamine due to its lower cost and ease of availability compared to L-penicillamine. The first attempt toward the formation of the penicillamide using ammonolysis of D-Pen-OMe in anhydrous MeOH failed (Scheme 6.13.). Although the rationale for this failure was not easily discernable, we hypothesized that the free amine and thiol groups had a role to play in this failure. That is, we hypothesized that in order for the amidation to be successful, the amine and thiol functionalities would have to be masked (or protected). Rather than using two different protecting groups for the $–NH_2$ and –SH groups, we were inspired by: (1) the possibility of using the thiazolidine ring to achieve simultaneous masking of both reactive groups,[176] and (2) literature precedent for the synthesis of C-2 substituted thiazolidines derived from penicillamines.[177]

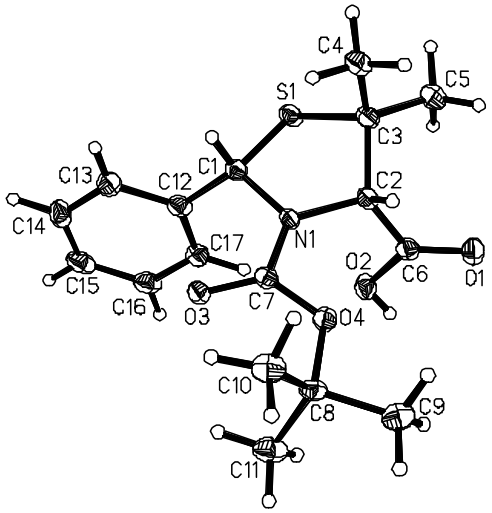
Since the use of an unsubstituted thiazolidine (at C-2 position) would lead to a robust molecule, we decided to synthesize a C-2-phenyl substituted thiazolidine from D-penicillamine, which would in effect install a C-2 carbon that was fairly labile under reducing conditions and capable of undergoing simultaneous deprotection to unravel the free $–NH_2$ and –SH groups at the end of the sequence.[176] As shown in Scheme 6.13., D-penicillamine was condensed with benzaldehyde to give a diastereomeric mixture of the C-2-phenyl thiazolidine **6.24**. The two diastereomers appear to be constantly interconverting as was observed by muted $^{13}C$ NMR intensities under ambient

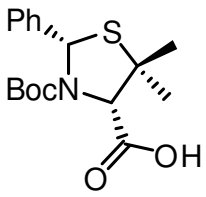
**Scheme 6.13.** Synthesis of penicillamine carboxamide




conditions. *N*-Boc protection of the diastereomeric mixture under prolonged reaction times led to the synthesis of the *N*-protected thiazolidine **6.25** as a single diastereomer; the *cis*-2,4-substituted thiazolidine. This was confirmed by X-ray structure determination and there exists literature precedent for this observation with respect to thiazolidines derived from penicillamines.[177a, 177c] It can be argued that although an equilibrium exists in the interconversion of the two diastereomeric forms, the sterically less encumbered *cis*-form perhaps, reacts faster with $Boc_2O$ to form the *N*-Boc derivative. This constantly

pushes the equilibrium toward the *cis*-form and eventually leads to almost complete diastereospecific formation of **6.25**. The acid was then converted to the primary carboxamide **6.26** in the presence of ammonia and coupling agent HATU. Dissolving metal reduction using lithium metal (Li°) in ammonia[178, 179] reductively led to the formation of the *N*-Boc penicillamide **6.27** which was subsequently *N*-deprotected using 4 N HCl in dioxane in the presence of anisole as the cation scavenger to give the desired penicillamide **6.23** as the hydrochloride salt.[178]



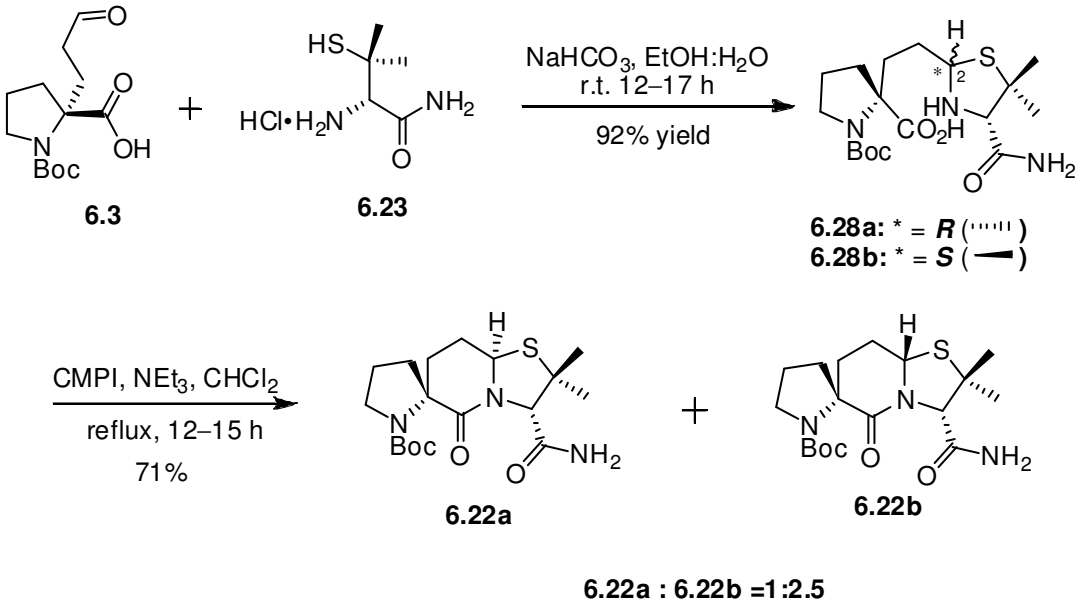

**6.25**

**Figure 6.4.** X-ray structure of **6.25**

We next proceeded to utilize **6.23** toward the formation of the 5.6.5-spirobicyclic lactams (Scheme 6.14).The condensation and lactamization sequence proceeded smoothly. The overall two-step yield was significantly higher as compared to the yield for the reaction with the analogous D-penicillamine methyl ester (68% vs. 46%). The diastereoisomeric ratio however was initially surprising. The 8a'β-H epimer was formed in three times the amount as the 8a'α-H epimer. Although this ratio is *opposite* to that

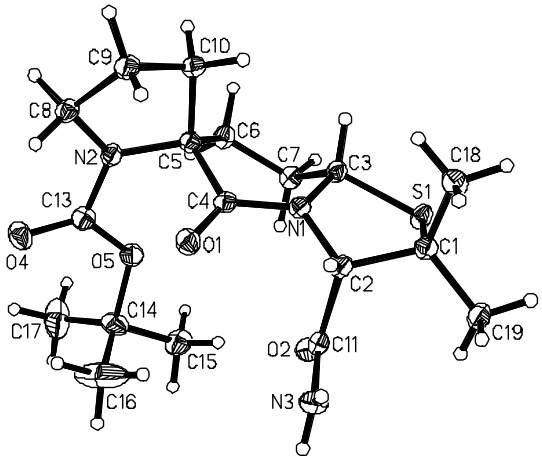
obtained with L-Pen-OMe•HCl, on closer examination, it can be observed that just as in the case of L-penicillamine methyl ester, it is the *cis*-3',8a'-substituted derivative that is the major product. Relative and absolute stereochemistries were assigned as per X-ray crystallographic data for **6.22b**.

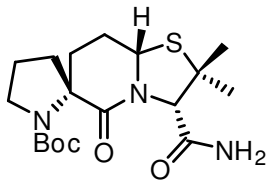
**Scheme 6.14** Condensation/lactamization using penicillamide




If we were to structurally equate the 2,4-disubstituted-thiazolidine intermediate with a 2,5-disubstituted-pyrrolidine, it could be anticipated that the *cis*-disubstituted analogue is better poised to react at the NH compared to the *trans*-disubstituted derivative; the latter poses steric hindrance irrespective of the plane of nucleophilic attack (vis à vis above or below the pyrrolidine ring). Although this can be used as a general model to explain the diastereoselectivity in the formation of the 5.6.5-spiro bicyclic lactams, it is at odds with the observation that in case of the 8a'-(*S*)-configuration (at least for the D-Cys-OMe derivative), lactamization under thermal conditions gave rise

to the 8a'α-H epimer almost exclusively. In view of this anomaly, it is hypothesized that the carboxamide $CONH_2$ is involved in intramolecular hydrogen bonding with the thiazolidine NH, a phenomenon that can be supported in a dichloromethane reaction milieu and that can possibly explain both improved yields and perhaps diastereoselectivity.




**6.22b**

**Figure 6.5.** X-ray structure of **6.22b**

### 6.10. Pharmacological testing of 5.2b: Experiments and observations

At present, the pharmacological studies involving **5.2b** have been completed and the other synthesized molecules, **5.4b** and **5.5a** will be eventually tested in a similar manner by our collaborator, Dr. Ram K. Mishra and his group at the Department of Psychiatry and Behavioral Neuroscience at McMaster University in Ontario, Canada.

Compound **5.2b** was tested in a tritiated *N*-propylnorapomorphine ([$^3$H]NPA) binding assay with bovine striatal dopamine $D_2$ receptors in a manner similar to the diastereomeric modulator, **5.2a** (this discussion includes **5.2a** attributes to compare and contrast)**.** Each PLG peptidomimetic, at various concentrations, was incubated with the

membrane preparation containing [$^3$H]NPA and the percent change in [$^3$H]NPA binding was measured. The results are depicted in Figure 6.6.A, 6.6.B. for **5.2a** and **5.2b**, respectively.

Compound **5.2a** behaved like PLG and typical PLG peptidomimetics, wherein it enhanced the binding of [$^3$H]NPA to isolated dopamine $D_2$ receptors and exhibited the characteristic bell-shaped dose response curve. The maximum effect for **5.2a** was at a concentration of $10^{-8}$ M where it significantly enhanced the binding of [$^3$H]NPA binding by 14.6 ± 3.9%. A previously synthesized PLG analogue derived from L-cysteine **5.1a** maximally enhanced [$^3$H]NPA binding by 21.4 ± 6.1% at a concentration of $10^{-7}$ M. As anticipated, **5.2b** the diastereoisomer of **5.2a**, *decreased* the binding of [$^3$H]NPA to isolated dopamine $D_2$ receptors. Compound **5.2b** significantly decreased the binding of [$^3$H]NPA to dopamine $D_2$ receptors by 11.5 ± 3.1 % at a concentration of $10^{-8}$ M. It should be noted that the corresponding L-cysteine derived PLG peptidomimetics **5.1b**, significantly decreased the binding of [$^3$H]NPA to dopamine $D_2$ receptors by 9.2 ± 3.4% at a concentration of $10^{-6}$ M. Like the positive allosteric modulators, both **5.2b** and **5.1b** possessed bell-shaped dose response curves. Compound **5.2b** was also evaluated for its ability to displace [$^{125}$I]**6.29** (Figure 6.7) in a competition binding assay. PLG peptidomimetic **6.29** and its derivatives were previously shown to be positive modulators of the dopamine receptor and it was developed as a radioligand for the PLG allosteric modulatory site on the dopamine $D_2$ receptor. The results depicted in the same figure show that both spiro bicyclic PLG peptidomimetics **5.2a** and **5.2b** are able to displace [$^{125}$I]**6.29** from its binding site. This assay suggests that the positive and negative allosteric activity of the PLG analogues is brought about via the *same site* on the dopamine $D_2$ receptor.

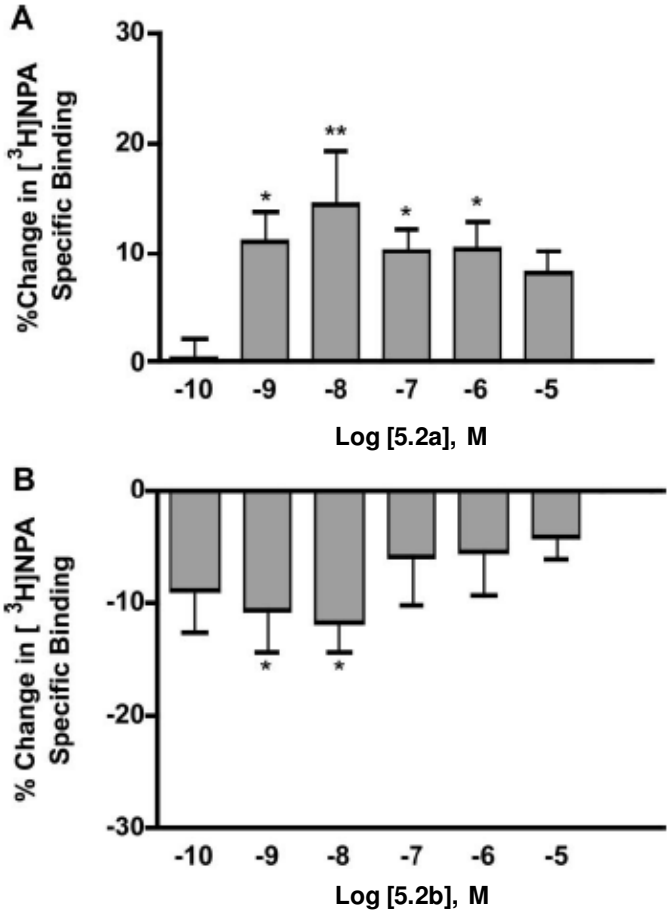



**Figure 6.6.** Modulation of [$^3$H]NPA binding to dopamine $D_2$ receptors by PLG peptidomimetics **5.2a** (A), **5.2b** (B). Data represent the percent change in specific [$^3$H]NPA binding relative to the control value when the indicated concentration of peptidomimetics was added directly to the assay buffer. *Results are the mean ±SEM of five separate experiments carried out in triplicate. The data were analyzed by a one way analysis of variance followed by a Dunnett's post hoc test: (*) significantly different ($p < 0.05$) from the control value; (**) significantly different ($p < 0.01$) from the control value.*

(Reprinted with permission from Raghavan, B.; Skoblenick, K.; Bhagwanth, S.; Argintaru, N.; Mishra, R. K.; Johnson, R. L. Allosteric Modulation of the Dopamine $D_2$ Receptor by Pro-Leu-Gly-$NH_2$ Peptidomimetics Constrained in Either a Polyproline II Helix or a Type II *β*-Turn Conformation. *J. Med. Chem.* **2009,** *52*, 2043–2051. Copyright 2009, American Chemical Society)

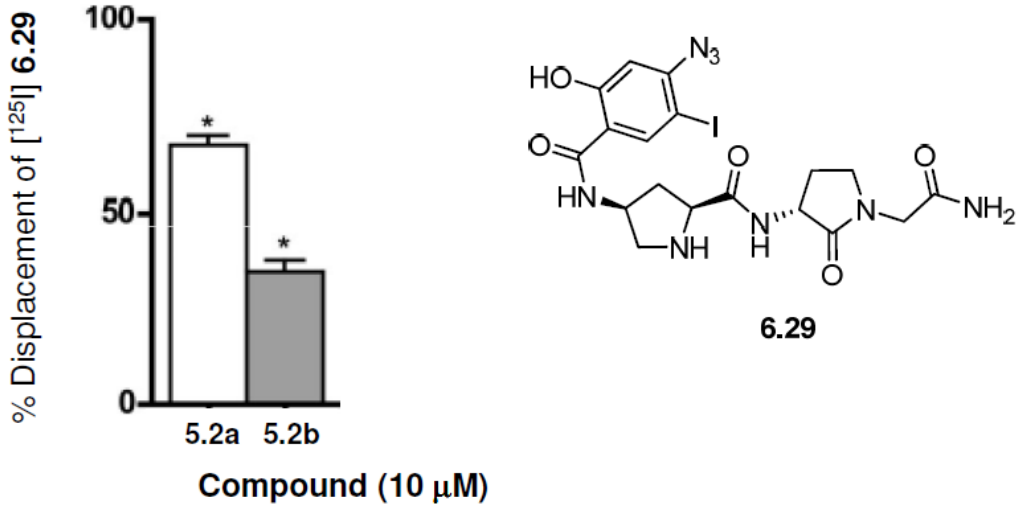



**Figure 6.7.** Displacement of [$^{125}$I]**6.29** from the PLG allosteric modulatory site on the dopamine $D_2$ receptor by analogues **5.2a** and **5.2b**. Data represent the percent displacement of [$^{125}$I]**6.29** from its binding site on bovine striatal membranes. Values graphed are relative to controls in which maximum specific binding was calculated as the amount of [$^{125}$I]**6.29** bound in the presence and absence of 10 mM PLG. *Results are the mean ± SEM of five separate experiments carried out in triplicate. The data were analyzed by a one way analysis of variance followed by a Dunnett's post-hoc test. *Significantly different ($p<0.05$) from the control value.*

(Reprinted with permission from Raghavan, B.; Skoblenick, K.; Bhagwanth, S.; Argintaru, N.; Mishra, R. K.; Johnson, R. L. Allosteric Modulation of the Dopamine $D_2$ Receptor by Pro-Leu-Gly-$NH_2$ Peptidomimetics Constrained in Either a Polyproline II Helix or a Type II *β*-Turn Conformation. *J. Med. Chem.* **2009,** *52*, 2043–2051. Copyright 2009, American Chemical Society)

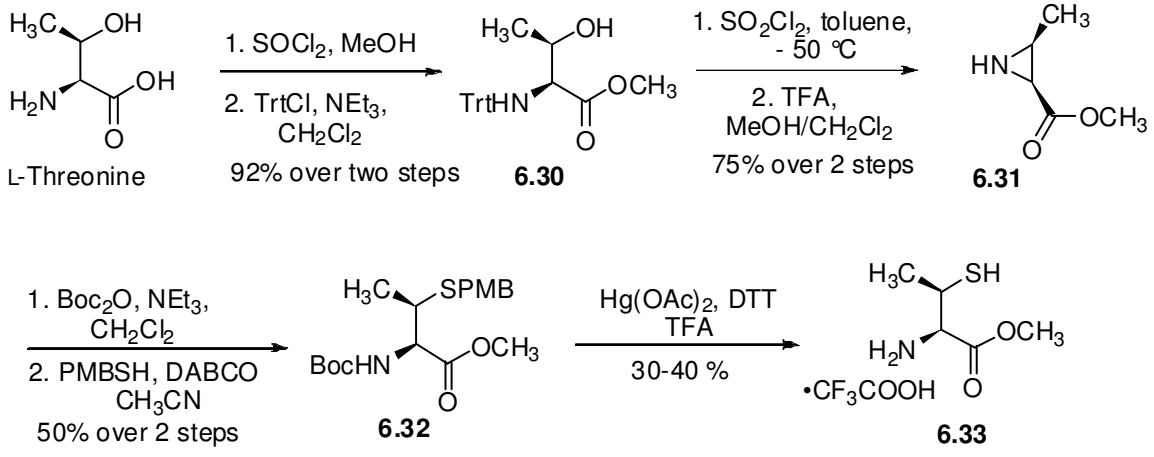
### 6.11. Future experiments and studies

Synthesis of compound **5.5b** will complete the sequence of reactions for the penicillamine derivatives. It will also be instructive to obtain the *N*-Boc proline coupled analogues of **25a** and **25b**. This will effectively prove that incorporating the carboxamide prior to the lactamization is the best course of action in synthesizing PLG analogues

containing the 5.6.5-spiro bicyclic lactams. Hopefully, it will also provide a predictable route to synthesize **5.4a**, which was impossible using the existing strategy due to the base-lability of the key intermediate **6.17a** as shown in Scheme 6.10.

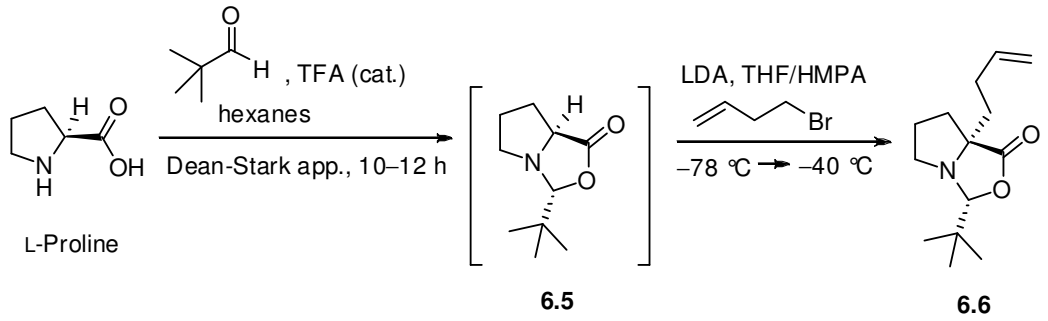
As discussed in Chapter 5, the synthesis of another series of potentially negative modulators was also originally targeted. The syntheses of C-2'-monomethyl substituted 5.6.5-spiro bicyclic PLG analogues required large quantities of *β*-methyl cysteine derivatives (both (*R*)- and (*S*)- configurations about the α-position). Although the synthesis of one of the intermediates was explored, it requires completion. The synthetic route explored toward the *β*-methyl cysteine derivative is depicted in Scheme 6.15. We anticipated that commercially available threonine/allo-threonine could be used as the the starting material and sulfur could be installed following a double $S_N2$ reaction sequence which proceeds via an aziridine intermediate **6.31** capable of isolation. Although this proved to be largely possible, the last two steps of the sequence require considerable optimization, especially based on the outcome of carboxamide lactamization studies as shown in Scheme 6.14.

**Scheme 6.15.** Synthesis of the key *β*-methyl cysteine derivative



**6.12.** Experimental section:

**(2*R*, 5*S*)-1-Aza-5-(3'-butenyl)-2-*tert*-butyl-3-oxabicyclo[3.3.0]octan-4-one (6.6)**




L-Proline (10 g, 86.9 mmol, 1 equiv.) was finely ground with a mortar and pestle. To this, a solution of TFA (0.3 mL, approx. 4 mol%) in 2 mL hexanes was added and the mixture was triturated well. The excess solvent was allowed to evaporate and the free-flowing powder was transferred to a dry flask flushed with argon. Hexanes (250 mL) was then added to the vessel. Pivalaldehyde (25 mL, 230 mmol, 2.6 equiv.) was added while the mixture was stirred vigorously (Note 1). The reaction flask was equipped with a Dean-Stark trap and then heated to reflux (Note 2). The reaction was allowed to proceed till the mixture became clear with the formation of a light yellow color. Quantitative collection of water was observed. This was typically observed after 11–14 h (Note 3). The solvent was then removed *in vacuo* and utmost care was exercised to ensure that the intermediate oxazolidinone was *only* exposed to inert atmosphere (Note 4). The product was obtained as a yellow-colored waxy solid which was immediately subjected to enolate formation and alkylation (Note 5).

A 2.5 M solution of n-BuLi in hexanes (42 mL, 104 mmol, 1.2 equiv.) was added dropwise to a 250 mL flask containing *N*,*N*-diisopropylamine (16.1 mL, 115 mmol, 1.3 equiv.) in 75 mL anhyd. THF that had been cooled to –78 °C and stirred under argon. The reaction was stirred at –78 °C for 10 min and then warmed to 0 °C. After 15 min, the reaction was cooled back to –78 °C. The resulting 0.9 M LDA solution was cannulated

into a flask containing the intermediate oxazolidinone **6.5** (15.9 g, 86.9 mmol, 1 equiv.) in 75 mL anhyd. THF at –78 °C and the reaction was stirred for 15 min after which freshly distilled HMPA (16.6 mL, 95.5 mmol, 1.1 equiv.) (Note 6) was added. The reaction turned a dark orange color after the addition of HMPA. After 15 min, 4-bromo-1-butene (17.6 mL, 173.7 mmol, 2 equiv.) was added. The reaction was stirred at –78 °C for an hour and then warmed to –40 °C (using $CH_3CN$/dry ice) where it was stirred for 6 h. The intensity of the reaction color reduced over the course of 6 h. The unreacted base was quenched using saturated $NH_4Cl$ and THF removed *in vacuo*. The waxy residue was partitioned between $Et_2O$ and 1N HCl (400 mL each). The ether extract was washed with a second portion of 1 N HCl to ensure maximum removal of HMPA. The ether layer was then washed sequentially with sat. $NaHCO_3$ and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to yield 12 g of the alkylated oxazolidinone **6.6**, representing a 60% yield over two steps. This crude product was taken to the next step without further purification. An analytical sample can be prepared by passing 1 g of the crude material through a Luknova® 4 g normal phase $SiO_2$ gel column with elution by hexanes. This material is a colorless oil and its spectral properties are consistent with those reported in the literature.[170]

TLC $R_f$ 0.56 (EtOAce/hexanes, 3:1)

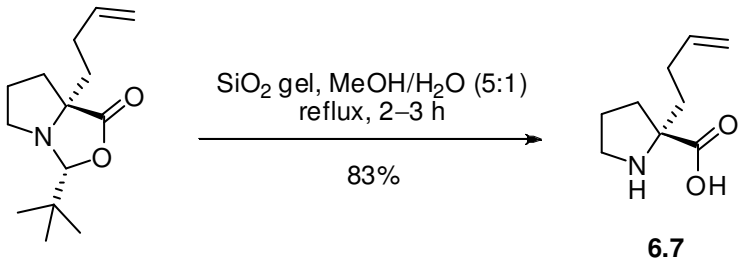
$^1$H NMR (400 MHz, $CDCl_3$) δ 5.81(dddd or app. ddt, 1H, $J_1$ = 16.8 Hz, $J_2$ = 10.2 Hz, $J_3$ = $J_4$ = 6.5 Hz, C*H*=$CH_2$), 5.05–4.92 (m, 2H, CH=C$H_2$), 4.22 (s, 1H, N-C*H*-O), 3.02–2.82 (m, 2H, δ-C$H_2$), 2.26–2.07 (m, 3H, C$H_2$CH=$CH_2$, β-C$H_2$), 1.88–1.60 (m, 5H, γ-C$H_2$, β-C$H_2$, C$H_2$$CH_2$CH=$CH_2$), 0.91 (s, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$), 178.4 (*C*=O), 138.1 (*C*H=$CH_2$), 114.8 (CH=*C*$H_2$), 105.2 (N-*C*-O), 71.8 (α-*C*), 57.6 (δ-*C*), 36.9 (*C*(C$H_3$)$_3$), 36.5, 35.8 (β-*C*, *C*$H_2$$CH_2$CH=$CH_2$), 28.3 (*C*$H_2$CH=$CH_2$), 24.9 (γ-*C*), 24.4 (C(*C*$H_3$)$_3$)

Notes:

1. The reaction should be stirred vigorously to prevent reactants from depositing on the sides of the flask and thereby decomposing due to localized superheating over the course of the reaction.
2. The reaction mixture should be heated only after pivalaldehyde addition. This prevents issues discussed in Note 1.
3. The time for completion has been observed to depend on reaction scale. Less than 10 g proline is consumed faster.
4. The intermediate oxazolidinone is *extremely* moisture-sensitive. Ar should be used when the rotary evaporator vacuum is equalized to atm. pressure.
5. Complete conversion was assumed.
6. HMPA was distilled from $CaH_2$ using vacuum distillation. The fraction was collected at 92 °C and at 5 mmHg (or 5 Torr).

**(*S*)-2-(3'-Butenyl)pyrrolidine-2-carboxylic Acid (6.7)**




**6.6** **6.7**

The alkylated pivaloyl oxazolidinone **6.6** (10 g, 42.1 mmol) was dissolved in 5:1 methanol/water (240 mL). Silica gel (20 g) was then suspended in this reaction mixture and the reaction was refluxed for 2 h. The mixture was then cooled to r.t. and the solvent removed *in vacuo.* The dry residue was suspended in $CH_2Cl_2$/MeOH (20:1) and filtered

to remove the silica gel which was washed with $CH_2Cl_2$. The clear filtrate was then concentrated to a pale yellow-colored solid which was triturated thrice with hot $Et_2O$ to give a white solid. The solid was re-crystallized (Note 1) from iPrOH/MeOH/$H_2O$ to give 5.9 g of amino acid **6.7** (83% yield).

mp > 250 °C (dec.)

$[\alpha]_D$ –73.0 (*c* 0.12, $CH_3OH$) [lit.[170] $[\alpha]_D$ –73.6 (*c* 0.88, $CH_3OH$); lit.[175] $[\alpha]_D$ –73.4 (*c* 0.095, $CH_3OH$)]

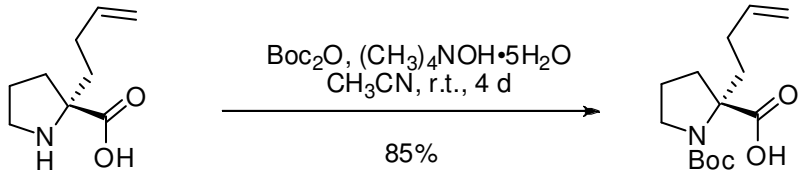
$^1H$ NMR (400 MHz, $D_2O$, gCOSY assignments) δ 5.73 (dddd, 1H, $J_1$ = 12.7 Hz, $J_2$ = 10.2 Hz, $J_3$ = 6.4 Hz, $J_4$ = 6.4 Hz, C*H*=$CH_2$), 5.12–4.90 (m, 2H, CH=C*H*$_2$), 3.33–3.17 (m, 2H, δ-C*H*$_2$), 2.29–2.22 (m, 1H, γ-C*H*$_2$), 2.09–1.71 (m, 7H, C*H*$_2$C*H*$_2$CH=$CH_2$, β-C*H*$_2$, γ-C*H*$_2$)

$^{13}C$ NMR (100 MHz, $D_2O$, gHMQC assignments) δ 175.9 (C=O), 136.9 (*C*H=$CH_2$), 115.8 (*C*H=$CH_2$), 74.5 (α-*C*), 45.9 (δ-*C*), 35.7 (γ-*C*), 34.6 (*C*$H_2CH_2$CH=$CH_2$), 28.9 ($CH_2$*C*$H_2$CH=$CH_2$), 22.8 (β-*C*)

Notes:

1. The white solid obtained after hot $Et_2O$ treatment can be used directly without further purification.

***N*-(*tert*-Butyloxycarbonyl)-(*S*)-2-(3'-butenyl)pyrrolidine-2-carboxylic Acid**




**6.7**

(*S*)-2-(3'-Butenyl)proline **6.7** (5.9 g, 35.0 mmol) and tetramethylammonium hydroxide pentahydrate (6.5 g, 35.0 mmol, 1.0 equiv.) were suspended in 350 mL of anhyd. acetonitrile (Note 1) under argon. The reaction mixture was stirred for 20 min during which a clear solution was obtained indicating complete salt formation. $Boc_2O$ (11.5 g, 52.5 mmol, 1.5 equiv.) was added and the reaction was stirred under Ar for 3 days after which an additional amount of $Boc_2O$ (3.8 g, 17.5 mmol, 0.5 equiv.) was added. The reaction was allowed to proceed for another day. The reaction mixture was then concentrated under vacuum and the residue was partitioned between 200 mL 1N NaOH and 200 mL $Et_2O$. The aqueous layer was extracted again with $Et_2O$ (100 mL). The aqueous layer was cooled to 0 °C and then acidified with 3N HCl to pH 2–3. This was then extracted with EtOAc (3 x 150 mL). The combined ethyl acetate extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a white solid. The product was crystallized from $Et_2O$/hexanes to give white crystals weighing 8.0 g (85 % yield). This compound can also be carried on to the next step without crystallization.

mp 91–92 °C [lit.[175] 92 °C]

[α]D +38.7 (*c* 1.8, $CH_3OH$) [lit.[170] $[\alpha]_D$ +36.2, (*c* 2.13, $CH_3OH$); lit.[175] $[\alpha]_D$ +37.4 (c 1.35, $CH_3OH$)]

TLC $R_f$ 0.60 (2-propanol/$NH_4OH$, 4:1)

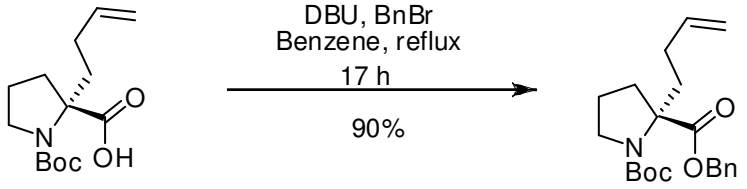
$^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.84–5.73 (m, 1H, C*H*=$CH_2$), 5.04–4.94 (m, 2H, C*H*=C*H*$_2$), 3.76–3.71 (m, 0.4H, δ-C*H*$_2$), 3.55–3.28 (m, 1.6H, δ-C*H*$_2$), 2.68–2.6 (m, 0.5H, β-C*H*$_2$), 2.30–1.74 (m, 7.5H, C*H*$_2$$CH_2$CH=$CH_2$, $CH_2$C*H*$_2$CH=$CH_2$, γ-C*H*$_2$, β-C*H*$_2$), 1.48 and 1.41 (s, 9H, C(C*H*)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, rotamers observed about carbamate bond in a 1–2:1 ratio) δ 180.9 and 175.3 ((C=O)–OH), 157.1 and 153.9 (NH–(C=O)), 138.2 and 137.4 (*C*H=$CH_2$), 115.3 and 114.8 (CH=*C*H$_2$), 82.1 and 80.6 (α-*C*), 49.4 and 48.7 (δ-*C*), 37.6 and 35. 3 (β-*C*), 34.1 and 33.6 (*C*H$CH_2$CH=$CH_2$), 28.6 and 28.0 ($CH_2$*C*H$_2$CH=$CH_2$) 28.5 and 28.4 (C(*C*H$_3$)$_3$), 22.9 and 22.8 (γ-*C*)

Notes:

1. Acetonitrile was rendered anhydrous by distilling from $CaH_2$.

**Benzyl *N*-(*tert*-Butyloxycarbonyl)-(*S*)-2-(3'-butenyl)pyrrolidine-2-carboxylate (6.8)**




**6.8**

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 4.6 mL, 30.9 mmol, 1.0 equiv.) was added to a solution of Boc-protected amino acid in 300 mL benzene. This was followed by the addition of BnBr (5.5 mL, 46.4 mmol, 1.5 equiv.). The solution was stirred under Ar for 20 min. following which a thick off-white precipitate formed. The suspension was then refluxed with vigorous stirring under Ar for 6 h. An additional amount of BnBr (1.9 mL, 15.5 mmol, 0.5 equiv.) was incorporated into the reaction mixture. Following reflux

for another 12 h, TLC indicated complete consumption of starting material. The mixture was cooled to r.t. and filtered to remove the DBU•HBr precipitate. The residue was washed with EtOAc and the filtrate washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to give a yellow-colored oil which was further purified by flash chromatography in two portions using the Isco Combiflash Retrieve system with an 80 g Luknova® normal phase $SiO_2$ column (gradient elution: 0–10% EtOAc in hexanes). The product is obtained as a clear, colorless oil weighing 10.0 g (90% yield).

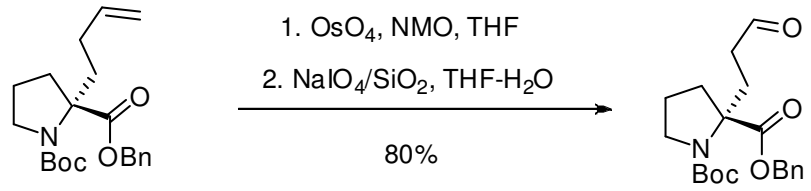
TLC $R_f$ 0.42 (Hexanes/EtOAc, 5:1) [lit.[175] $R_f$ 0.23 (hexanes/EtOAc, 10:1)]

$[\alpha]_D$ +9.2 (*c* 1.18, $CHCl_3$) [lit.[170] $[\alpha]_D$ +8.4 (*c* 1.1, $CHCl_3$); lit.[175] $[\alpha]_D$ +8.6 (*c* 1.34, $CHCl_3$)]

$^1$H NMR (400 MHz, $CDCl_3$) δ 7.34–7.29 (m, 5H, Ar-C*H*), 5.86–5.76 (m, 1H, C*H*=$CH_2$), 5.19–4.91 (m, 4H, C$H_2$Ph, CH=C$H_2$), 3.76–3.69 (m, 0.7H, δ-C$H_2$), 3.62–3.56 (m, 0.3H, δ-C$H_2$), 3.44–3.33 (m, 1H, δ-C$H_2$), 2.49–2.43 (m, 0.3H, β-C$H_2$), 2.32–2.20 (m, 0.7H, β-C$H_2$), 2.10–1.73 (m, 7H, C$H_2$$CH_2$CH=$CH_2$, $CH_2$C$H_2$CH=$CH_2$, γ-C$H_2$, β-C$H_2$), 1.41 and 1.33 (s, 9H, C(C*H*)$_3$)

$^{13}$CNMR (100 MHz, $CDCl_3$, rotamers observed about the carbamate bond in a 2:1 ratio) δ 174.6 and 174.5 ((C=O)–OH), 154.0 and 153.8 (NH–(C=O)), 138.6 and 138.2 (Ar-$C_p$), 136.2 and 135.8 (*C*H=$CH_2$), 128.7 and 128.5 (Ar-*C*), 128.3 and 128.1 (Ar-*C*), 128.0 and 127.9 (Ar-$C_q$), 114.7 and 114.4 (CH=*C*$H_2$), 80.1 and 79.5 (NH–(C=O)–*C*$(CH_3)_3$), 67.9 and 67.4 (α-*C*), 66.8 and 66.7 (*C*$H_2$Ph), 48.7 (δ-*C*$H_2$), 37.5 and 36.2 (β-*C*), 34.3 and 33.4 (*C*H$CH_2$CH=$CH_2$), 28.5 and 28.0 ($CH_2$*C*$H_2$CH=$CH_2$), 28.3 (NH–(C=O)–C(*C*$H_3$)$_3$), 23.3 and 22.7 (γ-*C*)

**Benzyl *N*-(*tert*-Butyloxycarbonyl)-(*S*)-2-((propan-3'-al)yl)pyrrolidine-2-carboxylate (6.9)**




**6.8** **6.9**

The alkene **6.8** (13 g, 36.2 mmol, 1.0 equiv.) was dissolved in 500 mL volume of a 5:1 THF-$H_2O$ mixture. This was followed by a sequential addition of a solution of $OsO_4$ (462 mg, 1.8 mmol, 5 mol%) in *tert*-butanol and *N*-methylmorpholine *N*-oxide (NMO, 10.6 g, 90.5 mmol, 2.5 equiv.). The dark amber-colored solution was stirred at room temperature under Ar for 17 h. A few mLs of a 10% sodium sulfite solution was added to quench excess oxidizing agents and then THF was removed *in vacuo.* The aqueous residue was washed with EtOAc and the organic layer dried over $MgSO_4$, filtered and concentrated to a yellow to brown-colored oil that was used without further purification.

To a solution of the resulting diol in 400 mL $CH_2Cl_2$, $NaIO_4$ supported on $SiO_2$ gel[174] (72 g, Note 1) was added in one portion. The reaction was stirred for 1–2 h at r.t. until the starting diol was no longer detected by TLC analysis. The reaction mixture was filtered twice through a sintered glass funnel and the $SiO_2$ was washed well with $CH_2Cl_2$. The filtrate was concentrated to a pale yellow oil which was purified by flash chromatography (gradient elution:0–20% EtOAc/hexanes) to give the product **6.9** as a colorless oil weighing 10 g (80% yield)

TLC $R_f$ 0.3 (EtOAc/hexanes, 1:3)

$[\alpha]_D$ +11.1 (*c* 1.33, $CHCl_3$) [lit.[170] +11.5 (*c* 1.7, $CHCl_3$)]

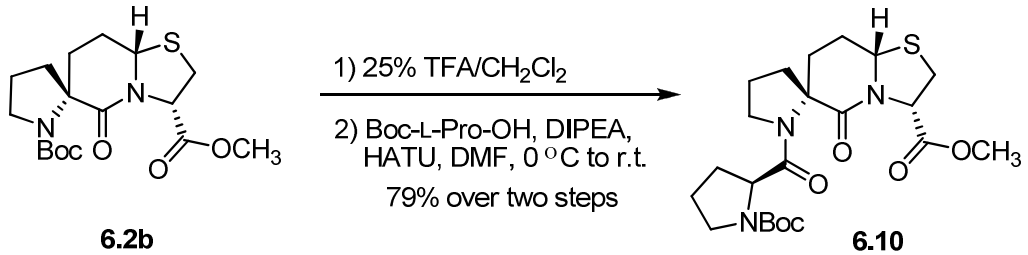
$^{1}$H NMR (400 MHz, $CDCl_3$, rotamers observed about the carbamate bond in a 1:1 ratio) δ 9.75 and 9.67 (s, 1H, (C=O)-*H*), 7.37–7.29 (m, 5H, aromatic-C*H*), 5.22–5.04 (m, 2H, C$H_2$Ph), 3.76–3.67 (m, 0.5H, Pro δ-C$H_2$), 3.50–3.59 (m, 0.5H, Pro δ-C$H_2$), 3.45–3.30 (m, 1H, Pro δ-C$H_2$), 2.72–2.22 (m, 4H, C$H_2$C$H_2$-(C=O)-H), 2.15–1.75 (m, 4H, Pro β-C$H_2$, Pro γ-C$H_2$), 1.39 and 1.34 (C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, rotamers observed about the carbamate bond in approximately 1:1 ratio) 202.5 and 201.4 ((*C*=O)-H), 174.1 and 173.9 (ester *C*=O), 154.4 and 153.8 (carbamate *C*=O), 135.9 and 135.6 (benzyl Ar-$C_q$), 128.8, 128.6, 128.5, 128.2 and 128.1 (benzyl Ar-*C*H), 80.8 and 80.1 (*C*(CH$_3$)$_3$), 67.6 and 67.1 (Pro α-*C*), 67.1 and 67.0 (*C*H$_2$Ph), 48.7 and 48.6 (Pro δ-*C*), 39.5 and 39.1 (CH$_2$*C*H$_2$-(C=O)-H), 37.5 and 36.6 (Pro β-*C*), 28.4 and 28.3 (C(*C*H$_3$)$_3$), 27.8 and 27.7 (*C*H$_2$CH$_2$-(C=O)-H), 23.3 and 22.7 (Pro γ-*C*)

Notes:

1. Typically, 2g $NaIO_4$ in $SiO_2$ per mmol diol is used.

**Methyl [3'*S*-(3'α,6'α,8'aβ)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.10)**




**6.2b** **6.10**

Trifluoroacetic acid (2.5 mL, 34 mmol, 40 equiv.) was added to an ice-cooled anhydrous dichloromethane solution of the spirocycle (313 mg, 0.85 mmol, 1.0 equiv.). The final concentration of TFA in dichloromethane was 20–25%. The reaction was monitored by thin layer chromatography (TLC) using 25% ethyl acetate in hexanes. After 45–60 minutes, the starting material was consumed completely. Solvent and excess TFA were removed *in vacuo*. The pale-yellow colored oil was used in the next step without further purification.

Boc-L-proline (220 mg, 1.0 mmol, 1.2 equiv.) was dissolved in anhydrous DMF (5 mL). The solution was cooled in an ice-salt bath, followed by the addition of DIPEA (0.2 mL, 1.3 mmol, 1.5 equiv.) and HATU (388 mg, 1.0 mmol, 1.2 equiv.). The solution was stirred under Ar at this temperature for 30 minutes. In another 10 mL flask, the deprotected amine from the first step was suspended in anhydrous DMF (5mL) and the trifluoroacetate salt neutralized with DIPEA (0.2 mL, 1.3 mmol, 1.5 equiv.). The clear solution obtained was added to the activated acid. The mixture was stirred under Ar for 20 hours after which the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and washed sequentially with 10% citric acid, saturated $NaHCO_3$, and brine solutions.The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow-colored oil which was purified by flash chromatography starting with 100% $CH_2Cl_2$ and increasing the polarity in discrete steps to 4% MeOH in $CH_2Cl_2$ to give the purified compound as a colorless oil in 79% yield.

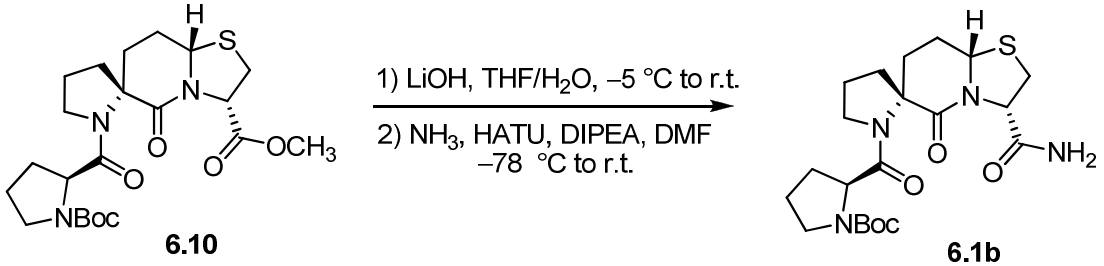
$[\alpha]_D$ -40.7 (*c* 0.43, $CDCl_3$)

TLC $R_f$ 0.65 ($CH_2Cl_2$ / MeOH, 10:1)

$^1H$ and $^{13}C$ NMR spectra indicate presence of two rotameric forms of the molecule about the carbamate bond in a 1:1 ratio. Several peaks display the rotamers.

$^1$H NMR (300 MHz, $CDCl_3$, gCOSY) δ 4.80–4.74 (m, 1H, 8'a-C*H*), 4.68–4.65 (m, 1H, 3'-C*H*), 4.43 (*app*. t, 0.5 H, *J* = 4.8, 9.9 Hz, Pro α-C*H*), 4.30 (dd, 0.5H, *J* = 1.2, 5.7 Hz, Pro α-C*H*), 3.86–3.78 (AB qt. 0.5 H, *J* = 9, 15 Hz, Pro δ-C$H_2$), 3.74–3.49 (m, 2.5H, Pro δ-C$H_2$, 5-C$H_2$), 3.67 (s, OC$H_3$), 3.39–3.26 (m, 2H, Pro δ-C$H_2$, 2'-C$H_2$), 3.15–3.09 (dd, 1H, *J* = 4.5, 12.6 Hz, 2'-C$H_2$), 2.68–2.52 (m, 2H, 8'C$H_2$), 2.12–1.84 (m, 9H, Pro β-C$H_2$, Pro γ-C$H_2$, 3-C$H_2$, 4-C$H_2$, 7'-C$H_2$), 1.80–1.70 (m, 1H, 7'-C$H_2$), 1.39 and 1.37 (s, 9H, Boc-C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$, HMQC) δ 171.4, 171.1, 170.8, 170.6 (amide and ester *C*=O), 154.7 and 153.9 (Boc *C*=O), 79.5 and 79.3 (Boc *C*$(CH_3)_3$), 65.0 and 64.9 (6'-C), 63.6, 63.5, 63.4 (8'a-*C* and 3'-*C*), 58.3 (Pro α-*C*), 52.6 and 52.5 (O-*C*$H_3$), 48.1 (Pro δ-*C*), 47.2 and 46.9 (5-*C*), 42.0 and 41.9 (3-*C*), 37.0 (8'-*C*), 33.1(2'-*C*), 29.8 (Pro β-*C*), 28.9 and 28.8 (7'-*C*), 28.7 and 28.6 (Boc *C*$H_3$), 24.3, 23.8 and 23.8, 23.6 (Pro γ-*C* and 4-*C*)
ESI HRMS *m/z* 490.1978 $[M+Na]^+$, ($C_{22}H_{33}N_3O_6S$+ $Na^+$) requires 490.1988.

**[3'*S*-(3'α,6'α,8'aβ)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (6.1b)**



**6.10** **6.1b**

A solution of the ester **6.10** (240 mg, 0.5 mmol, 1.0 equiv.) in 3:1 THF-water was cooled to -5 °C in an ice-salt bath. Lithium hydroxide (37 mg, 1.5 mmol, 3.0 equiv.) was added at this temperature. The final molarity of LiOH was 0.5 M. The reaction was

gradually warmed to room temperature. Ester hydrolysis was determined to be complete in less than 4 hours by thin layer chromatography. (Note 1)

The carboxylic acid obtained (211 mg, 0.5 mmol, 1.0 equiv.) was dissolved in anhydrous DMF (3 mL) in the presence of DIPEA (0.12 mL, 0.8 mmol, 1.6 equiv.) and the solution cooled to –5 °C in an ice-salt bath. HATU (179 mg, 0.5 mmol, 1.0 equiv.) was added to the solution which was then warmed to room temperature for 20 minutes. The pale-yellow colored solution obtained was cooled to –78 °C and a saturated solution of ammonia in anhydrous $CH_2Cl_2$ was added (8–10 mL). The solution turned into a bright canary yellow color. An empty balloon was fitted onto the flask via an adaptor and the reaction warmed to room temperature over 1 hour. The reaction mixture was stirred at ambient temperature for 24 hours, following which an additional amount of ammonia in $CH_2Cl_2$ (3–4 mL) was added. Following another 24 hours, the solvent and excess ammonia were removed *in vacuo.* The residue was dissolved in EtOAc and washed sequentially with 10% citric acid, saturated $NaHCO_3$, and brine solutions. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to give a pale yellow oil which was purified by flash chromatography using gradient elution (100% $CH_2Cl_2$ first and increasing to 20 % MeOH in $CH_2Cl_2$) to give an overall 85% yield. (Note 2)

TLC *Rf* 0.49 ($CH_2Cl_2$/MeOH, 10:1)]

$[\alpha]_D$ –35.8 (*c* 0.81, $CDCl_3$)

$^1H$ and $^{13}C$ NMR spectra indicate presence of two rotameric forms of the molecule about the carbamate bond in a 1:1 ratio. Several peaks display the rotamers.

$^1H$ NMR (300 MHz, $CDCl_3$, gCOSY) δ 7.49 and 7.59 (s, 1H, carboxamide N*H*), 5.36 (br. s, 1H, carboxamide N*H*), 4.81–4.75 (m, 2H, 8'a-C*H* and 3'-C*H*), 4.45–4.41 (dd, 0.5H, *J* = 3.0 and 8.1 Hz, Pro α-C*H*), 4.37–4.33 (dd, 0.5H, *J* = 3.0 and 8.7 Hz, Pro α-C*H*), 3.98–3.91 (ddd, 0.5H, *J* = 3.9, 8.1, and 9.3 Hz, Pro δ-C*H*), 3.79–3.72 (ddd, 0.5H, *J* = 3.0, 7.8,

and 8.7 Hz, Pro δ-C*H*), 3.62–3.22 (m, 5H, Pro δ-C*H*, 5-C$H_2$, 2'-C$H_2$), 2.69–2.39 (m, 1H, 8'-C*H*), 2.33–1.79 (m, 11H, Pro β-C$H_2$, Pro γ-C$H_2$, 3-C$H_2$, 4-C$H_2$, 7'-C$H_2$, 8'-C*H*), 1.43 and 1.41 (s, 9H, Boc-C$H_3$).

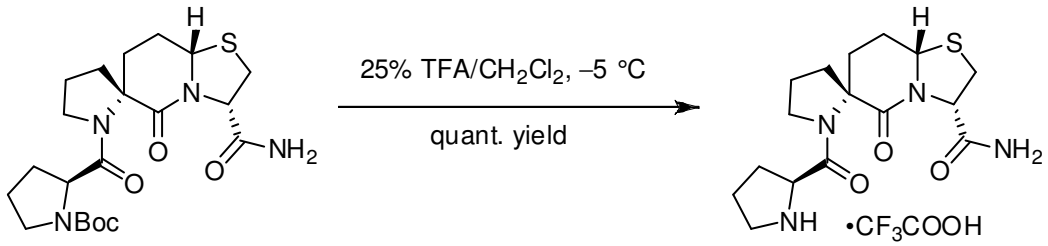
$^{13}$C NMR (75 MHz, $CDCl_3$, HMQC) δ 172.5, 172.3, 172.2, 172.1, 170.5, and 170.2 (amide *C*=O), 154.8 and 153.9 (Boc *C*=O), 79.7 and 79.5 (Boc *C*($CH_3$)$_3$), 64.5, 64.4, 64.1, 63.9 (6'-*C*, 8'a-*C* and 3'-*C*), 58.1 and 58.0 (Pro α-*C*), 47.96 and 47.94 (Pro δ-*C*), 47.1 and 46.9 (5-*C*), 41.2 and 41.1 (3-*C*), 38.1 and 38.0 (8'-*C*), 33.9 and 33.8 (2'-*C*), 29.5 (Pro β-*C*), 28.7 and 28.6 (Boc C(*C*$H_3$)$_3$), 28.1 and 27.8 (7'-*C*), 24.6, 24.5, 23.5 (Pro γ-*C* and 4-*C*)

ESI HRMS *m/z* 475.1987 $[M+Na]^+$, ($C_{21}H_{32}N_4O_5S$+ $Na^+$) requires 475.1991.

Notes:

1. Two distinct spots were observed by TLC, indicative of possible epimerization of the thiazolidine stereocenter. The mixture of carboxylic acids was used in the subsequent coupling step.
2. Combined yield of both diastereomers differing in stereochemistry about the 3' carbon was 85%. A 1:1 ratio of the two carboxamides were obtained.

**[3'*S*-(3'α,6'α,8'aβ)]-1-[2"(*S*)-Pyrrolidinyl]carbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (5.2b)**




**6.1b** **5.2b**

The Boc-protected molecule **6.1b** (50 mg, 0.1 mmol, 1.0 equiv.) was dissolved in 1–2 mL anhydrous $CH_2Cl_2$ and cooled to –5 °C using an ice-salt bath. Trifluoroacetic acid (0.2 mL, 2.2 mmol, 20 equiv.) was added drop-wise to this solution, which was stirred at that temperature for 45–60 minutes. The starting material was completely consumed at this point (as determined by thin layer chromatography). Solvent and excess TFA were removed *in vacuo.* The colorless oil obtained was suspended in $CH_2Cl_2$ and solvent removed in the rotary evaporator. This process was repeated several times until a semi-solid material was obtained. This material was triturated with diethyl ether and the ether decanted to remove any soluble impurities. The pure product **5.2b** was obtained as a white foam/semi-solid weighing 45 mg, indicating quantitative yield. A solution of the molecule in 4:1 acetonitrile-water was subjected to HPLC analysis on a Supelco Ascentis® C18 column (5 µm particle size, 250 mm x 10 mm dimension), using an isocratic 98:2 acetonitrile/ 0.1% TFA aq. solution. A retention time of 3.75 min. was obtained (100% compared to a blank composed of 4:1 acetonitrile-water).

TLC *Rf* 0.53 (2-propanol / $NH_4OH$, 4:1)

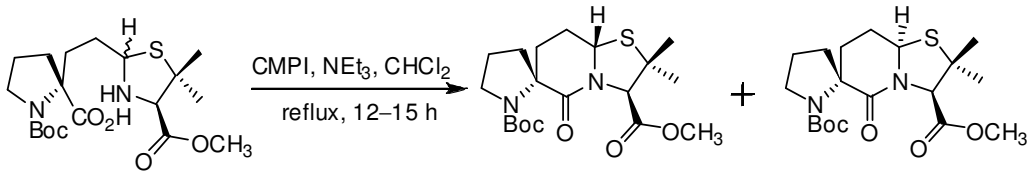
$[\alpha]_D$ -11.5 (*c* 0.13, $CD_3OD$)

$^1$H NMR (300 MHz, $CDCl_3$) δ 4.89–4.84 (dd, 1H, *J* = 2.4 and 10.8 Hz, 8'a-C*H*), 4.56 (d, 1H, *J* = 6.6 Hz, 3'-C*H*), 4.47–4.42 (m, 1H, Pro α-C*H*), 3.72–3.65 (m, 1H, Pro δ-C$H_2$), 3.49–3.41 (m, 1H, Pro δ-C$H_2$),3.34–3.14 (m, 4H, 2'-C$H_2$, 5-C$H_2$), 2.43–2.26 (m, 3H, 7'-C*H*, 8'-C$H_2$), 2.15–1.86 (m, 9H, Pro β-C$H_2$, Pro γ-C$H_2$, 3-C$H_2$, 4-C$H_2$, 7'-C*H*)

$^{13}$C NMR (75 MHz, $CDCl_3$, HMQC) δ 174.8, 171.6, 168.5 (amide *C*=O), 66.7 (6'-*C*), 65.9, 65.1 (8'a-*C* and 3'-*C*), 60.5 (Pro α-*C*), 49.2 (Pro δ-*C*), 47.8 (5-*C*), 41.9 (3-*C*), 38.0 (8'-*C*), 34.3 (2-*C*), 29.1(Pro β-*C*), 28.7 (7'-*C*), 25.3, 25.0 (Pro γ-*C* and 4-*C*).

ESI HRMS *m/z* 353.1627 $[M+H]^+$, ($C_{16}H_{25}N_4O_3S+ H^+$) requires 353.1642.

**Methyl [3'*R*-(3'β, 6'α,8'aβ)]-1-(*tert*-Butyloxycarbonyl)-2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.13b and Methyl [3'*R*-(3'β,6'α,8'aα)]-1-(*tert*-Butyloxycarbonyl)-2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.13a**




**6.12** **6.13b** **6.13a**

The diastereomeric thiazolidine mixture (2.1 g, 5.0 mmol, 1 equiv.) was dissolved in 150 mL anhydrous $CH_2Cl_2$ (Note 1). Triethylamine (1.7 mL, 12.5 mmol, 2.5 equiv.) was added in one portion to the solution followed by CMPI (1.5 g, 6.0 mmol, 1.2 equiv.). A bright yellow-colored suspension was obtained. This suspension was refluxed for 17 h at the end of which a dark amber-colored solution was obtained. The reaction mixture was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to an orange-colored syrup that was purified using flash chromatography (gradient elution: 10–66% EtOAc in

hexanes; very slow increments in polarity and using a column diameter that would be used for a 7–8 g reaction scale). The two diastereomers, **6.13b** and **6.13a**, were obtained in a 1:3 ratio weighing 300 mg (white solid) and 900 mg (oil) respectively (63% yield).

Diastereomer **6.13b**

mp 160–162 °C (dec.)

TLC $R_f$ 0.42 (EtOAc/hexanes, 1:1)

$[\alpha]_D$ –198.5 (*c* 0.4, $CH_2Cl_2$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.08 (dt, $J_1$ = 10.2 Hz, $J_2$ = 3.1 Hz, 1H, 8'a-C*H*), 4.75 and 4.63 (s, 1H, 3'-C*H*), 3.72 and 3.71 (s, 3H, OC$H_3$), 3.64–3.38 (m, 2H, 5-C$H_2$), 2.58–1.75 (m, 8H, 7'-C$H_2$, 8'-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.60 and 1.59 (s, 3H, 2'-C(C$H_3$)$_2$), 1.41 (app.s, 12H, C(C$H_3$)$_3$, 2'-C(C$H_3$)$_2$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment, rotamers observed about the carbamate bond) δ 171.2 and 170.8 (lactam *C*=O), 169.2 and 169.0 (ester *C*=O), 154.4 and 154.0 (carbamate *C*=O), 80.4 and 79.3 (*C*($CH_3$)$_3$), 72.1 and 71.8 (3'-*C*), 63.9 and 63.5 (2'-*C*), 62.7 and 62.3 (8'a-*C*), 52.2 and 52.1 (6'-*C*), 51.1 (OC$H_3$), 48.6 and 48.4 (5-*C*), 43.2 and 42.2 (3-*C*), 36.0 and 35.8 (7'-*C*), 28.9, 28.8, 28.7, 28.6, 28.5 (C(*C*$H_3$)$_3$, 2'-C(*C*$H_3$)$_2$), 27.8 and 26.9 (8'-*C*), 22.9 and 22.4 (4-*C*)

ESI HRMS *m/z* 421.1755 $[M+Na]^+$ $C_{19}H_{30}N_2O_5S$+Na requires 421.1768

Diastereomer **6.13a**

TLC $R_f$ 0.40 (EtOAc/hexanes, 1:1)

$[\alpha]_D$ +13.7 (*c* 0.75, $CH_2Cl_2$)

$^{1}$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.11 (dd, $J_1$ = 11.2 Hz, $J_2$ = 3.4 Hz, 0.4H, 8'a-C*H*), 4.97 (dd, $J_1$ = 11.4 Hz, $J_2$ = 3.4 Hz, 0.6H, 8'a-C*H*), 4.37 and 4.31 (s, 1H, 3'-C*H*), 3.68 and 3.67 (s, 3H, OC$H_3$), 3.57–3.40 (m, 2H, 5-C$H_2$), 2.61 (ddd, $J_1$ = $J_2$ = 13.7 Hz, $J_3$ = 3.1 Hz, 0.4H, 3-C$H_2$), 2.37 (m, ddd, $J_1$ = $J_2$ = 13.7 Hz, $J_3$ = 2.6 Hz, 0.6H, 7'-C$H_2$), 2.29–2.14 (m, 2H, 8'-C$H_2$,3-C$H_2$), 2.02–1.71 (m, 5H, 8'-C$H_2$, 7'-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.61 and 1.57 (s, 3H, 2'–C(C$H_3$)$_2$), 1.38 (s, 9H, C(C$H_3$)$_3$), 1.34 and 1.32 (s, 3H, 2'-C–(C$H_3$)$_2$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment, rotamers observed about the carbamate bond) δ 171.6 and 171.4 (lactam *C*=O), 170.0 and 169.8 (ester *C*=O), 153.9 and 153.3 (carbamate *C*=O), 80.1 and 79.5 (*C*(CH$_3$)$_3$), 72.2 and 71.8 (3'-*C*), 64.8 and 64.7 (8'a-*C*), 62.3 and 62.0 (2'-*C*), 52.1 and 51.9 (O*C*H$_3$), 51.3 and 51.2 (6'-*C*), 48.7 and 48.4 (5-*C*), 39.6 and 38.8 (8'-*C*), 33.1 and 32.3 (7'-*C*), 32.2 and 31.9 (2'-C(*C*H$_3$)$_2$), 28.7 and 28.6 (C(*C*H$_3$)$_3$), 27.2 and 27.1 (3-*C*), 24.6 and 24.5 (2'-C(*C*H$_3$)$_2$), 23.4 and 22.8 (4-*C*)

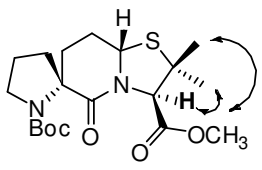
ESI HRMS *m/z* 421.1781 $[M+Na]^+$ $C_{19}H_{30}N_2O_5S$+Na requires 421.1768

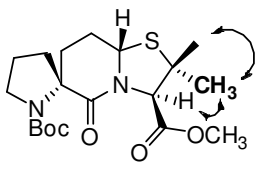
Notes:

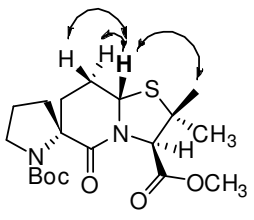
1. DCM was rendered anhydrous by distilling from $CaH_2$.
2. Detailed 1-D NOE NMR studies have indicated the respective stereochemistry about *C*-8'a (the bridgehead carbon).

NOE NMR analysis results:

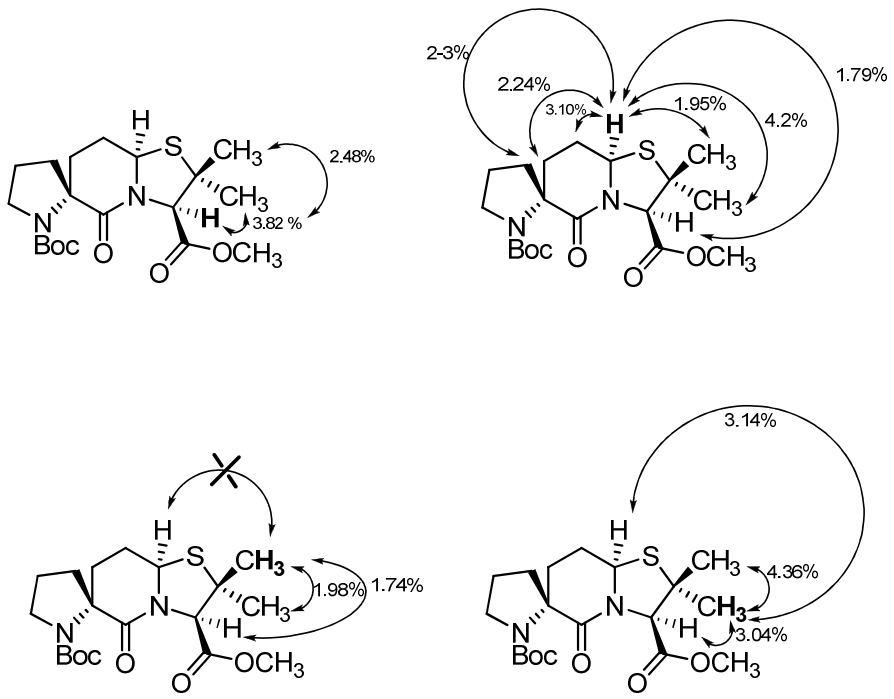
1. Irradiation of protons in **6.13b** (**bolded** in each structure)



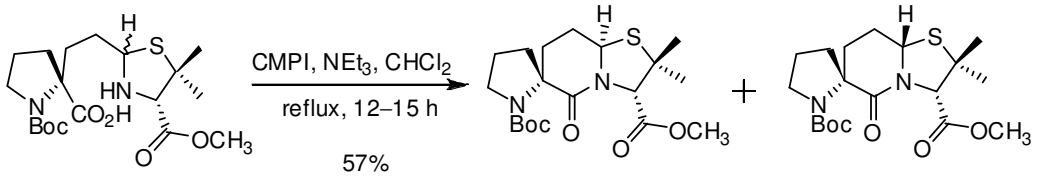



2. Irradiation of protons in **6.13a** (**bolded** in each structure)




**Methyl [3'*S*-(3'α, 6'α,8'aα)]-1-(*tert*-Butyloxycarbonyl)-2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.16a)**
**and**
**Methyl [3'*S*-(3'α,6'α,8'aβ)]-1-(*tert*-Butyloxycarbonyl)-2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.16b).**




**6.15** **6.16a** **6.16b**

The diastereomeric thiazolidine mixture **6.15** (1.9 g, 4.7 mmol, 1 equiv.) was dissolved in 150 mL anhydrous $CH_2Cl_2$ (Note 1). Triethylamine (1.6 mL, 11.7 mmol, 2.5 equiv.) was added in one portion to the solution followed by CMPI (1.4 g, 5.6 mmol, 1.2

equiv.). A bright yellow-colored suspension was obtained. This suspension was refluxed for 17 h at the end of which a dark amber-colored solution was obtained. The reaction mixture was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to an orange-colored syrup that was purified using flash chromatography (gradient elution: 10–66% EtOAc in hexanes; very slow increments in polarity and using a column diameter that would be used for a 7–8 g reaction scale). The two diastereomers **6.16a** and **6.16b** were obtained in a 1:1 ratio weighing 500 mg each (57% yield).

Diastereomer **6.16a**

mp 166–167 °C (dec.)

TLC $R_f$ 0.34 (EtOAc/hexanes, 1:1)

$[\alpha]_D$ +138.4 (*c* 0.5, $CH_2Cl_2$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.32 (dd, $J_1$ = 10.8 Hz, $J_2$ = 3.9 Hz, 0.4H, 8'a-C*H*), 5.23 (dd, $J_1$ = 11.0 Hz, $J_2$ = 4.2 Hz, 0.6H, 8'a-C*H*), 4.61 and 4.57 (s, 1H, 3'-C*H*), 3.76 and 3.71 (s, 3H, OC$H_3$), 3.68–3.44 (m, 2H, 5-C$H_2$), 2.79–2.64 (m, 1H, 7'-C$H_2$), 2.32–2.20 (m, 2H, 8'-C$H_2$, 3-C$H_2$), 2.04–1.71 (m, 5H, 8'-C$H_2$, 7'-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.59 and 1.56 (s, 3H, 2'-C(C$H_3$)$_2$), 1.49–1.42 (m, 12H, 2'-C(C$H_3$)$_2$, C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 171.7 and 171.0 (lactam *C*=O), 169.0 and 168.7 (ester *C*=O), 153.6 and 153.5 (carbamate *C*=O), 80.5 and 79.3 (NH–(C=O)–*C*(CH$_3$)$_3$), 72.3 and 72.1 (3'-*C*), 64.6 and 64.5 (2'-*C*(CH$_3$)$_2$), 62.4 and 62.3 (8'a-*C*), 51.9 and 51.8 (O–*C*H$_3$), 51.4 and 51.1 (6'-*C*), 48.6 (5-*C*), 41.0 and 38.9 (3-*C*), 32.5 and 31.5 (7'-*C*), 30.3 and 30.0 (2'-C(*C*H$_3$)$_2$, 28.9, 28.5, 28.2, 27.8 (8'-*C* and C(*C*H$_3$)$_3$), 27.1 and 26.6 (4-*C*).

ESI HRMS *m/z* 421.1792 [M+Na]$^+$ $C_{19}H_{30}N_2O_5S$+Na requires 421.1768

Diastereomer **6.16b**

mp 163–164 °C (dec.)

TLC $R_f$ 0.26 (EtOAc/hexanes, 1:1)

$[\alpha]_D$ –130.5 (*c* 0.5, $CH_2Cl_2$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 4.93 (dd, $J_1$ = 11.1 Hz, $J_2$ = 2.1 Hz, 1H, 8'a-C*H*), 4.27 and 4.26 (s, 1H, 3'-C*H*), 3.74 and 3.69 (s, 3H, OC$H_3$), 3.67–3.55 (m, 1H, 5-C$H_2$), 3.52–3.39 (m, 1H, 5-C$H_2$), 2.68–2.57 (m, 1.2H, 7'-C$H_2$), 2.36–2.25 (m, 0.8H, 8'-C$H_2$), 2.19–1.74 (m, 6H, 8'-C$H_2$, 7'-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.59 (s, 3H, 2'-C(C$H_3$)$_2$), 1.48–1.38 (m, 12H, 2'-C(C$H_3$)$_2$, C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 172.3 and 171.8 (lactam *C*=O), 169.8 and 169.7 (ester *C*=O), 154.4 and 154.1 (carbamate *C*=O) 80.5 and 79.1 (NH–(C=O)–*C*(CH$_3$)$_3$), 73.5 and 73.4 (3'-*C*), 72.4 and 72.3 (6'-*C*), 63.9 and 63.6 (8'a-*C*), 61.5 and 61.47 (2'-*C*(CH$_3$)$_2$), 52.0 and 51.9 (O–*C*H$_3$), 48.3 (5-*C*), 44.6 and 42.9 (3-*C*), 36.7 (7'-*C*), 31.8 and 31.7 (2'-C(*C*H$_3$)$_2$), 28.9 and 28.5 (8'-*C*), 28.3 and 28.28 (C(*C*H$_3$)$_3$), 23.5 and 23.4 (2'-C(*C*H$_3$)$_2$), 22.7 and 22.0 (4-*C*).

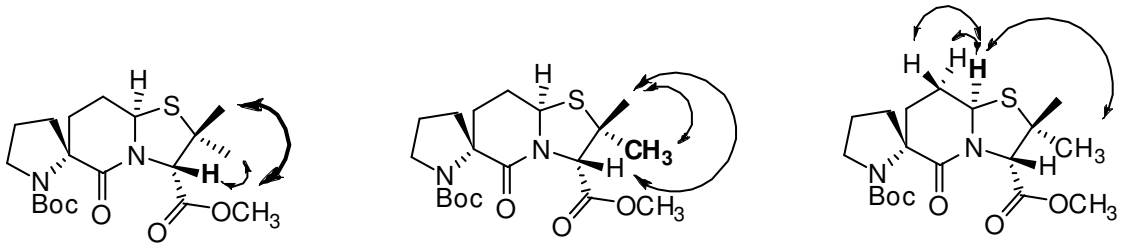
ESI HRMS *m/z* 421.1778 $[M+Na]^+$ $C_{19}H_{30}N_2O_5S$+Na requires 421.1768

Notes:

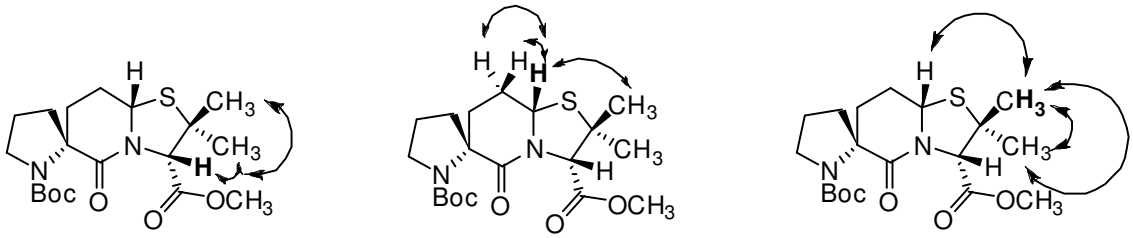
1. DCM was rendered anhydrous by distilling from $CaH_2$.
2. Detailed 1-D NOE NMR studies have indicated the respective stereochemistry about *C*-8'a (the bridgehead carbon).
3. Single molecule X-ray crystal structure for both **6.16a** and **6.16b** was performed and relative stereochemistry confirmed.

NOE NMR analysis results:

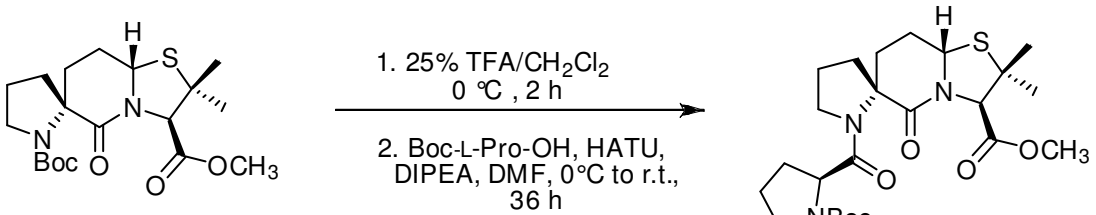
1. Irradiation of protons in **6.16a** (**bolded** in each structure)



2. Irradiation of protons in **6.16b** (**bolded** in each structure)



**Methyl [3'*R*-(3'β,6'α,8'aβ)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.17b)**




**6.13b** **6.17b**

A solution of the spirocycle **6.13b** (115 mg, 0.3 mmol, 1.0 equiv.) in 3 mL anhydrous $CH_2Cl_2$ (Note 1) was cooled to 0 °C using an ice bath. Trifluoroacetic acid

(0.9 mL, 11.6 mmol, 40 equiv.) was then added dropwise to this solution which was then stirred at 0 °C until Boc deprotection was determined to be complete by TLC. This was usually between 1.5 and 2 h. The reaction was warmed up to r.t. and the volatiles removed by rotary evaporation. The residue was re-dissolved in $CH_2Cl_2$ and the solvent removed by evaporation twice until the excess TFA was removed. The pale yellow-colored residue was dried to a constant weight for 5–6 h under vacuum.

A solution of Boc-L-proline (75 mg, 0.4 mmol, 1.2 equiv.) in 2–3 mL DMF (Note 2) was cooled to –5 °C using an ice/salt bath. DIPEA (0.1 mL, 0.6 mmol, 2.0 equiv.) was added drop-wise to this cooled solution followed by HATU (133 mg, 0.4 mmol, 1.2 equiv.). The clear solution was stirred for 30 min after which a solution of the spirocycle free amine in 2 mL DMF [obtained by neutralizing the TFA salt (120 mg, 0.3 mmol, 1.0 equiv.) with DIPEA (0.1 mL, 0.6 mmol, 2.0 equiv.)] was added to the active ester. The yellow colored solution was warmed to r.t. and stirred there under Ar for 36 h. At the end of this time, DMF and other volatiles were removed *in vacuo.* The residue was dissolved in 50 mL EtOAc and was sequentially washed with 10% citric acid, sat. $NaHCO_3$ and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to give yellow-colored residue which was purified by flash chromatography (gradient elution: 0–6% MeOH/$CH_2Cl_2$ in discrete steps) to give the product **6.17b** clear oil weighing 110 mg (77% yield).

TLC $R_f$ 0.55 ($CH_2Cl_2$/MeOH, 20:1)

$[\alpha]_D$ –132 (*c* 3.02, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 5.03 (dt, 1H, 8'a-C*H*), 4.69 and 4.67 (s, 1H, 3'-C*H*), 4.38 (dd, 0.5H, $J_1$ = 7.9 Hz , $J_2$ = 3.5 Hz, Pro α-C*H*) 4.30 (dd, 0.5H, $J_1$ = 8.4 Hz, $J_2$ = 3.6 Hz, Pro α-C*H*), 3.85–3.79 (m, 0.5H, Pro δ-C$H_2$), 3.68 and 3.67 (s, 3H, OC$H_3$), 3.68–3.64 (ovlp. m, 0.5H,

Pro δ-C$H_2$), 3.58–3.46 (m, 2H, Pro δ-C$H_2$, 5-C$H_2$), 3.41–3.28 (m, 1H, 5-C$H_2$), 2.56–2.42 (m, 2H, 8'-C$H_2$, 7'-C$H_2$), 2.28–1.82 (m, 9H, 3-C$H_2$, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 4-C$H_2$), 1.79–1.69 (m, 1H, 4-C$H_2$), 1.56 and 1.55 (s, 3H, 2'-C(C$H_3$)$_2$), 1.39–1.34 (m, 12H, 2'-C(C$H_3$)$_2$, C(C$H_3$)$_3$)

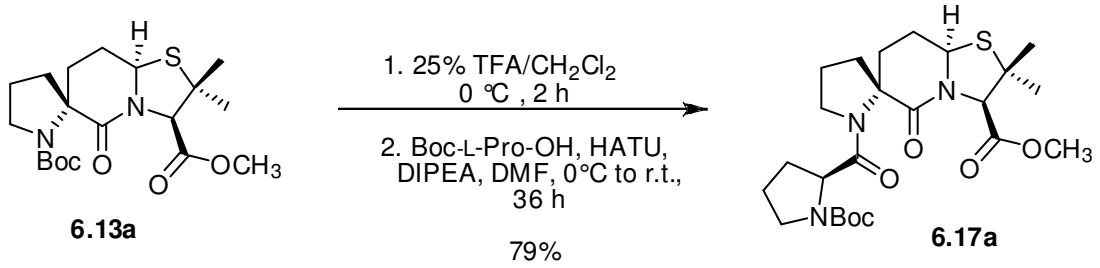
$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 171.2 and 170.9 (lactam *C*=O), 170.5 and 170.2 (amide *C*=O), 169.13 and 169.11 (ester *C*=O), 154.6 and 153.7 (carbamate *C*=O), 79.3 and 79.2 (*C*($CH_3$)$_3$), 71.9 and 71.8 (3'-*C*), 64.9 and 64.8 (6'-*C*), 62.9 and 62.8 (8'a-*C*), 58.1 and 58.0 (Pro α-*C*), 52.1 and 52.0 (O*C*$H_3$), 50.9 and 50.7 (2'-*C*), 48.1 and 48.0 (Pro δ-*C*), 47.1 and 46.8 (5-*C*), 41.5 and 41.4 (3-*C*), 36.5 and 36.3 (8'-*C*), 29.7 (Pro β-*C*), 28.9, 28.8, 28.7 and 28.6 (7'-*C*, 2'-C(*C*$H_3$)$_2$), 28.5 (C(*C*$H_3$)$_3$), 26.8 and 26.7 (2'-C(*C*$H_3$)$_2$), 24.4, 23.69, 23.68, 23.6 (Pro γ-*C*, 4-*C*)

ESI HRMS *m/z* 518.2298 $[M+Na]^+$ $C_{24}H_{37}N_3O_6S$+Na requires 518.2295

Notes:

1. $CH_2Cl_2$ was rendered anhydrous by distilling from $CaH_2$
2. DMF that was purchased was stored over 4 Å molecular sieves and used directly without further purification.

**Methyl [3'*R*-(3'β,6'α,8'aα)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.17a)**




**6.13a** **6.17a**

A solution of the spirocycle **6.13a** (500 mg, 1.3 mmol, 1.0 equiv.) in 11 mL anhydrous $CH_2Cl_2$ (Note 1) was cooled to 0 °C using an ice bath. Trifluoroacetic acid (3.8 mL, 50 mmol, 40 equiv.) was then added dropwise to this solution which was then stirred at 0 °C until Boc deprotection was determined to be complete by TLC. This was usually between 1.5 and 2 h. The reaction was warmed up to r.t. and the volatiles removed by rotary evaporation. The residue was re-dissolved in $CH_2Cl_2$ and the solvent removed by evaporation twice until the excess TFA was removed. The pale yellow-colored residue was dried to a constant weight for 5–6 h under vacuum.

A solution of Boc-L-proline (437 mg, 2.0 mmol, 1.2 equiv.) in 5 mL DMF (Note 2) was cooled to –5 °C using an ice/salt bath. DIPEA (0.6 mL, 3.4 mmol, 2.0 equiv.) was added drop-wise to this cooled solution followed by HATU (772 mg, 2.0 mmol, 1.2 equiv.). The clear solution was stirred for 30 min after which a solution of the spirocycle free amine in 5 mL DMF [obtained by neutralizing the TFA salt (700 mg, 1.7 mmol, 1.0 equiv.) with DIPEA (0.6 mL, 3.4 mmol, 2.0 equiv.)] was added to the active ester. The yellow colored solution was warmed to r.t. and stirred there under Ar for 36 h. At the end of this time, DMF and other volatiles were removed *in vacuo.* The residue was dissolved in 80 mL EtOAc and was sequentially washed with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to

give yellow-colored residue which was purified by flash chromatography (gradient elution: 0–6% MeOH/$CH_2Cl_2$ in discrete steps) to give a clear oil weighing 492 mg (79% yield).

TLC $R_f$ 0.47 ($CH_2Cl_2$/MeOH, 20:1)

$[\alpha]_D$ –33 (*c* 1.11, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 5.17 (dt, 1H, $J_1$ = 11.0 Hz, $J_2$ = 3.0 Hz, 8'a-C*H*), 4.44 (dd, 0.5H, $J_1$ = 7.8 Hz , $J_2$ = 3.4 Hz, Pro α-C*H*), 4.40 and 4.39 (s, 1H, 3'-C*H*), 4.32 (dd, 0.5H, $J_1$ = 8.5 Hz, $J_2$ = 4.0 Hz, Pro α-C*H*), 3.89–3.85 (m, 0.5H, Pro δ-C$H_2$), 3.78–3.67 (ovlp. m, 0.5H, Pro δ-C$H_2$), 3.71 (s, 3H, OC$H_3$), 3.60–3.49 (m, 2H, Pro δ-C$H_2$, 5-C$H_2$), 3.46–3.33 (m, 1H, 5-C$H_2$), 2.74–2.66 (m, 1H, 7'-C$H_2$), 2.29–1.84 (m, 9H, 8'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.83–1.76 (m, 2H, 4-C$H_2$, 7'-C$H_2$), 1.65 and 1.64 (s, 3H, 2'-C(C$H_3$)$_2$), 1.43 and 1.41 (s, 9H, C(C$H_3$)$_3$), 1.36 and 1.35 (s, 3H, 2'-C(C$H_3$)$_2$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 171.3 and 170.8 (lactam *C*=O), 170.7 and 170.6 (amide *C*=O), 170.1 (ester *C*=O), 154.6 and 153.7 (carbamate *C*=O), 79.5 and 79.4 (*C*($CH_3$)$_3$), 71.9 and 71.8 (3'-*C*), 66.1 and 66.0 (6'-*C*), 62.0 and 61.9 (8'a-*C*), 58.1 and 58.0 (Pro α-*C*), 52.0 (O*C*$H_3$), 51.3 and 51.2 (2'-*C*), 48.4 and 48.3 (Pro δ-*C*), 47.2 and 46.9 (5-*C*), 37.7 and 37.6 (8'-*C*), 32.3 and 32.2 (2'-C(*C*$H_3$)$_2$), 31.8 and 31.5 (7'-*C*), 29.7 and 28.7 (3-*C*), 28.6 and 28.5 (C(*C*$H_3$)$_3$), 27.4 and 27.3 (Pro β-*C*), 24.6 and 24.5 (2'-C(*C*$H_3$)$_2$), 24.5, 24.3, 24.2 and 23.8 (Pro γ-*C*, 4-*C*)

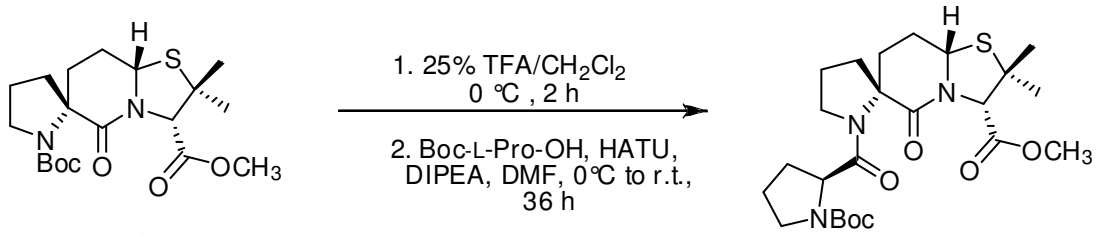
ESI HRMS *m/z* 518.2312 $[M+Na]^+$ $C_{24}H_{38}N_3O_6S$+Na requires 518.2295

Notes:

1. $CH_2Cl_2$ was rendered anhydrous by distilling from $CaH_2$

2. DMF that was purchased was stored over 4 Å molecular sieves and used directly without further purification.

**Methyl [3'*S*-(3'α,6'α,8'aβ)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.18b)**




**6.16b** **6.18b**

A solution of the spirocycle **6.16b** (380 mg, 0.9 mmol, 1.0 equiv.) in 8 mL anhydrous $CH_2Cl_2$ (Note 1) was cooled to 0 °C using an ice bath. Trifluoroacetic acid (2.9 mL, 38 mmol, 40 equiv.) was then added dropwise to this solution which was then stirred at 0 °C until Boc deprotection was determined to be complete by TLC. This was usually between 1.5 and 2 h. The reaction was warmed up to r.t. and the volatiles removed by rotary evaporation. The residue was re-dissolved in $CH_2Cl_2$ and the solvent removed by evaporation twice until the excess TFA was removed. The pale yellow-colored residue was dried to a constant weight for 5–6 h under vacuum.

A solution of Boc-L-proline (323 mg, 1.5 mmol, 1.2 equiv.) in 4 mL DMF (Note 2) was cooled to –5 °C using an ice/salt bath. DIPEA (0.6 mL, 3.8 mmol, 3.0 equiv.) was added drop-wise to this cooled solution followed by HATU (570 mg, 1.5 mmol, 1.2 equiv.). The clear solution was stirred for 30 min after which a solution of the spirocycle free amine in 4 mL DMF [obtained by neutralizing the TFA salt (520 mg, 1.2 mmol, 1.0 equiv.) with DIPEA (0.6 mL, 3.8 mmol, 3.0 equiv.)] was added to the active ester. The

yellow colored solution was warmed to r.t. and stirred there under Ar for 36 h. At the end of this time, DMF and other volatiles were removed *in vacuo.* The residue was dissolved in 80 mL EtOAc and was sequentially washed with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to give yellow-colored residue which was purified by flash chromatography (gradient elution: 0–10% MeOH/$CH_2Cl_2$ in discrete steps) to give a lightly pink-colored foam/solid which was crystallized from hexanes/EtOAc to give a white solid weighing 400 mg (84% yield).

mp 162–163 °C

TLC $R_f$ 0.40 ($CH_2Cl_2$/MeOH, 20:1)

$[\alpha]_D$ –125 (*c* 0.95, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 4.96–4.91 (m, 1H, 8'a-C*H*), 4.45 (app. t, 0.5H, Pro α-C*H*) 4.32 (dd, 0.5H, $J_1$ = 7.9 Hz, $J_2$ = 3.0 Hz, Pro α-C*H*), 4.21 and 4.20 (s, 1H, 3'-C*H*), 3.87–3.81 (m, 0.5H, Pro δ-C$H_2$), 3.72–3.65 (ovlp. m, 0.5H, Pro δ-C$H_2$), 3.683 and 3.681 (s, 3H, OC$H_3$), 3.62–3.52 (m, 2H, Pro δ-C$H_2$, 5-C$H_2$), 3.41–3.29 (m, 1H, 5-C$H_2$), 2.67–2.57 (m, 2H, 8'-C$H_2$, 7'-C$H_2$), 2.19–1.73 (m, 10H, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.59 and 1.58 (s, 3H, 2'-C(C$H_3$)$_2$), 1.43–1.40 (s, 9H, C(C$H_3$)$_3$), 1.37 and 1.36 (s, 3H, 2'-C(C$H_3$)$_2$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 171.7 and 170.4 (lactam *C*=O), 171.0 and 170.8 (amide *C*=O), 169.8 and 169.7 (ester *C*=O), 154.7 and 153.8 (carbamate *C*=O), 79.4 and 79.2 (*C*($CH_3$)$_3$), 73.7 and 73.6 (3'-*C*), 64.9 and 64.8 (6'-*C*), 62.6 and 62.5 (8'a-*C*), 58.2 (Pro α-*C*), 52.1 and 51.9 (2'-*C*), 51.04 and 51.01 (O*C*$H_3$), 47.9 (Pro δ-*C*), 47.1 and 46.9 (5-*C*), 42.2 and 42.1 (3-*C*), 37.2 and 37.1 (8'-*C*), 32.2 and 31.1 (2'-C(*C*$H_3$)$_2$), 29.7

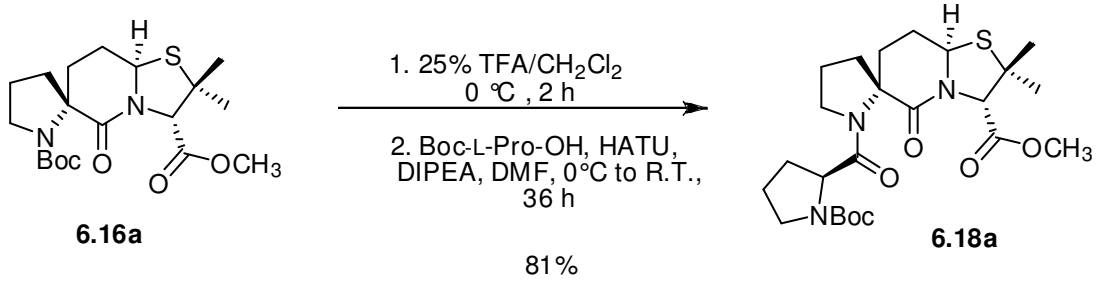
(Pro β-*C*), 28.9 and 28.7 (7'-*C*), 28.6 and 28.5 (C(*C*$H_3$)$_3$), 24.2, 23.8 and 23.6 (Pro γ-*C*, 2'-C(*C*$H_3$)$_2$), 23.6 and 23.5 (4-*C*)

ESI HRMS *m/z* 518.2293 $[M+Na]^+$ $C_{24}H_{38}N_3O_6S$+Na requires 518.2295

Notes:

1. $CH_2Cl_2$ was rendered anhydrous by distilling from $CaH_2$
2. DMF that was purchased was stored over 4 Å molecular sieves and used directly without further purification.

**Methyl [3'*S*-(3'α,6'α,8'aα)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (6.18a)**




**6.16a** **6.18a**

A solution of the spirocycle **6.16a** (380 mg, 0.9 mmol, 1.0 equiv.) in 8 mL anhydrous $CH_2Cl_2$ (Note 1) was cooled to 0 °C using an ice bath. Trifluoroacetic acid (2.9 mL, 38 mmol, 40 equiv.) was then added dropwise to this solution which was then stirred at 0 °C until Boc deprotection was determined to be complete by TLC. This was usually between 1.5 and 2 h. The reaction was warmed up to r.t. and the volatiles removed by rotary evaporation. The residue was re-dissolved in $CH_2Cl_2$ and the solvent removed by evaporation twice until the excess TFA was removed. The pale yellow-colored residue was dried to a constant weight for 5–6 h under vacuum.

A solution of Boc-L-proline (351 mg, 1.6 mmol, 1.2 equiv.) in 4 mL DMF (Note 2) was cooled to –5 °C using an ice/salt bath. DIPEA (0.7 mL, 4.1 mmol, 3.0 equiv.) was added drop-wise to this cooled solution followed by HATU (620 mg, 1.6 mmol, 1.2 equiv.). The clear solution was stirred for 30 min after which a solution of the spirocycle free amine in 4 mL DMF [obtained by neutralizing the TFA salt (560 mg, 1.4 mmol, 1.0 equiv.) with DIPEA (0.7 mL, 4.1 mmol, 3.0 equiv.)] was added to the active ester. The yellow colored solution was warmed to r.t. and stirred there under Ar for 36 h. At the end of this time, DMF and other volatiles were removed *in vacuo.* The residue was dissolved in 80 mL EtOAc and was sequentially washed with 10% citric acid, sat. $NaHCO_3$ and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to give yellow-colored residue which was purified by flash chromatography (gradient elution: 0–10% MeOH/$CH_2Cl_2$ in discrete steps) to give a lightly pink-colored foam/solid which was crystallized from hexanes/EtOAc to give a white solid weighing 380 mg (81% yield).

mp 140–142 °C

TLC $R_f$ 0.35 ($CH_2Cl_2$/MeOH, 20:1)

$[\alpha]_D$ +57.9 (*c* 0.63, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 5.33 (dt, 1H, 8'a-C*H*), 4.52 (s, 1H, 3'-C*H*), 4.47 (dd, 0.5H, $J_1$ = 7.5 Hz , $J_2$ = 3.4 Hz, Pro α-C*H*) 4.33 (dd, 0.5H, $J_1$ = 8.0 Hz, $J_2$ = 3.2 Hz, Pro α-C*H*), 3.88 (dt, 0.5H, $J_1$ = 7.7 Hz, $J_2$ = 3.1 Hz, Pro δ-C$H_2$), 3.74 (dt, 0.5H, $J_1$ = 8.9 Hz, $J_2$ = 3.1 Hz, Pro δ-C$H_2$), 3.69 (s, 3H, OC$H_3$), 3.61–3.50 (m, 2H, Pro δ-C$H_2$, 5-C$H_2$), 3.41–3.29 (m, 1H, 5-C$H_2$), 2.88–2.74 (m, 1H, 7'-C$H_2$), 2.27–1.69 (m, 11H, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 3-C$H_2$, 4-C$H_2$), 1.57 and 1.56 (s, 3H, 2'-C(C$H_3$)$_2$), 1.45–1.44 (m, 3H, 2'-C(C$H_3$)$_2$), 1.42 and 1.39 (s, 9H, C(C$H_3$)$_3$)

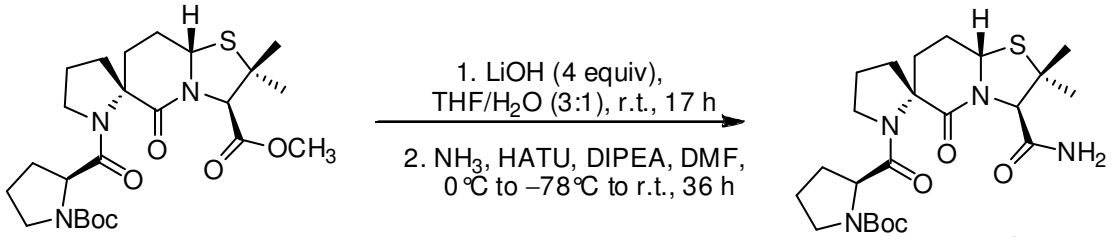
$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 171.0 and 170.6 (lactam *C*=O), 170.5 and 170.3 (amide *C*=O), 169.1 and 169.0 (ester *C*=O), 154.6 and 153.7 (carbamate *C*=O), 79.4 and 79.3 ($C(CH_3)_3$), 72.5 and 72.4 (3'-*C*), 66.1 and 66.0 (6'-*C*), 62.4 and 62.3 (8'a-*C*), 58.0 and 57.9 (Pro α-*C*), 51.9 and 51.8 ($OCH_3$), 51.4 and 51.3 (2'-*C*), 48.4 and 48.3 (Pro δ-*C*), 47.0 and 46.8 (5-*C*), 37.94 and 37.93 (8'-*C*), 31.0 and 30.8 (3-*C*), 30.3 and 30.2 (2'-$C(CH_3)_2$), 29.7 (Pro β-*C*), 28.7, 28.6, 28.5 and 28.3 (7'-*C*, $C(CH_3)_3$), 27.13 and 27.10 (2'-$C(CH_3)_2$), 24.3, 24.1 and 23.5 (Pro γ-*C*, 4-*C*)

ESI HRMS *m/z* 518.2293 $[M+Na]^+$ $C_{24}H_{38}N_3O_6S$+Na requires 518.2295

Notes:

1. $CH_2Cl_2$ was rendered anhydrous by distilling from $CaH_2$
2. DMF that was purchased was stored over 4 Å molecular sieves and used directly without further purification.

**[3'*R*-(3'β,6'α,8'aβ)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (6.19b)**




**6.17b** **6.19b**

The spirobicyclic methyl ester **6.17b** (430 mg, 0.86 mmol, 1equiv.) was dissolved in 9 mL THF. LiOH (81.6 mg, 3.4 mmol, 4 equiv.) dissolved in 3 mL $H_2O$ was added to the above solution and the reaction stirred under Ar for 17 h at which time analysis by TLC indicated complete consumption of the starting material. THF was removed by

rotary evaporation. The remaining aqueous residue was diluted with water while the pH was adjusted to 12–14 using 1 N NaOH. The solution was washed with 50 mL EtOAc. The pH of the aqueous layer was then adjusted to 2–3 using dilute HCl and the solution washed with EtOAc (2 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give a colorless oil which converted to a foam/solid under high vacuum. The pure carboxylic acid weighed 413 mg (quantitative yield). $^1$H NMR analysis indicated that the product was a single diastereoisomer and that the ester hydrolysis had taken place without any epimerization/racemization of any stereocenters in the molecule. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.98 (br. s, 1H, COOH), 5.06 (dt, $J_1$ = 12.6 Hz, $J_2$ = 2.9 Hz, 1H, 8'a-C*H*), 4.69 and 4.66 (s, 1H, 3'-C*H*), 4.41 (dd, $J_1$ = 7.7 Hz, $J_2$ = 3.5 Hz, 0.5H, Pro α-*C*), 4.32 (dd, $J_1$ = 8.3 Hz, $J_2$ = 4 Hz, 0.5H, Pro α-C*H*), 3.88–3.83 (m, 0.5H, Pro δ-C$H_2$), 3.71–3.66 (m, 0.5H, Pro δ-C$H_2$), 3.61–3.30 (m, 3H, Pro δ-C$H_2$, 5-C$H_2$), 2.60–2.40 (m, 2H, 8'-C$H_2$, 7'-C$H_2$), 2.30–1.85 (m, 9H, 3-C$H_2$, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 4-C$H_2$), 1.80–1.70 (m, 1H, 4-C$H_2$), 1.60 and 1.59 (s, 3H, 2'-C(C$H_3$)$_2$), 1.47 and 1.46 (s, 3H, 2'-C(C$H_3$)$_2$), 1.40 and 1.38 (m, 9H, C(C$H_3$)$_3$)

A solution of the carboxylic acid (413 mg, 0.86 mmol, 1 equiv.) in 3 mL DMF was cooled to 0 °C. This was followed by the sequential addition of DIPEA (0.3 mL, 1.72 mmol, 2.0 equiv.) and HATU (392 mg, 1.03 mmol, 1.2 equiv.). The reaction was stirred for 20 min. at this temperature under Ar. The solution was then cooled to –78 °C. In a cooled measuring cylinder, 2–3 mL anhydrous ammonia was condensed into 5 mL anhydrous $CH_2Cl_2$. This solution was added to the previous solution of the active ester. A canary yellow-colored solution was formed instantly. A glass adapter fitted with a balloon was attached to the reaction flask. The reaction was stirred at –78 °C for 7 h and then warmed to r.t. over the next 17 h. The next day, another 2 mL portion of liquid

ammonia was added and the reaction stirred for another 17 h. At the end of this time, the reaction mixture was diluted with 50 mL $CH_2Cl_2$ and filtered to remove the yellow-colored precipitate. The almost colorless filtrate was concentrated and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was further purified by flash chromatography (gradient elution:0–15% MeOH/$CH_2Cl_2$, pre-packed Luknova® 40g $SiO_2$ cartridge, Combiflash Retrieve® system, 25 mL/min. flow-rate) to give a cloudy and colorless oil. The precipitated tetramethyl urea (TMU) was removed by filtering the product through a 0.45 μm membrane filter disc to give 80 mg of pure product **6.19b** (20% yield).

TLC $R_f$ 0.35 ($CH_2Cl_2$/MeOH, 10:1)

$[\alpha]_D$ –162.4 (*c* 1.15, $CHCl_3$)

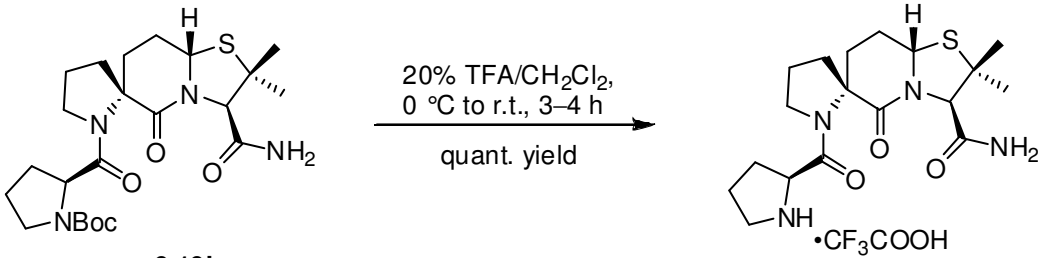
$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 6.28 (app. br. t, 1H, N*H*), 5.95 (app. br. d, 1H, N*H*), 5.11 (dt, 1H, 8'a-C*H*), 4.56 and 4.54 (s, 1H, 3'-C*H*), 4.41 (m, 0.5H, Pro α-C*H*), 4.30 (m, 0.5H, Pro α-C*H*), 3.86–3.80 (m, 0.5H, Pro δ-C$H_2$), 3.71–3.61 (m, 0.5H, Pro δ-C$H_2$), 3.55–3.45 (m, 2H, Pro δ-C$H_2$, 5-C$H_2$), 3.43–3.30 (m, 1H, 5-C$H_2$), 2.55–2.40 (m, 2H, 8'-C$H_2$, 7'-C$H_2$), 2.30–1.85 (m, 9H, 3-C$H_2$, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 4-C$H_2$), 1.77–1.70 (m, 1H, 4-C$H_2$), 1.57, 1.47 (s, 3H, 2'-C(C$H_3$)$_2$), 1.40 and 1.37 (m, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 171.3 and 170.9 (lactam *C*=O), 170.9, 170.5, 170.2 (amide *C*=O), 154.6 and 153.8 (carbamate *C*=O), 79.5 and 79.3 (*C*($CH_3$)$_3$), 72.9 and 72.8 (3'-*C*), 65.0 and 64.9 (6'-*C*), 63.3 and 63.1 (8'a-*C*), 58.2 and 58.1 (Pro α-*C*), 50.8 and 50.6 (2'-*C*), 48.1 and 48.0 (Pro δ-*C*), 47.1 and 46.9 (5-*C*), 41.3 and 41.2 (3-*C*), 36.3

and 36.1 (8'-*C*), 29.7 (Pro β-*C*), 29.1, 29.0, 28.8, 28.7, 28.5 and 28.4 (2'-C($CH_3$)$_2$, 7'-*C*, C($CH_3$)$_3$), 26.5 (2'-C($CH_3$)$_2$), 24.5, 23. 9, 23.7 (Pro γ-*C*, 4-*C*)

ESI HRMS *m/z* 503.2317 $[M+Na]^+$ $C_{23}H_{36}N_4O_5S$+Na requires 503.2299

**[3'*R*-(3'β,6'α,8'aβ)]-1-[[2''(*S*)-Pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide Trifluoroacetate Salt (5.4b)**




**6.19b** **5.4b**

The spiro-bicyclic carboxamide **6.19b** (50 mg, 0.1 mmol, 1 equiv.) was dissolved in 2 mL anhydrous $CH_2Cl_2$ and the solution cooled to 0 °C. Trifluoroacetic acid (0.4 mL, 5.1 mmol, 51 equiv.) was added dropwise to this solution which was then stirred at this temperature for 1 h and then allowed to warm to room temperature until the reaction was complete. The pale yellow-colored solution was concentrated to give an oil which was repeatedly triturated with a $CH_2Cl_2$/diethyl ether mixture and concentrated until a crystalline white-colored solid was obtained. The solid was dried well under high vacuum to give the final compound **5.4b** weighing 49 mg (quantitative yield). A solution of the molecule (1.6 mg/mL in 4:1 acetonitrile-water) was diluted (2 x by volume) with 0.05% ammonium formate buffer solution and 10–20 µL was subjected to HPLC analysis at 214 nm using a Varian Pursuit® C18 column (3 µm particle size, 100 mm x 2 mm dimension) and using a gradient 10–90% acetonitrile in 0.05% ammonium formate buffer solution. A retention time of 4.24 min. was obtained in a 10 min run and purity was 96.1%.

mp 260 °C (dec.)

TLC $R_f$ 0.75 (iPrOH/$NH_4OH$, 4:1)

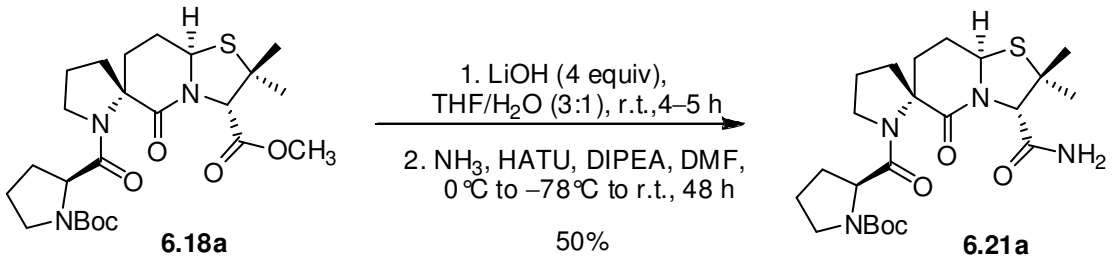
$[\alpha]_D$ –163 (*c* 0.5, $CH_3OH$)

$^1H$ NMR (400 MHz, $CD_3OD$, gCOSY assignment) δ 5.23 (dd, $J_1$ = 10 Hz, $J_2$ = 3.6 Hz, 1H, 8'a-C*H*), 4.52 (s, 1H, 3'-C*H*), 4.52–4.66 (ovlp. m, 1H, Pro α-C*H*), 3.86–3.76–3.71 (m, 1H, Pro δ-C$H_2$), 3.57–3.51 (m, 1H, Pro δ-C$H_2$), 3.40–3.25 (m, 2H, 5-C$H_2$), 2.50–2.34 (m, 3H, 8'-C$H_2$, 7'-C$H_2$, 3-C$H_2$), 2.26–1.99 (m, 9H, 3-C$H_2$, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 4-C$H_2$), 1.59, 1.50 (s, 3H, 2'-C(C$H_3$)$_2$)

$^{13}C$ NMR (100 MHz, $CD_3OD$, gHMQC and HMBC assignment) δ 170.6, 169.8, 165.8 (amide *C*=O), 74.0 (3'-*C*), 66.7 (6'-*C*), 63.9 (8'a-*C*), 60.4 (Pro α-*C*), 51.6 (2'-*C*), 48.7 (Pro δ-*C*, overlapped by residual solvent peaks, gHMQC assignment), 47.7 (5-*C*), 41.9 (3-*C*), 36.5 (Pro β-*C*), 29.7 (2'-C(*C*$H_3$)$_2$), 29.3 (8'-*C*), 29.2 (7'-*C*), 27.4 (2'-C(*C*$H_3$)$_2$), 25.5, 25.2 (Pro γ-*C*, 4-*C*)

ESI HRMS *m/z* 381.1961 $[M+H]^+$ $C_{18}H_{29}N_4O_3S$ requires 381.1955

**[3'*S*-(3'α,6'α,8'aα)]-1-[[1''-(*tert*-Butyloxycarbonyl)-2''(*S*)-pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (6.21a)**




**6.18a** **6.21a**

The spirobicyclic methyl ester (370 mg, 0.75 mmol, 1 equiv.) was dissolved in 9 mL THF. LiOH (72 mg, 3 mmol, 4 equiv.) dissolved in 3 mL $H_2O$ was added to the above solution and the reaction stirred under Ar for 4–5 h at which time analysis by TLC indicated complete consumption of the starting material. THF was removed by rotary evaporation. The remaining aqueous residue was diluted with water while the pH was adjusted to 12–14 using 1 N NaOH. The solution was washed with 50 mL EtOAc. The pH of the aqueous layer was then adjusted to 2–3 using dilute HCl and the solution washed with EtOAc (2 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give a colorless oil which converted to a foam/solid under high vacuum. The pure carboxylic acid weighed 360 mg (quantitative yield). $^1$H NMR analysis indicated that the product was a single diastereoisomer and that the ester hydrolysis had taken place without any epimerization/racemization of any stereocenters in the molecule. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.00 (br. s, 1H, COOH), 5.23 (dt, $J_1$ = 10.4 Hz, $J_2$ = 3.2 Hz, 1H, 8'a-C*H*), 4.52 (s, 1H, 3'-C*H*), 4.41 (dd, $J_1$ = 7.6 Hz, $J_2$ = 2.8 Hz, 0.5H, Pro α-*C*), 4.31 (dd, $J_1$ = 8.0 Hz, $J_2$ = 3.6 Hz, 0.5H, Pro α-C*H*), 3.961 (t, $J$ = 8.4 Hz, 0.5H, Pro δ-C$H_2$), 3.80 (t, $J$ = 10.4 Hz, 0.5H, Pro δ-C$H_2$), 3.60–3.30 (m, 3H, Pro δ-C$H_2$, 5-C$H_2$), 2.51–1.67 (m, 12H, 3-C$H_2$, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 4-C$H_2$), 1.80–1.70 (m, 1H, 4-C$H_2$), 1.64 (s, 3H, 2'-C(C$H_3$)$_2$), 1.54 (s, 3H, 2'-C(C$H_3$)$_2$), 1.41 and 1.39 (m, 9H, C(C$H_3$)$_3$)

A solution of the carboxylic acid (262 mg, 0.54 mmol, 1 equiv.) in 2 mL DMF was cooled to 0 °C. This was followed by the sequential addition of DIPEA (0.2 mL, 1.1 mmol, 2.0 equiv.) and HATU (247 mg, 0.65 mmol, 1.2 equiv.). The reaction was stirred for 20 min. at this temperature under Ar. The solution was then cooled to –78 °C. In a cooled measuring cylinder, 4 mL anhydrous ammonia was condensed into 5 mL anhydrous $CH_2Cl_2$. This solution was added to the previous solution of the active ester. A canary yellow-colored solution was formed instantly. A glass adapter fitted with a balloon was attached to the reaction flask. The reaction was stirred at –78 °C for 7 h and then warmed to r.t. over the next 48 h. At the end of this time, the reaction mixture was diluted with 50 mL $CH_2Cl_2$ and filtered to remove the yellow-colored precipitate. The almost colorless filtrate was concentrated and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was further purified by flash chromatography (gradient elution:0–6% MeOH/$CH_2Cl_2$, pre-packed Luknova® 40g $SiO_2$ cartridge, Combiflash Retrieve® system, 30 mL/min. flow-rate) to give the pure product as a colorless oil weighing 130 mg (50% yield).

TLC $R_f$ 0.55 ($CH_2Cl_2$/MeOH, 10:1)

$[\alpha]_D$ +59.3 (*c* 0.5, $CHCl_3$)

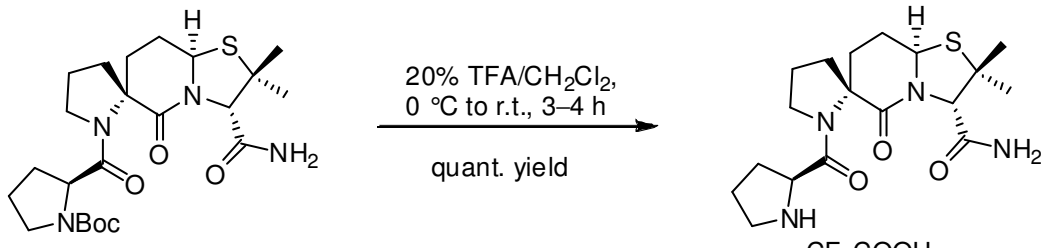
$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment, rotamers observed about the carbamate bond in a 1:1 ratio) δ 7.39 and 7.32 (br. s, 1H, N$H_{\text{hydrogen bonded}}$), 5.15–5.11 (m, 2H, 8'a-C*H*, N*H* ), 4.43 (dd, $J_1$ = 8.8 Hz, $J_2$ = 2.8 Hz, 0.5H, Pro α-C*H*), 4.36 (s, 1H, 3'-C*H*), 4.31 (dd, $J_1$ = 8.0 Hz, $J_2$ = 2.4 Hz, 0.5H, Pro α-C*H*), 3.95 (t, 0.5H, Pro δ-C$H_2$), 3.79 (t, 0.5H, Pro δ-C$H_2$), 3.60–3.31 (m, 3H, Pro δ-C$H_2$, 5-C$H_2$), 2.59–2.47 (m, 1H, 3-C$H_2$), 2.36–2.22 (m, 2H, 8'-C$H_2$, 4-C$H_2$), 2.15–1.72 (m, 9H, 3-C$H_2$, 8'-C$H_2$, 7'-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$,

4-C$H_2$), 1.66 (s, 3H, 2'-C(C$H_3$)$_2$), 1.54 (s, 3H, 2'-C(C$H_3$)$_2$), 1.41 and 1.39 (m, 9H, C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment, rotamers observed about the carbamate bond in a 1.5:1 ratio) δ 170.9, 170.5, 169.7, 169.5, 169.0, 168.8 (amide *C*=O), 153.3 and 152.3 (carbamate *C*=O), 79.7 and 79.6 (*C*(CH$_3$)$_3$), 73.3 (3'-*C*), 66.6 and 66.6 (6'-*C*), 62.4 and 62.3 (8'a-*C*), 58.2 (Pro α-*C*), 52.6 and 52.5 (2'-*C*), 48.9 (Pro δ-*C*), 47.5 and 47.2 (5-*C*), 37.3 (3-*C*), 31.3 and 31.1 (7'-*C*), 30.3 (Pro β-*C*), 29.6 and 29.5 (2'-C(*C*H$_3$)$_2$), 29.3 and 29.2 (8'-*C*,C(*C*H$_3$)$_3$), 27.7 and 27.6 (2'-C(*C*H$_3$)$_2$), 25.3, 25.0, 24.9, 24.5 (Pro γ-*C*, 4-*C*)

ESI HRMS *m/z* 503.2317 [M+Na]$^+$ $C_{23}H_{36}N_4O_5S$+Na requires 503.2299

**[3'*S*-(3'α,6'α,8'aα)]-1-[[2''(*S*)-Pyrrolidinyl]carbonyl]- 2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide Trifluoroacetate salt (5.5a)**




**6.21a** **5.5a**

The spiro-bicyclic carboxamide (84 mg, 0.17 mmol, 1 equiv.) was dissolved in 2 mL anhydrous $CH_2Cl_2$ and the solution cooled to 0 °C. Trifluoroacetic acid (0.6 mL, 7.7 mmol, 45 equiv.) was added dropwise to this solution which was then stirred at this temperature for 1 h and then allowed to warm to room temperature until the reaction was complete. The pale yellow-colored solution was concentrated to give an oil which was

repeatedly triturated with a $CH_2Cl_2$/diethyl ether mixture and concentrated until a crystalline white-colored solid was obtained. The solid was dried well under high vacuum to give the final compound weighing 71 mg (85% yield). A solution of the molecule (1.6 mg/mL in 4:1 acetonitrile-water) was diluted (2 x by volume) with 0.05% ammonium formate buffer solution and 10–20 µL was subjected to HPLC analysis at 214 nm using a Varian Pursuit® C18 column (3 µm particle size, 100 mm x 2 mm dimension) and using a gradient 10–90% acetonitrile in 0.05% ammonium formate buffer solution. A retention time of 5.29 min. was obtained in a 10 min run and purity was 99.1%.

mp 260 °C (dec.)

TLC $R_f$ 0.68 (iPrOH/$NH_4OH$, 5:1)

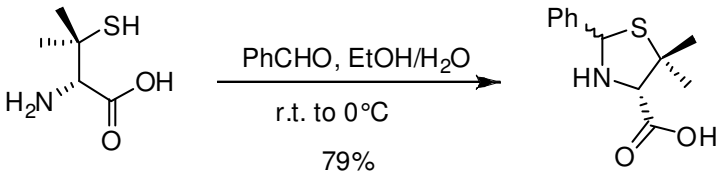
$[\alpha]_D$ +80.4 (*c* 0.45, $CH_3OH$)

$^1H$ NMR (400 MHz, $CD_3OD$, gCOSY assignment) δ 5.20 (dd, $J_1$ = 10.8 Hz, $J_2$ = 4.0 Hz, 1H, 8'a-C*H*), 4.87 (dd, $J_1$ = 8.8 Hz, $J_2$ = 5.2 Hz, 1H, Pro α-C*H*), 4.27 (s, 1H, 3'-C*H*), 3.76–3.72 (m, 1H, 5-C$H_2$), 3.53–3.47 (m, 1H, 5-C$H_2$), 3.33–3.22 (m, 2H, Pro δ-C$H_2$), 2.45–2.25 (m, 3H, 7'-C$H_2$, Pro β-C$H_2$, 8'-C$H_2$), 2.20–1.86 (m, 8H, 3-C$H_2$, 4-C$H_2$, Pro β-C$H_2$, Pro γ-C$H_2$, 7'-C$H_2$), 1.57, 1.49 (s, 3H, 2'-C(C$H_3$)$_2$)

$^{13}C$ NMR (100 MHz, $CD_3OD$, gHMQC and HMBC assignment) δ 171.1, 170.1, 166.3 (amide *C*=O), 74.4 (3'-*C*), 68.3 (6'-*C*), 63.4 (8'a-*C*), 60.3 (Pro α-*C*), 52.4 (2'-*C*), 49.5 (5-*C*, overlapped by residual solvent peaks, gHMQC assignment), 47.7 (Pro δ-*C*), 37.9 (3-*C*), 31.1 (7'-*C*), 29.9 (2'-C(*C*$H_3$)$_2$), 29.4 (Pro β-*C*), 28.1 (8'-*C*), 27.3 (2'-C(*C*$H_3$)$_2$), 25.5, 25.3 (Pro γ-*C*, 4-*C*)

ESI HRMS *m/z* 381.1946 $[M+H]^+$ $C_{18}H_{29}N_4O_3S$ requires 381.1955

**(4*S*)-5,5-Dimethyl-2-phenylthiazolidine-4-carboxylic Acid (6.24)**




D-Penicillamine **6.24**

D-Penicillamine (746 mg, 5 mmol, 1.0 equiv.) was dissolved in 10 mL water with vigorous stirring. A solution of benzaldehyde (0.5 mL, 5 mmol, 1.0 equiv.) in 3 mL EtOH was added drop-wise to the aqueous solution. A thick-white precipitate formed almost immediately. Once addition was complete, the solution was further diluted (Note 1) with an EtOH-$H_2O$ mix (4:1) and stirred vigorously for 1 h at r.t. and 4 h at 0 °C. The white-colored precipitate was filtered and washed with water. The precipitate was then suspended in $CH_2Cl_2$ first and then xylenes and subjected to rotary evaporation in order to remove most of the water azeotropically. The slightly wet precipitate was dried under high vacuum to give the product **6.24** as a free-flowing white powder weighing 940 mg (79 % yield, Notes 2–3).

mp 165 °C (dec.)

TLC $R_f$ 0.64 (iPrOH/$NH_4OH$, 4:1)

$[\alpha]_D$ +43.6 (*c* 1.2, MeOH)

$^1$H NMR (400 MHz, DMSO-$d_6$, diastereomers present in a 1.5–1 ratio, major diastereomer indicated by *) δ 7.60–7.50 (m, 2H, aromatic *C*H), 7.45–7.20 (m, 6.3H, aromatic *C*H), 5.91 (s, 0.7H, 2-C*H*) , 5.69* (s,1H, 2-C*H*), 3.73* (s, 1H, 4-C*H*), 3.65 (s, 0.7H, 4-C*H*), 1.72* (s, 3H, C$H_3$), 1.60 (s, 2.1H, C$H_3$), 1.42* (s, 3H, C$H_3$), 1.36 (s, 2H, C$H_3$).

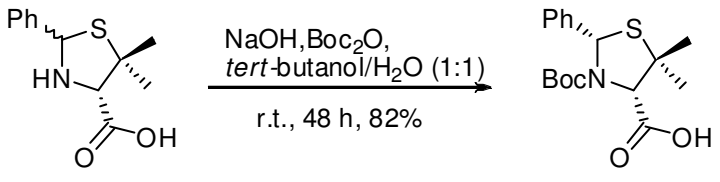
$^{13}$C NMR (100 MHz, DMSO-$d_6$, diastereomers present in a 1.5:1 ratio, major diastereomer indicated by *) δ 170.7, 170.4* (*C*=O), 144.1, 139.1* (aromatic $C_q$), 128.5*, 126.2 (aromatic *C*H), 128.3*, 126.9 (aromatic *C*H), 128.2, 127.4* (aromatic *C*H), 74.1*, 73.2 (4-*C*), 69.1*, 67.7 (2-*C*), 60.1, 59.5* (5-*C*), 29.1*, 27.7, 28.2 *, 26.8 ((*C*$H_3$)$_2$)

ESI HRMS *m/z* 260.0718 $[M+Na]^+$ $C_{12}H_{15}NO_2S$+Na requires 260.0716

Notes:

1. The precipitate formed is very thick and interferes with the stirring efficiency of the magnetic stir bar. On large scale, the starting volumes of EtOH and $H_2O$ should be substantially adjusted to prevent this.
2. On a small scale, NMR analysis of the product in DMSO-$d_6$ immediately following the drying process indicated the presence of the two diastereomers (C-2) in a 5:1 ratio. When the *same solution* was subjected to NMR analysis again after 12 h, the diastereomeric ratio had changed to approximately 1.5:1. It is possible that the formation of the thiazolidine is an equilibrium reaction. When the reaction was conducted on a large scale (30 mmol vs. 5 mmol), the initial diastereomeric ratio was > 13–15:1.
3. Caution: *Stench!*

**(2*S*,4*S*)-*N*-*tert*-Butyloxycarbonyl-5,5-Dimethyl-2-phenylthiazolidine-4-carboxylic Acid (6.25)**




**6.24** **6.25**

Sodium hydroxide (80 mg, 2 mmol, 1 equiv.) was added as a powder to a suspension of the amino acid **6.24** (475 mg, 2 mmol, 1 equiv.) in water (3 mL). Within 10 minutes, a clear solution was obtained. A solution of $Boc_2O$ (445 mg, 2.04 mmol, 1.02 equiv.) in *tert*-butanol (3 mL) was added to the aqueous solution. The cloudy solution was stirred under Ar for 48 h during which a white-colored suspension was obtained. *tert*-Butanol was removed by rotary evaporation and the residue diluted with $H_2O$. The pH of this solution was adjusted to 11–12 and it was washed with $Et_2O$. The pH of the basic solution was adjusted to 2–3 using HCl to give rise to a white precipitate which was extracted into EtOAc (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give rise to the pure product as a white solid weighing 551 mg (82% yield) which was further crystallized using hexanes/EtOAc/2–3 drops of MeOH to give needle-shaped crystals. The product **6.25** was entirely a single diastereomer (Note 1).

mp 215–217 °C (dec.)

TLC $R_f$ 0.60 (iPrOH/$NH_4OH$, 4:1)

$[\alpha]_D$ –75 (*c* 1.0 $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, Note 2) 7.66 (app. d, 2H, Ar-C*H*), 7.35–7.24 (m, 3H, Ar-C*H*), 5.98 (br. s, 1H, 2-C*H*), 4.54 (s, 1H, 4-C*H*), 1.68 (s, 3H, 5-C(C$H_3$)$_2$), 1.47–1.55 (m, 12H, C(*C*$H_3$)$_3$, 5-C(C$H_3$)$_2$)

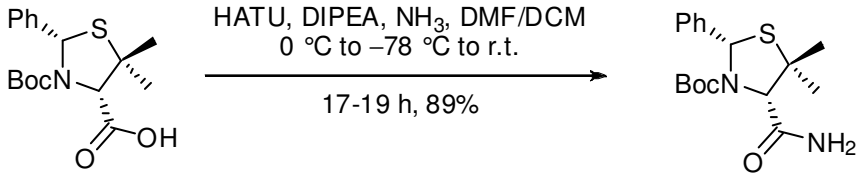
$^{13}$C NMR (100 MHz, DMSO-$d_6$ Note 3) 171.0 (acid *C*=O), 152.7 (carbamate *C*=O), 141.2 (Ar-$C_q$), 127.7, 127.2, 126.6 (Ar-*C*H), 79.9 (*C*($CH_3$)$_3$), 72.8 (4-*C*), 64.9 (2-*C*), 51.2 (5-*C*), 30.9 (5-C(*C*$H_3$)$_2$), 27.5 (C(*C*$H_3$)$_3$), 24.4 (5-C(*C*$H_3$)$_2$)

ESI HRMS *m/z* 360.1243 $[M+Na]^+$ $C_{17}H_{23}NO_4S$+Na requires 360.1240

Notes:

1. The pure product was crystallized and stereoselectivity was determined by X-ray structure.
2. $^{1}H$ NMR conducted in $CDCl_3$ depicts mostly one diastereomer in the product except for some peak integrations. This property is reflected in the $^{13}C$ NMR in $CDCl_3$ except the intensities of some aliphatic region peaks are highly attenuated.
3. $^{13}C$ NMR reported above was performed in DMSO-$d_6$ which contained traces of water. The chemical shifts reported are those corresponding to the major diastereomer. The product appears to be in constant equilibrium with its diastereomer (via opening and closing of the thiazolidine ring) at the NMR time-scale, specifically when there is water present in the NMR solvent. Due to this reason, the aliphatic peaks appear with attenuated intensity.

**(2*S*,4*S*)-*N-tert*-Butyloxycarbonyl-5,5-Dimethyl-2-phenylthiazolidine-4-carboxamide (6.26)**




**6.25** **6.26**

A solution of the carboxylic acid **6.25** (337 mg, 1 mmol, 1 equiv.) in 2 mL anhydrous DMF (Note 1) was cooled to 0 °C. This was followed by the sequential addition of DIPEA (0.3 mL, 2 mmol, 2 equiv.) and HATU (456 mg, 1.2 mmol, 1.2 equiv.). The pale yellow-colored solution was stirred at this temperature for 20 min. following which it was cooled to –78 °C. In a cooled measuring cylinder, 2 mL anhydrous

ammonia (Note 2) was condensed into 5 mL anhydrous $CH_2Cl_2$ (Note 3). This solution was added to the former active ester resulting in a canary yellow precipitate formation almost immediately. An adapter fitted with a balloon was attached to the reaction flask. The reaction mixture was stirred at –78 °C for 7 h and then allowed to warm to room temperature over a 12 h period. The next morning, the reaction mixture was diluted with $CH_2Cl_2$ (40 mL) and filtered to give a nearly colorless filtrate. The volatiles were removed by rotary evaporation, including majority of DMF and the residue was dissolved in EtOAc (60 mL) and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer which was opalescent at this time was dried over $MgSO_4$, filtered and concentrated to give a colorless oil and some solid (Note 4) which was almost 80% pure by NMR. It was further purified by flash chromatography (gradient elution: 0–5% MeOH/$CH_2Cl_2$) to give the product **6.26** as a colorless oil which solidified (foam to solid) under vacuum and which weighed 300 mg (89% yield).

mp 103–105 °C

TLC $R_f$ 0.6 ($CH_2Cl_2$/MeOH, 20:1)

$[\alpha]_D$ –9.4 (*c* 1.03, $CDCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$) 7.46 (d, $J$ = 7.6 Hz, 2H, aromatic C$H_{ortho}$), 7.29–7.18 (m, 3H, aromatic C$H_{meta}$ , C$H_{para}$), 6.32 (br. s, 1H, N*H*), 6.00 (s, 1H, 2-C*H*), 5.89 (br. s, 1H, N*H*), 4.3 (s, 1H, 4-C*H*), 1.56 (s, 3H, C(C$H_3$)$_2$), 1.37 (3H, C(C$H_3$)$_2$), 1.2 (s, 9H, Boc C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$) δ 171.1 (amide *C*=O), 153.0 (carbamate *C*=O), 139.1 (aromatic $C_q$), 127.8, 127.2, 125.7 (aromatic *C*H), 81.9 (*C*(CH$_3$)$_3$), 74.9 (4-*C*), 66.1 (2-*C*), 52.6 (5-*C*), 32.0 (5-C(*C*H$_3$)$_2$), 28.9 (C(*C*H$_3$)$_3$), 25.0 (5-C(*C*H$_3$)$_2$).

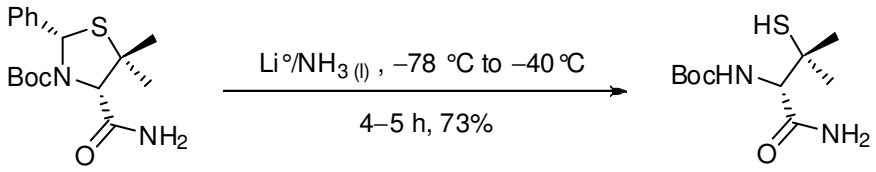
ESI HRMS *m/z* 359.1405 $[M+Na]^+$ $C_{17}H_{24}N_2O_3S$+Na requires 359.1400

Notes:

1. DMF was stored over 4 Å molecular sieves and used without further purification.

2. Anhydrous ammonia was purchased from Matheson Trigas or Sigma Aldrich in a lecture bottle.
3. $CH_2Cl_2$ was rendered anhydrous by distilling from $CaH_2$.
4. As per $^1H$ and $^{13}C$ NMR, the peaks at 2.8 ppm and 39.5 ppm respectively belong to tetramethyl urea (TMU), which is a by-product of amide bond formations using HATU. Even after flash chromatography, the product was contaminated to an extent of 11% with TMU.

**(*S*)-N-*tert*-Butyloxycarbonyl penicillamine carboxamide (6.27)**




**6.26** **6.27**

The diastereomerically pure thiazolidine **6.26** (267 mg, 0.8 mmol, 1 equiv.) was taken in a 100 mL RBF and purged with Ar. The flask was cooled to –78 °C and equipped with an efficient teflon-coated stir bar. Approximately 25 mL anhydrous ammonia (Note 1) was directly condensed into the flask. The substrate dissolved in liquid ammonia even at this temperature. An adapter fitted with a balloon was then attached to the flask. Lithium metal (Note 2) was cut into 2 mm cubes and added to the reaction flask. After 2 min., the entire reaction mixture took on an intense royal blue color. The reaction was stirred vigorously at –78 °C for 2 h and then warmed to –40 °C for approximately 15 min. before being cooled back to –78 °C. The reaction was stirred for a further 2–3 h during which time Li metal was added to maintain the blue color. At the end of a total 5 h, the reaction was quenched by addition of solid ammonium chloride

($NH_4Cl$) until the blue color disappeared and a white suspension was obtained. Ammonia was removed under a gentle stream of Ar over 20 min. to leave behind a free-flowing chalky white solid (Note 3). The solid was partitioned between water (pH adjusted to 7) and $CH_2Cl_2$ (60 mL each). The aqueous layer was washed 2 more times with 70 mL portions of $CH_2Cl_2$. The organic layers were pooled together, dried over $MgSO_4$, filtered and concentrated to give a colorless oil/foam with was 90% pure by NMR analysis. It was further purified by flash chromatography (gradient elution: 0–10% MeOH/$CH_2Cl_2$) to give the product **6.27** as a white foam/solid weighing 145 mg (73%).

mp 55–57 °C

TLC $R_f$ 0.58 ($CH_2Cl_2$/MeOH, 10:1)

$[\alpha]_D$ –11.5 (*c* 0.6, MeOH)

$^1$H NMR (400 MHz, $CDCl_3$), δ 6.40 (br.s, 1H, N*H*$_2$), 5.74 (br.s, 1H, N*H*$_2$), 5.62 (d, *J* = 8 Hz, 1H, Boc N*H*), 4.13 (d, *J* = 9.2 Hz, 1H, α-C*H*), 1.54 (s, 3H, S-C(C*H*$_3$)$_2$), 1.44 (Boc C(C*H*$_3$)$_3$), 1.34 (s, 3H, S-C(C*H*$_3$)$_2$)

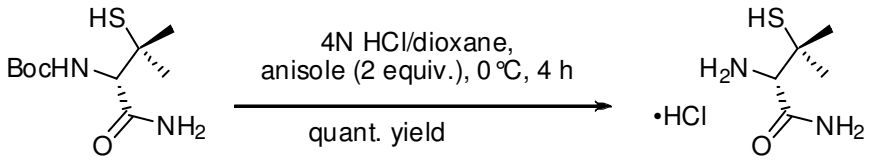
$^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.9 (amide *C*=O), 154.8 (carbamate *C*=O), 80.5 (*C*(CH$_3$)$_3$), 62.3 (α-*C*), 46.4 (β-*C*), 31.9 (C(*C*H$_3$)$_2$), 29.3 (Boc C(*C*H$_3$)$_3$)

ESI HRMS *m/z* 271.1091 $[M+Na]^+$ $C_{10}H_{20}N_2O_3S$+Na requires 271.1087

Notes:

1. Anhydrous ammonia was purchased from Matheson Trigas or Sigma Aldrich in a lecture bottle.
2. Lithium wire purchased from Sigma Aldrich was used after the residual paraffin was washed off using hexanes. On a large scale (13 mmol substrate), approximately 8–10 equiv. Li metal was required to maintain the blue color of the reaction.
3. Caution: *Particles are easily aerosolized!*

**D-Penicillamine carboxamide•Hydrochloride (6.23)**




**6.27** **6.23**

The Boc-protected penicillamine carboxamide **6.27** (145 mg, 0.6 mmol, 1 equiv.) was dissolved in 4 N HCl/dioxane (8 mL, Note 1) and the solution cooled to 0 °C. While the solution was vigorously stirred, anisole (0.2 mL, 2.4 mmol, 4 equiv.) was added drop-wise to the solution (Note 2). The reaction mixture was stirred until the starting material had been completely consumed as determined by periodic analysis by TLC. At the end of 4 h, when all the starting material was consumed, the volatiles were removed by rotary evaporation. The white solid residue was triturated 4 times with hot $Et_2O$ and the organic supernatant layer decanted each time to remove most of the anisole. The residue was dried under vacuum to give the pure product **6.23** weighing 110 mg (quantitative yield) as a free-flowing powder.

mp > 210 °C (dec.)

TLC $R_f$ 0.73 (iPrOH/$NH_4OH$, 4:1)

$[\alpha]_D$ –84.7 (*c* 1.0, $CD_3OD$)

$^1H$ NMR (400 MHz, $CD_3OD$) δ 3.84 (s, 1H, α-C*H*), 1.48, 1.39 (s, 3H, $C(CH_3)_2$)

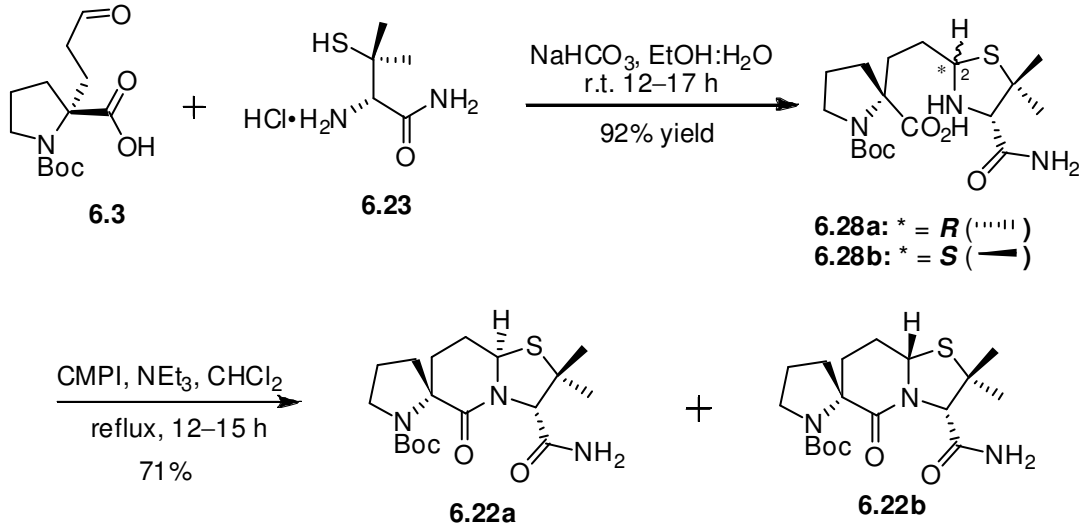
$^{13}C$ NMR (100 MHz, $CD_3OD$) δ 167.7 (*C*=O), 63.2 (α-*C*), 44.8 (β-*C*), 31.0, 28.64 ($C(CH_3)_2$)

ESI HRMS *m/z* 149.0749 $[M+H]^+$ $C_5H_{13}N_2OS$+Na requires 149.0743

Notes:

1. The volume of 4 N HCl/dioxane required for complete conversion depends on the concentration of dissolved HCl and that in turn depends on the age of the opened bottle. Lesser volume can be used from a freshly-opened bottle.
2. Anisole is commonly used as a *tert*-butyl cation scavenger; in this case particularly to prevent any reaction of the latter with the sulfhydryl functionality.

**[3'*S*-(3'α,6'α,8'aα)]-1-(*tert*-Butyloxycarbonyl)-2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (6.22a) and [3'*S*-(3'α,6'α,8'aβ)]-1-(*tert*-Butyloxycarbonyl)-2',2'-dimethyl-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (6.22b)**




**6.3** **6.23** **6.28a:** * = ***R*** ( ) **6.28b:** * = ***S*** ( )

**6.22a** **6.22b**

**6.22a : 6.22b =1 :2.5**

*N*-Boc-α-butenylproline **6.4** (1.65 g, 6.1 mmol, 1.01 equiv.) was dissolved in a 60 mL mixture of EtOH and $H_2O$ (1:1). The solution was then cooled using an ice/salt bath. Sodium bicarbonate (504 mg, 6.0 mmol, 1 equiv.) was then added to the solution followed by D-penicillamide•HCl (1.1 g, 6.0 mmol, 1 equiv.). The pH of the resulting solution was adjusted to 6.5 and the colorless solution was stirred for 17 h at room temperature under Ar. After this time period, EtOH was removed *in vacuo* and the

resulting aqueous residue was washed with EtOAc (3 x 100 mL). The pH of the aqueous layer was adjusted to 4.0-4.5 between the washes to ensure maximum product precipitation. The organic layers were combined and dried over $MgSO_4$, filtered and concentrated to give the intermediate thiazolidine mixture of **6.28a** and **6.28b** as a white-colored foam weighing 2.2 g (91% yield).

The diastereomeric thiazolidine mixture (2.2 g, 5.5 mmol, 1 equiv.) was dissolved in 165 mL anhydrous $CH_2Cl_2$ (Note 1). Triethylamine (1.9 mL, 13.7 mmol, 2.5 equiv.) was added in one portion to the solution followed by CMPI (1.7 g, 6.6 mmol, 1.2 equiv.). A bright yellow-colored suspension was obtained. This suspension was refluxed for 17 h at the end of which an amber-colored solution was obtained. The reaction mixture was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated to an orange-colored syrup that was purified using flash chromatography (gradient elution: 40–100% EtOAc/hexanes followed by 1–5% MeOH/EtOAc; Luknova 80g $SiO_2$ gel pre-packed column). The two diastereomers, **6.22a** and **6.22b**, were obtained in a 1:2.5 ratio weighing 425 mg (white foam/solid) and 1100 mg (white solid) respectively (71% yield).

<u>Diastereomer **6.22a**</u>

mp 68–78 °C

TLC $R_f$ 0.61 (MeOH/EtOAc, 2.5%)

$[\alpha]_D$ +145.1 (*c* 1.11, $CHCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 7.47 (br.s, 1H, N$H_2$), 5.16 (dd, 1H, $J_1$ = 10.8 Hz, $J_2$ = 3.6 Hz, 8a'-C$H$), 5.07 (br. s, 1H, N$H_2$), 4.44 (s, 1H, 3'-C$H$), 3.55–3.44 (m, 2H, 5-C$H_2$), 2.45 (app. t, 1H, 7'-C$H_2$), 2.38–2.24 (m, 2H, 3-C$H_2$, 8'-C$H_2$), 2.02–1.74 (m, 5H, 4-C$H_2$, 3-C$H_2$, 7'-C$H_2$, 8'-C$H_2$), 1.63 (2'-C(C$H_3$)$_2$), 1.52 (2'-C(C$H_3$)$_2$), 1.33 (C(C$H_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 169.9, 169.8 (lactam and carboxamide *C*=O), 153.4 (carbamate *C*=O), 80.4 (*C*($CH_3$)$_3$), 73.4 (3'-*C*), 65.2 (6'-*C*), 62.6 (8'a-*C*), 52.6 (2'-*C*($CH_3$)$_2$), 48.9 (5-*C*), 38.4 (3-*C*), 32.2 (7'-*C*), 29.6 (2'-C(*C*$H_3$)$_2$), 29.4 (C(*C*$H_3$)$_3$), 27.8 (8'-*C*), 27.6 (2'-C(*C*$H_3$)$_2$), 24.4 (4-*C*)

ESI HRMS *m/z* 406.1789 $[M+Na]^+$ $C_{18}H_{29}N_3O_4S$+Na requires 406.1771

Diastereomer **6.22b**

mp 238–241 °C

TLC $R_f$ 0.38 (MeOH/EtOAc, 2.5%)

$[\alpha]_D$ – 43.9 (*c* 1.09, $CDCl_3$)

$^1$H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 7.18 (br. s, 1H, N*H*$_2$), 5.09 (br. s, 1H, N*H*$_2$), 4.92 (dd, 1H, $J_1$ = 11.2 Hz, $J_2$ = 2.4 Hz, 8a'-C*H*), 4.15 (s, 1H, 3'-C*H*), 3.57–3.51 (m, 1H, 5-C*H*$_2$), 3.45–3.39 (m, 1H, 5-C*H*$_2$), 2.77 (dddd or dq, 1H, $J_1$ = $J_2$ = $J_3$ = 15.6 Hz, $J_4$ = 4 Hz, 8'-C*H*$_2$), 2.45–2.33 (m, 2H, 7'-C*H*$_2$, 3-C*H*$_2$), 2.11–1.94 (m, 3H, 8'-C*H*$_2$, 7'-C*H*$_2$, 4-C*H*$_2$), 1.86–1.76 (m, 2H, 4-C*H*$_2$, 3-C*H*$_2$), 1.60 (s, 3H, 2'-C(C*H*$_3$)$_2$), 1.48 (s, 3H, 2'-C(C*H*$_3$)$_2$), 1.37 (s, 9H, C(C*H*$_3$)$_3$)

$^{13}$C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) δ 170.0, 169.2 (lactam and carboxamide *C*=O), 153.9 (carbamate *C*=O), 80.1 (*C*($CH_3$)$_3$), 74.3 (3'-*C*), 62.9 (6'-*C*), 62.7 (8a'-*C*), 51.4 (2'-*C*($CH_3$)$_2$), 48.9 (5-*C*), 42.9 (3-*C*), 38.9 (7'-*C*), 32.7 (2'-C(*C*$H_3$)$_2$), 29.3 (C(*C*$H_3$)$_3$), 27.8 (8'-*C*), 25.1 (2'-C(*C*$H_3$)$_2$), 24.7 (4-*C*)

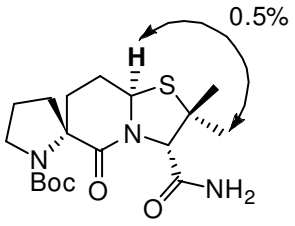
ESI HRMS *m/z* 406.1781 $[M+Na]^+$ $C_{18}H_{29}N_3O_4S$+Na requires 406.1771

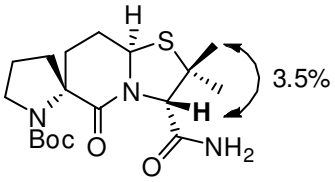
Comments:

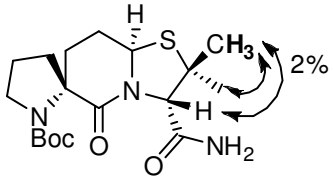
Relative stereochemistry at C-8a' assigned on the basis of 1-D NOE experiments. Compounds will be crystallized and X-ray structure determined for absolute configurations.

1-D NOE NMR analysis results:

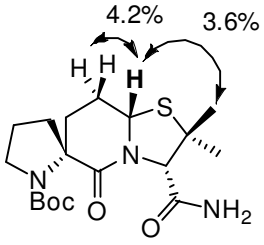
1. Irradiation of protons in **6.22a** (**bolded** in each structure)

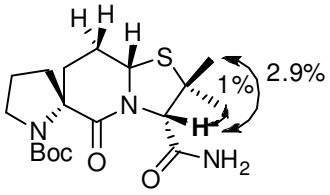


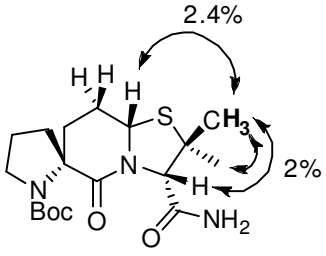




2. Irradiation of protons in **6.22b** (**bolded** in each structure)



## Bibliography


1. Stewart, D.E., Sarkar, A.; Wampler, J.E. Occurrence and Role of *cis*-Peptide Bonds in Protein Structures. *J. Mol. Biol.* **1990**, *214*, 253–260.
2. Kofron, J.L.; Kuzmic, P.; Kishore, V.; Colon-Bonilla, E.; Rich, D.H. Determination of Kinetic Constants for Peptidyl Prolyl *Cis*-*Trans* Isomerases by an Improved Spectrophotometric Assay. *Biochemistry* **1991**, *30*, 6127–6134.
3. Sali, A.; Overington, J.P. Derivation of Rules for Comparative Protein Modeling from a Database of Protein Structure Alignments. *Protein Sci.* **1994**, *3*, 1582–1596.
4. L-Pro can be found in the i+2 position of other type *β*-turns, such as the type I, type II' etc.
5. The angles for the type VI turns was originally defined by Richardson more than a decade after the torsion angles of the other *β*-turns were defined. Richardson, J.S. The anatomy and taxonomy of protein structure. *Adv. Protein Chem*. **1981**, *34*, 167-339.
6. Reed, L.R.; Johnson, P.L. Solid State Conformation of the *C*-Terminal Tripeptide of Oxytocin, L-Pro-L-Leu-Gly-$NH_2$. *J. Am. Chem. Soc.* **1973**, *95*, 7523–7524.
7. Higashijima, T.; Tasumi, M.; Miyazawa, T.; Miyoshi, M. Nuclear-Magnetic-Resonance Study of Aggregations and Conformations of Melanostatin and Related Peptides. *Eur. J. Biochem*. **1978**, *89*, 543–556.
8. Hruby V.J. in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins (ed. Weinstein B.) 1974, pp.1–188. Marcel Dekker, New York.
9. Deslaures, R.; Walter, R.; Smith, I.C.P.; Intramolecular Motion in Peptides Deptermined by $^{13}C$ NMR: A Spin-Lattice Relaxation Time-Study on MSH-Release-Inhibiting Factor, **1973**, *37*, 27–32.
10. Schwartz, R.W.; Mattice W.L.; Sprites, M.A. Melanostatin Conformations in Solution. *Biopolymers* **1979***, 18*, 1835–1848.
11. Hutchinson, E.G.; Thornton, J.M. A Revised Set of Potentials for Beta Turn Formation in Proteins. *Protein Sci.* **1994**, *3*, 2207–2216.
12. (a) Gramberg, D.; Weber, C.; Beelis, R.; Inglis, J.; Bruns, C.; Robinson, J.A. Synthesis of a Type VI *β*-Turn Peptide Mimetic and Its Incorporation into a High Affinity Somatostatin Receptor Ligand. *Helv. Chim. Acta.* **1995**, *78*, 1588–1606. (b) Kim. K.; Dumas, J.-P; Germanas, J.P. Novel Bicyclic Lactams as XaaPro

Type VI *β*-Turn Mimics: Design, Synthesis and Evaluation. *J. Org. Chem.* **1996**, *61*, 3138–3144.

13. Rogiers, J.; Borggraeve, W.M.; Toppet, S.M.; Compernolle, F.; Hoornaert, G. Stereoselective Transformation of Pyrazinones into Substituted Analogues of *cis*-5-Amino-6-oxo-2-piperidinemethanol and *cis*-5-Amino-2-piperidinemethanol. *Tetrahedron* **2003**, *59*, 5047–5054.
14. Belec, L.; Slaninova, J.; Lubell, W.D. *J. Med. Chem.* **2000**, *43*, 1448–1455.
15. Vartak, A.P. Design and Synthesis of Type VI *β*-Turn Mimics of L-Prolyl-L-Xaa-L-Prolinamide. Ph.D. Dissertation, University of Minnesota. **2006**, p.28.
16. Vartak, A.P.; Johnson, R.L. Concerted Synthesis of a Type VI *β*-Turn Mimic of Pro-Pro-Pro-$NH_2$. *Org. Lett.* **2006**, *8*, 983–986.
17. Hoffman, T.; Lanig, H.; Waibel, R.; Gmeiner, P. Rational Molecular Design and EPC Synthesis of a Type VI *β*-Turn Inducing Peptide Mimic. *Angew. Chem. Int. Ed.* **2001**, *40*, 3361–3364.
18. Vartak, A.P. Design and Synthesis of Type VI *β*-Turn Mimics of L-Prolyl-L-Xaa-L-Prolinamide. Ph.D. Dissertation, University of Minnesota. **2006**, p.21–22.
19. Derrer. S.; Davies, J.E.; Holmes, A.B. Synthesis and Conformational Analysis of a Type VIb *β*-Turn Mimetic Based on an Eight-Membered Lactam. *J. Chem. Soc. Perkin Trans.1* **2000**, *30*, 2957–2967.
20. Afzali-Ardakani, A.; Rappoport, H. L-Vinylglycine. *J.Org. Chem.* **1980,** *45*, 4817–4820.
21. Baldwin, J. E.; Haber, S. B.; Hoskins, C.; Kruse, L. I. Synthesis of *β*, *γ*-Unsaturated Amino Acids. *J.Org. Chem.* **1977,** *42*, 1239–1241.
22. Hanessian, S.; Sahoo, S. P. A Novel and Efficient Synthesis of L-Vinylglycine. *Tet. Lett.* **1984,** *25*, 1425–1428.
23. Beaulieu, P. L.; Duceppe, J.-S.; Johnson, C. Synthesis of Chiral Vinylglycines. *J. Org. Chem.* **1991,** *56*, 4196–4204.
24. Crisp, G. T.; Glink, P. T. Palladium-Catalyzed Heck Couplings of L-Vinylglycine Derivatives with Vinyl and Aryl Halides and Triflates. *Tetrahedron* **1992,** 48, 3541–3556.
25. Myers, A. G. Oxidation. In *Chem 215*, pp 18–19.
26. Tojo, G.; Fernandez, M. *Oxidation of Primary Alcohols to Carboxylic Acids-A Guide to Current Common Practice*. Springer: New York, 2007; p 13–31.

27. (a) Garner, P.; Park, J.-M. *Org. Synth.* Coll. Vol. 9, **1998**, 300. (b) Garner, P.; Park, J.-M. *J. Org. Chem.* **1987**, *52*, 2361 (c) McKillop, A.; Taylor, R. J. K.; Watson, R. J.; Lewis, N. An Improved Procedure for the Preparation of the Garner Aldehyde and Its Use for the Synthesis of *N*-Protected 1-Halo-2-(*R*)-Amino-3-Butenes. *Synlett* **1994,** *1*, 31–33.
28. Dondoni, A.; Massi, A.; Minghini, E.; Sabbatini, S.; Bertolasi, V. Model Studies Toward the Synthesis of Dihydropyrimidinyl and Pyridyl alpha amino acids via Three Component Biginelli and Hantzsch Cyclocondensations. *J. Org. Chem.* **2003,** *68*, 6172–6183.
29. Trost, B. M.; Bunt, R. C.; Lemoine, R. C.; Calkins, T. L. Dynamic Kinetic Asymmetric Transformation of Diene Monoepoxides: A Practical Asymmetric Synthesis of Vinylglycinol, Vigabatrin, and Ethambutol. *J. Am. Chem. Soc.* **2000,** *122*, 5968–5976.
30. Butts, C. P.; Fiali, E.; Llyod-Jones, G. C.; Norrby, P.-O.; Sale, D. A.; Schramm, Y. Structure-Based Rationale for Selectivity in the Asymmetric Allylic Alkylation of Cycloalkenyl Esters Employing the Trost 'Standard Ligand' (TSL): Isolation, Analysis and Alkylation of the Monomeric form of the Cationic $\eta^3$-Cyclohexenyl Complex [($\eta^3$-c-$C_6H_9$)Pd(TSL)]. *J. Am. Chem.Soc.* **2009,** *131*, 9945–9957.
31. Trost, B. M.; Fandrick, D. R.; Brodmann, T.; Stiles, D. T. Dynamic Kinetic Asymmetric Allylic Amination and Acyl Migration of Vinyl Aziridines with Imido Carboxylates. *Angew.Chem. Int. Ed.* **2007,** *46*, 6123–6125.
32. Bittermann, H.; Gmeiner, P. Chirospecific Synthesis of Spirocyclic *β*-Lactams and Their Characterization as Potent Type II *β*-Turn Inducing Peptide Mimetics. *J. Org.Chem.* **2006,** *71*, 97-102.
33. (a) Wang, H.; Germanas, J. P. 4-Alkyl-2-trichloromethyloxazolidin-5-ones: Valuable Precursors to Enantiomerically Pure C- and N-Protected α-Alkyl Prolines. *Synlett* **1999,** *1*, 33–36. (b) Poloński, T. "Optical Activity of Lactones and Lactams-III: Circular Dichroism Spectra of 5-Oxazolidinones" *Tetrahedron* **1985**, *41*, 603–609.
34. Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. Alkylation of Amino Acids without Loss of the Optical Activity: Preparation of a-Substituted Proline Derivatives. A Case of Self-Reproduction of Chirality. *J. Am. Chem. Soc.* **1983,** *105*, 5390–5398.

35. Lu, X.; Arthur, G.; Bittman, R. Synthesis of a Novel Ceramide Analogue via Tebbe Methylenation and Evaluation of Its Antiproliferative Activity. *Org. Lett.* **2005,** *7*, 1645–1648.
36. Hartley, R. C.; McKiernan, G. J. Titanium reagents for the alkylidenation of carboxylic acid and carbonic acid derivatives. *J. Chem. Soc. Perkin Trans. 1* **2002**, 2763–2793.
37. Khalil, E. M.; Subasinghe, N. L.; Johnson, R. L. An Efficient and High Yield Method for the N-*tert*-Butoxycarbonyl Protection of Sterically Hindered Amino Acids. *Tetrahedron Lett.* **1996,** *37*, 3441–3444.
38. Vartak, A. P.; Skoblenick, K.; Thomas, N.; Mishra, R. K.; Johnson, R. L. Allosteric Modulation of the Dopamine Receptor by Conformationally Constrained Type VI *β*-Turn Peptidomimetics of Pro-Leu-Gly-$NH_2$. *J. Med. Chem.* **2007,** *50*, 6725–6729.
39. Artman, G. D. I.; Grubbs, A. W.; Williams, R.M. Concise, Asymmetric, Stereocontrolled Total Synthesis of Stephacidins A, B and Notoamide B. *J. Am. Chem. Soc.* **2007,** *129*, 6336–6342.
40. Katritzky, A. R.; Todadze, E.; Angrish, P.; Draghizi, B. Efficient Peptide Coupling Involving Sterically Hindered Amino Acids. *J. Org. Chem.* **2007,** *72*, 5794–5801.
41. Beal, L. M.; Liu, B.; Chu, W.; Moeller, K. D. Anodic Amide Oxidation/Olefin Metathesis Strategies: Developing a Unified Approach to the Synthesis of Bicyclic Lactam Peptidomimetics. *Tetrahedron* **2000,** *56*, 10113–10125.
42. Hoffmann, T.; Waibel, R.; Gmeiner, P. A General Approach to Dehydro-Freidinger Lactams: Ex-Chiral Pool Synthesis and Spectroscopic Evaluation as Potential Reverse Turn Inducers. *J. Org. Chem.* **2003,** *68*, 62–69.
43. Harris, P. W. R.; Brimble, M. A.; Muir, V. J.; Lai, M. Y. H.; Trotter, N. S.; Callis, D. J. Synthesis of Proline-Modified Analogues of the Neuroprotective Agent Glycyl-L-Prolyl-Glutamic acid (GPE). *Tetrahedron* **2005,** *61*, 10018–10035.
44. Baran, P. S.; Guerrero, C. A.; Ambhaikar, N. B.; Hafensteiner, B. D. Short, Enantioselective Total Synthesis of Stephacidin A. *Angew.Chem. Int. Ed.* **2005,** *44*, 606–609.
45. White, J. M.; Tunoori, A. R.; Turunen, B. J.; Georg, G. I. [Bis(2-methoxyethyl)amino]sulfur Trifluoride, the Deoxo-Fluor Reagent: Application

toward One-Flask Transformations of Carboxylic Acids to Amides. *J.Org. Chem.* **2004,** *69*, 2573–2576.

46. Sole, N.; Torres, J. L.; Garcia Anton, J. M.; Valencia, G.; Reig, F. Mixed Anhydrides in Peptide Synthesis. Factors Affecting Urethane Formation and Racemization. *Tetrahedron* **1986,** *42*, 193–198.
47. Valeur, E.; Bradley, M. PS-IIDQ: A Supported Coupling Reagent for Efficient and General Amide Bond Formation. *Tetrahedron* **2007,** *63*, 8855–8871.
48. Tung, R. D.; Rich, D. H. Bis(2-oxo-3-oxazolidinyl)phosphinic Chloride as a Coupling Reagent for N-Alkyl Amino Acids. *J. Am. Chem. Soc.* **1985,** *107*, 4342–4343.
49. Carpino, L. A.; Xia, J.; El-Faham, A. 3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene. *J. Org. Chem.* **2004,** *69*, 54–61.
50. Carpino, L. A. 1-Hydroxy-7-Azabenzotriazole. An Efficient Peptide Coupling Additive. *J. Am. Chem. Soc.* **1993,** *115*, 4397–4398.
51. Kappe, C. O. Controlled Microwave Heating in Modern Organic Synthesis. *Angew.Chem. Intl. Ed.* **2004,** *43*, 6250–6284.
52. Nosse, B.; Schall, A.; Jeong, W. B.; Reiser, O. Optimization of Ring-Closing Metathesis: Inert Gas Sparging and Microwave Irradiation. *Adv. Synth. Catal.* **2005,** *347*, 1869–1874.
53. Chapman, R.; Arora, P. S. Optimized Synthesis of Hydrogen-Bond Surrogate Helices: Surprising Effects of Microwave Heating on the Activity of Grubbs Catalysts. *Org. Lett.* **2006,** *8*, 5825–5828.
54. Hong, S. H.; Wenzel, A. G.; Salguero, T. T.; Day, M. W.; Grubbs, R. H. Decomposition of Ruthenium Olefin Metathesis Catalysts. *J. Am. Chem. Soc.* **2007,** *129*, 7961–7968.
55. McMurry, J. E.; Fleming, M. P.; Kees, K. L.; Krepski, L. R. Titanium-Induced Reductive Coupling of Carbonyls to Olefins. *J. Org. Chem.* **1978,** *43*, 3255–3266.
56. Hoye, T. R.; Jeffrey, C. S.; Tenakoon, M. A.; Wang, J.; Zhao, H. Relay Ring-Closing Metathesis (RRCM): A Strategy for Directing Metal Movement Throughout Olefin Metathesis Sequences. *J. Am. Chem. Soc.* **2004,** *126*, 10210–10211.
57. Wang, X.; Bowman, E. J.; Bowman, B. J.; Porco Jr., J. A. Total Synthesis of the Salicylate Enamide Macrolide Oximidine III: Application of Relay Ring-Closing

Metathesis. *Angew.Chem. Int. Ed.* **2004,** *43*, 3601–3605.

58. Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. Rational Molecular Design and EPC Synthesis of a Type VI *β*-Turn Inducing Peptide Mimetic. *Angew.Chem. Int. Ed.* **2001,** *40*, 3361–3364.
59. Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. A General Model for Selectivity in Olefin Cross Metathesis. *J. Am. Chem. Soc.* **2003,** *125*, 11360–11370.
60. Creighton, C. J.; Reitz, A. B. Synthesis of an Eight-Membered Cyclic Pseudo-Dipeptide Using Ring Closing Metathesis. *Org. Lett.* **2001,** *3*, 893–895.
61. Furstner, A.; Langemann, K. Total Syntheses of (+)-Ricinelaidic Acid Lactone and of (-)-Gloeosporone Based on Transition-Metal-Catalyzed C-C Bond Formations. *J. Am. Chem. Soc.* **1997,** *119*, 9130–9136.
62. Grubbs, R. H.; Miller, S. J.; Fu, G. C. Ring-Closing Metathesis and Related Processes in Organic Synthesis. *Acc. Chem. Res.* **1995,** *28*, 446–452.
63. Vartak, A.P. Ph.D. dissertation. University of Minnesota, **2006**.
64. Genin, M.J.; Johnson, R.L. Design, Synthesis, and Conformational Analysis of a Novel Spiro-Bicyclic System as a Type II *β*-Turn Peptidomimetic. *J. Am. Chem. Soc.* **1992**, *112*, 8778–8783.
65. Kerr, J. F.; Wyllie, A. H.; Currie, A. R. Apoptosis: A Basic Biological Phenomenon with Wide-Ranging Implications in Tissue Kinetics. *Br. J. Cancer* **1972,** *26*, 239–257.
66. Shi, Y. A Structural View of Mitochondria-Mediated Apoptosis. *Nat. Struc. Biol.* **2001,** *8*, 394–401.
67. Stellar, H. Mechanisms and Genes of Cellular Suicide. *Science* **1995,** *267*, 1445–1449.
68. Jacobson, M. D.; Weil, M.; Raff, M. C. Programmed Cell Death in Animal Development. *Cell* **1997,** *88*, 347–354.
69. Horvitz, H. R. Genetic Control of Programmed Cell Death in the Nematode, *Caenorhabditis Elegans*. *Cancer Res.* **1999,** *59*, 1701–1706.
70. Fesik, S. W. Insights into Programmed Cell Death through Structural Biology. *Cell* **2000,** *103*, 272–282.
71. Green, D. R.; Martin, S. J. The Killer and the Executioner: How Apoptosis Controls Malignancy. *Curr. Opin. Immunol.* **1995,** *7*, 694–703.

72. Lowe, S. W.; Lin, A. W. Apoptosis in Cancer. *Carcinogenesis* **2000,** *20*, 485–495.
73. Nicholson, D. W. From Bench to Clinic with Apoptosis-Based Therapeutic Agents. *Nature* **2000,** *407*, 810–816.
74. Ponder, B. A. Cancer Genetics. *Nature* **2001,** *411*, 336–341.
75. Riedl, S. J.; Shi, Y. Molecular Mechanisms of Caspase Regulation During Apoptosis. *Nat. Rev. Mol. Cell Bio.* **2004,** *5*, 897–907.
76. Hanahan, D.; Weinberg, R. A. The Hallmarks of Cancer. *Cell* **2000,** *100*, 57–70.
77. Earnshaw, W. C.; Martins, L. M.; Kaufmann, S. H. Mammalian Caspases: Structure, Activation, Substrate, and Function During Apoptosis. *Annu. Rev. Biochem.* **1999,** *68*, 383–424.
78. Deveraux, Q. L.; Reed, J. C. IAP Family Proteins–Suppressors of Apoptosis. *Gene. Dev.* **1999,** *13*, 239–252.
79. Salvesen, G. S.; Duckett, C. S. IAP Proteins: Blocking the Road to Death's Door. *Nat. Rev. Mol. Cell Bio.* **2002,** *3*, 401–410.
80. Shi, Y. Mechanisms of Caspase Inhibition and Activation During Apoptosis. *Mol. Cell* **2002,** *9*, 459–470.
81. Holcik, M.; Korneluk, R. G. XIAP, The Guardian Angel. *Nature Rev. Mol. Cell. Biol.* **2001,** *2*, 550–556.
82. Shiozaki, E. N.; Chai, J.; Rigotti, D. J.; Riedl, S. J.; Li, P.; Srinivasula, S. M.; Alnemri, E. S.; Fairman, R.; Shi, Y. Mechanism of XIAP-Mediated Inhibition of Caspase-9. *Mol. Cell* **2003,** *11*, 519–527.
83. Sun, C.; Cai, M.; Meadows, R. P.; Xu, N.; Gunasekera, A. H.; Herrmann, J.; Wu, J. C.; Fesik, S. W. NMR Structure and Mutagenesis of the Third BIR Domain of the Inhibitor of Apoptosis Protein XIAP. *J. Biol. Chem.* **2000,** *275*, 33777–33781.
84. Schimmer, A. D.; Dalili, S.; Batey, R. A.; Riedl, S. J. Targeting XIAP for the Treatment of Malignancy. *Cell Death Differ.* **2006,** *13*, 179–188 and references therein.
85. Shi, Y. A Conserved Tetrapeptide Motif: Potentiating Apoptosis through IAP Binding. *Cell Death Differ.* **2002,** *9*, 93–95.
86. Du, C.; Fang, M.; Li, Y.; Wang, X. Smac, A Mitochondrial Protein That

Promotes Cytochrome *c*-Dependent Caspase Activation During Apoptosis. *Cell* **2000,** *102*, 33–42.

87. Verhagen, A. M. Identification of DIABLO, a Mammalian Protein That Promotes Apoptosis by Binding to and Antagonizing IAP Proteins. *Cell* **2000,** *102*, 43–53.
88. Liu, Z.; Sun, C.; Olejniczak, E. T.; Meadows, R. P.; Betz, S. F.; Oost, T.; Hermann, J.; Wu, J. C.; Fesik, S. W. Structural Basis for Binding of Smac/Diablo to the XIAP BIR3 Domain. *Nature Rev. Mol. Cell. Biol.* **2000,** *408*, 1004–1008.
89. Chai, J.; Du, C.; Wu, J.-W.; Kyin, S.; Wang, X.; Shi, Y. Structural and Biochemical Basis of Apoptotic Activation by Smac/Diablo. *Nature* **2000,** *406*, 855–862.
90. Wu, G.; Chai, J.; Suber, T. L.; Wu, J.-W.; Du, C.; Wang, X.; Shi, Y. Structural Basis of IAP Recognition by Smac/Diablo. *Nature Rev. Mol. Cell. Biol.* **2000,** *408*, 1008–1012.
91. Srinivasula, S. M.; Hegde, R.; Saleh, A.; Datta, P.; Shiozaki, E.; Chai, J.; Lee, R.-A.; Robbins, P. D.; Fernandes-Alnemri, T.; Shi, Y.; Alnemri, E.S. A Conserved XIAP Interaction Motif in Caspase-9 and Smac/Diablo Regulates Caspase Activity and Apoptosis. *Nature* **2001,** 410, 112–116.
92. Arnt, C. R.; Chiorean, M. V.; Heldebrant, M. P.; Gores, G. J.; Kaufmann, S. H. Synthetic Smac/Diablo Peptides Enhance the Effects of Chemotherapeutic Agents by Binding XIAP and cIAP1 *in Situ*. *J. Biol. Chem.* **2002,** *277*, 44236–44243.
93. Fulda, S.; Wick, W.; Weller, M.; Debatin, K. M. Smac Agonists Sensitize for Apo2L/Trail- or Anticancer Drug-Induced Apoptosis and Induce Regression of Malignant Glioma *in vivo*. *Nature Med.* **2002,** *8*, 808–815.
94. Yang, L.; Mashima, T.; Sato, S.; Mochizuki, M.; Sakamoto, H.; Yamori, T.; Oh-Hara, T.; Tsuruo, T. Predominant Suppression of Apoptosome by Inhibitor of Apoptosis Protein in Non-Small Cell Lung Cancer H460 Cells: Therapeutic Effect of a Novel Polyarginine-Conjugated Smac Peptide. *Cancer Res.* **2003,** *63*, 831–837.
95. Sharma, S. K.; Straub, C.; Zawel, L. Development of Peptidomimetics Targeting IAPs. *Int. J. Pept. Res. Ther.* **2006,** *12*, 21–32.

96. Kipp, R. A.; Case, M. A.; Wist, A. D.; Cresson, C. M.; Carrell, M.; Griner, E.; Wilta, A.; Albiniak, P. A.; Chai, J.; Shi, Y.; Semmelhack, M. F.; McLendon, G. L. Molecular Targeting of Inhibitors of Apoptosis Proteins Based on Small Molecule Mimics of Natural Binding Partners. *Biochemistry* **2002,** *41*, 7344–7349.
97. Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Qian, D.; Lu, J.; Qiu, S.; Bai, L.; Peng, Y.; Cai, Q.; Wang, S. Design of Small-Molecule Peptidic and Nonpeptidic Smac Mimetics. *Acc. Chem. Res.* **2008,** *41*, 1264–1277.
98. Oost, T. K.; Sun, C.; Armstrong, R. C.; Al-Assaad, A. S.; Betz, S. F.; Deckwerth, T. L.; Ding, H.; Elmore, S. W.; Meadows, R. P.; Olejniczak, E. T.; Oleksijew, A.; Oltersdorf, T.; Rosenberg, S. H.; Shoemaker, A. R.; Tomaselli, K. J.; Zou, H.; Fesik, S. W. Discovery of Potent Antagonists of the Antiapoptotic Protein XIAP for the Treatment of Cancer. *J. Med. Chem.* **2004,** *47*, 4417–4426.
99. Gaither, A.; Porter, D.; Yao, Y.; Borawski, J.; Yang, G.; Donovan, J.; Sage, D.; Slisz, J.; Tran, M.; Straub, C.; Ramsey, T.; Iourgenko, V.; Huang, A.; Chen, Y.; Schlegel, R.; Labow, M.; Fawell, S.; Sellers, W. R.; Zawel, L. A Smac Mimetic Rescue Screen Reveals Roles for Inhibitor of Apoptosis Proteins in Tumor Necrosis Factor-Alpha Signaling. *Cancer Res.* **2007,** *67*, 11493–11498.
100. Zobel, K.; Wang, L.; Varfolomeev, E.; Franklin, M. C.; Elliott, L. O.; Wallweber, H. J. A.; Okawa, D. C.; Flygare, J. A.; Vucic, D.; Fairbrother, W. J.; Deshayes, K. Design, Synthesis, and Biological Activity of a Potent Smac Mimetic That Sensitizes Cancer Cells to Apoptosis by Antagonizing IAPS. *ACS Chem. Biol.* **2006,** *1*, 525–533.
101. Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Qiu, S.; Yang, C.-Y.; Gao, W.; Meagher, J.; Stuckey, J.; Wang, S. Design, Synthesis, and Evaluation of A Potent, Cell-Permeable, Conformationally Constrained Second Mitochondria Derived Activator of Caspase (Smac) Mimetic. *J. Med. Chem.* **2006,** *49*, 7916–7920.
102. Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Xu, L.; Liu, M.; Tomita, Y.; Pan, H.; Yoshioka, Y.; Krajewski, K.; Roller, P. P.; Wang, S. Structure-Based Design of Potent, Conformationally Constrained Smac Mimetics. *J. Am .*

*Chem. Soc.* **2004,** *126*, 16686–16687.

103. Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Xu, L.; Tomita, Y.; Krajewski, K.; Roller, P. P.; Wang, S. Structure-Based Design, Synthesis, and Evaluation of Conformationally Constrained Mimetics of the Second Mitochondria-Derived Activator of Caspase That Target the X-Linked Inhibitor of Apoptosis Protein/Caspase-9 Interaction Site. *J. Med. Chem.* **2004,** *47*, 4147–4150.
104. Li, L.; Thomas, R. M.; Suzuki, H.; De Brabander, J. K.; Wang, X.; Harran, P. G. A Small Molecule Smac Mimic Potentiates TRAIL- and TNFα-Mediated Cell Death. *Science* **2004,** *305*, 1471–1474.
105. Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Meagher, J. L.; Yang, C.-Y.; Qiu, S.; Tomita, Y.; Ueda, Y.; Jiang, S.; Krajewski, K.; Roller, P. P.; Stuckey, J. A.; Wang, S. Design, Synthesis, and Characterization of a Potent, Nonpeptide, Cell-Permeable, Bivalent Smac Mimetic That Concurrently Targets Both the BIR2 and BIR3 Domains in XIAP. *J. Am. Chem. Soc.* **2007,** *129*, 15279–15294.
106. Subasinghe, N. L.; Bontems, R. J.; McIntee, E.; Mishra, R. K.; Johnson, R. L. Bicyclic Thiazolidine Lactam Peptidomimetics of the Dopamine Receptor Modulating Peptide Pro-Leu-Gly-$NH_2$. *J. Med. Chem.* **1993,** *36*, 2356–2361.
107. Khalil, E. M.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Synthesis and Dopamine Receptor Modulating Activity of Substituted Bicyclic Thiazolidine Lactam Peptidomimetics of L-Prolyl-L-Leucyl-Glycinamide. *J. Med. Chem.* **1999,** *42*, 2977–2987.
108. Keller, M.; Boissard, C.; Patiny, L.; Chung, N. N.; Lemieux, C.; Mutter, M.; Schiller, P. W. Pseudoproline-Containing Analogues of Morphiceptin and Endomorphin-2: Evidence for a *Cis* Tyr-Pro Amide Bond in the Bioactive Conformation. *J. Med. Chem.* **2001,** *44*, 3896–3903.
109. Baldwin, J. E.; Lee, E. Synthesis of Bicyclic γ-Lactams Via Oxazolidinones. *Tetrahedron Lett.* **1986,** *42*, 6551–6554.
110. Subasinghe, N. L.; Bontems, R. J.; McIntee, E.; Mishra, R. K.; Johnson, R. L. Bicyclic Thiazolidine Lactam Peptidomimetics of the Dopamine Receptor Modulating Peptide Pro-Leu-Gly-$NH_2$. *J. Med. Chem.* **1993,** *36*, 2356–2361.
111. Huang, H.; Iwasawa, N.; Mukaiyama, T. A. A Convenient Method for the

Construction of *β*-Lactam Compounds from *β* Amino Acids Using 2-Chloro-1-Methylpyridinium Iodide as Condensing Agent. *Chem. Lett.* **1984,** *13*, 1465–1466.

112. Khalil, E. M.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Synthesis and Dopamine Receptor Modulating Activity of Substituted Bicyclic Thiazolidine Lactam Peptidomimetics of L-Prolyl-L-Leucyl-Glycinamide. *J. Med. Chem.* **1999,** *42*, 2977–2987.
113. Myers, A. G.; Yoon, T.; Gleason, J. L. A One-Step Synthesis of Pseudoephedrine Glycinamide, a Versatile Precursor for the Synthesis of α-Amino Acids. *Tetrahedron Lett.* **1995,** *36*, 4555–4558.
114. Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. Highly Practical Methodology for the Synthesis of D- and L-α-Amino Acids, N-Protected α-Amino Acids, and N-Methyl- α-Amino Acids. *J. Am. Chem. Soc.* **1997,** *119*, 656–673.
115. Myers, A. G.; Schnider, P.; Kwon, S.; Kung, D. W. Greatly Simplified Procedures for the Synthesis of α-Amino Acids by the Direct Alkylation of Pseudoephedrine Glycinamide Hydrate. *J. Org. Chem.* **1999,** *64*, 3322–3327.
116. Myers, A. G.; Gleason, J. L. Asymmetric Synthesis of α-Amino Acids by the Alkylation of Pseudoephedrine Glycinamide: L-Allylglycine and Boc-L-Allylglycine. *Org. Synth.* **1999,** *76*, 57.
117. Broadrup, R. L.; Wang, B.; Malachowski, W. P. A General Strategy for the Synthesis of Azapeptidomimetic Lactams. *Tetrahedron* **2005,** *61*, 10277–10284.
118. Larcheveque, E.; Ignatova, T.; Cuvigny, T. Asymmetric Synthesis of α-Substituted Ketones and Acids Via Chiral *N,N*-Substituted Amides. *Tetrahedron Lett.* **1978,** *19*, 3961–3964.
119. Brenner, M.; Huber, W. Production of α-Amino Acid Esters by Alcoholysis of the Methyl Ester. *Helv. Chim. Acta.* **1953,** *36*, 1109–1115.
120. Seebach, D.; Thaler, A.; Beck, A. K. Solubilization of Peptides in Non-Polar Organic Solvents by the Addition of Inorganic Salts: Facts and Implications. *Helv. Chim. Acta.* **1989,** *72*, 857–867.
121. Hoffman, R. W. Allylic 1,3-Strain as a Controlling Factor in Stereoselective Transformations. *Chem. Rev.* **1989,** *89*, 1841–1860.

122. Wunsch, T.; Meyers, A. I. Asymmetric Synthesis of 4,4-Disubstituted 1-Naphthalenones. Diastereoselectivity as a Function of Metal Alkoxides. *J. Org. Chem.* **1990,** *55*, 4233–4235.
123. Evans, D. A.; Takacs, J. M. Enantioselective Alkylation of Chiral Enolates. *Tetrahedron Lett.* **1980,** *21*, 4233–4236.
124. Pappo, R.; Allen, D. S.; Lemieux, R. U.; Johnson, W. S. Osmium Tetroxide-Catalyzed Periodate Oxidation of Olefinic Bonds. *J. Org. Chem.* **1956,** *21*, 478–479.
125. Funk, R. L.; Abelman, M. M.; Jellison, K. M. Generation of Ketenes from Carboxylic Acids Using the Mukaiyama Reagent (1-Methyl-2-Chloropyridinium Iodide). *Syn. Lett.* **1989,** *1*, 36–37.
126. Han, C.; Lee, J. P.; Lobkovsky, E.; Porco, J. A., Jr. Catalytic Ester-Amide Exchange Using Group (IV) Metal Alkoxide-Activator Complexes. *J. Am. Chem. Soc.* **2005,** *126*, 10039–10044.
127. Basha, A.; Lipton, M.; Weinreb, S. M. A Mild, General Method for Conversion of Esters to Amides. *Tetrahedron Lett.* **1977,** *18*, 4171–4174.
128. Mishra, R. K.; Chiu, S.; Chiu, P.; Mishra, C. P. Pharmacology of L-Prolyl-L-Leucyl-Glycinamide (PLG): A Review. *Meth. Find. Expt. Clin. Pharmacol.* **1983,** 5, 203–233.
129. Srivastava, L. K.; Bajwa, S. B.; Johnson, R. L.; Mishra, R. K. Interaction of L-Prolyl-L-Leucyl-Glycinamide (PLG) with Dopamine $D_2$ Receptor: Evidence for Modulation of Agonist Affinity States in Bovine Striatal Membranes. *J. Neurochem.* **1988,** *50*, 960–968.
130. Genin, M. J.; Mishra, R. K.; Johnson, R. L., Dopamine Receptor Modulation by a Highly Rigid Spiro Bicyclic Peptidomimetic of Pro-Leu-Gly-$NH_2$. *J. Med. Chem.* **1993,** *36*, 3481–3483.
131. Genin, M. J.; Ojala, W. H.; Gleason, W. B.; Johnson, R. L. Synthesis and Crystal Structure of a Peptidomimetic Containing the (*R*)-4.4-Spirolactam Type II *β* Turn Mimic. *J.Org. Chem.* **1993,** *58*, 2334–2337.
132. Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Nair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, Synthesis and Dopamine Receptor Modulating Activity of Spiro Bicyclic Peptidomimetics of L-Prolyl-L-Leucyl-Glycinamide. *J. Med. Chem.* **1999,** *42*, 628–637.

133. Raghavan, B.; Skoblenick, K.; Bhagwanth, S.; Argintaru, N.; Mishra, R. K.; Johnson, R. L. Allosteric Modulation of the Dopamine $D_2$ Receptor by Pro-Leu-Gly-$NH_2$ Peptidomimetics Constrained in Either a Polyproline II Helix or a Type II *β*-Turn Conformation. *J. Med. Chem.* **2009,** *52*, 2043–2051.
134. George, S. R.; O'Dowd, B. F.; Lee, S. P. G-Protein-Coupled Receptor Oligomerization and Its Potential for Drug Discovery. *Nature Rev. Drug Discov.* **2002,** *1*, 808–820.
135. Crocker, A. D. Dopamine-Mechanisms of Action. *Aust. Prescr.* **1994,** *17*, 17-21.
136. Creese, I.; Fraser, C. M. *Dopamine Receptors*. A.R. Liss: New York, 1987; Vol. 8, p 261.
137. Civelli, O.; Bunzow, J. R.; Grandy, D. K. Molecular Diversity of the Dopamine Receptors. *Annu. Rev. Pharmacol. Toxicol.* **1993,** *32*, 281–307.
138. Wichmann, T.; DeLong, M., R. Models of Basal Ganglia Function and Pathophysiology of Movement Disorders. *Neurosurg. Clin. N. Am.* **1998,** *9*, 223–236.
139. Wichmann, T.; DeLong, M. R. Functional and Pathophysiological Models of the Basal Ganglia. *Curr. Opin. Neurobiol.* **1996,** *6*, 751–758.
140. Poewe, W. H.; Lees, A. J.; Stern, G. M. Low-Dose L-DOPA Therapy in Parkinson's Disease: A 6-Year Follow-up Study. *Neurology* **1986,** *36*, 1528–1530.
141. Jenner, P. Molecular Mechanisms of L-DOPA-Induced Dyskinesia. *Nature Rev. Neurosci.* **2008,** *9*, 665–677.
142. Conn, P. J.; Christopoulos, A.; Lindsley, C. W. Allosteric Modulators of GPCRs: A Novel Approach for the Treatment of CNS Disorders. *Nature Rev. Drug Discov.* **2009,** *8*, 41–54.
143. Langmead, C. J.; Christopoulos, A. Allosteric Agonists of 7-TM Receptors:Expanding the Pharmacological Toolbox. *Trends Pharmacol. Sci.* **2006,** *27*, 475–481.
144. May, L. T.; Leach, K.; Sexton, P. M.; Christopoulos, A. Allosteric Modulation of G-Protein-Coupled Receptors. *Annu. Rev. Pharmacol. Toxicol.* **2007,** *47*, 1–51.
145. Christopoulos, A. Allosteric Binding Sites on Cell-Surface Receptors: Novel

Targets for Drug Discovery. *Nature Rev. Drug Discov.* **2002,** *1*, 198–210.

146. Lazareno, S.; Dolezal, V.; Popham, A.; Birdsall, N. J. Thiochrome Enhances Acetylcholine Affinity at Muscarinic $M_4$ Receptors: Receptor Sub–Type Selectivity Via Cooperativity Rather Than Affinity. *Mol. Pharmacol.* **2004,** *65*, 257–266.S
147. Nair, R. M. G.; Kastin, A. J.; Schally, A. V. Isolation and Structure of Hypothalamic MSH Release-Inhibiting Hormone. *Biochem. Biophy. Res. Commun.* **1971,** *43*, 1376–1381.
148. Miller, L.; Kastin, A. J. MIF-1 and Tyr-MIF-1 Do Not Alter GABA Binding on the $GABA_A$ Receptor. *Brain Res. Bull.* **1990,** *25*, 917–919.
149. Gulati, A.; Bhargava, H. N. Effect of Melatropin Release Inhibiting Factor on Changes by Haloperidol and Centbutindole in Cerebral Cortical 5-Hydroxytryptamine Receptors. *Pharmacology* **1990,** *41*, 98–106.
150. Verma, V.; Mann, A.; Costain, W.; Pontoriero, G.; Castellano, J. M.; Skoblenick, K.; Gupta, S. K.; Pristupa, Z.; Niznik, H. B.; Johnson; L.R.; Nair, V. D.; Mishra, R. K. Modulation of Agonist Binding to Human Dopamine Receptor Subtypes by L-Prolyl-L-Leucyl-Glycinamide and a Peptidomimetic Analogue. *J. Pharmacol. Exp. Ther.* **2005,** *315*, 1228–1236.
151. Kostrezewa, R. M.; Kastin, A. J.; Spirtes, M. A. α-MSH and MIF-1 Effects on Catecholamine Levels and Synthesis in Various Rat Brain Areas. *Pharmacol. Biochem. Behav.* **1975,** *3*, 1017–1023.
152. Reed, L. R.; Johnson, P.L. Solid State Conformation of the C-Terminal Tripeptide of Oxytocin, L-Pro-L-Leu-Gly-$NH_2$. *J. Am. Chem. Soc.* **1973,** *95*, 7523–7524.
153. Schwartz, R. W.; Mattice, W. L.; Sprites, M. A. Melanostatin Conformations in Solution. *Biopolymers* **1979,** *18*, 1835–1848.
154. Johnson, R. L.; Rajakumar, G.; Mishra, R. K., Dopamine Receptor Modulation by Pro-Leu-Gly-$NH_2$ Analogues Possessing Cyclic Amino Acid Residues at the C-Terminal Position. *J. Med. Chem.* **1986,** *29*, 2100–2104.
155. Johnson, R. L.; Rajakumar, G.; Yu, K. L.; Mishra, R. K. Synthesis of Pro-Leu-Gly-$NH_2$ Analogues Modified at the Prolyl Residue and Evaluation of Their Effects on the Receptor Binding Activity of the Central Dopamine Receptor Agonist, ADTN. *J. Med. Chem.* **1986,** *29*, 2104–2107.

156. Chiu, S.; Paulose, C. S.; Mishra, R. K. Effect of L-Prolyl-L-Leucyl-Glycinamide (PLG) on Neuroleptic-Induced Catalepsy and Dopamine/Neuroleptic Receptor Binding. *Peptides* **1981,** *2*, 105–111.
157. Mycroft, F. J.; Bhargava, H. N.; Wei, E. T. Pharmacological Activities of the MIF-1 Analogues Pro-Leu-Gly, Tyr-Pro-Leu-Gly and Pareptide. *Peptides* **1987,** *8*, 1051–1055.
158. Bhargava, H. N. The Effect of Melanotropin Release-Inhibiting Factor, Its Metabolites and Analogues on [$^3$H]-Spiroperidol and [$^3$H]-Apomorphine Binding Sites. *Gen. Pharmacol.* **1983,** *14*, 609–614.
159. Das, S.; Bhargava, H. N. Effect of Chronic Treatment with Morphine on the Binding of $^3$H-*N*-n-Propylnorapomorphine to Striatal Membranes of Rats: Influence of Prolyl-Leucyl-Glycinamide. *Pharmacology* **1985,** *31*, 241-247.
160. Mishra, R. K.; Makman, M. H.; Costain, W. J.; Nair, V. D.; Johnson, R. L. Modulation of Agonist Stimulated Adenylyl Cyclase and GTPase Activity by L-Pro-L-Leu-Glycinamide and Its Peptidomimetic Analogue in Rat Striatal Membranes. *Neurosci. Lett.* **1999,** *269*, 21–24.
161. Plotnikoff, N. P.; Kastin, A. J.; Anderson, M. J.; Schally, A. V. DOPA Potentiation by a Hypothalamic Factor, MSH-Release-Inhibiting Hormone (MIF). *Life Sci.* **1971,** *10*, 1279-1283.
162. Plotnikoff, N. P.; Kastin, A. J. Pharmacological Studies with a Tripeptide, Prolyl-Leucyl-Glycine Amide. *Arch. Int. Pharmacodyn.* **1974,** *211*, 211–224.
163. Kostrzewa, R. M.; Kastin, A. J.; Spirtes, M. A. α-MSH and MIF-1 Effects on Catecholamine Levels and Synthesis in Various Rat Brain Areas. *Pharmacol. Biochem. Behav.* **1975,** *3*, 1017–1023.
164. Smith, J. R.; Morgan, M. The Effects of Prolyl-Leucyl-Glycine Amide on Drug-Induced Rotation in Lesioned Rats. *Gen. Pharmacol.* **1982,** *13*, 203–207.
165. Ott, M.; Mishra, R. K.; Johnson, R. L. Modulation of Dopaminergic Neurotransmission in the 6-Hydroxydopamine Lesioned Rotational Model by Peptidomimetic Analogues of L-Prolyl-L-Leucyl-Glycine Amide. *Brain Res.* **1996,** *737*, 287–291.
166. Sheng, J. G.; Xu, D. L.; Yu, H. Z.; Xu, X. R.; Tang, Q. M. Partial Protection from the Dopaminergic Neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-

Tetrahydropyridine (MPTP) by Pro-Leu-Gly-$NH_2$ (PLG, MIF-1). *Life Sci.* **1987,** *40*, 2007–2010.

167. Marcotte, E. R.; Chugh, A.; Mishra, R. K.; Johnson, R. L. Protection against MPTP Treatment by an Analogue of Pro-Leu-Gly-$NH_2$ (PLG, MIF-1). *Peptides* **1998,** *19*, 403–406.
168. Baures, P. W.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Synthesis and Dopamine Receptor Modulating Activity of Unsubstituted and Substituted Triproline Analogues of L-Prolyl-L-Leucyl-Glycinamide (PLG). *Bioorg. Med. Chem. Lett.* **1999,** *9*, 2349–2352.
169. Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. Alkylation of Amino Acids without Loss of the Optical Activity: Preparation of α-Substituted Proline Derivatives. A Case of Self-Reproduction of Chirality. *J. Am. Chem. Soc.* **1983,** *105*, 5390–5398.
170. Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Nair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, Synthesis and Dopamine Receptor Modulating Activity of Spiro Bicyclic Peptidomimetics of L-Prolyl-L-Leucyl-Glycinamide. *J. Med. Chem.* **1999,** *42*, 628–637.
171. Raghavan, B.; Skoblenick, K.; Bhagwanth, S.; Argintaru, N.; Mishra, R. K.; Johnson, R. L. Allosteric Modulation of the Dopamine $D_2$ Receptor by Pro-Leu-Gly-$NH_2$ Peptidomimetics Constrained in Either a Polyproline II Helix or a Type II *β*-Turn Conformation. *J. Med. Chem.* **2009,** *52*, 2043–2051.
172. Seebach, D.; Mukhopadhyay, T. Substitution of HMPA by the Cyclic Urea DMPU as a Cosolvent for Highly Reactive Nucleophiles and Bases. *Helv. Chim. Acta.* **1982,** *65*, 385–391.
173. Khalil, E. M.; Subasinghe, N. L.; Johnson, R. L. An Efficient and High Yield Method for the *N-tert*-Butoxycarbonyl Protection of Sterically Hindered Amino Acids. *Tetrahedron Lett.* **1996,** *37*, 3441–3444.
174. Zhong, Y.-L.; Shing, T. K. M. Efficient and Facile Glycol Cleavage Oxidation Using Improved Silica Gel-Supported Sodium Metaperiodate. *J. Org. Chem.* **1997,** *62*, 2622–2624.
175. Raghavan, B. Ph.D. Dissertation, University of Minnesota
176. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*. Third ed.; John Wiley & Sons Inc.: New York, 1998; p 474.

177. (a). Magnin, D. R.; Gordon, E. M. US Patent 4970221,1990. (b) Bekhit, A. A.; Fahmy, H. T. Y. Design and Synthesis of Some Substituted 1*H*-Pyrazolyl-Oxazolidines or 1*H*-Pryazolyl-Thiazolidines as Anti-Inflammatory- Antimicrobial Agents. *Arch. Pharm. Pharm. Med. Chem.* **2003,** *2*, 111–118. (c) Fujita, M.; Ota, A.; Ito, S.; Yamamoto, K.; Kawashima, Y.; Iso, T.; Iwao, J.-I., Synthesis and Calcium Antagonistic Activity of (+)-(*R*)- and (*S*)-3-Acetyl-2-[5-Methoxy-2-[4-[*N*-Methyl-*N*-(3,4,5-Trimethoxyphenethyl)Amino]Butoxy]Phenyl]Benzothiazoline Hydrochloride. *Chem. Pharm. Bull.* **1990,** *38*, 936–941. (d) Samanen, J.; Narindray, D.; Cash, T.; Brandeis, E.; Adams, W. J.; Yellin, T.; Eggleston, D.; DeBrosse, C.; Regoli, D. Potent Angiotensin II Antagonists with Non-Beta Branched Amino Acids in Position 5. *J. Med. Chem.* **1989,** *32*, 466–472.

178. Deroose, F. C.; De Clercq, P. J. Novel Enantioselective Syntheses of (+)-Biotin. *J.Org. Chem.* **1995,** *60*, 321–330.

179. Suyama, T. L.; Gerwick, W. H. Stereospecific Total Synthesis of Somocystinamide A. *Org. Lett.* **2008,** *10*, 4449–4452.