May 2015

Asymmetric Synthesis of Tryptophan Derivatives and Its Application to Streamlined Synthesis of Tryprosatin A and B

Matthew Huisman

University of Wisconsin-Milwaukee

Follow this and additional works at: https://dc.uwm.edu/etd

Part of the Organic Chemistry Commons

Recommended Citation


https://dc.uwm.edu/etd/880

This Dissertation is brought to you for free and open access by UWM Digital Commons. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of UWM Digital Commons. For more information, please contact open-access@uwm.edu.
ASYMmetric SYNthesis of TRYPTOPHAn DRIViATivEs AND ITS APPLICATION TO STREAMLiNED

SYNTHESIS OF TRYPROSATAiN A AND B.

by

Matthew Marcus Huisman

A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

In Chemistry

at

The University of Wisconsin-Milwaukee

May 2015
Tryprostatins have been shown to be potential antitumor antimitotic agents. Tryprostatins have been isolated from the fermentation broth of marine fungal strain *Aspergillus fumigatus* in trace amounts. Our lab has developed a phase-transfer-catalyzed asymmetric alkylation reaction to produce protected tryptophans (Trp) with high enantioselectivity (90-95% ee) as synthetic precursors to Tryprostatins. Studies of Tryprostatins indicate that manipulation of ring-A may cause enhanced activity. We propose a general synthetic route to several new tryprostatins that may be tolerant to ring-A analogues of gramine utilizing achiral reactants. The synthesis of Tryprostatin B has been completed with 20% overall yield in 7 steps. In the future our group will hopefully be able to utilize this chemistry to develop a large number of Tryprostatin analogs. We hope that one of these derivatives will be selective against cancer cells, with therapeutic concentrations in the nanomolar region.
TABLE OF CONTENTS

CHAPTER

1. INTRODUCTION ............. 1
   1.1. Tryprostatin A and B and their Biological Activity ......................... 1
   1.2. Development of this work by the Cook Group ................................ 6
   1.3. Fukuyama’s synthesis ....................................................................... 12
   1.4. Cell Cycle and Anticancer Drugs ...................................................... 15
   1.5. Inhibitors of Chromatin Function ....................................................... 18
   1.6. Inhibitors of Breast Cancer Resistance Protein ................................... 21
   1.7. Benzophenone Imine Glycine Schiff Base .......................................... 21
   1.8. Asymmetric Phase-Transfer Catalysis Utilizing Chiral Quaternary Ammonium Salts: Asymmetric ................................................................. 25
   1.9. Diketopiperazine rings and their significance ..................................... 28

2. BACKGROUND ................. 31
   2.1. Development of the Acrylates ............................................................ 31
   2.2. Acrylates to 3-ethylesterindoles ......................................................... 33
   2.3. 3-ethylesterindoles to gramines ......................................................... 35

3. OBJECTIVE .................... 44
   3.1. Optically active tryptophan derivatives .............................................. 44
   3.2. Using optically active tryptophan to synthesize natural product tryprostatin A and B ............................ 44
   3.3. Utilizing enantio-enriched tryptophan and tryprostatin synthesis to make derivatives of tryprostatin ................................................................. 44

4. RESULTS ...................... 45
   4.1. Synthesis of optically active tryptophan and three analogs .................. 45
   4.2. Utilization of optically active tryptophan to synthesize natural product tryprostatin B .................. 46
   4.3. Previous Tryprostatin syntheses ......................................................... 48
   4.4. Our proposed synthesis ..................................................................... 51
4.5. Alternative proposed synthesis ................................................................. 55
4.6. Monitoring the effectiveness of the C-2 isoprenyl quaternary ammonium bromide salt . 61
4.7. Attempts to synthesis Tryprostatin B .................................................................. 64
4.8. DKP ring closure by microwave in water .............................................................. 73
5. CONCLUSION ......................................................................................................... 79
  5.1. Importance of tryptophan and asymmetric synthesis in medicinal chemistry .......... 79
  5.2. Our initial goals: ................................................................................................. 80
  5.3. Utilization asymmetric synthesis to make natural products tryprostatin A and B .......... 82
  5.4. The future plans of this project: ........................................................................... 83
6. EXPERIMENTAL SECTION ..................................................................................... 84
7. DATA .................................................................................................................... 145
8. VITA ...................................................................................................................... 259
9. REFERENCE ......................................................................................................... 256
LIST OF FIGURES

Figure 1. Tryprostatin A and B ............................................................................................................. 1
Figure 2. Spirotryprostatins .............................................................................................................. 2
Figure 3. Cyclotryprostatins ............................................................................................................ 3
Figure 4. Fumitremorgins ............................................................................................................... 3
Figure 5. V-70 radical initiator ......................................................................................................... 13
Figure 6. The cell cycle .................................................................................................................. 15
Figure 7. Cell cycle representing G1, S, and G2 phases. ................................................................. 16
Figure 8. Cell cycle showing mitosis. ............................................................................................. 17
Figure 9. Microtubules roll in cell division ..................................................................................... 19
Figure 10. Chromosome division along microtubules ..................................................................... 20
Figure 11. Comparison of Sörensen’s Glycine Anion and O’Donnell’s Glycine Anion .............. 22
Figure 12. Comparison of acidity of protons on alpha carbon when not alkylated and when mono alkylated. ................................................................................................................. 22
Figure 13. First Generation Cinchona Alkaloids .......................................................................... 23
Figure 14. Second Generation Cinchona Alkaloids ..................................................................... 23
Figure 15. Free OH compared with alkylated oxygen on the phase transfer catalyst .................... 24
Figure 16. Stereoview of the ion pair between the enolate of the O’Donnell Schiff base and the phase transfer catalyst ........................................................................................................... 25
Figure 17. Phase-transfer catalyst diagram of ion exchange ............................................................ 26
Figure 18. Ion pair between phase-transfer catalyst and O’Donnell Schiff base ......................... 28
Figure 19. Numbering system of 2,5-DKPs ................................................................................... 29
Figure 20. Most common synthetic procedure for closing 2,5-DKP ................................................. 30
Figure 21. Newman projection of possible transition states between acrylate and beta-keto ester .............................................................................................................................................. 33
Figure 22. Mechanism demonstrating reduction of acrylate and ring closing of indole ............. 35
Figure 23. Phase-transfer catalysts that were screened .................................................................. 38
Figure 24. Screening of quaternization reagents .......................................................................... 39
Figure 25. Tryprostatin A and B .................................................................................................... 48
Figure 26. Comparison of resonance stabilization between two proposed transition states ......... 54
Figure 27. Cook’s Schöllkopf Chiral Auxiliary protected indole .................................................... 55
Figure 28. Crystal structure of C-2 isoprenylated isoprenyl quaternary ammonium salt ........... 59
Figure 29. Reducing agents in live cells change Owen’s reagent into purple dye ....................... 76
Figure 30. Boc protection of gramine ............................................................................................. 88
Figure 31. Disoprenylation of Boc gramine .................................................................................... 90
Figure 32. TLC plate of fractions eluted from column chromatography ....................................... 91
Figure 33. Structure of glycine Schiff base .................................................................................... 92
Figure 34. NMR of Glycine Schiff base ........................................................................................ 93
Figure 35. Structure of protected tryptophan ............................................................................... 94
Figure 36. NMR of protected tryptophan .................................................................................... 94
Figure 37. Structure of disoprenylated quaternary ammonium bromide salt ............................. 95
Figure 38. NMR of diisoprenylated quaternary ammonium bromide salt ........................................... 95
Figure 39. TLC of Fmoc deprotection ........................................................................................................ 102
Figure 40. TLC of reagents and crude reaction mixture .............................................................................. 105
Figure 41. Apparatus for synthesis of glycine Schiff base .......................................................................... 107
Figure 42. Charred reaction product ........................................................................................................... 110
Figure 43. TLC plate of crude reaction mixture .......................................................................................... 121
LIST OF TABLES

Table 1. Comparison of acrylate and beta-keto ester using Iron Lewis catalyst vs HBF₄ ............. 32
Table 2. Yield of benzaldehyde to acrylate ..................................................................................... 34
Table 3. Yields of acrylates to 3-ethylesterindole ......................................................................... 34
Table 4. Yield of 3-ethylesterindoles to gramines with ring A substitutions .................................... 36
Table 5. Screening of optically active tryptophan with bases at varying concentrations .............. 40
Table 6. Screening of various amounts of phase-transfer catalyst loading ....................................... 41
Table 7. Solvent screening to make optically active tryptophan .................................................... 42
Table 8. Monitoring the effect of temperature depression on the synthesis of optically active tryptophan ......................................................................................................................... 42
Table 9. Monitoring the effect of number of equivalents of water on the synthesis of optically active tryptophan ......................................................................................................................... 43
Table 10. Synthesis of 5 and 6 indole ring position tryptophan analogs ............................................ 46
Table 11. Comparison between dichloromethane and dioxane in 10 and 45% aq. KOH .................. 61
Table 12. Solvent screening of phase-transfer catalyst ..................................................................... 62
Table 13. Screening the effect of equivalents of water ..................................................................... 63
Table 14. Screening temperature and catalyst loading ...................................................................... 64
Table 15. Shows chromosome content and percent cell survival, indicating tryprostatin A inhibits cell progression after chromosomes double in cell division ................................................................. 77
LIST OF SCHEMES

Scheme 1. Danishefsky’s 1996 synthesis of tryprostatin B ........................................... 5
Scheme 2. Synthesis of starting material for Cook’s synthesis ....................................... 6
Scheme 3. Cook’s tryprostatin synthesis ....................................................................... 7
Scheme 4. Cook’s synthesis of diastereomers ................................................................. 10
Scheme 5. Cook’s synthesis of enantiomers of tryprostatin ........................................... 11
Scheme 6. Cook’s synthesis of diastereomers of tryprostatin ......................................... 12
Scheme 7. Fukuyama’s synthesis of tryprostatins ......................................................... 14
Scheme 8. Synthetic equation using iron Lewis acid catalysis to make acrylates ......... 31
Scheme 9. Comparison between Iron Lewis acid and HBF₄ to make acrylate ............. 31
Scheme 10. 2-nitrobenzaldehyde through aldehyde to 3-ethylesterindole ................. 33
Scheme 11. 3-ethylester indole to gramine ..................................................................... 35
Scheme 12. Synthesis of quaternary ammonium salt to racemic tryptophan ............. 36
Scheme 13. Initial screening to make optically active protected tryptophan .............. 37
Scheme 14. Synthesis of optically active tryptophan screening various bases and concentration of bases .............................................................. 39
Scheme 15. Screening of amount of phase transfer catalyst that was used .................. 40
Scheme 16. Solvent screening to make optically active tryptophans ......................... 41
Scheme 17. Monitoring the effect of temperature when making optically active tryptophan .... 42
Scheme 18. Monitoring the effect of number of equivalents of water on the synthesis of optically active tryptophan ................................................................. 43
Scheme 19. Synthesis of 5 and 6 indole ring position tryptophan analogs ................. 45
Scheme 20. Initial proposed synthesis of tryprostatin B ............................................... 47
Scheme 21. Danishefsky’s 1996 Tryprostatin B synthesis ............................................. 49
Scheme 22. Cook’s 2002 Tryprostatin B synthesis. Further developed in 2008 to include diastereomers, enantiomers and a number of derivatives .............................. 50
Scheme 23. Synthesis of Schöllkopf Chiral Auxiliary .................................................. 50
Scheme 24. Boc protection of skatole .......................................................................... 51
Scheme 25. Fukuyama’s 2010 Tryprostatin A synthesis ................................................ 51
Scheme 26. Our proposed stream-lined tryprostatin B synthesis ................................ 51
Scheme 27. Isoprenylation at the alpha carbon position instead of the C-2 position ...... 53
Scheme 28. Alternative proposed synthesis of tryprostatin B ..................................... 55
Scheme 29. Boc protection of gramine ......................................................................... 56
Scheme 30. Proposed C-2 isoprenylation ..................................................................... 56
Scheme 31. Actual isoprenylation to make isoprenyl quaternary ammonium salt ...... 57
Scheme 32. Utilization of isoprenyl quaternary ammonium salt to synthesis protected tryptophan .................................................................................. 57
Scheme 33. Synthesis of C-2 isoprenylated and isoprenyl quaternary ammonium salt, which was able to undergo the phase-transfer catalyst reaction to make C-2 isoprenylated protected tryptophan ................................................................. 58
Scheme 34. Comparison between dichloromethane and dioxane in 10 and 45% aq. KOH .... 61
Scheme 35. Solvent screening of phase-transfer catalyst ............................................. 62
Scheme 36. Screening the effect of equivalents of water .................................................. 63
Scheme 37. Screening temperature and catalyst loading .................................................. 63
Scheme 38. Proposed synthesis of tryprostatin B ............................................................ 65
Scheme 39. Dipeptide synthesis using proline acid chloride ........................................... 66
Scheme 40. Dipeptide synthesis using peptide coupling reagent ...................................... 67
Scheme 41. Deprotection of Fmoc from dipeptide ........................................................... 68
Scheme 42. Alternative method of Fmoc deprotection ....................................................... 68
Scheme 43. First attempt to close DKP ring ...................................................................... 69
Scheme 44. Failed DKP ring closure without deprotecting Fmoc dipeptide ....................... 70
Scheme 45. Attempt to remove t-butyl using lithium hydroxide ...................................... 71
Scheme 46. Attempt to remove t-butyl group using 6N HCl at reflux ............................... 71
Scheme 47. Attempt to remove t-butyl using 5N HCl at room temp ................................. 72
Scheme 48. Attempt to remove t-butyl group using phosphoric acid at room temp ......... 72
Scheme 49. Model for DKP ring closure ......................................................................... 73
Scheme 50. Total synthesis tryprostatin B ..................................................................... 73
Scheme 51. Total synthesis tryprostatin A ..................................................................... 75
Scheme 52. Proposed alternative method to tryprostatins ............................................... 78
Scheme 53. Synthesis of protected tryptophan ................................................................. 85
Scheme 54. Boc protection of tryptophan ........................................................................ 86
Scheme 55. Boc protection of gramine ............................................................................ 86
Scheme 56. Diisoprenylation of Boc gramine ................................................................... 88
Scheme 57. Phase-transfer catalyst using diisoprenylated quaternary ammonium bromide salt 91
Scheme 58. Phase-transfer catalyst screening procedure ................................................. 96

Scheme 59. Deprotection of tryptophan amine ............................................................... 97
Scheme 60. Attempted isolation of ammonium chloride salt ........................................... 98
Scheme 61. Synthesis of Fmoc proline acid chloride ....................................................... 99
Scheme 62. Synthesis of dipeptide using acid chloride .................................................... 100
Scheme 63. Deprotection of Fmoc .................................................................................. 101
Scheme 64. Peptide coupling of proline and ethyl ester glycine ....................................... 102
Scheme 65. Attempted DKP ring closure in DMF .......................................................... 103
Scheme 66. Phase-transfer catalyst reaction using ethyl ester glycine ........................... 104
Scheme 67. Synthesis of glicine schiff base .................................................................... 106
Scheme 68. Deprotection of amine using 1 N HCl .......................................................... 107
Scheme 69. Attempted deprotection of t-butyl group ..................................................... 108
Scheme 70. Attempted deprotection of t-butyl group ..................................................... 109
Scheme 71. Attempted deprotection of t-butyl group using 5 N HCl ............................... 110
Scheme 72. Attempted synthesis of dipeptide proline salt .............................................. 111
Scheme 73. Attempted deprotection of t-butyl group ..................................................... 112
Scheme 74. Deprotection of Glycine Schiff base ............................................................. 112
Scheme 75. Deprotection of glycine Schiff base using 15% citric acid ............................ 113
Scheme 76. Synthesis of glycine proline using acid chloride .......................................... 114
Scheme 77. Deprotection of dipeptide ............................................................................. 115
Scheme 78. Synthesis of ethyl ester glycine Schiff base .............................................. 115
Scheme 79. Peptide coupling of proline and glycine ethyl ester ............................... 116
Scheme 80. Phase-transfer catalyst with glycine ethyl ester ..................................... 117
Scheme 81. Attempted DKP ring closure .................................................................... 118
Scheme 82. Attempted DKP ring closure .................................................................... 119
Scheme 83. Peptide coupling between t-butyl glycine and proline ......................... 120
Scheme 84. Attempted DKP ring closure .................................................................... 121
Scheme 85. Attempted DKP ring closure .................................................................... 122
Scheme 86. Peptide coupling of isoprenyl tryptophan and proline ......................... 123
Scheme 87. Attempted synthesis of tryprostatin B ...................................................... 124
Scheme 88. Synthesis of tryprostatin B ..................................................................... 125
Scheme 89. Attempted synthesis of tryprostatin B ...................................................... 126
Scheme 90. Attempted synthesis of tryprostatin B ...................................................... 127
Scheme 91. Synthesis of tryptophan amine ................................................................. 128
Scheme 92. Attempted peptide coupling with DCC ..................................................... 129
Scheme 93. Synthesis of 6-methoxygramine ............................................................... 131
Scheme 94. Synthesis on diisoprenyl 6-methoxygramine salt .................................... 132
Scheme 95. Attempted synthesis using sec-BuLi ......................................................... 134
Scheme 96. Single isoprenylation .................................................................................. 136
Scheme 97. Synthesis of diisoprenyl quaternary ammonium salt .............................. 137
Scheme 98. PTC and deprotection steps combined ..................................................... 138
Scheme 99. Synthesis of 6-MeOtryptophan ............................................................... 139
Scheme 100. Attempted deprotection of benzophenone imine ................................. 140
Scheme 101. Deprotection of benzophenone imine using citric acid ......................... 141
Scheme 102. Peptide coupling of 6MeOtryptophan and proline ............................... 142
Scheme 103. Deprotection of Fmoc .............................................................................. 143
Scheme 104. Synthesis of tryprostatin A ................................................................. 144
To

My family

Acknowledgments

I wish to express my sincere gratitude to Professor M. Mahmun Hossain for his guidance, support and encouragement during the course of my work.

I would like to thank professors: Alexander E Arnold, Arsenio Andrew Pacheco, Xiaohua Peng, Nicholas R Silvagg, James M. Cook and Alan Schwabacher.

I would like to thank these researchers: Dr. Robert Todd, Dr. Matthew Dudley, Dr. Monzu Morshed, Sharif Md. Asad, Masha Shevyrev-Shteynbuk, Nazim Md. Uddin, Joeseph S. Ulick, Shamzul Amed, Mizzanor Md. Rahaman, Dan Murphy and Brian Spindler.

I would like to thank my brother Eric James Huisman, my deepest appreciation and thanks go to my father Steven Kenneth Huisman and my mother Michelle Marie Huisman who were always there for me.

I would like to thank Sarah Anne Oehm. Sarah has been very close to me in my time at University of Wisconsin-Milwaukee. This project could never have been completed without her never-ending support.

Finally, I wish to thank the Graduate school (UWM) and funding sources as well as the office staff of the Department of Chemistry (UWM) for their assistance.
1. INTRODUCTION

1.1. Tryprostatin A and B and their Biological Activity.

![Chemical structure of Tryprostatin A and B]

R= OMe, Tryprostatin A (1)

R= H, Tryprostatin B (2)

Figure 1. Tryprostatin A and B

Tryprostatins A 1 and B 2 (Figure 1) are natural products with therapeutic activity against breast cancer. The natural source for these compounds is a marine fungus, specifically, strain BM939 of Aspergillus fumigates. Tryprostatins A and B were isolated as secondary metabolites from fermentation broth. Tryprostatins A 1 and B 2 were found to have high activity in tsFT210 cells with inhibitory concentrations of 50 µg/ml of 1 and 12.5 µg/ml of 2, respectively. These molecules function to completely arrest microtubule formation during the G2/M phase, thus inhibiting cell cycle progression. Tryprostatins A 1 and B 2 contain a 2-isoprenyl tryptophan moiety and a proline residue, the latter of which was located in the diketopiperazine unit.
In addition to tryprostatin A \(1\) and B \(2\), spirotryprostatins A \(3\) and B \(4\) (Figure 2) and cyclotryprostatins A-D \(5\) (Figure 3) were isolated from the same species \(4\) by Osada et al. Spirotryprostatins have the same biological function, arresting the cell cycle at the G2/M, but were less potent than Tryprostatins: IC\(_{50}\) values of 197.5 (3) and 14.0 µM (4). On the other hand, cyclotryprostatins A-D \(5\), which belong to the family of Fumitregorins (Figure 4), \(^{6-11}\) showed good potency with IC\(_{50}\) values of 5.6µM, 19.5µM, 23.4µM, and 25.3µM, respectively. Like Tryprostatins, these function medicinally to inhibit tsFT210 cell cycle progression during the G2/M phase. \(^2\)

![Figure 2. Spirotryprostatins](image)

3 Spirotryprostatin A

4 Spirotryprostatin B

R=H  Cyclotryprostatin A  Cyclotryprostatin C

R=CH\(_3\)  Cyclotryprostatin B.
The interesting biological activity of this family of alkaloids has piqued curiosity in their total synthesis. The first total synthesis of the parent, tryprostatin B was reported by Danishefsky et al. Via the chloroindolenine/borane approach, illustrated by the scheme below. The N-phthaloyl-L-tryptophan methyl ester was treated with tert-butyl hypochlorite to generate the
chloroindolenine intermediate at 0°C. This intermediate was then treated with prenyl stannane and followed by rapid addition of boron trichloride (two equivalents) to provide the desired 2-isoprenyl tryptophan derivative. It is thought that the reaction of prenyl stannane with boron trichloride generated a nucleophilic prenylation species in situ. This species is believed to react with the chloroindolenine to provide the “ate” like structure complexed to the indolenine Nα-nitrogen atom. This step was followed by intramolecular delivery of the isoprenyl moiety to the indole C(2) position. Smooth removal of the N-phthaloyl protecting group generated the required L-2-isoprenyltryptophan methyl ester. The coupling reaction between the 2-isoprenyl tryptophan unit and the N-Boc-protected L-prolinyl acid fluoride furnished dipeptide as illustrated. The Boc protecting group was removed on treatment of material with trimethylsilyl iodide to afford the free amine. The free amine was stirred in a solution of ammonia/methanol for 24 h, the formation of the diketopiperazine unit resulted in tryprostatin B identical to the natural material. In 1996 Danishefsky’s group was able to accomplish the synthesis of Tryprostatin B in eight steps with 46% over all yield.
Scheme 1. Danishefsky’s 1996 synthesis of tryprostatin B

Danishefsky’s 1996 synthesis of tryprostatin B
Microtubules have important roles in cell growth and division, making them promising targets for cancer therapeutics. Tryprostatins target and inhibit microtubule growth and therefore have considerable value as potential drugs for treating cancer.\textsuperscript{2}

\section*{1.2. Development of this work by the Cook Group}

Synthesis of the starting material, Boc protected 6-methoxy-3-methylindole is shown below with a 67\% yield.

\textbf{Scheme 2.} Synthesis of starting material for Cook’s synthesis

In 1997 the first total synthesis of tryprostatin A was completed by Gan in Cook’s group via a regiospecific bromination process coupled with the Schöllkopf chiral auxiliary.\textsuperscript{4} This approach provided the 2-bromo-6-methoxytryptophan as a key intermediate in good yield. 1-\textit{tert-}...
butyloxy carbonyl-3-methylindole was prepared in four steps from m-anisidine via a Japp-Klingemann/Fischer Indole protocol. The azobisisobutyronitrile (AIBN) initiated regiospecific bromination of 3-methylindole at the allylic position was accomplished using N-bromosuccinimide (NBS) as the brominating agent via a radical process. The coupling reaction between the benzylic bromide which resulted and the anion of the Schöllkopf chiral auxiliary provided the stable dihydropyrazine with diastereoselectivity. Electrophilic, regiospecific brominating of pyrazine with NBS at the indole C(2)-position generated under conditions of electrophilic substitution. Using lithium-halogen exchange followed by addition of isoprenyl bromide, the desired C2-functionality was achieved. Hydrolysis of the pyrazine unit in acidic conditions (THF, aq 2 NHCl) provided the ethyl ester. Treatment of the tryptophan derivative with N-Troc-L-prolinyl chloride afforded the desired dipeptide after reductive cleavage of the protecting Troc group. Cyclization to form the diketopiperazine and removal of the Boc protecting group generated tryprostatin A in a one pot process. The optical rotation and spectral data of synthetic tryprostatin A were in agreement with those of the natural product. In 1997 Cook’s group was able to synthesis tryprostatin A in an enantiospecific fashion using sixteen steps 9.15 % yield.

Scheme 3. Cook’s tryprostatin synthesis
In 2008, the synthesis of Tryprostatin A and B as well as their enantiomers was developed by Zhao in Cook’s group. In order to introduce the isoprenyl group at the indole C(2) position of and decrease the number of steps earlier reported by Gan et al. LDA was employed to form the
anion at C(2). The indole was stirred with LDA at -78 °C followed by the addition of dry, pure isoprenyl bromide to furnish 2-isoprenylpyrazine. This was an improvement in the synthesis of 2-isoprenylpyrazine, this procedure was used for tryprostatin B. Since the Schöllkopf chiral auxiliary can tolerate strongly alkaline conditions, it served well as a protecting group for the amino acid functional group and prevented racemization. The pyrazine moiety was removed under acidic conditions (aq HCl, THF) in 92% yield to provide L-valine ethyl ester and 2-isoprenyl tryptophan. Using the same conditions, the enantiomeric 2-isoprenyl tryptophans and were synthesized and employed for the enantiospecific synthesis of tryprostatins A and B. In 2008 Cook’s group was able to synthesize tryprostatin B in 40% yield as well as the enantiomers and diastereomers in through the same procedure using the corresponding amino acids.
Scheme 4. Cook’s synthesis of diastereomers

With the key 2-isoprenyltryptophan derivatives in hand, the diketopiperazine unit was built on as illustrated. The various 2-isoprenyl-tryptophans were stirred with N-Fmoc-L-proline chloride in the presence of triethylamine in chloroform at room temperature this was followed by removal of the solvent. The Fmoc protecting group was then removed by addition of diethylamine (DEA) in acetonitrile. Solvents were removed under reduced pressure. Formation of the diketopiperazine as well as the removal of the Boc protecting group from the indole N(H)
were achieved by heating in refluxing xylenes in high dilution. A stereospecific, enantiospecific total synthesis of Tryprostatin A and B was accomplished via alkylation of the corresponding 2-lithioindole derivatives. This procedure was also applied to the enantiomers of tryprostatin A and tryprostatin B. The optical rotations of the natural products and the enantiomers were in agreement with those reported by Osada et al. for the natural products. This route was used for the total synthesis of the mismatched pairs of Tryprostatin A and B for biological screening.

**Scheme 5.** Cook’s synthesis of enantiomers of tryprostatin
1.3. Fukuyama’s synthesis

In 2010 Fukuyama’s group decide to pursue the synthesis of Tryprostatins, interest from this sprung from the history of exploring radical mediated cyclization in the formation of the 2, 3 substituted indole rings. They further speculated that they may be able use that chemistry to make natural products Tryprostatin A and Tryprostatin B.

Fukuyama and co-workers began by making an aromatic iodide that they would be able to use a Sonoashira coupling, a palladium-mediated coupling to attach the aromatic ring to the desired alkyne. From this intermediate they were able to make the ortho-alkenyl isocyanide in two steps.
Next the Fukuyama group proceeded to demonstrate their expertise over radical mediated cycliation of indoles by using a unique radical initiator to close the between the newly formed 2 and 3 carbons. This step also utilized tri butyl tin as a leaving group for the isoprenyl group undergoing addition at the indole C2 position.

![Figure 5. V-70 radical initiator](image)

Next steps include a ring opening, oxidation and peptide coupling. This was followed by reflux in high boiling solvent N-methyl-2-pyrrolidone (NMP) to close the diketopiperzine ring. The synthesis was achieved in ten steps in 39 percent yield.
Scheme 7. Fukuyama’s synthesis of tryprostatins
The cell cycle is the process of one cell turning into two daughter cells. This process is often broken into four parts consisting of G1, S, G2 and M.\textsuperscript{6} Interphase is often times used to describe the combination of G1, S, and G2. During interphase the cell grows and prepares for cell division by duplicating its DNA. The remaining mitotic (M) phase splits the cell into two daughter
cells. M phase is followed by cytokinesis where the cell completely divides. See figure 6.

![Cell cycle diagram]

**Figure 7.** Cell cycle representing G1, S, and G2 phases.

Gap 0 (G₀) Resting phase cell is not dividing. Gap 1 (G₁) Cells increase in size. G₁ check point ensures that the DNA is ready for replication. Synthesis (S) DNA replication occurs. Gap 2 (G₂) the cell grows G₂ checkpoint ensures that cell is ready to enter the M phase and divide. Mitosis (M) Cell growth stops cell divides into two daughter cells, Metaphase checkpoint ensures that the cell is ready to complete cell division. See figure 7.
Furthermore, the Mitotic phase is broken down into five phases. Furthermore, the Mitotic phase is broken down into five phases demonstrated by the acronym IPMAT. Interphase as discussed earlier is the phase leading up to cell division. During this resting phase gaining nutrients and the cell is not dividing. During prophase the chromatin condenses and membrane surrounding the nucleus disappears. During metaphase telomeres appear and the
chromosomes line up on the equatorial plane. In anaphase the chromosomes divide and separate to opposite sides of the cell. During Telophase the cell divides into two different cells.

Cyclin-dependent kinases (CDKs) are a family of protein kinases, which regulate the cell. They are present in all eukaryotes and their regulatory function in the cell cycle has been conserved over time. They bind cyclin and without cyclin CDK has little kinase activity. CDKs phosphorylate their substrates on serines and threonines. Animal cells contain at least nine CDKs four of which are directly involved in cell cycle regulation.

1.5. Inhibitors of Chromatin Function
Topoisomerase inhibitors

Topoisomerase inhibitors interfere with the enzymes that control the changes in DNA structure via a mechanism that involves catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the cell cycle.

Microtubule Inhibitors

Microtubules are a structural part of the cytoskeleton and are found in the cytoplasm. Microtubules are formed by the combination of alpha and beta tubulin protein segments. The formation and deconstruction of the microtubules are very rapid. The purpose of microtubules is to maintain the structure of the cell. Microtubules provide platforms for intracellular transport, by forming the structures on which motor proteins, dynein and kinesin move. They act as the metaphorical train track and train cars of the cell. Lastly the microtubules are used in cell division connecting to the mitotic spindles to the telomers on which the motor proteins pull the chromosomes apart. Microtubules are long hollow tubes which polymerize end to end. In order for polymerization to occur dimers must be present above an established concentration.
Microtubules have polarity, the end with the alpha subunit exposed is (-) while the beta subunit is the (+). Elongation only occurs from the (+) end.

Figure 9. Microtubules roll in cell division

Mitotic spindles also called spindle apparatus are present in all eukaryotes. The job of the microtubules is to separate the cell’s sister chromosomes during anaphase in the process of cell division. This spindle microtubules, microtubule-associated proteins (MAPS) and the microtubule organization center (MTOC) are all involved in this dynamic process.
Cytoskeletal drugs are molecules that interact with actin or tubulin.\textsuperscript{10} Some such as taxol stabilize the microtubules, while others prevent polymerization. Cytochalasin D binds to actin monomers and prevents polymerization of actin filaments; this is an example of a destabilizing agent.

1.5.1 Microtubules as drugable targets

Many drugs have been able to bind to tubulin by modifying its activation site, the effect of this is that the microtubule dynamics are manipulated.\textsuperscript{11} This interference can prevent a cell from going into a cell cycle and can lead to programmed cell death or apoptosis.\textsuperscript{12} Both microtubule stabilizer and destabilizers can suppress microtubule dynamics. The Taxane family of anti-cancer drugs, which contains Taxol, is a well-known example of a member of this family. These

\textbf{Figure 10.} Chromosome division along microtubules
compounds work by stabilizing the GDP bound tubulin, stopping depolymerization. Vincristine and Colchicine have the opposite effect, blocking the polymerization of tubulin to microtubules.

1.6. Inhibitors of Breast Cancer Resistance Protein
Multidrug resistance (MDR) has been shown to be one of the most difficult obstacles to overcome in treating cancer. This resistance is often due to membrane bound proteins, driven by ATP push anticancer therapeutics out of the cell. Breast Cancer Resistance Protein (BCRP) an ATP-binding cassette transporter has been shown to be one of these types of “problematic proteins.” Inhibition of this class of proteins has been shown to increase the intracellular drug accumulation and reverses BCRP-mediated multidrug resistance. A better understanding of molecules that interact with multidrug resistance proteins such as BCRP or better understanding of the binding site is critical in the advancement of designing more effective therapeutic strategies. BCRP was initially discovered as a placenta-specific adenosine triphosphate ATP) binding cassette transporter (ABCP), but was later found in an assortment of tumor types. Since BCRP is involved in exporting substrates from the cell, the pharmacological efficacy of drugs that are substrates of BCRP are compromised. BCRP has an ability to remove a wide variety of molecules from the cells. Multidrug resistance protein is considered one of the major transporters causing drug resistance in mammalian cells.

1.7. Benzophenone Imine Glycine Schiff Base
Catalytic asymmetric synthetic reactions are attractive because they don’t use the often times more expensive chiral control reagent in more than the standard twenty mole percent. Chiral phase-transfer catalysis (PTC) are often preferred by organic chemists due to their mild conditions, simple reaction procedures, safe and inexpensive reagents and solvents, furthermore the PTC reactions have been shown to be tolerant to scale up, making them
incredibly useful for production of products on gram to kilogram scale. O’Donnell’s laboratory originally developed benzophenone imines of glycine alkyl esters in 1978 as an alternative method to obtain diethylacetamidomalonate, which is the starting material for the classical 1903 Sörensen method for the synthesis of racemic α-amino acids.”

![Glycine Anion](image)

**Figure 11.** Comparison of Sörensen’s Glycine Anion and O’Donnell’s Glycine Anion

Since its development the O’Donnell Schiff base or the benzophenone imine glycine ester has found applications in both chiral and racemic amino acids. A critical characteristic of the O’Donnell Schiff base is the selective monoalkylation of the substrate in base, due to the difference in acidity of the α-carbon’s proton in the starting material and the monoalkylated product. This change in acidity [pKa (DMSO)] is essential for the stereoselective addition of an alkyl group, without causing base induced racimization or making the dialkylated product.

![Glycine Anion](image)

**Figure 12.** Comparison of acidity of protons on alpha carbon when not alkylated and when mono alkylated.

1.7.1 Entry into Eantioselective PTC utilizing *Cinchona* Alkaloids
The *Cinchona* alkaloids have played a major role in the development of phase-transfer catalysis. Cinchonine and cinchonidine derived catalysts have been used commonly in chiral PTC due to the parent alkaloids being inexpensive and easily converted into effective phase-transfer catalysts.\(^\text{17}\)

**Figure 13. First Generation Cinchona Alkaloids**

The first generation of Cinchona alkaloids gave enantioslective alkylations on the order of 66%ee using the O’Donnell Schiff base and Sodium Hydroxide. The O’Donnell group made a large improvement to enantioselectivity by suggesting the O-alkylation of the Cinchona quaternary ammonium salt was the active catalyst. From this idea came the second generation of Cinchona-Derived Catalysts.

**Figure 14. Second Generation Cinchona Alkaloids**
The highest reported enantioselectivity for this generation of catalyst was 81%ee. Solvent mixtures for the second generation of catalyst have been reported using toluene:dichloromethane in ratios of (7:3). This solvent ratio has not changed much from this generation into the future.

A number of groups have tried to further change the catalyst, these groups include Lygo,\textsuperscript{20} Corey, who were simultaneously reported the third generation of catalyst which employed a larger and therefore more stericly locked aromatic ring as the quaternary portion of the ammonium salt, for this they used N-9-anthracenylmenthyl.

![Free OH Catalysts](image1)

![O-Alkyl Catalysts](image2)

**Figure 15.** Free OH compared with alkylated oxygen on the phase transfer catalyst

Corey et al. suggested the enantioselectivity may be due to the key ion pair between enolate and catalyst, where the alkyl halide approaches the ion pair from the dashed arrow leading to
the S product in the case of the Cinchonindine catalyst. Depicted on the top is the anion of the Schiff Base and on the bottom is the cation of the 3rd generation catalyst.

**Figure 16.** Stereoview of the ion pair between the enolate of the O’Donnell Schiff base and the phase transfer catalyst

Phase-transfer catalysis has been proven to be a powerful tool in synthetic organic chemistry because of its simplicity, mild conditions, and suitability for scale up. This field of highly enantioselective alkylation has become a promising area of green sustainable chemistry.

Asymmetric transformations catalyzed by chiral onium salts and crown ethers have been used to synthesis an array of compounds from amino acids to natural product to synthetic drugs. 

1.8. Asymmetric Phase-Transfer Catalysis Utilizing Chiral Quaternary Ammonium Salts: Asymmetric

Phase-transfer catalysts (PTCs) by definition help transfer a substrate molecule or ion from the aqueous phase to the organic phase. The decrease in the amount of energy required to cross the phase barrier
Due to the shuttling of the PTC greatly increases the rate of the reaction. Quaternary ammounium salts are the most commonly utilized PTC. This reaction often proceeds due to the formation of an anion ($Y^-$) in the organic phase due to the hydrophobic nature of organic phases in comparison to aqueous solutions the anion. Substrate ($Y^-$) is more reactive in the organic phase when ion paired to $Q^+Y^-$ due to the ion pairs greater charge separation and lower hydration, this effect causes greatly increased reaction rates when compared to not using the PTC.\textsuperscript{22}

The controlled delivery of anion to the substrate causes greater selectivity when compared to PTCs alternative homogeneous reactions. The reaction conditions are tolerant to most water-immiscible organic solvents. Although many times there are more reagents added to phase-transfer catalyzed reactions, the reactions are often times easy to purify due to the two phase nature of the reactions, organic products into organic layer and ionic salts and other water-miscible materials in the water layer. Lastly the catalysts are usually cheap and environmentally benign.

\begin{figure}
\centering
\begin{tikzpicture}[auto, node distance=2cm]
    \node (substrate) {$\text{Substrate-}X + Q^+Y^- \xrightarrow{k_2} Q^+X^- + \text{Product-}Y$};
    \node (interface) [below of=substrate] {$k_1 \ll k_2 \ll k_3$};
    \node (aqueous) [right of=interface] {$Q^+ = \text{phase-transfer catalyst}$};
    \node (organic) [left of=interface, xshift=-1cm] {$k_1 \ll k_2 \ll k_3$};
    \node (byproduct) [below of=substrate, xshift=1cm] {$\text{By-product}^+X^- + Q^+Y^- \xrightarrow{k_4} Q^+X^- + \text{Reagent}^+Y^-$};
    \draw[->] (substrate) -- (interface) node[midway, above] {};\draw[->] (interface) -- (aqueous) node[midway, above] {};\draw[->] (interface) -- (organic) node[midway, above] {};\draw[->] (byproduct) -- (interface) node[midway, above] {};\draw[->] (byproduct) -- (aqueous) node[midway, above] {};\node (organic) [right of=interface, xshift=1cm] {$Q^+ = \text{phase-transfer catalyst}$};
\end{tikzpicture}
\caption{Phase-transfer catalyst diagram of ion exchange}
\end{figure}

1.8.1 Further development of Glycine Imines
O’Donnell reported the first asymmetric alkylation of the glycine imine ester utilizing phase-transfer catalysis in 1989. These studies using the first generation N-benzyl Cinchona alkaloids were used, resulting in enantioselectivities ranging from 42-66% ee. These studies led to the conformation that tert-butyl ester imine was the best substrate in terms of enantioselectivity and that the diastereoisomeric catalyst of cinchonine and cinchonidine were enantio-complementary meaning they lead to the opposite chirality in the products.

The phase-transfer catalysts, stemming from the Cinchona alkaloids, were found to undergo O-benzylolation under the reaction conditions. This led to the development of prealkylated salts which gave similar enantioselectivity. Ion-pair arrangement A accounts for the enantioselectivities obtained using Cinchona based phase-transfer catalysts. “In this arrangement the Re-face of the enolate carbon is blocked by the quinolone ring of the quaternary ammonium salt, so preferential reaction via the Si-face would be expected. Alternate ion pair’s inspection of structure in ion-pair B suggested it should be less favored due to the increased charge separation required to accommodate the tert-butyl group in the “groove” between the quinolone and anthracene rings.”
Figure 18. Ion pair between phase-transfer catalysit and O’Donnell Schiff base

The drive to develop asymmetric phase-transfer alkylation reactions as a “green” alternative to its homogeneous counter parts and the reactions tolerance to non-chlorinated solvents, ambient temperature and aqueous base makes them environmentally benign in comparison. Good yields and above 90 percent enantioselectivity have been reported for the synthesis of a wide assortment of amino acids.\(^{23}\) Often times these products can be crystallized to reach high levels of enantiomerically pure products.

1.9. Diketopiperizine rings and their significance

2,5-Diketopiperazines (2,5-DKPs) are formed by closing a six membered ring made up of the backbones of two amino acids. These cyclodipeptides are prevalent in nature. The combination of these two things have allowed DKPs to become a unique class of naturally occurring privileged structures, allowing significant diversity due the readily available number and wide
range of characteristics including functionality, hydrophobicity and charge at physiological pH throughout amino acids. This family of compounds can bind to a wide assortment of biological receptors; this six membered cyclic dipeptide is constrained four, of the positions can be manipulated in terms of stereochemistry. Due to the manipulation potential in terms of stereochemistry DKP are quite easy to chirally enrich using only amino acids in the synthesis.  

![Diagram](image)

**Figure 19.** Numbering system of 2,5-DKPs

2,5-DKPs have been extensively examined using crystal and molecular structures. Some of the DKP’s ability to bind to enzymes and receptors is due to the two H-bond acceptor and two H-bond donor sites they contain that stem from the two cis-amide bonds. 2,5-DKPs exist as flat and slightly puckered boat isomers, separated by only a few kcal/mol because of it slightly rigid yet flexible conformation. All of the known active Typrostatins, Fumitremorgins and Spirotryprostatins contain both an indole ring and a diketopiperizine ring.

Dipeptides can spontaneously cyclize to form a 2,5-DKP, although this requires an amine at one terminus and an ester at the other. Coupling a nitrogen-protected α-amino acid and an α-amino acid ester the most commonly used synthetic procedure.
Figure 20. Most common synthetic procedure for closing 2,5-DKP

Deprotection of the nitrogen protecting group yields the dipeptide ester, now the nitrogen can act as a nucleophile and attack the carbonyl carbon displacing the ester \textit{insitu} to make an amide. \textit{Cis}-orientation of the amide bond is required for the closing of the six membered ring. Ring closing is difficult if the \textit{cis}-orientation is prevented due to steric or electronic effects.\textsuperscript{24-25} Other strategies that have been used to close dipeptide rings have involved refluxing in high boiling solvents for extended amounts of time. An example of this was reported by Cooks group to close the DKP ring on Tryprostatin, conditions include refluxing in Xylenes for 48 hours.\textsuperscript{1d,26} As of 2006 Tullberg’s group has demonstrated DKP ring closures utilizing microwave heating. This procedure utilizes water as a solvent and has shown no epimerization and most importantly is tolerant to amino acid sequence.\textsuperscript{27} Due to the Boc protecting groups thermally labiality, Boc nitrogen protected dipeptides became standard in this synthetic procedure, leading to the deprotection and cyclization in one step.
2. BACKGROUND

2.1. Development of the Acrylates
The Hossain group has been working on acrylate chemistry since 1998 when they published a paper on the topic of catalytic iron Lewis acid catalyst activation of benzaldehydes to form acrylates. The products of this reaction are in competition with the minor product the beta keto ester.

**Scheme 8.** Synthetic equation using iron Lewis acid catalysis to make acrylates

In 2004, the group screened the reaction against other Lewis acids to evaluate the efficiency of the iron Lewis acid. HBF₄·OEt₂ is used in the production of the iron Lewis acid, so it was questioned whether residual HBF₄·OEt₂ in the iron Lewis acid could be catalyzing this reaction.

**Scheme 9.** Comparison between Iron Lewis acid and HBF₄ to make acrylate
Table 1. Comparison of acrylate and beta-keto ester using Iron Lewis catalyst vs HBF₄

<table>
<thead>
<tr>
<th>A</th>
<th>R¹</th>
<th>Cat</th>
<th>°C</th>
<th>Yield B</th>
<th>Yield C</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>HBF₄</td>
<td>rt</td>
<td>42</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>-78</td>
<td>74</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fp⁺BF₄</td>
<td>rt</td>
<td>58</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fp⁺BF₄</td>
<td>0</td>
<td>70</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4-MeO</td>
<td>HBF₄</td>
<td>0</td>
<td>75</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>-78</td>
<td>90</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fp⁺BF₄</td>
<td>0</td>
<td>60</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2-Me</td>
<td>HBF₄</td>
<td>0</td>
<td>60</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fp⁺BF₄</td>
<td>0</td>
<td>74</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>4-Br</td>
<td>HBF₄</td>
<td>0</td>
<td>55</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fp⁺BF₄</td>
<td>0</td>
<td>62</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

Letters in table correspond to the letter assignment of the molecules in the synthetic scheme.

This reaction is expected to proceed in favor of the acrylate over the beta-keto ester due to the lowest energy Newman projection, compared to the second lowest energy projection, the temperature depression favors the lowest energy Newman projection in turn leading to higher acrylate yields. This reaction proceeds through a unique 1,2-aryl shift instead of by a hydride
migration which has been shown in 1998 in the Hossain’s group. The changes of in fine tuning of ratios of observed B and C were varying electron donating group which supports 1,2-aryl migration over hydride migration.

Figure 21. Newman projection of possible transition states between acrylate and beta-keto ester

2.2. Acrylates to 3-ethylesterindoles
In 2006, after identifying that HBF$_4$∙OEt$_2$ is the best catalyst for the reaction, and that -78°C is the optimum temperature for regioselectivity of the acrylate over the beta-keto ester, the Hossain group began work on making the ortho-nitro-acrylates into 3-ethylesterindoles.$^{30}$

Scheme 10. 2-nitrobenzaldehyde through aldehyde to 3-ethylesterindole
Table 2. Yield of benzaldehyde to acrylate

<table>
<thead>
<tr>
<th>A R</th>
<th>Catalyst</th>
<th>°C</th>
<th>Yield B</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Fp⁺BF₄⁻</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>-78</td>
<td>75</td>
</tr>
<tr>
<td>5-OCH₃</td>
<td>Fp⁺BF₄⁻</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>-78</td>
<td>75</td>
</tr>
<tr>
<td>4,5-OCH₃</td>
<td>HBF₄</td>
<td>-78</td>
<td>76</td>
</tr>
<tr>
<td>4-OCH₂O-5</td>
<td>HBF₄</td>
<td>-78</td>
<td>86</td>
</tr>
<tr>
<td>5-Cl</td>
<td>Fp⁺BF₄⁻</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>HBF₄</td>
<td>-78</td>
<td>50</td>
</tr>
</tbody>
</table>

Letters in table correspond to the substrate in the synthetic diagram.

Table 3. Yields of acrylates to 3-ethylesterindole

<table>
<thead>
<tr>
<th>B</th>
<th>Yield C</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>90</td>
</tr>
<tr>
<td>5-OCH₃</td>
<td>62</td>
</tr>
<tr>
<td>4,5-OCH₃</td>
<td>76</td>
</tr>
<tr>
<td>4-OCH₂O-5</td>
<td>86</td>
</tr>
<tr>
<td>5-Cl</td>
<td>66</td>
</tr>
</tbody>
</table>

It is proposed that this reaction proceeded via the following mechanism.
Figure 22. Mechanism demonstrating reduction of acrylate and ring closing of indole

2.3. 3-ethylesterindoles to gramines
In 2009 the group published a procedure to convert the protected 3-ethylesterindole into a 3-carboxamide using an amidoaluminum mediated mechanism. From 3-ethylesterindole using DIBAL-H it was possible to convert the carboxamide to gramine.31

Scheme 11. 3-ethylester indole to gramine
Table 4. Yield of 3-ethylesterindoles to gramine with ring A substitutions

<table>
<thead>
<tr>
<th>A R¹</th>
<th>Yield B</th>
<th>Yield C</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>77</td>
<td>94</td>
</tr>
<tr>
<td>5-MeO</td>
<td>73</td>
<td>87</td>
</tr>
<tr>
<td>6-MeO</td>
<td>64</td>
<td>90</td>
</tr>
<tr>
<td>5-Br</td>
<td>61</td>
<td>65</td>
</tr>
</tbody>
</table>

This leads us to investigation of what has been done previously with gramine. In the mid-1940s various groups were researching the conversion of gramine to racemic tryptophan.³²

Scheme 12. Synthesis of quaternary ammonium salt to racemic tryptophan

We wondered if it would be possible to make optically-pure tryptophan through a chiral phase transfer catalyst reaction using organo-catalyst. We thought this would be interesting chemistry and would be likely to find industrial use, as tryptophans are important building blocks for
indoles a novel class of compounds. The Hossain group developed the following reaction to make tryptophan.

**Scheme 13.** Initial screening to make optically active protected tryptophan

![Scheme 13](image)

At this point we began screening for a catalyst that would give high enantiomeric excess (%ee). The catalysts that were screened are shown below. All of the catalysts are derived from the 3rd generation of cinchonidine catalysts.³¹
From this screening process found that O-Allyl-N-Anthrcenyl- bromide gave the highest % ee.

The next thing that needed to be screened was the quaternization reagents. Quaternization reagents screened are shown below. Note that it is important for the reaction that the substrate is as soluble in the organic layer as possible; this forces the reaction to proceed via the phase transfer catalyst.\textsuperscript{33}
Figure 24. Screening of quaternization reagents

Next the amount of base in the aqueous layer was varied and the results monitored. Results are shown in the table below.

Scheme 14. Synthesis of optically active tryptophan screening various bases and concentration of bases
Table 5. Screening of optically active tryptophan with bases at varying concentrations

<table>
<thead>
<tr>
<th>Run</th>
<th>Conc(1a)[c]</th>
<th>% Base (% aq)</th>
<th>Time (h)</th>
<th>% Yield[d]</th>
<th>% ee[e]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>10% NaOH</td>
<td>&gt; 24</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>50% NaOH</td>
<td>8</td>
<td>47</td>
<td>75</td>
</tr>
<tr>
<td>3[b]</td>
<td>0.01</td>
<td>50% NaOH</td>
<td>16</td>
<td>42</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>10% KOH</td>
<td>5</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>45% KOH</td>
<td>2</td>
<td>&gt;95</td>
<td>84</td>
</tr>
<tr>
<td>6</td>
<td>0.1</td>
<td>10% KOH</td>
<td>2</td>
<td>97</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>10% CsOH</td>
<td>3</td>
<td>18</td>
<td>59</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>10% Ba(OH)₂</td>
<td>13</td>
<td>16</td>
<td>46</td>
</tr>
<tr>
<td>9</td>
<td>0.1</td>
<td>25% K₂CO₃</td>
<td>N.R.[f]</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Next, the catalyst loading was varied and monitored as shown in the reaction below; the results are shown in the table.³³

Scheme 15. Screening of amount of phase transfer catalyst that was used
Table 6. Screening of various amounts of phase-transfer catalyst loading

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate Conc.</th>
<th>Base</th>
<th>Catalyst Loading</th>
<th>Reaction Time (h)</th>
<th>% Yield$^a$</th>
<th>% ee$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>NaOH</td>
<td>0.2</td>
<td>11</td>
<td>18</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td></td>
<td>0.6</td>
<td>4</td>
<td>47</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>KOH</td>
<td>0.2</td>
<td>22</td>
<td>9</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td></td>
<td>0.6</td>
<td>2</td>
<td>97</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td></td>
<td>0.6</td>
<td>1</td>
<td>99</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>0.1</td>
<td>CsOH•H2O</td>
<td>0.2</td>
<td>3</td>
<td>39</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>0.6</td>
<td>3</td>
<td>81</td>
<td>71</td>
</tr>
<tr>
<td>8</td>
<td>Ba(OH)2•8H2O</td>
<td>0.2</td>
<td>13</td>
<td>16</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>0.6</td>
<td>3</td>
<td>36</td>
<td>67</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
<td>K2CO3</td>
<td>0.6</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Following the same screening process we screened various organic solvents.

Scheme 16. Solvent screening to make optically active tryptophans
**Table 7.** Solvent screening to make optically active tryptophan

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>% Yield[[b]]</th>
<th>% ee[[c]]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂Cl₂</td>
<td>1</td>
<td>99</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>1,4-Dioxane</td>
<td>2</td>
<td>95</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>THF</td>
<td>4</td>
<td>85</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>PhCH₃</td>
<td>3</td>
<td>62</td>
<td>71</td>
</tr>
</tbody>
</table>

The next thing we wanted to monitor was the effect of temperature on the reaction.

**Scheme 17.** Monitoring the effect of temperature when making optically active tryptophan

![Scheme 17](image)

**Table 8.** Monitoring the effect of temperature depression on the synthesis of optically active tryptophan

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temp C</th>
<th>Rxn time (hrs)</th>
<th>% Yield</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>1</td>
<td>86</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>-30</td>
<td>8</td>
<td>80</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>-78</td>
<td>15</td>
<td>81</td>
<td>83</td>
</tr>
</tbody>
</table>

Next we wanted to monitor the effect the number of equivalents of water had on the synthesis of optically active tryptophan.
**Scheme 18.** Monitoring the effect of number of equivalents of water on the synthesis of optically active tryptophan

![Scheme 18](image_url)

**Table 9.** Monitoring the effect of number of equivalents of water on the synthesis of optically active tryptophan

<table>
<thead>
<tr>
<th>Entry</th>
<th>Water (equiv)</th>
<th>Time (h)</th>
<th>% Yield</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>8</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>18</td>
<td>80</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>19</td>
<td>&gt;95</td>
<td>83</td>
</tr>
</tbody>
</table>

Summary of the screening shows that O-allyl-N-anthrcenyl-cinchonadinium bromide is the best catalyst, 4-trifluoromethoxybenzyl bromide is the best quaternization reagent, KOH best yielding base, a minimum 6 eq. water for optimal %ee, dioxane is the best solvent in regards to %ee, and dichloromethane is the best solvent in terms of yield.\(^{33}\)
3. OBJECTIVE

3.1. Optically active tryptophan derivatives
The primary goal was to develop a method to asymmetrically synthesize tryptophan from
gramines in a fashion that was tolerant to variations on the 4, 5, 6 and 7 position of the indole
ring. To make this objective an accomplished goal, we applied this procedure to 5-bromo, 5-
methoxy and 6-methoxy gramines to make the corresponding tryptophan derivatives.

3.2. Using optically active tryptophan to synthesize natural product tryprostatin A and B
After this goal was accomplished, our next goal was to develop a method that could utilize this
asymmetric reaction yielding enantiopure tryptophans into a total synthesis of a natural
product. We decided our targets would be tryprostatin A and B. These natural products have
low natural abundance, lengthy synthesis, and the synthesis utilizes a protected L-tryptophan as
starting material.

3.3. Utilizing enantio-enriched tryptophan and tryprostatin synthesis to make derivatives of tryprostatin
In the big picture we set out to develop a method that would asymmetrically synthesize
tryptophan and derivatives of tryptophan. We then utilized this newly developed method to
streamline the synthesis of tryprostatin B and tryprostatin A. We believe we have developed a
procedure that is tolerant to ring-A gramine analogs and reaches far beyond the scope of
previous syntheses because of this tolerance to ring-A substitution possibilities. Lastly, although
it was not a goal we initially set out to accomplish, we have also developed synthesis that very
possibly has the potential to give an entry way into making C2-derivatized tryptophan.
4. RESULTS

4.1. Synthesis of optically active tryptophan and three analogs
We wanted to evaluate how tolerant the phase transfer catalyzed asymmetric reaction was to various substitution patterns on the indole ring. HPLC was used to determine enantiomeric excess mobile phase was 6\% Isopropyl alcohol 94 \% Hexane at a flow rate of 1 mL/min using a Chiralcel OD column.\(^{34}\)

**Scheme 19.** Synthesis of 5 and 6 indole ring position tryptophan analogs

\[
\begin{align*}
\text{R}^1 & \quad \text{N} \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{Ph} & \quad \text{Ph} \\
\text{81-87} \% \text{ isolated yield} \\
\text{90-96} \% \text{ ee}
\end{align*}
\]
Table 10. Synthesis of 5 and 6 indole ring position tryptophan analogs

<table>
<thead>
<tr>
<th>Tryptophan</th>
<th>Yield</th>
<th>%ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>R¹</td>
<td>80</td>
<td>92</td>
</tr>
<tr>
<td>H</td>
<td>80</td>
<td>92</td>
</tr>
<tr>
<td>5-MeO</td>
<td>75</td>
<td>91</td>
</tr>
<tr>
<td>6-MeO</td>
<td>65</td>
<td>95</td>
</tr>
<tr>
<td>5-Br</td>
<td>73</td>
<td>90</td>
</tr>
</tbody>
</table>

4.2. Utilization of optically active tryptophan to synthesis natural product tryprostatin B
From here we looked for an application of our newly developed chiral phase transfer catalyst reaction. We proposed that we use this new reaction and apply it to the synthesis of tryprostatin and analogs of the parent structure. Shown below is our proposed synthesis.
Scheme 20. Initial proposed synthesis of tryprostatin B

At this point we would have the shortest most concise synthesis of Tryprostatin B. Our objective at this point was to shorten the synthesis making it viable for commercial applications and improve on the anticancer activity by making analogs. The whole time we had to keep in mind that the reaction scheme has to be analog tolerant.

Like other anti-cancer microtubule inhibitors such as the vinka alkaloids including the vinblastine family, Tryprostatin has very low abundance in nature. We chose to target the tryprostatin family of compounds due to their simpler synthesis when compared to the Vinblastine family of compounds.
Figure 25. Tryprostatin A and B

4.3. Previous Tryprostatin syntheses
The first synthesis of Tryprostatin B was completed by the Danishefsky’s group in 1996 using the following procedure.\textsuperscript{3a}
Scheme 21. Danishefsky's 1996 Tryprostatin B synthesis

Five years later the Cook group synthesized Tryprostatin A in 2002 and followed that by synthesizing a number of enantiomers, diastereomers and other substituted analogs in 2008 using very similar procedures developed in 2002. In 2008 they substituted in unnatural amino acids or other substituted starting materials. They achieved the synthesis of these new compounds using the reaction scheme shown below.  

\[^{14, 2}\]
**Scheme 22.** Cook’s 2002 Tryprostatin B synthesis. Further developed in 2008 to include diastereomers, enantiomers and a number of derivatives

One of the drawbacks to Cook’s synthesis is that it uses triphosgene to make the Schöllkopf Chiral Auxiliary. Triphosgene is a chemical that is not preferred to be used by most chemists due to its decomposition to phosgene, which gained infamy due to its use as a chemical weapon during World War 1. Therefore we desired to skip the use of triphosgene altogether.

**Scheme 23.** Synthesis of Schöllkopf Chiral Auxiliary

In the synthesis they also have to Boc protect skatole shown below which is not reported as a step in the synthesis. There are very few substituted skatoles commercially available to make Tryprostatin derivatives with.
**Scheme 24.** Boc protection of skatole

![Boc protection of skatole](image)

One of the more current syntheses from 2010 is shown in the synthetic scheme shown below.\(^5\)

**Scheme 25.** Fukuyama’s 2010 Tryprostatin A synthesis

![Tryprostatin A synthesis](image)

This synthesis has 11 steps a 30% yield and utilizes a toxic tin coupling reagent and triphosgene.

**4.4. Our proposed synthesis**

We proceeded with our proposed procedure which is complementary to the Cook group synthesis.

**Scheme 26.** Our proposed stream-lined tryprostatin B synthesis

![Tryprostatin B synthesis](image)
This procedure was proceeding smoothly until the lithiation isoprenyl bromination reaction, at this point it appeared that we isolated a compound that had the isoprenylation on the amino acids α-carbon.
Two compounds could have been expected. We desired to identify proton NMR peaks for the C2 isoprenylated compound include the α-carbon’s proton at one proton at 4.35 ppm (Figure shown above indicated as H), and the carbon at 14 ppm (indicated by CH2). Unfortunately we found the α-carbon had been isoprenylated, this was determined by three signatures that indicated α-carbon isoprenylation: 1) lack of the α-carbon’s proton, 2) two sets of diastereotopic protons and 3) a carbon peak at about 36 ppm. Points are indicated by 1 2 and 3 in the diagram.
This alkylation is consistent with O’Donnell’s work making unnatural amino acids. 19,35 This α-carbon isoprenylation was not seen in Cook’s procedure due to the difference in acidity of the proton in the Schöllkopf Chiral Auxiliary protected indole, compared to our very differently protected indole. We think that resonance stability and migration of electrons to stabilizes the anion shown below.

Figure 26. Comparison of resonance stabilization between two proposed transition states

Compared to Cook’s compound which cannot undergo this type of resonance stabilization due difference in the chosen protecting group. Cook’s compound is shown in Figure 27.
4.5. **Alternative proposed synthesis**

After this disappointing finding, we decided that we must take a new approach to adding the isoprenyl group to the C-2 position of the indole ring. We decided we would attempt to put the isoprenyl group on before the phase transfer reaction following the outlined procedure below.

**Scheme 28. Alternative proposed synthesis of tryprostatin B**
This procedure starts with the boc protection of gramine to protect the indolic nitrogen from the lithiating agent. This reaction went as expected in over 90% yield.

**Scheme 29. Boc protection of gramine**

![Scheme 29](image)

The next reaction had some challenges; we proposed the reaction would work as depicted in equation 30.

**Scheme 30. Proposed C-2 isoprenylation**

![Scheme 30](image)

What we had actually saw was that the isoprenyl bromide added to the graminic nitrogen instead of the indolic C-2 position as expected, this is depicted below.
Scheme 31. Actual isoprenylation to make isoprenyl quaternary ammonium salt

After identifying this compound we wondered if it would be possible to use the isoprenyled nitrogen salt as our substrate for the phase transfer catalysis reaction. This idea is shown in equation 32.

Scheme 32. Utilization of isoprenyl quaternary ammonium salt to synthesis protected tryptophan

The identification of this protected tryptophan was exciting for us because it showed that we could use the isoprenyled nitrogen salt to carry out the phase transfer catalysis reaction. At this point we decided that we should try to put two isoprenyl groups on the substrate and then try the phase transfer reaction as indicated below. Important to point out at this we now had a procedure that used three steps to get to the “key” 2-isoprenylimineprotectedt-butylestertryptophan.
Scheme 33. Synthesis of C-2 isoprenylated and isoprenyl quaternary ammonium salt, which was able to undergo the phase-transfer catalyst reaction to make C-2 isoprenylated protected tryptophan

Due to the magnitude of our entry to the most important intermediate in our pathway we later obtained a crystal structure of the diisopropyl boc protected gramine salt.

Crystal Structure data
CCDC 922382
Figure 28. Crystal structure of C-2 isoprenylated isoprenyl quaternary ammonium salt

Blocks grown using slow diffusion method: Ethyl Acetate/Hexane
Analyzed by Xray diffraction at UCSD with Arnie Rheingold

Unit Cell Dimensions:  
\[ a=8.5784(2) \text{ Å}; \quad b=12.9668(3) \text{ Å}; \quad c=13.5267(3) \text{ Å} \]
\[ \alpha=109.266(2) ^\circ; \quad \beta=103.084(2) ^\circ; \quad \gamma=107.596(2) ^\circ \]

Triclinic lattice, P1 space group, Z = 2 molecules per unit cell. R1 = 4.39%

Contact: Matthew Huisman, mhuisman@uwm.edu
Authors: Matthew M. Huisman, Sarah Oehm M. Mahmun Hossain, Arnold L. Rheingold

Table 1 Crystal data and structure refinement for Hossain01_0m

<table>
<thead>
<tr>
<th>Identification code</th>
<th>Hossain01_0m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C26H39N2O2Br</td>
</tr>
<tr>
<td>Formula weight</td>
<td>491.50</td>
</tr>
<tr>
<td>Temperature/K</td>
<td>273.15</td>
</tr>
<tr>
<td>Crystal system</td>
<td>triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P1</td>
</tr>
<tr>
<td>( a/\text{Å} )</td>
<td>8.5784(2)</td>
</tr>
<tr>
<td>( b/\text{Å} )</td>
<td>12.9668(3)</td>
</tr>
<tr>
<td>( c/\text{Å} )</td>
<td>13.5267(3)</td>
</tr>
<tr>
<td>( \alpha/^\circ )</td>
<td>109.266(2)</td>
</tr>
<tr>
<td>( \beta/^\circ )</td>
<td>103.084(2)</td>
</tr>
<tr>
<td>( \gamma/^\circ )</td>
<td>107.596(2)</td>
</tr>
<tr>
<td>Volume/Å³</td>
<td>1261.86(5)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>( \rho_{\text{calc}}/\text{g/m3} )</td>
<td>1.294</td>
</tr>
<tr>
<td>( m/\text{mm}^1 )</td>
<td>1.653</td>
</tr>
<tr>
<td>( F(000) )</td>
<td>520.0</td>
</tr>
<tr>
<td>Crystal size/µm³</td>
<td>0.3 × 0.24 × 0.18</td>
</tr>
<tr>
<td>( 2\Theta ) range for data collection</td>
<td>3.42 to 63.92°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-12 ≤ h ≤ 12, -19 ≤ k ≤ 19, -20 ≤ l ≤ 20</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>23123</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>16463[R(int) = 0.0238]</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>16463/3/577</td>
</tr>
<tr>
<td>Goodness-of-fit on ( F^2 )</td>
<td>0.917</td>
</tr>
<tr>
<td>Final R indexes [I&gt;=2( \sigma (I) )]</td>
<td>R1 = 0.0440, ( wR2 = 0.1103 )</td>
</tr>
<tr>
<td>Final R indexes [all data]</td>
<td>R1 = 0.0729, ( wR2 = 0.1451 )</td>
</tr>
<tr>
<td>Largest diff. peak/hole / e Å³</td>
<td>0.94/-0.52</td>
</tr>
<tr>
<td>Flack parameter</td>
<td>0.21(2)</td>
</tr>
</tbody>
</table>

chemical_name_systematic: N-[(1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl]-N,N,3-trimethylbut-2-en-1-aminium bromide

chemical_name_common: Compound synonym: boc protected 2 isoprenyl N isoprenyl gramine salt

data_hossain1
4.6. Monitoring the effectiveness of the C-2 isoprenyl quaternary ammonium bromide salt

We questioned the effectiveness of the phase transfer reaction with an altered substrate. We compared our reaction to similar reactions we have done in the past screening dichloromethane against 1,4 dioxane using different concentrations of KOH to push this reaction.

Scheme 34. Comparison between dichloromethane and dioxane in 10 and 45% aq. KOH

Results indicated in table 11.

Table 11. Comparison between dichloromethane and dioxane in 10 and 45% aq. KOH

<table>
<thead>
<tr>
<th>Rxn #</th>
<th>% KOH in water</th>
<th>Solvent</th>
<th>Time in hours</th>
<th>% Conversion</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10%</td>
<td>CH₂Cl₂</td>
<td>18</td>
<td>Trace</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>10%</td>
<td>1,4 Dioxane</td>
<td>18</td>
<td>Trace</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>45%</td>
<td>CH₂Cl₂</td>
<td>18</td>
<td>5</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>45%</td>
<td>1,4 Dioxane</td>
<td>18</td>
<td>95</td>
<td>42</td>
</tr>
</tbody>
</table>

Percent conversion was determined by NMR monitoring the disappearance of the α-carbons protons on the Glycine at 4.1 ppm and comparing it to the formation of the α-carbons proton
at 4.3 ppm. Percent ee was determined by HPLC using Chiralcel OD and Hexane/IPA mobile phase.

These results indicated that diluted solutions of KOH yielded little product, but when using high concentrations of KOH in water the reaction’s percent conversion was greatly improved.

Next we wanted to observe the effect of solvent on the percent conversion and percent ee, this led us to solvent screening of the reaction.

**Scheme 35.** Solvent screening of phase-transfer catalyst

<table>
<thead>
<tr>
<th>Solvent Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rxn #</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Percent conversion determined by NMR, percent ee determined by HPLC using Chiralcel OD and Hexane/IPA mobile phase.
We then screened the equivalents of water that were used in the reaction.

**Scheme 36. Screening the effect of equivalents of water**

![Scheme 36](image)

**Table 13. Screening the effect of equivalents of water**

<table>
<thead>
<tr>
<th>Rxn #</th>
<th>Water Equiv</th>
<th>Time (hr)</th>
<th>% Conversion</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>22</td>
<td>79</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>22</td>
<td>51</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>22</td>
<td>55</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>22</td>
<td>54</td>
<td>62</td>
</tr>
</tbody>
</table>

Next we wanted to observe the effect on percent yield and percent ee varying catalyst loading and temperature would have.

**Scheme 37. Screening temperature and catalyst loading**

![Scheme 37](image)
Table 14. Screening temperature and catalyst loading

<table>
<thead>
<tr>
<th>Rxn #</th>
<th>C</th>
<th>eq. cat</th>
<th>% Conversion</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rt</td>
<td>0.2</td>
<td>100</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>rt</td>
<td>0.6</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.2</td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0.6</td>
<td>81</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>rt</td>
<td>0.2</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>rt</td>
<td>0.1</td>
<td>100</td>
<td>41</td>
</tr>
<tr>
<td>7</td>
<td>rt</td>
<td>0.05</td>
<td>100</td>
<td>79</td>
</tr>
<tr>
<td>8</td>
<td>rt</td>
<td>0.025</td>
<td>100</td>
<td>47</td>
</tr>
<tr>
<td>*9</td>
<td>rt</td>
<td>0.2</td>
<td>90</td>
<td>18</td>
</tr>
</tbody>
</table>

*Solid KOH

4.7. Attempts to synthesis Tryprostatin B

We then began pursuing the synthesis of tryprostatin using the procedure below.
We desired to accomplish three goals in our synthesis: 1) to make the synthesis of tryprostatin highly stream-lined 2) make the synthesis derivative tolerant and 3) make the synthesis more environmentally benign.

After producing the free amine we approached a variety of methods to couple the Fmoc-L-Pro to the Trp amine. One procedure we decided to pursue was Cook’s procedure using the Fmoc-LPro-Cl. \(^{36}\) Shown in Equation 39.
Another possible synthetic route to the desired product was to use a peptide coupling reagent such as PyBOP and couple the peptides together. We found that the peptide coupling reaction was our preferred method due to thionyl chlorides aggressive nature towards the equipment, especially the Tygon tubing.
Scheme 40. Dipeptide synthesis using peptide coupling reagent

After synthesis of the protected 2-isoprenyltryptophan fmoc proline was completed, it was deprotected using a procedure modeled after the one described in \textsuperscript{1d} for the deprotection of fmoc.
**Scheme 41.** Deprotection of Fmoc from dipeptide

This was successful in and the shown product was purified using column chromatography. This was reattempted using the procedure for fmoc deprotection utilizing piperidine instead of diethyl amine. This procedure formed the fmoc deprotection product as a solid, making it easier to purify, via filtration.\(^{37}\)

**Scheme 42.** Alternative method of Fmoc deprotection

After this synthesis of the tbutylester2-isoprenyltryptophanproline dipeptide was completed we evaluated a number of ways to close the diketopiperizine (DKP) ring. Initially we attempted the
ring closing diketopiperzine formation following the method used by the Cook\textsuperscript{1d} group which had successfully closed the ethyl ester of this compound. This reaction returned a burn charred material, without any promising NMR peaks to indicate the closing of the ring, such as the loss of the t-butyl protecting group in the proton or the change of the ester to the amide in the carbon NMR.

**Scheme 43. First attempt to close DKP ring**

Next we tried a slightly altered version using a more polar dimethylformide (DMF) solvent\textsuperscript{37} utilizing the polarity of the solvent to help stabilize the partial positive carbonyl carbon, in turn making it more susceptible to nucleophilic attack. Unfortunately this returned the starting material after the DMF was laboriously removed.

At this point we became concerned that the tert-butyl group may require much more energy to overcome the larger activation energy and thus act as a leaving group.
Next we tried using microwave synthesis to get the ring to close in a 20% piperidine/DMF mixture in the microwave. Reaction deprotected the fmoc protecting group but unfortunately did not close the DKP ring.

**Scheme 44.** Failed DKP ring closure without deprotecting Fmoc dipeptide

We then made an effort to try and remove the t-butyl group using lithium hydroxide using a similar procedure that had been reported to work for different ester deprotection reactions. This reaction either did not yield well or the material got stuck in the water layer. Acidification of this material did not result in it being organic soluble. Water was removed and the material did not appear to be there either.
Scheme 45. Attempt to remove t-butyl using lithium hydroxide

After this attempt we decided to pursue the deprotected t-butyl material using a different approach. This procedure used a refluxing 6 N hydrochloric acid (HCl) solution to remove the t-butyl group. This resulted in what appeared to be an acid charred material. This reaction mixture showed little promise via proton NMR due to a strong t-butyl peak.

Scheme 46. Attempt to remove t-butyl group using 6N HCl at reflux

Next we attempted to deprotect the t-butyl peak using a 5N HCl solution at room temperature in chloroform. This crude reaction mixture also showed a strong t-butyl ester presence.
Scheme 47. Attempt to remove t-butyl using 5N HCl at room temp

Next we tried to deprotect the t-butyl group by using phosphoric acid in dichloromethane. The proton NMR spectrum of this material indicated that the stubborn t-butyl group was still present.

Scheme 48. Attempt to remove t-butyl group using phosphoric acid at room temp
Convinced deprotection of the t-butyl group was not going be an effective process to achieve the DKP cyclization. We began pursuing other routes to close the diketopiperizing ring. After many frustrating failures at attempts to close the DKP ring we decided that it may be advantageous to use a model reaction to find a procedure that was capable of cyclizing the DKP ring. This route was pursued using a model reaction of the glycine Schiff base and Fmoc protected proline.

### 4.8. DKP ring closure by microwave in water

To begin this we synthesized the glycine Schiff on multigram scale.\(^4\)

**Scheme 49.** Model for DKP ring closure

After we found a procedure that was capable of closing the DKP ring we successfully applied this procedure to the synthesis of tryprostatin B.

**Scheme 50.** Total synthesis tryprostatin B
After completion of screening, synthesis of TPS A was pursued via the analogous scheme.
Now that we have shown the synthesis of tryprostatins is possible through our unpresidented procedure we would like to compare their activity against the past IC50 screenings to insure that
our synthesized compounds behave as the naturally isolated material. In the past evaluation of tryprostatin has been done using percent cell survival compared to the phase of the cells that survived.\textsuperscript{1a} Turbimetric assays have been used to determine the stabilization or destabilization of microtubules. Cell titer 96 AQueous from Promega has been used by the Cook group to measure the survival of cells in a solution of tryprostatin.\textsuperscript{1d} This microtiter plate has a MTS like compound in each of the wells this MTS is reduced by living cells to Formazan which has a purple/violet color to it; this color is used to determine the amount of living cells and lack of color to determine the amount of dead cells.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{MTS_formazan.png}
\caption{Reducing agents in live cells change Owen’s reagent into purple dye}
\end{figure}

Osada’s group presented data comparing a control, various compounds and tryprostatin A and B at 25 and 50 µM concentrations to observe the number of living cells after they were incubated and grown for 24 hours. They also observed the amount of DNA in the cells to predict what phase of the cell cycle the cells were in.\textsuperscript{1a,1b,1c}
Table 15. Shows chromosome content and percent cell survival, indicating tryprostatin A inhibits cell progression after chromosomes double in cell division

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (microM)</th>
<th>DNA content</th>
<th>2C</th>
<th>4C</th>
<th>4C</th>
<th>Cell number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>65.4</td>
<td>12.3</td>
<td>22.3</td>
<td></td>
<td>161.8</td>
</tr>
<tr>
<td>Stauroporine</td>
<td>0.02</td>
<td>76.9</td>
<td>8.5</td>
<td>11.1</td>
<td></td>
<td>143.1</td>
</tr>
<tr>
<td>Colchicine</td>
<td>1</td>
<td>9.4</td>
<td>11.5</td>
<td>65.9</td>
<td></td>
<td>69.4</td>
</tr>
<tr>
<td>TPS A</td>
<td>25</td>
<td>29.2</td>
<td>16.2</td>
<td>49.4</td>
<td></td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>9</td>
<td>15.2</td>
<td>70.5</td>
<td></td>
<td>68.7</td>
</tr>
<tr>
<td>TPS B</td>
<td>25</td>
<td>28.6</td>
<td>19.1</td>
<td>24.8</td>
<td></td>
<td>57.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>31.8</td>
<td>20.4</td>
<td>15.2</td>
<td></td>
<td>57.8</td>
</tr>
</tbody>
</table>

Exponentially growing 3Y1 cells were treated with various compounds for 24 h and the distribution of DNA content and relative cell numbers were determined. The cell number is the ratio of the number of cells at 24 h to that at 0 h expressed as a percentage. We plan to measure cell toxicity using a similar screening process.

We are interested to see if it would be possible to make the diisoprenylgramine salt apply the phase-transfer procedure to a closed DKP ring. Concerns with that procedure include the
glycine’s α-carbons proton may have reduced acidity than the starting material. The acidity may be increased by adding an ester group as shown below.

**Scheme 52.** Proposed alternative method to tryprostatins
5. CONCLUSION

5.1. Importance of tryptophan and asymmetric synthesis in medicinal chemistry
Tryptophan is a natural amino acid, famous for making people tired after thanksgiving, although that is myth. One of the most interesting things about tryptophan is its unique side chain. Like all amino acids, tryptophan has a carboxylic acid connected to an alpha carbon connected to an amine. From the twenty natural amino acids organisms can make a wide assortment of peptides and proteins.

The nitrogen-containing heterocycle that makes up the tryptophan side chain attached to its alpha carbon is called an indole. Indoles make up a large percent of neurotransmitters, prescription and recreational drugs. Due to unique characteristics of indoles, tryptophan derivatives have been at the center of extensive research for the last century.

Furthermore in the area of medicinal chemistry, the discovery that in many drugs one enantiomer shows high biological activity compared to the low biological activity or even harmful effects of its mirror image has sparked a great deal of interest in asymmetric synthesis. A classic example of the importance of enantiomeric selectivity is demonstrated in thalidomide. The R-isomer is active against morning sickness, while its S-enantiomer causes severe birth defects. The unfortunate administration of its racemic form caused tragic limb malformation in over ten thousand babies before it was pulled from the market. Later it was discovered that the R-isomer racemizes upon metabolism and was not suitable during pregnancy. Similarly, naproxen, a common pain reliever and anti-inflammatory is sold commercially as the optically pure s-enantiomer, as its
mirror image causes liver damage. The interest to synthesize enantio-pure tryptophan building blocks is a well-established concern for discovery of new medicines.

5.2. Our initial goals:
We set out to develop an asymmetric procedure that could make optically active tryptophans.

We were able to synthesize four ring-A substituted tryptophan derivatives in more than 90% ee.

Next we desired to find an application of our new synthesis. The 2-isoprenyl tryptophan moiety is an essential intermediate for the formation of tryprostatins. This intermediate has been present as an intermediate in every known synthesis of tryprostatins to date. The protecting groups vary from synthesis to synthesis, but the 2-isoprenyl tryptophan remains the most crucial synthetic intermediate.

In the first synthesis of tryprostatin\textsuperscript{3a} Danishefsky’s group used tributyl tin to couple boron dichloride-3-methyl-1-butene with an amino protected tryptophan to the indolic nitrogen, which then relied on a rearrangement to form the protected 2-isoprenyl tryptophan.

Alternatively in Cook’s group synthesis\textsuperscript{4} the isoprenyl group was added by using the Schöllkopf chiral auxiliary followed by LDA in THF and adding isoprenyl bromide, resulting in a protected 2-isoprenyl tryptophan.

Fukuyama’s group became interested in the synthesis due to their history of using radical chemistry to make indole rings.\textsuperscript{5} Fukuyama’s group built the indole ring and then
utilized the resulting protected tryptophan to make tryprostatin. The synthesis was achieved in ten steps in 39 percent yield.

Our next and most challenging goal was removing steps in the already known procedures of the established syntheses. We were able to reduce number of steps to six compared to the most current synthesis which is ten steps.

We attempted to achieve the 2-isoprenyl tryptophan using a similar approach to the Cook group approach, by making the protected tryptophan and then attempting the alkylation using a lithiating reagent.

The major isolated product had the isoprenyl group on the alpha carbon instead of the 2-position of the indole ring. To overcome this hurdle, we attempted to put the isoprenyl group on the C2-position using n-butyl lithium and isoprenyl bromide before doing the phase-transfer reaction to make tryptophan. When attempting this synthesis with one equivalent of isoprenyl bromide only the graminic isoprenyl ammonium bromide was formed. After this attempt we tried this with 2.25 eq of n-butyl lithium and an excess of isoprenyl bromide, we were able to isolate the 2-isoprenyl quaternary ammonium graminic nitrogen bromide salt. This molecule was able to proceed through the phase-transfer reaction as expected to make C2-isoprenyl tryptophan but with low enantiomeric excess.
Our procedure is one step shorter than Danishefsky’s, one step shorter than Cook’s (without including the synthesis of Schöllkopf chiral auxiliary which is three steps) and three steps shorter than Fukuyama’s ten step synthesis which utilizes V-70 as a radical initiator. Danishefsky’s procedure is seven steps and has not been shown to work for the 6-methoxy indole, which leads to tryprostatin A.

This synthesis also starts from L-tryptophan. It is well known that 6-methoxy tryptophan is difficult to obtain and very expensive. Economics is likely the reason this procedure has not been used to make tryprostatin A. To further support this claim, all the known tryprostatin A syntheses start from smaller building blocks than tryptophan. Although Danishefsky's synthesis is elegant in its simplicity, what it lacks is tolerance to ring-A substitution, which seriously limits its synthetic scope.

### 5.3. Utilization asymmetric synthesis to make natural products tryprostatin A and B

We then moved on and attempted to synthesize two known natural products, tryprostatins A and B. These goals were also accomplished. At this point we are able to obtain enatiomeric excesses in the 50-60 % range. Although this is not as high as we have reported for the phase-transfer catalyzed reactions without the isoprenyl group on the 2 position of the indole ring, it does leave quite a bit of room for improvement. The problem may be that the 2-isoprenyl group acts as a steric blocker and limits the amount of enantioselective alkylation. It has been reported that sterically congested substrates yield lower %ee than their uncongested counterparts. Our unprecedented PTC still may have the potential to yield high enantiomeric excess with further screening during the crucial chiral center forming step.
5.4. The future plans of this project:
In the future, the Hossain group is expected to publish the shortest known synthesis of tryprostatins A and B. Furthermore, they are expected to use the method developed in our lab to make a variety of tryprostatins with ring-A substitutions and screen them in MTT assays to test cell viability. If any of the newly synthesized compounds have higher cell toxicity than the previously known tryprostatins, the compounds will be tested further, along the lines of mechanism of action, target protein interaction, concentration of toxicity and selectivity for cancer cells. Through Milwaukee Institute Drug Discovery (MIDD) and Open Innovation Drug Design (OIDD) Lilly has expressed interest in screening the new tryprostatin targets.

We have shown that we can make ring-A substituted tryprostatins using our unprecedented shortest known synthesis. We have demonstrated that this synthesis is shorter and more tolerant than any of its predecessors.

It would be an effective use of time and energy to synthesize the material in a non-asymmetric fashion then separate each diastereomer and measure its biological activity. Another interesting idea would be to make the 2-isoprenyl salt and see if it would be possible to couple the diketopiperazine ring directly.

Lastly, it is important to identify the problem we are having with the asymmetry of this reaction. One plausible reason that the 2-isoprenyl gramine salt may not undergo the phase-transfer reaction as well as the 4-trifluoromethoxybenzyl salt may be caused simply by the added steric hindrance of the C2-isoprenyl on the indole ring.

A good way to clarify if the 2-isoprenyl group were acting as a steric blocker would be to make the same isoprenyl ammonium salt without the isoprenyl indole C2-position and see if the phase-transfer reaction yields a high enantiomers excess.
6. EXPERIMENTAL SECTION

General Procedure

All procedures were performed under a dry nitrogen atmosphere using standard Schlenk techniques unless otherwise noted. All reaction vessels were flame dried under vacuum and filled with nitrogen prior to use. Reagents were purchased from Aldrich Chemicals and used as is. Flash chromatography was performed using EM Science F_{254} silica gel 60. N-(diphenylmethylene) glycine tert-butyl ester, sodium hydroxide, phase transfer catalysts and anhydrous sodium sulfate were purchased from Aldrich. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane. CDCl_{3} was used as the solvent. Previously ¹H NMR or GC identified reported compounds. All new compounds were additionally characterized by ¹H NMR, ¹³C NMR and GCMS.

Hexanes a mixture of isomers was purchased Aldrich in 200 L drums this solvent was similar to petroleum ether in Purification of Laboratory Chemicals 2nd Edition Perrin page 375. Hexane 4 L was stirred over 75 mL of conc. H_{2}SO_{4} for 24 to 48 hours. 500 mL of this was put into a 1 L separatory funnel along with 50 mL of 10 % H_{2}SO_{4} (10 mL H_{2}SO_{4} 90 mL water) and 50 mL of 1% KnMnO_{4} (1 g KMnO_{4} in 99 mL of water 0.0063 M) and shaken. (To remove unsaturated, including aromatic, hydrocarbons) until permanganate color persists. Wash with water (50 mL), aq. Na_{2}CO_{3} (sat. 50 mL) and again with water (50 mL). Dried over Na_{2}SO_{4}, and distilled over phosphorus pentoxide.

5.2. Instrumentation

All ¹H (300 MHz), and ¹³C (75.5 MHz) NMRs were performed with a Burker 300 and samples dissolve in CDCl_{3} unless otherwise noted. Enantioselectivity was obtained via chiral HPLC using a
Waters setup including an Inline Degasser AF, 2998 Photodiode Array Detector, 1525 Binary HPLC Pump equipped with Breeze Software. This was equipped with a Chiralcel OD (column no. OD00CE-FF071) column using hexane and isopropanol at 254nm and a broad range channel from 200-600nm column temperature was room temperature flow rate was 1 mL/min unless otherwise stated. HPLC grade solvents were used in all HPLC analysis.

**Synthetic Procedure for Amine Ester Protected Tryptophan**

**Scheme 53.** Synthesis of protected tryptophan

Gramine (.15 g 0.862 mmol 1 eq) was dissolved in a solution of dichloromethane (6 mL) 4-(Trifloromethoxy)-benzyl bromide (0.15 mL 0.938 mmol 1.1 eq) was added forming a solid
precipitate. To this mixture O- Allyl-N-(9-anthracenyl-methyl)cinchonidinium bromide(0.100 g 0.165 mmol 0.193 eq), N-(Diphenylmethylene)-glycine tert-butyl ester( 0.265 g 0.897 mmol 1.05 eq), and 45% potassium hydroxide in water (2 mL) was added to the mixture and allowed to stir until the solution became clear and two layers could be seen. Layers were separated and the organic layer was washed three times with water then dried over sodium sulfate. Then compound was purified by column chromatography using 10% ethyl acetate and 90% pentane.

**Synthetic Procedure for N Boc Protected Tryptophan**

**Scheme 54.** Boc protection of tryptophan

![Chemical structure of Boc protection of tryptophan](image)

Tryptophan N-diphenylmethyamine t-butyyl ester (0.745g 1.755 mmol 1 eq) was dissolved in acetonitrile a catalytic amount of DMAP (0.043 g 0.35 mmol 0.2 eq) was added along with di tert-butyl dicarbonate (0.575g 02.63 mmol 1.5 eq) and stirred for 24 hours. Product was purified using column chromatography with 10% ethyl acetate and 90% pentane.

**Synthetic procedure for N Boc Gramine**

**Scheme 55.** Boc protection of gramine
This reaction was modeled after a very similar reaction discussed in Tetrahedron 55 (1999) 10989-11000, compound 8 to 9. A solution of gramine (3.7 g 21 mmol 1 eq.) in THF (90 mL) was made. This solution was put into an addition funnel on a 250 mL three necked reaction vessel in an ice water cooling bath and added dropwise to a stirred solution of di-t-butyl dicarbonate (5.50 g 25 mmol 1.2 eq.), 4-(dimethylamino)pyridine (257 mg, 2.1 mmol 0.1 eq.), triethylamine (3.5 mL, 2.5 mmol, 0.12 eq.) in THF (50 mL). After stirring for 1.5 hours at room temperature, water (50) mL was added to the reaction mixture and the solvent was removed via roto vap. The organic layer was separated and the aqueous layer was extracted twice with ether (50 mL). The combined extract was washed three times with water and then with brine solution and dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel using (could probably use ethyl acetate alone as mobile phase) hexane:ethyl acetate (1:2) as an eluent to give 5.18 g of Boc Gramine product in 90.0% yield. Theoretical yield is 5.75 g.

Apparatus set up shown below.
Figure 30. Boc protection of gramine

Synthetic procedure for N+ diisoprenyl bromide salt of gramine

Scheme 56. Diisoprenylation of Boc gramine

5.0 g (18.2 mmol, 1 eq.) Boc Gramine was weighed out in a beaker. 500 mL three-neck with thermometer adapter round bottom flask was oven dried for 3 hours with stir bar inside. It was removed from the oven and clamped. On one neck rubber septum was inserted, in the other nitrogen outlet was inserted, at this point the boc gramine was charged to the flask via a powder funnel, and in the last neck nitrogen inlet was inserted, as quickly as possible. To the reaction
vessel blue distilled THF (245) mL was charged via syringe. The reaction mixture was allowed to 
stir for 1 hour to insure that all of the starting material was dissolved in the solution. At this 
point solution is orange/peach in color. Reaction vessel was cooled in a dry ice/acetone bath 
until reaction was -70 °C. At this time n-butyl lithium (14.58 mL 2.5 M 36.44 mmol 2.0 eq.) was 
added dropwise to the reaction vessel via a syringe over 1 hour, maintaining a temperature 
range between -65 and -70 °C. At this point the reaction is bright red/orange. After addition of 
n-butyl lithium reaction was let stir undisturbed for 1 hour and 30 minutes at -70 °C. Isoprenyl 
bromide (9.4 mL 81.99 mmol 4.5 eq.) was added to the reaction dropwise. After addition of 
isoprenyl bromide the color of the reaction mixture is orange. At this point the reaction was left 
to warm overnight. When returning the next day color of the reaction mixture was clear 
orange. Deionized water (5 mL) was added to the reaction vessel, no reaction indicated that the 
n-butyl lithium was quenched. At this point the solvent was removed using the roto vap. After 
organic solvent was removed the water and residue was poured into a separatory funnel and 
extracted with dichloromethane three times (50 mL). Organic layer was dried over sodium 
sulfate. Solvent was removed via roto vap and high vac with cold finger. (Purify immediately or 
freeze left on bench it turns an undesirable brown/black heat from the roto vap bath may also 
be the cause) Residue was purified using flash chromatography (10 x 6 cm silica gel) eluent was 
5% methanol: 95% dichloromethane to provide a light brown solid 6.14 g in 69% yield. See TLC 
plate developed in 9:1 Dichloromethane:Methanol observed with short range UV lamp and 
stained with ninhydrin stain and heated on a hot plate until colored. Spot with Rf of 0.5 is 
product and has a purple/violet color when the TLC plate is developed in the ninhydrin stain. 
Recrystallization solvents that have been tried, material is soluble in ethanol, material with 
water and heat forms a white cloudy solution, attempts at purification by recrystallization has 
failed.
Figure 31. Diisoprenylation of Boc gramine
Synthesis of 2-isoprenyl-N-diphenylmethylene-t-butylerstertryptophan

Scheme 57. Phase-transfer catalyst using diisoprenylated quaternary ammonium bromide salt

N-isoprenyl-2-isoprenylbocgramine (2.00 g, 4.069 mmol, 1 eq.) N-(diphenylmethylene) glycine tert-butyl ester (1.202 g, 4.069 mmol) and O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (0.4961 g 0.8192 mmol, 0.2 eq) dissolved in acetonitrile (30 mL) in a 250 mL round bottom flask with a stir bar. Reaction mixture was allowed to stir for 30 minutes. At this point the reaction mixture is a dark brown color with a light yellow precipitate that appears to be the phase transfer catalyst. At this point 20 mL 45% KOH solution was added to the reaction mixture and allowed to stir, the aqueous layer was on the bottom and was clear and light yellow and the organic layer was dark brown and on the top. Reaction was allowed to stir for 20 hours; at this point the reaction has a dark layer on top and a clear orange layer on bottom. A small
sample was pulled from the reaction vessel, dissolved in CDCl₃ and taken to the 300 NMR where I looked for the singlet at 4.1 representing the CH₂ peak from the glycinate, disappearance of this peak indicates that the reaction has gone to completion. From previous attempts at this experiment if the singlet at 4.1 remains add more KOH and let the reaction continue. After confirmation that the reaction has gone to completion, solvent was removed from the reaction by rotovap leaving water and an orange residue on top of the water. Dichloromethane (3x 50 mL) was added to the reaction vessel this solution was put into a separatory funnel with 50 mL deionized water diluting the water layer enough that the density becomes less than the dichloromethane. Organic layer was collected and dried over sodium sulfate. Solvent was removed weight of this portion is 2.9158 g. Thin layer chromatography (TLC) was used to identify a solvent system for column chromatography, TLC indicated that mobile phase for the column should be 9:1 Hexane:Ethyl Acetate. Column used was 9 cm tall by 6 cm wide, isolating 1.21 g of product in 60.5 % yield.

![Figure 33. Structure of glycine Schiff base](image-url)
Figure 34. NMR of Glycine Schiff base
Figure 35. Structure of protected tryptophan

Figure 36. NMR of protected tryptophan
Figure 37. Structure of diisoprenylated quaternary ammonium bromide salt

\[
\text{C}_{26}\text{H}_{39}\text{N}_{2}\text{O}_{2}^+ \\
\text{Mol. Wt.: 411.6}
\]

Figure 38. NMR of diisoprenylated quaternary ammonium bromide salt
Screening of %ee N+ isoprenyl salt

**Scheme 58.** Phase-transfer catalyst screening procedure

(0.1 g 0.203 mmol 1 eq.) of N+ salt was added to a 7.5 mL vial with a mini stir bar. To this (0.07 g 0.236 mmol 1.16 eq) of Schiff base and (0.03 g 0.04954 mmol 0.2440 eq) of phase transfer catalyst was added. To the reaction mixture 2 mL of solvent was added and reaction mixture was let stir 30 min. 1 mL of 45% KOH was added to the reaction vessel. This was let stir for 18 hours. Crude reaction mixture was run through a short silica plug using 25 mL of 20 % Ethyl Acetate and Hexane. Percent conversion was monitored via proton NMR of organic layer by comparing the integration of the multiplet at 4.2 and the singlet at 4.1.
Synthesis of 2-Isoprenylt-N-aminebutylestertryptophan

Scheme 59. Deprotection of tryptophan amine

Procedure adapted from Journal of Organic Chemistry Vol. 68, No. 11, 2003. 2-isoprenyl-N-diphenylmethylene-t-butylestertryptophan (1.04 g 2.111 mol) was dissolved in THF (12.66 mL) reaction mixture is clear and orange in color. To the reaction mixture 1 N HCl was added, upon addition of the HCl color changed from clear orange to dark red, and the reaction mixture was allowed to stir 2 hours. The reaction was monitored by TLC (mobile phase 1:2 Hexane:Ethyl Acetate) after 2 hours small amount of starting material in reaction mixture on the TLC indicated the reaction was not complete. Reaction was let stir overnight for 16 hours. TLC at this time indicated no starting material present in the reaction mixture. The resulting mixture was washed with hexanes (2 x 43 mL) and then the aqueous phase was basified with solid sodium bicarbonate and extracted with dichloromethane (4 x 50 mL). Dichloromethane extracts were dried over sodium sulfate and concentrated under reduced pressure (yielding 0.30 g of material, 43.3%). NMR of this product indicated the product was present but contained minor impurities. Reaction mixture was further purified by column chromatography 9:1 Dichloromethane:Methanol.
Also there is similar chemistry in *Organic Letters* 2010 Vol. 12, No. 8 1688-1691 Supporting documents S-13. 2-isoprenyl-N-diphenylmethylenet-butylestertryptophan simi pure was dissolved in THF (50 mL) and 1 N HCl (50 mL) was added at 0 °C. After stirred for 4 hours, THF was removed under reduced pressure. The resulting solution was washed with ether (3 x 25 mL) and aqueous layer was neutralized with NaHCO₃. The mixture was then extracted with CH₂Cl₂ (3 x 50 mL). The ether and dichloromethane layers were dried over anhydrous Na₂SO₄. Ether layer contained benzophenone and dichloromethane layer contained amine. After filtration and concentration under reduced pressure, the product was obtained in % yield after purification by flash column chromatography using mixtures of CH₂Cl₂/MeOH (50:1) as eluent. Also Ethyl Acetate:Hexane 3:7 may be used. Material has an Rf value of 0.4 to 0.5. This is especially useful if the PTC has not been removed previously.

**Synthesis of 2-isoprenyl tryptophan t-butyl ester hydrochloride amine**

**Scheme 60.** Attempted isolation of ammonium chloride salt

Also there is similar chemistry in *Organic Letters* 2010 Vol. 12, No. 8 1688-1691 Supporting documents S-13. 2-isoprenyl-N-diphenylmethylenet-butylestertryptophan simi pure (0.61 g
1.24 mmol 1 eq.) was dissolved in THF (20 mL) and 1 N HCl (20 mL) was added at 0 °C. After stirred for 4 hours, THF was removed under reduced pressure. The resulting solution was washed with ether (3 x 50 mL) and aqueous layer roto vaped to dryness. NMR of this aqueous layer was taken in d₂O ether extraction taken in CDCl₃. Aqueous layer mass 0.15 g and organic layers mass is 0.67 g. Aqueous layer did not agree with product spectrum, organic layer looked like benzophenone and the amine.

**Synthesis of Fmoc Proline Acid Chloride**

**Scheme 61.** Synthesis of Fmoc proline acid chloride

Fmoc-L-proline (1.53 g 3.01 mmol 1 eq) was dissolved in thionyl chloride (12.09 mL). The solution which resulted was stirred overnight at rt. Excess thionyl chloride was removed under reduced pressure, yielding 1.70 g of white yellow solid.
Synthesis of 2-isoprenyltryptophan t-butylester fmoc proline

Scheme 62. Synthesis of dipeptide using acid chloride

This procedure was done following the procedure for a similar compound in *H.D. Jain et al* Bioorg. Med. Chem. 16 2008 4626-4651. Fmoc-L-proline chloride (1.70 g 4.78 mol 1.59 eq) which resulted was dissolved in dry CHCl₃ (12.09 mL). This solution was added dropwise at 0° C to a solution of 2-isoprenyltryptophan-butylamine (0.99 g 3.01 mmol 1 eq) and triethylamine (0.762 g 0.00726mol 1.05 mL 2.5 eq) in dry CHCl₃ (72.5 mL). The mixture that resulted was stirred at 0 °C for 0.5 hr and then at rt overnight. Solvent was remove under rotovap and oil pump to remove solvent producing 3.78 g of orange solid in 194 % yield purity determined by NMR in CDCl₃.
Deprotection of the Fmoc protecting group on the 2-isoprenyltryptophan-t-butylester-fmocproline

Scheme 63. Deprotection of Fmoc

This procedure was done following the procedure for a similar compound in H.D. Jain et al Bioorg. Med. Chem. 16 2008 4626-4651. Crude solid material from the acid chloride reaction (see above) was dissolved in Acetonitrile (7.75 mL) and stirred via a stir bar until it made a homogeneous solution. To this solution Diethyl amine (7.75 mL) was added dropwise to the reaction flask using an addition funnel. The reaction was let stir overnight and progress was monitored by TLC. Mobile Phase is 9:1 Dichloromethane:Methanol. Fraction 1 is Flourne amine Fmoc deprotection side product Rf = 0.9. Fraction 2 and 3 are undistinguishable by NMR unknowns Rf = 0.55-0.50. Fraction 4 and 5 are product Rf = 0.50. Fraction 6 and 7 are Proline derivatives Rf = 0.35. It is highly suggested to use the smallest collection vessels possible to collect fractions from Rf 0.6-0.4.
Figure 39. TLC of Fmoc deprotection

Synthesis of Fmoc Proline Gycine ethyl ester

Scheme 64. Peptide coupling of proline and ethyl ester glycine

Following procedure found in V.L. Campo et al. Tetrahedron 65 (2009) 5343. Also procedure in Org. Lett. 2011 vol. 13, No. 24 6334. To a solution of FmocProline-OH (1.00g 2.96 mmol 1 eq)
in Dichloromethane (20 mL) at room temp, PyBOP (1.85 g 3.55 mole 1.2 eq) and Diisopropyl ethyl amine (DIEA) (1.15 g 8.90 mmol 3 eq) were added. The reaction mixture was stirred for 10 min before the glycineethylester HCl (0.413 g 2.96 mmol 1 eq) was added to the reaction vessel. The reaction mixture was stirred overnight and concentrated on the rotovap and oil pump. The crude material was purified by column chromatography using Hexane/EtOAc (7:3) to give FmocProlineGlycineethylester. Material eluted with the byproduct of the coupling reagent, this gave an NMR that appeared to have THF like spectra. It was not purified further.

**Synthesis of diketopiperazine**

**Scheme 65.** Attempted DKP ring closure in DMF

![Scheme 65](image)

Procedure adapted from V.L. Campo et al. Tetrahedron 65 (2009) 5343-5349. The protected dipeptide (0.25 g 0.555 mmol 1 eq.) was treated with 20% piperidine 0.32 mL /DMF 1.28 mL (6 eq.) and allowed to stir at room temperature for 18 hours. After concentration in vacuo, the residue was purified by column chromatography [EtOAc/hexane 1:1v/v, DCM/MeOH 9:1 v/v).
Synthesis of 2-isoprenyl tryptophan ethyl ester benzophenone imine

Scheme 66. Phase-transfer catalyst reaction using ethyl ester glycine

2-isoprenyl Indole N+dimethyl isoprenyl bromide salt (0.1 g 0.2034 mmol 1 eq) N-(diphenylmethylene) glycine ethyl ester (0.05438 g 0.2034 mmol 1 eq) and O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (0.02464 g 0.04069 mmol 0.2 eq) was dissolved in solvent (2 mL) in a 5 mL vial round bottom flask with stir bar stirring. Reaction mixture was allowed to stir for 30 minutes. At this point the reaction mixture is a dark brown color with a light yellow precipitate that appears to be the phase transfer catalyst. TLC plate was taken of starters and crude mixture (see bleow). TLCs were exposed to both cerium (IV) ammonium sulfate stain for alkaloids where the N+ salt turned red no effect on the other spots. Ninhydrin stain was also used on TLC plate and two spots were sensitive to it, N+ salt purple, Schiff base is pink, and PTC shows no reaction. At this point 1 mL 45% KOH was added to the reaction mixture and allowed to stir, the aqueous layer was on the bottom and was clear and light yellow and the organic layer was dark brown and on the top. After about 10 minutes the organic layer turned from brown to dark red. After 30 min the KOH layer was removed and replaced and the
reaction was continued to stir. Shortly after this time color of organic layer was red and clear.

Reaction was allowed to stir for 20 hours; at this point the reaction has a dark layer on top and a clear orange/yellow layer on bottom. At this point the crude reaction mixture was monitored by TLC. After 20 hours TLC was run Cerium (IV) ammonium sulfate stain indicates presence of indole in lane 4, ninhydrin stain indicated very weak signal for amines in lane 4. NMR was taken of crude reaction mixture, which suggested that the reaction had gone at least to some extent to product. Gradient column was run on the reaction mixture using Hexane:EtOAc 9:1, 8:2, 7:3 in 100 mL portions product appears to be purple on TLC in high concentrations.

Figure 40. TLC of reagents and crude reaction mixture
Synthesis of tert-butyl N-(Diphenylmethylene)glycinate

**Scheme 67. Synthesis of glicine schiff base**


A solution of tert-butyl 2-bromoacetate (5.8 mL 7.7 g, 39.5 mmol 1 eq) in acetonitrile (44 mL) was treated with benzophenonimine (6.6 mL 7.1 g, 39.3 mmol 1 eq) and diisopropylethylamine (6.8 mL, 5.0 g, 35.0 mmol 0.90 eq), and the mixture was then heated at reflux for 12 hours.

After the system had cooled to room temperature, most of the acetonitrile was removed in vacuo. The residue was partitioned between water (40 mL) and diethyl ether (60 mL) and the phases were separated. The organic layer was dried with Na$_2$SO$_4$, filtered and concentrated in vacuo until the mixture became turbid. Crystallization was done using ethanol/petroleum ether (in our case we substituted hexane for petroleum ether) 1:4. The yield was 86 % slightly yellow solid.
**Figure 41.** Apparatus for synthesis of glycine schiff base

**Synthesis of 2-Isoprenyl-N-aminebutylestertryptophan**

**Scheme 68.** Deprotection of amine using 1 N HCl

![Scheme 68](image.png)

Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691. Crude reaction mixture forming 2-isoprenyl-N-diphenylmethylenet-butylestertryptophan (100.0 mg 0.24 mmol) was dissolved in THF (2 mL) and 1 N HCl (2 mL) was added at 0 °C. After stirred for 4 hours, THF was
removed under reduced pressure. The resulting aqueous solution was washed with ether (3 x 20 mL) and neutralized with NaHCO₃. The mixture was then extracted with CH₂CCl₂ (3 x 20 mL). The organic layers were combined and dried over anhydrous Na₂SO₄. After filtration and concentration under reduced pressure, the product was obtained after purification by flash column chromatography using gradient mixtures of CH₂CCl₂/MeOH (98:2 95:5 90:10) as the eluent.

Scheme 69. Attempted deprotection of t-butyl group

\[
\begin{align*}
\text{C}_{28}\text{H}_{35}\text{N}_3\text{O}_3 & \quad \text{Exact Mass: } 425.27 \\
\text{Mol. Wt.: } 425.56 \\
\text{THF: } \text{H}_2\text{O}, 4:1 & \quad \text{LiOH } 25 \text{ eq} \\
\Delta 50^\circ \text{C} & \\
\text{C}_{21}\text{H}_{26}\text{LiN}_3\text{O}_3 & \quad \text{Exact Mass: } 375.21 \\
\text{Mol. Wt.: } 375.39
\end{align*}
\]

tert-butyl 3-{2-(3-methylbut-2-enyl)-1H-indol-3-yl}-2-{pyrrolidine-2-carboxamido}propanoate (0.01 g 0.234 mmol 1 eq) in a round bottom flask. 4 mL of tetrahydrofuran was added to the reaction vessel along with 1 mL of water. To this Lithium hydroxide (0.14 g 5.87 mmol 25 eq) were added as a solid. This reaction was heated to and let stir. Reaction was monitored by TLC and cerium ammonium sulfate (indole stain) using a 9:1 Dichloromethane:Methanol mobile phase until the stained spot changed Rf value from 0.5 to 0.1. At this point the reaction was let cool then it was put on the roto vap to remove tetrahydrofuran and water. To this deionized water was added to the reaction vessel and pH was taken indicating the mixture was strongly basic. KHSO₄ was added as a solid until the reaction mixture indicated a pH in between 2 and 3. At this point dichloromethane was added to the reaction mixture, and then the mixture was
poured into a separatory funnel. Organic layer was removed and dried over Na$_2$SO$_4$. Solvent was removed via roto vap and high vac on oil pump. NMR was taken of the material in the organic layer this did not appear to be the product.

**Synthesis of 2-isoprenyl tryptophan hydrochloride**

**Scheme 70.** Attempted deprotection of t-butyl group

Procedure adapted from a similar reaction discussed in in Organic Letters 2010 Vol. 12, No.8 1688-1691 N diphenylmethylenet-butyl ester 2-isoprenyl tryptophan (0.18 g 0.3659 mmol 1 eq.) in a round bottom flask was put into a was heated to reflux with stirring in 6 M HCl (10 mL) under an N$_2$ atmosphere for 24 hours. After it was cooled, the reaction mixture was washed successively with CH$_2$Cl$_2$ (2 x 5 mL) and ether (2 x 5 mL) before concentration to dryness under vacuum and in 5 mL methanol. Organic layer was evaluated with proton NMR this indicated the material was benzophenone. Aqueous layer was evaporated and evaluated via proton NMR which did not appear to be the product.
Figure 42. Charred reaction product

Synthesis of 2-isoprenyl tryptophan hydrochloride

Scheme 71. Attempted deprotection of t-butyl group using 5 N HCl

Procedure adapted from a similar reaction in J. Org. Chem., Vol. 62, No. 12, 1997. To a round bottom flask 2-isoprenylN-diphenylmethylenet-butylester tryptophan (.05 g 0.0101 mmol 1 eq.),
0.1 mL 5N HCl and 1 mL of CHCl₃ was added. The reaction was stirred at room temperature for 4 hours until tlc showed disappearance of starting material. The CHCl₃ was then removed, the aqueous layer was washed with CHCl₃ (3 x 2.5 mL) and then separated, and the solvent was evaporated to give 2-isoprenylN-diphenylmethylenet-butylester tryptophan. Reaction did not appear to deprotect t-butyl group.

**Synthesis of 2-isoprenyl proline tryptophan**

**Scheme 72.** Attempted synthesis of dipeptide proline salt

Procedure adapted from J. Org. Chem., Vol. 62, No. 12, 1997. 2-isoprenyl t-butyl ester tryptophan proline was dissolved in 4 mL of CHCl₃ to this a 0.25 mL of 4 N HCl was added and reaction was stirred for 7 hours TLC plate at this time indicated very little change. Half of this reaction was worked up at this point, remaining reaction mixture was heated to 45 °C for an hour then the reaction mixture was let cool to room temperature and mixture was stirred for 16 hours. Both first half and second half were worked up in the same way organic layer was washed with water and then dried over Na₂SO₄. NMRs were taken of the samples both looked like they contained the t-butyl ester peak.
**Synthesis of 2-isoprenyl proline tryptophan**

**Scheme 73.** Attempted deprotection of t-butyl group

![Chemical structure](image)

Reaction adapted from J. Org. Chem., Vol. 71, No. 24, 2006 4675. 70 mg of 2-isoprenyl t-butyl ester tryptophan proline dissolved in 1 mL of dichloromethane and stirred. To this 0.1 mL of phosphoric acid 85 wt % was added dropwise vial syringe dropwise. The reaction was stirred for 14 hours. NMR of material indicated t-butyl group was still present.

**Synthesis of t-butyl glycinate**

**Scheme 74.** Deprotection of Glycine Schiff base

![Chemical structure](image)
Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691. N-diphenylmethylene-t-butylestertryptophan (0.5 g 1.69 mmol 1 eq) dissolved in 10.5 mL THF along with 10.5 mL of 1 N HCl solution. Reaction mixture was stirred at room temperature for 1 hour. THF was rotovaped off. Reaction mixture was washed with hexane three times; this layer was dried over Na₂SO₄ and rotovaped to dryness. NMR indicates this is benzophenone. Aqueous layer was basified using sodium bicarbonate, until adding solid gave no more bubbles. Aqueous layer was extracted with dichloromethane three times. Dichloromethane layer was dried over Na₂SO₄ and rotovaped to dryness.

**Synthesis of t–butyl glycinate**

**Scheme 75.** Deprotection of glycine Schiff base using 15% citric acid

3-26-13 Procedure adapted from Tetrahedron Letters 43 (2002) 6677-6679. Glycine imine (1.4 g 4.7 mmol 1 eq.) was dissolved in (23.3 mL) of tetrahydrofuran and (8.7 mL) of 15% aqueous citric acid. Reaction mixture was stirred vigorously at room temperature for 18 h, and then diluted with 1 M hydrochloric acid (5.83 mL). THF remove via rotovap. The mixture is extracted with diethyl ether (2x11.66 mL) to remove the benzophenone, then the aqueous layer was basified (K₂CO₃) until no more K₂CO₃ would dissolve. Extraction with chloroform (5x17.5 mL) followed by drying of the extracts (Na₂SO₄) and concentration under reduced pressure gives the
crude amino acid tert-butyl ester which can generally be purified by passing through a plug of silica. Amine looked clean. 96 % Yield 0.6 g theoretical was 0.62 g.

**Synthesis of Fmoc-Proline glycine t-butylester dipeptide**

**Scheme 76.** Synthesis of glycine proline using acid chloride

This procedure was done following the procedure for a similar compound in *H.D. Jain et al Bioorg. Med. Chem. 16 2008 4626-4651*. Fmoc-L-proline chloride (1.71 g 0.00481 mol 1.85 eq) which resulted was dissolved in dry CHCl₃ (12.09 mL). This solution was added dropwise at 0° C to a solution of t-butyl ester glycine amine (0.34 g 0.00259 mol 1eq) and triethylamine (0.0.655 g 0.00655 mol 0.90 mL 2.5 eq) in dry CHCl₃ (72.5 mL). The mixture that resulted was stirred at 0 °C for 0.5 hr and then at rt overnight. Solvent was remove under rotovap and oil pump to remove solvent producing 2.54 g of solid in 218% yield purity determined by NMR in CDCl₃. Theoretical yield was 1.16 g. NMR showed two large peaks at ca. 3.0 and 1.5 ppm.
Synthesis of t-butyl glycine proline

Scheme 77. Deprotection of dipeptide

Reaction was modeled after H. D. Jain Bioorg. Med. Chem. 16 2008 4626-4651. 0.5 g of the t-butyl glycine fmoc proline was dissolved in acetonitrile (10 mL) and diethylamine (10 mL). The reaction mixture was stirred for two hours at room temperature.

Synthesis of benzophenone imine glycine ethyl ester

Scheme 78. Synthesis of ethyl ester glycine Schiff base

Procedure from Chem. Eur. J. 2010, 16, 1153-1157. A mixture of the corresponding benzophenone NH-imine (0.181 g 0.167 mL mmol 1 eq. 1.08 g/mL), amino acid ester hydrochloride (0.153 g 1.1 mmol 1.1 eq) and MgSO₄ (0.181 g 1.5 mmol 1.5 eq) were stirred in
dichloromethane (10 mL) at room temperature for 24 hours. The reaction was filtered and the filtrate was washed with water and brine, and dried over MgSO₄. Filtration and solvent removal afford α-ketiminoesters which were used without further purification. Theoretical yield 0.267 g.

**Synthesis of Fmoc proline glycine ethyl ester**

**Scheme 79.** Peptide coupling of proline and glycine ethyl ester

4-10-13 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (2.09 g 4.02 mmol 1.36 eq) and i-Pr₂Net (0.8682 g 1.17 mL 6.72 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of proline (1.36 g 3.93 mmol 1.33 eq) and ethyl ester glycine (0.412 g 2.95 mmol 1 eq) in CH₃CN (30 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH₂Cl₂ (100 mL) and 1 M HCl (100 mL). The layers were separated,
and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 25 mL). The combined organic layers were dried (MgSO$_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with 60 % EtOAc/Hexane to give 1.18 g (95 % yield) of dipeptide as clear viscous oil.

**Synthesis of 2-isoprenyl tryptophan ethyl ester**

**Scheme 80.** Phase-transfer catalyst with glycine ethyl ester

4-10-13 2-isoprenyl Indole N+dimethyl isoprenyl bromide salt (1.0 g 0.002034 mol 1 eq) N-(diphenylmethylene) glycine ethyl ester (0.5438 g 0.002034 mol 1 eq) and O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (0.2464 g 0.4069 mmol 0.2 eq) was dissolved in 1,4 dioxane (20 mL) in a round bottom flask with stir bar stirring. Reaction mixture was allowed to stir for 30 minutes. At this point the reaction mixture is a dark brown color with a light yellow precipitate that appears to be the phase transfer catalyst. At this point 20 mL 45% KOH was added to the reaction mixture and allowed to stir, the aqueous layer was on the bottom and
was clear and light yellow and the organic layer was dark brown and on the top. After about 10 minutes the organic layer turned from brown to dark red. Shortly after this time color of organic layer was red and clear. Reaction was allowed to stir for 20 hours; at this point the reaction has a dark layer on top and a clear orange/yellow layer on bottom. At this point the crude reaction mixture was monitored by TLC. After 20 hours TLC was run Cerium (IV) ammonium sulfate stain indicates presence of indole in lane 4, ninhydrin stain indicated very weak signal for amines in lane 4. NMR was taken of crude reaction mixture, which suggested that the reaction had gone at least to some extent to product. Gradient column was run on the reaction mixture using Hexane:EtOAc 9:1, 8:2, 7:3 in 100 mL portions product appears to be purple on TLC in high concentrations.

**Synthesis of diketopiperazine**

**Scheme 81.** Attempted DKP ring closure

---

Procedure adapted from Org. Lett., Vol. 15 No.1, 2013 pg. 22-25 Procedure on S21 of supporting documents. 1.34 times scale. Et₃N (2.80 g 3.86 mL 0.7255 g/mL 10 eq.) and 2-hydroxypyridine
(0.058 g 0.615 mmol 0.22 eq.) were added to a solution of dipeptide (1.18 g 2.79 mmol 1 eq.) in CH$_3$CN (53.6 mL) and the reaction was heated under reflux for 21 hrs. The reaction was cooled to room temperature and then concentrated to reduced pressure. The residue was partitioned between 1 M HCl (67 mL) and CH$_2$Cl$_2$ (134 mL). The organic phase was removed, and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 33.5 mL). The organics were combined, washed with saturated aqueous NaCl (134 mL), then dried (MgSO$_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with MeOH/CHCl$_3$ to give no promising looking material by NMR.

**Synthesis of Diketopiperazine**

**Scheme 82.** Attempted DKP ring closure

T-butyl glycine Fmoc proline was dissolved in a 4:1 ratio of THF/water (2.5 mL). Next Lithium hydroxide was added to the reaction vessel (25 eq.) The solution was heated at 50 °C for 15 hours. The reaction was diluted with water (10 mL) then was acidified to a pH of 5 with KH$_2$SO$_4$. The aqueous layer was extracted with ethyl acetate (10 mL) four times. The organic layers were combined and washed with water, brine, and dried over Na$_2$SO$_4$. Material was concentrated in vacuo. Organic layer contained no material. Aqueous layer was evaporated and NMR was taken in d$_2$O. HMBC indicated that the material was the open dipeptide, due to the carbonyl carbon.
not seeing the alpha carbons proton or the other protons on the other side of the proline nitrogen.

**Scheme 83.** Peptide coupling between t-butyl glycine and proline

5-8-13 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (2.49 g 4.78 mmol 1.36 eq) and i-Pr₂NEt (1.03 g 1.39 mL 7.97 mmol) Density 0.742 g/mL 2.27 eq) were added to a solution of proline (1.61 g 4.77 mmol 1.33 eq) and t-butyl ester glycine (0.63 g 4.80 mmol 1 eq) in CH₃CN (35.7 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH₂Cl₂ (100 mL) and 1 M HCl (100 mL). The layers were separated, and the aqueous phase was extracted with CH₂Cl₂ (2 x 25 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was
purified by flash chromatography eluting with 60 % EtOAc/Hexane to give dipeptide as clear viscous oil. Dipeptide is the spot at about 0.5 Rf in 1:2 Hex:EtOAc.

**Figure 43.** TLC plate of crude reaction mixture

**Synthesis of Diketopiperazine Ring**

**Scheme 84.** Attempted DKP ring closure

Reaction was modeled after Molecules 2009, 14, 2836-2849. Each dipeptidyl ester (0.25 mmol 0.113 g 1 eq.) was suspended in water:diethylamine (1 mL) and heated for 10 minutes at 250 °C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was
filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. Reaction did not work as well as the reaction in just water.

Synthesis of Diketopiperazine Ring

Scheme 85. Attempted DKP ring closure

5-13-13 Reaction was modeled after Molecules 2009, 14, 2836-2849. Each dipeptidyl ester (0.25 mmol 0.113 g 1 eq.) was suspended in water (1 mL) and heated for 10 minutes at 250 °C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. The aqueous layer was dissolved in deuterated MeOH, the proton NMR was consistent with proton spec reported in J. Braz. Chem. Soc. Vol. 16, No. 6B, 1448-1453, 2005. The carbon showed signature peaks of the amide carbons at 165.3, and 170.8 ppm, for carbon 1 and 7 respectively. What appear to be Fmoc fragments were attempted to be removed by washing with hexane.
Synthesis of 2-isoprenyltryptophant-butyl ester fmoc proline

Scheme 86. Peptide coupling of isoprenyl tryptophan and proline

Chemical Formula: $C_{16}H_{20}F_{6}N_{6}OP_{2}$
Exact Mass: 520.17
Molecular Weight: 520.39

Chemical Formula: $C_{26}H_{22}N_{2}O_{2}$
Exact Mass: 328.22
Molecular Weight: 328.45

Chemical Formula: $C_{26}H_{21}NO_{4}$
Exact Mass: 337.13
Molecular Weight: 337.37

Chemical Formula: $C_{46}H_{48}N_{3}O_{5}$
Exact Mass: 647.34
Molecular Weight: 647.80

Chemical Formula: $C_{5}H_{16}N$
Exact Mass: 129.15
Molecular Weight: 129.24

5-21-13 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (0.642 g 1.24 mmol 1.36 eq) and i-Pr$_2$NEt (0.270 g 0.361 mL 2.07 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of proline (0.410 g 1.21 mmol 1.33 eq) and 2-isoprenyl tryptophan t-butyl ester (0.30 g 0.913 mmol 1 eq) in CH$_3$CN (9 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH$_2$Cl$_2$ (30 mL) and 1 M HCl (30 mL). The layers were separated, and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 7.5 mL). The combined organic layers were dried (MgSO$_4$), filtered and concentrated under reduced pressure. The
residue was purified by flash chromatography eluting with 1:1 EtOAc:Hexane to give 0.39 g 66% yield of dipeptide as yellow oil. Dipeptide is the spot at about 0.5 Rf in 1:1 Hex:EtOAc.

Synthesis of Tryprostatin B

Scheme 87. Attempted synthesis of tryprostatin B

Reaction was modeled after Molecules 2009, 14, 2836-2849. 2-isoprenyltryptophanproline fmoc t-butyl ester (0.162 g 0.25 mmol 1 eq.) was suspended in water (1 mL) and heated for 10 minutes at 250 °C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material was purified by column chromatography material that was recovered did not look like Tryprostatin it looked more like the dipeptide with no t- butyl group.
Synthesis of Tryprostatin B

Scheme 88. Synthesis of tryprostatin B

Reaction was modeled after Molecules 2009, 14, 2836-2849. 2-isoprenyltryptophanproline t-butyl ester (0.097 g 0.27 mmol 1 eq.) was suspended in water (1 mL) and heated for 10 minutes at 250 °C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material contained no t-butyl peak at 1.3 ppm in the proton and also contained 169.5 and 165.8 in the carbon NMR which is very close to the reported values of the amides for Tryprostatin B. The starting material for this reaction has diastereomeric esters and amides which come at 175.1 174.8 and 171.7 171.3 respectively. This material was dry loaded onto a column and a gradient column was run on it using methanol:dichloromethane solutions from 0:100 to 15:85. Material eluted with about 2% methanol:dichloromethane. Theoretical yield 0.08016g. Diastereomers isolated 0.05/0.08 = 62.5% ca. 63% of desired products Tryprostatin B (0.01 g) 13 % yield diastereomers of Tryprostatin B (0.04 g) in 38% yield.
Synthesis of Tryprostatin B

**Scheme 89. Attempted synthesis of tryprostatin B**

Reaction was modeled after Molecules 2009, 14, 2836-2849 and V.L. Campo et al. Tetrahedron 65 (2009) 5343-5349. 2-isoprenyltryptophanproline fmoc t-butyl ester (0.162 g 0.25 mmol 1 eq.) was suspended in water (1 mL) and piperidine (0.25 mL) this was let stir at room temp for 18 hours. This material was then heated for 10 minutes at 250 °C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material was purified by column chromatography material that was recovered did not look like Tryprostatin it looked more like the dipeptide with not butyl group.
Synthesis of Tryprostatin B

Scheme 90. Attempted synthesis of tryprostatin B

2-isoprenyltryptophanproline fomoc t-butyl ester (0.162 g 0.25 mmol 1 eq.) was suspended in water (1 mL) and piperidine (0.25 mL). This material was then heated for 10 minutes at 250 °C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material was purified by column chromatography material that was recovered did not look like Tryprostatin it looked more like the dipeptide with no t-butyl group.
Synthesis of 2-isoprenyl tryptophan t–butyl ester amine

Scheme 91. Synthesis of tryptophan amine

Procedure adapted from Tetrahedron Letters 43 (2002) 6677-6679. 2-isoprenyltryptophan-t-butyl ester diphenyl imine (g mmol 1 eq.) was dissolved in (mL) of tetrahydrofuran and (mL) of 15% aqueous citric acid. Reaction mixture was stirred vigorously at room temperature for 18 h, and then diluted with 1 M hydrochloric acid (mL). The mixture is extracted with diethyl ether (2x mL) to remove the benzophenone, then the aqueous layer was basified (K$_2$CO$_3$) until no more K$_2$CO$_3$ would dissolve. Extraction with chloroform (5x mL) followed by drying of the extracts (Na$_2$SO$_4$) and concentration under reduced pressure gives the crude amino acid tert-butyl ester which can generally be purified by passing through a plug of silica. Amine looked clean. 97 % yield 0.6 g theoretical was 0.62 g.
Synthesis of ethyl ester glycine proline

Scheme 92. Attempted peptide coupling with DCC

7-12-13 Procedure adapted from Eur. J. Org. Chem. 2009, 5717. 32 times scale. DIC we did not have so we substituted DCC (1.97 g 9.55 mmol 1.1 eq.) and triethylamine (0.879 g 0.726 g/mL 1.211 mL 1 eq.) and glycine ethyl ester hydrochloride (1.21 g 8.69 mmol 1 eq.) in DCM (50 mL) were successively added at room temperature to a stirred solution of L-Proline (1.0 g 8.69 mmol 1 eq.) in DCM (100 mL). Reaction mixture was stirred for 3 days and then diluted with DCM (mL) and HCl (0.1 N mL) the layers were separated the aqueous phase was extracted with DCM (3x mL) and the combined chlorinated extracts were washed with water, dried with MgSO₄, filtered and concentrated under reduced pressure. Crude residue was purified by flash chromatography hexane:EtOAc 80:20.

N+ salt to amine
Procedure to attempt skipping the isolation step of the 2isopropyltryptophan Schiff base was tried using HCl and 15% citric acid. Phase transfer catalyst reaction was done on a 2.0 g scale. Crude reaction material weighed 2.75 g, about 10% of the material was used for each of these screenings. Theoretically this should yield about 0.2 of material.

Reaction 1) Followed procedure for Tett Lett 43 (2002) 6677-6679. 0.275 g crude reaction material from the N+ salt, PTC, Schiff base, 45% KOH reaction. Material was dissolved in THF (2 mL) and (0.75 mL) of 15% citric acid, reaction vessel was allowed to stir for 18 hours. Then it was diluted with 1 M HCl (0.5 mL). Mixture was extracted with diethyl ether (2 x 1.5 mL) to remove benzophenone then basified with K$_2$CO$_3$. This was extracted with dichloromethane (5 x 1.5 mL) and then dried over Na$_2$SO$_4$ and concentrated via rotovap. NMR of material did not show any tryptophan amine.

Reaction 2) Reaction was modeled after Org. Lett. 2010 vol 12 No. 8 pg. 1688. 0.275 g crude material reaction material from the N+ salt, PTC, Schiff base, 45% KOH reaction. Material was dissolved in THF (0.5 mL) and 1 N HCl (0.5 mL) at 0° C. After the reaction was stirred for 4 hours the THF was removed under reduced pressure. The resulting aqueous layer was washed with ether (3 x 5 mL) and neutralized with NaHCO$_3$. Mixture was then extracted with dichloromethane (3x 5 mL) organic layers dried over anhydrous MgSO$_4$. The NMR of this material indicated that the product was there, but contained impurities mass of this material was 0.10 g. This was attempted to be purified by running through a pipet column, mobile phase 9:1 DCM:MeOH.
Synthesis of 6-Methoxybocgramine

Scheme 93. Synthesis of 6-methoxygramine

All glassware was oven dried for 4 hours previous to use. A solution of 6-methoxygramine (0.37 g 1.8 mmol 1 eq) was made in an addition funnel using THF (9 mL). This addition funnel was put in top of a 100 mL three neck round bottom flask which was placed in an ice water cooling bath and added dropwise to a solution of ditert-butyldicarbonate (0.47 g 2.2 mmol 1.2 eq) 4-Dimethylaminopyridine (22 mg 0.18 mmol 0.1 eq) and triethylamine (0.30 mL 0.25 mmol 0.12 eq 0.726 g/mL) in THF (5 mL). About half way through the addition the reaction mixture changed from clear to cloudy. After stirring for one and a half hours the reaction water was added to the reaction mixture. Solvent was removed via roto vap and the material was extracted with ether three times. The extract was washed with brine and dried over sodium sulfate. The mixture was run through a cotton plugged funnel to remove sodium sulfate and the solvent was removed via roto vap. 9-3-13 80% yield.
All glassware was oven dried for 4 hours previous to use. A solution of 6-methoxygramine (2.27 g, 11.1 mmol, 1 eq) was made in an addition funnel using THF (55 mL). This addition funnel was put in top of a 500 mL three neck round bottom flask which was placed in an ice water cooling bath and added dropwise to a solution of ditert-butyldicarbonate (2.91 g, 13.3 mmol, 1.2 eq) 4-Dimethylaminopyridine (0.136 g, 11.1 mmol, 0.1 eq) and triethylamine (0.135 g, 0.186 mL, 1.33 mmol, 0.12 eq, 0.726 g/mL) in THF (30 mL). About half way through the addition the reaction mixture changed from clear to cloudy. After stirring for 16 hours water (25 mL) was added to the reaction mixture. Solvent was removed via roto vap and the material was extracted with ether two times. The extract was washed with brine and dried over sodium sulfate. The mixture was run through a cotton plugged funnel to remove sodium sulfate and the solvent was removed via roto vap. 

12-12-13 3.28 g/3.38 g = 97% yield.

**Synthesis of N+ isoprenyl-6-methoxy-2-isoprenylbocgramine**

**Scheme 94.** Synthesis on diisoprenyl 6-methoxygramine salt
6-methoxy boc gramine (0.20 g 0.657mmol 1 eq.) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is clear with a brown tint. Reaction vessel was cooled in a dry ice acetone bath until the reaction mixture was at -70°C. n-Butyl Lithium (1.05 0.5256 mL 2.5 M 1.314 mmol 2.0 eq) was added dropwise over an hour maintaining a temperature range between -65 and -70°C to the reaction mixture leaving the reaction mixture a bright red orange. After the addition of n-Butyl Lithium the reaction was let stir undisturbed for one and a half hours at -70°C. Isoprenyl bromide (0.314 mL 0.399 g 2.6 mmol 4 eq. 1.27 g/mL) was added dropwise to the reaction vessel. At this point the reaction mixture was orange. The reaction was then let warm to room temp overnight. When returning the next day the color of the reaction mixture was clear orange. Deionized water (5 mL) was added to the reaction mixture, no reaction from this indicated that the b-butyl lithium was quenched. The solvent was removed using the rotovap. After the solvent was removed the water and residue was poured into a separatory funnel and extracted with dichloromethane three times (10 mL). Organic layer was dried over sodium sulfate. Solvent was removed via rotovap and oil pump. NMR was taken to see if material was present. Material was purified with a pipet column using neutral alumina gel as stationary phase and DCM as mobile phase. 0.15 g of product was isolated in 40 % yield.

Scale up
4-24-14 6-Methoxybocgramine (2.24 g 7.36 mmol 1 eq.) weighted out. A 500 mL three neck round bottom flask was oven dried over night with stir bar inside was removed from oven and clamped. To this vessel 6-Methoxybocgramine was charged via a powder funnel. Nitrogen inlet, oil bubbler, and rubber septum were used to fill necks in round bottom flask. Tetrahydrofuran 100 mL (dry blue distilled) was used to solvate the material. This was let stir for thirty minutes. Reaction vessel was submerged in an acetone/dry ice bath for one half to an hour until temperature was constant. To the reaction mixture n–butyllitium (5.9 mL 2.5 M 14.75 mmol 2.00 eq.) was added in a drop wise fashion using a syringe through the septum. Reaction mixture was orange in color. Reaction was let stir for one hour. Isoprenyl Bromide (3.45 mL 4.38 g 29.40 mmol 4.00 eq 1.27 g/mL) was added to the reaction mixture in a drop wise fashion. Reaction mixture was yellow/orange in color and was left to stir overnight for 16 hours. Upon returning the next day reaction mixture was orange in color. Reaction mixture was poured into a single neck round bottom flask and solvent was removed via roto vap and oil pump. Column diameter was 6 cm x 9 cm tall. Column was run on the material by slurry loading the silica gel on to the column and dry loading the crude sample. Column was run using 9:1 Dichloromethane:Methanol mobile phase void volume collected in beakers and fractions were collected in test tubes until they were half way full. Fractions 2-5 mass 0.61 g, Frac 6-8 1.02 g, Frac 9-10 0.86 g, and Frac 11-12 0.83 g. Totaling 3.32 g actual yield / 3.84 g theoretical yield = 86% yield.

**Synthesis of N+ isoprenyl-6-methoxy-2-isoprenylbocgramine**

**Scheme 95.** Attempted synthesis using sec-BuLi
6-methoxy boc gramine (0.20 g 0.657 mmol 1 eq.) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is clear with a brown tint. Reaction vessel was cooled in a dry ice acetone bath until the reaction mixture was at -70°C. Sec Butyl Lithium (4.26 0.938 mL, 1.4 M 1.314 mmol 2.0 eq.) was added dropwise to the reaction mixture was orange and clear. After one hour of stirring isoprenyl bromide (0.3084 mL 0.3917 g 2.6284 mmol 4.0 eq) was added to the reaction mixture in a dropwise fashion this gives off a white gas reaction mixture at this point is light yellow. This reaction mixture was left to warm overnight. Upon returning the next day the reaction color was red orange brown and clear. Reaction was let stir overnight. Upon returning in the morning water was added (5 mL) the reaction was put on the rotovap to remove the THF. Organic layer was extracted with DCM (3 x 10 mL) dried over sodium sulfate filtered using cotton plug and concentrated via roto vap, NMR indicated that the single isoprenylation salt was found.
Isoprenylated boc gramine salt

Scheme 96. Single isoprenylation

6-methoxy boc gramine (0.20 g 0.657 mmol 1 eq.) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is clear. After stirring for one hour isoprenyl bromide (0.3084 mL, 0.3917 g 2.6284 mmol 4 eq.) was added in a dropwise fashion. Reaction was let stir overnight.
Synthesis of diisoprenylated boc gramine

Scheme 97. Synthesis of diisoprenyl quaternary ammonium salt

Reaction is being done to test if this reaction is not working because of the electronics of the 6meogramine or if it is the procedure. Boc gramine (0.20 g 0.7290 mmol 1 eq) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is light yellow and clear.

Reaction vessel was cooled in a dry ice acetone bath until the reaction mixture was at -70°C. n-Butyl Lithium (0.583 mL 2.5 M 1.458 mmol 2.0 eq) was added dropwise over an hour maintaining a temperature range between -65 and -70 °C to the reaction mixture leaving the reaction mixture a bright red orange. After the addition of n-Butyl Lithium the reaction was let stir undisturbed for one and a half hours at -70°C. Isoprenyl bromide (0.314 mL 0.399 g 2.6 mmol 4 eq. 1.27g/mL) was added dropwise to the reaction vessel.
Synthesis of 2-IsoprenylN-amine t-butylerstertryptophan

Scheme 98. PTC and deprotection steps combined

Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691. Crude reaction mixture starting with N-isoprenyl-2-isoprenylboc gramine (100.0 mg 0.203 mmol) was dissolved in THF (2 mL) and 1 N HCl (2 mL) was added at 0 °C. After stirred for 4 hours, THF was removed under reduced pressure. The resulting aqueous solution was washed with ether (3 x 20 mL) and neutralized with NaHCO₃. The mixture was then extracted with CH₂Cl₂ (3 x 20 mL). The organic layers were combined and dried over anhydrous Na₂SO₄. After filtration and concentration under reduced pressure, the product was obtained after purification by flash column chromatography using gradient mixtures of CH₂Cl₂/MeOH (98:2 95:5 90:10) as the eluent.
Synthesis of 6-MeO-2-isoprenyl t-buesterdiphenyl amine tryptophan

Scheme 99. Synthesis of 6-MeOtryptophan

6-MeON+ salt (0.1 g 0.192 mmol 1 eq.) was added to a 7.5 mL vial with a mini stir bar. To this (0.07 g 0.237 mmol 1.23 eq) of Schiff base and (0.03 g 0.04954 mmol 0.2580 eq) of phase transfer catalyst was added. To the reaction mixture 2 mL of toluene was added and reaction mixture was let stir 30 min. 1 mL of 45% KOH was added to the reaction vessel. Crude reaction mixture was run through a short neutral alumina plug using 3:1 Hexane:Ethyl Acetate and gradient to higher polarity. Percent conversion was monitored via proton NMR of organic layer by comparing the integration of the multipet at 4.2 and the singlet at 4.1.
Synthesis of 6-Methoxy-2-isoprenyl-t-butylestertryptophanamine

Scheme 100. Attempted deprotection of benzophenone imine

Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691 (S-13). 6-Methoxy-2-isoprenyl-t-butylestertryptophandiphenylmethylene (0.03 g 0.00574 mmol 1 eq) was dissolved in 1 mL THF along with 1 mL of 1 N HCl solution. Reaction mixture was stirred at 0°C for 4 hours. THF was rotovaped off. Reaction mixture was washed with hexane three times; this layer was dried over Na₂SO₄ and rotovaped to dryness. NMR indicates this is benzophenone. Aqueous layer was basified using sodium bicarbonate, until adding solid gave no more bubbles. Aqueous layer was extracted with dichloromethane three times. Dichloromethane layer was dried over Na₂SO₄ and rotovaped to dryness.
Synthesis of 6Methoxy-2-isoprenyltryptophan t-butyl ester amine

Scheme 101. Deprotection of benzophenone imine using citric acid

5-19-14 Procedure adapted from Tetrahedron Letters Vol 43 Iss 37(2002) 6677-6679. 6-Methoxy-2-isoprenyltryptophan-t-butyl ester diphenyl imine partially purified most of which is benzophenone (1.22 g mmol 1 eq.) was dissolved in tetrahydrofuran (4 mL) and of 15% aqueous citric acid (1.5 mL) added. Reaction mixture was stirred vigorously at room temperature for 18 h, and then diluted with 1 M hydrochloric acid (1 mL). The mixture is extracted with diethyl ether (3 x 5 mL) to remove the benzophenone, then the aqueous layer was basified (K$_2$CO$_3$) until no more K$_2$CO$_3$ would dissolve. Extraction with dichloromethane (5 x 5 mL) followed by drying of the extracts (Na$_2$SO$_4$) and concentration under reduced pressure gives the crude amino acid tert-butyl ester which can generally be purified by passing through a plug of silica or alumina. Gradient column was run using Hexane:Ethyl acetate 9:1, 7:3, 1:1 Dichloromethane, Dichloromethane:Methanol 9:1. Appeared to come out with 1:1 Hex:EtOAc or DCM, but make sure to check earlier fractions.
Synthesis of 6Methoxy-2-isoprenylbutylestertryptophanfmocprolindedipeptide

**Scheme 102.** Peptide coupling of 6MeOtryptophan and proline

- **Chemical Formula:** C_{18}H_{28}F_{6}N_{6}OP_{2}
  - **Exact Mass:** 520.17
  - **Molecular Weight:** 520.39

- **Chemical Formula:** C_{20}H_{19}NO_{4}
  - **Exact Mass:** 337.13
  - **Molecular Weight:** 337.37

- **Chemical Formula:** C_{21}H_{19}N
  - **Exact Mass:** 129.15
  - **Molecular Weight:** 129.24

7-2-14 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25 Supporting docs S20

Dipeptide 13. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (0.197 g 0.379 mmol 1.36 eq) and i-Pr_{2}NEt (0.0818 g 0.110 mL 0.633 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of Fmoc proline (0.125 g 0.371 mmol 1.33 eq) and 6-Methoxy2-isoprenyl tryptophan t-butyl ester amine (0.1 g 0.279 mmol 1 eq) in CH_{3}CN (3 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH_{2}Cl_{2} (10 mL) and 1 M HCl (10 mL). The layers were separated, and the aqueous phase was extracted with CH_{2}Cl_{2} (2 x 5 mL). The combined organic layers were dried (MgSO_{4}), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting
with 1:1 EtOAc:Hexane to give 0.39 g 66 % yield of dipeptide as yellow oil. Dipeptide is the spot at about 0.5 Rf in 1:1 Hex:EtOAc.

**Synthesis of 6-Methoxy-2-isoprenyltryptophanprolinetbutylester**

**Scheme 103.** Deprotection of Fmoc

7-7-14Following H.D Jai et al. Bioorg. Med. Chem. 16 (2008) 4626-4651. Crude reaction mixture was dissolved in acetonitrile (3 ml) and to this diethylamine (3 mL). The reaction mixture was stirred overnight. The next day the mixture was TLCed and roto vaped to dryness.
Procedure was modeled after a similar procedure in Molecules 2009, 14, 2836-2849. Material was transferred from previous vessel and evaporated to dryness using roto vap in microwave reaction vessel. Water (1 mL) was then added to this reaction vessel, at this point the material made an orange milky suspension. Microwave maximums were set to 250 °C and 150 PSI. Actual values were about 195 °C and 140 PSI. After the reaction was complete the material was a dark brown oil. This was submitted for mass spec to determine if product was present. It may have been in a very small amount. Mass spec provided evidence that tryprostatin A was present.
7. DATA

Tryprostatain A

Chemical Formula: $C_{22}H_{27}N_{3}O_{3}$
Molecular Weight: 381.47

==== Shimadzu LabSolutions Data Report ====

<Spectrum>
6-Methoxy-2-isoprenyl-butylestertryptophanamine

Chemical Formula: $C_{21}H_{38}N_{2}O_{3}$
Molecular Weight: 358.47

$^{1}H$ Name: huis7-31-14 Expno: 1 Procno: 1

$^{13}C$ Name: huis7-31-14 Expno: 3 Procno: 1

HSQC Name: huis7-31-14 Expno: 2 Procno: 1

HRMS Name: Huis7-31-14 Date: 8-5-2014 Time 11:31:00 PM
6-Methoxy-2-isoprenyl-butylesterdephenylmethylenetryptopan

Chemical Formula: $C_{34}H_{38}N_2O_3$
Molecular Weight: 522.68

$^1$H Name: huis3-8-14 Expno: 2 Procno: 1

$^{13}$C Name: huis3-8-14 Expno: 3 Procno: 1

LRMS Name: Matt Date: 3-11-2014 Time 3:09:50 PM

HRMS Name: Huis 8-11-14 (3)
Shimadzu LabSolutions Data Report

<Spectrum>

Lineth 1 R:Time/0.133(Scan#)/0
MassPeaks:002
RawMode:Single 0.133(0) BasePeak:626(122807)
SO Mode:None Segment 1 - Event 1
Shimadzu LCMS-IT-TOF Analysis Report
Aug 12, 2014

Acquired by: Mark Wang, Department of Chemistry and Biochemistry, UW-Milwaukee
Sample Name: Huis 8-11-14(3)
Sample ID: Huis 3-11-14(3)
Vial #: 4
Injection Volume: 5 µL
Data File Name: Huis 9-11-14(3) 01.lcd
Method File Name: MW Manual (MS-MS, +, -).lcm
Batch File Name: 
Report File Name: DefaultLCMS.lcr
Data Acquired: 6/12/2014 11:34:35 AM
Data Processed: 6/12/2014 11:37:36 AM

Formula Prediction Results
6-Methoxydiisoprenylbocgramine

Chemical Formula: $C_{27}H_{41}BrN_2O_3$
Molecular Weight: 521.53

$^1H$ Name: huis11-4-13 Expno:7 Procno: 1

$^{13}C$ Name: huis11-4-13 Expno: 8 Procno: 1

HSQC Name: huis11-4-13 Expno: 9 Procno: 1

LRMS Name: Date 12-19-2013 Huis-010 Time 11:26:12 AM

HRMS Name: Huis 8-11-14 (2)
==== Shimadzu LabSolutions Data Report ==== 

<Spectrum> 
Line 1: RT 0.130 (Scan #6) 
Mass peaks: 507 
RawMode: Single 0.1336 (Base Peak: 440(278581)) 
B0 Mode: None 
Segment 1 - Event 1
6-Methoxybocgramine

Chemical Formula: C_{17}H_{24}N_{2}O_{3}
Molecular Weight: 304.38

^1H Name: huis9-5-13 Expno: 3 Procno: 1

^13C Name: huis9-5-13 Expno: 3 Procno: 1

HSQC Name: huis9-5-13 Expno: 5 Procno: 1

LRMS Name: Matt-Frac-3 Date: 9-30-13 Time: 4:02

HRMS Name: Huis 8-11-14 (1)
Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample: Malt-Frac-3

Operator: Mark Wang

Data filename: C:\Lab\Solutions\Data\Hossain Mahmoud\Malt-Frac-3.pec
Spectrum Mode: Single
Retention Time: 0.133 min.
Interface Type (ESI, APCl, DURS): DUB
Acquision Mode (Scan, SIM, Profile): Scan
Polarity (+,-): +

[Graph of mass spectrum with m/z values]
Shimadzu LCMS-IT-TOF Analysis Report
Aug 12, 2014

Acquired by : Mark Wang, Department of Chemistry and Biochemistry, UW Milwaukee
Sample Name : Hsiu 8-11-14(1)
Sample ID : Hsiu 8-11-14(1)
Visit # : 2
Injection Volume : 5 µL
Data File Name : Hsiu 8-11-14(1) 002.lcd
Method File Name : MW Manual (MS-MS, +,-).lc
Batch File Name :
Report File Name : DefaultLCMS.lcr
Data Acquired : 8/12/2014 11:21:19 AM
Data Processed : 8/12/2014 11:24:10 AM

Formula Prediction Results

![Formula Prediction Results Image]
$C_{28}H_{28}N_2O_2$
Mol. Wt.: 424.53

$^1H$ Name: huis Expno: 1 Procno: 1 Date: 20091117

$^{13}C$ Name: huis Expno: 3 Procno: 1 Date: 20091117

$^{13}C$ HSQC Name: huis Expno: 2 Procno: 1 Date: 20091117
C\textsubscript{33}H\textsubscript{36}N\textsubscript{2}O\textsubscript{2}
Mol. Wt.: 492.65

\textsuperscript{1}H Name: huis10-13-11 Expno: 1 Procno: 1

\textsuperscript{13}C Name: huis10-13-11 Expno: 3 Procno: 1

LRMS

HRMS
Display Report - All Windows Selected Analy

Analysis Name: 1215107.D
Method: Copy of MARX02.M
Sample Na: M Husnan
Analysis Int

Instrument: LC MSQ-Trap-SE
Operator: Mark Wong
Print Date: 12/15/11 15:14:55
Acq. Date: 12/15/11 15:06:47
$\text{C}_{26}\text{H}_{39}\text{N}_2\text{O}_2^+$

Mol. Wt.: 411.6

$^1\text{H}$ Name: huis1-23-13 Expno: 3 Procno: 1

$^{13}\text{C}$ Name: huis1-23-13 Expno: 5 Procno: 1

$^{13}\text{C}$ Dept135 Name: huis1-23-13 Expno: 6 Procno: 1
UNIVERSITY OF WISCONSIN-MILWAUKEE
CHEMISTRY DEPARTMENT

ELEMENTAL ANALYSIS REQUEST (C. H. N.)

1. Sample size for a single analysis is approximately 2 mg. Multiple runs on the same sample can be done. Request it in the comments section. Ash weight is not available.
2. Solids and non-volatile liquids are analyzed in the same manner. Volatile liquids are analyzed differently and may require prior arrangements.
3. Normal error tolerance is ±0.3%
4. The sample ID is limited to 13 characters and no extensions. ID characters may be any combination of letters, numbers, and the underscore character. Do not use Roman numerals.
5. Do not reuse ID codes, as a new result may overwrite an older one.
6. Combustion conditions are; pure oxygen at 30 psi, and 1800° C.

FILL IN THE ITEMS BELOW

Faculty name (print) 
Dr. Mahmud Hossain

Student name (print) 
Md. Shuvit Asad

Sample ID 
Sa 061711

Date submitted 
06/20/11

Molecular formula 
C_{26}H_{29}BrN_{2}O_{2}

Calculated N% by wt. 
5.70 (with Br) / 6.81 (without Br) 5.7210

Calculated C% by wt. 
63.54 (%) / 75.87 (%) 63.3038

Calculated H% by wt. 
8.00 (%) / 19.55 (%) 8.3621

Comments: 
Good Job!

Mark 
06/22/2011

A separate page will be returned showing the results.
### C.H.N Elemental Analysis

**Page:** 1  **Sample:** SA-061711 (J221111)

- **S/W version:** 1.06
- **Operator ID:** Mark Wang
- **Sample ID:** SA-061711 (#11)
- **Sample Name:** UWM Chemistry
- **Method Name:** Minutes
- **Method File:** MMBU111.MTH
- **Analysis Type:** Unknown (Area)
- **Sample Weight:** 2.326 g
- **Chromatogram:** C:\EAM\MARKDA\J221111.DAT
- **Printed:** 6/22/2011 17:21

**Calib. method:** using 'K Factors'

<table>
<thead>
<tr>
<th>Element Name</th>
<th>Element %</th>
<th>Ret.Time</th>
<th>Area</th>
<th>BC</th>
<th>Area ratio</th>
<th>K factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>5.7210</td>
<td>1.11</td>
<td>414285</td>
<td>20.07345</td>
<td>311330E+07</td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>63.3038</td>
<td>1.41</td>
<td>8316138</td>
<td>1.000000</td>
<td>564378E+07</td>
<td></td>
</tr>
<tr>
<td>Hydrogen</td>
<td>8.3621</td>
<td>3.92</td>
<td>2895564</td>
<td>2.872047</td>
<td>148870E+08</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>77.3868</td>
<td></td>
<td>11625970</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Crystal Structure data
CCDC 922382
Blocks grown using slow diffusion method: Ethyl Acetate/Hexane

Analyzed by X-ray diffraction at UCSD with Arnie Rheingold
Unit Cell Dimensions:  
\( a=8.5784(2) \); \( b=12.9668(3) \); \( c=13.5267(3) \)\( \text{Å} \)
\( \alpha=109.266(2)^\circ \); \( \beta=103.084(2)^\circ \); \( \gamma=107.596(2)^\circ \)

Triclinic lattice, P1 space group, \( Z = 2 \) molecules per unit cell. \( R_1 = 4.39\% \)

Contact: Matthew Huisman, \( mhuisman@uwm.edu \)
Authors: Matthew M. Huisman, Sarah Oehm M. Mahmun Hossain, Arnold L. Rheingold
Table 1 Crystal data and structure refinement for Hosain01_0m

<table>
<thead>
<tr>
<th>Identification code</th>
<th>Hosain01_0m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C26H39N2O2Br</td>
</tr>
<tr>
<td>Formula weight</td>
<td>491.50</td>
</tr>
<tr>
<td>Temperature/K</td>
<td>273.15</td>
</tr>
<tr>
<td>Crystal system</td>
<td>triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P1</td>
</tr>
<tr>
<td>a/Å</td>
<td>8.5784(2)</td>
</tr>
<tr>
<td>b/Å</td>
<td>12.9668(3)</td>
</tr>
<tr>
<td>c/Å</td>
<td>13.5267(3)</td>
</tr>
<tr>
<td>( \alpha/^\circ )</td>
<td>109.266(2)</td>
</tr>
<tr>
<td>( \beta/^\circ )</td>
<td>103.084(2)</td>
</tr>
<tr>
<td>( \gamma/^\circ )</td>
<td>107.596(2)</td>
</tr>
<tr>
<td>Volume/Å³</td>
<td>1261.86(5)</td>
</tr>
<tr>
<td>( Z )</td>
<td>2</td>
</tr>
<tr>
<td>( \rho_{\text{calc}}/\text{mg/mm}^{3} )</td>
<td>1.294</td>
</tr>
<tr>
<td>( m/\text{mm}^{1} )</td>
<td>1.653</td>
</tr>
<tr>
<td>( F(000) )</td>
<td>520.0</td>
</tr>
<tr>
<td>Crystal size/mm³</td>
<td>0.3 ( \times ) 0.24 ( \times ) 0.18</td>
</tr>
<tr>
<td>2Θ range for data collection</td>
<td>3.42 to 63.92°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>(-12 \leq h \leq 12, -19 \leq k \leq 19, -20 \leq l \leq 20)</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>23123</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>16463[R(int) = 0.0238]</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>16463/3/577</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>0.917</td>
</tr>
<tr>
<td>Final R indexes [I ( \geq ) 2( \sigma ) (I)]</td>
<td>( R_1 = 0.0440, wR₂ = 0.1103 )</td>
</tr>
<tr>
<td>Final R indexes [all data]</td>
<td>( R_1 = 0.0729, wR₂ = 0.1451 )</td>
</tr>
<tr>
<td>Largest diff. peak/hole / e Å⁻³</td>
<td>0.94/-0.52</td>
</tr>
<tr>
<td>Flack parameter</td>
<td>0.21(2)</td>
</tr>
</tbody>
</table>

c_chemical_name_systematic : N-{[1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl}-N,N,3-trimethylbut-2-en-1 aminium bromide

_chemical_name_common      Compound synonym: boc protected 2 isoprenyl N isoprenyl gramine salt

data_hossain1

_audit_creation_method     SHELXL-97
_chemical_name_systematic
;

_chemical_name_common
?

_chemical_melting_point
?

_chemical_formula_moiety
?

_chemical_formula_sum
'C26 H39 Br N2 O2'

_chemical_formula_weight
491.50

loop_
_atom_type_symbol
_atom_type_description
_atom_type_scat_dispersion_real
_atom_type_scat_dispersion_imag
_atom_type_scat_source
'C'  'C'  0.0033  0.0016
'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
'H'  'H'  0.0000  0.0000
'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
'N'  'N'  0.0061  0.0033
'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
'O'  'O'  0.0106  0.0060
'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
'Br'  'Br' -0.2901  2.4595
'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'

_symmetry_cell_setting
?

_symmetry_space_group_name_H-M
?

loop_
_symmetry_equiv_pos_as_xyz
'x, y, z'
'x, -y, -z'

_cell_length_a
8.5784(2)

_cell_length_b
12.9668(3)

_cell_length_c
13.5267(3)

_cell_angle_alpha
109.266(2)

_cell_angle_beta
103.084(2)

_cell_angle_gamma
107.596(2)

_cell_volume
1261.86(5)

_cell_formula_units_Z
2

_cell_measurement_temperature
100(2)

_cell_measurement_reflns_used
?

_cell_measurement_theta_min
?

_cell_measurement_theta_max
?

_exptl_crystal_description
?

_exptl_crystal_colour
?

_exptl_crystal_size_max
0.20

_exptl_crystal_size_mid
0.15

_exptl_crystal_size_min
0.15
Refinement of $F^2$ against ALL reflections. The weighted R-factor $wR$ and
goodness of fit $S$ are based on $F^2$, conventional R-factors $R$ are based
on $F$, with $F$ set to zero for negative $F^2$. The threshold expression of
F^2 > 2\sigma(F^2) is used only for calculating R-factors (gt) etc. and is not relevant to the choice of reflections for refinement. R-factors based on F^2 are statistically about twice as large as those based on F, and R-factors based on ALL data will be even larger.

; _refine_ls_structure_factor_coef Fsqd _refine_ls_matrix_type full _refine_ls_weighting_scheme calc _refine_ls_weighting_details 'calc w=1/\sqrt{(F0^2)+(0.0514P)^2+0.0082P} where P=(F0^2+2Fc^2)/3' _atom_sites_solution_primary direct _atom_sites_solution_secondary difmap _atom_sites_solution_hydrogens geom _refine_ls_hydrogen_treatment mixed _refine_ls_extinction_method none _refine_ls_extinction_coef ? _refine_ls_number_reflns 8628 _refine_ls_number_parameters 288 _refine_ls_number_restraints 0 _refine_ls_R_factor_all 0.0588 _refine_ls_R_factor_gt 0.0383 _refine_ls_wR_factor_ref 0.0932 _refine_ls_wR_factor_gt 0.0851 _refine_ls_goodness_of_fit_ref 1.002 _refine_ls_restrained_S_all 1.002 _refine_ls_shift/su_max 0.005 _refine_ls_shift/su_mean 0.000

loop _atom_site_label _atom_site_type_symbol _atom_site_fract_x _atom_site_fract_y _atom_site_fract_z _atom_site_U_iso_or_equiv _atom_site_adp_type _atom_site_occupancy _atom_site_symmetry_multiplicity _atom_site_calc_flag _atom_site_refinement_flags _atom_site_disorder_assembly _atom_site_disorder_group Br1 Br 0.26156(2) 0.907615(15) 0.128951(14) 0.02256(6) Uani 1 1 d . . . O1 O 0.09717(16) 0.64626(11) 0.35352(10) 0.0225(2) Uani 1 1 d . . . O2 O -0.14268(17) 0.66066(13) 0.26027(10) 0.0300(3) Uani 1 1 d . . . N1 N -0.00475(17) 0.77056(11) 0.44977(11) 0.0160(2) Uani 1 1 d . . . N2 N 0.12175(16) 0.96019(11) 0.83787(11) 0.0154(2) Uani 1 1 d . . . C1 C -0.1248(2) 0.82304(14) 0.46308(13) 0.0162(3) Uani 1 1 d . . .
<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Uiso</th>
<th>Occupancy</th>
<th>Site</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>-0.2837(2)</td>
<td>0.80211(15)</td>
<td>0.38790(14)</td>
<td>0.0198(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H2A</td>
<td>-0.3311</td>
<td>0.7422</td>
<td>0.3128</td>
<td>0.024</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>-0.3704(2)</td>
<td>0.87209(15)</td>
<td>0.42681(14)</td>
<td>0.0213(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H3A</td>
<td>-0.4788</td>
<td>0.8600</td>
<td>0.3770</td>
<td>0.026</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>-0.3024(2)</td>
<td>0.95980(15)</td>
<td>0.53715(14)</td>
<td>0.0208(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H4A</td>
<td>-0.3648</td>
<td>1.0065</td>
<td>0.5610</td>
<td>0.025</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>-0.1464(2)</td>
<td>0.97930(15)</td>
<td>0.61168(14)</td>
<td>0.0183(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H5A</td>
<td>-0.1004</td>
<td>1.0388</td>
<td>0.5093</td>
<td>0.022</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>0.05673(19)</td>
<td>0.90973(13)</td>
<td>0.57168(13)</td>
<td>0.0153(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>0.10789(19)</td>
<td>0.90980(13)</td>
<td>0.62936(12)</td>
<td>0.0148(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C8</td>
<td>0.13886(19)</td>
<td>0.82623(13)</td>
<td>0.55342(13)</td>
<td>0.0153(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C9</td>
<td>0.2969(2)</td>
<td>0.79779(14)</td>
<td>0.57135(13)</td>
<td>0.0177(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>0.2635(2)</td>
<td>0.67882(15)</td>
<td>0.57709(14)</td>
<td>0.0195(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H10A</td>
<td>0.1449</td>
<td>0.6222</td>
<td>0.5468</td>
<td>0.023</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C11</td>
<td>0.3849(2)</td>
<td>0.64669(15)</td>
<td>0.62055(15)</td>
<td>0.0226(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C12</td>
<td>0.3380(3)</td>
<td>0.52332(17)</td>
<td>0.61499(18)</td>
<td>0.0306(4)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H12A</td>
<td>0.2114</td>
<td>0.4761</td>
<td>0.5749</td>
<td>0.046</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H12B</td>
<td>0.3704</td>
<td>0.5286</td>
<td>0.6913</td>
<td>0.046</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H12C</td>
<td>0.4015</td>
<td>0.4846</td>
<td>0.5751</td>
<td>0.046</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C13</td>
<td>0.5772(2)</td>
<td>0.72660(18)</td>
<td>0.6740(2)</td>
<td>0.0356(5)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H13A</td>
<td>0.5940</td>
<td>0.8090</td>
<td>0.6878</td>
<td>0.053</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H13B</td>
<td>0.6370</td>
<td>0.6993</td>
<td>0.6237</td>
<td>0.053</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H13C</td>
<td>0.6263</td>
<td>0.7238</td>
<td>0.7454</td>
<td>0.053</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C14</td>
<td>0.2305(2)</td>
<td>0.99903(14)</td>
<td>0.74480(12)</td>
<td>0.0159(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H14A</td>
<td>0.2119</td>
<td>1.0737</td>
<td>0.7611</td>
<td>0.019</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H14B</td>
<td>0.3522</td>
<td>1.0179</td>
<td>0.7466</td>
<td>0.019</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C15</td>
<td>0.0274(2)</td>
<td>0.99930(14)</td>
<td>0.74480(12)</td>
<td>0.0159(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H15A</td>
<td>0.0218</td>
<td>0.8885</td>
<td>0.8922</td>
<td>0.031</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>0.2782(2)</td>
<td>0.86466(14)</td>
<td>0.83030(14)</td>
<td>0.0198(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H16A</td>
<td>0.2072</td>
<td>0.7948</td>
<td>0.7580</td>
<td>0.030</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H16B</td>
<td>0.2693</td>
<td>0.8416</td>
<td>0.8916</td>
<td>0.030</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H16C</td>
<td>0.4010</td>
<td>0.8948</td>
<td>0.8364</td>
<td>0.030</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C17</td>
<td>0.3288(2)</td>
<td>1.06740(14)</td>
<td>0.95033(13)</td>
<td>0.0182(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H17A</td>
<td>0.3337</td>
<td>1.0402</td>
<td>1.0108</td>
<td>0.022</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H17B</td>
<td>0.4495</td>
<td>1.0995</td>
<td>0.9507</td>
<td>0.022</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C18</td>
<td>0.2668(2)</td>
<td>1.16523(15)</td>
<td>0.97513(14)</td>
<td>0.0216(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H18A</td>
<td>0.1619</td>
<td>1.1499</td>
<td>0.9910</td>
<td>0.026</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C19</td>
<td>0.3441(2)</td>
<td>1.27206(15)</td>
<td>0.97723(14)</td>
<td>0.0226(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C20</td>
<td>0.2701(3)</td>
<td>1.36419(18)</td>
<td>1.00961(17)</td>
<td>0.0341(4)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H20A</td>
<td>0.1613</td>
<td>1.3294</td>
<td>1.0214</td>
<td>0.051</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H20B</td>
<td>0.2454</td>
<td>1.3897</td>
<td>0.9494</td>
<td>0.051</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H20C</td>
<td>0.3554</td>
<td>1.4335</td>
<td>1.0791</td>
<td>0.051</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C21</td>
<td>0.5024(3)</td>
<td>1.31118(17)</td>
<td>0.94698(17)</td>
<td>0.0314(4)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H21A</td>
<td>0.5436</td>
<td>1.2470</td>
<td>0.9283</td>
<td>0.047</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H21B</td>
<td>0.5955</td>
<td>1.3832</td>
<td>1.0108</td>
<td>0.047</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>H21C</td>
<td>0.4717</td>
<td>1.3289</td>
<td>0.8820</td>
<td>0.047</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>-0.0258(2)</td>
<td>0.68790(14)</td>
<td>0.34429(13)</td>
<td>0.0178(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C23</td>
<td>0.1041(2)</td>
<td>0.55761(15)</td>
<td>0.25343(14)</td>
<td>0.0217(3)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>0.2593(3)</td>
<td>0.5350(2)</td>
<td>0.30555(17)</td>
<td>0.0400(5)</td>
<td>Uani 1</td>
<td>1 d . . .</td>
<td></td>
</tr>
<tr>
<td>H24A</td>
<td>0.3653</td>
<td>0.6100</td>
<td>0.3427</td>
<td>0.060</td>
<td>Uiso 1</td>
<td>calc R . .</td>
<td></td>
</tr>
</tbody>
</table>
H24B  H  0.2757  0.4755  0.2467  0.060  Uiso  1  1  calc  R . .
H24C  H  0.2371  0.5050  0.3609  0.060  Uiso  1  1  calc  R . .
C25    C -0.1615  0.4052  0.0440  0.0323  (5)  Uani  1  1  d . .
H25A  H  -0.1988  0.4466  0.1558  0.060  Uiso  1  1  calc  R . .
H25B  H  -0.1792  0.4461  0.2562  0.063  Uiso  1  1  calc  R . .
H25C  H  -0.1596  0.4147  0.3573  0.063  Uiso  1  1  calc  R . .
C26    C  0.1391(3)  0.61289(19)  0.17451(17)  0.0333(4)  Uani  1  1  d . .
H26A  H  0.0339  0.6300  0.2142  0.050  Uiso  1  1  calc  R . .
H26B  H  0.1552  0.5572  0.1124  0.050  Uiso  1  1  calc  R . .
H26C  H  0.2455  0.6876  0.2151  0.050  Uiso  1  1  calc  R . .

loop_
_atom_site_aniso_label
_atom_site_aniso_U_11
_atom_site_aniso_U_22
_atom_site_aniso_U_33
_atom_site_aniso_U_23
_atom_site_aniso_U_13
_atom_site_aniso_U_12
Br1  0.02199(9)  0.02818(10)  0.02860(10)  0.01664(7)  0.01626(7)
     0.01419(7)
O1   0.0257(6)  0.0246(6)  0.0174(6)  0.0050(5)  0.0070(5)  0.0157(5)
O2   0.0271(6)  0.0448(8)  0.0162(6)  0.0063(5)  0.0055(5)  0.0216(6)
N1   0.0161(6)  0.0158(6)  0.0153(6)  0.0056(5)  0.0055(5)  0.0067(5)
N2   0.0134(6)  0.0171(6)  0.0161(6)  0.0079(5)  0.0057(5)  0.0056(5)
C1   0.0153(7)  0.0169(7)  0.0191(7)  0.0093(6)  0.0081(6)  0.0068(6)
C2   0.0182(7)  0.0213(8)  0.0189(7)  0.0087(6)  0.0055(6)  0.0078(6)
C3   0.0160(7)  0.0259(8)  0.0250(8)  0.0142(7)  0.0065(6)  0.0096(7)
C4   0.0198(7)  0.0236(8)  0.0264(8)  0.0140(7)  0.0115(6)  0.0124(7)
C5   0.0196(7)  0.0200(8)  0.0205(8)  0.0110(6)  0.0099(6)  0.0104(6)
C6   0.0155(7)  0.0163(7)  0.0173(7)  0.0094(6)  0.0076(6)  0.0071(6)
C7   0.0135(6)  0.0157(7)  0.0158(7)  0.0078(6)  0.0061(5)  0.0051(6)
C8   0.0137(6)  0.0154(7)  0.0172(7)  0.0080(6)  0.0062(5)  0.0050(6)
C9   0.0143(7)  0.0198(8)  0.0194(7)  0.0080(6)  0.0073(6)  0.0071(6)
C10  0.0146(7)  0.0205(8)  0.0215(8)  0.0075(6)  0.0066(6)  0.0063(6)
C11  0.0199(8)  0.0238(8)  0.0260(8)  0.0113(7)  0.0099(7)  0.0096(7)
C12  0.0266(9)  0.0301(10) 0.0414(11)  0.0202(9)  0.0114(8)  0.0146(8)
C13  0.0199(8)  0.0329(10) 0.0545(13)  0.0206(10) 0.0081(8)  0.0131(8)
C14  0.0167(7)  0.0172(7)  0.0153(7)  0.0081(6)  0.0075(6)  0.0065(6)
C15  0.0138(7)  0.0278(9)  0.0236(8)  0.0142(7)  0.0091(6)  0.0063(6)
C16  0.0219(8)  0.0184(8)  0.0207(8)  0.0094(6)  0.0077(6)  0.0093(6)
C17  0.0172(7)  0.0188(7)  0.0156(7)  0.0066(6)  0.0045(6)  0.0054(6)
C18  0.0243(8)  0.0245(8)  0.0180(7)  0.0079(6)  0.0112(6)  0.0114(7)
C19  0.0286(9)  0.0215(8)  0.0149(7)  0.0052(6)  0.0070(6)  0.0103(7)
C20  0.0508(12) 0.0290(10)  0.0291(10)  0.0110(8)  0.0172(9)  0.0242(9)
C21  0.0311(10)  0.0230(9)  0.0340(10)  0.0109(8)  0.0116(8)  0.0048(8)
C22  0.0171(7)  0.0187(7)  0.0193(7)  0.0090(6)  0.0086(6)  0.0071(6)
C23  0.0255(8)  0.0209(8)  0.0191(8)  0.0048(6)  0.0098(6)  0.0128(7)
C24  0.0516(13)  0.0498(13)  0.0276(10)  0.0100(9)  0.0125(9)  0.0407(11)
C25  0.0417(12)  0.0240(10)  0.0461(13)  -0.0002(9)  0.0241(10)  0.0057(9)
C26  0.0450(11)  0.0405(11)  0.0355(10)  0.0220(9)  0.0273(9)  0.0286(10)

_geom_special_details
;
All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

; loop_
  _geom_bond_atom_site_label_1
  _geom_bond_atom_site_label_2
  _geom_bond_distance
  _geom_bond_site_symmetry_2
  _geom_bond_publ_flag
O1 C22 1.3225(19) . ?
O1 C23 1.4860(19) . ?
O2 C22 1.195(2) . ?
N1 C22 1.405(2) . ?
N1 C1 1.4084(19) . ?
N1 C8 1.4202(19) . ?
N2 C16 1.4909(19) . ?
N2 C15 1.4923(19) . ?
N2 C14 1.5234(19) . ?
N2 C17 1.523(2) . ?
C1 C2 1.390(2) . ?
C1 C6 1.400(2) . ?
C2 C3 1.386(2) . ?
C2 H2A 0.9500 . ?
C3 C4 1.395(2) . ?
C3 H3A 0.9500 . ?
C4 C5 1.374(2) . ?
C4 H4A 0.9500 . ?
C5 C6 1.398(2) . ?
C5 H5A 0.9500 . ?
C6 C7 1.439(2) . ?
C7 C8 1.365(2) . ?
C7 C14 1.487(2) . ?
C8 C9 1.499(2) . ?
C9 C10 1.513(2) . ?
C9 H9A 0.9900 . ?
C9 H9B 0.9900 . ?
C10 C11 1.323(2) . ?
C10 H10A 0.9500 . ?
C11 C12 1.498(3) . ?
C11 C13 1.500(3) . ?
C12 H12A 0.9800 . ?
C12 H12B 0.9800 . ?
C12 H12C 0.9800 . ?
C13  H13A 0.9800 . ?
C13  H13B 0.9800 . ?
C13  H13C 0.9800 . ?
C14  H14A 0.9900 . ?
C14  H14B 0.9900 . ?
C15  H15A 0.9800 . ?
C15  H15B 0.9800 . ?
C15  H15C 0.9800 . ?
C16  H16A 0.9800 . ?
C16  H16B 0.9800 . ?
C16  H16C 0.9800 . ?
C17  C18 1.488(2) . ?
C17  H17A 0.9900 . ?
C17  H17B 0.9900 . ?
C18  C19 1.330(2) . ?
C18  H18A 0.9500 . ?
C19  C21 1.497(3) . ?
C19  C20 1.504(2) . ?
C20  H20A 0.9800 . ?
C20  H20B 0.9800 . ?
C20  H20C 0.9800 . ?
C21  H21A 0.9800 . ?
C21  H21B 0.9800 . ?
C21  H21C 0.9800 . ?
C23  C26 1.504(3) . ?
C23  C25 1.507(3) . ?
C23  C24 1.518(3) . ?
C24  H24A 0.9800 . ?
C24  H24B 0.9800 . ?
C24  H24C 0.9800 . ?
C25  H25A 0.9800 . ?
C25  H25B 0.9800 . ?
C25  H25C 0.9800 . ?
C26  H26A 0.9800 . ?
C26  H26B 0.9800 . ?
C26  H26C 0.9800 . ?

loop_
  _geom_angle_atom_site_label_1
  _geom_angle_atom_site_label_2
  _geom_angle_atom_site_label_3
  _geom_angle
  _geom_angle_site_symmetry_1
  _geom_angle_site_symmetry_3
  _geom_angle_publ_flag
C22  O1  C23  121.20(13) . . ?
C22  N1  C1  121.65(13) . . ?
C22  N1  C8  129.42(13) . . ?
C1  N1  C8  108.51(12) . . ?
C16  N2  C15  108.57(12) . . ?
C16  N2  C14  109.87(11) . . ?
C15  N2  C14  112.22(12) . . ?
C16  N2  C17  107.51(12) . . ?
C15  N2  C17  110.41(12) . . ?
C14 N2 C17 108.15(11) . . ?
C2 C1 C6 121.28(14) . . ?
2 C1 N1 131.41(14) . . ?
C6 C1 N1 107.31(13) . . ?
C3 C2 C1 117.41(15) . . ?
C3 C2 H2A 121.3 . . ?
C1 C2 H2A 121.3 . . ?
C2 C3 C4 121.74(15) . . ?
C2 C3 H3A 119.1 . . ?
C4 C3 H3A 119.1 . . ?
C5 C4 C3 120.72(15) . . ?
C5 C4 H4A 119.6 . . ?
C3 C4 H4A 119.6 . . ?
C4 C5 C6 118.58(15) . . ?
C4 C5 H5A 120.7 . . ?
C6 C5 H5A 120.7 . . ?
C5 C6 C1 120.26(14) . . ?
C5 C6 C7 132.10(15) . . ?
C1 C6 C7 107.61(13) . . ?
C8 C7 C6 108.49(13) . . ?
C8 C7 C14 127.13(14) . . ?
C7 C8 N1 108.08(13) . . ?
C7 C8 C9 127.56(14) . . ?
N1 C8 C9 124.34(13) . . ?
C8 C9 C10 114.05(12) . . ?
C8 C9 H9A 108.7 . . ?
C10 C9 H9A 108.7 . . ?
C8 C9 H9B 108.7 . . ?
C10 C9 H9B 108.7 . . ?
H9A C9 H9B 107.6 . . ?
C11 C10 C9 125.73(15) . . ?
C11 C10 H10A 117.1 . . ?
C9 C10 H10A 117.1 . . ?
C10 C11 C12 121.22(16) . . ?
C10 C11 C13 123.70(16) . . ?
C12 C11 C13 115.03(15) . . ?
C11 C12 H12A 109.5 . . ?
C11 C12 H12B 109.5 . . ?
H12A C12 H12B 109.5 . . ?
C11 C12 H12C 109.5 . . ?
H12A C12 H12C 109.5 . . ?
H12B C12 H12C 109.5 . . ?
C11 C13 H13A 109.5 . . ?
C11 C13 H13B 109.5 . . ?
H13A C13 H13B 109.5 . . ?
C11 C13 H13C 109.5 . . ?
H13A C13 H13C 109.5 . . ?
H13B C13 H13C 109.5 . . ?
C7 C14 N2 115.32(12) . . ?
C7 C14 H14A 108.4 . . ?
N2 C14 H14A 108.4 . . ?
C7 C14 H14B 108.4 . . ?
N2 C14 H14B 108.4 . . ?
H14A C14 H14B 107.5 . . ?
N2 C15 H15A 109.5 . . ?
N2 C15 H15B 109.5 . . ?
H15A C15 H15B 109.5 . . ?
N2 C15 H15C 109.5 . . ?
H15A C15 H15C 109.5 . . ?
H15B C15 H15C 109.5 . . ?
N2 C16 H16A 109.5 . . ?
N2 C16 H16B 109.5 . . ?
H16A C16 H16B 109.5 . . ?
N2 C16 H16C 109.5 . . ?
H16A C16 H16C 109.5 . . ?
H16B C16 H16C 109.5 . . ?
C18 C17 N2 113.48(13) . . ?
C18 C17 H17A 108.9 . . ?
N2 C17 H17A 108.9 . . ?
C18 C17 H17B 108.9 . . ?
N2 C17 H17B 108.9 . . ?
H17A C17 H17B 107.7 . . ?
C19 C18 C17 126.26(16) . . ?
C19 C18 H18A 116.9 . . ?
C17 C18 H18A 116.9 . . ?
C18 C19 C21 125.49(16) . . ?
C18 C19 C20 119.98(17) . . ?
C21 C19 C20 114.51(16) . . ?
C19 C20 H20A 109.5 . . ?
C19 C20 H20B 109.5 . . ?
H20A C20 H20B 109.5 . . ?
C19 C20 H20C 109.5 . . ?
H20A C20 H20C 109.5 . . ?
H20B C20 H20C 109.5 . . ?
C19 C21 H21A 109.5 . . ?
C19 C21 H21B 109.5 . . ?
H21A C21 H21B 109.5 . . ?
C19 C21 H21C 109.5 . . ?
H21A C21 H21C 109.5 . . ?
H21B C21 H21C 109.5 . . ?
O2 C22 O1 126.88(15) . . ?
O2 C22 N1 122.37(14) . . ?
O1 C22 N1 110.75(13) . . ?
O1 C23 C26 109.67(14) . . ?
O1 C23 C25 109.57(14) . . ?
C26 C23 C25 113.22(17) . . ?
O1 C23 C24 101.88(13) . . ?
C26 C23 C24 111.06(16) . . ?
C25 C23 C24 110.85(17) . . ?
C23 C24 H24A 109.5 . . ?
C23 C24 H24B 109.5 . . ?
H24A C24 H24B 109.5 . . ?
C23 C24 H24C 109.5 . . ?
H24A C24 H24C 109.5 . . ?
H24B C24 H24C 109.5 . . ?
C23 C25 H25A 109.5 . . ?
C23 C25 H25B 109.5 . . ?
H25A C25 H25B 109.5 . . ?
C23 C25 H25C 109.5 . . ?
H25A C25 H25C 109.5 . . ?
H25B C25 H25C 109.5 . . ?
C23 C26 H26A 109.5 . . ?
C23 C26 H26B 109.5 . . ?
H26A C26 H26B 109.5 . . ?
C23 C26 H26C 109.5 . . ?
H26A C26 H26C 109.5 . . ?
H26B C26 H26C 109.5 . . ?

_diffrn_measured_fraction_theta_max    0.988
_diffrn_reflns_theta_full              31.96
_diffrn_measured_fraction_theta_full   0.988
_refine_diff_density_max    0.882
_refine_diff_density_min    -0.533
_refine_diff_density_rms    0.075

\( \text{Mol. Wt.: } 423.39 \)

\( \text{C}_{21}\text{H}_{31}\text{BrN}_{2}\text{O}_{2} \)

\( ^1\text{H Name: huis4-25-11 Expno: 4 Procno: 1 } \)
$\text{C}_{16}\text{H}_{22}\text{N}_{2}\text{O}_{2}$

Mol. Wt.: 274.36

$^1\text{H}$ Name: huis5-15-12 Expno: 2 Procno: 1

$^{13}\text{C}$ Name: huis5-15-12 Expno: 3 Procno: 1
$^{13}$C HSQC Name: huis5-15-12 Expno: 4 Procno: 1

HRMS
$^1$H NMR of Boc protected Tryptophan N-diphenyl methylene t-butyl ester

N+ salt characterization
\[
\text{C}_{26}\text{H}_{25}\text{BrN}_3^+
\]

**Exact Mass:** 383.1

**Mol. Wt.:** 384.31

\[
\text{m/e: 383.10 (100.0%), 385.10 (97.5%), 384.10 (22.7%), 386.10 (21.3%), 387.10 (2.4%), 385.11 (2.3%), 386.09 (1.1%)}
\]

**C, 62.50; H, 5.77; Br, 20.79; N, 10.93**

\[
\text{C}_{19}\text{H}_{29}\text{F}_3\text{N}_2\text{O}^+
\]

**Exact Mass:** 349.15

**Mol. Wt.:** 349.37

\[
\text{m/e: 349.15 (100.0%), 350.16 (20.8%), 351.16 (2.3%)}
\]

**C, 65.32; H, 5.77; F, 16.31; N, 8.02; O, 4.38**
Carbon Spectrum
N+ salt rinsed CH2Cl2 and filtered over vac in d6 DMSO
Proton Spectrum
gramine N+ salt in d6 DMSO
Washed over vac filtration

$\frac{1.99}{3.82} = \frac{56833}{382}$ yield

$6$ Aromatic Phenyl

$4$ Aromatic Indole

---

205
**Eager 200 Strip-Chart**

Filename: C:\EAW\J200911.DAT
Sample name: HN001 Analyzed: 10-28-09 11:57

**Eager 200 Report**

S. version: 1.06
Operator ID: LAB
Method Name: Minute8
Analysed: 10-28-09 11:57

Company Name: UWM Chemistry
Method File: MINUTE8.MTH
Printed: 10/28/2009 12:06

Sample ID: HN001 (# 11)
Analysis Type: UnKOWN (Area)
Chromatogram: C:\EAW\J200911.DAT
Channel: E.A. Channel A
Sample weight: 2.006

Calib. method: using 'K Factors'

<table>
<thead>
<tr>
<th>Element Name</th>
<th>Element %</th>
<th>Ret. Time</th>
<th>Area</th>
<th>BC</th>
<th>Area ratio</th>
<th>K factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>10.0081</td>
<td>0.74</td>
<td>498618</td>
<td>FU</td>
<td>13.387040</td>
<td>.247999E+07</td>
</tr>
<tr>
<td>Carbon</td>
<td>59.4638</td>
<td>1.06</td>
<td>6675412</td>
<td>FU</td>
<td>1.000000</td>
<td>.559125E+07</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>5.6060</td>
<td>3.31</td>
<td>1841161</td>
<td>RS</td>
<td>3.625653</td>
<td>.163442E+08</td>
</tr>
</tbody>
</table>

\[ C_{38}H_{44}N_{2}O_{4} \]
Mol. Wt.: 592.77

\^1H Name: huis7-20-10 Expno: 6 Procno: 1

\^13C Name: huis7-20-10 Expno: 7 Procno: 1

\^13C HSQC Name: huis7-20-10 Expno: 8 Procno: 1
proton spectrum
bottom spot from column from 3 run
carbon spectrum
bottom spot isolated from H-exchange
N\H\NH_2\O\O\C_20H_28N_2O_2\Mol. Wt.: 328.45
\(^1\)H Name: huis2-20-12 Expno: 1 Procno: 1

\(^{13}\)C Name: huis2-20-12 Expno: 2 Procno: 1

HSQC Name: huis2-20-12 Expt: 3 Procno: 1
$\text{C}_{26}\text{H}_{35}\text{N}_{3}\text{O}_{3}$

Mol. Wt.: 425.56

$^1\text{H Name: huis4-9-12 Expno: 3 Procno: 1}$

$^{13}\text{C Name: huis4-9-12 Expno: 4 Procno: 1}$

$^{13}\text{C HSQC Name: huis4-9-12 Expno: 5 Procno: 1}$

$^{13}\text{C Dept135 Name: huis4-9-12 Expno: 6 Procno: 1}$

HRMS
$^1$H Name: huis--13 Expno: 3 Procno: 1

$^{13}$C Name: huis--13 Expno: 4 Procno: 1

$^{13}$C HSQC Name: huis--13 Expno: 5 Procno: 1

HPLC Racemic 9-28-2010 11 27 33
**SAMPLE INFORMATION**

- **Sample Name:** Unk
- **Sample Type:** Unknown
- **Vial:** 1
- **Injection #:** 1
- **Injection Volume:** 5.00 ul
- **Run Time:** 25.00 Minutes
- **Sampling Rate:** 10.00 per sec

**Sample Values:**
- **Injection Volume =** 5.00
- **Sample Weight =** 1.00000
- **Dilution =** 1.00000

**Sample Set Name:** racemic tip

**Peak Name** | **RT (min)** | **Peak Type** | **Area (µV*sec)** | **% Area** | **Height (µV)** | **% Height** | **Integration Type** | **Response** | **Peak Codes**
---|---|---|---|---|---|---|---|---|---
1 | Rt | 15.999 | Found | 566447 | 50.15 | 14127 | 55.70 | BV | 5.664e+005 | Q20
2 | St | 17.747 | Found | 563089 | 49.85 | 11235 | 44.30 | VB | 5.631e+005 | Q20
Racemic Trp

**Acquisition Log**

- **Acquired By**: Breeze
- **Injection**: 1
- **Date Acquired**: 9/28/2010 10:30:22 AM CDT
- **Run Time**: 25.00 (Minutes)
- **Acq Method Set**: Chiralcel OD 09022010
- **Injection Volume**: 5.00 (µL)
- **Injection Id**: 1685
- **Instrument Method Name**: Chiralcel OD
- **Suppressed**: No

**Table**

<table>
<thead>
<tr>
<th>Points Across Peak</th>
<th>Start Time (min)</th>
<th>End Time (min)</th>
<th>Baseline Start (min)</th>
<th>Baseline End (min)</th>
<th>Slope (µV/sec)</th>
<th>Offset (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.16</td>
<td>15.153</td>
<td>16.847</td>
<td>15.153</td>
<td>3.831099e-005</td>
<td>-3.128393e-004</td>
</tr>
</tbody>
</table>
HPLC of chiral trp 9-21-2010 time 3 32 18
Sample Information

Sample Name: Chiral Trp 20x in mp
Sample Type: Unknown
Vial: 1
Injection #: 1
Injection Volume: 20.00 ul
Run Time: 22.00 Minutes
Sampling Rate: 10.00 per sec

Acquired By: Breeze
Date Acquired: 9/21/2010 2:43:16 PM CDT

Processed By: Breeze/Breeze
Date Processed: 9/21/2010 3:32:18 PM CDT
Channel Name: 2598 Ch1 254nm@1.2nm
Channel Desc.: 2098 Ch1 254nm@1.2nm
Sample Set Name: Chiral OD 09212010

Sample Values
Injection Volume = 20.00 Sample Weight = 1.00000 Dilution = 1.00000

Used in Calculation:

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (uV*sec)</th>
<th>% Area</th>
<th>Height (uV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak1</td>
<td>3.558</td>
<td>Found</td>
<td>35279369</td>
<td>72.13</td>
<td>3018113</td>
<td>79.42</td>
<td>VV</td>
<td>3.528e+007</td>
</tr>
<tr>
<td>Peak2</td>
<td>3.959</td>
<td>Found</td>
<td>1568257</td>
<td>3.21</td>
<td>110235</td>
<td>2.90</td>
<td>VV</td>
<td>1.568e+006</td>
</tr>
<tr>
<td>Peak3</td>
<td>5.003</td>
<td>Found</td>
<td>1673874</td>
<td>3.42</td>
<td>154911</td>
<td>4.08</td>
<td>VV</td>
<td>1.674e+006</td>
</tr>
<tr>
<td>Peak4</td>
<td>5.417</td>
<td>Found</td>
<td>4071960</td>
<td>6.33</td>
<td>374097</td>
<td>9.84</td>
<td>VV</td>
<td>4.072e+006</td>
</tr>
</tbody>
</table>

Report Method: Detailed Individual Report
Printed: 4/12/2012 12:54:00 PM US/Central
### UV - Milwaukee

**Project Name:** M Hulsman  
**Reported by User:** Breeze user (Breeze)

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (µV * sec)</th>
<th>% Area</th>
<th>Height (µV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 5</td>
<td>6.483</td>
<td>Found</td>
<td>240682</td>
<td>0.49</td>
<td>5680</td>
<td>0.15</td>
<td>VV</td>
<td>2.407e+005</td>
</tr>
<tr>
<td>Peak 6</td>
<td>7.702</td>
<td>Found</td>
<td>128651</td>
<td>0.26</td>
<td>2511</td>
<td>0.07</td>
<td>VV</td>
<td>1.287e+005</td>
</tr>
<tr>
<td>Peak 7</td>
<td>9.708</td>
<td>Found</td>
<td>197977</td>
<td>0.41</td>
<td>5727</td>
<td>0.15</td>
<td>VV</td>
<td>1.968e+005</td>
</tr>
<tr>
<td>Peak 8</td>
<td>10.665</td>
<td>Found</td>
<td>201239</td>
<td>0.41</td>
<td>5806</td>
<td>0.15</td>
<td>VB</td>
<td>2.012e+005</td>
</tr>
<tr>
<td>Peak 9</td>
<td>14.573</td>
<td>Found</td>
<td>216465</td>
<td>0.44</td>
<td>5107</td>
<td>0.13</td>
<td>BV</td>
<td>2.155e+005</td>
</tr>
<tr>
<td>Peak 10</td>
<td>15.889</td>
<td>Found</td>
<td>3585419</td>
<td>7.33</td>
<td>77144</td>
<td>2.03</td>
<td>VV</td>
<td>3.585e+006</td>
</tr>
<tr>
<td>Peak 11</td>
<td>17.515</td>
<td>Found</td>
<td>1076641</td>
<td>2.20</td>
<td>19664</td>
<td>0.52</td>
<td>VB</td>
<td>1.077e+006</td>
</tr>
<tr>
<td>Peak 12</td>
<td>21.617</td>
<td>Found</td>
<td>668238</td>
<td>1.37</td>
<td>26632</td>
<td>0.55</td>
<td>BB</td>
<td>6.862e+005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak Code</th>
<th>Points Across Peak</th>
<th>Start Time (min)</th>
<th>End Time (min)</th>
<th>Baseline Start (min)</th>
<th>Baseline End (min)</th>
<th>Slope (µV/µsec)</th>
<th>Offset (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q20</td>
<td>344</td>
<td>3.262</td>
<td>3.835</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>552</td>
<td>3.835</td>
<td>4.755</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>283</td>
<td>4.755</td>
<td>5.227</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>636</td>
<td>5.227</td>
<td>6.287</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>667</td>
<td>6.287</td>
<td>7.398</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>647</td>
<td>7.398</td>
<td>8.477</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>638</td>
<td>9.152</td>
<td>10.215</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>912</td>
<td>10.215</td>
<td>11.735</td>
<td>2.573</td>
<td>11.735</td>
<td>2.794361e-004</td>
<td>-1.081062e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>970</td>
<td>13.460</td>
<td>15.077</td>
<td>13.460</td>
<td>20.165</td>
<td>-1.624012e-005</td>
<td>2.170612e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>1081</td>
<td>15.077</td>
<td>16.878</td>
<td>13.460</td>
<td>20.165</td>
<td>-1.624012e-005</td>
<td>2.170612e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>1972</td>
<td>16.878</td>
<td>20.165</td>
<td>13.460</td>
<td>20.165</td>
<td>-1.624012e-005</td>
<td>2.170612e-003</td>
</tr>
<tr>
<td>Q20</td>
<td>599</td>
<td>21.000</td>
<td>21.996</td>
<td>21.000</td>
<td>21.996</td>
<td>6.419375e-002</td>
<td>-1.333622e+000</td>
</tr>
</tbody>
</table>

### Acquisition Log

Acquired By: Breeze  
Injection: 1  
Date Acquired: 9/21/2010 2:43:16 PM CDT  
Run Time: 22.00 (Minutes)  
AIC Method Set: Chiralcol CD 09212010  
Injection Volume: 20.00 (µl)

Report Method: Detailed Individual Report  
Printed: 4/12/2012  
Page: 2 of 3  
12:54:00 PM US/Central
Chiral Trp
<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>% Area</th>
<th>Height</th>
<th>Int Type</th>
<th>Amount</th>
<th>Units</th>
<th>Peak Type</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak10</td>
<td>15.869</td>
<td>35854</td>
<td>19</td>
<td>7.33</td>
<td>VV</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
<tr>
<td>Peak11</td>
<td>17.515</td>
<td>10766</td>
<td>41</td>
<td>2.20</td>
<td>VB</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
</tbody>
</table>
Carbon Spectrum

Current Data Parameters
NAME: mu072710
PROCID: 1

F2 - Acquisition Parameters
Date: 20100727
Time: 11:02
INSTRUM: dpx300
PROCEDURE: 5 mm Multin
POLARIZATION: zpgc
TD: 45000
SOYENT: CDCl3
NS: 1024
DS: 0
SM: 18115.941 Hz
NOCRES: 0.276427 Hz
NQ: 1.8088436 sec
EG: 616
DW: 27.600 usec
DE: 6.00 usec
TR: 360.0 s
TD1: 0.10000000 sec
dl1: 0.00000000 sec

----------- CHANNEL f1 -----------
NC1: 12C
P1: 6.380 usec
PL1: -6.00 dB
SF1: 75.4078669 MHz

----------- CHANNEL f2 -----------
CT1: 16
NC2: 16
CT2: 100.00 usec
PL2: -6.00 dB
PH12: 16.00 dB
SF2: 360.1312058 MHz

F2 - Processing parameters
SI: 32768
SP: 75.4577867 MHz
MDM: BM
SSN: 0
LB: 3.00 Hz
GR: 0
PC: 1.40

1D NMR plot parameters
CT: 22.00 cm
F1P: 145.000 ppm
F2P: 10.488 s
FT: 100.000 ppm
F1: 7546.78 Hz
F2: 2.04845 ppm/cm
M: 154.35688 Hz/cm
5MeOTRP

Theoretical (M+H)+ = 465.2235
Accuracy = 4.0 ppm

Mel: 56.760 counts.
Sample Information

Sample Name: Unk
Sample Type: Unknown
Vial: 1
Injection #: 1
Injection Volume: 5.00 ul
Run Time: 90.00 Minutes
Sampling Rate: 10.00 per sec

Acquired By: Breeze
Date Acquired: 10/12/2010 6:33:57 PM CDT
Acq. Method: Chiralcel OD

Processed By: Breeze
Date Processed: 11/22/2010 12:24:37 PM CST
Channel Name: 2998 Ch1 254nm@1.2nm
Channel Desc.: 2998 Ch1 254nm@1.2nm
Sample Set Name: 5 methoxy trp

Sample Values
Injection Volume = 5.00
Sample Weight = 1.00000
Dilution = 1.00000

Used in Calculation:

Peak Name | RT (min) | Peak Type | Area (µV*sec) | % Area | Height (µV) | % Height | Integration Type | Response
---|---|---|---|---|---|---|---|---
1 | S 5 methoxy trp | 58.480 | Found | 1131332 | 49.01 | 7599 | 52.28 | BV | 1.131e+006
2 | R 5 methoxy trp | 63.288 | Found | 1176911 | 50.99 | 6935 | 47.72 | VB | 1.177e+006
Acquisition Log

Acquired By: Breeze
Injection: 1
Date Acquired: 10/12/2010 6:33:57 PM CDT
Run Time: 90.00 (Minutes)
Acq Method Set: Chiralcel OD
Injection Volume: 5.00 (µL)
Injection Id: 2119
Instrument Method Name: Chiralcel OD
Superseded: No
### Table

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>% Area</th>
<th>Height</th>
<th>Int Type</th>
<th>Amount</th>
<th>Units</th>
<th>Peak Type</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 5 methoxy trp</td>
<td>58.480</td>
<td>1131332</td>
<td>49.01</td>
<td>7599</td>
<td>BV</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
<tr>
<td>S 5 methoxy trp</td>
<td>63.288</td>
<td>1176911</td>
<td>50.99</td>
<td>6935</td>
<td>VB</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
</tbody>
</table>

**Racemic 5-methoxy trp**

Solvent: 2% IPA/Hexane; Flow rate: 1mL/min
**Sample Information**

**Sample Name:** Unknown  
**Sample Type:** Unknown  
**Injection #:** 1  
**Injection Volume:** 5.00 ml  
**Run Time:** 90.00 Minutes  
**Sampling Rate:** 10.00 per sec  

**Acquired By:** Breeze  
**Date Acquired:** 10/12/2010 4:33:54 PM CDT  
**Acq. Method:** Chiral OD  
**Date Processed:** 11/16/2011 3:52:03 PM CST  
**Channel Name:** 2998 Ch1 254nm@1.2nm  
**Channel Desc.:** 2998 Ch1 254nm@1.2nm  
**Sample Set Name:** 5 methoxy trp  

**Sample Values:** Injection Volume = 5.00 Sample/Weight = 1.00000 Dilution = 1.00000

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area</th>
<th>% Area</th>
<th>Height (µV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak1</td>
<td>5.084</td>
<td>Found</td>
<td>221592</td>
<td>9.97</td>
<td>4354</td>
<td>10.54</td>
<td>BV</td>
<td>2.216e+006</td>
</tr>
<tr>
<td>Peak2</td>
<td>6.450</td>
<td>Found</td>
<td>72004</td>
<td>3.24</td>
<td>4466</td>
<td>19.01</td>
<td>VV</td>
<td>7.200e+004</td>
</tr>
<tr>
<td>Peak3</td>
<td>7.214</td>
<td>Found</td>
<td>13569</td>
<td>0.61</td>
<td>190</td>
<td>0.81</td>
<td>VV</td>
<td>1.357e+004</td>
</tr>
<tr>
<td>Peak4</td>
<td>12.021</td>
<td>Found</td>
<td>37427</td>
<td>1.68</td>
<td>1112</td>
<td>4.74</td>
<td>VV</td>
<td>3.743e+004</td>
</tr>
</tbody>
</table>

Report Method: Detailed Individual Report  
Printed: 11/16/2011 3:52:23 PM U/0/Central  
Page: 1 of 2
UW - Milwaukee

Project Name: M Hulman
Reported by User: Breeze user (Breeze)

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (µV·sec)</th>
<th>% Area</th>
<th>Height (µV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 5</td>
<td>13.714</td>
<td>Found</td>
<td>16608</td>
<td>0.75</td>
<td>364</td>
<td>1.55</td>
<td>VB</td>
<td>1.661e+004</td>
</tr>
<tr>
<td>Peak 6</td>
<td>29.521</td>
<td>Found</td>
<td>190632</td>
<td>8.55</td>
<td>2080</td>
<td>8.86</td>
<td>BB</td>
<td>1.906e+005</td>
</tr>
<tr>
<td>R 5 MeO tp</td>
<td>55.202</td>
<td>Found</td>
<td>59332</td>
<td>2.67</td>
<td>507</td>
<td>2.16</td>
<td>BV</td>
<td>5.933e+004</td>
</tr>
<tr>
<td>S 5 MeO tp</td>
<td>62.282</td>
<td>Found</td>
<td>1610677</td>
<td>72.49</td>
<td>10415</td>
<td>44.34</td>
<td>VB</td>
<td>1.611e+006</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak Codes</th>
<th>Points Across Peak</th>
<th>Start Time (min)</th>
<th>End Time (min)</th>
<th>Baseline Start (min)</th>
<th>Baseline End (min)</th>
<th>Slope (µV/sec)</th>
<th>Offset (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Q20</td>
<td>2037</td>
<td>2.255</td>
<td>5.650</td>
<td>2.255</td>
<td>15.425</td>
<td>8.321954e+006</td>
<td>-1.356598e-005</td>
</tr>
<tr>
<td>2 Q20</td>
<td>670</td>
<td>5.650</td>
<td>5.757</td>
<td>2.255</td>
<td>15.425</td>
<td>8.321954e+006</td>
<td>-1.356598e-005</td>
</tr>
<tr>
<td>3 Q20</td>
<td>1608</td>
<td>6.767</td>
<td>9.447</td>
<td>2.255</td>
<td>15.425</td>
<td>8.321954e+006</td>
<td>-1.356598e-005</td>
</tr>
<tr>
<td>4 Q20</td>
<td>2151</td>
<td>9.447</td>
<td>13.032</td>
<td>2.255</td>
<td>15.425</td>
<td>8.321954e+006</td>
<td>-1.356598e-005</td>
</tr>
<tr>
<td>5 Q20</td>
<td>1436</td>
<td>13.032</td>
<td>15.425</td>
<td>2.255</td>
<td>15.425</td>
<td>8.321954e+006</td>
<td>-1.356598e-005</td>
</tr>
<tr>
<td>6 Q20</td>
<td>2396</td>
<td>27.888</td>
<td>31.882</td>
<td>27.888</td>
<td>31.882</td>
<td>9.040270e+006</td>
<td>2.875150e+007</td>
</tr>
<tr>
<td>7 Q20</td>
<td>1800</td>
<td>56.123</td>
<td>59.123</td>
<td>56.123</td>
<td>67.407</td>
<td>1.231906e-005</td>
<td>-1.319265e-004</td>
</tr>
<tr>
<td>8 Q20</td>
<td>4970</td>
<td>59.123</td>
<td>67.407</td>
<td>56.123</td>
<td>67.407</td>
<td>1.231906e-005</td>
<td>-1.319265e-004</td>
</tr>
</tbody>
</table>

Acquisition Log

Acquired By: Breeze
Injection: 1
Date Acquired: 10/12/2010 4:33:54 PM CST
Run Time: 50.00 (Minutes)
Acq Method Set: Chiralcel OD
Injection Volume: 6.00 (µL)
Injection Id: 2101
Instrument Method Name: Chiralcel OD
Superseded: No
<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>% Area</th>
<th>Height</th>
<th>Int Type</th>
<th>Amount</th>
<th>Units</th>
<th>Peak Type</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 5 MeO trp</td>
<td>58.202</td>
<td>76088</td>
<td>4.41</td>
<td>584</td>
<td>VV</td>
<td>Found</td>
<td>Q20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S 5 MeO trp</td>
<td>62.282</td>
<td>1650931</td>
<td>95.59</td>
<td>10491</td>
<td>VB</td>
<td>Found</td>
<td>Q20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Compound with 91 % ee
6MeOTRP

Theoretical (M+H+) = 456.2335
Accuracy = 3.1 ppm
## Peak Table

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (μV/sec)</th>
<th>% Area</th>
<th>Height (μV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak5</td>
<td>21.669</td>
<td>Found</td>
<td>94079</td>
<td>2.74</td>
<td>2305</td>
<td>5.77</td>
<td>BB</td>
<td>9.409e+004</td>
</tr>
<tr>
<td>S MeOtrp</td>
<td>38.472</td>
<td>Found</td>
<td>1474660</td>
<td>42.96</td>
<td>19221</td>
<td>38.16</td>
<td>BV</td>
<td>1.474e+006</td>
</tr>
<tr>
<td>R MeOtrp</td>
<td>40.912</td>
<td>Found</td>
<td>1499449</td>
<td>43.69</td>
<td>18230</td>
<td>36.19</td>
<td>VB</td>
<td>1.496e+006</td>
</tr>
</tbody>
</table>

## Acquisition Log

- **Acquired By:** Breeze
- **Injection:** 1
- **Date Acquired:** 11/1/2010 11:32:26 AM CST
- **Run Time:** 60.00 (Minutes)
- **Acq Method Set:** Chiralcel OD
- **Injection Volume:** 5.00 (μL)
- **Injection Id:** 3002
- **Instrument Method Name:** Chiralcel OD
- **Superseded:** No
Racemic 6-methoxy trp

Solvent: 10% IPA Hexane; Flow rate : 0.3mL/min
### SAMPLE INFORMATION

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Unknown</th>
<th>Acquired By</th>
<th>Breeze</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
<td>Unknown</td>
<td>Date Acquired</td>
<td>11/1/2010 12:34:48 PM CDT</td>
</tr>
<tr>
<td>Vial</td>
<td>1</td>
<td>Acq. Method</td>
<td>Chiral OD</td>
</tr>
<tr>
<td>Injection</td>
<td>1</td>
<td>Processed By</td>
<td>Breeze</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>5.00 ul</td>
<td>Date Processed</td>
<td>11/16/2011 3:45:46 PM CST</td>
</tr>
<tr>
<td>Run Time</td>
<td>60.00 Minutes</td>
<td>Channel Name</td>
<td>2998 Ch1 <a href="mailto:254nm@1.2nm">254nm@1.2nm</a></td>
</tr>
<tr>
<td>Sampling Rate</td>
<td>10.00 per sec</td>
<td>Channel Desc.</td>
<td>2998 Ch1 <a href="mailto:254nm@1.2nm">254nm@1.2nm</a></td>
</tr>
<tr>
<td>Sample Values</td>
<td>Injection Volume = 5.00 Sample Weight = 1.00000 Dilution = 1.00000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used In Calculation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Chromatogram

#### Peak Table

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (µV sec)</th>
<th>% Area</th>
<th>Height (µV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak1</td>
<td>10.134</td>
<td>Found</td>
<td>69447</td>
<td>0.42</td>
<td>2232</td>
<td>0.81</td>
<td>BV</td>
<td>6.545e+04</td>
</tr>
<tr>
<td>Peak2</td>
<td>10.972</td>
<td>Found</td>
<td>11185</td>
<td>0.68</td>
<td>4526</td>
<td>1.68</td>
<td>VV</td>
<td>1.119e+05</td>
</tr>
<tr>
<td>Peak3</td>
<td>11.456</td>
<td>Found</td>
<td>258867</td>
<td>1.57</td>
<td>9223</td>
<td>3.35</td>
<td>VV</td>
<td>2.586e+05</td>
</tr>
<tr>
<td>Peak4</td>
<td>12.766</td>
<td>Found</td>
<td>321070</td>
<td>1.95</td>
<td>5569</td>
<td>2.02</td>
<td>VV</td>
<td>3.211e+05</td>
</tr>
</tbody>
</table>

Report Method: Detailed Individual Report

Printed: 11/16/2011 3:46:12 PM U/0/Central

Page: 1 of 3
**UW - Milwaukee**

**Project Name:** M. Hulians

**Reported by User:** Breeze user (Breeze)

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (µV/sec)</th>
<th>% Area</th>
<th>Height (µV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 5</td>
<td>13.25</td>
<td>Found</td>
<td>123295</td>
<td>0.75</td>
<td>4927</td>
<td>1.79</td>
<td>VV</td>
<td>1.239e+005</td>
</tr>
<tr>
<td>Peak 6</td>
<td>13.74</td>
<td>Found</td>
<td>129256</td>
<td>0.78</td>
<td>5154</td>
<td>1.87</td>
<td>VV</td>
<td>1.239e+005</td>
</tr>
<tr>
<td>Peak 7</td>
<td>14.39</td>
<td>Found</td>
<td>257354</td>
<td>1.66</td>
<td>5501</td>
<td>2.00</td>
<td>VV</td>
<td>2.575e+005</td>
</tr>
<tr>
<td>Peak 8</td>
<td>15.44</td>
<td>Found</td>
<td>134944</td>
<td>0.82</td>
<td>4500</td>
<td>1.64</td>
<td>VV</td>
<td>1.349e+005</td>
</tr>
<tr>
<td>Peak 9</td>
<td>16.39</td>
<td>Found</td>
<td>1271544</td>
<td>7.71</td>
<td>49415</td>
<td>17.96</td>
<td>VV</td>
<td>1.272e+005</td>
</tr>
<tr>
<td>Peak 10</td>
<td>17.77</td>
<td>Found</td>
<td>43665</td>
<td>0.26</td>
<td>1126</td>
<td>0.41</td>
<td>VB</td>
<td>4.361e+004</td>
</tr>
<tr>
<td>Peak 11</td>
<td>20.77</td>
<td>Found</td>
<td>397992</td>
<td>2.17</td>
<td>6135</td>
<td>2.39</td>
<td>BV</td>
<td>3.572e+005</td>
</tr>
<tr>
<td>Peak 12</td>
<td>21.72</td>
<td>Found</td>
<td>172180</td>
<td>1.04</td>
<td>4531</td>
<td>1.65</td>
<td>VV</td>
<td>1.722e+005</td>
</tr>
<tr>
<td>Peak 13</td>
<td>23.16</td>
<td>Found</td>
<td>8202</td>
<td>0.06</td>
<td>320</td>
<td>0.12</td>
<td>VB</td>
<td>8.202e+003</td>
</tr>
<tr>
<td>Peak 14</td>
<td>24.16</td>
<td>Found</td>
<td>48657</td>
<td>0.30</td>
<td>1954</td>
<td>0.55</td>
<td>BB</td>
<td>4.859e+004</td>
</tr>
<tr>
<td>Peak 15</td>
<td>33.34</td>
<td>Found</td>
<td>190547</td>
<td>11.59</td>
<td>26490</td>
<td>9.61</td>
<td>BB</td>
<td>1.510e+005</td>
</tr>
<tr>
<td>Peak 16</td>
<td>36.60</td>
<td>Found</td>
<td>35832</td>
<td>0.22</td>
<td>955</td>
<td>0.31</td>
<td>BV</td>
<td>3.556e+004</td>
</tr>
<tr>
<td>S 5 MeO</td>
<td>38.50</td>
<td>Found</td>
<td>10099731</td>
<td>61.23</td>
<td>128171</td>
<td>46.59</td>
<td>VV</td>
<td>1.010e+007</td>
</tr>
<tr>
<td>R 5 MeO</td>
<td>41.24</td>
<td>Found</td>
<td>505093</td>
<td>3.06</td>
<td>6235</td>
<td>2.27</td>
<td>VV</td>
<td>5.051e+005</td>
</tr>
<tr>
<td>Peak 19</td>
<td>43.64</td>
<td>Found</td>
<td>634727</td>
<td>3.95</td>
<td>6038</td>
<td>2.19</td>
<td>VB</td>
<td>6.347e+005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak Codes</th>
<th>Points Across Peak</th>
<th>Start Time (min)</th>
<th>End Time (min)</th>
<th>Baseline Start (min)</th>
<th>Baseline End (min)</th>
<th>Slope (µV/sec)</th>
<th>Offset (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q30</td>
<td>661</td>
<td>10.418</td>
<td>11.159</td>
<td>9.347</td>
<td>15.608</td>
<td>7.3420906e-005</td>
<td>-3.492407e+004</td>
</tr>
<tr>
<td>Q20</td>
<td>475</td>
<td>11.159</td>
<td>11.950</td>
<td>9.347</td>
<td>15.608</td>
<td>7.3420906e-005</td>
<td>-3.492407e+004</td>
</tr>
<tr>
<td>Q20</td>
<td>656</td>
<td>13.977</td>
<td>15.053</td>
<td>9.347</td>
<td>15.608</td>
<td>7.3420906e-005</td>
<td>-3.492407e+004</td>
</tr>
<tr>
<td>Q20</td>
<td>486</td>
<td>15.053</td>
<td>15.863</td>
<td>9.347</td>
<td>15.608</td>
<td>7.3420906e-005</td>
<td>-3.492407e+004</td>
</tr>
<tr>
<td>Q20</td>
<td>922</td>
<td>15.863</td>
<td>17.400</td>
<td>9.347</td>
<td>15.608</td>
<td>7.3420906e-005</td>
<td>-3.492407e+004</td>
</tr>
<tr>
<td>Q20</td>
<td>725</td>
<td>17.400</td>
<td>18.608</td>
<td>9.347</td>
<td>15.608</td>
<td>7.3420906e-005</td>
<td>-3.492407e+004</td>
</tr>
<tr>
<td>Q20</td>
<td>924</td>
<td>19.788</td>
<td>21.328</td>
<td>19.788</td>
<td>23.565</td>
<td>1.0297444e+04</td>
<td>-1.116991e+003</td>
</tr>
<tr>
<td>Q20</td>
<td>832</td>
<td>21.328</td>
<td>22.715</td>
<td>19.788</td>
<td>23.565</td>
<td>1.0297444e+04</td>
<td>-1.116991e+003</td>
</tr>
</tbody>
</table>

**Report Method:** Detailed Individual Report

**Printed:** 11/16/2011

Page: 3 of 3

3:46:13 PM US/Central
### Acquisition Log

<table>
<thead>
<tr>
<th>Acquired By</th>
<th>Breeze</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection</td>
<td>1</td>
</tr>
<tr>
<td>Date Acquired</td>
<td>11/1/2012 12:34:48 PM CDT</td>
</tr>
<tr>
<td>Run Time</td>
<td>60.00 (Minutes)</td>
</tr>
<tr>
<td>Acq Method Set</td>
<td>Chiralcel OD</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>5.00 (µL)</td>
</tr>
<tr>
<td>Instrument Method Name</td>
<td>Chiralcel OD</td>
</tr>
<tr>
<td>Superseded</td>
<td>No</td>
</tr>
</tbody>
</table>

User stopped flow at 59.45 minutes
<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>% Area</th>
<th>Height</th>
<th>Int Type</th>
<th>Amount</th>
<th>Units</th>
<th>Peak Type</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 6 MeO trp</td>
<td>38.508</td>
<td>1009973</td>
<td>70.04</td>
<td>128171</td>
<td>VV</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
<tr>
<td>R 6 MeO trp</td>
<td>41.243</td>
<td>505093</td>
<td>3.50</td>
<td>6235</td>
<td>VV</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
</tbody>
</table>

Compound with 95 %ee
### UW - Milwaukee

**Project Name:** M Hulahan  
**Reported by User:** Breeze user (Breeze)

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (μV·sec)</th>
<th>% Area</th>
<th>Height (μV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Peak5</td>
<td>3.917</td>
<td>Found</td>
<td>12322</td>
<td>0.62</td>
<td>1366</td>
<td>1.50</td>
<td>VV</td>
<td>1.232e+004</td>
<td>020</td>
</tr>
<tr>
<td>6 Peak6</td>
<td>4.181</td>
<td>Found</td>
<td>7180</td>
<td>0.36</td>
<td>703</td>
<td>0.76</td>
<td>VB</td>
<td>7.180e+003</td>
<td>020</td>
</tr>
<tr>
<td>7 Peak7</td>
<td>4.556</td>
<td>Found</td>
<td>119943</td>
<td>6.02</td>
<td>13010</td>
<td>14.12</td>
<td>BV</td>
<td>1.199e+005</td>
<td>020</td>
</tr>
<tr>
<td>8 Peak8</td>
<td>4.846</td>
<td>Found</td>
<td>59473</td>
<td>2.99</td>
<td>4750</td>
<td>5.15</td>
<td>VV</td>
<td>5.947e+004</td>
<td>020</td>
</tr>
<tr>
<td>9 Peak9</td>
<td>5.462</td>
<td>Found</td>
<td>232109</td>
<td>11.66</td>
<td>25361</td>
<td>27.45</td>
<td>VB</td>
<td>2.321e+005</td>
<td>020</td>
</tr>
<tr>
<td>10 Peak10</td>
<td>6.837</td>
<td>Found</td>
<td>3969</td>
<td>0.20</td>
<td>269</td>
<td>0.29</td>
<td>BB</td>
<td>3.969e+003</td>
<td>020</td>
</tr>
<tr>
<td>11 Peak11</td>
<td>5.364</td>
<td>Found</td>
<td>90131</td>
<td>3.32</td>
<td>4622</td>
<td>5.02</td>
<td>BE</td>
<td>5.613e+004</td>
<td>020</td>
</tr>
<tr>
<td>12 Peak12</td>
<td>5.952</td>
<td>Found</td>
<td>11482</td>
<td>0.58</td>
<td>428</td>
<td>0.46</td>
<td>VB</td>
<td>1.148e+004</td>
<td>020</td>
</tr>
<tr>
<td>14 S.B. B.P</td>
<td>15.100</td>
<td>Found</td>
<td>736576</td>
<td>35.99</td>
<td>17894</td>
<td>19.42</td>
<td>VB</td>
<td>7.366e+005</td>
<td>020</td>
</tr>
</tbody>
</table>

### Acquisition Log

**Acquired By:** Breeze  
**Report Method:** Detailed Individual Report  
**Printed:** 11/16/2011  
**Page:** 3 of 3  
**3:31:55 PM US/Central**
<table>
<thead>
<tr>
<th><strong>UW - Milwaukee</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Name</strong></td>
<td>M Hulanek</td>
</tr>
<tr>
<td><strong>Reported by User</strong></td>
<td>Breeze user (Breeze)</td>
</tr>
<tr>
<td><strong>Injection</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Date Acquired</strong></td>
<td>10/27/2010 11:17:14 AM CDT</td>
</tr>
<tr>
<td><strong>Run Time</strong></td>
<td>25.00 (Minutes)</td>
</tr>
<tr>
<td><strong>Acq Method Set</strong></td>
<td>Chiralcel OD</td>
</tr>
<tr>
<td><strong>Injection Volume</strong></td>
<td>5.00 (uL)</td>
</tr>
<tr>
<td><strong>Injection Id</strong></td>
<td>2644</td>
</tr>
<tr>
<td><strong>Instrument Method Name</strong></td>
<td>Chiralcel OD</td>
</tr>
<tr>
<td><strong>Superseded</strong></td>
<td>No</td>
</tr>
</tbody>
</table>

---

**Report Method:** Detailed Individual Report  
**Printed:** 11/16/2011  
**Page:** 3 of 3  
**3:31:48 PM UST/Central**
<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>% Area</th>
<th>Height</th>
<th>Int Type</th>
<th>Amount</th>
<th>Units</th>
<th>Peak Type</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 R 5 br trp</td>
<td>13.749</td>
<td>719288</td>
<td>49.41</td>
<td>19144</td>
<td>BV</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
<tr>
<td>2 S 5 br trp</td>
<td>15.100</td>
<td>736576</td>
<td>50.59</td>
<td>17894</td>
<td>VB</td>
<td></td>
<td></td>
<td>Found</td>
<td>Q20</td>
</tr>
</tbody>
</table>

Racemic 5-bromo trp

Solvent: 5% IPA Hexane; Flow rate: 1mL/min
### Sample Information

**Sample Name:** Unknown  
**Acquired By:** Breeze  
**Sample Type:** Unknown  
**Date Acquired:** 10/27/2010 12:56:33 PM CDT  
**Vial:** 1  
**Acq. Method:** Chiral OD  
**Injection #:** 1  
**Date Processed:** 11/16/2011 3:26:15 PM CDT  
**Run Time:** 25.00 Minutes  
**Channel Name:** 2988 Chn 254nm@1.2nm  
**Sampling Rate:** 10.00 per sec  
**Channel Desc.:** 2988 Chn 254nm@1.2nm  
**Sample Set Name:** 5 or 10 or 10  
**Sample Values:**  
**Injection Volume:** 5.00 uL  
**Sample Weight:** 1.00000  
**Dilution:** 1.00000  
**Used in Calculation:**

---

### Chromatogram

![Chromatogram](image_url)  

### Integration Data

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Peak Type</th>
<th>Area (µV/sec)</th>
<th>% Area</th>
<th>Height (µV)</th>
<th>% Height</th>
<th>Integration Type</th>
<th>Response</th>
<th>Peak Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.532</td>
<td>Found</td>
<td>3925</td>
<td>0.25</td>
<td>1750</td>
<td>0.98</td>
<td>BV</td>
<td>3.525e+003</td>
<td>Q20</td>
</tr>
<tr>
<td>2</td>
<td>3.012</td>
<td>Found</td>
<td>1057</td>
<td>0.07</td>
<td>440</td>
<td>0.23</td>
<td>VV</td>
<td>1.057e+003</td>
<td>Q20</td>
</tr>
<tr>
<td>3</td>
<td>3.070</td>
<td>Found</td>
<td>697</td>
<td>0.04</td>
<td>250</td>
<td>0.14</td>
<td>VB</td>
<td>6.971e+002</td>
<td>Q20</td>
</tr>
<tr>
<td>4</td>
<td>3.575</td>
<td>Found</td>
<td>649598</td>
<td>41.44</td>
<td>110650</td>
<td>67.47</td>
<td>BV</td>
<td>6.495e+005</td>
<td>Q20</td>
</tr>
</tbody>
</table>

---

**Report Method:** Detailed Individual Report  
**Printed:** 11/16/2011  
**Page:** 1 of 2  
**Time:** 3:29:28 PM U/0/Central
## Acquisition Log

Acquired By: Breeze  
Injection: 1  
Date Acquired: 10/27/2010 12:56:33 PM CDT  
Run Time: 25.00 (Minutes)  
Acq Method Set: Chiralcel OD  
Injection Volume: 5.00 (uL)  
Injection Id: 2712  
Instrument Method Name: Chiralcel OD  
Superseded: No  

---

<table>
<thead>
<tr>
<th>Points Across Peak</th>
<th>Start Time (min)</th>
<th>End Time (min)</th>
<th>Baseline Start (min)</th>
<th>Baseline End (min)</th>
<th>Slope (µV/sec)</th>
<th>Offset (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>2.883</td>
<td>2.977</td>
<td>3.147</td>
<td>6.3316×10^-6</td>
<td>-1.941391×10^-3</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>2.977</td>
<td>3.043</td>
<td>3.147</td>
<td>6.3316×10^-6</td>
<td>-1.941391×10^-3</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>3.043</td>
<td>3.147</td>
<td>3.147</td>
<td>6.3316×10^-6</td>
<td>-1.941391×10^-3</td>
</tr>
<tr>
<td>4</td>
<td>267</td>
<td>3.342</td>
<td>3.432</td>
<td>4.055</td>
<td>5.3559×10^-6</td>
<td>-1.642985×10^-3</td>
</tr>
<tr>
<td>5</td>
<td>107</td>
<td>3.877</td>
<td>4.055</td>
<td>4.055</td>
<td>5.3559×10^-6</td>
<td>-1.642985×10^-3</td>
</tr>
<tr>
<td>6</td>
<td>252</td>
<td>4.367</td>
<td>4.407</td>
<td>4.357</td>
<td>3.5940×10^-6</td>
<td>-1.928999×10^-3</td>
</tr>
<tr>
<td>7</td>
<td>244</td>
<td>4.807</td>
<td>5.213</td>
<td>4.337</td>
<td>3.5646×10^-6</td>
<td>-1.928999×10^-3</td>
</tr>
<tr>
<td>8</td>
<td>363</td>
<td>5.213</td>
<td>5.802</td>
<td>4.337</td>
<td>3.5646×10^-6</td>
<td>-1.928999×10^-3</td>
</tr>
<tr>
<td>9</td>
<td>167</td>
<td>6.577</td>
<td>6.855</td>
<td>5.802</td>
<td>3.5646×10^-6</td>
<td>-1.928999×10^-3</td>
</tr>
<tr>
<td>Name</td>
<td>Retention Time</td>
<td>Area</td>
<td>% Area</td>
<td>Height</td>
<td>Int Type</td>
<td>Amount</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>R 5 br trp</td>
<td>13.874</td>
<td>49506</td>
<td>3.25</td>
<td>1611</td>
<td>BV</td>
<td></td>
</tr>
<tr>
<td>S 5 br trp</td>
<td>15.122</td>
<td>450865</td>
<td>29.57</td>
<td>11170</td>
<td>VB</td>
<td></td>
</tr>
</tbody>
</table>

**Compound with 90 % ee**
8. REFERENCE


33. Todd, R. Powerful three component/one-pot asymmetric protocol for synthesizing chiral tryptophan derivatives and an investigation into the decomposition of borane-tetrahydrofuran through accelerated thermal decomposition studies and stabilization by Lewis base University of Wisconsin-Milwaukee 2008.


9. VITA

CURRICULIM VITA

Matthew M. Huisman

Place of birth: Appleton, WI

Education
B.S., University of Wisconsin-Whitewater May 2008
Major: Biology

PhD. University of Wisconsin-Milwaukee May 2014
Internship

Dissertation Title: ASYMMETRIC SYNTHESIS OF TRYPTOPHAN DERIVATIVES AND ITS
APPLICATION TO STREAMLINED SYNTHESIS OF TRYPROSATAIN A AND B.

R&D Chemist Sigma Aldrich Milwaukee

Publications
Robert Todd, Matthew Huisman, Nazim Uddin, Sarah Oehm, M. Mahmun Hossain. “One-
Pot Enatioselective Synthesis of Tryptophan Derivatives via Phase-Transfer Catalytic

Teaching Experience

Led discussions for Chemistry (100) Chemical Science, (103) Survey of Biochemistry, (104)
General Chemistry and Qualitative Analysis, and (105) General Chemistry for Engineers.
Also taught Chemistry 103, 104, 105, 342 Introductory to Organic Chemistry and 344
Organic Chemistry labs.

Research Experience: Knowledgeable about synthetic organic chemistry skills include:
synthesis, purification procedures, \(^1\)H, \(^13\)C and 2D NMR, HPLC, Mass spec, chemdraw,
scifinder, word, endnote, powerpoint.

Awards/Honors 2014 Moczynski Outstanding Teaching Assistant Award (Recommended by professors)

First Year Student Success Award 2011-2012 (Named by UWM first year students as person
on campus who helped them most in their college success)