EFFORTS TOWARDS THE TOTAL SYNTHESIS OF NOSIHEPTIDE

by

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Abstract

This thesis described here aims to achieve the total synthesis of nosiheptide, a representative member of the so-called E-series of thiopeptide antibiotic. Our group has established various techniques for the assembly of their complex molecular framework, and demonstrated the total syntheses of two D-series thiopeptide antibiotics. However, there is no precedent total synthesis of compound belonging to E-series. The part of the reason is that the presence of an additional 3-OH substituent not only adds significant synthetic complications, but also bars the use of the previously established technology. The present work details the assembly of the pyridine-thiazole cluster of E-series thiopeptide substance through a modified Hantzsch reaction that delivers the complete pyridine segment in a triply convergent fashion. Optimization studies related to this pyridine formation have further enhanced the conciseness and simplicity of the method. This chemistry thus developed can be applied to medicinal chemistry investigations in thiopeptide antibiotic field; an endeavor that will possibly unveil valuable new antibiotics.

Preface

Hee Jong (Jason) Hwang has performed all experiments reported in this document, planned tactical synthetic routes described, and wrote this dissertation. Marco A. Ciufolini has provided technical suggestions.

The work described in Chapter 3 has been published. Hwang, H-J. and Ciufolini, Marco. A. (**2015**) A Route to the Heterocyclic Cluster of the E-series of Thiopeptide Antibiotics. *Journal of Organic Chemistry*, 80: 4184-4188.

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[α]	specific rotation
Ac	acetyl
(acac)	acetylacetonate
aq	aqueous
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
b	broad
Bu	butyl
^{o}C	degrees Celsius
cat.	catalytic
cald	calculated
cm^{-1}	wavenumbers
δ	chemical shift (parts per million down from tetramethylsilane)
d	doublet
D-A	Diels-Alder reaction
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DIBAL	diisobutylaluminum hydride
DMAP	4-N,N-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
dppp	1,3-bis(diphenylphosphino)propane
EBP	ethyl bromopyruvate
Ef	elongation factor
eq	equivalents
Et	ethyl
FCC	Flash Column Chromatography
g	gram(s)
h	hour(s)
HFIP	1,1,1,3,3,3,-hexafluoro-2-propanol
HRMS	high resolution mass spectrometry
Hz	Hertz (s^{-1})
i	iso (as an alkyl group)
IR	Infrared
J	coupling constant
LDA	lithium diisopropylamide
LRMS	loss resolution mass spectrometry
m	multiplet

M	molar (moles per liter); mega
mCPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
min	minute(s)
MOM	methoxymethyl
mp	melting point
MP	micrococcin P
n	normal (as an alkyl group)
NBS	N-bromosuccinimide
n-BuLi	*n*-butyllithium
NMR	nuclear magnetic resonance
p	para (as a benzene substituent)
Pg	unspecified protecting group
Ph	phenyl
PPA	polyphosphoric acid
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
py	pyridine
q	quartet
R_f	retention factor

RNA	ribonucleic acid
r.t	room temperature
s	singlet
sat.	saturated
t	triplet
t	tertiary (as an alkyl group)
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
t-BuLi	*tert*-butyllithium
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFAA	trifluoroacetic anhydride
TFE	2,2,2-trifluoroethanol
THF	tetrahydrofuran
THP	tetrahydropyran
Thz	unspecified thiazole units
TIPS	triisopropylsilyl
TLC	Thin Layer Chromatography
TMS	trimethylsilyl
μ	micro

Acknowledgements

I would like to thank my supervisor, Marco A. Ciufolini, for taking me into his group and for letting me explore the world of chemistry with intellectual freedom. Years spent his group not only enabled me to re-evaluate the true meaning of passion and dedication that Marco emanates, but also taught me to appreciate many aspects of life. I feel truly fortunate to work under his supervision.

I would also like to extend my gratitude towards my fabulous lab members, past and present. Memories I have engraved with you have been a great journey: from chemistry consultation to life in general. Dr. Matthew Smith, who taught me a great deal of practical lab experience, Dr. Josh Zaifman for enormous consultation and joyful conversation, Taka for sharing his chemistry wisdom and random chit-chatting filled with laughter, Leanne for her funny jokes and support, Patrick for his confidence which I have applied in my experiments, Marco for properly introducing me great Italian food and culture, Dimitri for regular coffee with croissant and unforgettable chats, and Nikita for being super understanding and bearing with me in A322.

This journey could not have been achieved with the support of my family: My father who always listens, and gives unconditional support, my mother who always provides emotional support, and my brother for being my best friend.

Thank you my love, Bonnie Park, who has given me strength and reason to excel. I could not have gone this far without you. Thank you for her family for countless support.

Lastly, I am indebted for many of my friends who have been a great support.

1. Introduction

1.1 Background

The discovery of penicillin by Sir Alexander Fleming, in the 1920's, ushered in the so-called antibiotic era, and for the first time in human history provided the medical establishment with effective weapons to combat a wide variety of bacterial ailments. This not only saved millions of lives, but also stimulated intense research in the field of antibiotics. Soon after the introduction of the first antibiotics, however, it became apparent that bacterial pathogens become resistant to such drugs over time.[1] It is now established that microorganisms gain resistance through genetic mutations.[2] That disturbing observation should have stimulated a quest for new antibiotics; yet – paradoxically – research in the field, both in the public and the private sectors, abated greatly in the 1970's and 1980's, and very few new antibiotics have been discovered and developed since. The inevitable – if entirely predictable – consequence is that the world is now facing an "antibiotic crisis,"[3] which threatens to return humankind to the "dark age" of pre-antibiotic times. The problem is especially acute in hospitals, where patients already weakened by various conditions tend to contract lethal infections caused by strains of *Staphylococcus*, *Enterococcus*, and other bacterial species that have become resistant even to vancomycin: an antibiotic that is reserved as a drug of last resort.[4] Such "nosocomial infections" constitute a major public health concern.[5]

1.2 The Thiopeptide Antibiotics

The foregoing state of affairs has rekindled interest in the search of new antibacterial resources. In that connection, the so-called thiopeptide antibiotics are actively being investigated as possible sources of new anti-infective drugs.[6] Thiopeptides are highly modified, sulfur-rich,

cyclic peptides produced by *Streptomyces* and *Bacillus* species, and exemplified below by micrococcin P1 [MP1], **1**, thiocillin I, **2**, nosiheptide, **3**, and nocathiacin I, **4**. They are characterized by the a heterocyclic core consisting of pyridine, which may be present in partially– or completely reduced form, decorated with various thiazole and/or oxazole residues, and embedded within a macrocyclic array.[7] Various dehydroamino acid subunits are also present. The first thiopeptide to be discovered is **1**, which was described in 1948. Since then, approximately 80 members of the family have been reported.

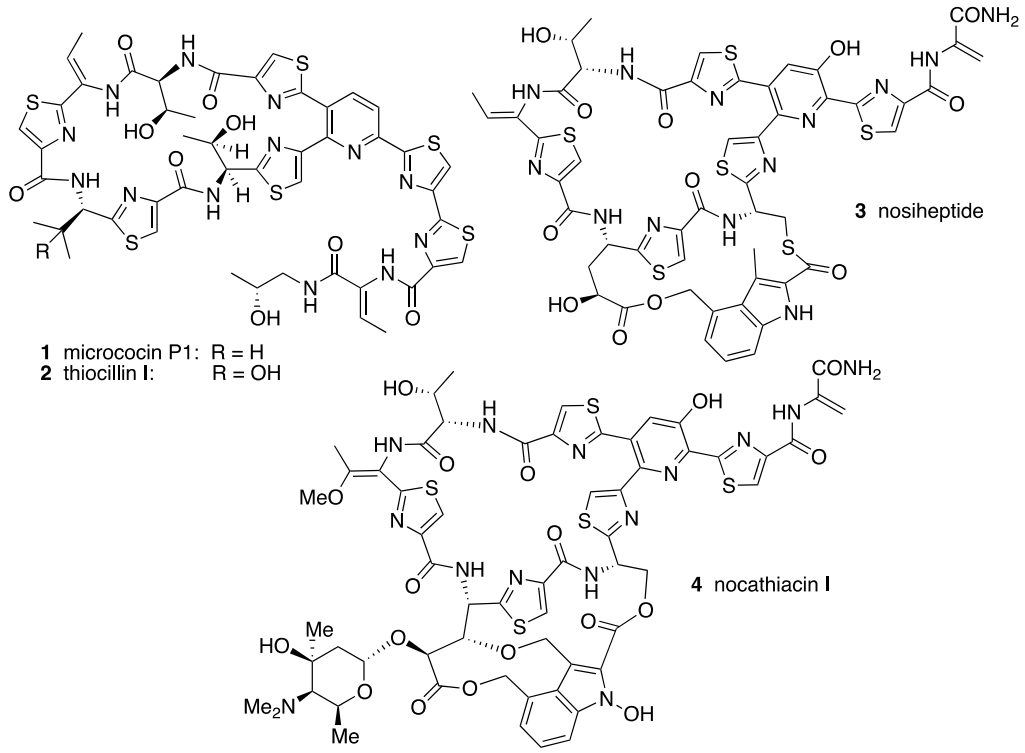


Figure 1. Representative Thiopeptide Antibiotics

All thiopeptides are potent inhibitors of protein synthesis, seemingly as a consequence of their tight binding to particular regions of the ribosome, ultimately impairing the proper functioning thereof. In that respect, they may be distinguished into 2 classes: those that target the

L11 domain of the 23S ribosomal RNA subunit,[8] and those that bind to the elongation factor (Ef).[9] The first class of thiopeptides are believed to stabilize a particular ribosomal conformation, thereby hindering the conformational changes required for the elongation step of protein synthesis. The second group of compounds bind strongly to the elongation factor known as Ef-Tu factor. This prevents the transfer of aminoacyl residues to the nascent peptide chain. In either case, protein synthesis is halted, resulting in the death of the organism. Therefore, thiopeptides are potent antiprotozoal, antifungal, and even antitumor agents.[6] In addition, they possess elevated antimicrobial activity, but only against Gram-positive bacteria. Even so, their potency against methicillin-resistant *Staphylococcus aureus* (MRSA; a strain of great current concern) makes them attractive as starting points for the development of new antibiotics.

Hensens suggested a classification of thiopeptide antibiotics into five different groups, A-E, depending on the oxidation state of the pyridine nucleus.[10] Group A includes compounds that incorporate a fully reduced pyridine; e.g., thiopeptin A3a, **5**. The B series encompasses substances that display a tetrahydropyridine core; e.g., thiostrepton A, **6**. The compound, Sch 40832, **7**, is the only member of the C family, which is characterized by the presence of an imidazopiperidine core (Figure 2). The D class thiopeptides counts the largest number of members, all of which exhibit a 2,3,6-trisubstituted pyridine, as seen in micrococcin P1, **1**, and thiocillin I, **2**. Finally, a 3-hydroxypyridine core defines the E series of antibiotics, exemplified by nosiheptide, **3**, and nocathiacin, **4**.

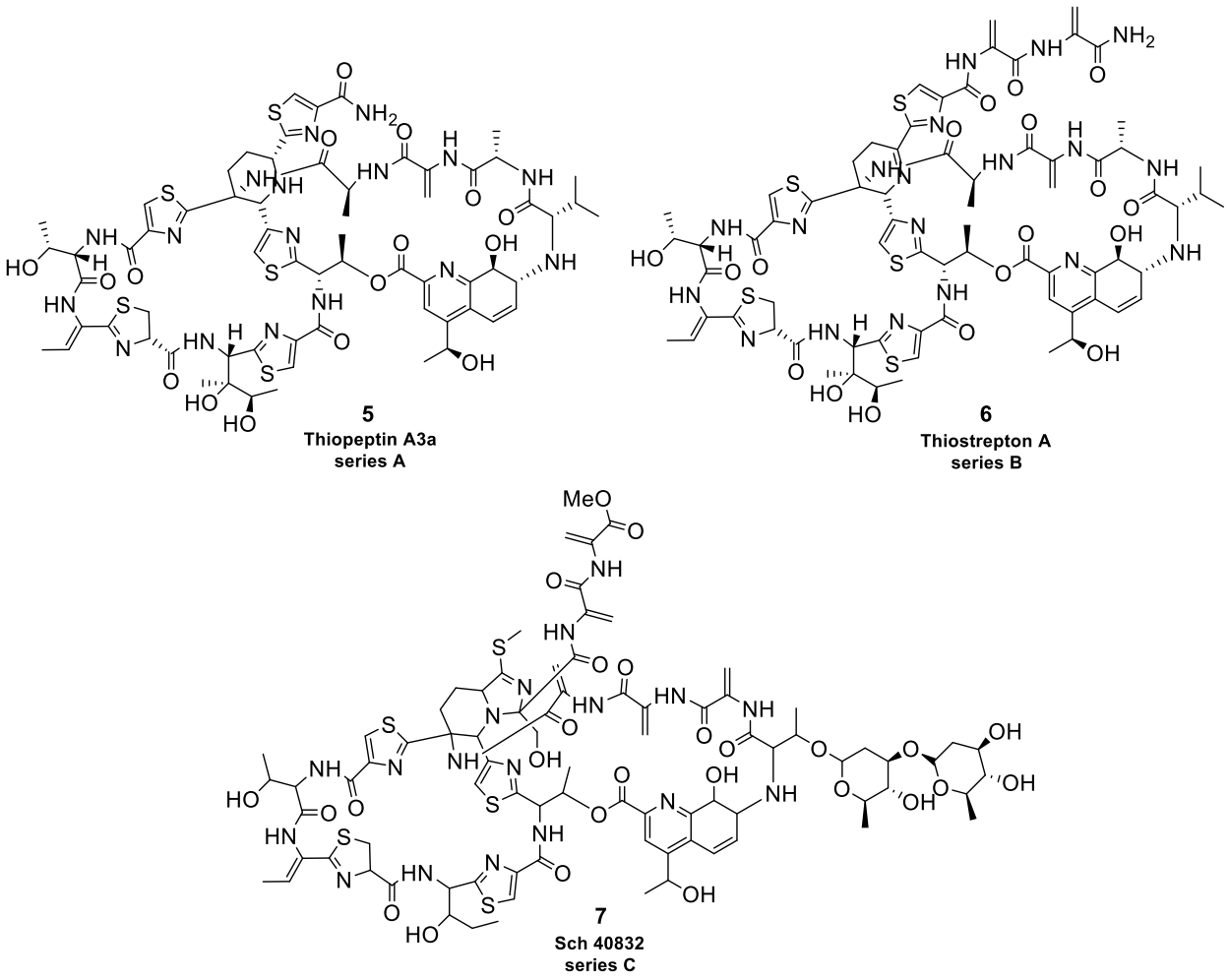


Figure 2. Examples of A, B, and C-type Thiopeptides Under Hansen's Classification

The biosynthesis of thiopeptides is believed to involve a series of post-translational modifications of a large peptide. Bycroft and Gowland first suggested in 1978 that an aza Diels-Alder reaction may be involved in the biosynthesis of the pyridine core in the thiopeptide antibiotics[11]. This hypothesis was confirmed only recently, thanks to the work of Walsh[12] and others. Micrococcin P1, **1**, and thiocillin I, **2**, are now accepted to emanate from a polypeptide

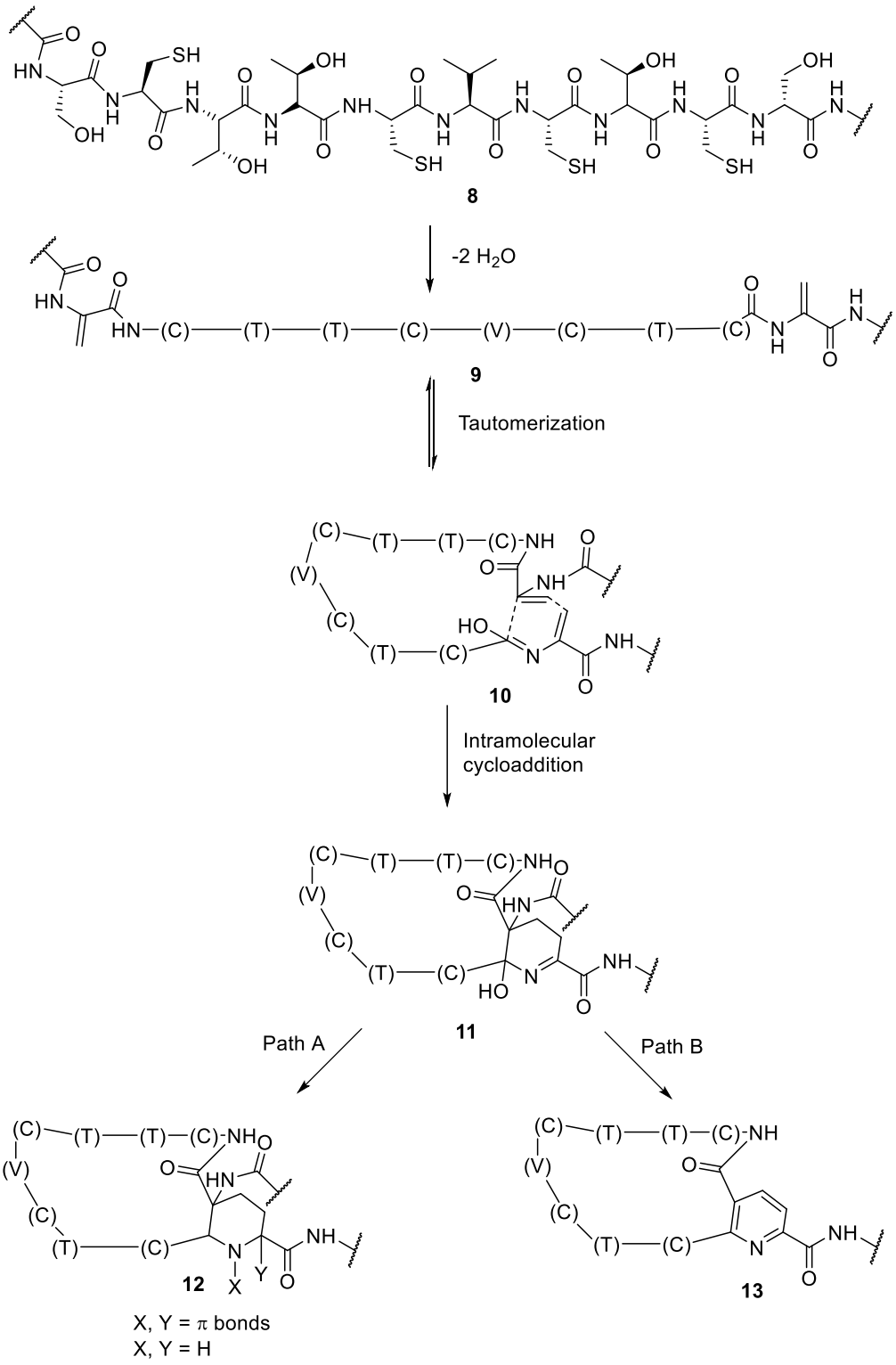
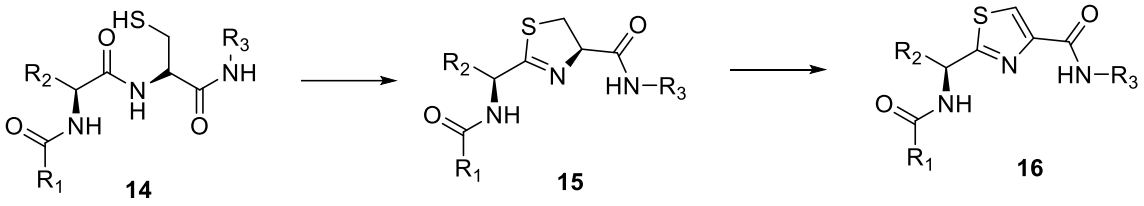


Figure 3. Biosynthesis of Pyridine and Tetrahydropyridine Units of Thiopeptide Antibiotics

such as **8**, which undergoes a double dehydration leading to **9**, wherein the letters in parentheses [(C), (T), (V)] represent either actual amino acid residues, or amino acid-derived thiazolines or thiazoles.[13] Within an appropriate enzyme, peptide **9** undergoes amide tautomerization and folding into conformation **10**, which promotes intramolecular cycloaddition leading to **11**. Two possible fates now await the newly formed **11**: dehydration and reduction (path A) lead to the A and B series of natural products (structure **12**), whereas dehydration and elimination of the acylamino moiety (path B) yield the fully aromatic pyridine **13** seen in the D series. Interestingly, Nicolaou and Moody have duplicated these biosynthetic routes to the pyridine core of thiopeptides in their own synthetic work (*vide infra*).

On the other hand, the thiazole residues are likely to arise through the cyclization of N-acyl cysteine segments **14** to thiazolines **15**, which subsequently undergo aromatization to form thiazole **16**[14] (Scheme 1).



Scheme 1. Biosynthesis of Thiazole Moieties of Thiopeptide Antibiotics

1.3 Objective of the Present Study

The present study aimed to devise a rapid synthesis of the 3-hydroxypyridine core of the E-series of thiopeptide antibiotics, in a form suitable for the conduct of a total synthesis of representative members of the family. A plausible form of the desired pyridine would thus be **17**.

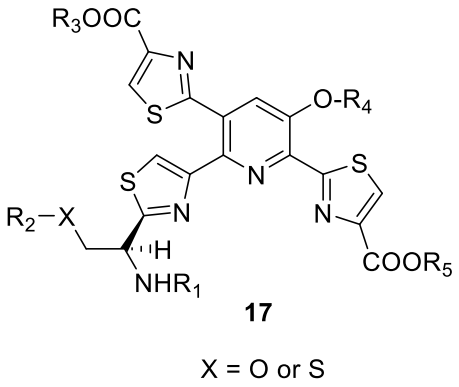


Figure 4. Hypothetical Desired Pyridine Core of E-series Thiopeptides

It should be noted that very few thiopeptides have succumbed to total synthesis, and that no syntheses of E-type compounds have yet been achieved. Even exploratory studies in the area are few. There can be little doubt that this is a consequence of the difficulties encountered in the assembly of pyridines **17**. The section that follows elaborates the point by outlining key aspects of known syntheses of thiopeptides; by illustrating how the technology is devised in that connection shows how entirely inadequate it is for the construction of compound **17**.

2. Previous Syntheses of Thiopeptide Antibiotics

2.1 Background

Thiopeptide antibiotics began to attract attention from the synthetic community only in the late 1990's. Even so, only seven thiopeptides have fallen to total synthesis as of this writing: promothiocin A, **18**,[15] in 1998, amythiamicin D, **19**,[16] and thiostrepton A, **6**,[17] in 2005, Siomycin A , **20**[18] and GE2270A[22], **21**, in 2007, micrococcin P1, **1**,[19] in 2009, and thiocillin I, **2**,[20] in 2011 (Figure 5).

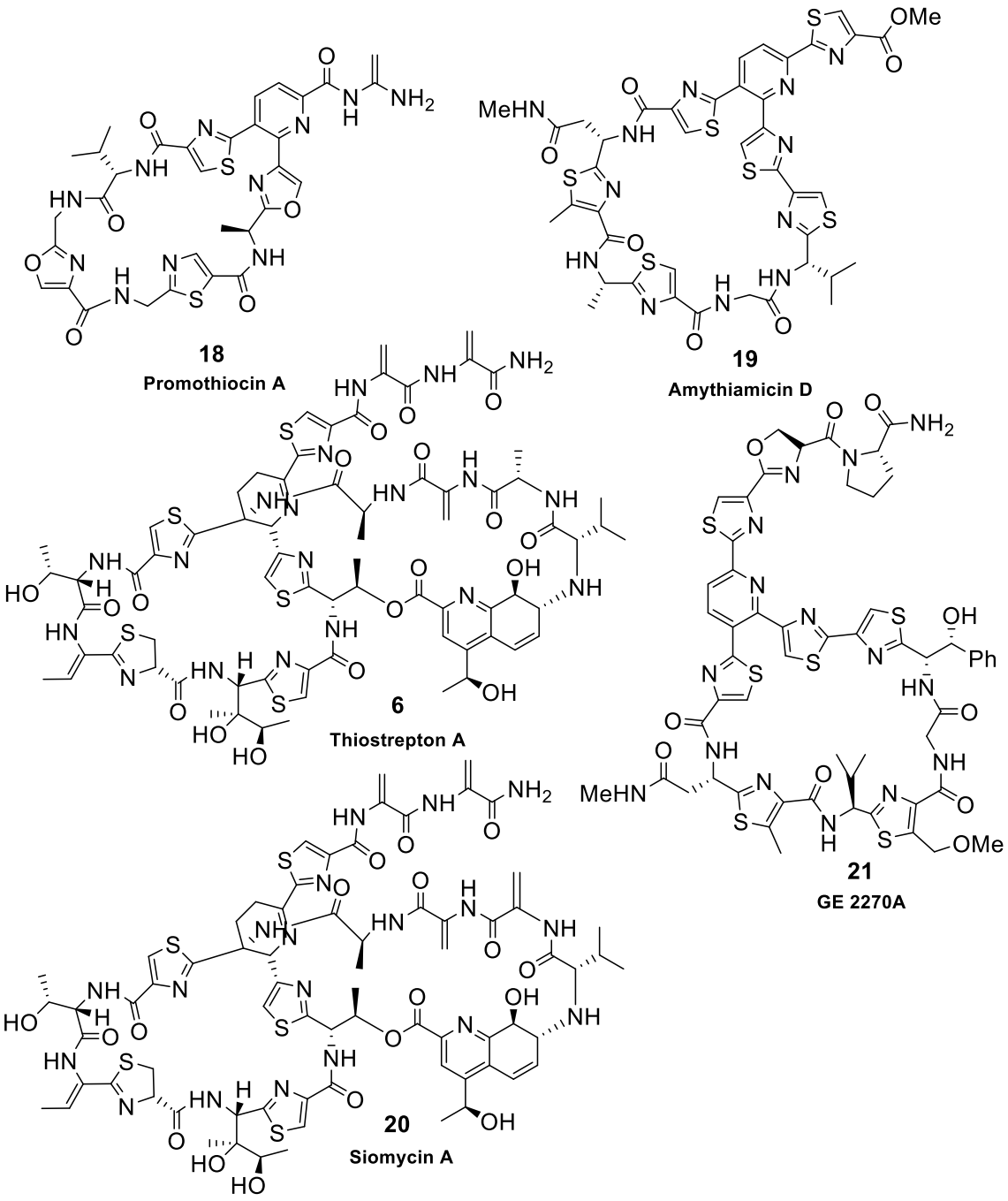


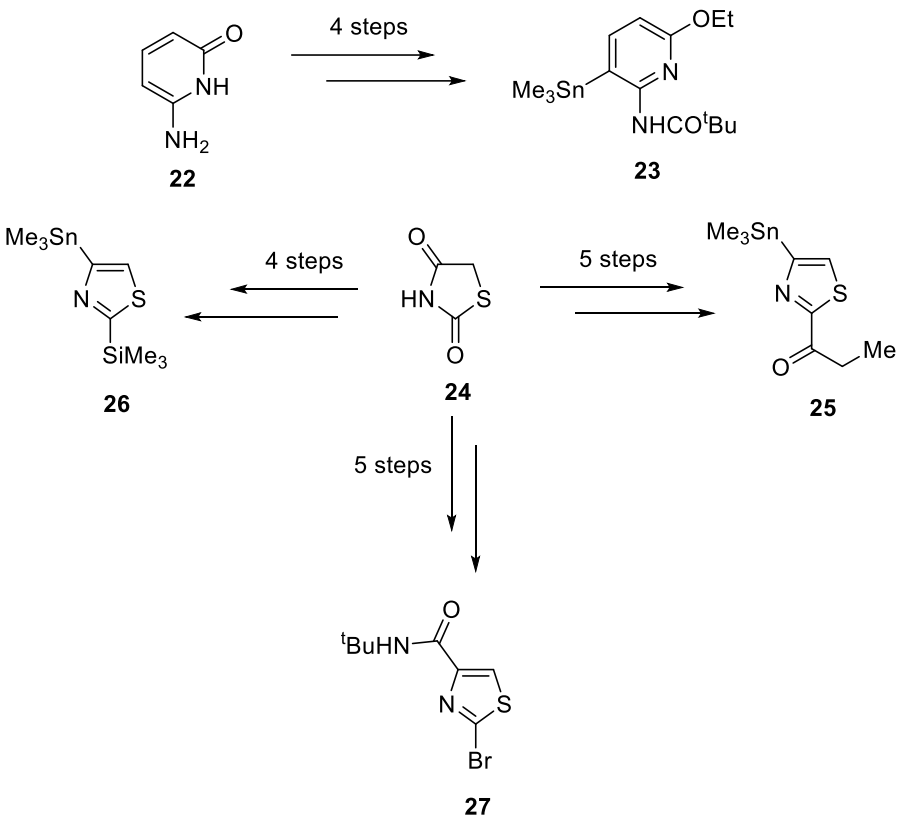
Figure 5. Some Thiopeptides that Have Been Succumbed by Total Synthesis

Early synthetic efforts in the area uncovered an important strategic principle: the key to a successful synthesis of thiopeptide compounds is the rapid assembly of their pyridine-thiazole

core cluster. Despite the fact that the synthesis of pyridines has been studied for a long time, the preparation of such complex pyridine-thiazole arrays remains a challenging proposition. Furthermore, the numerous difficulties that it presents are best addressed through the development of new chemical methodology. Indeed, the main players in the field, C. J. Moody, K. C. Nicolaou, and M. A. Ciufolini, have all devised new techniques for the construction of those crucial subunits. The nature of such problems, and some noteworthy solutions developed in response to these, are highlighted in paragraphs that follow.

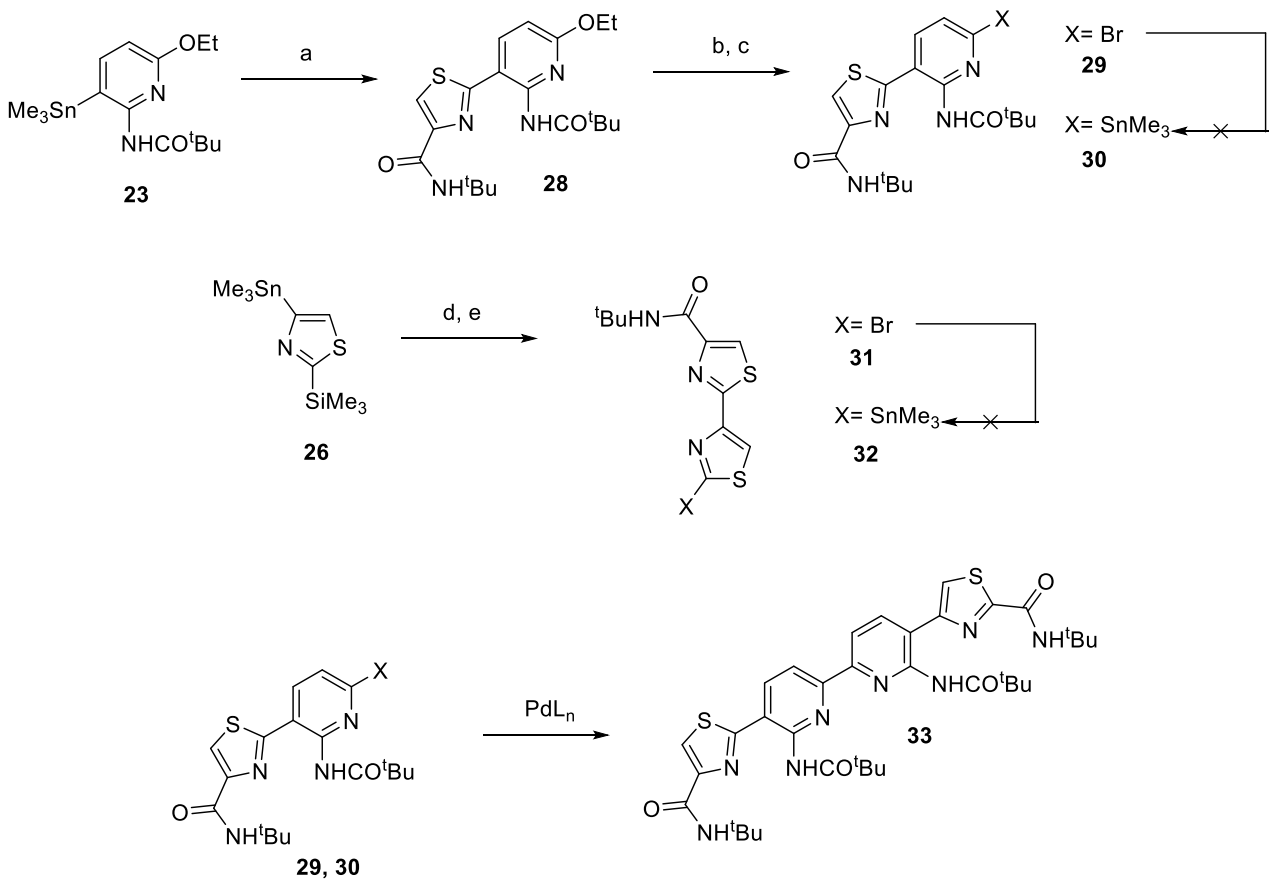
2.2 Background on Pyridine Synthesis

Two major strategies have been followed for the assembly of thiopeptide core clusters: the modification of a pre-existing pyridine through metal-mediated coupling reactions,[21,22] and the *de novo* assembly of the pyridine with the full complement of thiazoles. The first approach is apparent in the earliest recorded study in the thiopeptide field: the Kelly synthesis of micrococcinic acid, **35**,[21] a product of acid hydrolysis of micrococcin P1. The route to **35** relied upon sequential Stille coupling[23] of thiazole and pyridine units. This required fairly complex pyridyl- and thiazolylstannanes, the preparation of which proved to be lengthy and arduous. Even, the preparation of building blocks required 18 independent operations (Scheme 2).



Scheme 2. Building Blocks of Kelly's Synthesis of Microccocinic Acid

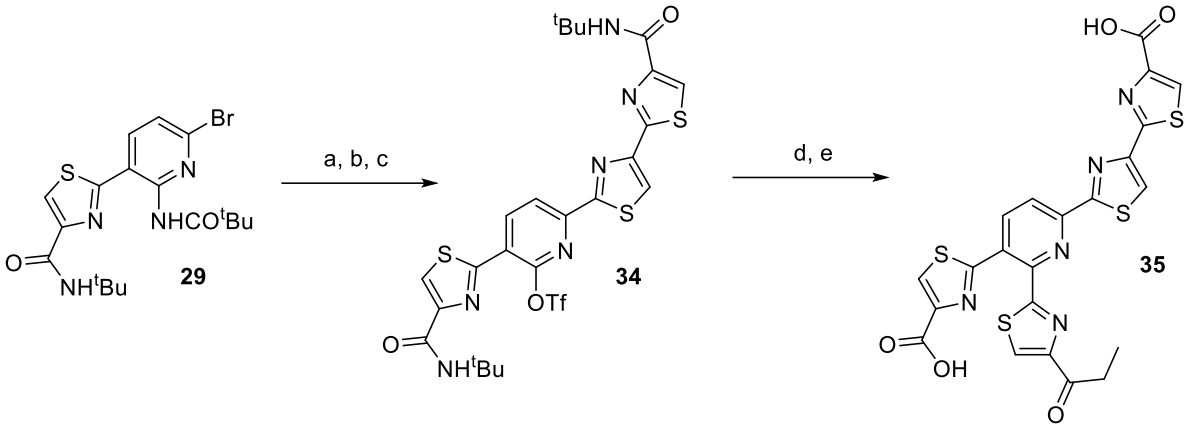
Significant complications arose when all attempts to advance **29** to stannane **30**, or **31** to stannane **32**, were unsuccessful. The problem was that bromides **29** and **31** were largely converted into the corresponding homodimers in the presence of Pd catalysts. Evidently, species such as **30** are so electrophilic that most of the initially formed stannane reacts with more **29** to yield **33** (Scheme 3).



(a) 5mol % $(Ph_3P)_2PdCl_2$, 2 eq **27**, 55% (b) Me_3SiI, 72% (c) $POBr_3$, 88%, (d) 5mol % $(Ph_3P)_2PdCl_2$, 2 eq **27**, 65%, (e) Br_2, 68%,

Scheme 3. Kelly's Attempt to Synthesize Aryl Stannyl Derivatives and Formation of Homodiers

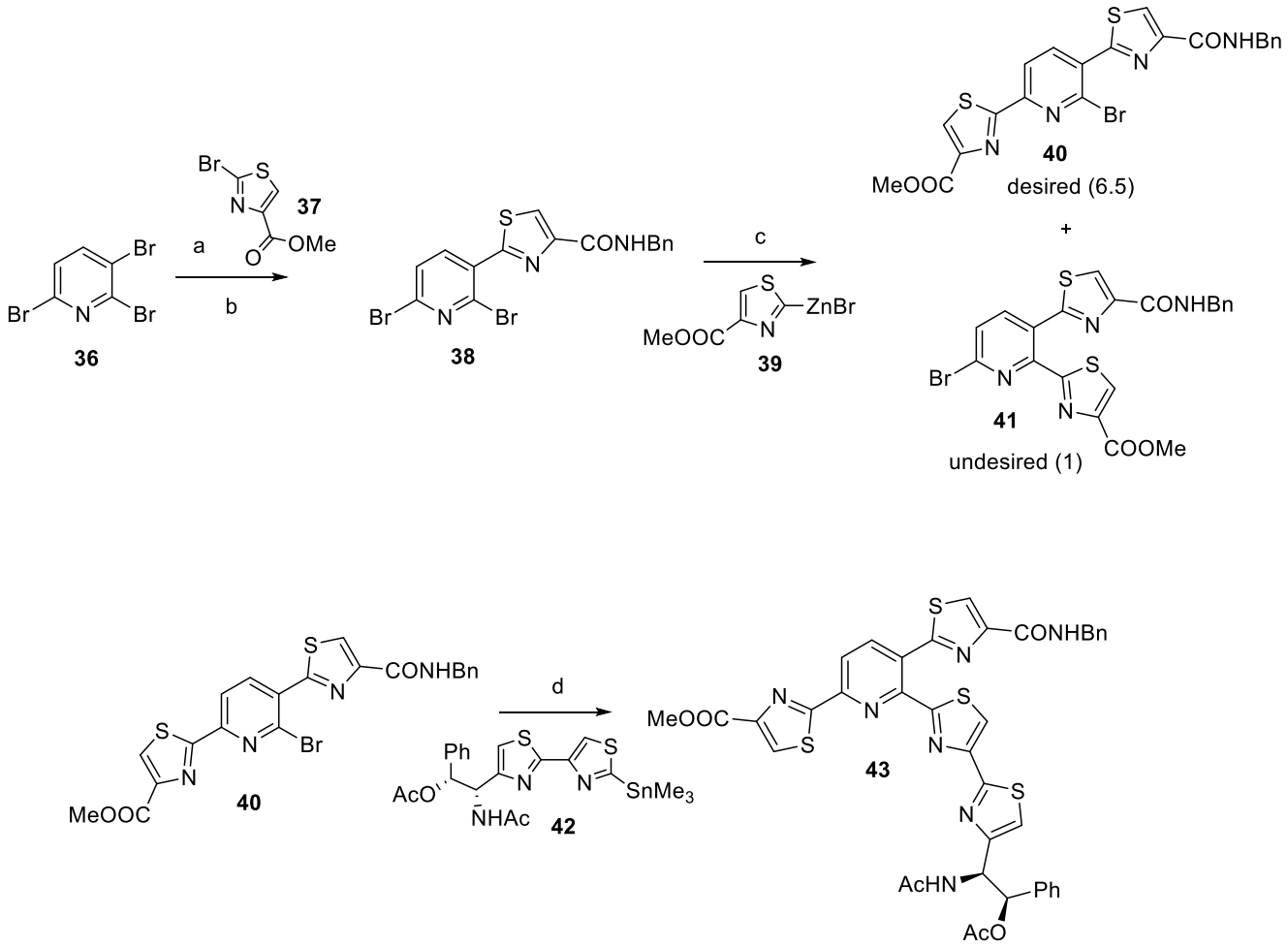
A moderately successful stratagem to bypass such obstacles entailed the reaction of a 1:1 mixture of **29** and **31** with $(Me_3Sn)_2$ in the presence of a catalytic amount of $(Ph_3P)_2PdCl_2$, whereupon the desired product **34** was isolated in 25% yield over 3 steps (Scheme 4). From the standpoint of process chemistry, Kelly's methodology is less than appealing.



(a) 4eq $(Me_3Sn)_2$, **31**, 11mol % $(Ph_3P)_2PdCl_2$, 49%, (b) aq. HNO_2, 97% (c) Tf_2O, 58%, (d) 10mol % $(Ph_3P)_4Pd$, **25**, 89% (e) H_3O^+, 80%

Scheme 4. Kelly's Synthesis of the Pyridine Core of Micrococcinic Acid

Similar difficulties have plagued a more recent synthesis of GE2270A by Bach and coworkers[22] (Scheme 5). Key steps in this work are two Negishi and one Stille cross-coupling reactions to elaborate 2,3,6-tribromopyridine **36** into the trisubstituted pyridine core **43**. Complete C-3 regioselectivity was observed during an initial halogen-lithium exchange of tribromopyridine **36**, and the resultant 3-pyridyllithium was successfully converted into the corresponding Zn organometallic. The first Negishi coupling with **37** performed well to afford **38** in 81% yield. However, the second Negishi coupling of **38** with **39** occurred with moderate C-6 regioselectivity (6:5:1) and produced considerable quantities of homodimers. This led to complex mixtures of products, which were difficult to separate. Laborious purification efforts and the use of expensive transition metal catalysts clearly overshadow this approach.

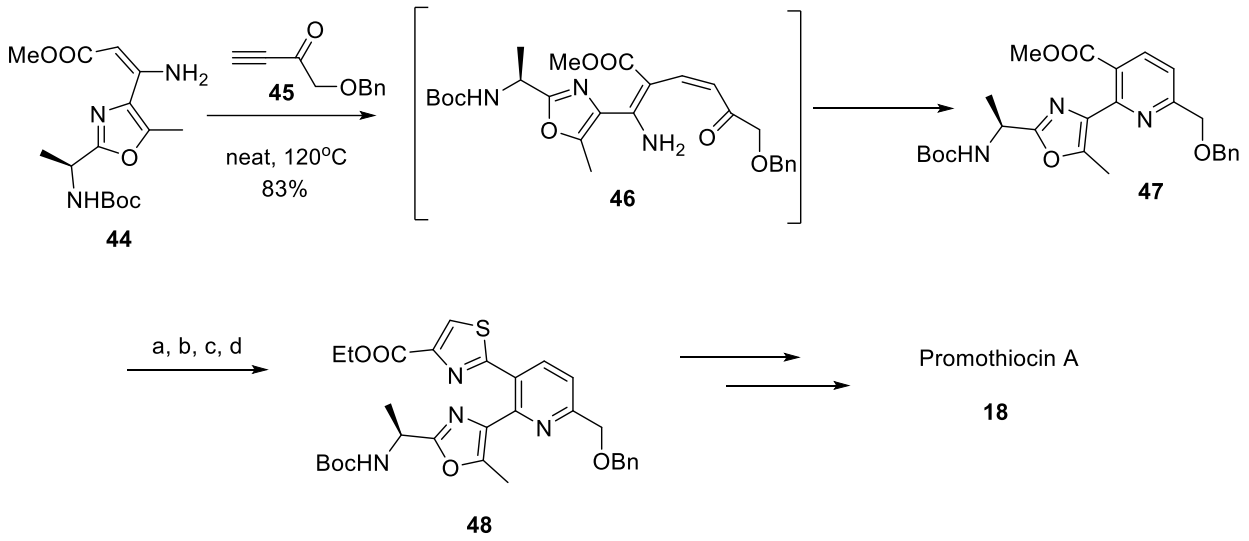


(a) n-BuLi, THF, $ZnCl_2$, $PdCl_2(PPh_3)_2$, 81% (b) $BnNH_2$, DIBAL-H, THF, DCM, 86% (c) $PdCl_2(PPh_3)_2$, THF, 78% (d) $Pd(PPh_3)_4$, dioxane, 80°C, 61%

Scheme 5. Bach Synthesis of Pyridine Core of *ent*-GE2270A

A *de novo* construction of the pyridine unit can circumvent many of the foregoing difficulties. On the other hand, it requires the development of appropriate pyridine-forming methodology. A convincing demonstration is provided by the Moody synthesis of Promothiocin A **18**.[15] Thus, a modified Bohlmann-Rahtz reaction involving the union of ynone **45** with enamine **44** created pyridine **47** in a single step. The process is likely to involve Michael-type addition of the enamine to the ynone, followed by cyclization / aromatization of the resultant **46**.

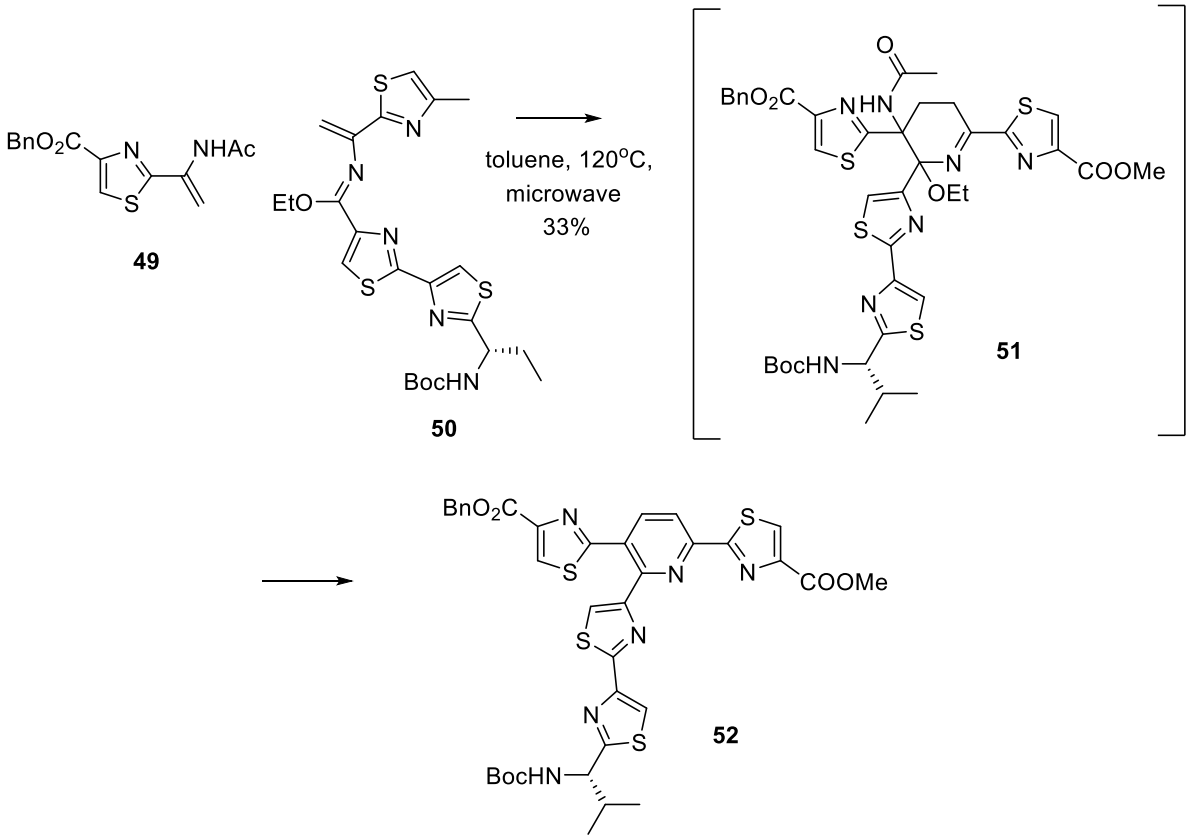
Compound **47** was rapidly advanced to **48**, which was then elaborated to promothiocin A (Scheme 6).



(a) LiOH, 92% (b) EtO_2CCl, Et_3N, then NH_4OH, 85% (c) Lawesson's Reagent, 59% (d) EBP, 72%

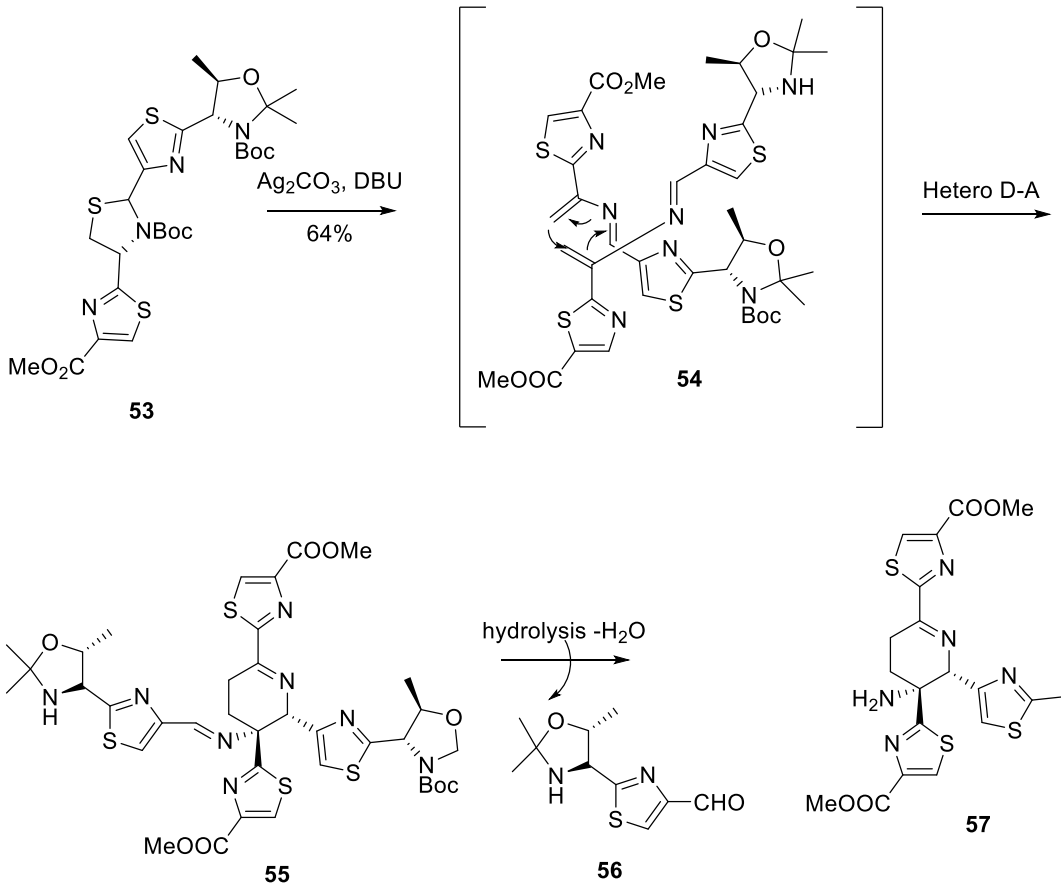
Scheme 6. Moody Synthesis of Pyridine Core of Promothiocin A

Moody and Nicolaou independently described a remarkable biomimetic avenue to thiopeptide cores through aza-Diels-Alder reaction. The centerpiece of Moody's synthesis of amythiamicin D, **19**,[16] is the cycloaddition of **49** to **50**, followed by eliminative aromatization of intermediate dihydropyridine **51** (Scheme 7).



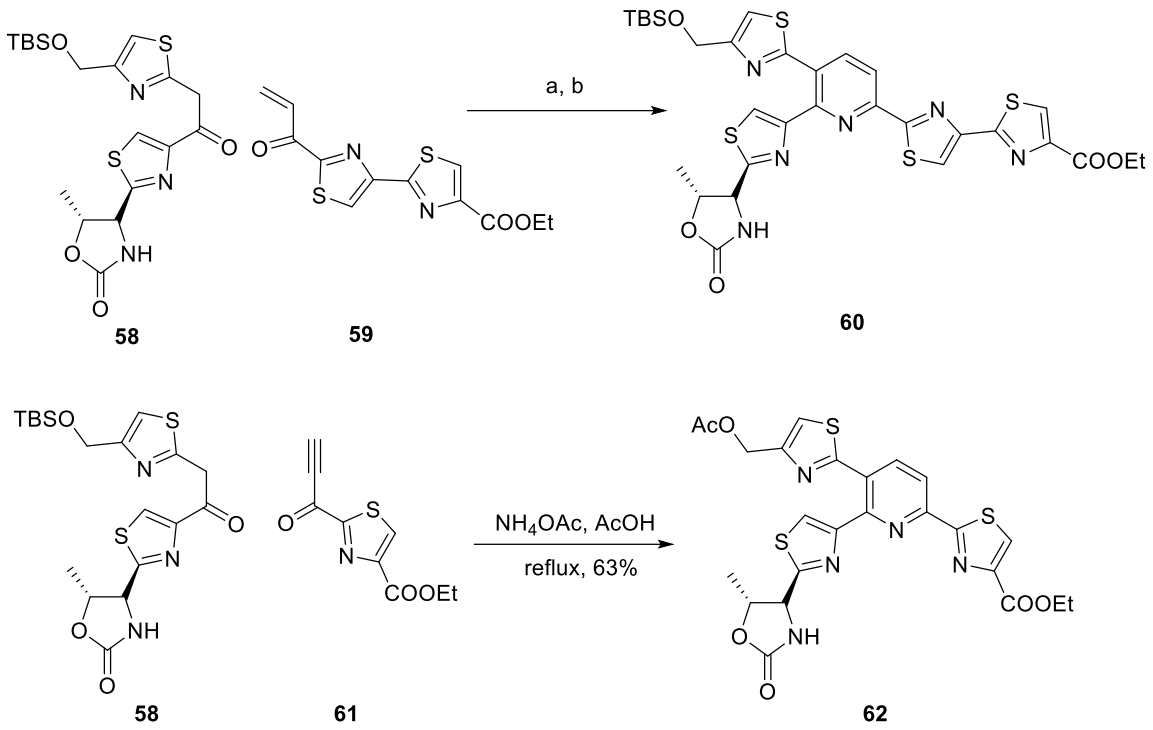
Scheme 7. Moody's Biomimetic Synthesis of Pyridine Core of Amythiamicin D

The technology is especially valuable for the construction of the partially reduced pyridines found in thiostrepton and related antibiotics. The Nicolaou synthesis of thiostrepton embodies an especially impressive application of the technique.[17] Thus, treatment of thiazolidine **53** with Ag_2CO_3 and DBU produced transient azadiene **54**, which underwent sequential hetero Diels-Alder dimerization – imine hydrolysis to afford the desired aminopiperidine **57** in a single step (Scheme 8).



Scheme 8. Nicolaou's Biomimetic Synthesis of Pyridine Core of Thiostrepton

Finally, the Ciufolini synthesis of Micrococcin P1[19] introduced a modification of the classical Hantzch pyridine synthesis that enabled the merger of fragments **58** and **59** into pyridine **60** in two steps, while the synthesis of Thiocillin I[20,24] demonstrated an even more concise route to pyridine **62** through yet another modification of the Bohlmann-Rahtz reaction (Scheme 9).



(a) cat Li_2CO_3, EtOAc, 99% (b) NH_4OAc, EtOH then DDQ, toluene, 98%

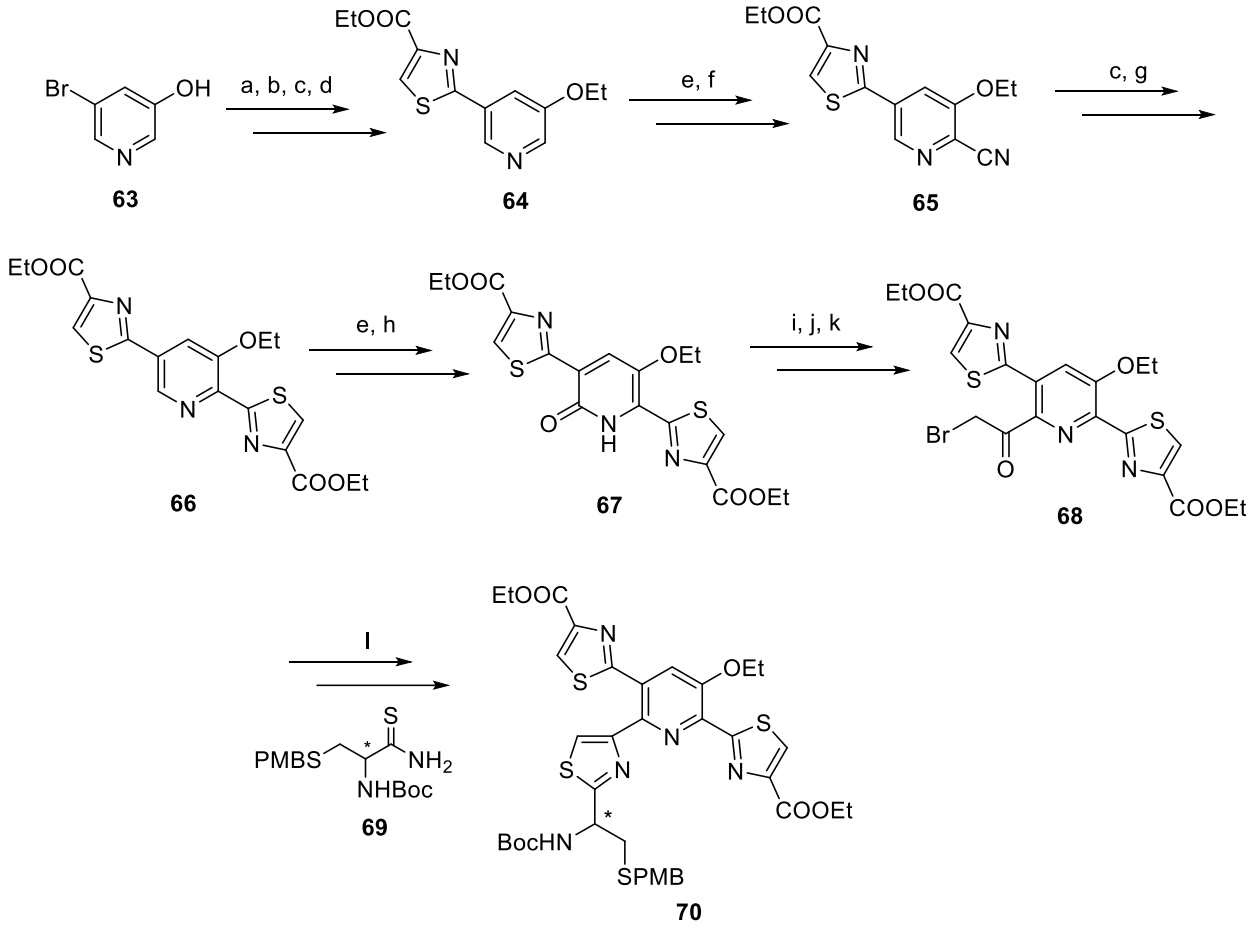
Scheme 9. Ciufolini's *de novo* Pyridine Synthesis to Micrococcin P1 and Thiocillin I

2.3 Synthesis of 3-hydroxypyridine Cores of the Type found in E-series

The pyridine-forming techniques outlined above lead to 2,3,6-trisubstituted pyridines. However, the heterocyclic core of the E-series of thiopeptides, such as nocathiacin, **4**, and nosiheptide, **3** (Figure 1), is based on a tetrasubstituted, 3-hydroxypyridine. The presence of the OH substituent greatly complicates the assembly of the target pyridines and bars the use of previously developed technology. Perhaps it is for this reason that no total synthesis of any E-type thiopeptide has been achieved to date. Indeed, few chemists have attempted the synthesis of

such compounds, and activity in the area has been largely confined to the preparation of simpler building blocks.[25, 26, 27,28]

Routes to E-type, 3-hydroxypyridine thiopeptide cores have been described by Shin and by Arndt. Shin and collaborators favored a linear approach that involves the modification of a preformed pyridine through classical transformations. Their route (Scheme 10) leads to compound **70** through a lengthy, 14-step sequence. Furthermore, product **70** suffers from two major shortcomings. First, the substance is obtained as a bis-ethyl ester. These two ester functions will have to be differentiated at a later stage of the synthesis, but as demonstrated by Nicolaou,[29] this is extremely difficult to do with materials of the type **70**. Translation of the Shin avenue to **70** into a total synthesis will necessitate a retailoring of the sequence, in such a way that the two ester groups (or their equivalents) emerge in a suitably differentiated form. Second, the 3-pyridinol segment in **70** is O-protected as an ethyl ether. Our own experience (*vide infra*) strongly suggests that this form of protection is entirely inappropriate, in that attempts to cleave the ethyl ether and release the free pyridinol have uniformly resulted in destruction the molecule.

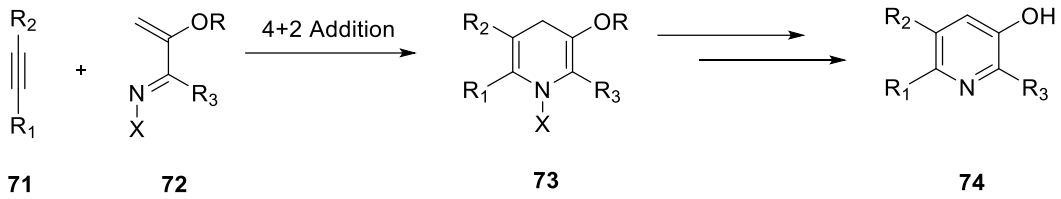


(a) CuCN/DMF, 85% (b) Et_2SO_4, K_2CO_3/THF, (c) H_2S/py, Et_3N, 80% over 2 steps (d) EBP/EtOH, 81% (e) m-CPBA/CH_2Cl_2, 100% (f) TMSCN, Et_3N/MeCN, 83% (g) EBP, K_2CO_3/THF, TFAA-py/THF (h) Ac_2O, 81% over 2 steps (i) Tf_2O, $iPrNEt_2$/DMAP (j) ethyl vinyl ether, $Pd(OAc)_2$, dppp, Et_3N/DMF, 64% (k) NBS, H_2O/THF, (l) EtOH

Scheme 10. Shin's Routes to the Pyridine Core of Nosiheptide

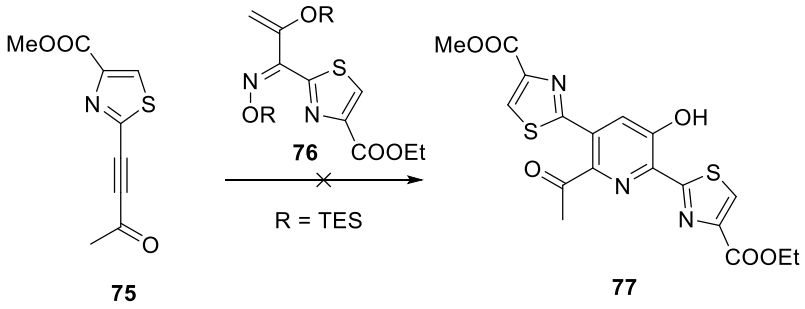
It should be mentioned that Shin and collaborators employed similar linear strategies and classical transformations also in their synthesis of micrococcin-type cores.[30] The length of the resulting sequences render their approach less attractive relative to the ones delineated in Schemes 2 to 5 above.

Arndt and his group have focused on a convergent route that relies on 1-azadiene cycloaddition technology. Thus, the union of readily available oxime **72** with appropriate acetylenes **71** led directly to **74**[26,31] (Scheme 11).



Scheme 11. Arndt's Hetero Diels-Alder Approach to the Pyridine Core of Nosiheptide

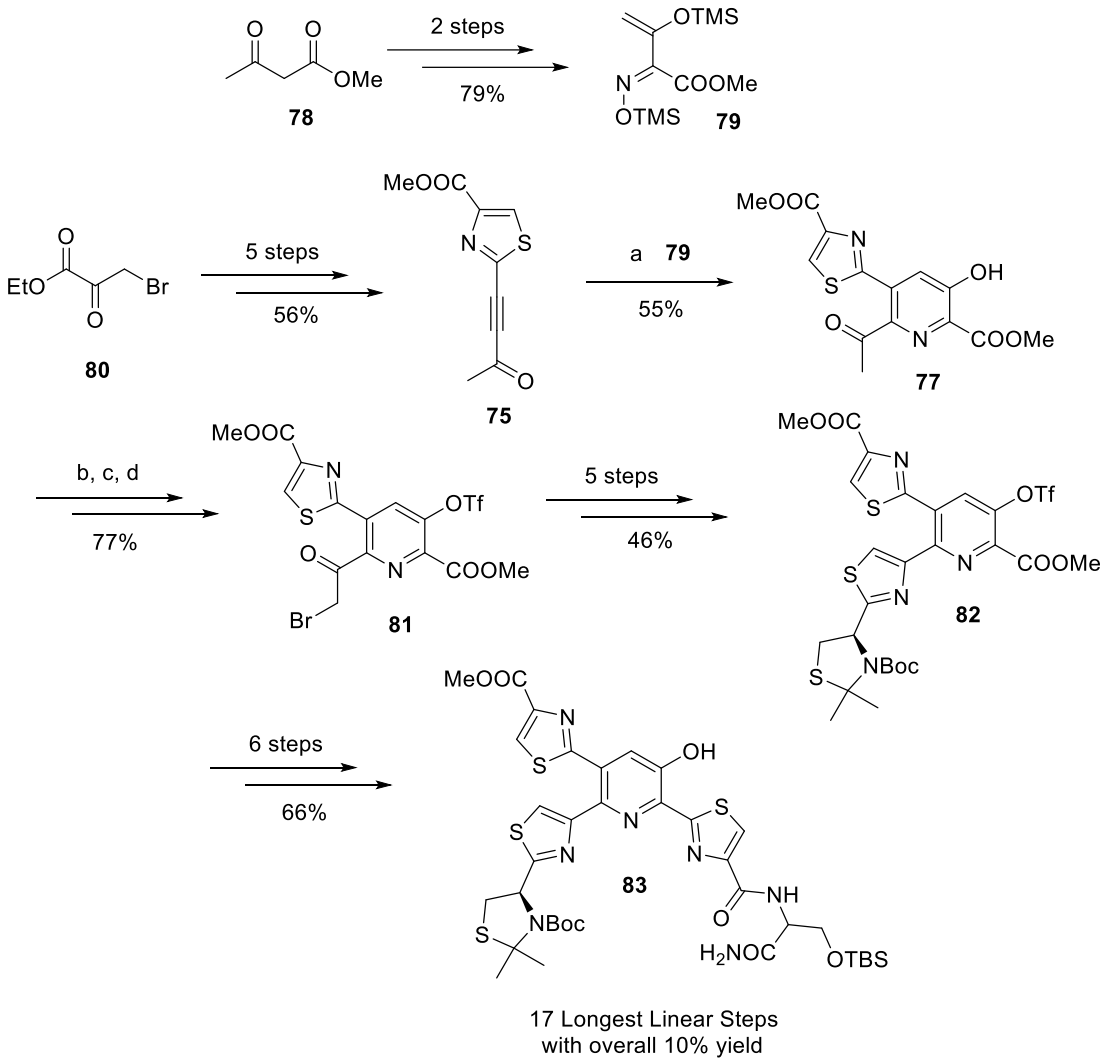
Two limitations plague this elegant strategy. First, the cycloaddition step proceeds with good regioselectivity only when the acetylenic R_1 substituent is the carbonyl group of a ketone or an ester. Furthermore, the R_3 group can only be an ester. Thus, Arndt's original plan for the assembly of **77** through the union of **75** and **76** was found to be unworkable, seemingly due to unfavorable steric interactions involving the thiazolyl group in azadiene **76** (Scheme 12). The methodology thus fails to provide access to fully substituted E-type pyridines.



Scheme 12. Arndt's Failed to Attempt for the Construction of Bis-Thiazolo-Pyridine

To overcome such limitations, the authors resorted to a hetero-Diels-Alder reaction of ynone **75** and oxime **79**, leading to pyridine **77** (Scheme 13). Eight additional steps advanced **77**

to **82**. The final incorporation of third thiazole was done through additional 6 steps to furnish **83**. The longest linear step entailed in this synthesis is a long 17 steps.



(a) Toluene, 180°C, 3eq **79** (b) Tf_2O, NEt_3 (c) TIPSOTf, NEt_3 (d) NBS, THF/H_2O

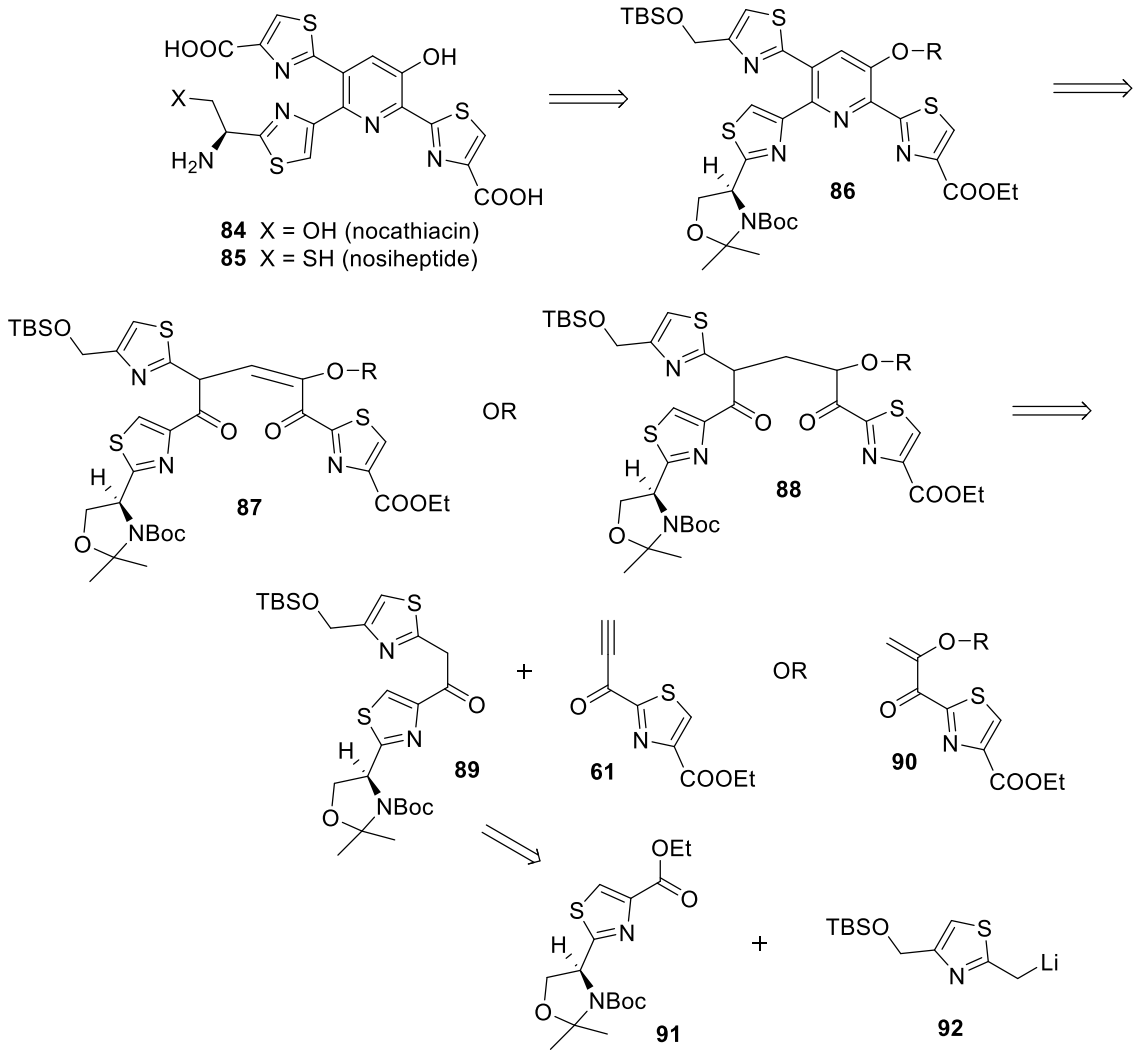
Scheme 13. Arndt's Hetero Diels-Alder to Pyridine Core of Nosiheptide

It is apparent from the foregoing that a *de novo* pyridine construction, especially one involving new methodology, generally results in a shorter and more efficient avenue to

thiopeptide cores. It is with this guiding principle in mind that we set out to devise a concise route to the core of E-type thiopeptides.

3. Synthesis of the Pyridine Core of the E-series of Thiopeptides

The logic that governed our approach to the E-series of thiopeptide hydroxypyridines rested on three principles. First, nosiheptide-type cores, **85**, would derive from nocathiacin-like ones, **84**, by OH → SH interconversion, perhaps through a Mitsunobu reaction.[32] Second, previous synthesis of MP1, **1**, and thiocillin I, **2**, had shown that differentiation of the carboxy groups is best achieved by introducing the "northwestern" COOH as a protected primary alcohol.

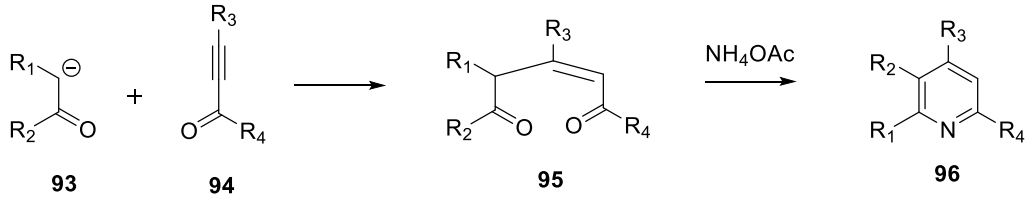


Scheme 14. Hypothetical Approaches to the Pyridine Core of Nocathiacin and Nosiheptide

Therefore, the target structures **84, 85** would be obtained from **86** (Scheme 14). Third, our routes to the pyridines cores of MP1 **1** and thiocillin I **2** relied on a sequence characterized by a high degree of convergency, which translated into a rather concise synthesis. It was essential to retain such a desirable feature in an avenue to E-series hydroxypyridines. Such an objective seemed attainable though a Hantzsch-like construction, which required either precursor **87** or **88**. The latter would be prepared through the union of ketone **89** either with ynone **61** or enone **90**. In turn, fragment **89** would emanate from the condensation of ester **91** with organolithium species **92**. In this manner, pyridine **86** would arise via a triply convergent sequence that involves the merger of segments **89** and **61**, or either **89** and **90**.

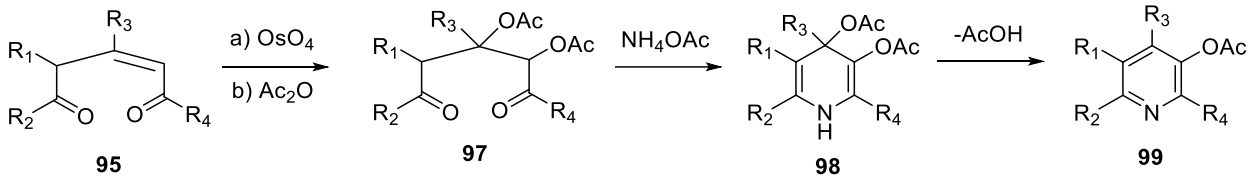
3.1 Eiden-Herdeis Approach

The elaboration of ketone **89** and ynone **61** into **87** was envisioned to proceed by a variant of the Eiden-Herdeis pyridine synthesis.[33] In its original form, this useful transformation starts with a 1,4 addition of the enolate of a ketone **93** to an ynone **94**, leading to 1,5-dicarbonyl compound **95** (Scheme 15). Reaction of the latter with — typically — NH_4OAc then furnishes pyridine **96**. Whereas the process cannot directly deliver 3-hydroxy (or alkoxy) pyridines, it was



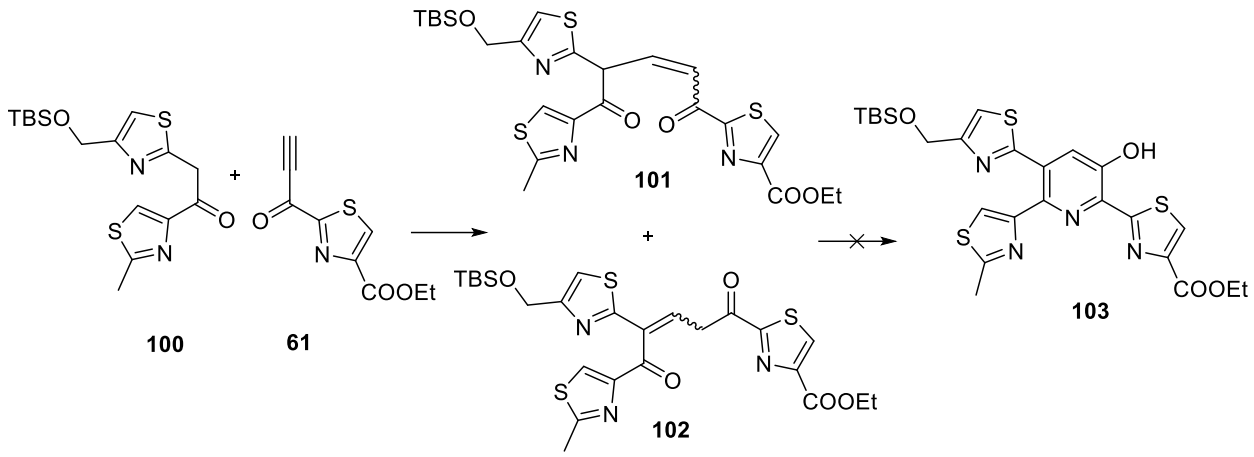
Scheme 15. Representative Eiden-Herdeis Reaction

surmised that dihydroxylation of adduct **95** and acetylation of the emerging diol might produce **97**, treatment of which with NH_4OAc in all likelihood would lead to acetoxypyridine **99** (Scheme 16).



Scheme 16. Modified Eiden-Herdeis Synthesis that May Lead to a 3-oxygenated Pyridine

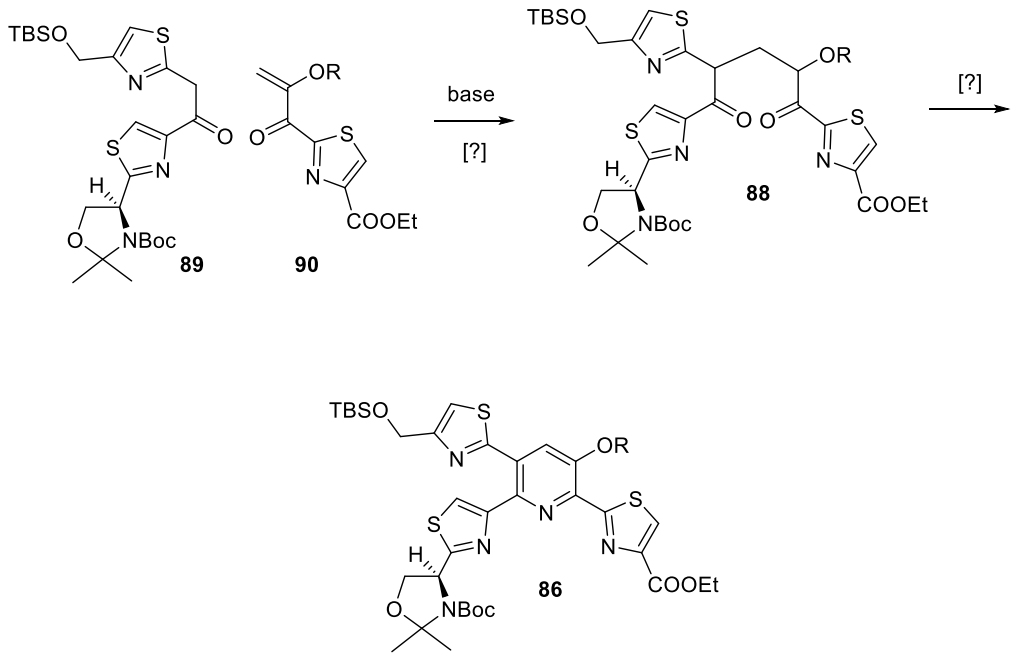
As it happens, fragments **100** and **61** were already available in our laboratory, having been prepared during earlier investigations in the thiopeptide field. Therefore, they were conveniently employed in an exploratory study that aimed to ascertain the feasibility of the above modification of the Eiden-Herdeis reaction. To our dismay, it quickly became apparent that such an approach suffered from numerous chemical and technical complications. Compound **101** was sensitive to silica gel and chromatographic purification was not possible. In addition, it appeared to equilibrate with isomer **102**, which in turn existed as two geometric isomers. Attempted osmylation of this mixture of isomers produced an even more complex mixture of products. Clearly, the Eiden-Herdeis approach was neither chemically nor technically viable. Therefore, it was abandoned and our focus shifted to a route to **86** from **89** and **90** (Scheme 17).



Scheme 17. Failed Attempt of Eiden-Herdeis Reaction

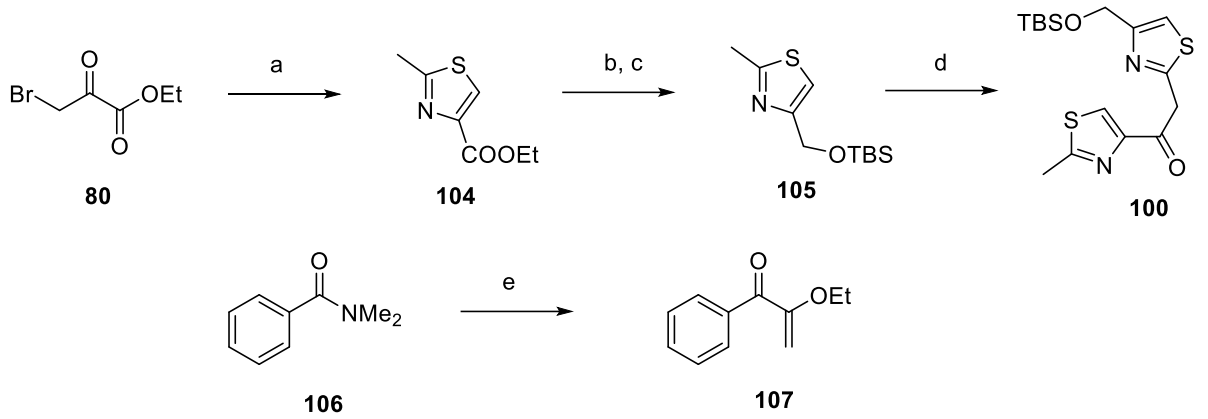
3.2 The Hantzsch Route

As seen previously in Scheme 9, Ciufolini employed a modified Hantzsch pyridine synthesis for the construction of the heterocyclic core cluster of MP1. By extension of that logic, one may surmise that the union of ketone **89** and enone **90** should lead to an ultimate pyridine of the kind found in the E-series of thiopeptides (Scheme 18).



Scheme 18. Retrosynthetic Hypothesis for the Construction of Pyridine **86**

The feasibility of such an approach was explored with model substrates **100** and **107**, both of which were readily prepared as shown in Scheme 19. Condensation of ethyl bromopyruvate with thioacetamide, reduction of the emerging thiazole **104** (DIBAL), and protection of the resultant alcohol afforded **105**. This material can be regioselectively metallated at the methyl group.[35] Condensation of lithiated **106** with ester **104** thus returned the model ketone **100** in 56% yield over 3 steps. Model enone **107** was made in just one step by addition of lithiated ethyl vinyl ether to commercial amide **106**.[34]

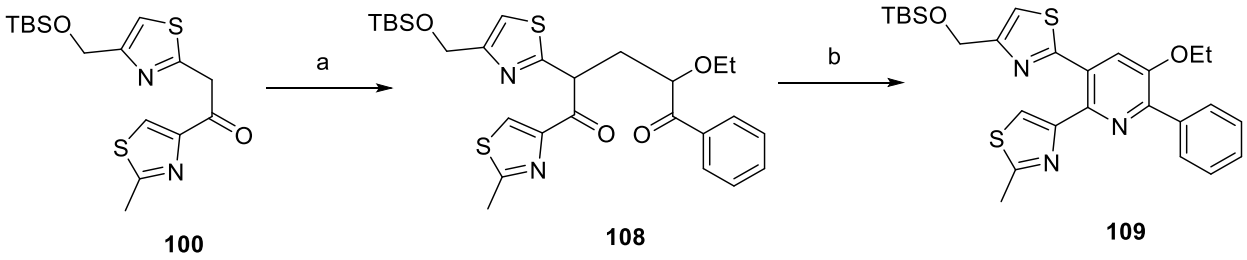


80 104 105 100 106 107

(a) thioacetamide, EtOH, 100% (b) DIBAL-H, THF (c) TBS-Cl, imidazole, DCM, 85% over 2 steps (d) n-BuLi, THF, **104**, 60% (e) t-BuLi, THP/THF, ethyl vinyl ether, 65%

Scheme 19. Preparation of Model Substrates

The lithium enolate of ketone **100**, prepared by deprotonation with LDA, smoothly underwent Michael addition to enone **107** to furnish 1,5 diketone **108** as a mixture of diastereomers and of keto-enol– as well as ring-chain tautomers. Subjection of **108** to NH_4OAc in refluxing ethanol under an O_2 atmosphere (balloon) afforded pyridine **109** in a moderate, but satisfactory, 38% yield (Scheme 20).

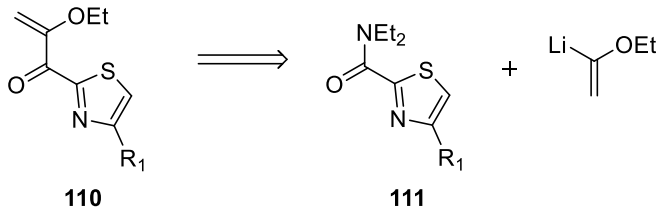


100 108 109

(a) LDA, THF, **107**, -78°C to 0°C (b) NH_4OAc, EtOH, O_2, 60°C, 38% over 2 steps

Scheme 20. Preparation of Model 3-ethoxypyridine

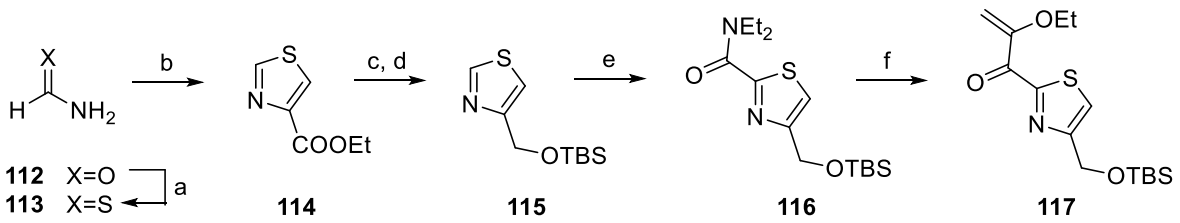
This encouraging result induced us to research whether the technique could be extended to the preparation of a tris-thiazolyl pyridine more closely resembling the core of thiopeptides; i.e., whether a thiazolyl enone of general structure **110** might be serviceable in the above sequence (Scheme 21).



110 **111**

Scheme 21. Retrosynthetic Analysis of Thiazole Enone Fragment

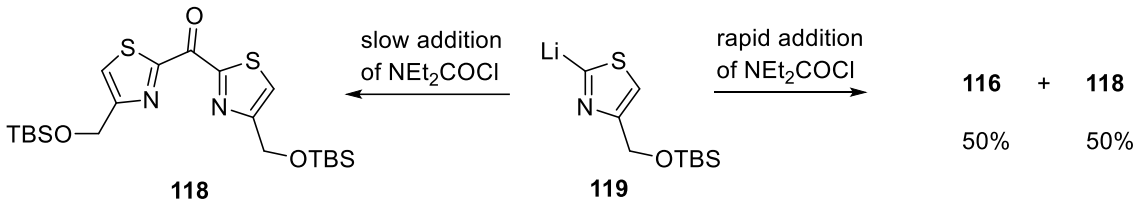
Past experience had shown that enones similar to **110**, but lacking the ethoxy functionality, are problematic substrates in Michael chemistry. They are extremely electrophilic, and this causes them to polymerize easily under a variety of nucleophilic conditions.[35] At the present juncture, it was unclear whether the alkoxy group might alleviate or exacerbate such unpleasant tendencies. Indeed, the ethoxy unit might moderate the electrophilic reactivity of the enone,[36] thus retarding Michael polymerization, or it might destabilize the enolate resulting from an initial 1,4-addition,[37] so as to trigger a number of possible side reactions. Regardless, a suitable form of **110** seemed to be available by reaction of amide **111** with lithiated ethyl vinyl ether. Accordingly, a sequence very similar to that seen in Scheme 19, but employing thioformamide in lieu of thioacetamide, led to thiazole **115** in 20% overall yield (Scheme 22). Deprotonation of the latter and reaction with *N*,*N*-diethyl carbamoyl chloride produced **116**, which upon addition of lithiated ethyl vinyl ether advanced to **117**.



(a) P_2S_5, Et_2O. (b) EBP, EtOH, 2 steps over 37%. (c) DIBAL-H, THF, -78°C to r.t (d) TBS-Cl, imidazole, DCM, 90% over 2 steps. (e) n-BuLi, Et_2NCOCl, 50%. (f) t-BuLi, THP/THF, ethyl vinyl ether, 77%

Scheme 22. Preparation of Thiazolyl Enone **117**

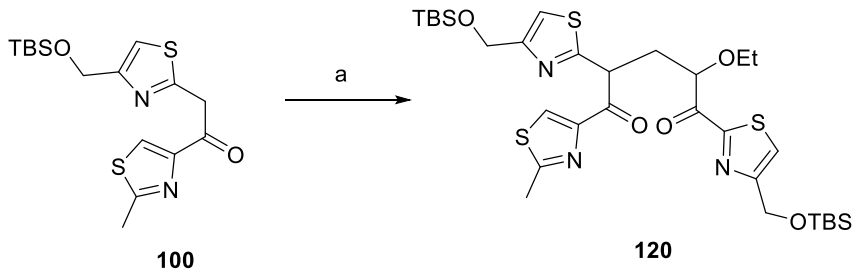
An unpleasant problem was uncovered during the transformation of **115** into **116**: the reaction of lithiated **115** with *N*,*N*-diethyl carbamoyl chloride returned significant quantities of ketone **118**: the product of double addition of **119** to the electrophile (Scheme 23). A slow addition of carbamoyl chloride to a solution of **119** (–78 °C) resulted in exclusive formation of **118**. A rapid addition of the electrophile afforded an essentially 1:1 mixture of undesired **118** and desired **116**. Luckily, no such complication was observed in the addition of lithiated vinyl ether to amide **116**, and the desired enone **117** was obtained smoothly and in good yield.



Scheme 23. Unexpected Thiazole Anion Behavior

We were pleasantly surprised to discover that enone **117** was a well-behaved substrate in Michael chemistry. Indeed, it transpired that the best way to carry out the Michael step was to add **117**, at room temperature, to a solution of the sodium enolate of **100**, prepared by reaction of

the ketone with sodium hydride. The lithium enolate of **100**, prepared with LDA as shown earlier in Scheme 20, produced inferior results due to incomplete conversion after longer reaction times. Diketone **120** was again obtained as a mixture of tautomers and diastereomers (Scheme 24).

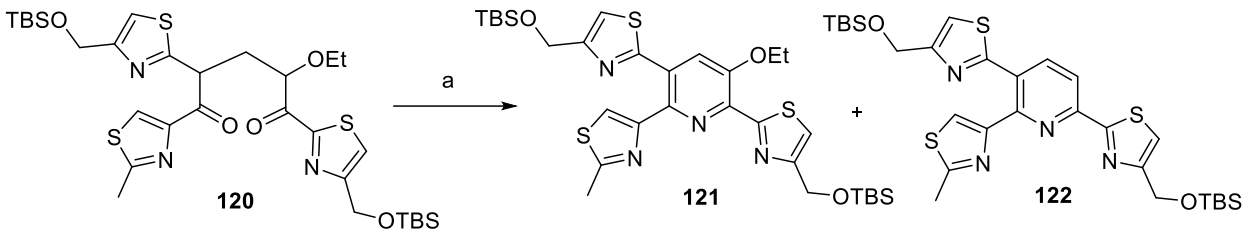


100 **120**

(a) NaH, THF, enone **117**

Scheme 24. Formation of 1,5 Diketone of Model Tris-Thiazole System

To our surprise, subjection of **120** to the pyridine-forming conditions described above for model system **108** (Scheme 20) furnished desethoxy pyridine **122** as the major product (Scheme 25). The desired **121** was present in the reaction mixture (^{1}H NMR, mass spectrometry), but the extent of its formation was less than 5%. The formation of **122** must be due to aromatization of the sensitive dihydropyridine intermediate through elimination of ethanol. It was surmised that metallic oxidants, such as Fe(III)[acac]$_3$ or Cu(II)(OAc)$_2$, might accelerate the aromatization of the Hantzsch dihydropyridine and suppress the elimination of ethanol. However, reactions carried out in the presence of such metallic agents produced a greater amount of undesired pyridine.



(a) NH_4OAc, EtOH, O_2, 60°C (Table 1)

Scheme 25. Unexpected Outcome of Condensation of Pyridine **121**

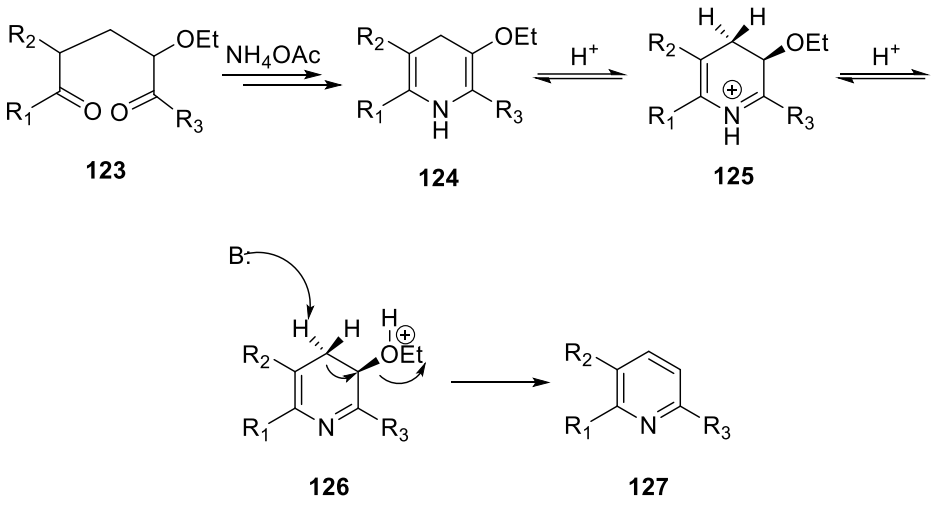
Additional investigations revealed that the formation of desethoxy pyridine **122** could be suppressed by running the reaction in a slightly acidic media. Table 1 below summarizes the results of representative optimization experiments. The best solvent was a 5:8 mixture of 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), and best result were obtained by operating at 60 °C. Slow addition of 1,5 diketone **120** (syringe pump) into the reaction vessel was beneficial. Also, NH_4OAc was optimal among the various ammonia sources (NH_4Cl, NH_4COOCF_3, $(NH_4)_2CO_3$) examined during this study. The moderate yield of **121** thus obtained, 35% after chromatography, was deemed satisfactory at this stage, especially in light of the conciseness and simplicity of the method.

Solvent	Temperature	Medium condition	Yield (%) "121" + "122"
EtOH	60 °C	O_2	9% + 15%
AcOH	60 °C	O_2	3% + 0%
TFE	60 °C	O_2	19% + 0%
HFIP	60 °C	O_2	6% + 0%
TFE/HFIP(5:8)	60 °C	O_2	28% + 0%
TFE/HFIP(5:8)	60 °C	Under O_2, slow addition	35% + 0%

Table 1. Optimization Studies of the Pyridine Formation

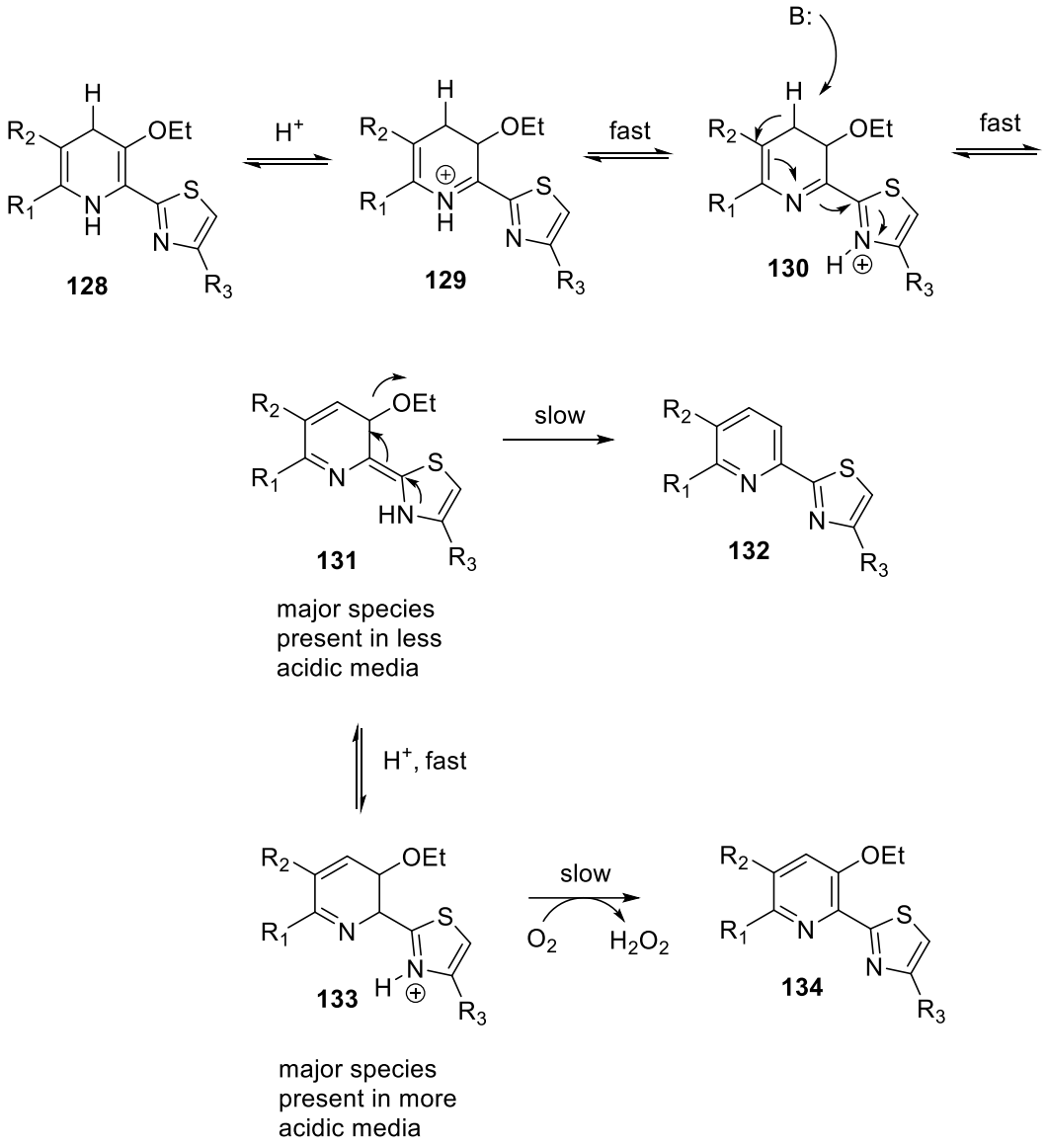
It is interesting to speculate as to the reason(s) why acidic media disfavor the formation of desethoxypyridine **122**. One possible — if naïve — explanation is that a basic agent (B: in

Scheme 26; acetate ion) might be involved in the elimination of EtOH from dihydropyridine isomer **126**. Acidic media would neutralize such basic species and retard the rate of eliminative aromatization (Scheme 26).



Scheme 26. Hypothetical Mechanism of Elimination of the Pyridine

However, this fails to account for the fact that desethoxy pyridine formation was observed only with substrate **121**: no such problem subsisted with analog **109**. The implication then is that the thiazole segment adjacent to the OEt group somehow promotes formation of **122**. A rationale for this may be ventured as follows. Because the reaction medium is always acidic (excess NH_4OAc), Hantzsch dihydropyridine **128** may exist in fast prototropic equilibrium with species **129** and **130**. The latter, in turn, may equilibrate with **131**, the enamine segment in which could promote slow expulsion of the ethoxy group, leading to **132**. Progressively more acidic media would favor protonation of **131** to give **133**, which may well become a/the major equilibrium species in the presence of acid. Loss of ethanol from **133** is unlikely: the only fate awaiting it would be slow aerobic oxidation to **134** (Scheme 27).

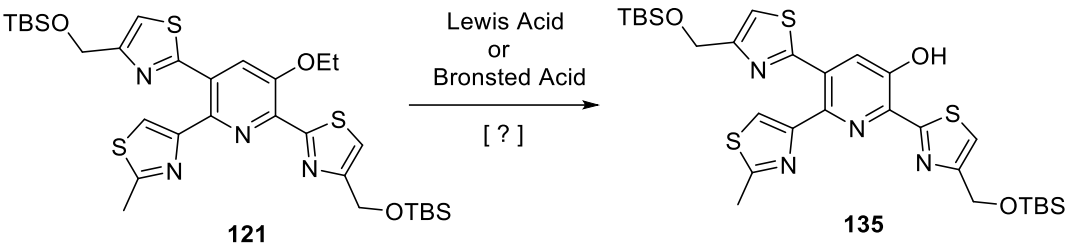


Scheme 27. Hypothetical Mechanism of Desethoxypyridine Formation in Tris-Thiazole System

3.3 Model Study to Restore Hydroxypyridine

The sequence detailed above delivered pyridine **121**, wherein the phenolic OH group is present as ethyl ether. A search of the literature failed to uncover precedent for the deblocking of

similar heterocyclic phenolic ethers. Therefore, compound **121** served to explore deprotection methods that might lead to free pyridinol **135** (Scheme 28).

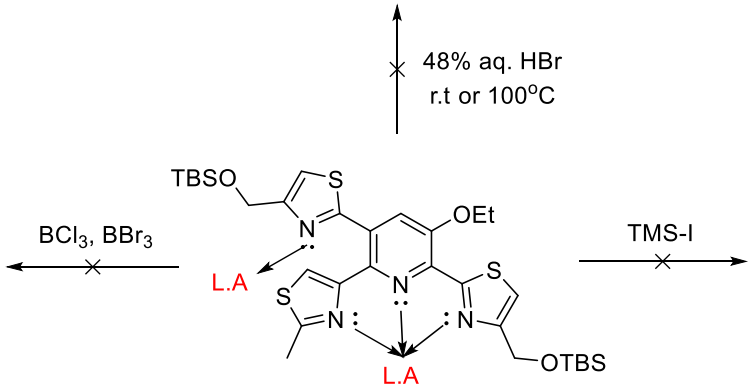


Scheme 28. Envisioned Deprotection Sequence

Initial efforts centered on classical methods to effect the foregoing transformation. A time-honored approach to the cleavage of phenolic ethers is the use of 48% aqueous HBr.[38] Exposure of **121** to this reagent, at room temperature, resulted in clean release of the TBS groups, but the ethyl ether remained intact even after 12 h at 25 °C. Heating of the reaction mixture at temperatures above 50 °C, in an attempt to force the issue, resulted in rapid destruction of the substrate. Evidently, the molecule was intolerant of vigorous treatment with Bronsted acids.

Other conventional deblocking methods involve the use of Lewis acidic reagents, e.g., BBr_3,[39] TMSI,[40] and so on. The action of one equivalent of BBr_3 upon pyridine **121** induced the release of a single TBS group. The addition of two more equivalents of BBr_3 had virtually no effect: the desilylated derivative of pyridine **121** seemed to be perfectly stable under these conditions. However, the introduction of a total of more than 3 equivalent of BBr_3 prompted rapid destruction of the substrate. Similar results were obtained when Me_3SiI was employed in lieu of BBr_3, indicating that the molecule was also intolerant of Lewis acidic reagents.

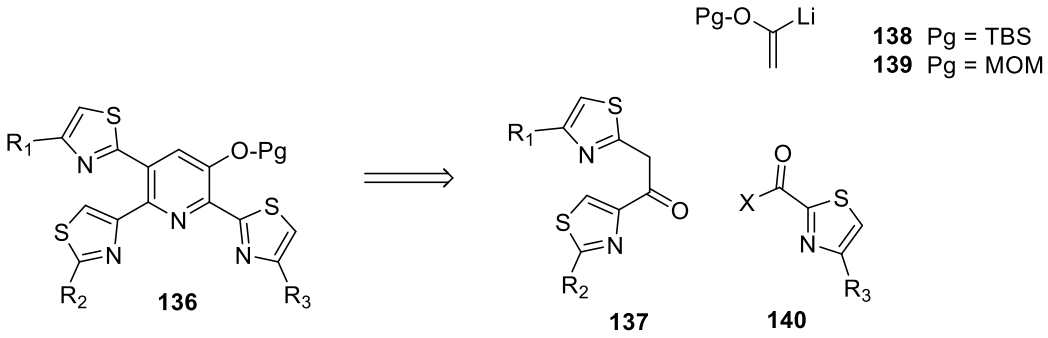
The reasons for this behavior are unclear. Compound **121** obviously presents numerous Lewis basic sites, which could sequester multiple equivalents of Lewis acidic species (Scheme 29). It thus seems reasonable that more than 2-3 equivalents of a deblocking agent would be required to release the ethyl group. What escapes a simplistic explanation is the rapid and complete obliteration of the substrate upon contact with more than 3 equivalents of such reagents.



Scheme 29. Unfruitful Deprotection Sequences Attempted

The foregoing observations mandated the use of a more labile pyridinol protecting group the "real" system. Recall that the latter would incorporate additional acid-sensitive functionality, which in any event barred the use of vigorous, poorly selective reagents. The choice of a suitable blocking group was also conditioned by the fact that such a segment would have to withstand the formation of organolithium species. Labile forms of protection, such as ester and silyl ethers, would be entirely inadequate in the present context. To illustrate, attempted lithiation of the TBS enol ether of acetaldehyde to give organometallic agent **138** Pg = TBS, followed by interception thereof by addition to an aldehyde, afforded intractable mixtures of products. No attempt was made to determine the nature of these. In the end, we opted for Pg = CH_2OMe (methoxymethoxy, MOM). The acetal nature of such a group rendered it potentially releasable under very mild

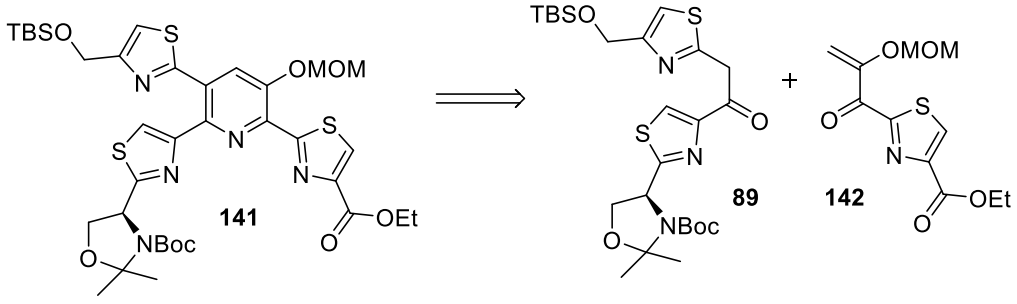
acidic conditions. Furthermore, organometallic compound **139**, Pg = MOM, is known.[41] Our confidence in the pyridine-forming sequence, and in our ability to successfully deblock a MOM-protected pyridinol, was sufficiently high that the next phase of the research focused directly on the synthesis of the actual nocathiacin / nosiheptide core (Scheme 30).



Scheme 30. Retrosynthetic Analysis of Pyridine Core Exhibiting More Labile Protecting Group

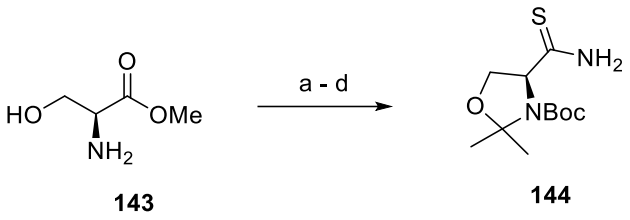
3.4 Approaches to the Assembly of the Real Pyridine

The observations summarized in the preceding sections, as well as past experience in the thiopeptide field, led us to envision compound **141** (Scheme 31) as a particularly convenient form of the pyridine-thiazole core cluster of nocathiacine and nosiheptide. The synthesis of **141** would be achieved from fragments **89** and **142**.



Scheme 31. Retrosynthetic Analysis of the 'Real' Pyridine

A route to **89** was charted from L-serine methyl ester, which was converted into the known[42] thioamide **144** in 4 steps and in 54% yield (Scheme 32). This thioamide would function as a nucleophile in a subsequent Hantzsch thiazole synthesis: an especially practical method to for the preparation of thiazoles first described in 1887.[43] The process entails a generally

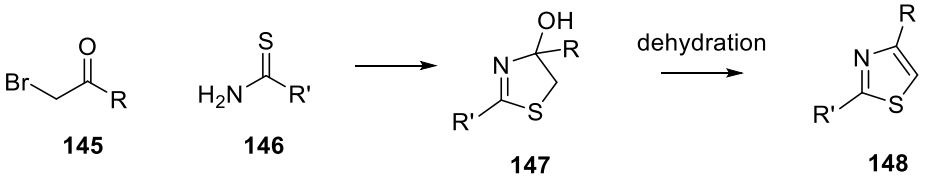


143 **144**

(a) Boc_2O, Et_3N, DCM, 0°C to r.t, 85% (b) Dimethoxypropane, PPTS, THF, reflux (c) NH_4OH/MeOH, 35°C, 85% over 2 steps (d) Lawesson's reagent, THF, reflux, 66%

Scheme 32. Preparation of Thioamide **144**

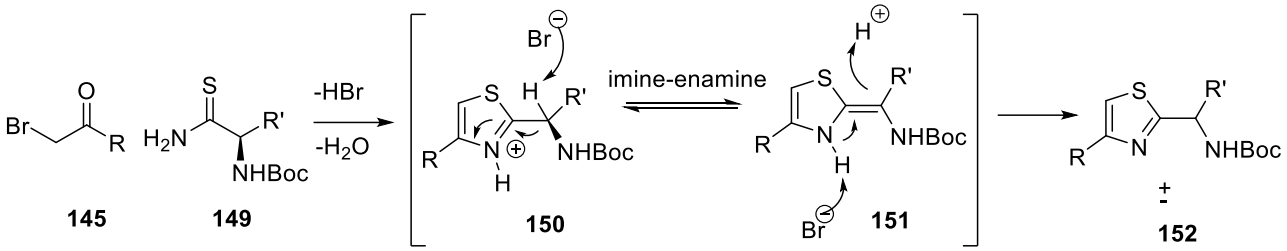
high-yielding condensation of a thioamide with an α-halocarbonyl compound, commonly in refluxing ethanol (Scheme 33). The reaction is also very simple to implement.



145 **146** **147** **148**

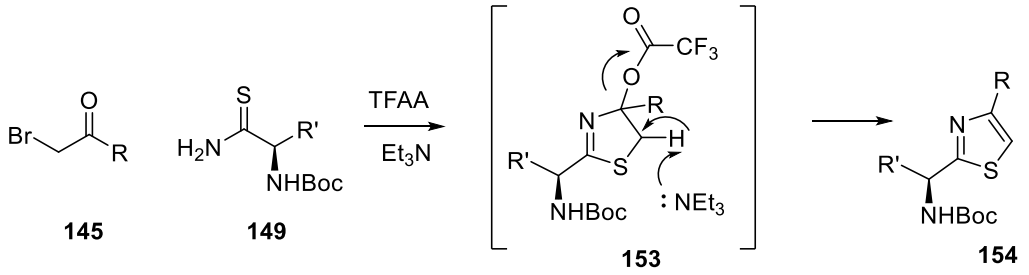
Scheme 33. Hantzsch Thiazole Synthesis

However, enantioenriched thioamides derived from α-amino acids suffer substantial or complete racemization during the reaction. Available evidence[44] indicates that the problem occurs at the stage of thiazole **150**, which upon protonation by the HBr released into the medium equilibrates with enamine **151**, resulting in loss configuration (Scheme 34).



Scheme 34. Racemization of α-amino Thiazoles

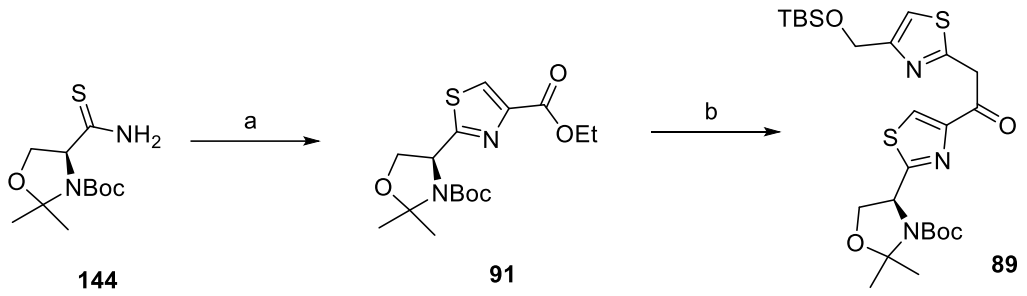
Such a difficulty may be circumvented by condensing amino acid derived thioamides **145** with an α- halocarbonyl compound at low temperature, and under gently basic conditions, and by dehydrating the emerging hydroxythiazoline **154** with trifluoroacetic anhydride (TFAA) in the presence of pyridine and triethylamine (Scheme 35). This procedure is commonly described as the Holzapfel-Meyers-Nicolaou (HMN) modification of the Hantzsch thiazole synthesis.[17] Accordingly, reaction of **145** with ethyl bromopyruvate afforded thiazole **91** with no erosion of stereochemical purity.



Scheme 35. The Holzapfel-Meyers-Nicolaou (HMN) Variant of Hantzsch Thiazole Synthesis

Reaction of **91** with lithiated thiazole **92**, as seen previously in Scheme 19, then afforded ketone **89** (Scheme 36). It should be mentioned that compound **89** existed as a mixture of keto- and enol tautomers (^{1}H NMR) in $CDCl_3$ at room temperature. Furthermore each tautomer gave rise to a pair of BOC group rotamers that interconverted slowly on the NMR time scale at 25 °C:

this produced appreciable broadening of the ^{1}H and ^{13}C NMR signals of the molecule. Much sharper spectra were obtained at 60 – 80 °C from acetonitrile-d_3 or DMSO-d_6 solutions.



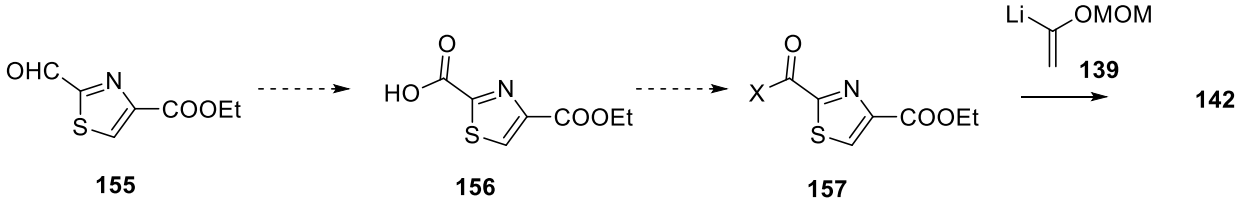
144 **91** **89**

(a) EBP, $KHCO_3$, Et_3N, Pyridine, TFAA, 97% (b) n-BuLi, THF, **105**, 74%

Scheme 36. Preparation of Ketone **89**

Analogous line broadening was observed for all subsequent compounds derived from **89**. Therefore, NMR spectra of derivatives of **89** were recorded both at 25 °C and at appropriately higher temperatures.

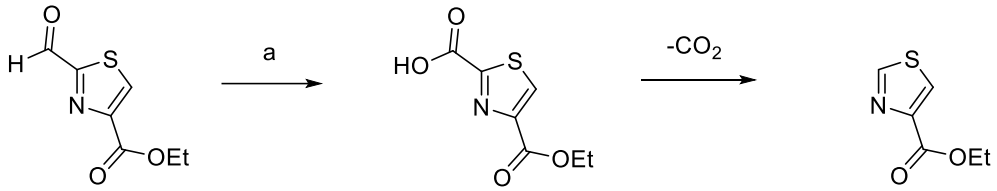
The preparation of enone **142** was less straightforward. A reasonable precursor of **142** was aldehyde **155**, which had previously been utilized in the total synthesis of thiocillin I.[20] An initial route to **142** envisaged oxidation of **155** to carboxylic acid **156**, which after suitable activation (cf. **157**, L = leaving group) would react with **139** (Scheme 37).



155 **156** **157** **142**

Scheme 37. Envisioned Synthesis of Enone from the Corresponding Carboxylic Acid

However, whereas the Pinnick oxidation of **155** afforded **156** in good yield, the tendency of the latter to undergo decarboxylation to **114** (Scheme 38) precluded its conversion into an acyl chloride or an acyl imidazole, in preparation for the organometallic step. Therefore, this



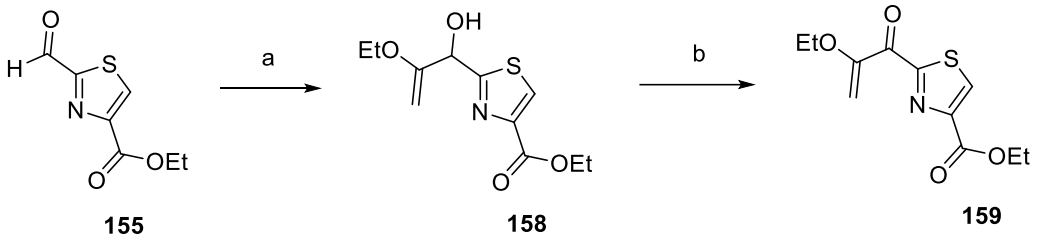
155 **156** **114**

(a) $NaClO_2$, NaH_2PO_4, t-BuOH, 2-methyl-2-butene, 80%

Scheme 38. Unsuccessful Functional Group Interconversion Due to Rapid Decarboxylation

approach was abandoned, in favor of one involving the postponement of the oxidative step after the reaction with a suitable organolithium species.

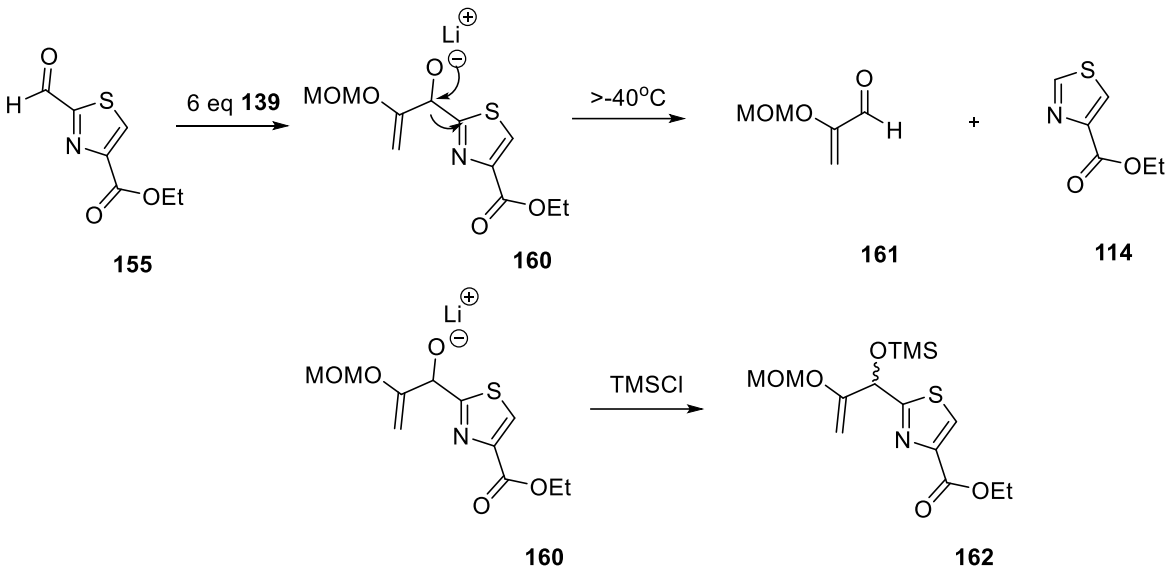
Lithiated ethyl vinyl ether underwent smooth addition to **155** to afford **158**. There was no interference from the ester functionality. On the other hand, attempts to purify sensitive alcohol **158** resulted in unacceptable material loss (mass recovery less than 10%). Fortunately, immediate MnO_2 oxidation of crude **158** delivered enone **159** in excellent yield. Although **159** was not useful for the assembly of the real pyridine (it incorporates an O-Et, instead of an O-MOM, group), it served to validate the general approach (Scheme 39).



(a) t-BuLi, THP/THF, ethyl vinyl ether, 89%, (b) MnO_2, DCM, 85%

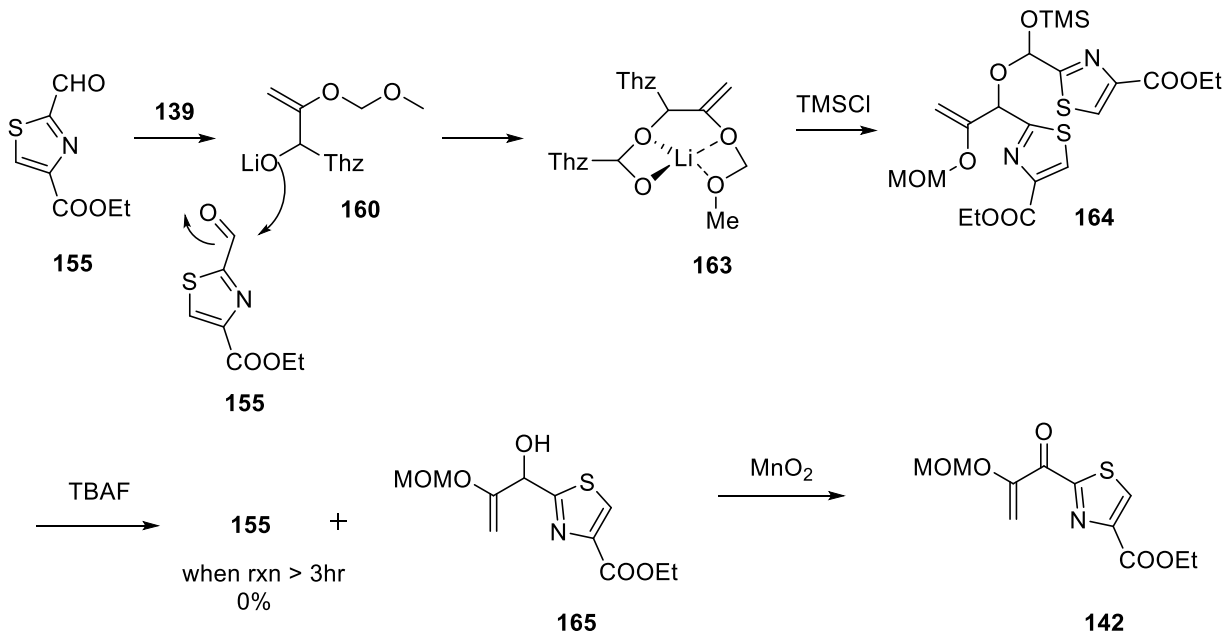
Scheme 39. Synthesis of Enone **159**

Our attention thus refocused on the reaction of **155** with lithiated vinyl MOM ether.[41] Unlike the sequence of Scheme 22, the new process suffered from a number of complications. First, six equivalents of **139** were necessary to force the reaction to completion. Second, the alkoxide **160** thus formed underwent fragmentation to give **161** and **114** upon warming above -40 °C. This mandated the quenching of the reaction mixture containing **160** with TMSCl, at -78 °C, prior to work-up. The product thus obtained, initially assumed to be TMS-protected alcohol **162**, was found to be very sensitive to both acid and base, but deprotection with TBAF reproducibly afforded **165**.



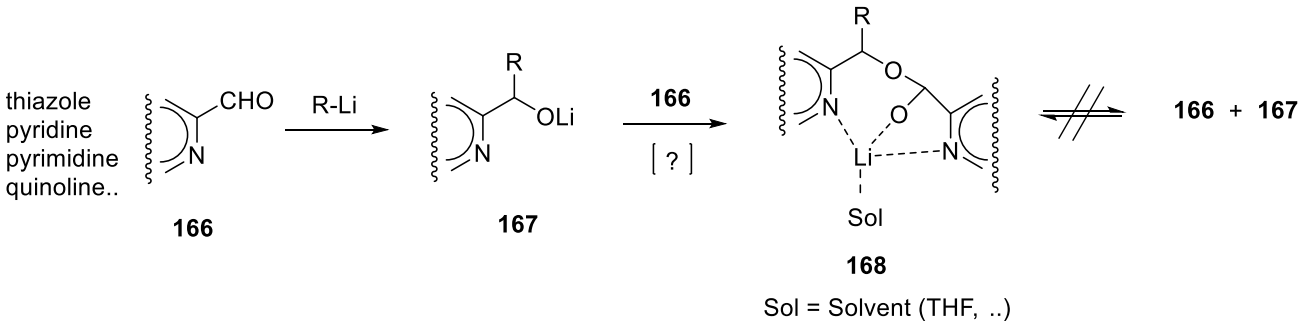
Scheme 40. Unexpected Outcomes of Elimination Pathways and Circumvention

However, this step also produced equal amounts of aldehyde **155**, which was definitely not present in crude **162**. Scrutiny of reaction mixtures (^{1}H NMR) revealed that the presumed **162** was actually a mixture of diastereomers of compound **164** (Scheme 41). A plausible mechanism for the formation of this material involves addition of lithiated **139** to **155**, followed by rapid addition of alkoxide **160** to a second molecule of the extremely electrophilic aldehyde **155**. The resultant hemiacetal anion appears to be unusually stable under the conditions of this reaction (it does not release starting **155**), perhaps due to its existence as chelated complex **163**. Interception of this intermediate by TMS-Cl leads to mixed acetal **164**. Exposure of the latter to TBAF causes unraveling to an equimolar mixture of **165** and starting **155**. Attempts to contain the formation of **165** by simultaneous addition of **155** and TMSCl to a solution of **139**, in the hope of capturing alkoxide **160** before it might add to a second molecule of **155**, were uniformly unsuccessful.



Scheme 41. Formation of Dimeric Adduct **164** and Formation of Enone **142**

It should be noted that the literature records numerous cases, in which the addition of an organometallic species to a highly electrophilic, thiazole-, quinoline-, or pyrimidine-derived heteroaromatic aldehyde takes place in low / moderate yield.[45] In one case, this has been attributed to the "reduced electrophilicity" of the aldehyde [46] even though all indications point to the conclusion that the aldehyde in question is quite electrophilic. It is tempting to speculate that the diminished yields often observed with the above substrates may be due to the rapid formation of stabilized chelates such as **168** (Scheme 42), which do not readily release the free aldehyde under typical reaction conditions.

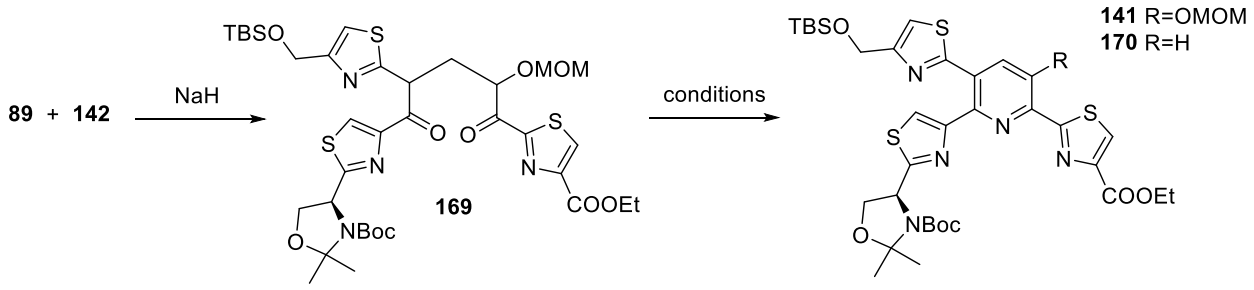


Scheme 42. Possible Insights into the Reactivity of Aromatic Aldehyde

Just as indicated earlier for **158**, any method to purify **165** resulted in a massive loss of material. Therefore, this sensitive alcohol was immediately subjected to allylic oxidation with MnO_2 to furnish enone **142**. The oxidation step proceeded smoothly, but the product enone **142** was contaminated with starting aldehyde **155**. In all likelihood, the aldehyde would interfere with the subsequent Michael step, by reacting with the enolates involved in the reaction. It was thus essential to remove it from **164**. Attempted chromatographic purification of the latter resulted in major loss of material. Indeed, enone **142** proved to be quite intolerant of chromatographic supports such as silica and alumina. The search for a way to remove **155** from **164** without resorting to chromatography revealed that longer contact time between **164** and TBAF (3 hrs instead of 30 mins**)** affords **165** free from **155**. We believe that the small amounts of water and/or hydroxide impurities present in TBAF react with aldehyde to form the corresponding hydrate, which is likely to be water-soluble, and is therefore readily removed during the subsequent aqueous work up. Aldehyde-free enone thus obtained degraded fairly rapidly at room temperature; therefore, it was promptly advanced to the subsequent Hantzsch reaction without further purification.

To our surprise, the seemingly minor change in O-protecting groups between enones **117** and **142** had a significant impact on the efficiency of the pyridine-forming sequence. Thus, the

merger of ketone **89** and enone **142** under the conditions detailed earlier in Scheme 24 and 25 returned pyridine **141** in only 4% yield (Table 2). Such a poor result was attributable to the diminished efficiency of both the Michael reaction and the pyridine-forming step. Concerning the latter, it was determined that a major byproduct of the reaction was desoxypyridine **170**. Observation made during model work suggested that more acidic reaction media should prevent the formation of **170**. Ultimately, it was determined that conduct of the reaction in a mixture of TFE/AcOH (4:1), and in the presence of 1 mol eq. of PPTS,[47] suppressed the formation of **170** and tripled the yield of desired **141** (10% over two steps).

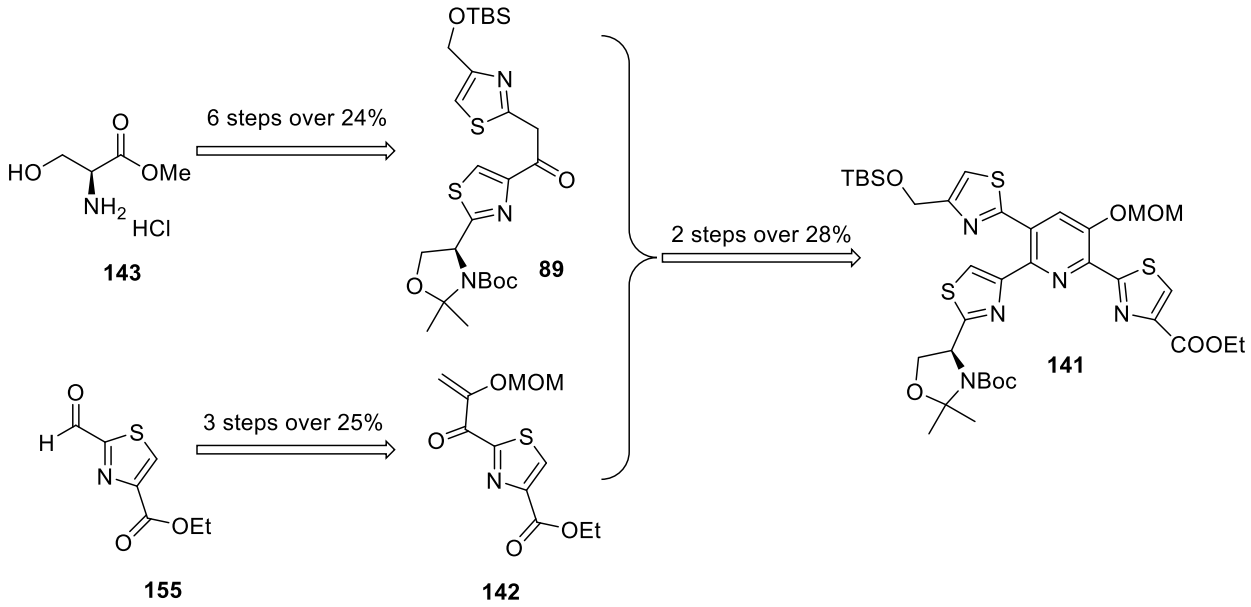


Solvent	Enone	Additive	Yield of 141	Yield of 170
5:8 TFE/HFIP	Crude	None	4%	10%
AcOH	Crude	None	4%	0
HFIP	Crude	None	5%	0
4:1 TFE/AcOH	Crude	PPTS	10%	0
4:1 TFE/AcOH	Purified	PPTS	28%	0

Table 2. Attempted Optimization Sequences Leading to the OMOM Pyridine

As far as the Michael step is concerned, it rapidly transpired that the use of enone of greater purity was the key to a cleaner, more efficient reaction, but as mentioned previously, the purification of **142** is a delicate proposition. We presumed that this was due to the acid-sensitive nature of that material. We thus developed a chromatographic method that entails a rapid elution

of **142** through silica gel that had been strongly deactivated with Et_3N. To our relief, the method afforded enone of good (ca. 90% by NMR) purity, while containing material loss to an acceptable level. When this enone of greater purity was employed in the pyridine-forming reaction, under the optimized condition developed earlier, the desired **141** emerged in 28% chromatographed yield over 2 steps. Despite its moderate yield, this route to **141** is short (11 operation total, with the longest linear sequence encompassing 6 steps and with overall yield of 11% along this path; Scheme 43) and it employs inexpensive starting materials and reagents.

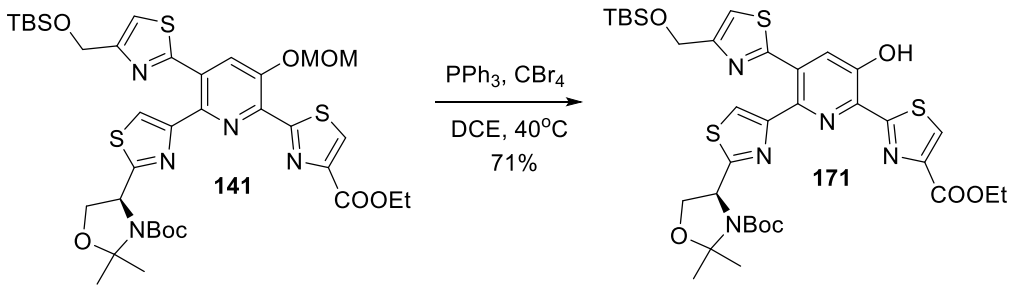


Scheme 43. Summary of the Route to Pyridine **141**

We conclude this section with a reminder that the BOC unit in **141** gave rise to a pair of slow-interconverting (25 °C) rotamers. This caused broadening of ^{1}H and ^{13}C NMR signals. Spectra recorded at 60 – 80 °C from acetonitrile-d_3 or DMSO-d_6 solutions were much sharper and more readily interpreted.

3.5 Restoration of 3-hydroxypyridine

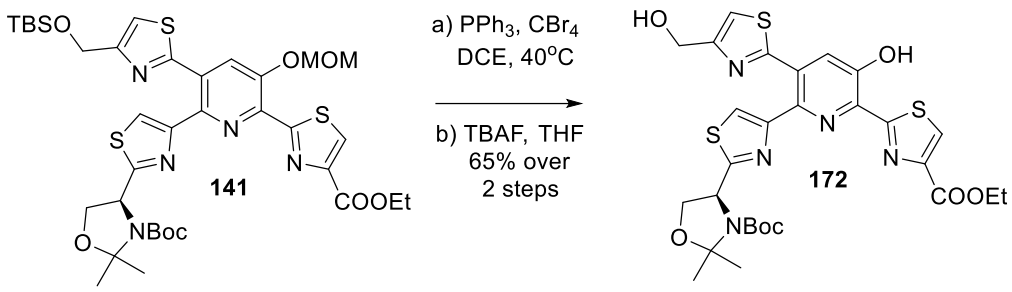
A final aspect of this phase of the research centered on the selective deblocking of **141** to a free 3-hydroxypyridine. Once again, conventional techniques for MOM group cleavage, by the use of the acidic reagents listed in section 3.3, led either to destruction of the substrate or release of the other protecting groups. A literature search aiming to identify the mildest possible



Scheme 44. Successful Peng Deprotection of Phenolic MOM-Ether

methods for phenolic MOM ether deprotection led to a noteworthy paper by Peng and coworkers. These researchers discovered that MOM derivatives of phenols are cleanly deblocked upon treatment with 25 mol % each of PPh_3 and CBr_4 at 40 ^oC in DCE.[48] Despite the complexity, and the observed sensitivity of pyridine **141**, the Peng deprotection performed extremely well and delivered **171** in 71% yield (Scheme 44). The survival of the TBS group under these conditions is remarkable. Line broadening due to the slow interconversion of BOC rotamers was apparent also in the room temperature NMR spectra of **171**. An unpleasant surprise materialized upon heating a solution of **171** in DMSO-d_6 in an NMR probe, in an attempt to obtain sharper spectra: partial release of the TBS group occurred (ca. 20% after 30 min), preventing proper characterization of what was now a mixture of **171** and **172** (Scheme 45). It was thus decided to

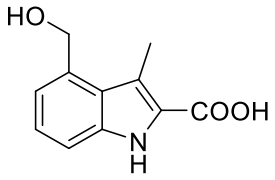
fully deblock **171** and to carry out a full characterization at the stage of **172**, obtained in 65% yield from **141** over 2 steps.



Scheme 45. Fully Deblocked Hydroxypyridine for Full Characterization

4. Indole Segment of Nosiheptide

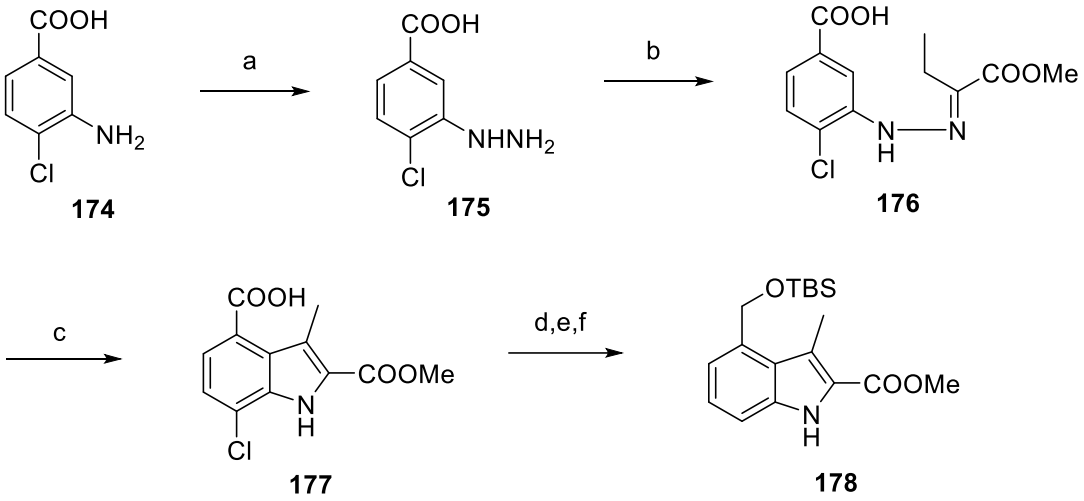
One of the other key heterocyclic units of nosiheptide is indole **173** (Scheme 46). A good route to a differentially protected form of **173** has been described by Moody and collaborators.[28]



173

Scheme 46. The Desired Indole Fragment of Nosiheptide

Their approach rests upon a Fischer indole synthesis, which employs the hydrazine derived from commercial aniline **174** and methyl α-oxobutyrate (Scheme 47). The Moody sequence was duplicated without difficulty. Indeed, indole **177** thus obtained was of excellent purity and required no chromatography. Straightforward functional group manipulations, as detailed by Moody, were served to convert **177** into the desired **178**. This fragment was thus assembled in an efficient manner with 6 steps over 30% yield.

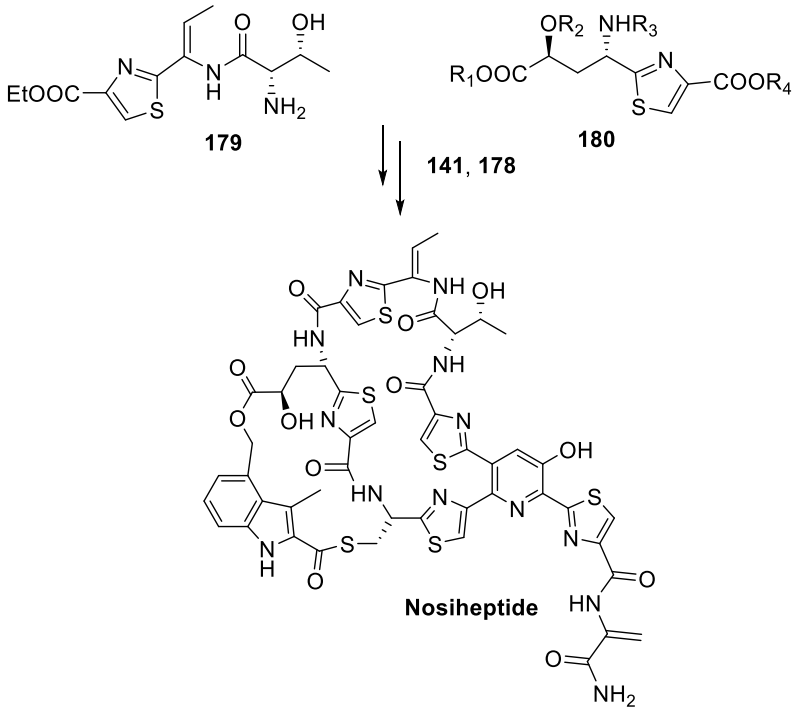


(a) $NaNO_2$, HCl, then $SnCl_2$, -10 °C (b) methyl 2-oxobutanoate, AcOH/H_2O, 70 °C (c) AcOH, PPA, 90 °C, 60% over 3 steps (d) H_2, Pd/C, MeOH (e) BH_3-SMe_2, THF (f) TBS-Cl, imidazole, DMF, 50% over 3 steps

Scheme 47. Synthesis of Indole Fragment of Nosiheptide **178**

5. Conclusion

The synthesis of the pyridine core of nocathiacin and nosiheptide has been demonstrated. The route entails twelve distinct operations and six steps along the longest linear sequence. This approach is by far the most competitive one compared to known alternatives, in terms of length, yield and cost of materials. In order to complete the total synthesis of nosiheptide, two additional fragments are required: **179** and **180** (Scheme 48). Segment **179** is also found in MP1 and thiocillin I, and so it has already been synthesized in this laboratory. A good approach to compound **180** has been described by Moody and collaborators.[27] Therefore, few, if any, difficulties are anticipated with the preparation of **179** and **180**. Once these are in hand, strategic peptide couplings established earlier in connection with the synthesis of **141** and **178** will be used to assemble the target nosiheptide.



Scheme 48. Anticipated Completion of Nosiheptide

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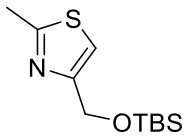
Appendix

Experimental Protocols

Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were recorded on Bruker model AVANCE II+ 300 (300 MHz for ^{1}H and 75 MHz ^{13}C) spectrometer using $CDCl_3$ as the solvent at room temperature. Chemical shifts are reported in parts per million (ppm) on the δ scale and coupling constants, J, are in hertz (Hz). Multiplicities are reported as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "dd" (doublet of doublets), "m" (multiplet), "b" (broad). Infrared (IR) spectra (cm^{-1}) were recorded on a Perkin-Elmer model 1710 Fourier transform spectrophotometer on a Universal Sampling Accessories while optical rotations were measured on a Jasco P-1010 polarimeter at the sodium D line (589nm). Unless otherwise stated, low-resolution mass spectra (m/z) were obtained in the electrospray (ESI) mode on a Waters Micromass ZQ mass spectrometer. High-resolution mass spectra (m/z) were recorded in the electrospray (ESI) mode on a Micromass LCT mass spectrometer by the UBC Mass Spectrometry Laboratory. Melting points were measured on a Mel-Temp apparatus. All reagents and solvents were commercial products and used without further purification except THF (freshly distilled from Na/benzophenone under nitrogen), THP (small amount (~25-50ml) freshly distilled from Na), and CH_2Cl_2 (freshly distilled from CaH_2 under nitrogen). Commercial n-BuLi, and t-BuLi were titrated against N-benzylbenzamide in THF at -40°C until persistence of a blue color was observed. Flash chromatography was performed on a Silicycle 230-400 mesh silica gel. Analytic and preparative TLC was carried out with Merck silica gel 60 plates with fluorescent indicator. Spots were visualized with UV light. Unless otherwise stated, reactions were performed under dry argon in flame- or oven-dried flasks equipped with Teflon™ stirbars. All

flasks were fitted with rubber septa for the introduction of substrates, reagents and solvents via syringe. Solvents, pure liquid reagents or reagents in solution, and solids were added in one portion unless otherwise stated.

A.1 Preparation of 4-(((tert-butyldimethylsilyl)oxy)methyl)-2-methylthiazole (105)



Ethyl bromopyruvate (50mmol, 7.00ml, 9.69g, 1 eq) was added to the solution of thioacetamide (51.5mmol, 3.87g, 1.03 eq) in EtOH (30ml) in portions over 5 minutes. The mixture was stirred overnight at r.t. The reaction was then poured onto 2.5N HCl (30ml), stirred 30 mins, and washed with diethyl ether (3 x 30ml). The aqueous solution was then neutralized with excess solid $NaHCO_3$ until the pH of the aqueous wash (pH paper) stabilized at 8, and extracted with EtOAc (3 x 70ml). Then, organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford compound **104** as a yellow solid. The solution of compound **104** (50mmol, 8.56g, 1 eq) in THF (50ml) was slowly added to the flask containing DIBAL-H in hexane (1M, 120mmol, 120ml, 2.5 equiv) at -78^{o}C over period of 5 minutes. Then, the mixture was stirred at 45 minutes at -78^{o}C, and brought to r.t for 1 hr. The mixture was diluted with 350ml diethyl ether, and Fieser work up was performed to get rid of excess aluminum salts. Solid residue was filtered, and further washed with 2 x 100ml diethyl ether. The filtrate was then concentrated *in vacuo* to afford alcohol as brown oil. The resulting alcohol (41.8mmol, 5.40g, 1 eq) was immediately dissolved in 60ml DCM and TBS-Cl (46mmol, 6.96g, 1.1 eq) and imidazole (83.6mmol, 5.69g, 2 eq) were added to the mixture and stirred overnight at r.t. The mixture was then diluted with 120ml DCM and transferred to a separatory funnel. Organic layer was washed with sat. aq. $NaHCO_3$ (40ml), and brine (20ml). Organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (10% EtOAc:90% Hex, R_f = 0.37) of the residue afforded a known compound **105** [Lefranc, D; Ciufolini, M. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198] as a brown oil.

Yield: 10.3g (85% over 3 steps)

^{1}H NMR: 7.00 (t, 1H, J=1.2Hz), 4.82 (d, 2H, J=1.1Hz), 2.09 (s, 3H), 0.95 (s, 9H), 0.13 (s, 6H)

^{13}C NMR: 166.0, 152.0, 112.9, 62.3, 25.9, 19,1 18.4, -5.4

IR: 1717, 1097, 835cm^{-1}

LRMS: 266.4 [M+Na^{+}]

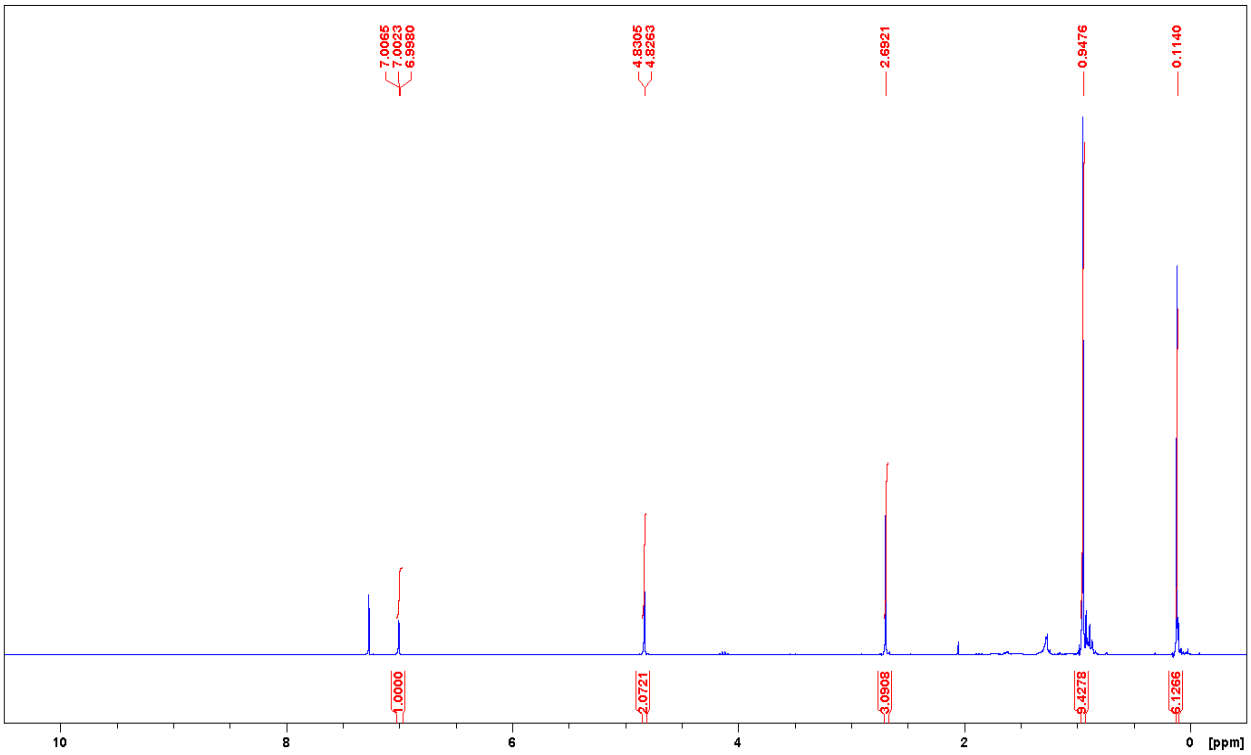


Figure A1. ^{1}H NMR of **105**

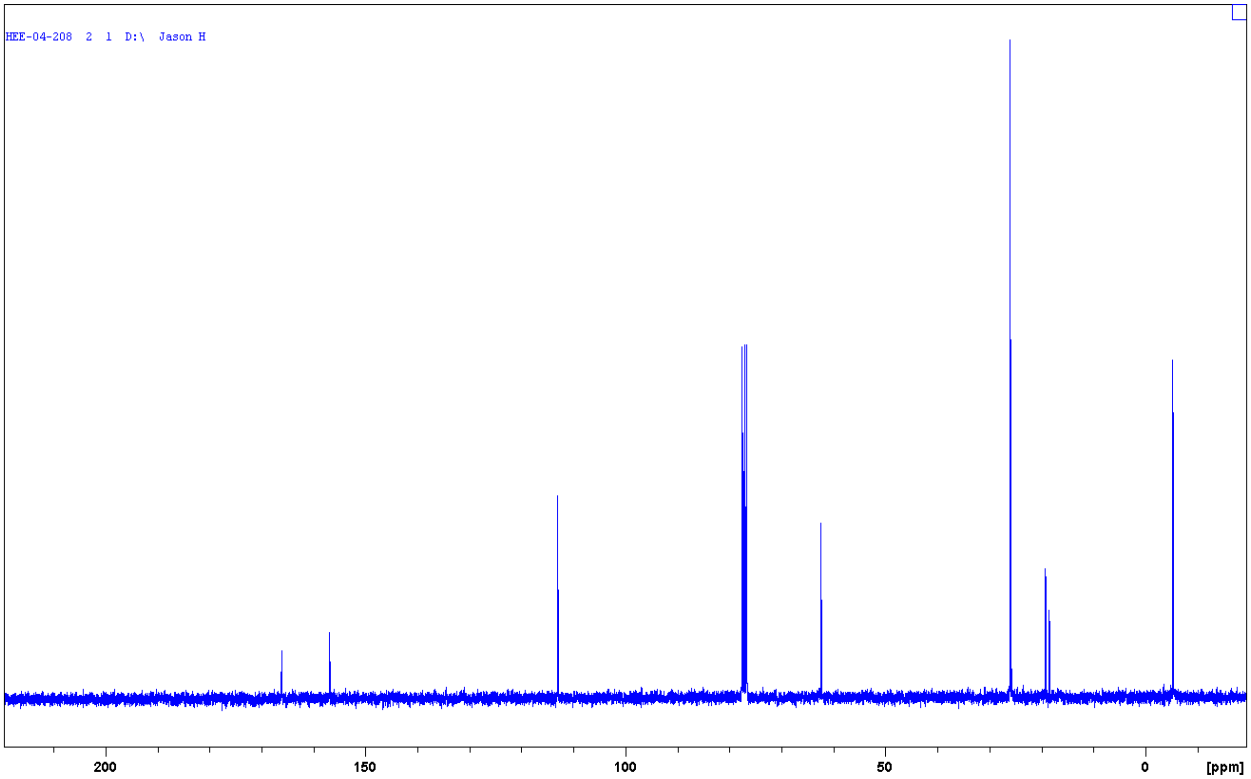
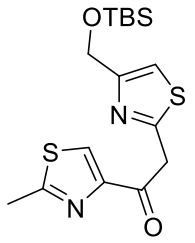


Figure A2. ^{13}C NMR of **105**

A.2 Preparation of 2-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)-1-(2-methylthiazol-4-yl)ethan-1-one (100)



Commercial n-BuLi solution (1.23 M in hexanes, 6.10 mL, 7.50 mmol) was added over 3 min to a cold (–78 °C) solution of **105** (1.82 g, 7.50 mmol, 2 eq) in THF (10 mL), the mixture was stirred at –78 °C for 40 min, then a solution of compound **104** (0.590 g, 3.75 mmol, 1 equiv) in THF (4 mL) was slowly added over a period of 3 min. The mixture was slowly brought up to r.t over a period of 2 hours, then it was quenched with aq. sat. NH_4Cl (5 mL). The mixture was diluted with EtOAc (50 mL), transferred to a separatory funnel, and carefully acidified with 0.5M HCl to pH 5. The organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (5% EtOAc : 95% Hexane, R_f=0.15) of the residue afforded the desired ketone **100** as a yellow low melting solid, which existed (^1H and ^{13}C NMR) as a mixture of the keto- (major) and enol forms (minor); (ca. 2:1 ratio). Unreacted 2-methyl-4-(tert-butyldimethylsilyl-oxy)methyl thiazole **105** was recovered. (450 mg, 1.84 mmol, 49%, R_f = 0.17)

Yield: 828mg (60%)

^1H: [8.12(s, enol), 7.60 (s, keto) (1H)], [7.16 (s, enol), 6.96 (s, keto) (1H)], [6.72 (s, keto) (0.6H)], [4.86-4.80 (m, contains enol), (2.4H)], [2.76 (s, enol), 2.74 (s, keto) (3H)], [0.96(s, keto), 0.94 (s, enol), (9H)], [0.14 (s, keto), 0.11 (s, enol) (6H)]

^{13}C: 189.2, 168.1, 166.4, 166.1, 162.0, 156.8, 155.2, 154.9, 153.4, 150.7, 126.6, 117.4, 114.6, 110.1, 93.2, 62.3, 61.8, 44.1, 25.9, 19.3, 18.4, -5.3, -5.4

IR: 3126, 1694, 1630, 1090, 837cm^{-1}

LRMS: 391.3 [$M+Na^+$]

HRMS: cald for 391.0946 $C_{16}H_{24}N_2O_2S_2SiNa$; found: 391.0950 [$M+Na^+$]

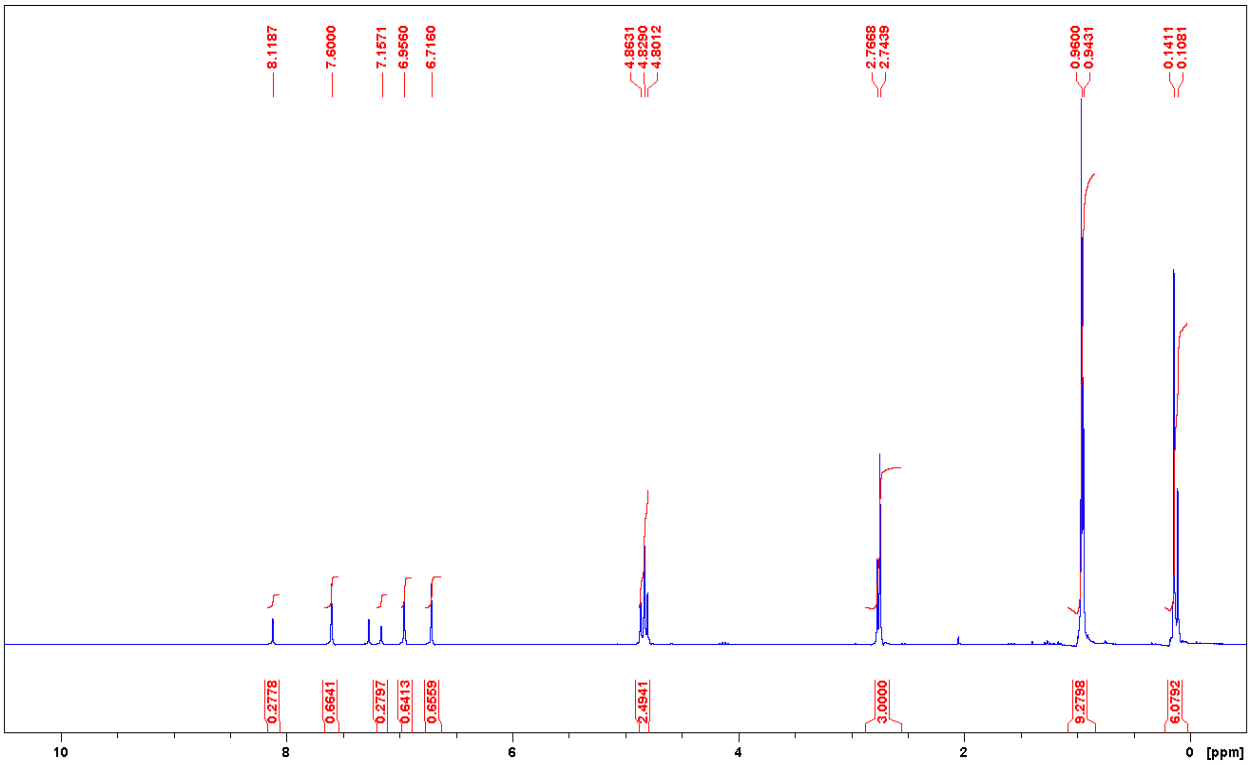


Figure A3. ^{1}H NMR of **100**

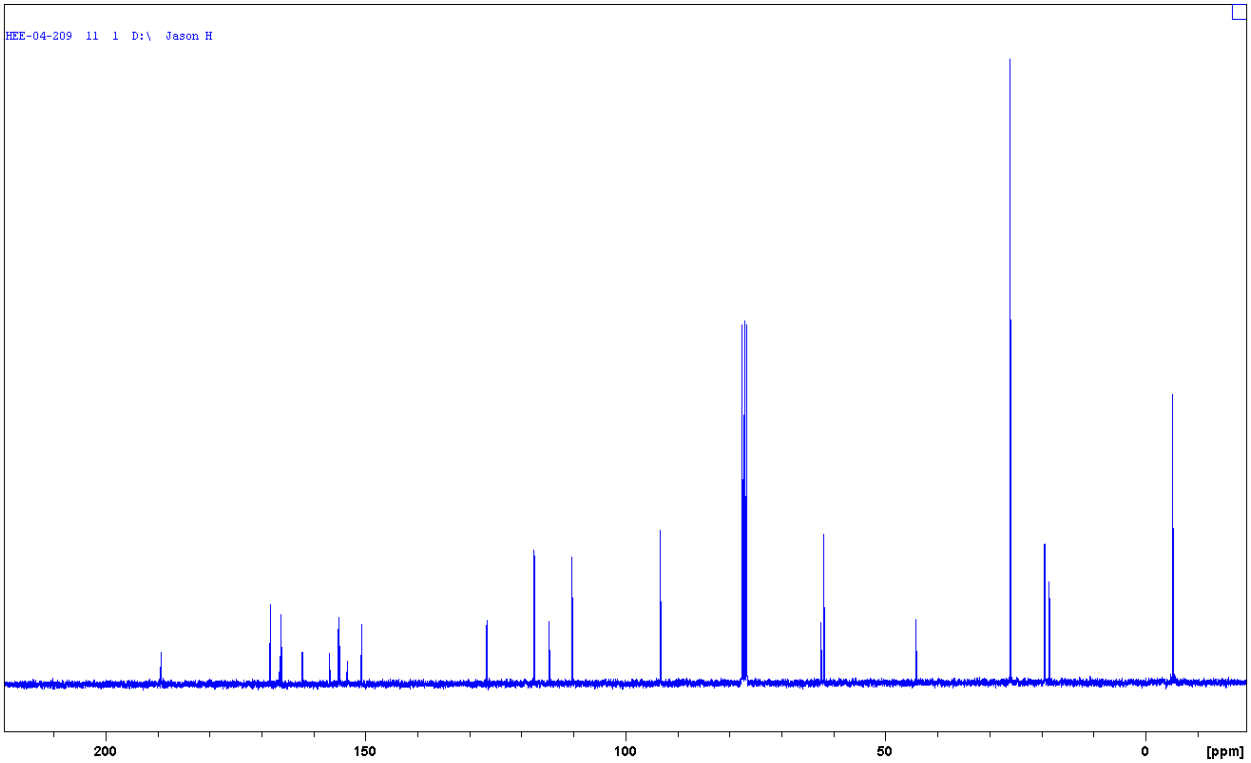
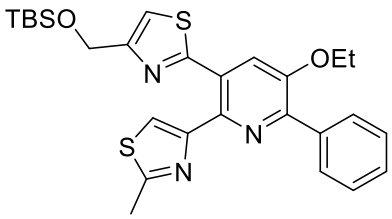


Figure A4. ^{13}C NMR of **100**

A.3 Preparation of 4-(((tert-butyldimethylsilyl)oxy)methyl)-2-(5-ethoxy-2-(2-methylthiazol-4-yl)-6-phenylpyridin-3-yl)thiazole (109)



Commercial n-BuLi in pentane (1.42 M, 0.105 ml, 0.149mmol, 1.1 eq) was carefully added over 3 min to a cold (–78 °C) solution of diisopropylamine (0.149mmol, 0.021ml, 1.1 eq) in THF (0.5ml), and stirred at -78^{o}C for 10 minutes. Ketone **100** (50mg, 0.136mmol, 1 eq) in THF (0.1ml) was slowly added to the flask, and stirred at -78^{o}C for 30 minutes. Then, the solution of the enone **107** (0.156mmol, 27.5mg, 1.15 eq) in THF (0.1ml) was added dropwise to the flask and slowly brought it back to 0^{o}C for 2 hours. Then, the mixture was quenched with 0.5ml sat. aq. NH_4Cl and further diluted with EtOAc (8ml) and transferred to a separatory funnel. The organic layer was washed with extra 1ml sat. aq. NH_4Cl and was collected and dried over Na_2SO_4. The mixture was then concentrated *in vacuo.* The resulting diketone **108** was then dissolved in 3ml EtOH, and NH_4OAc was added (1.36mmol, 106mg, 10 eq). The mixture was then brought up to 60^{o}C and stirred for 14 hours under O_2 balloon. The mixture was then concentrated *in vacuo* to get rid of excess solvent, diluted with 10ml EtOAc, and transferred to a separatory funnel. The organic layer was washed with portions of 1ml sat. aq. $NaHCO_3$ until the pH of the aqueous washes (pH paper) stabilized at 7. The organic layer was collected, dried over Na_2SO4, and concentrated *in vacuo.* The crude was subjected to flash column chromatograph (15% EtOAc:85% Hexane, R_f=0.30) to afford the pyridine **109** as a yellow solid.

Yield: 27mg (38%)

Melting point: 98-101^{o}C

^{1}H NMR: 8.08-8.05 (m, 2H), 7.89 (s, 1H), 7.46-7.37 (m, 4H), 7.19 (s, 1H), 4.92 (s, 2H), 4.20 (q, 2H, J=7.0Hz), 2.69 (s, 3H), 1.48 (t, 3H, J=7.0Hz), 0.96 (s, 9H), 0.14 (s, 6H)

^{13}C NMR: 165.4, 164.7, 157.1, 153.2, 152.3, 147.8, 143.1, 137.1, 129.7, 128.8, 128.6, 127.8, 120.2, 119.3, 115.9, 64.6, 62.4, 25.9, 19.1, 18.4, 14.6, -5.3

IR: 1366, 1088, 834cm^{-1}

LRMS: 524.3 [M+H^{+}]

HRMS: cald for 523.1783 $C_{27}H_{34}N_3O_2S_2Si$; found: 523.1791 [M+H^{+}]

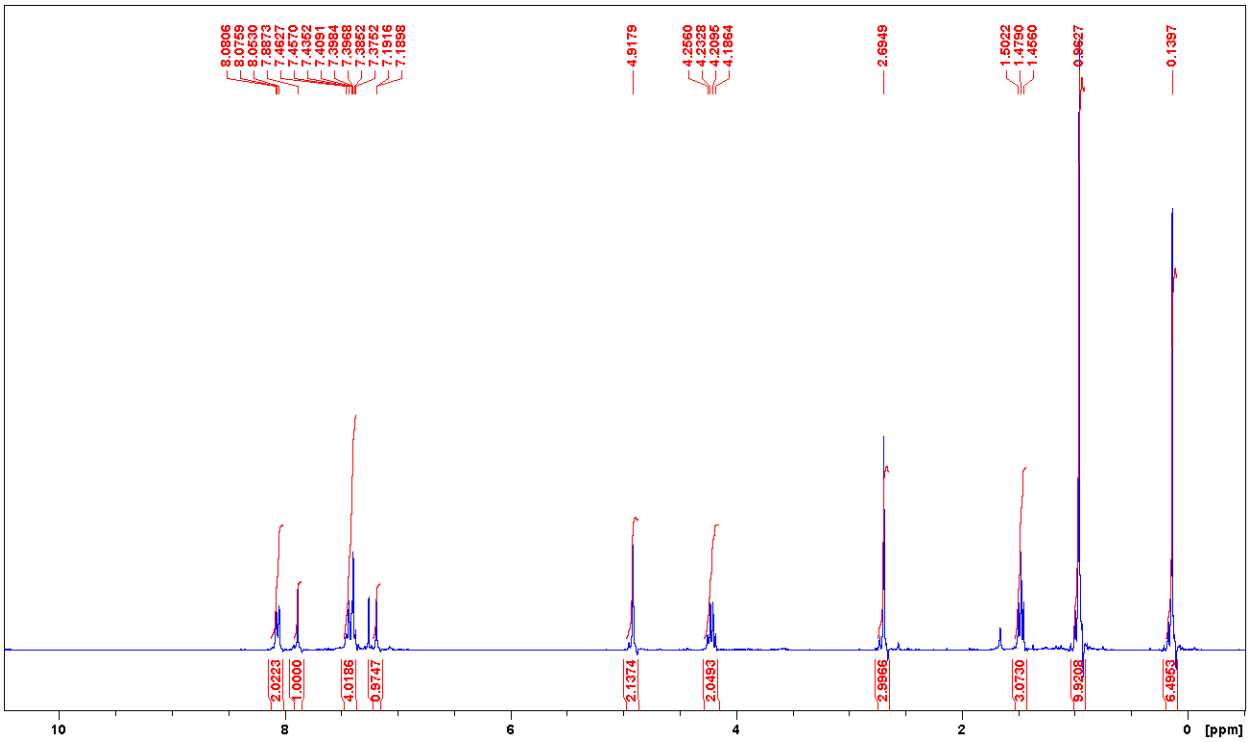


Figure A5. ^{1}H NMR of **109**

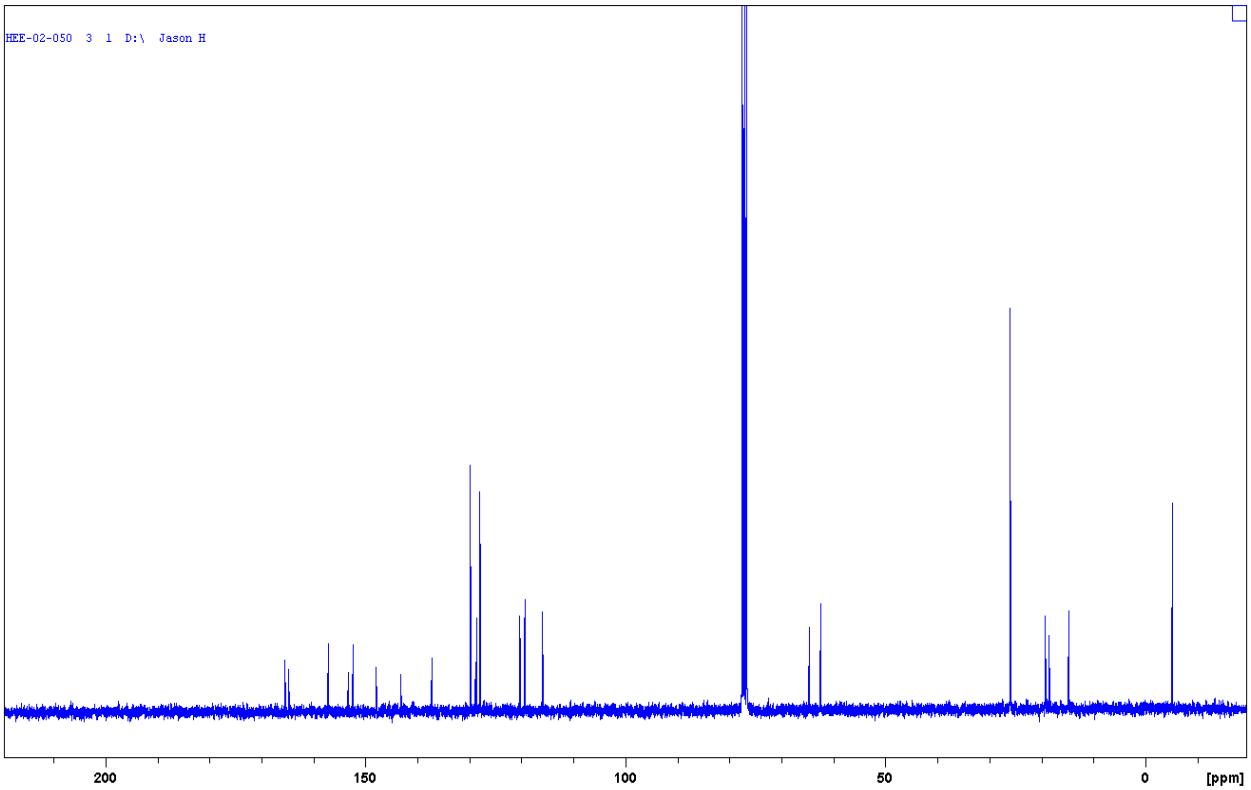
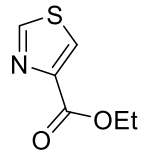


Figure A6. ^{13}C NMR of **109**

A.4 Preparation of ethyl thiazole-4-carboxylate (114)



P_4S_{10} (30mmol, 13.35g, 0.4 eq) was added to the solution of formamide (75mmol, 3.00ml, 1 eq) in Et_2O (60ml) at 0°C in small portions over 3 minutes. The reaction mixture was allowed to warm to r.t for 2 hours. The flask was diluted with 250ml of diethyl ether, and the solid residue was filtered, and filtrate was concentrated *in vacuo* to afford thioformamide as yellow smelly foam. The resulting thioformamide (1.96g, 32mmol, 1 eq) was immediately redissolved in 35mol EtOH, and neat ethyl bromopyruvate was slowly added to the flask (32mmol, 4.51ml, 1 eq) in portions over 5 minutes, and stirred overnight at r.t. Then, 25ml of 2.5N HCl was poured, stirred over 30 mins. Then, the mixture was washed with 2 x 30ml diethyl ether, and aqueous layer was neutralized with solid $NaHCO_3$ until the pH of the aqueous washes (pH paper) stabilized at 8. The aqueous layer was extracted with diethyl ether (3 x 50ml), and washed with brine (15ml). The combined organic layer is dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography (50% EtOAc: 50% Hex, R_f = 0.50) of the residue afforded **114** as a brown solid.

Yield: 4.35g (2 steps over 37%)

^{1}H NMR: 8.85 (d, 1H, J=2.1Hz), 8.23 (d, 1H, J=2.1Hz), 4.41 (q, 2H, J=7.1Hz), 1.40 (t, 3H, J=7.1Hz)

^{13}C NMR: 161.2, 153.5, 148.1, 127.2, 61.5, 14.3

IR: 1717, 1264cm^{-1}

LRMS: 180.2 [M+Na$^+$], HRMS: cald for 180.0090 $C6H7NO_2SNa$; found: 180.0098 [M+Na$^+$]

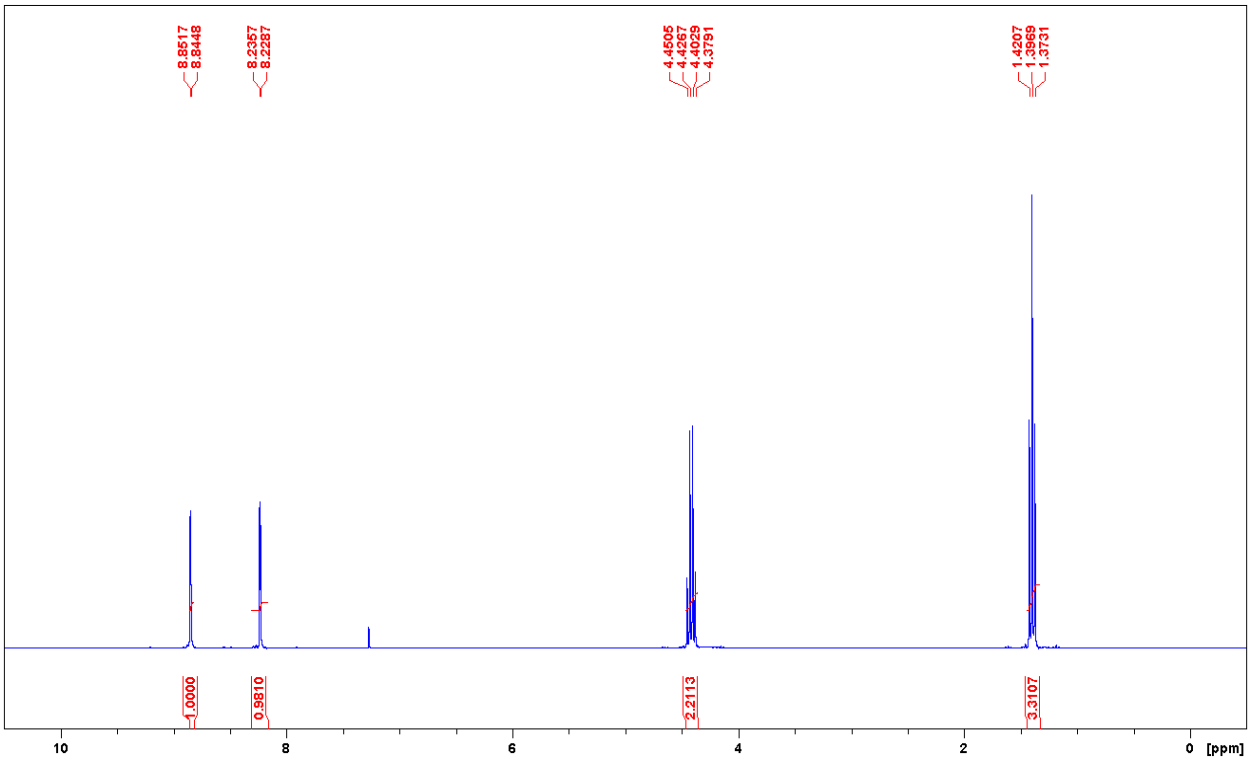


Figure A7. ^{1}H NMR of **114**

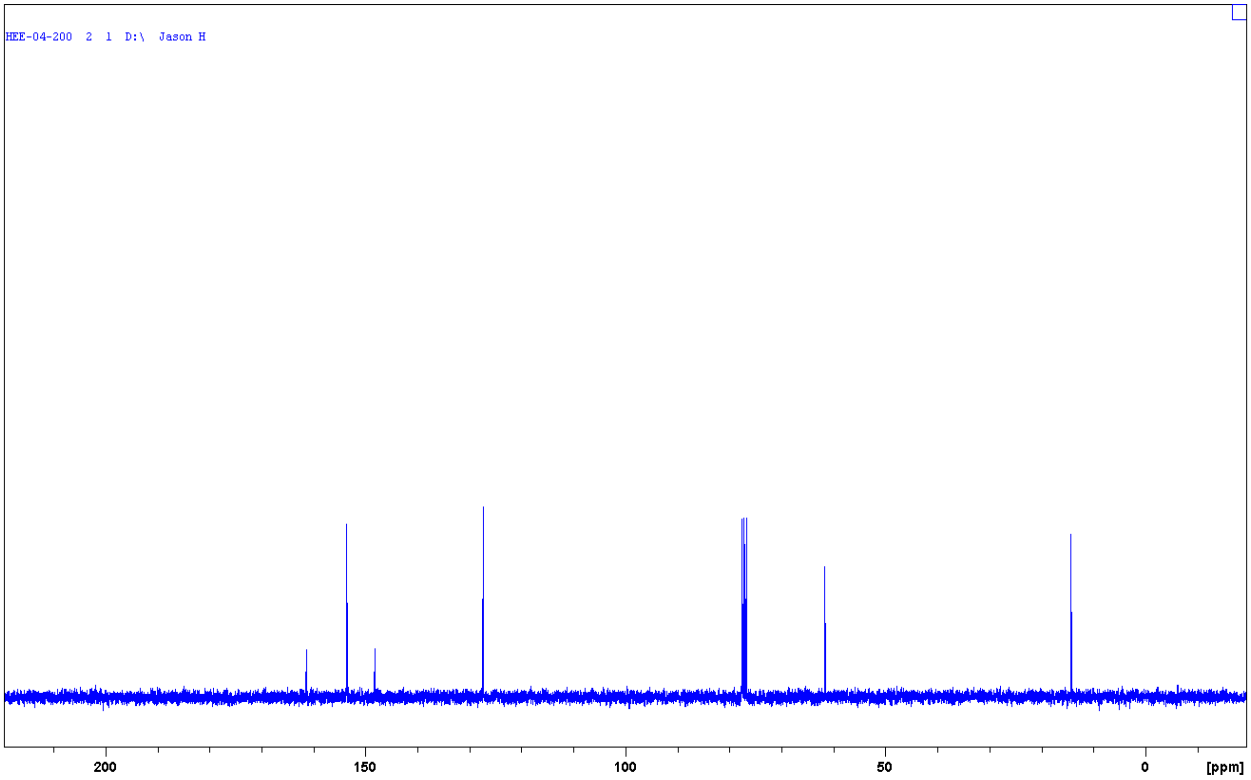
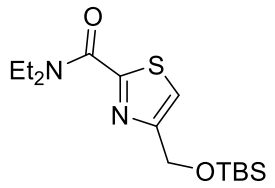


Figure A8. ^{13}C NMR of **114**

A.5 Preparation of 4-(((tert-butyldimethylsilyl)oxy)methyl)-N,N-diethylthiazole-2-carboxamide (116)



Compound **114** dissolved in 18ml THF (17.6mmol, 2.77g, 1 eq) was added dropwise to the flask containing DIBAL-H in hexane (1M, 44mmol, 44ml, 2.5 eq) at -78^{o}C over a period of 3 minutes. Then, the mixture was stirred for 45 minutes at -78^{o}C, and brought to r.t for 1 hour. The mixture was diluted with 100ml diethyl ether, and Fieser work up was performed to get rid of excess aluminum salts. Solid residue was filtered, and further washed with 2 x 50ml diethyl ether. The collected organic layer was then concentrated *in vacuo* to afford alcohol as brown oil. The resulting alcohol (11.5mmol, 1.33g, 1 eq) was immediately dissolved in 15ml DCM, and TBSCl (12.7mmol, 1.92g, 1.1 eq) and imidazole (23.0mmol, 1.58g, 2 eq) were added to the mixture and stirred overnight at r.t. The mixture was then diluted with 30ml DCM, and transferred to a separatory funnel. Organic layer was washed with sat. aq. $NaHCO_3$ (15ml), and brine (10ml). Organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromagography (10% EtOAc:90% Hex, R_f = 0.40) of the residue afforded **115** as a faint yellow oil. (15.8mmol, 3.63g, 90% over 2 steps). Commercial n-BuLi solution (1.36 M in hexanes, 11.6 mL, 15.8 mmol) was added over 3 min to a cold (–78 °C) solution of **115** (15.8mmol, 3.63g, 1 eq) in THF (20ml), the mixture was stirred at –78 °C for 30 min, then neat diethylcarbamyl chloride (47.4mmol, 6.00ml, 3 eq) was added rapidly at once and slowly warmed to -40^{o}C for 1 hour. The resulting mixture was then quenched with sat. aq. NH_4Cl (15ml), and brought up to r.t immediately with gentle warm water bath. Extra 10ml sat. aq. NH_4Cl was administrated concomitantly throughout the warming process. The mixture was then diluted with EtOAc (90ml)

and transferred to a separatory funnel. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo.* Flash column chromatography (5% EtOAc : 95% Hexane) of the residue afforded the desired amide **116** as a faint yellow oil. TLC monitored by (10% EtOAc: 90% Hex, R_f=0.21).

Yield: 5.77g (45% over 3 steps)

^{1}H NMR: 7.36 (t, 1H, J=1.0Hz), 4.84 (d, 2H, J=1.0Hz), 4.01 (q, 2H, J=7.0Hz), 3.54 (q, 2H, J=7.0Hz), 1.25 (t, 6H, J=7.0Hz), 0.95 (s, 9H), 0.12 (s, 6H)

^{13}C NMR: 165.2, 160.1, 157.8, 118.8, 62.1, 42.9, 42.2, 25.9, 18.4, 14.5, 12.7, -5.3

IR: 1621, 1068, 835cm^{-1}

LRMS: 351.3 [M+Na$^+$]

HRMS: cald for 351.1538 $C_{15}H_{20}N_2O_2NaSiS$; found: 351.1539 [M+Na$^+$]

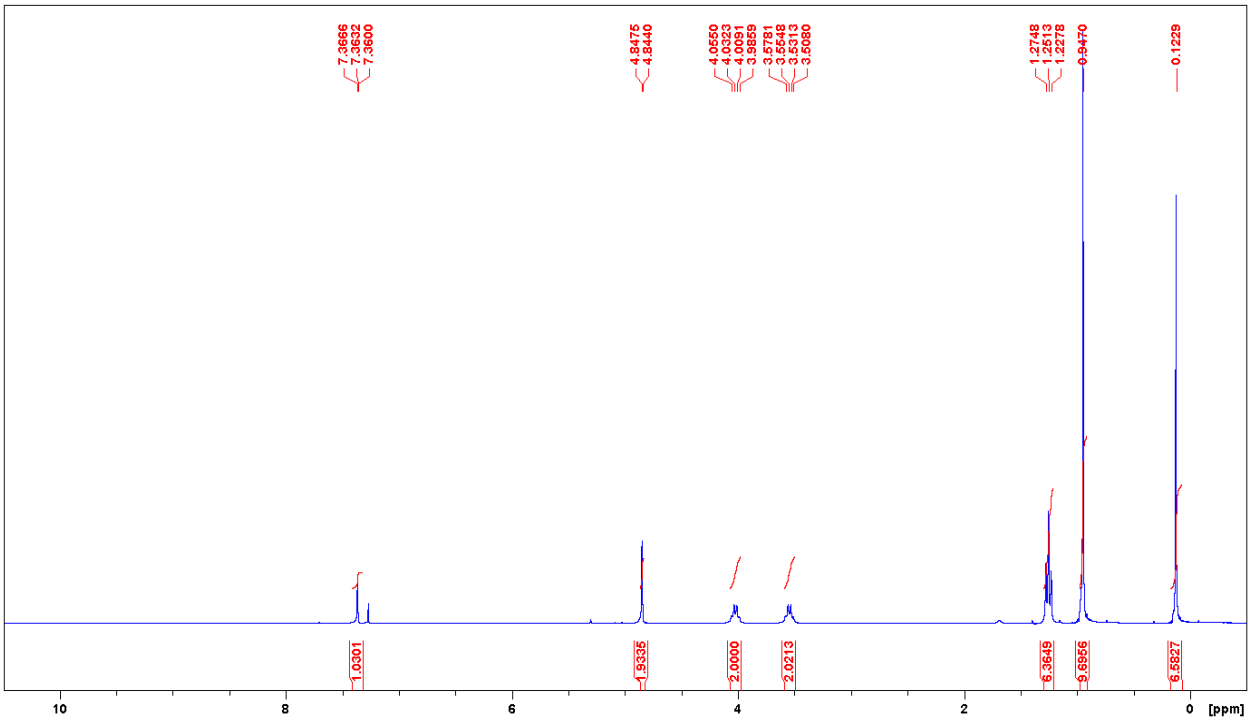


Figure A9. ^{1}H NMR of **116**

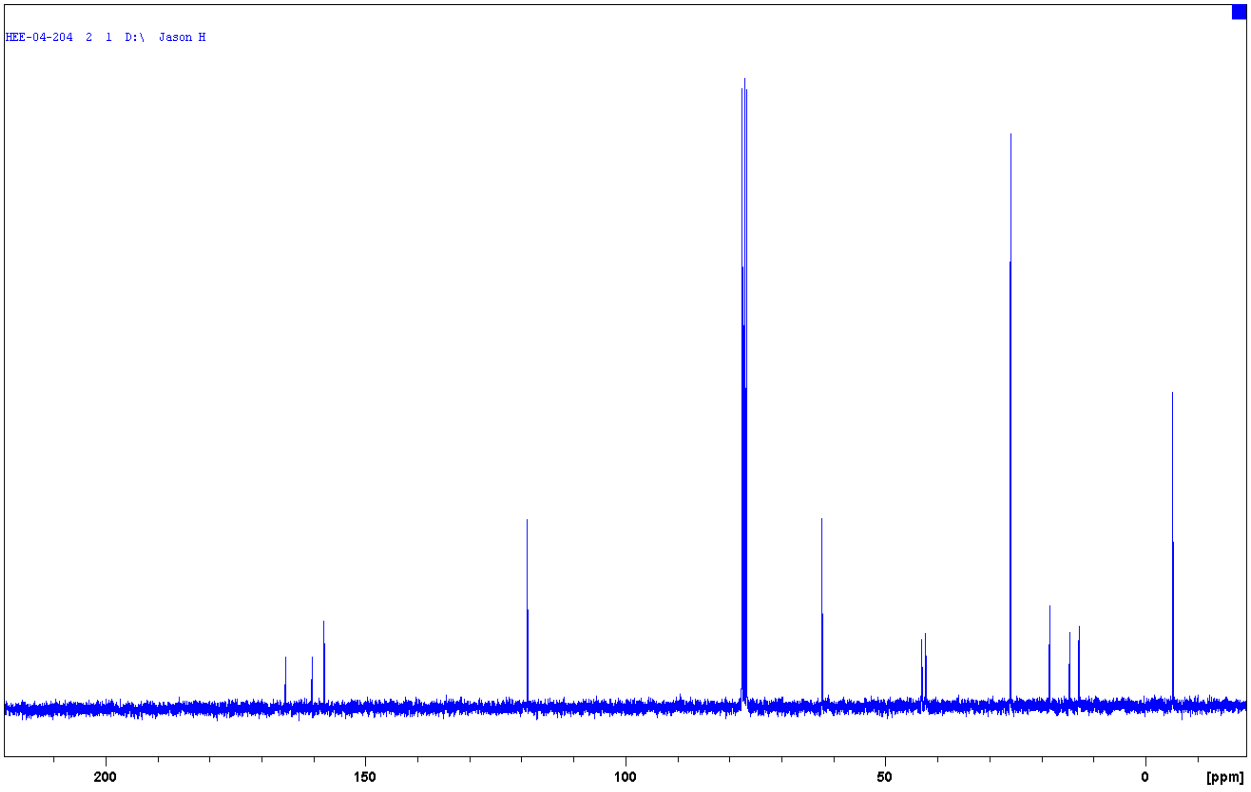
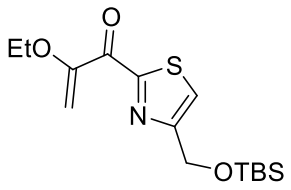


Figure A10. ^{13}C NMR of **116**

A.6 Preparation of 1-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)-2-ethoxyprop-2-en-1-one (117)



Commercial *tert*-BuLi in pentane (0.97 M, 3.35 ml, 3.26mmol, 1.4 eq) was carefully added over 3 min to a cold (–78 °C) solution of ethyl vinyl ether (0.394ml, 4.15mmol, 1.8 eq) in dry tetrahydropyran (1.5 mL). A bright yellow solution resulted. The mixture was stirred at –78 °C for 10 min, then it was warmed to –10 °C (NaCl/ice bath) and stirred at that temperature for 23 min, during which time the bright yellow color disappeared. The mixture was then cooled back to –78 °C and diluted with THF (2.5 mL). Then, the solution of amide **116** (2.31mmol, 760mg, 1 eq) in THF (2.5ml) was added to the mixture. The mixture was stirred at -78^{o}C for 3 hours, and was slowly warmed to room temperature and quenched with H_2O (3ml) and stirred for 1 minute. The mixture was diluted with EtOAc (20ml), and transferred to a separatory funnel. Aqueous layer was further extracted with EtOAc (2 x 10ml). The combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography (1% Et_3N: 10% EtOAc: 89% Hex, R_f = 0.34) of the residue afforded the desired enone **117** as a yellow oil.

Yield: 580mg (77%)

^{1}H NMR: 7.57 (t, 1H, J=1.0Hz), 6.05 (d, 1H, J=2.8Hz), 5.00 (d, 1H, J=2.8Hz), 4.95 (d, 2H, J=1.0Hz), 3.95 (q, 2H, J=7.0Hz), 1.47 (t, 3H, J=7.0Hz), 0.95 (s, 9H), 0.13 (s, 6H)

^{13}C NMR: 179.2, 164.0, 159.6, 155.6, 121.3, 99.0, 64.1, 62.3, 25.9, 18.3, 14.2, -5.4

IR: 1746, 1695, 1135, 1095, 836cm^{-1}

LRMS: 350.3 [M+Na^{+}], HRMS: cald for 350.1222 $C_{15}H_{25}NO_3NaSiS$; found: 350.1222 [M+Na^{+}]

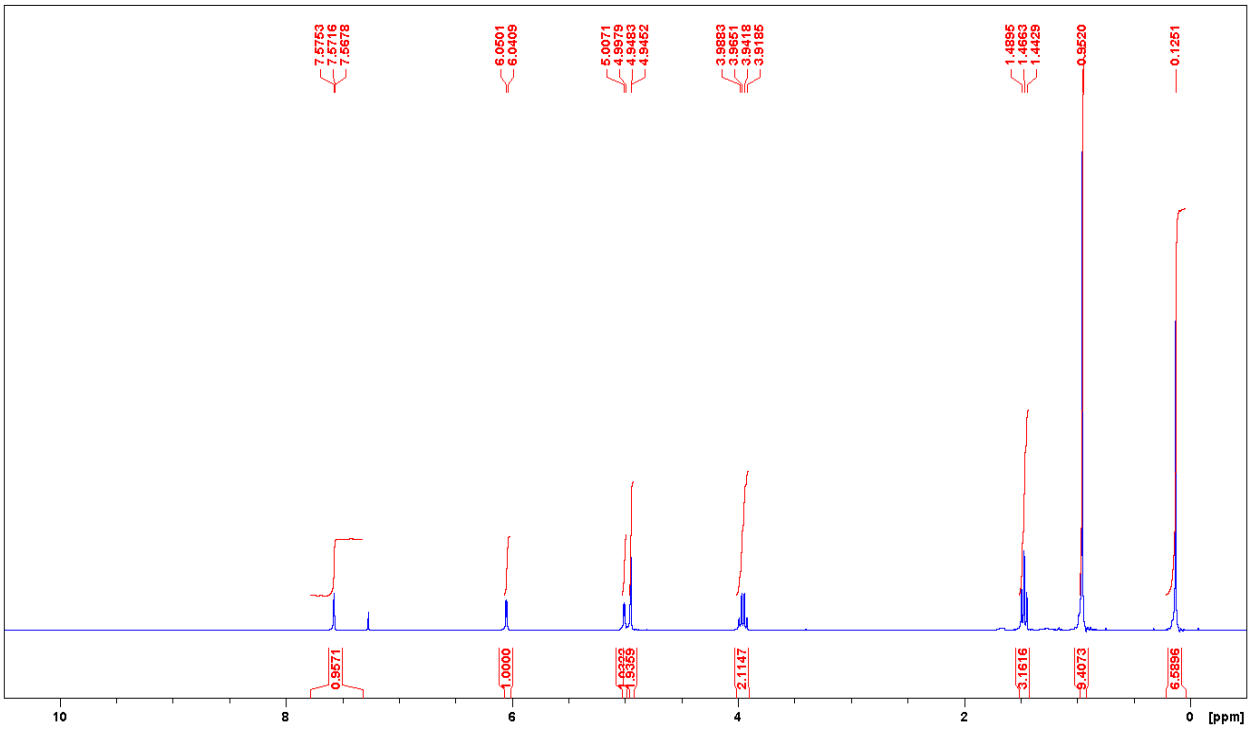


Figure A11. ^{1}H NMR of **117**

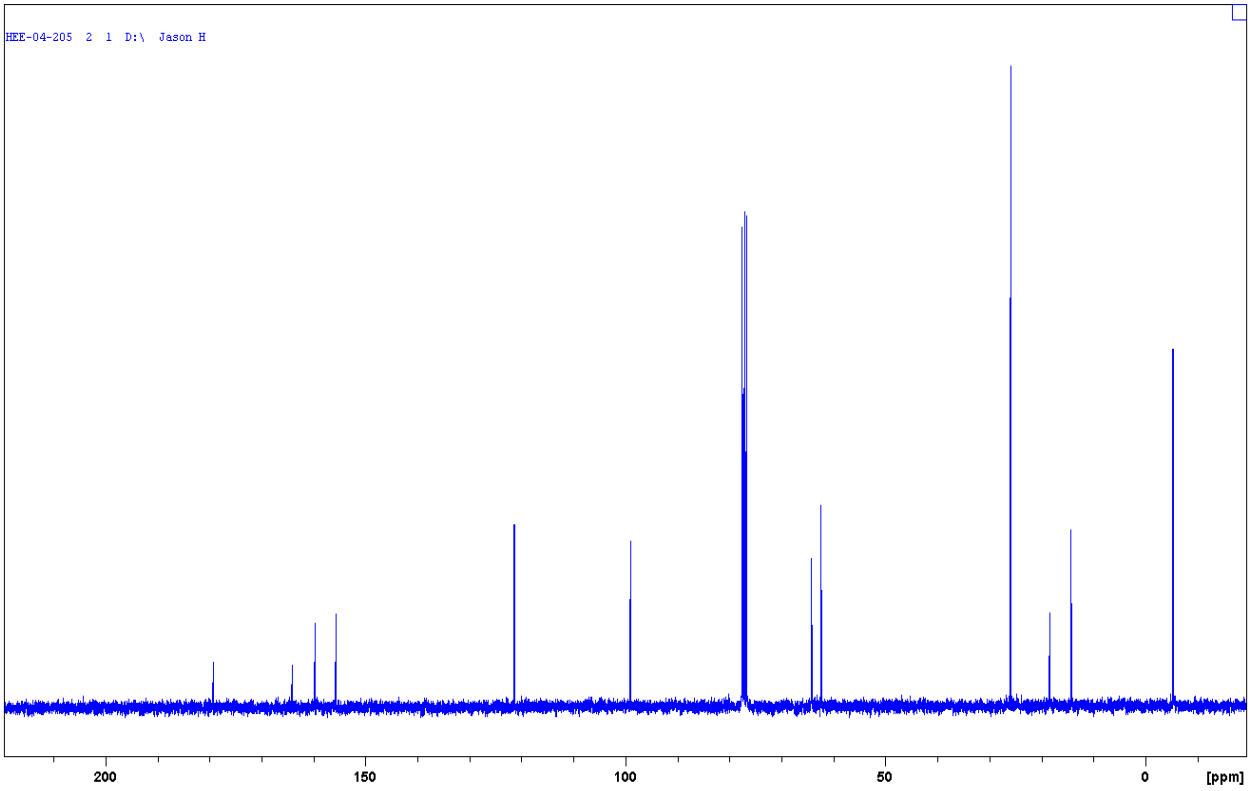
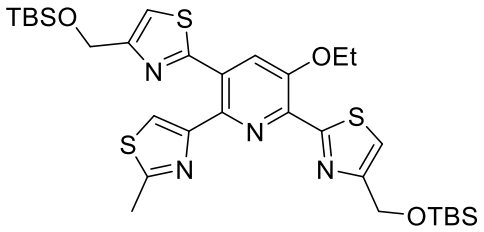


Figure A12. ^{13}C NMR of **117**

A.7 Preparation of 2,2'-(3-ethoxy-6-(2-methylthiazol-4-yl)pyridine-2,5-diyl)bis(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazole) (121)



Commercial NaH (60% oil dispersion, 7.8 mg, 0.195 mmol, 1.08 eq) was dispensed into a flask maintained under inert atmosphere (Ar balloon), washed with hexane (3 x 0.2 mL) to remove excess oil, and suspended in THF (0.2 mL). A solution of ketone **100** (66 mg, 0.180 mmol, 1 eq) in THF (0.2 mL) was slowly added (syringe) at r.t over ca. 2 min. Evolution of H_2 was observed, and the color changed from faint green to yellow upon stirring at r.t for 15 minutes. A solution of enone **117** (67 mg, 0.205 mmol, 1.15 eq) in THF (0.2 mL) was added at r.t over a period of 1 min, whereupon the color of the solution turned from yellow to brown. The mixture was stirred for 90 min at r.t, then it was quenched with aq. sat. NH_4Cl (0.2 mL), diluted with EtOAc (5 mL) and transferred to a separatory funnel. The aqueous layer was discarded, while the organic phase was washed with more aq. sat. NH_4Cl (0.5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting diketone **120** was then dissolved in 8:5 mixture of TFE:HFIP (1ml:0.65ml), and slowly syringe pumped over a period of 2.5 hours to the flask containing NH_4OAc (2mmol, 156mg, 11 equiv) in 8:5 mixture of TFE:HFIP (0.8ml:0.5ml) under O_2 balloon at 60^{o}C. The mixture was stirred at 60^{o}C (oil bath temperature) for 16 hours. The mixture was then concentrated *in vacuo* to get rid of excess solvent, diluted with 7ml EtOAc, and transferred to a separatory funnel. This solution was washed 2-3 times with 1 mL portions of aq. sat. $NaHCO_3$ until the pH of the aqueous washes (pH paper) stabilized at 7, then it was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography of the residue (1.5% Et_3N: 18% EtOAc:

81.5% Hexane, R_f = 0.21) afforded pyridine **121** as a yellow solid. This compound was highly UV active (purple under short wavelength, and sky blue under long wavelength)

Yield: 43mg (35% over 2 steps)

Melting point: 98-101^{o}C

^{1}H NMR: 7.98 (s, 1H), 7.46 (s, 1H), 7.40 (s, 1H), 7.23 (s, 1H), 5.04 (s, 2H), 4.92 (s, 2H), 4.38 (q, 2H, J=7.1Hz), 2.69 (s, 3H), 1.63 (t, 3H, J=7.1Hz), 0.97 (s, 9H), 0.96 (s, 9H), 0.14 (s, 6H), 0.13 (s, 6H)

^{13}C NMR: 165.4, 164.1, 162.4, 158.4, 157.3, 152.4, 151.8, 143.6, 139.9, 130.2, 121.0, 120.0, 116.4, 116.3, 65.5, 62.8, 62.3, 26.0, 25.9, 19.1, 18.41, 18.39, 14.6, -5.29, -5.31

IR: 1366, 1098, 836cm^{-1}

LRMS: 697.4 [M+Na^{+}]

HRMS: cald for 697.2168 $C_{31}H_{46}N_4O_3S_2Si_2Na$; found: 697.2178 [M+Na^{+}]

2,2'-(6-(2-methylthiazol-4-yl)pyridine-2,5-diyl)bis(4-(((tert-butyldimethylsilyl)oxy)methyl)-thiazole) (122). This material was not purified to homogeneity and it was characterized only by ^{1}H NMR and low-resolution mass spectrometry. **^{1}H**: 8.32 (d, 1H, J=8.2Hz), 8.19 (d, 1H, J=8.2Hz), 7.60 (s, 1H), 7.34 (s, 1H), 7.23 (s, 1H), 4.96 (s, 2H), 4.91 (s, 2H), 2.68 (s, 3H), 0.98 (s, 9H), 0.16 (s, 6H), 0.13 (s, 6H). LRMS: 654.7 [M+Na^{+}]

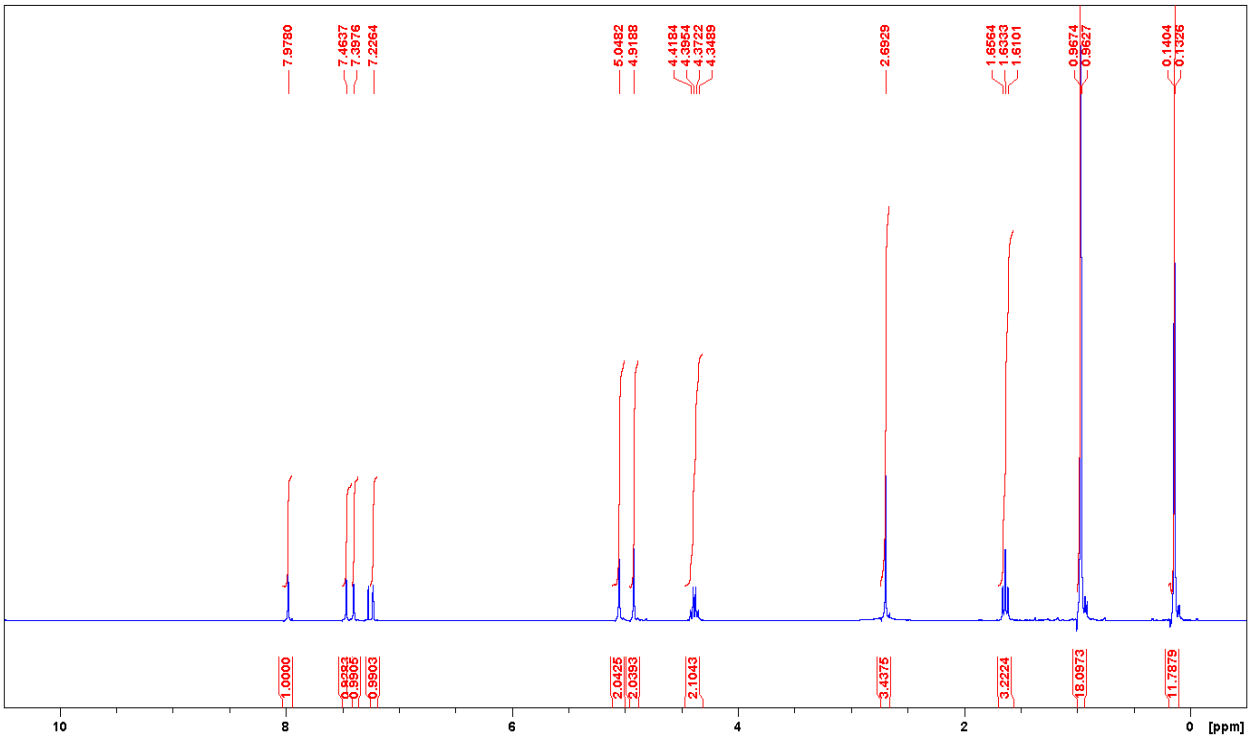


Figure A13. ^{1}H NMR of **121**

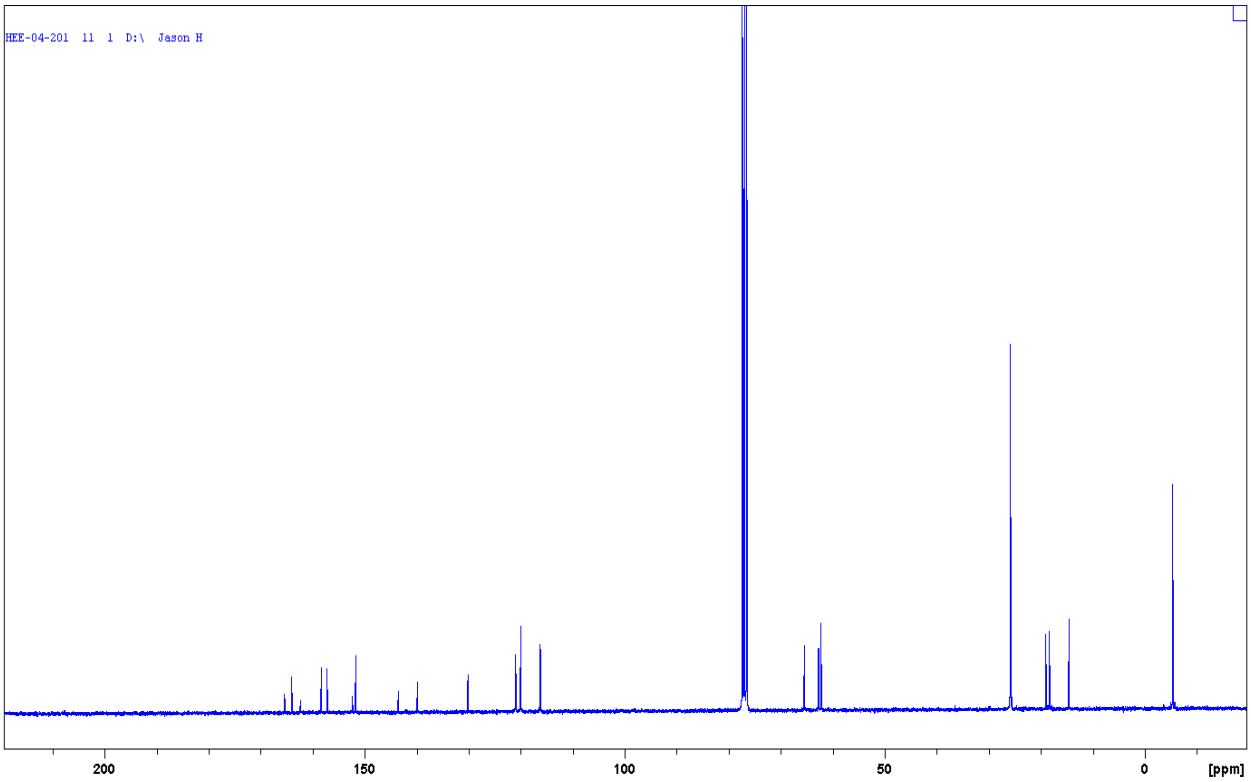


Figure A14. ^{13}C NMR of **121**

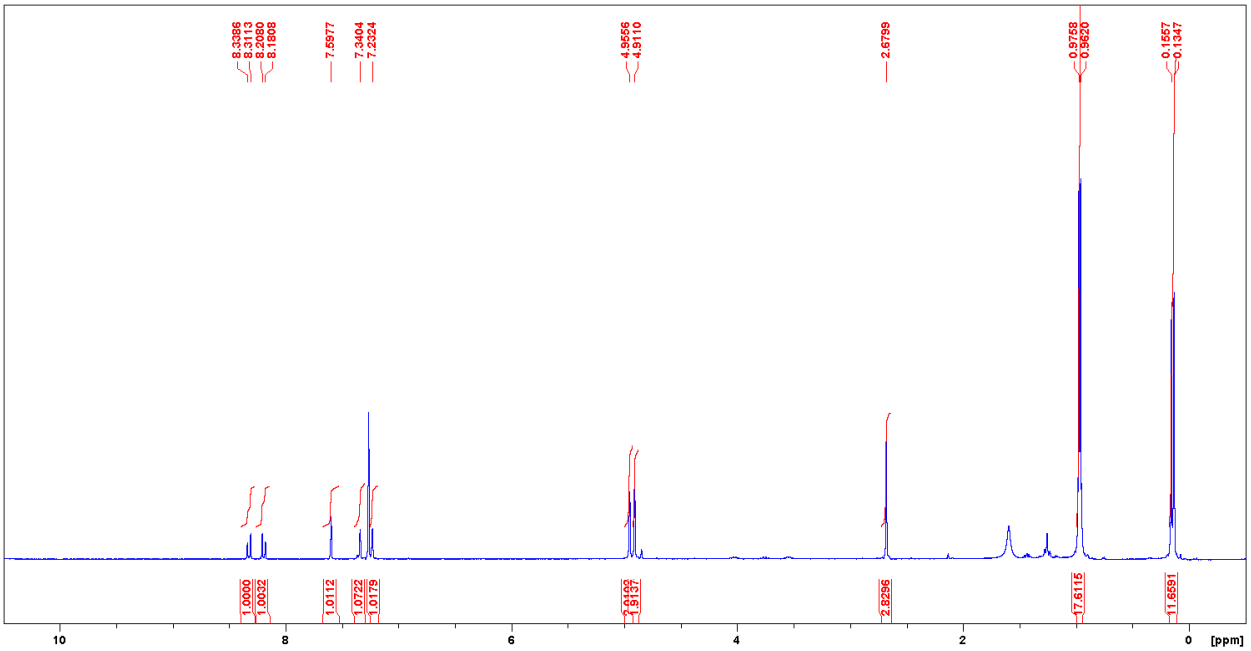
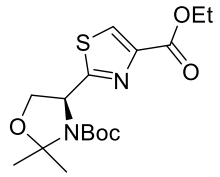


Figure A15. ^{1}H NMR of **122**

A.8 Preparation of Tert-butyl (S)-4-(4-(2-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)acetyl)thiazol-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (91)



A solution of known tert-butyl (S)-4-carbamothioyl-2,2-dimethyloxazolidine-3-carboxylate **(144)** [Lin, C-C.; Tantisantisom, W.; McAlpine, S. R. *Org Lett*. **2013**, *15*, 3574] (1.28 g, 5 mmol) in DME (30 mL) containing suspended $KHCO_3$ (3.59 g, 35 mmol, 7 eq) was stirred at r.t for 10 min. Neat ethyl bromopyruvate (2.92 g, 15 mmol, 3 eq, 2.11 mL) was added dropwise over a period of 3 minutes, and the mixture was stirred for 16 h at r.t. The solvent was removed *in vacuo*, the residue was redissolved in EtOAc (65 mL) and the solution was sequentially washed with brine (20mL) and water (15mL), dried (Na_2SO_4), and evaporated *in vacuo*. The residue was placed under high vacuum to remove all remaining EtOAc, then it was redissolved in DME (30 mL). The solution was cooled to 0°C and pyridine (3.05 mL, 3.00 g, 37.5 mmol, 7.5 eq) was added slowly over a period of 3 minutes. After 5 minutes, TFAA (4.14 g, 20 mmol, 2.78 mL, 4 eq) was added slowly. The mixture was stirred at 0°C for 3 h, then it was brought to r.t. Triethylamine (1.21 g, 10 mmol, 1.69 mL, 2 eq) was slowly added and stirring at r.t was continued for another hour, whereupon the reaction was complete (TLC). The mixture was evaporated and the residue was dissolved in EtOAc (70mL), washed with 1M HCl (15mL), sat. aq. $NaHCO_3$ (15mL), and brine (15mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (25% EtOAc : 75% Hex, $R_f = 0.31$) of the residue afforded **91** as a yellow solid.

Yield: 1.72g (97%)

^{1}H NMR: 8.09 (s, 1H), 5.36 (bs, minor rotamer) & 5.29-5.25 (m, major rotamer; 1H), 4.42 (q, 2H, J=7.0 Hz), 4.32-4.28 (m, major rotamer) & 4.17-4.14 (m, minor rotamer; 2H), 1.79 (bs, major rotamer) & 1.73 (bs, minor rotamer, 3H), 1.57 (bs, major rotamer) & 1.50 (bs, minor rotamer; 6H), 1.38 (t, 3H, J=7.0 Hz), 1.30 (bs, 6H).

^{13}C NMR: 175.3, 161.3, 151.5, 147.2, 127.1, 95.2 (major rotamer) & 94.8 (minor rotamer), 81.4 (minor rotamer) & 81.0 (major rotamer), 69.3 (major rotamer) & 68.9 (minor rotamer), 61.5, 59.4, 28.2, 27.3 (minor rotamer) & 26.5 (major rotamer), 23.9 (minor rotamer) & 22.7 (major rotamer), 14.4.

Melting Point: 105-109ºC

Optical Rotation: $[\alpha]_D^{22}$ = -13.1º (CH_2Cl_2, c = 1.05)

IR: 1701, 1364cm^{-1}

LRMS: 379.1 [M+Na^{+}]

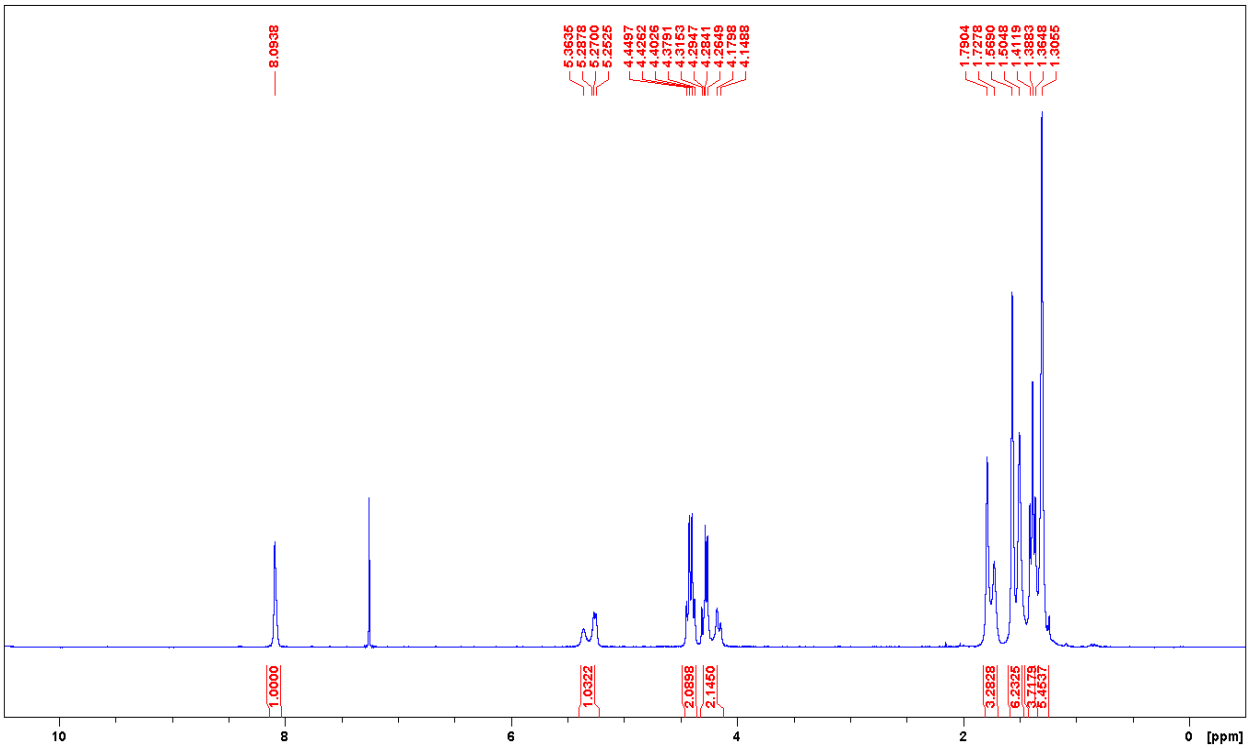


Figure A16. ^{1}H NMR of **91**

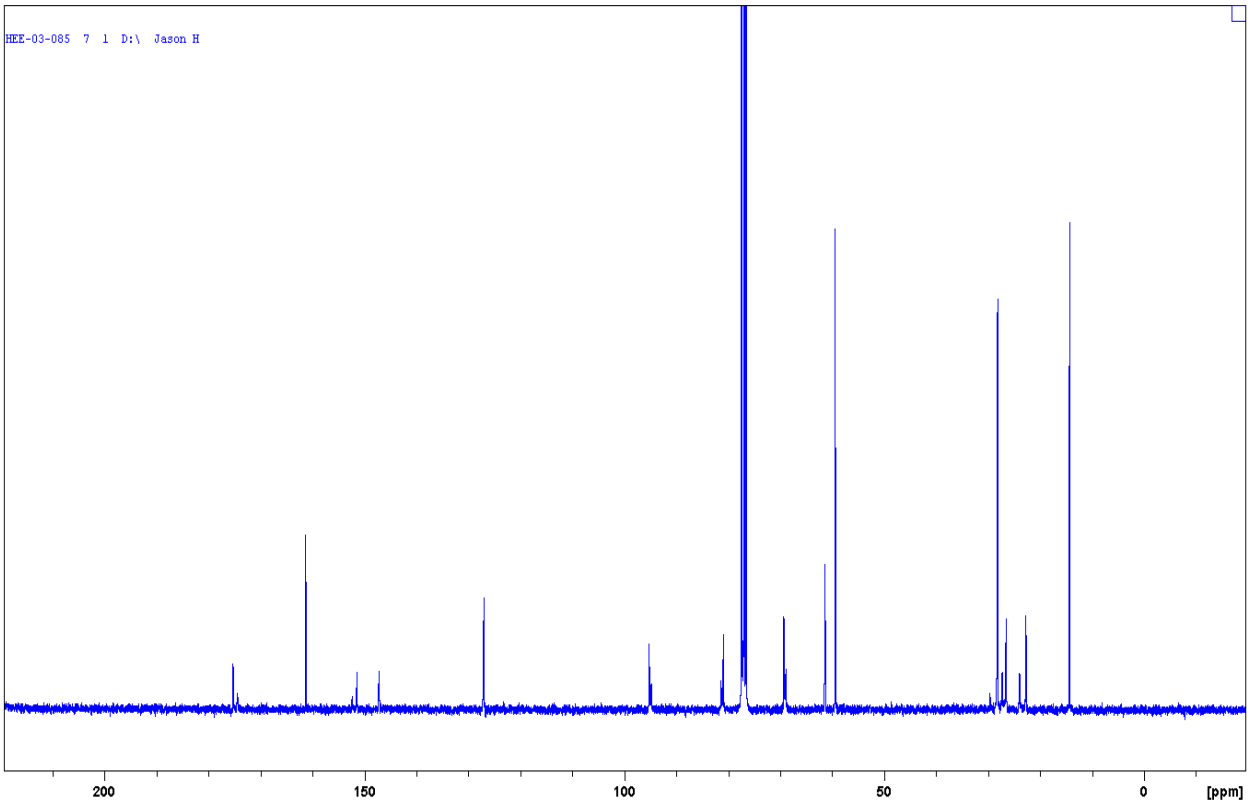
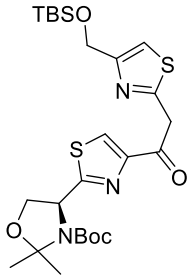


Figure A17. ^{13}C NMR of **91**

A.9 Preparation of Tert-butyl (S)-4-(4-(2-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)acetyl)thiazol-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (89)



Commercial n-BuLi solution (1.08 M in hexanes, 7.16 mL, 7.73 mmol) was added over 3 min to a cold (–78 °C) solution of 2-methyl-4-(tert-butyldimethylsilyl-oxy)methyl thiazole (1.88 g, 7.73 mmol, 2.1 eq) **105** in THF (12 mL), the mixture was stirred at –78 °C for 40 min, then a solution of compound **91** (1.30 g, 3.63 mmol, 1 eq) in THF (4 mL) was slowly added over a period of 3 min. The mixture was slowly brought up to r.t over a period of 2 hours, then it was quenched with aq. sat. NH_4Cl solution (5 mL). The mixture was diluted with EtOAc (50 mL), transferred to a separatory funnel, and carefully acidified with 0.5M HCl to pH 5. The organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (15% EtOAc : 85% Hexane, R_f= 0.33) of the residue afforded unreacted 2-methyl-4-(tert-butyldimethylsilyl-oxy)methyl thiazole (378 mg, 1.56 mmol, 38%, R_f = 0.50) and the desired ketone **89** as a thick yellow film. Proton and ^{13}C NMR spectra of this material revealed that it existed as a mixture BOC rotamers of the keto- (minor) and enol forms (major; ca. 1:2 ratio).

Yield: 1.49g (74%)

^1H NMR: [8.20(br. s, keto), 7.67 (br. s, enol) (1H)], [7.16 (br. s, keto), 6.95 (br. s, enol) (1H)], [6.69 (s, enol), (0.6H)], [5.24 (br. s, keto), 5.21 (br. s, enol) (1H)], [4.85-4.80 (m, contains keto form of enol at 6.69), (2.5H)], [4.34-4.27 (m, enol), 4.22-4.16 (m, keto) (2H)], [1.81-1.77 (m, enol), 1.76-1.71 (m, keto) (3H)], [1.59, 1.51 (br. 2s of equal intensity, (6H)], 1.32 (br. s, enol and keto), (6H), [0.95(s, enol), 0.93 (s, keto), (9H)], [0.13 (s, enol), 0.09 (s, keto) (6H)].

^{13}C NMR (100 MHz, CD_3CN, 65ºC): 190.4, 175.3, 175.0, 169.4, 163.6, 157.9, 156.7, 156.3, 154.6, 153.1, 151.7, 128.1, 119.2, 116.6, 112.4, 96.1, 96.0, 94.0, 81.7, 69.8, 62.9, 62.4, 60.6, 60.5, 45.0, 28.8, 27.4, 26.5, 24.1, 19.2, -4.8

IR: 3126, 1694, 1630, 1090, 837cm^{-1}

Optical Rotation: $[\alpha]_D^{22}$ = -6.09º (CH_2Cl_2, c = 1.78)

LRMS: 554.3 [$M+H^+$]

HRMS: cald for 554.2179 $C_{25}H_{40}N_3O_5SiS_2$; found: 554.2179 [$M+H^+$]

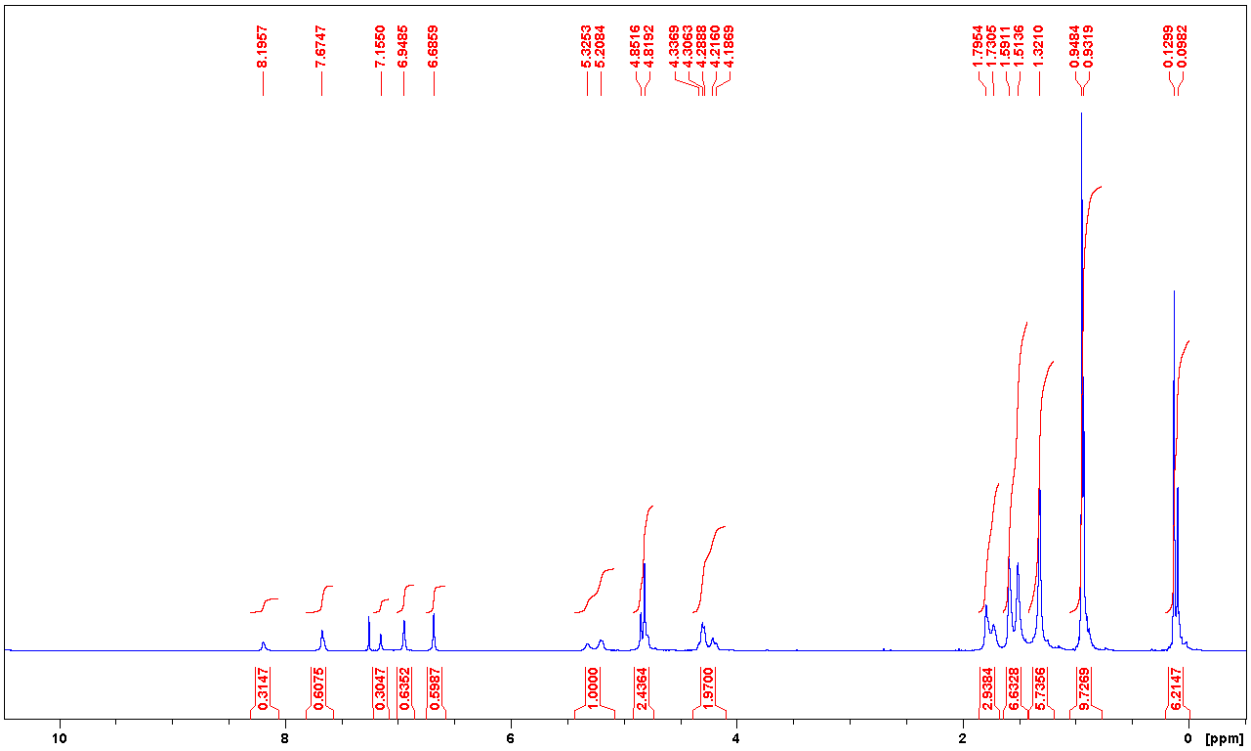


Figure A18. ^{1}H NMR of **89** at Room Temperature

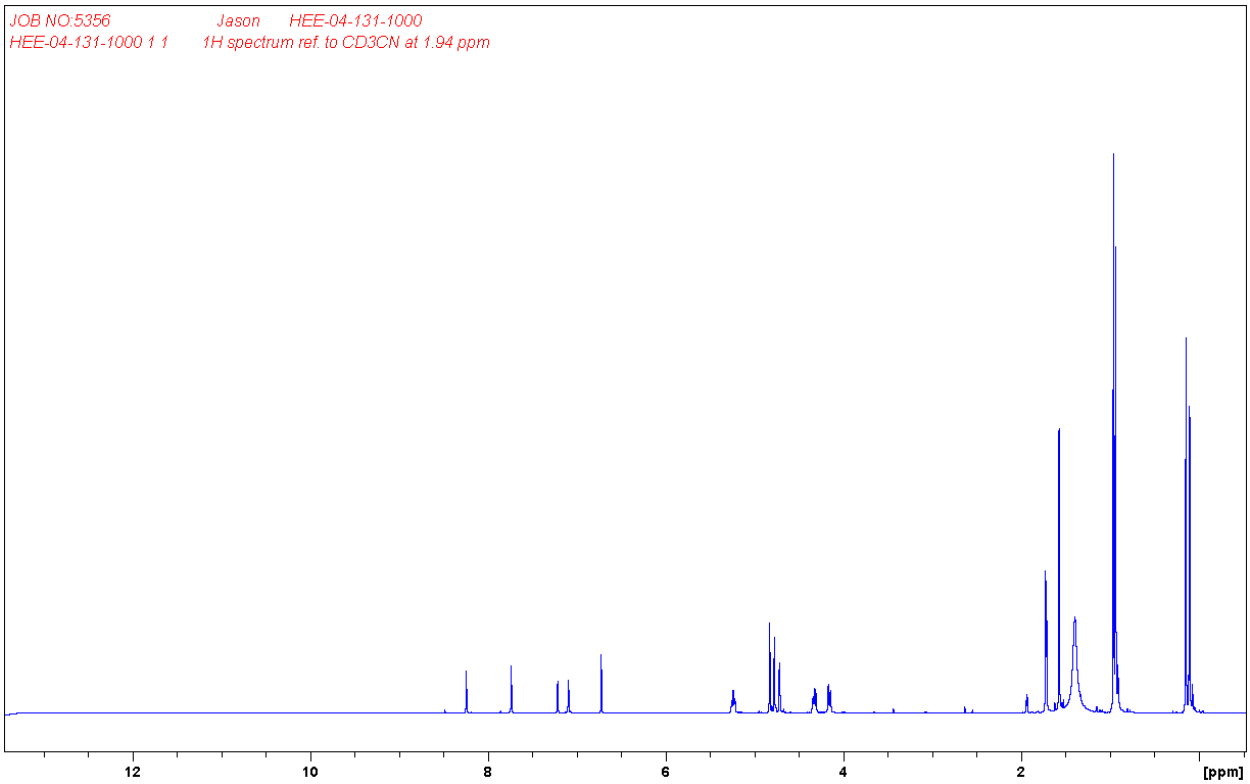


Figure A19. ^{1}H NMR of **89** at 65°C in d_3-acetonitrile (400MHz)

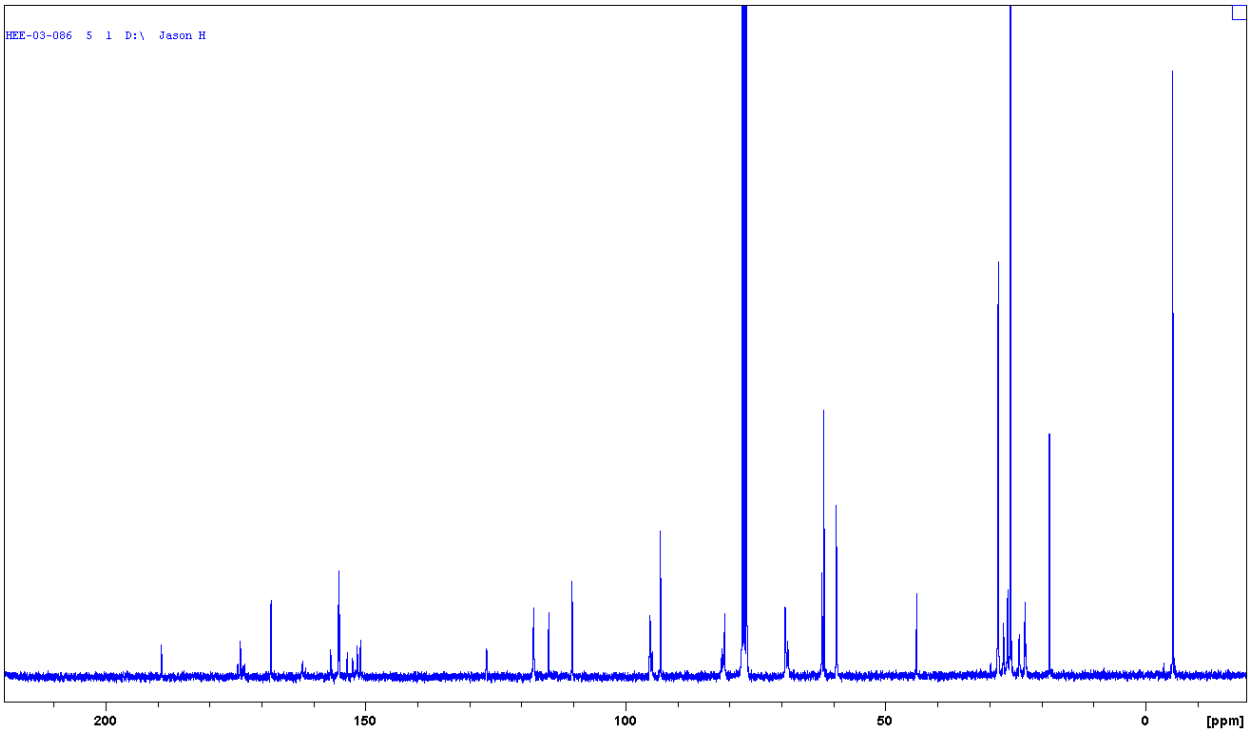


Figure A20. ^{13}C NMR of **89** at Room Temperature

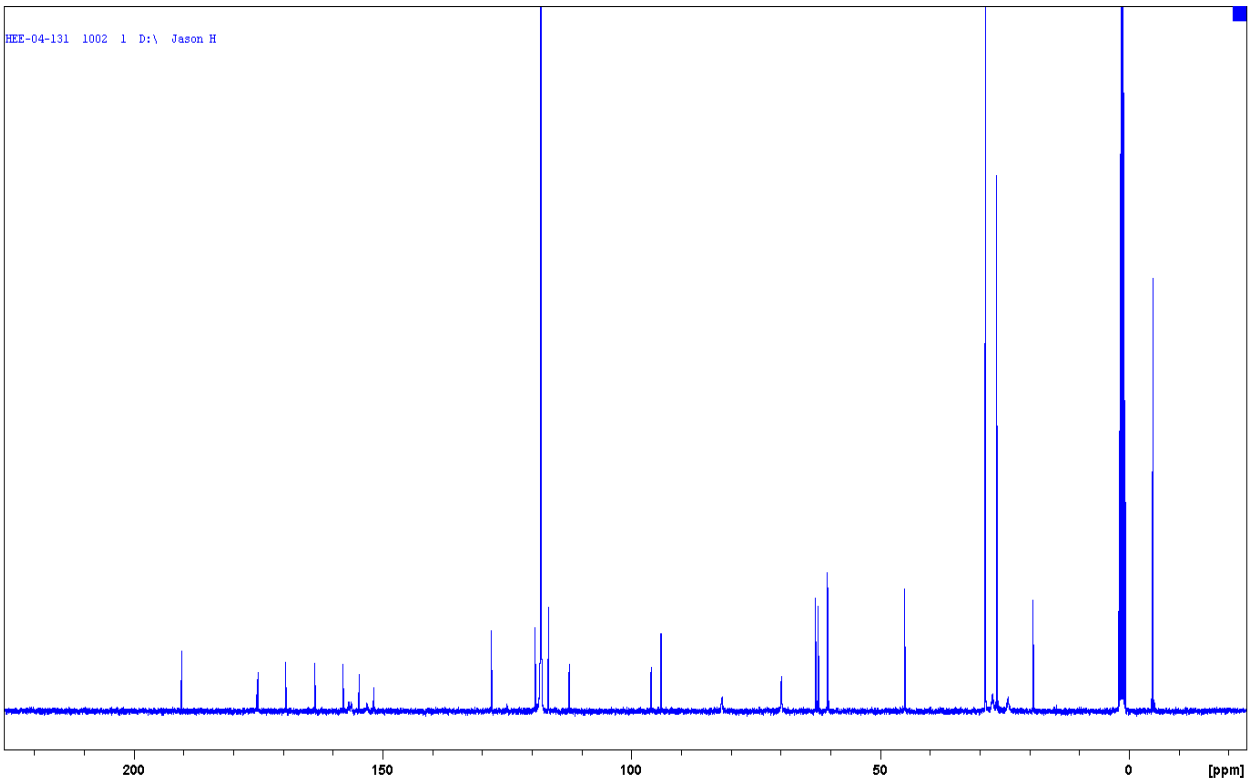
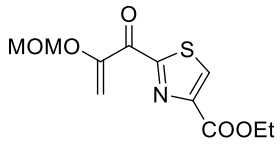


Figure A21. ^{13}C NMR of **89** at 65°C in d_3-acetonitrile (100MHz)

A.10 Preparation of ethyl 2-(2-(methoxymethoxy)acryloyl)thiazole-4-carboxylate (142)



Commercial *tert*-BuLi in pentane (1.24 M, 7.0 mL, 8.7 mmol, 5.8 eq) was carefully added over 3 min to a cold (–78 °C) solution of known methoxymethyl vinyl ether (385 mg, 8.7 mmol, 5.8 eq) [Tamao, K.; Nakagawa. Y.; Ito, Y. *Org Syn.* **1996**, *73*, 94] in dry tetrahydropyran (3.5 mL). A bright yellow solution resulted. The mixture was stirred at –78 °C for 10 min, then it was warmed to –10 °C (NaCl/ice bath) and stirred at that temperature for 23 min, during which time the bright yellow color disappeared. The mixture was then cooled back to –78 °C and diluted with THF (2.5 mL). A solution of aldehyde **155** (277 mg, 1.5 mmol, 1 equiv) in THF (1.5 mL) was added dropwise, whereupon the color of the solution turned light red. The mixture was stirred at –78 °C for 20 min, then it was quenched with TMSCl (1.04 mL, 8.20 mmol, 5.5 eq) and stirred for 10 more min. Aqueous sat. NH_4Cl (1.5 mL) was added, and the mixture was rapidly warmed to r.t (warm water bath). Extra aq. sat. NH_4Cl (3 mL) was added during the warming process. The mixture was then diluted with EtOAc (25 mL) and transferred to a separatory funnel and the aqueous layer was discarded. The organic phase was washed with more aq. sat. NH_4Cl (3 mL), dried over Na_2SO_4 and concentrated *in vacuo*. In crude form, the sensitive product **164** (not fully characterized; a proton NMR is provided) was immediately taken up in THF (6 mL), treated with 1M TBAF in THF (1.80 mL, 1.80 mmol) and stirred at r.t for 3 h. The mixture was quenched with aq. sat. NH_4Cl (3 mL), diluted with EtOAc (25 mL) and transferred to a separatory funnel. Water (5 mL) was added to remove ammonium salts completely, then the organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to afford crude

alcohol **165** (410 mg, 1.50 mmol). This compound was immediately dissolved in CH_2Cl_2 (15 mL) and treated with MnO_2 (1.30 g, 15 mmol, 10 eq relative to **165**). The mixture was stirred at r.t for 48 hours, then it was filtered over Celite. The filtrate was evaporated *in vacuo* and the residue was immediately applied to a column of silica gel (10 g) that had been deactivated by flushing with 3.5% Et_3N in hexane (20 mL). Elution with 3.5% Et_3N: 50% EtOAc: 46.5% hexanes yielded enone **142** as a yellow oil (R_f = 0.63).

Yield: 101mg (25% over 3 steps)

^{1}H NMR: 8.43 (s, 1H), 6.57 (d, 1H, J=3.0Hz), 5.63 (d, 1H, J=3.0Hz), 5.17 (s, 2H), 4.43 (q, 2H, J=7.3Hz), 3.50 (s, 3H), 1.41 (t, 3H, J=7.3Hz)

^{13}C NMR: 179.0, 165.9, 160.8, 153.1, 148.6, 132.9, 107.2, 94.6, 61.8, 56.5, 14.3

IR: 1733, 1721, 1658, 1610, 1152, 1011cm^{-1}

LRMS: 272.2 [M+H$^+$], 294.2 [M+Na$^+$]

HRMS: cald for 272.0593 $C_{11}H_{14}NO_5S$; found: 272.0590 [M+H$^+$]

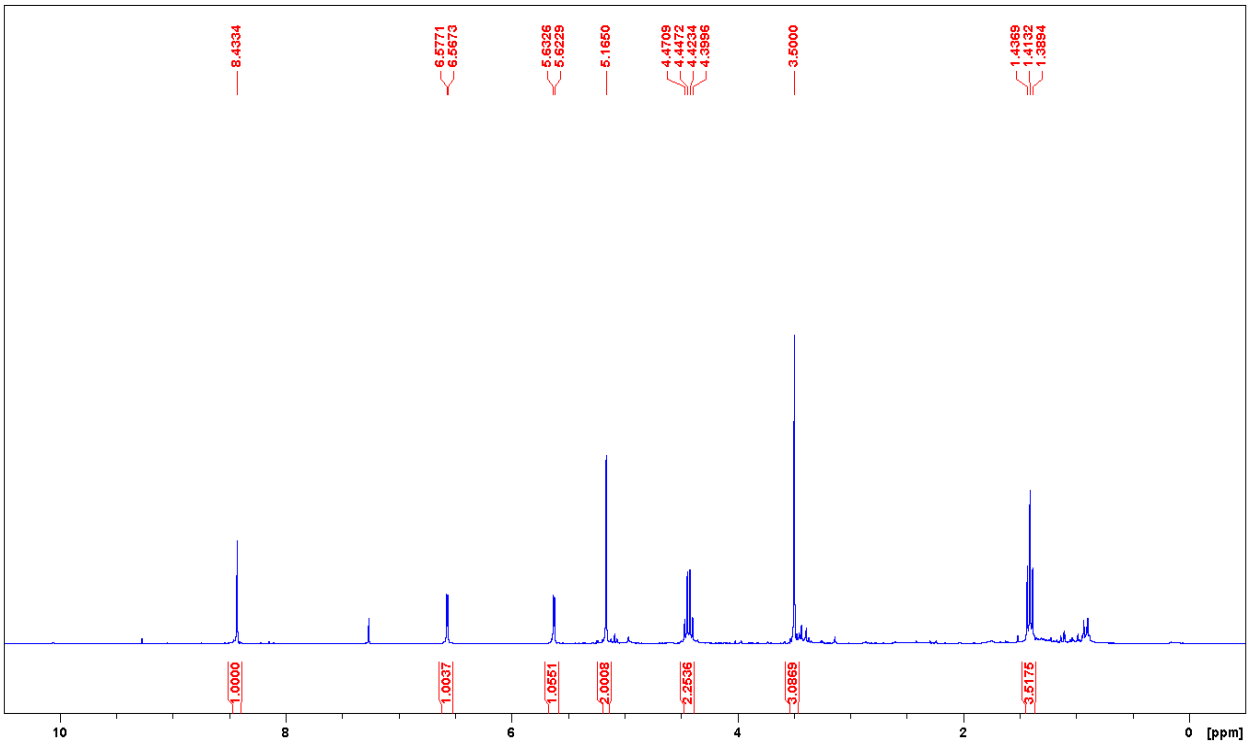


Figure A22. ^{1}H NMR of **142**

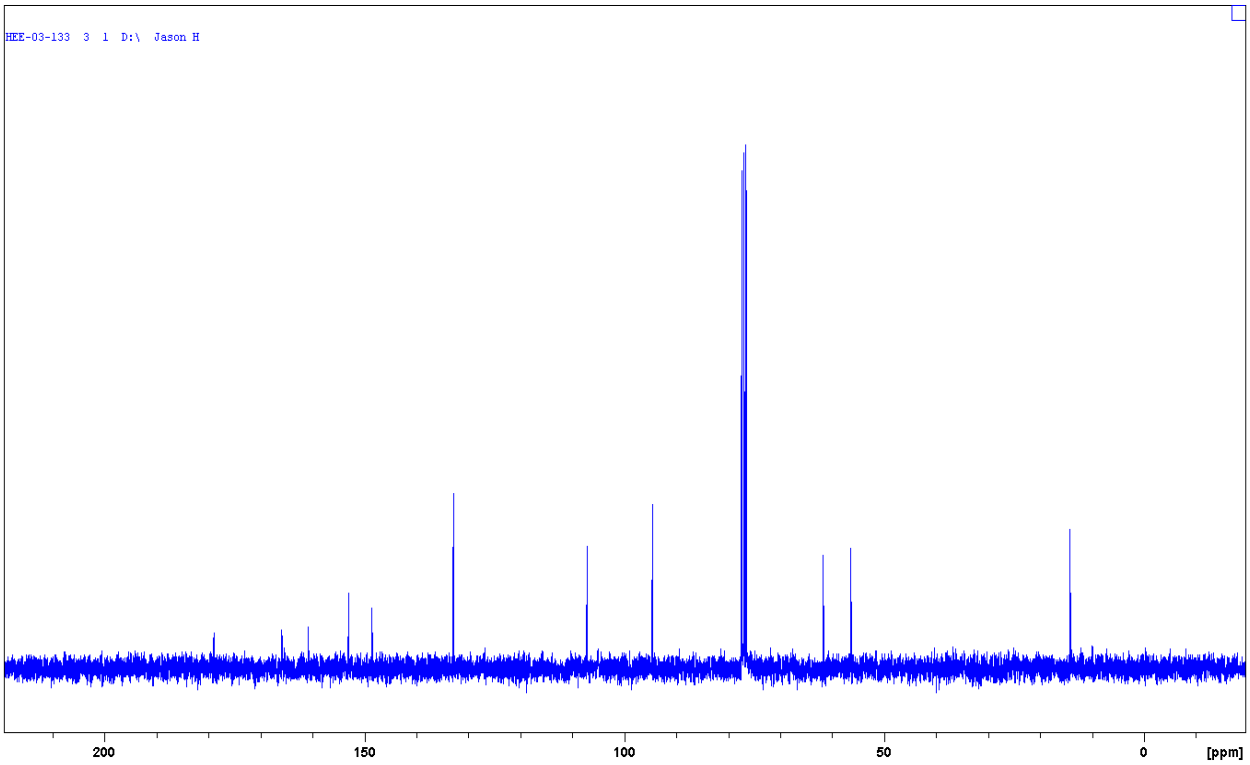


Figure A23. ^{13}C NMR of **142**

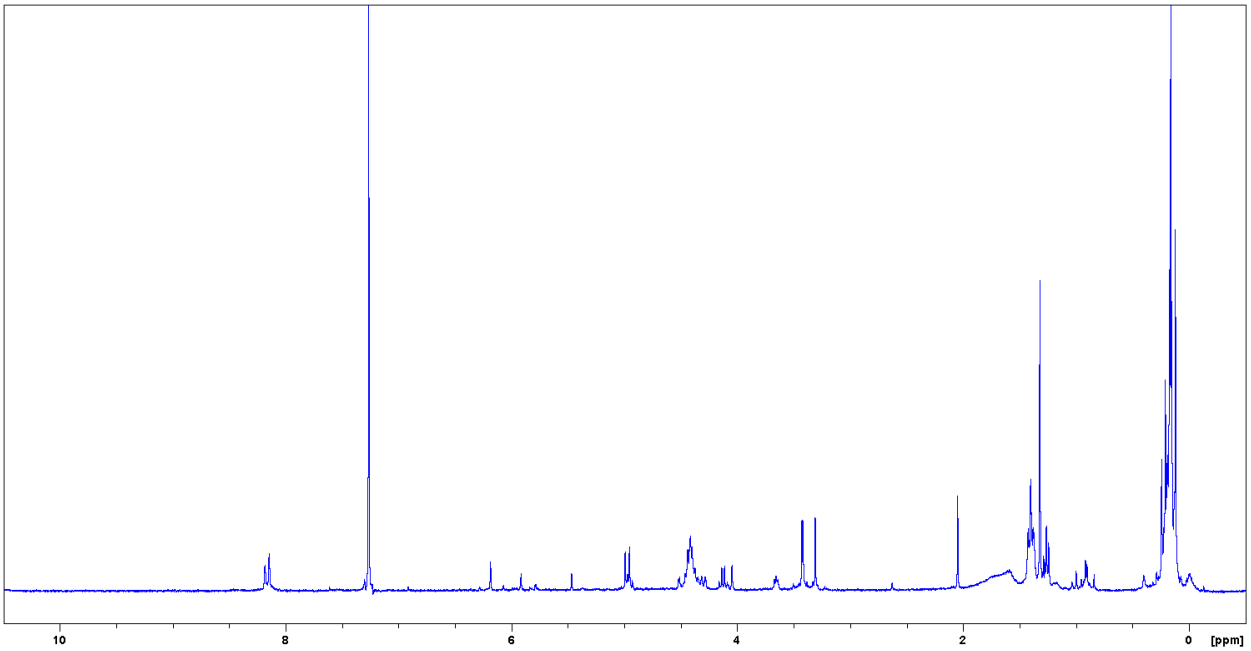
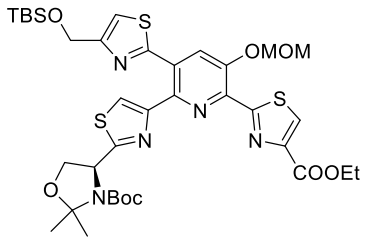


Figure A24. ^{1}H NMR of Suspected Dimer of Crude **164**

A.11 Preparation of tert-butyl (S)-4-(4-(3-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)-6-(4-(ethoxycarbonyl)thiazol-2-yl)-5-(methoxymethoxy)pyridin-2-yl)thiazol-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (141)



Commercial NaH (60% oil dispersion, 16 mg, 0.40 mmol, 1.3 eq) was dispensed into a flask maintained under inert atmosphere (Ar balloon), washed with hexane (3 x 0.4 mL) to remove excess oil, and suspended in THF (0.4 mL). A solution of ketone **89** (162 mg, 300 μmol, 1 eq) in THF (0.5 mL) was slowly added (syringe) at r.t over ca. 2 min. Evolution of H_2 was observed, and the color changed from faint green to yellow upon stirring at r.t for 15 minutes. A solution of enone **142** (126 mg, 0.46 mmol, 1.5 eq) in THF (0.4 mL) was added at r.t over a period of 1 min, whereupon the color of the solution turned from yellow to brown. The mixture was stirred for 90 min at r.t, then it was quenched with aq. sat. NH_4Cl (0.5 mL), diluted with EtOAc (10 mL) and transferred to a separatory funnel. The aqueous layer was discarded, while the organic phase was washed with more aq. sat. NH_4Cl (0.5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude diketone **169** was immediately taken up in a 4:1 mixture of TFE (1.8 mL) and AcOH (0.45 mL), and the solution was added over a period of 2.5 h (syringe pump) to a flask containing a warm (60 °C, oil bath temperature) solution of NH_4OAc (288 mg, 3.75 mmol, 12.5 eq) and PPTS (75 mg, 300 μmol) in 4:1 TFE (2 mL) - AcOH (0.5 mL), maintained under O_2 atmosphere (balloon). The mixture was stirred at 60^{o}C for 14 h, then it was concentrated *in vacuo* and the residue was taken up with EtOAc (15 mL). This solution was washed 2-3 times with 2 mL portions of aq. sat. $NaHCO_3$ until the pH of the aqueous washes (pH paper) stabilized at 7, then it

was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography of the residue (1% Et_3N: 25% EtOAc: 74% Hexane) afforded pyridine **141** as a yellow solid. This compound was highly UV active (purple under short wavelength, and sky blue under long wavelength), and its elution was readily monitored by TLC (50% EtOAc: 50% Hexane; R_f=0.40). The room temperature NMR spectra of **141** exhibited broad lines and revealed the presence of BOC rotamers. Therefore, NMR spectra were recorded from DMSO-d_6 solutions at 80 °C.

Yield: 22mg (28% over 2 stpes)

Melting Point: 58 – 61^oC

^1H NMR (400 MHz, DMSO-d_6, 80 °C): 8.61 (s, 1H), 8.20 (s, 1H), 7.91 (s, 1H), 7.48 (s, 1H), 5.51 (s, 2H), 5.15 (d of d, 1H,J=6.2Hz J=1.7Hz), 4.82 (s, 2H), 4.37 (q, 2H, J=7.1Hz), 4.22 (d of d, 1H, J=9.0Hz, J=6.2Hz), 3.96 (d of d, 1H, J=9.0Hz, J= 1.7Hz), 3.57(s, 3H), 1.63 (s, 3H), 1.52 (s, 3H), 1.41 (s, 9H), 1.36 (t, 3H, J=7.1Hz), 0.94 (s, 9H), 0.12 (s, 6H).

^{13}C NMR (100 MHz, DMSO-d_6, 80 °C): 172.0, 164.0, 162.3, 160.5, 156.3, 151.4, 150.9, 149.4, 147.1, 143.9, 139.2, 130.3, 130.2, 125.0, 120.3, 117.4, 95.4, 93.8, 79.7, 68.0, 61.0, 60.3, 58.3, 56.1, 27.6, 26.1, 25.4, 23.1, 17.5, 13.7, -5.7.

IR: 1705, 1154, 1088cm^{-1}

Optical Rotation: $[\alpha]_D^{21}$ = -11.3^o (CH_2Cl_2, c = 1.65)

LRMS: 826.4 [$M+Na^+$]

HRMS: cald for 826.2410 $C_{36}H_{49}N_5O_8SiS_3Na$; found: 826.2411 [$M+Na^+$]

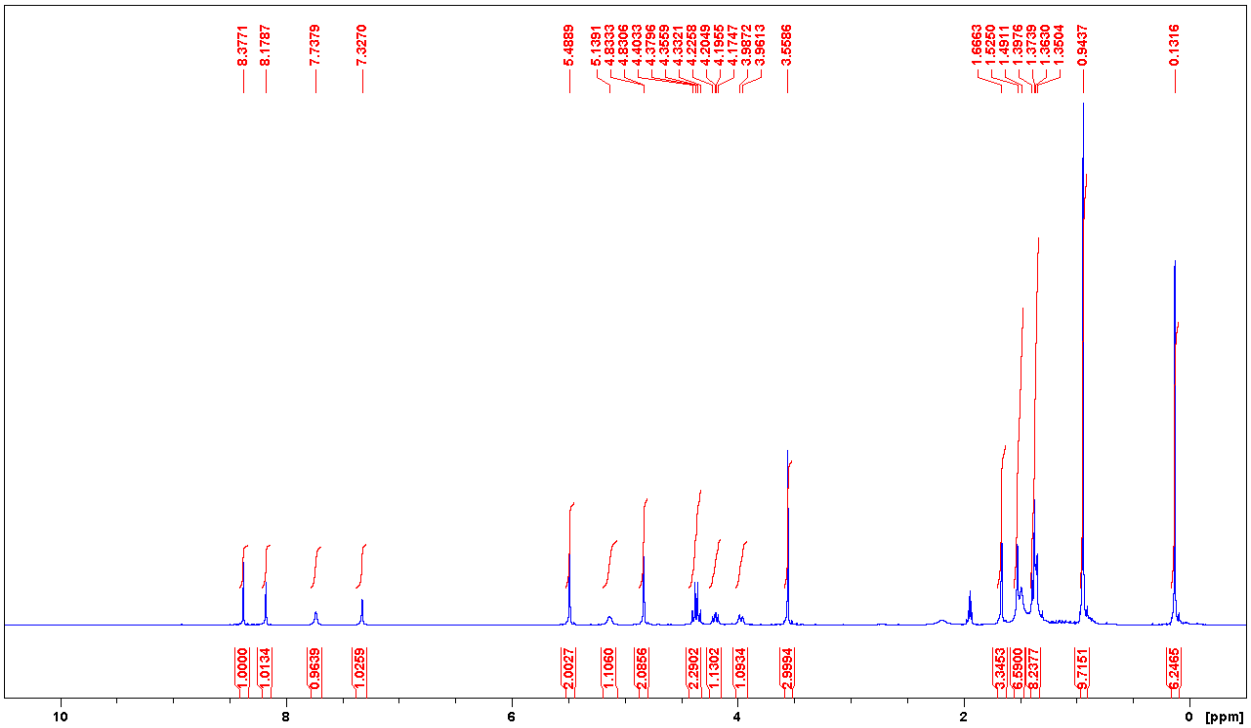


Figure A25. ^{1}H NMR of **141** at Room Temperature in d_3-acetonitrile

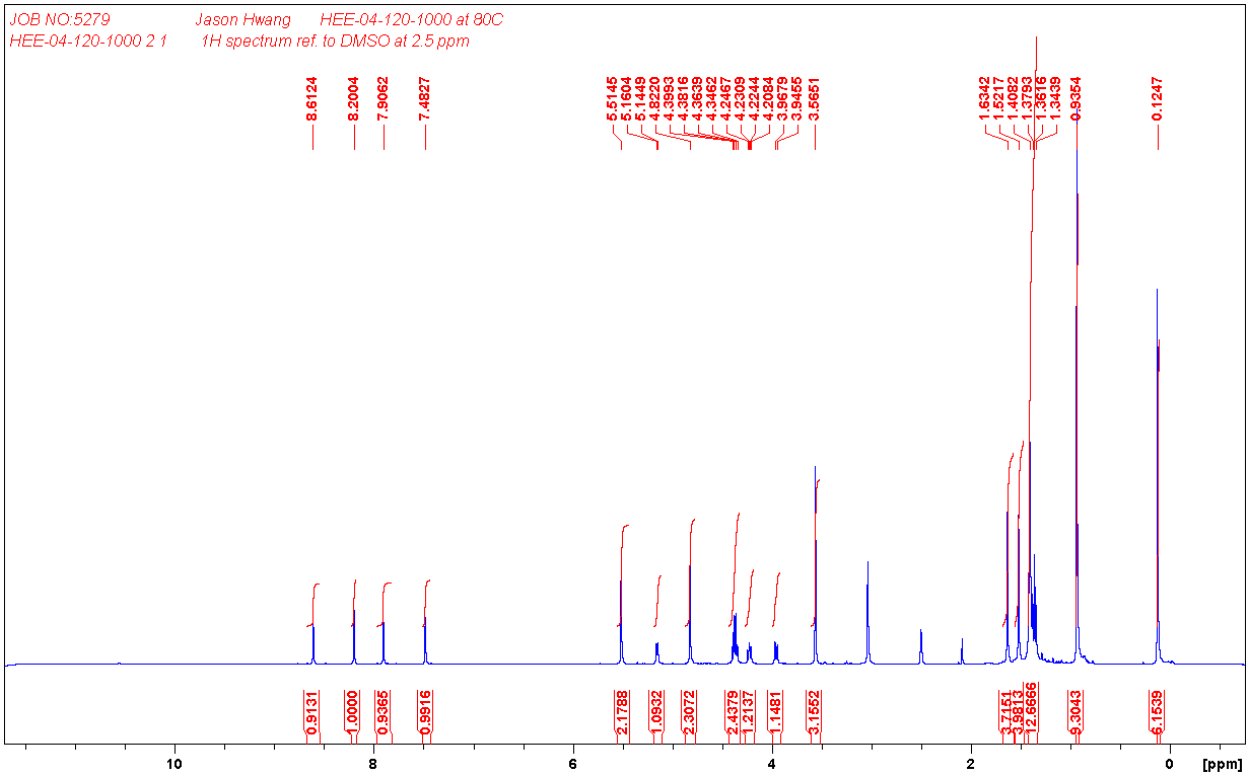


Figure A26. ^{1}H NMR of **141** at 80^{o}C in d_6-DMSO (400MHz)

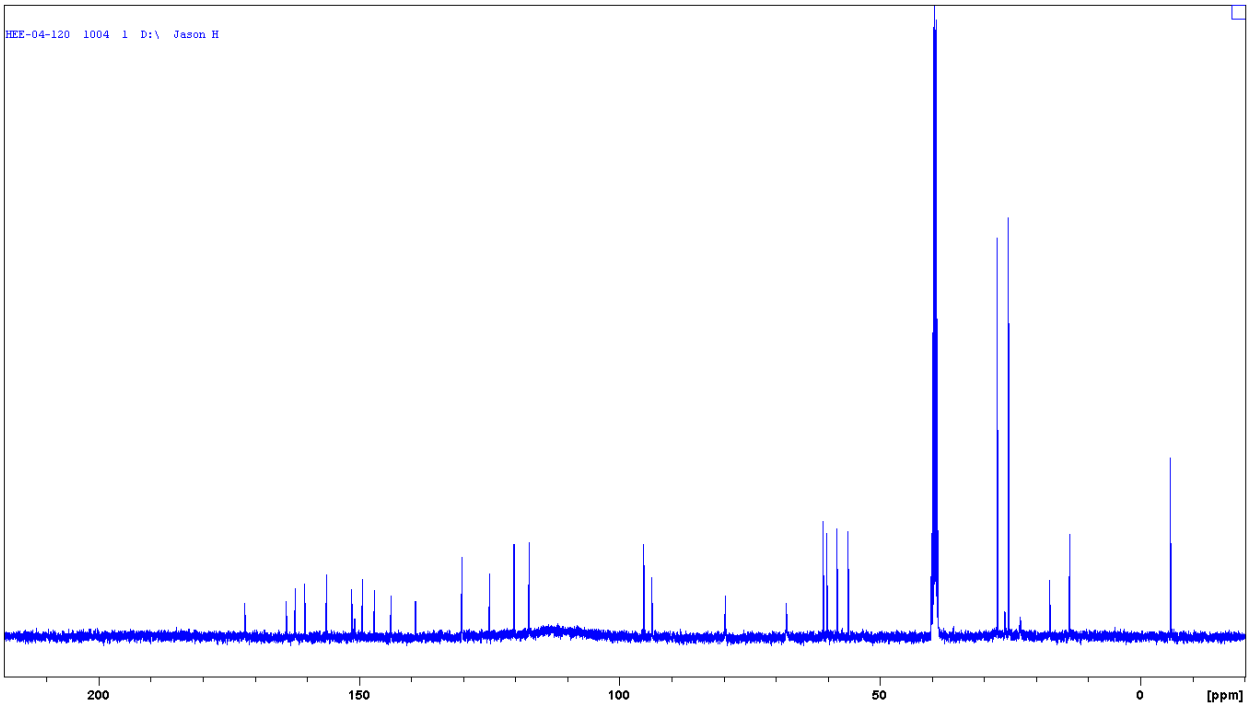


Figure A27. ^{13}C NMR of **141** at 80°C in d_6-DMSO (100MHz)

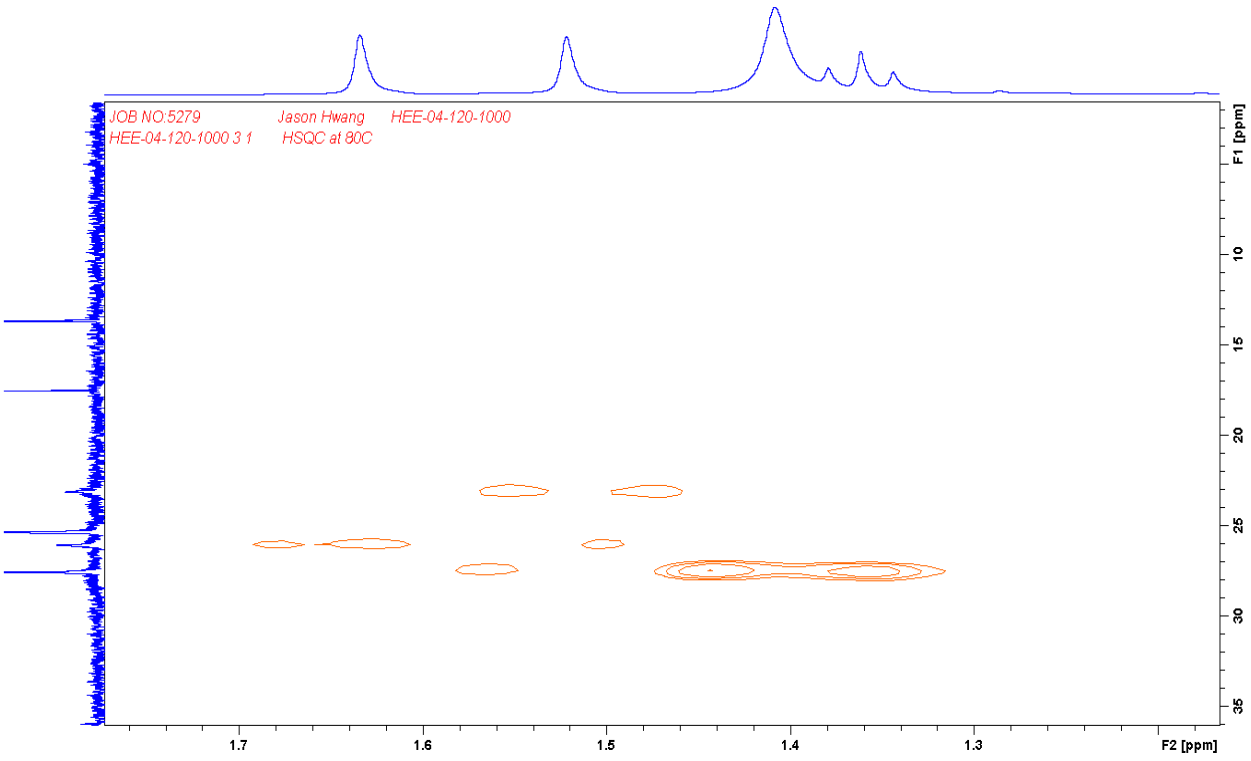


Figure A28. Expanded HSQC of **141**

Column: Agilent ZORBAX Eclipse XDB-C18, 5µm, 4.6mm x 150mm
Solvent: MeOH (5%), H_2O (95%)
Flow rate: 0.7mL/min
Detection: UV, 254nm

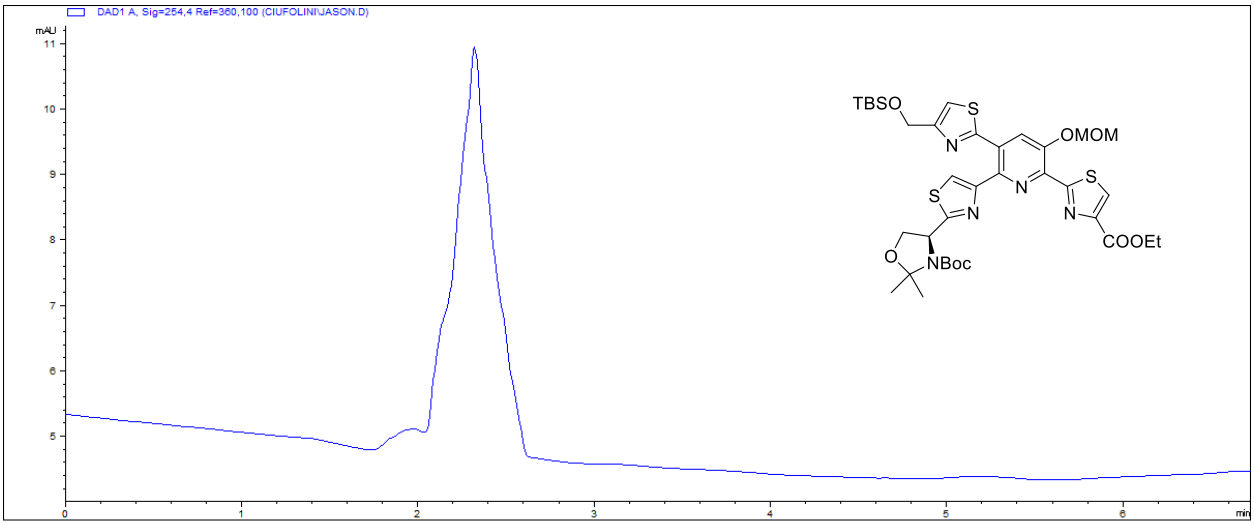
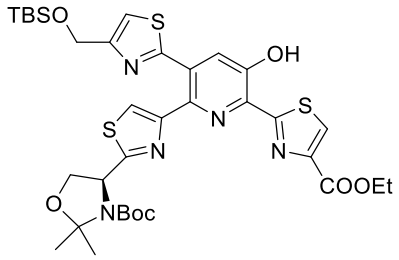


Figure A29. HPLC Trace of Pyridine **141**

A.12 Preparation of tert-butyl (S)-4-(4-(3-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)-6-(4-(ethoxycarbonyl)thiazol-2-yl)-5-hydroxypyridin-2-yl)thiazol-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (171)



Solid PPh_3 (2.6 mg, 10 μmol, 0.25 eq) and CBr_4 (3.3 mg, 10 μmol, 0.25 eq) were added to a solution of pyridine **141** (32 mg, 40 μmol) in 1,2-dichloroethane (0.7 mL) and the mixture was heated to 40 °C (oil bath temperature), with good stirring, for 3 h, whereupon TLC (50% EtOAc:50% Hexane) showed complete conversion of **141** into **171**. The solution was then diluted with CH_2Cl_2 (4 mL), washed with aq. sat. $NaHCO_3$ (1 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (20% EtOAc:80% Hexane) of the residue afforded pyridine **171** as a yellow film. The compound, which was very streaky on TLC (R_f = 0.51-0.73 in 50% EtOAc:50% Hexane), is highly UV active. It can be visualized on TLC as a green spot under short wavelength, and a bright green one under long wavelength.

Yield: 22mg (71%)

^{1}H NMR(CD_3CN): 11.58 (bs, 1H), 8.26 (s, 1H), 7.95 (s, 1H), 7.56 (s, 1H), 7.23 (s, 1H), [5.27-5.22 (m, minor rotamer), 5.16-5.11 (m, major rotamer) (1H)], 4.90 (s, 2H), 4.45 (q, 2H, J=7.1Hz), [4.21-4.13 (m, major rotamer), 4.11-4.03 (m, minor rotamor) (2H)], [1.79 (bs, major rotamer), 1.72 (bs, minor rotamer) (3H)], [1.58, 1.54 (bs, rotamers of equal intensity, 6H)], 1.46-1.40 (m, 9H), 0.96 (s, 9H), 0.13 (s, 6H)

IR: 3126, 2932 1704, 1365, 839cm^{-1}

Optical Rotation: $[\alpha]_D^{25}$ = -8.89^o (CH_2Cl_2, c =0.350)

LRMS: 760.3 [M+H$^+$]

HRMS: cald for 760.2329 $C_{34}H_{46}N_5O_7SiS_3$; found: 760.2324 [$M+H^+$].

Heating a solution of **171** in DMSO-d_6 to 80 ^oC in an NMR probe caused partial release of the TBS group. An NMR spectrum of the resulting mixture is provided.

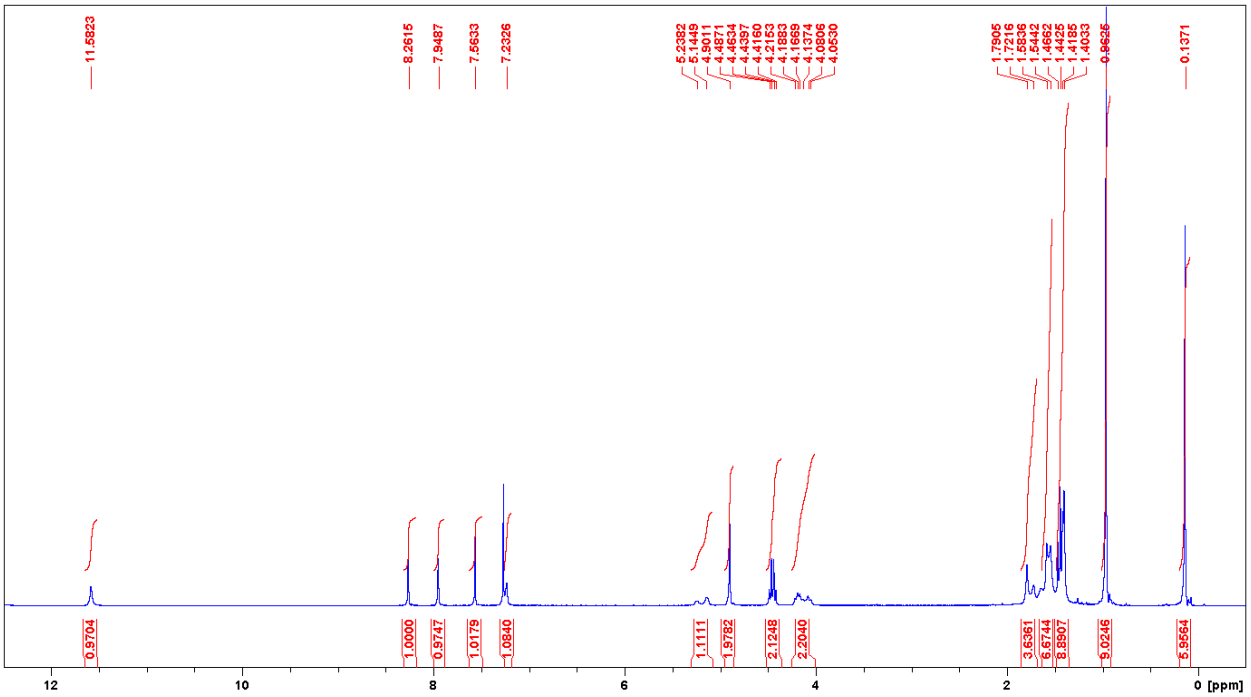


Figure A30. ^{1}H NMR of **171** at Room Temperature in d_3-acetonitrile

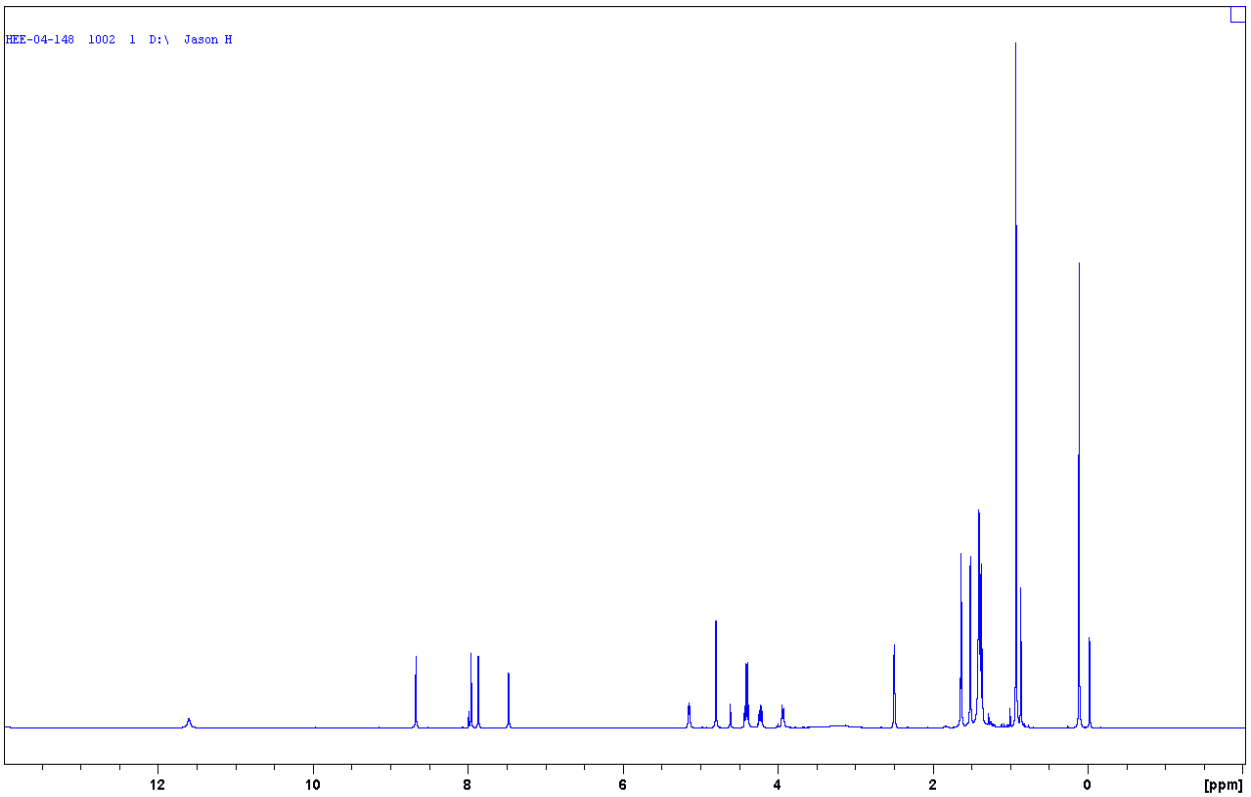
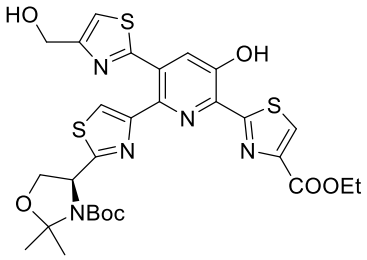


Figure A31. ^{1}H NMR of partially released **171** at 80^{o}C in d_6-DMSO (400MHz)

A.13 Preparation of tert-butyl (S)-4-(4-(6-(4-(ethoxycarbonyl)thiazol-2-yl)-5-hydroxy-3-(4-(hydroxymethyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (172)



The partially desilylated pyridine described above was redissolved in THF (0.1 mL) and was with TBAF (1M, 0.06 mmol, 0.06 mL), and was stirred at room temperature for 20 minutes. The mixture was then diluted with EtOAc (2mL), washed with sat. aq. NH_4Cl (0.5mL), and water (2 x 0.5mL) to remove excess ammonium salts. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was subject to flash column chromatography (40% EtOAc: 60% Hexane) and the elution of the desired product was monitored by TLC with (70% EtOAc:30% Hexane, Rf = 0.14-0.31, extremely streaky) as the eluent. Compound **172** appeared as purple spot under short wavelength, and as a yellow one under long wavelength. The product was isolated as yellow foam.

Yield: 17mg (66% over 2 steps)

^{1}H NMR(CD_3CN): 11.71 (bs, 1H), 8.37 (s, 1H), 7.90 (s, 1H), 7.71 (s, 1H), 7.34 (s, 1H), 5.10 (d, 1H, J=5.9Hz), 4.64 (s, 2H), 4.39 (q, 2H, J=7.1Hz), 4.19 (d of d, 1H, J=9.0Hz, J=6.3Hz), 3.92 (d of d, 1H, J=9.0Hz, J=1.6Hz), 1.67 (s, 3H), 1.52 (s, 3H), 1.49 (bs, 3H), 1.41-1.37 (m, 9H)

^{13}C (100 MHz, DMSO-d_6, 65 °C): 172.3, 168.2, 162.0, 159.7, 157.8, 151.5, 151.2, 151.0, 145.4, 142.7, 133.8, 131.5, 130.1, 125.0, 120.2, 117.2, 93.9, 79.8, 68.2, 60.9, 59.4, 58.3, 27.7, 26.3, 23.1, 13.8

IR: 3424, 3115, 2934, 1702, 1366, 1101cm^{-1}

Optical Rotation: $[\alpha]_D^{21}$ = -12.1^o (CH_2Cl_2, c =0.850)

LRMS: 646.4 [$M+H^+$]

HRMS: cald for 646.1464 $C_{28}H_{32}N_5O_7S_3$; found: 646.1470 [$M+H^+$]

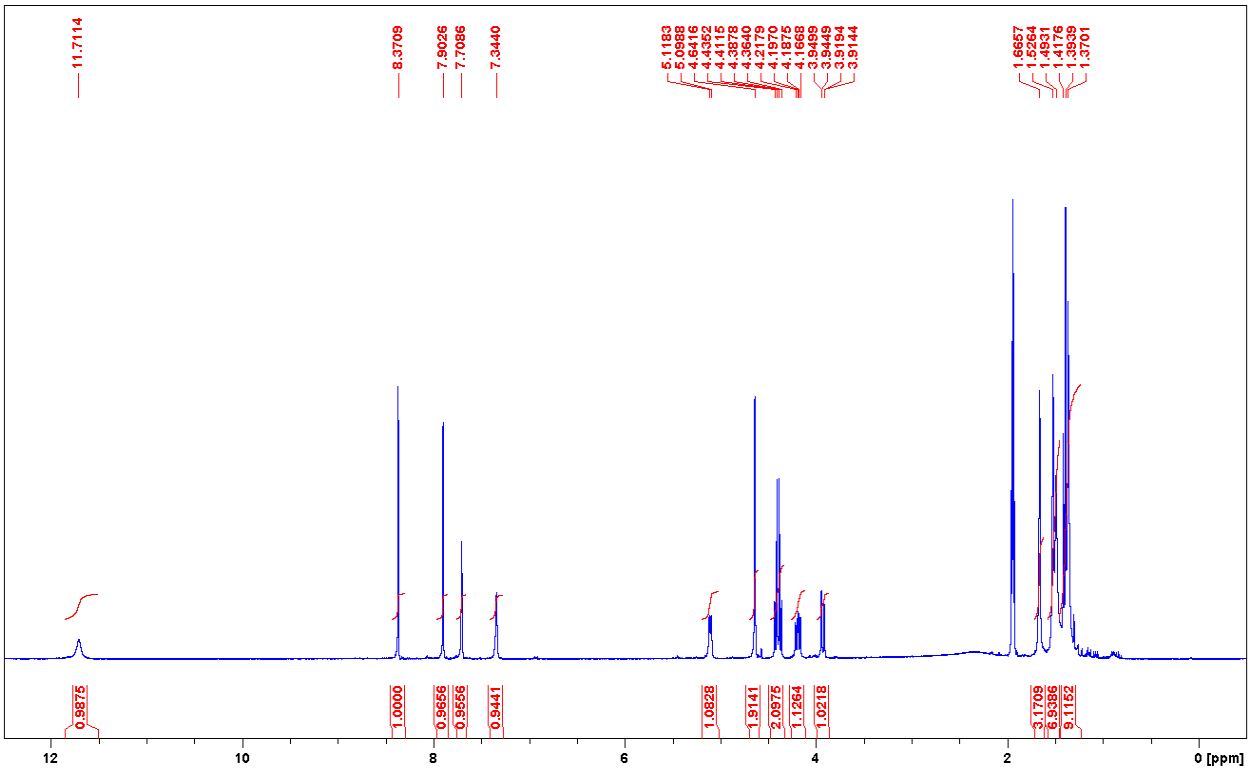


Figure A32. ^{1}H NMR of **172** at room temperature in d_3-acetonitrile

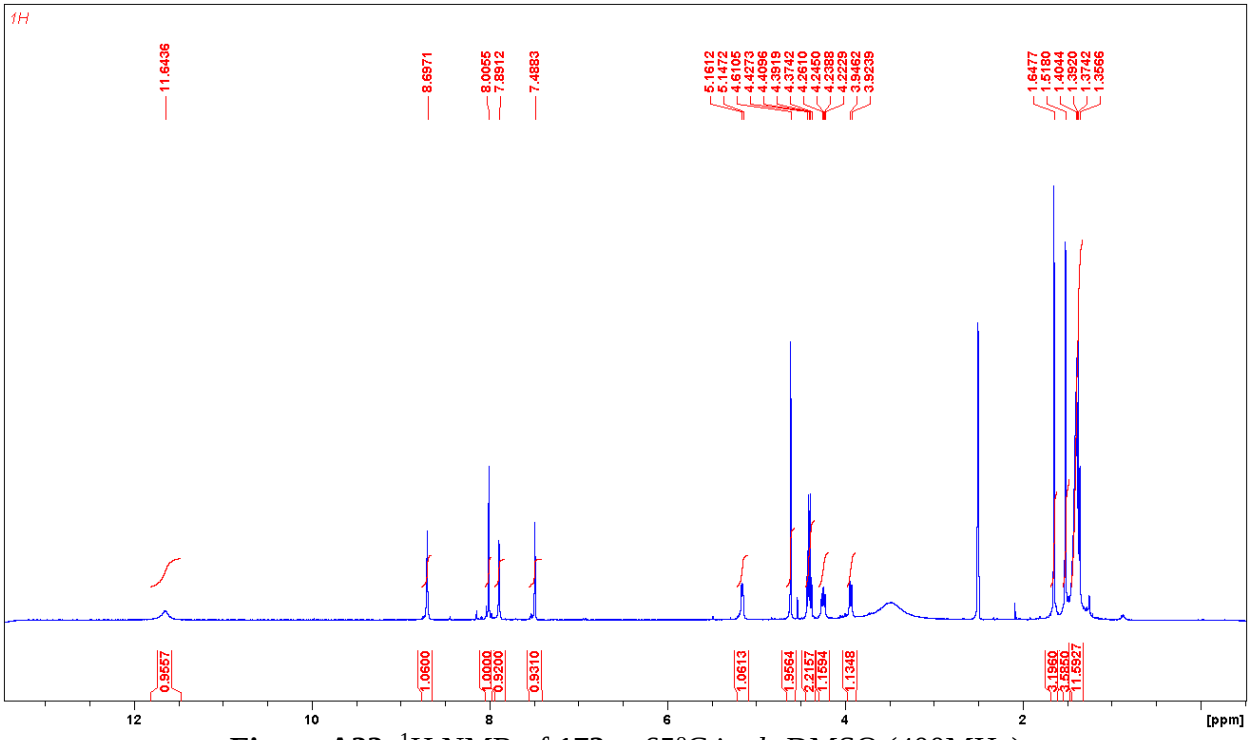


Figure A33. ^{1}H NMR of **172** at 65°C in d_6-DMSO (400MHz)

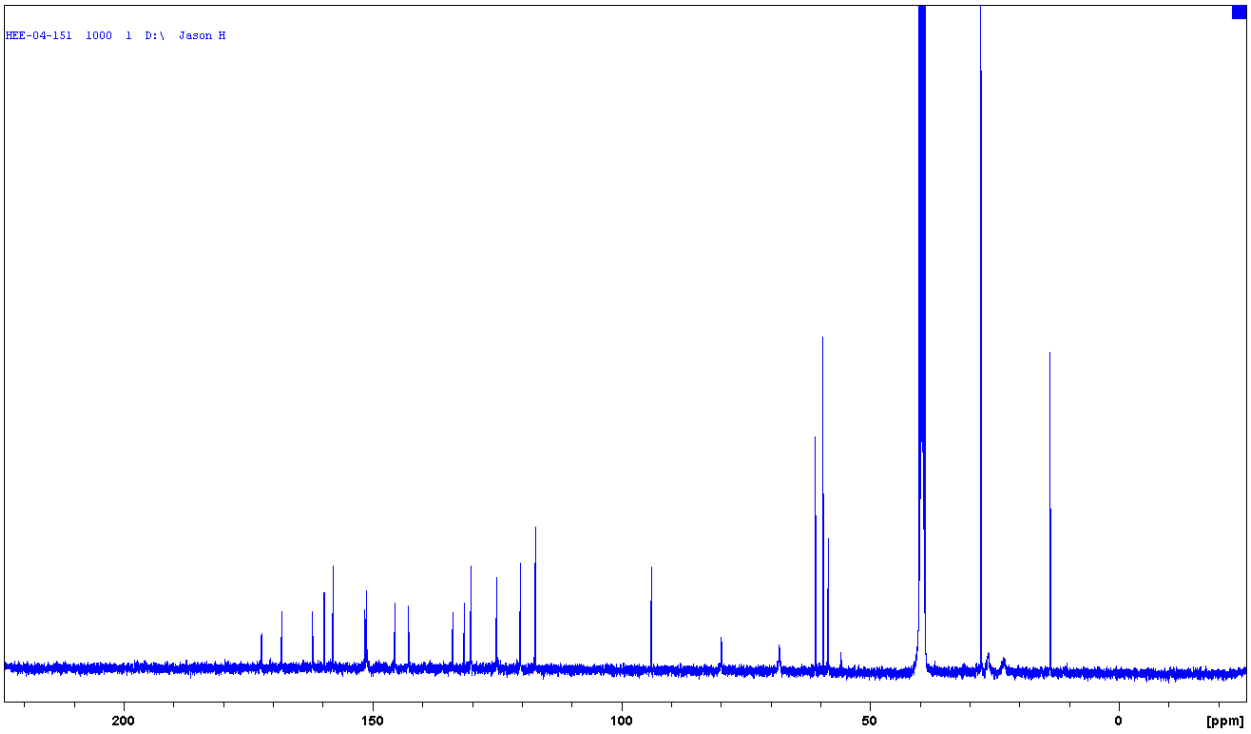
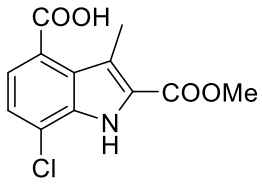


Figure A34. ^{13}C NMR of **172** at 65°C in d_6-DMSO (100MHz)

A.14 Preparation of 7-chloro-2-(methoxycarbonyl)-3-methyl-1H-indole-4-carboxylic acid (177)



$NaNO_2$ (7mmol, 0.484g) in H_2O (3ml) was slowly added to the solution of **174** in conc. HCl (8ml) and H_2O (5ml) very carefully over 10 minutes at -10^oC (NaCl/ice bath), and the mixture was stirred at -5^oC for 15 minutes. Then, the solution of $SnCl_2$ (3.96g, 8.75mmol) in conc. HCl (4.5ml) was slowly added to the mixture, and mixture was further stirred at -5^oC for 15 minutes. Then, the resulting solid was filtered using EtOH (15ml)/Et_2O (15ml) and dried under high vac to remove excess solvents to afford **175** as a brown solid. Then, hydrazine **174** (6.8mmol, 1.27g) was re-dissolved in the mixture of AcOH/H_2O (24ml:4ml), and methyl 2-oxobutanoate (6.6mmol, 0.680ml) was added. The mixture was brought up to 70^oC and stirred for 1 hour. The reaction mixture was diluted with additional 40ml H_2O and transferred to a separatory funnel, and aqueous layer was extracted with EtOAc (70ml x 3 ml). The combined organic layer was washed with 10ml H_2O, dried over Na_2SO_4, and concentrated *in vacuo* to afford hydrazone **176** as a white solid. This white solid was redissolved in 25ml AcOH, and PPA (~2ml) was added, and the mixture was brought up to 90^oC and stirred for 2 hours. The excess AcOH was removed *in vacuo*, and the residue was suspended in water, filtred, and dried in high vac to afford the known indole **177** as a brown solid. [Bentley, D. J.; Fairhurst, J.; Gallagher, P. T.; Manteuffel, A. L.; Moody, C. J.; Pinder, J. L. *Org. Biomol. Chem.* **2004,** *2*, 701]

Yield: 1.12g (60% over 3 steps)

^{1}H NMR (Acetone-d_6): 10.80 (bs, 1H), 7.59 (d, 1H, J=7.9Hz), 7.43 (d, 1H, J=7.9Hz), 3.93 (s, 3H), 2.72 (s, 3H)

^{13}C NMR (Acetone-d_6): 167.8, 161.7, 134.2, 134.1, 126.5, 126.2, 123.5, 123.1, 120.9, 120.7, 51.1, 11.4

IR: 3254, 1713, 1687, 1229cm^{-1}

LRMS: 268.7 [$M^{35}+H^{+}$], 270.7 [$M^{37}+H^{+}$]

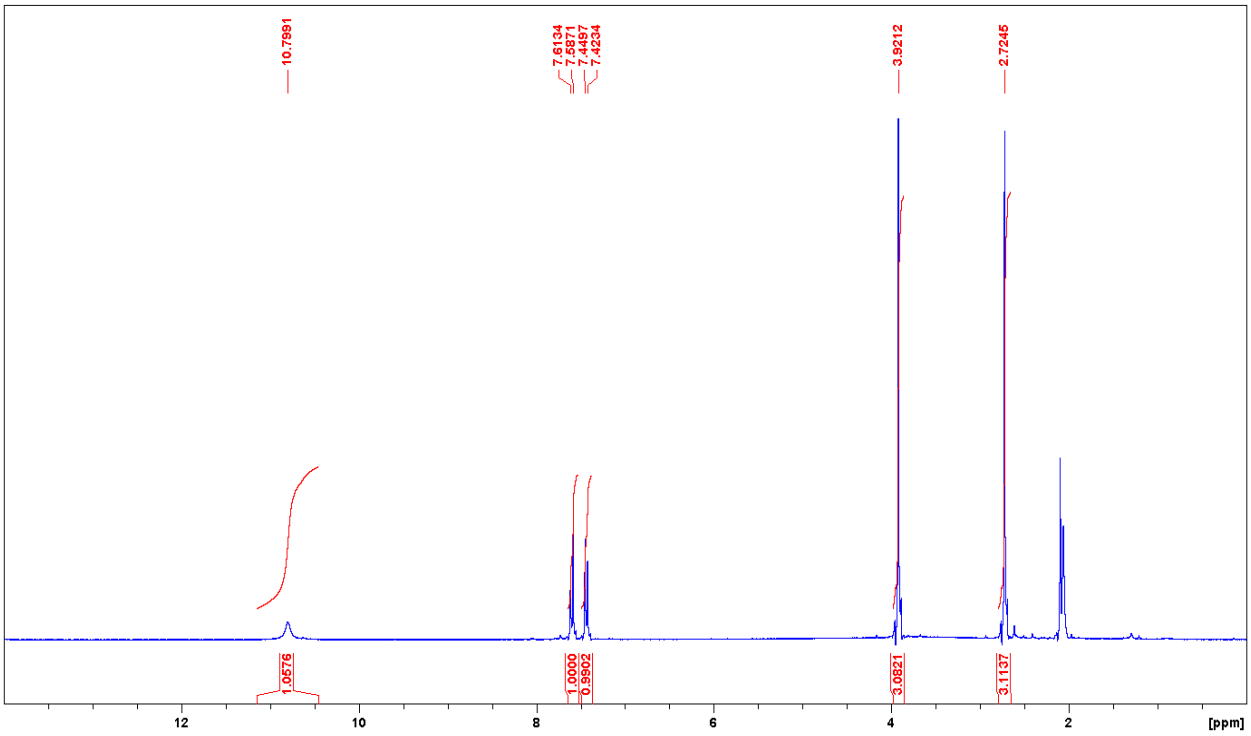


Figure A35. ^{1}H NMR of **177**

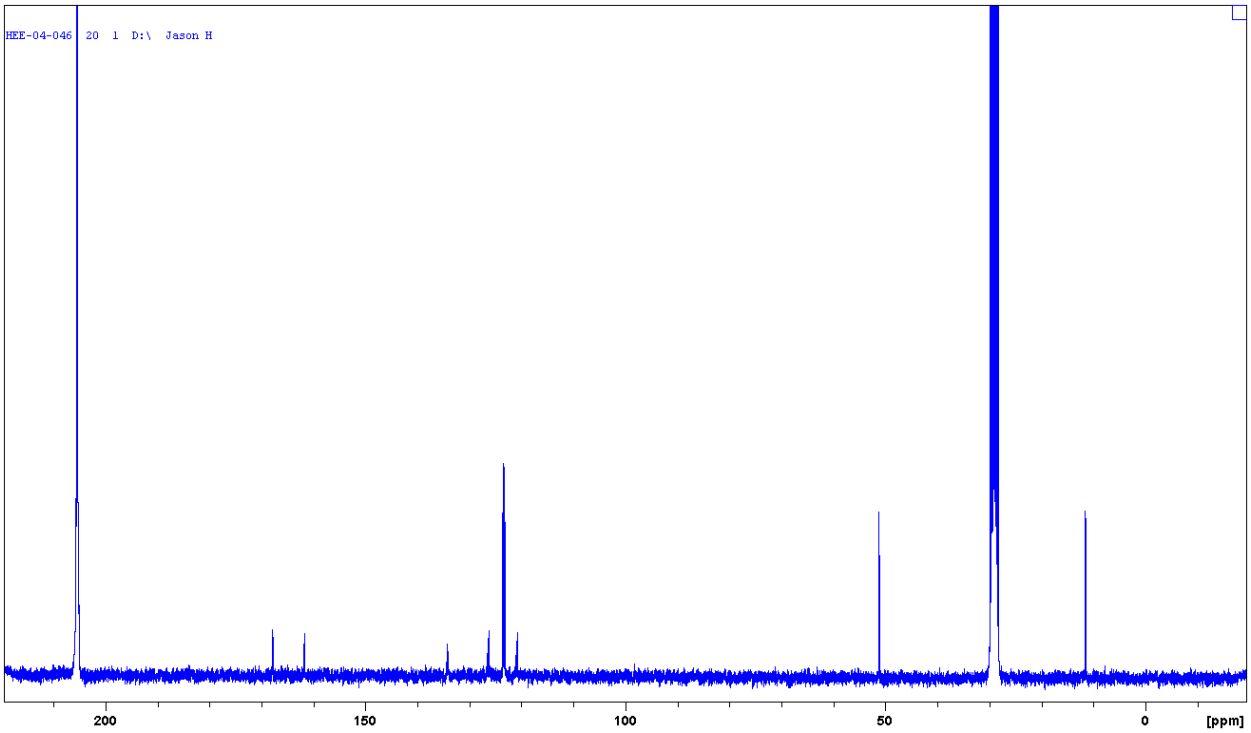
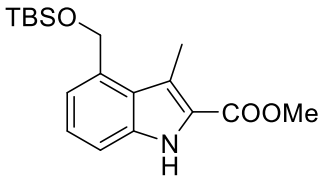


Figure A36. ^{13}C NMR of **177**

A.15 Preparation of 7-chloro-2-(methoxycarbonyl)-3-methyl-1H-indole-4-carboxylic acid (178)



The indole **177** was dissolved (267mg, 1mmol) in 10ml MeOH, and 27mg of (10% weight) Pd/C was carefully added to the mixture, and subsequently H_2 was introduced (balloon). The mixture was stirred for 72 hours at r.t, by the time starting indole **177** was fully converted. (monitored by NMR). The resulting dechlorinated indole (0.8mmol, 176mg) was dissolved in 3ml THF, and cooled to 0^oC. BH_3-SMe_2 (10M, 1mmol, 0.100ml, 1.25 eq) complex was added to the mixture dropwise over 3 minutes, and slowly brought up to r.t for 3 hours. The excess solvent was evaporated *in vacuo,* and the resulting residue was diluted with 20ml EtOAc, and sat. aq. $NaHCO_3$ (8ml) was poured, and transferred to a separatory funnel. Organic layer was collected, and aqueous layer was further extracted with 10ml EtOAc. Combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo* to afford the alcohol as a slightly yellow solid. The resulted alcohol was redissolved in 5ml DMF, and TBS-Cl (1mmol, 150mg, 1.25 eq) and imidazole (1.2mmol, 80mg, 1.5 eq) were added, and stirred overnight. The mixture was then diluted with 30ml EtOAc, and transferred to a separatory funnel. The organic layer was washed with H_2O (5ml x 5). Combined organic layer was further washed with brine (3ml), and dried over Na_2SO_4, and concentrated *in vacuo*. The crude was subjected to flash column chromatograph (20% EtOAc:80% Hex, R_f=0.47) to afford the known indole **178** a white solid. [Bentley, D. J.; Fairhurst, J.; Gallagher, P. T.; Manteuffel, A. L.; Moody, C. J.; Pinder, J. L. *Org. Biomol. Chem.* **2004,** *2*, 701]

Yield: 167mg (50% over 3 steps)

^{1}H NMR (Acetone-d_6): 7.42 (d, 1H, J=8.2Hz), 7.23 (m, 1H, J=8.2Hz, 7.1Hz), 7.11 (d, 1H, J=7.1Hz), 5.19 (s, 2H), 3.90 (s, 3H), 2.86 (s, 3H), 0.94 (s, 9H), 0.11 (s, 6H)

^{13}C NMR (Acetone-d_6): 162.4, 137.1, 135.8, 125.6, 124.7, 123.2, 120.0, 119.0, 111.8, 63.7, 50.7, 25.4, 18.0, 11.2, -5.9

IR: 3246, 1695, 1662, 1181cm^{-1}

LRMS: 334.8 [M+H^{+}]

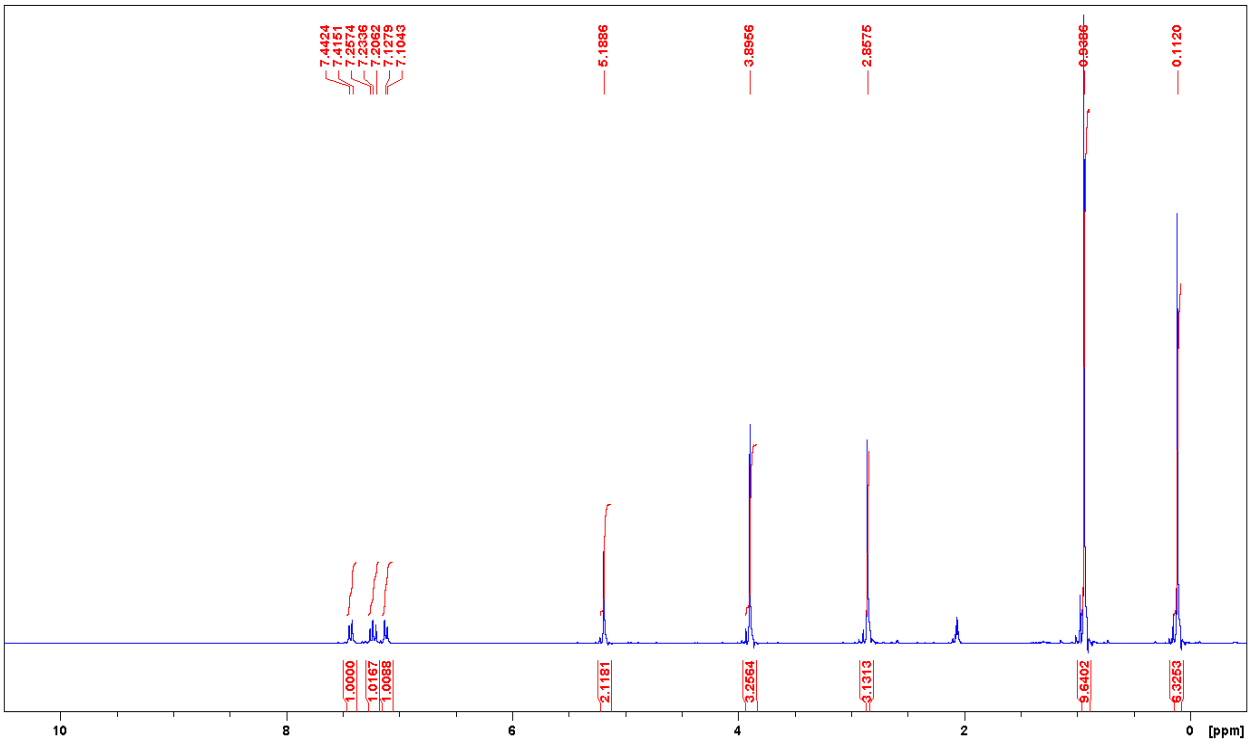


Figure A37. ^{1}H NMR of **178**

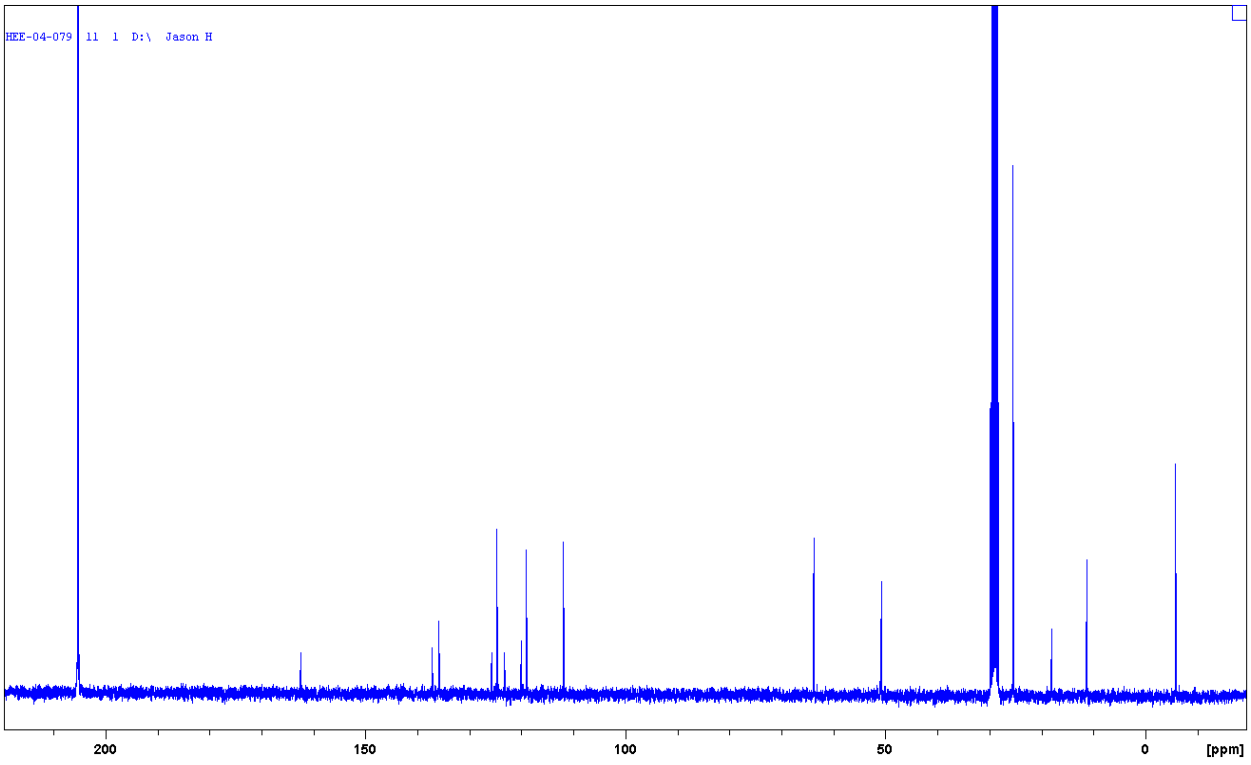


Figure A38. ^{13}C NMR of **178**