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A Stereodivergent Synthesis of the Anti-Inflammatory Agent BIRT-377. Approaches to the Synthesis of Gephyrotoxin

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A Stereodivergent Synthesis of the Anti-Inflammatory Agent BIRT-377. Approaches to the Synthesis of Gephyrotoxin

by

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A THESIS

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Abstract

The potent anti-inflammatory agent, BIRT-377, contains an α-quaternary center in a hydantoin ring. Due to requirements for clinical testing on enantiopure compounds, the need for enantioselective routes to chiral compounds, such as BIRT-377, are highly sought. Enantioselective syntheses of compounds containing α-quaternary amine moieties are difficult because steric hinderance precludes the use of many standard synthetic procedures.

Enzymatic desymmetrization of α,α-disubstituted malonic esters, followed by Curtius rearrangement, has been shown to provide access to α-quaternary amino acid derivatives, often with high enantiopurity. Thus, using this methodology a stereodivergent synthesis of BIRT-377 was accomplished, affording both enantiomers in high optical purity.

Acetylenic sulfones have been employed in sequential conjugate addition and cyclization reactions in our group to synthesize various nitrogen-containing heterocycles. We attempted to extend this methodology to the synthesis of the alkaloid gephyrotoxin; however, we were unable to obtain a β-amino ester compound required for a key conjugate addition step.

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Dedication

To my Mam

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List of Abbreviations

Å	Ångstroms
Ac	acetyl
AIBN	2,2'-azobis(2-isobutyronitile)
Ar	aryl
atm	atmospheres
ATR	attenuated total reflectance
BHT	butylated hydroxytoluene
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br s	broad singlet
BRSM	Based on recovered starting material
BSA	bis(trimethylsilyl)acetamide
Bu	butyl
°C	degrees Celcius
calc'd	calculated
Cbz	carbobenzyloxy
CDI	*N*,*N*-carbonyldiimidazole
CM	complex mixture
cm^{-1}	wavenumbers
Cp	cyclopentyl
Cy	cyclohexyl

Δ	heat
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublet of doublets
de	diastereomeric excess
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarization transfer
DHQ	decahydroquinoline
DIBAL	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DPPA	diphenylphosphoryl azide
E^+	electrophile
ee	enantiomeric excess
EI	electron impact ionization
ent	enantiomer
equiv.	equivalent

ESI	electronspray ionization
Et	ethyl
eV	electron volts
FT-ICR	Fourier transform ion cyclotron resonance
g	grams
H_L	hydrophobic large pocket
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectroscopy
H_S	hydrophobic small pocket
HSQC	heteronuclear multiple quantum coherence
hν	light
Hz	Hertz
i	*iso*
ICAM-1	intercelluar adhesion molecule 1
Im	imidazole
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
J	coupling constant
LDA	lithium diisopropylamide
LFA-1	leukocyte function antigen 1
liq	liquid
LUMO	lowest unoccupied molecular orbital

M	molar
m/z	mass to charge ratio
Me	methyl
MeCN	acetonitrile
mg	milligrams
MHz	megaHertz
mL	milliliters
mmol	millimoles
mol	moles
MOM	methoxymethyl
MP	melting point
Ms	methanesulfonyl
MS	mass spectrometry
n	normal chain
N	normal
nm	nanometer
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
Nuc	nucleophile
p	*para*
P_B	polar back pocket
PCC	pyridinium chlorochromate

P_F	polar front pocket
Ph	phenyl
PLE	porcine liver esterase
ppm	part per million
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
Py	pyridine
Quant.	quantitative
R	generalized substituent
R_f	retention factor
R_L	large substituent
R_S	small substituent
RT	room temperature
s	singlet
SDBBA	sodium diisobutyl-*t*-butoxyaluminum hydride
Ser	serine
SM	starting material
t	triplet
t	*tert*
TBAB	tetra-*n*-butylammonium bromide
TBAF	tetra-*n*-butylammonium fluoride
TBDPS	tert-butyldiphenylsilyl

TBS	*t*-butyldimethylsilyl
td	triplet of doublets
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Tof	time of flight
tris	tris(hydroxymethyl)aminomethane
Ts	*p*-toluenesulfonyl
UV	ultraviolet
v	volume
δ	chemical shift in ppm
μL	microliters
μm	micrometer

Chapter One: Introduction

1.1 Overview

This thesis presents the work carried out on two distinct projects: one involves the employment of a method that combines an enzymatic (porcine liver esterase) desymmetrization of dimethyl malonate esters with a Curtius rearrangement to synthesize α-quaternary amino acid derivatives, in particular the anti-inflammatory agents (+) and (-) BIRT-377, through a stereodivergent pathway. The second is the use of conjugate addition and cyclization methodology based on acetylenic sulfones using amino esters in a new approach to the synthesis of the dendrobatid alkaloid gephyrotoxin.

The introduction starts with a description of how α-quaternary amino acids can be synthesized by enzymatic desymmetrization and Curtius rearrangement reactions, as well as an explanation of how the active site of porcine liver esterase controls the enantioselectivity of ester hydrolysis and a summary of previous enantioselective syntheses of BIRT-377. The second part of this chapter includes an overview of dendrobatid alkaloids, which is followed by a brief review of acetylenic sulfone chemistry and its use in previous alkaloid syntheses by our group. Finally a review of gephyrotoxin and a synopsis of previous syntheses of the alkaloid are provided.

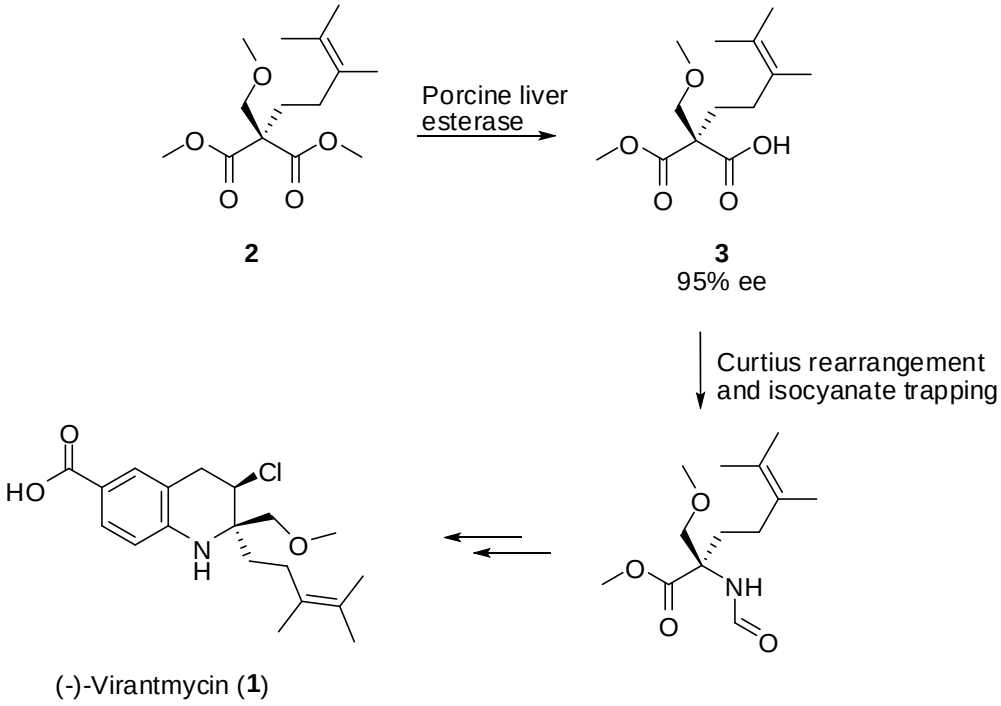
1.2 BIRT-377

1.2.1 *α*-Quaternary amino acid derivatives

Peptides and proteins are essential for life due to the diversity in their structural and functional attributes. In biological systems they have a myriad of roles ranging from enzymes, structural components of cells, secondary messengers and regulators to name a few. Linear peptides can adopt a broad variety of conformations based on their intramolecular interactions between internal residues, as well as interactions with their environment.[1,2]

Over the past few decades, designer peptides and proteins have become a very attractive area of study because they allow for examination of the effects of structural alterations upon interactions with ligands or receptors and the regulation of biological processes dependent on such interactions. All naturally occurring amino acids, except for glycine, contain a single α-substituent; therefore *α,α*-disubstituted (quaternary) derivatives are a potentially useful class of compounds and could be incorporated into designer peptides and proteins. This structural feature also occurs widely among alkaloids and various medicinal compounds. Due to this, there has been considerable effort into developing novel methods for the enantioselective synthesis of α-quaternary amino acids.[3-11]

Our group's interest in this subject was initiated by the synthesis of the antiviral alkaloid (-)-virantmycin (**1**).[12] The *α*-quaternary amino acid derivative required for this synthesis was achieved via a combination of porcine liver esterase (PLE) desymmetrization of disubstituted malonic ester **2** followed by Curtius rearrangement of half-ester product **3** (Scheme 1.1).



Scheme 1.1

1.2.1.1 Desymmetrization using porcine liver esterase

Porcine liver esterase (PLE) is a serine-type esterase, which is to say that it is the serine residue that acts as the nucleophilic site for the required cleavage. The mechanism of action is through attack of the serine residue on the substrate, then formation of an *O*-acyl intermediate, which is subsequently hydrolyzed. PLE's advantages are that it is commercially available and cheap, it hydrolyzes a broad range of esters and it can tolerate relatively high levels of organic solvent before denaturing, which is important because dissolution of the substrate usually requires an organic medium. These characteristics make PLE attractive for synthetic applications.

Another attribute of this enzyme is that there has been a significant amount of investigation carried out on the structural features that control its stereoselectivity, despite there being no published X-ray structure on the enzyme's active site to date. This is

crucial as it makes the outcome of reactions more predictable, thus providing a logical use of the enzyme for synthetic desymmetrization. The use of PLE for the enantioselective cleavage of diesters, in particular, has been extensively explored.[13-18] In 1985, Björkling *et al.* noticed a reversal of enantioselectivity with variation in the length of the larger alkyl substituent on propanedioic acid diesters (Figure 1.1).[19] When the large alkyl chain was extended beyond four carbons there was a complete inversion of stereochemistry.

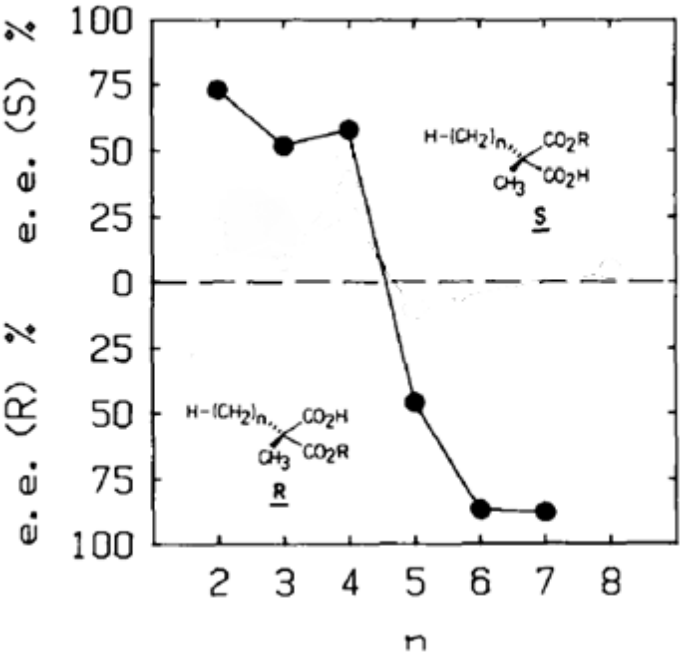


Figure 1.1: Björkling's study on PLE desymmetrization of α-methyl-α-*n*-alkyl diesters (reproduced from reference 19 with permission from the publisher)

Subsequently, Jones *et al.* published several papers on studies of structure-activity relationships of PLE desymmetrizations of malonate esters, which concluded with a model of the enzyme active site.[20-23] This model was based upon an empirical approach that used a sample set of roughly one hundred malonic esters and the enantiomeric excesses of the products formed. All of the substrates used were methyl esters, the reason being they were the simplest leaving group possible, gave the best yields and fastest reactions, and are widely used in synthesis.[20]

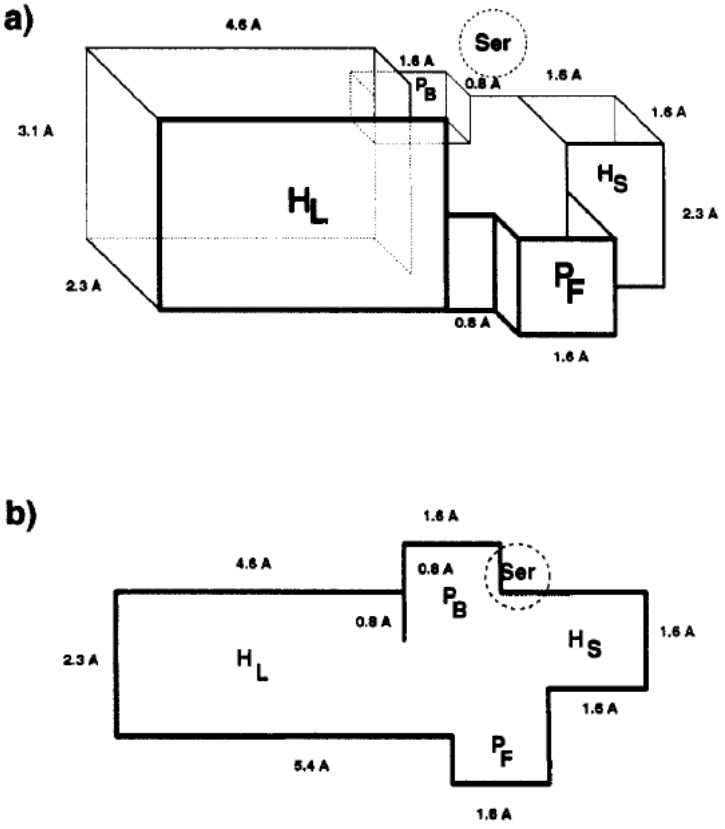
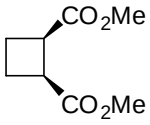
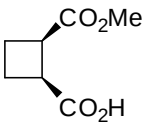
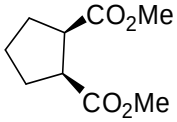
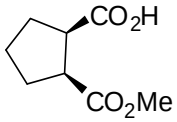
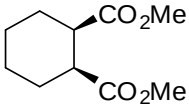
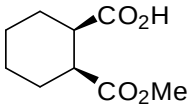
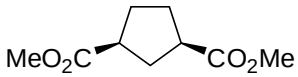
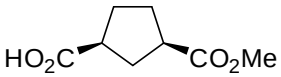
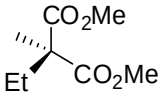
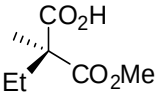
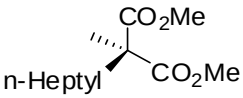
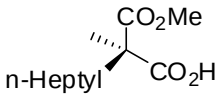
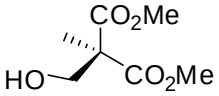
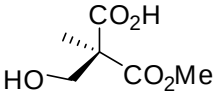
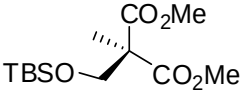
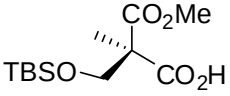
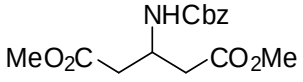
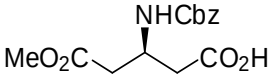
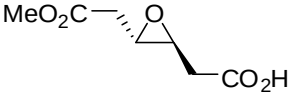


Figure 1.2: Jones' model of the PLE active site (reproduced from reference 20)

a) = oblique view; b) = top view; Ser = catalytic serine residue; P_B = polar back pocket; P_F = polar front pocket; H_L = hydrophobic large pocket; H_S = hydrophobic small pocket.

As shown in Figure 1.2, the active site has five binding pockets (Ser, P_B, P_F, H_L and H_S). The one that contains the serine residue (Ser) has a diameter of 1 $Å^3$. The other four pockets control the stereochemistry. The back polar pocket (P_B) is located next to the serine residue and can fit a group that is capable of hydrogen bonding. This pocket is where the ester group that is cleaved fits. Unlike the rest of the pockets, its rear boundary is open, allowing for groups to stretch beyond this region. The top face of the active site is also open. The polar front pocket (P_F) can accommodate a polar group, which is usually the second ester group but it can also accommodate a non-polar group. Out of the two hydrophobic pockets one is large (H_L) and one small (H_S). Using the experimental data obtained, the limits determined for the large and small hydrophobic pockets were set as ~33 and 5.5 $Å^3$, respectively. Polar groups like carbonyl, nitro, amino and hydroxyl won't bind in these pockets; however less polar moieties such as halogens, ethers and ketals are tolerated. The pocket sizes were determined by first placing the serine residue at a fixed position in space. Secondly, the ester to be cleaved was placed next to this residue and finally the other substituents were arranged into the various pockets according to their polarity and size. The model was based on cubic space as it was also widely used at the time for other enzymes because of its simplicity.

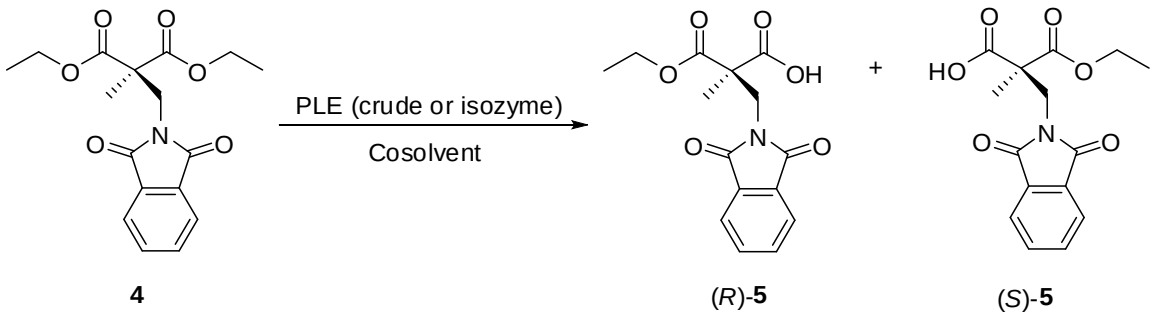
Table 1.1: Some diesters used in designing the PLE model

Entry	Substrate	Major product	% ee
1			>97
2			17
3			>97
4			34
5			73
6			88
7			6
8			95
9			93
10			>95

Shown above in Table 1.1 are a few of the diesters that Jones used to design the model. As can be seen there are numerous diester substrates in addition to malonates with varying affinities for the enzyme, which can be reflected in the enantiomeric excesses. Like in Björkling's observations, the methyl-containing substrates resulted in less than desirable ees. This is due to the loose fit of the methyl group in the H_S pocket (Entries 5 and 6). Only when the R_L group is substantially larger, do the methyl group-containing substrates produce more favourable ees, which is due to a tighter fit of the entire substrate into the active site (entry 8).

Masterson *et al.*[24] published a paper in 2012 on the cosolvent effect, which has been shown to cause changes in the enantioselective desymmetrization of disubstituted malonic esters with PLE. These experiments used several cosolvents: *i*-PrOH, *t*-BuOH, MeCN, dioxane, DMSO, DMF and THF at varying concentrations between 0-15% (v/v) in buffer (pH 7.4). PLE comes as a crude mixture but isolated pure isozymes are also available. Isozymes slightly differ in amino acid sequence but catalyze the same reaction. Six pure isozymes were tested as well as crude PLE in Masterson's experiments.

For a better understanding, Scheme 1.2 displays how malonic ester (**4**) is enantioselectivily desymmetrized to give a mixture of (*R*) and (*S*) half esters (**5**). Figure 1.3 shows the results of these reactions in a variety of solvents at different concentrations against the % ee of the products formed. There is no linear trend for the solvent concentration and ee observed. This is due to the different selectivities of the isozymes within the crude mixture of PLE in the various solvents.



Scheme 1.2

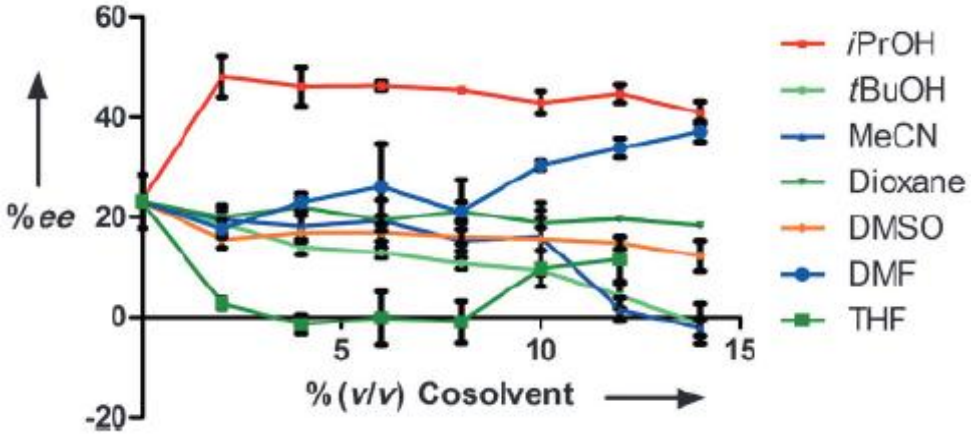
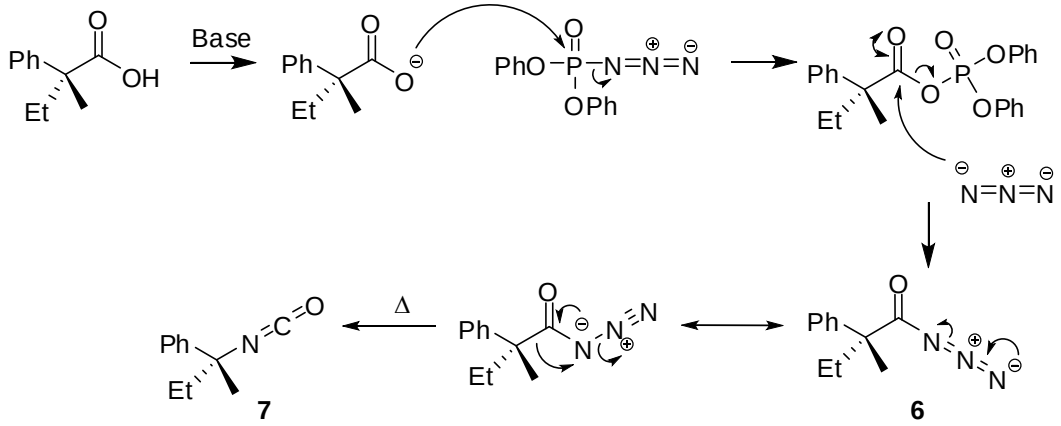


Figure 1.3: Crude PLE hydrolysis of 4 using a variety of non-nucleophilic cosolvents (reproduced from reference 24 with permission from the publisher)

Buffer used was sodium phosphate (0.1N, pH 7.4). Positive % ee values refer to (*R*)-preference.

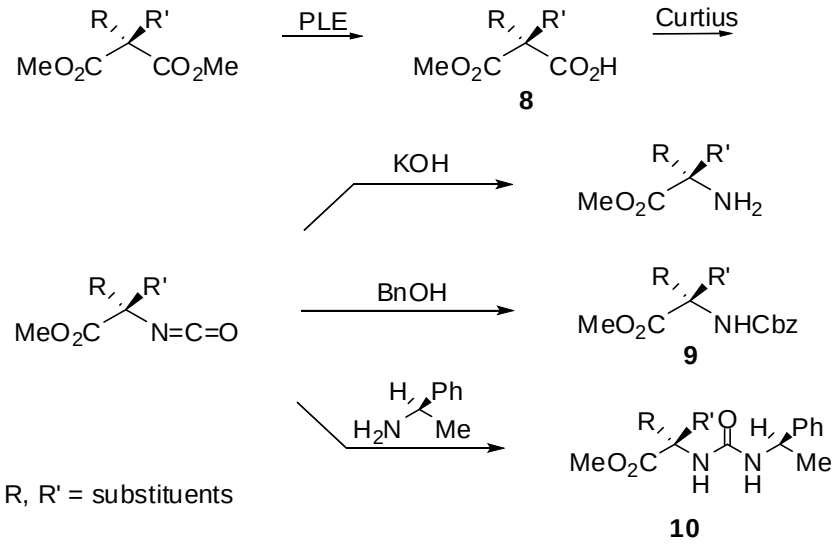
1.2.1.2 Sequential PLE desymmetrization/Curtius rearrangement reactions

The Curtius rearrangement, which was later modified by Shioiri *et al*[25] with the use of diphenylphosphoryl azide (DPPA), is the transformation of an acyl azide (**6**) into its corresponding isocyanate (**7**) (Scheme 1.3). The Curtius rearrangement is a transformation that is especially effective for hindered molecules and proceeds with retention of stereochemistry.[26-29]



Scheme 1.3

Our group investigated the use of sequential PLE desymmetrization/Curtius rearrangement reactions in combination for the synthesis of α-quaternary amino acid derivatives.[30] Drawing from the respective known methodology on PLE hydrolysis and Curtius rearrangements, a wide range of α-quaternary amino acids was synthesized from prochiral starting materials, often with high enantiomeric excesses and predictable absolute configurations (Scheme 1.4 and Table 1.2). It shows how disubstituted malonate esters can be first cleaved to half-esters (**8**) enantioselectivily and then converted into free amines, carbamate protected amines (**9**) or ureas (**10**). This methodology could be further applied to the synthesis of a broad variety of α-quaternary amine-containing natural products and biologically interesting molecules. Note in Table 1.2, as expected from Jones' and Björkling's work, the substrates with R' = Me display diminished ees unless the R group is of a substantial size.



Scheme 1.4

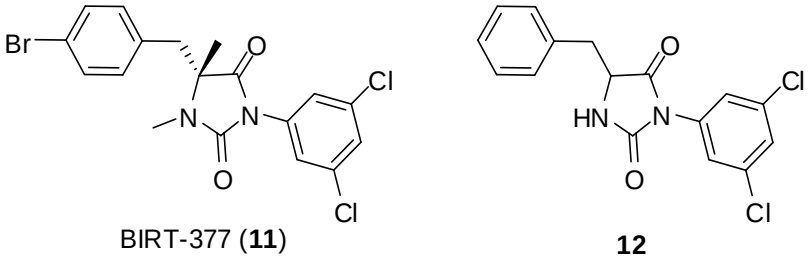
Table 1.2: Preparation of half-esters and α-quaternary amino acid derivatives[30]

		ee or de	
R	R'	half-ester **8**[a]	product **9** or **10**[b]
$CH_3(CH_2)_5$	Me	**8a** 67	**9a**
i-Pr	Me	**8b** 42	**9b** 37
Me	$HC{\equiv}CCH_2$	**8c** 63	**9c** 63
Cy	Me	**8d** >98	**10d** >98
$CyCH_2$	Et	**8e** >98	**10e** >98
Ph	Me	**8f** >98	**9f**
$PhCH_2$	Et	**8g**	**10g** 52
$PhCH_2$	*n*-Pr	**8h**	**10h** 79
$PhCH_2CH_2$	Me	**8i**	**10i** 80
$PhCH_2CH_2$	Et	**8j**	**10j** 93
$PhCH_2CH_2$	*n*-Pr	**8k**	**10k** 94
$PhCH_2CH_2$	*n*-Bu	**8l**	**10l** >98

[a]Enantiomeric excess (ee) was measured by NMR integration of salts formed with (*R*)-(+)-*α*-methylbenzylamine. [b]Enantiomeric excesses of **9b** and **9c** were measured by HPLC on a chiral column. Diastereomeric excesses (de) of **10** were measured by NMR integration of the crude reaction mixture.

1.2.2 BIRT-377 Background

The hydantoin BIRT-377 (**11**) is a member of a class of small molecules that are potent anti-inflammatory agents. BIRT-377 was identified as an early lead compound by Boehringer-Ingelheim Inc. after further optimization of compound **12**, which had been identified earlier as possessing the desired type of bioactivity.[31] Its binding mode was elucidated from X-ray structural data. These compounds inhibit interactions between leukocyte function antigen 1 (LFA-1) with the binding ligand, intercelluar adhesion molecule 1 (ICAM-1) via non-covalent binding at an allosteric site on the CD11a alpha protein chain.[32,33] People who cannot express these leukocyte integrins (transmembrane receptors) tend to have recurring infections because they are not capable of mounting an effective immune response.[34] On the other extreme, in patients with overactive immune responses and inflammatory issues, the inhibition of LFA-1 mediated cell adhesion presents a therapy for these disease states.[35,36] Antagonists such as BIRT-377 could potentially be used for the treatment of allograft rejections and autoimmune illnesses such as Crohn's disease. BIRT-377 is orally bioactive and inhibits leukocyte activity *in vitro* and *in vivo* in functional assays.[37]



BIRT-377 (**11**) **12**

Figure 1.4: Hydantoin based potential anti-inflammatory agents

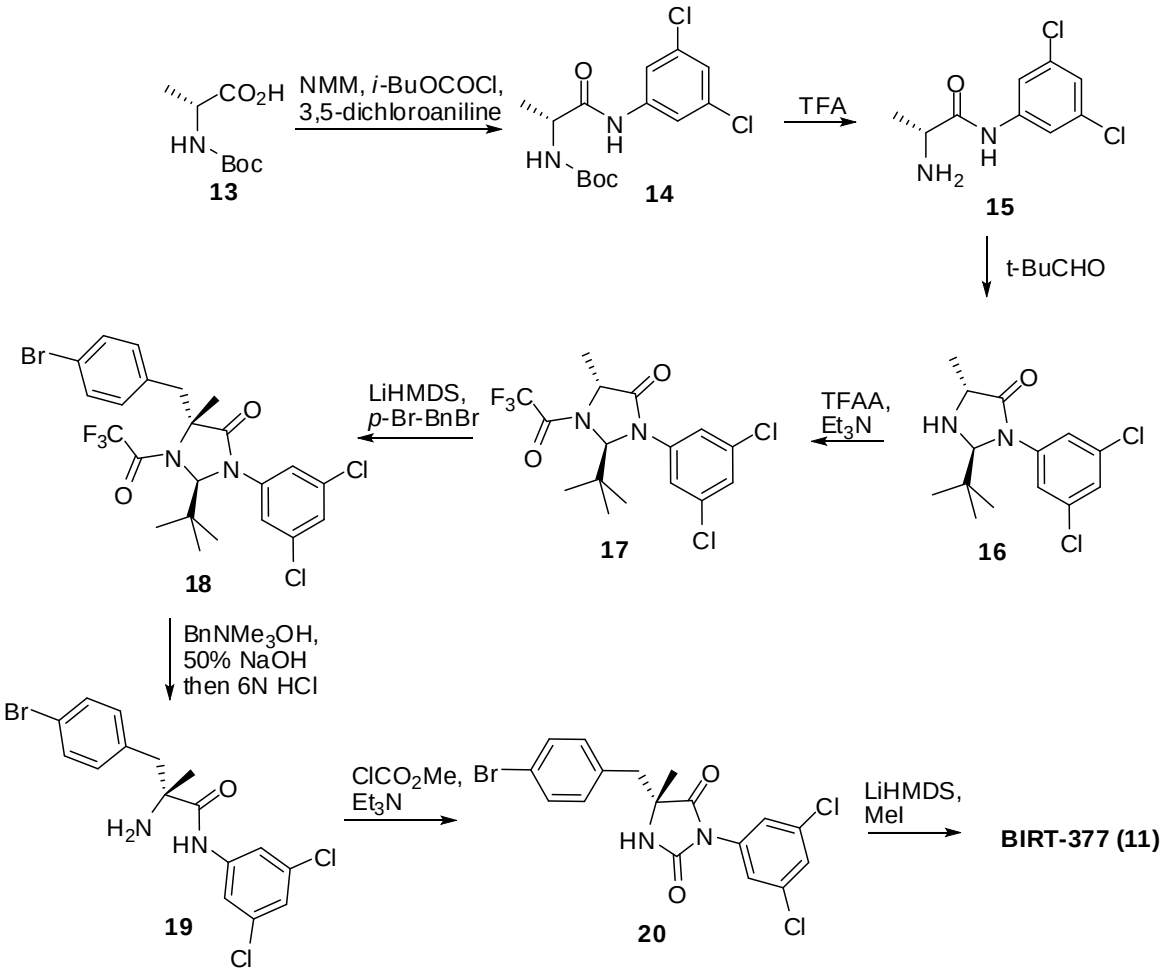
1.2.3 Previous syntheses

Due to the requirement for enantiopure compound testing by governing administrative bodies, the need for highly enantioselective syntheses of these drugs is crucial. In the case of BIRT-377, it contains an α-quaternary amino acid moiety within its hydantoin ring. These compounds are particularly challenging to synthesize because installation of these centers, as well as reactions attempted in their proximity, are impeded by steric constraints.

1.2.3.1 Yee's synthesis

The first synthesis of BIRT-377 was carried out by Nathan Yee at Boehringer-Ingelheim Inc. in 2000.[38] The synthesis began with the amide coupling of commercially available *D*-*N*-Boc-alanine (**13**) with 3,5-dichloroaniline (Scheme 1.5). This was followed with deprotection of amide **14** under acidic conditions to afford primary amine **15**. Seebach's "Self-Regeneration of Stereocenters" protocol[39] was then employed to install the chiral quaternary center. First, amino amide **15** was treated with pivalaldehyde. The resulting crystalline solid was identified as the desired *trans* imidazolidinone, produced as a single diastereomer. Key intermediate **16** was then protected with triflouroacetic anhydride to give diamide **17**, which was alkylated on the opposite face to the *t*-butyl group to afford the quaternary center on compound **18**. Hydrolysis under harsh conditions produced amino amide **19**, which was then treated with methyl chloroformate to give hydantoin **20**. This was then methylated to afford BIRT-377 (**11**) in 43% overall yield with an optical purity >99.9% ee. Yee *et al.* later published two papers on the production of BIRT-377 on a multi-kilogram scale.[40,41] This

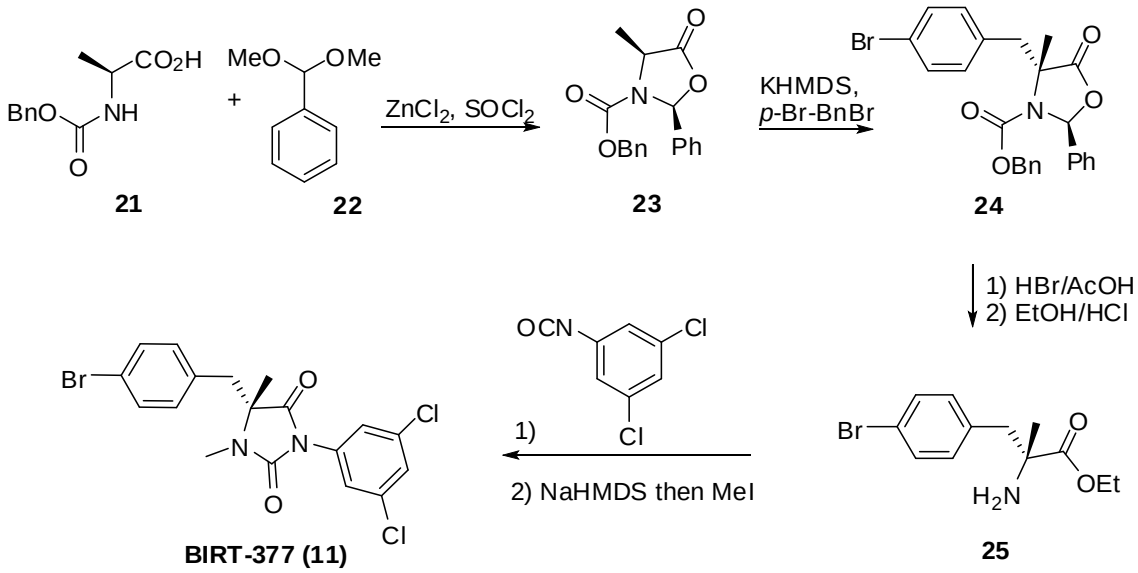
remains the most practical and cost effective multi-kilogram route to date, albeit under harsh conditions.



Scheme 1.5

1.2.3.2 Kapadia's synthesis

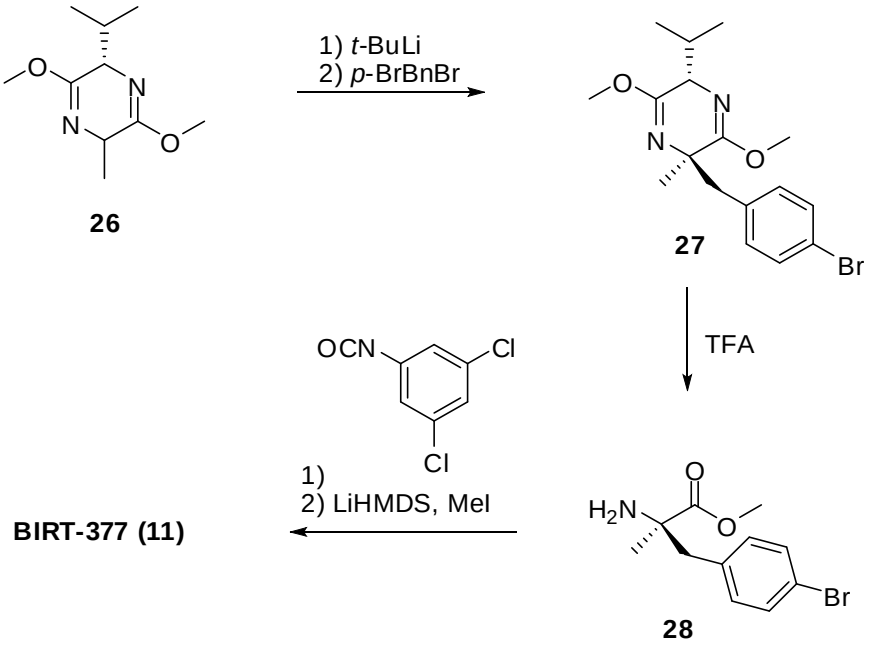
In late 2000, Kapadia *et al.*, also from Boehringer-Ingelheim Inc., published an improved synthesis of BIRT-377.[42] This synthesis also involved Seebach's protocol and was carried out on a multigram scale. Amino acid **21** and acetal **22** in the presence of thionyl chloride and zinc (II) chloride formed oxazolidinone **23** in a 8:1 *cis*/*trans* ratio (Scheme 1.6). This was then alkylated to give intermediate **24**. Hydrolysis of the crude product led to amino ester **25**, which underwent sequential reaction with 3,5-dichlorophenyl isocyanate and *N*-methylation to afford BIRT-377 (**11**) in 41% overall yield and >99% enantiopurity. Napolitano *et al.*[43] also published a similar synthesis in 2001. This approach is very similar to that of Yee's but not as efficient on a large scale.



Scheme 1.6

1.2.3.3 Magriotis' synthesis

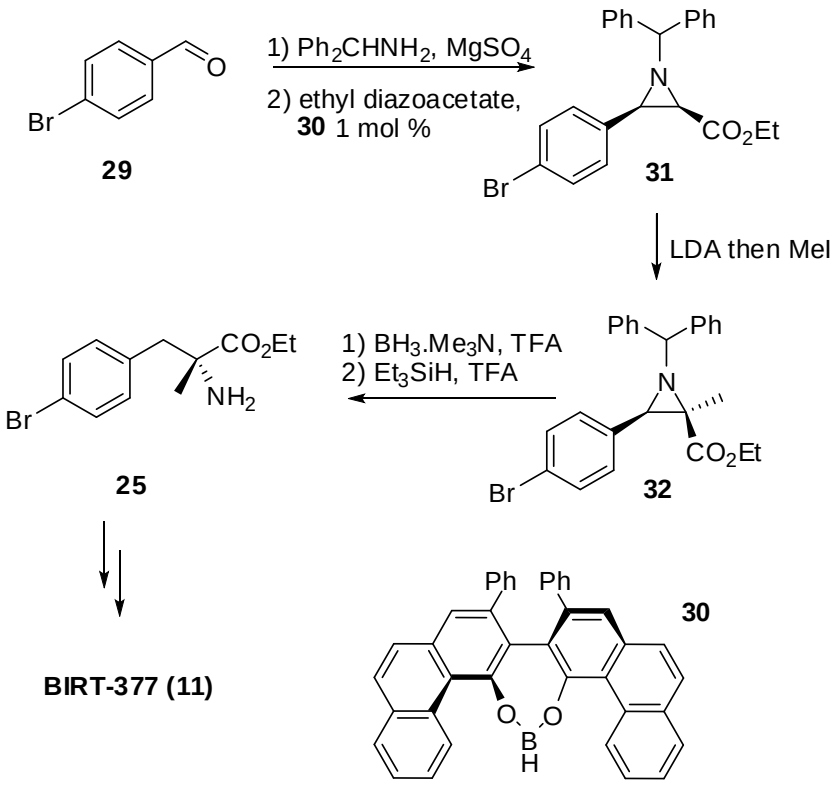
In 2006, Magriotis *et al.*[44] synthesized BIRT-377 via a very short and efficient route by employing Schöllkopf's methodology on creating quaternary α-amino acids.[45] Starting with alkylation of chiral auxiliary **26**, which was first synthesized by Schöllkopf *et al.*,[45] the quaternary center in compound **27** was installed with a diastereoselectivity >95% (Scheme 1.7). Removal of the auxiliary under acidic conditions afforded amino ester **28**, which was carried through the same steps as in Kapadia's synthesis to obtain BIRT-377 in 77% yield. This route is very short although only a small number of vendors sell starting material **26**, which is also quite expensive.



Scheme 1.7

1.2.3.4 Wulff's synthesis

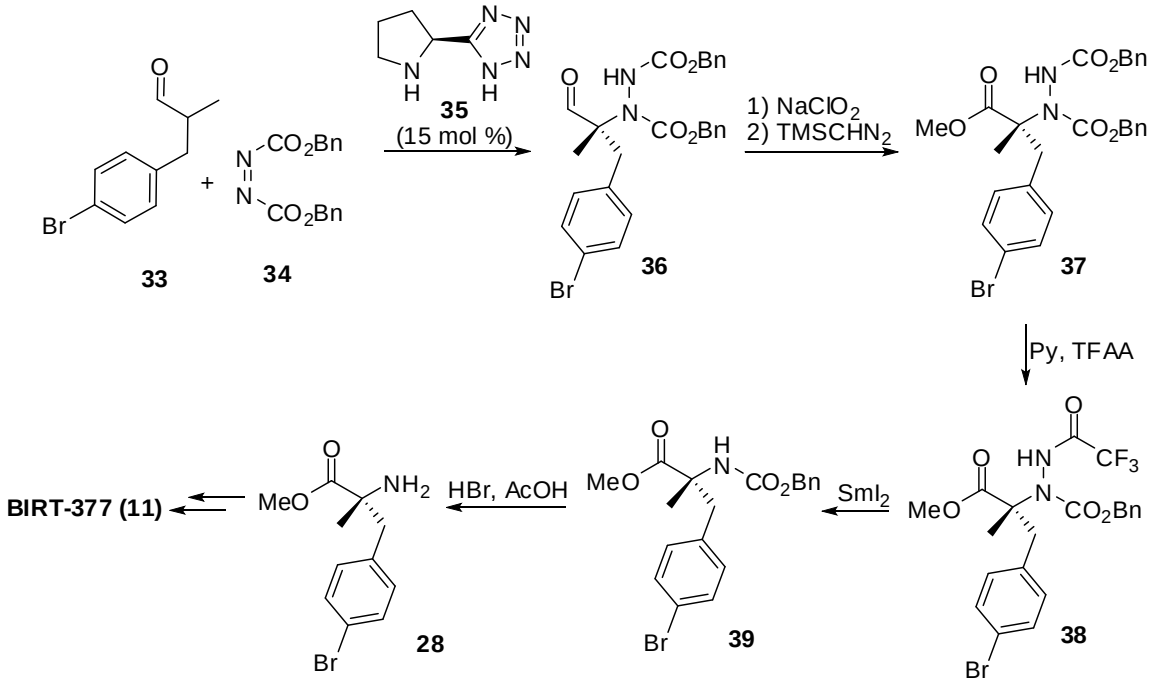
Wulff *et al.*[46] synthesized BIRT-377 using a boron-based chiral Lewis acid **30**, which is made from a commercially available ligand, as part of their methodology paper on diastereoselective aziridine alkylation. Aldehyde **29** was converted to an imine species before the cyclization reaction was carried out to form aziridine **31** with >99% ee (Scheme 1.8). This was then methylated from the less hindered face to afford α-quaternary amine **32** with a 99:1 diastereomeric ratio. Reductive ring-opening and amine deprotection produced amino ester **25**, which was carried down the same route as shown previously, in Scheme 1.6, to give BIRT-377 in 42% overall yield with an enantiomeric excess of >99%. While the route provided and excellent ee, the formation of explosive diazo-intermediates, the use of pyrophoric triethylsilane and the reliance upon the costly catalyst **30** create an impediment to scale-up.



Scheme 1.8

1.2.3.5 Barbas' synthesis

Barbas *et al.*,[47] published a synthesis in 2004 which used a proline derivative-catalyzed amination to install the α-quaternary amine. The tetrazole catalyst **35** was used to form the enamine intermediate of aldehyde **33**, which underwent reaction with diazene **34** (Scheme 1.9). The transition state (Figure 1.5) allows for a hydrogen bond-coordinated approach of the diazene from the top face.[48,49]



Scheme 1.9

Hydrazine **36**, which was obtained in >99% ee, was then oxidized to afford ester **37**. The protected hydrazine **38** was reductively cleaved with samarium (II) iodide to give carbamate **39**, which was deprotected to produce amino ester **28**. BIRT-377 was obtained in 47% yield over 11 steps with an optical purity of >99%. This synthesis, although long, contains some very interesting chemistry to obtain BIRT-377 in a high ee with the use of a relatively cheap organocatalyst **35**.

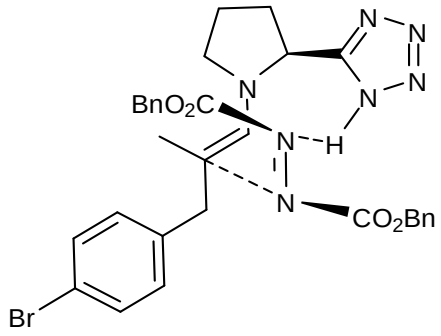
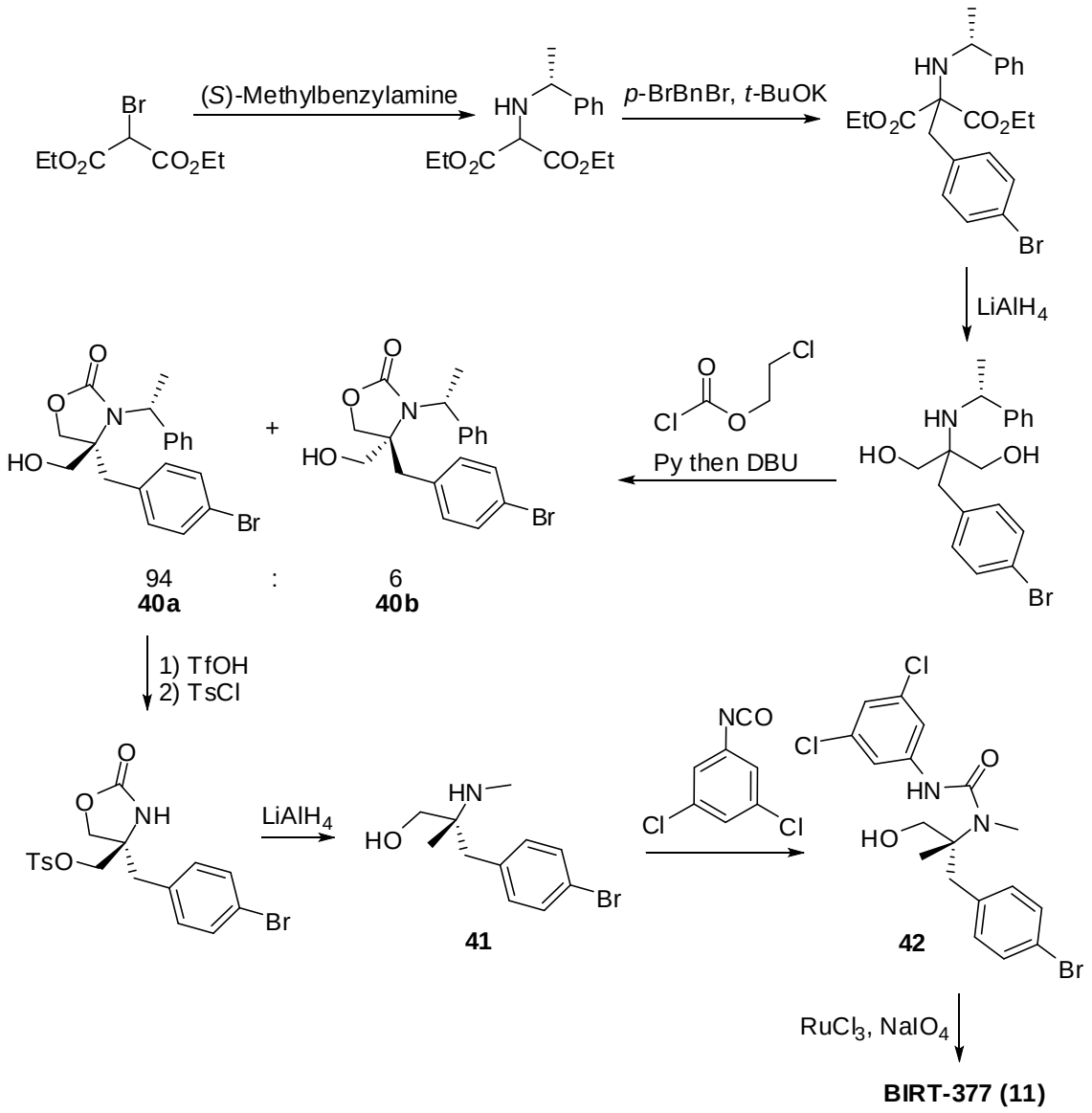


Figure 1.5: Transition state for organocatalytic amination

1.2.3.6 Sugiyama's synthesis

In 2012 Sugiyama *et al.*,[50] published a synthesis of BIRT-377 which employed a non-enzymatic diastereoselective asymmetric desymmetrization reaction. Installation of the pseudo auxiliary, (*S*)-methylbenzylamine was used to give the diastereomeric ratio of 94:6 in oxazolidinone **40** (Scheme 1.10). Cleavage of part of the chiral auxiliary and several functional group conversions produced secondary amine **41**. This underwent reaction with the 3,5-dichlorophenyl isocyanate to install the urea moiety in **42**, which was followed by a one pot oxidation and ring closure to afford BIRT-377 in a 6% overall yield. The levorotatory specific rotation reported in this paper is the opposite to that obtained via the other syntheses. Furthermore, Sugiyama *et al*. cite a literature rotation from a paper by Yee *et al*. that they claim is comparable and of the same sign to their own. However, scrutiny of this paper reveals that no specific rotation is reported, while in other papers by this group the rotation is given as dextrorotatory.[41]

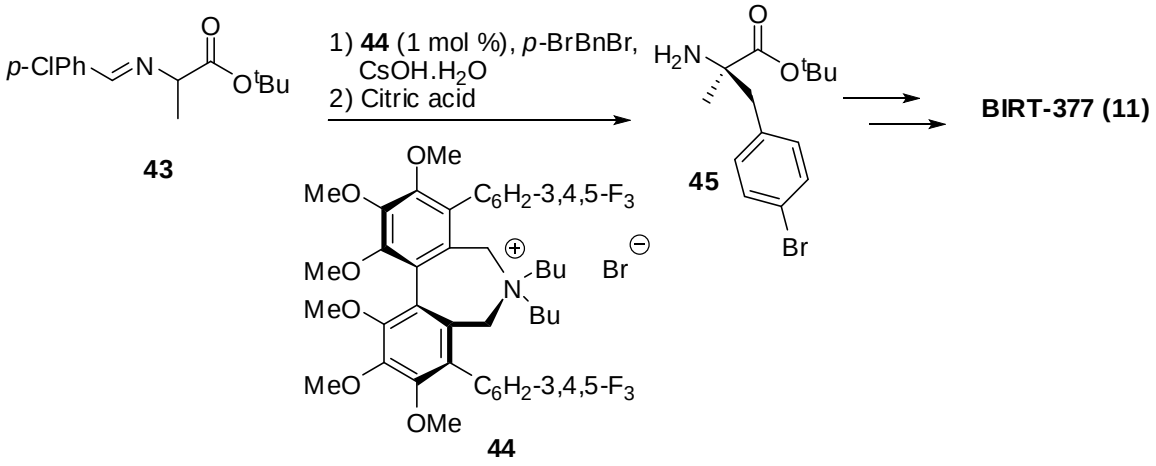


Scheme 1.10

1.2.3.7 Maruoka's synthesis

Maruoka's synthesis employed the use of the chiral phase transfer catalyst **44** to install the quaternary center in BIRT-377.[51] Imino ester **43** was deprotonated in the presence of chiral ammonium species **44** and alkylated to form the chiral center in 97% ee (Scheme 1.11). This was then acidified to afford amino ester **45**, which then followed

the same route as Kapadia's synthesis to reach the final product. The use of the *t*-butyl group on ester **43** improved the enantioselectivity of the reaction by adding steric bulk to the ester substituent. BIRT-377 was obtained in a very high 66% overall yield; however the downfall of this synthesis is in the catalyst, which is not commercially available and takes several steps to prepare.



Scheme 1.11

1.2.4 Objectives

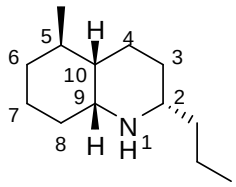
The objectives of the first part of this thesis are to showcase and validate the enzymatic desymmetrization/Curtius rearrangement combination, by synthesizing both BIRT-377 and (*ent*)-BIRT-377 stereodivergently, which has not been previously reported. This methodology could then be used as a general approach to the enantioselective synthesis of unsymmetrical hydantoin compounds. Furthermore, it could be used for a stereodivergent variation to obtain both enantiomers, which could potentially have drastically different medicinal properties. The optimization of the enantioselective enzymatic hydrolysis step, and of other steps as required, was also planned.

1.3 Gephyrotoxin

1.3.1 Dendrobatid alkaloids

Many natural products have biological activity that can be used for the treatment of a myriad of diseases and disorders. Many of the drugs on the market today were isolated from natural materials or are derivatives thereof. Alkaloids are found in a large number of plant and animal species and comprise a huge group of biogenetically diverse families of organic nitrogenous bases. Dendrobatid alkaloids are produced by several species of neotropical frogs of the genera *Phyllobates* and *Dendrobates*,[52-56] the Madagascan frogs *Mantella aurantiaca* and *Mantella madagascariensis*,[57,58] the Australian frog *Pseudophryne semimarmorata*[57,59] as well as the Brazilian toad *Melanophyryniscus moreirae*.[57] Over 800 alkaloids have been detected in skin extracts of the frog family *Dendrobatidae.*[60] In some cases, they have been acquired through their food source, which is usually based on arthropods.[61] The decahydroquinoline (DHQ) alkaloids are a small fraction of frog alkaloids. They have a bicyclic structure, as exemplified by pumiliotoxin C (**46**). A sizable portion of these decahydroquinolines are 2,5-disubstituted and have either *cis* or *trans* ring fusions.[61] Pumiliotoxin C is a potent neurotoxin that acts as a non-competitive blocker for acetylcholine receptor channels and therefore has attracted considerable attention from a pharmaceutical standpoint.[62-68] For the characterization of pumiliotoxin C, 2540 frogs from the species *Dendrobatides pumilio*, or more commonly known as the blue jean frog, were harvested. It has also been extracted from *Solenopsis azteca* ants in Central America. The compound's detection in ants strengthened a dietary hypothesis for the origin of the approximately 30 DHQs that have been detected in extracts of frog skin. Taking the low natural abundance of

(-)-pumiliotoxin C and other derivative alkaloids into consideration, a general synthetic means to obtain these alkaloids is highly sought to enable further biological testing without devastating frog populations.

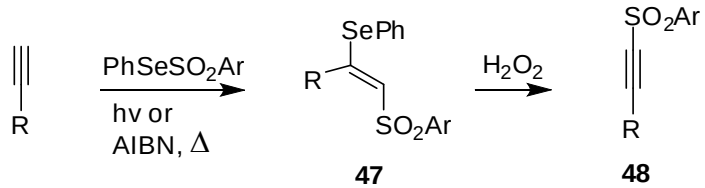


46

Figure 1.6: (-)-Pumiliotoxin C with atom numbering for decahydroquinolines

1.3.2 Acetylenic sulfone chemistry for the synthesis of dendrobatid and similar alkaloids

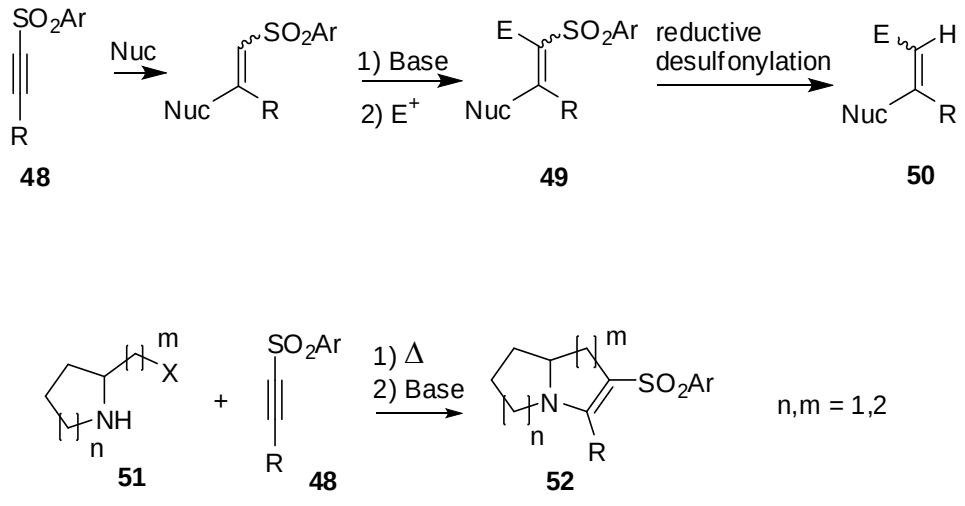
The employment of sequential conjugate addition and cyclization using an acetylenic sulfone could potentially be used to access decahydroquinoline cores. Cyclizations and cycloadditions of *α,β*-unsaturated sulfones have been investigated by our group for several years. There have been several reviews on the reactivity of acetylenic and allenic sulfones.[69-71] Shown in Scheme 1.12 is our group's methodology for the synthesis of acetylenic sulfones.[72-74]



47 **48**

Scheme 1.12

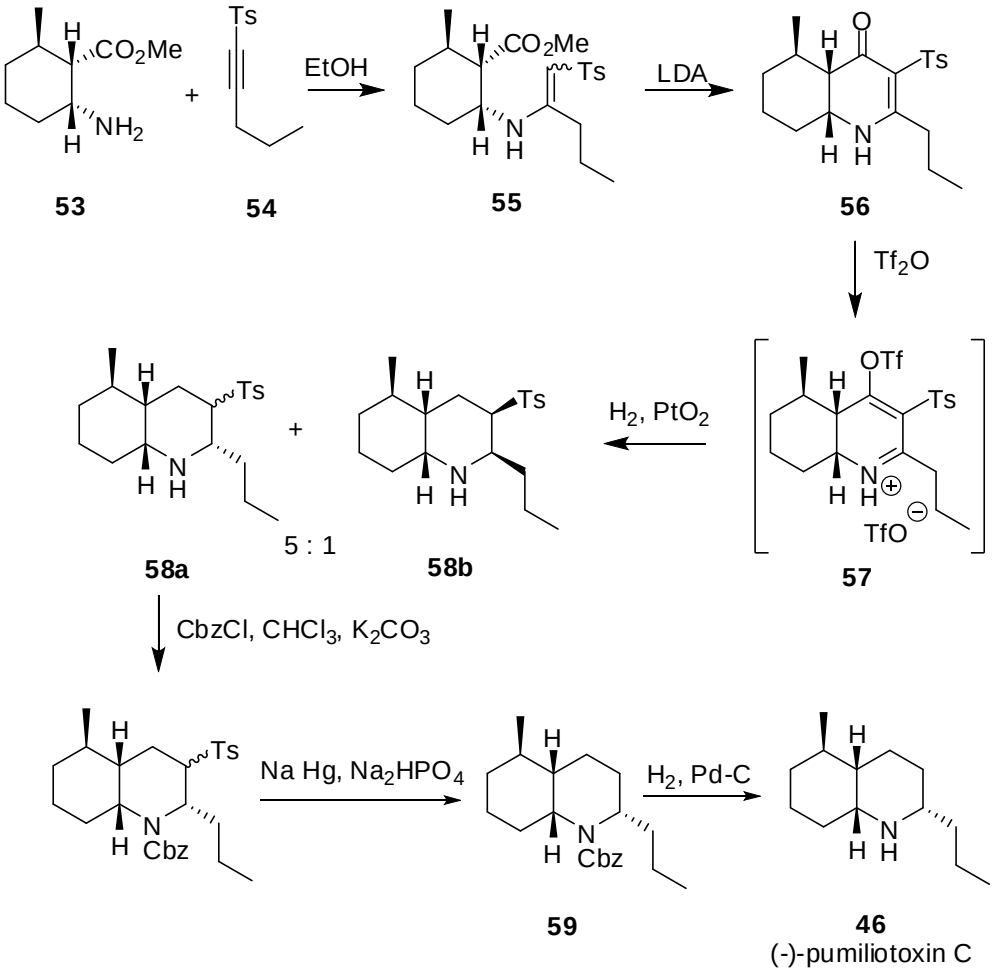
Compounds **48** can be obtained by the free-radical addition of phenyl arylselenosulfonates to alkynes. This can be initiated by light or by heat in the presence of a radical initiator (AIBN).[75] The *syn* configuration between the vinyl hydrogen and the phenylseleno group in compound **47** then allows for production of acetylenic sulfones **48** via selenoxide elimination. Compound **48** can be used as a Michael acceptor that can readily react with nucleophiles. This is followed by an alkylation or acylation, brought about by the sulfone's ability to stabilize α or γ carbanions, to afford the substituted olefin **49** and finally **50** by a reductive cleavage[76] of the sulfone (Scheme 1.13). Providing the amine nucleophile also contains an alkylating or acylating moiety, as in **51**, the cyclization can then be used to form a ring system with varying sizes where n,m = 1,2, as in **52**.



Scheme 1.13

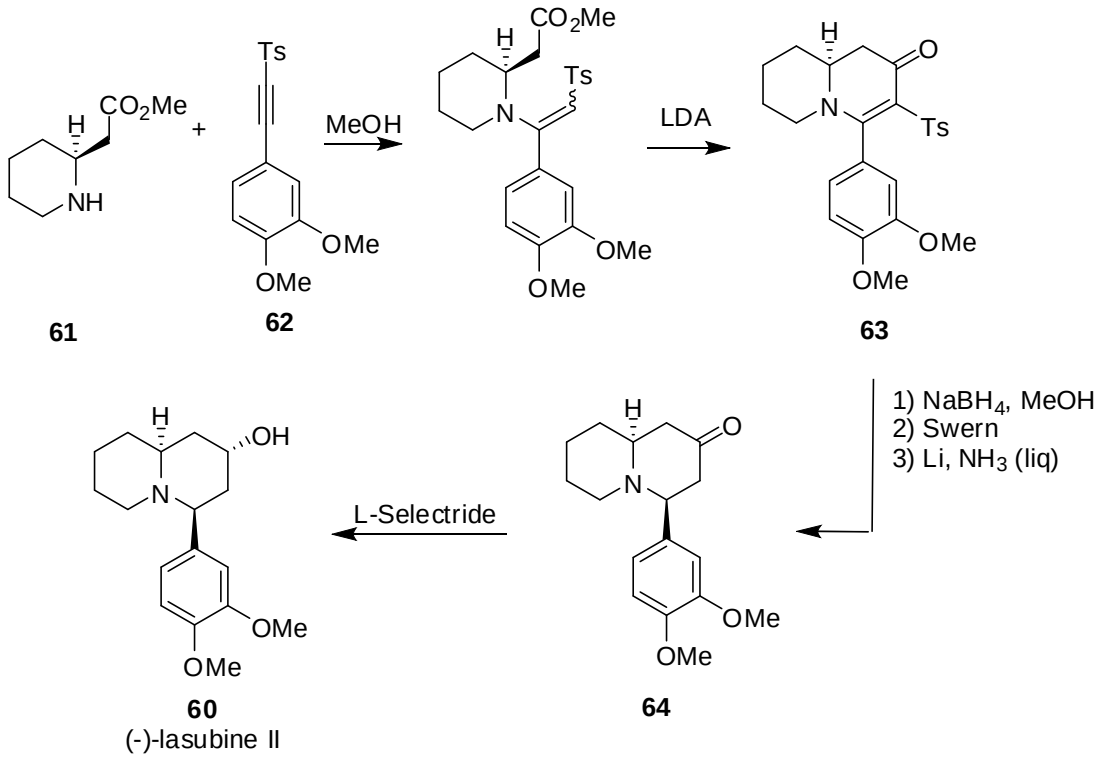
Our group has previously used this methodology to synthesize various alkaloids of biological interest, including (-)-pumiliotoxin C[77] (Scheme 1.14), (-)-lasubine II[78] (Scheme 1.15), which contains a quinolizidine ring, and many others.[79-83]

For example, amino ester **53**, which was synthesized via a Diels-Alder reaction and enzymatic desymmetrization, underwent reaction with acetylenic sulfone **54** by Michael addition to afford product **55**. This was then deprotonated and cyclized to afford heterocycle **56**. Compound **56** could be obtained in one-pot; however, acetylenic sulfones are prone to polymerization. Reduction of the double bond proved difficult due to the required *endo* orientation of the propyl chain. This was achieved by trapping of the enaminone moiety as triflate salt **57**, followed by hydrogenation and deoxygenation to give **58** in favour of the correct diastereomer. Cleavage of the sulfone group with sodium amalgam afforded decahydroquinoline **59**, which was *N*-deprotected to give (-)-pumiliotoxin C.



Scheme 1.14

(-)-Lasubine II (**60**) was obtained through the same use of acetylenic sulfone chemistry using amino ester **61** and acetylenic sulfone **62** to give heterocycle **63** (Scheme 1.15). Stereoselective reduction to obtain the correct orientation for the disubstituted phenyl group, subsequent Swern oxidation and reductive desulfonylation gave quinolizidine **64**. L-Selectride was then used to obtain the final product **60**.

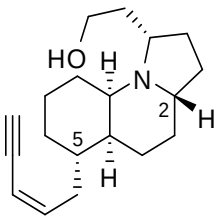


Scheme 1.15

1.3.3 Gephyrotoxin background

Gephyrotoxin (**65**) (Figure 1.7) has a more complex structure than the alkaloids previously synthesized in our group; however, it still contains the same *cis*-fused decahydroquinoline core with substitutions at the C-2 and C-5 positions. This makes it the next logical step in the advancement of our acetylenic sulfone chemistry for applications to the synthesis of nitrogen-containing heterocycles of biological interest. Gephyrotoxin was first isolated by Daly and coworkers in 1977. It is a naturally occurring product which is secreted by the harlequin poison frog, *Dendrobates histrionicus*.[84] Gephyrotoxin is a member of the class of compounds known as histrionicotoxins. These toxins are less powerful compared to many other alkaloids found

in poison frogs, such as batrachotoxins. Muscarinic antagonism was first reported for gephyrotoxin, the potency of which was inconclusive due to the small amount of alkaloid that was obtained from frog specimens.[54,55,84,85] Gephyrotoxin was later found to be also neurologically active.[86]



65

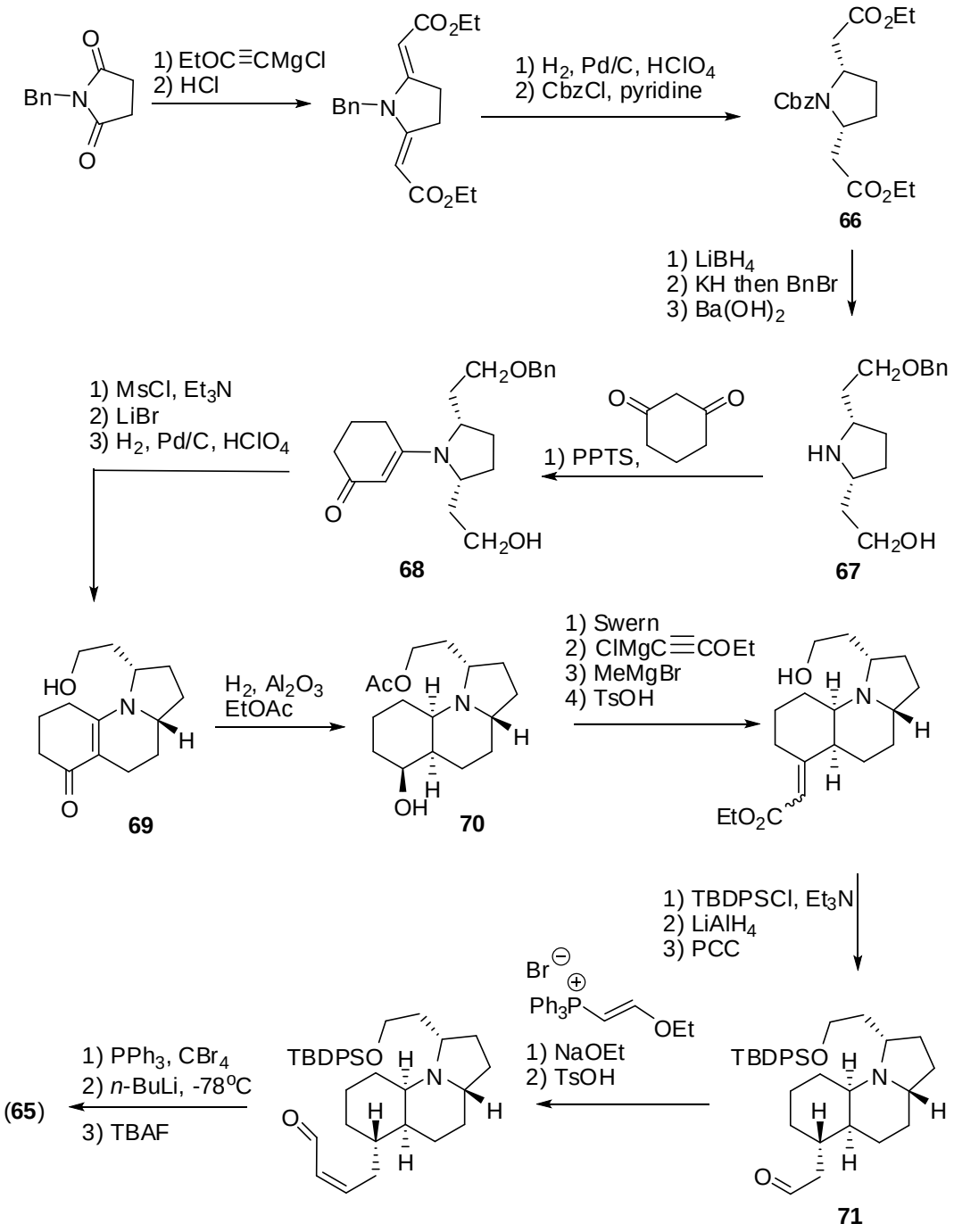
Figure 1.7: Gephyrotoxin

1.3.4 Previous syntheses

1.3.4.1 Kishi's synthesis

The first racemic total synthesis of gephyrotoxin was published by Kishi *et al.*,[87] in 1980 from succinimide in <4% yield. Their synthesis involved the use of hydrogenation to install the relative *cis* stereochemistry in diester **66** (Scheme 1.16). The ester groups were then reduced, and the resulting diol was mono-benzylated to give key intermediate **67**. Under acidic conditions with 1,3-cyclohexanedione enaminone **68** was formed. Cyclization to the carbonyl α-position led to core structure **69**. Various hydrogenation approaches were attempted and aluminum oxide in ethyl acetate successfully afforded acyl-protected key intermediate **70**. A similar approach to that at the start of the synthesis was used to install the unsaturated ester, which was reduced with lithium aluminum hydride and sequentially oxidised with PCC to give the aldehyde chain

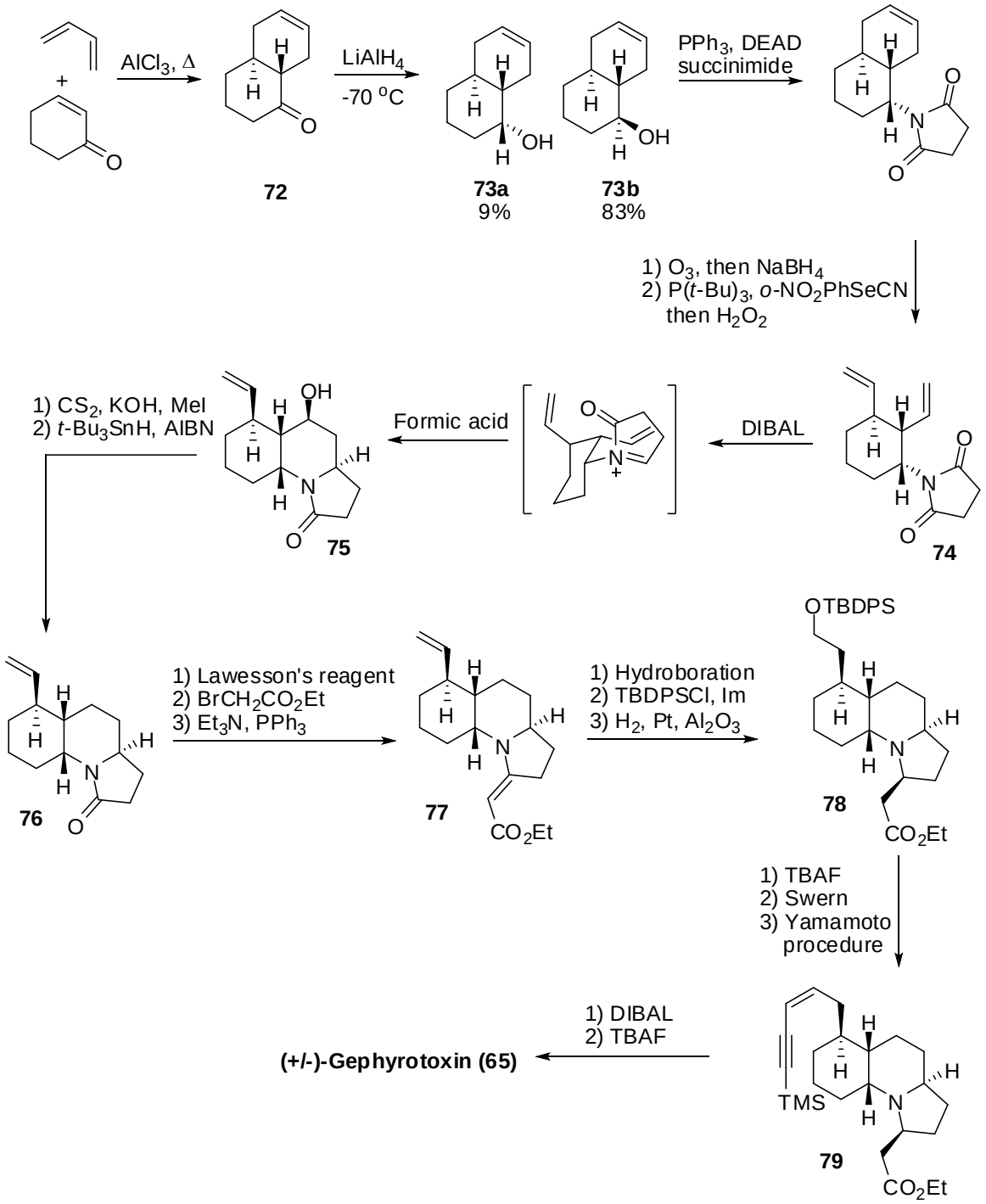
in the more thermodynamic *exo* position on decahydroquinoline **71**. Wittig and Corey-Fuchs[88] reactions installed the enyne side-chain and cleavage of the silyl group afforded gephyrotoxin in 24 steps. A year later Kishi *et al*. published an enantioselective synthesis using commercially available *L*-pyroglutamic acid to prepare **67**, followed by the same route for the remainder of the synthesis.[89]


1) EtOC≡CMgCl
2) HCl
1) H2, Pd/C, HClO4
2) CbzCl, pyridine
66
1) LiBH4
2) KH then BnBr
3) Ba(OH)2
1) MsCl, Et3N
2) LiBr
3) H2, Pd/C, HClO4
1) PPTS,
68
67
H2, Al2O3
EtOAc
69
70
1) Swern
2) ClMgC≡COEt
3) MeMgBr
4) TsOH
1) TBDPSCl, Et3N
2) LiAlH4
3) PCC
1) NaOEt
2) TsOH
1) PPh3, CBr4
2) n-BuLi, -78°C
3) TBAF
(65)
71

Scheme 1.16

1.3.4.2 Hart's synthesis

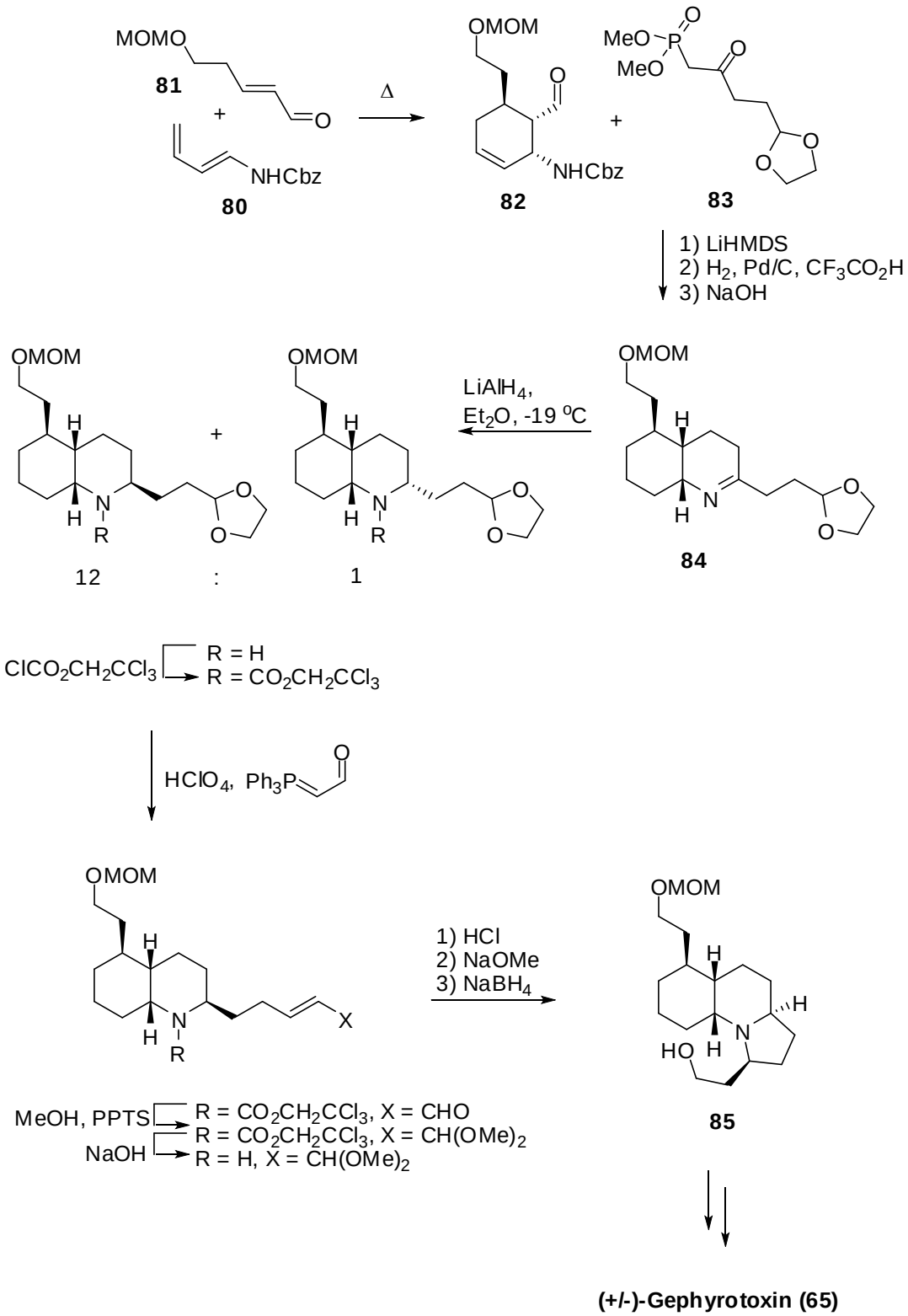
Hart *et al.*,[90] published a racemic synthesis of gephyrotoxin in 1982. Ketone **72** was synthesized via a Diels-Alder reaction (Scheme 1.17). Alcohol **73b** was favoured in the reduction of **72** with lithium aluminum hydride due to the preferential axial approach of the hydride at -70 °C. After several routine steps, succinimide **74** was reduced to form an *N*-acyliminium intermediate, which in the presence of formic acid cyclized to form the gephyrotoxin backbone **75** with the correct stereochemistry. The unwanted alcohol was then cleaved using Barton-McCombie deoxygenation to afford key intermediate **76**.[91] This was then treated with Lawesson's reagent and converted to unsaturated ester **77** via the corresponding thiolactam.[92] After hydroboration of the vinyl group the trisubstituted olefin underwent hydrogenation to give ester **78** diastereoselectively. It is noteworthy that the selectivity was diminished without the prior installation of the bulky silyl group. Deprotection, oxidation and Yamamoto's procedure[93] delivered protected enyne **79**. Reduction and deprotection finally afforded gephyrotoxin in 23 steps.


AlCl3, Δ
LiAlH4
-70 °C
PPh3, DEAD
succinimide
72
73a
9%
73b
83%
1) O3, then NaBH4
2) P(t-Bu)3, o-NO2PhSeCN
then H2O2
74
DIBAL
Formic acid
75
1) CS2, KOH, MeI
2) t-Bu3SnH, AIBN
76
1) Lawesson's reagent
2) BrCH2CO2Et
3) Et3N, PPh3
77
1) Hydroboration
2) TBDPSCl, Im
3) H2, Pt, Al2O3
78
1) TBAF
2) Swern
3) Yamamoto
procedure
79
1) DIBAL
2) TBAF
(+/-)-Gephyrotoxin (65)

Scheme 1.17

1.3.4.3 Overman's synthesis

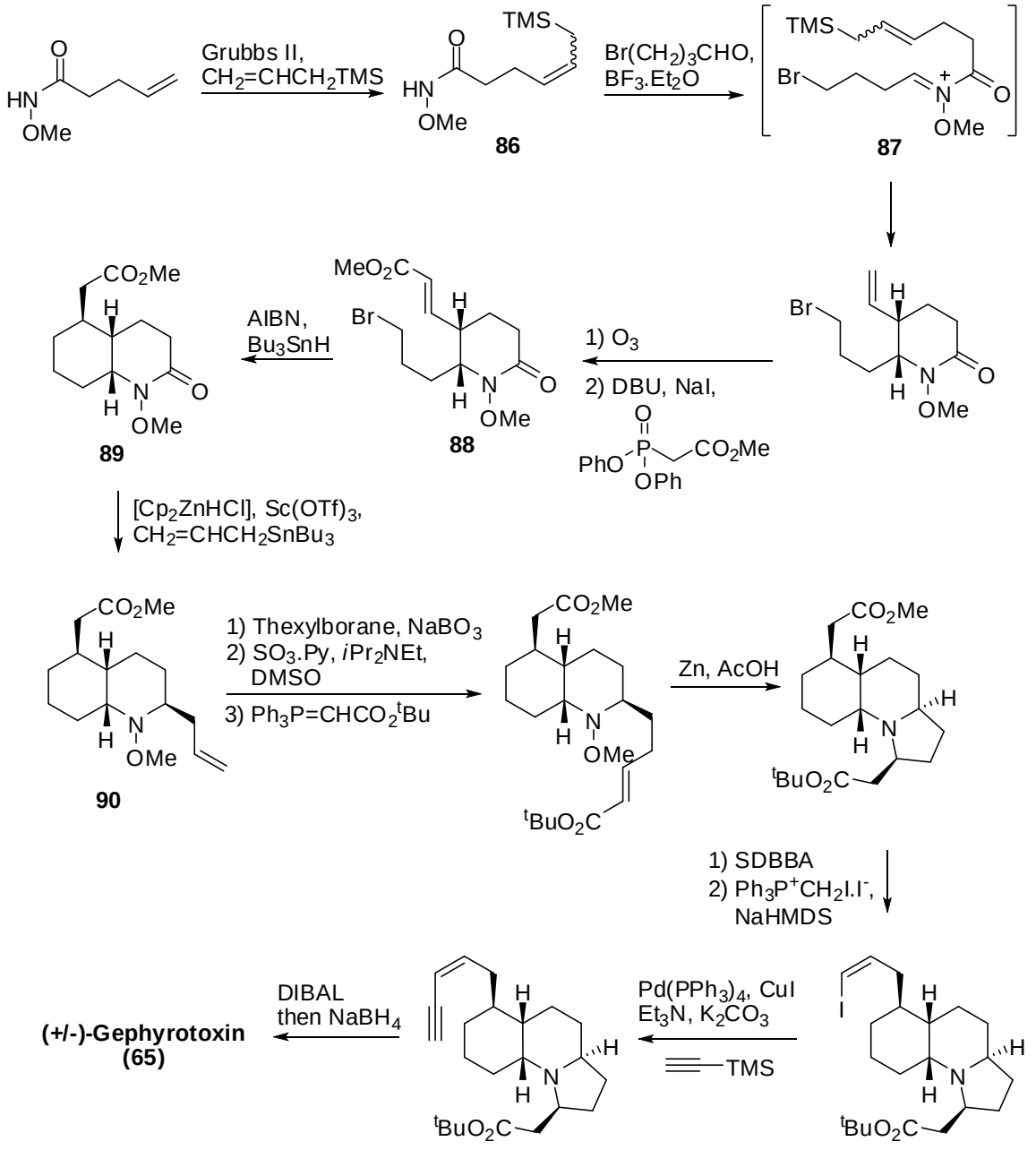
Overman *et al.*'s[94] racemic synthesis of gephyrotoxin used a Diels-Alder reaction with diene **80**[95] and unsaturated aldehyde **81** to install three contiguous stereocenters in aldehyde **82** in a predictable fashion (Scheme 1.18).[96] This then underwent a Horner-Wadsworth-Emmons olefination reaction. The subsequent hydrogenation was carried out under aqueous acidic conditions, in order to deprotect the amine group as well as protonate it. This was done to suppress an intramolecular condensation side reaction of the nitrogen and ketone groups which would eventually result in imine formation, hydrogenation of which would give an undesired epimer. Compound **84** underwent very specific reduction conditions to afford the correct decahydroquinoline diastereomer in an acceptable ratio. Modification of the chain at the C-2 position concluded with a Michael addition and reduction to install the third ring of intermediate **85**. The enyne side chain at the C-5 position was finally installed to afford the final product **65** using a method developed by Corey and Rücker.[97] The total synthesis was accomplished in 15 steps and 6.5% overall yield.


MOMO
81
+
O
NHCbz
80
Δ
OMOM
O
NHCbz
82
+
MeO
P
O
MeO
O
O
O
83
1) LiHMDS
2) H2, Pd/C, CF3CO2H
3) NaOH
OMOM
H
+
N
H
R
O
O
12
:
OMOM
H
N
H
R
O
O
1
LiAlH4,
Et2O, -19 °C
OMOM
H
N
H
O
O
84
ClCO2CH2CCl3
R = H
R = CO2CH2CCl3
HClO4, Ph3P
O
OMOM
H
N
H
R
X
1) HCl
2) NaOMe
3) NaBH4
OMOM
H
H
N
HO
H
85
MeOH, PPTS
R = CO2CH2CCl3, X = CHO
R = CO2CH2CCl3, X = CH(OMe)2
NaOH
R = H, X = CH(OMe)2
(+/-)-Gephyrotoxin (65)

Scheme 1.18

1.3.4.4 Chida's synthesis

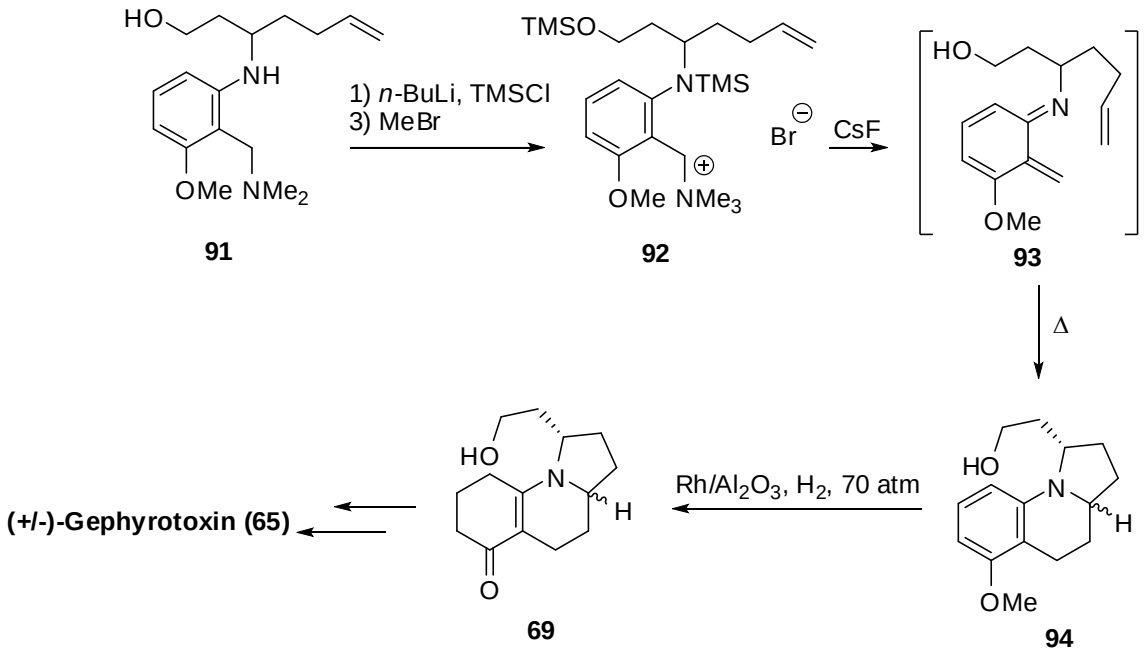
The most recent total synthesis of gephyrotoxin was carried out by Chida *et al*.[98] in 2014. The key reactions in this synthesis begin with iminium ion formation from amide **86**, which was stabilized by electron-donation from the methoxy group (Scheme 1.19). Iminium ion **87** then underwent a cyclization reaction assisted by the silyl group's *β*-stabilizing effect. After ozonolysis and olefination, ester **88** afforded *exo* product **89** in a 4.6:1 diastereomeric ratio through radical cyclization. This was followed by a zirconium-based reductive nucleophilic addition, which was selective for the amide carbonyl group, to produce key intermediate **90**.[99,100] This reaction was also diastereoselective as it oriented the allyl group at the C-2 position on the more thermodynamically stable convex face. Selective reduction of the methyl ester with sodium diisobutyl-*t*-butoxyaluminum hydride (SDBBA) and several more steps afforded gephyrotoxin in 9% total yield.


Grubbs II,
CH2=CHCH2TMS
Br(CH2)3CHO,
BF3.Et2O
86
87
1) O3
2) DBU, NaI,
AIBN,
Bu3SnH
88
89
[Cp2ZnHCl], Sc(OTf)3,
CH2=CHCH2SnBu3
90
1) Thexylborane, NaBO3
2) SO3.Py, iPr2NEt,
DMSO
3) Ph3P=CHCO2tBu
Zn, AcOH
1) SDBBA
2) Ph3P+CH2I.I-,
NaHMDS
Pd(PPh3)4, CuI
Et3N, K2CO3
TMS
DIBAL
then NaBH4
(+/-)-Gephyrotoxin
(65)

Scheme 1.19

1.3.4.5 Saegusa's formal synthesis

Saegusa *et al*,[101] completed the first formal synthesis of gephyrotoxin in 1983 by reaching one of Kishi's key intermediates. The hydroxyl group of aniline **91** was initially protected, followed by methylation to afford the ammonium salt **92**. Treatment with a fluoride source produced hetero-diene intermediate **93**, which when heated underwent a Diels-Alder reaction. This afforded the desired diastereomer as the major compound (4.5:1). Heterocycle **94** was then subjected to harsh hydrogenation conditions to arrive at Kishi's intermediate **69**. Other formal syntheses include approaches by Pearson,[102] Hsung,[103] Lhommet[104] and Trudell.[105]



Scheme 1.20

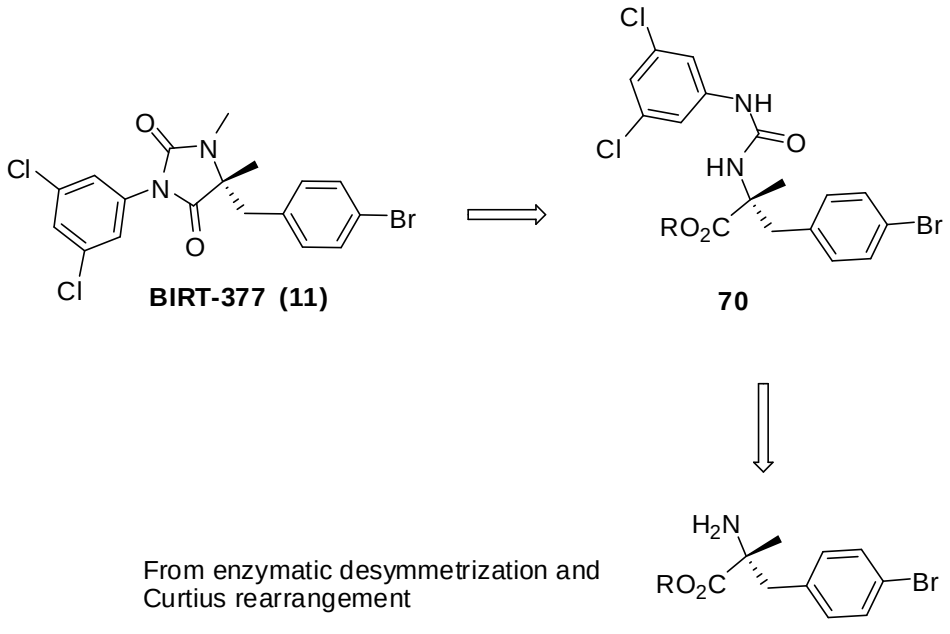
1.3.5 Objectives

The second set of objectives was to establish a route for the synthesis of gephyrotoxin (**65**) using the acetylenic sulfone conjugate addition and cyclization methodology developed by our group. The amino ester moiety required for such a reaction would be synthesized via a Diels-Alder reaction, which would install the relative stereochemistry.

Chapter Two: Synthesis of BIRT-377 and an Approach to the Synthesis of Gephyrotoxin

2.1 Stereodivergent synthesis of BIRT-377

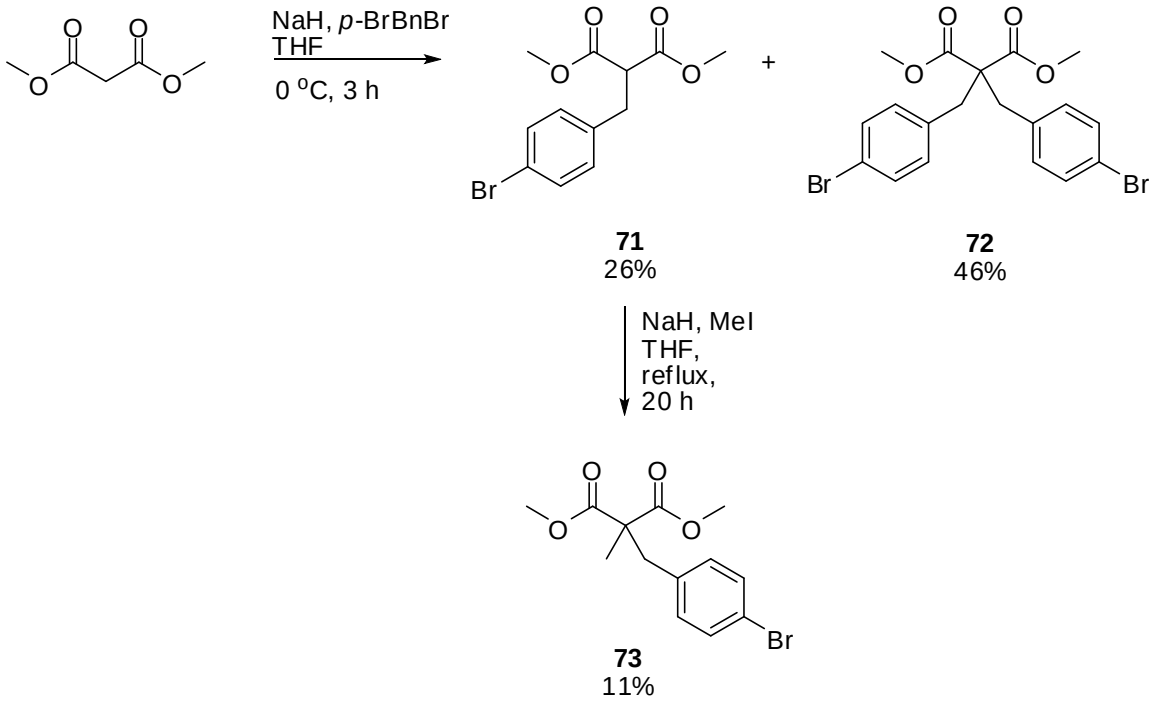
Using the methodology based on PLE desymmetrization and the Curtius rearrangement explained in Chapter One, both enantiomers of BIRT-377 were successfully synthesized in high enantiomeric excess. As shown in Scheme 2.1, the synthesis of BIRT-377 was attempted via compound **70**, which is an α-quaternary amino acid derivative that can be synthesized from the corresponding amino ester. The first half of this chapter explicates each step employed in this synthesis, including a discussion of how some of the reactions were developed and optimized.



Scheme 2.1

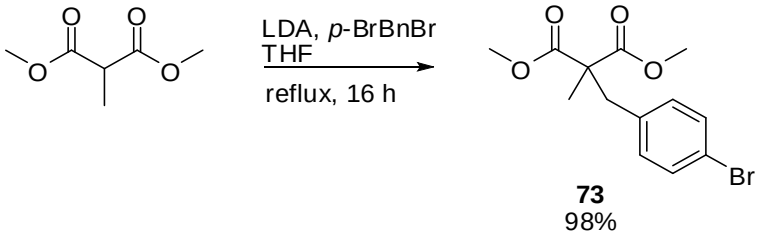
2.1.1 Preparation of an *α,α*-disubstituted malonate diester

Dimethyl malonate was treated with sodium hydride and *p*-bromobenzyl bromide to afford the undesired α,α-disubstituted malonic ester **72** as the major product, rather than the desired monosubstituted derivative **71** (Scheme 2.2). Even at 0 °C with the use of a syringe pump to administer the electrophile over the course of 3 hours, the undesired product remained dominant. The desired malonate **73** was then synthesized by methylation of **71**. Due to the unsatisfactory yield of this route, another approach was attempted based on the opposite order of attachment of the methyl and *p*-bromobenzyl substituents.



Scheme 2.2

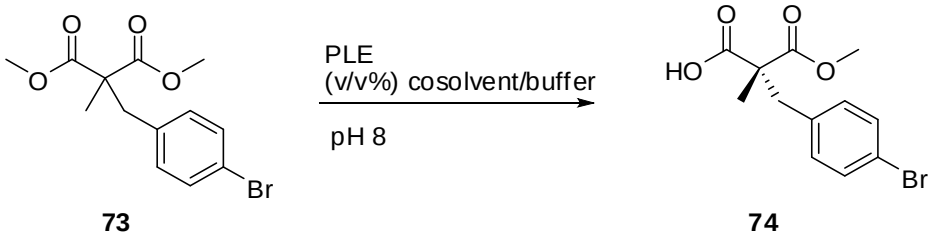
The failure of the approach shown in Scheme 2.2 is attributed to the high reactivity of *p*-bromobenzyl bromide and the enhanced nucleophilicity of the anion of **71**, which made it difficult to avoid the unwanted second alkylation. Fortunately, dimethyl 2-methylmalonate was commercially available, albeit at a slightly higher cost than dimethyl malonate; however, the route consisted of only one step and gave a considerably higher yield for compound **73** (Scheme 2.3). Using lithium diisopropylamide (LDA) as a base, α,α-disubstituted malonate **73** was obtained in 98% yield.



Scheme 2.3

2.1.2 Preparation of half-ester 74 by PLE enzymatic desymmetrization

Diester **73** was initially subjected to PLE hydrolysis conditions that had proved successful in our group's previous studies.[12,30] Unfortunately, these conditions gave a disappointing enantiomeric excess of only 30%.



Scheme 2.4

With Masterson's[24] research on solvent effects on PLE reactions in mind, extensive optimization of this reaction was carried out using a broad range of cosolvents at varying concentrations (Scheme 2.4, Table 2.1). Unlike Masterson's work, commercial PLE, which consists of an unspecified mixture of isozymes, was used instead of individual isozymes due to its low cost.

In general, the process tolerated a variety of cosolvents at concentrations in the range of 2.5-15% in an aqueous sodium phosphate buffer at pH 8. Similar buffers at pH 7 and 7.5, as well as tris.HCl buffer at pH 7.5 gave marginally lower ee values.

Fluctuation in yield can be attributed to two issues. First, at low cosolvent ratios the substrate had poor solubility resulting in heterogeneous reactions, which are inherently slower. Second, the enzyme cannot tolerate higher cosolvent concentrations and denatures, thereby slowing and eventually halting the reaction.

Just as expected, there was no linear relationship between enantioselectivity and the ratio of cosolvent to buffer solution due to the solvent's varying effects on the different isozymes that make up the crude PLE mixture.[24] It was noted that when the reaction was cooled to 0 °C the enantioselectivity increased slightly, albeit a longer reaction time of 7 days was required (Entries 6 and 20). Furthermore, when the temperature was raised to 40 °C there was a loss of enantioselectivity, but an increase in reactivity (Entries 7, 21 and 22). The highest enantioselectivities were obtained at 0 °C in 5% *i*-propanol (ee: 75%; isolated yield: 59%; Entry 6) or in 2.5% *t*-butanol (ee: 73%; isolated yield: 57%; Entry 20) as the cosolvent after 7 days. When the former experiment in *i*-propanol was conducted at room temperature for 2 days, the ee was only slightly reduced (ee: 70%; isolated yield: 64%; Entry 2) and these conditions were preferred for

larger scale experiments. The use of THF, acetonitrile or trifluoroethanol afforded lower enantioselectivities (Entries 8-29), while ethanol (not shown), for the most part, provided very poor enantioselectivities and yields.

A range of other enzymes were also tested. These included: α-chymotrypsin,[19,106] as well as lipases from *Thermomyces lanuginosus* (lipolase), *Rhizopus Niveus* and *Pseudomonas Cepacia*, [107,108] but all either failed to produce **74** or afforded **74** in significantly lower enantioselectivities. After the first few days, the PLE-mediated hydrolysis slowed and the unreacted starting material was easily recovered and recycled. Thus, the yield in Entry 2 was 97%, based on recovered starting material (BRSM).

The enantiomeric excesses of half-esters **74** were measured by ^{1}H NMR integration of a solution of the diastereomeric salts formed by mixing the product with an equimolar amount of (*R*)-(+)-α-methylbenzylamine in $CDCl_3$. Other deuterated solvents such as CD_3OD, benzene-d_6 and DMSO-d_6 were also tested, but $CDCl_3$ gave the clearest results with cleanly separated peaks of the resulting diastereomers.

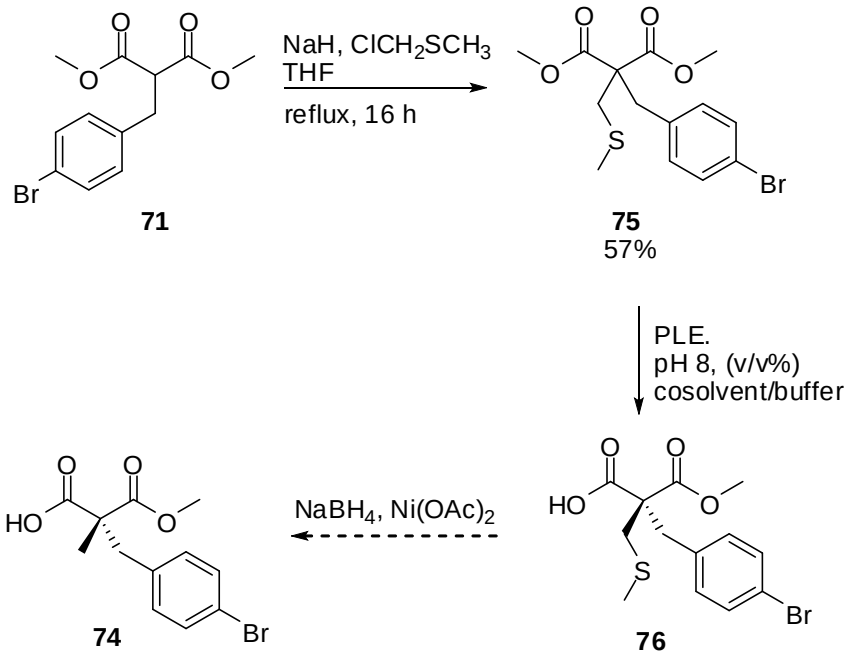
Table 2.1: Optimization of PLE-mediated desymmetrization of diester 73

Entry	Cosolvent	Cosolvent (%)[a]	Temperature (°C)	Time (days)	Yield (%)[b]	ee (%)
1	*i*-Propanol	2.5	16	2	31	64
2		5	16	2	64 (97)[c]	70
3		7.5	16	2	45	68
4		10	16	2	61	64
5		15	16	2	47	58
6		5	0	7	59	75
7		5	40	1	49	68
8	THF	2.5	16	2	36	50
9		5	16	2	39	50
10		7.5	16	2	62	44
11		10	16	2	25	46
12		12.5	16	2	28	46
13		15	16	2	15	40
14	*t*-Butanol	2.5	16	2	32	68
15		5	16	2	26	64
16		7.5	16	2	23	62
17		10	16	2	34	60
18		12.5	16	2	58	50
19		15	16	2	51	46
20		2.5	0	7	57	73
21		2.5	40	1	37	63
22		5	40	1	36	64
23	Acetonitrile	5	16	2	26	50
24		7.5	16	2	33	40
25		10	16	2	55	30
26		12.5	16	2	47	20
27	Trifluoroethanol	2.5	16	2	43	64
28		5	16	2	19	58
29		10	16	2	SM	-

[a]The cosolvent was mixed with a pH 8 sodium phosphate buffer 0.2 M. [b]Isolated yields of half-ester **74** are reported; SM indicates that only starting material was recovered. [c]Yield based on recovered starting material.

2.1.3 Attempts to improve the enantioselectivity of the PLE-mediated hydrolysis

Initial attempts to improve the enantioselectivity involved extending the length of the small alkyl chain at the quaternary centre with a tether that could be cleaved at a later stage. The theory behind this came from the observations in our group's methodology on PLE hydrolysis/Curtius rearrangement reactions[30] and Jones' work on the PLE model,[20] which indicated that a substrate with the small alkyl chain (R_S) = 3 carbons atoms improved enantioselectivity, compared to a R_S = 1 carbon atom. With R_S = 3 there was potential for a better fit in the H_S pocket (Refer to Figure 1.2). A thioether linker was chosen as it could be selectively cleaved by using nickel boride, a reagent developed for reductive desulfurizations by our group previously, to obtain the desired methyl group.[109]



Scheme 2.5

Thus, previously synthesized diester **71** was alkylated with chloromethyl methyl sulfide (Scheme 2.5). Much to our surprise and disappointment, the tethered substrate **75** gave substantially lower yields and enantioselectivities of the half-ester **76** than in the conversion of the original substrate **73** to **74**. The enantioselectivities again were determined by ^{1}H NMR integration of a solution of the diastereomeric salts formed by mixing the product with an equimolar amount of (*R*)-(+)-*α*-methylbenzylamine in benzene-d_6 (Table 2.2). Due to these low enantioselectivities, this approach was abandoned.

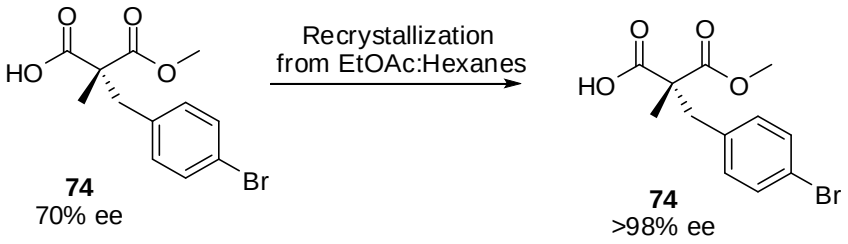
Table 2.2: Optimization of PLE-mediated desymmetrization of diester 75

Entry	Cosolvent (%)[a]	Temperature (°C)[b]	Time (days)	Yield (%)[c]	ee (%)
1	2.5	RT	2	8	21
2	5	RT	2	1	28
3	7.5	RT	2	1	28
4	10	RT	2	7	41
5	12.5	RT	2	16	22
6	15	RT	2	11	40

[a]The cosolvent, 2-propanol, was mixed with a pH 8 sodium phosphate buffer. [b]RT indicates room temperature. [c]Isolated yields of half-ester **76** are reported.

We therefore returned to the original approach, where it may be recalled the best yield and enantioselectivity were obtained (Table 2.1, Entry 2; 64% yield, 70% ee). Fortunately, half-ester **74** of Entry 2 could be recrystallized from ethyl acetate:hexanes to produce two crops of an enantiopure product (ee >98%), which was verified by NMR

analysis of the half-ester salt formed with (*R*)-(+)-*α*-methylbenzylamine in $CDCl_3$ (Scheme 2.6). Furthermore, 88% of the desired enantiomer was recovered during the recrystallization. This aspect of the work was conducted in part by undergraduate student Matthew Saunders.



Scheme 2.6

The benzylic protons of salt **77** split to give two sets of two of doublets, which can be seen in the spectrum of the racemic material in Figure 2.1a. An expansion of these benzylic peaks is shown in Figure 2.1b. Two doublets belong to each diastereomer, which were present in a 50:50 mixture, and represent the "*S*" and "*R*" enantiomers of the original **74**. When partial hydrolysis was mediated by PLE one of the diastereomers was formed in excess (Figure 2.1c). After the resulting product was enantioenriched by recrystallization, the minor enantiomer was gone within the limits of detection (Figure 2.1d). Determination of which peaks belonged to which diastereomer was determined by the optical rotation of the final compound **11** in comparison with literature values, which will be shown later in the chapter. Enantiomeric excess shown on the right hand side of Figure 2.1 pertains to the (*R*) enantiomer. With enantiopure half-ester **74** in hand the synthesis of BIRT-377 could now proceed.

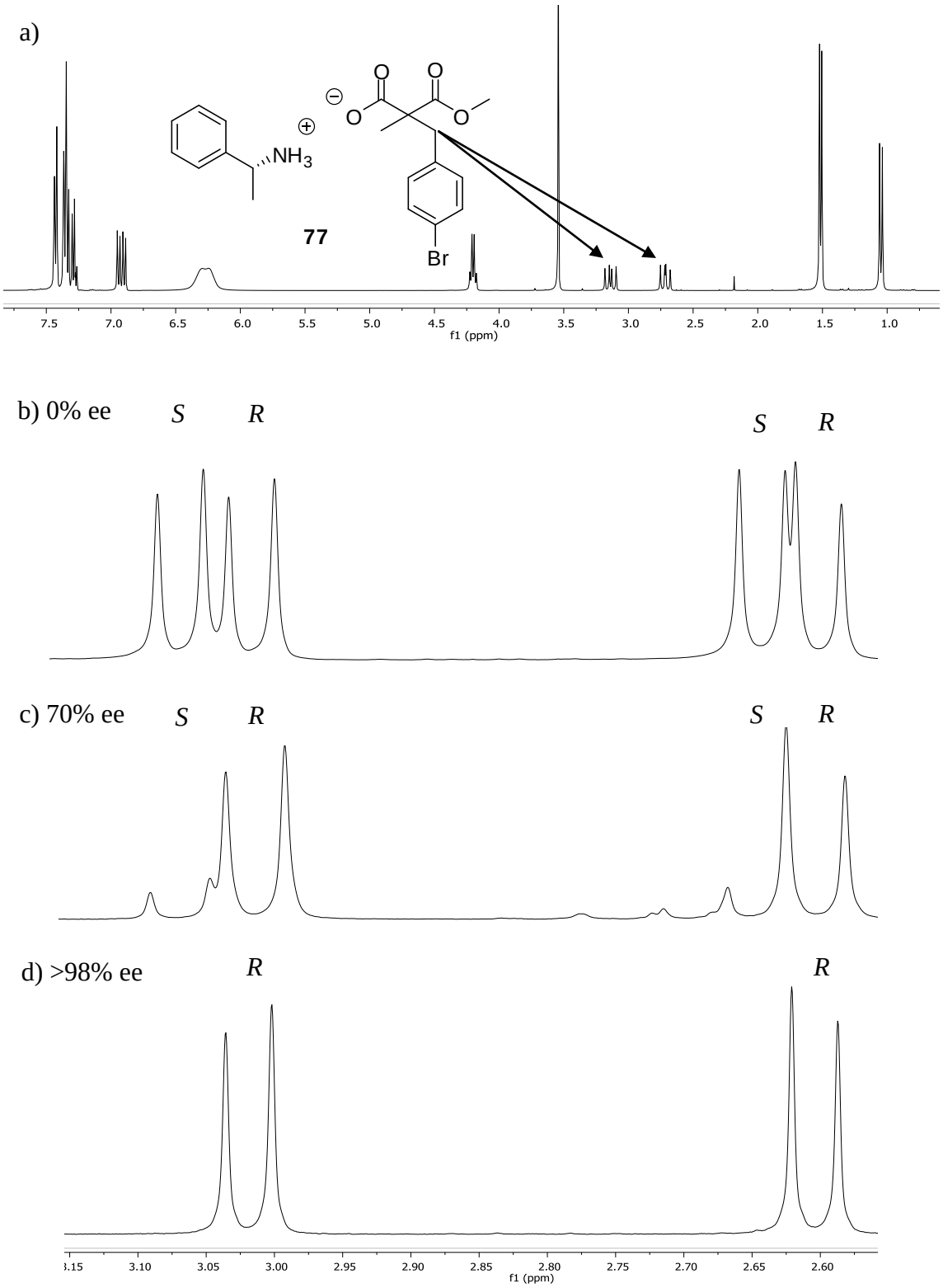
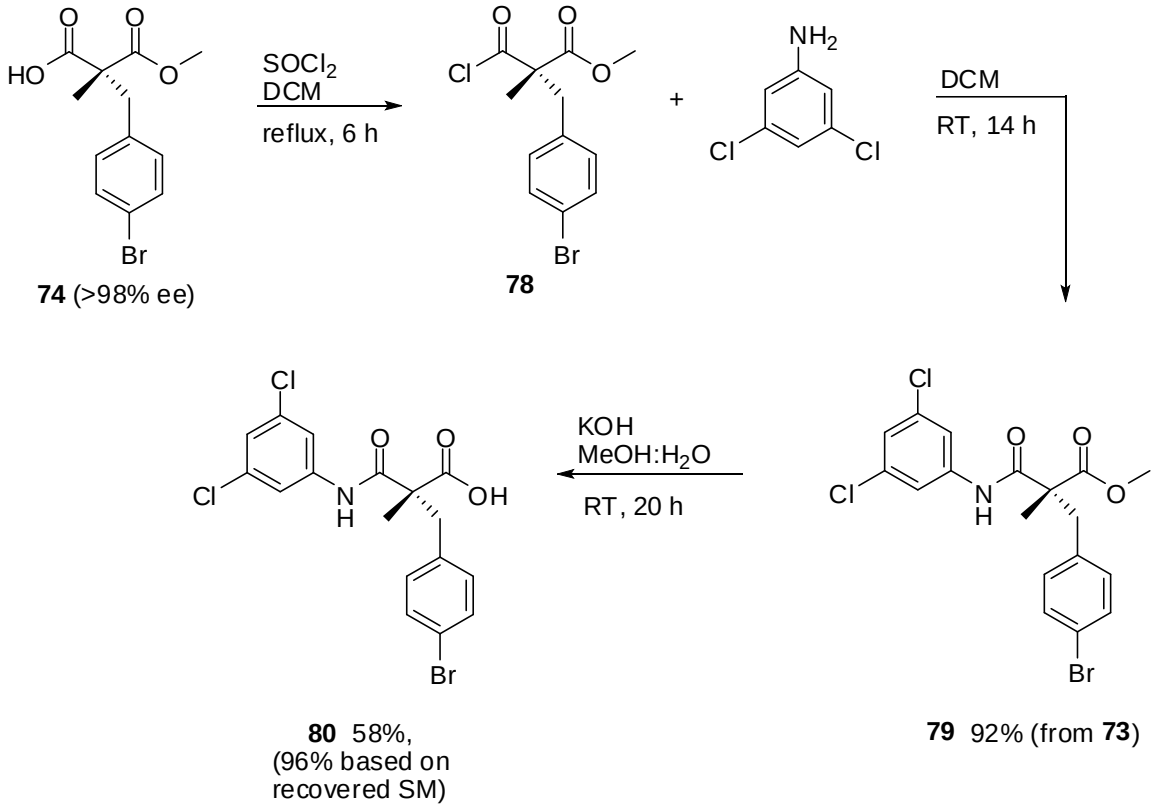


Figure 2.1: Determination of enantiomeric excess of 74 by NMR analysis of salt 77

2.1.4 Synthesis of BIRT-377

2.1.4.1 Preparation of carboxylic acid 80

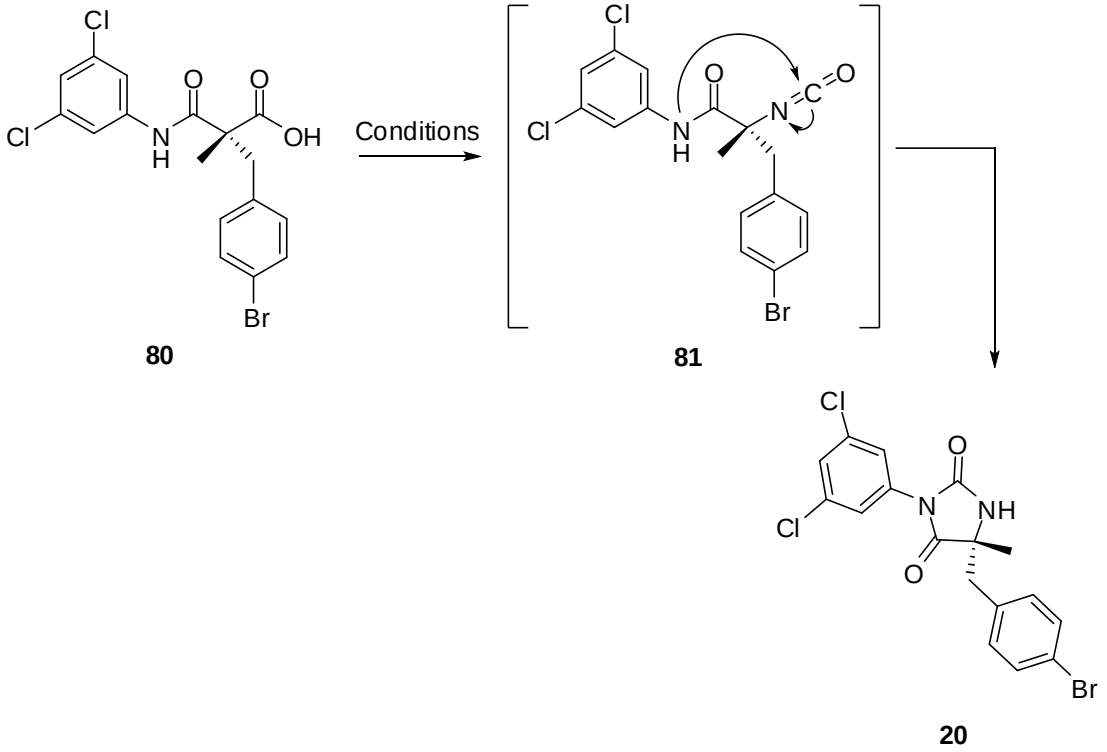
Enantiopure half-ester **(*R*)-74** was then treated with thionyl chloride to afford acid chloride **78** and subsequent amide coupling with 3,5-dichloroaniline produced amide **79** in high yield, even in the absence of a scavenger for HCl. The presence of triethylamine had no significant effect on the reaction (Scheme 2.7). The ester group was gently hydrolyzed to give acid **80** in 58% yield. The reaction was carried out under mild conditions (room temperature) due to potential decarboxylation of the product and was stopped before completion to prevent a build up of side products. The remaining ester **79** was recycled to give a 96% yield based on recovered starting material.



Scheme 2.7

2.1.4.2 Synthesis of the hydantoin core

Acid **80** was then poised to undergo a Curtius rearrangement reaction to isocyanate **81**, followed by intramolecular trapping to afford hydantoin **20** (Scheme 2.8). Unfortunately, this was not the case under Shioiri-modified Curtius rearrangement conditions,[25] which are shown in Scheme 1.3 and are the typical modern conditions used, and only resulted in decomposition of the starting material (Entry 1, Table 2.3). This was possibly due to complications from the starting material's poor solubility in toluene, which is a typical solvent in Curtius rearrangements. A variety of attempts were made to remedy this issue. One approach was a two-pot process, which converted acid **80** into the lipophilic acid chloride in 1,2-dichloroethane (DCE) before transfer of this intermediate into a more commonly used solvent for the Curtius rearrangement. This resulted in a much more promising yield of 51% (Entry 2). Dioxane was tested because it is more polar than toluene but has a similar boiling point; however, it resulted in a complex reaction mixture (Entry 3). The use of silver carbonate has been shown to work well with carbohydrates in these rearrangements, which are generally even more polar than **80**.[110] Reaction in dioxane with silver carbonate and potassium carbonate gave the best yield of 56% (Entry 4). Attempts to use an even more polar solvent, such as DMF, gave a marginally lower yield, which was most likely due to extraction difficulties (Entry 5).



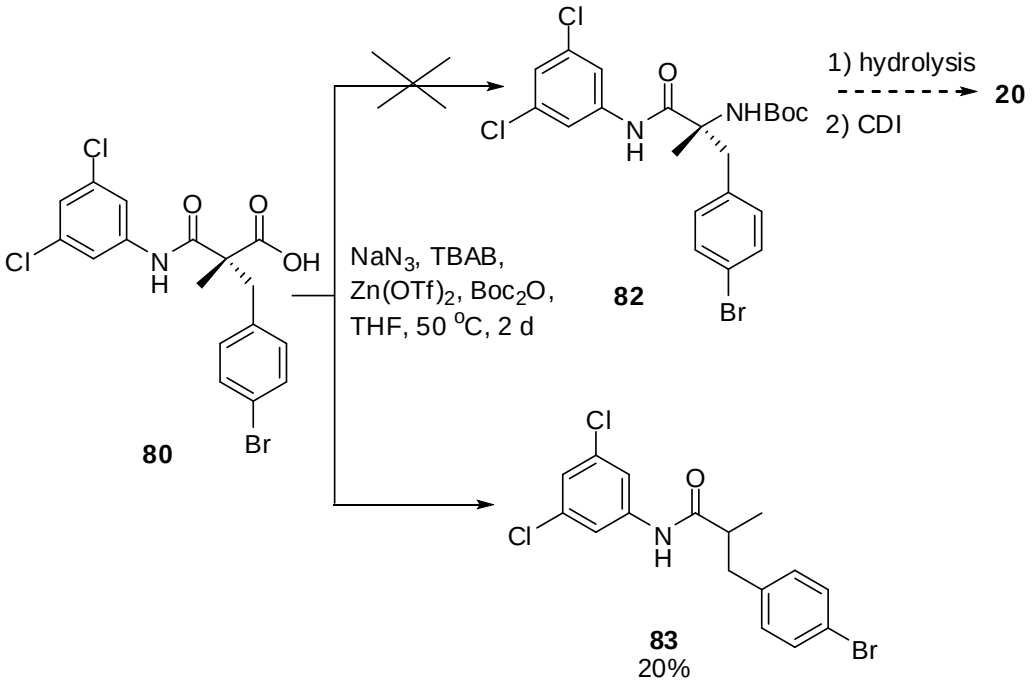
Scheme 2.8

Table 2.3: Optimization of isocyanate formation/trapping reaction

Entry	Solvent	Additives	Tempera-ture (°C)	Time (hours)	Yield (%)[a]
1	Toluene	DPPA, Et_3N	Reflux	22	CM
2	1) DCE 2) THF[b]	1) $SOCl_2$ 2) NaN_3, Et_3N[b]	Reflux	5, 14[b]	51
3	Dioxane	DPPA, Et_3N	Reflux	22	CM
4	Dioxane	DPPA, K_2CO_3, Ag_2CO_3	Reflux	22	56
5	DMF	DPPA, K_2CO_3, Ag_2CO_3	110	22	54

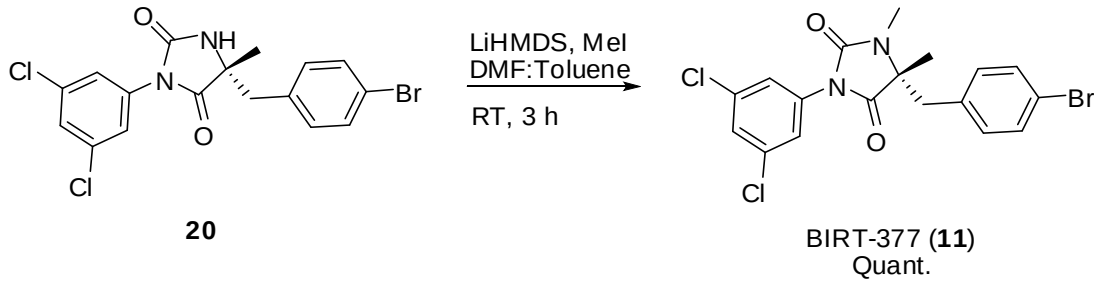
[a]Isolated yields of hydantoin **20**; CM indicates a complex reaction mixture. [b]This was a two-pot reaction and Entry 2 indicates the solvents, additives and reaction times of two separate steps.

In an attempt to improve the yield, the method of Lebel *et al.*[111] was investigated (Scheme 2.9). This involves the use of Boc-anhydride and a zinc-based Lewis acid to activate the carboxylic acid to allow attack by sodium azide and formation of the acyl azide, which can then undergo a Curtius rearrangement. The isocyanate intermediate can be attacked by the *t*-butyl alcohol to produce carbamate **82**. Subsequent deprotection and cyclization with *N*,*N*-carbonyldiimidazole (CDI) then affords hydantoin **20**. Regrettably, a side reaction gave compound **83** via decarboxylation. This demonstrates the instability of acid **80** and the need for mild conditions.



Scheme 2.9

With hydantoin **20** in hand (Table 2.3, Entry 4), *N*-methylation was carried out via a literature method by deprotonation with LiHMDS and treatment with iodomethane in dry DMF and toluene to afford BIRT-377 (**11**) in quantitative yield (Scheme 2.10).[44]



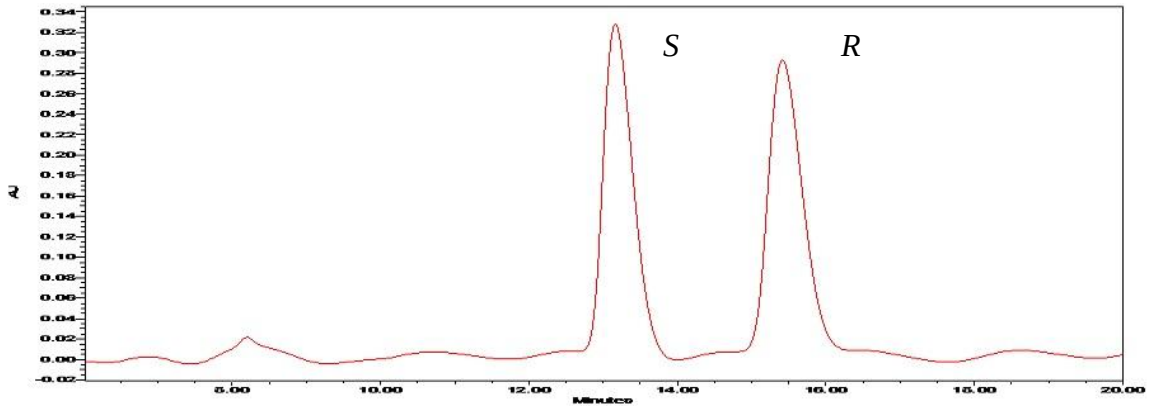
Scheme 2.10

2.1.5 Determination of enantiopurity of hydantoin 11

Optical rotation for hydantoin (*R*)-(+)-BIRT-377 was similar, if not higher, than those reported in the literature. For example, the observed rotation of our compound **11** was $[\alpha]_D^{20}$ +135 (c 1.50, ethanol), whereas the literature rotations for **11** are as follows: $[\alpha]_D^{25}$ +127.1 (c 1, ethanol),[44] $[\alpha]_D^{25}$ +131.6 (c 1.0, ethanol)[47] and $[\alpha]_D^{25}$ +134.3 (c 1.0, ethanol).[42] As mentioned earlier, this confirmed that the enantiomer obtained had the *R*-configuration and enantioenrichment of **74** as displayed in Figure 2.1.

Enantiomeric excess was also confirmed by chiral HPLC, using a Chiralpak AD column with an isocratic solvent system of *i*-propanol:hexanes, the details of which is further explained in the experimental chapter. The chiral HPLC trace shown in Figure 2.2 shows that both enantiomers separated cleanly in: a) the racemic sample and b) BIRT-377 (**11**), which contains only one substantial peak (*R*), thus showing an enantiomeric excess of >98%. This in combination with the optical rotations obtained for **11** prove the enantioselectivity of this synthesis.

a)



b)

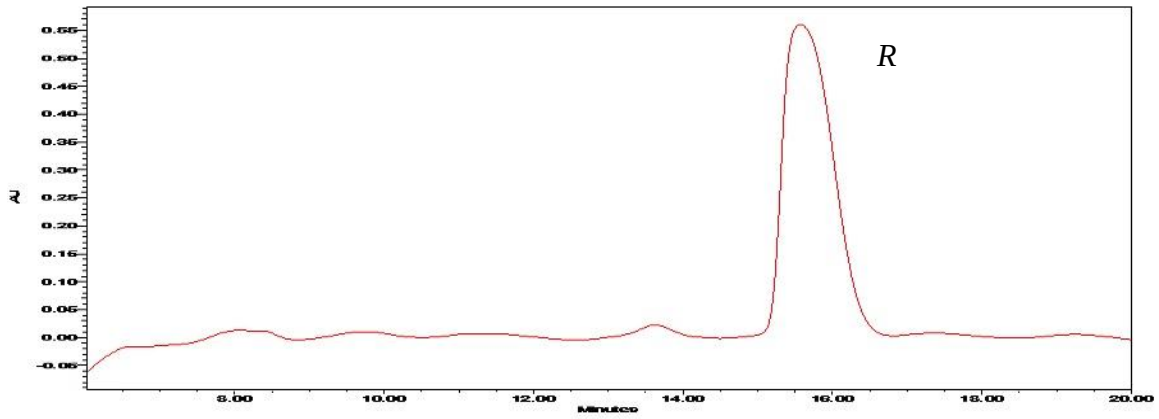
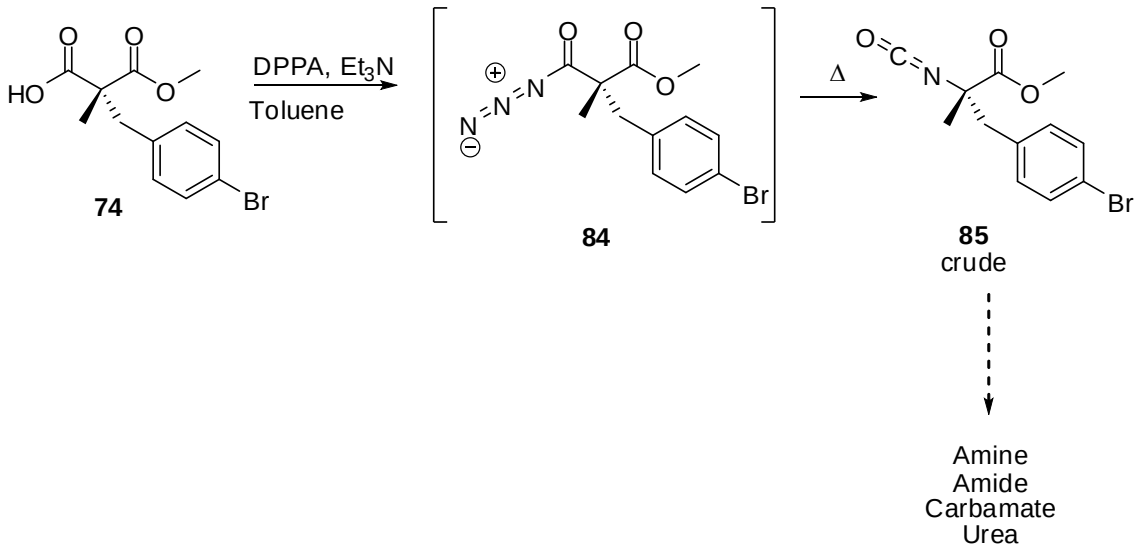


Figure 2.2: Chiral HPLC data for a) Racemic BIRT-377, b) BIRT-377 (11)

2.1.6 Synthesis of (*ent*)-BIRT-377

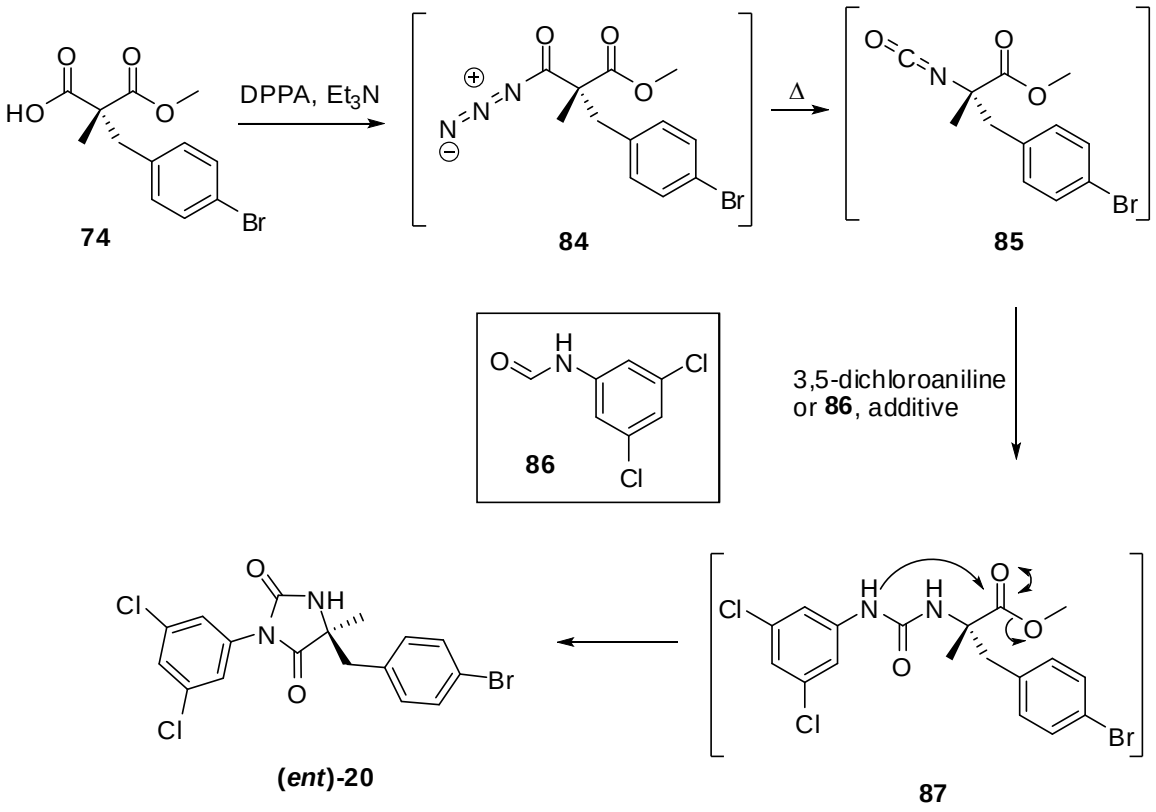
(*Ent*)-BIRT-377 was also synthesized from half ester **74** by reversal of the order of the steps involving Curtius rearrangement and addition of the 3,5-dichloroaniline component. Enantiopure half-ester **74** first underwent a Shioiri-modified Curtius rearrangement[25] in the presence of triethylamine and diphenylphosphoryl azide to give acyl azide **84**. The latter was then heated to afford crude isocyanate **85**, which was initially isolated to verify its formation. This relatively stable intermediate could potentially be converted directly to amine **28** or protected as an amide, carbamate or urea (Scheme 2.11).



Scheme 2.11

If isocyanate **85** could be trapped with 3,5-dichloroaniline as urea **87**, then it might be expected to undergo a ring-closing reaction to afford the hydantoin core in one step (Scheme 2.12). This one-pot Curtius rearrangement, isocyanate trapping and cyclization reaction was attempted, but disappointingly, this proved very troublesome due to the sterically hindered quaternary center adjacent to the isocyanate moiety and the

resonance-deactivated nucleophile, 3,5-dichloroaniline, which also has two inductively electron-withdrawing chlorine atoms at the *meta* positions.



Scheme 2.12

Reaction of **74** with DPPA and triethylamine gave no reaction (Entry 1), unless the nucleophilic catalyst dimethylaminopyridine was added (Entry 2). Deprotonation of 3,5-dichloroaniline with strong bases prior to its addition to the isocyanate afforded only complex reaction mixtures (Entries 3 and 4). Methylaluminum dichloride and boron trifluoride diethyl etherate were also tested, in the hope of activating the isocyanate. These are also hard Lewis acids and therefore would be less likely to deactivate the nucleophile, 3,5-dichloroaniline. Unfortunately use of Lewis acids yielded little or no product (Entries 5 and 6).

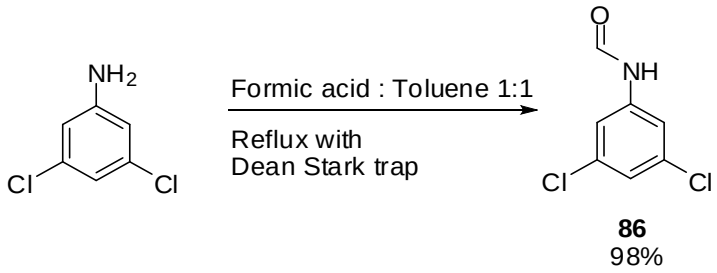
The *N*-formylaniline **86** was synthesized from 3,5-dichloroaniline by refluxing with formic acid in a Dean-Stark apparatus (Scheme 2.13). *N*-Deprotonation of aniline **86** was assisted by the electron-withdrawing formyl group. The resulting anion then functioned as a more powerful nucleophile than the original aniline to attack isocyanate **85**. Upon work-up, the *N*-formyl amide can hydrolyze to afford **87** and cyclization can occur; however use of this *N*-formyl nucleophile gave a poor yield (Entry 7).

Increasing the temperature of the reaction by using higher-boiling chlorobenzene as a solvent only caused the reagents to decompose (Entry 8). Due to the disappointing yields, a stepwise approach was favoured due to the by-products in the isocyanate-forming stage potentially reacting in the trapping or cyclization steps.

Table 2.4: Optimization of the synthesis of (*ent*)-20 via one-pot method

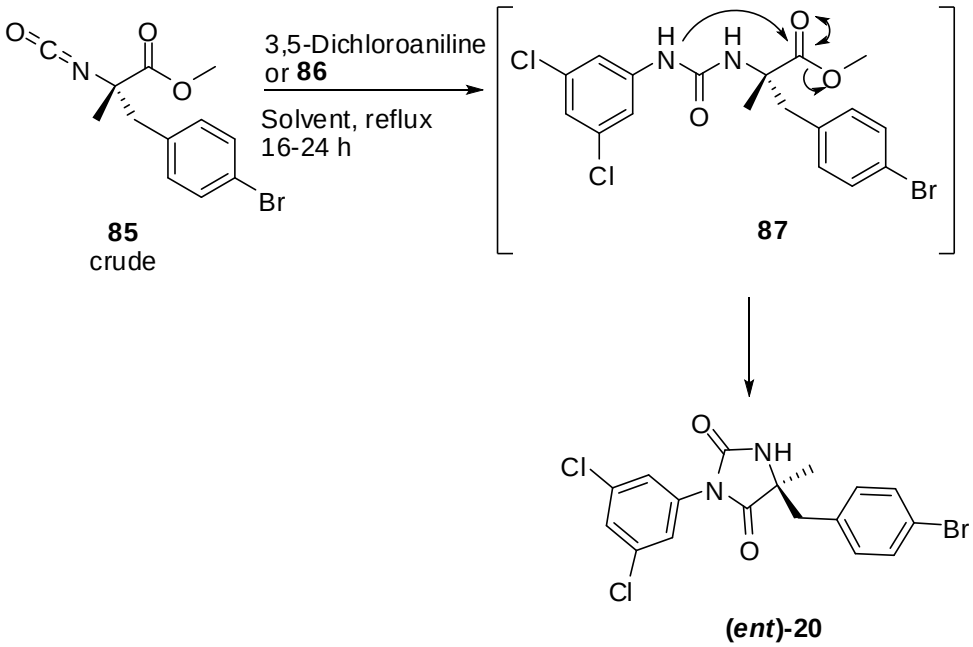
Entry	Solvent	Additive	Temperature (°C)	Time (hours)	Yield (%)[a]
1	Toluene	Et_3N	Reflux	20	SM
2	Toluene	Et_3N, DMAP	Reflux	20	26
3	Toluene	LDA, DMAP	Reflux	12	CM
4	Toluene	NaH, DMAP	Reflux	16	CM
5	Toluene	$MeAlCl_2$	Reflux	22	3
6	Toluene	$BF_3.OEt_2$	Reflux	20	CM
7	Toluene	Et_3N ,DMAP, **86**	Reflux	20	13
8	Chlorobenzene	DMAP	Reflux	20	CM

[a]Isolated yields of hydantoin **(*ent*)-20**; SM indicates that only starting material was recovered; CM indicates a complex mixture.



Scheme 2.13

The stepwise approach with isocyanate trapping and cyclization of the isolated **85** performed slightly better than the one-pot method, which suggested that the by-product, diphenyl phosphate, did interact in the subsequent reaction somewhat (refer to Scheme 1.3). Nevertheless, the yield over the two steps was still quite low (Table 2.5, Entry 1). Refluxing in higher-boiling solvents only resulted in decomposition (Entries 2-4), as did the use of other bases (Entries 5-9). The addition of Lewis acids, for the most part, gave only complex reaction mixtures (Entries 10-15). Lanthanides and transition metals resulted in no product formation (Entries 16-18). The use of DMF, which is far more polar, afforded some product when used with mild bases (Entries 20-22) and stronger bases resulted in decomposition (Entries 23 and 24). Overall the conditions described in Entry 10 gave the best yield of 36% over two steps with the use of boron trifluoride diethyl etherate.


3,5-Dichloroaniline
or 86
Solvent, reflux
16-24 h
85
crude
87
(ent)-20

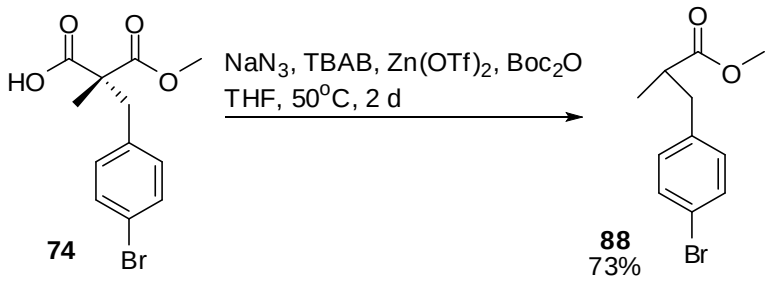
Scheme 2.14

Table 2.5: Optimization of isocyanate trapping and cyclization

Entry	Solvent	Additive	Temperature (°C)	Yield (%)[a]
1	Toluene	Et_3N	Reflux	26
2	*p*-Xylene	Et_3N	Reflux	Trace
3	Chlorobenzene	Et_3N	Reflux	Trace
4	Dichlorobenzene	Et_3N	140	CM
5	Toluene	Pyridine	Reflux	SM
6	Toluene	NaH	Reflux	CM
7	Toluene	LDA	Reflux	CM
8	Toluene	K_2CO_3, $AgCO_3$	Reflux	Trace
9	Toluene	Et_3N, Compound (**86**)	Reflux	12
10	Toluene	$BF_3.Et_2O$	Reflux	36
11	Toluene	CuCl	Reflux	18
12	Toluene	$EtAlCl_2$	Reflux	SM
13	Toluene	Amberlyst	Reflux	CM
14	Toluene	$MgSO_4$	Reflux	CM
15	Toluene	$MeAlCl_2$	Reflux	3
16	Toluene	$Tb(OTf)_3$	Reflux	CM
17	Toluene	$LaCl_3$	Reflux	SM
18	Toluene	Pd(II)Acetate	Reflux	CM
19	Dry DMF	CuCl	Reflux	CM
20	DMF	Pyridine	110	25
21	Dry DMF	Pyridine	110	28
22	Dry DMF	Et_3N	110	30
23	Dry DMF	$CsCO_3$,	110	CM
24	Dry DMF	$CsCO_3$, Compound (**86**)	110	CM

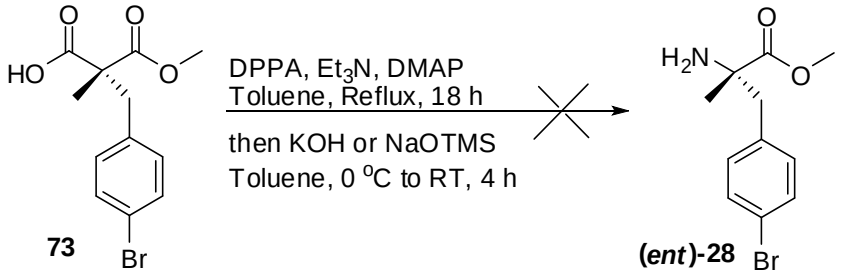
[a]Isolated yields of hydantoin (**(*ent*)-20**) over 2 steps; SM indicates that only starting material was recovered; CM indicates a complex mixture.

Several alternative approaches were attempted as follows. Lebel's method was attempted initially to give the Boc-protected amine, as shown in Scheme 2.15 with half-ester **74**.[111] This unfortunately, yet again, resulted in decarboxylation of the reactant to give undesired ester **88** in a relatively high yield.



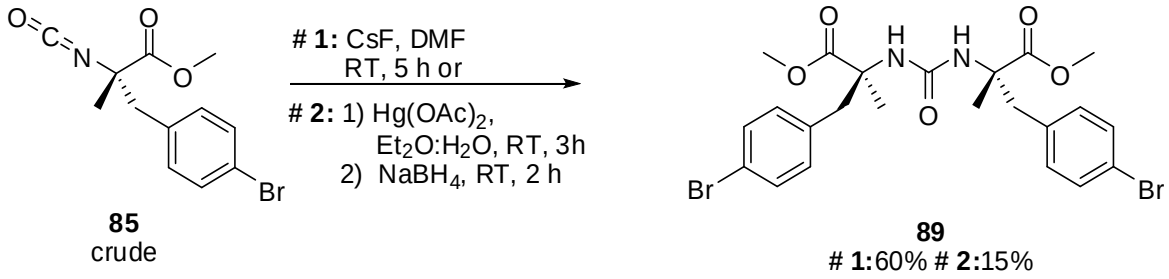
Scheme 2.15

Another approach was to isolate free amine **(*ent*)-28** first. Initially this was attempted with potassium hydroxide, which gave a complex mixture. Sodium trimethylsilanolate was also tested, which was expected to form a TMS-protected carbamic acid. This would undergo silyl-deprotection upon workup and then decarboxylate to afford the free amine.[112] Sadly this was not the case as no product was observed (Scheme 2.16).



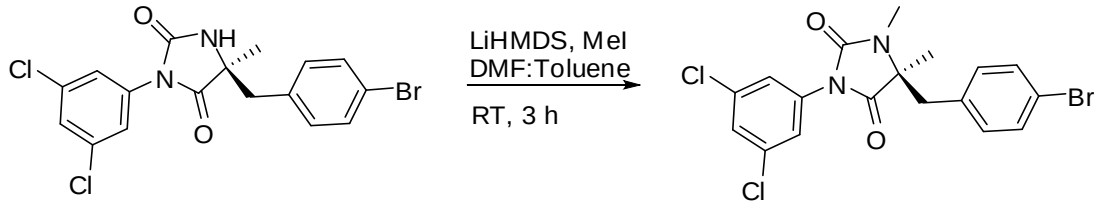
Scheme 2.16

Alternatively, isocyanate **85** could be attacked by fluoride ion in order to produce **(*ent*)-28**. Cesium fluoride was selected as an appropriate reagent; however, the major product that was obtained was a urea-linked dimer **89**, which was verified by mass spectrometry. This was the result of the free amine product (**28**) attacking the isocyanate starting material **85**. A mercuration/demercuration reaction was also attempted under reportedly mild conditions for the formation of amines; however it resulted in the same dimer side-product (Scheme 2.17).[113]



Scheme 2.17

In view of our inability to further improve the yield of **(*ent*)-20**, we returned to the product from Table 2.5, Entry 10. Methylation was carried out by the same literature procedure used for BIRT-377 itself to afford *ent*-BIRT-377 (**(*ent*)-11**) in a quantitative yield (Scheme 2.18).[44] (*S*)-(-)-BIRT-377 (**(*ent*)-11**) gave a respectable optical rotation of $[\alpha]_D^{20}$ -128 (c 1.00, ethanol), which is slightly lower in magnitude than that of the obtained and literature rotations of (*R*)-(+)-BIRT-377.



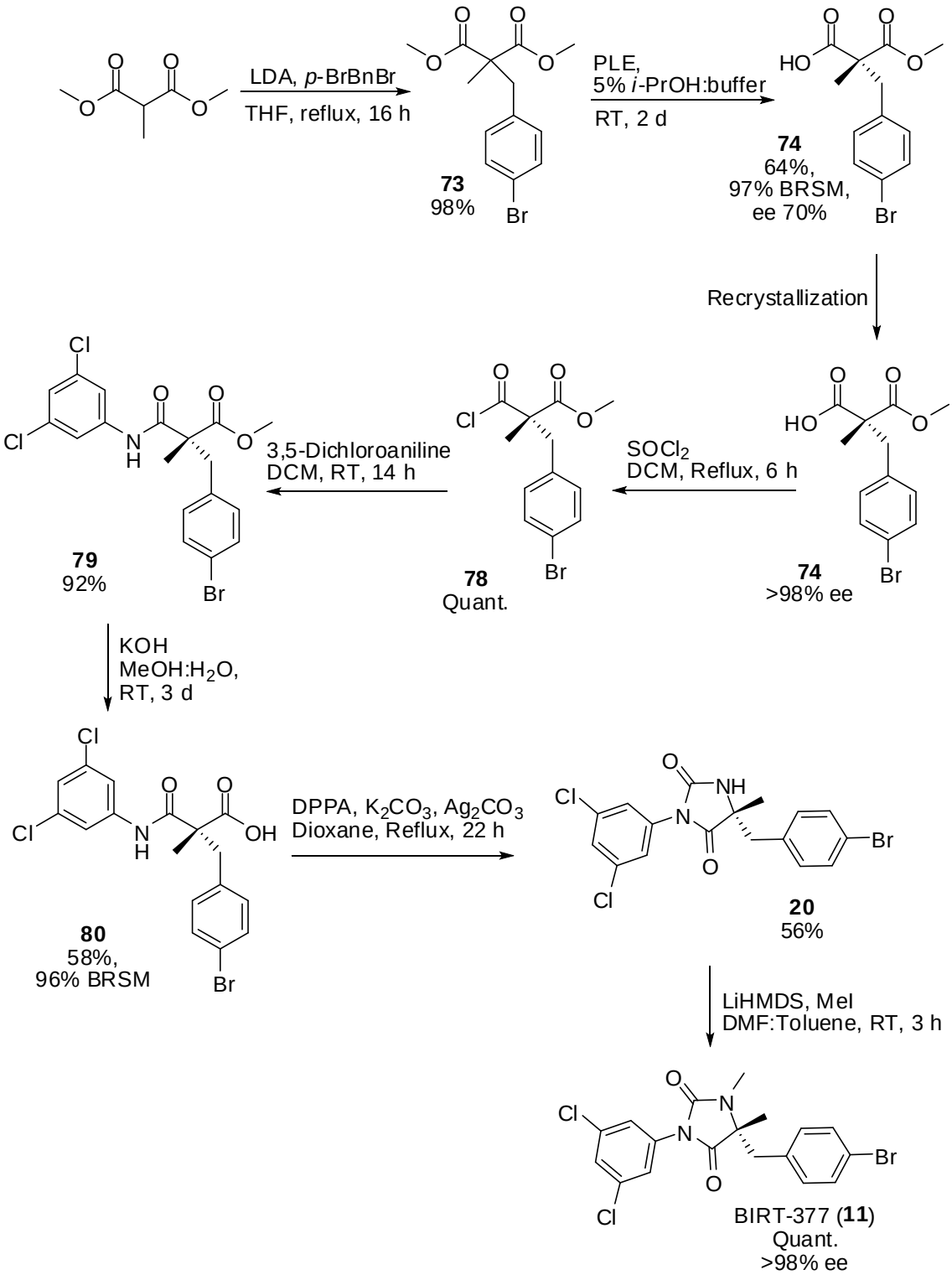
(*ent*)-20

(*ent*)-11
Quant.

Scheme 2.18

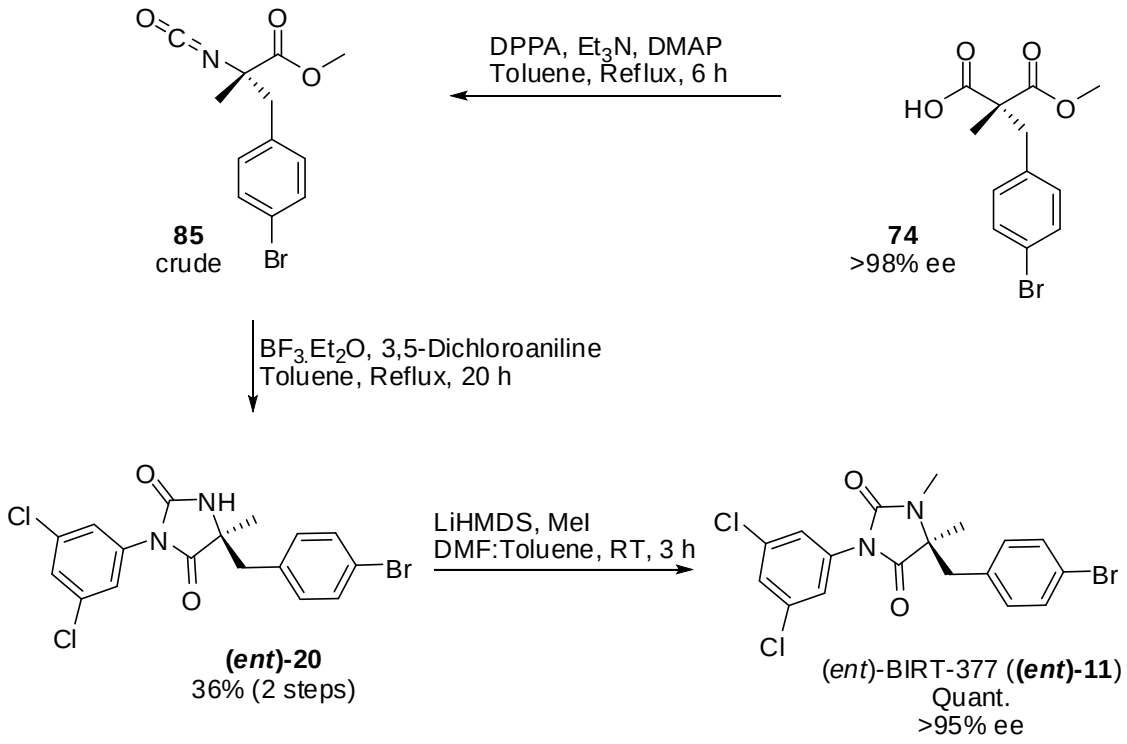
2.1.7 Summary of stereodivergent synthesis

In summary, BIRT-377 (**11**) was synthesized from dimethyl 2-methylmalonate in 7 steps following the route shown in Scheme 2.19, with an overall chemical yield of 19% or 47% based on recovered starting material. With the exclusion of the lost product during recrystallization, the overall enantiopure yield was 14%.


LDA, p-BrBnBr
THF, reflux, 16 h
73
98%
PLE,
5% i-PrOH:buffer
RT, 2 d
74
64%,
97% BRSM,
ee 70%
Recrystallization
74
>98% ee
SOCl2
DCM, Reflux, 6 h
78
Quant.
3,5-Dichloroaniline
DCM, RT, 14 h
79
92%
KOH
MeOH:H2O,
RT, 3 d
80
58%,
96% BRSM
DPPA, K2CO3, Ag2CO3
Dioxane, Reflux, 22 h
20
56%
LiHMDS, MeI
DMF:Toluene, RT, 3 h
BIRT-377 (11)
Quant.
>98% ee

Scheme 2.19

(*Ent*)-BIRT-377 (**(*ent*)-11**) was synthesized from the same intermediate, enantiopure half-ester **74**, in 3 steps, in 36% yield. The overall chemical yield was 23% or 34% based on recovered starting material. With the exclusion of the lost product during recrystallization, the overall enantiopure yield was 17% (Scheme 2.20).



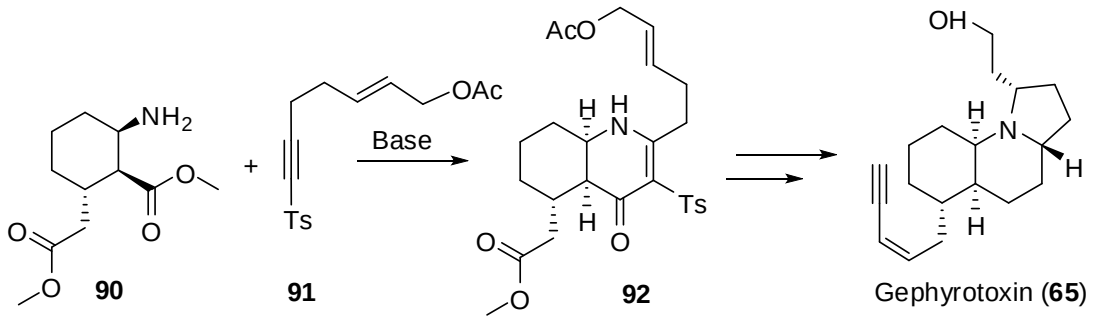
Scheme 2.20

In conclusion, our synthesis of BIRT-377 is not the shortest route published; however it obtains **11** in a respectable overall yield. Our route requires easy to use cheap starting materials relative to previous syntheses, which employ either specialist starting materials or complex catalysts to install the chiral center, in most cases. A drawback of our synthesis results from the poor scalability of the enzymatic desymmetrization step, which requires high dilution to obtain the required cosolvent/buffer ratio and therefore is not feasible on a kilogram scale. To date the Yee *et al.*[40] synthesis still is the most efficient large scale synthesis of BIRT-377. However, ours is the first stereodivergent synthesis to date and makes (*ent*)-BIRT-377 readily available for comparison with other medicinal hydantoin products related to BIRT-377

2.2 Approaches to the synthesis of gephyrotoxin

2.2.1 Introduction

As discussed in Chapter One, our proposed synthesis of gephyrotoxin (**65**) involves the conjugate addition and α-acylation of amino ester **90** and acetylenic sulfone **91**, as shown in Scheme 2.21. Enaminone **92** could then undergo *O*-deprotection of the allylic acetate, followed by oxidation, which should trigger an intramolecular conjugate addition to form the third ring. Several more functional group conversions would then deliver gephyrotoxin (**65**).

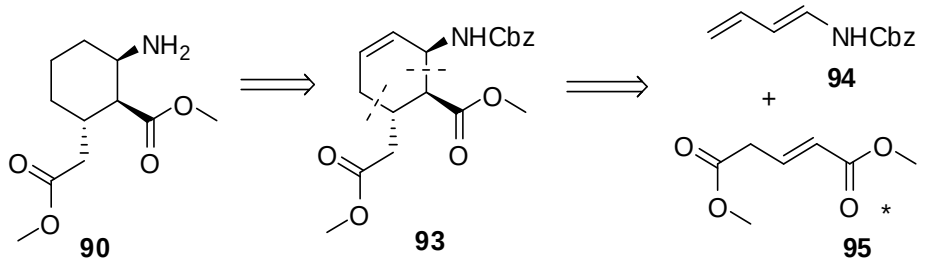


Scheme 2.21

2.2.2 Attempted synthesis of amino ester 90

Amino ester **90** might be synthesized in a number of ways; these include Diels-Alder reactions to prepare the corresponding cyclohexene compound **93**, which would install the relative stereochemistry in one step. *trans*-Diene **94** is expected to react with diester **95** (Scheme 2.22) to afford the cyclohexene derivative **93**, which after hydrogenation affords **90**. Due to the electron-rich diene and electron-deficient dienophile's substituents, the correct regiochemistry should be favoured. The reaction

should go through an *endo* transition state due to the secondary orbital interactions between the ester moiety designated by the * in **95** and the diene π-system in **94** (Figure 2.3a).



Scheme 2.22

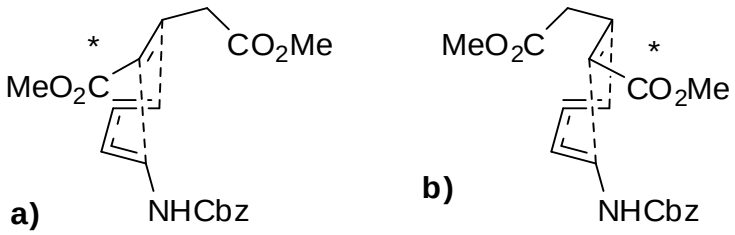


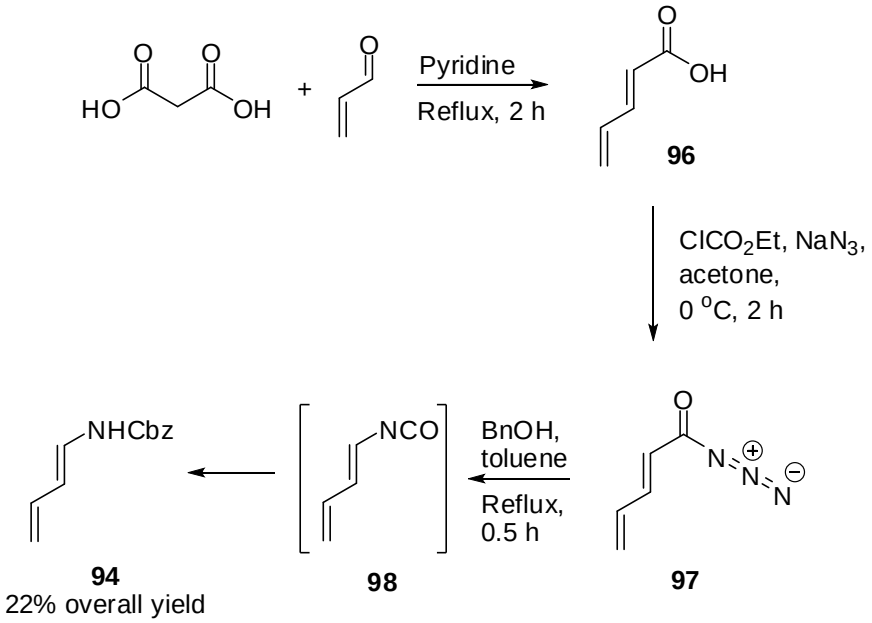
Figure 2.3: The a) *endo* and b) *exo* transition states for the formation of amino ester 93

2.2.2.1 Synthesis of diene 94

Benzyl *trans*-1,3-butadiene-1-carbamate (**94**) was reported by Overman, who employed it in other Diels-Alder reactions.[114] According to Overman *et al*.[96] the ability of diene **94** to participate successfully in [4+2] cycloadditions is due to its relatively good thermal stability, since most Diels-Alder reactions require heating, and its reactivity

toward dienophiles. Unfortunately, diene **94** readily decomposes in the presence of even mild acid, which is sometimes employed to catalyze Diels-Alder reactions.[95]

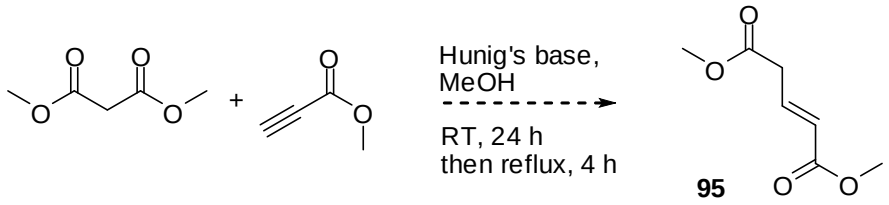
The synthesis of diene **94**[95] started with the Knoevenagel condensation between malonic acid and acrolein in the presence of pyridine, followed by decarboxylation to afford *trans*-2,4-pentadienoic acid (**96**), which is shown in Scheme 2.23. Reaction of this with ethyl chloroformate followed by sodium azide afforded acyl azide **97**. Formation of isocyanate **98** by heating and finally trapping with benzyl alcohol provided diene **94**. One serious problem with the Overman synthesis of **94** is the requirement for the evaporation of solvent to almost dryness to concentrate the acyl azide **97**, which is particularly explosive when concentrated. Thick gloves were worn and a blast shield used as a precaution when carrying out this reaction.



Scheme 2.23

2.2.2.2 Attempted synthesis of dienophile 95

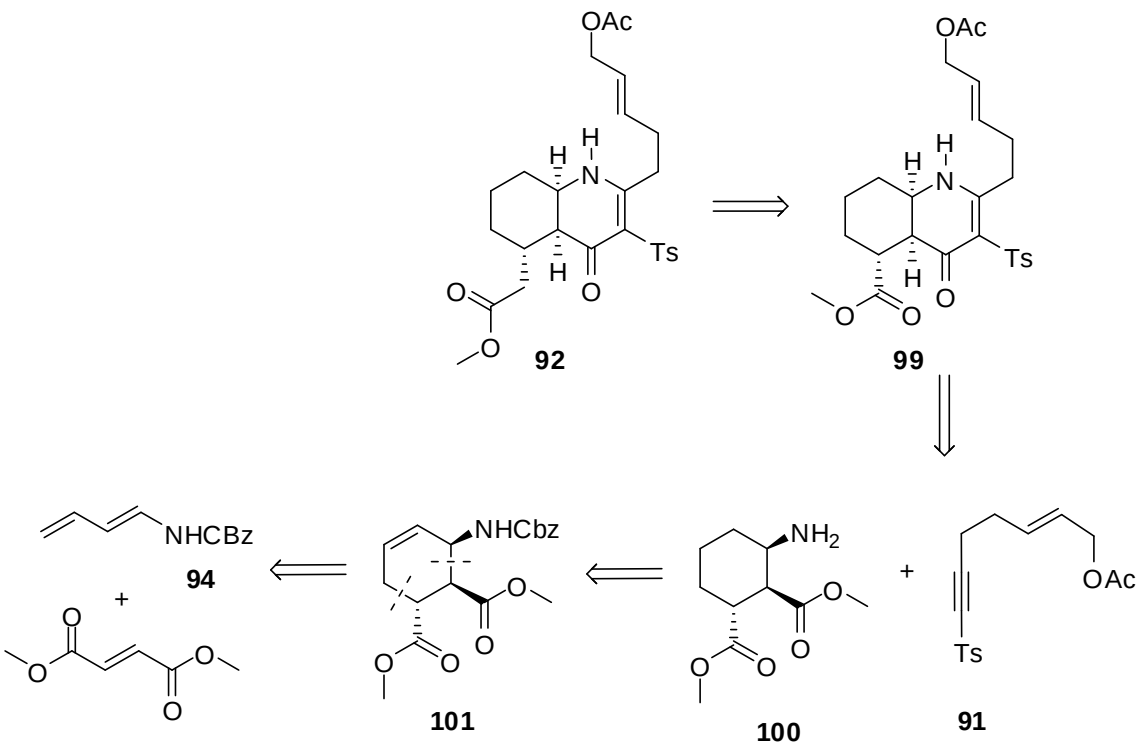
trans-Glutaconate methyl ester (**95**) is commercially available; however it is expensive and it is not feasible to buy the required amount needed for a total synthesis. An alternative means to obtain it was proposed by modifying a procedure by Watts *et al*.[115] Conjugate addition of dimethyl malonate to methyl propiolate in the presence of Hunig's base, followed by decarboxylation upon heating should have formed diesters **95**. Unfortunately, this reaction did not produce the desired product (Scheme 2.24). Thus, due to the inability to access **95** in a reasonable quantity another route was devised.



Scheme 2.24

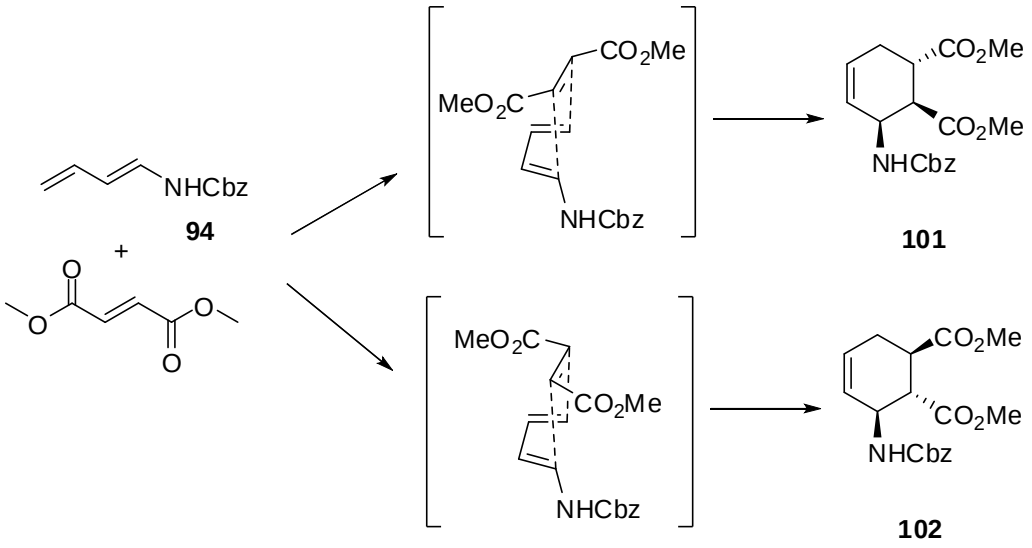
2.2.3 First revised retrosynthesis

We next considered that enaminone **92** could be formed by homologation of the methyl ester group of **99** by an Arndt-Eistert reaction of the corresponding acid derivative, which has literature precedence for retention of stereochemistry of chiral acids (Scheme 2.25).[116] Enaminone **99** would be synthesized by conjugate addition and cyclization of **91** and **100** by our group's acetylenic sulfone methodology. Amino ester **100** would be obtained by the cycloaddition reaction of diene **94** and dimethyl fumarate, followed by hydrogenation.



Scheme 2.25

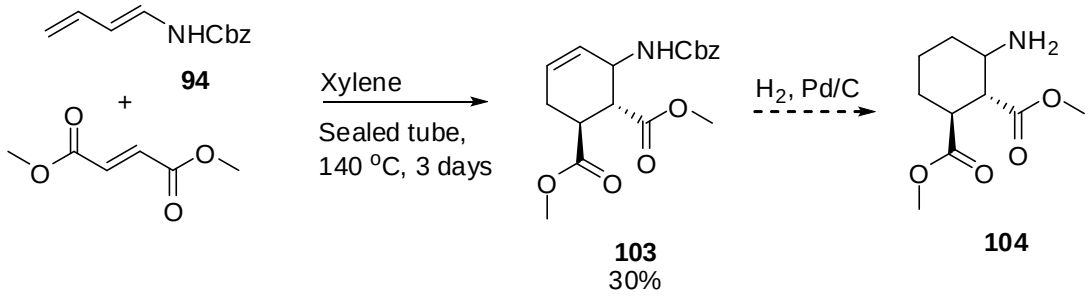
This would provide a cheap, commercially available dienophile, which is more reactive than **95**. Dimethyl fumarate is a popular choice for Diels-Alder reactions because of its low LUMO level due to the two electron-withdrawing ester groups directly bonded to the olefin moiety. A potential problem, however, might be the simultaneous formation of diastereomeric carbamate **102** as a side product (Scheme 2.26).



Scheme 2.26

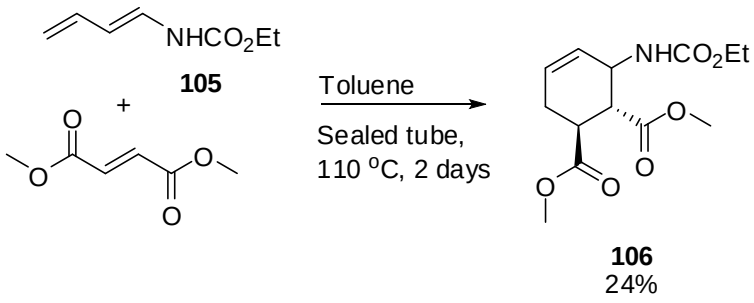
2.2.3.1 Attempted synthesis of amino ester 101

Dimethyl fumarate and diene **94** underwent cycloaddition to form a mixture of two diastereomers (Scheme 2.27). One diastereomer was isolated in 30% yield (**103**). Unfortunately its relative stereochemistry could not be elucidated by NMR due to interference by long range allylic coupling with the α-carbamate proton. Hydrogenation of unknown diastereomer **103** was carried out on a small scale in an attempt to determine the relative stereochemistry of amino ester **104** and in turn **103**; however this was not successful. Attempts to scale-up the reaction to form **103** also failed.



Scheme 2.27

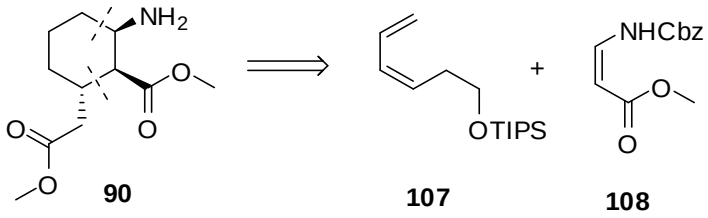
Ethyl *trans*-1,3-butadiene-1-carbamate (**105**), which was also first synthesized by Overman *et al*.,[95] was similarly tested in case steric hindrance of the Cbz-moiety of analogue **94** impeded the reaction (Scheme 2.28). Amino ester **106** was synthesized in 24% yield but also had the same issue with relative stereochemistry elucidation as carbamate **103**. This route was eventually abandoned due to the low yielding Diels-Alder reactions, unpredictability of relative stereochemistry and failure to scale-up.



Scheme 2.28

2.2.4 Second revised retrosynthesis

Amino ester **90** can also be retrosynthetically bisected as shown in Scheme 2.29. Compound **108** will be strongly polarized due to the push-pull interactions of the amino and ester functional groups, respectively. Diene **107** is substituted by a single alkyl group, which should promote the correct regiochemistry. Olefin **108** requires the *cis* geometry to ensure the correct relative stereochemistry, providing that the reaction goes through an *endo* transition state, which would be expected due to secondary orbital interactions (Figure 2.4).

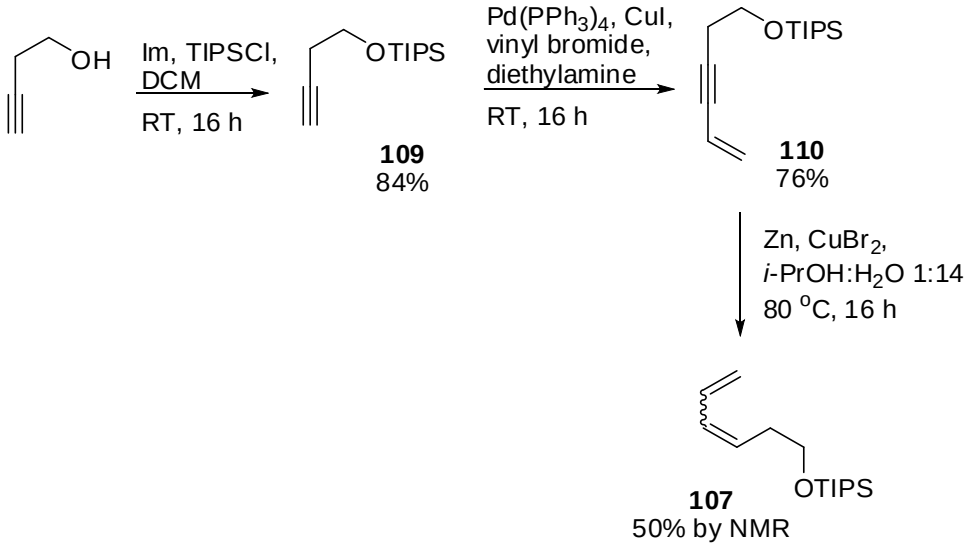


Scheme 2.29

Figure 2.4 a) *endo* and b) *exo* transition states for cycloaddition reaction of 107 and 108

2.2.4.1 Synthesis of diene 107

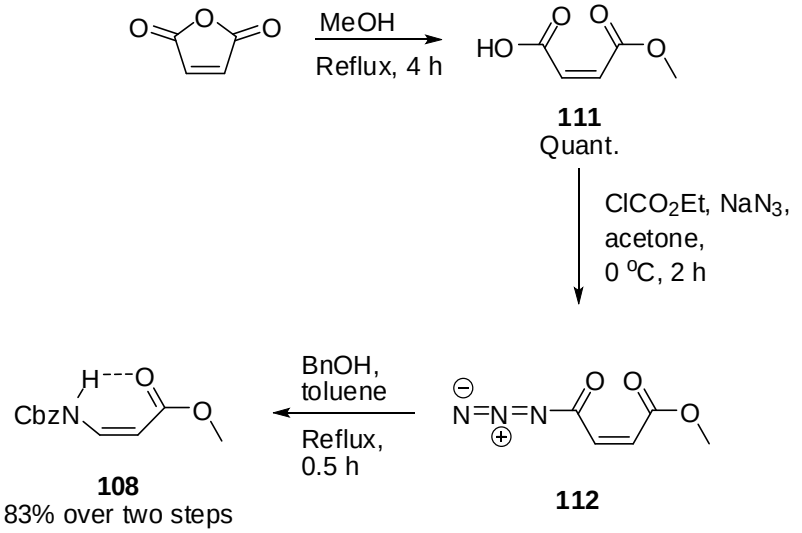
Silylation of but-3-yn-1-ol afforded TIPS-protected alcohol **109** in good yield (Scheme 2.30). This then underwent a Sonogashira reaction with vinyl bromide to produce enyne **110** cleanly on a 5 gram scale. Partial hydrogenation of **110** using Lindlar's catalyst resulted in an inseparable mixture of products with varying levels of saturation, even with optimization. Use of an activated Zn-Cu catalyst[117] was also used to produce crude **107** in 50% yield, which was indicated by NMR spectroscopy. Unfortunately, this product was inseparable from the side products and the *cis*-olefin orientation could not be verified by NMR spectroscopy. However, this catalyst reportedly gives only the corresponding *cis*-alkene derivative according to the literature.[117]



Scheme 2.30

2.2.4.2 Synthesis of dienophile 108

Refluxing maleic anhydride in methanol for 4 hours afforded a quantitative yield of half ester **111** (Scheme 2.31). Conversion to acyl azide **112** was followed by isocyanate formation and trapping to give amino ester **108**, which was obtained exclusively as the *cis* isomer, presumably due to intramolecular hydrogen bonding. The geometry and hydrogen bonding were confirmed by the coupling constants of the vinyl protons by ^{1}H-NMR spectroscopy (J = 8.8 Hz) and the downfield signal of the N-H proton at δ 9.79 ppm. Due to the high yield, high temperature required for formation and intramolecular hydrogen bonding, compound **108** was presumed to be the thermodynamic isomer.

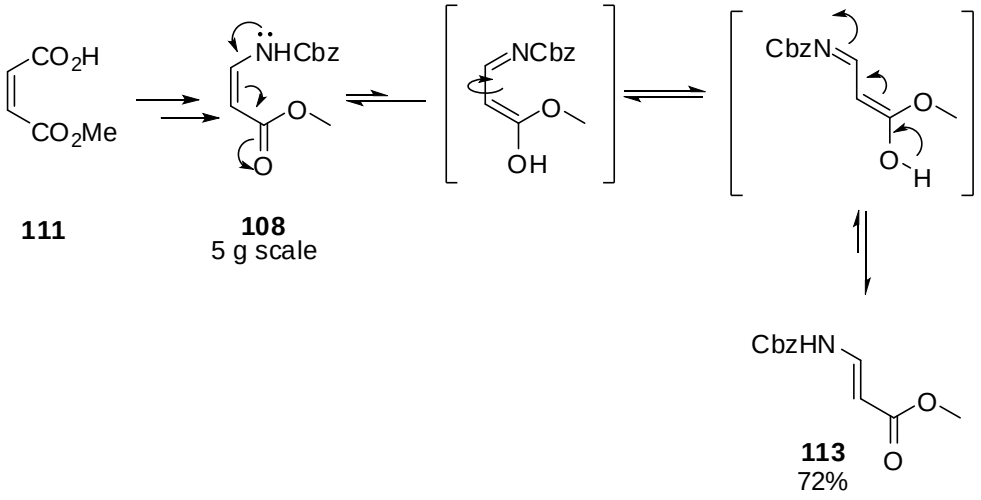


Scheme 2.31

Surprisingly, upon scale-up of this reaction, which required longer heating times to push the reaction to completion, only the *trans* isomer was isolated, albeit again in high yield. This is hypothesized to form via tautomerization, followed by free rotation and reformation as the *trans*-isomer, as proposed in Scheme 2.32. Alternatively, free

rotation in the corresponding zwitterion would also produce the *trans*-isomer. The driving force for this isomerization is attributed to the more thermodynamically stable *trans*-olefin, which appears to be even more stable than the intramolecularly hydrogen-bonded *cis*-olefin **108**. The formation of the *trans*-isomer was confirmed by ^{1}H-NMR spectroscopy from the coupling constants of the vinyl protons (J = 14.0 Hz).

To prove this hypothesis, *cis*-olefin **108** was refluxed in toluene for 2 hours, which resulted in full conversion to *trans*-olefin **113**. Due to this issue and the inability to obtain diene **107** in a pure form, an alternative approach would be required.



Scheme 2.32

2.2.5 Conclusion

A variety of routes for the synthesis of gephyrotoxin (**65**) were investigated; however, none of these routes were successful in affording the desired product due to difficulties imposed by obtaining the required starting materials and scaling-up of Diels-Alder reactions. Other potential Diels-Alder approaches need to be investigated, which will be discussed further in Section 2.3.2 on future directions toward gephyrotoxin.

2.3 Future work

2.3.1 BIRT-377

BIRT-377 and (*ent*)-BIRT-377 were successfully synthesized enantioselectivily through the PLE-mediated desymmetrization/Curtius rearrangement combination. Future work on this approach could involve the synthesis of more complex hydantoins, such as sorbinil (**114**), which is used for the treatment of diabetic complications, and the later stage BIRT compound, BIRT-2584 (**115**) (Figure 2.5).[37] This approach is not just limited to hydantoin-containing compounds but could include many other α-quaternary amino acid derivatives. The use of the PLE desymmetrization/Curtius rearrangement combination has also been employed in the attempted synthesis of (-)-adalinine (**116**), which only ran into difficulties in the late stages of its synthesis but achieved the highly enantioselective (>98% ee) formation of the α-quaternary center.[118]

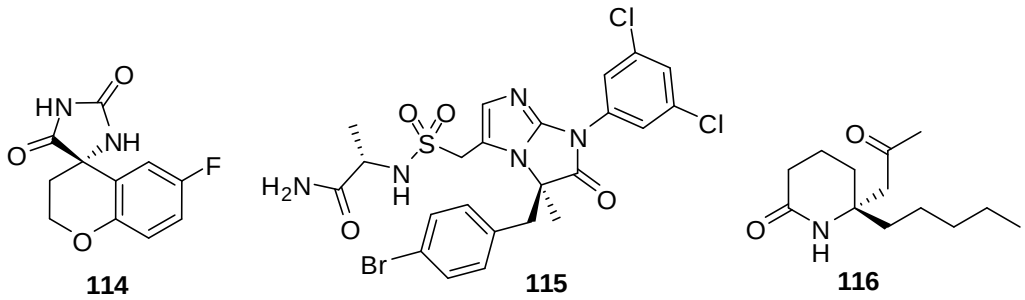
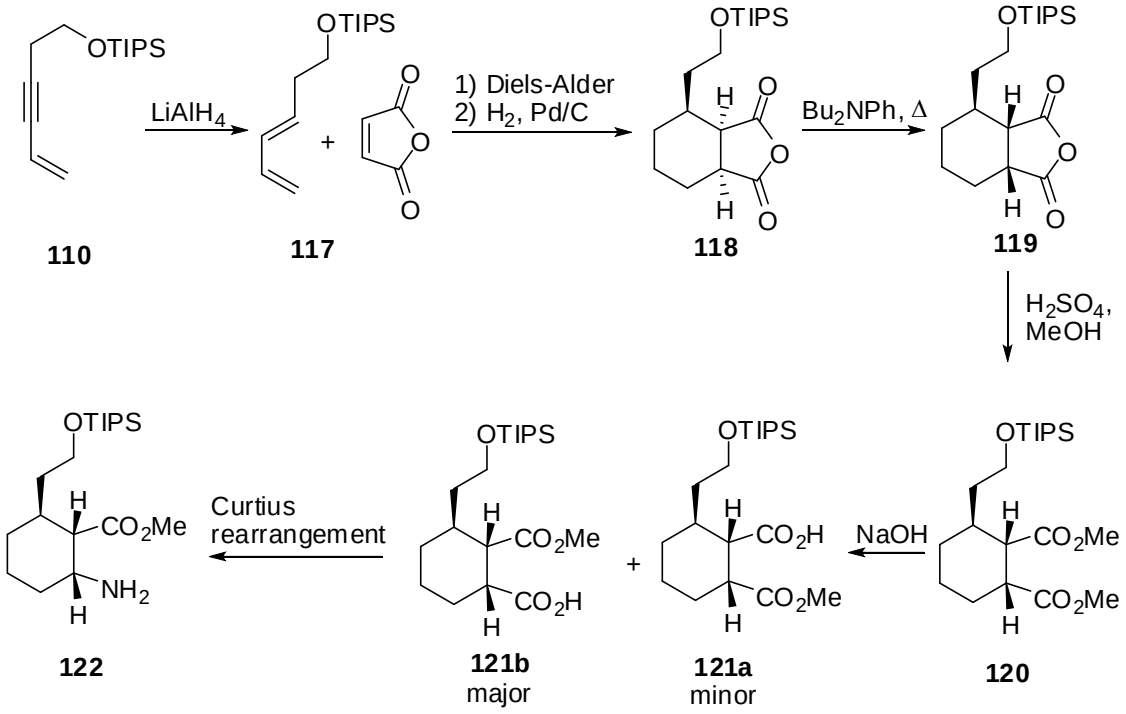


Figure 2.5: Potential future targets

2.3.2 Gephyrotoxin

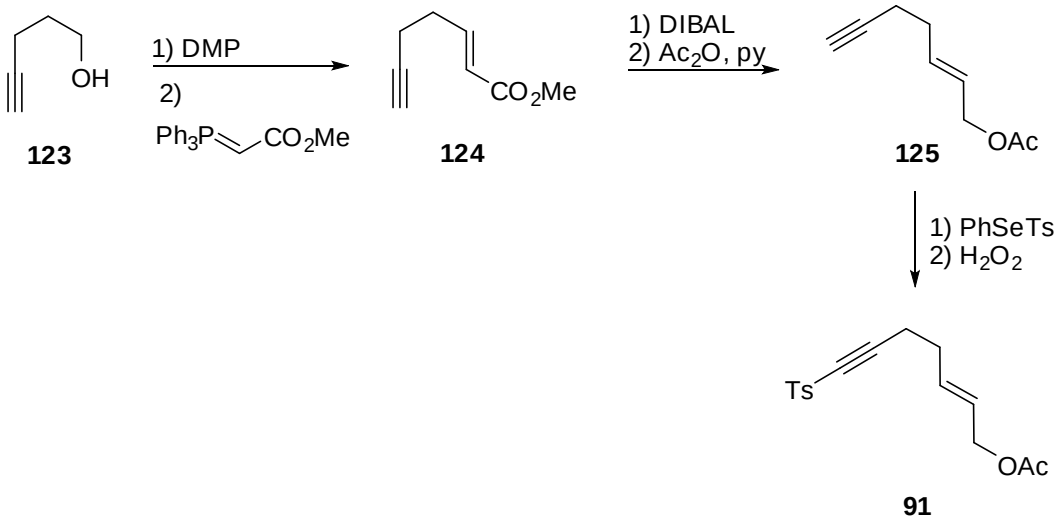
Other future work could be pursued toward the completion of the synthesis of gephyrotoxin, providing a successful Diels-Alder reaction can be carried out to establish the relative stereochemistry and obtain a practical key intermediate. The most obvious approach would be to synthesize dienophile **95** by the method of Tamelen *et al.*[119] and carry out the Diels-Alder reaction as shown retrosynthetically in Scheme 2.22.

Shown in Scheme 2.33 is a potential Diels-Alder approach adapted from our group's previous work on pumiliotoxin C.[77,120] Note that the approach to the latter compound also employed PLE-mediated desymmetrization to make the route enantioselective.[77] Starting with previously synthesized enyne **110**, reduction by lithium aluminum hydride would favor the formation of *trans*-olefin **117**. Cycloaddition with maleic anhydride and hydrogenation should afford product **118** with the undesired relative stereochemistry. Fortunately, based on our experience with the pumiliotoxin C synthesis, this can be epimerized to give the more thermodynamically stable product **119**. Opening of the anhydride moiety and hydrolysis of the less hindered ester group of **120** would afford half-ester **121b**, hopefully with high regioselectivity. Curtius rearrangement would then afford the elusive amino ester **122**.



Scheme 2.33

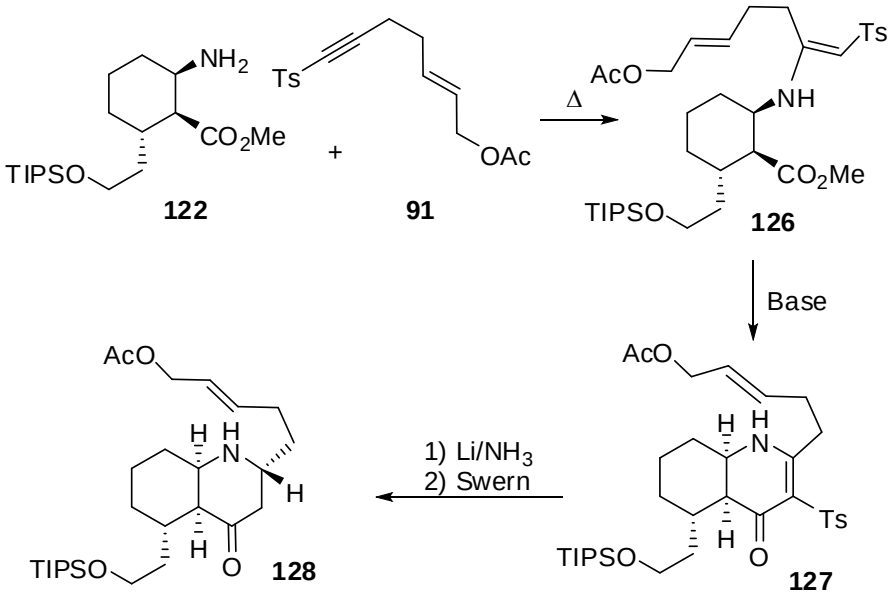
Acetylenic sulfone **91** could be synthesized from commercially available alcohol **123** (Scheme 2.34). Oxidation and Wittig reaction is expected to afford unsaturated ester **124**, followed by selective reduction and protection to give the known compound **125**.[121] Selenosulfonation would then produce acetylenic sulfone **90** by the method shown in Scheme 1.12.



123 **124** **125** **91**

Scheme 2.34

Finally the conjugate addition and cyclization of amino ester **122** to acetylenic sulfone **91** would afford enaminone **127** as shown in Scheme 2.35. Birch reduction of the enaminone group should give the correct diastereomer, which is the thermodynamically favored *exo*-product, as well as cleave the tosyl group. Reoxidation then affords ketone **128**.

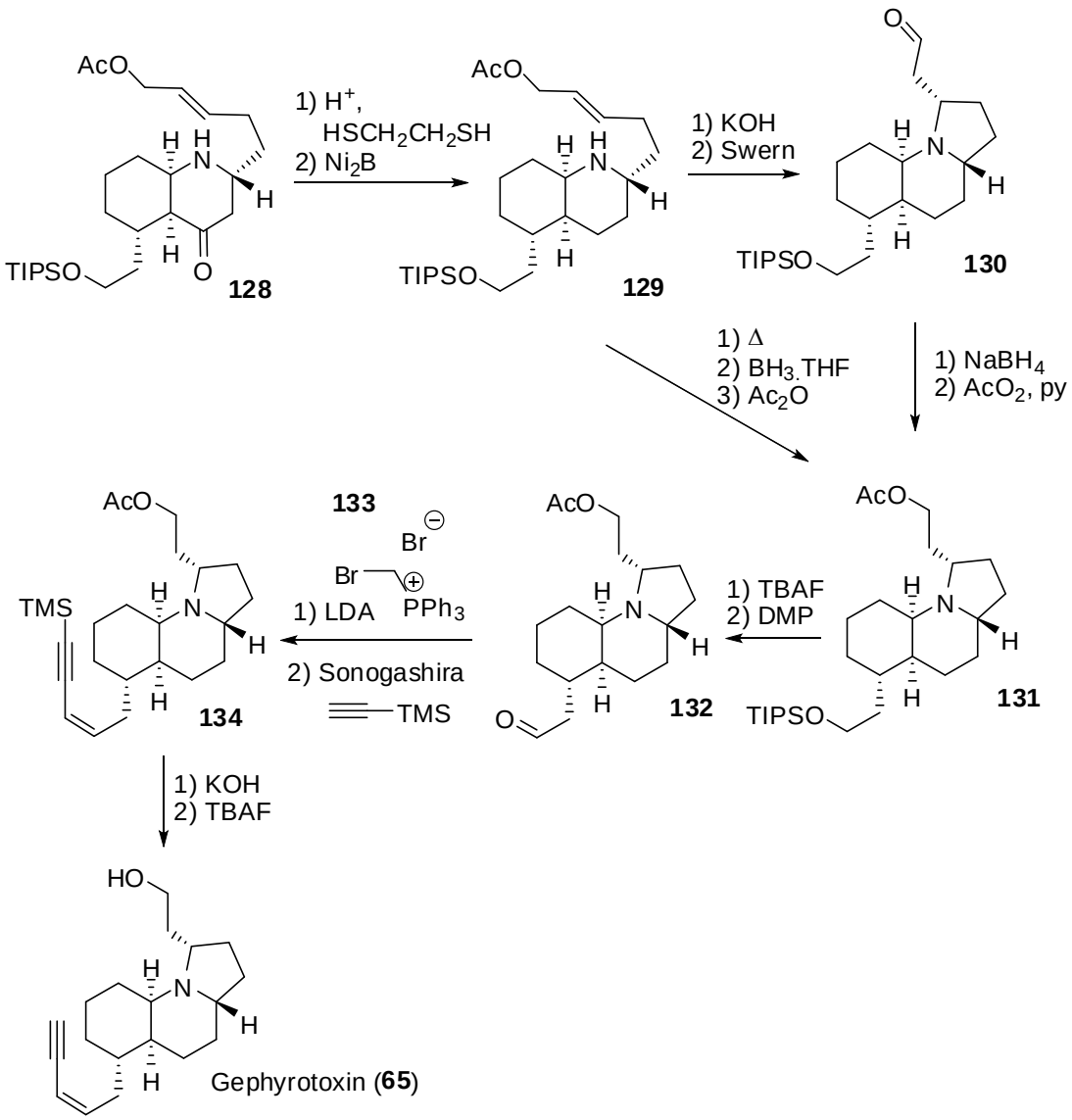


Scheme 2.35

The ketone moiety could be reduced via the corresponding dithiolane, followed by cleavage with nickel boride to afford key intermediate **129** (Scheme 2.36).[109] Hydrolysis of acyl alcohol **129**, followed by conversion to an *α,β*-unsaturated aldehyde group will allow for intramolecular conjugate addition to form aldehyde **130**. The stereochemical outcome of the aldehyde side-chain should favour the less congested *exo*-orientation. Reduction to the free alcohol with sodium borohydride and then protection should produce compound **131**.

Alternatively, the conversion of **129** to **131** might be carried out by S_N2' displacement of the acetate, followed by hydroboration and reacetylation. The silyl ether moiety on compound **131** can be removed using TBAF followed by oxidation, from which the exposed aldehyde could undergo a Wittig reaction to afford **134**. The *Z*-configuration would be accomplished with the use of the indicated ylide **133**, which is a non-stabilized ylide and commercially available. Sonogashira coupling would afford

the enyne moiety. Finally, the resulting enyne **134** ccould be deprotected in the presence of base and TBAF to afford gephyrotoxin (**65**).



Scheme 2.36

Chapter Three: Experimental

3.1 General remarks

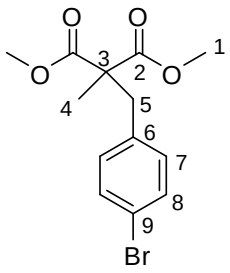
All reagents, unless otherwise noted, were obtained from commercial sources and purified by standard methods as necessary. THF, dioxane and ether were distilled over $LiAlH_4$ immediately prior to use or THF was obtained from an MBraun MB-SPS solvent purification system. Toluene and dichloromethane were dried by distillation over CaH_2. DMF was dried by distillation over $MgSO_4$ under vacuum. Alkyllithium reagents were titrated with menthol and 2,2'-bipyridyl immediately prior to use. Flash chromatography was performed on silica gel (230-400 mesh). Analytical TLC was carried out on aluminum backed plates coated with Merck silica gel 60 F-254, with detection by UV light or staining with phosphomolybdic acid solution in methanol. Melting points were measured using an A. H. Thomas hot-stage apparatus. IR spectra were recorded on a Nicolet Nexus 470 spectrometer, using attenuated total reflectance if stated. Porcine liver esterase (PLE) was obtained from Sigma-Aldrich Co. as a crude lyophilized powder from porcine liver with less than 5% buffer salt and an activity of 17 units/mg of solid. Specific rotations were obtained on a Rudolf Research Autopole IV.

NMR spectra were recorded in deuteriochloroform unless otherwise indicated. Referencing of chemical shifts was relative to the residual solvent (^{1}H NMR δ 7.26; ^{13}C δ 77.1 ppm). Assignments of primary, secondary, tertiary, and quaternary carbons, where indicated, were based upon DEPT-135 or HSQC analyses. Numbering of atoms in structures shown in this chapter was made for convenience in indicating spectral assignments, and does not necessarily reflect IUPAC nomenclature. ^{1}H NMR, ^{13}C NMR and HSQC data were collected on a Bruker DMX-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz), a

Bruker Avance III 400 MHz (^{1}H, 400 MHz; ^{13}C, 101 MHz) and a Bruker Avance 400 MHz (^{1}H, 400 MHz; ^{13}C, 101 MHz). Low and high resolution mass spectra were obtained on a Waters GCT Premier, a Thermo Finnigan SSQ7000, a Bruker Esquire 3000, a Agilent 6520 Q-Tof, or a Bruker FT-ICR MS Apex mass spectrometer by Ms. Q. Wu, Ms. D. Fox, Mr. J. Li, or Mr. W. White. All mass spectra were obtained by electron impact ionization at 70 eV with direct probe sample introduction unless otherwise indicated. Elemental analyses were determined by Mr. J. Li using a Perkin Elmer Series II 2400 CHNS/O Analyzer.

3.2 Experiments pertaining to BIRT-377

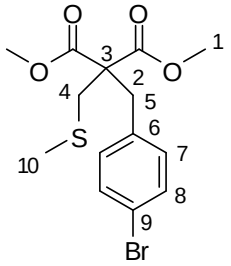
3.2.1 Dimethyl 2-(4-bromobenzyl)-2-methylmalonate (73)



Diisopropylamine (2.43 g, 3.37 mL, 24.1 mmol) was dissolved in dry THF (100 mL) at 0 °C under argon. *n*-Butyllithium (9.6 mL, 2.5 M in hexanes, 24 mmol) was added dropwise over 5 minutes. Dimethyl 2-methylmalonate (3.5 g, 3.2 mL, 24 mmol) was added dropwise over 20 minutes. The mixture was stirred at room temperature for 1 hour and then 1-bromo-4-(bromomethyl)benzene (7.212 g, 28.84 mmol) dissolved in dry THF (50 mL) was added to the mixture via cannula transfer. The mixture was refluxed for 16 h. It was then quenched with brine and extracted with diethyl ether. The diethyl ether extracts were dried with magnesium sulfate and concentrated *in vacuo* to afford **73** (7.50

g, 23.8 mmol, 98 %) as a pale yellow solid: R_f 0.16 (ethyl acetate:hexanes, 1:2); MP 55 – 56 °C; IR (film) 1733 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.38 (d, *J* = 8.4 Hz, 2 H, H-8), 6.98 (d, *J* = 8.4 Hz, 2 H, H-7), 3.72 (s, 6 H, H-1), 3.17 (s, 2 H, H-5), 1.34 (s, 3 H, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ 172.2 (C-2), 135.2 (C-6), 132.0 (C-8), 131.5 (C-7), 121.2 (C-9), 54.9 (C-3), 52.7 (C-1), 40.8 (C-5), 19.9 (C-4); mass spectrum (EI), (*m/z*, %) 316 (30, M^+, ^{81}Br), 314 (32, M^+, ^{79}Br), 256 (89), 254 (91), 196 (22), 194 (22), 171 (100), 169 (98), 115 (51); HRMS (EI) calc'd for $C_{13}H_{15}{}^{81}BrO_4$: 316.0133; found: 316.0124; calc'd for $C_{13}H_{15}{}^{79}BrO_4$: 314.0154; found: 314.0143.

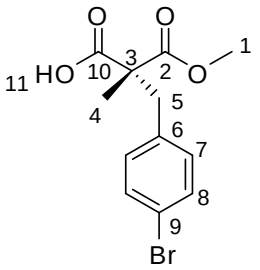
3.2.2 Dimethyl 2-(4-bromobenzyl)-2-(methylthiomethyl)malonate (75)



Sodium hydride (199 mg, 60% dispersion in mineral oil, 4.98 mmol) was dissolved in dry THF (100 mL) at 0°C under argon. Compound **71** (1.00 g, 3.32 mmol) dissolved in dry THF (15 mL) was added dropwise and the mixture was stirred for 2 hours. Chloromethyl methyl sulfide (818 mg, 710 µL, 8.30 mmol) dissolved in THF (2 mL) was then added and the mixture was refluxed for 16 hours. It was quenched with saturated ammonium chloride solution and extracted with diethyl ether. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to afford **75** (506 mg, 1.39 mmol, 42 %) as a pale yellow oil: R_f 0.35 (ethyl acetate:hexanes, 1:4); IR (film) 1733 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.39 (d, *J* = 8.4 Hz, 2 H, H-8), 7.01 (d, *J*

= 8.4 Hz, 2 H, H-7), 3.34 (s, 6 H, H-1), 3.31 (s, 2 H, H-4 or 5), 2.92 (s, 2 H, H-4 or 5), 2.10 (s, 3 H, H-10); ^{13}C NMR (400 MHz; $CDCl_3$) δ 170.4 (C-2), 134.7 (C-6), 131.7 (C-7 and C-8), 121.4 (C-9), 59.4 (C-3), 52.9 (C-1), 37.1 (C-4 or 5), 36.7 (C-4 or 5), 17.1 (C-10); mass spectrum (EI), (*m/z*, %) 362 (5, M^+, ^{81}Br), 360 (4, M^+, ^{79}Br), 269 (15), 267 (14), 191 (100), 159 (91); HRMS (EI) calc`d for $C_{14}H_{17}{}^{81}BrO_4S$: 362.0010; found: 362.0008; calc`d for $C_{14}H_{17}{}^{79}BrO_4S$: 360.0031; found: 360.0046.

3.2.3 (*R*)-2-(4-Bromobenzyl)-3-methoxy-2-methyl-3-oxopropanoic acid (74)



Diester **73** (400 mg, 1.27 mmol) in 2-propanol (4 mL) was added to sodium phosphate buffer (76 mL, 0.2 M, pH 8). PLE (50 mg) was added to the 2-propanol solution of **73**. The mixture was stirred for 1 week at 0 ^{O}C. It was then acidified with 1 M hydrochloric acid and extracted with dichloromethane. The organic fractions were filtered through Celite and then extracted with 1 M potassium hydroxide. Unreacted starting material **73** (152 mg, 38%) was recovered by evaporation of the organic layer and chromatography over silica-gel (ethyl acetate:hexanes, 1:2). The aqueous layer was reacidified with 1 M hydrochloric acid and extracted with dichloromethane. The combined organic phases were dried with magnesium sulfate and concentrated *in vacuo* to yield **74** (225 mg, 0.747 mmol, 59%, 97% BRSM) as an off white solid: R_f 0.18 (ethyl acetate:hexanes + acetic acid, 1:2 + 5%); MP 61 – 63 °C; IR (film) 2995, 1705 cm^{-1}; ^{1}H

NMR (300 MHz; $CDCl_3$) δ 11.24 (br s, 1 H, H-11), 7.40 (d, *J* = 8.4 Hz, 2 H, H-8), 7.03 (d, *J* = 8.4 Hz, 2 H, H-7), 3.76 (s, 3 H, H-1), 3.25 (d, *J* = 13.7 Hz, H-5α), 3.15 (d, *J* = 13.7 Hz, H-5β), 1.39 (s, 3 H, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ 177.7 (C-10), 172.0 (C-2), 134.8 (C-6), 132.0 (C-8), 131.6 (C-7), 121.4 (C-9), 54.9 (C-3), 53.0 (C-1), 40.8 (C-5), 19.9 (C-4); mass spectrum (EI), (*m/z*, %) 302 (12, M^+, ^{81}Br), 300 (10, M^+, ^{79}Br), 256 (22), 254 (20), 171 (100), 169 (98); HRMS (EI) calc'd for $C_{12}H_{13}{}^{81}BrO_4$: 301.9977; found: 301.9991; calc'd for $C_{12}H_{13}{}^{79}BrO_4$: 299.9997; found: 299.9987; ee 75%.

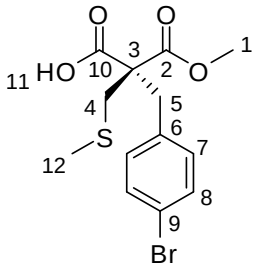
A larger batch of half-ester **74** (2.64 g, ee 70%) was prepared similarly, except at room temperature for 2 days. Recrystallization from ethyl acetate-hexane afforded 1.96 g of product in two crops that were combined (88% recovery of the major enantiomer); MP 71-72 °C; $[\alpha]_D^{20}$ -5.4 (c 4.6, dichloromethane); ee > 98%.

The ees for the above products were measured by integration of well separated methyl and benzylic signals in the ^1H NMR spectrum of an equimolar mixture of **74** and *R*-(+)-*α*-methylbenzylamine in $CDCl_3$.

A racemic sample of **74** was prepared by dissolving diester **73** (200 mg, 0.635 mmol) in methanol (20 mL). Potassium hydroxide (355 mg, 6.35 mmol) dissolved in water (10 mL) was added and the mixture was stirred for 4 hours at 16 °C. The mixture was then washed with dichloromethane, acidified with 1 M hydrochloric acid and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to afford the racemic half-ester **74** (162 mg, 0.530 mmol, 85%) as a yellow oil with NMR spectra identical to the sample prepared by PLE hydrolysis. Racemic half-ester **74** was treated with an equimolar amount of

R-(+)-*α*-methylbenzylamine in $CDCl_3$ to ensure that NMR signals were well separated and identified in the diastereomeric salt.

3.2.4 (*S*)-2-(4-Bromobenzyl)-3-methoxy-2-(methylthiomethyl)-3-oxopropanoic acid (76)



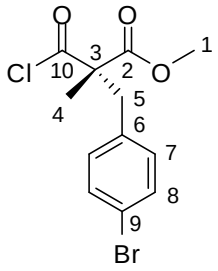
Diester **75** (100 mg, 0.28 mmol) in 2-propanol (4 mL) was added to sodium phosphate buffer (36 mL, 0.2 M, pH 8). PLE (14 mg) was added to the 2-propanol solution of **75**. The mixture was stirred for 2 days at room temperature. It was then acidified with 1 M hydrochloric acid and extracted with dichloromethane. The organic fractions were filtered through Celite and then extracted with 1 M potassium hydroxide. The aqueous layer was reacidified with 1 M hydrochloric acid and extracted with dichloromethane. The combined organic phases were dried with magnesium sulfate and concentrated *in vacuo* to yield **76** (7 mg, 0.02 mmol, 7%) as a pale yellow oil: R_f 0.10 (ethyl acetate:hexanes + acetic acid, 1:9 + 1%); IR (film) 1742, 1700 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 10.74 (br s, 1 H, H-11), 7.40 (d, *J* = 8.4 Hz, 2 H, H-8), 7.03 (d, *J* = 8.4 Hz, 2 H, H-7), 3.80 (s, 3 H, H-1), 3.35 (d, *J* = 13.8 Hz, H-4α or 5α), 3.24 (d, *J* = 13.8 Hz, H-4β or 5β), 3.04 (d, *J* = 13.4 Hz, H-4α or 5α), 2.96 (d, *J* = 13.4 Hz, H-4β or 5β), 2.14 (s, 3 H, H-12); ^{13}C NMR (400 MHz; $CDCl_3$) δ 174.7 (C-10), 171.5 (C-2), 134.1 (C-6), 131.9 (C-8), 131.5 (C-7), 121.7 (C-9), 59.9 (C-3), 53.3 (C-1), 38.6 (C-4 or 5), 37.6 (C-4 or 5),

17.1 (C-12); mass spectrum (EI), (*m/z*, %) 348 (2, M^+, ^{81}Br), 346 (2, M^+, ^{79}Br), 256 (35), 254 (37), 196 (48), 194 (52), 115 (100); HRMS (EI) calc`d for $C_{13}H_{15}{}^{81}BrO_4S$: 347.9854; found: 347.9856; calc`d for $C_{13}H_{15}{}^{79}BrO_4S$: 345.9874; found: 345.9866; $[\alpha]_D^{20}$ -3.2 (c 0.5, dichloromethane); ee 41%.

The ee was measured by integration of well separated methyl and benzylic signals in ^1H NMR spectrum of an equimolar mixture of **76** and *R*-(+)-*α*-methylbenzylamine in benzene-d_6.

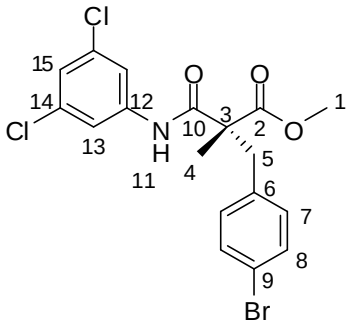
A racemic sample of **76** was prepared by dissolving diester **75** (90 mg, 0.25 mmol) in methanol (20 mL). Potassium hydroxide (189 mg, 2.48 mmol) dissolved in water (10 mL) was added and the mixture was stirred for 4 hours at 16 °C. The mixture was then washed with dichloromethane, acidified with 1 M hydrochloric acid and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to afford the racemic half-ester **76** (39 mg, 0.11 mmol, 45%) as a yellow oil with NMR spectra identical to the sample prepared by PLE hydrolysis. Racemic half-ester **76** was treated with an equimolar amount of *R*-(+)-α-methylbenzylamine in benzene-d_6 to ensure that NMR signals were well separated and identified in the diastereomeric salt.

3.2.5 (*S*)-Methyl 2-(4-bromobenzyl)-3-chloro-2-methyl-3-oxopropanoate (78)



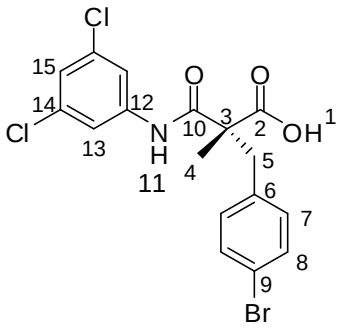
Half-ester **74** (145 mg, 0.480 mmol) was dissolved in dichloromethane (50 mL). Thionyl chloride (295 mg, 180 µL, 2.48 mmol) was then added and the mixture was refluxed for 6 hours under argon. The mixture was then concentrated *in vacuo* to afford acid chloride **78** (150 mg, 0.477 mmol, >99%) as a light brown oil: R_f 0.82 (ethyl acetate:hexanes, 1:2); IR (film) 1788, 1746 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.42 (d, *J* = 8.4 Hz, 2 H, H-8), 7.02 (d, *J* = 8.4 Hz, 2 H, H-7), 3.80 (s, 3 H, H-1), 3.27 (t, *J* = 15 Hz, H-5), 1.45 (s, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ 173.2 (C-10), 169.8 (C-2), 133.7 (C-6), 132.0 (C-8), 131.8 (C-7), 121.9 (C-9), 64.7 (C-3), 53.4 (C-1), 40.8 (C-5), 20.1 (C-4); mass spectrum (EI), (*m/z*, %) 320 (12, M^+, ^{81}Br), 318 (10, M^+, ^{79}Br), 256 (32), 254 (34), 171 (100), 169 (94); HRMS (EI) calc'd for $C_{12}H_{12}{}^{81}Br^{35}ClO_3$: 319.9638; found: 319.9642; calc'd for $C_{12}H_{12}{}^{79}Br^{35}ClO_3$: 317.9658; found: 317.9667.

3.2.6 (*R*)-Methyl 2-(4-bromobenzyl)-3-(3,5-dichlorophenylamino)-2-methyl-3-oxopropanoate (79)



Acid chloride **78** (215 mg, 0.673 mmol) and 3,5-dichloraniline (109 mg, 0.673 mmol) were dissolved in dichloromethane (5 mL) and stirred at room temperature for 14 hours. The mixture was washed with saturated sodium bicarbonate, followed by brine and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to yield **79** (274 mg, 0.616 mmol, 92%) as a pale pink oil: R_f 0.63 (ethyl acetate:hexanes, 1:2); IR (film) 3388, 1715, 1645 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 9.61 (br s, 1 H, H-11), 7.48 (d, *J* = 1.8 Hz, 2 H, H-13), 7.36 (d, *J* = 8.4 Hz, 2 H, H-8), 7.10 (t, *J* = 1.8 Hz, 1 H, H-15), 6.95 (d, *J* = 8.4 Hz, 2 H, H-7) 3.77 (s, 3 H, H-1), 3.38 (d, *J* = 13.5 Hz, H-5α), 3.08 (d, *J* = 13.5 Hz, H-5β), 1.55 (s, 3 H, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ 175.6 (C-10), 169.2 (C-2), 139.4 (C-12), 135.3 (C-14), 135.2 (C-6), 131.7 (C-8), 131.3 (C-7), 124.6 (C-15), 121.5 (C-9), 118.6 (C-13), 55.5 (C-3), 53.1 (C-1), 44.1 (C-5), 22.1 (C-4); mass spectrum (EI), (*m/z*, %) 445 (18, M^+, ^{81}Br), 443 (16, M^+, ^{79}Br), 256 (36), 254 (32), 225 (38), 223 (42), 171 (98), 169 (100); HRMS (EI) calc'd for $C_{18}H_{16}{}^{81}Br^{35}Cl_2NO_3$: 444.9670; found 444.9673; calc'd for $C_{18}H_{16}{}^{79}Br^{35}Cl_2NO_3$: 442.9691; found: 442.9699; $[\alpha]_D^{20}$ +60.1 (c 3.0, dichloromethane).

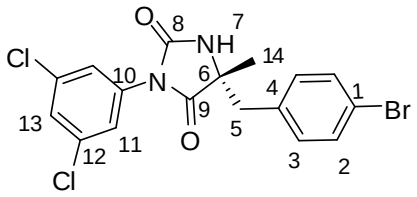
3.2.7 (*R*)-2-(4-Bromobenzyl)-3-(3,5-dichlorophenylamino)-2-methyl-3-oxopropanoic acid (80)



Amide **79** (240 mg, 0.539 mmol) was dissolved in methanol (5 mL). Potassium hydroxide (302 mg, 5.39 mmol) in water (6 mL) was added and the solution was stirred for 20 h at room temperature. The mixture was then extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to obtain recovered starting material **79** (91 mg, 38%). The aqueous layer was acidified with 1 M HCl and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to afford **80** (135 mg, 0.313 mmol, 58%, 96% BRSM) as a pale pink solid: R_f 0.18 (ethyl acetate:hexanes + acetic acid, 1:2 + 5%); MP 158 – 159 °C; IR (ATR) 3080, 1712, 1655 cm^{-1}; ^1H NMR (400 MHz; CD_3OD) δ 7.58 (d, *J* = 1.9 Hz, 2 H, H-13), 7.39 (d, *J* = 8.4 Hz, 2 H, H-8), 7.17 (t, *J* = 1.9 Hz, 1 H, H-15), 7.10 (d, *J* = 8.4 Hz, 2 H, H-7), 3.29 (d, *J* = 13.6 Hz, H-5α), 3.23 (d, *J* = 13.6 Hz, H-5β), 1.45 (s, 3 H, H-4); ^{13}C NMR (400 MHz; CD_3OD) δ 176.0 (C-2), 172.4 (C-10), 141.7 (C-12), 137.1 (C-6), 136.0 (C-14), 133.3 (C-8), 132.3 (C-7), 124.9 (C-15), 121.9 (C-9), 120.1 (C-13), 56.9 (C-3), 42.6 (C-5), 21.0 (C-4); mass spectrum (EI), (*m/z*, %) 431 (<1, M^+, ^{81}Br), 429 (<1, M^+, ^{79}Br), 387 (33), 199 (56), 197 (53), 171 (100), 169 (90), 161 (74);

HRMS (EI) calc'd for $C_{17}H_{14}{}^{81}Br^{35}Cl_2NO_3$: 430.9514; found: 430.9506; calc'd for $C_{17}H_{14}{}^{79}Br^{35}Cl_2NO_3$: 428.9534; found: 428.9554; $[\alpha]_D{}^{20}$ +83 (c 1.9, dichloromethane).

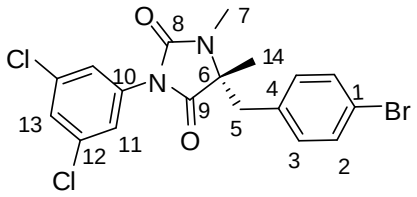
3.2.8 (*R*)-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methylimidazolidine-2,4-dione (20)



Diphenylphosphoryl azide (100 µL, 126 mg, 0.464 mmol) was added to a mixture of **80** (100 mg, 0.232 mmol), potassium carbonate (35 mg, 0.26 mmol) and silver carbonate (64 mg, 0.23 mmol) dissolved in dry dioxane (10 mL). The mixture was stirred at room temperature under argon for 1 hour followed by 22 hours at reflux. It was washed with 2M hydrochloric acid then saturated sodium bicarbonate and finally brine. The mixture was extracted with ethyl acetate. The organic layers were combined and dried over magnesium sulfate. This was then concentrated in *vacuo* to afford **20** as a colourless oil (56 mg, 0.13 mmol, 56%). Rf 0.21 (ethyl acetate:hexanes, 1:2); IR (film) 3276, 1771, 1714 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 7.43 (d, *J* = 8.4 Hz, 2 H, H-2), 7.34 (t, *J* = 1.7 Hz, 1 H, H-13), , 7.14 (br s, 1 H, H-7), 7.05 (d, *J* = 8.4 Hz, 2 H, H-3), 6.96 (d, *J* = 1.9 Hz, 2 H, H-11), 3.13 (d, *J* = 13.7 Hz, H-5α), 2.88 (d, *J* = 13.7 Hz, H-5β), 1.59 (s, 3 H, H-14); ^{13}C NMR (400 MHz; $CDCl_3$) δ 174.3 (C-9), 155.1 (C-8), 135.4 (C-10), 133.0 (C-13), 133.0 (C-4), 131.9 (C-2), 131.8 (C-3), 128.7 (C-12), 124.7 (C-11), 122.1 (C-1), 63.0 (C-6), 43.7 (C-5), 23.5 (C-14); mass spectrum (EI), (*m/z*, %) 428 (18, M^+, ^{81}Br), 426 (12, M^+, ^{79}Br), 259 (23), 257 (32), 171 (100), 169 (94); HRMS (EI) calc`d for

$C_{17}H_{13}{}^{81}Br^{35}Cl_2N_2O_2$: 427.9517; found: 427.9521; calc`d for $C_{17}H_{13}{}^{79}Br^{35}Cl_2N_2O_2$: 425.9537; found: 425.9544. $[\alpha]_D{}^{20}$ +120 (c 2.00, dichloromethane).

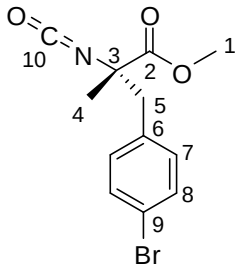
3.2.9 (*R*)-5-(4-Bromobenzyl)-3-(3,5-dichlorophenyl)-1,5-dimethylimidazolidine-2,4-dione, BIRT-377 (11)



The procedure of Magriotis *et al*.[44] was followed. Hydantoin **20** (22 mg, 0.051 mmol) was dissolved in dry *N*,*N*-dimethylformamide (0.5 mL) at 0°C. A mixture of Bis(trimethylsilyl)amine (10 mg, 13 µL, 0.062 mmol) and *n*-butyllithium (5.2 mg, 25 µL, 2.5 M in hexanes, 0.062 mmol) in toluene (0.5 mL) was then added drop wise to the hydantion solution and stirred for 30 minutes. Iodomethane (22mg, 10 µL, 0.16 mmol) was added. The mixture was stirred for a further 3 hours at room temperature. It was quenched with water and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to afford BIRT-377 (**11**) (22 mg, 0.050 mmol, 97%). R_f 0.23 (ethyl acetate:hexanes, 1:2); MP 136 – 137 °C; IR (film) 1776, 1724 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 7.42 (d, *J* = 8.4 Hz, 2 H, H-2), 7.29 (t, *J* = 1.8 Hz, 1 H, H-13), 6.95 (d, *J* = 8.4 Hz, 2 H, H-3), 6.85 (d, *J* = 1.9 Hz, 2 H, H-11), 3.10 (d, *J* = 14.0 Hz, H-5α), 3.07 (s, 3 H, H-7), 2.97 (d, *J* = 14.0 Hz, 1 H, H-5β), 1.62 (s, 3 H, H-14); ^{13}C NMR (400 MHz; $CDCl_3$) δ 173.6 (C-9), 153.7 (C-8), 135.3 (C-10), 133.3 (C-13), 133.1 (C-4), 132.1 (C-2), 131.3 (C-3), 128.6 (C-12), 124.7 (C-11), 122.2 (C-1), 65.9 (C-6), 41.0 (C-5), 25.5 (C-7), 21.3 (C-14); mass spectrum (EI), (*m/z*, %) 442 (3, M^+,

^{81}Br), 440 (2, M^+, ^{79}Br), 271 (100), 56 (71); HRMS (EI) calc'd for $C_{18}H_{15}{}^{81}Br^{35}Cl_2N_2O_2$: 441.9673; found: 441.9656; calc'd for $C_{18}H_{15}{}^{79}Br^{35}Cl_2N_2O_2$: 439.9694; found: 439.9688. Elemental analysis calc'd for $C_{18}H_{15}BrCl_2N_2O_2$: C, 48.90; H, 3.42; N, 6.34; found: C, 49.09; H, 3.42; N, 6.38. $[\alpha]_D^{20}$ +135 (c 1.00, ethanol); ee >98% based on HPLC analysis (Chiralpak AD column; 250 mm x 4.6 mm; 10 μm particle size. Solvent system: isocratic conditions using 95:5 hexanes-(*i*-PrOH + 10% diethylamine); injection solvent: 9:1 hexanes–ethyl acetate. Detector: UV, 254 nm).

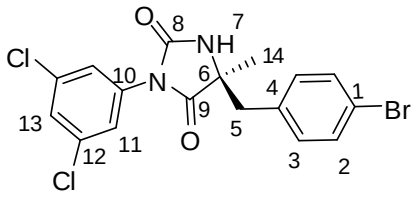
3.2.10 (*S*)-Methyl 3-(4-bromophenyl)-2-isocyanato-2-methylpropanoate (85)



Half-ester **74** (480 mg, 1.59 mmol), diphenylphosphoryl azide (412 mg, 325 μL, 1.51 mmol), triethylamine (177 mg, 254 μL, 1.75 mmol) and 4-dimethylaminopyridine (214 mg, 1.75 mmol) were dissolved in toluene (40 mL) and refluxed for 6 hours under argon. The mixture was then washed with saturated sodium bicarbonate, followed by brine and extracted with diethyl ether. The combined organic layers were dried over magnesium sulfate to afford crude isocyanate **85** (482 mg, 1.62 mmol) as a colourless oil: R_f 0.76 (ethyl acetate:hexanes, 1:2); IR (film) 2248, 1738 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.44 (d, *J* = 8.4 Hz, 2 H, H-8), 7.05 (d, *J* = 8.4 Hz, 2 H, H-7), 3.78 (s, 3 H, H-1), 3.11 (d, *J* = 13.5 Hz, H-5α), 2.85 (d, *J* = 13.5 Hz, H-5β), 1.55 (s, H-4); ^{13}C NMR

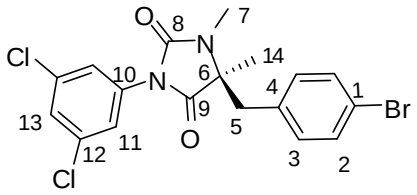
(400 MHz; $CDCl_3$) δ 173.0 (C-2), 134.0 (C-6), 131.7 (C-8), 131.5 (C-7), 121.7 (C-9), 65.0 (C-10), 53.0 (C-3), 45.5 (C-1), 29.7 (C-5), 26.9 (C-4).

3.2.11 (*S*)-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methylimidazolidine-2,4-dione ((*ent*)-20)



Boron trifluoride diethyl etherate (36 μL, 42 mg, 0.30 mmol) was added to a mixture of crude **85** (88 mg, 0.30 mmol) and 3,5-dichloroaniline (48 mg, 0.30 mmol) dissolved in dry toluene (15 mL). The mixture was then stirred under argon at room temperature for 3 hours followed by reflux for 20 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layers were dried over magnesium sulfate and concentrated in *vacuo* to afford hydantoin **(*ent*)-20** (45 mg, 0.11 mmol, 36%) as a colourless oil. R_f 0.21 (ethyl acetate:hexanes, 1:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.44 (d, *J* = 8.4 Hz, 2 H, H-2), 7.34 (t, *J* = 1.7 Hz, 1 H, H-13), 7.05 (d, *J* = 8.4 Hz, 2 H, H-3), 6.98 (d, *J* = 1.9 Hz, 2 H, H-11), 6.93 (br s, 1 H, H-7), 3.13 (d, *J* = 13.7 Hz, H-5α), 2.89 (d, *J* = 13.7 Hz, H-5β), 1.59 (s, 3 H, H-14); ^{13}C NMR (400 MHz; $CDCl_3$) δ 174.3 (C-9), 154.9 (C-8), 135.4 (C-10), 133.0 (C-4), 131.9 (C-2, C-3), 128.7 (C-12), 124.7 (C-11), 122.2 (C-1), 62.9 (C6), 43.4 (C-5), 23.6 (C-14); $[\alpha]_D^{20}$ -119 (c 1.30, dichloromethane).

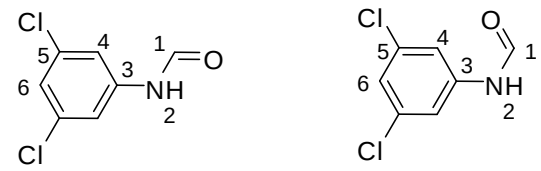
3.2.12 (*S*)-5-(4-Bromobenzyl)-3-(3,5-dichlorophenyl)-1,5-dimethylimidazolidine-2,4-dione, *ent*-BIRT-377 ((*ent*)-11)



The procedure of Magriotis *et al*.[44] was followed. Hydantoin **(*ent*)-20** (22 mg, 0.051 mmol) was dissolved in dry *N*,*N*-dimethylformamide (0.5 mL) at 0 °C. A mixture of Bis(trimethylsilyl)amine (10 mg, 13 µL, 0.062 mmol) and *n*-butyllithium (5.2 mg, 25 µL, 2.5 M in hexanes, 0.062 mmol) in toluene (0.5 mL) was then added drop wise to the hydantion solution and the mixture was stirred for 30 minutes. Iodomethane (22 mg, 10 µL, 0.16 mmol) was added. The mixture was stirred for a further 3 hours at room temperature. It was quenched with water and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to afford (*ent*)-BIRT-377 **((*ent*)-11)** (22mg, 0.050 mmol, 97%). R_f 0.23 (ethyl acetate:hexanes, 1:2); MP 134 - 135 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.42 (d, *J* = 8.4 Hz, 2 H, H-2), 7.29 (t, *J* = 1.8 Hz, 1 H, H-13), 6.94 (d, *J* = 8.4 Hz, 2 H, H-3), 6.85 (d, *J* = 1.9 Hz, 2 H, H-11), 3.09 (d, *J* = 17.0 Hz, H-5α), 3.07 (s, 3 H, H-7), 2.96 (d, *J* = 14.0 Hz, 1 H, H-5β), 1.62 (s, 3 H, H-14); ^{13}C NMR (400 MHz; $CDCl_3$) δ 173.5 (C-9), 153.6 (C-8), 135.2 (C-10), 133.2 (C-13), 133.0 (C-4), 132.0 (C-2), 131.3 (C-3), 128.5 (C-12), 124.7 (C-11), 122.2 (C-1), 65.8 (C-6), 40.9 (C-5), 25.5 (C-7), 21.2 (C-14); $[\alpha]_D^{20}$ -128 (c 1.00, ethanol); 94% ee based on rotation observed for BIRT-377 (+135°).

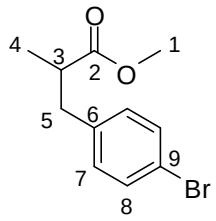
3.2.13 N-(3,5-dichlorophenyl)formamide (86)

a) **b)**



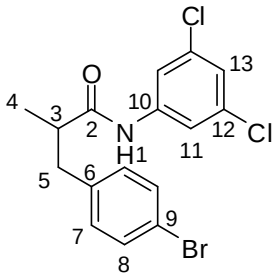
3,5-Dichloroaniline (405 mg, 2.50 mmol) was dissolved in toluene (20 mL). Formic acid (25 mL) was then added and the mixture was stirred at refluxed in a round bottom flask equipped with a Dean-Stark trap for 7 hours. The mixture was then concentrated in vacuo to afford **86** (465 mg, 2.45 mmol, 98%) as a pale pink solid. R_f 0.28 (ethyl acetate: hexanes, 1:9); MP 124 – 125 °C; IR (film) 3047, 1658 cm^{-1}; **a)** ^1H NMR (400 MHz; $CDCl_3$) δ 8.70 (d, *J* = 11.1 Hz, 1 H, H-1), 8.25 (br d, *J* = 10.0 Hz 1 H, H-2), 7.18 (t, *J* = 1.7, 1 H, H-6), 7.00 (d, *J* = 1.7 Hz, 2 H, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ 161.4 (C-1),138.8 (C-3), 136.4 (C-5), 125.4 (C-6), 117.0 (C-4); **b)** ^1H NMR (400 MHz; $CDCl_3$) δ 8.38 (d, *J* = 1.3 Hz, 1 H, H-2), 7.51 (d, *J* = 1.8 Hz, 2 H, H-4), 7.40 (br s, 1 H, H-1), 7.14 (t, *J* = 1.8, 1 H, H-6); ^{13}C NMR (400 MHz; $CDCl_3$) δ 159.0 (C-2), 138.6 (C-3), 135.6 (C-5), 125.0 (C-6), 118.3 (C-4); mass spectrum (EI), (m/z, %) 189 (74, M^+), 161 (100), 126 (22), 99 (30), 90 (32), 63 (34). HRMS (EI) calc`d for $C_7H_5{}^{35}Cl_2NO$: 188.9748; found: 188.9741.

3.2.14 Methyl 3-(4-bromophenyl)-2-methylpropanoate (88)



Half-ester **74** (100 mg, 0.330 mmol), sodium azide (76 mg, 1.2 mmol), tetrabutylammonium bromide (16 mg, 0.050 mmol), zinc triflate (4 mg, 0.01 mmol) and di-*tert*-butyl dicarbonate (80 mg, 0.37 mmol) were dissolved in dry THF (10 mL) and stirred at 50 °C for 2 days. The reaction mixture was quenched with 10% $NaNO_2$. The product was extracted with ethyl acetate and washed with saturated sodium bicarbonate and then brine. The organic layers were combined, dried over magnesium sulfate and concentrated in vacuo to afford **88** (62 mg, 0.25 mmol, 73%) as a pale yellow liquid. R_f 0.27 (ethyl acetate:hexanes, 1:4); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.39 (d, *J* = 8.4, 2 H, H-8), 7.03 (d, *J* = 8.4 Hz, 2 H, H-7), 3.63 (s, 3 H, H-1), 2.96 (dd, *J* = 6.6, 12.8 Hz, 1 H, H-5α), 2.66 (m, 2 H, H-5β and H-3), 1.15 (d, *J* = 6.8 Hz, 3 H, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ 176.3 (C-2), 138.5 (C-6), 131.6 (C-8), 130.8 (C-7), 120.3 (C-9), 51.8 (C-1), 41.4 (C-3 or 5), 39.2 (C-3 or 5), 16.9 (C-4); mass spectrum (EI), (m/z, %) 258 (84, M^+, ^{81}Br), 256 (82, M^+, ^{79}Br), 198 (100), 196 (94). HRMS (ESI, $[M+H]^+$) calc`d for $C_{11}H_{14}{}^{81}BrO_2$: 259.0148; found: 259.0152; $C_{11}H_{14}{}^{79}BrO_2$: 257.0171; found: 257.0166.

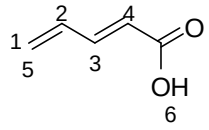
3.2.15 3-(4-Bromophenyl)-N-(3,5-dichlorophenyl)-2-methylpropanamide (83)



Amide **80** (100 mg, 0.230 mmol), sodium azide (53 mg, 0.81 mmol), tetrabutylammonium bromide (11 mg, 0.030 mmol), zinc triflate (3 mg, 0.010 mmol) and di-*tert*-butyl dicarbonate (57 mg, 0.26 mmol) were dissolved in dry THF (10 mL) and stirred at 50 °C for 2 days. The reaction mixture was quenched with 10% $NaNO_2$. The product was extracted with ethyl acetate and washed with saturated sodium bicarbonate and then brine. The organic layers were combined, dried over magnesium sulfate and concentrated in vacuo to afford **83** (18 mg, 0.050 mmol, 20%) as a pale yellow solid. R_f 0.42 (ethyl acetate:hexanes, 1:4); MP 122-123 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.40 (d, *J* = 8.3, 2 H, H-8), 7.35 (d, *J* = 1.5 Hz, 2 H, H-11), 7.08 (t, *J* = 1.8 Hz, 2 H, H-13), 7.05 (d, *J* = 8.3 Hz, 2 H, H-7), 6.98 (br s, 1 H, H-1), 2.98 (dd, *J* = 8.6, 13.5 Hz, 1 H, H-5), 2.70 (dd, *J* = 6.1, 13.6 Hz, 1 H, H-5), 2.53 (m, 1 H, H-3), 1.27 (d, *J* = 6.8 Hz, 3 H, H-4); ^{13}C NMR (400 MHz; $CDCl_3$) δ173.9 (C-2), 139.4 (Ar), 138.4 (Ar), 135.4 (Ar), 131.9 (Ar), 130.8 (Ar), 124.5 (Ar), 120.7 (Ar), 118.3 (Ar), 45.0 (C-3 or 5), 39.9 (C-3 or 5), 18.0 (C-4); mass spectrum (EI), (m/z, %) 387 (100, M^+, ^{81}Br), 385 (60, M^+, ^{79}Br), 199 (33), 197 (34), 171 (56), 169 (50). HRMS (ESI, $[M+H]^+$) calc`d for $C_{16}H_{15}{}^{81}Br\ {}^{35}Cl_2NO$: 387.9683; found: 387.9685; $C_{16}H_{15}{}^{79}Br\ {}^{35}Cl_2NO$: 385.9709; found: 385.9703.

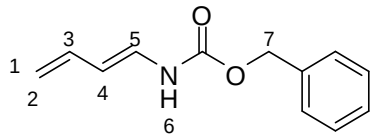
3.3 Experiments pertaining to gephyrotoxin

3.3.1 (*E*)-Penta-2,4-dienoic acid (96)



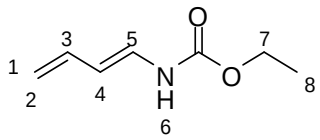
The procedure of Overman *et al*.[95] was followed. Malonic acid (8.26 g, 79.4 mmol) was dissolved in pyridine (10 mL) at 0 °C. Acrolein (5.95 mL, 5.00 g, 89.3 mmol) was then added dropwise. The reaction mixture was refluxed for 1 hour. The reaction was quenched with water (50 mL) and the mixture was acidified with concentrated sulfuric acid. The product was extracted with ethyl acetate. The ethyl acetate solution was then extracted with 1M KOH and washed with ethyl acetate. The mixture was reacidified with 1M HCl and extracted with ethyl acetate. The organic layers were combined and dried over magnesium sulfate and concentrated *in vacuo* to afford crude **96** as an off-white solid. MP 67 - 69 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ 11.21 (s, 1 H, H-6), 7.36 (dd, *J* = 15.4, 11.0 Hz, 1 H, H-3), 6.49 (dt, *J* = 16.9, 10.1 Hz, 1 H, H-2), 5.92 (d, *J* = 15.4 Hz, 1 H, H-4), 5.66 (d, *J* = 16.2 Hz, 1 H, H-5), 5.56 (d, *J* = 10.0 Hz, 1 H, H-1). Compound **96** was used in its crude form for subsequent reactions due to its tendency to decompose.

3.3.2 (*E*)-Benzyl buta-1,3-dienylcarbamate (94)



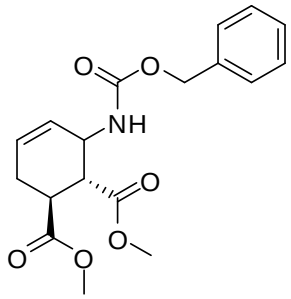
The procedure of Overman *et al*.[95] was followed. Crude acid **96** (200 mg, 2.04 mmol) was dissolved in acetone (30 mL) and cooled to 0 °C. Triethylamine (356 µL, 258 mg, 2.55 mmol) and ethyl chloroformate (204 µL, 232 mg, 2.14 mmol) were then added and stirred for 30 minutes. Sodium azide (265 mg, 4.08 mmol) dissolved in water (15 mL) was then added dropwise over 30 minutes and stirred for another 30 minutes. The acyl azide intermediate was then extracted with toluene. The organic layers were combined and dried over magnesium sulfate. The reaction mixture was then concentrated to ~70 mL *in vacuo*. This was added to benzyl alcohol (211 µL, 221 mg, 2.04 mmol) dissolved in toluene (10 mL) and refluxed for 5 hours under argon. The mixture was quenched with brine and extracted with diethyl ether. The organic layers were combined and dried over magnesium sulfate. The mixture was then concentrated in vacuo to afford **94** (70 mg, 0.44 mmol, 22%) as a pale yellow solid. R_f 0.24 (ethyl acetate:hexanes, 1:9); MP 69 - 71 °C ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.36 (m, 5 H, Ar), 6.75 (m, 1 H), 6.62 (br s, 1 H, H-6), 6.28 (m, 1 H), 5.71 (m, 1 H), 5.16 (s, 2 H, H-7), 5.04 (d, *J* = 16.9 Hz, 1 H, H-2), 4.92 (d, *J* = 10.2 Hz, 1 H, H-1).

3.3.3 (*E*)-Ethyl buta-1,3-dienylcarbamate (105)



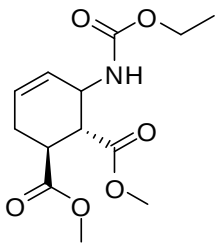
The procedure of Overman *et al*.[95] was followed. Crude acid **96** (13 mg, 130 mmol) was dissolved in acetone (70 mL) and cooled to 0 °C. Triethylamine (23.1 mL, 1.67 g, 16.6 mmol) and ethyl chloroformate (13.3 mL, 15.1 g, 139 mmol) were then added and stirred for 30 minutes. Sodium azide (17.2 g, 265 mmol) dissolved in water (60 mL) was then added dropwise over 30 minutes and the mixture was stirred for another 30 minutes. The acyl azide intermediate was then extracted with toluene. The organic layers were combined and dried over magnesium sulfate. The reaction mixture was then concentrated to ~200 mL *in vacuo.* This was then added to ethanol (20 mL) and refluxed for 5 hours under argon. The mixture was quenched with brine and extracted with diethyl ether. The organic layers were combined and dried over magnesium sulfate. The mixture was then concentrated in vacuo to afford **105** (2.619 g, 18.55 mmol, 14%) as a pale yellow oil. R_f 0.46 (ethyl acetate:hexanes, 1:4); ^{1}H NMR (400 MHz; $CDCl_3$) δ 6.74 (t, *J* = 12.6 Hz, 1 H), 6.38 (br s, 1 H, H-6), 6.27 (dt, *J* = 17.0, 10.5 Hz, 1 H), 5.67 (m, 1 H), 5.03 (d, *J* = 16.9 Hz, 1 H, H-2), 4.90 (d, *J* = 10.2 Hz, 1 H, H-1), 4.18 (q, *J* = 7.3 Hz, 1 H, H-7), 1.27 (t, *J* = 7.3 Hz, 1 H, H-8).

3.3.4 Dimethyl 3-(benzyloxycarbonylamino)cyclohex-4-ene-1,2-dicarboxylate (103)



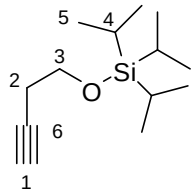
Dimethyl fumarate (50 mg, 0.35 mmol) and **94** (71 mg, 0.35 mmol) and a small amount of BHT were dissolved in a minimum amount of *p*-xylene in a sealed tube and stirred at 140 °C for 2 days under an inert atmosphere. The reaction mixture was then cooled and concentrated *in vacuo* to give **103** (14 mg, 0.040 mmol, 11 %). R_f 0.24 (ethyl acetate:hexanes, 1:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.31 (m, 5 H, Ar-H), 5.79 (m, 2 H), 5.04 (s, 2 H, Ar-CH_2), 4.71 (m, 2 H), 3.68 (s, 3 H, O-CH_3), 3.58 (s, 3 H, O-CH_3), 3.04 (dd, *J* = 4.6, 11.7 Hz, 1 H), 2.81 (dt, *J* = 5.6, 11.3 Hz, 1 H), 2.42 (td, *J* = 5.1, 17.6 Hz, 1 H), 2.10 (m, 1 H); ^{13}C NMR (400 MHz; $CDCl_3$) δ 176.1, 173.9, 156.5, 152.42, 137.4, 129.5, 129.1, 129.1, 127.05, 67.5, 52.7, 52.6, 46.4, 46.4, 37.9, 28.4; HRMS (ESI, $[M+Na]^+$) calc`d for $C_{18}H_{21}NNaO_6$: 370.1261; found 370.1256.

3.3.5 Dimethyl 3-(ethoxycarbonylamino)cyclohex-4-ene-1,2-dicarboxylate (106)



Dimethyl fumarate (51 mg, 0.35 mmol) and **105** (50 mg, 0.35 mmol) and a small amount of BHT were dissolved in a minimum amount of toluene in a sealed tube and stirred at 110°C for 2 days under an inert atmosphere. The reaction mixture was then cooled and concentrated *in vacuo* to give **106** (24 mg, 0.080 mmol, 24 %). R_f 0.30 (ethyl acetate: hexanes, 1:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ 5.81 (ddd, *J* = 2.1, 4.6, 9.8 Hz, 1 H), 5.78 (m, 2 H), 4.63 (br s, 2 H), 4.04 (q , *J* = 7.0 Hz, 2 H), 3.68 (s, 3 H, O-CH_3), 3.65 (s, 3 H, O-CH_3), 3.01 (m, 1 H), 2.81 (td, *J* = 5.6, 11.3 Hz, 1 H), 2.40 (ddt, *J* = 1.3, 18.1, 5.3 Hz, 1 H), 2.10 (t, *J* = 7.0 Hz, 3 H).

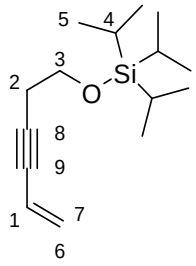
3.3.6 1-Triisopropylsilyloxy-3-butyne (109)



1-Butyn-4-ol (200 mg, 216 µL, 2.85 mmol), triisopropylsilyl chloride (605 mg, 672 µL, 3.14 mmol) and imidazole (233 mg, 3.42 mmol) were dissolved in DCM (10 mL) and stirred at room temperature overnight. The mixture was then quenched with water and extracted with DCM. The organic layers were combined, dried over magnesium sulfate and concentrated *in vacuo* to give **109** (5.463 g, 24.13 mmol, 85 %) as

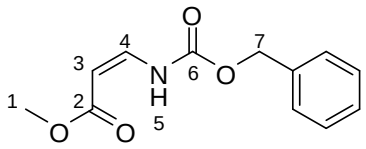
a brown oil. R_f 0.69 (ethyl acetate: hexanes, 1:2); IR (film) 3054, 2982, 1263 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 3.80 (t, *J* = 7.3 Hz, 2 H, H-3), 2.42 (td, *J* = 2.7, 7.3 Hz, 2 H, H-2), 1.94 (t, *J* = 2.7 Hz, 1 H, H-1), 1.05 (m, 21 H, H-4, H-5); ^{13}C NMR (400 MHz; $CDCl_3$) δ 81.8 (C-6), 69.5 (C-1), 62.3 (C-3), 23.1 (C-2), 18.2 (C-5), 12.2 (C-4).

3.3.7 1-Triisopropylsilyloxy-5-hexen-3-yn (110)



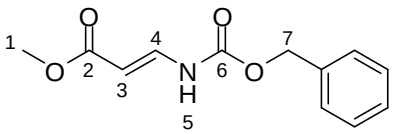
Alkyne **109** (5.46 g, 24.1 mmol), tetrakis(triphenylphosphine) palladium (55 mg, 0.050 mmol), copper(I) iodide (66 mg, 0.34 mmol) and vinyl bromide (31 mL, 1.0 M in THF, 31 mmol) were dissolved in dry diethylamine (10 mL) and stirred at room temperature for 18 hours. The mixture was then quenched with water and extracted with diethyl ether. The organic layers were combined and washed with 1 M HCl. The organic layer was dried with magnesium sulfate and concentrated *in vacuo* to give **110** (4.65 g, 18.4 mmol, 76 %) as a brown oil. R_f 0.77 (ethyl acetate: hexanes, 1:2); IR (film) 2260 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 5.75 (tdd, *J* = 2.1, 10.9, 17.5 Hz, 1 H, H-1), 5.53 (dd, *J* = 2.4, 17.4 Hz, 1 H, H-7), 5.37 (dd, *J* = 2.4, 10.9 Hz, 1 H, H-6), 3.80 (t, *J* = 7.3 Hz, 2 H, H-3) 2.54 (td, *J* = 2.1, 7.3 Hz, 2 H, H-2), 1.05 (m, 21 H, H-4, H-5); ^{13}C NMR (400 MHz; $CDCl_3$) δ 126.0 (C-6), 117.7 (C-1), 88.15 (C-8 or 9), 80.5 (C-8 or 9), 62.4 (C-3), 24.0 (C-2), 18.2 (C-5), 12.2 (C-4).

3.3.8 (*Z*)-Methyl 3-(benzyloxycarbonylamino)acrylate (108)



Monomethyl maleate (200 mg, 1.57 mmol) and triethylamine (195 mg, 268 µL, 1.92 mmol) were dissolved in acetone (30 mL) at 0 °C, followed by ethyl chloroformate (350 mg, 308 µL, 3.23 mmol). The reaction mixture was stirred for 30 minutes. Sodium azide (200 mg, 3.07 mmol) dissolved in water (10 mL was then added and the mixture was stirred for 1 hour. The acyl azide was then extracted with toluene. The organic layers were combined and dried over magnesium sulfate and concentrated *in vacuo* to almost dryness. The oily mixture was then flushed with argon for 1 hour. Dry toluene (10 mL) and benzyl alcohol (166 mg, 159 µL, 1.54 mmol) were added and the mixture was stirred at 80 °C for 2 hours. It was then stirred for 2 days at room temperature. The mixture was quenched with brine and extracted with diethyl ether. The organic layers were combined, dried over magnesium sulfate and concentrated *in vacuo* to give **108** (250 mg, 1.31 mmol, 83%) as a pale yellow oil. R_f 0.60 (ethyl acetate:hexanes, 1:2); IR (film) 3333, 2952, 1741, 1691 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 9.79 (br d, *J* = 7.3 Hz, 1 H, H-5), 7.35 (m, 6 H, Ar-H + H-4), 5.19 (s, 2 H, H-7), 5.06 (d , *J* = 8.8 Hz, 1 H, H-3), 3.69 (s, 3 H, H-1); ^{13}C NMR (400 MHz; $CDCl_3$) δ 170.5 (C-2), 154.5 (C-6), 141.1 (Ar or C-4), 136.4 (Ar or C-4), 129.6 (Ar or C-4), 129.6 (Ar or C-4), 129.3 (Ar or C-4), 95.6 (C-3), 68.6 (C-7), 51.7 (C-1); HRMS (ESI, $[M+Na]^+$) calc`d for $C_{12}H_{13}NNaO_4$: 258.0742; found 258.0729.

3.3.9 (*E*)-Methyl 3-(benzyloxycarbonylamino)acrylate (113)



Monomethyl maleate (2.000 g, 15.73 mmol) and triethylamine (1.95 g, 2.68 mL, 19.2 mmol) were dissolved in acetone (300 mL) at 0 °C, followed by ethyl chloroformate (3.50 g, 3.08 mL, 32.3 mmol). The reaction mixture was stirred for 30 minutes. Sodium azide (2.000 g, 30.72 mmol) dissolved in water (100 mL) was then added and the mixture was stirred for 1 hour. The acyl azide was then extracted with toluene. The organic layers were combined and dried over magnesium sulfate and concentrated *in vacuo* to almost dryness. The oily mixture was then flushed with argon for 1 hour. Dry toluene (100 mL) and benzyl alcohol (1.66 g, 1.59 mL, 15.4 mmol) were added and the mixture was stirred at 80 °C for 3 hours. It was then stirred for 2 days at room temperature. The mixture was quenched with brine and extracted with diethyl ether. The organic layers were combined, dried over magnesium sulfate and concentrated *in vacuo* to give **113** (2.23 g, 9.48 mmol, 60%) as a pale yellow solid. R_f 0.54 (ethyl acetate: hexanes, 1:2); IR (ATR) 3251, 1731, 1701, 1631 cm^{-1}; ^{1}H NMR (400 MHz; DMSO-d_6) δ 10.50 (br d, *J* = 10.7 Hz, 1 H, H-5), 7.63 (dd, *J* = 11.1, 14.0 Hz, 1 H, H-4), 7.40 (m, 5 H, Ar-H), 5.39 (d, *J* = 14.0 Hz, 1 H, H-3), 5.17 (s, 3 H, H-1); ^{13}C NMR (400 MHz; $CDCl_3$) δ 169.0 (C-2), 153.9 (C-6), 140.6 (Ar or C-4), 136.2 (Ar or C-4), 129.7 (Ar or C-4), 129.7 (Ar or C-4), 129.5 (Ar or C-4), 100.6 (C-3), 68.8 (C-7), 51.9 (C-1); HRMS (ESI, $[M+H]^+$) calc`d for $C_{12}H_{14}NO_4$: 236.0917; found 236.0921.

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