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Department of Organic Chemistry

Stereoselective Synthesis of Epoxides and Diepoxides

Applications in Total Synthesis of Natural Products and Analogues

Síntesis Estereoselectiva de Epóxidos y Diepóxidos

Aplicaciones en Síntesis Total de Productos Naturales y Análogos

Memoria que para optar al grado de
Doctor en Química (Mención Internacional)
por la Universidad de Málaga
presenta

Cristina García Ruiz

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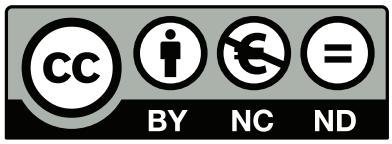

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D. FRANCISCO SARABIA GARCÍA, CATEDRÁTICO DEL DEPARTAMENTO DE QUÍMICA ORGÁNICA DE LA FACULTAD DE CIENCIAS DE LA UNIVERSIDAD DE MÁLAGA,

CERTIFICA:

Que la memoria adjunta, titulada "STEREOSELECTIVE SYNTHESIS OF EPOXIDES AND DIEPOXIDES. APPLICATIONS IN TOTAL SYNTHESIS OF NATURAL PRODUCTS AND ANALOGUES", que para optar al grado de Doctor en Química (Mención Internacional) presenta Dª. María Cristina García Ruiz, ha sido realizada bajo mi dirección en los laboratorios del Departamento de Química Orgánica de la Universidad de Málaga.

Considerando que constituye trabajo de Tesis Doctoral, autorizo su presentación en la Facultad de Ciencias de la Universidad de Málaga.

Y para que así conste, firmo el siguiente certificado, en Málaga a 30 de Octubre de Dos Mil Catorce.

Fdo. Francisco R. Sarabia García

Abstract

The present work is on the total synthesis of the natural compounds and/or analogues of Bengamides, Gummiferol and Depudecin. The target molecules, selected in virtue of their prominent biological activity as antibiotic and antitumor agents are featured by the presence of one or two epoxide groups and were synthesized by application of a novel methodology of asymmetric epoxidation based on the use of a new class of chiral sulfur ylides. The synthetic routes described in this thesis employed cheap and readily available starting materials and all the targeted molecules were reached in a moderate to good overall yield, except for the case of Depudecin, whose final step has not yet been accomplished.

Keys words: chiral sulfur ylides, epoxides, total synthesis, asymmetric synthesis, natural products, analogues, bengamides, gummiferol, depudecin

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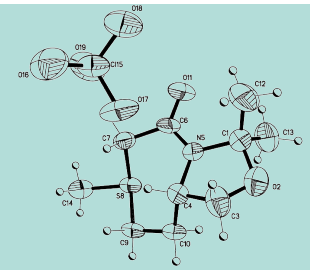
List of Abbreviations

$[\alpha]_D$	specific rotation at wavelength of sodium D line
Å	anstrong
Ac	acetyl
AcO	acetate
aq	aqueous
BAIB	[bis(acetoxy)iodo]benzene
BAE	bovine aortic endothelial
Bn	benzyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BPO	benzoyl peroxide
br	broad
Bu	butyl
c	concentration for specific rotation measurements
°C	degrees Celsius
^{13}C NMR	carbon nuclear magnetic resonante
COSY	correlation spectroscopy
CTC	2-chlorotrityl chloride
δ	NMR chemical shift in parts per million downfield from a standard
DIBAL-H	diisobutyl aluminum hydride
DIC	*N*,*N*-diisopropylcarbodiimide
DIPEA	diisopropyl ethyl amine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EC_{50}	50% effective concentration
ee	enantiomeric excess
equiv	equivalent
Et	ethyl

et al.	and others
etc.	and so forth
FAB	fast atom bombardment
g	gram(s)
h	hour(s)
^{1}H NMR	proton nuclear magnetic resonance
HOBt	1-hydroxy-benzotriazole
HsMetAp1	human methionine aminopeptidase type I
HsMetAp2	human methionine aminopeptidase type II
Hz	Hertz
IC_{50}	inhibitory concentration 50
ImH	imidazol
IR	infrared (spectroscopy)
$\mathcal{J}$	coupling constant
L	litro
Lys	lysine
m	multiplet or milli
μ	micro
MetAp	methionine aminopeptidase
MHz	megaHertz
min	minute(s)
mol	mole(s)
mp	melting point
Mtr	2,3,5-trimethyl-4-methoxybenzenesulfonyl
MS	mass spectrometry
M_w	weight-average molecular weight
m/z	mass-to-charge ratio
N	normal
n	nano
NMR	nuclear magnetic resonance
no.	number
PCC	pyridinium chlorochromate
pH	hydrogen ion concentration in aqueous solution

ppm	parts per million
PPTS	*p*-toluensulfonic acid
Pyr	pyridine
q	quartet
rt	room temperature
R_f	retention factor
Red-Al	sodium bis(2-methoxyethoxy)aluminum hydride
s	singlet or strong
SAE	Sharpless asymmetric epoxidation
sp.	species
Super-H	lithium triethylborohydride
t	triplet
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBDPSCl	*tert*-butyldiphenylsilyl chloride
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride
TEMPO	2,2,6,6-tetramethyl-1-pyperidineiloxy
tert	tertiary
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSCl	trimethylsilyl chloride
w	weak
wt %	weight percent



CHAPTER 1

Introduction

Natural products have been widely used to study the cellular functions of proteins in several ways. Identification of the cellular receptors led to discover new proteins with functions linked to the effects that natural products have on cells.

1.1. Natural Products in Organic Synthesis

Selection of a particular target molecule for total synthesis is frequently motivated by potential properties of the final product such as physical qualities, biological activity or medicinal applications (**Figure 1.1**). For those target molecules with an interesting biological or medicinal profile, *Nature* remains an important source of inspiration;[1] given the infinite efficiency of biologically active compounds, Natural Products might be then considered as "privileged structures", because of their characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them suitable as lead structure for drug discovery.[1]

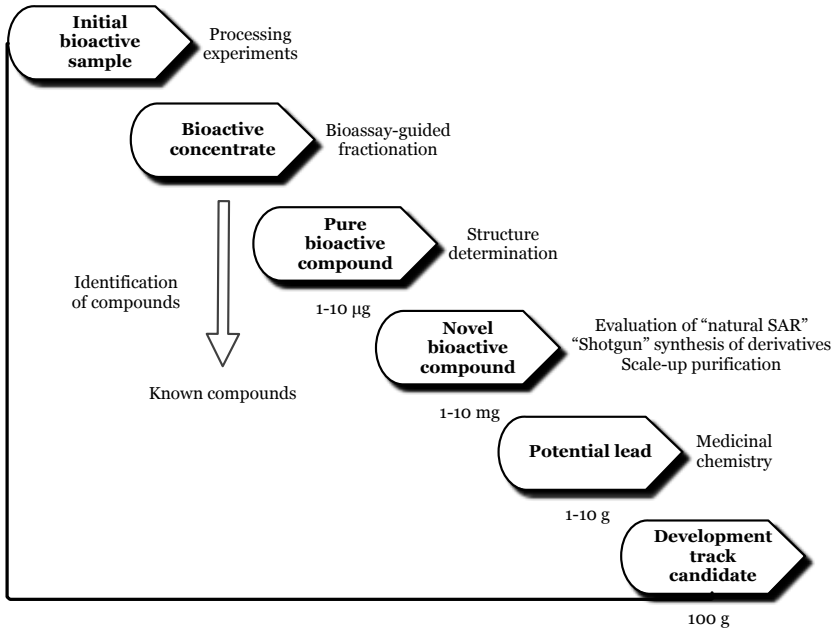


Figure 1.1. *Chemical process for natural products discovery*[1]

Playing an important role in drug discovery, nowadays, more than 50 percent of FDA-approved drugs were NPs or NP derivatives.[2] This fact might be due to several "quantifiable" advantages that must be highlighted.[3,4]

(i) NPs offer unmatched chemical diversity with structural complexity and biological potency. Although it is certainly difficult to calculate the scope of this advantage, a recent review estimates a ~100-fold higher hit rate for NPs over synthetic compounds.[5]

(ii) When compared with synthetic compounds, NPs databases contain not only more scaffolds, but also an important source of unexploited starting points in drug discovery.

(iii) NPs compounds serve as drugs as well as templates for drugs, leading to the discovery and better understanding of targets and pathways involved in the disease process.

As an example of the above mentioned advantages, the **Table 1.1**[3] illustrates a list of some anticancer agents isolated from natural sources undergoing clinical development.

Table 1.1. *Natural products of microbial origin undergoing clinical development as anticancer agents*[3]

Compound	Source	Status
Becatecarin	Rebeccamycin from *Lechevalieria aerocolonigenes*	Phase III
CKD-732	Fumagillin from *Aspergillus fumigatus*	Phase II
ECO-4601	*Micromonospora* sp.	Phase I
Elsamitrucin	unidentified actinomycete strain	Phase II
Irofulven	Illudin S from *Clitocybe illudens*	Phase II
Ixabepilone	Epothilone B from *Sorangium cellulosum*	Phase III
KOS-953	Geldanamycin from *Streptomyces hygroscopicus*	Phase II
NPI-0052	*Salinipora tropica*	Phase I
NPI-2358	Halimide from *Aspergillus* sp.	Phase I
Romidepsin	*Chromobacterium violaceum*	Phase II
Vorinostat	Trichostatin from *Streptomyces hygroscopicus*	Phase II
Temsirolimus	Sirolimus from *Streptomyces hygroscoicus*	Phase III

Given the determining role of natural products in the discovery and development of drug-like compounds, and taking into account the availability of new reagents and synthetic methods, it seems worthy appropriate to dedicate our efforts to the synthesis of novel natural products and analogues, exhibiting an intriguing biological profile.[6]

1.2. Total synthesis of Natural Products

Organic synthesis involves the assembly of complex molecules (usually containing the elements: C, H, O, N, S, P, B and halogens) from relatively simple starting materials and reagents through the formation and breaking of covalent bonds.

Traditionally, organic synthesis is divided into method-oriented synthesis and target-oriented synthesis, which is more commonly referred to as total synthesis.[7] The main issue of method-oriented synthesis is the invention, discovery and development of new synthetic reactions, reagents and catalysts. In contrast, the ultimate goal of total synthesis is the construction of a defined target molecule *via* a sequence of consecutive reactions (**Figure 1.2**).

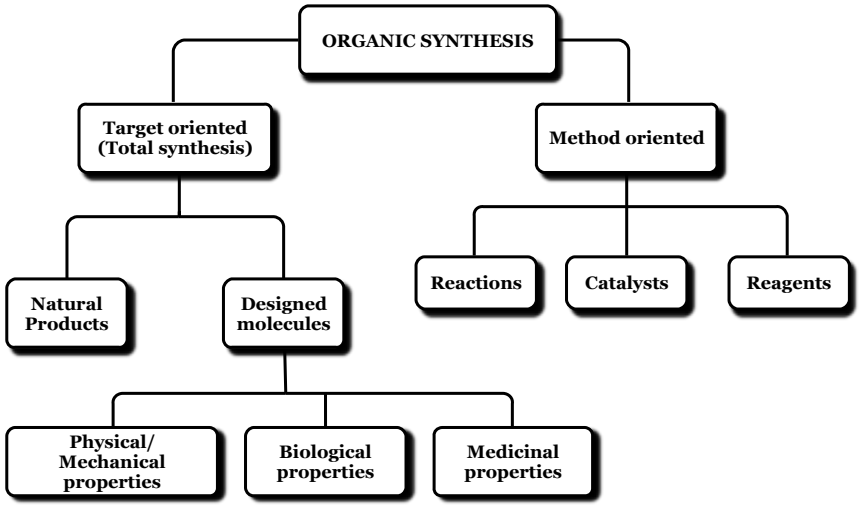


Figure 1.2. *Organic synthesis in perspective*[7]

As rightly discussed by Nicolaou and Sorensen in "Classics in Total Synthesis",[7] although at the beginning the purpose of a total synthesis was to confirm the molecular structure of a natural product, nowadays, other reasons for total synthesis have emerged. Among the most important are: (a) the challenge of synthesizing a naturally occurring substance of novel architecture; (b) the opportunity to discover and develop new synthetic chemistry; and (c) the ability to make contributions in biology by providing the natural substance as well as designed molecules that may mimic or inhibit the action of the natural product.[7]

Indisputably, total synthesis has indeed contributed to advances in a number of adjacent fields. In fact, almost half of the drugs on the market are natural products or derivatives thereof. Nevertheless, in the last decade, research on natural products by the pharmaceutical industry has declined. This is mainly due to a strategic shift toward combinatorial library synthesis of small organic molecules in combination with high-throughput screening for lead discovery as well as optimization.[3] However, the latest insights indicate that the generation and screening of a large number of synthetic compounds can at best complement but certainly not replace lead discovery *via* investigation of natural products, which have been carefully selected by evolutionary pressure.[1]

1.3. Epoxide in Organic Chemistry

Among the different functional groups, the oxirane group represents one of the most versatile and useful in Organic Chemistry, due to its capability of transformation into 1,2-bifunctional compounds, which are extremely appreciated in drugs and natural products synthesis, by means of ring opening reactions with nucleophiles.[8]

Moreover, the oxirane ring can be found in a wide range of natural products containing epoxides in their structures. Relevant examples of natural products containing one oxirane ring and with prominent cytotoxic activities are depicted in **Figure 1.3**. This is currently the case of epothilone B, isolated from *Sorangium cellulosum* cultures and that promotes the polymerization of tubulines.[9] Another such example is (-)-laulimalide, a potent antitumoral agent due to its potent microtubule-stabilizing properties that also inhibits the P-glycoprotein, which is active against multiple-drug resistance tumor cells.[10] A few other shining examples are cryptophycin 1, a cytotoxin possessing very potent antitumoral activity,[11] (-)-cycloepoxydon, which inhibits the tumor necrosis

factor NF-kB in 3T3 mouse cells,[12] or N1999-A2 that exhibits antitumor and antibacterial activities (**Figure 1.3**).[13]

Equally remarkable is the case of eponemycin[14] a novel antibiotic with specific activity against B16 melanoma, isolated from *Streptomyces hygroscopic* us No. P247-71.

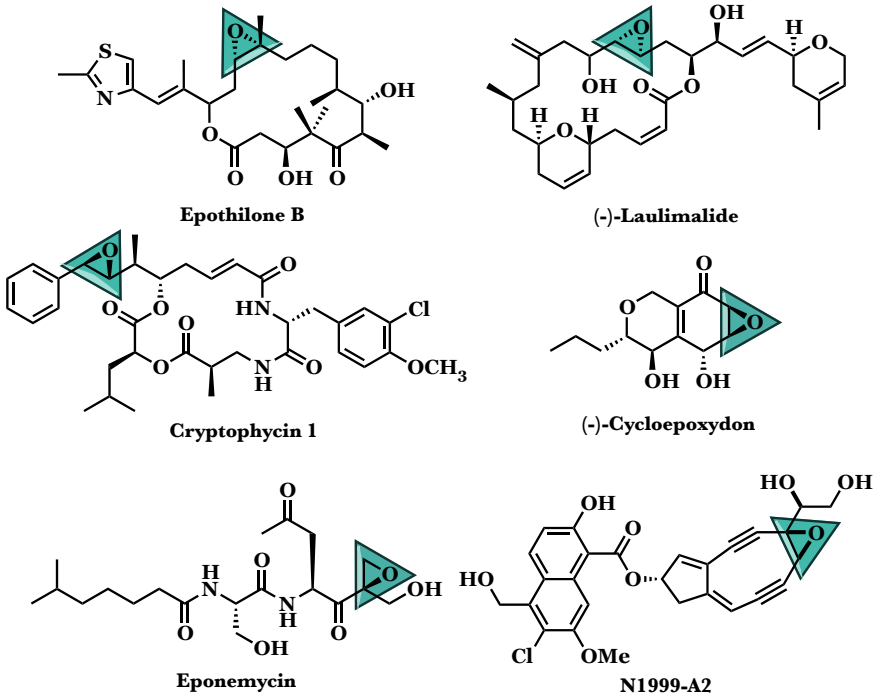


Figure 1.3. *Some examples of epoxide groups in natural products*

On the other hand, the occurrence of a diepoxide system is not so usual as the monoepoxide-containing natural products. Then it is worthy to mention (±)-leucomalure, a major sex pheromone component of the Satin moth *Leucoma salicis L.* (Lepidoptera: Lymantriidae)[15] or squalene tetraepoxide, a biosynthetic precursor of different oxacyclic triterpenoids (**Figure 1.4-A**).[16]

Among those striking examples exhibiting a promising biological activity, certain antitumor metabolites isolated from the fungus *Nattrassia mangiferae* (Sch 49210, Sch 53514 and Sch 53516),[17] or the complex metabolite Spatol, produced by a marine tropical alga, which displays a potent cytotoxicity against skin and brain tumour cells, by complete inhibition of cell division in human T242 Melanoma and 224C Astrocytoma neoplastic cell lines (**Figure 1.4-C**).[18-20]

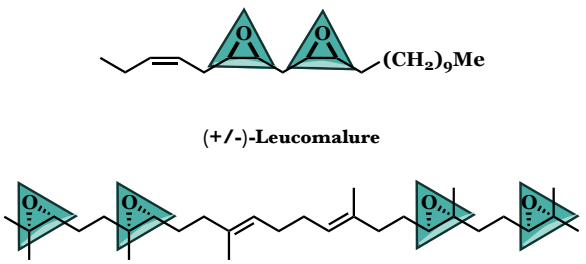


Figure 1.4-A. *Structural metabolites containing diepoxide systems*

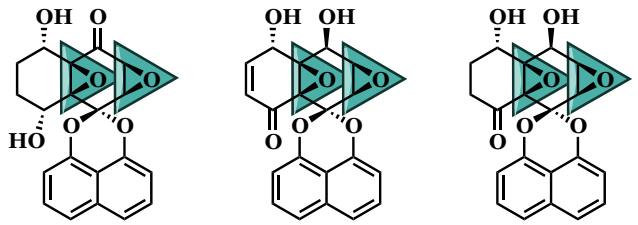


Figure 1.4-B. *Metabolites containing diepoxide systems*

It should be also mentioned the polyketide depudecine, an histone deacetylase inhibitor (HDAC) isolated from the fungus *Alternaria brassicicola* (**Figure 1.4-C**).[21,22]

Finally, a novel inhibitor of angiogenesis, rhizoxin, a microbial metabolite that contains two epoxide groups and exhibits anti-tubulin activity and significantly suppressed neovascularization (**Figure 1.4-C**).[23]

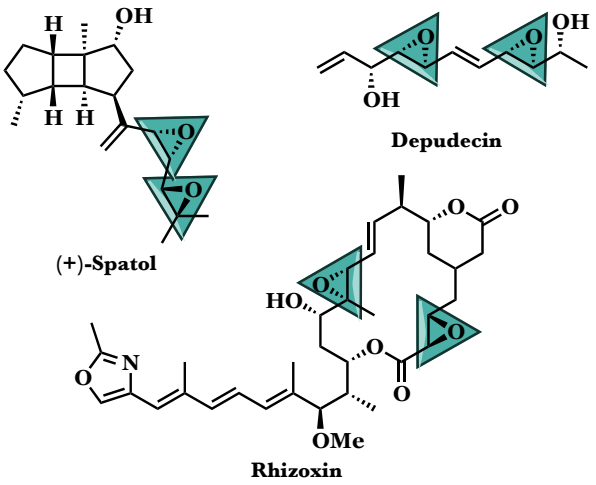


Figure 1.4-C. *Metabolites containing diepoxide systems*

1.4. Asymmetric Epoxidation Methodologies

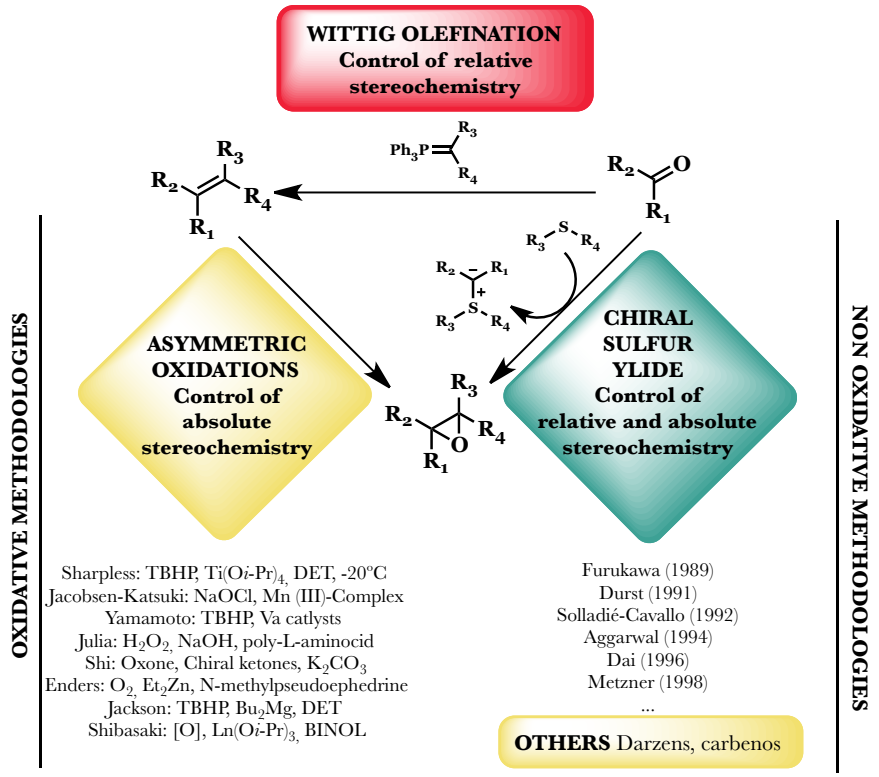
In the realm of asymmetric synthesis, methods that are directed towards the stereoselective construction of an oxirane ring constitute an important area of research, owing to the versatility and usefulness of this functional group.[8]

Chief among the different methodologies of epoxidation it is worth stressing those generating the oxirane ring from carbonyl compounds either from Wittig olefination followed by enantioselective oxidation of the prochiral olefin (oxidative methods) or by use of ylides, carbenes or Darzen's reagents (non oxidative methods) through enantioselective cycloaddition to a prochiral carbonyl group (**Scheme 1.1**).[24,25]

Among the flurry of asymmetric methods that have been developed to date, there is no doubt that the Sharpless asymmetric epoxidation reactions,[26] which rendered its inventor the Nobel Prize, has occupied a privileged position, being recognized as one of the most

important and most powerful reactions in organic synthesis by virtue of its high efficiency and generality for the enantiocontrolled synthesis of oxirane rings.

Scheme 1.1. *Main asymmetric epoxidation methodologies*



However, oxidative methodologies present some structural requirements (allylic alcohols, *cis*-alkenes, enones, etc.) and drawbacks (long reaction time, moderated enantiomeric excess, continue addition of oxidants, etc.). Nonetheless all these methods are synthetically valid for the synthesis of enantiomerically pure epoxides.

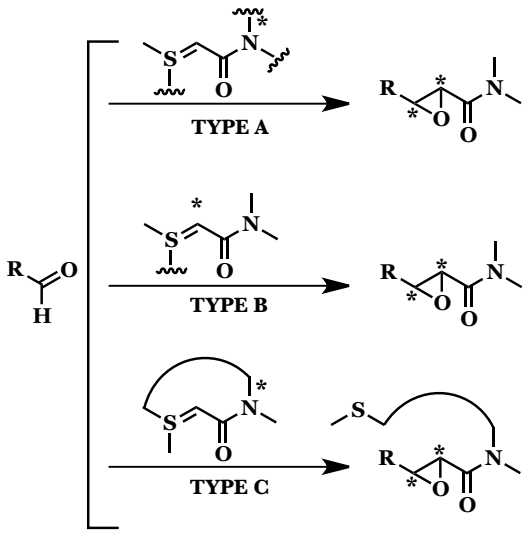
In the exploration of new methods for asymmetric epoxidation, chiral sulfur ylides have emerged as efficient synthetic tools not only for the asymmetric synthesis of epoxides,[27,28] but also for aziridines[29-32] and cyclopropanes.[33-38] In this sense, the contributions by Aggarwal's group[39-41] have reaped the benefits of this type of reagent in the synthesis of natural products[42-44] and drug-like compounds.[45-48]

To this aim, amide-stabilized sulfur ylides[49-55] are particularly valuable for three reasons: i) they only require very mild conditions and simple procedures for their reactions with carbonyl compounds, ii) they show high diastereocontrol in favor of the *trans* epoxides, and 3) the synthetic potential of the resulting glycidic amides, which displays exquisite regiocontrol for the C2 position in reactions with nucleophiles.[56-59]

In the case of amide-stabilized sulfur ylides, the chirality can be introduced in the amide fragment (type A), or in the sulfur fragment (type B). A third case, which has not been studied so far, can be represented by cyclic sulfur ylides of type C, which in our own experience render a high degree of diastereoselection in their reactions with carbonyl compounds owing to the presence of a chiral bicyclic system (**Scheme 1.2**).[57]

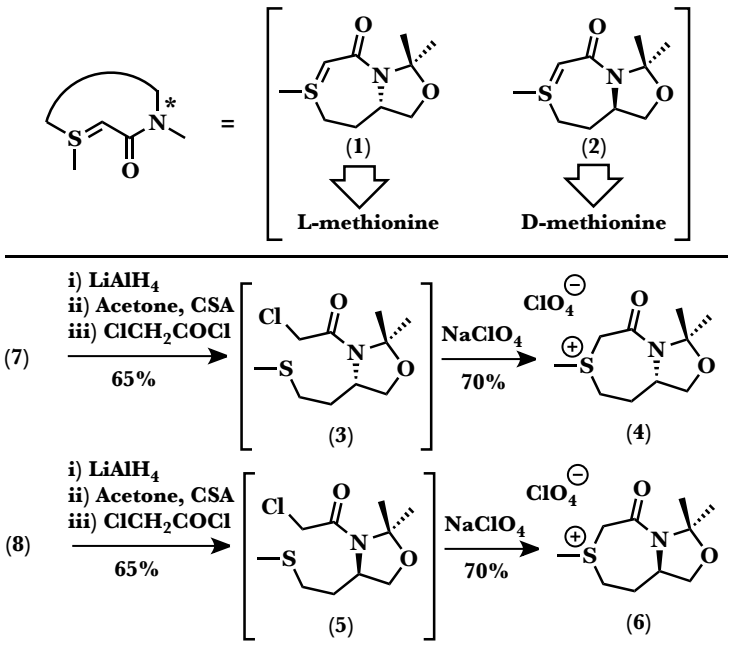
These new chiral sulfur ylides should yield epoxy amides of the type C depicted in **Scheme 1.2** and offer diverse synthetic possibilities, including oxirane ring-opening reactions that are combined with the reduction or hydrolysis of the amide functionality.[57]

Scheme 1.2. *General structures of sulfur ylides and reactions with carbonyl compounds*



Our experience with the synthesis of glycidic amides *via* amide-stabilized sulfur ylide[51,56,57,60] prompted us to consider the asymmetric version *via* this new potential class of chiral sulfur ylides (type C) which where succesfully synthesized from the corresponding commercially available amino acids. Thus, for the synthesis of the sulfonium salts **4** and **6**, which are precursor of sulfur ylides **1** and **2**, L- and D-methionines were subjected to treatment with $LiAlH_4$, followed by reaction with acetone under acidic catalysis with camphorsulfonic acid (CSA). The subsequent treatment with 2-chloroacetyl chloride furnished 2-chloroacetamides **3** and **5**, which were not isolated and were treated with sodium perchlorate in acetone to obtain, after crystallization, cyclic sulfonium salts **4** and **6** in 70% overall yields from L- and D- methionines, respectively (**Scheme 1.3**).

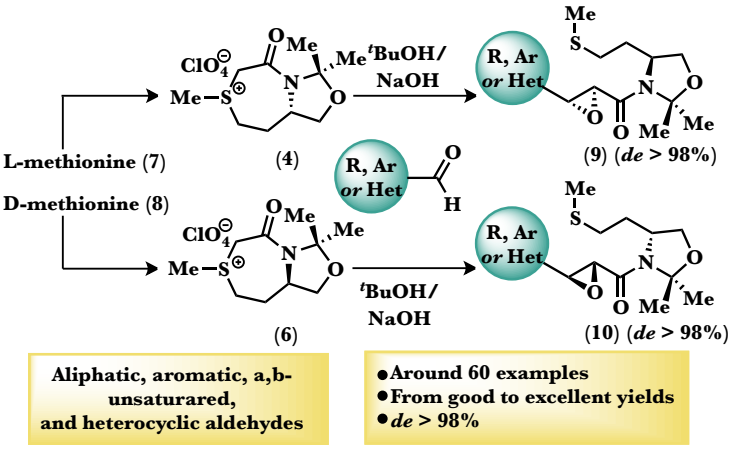
Scheme 1.3. *Design and synthesis of cyclic sulfur ylides* ***1*** *and* ***2***



It is important to highlight that sulfonium salts **4** and **6** were isolated as single diastereoisomers, which was essential for obtaining high asymmetric induction in their reactions with aldehydes.[54]

With regard to the reactivity, *in situ* preparation of ylides **1** and **2** by treatment of their corresponding sulfonium salts (**4** and **6**) with an aqueous solution of NaOH in *t*BuOH, followed by the addition of the aldehyde (one-phase method) or with an aqueous solution of NaOH in CH_2Cl_2-H_2O (two-phase method)[61] when the aldehyde was basic sensitive, afforded their corresponding epoxy amides, isolated as single diastereoisomers, which indicated an excellent level of stereochemical control. These epoxyamides were easily converted into the epoxy alcohols or epoxy aldehydes, as appropriate (**Scheme 1.4**).

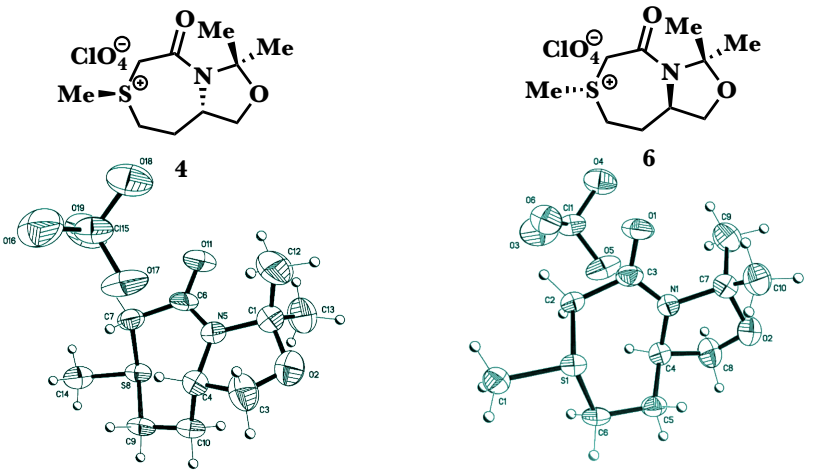
Scheme 1.4. *Reaction of sulfonium salts* ***4*** *and* ***6*** *with aldehydes*



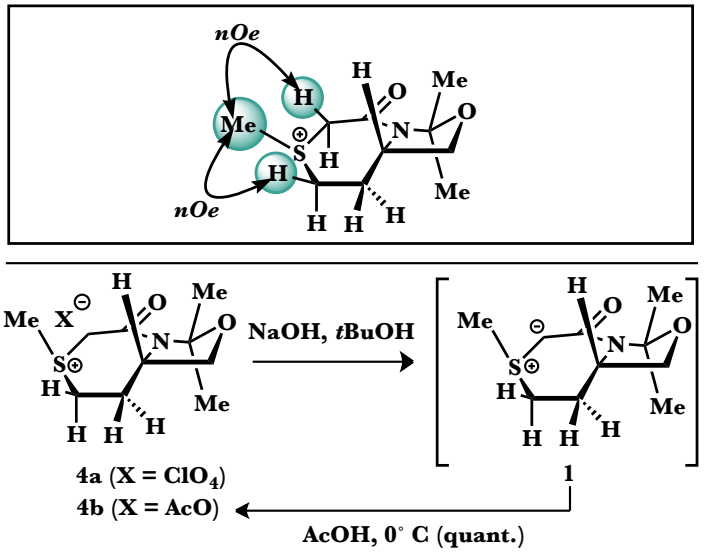
The high asymmetric induction provided by sulfonium salts **4** and **6**, together with the resulting stereochemistry was tentatively justified based on the structure of these sulfonium salts, by which the configuration at the sulphur atom might be established. In these previous studies, this configuration assignement was achieved by a combination of spectroscopic and theoretical studies since X-rays of these compounds were not possible to be obtained during those studies. Recently, we were able to obtain the X-rays of both **4** and **6** and the results demonstrated that we failed in our initial configuration assignments.* As showed in **Schemes 1.5-A** and **1.5-B**, the configurations at the sulphur atoms were the opposite in a conformation that could be match with the NOE correlations found during the spectroscopic studies.

* X-ray experiments were registered on a Nonius Kappa CCD diffractometer by Dr. John Davies in collaboration with Prof. S. V. Ley. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge (CB2 1EW)

Scheme 1.5-A. *Demonstration of the stereochemistry at the sulfur atom of sulfonium salts* ***4*** *and* ***6*** *- ORTEP's figures*



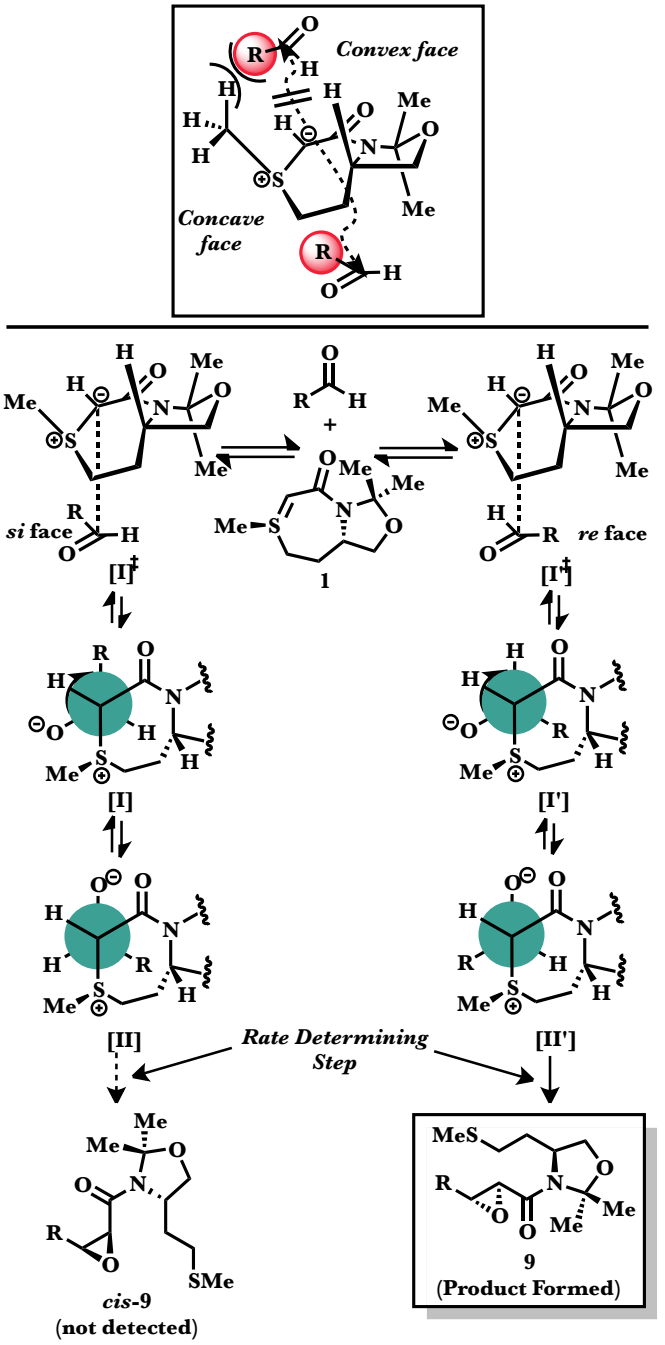
Scheme 1.5-B. *Demonstration of the stereochemistry at the sulfur atom of sulfonium salts* ***4*** *and* ***6*** *- NOE correlations and chemical experiments*



Assuming that the stereochemistry at the sulphur atom of the ylide remains the same with respect to its sulfonium salt precursor, as we experimentally demonstrated by the formation of sulphur ylide and

quenching with acetic acid that afforded sulfonium salt with similar diastereomeric purity, we then rationalized the resulting stereoselectivity as described in **Scheme 1.6**. This rationale would then support a concave approach as the most favourable pathway, in contrast to the transition state that is derived from a convex approach that would exhibit an important steric hindrance between the starting aldehyde with the methyl group at the sulphur atom. Combining this concave approach with a preferred cisoid arrangement between the reactants, ylide **1** could attack the aldehyde on its *re* or *si* faces. Initially, the preferred *si* face attack should proceed through intermediates I and II, after rotation of the C-C bond. This pathway should lead to the *cis* epoxide not detected due to the high barrier for ring closure being this step the rate determing step. Taking into account the reversibility of the betaine formation, as demonstrated by Aggarwal in crossover experiments, makes this pathway non-productive instead reverting back to the starting aldehyde and ylide. Then, the attack on the *re* face should led us to intermediates I' and II' being the ring closure step more favourable that should deliver the observed *trans* epoxy amide.

Scheme 1.6. *Theoretical rationale of the epoxidation*



Hence, with the development of this new methodology of asymmetric epoxidation,[56] natural products of biological interest, such as the case of bengamides (**11**),[62] the cyclodepsipeptides globomycin (**12**) and SF-1902 A5 (**13**),[63] or the sphingoid-type bases such as clavaminol H (**14**), phytosphingosine (**15**), sphinganine (**16**) or sphingosine (**17**) have been synthesized (**Figure 1.5**).[64]

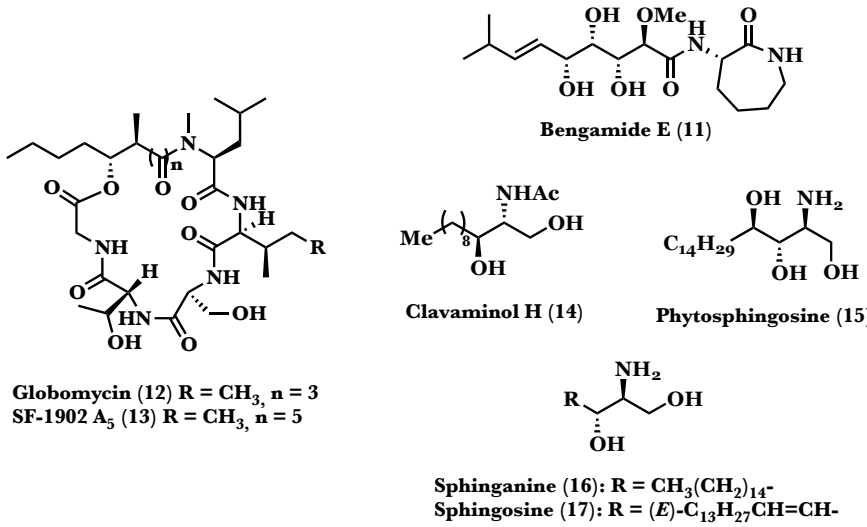


Figure 1.5. *Natural products synthesized via chiral sulfur ylide*

From this point, the next step and main goal of this thesis was the use of this emerging approach to the synthesis of new scaffolds and their application in synthesis of new natural products and analogues in efficient and stereoselective manners.

1.5. Aims and Outline of This Thesis

The remainder of this thesis will be the result of my efforts in the field of asymmetric total synthesis. The aim of every total synthesis discussed in this thesis is to develop an efficient route to the final product in relation to the introduction of stereogenic elements. The study objectives include the preparation and characterization of natural products and analogues with biological activity as antibiotic and/or antitumor agents. The biological background as well as the synthetic history of each target compounds will be introduced prior to discussion of the actual synthesis.

Chapter 2 provides a comprehensive review of the current research status of bengamides and includes a brief introduction to the structure, classification and precedents together with their eminent role as antitumor agents. As main objective, the major part of this chapter summarizes the strategies and experimental approaches to a new class of bengamide analogues containing either one or two epoxides throughout the polyketide chain, that might mimic the mode of action of fumagillin. With the purpose of better understanding the mechanism of action exhibited by bengamides, azido derivative in position C4 will be also described. Finally, the biological evaluation of some of the analogues synthesized will be discussed.

Chapter 3 describes the synthesis of gummiferol, a cytotoxic metabolite with antitumor activity that possesses two consecutive oxirane rings conjugated to a triacetylene moiety, by means of the use of chiral sulfur ylides. The isolation and characterization is described across this chapter as well as the synthesis of the natural product and analogues reported to date. In this way, the second objective will be to develop an alternative to the previous synthesis of gummiferol by generating the

epoxides in a stereoselective fashion by means of the chiral sulfur ylide methodology.

In **Chapter 4**, depudecin, a new microbial metabolite with promising antiangiogenic activity is presented together with its biological synthesis. Hence, our ultimate goal will be to stablish an efficient and straightforward route toward its total synthesis.

A further chapter (**Chapter 5**) has been included, displaying additional synthetic projects carried out during this thesis. Hence, contributions to the synthesis of the natural products globomycin, epibestatin and celebeside A, as well as the protease inhibitor AcArgValArgArgCMK, are described by using the solid phase synthesis methodology.

Finally, a summary of the outcome of this work, a general discussion and perspectives are provided in **Chapter 6**.

1.6. Notes and References

(1) Koehn, F. E. Carter, G. T. *Nat. Rev. Drug Discov.* **2005**, *4*, 206-220.

(2) Harvey, A. L. *Drug Discov. Today.* **2008**, *13*, 894-901.

(3) Lam, K. S. *Trends Microbiol.* **2007**, *15*, 279-289.

(4) Gu, J.; Gui, Y.; Chen, L.; Yuan, G.; Lu, H. -Z.; Xu, X. *PLoS One.* **2013**, *8*, 1-10.

(5) Bérdy, J. *J. Antibiot.* **2005**, *58*, 1-26.

(6) Nicolaou, K. C. *Proc. Natl. Acad. Sci.* **2004**, *101*, 11928-11936.

(7) Nicolaou, K. C. Sorensen, E. J. *Classics in total synthesis,* VCH, New York.; 1996.

(8) Yudin, A. K. *Aziridines and epoxides in organic synthesis,* Wiley-VCH Verlag GMBH&Co. KGaA, Wienheim, Germany; 2006.

(9) Ermolenko, M. S. Potier, P. *Tetrahedron Lett.* **2002**, *43*, 2895-2898.

(10) Mulzer, J. Hanbauer, M. *Tetrahedron Lett.* **2002**, *43*, 3381-3383.

(11) Li, L. -H. Tius, M. A. *Org. Lett.* **2002**, *4*, 1637-1640.

(12) Li, C.; Pace, E. A.; Liang, M. -C.; Lobkovsky, E.; Gilmore, T. D.; Porco, J. A. *J. Am. Chem. Soc.* **2001**, *123*, 11308-11309.

(13) Kobayashi, S.; Ashizawa, S.; Takahashi, Y.; Sugiura, Y.; Nagaoka, M.; Lear, M. J.; Hirama, M. *J. Am. Chem. Soc.* **2001**, *123*, 11294-11295.

(14) Sugawara, K.; Hatori, M.; Nishiyama, Y.; Tomita, K.; Kamei, H.; Konishi, M.; Oki, T. *J. Antibiot.* **1990**, *43*, 8-18.

(15) Gries, R.; Holden, D.; Gries, G. *Naturwissenschaften* **1997**, *84*, 219-221.

(16) Tong, R.; Boone, M. A.; McDonald, F. E. *J. Org. Chem.* **2009**, *74*, 8407-8409.

(17) Chu, M.; Truumees, I.; Patel, M. G.; Gullo, V. P.; Blood, C.; King, I.; Pai, J. -K.; Puar, M. S. *Tetrahedron Lett.* **1994**, *35*, 1343-1346.

(18) Gerwick, W. H. Fenical, W. *J. Org. Chem.* **1981**, *46*, 2233-2241.

(19) Gerwick, W. H. Fenical, W. *J. Org. Chem.* . **1983**, *48*, 3325-3329.

(20) Salomon, R. G.; Basu, B.; Roy, S.; Sharma *Tetrahedron Lett.* **1989**, *30*, 4621-4624.

(21) Matsumoto, M.; Matsutani, S.; Sugita, K.; Yoshida, H.; Hayashi, F.; Terui, Y.; Nakai, H.; Uotani, N.; Kawamura, Y.; Matsumoto, K.; Shoji, J.; Yoshida, T. *J. Antibiot.* **1991**, *45*, 879-885.

(22) Wight, W. D.; Kim, K. H.; Lawrence, C. B.; Walton, J. D. *MPMI.* **2009**, *22*, 1258-1267.

(23) Onozawa, C.; Shimamura, M.; Iwasaki, S.; Oikawa, T. *Jpn. J. Cancer Res.* **1997**, *88*, 1125-1129.

(24) Li, A. -H.; Dai, L. -X.; Aggarwal, V. K. *Chem. Rev.* **1997**, *97*, 2341-2372.

(25) Aggarwal, V. K. Winn, C. L. *Acc. Chem. Res.* **2004**, *37*, 611-620.

(26) Katsuki, T. Sharpless, K. B. *J. Am. Chem. Soc.* . **1980**, *102*, 5974-5976.

(27) Aggarwal, V. K.; Abdel-Rahman, H.; Jones, R. V. H.; Lee, H. Y.; Reid, B. D. *J. Am. Chem. Soc.* **1994**, *116*, 5973-5974.

(28) Aggarwal, V. K.; Kalomiri, M.; Thomas, A. P. *Tetrahedron: Asymm.* **1994**, *5*, 723-730.

(29) Aggarwal, V. K.; Thompson, A.; Jones, R. V. H.; Standen, M. C. H. *J. Org. Chem.* **1996**, *61*, 8368-8369.

(30) Aggarwal, V. K.; Ferrara, M.; O'Brien, C. J.; Thompson, A.; Jones, R. V. H.; Fieldhouse, R. *J. Chem. Soc. Perkin Trans. 1.* **2001**, 1635-1643.

(31) Aggarwal, V. K.; Charmant, J. P. H.; Ciampi, C.; Hornby, J. M.; O'Brien, C. J.; Hynd, G.; Parsons, R. *J. Chem. Soc. Perkin Trans. 1.* **2001**, 3159-3166.

(32) Aggarwal, V. K. Vasse, J. L. *Org. Lett.* **2003**, *5*, 3987-3990.

(33) Aggarwal, V. K.; Smith, H. W.; Hynd, G.; Jones, R. V. H.; Fieldhouse, R.; Spey, S. E. *J. Chem. Soc. Perkin Trans. 1.* **2000**, 3267-3276.

(34) Aggarwal, V. K.; Smith, H. W.; Jones, R. V. H.; Fieldhouse, R. *Chem. Commun.* **1997**, 1785-1786.

(35) Aggarwal, V. K.; Alonso, E.; Fang, G.; Ferrara, M.; Hynd, G.; Porcelloni, M. *Angew. Chem. Int. Ed.* . **2001**, *40*, 1433-1436.

(36) Ye, S.; Huang, Z. -Z.; Xia, C. -A.; Tang, Y.; Dai, L. -X. *J. Am. Chem. Soc.* **2002**, *124*, 2432-2433.

(37) Deng, X. -M.; Cai, P.; Ye, S.; Sun, X. -L.; Liao, W. -W.; Li, K.; Tang, Y.; Wu, Y. -D.; Dai, L. -X. *J. Am. Chem. Soc.* **2006**, *128*, 9730-9740.

(38) Riches, S. L.; Saha, C.; Filgueira, N. F.; Grange, E.; McGarrigle, E. M.; Aggarwal, V. K. *J. Am. Chem. Soc.* **2010**, *132*, 7626-7630.

(39) Fulton, J. R.; Aggarwal, V. K.; de Vicente, J. *Eur. J. Org. Chem.* **2005**, *2005*, 1479-1492.

(40) McGarrigle, E. M.; Myers, E. L.; Illa, O.; Shaw, M. A.; Riches, S. L.; Aggarwal, V. K. *Chem. Rev.* **2007**, *107*, 5841-5883.

(41) Badine, D. M.; Hebach, C.; Aggarwal, V. K. *Chem. Asian J.* **2006**, *1*, 438-444.

(42) Illa, O.; Arshad, M.; Ros, A.; McGarrigle, E. M.; Aggarwal, V. K. *J. Am. Chem. Soc.* **2010**, *132*, 1828-1830.

(43) Arshad, M.; Fernández, M. A.; McGarrigle, E. M.; Aggarwal, V. K. *Tetrahedron: Asymm.* **2010**, *21*, 1771-1776.

(44) Unthank, M. G.; Hussain, N.; Aggarwal, V. K. *Angew. Chem. Int. Ed.* **2006**, *45*, 7066-7069.

(45) Aggarwal, V. K.; Bae, I.; Lee, H. Y.; Richardson, J.; Williams, D. T. *Angew. Chem. Int. Ed.* **2003**, *42*, 3274-3278.

(46) Lei, H.; Yan, J.; Yu, J.; Liu, Y.; Wang, Z.; Xu, Z.; Ye, T. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 6533-6537.

(47) Aggarwal, V. K.; Bae, I.; Lee, H. -Y. *Tetrahedron* **2004**, *60*, 9725-9733.

(48) Aggarwal, V. K. Grange, E. *Chem. Eur. J.* **2005**, *12*, 568-575.

(49) Speziale, A. J.; Tung, C. C.; Ratts, K. W.; Yao, A. *J. Am. Chem. Soc.* **1965**, *87*, 3460-3462.

(50) Ratts, K. W. Yao, A. N. *J. Org. Chem.* **1966**, *31*, 1185-1188.

(51) Valpuesta-Fernández, M.; Durante-Lanes, P.; López-Herrera, F. J. *Tetrahedron* **1990**, *46*, 7911-7922.

(52) Aggarwal, V. K.; Blackburn, P.; Fieldhouse, R.; Jones, R. V. H. *Tetrahedron Lett.* **1998**, *39*, 8517-8520.

(53) Aggarwal, V. K.; Hynd, G.; Picoul, W.; Vasse, J. -L. *J. Am. Chem. Soc.* **2002**, *124*, 9964-9965.

(54) Aggarwal, V. K.; Charmant, J. P.; Fuentes, D.; Harvey, J. N.; Hynd, G.; Ohara, D.; Picoul, W.; Robiette, R.; Smith, C.; Vasse, J. -L.; Winn, C. L. *J. Am. Chem. Soc.* **2006**, *128*, 2105-2014.

(55) Sarabia, F.; Vivar-García, C.; García-Castro, M.; Martín-Ortiz, J. *J. Org. Chem.* **2011**, *76*, 3139-3150.

(56) Sarabia, F.; Chammaa, S.; García-Castro, M.; Martín-Gálvez, F. *Chem. Commun.* **2009**, 5763-5765.

(57) Sarabia, F.; Vivar-García, C.; García-Castro, M.; García-Ruiz, C.; Martín-Gálvez, F.; Sánchez-Ruiz, A.; Chammaa, S. *Chem. Eur. J.* **2012**, *18*, 15190-15201.

(58) Cardellach, J.; Font, J.; Ortuño, R. M. *Tetrahedron Lett.* **1985**, *26*, 2815-2816.

(59) Martín-Ortiz, L.; Chammaa, S.; Pino-González, M. S.; Sánchez-Ruiz, A.; García-Castro, M.; Assiego, C.; Sarabia, F. *Tetrahedron Lett.* **2004**, *45*, 9069-9072.

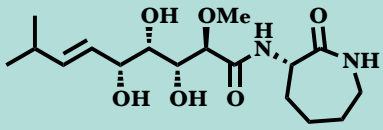
(60) López-Herrera, F. J.; Sarabia-García, F. R.; Pino-González, M. S. *Recent Res. Dev. Org. Chem.* **2000**, *4*, 465-490.

(61) López-Herrera, F. J.; Pino-González, M. S.; Sarabia-García, F.; Heras-López, A.; Ortega-Alcántara, J. J.; Pedraza-Cebrián, M. G. *Tetrahedron: Asymm.* **1996**, *7*, 2065-2071.

(62) Sarabia, F.; Martín-Gálvez, F.; Chammaa, S.; Martín-Ortiz, L.; Sánchez-Ruiz, A. *J. Org. Chem.* **2010**, *75*, 5526-5532.

(63) Sarabia, F.; Chammaa, S.; García-Ruiz, C. *J. Org. Chem.* **2011**, *76*, 2132-2144.

(64) Sarabia, F.; Vivar-García, C.; García-Ruiz, C.; Sánchez-Ruiz, A.; Pino-González, M. S.; García-Castro, M.; Chammaa, S. *Eur. J. Org. Chem.* **2014**, *2014*, 3847-3867.



CHAPTER 2

Bengamides

Bengamides, isolated from marine sponges, have proved to be a prolific family of natural products by virtue of their unprecedented molecular architectures and impressive biological profiles, including antitumor, antibiotic and anthelmintic properties. Such antitumor activity is related to the inhibition of methionine aminopeptidase enzymes involved in the cell cycle of endothelial cells and angiogenesis.

In 1986, Crews et al. described how "*The methanol extract of an undescribed Fiji sponge contains the novel seven-membered ring heterocycles, bengamide A and bengamide B, which are cyclized by a γ-hydroxylysine. [...] These compounds are biotoxic to eukaryotic cells, nematodes, and bacteria*".[1] Within the huge variety of natural products with potential biological applications,[2,3] those containing an α-amino-ε-caprolactam moiety have attracted great interest in the pharmaceutical industry,[4] which is currently the case of bengamides.

2.1. Structure and Classification of Bengamides

Bengamides[5] (**Table 2.1**) comprise a family of natural products isolated from marine sponge of the *Jaspidae* family, discovered in 1986 by Crews and co-workers, with a wide range of biological activities as

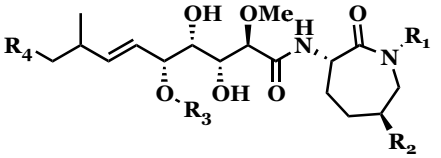
antitumoral, antibiotic and anthelminthic.[6,7] Some new members of the bengamides family (bengamides E, E' and F'), have been recently identified and isolated from *Myxococcus virescens* bacteria and reported by Crews et al.[8]

Harvesting bengamide-producing sponges or finding a bengamide-producing organism that could be growth in culture have been completely unsuccessful so far,[9] stirring up great interest in total synthesis that could provide suitable amounts of these compounds. In this way, numerous syntheses of bengamides have been reported, all of them utilizing an amide coupling reaction between a protected polyhydroxylated side-chain intermediate and a cyclolysine intermediate, and employing different starting materials, such as L- and D- glucose,[10,11] L-quebrachitol[12] or D-tartaric acid,[13] among others.[14]

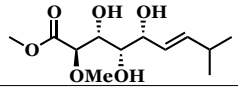
2.2. Bengamides as Inhibitors of MetAP

Within all the members of the bengamides family, bengamides A (**18**) and B (**19**) are the most potent, with IC_{50} values against larynx epithelial carinoma of 0.001 and 0.0024 μM, respectively, followed by bengamide E (**11**), with an IC_{50} value of 3.3 μM.[15] Whereas *in vivo* studies demonstrated cytotoxicity profile against MDA-MB-435 human mammary carcinoma,[16] *in vitro* anti tumour activity of bengamide B (**19**) disclosed a unique profile in the NCI 60 cell line panel compared to standard anti tumor agents, revealing arrest at both G1 and G2/M phases of the cell cycle by FACS (Fluoresecence-activated cell sorter), which suggests that the cytotoxicity exhibited by the bengamides was due to inhibition of a novel target.[6]

Table 2.1. *Molecular structure of bengamides*



TYPE I

Bengamide	R_1	R_2	R_3	R_4
A (**18**)	H	$OCO(CH_2)_{12}CH_3$	H	H
B (**19**)	CH_3			
G (**20**)	H	$OCO(CH_2)_{11}CH_3$	H	H
H (**21**)	CH_3			
I (**22**)	H	$OCO(CH_2)_{13}CH_3$	H	H
J (**23**)	CH_3			
L (**24**)	H	$OCO(CH_2)_{11}CH(CH_3)_2$	H	H
M (**25**)	CH_3			
N (**26**)	H	$OCO(CH_2)_{10}CH(CH_3)_2$	H	H
O (**27**)	CH_3			
Y (**28**)	H	OH	H	H
Z (**29**)	CH_3	OH	H	H
C (**30**)	H		H	H
D (**31**)	CH_3			

TYPE II

Bengamide	R_1	R_2	R_3	R_4
E (**11**)	H	H	H	H
F (**32**)	CH_3	H	H	H
E' (**33**)	H	H	H	CH_3
F' (**34**)	CH_3	H	H	CH_3
P (**35**)	H	H	$C(=O)(CH)_{12}CH_3$	H
Q (**36**)	CH_3	H	$C(=O)(CH)_{12}CH_3$	H
R (**37**)	H	H	$C(=O)(CH)_{14}CH_3$	H

Proteomic studies carried out by Towbin et al. allowed to establish MetAPs, enzymes related to the protein biosynthesis, as the molecular targets for bengamides[5] inhibiting the tumor growth both *in vitro* and *in vivo*,[17] which marked a turning point in the synthesis of potent and selective analogues.

2.3. Methionine Aminopeptidases

Methionine aminopeptidase enzymes represent a unique class of metal dependent amino peptidases that remove unblocked *N*-terminal initiator methionine on either peptides or proteins,[18] both in a co-translational or post-translational mode,[19] suggesting a role in regulating processes rather than general protein degradation.[18] The removal of the *N*-terminal methionine by MetAPs is a critical step in the maturation of many proteins and is essential for further amino terminal modifications.[20] Therefore, its inhibition has acquired a special importance since it has been demonstrated that MetAP2 is involved in the development of a certain number of tumours.[21]

2.3.1. Comparison between MetAP Type I and II

There are two isoforms of methionine aminopeptidases, type I (MetAP1) and II (MetAP2),[22,23] differing from one another by particular differential sequences. Thus, the type II enzymes present an α-helical domain of 60 residues inserted in a surface loop of the *C*-terminal half of the molecule.[24] The proximity of this domain to the active site suggests that it is the key for the differentiation in specifity of the two classes. Also, the modifications due to the presence of *N*-terminal extensions further differentiate the enzymes.[18] Moreover, type I is further divided into types Ia, Ic (prokaryotes) and Ib (only in eukaryotes) (**Figure 2.1**).[25,26] The eukaryotic MetAPs are differentiated from their prokaryotic counterparts by an additional *N*-terminal extension.[24] The eukaryotic MetAP2 has two putative zinc finger motifs at the extreme *N*-terminus and a highly charged *N*-terminus with alternating polyacidic and polybasic stretches in a similarly sized segment. Although the catalytic domains of both MetAPs possess very similar structures, all the residues that form the methionine-binding pocket are different, but the shape of the pocket is conserved. Furthermore, MetAP2 also contains an inserted region contacting the

catalytic domain in some of the same area covered by the connector of the type I.[25] These data support the proposal that MetAP types I and II may present a common functional role.

From the biomedical point of view, MetAP2 has attracted much more attention that MetAP1 due to the discovery of MetAP2 and not MetAP1 as the biological target of some of the anti-antiogenic compounds, such as the aforementioned fumagillin (**38**) and ovalicin (**39**)[27] (**Figure 2.2**), together with their synthetic analogues and other synthetic molecules.[28] One reason is the smaller active site pocket of the type I enzyme, which limits accessibility. A second reason is that inhibitor binding to *Hs*MetAP1 requires ~120º rotation of His^{210} and disruption of hydrogen bonding to Tyr^{195}.[25] In HsMetAP2, a histidine (His^{339}) undergoes a related conformational change, but there is no loss of hydrogen bonding.[27] Taking into account that methionine amino peptidases have been identified as antitumor target for these anti-angiogenic agents, it would be interesting to investigate the role of these enzymes in angiogenesis. However, despite numerous studies stressing the role of aminopeptidases in the formation of new blood vessels, it is still unclear what role MetAP2 plays in regulating angiogenesis.[29]

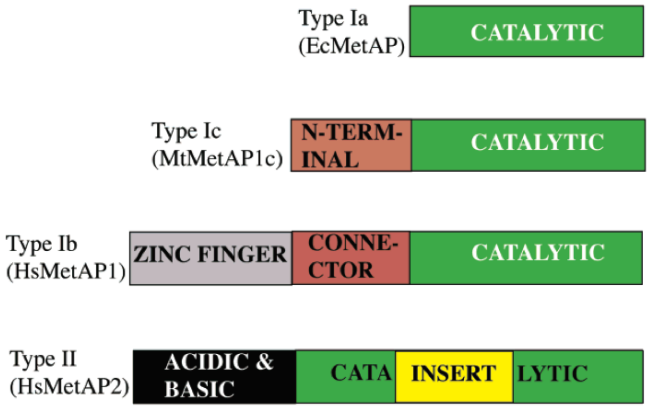
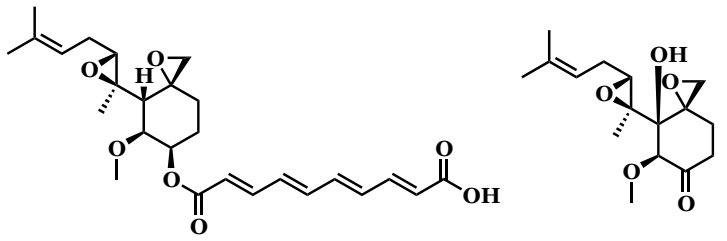


Figure 2.1. *Domain organization of type I and type II MetAPs*[25]



Fumagillin
(38)

Ovalicin
(39)

Figure 2.2. *Fungal metabolites inhibitors of angiogenesis*

The overall relationship between the structures of type I and type II human MetAP is illustrated in **Figure 2.3**.[25] The catalytic domains, with only 27% sequence identity, however, have very similar structures. All the metal-binding residues in the active site are conserved, whereas almost all of the residues that form the methionine-binding pocket are different. In spite of these differences, the shape of the pocket is conserved (**Figure 2.3**).[25]

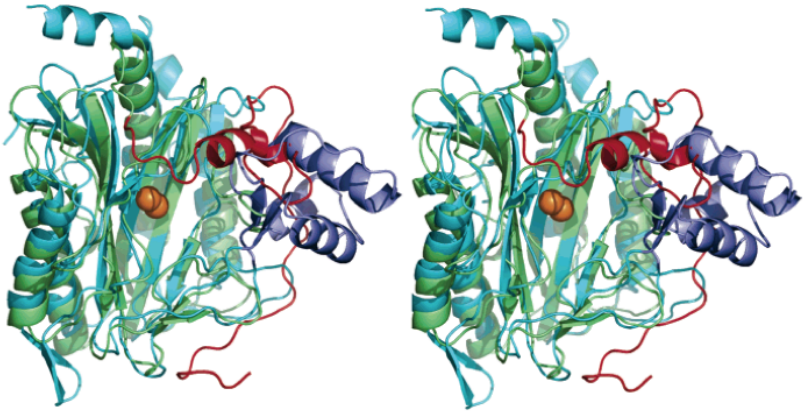


Figure 2.3. *Stereo diagram showing the relationship of* t*HsMetAP1 (green) and HsMetAP2 (cyan) structures. The catalytic domains are very similar. The connector region of tHsMetAP2 (red) and the insert domain (blue) of HsMetAP2 are located in similar regions of the surface of the catalytic domains*[25]

In the presence of excess Co^{+2}, a third cobalt ion binds in the active site region. This fact explains why excess of metal ions can be inhibitory.[25] Also, the *N*-terminal region of the protein contains three distinct Pro-x-x-Pro motifs, supporting the prior sugggestion that this region of the protein may participate in binding to the ribosome.

The recently reported structure of *M. tuberculosis* MetAP provided the first example of a MetAP with a structured extension at the amino-terminus. Within this extension, there is a sequence element Pro-X-X-Pro motif that is arranged on the surface of the protein in such a way that it seems poised to bind an SH_3 protein domain. This led to the suggestion that the PxxP motif of *M. tuberculosis* MetAP might be the target site for mediating MetAP binding to ribosome.[30]

2.3.2. Structure of the Complex MetAP2-Bengamide

In order to shed light to the mechanism of inhibition of methionine aminopeptidases (MetAP) by bengamides, *Hs*MetAP2 was co-crystallized together with the synthetic inhibitor LAF153 (**40**), a non-acylated derivative from LAF389 (**41**), that also inhibits *Hs*MetAP2 both *in vitro* and *in vivo* (**Figure 2.4**).[6]

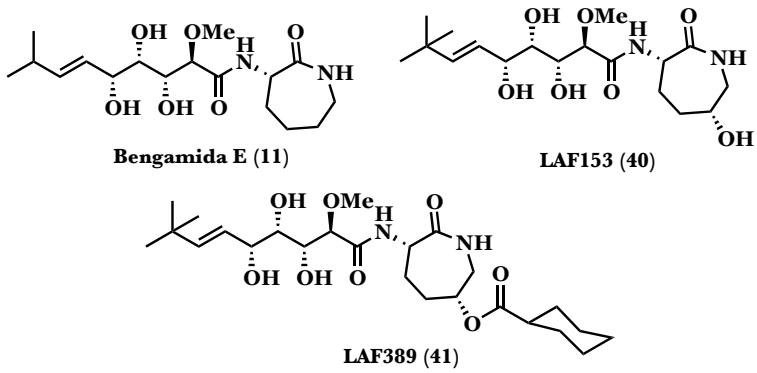


Figure 2.4. *Structures of bengamide E (**11**), LAF153 (**40**) and LAF389 (**41**)*

This enzyme-substrate complex structure proved the mode of interaction of these bioactive compounds at the active site of the methionine aminopeptidases (**Figure 2.5-A**). Hence, the X-ray structure revealed a critical dinuclear metal center placed as a deep invagination in the surface of the enzyme.[18] On the other hand, the hydrophobic pocket P1, in the innermost portion of the active-site, interacted with the terminal alkyl group of the olefin, while pocket P2, formed at the solvent-exposed surface, hold the caprolactam ring. The coordination of the cobalt ions with the hydroxyl groups at C3, C4, and C5 occurred in a similar way to that observed for peptidic inhibitors of aminopeptidases (**Figure 2.5-B**). [31]

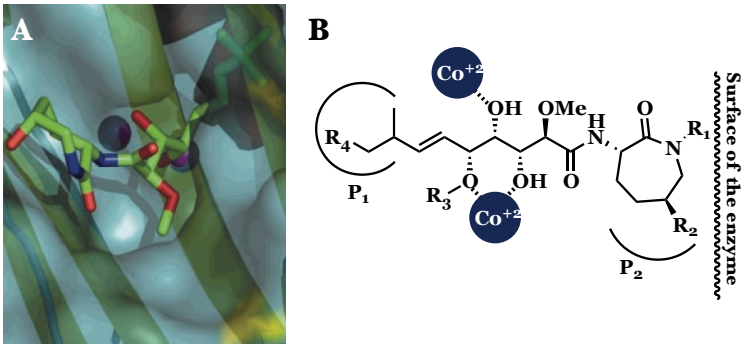


Figure 2.5. ***(A)*** *Structure of human MetAP2 in complex with the bengamide analogue LAF153* ***(B)*** *Mechanism of action of bengamides with MetAPs*

Once the mechanism of interaction was known, considerable variations of the structure of bengamides have been made and numerous analogues owning better bioactivity profile synthesized, such LAF-389 (**41**)[32] and its synthetic analogues (**42**) and (**43**),[33] which exhibited an increased antitumor activity compared to bengamide A (IC_{50} = 0.001 ± 0.0006 μM) and stressed the influence of the alkyl group at P1 and the caprolactam moiety in the biological activity (**Figure 2.6**).

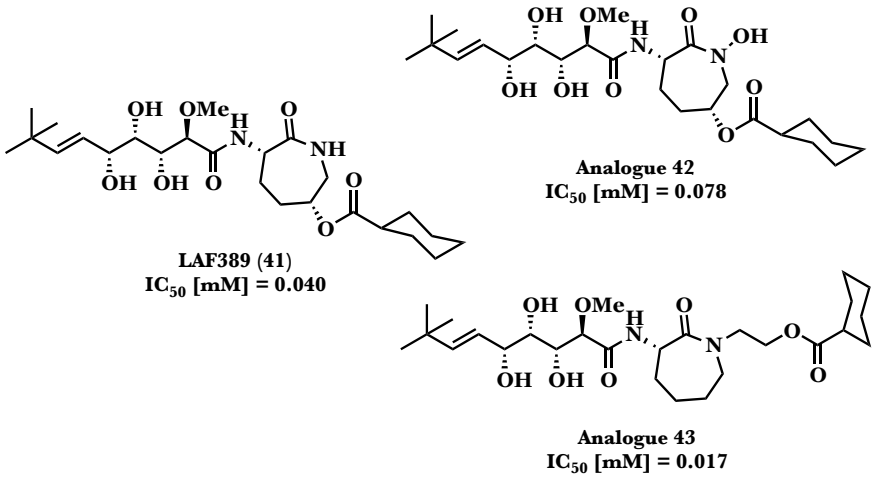


Figure 2.6. *Potent bioactive analogues of bengamides*

2.4. Angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing microvasculature takes place during physiologic and reparative processes.[34] Neovascularization is critical for the growth of tumours and is a dominant feature in a variety of angiogenic diseases such as diabetic retinopathy, haemangiomas, arthritis and psoriasis.[35] Frequently described as one of the hallmarks of cancer, because of its role in tumour growth, invasion and metastasis,[36] the discovery of fumagillin, a fungal metabolite isolated from a species of *Aspergillus sp*. that can potentially suppress tumour growth, supported the development of new angiogenesis inhibitors.[28,37] Different types of anti-angiogenic agents have been shown to affect angiogenesis, and some of these agents are now in clinical trials in patients with cancer or AIDS.[38]

2.4.1. Angiogenesis Process

In their book *Angiogenesis*, A. Rodríguez-Quesada and M. A. Medina pointed out that "*the growth of new blood vessels from the existing vascular bed is a process that occurs under strict temporal and spatial control. [...] Any*

of the steps involved in angiogenesis may be a potential target for pharmacological intervention of angiogenesis-dependent diseases".[39]

The angiogenesis process is divided into four steps, each of which can be considered as a pharmacological target (**Figure 2.7**).[36]

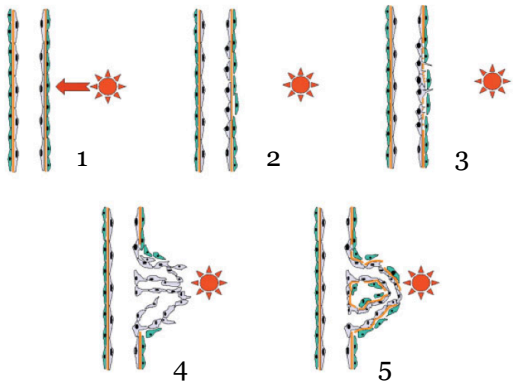


Figure 2.7. *Scheme of the steps of the angiogenic process*[39]

The first step covers an activating proangiogenic signal that elicits the switching on of the angiogenic phenotype of resting endothelial cells. During the second step, those activated cells cause the degradation of the basal membrane, extracellular matrix remodeling, proliferation and migration. Finally, morphogenesis contributes to the alignment of endothelial cells by forming a new micro vessel that is stabilized by the recruitment of pericytes and smooth muscle cells.

2.4.2. Angiogenesis and Fumagillin

Fumagillin (**38**), isolated during routine culturing of capillary endothelial cells contaminated by the fungus *Aspergillus fumigatus* was one of the first reported inhibitors of the endothelial cell proliferation.[28]

Despite fumagillin inhibits endothelial cell proliferation *in vitro* and tumour-induced angiogenesis *in vivo*, its prolonged administration was

limited because it caused severe weight loss. This result has led to develop new derivatives displaying more potent activity and without the usual toxic side-effects.[28] One of this fumagillin analogues, TNP-470 (**44**) (also known as AGM-1470) (TAP Pharmaceuticals Inc., Deerfield, IL, EUA), exhibits an IC_{50} value 50-fold more active than the fumagillin parent and a reasonably better selectivity (**Figure 2.8**).

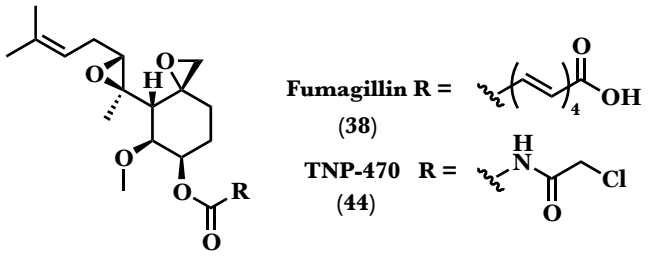


Figure 2.8. *Inhibitors of endothelial cell proliferation*

The molecular mechanism of the inhibition of angiogenesis by AGM-1470 (**44**) is specifically related to the type II methionine aminopeptidase. It also inhibits the activation of the cyclin-dependent kinases and the phosphorylation of the retinoblastoma gene product.[40] This compound is currently undergoing in phase II clinical trials for a variety of cancers, including Kaposi's sarcoma, prostate cancer, cervical carcinoma, glioblastoma, pancreatic cancer and renal cancer.[41]

2.4.2.1. Structure of *Hs*MetAP2 Complexed with Fumagillin

The relationship between the antiproliferative activity of various fumagillin analogues with their ability to inhibit MetAP2 activity *in vitro* suggests that MetAP2 is the physiologically relevant target of fumagillin-based therapeutic agent.[40] This proposal is supported by a recent report that human endothelial cells are specially sensitive to fumagillin and that proliferation of these cells can be blocked by human MetAP2 antisense oligonucleotides.[27]

The X-ray analysis of the complex MetAP2-fumagillin showed a covalent bond between an imidazole nitrogen atom of His[231] and the carbon of the spirocyclic epoxide (**Figure 2.9**).[27,40] This covalent bond formation is responsible for fumagillin's irreversible inhibition.[27]

Although not predicted, the formation of this C-N bond is analogous to the alkylation of a catalytic histidine by α-chloroketone-type inhibitors of serine proteases.[42]

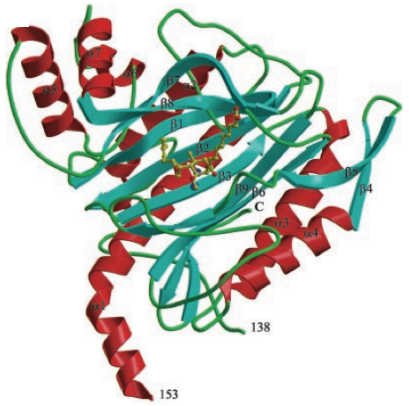


Figure 2.9. *Overall structure of the complex HsMetAP2 and fumagillin*[27]

The residue of His[231] does not move considerably upon bond formation; its nucleophilic imidazole nitrogen is perfectly positioned to bind with the methylene of the epoxide **(Figure 2.10-A).** The oxygen, liberated from the breaking of the epoxide is coordinated with cobalt (3.28 Å), and it occupies the approximate position of the cobalt-associated water molecule in the uncomplexed structure.[27] A molecule of water, equidistant from both cobalts, forms a hydrogen bond with this fumagillin oxygen **(Figure 2.10-A)**. The only residue that moves significantly upon fumagillin complexation is His[330], which rotates its side chain to avoid close contacts with fumagillin **(Figure 2.10-B)**.[27]

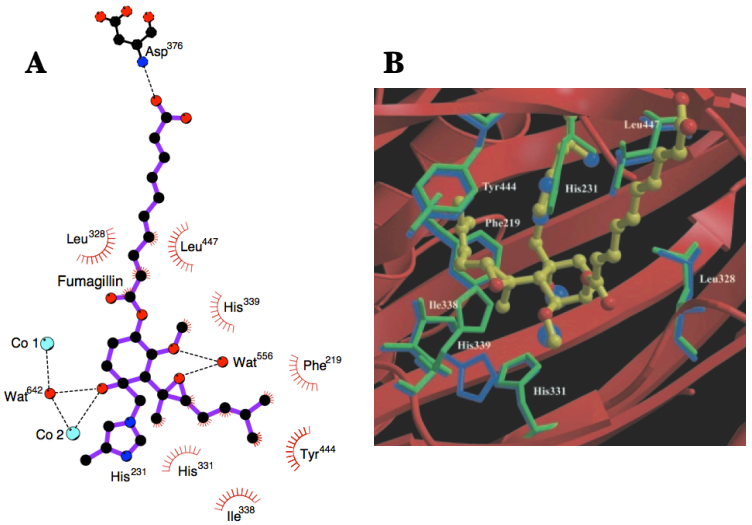


Figure 2.10. **(A)** *LIGPLOT of fumagillin in the binding pocket* **(B)** *Fumagillin in the active site of HsMetAP2*[27]

The epoxide-bearing side chain of fumagillin occupies the completely covered pocket near the active site (**Figure 2.10-B**).[27] It has hydrophobic contacts with His^{231} at the mouth of the pocket, Tyr^{444}, Ile^{338}, His^{339}, and Phe^{219}. A well-defined water molecule forms hydrogen bonds with the side chain epoxide and the methoxyl group at C5 (**Figure 2.10-A**).[27] The long unsaturated side chain protrudes from the binding pocket and makes two hydrophobic contacts with Leu^{328} and Leu^{447}, and both residues are conserved in the MetAP2 family. Leu^{447} lies near the end of the insertion that defines the MetAP2 family and the constriction formed by the Leu^{447}-Leu^{328} pair provides a structural basis for the MetAP2 family's requirement for a substrate with a small side chain at P2.[43] The terminal carboxyl of the side chain makes a hydrogen bond with Asp^{376}.[27]

2.4.2.2. Differences between Bengamides and Fumagillin and Ovalicin

Bengamides differ from the known MetAPs inhibitors, fumagillin and ovalicin in several aspects. Inhibition by fumagillin and ovalicin is achieved at the molecular level by the presence of two epoxide groups and the subsequent covalent binding to His^{231} located in the active site of MetAP2 as mentioned before (**Figure 2.11**).[27,44] Additionally, both compounds are selective inhibitors of endothelial cell proliferation, whereas bengamides inhibit all the tested cells (endothelial and epithelial). As depicted in **Figure 2.11**, the highly specific affinity of the bengamides towards methionine aminopeptidases is the result of multiple interactions that include hydrophobic and polar interactions of the terminal alkyl group of the olefin and the caprolactam moiety with the P1 pocket and the solvent-exposed P2 region, respectively, together with a coordination of the cobalt ions, present at the active site, with the hydroxyls at the C-3, C-4 and C-5 positions.[6]

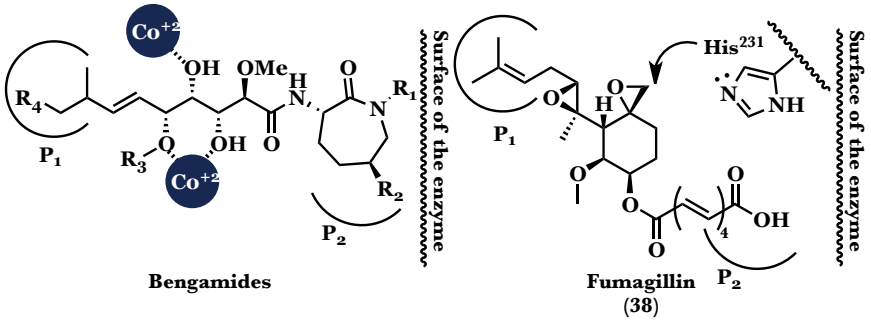


Figure 2.11. *Interaction of bengamides and fumagillin with MetAP2*

2.5. Synthesis of Bengamide Analogues

The intriguing biological activity exhibited by bengamides as promising new anticancer compounds led to our research group to delineate a synthetic strategy for bengamides and analogues, based on the above mentioned novel asymmetric epoxidation methodology.[45,46]

This synthetic strategy, consisted of three key steps detailed below, provided ready access to a set of analogues by modifications at C-2 and olefinic positions, as well as by modification of the lactam residue (**Figure 2.12**) by means of (1) an epoxide opening for the introduction of the methyl group at the C-2 position, (2) an olefin cross metathesis, as a means for the introduction of the isopropyl substituent of the olefin, and (3) an amide coupling for the incorporation of the ε-caprolactam residue.

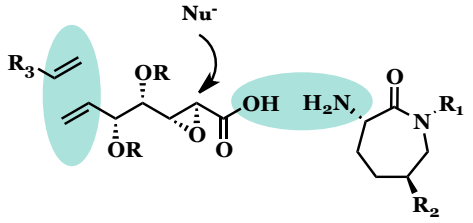


Figure 2.12. *Divergent strategy to bengamides via sulfur ylides*

Interestingly, this strategy was envisioned as a diversity-oriented synthetic approach, capable of delivering a wide array of analogues, allowing the incorporation of structural modifications at positions essential in the interaction of the molecules with the active site of methionine amino peptidases.[47] In this way, the synthesis of new derivatives of bengamides has allowed to deepen in the extensive structure-activity relationship study, as in the case of the potent cyclopentyl analogue, recently described and that possess a three- to fourfold more potent IC_{50} than its natural counterpart bengamide E against different tumour cell lines.[15] It is similarly remarkable the contributions by Prof. Nan, who

synthesized symplified analogues containing an open amide instead of a caprolactam ring, exhibiting potent cytotoxicity and better aqueous solubility compared with natural bengamides.[48]

2.5.1. New Analogues of Bengamides

The first objective of this thesis is the total synthesis of new analogues of bengamides containing oxirane rings through the polyketide chain, that could simulate the carbon skeleton of fumagillin and mimic its interaction with His[231], by means of a covalent interaction between the nitrogen atom in the imidazol ring and one of the epoxide carbons. The consequent biological evaluation of each of the synthesized analogues will provide new results about the structure/activity relationship which could lead to the design of more potent and efficient derivatives (**Figure 2.13**).

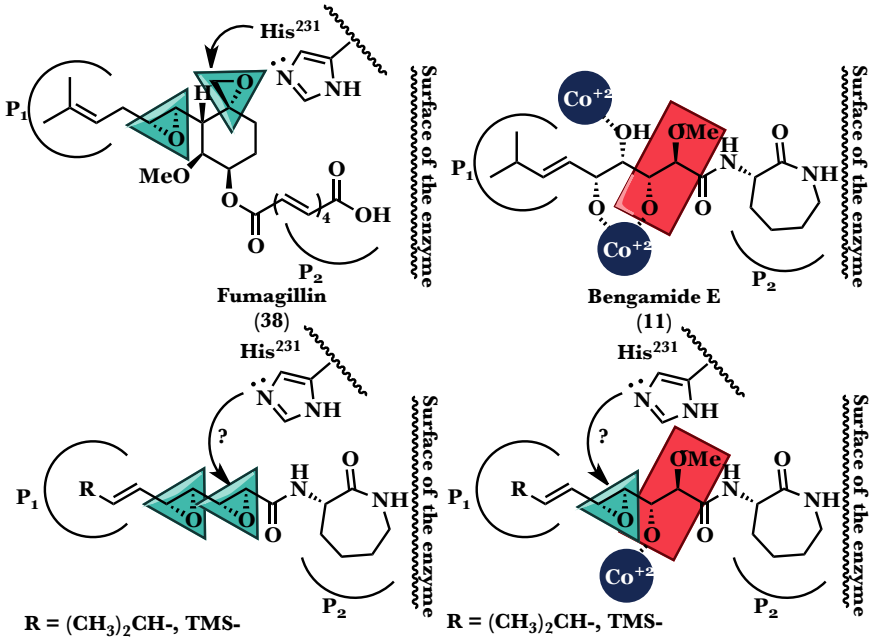


Figure 2.13. *Epoxy bengamides as new potential fumagilllin-like inhibitors*

Taking advantadge of this approach, we found also interesting to extend this methodology to the synthesis of amino derivatives of bengamides. The propitious interaction between amine groups and Co^{+2} cations present at the active site might involve a significant increase in the biological activity of these new analogues. In this way, the initial steps towards the synthesis of 4-amino derivatives have been established (**Figure 2.14**).

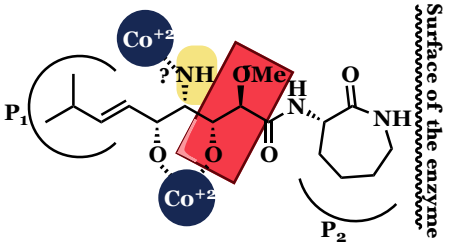
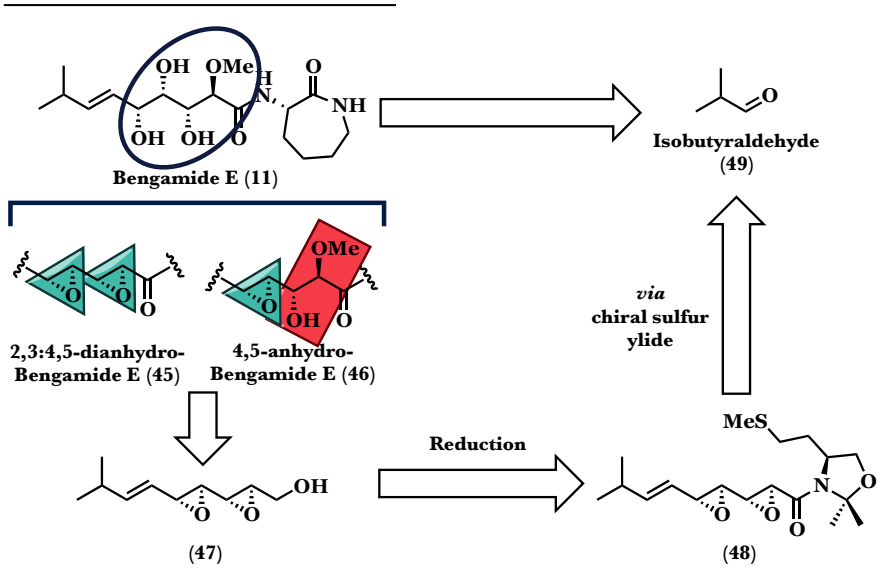


Figure 2.14. *Amino bengamides as new potential inhibitors*

2.5.2. Retrosynthesis

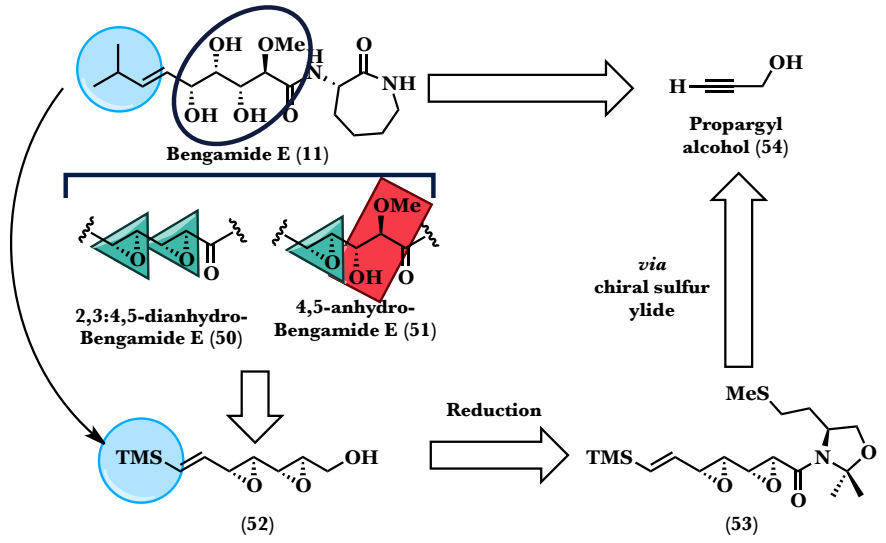
The synthesis of the 2,3:4,5-diepoxi (**45**) and 4,5-epoxi (**46**) derivatives of bengamides has been established from readily available starting material such as isobutyraldehyde (**49**), that easily provides the isopropyl group at the terminal olefinic position of bengamides (**Scheme 2.1**). The key intermediate, epoxy amide **48** can be easily obtaied *via* chiral sulfur ylide.

Scheme 2.1. *Retrosynthetic analysis of epoxy bengamide E analogues derived from isobutyraldehyde*



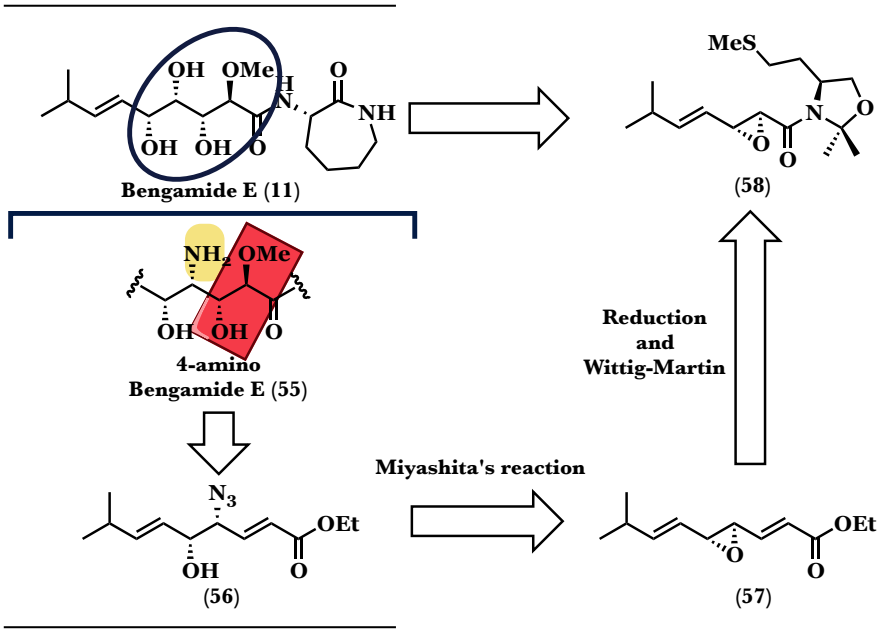
With the aim of extending the number of analogues, propargyl alcohol (**54**) was also considered as starting material, since it allows the introduction of a TMS- group at the terminal olefinic position, an isosteric group of the isopropyl one (**Scheme 2.2**).[49] Additionally, these silyl derivatives could be easily transformed into new functional groups by taking advantage of the vinyl silanes reactivity.[50,51] For the introduction of the stereogenic centers at the main skeleton of bengamides, the chiral sulfur ylides protocol was employed resulting in the appropriate setting-up of the mono- and di-epoxi groups.[52,53]

Scheme 2.2. *Retrosynthetic analysis of epoxy bengamide E analogues derived from propargyl alcohol*



Finally, for the synthesis of the amino derivative (**55**), a strategy involving the azido opening of the corresponding α,β-unsaturated ɣ,δ-epoxy ester has been planned, as depicted in **Scheme 2.3**. Thus, starting from epoxy amide **58**, by conversion into the corresponding α,β-unsaturated ester **57** and subsequent oxirane ring opening, *syn* azido alcohol **56** can be synthesized, from which 4-amino bengamide E (**55**) would be accomplished.

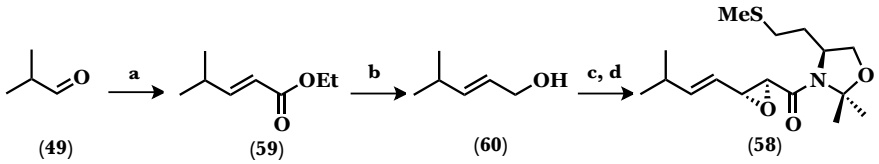
Scheme 2.3. *Retrosynthesis for C4 amino bengamide E analogue*



2.5.3. Synthesis of Epoxy Bengamide Analogues Derived from Isobutyraldehyde

The preparation of the main fragment of bengamides (**58**) started with the formation of the allylic alcohol **60** *via* reduction of the corresponding α,β-unsaturated ester **59** (75% isolated yield), which was synthesized from isobutyraldehyde by a Wittig reaction with (carbethoxymethylene)triphenylphosphorane (87%). Subsequent oxidation into the α,β-unsaturated aldehyde by means of MnO_2, followed by reaction with the sulfonium salt **4** under basic conditions afforded epoxyamide **58**, which was obtained as a single diastereoisomer (in 58% yield over 2 steps) after purification by flash column chromatography (**Scheme 2.4**). From this key intermediate both mono- and di-epoxi analogues can be synthesized as described below.

Scheme 2.4. *Synthesis of the epoxy amide* ***58***



Reagents and conditions: (a) Ph_3PCHCO_2Et, DCM, 25ºC, 87%. (b) DIBAL-H, -78ºC, 30 min, 75%. (c) MnO_2, DCM, 25ºC, 12 h. (d) **4**, 3.0 M NaOH, *t*BuOH, 25ºC, 16 h, 58% (over 2 steps).

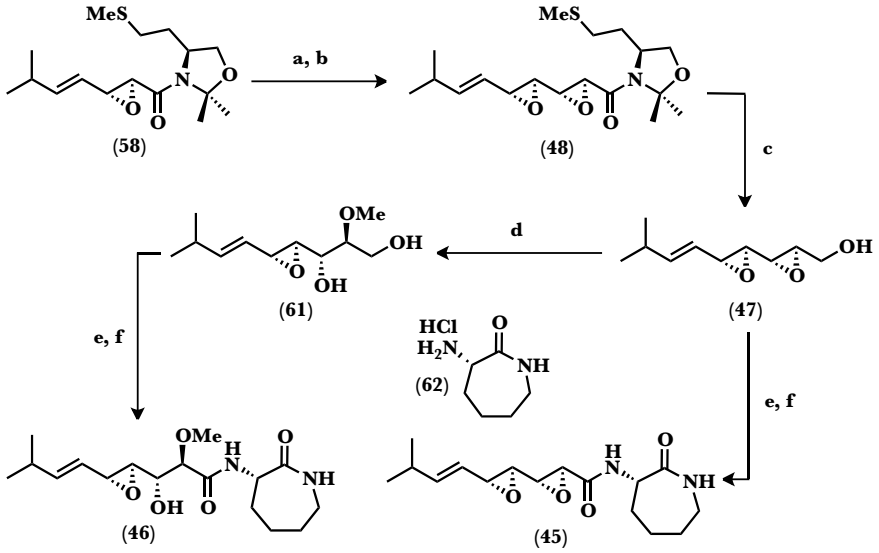
Once the first oxirane ring was installed in a stereoselective fashion, the construction of a second oxirane group was undertaken *via* direct reduction of **58** to the epoxy aldehyde by the action of sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al)[54] followed by a second reaction with sulfonium salt **4**, according to a two-phase protocol,[55] due to the presence of the first oxirane ring contiguous to the carbonyl group, to obtain diepoxy amide **48** in an excellent 85% overall yield from **58** (**Scheme 2.5**).

Reduction of **48** with Super-H yielded the expected diepoxy alcohol **47** which was considered a key product for the consecution of both coveted epoxy analogues of bengamide E (**Scheme 2.5**).

In a first set, diepoxy alcohol **47** was oxidized to the corresponding diepoxy acid by the action of TEMPO/BAIB conditions and coupled with amino lactam **62** in the presence of BOP and DIPEA, to furnish the diepoxy bengamide E analogue **45**. On the other hand, **47** was treated with methanol in the presence of $B(OMe)_3$ and DBU to yield the corresponding 2-methoxyl derivative **61** in both chemo- and regioselective manners. Selective oxidation of the primary alcohol and coupling of the resulting acid with amino lactam **62** were carried out

without problems to obtain the desired 5,6-epoxy bengamide E **46** (**Scheme 2.5**).

Scheme 2.5. *Synthesis of the analogues **45** and **46***



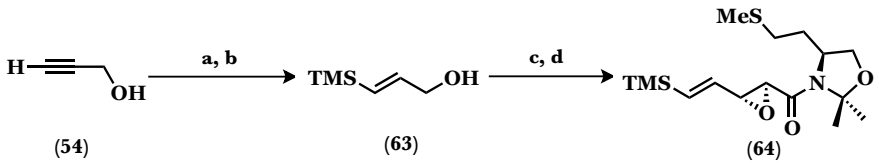
Reagents and conditions: (a) Red-Al, THF, 0ºC, 45 min. (b) **4**, 5.0 M NaOH, CH_2Cl_2-H_2O, 25ºC, 85% (over 2 steps). (c) Super-H, THF, 0ºC, 30 min, 70%. (d) $B(OMe)_3$, DBU, MeOH, reflux, 12 h.(e) TEMPO/BAIB, CH_3CN-H_2O, 25ºC, 16 h. (f) **62**, BOP, DIPEA, DMF, 25ºC, 16 h, 55% for **45** (from **47**), 47% for **46** (from **47**).

2.5.4. Synthesis of Epoxy Bengamide Analogues Derived from Propargyl Alcohol

Given the positive results obtained in the synthesis of the epoxy and diepoxy bengamide analogues, we deemed it of interest to extend this chemistry to the trimethyl silyl derivatives **50** and **51** because the presence of this trimethylsilyl moiety can serve as an isostere for the isopropyl group found in the natural bengamides, as well as a handle to expand the synthetic possibilities for further structural modifications at the terminal

olefinic position in virtue of the activity of vinylsilanes.[50,51] The main fragment of these TMS-derivatives was accomplished from propargyl alcohol in a four-steps sequence involving introduction of the silyl ether, reduction to the corresponding allylic alcohol under Red-Al conditions[56] and subsequent oxidation and coupling with the sulfur ylide derived from L-Met **(1)**. In this way, the epoxy amide **64** was obtained in a gratifying 75% overall yield (**Scheme 2.6**).

Scheme 2.6. *Synthesis of the epoxyamide* ***64***



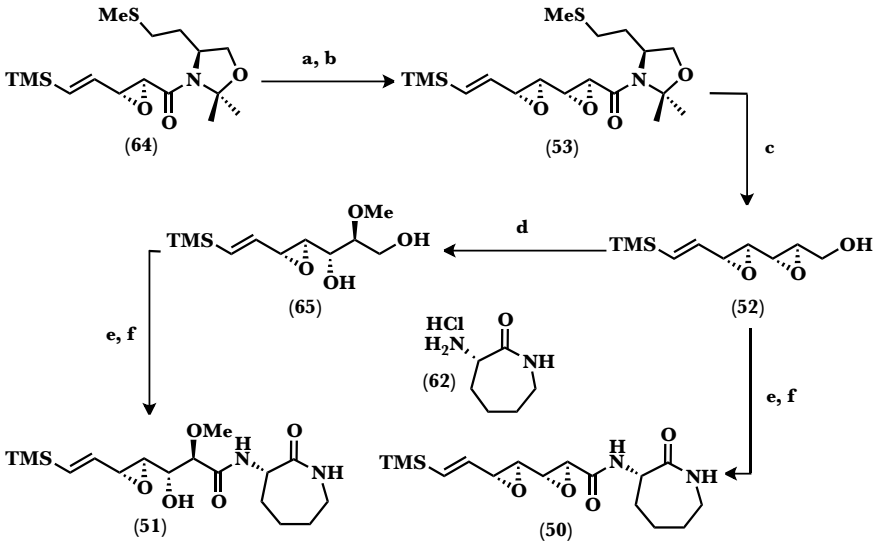
(54) **(63)** **(64)**

Reagents and conditions: (a) EtMgBr, TMSCl, 0ºC. (b) Red-Al, THF, 0ºC, 45 min. (c) MnO_2, DCM, 16 h. (d) **4**, 5.0 M NaOH, *t*BuOH, 25ºC, 75% (overall yield).

The key epoxy amide **64** was then converted into the corresponding diepoxy amide *via* direct reduction to the epoxy aldehyde by the action of sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al)[54] followed by a second reaction with sulfonium salt **4**, according to the two-phase method,[55] proceeding in a 84% yield over 2 steps (**Scheme 2.7**).

Reduction of **53** with Super-H to yield the diepoxy alcohol **52** and subsequent reactions sequence as proceeded above rendered both coveted epoxy analogues of bengamide E (**Scheme 2.7**).

Scheme 2.7. *Synthesis of the analogues* ***50*** *and* ***51***



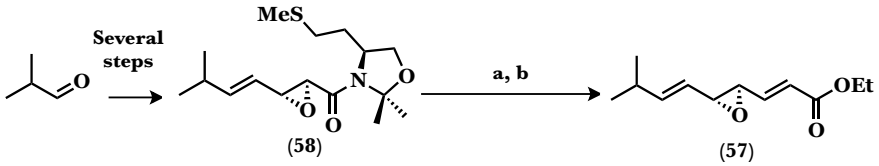
Reagents and conditions: (a) Red-Al, THF, 0ºC, 45 min. (b) **4**, 5.0 M NaOH, CH_2Cl_2-H_2O, 25ºC, 84% (over 2 steps). (c) Super-H, THF, 0ºC, 30 min, 55%. (d) $B(OMe)_3$, DBU, MeOH, reflux, 12 h. (e) TEMPO/BAIB, CH_3CN-H_2O, 25ºC, 16 h. (f) **62**, BOP, DIPEA, DMF, 25ºC, 16 h, 30% for **50** (from **52)**, 26% for **51** (from **52).**

Thus, proceeding in a similar manner as for analogues **45** and **46**, epoxy and diepoxy bengamide analogues **50** and **51** were obtained, in similar, or even better yields compared with the isopropyl series.

2.5.5. Synthesis of Amino Derivatives of Bengamide E

The synthesis of the amino derivative **55** started from the previously described epoxy amide **58**, which was subjected to reduction and Wittig-Martin reaction to yield the γ,δ-epoxy α,β-unsaturated ester (**57)** in 82% yield over 2 steps (**Scheme 2.8**).

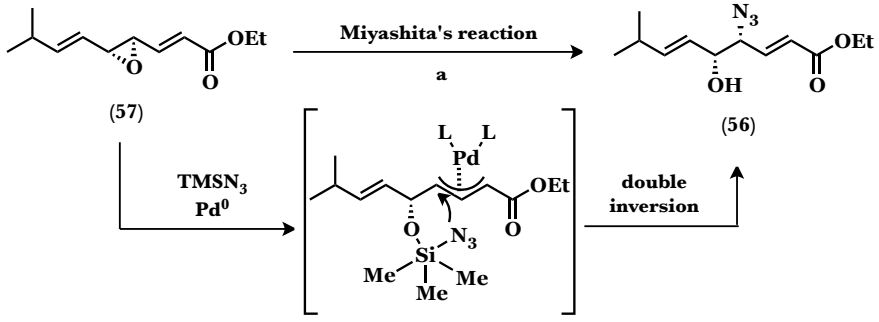
Scheme 2.8. *Synthesis of the γ,δ-epoxy α,β-unsaturated ester* ***(57)***



Reagents and conditions: (a) Red-Al, THF, 0ºC, 45 min. (b) $Bu_3P{=}CHCO_2Et$, CH_2Cl_2, 12 h, 82% (over 2 steps).

With the unsaturated-epoxy system in hand, the next step was the palladium-mediated oxirane ring opening, that should proceed with double inversion of configuration as described by Miyashita et al. to render the *syn*-azido alcohol **56** in 82% yield (**Scheme 2.9**).[57]

Scheme 2.9. *Pd^0-catalized stereospecific azide substitution reaction*

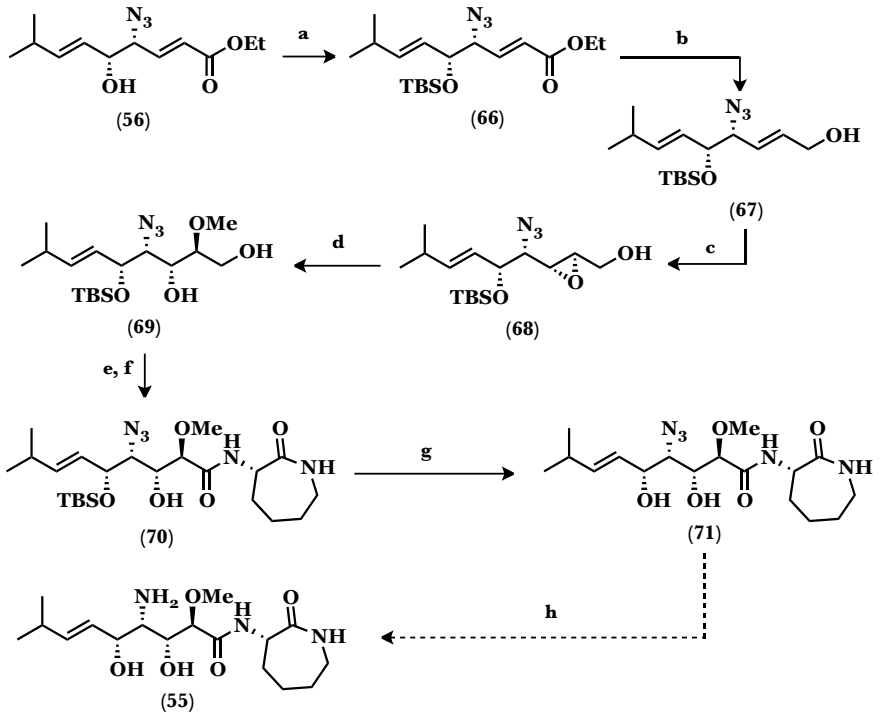


Reagents and conditions: (a) $TMSN_3$, $Pd[PPh_3]_4$, 25ºC, 5 h, then citric acid 10% in MeOH, 82%.

Syn-azido alcohol **56** was then protected as the silyl ether by reaction with TBSOTf and 2,6-lutidine and treated with DIBAL-H at -78ºC to afford the allylic alcohol **67** in 85% yield over 2 steps. With the azido allylic alcohol in hand, we proceeded with the epoxidation of the double bond. To this aim, **67** was subjected to a Sharpless asymmetric

epoxidation by treatment with TBHP in the presence of D-(-)-DET. However, the resulting epoxy alcohol **68** was obtained in a discouraging 10% yield. Further attemps to improve the yield of this epoxidation were unsuccessfull. Nevertheless, we were able to bring enought amount of azido epoxy alcohol to continue the delineated synthetic sequence. Thus, azido derivative **68** was treated with methanol in the presence of $B(OMe)_3$ and DBU to yield the corresponding 2-methoxyl derivative **69** in both chemo- and regioselective manners. Selective oxidation and coupling of the crude carboxylic acid with amino lactam **62** were carried out in the same manner as described before, to obtain the amide **70** in 86% over 3 steps. Deprotection of the silyl ether under TBAF conditions yielded the desired 4-azido-bengamide derivative **71** in 55% yield (**Scheme 2.10**). Although the reduction of the azide into the amine has not been tested yet, the azido derivative **71** represents a valuable precursor of the amino analogue of bengamide E and we feel confident this new derivative will be soon obtained.

Scheme 2.10. *Synthesis of C4 azido bengamide E and C4 amino bengamide E*



Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 25ºC, quant. (b) DIBAL-H, -78ºC, 45 min, 82%. (c) SAE, D-(-)-DET, 10%. (d) $B(OMe)_3$, DBU, MeOH, reflux, 16 h. (e) TEMPO/BAIB, CH_3CN-H_2O, 25ºC, 16 h. (f) **62**, BOP, DIPEA, DMF, 25ºC, 16 h, 86% (over 3 steps). (g) TBAF, THF, 0ºC, 40 min, 55%. h. Ph_3P, THF-H_2O (2:1), 25ºC, 16 h.

2.6. Biological Evaluation of Bengamide E Analogues

Having prepared the analogues of bengamide E, **45-46** and **50-51**, our next goal in this research was to evaluate their antitumor properties to determine the influence of the described structural modifications against the anti proliferative potency. The determination of

the cytotoxic properties of all these compounds was performed by measuring their IC_{50} values against a panel of different tumour cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay. This cytotoxicity was examined in four different cancer cell lines, namely, HL 60 (human promyelocytic leukemia), MDA-MB-231 (human breast carcinoma), HT1080 (human fibrosarcoma), and HT29 (human colon adenocarcinoma), and in a primary culture of non transformed bovine aorta endothelial (BAE) cells. Bengamide E and fumagillin were used as controls to compare the activity of the new synthesized analogues. The results of this investigation are summarized in **Table 2.2.**

According to these results, bengamide E analogues tested displayed a well-defined cytotoxicity in tumour cells as well as in endothelial cells. This is in agreement with previous observations indicating that inhibition of MetAP2 by bengamides does not result in selective inhibition of endothelial cell proliferation.[7] Dose-response curves obtained with bengamide E and its analogues showed a sharp decrease in cell survival at concentrations that are around the IC_{50}.

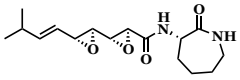
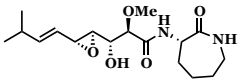
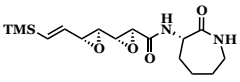
In contrast, fumagillin showed a biphasic effect on the growth of proliferating endothelial cells, indicating that fumagillin antiproliferative activity is not endothelial specific, what is in agreement with previously reported data.[58]

Fumagillin (**38**), the fungal metabolite that potently inhibits angiogenesis by blocking endothelial cell proliferation and has advanced into clinical trials for multiple cancers, showed a biphasic effect on the growth of proliferating endothelial cells. At owed concentrations there is a first decrease in cell number, probably due to a cytostatic effect, and after a plateau covering several orders of concentration, a second cytotoxic effect

is observed. These biphasic dose-response curves, typical of fumagillin and its derivatives, were obtained with the tumor cell lines studies, indicating that fumagillin anti proliferative activity is not endothelial specific, which is in agreement with previously reported data.[58]

Dose-dependent effect on the *in vitro* growth of tumor and endothelial cells of bengamide E (**11**), fumagillin (**38**) and compounds (**45**), (**46**) and (**50**) is shown in **Table 2.3**.

Table 2.2. *In vitro antitumoral activity (μM) of bengamide E (**11**), fumagillin (**38**) and synthetic analogues (**45**), (**46**) and (**50**) against different tumor cell lines and BAEC*

Compound	Cell Lines				
	MDAMB 231	HT29	HT1080	HL60	BAE
Bengamide E (**9**)	1.6±0.54	0.95±0.16	0.29±0.03	0.68±0.10	0.28±0.03
Fumagillin (**36**)	54.3±10.2	38.3±12.5	Biphasic curve	36±7.5	Biphasic curve
 (**45**)	100	100	>100	nd	100
 (**46**)	100	100	>100	nd	100
 (**50**)	100	100	>100	72	78

Cells were incubated for 72 h in the presence of each compound, and the ratios of viable cells were determined by MTT assay. The drug concentration required to inhibit cell growth by 50% (IC_{50}) was determined from semilogarithmic dose-response plots, and results represent the means ± SDs of three independent experiments.

These biological evaluations revealed that the epoxy and diepoxy bengamide analogues **45-47** and **50-51** were completely inactive in the cytotoxic studies, indicating that the replacement of the 1,2-hydroxyl systems by an oxirane ring produced a complete lack of interaction with the active site of the enzymes, not resulting in a fumagillin-like interaction as we initially surmised.

2.7. Summary and Concluding Remarks

In this chapter, the implementation of the novel asymmetric epoxidation methodology, based on the use of a new class of chiral sulfonium salts in the synthesis of epoxy and diepoxy bengamides derivatives has proved to be useful and efficient. The synthetic value of this protocol has been demonstrated by its application in the concise total synthesis of analogues **45-46** and **50-51** in good yield (12% for **45**, over 9 steps; 11% for **46**, over 10 steps; 10% for **50** over 9 steps and 9% for **51** over 10 steps) and with high selectivity (ee> 98%). In theory, **50** and **51** can also be utilized in future cross-coupling reactions to access bengamides of any desired alkyl substitution at the terminal olefinic position.

Additionally, cytotoxic studies have been carried out over compounds **45-46** and **50**, revealing that replacement of the hydroxyl groups by an oxirane ring throughout the polyketide chain of bengamides results in a total lack of antiproliferative activity.

Finally, the first steps towards new amino bengamide derivatives, considered promising new bengamide analogues, have been undertaken with the synthesis of the precursor **71**, containing the azido group at position C4.

2.8. Experimental Section

2.8.1. General Procedures for the Synthesis of Epoxy and Diepoxy Amides

2.8.1.1. Synthesis of Epoxy Amides

To a solution of sulfonium salt (**4** or **6**) (1.1 equiv) in *t*BuOH (~ 0.08 M) was added a 3.0 M aqueous NaOH solution (1.1 equiv). After 10 min at 25ºC, a solution of the corresponding aldehyde (1.0 equiv), in *t*BuOH (~ 0.1 M) was added, and the crude reaction mixture was vigorously stirred overnight at 25ºC. After this time, both phases were separated, and the aqueous layer was extracted twice with EtOAc. Combined organic extracts were then washed with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. Flash column chromatography (silica gel, EtOAc 20-30% in hexanes) afforded the corresponding epoxy amide.

2.8.1.2. Synthesis of Diepoxy Amides

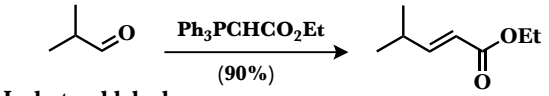
To a solution of the corresponding epoxy amide (1.0 equiv) in dry THF (0.08 M) was added dropwise Red-Al 70% w/v in toluene (2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

To obtain the corresponding diepoxy amide**,** a 5.0 M aqueous NaOH solution (1.0 equiv) was added to a solution of sulfonium salt (**4** or **6**) (1.1 equiv) and the crude aldehyde in CH_2Cl_2-H_2O (1:1) (~ 0.4 M). The reaction mixture was vigorously stirred overnight at 25ºC. After this time,

both phases were separated, and the aqueous layer was extracted twice with EtOAc. Combined organic extracts were then washed with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification of the crude product by flash column chromatography (silica gel, 20-30% EtOAc in hexanes) provided corresponding diepoxy amide.

2.8.2. Epoxy Bengamides Derived from Isobutyraldehyde

2.8.2.1. Synthesis of the Ester **59** from Isobutyraldehyde (**49**)

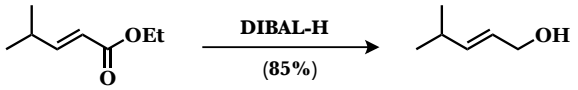


Isobutyraldehyde (49) **(59)**

Isobutyraldehyde (**49**) (2.0 g, 28.0 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (140 mL) and over this solution was added $Ph_3P{=}CHCO_2Et$ (19.5 g, 55.4 mmol 2.0 equiv). After 30 minutes the reaction was complete and the solvent was removed under reduced pressure. Flash column chromatography (silica gel, 10% EtOAc in hexanes) of the crude product furnished α,β-insaturated ester **59** (3.6 g, 90% yield) as a white solid and whose data matched with those reported in the literature.[59]

R_f = 0.78 [Silica gel, 40% EtOAc in hexanes]. ^{1}H RMN (400 MHz, $CDCl_3$), δ (ppm): 6.88 (dd, $\mathcal{J}$ = 15.7, 6.6 Hz, 1H, CHC*H*=CH), 5.71 (dd, $\mathcal{J}$ = 15.7, 1.5 Hz, 1H, CHCH=C*H*), 4.06 (q, $\mathcal{J}$ = 7.1 Hz, 2H, COOC*H*$_2$CH$_3$), 2.48-2.32 (m, 1H, (CH$_3$)$_3$C*H*), 1.20 (t, $\mathcal{J}$ = 6.4 Hz, 3H, COOCH$_2$C*H*$_3$), 1.01 (d, $\mathcal{J}$ = 6.8 Hz, 6H, (C*H*$_3$)$_2$CH).

2.8.2.2. Reduction of Ester **59** to Allylic Alcohol **60**

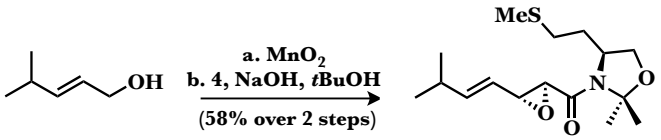


(59) **(60)**

At -78ºC, DIBAL-H 1.0 M in toluene (25 mL, 25.0 mmol, 2.5 equiv) was added over a solution of ester **59** (1.4 g, 9.9 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL). After 30 minutes, the reaction was complete and it was allowed to warm at 0ºC. At this temperature, the mixture was diluted with EtOAc and a saturated aqueous Na^+/K^+ tartrate solution. After 2 hours of vigorous stirring, the aqueous phase was extracted twice with EtOAc and the organic layer washed with water and brine, then dried over anhydrous $MgSO_4$ and filtered. The solvent was then removed carefully under reduced pressured at low temperature. Flash column chromatography (silica gel, 30% de EtOAc in hexanes) rendered allylic alcohol **60** (840 mg, 85% yield) as a colourless oil.

R_f = 0.23 [Silica gel, 40% EtOAc in hexanes]. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.70-5.63 (m, 1H, CH=C*H*CH_2), 5.58 (dtd, $\mathcal{J}$ = 15.4, 5.5, 0.8 Hz, 1H, CHC*H*=CH), 4.08 (d, $\mathcal{J}$ = 5.5 Hz, 2H, CH=CHCH_2), 2.34-2.25 (m, 1H, $(CH_3)_2$C*H*), 1.92 (bs, 1H, O*H*), 1.01 (d, $\mathcal{J}$ = 6.8 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 138.75, 128.67, 62.96, 31.36, 20.37.

2.8.2.3. Synthesis of the Epoxy Amide **58**



(60) **(58)**

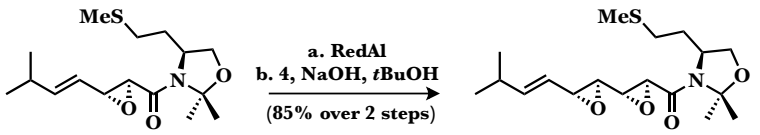
To a solution of allylic alcohol (**60**) (102 mg, 1.0 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) was added MnO_2 (1.4 g, 16.0 mmol, 16.0 equiv). After

stirring for 12 hours at 25ºC, the crude mixture was filtered through Celite, and the resulting clear solution was concentrated under reduced pressure at 20ºC to obtain the corresponding α,β-unsaturated aldehyde, which was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (350 mg, 1.12 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 0.34 mL, 1.02 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of epoxy amides to yield epoxy amide **58** (184 mg, 58% over two steps) as a yellow oil.

R_f = 0.18 [Silica gel, 30% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +15.9 (c 0.4, CH_2Cl_2) . ^{1}H NMR(400 MHz, $CDCl_3$), δ (ppm): 6.02 (dd, $\mathcal{J}$ = 15.6, 6.5 Hz, 1H, CH=C*H*CH(O)), 5.13 (ddd, $\mathcal{J}$ = 15.7, 8.0, 1.4 Hz, 1H, CHC*H*=CH), 4.27 (ddd, $\mathcal{J}$ = 8.5, 4.8, 3.2 Hz, 1H, NHC*H*), 4.01 (ddd, $\mathcal{J}$ = 9.1, 5.3, 1.4 Hz, 1H, OCH_2CH), 3.88 (d, $\mathcal{J}$ = 9.3 Hz, 1H, OCH_2CH), 3.54-3.49 (m, 2H, =CHC*H*(O) and COC*H*(O)), 2.59-2.51 (m, 1H, CH_3SCH_2), 2.46-2.37 (m, 1H, CH_3SCH_2), 2.37-2.28 (m, 1H, $(CH_3)_2CH$), 2.07 (s, 3H, CH_3S), 2.05-1.97 (m, 1H, SCH_2CH_2), 1.80-1.72 (m, 1H, SCH_2CH_2), 1.63 (s, 3H, CH_3C), 1.53 (s, 3H, CH_3C), 0.99 (d, $\mathcal{J}$ = 6.8 Hz, 3H, CH_3CH), 0.98 (d, $\mathcal{J}$ = 6.7 Hz, 3H, CH_3CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 163.57, 146.05, 122.52, 95.91, 67.03, 58.53, 55.83, 55.48, 34.27, 30.95, 30.81, 26.29, 23.02, 21.80, 21.77, 15.84. HRMS (ESI-TOF) *m/e* 314.1790 calcd for $C_{16}H_{27}NO_3S$ $[M + H]^+$, found 314.1784.

2.8.2.4. Synthesis of the Diepoxy Amide **48**



(58) **(48)**

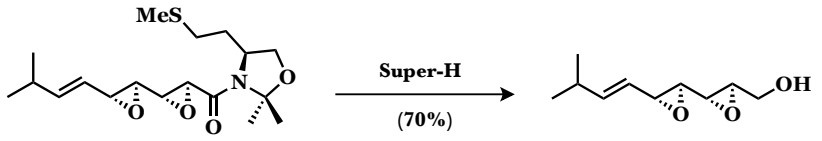
To a solution of epoxy amide **58** (320 mg, 1.02 mmol, 1.0 equiv) in dry THF (20 mL) was added dropwise Red-Al (0.7 mL, 2.24 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (350 mg, 1.12 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.20 mL, 1.02 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **48** (310 mg, 85% over two steps) as a yellow oil.

R_f = 0.37 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +35.1 (*c* 0.7, CH_2Cl_2) . ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.00-5.92 (m, 1H, $(CH_3)_2CHCH$=), 5.09 (ddd, J = 15.6, 8.3, 1.4 Hz, 1H, $(CH_3)_2CHCH$=C*H*), 4.31 (ddd, J = 8.5, 4.8, 3.3 Hz, 1H, NC*H*), 4.00 (ddd, J = 9.2, 5.2, 1.4 Hz, 1H, OCH_2CH), 3.90 (dd, J = 9.2, 0.6 Hz, 1H, OCH_2CH), 3.58 (d, J = 2.0 Hz, 1H, COC*H*(O)), 3.36 (dd, J = 8.3, 1.7 Hz, 1H, COCH(O)C*H*), 3.34 (dd, J = 3.6, 1.9 Hz, 1H, =CHC*H*(O)), 3.01 (dd, J = 3.5, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.57 (ddd, J = 13.0, 7.7, 5.1 Hz, 1H, CH_3SCH_2), 2.47 (ddd, J = 13.2, 8.4, 7.2 Hz, 1H, CH_3SCH_2), 2.37-2.27 (m, 1H, $(CH_3)_3CH$), 2.11 (s, 3H, CH_3S), 2.09-2.01 (m, 1H, SCH_2CH_2), 1.86-1.76 (m, 1H, SCH_2CH_2), 1.63 (s, 3H, CH_3C), 1.52 (s,

3H, C*H*$_3$C), 0.99 (d, $\mathcal{J}$ = 6.8 Hz, 6H, (C*H*$_3$)$_2$CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.96, 145.36, 122.84, 95.94, 67.05, 56.54, 56.43, 55.98, 55.55, 51.20, 34.45, 30.90, 30.71, 26.22, 22.96, 21.86, 21.83, 15.85. HRMS (ESI-TOF) *m/e* 356.1896 calcd for $C_{18}H_{29}NO_4S$ [M + H]$^+$, found 356.1872.

2.8.2.5. Synthesis of the Diepoxy Alcohol **47**

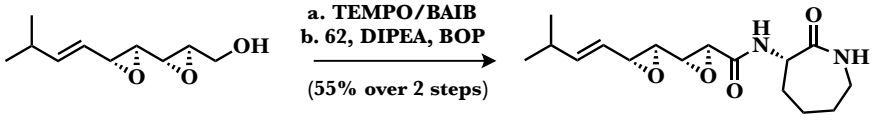


(48) **(47)**

Diepoxy amide **48** (100 mg, 0.28 mmol, 1.0 equiv) in THF (6 mL) was reduced by treatment with Super-H 1.0 M in THF (0.7 mL, 0.7 mmol, 2.5 equiv) at 0ºC. After 1 h at this temperature, the reaction mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was separated, extracted with Et_2O twice and the combined organic phase washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, 30% EtOAc in hexanes) provided diepoxy alcohol **47** (36 mg, 70%) as a pale yellow oil.

R_f = 0.26 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +27.2 (*c* 0.9, CH_2Cl_2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.95 (ddd, $\mathcal{J}$ = 15.6, 6.5, 3.3 Hz, 1H, (CH$_3$)$_2$CHC*H*=), 5.10 (ddd, $\mathcal{J}$ = 15.6, 8.3, 1.3 Hz, 1H, (CH$_3$)$_2$CHCH=C*H*), 4.01-3.93 (m, 1H, C*H*$_2$OH), 3.75-3.65 (m, 1H, C*H*$_2$OH), 3.33 (dd, $\mathcal{J}$ = 8.2, 2.0 Hz, 1H, =CHC*H*(O)), 3.20-3.16 (m, 1H, =CHCH(O)C*H*), 3.09-2.97 (m, 1H, (O)C*H*CH$_2$), 2.91 (dd, $\mathcal{J}$ = 4.6, 2.1 Hz, 1H, C*H*(O)CHCH$_2$), 2.40-2.27 (m, 1H, (CH$_3$)$_2$C*H*), 1.00 (d, $\mathcal{J}$ = 6.8 Hz, 6H, (C*H*$_3$)$_2$CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 145.06, 123.17, 60.57, 57.87, 56.26, 55.72, 53.53, 30.90, 21.88, 21.86. HRMS (ESI-TOF) *m/e*. 185.1178 calcd for $C_{10}H_{16}O_3$ [M + H]$^+$, found 185.1206.

2.8.2.6. Synthesis of the Diepoxy Amide **45**



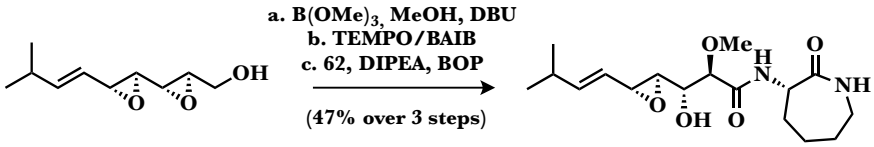
(47) **(45)**

Diepoxy alcohol **47** (50 mg, 0.27 mmol, 1.0 equiv) was dissolved in a 1:1 mixture of CH_3CN/H_2O (8 mL) and the resulting solution was treated with BAIB (535 mg, 1.6 mmol, 6.0 equiv), followed by TEMPO (40 mg, 0.22 mmol, 0.8 equiv) at 25ºC. After 5 h, the crude mixture was diluted with EtOAc, quenched by the addition of a saturated aqueous $Na_2S_2O_3$ solution and, after separation of both layers, the aqueous phase was then extracted with EtOAc. The organic solution was washed again with a saturated aqueous $Na_2S_2O_3$ solution, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude acid was dissolved in DMF (3 mL) and treated with DIPEA (0.1 mL, 0.5 mmol, 2.0 equiv), amino lactam **62** (67 mg, 0.41 mmil, 1.5 equiv) and BOP (150 mg, 0.32 mmol, 1.2 equiv) at 25ºC. After being stirred at this temperature overnight, the crude mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. Purification of the obtained crude product by flash column chromatography (silica gel, 80% EtOAc in hexanes) provided diepoxy amide **45** (3 mg, 55% over 2 steps) as a white amorphous solid.

R_f = 0.43 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +49.4 (*c* 0.4, DMSO) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.46 (d, $\mathcal{J}$ = 5.5 Hz, 1H, CON*H*CH), 6.10 (t, $\mathcal{J}$ = 6.0 Hz, 1H, CON*H*CH_2), 5.95 (dd, $\mathcal{J}$ = 15.6, 6.6 Hz, 1H, $(CH_3)_2$CHC*H*=CH), 5.09 (ddd, $\mathcal{J}$ = 15.6, 8.2, 1.4 Hz, 1H, $(CH_3)_3$CHCH=C*H*), 4.50 (ddd, $\mathcal{J}$ = 11.4, 6.0, 1.4 Hz, 1H, C*H*NHCO), 3.45 (d, $\mathcal{J}$ = 2.1 Hz, 1H, (O)C*H*CONH), 3.35 (dd, $\mathcal{J}$ = 8.2, 1.8 Hz, 1H,

=CHC*H*(O)), 3.30-3.24 (m, 2H, CONHCH_2,), 3.05 (dd, $\mathcal{J}$ = 4.5, 2.1 Hz, 1H, C*H*(O)CHCO), 2.90 (dd, $\mathcal{J}$ = 4.5, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.38-2.28 (m, 1H, $(CH_3)_2$C*H*), 2.04-1.96 (m, 2H, CH_2), 1.90-1.79 (m, 2H, CH_2), 1.50-1.36 (m, 2H, NHCHCH_2), 1.00 (d, $\mathcal{J}$ = 6.8 Hz, 6H, $(CH_3)_2$CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 174.62, 166.83, 145.43, 122.65, 57.19, 56.99, 56.40, 52.67, 51.73, 42.15, 31.38, 30.92, 28.87, 27.86, 21.83. HRMS (ESI-TOF) *m/e* 309.1814 calcd for $C_{16}H_{24}N_2O_4$ $[M + H]^+$, found 309.1802.

2.8.2.7. Synthesis of the Epoxy Amide **46**



(47) **(46)**

Epoxy alcohol **47** (15 mg, 0.08 mmol, 1.0 equiv) was dissolved in a 1:1 mixture of MeOH/$B(OMe)_3$ (3 mL) and the resulting solution was treated with DBU (10 μL, 0.08 μmol 1.0 equiv) and heated at 65ºC for 12 hours. After this time, the reaction mixture was allowed to reach room temperature, cooled to 0ºC, and then treated with a saturated aqueous $NaHCO_3$ solution. After stirring for 30 min at 0ºC, EtOAc was added and both phases were separated. The aqueous phase was extracted with EtOAc and the combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting crude product was used in the next step without further purification.

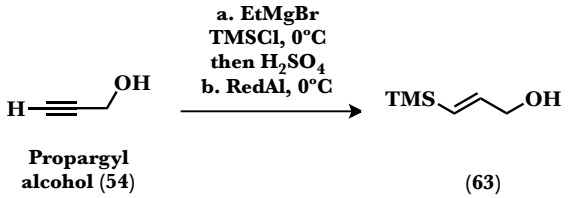
The crude of the diol (0.08 mmol, 1.0 equiv) was dissolved in a 1:1 mixture of CH_3CN/H_2O (4 mL) and the resulting solution was treated with BAIB (160 mg, 0.49 μmol, 6.0 equiv) followed by TEMPO (10 mg, 0.065 mmol, 0.8 equiv) at 25ºC. After 5 h, the crude mixture was diluted with EtOAc, quenched by the addition of a saturated aqueous $Na_2S_2O_3$

solution and, after separation of both layers, the aqueous phase was then extracted with EtOAc. The organic solution was washed again with a saturated aqueous $Na_2S_2O_3$ solution, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude acid (0.08 mmol, 1.0 equiv) was dissolved in DMF (5 mL) and treated with DIPEA (30 μL, 0.16 mmol, 2.0 equiv), amino lactam **62** (20 mg, 0.12 μmol, 1.5 equiv) and BOP (44 mg, 0.096 mmol, 1.2 equiv) at 25ºC. After being stirred at this temperature overnight, the crude mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. Purification of the obtained crude product by flash column chromatography (silica gel, 80% EtOAc in hexanes) provided amide **46** (13 mg, 47% over 3 steps) as a colourless oil.

R_f = 0.21 [Silica-gel, EtOAc]. $[\alpha]^{25}_D$ = +111.0 (*c* 0.1, CH_2Cl_2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.78 (d, $\mathcal{J}$ = 12.3 Hz, 1H, CON*H*CH), 5.94-5.87 (m, 1H, CON*H*CH_2), 5.84 (ddd, $\mathcal{J}$ = 15.5, 6.5, 1.0 Hz, 1H, $(CH_3)_2$CHC*H*=CH), 5.36 (ddd, $\mathcal{J}$ = 15.5, 7.1, 1.4, 1H, $(CH_3)_3$CHCH=C*H*), 4.59-4.50 (m, 2H, C*H*NHCO and C*H*OH), 4.05-4.02 (m, 1H, O*H*), 3.86 (s, 3H, CHOCH_3), 3.40-3.19 (m, 4H, CONHCH_2,=CHC*H*(O) and CH_3OC*H*), 2.39-2.30 (m, 1H, $(CH_3)_2$C*H*), 2.09-1.95 (m, 2H, CH_2), 1.91-1.19 (m 2H, CH_2), 1.67-1.56 (m, 2H, NHCHCH_2), 1.02 (d, $\mathcal{J}$ = 6.8, 6H (CH_3)$_2$CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 175.6, 175.2, 143.4, 122.4, 82.9, 57.8, 57.0, 53.4, 52.1, 51.6, 42.1, 31.4, 30.2, 28.9, 27.9, 22.1, 22.0. HRMS (ESI-TOF) *m/e* 341.2077 calcd for $C_{17}H_{28}N_2O_5$ $[M + H]^+$, found 341.2058.

2.8.3. Epoxy Bengamides Derived from Propargyl Alcohol

2.8.3.1. Synthesis of Allylic Alcohol **63**



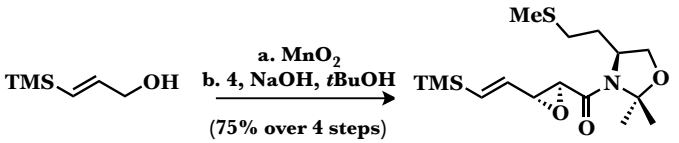
A solution of propargyl alcohol **54** (1.0 g, 18.3 mmol, 1.0 equiv) in THF (20 mL) was added dropwise over a solution of EtMgBr (40% in 2-methyltetrahydrofurane) (18 mL, 55.0 mmol, 3.0 equiv) in THF (100 mL) at 0ºC. Once the addition was completed, the reaction mixture was allowed to warm to room temperature and stirred for 3 hours. After this time, TMSCl (5.4 mL, 42.2 mmol, 2.3 equiv) was added dropwise and carefully keeping temperature under 25ºC. The reaction was stirred overnight and then quenched by the dropwise addition of 3.6 M H_2SO_4 (27 mL, 97.2 mmol, 5.3 equiv) at 0ºC. The organic layer was separated and the aqueous phase was extracted with Et_2O. All the organic extracts were combined, dried over $MgSO_4$ and concentrated in vacuo. The residual yellow liquid was used in the next step without further purification.[56]

In a round flask was dissolved Red-Al (sodium bis (methoxyethoxy)aluminum hydride in 60% w/v in toluene (10 mL, 29.3 mmol, 1.6 equiv) in anhydrous Et_2O (40 mL). The solution was cooled to 0ºC and treated dropwise with a solution of the crude above obtained (18.3 mmol, 1.0 equiv) in Et_2O (34 mL). Ten minutes after complete addition the ice bath was removed, and the reaction was complete within 1 hour. The reaction was quenched by the dropwise addition of 3.6 M H_2SO_4 (37 mL, 132.0 mmol, 7.2 equiv). The organic layer was separated and the aqueous layer was extracted with Et_2O. All the organic extracts were combined, dried over $MgSO_4$ and concentrated in vacuo. The

residual colourless oil, whose data correspond to those described in literature,[56] was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.18 (dt, $\mathcal{J}$ = 18.8, 4.4 Hz, 1H, SiCH=C*H*), 5.92 (dt, $\mathcal{J}$ = 18.8, 1.7 Hz, 1H, SiC*H*=CH), 4.18 (dd, $\mathcal{J}$ = 4.4, 1.7 Hz, 2H, CH_2OH), 2.10 (sa, 1H, O*H*), 0.08 (s, 9H, (CH_3)$_3$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 145.08, 129.05, 64.99, -1.39.

2.8.3.2. Synthesis of Epoxy Amide **64**



(63) **(64)**

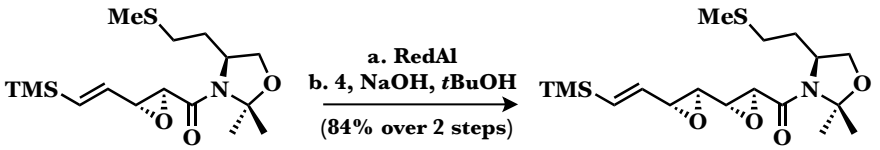
Allylic alcohol (**64**) (880 mg, 6.7 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (40 mL) and over this solution was added MnO_2 85% (9.4 g, 107.0 mmol, 16.0 equiv). After 12 hours the reaction was completed and the mixture was filtered off thought a pad of celite. The solvent was then removed at low temperature under vacuum, and the resulting crude aldehyde was then used immediately in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (1.9 g, 6.1 mmol, 1.0 equiv) and NaOH (3.0 M aqueous solution, 1.8 mL, 5.6 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of epoxy amides to yield epoxy amide **64** (1.2 g, 75% over 4 steps) as a yellow oil.

R_f = 0.44 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +25.1 (*c* 0.3, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.30-6.24 (m, 1H, SiC*H*=CH), 5.72-5.65 (m, 1H, SiCH=C*H*), 4.25 (ddd, $\mathcal{J}$ = 10.2, 4.9, 3.2 Hz, 1H, NC*H*), 3.98 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.4 Hz, 1H, OCH_2CH), 3.85 (d, $\mathcal{J}$

= 9.2 Hz, 1H, OCH_2CH), 3.53-3.48 (m, 2H, =CHCH(O) y COCH(O)), 2.52 (ddd, $\mathcal{J}$ = 13.2, 7.0, 5.1 Hz, 1H, CH_3SCH_2), 2.38 (ddd, $\mathcal{J}$ = 13.4, 8.9, 6.7 Hz, 1H, CH_3SCH_2), 2.02 (s, 3H, SCH_3), 2.01-1.94 (m, 1H, SCH_2CH_2), 1.75-1.69 (m, 1H, SCH_2CH_2), 1.59 (s, 3H, $C(CH_3)_3$), 1.49 (s, 3H, $C(CH_3)_3$), 0.03 (s, 9H, $(CH_3)_3Si$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 166.07, 140.44, 138.61, 95.76, 68.69, 66.96, 59.49, 55.81, 55.74, 30.68, 26.20, 22.91, 15.74, -1.64. HRMS (ESI-TOF) *m/e* 344.1716 calcd for $C_{16}H_{29}NO_3SSi$ $[M + H]^+$, found 344.1709.

2.8.3.3. Synthesis of the diepoxy amide **53**



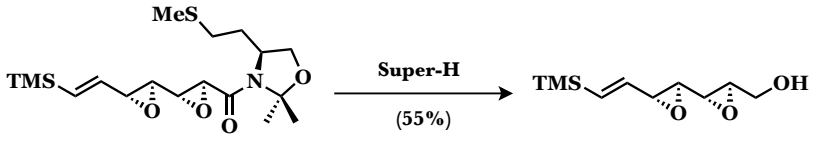
(64) **(53)**

Epoxy amide **64** (110 mg, 0.32 mmol, 1.0 equiv) in dry THF (5 mL) was treated dropwise with Red-Al 60% w/v in toluene (0.22 mL, 0.70 mmol, 2.2 equiv) at 0ºC. After 1 hour at this temperature, the reaction mixture was diluted with a saturated aqueous NH_4Cl solution. The aqueous phase was then separated, extracted twiced with EtOAc and the combined organic phase washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The resulting crude aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (111 mg, 0.35 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 0.06 mL, 0.32 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **53** (102 mg, 84% over two steps) as a yellow oil.

R_f = 0.19 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +31.4 (*c* 0.1, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.23 (dd, $\mathcal{J}$ = 18.7, 0.5 Hz, 1H, SiC*H*=CH), 5.67 (dd, $\mathcal{J}$ = 18.7, 7.6 Hz, 1H, SiCH=C*H*), 4.34-4.28 (m, 1H, NC*H*), 4.00 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.2 Hz, 1H, OCH_2CH), 3.89 (d, $\mathcal{J}$ = 9.2 Hz, 1H, OCH_2CH), 3.58 (d, $\mathcal{J}$ = 1.9 Hz, 1H, COC*H*(O)), 3.40-3.36 (m, 1H, COCH(O)C*H*), 3.35 (dd, $\mathcal{J}$ = 3.6, 2.0 Hz, 1H, =CHC*H*(O)), 3.03 (dd, $\mathcal{J}$ = 3.6, 2.0 Hz, 1H, =CHCH(O)C*H*), 2.58 (ddd, $\mathcal{J}$ = 13.0, 7.5, 5.1 Hz, 1H, SCH_2CH$_2$), 2.46 (ddd, $\mathcal{J}$ = 13.3, 8.4, 7.2 Hz, 1H, SCH_2CH$_2$), 2.10 (s, 3H, CH_3S), 2.08-2.02 (m, 1H, SCH$_2$CH_2), 1.85-1.76 (m, 1H, SCH$_2$CH_2), 1.63 (s, 3H, CH_3(C)), 1.52 (s, 3H, CH_3(C)), 0.06 (s, 9H, (CH_3)$_3$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.87, 140.85, 137.86, 95.94, 67.04, 57.56, 56.68, 55.96, 55.46, 51.20, 34.43, 30.71, 26.22, 22.94, 15.87, -1.58. HRMS (ESI-TOF) *m/e* 386.1821 calcd for $C_{18}H_{31}NO_4SSi$ $[M + H]^+$, found 386.1820.

2.8.3.4. Synthesis of the Diepoxy Alcohol **52**

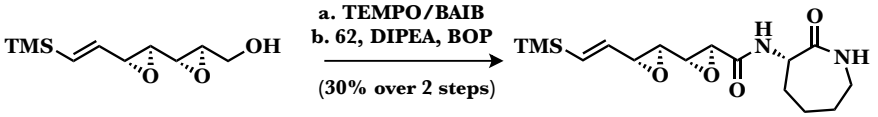


(53) **(52)**

Epoxy amide **53** (50 mg, 0.13 mmol, 1.0 equiv) in THF (4 mL) was treated with Super-H 1.0 M in THF (0.33 mL, 0.33 mmol, 2.5 equiv) at 0ºC. After 1 h at this temperature, the reaction mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was separated, extracted with Et_2O twice and the combined organic phase washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, 40% EtOAc in hexanes) provided epoxy alcohol **52** (15 mg, 55%) as a pale yellow oil.

R_f = 0.22 [Silica gel, 60% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +16.4 (*c* 0.2, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.23 (d, $\mathcal{J}$ = 18.7, 1H, SiC*H*=CH), 5.69 (dd, $\mathcal{J}$ = 18.7, 7.6 Hz, 1H, SiCH=C*H*), 4.00-3.94 (m, 1H, CH_2OH), 3.75-3.67 (m, 1H, CH_2OH), 3.38-3.33 (m, 1H, =CHC*H*(O)), 3.19 (dd, $\mathcal{J}$ = 5.7, 2.3 Hz, 1H, =CHCH(O)C*H*), 3.08 (dd, $\mathcal{J}$ = 4.7, 2.2 Hz, 1H, (O)C*H*CH_2), 2.93 (dd, $\mathcal{J}$ = 4.7, 2.1 Hz, 1H, C*H*(O)$CHCH_2$), 0.08 (s, 9H, (CH_3)$_3$Si). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 141.17, 137.51, 60.41, 57.99, 57.45, 55.74, 53.43, -1.58. HRMS (ESI-TOF) *m/e* 215.110 calcd for $C_{10}H_{18}O_3Si$ $[M + H]^+$, found 215.1112.

2.8.3.5. Synthesis of the Diepoxy Amide **50**



(52) **(50)**

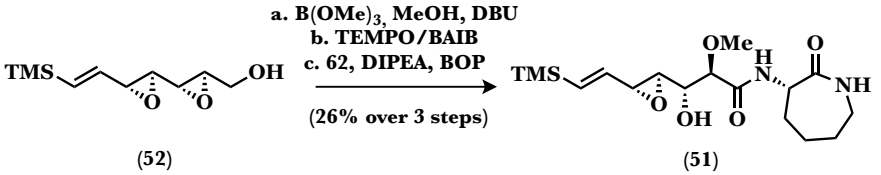
Diepoxy alcohol **52** (40 mg, 0.2 mmol, 1.0 equiv) was dissolved in a 1:1 mixture of CH_3CN/H_2O (8 mL) and the resulting solution was treated with BAIB (370 mg, 1.12 mmil, 6.0 equiv) followed by TEMPO (24 mg, 0.15 mmol, 0.8 equiv) at 25ºC. After 5 h, the crude mixture was diluted with EtOAc, quenched by the addition of a saturated aqueous $Na_2S_2O_3$ solution and, after separation of both layers, the aqueous phase was then extracted with EtOAc. The organic solution was washed again with saturated aqueous $Na_2S_2O_3$ solution then dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure.

The crude acid was dissolved in DMF (4 mL) and treated with DIPEA (0.06 mL, 0.4 mmol, 2.0 equiv), amino lactam **62** (46 mg, 0.27 mmol, 1.5 equiv) and BOP (100 mg, 0.22 mmol, 1.2 equiv) at 25ºC. After being stirred at this temperature overnight, the crude mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic phases

were washed with brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. Purification of the obtained crude product by flash column chromatography (silica gel, 80% EtOAc in hexanes) provided diepoxy amide **50** (20 mg, 30% over 2 steps) as a colourless oil.

R_f = 0.33 [Silica gel, EtOAc]. $[\alpha]^{25}_D$ = +33.1 (*c* 0.4, CH_2Cl_2). ^{1}H RMN (400 MHz, $CDCl_3$), δ (ppm): 7.46 (d, $\mathcal{J}$ = 5.9 Hz, 1H, N*H*CHCO), 6.23 (dd, $\mathcal{J}$ = 18.7, 0.6 Hz, 1H, SiC*H*=CH), 6.17 (t, $\mathcal{J}$ = 7.2 Hz, 1H, CON*H*CH_2), 5.67 (dd, $\mathcal{J}$ = 18.7, 7.5 Hz, 1H, SiCH=C*H*), 4.50 (ddd, $\mathcal{J}$ = 11.4, 5.8, 1.3 Hz, 1H, NHC*H*CO), 3.46 (d, $\mathcal{J}$ = 2.1 Hz, 1H, COC*H*(O)), 3.37 (ddd, $\mathcal{J}$ = 7.5, 2.0, 0.5 Hz, 1H, =CHC*H*(O)), 3.30-3.23 (m, 2H, CONHC*H*$_2$), 3.06 (dd, $\mathcal{J}$ = 4.5, 2.0 Hz, 1H, COCH(O)C*H*), 2.93 (dd, $\mathcal{J}$ = 4.5, 2.0 Hz, 1H, =CHCH(O)C*H*), 2.07-1.95 (m, 2H, C*H*$_2$), 1.91-1.78 (m, 2H, C*H*$_2$), 1.70-1.55 (m, 2H, NHCHC*H*$_2$), 0.08 (s, 9H, (C*H*$_3$)$_3$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 174.6, 162.54, 140.61, 137.95, 57.52, 57.28, 56.83, 52.67, 51.73, 42.10, 31.42, 28.84, 27.85, -1.62. HRMS (ESI-TOF) *m/e* 339.1740 calcd for $C_{16}H_{26}N_2O_4Si$ $[M + H]^+$, found 339.1725.

2.8.3.6. Synthesis of the Epoxy Amide **51**



Diepoxy alcohol **52** (50 mg, 0.21 mmol, 1.0 equiv) was dissolved in a 1:1 mixture of MeOH/$B(OMe)_3$ (5 mL) and the resulting solution was treated with DBU (33 μL, 0.26 mmol 1.0 equiv) and heated at 65ºC for 12 hours. After this time, the reaction mixture was allowed to reach room temperature, cooled to 0ºC, and then treated with a saturated aqueous $NaHCO_3$ solution. After stirring for 30 min at 0ºC, EtOAc was added and both phases were separated. The aqueous phase was extracted with EtOAc and the combined organic extracts were washed with water

and brine, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting crude product was used in the next step without further purification.

The crude of the corresponding epoxy diol (0.21 mmol, 1.0 equiv) was dissolved in 1:1 mixture of CH_3CN/H_2O (8 mL) and the resulting solution was treated with BAIB (415 mg, 1.26 μmol, 6.0 equiv) followed by TEMPO (27 mg, 0.17 mmol, 0.8 equiv) at 25ºC. After 5 h, the crude mixture was diluted with EtOAc , quenched by the addition of a saturated aqueous $Na_2S_2O_3$ solution, and, after separation of both layers, the aqueous phase was then extracted with EtOAc. The organic solution was washed with saturated aqueous $Na_2S_2O_3$ solution, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure.

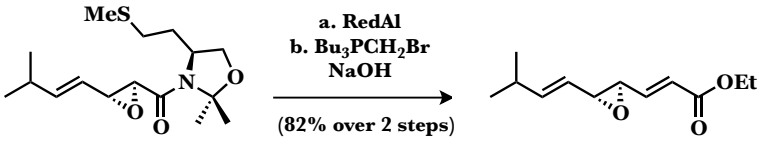
The crude acid (0.21 mmol, 1.0 equiv) was dissolved in DMF (5 mL) and treated with DIPEA (0.1 mL, 0.42 mmol, 2.0 equiv), amino lactam **62** (52 mg, 0.32 mmol, 1.5 equiv) and BOP (114 mg, 0.25 mmol, 1.2 equiv) at 25ºC. After being stirred at this temperature overnight, the crude mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. Purification of the obtained crude product by flash column chromatography (silica gel, 80% EtOAc in hexanes) provided epoxy amide **51** (20 mg, 26% over 3 steps) as a colourless oil.

R_f = 0.21 [Silica gel, EtOAc]. $[\alpha]^{25}_D$ = +11.0 (*c* 0.1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.74 (d, $\mathcal{J}$ = 7.1 Hz, 1H, NHC*H*CO), 6.10 (dd, $\mathcal{J}$ = 18.8, 1.2 Hz, 1H, SiC*H*=CH), 6.00 (dd, $\mathcal{J}$ = 18.7, 4.9 Hz, 1H, SiCH=C*H*), 5.96-5.88 (m, 1H, CON*H*CH_2), 4.55 (ddd, $\mathcal{J}$ = 11.3, 6.6, 1.6

Hz, 1H, NHC*H*CO), 4.10 (dd, $\mathcal{J}$ = 4.9, 1.2 Hz, 1H, C*H*OH), 3.42 (s, 3H, OCH_3), 3.37-3.19 (m, 4H, CONHCH_2, =CHC*H*(O) y CH_3OC*H*), 2.24-2.18 (m, 1H, CH(O)C*H*CHOH), 2.04-1.95 (m, 2H, CH_2), 1.91-1.74 (m, 2H, CH_2), 1.55-1.35 (m, 2H, NHCHCH_2), 0.08 (s, 9H, (CH_3)$_3$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 175.18, 169.74, 140.22, 133.81, 84.47, 77.20, 57.71, 51.56, 42.18, 31.60, 29.69, 28.98, 27.98, 14.10, -1.39. HRMS (ESI-TOF) *m/e* 371.2002 calcd for $C_{17}H_{30}N_2O_5Si$ $[M + H]^+$, found 371.1986.

2.8.4. Amino Bengamide Derivative

2.8.4.1. Synthesis of the Epoxy α,β-unsaturated Ester **57**



(58) **(57)**

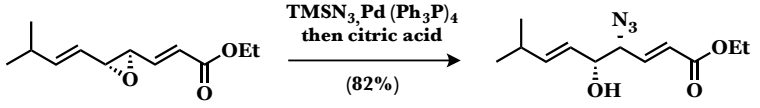
Epoxy amide **58** (170 mg, 0.5 mmol, 1.0 equiv) in dry THF (10 mL) was treated dropwise with Red-Al 60% w/v in toluene (0.4 mL, 1.1 mmol, 2.2 equiv) at 0ºC. After 1 hour at this temperature, the reaction mixture was diluted with a saturated aqueous NH_4Cl solution. The aqueous phase was then separated, extracted twice with EtOAc and the combined organic phase washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The resulting crude aldehyde was used in the next without further purification.

A solution of tributyl(ethoxycarbonylmethylene)phosphonium bromide (230 mg, 0.6 mmol, 1.5 equiv) in CH_2Cl_2 (10 mL) was washed twice with a 1.0 M NaOH aqueous solution, dried over anhydrous $MgSO_4$, and diluted with toluene (10 mL). After CH_2Cl_2 was removed, the resulting solution was then added to a stirred solution of the corresponding aldehyde and benzoic acid (13 mg, 0.1 mmol, 0.2 equiv) in

toluene (10 mL) at 95ºC. After 30 min, the solvent was evaporated and the residue was purified by flash column chromatography (silica gel, 20% EtOAc in hexanes) to provide the corresponding α,β-unsaturated ester **57** (85 mg, 82% over 2 steps) as a pale yellow oil.

R_f = 0.7 [Silica gel, 30% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +20.6 (*c* 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.71 (dd, $\mathcal{J}$ = 15.7, 6.9 Hz, 1H, CH(O)CHC*H*=), 6.12 (dd, $\mathcal{J}$ = 15.7, 0.7 Hz, 1H, CH=C*H*CO), 5.94 (dd, $\mathcal{J}$ = 15.6, 6.5 Hz, 1H, $(CH_3)_2$CHC*H*=), 5.15 (ddd, $\mathcal{J}$ = 15.6, 8.0, 1.4 Hz, 1H, $(CH_3)_2$CHCH=C*H*), 4.20 (q, $\mathcal{J}$ = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 3.35 (ddd, $\mathcal{J}$ = 6.9, 1.9, 0.7 Hz, 1H, CH=CHCH(O)C*H*), 3.27 (dd, $\mathcal{J}$ = 8.0, 1.9 Hz, 1H, CH=CHC*H*(O)), 2.34 (dqd, $\mathcal{J}$ = 13.5, 6.7, 1.3 Hz, 1H, $(CH_3)_2$C*H*), 1.28 (t, $\mathcal{J}$ = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 1.01 (d, $\mathcal{J}$ = 6.8 Hz, 3H, $(CH_3)_2$CH), 0.99 (d, $\mathcal{J}$ = 6.8 Hz, 3H, $(CH_3)_2$CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.65, 144.91, 144.07, 123.59, 123.09, 61.44, 60.57, 58.07, 30.91, 21.86, 14.20. HRMS (H-ESI) *m/e* 211.13287 calcd for $C_{12}H_{18}O_3$ $[M + H]^+$, found 211.13275.

2.8.4.2. Synthesis of the Azido Alcohol **56**



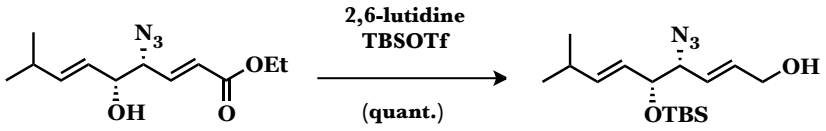
(57) **(56)**

Epoxy unsaturated ester **57** (105 mg, 0.5 mmol, 1.0 equiv) was dissolved in THF (5 mL) and over this solution was added $TMSN_3$ (0.13 mL, 1.0 mmol, 2.0 equiv) and $Pd[PPh_3]_4$ (30 mg, 0.02 mmol, 0.05 equiv) at rt. After 5 hours, the mixture was treated at 0ºC with a 10% citric acid solution in MeOH and was stirred at the same temperature for 1 hour more. Solid $NaHCO_3$ was then added and the mixture stirred for 1 min and filtered. MeOH was completely removed in vacuo and the crude was

purified by flash column chromatography (silica gel, 10% EtOAc in hexanes) to yield *syn*-azido alcohol **56** (103 mg, 82%) as a pale yellow oil.

R_f = 0.34 [Silica-gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -12.0 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.81 (dd, $\mathcal{J}$ = 15.6, 6.7 Hz, 1H, CH(OH)CHN$_3$C*H*=), 6.08 (dd, $\mathcal{J}$ = 15.6, 1.3 Hz, 1H CH=C*H*CO), 5.76 (ddd, $\mathcal{J}$ = 15.5, 6.7, 1.0 Hz, 1H, $(CH_3)_2$CHC*H*=), 5.40 (ddd, $\mathcal{J}$ = 15.5, 6.9, 1.4 Hz, 1H, $(CH_3)_2$CHCH=C*H*), 4.22 (q, $\mathcal{J}$ = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 4.09 (t, $\mathcal{J}$ = 7.4 Hz, 1H, CH=CHC*H*OH), 4.02 (td, $\mathcal{J}$ = 6.5, 1.3 Hz, 1H,CH=CHCH(OH)C*H*N$_3$), 2.32 (dqd, $\mathcal{J}$ = 13.4, 6.7, 1.3 Hz, 1H, $(CH_3)_2$C*H*), 1.30 (t, $\mathcal{J}$ = 7.1 Hz, 3H, OCH$_2$C*H*$_3$),1.00 (d, $\mathcal{J}$ = 6.8 Hz, 3H, (C*H*$_3$)$_2$CH), 0.99 (d, $\mathcal{J}$ = 6.8 Hz, 3H, (C*H*$_3$)$_2$CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.49, 142.94, 141.21, 124.90, 124.51, 74.68, 67.39, 60.77, 30.87, 22.02, 22.00, 14.18. HRMS (H-ESI) *m/e* 276.13186 calcd for $C_{12}H_{19}N_3O_3$ $[M + H]^+$, found 276.13168.

2.8.4.3. Synthesis of the Silyl Ether **66**

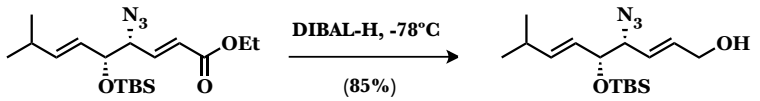


(56) **(66)**

To a solution of azido alcohol **66** (20 mg, 0.07 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added at 0ºC 2,6-lutidine (0.02 mL, 0.09 mmol, 1.2 equiv) and TBSOTf (0.02 mL, 0.16 mmol, 2.0 equiv). After 1 hour at this temperature, the mixture was quenched with MeOH, diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was subjected to purification by flash column chromatography (silica gel, 15% EtOAc in hexanes) to yield **66** (29 mg, quant.) as a pale yellow oil.

R_f = 0.80 [Silica gel, 20% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 29.0 (*c* 0.2, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.81 (dd, $\mathcal{J}$ = 15.7, 6.7 Hz, 1H, CHC*H*=CHCO), 6.08 (dd, $\mathcal{J}$ = 15.6, 1.3 Hz, 1H, CHCH=C*H*CO), 5.64 (dd, $\mathcal{J}$ = 15.5, 6.8 Hz, 1H, $(CH_3)_2$CHC*H*=), 5.37 (ddd, $\mathcal{J}$ = 15.5, 7.4, 1.3 Hz, 1H, $(CH_3)_2$CHCH=C*H*), 4.21 (q, $\mathcal{J}$ = 7.1 Hz, 2H, OCH_2CH$_3$), 4.09 (ddd, $\mathcal{J}$ = 6.8 Hz, 1H, CH=CHC*H*O), 3.86 (td, $\mathcal{J}$ = 6.1, 1.5 Hz, 1H, CH=CHCHC*H*N_3), 2.31 (dqd, $\mathcal{J}$ = 13.5, 6.7, 1.4 Hz, 1H, $(CH_3)_2$C*H*), 1.29 (t, $\mathcal{J}$ = 7.1 Hz, 3H, OCH$_2$CH_3), 0.99 (dd, $\mathcal{J}$ = 6.8, 2.5 Hz, 3H, (CH_3)$_2$CH), 0.98 (dd, $\mathcal{J}$ = 6.8, 2.5 Hz, 3H, (CH_3)$_2$CH) 0.87 (s, 9H, OSiCH(CH_3)$_3$), 0.09 (s, 3H, OSiCH(CH_3)$_2$), 0.04 (s, 3H, OSiCH(CH_3)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.72, 142.29, 141.81, 125.89, 123.86, 76.79, 67.34, 60.56, 30.83, 25.76, 22.04, 18.13, 14.19, -2.95, -4.17, -4.88. HRMS (H-ESI) *m/e* 390.21834 calcd for $C_{18}H_{33}N_3O_3Si$ $[M + Na]^+$, found 390.21799.

2.8.4.4. Synthesis of the Allylic Alcohol **67**

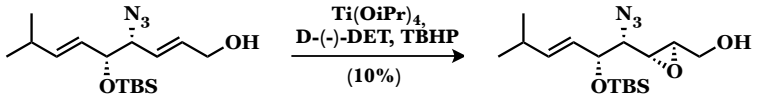


(66) **(67)**

A solution of **66** (80 mg, 0.22 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was cooled at -78°C and then treated with DIBAL-H 1.0 M solution in toluene (0.54 mL, 0.54 mmol, 2.5 equiv) After 20 min, the reaction was quenched by adding of EtOAc at -78°C and the mixture was allowed to reach rt and treated with a saturated aqueous Na^+/K^+ tartrate solution. The resulting mixture was vigorously stirred until a clear separation of both organic and aqueous phases. The aqueous phase was then separated, the organic extract washed with water and brine, dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The crude of the alcohol was then purified by flash column chromatography (silica gel, 15% EtOAc in hexanes) to afford allylic alcohol **67** (60 mg, 85%) as a colourless oil.

R_f = 0.71 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +5.0 (*c* 0.5, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.91 (dtd, $\mathcal{J}$ = 15.5, 5.2, 1.0 Hz, 1H CH=C*H*CO), 5.68 (ddt, $\mathcal{J}$ = 15.5, 7.3, 1.6 Hz, 1H CH(OH)CHN$_3$C*H*=), 5.60 (ddd, $\mathcal{J}$ = 15.5, 6.7, 0.9 Hz, 1H $(CH_3)_2$CHC*H*=), 5.38 (ddd, $\mathcal{J}$ = 15.5, 7.3, 1.3 Hz, 1H $(CH_3)_2$CHCH=C*H*), 4.22 – 4.15 (m, 2H, C*H*$_2$OH), 4.03 (t, $\mathcal{J}$ = 6.3 Hz, 1H, CH=CHC*H*OSi)), 3.71 (t, $\mathcal{J}$ = 6.6 Hz, 1H CH=CHCH(OH)C*H*N$_3$), 2.30 (dqd, $\mathcal{J}$ = 13.4, 6.7, 1.1 Hz, 1H $(CH_3)_2$C*H*)), 0.99 (d, $\mathcal{J}$ = 6.7 Hz, 3H $(CH_3)_2$CH), 0.98 (d, $\mathcal{J}$ = 6.7 Hz, 3H $(CH_3)_2$CH), 0.90 (s, 9H, OSiCH(C*H*$_3$)$_3$), 0.08 (s, 3H, OSiCH(C*H*$_3$)$_2$), 0.04 (s, 3H, OSiCH(CH$_3$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 140.94, 133.66, 126.47, 126.27, 76.81, 68.32, 62.88, 30.80, 25.80, 22.15, 22.08, 18.18, -4.13, -4.81.

2.8.4.5. Synthesis of the Epoxy Alcohol **68**

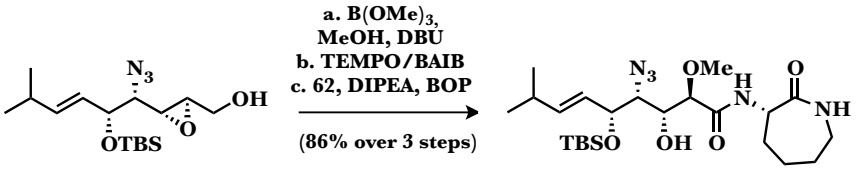


(67) **(68)**

To a solution of titanium tetraisopropoxide (0.02 mL, 0.08 mmol, 0.35 equiv) in CH_2Cl_2 (5 mL) was added D(-)-DET (0.01 mL, 0.08 mmol, 0.35 equiv) at -20ºC. After 15 min at this temperature, a solution of allylic alcohol **67** (70 mg, 0.22 mmol, 1.0 equiv) in CH_2Cl_2 (3 mL) was added dropwise, followed by the addition, after additional 30 min, of TBHP 5.5 M in decane (0.06 mL, 0.34 mmol, 1.6 equiv) at the same temperature. After 8 h at this temperature, the reaction mixture was quenched with Me_2S (0.07 mL, 0.98 mmol, 4.6 equiv) at 0ºC filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 20% EtOAc in hexanes) to obtained epoxy alcohol **68** (8 mg, 10%) as a colourless oil.

R_f = 0.63 [Silica-gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +29.5 (*c* 0.4, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.70 (ddd, $\mathcal{J}$ = 15.5, 6.6, 0.9 Hz, 1H $(CH_3)_2CHCH$=), 5.41 (ddd, $\mathcal{J}$ = 15.6, 7.6, 1.4 Hz, 1H, $(CH_3)_2CHCH$=C*H*), 4.21 - 4.13 (m, 1H, CH=CHC*H*O), 3.94 (d, $\mathcal{J}$ = 12.6 Hz, 1H, CHN_3CH(O)CH), 3.62 (d, $\mathcal{J}$ = 13.4 Hz, 1H, CHN_3CH(O)C*H*), 3.15 -3.11 (m, 1H, =CHCHOC*H*N_3), 3.1 -3.09 (m, 2H, CH(O)CHCH_2), 2.39 - 2.25 (m, 1H, $(CH_3)_2CH$), 1.68 (bs, 1H, O*H*), 1.01 (d, $\mathcal{J}$ = 6.7, 3H, $(CH_3)_2CH$), 0.97 (d, $\mathcal{J}$ = 6.7, 3H, $(CH_3)_2CH$), 0.90 (s, 9H, $(CH_3)_3CSi$), 0.10 (s, 3H, $(CH_3)_2Si$, 0.06 (s, 3H, $(CH_3)_2Si$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 141.60, 125.92, 75.36, 67.84, 60.56, 56.99, 54.61, 30.76, 25.76, 22.07, 21.92, 18.08, -4.11, -4.87.

2.8.4.6. Synthesis of the Amide **70**



Epoxy alcohol **68** (10 mg, 30 μmol, 1.0 equiv) was dissolved in a 1:1 mixture of MeOH/$B(OMe)_3$ (2 mL) and the resulting solution was treated with DBU (5 μL, 30 μmol, 1.0 equiv) and heated at 70ºC for 12 hours. After this time, the reaction mixture was allowed to reach room temperature, cooled to 0ºC, and then treated with a saturated aqueous $NaHCO_3$ solution. After stirring for 30 min at 0ºC, EtOAc was added and both phases were separated. The aqueous phase was extracted with EtOAc and the combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting crude product was used in the next step without further purification.

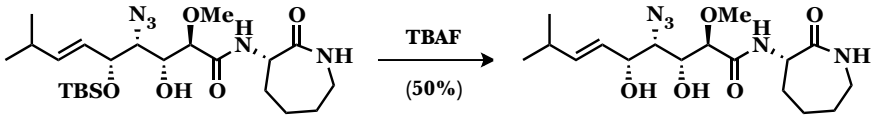
The crude of the corresponding diol was dissolved in 1:1 mixture of CH_3CN/H_2O (4 mL) and the resulting solution was treated with BAIB (55 mg, 168 μmol, 6.0 equiv) followed by TEMPO (4 mg, 30 μmol, 0.8 equiv) at 25ºC. After 5 h, the crude mixture was diluted with EtOAc, quenched by the addition of a saturated aqueous $Na_2S_2O_3$ solution and, after separation of both layers, the aqueous phase was then extracted with EtOAc. The organic solution was washed again with a saturated aqueous $Na_2S_2O_3$ solution, then dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure.

The crude acid was dissolved in DMF (4 mL) and treated with DIPEA (10 μL, 56 μmol, 2.0 equiv), amino lactam **62** (7 mg, 42 μmol, 1.5 equiv) and BOP (15 mg, 34 μmol, 1.2 equiv) at 25ºC. After being stirred at this temperature overnight, the crude mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. Purification of the obtained crude product by flash column chromatography (silica gel, 60% EtOAc in hexanes) provided amide **70** (12 mg, 86% over 3 steps) as a colourless oil.

R_f = 0.33 [Silica-gel, 80% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 18.4 (*c* 0.3, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.93 (d, J = 6.2 Hz, 1H, CON*H*CH), 6.09 (t, J = 6.7 Hz, 1H, CON*H*CH_2), 5.72 (ddd, J = 15.5, 6.5, 0.8 Hz, 1H, $(CH_3)_2$CHC*H*=CH), 5.34 (ddd, J = 15.6, 7.7, 1.4 Hz, 1H, $(CH_3)_3$CHCH=C*H*), 4.54 (ddd, J = 11.3, 6.2, 1.8 Hz, 1H, C*H*NHCO), 4.44 - 4.36 (m, 1H, CH=CHC*H*O), 3.76 - 3.73 (m, 1H, C*H*OH), 3.54 (s, 3H, CHOC*H*$_3$), 3.38 (dd, J = 8.4, 1.6 Hz, 1H, CH=CHCHOC*H*N_3), 3.34 - 3.19 (m, 3H, CONHCH_2 and CH_3OC*H*), 2.37 - 2.23 (m, 1H, $(CH_3)_2$C*H*), 2.15 - 1.98 (m, 2H, CH_2), 1.92 - 1.75 (m, 2H, CH_2), 1.62 - 1.36 (m, 2H, NHCHCH_2), 0.98 (d, J = 6.8, Hz, 3H,

(C*H*$_3$)$_2$CH), 0.97 (d, $\mathcal{J}$ = 6.8, 3H, (C*H*$_3$)$_2$CH), 0.92 (s, 9H, (C*H*$_3$)$_3$CSi), 0.13 (s, 3H, (C*H*$_3$)$_2$Si), 0.06 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 174.78, 171.79, 141.62, 126.39, 80.76, 77.20, 75.05, 71.24, 67.68, 59.95, 51.95, 42.16, 31.23, 30.70, 28.89, 27.95, 25.82, 25.75, 22.13, 21.77, 18.08.

2.8.4.7. Synthesis of the Azido Analogue of Bengamide E **71**



(70) **(71)**

Silyl ether **70** (15 mg, 0.03 mmol, 1.0 equiv) was dissolved in THF (2 mL) and over this solution was added 1.0 M TBAF in THF (0.04 mL, 0.04 mmol, 1.2 equiv) at rt. After 30 min, the reaction crude was diluted with EtOAc and a saturated aqueous NH_4Cl solution. The aqueous phase was then extracted with EtOAc and the organic extracts washed with H_2O, brine and dried over $MgSO_4$. The solvent was removed under vaccuum and the crude product was purified by flash column chromatography (silica gel, EtOAc) to afford azido analogue of bengamide E **71** (6 mg, 50%) as a colourless oil.

R_f= 0.23 [Silica gel, EtOAc]. $[\alpha]^{25}_D$ = +20.6 (*c* 0.2, CH_2Cl_2). ^{1}H RMN (400 MHz, $CDCl_3$), δ (ppm): 7.94 (d, *J* = 6.3 Hz, 1H, CON*H*CH), 6.01 (t, *J* = 6.6 Hz, 1H, CON*H*CH$_2$), 5.85 (ddd, *J* = 15.5, 6.5, 1.2 Hz, 1H, (CH$_3$)$_2$CHC*H*=CH), 5.51 (ddd, *J* = 15.5, 6.5, 1.4 Hz, 1H, (CH$_3$)$_3$CHCH=C*H*), 4.60 – 4.47 (m, 2H, C*H*NHCO and =CHC*H*OH), 4.29 (d, *J* = 2.6 Hz, 1H, O*H*), 3.92 (dt, *J* = 7.9, 2.8 Hz, 1H, CHOHCHN$_3$C*H*OH), 3.84 (d, *J* = 7.9 Hz, 1H, C*H*OCH$_3$), 3.55 (s, 3H, CHOC*H*$_3$), 3.41 (dd, *J* = 5.0, 3.0 Hz, 1H, =CHCHOHC*H*N$_3$), 3.37 – 3.23 (m, 2H, C*H*$_2$), 2.95 (d, *J* = 2.9 Hz, 1H, O*H*), 2.42 – 2.28 (m, 1H, (CH$_3$)$_2$C*H*), 2.16 – 1.99 (m, 2H, C*H*$_2$), 1.94 – 1.77 (m, 2H, C*H*$_2$), 1.51 –

1.39 (m, 2H, NHCHCH_2), 1.02 (d, J = 6.7, 3H, (CH_3)$_2$CH), 1.02 (d, J = 6.7 Hz, 3H, (CH_3)$_2$CH). ^{13}C RMN (100 MHz, $CDCl_3$), δ (ppm): 174.63, 171.61, 141.37, 125.49, 80.26, 74.26, 73.73, 65.18, 59.99, 52.05, 42.17, 31.10, 30.76, 29.70, 28.89, 22.14, 21.98.

2.9. Notes and References

(1) Quiñoà, E.; Adamczeski, M.; Crews, P. *J. Org. Chem.* **1986**, *51*, 4497-4498.

(2) Nicolaou, K. C.; Chen, J. S.; Dalby, S. M. *Bioorg. Med. Chem.* **2009**, *17*, 2290-2303.

(3) Sashidhara, K. V.; White, K. N.; Crews, P. *J. Nat. Prod.* **2009**, *72*, 588-603.

(4) Liu, G.; Tai, W. -Y.; Li, Y. -L.; Nan, F. -J. *Tetrahedron Lett.* **2006**, *47*, 3295-3298.

(5) García-Ruiz, C. Sarabia, F. *Mar. Drugs.* **2014**, *12*, 1580-1622.

(6) Towbin, H.; Bair, K. W.; DeCaprio, J. A.; Eck, M. J.; Kim, S.; Kinder, F. R.; Morollo, A.; Mueller, D. R.; Schindler, P.; Song, H. K.; van Oostrum, J.; Versace, R. W.; Voshol, H.; Wood, J.; Zabludoff, S.; Phillips, P. E. *J. Biol. Chem.* **2003**, *278*, 52964-52971.

(7) Kim, S.; LaMontagne, K.; Sabio, M.; Sharma, S.; Versace, R. W.; Yusuff, N.; Phillips, P. E. *Cancer Res.* **2004**, *64*, 2984-2987.

(8) Johnson, T. A.; Sohn, J.; Vaske, Y. M.; White, K. N.; Cohen, T. L.; Vervoort, H. C.; Tenney, K.; Valeriote, F. A.; Bjeldanes, L. F.; Crews, P. *Bioorg. Med. Chem.* **2012**, *20*, 4348-4355.

(9) Kinder Jr., F. R. *Org. Prep. Proc. Int.* **2002**, *34*, 559-583.

(10) Broka, C. A. Ehrler, J. *Tetrahedron Lett.* **1991**, *32*, 5907-5910.

(11) Kishimoto, H.; Ohrui, H.; Meguro, H. *J. Org. Chem.* **1992**, *57*, 5042-5044.

(12) Chida, N.; Tobe, T.; Ogawa, S. *Tetrahedron Lett.* **1991**, *32*, 1063-1066.

(13) Mukai, C.; Moharram, S. M.; Kataoka, O.; Hanaoka, M. *J. Chem. Soc. Perkin Trans. 1.* **1995**, 2849-2854.

(14) Metri, P. K.; Schiess, R.; Prasad, K. R. *Chem. Asian J.* **2013**, *8*, 488-493.

(15) Martín-Gálvez, F.; García-Ruiz, C.; Sánchez-Ruiz, A.; Valeriote, F. A.; Sarabia, F. *ChemMedChem.* **2013**, *8*, 819-831.

(16) Thale, Z.; Kinder, F. R.; Bair, K. W.; Bontempo, J.; Czuchta, A. M.; Versace, R. W.; Phillips, P. E.; Sanders, M. L.; Wattanasin, S.; Crews, P. *J. Org. Chem.* **2001**, *66*, 1733-1741.

(17) Bergers, G.; Javaherian, K.; Lo, K. -M.; Folkman, J.; Hanahan, D. *Science* **1999**, *284*, 808-812.

(18) Lowther, W. T. Matthews, B. W. *Biochim. Biophys. Acta.* **2000**, *1477*, 157-167.

(19) Giglione, C.; Boularot, A.; Meinnel, T. *Cell. Mol. Life Sci.* **2004**, *61*, 1455-1474.

(20) Ben-Bassat, A.; Bauer, K.; Chang, S. Y.; Myambo, K.; Boosman, A. *J. Bacteriol.* **1987**, *169*, 751-757.

(21) Levine, A. J. *Cell* **1997**, *88*, 323-331.

(22) Bazan, J. F.; Weaver, L. H.; Roderick, S. L.; Huber, R.; Matthews, B. W. *Proc. Natl. Acad. Sci.* **1994**, *91*, 2473-2477.

(23) Arfin, S. M.; Kendall, R. L.; Hall, L.; Weaver, L. H.; Stewart, A. E.; Matthews, B. W.; Bradshaw, R. A. *Proc. Natl. Acad. Sci. USA.* **1995**, *92*, 7714-7718.

(24) Bradshaw, R. A.; Brickey, W. W.; Walker, K. W. *Trends Biochem. Sci.* **1998**, *23*, 263-267.

(25) Addlagatta, A.; Hu, X.; Liu, J. O.; Matthews, B. W. *Biochemistry* **2005**, *44*, 14741-14749.

(26) Hu, X.; Addlagatta, A.; Lu, J.; Matthews, B. W.; Liu, J. O. *Proc. Natl. Acad. Sci. USA.* **2006**, *103*, 18148-18153.

(27) Liu, S.; Widom, J.; Kemp, C. W.; Crews, C. M.; Clardy, J. *Science* **1998**, *282*, 1324-1327.

(28) Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* **1990**, *348*, 555-557.

(29) Sato, Y. *Biol. Pharm. Bull.* **2004**, *27*, 772-776.

(30) Addlagatta, A.; Quillin, M. L.; Omotoso, O.; Liu, J. O.; Matthews, B. W. *Biochemistry* **2005**, *44*, 7166-7174.

(31) Lowther, W. T.; Orville, A. M.; Madded, D. T.; Lim, S.; Rich, D. H.; Matthews, B. W. *Biochemistry* **1999**, *38*, 7678-7688.

(32) Liu, G.; Ma, Y. M.; Tai, W. Y.; Xie, C. M.; Li, Y. L.; Li, J.; Nan, F. J. *ChemMedChem* **2008**, *3*, 74-78.

(33) Dumez, H.; Gall, H.; Capdeville, R.; Dutreix, C.; van Oosterom, A. T.; Giaccone, G. *Anti-cancer Drugs.* **2007**, *18*, 219-225.

(34) Folkman, J.; Itzhak, D.; Goldberg, M. D.; Rosen, E. M. In; 1997.

(35) Folkman, J. *Nature Med.* **1995**, *1*, 27-30.

(36) Quesada, A. R.; Muñoz-Chápuli, R.; Medina, M. A. *Med. Res. Rev.* **2006**, *26*, 483-530.

(37) McCowen, C.; Callender, E.; Lawlis, F. *Science* **1951**, *113*, 202-203.

(38) Oikawa, T.; Hirotani, K.; Nakamura, O.; Shudo, K.; Hiragun, A.; Iwaguchi, T. *Cancer Lett.* **1989**, *48*, 157-162.

(39) Rodríguez-Quesada, A. R.; Medina, M. A.; Muñoz-Chápuli, O. *Angiogenesis,* Universidad de Málaga-Servicio de Publicaciones e intercambio; 2004.

(40) Griffith, E. C.; Su, Z.; Turk, B. E.; Chen, S.; Chang, Y. H.; Wu, Z.; Biemann, K.; Liu, J. O. *Chem. Biol.* **1997**, *4*, 461-471.

(41) Turk, B. E.; Griffith, E. C.; Wolf, S.; Biemann, K.; Chang, Y. H.; Liu, J. O. *Chem. Biol.* **1999**, *6*, 823-833.

(42) Kraut, J. *Annu. Rev. Biochem.* **1977**, *46*, 331-358.

(43) Kendall, R. L. Bradshaw, R. A. *J. Biol. Chem.* **1992**, *267*, 20667-20673.

(44) Griffith, E. C.; Su, Z.; Niwayama, S.; Ramsay, C. A.; Chang, Y. H.; Liu, J. O. *Proc. Natl. Acad. Sci USA.* **1998**, *95*, 15183-15188.

(45) Sarabia, F. Sánchez-Ruiz, A. *Tetrahedron Lett.* **2005**, *46*, 1131-1135.

(46) Sarabia, F. Sánchez-Ruiz, A. *J. Org. Chem.* **2005**, *70*, 9514-9520.

(47) Sarabia, F.; Martín-Gálvez, F.; Chammaa, S.; Martín-Ortiz, L.; Sánchez-Ruiz, A. *J. Org. Chem.* **2010**, *75*, 5526-5532.

(48) Tai, W. Y.; Zhang, R. T.; Ma, Y. M.; Gu, M.; Liu, G.; Li, J.; Nan, F. J. *ChemMedChem* **2011**, *6*, 1555-1558.

(49) Mohamed, M. Brook, M. A. *Helv. Chim. Acta.* **2002**, *85*, 4165-4181.

(50) Ilardi, E. A.; Stivala, C. E.; Zakarian, A. *Org. Lett.* **2008**, *10*, 1727-1230.

(51) Sidera, M.; Costa, A. M.; Vilarrasa, J. *Org. Lett.* **2011**, *13*, 4934-4937.

(52) Sarabia, F.; Chammaa, S.; García-Castro, M.; Martín-Gálvez, F. *Chem. Commun.* **2009**, 5763-5765.

(53) Sarabia, F.; Vivar-García, C.; García-Castro, M.; García-Ruiz, C.; Martín-Gálvez, F.; Sánchez-Ruiz, A.; Chammaa, S. *Chem. Eur. J.* **2012**, *18*, 15190-15201.

(54) López-Herrera, F. J.; Sarabia-García, F.; Pedraza-Cebrián, G. M.; Pino-González, M. S. *Tetrahedron Lett.* **1999**, *40*, 1379-1380.

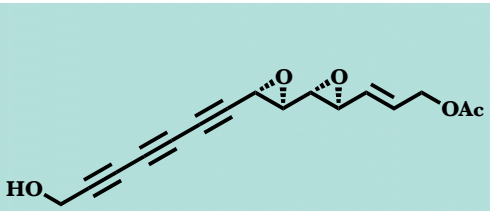
(55) López-Herrera, F. J.; Pino-González, M. S.; Sarabia-García, F.; Heras-López, A.; Ortega-Alcántara, J. J.; Pedraza-Cebrián, M. G. *Tetrahedron: Asymm.* **1996**, *7*, 2065-2071.

(56) Denmark, S. E. Jones, T. K. *J. Org. Chem.* . **1982**, *47*, 4595-4597.

(57) Miyashita, M.; Mizutani, T.; Tadano, G.; Iwata, Y.; Miyazawa, M.; Tanino, K. *Angew. Chem. Int. Ed.* **2005**, *44*, 5094-5097.

(58) Rodríguez-Nieto, S.; Medina, M. A.; Quesada, A. R. *Anticancer Res.* **2001**, *5*, 3457-3460.

(59) Gung, B. W. Yanik, M. M. *J. Org. Chem.* **1996**, *61*, 947-951.



CHAPTER 3

Gummiferol

The structure of Gummiferol, isolated from the leaves of Adenia gummifera contains apart from a conjugated triacetylene moiety two oxirane rings, exhibiting significant activity against the KB human cell line and a broad cytotoxic spectrum against other human cancer cell lines.

3.1. Introduction

Adenia gummifera (Passifloraceae) is a species that grows in various regions of Africa. The juice from the leaves of this plant is used in Tanzania to treat colic, while the crushed leaves are applied externally in the treatment of broken bones.[1] The roots are employed as a diuretic, to treat filariasis, and against infertility,[2] although these are just a few examples of the many medicinal uses traditionally related to *A. gummifera*.[3] Despite the fact that polyacetylenes are in general known to be labile, the concomitant presence of high unsaturation and epoxide functionalities might well confer the observed cytotoxic activity.

Conjugated acetylene moieties are common structural units found on an extensive number of natural products, many of which display an impressive biological activity as antibacterial (**72**),[4] antiparisitic (**73**)[5] and insecticidal agents (**74**).[6] This class of polyacetylenes shows in addition an inhibitory effect on HIV-1 integrase[7] and cytotoxicity against a wide range of cell lines.[8, 9-11]

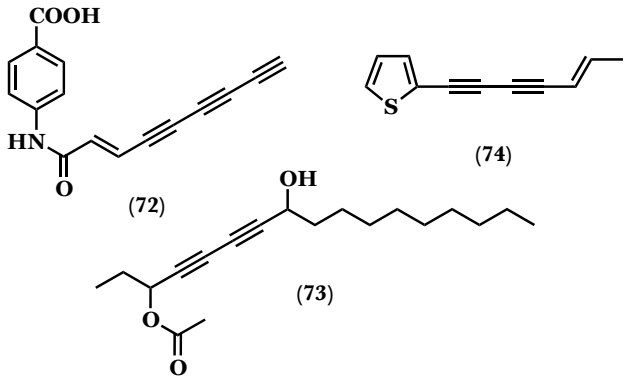


Figure 3.1. *Some examples of conjugated acetylene moieties in Natural Products*

3.2. Biological Context

In 1995, Wall et al. isolated from the leaves of *Adenia gummifera* by treatment with a 50% MeOH/$CHCl_3$ an organic portion that exhibited an ED_{50} value of 1.1 μg/mL in the human epidermoid carcinoma (KB) cell line assay.[12] Subsequent purification by flash column chromatography rendered (-)-gummiferol, whose structure was still unknown.

3.2.1. Cytotoxicity Tests

Although a diverse array of polyacetylenes has been isolated mainly from species of the *Compositae* and *Umbelliferae* families and to a lesser extent from plants of other families,[13] the polyacetylene diepoxide structural feature of gummiferol appears to be unique. Its closest

structural variant is a C14-monoepoxy acetylene compound previously isolated from *Panax quinqufolium* (Araliaceae) as a cytotoxic constituent.[14]

The concomitant presence of high unsaturation and epoxide functionalities might well confer the observed cytotoxic activity, shown in **Table 3.1**.[12]

Table 3.1. *Cytotoxicity of gummiferol*

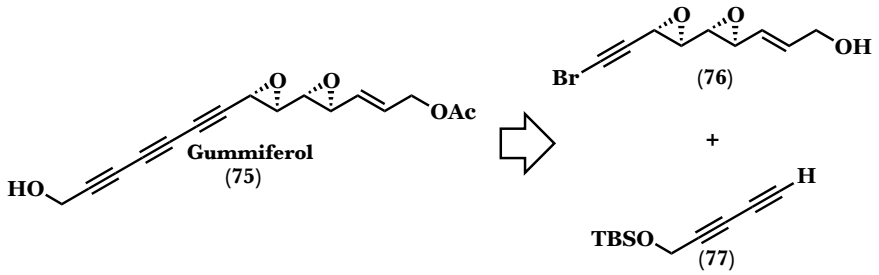
Cell line	ED_{50} (μg/mL)	Cell line	ED_{50} (μg/mL)
BC1	0.2	P-388	**0.03**
HT	0.1	A-431	0.5
Lul	0.9	LNCaP	0.2
Mel2	1.3	ZR-75-1	0.2
Col2	0.6	U-373	**0.05**
KB	0.3	KB-V(-VLB)	0.4
KB-V(+VLB)	0.3		

Particular striking is its significant activity against various cell lines including strong activity against P388 (mouse leukaemia) and U373 (human glioblastoma). This antitumoral profile renders gummiferol as an interesting synthesis and biomedical endeavour.

3.3. Structure and Synthesis of Gummiferol

Until today, only Takamura and co-workers have reported a total synthesis of gummiferol that let to establish its absolute configuration (**Scheme 3.1**).[15] Prior to that, Wall et al. described the structure of the natural product on the basis of its spectra,[12] although the stereochemistry of the two oxirane rings remained unknown until Takamura's publication. The unambiguous stereochemical elucidation of (-)-gummiferol (**75**) was performed by the modified Mosher method.[16]

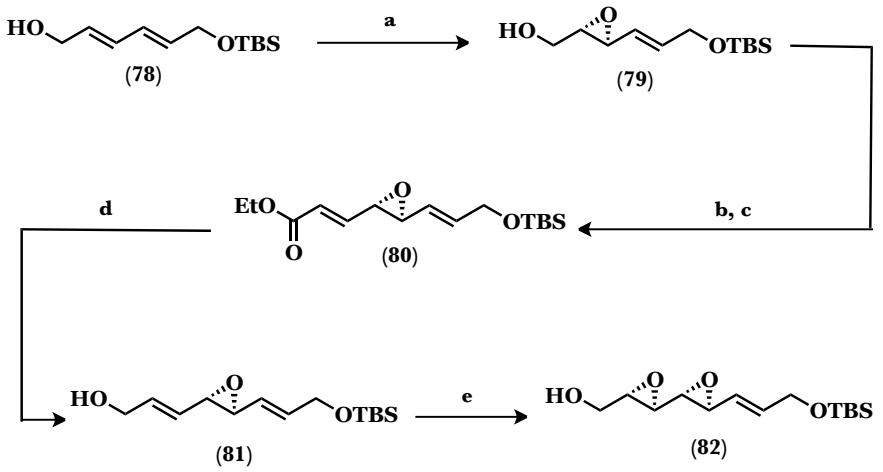
Scheme 3.1. *Retrosynthetic analysis of gummiferol by Takamura et al.*



The main core of gummiferol was assembled by connection of chiral building block **76** and the dicetylene moiety **77** *via* a Cadiot-Chodkiewicz coupling.[17]

For the preparation of enantiopure fragment **76**, dienol **78** was selected as starting material,[18] which was subjected to a first Sharpless asymmetric epoxidation with a significant recovery of starting material. Epoxy alcohol **79** was then converted into the allylic alcohol **81** by a 3-step sequence involving Parikh-Doering oxidation,[10] Horner-Wadsworth-Emmons reaction[19] and DIBAL-H reduction. A second Sharpless epoxidation delivered the desired diepoxy alcohol **82** in a 80% yield (**Scheme 3.2**).

Scheme 3.2. *Synthesis of the diepoxy alcohol* ***82***

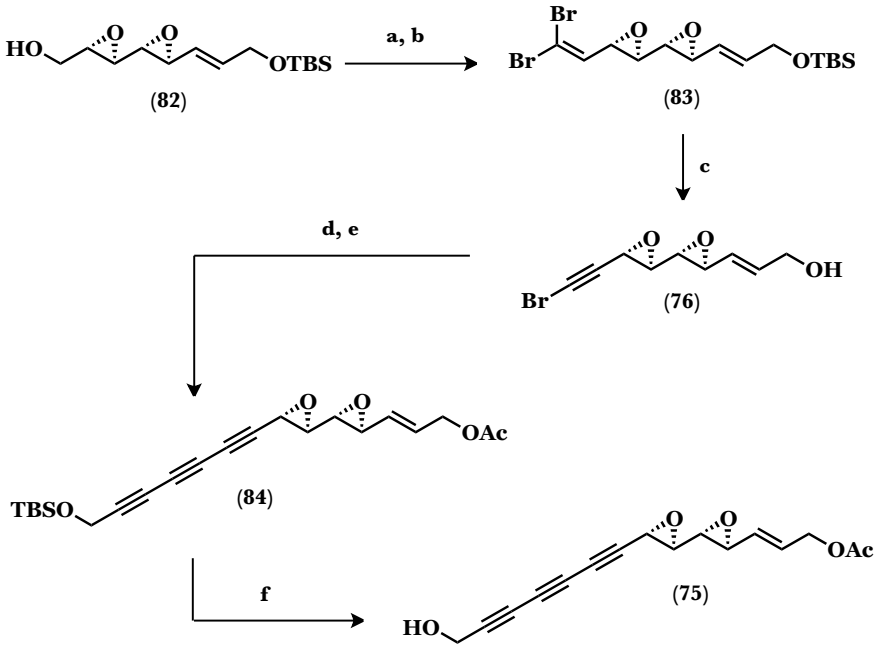


Reagents and conditions: (a) SAE, (+)-DIPT, 42% (71% brsm). (b) SO_3·Pyr, Et_3N, DMSO, CH_2Cl_2, 0ºC to rt. (c) $(EtO)_2P(O)CH_2CO_2Et$, DIPEA, LiCl, CH_3CN, 0ºC to rt, 80% (over 2 steps). (d) DIBAL-H, CH_2Cl_2, -78ºC, 80% (2 cycles). (e) SAE, (+)-DIPT, 80%.

Conversion of the diepoxy alcohol **82** into the bromo derivative **76** commenced with a Parikh-Doering oxidation,[10] followed by one-carbon homologation with $CBr_4/Ph_3P/Et_3N$[20] and subsequent treatment with TBAF to afford the bromoacetylenic alcohol **76** (**Scheme 3.3**).[21]

The assembly of the diacetylene unit and the bromoacetylenic alcohol **76** proceeded *via* a Cadiot-Chodkiewicz reaction,[17] after which the synthesis of gummiferol was completed by acetylation and deprotection of the remaining hydroxyl moiety by treatment with buffered HF-pyridine to give **75** in a 83% overall yield (**Scheme 3.3**).

Scheme 3.3. *Synthesis of gummiferol by Takamura et al.*



Reagents and conditions: (a) SO_3·Pyr, Et_3N, DMSO, CH_2Cl_2, 0ºC to rt. (b)CBr_4, Ph_3P, Et_3N, CH_2Cl_2, -78ºC. (c) TBAF, THF, 0ºC to rt, 70% (over 3 steps). (d) **77**, CuCl, NH_2OH·HCl, Et_3N, MeOH, -78ºC. (e) Ac_2O, Pyr, DMAP, CH_2Cl_2, 0ºC, 56% (over 2 steps). (f) HF·Pyr, THF, 0ºC, quant.

This methodology described by Takamura was extended to the synthesis of the enantiomer *ent*-(**75**) and the two possible diastereoisomers of (-)-gummiferol, diepoxides **(85)** and *ent*-(**85)**.

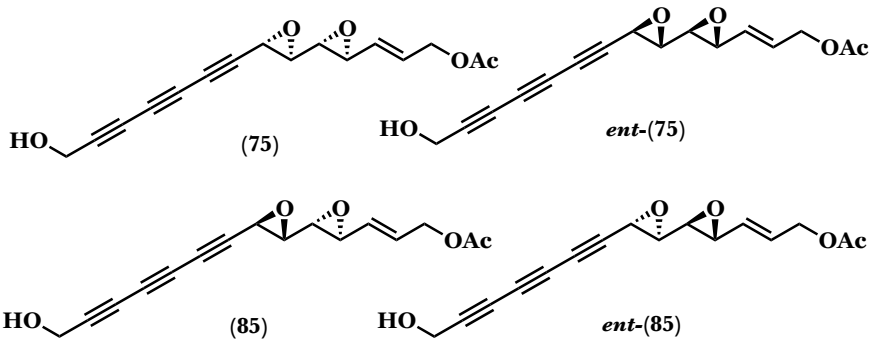


Figure 3.2. *Isomers of (-)-gummiferol*

More recently, in 2013, the same authors improved the procedure above described and applied it to the synthesis of both enantiomers of (**75**) and *ent*-(**75**) (**Figures 3.2**).[22] Furthermore, they prepared a series of gummiferol analogues that allowed a detailed structure-activity relationship study (**Figure 3.3**).

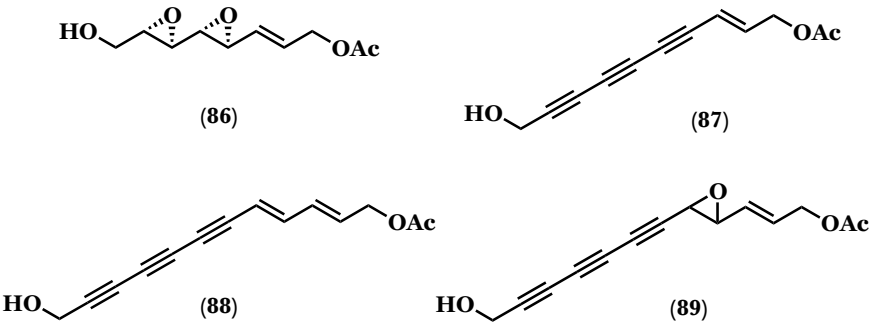


Figure 3.3. *Truncated isomers of gummiferol*

The growth-inhibitory activity of all these synthetic products against HL60 and HeLa S3 cells allowed to determine that the stereochemistry of the diepoxide unit has little effect on the cytotoxic activity and how crucial was the presence of the triacetylene moiety for the biological activity [22] (**Table 3.2**).

Table 3.2. *Growth-inhibitory activity (μM) of (-)-gummiferol and its synthetic isomers*

Compound	HL60	HeLa S_3	Compound	HL60	HeLa S_3
(**75**)	1.22	6.76	(**86**)	>100	>100
ent-(**75**)	1.23	6.68	(**87**)	12.3	31.6
(**85**)	1.62	8.61	(**88**)	4.63	22.4
ent-(**85**)	3.61	19.1	(**89**)	4.75	17.4

An important aspect of the synthesis of the (-)-gummiferol, as described above, is that it constitutes a general method for the preparation of any desired diastereoisomer. However there is still room for improvement, for instance in the yields of the first Sharpless epoxidation, that still remain in 42% (71% based on recovered starting material).

3.4. Synthesis of Gummiferol

The first total synthesis of Gummiferol offers new opportunities both in the biological and medical fields. However, even though the synthesis previously described constitutes a general method capable of producing a range of (non-natural) gummiferol isomers, alternative (more efficient) approaches remain a continuing area of research.

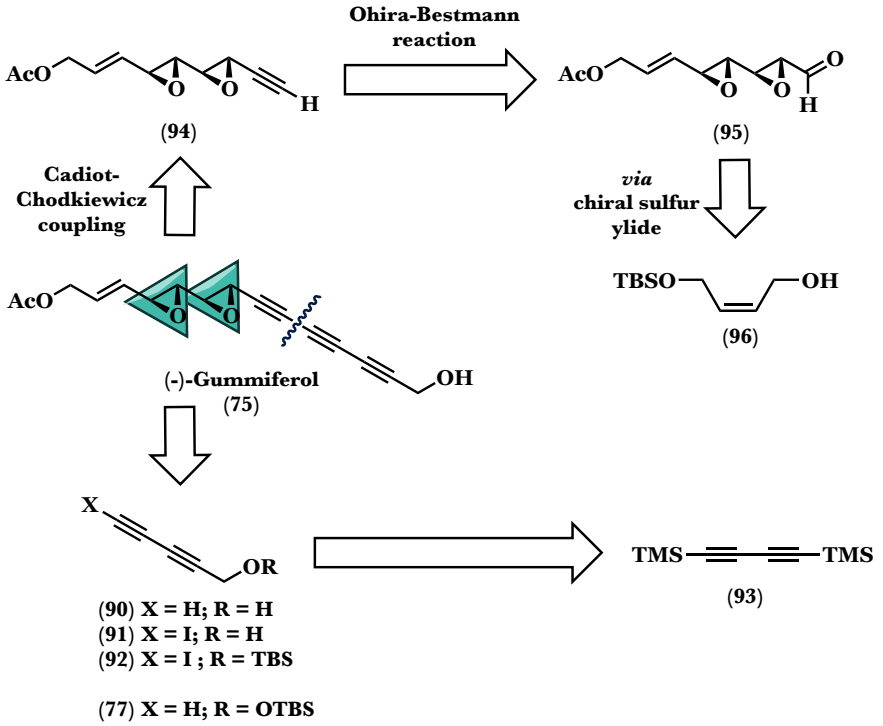
Considering the promising biological profile displayed by gummiferol and with the aim of offering an alternative to the only synthesis reported, a more efficient synthetic strategy has been postulated based on the use of chiral sulfur ylides,[23] that opens up access to all the diastereoisomers of gummiferol by reducing the number of steps and offers an unexplored option to the Sharpless asymmetric epoxidation for these particular systems.

3.4.1. Retrosynthetic Analysis of Gummiferol

The retrosynthetic strategy for the synthesis of gummiferol is described in **Scheme 3.4**. Thus, disconnection of the triacetylene moiety

in the natural product provided diepoxy alkyne **94** and some ot the diacetylenes **90-92** as suitable building blocks. Both key fragments would be assembled *via* a Cadiot-Chodkiewicz coupling.[17]

Scheme 3.4. *Retrosynthesis of gummiferol*

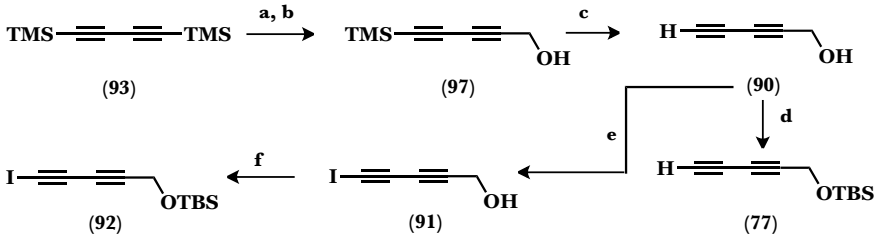


3.4.2. Synthesis of the Diacetylene Fragment

According to the above retrosynthetic analysis, synthesis of gummiferol commenced with the preparation of alkynes **90-92**. This synthetic task was accomplished from the commercial dialkyne **93**, following the procedure described by V. Fiandenese et al. for the synthesis of the alcohol **97**.[24] The subsequent deprotection of the terminal alkyne and iodation under basic conditions provided compound **91**,[25] that did not required any further purification (**Scheme 3.5**). Subsequent protection

into the corresponding silyl ether by action of TBSOTf and 2,6-lutidine provided iodide **92** in 68% overall yield.

Scheme 3.5. *Synthesis of the diacetylen moiety*



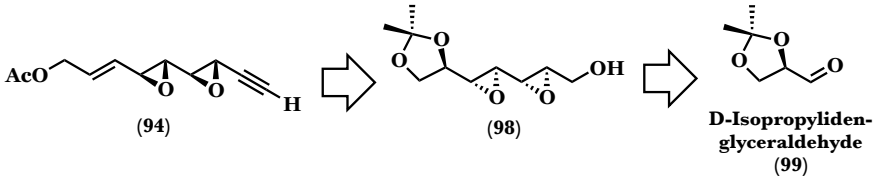
Reagents and conditions: (a) MeLi-LiBr, Et_2O, 0ºC, 8 h. (b) $(CH_2O)n$, Et_2O, 0ºC, 8 h. (c) K_2CO_3, MeOH, 25ºC,12 h. (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0ºC to rt, 3 h. (e) KOH, I_2, H_2O-MeOH, 0ºC to rt. (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0ºC to rt, 3 h, 68% overall yield.

3.4.3. Synthesis of the Diepoxy Alkyne

3.4.3.1. First Approach to the Synthesis of the Diepoxy Alkyne **94**

We first envisioned the synthesis of the diepoxy alkyne **94** from readily available isopropyliden-D-glyceraldehyde (IDG) (**99**) as depicted in **Scheme 3.6**. This suitable starting material would provide an stereochemical induction over the introduction of the first oxirane ring.

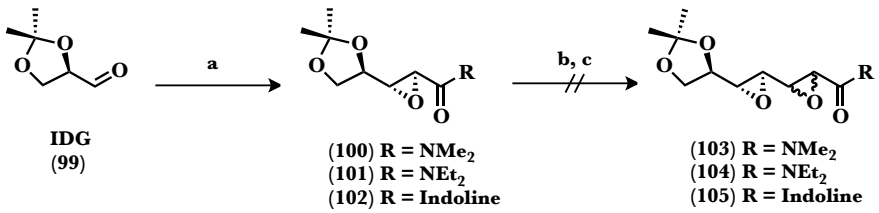
Scheme 3.6. *First approach to the synthesis of the diepoxy alkyne **94** from IDG*



However, our initial efforts were made in vain since all the conditions tested for the synthesis of the diepoxy amide, by reduction of

the known epoxy amides **100-102** and subsequent reaction with the corresponding non-chiral sulfur ylide, provided complex crude mixtures and very low yields. In light of these discouraging results, we turned our attention to an alternative strategy (**Scheme 3.7**).

Scheme 3.7. *First approach to the synthesis of the diepoxy alkyne* ***94***



Reagents and conditions: (a) Me_2S=CHCOR (with R = NMe_2, NEt_2, Indoline), 5.0 M NaOH, CH_2Cl_2- H_2O, 25ºC, 8 to 12 h. (b) Red-Al, THF, 0ºC, 1 h. (c) Me_2S=CHCOR (with R = NMe_2, NEt_2, Indoline), 5.0 M NaOH, CH_2Cl_2-H_2O, 25ºC, 8 to 12 h.

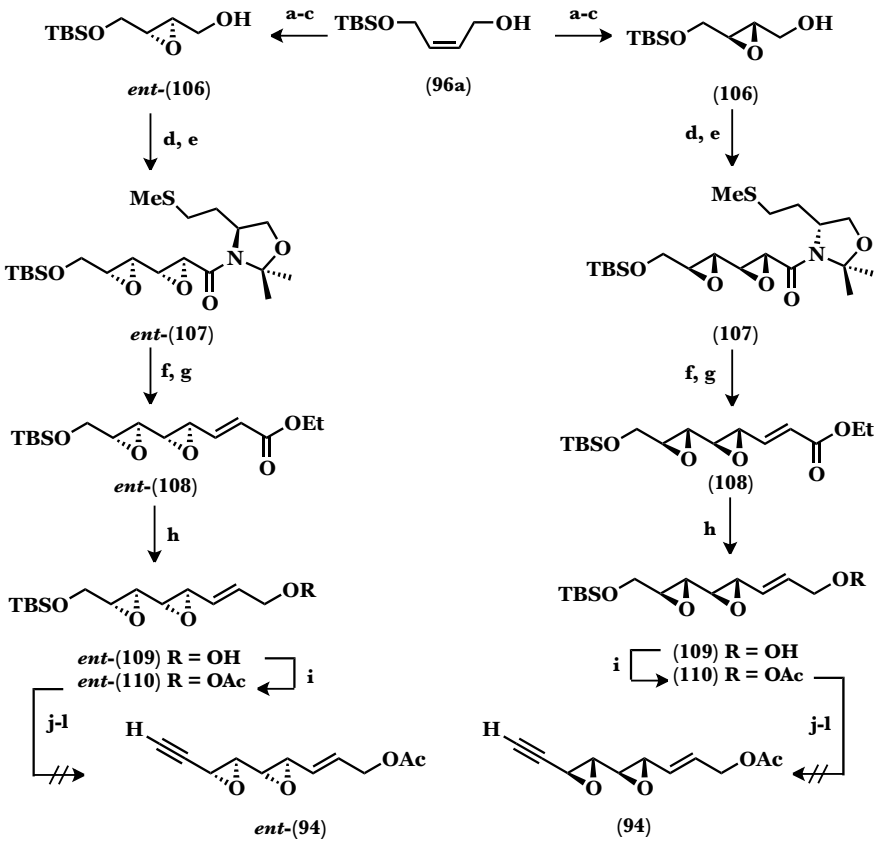
3.4.3.2. Second Approach to the Synthesis of the Diepoxy Alkyne **94**

In a second attemp towards our synthetic target, we planned the synthesis of the building block **94** from alcohol **96** in a strategy that combined the Sharpless asymmetric epoxidation (SAE) together with the use of our chiral sulfur ylides, as depicted in **Scheme 3.8**. This new starting material would provide (-)-gummiferol (**75**) as well as its non-natural enantiomer, (+)-gummiferol, *ent*-(**75**).

From the epoxy alcohols *ent*-**106** and **106**, the epoxy amides *ent*-**107** and **107** were obtained by Parikh-Doering oxidation,[10] followed by treatment with the sulfur ylides derived from L- and D- methionine (**1**) and (**2**), respectively. Thus, epoxy amides *ent*-**107** and **107** were obtained in 60 and 57% yields respectively from the correspoding epoxy alcohols *ent*-**106** and **106**. Subsequent conversion into the corresponding allylic

alcohols and protection with Ac_2O/Pyr furnished the diepoxy alkenes *ent*-**110** and **110**, 73 and 30% overall yields respectively (**Scheme 3.8**).

Scheme 3.8. *Second approach to the synthesis of diepoxy alkyne ent-**94** and **94***

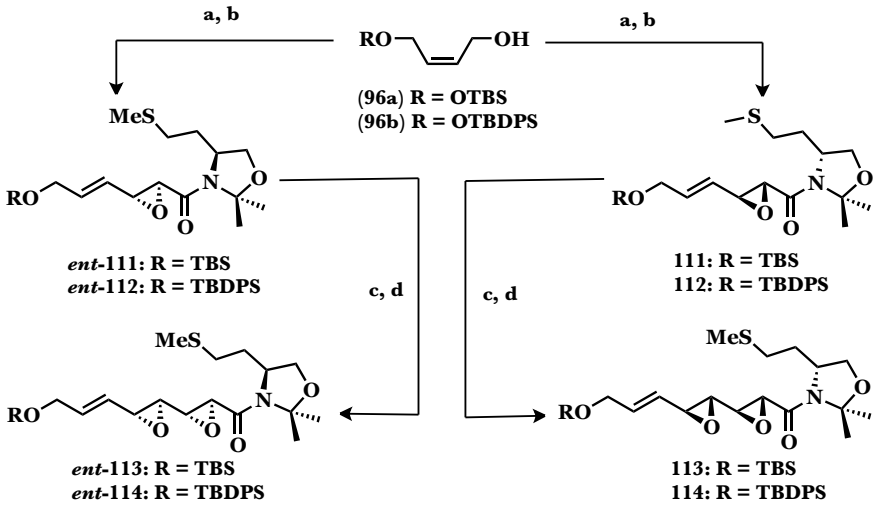


Reagents and conditions: (a) PCC, NaOAc, CH_2Cl_2, 25ºC, 16 h. (b) DIBAL-H, CH_2Cl_2, -78ºC, 45 min (c) SAE, 73% for *ent*-**106**, 65% for **106** (over 2 steps). (d) SO_3·Pyr, CH_2Cl_2, DMSO, 0ºC to rt. (e) **4** or **6**, 5.0 M NaOH, CH_2Cl_2-H_2O, 57% for *ent*-**107**, 60% for **107** (over 2 steps). (f) Red-Al, THF, 0ºC, 45 min. (g) Ph_3PCHCO_2Et, CH_2Cl_2, 25ºC, 57% for *ent*-**108**, 60% for **108** (over 2 steps). (h) DIBAL-H, CH_2Cl_2, -78ºC, 45 min, 76% for *ent*-**109**, 36% for **109**. (i) Ac_2O/Pyr, 25ºC, 12 h, 73% for *ent*-**110**, 30% for **110**. (j) TBAF (k) SO_3·Pyr, CH_2Cl_2, DMSO, 0ºC to rt or Swern oxidation or TEMPO/BAIB, CH_3CN-H_2O, 25ºC, 16 h or PCC, NaOAc, CH_2Cl_2, 25ºC, 16 h. (l) OB-reagent, K_2CO_3.

As depicted in **Scheme 3.8**, chiral sulfur ylides methodology enabled us to synthesize each of the epoxides *ent*-**110** and **110** in a good overall yield (slightly better for *ent*- isomer) and in a stereospecific fashion. reducing the number steps with regard to Takamura's synthesis. Nevertheless, we found several inconveniences during the insertion of the alkyne *via* Ohira-Bestmann reaction. Thus, despite deprotection of silyl ethers *ent*-**110** and **110** proceeded in a very good yield, subsequent oxidations to the aldehyde by treatment with either SO_3·Pyr, Swern conditions, TEMPO/BAIB or PCC, followed by reaction with the O-B reagent resulted in the not obtention of the desired alkynes and although several oxidation conditions were tested to obtain the corresponding aldehyde, we were not able to isolate alkynes *ent*-**94** and **94**. The failure of this key step forced us to consider a new approach and we finally decided to establish to this aim a new and shorter strategy.

Starting again from alcohol **96a**, which was converted into the corresponding aldehyde by action of PCC and subsequent reaction with salts **4** and **6** in basic media, epoxy amides *ent*-**111** and **111** were obtained in a 40% yield over 2 steps, according to the one-phase procedure. Consequent reduction by action of Red-Al, followed by treatment with chiral sulfonium salts once again, supplied diepoxy amides *ent*-**113** and **113** in a 95% and 61% yield over 2 steps, respectively (**Scheme 3.9**).

Scheme 3.9. *Synthesis of the diepoxy amides ent-**113**, **113**, ent-**114** and **114***



Reagents and conditions: (a) PCC, NaOAc, CH_2Cl_2, 25ºC, 16 h. (b) **4** or **6**, 3.0 M NaOH, *t*BuOH, 25ºC, 12 h, 40% for *ent-**111***, 40% for **111**, 40% for *ent-**112***, 40% for *ent*-**112** (over 2 steps). (c) Red-Al, THF, 0ºC, 45 min to 1 h. (d) **4** or **6**, 5.0 M NaOH, CH_2Cl_2, rt, 95% for *ent*-**113**, 61% for **113,** 95% for *ent*-**114**, 70% for **114** (over 2 steps).

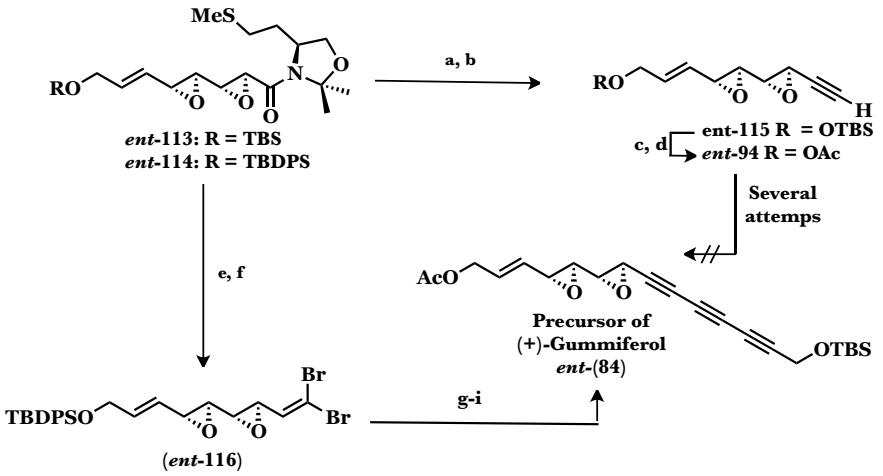
We decided also to assume a last modification and we changed the TBS- group by the TBDPS- one which not only increases the molecular weight of the compounds but also allows to easily monitor the reaction by UV, and to our delight, better yields were obtained in all the cases in the reactions performed with TBDPS- groups.

From these compounds *ent*-**113**, **113** and *ent*-**114** and **114,** diepoxy alkynes were synthesized through succesive reduction under Red-Al and Ohira-Bestmann coupling reactions. Thus, alkynes *ent*-**94** and **94** were obtained in a 12% and 44% yields (over 2 steps), respectively. In terms of yield, it should be mentioned the differences in results between both enantiomeric series, which might be explained because of the lability of the aldehydes, despite the coupling reaction was set up inmediately (**Schemes 3.10-A and 3.10-B**).

Albeit all our best efforts were made to couple diepoxy alkenes *ent*-**94** and **94** with alkynes **90-92** we were not able to obtain gummiferol by this methodology, even though different conditions were tested (**Table 3.3**).

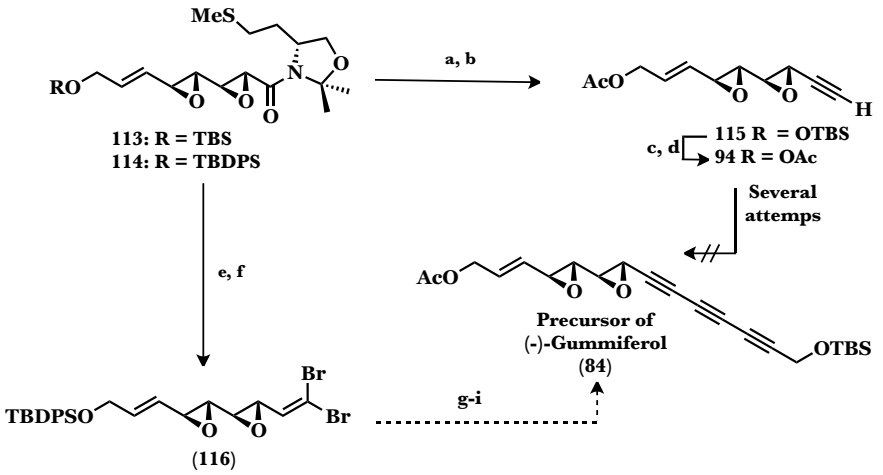
With these discouraging results in hand, we finally resolved to reproduce the conditions reported by Takamura and finally, the coupling between alcohols *ent*-**116** and **116** and alkyne **77** was accomplished in the presence of Cu (I) and $EtNH_2$. In this way, precursor of (+) -gummiferol was obtained in a 55% overall yield (**Scheme 3.10-A**). In the case of (-)-gummiferol, the same scheme is being accomplished to afford precursor **84** (**Scheme 3.10-B**).

Scheme 3.10-A. *Synthesis of the precursor of (+)-gummiferol*



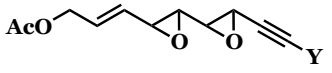
Reagents and conditions: (a) Red-Al, THF, 0ºC, 45 min. (b) Ohira-Bestmann reagent, K_2CO_3, MeOH, 25ºC, 16 h. (c) TBAF, THF, 25ºC, 12 h. d) Ac_2O/Pyr, 25ºC, 2h, 30% (over 2 steps). (e) Red-Al, THF, 0ºC, 45 min. (f) CBr_4, Ph_3P, Et_3N, 25ºC, min, 40% (over 2 steps). (g) TBAF, THF, 0ºC, 1 h. (h) **77**, CuI, $EtNH_2$, .(i) Ac_2O/Pyr, DMAP, 51% (over 3 steps).

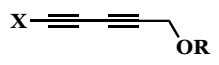
Scheme 3.10-B. *Synthesis of the precursor of (-)-gummiferol*



Reagents and conditions: (a) Red-Al, THF, 0ºC, 45 min. (b) Ohira-Bestmann reagent, K_2CO_3, MeOH, 25ºC, 16 h, 44%. (c) TBAF, THF, 25ºC, 12 h (d) Ac_2O,/Pyr, 25ºC, 2 h, 25% (over 2 steps). (e) Red-Al, THF, 0ºC, 45 min. (f) CBr_4, Ph_3P, Et_3N, 25ºC, min, 40%. (g) TBAF, THF, 0ºC, 1 h. (h) **77**, CuI, $EtNH_2$. (i) Ac_2O/Pyr, DMAP.

Tablel 3.3. *Coupling essays*



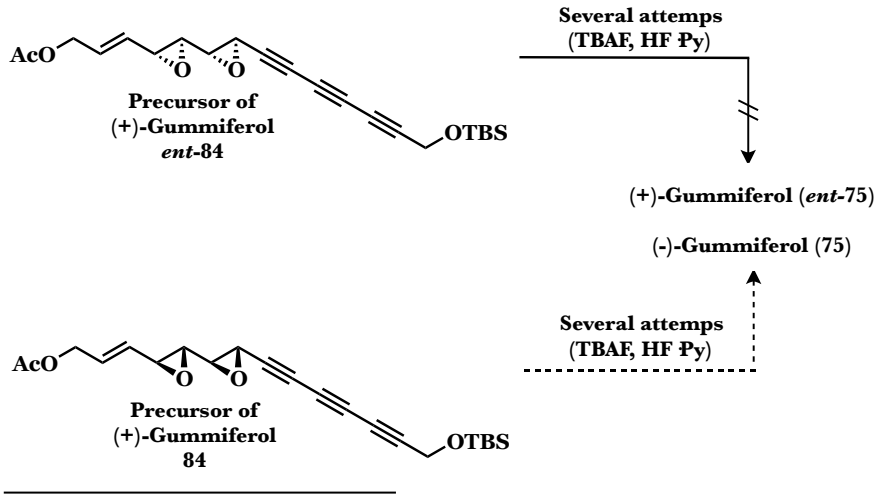


1	**X = H**	**Y = H**	**R = H**
2	**X = H**	**Y = I**	**R = H**
3	**X = H**	**Y = I**	**R = OTBS**
4	**X = Br**	**Y = H**	**R = OTBS**

Reagents and Conditions: (1) $Cu(OAc)_2$, Piperidine, CH_2Cl_2, 25ºC, 10-20 h. (2) $(Ph_3P)PdCl_2$, CuI, DIPA,THF, 2 h. (3) X, CuCl, NH_2OH, 0ºC to -78ºC, $EtNH_2$, MeOH or CuI, Piperidine, 25ºC, 1 h. (4) CuCl, NH_2OH, 0ºC to -78ºC, $EtNH_2$, MeOH, 3 h.

The final deprotection of both precursors *ent*-**84** and **84** was tested in different standard desilylation protocols (TBAF, HF·Pyr). In both cases, we could not detect any trace of gummiferol, and we suspect that the products were decomposed. Nevertheless, we keep working on this final step and several conditions are being tried. (**Scheme 3.11**).

Scheme 3.11. *Towards the completions of the synthesis of (+) and (-)-gummiferol*



3.5. Summary and Concluding Remarks

In this chapter, new approaches towards the synthesis of natural and non-natural gummiferol were described. In particular, the development of an alternative route to construct the diepoxy alkyne was discussed. It was shown, that the application of our chiral sulfur ylides methodology is a feasible and efficient alternative to asymmetric catalysis for the synthesis of the chiral part of the chain.

Compound *ent*-**84** was prepared from alcohol **96b** in 10 steps and 8% overall yield and with complete stereo control. In comparison, Takamura and co-workers synthesized a similar building block in 11 steps and 8% overall yield. In their case, the stereoselective introduction of the epoxide moieties was accomplished with moderate diastereoselectivity and modest yields using a Sharpless asymmetric epoxidation reaction.

From readily available starting material, this new route is in principle suitable for the synthesis of all the diastereoisomers of gummiferol together with other analogues that could be synthesized by taking advantage of the chiral sulfur ylide methodology.

Unfortunately, although several attemps have being made, deprotection of precursos *ent*-**84** and **84** is currently obstructed by the lack of an efficient method for the final desilylation.

3.6. Experimental section

3.6.1. General Procedures for the Synthesis of Epoxy and Diepoxy Amides

3.6.1.1. Synthesis of Epoxy Amides

To a solution of sulfonium salt (**4** or **6**) (1.1 equiv) in *t*BuOH (~ 0.08 M) was added a 3.0 M aqueous NaOH solution (1.1 equiv). After 10 min at 25ºC, a solution of the corresponding aldehyde (1.0 equiv), in *t*BuOH (~ 0.1 M) was added, and the crude reaction mixture was vigorously stirred overnight at 25ºC. After this time, both phases were separated, and the aqueous layer was extracted twice with EtOAc. Combined organic extracts were then washed with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. Flash column chromatography (silica gel, EtOAc 30-50% in hexanes) afforded the corresponding epoxy amide.

3.6.1.2. Synthesis of Diepoxy Amides

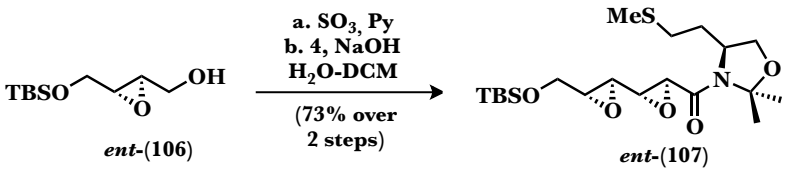
To a solution of the corresponding epoxy amide (1.0 equiv) in dry THF (0.08 M) was added dropwise Red-Al 70% w/v in toluene (2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

To obtain the corresponding diepoxy amide**,** a 5.0 M aqueous NaOH solution (1.0 equiv) was added to a solution of sulfonium salt (**4** or **6**) (1.1 equiv) and the crude aldehyde in CH_2Cl_2-H_2O (1:1) (~ 0.4 M). The reaction mixture was vigorously stirred overnight at 25ºC. After this time,

both phases were separated, and the aqueous layer was extracted twice with EtOAc. Combined organic extracts were then washed with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification of the crude product by flash column chromatography (silica gel, 30-50% EtOAc in hexanes) provided corresponding diepoxy amide.

3.6.2. Synthesis of (+)-Gummiferol

3.6.2.1. Synthesis of the Diepoxy Amide *ent*-**107**



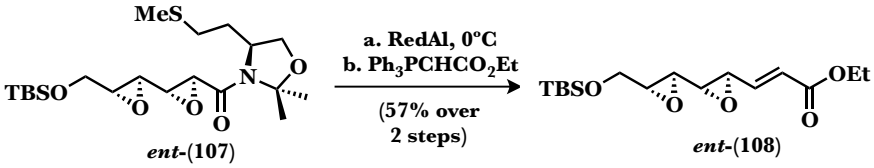
ent-(106) *ent*-(107)

Epoxy alcohol *ent*-**106** (320 mg, 1.47 mmol 1.0 equiv) was dissolved in a CH_2Cl_2/DMSO (1:1) mixture (6 mL) and cooled at 0°C. At this temperature, Et_3N (0.6 mL, 4.4 mmol, 3.0 equiv) was added followed by SO_3·Pyr (420 mg, 2.6 mmol, 1.8 equiv). The mixture was allowed to reach rt and after 5 hours was quenched by addition of buffer pH = 7 and diluted with Et_2O. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure. The resulting crude of the aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (350 mg, 1.12 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.27 mL, 1.36 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide *ent*-**107** (460 mg, 73% over 2 steps) as a yellow oil.

R_f = 0.20 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 26.7 (*c* 0.4, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 4.34-4.28 (m, 1H, CONC*H*), 4.02 (ddd, $\mathcal{J}$ = 9.2, 5.2, 1.4 Hz, 1H, OCH_2CH), 3.93-3.88 (m, 2H, OCH_2CH and SiOCH_2), 3.74 (dd, $\mathcal{J}$ = 12.3, 3.6 Hz, 1H, CH_2C*H*(O)CH), 3.58 (d, $\mathcal{J}$ = 2.0 Hz, 1H, CH(O)C*H*CO), 3.37 (dd, $\mathcal{J}$ = 3.3, 2.0 Hz, 1H, C*H*(O)CHCO), 3.16-3.11 (m, 2H, CH_2CH(O)C*H* and SiOCH_2), 2.59 (ddd, $\mathcal{J}$ = 13.0, 7.7, 5.1 Hz, 1H, SC$H_2$$CH_2$), 2.52-2.43 (m, 1H, SC$H_2$$CH_2$), 2.13 (s, 3H, C$H_3$S), 2.10 -2.05 (m, 1H, SCH_2CH_2), 1.84 (d, $\mathcal{J}$ = 5.4 Hz, 1H, SCH_2CH_2), 1.65 (s, 3H, (CH_3)$_2$C), 1.53 (s, 3H, (CH_3)$_2$C), 0.88 (s, 9H, (CH_3)$_3$CSi), 0.06 (s, 3H, (CH_3)$_2$Si), 0.05 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.94, 95.86, 67.01, 61.57, 56.29, 55.95, 55.50, 51.69, 51.23, 34.40, 30.61, 26.17, 25.78, 25.65, 18.27, 15.70, -5.42, -5.44.

3.6.2.2. Synthesis of the γ,δ-Diepoxy α,β-Unsaturated Ester *ent*-**108**

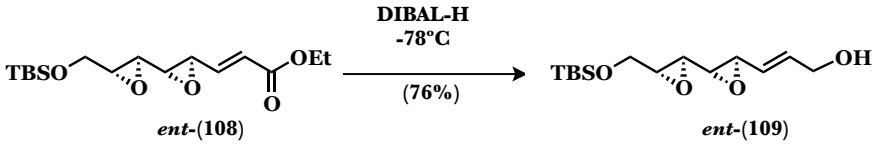


Diepoxiamide *ent*-**107** (230 mg, 0.53 mmol, 1.0 equiv) in dry THF (8 mL) was treated dropwise with Red-Al 60% w/v in toluene (0.33 mL, 1.17 mmol, 2.2 equiv) at 0ºC. After 1 hour at this temperature, the reaction mixture was diluted with a saturated aqueous NH_4Cl solution. The aqueous phase was then separated, extracted twiced with EtOAc and the combined organic phase washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The resulting crude aldehyde was used in the next step without further purification.

The crude aldehyde was dissolved in CH_2Cl_2 (10 mL) and over this solution was added the phophorous ylide $Ph_3P{=}CHCO_2Et$ (280 mg, 0.8 mmol 1.5 equiv). After 30 minutes the reaction is complete and the solvent was removed under reduced pressure. Flash column chromatography (silica gel, 20% EtOAc in hexanes) afforded the α,β-unsaturated diepoxy ester *ent*-**108** (100 mg, 57% over 2 steps) as a pale yellow oil.

R_f = 0.58 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 8.1 (*c* 0.4, CH_2Cl_2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.63 (dd, $\mathcal{J}$ = 15.7, 7.2 Hz, 1H, C*H*=CHCO), 6.14 (dd, $\mathcal{J}$ = 15.7, 0.7 Hz, 1H, CH=C*H*CO), 4.18 (q, $\mathcal{J}$ = 6.8 Hz, 2H, CH_2CH$_3$), 3.86 (dd, $\mathcal{J}$ = 12.3, 2.8 Hz, 1H, SiOCH_2), 3.72 (dd, $\mathcal{J}$ = 12.3, 3.9 Hz, 1H, SiOCH_2), 3.45 (ddd, $\mathcal{J}$ = 7.2, 1.9, 0.7 Hz, 1H, CH(O)C*H*CH=), 3.10-3.07 (m, 1H, CH$_2$C*H*(O)), 3.03 (dd, $\mathcal{J}$ = 4.0, 2.1 Hz, 1H, CH$_2$CH(O)C*H*), 2.99 (dd, $\mathcal{J}$ = 4.0, 1.9 Hz, 1H, C*H*(O)CHCH=), 1.26 (t, $\mathcal{J}$ = 7.1 Hz, 3H, CH$_2$CH_3), 0.86 (s, 9H, (CH_3)$_3$CSi), 0.04 (s, 3H, (CH_3)$_2$Si), 0.05 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.39, 143.03, 124.68, 61.81, 60.66, 58.49, 56.22, 53.76, 52.60, 25.81, 18.30, 14.17, -5.38, -5.40.

3.6.2.3. Synthesis of the Allylic Alcohol *ent*-**109**

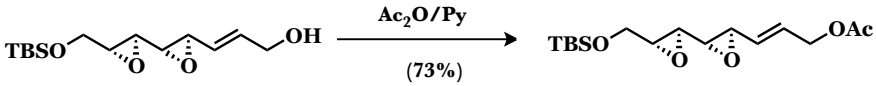


At -78°C, DIBAL-H 1.0 M in toluene (1.15 mL, 1.15 mmol, 2.5 equiv) was added over a solution of ester *ent*-**108** (151 mg, 0.46 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL). After 30 minutes, the reaction was complete and it was allowed to warm at 0°C. At this temperature, the mixture was diluted with EtOAc and a saturated aqueous Na^+/K^+ tartrate solution. After 2 hours of vigorous stirring, the aqueous phase was extracted twice

with EtOAc and the organic layer washed with water and brine, then dried over anhydrous $MgSO_4$ and filtered. The solvent was then removed carefully under reduced pressured at low temperature. Purification by flash column chromatography (silica gel, 20% EtOAc in hexanes) rendered the allylic alcohol *ent*-**109** (100 mg, 76% yield) as a pale yellow oil.

R_f = 0.40 [Silica gel, 30% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +14.4 (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.10 (dtd, $\mathcal{J}$ = 15.6, 5.1, 0.6 Hz, 1H, CH=C*H*CH_2), 5.47 (ddt, $\mathcal{J}$ = 15.6, 7.9, 1.7 Hz, 1H, C*H*=CHCH_2), 4.17 (dd, $\mathcal{J}$ = 5.2, 1.7 Hz, 2H, =CHC*H*$_2$OH), 3.87 (dd, $\mathcal{J}$ = 12.2, 2.8 Hz, 1H, SiOC*H*$_2$), 3.73 (dd, $\mathcal{J}$ = 12.2, 4.0 Hz, 1H, SiOC*H*$_2$), 3.39 (ddd, $\mathcal{J}$ = 7.9, 2.1, 0.6 Hz, 1H, (O)C*H*CH=), 3.09 (ddd, $\mathcal{J}$ = 4.1, 2.8, 2.3 Hz, 1H, CH_2C*H*(O)CH), 2.99 (dd, $\mathcal{J}$ = 4.4, 2.2 Hz, 1H, CH_2CH(O)C*H*), 2.92 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, C*H*(O)CHCH=), 1.74 (bs, 1H, O*H*), 0.88 (s, 9H, (C*H*$_3$)$_3$CSi)), 0.06 (s, 3H, (C*H*$_3$)$_2$Si), 0.05 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 135.40, 127.12, 62.50, 62.04, 58.00, 56.06, 55.29, 53.26, 25.84, 18.33, -5.37, -5.36.

3.6.2.4. Synthesis of Acetyl Derivative *ent*-110



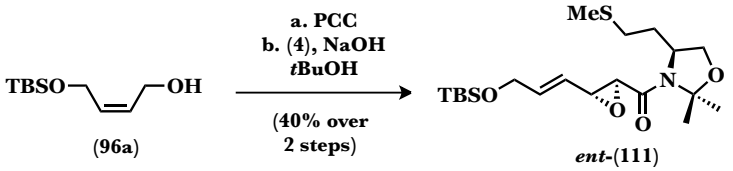
ent-(109) *ent*-(110)

Over a solution of the allylic alcohol *ent*-**109** (32 mg, 0.1 mmol, 1.0 equiv) in pyridine (3 mL) was added Ac_2O (0.4 mL, 4.5 mmol, 40 equiv) at rt. After 12 h at the same temperature, the solvent was removed under vacuum and the crude product was purified by flash column chromatography (silica gel, 10 % EtOAc in hexanes) to furnish acetate *ent*-**110** (27 mg, 73% yield) as a pale yellow oil.

R_f = 0.91 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 10.2 (*c* 0.3, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.02 (dtd, $\mathcal{J}$ = 15.6, 5.8,

0.6 Hz, 1H, CH=C*H*CH$_2$), 5.50 (ddt, $\mathcal{J}$ = 15.6, 7.8, 1.5 Hz, 1H, C*H*=CHCH$_2$), 4.58 (dd, $\mathcal{J}$ = 5.8, 1.5 Hz, 2H, =CHC*H*$_2$OAc), 3.89-3.85 (ddd, $\mathcal{J}$ = 12.2, 2.8, 0.4, 1H, SiOC*H*$_2$), 3.73 (dd, $\mathcal{J}$ = 12.2, 4.0 Hz, 1H, SiOC*H*$_2$), 3.37 (ddd, $\mathcal{J}$ = 7.8, 2.1, 0.6 Hz, 1H, CH(O)C*H*CH=), 3.09 (ddd, $\mathcal{J}$ = 4.0, 2.8, 2.2 Hz, 1H, CH$_2$C*H*(O)CH), 2.99 (dd, $\mathcal{J}$ = 4.3, 2.2 Hz, 1H, CH$_2$CH(O)C*H*), 2.93 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, C*H*(O)CHCH=), 2.07 (s, 3H, OCOC*H*$_3$), 0.88 (s, 9H, (C*H*$_3$)$_3$CSi)), 0.06 (s, 3H, (C*H*$_3$)$_2$Si), 0.05 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, CDCl$_3$), δ (ppm): 170.58, 130.20, 129.84, 63.62, 61.98, 57.97, 56.07, 54.93, 53.10, 25.84, 20.85, 18.33, -5.35, -5.37.

3.6.2.5. Synthesis of the Epoxy Amide *ent*-**111**

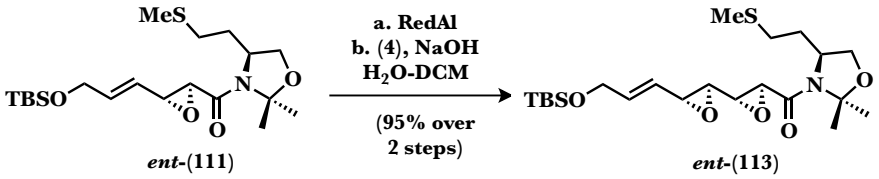


Over a suspension of PCC (1.9 g, 8.9 mmol, 1.5 equiv) in CH_2Cl_2 (20 mL) was added NaOAc anhydrous (1.9 g, 23.7 mmol, 4.0 equiv) followed by *cis*-allylic alcohol **96a** (1.1 mL, 5.93 mmol, 1.0 equiv) dissolved in CH_2Cl_2 (10 mL). The reaction mixture was stirred at rt for 6 h and the resulting crude was filtered off through a pad of SiO_2 and washed with Et_2O. The solvent was then removed under vacuum and the crude aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (1.2 g, 3.93 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 1.2 mL, 3.57 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of epoxy amides to yield epoxy amide *ent*-**111** (600 mg, 40% over two steps) as a yellow oil.

R_f = 0.37 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +42.6 (*c* 0.1, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.08 (dtd, $\mathcal{J}$ = 15.4, 4.2, 0.6 Hz, 1H, CH_2CH=CH), 5.47 (ddt, $\mathcal{J}$ = 15.4, 8.1, 1.9 Hz, 1H, CH_2CH=C*H*), 4.26 (ddd, $\mathcal{J}$ = 10.2, 4.9, 3.2 Hz, 1H, CONC*H*), 4.15 (dt, $\mathcal{J}$ = 3.8, 1.8 Hz, 2H, SiOCH_2), 3.98 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.3 Hz, 1H, OCH_2CH), 3.85 (dd, $\mathcal{J}$ = 9.2, 0.8 Hz, 1H, OCH_2CH), 3.56 (dd, $\mathcal{J}$ = 8.1, 1.7 Hz, 1H, =CHC*H*(O)CH), 3.50 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)C*H*CO), 2.51 (ddd, $\mathcal{J}$ = 13.3, 7.0, 5.0 Hz, 1H, SC$H_2$$CH_2$), 2.39 (ddd, $\mathcal{J}$ = 13.4, 8.9, 6.7 Hz, 1H, SC$H_2$$CH_2$), 2.04 (s, 3H, C$H_3$S), 2.02-1.96 (m, 1H, SCH_2CH_2), 1.77-1.70 (m, 1H, SCH_2CH_2), 1.60 (s, 3H, (CH_3)$_2$C), 1.50 (s, 3H, (CH_3)$_2$C), 0.86 (s, 9H, (CH_3)$_3$CSi), 0.02 (s, 6H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 163.39, 137.11, 124.44, 95.83, 66.99, 62.48, 57.78, 55.83, 55.38, 34.25, 30.75, 26.25, 25.85, 22.96, 18.29, 15.77, -5.39, -5.41. HRMS (H-ESI) *m/e* 416.22853 calcd for $C_{20}H_{37}NO_4SSi$ $[M + H]^+$, found 416.22855.

3.6.2.6. Synthesis of the Diepoxy Amide *ent*-**113**



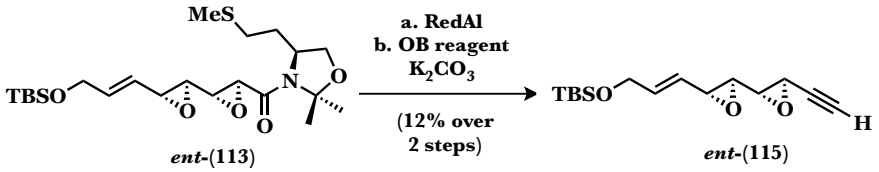
ent-(**111**) *ent*-(**113**)

To a solution of epoxy amide *ent*-**111** (100 mg, 0.24 mmol, 1.0 equiv) in dry THF (6 mL) was added dropwise Red-Al 60% w/v (0.15 mL, 0.53 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (85 mg, 0.26 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.04 mL, 0.24 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide *ent*-**113** (36 mg, 95% over two steps) as a yellow oil.

R_f = 0.26 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +51.9 (*c* 1.0, CH_2Cl_2). ^{1}H RMN (400 MHz, $CDCl_3$), δ (ppm): 6.04 (dtd, $\mathcal{J}$ = 15.4, 4.3, 0.7 Hz, 1H, CH_2CH=CH), 5.44 (ddt, $\mathcal{J}$ = 15.4, 8.0, 1.9 Hz, 1H, CH_2CH=C*H*), 4.33-4.26 (m, 1H, CONC*H*), 4.17 (dd, $\mathcal{J}$ = 4.2, 1.8 Hz, 2H, SiOCH_2), 4.02- 3.97 (m, 1H, OCH_2CH), 3.89 (d, $\mathcal{J}$ = 9.2 Hz, 1H, OCH_2CH), 3.57 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)C*H*CO), 3.42 (dd, $\mathcal{J}$ = 7.9, 1.7 Hz, 1H, =CHC*H*(O)CH), 3.35 (dd, $\mathcal{J}$ = 3.3, 1.9 Hz, 1H, C*H*(O)CHCO), 3.02 (dd, $\mathcal{J}$ = 3.3, 2.1 Hz, 1H, =CHC*H(*O)C*H*), 2.57 (ddd, $\mathcal{J}$ = 13.0, 7.7, 5.1 Hz, 1H, SCH_2CH$_2$), 2.47 (dt, $\mathcal{J}$ = 13.3, 7.9 Hz, 1H, SCH_2CH$_2$), 2.11 (s, 3H, CH_3S), 2.08-2.01 (m, 1H, SCH$_2$CH_2), 1.86-1.75 (m, 1H, SCH$_2$CH_2), 1.63 (s, 3H, (CH_3)$_2$C), 1.51 (s, 3H, (CH_3)$_2$C), 0.88 (s, 9H, (CH_3)$_3$CSi), 0.04 (s, 6H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.92, 136.24, 125.09, 95.94, 67.05, 62.64, 56.56, 55.99, 55.67, 55.40, 51.24, 34.46, 30.69, 26.21, 25.89, 22.95, 18.36, 15.83, -5.32, -5.34. HRMS (H-ESI) *m/e* 458.23910 calcd for $C_{22}H_{39}NO_5SSi$ [M + H]$^+$, found 458.23907.

3.6.2.7. Synthesis of the Alkyne *ent*-**115**



ent-(113) *ent*-(115)

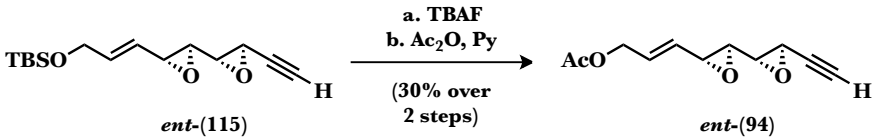
To a solution of epoxy amide *ent*-**113** (170 mg, 0.38 mmol, 1.0 equiv) in dry THF (10 mL) was added dropwise Red-Al 60% w/v (0.20 mL, 0.87 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture

was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

To a solution of crude of aldehyde in dry MeOH (10 mL) was added K_2CO_3 (100 mg, 0.7 mmol, 2.0 equiv) at rt followed by a solution of the Ohira-Bestmann reagent [(dimethyl(diazomethyl)phosphonate, $CH_3COC(N_2)PO(OMe)_2$)] (140 mg, 0.7 mmol, 2.0 equiv) in MeOH (5 mL). The progress of the reaction was monitored by TLC. After the reaction was complete, it was then diluted with H_2O and Et_2O. The aqueous phase was extracted with Et_2O, dried over $MgSO_4$ and the solvent removed under pressure. Flash column chromatography (silica gel, 10% EtOAc en hexanes) rendered the alkyne *ent*-**115** as a pale yellow oil (12 mg , 12 % over 2 steps).

R_f = 0.54 [Silica gel, 20% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +66.0 (*c* 0.25, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 6.05 (dtd, $\mathcal{J}$ = 15.4, 4.3, 0.5 Hz, 1H, CH_2CH=CH), 5.45 (ddt, $\mathcal{J}$ = 15.4, 8.0, 1.9 Hz, 1H, CH_2CH=C*H*), 4.19 (dd, $\mathcal{J}$ = 4.3, 1.9 Hz, 2H, $SiOCH_2$), 3.41-3.37 (ddd, $\mathcal{J}$ = 8.0, 2.1, 0.6 Hz, 1H, =CHC*H*(O)CH), 3.36 (dd, $\mathcal{J}$ = 1.7 Hz, 1H, CH(O)C*H*CCH), 3.26 (dd, $\mathcal{J}$ = 3.5, 2.1 Hz, 1H, C*H*(O)CHCCH), 2.97 (dd, $\mathcal{J}$ = 3.5, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.34 (d, $\mathcal{J}$ = 1.7 Hz, 1H, CC*H*), 0.90 (s, 9H, $(CH_3)_3CSi$), 0.07 (s, 6H, $(CH_3)_2Si$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 136.23, 125.14, 79.32, 72.41, 62.65, 57.24, 56.61, 55.49, 42.59, 25.90, 18.38, -5.31, -5.33. HRMS (H-ESI) *m/e* 281.15675 calcd for $C_{15}H_{24}O_3Si$ $[M + H]^+$, found 281.15686.

3.6.2.8. Synthesis of the Diepoxy Alkyne *ent*-**94**



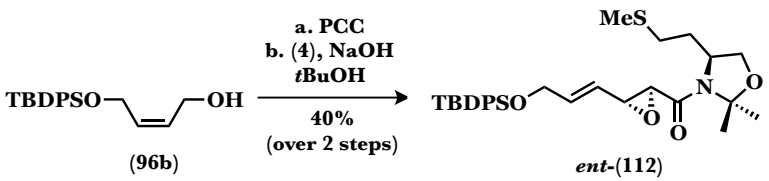
***ent*-(115)** **(30% over 2 steps)** ***ent*-(94)**

Alkyne *ent*-**115** (15 mg, 0.05 mmol, 1.0 equiv) was dissolved in THF (5 mL) and over this solution was added 1.0 M TBAF in THF (0.08 mL, 0.08 mmol, 1.5 equiv) at rt. After 45 min, the reaction mixture was diluted with Et_2O and a saturated solution of NH_4Cl. The aqueous phase was then extracted with EtOAc and the organic extract washed with H_2O, brine and dried over $MgSO_4$. The solvent was removed under vacuum and the crude alcohol was used in the next step without further purification.

Over a solution of the allylic alcohol in pyridine (2 mL) was added Ac_2O (0.2 mL, 2.0 mmol, 40 equiv) at rt. After 12 h at the same temperature, the solvent was removed under vacuum and the crude product was purified by flash column chromatography (silica gel, 10 % EtOAc in hexanes) to furnish alkyne *ent*-**94** (3 mg, 30% yield over 2 steps) as a pale yellow oil.

R_f = 0.54 [Silica gel, 20% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +50.7 (*c* 0.1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.04 (dtd, $\mathcal{J}$ = 15.6, 5.8, 0.6 Hz, 1H, CH_2CH=CH), 5.50 (ddt, $\mathcal{J}$ = 15.6, 7.8, 1.5 Hz, 1H, CH_2CH=C*H*), 4.59 (dd, $\mathcal{J}$ = 5.7, 1.5 Hz, 2H, OCH_2), 3.39 (dd, $\mathcal{J}$ = 7.9, 1.9 Hz, 1H, CH=CHC*H*(O)CH), 3.37-3.35 (m, 1H, CH(O)C*H*C), 3.27 (dd, $\mathcal{J}$ = 3.4, 2.1 Hz, 1H, C*H*(O)CHC), 2.99 (dd, $\mathcal{J}$ = 3.4, 2.0 Hz, 1H, =CHCH(O)C*H*), 2.34 (d, $\mathcal{J}$ = 1.7 Hz, 1H, CC*H*), 2.08 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 170.53**,** 130.34, 129.59, 79.18, 72.50, 63.51, 56.98, 56.53, 54.93, 42.65, 20.83.

3.6.2.9. Synthesis of the Diepoxy Amide *ent*-**112**



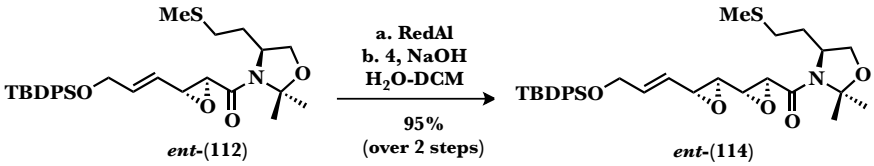
Over a suspension of PCC (3.2 g, 14.8 mmol, 1.5 equiv) in CH_2Cl_2 (20 mL) was added NaOAc anhydrous (3.2 g, 39.5 mmol, 4.0 equiv) followed by *cis*-allylic alcohol **96b** (2.0 mL, 9.9 mmol, 1.0 equiv) dissolved in CH_2Cl_2 (10 mL). The reaction mixture was stirred at rt for 6 h and the resulting crude was filtered off through a pad of SiO_2 and washed with Et_2O. The solvent was then removed under vacuum and the crude aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (3.4 g, 10.9 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 3.3 mL, 9.9 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of epoxy amides to yield epoxy amide *ent*-**112** (2.1 g, 40% over two steps) as a pale yellow oil.

R_f = 0.55 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +23.0 (*c* 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.68 – 7.64 (m, 4H, C*H* aromatic), 7.43 – 7.36 (m, 6H, C*H* aromatic), 6.12 (dt, $\mathcal{J}$ = 15.4, 4.0 Hz, 1H, CH_2CH=CH), 5.65 (ddt, $\mathcal{J}$ = 15.4, 8.1, 2.0 Hz, 1H, CH_2CH=C*H*), 4.32 (ddd, $\mathcal{J}$ = 8.3, 4.7, 3.2 Hz, 1H, CONC*H*), 4.23 (dt, $\mathcal{J}$ = 3.7, 1.7 Hz, 2H, SiOCH_2), 4.03 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.3 Hz, 1H, OCH_2CH), 3.90 (d, $\mathcal{J}$ = 9.1 Hz, 1H, OCH_2CH), 3.64 (dd, $\mathcal{J}$ = 8.1, 1.6 Hz, 1H, =CHC*H*(O)CH), 3.57 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)C*H*CO), 2.57 (ddd, $\mathcal{J}$ = 13.2, 6.9, 5.1 Hz, 1H, SCH_2CH$_2$), 2.43 (ddd, $\mathcal{J}$ = 13.4, 9.0, 6.6 Hz, 1H, SCH_2CH$_2$), 2.11 – 2.05 (m, 1H, SCH$_2$CH_2), 2.04 (s, 3H, CH_3S), 1.84 – 1.76 (m, 1H, SCH$_2$CH_2), 1.66 (s, 3H, (CH_3)$_2$C), 1.56 (s, 3H, (CH_3)$_2$C), 1.06 (s, 9H, (CH_3)$_3$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 163.46,

136.74, 135.48, 133.32, 129.81, 127.77, 124.52, 95.94, 67.07, 63.29, 60.37, 57.90, 55.87, 55.55, 34.33, 30.84, 26.83, 26.32, 23.05, 19.26, 15.83.

3.6.2.10. Synthesis of the Diepoxy Amide *ent*-**114**



ent-(112) *ent*-(114)

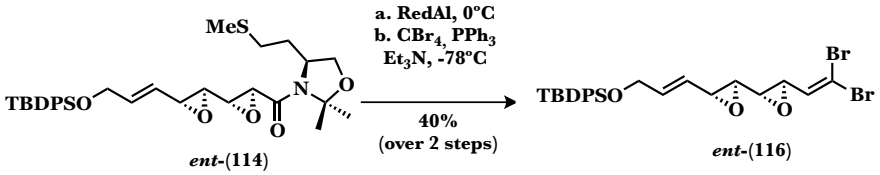
To a solution of epoxy amide *ent*-**111** (166 mg, 0.31 mmol, 1.0 equiv) in dry THF (10 mL) was added dropwise Red-Al 60% w/v (0.2 mL, 0.62 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (110 mg, m 0.34 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.01 mL, 0.31 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide *ent*-**114** (170 mg, 95% over two steps) as a pale yellow oil.

R_f = 0.45 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +35.1 (*c* 0.4, CH_2Cl_2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.71 – 7.67 (m, 4H, C*H* aromatic), 7.47 – 7.40 (m, 6H, C*H* aromatic), 6.08 (dt, $\mathcal{J}$ = 15.4, 4.2 Hz, 1H, CH_2CH=CH), 5.59 (ddt, $\mathcal{J}$ = 15.4, 8.0, 1.9 Hz, 1H, CH_2CH=C*H*), 4.36 (ddd, $\mathcal{J}$ = 9.9, 4.8, 3.6 Hz, 1H, CONC*H*), 4.25 (dd, $\mathcal{J}$ = 4.1, 1.9 Hz, 2H, SiOCH_2), 4.06 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.3 Hz, 1H, OCH_2CH), 3.95 (d, $\mathcal{J}$ = 9.2 Hz, 1H, OCH_2CH), 3.63 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)C*H*CO), 3.47

(dd, $\mathcal{J}$ = 7.9, 2.0 Hz, 1H, =CHC*H*(O)CH), 3.43 (dd, $\mathcal{J}$ = 3.3, 1.9 Hz, 1H, C*H*(O)CHCO), 3.08 (dd, $\mathcal{J}$ = 3.4, 2.1 Hz, 1H, =CHCH*(*O)C*H*), 2.63 (ddd, $\mathcal{J}$ = 13.0, 7.7, 5.1 Hz, 1H, SC*H*$_2$CH$_2$), 2.52 (ddd, $\mathcal{J}$ = 13.2, 8.2, 7.3 Hz, 1H, SC*H*$_2$CH$_2$), 2.15 (s, 3H, C*H*$_3$S), 2.14 – 2.09 (m, 1H, SCH$_2$C*H*$_2$), 1.92 – 1.82 (m, 1H, SCH$_2$C*H*$_2$), 1.69 (s, 3H, (C*H*$_3$)$_2$C), 1.58 (s, 3H, (C*H*$_3$)$_2$C), 1.09 (s, 9H, (C*H*$_3$)$_3$CSi). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.94**,** 135.79, 135.50, 133.39, 129.77, 127.74, 125.15, 95.98, 67.08, 63.39, 56.61, 56.01, 55.74, 55.40, 51.31, 34.49, 30.74, 26.82, 26.24, 22.98, 19.24, 15.86. HRMS (H-ESI) *m/e* 582.27040 calcd for $C_{32}H_{43}NO_5SSi$ $[M + H]^+$, found 582.27057.

3.6.2.11. Synthesis of the Dibromo Alkene *ent*-**116**



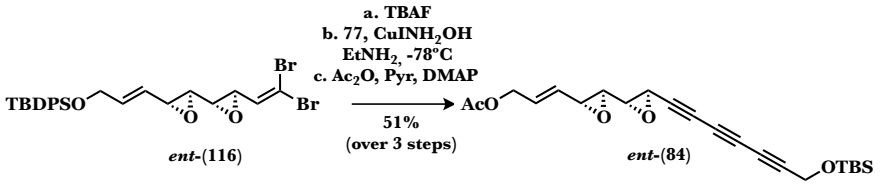
To a solution of epoxy amide *ent*-**114** (80 mg, 0.14 mmol, 1.0 equiv) in dry THF (5 mL) was added dropwise Red-Al 60% w/v (0.08 mL, 0.27 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

To a solution of CBr_4 (130 mg, 0.37 mmol, 2.7 equiv) in CH_2Cl_2 (5 mL) was added Ph_3P (195 mg, 0.74 mmol, 5.3 equiv) at 0ºC. The mixture was stirred at the same temperature for 15 min, and Et_3N (0.1 mL 0.7 mmol, 5.3 equiv) was added to the resulting mixture at 0ºC. After the mixture was stirred at the same temperature for 5 min, the aldehyde obtained above dissolved in CH_2Cl_2 (5 mL) was added at -78ºC. The

mixture was stirred at the same temperature for 2 h. The reaction was quenched with a saturated aqueous $NaHCO_3$ and the mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4. Concentration and purification by flash column chromatography (silica gel, 10% EtOAc in hexanes) gave the corresponding dibromo alkene *ent*-**116** (32 mg, 40% over 2 steps) as a pale yellow oil.

R_f = 0.75 [Silica gel, 10% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +26.6 (*c* 0.8, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.69 - 7.63 (m, 2H, C*H* aromatic), 7.46 - 7.33 (m, 8H, C*H* aromatic), 6.16 (dd, $\mathcal{J}$ = 7.8, 0.7 Hz, 1H, C*H*=CHBr$_2$), 6.08 - 5.99 (m, 1H, CH_2C*H*=), 5.60 - 5.51 (m, 1H, CH_2CH=C*H*), 4.23 (dd, $\mathcal{J}$ = 4.2, 2.0 Hz, 2H, CH_2CH=), 3.64 (ddd, $\mathcal{J}$ = 7.8, 2.0, 0.7 Hz, 1H,(O)C*H*CH=CHBr$_2$), 3.41 (dd, $\mathcal{J}$ = 7.9, 2.2 Hz, 1H, CH=CHC*H*(O)CH), 3.07 (ddd, $\mathcal{J}$ = 3.9, 2.0, 0.7 Hz, 1H, C*H*(O)CHCH=CHBr$_2$), 2.98 (ddd, $\mathcal{J}$ = 3.9, 2.1, 0.7 Hz, 1H, CH=CHCH(O)C*H*), 1.06 (s, 9H, $(CH_3)_3$CSi). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 135.62, 135.50, 134.46, 133.41, 133.28, 129.76, 127.72, 125.23, 63.39, 57.17, 56.66, 55.61, 54.98, 26.82, 19.25.

3.6.2.12. Synthesis of the Precursor of Gummiferol *ent*-**84**



To a solution of the dibromo alkene *ent*-**116** (51 mg, 0.09 mmol, 1.0 equiv) in THF (5 mL) was added TBAF (1.0 M solution in THF, 0.4 mL, 0.36 mmol, 4.0 equiv) at 0°C. The mixture was stirred at room temperature for 1 h and the solvent was then removed under vacuum. Corresponding bromo alkyne was obtained as a colourless amorphous solid and used in the next step without further purification.

To a 70% aqueous solution of $EtNH_2$ (2.25 mL) in MeOH (3 mL) was added CuCl (4 mg, 45.0 μmol, 0.5 equiv) at room temperature that resulted in the formation of a blue solution. To the resulting mixture was added $NH_2OH \cdot HCl$ (19 mg, 270.0 μmol, 3.0 equiv) at the same temperature to discharge the blue color. To the resulting mixture was added diacetylene **77** (19 mg, 99.0 μmol, 1.1 equiv) in MeOH (1.0 mL) at room temperature, and the mixture was stirred at the same temperature for 20 min that resulted in the formation of a yellow suspension. To the resulting mixture was added bromo acetylene above obtained in MeOH (1.0 mL) at -78ºC, and the mixture was stirred at the same temperature for 20 min. The mixture was diluted with Et_2O, washed with H_2O and brine, dried over Na_2SO_4 and the solvent removed under vacuum. The resulting trialkyne was used in the next step without any further purification.

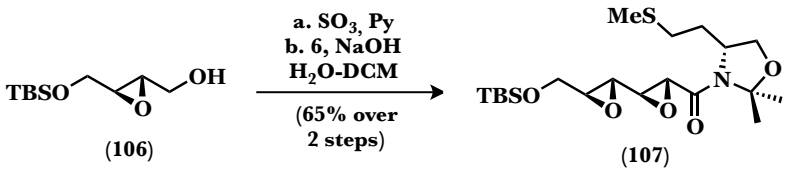
To a solution of the obtained trialkyne in CH_2Cl_2 (4 mL) were added pyridine (0.05 mL, 0.63 mmol, 7.0 equiv), Ac_2O (0.04 mL, 0.45 mmol, 5.0 equiv) and 4-DMAP (4 mg, 0.03 mmol, 0.3 equiv) at 0ºC. The mixture was stirred at the same temperature for 20 min. The mixture was then diluted with Et_2O, washed with H_2O and brine, and then dried over $MgSO_4$. Concentration and purification by flash column chromatography (silica gel, 30% EtOAc in hexanes) gave acetate *ent*-**84** (20 mg, 51% yield over 3 steps) as a yellow oil.

R_f = 0.7 [Silica gel, 60% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +11.2 (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.04 (ddd, $\mathcal{J}$ = 15.2, 6.1, 5.5 Hz, 1H, $AcOCH_2CH$=), 5.49 (ddt, $\mathcal{J}$ = 15.6, 7.8, 1.5 Hz, 1H, $AcOCH_2CH$=C*H*), 4.59 (dd, $\mathcal{J}$ = 5.7, 1.5 Hz, 2H, $AcOCH_2$), 4.39 (s, 2H, CH_2OTBS), 3.44 (d, $\mathcal{J}$ = 2.0 Hz, 1H, C*H*(O)CHC), 3.38 (dd, $\mathcal{J}$ = 8.1, 1.8 Hz, 1H, =CHC*H*(O)), 3.33 (dd, $\mathcal{J}$ = 3.2, 2.0 Hz, 1H, C*H*(O)CHC), 3.01 (dd, $\mathcal{J}$ = 3.2, 2.0 Hz, 1H,=CHCH(O)C*H*), 2.08 (s, 3H, CH_3CO), 0.90 (s, 9H, $(CH_3)_3$CSi), 0.12 (s, 6H $(CH_3)_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ

(ppm): 170.54, 130.50, 129.43, 77.80, 73.63, 69.49, 69.14, 63.48, 63.14, 62.01, 57.48, 56.24, 55.07, 52.11, 43.05, 25.72, 20.83, 18.25, -5.22. HRMS (H-ESI) *m/e* 359.31559 calcd for $C_{22}H_{28}O_5Si$ $[M - CH_3CO + H]^+$, found 359.31555.

3.6.3. Synthesis of (-)-Gummiferol

3.6.3.1. Synthesis of the Diepoxy Amide **107**



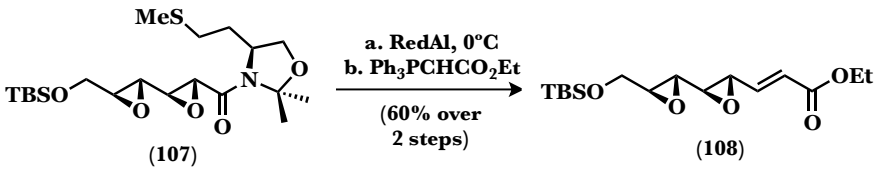
Epoxy alcohol **106** (340 mg, 1.56 mmol 1.0 equiv) was dissolved in a CH_2Cl_2/DMSO (1:1) mixture (6 mL) and cooled at 0ºC. At this temperature, Et_3N (0.7 mL, 4.7 mmol, 3.0 equiv) was added followed by SO_3·Pyr (450 mg, 2.8 mmol, 1.8 equiv). The mixture was allowed to reach rt and 5 hours later was quenched by addition of buffer pH = 7 and diluted with Et_2O. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure. The resulting crude of the aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **6** (530 mg, 1.7 mmol 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.31 mL, 1.53 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **107** (426 mg, 65% over 2 steps) as a pale yellow oil.

R_f = 0.2 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = - 19.2 (*c* 0.4, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 4.27 (ddd, $\mathcal{J}$ = 8.8, 4.7,

3.5 Hz, 1H, CONC*H)*, 3.97 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.3 Hz, 1H, OCH_2CH), 3.89-3.86 (m, 1H, OCH_2CH), 3.85 (d, $\mathcal{J}$ = 2.0 Hz, 1H, SiOCH_2), 3.69 (dd, $\mathcal{J}$ = 12.3, 3.7 Hz, 1H, CH_2CH(O)CH), 3.54 (d, $\mathcal{J}$ = 2.0 Hz, 1H, CH(O)C*H*CO), 3.30 (dd, $\mathcal{J}$ = 3.4, 2.0 Hz, 1H, C*H*(O)CHCO), 3.11 – 3.06 (m, 2H, SiOCH_2 and CH_2CH(O)C*H*), 2.59-2.50 (m, 1H, SCH_2CH_2), 2.48-2.39 (m, 1H, SCH_2CH_2), 2.08 (s, 3H, CH_3S), 2.05-2.00 (m, 1H, SCH_2CH_2), 1.83-1.73 (m, 1H, SCH_2CH_2), 1.59 (s, 3H, CH_3C), 1.48 (s, 3H, CH_3C), 0.84 (s, 9H, (CH_3)$_3$CSi), 0.02 (s, 3H, (CH_3)$_2$Si), 0.00 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.92, 95.87, 67.03, 61.59, 56.29, 55.96, 55.50, 51.73, 51.25, 34.42, 30.64, 26.19, 25.80, 22.93, 18.28, 15.73, -5.39, -5.41.

3.6.3.2. Synthesis of the γ,δ-Diepoxy α,β-Unsaturated Ester **108**



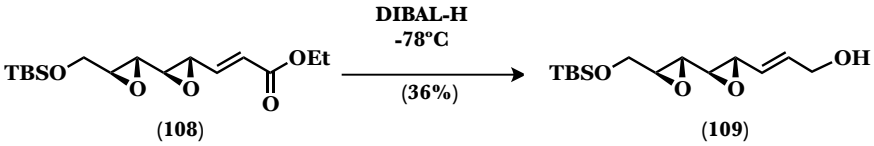
Diepoxy amide **107** (213 mg, 0.5 mmol, 1.0 equiv) in dry THF (8 mL) was treated dropwise with Red-Al 60% w/v in toluene (0.31 mL, 1.08 mmol, 2.2 equiv) at 0ºC. After 1 hour at this temperature, the reaction mixture was diluted with a saturated aqueous NH_4Cl solution. The aqueous phase was then separated, extracted twiced with EtOAc and the combined organic phase washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The resulting crude aldehyde was used in the next step without further purification.

The crude aldehyde was dissolved in CH_2Cl_2 (10 mL) and over this solution was added the phophorous ylide $Ph_3P{=}CHCO_2Et$ (260 mg, 0.75 mmol 1.5 equiv). After 30 minutes the reaction is over and the solvent was removed under reduced pressure. Flash column chromatography

(silica gel, 20% EtOAc in hexanes) gave the α,β-unsaturated diepoxy ester **108** (100 mg, 60% yield over 2 steps) as a pale yellow oil.

R_f = 0.64 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = - 12.4 (*c* 0.7, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.64 (dd, $\mathcal{J}$ = 15.7, 7.2 Hz, 1H, C*H*=CHCO), 6.15 (dd, $\mathcal{J}$ = 15.7, 0.7 Hz, 1H, CH=C*H*CO), 4.19 (q, $\mathcal{J}$ = 7.1 Hz, 2H, CH_2CH$_3$), 3.87 (dd, $\mathcal{J}$ = 12.3, 2.8 Hz, 1H, SiOCH_2), 3.74 (dd, $\mathcal{J}$ = 12.3, 3.9 Hz, 1H, SiOCH_2), 3.47 (ddd, $\mathcal{J}$ = 7.2, 2.0, 0.7 Hz, 1H, CH(O)C*H*CH=), 3.10 (ddd, $\mathcal{J}$ = 3.9, 2.7, 2.1 Hz, 1H, CH$_2$C*H*(O)), 3.04 (dd, $\mathcal{J}$ = 4.0, 2.1 Hz, 1H, CH$_2$CH(O)C*H*), 3.01 (dd, $\mathcal{J}$ = 4.0, 2.0 Hz, 1H, C*H*(O)CHCH=), 1.28 (t, $\mathcal{J}$ = 7.1 Hz, 3H, CH$_2$CH_3), 0.88 (s, 9H, (CH_3)$_3$CSi), 0.06 (s, 3H, (CH_3)$_2$Si), 0.05 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.41, 143.02, 124.71, 61.82, 60.69, 58.50, 56.24, 53.77, 52.62, 25.82, 18.32, 14.18, -5.37, -5.39.

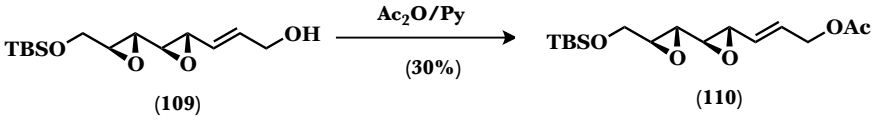
3.6.3.3. Synthesis of the Allylic Alcohol **109**



At -78ºC, DIBAL-H 1.0 M in toluene (0.53 mL, 0.53 mmol, 2.5 equiv) was added over a solution of ester **108** (70 mg, 0.21 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL). After 30 minutes, the reaction was complete and it was allowed to warm at 0ºC. At this temperature, the mixture was diluted with EtOAc and a saturated aqueous Na^+/K^+ tartrate solution. After 2 hours of vigorous stirring, the aqueous phase was extracted twice with EtOAc and the organic layer washed with water and brine, then dried over anhydrous $MgSO_4$ and filtered. The solvent was then removed carefully under reduced pressured at low temperature. Purification by flash column chromatography (silica gel, 20% EtOAc in hexanes) rendered the allylic alcohol **109** (22 mg, 36% yield) as a pale yellow oil.

R_f = 0.35 [Silica-gel, 30% EtOAc in hexanes]. $[\alpha]^{25}_D$ = - 20.2 (*c* 0.4, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.11 (dt, $\mathcal{J}$ = 15.6, 5.1 Hz, 1H, CH=C*H*CH_2), 5.49 (ddt, $\mathcal{J}$ = 15.6, 7.9, 1.7 Hz, 1H, C*H*=CHCH_2), 4.19 (d, $\mathcal{J}$ = 3.9 Hz, 2H, =CHC*H*$_2$OH), 3.88 (dd, $\mathcal{J}$ = 12.2, 2.8 Hz, 1H, SiOC*H*$_2$), 3.74 (dd, $\mathcal{J}$ = 12.2, 4.0 Hz, 1H, SiOC*H*$_2$), 3.40 (dd, $\mathcal{J}$ = 7.9, 2.1 Hz, 1H, (O)C*H*CH=), 3.09 (ddd, $\mathcal{J}$ = 4.0, 2.8, 2.1 Hz, 1H, CH_2C*H*(O)CH), 3.00 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, CH_2CH(O)C*H*), 2.93 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, C*H*(O)CHCH=), 1.61 (bs, 1H, O*H*), 0.89 (s, 9H, (C*H*$_3$)$_3$CSi)), 0.06 (s, 3H, (C*H*$_3$)$_2$Si), 0.05 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 135.29, 127.24, 62.57, 62.05, 58.00, 56.05, 55.25, 53.25, 25.84, 18.34, -5.35, -5.36.

3.6.3.4. Synthesis of the Acetyl Derivative **110**



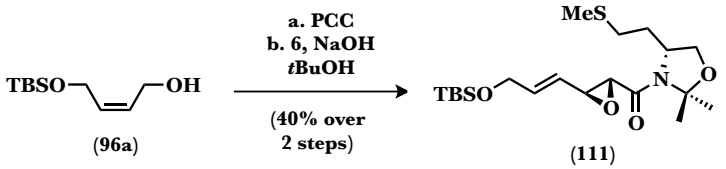
(109) **(110)**

Over a solution of the allylic alcohol **109** (43 mg, 0.15 mmol, 1. equiv) in pyridine (4 mL) was added Ac_2O (0.6 mL, 6.0 mmol, 40 equiv) at rt. After 12 h at the same temperature, the solvent was removed under vacuum and the crude product was purified by flash column chromatography (silica gel, 10 % EtOAc en hexanes) to furnish acetate **110** (15 mg, 30% yield) as a pale yellow oil.

R_f = 0.91 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -15.3 (*c* 0.4, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.03 (dtd, $\mathcal{J}$ = 15.6, 5.8, 0.6 Hz, 1H, CH=C*H*CH_2), 5.51 (ddt, $\mathcal{J}$ = 15.6, 7.8, 1.5 Hz, 1H, C*H*=CHCH_2), 4.58 (dd, $\mathcal{J}$ = 5.8, 1.5 Hz, 2H, =CHC*H*$_2$OH), 3.92 - 3.83 (m, 1H, SiOC*H*$_2$), 3.74 (dd, $\mathcal{J}$ = 12.2, 4.0 Hz, 1H, SiOC*H*$_2$), 3.38 (dd, $\mathcal{J}$ = 8.0, 1.9 Hz, 1H, CH(O)C*H*CH=), 3.09 (ddd, $\mathcal{J}$ = 4.0, 2.8, 2.1 Hz, 1H, CH_2C*H*(O)CH), 2.99 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, CH_2CH(O)C*H*), 2.93 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, C*H*(O)CHCH=), 2.17 (s, 3H, OCOC*H*$_3$), 0.89 (s,

9H, (CH_3)$_3$CSi)), 0.07 (s, 3H, (CH_3)$_2$Si), 0.06 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 130.19, 129.83, 63.61, 61.98, 57.97, 56.07, 54.93, 53.10, 30.91, 25.84, 20,93 18.33, -5.36. -5.37.

3.6.3.5. Synthesis of the Epoxy Amide **111**



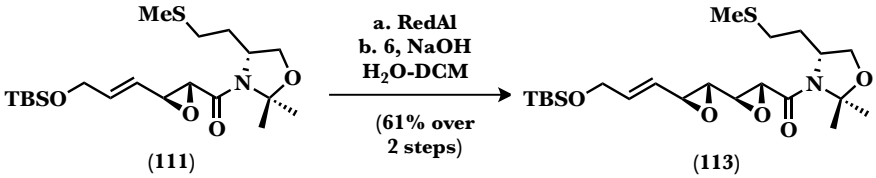
Over a suspension of PCC (1.6 g, 7.4 mmol, 1.5 equiv) in CH_2Cl_2 (20 mL) was added NaOAc anhydrous (1.6 g, 19.8 mmol, 4.0 equiv) followed by *cis*-alcohol **96a** dissolved in CH_2Cl_2 (1.0 g, 4.94 mmol, 1.0 equiv). The reaction mixture was stirred at rt for 6 h and the resulting crude was filtered off through a pad of SiO_2 and washed with Et_2O. The solvent was then removed under vacuum and the crude aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **6** (1.3 g, 4.0 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 1.2 mL, 3.6 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of epoxy amides to yield epoxy amide **111** (800 mg, 40% over two steps) as a pale yellow oil.

R_f = 0.43 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -11.1 (*c* 0.8, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.09 (ddd, $\mathcal{J}$ = 15.4, 4.4, 4.0 Hz, 1H, CH_2CH=CH), 5.48 (ddt, $\mathcal{J}$ = 15.4, 8.1, 2.0 Hz, 1H, CH_2CH=CH), 4.27 (ddd, $\mathcal{J}$ = 10.2, 4.9, 3.1 Hz, 1H, CONCH), 4.17 (dt, $\mathcal{J}$ = 3.7, 1.7 Hz, 2H, SiOCH_2), 3.99 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.4 Hz, 1H, OCH_2CH), 3.89 – 3.84 (m, 1H, OCH_2CH), 3.58 (dd, $\mathcal{J}$ = 8.1, 1.7 Hz, 1H, =CHCH(O)CH), 3.51 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)CHCO), 2.53 (ddd, $\mathcal{J}$ = 13.2, 7.0, 5.1 Hz, 1H, SCH_2CH$_2$), 2.40 (ddd, $\mathcal{J}$ = 13.4, 8.9, 6.6 Hz, 1H,

SC*H*$_2$CH$_2$), 2.05 (s, 3H, C*H*$_3$S), 2.03-1.96 (m, 1H, SCH$_2$C*H*$_2$), 1.78-1.71 (m, 1H, SCH$_2$C*H*$_2$), 1.61 (s, 3H, CH$_3$C), 1.51 (s, 3H, CH$_3$C), 0.87 (s, 9H, (CH$_3$)$_3$CSi), 0.03 (s, 6H, (CH$_3$)$_2$Si). ^{13}C NMR (100 MHz, CDCl$_3$), δ (ppm): 163.42, 137.15, 124.45, 95.89, 67.01, 62.51, 57.82, 55.85, 55.44, 34.27, 30.79, 26.27, 25.88, 22.99, 18.32, 15.81, -5.37, -5.38.

3.6.3.6. Synthesis of the Diepoxy amide **113**



(111) **(113)**

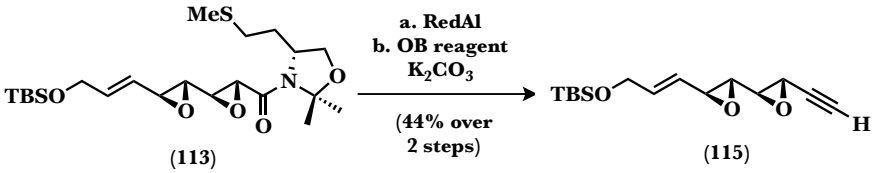
To a solution of epoxy amide **111** (112 mg, 0.27 mmol, 1.0 equiv) in dry THF (6 mL) was added dropwise Red-Al 60% w/v (0.15 mL, 0.54 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **6** (100 mg, 0.30 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.06 mL, 0.30 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **113** (76 mg, 61% over two steps) as a pale yellow oil.

R_f = 0.26 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -21.4 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.04 (dt, J = 15.4, 4.3 Hz, 1H, CH$_2$C*H*=CH), 5.50-5.40 (m, 1H, CH$_2$CH=C*H*), 4.34-4.27 (m, 1H, CONC*H*), 4.18 (dd, J = 4.2, 1.9 Hz, 2H, SiOC*H*$_2$), 4.01 (dd, J = 8.7, 5.7 Hz, 1H, OC*H*$_2$CH), 3.90 (d, J = 9.2 Hz, 1H, OC*H*$_2$CH), 3.58 (d, J =

1.9 Hz, 1H, CH(O)C*H*CO, 3.43 (dd, $\mathcal{J}$ = 8.0, 1.9 Hz, 1H, =CHC*H*(O)CH), 3.36 (dd, $\mathcal{J}$ = 3.3, 1.9 Hz, 1H, C*H*(O)CHCO), 3.05-3.02 (m, 1H, =CHCH*(*O)C*H*), 2.58 (ddd, $\mathcal{J}$ = 12.9, 7.7, 5.2 Hz, 1H, SC*H*$_2$CH$_2$), 2.52 – 2.41 (m, 1H, SC*H*$_2$CH$_2$), 2.11 (s, 3H, C*H*$_3$S), 2.05 (ddd, $\mathcal{J}$ = 20.8, 9.3, 5.1 Hz, 1H, SCH$_2$C*H*$_2$), 1.81 - 1.70 (m, 1H, SCH$_2$C*H*$_2$), 1.63 (s, 3H, (C*H*$_3$)$_2$C), 1.52 (s, 3H, (C*H*$_3$)$_2$C), 0.89 (s, 9H, (CH$_3$)$_3$CSi), 0.05 (s, 6H, (CH$_3$)$_2$Si.

3.6.3.7. Synthesis of the Alkyne **115**



(113) (115)

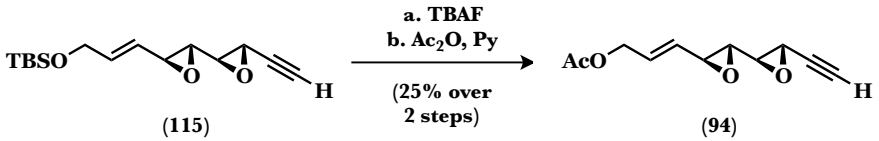
To a solution of epoxy amide **113** (76 mg, 0.17 mmol, 1.0 equiv) in dry THF (5 mL) was added dropwise Red-Al 60% w/v (0.1 mL, 0.37 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

The crude of the aldehyde was dissolved in dry MeOH (5 mL) and over this solution was added K_2CO_3 (50 mg, 0.33 mmol, 2.0 equiv) at rt followed by a solution of the Ohira-Bestmann reagent [(dimethyl(diazomethyl)phosphonate, $CH_3COC(N_2)PO(OMe)_2$)] (70 mg, 0.33 mmol, 2.0 equiv) in MeOH (2 mL). The progress of the reaction was monitored by TLC. After completion, the reaction crude was diluted with H_2O and Et_2O. The aqueous phase was extracted with Et_2O, dried over $MgSO_4$ and the solved removed under reduced pressure. Purification by

flash column chromatography (silica gel, 20% EtOAc en hexanes) rendered the alkyne **115** (20 mg, 44 % over 2 steps) as a pale yellow oil.

R_f = 0.8 [Silica-gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -38.0 (*c* 0.2, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.05 (dtd, $\mathcal{J}$ = 15.4, 4.3, 0.6 Hz, 1H, CH_2CH=CH), 5.45 (ddt, $\mathcal{J}$ = 15.4, 8.0, 1.9 Hz, 1H, CH_2CH=C*H*), 4.20 (dd, $\mathcal{J}$ = 4.2, 1.8 Hz, 2H, $SiOCH_2$), 3.40 (dd, $\mathcal{J}$ = 8.0, 1.6 Hz, 1H, =CHC*H*(O)CH), 3.36 (dt, $\mathcal{J}$ = 3.3, 1.6 Hz, 1H, CH(O)C*H*CCH), 3.26 (dd, $\mathcal{J}$ = 3.5, 2.1 Hz, 1H, C*H*(O)CHCCH), 2.97 (dd, $\mathcal{J}$ = 3.5, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.34 (d, $\mathcal{J}$ = 1.7 Hz, 1H, CC*H*), 0.91 (s, 9H, $(CH_3)_3CSi$), 0.07 (s, 6H, $(CH_3)_2Si$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 136.24, 125.14, 79.29, 72.30, 62.66, 57.25, 56.61, 55.52, 42.59, 25.90, 18.20, -5.31, -5.33.

3.6.3.8. Synthesis of the Diepoxy Alkyne **94**



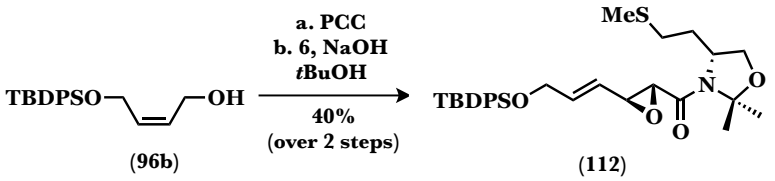
Alkyne **115** (20 mg, 0.07 mmol, 1.0 equiv) was dissolved in THF (5 mL) and over this solution was added 1.0 M TBAF in THF (0.11 mL, 0.11 mmol, 1.5 equiv) at rt. After 45 min, the reaction mixture was diluted with Et_2O and NH_4Cl. The aqueous phase was then extracted with EtOAc and the organic extract washed with H_2O, brine and dried over $MgSO_4$. The solvent was removed under vacuum and the crude alcohol was used in the next step without further purification.

Over a solution of the allylic alcohol in pyridine (2 mL) was added Ac_2O (0.2 mL, 2.0 mmol, 40 equiv) at rt. After 12 h at the same temperature, the solvent was removed under vacuum and the crude product was purified by flash column chromatography (silica gel, 10 %

EtOAc in hexanes) to furnish acetate **94** (4 mg, 25% yield over 2 steps) as a pale yellow oil.

R_f = 0.52 [Silica gel, 20% EtOAc in hexanes]. $[\alpha]^{25}_D$ = - 45.3 (c 0.5, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.04 (dtd, $\mathcal{J}$ = 15.6, 5.8, 0.7 Hz, 1H, CH_2CH=CH), 5.59 - 5.42 (m, CH_2CH=C*H*), 4.61 (dd, $\mathcal{J}$ = 5.8, 1.6 Hz, 2H, OCH_2), 3.42 - 3.33 (m, 2H, CH=CHC*H*(O)CH and CH(O)C*H*C), 3.27 (dd, $\mathcal{J}$ = 3.4, 2.0 Hz, 1H, C*H*(O)CHC), 2.99 (dd, $\mathcal{J}$ = 3.4, 2.0 Hz, 1H, =CHCH(O)C*H*), 2.34 (d, $\mathcal{J}$ = 1.7 Hz, 1H, CC*H*), 2.13 - 2.03 (s, 3H, CH_3CO).

3.6.3.9. Synthesis of the Diepoxy Amide **112**

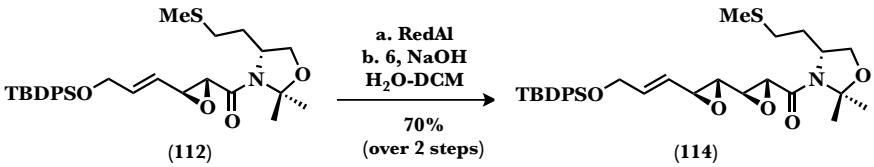


Over a suspension of PCC (1.6 g, 7.4 mmol, 1.5 equiv) in CH_2Cl_2 (20 mL) was added NaOAc anhydrous (1.6 g, 20.0 mmol, 4.0 equiv) followed by *cis*-allylic alcohol **96b** (0.9 mL, 5.0 mmol, 1.0 equiv) dissolved in CH_2Cl_2 (10 mL). The reaction mixture was stirred at rt for 6 h and the resulting crude was filtered off through a pad of SiO_2 and washed with Et_2O. The solvent was then removed under vacuum and the crude aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **6** (1.7 g, 5.4 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 1.7 mL, 5.0 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of epoxy amides to yield epoxy amide **112** (1.0 g, 40% over two steps) as a pale yellow oil.

R_f = 0.6 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -9.4 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.67 – 7.64 (m, 4H, C*H* aromatic), 7.44 – 7.36 (m, 6H, C*H* aromatic), 6.12 (dt, $\mathcal{J}$ = 15.4, 4.0 Hz, 1H, CH_2CH=CH), 5.65 (ddt, $\mathcal{J}$ = 15.4, 8.1, 1.9 Hz, 1H, CH_2CH=C*H*), 4.31 (dt, $\mathcal{J}$ = 8.2, 5.0 Hz, 1H, CONC*H*), 4.23 (dt, $\mathcal{J}$ = 3.6, 1.7 Hz, 2H, $SiOCH_2$), 4.03 (ddd, $\mathcal{J}$ = 9.1, 5.2, 1.3 Hz, 1H, OCH_2CH), 3.90 (d, $\mathcal{J}$ = 9.2 Hz, 1H, OCH_2CH) 3.63 (dd, $\mathcal{J}$ = 8.1, 1.8 Hz, 1H, =CHC*H*(O)CH), 3.56 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)C*H*CO), 2.61 – 2.51 (m, 1H, SCH_2CH_2), 2.43 (ddd, $\mathcal{J}$ = 13.4, 9.0, 6.6 Hz, 1H, SCH_2CH_2), 2.14 – 2.07 (m, 1H, SCH_2CH_2), 2.04 (s, 3H, CH_3S), 1.84 – 1.70 (m, 1H, SCH_2CH_2), 1.66 (s, 3H, $(CH_3)_2C$), 1.56 (s, 3H, $(CH_3)_2C$), 1.06 (s, 9H, $(CH_3)_3Si$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 163.46, 136.74, 135.48, 133.32, 133.23, 129.79, 127.76, 124.52, 95.95, 67.07, 63.29, 60.37, 57.89, 55.87, 55.57, 34.33, 30.84, 26.82, 26.31, 23.04, 19.25, 15.83.

3.6.3.10. Synthesis of the Diepoxy Amide **114**



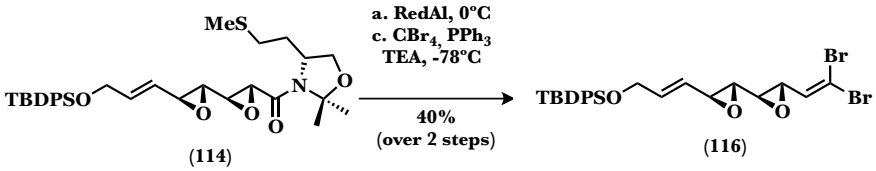
To a solution of epoxy amide **112** (250 mg, 0.46 mmol, 1.0 equiv) in dry THF (10 mL) was added dropwise Red-Al 60% w/v (0.3 mL, 1.0 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **6** (160 mg, 0.51 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.1 mL, 0.46

mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **114** (187 mg, 70% over two steps) as a pale yellow oil.

R_f = 0.40 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = -23.0 (*c* 1.0, CH_2Cl_2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.68 – 7.64 (m, 4H, C*H* aromatic), 7.44 – 7.36 (m, 6H, C*H* aromatic), 6.05 (ddd, $\mathcal{J}$ = 15.4, 4.5, 3.9 Hz, 1H, CH_2CH=CH), 5.56 (ddt, $\mathcal{J}$ = 15.4, 7.9, 1.9 Hz, 1H, CH_2CH=C*H*), 4.36 – 4.29 (m, 1H, CONC*H*), 4.23 (dd, $\mathcal{J}$ = 4.2, 1.9 Hz, 2H, SiOCH_2), 4.06 – 4.00 (ddd, $\mathcal{J}$ = 9.2, 5.3, 1.4 Hz, 1H, OCH_2CH), 3.92 (dd, $\mathcal{J}$ = 9.2, 0.8 Hz, 1H, OCH_2CH), 3.60 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH(O)C*H*CO), 3.44 (dd, $\mathcal{J}$ = 7.9, 1.9 Hz, 1H, =CHC*H*(O)CH), 3.40 (dd, $\mathcal{J}$ = 3.3, 2.0 Hz, 1H, C*H*(O)CHCO), 3.04 (dd, $\mathcal{J}$ = 3.3, 2.1 Hz, 1H, =CHC*H(*O)C*H*), 2.65 – 2.55 (m, 1H, SCH_2CH_2), 2.49 (dt, $\mathcal{J}$ = 12.4, 6.6 Hz, 1H, SCH_2CH_2), 2.12 (s, 3H, CH_3S), 2.11 – 2.05 (m, 1H, SCH_2CH_2), 1.90 – 1.76 (m,1H, SCH_2CH_2), 1.66 (s, 3H, $(CH_3)_2$C), 1.54 (s, 3H, $(CH_3)_2$C), 1.09 – 1.03 (s, 9H, $(CH_3)_3$CSi). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.94**,** 135.79, 135.50, 133.39, 129.77, 127.74, 125.15, 95.98, 67.08, 63.39, 56.61, 56.01, 55.74, 55.40, 51.31, 34.49, 30.74, 26.82, 26.24, 22.98, 19.24, 15.86.

3.6.3.11. Synthesis of the Dibromo Alkene **116**



(114) **(116)**

To a solution of diepoxy amide **114** (100 mg, 0.17 mmol, 1.0 equiv) in dry THF (10 mL) was added dropwise Red-Al 60% w/v (0.12 mL, 0.38 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc,

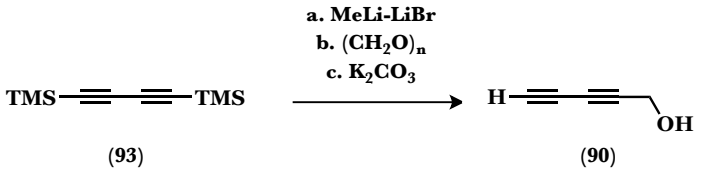
the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

To a solution of CBr_4 (115 mg, 0.34 mmol, 2.0 equiv) in CH_2Cl_2 (10 mL) was added Ph_3P (90 mg, 0.34 mmol) at 0ºC. The mixture was stirred at the same temperature for 15 min, and Et_3N (0.02 mL, 0.17 mmol, 1.0 equiv) was added to the resulting mixture at 0ºC. After the mixture was stirred at the same temperature for 5 min, the aldehyde obtained above, dissolved in CH_2Cl_2 (5 mL), was added at -78ºC. The mixture was stirred at the same temperature for 2 h. After this time, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over $MgSO_4$. Concentration and purification by flash column chromatography (silica gel, 10% EtOAc in hexanes) gave the corresponding dibromoalkene **116** (40 mg, 40% yield) .

R_f = 0.7 [Silica gel, 10% EtOAc in hexanes]. $[\alpha]^{25}D$ = -35.1 (c 0.7, CH_2Cl_2). ^1H RMN (400 MHz, $CDCl_3$), δ (ppm): 7.68 – 7.64 (m, 2H), 7.44 – 7.36 (m, 8H), 6.16 (d, $\mathcal{J}$ = 7.8 Hz, 1H, C*H*=CBr_2), 6.07 – 6.00 (m, 1H, CH_2C*H*=), 5.55 (ddt, $\mathcal{J}$ = 15.4, 7.8, 1.9 Hz, 1H, CH_2CH=C*H*), 4.23 (dd, $\mathcal{J}$ = 4.2, 1.9 Hz, 2H, C*H*$_2$CH=), 3.64 (dd, $\mathcal{J}$ = 7.8, 2.0 Hz, 1H, (O)C*H*CH=CBr_2), 3.40 (dd, $\mathcal{J}$ = 7.9, 2.0 Hz, 1H, CH=CHC*H*(O)CH)), 3.07 (dd, $\mathcal{J}$ = 3.9, 2.0 Hz, 1H, C*H*(O)CHCH=CBr_2), 2.97 (dd, $\mathcal{J}$ = 3.9, 2.1 Hz, 1H, CH=CHCH(O)C*H*), 1.06 (s, 9H, $(CH_3)_3$CSi). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 135.63, 135.57, 135.51, 134.80, 134.47, 129.77, 127.74, 125.24, 63.41, 57.18, 56.68, 55.61, 54.99, 26.84, 19.27.

3.6.4. Synthesis of the Dialkyne Fragment

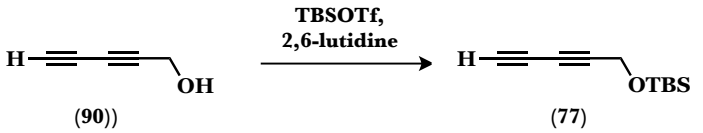
3.6.4.1. Synthesis of the Dialkynyl Alcohol **90**



Over a solution of 1,4-bis(trimethylsilyl)butadiyne (**93**) (500 mg, 2.6 mmol, 1.0 equiv) in Et_2O (10 mL) was added MeLi·LiBr at rt. The mixture was then stirred at the same temperature for 8 h. Subsequently, paraformaldehyde $(CH_2O)n$ (78 mg, 2.6 mmol, 1.0 equiv) in Et_2O (10 mL) was then added at 0ºC, and the reaction allowed to reach rt and then stirred over 8 h. The reaction mixture was then diluted with EtOAc and quenched with a saturated solution of NH_4Cl. The organic phase was dried over $MgSO_4$ and the solvent removed under vacuum. The corresponding alcohol was used in the next step without further purification.

The resulting alcohol was dissolved in MeOH (10 mL) and over this solution solid K_2CO_3 (180 mg, 1.3 mmol, 1.2 equiv) was added, at rt. The reaction mixture was stirred over 12 h, and then quenched with NH_4Cl. Extractions with Et_2O and filtration through SiO_2 provided the corresponding dialkynol **90** that was used in the next step without any further purification.

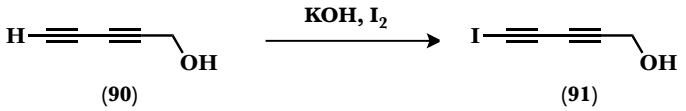
3.6.4.2. Synthesis of the Dialkyne **77**



Alcohol **90** (150 mg, 1.9 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (10 mL) and the mixture cooled at 0ºC. 2,6-lutidine (0.4 mL, 2.2

mmol, 2.0 equiv) was then added, followed by TBSOTf (0.4 mL, 3.8 mmol, 1.2 equiv). The reaction was monitored by TLC and after 3 h, no trace of the starting material was detected. The crude reaction was quenched by addition of H_2O and the aqueous phase extracted with EtOAc. The organic phase was then washed with brine, dried over $MgSO_4$ and the solvent removed under vacuum. The dialkyne **77** was used in the next step without further purification.

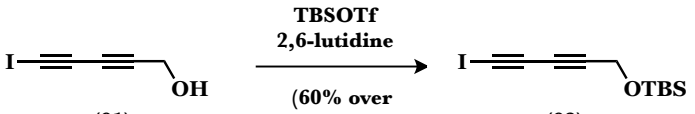
3.6.4.3. Synthesis of the Iodo Alcohol **91**



(90) **(91)**

For the synthesis of the iodo derivative, KOH (220 mg, 2.6 mmol, 2.5 equiv) was dissolved in H_2O (~ 0.5 mL) and cooled at 0ºC. Dialkynol **90** was dissolved in MeOH (5 mL) and this solution was added over dropwise. After 10 min stirring at the same temperature, I_2 (295 mg, 1.2 mmol, 1.1 equiv) was added portionwise and the reaction mixture allowed to reach rt. After 3 h, Et_2O and an aqueous solution of Na_2SO_3 were added and the aqueous phase extracted with Et_2O. The organic phase was then washed with brine and the solvent removed under vacuum. The iode alkyne was used in subsequent step withouth further purification.

3.6.4.4. Synthesis of the Silyl Ether **92**



(91) **(92)**

Alcohol **91** above obtained was dissolved in CH_2Cl_2 (10 mL) and the mixture cooled at 0ºC. 2,6-lutidine (0.25 mL, 2.1 mmol, 2.0 equiv) was then added, followed by TBSOTf (0.28 mL, 1.3 mmol, 1.2 equiv). The reaction was monitored by TLC and after 3 h, no trace of the starting material was detected. The crude reaction was quenched by

addition of H_2O and the aqueous phase extracted with EtOAc. The organic phase was then washed with brine, dried over $MgSO_4$ and the solvent removed under vacuum. Purification by flash column chromatography (silica gel, 10% EtOAc in hexanes) render **92** (230 mg, 68% overall yield) as a pale yellow oil.

Rf = 0.63 [Silica gel, 40% EtOAc in hexanes]. 1H RMN (400 MHz, CDCl3), δ (ppm): 4.41 (s, 2H, CH_2), 0.91 (s, 9H, (CH_3)$_3$CSi), 0.12 (s, 6H, (CH_3)$_2$Si). 13C RMN (100 MHz, CDCl3), δ (ppm): 77.96, 73.60, 70.41, 51.79, 25.75, 18.25, -5.19.

3.7. Notes and References

(1) Hedberg, I.; Hedberg, O.; Madati, P. J.; Mshigeni, K. E.; Mshiu, E. N.; Samuelsson, G. *J. Ethnopharmacol.* **1983**, *9*, 237-260.

(2) Comely, J. C. W. *Trop. Med. Parasitol.* **1980**, *41*, 1-9.

(3) Neame, P. B. Pillay, V. K. G. *S. Afr. Med. J.* **1964**, *38*, 729-732.

(4) Parish, C. A.; Huber, J.; Baxter, J.; González, A.; Collado, J.; Platas, G.; Diez, M. T.; Vicente, F.; Dorso, K.; Abruzzo, G.; Wilson, K. *J Nat Prod.* **2004**, *67*, 1900-1902.

(5) Senn, M.; Gunzenhauser, S.; Brun, R.; Séquin, U. *J Nat Prod.* **2007**, *70*, 1565-1569.

(6) Tian, Y.; Wei, X.; Xu, H. *J. Nat. Prod.* . **2006**, *69*, 1241-1244.

(7) Lerch, M. L.; harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **2003**, *66*, 667-670.

(8) Bernart, M. W.; Cardellina, J. H.; Balaschak, M. S.; Alexander, M. R.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Prod.* **1996**, *59*, 748-753.

(9) Ito, A.; Cui, B.; Chávez, D.; Chai, H. B.; Shin, Y. G.; Kawanishi, K.; Kardono, L. B. S.; Riswan, S.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2001**, *64*, 246-248.

(10) Parikh, J. R. Doering, W. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505-5507.

(11) Okamoto, C.; Nakao, Y.; Fujita, T.; Iwashita, T.; van Soest, R. W.; Fusetani, N.; Matsunaga, S. *J. Nat. Prod.* **2007**, *70*, 1816-1819.

(12) Fullas, F.; Brown, D. M.; Wani, M. C.; Wall, M. E. *J. Nat. Prod.* **1995**, *58*, 1625-1628.

(13) Bohlmann, F.; Burkhardt, T.; Zdero, C. *Phytochemistry* **1973**, *3*, 112-131.

(14) Fujimoto, Y.; Wang, H.; Kirisawa, M.; Satoh, M.; Takeuchi, N. *Phytochemistry* **1992**, *31*, 3499-3501.

(15) Takamura, H.; Wada, H.; Lu, N.; Kadota, I. *Org. Lett.* **2011**, *13*, 3644-3647.

(16) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092-4096.

(17) Siemsen, P.; Livingston, R. C.; Diederich, F. *Angew. Chem. Int. Ed.* **2000**, *39*, 2632-2657.

(18) Zhang, P. Morken, J. P. *J. Am. Chem. Soc.* **2009**, *131*, 12550-12551.

(19) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183-2186.

(20) Corey, E. J. Fuchs, P. L. *Tetrahedron Lett.* **1972**, *36,* 3769-3772.

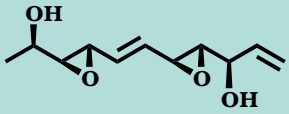
(21) González, I. C. Forsyth, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 9099-9108.

(22) Takamura, H.; Wada, H.; Lu, N.; Ohno, O.; Suenaga, K.; Kadota, I. *J. Org. Chem.* **2013**, *78*, 2443-2454.

(23) Sarabia, F.; Chammaa, S.; García-Castro, M.; Martín-Gálvez, F. *Chem. Commun.* **2009**, 5763-5765.

(24) Fiandanese, V.; Bottalico, D.; Marchese, G.; Punzi, A. *J. Organom. Chem.* **2005**, *690*, 3004-3008.

(25) Xu, R.; Gramlich, V.; Frauenrath, H. *J. Am. Chem. Soc.* **2006**, *128*, 5541-5547.



CHAPTER 4

Depudecin

The microbial metabolite depudecin is a linear polyketide characterized by a highly oxidized 11-carbon chain containing two epoxides conjugated through a trans-disubstituted olefin. Depudecin was first reported in 1991 by Matsumoto et al. and inhibits histone deacetylase activity effectively both in vivo and in vitro.

In 1991, Matsumoto and colleagues found during a screening work from microbial cultures for antitumor agents with detransforming activity, a new metabolite that exhibited cell differentiation-modulation activity.[1,2] The microbial metabolite, isolated from a soil sample collected in Okinawa (Japan) identified as the fungus *Alternaria brassicicola*, RF-328 exhibited an unprecedented structure containing two epoxide groups separated by a double bond which was determined and confirmed by the same author by X-ray diffraction analysis of the corresponding bis-(1S)-(-)-camphanate derivative.[2] This novel compound was also isolated from the weed pathogen *Nimbya scirpicola* by Tanaka et al., who demonstrated its phytotoxicity toward the host plant of the fungus, *Eleocharis kuroguwai*, and toward other tested plants.[3]

4.1. Biological Context

Cytoskeletal changes are often a consequence of malignant transformation. One of the most characteristic is the loss of actin stress fibers, which suggests that the actin-containing microfilament system is a critical target during tumorigenesis.[4]

Several natural products such as herbimycin A,[5] radicicol[6] or azatyrosine[7] have been reported to detransforming activity using oncogene-transformed cells (v-sis, v-src or v-ras oncogenes as well as other cells derived from tumours). Although it remains unclear how these compounds show a broad spectrum of detransforming activity in tumor cells, there is sufficient evidence suggesting that histone deacetylases (HDACs) are likely molecular targets of these agents.[8] The broad spectrum of detransforming activities displayed by HDAC inhibitors implies a role for HDACs in stress fiber formation and cell growth control, meaning that HDACs may repress the transcription of anti-tumor genes.[9]

In contrast to those previously identified inhibitor of histone deacetylase such as trapoxin (**117**)[10,11] and trichostatin A (**118**)[12] or largazole (**119**),[13] depudecin (**120**) possesses a different chemical structure that might increase its selectivity towards these biological targets. This fact makes depudecin a useful tool to better understand the enzymatic mechanisms of action of this class of deacetylases (**Figure 4.1**).[14]

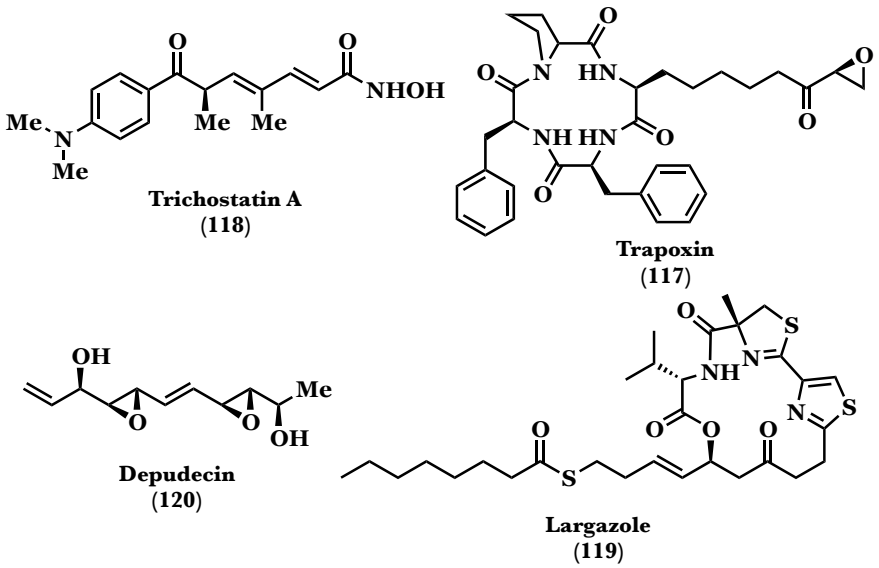


Figure 4.1. *Chemical structure of depudecin and other HDACs inhibitors*

Although depudecin can reverse the phenotype transformation of ras-oncogene which has been reported to be expressed in many tumours and tumour cell lines,[15] it does not suppress the expression of the *ras* gene, suggesting that its mode of action does not relate directly to Ras function. It also shows a spectrum of detransforming activity, both in v-*raf*-transformed cells and in a human osteosarcoma cell line, MG63. This molecule induces production of the flat phenotype of ras-NIH3T3 cells at a very low concentration (1.0 μM), revealing that depudecin should therefore be involved in the signal transduction after the signal of raf-oncogene.[16]

In 1995, Oikawa et al. described its anti-angiogenic activity *in vivo* involving an inhibitory effect on vascular endothelial cell growth,[17] with and ID_{50} of 320 ng per egg and a maxima inhibitory activity of 10 μg per egg, in the *in vivo* assay system involving chick embryo CAM. Likewise, it inhibited the proliferation of HUVECs in a concentration-dependent manner with an IC_{50} of 13 μM, suggesting that the inhibitory effect on

vascular endothelial cell growth by depudecin contributes its anti-angionic action.

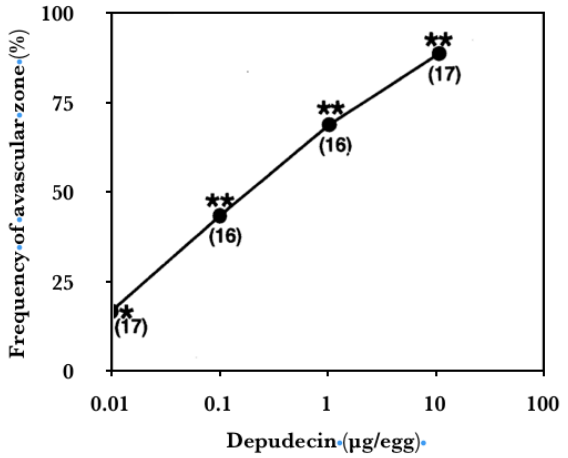


Figure 4.2. *Inhibitory effect of depudecin on embryonic angiogenesis*[17]

These results indicate depudecin might be a promising anti-angiogenic and would suggest that an epoxide group could be important in the search for a new angiogenesis inhibitor.[17]

4.1.1. Biosynthesis of Depudecin

Nabeta and co-workers in their interest to understand the biosynthesis of depudecin, studied the diferential hydrogen exchange occurred in the enzymatic fatty acid elongation process reported previously by Saito et al.[18] and suggested that although the starter acetate unit came from acetyl CoA, the biosynthesis of depudecin may imply another mechanism apart of decarboxylative condensation between polyacetoacetyl CoA and malonyl CoA, with subsequent decarboxylation of the end carbonyl group and that still remained unknown.[19,20]

In 2005, Amnuaykanjanasin and co-workers extracted depudecin from the fungus *Xylaria* sp. BCC 1067 together with the polyketide 19-20-

epoxycytochalasin Q, and tried to reveal the PKS (polyketide synthases) genes responsible for their biosynthesis.[21]

But it was not until the year 2009 when Walton et al. identified the genes for depudecin biosynthesis and created depudecin-minus mutants in order to test whether depudecin was a virulence factor for *A. Brassicicola* as happened to a chemically unrelated HDAC inhibor, HC toxin, that exhibits a major virulence factor in the interaction between *Cochliobolus carbonum*and and its host.[22] They reported the six-genes cluster of depudecin (DEP1 to DEP6), which are predicted to encode a polyketide synthase (AbPKS9 or DEP5), a transcription factor (DEP6), two monooxygenases (DEP2 and DEP4), a transporter of the major facilitator superfamily (DEP3), and one protein of unknown function (DEP1).[22] With the exception of DEP1, the genes of the depudecin cluster have plausible roles in depudecin biosynthesis based in the knowledge of similar genes in other secondary metabolite pathways.[22]

4.1.2. Histone Deacetylase Inhibitors

Histones are small basic proteins rich in amino-acids, lysine and arginine, that form the nucleosome core by complexing with DNA. Repetitive units of this nucleosome leads to the chromatin, in which all the human genome is packaged.

There are two antagonist forms of histones, acetylated or deacetylated, and the enzymes that regulate this equilibrium are histone acetyl transferases (HTAs), producing the acetylation and histone deacetylases (HDACs) which reverse this process.[23]

Whereas acetylation of histone tails correlates with nucleosome remodeling and transcriptional activation, deacetylation induces transcriptional suppression through chromatin condensation.[24] Monneret

explains this fact due to the neutralization of the positive charge of lysine residues in the *N*-terminal tail during the acetylation process, that implies a relaxation in the histone-DNA contacts that facilitates the accessibility of a variety of factors to DNA.[23] Thought, deacetylation of histone tails causes chromatin condensation, electrostatic interactions of neighbouring nucleosomes and as a consequence, access of the transcriptional machinery to DNA target sequences is blocked.[25]

Therefore, inhibition of HDACs, potent inducers of growth arrest, differentiation and apoptosis of tumour cell lines, represents a novel strategy in human cancer therapy because of the key role these enzymes plays in regulation gene expression and chromatin assembly.[23]

4.1.3. Inhibition of Histone Deacetylases by Depudecin

Kwon et al. identified the molecular target(s) of depudecin and found evidence that this potential anti-tumor drug belongs to an expanding group of pharmaceuticals that inhibit histone deacetylases (HDAC): (I) depudecin is a competitive inhibitor of trapoxin, a known inhibitor of histone deacetylases, (ii) depudecin inhibits histone deacetylases *in vitro*, and (iii) treatment of cells with depudecin leads to histone hyperacetylation *in vivo*.[9]

Depudecin induces hyperacetylation of histones in a dose-dependent manner and inhibit 50% of the purified recombinant HDAC1 at a concentration of 47 μM.[9] This result taken as a whole, provides a plausible molecular basis for the actions of depudecin, and suggests, if somewhat more tentatively, that changes in gene expression lie behind the morphological reversion induced in transformed fibroblasts by depudecin.

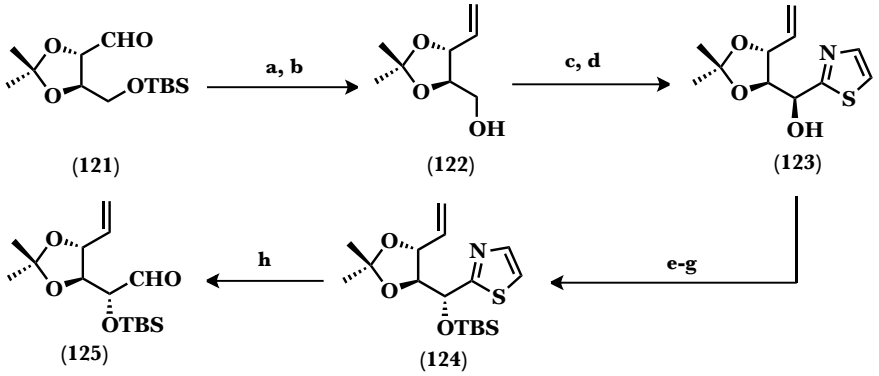
4.2. Previous Synthesis of Depudecin

The first synthesis of (-)-depudecin was reported by the group of Schreiber and co-workers in 1995 according to a strategy that also provides access to depudecin-related compounds.[26]

This synthetic strategy was based on an asymmetric methodology that uses a one-pot procedure developed by Sharpless and others for the stereoselective conversion of *syn*-vicinal diols into *trans*-epoxides.[27-30]

The synthesis of the key intermediate **125** started from a D-threose derivative **121**, which was easily transformed into the *anti*-alcohol **123** by reaction of the corresponding aldehyde with 2-trimethylsilylthiazole. Conversion of the anti-alcohol **123** into the *syn* diastereomer **124** was achieved through an oxidation-reduction sequence in a 90% overall yield. Subsequent cleavage of the thistle ring provided the aldehyde **125** that represented the left part of depudecin, in 88% yield (**Scheme 4.1**).

Scheme 4.1. *Synthesis of the aldehyde* ***125***



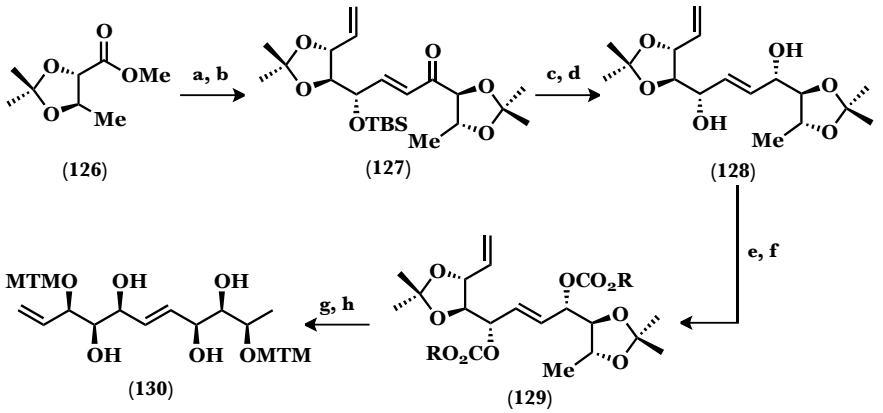
(121) (122) (123) (124) (125)

Reagents and conditions: (a) Ph_3PMeI, *t*BuOK, 23ºC, 91% (b) TBAF, THF, 23ºC, 99%. (c) Swern oxidation, quant. (d) 2-trimethylsilylthiazole, THF, 23ºC, then TBAF, 71%. (e) Swern oxidation, 76%. (f) $NaBH_4$, MeOH, -78ºC to -20ºC,

99%. (g) TBSCl, imidazole, DMF, 70ºC, 100%. (h) MeI, MeCN, reflux then CuO, $CuCl_2$, CH_3CN-H_2O, 88%.

Aldehyde **125** was then subjeted to condensation with the ketophosphonate derived from **126** affording enone **127** in 88% yield. From enone **127**, a diastereoselective reduction with sodium borohydride in the presence of cerium chloride, followed by deprotection of the silyl group and conversion of diol **128** into the tetraol, in a 4 steps sequence afforded the key intermediate **130** that contained the complete depudecin framework in a 60% overall yield. (**Scheme 4.2**)

Scheme 4.2. *Synthesis of the key intermediate tetraol* ***130***



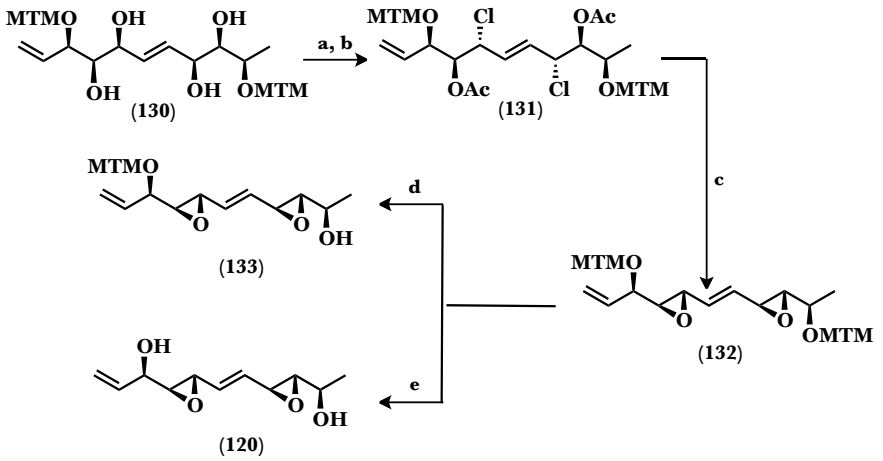
Reagents and conditions: (a) $LiCH_2P(O)(OMe)_2$, THF, 0ºC, 92%. (b) NaH, THF, 23ºC, then compound **125**, 23ºC, 88%. (c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, -78ºC to -20ºC, 96%. (d) TBAF, then separation of stereoisomers, 74%. (e) $ClCO_2CH_2CCl_3$, 2,6-lutidine, CH_2Cl_2, 23ºC, 99%. (f) HCl (anhyd.), MeOH, 23ºC, 6 h, 44%. (g) Me_2S, benzoyl peroxide (BPO), CH_3CN, 0ºC, 34%. (h) 1 M LiOH, THF, 23ºC, 93%.

The transformation of the tetraol **130** into the desired diepoxide was achieved through formation of the corresponding diacetoxy dichloride **131**, whose deprotection under excess of mercuric chloride in

the presence of calcium carbonate gave depudecin in 52% yield (**Scheme 4.3**).

This synthetic strategy proceeded in 22 steps with an overall yield of 1.4%. Although the construction of the two epoxide moieties took place in a simultaneuos fashion, this strategy presented the drawback of using an excess of mercuric chloride. Also the necessity of an oxidation-reduction sequence to obtain the *syn*-alcohol made the synthesis longer.

Scheme 4.3. *Synthesis of depudecin (**120**)*



Reagents and conditions: (a) $MeC(OMe)_3$, cat PPTS, 23ºC. (b) TMSCl, Et_3N, 23ºC. (c) K_2CO_3, MeOH, 23ºC, 74% (over 3 steps). (d) 10.0 equiv. $HgCl_2$-$CaCO_3$, CH_3CN-H_2O, 23ºC, 3.5 h, 73%. (e) 50.0 equiv. $HgCl_2$-$CaCO_3$, CH_3CN-H_2O, 23ºC, 3.5 h, 52%.

The same author analysed the biological activity of the related derivatives, mono-methylthiomethyl-protected depudecin **133** and the bis-methylthiomethyl-protected depudecin **132** and concluded they were both inactive at the same concentration, suggesting that the epoxide and hydroxyl groups are essential for the detransforming activity of depudecin.

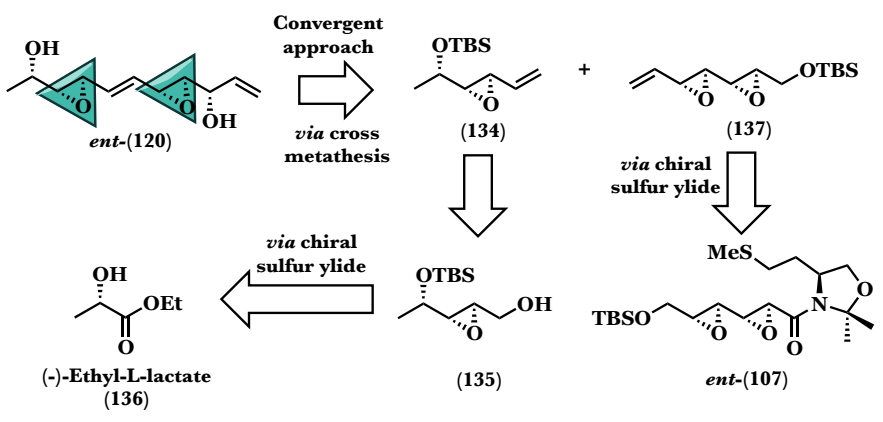
4.3. Synthesis of Depudecin

The biological profile exhibited by depudecin indicates that it might be a promising anti-angiogenic target and would suggest that an epoxide group could be in the search for new angiogenesis inhibitor.[22] Under this premise, we planned the total synthesis of the enantiomer of (-)-depudecin, that still remains unknown and might present a similar cytotoxicity, employing once again the novel methodology of asymmetric epoxidation based on the use of chiral sulfur ylides.

4.3.1. Retrosynthetic analysis of (+)-Depudecin

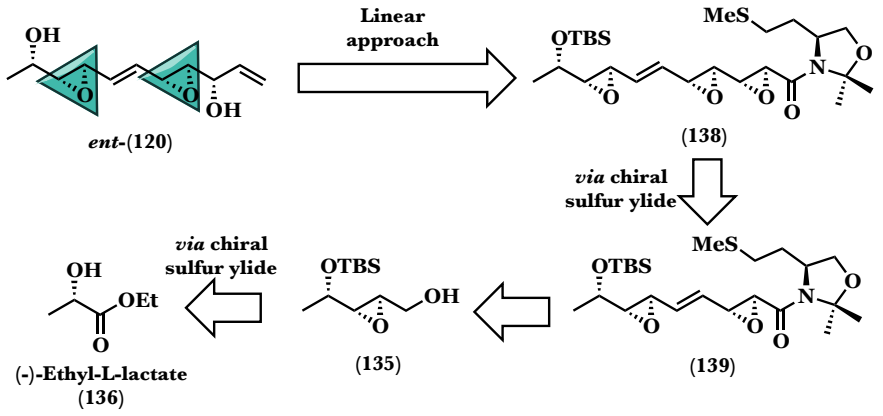
Two retrosynthetic strategies for the synthesis of the (+) enantiomer of depudecin are presented in **Scheme 4.4-A** and **4.4-B**. Both routes relied on the same epoxy alcohol, which was readily available from the starting material (-)-ethyl-L-lactate, that provides the only stereocenter not generated through this approach. In a first attempt, we planned a convergent synthesis of depudecin through the linkage of two functionalized epoxide units *via* a cross metathesis of alkenes **134** and **137** under the influence of the Hoveyda-Grubbs catalyst.

Scheme 4.4-A. *Retrosynthetic analysis of (+)-depudecin. Convergent approach*



We planned the synthesis of key fragment **137** from *ent*-**107**, which was previously obtained during the synthesis of gummiferol. Aditionally, epoxy alcohol **135** could be easily synthesized from readily commercial starting material such as (-)-ethyl L-lactate (**136**). Unfortunately, the low yields obtained by this way led us to design a second approach. Thus, the second strategy consists of a linear sequence with the epoxy amide **139** as key intermediate.

Scheme 4.4-B. *Retrosynthetic analysis of (+)-depudecin. Linear Approach*



It should be highlighted that, in both cases, the epoxides through the skeleton of depudecin would be synthesized by using the chiral sulfur ylides obtained from sulfonium salt **4**.

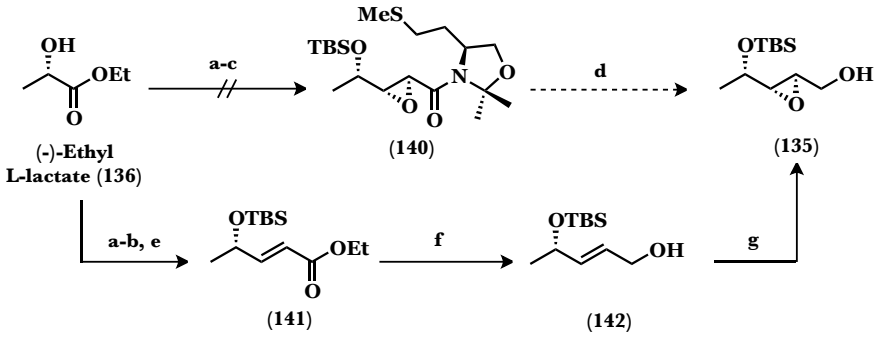
4.3.2. Towards the Total Synthesis of (+)-Depudecin

4.3.2.1. Convergent Approach

Our initial efforts toward the total synthesis of (+)-depudecin *ent*-(**120**) focused on the convergent approach. To this aim, (-)-ethyl-L-lactate was chosen as an appropriate starting material. This compound was first converted into the silyl ether and the carboxylic acid reduced into the aldehyde by the action of 1.0 equiv of DIBAL-H at -78ºC. The crude

aldehyde was then subjected to reaction with the sulfonium salt from L-methionine (**4**). However, although several attempts were made to obtain the epoxy amide **140**, the reaction crude resulted in a complex mixture, isolating epoxy amide in a poor yield. Even though the desired product was likely to be in the mixture, this could not be confirmed by isolation and full characterization which led us to use the Sharpless methodology (**Scheme 4.5**). Thus, the crude aldehyde was subjected to a Wittig-Martin reaction to generate the corresponding *trans*-α,β-unsaturated ester in a 87% overall yield. The synthesis of the key epoxy alcohol proceeded by reduction of the ester **141** into the allylic alcohol **142** by treatment with DIBAL-H at -78ºC and Sharpless asymmetric epoxidation with D-(-)-DET to afford the desired epoxy alcohol **135** in a 90% yield.

Scheme 4.5. *Synthesis of the epoxy alcohol* ***135***



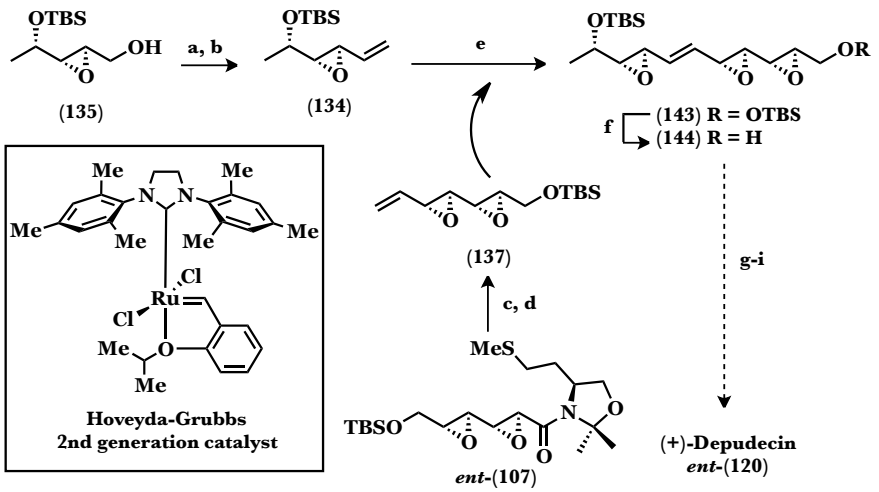
Reagents and conditions: (a) TBSCl, imidazol, CH_2Cl_2, 0ºC. (b) DIBAL-H, CH_2Cl_2, -78ºC, 1 h. (c) **4**, 5.0 M NaOH, CH_2Cl_2-H_2O, 25ºC, 16 h. (d) Super-H, THF, 0ºC, 15 min. (e) $Bu_3PCH_2CO_2Et$, NaOH, toluene-CH_2Cl_2, 87% (over 3 steps). (f) DIBAL-H, CH_2Cl_2, -78ºC, 1 h, 91%. (g) SAE, D-(-)-DET, 90%.

We continued the synthesis of the epoxy alkene **134** from epoxy alcohol **135** by a two steps sequence involving oxidation and Wittig reaction (**Scheme 4.6**). For key fragment **137**, we planned to start from diepoxy amide *ent*-(**107**), that was previously obtained for the synthesis of

gummiferol. Thus, reduction of the mentioned diepoxy amide *ent*-(**107**) under Red-Al, followed by Wittig reaction, afforded alkene **137** in 50% yield over 2 steps.

With the both key fragments in hand, we investigated the viability of the metathesis reaction. For the assembly of both epoxy olefins, a cross-metathesis with Hoveyda-Grubbs 2nd generation was initially atempted. However, this reaction afforded the coupling product albeit in a very low yield (15%), due to the formation of sub-products during the metathesis reactions, such as the homo-coupling product in 60% yield. Different conditions and equivalents were tested, including second cycles of metathesis but we did not get an improvement of the yield of the desired product. However, despite the yield of this reaction was variable and generally modest, we were able to prepare **143** in sufficient amounts to continue with the synthesis. In this way, selective deprotection step of the primary alcohol was accomplished in the presence of TsOH resulting in triepoxy alcohol **144**, in a 50% yield (**Scheme 4.6**). Subsequent Apple[31] reaction followed by reductive elimination by treatment of the resulting bromo derivative with Zn would led us to the precursor of depudecin. Unfortunately, this procedure still remains incomplete due to lack of time.

Scheme 4.6. *Convergent approach to the synthesis of (+)-depudecin*



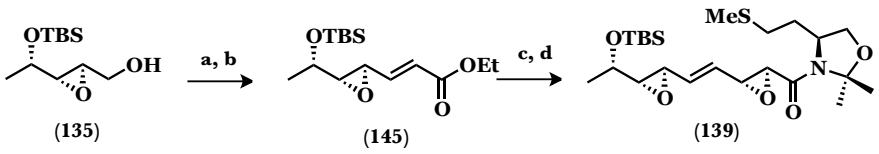
Reagents and conditions: (a) SO_3·Pyr, Et_3N, CH_2Cl_2, DMSO, 0ºC to rt, 4 h. (b) Ph_3PCH_3Br, NaHMDS, 0ºC to rt, 12 h, 50% (over 2 steps). (c) Red-Al, THF, 0ºC, 45 min. (d) Ph_3PCH_3Br, NaHMDS, 0ºC to rt, 12 h, 30% (over 2 steps). (e) **137**, Hoveyda-Grubbs 2nd generation catalyst, CH_2Cl_2, 45ºC, 72 h, 15%. (f) TsOH, 50%. (g) CBr_4, Ph_3P. (h) Zn (i) TBAF.

4.3.2.2. Linear Approach

As an efficient alternative to the convergent synthesis described above, a second route to depudecin, involving the synthesis of the diepoxy amide **138** was developed.

To this aim, we first proceeded to obtain the epoxy amide **139** *via* chiral sulfur ylide by a sequence involving: (i) oxidation of epoxy alcohol **135** into the aldehyde by treatment with SO_3-Pyr complex, (ii) a Wittig-Martin reaction, (iii) reduction to the α,β-unsaturated aldehyde under DIBAL-H and (iv) reaction with the sulfur ylide resulting from sulfonium salt **4**. To our delight, this four-steps sequence was accomplished in a 75% overall yield and provided the essential fragment **139** as a single diastereoisomer (**Scheme 4.7**).

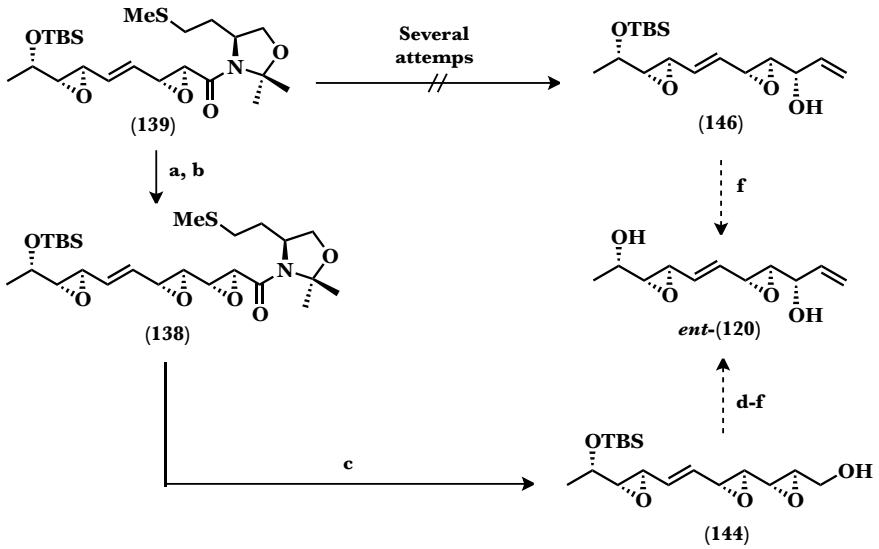
Scheme 4.7. *Synthesis of the key intermediate epoxy amide* ***139***



Reagents and conditions: (a) SO_3·Pyr, CH_2Cl_2-DMSO, Et_3N, 0ºC. (b) $Bu_3PCH_2CO_2Et$, NaOH, toluene-CH_2Cl_2, 62% (over 2 steps) (c) DIBAL-H, CH_2Cl_2, -78ºC, 1 h. d) **4**, NaOH, *t*BuOH, 12 h, 55% (over 2 steps).

From compound **139**, two approaches were explored. In our efforts to shorten the number of steps, a straightforward addition of the appropriate vinyl Grignard reagent was planned. To this aim, epoxy amide **139** was reduced into the corresponding epoxy aldehyde by the action of Red-Al, as previously reported, followed by addition of vinyl magnesium bromide. Even though several conditions were tested all of them resulted in complex mixture and decomposition of the starting epoxy aldehyde. This disappointing and unexpected results led us to change our initial strategy by synthesizing the triepoxy amide **138** which was obtained as a single diasteroisomer in a moderate 30% yield over 2 steps by reaction of corresponding epoxy aldehyde derived from **139** with sulfonium salt **4** employing our two phase method (**Scheme 4.8**).[32] This highly valuable tri-epoxide fragment was then converted into the corresponding tri-epoxy alcohol **144** by reaction with Super-H in 60% yield.

Scheme 4.8. *Towards the synthesis of (+)-depudecin*



Reagents and conditions: (a) Red-Al, THF, 0ºC, 45 min. (b) **4**, NaOH, CH_2Cl_2-H_2O, 30% (over 2 steps). (c) Super-H, THF, 0ºC, 30 min, 60%. (d) CBr_4, Ph_3P. (e) Zn (f) TBAF

With this approach, we have provided a synthetic alternative to the synthesis to the tri-epoxy alcohol **144** and have established a valuable methodology for the synthesis of the carbon skeleton of depudecin. Although three steps remains still unaffordable for completing the total synthesis of (+)-depudecin, previously reported conversion of epoxy alcohols into vinyl alcohol makes us to be confident on this strategy.[33]

4.4. Summary and Concluding Remarks

In this chapter, new approaches towards the synthesis of the non-natural enantiomer of depudecin were described. Particularly, the development of an alternative route to build the oxigenated carbon skeleton of depudecin was discussed. In comparison to the synthesis of Schreiber, the precursor of the depudecin **144** was synthesized in a stereoselective fashion using the chiral sulfur ylide derived from L-Met in a sequence involving 12 steps, with an overall yield of 4%. Additionally, a more convergent efficient route for the rapid assembly of the building blocks **134** and **137** *via* cross metathesis was described, although alternative cross-coupling methods should be tested in order to increase the yield of the targeted product. Due to the lack of time, new ways to accomplish the total synthesis have to be explored, that could provide an efficient route towards depudecin and its analogues.

4.5. Experimental section

4.5.1. General Procedures for the Synthesis of Epoxy and Diepoxy Amides

4.5.1.1. Synthesis of Epoxy Amides

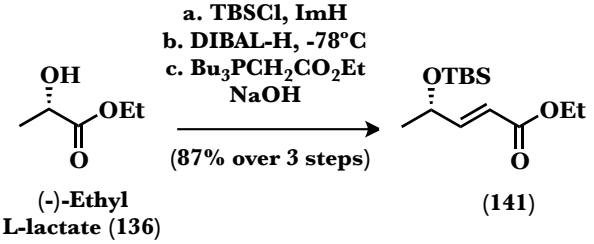
To a solution of sulfonium salt (**4** or **6**) (1.1 equiv) in *t*BuOH (~ 0.08 M) was added a 3.0 M aqueous NaOH solution (1.1 equiv). After 10 min at 25ºC, a solution of the corresponding aldehyde (1.0 equiv), in *t*BuOH (~ 0.1 M) was added, and the crude reaction mixture was vigorously stirred overnight at 25ºC. After this time, both phases were separated, and the aqueous layer was extracted twice with EtOAc. Combined organic extracts were then washed with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. Flash column chromatography (silica gel, EtOAc 30-40% in hexanes) afforded the corresponding epoxy amide.

4.5.1.2. Synthesis of Diepoxy Amides

To a solution of the corresponding epoxy amide (1.0 equiv) in dry THF (0.08 M) was added dropwise Red-Al 70% w/v in toluene (2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude epoxy aldehyde was used for the next step without further purification.

4.5.2. Convergent approach

4.5.2.1. Synthesis of the α,β-Unsaturated Ester **141**



(-)-Ethyl L-lactate (136) **(141)**

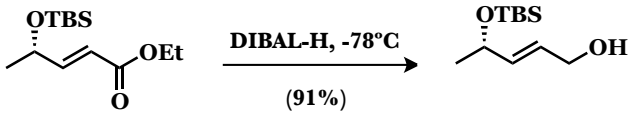
To a solution of **136** (4.8 mL, 42.3 mmol, 1.0 equiv) in CH_2Cl_2 (45 mL) was added, at 0ºC, imidazol (3.5 g, 51.0 mmol, 1.2 equiv) and TBSCl (7 g, 46.5 mmol, 1.1 equiv) and the reaction was allowed to reach rt. After 5 hours the mixture was quenched with H_2O and diluted with Et_2O. The aqueous phase was extracted with Et_2O and the organic layers were washed with brine, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The corresponding silyl ether, whose data match with those reported in bibliography, was used in the next step without further purification.[34]

The crude silyl ether dissolved in CH_2Cl_2 (50 mL) was cooled at -78ºC and then treated with DIBAL-H 1.0 M solution in toluene (25.8 mL, 25.8 mmol, 1.0 equiv) After 20 min, the reaction was quenched by addition of EtOAc at -78ºC and the mixture was allowed to reach rt and treated with a saturated aqueous Na^+/K^+ tartrate solution. The resulting mixture was vigorously stirred until a clear separation of both organic and aqueous phases. The aqueous phase was then separated, the organic extract washed with water and brine, dried and the solvent evaporated under reduced pressure. The crude of the aldehyde was used in the next step without further purification.

A solution of tributyl(ethoxycarbonylmethylene)phosphonium bromide (5.8 g, 16.0 mmol, 1.25 equiv) in CH_2Cl_2 (15 mL) was washed twice with a 1.0 M aqueous NaOH solution (30 mL), dried and diluted with toluene (20 mL). After removing CH_2Cl_2, the resulting solution was then added to a stirred solution of crude aldehyde and benzoic acid (310 mg, 2.5 mmol, 0.2 equiv) in toluene (50 mL) at 95ºC. After 30 min the solvent was evaporated and the residue was purified by flash column chromatography (silica gel 10% EtOAc in hexanes) to provide the corresponding α,β-unsaturated ester **141** (2.9 g, 87% over 3 steps) as a pale yellow oil.

R_f = 0.73 [Silica gel, 20% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 8.3 (*c* 0.7, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.92 (dd, $\mathcal{J}$ = 15.5, 4.1 Hz, 1H, C*H*=CHCO$_2$), 5.98 (dd, $\mathcal{J}$ = 15.5, 1.8 Hz, 1H, CH=C*H*CO$_2$), 4.51 - 4.39 (m, 1H, CH$_3$C*H*), 4.19 (qd, $\mathcal{J}$ = 7.1, 3.1 Hz, 2H, OC*H*$_2$CH$_3$), 1.29 (t, $\mathcal{J}$ = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 1.25 (d, $\mathcal{J}$ = 6.6 Hz, 3H, C*H*$_3$CH), 0.90 (s, 9H, (C*H*$_3$)$_3$CSi)), 0.06 (s, 3H, (C*H*$_3$)$_2$Si), 0.06 (s, 3H, (C*H*$_3$)$_2$Si)). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 166.85, 151.88, 118.96, 67.70, 60.29, 25.81, 23.54, 18.21, 14.26, -4.85, -4.87.

4.5.2.2. Synthesis of the Allylic Alcohol **142**



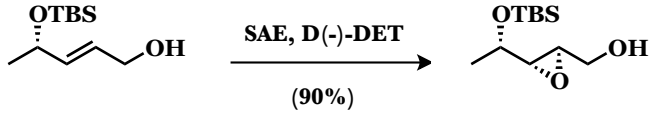
(141) **(142)**

A solution of **141** (2.2 g, 8.5 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL) was cooled at -78ºC and then treated with DIBAL-H 1.0 M solution in toluene (22 mL, 22.0 mmol, 2.5 equiv). After 40 min, the reaction was quenched by adding of EtOAc at -78ºC and the mixture was allowed to reach rt and treated with a saturated aqueous Na^+/K^+ tartrate solution. The resulting mixture was vigorously stirred until a clear separation of

both organic and aqueous phases. The aqueous phase was then separated, the organic extract washed with water and brine, dried and the solvent evaporated under reduced pressure. The crude was purified by flash column chromatography (silica gel, 10% EtOAc in hexanes) to obtain **142** (1.67 g, 91%) as a colourless oil.

R_f = 0.55 [Silica gel, 20% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 16.4 (*c* 0.8, CH_2Cl_2). ^1H RMN (400 MHz, $CDCl_3$), δ (ppm): 5.78 - 5.71 (m, 2H, CHC*H*=CH and CH=C*H*CH_2), 4.36 - 4.27 (m, 1H, CH_3C*H*CH=), 4.13 (dd, $\mathcal{J}$ = 4.2, 0.9 Hz, 2H, =CHCH_2), 1.57 (bs, 1H, O*H*), 1.21 (d, $\mathcal{J}$ = 6.4 Hz, 3H, CH_3CH), 0.89 (s, 9H, (CH_3)$_3$CSi), 0.06 (s, 3H, (CH_3)$_2$Si), 0.05 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 136.35, 127.30, 68.48, 63.15, 25.88, 24.32, 18.26, -4.62, -4.75.

4.5.2.3. Synthesis of the Epoxy Alcohol **135**

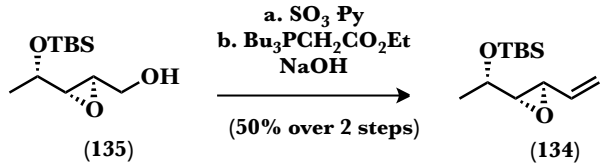


(142) **(135)**

To a solution of titanium tetraisopropoxide (0.7 mL, 2.3 mmol, 0.35 equiv) in CH_2Cl_2 (30 mL) was added D-(-)-DET (0.4 mL, 2.3 mmol, 0.35 equiv) at -20ºC. After 15 min at this temperature, a solution of alyllic alcohol **142** (1.4 g, 6.6 mmol, 1.0 equiv) in CH_2Cl_2 (40 mL) was added dropwise, followed by the addition, after additional 30 min, of a 5.5 M TBHP solution in decane (2 mL, 10.5 mmol, 1.6 equiv) at the same temperature. After 8 h at this temperature, the reaction mixture was quenched with Me_2S (2.3 mL, 30.0 mmol, 4.6 equiv) at 0ºC, filtered off and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 20% EtOAc in hexanes) to obtained epoxy alcohol **135** (1.3 g, 90%) as a colourless oil.

R_f = 0.36 [Silica-gel, 30% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 21.0 (*c* 0.9, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 3.90 (ddd, $\mathcal{J}$ = 12.7, 5.5, 2.5 Hz, 1H, C*H*$_2$OH), 3.66 - 3.55 (m, 2H, CH_3CH and C*H*$_2$OH), 3.01 (dt, $\mathcal{J}$ = 4.7, 2.4 Hz, 1H, C*H*(O)CHCH$_2$), 2.95 (dd, $\mathcal{J}$ = 5.8, 2.4 Hz, 1H, CH(O)C*H*CH$_2$), 2.29 (t, $\mathcal{J}$ = 6.4 Hz, 1H, O*H)*, 1.18 (d, $\mathcal{J}$ = 6.4 Hz, 3H, C*H*$_3$CH), 0.88 (s, 9H, (C*H*$_3$)$_3$CSi)), 0.07 (s, 3H, (C*H*$_3$)$_2$Si), 0.05 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 69.12, 61.51, 60.06, 56.15, 25.79, 20.34, 18.13, -4.69, -4.87. HRMS (H-ESI) *m/e* 233.15675 calcd for $C_{11}H_{24}O_3Si$ $[M + H]^+$, found 233.15669.

4.5.2.4. Synthesis of the Epoxy Olefin **134**



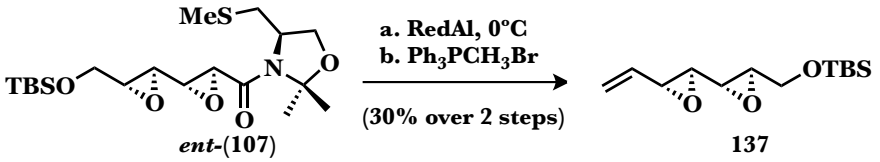
Epoxy alcohol **135** (220 mg, 0.95 mmol, 1.0 equiv) was dissolved in a CH_2Cl_2/DMSO (1:1) mixture (20 mL) and cooled at 0ºC. At this temperature, Et_3N (0.4 mL, 14.2 mmol, 3.0 equiv) was added followed by SO_3·Pyr (270 mg, 1.71 mmol, 1.8 equiv). The mixture was allowed to reach rt and 5 hours later was quenched by addition of a buffer solution pH = 7 and diluted with Et_2O. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$ and the solvent removed under pressure. The resulting crude of the aldehyde was used in the next step without further purification.

Over a solution of Ph_3PCH_3Br (693 mg, 1.9 mmol, 2.0 equiv) in THF (10 mL) was added NaHMDS 2.0 M in THF (0.7 mL, 1.4 mmol, 1.5 equiv) at 0ºC. The reaction was stirred at the same temperature over 10 min and then, the crude of the aldehyde above obtained dissolved in THF (30 mL) was added at once. The reaction crude was allowed to reach rt

and stirred overnight. The solvent was then removed under vacuum and the crude purified by flash column chromatography (silica gel, 10% EtOAc in hexanes) to afford **134** (100 mg, 50% over 2 steps) as a pale yellow oil.

R_f = 0.74 [Silica gel, 10% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 35.2 (*c* 0.4, CH_2Cl_2) . ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.58 (ddd, $\mathcal{J}$ = 17.3, 10.1, 7.5 Hz, 1H, CH(O)CHC*H*=), 5.45 (ddd, $\mathcal{J}$ = 17.2, 1.6, 0.4 Hz, 1H, CH=CH_2), 5.26 (ddd, $\mathcal{J}$ = 10.2, 1.5, 0.6 Hz, 1H, CH=CH_2), 3.62 (dt, $\mathcal{J}$ = 12.3, 6.3 Hz, 1H, CH_3C*H*), 3.20 (dd, $\mathcal{J}$ = 7.5, 2.2 Hz, 1H, CH_3CHCH(O)C*H*), 2.85 (dd, $\mathcal{J}$ = 5.8, 2.2 Hz, 1H, CH_3CHC*H*(O)), 1.21 (d, $\mathcal{J}$ = 6.4 Hz, 3H, CH_3CH), 0.90 (s, 9H, (CH_3)$_3$CSi), 0.10 (s, 3H, (CH_3)$_2$Si), 0.07 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 135.26, 119.22, 69.42, 64.37, 56.28, 25.81, 20.28, 18.16, -4.66, -4.88. HRMS (APCI) *m/e* 229.16183 calcd for $C_{12}H_{24}O_2Si$ $[M + H]^+$, found 229.16194.

4.5.2.5. Synthesis of the Diepoxy Olefin **137**

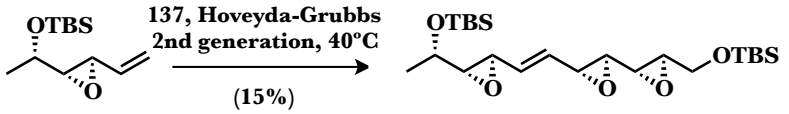


Diepoxiamide *ent*-**107** (350 mg, 0.8 mmol, 1.0 equiv) in dry THF (10 mL) was treated dropwise with Red-Al 60% w/v in toluene (0.54 mL, 1.8 mmol, 2.2 equiv) at 0ºC. After 1 hour at this temperature, the reaction mixture was diluted with a saturated aqueous NH_4Cl solution. The aqueous phase was then separated, extracted twiced with EtOAc and the combined organic phase washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The resulting crude aldehyde was used in the next without further purification.

The crude aldehyde dissolved in THF (10 mL) was cooled at 0ºC and over this solution was added NaHMDS 2.0 M in THF (0.6 mL, 1.2 mmol, 1.5 equiv). The reaction was stirred at the same temperature over 10 min and then Ph_3PCH_3Br (590 mg, 1.6 mmol, 2.0 equiv) dissolved in THF (5 mL) was added at once. The reaction crude was allowed to reach rt and stirred overnight. The solvent was then removed under vacuum and the crude purified by flash column chromatography (silica gel, 20% EtOAc in hexanes) to afford **137** (70 mg, 30% over 2 steps) as a pale yellow oil.

R_f = 0.33 [Silica gel, 10% EtOAc in hexanes]. $[\alpha]^{25}_D$ = - 25.4 (*c* 0.8, CH2Cl2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.60 - 5.48 (m, 2H, C*H*$_2$=CH and CH$_2$=C*H*), 5.35 - 5.30 (m, 1H, C*H*$_2$=CH), 3.88 (dd, $\mathcal{J}$ = 12.2, 2.8 Hz, 1H, CHCH(O)CHC*H*$_2$), 3.75 (dd, $\mathcal{J}$ = 12.2, 4.0 Hz, 1H, CHCH(O)CHC*H*$_2$), 3.37 (dd, $\mathcal{J}$ = 7.1, 2.1 Hz, 1H, =CHC*H*(O)CH), 3.11 (ddd, $\mathcal{J}$ = 4.0, 2.9, 2.1 Hz, 1H, CHCH(O)C*H*CH$_2$), 3.00 (dd, $\mathcal{J}$ = 4.4, 2.1 Hz, 1H, CHC*H*(O)CHCH$_2$), 2.92 (dd, $\mathcal{J}$ = 4.4, 2.1 Hz, 1H, =CHCH(O)C*H*), 0.90 (s, 9H, (C*H*$_3$)$_3$CSi), 0.08 (s, 3H, (C*H*$_3$)$_2$Si), 0.07 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 134.43, 120.17, 62.07, 57.90, 56.03, 55.96, 53.29, 29.69, 25.84, 18.33, -5.36. HRMS (APCI) *m/e* 257.15675 calcd for $C_{13}H_{24}O_3Si$ [M + H]$^+$, found 257.15674.

4.5.2.6. Cross metathesis of Olefins **134** and **137**



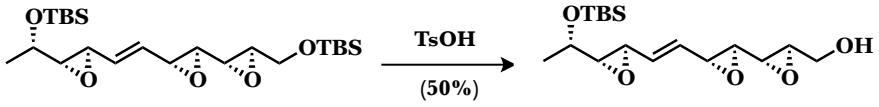
(134) **(143)**

Alkene **134** (60 mg, 0.27 mmol, 3.0 equiv) and diepoxy alkene **137** (23 mg, 0.09 mmol, 1.0 equiv) were dissolved in CH_2Cl_2 (8 mL) and Hoveyda-Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 0.2 equiv) was added. The flask was then capped and heated at 40ºC overnight, after which the crude mixture was concentrated and purified by flash column

chromatography (silica gel, 5% EtOAc in hexanes) to yield **143** (6 mg, 15%) as a pale yellow oil.

R_f = 0.57 [Silica gel, 10% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 31.4 (*c* 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.72 (dd, $\mathcal{J}$ = 15.7, 7.0 Hz, 1H, (O)CHC*H*=CH), 5.64 (dd, $\mathcal{J}$ = 15.6, 7.0 Hz, 1H, (O)CHCH=C*H*), 3.88 (dd, $\mathcal{J}$ = 12.2, 2.7 Hz, 1H, (O)CHCH_2O), 3.74 (dd, $\mathcal{J}$ = 12.2, 4.0 Hz, 1H, (O)CHCH_2O), 3.63 (dd, $\mathcal{J}$ = 6.5, 5.8 Hz, 1H, CH_3C*H*), 3.37 (dd, $\mathcal{J}$ = 7.1, 2.0 Hz, 1H, =CHC*H*(O)), 3.23 (dd, $\mathcal{J}$ = 7.0, 2.1 Hz, 1H, CH_3CHCH(O)C*H*), 3.10 (ddd, $\mathcal{J}$ = 4.0, 2.8, 2.1 Hz, 1H, CH(O)CHCH(O)C*H*), 3.00 (dd, $\mathcal{J}$ = 4.2, 2.1 Hz, 1H, CH(O)CHC*H*(O)CH), 2.93 (dd, $\mathcal{J}$ = 4.2, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.86 (dd, $\mathcal{J}$ = 5.7, 2.1 Hz, 1H, CH_3CHC*H*(O)), 1.20 (d, $\mathcal{J}$ = 6.4 Hz, 3H, C*H*$_3$CH), 0.90 (s, 9H, (C*H*$_3$)$_3$CSi), 0.89 (s, 9H, (C*H*$_3$)$_3$CSi), 0.09 (s, 3H, (C*H*$_3$)$_2$Si), 0.07 (s, 3H, (C*H*$_3$)$_2$Si), 0.07 (s, 3H, (C*H*$_3$)$_2$Si) 0.06 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 132.64, 130.77, 69.16, 64.68, 61.98, 58.08, 56.07, 54.85, 53.03, 32.06, 29.70, 25.84, 25.80, 20.26, 18.33, 18.16, -4.67, -4.84, -5.36. HRMS (APCI) *m/e* 479.26195 calcd for $C_{23}H_{44}O_5Si_2$ $[M + H]^+$, found 479.26208.

4.5.2.7. Synthesis of Triepoxy Alcohol **144**



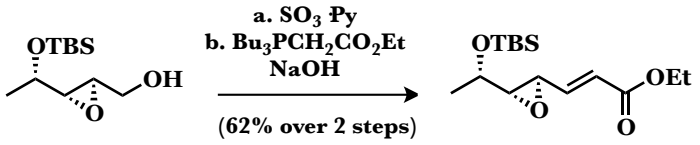
(143) **(144)**

Silyl ether **143** (30 mg, 0.07 mmol, 1.0 equiv) was dissolved in a mixture THF-H_2O (20:1) (5 mL) and over this solution was added TsOH (2 mg, 0.01 mmol, 0.1 equiv) at 25ºC. After stirring for 40 min at the same tempearture, the solvent was removed under vacuum and the crude mixture purified by flash column chromatography (silica gel, 40% EtOAc in hexanes) to afford **144** (11 mg, 50%) as a colourless oil.

R_f = 0.38 [Silica gel, 60% EtOAc in hexanes]. $[\alpha]^{25}_D$ = +35.2 (*c* 0.4, CH_2Cl_2) . ^1H RMN (400 MHz, $CDCl_3$), δ (ppm) (5.73 (dd, $\mathcal{J}$ = 15.6, 7.1 Hz, 1H, CH(O)CHC*H*=), 5.64 (dd, $\mathcal{J}$ = 15.7, 7.2 Hz, 1H, CHCH=C*H*), 3.97 (dd, $\mathcal{J}$ = 12.9, 2.1 Hz, 1H, CH(O)CHCH_2), 3.72 (dd, $\mathcal{J}$ = 13.1, 3.2 Hz, 1H, CH(O)CHCH_2), 3.66 – 3.61 (m, 1H, CH_3C*H*), 3.39 (dd, $\mathcal{J}$ = 7.2, 2.1 Hz, 1H, CH=CHC*H*(O), 3.24 (dd, $\mathcal{J}$ = 7.1, 2.0 Hz, 1H, CH_3CHCH(O)C*H*), 3.20 – 3.16 (m, 1H, CH(O)CHCH(O)C*H*), 3.10 (dd, $\mathcal{J}$ = 4.3, 2.2 Hz, 1H, =CHCH(O)CHC*H*(O)), 3.03 (bs, 1H, O*H*), 2.94 (dd, $\mathcal{J}$ = 4.3, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.87 (dd, $\mathcal{J}$ = 5.7, 2.1 Hz, 1H, CH_3CHC*H*(O)), 1.20 (d, $\mathcal{J}$ = 6.4 Hz, 3H, CH_3CH), 0.90 (s, 9H, (CH_3)$_3$CHSi), 0.09 (s, 3H, (CH_3)$_2$Si), 0.07 (s, 3H, (CH_3)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 132.85, 130.55, 69.14, 64.71, 60.44, 57.94, 55.74, 54.80, 53.00, 29,70, 25.80, 20.26, 18.16, -4.67, -4.84.

4.5.3. Linear approach

4.5.3.1. Synthesis of the α,β-Unsaturated Ester **145**



(135) **(145)**

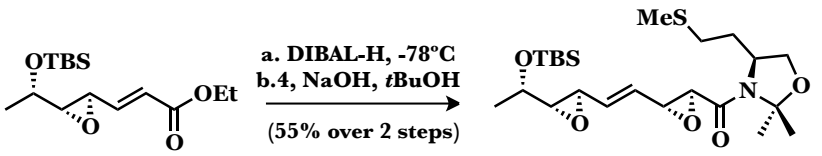
Epoxy alcohol **135** (1.1 g, 4.7 mmol, 1.0 equiv) was dissolved in a CH_2Cl_2/DMSO (1:1) mixture (20 mL) and cooled at 0ºC. At this temperature, Et_3N (2 mL, 14.2 mmol, 3.0 equiv) was added followed by SO_3·Pyr complex (1.4 g, 8.5 mmol, 1.8 equiv). The mixture was allowed to reach rt and 5 hours later was quenched by addition of a buffer pH = 7 solution and diluted with Et_2O. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$ and the solvent removed under

reduced pressure. The resulting crude aldehyde was used in the next step without further purification.

A solution of tributyl(ethoxycarbonylmethylene)phosphonium bromide (2.2 g, 4.7 mmol, 1.25 equiv) in CH_2Cl_2 (10 mL) was washed twice with a 1.0 M aqueous NaOH solution, dried over anhydrous $MgSO_4$, and diluted with toluene (10 mL). After CH_2Cl_2 was removed, the resulting solution was then added to a stirred solution of the corresponding aldehyde (4.7 mmol, 1.0 equiv) and benzoic acid (115 mg, 0.9 mmol, 0.2 equiv) in toluene (20 mL) at 95°C. After 30 min, the solvent was evaporated and the residue was purified by flash column chromatography (silica gel, 20% EtOAc in hexanes) to provide the corresponding α,β-unsaturated ester **145** (880 mg, 62% over 2 steps) as a pale yellow oil. HRMS (H-ESI) *m/e* 301.18296 calcd for $C_{15}H_{28}O_4Si$ [M + H]$^+$, found 301.18292.

R_f = 0.84 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 3.8 (*c* 0.5, CH_2Cl_2) . ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 6.65 (dd, $\mathcal{J}$ = 15.7, 7.2 Hz, 1H, C*H*=CHCO), 6.11 (d, $\mathcal{J}$ = 15.7 Hz, 1H, CH=C*H*CO), 4.18 (q, $\mathcal{J}$ = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 3.72 – 3.62 (m, 1H, CH$_3$C*H*), 3.33 (dd, $\mathcal{J}$ = 7.2, 1.9 Hz, 1H, (O)C*H*CH=), 2.91 (dd, $\mathcal{J}$ = 5.5, 2.0 Hz, 1H, C*H*(O)CHCH=), 1.27 (t, $\mathcal{J}$ = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 1.20 (d, $\mathcal{J}$ = 6.4 Hz, 3H, C*H*$_3$CH), 0.88 (s, 9H, (C*H*$_3$)$_3$CSi), 0.08 (s, 3H, (C*H*$_3$)$_2$Si), 0.05 (s, 3H, (C*H*$_3$)$_2$Si). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.53, 144.05, 123.92, 68.83, 65.10, 60.57, 53.92, 25.77, 20.23, 18.13, 14.18, -4.72, -4.85.

4.5.3.2. Synthesis of the Diepoxy Amide **139**



(145) **(139)**

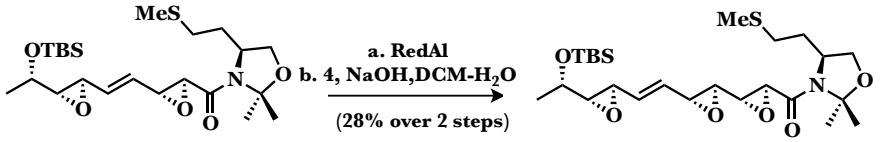
A solution of **145** (175 mg, 0.6 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) was cooled at -78ºC and then treated with DIBAL-H 1 M solution in toluene (0.6 mL, 0.6 mmol, 1.0 equiv) After 20 min, the reaction was quenched by addition of EtOAc at -78ºC and the mixture was allowed to reach rt and treated with a saturated aqueous Na^+/K^+ tartrate solution. The resulting mixture was vigorously stirred until a clear separation of both organic and aqueous phases. The aqueous phase was then separated, the organic extract washed with water and brine, dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The crude of the aldehyde was used in the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (200 g, 0.6 mmol, 1.1 equiv) and NaOH (3.0 M aqueous solution, 0.2 mL, 0.6 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **139** (80 mg, 55% over two steps) as a white solid.

R_f = 0.84 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 28.33 (*c* 0.5, CH_2Cl_2). mp = 76 - 77ºC. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.83 (dd, $\mathcal{J}$ = 15.7, 7.2 Hz, 1H, (O)CHCH=C*H*), 5.67 (dd, $\mathcal{J}$ = 15.7, 7.5 Hz, 1H, CH(O)CHC*H*=), 4.34 - 4.23 (m, 1H, NC*H*), 4.02 (ddd, $\mathcal{J}$ = 9.2, 5.3, 1.4 Hz, 1H, OC*H*$_2$CH), 3.90 (d, $\mathcal{J}$ = 9.2 Hz, 1H, OC*H*$_2$CH), 3.71 - 3.57 (m, 2H, CH_3CHC*H*(O) and CH_3C*H*), 3.53 (d, $\mathcal{J}$ = 1.9 Hz, 1H, CH_3CHCH(O)C*H*), 3.24 (dd, $\mathcal{J}$ = 7.2, 2.1 Hz, 1H, =CHC*H*(O)), 2.87 (dd, $\mathcal{J}$ = 5.6, 2.1 Hz, 1H, =CHCH(O)C*H*), 2.64 - 2.51 (m, 1H, CH_3SC*H*$_2$),

2.43 (ddd, $\mathcal{J}$ = 13.2, 8.8, 6.7 Hz, 1H, CH_3SCH_2), 2.10 (s, 3H, CH_3S), 2.08 - 1.99 (m, 1H, $CH_3SCH_2CH_2$), 1.82 - 1.68 (m, 1H, $CH_3SCH_2CH_2$), 1.65 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.20 (d, $\mathcal{J}$ = 6.4 Hz, 3H, CH_3CH), 0.89 (s, 9H, $(CH_3)_3CSi$), 0.09 (s, 3H, $(CH_3)_2Si$), 0.06 (s, 3H, $(CH_3)_2Si$). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.98, 133.97, 129.54, 95.99, 69.03, 67.05, 64.81, 57.02, 55.87, 55.62, 54.64, 34.42, 30.82, 26.28, 25.80, 22.99, 20.27, 18.16, 15.94, -4.67, -4.83. HRMS (H-ESI) *m/e* 472.25475 calcd for $C_{23}H_{41}NO_5SSi$ $[M + H]^+$, found 472.25504.

4.5.3.3. Synthesis of the Triepoxy Amide **138**



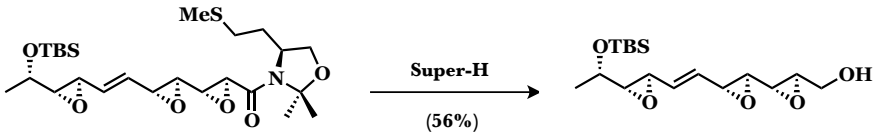
(139) **(138)**

To a solution of diepoxy amide **139** (65 mg, 0.14 mmol, 1.0 equiv) in THF (5 mL) was added dropwise Red-Al (0.09 mL, 70% w/v in toluene, 0.30 mmol, 2.2 equiv) at 0ºC. After 1 h at 0ºC, the reaction mixture was quenched by addition of a saturated aqueous NH_4Cl solution. After separation of both layers, the aqueous phase was extracted with EtOAc, the organic extracts were washed with brine and dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The crude diepoxy aldehyde was used for the next step without further purification.

The crude aldehyde was reacted with sulfonium salt **4** (49 mg, 0.15 mmol, 1.1 equiv) and NaOH (5.0 M aqueous solution, 0.03 mL, 0.14 mmol, 1.0 equiv) according to the general procedure described above for the synthesis of diepoxy amides to yield diepoxy amide **138** (20 mg, 28% over 2 steps) as a colourless oil.

R_f = 0.25 [Silica gel, 40% EtOAc in hexanes]. $[\alpha]^{25}_D$ = + 41.6 (*c* 0.5, CH_2Cl_2) . ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.75 (dd, $\mathcal{J}$ = 15.7, 7.2 Hz, 1H,C*H*=CHCH(O), 5.64 (dd, $\mathcal{J}$ = 15.7, 7.3 Hz, 1H, CH=C*H*CH(O)), 4.34 – 4.28 (m, 1H, NC*H*), 4.05 – 3.99 (m, 1H, OCH_2), 3.91 (d, $\mathcal{J}$ = 9.2 Hz, 1H, OCH_2), 3.66 – 3.61 (m, 1H, CH_3C*H*), 3.59 (d, $\mathcal{J}$ = 1.9 Hz, 1H, =CHCH(O)CHCH(O)C*H*), 3.42 (dd, $\mathcal{J}$ = 7.3, 1.9 Hz, 1H,CH=CHC*H*(O)CH), 3.39 (dd, $\mathcal{J}$ = 3.1, 1.9 Hz, 1H, =CHCH(O)CHC*H*), 3.24 (dd, $\mathcal{J}$ = 7.2, 2.0 Hz, 1H, CH(O)C*H*CH=), 3.06 (dd, $\mathcal{J}$ = 3.2, 2.0 Hz, 1H, CH=CHCH(O)C*H*), 2.86 (dd, $\mathcal{J}$ = 5.6, 2.1 Hz, 1H, C*H*(O)CHCH=), 2.59 (ddd, $\mathcal{J}$ = 12.9, 7.5, 5.1 Hz, 1H, NCHCH$_2$CH_2), 2.48 (ddd, $\mathcal{J}$ = 13.2, 8.3, 7.3 Hz, 1H, NCHCH$_2$CH_2), 2.13 (s, 3H, CH_3S), 2.10 – 2.05 (m, 1H, NCHCH_2CH$_2$), 1.87 – 1.76 (m, 1H, NCHCH_2CH$_2$), 1.65 (s, 3H, (CH_3)$_2$C), 1.53 (s, 3H, (CH_3)$_2$C), 1.20 (d, $\mathcal{J}$ = 6.4 Hz, 3H, CH_3CH), 0.89 (s, $\mathcal{J}$ = 3.3 Hz, 9H, (CH_3)$_3$CSi), 0.09 (s, 3H, (CH$_3$)$_2$Si), 0.06 (s, 3H, (CH$_3$)$_2$Si. ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 162.77, 133.17, 130.17, 95.97, 69.10, 67.05, 64.72, 56.63, 55.99, 55.09, 55.01, 54.77, 51.28, 34.49, 30.72, 26.22, 25.80, 22.96, 20.26, 18.16, 15.89, -4.67, -4.83. HRMS (H-ESI) *m/e* 514.26531 calcd for $C_{25}H_{43}NO_6SSi$ $[M + H]^+$, found 514.26556.

4.5.3.4. Synthesis of Triepoxy Alcohol **144**



(138) **(144)**

Triepoxy amide **138** (40 mg, 0.07 mmol, 1.0 equiv) in THF was treated with Super-H 1.0 M in THF (0.2 mL, 0.2 mmol, 2. equiv) at 0ºC. After 1 h at this temperature, the reaction mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was separated, extracted with Et_2O twice and the combined organic layers washed with water and brine. dried over anhydrous $MgSO_4$ and

concentrated under reduced pressure. Purification by flash column chromatography (silica gel, 40% EtOAc in hexanes) provided triepoxy alcohol **144** (15 mg, 56%) as a colourless oil previously described.

4.6. Notes and References

(1) Itazaki, H.; Nagashima, K.; Sugita, K.; Yoshida, H.; Kawamura, Y.; Yasuda, Y.; Matsumoto, K.; Ishii, K.; Uotani, N.; Nakai, H.; Terui, A.; Yoshimatsu, S.; Ikenishi, Y.; Nakagawa, Y. *J. Antibiot.* **1990**, *43*, 1524-1532.

(2) Matsumoto, M.; Matsutani, S.; Sugita, K.; Yoshida, H.; Hayashi, F.; Terui, Y.; Nakai, H.; Uotani, N.; Kawamura, Y.; Matsumoto, K.; Shoji, J.; Yoshida, T. *J. Antibiot.* **1991**, *45*, 879-885.

(3) Tanaka, M.; Ohra, J.; Tsujino, Y.; Sawaji, Y.; Fujimori, T. *Biosci. Biotech. Biochem.* **1994**, *58*, 565-566.

(4) Wang, E. Goldberg, A. R. *Proc. Natl. Acad. Sci. USA.* **1976**, *73*, 4065-4069.

(5) Uchara, Y.; Hori, M.; Takeuchi, O.; Umezawa, H. *Mol. Cell. Biol.* **1986**, *6*, 2198-2296.

(6) Kwon, H. J.; Yoshida, M.; Fukui, Y.; Horinouchi, S.; Beppu, T. *Cancer Res.* **1992**, *52*, 6926-6930.

(7) Shindo, O. N.; Makabe, O.; Nagahara, H.; Nishimura, S. *Basic Life Sci.* **1990**, *522*, 309-312.

(8) Yoshida, M.; Horinouchi, S.; Beppu, T. *Bioessays.* **1995**, *17*, 423-430.

(9) Kwon, H. J.; Owa, T.; Hassig, C. A.; Shimada, J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 3356-3361.

(10) Itazaki, H.; Nagashima, K.; Sugita, K.; Yoshida, H.; Kawamura, Y.; Yasuda, Y.; Matsumoto, K.; Ishii, K.; Uotani, N.; Nakal, H.; Terui, A.; Yoshimatsu, S.; Ikenishi, Y.; Nakagawa, Y. *J. Antibiot.* **1990**, *43*, 1524-1532.

(11) Yoshida, H. Sugita, K. *Jpn. J. Cancer Res.* **1992**, *83*, 324-328.

Sugita, K.; Koizumi, K.; Yoshida, H. *Cancer Res.* **1992**, *52*, 168-172.

(13)Ying, Y.; Taori, K.; Kim, H.; Hong, J.; Luesch, H. *J. Am. Chem. Soc.* **2008,** *130*, 8455-8459.

(14) Privalsky, M. L. *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 3335-3337.

(15) Bos, J. L. *Cancer Res.* **1989**, *49*, 4682-4689.

(16) Sugita, k.; Yoshida, H.; Matsumoto, M.; Matsutani, S. *Biochem. Biophys. Res. Commun.* **1992**, *182*, 379-387.

(17) Oikawa, T.; Onozawa, C.; Inose, M.; Sasai, M. *Biol. Pharm. Bull.* **1995**, *18*, 1305-1307.

(18) Saito, K.; Kawaguchi, A.; Nozoe, S.; Seyama, Y.; Okuda, S. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 995-1001.

(19) Nakatsuka, S. I.; Feng, B. N.; Goto, T.; Tsuge, T.; Nishimura, S. *Phytochemistry* **1990**, *29*, 1529-1531.

(20) Ayer, W. A. Craw, P. A. *Can. J. Chem.* **1992**, *70*, 1348-1355.

(21) Amnuaykanjanasin, A.; Puny, J.; Paungmoung, P.; Rungrod, A.; Tachaleat, A.; Pongpattanakitshote, S.; Cheevadhanarak, S.; Tanticharoen, M. *FEMS Microbial Lett.* **2005**, *251*, 125-136

(22) Wight, W. D.; Kim, K. H.; Lawrence, C. B.; Walton, J. D. *MPMI.* **2009**, *22*, 1258-1267.

Monneret, C. *Eur. J. Med. Chem.* **2005**, *40*, 1-13.

Norton, V. G.; Imai, B. S.; Yau, P.; Bradbury, E. M. *Cell* **1989**, *57*, 449-457.

(25) Brandl, A.; Heinzel, T.; Krämer, O. H. *Biol. Cell.* **2009**, *101*, 193-205.

(26) Shimada, J.; Kwon, H. J.; Sawamura, M.; Schreiber, S. L. *Chem. Biol.* **1995**, *2*, 517-525.

(27) Kolb, H. C. Sharpless, K. B. *Tetrahedron* **1992**, *48*, 10515-10530.

(28) Sunay, U.; Mootoo, D.; Molino, B.; Fraser-Reid, B. *Tetrahedron Lett.* **1986**, *27*, 4697-4700.

(29) Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.* **1984**, *25*, 367-370.

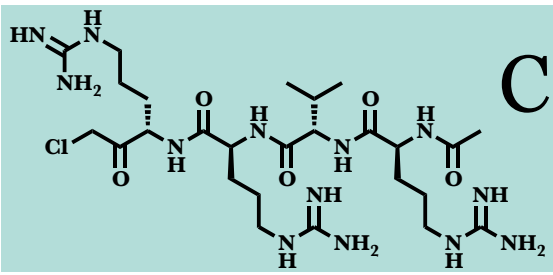
(30) Russel, A. F.; Greengerg, S.; Moffatt, J. G. *J. Am. Chem. Soc.* **1973**, *95*, 4025-4030.

(31) Baughman, T. W.; Swore, J. C.; Wagener, K. B. T. *Tetrahedron* **2004**, *60,* 10943-10948.

(32) López-Herrera, F. J.; Pino-González, M. S.; Sarabia-García, F.; Heras-López, A.; Ortega-Alcántara, J. J.; Pedraza-Cebrián, M. G. *Tetrahedron: Asymm.* **1996**, *7*, 2065-2071.

(33) Sabitha, G.; Reddy, C. N.; Gopal, P.; Yadav, S. *Tet. Lett.* **2010,** *51*, 5736-5739.

(34) Jamieson, A. G.; Sutherland, A. *Org. Biomol. Chem.* **2005**, *3*, 735-736.



CHAPTER 5

Other Contributions

During the course of this thesis, we also challenged several synthetic projects, most of them related to solid phase peptide synthesis (SPPS). Consequently, as a result of the above mentioned collaborations, the contributions to the synthesis of several natural products containing peptidic fragments will be briefly discussed throughout this chapter.

5.1. Solid Phase Synthesis of Globomycin

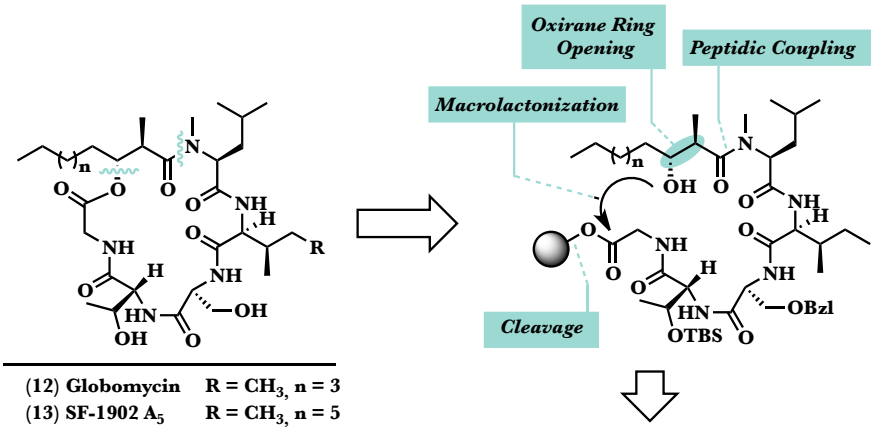
The lipocyclodepsipeptide glomobycin (**12**) and its congeners (**Scheme 5.1**), isolated from four different strains of actinomycetes in 1978,[1-3] represent an interesting family of natural products distinguished by their striking antibiotic activities against Gram-negative bacteria[4-6] and represent an attractive biological target for the development of new antibiotics.

Comparatively, globomycin (**12**) and SF-1902 A_5 (**13**) display greater antibiotic activities when compared to other antibiotics,[7] such as ampicilllin or streptomycin, against *E. coli, Salmonella enteriditis*, and *Enterobacter cloacae*. On the other hand, SF-1902 A_5 is more active than globomycin (MIC against *E. coli* NIHJJC-2: 6.25 μg/mL for **12**, 1.56 μg/

mL for **13**), revealing that the lipidic chain plays an important role in the biological activity. These biological properties render these cyclodepsipeptides as attractive synthetic endeavors in the search for new antibiotics.

With the structure of globomycin established by Haneishi et al.[3] and a first total synthesis reported by the Kogen group in 2000,[8] our research group decided to embark on a research program directed to the design of an efficient and readily accesible route toward this class of natural products and analogues. Hence, in 2011 the synthesis of globomycin and SF-1902 A_5 was accomplished utilizing solid phase synthesis for the peptidic fragment and the chiral sulfur ylide as asymmetric methodology of epoxidation for the lipidic chain.[9]

Scheme 5.1. *Molecular structures of globomycin and its congeners and retrosynthesis of globomycin*



(12) Globomycin R = CH_3, n = 3
(13) SF-1902 A_5 R = CH_3, n = 5
(147) SF-1902 A_{4a} R = CH_3, n = 4
(148) SF-1902 A_2 R = CH_3, n = 1
(149) SF-1902 A_3 R = H, n = 3
(150) SF-1902 A_{4b} R = H, n = 5

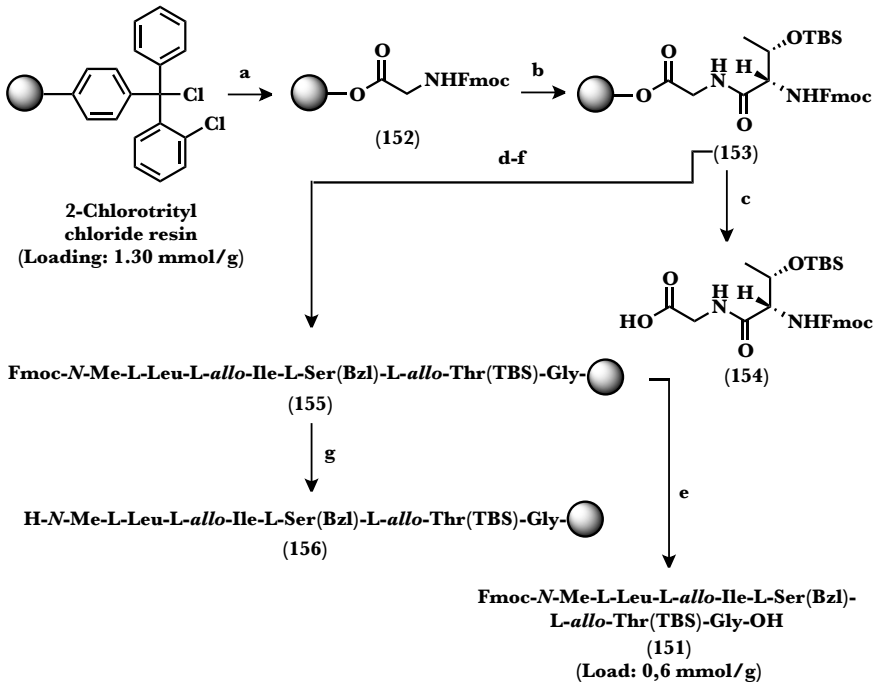
Fmoc-N-Me-L-Leu-L-allo-Ile-L-Ser(Bzl)-L-allo-Thr(TBS)-Gly-OH (151)

Here we would like to describe the synthesis of the linear peptidic chain (**151**) common to globomycin and SF-1902 A_5, that was achieved in

10 steps by taking advantage of the solid phase methodology and revealing a load of 0.6 mmol/g for resin **155** (**Scheme 5.2**).

Thus, Fmoc-Gly was linked onto the 2-chlorotrityl chloride (CTC) resin by esterification with DIPEA to obtain resin **152.** After removing the Fmoc group by treatment with 20% of piperidine in DMF, the following Fmoc aminoacid derivative, Fmoc-L-*allo*-Thr(TBS)-OH, was loaded onto the resulting resin by the action of DIC in the presence of HOBt in DMF. To check the loading of the amino acid and to ensure the effectiveness of the chosen coupling procedure, we decided to cleave the dipeptide by treatment of **153** with CH_2Cl_2/AcOH/CF_3CH_2OH (TFE) (7:2:1), which gave pure dipeptide **154**. The synthesis was continued from **153**, repeating the procedure of coupling and Fmoc deprotection steps for each amino acid being loaded in the following order: (1) Fmoc-L-Ser(Bzl)-OH, (2) Fmoc-L-*allo*-Ile-OH, and (3) Fmoc-*N*-Me-L-Leu-OH to obtain resin **155**. At this stage, we again checked the overall loading of the resin, and thus **155** was treated with CH_2Cl_2/AcOH/CF_3CH_2OH (7:2:1) to provide pure Fmoc protected pentapeptide **151** in an amount that revealed a load of 0.6 mmol/g. Finally, resin **155** was treated with piperidine to remove the Fmoc group and providing the desired pentapeptide **156** (**Scheme 5.2**).

Scheme 5.2. *Solid phase synthesis of the peptidic chain of globomycin*



Reagents and Conditions: (a) Fmoc-Gly-OH, DIPEA, DMF, 25ºC, 30 h. (b) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-*allo*-Thr(TBS)-OH, HOBt, DIC, DMF, 25ºC, 24 h. (c) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 25ºC, 30 min. (d) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-Ser(Bzl)-OH, HOBt, DIC, DMF, 25ºC, 24 h. (e) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-*allo*-Ile-OH, HOBt, DIC, DMF, 25ºC, 24 h. (f) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-*N*-Me-L-Leu-OH, HOBt, DIC, DMF, 25ºC, 24 h. (g) 20% Piperidine in DMF (Load: 0.6 mmol/g for resin **155**).

5.1.1. Experimental Section

5.1.1.1. Fmoc-Gly-OH Loaded 2-Chlorotrityl Resin (**152**)

A 5 mL polypropylene syringe fitted with polyethylene porous disk charged with 2-chlorotrityl chloride resin (300 mg, L = 1.3 mmol/g, 0.39 mmol, 1.0 equiv), was loaded with a solution of Fmoc-Gly-OH (348 mg,

1.17 mmol, 3.0 equiv) and DIPEA (235 μL, 1.37 mmol, 3.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 280 rpm for 30 h, the solution was unloaded, and the resin was washed by shaking with dry DMF (5 x 3 mL). The resulting swelled resin was used in the next step.

5.1.1.2. 2-Chlorotrityl-Gly-L-*allo*-Thr(TBS)-Fmoc resin (**153**)

The polypropylene syringe loaded with the swelled resin **152** was treated with 20% piperidine in DMF (3 x 3 mL x 20 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-L-*allo*-Thr(TBS)-OH (355 mg, 0.78 mmol, 2.0 equiv), HOBt (107 mg, 0.78 mmol, 2.0 equiv) and DIC (155 μL, 1.0 mmol, 2.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 280 rpm for 24 h, and then, the solution was unloaded, and the resin washed with dry DMF (5 x 3 mL). The resulting swelled resin was used in the next step.

5.1.1.3. 2-Chlorotrityl-Gly-L-*allo*-Thr(TBS)-L-Ser(Bzl)-L-*allo*-Ile-Me-L-Leu-Fmoc resin (**155**)

The polypropylene syringe loaded with the swelled resin **153** was treated with 20% piperidine in DMF (3 x 3 mL x 20 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-L-Ser(Bzl)-OH (559 mg, .1.18 mmol, 3.0 equiv), HOBt (161 mg, 1.17 mmol, 3.0 equiv) and DIC (216 μL, 1.37 mmol, 3.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 280 rpm for 24 h, and then, the solution was unloaded, and the resin washed with dry DMF (5 x 3 mL). The resulting swelled resin was used in the next step. This sequence was repeated with Fmoc-L-*allo*-Ile-OH (276 mg, 0.78 mmol, 2.0 equiv), HOBt (107 mg, 0.78 mmol, 2.0 equiv) and DIC (155 μL, 1.0 mmol, 2.5 equiv), followed by treatment with 20% piperidine in DMF and then loading of Fmoc-*N*-Me-L-Leu-OH (358 mg, 0.98 mmol,

2.5 equiv), HOBt (135 mg, 0.98 mmol, 2.5 equiv) and DIC (195 μL, 1.25 mmol, 3.2 equiv). Finally, the resin was washed with DMF (5 x 3 mL), CH_2Cl_2 (3 x 3 mL), MeOH (3 x 3 mL) and Et_2O (3 x 3 mL). The resulting resin was dried under vacuum to recover 626 mg of polymer bound *N*-Fmoc protected pentapeptide **155**.

5.1.1.4. Fmoc-*N*-Me-L-Leu-L-*allo*-Ile-L-Ser(Bzl)-L-*allo*-Thr(TBS)-Gly-OH (**151**)

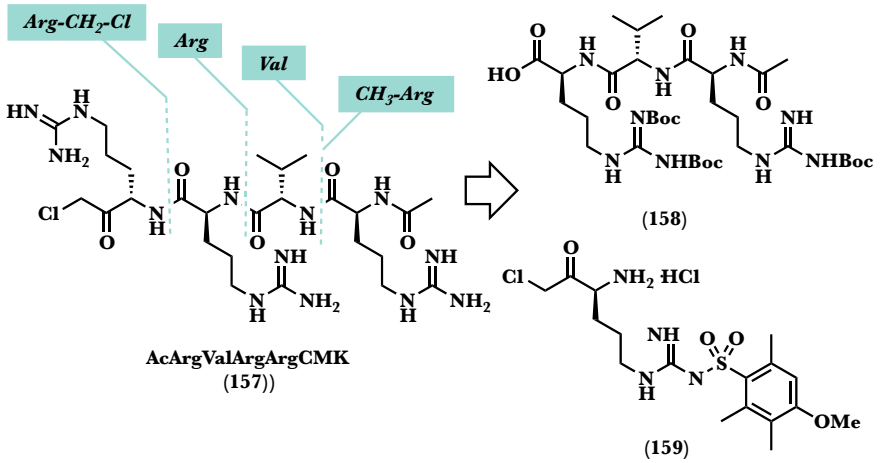
Relesase of a small amount of peptide from resin x (17.5 mg) by treatment with CH_2Cl_2/AcOH/TFE (1.0 ml, 7:2:1) gave the *N*-Fmoc protected pentapeptide **151** (9.6 mg), which revealed a load of 0.6 mmol/g for resin **155**.

Data for **151** (two rotamers in a 2.9:1 ratio): ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm) major rotamer,: 8.16 (t, $\mathcal{J}$ = 5.0 Hz, 1H), 8.05 (d, $\mathcal{J}$ = 7.6 Hz, 1H), 7.99 (d, $\mathcal{J}$ = 9.0 Hz, 1H), 7.89 (d, $\mathcal{J}$ = 7.5 Hz, 2H), 7.63 (d, $\mathcal{J}$ = 7.1, 2H), 7.62-7.70 (m, 1H), 7.41 (t, $\mathcal{J}$ = 7.5 Hz, 2H), 7.24 - 7.33 (m, 7H), 4.65-4.70 (m, 2H), 4.48 (m, 2H), 4.38 (dd, $\mathcal{J}$ = 9.0, 7.2 Hz, 1H), 4.25 - 4.45 (m, 4H), 4.01 (q, $\mathcal{J}$ = 6.5 Hz, 1H), 3.77 (dd, $\mathcal{J}$ = 17.5, 6.2, 1H), 3.52 - 3.59 (m, 3H), 2.79 (s, 3/4H), 2.68 (s, 9/4H), 1.73 - 1.84 (m, 3/4H), 1.47 -1.61 (m, 2H), 1.21 - 1.36 (m, 2H), 1.05 (d, $\mathcal{J}$ = 6.2 Hz, 3H), 0.95 - 1.07 (m, 1H), 0.88 (d, $\mathcal{J}$ = 6.6 Hz, 3H), 0.80 (s, 9H), 0.71-0.89 (m, 9H), 0.01 (s, 3H), 0.03 (s, 3H). FAB HRMS (NBA) *m/e* 930.5049, calcd for $C_{50}H_{71}N_5O_{10}Si$ $[M + H]^+$, found 930.5043.

5.2. Solid Phase Synthesis of Peptide 157

As part of a collaboration with the Department of Ecology of the University of Málaga, the solid phase synthesis of the inhibitor AcArgValArgArgCMK, that is currently under biological evaluation, was also accomplished according to the retrosynthetic analysis depicted in **Scheme 5.3**.

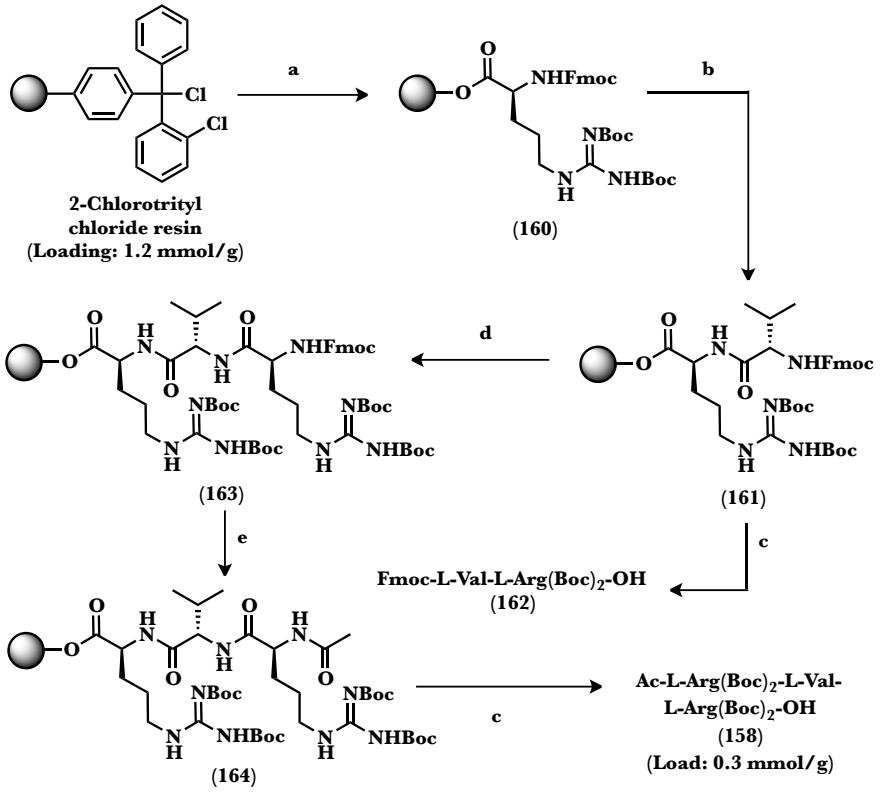
Scheme 5.3. *Retrosynthetic analysis for tetrapeptide* ***157***



Thus, for the tripeptide **158**, Fmoc-L-Arg(Boc)$_2$-OH was linked onto the 2-chlorotrityl chloride (CTC) resin by esterification with DIPEA to afford resin **160**. Once the Fmoc group was removed by treatment with a 20% solution of piperidine in DMF, the following Fmoc aminoacid derivative, Fmoc-L-Val-OH, was loaded onto the resulting resin by the action of DIC in the presence of HOBt in DMF. To check the loading of the amino acid, we decided to cleave the dipeptide by treatment of **161** with CH_2Cl_2/AcOH/CF_3CH_2OH (TFE) (7:2:1), which gave pure dipeptide **162**. The synthesis was continued by repeating the procedure of coupling and Fmoc deprotection steps for Fmoc-L-Arg(Boc)$_2$-OH. The treatment with piperidine was accomplished to remove the Fmoc group

prior to the subsequent acetylation with Ac_2O and pyridine that afforded the acyl resin **164**, which was then treated with CH_2Cl_2/AcOH/ CF_3CH_2OH (7:2:1) to provide pure tripeptide **158** in an amount that revealed a load of 0.3 mmol/g for resin **164**.

Scheme 5.4. *Solid phase synthesis of tripeptide* ***158***

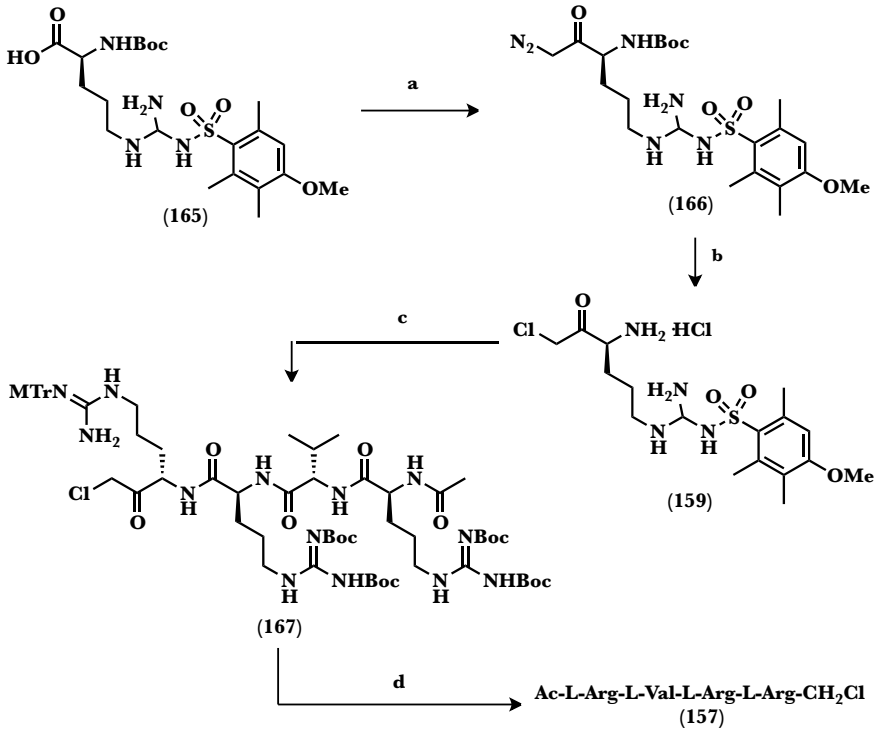


Reagent and Conditions: (a) Fmoc-L-Arg(Boc)$_2$-OH, DIPEA, DMF, 25ºC, 24 h. (b) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-Val-OH, DIC, HOBt, DMF, 25ºC, 24 h. (c) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 30 min. (d) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-Arg(Boc)$_2$-OH, DIC, HOBt, DMF, 25ºC, 24 h. (e) 20% Piperidine in DMF, 25ºC, 30 min; Ac_2O/Pyr, DMF, 25ºC, 24 h (Load: 0.3 mmol/g for resin **164**).

For the preparation of the chloromethyl ketone **159** we followed the procedure described by Sun and co-workers,[10] in which the protected

amino acid Boc-L-Arg(Mtr)-OH was reacted with diazomethane[11] to afford diazo ketone Boc-L-Arg(Mtr)-CHN_2 (**165**), which was subsequently subjected to the action of HCl in methanol, which provided the desired Arg-(Mtr)-chloromethyl ketone **159** as its hydrochloride salt (**Scheme 5.5**). The final coupling of **159** with tripeptide **158** by the action of DIC in the presence of HOBt in DMF followed by treatment with thioanisol in TFA provided the coveted tetrapeptide **157** in 64% yield over 3 steps, as depicted in **Scheme 5.5**.

Scheme 5.5. *Solid phase synthesis of tetrapeptide* ***157***



Reagents and Conditions: (a) $(CH_3)_2CHOCOCl$, NMM, CH_2N_2, THF -20°C to 0°C, 4 h, 52%. (b) MeOH·HCl, THF, 25°C, 24 h. (c) **158**, DIPEA, DIC, HOBt, CH_2Cl_2, 25°C, 24 h. (d) TFA, thioanisol, 25°C, 20 h, 64% (over 3 steps).

5.2.1. Experimental Section

5.2.1.1. 2-Chlorotrityl-L-Arg(Boc)$_2$-Fmoc Resin (**160**).

A 5 mL polypropylene syringe fitted with polyethylene porous disk charged with 2-chlorotrityl chloride resin (200 mg, Load = 1.2 mmol/g, 0.24 mmol, 1.0 equiv), was loaded with a solution of Fmoc-L-Arg(Boc)$_2$-OH (286 mg, 0.48 mol, 2.0 equiv) and DIPEA (105 μL, 0.6 mmol, 2.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 300 rpm for 24 h, the solution was unloaded, and the resin was washed by shaking with dry DMF (5 x 3 mL). The resulting swelled resin was used in the next step.

5.2.1.2. 2-Chlorotrityl-L-Arg(Boc)$_2$-L-Val-Fmoc Resin (**161**).

The polypropylene syringe loaded with the swelled resin **160** was treated with a 20% piperidine solution in DMF (2 x 3 mL x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-L-Val-OH (163 mg, 0.48 mmol, 2.0 equiv), HOBt (66 mg, 0.48 mmol, 2.0 equiv) and DIC (94 μl, 0.6 mmol, 2.5 mmol) in dry DMF (3 mL). The resulting suspension was shaken at 300 rpm for 24 h, and then, the solution was unloaded and the resin washed with dry DMF (5 x 3 mL). The corresponding swelled resin was used in the next step.

5.2.1.3. 2-Chlorotrityl-L-Arg(Boc)2-L-Val-L-Arg(Boc)$_2$-Fmoc Resin (**163**).

The polypropylene syringe loaded with the swelled resin **161** was treated with a 20% piperidine solution in DMF (2 x 3 mL x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-L-Arg(Boc)$_2$-OH (286 mg, 0.48 mmol, 2.0 equiv), HOBt (66 mg, 0.48 mmol, 2.0 equiv) and DIC (105 μl, 0.6 mmol, 2.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 300

rpm for 24 h, and then, the solution was unloaded and the resin washed with dry DMF (5 x 3 mL). The corresponding swelled **163** resin was used in the next step.

5.2.1.4. 2-Chlorotrityl-L-Arg(Boc)2-L-Val-L-Arg(Boc)$_2$-acetyl Resin (**164**).

The polypropylene syringe loaded with the swelled resin **163** was treated with a 20% piperidine solution in DMF (2 x 3 mL x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Ac_2O (110 μL, 1.2 mmol, 5.0 equiv) and pyridine (96 μL, 1.2 mmol, 5.0 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 300 rpm for 24 h, and the solution was then unloaded and the resin washed with dry DMF (5 x 3 mL). The resulting swelled resin **164** was used in the next step.

5.2.1.5. Ac-L-Arg(Boc)$_2$-L-Val-L-Arg(Boc)$_2$-OH (**158**)

The polypropylene syringe loaded with the swelled resin **164** was treated with a 20% piperidine solution in DMF (2 x 3 mL x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL). Release of a small amount of peptide from resin 4 (47 mg) by treatment with CH_2Cl_2/ AcOH/TFE (1.0 mL, 7:2:1) gave the unprotected tripeptide 5 (27 mg) which revealed a load of 0.3 mmol/g for resin **158**.

^{1}H RMN (400 MHz, $CDCl_3$), δ (ppm) 8.40 (d, $\mathcal{J}$ = 31.6 Hz, 2H), 7.62 (d, $\mathcal{J}$ = 8.0 Hz, 1H), 7.45 (d, $\mathcal{J}$ = 8.6 Hz, 1H), 7.21 (d, $\mathcal{J}$ = 7.1 Hz, 1H), 4.55 (td, $\mathcal{J}$ = 8.1, 5.2 Hz, 1H), 4.48 (dt, $\mathcal{J}$ = 7.7, 6.0 Hz, 1H), 4.30 (dd, $\mathcal{J}$ = 8.6, 6.4 Hz, 1H), 3.55 - 3.25 (m, 5H), 2.04 (s, 3H), 1.97 - 1.57 (m, 11H), 1.51 - 1.42 (m, 36H), 0.91 (d, $\mathcal{J}$ = 11.9 Hz, 3H), 0.90 (d, $\mathcal{J}$ = 12.0 Hz, 3H).

5.2.1.6. BocArg(MTr)CHN2 (**166**)

Commercially available BocArg(MTr)-OH (**165**) (500 mg, 1.03 mmol, 1.0 equiv) was dissolved in THF (10 mL) and cooled at -20ºC. Over this solution NMM (0.17 mL, 1.54 mmol, 1.5 equiv) was added, followed by isopropyl chloroformate 1.0 M in toluene (1.54 mL, 1.54 mmol, 1.5 equiv) and the reaction mixture was stirred for 4 h at the same temperature. The mixture was then filtered off and an ethereal solution of diazomethane[11] (4 mL) was added at -20ºC. The reaction was allowed to reach 0ºC and after 1 h, the solvent was removed under vacuum and the corresponding crude diazo ketone was purified by flash column chromatography (EtOAc) to obtain pure diazo **166** (270 mg, 52%) as white needles, whose spectral data matched literature values.[10]

5.2.1.7. H-Arg(MTr)CH_2Cl·HCl (**159**)

BocArg(MTr)CHN_2 (270 mg, 0.53 mmol, 1.0 equiv) was dissolved in THF (5 mL) and allowed to react with methanolic HCl 1.8 N (1.5 mL, 2.6 mmol, 5.0 equiv) at rt until nitrogen evolution ceased (~40 min). The solvent was removed by evaporation at rt and the residue was taken up in 5 mL of 1.8 N methanolic HCl under N_2. After stirring the solution for 30 min at rt, 240 mg of a white solid was obtained by evaporating the solvent. The residual solvent was removed in vacuo over a 24 h period and the resulting product was used in subsequent reactions without further purification.[10]

5.2.1.8. Ac-L-Arg(Boc)-2-L-Val-L-Arg(Boc)-2-$ClCH_2$-Arg(Boc)(MTr) (**167**)

The corresponding acid **158** (27 mg, 31 μmol, 1.0 equiv) was then dissolved in dry CH_2Cl_2 (1 mL) and over this solution HOBt (5 mg, 33 μmol, 1.0 equiv) and DIC (8 μL, 50 μmol, 1.5 equiv) were added at rt. The

reaction mixture was stirred for 10 min after which the hydrochloride **159** (15 mg, 33 μmol, 1.0 equiv) dissolved in CH_2Cl_2 (1 mL) was added, followed by DIPEA (23 μL, 132 μmol, 4.0 equiv). The reaction mixture was stirred overnight at 25ºC and then diluted with CH_2Cl_2 and H_2O. Both phases were separated, and the aqueous layer extracted with CH_2Cl_2 twice. Combined organic extracts were then washed with water and brine, filtered and concentrated to obtain protected tetrapeptide **167** that was used in the next step without further purification.

5.2.1.9. Ac-L-Arg-L-Val-L-Arg-L-Arg-CH_2Cl (**157**)

Tetrapeptide **167** was dissolved in a 95% aqueous TFA solution (160 μl) and treated dropwise with thioanisole (8 μL, 66 μmol, 14.0 equiv). The resulting dark solution was stirred for 20 h at rt and then concentrated under vacuum. The corresponding unprotected peptide was purified by preparative HPLC (30% CH_3CN/H_2O) to afford 3 mg of the tris-trifluoracetate salt **157** as a colourless oil (64% yield over 3 steps).

^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.49 (d, $\mathcal{J}$ = 6.9 Hz, 1H), 8.37 (d, $\mathcal{J}$ = 6.7 Hz, 1H), 8.35 8.23 (m, 2H), 8.10 (dd, $\mathcal{J}$ = 24.7, 7.8 Hz, 2H), 7.71 - 7.57 (m, 3H), 7.56 7.52 (m, 1H), 7.49 - 6.77 (m, 10H), 4.54 (s, 1H), 4.34 - 4.15 (m, 4H), 4.13 - 4.05 (m, 2H), 3.15 (d, $\mathcal{J}$ = 4.3 Hz, 4H), 3.13 - 3.10 (m, 2H), 2.03 - 1.87 (m, 2H), 1.84 (d, $\mathcal{J}$ = 3.8 Hz, 3H), 1.69 - 1.56 (m, 3H), 1.54-1.37 (m, 5H), 0.87 - 0.80 (m, 4H); HRMS (ESI) *m/e* 1002.90 calcd for $C_{32}H_{53}ClF_9N_{13}O_{11}$ $[M + H]^+$, found 1003.2596.

5.3. Solid Phase Synthesis of Epibestatin

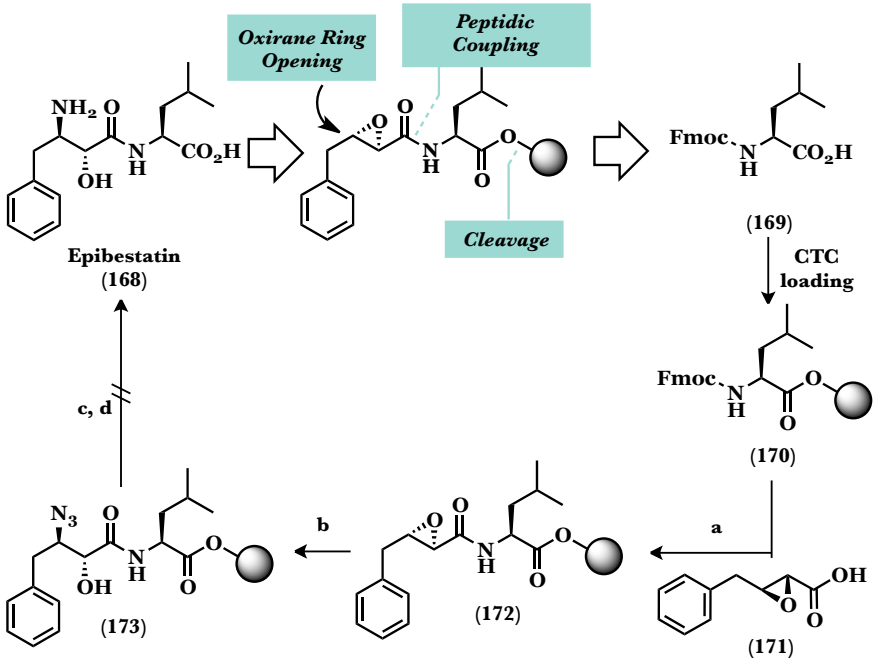
Bestatin analogue [(2R,3R)-3-amino-2-hydroxy-4-phenyl-butanoyl]-L-leucine (**168**), also known as epibestatin, was first described by Takita et al. who isolated it from a culture of *Streptomyces olivoreticuli* in 1976.[12,13] Although bestatin, as well as the others analogues, is a dipeptide with potent cytotoxicity against aminopeptidase B and leucine aminopeptidase, the case of epibestatin is particularly interesting. If the inhibitory activity of bestatin is attributable to five-membered chelate ring formation by a pair of adjacent amino and hydroxyl groups of bestatin and a metal ion of the enzyme, the isomers possessing erythro-AHPA, as for the case of epibestatin, do not form the chelate ring, resulting in a loss of the inhibitory activity.[12]

Nevertheless, epibestatin was recently identified as one of the molecules that selectively stabilize the 14-3-3/PMA2 complex,[14] involved in the regulation of several hundred proteins, among them important pharmaceutical targets such as Raf, p53, Cdc25, Cdk2, and histone deacetylases (HDACs).[15] This remarkable activity makes epibestatin a valuable tool in investigating the biology of 14-3-3 protein-protein interactions and migh be promising starting points for the development of medicinally active agents.[14]

Although a bunch of synthesis of epibestatin has been already described, we decided to apply the solid phase peptide synthesis in combination with the chiral sulfur ylide methodology to prepare it in efficient, stereoselective and expedient manners. Thus, the route depicted in **Scheme 5.6** constitutes a straightforward procedure for making epibestatin (**168)** in solid phase. The process involves the linking of the starting amino acid Fmoc-L-Leu-OH onto the 2-chlorotrityl chloride (CTC) resin by esterification with DIPEA to afford resin **170**. Subsequent removal of the Fmoc group by treatment with piperidine in DMF and coupling with the epoxy acid **171** prepared according to our asymmetric

epoxidation methodology, by the action of DIC in the presence of HOBt in DMF provided resin **172**. For the azide opening of the corresponding epoxy peptide **172** different conditions were tested and we found that opening of the oxirane ring with $Mg(N_3)_2$, prepared *in situ* by combination of NaN_3 and $MgSO_4$ in DMF at 65ºC seemed to be the most efficient, as described by Sharpless et al.[16] Despite several attemps were tested to reduce the azido group *via* Staudinger's reaction, no signicant trace of epibetatin was obtained after resin cleavage (**Scheme 5.6**).

Scheme 5.6. *Synthesis of epibestatin in solid phase*

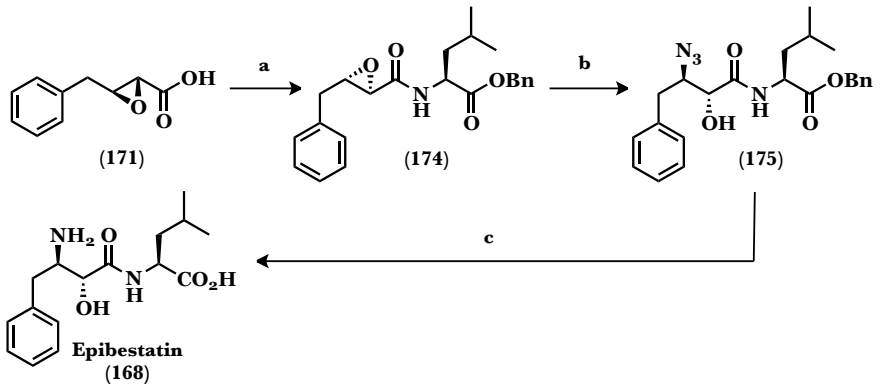


Reagents and Conditions: (a) 20% Piperidine in DMF, 25ºC, 30 min; **171**, DIC, HOBt, DMF, 25ºC, 16 h. (b) NaN_3, $MgSO_4$, DMF, 65ºC, 16 h. (c) Ph_3P, THF-H_2O (2:1), 25ºC, 16 h. (d) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 30 min.

For the synthesis of the epoxy acid **171**, known corresponding epoxy amide[17] was used as starting material, reduced into the epoxy alcohol by the action of Super-H and converted into the carboxylic acid under TEMPO/BAIB.

We therefore decided to continue with the synthesis even if solid phase methodology was discarded. Hence, epoxy acid **171** was coupled with protected Fmoc-L-Leu(OBn) by the action of DIC in the presence of HOBt, to obtain epoxy amide **174** in 94%. Then the oxirane ring opening reaction with $Mg(N_3)_2$ proceeded in a regioselective way in 40% yield by using the same conditions described above.[16] Reduction of the azide group and benzyl ester was undertaken in one step by treatment of protected azido derivative **175** with H_2 in the presence of catalyst to afford epibestatin, whose spectral data matched literature values (**Scheme 5.7**).[12]

Scheme 5.7. *Synthesis of epibestatin*



Reagents and conditions: (a) Fmoc-L-Leu(Obn), DIC, HOBt, DMF, 25ºC, 16 h, 94%. (b) NaN_3, $MgSO_4$, MeOH, 65ºC, 16 h, 40%. (c) H_2, Pd/C, EtOAc, 25ºC, 16 h, 67%.

5.3.1. Experimental Section

5.3.1.1. Synthesis of Epoxy Amide **174**

The epoxy acid **171** (60 mg, 0.33 mmol, 1.0 equiv) and the amino acid Fmoc-L-Leu(OBn) (111 mg, 0.5 mmol, 1.5 equiv) were dissolved in DMF (5 mL) and treated with DIC (0.1 mL, 0.8 mmol, 2.5 equiv), and HOBt (95 mg, 0.7 mmol, 2.0 equiv) at 25ºC. After stirring overnight at

this temperature, the crude mixture was diluted with Et_2O and washed with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O and the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. Purification of the obtained crude product by flash column chromatography (silica gel, 30% EtOAc in hexanes) provided epoxy amide **174** (118 mg, 94%) as a colourless oil.

R_f = 0.65 (Silica gel, 40% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.38 - 7.28 (m, 8H), 7.24 - 7.20 (m, 2H), 6.39 (d, $\mathcal{J}$ = 8.9 Hz, 1H), 5.20 - 5.13 (m, 1H), 4.71 - 4.59 (m, 1H), 3.30 (d, $\mathcal{J}$ = 2.1 Hz, 1H), 3.21 (ddd, $\mathcal{J}$ = 6.2, 3.9, 2.1 Hz, 1H), 3.06 (dd, $\mathcal{J}$ = 14.8, 3.9 Hz, 1H), 2.81 (dd, $\mathcal{J}$ = 14.8, 6.3 Hz, 1H), 1.75 - 1.58 (m, 3H), 1.52 (dd, $\mathcal{J}$ = 9.6, 8.0 Hz, 1H), 0.92 (d, $\mathcal{J}$ = 6.2 Hz, 3H), 0.91 (d, $\mathcal{J}$ = 6.4 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 172.19, 168.22, 135.73, 135.24, 129.13, 128.66, 128.61, 128.46, 128.24, 127.06, 67.14, 59.25, 54.90, 50.07, 41.04, 37.73, 24.81, 22.84, 21.65.

5.3.1.2. Synthesis of the Azido Alcohol **175**

A mixture of epoxy amide **174** (41 mg, 0.1 mmol, 1.0 equiv), NaN_3 (15 mg, 0.2 mmol, 2.0 equiv) and $MgSO_4$ (15 mg, 0.1 mmol, 1.0 equiv) was refluxed in MeOH overnight. After removal of the solvent, the reaction crude was purified by flash column chromatography (silica gel, 20% EtOAc in hexanes) to afford **175** (17 mg, 40%) as an amorphous white solid.

R_f = 0.60 (Silica gel, 40% EtOAc in Hexane). $[\alpha]^{25}_D$ = +7.3 (*c* 0.7, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.37 - 7.21 (m, 10H), 6.98 (d, $\mathcal{J}$ = 8.4 Hz, 1H), 4.68 - 4.57 (m, 1H), 4.34 - 4.28 (m, 1H), 3.99 (dt, $\mathcal{J}$ = 10.0, 3.8 Hz, 1H), 3.30 (d, $\mathcal{J}$ = 4.8 Hz, 1H), 3.02 (dd, $\mathcal{J}$ = 14.2, 3.6 Hz, 1H), 2.89 (dd, $\mathcal{J}$ = 14.2, 10.0 Hz, 1H), 1.76 - 1.56 (m, 5H), 0.96 (d, $\mathcal{J}$ = 6.2

Hz, 6H).^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 172.75, 170.16, 137.24, 129.47, 128.61, 126.89, 73.47, 66.19, 52.41, 50.65, 41.26, 35.01, 24.95, 22.80, 21.81.

5.3.1.3. Synthesis of Epibestatin **168**

Over a solution of the azido alcohol **175** (17 mg, 0.04 mmol, 1.0 equiv) in EtOAc (3 mL) was added 10% Pd/C (~ 5 mg, 0.04 mmol, 0.1 equiv). The reaction was stirred under H_2 atmosphere overnight, and then filtered off through a pad of silica gel. The crude was purified by flash column chromatography (silica gel, 5% MeOH in CH_2Cl_2) to afford **168** (8 mg, 67% as an amorphous white solid and whose spectral data matched literature values.[12]

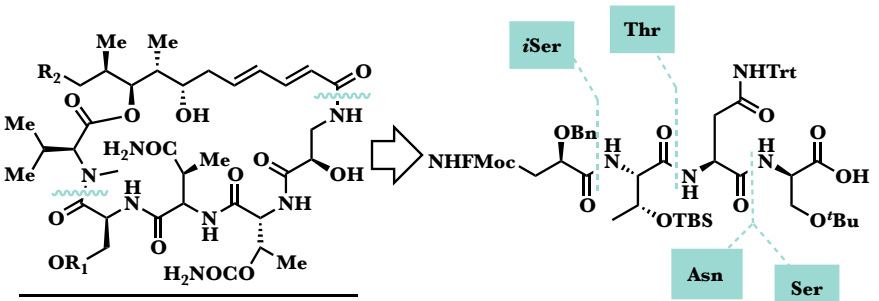
R_f = 0.4 (Silica gel, 10% MeOH in CH_2Cl_2). $[\alpha]^{25}_D$ = -1.0 (*c* 0.6, CH_2Cl_2). ^{1}H NMR (400 MHz, D_2O): 7.49 - 7.41 (m, 2H), 7.42 - 7.30 (m, 3H), 4.52 (dd, $\mathcal{J}$ = 9.4, 5.4 Hz, 1H), 4.41 (d, $\mathcal{J}$ = 3.4 Hz, 1H), 3.57 (d, $\mathcal{J}$ = 9.8 Hz, 1H), 2.95 (dd, $\mathcal{J}$ = 13.9, 4.1 Hz, 1H), 2.72 (dd, $\mathcal{J}$ = 14.0, 10.1 Hz, 1H), 1.84 - 1.67 (m, 4H), 1.00 (d, $\mathcal{J}$ = 6.1 Hz, 3H), 0.96 (d, $\mathcal{J}$ = 6.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): 173.20, 129.55, 128.68, 126.62, 124.87, 53.40, 52.29, 50.61, 29.70, 25.82, 24.96, 22.86, 21.75, 14.20. IR 2936, 2846, 2110, 1667, 1511, 1464, 1432, 1299, 1117, 1075 cm^{-1}. HRMS (H-ESI) *m/e* 309.1808 calcd for $C_{16}H_{24}N_2O_4$ $[M + H]^+$, found 309.1807.

5.4. Solid Phase Synthesis of Celebeside A

Marine sponges belonging to the *Teonellidae* family have yielded a number of unique compounds with a broad spectrum of biological activities, including antifungal, antibiotic and antitumoral.[18] Among these families and despite little has been studied, the genus of *Siliquariaspongia* possesses a wide number of biologically active natural products, such as mirabalin,[19] a potent antitumor macrolide and mirabamides A-D, glycosylated depsipeptides that inhibit HIV-1 fusion.[20]

Celebesides are a family of unusual cyclodepsipeptides recently isolated from the marine sponge *Siliquariaspongia mirabilis* by Bewley and co-workers, that contain a polyketide moiety and five amino acid residues. This compounds showed activity in HIV-1 neutralization, antifungal, and cytotoxicity assays.[21] To date six new depsipeptides of two different structural classes (celebesides A-C and theopapuamides B-D) have been isolated and the absolute configurations elucidated. (**Scheme 5.8**). Interestingly, celebeside A (**176)** exhibited biological activity as HIV-1 inhibitor, with an IC_{50} value of 1.9 ± 0.4 μg/mL as well as cytotoxicity against a human colon tumor cell line (HCT-116) with IC_{50} value of 2.1 μg/mL. As part of a research programme directed towards the total synthesis of celebeside A, we targeted the modified peptidic fragment of celebeside A (**179)**, which lacks the methyl group at the Asp residue, in order to check the viability of the proposed synthetic strategy. Having been described the synthesis of the polyketide chain by Sarabia et al.[22] herein we report the solid phase synthsis of the modified peptide fragment **179** (**Scheme 5.8**).

Scheme 5.8. *Retrosynthesis of the peptide fragment of celebeside*

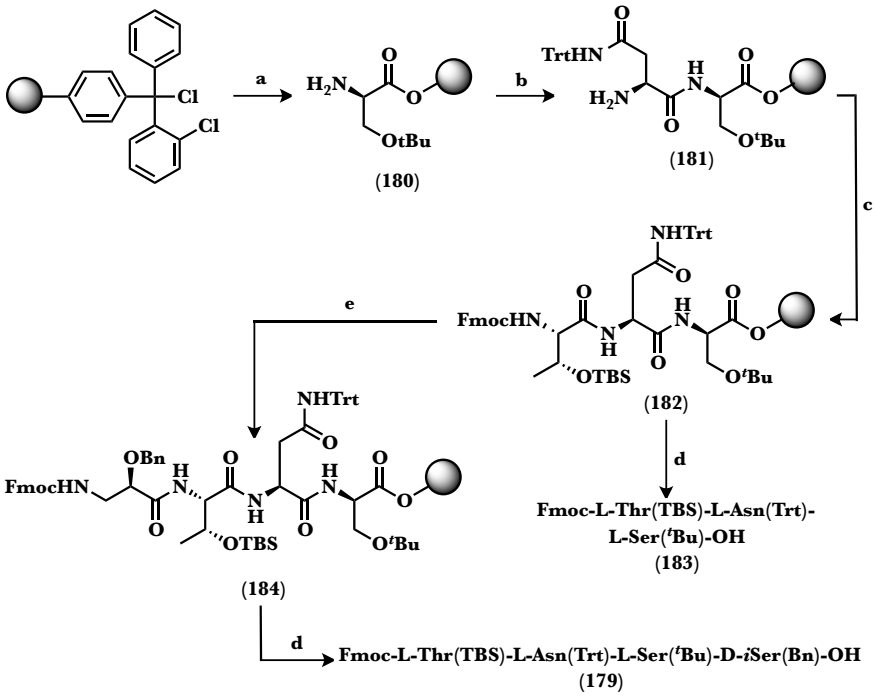


(176) Celebeside A $R_1 = PO_3H_2$; $R_2 = C_2H_5$
(177) Celebeside B $R_1 = PO_3H_2$; $R_2 = CH_3$
(178) Celebeside C $R_1 = H$; $R_2 = C_2H_5$

Modified peptide (179)

In this manner, for the synthesis of the tetrapeptide **179**, Fmoc-L-Ser(*t*Bu)-OH was linked onto the 2-chlorotrityl chloride (CTC) resin by esterification with DIPEA to afford resin **180**. The removal of the Fmoc group by treatment of piperidine in DMF, and subsequent peptide coupling with Fmoc-L-Asn(Trt)-OH by the action of DIC in the presence of HOBt in DMF afforded Fmoc resin **181**. A second treatment with piperidine in DMF followed by coupling with Fmoc-L-Thr(OTBS), under the same conditions, provided Fmoc resin **182**. In order to check the loading of the amino acid we decided to cleave the tripeptide at this stage by treatment of **182** with CH_2Cl_2/AcOH/CF_3CH_2OH (TFE) (7:2:1), which gave pure tripeptide **183**. The synthesis was continued by repeating the procedure of Fmoc deprotection and coupling steps for Fmoc-D-*i*Ser(Bn)-OH. The treatment with piperidine to remove the Fmoc group and subsequent cleavage with CH_2Cl_2/AcOH/CF_3CH_2OH (7:2:1) provided pure tetrapeptide **179** in an amount that revealed a load of 0.2 mmol/g.

Scheme 5.9. *Synthesis of tetrapeptide* ***179***



Reagents and Conditions: (a) Fmoc-L-Ser(*t*Bu)-OH, DIPEA, DMF, 25ºC, 24 h. (b) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-Asn(Trt)-OH, DIC, HOBt, DMF, 25ºC, 24 h. (c) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-L-Thr(OTBS)-OH, DIC, HOBt, DMF, 25ºC, 24 h. (d) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 25ºC, 30 min. (e) 20% Piperidine in DMF, 25ºC, 30 min; Fmoc-D-*i*Ser(Bn)-OH, DIC, HOBt, DMF, 25ºC, 24 h.

5.4.1. Experimental Section

5.4.1.1. 2-Chlorotrityl-L-Ser(ᵗBu) Fmoc Resin (**180**)

A 5 mL polypropylene syringe fitted with polyethylene porous disk charged with 2-chlorotrityl chloride resin (200 mg, L = 1.2 mmol/g, 0.24 mmol, 1.0 equiv), was loaded with a solution of Fmoc-L-Ser(ᵗBu)-OH (184 mg, 0.48 mol, 2.0 equiv) and DIPEA (0.1 mL, 0.6 mmol, 2.5 equiv) in dry DMF (3 ml). The resulting suspension was shaken at 300 rpm for 24 h, the solution was unloaded, and the resin was washed by shaking with dry DMF (5 x 3 mL). The resulting swelled resin was used in the next step.

5.4.1.2. 2-Chlorotrityl-L-Ser(ᵗBu)-L-Asn(Trt) Fmoc Resin (**181**)

The polypropylene syringe loaded with the swelled resin **180** was treated with a 20% piperidine solution in DMF (2 x 3 ml x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-L-Asn(Trt)-OH (286 mg, 0.48 mmol, 2.0 equiv), HOBt (66 mg, 0.48 mmol, 2.0 equiv) and DIC (0.1 mL, 0.6 mmol, 2.5 mmol) in dry DMF (3 mL). The resulting suspension was shaken at 300 rpm for 24 h, and then, the solution was unloaded and the resin washed with dry DMF (5 x 3 mL). The corresponding swelled resin was used in the next step.

5.4.1.3. 2-Chlorotrityl-L-Ser(ᵗBu)-L-Asn(Trt)-L-Thr(TBS) Fmoc resin (**182**)

The polypropylene syringe loaded with the swelled resin **181** was treated with a 20% piperidine in DMF (2 x 3 mL x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-L-Thr(TBS)-OH (220 mg, 0.48 mmol, 2.0 equiv), HOBt (66 mg, 0.48 mmol, 2.0 equiv) and DIC (105 μL, 0.6 mmol, 2.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 300 rpm for 24

h, and then, the solution was unloaded and the resin washed with dry DMF (5 x 3 mL). The corresponding swelled resin was used in the next step. Release of a small amount of peptide from resin **181** (17.5 mg) by treatment with CH_2Cl_2/AcOH/TFE (1.0 mL, 7:2:1) gave the *N*-Fmoc protected tripeptide **183** (11 mg), which revealed a load of 0.4 mmol/g for resin **182**.

Data for **183**: ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.95 (d, $\mathcal{J}$ = 7.3 Hz, 1H), 7.76 (dd, $\mathcal{J}$ = 7.6, 2.2 Hz, 2H), 7.58 (t, $\mathcal{J}$ = 7.3 Hz, 1H), 7.52 (d, $\mathcal{J}$ = 7.5 Hz, 1H), 7.43 - 7.33 (m, 2H), 7.32 - 7.13 (m, 19H), 6.97 (bs, 1H), 5.71 (d, $\mathcal{J}$ = 7.1 Hz, 1H), 4.88 - 4.74 (m, 1H), 4.59 - 4.46 (m, 1H), 4.41 (dd, $\mathcal{J}$ = 6.4, 3.1 Hz, 1H), 4.36 - 4.29 (m, 2H), 4.21 - 4.11 (m, 2H), 3.75 (dd, $\mathcal{J}$ = 8.8, 4.2 Hz, 1H), 3.44 (t, $\mathcal{J}$ = 7.6 Hz, 1H), 3.01 - 2.87 (m, 1H), 2.70 (dd, $\mathcal{J}$ = 15.4, 6.9 Hz, 1H), 1.13 (s, 9H), 1.12 (d, $\mathcal{J}$ = 6.1 Hz, 1H), 0.89 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 172.07, 171.03, 170.04, 169.85, 156.31, 144.30, 144.04, 143.64, 141.30, 141.26, 128.72, 127.93, 127.71, 127.67, 127.04, 125.23, 125.15, 119.92, 74.56, 70.91, 68.14, 67.22, 60.76, 60.04, 52.94, 49.79, 47.12, 38.47, 29.70, 27.22, 25.87, 19.29, -4.66, -4.92.

5.4.1.4. Fmoc-D-*i*Ser(OBn)-L-Thr(TBS)-L-Asn(Trt)-L-Ser(*t*Bu) (179)

The polypropylene syringe loaded with a portion of the swelled resin **182** (85 mg) was treated with a 20% piperidine in DMF (2 x 3 mL x 10 min). After the last run, the resin was washed with dry DMF (5 x 3 mL) and loaded with a solution of Fmoc-D-*i*Ser(OBn) (28 mg, 0.07 mmol, 2.0 equiv), HOBt (10 mg, 0.07 mmol, 2.0 equiv) and DIC (10.0 μL, 0.08 mmol, 2.5 equiv) in dry DMF (3 mL). The resulting suspension was shaken at 300 rpm for 24 h, and then, the solution was unloaded and the resin washed with dry DMF (5 x 3 mL). The corresponding swelled resin was used in the next step. Release of peptide from resin **184** by treatment

with CH_2Cl_2/AcOH/TFE (1.0 mL, 7:2:1) gave the *N*-Fmoc protected tripeptide **179** (20 mg), which revealed a load of 0.2 mmol/g for resin **184**.

Data for **179**: ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.69 (d, $\mathcal{J}$ = 15.1 Hz, 1H), 8.10 (d, $\mathcal{J}$ = 8.6 Hz, 1H), 7.87 (dd, $\mathcal{J}$ = 7.5, 2.4 Hz, 1H), 7.69 (dd, $\mathcal{J}$ = 7.7, 3.0 Hz, 1H), 7.50 (dd, $\mathcal{J}$ = 14.3, 7.3 Hz, 2H), 7.42 - 7.02 (m, 26H), 5.00 - 4.86 (m, 1H), 4.66 - 4.54 (m, 1H), 4.51 (d, $\mathcal{J}$ = 11.8 Hz, 1H), 4.42 (d, $\mathcal{J}$ = 8.5 Hz, 1H), 4.31 - 4.21 (m, 2H), 4.17 (dd, $\mathcal{J}$ = 15.0, 7.7 Hz, 1H), 4.07 - 3.89 (m, 2H), 3.60 - 3.40 (m, 4H), 3.12 (td, $\mathcal{J}$ = 14.3, 13.4, 6.2 Hz, 1H), 2.75 (d, $\mathcal{J}$ = 15.0 Hz, 1H), 2.62 (d, $\mathcal{J}$ = 6.3 Hz, 1H), 2.38 - 2.24 (m, 1H), 1.03 (s, 9H), 0.82 (s, 3H), 0.77 (s, 9H), 0.02 (s, 3H), -0.02 (s, 3H). HRMS (H-ESI) *m/e* calcd for $C_{65}H_{77}N_5O_{11}$ 1154.52811 $[M + Na]^+$, found 1154.52905.

5.5. Notes and References

(1) Inukai, M.; Enokita, R.; Torikata, A.; Nakahara, M.; Iwado, S.; Arai, M. *J. Antibiot.* **1978**, *31*, 410-420.

(2) Inukai, M.; Nakajima, M.; Osawa, M.; Haneishi, T.; Arai, M. *J. Antibiot.* **1978**, *31*, 421-425.

(3) Nakajima, M.; Inukai, M.; Haneishi, T.; Terahara, A.; Arai, M.; Kinoshita, T.; Tamura, C. *J. Antibiot.* . **1978**, *31*, 426-432.

(4) Inukai, M.; Takeuchi, M.; Shimizu, K. *Agric. Biol. Chem.* **1984**, *48*, 513-518.

(5) Mallea, M.; Chevalier, J.; Eyraud, A.; Pages, J. -M. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 1370-1373.

(6) Fassi Fehri, L.; Wróblewski, H.; Blanchard, A. *Antimicrob. Agents Chemother.* **2007**, *51*, 468-474.

(7) Kiho, T.; Nakayama, M.; Yasuda, K.; Miyakoshi, S.; Inukai, M.; Kogen, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2315-2318.

(8) Kogen, H.; Kiho, T.; Nakayama, M.; Furukawa, Y.; Kinoshita, T.; Inukai, M. *J. Am. Chem. Soc.* **2000**, *122*, 10214-10215.

(9) Sarabia, F.; Chammaa, S.; García-Ruiz, C. *J. Org. Chem.* **2011**, *76*, 2132-2144.

Sun, A.; Shoji, M.; Lu, Y. J.; Liotta, D. C.; Snyder, P. *J. Med. Chem.* **2006**, *49*, 3153-3158.

(11) Preparation of diazomethane: In a two-necked round botton flask was dissolved KOH 85% (0.92 g, 14 mmol, 1.0 equiv) in a mixture of H_2O (1.3 mL), di(ethylene glycol) ethyl ether (4.7 mL) and Et_2O (1.3 mL). The reaction mixture was warmed at 70-80ºC and a solution of diazald (3.0 g, 14 mmol, 1.0 equiv) in Et_2O (15 mL) was then added dropwise to the mixture. After few minutes, the colourless ethereal solution of CH_2N_2 is collected by destillation in a round flask and the reaction finishes when the destillated solution turns to pale yellow.

(12) Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. *Arch. Biochem. Biophy.* **1976**, *177*, 196-200.

(13) Nishizawa, R. Saino, T. *J. Med. Chem.* **1977**, *20*, 510-515.

(14) Rose, R.; Erdmann, S.; Bovens, S.; Wolf, A.; Rose, M.; Hennig, S.; Waldmann, H.; Ottmann, C. *Angew. Chem. Int. Ed.* **2010**, *49*, 4129-4132.

(15) Hermeking, H. *Nat. Rev. Cancer.* **2003**, *3*, 931-943.

(16) Behrens, C. H. Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696-5704.

(17) Sarabia, F.; Chammaa, S.; García-Castro, M.; Martín-Gálvez, F. *Chem. Commun.* **2009**, 5763-5765.

(18) Matsunaga, S. Fusetani, N. *Curr. Org. Chem.* **2003**, *7*, 945-966.

(19) Plaza, a.; Baker, H. L.; Bewley, C. A. *J. Nat. Prod.* **2008**, *71*, 473-477.

(20) Plaza, A.; Gustchina, E.; Baker, H. L.; Kelly, M.; Bewley, C. A. *J. Nat. Prod.* **2007**, *70*, 1753-1760.

(21) Plaza, A.; Bifulco, G.; Keffer, J. L.; Lloyd, J. R.; Baker, H. L.; Bewley, C. A. *J. Org. Chem.* **2009**, *74*, 504-512.

(22) Sarabia, F.; Vivar-García, C.; García-Ruiz, C.; Sánchez-Ruiz, A.; Pino-González, M. S.; García-Castro, M.; Chammaa, S. *Eur. J. Org. Chem.* **2014**, 3847-3867.

CHAPTER 6

Conclusions and Future Work

In summary, the versatility and utility of the chiral sulfonium salts have been demonstrated once again with the synthesis of natural products gummiferol and depudecin, and the synthetic epoxy analogues of bengamides. The synthetic value of this protocol has been applied to the construction of epoxy and di-epoxy key fragments that provide direct access to each of the targeted molecules. To this end, a general and efficient method for the preparation of enantiopure *trans* di-epoxides in 4 steps within moderate to good yields was developed.

Biological evaluation of bengamide analogues **45-46** and **50** did not reveal a fumagillin-like interaction with MetAP, as we initially assumed. The latter result implicates that the polyol structure of bengamides is not amenable to modification, which is in accordance with previous reported works.

Additionally, the first steps towards a new type of bengamide analogues (aminobengamides) have been established and consolidated with the synthesis of the azido derivative **71**, in which the azido group can be easily inserted by using the Miyashita's procedure that rendered *syn* azido alcohols.

From a biological point of view, testing of a library of amino bengamides analogues with variations in both the caprolactam moiety as well as the olefin may provide more potent derivatives in virtue of the known affinity of Co^{+2} for amino groups. Hence, cytotoxicity essays would be very helpful in acquiring detailed knowledge regarding the relationship between structure and activity. As a continuation of this work, we are pursuing efforts to increase the number of new analogues and at present, attemps are being made to synthesize 2- and 4- amino bengamides.

The synthesis of the precursor of gummiferol, has been completed in 8 steps and 8% overall yield. In this way, an alternative formal synthesis to the procedure described by Takamura and co-workers has been accomplished. Although the last steps towards gummiferol remains still innaccesible, we feel confidents in deprotecting the silyl ether and complete the total synthesis.

The synthetic value of the epoxidation protocol based on sulfur ylide has been also demonstrated by its application in the concise total synthesis of the non-natural enantiomer of depudecin. Although a final step still remains unaffordable, we trust that the total synthesis could be complete, by conversion of the key fragment triepoxy alcohol **144** into the final product. This approach should show broad application in the total synthesis of a range of depudecin analogues thereof.

In order to achieve further knowledge in other kind of synthesis, throughout this time the solid phase peptide syntheses (SPPS) of several natural products have been carried out, which has allowed us to gain insight into peptide synthesis of molecules such as globomycin, epibestatin or celebeside A. This efficient methodology, together with the use of chiral sulfur ylides will open new doors to the total synthesis of more complex an active natural products.

Resumen

La identificación de compuestos capaces de interaccionar de forma selectiva con objetivos biológicos específicos y predeterminados, con el fin de inhibirlos o activarlos, representa la base para el descubrimiento y desarrollo de nuevos fármacos. Además, esta capacidad permite obtener una valiosa información para la comprensión del mecanismo de acción biológica. Surge así la definida como Biología Química, que incluye el uso de compuestos bioactivos como sondas de dianas biológicas, fuente de nuevos cabezas de serie en el tratamiento de una determinada enfermedad, o como moléculas usadas en la comunicación celular y procesos de señalización.

Entre todos los grupos funcionales, el grupo oxirano representa uno de los más versátiles en química orgánica, debido a su posible funcionalización estereoespecífica y regioselectiva por apertura del anillo con diferentes nucleófilos, dando lugar a compuestos bifuncionales, resultando muy útil para la síntesis de fármacos y productos naturales.

Dada la importancia química y biológica de la función del anillo de oxirano, se han desarrollado múltiples métodos de epoxidación. De entre todos los métodos destacan aquellos que generan el epóxido de forma enantioselectiva desde compuestos carbonílicos: bien por

olefinación de Wittig seguida de una oxidación enantioselectiva del doble enlace proquiral (métodos oxidativos), o mediante el empleo de un iluro, un carbeno o el reactivo de Darzens (métodos no oxidativos) a través de una cicloadición enantioselectiva con un carbonilo proquiral.

Dentro de las metodologías oxidativas, la epoxidación asimétrica de Sharpless representa uno de los mayores hitos de la síntesis orgánica moderna. Sin embargo, los métodos oxidativos presentan ciertos requerimientos estructurales (alcoholes alílicos, *cis*-alquenos, enonas, etc.) y limitaciones experimentales (tiempos de reacción elevados, contínua adición de agentes oxidantes, excesos enantioméricos moderados, etc.). No obstante, a pesar de estas limitaciones, todas estas metodologías siguen siendo herramientas sintéticas útiles para obtener epóxidos enantioméricamente puros.

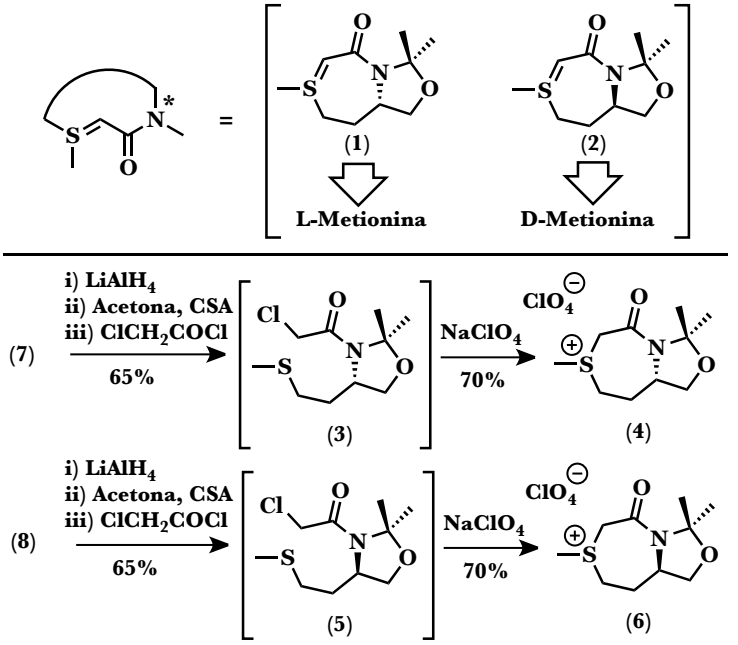
En relación a los métodos no oxidativos, de especial interés resulta el uso de iluros de azufre, que presentan geometría tetraédrica, y son capaces de inducir quiralidad en los compuestos carbonílicos. A pesar de ser reacciones en una etapa, no han sido tan ampliamente descritas como las metodologías oxidativas, aunque diferentes grupos de investigación han contribuído en este área, siendo el grupo del profesor Aggarwal el más prolífero.

Especialmente nos centraremos en los iluros de azufre estabilizados por amidas, particularmente útiles por tres razones: 1) requieren condiciones de reacción suaves y procedimientos simples para reaccionar con compuestos carbonílicos, 2) muestran un elevado diastereocontrol a favor de los epóxidos *trans*, y 3) el potencial sintético de las amidas glicídicas generadas, que presentan un excelente regiocontrol en la posición C2 en las reacciones de apertura con nucleófilos.

Tras los antecedentes sobre la síntesis de amidas glicídicas vía iluros de azufre estabilizados, nuestro grupo de investigación abordó la versión asimétrica de la síntesis de estas 2,3-epoxiamidas a través del

diseño y síntesis de una nueva clase de iluros de azufre cíclicos quirales. Esta nueva propuesta de iluro de azufre, cuya inducción asimétrica es debida al fragmento de amida, preparado desde los aminoácidos comerciales L- y D- metionina, originó la síntesis de las correspondientes sales de sulfonio, precursoras de los mencionados iluros, según la secuencia descrita en el **Esquema 1.3**, en la que, a través del intermedio de cloroacetamida (**3**) o (**5**), mediante ciclación en presencia de perclorato sódico, y después de recristalización, se obtienen sendas sales de sulfonio enantioméricamente puras (**4**) y (**6**).

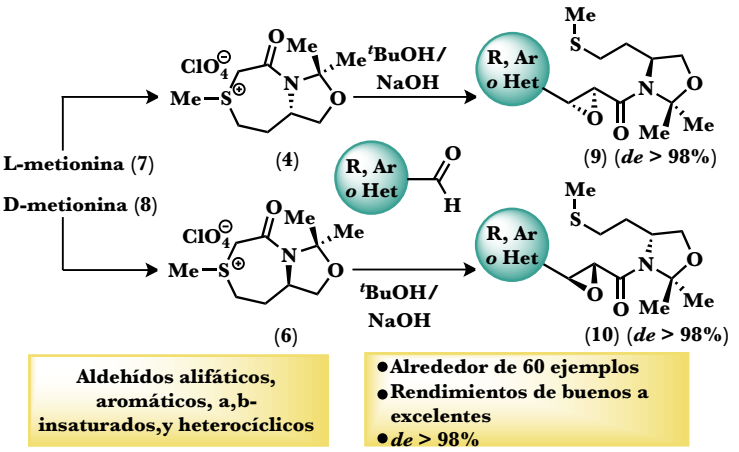
Esquema 1.3. *Diseño y síntesis de iluros quirales de azufre* ***1*** *y* ***2***



En cuanto a la reactividad, la preparación *in situ* del correspondiente iluro derivado de las sales de derivadas de L- y D-metionina (**4**) y (**6**), y su posterior reacción con aldehídos, empleando bien la metodología en una fase (*t*BuOH como disolvente y solución acuosa de NaOH) o el método de dos fases (mezcla DCM/H_2O como disolvente y solución acuosa de NaOH), empleado particularmente cuando se

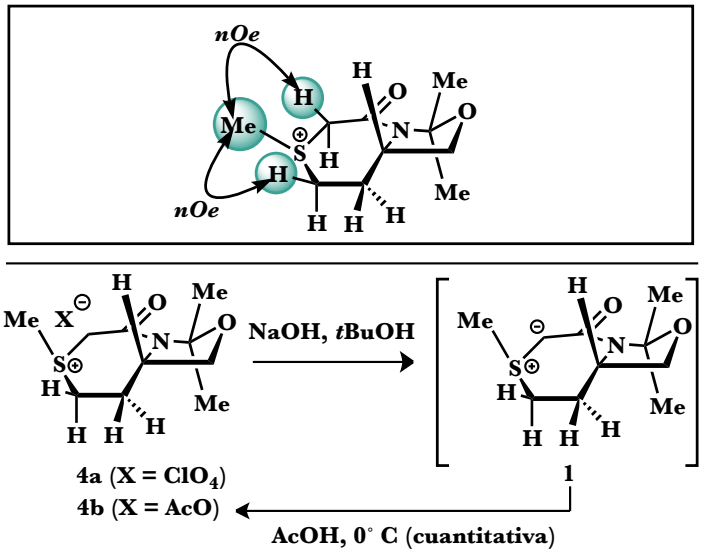
encuentran grupos hidroxilo en posiciones adyacentes al carbonilo, proporciona sólo uno de los diastereoisómeros posibles de las epoxiamidas, con un excelente control estereoquímico como muestra el **Esquema 1.4.**

Esquema 1.4. *Reacción de sales de sulfonio* ***4*** *y* ***6*** *con aldehídos*



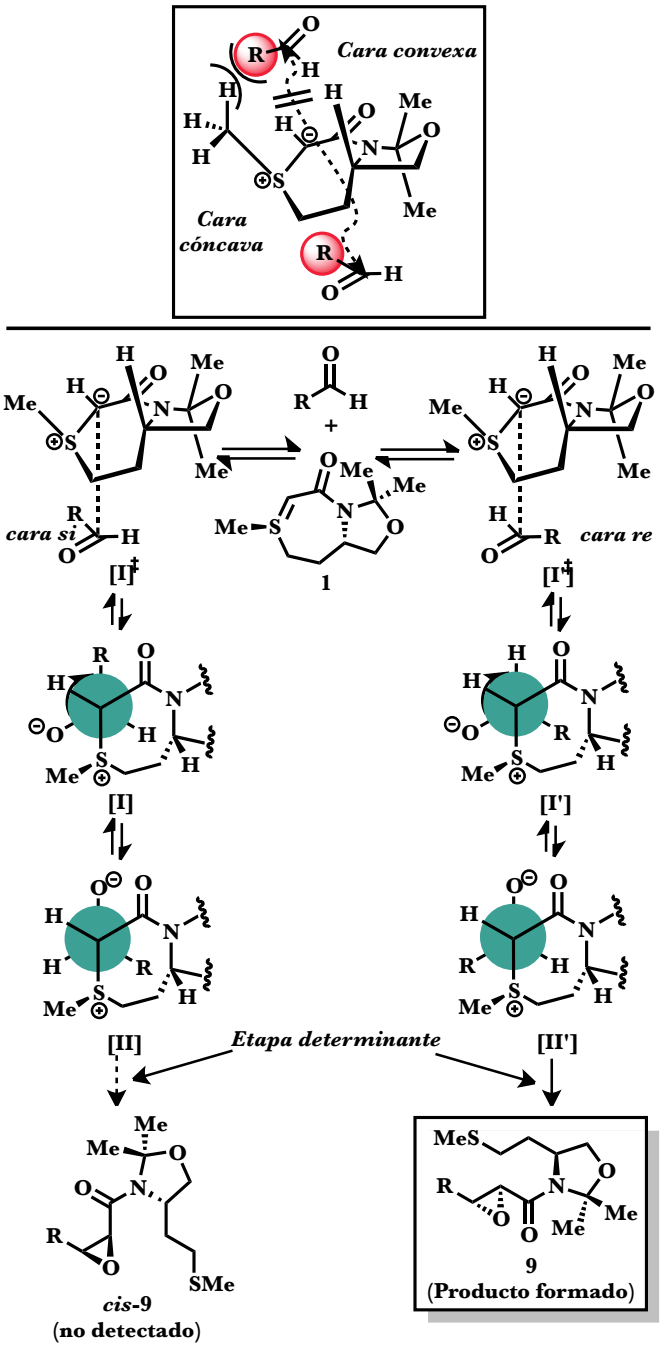
Recientemente, se ha realizado la asignación de la configuración de estas sales de sulfonio quirales, gracias a estudios de rayos-X. Asumiendo que la estereoquímica en el átomo de azufre del iluro permanece intacta con respecto a la sal de sulfonio precursora, como demostramos experimentalmente por la formación del correspondiente iluro de azufre y tratamiento con ácido acético para obtener la sal de sulfonio, con similar pureza diastereomérica, racionalizamos la estereoselectividad resultante, como se muestra en el **Esquema 1.5-B.**

Scheme 1.5-B. *Demostración de la estereoquímica en el átomo de azufre de las sales de sulfonio* ***4*** *y* ***6*** *- correlaciones NOE y experimentos químicos*



La aproximación por la cara cónvava junto con una disposición cisoide entre los reactantes, permitiría al iluro **1** atacar al aldehído por su cara *re* o *si*. Inicialmente, el ataque hacia la cara *si* preferida, debería proceder a través de los intermedios I y II, tras la rotación del enlace C-C. Este mecanismo conduciría a un epóxido tipo *cis* no detectado debido posiblemente a la alta barrera requerida para el cierre del anillo. El ataque se produce por tanto por la cara *re* originando los intermedios I' y II', siendo el cierre del anillo más favorable en este caso, y proporcionando las *trans* epoxi amidas observadas.

Esquema 1.6. *Razonamiento teórico de la epoxicación*



De esta forma, tras haber desarrollado recientemente una metodología válida para la síntesis de epóxidos, el siguiente paso era la aplicación de la misma a la síntesis de productos naturales y análogos que presenten una actividad biológica interesante como agentes antitumorales y/o antibióticos, como ya se hizo en trabajos anteriores para la síntesis de análogos de bengamidas o de los ciclodepsipéptidos naturales Globomicina y SF-1904 A_5.

El principal objetivo del trabajo desarrollado en esta Tesis Doctoral ha sido la síntesis total de productos naturales y/o análogos de las familias de **bengamidas, gummiferol** y **depudecina**, conteniendo grupos epóxido cuya construcción se realizaría aplicando la metodología anteriormente descrita. Adicionalmente, durante el transcurso de esta investigación se ha llevado a cabo un proyecto paralelo de síntesis orgánica en fase sólida, completando la síntesis de las cadenas peptídicas de diferentes productos naturales de interés biológico como **globomicina**, **epibestatina**, y **celebésido A**, y el tetrapéptido sintético **AcArgValArgArgCMK**, que se encuentra actualmente bajo ensayos biológicos de actividad.

Bengamidas

Las Bengamidas, descubiertas en 1986 por Crews y col., comprenden una familia de productos naturales aislados de esponjas marinas de la familia *Jaspidae*, con una amplia e interesante actividad biológica como agentes antibióticos, antihelmínticos y antitumorales.

La actividad antitumoral está relacionada directamente con la inhibición de las enzimas metionina aminopeptidasas tipos I (MetAP1) y II (MetAP2), involucradas en la proliferación de células endoteliales y angiogénesis. Este mecanismo de acción es similar al exhibido por los compuestos fumagilina y ovalicina, agentes antitumorales con actividad anti-angiogénica, cuya estructura presenta notables diferencias respecto a

la de las bengamides. Particularmente, las bengamides se vinculan con el sitio activo de la enzima debido a una interacción hidrofóbica del grupo alquílico terminal de la olefina con la cavidad P1, una interacción polar de la caprolactama con la región P2 expuesta al disolvente y una coordinación entre los grupos hidroxilos C-3, C-4 y C-5 de su cadena policétida con iones cobalto presentes en el centro activo (**Figura 2.11**).

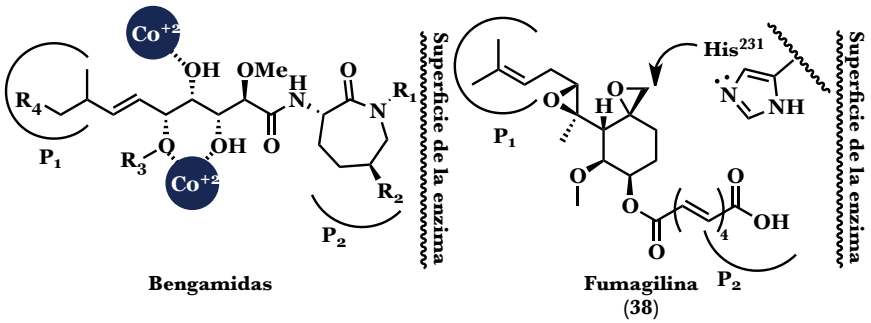


Figura 2.11. *Interacción de bengamidas y fumagilina con MetAP2*

Con el objetivo de diseñar derivados de bengamidas que puedan mimetizar la interacción de fumagilina en el centro activo de la enzima, mediante una unión covalente al átomo de nitrógeno del anillo de imidazol del residuo de His^{231}, tal y como muestra la **Figura 2.13**, se han planteado los epoxi- derivados de bengamida E como interesantes analógos. En todos ellos se han sustituído los grupos hidroxilo de la cadena policétida de las bengamidas por anillos de oxirano, cuya síntesis se plantearía mediante la metodología de epoxidación asimétrica *via* sales de sulfonio quirales. La evaluación biológica de éstos, permitiría completar estudios SAR sobre este tipo de compuestos.

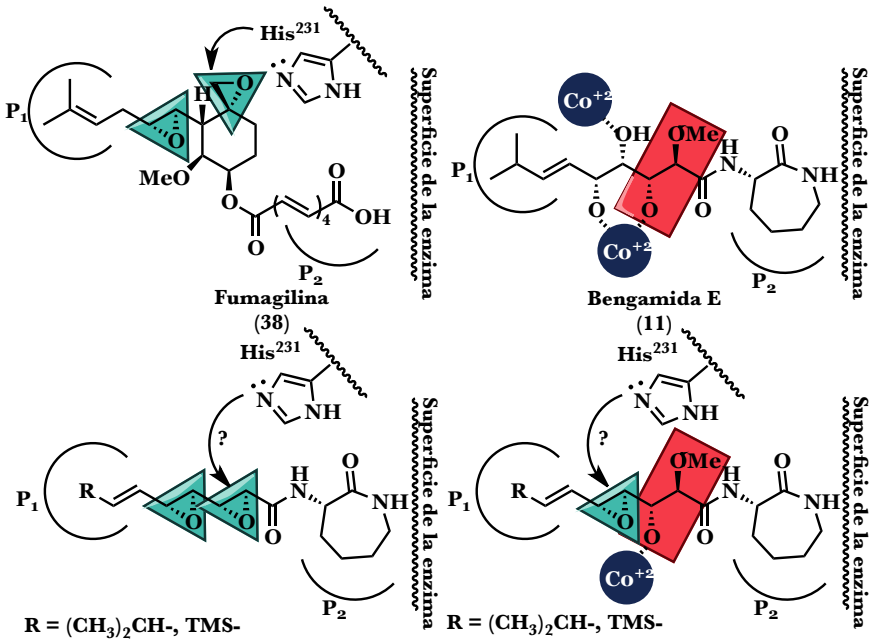


Figura 2.13. *Epoxi bengamidas como nuevos potenciales inhibidores tipo-fumagilina*

Por otro lado, se ha planteado una sustitución en la olefina terminal de las bengamidas. De esta forma, se ha reemplazado el grupo isopropilo característico de la bengamide E, por el grupo alostérico TMS- que permitiría nuevas funcionalizaciones en esta posición debida a la conocida reactividad de los vinil silanos. Adicionalmente, y aprovechando la metodología sintética descrita anteriormente, se ha iniciado la síntesis del amino derivado en C4, que puede presentar una mayor interacción con los cationes Co (II) presentes en el centro activo de la enzima, proporcionando un perfil biológico más potente (**Figura 2.14**).

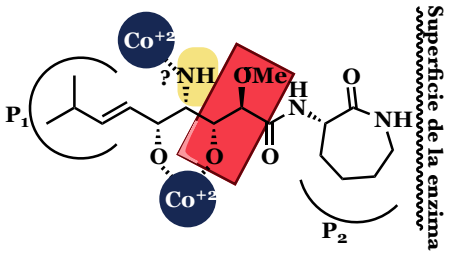
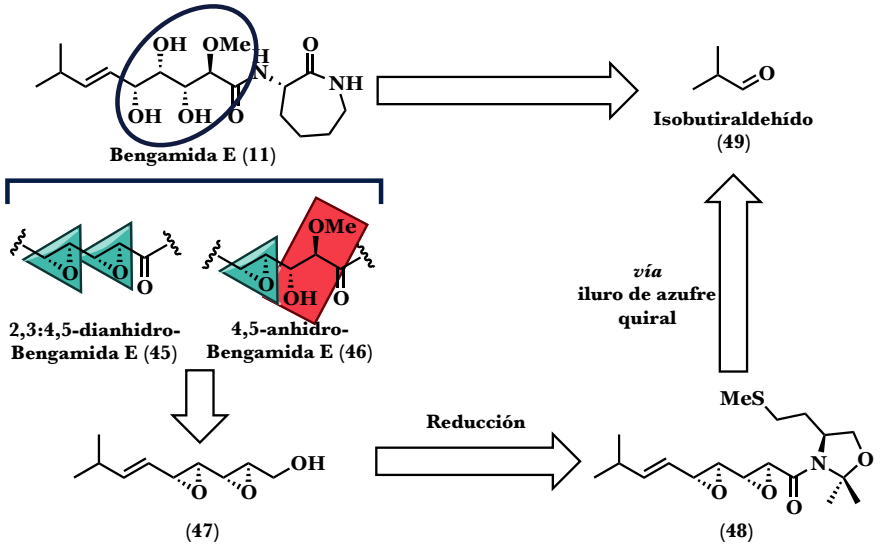


Figura 2.14. *Amino bengamidas como nuevos inhibidores*

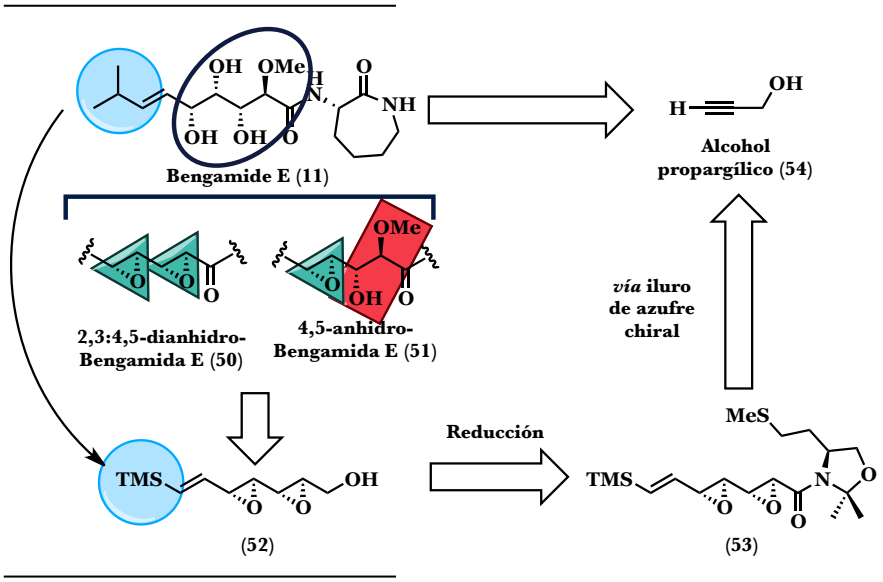
La síntesis de los 2,3:4,5-diepoxi- (**45**) y 4,5-epoxi- (**46**) derivados de bengamide E se ha establecido desde el reactivo comercial isobutiraldehído (**49)**, que proporciona el grupo isopropilo terminal presente en la parte olefínica de las bengamidas (**Esquema 2.1**).

Esquema 2.1 *Análisis retrosintético de epoxi análogos de bengamida E derivados de isobutiraldehído*



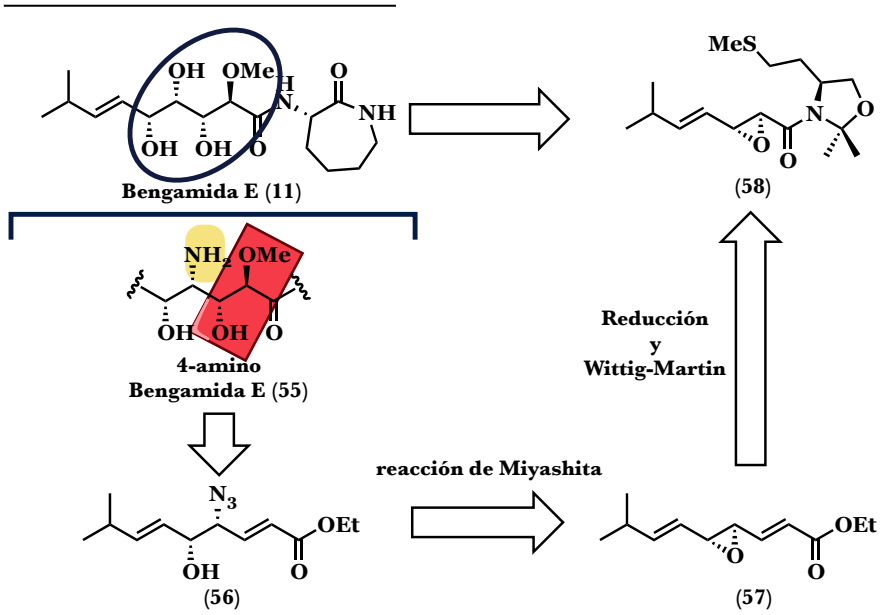
Por otra parte, los silil derivados pueden ser fácilmente obtenidos siguiendo un esquema sintético análogo empleando alcohol propargílico (**54)** como producto de partida (**Esquema 2.2**).

Esquema 2.2. *Análisis retrosintético de los epoxi- análogos de bengamida E derivados del alcohol propargílico*



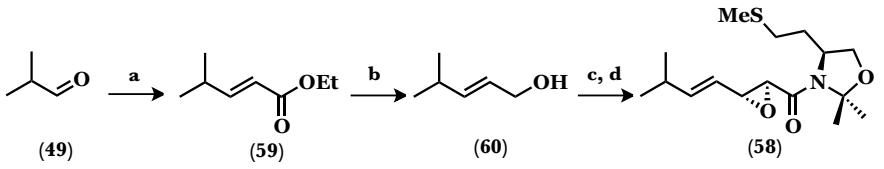
Para la síntesis del amino derivado en C4, se ha diseñado una estrategia que involucra la apertura del correspondiente ɣ,δ-epoxi éster α,β-insaturado (**57**) utilizando para ello la metodología descrita por Miyashita que proporciona *syn* azido alcoholes, tal y como muestra el **Esquema 2.3**.

Esquema 2.3. *Retrosíntesis para el análogo C4 amino bengamida E*



La síntesis de la epoxiamida **58** derivada de isobutiraldehído (**49**) y que representa un precursor común para los análogos **45**, **46** y **47** se completó tal como muestra el **Esquema 2.4**, mediante una secuencia de reacciones que implica formación y reducción del éster α,β-insaturado, posterior oxidación al aldehído y tratamiento con la sal de sulfonio procedente de L-metionina (**4**).

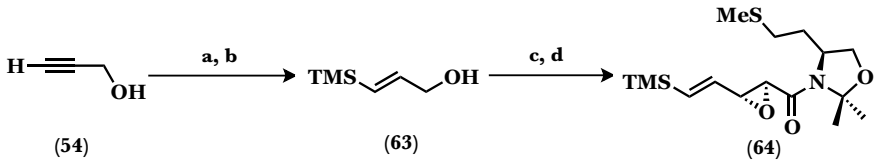
Esquema 2.4. *Síntesis de la epoxiamida **58***



Reactivos y condiciones: (a) Ph_3PCHCO_2Et, DCM, 25ºC, 87%. (b) DIBAL-H, -78ºC, 30 min, 75%. (c) MnO_2, DCM, 25ºC, 12 h. (d). **4**, 3 M NaOH, *t*BuOH, 25ºC, 16 h, 58% (sobre 2 etapas).

Por otra parte, la epoxiamida (**64**) derivada del alcohol propargílico (**54**) se sintetizó mediante la secuencia de reacciones descrita en el **Esquema 2.6**, con un rendimiento global de 75% sobre 4 etapas.

Esquema 2.6. *Sí´ntesis de la epoxiamida* ***64***

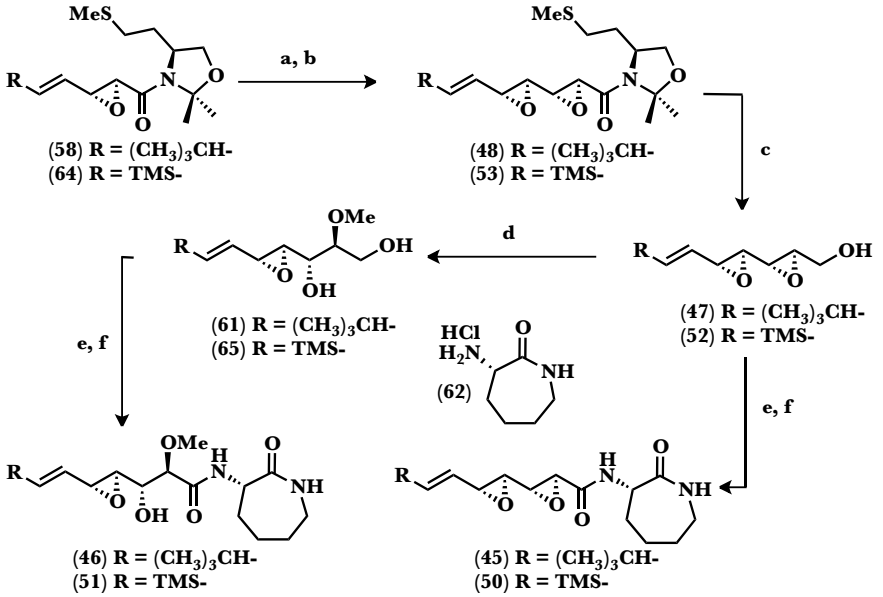


(54) **(63)** **(64)**

Reactivos y condiciones: (a) EtMgBr, TMSCl, 0ºC (b) Red-Al, THF, 0ºC, 45 min. (c) MnO_2, DCM, 16 h. (d) **4**, 5.0 M NaOH, *t*BuOH, 25ºC, 75% (rendimiento global).

La síntesis de los epoxi- y diepoxi- derivados de bengamida E se completó según muestran los **Esquemas 2.5** y **2.7**. La formación de las diepoxiamidas **48** y **53** se completó mediante reducción de las epoxiamidas **58** y **64** a los correspondientes epoxi- aldehídos, por acción de Red-Al, y posterior acoplamiento con la sal de sulfonio (**4**) en medio básico. La posterior reducción con Super-H rindió los diepoxi- alcoholes **47** y **52,** desde los que se obtuvieron los diepoxi- derivados **45** y **50**, mediante oxidación del alcohol al ácido carboxílico y posterior acoplamiento peptídico con **62**. La síntesis de los epoxi- derivados **46** y **51** requiere un paso adicional de apertura *anti* del epoxi alcohol bajo condiciones de Miyashita, completando posteriormente la síntesis como se ha descrito para los diepoxi- derivados.

Esquemas 2.5 y 2.7. *Síntesis de los epoxi- análogos de bengamida E (**45**), (**46**), (**50**) y (**51**)*



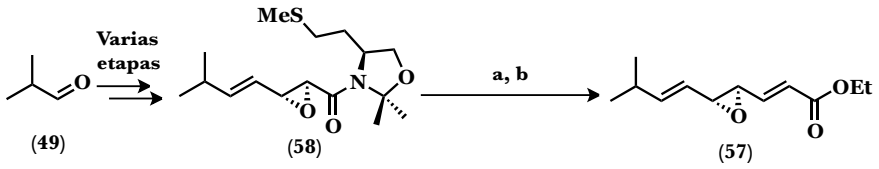
Reactivos y conditiones (a) Red-Al, THF, 0ºC, 45 min. (b) **4**, 5.0 M NaOH, CH_2Cl_2-H_2O, 25ºC, 85% para **48**, 84% para **53** (sobre 2 etapas). (c) Super-H, THF, 0ºC, 30 min, 70% para **46**, 55% para **51**. (d) $B(OMe)_3$, DBU, MeOH, reflux, 12 h. (e) TEMPO/BAIB, ACN-H_2O, 25ºC, 16 h. (f) **62**, BOP, DIPEA, DMF, 25ºC, 16 h, 55% para **45 (**desde **52**), 30% para **50** (desde **52)**, 47% para **46 (**desde **52**) ,26% para **51** (desde **52).**

Una vez obtenidos los epoxi- y diepoxi- análogos **45-46** y **50-51**, se procedió a evaluar la actividad antitumoral de los tres primeros, para determinar la influencia de las modificaciones estructurales realizadas en la actividad antiproliferativa. Así, las medidas de IC_{50} se realizaron frente a un panel de diferentes líneas de células tumorales (HL60, MDA-MB-231, HT1080, HT20 y BAE), empleando bengamide E (**11**) y fumagilina (**38)** como control. Desafortunadamente, los resultados biológicos revelaron que la sustitución en la cadena policétida por grupos oxirano producía una

pérdida total de actividad, indicando que no existe un modo de acción tipo fumagilina, como asumimos en un principio.

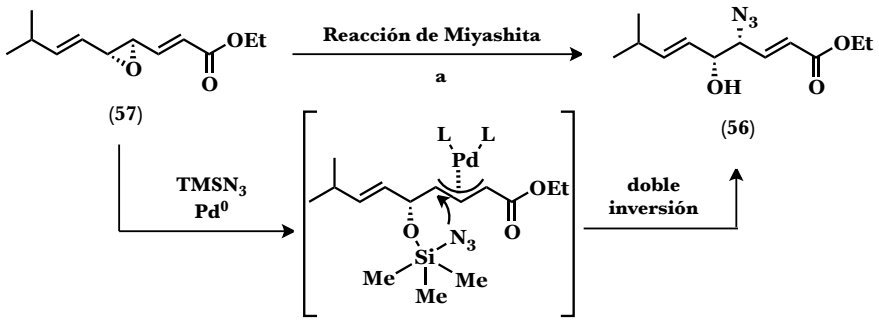
Por otro lado, para la síntesis de la C4 amino bengamida se procedió, desde la epoxiamida **58** a la obtención del epoxi- éster **57** (**Esquema 2.8**), desde el cuál, mediante la metodología de Miyashita se sintetizó el *syn* azido alcohol **56** (**Esquema 2.9**).

Esquema 2.8. *Síntesis del γ,δ-epoxi éster α,β-insaturado* ***(57)***



Reactivos y condiciones: (a) Red-Al, THF, 0ºC, 45 min. (b) $Bu_3P{=}CHCO_2Et$, CH_2Cl_2, 12 h, 82% (sobre 2 etapas).

Esquema 2.9. *Reacción de apertura estereoespecífica con azida catalizada por* Pd^0

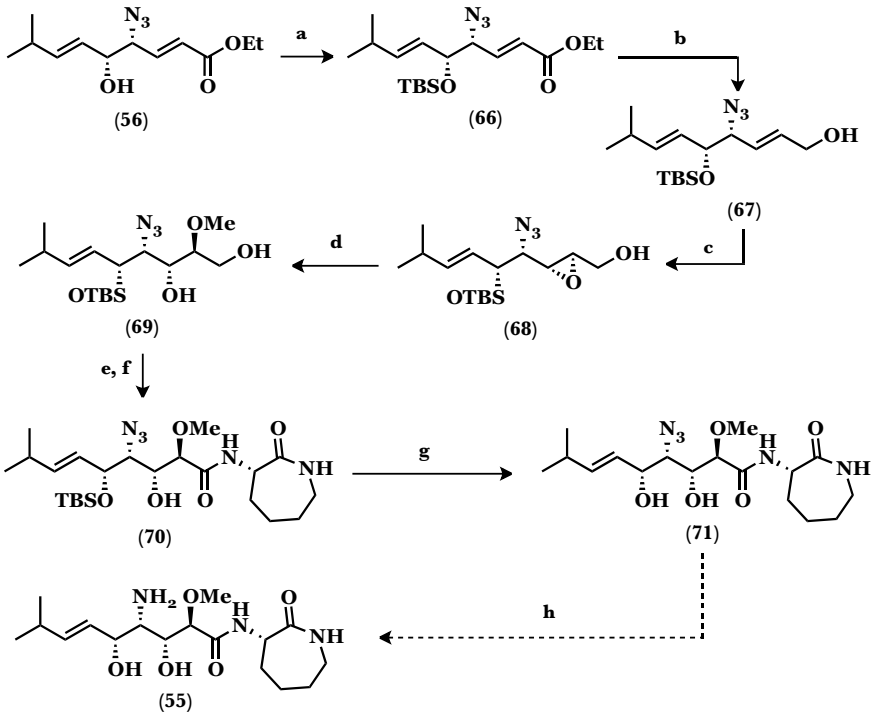


Reactivos y condiciones: (a) $TMSN_3$, $Pd(PPh_3)_4$, 25ºC, 5 h, después ácido cítrico 10% en MeOH, 82%.

Desde aquí, la síntesis se completa según describe el **Esquema 2.10**, mediante reacción de epoxidación asimétrica de Sharpless, y posterior apertura con MeOH, oxidación y acoplamiento peptídico, tal y como se realizó para los derivados anteriores. De esta forma, tras

desprotección del silil éter, se ha sintetizado el azido derivado **71**, del que se están realizando pruebas de reducción para obtener el amino análogo **55** (**Esquema 2.10**).

Esquema 2.10. *Síntesis del C4 azido y C4 amino derivado de bengamida E*



Reactivos y condiciones: (a) TBSOTf, 2,6-lutidina, CH_2Cl_2, 25ºC, cuant. (b) DIBAL-H, -78ºC, 45 min, 82%. (c) SAE, D-(-)-DET, 10%. (d) $B(OMe)_3$, DBU, MeOH, reflujo, 16 h. (e) TEMPO/BAIB, CH_3CN-H_2O, 25ºC, 16 h. (f) **62**, BOP, DIPEA, DMF, 25ºC, 16 h, 86% (sobre 3 etapas). (g) TBAF, THF, 0ºC, 40 min, 55%. h. Staudinger

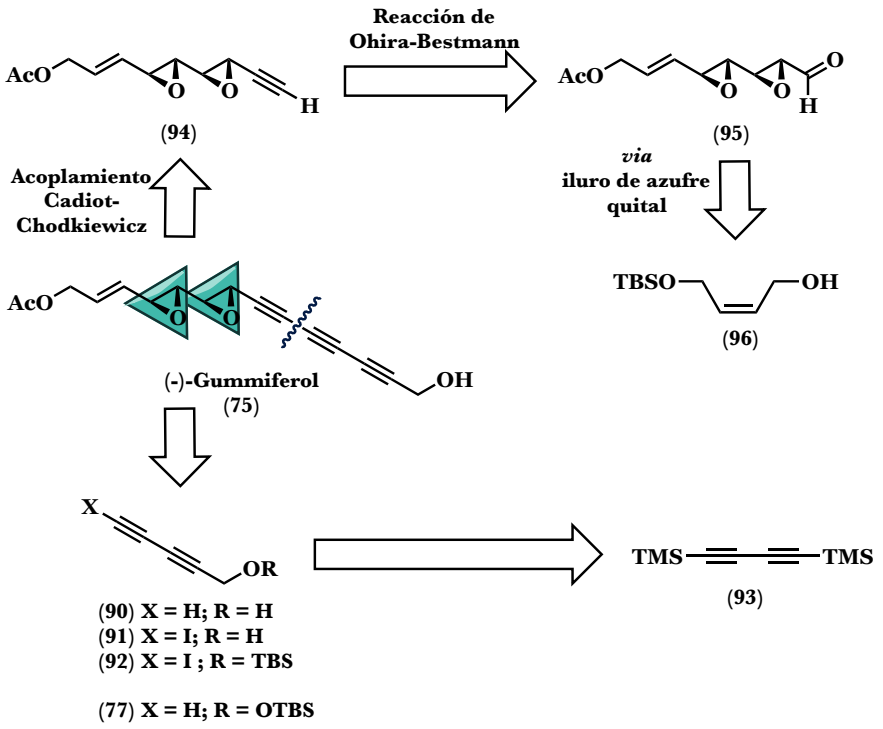
Gummiferol

El gummiferol, aislado de las hojas de *Adenia gummifera* en 1995 por Wall et al. presenta en su estructura, además de un sistema triacetilénico, dos epóxidos contiguos cuya configuración absoluta ha sido recientemente determinada por el grupo de Takamura et al. describiendo además la que es la única síntesis total hasta la fecha. Este producto exhibía un valor de ED_{50} de 1.1 μg/ml en el ensayo con células de carcinoma epidermoide humano (KB) siendo causa de la actividad citotóxica observada la concominante presencia de un sistema altamente insaturado así como los anillos contiguos de oxirano. Así, este compuesto exhibe una alta citotoxicidad frente a varias líneas celulares, destacando su fuerte actividad contra P-388 (leucemia linfocítica de ratón) y U-373 (carcinoma de mama humano), lo que le confiere un especial interés por la síntesis tanto del producto natural como de sus posibles análogos sintéticos.

Considerando la prometedora actividad biológica como anticancerígeno, y como alternativa a la primera síntesis del gummiferol, que emplea la metodología de epoxidación asimétrica de Sharpless, se ha desarrollado una nueva estrategia sintética, empleando para ello iluros de azufre quirales, como se describió anteriormente para la síntesis de los diepoxi- análogos de bengamides.

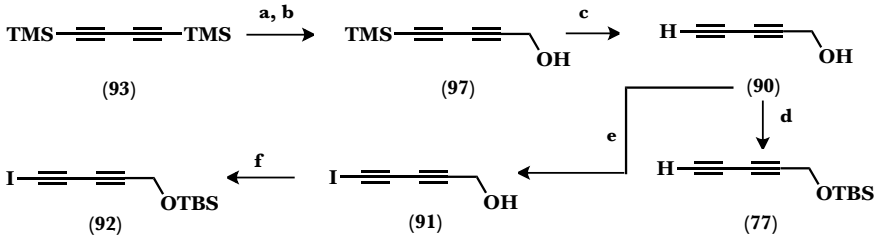
El análisis retrosintético del gummiferol se presenta en el **Esquema 3.4**, donde mediante desconexión del sistema triacetilénico se obtienen el diepoxi alquino **94** y el halo derivado **91** como principales *building blocks*, que podrían acoplarse mediante una reacción tipo Cadiot-Chodkiewicz.

Esquema 3.4. *Retrosíntesis de gummiferol*



La síntesis del halo derivado se planteó siguiendo la metodología descrito por Fiandenese et al. que permite obtener el yodo alcohol protegido **92** en 5 etapas (**Esquema 3.5**).

Esquema 3.5. *Síntesis del dialquino* ***92***



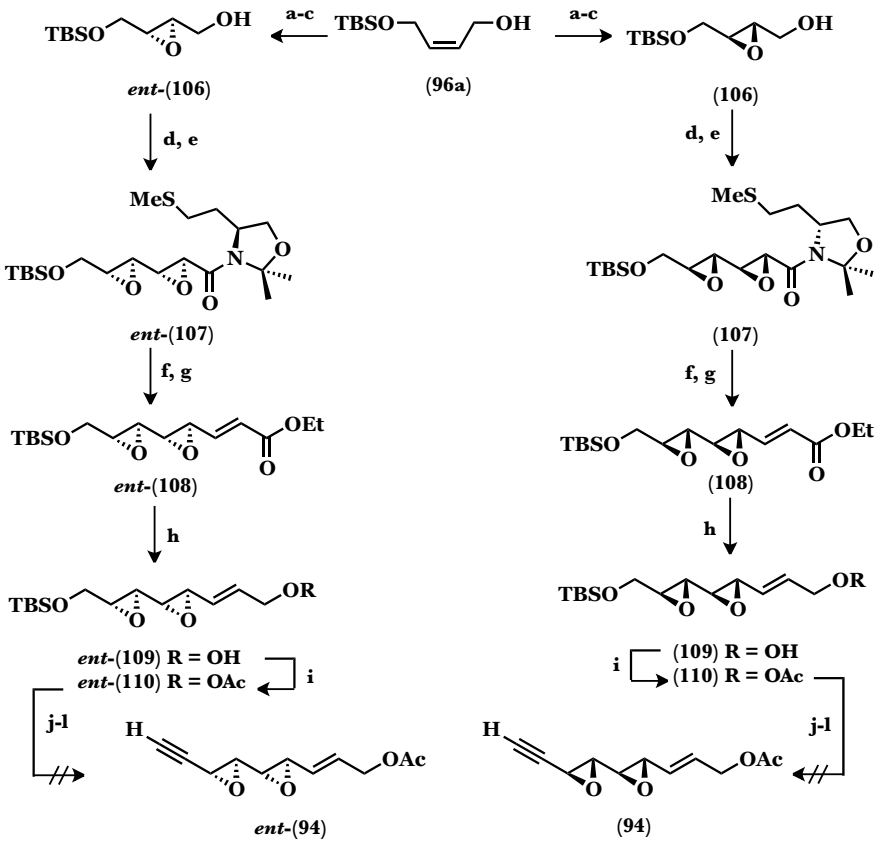
Reactivos y condiciones: (a) MeLi-LiBr, Et_2O, 0ºC, 8 h. (b) $(CH_2O)n$, Et_2O, 0ºC, 8 h. (c) K_2CO_3, MeOH, 25ºC,12 h. (d) TBSOTf, 2,6-lutidina, CH_2Cl_2, 0ºC a rt, 3 h. (e) KOH, I_2, H_2O-MeOH, 0ºC to rt. (f) TBSOTf, 2,6-lutidina, CH_2Cl_2, 0ºC a rt, 3 h, 68% rendimiento global.

En una primera aproximación, procedimos a realizar la síntesis del fragmento **94** desde el D-isopropilidén gliceraldehído (**99**), utilizando para tal fin diferentes sales de sulfonio, obteniendo en todos los casos crudos de reacción complejos y rendimientos extremadamente bajos, lo que nos llevó a cambiar de metodología.

Debido a los resultados anteriores, desarrollamos un esquema sintético que combinara la metodología de epoxidación asimétrica de Sharpless (SAE) y la de los iluros de azufre quirales. Se sintetizaron así las epoxiamidas **107** y ***ent*-107** en buen rendimiento, y desde las cuáles, se accedió a los diepóxidos **108** y ***ent*-108**, cuya desprotección, oxidación y acoplamiento rendiría el deseado diepoxi alquino **94** y ***ent*-94**.

Sin embargo, tras realizar diferentes pruebas de oxidación del alcohol resultante de la desprotección de los silil éteres **110** y *ent*-**110**, el acoplamiento posterior con el reactivo de Ohira-Bestmann no condujo en ninguno de los casos a los correspondientes alquinos, por lo que una nueva modificación se realizó en el esquema general de la síntesis de los dialquinos **94** y *ent*-**94**.

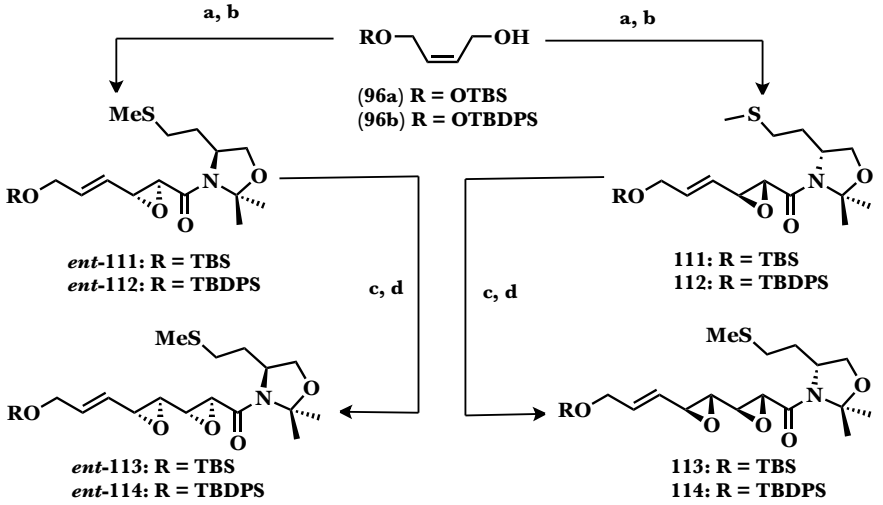
Esquema 3.8. *Segunda aproximación hacia la síntesis de los diepoxi- alquinos ent-**94** y **94***



Reactivos y condiciones: (a) PCC, NaOAc, CH_2Cl_2, 25ºC, 16 h. (b) DIBAL-H, CH_2Cl_2, -78ºC, 45 min (c) SAE, 73% para *ent*-**106**, 65% para **106** (sobre 2 etapas). (d) SO_3·Pyr, CH_2Cl_2, DMSO, 0ºC a rt. (e) **4** or **6**, 5.0 M NaOH, CH_2Cl_2-H_2O, 57% para *ent*-**107**, 60% para **107** (sobre 2 etapas). (f) Red-Al, THF, 0ºC, 45 min. (g) Ph_3PCHCO_2Et, CH_2Cl_2, 25ºC, 57% para *ent*-**108**, 60% para **108** (sobre 2 etapas). (h) DIBAL-H, CH_2Cl_2, -78ºC, 45 min, 76% para *ent*-**109**, 36% para **109**. (i) Ac_2O, Pyr, 25ºC, 12 h, 73% para *ent*-**110**, 30% para **110**. (j) TBAF (k) SO_3·Pyr, CH_2Cl_2, DMSO, 0ºC a rt; oxidación de Swern; TEMPO/BAIB, CH_3CN-H_2O, 25ºC, 16 h; PCC, NaOAc, CH_2Cl_2, 25ºC, 16 h. (l) reactivo OB, K_2CO_3.

Utilizando esta vez la metodología de los iluros de azufre como único método de epoxidación se sintetizaron sendos precursores de (+) y (-)-gummiferol, en una secuencia que comprende inicialmente la síntesis de las diepoxiamidas *ent*-**113** y **113**, y *ent*-**114** y **114** (**Esquema 3.9**). Para tal fin, se emplearon precursores sililados en forma de TBS- y TBDPS-, obteniendo los mejores resultados para este último grupo protector (**Esquema 3.9**).

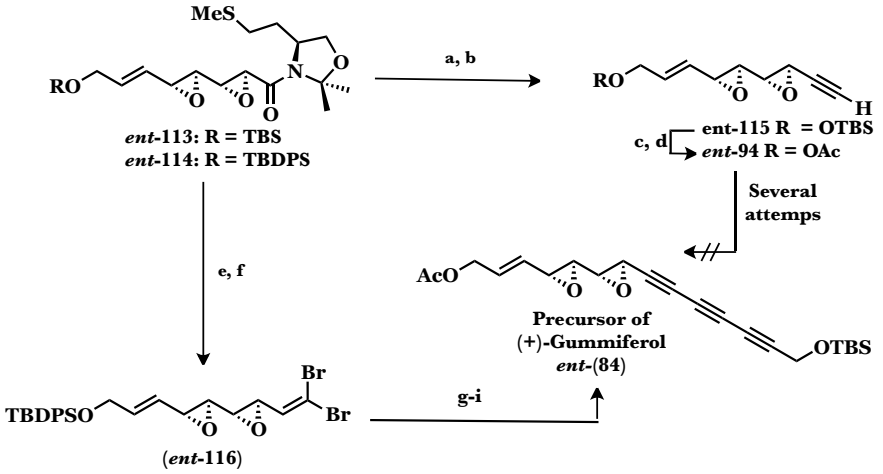
Esquema 3.9. *Síntesis de las diepoxiamidas ent-**113**, **113**, ent-**114** y **114***



Reactivos y condiciones: (a) PCC, NaOAc, CH_2Cl_2, 25ºC, 16 h. (b) **4** o **6**, 3.0 M NaOH, *t*BuOH, 25ºC, 12 h, 40% para *ent-**111***, 40% para **111**, 40% para *ent-**112***, 40% para *ent*-**112** (sobre 2 etapas). (c) Red-Al, THF, 0ºC, 45 min to 1 h. (d) **4** or **6**, 5.0 M NaOH, CH_2Cl_2, rt, 95% para *ent*-**113**, 61% para **113,** 95% para *ent*-**114**, 70% para **114** (sobre 2 etapas).

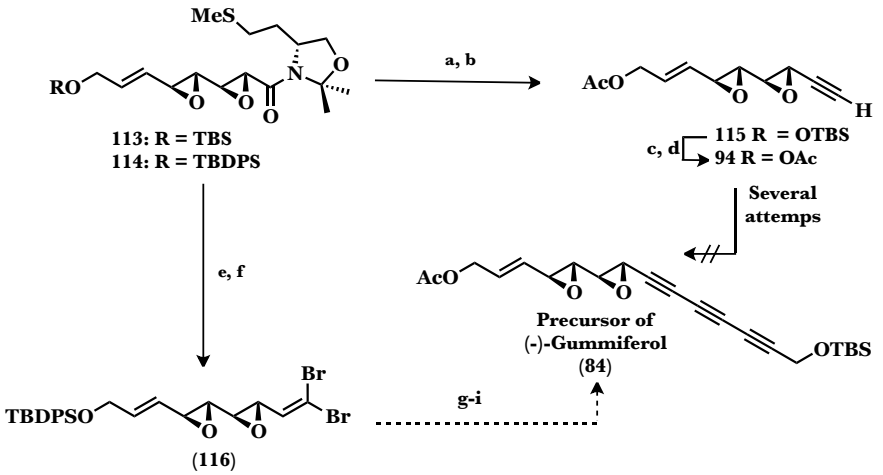
Posteriormente se procedió a la conversión en el correspondiente trialquino y con ello la finalización de la síntesis. Para ello, se emplearon las metodologías de Ohira-Bestmann y Corey-Fuchs para instalar el alquino terminal, consiguiendo el mejor resultado para ésta última, ya que permitió el acoplamiento con el yodo alquino **77**, en un 55% sobre 2 etapas (**Esquema 3.10-A** y **3.10-B**).

Esquema 3.10-A. *Síntesis del precursor de (+)-gummiferol*



Reactivos y condiciones: (a) Red-Al, THF, 0ºC, 45 min. (b) Reactivo Ohira-Bestmann, K_2CO_3, MeOH, 25ºC, 16 h. (c) TBAF, THF, 25ºC, 12 h. (d) Ac_2O,/ Pyr, 25ºC, 2 h, 5% (d) Ac_2O/Pyr, 30% (sobre 2 etapas). (e) Red-Al, THF, 0ºC, 45 min. (f) CBr_4, Ph_3P, Et_3N, 25ºC, min, 40% (sobre 2 etapas). (g) TBAF, THF, 0ºC, 1 h. (h) **77**, CuI, $EtNH_2$, .(i) Ac_2O/Pyr, DMAP, 51% (sobre 3 etapas).

Esquema 3.10-B. *Síntesis del precursor de (-)-gummiferol*

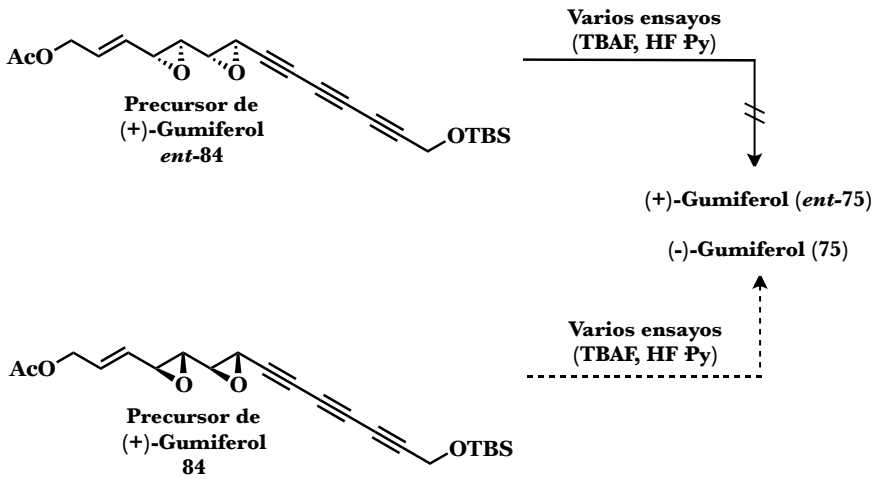


Reactivos y condiciones: (a) Red-Al, THF, 0ºC, 45 min. (b) Reactivo Ohira-Bestmann, K_2CO_3, MeOH, 25ºC, 16 h, 44%. (c) TBAF, THF, 25ºC, 12 h (d) Ac_2O,/Pyr, 25ºC, 2 h (d) Ac_2O/Pyr, 25% (sobre 2 etapas). (e) Red-Al, THF, 0ºC,

45 min. (f) CBr_4, Ph_3P, Et_3N, 25ºC, min, 40%. TBAF, THF, 0ºC, 1 h. (h) **77**, CuI, $EtNH_2$. (i) Ac_2O/Pyr, DMAP.

Por último, la desprotección del silil éter conduciría a (+) y (-)-gummiferol. Sin embargo, tras diferentes ensayos con diferentes condiciones (TBAF, HF·Pyr) y a pesar de los descrito por Takamura, no se ha detectado traza alguna del producto natural y en todos los casos se obtuvieron mezclas complejas y productos de descomposición. Actualmente, seguimos realizando diferentes pruebas que conduzcan al producto natural (**Esquema 3.11**).

Esquema 3.11. *Hacia la the síntesis de (+) y (-)-Gummiferol*



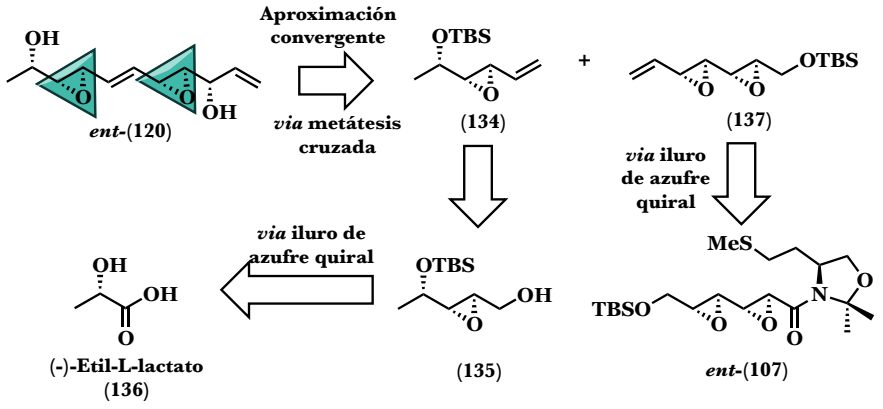
Depudecina

El metabolito microbiano depudecina, descrito por primera vez en 1991 por Matsumoto et al. es un policétido lineal caracterizado por una cadena carbonada altamene oxidada que contiene dos epóxidos conjugados a través de un sistema olefínico *trans*. Este producto natural inhibe eficientemente la actividad de la enzima histona deacetilasa tanto *in vitro* como *in vivo*.

En comparación a otros inhibidores de histona deacetilasa previamente descritos como trapoxina o tricostatina A, depudecina posee una estructura química diferente que le permite incrementar su selectividad sobre la diana biológica. Este hecho hace de depudecina un prometedor compuesto antiangiogénico y una herramienta útil para comprender mejor el mecanismo de acción de este tipo de enzimas deacetilasas. Bajo esta premisa, se ha diseñado la síntesis total del enantiómero de depudecina, aún desconocido, que podría exhibir una citotoxicidad similar, empleando para ello la metodología de epoxidación asimétrica basada en el uso de iluros quirales de azufre.

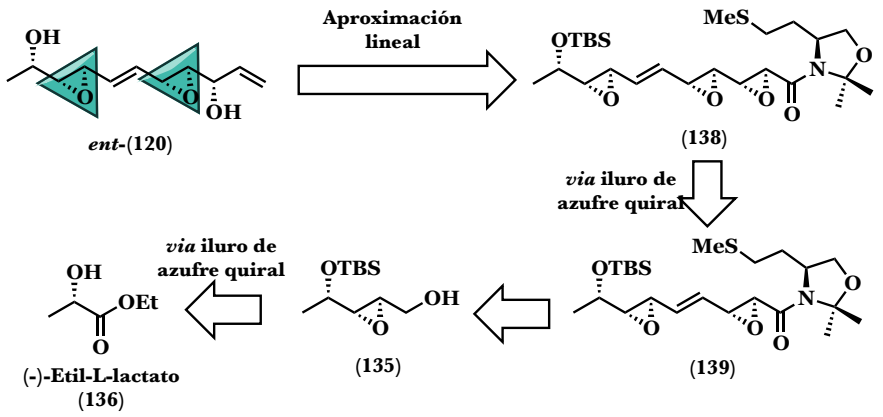
Para la síntesis del enantiómero no natural de depudecina *ent*-(**120**) se planteó, incialmente, una ruta convergente basada en una reacción de metátesis cruzada de las olefinas (**134**) y (**137**), mediada por el catalizador de Hoveyda-Grubbs de 2ª generación. Para tal fin, la olefina **134** podría ser sintetizada *vía* iluros de azufre quirales desde el reactivo comercial **136**, mientras que la olefina **137** se obtiene desde la diepoxiamida *ent*-**107**, previamente descrita para la síntesis del gummiferol (**Esquema 4.4-A**).

Esquema 4.4-A. *Análisis retrosintético de (+)-depudecin. Aproximación convergente*



Desafortunadamente, los bajos rendimientos obtenidos en la reacción de metátesis como se detallará más adelante, nos llevaron a realizar un cambio en la estrategia, desarrollando así una síntesis lineal, como muestra el **Esquema 4.4-B**.

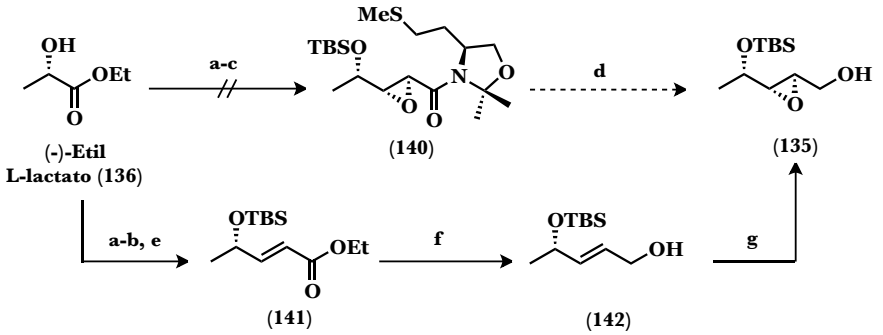
Esquema 4.4-B. *Análisis retrosintético de (+)-depudecin. Aproximación lineal*



Cabe señalar que en ambas aproximaciones, los epóxidos contenidos en el esqueleto de depudecina se generan *vía* iluros de azufre quirales con la sal de sulfonio **4**.

La síntesis del fragmento **135**, común a ambas aproximaciones sintéticas, procede tal y como muestra el **Esquema 4.5**. Inicialmente, la síntesis del epoxialcohol **135** se planteó por la *vía* de los iluros de azufre para generar la epoxiamida **140**; sin embargo, en todas las pruebas realizadas se obtuvieron bajos rendimientos y crudos de reacción complejos, por lo que se optó por realizar la epoxidación asimétrica de Sharpless del alcohol **142,** generado desde el éster **141**, como muestra el **Esquema 4.5**.

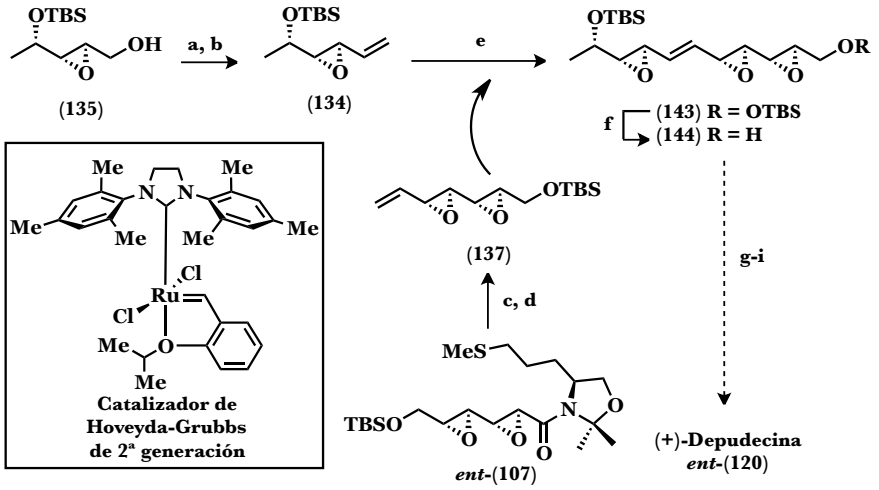
Esquema 4.5. *Síntesis del epoxialcohol* ***135***



Reactivos y condiciones: (a) TBSCl, imidazol, CH_2Cl_2, 0ºC. (b) DIBAL-H, CH_2Cl_2, -78ºC, 1 h. (c) **4**, 5.0 M NaOH, CH_2Cl_2-H_2O, 25ºC, 16 h. (d) Super-H, THF, 0ºC, 15 min. (e) $Bu_3PCH_2CO_2Et$, NaOH, tolueno-CH_2Cl_2, 87% (sobre 3 etapas). (f) DIBAL-H, CH_2Cl_2, -78ºC, 1 h, 91%. (g) SAE, D-(-)-DET, 90%.

Desde el epoxialcohol **135**, se obtiene la olefina **134**, mediante oxidación al correspondiente aldehído y posterior reacción de Wittig con la sal de fosfonio Ph_3PCH_3Br en medio básico. Por otro lado, la diepoxiamida *ent*-**107** se sometió a una reacción de reducción al diepoxi aldehído mediada por Red-Al y posterior acoplamiento con la sal de fosfonio Ph_3PCH_3Br, generando así la diepoxi olefina **137**. Una vez sintetizados ambos fragmentos, se procedió a la metátesis cruzada de olefinas catalizada por el reactivo de Hoveyda-Grubbs de segunda generación (**Esquema 4.6**), obeniendo así el triepóxido **143**, cuya desprotección mediada por TsOH rindió el triepoxi alcohol **144.**

Esquema 4.6. *Aproximación convergente a la síntesis de (+)-depudecina*



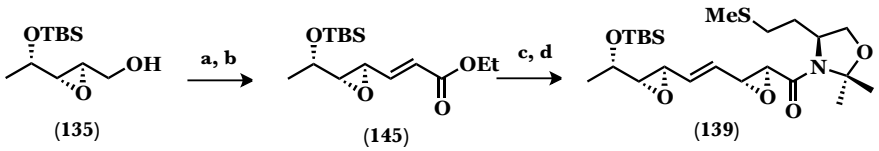
Reactivos y condiciones: (a) SO_3·Pyr, Et_3N, CH_2Cl_2, DMSO, 0ºC to rt, 4 h. (b) Ph_3PCH_3Br, NaHMDS, 0ºC a rt, 12 h, 50% (sobre 2 etapas). (c) Red-Al, THF, 0ºC, 45 min. (d) Ph_3PCH_3Br, NaHMDS, 0ºC a rt, 12 h, 30% (sobre 2 etapas). (e) **137**, catalizador de Hoveyda-Grubbs 2ª generación, CH_2Cl_2, 45ºC, 72 h, 15%. (f) TsOH, 50%. (g) CBr_4, Ph_3P. (h) Zn (i) TBAF

Desde el triepoxi- alcohol, la síntesis hacia (+)-depudecina se completaría mediante reacción de Corey-Fuchs y elminación reductora del correspondiente bromoderivado mediada por Zn. Por último, la desprotección del silil éter por medio de TBAF, conduciría al producto *ent*-**120**. Sin embargo, la falta de tiempo ha originado que el esquema no haya sido completado aún, y en la actualidad se están realizando pruebas para alcanzar el producto natural.

La aproximación lineal, por su parte, comparte con la convergente el epoxialcohol **135,** que fue transformado en el epoxi éster **145** mediante oxidación al correspondiente epoxi aldehído y posterior reacción de Wittig-Martin. Desde aquí, la oxidación directa desde el éster al aldehído, por medio de 1.0 equivalente de DIBAL-H a 78ºC, seguido de reacción

con la sal de sulfonio **4** rindió la epoxiamida **139** en un 55% de rendimiento sobre 2 etapas (**Esquema 4.7**).

Esquema 4.7. *Síntesis del intermedio epoxiamida* ***145***

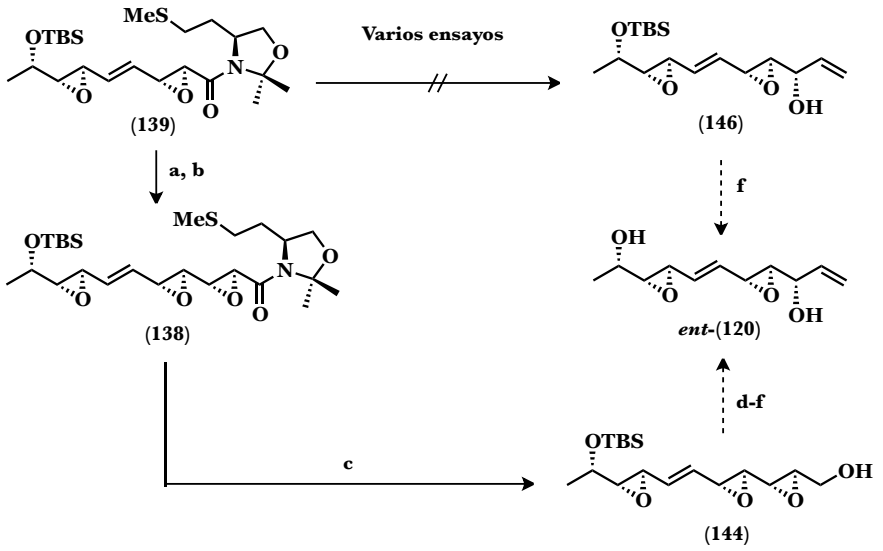


Reactivos y condiciones: (a) SO_3·Pyr, CH_2Cl_2-DMSO, Et_3N, 0ºC. (b) $Bu_3PCH_2CO_2Et$, NaOH, tolueno-CH_2Cl_2, 62% (sobre 2 etapas) (c) DIBAL-H, CH_2Cl_2, -78ºC, 1 h. d) **4**, NaOH, *t*BuOH, 12 h, 55% (sobre 2 etapas).

Una vez obtenida la epoxiamida **139**, se procedió por la vía lineal, a obtener la triepoxiamida **138**, desde la cuál, por acción de Super-H se alcanzó el triepoxi- alcohol **144** (**Esquema 4.8**).

Esta ruta proporciona una alternativa más eficiente a la convergente, en términos de rendimiento, e igualmente se obtiene el diepoxi alcohol **144**, desde el cuál se están realizando pruebas para su conversión en el producto natural (**Esquema 4.8**)

Esquema 4.8. *Hacia la síntesis de (+)-depudecina*

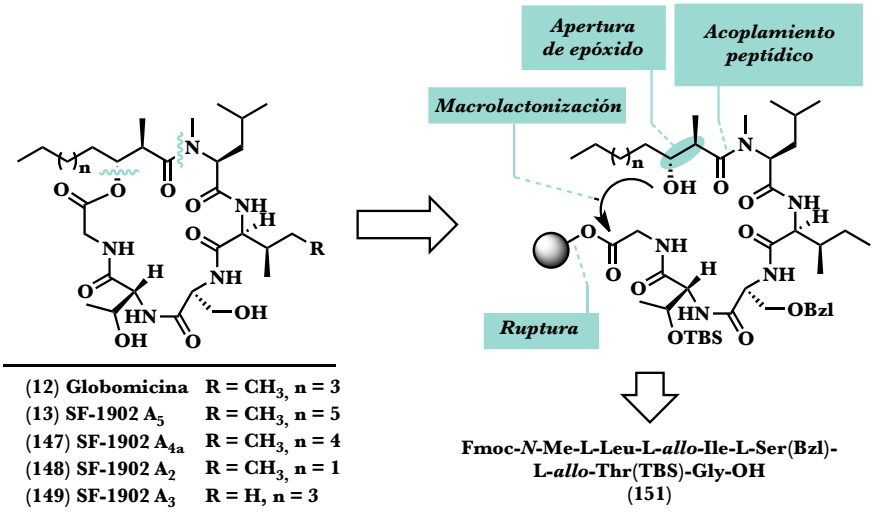


Reactivos y condiciones: (a) Red-Al, THF, 0ºC, 45 min. (b) **4**, NaOH, CH_2Cl_2-H_2O, 30% (sobre 2 etapas). (c) Super-H, THF, 0ºC, 30 min, 60%. (d) CBr_4, Ph_3P. (e) Zn (f) TBAF

Globomicina

El lipociclodepsipéptido glomomicina y sus congéneres (**Esquema 5.1**), aislados de cuatro líneas diferentes de actinomicetes en 1978, representa una interesante familia de productos naturales debido a su actividad antibiótica frente a bacterias Gram-negativa, convirtiéndolo, junto con su derivado SF-1902, en una diana para la búsqueda de nuevos antibióticos.

Esquema 5.1..*Estructura molecular de globomicina y sus congéneres y retrosíntesis de globomicina*

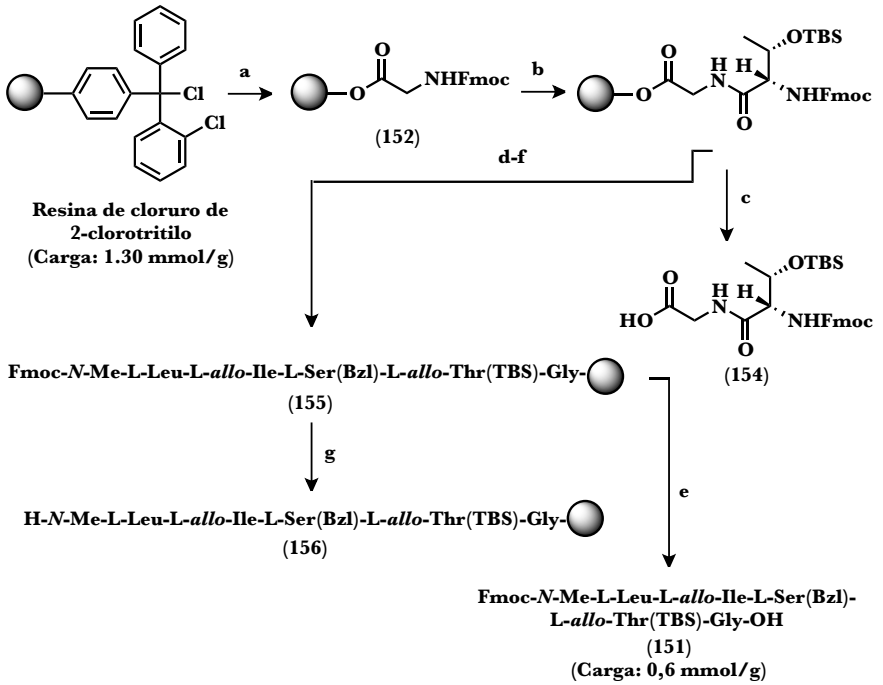


(12) Globomicina R = CH_3, n = 3
(13) SF-1902 A_5 R = CH_3, n = 5
(147) SF-1902 A_{4a} R = CH_3, n = 4
(148) SF-1902 A_2 R = CH_3, n = 1
(149) SF-1902 A_3 R = H, n = 3
(150) SF-1902 A_{4b} R = H, n = 5

Fmoc-*N*-Me-L-Leu-L-*allo*-Ile-L-Ser(Bzl)-L-*allo*-Thr(TBS)-Gly-OH (151)

La síntesis de la parte peptídica común a globomicina y SF-1902 se ha llevado a cabo en fase sólida, siguiendo la metodología Fmoc, empleando como resina el cloruro de 2-clorotritilo (**Esquema 5.2**). De esta forma se obtuvo el tetrapéptido **151**, en 7 etapas y con un rendimiento que revela una carga de 0.6 mmol/g de resina.

Esquema 5.2. *Síntesis en fase sólida de la parte peptídica de globomicina*

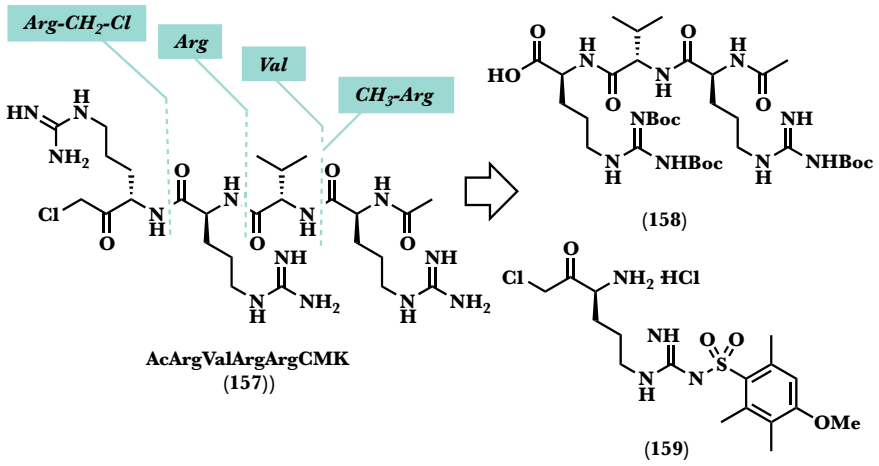


Reactivos y condiciones: (a) Fmoc-Gly-OH, DIPEA, DMF, 25ºC, 30 h. (b) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-*allo*-Thr(TBS)-OH, HOBt, DIC, DMF, 25ºC, 24 h. (c) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 25ºC, 30 min. (d) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-Ser(Bzl)-OH, HOBt, DIC, DMF, 25ºC, 24 h. (e) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-*allo*-Ile-OH, HOBt, DIC, DMF, 25ºC, 24 h. (f) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-*N*-Me-L-Leu-OH, HOBt, DIC, DMF, 25ºC, 24 h. (g) 20% Piperidina en DMF (Carga: 0.6 mmol/g para resina **155**).

Inhibidor AcArgValArgArgCMK

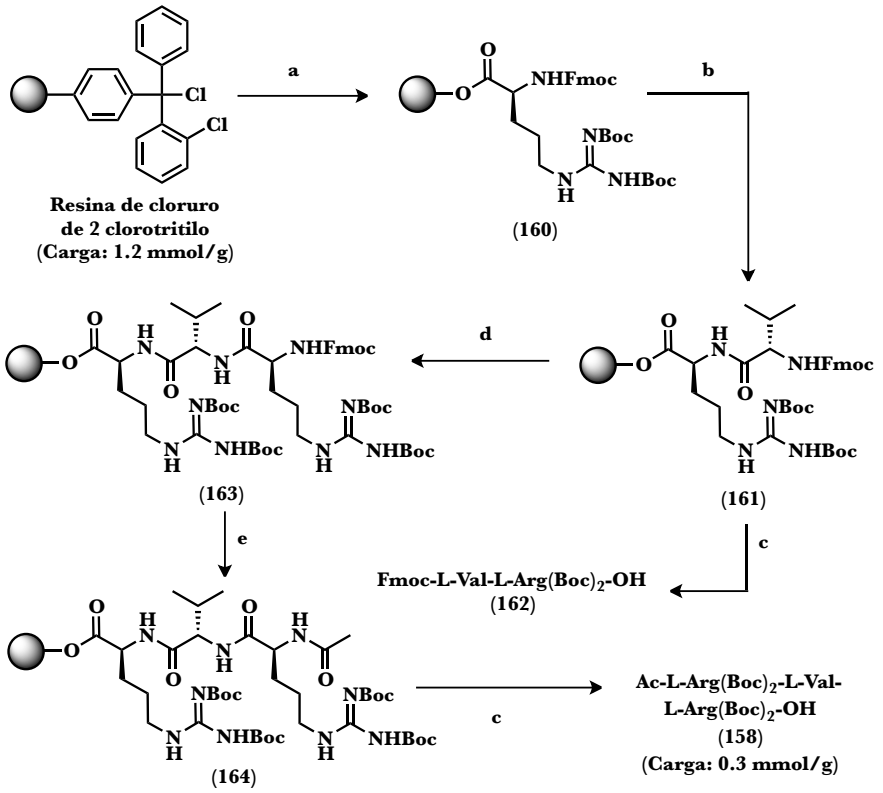
Como parte de una colaboración con el departamento de Ecología de la Universidad de Málaga, se ha desarrollado la síntesis del péptido de secuencia AcArgValArgArgCMK, que podría presentar una importante actividad como inhibidor enzimático de proteasas y que en la actualidad se encuentra bajo ensayos biológicos (**Esquema 5.3**).

Esquema 5.3. *Análisis retrosintético del tetrapéptido* ***157***



En este caso, y basándonos en la metodología Fmoc, se realizó la síntesis en fase sólida del tripéptido **158**, en 5 etapas y con un rendimiento que revela una carga de 0.3 mmol/g de resina, según muestra el **Esquema 5.4**.

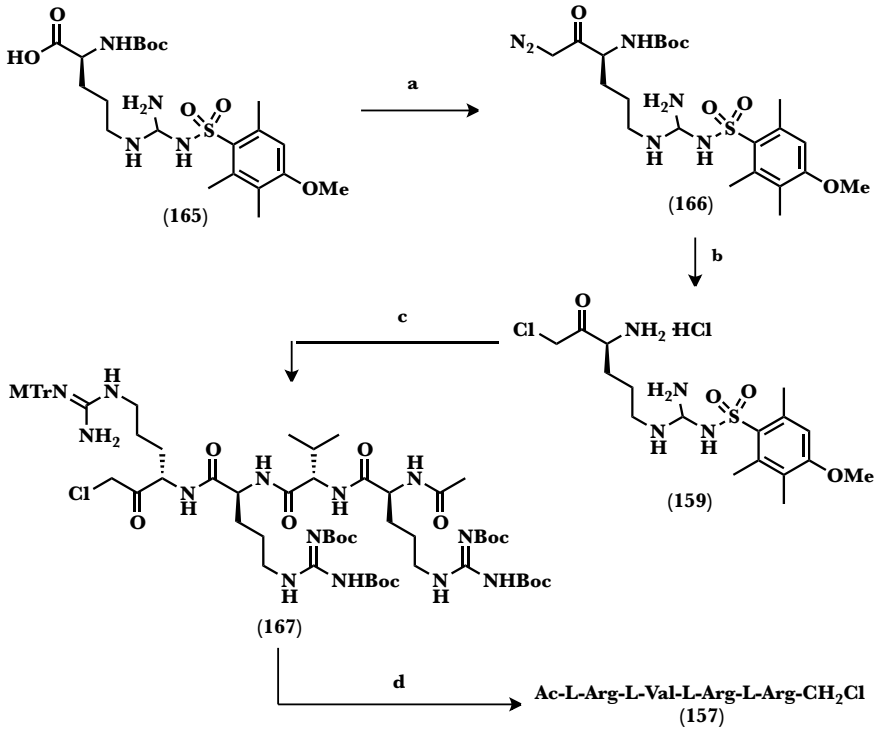
Esquema 5.4. *Síntesis en fase sólida del tripéptido* ***158***



Reactivos y condiciones: (a) Fmoc-L-Arg(Boc)$_2$-OH, DIPEA, DMF, 25ºC, 24 h. (b) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-Val-OH, DIC, HOBt, DMF, 25ºC, 24 h. (c). CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 30 min. (d) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-Arg(Boc)$_2$-OH, DIC, HOBt, DMF, 25ºC, 24 h. (e) 20% Piperidina en DMF, 25ºC, 30 min; Ac_2O/Pyr, DMF, 25ºC, 24 h (Carga: 0.3 mmol/g para resina **164**).

Paralelamente, se formó la clorocetona **159** desde el aminoácido comercial BocArg(MTr)OH, mediante el empleo de diazometano y posterior tratamiento con MeOH·HCl (**Esquema 5.5**). Finalmente, el acoplamiento del ácido **158** y la clorocetona **159**, seguido por la desprotección de los grupos Boc y MTr con ácido trifluoroacético proveyó el tetrapéptido objetivo **157** (**Esquema 5.5**).

Esquema 5.5. *Síntesis en fase sólida del tetrapéptido* ***157***



Reactivos y condiciones: (a) $(CH_3)_2CHOCOCl$, NMM, CH_2N_2, THF -20ºC to 0ºC, 4 h, 52%. (b) MeOH·HCl, THF, 25ºC, 24 h. (c) **158**, DIPEA, DIC, HOBt, CH_2Cl_2, 25ºC, 24 h. (d) TFA, tioanisol, 25ºC, 20 h, 64% (sobre 3 pasos).

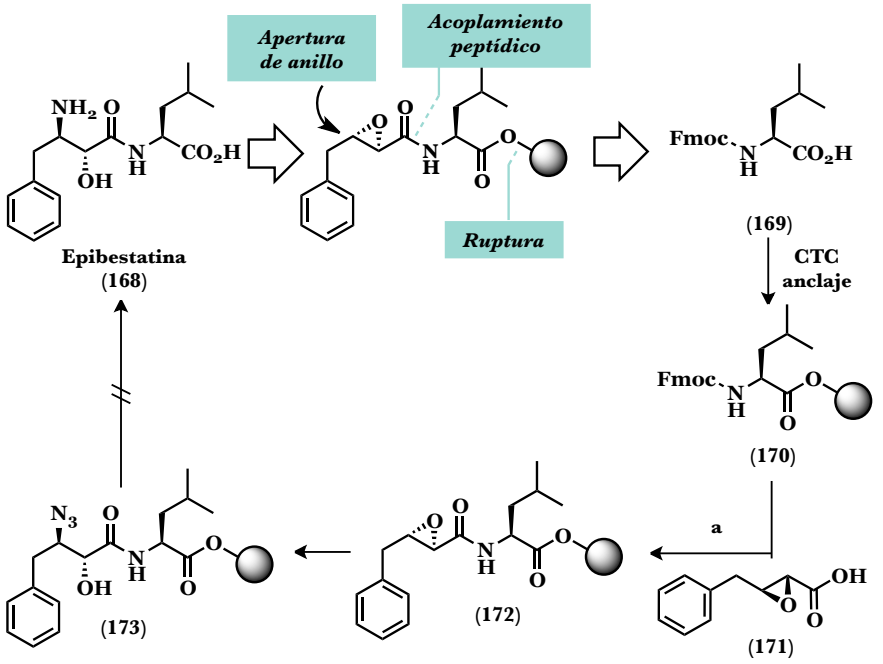
Epibestatina

Epibestatina (**168**), [2S,3R)-3-amino-2-hidroxi-4-fenil-butnoil]-L-leucina, aislada de cultivos de *Streptomyces olivoreticuli*, es un dipéptido con una potente citotoxicidad contra aminopeptidasa B y leucina aminopeptidasa.

Inicialmente se planteó la síntesis en fase sólida, por anclaje del aminoácido comercial **169** y acoplamiento del epoxiácido **171** (obtenido mediante la metodología de iluros de azufre quirales). Sin embargo, tras la apertura regioselectiva mediada por $MgSO_4$, la reducción del grupo azido

al amino en fase sólida, bajo diferentes condiciones de reacción, no pudo ser completada, por lo que recurrimos a la síntesis en disolución (**Esquema 5.6**).

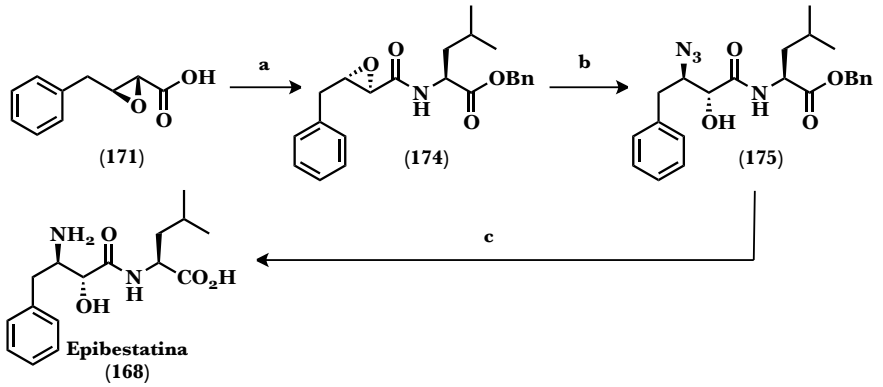
Esquema 5.6. *Síntesis de epibestatina en fase sólida*



Reactivos y condiciones: (a) 20% Piperidina en DMF, 25ºC, 30 min; **171**, DIC, HOBt, DMF, 25ºC, 16 h. (b) NaN_3, $MgSO_4$, DMF, 65ºC, 16 h. (c) Ph_3P, THF-H_2O (2:1), 25ºC, 16 h. (d) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 30 min.

La síntesis de epibestatina (**168**) en disolución pudo ser completada desde el azido éster **175**, que por reducción con H_2, Pd/C condujo al mencionado producto natural, en un total de 3 etapas con un rendimiento global de 25% (**Esquema 5.7**).

Esquema 5.7. *Síntesis de epibestatina*



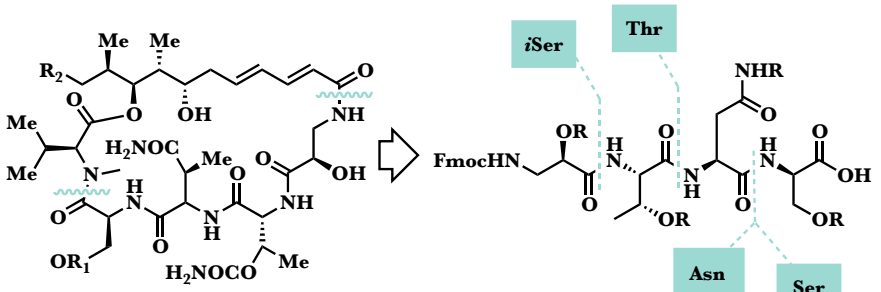
Reactivos y condiciones: (a) Fmoc-L-Leu(Obn), DIC, HOBt, DMF, 25ºC, 16 h, 94%. (b) NaN_3, $MgSO_4$, MeOH, 65ºC, 16 h, 40%. (c) H_2, Pd/C, EtOAc, 25ºC, 16 h, 67%.

Celebésido A

Los celebésidos son una familia inusual de ciclodepsipéptidos aislados recientemente de la esponja marina *Siliquariaspongia mirabilis* por Bewley et al., que contienen un sistema policétido y un fragmento peptídico de cinco aminoácidos.

Estos compuestos muestran actividad anti VIH, antifúngica, y antitumoral. De los 6 miembros aislados de la familia, celebésido A es el que presenta un valor más potente de IC_{50} en su ensayo como inhibidor del VIH. Tras haber descrito recientemente la síntesis de la cadena policétida, decidimos utilizar la metodología en fase sólida para la síntesis de la parte peptídica (**Esquema 5.8**).

Esquema 5.8. *Retrosíntesis del fragmento peptídico de celebésido A*

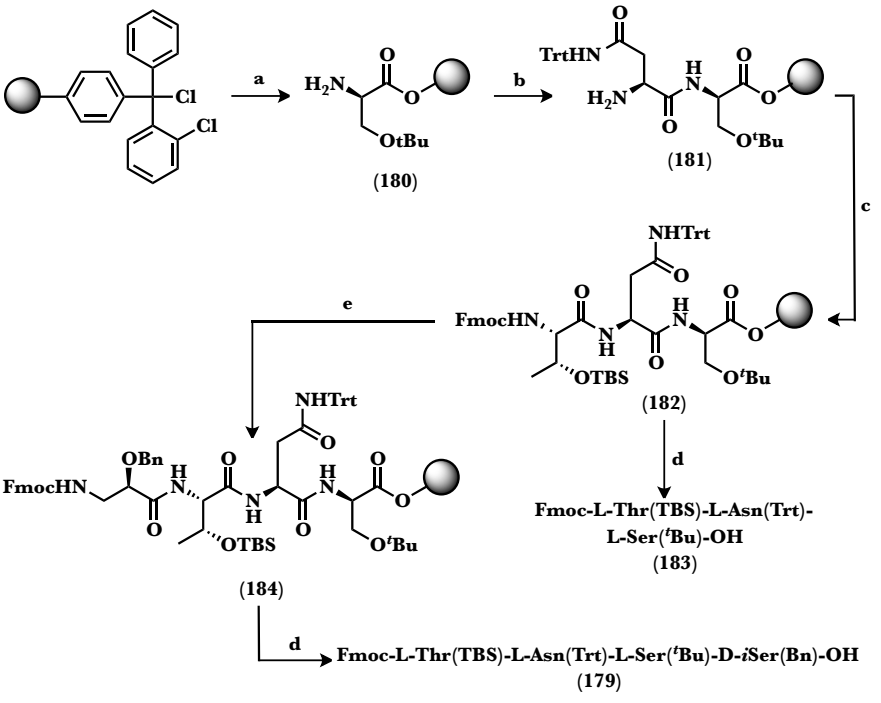


(176) Celebésido A $R_1 = PO_3H_2$; $R_2 = C_2H_5$
(177) Celebésido B $R_1 = PO_3H_2$; $R_2 = CH_3$
(178) CelebésidoC $R_1 = H$; $R_2 = C_2H_5$

Péptido modificado
(179)

De esta forma, la síntesis del tetrapéptido correspondiente se completó siguiendo un esquema sintético que se detalla en el **Esquema 5.9**.

Esquema 5.9. *Síntesis del tetrapéptido* ***179***



Reactivos y Condiciones: (a) Fmoc-L-Ser(*t*Bu)-OH, DIPEA, DMF, 25ºc, 24 h. (b) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-Asn(Trt)-OH, DIC, HOBt, DMF, 25ºC, 24 h. (c) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-L-Thr(OTBS)-OH, DIC, HOBt, DMF, 25ºC, 24 h. (d) CH_2Cl_2, AcOH, CF_3CH_2OH (7:2:1), 25ºC, 30 min. (e) 20% Piperidina en DMF, 25ºC, 30 min; Fmoc-D-*i*Ser(Bn)-OH, DIC, HOBt, DMF, 25ºC, 24 h.

Conclusiones

Durante la realización de esta Tesis Doctoral se ha llevado a cabo la síntesis tanto de productos naturales como análogos, que contienen anillos de oxirano en su estructura. En total, 4 análogos de bengamides fueron sintetizados y su actividad biológica evaluada, arrojando nuevos datos SAR a los ya conocidos. Se ha establecido además la primera estrategia para la síntesis de unos nuevos derivados, las amino bengamidas, que pueden presentar mayor interacción con las enzimas MetAP.

Se ha completado la síntesis del precursor del gummiferol, realizando una síntesis formal que emplea los iluros de azufre para la construcción de los epóxidos, ofreciendo una alternativa a la única síntesis total descrita por Takamura y basada en la metodología de Sharpless.

Por otro lado, se ha descrito una aproximación sintética hacia la depudecina, alcanzando un precursor avanzado de su enantiómero aún no descrito, y en la actualidad, seguimos trabajando para completar su síntesis total.

Además, se ha trabajado con la metodología de síntesis en fase sólida de las cadenas peptídicas de diferentes productos naturales de interés biológico, como son globomicina, epibestatina o celebésido A.

APPENDIX ONE

GENERAL METHODS

Chemical Techniques

All reactions were performed using oven-dried glassware (200ºC) under an atmosphere of dry argon unless using aqueous reagents or otherwise stated. All solvents used in reactions were dried and distilled using standard procedures. DMF and DMSO were purchased from Sigma-Aldrich. THF and toluene were distilled from sodium and CH_2Cl_2 and MeOH from calcium hydride prior to use. All other solvents were used as supplied unless otherwise stated.

All reagents were purchased from Aldrich, Acros Chimica, Merk or Alfa Aesar and were used as supplied or purified using standard procedures as necessary. All solutions used in workup procedures were saturated unless otherwise noted.

Flash column chromatography (FCC) was performed using E. Merk silica gel 60 Å, particle size 230-400 mesh under air pressure. All solvents used for chromatographic purification were distilled prior to use. Analytical TLC was performed using silica gel 60 F_{254} ALUGRAM® SII G/UV254 plates and visualized by ultraviolet radiation (254 nm), potassium permanganate or acidic ceric ammonium molybdate as appropriate. Preparative HPLC was performer in reversed-phase with a C_8 5 μL-Luna column (250 x 10.00 nm) with a flow rate 4.7 mL/min.

Quantities are reported to 3 significant figures and are rounded accordingly. Isolated yields are reported to 0 decimal places and "quant." signifies a yield of 99.5% or higher.

NMR spectra were recorded on Bruker DPX-400 (400 MHz). Chemical shifts are reported in ppm with the resonance resulting from incomplete deuteration of the solvent as the internal standard ($CDCl_3$: 7.26 ppm, DMSO-d_6, 2.49 ppm and D_2O, 4.80 ppm). ^1H NMR assignments were undertaken based on bidimentional NMR experiments of COSY (cosyp experiment). ^{13}C NMR spectra were recorded on Bruker DPX-400 (100 MHz) spectometer (with complete proton decoupling). Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($^{13}CDCl_3$: 77.0 ppm, t). Data are reported as follows: chemical shift δ/ppm (integration (^1H only), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, app = apparent, m = multiplet or combinations thereof). ^{13}C signals are singles (unless otherwise stated), coupling constants J (Hz, assignment).

High resolution mass spectrometry (HRMS) was performed on a Bruker HCT-ULTRA mass spectrometer under Fast Atom Bombardment (FAB) conditions and on a ESI-TOF and APCI mass spectrometer in positive mode. HRMS signals are reported to 4 decimal places and are within ± 5 ppm of theoretical values.

Infrared spectra were recorded neat on a Perkin Elmer Spectrum One FTIR spectrometer and only selected peaks are reported (s = strong, m = medium, w = weak, br = broad).

Specific optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a sodium halogen lamp (λ = 589 nm) and a cell path length of 100 nm (*c* given in g/100 mL).

Melting points were collected using a Gallenkamp or a Griffin melting point system using a gradient of 0.5ºC per min.

BIOLOGICAL TECHNIQUES

Material

Cell culture media were purchased from BLBco (GRand Island, NY, USA) and Cambrex (Walkersville, MD, USA). Fetal bovine serum (FBS) was a product of Harlan-Seralaba (Belton, UK). Supplements and other chemicals not listed in this section were obtained from Sigma Chemicals Co. (St. Louis, Mo., USA). Plasatics for cell culture were supplied by NUNC (Roskilde, Denmark). Bengamides and related compounds were dissolved in dimetylsulfoxide (DMSO) and stored at -20ºC until use.

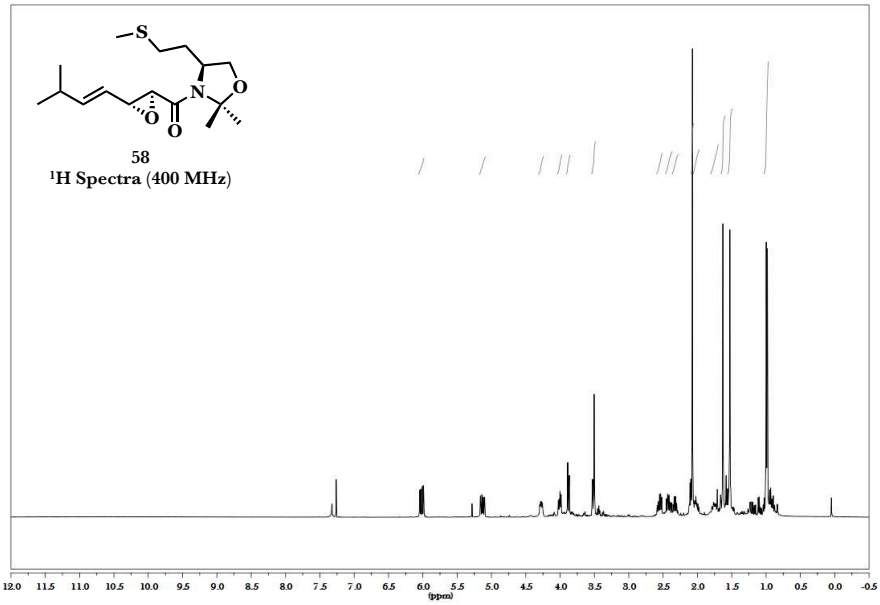
Cell culture

Bovine aortic endothelial (BAE) cells were obtained by collagenase digestion and maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (1 g/L), glutamine (2 mM), penicillin (50 IU/ml), streptomycin (50 μg/mL) and amphoterycin (1.25 μg/mL) supplemented with 10% FBS. All the cancer cell lines used in this study were obtained from the American Type culture collection (ATCC). human fibrosarcoma HT1080 cells were maintained in DMEM containing glucose (4.5 g/L), glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 μg/ml) and amphoterycin (1.25 μg/mL) supplemented with 10% FBS. Human colon adenocarcinoma HT29 cells were maintained in McCoy's 5A medium containing glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 μg/mL) and amphoterycin (1.25 μg/mL) supplemented with 10% FBS. Human breast cancer carcinoma MDA-MB-231 and human promyelocytic leukemia HL60 cells were maintained in RPMI1640 medium containing glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 IU/mL), streptomycin (50 μg/mL) and amphoterycin (1.25 μg/mL) supplemented with 10% and 20% FBS respectively.

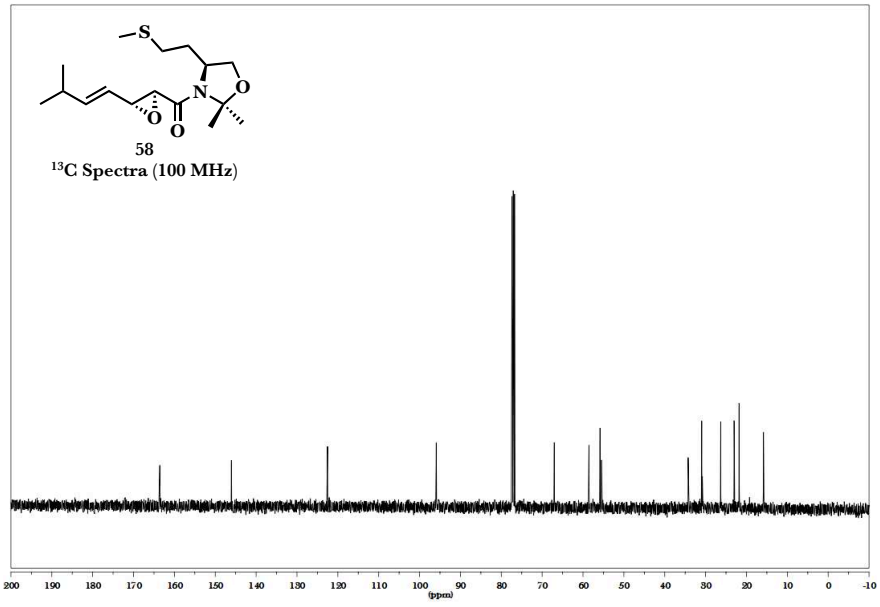
Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical co., S. Louis, MO) dye reduction assay in 96-well microplates was performed by Drug Discovery biotech S. L. (Málaga, Spain), using the method of Mossman. 3.10^{-3} BAE or 2.10exp3 tumor cells in a totla volum of 100 μL of their respective groth media were incubated with serial dilutions of the tested compounds. After 3 days of incubation (37ºC, 5% CO_2 in a humid atmosphere) 10 μL of MTT (5 mg/mL in PBS) were added to each well and the plate was incubated for a further 4 h (37ºC). The resulting formazan was dissolved in 150 μL of 0.04 N HC/2-propanol and read at 550 nm. all determinations were carried out in triplicate. IC_{50} value was calculated from semilogarithmic dose-response plots as the concentration of compound yielding a 50% of cell survival.

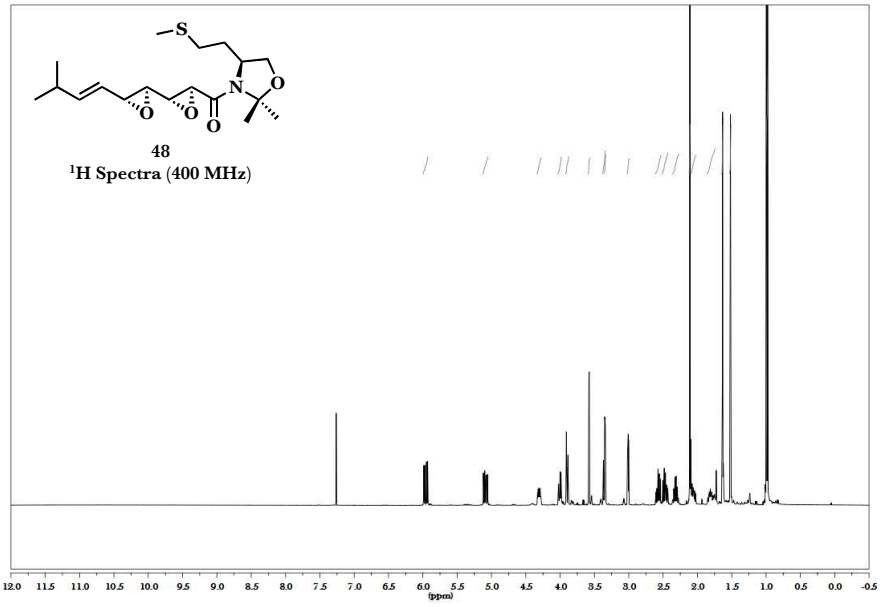
APPENDIX TWO: Spectra Relevant to Chapter Two

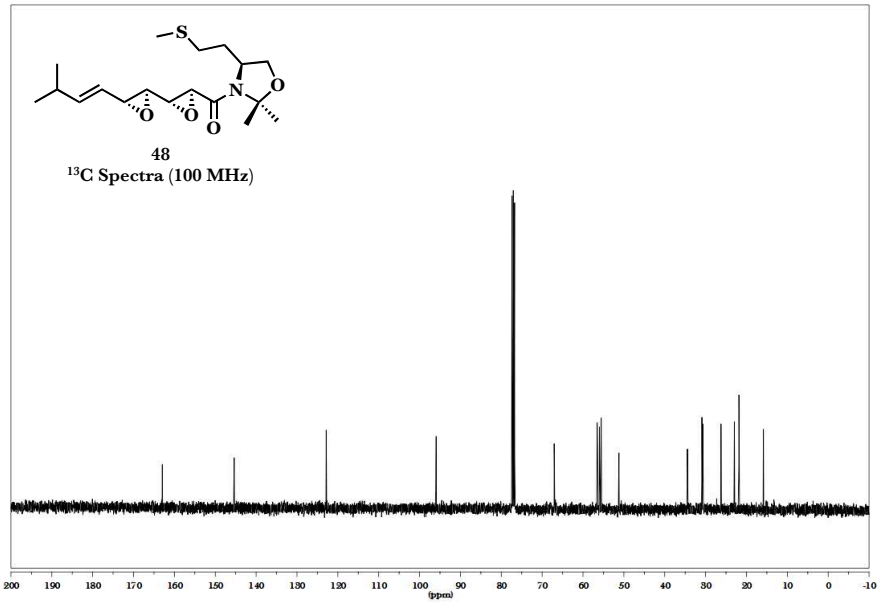


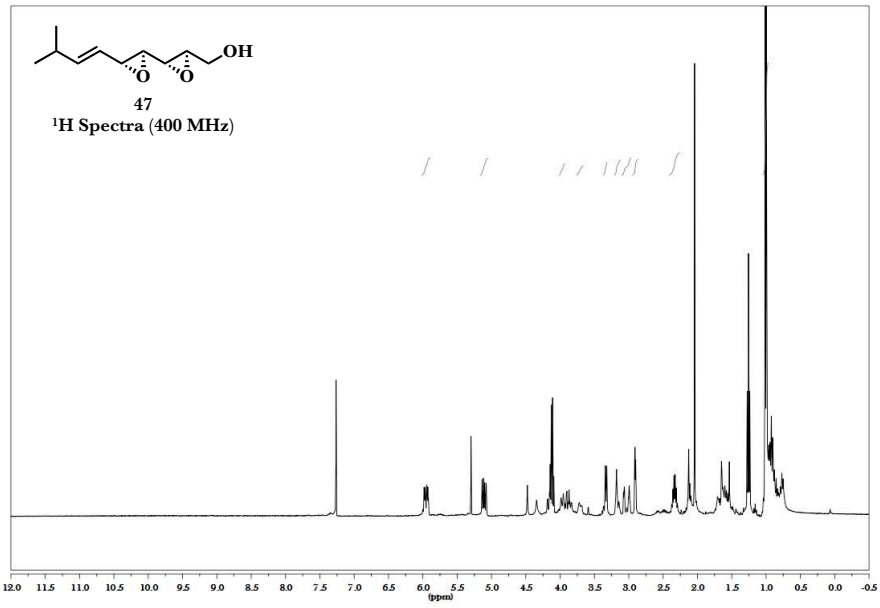
58
^{1}H Spectra (400 MHz)

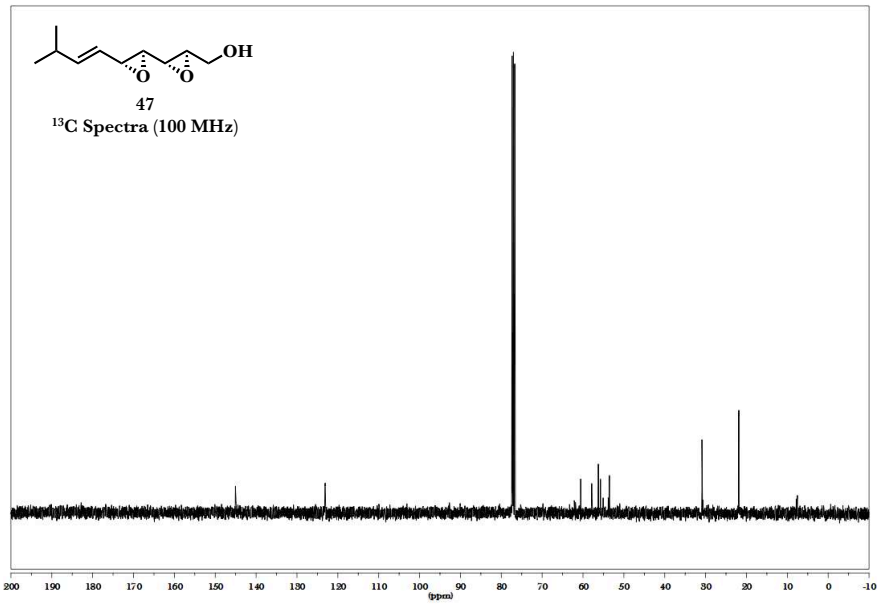


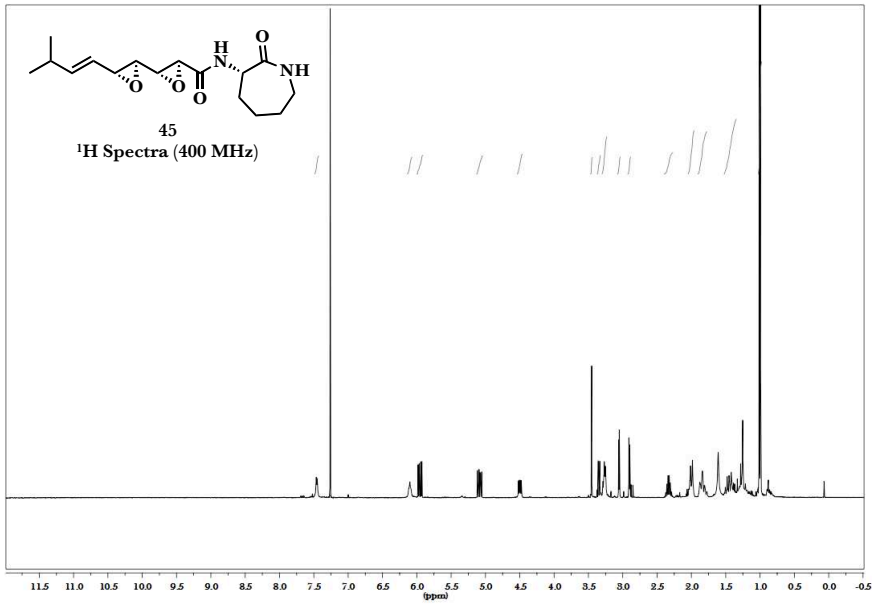
58
^{13}C Spectra (100 MHz)

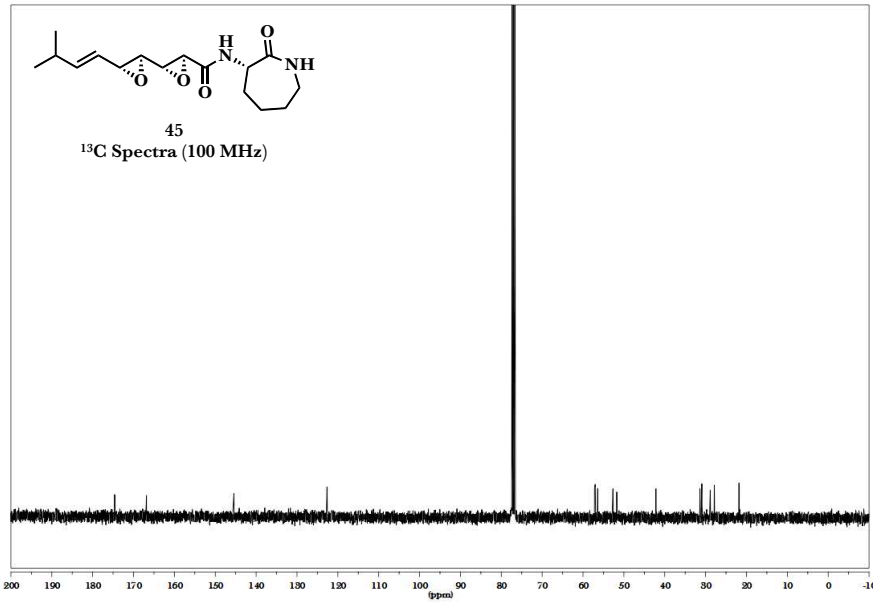


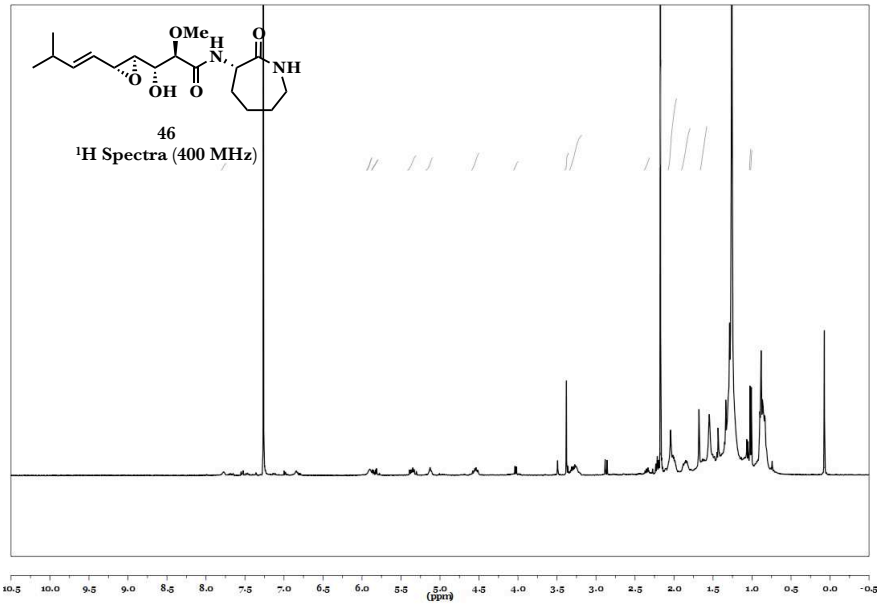




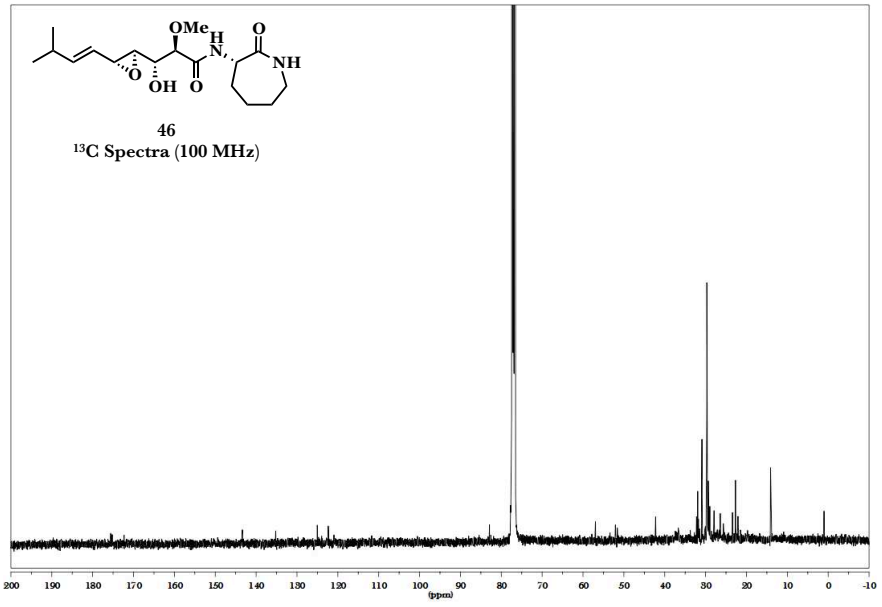




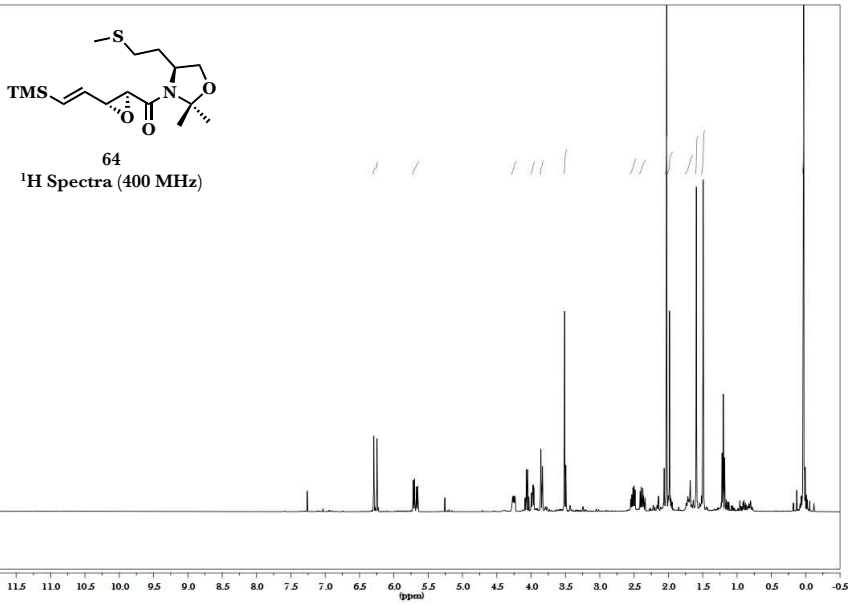




46
^{1}H Spectra (400 MHz)



46
^{13}C Spectra (100 MHz)



64
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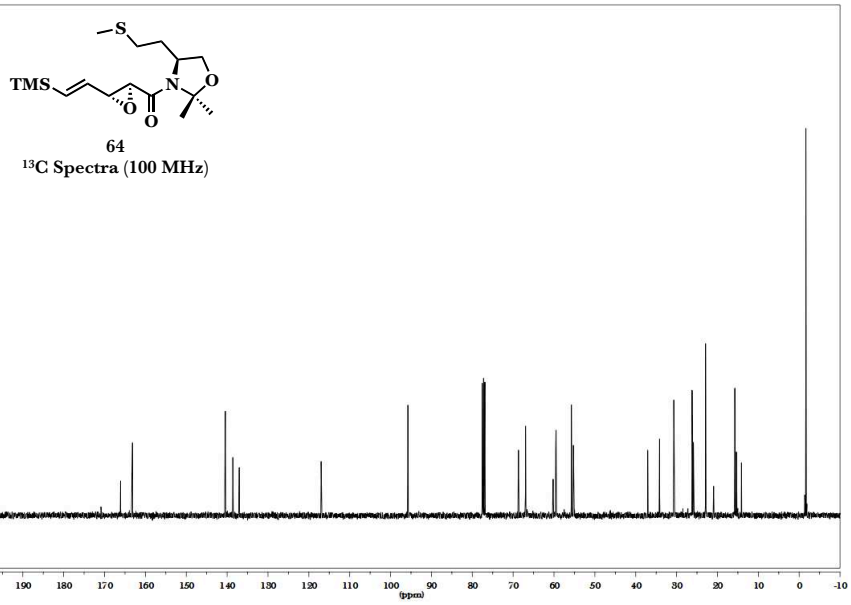

64
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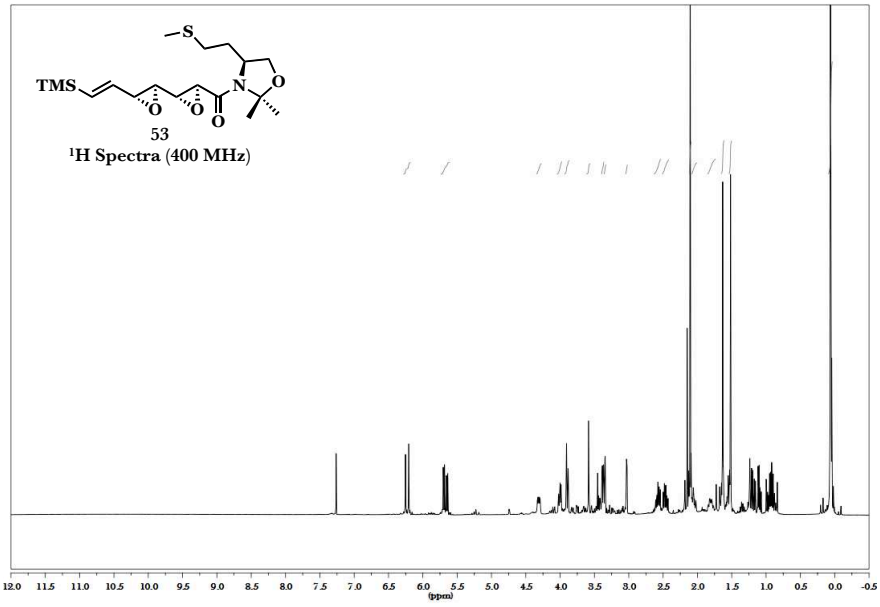
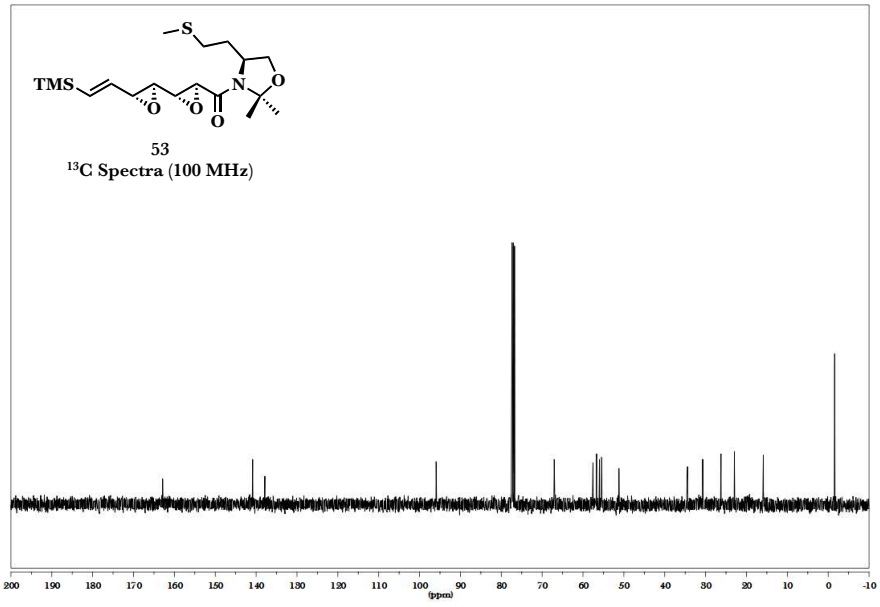
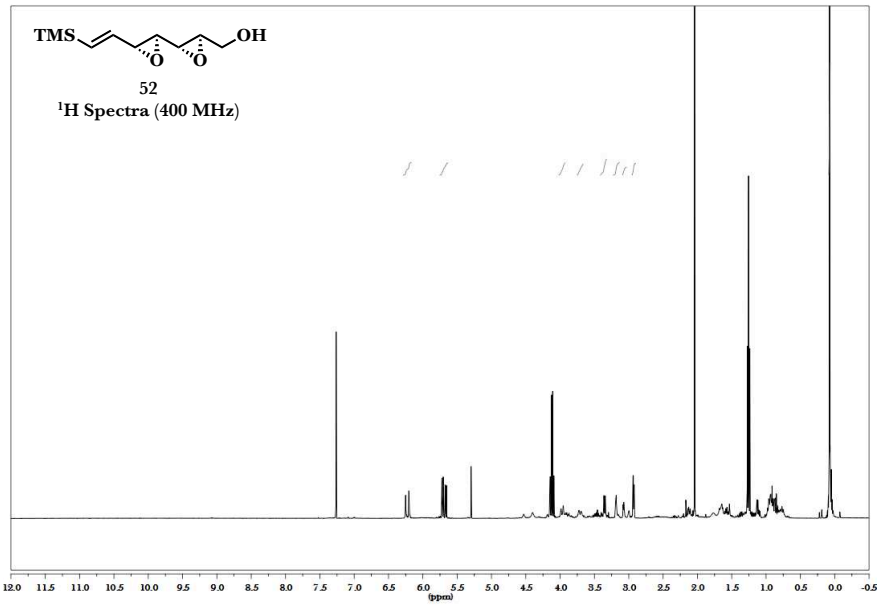
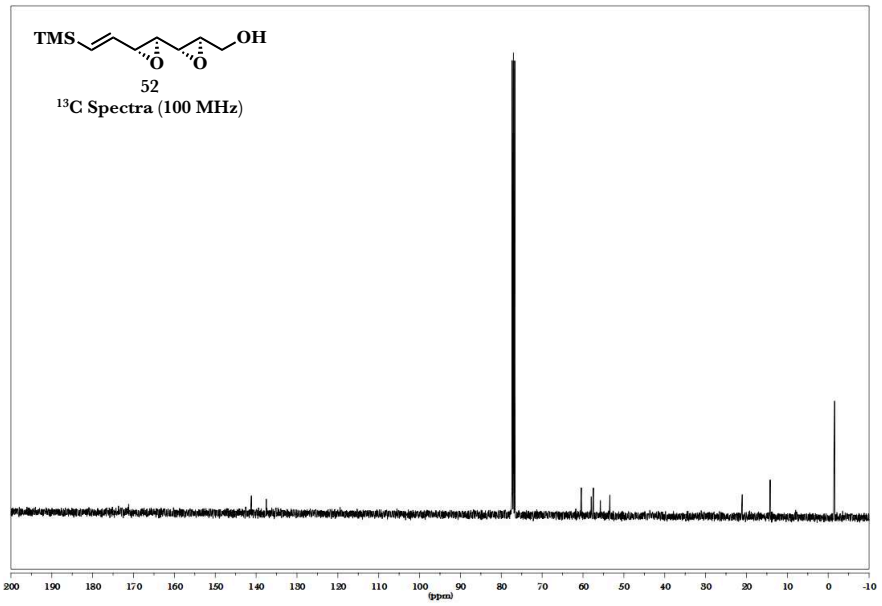
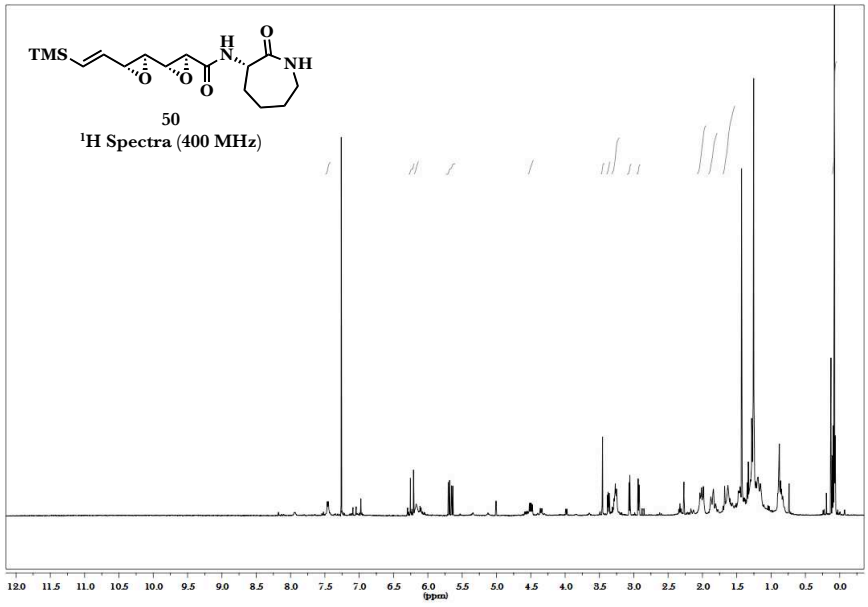




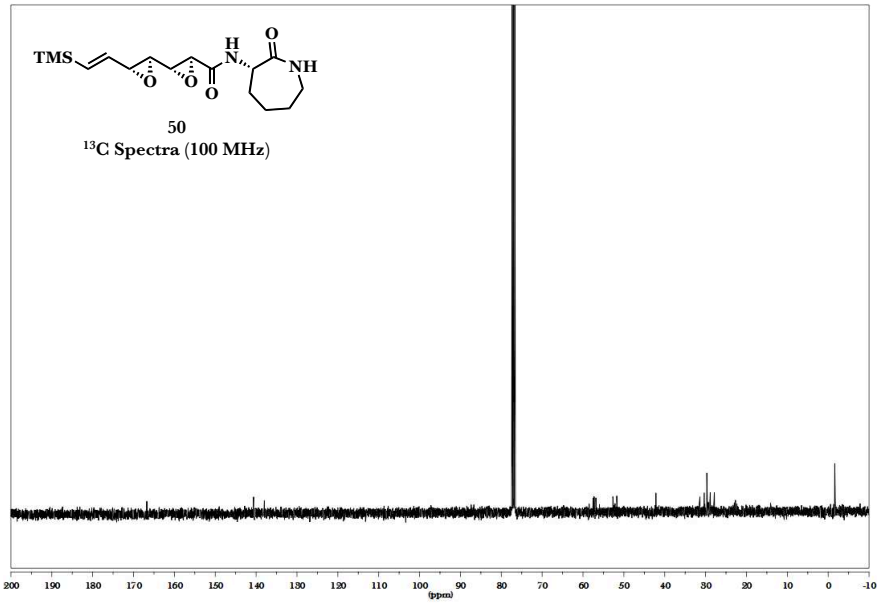






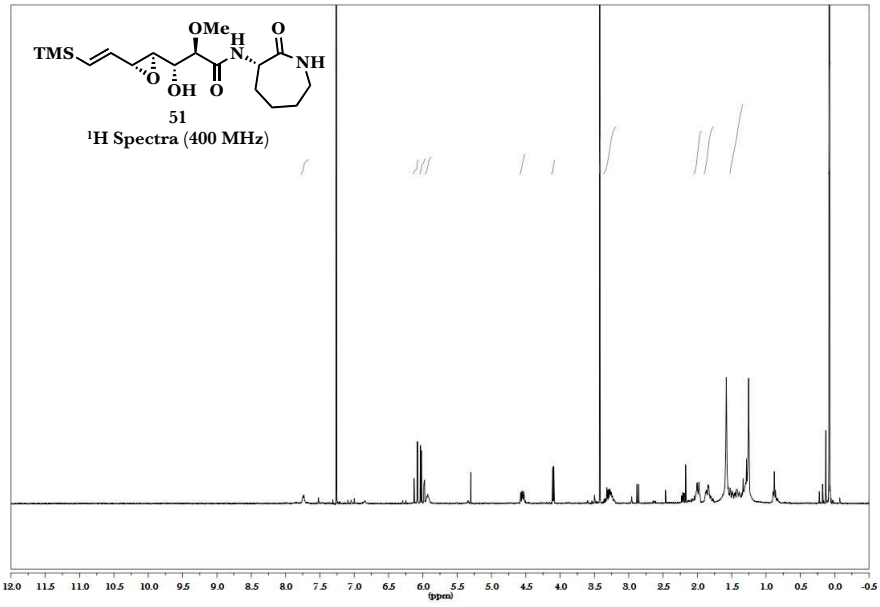
50

^{1}H Spectra (400 MHz)



50

^{13}C Spectra (100 MHz)



51

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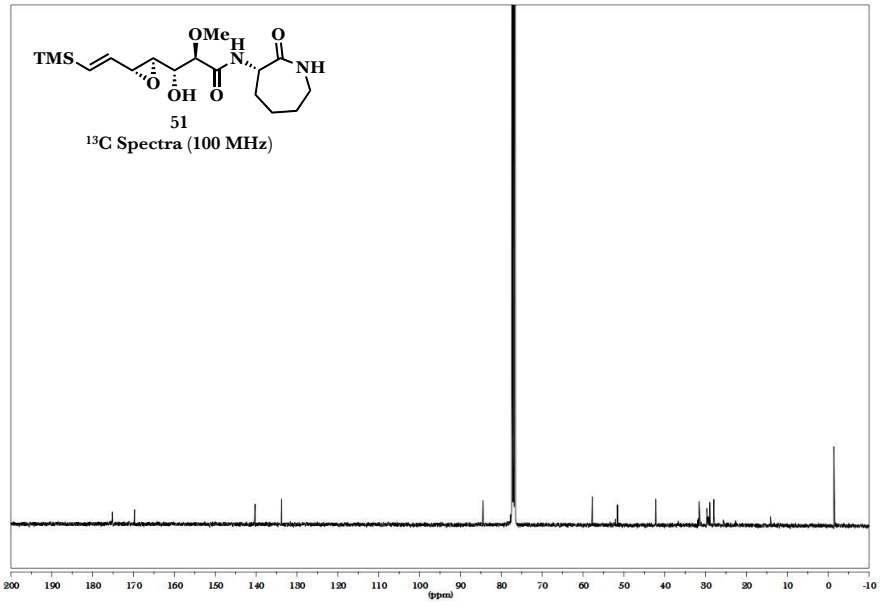
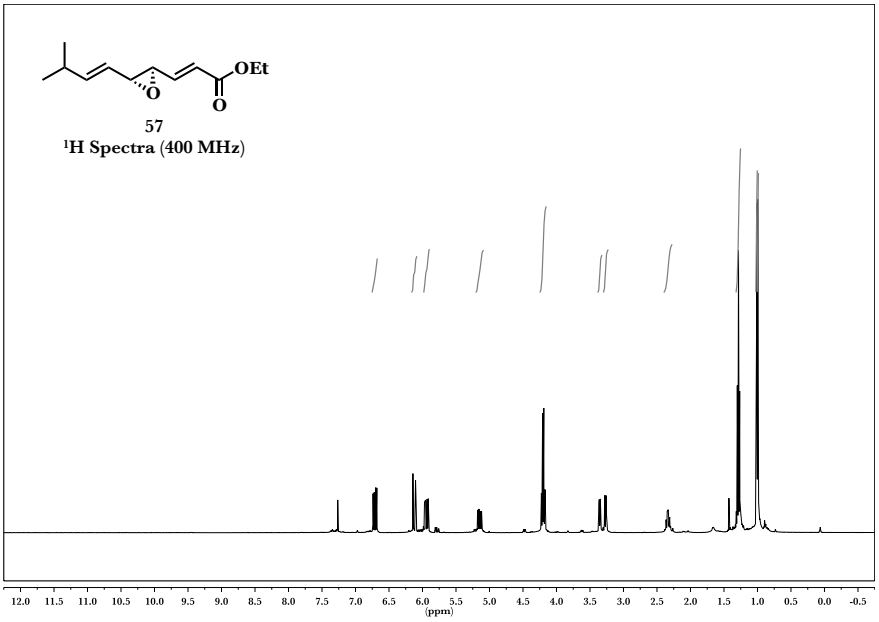
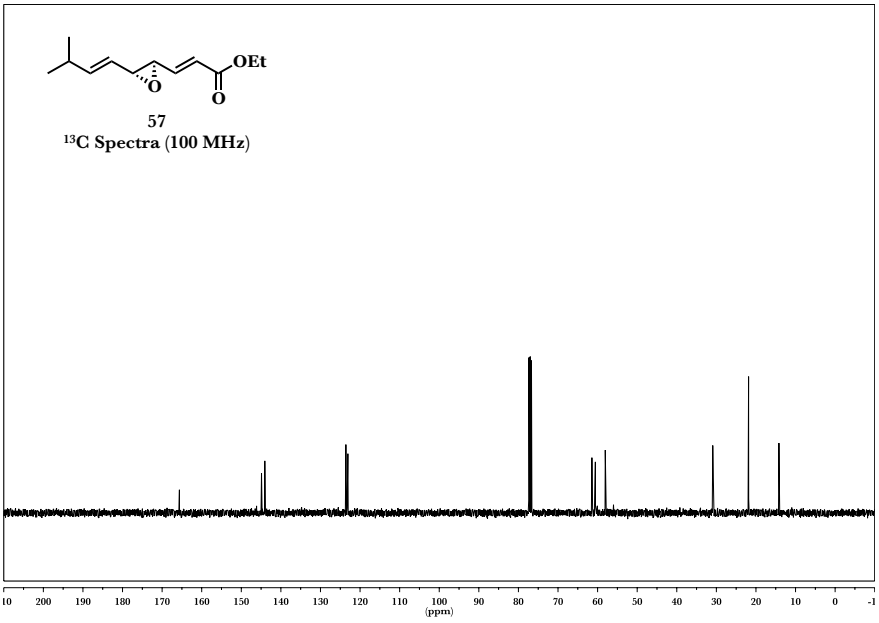
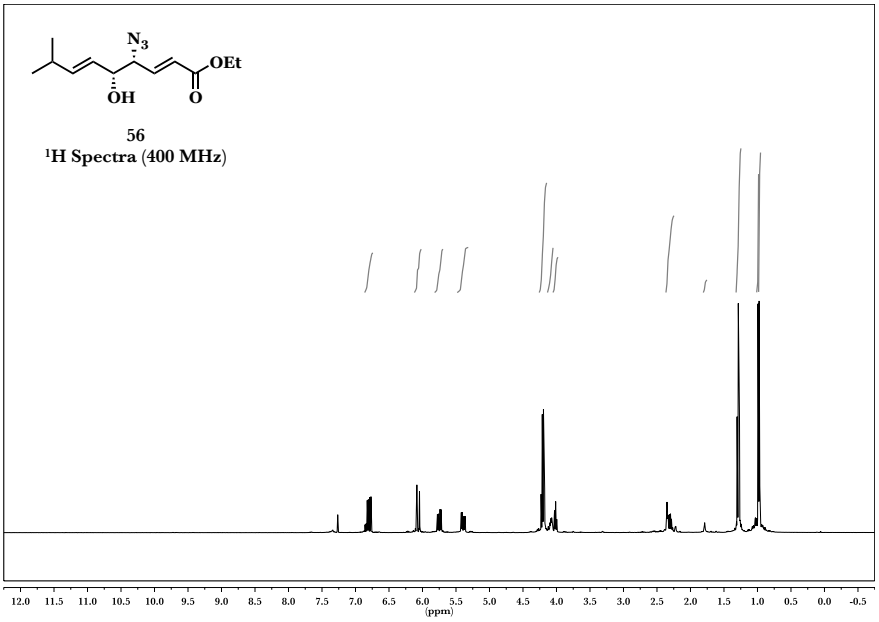


51

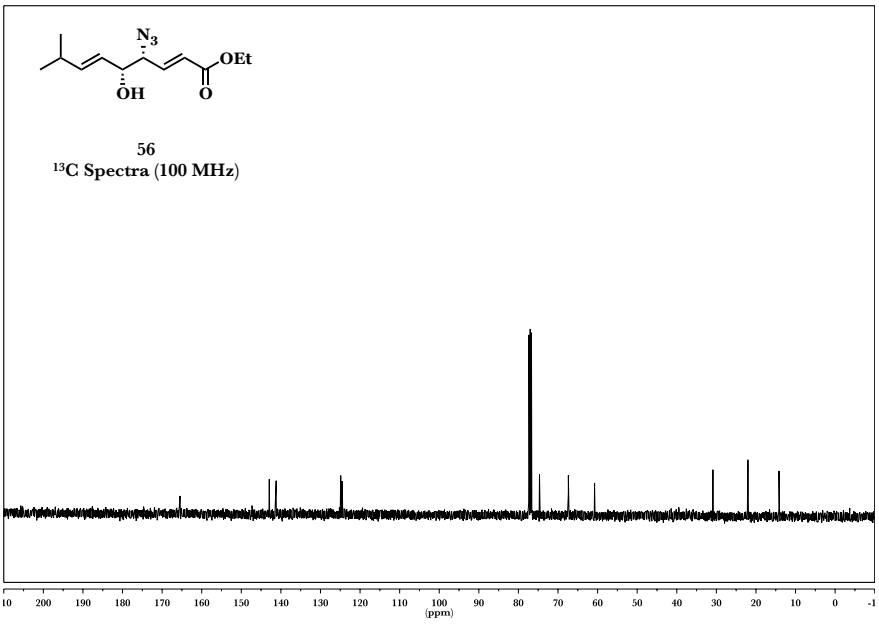
^{13}C Spectra (100 MHz)



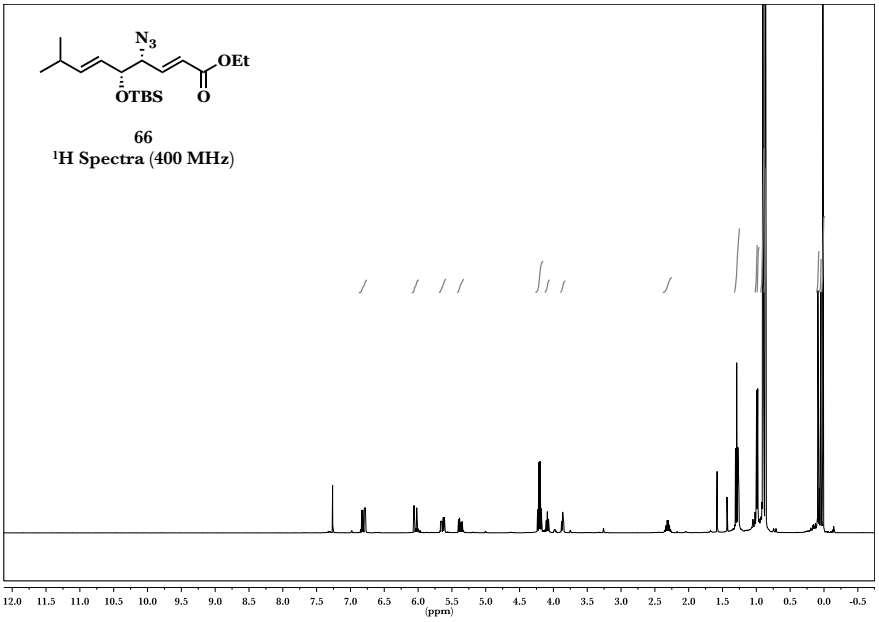




56
^{1}H Spectra (400 MHz)



56
^{13}C Spectra (100 MHz)



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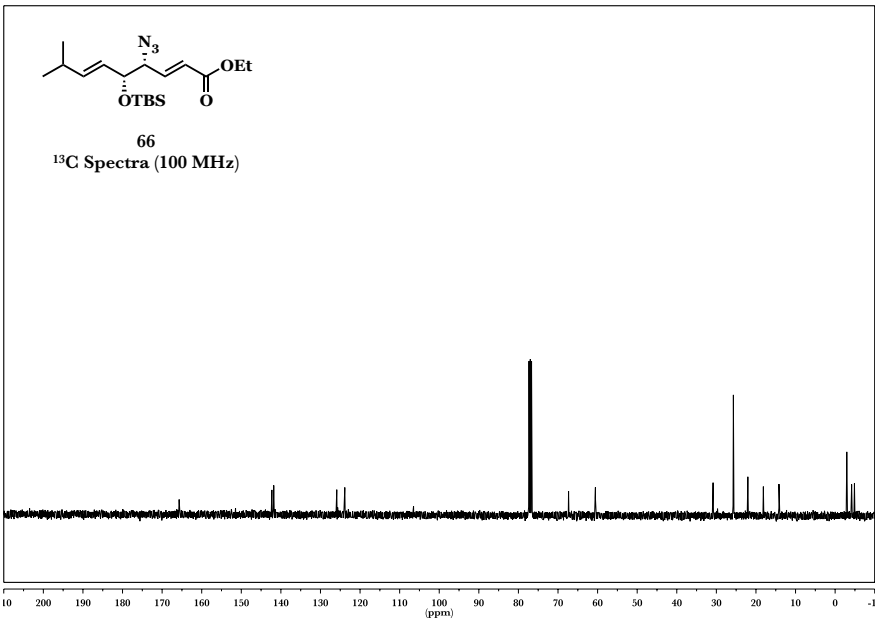

66
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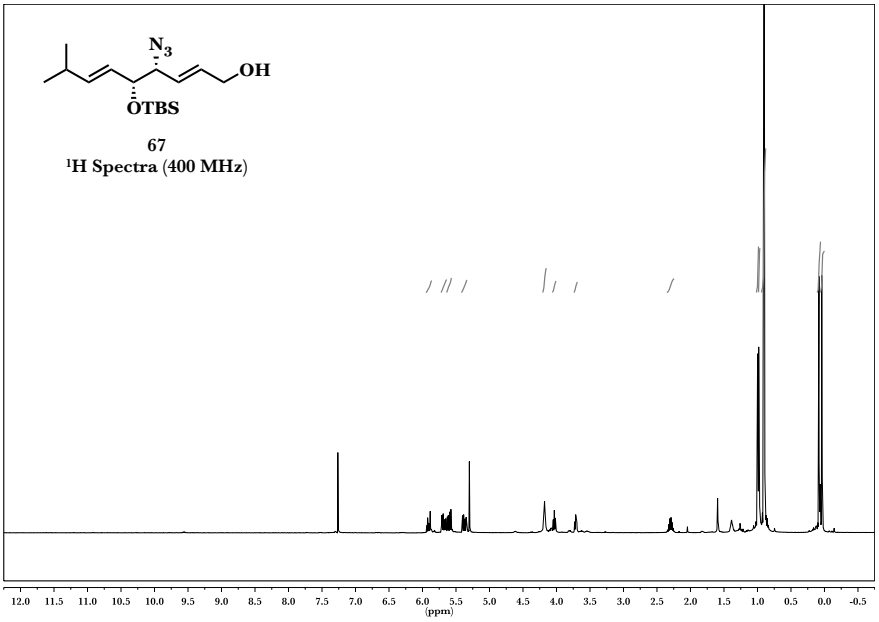
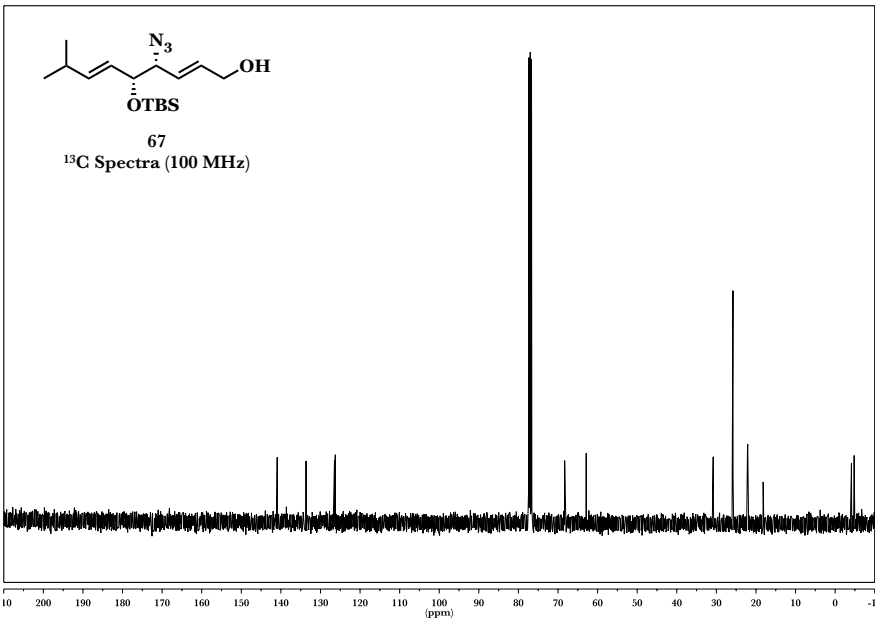
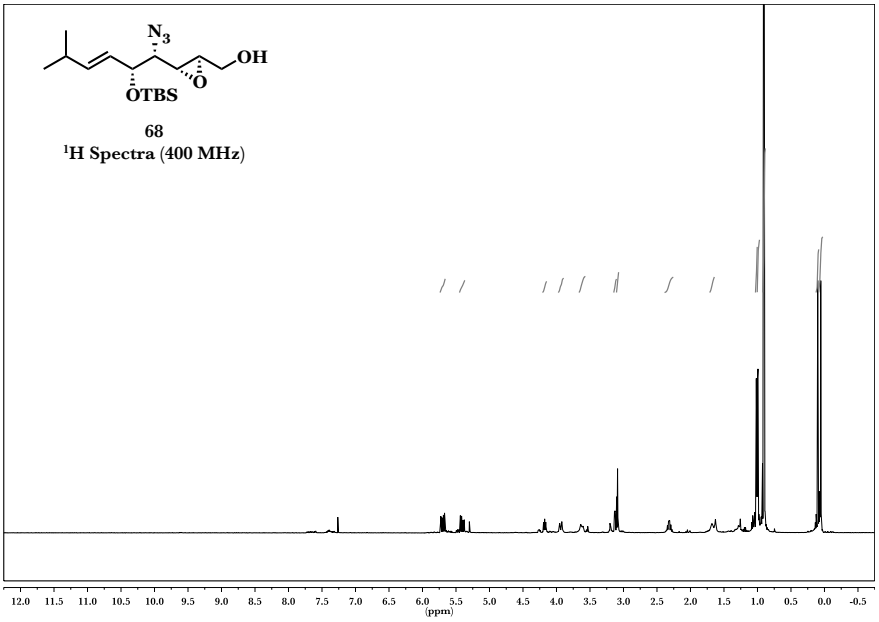
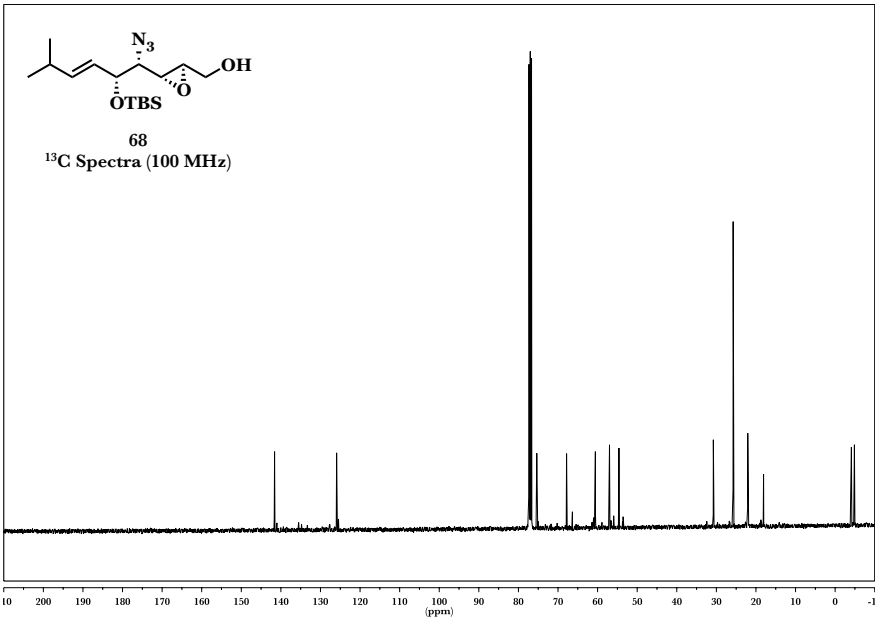
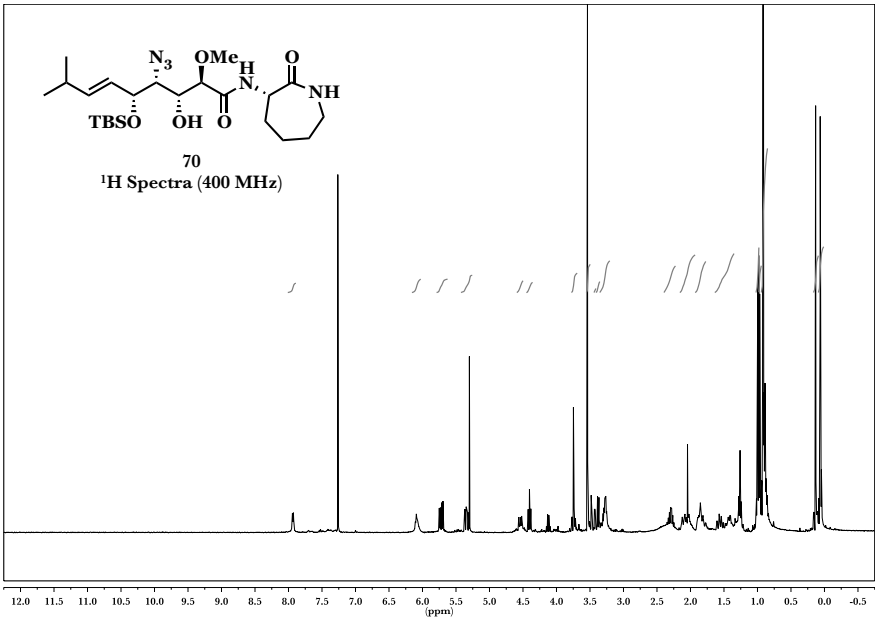


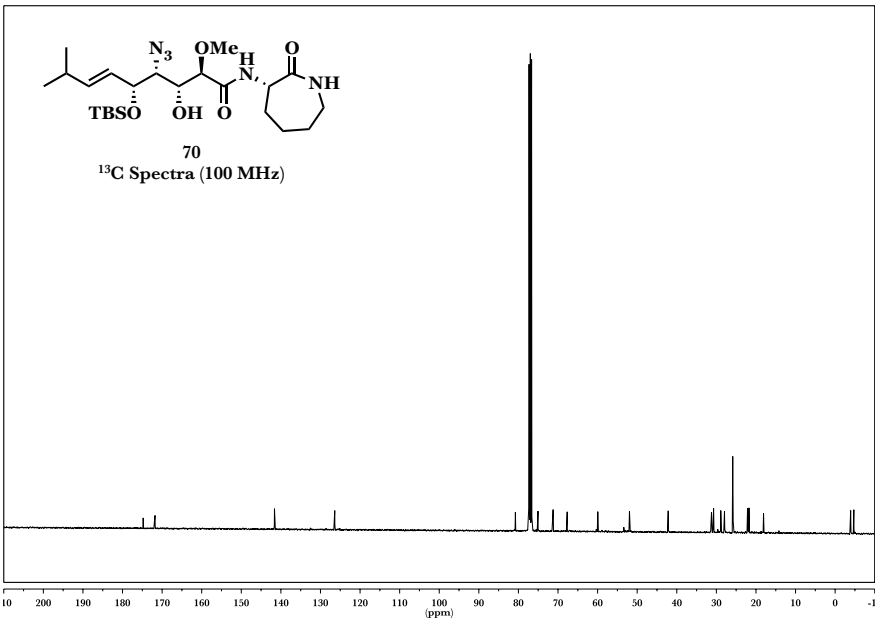




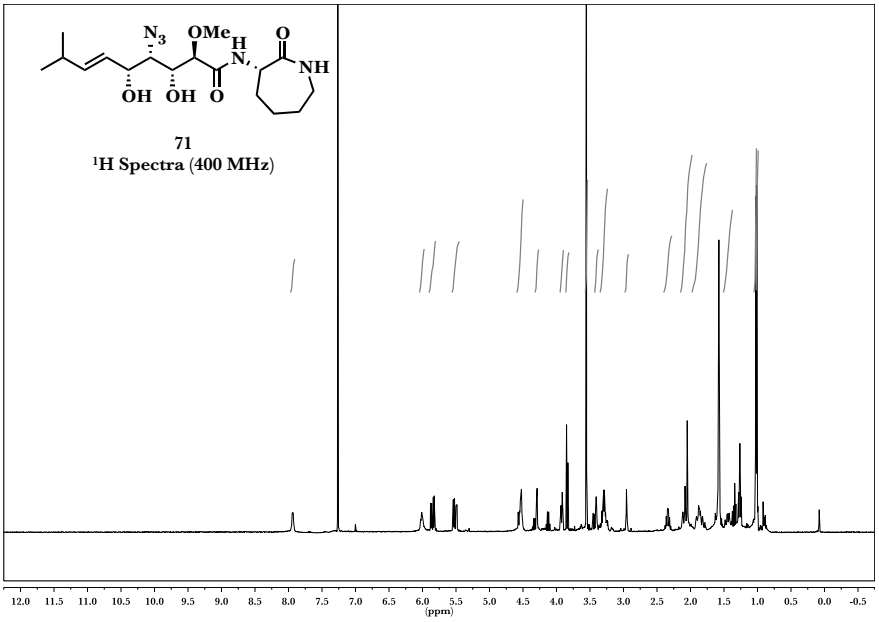




70
^{1}H Spectra (400 MHz)



70
^{13}C Spectra (100 MHz)



71
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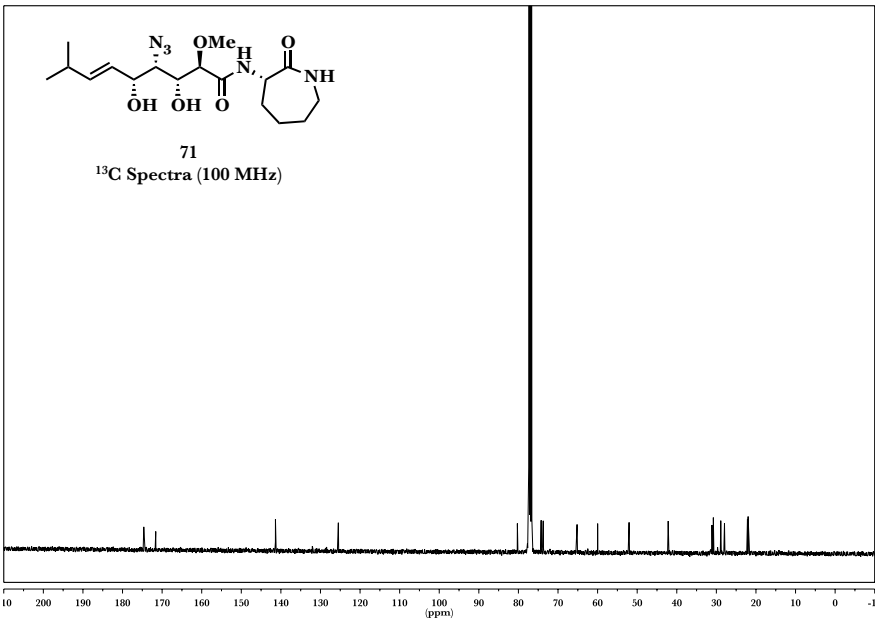
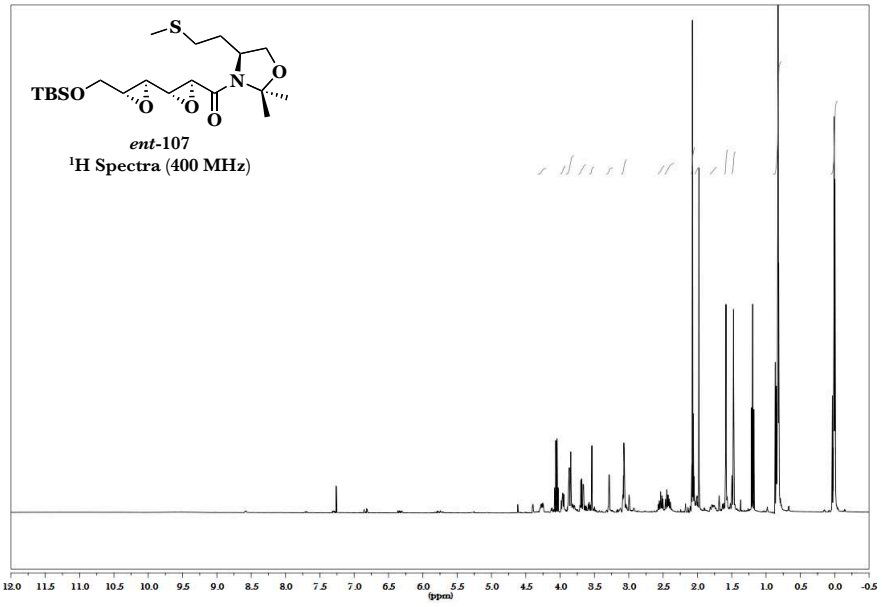
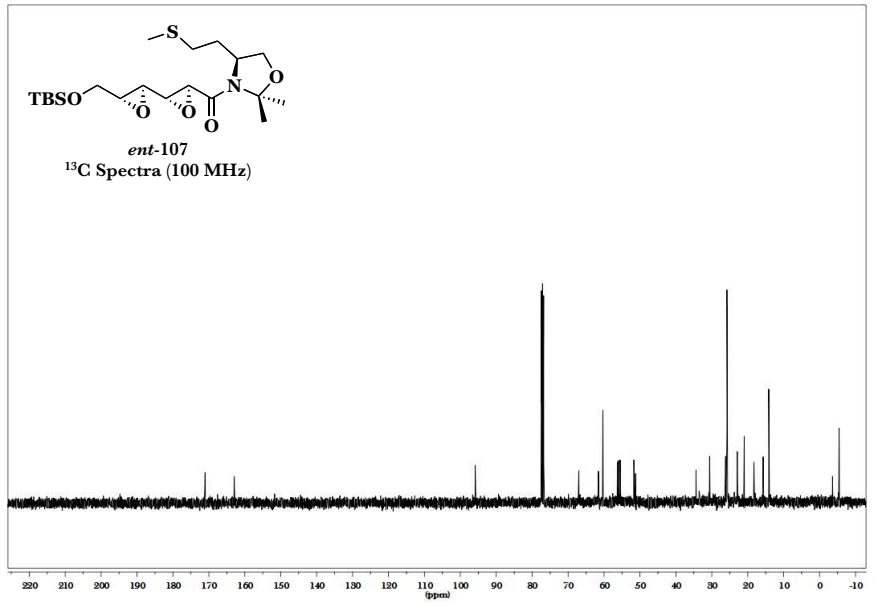
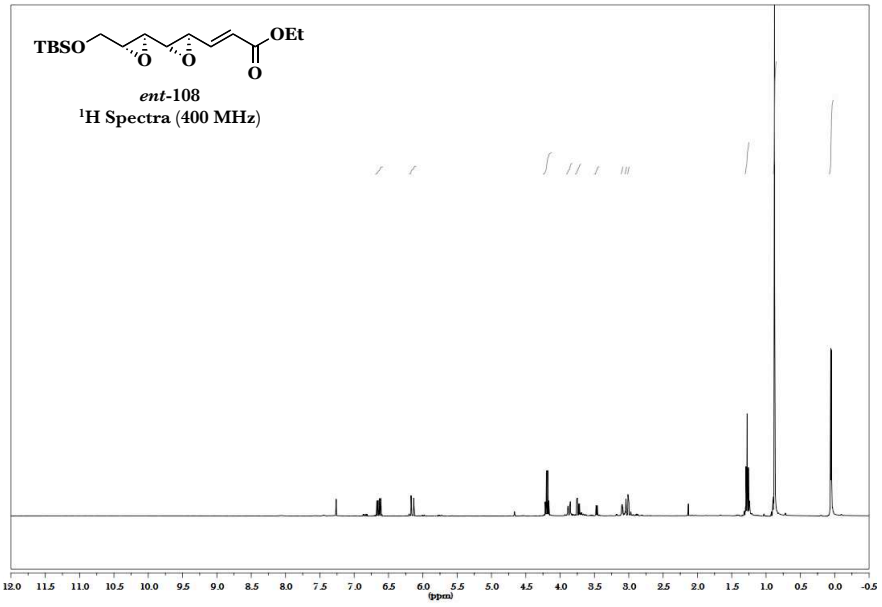


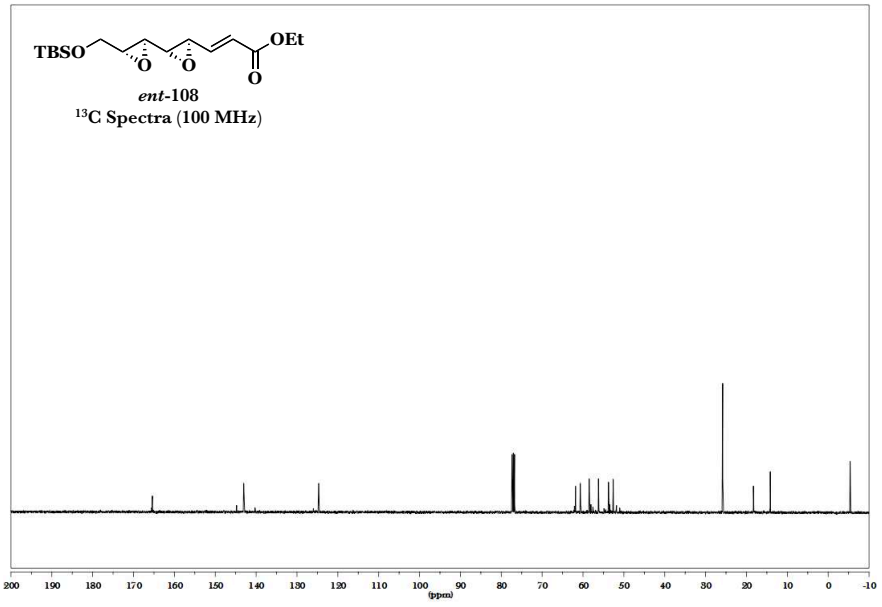
71
^{13}C Spectra (100 MHz)

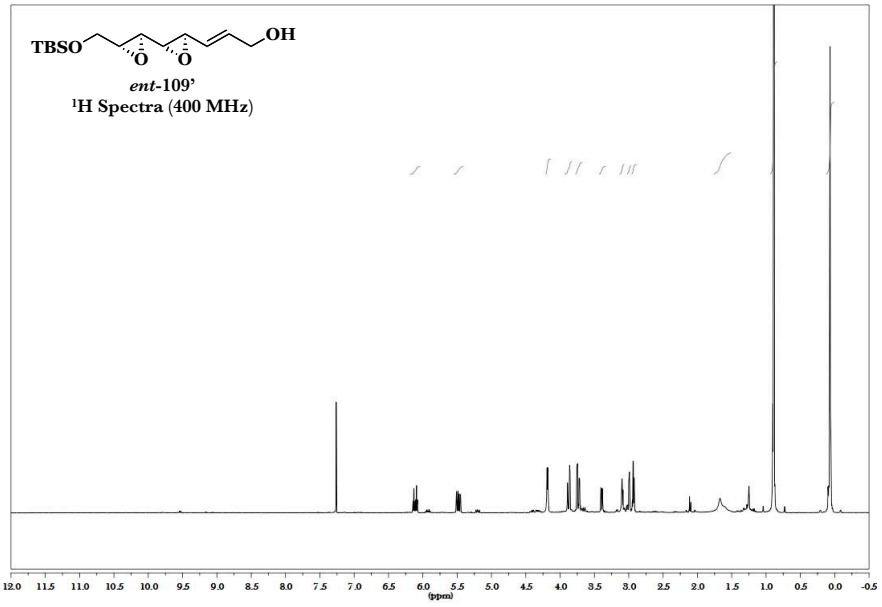
APPENDIX THREE: Spectra Relevant to Chapter Three

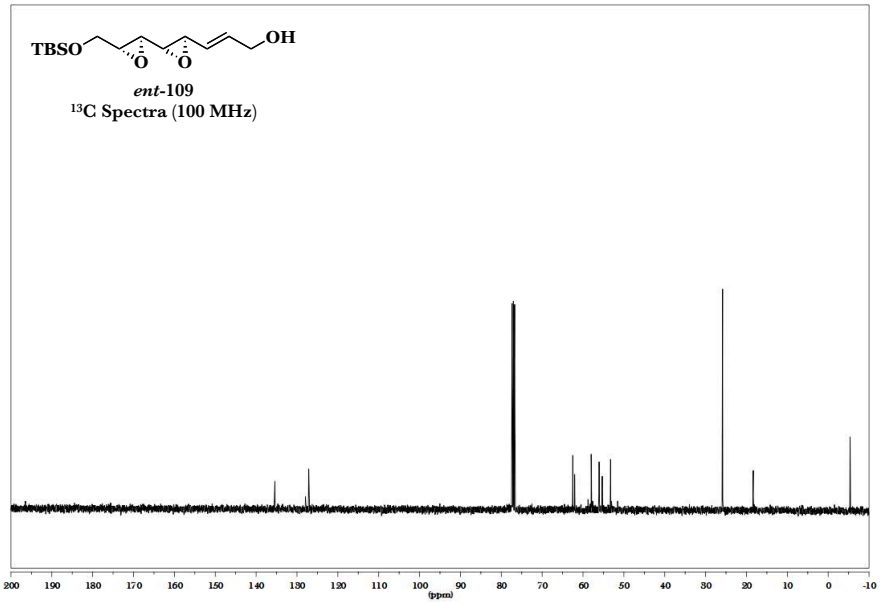


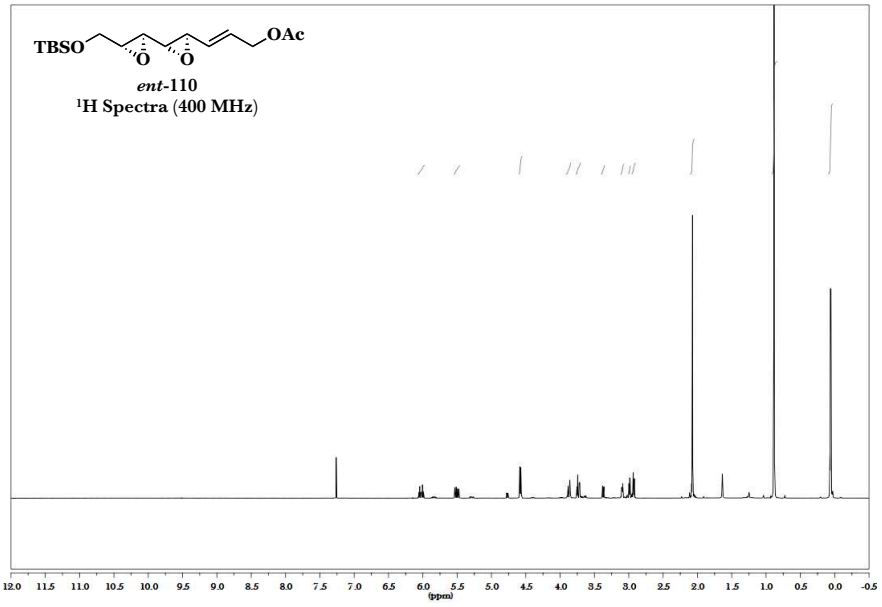


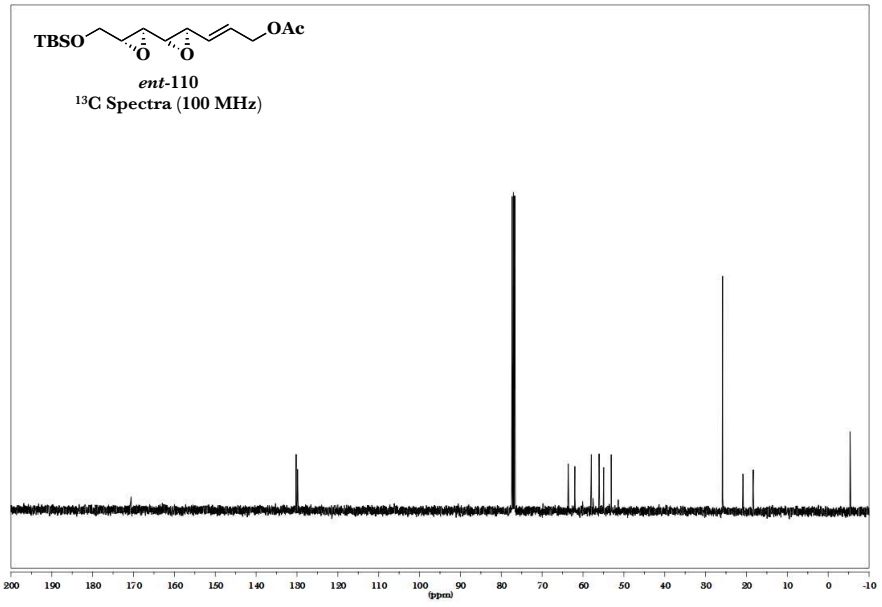


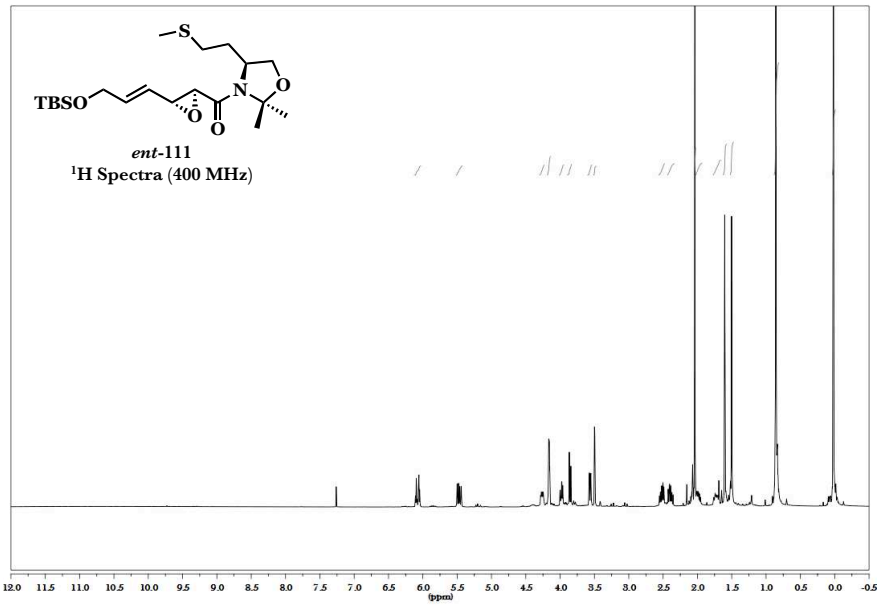




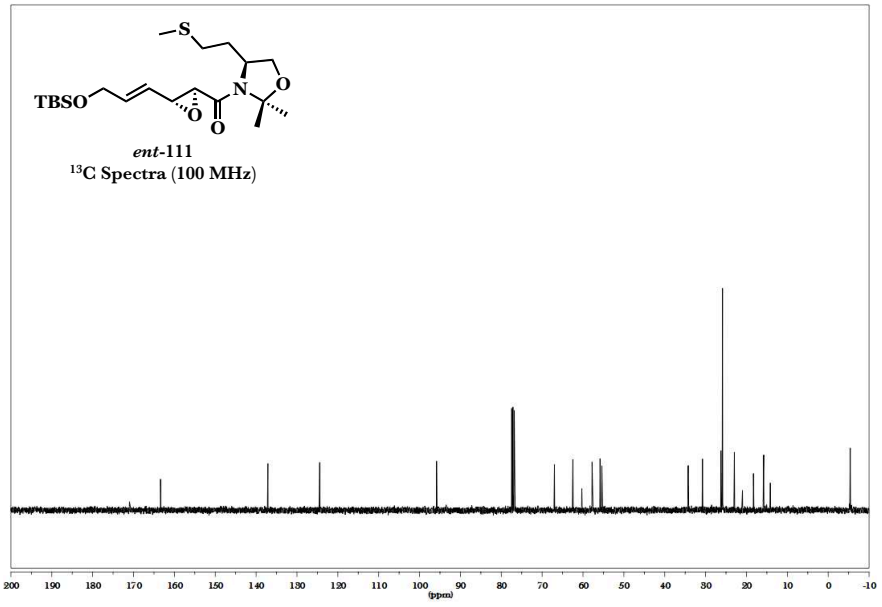




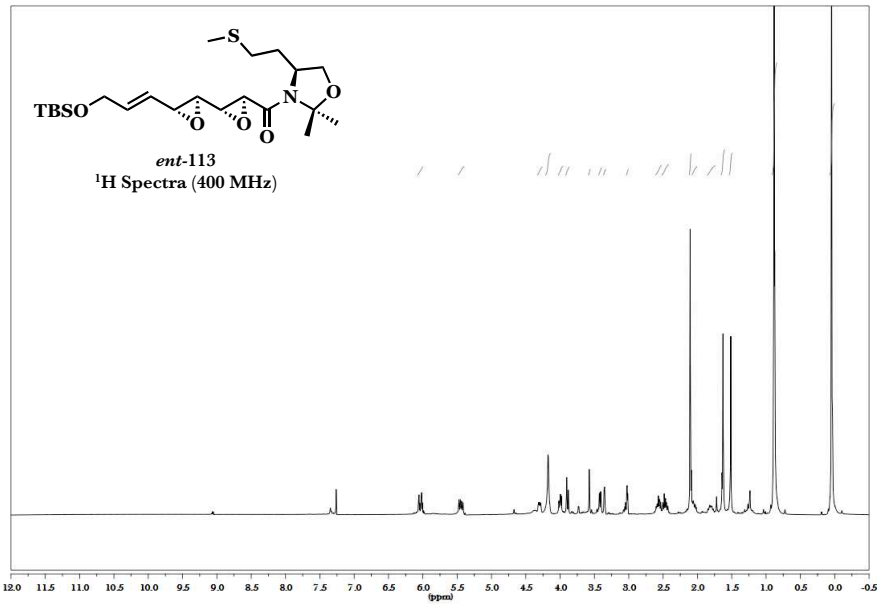




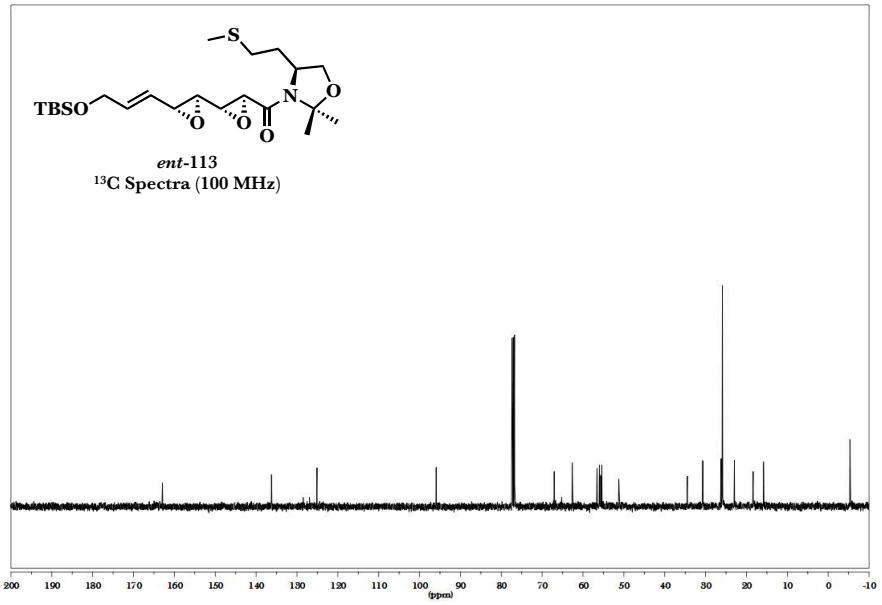
ent-111
^{1}H Spectra (400 MHz)



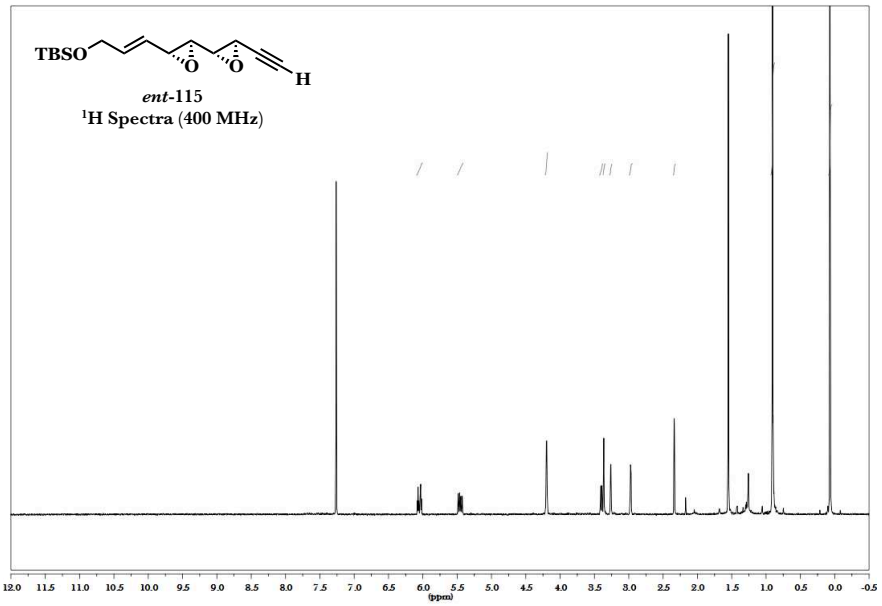
ent-111
^{13}C Spectra (100 MHz)

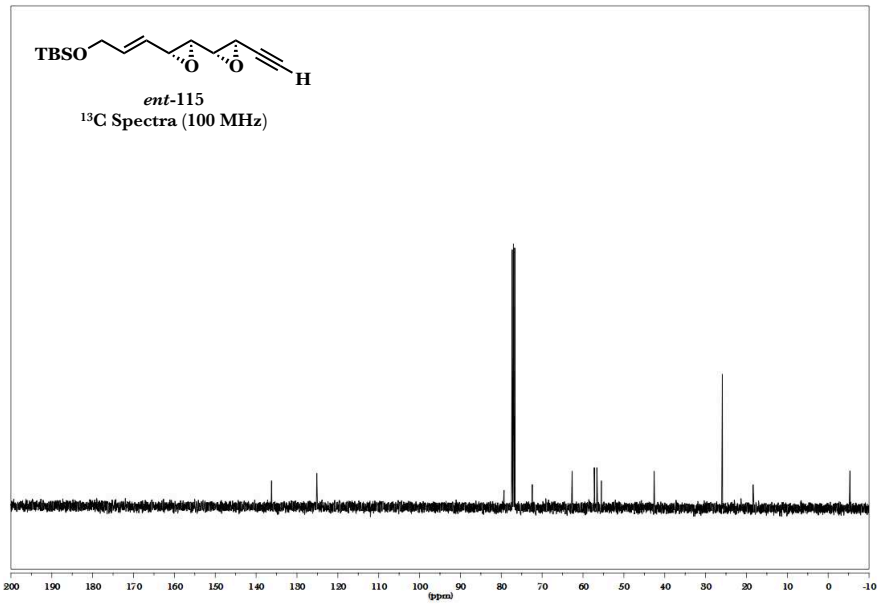


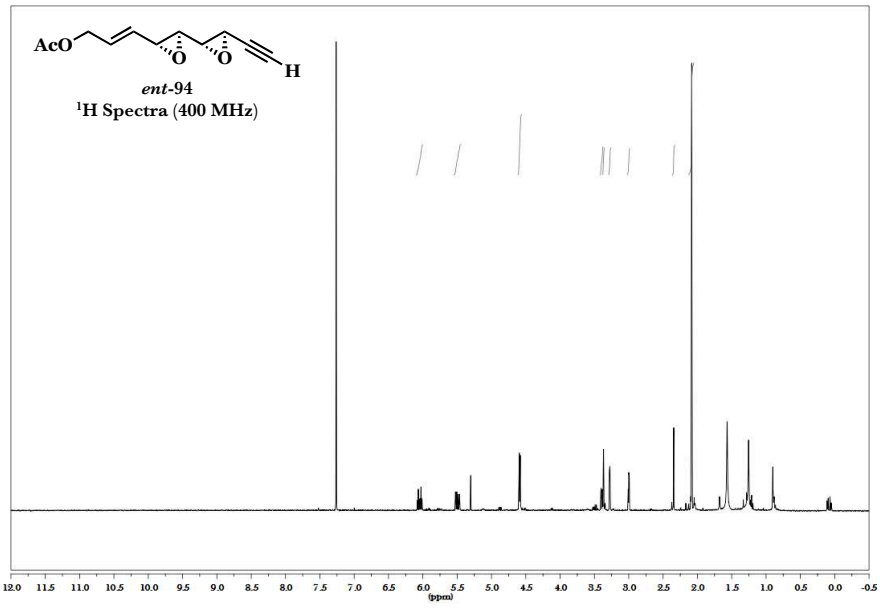
***ent*-113**
^{1}H Spectra (400 MHz)

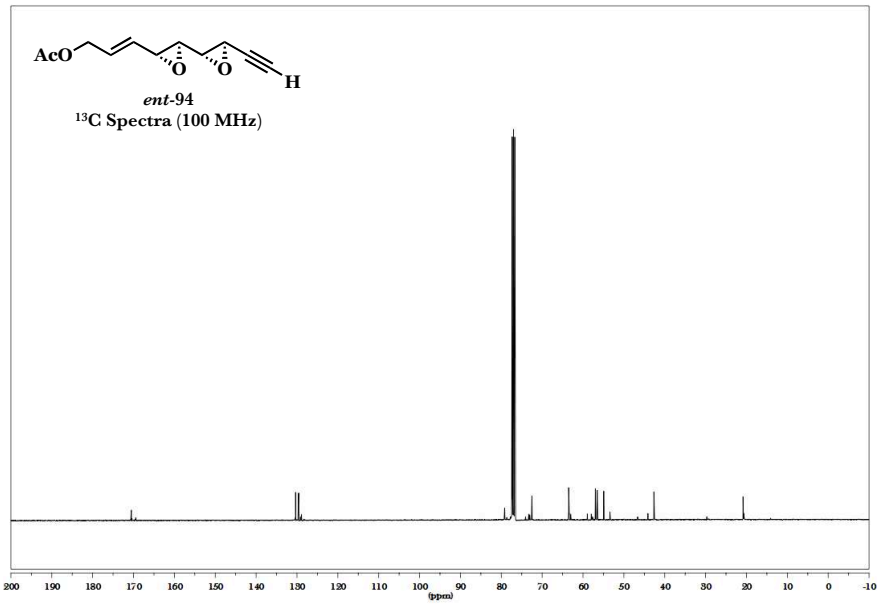


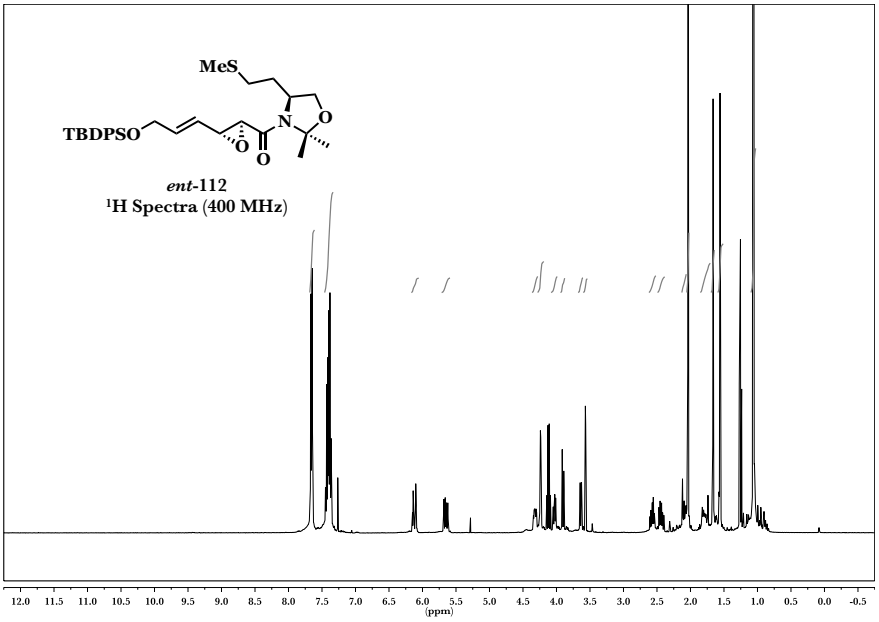
***ent*-113**
^{13}C Spectra (100 MHz)



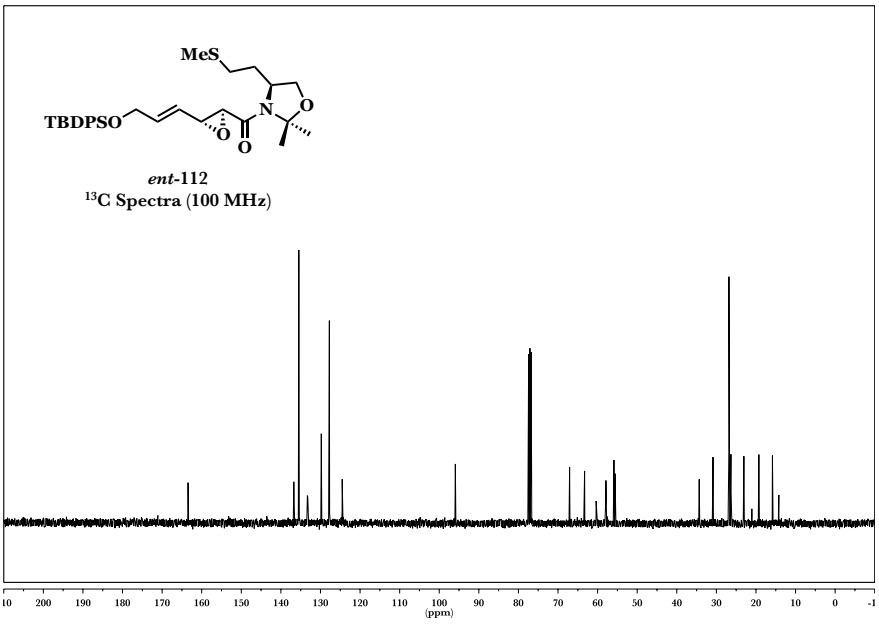




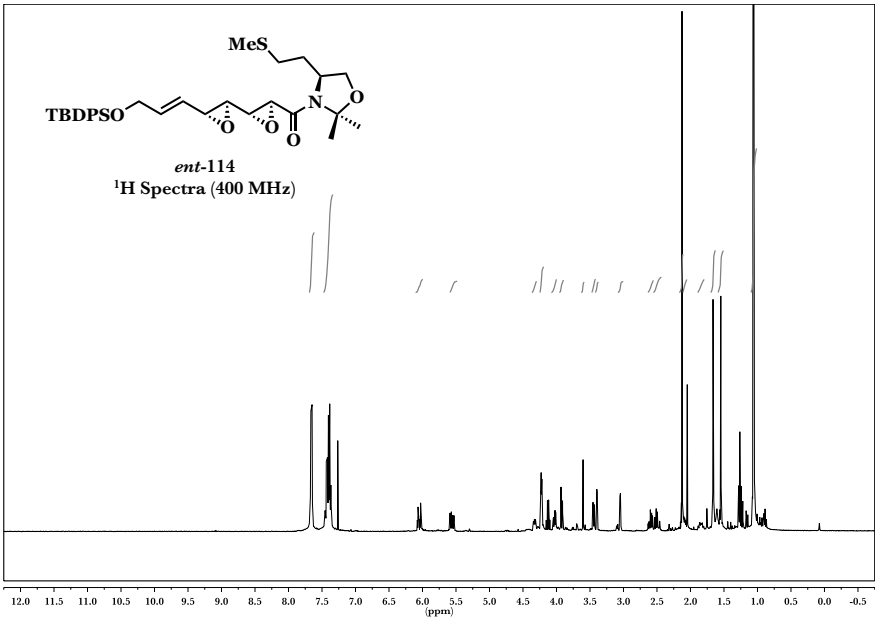


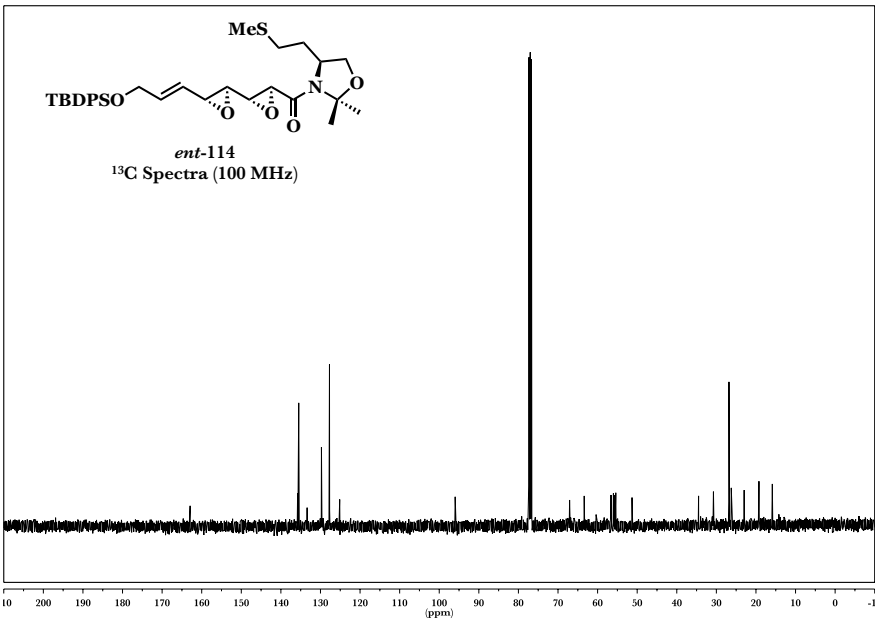


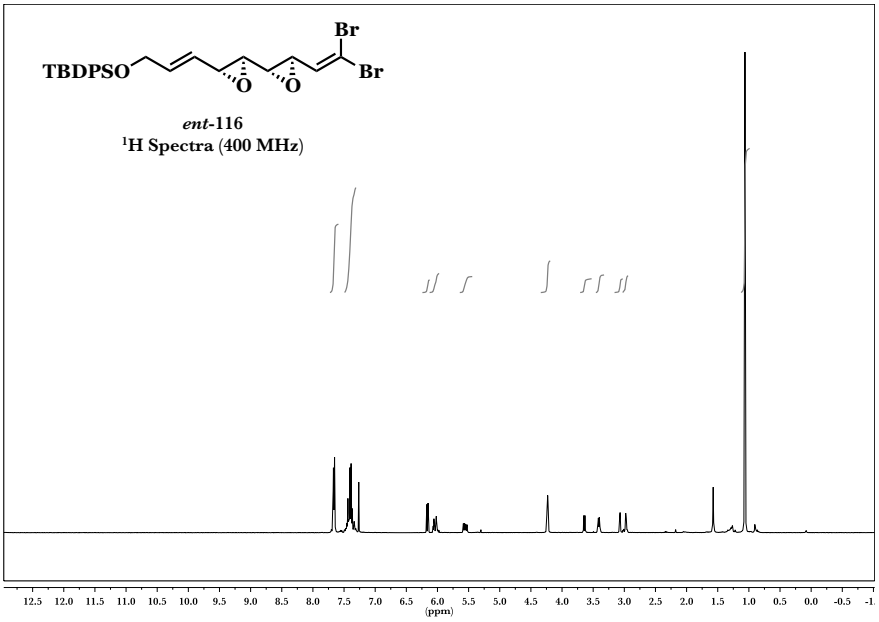
***ent*-112**
^{1}H Spectra (400 MHz)

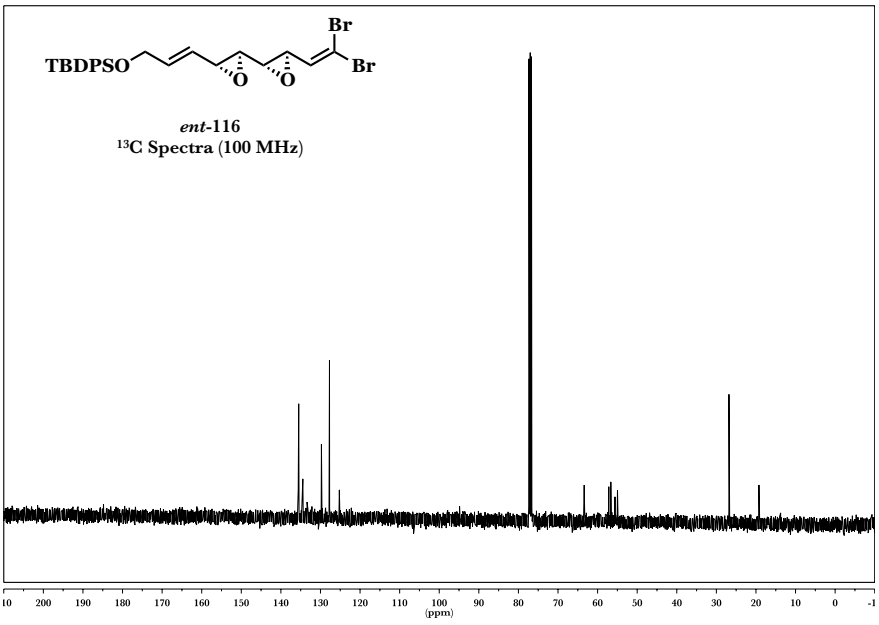


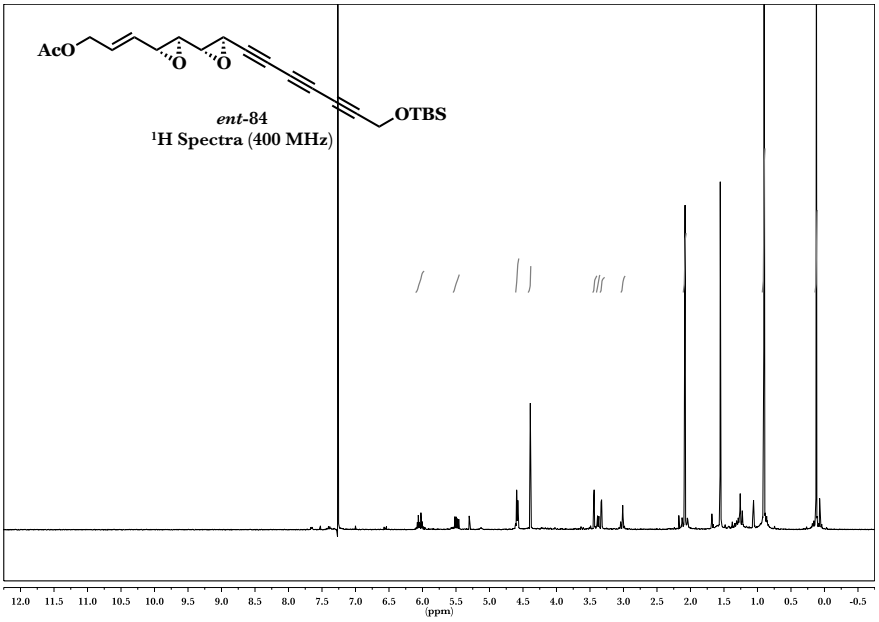
***ent*-112**
^{13}C Spectra (100 MHz)

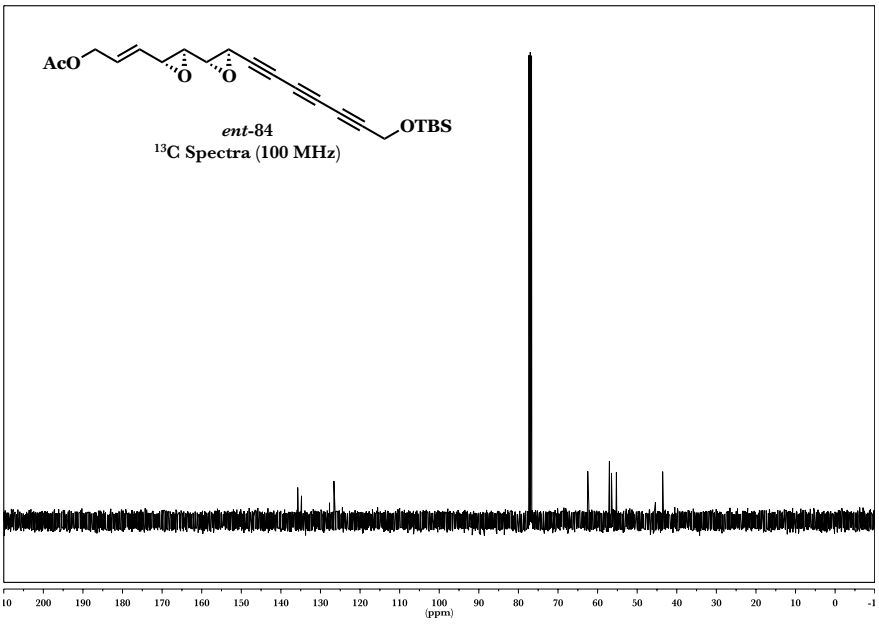


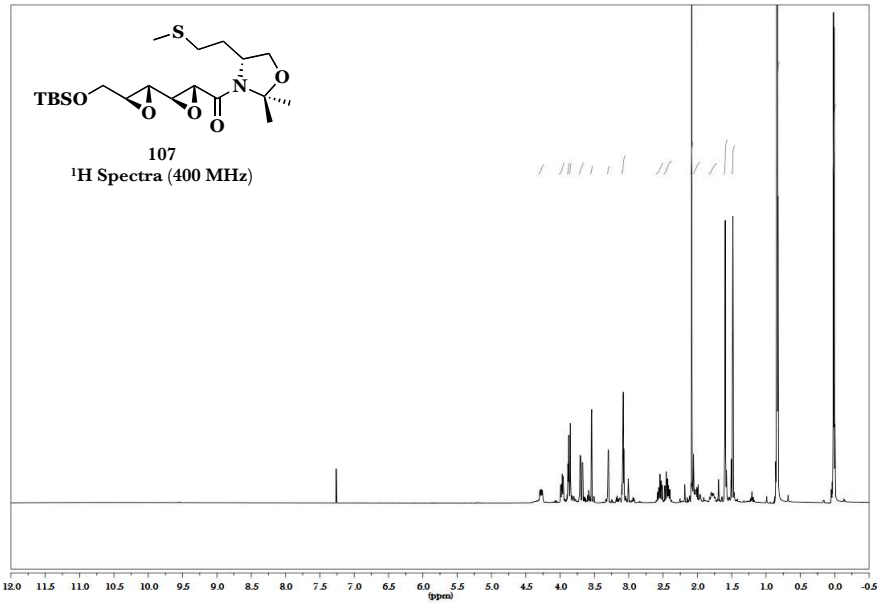




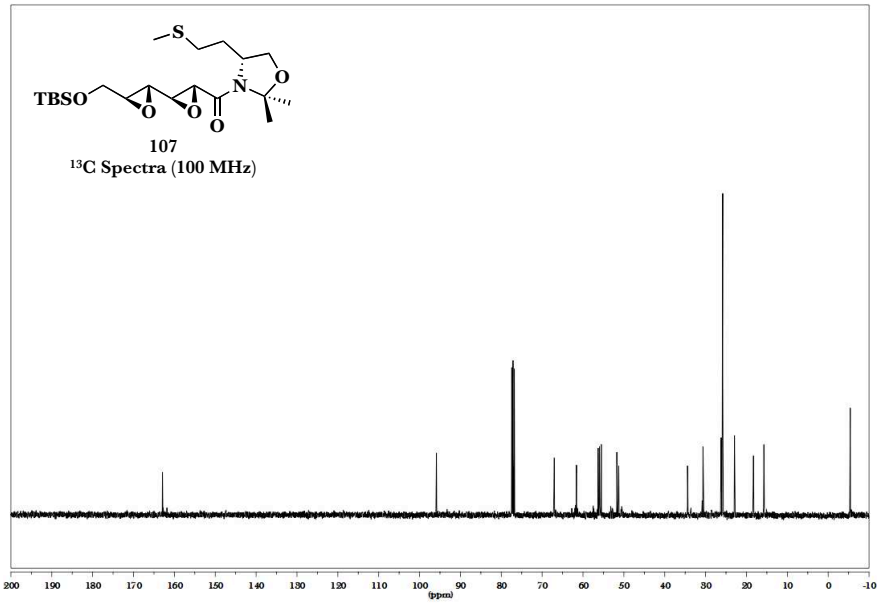




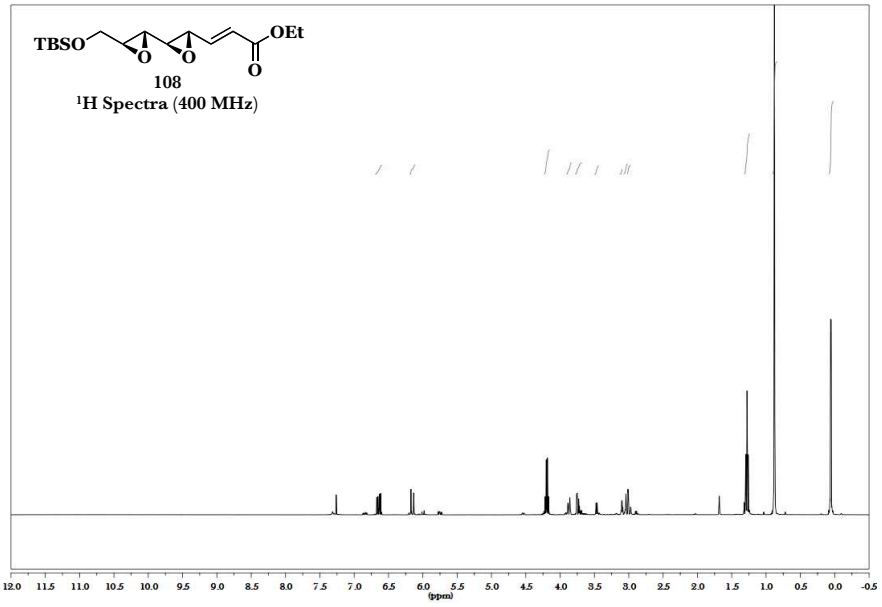


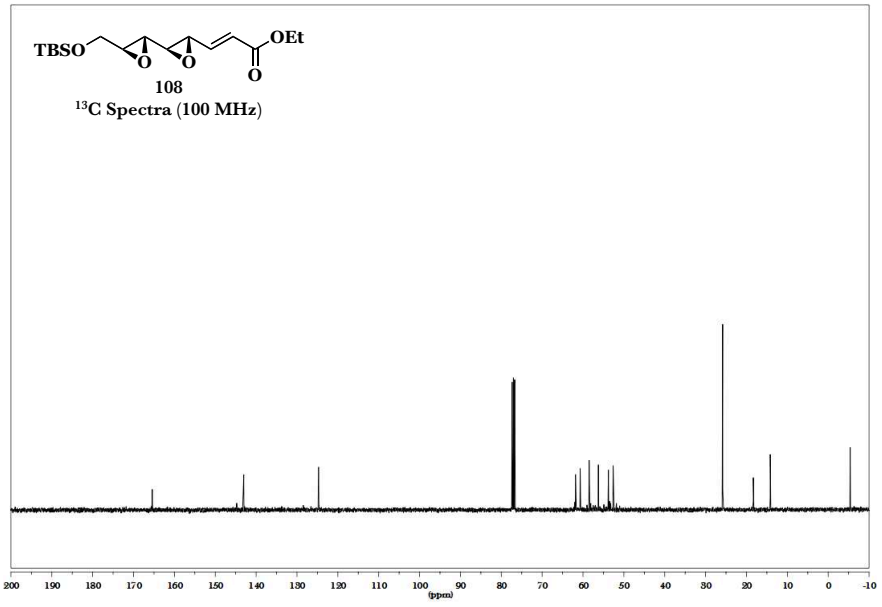


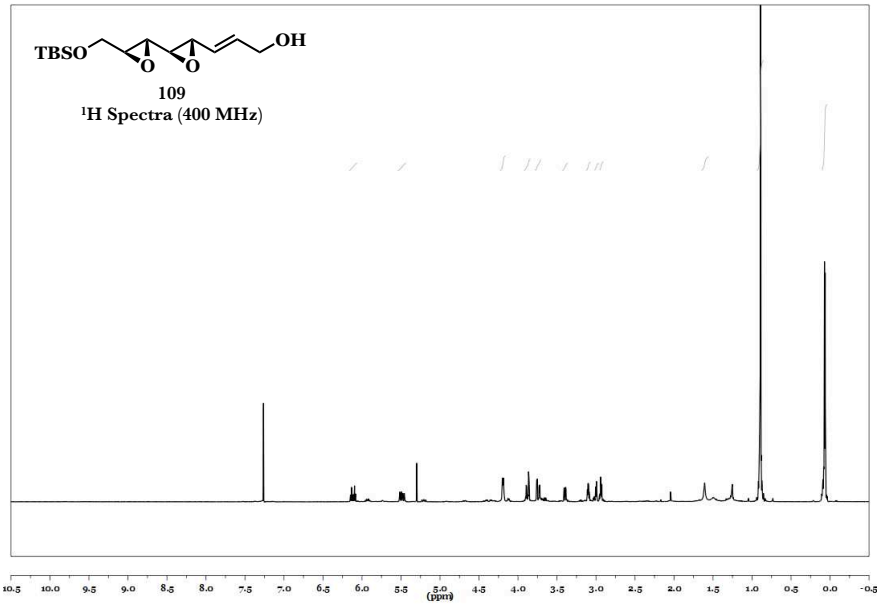
107
^{1}H Spectra (400 MHz)

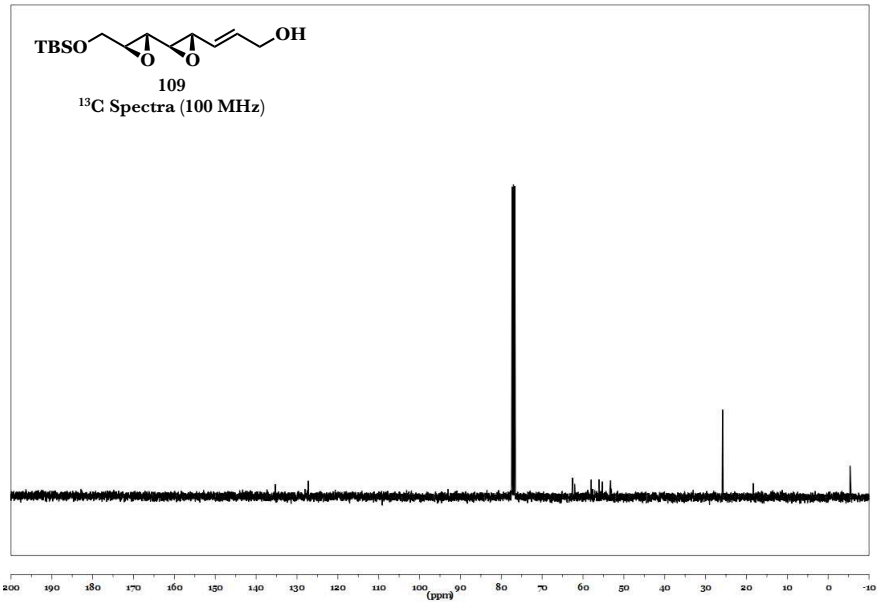


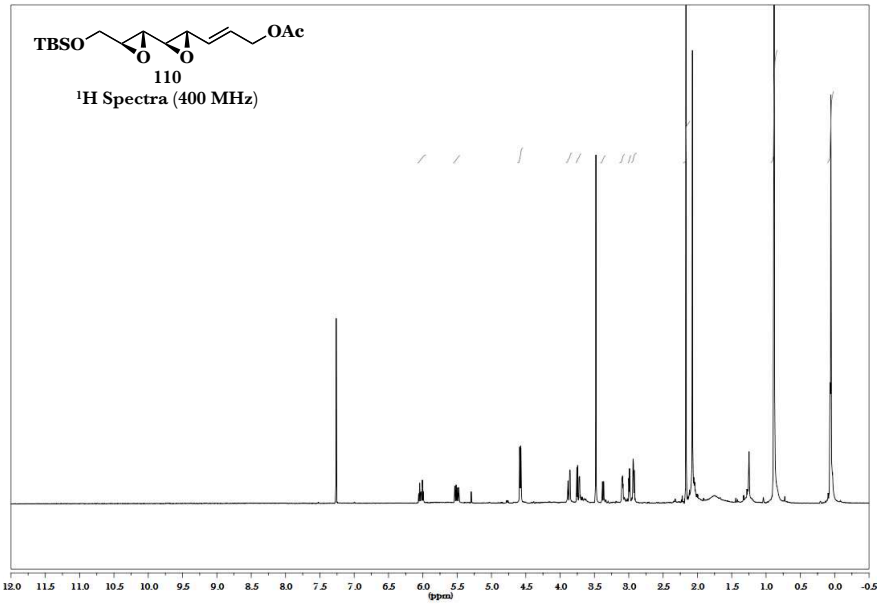
107
^{13}C Spectra (100 MHz)

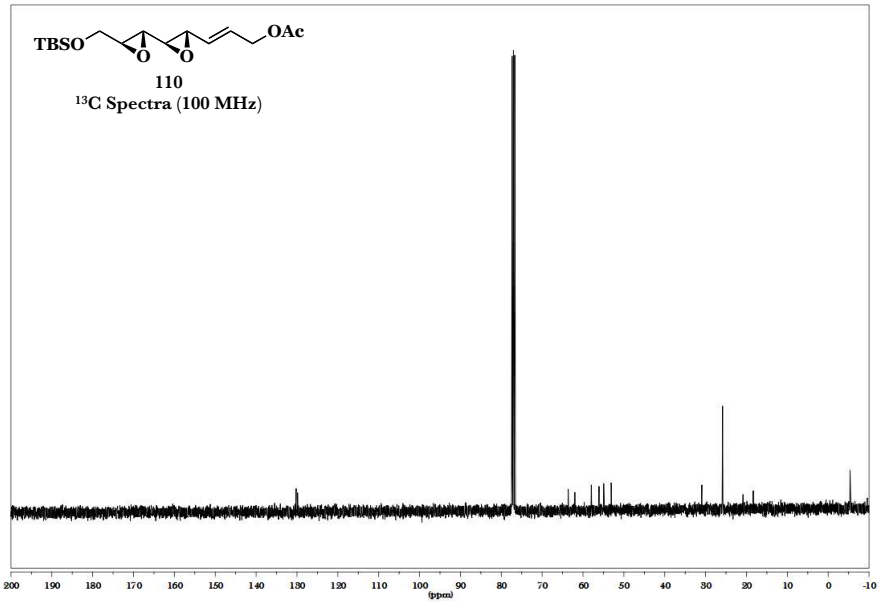


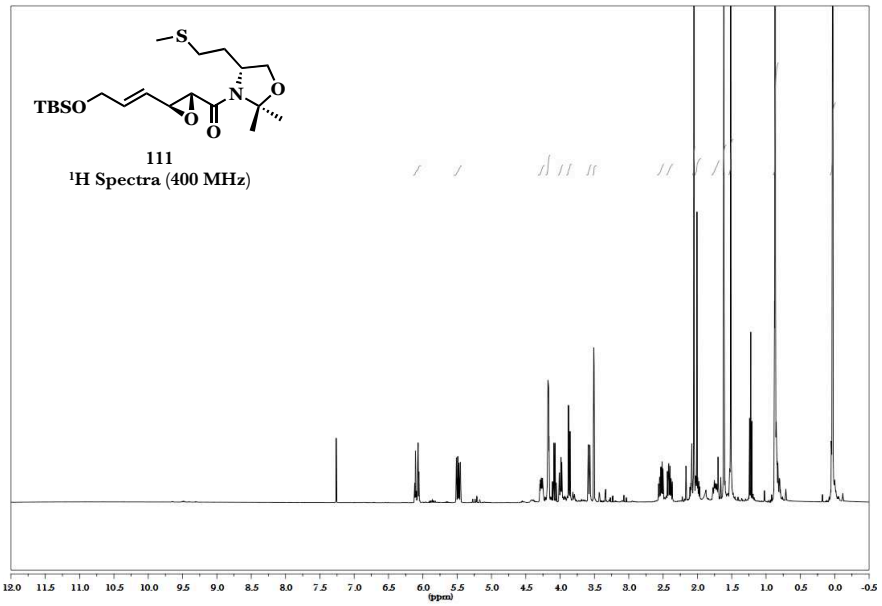




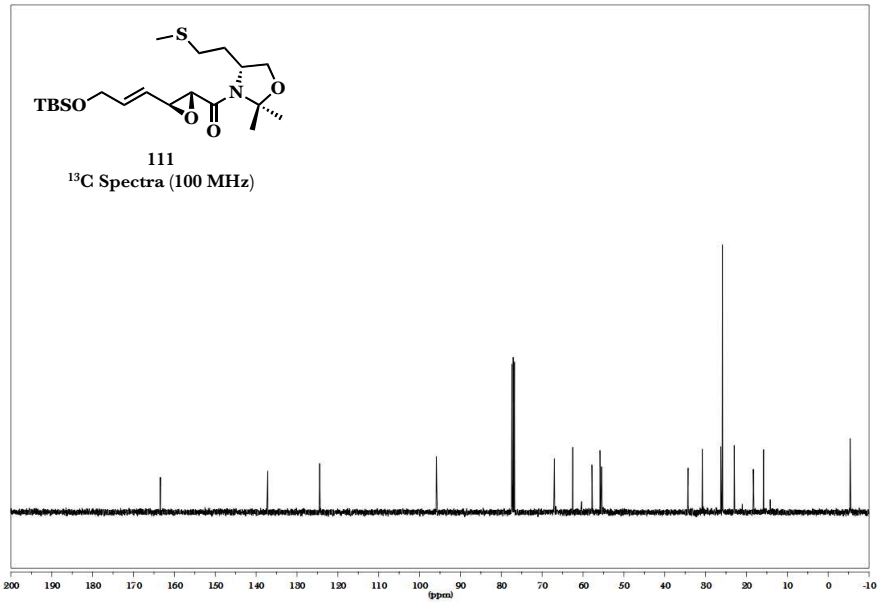




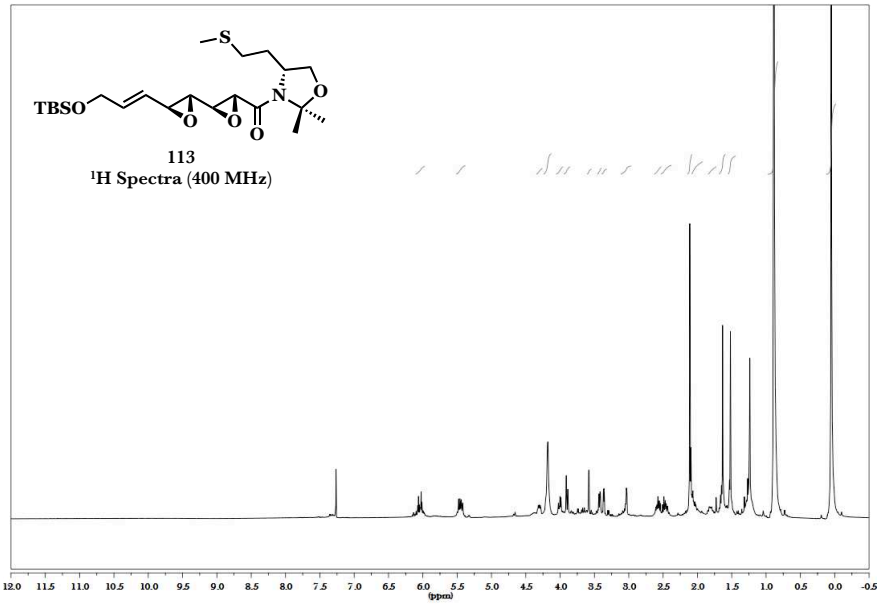




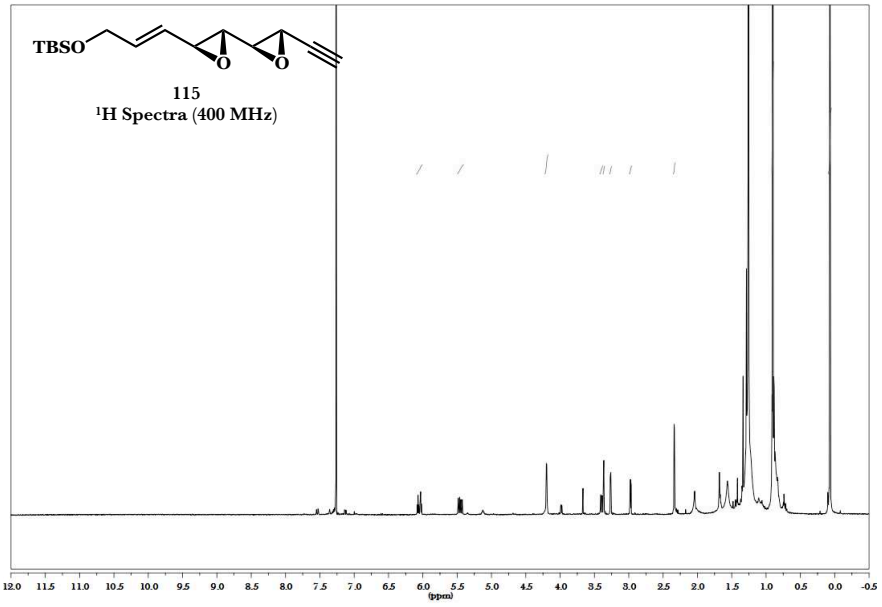
111
^{1}H Spectra (400 MHz)

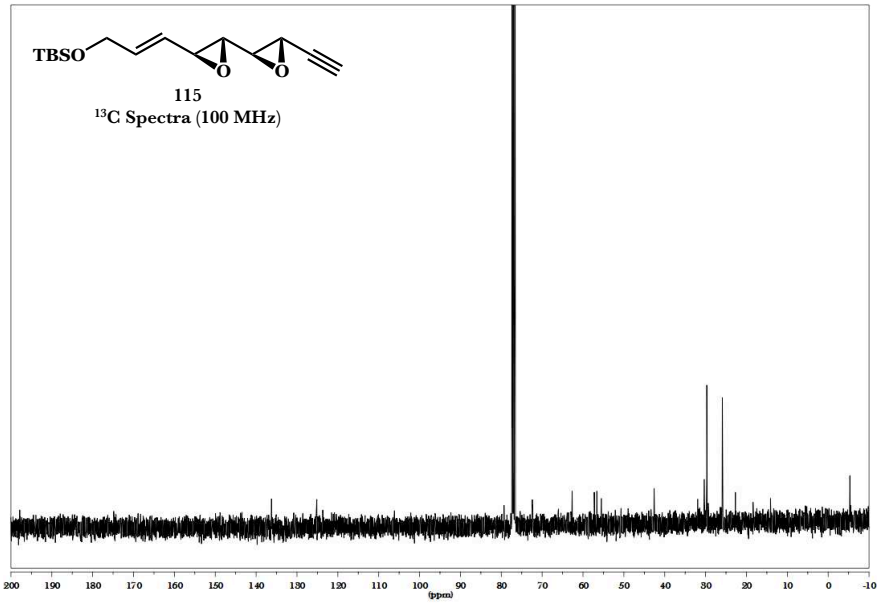


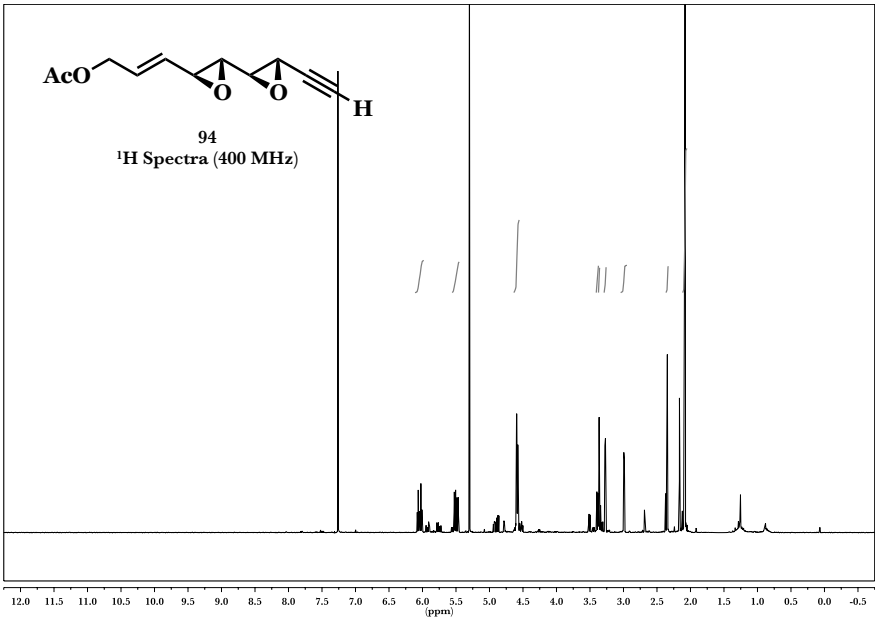
111
^{13}C Spectra (100 MHz)

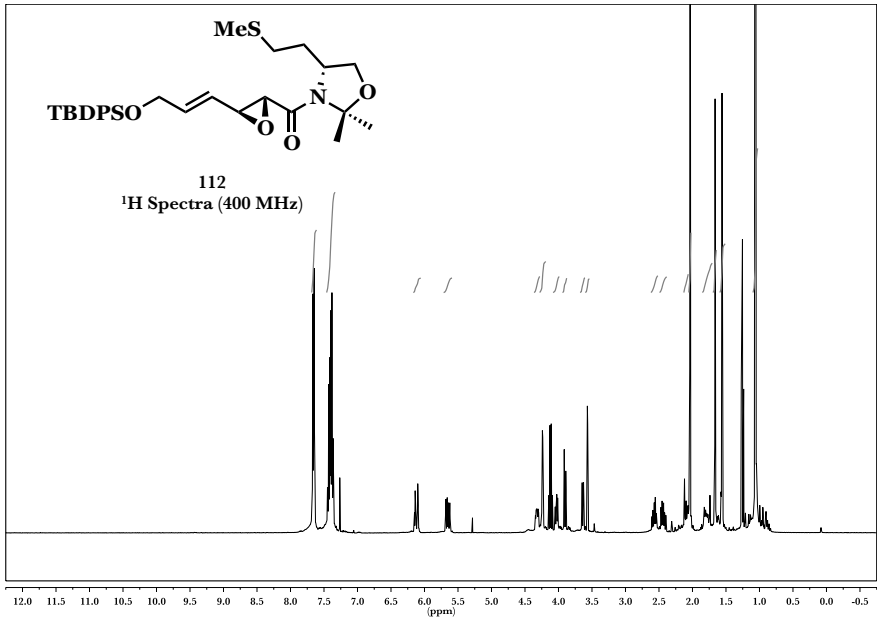


113
^{1}H Spectra (400 MHz)

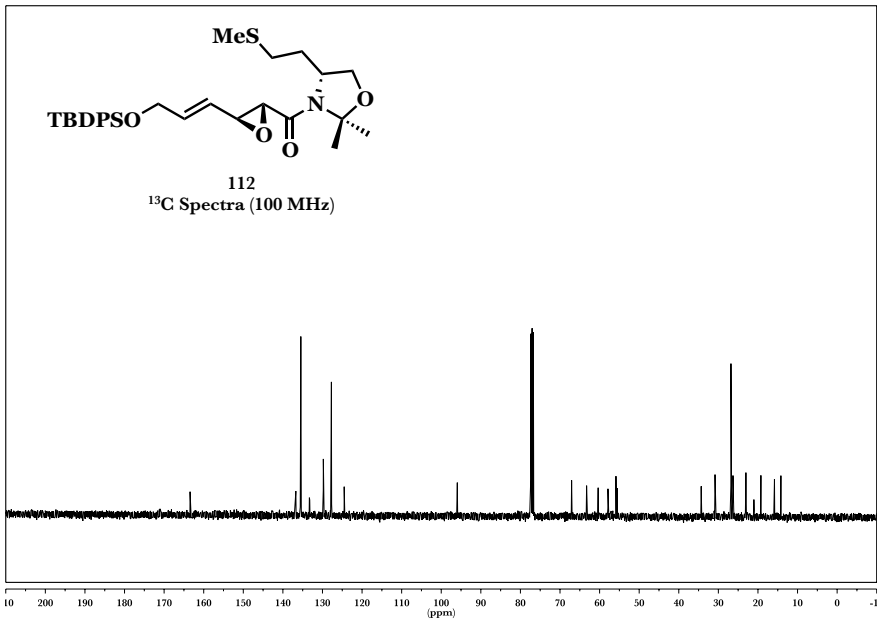




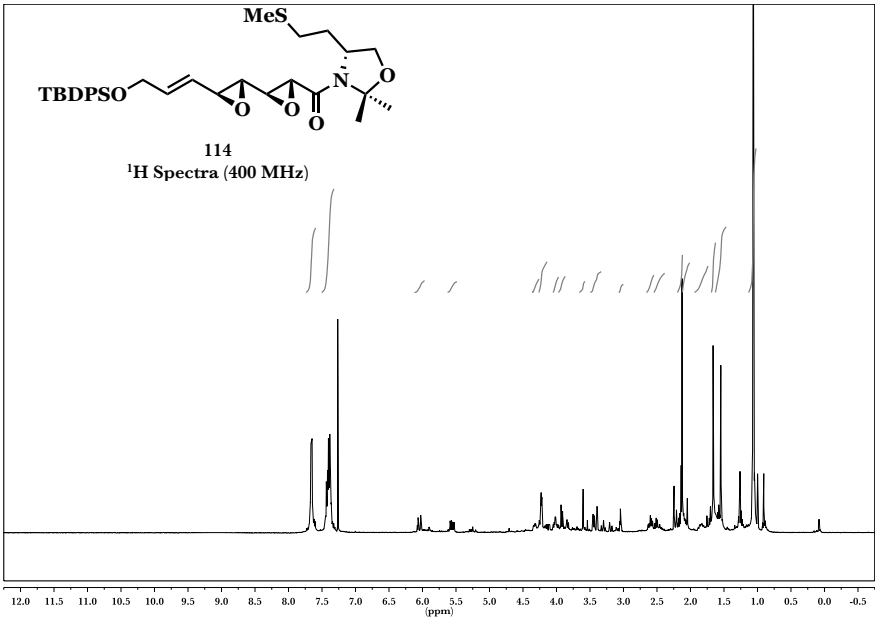




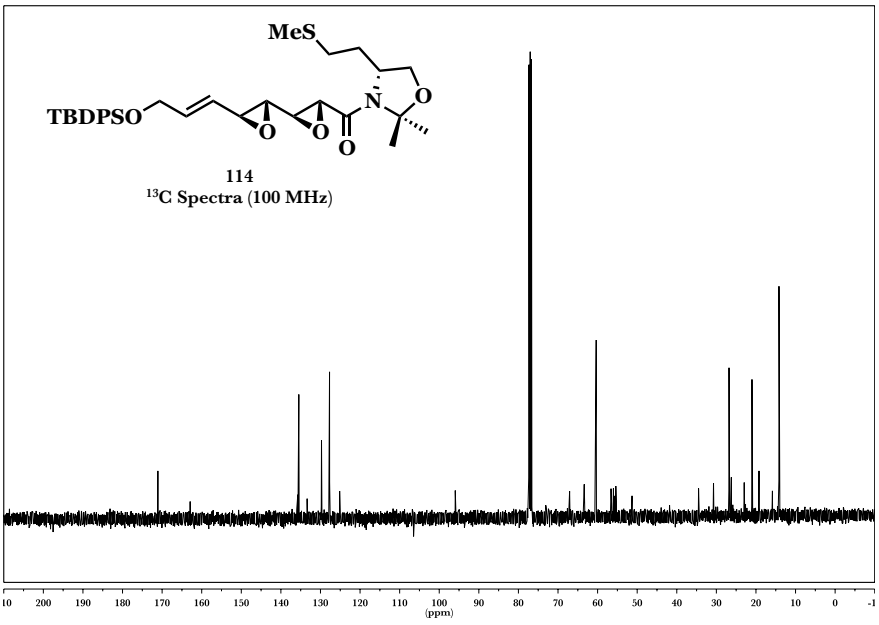
112
^{1}H Spectra (400 MHz)



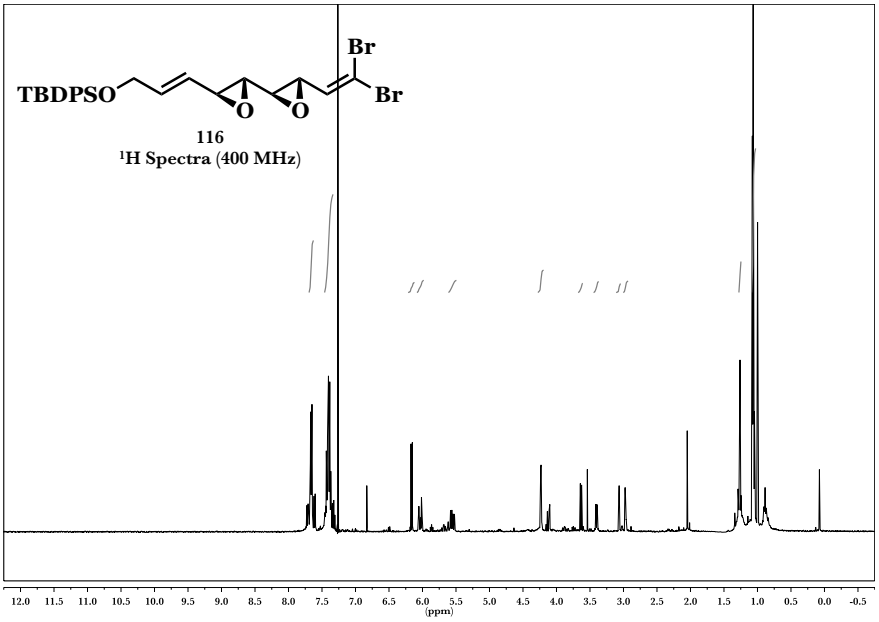
112
^{13}C Spectra (100 MHz)

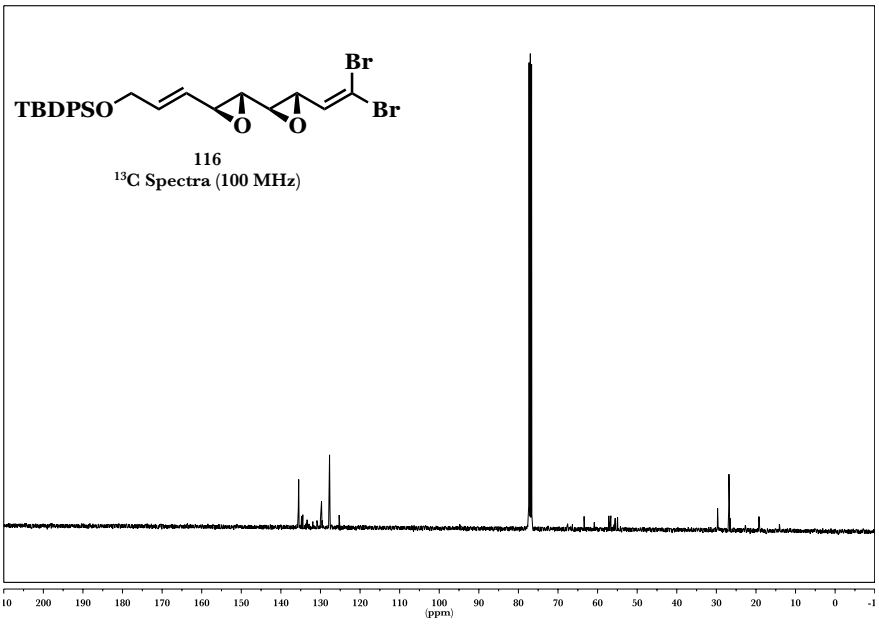


114
^{1}H Spectra (400 MHz)

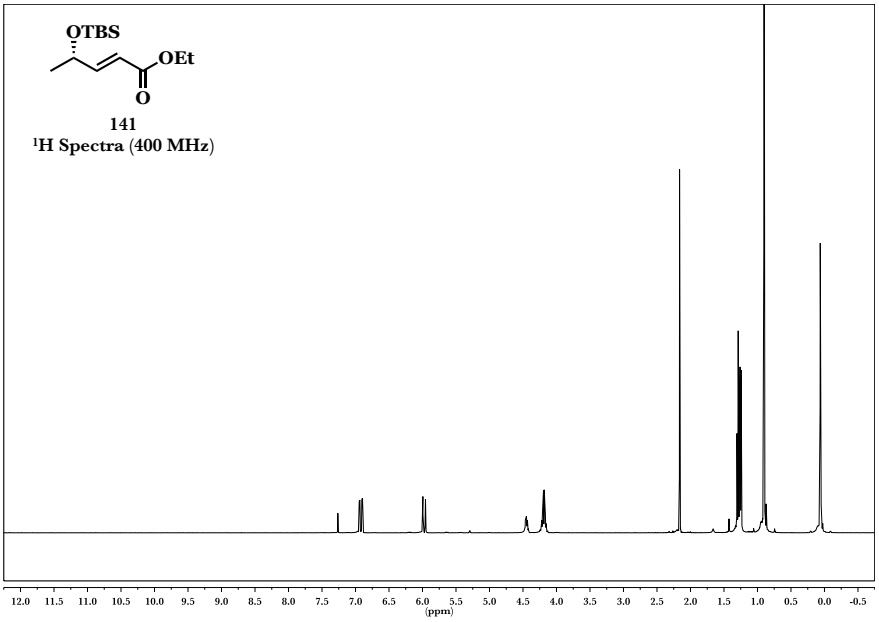


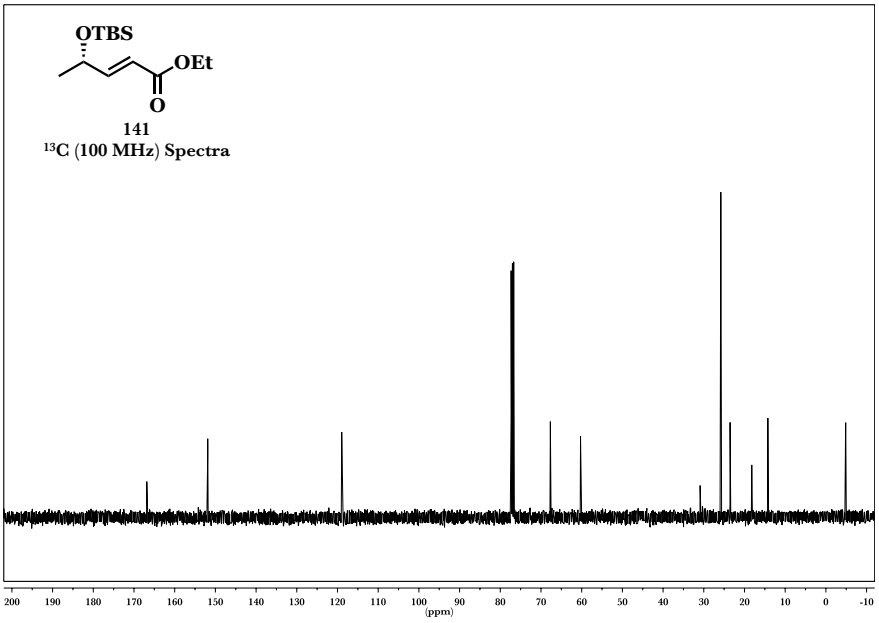
114
^{13}C Spectra (100 MHz)

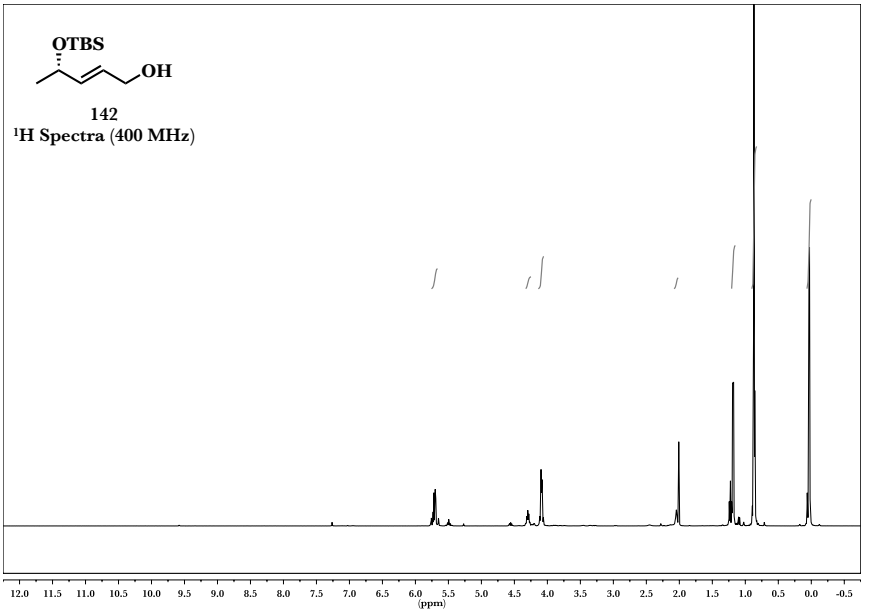


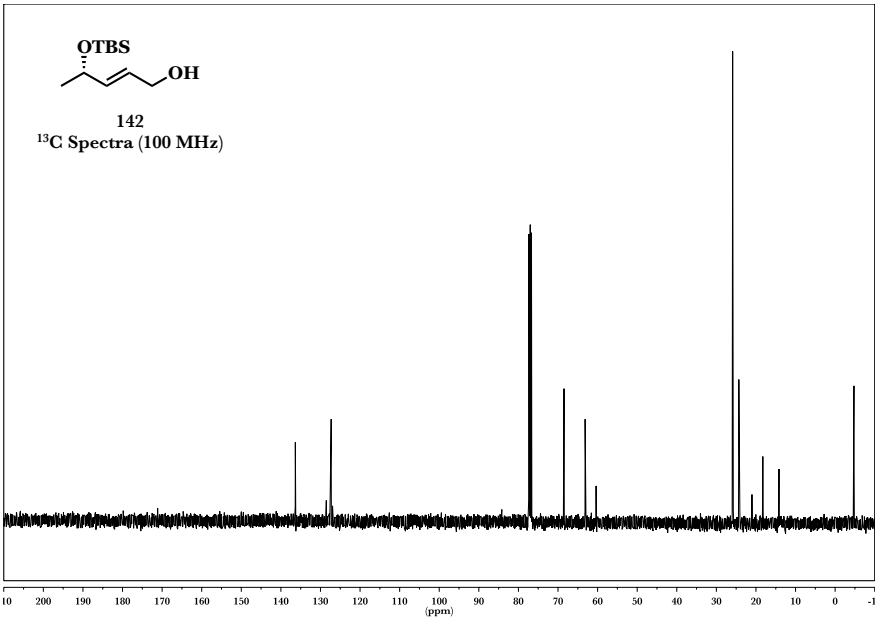


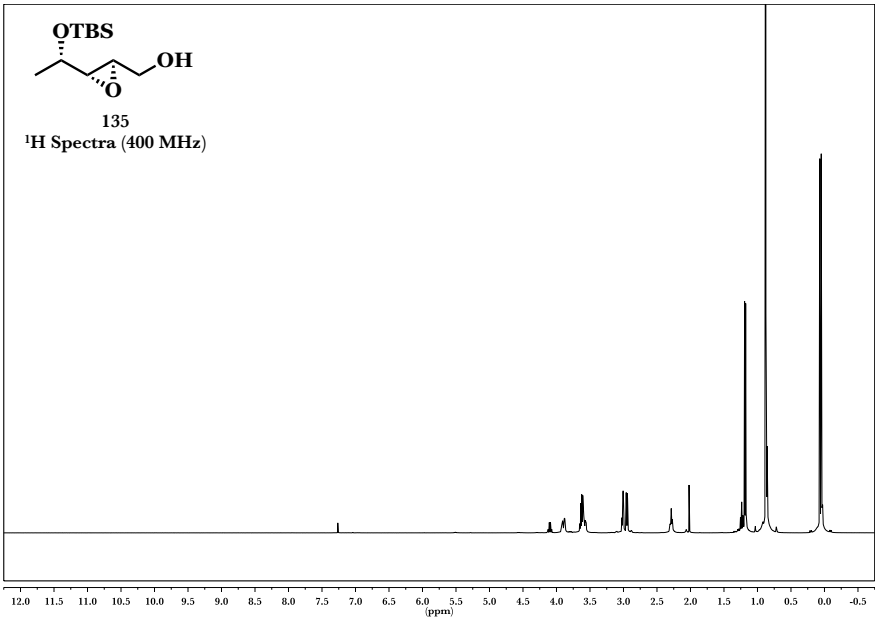
APPENDIX FOUR: Spectra Relevant to Chapter Four

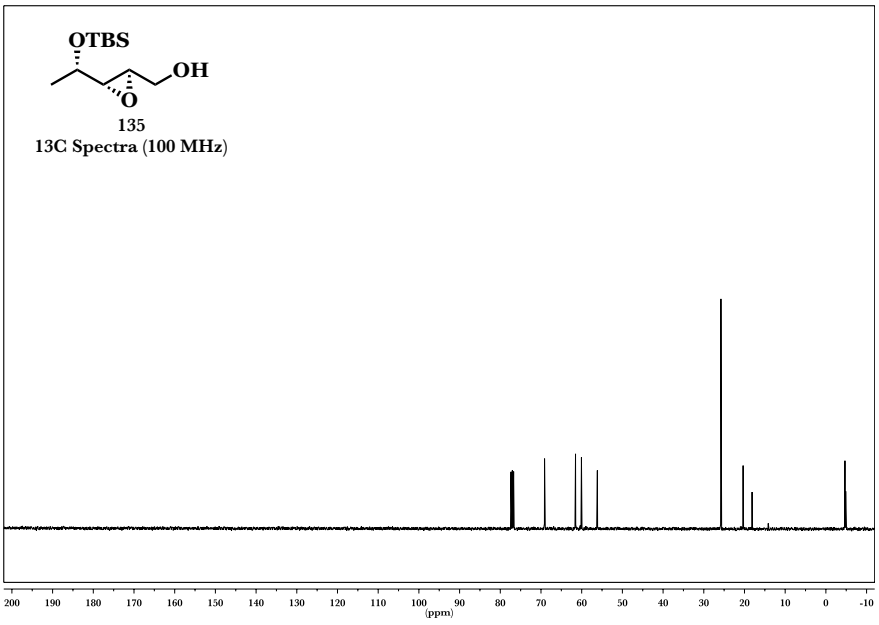


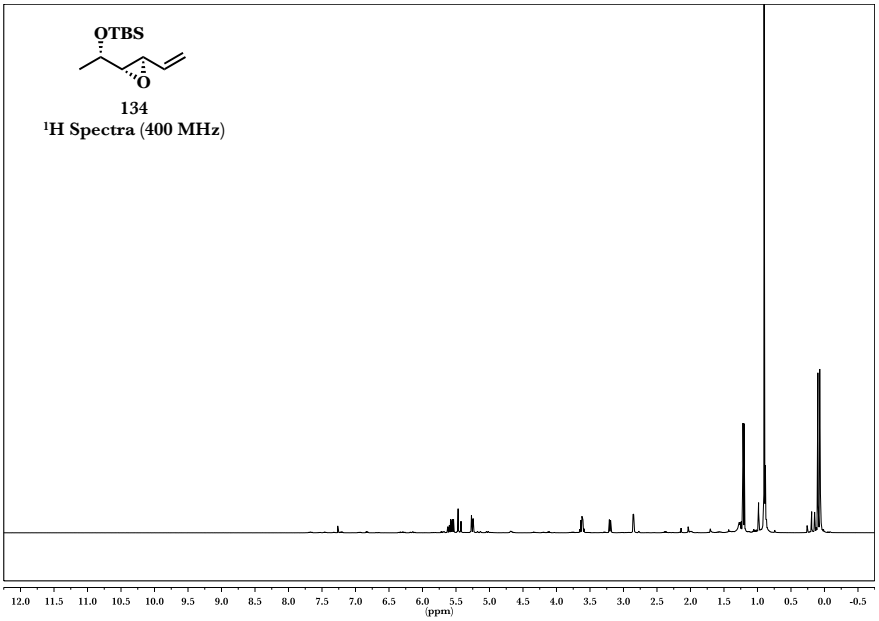






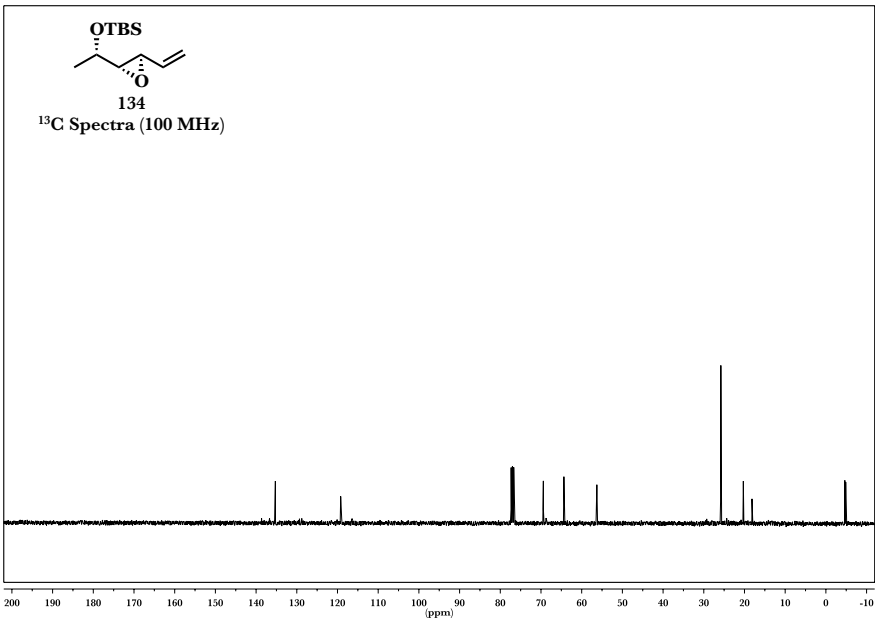






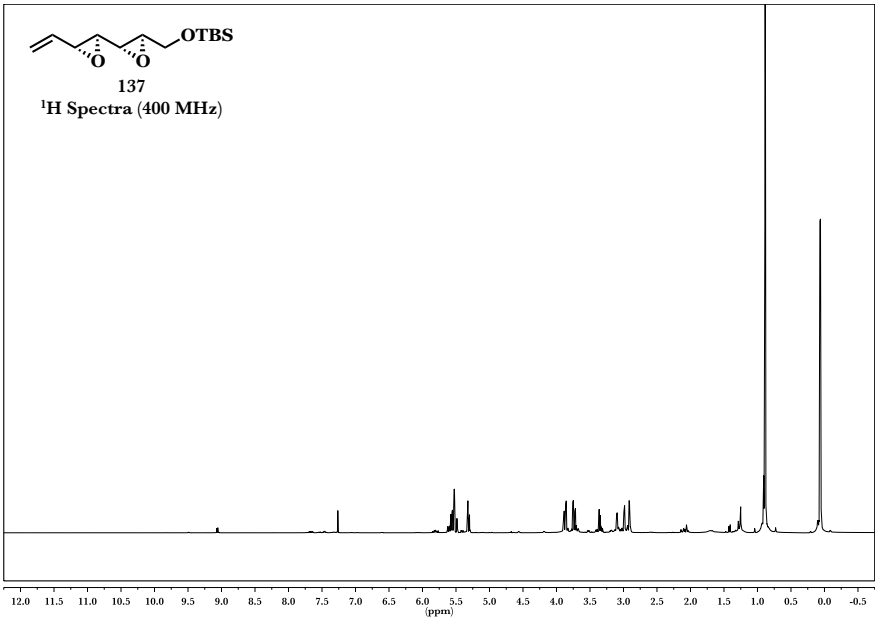
134

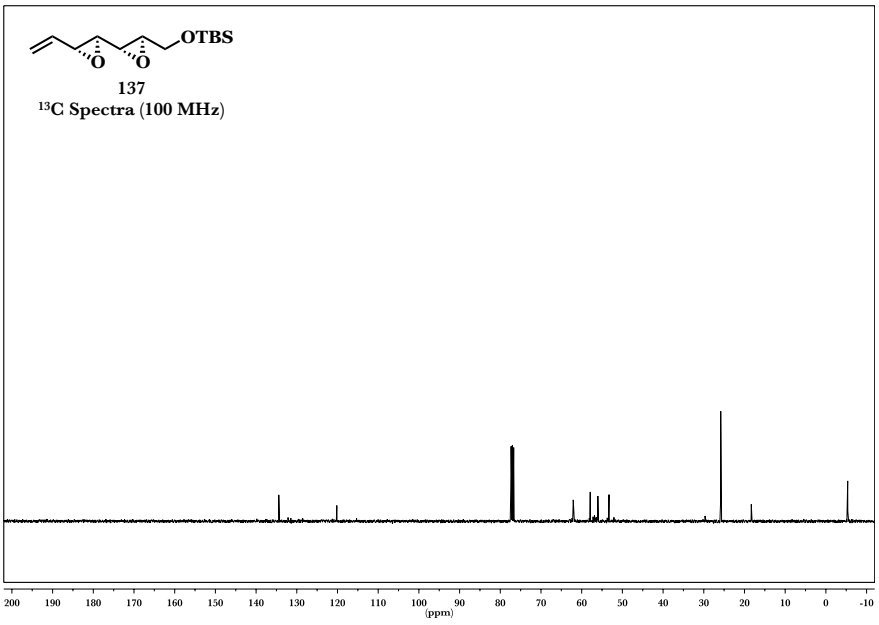
^{1}H Spectra (400 MHz)

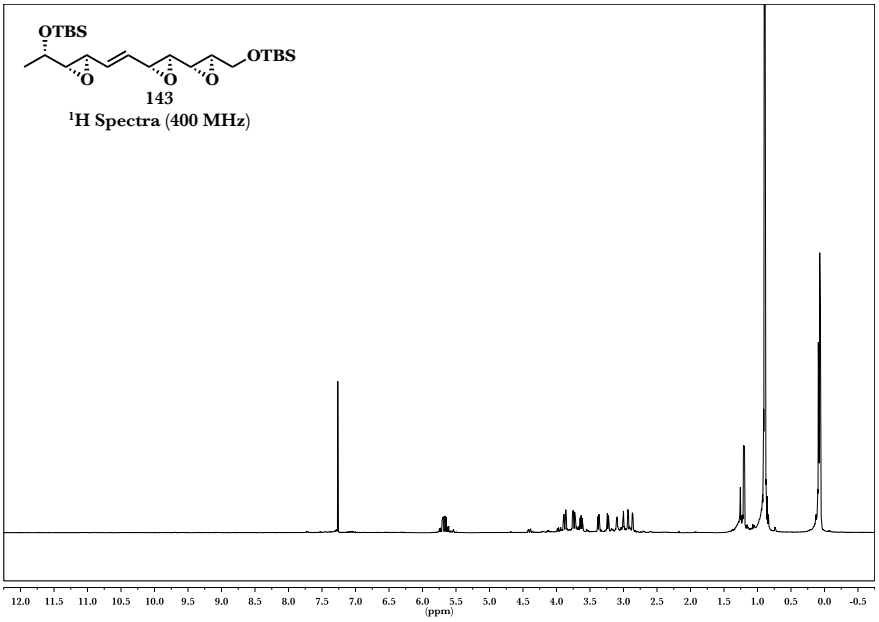


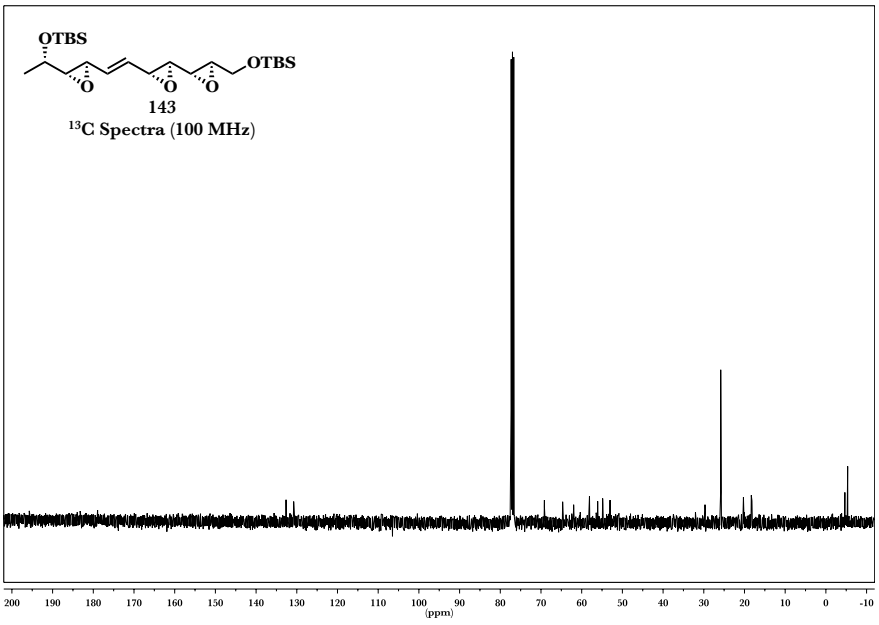
134

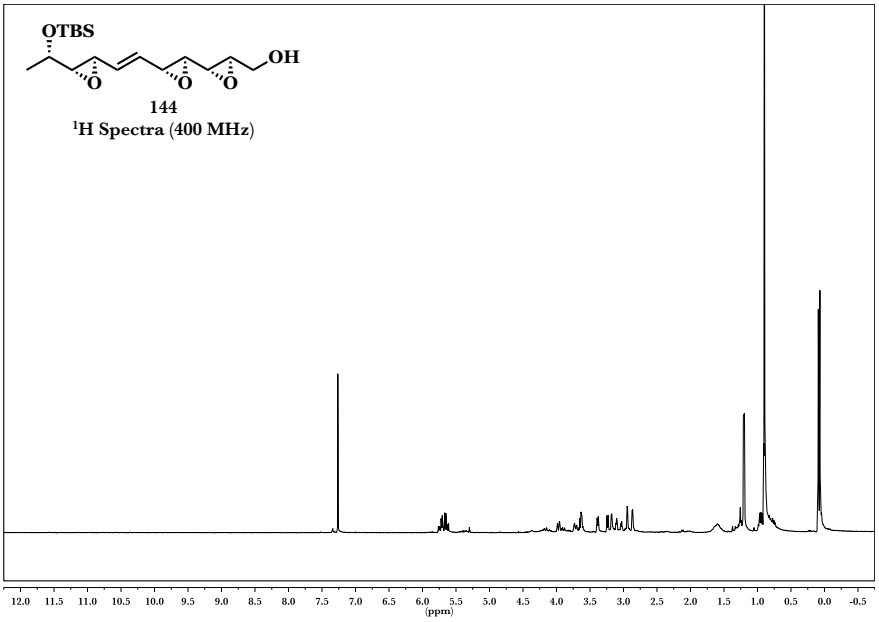
^{13}C Spectra (100 MHz)

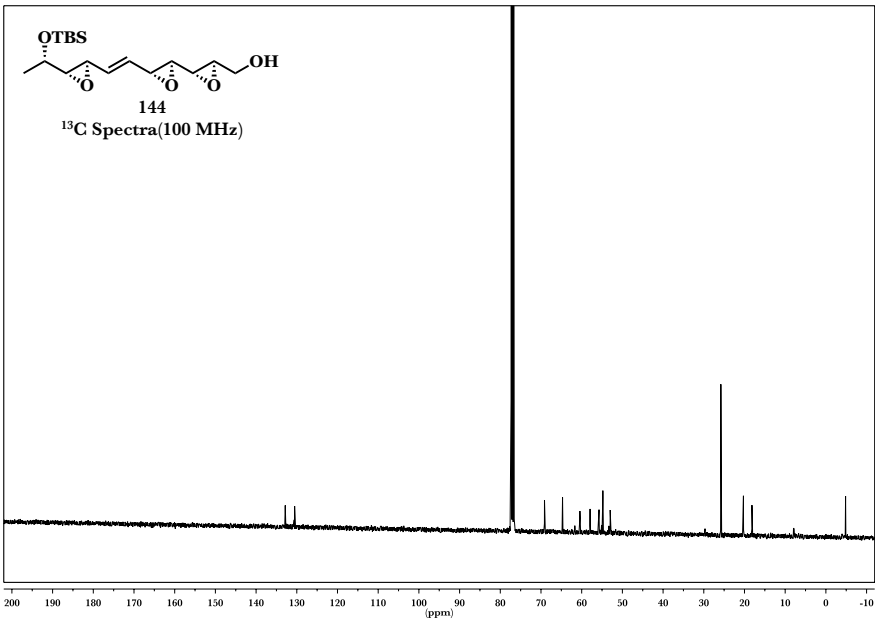


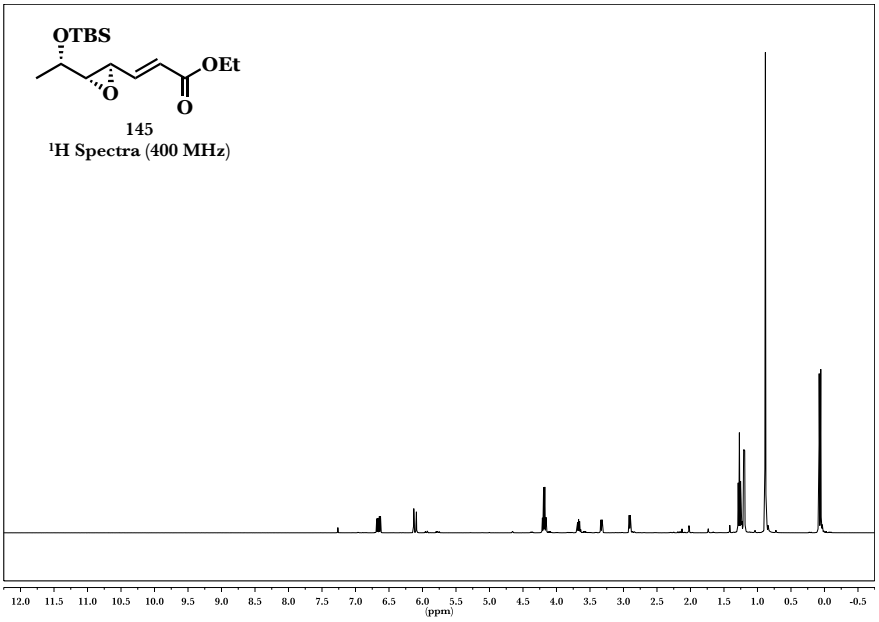


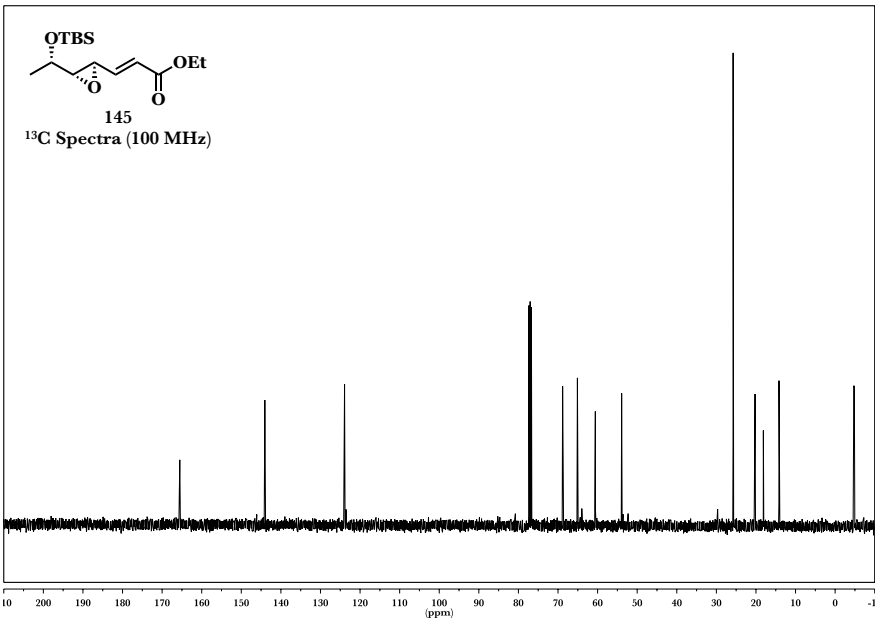


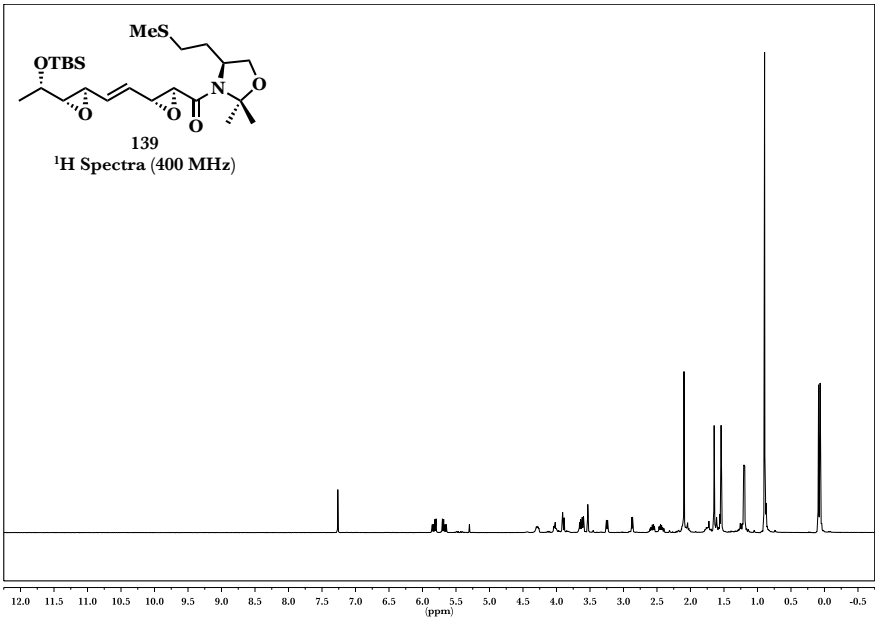






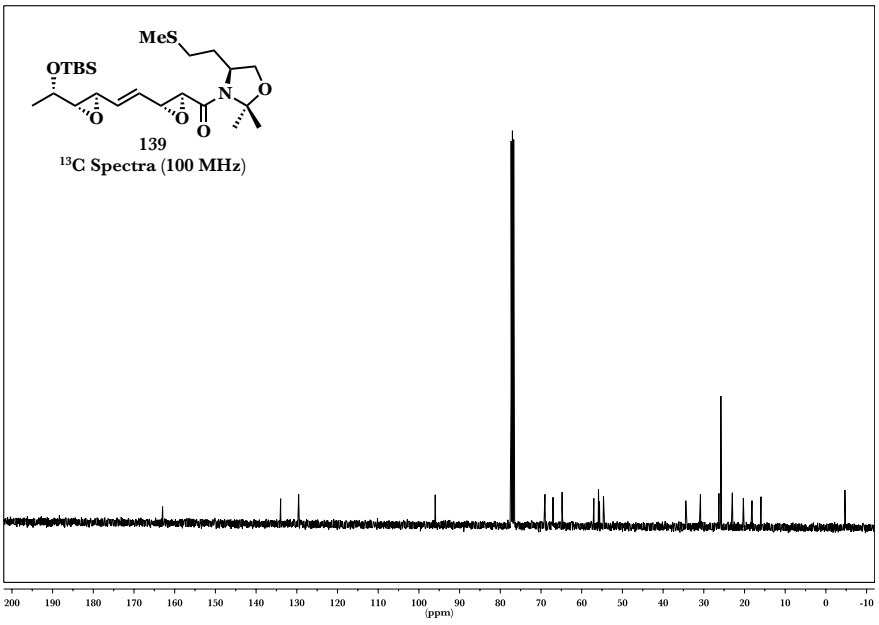






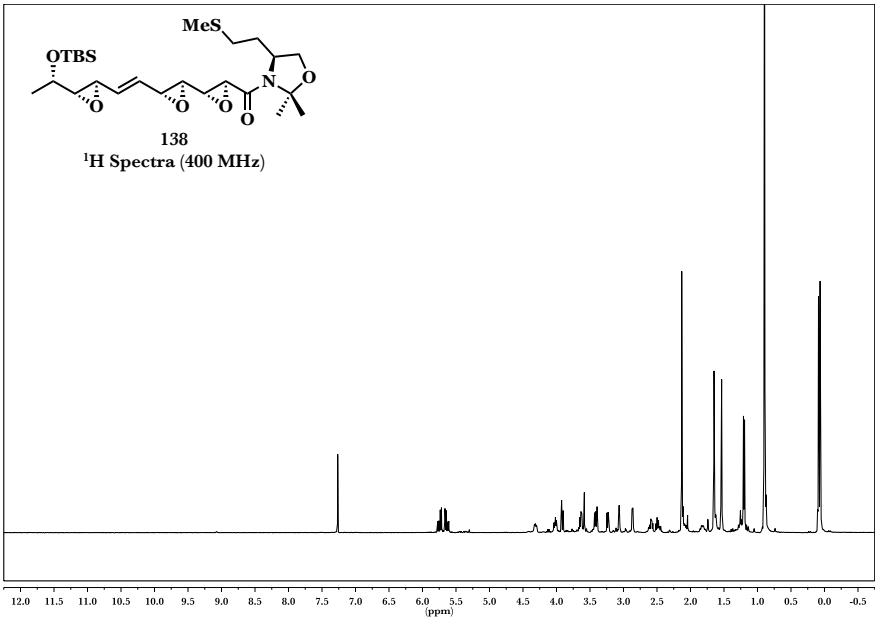
139

^{1}H Spectra (400 MHz)



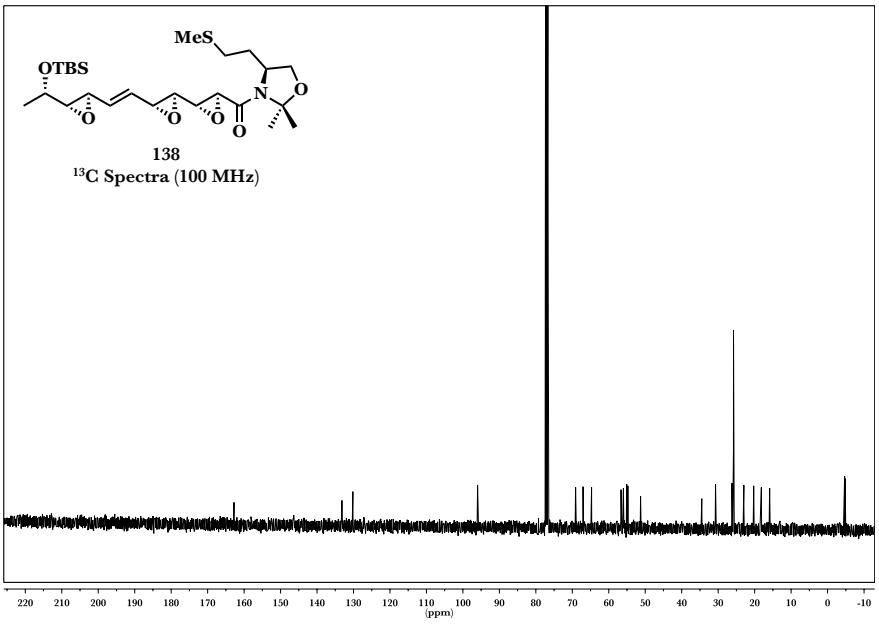
139

^{13}C Spectra (100 MHz)



138

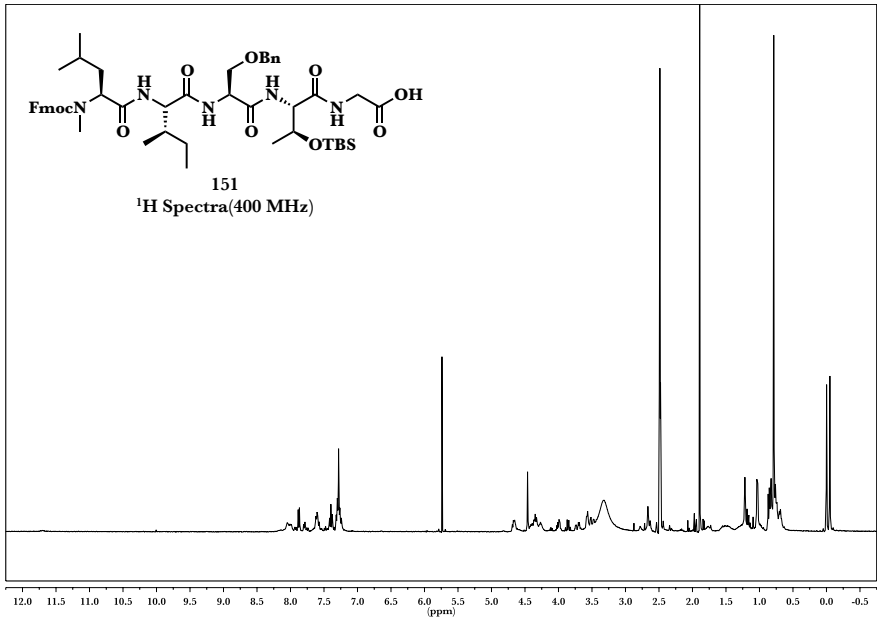
^{1}H Spectra (400 MHz)



138

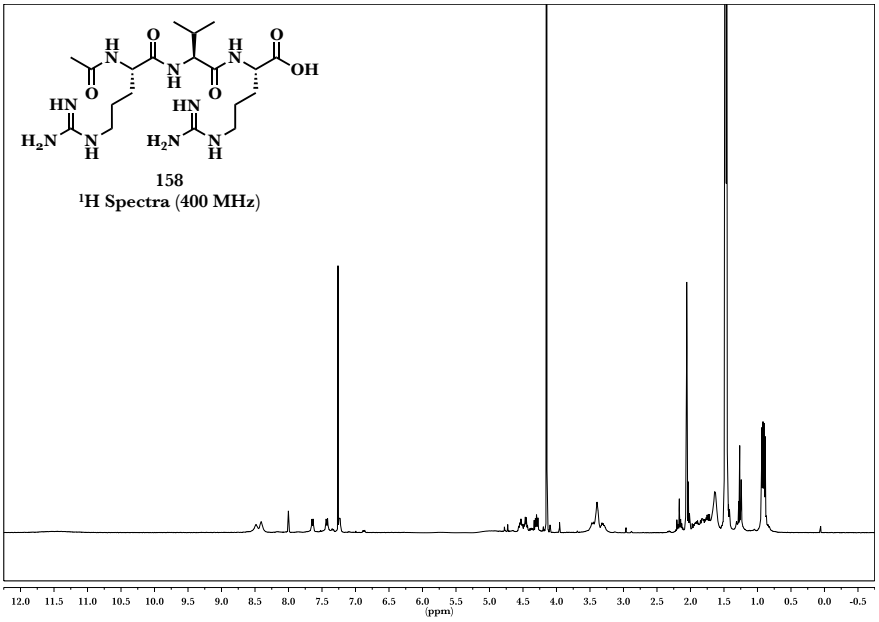
^{13}C Spectra (100 MHz)

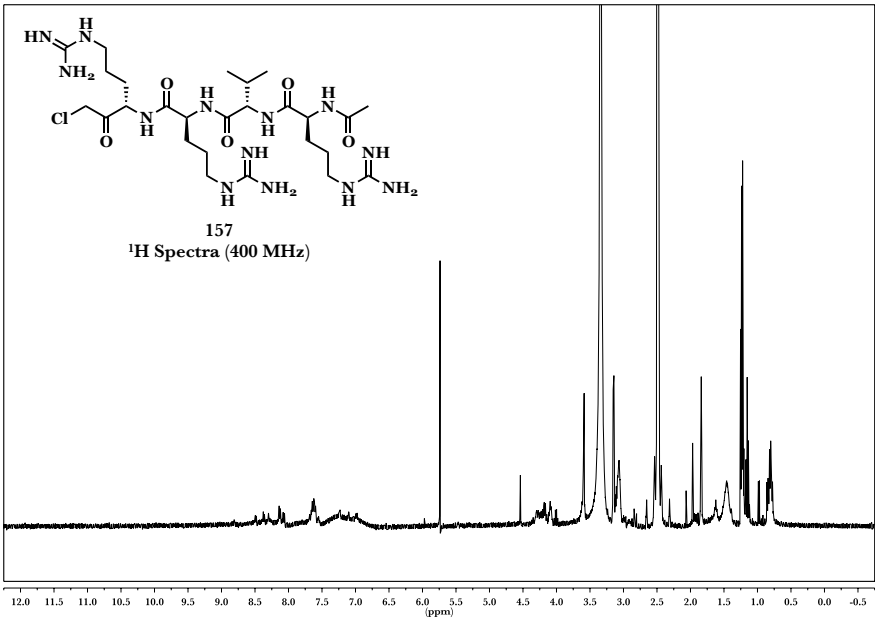
APPENDIX FIVE: Spectra Relevant to Chapter Five

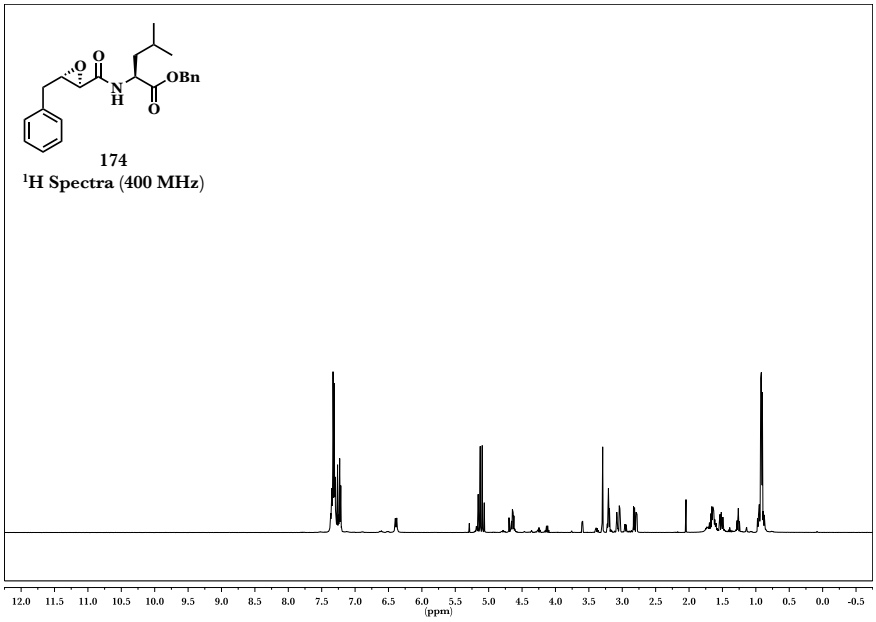


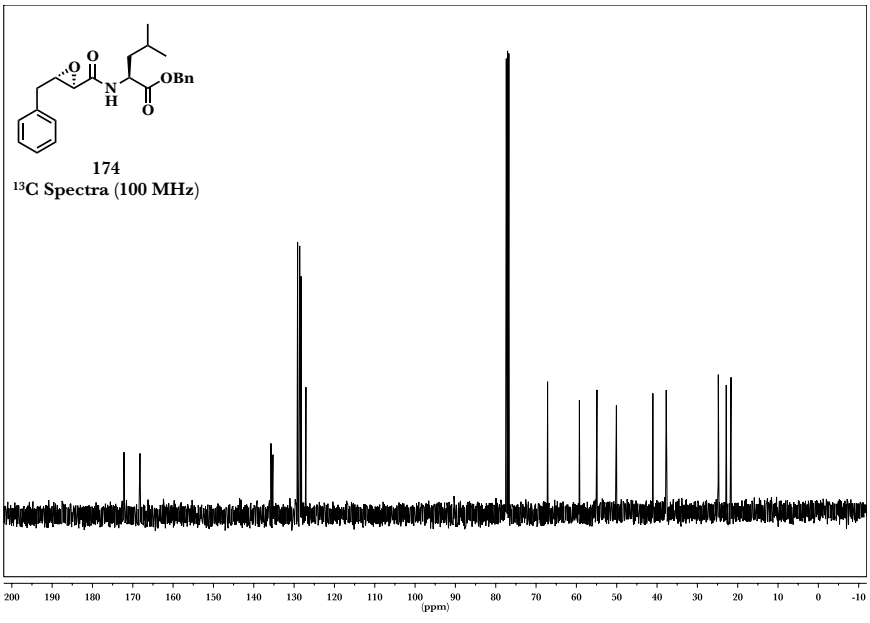
151

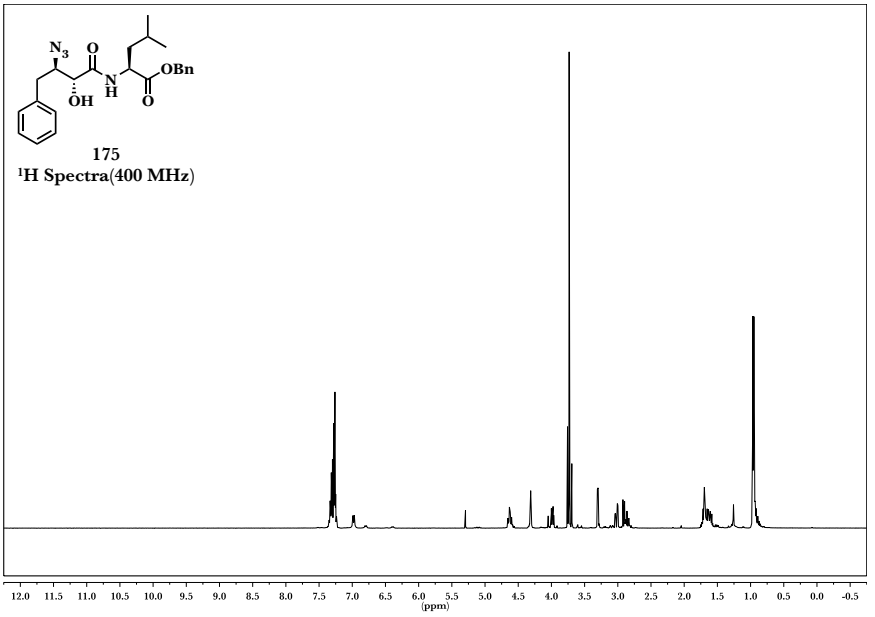
^{1}H Spectra(400 MHz)



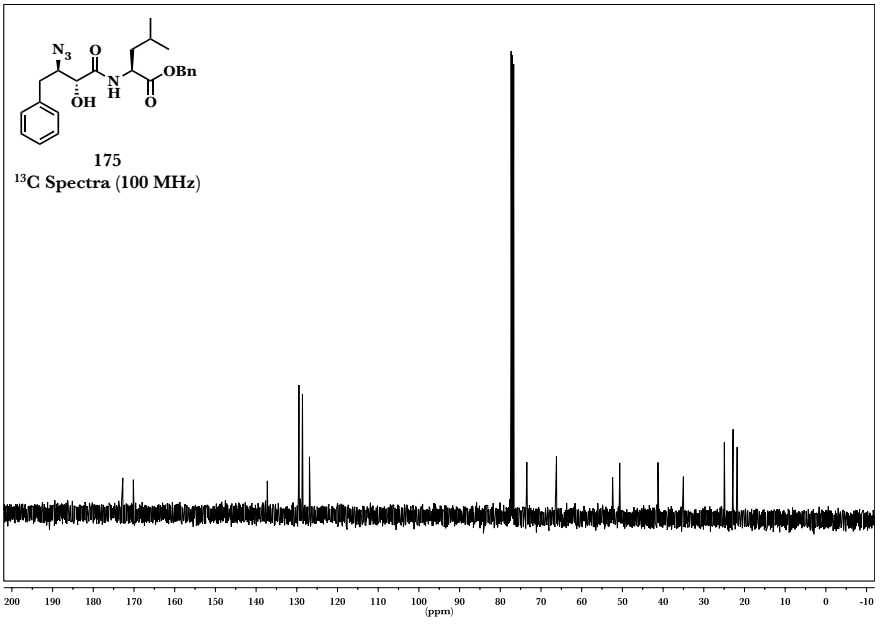




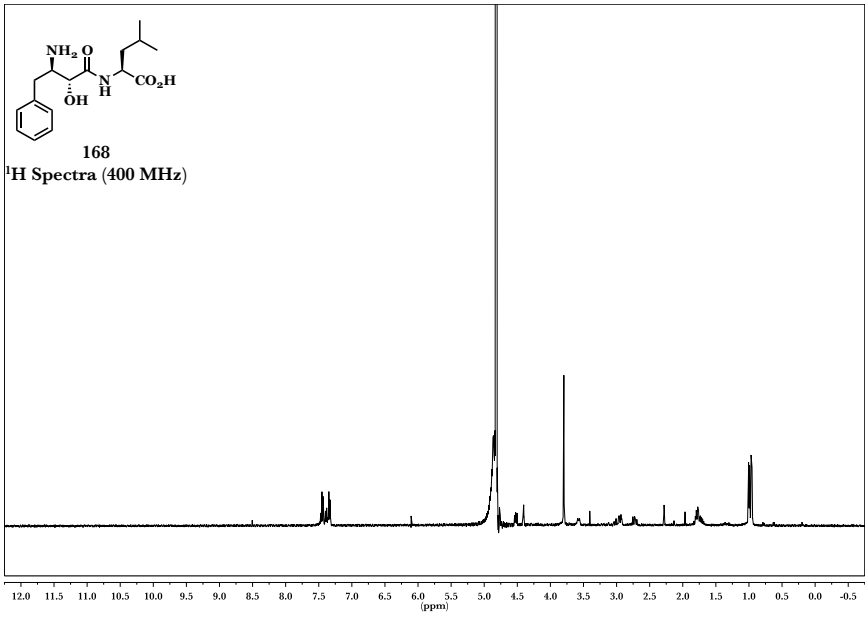


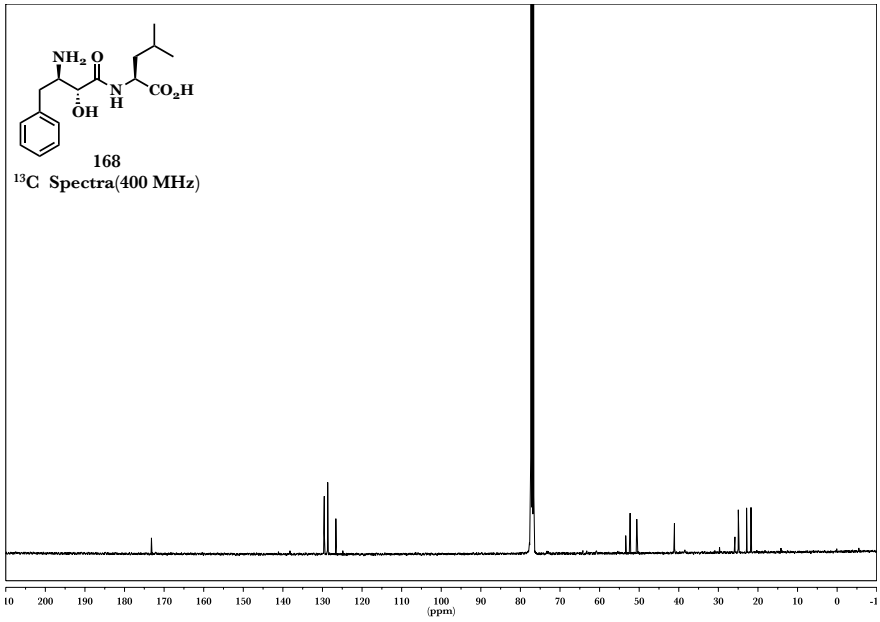


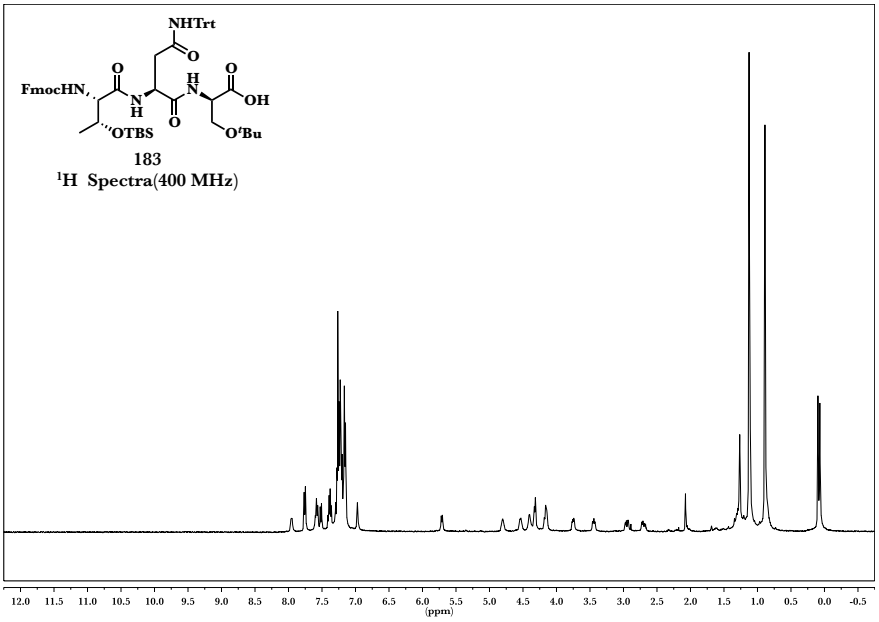
175
^{1}H Spectra(400 MHz)

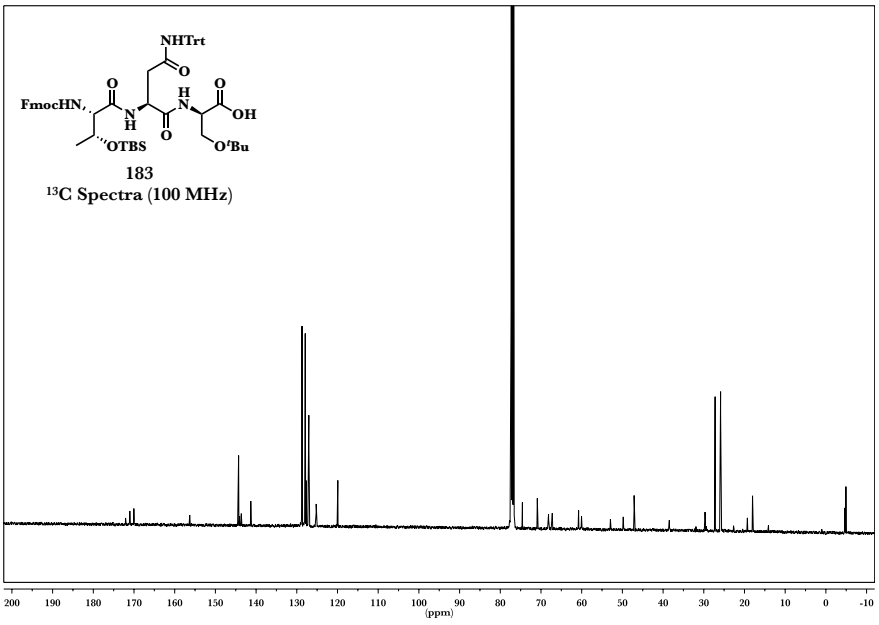


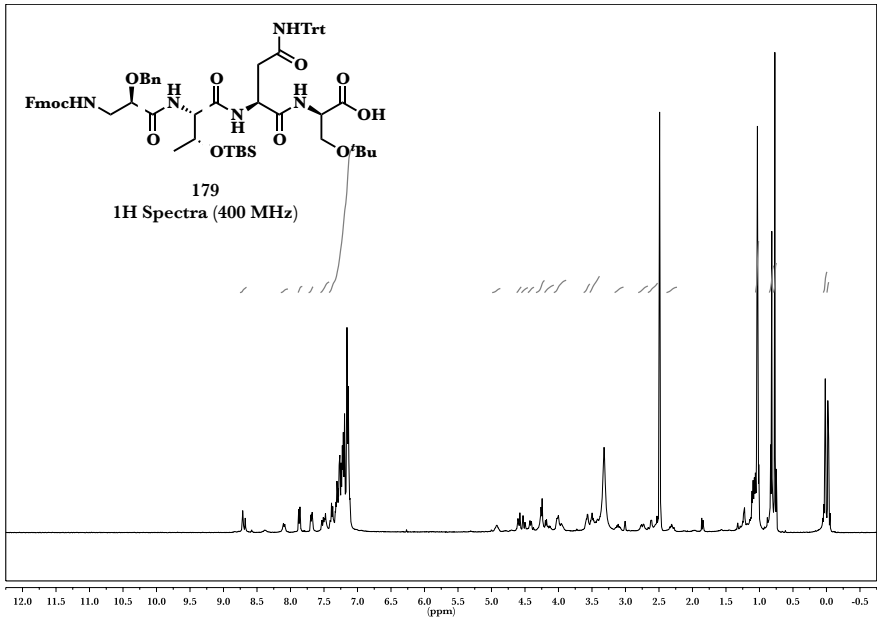
175
^{13}C Spectra (100 MHz)











179
1H Spectra (400 MHz)