BOLOGNA UNIVERSITY
DEPARTMENT OF CHEMISTRY "G. CIAMICIAN"

DESIGN AND SYNTHESIS OF ENZYMATIC INHIBITORS AND RECEPTOR LIGANDS

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Declaration

The work reported in this dissertation has been carried out in the Dipartimento di Chimica "G.Ciamician" of the Università di Bologna in between January 2005 and December 2007.

The candidate has been supervised throughout this period by Professor G.Cardillo, that must therefore be acknowledged as director and source of the whole research project. I would like to thank Professor Cardillo also for the professional improvement that she has motivated me to achieve. Dr. Tolomelli must be acknowledged for her active part in this project, for useful discussions and for her scientific (and not) support.

For the work presented in Chapter 1, Dr. Fabbroni and Dr. Perciaccante are deeply acknowledged, as well as Dr. Stenta that accomplished the computational section (working hard, especially on Sundays). I would like to thank Elisa Mosconi for her collaboration to the work described in Chapter 4, while Dr. Fabbroni, Dr. Perciaccante, Patrizia Galzerano, Riccardo Juris and the Professor Spampinato's group must be acknowledged for the work depicted in Chapter 5. Finally, I would like to thank Dr. Spring, the Spring group and particularly Dr.Gavilan of the Department of Chemistry, University of Cambridge for their support and help in the project illustrated in Chapter 6.

Some of the results presented here have been published on international journals, as clarified in the beginning of each chapter.

Fides Benfatti
Bologna, February 2008

Contents

List of Abbreviations

aq	aqueous
Ac	Acetyl
Boc	*t*-Butyloxycarbonyl
Bz	Benzoyl
Bn	Benzyl
c	concentration
Cbz	Carbobenzyloxy
CSA	Camphorsulfonic acid
Δ	reflux
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
de	diastereomeric excess
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
dr	diastereomeric ratio
E	Entgegen (opposite, trans)
eq.	equivalent
Et	Ethyl
g	gram
h	hours
iPr	isopropyl
LiHMDSA	Lithium Hexamethyldisilazide
M	molar mol/L
MCPBA	*meta*-Chloroperbenzoic Acid
Me	Methyl
min	minutes
mg	milligram
mL	millilitre
mp	melting point
Nu	nucleophile
Ph	Phenyl

PMB	*para*-Methoxybenzyl
ppm	parts per million
PPTS	Pyridinium *p*-toulensulfonate
Py	Pyridine
rt	room temperature
TBAF	tetra-*n*-butylammonium fluoride
TEA	Triethylamine
Tf	Triflate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl
Z	Zusammen (together, cis)

bs	broad singlet (NMR)
δ	Chemical shift (NMR)
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy (NMR)
d	doublet (NMR)
dd	doublet of a doublet (NMR)
DEPT	Distorsionless Enhancement by Polarisation Transfer (NMR)
EI	Electronic Impact (MS)
ESI	Electron Spray Ionisation
FID	Free Induction Decay (NMR)
FT	Fourier Tranform
Hz	Hertz
HETCOR	Heteronuclear Correlation (NMR)
HMQC	Heteronuclear Multiple Quantum Coherence (NMR)
HPLC	High Performance Liquid Chromatography
IR	Infrared
J	Coupling constant (NMR)
LC	Liquid Chromatography
m	multiplet (NMR)
MS	Mass Spectrum

NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect (Spectroscopy)
ORTEP	Oak Ridge Thermal Ellipse Program
q	quartet (NMR)
R_f	Retention Factor (chromatography)
s	singlet (NMR)
t	triplet (NMR)
TLC	Thin Layer Chromatography
t_r	retention time (HPLC)

AMBER	Assisted Model Building with Energy Refinement
COBRAMM	Suite of programs for computational chemistry (**CO**mputational **BR**idge from **A**b-initio and **M**olecular mechanics)
DFT	Density Functional Theory
Freq	Frequency calculation
GAFF	Generalised Amber Force Field
H	High level COBRAMM calculation type (pure QM system)
HF	Hartree-Fock
MM	Molecular Mechanics based method
MO	Molecular Orbital
MP2	Møller-Plesset second order perturbation theory
OPT	Optimisation (or minimisation)
PCM	Polarizable Continuum Model
PES	Potential Energy Surface
QM	Quantum Mechanics based method
SCC-DFTB	Self-Consistent-Charge Density-Functional Tight-Binding
SCF	Self Consistent Field
TS	Transition State

Introduction

Lessons from natural molecules[1]

Chemists have been learning from Nature for hundreds of years. A major achievement of mankind has been the creation of new compounds that did not exist in Nature, especially new medicines, new polymers, and simply new interesting compounds such as novel fragrances, for instance. Nowadays, well over 90% of all known chemical substances are unnatural products. They were often developed by imitating the general features of natural substances; a good example is synthetic polymers, which differ from but are intellectually related to the polymers of biology. The motivation for extending Nature is often simple curiosity about what else is possible, but there is always the hope that a useful new property might emerge.

In medicinal chemistry, natural products have at all times played a dominant role; in the past century, diverse classes of natural products have been isolated and their structures elucidated. These discoveries, along with the clarification of biological and biochemical mechanisms of therapeutic action, have been vital to the organic and medicinal chemistry research. Natural products have been priceless as tools for deciphering the biosynthesis' logic and as templates for developing novel drugs. Natural products are still major sources of innovative therapeutic agents, such as antibacterial antifungal and anticancer, however, obtaining a renewable supply of active compounds from biological sources can be problematic. Nevertheless, as the recent multigram, total synthesis of the potent anti-cancer natural product discodermolide[2-5] shows, the increasing efficiency of synthetic organic chemistry has reduced the barrier posed by limited natural supply, even for materials with very complex structures.

In **Figure 1a** the structures of four natural products that have been employed as drugs or leads are reported: vancomycin (1, an antibacterial), staurosporine (2, a protein kinases inhibitor), rapamycin (3, an immuno suppressor), and Taxol (4, a well-known anticancer). In contrast, **Figure 1b** shows the structures of four synthetic drug molecules that are broadly used: Viagra (5, treats erectile dysfunction), Prozac (6, antidepressant), Lipitor (7, hypocholesterolemic agent), and Gleevec (8, treats leukaemia). Two-dimensional and three-dimensional representations of these molecules are provided in **Figure 1** to highlight their topological characteristics. These comparisons stress several general distinctions between natural-products and synthetic drugs/drug candidates.

First, natural products typically have more stereogenic centres and more architectural complexity than synthetic molecules produced by medicinal chemists, although there are several exceptions (the very potent but structurally simple neurotransmitters, for instance).

Second, natural products contain relatively more carbon, hydrogen and oxygen, and less nitrogen and other elements than synthetic medicinal agents. Third, many useful natural products have molecular masses in excess of 500 daltons and high polarities (greater water solubility), and therefore violate Lipinski's "rule of five"[6]: this is a set of guidelines based on the characteristics of known drugs that provide an indication of whether a given small molecule is likely to have the desired pharmacokinetic properties to be an oral drug.

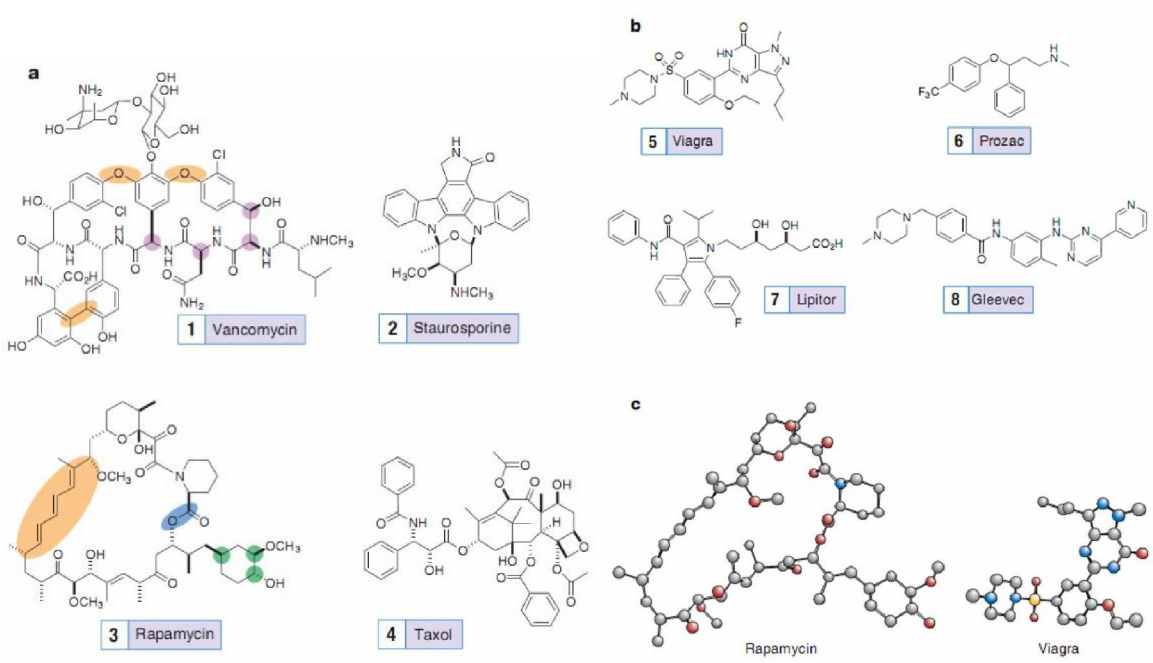


Figure 1[1]

Drug design

The term "rationale drug design[7,8]" has been used for more than 30 years to describe a marriage of experiment and technology intended to promote a more focussed approach toward drug discovery.

The term "rationale" means that this approach is based on the detailed knowledge of the pharmacological principle as well as the structure and function of the biological target (often using the *in silico* docking analysis of the protein X-ray crystal structures).

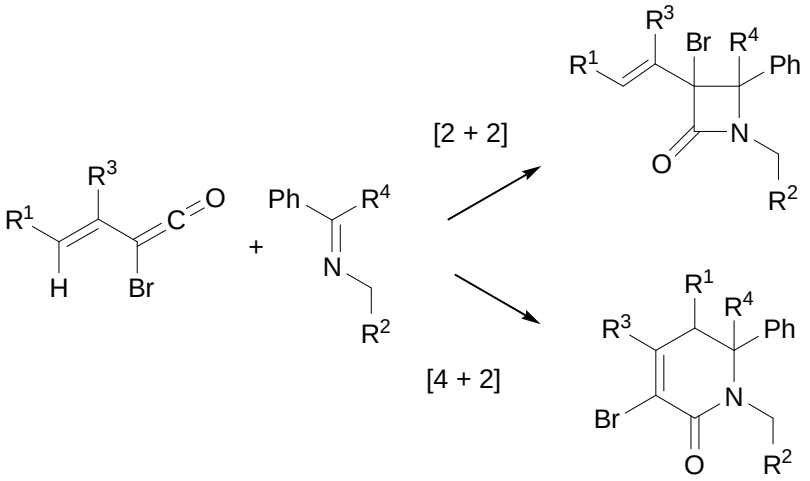
Many technological initiatives have been designed to provide an ever more detailed view of how potential drug molecules might interact with their macromolecular targets, and of how the effects they elicit might impact disease[9]. A huge number of small molecule libraries with the rationally determined properties and features have been be generated thanks to the combinatorial chemistry.

High-throughput screening of these libraries of compounds (including computer-based *in silico* screening), lead identification from hits and lead optimization have been central in drug discovery for the last two decades.

Nevertheless, the vast challenge represented by the discovery of new therapeutics with desirable physical properties and high levels of potency and selectivity is still open.
Therefore, new efficient, facile, reliable, economic and stereoselective synthetic methods are required to meet this challenge.

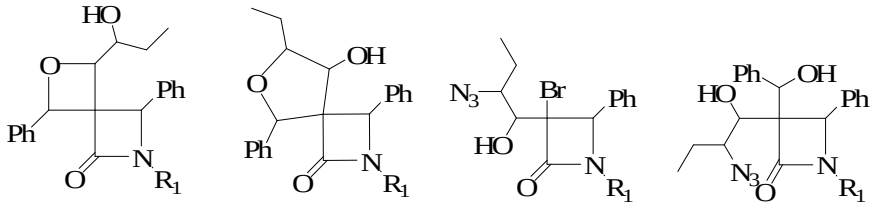
Thesis outline

In this dissertation , the design and synthesis of new cholesterol absorption inhibitors (CAI) as well as novel $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands and other potentially bioactive compounds is presented.
In Chapter 1, the scope of the [2+2] and [4+2] cycloaddition reaction between an imine and an α-bromo vinylketene leading to α-bromo azetidinones or dihydropyridinones, respectively, is shown, together with a detailed theoretical study on the periselectivity of these reactions.



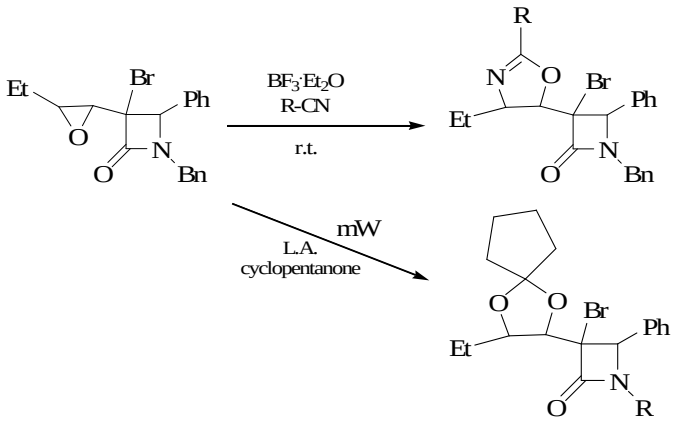
Scheme 1. Synthesis of 3-bromo-3-alkenyl-azetidin-2-ones and 3-bromo-4-alkyl-5,6-dihydropyridin-2-ones.

Chapter 2 illustrates the synthesis of new β-lactam-based cholesterol absorption inhibitors, with a special eye on synthetic issues. The results of the biological assays are reported therein, together with some structure-activity relationship (SAR) considerations.



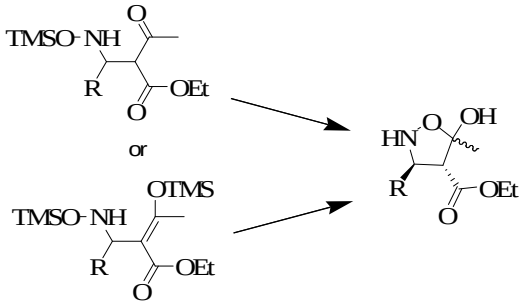
Scheme 2. Classes of β–lactamic molecules synthesised as CAI.

Another valuable α-bromo azetidinones derivatisation that allow to obtain dioxolanes and oxazolines is presented in Chapter 3.



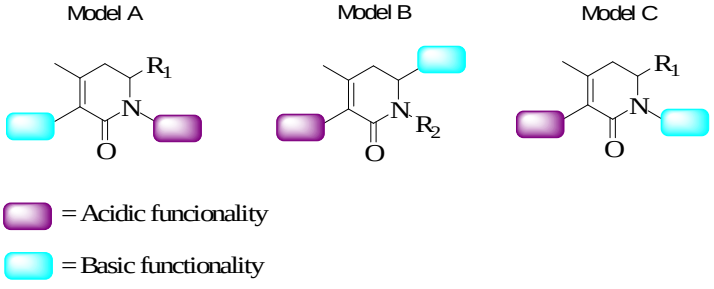
Scheme 3. Synthesis of β-lactam-based dioxolanes and oxazolines.

In Chapter 4, the synthesis of 5-hydroxy-4-carboxy isoxazolidines *via* Michael addition-intramolecular hemiketalisation is described. These molecules may represent constrained amino acids as well as pentose analogs. The mechanism of intramolecular hemiketalisation has been studied computationally, in order to give a rationale of the observed stereoselectivity.



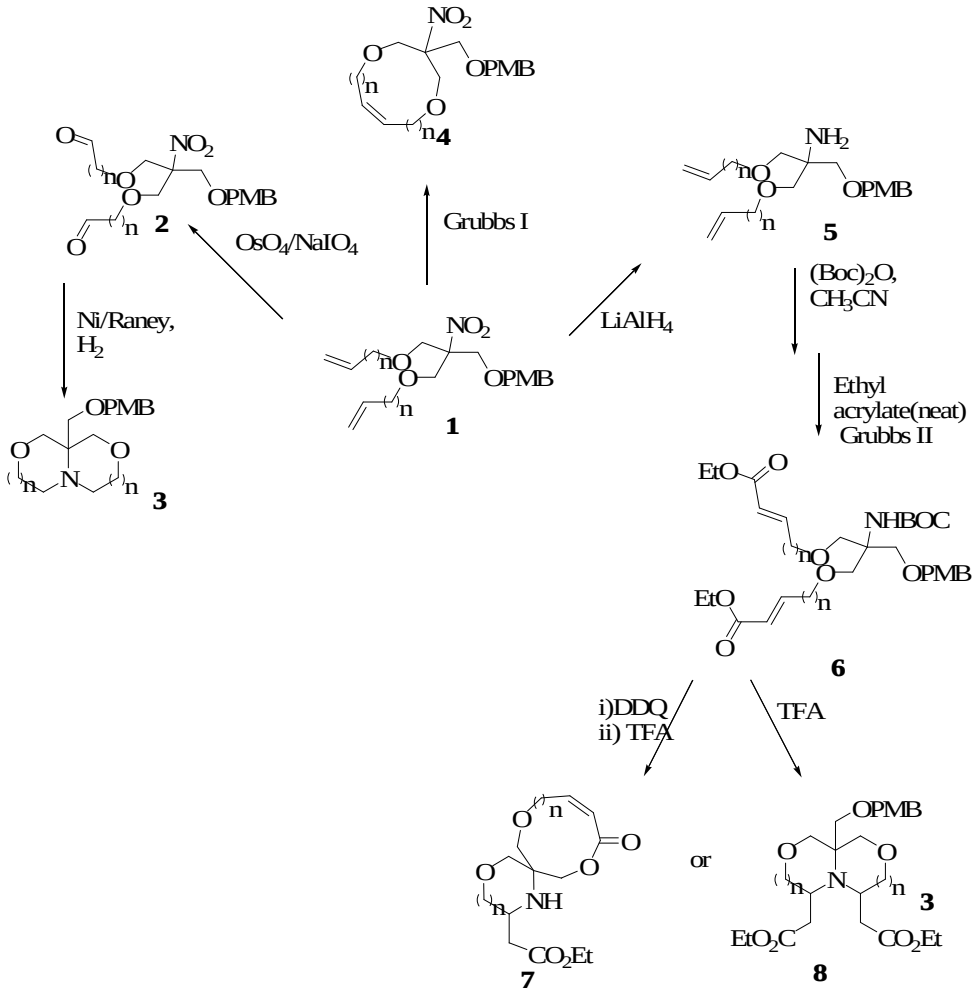
Scheme 4. The key step in the synthesis of 5-hydroxy-4-carboxy isoxazolidines.

The design and synthesis of a small library of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands sharing a 5,6-dihydropyridin-2-one scaffold are described in Chapter 5. All compounds in the library have been tested on both families of receptors and the biological assays results are reported therein, jointly with an account of the "rational drug design" strategy.



Scheme 5. Synthetic models of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands.

Chapter 6 reports the work carried out as guest PhD student in the Department of Chemistry of the University of Cambridge, under the supervision of Dr. Spring. The four-months research project described therein deals with the discovery of novel antibacterials using the Diversity-Oriented Synthesis (DOS) approach. The goal of DOS is to broadly populate the chemical space producing in a straightforward way a library of small and structurally diverse molecules, that can be tested afterwards to evaluate their bioactivity.



1 The cycloaddition reaction between α-bromo vinylketenes and imines

1.1 Introduction

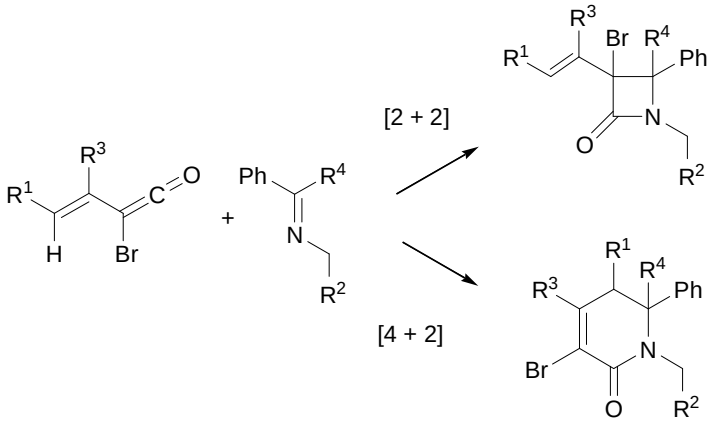
Ketenes exhibit a very peculiar cycloaddition chemistry because of their structural and electronic properties.[10] One of the most valuable and exploited pseudo-pericylic reaction of ketenes is certainly the reaction with imines to afford β-lactams, discovered by Staudinger at the beginning of the 20th century.[11] In view of the importance of β-lactams in medicinal chemistry as antibacterial agents[12-16] and as enzymatic inhibitors[17-20], Staudinger reaction has been extensively studied, both experimentally[21-33] and computationally.[34-44]

An interesting class of ketenes are the vinylketenes, that have proven to be versatile building blocks in cycloaddition reactions with various double bonds.[45-49] They are reported to behave as electron-deficient dienophiles in [2+2] cycloadditions with electron-rich partners and as diene component in [4+2] cycloadditions with electron-poor species.[50,51] Therefore, vinylketenes usually react with imines *via* Staudinger reaction. At the best of our knowledge, only silyl-vinylketenes have demonstrated to undergo [4+2] cycloaddition with imines.[52-55]

We carried out an experimental and a theoretical investigation on the reactivity of a new class of vinylketenes, the α-bromo-vinylketenes,[56] with imines. In this work successful synthetic routes toward α-bromo substituted 3-alkenyl-azetidin-2-ones and 4-alkyl-5,6-diyhdropyridin-2-ones have been described (**Scheme 1.1**). The halo substituent[30,57-63] dramatically affects the α-bromo-vinylketene's reactivity in the cycloaddition with an imine, promoting an unusual diene behaviour. Furthermore, a fine-tuning of the substituents on both vinylketene and imine moieties allows to selectively obtain [2+2] or [4+2] products. Moreover, these heterocycles are suitable for further elaborations, *via* the substitution of the halogen atom and the transformation of the double bond.[64-70] The derivatives of 3-bromo-3-alkenyl-azetidin-2-ones and 3-bromo-4-alkyl-5,6-diyhdropyridin-2-ones have shown inhibition of ACAT enzyme and antagonism of $\alpha_V\beta_3$ integrin respectively, thus demonstrating the versatility of these building blocks in the synthesis of biologically active compounds.[71-74]

A new synthetic approach was developed to obtain four and six membered lactams *via* cycloaddition reactions between α–bromo vinylketenes and imines and a theoretical investigation on the mechanism and on the stereoselectivity of these reactions, providing also a rationalisation for the unprecedented behaviour of this class of vinylketenes.

In the following sections we shall describe in details the experimental results since they are essential to understand the subsequent theoretical work.



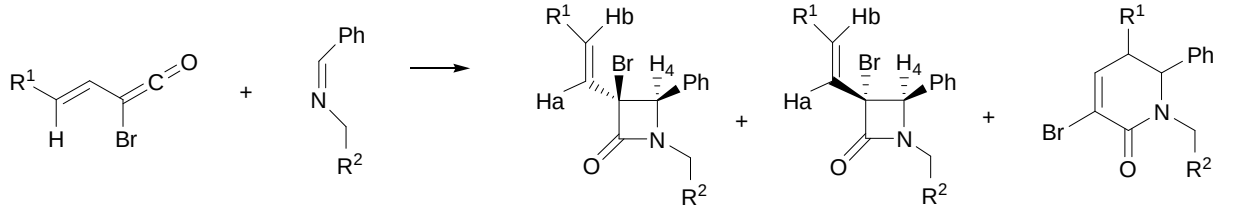
Scheme 1.1 Synthesis of 3-bromo-3-alkenyl-azetidin-2-ones and 3-bromo-4-alkyl-5,6-dihydropyridin-2-ones.

1.2 Experimental results

1.2.1 Reaction of linear α-bromo vinylketenes with imines.

Recently, we have investigated[75] the straightforward synthesis of 3-bromo-3-alkenyl-azetidin-2-ones *via* Staudinger reaction between α-bromo vinylketenes and an imine. The α-bromo vinylketenes **1a** and **1b** were prepared *in situ* starting from α-bromo hexenoyl chloride and α-bromo crotonyl chloride respectively in the presence of triethylamine.

The cycloaddition reaction of α-bromo vinylketenes **1a-b** with imines **2a-e** afforded, as major products, the *cis* β-lactams **3a-e** and **6a** but, unexpectedly, the formation of the six membered 5,6-dihydropyridin-2-ones **5a-e** and **8a** could not be avoided**,** even under a variety of experimental conditions (**Scheme 1.1**).



1a : R^1 = Et
1b: R^1 = H

2a: R^2 = Ph
2b: R^2 = *p*-MeO-Ph
2c: R^2 = CH=CH_2
2d: R^2 = CH_2COOEt

cis-**3a-d:** R^1 = Et
cis-**6a:** R^1 = H

trans-**4a-d:** R^1 = Et
trans-**7a:** R^1 = H

5a-d: R^1 = Et
8a: R^1 = H

Scheme 1.2 Reaction of **1a** and **1b** with imines **2a-d.**

The reaction of vinylketenes with imines, that affords 3-alkenyl-azetidin-2-ones, was reported in the past by Bose and Manhas,[76-79] but no traces of the six membered lactams was observed by the authors. This result prompted us to investigate the effect of the halogen on the reactivity of the intermediate ketene.

The cycloaddition reaction was performed on **1a** and **1b** with the imines derived from benzaldehyde and benzylamine (**2a**), *p*-methoxy-benzylamine (**2b**), allylamine (**2c**), β-alanine ethyl ester (**2d**) and (*S*)-1-phenylethylamine (**2e**).

The α-bromo vinylketene **1a** and the imine **2a** were reacted under several different conditions in order to increase yield and selectivity in the formation of 3-Br-3-alkenyl-β-lactam. The detailed investigation of the reaction conditions showed that the best results in diastereoselectivity could be obtained in CH_2Cl_2 at reflux by adding the proper acyl chloride to a hot solution of imine **2a** and TEA. Following this procedure, the β-lactams **3a-d** and **4a-d** and **6a-7a** were obtained in good yield and high selectivity in favour of the *cis* isomer, accompanied by a significant amount of **5a-d** and **8a** (Table 1.1). Product distributions were determined by ^{1}H NMR integration of distinctive key signals and by quantitation of the individual isomers obtained after chromatographic separation on silica gel, eluting with benzene.

Table 1.1 Reaction of α–bromo vinylketenes **1a-b** with imines **2b-d.**

Entry	Ketene	Imine	3 + 4 (%)[a]	3/4 (%)	5 (%)[a]
1	**1a**	**2a**	57	95 : 5	22
1	**1a**	**2b**	60	80 : 20	27
2	**1a**	**2c**	50	80 : 20	23
3	**1a**	**2d**	55	93 : 7	14
4	**1b**	**2a**	30	90 : 10	15

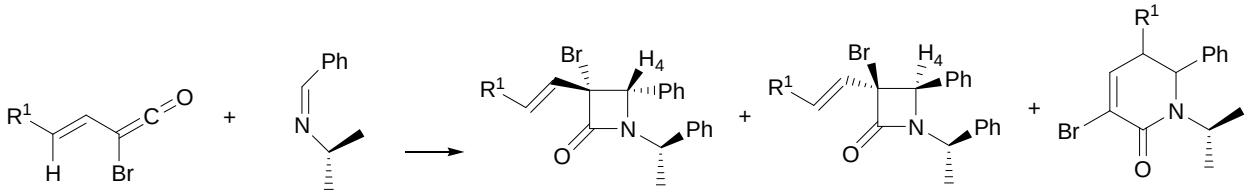
[a] Reported yields refer to isolated products. A small amount of amide was observed in all the reactions.

Compounds **3b-d** and **4b-d** were obtained with exclusive E configuration of the double bond in the side chain, as shown by the coupling constant (J = 15.6 Hz). The *cis* configuration of **3** was established by NOE experiments using DPFGSE pulse sequence.

The isomer **3** exihibited a strong NOE between H_4 and the protons of the double bond on C_3, thus suggesting a *cis* relationship between these two moieties. The same experiment performed on the minor isomer **4** did not afford any NOE effect, thus suggesting a *trans* geometry between H_4 and the alkenyl group.

The Staudinger reaction carried out with α-bromo vinylketenes **1a-b** and the imine **2e**, obtained starting from benzaldehyde and (*S*)-1-phenylethylamine, gave the β-lactams in

enantiomerically pure form (**Scheme 1.3**). Although four stereoisomers could possibly arise from this reaction, complete *cis* diastereoselectivity was observed and mixtures of *cis* β-lactams, together with a significative amount δ-lactams, were isolated both from the reaction of **1a** and **1b**.



1a : R^1 = Et
1b: R^1 = H

2e

3e: R^1 = Et
6e: R^1 = H

4e: R^1 = Et
7e: R^1 = H

5e: R^1 = Et
8e: R^1 = H

Scheme 1.3 Reaction of **1a** and **1b** with imine **2e**.

The mixtures were easily separated by flash chromatography on silica gel eluting with cyclohexane/diethyl ether. The major isomer **3e** was isolated as a gum, while the minor isomer **4e** is a solid that was crystallized from methanol. The DPFGSE-NOE experiments, performed on **3e** and **4e**, indicated for both compounds a 3,4-*cis* configuration, since a strong NOE effect was recorded between H_4 and the double bond protons.

The E configuration of the side chain was determined from the double bond coupling constant (J = 15.4 Hz). Furthermore, the (1*S*',3*S*,4*R*) absolute configuration of **4e** was established by X-ray diffraction. On the basis of these considerations, the assignment of the (1*S*', 3*R*,4*S*) absolute configuration to **3e** could be made. The comparison of the ^{1}H NMR data for **3e** and **4e** and the data for **6e** and **7e**, allowed us to find regularities when considering $H_{1'}$ and CH_3' of the phenylethyl moiety. The signal of H1', indeed, occurs at 5.10 ppm and 5.09 ppm for (1*S*', 3*R*,4*S*)-**3e** and for **6e** respectively, while it is observed at 4.33 ppm and 4.35 ppm for (1*S*', 3*S*,4*R*)-**4e** and for **7e** respectively. In a similar way, the signal of CH_3', occurs at 1.50 ppm and 1.52 ppm for (1*S*', 3*R*,4*S*)-**3e** and for **6e** respectively, and at 1.95 ppm both for (1*S*',3*S*,4*R*)-**4e** and **7e**.

On the basis of these observations, we attributed the (1*S*',3*R*,4*S*) configuration to **6e** and the (1*S*',3*S*,4*R*) configuration to **7e**.

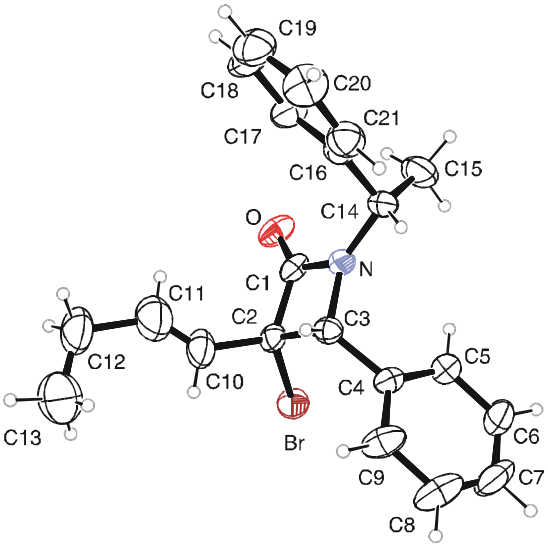
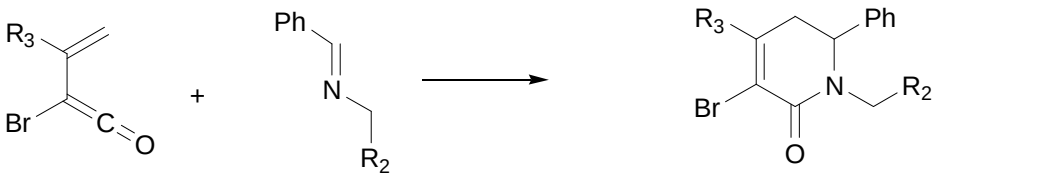


Figure 1.1 ORTEP diagram of **4e**.

1.2.2 Reaction of γ,γ-disubstituted-α-bromo vinylketenes with imines

Performing a modulation of the substituents of α-bromo vinylketenes, we discovered that their diene-behaviour could be enhanced with the introduction of a branch in β position. Actually, exclusively the [4+2] pathway is followed in the reactions of **1c-d** with imines **2a-d**, and the dihydropyridinones **9a-e** could be obtained in high yields, as previously reported.[64] Indeed, α-bromo vinylketene **1d** is prepared with excellent regioselectivity treating 2-bromo-3-methyl-2-hexenoyl chloride with 2 eq. of TEA, despite the possibility of deprotonation of the methylene group to give an isomeric vinylketene.[80]

No trace of the β-lactam was detected in the HPLC analysis and in the ^{1}H NMR spectra of the crude reaction (**Scheme 1.4**, Table 1.2).



1c: $R_3 = CH_3$
1d: $R_3 = n\text{-}C_3H_7$

2a-d

9a: R_3 =Me, R_2 = Ph
9b: R_3 =Me, R_2 = PMP
9c: R_3 =Me, R_2 = $CH{=}CH_2$
9d: R_3 =Me, R_2 = CH_2COOEt
9e: $R_3 = n\text{-}C_3H_7$, R_2 = Ph

Scheme 1.4 Reaction of γ,γ-disubstituted-α-bromo vinylketenes **1c-d** with imines **2a-d**.

The detailed investigation of the reaction conditions showed that the best results could be obtained when **1** and **2** were refluxed in CH_2Cl_2. Under these reaction conditions, **9a-e** were

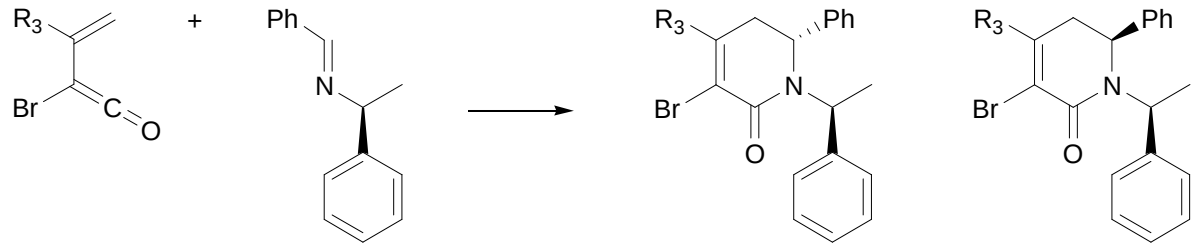
obtained in excellent yields (92-96%). Only the cycloaddition of **1c** and β-alanine derivative **2d** gave a lower yield, **9d** being isolated in 64% yield (Table 1.2, entry 4).

Table 1.2 Formation of 3-bromo-4-alkyl-5,6-dihydropyridin-2-one **3** via ketene-imine cyclisation.

Entry[a]	Ketene	Imine	Product	Yield (%)[b]
1	**1c**	**2a**	**9a**	98
2	**1c**	**2b**	**9b**	92
3	**1c**	**2c**	**9c**	96
4	**1c**	**2d**	**9d**	64
5	**1d**	**2a**	**9e**	94

[a]Reactions were performed in CH_2Cl_2.
[b]Yields correspond to the compounds purified by flash chromatography on silica gel.

Good yields and moderate diastereoselectivities were observed in the reactions of **1c** and **1d** with the chiral imine **2e** (98% yield and 62/38 d.r. for the reaction of **1c**, 55% yield and 68/32 d.r. for **1d**). The dihydropyridinones **10**/**11** and **12**/**13** were easily separated and fully characterised by NMR spectroscopy and LC-MS analysis. The (6*R*) absolute configuration of the newly created stereogenic centre in **12** was established by X-ray diffraction[64] , and the complete regularity of the ^{1}H NMR chemical shifts allowed us to confidently assign the stereochemistry to the other compounds of the class.

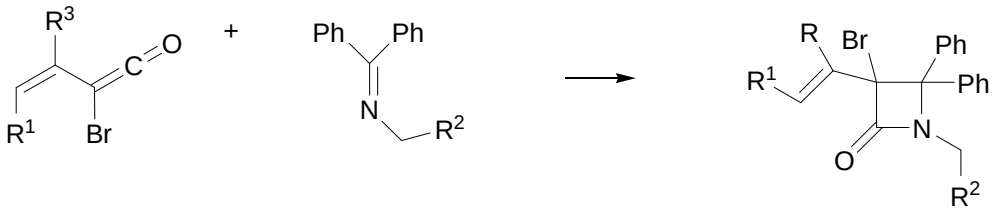


1c: $R_3 = CH_3$ **2e** **10**: $R_3 = CH_3$ **11**: $R_3 = CH_3$
1d: $R_3 = n\text{-}C_3H_7$ **12**: $R_3 = n\text{-}C_3H_7$ **13**: $R_3 = n\text{-}C_3H_7$

Scheme 1.5 Reaction of **1c** and **1d** with the chiral Schiff base **2e**.

1.2.3 Reaction of α-bromo vinylketenes with ketimines

The results reported above suggest that linear α-bromo vinylketenes **1a** and **1b** react with imines preferentially giving β-lactams, while γ,γ-disubstituted-α-bromo vinylketenes **1c** and **1d** afford exclusively six membered rings. In order to complete our investigation, we studied the influence of the imine substitution on the reactivity, treating α-bromo vinylketenes **1a**-**c** with the ketimines **2d** and **2f**, derived from benzophenone (**Scheme 1.6**).



1a: R^3 = H, R^1 = Et
1b: R^3 = H, R^1 = H
1c: R^3 = CH_3 R^1 = H

2d: R^2 = CH_2COOEt
2f: R^2 = COOEt

14d: R^3 = H, R^1 = Et, R^2=CH_2COOEt
14f: R^3 = H, R^1 = Et, R^2= COOEt
15d: R^3 = H, R^1 = H, R^2=CH_2COOEt
15f: R^3 = H, R^1 = H, R^2= COOEt
16d: R^3 = CH_3, R^1 = H, R^2=CH_2COOEt
16f: R^3 = CH_3, R^1 = H, R^2= COOEt

Scheme 1.6 Reaction of α-bromo vinylketenes with ketimines **2d** and **2f**.

The experimental results demonstrate that the substitution of the imine hydrogen with a phenyl group dramatically suppressed the strong preference of **1c** for [4+2] reaction, selectively leading to 3-bromo-3-alkenyl-azetidin-2-ones (Table 1.3).

Table 1.3 Formation of 3-bromo-3-alkenyl-azetidin-2-ones via ketene-ketimine cyclisation.

Entry[a]	Ketene	Imine	Product	Yield (%)[b]
1	**1a**	**2f**	**14f**	97
2	**1a**	**2d**	**14d**	84
3	**1b**	**2f**	**15f**	93
4	**1b**	**2d**	**15d**	90
5	**1c**	**2f**	**16f**	80
6	**1c**	**2d**	**16d**	87

[a]Reactions were performed in CH_2Cl_2. [b]Yields correspond to the compounds purified by flash chromatography on silica gel.

Therefore, in the cycloadditions between α-bromo vinylketenes and imines, variations of the substituents can be exploited to drive the reaction toward the [2+2] or the [4+2] path, in order to selectively obtain β-lactams or δ-lactams.

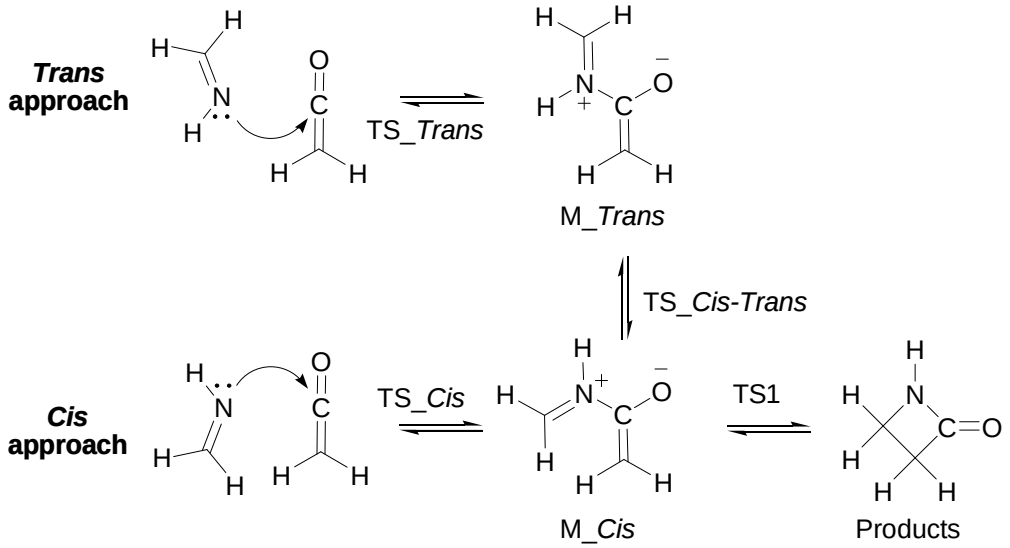
1.3 Computational Section

1.3.1 Choice of the model system and description of the computational method

To provide a rationale for the observed periselectivity, geometry optimisations of selected molecules in the presence of the solvent have been carried out. All computations have been performed at the DFT/B3LYP/DZVP[81-83] level, using Gaussian 03 package[84]; the solvent (dichloromethane) has been simulated by means of SCRF-CPCM[85-88] method. To validate this choice, the same level of calculation was used to study the addition of an unsubstituted imine to an unsubstituted ketene, in order to compare our results to those obtained by Venturini at

CASSCF-CASPT2 level.[36] The results obtained with the DFT method in gas phase are comparable to the CASSCF ones, both in terms of energy and geometry (results not reported here). Besides, the inclusion of the solvent (dichloromethane) effects *via* the SCRF method gave a quantitatively different pathway, according to Venturini.[36] The solvent helps stabilizing the structures with a charge separation (see **Scheme 1.7**), and the transition state TS_Cis_Trans connecting the minima M_Cis and M_Trans (and thus Cis and Trans pathways) could be easily located. Moreover, no influence of unpaired spin states have been found; single point calculation on all the found critical points, with both restricted and unrestricted methods (B3LYP and UB3LYP), have proved to give identical results and stable wavefunctions. For these reasons, a restricted DFT formulation was employed. The use of the hybrid B3LYP functional accounts for correlation effects with a lower computational demand with respect to CASSCF-CASPT2 or MP2 approaches. Furthermore, the inclusion of the solvent is of paramount importance to correctly reproduce the Potential Energy Surface (TS) related to the reactions under examination, due to the presence of critical points with consistent charge separation.

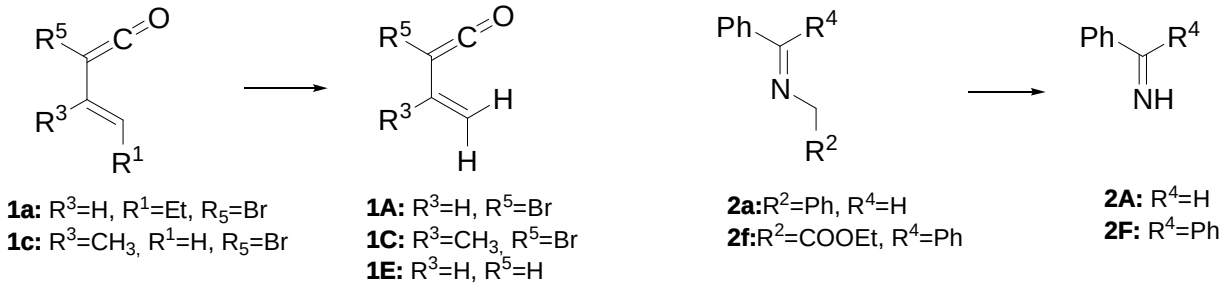
The geometries were optimised using redundant internal coordinates[89] and the nature of all the found critical points has been ascertained by means of frequency calculations, to check the presence of the correct number of negative eigenvalues. All optimisations were carried out in the presence of the solvent (see above) and the reported energy values include all the contribution due to solvent (both electrostatic and not).



Scheme 1.7 Pathways for the reaction of ketene and imine.

In order to decrease computational time, calculations have been carried out on simplified models of molecules, confidently assuming that these approximations do not affect the ability to reproduce experimental results (**Scheme 1.8**). The vinylketenes have been approximated

with **1A** (α-bromo-substituted), **1C** (both α-bromo-β-methyl-substituted) and **1E** (unsubstituted). Two molecules have been used to describe imines and ketimines (**2A** and **2F**, respectively). The nature of N-substituent has been considered irrelevant since experimentally it does not affect the reactions' outcome, therefore, it has been approximated with an H atom in all the calculations.



1a: R^3=H, R^1=Et, R_5=Br
1c: R^3=CH_3, R^1=H, R_5=Br

1A: R^3=H, R^5=Br
1C: R^3=CH_3, R^5=Br
1E: R^3=H, R^5=H

2a:R^2=Ph, R^4=H
2f:R^2=COOEt, R^4=Ph

2A: R^4=H
2F: R^4=Ph

Scheme 1.8 Model system used in the computational study.

1.4 Results and discussion

The [2+2] reaction between a ketene and an imine leading to β-lactams is unanimously considered a stepwise process; the first step is the nucleophilic attack of the imine nitrogen to the *sp*-carbon of the ketene, leading to a zwitterionic intermediate, that then undergo a conrotatory electrocyclic ring-closure to give the final product. Theoretical studies have provided an insight into the origins of the stereoselectivity,[39,41,44] which is without question the most intriguing problem; in particular, a detailed study recently reported by Xu and co-workers[34] clarified the role of the substituents in driving the stereoselectivity.

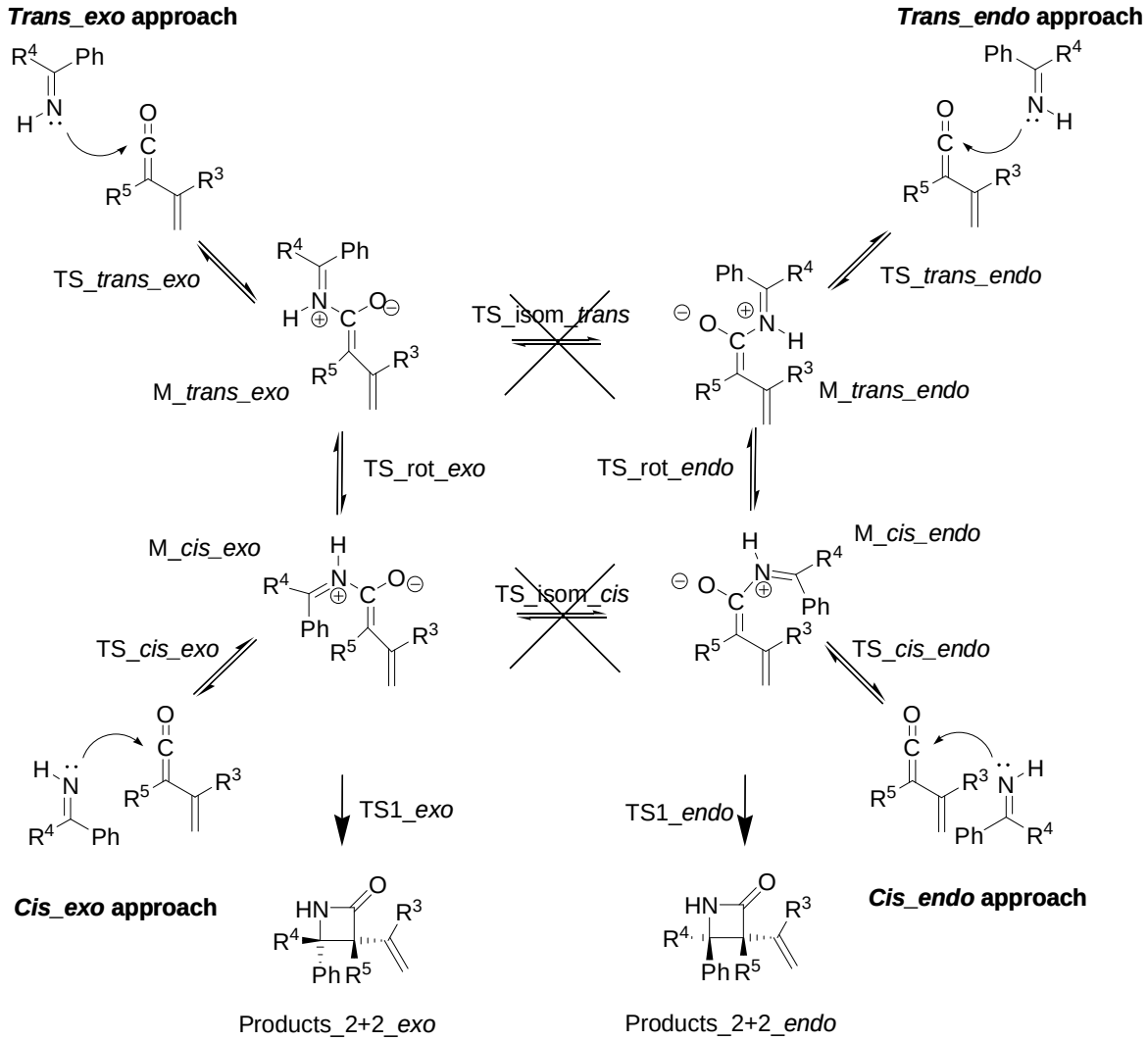
The conformations of the vinylketenes have been computationally studied;[50,51] the equilibrium from *s*-E-conformation to *s*-Z-conformation is of particular interest since it may be crucial for the discrimination between the [2+2] and the [4+2] pathway. The *s*-E-conformation is more stable than *s*-Z-conformation for all the vinylketenes (Table 1.4); however, the energies of the conformational TS are markedly lower for **1A** and **1C** than for **1E**, thus suggesting a role of the bromo substituent in the stabilisation of the unfavoured conformation. On the basis of these results it is clear that all the examined ketenes adopt a *s*-E-conformation at the equilibrium, but in RT conditions the rotation around the single bond is possible and is faster for **1C**.

Table 1.4 Energies of the conformational equilibrium of the ketenes.

molecule	E (kcal mol^{-1})[a]	Dihedral angle ϕ(°)
1A *s*-Z	3,09	0
1A_TS	5,81	108
1A *s*-E	0,00	180
1C *s*-Z	2,09	-43,39
1C_TS	5,07	-112,39
1C *s*-E	0,00	180
1E *s*-Z	2,18	0
1E_TS	7,01	98,24
1E *s*-E	0,00	180

The energies are referred to the *s*-E conformer

The possibility of an isomerisation from an (E) imine to a (Z) imine has been taken into account,[34] and finally the model imine **2A** was considered to be in the E configuration on the basis of both experimental and computational evidences. Our data suggest a barrier of about 30 kcal mol^{-1} for the E/Z isomerisation of **2A**.



Scheme 1.9 General scheme for the [2+2] pathways.

As previously reported in the literature[36,42], there are different approaches leading to β-lactams: two *endo* approaches, called *cis_endo* and *trans_endo*, and two *exo* approaches, called *cis_exo* and *trans_exo* (**Scheme 1.9**). In the following discussion, the *endo* suffix will be used for the imine approach on the vinyl group side of the ketene, while the *exo* suffix will be used for the imine approach on the R^5 group side of the ketene. As showed before the *cis* and *trans* approaches in both *exo* and *endo* pathways are connected by a rotational transition state between the minima M_*trans* and M_*cis*.

The energy of the critical points found on the PES for the [2+2] reaction between ketenes **1A, 1C** and **1E** and imine **2A** and ketimine **2F** are reported in table 1.5. In the four fully examined pathways (**1E**+**2A** *endo*/*exo* and **1A**+**2A** *endo*/*exo*), the rate determining step coincides with the electrocyclic ring closure of the M_cis minimum to give the β-lactam. The comparison of the barriers associated to TS1 in the *endo* and *exo* approaches (TS1_*endo* and TS1_*exo*) accounts for the preference of the system to give different β-lactam's diastereoisomers. A qualitative relation was observed between the $\Delta E^{TS1}_{(exo\text{-}endo)}$ values (Table 1.5) and the corresponding experimental ratio of products. In the case of the vinylketene **1E** (R^3=H) the greatest barrier (see Table 1.5 and Figure 1.2) is associated to the *endo* approach (TS1_*endo* = 15.75 kcal mol^{-1}), while the rate determining step of the *exo* approach was found to be 5.60 kcal mol^{-1} lower in energy (TS1_*exo* = 10.16 kcal mol^{-1}). This result is in good agreement with the observed experimental data[78], since *exo* approach leads to *cis* β–lactams. The opposite behaviour was observed in the reaction between α-bromo vinylketene **1A** and aldimine **2A,** since the [2+2] *endo* pathway, leading to the (Br-Ph)-*cis* β-lactam, is favoured over the *exo* of 4.46 kcal mol^{-1} (see Table 1.5 and experimental products ratio in Table 1.1). On the basis of the reported results, the analysis of the [2+2] pathway preference was extended to other reacting couples (**1C**+**2A, 1E**+**2F, 1A**+**2F, 1C**+**2F**), performing the calculations only on the most relevant critical point (TS1), confidently assuming the qualitative similarity of all the PES under examination.

Table 1.5 Energetics of the [2+2] pathways.

	1E+2A Energy (kcal mol^{-1})[a]	**1A+2A** Energy (kcal mol^{-1})[b]	**1C+2A** Energy (kcal mol^{-1})[b]	**1E+2F** Energy (kcal mol^{-1})[a]	**1A+2F** Energy (kcal mol^{-1})[b]	**1C+2F** Energy (kcal mol^{-1})[b]
TS_trans_endo	##[c]	##[c]				
M_trans_endo	-6.05	-12.62				
TS_rot_endo	-4.05	-11.13				
TS_cis_endo	3.95	2.01				
M_cis_endo	-4.19	-11.26				
TS1_endo	**15.75**	**6.72**	**10.83**	**11.01**	**2.95**	**9.56**
Products_endo	-28.05	-29.72				

TS_trans_exo	3.26	3.95				
M_trans_exo	-9.64	-13.21				
TS_Rot_exo	-4.94	-10.94				
TS_cis_exo	##[c]	##[c]				
M_cis_exo	-5.40	-10.61				
TS1_exo	**10.16**	**11.18**	**13.80**	**5.45**	**6.10**	**8.11**
Products_exo	-26.89	-29.86				
$\Delta E^{TS1}_{(exo\text{-}endo)}$	-5.60	+4.46	+2.97	-5.57	3.14	-1.45

a) Energies are referred to the sum of the energies of the reactants (1E and 2A)
b) Energies are referred to the sum of the energies of the reactants (1A and 2A)
c) The geometry obtained after the optimization was found to be a saddle of order higher than 1 ad so was considered irrelevant for a chemical point of view.

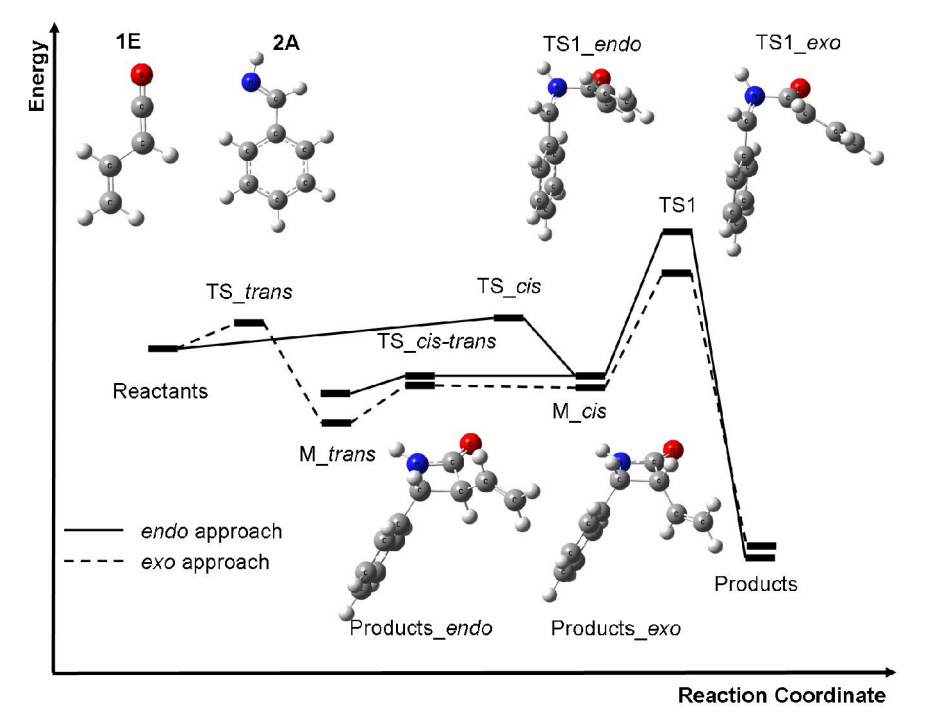


Figure 1.2 1E + **2A** [2+2] reaction profile.

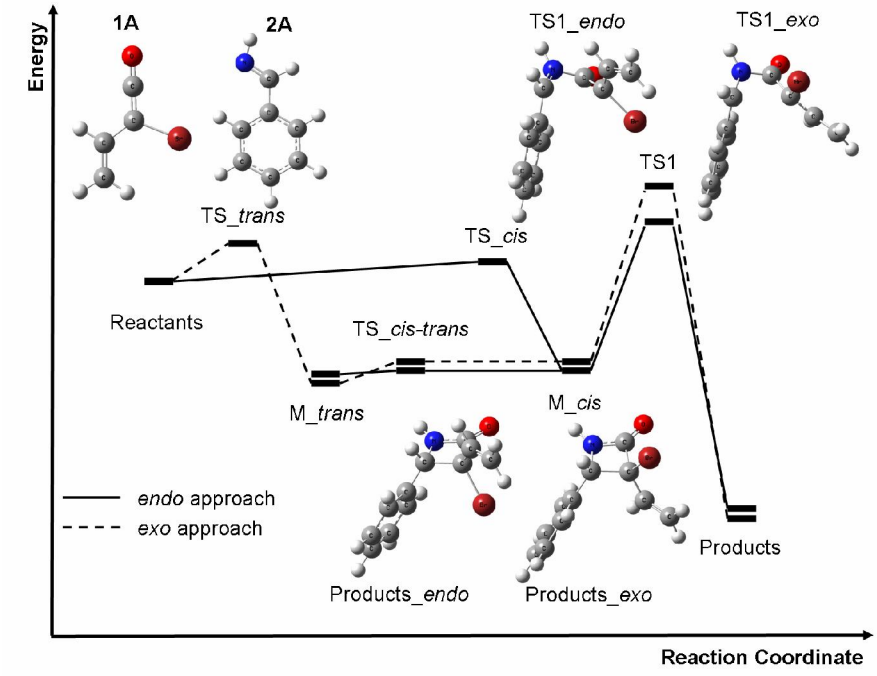
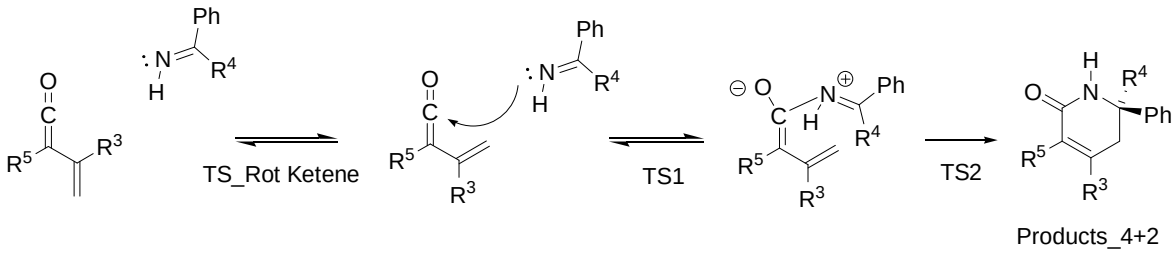


Figure 1.3 1A + 2A [2+2] reaction's profile.

We examined the isomerisation of the zwitterionic intermediates (see **Scheme 1.9**) from the more stable (E) imine geometry to the (Z); this pathway, if active, would connect the *endo* and *exo* pathways and result in an opposite diasteroselectivity. Even if some authors reported the occurrence of this mechanism in the case of benzaldehyde imines[90], no connection in between *endo* and *exo* pathways was found, since the location of the Transition States connecting M_*cis_endo* and M_*cis_exo* (TS_isom_*cis*) or M_*trans_endo* and M_*trans_exo* (TS_isom_*trans*) failed. Thus, we excluded the possibility of this isomerisation process in the case under examination.

To understand the peculiar diene-behaviour of α-bromo vinylketenes in depth, [4+2] pathways have been afterwards studied. First, on the basis of computational evidences, we excluded that the zwitterionic intermediates (M_*cis_endo* and M_*cis_exo*) of [2+2] pathway could give the six-membered product by way of rotation of vinyl group and subsequent ring-closure. In fact the Minima and the Transition States related to this mechanism were found in none of the examined PES. Instead, an independent two-step mechanism starting from the *s*-Z-vinylketene has been discovered in [4+2] cycloaddition; indeed, the capability of bromo substituent to stabilise *s*-Z-conformation of α-bromo vinylketenes enable a new reaction's pathway (**Scheme 1.10**).



Scheme 1.10 General scheme for the [4+2] pathway.

Table 1.6 Energetics of the [4+2] patways.

	1E+2A	**1A+2A**	**1C+2A**	**1C+2F**
Reactants	0.00	0.00	0.00	0.00
TS_rot_ketene	7.01	5.81	5.07	5.07
M0	2.18	3.09	2.09	2.09
TS1	10.63	8.26	7.27	8.68
M1	-1.30	-8.08	-8.11	-2.95
TS2	7.66	2.20	2.78	3.43
M2	-49.68	-53.93	-54.80	-46.76
TS3	-48.05	-52.02	-52.93	-43.43
M3	-51.51	-55.78	-56.72	-47.76

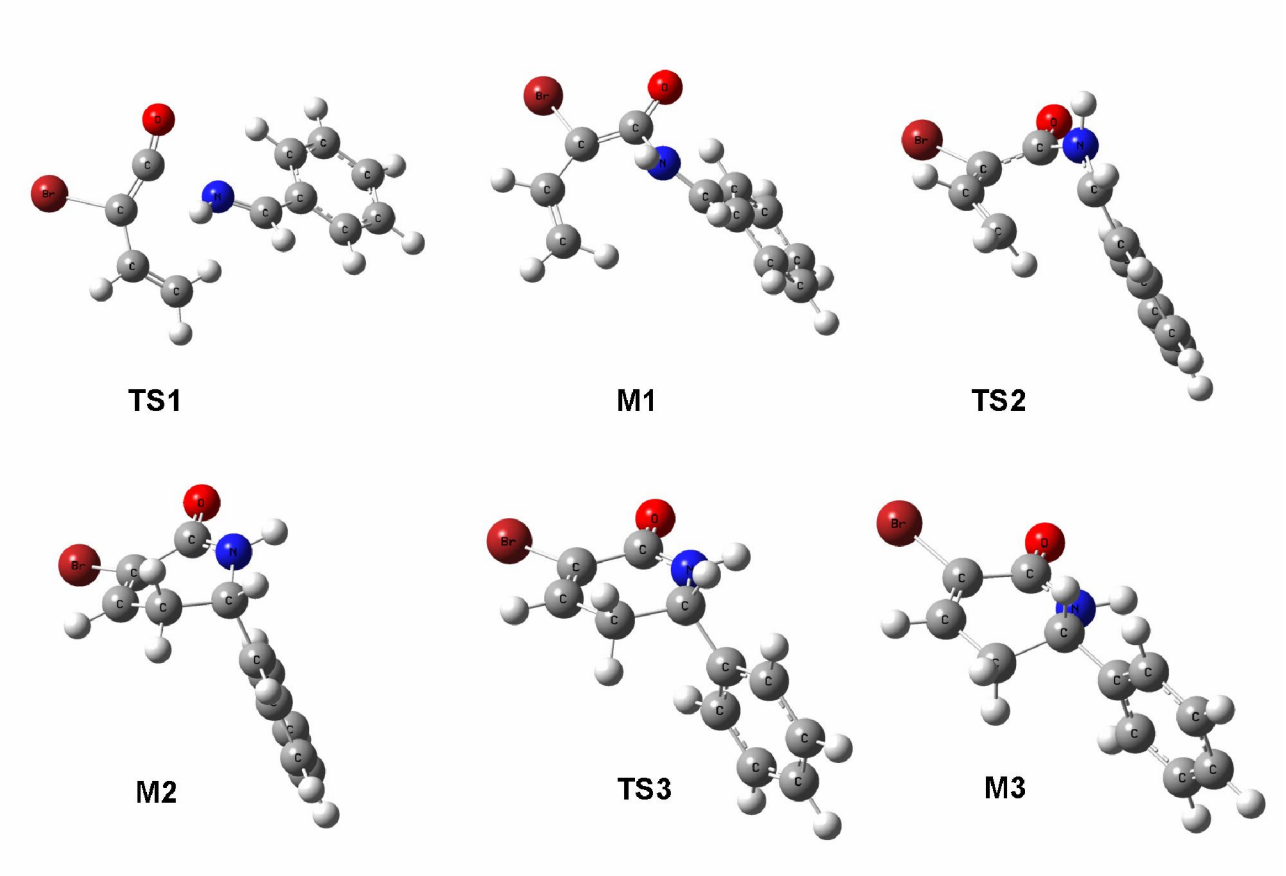


Figure 1.4 Geometries for [4+2] pathway (**1E**+**2A**)

The nucleophilic attack of the imine nitrogen to the *sp*-carbon of the α-bromo vinylketene in the *s*-Z-conformation is the first step of the reaction and it is also associated with the highest barrier (TS1);[50] the ring closure that follows from M1, affords through the transition state TS2 the final six-membered product (M2) with the phenyl group in axial position (M3); a low barrier isomerisation (TS3) gives the stable conformer with the phenyl group in equatorial position. A "ball & stick" representation of the [4+2] pathway for the **1A**+**2A** reaction is reported in Figure 1.4. In Figures 1.5-1.8, the energy diagrams for the [4+2] reaction of **2A** with **1E**, **1A** and **1C** are reported, compared with the highest barriers of the competitive [2+2] pathways. It is worthwhile to note that the diasteroselectivity predicted by the proposed [4+2] mechanism is consistent with the observed stereochemistry as previously reported.[64]

In the cycloaddition between **1E** and **2A**, the energies of [4+2] TS1 and [2+2] TS1_*exo* are similar; the exclusive formation of the β-lactam may be ascribed to the more favoured attack of the imine (TS_*trans_exo* = 3.26 kcal mol^{-1}) on the *s*-E-vinylketene upon its isomerisation to give the *s*-Z-vinylketene (TS_rot_ketene = 7.01 kcal mol^{-1}). In the reaction between **1A** and **2A**, the computed barriers for [2+2] (TS1_*exo* = 11.18 kcal $mol^{-1}$and TS1_*endo* = 6.72 kcal mol^{-1}) and [4+2] (TS1 = 8.26 kcal mol^{-1}) pathways are comparable; the experimental products ratio (Table 1.1) is in good qualitative agreement with the order of magnitude of the

corresponding rate-determining barriers. Concerning the cycloaddition of **1C** with **2A**, the preference for the [4+2] product is fully explained by the difference between TS1 (7.27 kcal mol^{-1}) and the higher barriers TS1_*exo* (13.80 kcal mol^{-1}) and TS1_*endo* (10.83 kcal mol^{-1}). Generally, the [4+2] pathway can be followed only by those vinylketenes that can easily populate the *s*-Z-conformation and it is unapproachable for other vinylketenes; in this sense, the unusual behaviour of the α-bromo vinylketenes in respect to unsubstituted vinylketenes may be rationalised.

However, when ketimine **2F** (R^4=Ph, **Scheme 1.8**) is involved, TS1 energy increases (8.68 kcal mol^{-1}) and the [4+2] pathway becomes therefore unfavoured compared to [2+2] pathway (TS1_*exo* = 8.11 kcal mol^{-1} and TS1_*endo* = 9.56 kcal mol^{-1}, Table 1.5), as represented in Figure 1.7.

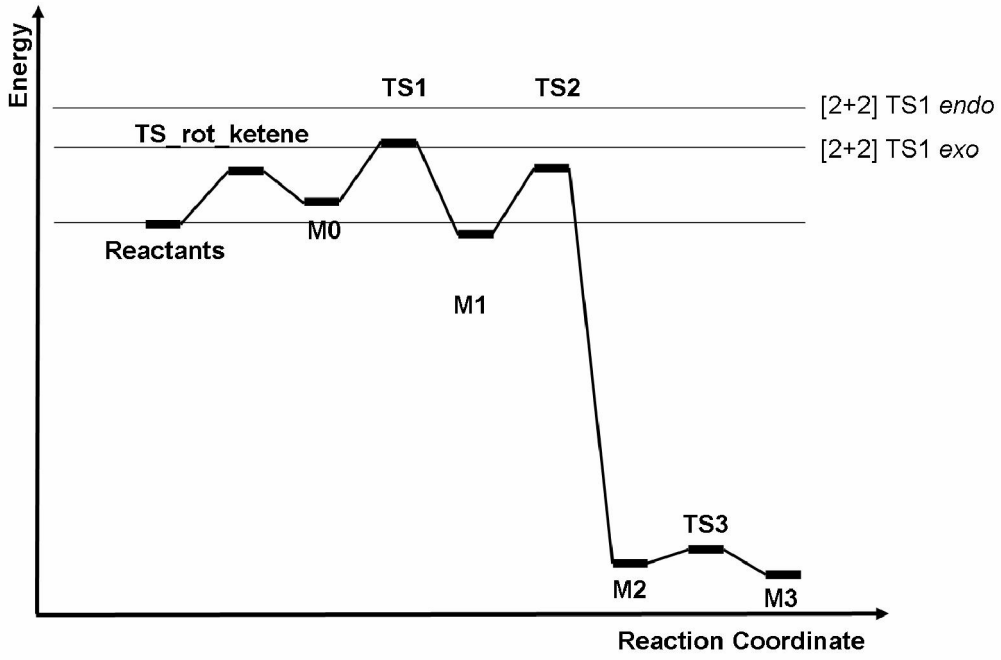


Figure 1.5 1E+**2A** [4+2] reaction profile.

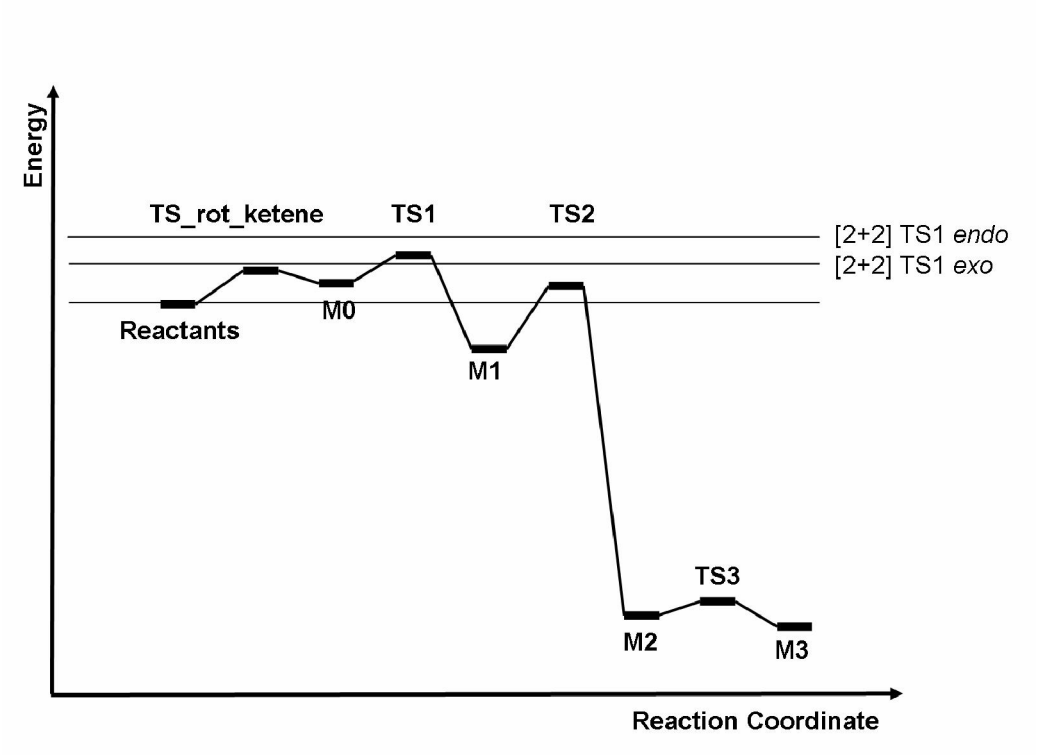


Figure 1.6 1A+**2A** [4+2] reaction profile.

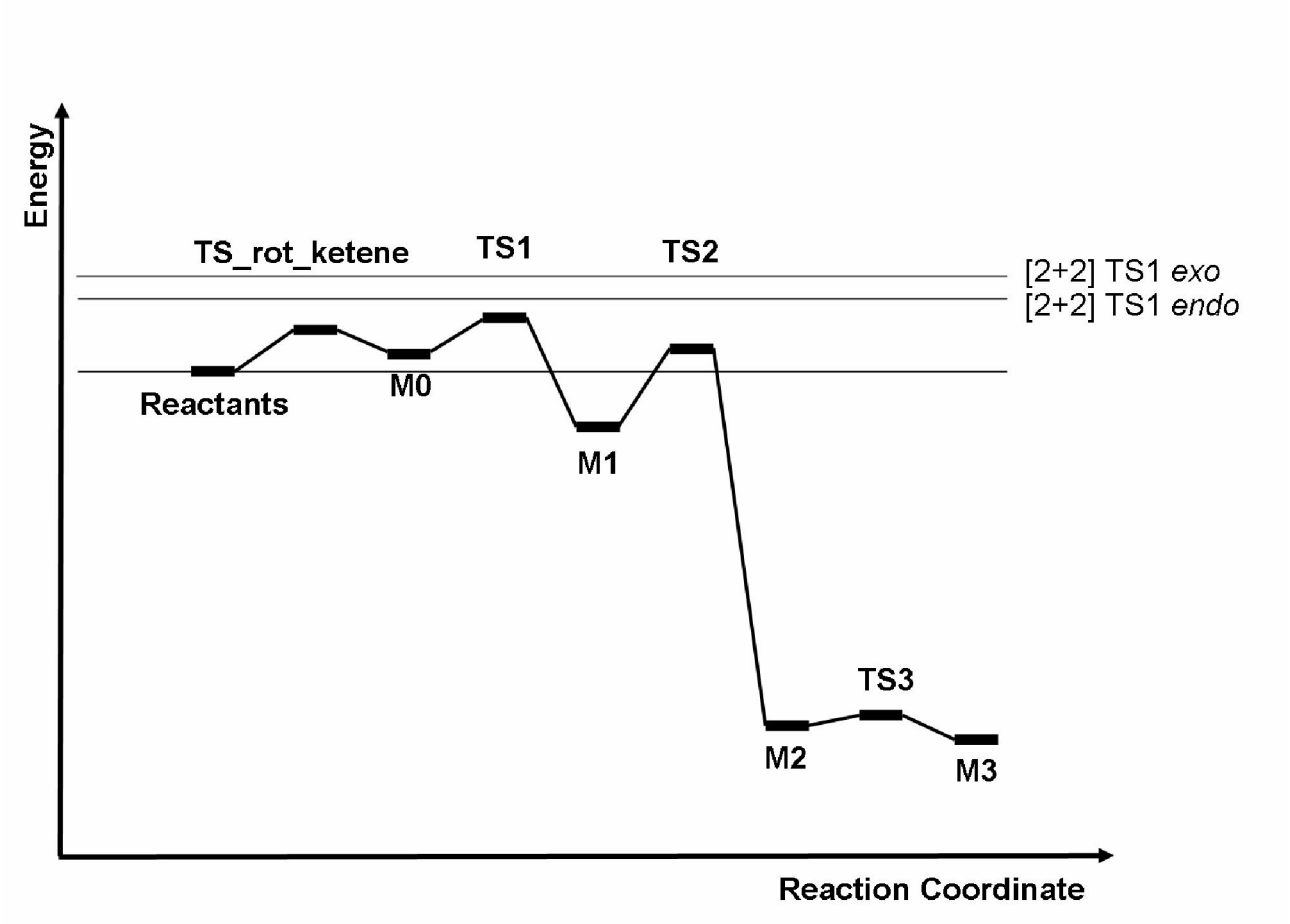


Figure 1.7 1C+**2A** [4+2] reaction profile.

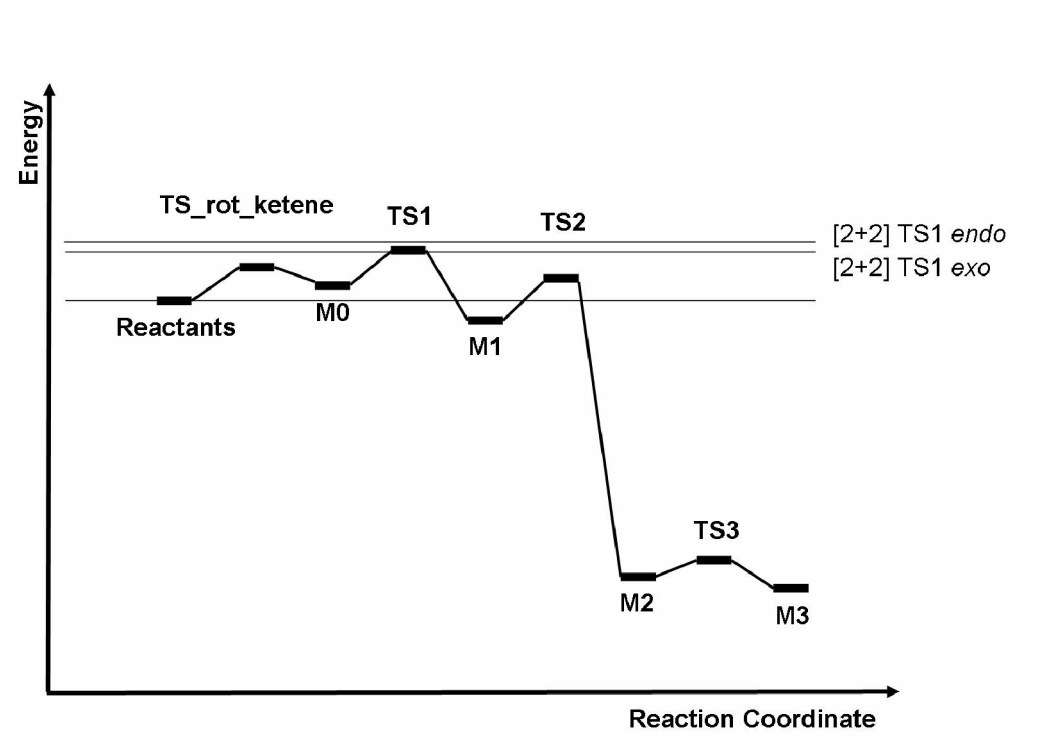


Figure 1.8 1C+2F [4+2] reaction profile.

1.5 Conclusions

In summary, we have documented the unprecedented behaviour of α-bromo vinylketenes in cycloadditions with imines; the singularity of this class of vinylketenes relies on the fact that they can act as dienophiles in [2+2] reactions, but they can also display an unusual diene reactivity in [4+2] reactions. Thus, α-bromo vinylketenes are versatile building blocks in the synthesis of highly functionalised heterocycles, since α-bromo substituted 3-alkenyl-azetidin-2-ones and 4-alkyl-5,6-diyhdropyridin-2-ones can be easily obtained from [2+2] and [4+2] cycloadditions with imines respectively. Interestingly, the reactivity of α-bromo vinylketenes can be modulated *via* a fine tuning of the substituents; the introduction of a methyl group in β-position, for example, completely suppress the dienophile reactivity and allow to obtain the [4+2] cycloaddition products exclusively. To switch again to the [2+2] cycloaddition, it is enough to employ a more hindered ketimine instead of an imine: both β-methyl substituted and unsubstituted α-bromo vinylketenes give azetidinones when reacted with ketimines.

The theoretical investigation carried out in the present thesis in order to rationalise these observations has provided interesting computational results that are in good qualitative agreement with the experimental ones.

Two pathways have been considered for the [2+2] reaction with an imine i.e. the *endo* and the *exo* paths. The former (*endo*) was found to be favoured for α-bromo vinylketenes, while the

latter (*exo*) was preferred in the case of unsubstituted vinylketenes. The bromo substituent is crucial for the discrimination between [2+2] and [4+2] mechanisms, since [4+2] cycloaddition occurs starting from a vinylketene in the *s*-Z-conformation. For the unsubstituted vinylketene, the barrier from the *s*-E-conformation to the *s*-Z-conformation is too high and, therefore, the favourite pathways are the [2+2] ones. For α-bromo vinylketenes, and especially for the β-methyl substituted compounds, this barrier can be overcame. In this way the [4+2] pathway becomes accessible and, in the latter case, preferred. In the reactions involving ketimines, the presence of another phenyl group especially affects the formation of the zwitterionic intermediate in the [4+2] pathway, in such a way that the [2+2] pathways become favoured with all α-bromo vinylketenes.

1.6 Experimental Section

DPFGSE-NOE (Double Pulse Field Gradient-Nuclear Overhauser Effect) experimets have been performed in $CDCl_3$ at 25°C on a Varian INOVA ® 400MHz (Oxford Magnet).

3a: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, *J* = 7.6 Hz, CH_2CH_3), 2.13 (2H, q, *J* = 6.4 Hz, CH_2CH_3), 3.89 (1H, d, *J* = 15.0 Hz, $PhCH_2$), 4.55 (1H, s, *H*C-Ph), 4.98 (1H, d, *J* = 15.0 Hz, $PhCH_2$), 5.80 (1H, dd, $J_{1,2}$ = 15.4 Hz, $J_{1,3}$ = 1.6 Hz, Br-C*CH*=CH), 6.13 (1H, dt, *J* = 15.4, 6.4 Hz, Br-C-CH=*CH*), 7.10-7.44 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.9, 25.4, 44.8, 66.9, 69.7, 125.5, 127.7, 127.9, 128.3, 128.4, 128.8, 128.9, 134.6, 136.8, 141.3, 165.7; LC-ESI-MS rt 14.4 min., m/z 370/372 (M+1), 392/394 (M+Na).

4a : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, t, *J* = 7.6 Hz, CH_2CH_3), 1.94 (2H, m, CH_2CH_3), 3.87 (1H, d, *J* = 15.2 Hz, $PhCH_2$), 4.78 (1H, s, *H*C-Ph), 4.95 (1H, d, *J* = 15.2 Hz, $PhCH_2$), 4.96 (1H, d, *J* = 15.6 Hz, Br-C*CH*=CH), 6.16 (1H, dt, *J* = 15.6, 6.4 Hz, Br-C-CH=*CH*), 7.10-7.44 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.6, 25.3, 44.2, 60.7, 70.8, 123.2, 127.8, 127.9, 128.3, 128.6, 128.8, 129.0, 133.1, 134.4, 139.5, 165.2; LC-ESI-MS rt 13.8 min., m/z 370/372 (M+1), 392/394 (M+Na).

5a : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.72 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.31 (1H, m, CH_2CH_3), 1.50 (1H, m, CH_2CH_3), 2.28 (1H, ddt, *J* = 6.6, 1.6, 6.5 Hz, *H*C-CH-Ph), 3.50 (1H, d, *J* = 14.2 Hz, $PhCH_2$), 4.39 (1H, bs, *H*C-Ph), 5.68 (1H, d, *J* = 14.2 Hz, $PhCH_2$), 6.73 (1H, dd, $J_{1,2}$ = 6.6 Hz, $J_{1,3}$ = 1.2 Hz, Br-C=C*H*), 7.05-7.30 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 10.8, 26.5, 46.3, 49.3, 60.8, 118.1, 126.1, 127.8, 128.2, 128.6, 128.8, 128.9, 136.6, 139.5, 141.3, 159.5; GC-MS m/z 369/371 (M, 10), 265 (8), 207 (12), 128 (20), 91 (100).

3b: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 2.11-2.22 (2H, m, CH_2CH_3), 3.80 (3H, s, OCH_3), 3.85 (1H, d, *J* = 14.9 Hz), 4.53 (1H, s, *H*C-Ph), 4.93 (1H, d, *J* = 14.9 Hz), 5.79 (1H, dt, $J_{1,2}$ = 15.3 Hz, $J_{1,3}$ = 1.5 Hz, Br-C*CH*=CH), 6.13 (1H, dt, *J* = 6.3, 15.3 Hz, Br-C-CH=*CH*), 6.8-7.4 (9H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.9, 25.3, 44.2, 55.2, 66.7, 69.6, 114.2, 125.5, 126.5, 127.7, 128.4, 128.9, 129.7, 134.7, 136.7, 159.3, 165.6; LC-ESI-MS rt 13.7 min., m/z 400-402 (M+1), 422-424 (M+Na).

4b: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 2.25-2.35 (2H, m, CH_2CH_3), 3.81 (3H, s, OCH_3), 3.82 (1H, d, *J* = 15.0 Hz), 4.75 (1H, s, *H*C-Ph), 4.91 (1H, d, *J* = 15.0 Hz), 4.95 (1H, dt, $J_{1,2}$ = 15.4 Hz, $J_{1,3}$ = 1.5 Hz, Br-C-*CH*=CH), 6.14 (1H, dt, *J* = 15.4, 6.3 Hz, Br-C-CH=*CH*), 6.85-6.89 (2H, m, ArH), 7.09-7.1189 (2H, m, ArH), 7.15-7.22 (2H, m, ArH), 7.32-7.40 (3H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.7, 24.9, 44.1, 55.2, 64.2, 70.1, 113.8, 123.6, 126.0, 127.8, 128.1, 128.7, 129.6, 133.2, 139.1, 159.2, 165.3; LC-ESI-MS rt 13.0 min., m/z 400-402 (M+1), 422-424 (M+Na).

5b : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.75 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.28 (1H, m, CH_2CH_3), 1.50 (1H, m, CH_2CH_3), 2.30 (1H, m, *H*C-CH-Ph), 3.45 (1H, d, *J* = 14.2 Hz, PhCH_2), 3.83 (3H, s, OCH_3), 4.40 (1H, bs, *H*C-Ph), 5.62 (1H, d, *J* = 14.2 Hz, PhCH_2), 6.73 (1H, d, *J* = 6.6 Hz, Br-C=*CH*), 6.87 (2H, m, ArH), 7.13-7.22 (4H, m, ArH), 7.35-7.40 (3H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 10.9, 26.6, 46.3, 48.7, 55.2, 60.6, 113.9, 126.1, 127.8, 128.3, 128.7, 128.8, 130.2, 139.6, 141.2, 159.2, 159.4; LC-ESI-MS rt 14.6 min., m/z 400-402 (M+1), 422-424 (M+Na).

3c : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t, *J* = 7.8 Hz, CH_2CH_3), 2.19 (2H, ddq, $J^{1,2}$ = 6.2, 7.8 Hz, $J^{1,3}$ = 1.5 Hz, CH_2CH_3), 3.48 (1H, dd, *J* = 15.2, 7.0 Hz, C*H*$_2$-CH=CH$_2$), 4.31 (1H, dd, *J* = 15.2, 5.2 Hz, C*H*$_2$-CH=CH$_2$), 4.81 (1H, bs, *H*C-Ph), 5.08-5.23 (2H, m, CH$_2$-CH=*CH*$_2$), 5.78 (1H, m, CH$_2$-*CH*=CH$_2$), 5.91 (1H, dt, $J^{1,2}$ = 15.4 Hz, $J^{1,3}$ = 1.5 Hz, Br-C-*CH*=CH), 6.20 (1H, dt, *J* = 15.4, 6.2 Hz, Br-C-CH=*CH*), 7.23-7.28 (2H, m, ArH), 7.38-7.45 (3H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.8, 25.3, 43.2, 67.3, 69.6, 119.1, 127.8, 128.3, 128.9, 130.3, 134.7, 136.7, 144.6, 165.5; LC-ESI-MS rt 12.40 min., m/z 320/322 (M+1), 342/344 (M+Na).

4c : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 1.91 (2H, q, *J* = 7.4 Hz, CH_2CH_3), 3.47 (1H, m, C*H*$_2$-CH=CH$_2$), 4.28 (1H, m, C*H*$_2$-CH=CH$_2$), 5.02 (1H, bs, *H*C-Ph), 5.10-5.24 (2H, m, CH$_2$-CH=*CH*$_2$), 5.83 (1H, dt, $J^{1,2}$ = 15.0 Hz, $J^{1,3}$ = 1.2 Hz, Br-C-*CH*=CH), 6.06-6.26 (2H, m, CH$_2$-*CH*=CH$_2$ + Br-C-CH=*CH*), 7.10-7.48 (5H, m, ArH). ^{13}C NMR ($CDCl_3$) δ 12.6, 25.4, 42.9, 68.0, 70.2, 120.3, 125.7, 127.6, 128.3, 128.9, 134.0, 139.4, 143.5, 166.0; LC-ESI-MS rt 12.85 min., m/z 320/322 (M+1), 342/344 (M+Na).

5c : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.09 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.86 (2H, dq, *J* = 14.5, 7.2 Hz, CH_2CH_3), 2.40 (1H, ddt, *J* = 6.6, 1.2, 14.5 Hz, *H*C-CH-Ph), 3.14 (1H, dd, *J* = 8.1, 14.5 Hz, CH_2-CH=CH_2), 4.53 (1H, bs, *H*C-Ph), 4.90 (1H, dd, *J* = 4.8, 14.5 Hz, CH_2-CH=CH_2), 5.15-5.26 (2H, m, CH_2-CH=CH_2), 5.74-5.87 (1H, m, CH_2-*C*H=CH_2), 6.76 (1H, dd, $J^{1,2}$ = 6.6 Hz, $J^{1,3}$ = 1.0 Hz, Br-C=C*H*), 7.12-7.16 (2H, m, ArH), 7.34-7.37 (3H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 11.2, 26.7, 46.3, 48.8, 61.5, 112.0, 126.0, 127.7, 128.8, 132.6, 139.7, 141.2, 144.6, 159.1; LC-ESI-MS rt 11.50 min., m/z 320/322 (M+1), 342/344 (M+Na).

3d : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 1.22 (3H, t, *J* = 7.5 Hz, OCH_2CH_3), 2.17 (2H, dq, *J* = 6.3, 7.4 Hz, CH_2CH_3), 2.58 (1H, dt, *J* = 16.8, 6.9 Hz, CH_2-COOEt), 2.73 (1H, dt, *J* = 16.8, 6.9 Hz, CH_2-COOEt), 3.28 (1H, dt, *J* = 14.4, 6.9 Hz, CH_2-N), 3.85 (1H, dt, *J* = 14.4, 6.9 Hz, CH_2-N), 4.05 (2H, q, *J* = 7.5 Hz, OCH_2CH_3), 4.82 (1H, bs, *H*C-Ph), 5.85 (1H, d, *J* = 15.3 Hz, BrC*H*=CH), 6.14 (1H, dt, *J* = 15.3, 6.3 Hz, BrCH=C*H*), 7.21-7.45 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.3, 14.0, 25.2, 32.3, 46.6, 60.8, 64.7, 68.3, 125.9, 127.8, 128.5, 128.9, 136.7, 144.6, 165.6, 171.8; LC-ESI-MS rt 13.40 min., m/z 380/382 (M+1), 402/404 (M+Na).

4d: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.12 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.26 (3H, t, *J* = 7.5 Hz, OCH_2CH_3), 2.36 (2H, dq, *J* = 6.3, 7.5 Hz, CH_2CH_3), 2.57 (1H, m, CH_2-COOEt), 2.75 (1H, m, CH_2-COOEt), 3.16 (1H, m, CH_2-N), 4.07 (3H, m, CH_2-N + OCH_2CH_3), 4.82 (1H, bs, *H*C-Ph), 5.78 (1H, d, *J* = 15.3 Hz, BrC*H*=CH), 6.10 (1H, dt, *J* = 15.3, 6.3 Hz, BrCH=C*H*), 7.25-7.47 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.4, 12.9, 25.3, 32.4, 46.5, 60.6, 65.3, 69.5, 123.1, 127.6, 128.3, 128.8, 138.3, 144.3, 166.0, 171.4; LC-ESI-MS rt 13.93 min., m/z 380/382 (M+1), 402/404 (M+Na).

5d : Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.12 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.24 (3H, t, *J* = 7.5 Hz, OCH_2CH_3), 1.71 (2H, m, CH_2CH_3), 2.40 (1H, m, *H*C-CH-Ph), 2.62 (1H, dt, *J* = 16.6, 6.0 Hz, CH_2-COOEt), 2.81 (1H, ddd, *J* = 16.6, 6.0, 8.5 Hz, CH_2-COOEt), 3.19 (1H, ddd, *J* = 14.4, 6.0, 8.5 Hz, CH_2-N), 4.02 (2H, q, OCH_2CH_3), 4.11 (1H, dt, *J* = 14.4, 6.0 Hz, CH_2-N), 4.74 (1H, bs, *H*C-Ph), 6.76 (1H, d, *J* = 6.0 Hz, Br-C=C*H*), 7.12-7.14 (2H, m, ArH), 7.34-7.42 (3H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.5, 12.7, 26.7, 32.9, 37.0, 44.1, 60.5, 69.6, 125.5, 127.6, 128.3, 128.7, 134.8, 141.5, 160.0, 170.6. LC-ESI-MS rt 12.81 min., m/z 380/382 (M+1), 402/404 (M+Na).

6a: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.92 (1H, d, *J* = 14.8 Hz, PhC*H*$_2$), 4.61 (1H, br s, *H*C-Ph), 5.02 (1H, d, *J* = 14.8 Hz, PhC*H*$_2$), 5.35 (1H, d, *J* = 10.7 Hz, Br-C-CH=C*H*$_2$), 5.65 (1H, d, *J* = 16.8 Hz, Br-C-CH=C*H*$_2$), 6.18 (1H, dd, *J* = 10.7, 16.8 Hz, Br-C-*CH*=CH$_2$), 7.1-7.5 (10H, m, Ph); ^{13}C NMR ($CDCl_3$) δ 44.7, 66.1, 69.5, 120.4, 127.6, 127.9, 128.2, 128.3, 128.7, 128.9, 131.8, 134.0, 134.3, 165.3; LC-ESI-MS rt 12.2 min., m/z 342/344 (M+1), 364/366 (M+Na).

7a: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.94 (1H, d, *J* = 14.6 Hz, PhC*H*$_2$), 4.83 (1H, s, *H*C-Ph), 5.00 (1H, d, *J* = 14.6 Hz, PhC*H*$_2$), 5.19 (1H, d, *J* = 10.5 Hz, Br-C-CH=C*H*$_2$), 5.41 (1H, dd, *J* = 10.5, 16.7 Hz, Br-C-*CH*=CH$_2$), 5.70 (1H, d, *J* = 16.7 Hz, Br-C-CH=C*H*$_2$), 7.1-7.5 (10H, m, Ph); ^{13}C NMR ($CDCl_3$) δ 44.3, 66.2, 69.8, 118.5, 127.6, 127.8, 128.2, 128.4, 128.7, 128.9, 133.9, 134.0, 134.2, 164.9; LC-ESI-MS rt 12.65 min., m/z 342/344 (M+1), 364/366 (M+Na).

8a: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.52 (1H, ddd, *J* = 2.4, 6.6, 17.7 Hz, C*H*$_2$CH-Ph), 2.97 (1H, ddd, *J* = 2.7, 7.8, 17.7 Hz, C*H*$_2$CH-Ph), 3.63 (1H, d, *J* = 15.0 Hz, C*H*$_2$-Ph), 4.65 (1H, dd, *J* = 2.4, 7.8 Hz, C*H*-Ph), 5.65 (1H, d, *J* = 15.0 Hz, C*H*$_2$-Ph), 6.74 (1H, dd, *J* = 2.7, 6.6 Hz, Br-C=C*H*), 7.18-7.42 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ$_C$ 33.9, 49.2, 57.4, 118.4, 126.2, 127.5, 128.0, 128.4, 128.6, 128.9, 136.8, 137.1, 139.2, 160.1; LC-ESI-MS rt 11.89 min., m/z 342/344 (M+1), 364/366 (M+Na).

(1S', 3*R*,4*S*)-3e : Isolated as a pale yellow oil; [α]$_D$ = +3 (c 1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, *J* = 7.5 Hz, CH_2C*H*$_3$), 1.50 (3H, d, *J* = 7.2 Hz, CH-C*H*$_3$), 2.05-2.22 (2H, m, C*H*$_2$CH$_3$), 4.50 (1H, s, *H*C-Ph), 5.10 (1H, q, *J* = 7.2 Hz, CH-C*H*$_3$), 5.74 (1H, dt, *J*^{1,2} = 15.4 Hz, *J*^{1,3} = 1.2 Hz, Br-C-*CH*=CH), 6.03 (1H, dt, *J* = 15.4, 6.6 Hz, Br-C-CH=C*H*), 7.25-7.42 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 13.0, 18.8, 25.4, 53.1, 67.5, 68.8, 125.4, 127.3, 128.0, 128.3, 128.4, 128.7, 129.0, 136.0, 136.5, 139.3, 166.2; LC-ESI-MS rt 15.57 min., m/z 384/386 (M+1), 406/408 (M+Na).

(1S', 3*S*,4*R*)-4e : Isolated as a white solid, m.p. 83-85 °C; [α]$_D$ = -10 (c 1.2 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t, *J* = 7.5 Hz, CH_2C*H*$_3$), 1.95 (3H, d, *J* = 7.0 Hz, CH-C*H*$_3$), 2.10-2.22 (2H, m, C*H*$_2$CH$_3$), 4.33 (1H, q, *J* = 7.0 Hz, CH-C*H*$_3$), 4.53 (1H, s, *H*C-Ph), 5.83 (1H, dt, *J*^{1,2} = 15.4 Hz, *J*^{1,3} = 1.5 Hz, Br-C-*CH*=CH), 6.18 (1H, dt, *J* = 15.4, 4.4 Hz, Br-C-CH=C*H*), 7.22-7.43 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 13.0, 20.3, 25.3, 55.4, 67.0, 69.1, 125.8, 126.7,

127.9, 128.0, 128.5, 128.8, 128.9, 135.0, 136.7, 140.8, 165.8; LC-ESI-MS rt 15.76 min., m/z 384/386 (M+1), 406/408 (M+Na).

Single crystal X-ray diffraction data of **4e** : $C_{21}H_{22}Br_1N_1O_1$, Fw.: 384.31, colorless platelet, size: 0.25 x 0.15 x 0.05 mm, monoclinic, space group $P2_1$, a = 6.3211(5) Å, b = 17.5402(13) Å, c = 8.9612(7) Å, ß = 98.877(3)°, V = 981.66(13) Å^3, theta range for data collection is from 2.30 to 30.17°, T=293(2) K, Z = 2, F (000) = 396, D_x = 1.300 Mg/m^3, μ = 2.100 mm^{-1}, data collected on a Bruker AXS CCD difractometer (Mo-Kα radiation, λ= 0.71073 Å) at 293(2) K, total of 12894 reflections, of which 5751 unique [$R_{(int)}$ = 0.0523]; 3128 reflections $I > 2\sigma$ (I). Empirical absorption correction was applied, initial structure model by direct methods. Anisotropic full-matrix least-squares refinement on F^2 for all non-hydrogen atoms yielded R_1 = 0.0514 and wR_2 = 0.1227 for 3128 [$I > 2\sigma$ (I)] and R_1 = 0.1035 and wR_2 = 0.1450 for all (5751) intensity data. Goodness-of-fit = 0.879, absolute structure parameter of the model: x = 0.008 (14), the max./mean shift/esd is 0.00 and 0.00, max./min. residual electron density in the final d.e.d. map was 0.563 and – 0.434 e Å^{-3}. CCDC number is 212779.

5e: Isolated as a white solid, m.p.= 150-152 °C; $[\alpha]^{19}_D$ –66 (c 1.14, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.49 (3H, t, J = 7.2 Hz, CH_2-CH_3), 0.82-1.02 (2H, m, CH_2-CH_3), 1.21 (3H, d, J = 7.2 Hz, CH-CH_3), 2.0-2.17 (1H, m, CH-CH_2-CH_3), 4.29 (1H, br s, CH-Ph), 6.29 (1H, q, J = 7.2 Hz, CH-CH_3), 6.61 (1H, dd, $J_{1,2}$ = 6.6 Hz, $J_{1,3}$ = 1.2 Hz, Br-C=CH), 7.13-7.16 (2H, m, ArH), 7.30-7.40 (8H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 10.7, 15.9, 25.7, 47.6, 52.2, 56.7, 118.6, 125.9, 127.1, 127.3, 127.9, 128.2, 128.4, 140.0, 140.5, 142.1, 159.0; LC-ESI-MS rt 15.1 min., m/z 384-386 (M+1), 406-408 (M+Na).

(1S', 3*R*,4*S*)-6e : Isolated as a sticky yellow oil; $[\alpha]_D$ = -12 (c 0.4 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.49 (3H, d, J = 7.2 Hz, CH-CH_3), 4.48 (1H, s, HC-Ph), 5.06 (1H, q, J = 7.2 Hz, CH-CH_3), 5.25 (1H, d, J = 10.6 Hz, Br-C-CH=CH_2), 5.51 (1H, d, J = 17.2 Hz, Br-C-CH=CH_2), 6.08 (1H, dd, J = 17.2, 10.6 Hz, Br-C-CH=CH_2), 7.26-7.42 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 18.9, 53.2, 66.9, 68.8, 118.2, 127.3, 127.4, 128.1, 128.3, 128.7, 129.1, 134.0, 135.6, 139.2, 165.6; LC-ESI-MS rt 12.5 min., m/z 356/358 (M+1), 378/380 (M+Na).

(1S', 3*S*,4*R*)-7e : Isolated as a sticky white oil; $[\alpha]_D$ = -54 (c 0.5 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.34 (3H, d, J = 7.0 Hz, CH-CH_3), 4.33 (1H, q, J = 7.0 Hz, CH-CH_3), 4.54 (1H, s, HC-Ph),

5.34 (1H, d, *J* = 10.4 Hz, Br-C-CH=*CH*$_2$), 5.64 (1H, d, *J* = 17.0 Hz, Br-C-CH=*CH*$_2$), 6.16 (1H, dd, *J* = 17.0, 10.4 Hz, Br-C*CH*=CH$_2$), 7.13-7.48 (10H, m, ArH); ^{13}C NMR (CDCl3) δ 17.5, 55.4, 66.2, 68.8, 118.4, 126.0, 126.6, 127.5, 127.8, 128.5, 128.9, 134.2, 135.6, 140.1, 165.3; LC-ESI-MS rt 12.6 min., m/z 356/358 (M+1), 378/380 (M+Na).

8e: Isolated as a pale yellow oil; ^{1}H NMR (CDCl$_3$) δ 1.24 (3H, d, *J* = 7.2 Hz, CH-*CH*$_3$), 2.31 (1H, ddd, *J* = 1.5, 7.2, 17.5 Hz, *CH*$_2$CH-Ph), 2.75 (1H, ddd, *J* = 2.4, 7.2, 17.5 Hz, *CH*$_2$CH-Ph), 4.47 (1H, bd, *J* = 7.2 Hz, *CH*-Ph), 6.21 (1H, q, *J* = 7.2 Hz, *CH*-CH$_3$), 6.59 (1H, dd, *J* = 2.4, 7.2 Hz, Br-C=*CH*), 7.18-7.29 (2H, m, ArH), 7.33-7.42 (8H, m, ArH); ^{13}C NMR (CDCl3) δ 16.2, 35.1, 52.5, 54.2, 119.1, 126.6, 127.5, 127.6, 127.8, 128.2, 128.5, 136.1, 141.2, 141.5, 159.8.; LC-ESI-MS rt 13.3 min., m/z 356/358 (M+1), 378/380 (M+Na).

Full characterisations of compounds **9a-e** and **10-13** can be found in:
Cardillo et al, *Tetrahedron* **2004**, *60*, 5031.

14d: Isolated as a pale yellow oil; ^{1}H NMR (CDCl$_3$) δ 0.80 (3H, t, *J* = 7.4 Hz, CH$_2$*CH*$_3$), 1.26 (3H, t, *J* = 7.2 Hz, OCH$_2$*CH*$_3$), 1.86-2.00 (2H, m, *CH*$_2$CH$_3$), 3.00 (2H, m, NCH$_2$*CH*$_2$), 3.49 (2H, m, N*CH*$_2$CH$_2$), 4.14 (2H, q, *J* = 7.2 Hz, O*CH*$_2$CH$_3$), 5.36 (1H, dt, *J*$_{1,2}$ = 15.4 Hz, *J*$_{1,3}$ = 1.4 Hz, Br-C*CH*=CH), 6.11 (1H, dt, *J* = 6.6, 15.4 Hz, Br-C-CH=*CH*), 7.2-7.5 (10H, m, ArH); ^{13}C NMR (CDCl3) δ 13.4, 19.3, 32.1, 39.2, 60.7, 77.1, 79.1, 118.2, 127.8, 127.9, 128.1, 128.3, 128.9, 129.4, 129.9, 135.9, 138.0, 140.0, 166.7, 170.7; LC-ESI-MS rt 10.8 min., m/z 442-444 (M+1), 464-466 (M+Na).

14f: Isolated as a pale yellow oil; ^{1}H NMR (CDCl$_3$) δ 0.79 (3H, t, *J* = 7.6 Hz, CH$_2$*CH*$_3$), 1.26 (3H, t, *J* = 7.0 Hz, OCH$_2$*CH*$_3$), 1.85-2.00 (2H, m, *CH*$_2$CH$_3$), 3.86 (1H, d, *J* = 17.6 Hz, N*CH*$_2$), 4.06 (1H, *J* = 17.6 Hz, N*CH*$_2$), 4.24 (2H, q, *J* = 7.0 Hz, O*CH*$_2$CH$_3$), 5.40 (1H, d, *J*$_{1,2}$ = 15.4 Hz, Br-C*CH*=CH), 6.10 (1H, dt, *J* = 6.6, 15.4 Hz, Br-C-CH=*CH*), 7.2-7.5 (10H, m, ArH); ^{13}C NMR (CDCl3) δ 12.4, 13.9, 25.1, 44.2, 61.7, 74.9, 78.5, 125.8, 127.6, 127.8, 128.2, 128.3, 128.7, 129.2, 136.3, 136.8, 138.0, 167.0 (2C); LC-ESI-MS rt 11.0 min., m/z 428-430 (M+1), 450-452 (M+Na).

15d: Isolated as a pale yellow oil; ^{1}H NMR (CDCl$_3$) δ 1.27 (3H, t, *J* = 7.4 Hz, CH$_2$*CH*$_3$), 3.02 (2H, m, NCH$_2$*CH*$_2$), 3.51 (2H, m, N*CH*$_2$CH$_2$), 4.16 (2H, q, *J* = 7.0 Hz, O*CH*$_2$CH$_3$), 5.12 (1H, dd, *J*$_{1,2}$ = 9.4 Hz, *J*$_{1,3}$ = 2.0 Hz, Br-C-CH=*CH*$_2$), 5.58-5.84 (2H, m, Br-C-CH=*CH*$_2$, Br-C-*CH*=CH$_2$); 7.2-7.5 (10H, m, ArH); ^{13}C NMR (CDCl3) δ 14.1, 32.4, 39.7, 60.8, 74.2, 77.2,

118.7, 127.7, 128.1, 128.3, 128.4, 128.8, 129.1, 134.2, 136.3, 137.9, 166.7, 170.7; LC-ESI-MS rt 9.7 min., m/z 428-430 (M+1), 450-452 (M+Na).

15f: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 3.86 (1H, d, *J* = 17.6 Hz, NCH_2), 4.14 (1H, *J* = 17.6 Hz, NCH_2), 4.26 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 5.15 (1H, dd, $J_{1,2}$ = 10.0 Hz, $J_{1,3}$ = 0.8 Hz, Br-C-CH=CH_2), 5.62-5.90 (2H, m, Br-C-CH=CH_2, Br-CCH=CH_2); 7.2-7.5 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 13.9, 44.3, 61.8, 74.5, 78.2, 118.9, 127.8, 128.1, 128.4, 128.5, 128.8, 128.9, 134.1, 136.1, 138.0, 166.4, 167.0; LC-ESI-MS rt 9.5 min., m/z 414-416 (M+1), 436-438 (M+Na).

16d: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.52 (3H, s, CCH_3), 2.80-3.14 (2H, m, NCH_2CH_2), 3.34-3.52 (2H, m, NCH_2CH_2), 4.13 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 5.09 (1H, s, C=CH_2), 5.92 (1H, m, C=CH_2); 7.2-7.5 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 14.0, 25.2, 32.4, 39.6, 60.8, 74.8, 77.5, 125.8, 127.6, 127.9, 128.2, 128.3, 128.7, 129.5, 136.5, 138.7, 138.0, 167.1, 170.8; LC-ESI-MS rt 11.7 min., m/z 442-444 (M+1), 464-466 (M+Na).

16f: Isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.52 (3H, s, CCH_3), 3.86 (1H, d, *J* = 17.6 Hz, NCH_2), 4.14 (1H, *J* = 17.6 Hz, NCH_2), 4.26 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 5.09 (1H, s, C=CH_2), 5.92 (1H, m, C=CH_2); 7.2-7.5 (10H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 13.9, 19.3, 44.0, 61.7, 77.9, 79.3, 118.5, 127.7, 127.9, 128.0, 128.3, 128.9, 129.6, 135.7, 138.0, 139.7, 166.5, 166.7; LC-ESI-MS rt 11.5 min., m/z 428-430 (M+1), 450-452 (M+Na).

2 Synthesis of unprecedented classes of functionalised β-lactams and their biological evaluation as acyl-CoA: cholesterol acyltransferase inhibitors[1]

2.1 Introduction

High serum cholesterol levels have been associated with cardiovascular disease (CD), a leading cause of death and disability in the Western world. Cholesterol metabolism has also been implicated in the development of Alzheimer's disease (AD), a neurogenerative condition that affects 5 million individuals. Cholesterol levels are affected by the rate of endogenous cholesterol synthesis, biliary cholesterol excretion, and dietary cholesterol absorption. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (statins) are currently the mainstay of hypercholesterolemia treatment and are the most widely used agents to lower LDL (Low Density Lipoprotein) cholesterol levels, demonstrating significant clinical outcomes. However, alternate and/or additional treatment strategies to further reduce LDL cholesterol and coronary artery disease risk are also being studied. Therefore, much effort is still being directed to finding agents with different hypocholesterolemic mechanism. Acyl-coenzyme A:cholesterol transferase (ACAT; EC 2.3.1.26)[91] is responsible of the intracellular esterification of cholesterol with fatty acids. This reaction takes place in the endoplasmic reticulum of a variety of cells and tissues, to facilitate both intracellular storage and intercellular transport of the otherwise toxic free cholesterol (a polar lipid). Therefore, ACAT inhibitors are being investigated as potent therapeutic agents for the treatment of hypercholesterolemia and atherosclerosis[92-94], since several studies have demonstrated that ACAT inhibitors limit cholesterol absorption in animal models. In recent years, a number of ACAT inhibitors have been reported[95-105]; unfortunately, in contrast to promising results in

[1] Result published in *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 1946-1950.
European Journal of Organic Chemistry **2007**, *2007*, 3199-3205.
Journal of Organic Chemistry **2006**, *71*, 9229-9232.

experimental animal models, all subsequent clinical studies in humans with ACAT inhibitors failed[106], mainly because of cell toxicity or low bioavailability.

Nevertheless, the discovery program developed by the Schering-Plough company that led to the discovery of Ezetimibe[107-109], the most famous non-statinic cholesterol-lowering drug, began as a program to discover novel ACAT inhibitors. Thus, ACAT assay may be employed on selected molecules to obtain preliminary indications on their inhibition of cholesterol absorption. In this chapter, the synthesis of different classes of β-lactam-based structures and their biological evaluation as ACAT inhibitors is reported. In the last decade, several molecules sharing a β-lactam *core* have been reported to be potent CAI (Cholesterol Absorption Inhibitors), the most important of them being Ezetimibe itself. SAR (Structure Activity Relationships) studies have established the β-lactam scaffold to be essential for the inhibitory activity. Actually, the β-lactam ring has a very peculiar geometry, since it is a rigid, almost planar heterocycle that defines out of plane vectors from the central core (**Figure 2.1**)[110].

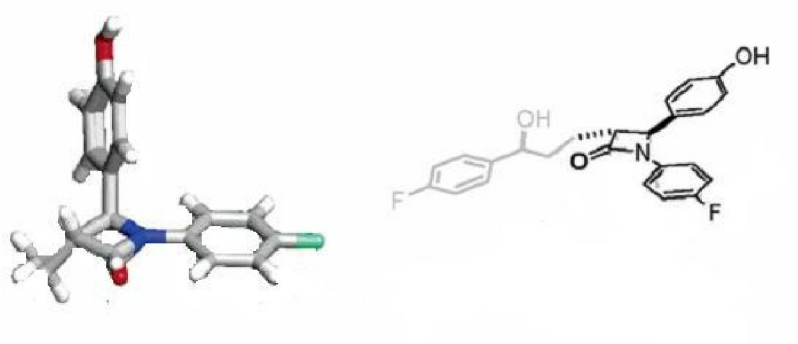
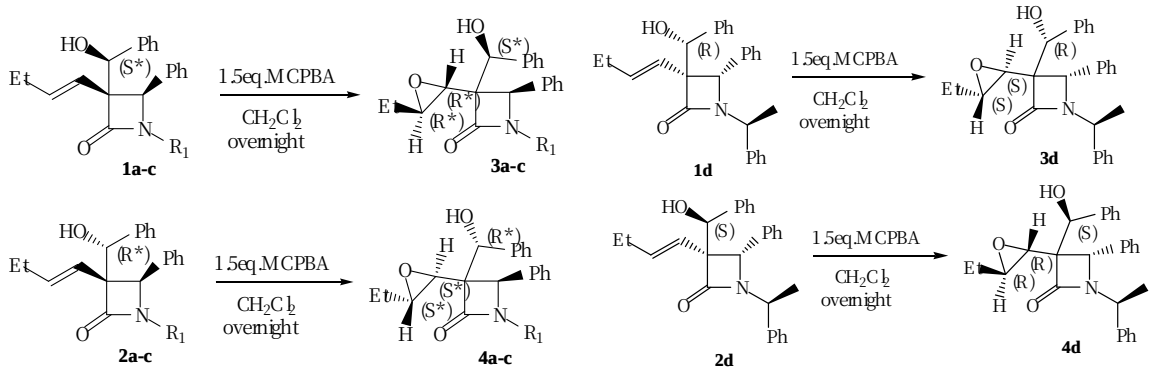


Figure 2.1.

2.2 Synthesis of highly functionalised β-lactams via intramolecular epoxide ring opening

Different classes of highly functionalised molecules can be obtained through epoxide ring opening[111] of compounds **3a-d** and **4a-d**, easily synthesised by MCPBA epoxidation of alkenyl azetidinones **1a-d** and **2a-d**[65] **(Scheme2.1, Table 2.1).** It is well known that the epoxidation with *m*-chloroperbenzoic acid of allylic and homoallylic alcohols occurs with high stereoselectivity[112-120], because of the complexation of the unsaturated alcohol with the peracid. Thus, the epoxidation of these compounds proceeded with excellent diastereoselectivities; unfortunately, the two isomers of epoxides **3a-d** and **4a-d** could not be separated by flash chromatography, but only by means of preparative HPLC.

Trans configuration can be assigned to epoxides **3** and **4** on the basis of the *j* coupling constants (j = 1.8÷2.4 Hz) in the ^{1}H-NMR spectra.



Scheme 2.1 Epoxidation of alkenyl azetidinones **1a-d** and **2a-d** with MCPBA. Major isomers of the products **3a-d** and **4a-d** are represented.

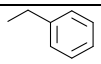
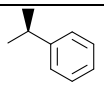
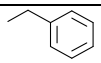
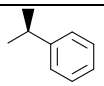
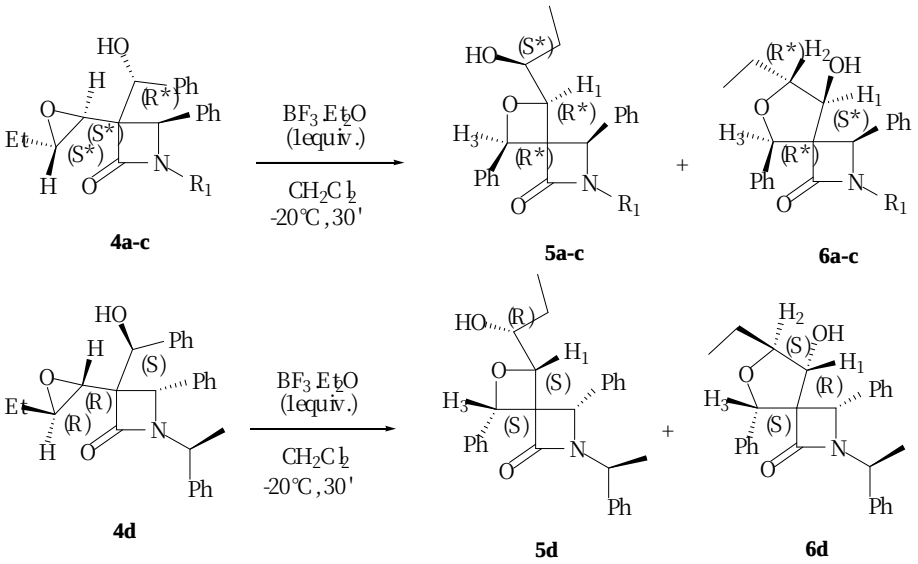
Entry	Epoxide	R_1	Yield (%)	d.r.
1	**3a**		84	84:16
2	**3b**		67	82:18
3	**3c**		78	90:10
4	**3d**	-	70	86:14
5	**4a**		69	84:16
6	**4b**		68	83:17
7	**4c**		69	86:14
8	**4d**	-	65	85:15

Table 2.1 Epoxidation of alkenyl azetidinones **1a-d** and **2a-d** with MCPBA giving compounds **3a-d** and **4a-d**.

The intramolecular Lewis acid-catalysed ring opening of these epoxides[121,122] represents a straightforward strategy to obtain new classes of interesting spiro β-lactams derivatives. In particular, depending on the regioselectivity of the ring opening, oxetane and tetrahydrofuran derivatives can be synthesised. Similar spiro-tetrahydrofuran derivatives have been generally prepared *via* ketene-imine cycloaddition starting from proper tetrahydrofuran-based acyl chloride, though a recent paper by Alcaide and co-workers[123] describes an alternative strategy founded on an intramolecular metal-catalysed cyclisation of unsaturated alcohols. Instead, to

the best of our knowledge, the synthesis of spiro-oxetane derivatives has never been reported previously. The reactions of epoxide ring opening were carried out on the mixture of epoxides' diastereoisomers with 1 equivalent of boron trifluoride diethyletherate complex, in different conditions; all the products arising from the ring opening of the major and the minor isomer of epoxide were easily separated by flash chromatography. The results are summarised in **Scheme 2.2** and **Table 2.2** for compounds **4a-d** and in **Scheme 2.3** and **Table 2.3** for compounds **3a-d.** Clearly, the ring opening of compounds **4a-d** is highly regioselective, and the oxetanes **5a-d** can be obtained as major products, although the yields are not excellent, because of the formation of a polimerised byproduct. The reaction is fast (after 30 minutes the starting material is consumed) and -20°C is the optimised temperature for achieving the best results in terms of yields and regioselectivities. As a matter of fact, the reaction is very slow at lower temperatures, and no reaction can be observed below -40°C; on the other hand, a more complex crude reaction mixture results increasing the temperature.



Scheme 2.2 Lewis acid induced intramolecular ring opening of epoxides **4a-d** .

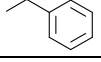
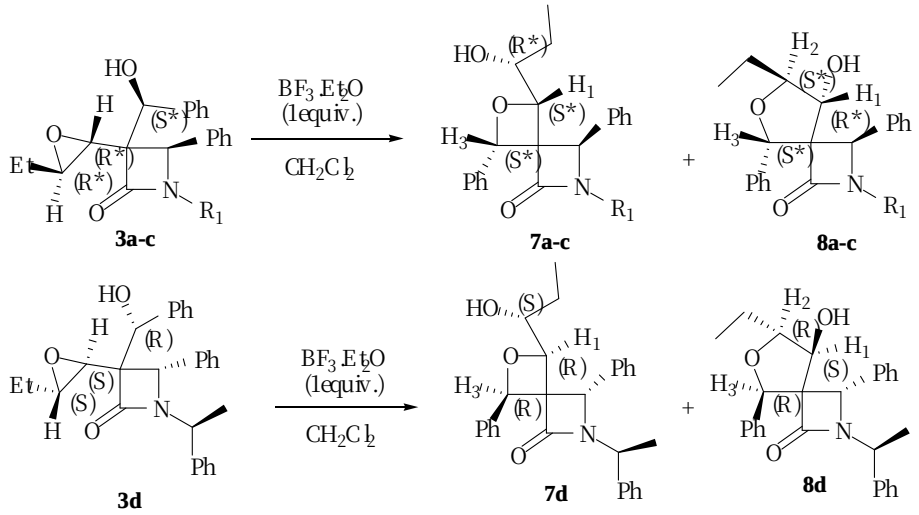
Entry	Epoxide	R_1	Yield (%)	5:6 ratio
1	4a		65	86:14
2	4b		60	72:28
3	4c	 COOEt	63	>99:1
4	4d	-	57	80:20

Table 2.2 Formation of spiro β-lactams **5a-d** and **6a-d** *via* Lewis acid-catalysed intramolecular epoxide ring opening.

Instead, the intramolecular ring opening of compounds **3a-d** occurred generally with poor regioselectivity, but it proved to be more sensitive to temperature variations. Actually, the tetrahydrofuran derivatives **8a-d** could be obtained as major products while performing the reaction in refluxing DCM, whereas the oxetane derivatives **7a-d** are the prevalent products while working at -20°C. To carry out the reactions at lower or higher temperature does not improve the results, since below -20°C the reaction does not occur and over +40°C polymerisation occurred.



Scheme 2.3 Lewis acid induced intramolecular ring opening of epoxides **3a-d**.

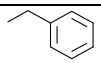
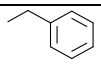
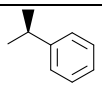
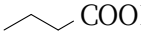
Entry	Epoxide	R_1	Temp. (°C)	Yield (%)	7:8 ratio
1	**3a**		-20	65	50:50
2	**3a**		+40	60	27:73
3	**3b**		-20	45	40:60
4	**3b**		+40	63	20:80
5	**3c**		-20	63	63:37
6	**3d**	-	-20	48	50:50
7	**3d**	-	+40	60	34:66

Table 2.3 Formation of spiro β-lactams **7a-d** and **8a-d** *via* Lewis acid-catalysed intramolecular epoxide ring opening.

A distinctive property of these classes of spiro β-lactams is the lactamic carbonyl's IR wavenumber: for the four membered compounds ν=1719÷1724 cm^{-1}, while for the five membered compounds ν=1743÷1750 cm^{-1}.

In order to provide a rationalisation for the different regioselectivities observed in the epoxide ring opening for compounds **3a-d** and **4a-d**, DFT calculations on the BF_3-complexes of the epoxides **3a** and **4a** have been performed. On the basis of the principle of the "collinearity requirement" proposed by Stork and co-workers, in the epoxide ring opening the C-O bond of the nucleophilic hydroxyl group and the epoxide's C-O bond that is going to be broken must be collinear. The minimised structure of BF_3 **3a** complex shows that the hydroxyl group can easily approach intramolecularly both carbon atoms of the epoxide; this evidence accounts for the low regioselectivity and for dependence of the crude composition on the temperature. On the contrary, the minimised structure of BF_3 **4a** complex shows that the *exo* approach leading to oxetane[124] **5** is more favoured than the *endo* approach, since in the former the collinearity can be achieved without geometry distortion (**Figure 2.2**).

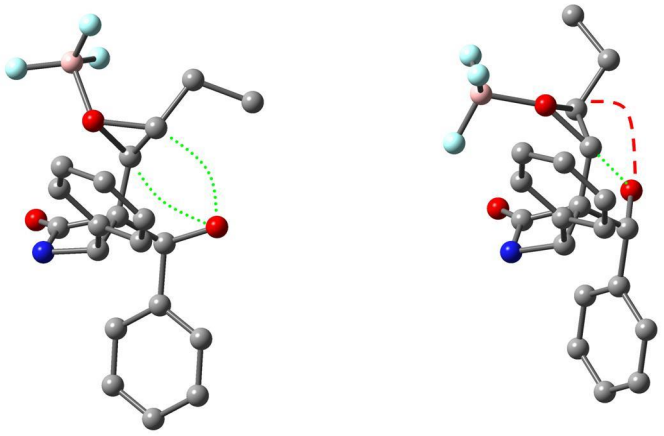


Figure 2.2.

Ring size determination has been made on the basis of ^{1}H-NMR analyses; the first evidence comes from the coupling constants: the ones for compounds **6a-d** and **8a-d** are diagnostic for *trans* tetrahydrofuran derivatives ($J_{H4'H5'}$=3.3÷5.7 Hz),[125-127] whereas the ones for compounds **5a-d** and **7a-d** are typical of open chain's couplings ($J_{H4'H5'}$=9.3÷11.4 Hz).

The relative configurations of the stereocenters in **8a** were assigned by NOESY-1D-DPFGSE. Irradiation of the $H^{4'}$ proton resulted in the major enhancement of $H^{2'}$, suggesting a *cis* relationship between the two protons, while a minor enhancement with the vicinal $H^{5'}$ was observed; no enhancement of the $H^{5'}$ signal could be observed upon irradiation of $H^{2'}$, whereas H^4 showed in this case an enhancement due to the peculiar geometry of this spiro compound (**Figure 2.3**). From these observations, the relative (4R*,2'S*,4R*,5'S*)

configuration, as shown in **Scheme 2.3**, could be attributed to the tetrahydrofuran derivative **8a**. The tetrahydrofuran ring had resulted from the intramolecular epoxide's ring opening, which had occurred with inversion of configuration, so the (1'R*,2'R*) relative configuration can be assigned to the stereocenters of the starting epoxide **3a** (See **Scheme 2.3**).

A NOESY-1D analysis performed on **5a** showed a strong enhancement of the $H^{2'}$ signal when $H^{4'}$ was irradiated and a medium nOe effect on H^{4}, thus suggesting a *cis* relationship between the two hydrogen atoms $H^{2'}$ and $H^{4'}$ (**Figure 2.4**).

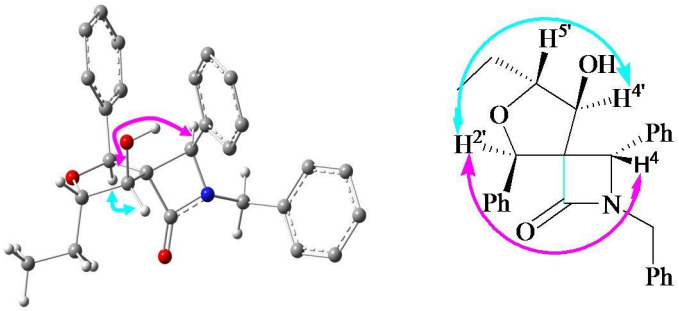


Figure 2.3.

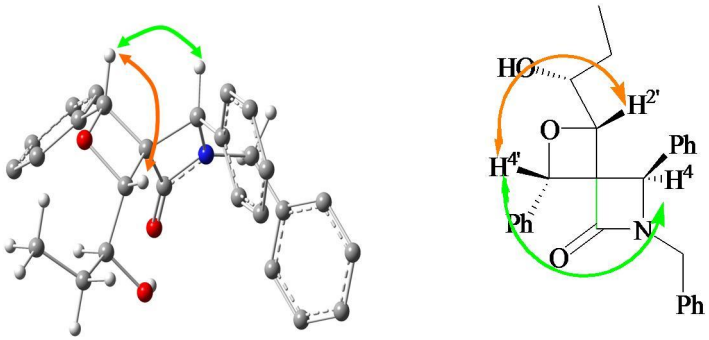


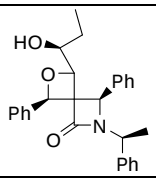
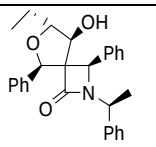
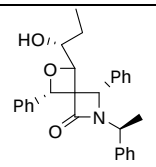
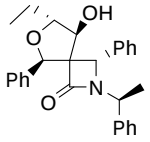
Figure 2.4.

These observations allowed the (4R*,2'R*,3'S*,4'R*) relative configuration to be attributed to the spiro compound **5a**, corresponding to a (1'S*,2'S*) configuration of the three-membered ring's stereocenters in the epoxide **4a** (See **Scheme 2.2**).

Finally, the complete chemical shifts' regularity observed in each class of compounds allow to attribute the relative or absolute configuration to every member of these classes.

2.3 Biological evaluation of spiro β-lactams as ACAT inhibitors

The enantiomerically pure spiro derivatives **5a**, **6a**, **5d**, and **8d** were tested as ACAT inhibitors, using Lovastatin as reference standard (IC_{50} = 12μM from the literature data, IC_{50} = 16.8 μM when concurrently tested). The results obtained from the enzymatic assays[2], performed following esterification of [^{14}C]-cholesterol with palmitoyl-CoA in the presence of the spiro-lactam (10μm), are reported in **Table 2.4**.

Entry	Compound	Structure	% Inhibition[a,b] (10μM)
1	5b		45
2	6b		23
3	5d		66
4	8d		27

[a] Measured by quantitation of [^{14}C]cholesterol esters by column chromatography.
[b] Lovastatin as reference standard (IC_{50} = 12μM from the literature data, IC_{50} = 16.8μM when concurrently tested).

Table 2.4 ACAT inhibition assays for spiroβ-lactams.

The biological evaluation displayed modest results (Entries 1–4), however interesting information may be inferred. Unfortunately, at this point an accurate SAR (Structure Activity Relationship) study can not be carried out because ACAT active site's structure is still missing. Nevertheless, some evidences on the catalytical function of ACAT may explain these data; it is well known that the ACAT active site is rich in His residues, that are frequently employed in general acid-base catalysis and in reaction intermediate stabilisation. It has been recently proposed that these His residues might function as general bases, interacting with the 3β-hydroxyl group of cholesterol, thus promoting the catalytic activity. On the basis of these

[2] Inhibition tests were performed by MDS Pharma Services on acyl CoA-cholesterol acyltransferase from New Zealand derived albino rabbit intestinal mucosa, using [^{14}C]palmitoyl CoA (18μM) as a substrate in 1% DMSO-0.2M potassium phosphate buffer (pH 7.4) and 1.5mg/mL bovine serum albumin at 25°C.

considerations, lipophilic compounds having free, well-exposed hydroxyl groups, available for interacting with the enzyme, are expected to be good inhibitors.

The comparison of the biological activities of oxetane spiro-derivatives **5b** and **5d** (Entries 1 and 3) with the corresponding tetrahydrofurane derivatives **6b** and **8d** (Entries 2 and 4) showed that four-membered rings are more active than five-membered ones.

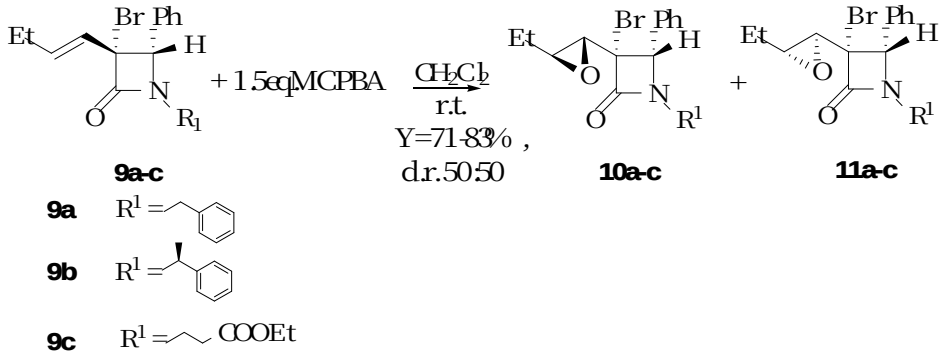
This fact can be ascribed to the greater accessibility of oxetane' hydroxyl group in respect to the tetrahydrofurane' one, which lies into the spirocyclic rigid structure. Furthermore, the comparison between the inhibitory activities of (4*S*)- and (4*R*)-spiro derivatives showed that the β-lactam ring's stereochemistry on C4 actually affects the bioactivity. In fact, while the compound (4*S*) **8d** (Entry 4, 27%) is slightly a better inhibitor than (4*R*) **6b** (Entry 2, 23%), the oxetane (4*S*) **5d** (Entry 3, 66%) is significantly more active against ACAT than (4*R*) **5b** (Entry 1, 45%).

2.4 Synthesis of azido- and aziridino-hydroxyl-β-lactams through stereo and regioselective epoxide ring opening

The development of new strategies for the introduction of amino and hydroxyl functionalities in the side chains of β-lactams is undoubtedly a crucial challenge, since it is well-documented that these substituents may enhance the bioactivity.

The epoxidation of the double bond of 3-bromo 3-alkenyl azetidinones and the subsequent epoxide ring opening with azides[128-130] represent a straightforward strategy for the introduction of C-O and C-N bonds in azetidinones' C-3 side chain. The reactions herein reported are characterized by complete stereocontrol in all steps and high overall yield; furthermore, only a few examples of C-3 side chain amino and azido function-containing azetidinones have been reported in the literature to date. The azido group can be easily elaborated to afford amines, aziridines, or cyclic nitrogen-containing compounds. Although the azido function is absent in most species' metabolism, it is quite stable in a biological environment, and lacking in toxicity, it has been introduced in a variety of drugs, the most important one being the well-known azidothymidine, AZT.

The treatment of substrates **9a**-**e**[65] with *meta* chloroperbenzoic acid (MCPBA), performed under concentrated conditions (2 M in DCM), afforded the corresponding epoxides in a 1:1 mixture and in good yields[75] (**Scheme 2.4**).



Scheme 2.4 Epoxidation of alkenyl azetidinones **9a-c** with MCPBA leading to compounds **10a-c** and **11a-c**.

Epoxides **10a c** and **11a c** were purified by flash chromatography. The first epoxide to be eluted (**10a**) was a solid that crystallise from chloroform, and its structure with the relative epoxide configuration was established by single-crystal X-ray analysis (**Figure 2.5**), thus allowing the (1*R**, 2*S**) configuration to be attributed to the newly introduced stereocenters. As a consequence, the (1*S**, 2*R**) configuration has been attributed to the isomer **11a**.

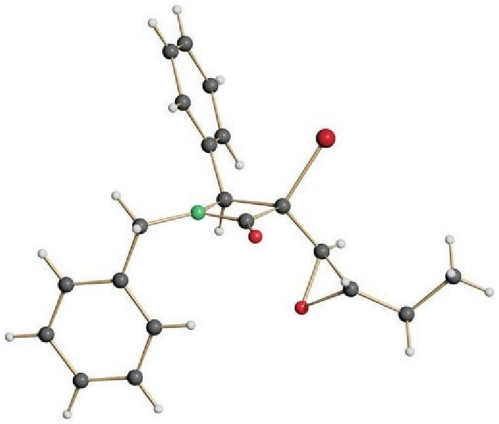


Figure 2.5.

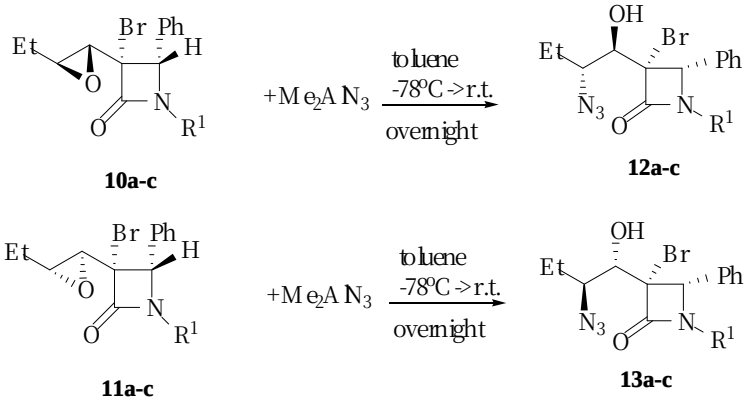
NOE analysis performed on compound **10a** is in agreement with the crystallographic structure; in fact, the irradiation of the hydrogen on the lactam ring's C4 enhances the signal relative to C1'-H. The same experiment performed on the isomer **11a** induces an enhancement on epoxide's C2'-H. Finally, the regularities observed in the ^{1}H NMR spectra of the pairs of compounds **10** and **11** allow to confidently attribute the relative configuration to **10b-11b** and the absolute configuration to **10c-11c** (**Table 2.5**).

Entry	Epoxide	δ H$^{1'}$ (ppm)	δ H$^{2'}$ (ppm)	$J_{H1'-H2'}$ (Hz)[b]
1	**10a**	3.27	3.57	2.2
2	**10b**	3.19	3.48	2.0
3	**10c**	3.26	3.49	2.1
4	**11a**	3.38	2.82	2.1
5	**11b**	3.37	2.81	2.3
6	**11c**	3.41	2.95	2.4

[a] Spectra recorded in $CDCl_3$ solution at 25 °C.
[b] The coupling constants account for a trans relationship.

Table 2.5 ^{1}H NMR data[a] for compounds **10a-c** and **11a-c.**

The regioselective ring opening of oxiranes provides a convenient way to prepare polyfunctionalized compounds. Successful ring openings of epoxy-alcohols with diethyl aluminium azide have been reported by Benedetti *et al*.[131], as a way to obtain an α-amino β-hydroxy sequence, which is an important structural feature in many classes of bioactive compounds. Therefore, the treatment of epoxides **10a-c** and **11a-c** with Me_2AlN_3, prepared in situ from sodium azide and Me_2AlCl, gave smoothly the azides **12a-c** or **13a-c** respectively in good yields and with complete stereo- and regioselectivity (**Scheme 2.5 Table 2.7**).



10a-c **12a-c**

11a-c **13a-c**

Scheme 2.5 Epoxide ring opening of **10a-c** and **11a-c** with Me_2AlN_3.

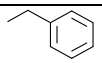
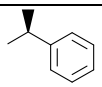
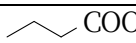
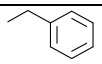
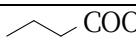
Entry	Compound	R_1	Yield (%)
1	12a		>95
2	12b		>95
3	12c		>95
4	13a		>95
5	13b		>95
6	13c		>95

Table 2.6

The ring opening of the epoxides occurred only on the C-2' position with inversion of the configuration; this selectivity could be explained suggesting the dissociation of Me_2AlN_3 and the subsequent formation of a cationic tetrahedral complex in which the metal coordinates both the epoxide's and the carbonyl group's oxigens. Concerning the stereochemistry, the ring opening of the epoxide with anionic azido group should occur with inversion of the configuration, as reported in the literature. Concerning the regiochemistry, the ring opening of

the epoxide does not seem to be ruled by charge effects[3]; it occurs on the C2' position exclusively since the collinear approach of azido group to C1' is forbidden by the ring's substituent on C4.

This is clearly shown by the geometry-optimized structures calculated *ab initio* (DFT/B3LYP/6.31G* minimization) for both the intermediate aluminium complexes of **10a-c** and **11a-c** (**Figure 2.6**).

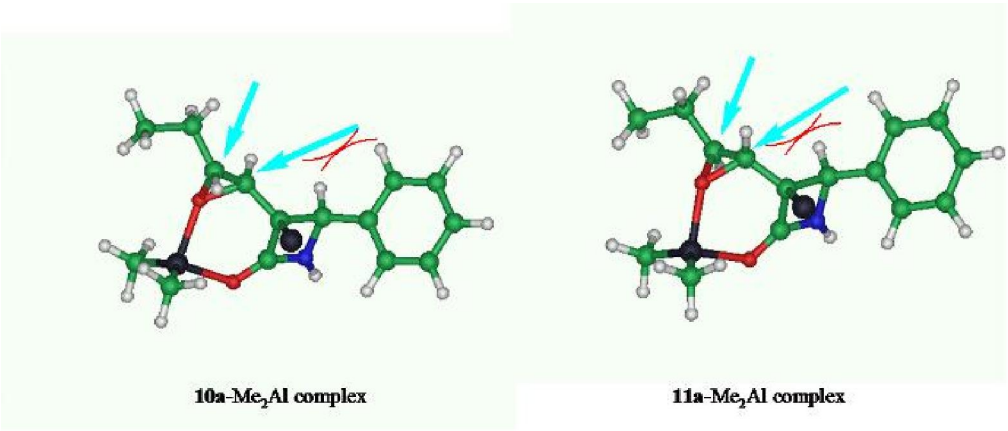
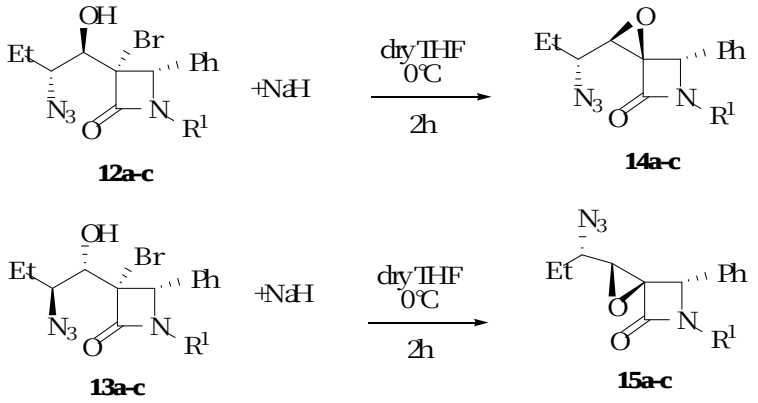


Figure 2.6

The subsequent treatment of halohydrins **12a-c** and **13a-c** with NaH in dry THF at 0°C gave cleanly unprecedented epoxide-spiro β-lactams **14a-c** and **15a-c** in almost quantitative yields (**Scheme 2.6** and **Table 2.7**). The bromide displacement took place *via* SN_2 mechanism, therefore inversion of configuration occurred on C3.



Scheme 2.6 Bromine displacement affording epoxide-spiro β-lactams **14a-c** and **15a-c**.

The conformationally rigid molecules **14a-c** and **15a-c** revealed symptomatic regularities in their ^{1}H-NMR spectra; as a matter of fact, in the spectra of the compounds **14a-c**, $H^{1'}$ was a doublet shielded by carbonyl group, while in the ones of the compounds **15a-c**, $H^{2'}$ was the more shielded (**Figure 2.7** and **Table 2.8**). It is noteworthy that the whole synthetic pathway from epoxides **10** and **11** to azides **12** and **13** and then to epoxide-spiro β-lactams **14** and **15**

[3] Computed atomic charges on C1' and C2' does not differ significantly.

does not require chromatographic purification, since the products can be obtained in high yield and without byproducts.

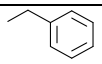
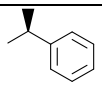
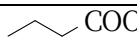
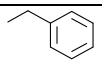
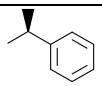
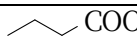
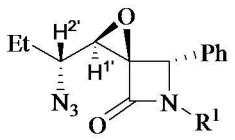
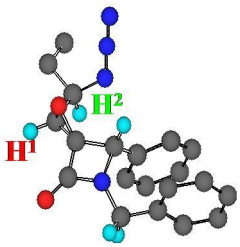
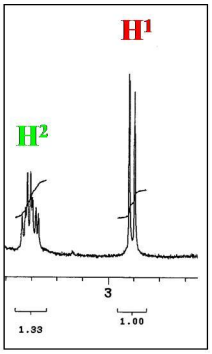
Entry	Compound	R_1	Yield (%)
1	14a		>95
2	14b		>95
3	14c		>95
4	15a		>95
5	15b		>95
6	15c		>95

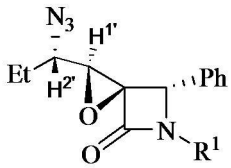
Table 2.7



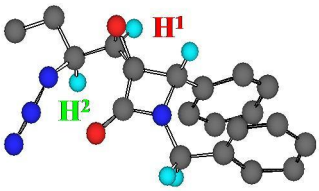
14a-c







15a-c



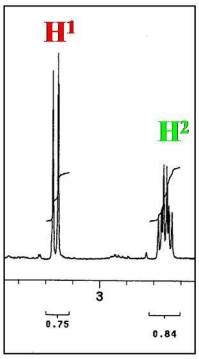


Figure 2.7.

Entry	Compound	δ $H^{1'}$(ppm)[a]	δ $H^{2'}$(ppm)
1	14a	2.81	3.61
2	14b	2.78	3.61
3	14c	2.78	3.55
4	15a	3.88	2.52
5	15b	3.32	2.40
6	15c	3.29	2.50

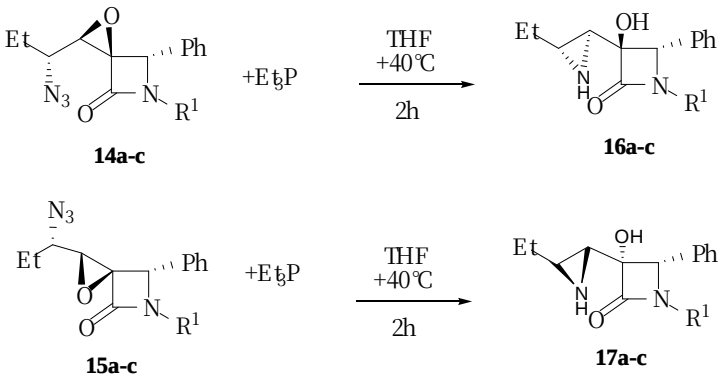
[a] Spectra recorded in $CDCl_3$ solution at 25 °C.

Table 2.8 Comparison of ^{1}H-NMR chemical shifts of $H^{1'}$ and $H^{2'}$ hydrogens in compounds **14a-c** and **15a-c**

Extensive experimentation has been carried out in order to reduce the azido group in compounds **12-13** and **14-15** avoiding the reaction of the other sensitive functions in the molecules (epoxide and bromo substituent). However, any attempt to reduce the azide **12a** with $NaBH_4$ in refluxing THF-methanol failed. Under these conditions, epoxide-spiro β-lactam **14a** was obtained in moderate yield, probably *via* deprotonation of the alcohol moiety and its consequent SN_2 reaction on the vicinal bromide.

The treatment of **12a** with BH_2Cl-SMe_2 as well as hydrogenation on Pd/C or on poisoned catalyst ($Pd/CaCO_3$) gave complex mixtures of products.

Excellent results were achieved by Staudinger reduction[132] of azides **14a-c** and **15a-c** with Ph_3P or Et_3P instead; the reaction were fast, highly reproducible and occurred in mild conditions. Aziridines **16a-c** and **17a-c** were obtained with yields ranging from 50-78%, *via* an aza-Payne[133-136]-like ring opening of the epoxide (**Scheme 2.7** and **Table 2.9**).



Scheme 2.7 Staudinger reduction of azides **14a-c** and **15a-c** followed by aza-Payne-like rearrangement.

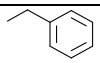
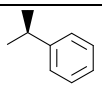
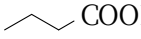
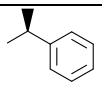
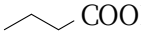
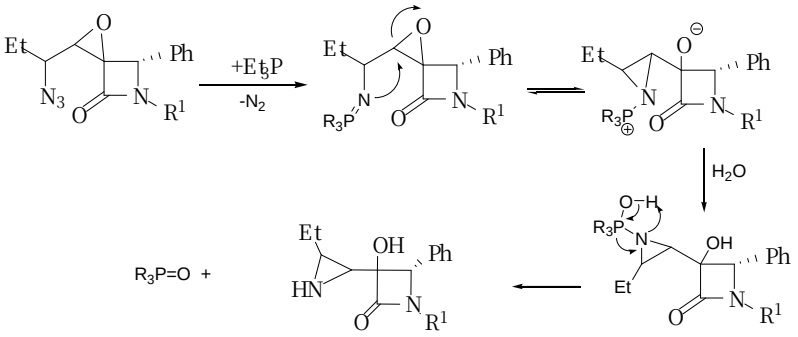
Entry	Compound	R_1	Yield (%)
1	16a		59
2	16b		62
3	16c		78
4	17a		51
5	17b		50
6	17c		57

Table 2.9

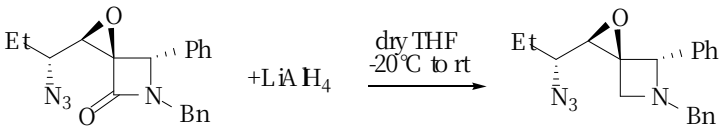
The ^{1}H NMR coupling constants of the aziridine[137] protons ($J_{2\text{-}3}$=2.0÷3.0 Hz) account for a *trans* relationship, thus confirming the stereochemistry previously attributed to the starting azides **14a c** and **15a c**. In the overall sequence from α-bromoepoxides to aziridines, the stereochemical configuration of both C-1' and C-2' carbon atoms has been inverted. The retention of the configuration at C-3, in the last step, is supported by mechanistic considerations. The proposed mechanism[138] for aza-Payne-like rearrangement is reported in **Scheme 2.8**.



Scheme 2.8 Proposed mechanism of aza-Payne-like rearrangement

The reaction proceeds through nucleophilic attack of the phospine on the azide, to form an aza-ylide intermediate. The nucleophilic nitrogen atom of the aza-ylide attacks the epoxide, inducing the ring opening. Hydrolysis of the adduct produces the aziridine-alcohol and releases the phosphine oxide.

Another interesting transformation performed on compound **14a** has been the chemoselective reduction of the β-lactamic carbonyl with $LiAlH_4$, that led to the azetidine **18**, without β-lactam ring opening and without affecting neither the epoxide nor the azido functionality (**Scheme 2.9**).



14a **18**

Scheme 2.9 Chemoselective reduction of azetidinone **14a** to azetidine **18.**

Azetidines have gained growing attention since it has been demonstrated that the azetidine ring, due to its peculiar geometry, could serve as an efficient replacement of the β-lactamic scaffold.

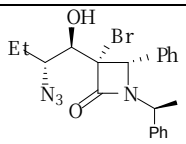
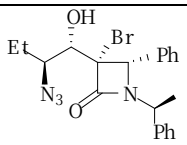
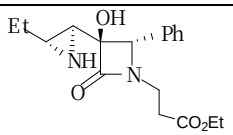
Hence, this reaction allows to obtain another promising class of rigid spirocyclic compounds, that might be employed as scaffolds.

2.5 Biological Evaluation of azido- and aziridino-hydroxyl-β-lactams

Representative examples of aziridines and their azido precursors were tested as acyl CoA-cholesterol acyltransferase (ACAT) inhibitors using Lovastatin as a reference standard, in the conditions described herein in Section 2.3. Assays' results are reported in **Table 2.10**.

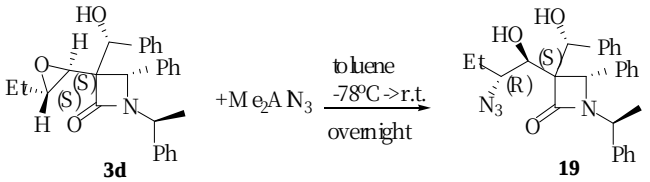
Once again, chiral compounds **12b** and **13b,** bearing more free hydroxyl groups, display a higher activity (Entry 1-2) than compound **16c** (Entry 3), which hydroxyl moiety is less accessible.

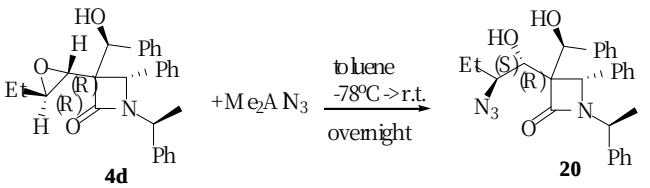
The percentages of ACAT inhibition of azido alcohols **12b** and **13b** are equivalent to the one of the oxetane spiro β-lactam **5d** (**Table 2.4**), that indeed has the same (4S) stereochemistry on the β-lactam ring.

Entry	Compound	Structure	% Inhibition[a,b] (10μM)
1	**12b**		**65**
2	**13b**		**60**
3	**16c**[c]		**22**

[a] Measured by quantitation of [^{14}C]Cholesterol esters by column chromatography.
[b] Lovastatin as reference standard (IC_{50} = 12μM from the literature data, IC_{50} = 16.8μM when concurrently tested).
[c] Racemic mixture of enantiomers.

Table 2.10 ACAT inhibition assays for azido and aziridino β-lactams.





Scheme 2.10 Epoxide ring opening of **3d** and **4d** with Me_2AlN_3.

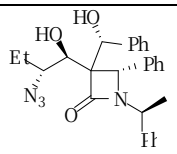
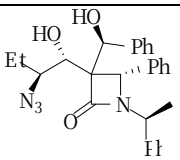
Entry	Compound	Structure	% Inhibition[a,b] (10μM)
1	19		79
2	20		87

Table 2.11 ACAT inhibition assays for azido diol β-lactams.

In order to check the reliability of SAR considerations on these classes of molecules, a pair of products having two hydroxyl groups and (4S) stereochemistry has been synthesised and tested afterwards. The ring opening of the enantiomerically pure epoxides **3d** and **4d** with Me_2AlN_3 led stereo- and regio-selectively to azido diols **19** and **20** in 65% and 67% yield respectively (**Scheme 2.10**).

As expected, both **19** and **20**, matching all the requirements deduced from the previous experimental observations, gave excellent results in the enzymatic assays (**Table 2.11**), displaying markedly higher percentages of ACAT inhibition, and therefore proving to be the best ACAT inhibitors among the compounds shown in this Chapter.

2.6 Experimental section

General : Unless stated otherwise, solvents and chemicals were obtained from commercial sources and were used without further purification. Flash chromatography was performed on silica gel (230–400 mesh). NMR spectra were recorded with 300 or 600 MHz spectrometers. Chemical shifts were reported as values (ppm) relative to the solvent peak of $CDCl_3$ set at =7.27 (^{1}HNMR) or 77.0 ppm (^{13}CNMR). Infrared spectra were recorded with an FT-IR spectrometer. Melting points are uncorrected. Microanalyses were performed with a FISONS EA 1108 CHNS-O Instrument.

MS analyses were performed o a liquid chromatograph coupled with an electrospray ionization-mass spectrometer (LC-ESI-MS), with H_2O/CH_3CN as solvent at 25 °C (positive scan, m/z 100–500, fragmentor 70 V). Preparative HPLC separations were performed on a Zorbax Eclipse XDB-C18 Prep HT column (21.2 150 mm, particle size 7 m, flow 12 mL/min) with water/acetonitrile 30:70 as eluting mixture. Retention factors (R_f) are relative to thin layer chromatography (TLC) performed on plastic sheets coated with silica gel 60-F_{254}, with a 1:1 cyclohexane/ethylacetate mixture as eluent. Complete characterization for compounds **1a–d**, **2a–d** and **9a-c** is reported in ref. 65.

Full geometry optimizations were performed with the Gaussian03 package of programs at the DFT B3LYP-STO-3G 2 level in order to achieve initial geometries, which were used to generate atomic "am1bcc" charges by use of the "antechamber" 3 module in Amber8.0 4 MM geometry optimization by use of the GAFF (Generalized Amber Force Field) in the "sander" module of Amber 8.0 was then applied.

General Procedure for the Preparation of Epoxides 3 and 4: m-Chloroperbenzoic acid (1.5 mmol, 1.5 equiv., 336 mg of commercial product, 77% purity) was added in one portion to a solution of 1 or 5 (1 mmol) in DCM (5 mL). The reaction mixture was stirred overnight at room temp. under inert atmosphere and was then diluted with DCM (10 mL). After having been washed twice with a saturated solution of K_2CO_3 (2 × 10 mL), the organic layer was separated and dried with Na_2SO_4, and the solvent was removed under reduced pressure. The products were purified by preparative HPLC with a Chiralcel OD column [cellulose tris(3,5-dimethylphenylcarbamate) coated on 10 m silica gel, hexane/iPrOH, 9:1, as eluent] or a Chiralcel OJ column [cellulose tris(4-methylbenzoate) coated on 10 m silica gel, hexane/iPrOH, 9:1, as eluent].

Compound 4a : Yield 64% (265 mg), dr 85:15, major isomer, yellow oil; Rf = 0.50. 1H NMR (CDCl3): = 0.73 (t, 1J = 7.5 Hz, 3H, CH3), 0.90-1.00 (m, 2H, CH2CH3), 1.85 (d, 1J = 2.1 Hz, 1H, CCHO), 3.25-3.29 (m, 1H, CH2CHO), 4.11 (d, 1J = 15.3 Hz, 1H, CH2Ph), 5.05 (d, 1J = 15.3 Hz, 1H, CH2Ph), 5.12 (s, 1H, NCHPh), 5.48 (s, 1H, CHOH), 6.86-6.89 (m, 2H, Ph), 7.20-7.40 (m, 11H, Ph), 7.50-7.60 (m, 2H, Ph) ppm. 13C NMR (CDCl3): = 9.5, 24.4, 44.8, 56.7, 57.2, 59.5, 68.2, 71.5, 127.0, 127.2, 127.5, 127.8, 128.1, 128.4, 128.5, 128.6, 128.8, 133.4, 134.9, 140.0, 169.7 ppm. IR (neat): = 3400, 3063, 3032, 2969, 2924, 1737, 1496, 1454, 1409, 1353, 1287, 1264 cm-1. LC-ESI-MS room temp. 14.2 min, m/z 414 [M + 1], 436 [M + Na]. C27H27NO3 (413.51): calcd. C 78.42, H 6.58, N 3.39; found C 78.51, H 6.49, N 3.18.

Compound 4b : Yield 70% (296 mg), dr 86:14, major isomer, yellow oil; Rf = 0.29. ^{1}H NMR (CDCl3): = 0.71 (t, 1J = 7.5 Hz, 3H, CHCH2CH3), 1.20-1.30 (m, 2H, CHCH2CH3), 1.25 (t, 1J = 6.9 Hz, 3 H, OCH2CH3), 1.79 (d, 1J = 2.4 Hz, 1 H, CCHO), 2.60-2.70 (m, 2 H, CH2CO), 3.13-3.17 (m, 1 H, CH2N), 3.27-3.38 (m, 1 H, CH2CHO), 3.88-3.97 (m, 1 H, CH2N), 4.11 (q, 1J = 6.9 Hz, 2H, OCH2CH3), 5.17 (s, 1H, NCHPh), 5.37 (s, 1H, CHOH), 6.93 (d, 1J = 7.5 Hz, 2H, Ph), 7.20-7.40 (m, 6H, Ph), 7.64 (d, 1J = 6.9 Hz, 2H, Ph) ppm. ^{13}C NMR (CDCl3): = 9.3, 14.0, 24.2, 32.6, 36.6, 56.3, 57.6, 59.2, 60.9, 67.8, 71.5, 126.6, 126.7, 128.0, 128.1, 128.3, 128.6, 135.0, 139.6, 169.1, 171.3 ppm. IR (neat): = 3422, 2963, 2910, 2851, 1749, 1643, 1460, 1383, 1260, 1024 cm-1. LC-ESI-MS room temp. 12.6 min, m/z 424 [M + 1], 446 [M + Na]. C25H29NO5 (423.5): calcd. C 70.90, H 6.90, N 3.31; found C 71.02, H 6.86, N 3.58.

Compound 4c : Yield 68% (290 mg), dr 83:17, major isomer, yellow oil; Rf = 0.55. [] = -80.0 (c = 0.7, CHCl3). 1H NMR (CDCl3): = 0.74 (t, 1J = 7.5 Hz, 3H, CH2CH3), 0.84-0.96 (m, 2H, CH2CH3), 1.75 (d, 1J = 2.1 Hz, 1H, CCHO), 1.84 (d, 1J = 7.0 Hz, 3H, CHCH3), 3.22-3.26 (m, 1H, CH2CHO), 4.58 (q, 1J = 7.0 Hz, 1H, CHCH3), 4.97 (s, 1H, NCHPh), 5.38 (s, 1H, CHOH), 6.64-6.77 (m, 2H, Ph), 7.10-7.40 (m, 11H, Ph), 7.57 (d, 1J = 7.2 Hz, 2H, Ph) ppm. 13C NMR (CDCl3): = 9.3, 20.0, 24.3, 54.2, 56.6, 56.8, 59.1, 66.8, 71.7, 126.7, 126.8, 126.9, 127.1, 127.5, 127.74, 127.9, 128.2, 128.4, 135.0, 139.8, 140.9, 169.0 ppm. IR (neat): = 3405, 3063, 3028, 2975, 2927, 1737, 1655, 1454, 1378, 1354, 1053 cm-1. LC-ESI-MS room temp. 14.7 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.20, H 6.63, N 3.25.

Compound 4d : Yield 68 % (289 mg), dr 83:17, major isomer, sticky oil; Rf = 0.60. [] = +44.5 (c = 0.5, CHCl3). 1H NMR (CDCl3): = 0.74 (t, 1J = 7.5 Hz 3H, CH2CH3), 1.2-1.4 (m, 2H, CH2CH3), 1.60 (d, 1J = 7.2 Hz 3H, CHCH3), 1.80 (d, 1J = 2.2 Hz, 1H, CCHO), 3.20-3.28 (m, 1H, CH2CHO), 4.84 (q, 1J = 7.2 Hz, 1H, CHCH3), 5.00 (s, 1H, NCHPh), 5.36 (s, 1H, CHOH), 6.80-6.90 (m, 2H, Ph), 7.22-7.65 (m, 13H, Ph) ppm. 13C NMR (CDCl3): = 9.3, 20.2, 24.3, 54.2, 56.5, 58.0, 59.3, 66.5, 71.7, 127.2, 127.3, 127.4, 127.5, 128.1, 128.2, 128.3, 128.4, 133.06, 135.9, 139.5, 167.1 ppm. IR (neat): = 3423, 3062, 3032, 2977, 2934, 1734, 1495, 1454, 1379, 1354, 1265, 1109, 1066, 1027 cm-1. LC-ESI-MS room temp. 15.2 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 79.00, H 6.77, N 3.46.

Compound 3a : Yield 84 % (346 mg), dr 84:16, major isomer, yellow oil; Rf = 0.43. 1H NMR (CDCl3): = 0.82 (t, 1J = 7.5 Hz, 3H, CH2CH3), 1.0-1.2 (m, 2H, CH2CH3), 2.69 (d, 1J = 2.4 Hz, 1H, CCHO), 3.34-3.38 (m, 1H, CH2CHO), 3.79 (d, 1J = 15.0 Hz, 1H, CH2Ph), 4.66 (s, 1H, NCHPh), 4.75 (d, 1J = 15.0 Hz, 1H, CH2Ph), 5.33 (s, 1H, CHOH), 6.34 (d, 1J = 7.2 Hz, 2H, Ph), 7.00-7.60 (m, 13H, Ph) ppm. 13C NMR (CDCl3): = 9.5, 24.5, 44.0, 57.3, 57.4, 58.9, 67.5, 72.3, 127.2, 127.4, 127.5, 128.0, 128.1, 128.3, 128.5, 128.6, 128.8, 134.0, 135.0, 139.2, 167.1 ppm. IR (neat): = 3436, 3059, 3025, 2959, 2926, 1733, 1487, 1460, 1408, 1355, 1262 cm-1. LC-ESI-MS room temp. 13.7 min, m/z 414 [M + 1], 436 [M + Na]. C27H27NO3 (413.51): calcd. C 78.42, H 6.58, N 3.39; found C 78.28, H 6.70, N 3.26.

Compound 3b : Yield 78 % (330 mg), dr 85:15, major isomer, yellow oil; Rf = 0.22. 1H NMR (CDCl3): = 0.83 (t, 1J = 7.5 Hz, 3H, CH2CH3), 1.18 (t, 1J = 6.9 Hz, 3H, OCH2CH3), 1.20-1.40 (m, 2H, CH2CH3), 1.59-1.67 (m, 1H, CH2CO), 1.79-1.89 (m, 1H, CH2CO), 2.63 (d, 1J = 2.4 Hz, 1H, CCHO), 2.90-3.00 (m, 1H, CH2N), 3.31-3.34 (m. 1H, CH2CHO), 3.59-3.68 (m, 1H, CH2N), 3.99 (q, 1J = 6.9 Hz, 2H, OCH2CH3), 4.87 (s, 1H, NCHPh), 5.29 (s, 1H, CHOH), 7.25-7.45 (m, 8H, Ph), 7.63 (d, 1J = 6.9 Hz, 2H, Ph) ppm. 13C NMR (CDCl3): = 9.4, 14.0, 24.4, 32.0, 35.5, 56.5, 58.4 (2 C), 60.6, 67.3, 72.5, 126.8, 127.2, 127.4, 128.2, 128.5, 128.9, 135.1, 138.5, 164.2, 167.1 ppm. IR (neat): = 3439, 3032, 2973, 2936, 1735, 1495, 1454, 1377, 1195, 1048, 1028 cm-1. LC-ESI-MS room temp. 12.0 min, m/z 424 [M + 1], 446 [M + Na]. C25H29NO5 (423.5): calcd. C 70.90, H 6.90, N 3.31; found C 70.68, H 7.02, N 3.17.

Compound 3c : Yield 67% (286 mg), dr 82:18, major isomer, white solid, m.p. 118-120 °C; Rf = 0.55. [] = -13.6 (c = 0.6, CHCl3). 1H NMR (CDCl3): = 0.85 (t, 1J = 7.5 Hz, 3 H, CH2CH3), 0.94-1.06 (m, 2 H, CH2CH3), 1.43 (d, 1J = 7.2 Hz, 3 H, CHCH3), 2.61 (d, 1J = 1.8 Hz, 1 H, CCHO), 3.30-3.40 (m, 1 H, CH2CHO), 4.39 (q, 1J = 7.2 Hz, 1 H, CHCH3), 4.62 (s, 1 H, NCHPh), 5.26 (s, 1 H, CHOH), 6.64 (d, 1J = 7.0 Hz, 2 H, Ph), 7.00-7.50 (m, 2 H, Ph) ppm. 13C NMR (CDCl3): = 9.4, 19.3, 24.4, 53.9, 57.0, 57.7, 58.2, 66.0, 72.6, 126.7, 127.1, 127.3, 127.4, 127.9, 128.1, 128.3, 128.4, 135.0, 138.8, 140.0, 166.9 ppm. IR (neat): = 3412, 3032, 2966, 2927, 1733, 1495, 1378, 1261, 1051, 1027 cm-1. LC-ESI-MS room temp. 14.7 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.62, H 6.99, N 3.11.

General Procedure for the Intramolecular Ring Opening of Epoxides 3 and 4: BF3·Et2O (1.1 mmol, 1.1 equiv., 156 mg, 0.139 mL) was added in one portion to a solution of epoxide 3 or 4 (1 mmol) in DCM (5 mL). The reaction mixture was stirred at the temperature of choice for 1 h and was then diluted with DCM (5 mL). After having been washed twice with water, the organic layer was separated and dried with Na_2SO_4, and the solvent was removed under reduced pressure. The products were purified by flash chromatography on silica gel (cyclohexane/EtOAc, 90:10 to 50:50).

Yield 5a + 6a = 80%, dr 5a/6a = 90:10.

Compound 5a : 297 mg, yellow oil; Rf = 0.27. 1H NMR (CDCl3): = 0.96 (t, J = 7.5 Hz, 3 H, CH2CH3), 2.04-2.09 (m, 1 H, CH2CH3), 2.20-2.24 (m, 1 H, CH2CH3), 2.83-2.86 (m, 1 H, CHOH), 3.50 (d, 1J = 11.4 Hz, 1 H, CHCHO), 3.59 (d, 1J = 15.6 Hz, 1 H, CH2Ph), 4.58 (s, 1 H, NCHPh), 4.63 (d, 1J = 15.6 Hz, 1 H, CH2Ph), 5.41 (s, 1 H, OCHPh), 6.84 (d, 1J = 7.8 Hz, 1 H, Ph), 6.90-6.92 (m, 2 H, Ph), 7.10-7.13 (m, 1 H, Ph), 7.20-7.33 (m, 9 H, Ph), 7.43 (d, 1J = 6.6 Hz, 2 H, Ph) ppm. 13C NMR: = 9.4, 18.2, 42.8, 43.5, 56.4, 65.8, 72.7, 74.0, 126.3, 126.5, 127.4, 128.0, 128.3, 128.5, 128.6, 128.9, 130.0, 134.7, 139.0, 139.6, 169.4 ppm. IR (neat): = 3337, 3018, 2972, 2919, 1719, 1620, 1487, 1461, 1408, 1334, 1261, 1070 cm-1. LC-ESI-MS room temp. 12.4 min, m/z 414 [M + 1], 436 [M + Na]. C27H27NO3 (413.51): calcd. C 78.42, H 6.58, N 3.39; found C 78.31, H 6.79, N 3.14.

Compound 6a : 33 mg, white oil; Rf = 0.44. 1H NMR (CDCl3): = 1.06 (t, J = 7.4 Hz, 3 H, CH2CH3), 2.06-2.32 (m, 2 H, CH2CH3), 3.53 (d, 1J = 15.4 Hz, 1 H, CH2Ph), 3.87 (m, 1 H,

CH2CHO), 4.21 (s, 1 H, NCHPh), 4.33 (br s, 1 H, CHOH), 4.72 (d, 1J = 15.4 Hz, 1 H, CH2Ph), 5.27 (s, 1 H, OCHPh), 6.21 (d, 1J = 7.0 Hz, 2 H, Ph), 6.98-7.60 (m, 13 H, Ph) ppm. 13C NMR (CDCl3): = 10.5, 25.1, 43.0, 57.2, 75.1, 76.2, 82.2, 88.4, 127.2, 127.5, 128.2, 128.5, 128.6, 128.7, 129.4, 129.9, 134.3, 135.2, 135.9, 169.3 ppm. IR (neat): = 3398, 3057, 2957, 2922, 1750, 1672, 1655, 1490, 1454, 1407 cm-1. LC-ESI-MS room temp. 13.8 min, m/z 414 [M + 1], 436 [M + Na]. C27H27NO3 (413.51): calcd. C 78.42, H 6.58, N 3.39; found C 78.55, H 6.51, N 3.48.

Yield 5b + 6b = 75%, dr 5b/6b > 99:1.

Compound 5b : 318 mg, sticky oil; Rf = 0.10. 1H NMR (CDCl3): = 0.96 (t, 1J = 7.4 Hz, 3 H, CH2CH3), 1.26 (t, 1J = 7.0 Hz, 3 H, OCH2CH3), 2.0-2.3 (m, 2 H, CH2CH3), 2.3-2.5 (m, 2 H, CH2CO), 2.83 (m, 1 H, CHOH), 3.02 (m, 1 H, NCH2), 3.38-3.49 (m, 2 H, CHCHO, NCH2), 4.10 (q, 1J = 7.0 Hz, 2 H, OCH2CH3), 4.75 (s, 1 H, NCHPh), 5.43 (s, 1 H, OCHPh), 7.2-7.5 (m, 10 H, Ph) ppm. 13C NMR: = 8.9, 13.7, 17.8, 32.4, 35.1, 42.4, 56.9, 60.4, 65.4, 71.8, 73.0, 126.2, 126.8, 127.9, 128.1, 128.5, 129.6, 131.3, 139.1, 164.1, 170.7 ppm. IR (neat): = 3439, 3056, 3034, 2960, 2924, 1724, 1451, 1377, 1192, 1043 cm-1. LC-ESI-MS room temp. 10.7 min, m/z 424 [M + 1], 446 [M + Na]. C25H29NO5 (423.5): calcd. C 70.90, H 6.90, N 3.31; found C 71.01, H 6.79, N 3.52.

Yield 5c + 6c = 60%, dr 5c/6c = 72:28.

Compound 5c : 185 mg, sticky oil; Rf = 0.36. [] = +25.5 (c = 2.0, CHCl3). 1H NMR (CDCl3): = 0.94 (t, 1J = 7.4 Hz, 3 H, CH2CH3), 1.61 (d, 1J = 7.2 Hz, 3 H, CHCH3), 1.98-2.22 (m, 2 H, CH2CH3), 2.84 (m, 1 H, CHOH), 3.43 (d, 1J = 11.2 Hz, 1 H, CHCHO), 3.50 (q, 1J = 7.2 Hz, 1 H, CHCH3), 4.59 (s, 1 H, NCHPh), 5.40 (s, 1 H, OCHPh), 6.8-7.5 (m, 15 H, Ph) ppm. 13C NMR: = 9.5, 18.2, 19.9, 42.9, 53.7, 56.2, 64.6, 72.9, 73.8, 126.0, 126.2, 126.3, 126.6, 127.3, 127.4, 128.2, 128.4, 128.5, 128.7, 129.7, 130.0, 131.8, 139.1, 139.7, 140.6, 169.5 ppm. IR (neat): = 3414, 3062, 2970, 2932, 1724, 1493, 1454, 1348, 1043 cm-1. LC-ESI-MS room temp. 13.1 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.81, H 6.68, N 3.11.

Compound 6c : 71 mg, white oil; Rf = 0.60. [] = +21.9 (c = 1.5, CHCl3). 1H NMR (CDCl3): = 1.06 (t, 1J = 7.6 Hz, 3 H, CH2CH3), 1.50 (d, 1J = 7.2 Hz, 3 H, CHCH3), 1.83-2.0 (m, 1 H,

CH2CH3), 2.0-2.23 (m, 1 H, CH2CH3), 3.87 (m, 1 H, CH2CHO), 4.07 (s, 1 H, NCHPh), 4.12 (q, 1J = 7.2 Hz, 1 H, CHCH3), 4.33 (br s, 1 H, CHOH), 5.26 (s, 1 H, OCHPh), 6.35 (d, 1J = 7.0 Hz, 2 H, Ph), 7.0-7.6 (m, 13 H, Ph) ppm. 13C NMR: = 10.5, 20.3, 25.0, 54.3, 57.1, 73.7, 76.0, 82.2, 88.3, 126.3, 127.2, 128.0, 128.2, 128.4, 128.5, 128.8, 129.2, 129.7, 135.6, 135.9, 140.0, 167.2 ppm. IR (neat): = 3352, 3062, 3023, 2968, 2929, 1743, 1494, 1454, 1377, 1026 cm-1. LC-ESI-MS room temp. 15.1 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.59, H 6.90, N 3.34.

Yield 5d + 6d = 57%, dr 5c/6c = 90:10.

Compound 5d: 219 mg, yellow oil; Rf = 0.38. [] = -10.9 (c = 1.3, CHCl3). 1H NMR (CDCl3): = 0.98 (t, 1J = 7.5 Hz, 3 H, CH2CH3), 1.15 (d, 1J = 7.2 Hz, 3 H, CHCH3), 2.0-2.2 (m, 1 H, CH2CH3), 2.2-2.4 (m, 1 H, CH2CH3), 2.90 (m, 1 H, CHOH), 3.47 (d, 1J = 9.3 Hz, 1 H, CHCHO), 4.48 (s, 1 H, NCHPh), 4.91 (q, 1J = 7.2 Hz, 1 H, CHCH3), 5.36 (s, 1 H, OCHPh), 6.70 (d, 1J = 8.6 Hz, 1 H, Ph), 6.95-7.45 (m, 14 H, Ph) ppm. 13C NMR: = 9.4, 18.0, 18.1, 42.8, 51.1, 56.4, 64.6, 73.2, 74.4, 126.3, 127.3, 127.4, 128.3, 128.5, 128.6, 128.8, 129.8, 132.8, 138.5, 139.6, 161.3 ppm. IR (neat): = 3418, 3064, 3027, 2968, 2930, 1724, 1492, 1452, 1381, 1045 cm-1. LC-ESI-MS room temp. 13.3 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.79, H 6.73, N 3.16.

Compound 6d: 26 mg, yellow oil; Rf = 0.59. [] = -21.0 (c = 1.0, CHCl3). 1H NMR (CDCl3): = 1.06 (t, J = 7.5 Hz, 3 H, CH2CH3), 1.12 (d, 1J = 7.2 Hz, 3 H, CHCH3), 1.8-2.0 (m, 1 H, CH2CH3), 2.0-2.2 (m, 1 H, CH2CH3), 3.87 (t, 1J = 7.5 Hz, 1 H, CH2CHO), 4.18 (s, 1 H, NCHPh), 4.34 (br s, 1 H, CHOH), 4.48 (q, 1J = 7.2 Hz, 1 H, CHCH3), 5.26 (s, 1 H, OCHPh), 6.57 (d, 1J = 8.4 Hz, 2 H, Ph), 7.0-7.6 (m, 13 H, Ph) ppm. 13C NMR: = 10.6, 19.5, 25.2, 52.7, 58.0, 73.5, 76.1, 82.2, 88.5, 127.1, 127.5, 128.2, 128.4, 128.5, 128.7, 128.8, 129.4, 129.7, 135.9, 137.0, 139.1, 167.0 ppm. IR (neat): = 3420, 3065, 3022, 2967, 2931, 1744, 1494, 1454, 1377, 1026 cm-1. LC-ESI-MS room temp. 15.0 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.48, H 7.00, N 3.09.

Yield 7a + 8a = 85%, dr 8a/7a = 34:66.

Compound 8a: 119 mg, white oil; Rf = 0.51. 1H NMR (CDCl3): = 0.99 (t, 1J = 7.2 Hz, 3 H, CH2CH3), 1.71-1.78 (m, 1 H, CH2CH3), 1.89-1.96 (m, 1 H, CH2CH3), 3.80 (d, 1J = 15.0

Hz, 1 H , CH2Ph), 3.96 (m, 1 H , CH2CHO), 3.97 (s, 1 H , NCHPh), 4.45 (d, 1J = 3.3 Hz, 1 H , CHOH), 4.85 (d, 1J = 15.0 Hz, 1 H , CH2Ph), 5.27 (s, 1 H , OCHPh), 6.95 (d, 1J = 6.0 Hz, 2 H , Ph), 7.08 (d, 1J = 6.0 Hz, 2 H , Ph), 7.22-7.38 (m, 11 H , Ph) ppm . 13C NMR (CDCl3): = 10.1, 24.2, 44.5, 59.7, 73.7, 77.1, 81.8, 87.3, 127.0, 127.7, 128.1, 128.3, 128.4, 128.5, 128.7, 129.0 129.5, 134.9, 135.1, 139.5, 169.2 ppm . IR (neat): = 3421, 3030, 2963, 2923, 1750, 1653, 1496, 1456, 1242 cm-1 . LC-ESI-MS room temp. 14.4 min, m/z 414 [M + 1], 436 [M + Na]. C27H27NO3 (413.51): calcd. C 78.42, H 6.58, N 3.39; found C 78.60, H 6.39, N 3.52.

Compound 7a : 231 mg, white oil; R f = 0.30 . 1H NMR (CDCl3): = 1.09 (t, 1J = 7.2 Hz, 3 H , CH2CH3), 2.19-2.26 (m, 1 H , CH2CH3), 2.27-2.38 (m, 1 H , CH2CH3), 2.90 (m, 1 H , CHOH), 3.26 (d, 1J = 15.6 Hz, 1 H , CH2Ph), 4.10 (d, 1J = 11.4 Hz, 1 H , CHCHO), 4.37 (s, 1 H , NCHPh), 4.50 (d, 1J = 15.6 Hz, 1 H , CH2Ph), 5.71 (s, 1 H , OCHPh), 6.35 (d, 1J = 6.9 Hz, 2 H , Ph), 6.86 (d, 1J = 6.9 Hz, 1 H , Ph), 7.09-7.59 (m, 12 H , Ph) ppm . 13C NMR (CDCl3): = 9.2, 18.6, 42.7, 55.7, 66.8, 70.4, 70.6, 86.0, 126.3, 126.7, 126.9, 127.2, 127.5, 128.2, 128.5, 128.6, 128.9, 131.4, 139.9, 169.1 ppm . IR (neat): = 3427, 3061, 3020, 2958, 2917, 1730, 1495, 1452, 1043 cm-1 . LC-ESI-MS room temp. 12.6 min, m/z 414 [M + 1], 436 [M + Na]. C27H27NO3 (413.51): calcd. C 78.42, H 6.58, N 3.39; found C 78.71, H 6.39, N 3.46.

Yield 7b + 8b = 65% , dr 8b/7b = 37:63 .

Compound 8b : 101 mg, sticky oil; R f = 0.31 . 1H NMR (CDCl3): = 1.01 (t, 1J = 7.5 Hz, 3 H , CH2CH3), 1.21 (t, 1J = 7.5 Hz, 3 H , OCH2CH3), 1.71-1.80 (m, 1 H , CH2CH3), 1.85-1.98 (m, 1 H , CH2CH3), 2.41 (m, 2 H , CH2CO), 3.09-3.19 (m, 1 H , NCH2), 3.75-3.84 (m, 1 H , NCH2), 3.97 (m, 1 H , CH2CHO), 4.04 (q, 1J = 7.5 Hz, 2 H , OCH2CH3), 4.19 (s, 1 H , NCHPh), 4.43 (br s, 1 H , CHOH), 5.32 (s, 1 H , OCHPh), 7.1-7.5 (m, 10 H , Ph) ppm . 13C NMR : = 10.1, 21.0, 24.4, 32.6, 36.1, 60.4, 60.8, 70.1, 73.6, 87.2, 126.6, 127.0, 127.1, 127.4, 128.1, 128.3, 128.6, 128.9, 129.1, 129.5, 130.5, 139.6, 169.2, 171.1 ppm . IR (neat): = 3396, 2962, 2923, 1729, 1655, 1451, 1372, 1188 cm-1 . LC-ESI-MS room temp. 12.7 min, m/z 424 [M + 1], 446 [M + Na]. C25H29NO5 (423.5): calcd. C 70.90, H 6.90, N 3.31; found C 70.84, H 7.02, N 3.08.

Compound 7b : 172 mg, sticky oil; R f = 0.09 . 1H NMR (CDCl3): = 1.06 (t, 1J = 7.2 Hz, 3 H , CH2CH3), 1.21 (t, 1J = 7.0 Hz, 3 H , OCH2CH3), 1.70-1.90 (m, 2 H , CH2CH3), 1.95 (m, 2 H , CH2CO), 2.61-2.76 (m, 1 H , NCH2), 2.85 (m, 1 H , CHOH), 3.14-3.32 (m, 1 H , NCH2), 3.90-

4.20 (m, 3H, CHCHO, OCH2CH3), 4.51 (s, 1H, NCHPh), 5.63 (s, 1H, OCHPh), 7.0-7.6 (m, 10H, Ph) ppm. 13C NMR: = 9.1, 14.1, 18.5, 32.6, 34.6, 57.1, 60.6, 66.4, 70.1, 71.3, 86.2, 126.2, 126.5, 126.6, 126.7, 127.4, 127.9, 128.2, 128.7, 128.9, 130.5, 138.8, 139.7, 169.5, 170.9 ppm. IR (neat): = 3423, 3051, 3031, 2969, 2928, 1735, 1454, 1376, 1190, 1044 cm-1. LC-ESI-MS room temp. 10.9 min, m/z 424 [M + 1], 446 [M + Na]. C25H29NO5 (423.5): calcd. C 70.90, H 6.90, N 3.31; found C 70.76, H 6.99, N 3.16.

Yield 7c + 8c = 65%, dr 8c/7c = 80:20.

Compound 8c: 222 mg, pale yellow oil; Rf = 0.69. [] = -17.8 (c = 1.8, CHCl3). 1H NMR (CDCl3): = 0.92 (t, 1J = 7.5 Hz, 3H, CH2CH3), 1.93 (d, 1J = 7.2 Hz, 3H, CHCH3), 1.7-2.0 (m, 2H, CH2CH3), 3.91 (s, 1H, NCHPh), 3.98 (m, 1H, CH2CHO), 4.43 (q, 1J = 7.2 Hz, 1H, CHCH3), 4.67 (d, 1J = 5.7 Hz, 1H, CHOH), 5.29 (s, 1H, OCHPh), 7.0-7.5 (m, 15H, Ph) ppm. 13C NMR: = 10.2, 19.7, 24.0, 54.6, 59.8, 72.8, 82.2, 82.4, 87.7, 126.8, 127.2, 127.7, 128.1, 128.4, 128.5, 128.7, 128.9, 129.4, 135.5, 139.7, 140.8, 169.5 ppm. IR (neat): = 3379, 3065, 3024, 2969, 2922, 1745, 1494, 1450, 1371, 1072 cm-1. LC-ESI-MS room temp. 15.8 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.83, H 6.67, N 3.09.

Compound 7c: 55 mg, yellow oil; Rf = 0.38. [] = +25.0 (c = 0.8, CHCl3). 1H NMR (CDCl3): = 1.08 (t, 1J = 7.2 Hz, 3H, CH2CH3), 1.40 (d, 1J = 7.2 Hz, 3H, CHCH3), 2.10-2.25 (m, 1H, CH2CH3), 2.25-2.40 (m, 1H, CH2CH3), 2.93 (m, 1H, CHOH), 3.95 (q, 1J = 7.2 Hz, 1H, CHCH3), 4.04 (d, 1J = 11.1 Hz, 1H, CHCHO), 4.31 (s, 1H, NCHPh), 5.69 (s, 1H, OCHPh), 6.45 (d, 1J = 6.9 Hz, 2H, Ph), 6.74 (d, 1J = 6.9 Hz, 1H, Ph), 7.0-7.6 (m, 12H, Ph) ppm. 13C NMR: = 9.2, 18.5, 20.3, 42.8, 53.8, 55.6, 60.4, 65.3, 70.6, 126.1, 126.2, 126.8, 126.9, 127.0, 128.0, 128.3, 128.4, 128.7, 138.9, 139.9, 140.3, 169.1 ppm. IR (neat): = 3420, 3065, 3024, 2962, 2928, 1721, 1494, 1449, 1385, 1042 cm-1. LC-ESI-MS room temp. 13.3 min, m/z 428 [M + 1], 450 [M + Na]. C28H29NO3 (427.53): calcd. C 78.66, H 6.84, N 3.28; found C 78.49, H 6.71, N 3.45.

Compound 7d: [α] = -72.4 (c=0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.93 (d, 3H, *J*=7.0 Hz), 1.06 (t, 3H, *J* =7.3 Hz), 2.1-2.4 (m, 2H), 2.90 (m, 1H), 4.01 (d, 1H, *J* =11.4Hz), 4.25 (s, 1H), 4.68 (q, 1H, *J* =7.0 Hz,), 5.64 (s, 1H), 6.48 (d, 2H, *J* =7.1 Hz), 6.68 (d, 1H, *J* =7.8Hz), 7.0-7.6 (m, 12H).; ^{13}C-NMR δ 9.4, 17.5, 18.6, 42.9, 50.4, 55.8, 65.9, 70.9, 65.7, 70.9(2C), 126.3, 126.5, 126.9, 127.2, 127.4, 128.0, 128.4, 128.5, 128.9,

130.1, 133.3, 128.0, 139.1, 140.0, 168.7; IR (neat): 3406, 3044, 3024, 2962, 2922, 1716, 1490, 1450, 1062 cm^{-1}; LC-ESI-MS rt 13.3 min., m/z 428 (M+1), 450 (M+Na).

Compound 8d: $[\alpha] = +0$ (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.99 (t, 3H, *J* =7.5 Hz), 1.33 (d, 3H, *J* =7.4 Hz), 1.60 (bs, 1H), 1.62-1.80 (m, 1H), 1.8-2.1 (m, 1H), 3.89 (s, 1H), 3.99 (m, 1H), 4.40 (dd, 1H, *J* =2.2, 3.6Hz), 4.91 (q, 1H, *J* =7.4 Hz), 5.15 (s, 1H), 7.0-7.4 (m, 15H); ^{13}C-NMR δ 10.3, 18.9, 23.8, 53.1, 60.0, 72.9, 76.7, 82.4, 88.3, 127.2, 127.9, 128.0, 128.1, 128.4, 128.7, 129.0, 129.1, 129.4, 136.9, 139.8, 140.0, 170.0; IR (neat): 3424, 3029, 2966, 2930, 1745, 1494, 1454, 1377, 1023 cm^{-1}; LC-ESI-MS rt 16.2 min., *m/z* 428 (M+1), 450 (M+Na).

General procedure for the epoxidation of 3-alkylidene-3-bromo-azetidin-2-ones 9a-c.

To a stirred solution of **9** (1 mmol) in CH_2Cl_2 at r.t., m-chloroperbenzoic acid (1.5 equiv., 0.258 g.) was added in one portion. The reaction was stirred overnight and then diluted with a saturated solution of K_2CO_3 (5 mL) and CH_2Cl_2 (5 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **2** and **3** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 9/1 as eluant).

Compound 10a: m.p. 145-147 °C; HPLC-MS r_t=14.5 min (M+1)=386/388 (M+Na)=408/410 m/z; IR (neat) ν 3073, 2959, 2930, 1771, 1654, 1455, 1395, 1355, 1157, 1124, 1077 cm^{-1}; ^{1}H-NMR (600MHz, $CDCl_3$) δ 1.05 (t, 3H, J=7.2Hz), 1.53-1.77 (m, 2H), 3.27 (1H, d, J=2.2Hz), 3.57 (1H, dt, J=2.2, 5.6Hz), 3.91 (d, 1H, J=15.0Hz), 4.78 (s, 1H), 4.99 (d, 1H, J=15.0Hz), 7.18-7.43 (m, 10H); ^{13}C-NMR (150MHz, $CDCl_3$) δ 9.6 (CH_3), 24.6 (CH_2), 44.9 (CH_2), 57.3 (CH), 58.7 (CH), 59.5 (CH), 68.4 (C), 127.7 (CH), 127.9 (CH), 128.2 (CH), 128.4 (CH), 128.7 (CH), 128.9 (CH), 133.7 (C), 133.9 (C), 163.5 (C).

Compound 11a: m.p. 110-112 °C; HPLC-MS r_t=15.2 min (M+1)=386/388 (M+Na)=408/410 m/z; IR (neat) ν 2962, 2928, 1775, 1494, 1454, 1392, 1351, 1147, 1072 cm^{-1}; ^{1}H-NMR (600MHz, $CDCl_3$) δ 1.02 (t, 3H, J=7.6Hz), 1.45-1.82 (m, 2H), 2.89 (1H, dt, J=1.8, 5.6Hz), 3.45 (1H, d, J=1.8Hz), 3.93 (d, 1H, J=15.0Hz), 4.59 (s, 1H), 4.97 (d, 1H, J=15.0Hz), 7.16-

7.44 (m, 10H); ^{13}C-NMR (150MHz, $CDCl_3$) δ 9.4(CH_3), 24.2(CH_2), 44.9(CH_2), 57.3(CH), 58.9(CH), 59.5(CH), 68.8(C), 127.8(CH), 127.9(CH), 128.1(CH), 128.4(CH), 128.7(CH), 128.9(CH), 133.8(C), 134.0(C), 163.4(C).

Compound 10b : $[\alpha]_D^{20}$ -0.3 *(c=1.0, $CHCl_3$)* ; MS-ESI: (M+1)=400/402 (M+Na)=422/424 m/z; IR (neat)ν 3386, 3065, 3031, 2969, 2922, 1763, 1654, 1456, 1376, 1152, 1069, 1025 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.02 (t, 3H, J=7.2Hz), 1.52 (d, 3H, J=7.2Hz), 1.5-1.7 (m, 2H), 3.21 (1H, d, J=2.2Hz), 3.49 (1H, dt, J=2.2, 5.8Hz) 4.70 (s, 1H), 4.95 (q, 1H, J=7.2Hz), 7.22-7.41 (m, 10H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.6(CH_3), 19.2(CH_3), 24.7(CH_2), 53.6 (CH), 59.0(CH), 59.4(CH), 63.9(CH), 67.4(C), 127.5(CH), 127.9(CH), 128.0(CH), 128.1(CH), 128.3(CH), 128.6(CH), 135.3(C), 138.7(C), 164.0(CH).

Compound 11b : $[\alpha]_D^{20}$ +24.1 *(c=0.6, $CHCl_3$)* ; ESI-MS (M+1)=400/402 (M+Na)=422/424 m/z; IR (neat)ν 3399, 3051, 3024, 2968, 2928, 1765, 1654, 1494, 1456, 1371, 1150, 1068 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.99 (t, 3H, J=7.6Hz), 1.50 (d, 3H, J=7.0Hz), 1.60-1.75 (m, 2HH_3), 2.81 (1H, dt, J=2.2, 5.4Hz), 3.37 (1H, d, J=2.2Hz), 4.53 (s, 1H), 4.93 (q, 1H, J=7.0Hz), 7.18-7.42 (m, 10H); ^{13}C-NMR (50MHz, $CDCl_3$) δ 9.4(CH_3), 19.2(CH_3), 24.1(CH_2), 53.7(CH), 57.4(CH), 58.6(CH), 60.1(CH), 67.8(C), 127.1(CH), 127.8(CH), 127.9(CH), 128.2(CH), 128.5(CH), 128.9(CH), 135.2(C), 139.0(C), 164.6(C).

Compound 10c :HPLC-MS r_t =13.3 min (M+1)=396/398 (M+Na)=418/420 m/z; IR (neat)ν 3399, 2967, 2922, 1771, 1730, 1658, 1617, 1456, 1375, 1193, 1027 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.01 (t, 3H, J=7.2Hz), 1.21 (t, 3H, J=7.5Hz), 1.56-1.70 (m, 2H), 2.60 (m, 2H), 3.09-3.18 (m,1H), 3.26 (1H, d, J=2.1Hz), 3.49 (1H, dt, J=2.1, 5.4Hz), 3.74-3.84 (m,1H), 4.09 (q, 2H, J=7.5Hz), 4.99 (s, 1H), 7.10 (d, 2H, J=6.6Hz), 7.23-7.44 (m, 3H), ^{13}C-NMR (50MHz, $CDCl_3$) δ 9.5(CH_3), 13.9(CH_3), 24.5(CH_2), 32.2(CH_2), 37.0(CH_2), 58.6(CH), 59.4(CH), 60.7(CH_2), 64.6(CH), 68.0(C), 127.5(CH), 128.3(CH), 129.0(CH), 134.2(C), 163.3(C), 170.6(C).

Compound 11c :HPLC-MS r_t =12.8 min (M+1)=396/398 (M+Na)=418/420 m/z ; IR (neat)ν 3427, 2970, 2928, 1772, 1734, 1456, 1395, 1376, 1351, 1193, 1028 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.04 (t, 3H, J=7.5Hz), 1.24 (t, 3H, J=7.2Hz), 1.65-1.76 (m, 2H), 2.64 (m, 2H), 2.95 (1H, dt, J=1.8, 5.4Hz), 3.22-3.31 (m, 1H), 3.41 (1H, d, J=1.8Hz), 3.14-3.32 (m, 1H), 3.78-

3.88 (s, 1H), 4.11 (q, 2H, J=7.5Hz), 4.75 (s, 1H), 7.22-7.30 (m, 2H), 7.38-7.48 (m, 3H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.5 (CH_3), 13.9 (CH_3), 24.2 (CH_2), 32.4 (CH_2), 37.1 (CH_2), 57.2 (CH), 58.9 (CH), 60.7 (CH_2), 60.8 (CH), 68.5 (C), 127.7 (CH), 128.4 (CH), 129.0 (CH), 134.2 (C), 164.5 (C), 170.6 (C).

General procedure for the ring opening of epoxides 10a-c and 11a-c.

To a stirred solution of NaN_3 (1 mmol) in toluene (3 mL) at 25 °C under nitrogen atmosphere, Me_2AlCl (1 mmol, 1 equiv., 1 mL solution 1M in hexane) was added dropwise. The reaction was stirred for 4 hours and then cooled to −78 °C. Epoxide **10** or **11** (0.5 equiv., 0.5 mmol) was diluted in toluene (0.5 ml) and then added to the reaction mixture. The solution was stirred overnight, slowly reaching room temperature and then was diluted with EtOAc, cooled to 5 °C and added to a aqueous solution (5 mL) containing NaF (1 equiv., 1 mmol., 42 mg). The two phases were stirred for 30 minutes and then were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **12** and **13** were used in the following step without further purification.

Compound 12a : m.p. 108-110 °C; HPLC-MS r_t = 13.8 min (M+1)=429/431 (M+Na)=451/453 m/z; IR (neat) ν 3420, 2966, 2925, 2108, 1752, 1647, 1457, 1399, 1356, 1264, 1107, 1170 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.08 (t, 3H, J=7.4Hz), 1.59-1.74 (m, 1H), 2.04-2.18 (m, 1H), 3.29 (bs, 1H), 3.50 (dt, 1H, J=3.0, 8.7Hz), 3.62 (d, 1H, J=8.7Hz), 3.88 (d, 1H, J=14.8Hz), 4.77 (s, 1H), 4.92 (d, 1H, J=14.8Hz), 7.14-7.20 (m, 2H), 7.30-7.47 (m, 8H); ^{13}C-NMR (50MHz, $CDCl_3$) δ 9.9 (CH_3), 24.5 (CH_2), 44.9 (CH_3), 64.3 (CH), 65.7 (C), 73.8 (CH), 75.1 (CH), 128.2 (CH), 128.4 (CH), 128.6 (CH), 128.7 (CH), 129.0 (CH), 129.2 (CH), 133.0 (C), 134.1 (C), 166.3 (C).

Compound 13a: m.p. 189-191 °C; HPLC-MS r_t = 14.0 min (M+1)=429/431 (M+Na)=451/453 m/z; IR (neat) ν 3398, 2962, 2914, 2098, 1751, 1456, 1405, 1354, 1310, 1256, 1106, 1025 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.06 (t, 3H, J=7.2Hz), 1.57-1.69 (m, 1H), 1.74-1.82 (m, 1H), 3.61 (dt, 1H, J=3.6, 10.8Hz), 3.69 (bs, 1H), 3.93 (d, 1H, J=15.0Hz), 4.40 (d, 1H, J=3.6Hz), 4.94 (d, 1H, J=15.0Hz), 5.08 (s, 1H), 7.20-7.44 (m, 10H); ^{13}C-NMR (50MHz, $CDCl_3$) δ 10.8 (CH_3), 22.2 (CH_2), 44.9 (CH_2), 61.6 (CH), 65.4 (C), 71.4 (CH), 74.0 (CH), 127.9 (CH), 128.2 (CH), 128.3 (CH), 128.7 (CH), 128.9 (CH), 129.1 (CH), 133.4 (C), 134.0 (C), 166.0 (C).

Compound 12b : $[\alpha]_D^{20}$ +49.9 *(c=1.0, $CHCl_3$)* ; HPLC-MS r_t =15.0min (M+1)=443/445 (M+Na)=465/467 m/z; IR (neat)ν 3416, 2962, 2928, 2107, 1751, 1654, 1456, 1378, 1150, 1102, 1061 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.06 (t, 3H, J=7.5Hz), 1.46 (d, 3H, J=7.2Hz), 1.60-1.70 (m, 1H), 2.00-2.15 (m, 1H), 3.48 (m, 2H, dt), 4.69 (s, 1H), 5.04 (q, 1H, J=7.2Hz), 7.26-7.42 (m, 10H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.7(CH_3), 18.6(CH_3), 24.3(CH_2), 53.2(CH), 64.4(CH), 65.4(C), 72.9(CH), 74.7(CH), 127.1(CH), 127.6(CH), 127.9(CH), 128.7(CH), 129.0(CH), 129.1(CH), 134.4(C), 138.7(C), 166.7(C).

Compound 13b : $[\alpha]_D^{20}$ -23.1 (c=1.2, $CHCl_3$); HPLC-MS r_t =14.7min (M+1)=443/445 (M+Na)=465/467 m/z; IR (neat)ν 3382, 2969, 2928, 2098, 1745, 1653, 1495, 1456, 1378, 1344, 1314, 1272, 1259, 1074 cm^{-1}; ^{1}H-NMR (200MHz, $CDCl_3$) δ 1.03 (t, 3H, J=7.2Hz), 1.51 (d, 3H, J=7.0Hz), 1.55-1.69 (m, 1H), 1.72-1.89 (m, 1H), 3.57 (1H, dt, J=3.6, 10.8Hz), 4.01 (bs, 1H), 4.39 (1H, d, J=3.6Hz), 4.86 (q, 1H, J=7.0Hz), 5.03 (s, 1HPh), 7.23-7.40 (m, 10Hr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 10.8(CH_3), 19.2(CH_3), 22.0(CH_2), 54.0(CH), 61.8(CH), 65.3(C), 70.2(CH), 74.2(CH), 127.4(CH), 127.8(CH), 128.4(2C, CH), 128.9(CH), 129.3(CH), 134.8(C), 138.9(C), 166.1(C).

Compound 12c: HPLC-MS r_t =12.4 min (M+1)=439/441(M+Na)=461/463 m/z IR (neat)ν 3446, 2974, 2930, 2108, 1759, 1642, 1458, 1399, 1377, 1263, 1188, 1108, cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.14 (t, 3H, J=7.2Hz), 1.27 (t, 3H, J=7.0Hz), 1.69-1.83 (m, 1H), 2.04-2.16 (m, 1H), 2.51-2.60 (m, 1H), 2.66-2.75 (m, 1H), 3.21-3.30 (m, 2H), 3.55 (m, 1H), 3.82 (m, 2H), 4.14 (q, 2H, J=7.0Hz), 5.07 (s, 1H), 7.35-7.47 (m, 5H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.4(CH_3), 14.0(CH_3), 24.2(CH_2), 32.0(CH_2), 37.0(CH_2), 60.9(CH_2), 65.3(CH), 65.5(C), 73.8(CH), 75.1(CH), 128.1(CH), 128.3(CH), 128.5(CH), 128.7(CH), 129.0(CH), 133.5(C), 166.3(C), 171.9(C).

Compound 13c: HPLC-MS r_t =12.5 min (M+1)=439/441(M+Na)=461/463 m/z; IR (neat)ν 3416, 2968, 2925, 2101, 1756, 1733, 1647, 1455, 1402, 1377, 1263, 1192, 1110, 1028 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.10 (t, 3H, J=7.2Hz), 1.26 (t, 3H, J=7.5Hz), 1.61-1.74 (m, 1H), 1.80-1.93 (m, 1H), 2.64 (m, 2HCO_2), 3.17-3.26 (m, 1H), 3.41 (1H, dt, J=3.6, 10.8Hz), 3.81-3.90 (m, 1H), 4.13 (q, 2H, J=7.5Hz), 4.43 (1H, d, J=3.6Hz), 5.27 (s, 1H), 7.34-7.50 (m, 5H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 10.9(CH_3), 14.0(CH_3), 22.0(CH_2), 32.2(CH_2), 37.0(CH_2),

61.1 (CH_2), 62.2 (CH), 65.4 (C), 71.0 (CH), 74.0 (CH), 128.4 (CH), 128.8 (CH), 129.2 (CH), 133.9 (C), 166.0 (C), 171.1 (C).

General procedure for the formation of epoxides 14a-c and 15a-c.

A solution of compound **12** or **13** (1 mmol) and NaH (1.2 equiv., 1.2 mmol, 29 mg) in dry CH_2Cl_2 (10 mL) was stirred at 0 °C for two hours. The reaction was quenched by adding cold water dropwise (1 ml), further diluted with water (10 mL) and then layers were separated. The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **14** and **15** were isolated pure and used in the following step without further purification.

Compound 14a: HPLC-MS r_t = 16.0 min (M+1) = 349 (M+Na) = 371 m/z; IR (neat) ν 3392, 3058, 3024, 2968, 2924, 2099, 1770, 1496, 1456, 1387, 1261, 1101, 1028 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.04 (t, 3H, J=7.4 Hz), 1.54-1.80 (m, 1H), 1.81-2.00 (m, 1H), 2.81 (d, 1H, J=8.4 Hz), 3.61 (dt, 1H, J=5.2, 8.4 Hz), 3.91 (d, 1H, J=15.0 Hz), 4.64 (s, 1H), 4.98 (d, 1H, J=15.0 Hz), 7.15-7.44 (m, 10H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 9.6 (CH_3), 29.7 (CH_2), 44.9 (CH_2), 59.9 (CH), 61.6 (CH), 62.7 (C), 74.3 (CH), 127.4 (CH), 128.0 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 129.2 (CH), 134.0 (C), 134.6 (C), 169.3 (C).

Compound 15a: HPLC-MS r_t = 15.0 min (M+1) = 349 (M+Na) = 371 m/z; IR (neat) ν 3058, 3031, 2969, 2105, 1770, 1496, 1456, 1391, 1355, 1261, 1181, 1075, 1028 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, J=7.4 Hz), 1.35-1.65 (m, 2H), 2.52 (dt, 1H, J=4.8, 7.8 Hz), 3.33 (d, 1H, J=7.8 Hz), 3.88 (d, 1H, J=15.0 Hz), 4.74 (s, 1H), 4.95 (d, 1H, J=15.0 Hz), 7.11-7.46 (m, 10H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 9.4 (CH_3), 25.5 (CH_2), 44.5 (CH_2), 58.3 (CH), 61.0 (CH), 63.1 (C), 74.6 (CH), 127.5 (CH), 128.0 (CH), 128.8 (CH), 129.0 (CH), 129.1 (CH), 129.6 (CH), 132.8 (C), 134.3 (C), 166.6 (C).

Compound 14b: $[\alpha]_D^{20}$ +2.7 *(c=1.0, $CHCl_3$)*; HPLC-MS r_t = 17.4 min (M+1) = 363 (M+Na) = 385 m/z; IR (neat) ν 3442, 2973, 2930, 2099, 1766, 1657, 1495, 1455, 1373, 1340, 1274, 1179, 1075 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.04 (t, 3H, J=7.5 Hz), 1.41 (d, 3H, J=7.2 Hz), 1.56-1.67 (m, 1H), 1.72-1.81 (m, 1H), 2.78 (d, 1H, J=8.4 Hz), 3.61 (1H, dt, J=5.1, 8.4 Hz), 4.53 (s, 1H), 5.19 (q, 1H, J=7.2 Hz), 7.24-7.41 (m, 10H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 9.7 (CH_3), 18.9 (CH_3), 29.7 (CH_2), 52.9 (CH), 61.7 (CH), 62.7 (CH), 63.9 (C), 73.6 (CH),

127.4(CH), 127.7(CH), 128.1(CH), 128.3(CH), 128.7(CH), 128.9(CH), 135.8(C), 138.9(C), 166.9(C).

Compound 15b: $[\alpha]_D^{20}$ -21.1 *(c=2.0, $CHCl_3$)*; HPLC-MS r_t =15.6 min (M+1)=363 (M+Na)=385 m/z; IR (neat) ν 3431, 3037, 2968, 2926, 2104, 1766, 1645, 1493, 1455, 1376, 1262, 1117 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.87 (t, 3H, J=7.2Hz), 1.42 (d, 3H, J=7.0Hz), 1.41-1.63 (m, 2H), 2.40 (1H, dt, J=4.8, 7.5Hz), 3.32 (1H, d, J=7.5Hz), 4.63 (s, 1H), 5.15 (q, 1H, J=7.0Hz), 7.24-7.43 (m, 10HHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.4(CH_3), 19.1(CH_3), 25.6(CH_2), 53.0(CH), 58.3(CH), 60.9(CH), 63.2(C), 74.0(CH), 127.4(CH), 127.7(CH), 128.0(CH), 128.6(CH), 128.7(CH), 128.5(CH), 134.8(C), 138.8(C), 166.9(C).

Compound 14c: HPLC-MS r_t =13.5 min (M+1)=359 (M+Na)=381 m/z; IR (neat) ν 2969, 2928, 2853, 2104, 1770, 1733, 1494, 1454, 1393, 1376, 1261, 1189 cm^{-1}; ^{1}H-NMR (200MHz, $CDCl_3$) δ 1.01 (t, 3H, J=7.5Hz), 1.24 (t, 3H, J=7.4Hz), 1.49-1.81 (m, 2H), 2.45-2.71 (m, 2H), 2.78 (d, 1H, J=8.4Hz), 3.26-3.40 (m, 1H), 3.55 (d, 1H, J=5.2, 8.4Hz), 3.77-3.90 (m, 1H), 4.10 (q, 2H, J=7.4Hz), 4.84 (s, 1H), 7.32-7.50 (m, 5H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.7(CH_3), 14.2(CH_3), 25.6(CH_2), 33.0(CH_2), 37.1(CH_2), 60.0(CH_2), 61.0(CH), 61.7(CH), 64.1(C), 74.4(CH), 127.4(CH), 129.4(CH), 129.5(CH), 134.5(C), 167.1(CH), 171.0(CH).

Compound 15c: HPLC-MS r_t =12.4 min (M+1)=359 (M+Na)=381 m/z; IR (neat) ν 3439, 2967, 2930, 2105, 1772, 1731, 1458, 1395, 1373, 1261, 1189, 1104, 1023 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.89 (t, 3H, J=7.2Hz), 1.25 (t, 3H, J=7.5Hz), 1.39-1.53 (m, 1H), 1.54-1.64 (m, 1H), 2.50-2.71 (m, 3H), 3.29 (d, 1H, J=7.5Hz), 3.23-3.33 (m, 1H), 3.79-3.88 (m, 1H), 4.13 (q, 2H, J=7.5Hz), 4.96 (s, 1H), 7.38-7.47 (m, 5H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.4(CH_3), 14.0(CH_3), 25.5(CH_2), 32.8(CH_2), 36.6(CH_2), 58.3(CH_2), 60.9(CH), 61.0(CH), 64.4(C), 74.6(CH), 127.5(CH), 129.1(CH), 129.7(CH), 133.2(C), 167.0(C), 170.8(C).

General procedure for the tandem reduction-aza-Payne rearrangement to compound 16a-c and 17a-c.

A solution of azido-epoxide **14** or **15** (1 mmol) and Et_3P (1.2 mmol, 1.2 equiv., 1.2 mL of 1M solution in THF) in dry THF (5 mL) was stirred at reflux under nitrogen atmosphere. After two hours the reaction was stopped by adding 6M HCl (2 mL), THF was removed under reduced pressure and the residue was diluted with EtOAc. The two phases were separated and

a 6M solution of NaOH was added to the aqueous layer to reach basic pH. The basic aqueous phase was then extracted twice with EtOAc (10 mL), the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure, to give pure compounds **16** and **17.**

Compound 16a : Yield 59%; HPLC-MS r_t =9.6 min (M+1)=323 (M+Na)=345 m/z; IR (neat) ν 3285, 3059, 3032, 1755, 1604, 1495, 1454, 1399, 1355, 1252, 1126, 1027 cm^{-1}; ^{1}H-NMR (200MHz, $CDCl_3$) δ 0.46 (t, 3H, J=7.4Hz), 0.74-0.93 (m, 2H), 1.47 (bs, 1H), 1.87 (bs, 1H), 4.03 (d, 1H, J=15.0Hz), 4.54 (s, 1H), 4.96 (d, 1H, J=15.0Hz), 7.17-7.40 (m, 10H); ^{13}C-NMR (50MHz, $CDCl_3$) δ 10.3 (CH_3), 20.2 (CH), 25.8 (CH_2), 29.7 (CH), 44.3 (CH_2), 67.0 (CH), 85.2 (C), 127.2 (CH), 127.8 (CH), 128.3 (CH), 128.5 (CH), 128.8 (2C, CH), 134.6 (C), 134.9 (C), 171.1 (C).

Compound 17a : Yield 51%; HPLC-MS r_t =9.7 min (M+1)=323 (M+Na)=345 m/z; IR (neat) ν 3401, 2965, 2925, 1745, 1645, 1494, 1454, 1400, 1353, 1273, 1259, 1115, 1072 cm^{-1}; ^{1}H-NMR (200MHz, $CDCl_3$) δ 0.90 (t, 3H, J=7.2Hz), 1.00-1.19 (m, 1H), 1.24-1.48 (m, 1H), 1.73 (bs, 1H), 2.21 (m, 1H), 4.05 (d, 1H, J=15.0Hz), 4.59 (s, 1H), 4.98 (d, 1H, J=15.0Hz), 7.20-7.45 (m, 10H); ^{13}C-NMR (50MHz, $CDCl_3$) δ 11.1 (CH_3), 20.3 (CH), 25.9 (CH_2), 29.7 (CH), 44.5 (CH_2), 68.6 (CH), 86.5 (C), 126.9 (CH), 127.8 (CH), 128.3 (CH), 128.5 (CH), 128.8 (CH), 128.9 (CH), 134.6 (C), 134.9 (C), 169.3 (C).

Compound 16b : Yield 62%; $[\alpha]_D^{20}$ +23.9 *(c=1.5, $CHCl_3$)* ; HPLC-MS r_t =11.2 min (M+1)=337 (M+Na)=359 m/z; IR (neat) ν 3291, 3048, 3026, 2967, 2930, 1749, 1602, 1495, 1454, 1377, 1355, 1252, 1115, 1023 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.45 (t, 3H, J=7.5Hz), 0.73-0.94 (m, 2H), 1.39 (m, 1H), 1.57 (d, 3H, J=7.4Hz), 1.89 (bs, 1H), 4.46 (s, 1H), 4.98 (q, 1H, J=7.4Hz), 7.19-7.37 (m, 10H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 10.2 (CH_3), 19.8 (CH_3), 25.6 (CH), 26.3 (CH_2), 29.7 (CH), 53.1 (CH), 65.3 (CH), 83.8 (C), 127.4 (CH), 127.6 (CH), 128.3 (CH), 128.4 (CH), 128.5 (CH), 128.7 (CH), 139.7 (C), 140.9 (C), 171.5 (C).

Compound 17b: Yield 50%; $[\alpha]_D^{20}$ +20.0 *(c=1.0, $CHCl_3$)*; HPLC-MS r_t =16.9 min (M+1)=337 (M+Na)=359 m/z; IR (neat) ν 3291, 3063, 3033, 2967, 2923, 1748, 1598, 1583, 1495, 1454, 1377, 1218, 1108, 1023 cm^{-1}; ^{1}H-NMR (200MHz, $CDCl_3$) δ 0.85 (t, 3H, J=7.0Hz), 0.88-1.05 (m, 1HCH_3), 1.22-1.45 (m, 1H), 1.58 (d, 3H, J=7.4Hz), 1.59 (bs, 1H),

2.12 (m, 1H), 4.50 (s, 1H), 4.89 (q, 1H, J=7.4Hz), 7.20-7.38 (m, 10H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.2(CH_3), 19.5(CH_3), 23.5(CH), 24.6(CH_2), 29.7(CH), 52.8(CH), 65.7(CH), 87.6(C), 127.1(CH), 127.4(CH), 128.3(CH), 128.6(CH), 128.7(CH), 129.0(CH), 139.4(C), 140.0(C), 170.4(C).

Compound 16c : Yield 78%; HPLC-MS r_t =8.0 min (M+1)=333 (M+Na)=355 m/z; IR (neat) ν 3284, 3063, 3033, 2967, 2923, 1753, 1736, 1495, 1458, 1399, 1377, 1190, 1125, 1023 cm^{-1}; ^{1}H-NMR (200MHz, $CDCl_3$) δ 0.48 (t, 3H, J=7.5Hz), 0.74-0.94 (m, 2H), 1.25 (t, 3H, J=7.2Hz), 1.49 (m, 1H), 1.89 (d, 1H, J=3.0), 2.68 (t, 2H, J=7.5), 3.32-3.42 (m, 1H), 3.86-3.96 (m, 1H), 4.11 (q, 2H, J=7.2Hz), 4.74 (s, 1H), 7.29-7.47 (m, 5H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 10.2(CH_3), 14.1(CH_3), 20.6 (CH), 24.6(CH_2), 29.7(CH), 36.5(CH_2), 59.4(CH_2), 59.8(CH_2), 68.2(CH), 85.3(C), 127.1(CH), 128.5(CH), 128.9(CH), 134.6(C), 167.4(C), 171.0(C).

Compound 17c : Yield 57%; HPLC-MS r_t =8.2 min (M+1)=333 (M+Na)=355 m/z; IR (neat) ν 3439, 2959, 2923, 1742, 1650, 1457, 1414, 1377, 1265, 1101, 1023 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.87 (t, 3H, J=7.0Hz), 1.15 (t, 3H, J=7.2Hz), 1.16-1.30 (m, 2H), 1.63 (bs, 1H), 2.10 (m, 1H), 2.66 (t, 2H, J=7.0), 3.27-3.41 (m, 1H), 3.84-3.98 (m, 1H), 4.10 (q, 2H, J=7.2Hz), 4.74 (s, 1H), 7.20-7.47 (m, 5H); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.0(CH_3), 14.2(CH_3), 20.2(CH), 25.1(CH_2), 27.3(CH), 38.2(CH_2), 58.6(CH_2), 60.1(CH_2), 67.5(CH), 84.9(C), 127.2(CH), 127.5(CH), 128.7(CH), 134.8(C), 167.6(C), 171.3(C).

19: [α] = +38.8 (c=1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.04 (d, 3H, *J* =7.2 Hz), 1.10 (t, 3H, *J* =7.2 Hz), 1.71 (m, 1H), 2.04 (m, 1H), 2.34 (bs, 1H), 3.71 (dt, 1H, *J* =1.2, 3.0 Hz), 4.02 (bs, 1H),), 4.10 (q, 1H, *J* =7.2 Hz), 4.26 (bs, 1H), 4.88 (s, 1H), 5.54 (s, 1H), 6.83 (d, 2H, *J* =7.5 Hz), 7.17 (m, 3H), 7.19-7.40 (m, 8H), 7.59 (d, 2H, *J* =7.5 Hz); ^{13}C-NMR δ 11.1, 19.8, 23.4, 54.8, 59.4, 66.5, 68.7, 72.8, 73.9, 127.3, 127.4, 127.5, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.7, 135.9, 138.2, 139.7, 166.3; IR (neat): 3362, 3063, 2971, 2931, 2103, 1743, 1494, 1453, 1261, 1069 cm^{-1} ; LC-ESI-MS rt 16.4 min., *m/z* 471 (M+1), 493 (M+Na).

20: ^{1}H-NMR ($CDCl_3$): δ 0.90 (t, 3H, J = 7.2 Hz), 1.38 (m, 1H), 1.47 (d, 3H, J = 6.9 Hz), 1.81 (m, 1H), 2.00 (d, 1H, J = 6.9 Hz), 3.28 (dt, 1H, J = 2.7, 7.8 Hz), 3.75 (dd, 1H, J = 6.9, 7.8 Hz), 3.90 (bs, 1H), 4.38 (s, 1H), 4.51 (q, 1H, J = 6.9 Hz), 5.32 (bs, 1H), 7.05-7.59 (m, 15H); ^{13}C-NMR δ 10.1, 19.9, 24.4, 54.4, 62.3, 65.3, 69.3, 71.9, 74.0, 127.3, 127.5, 127.7, 127.8, 128.0, 128.2, 128.3, 128.4, 128.5, 135.6, 139.6, 140.0, 169.0; IR (neat): 3417, 2923, 2853, 2103, 1731, 1456, 1376 cm^{-1}; LC-ESI-MS rt 11.83 min., m/z 963 (2M+1).

X-Ray structure of compound 10a

The crystallographic data for **10a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers **CCDC 286004**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk

3 Microwave-assisted synthesis of 1,3-dioxolanes and oxazolines via Lewis acid catalysed epoxide ring-opening[4]

3.1 Introduction

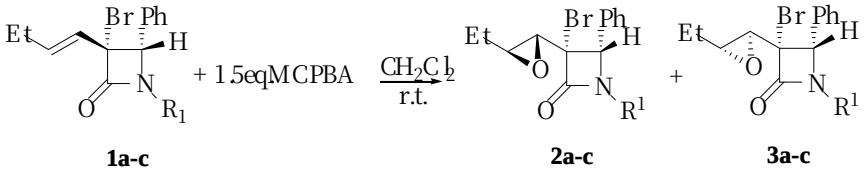
1,3-Dioxolanes are widely used as protecting groups for carbonyl functions and 1,2-diols in the synthesis of naturally occurring compounds, and represent useful intermediates and end-products in pharmaceutical, fragrance and polymer industries[139-141]. Moreover, to date, a variety of chiral cyclic acetals have been designed and employed as chiral auxiliaries, ligands, and catalysts in a broad range of asymmetric reactions[142-144]. Recently, the transformation of epoxides into 1,3-dioxolanes[145] with carbonyl compounds using several Lewis acids and other catalysts, has received increasing interest[146-151]. However, this methodology has been generally applied to terminal epoxides, that are known to be more reactive in respect to disubstituted ones. In these cases, the nucleophilic attack occurs preferentially on the less substituted methylene group, thus avoiding problems of regio- and stereoselectivity. Stereochemically, the reaction is known to proceed with inversion of the configuration at the reacting carbon position[152], while for disubstituted examples, the regioselectivity is strongly influenced by many factors.

The synthesis of 1,3-dioxolane and oxazolines from epoxides is described in this Chapter, as a part of our research focused on the development of new strategies for the introduction of amino and hydroxyl functionalities in the side chains of β-lactams. The protocols reported herein for the microwave-catalyzed transformation of enantiopure and racemic β-lactam-containing epoxides into the corresponding 1,3-dioxolanes and oxazolines are characterised by complete regio- and stereo-selectivity. Microwave assisted organic synthesis[153-156] is a rapidly expanding area of research, since it often offers the opportunity to reduce reaction times from hours to minutes and to increase product yield, performing solvent-free reactions in compliance with the green-chemistry's principles[157].

[4] Results published in *Advanced Synthesis & Catalysis* **2007**, *349*, 1256-1264.

3.2 Synthesis of 1,3-dioxolanes

As previously reported in **Section 2.4**, the treatment of substrates **1a-c** with *meta* chloroperbenzoic acid (MCPBA) afforded the corresponding epoxides **2a-c** and **3a-c** in a 1:1 mixture (**Scheme 3.1**).



1a-c **2a-c** **3a-c**

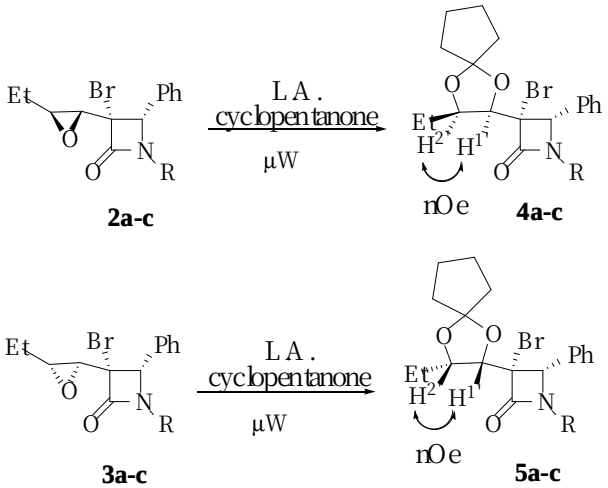
1a R = CH_2Ph
1b R = (*S*)-(-)-$CH(CH_3)Ph$
1c R = CH_2CH_2COOEt

Scheme 3.1 Epoxidation of alkenyl azetidinones **1a-c** with MCPBA leading to compounds **2a-c** and **3a-c**.

The first attempt meant to the preparation of 1,3 dioxolanes involved the reaction of rac **2a** and rac **3a** with an excess of propanone used both as reagent and solvent in the presence of 1 equiv. of BF_3-Et_2O at room temperaature for 48 h. The propanone has been widely used to convert epoxides into the corresponding dioxolanes under BF_3-Et_2O catalysis, while for other ketones only a few examples have been reported. Unfortunately, the reaction with propanone provided a mixture containing the unreacted starting material, the desired acetal and the corresponding diol.

Aiming to reduce reaction times and the formation of undesired derivatives, a microwave-assisted methodology was applied to this reaction. Under these new conditions, the carbonyl compound of choice was the cyclopentanone, more compatible with microwave irradiation but unreactive with our substrates both at room temperature or in refluxing solvents.

Therefore, compounds **2a–c** or **3a–c** and BF_3-Et_2O in a equimolar ratio and cyclopentanone (10 equivs.) were irradiated at 200 Watt for 5 min, giving 1,3-dioxolanes in good yield (**Scheme 3.2**).

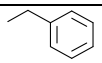
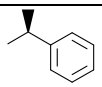
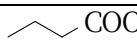
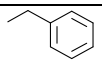
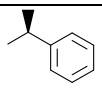
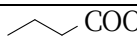


2a-c **4a-c**

3a-c **5a-c**

Scheme 3.2 Lewis acid-catalysed synthesis of 1,3-dioxolanes **4a-c** and **5a-c**.

The reaction occurred under complete regio- and stereo-control, as confirmed by the presence of a single diastereoisomer in the ^{1}H-NMR spectrum of the crude mixture. Although no by-products and no traces of unreacted starting material could be detected in the spectrum, the pure dioxolanes were not isolated by flash chromatography in quantitative yields, probably because of partial microwave-induced decomposition. The results are shown in **Table 3.1**.

Entry	Compound	R	Conversion[a] (%)	Yield[b] (%)
1	4a		90	65
2	4b		>95	60
3	4c		>95	70
4	5a		>95	70
5	5b		>95	60
6	5c		>95	90

[a] Conversion was calculated on the basis of ^{1}H NMR spectra signals.
[b] Yield of isolated product, after purification by chromatography on alumina.
Table 3.1 $BF_3 \cdot OEt_2$-catalysed epoxide ring opening under microwave conditions.

The regio- and stereo-chemistry of the ring opening were rigorously demonstrated. In fact, starting from the trans epoxides **2a** and **3a**, the *cis* acetals **4a** and **5a** were obtained, as established through NOE experiments. Furthermore, the X-ray analysis performed on **5a** confirmed that the ring opening occurred exclusively on the less hindered C2' position, with inversion of the configuration (**Figure 3.1**).

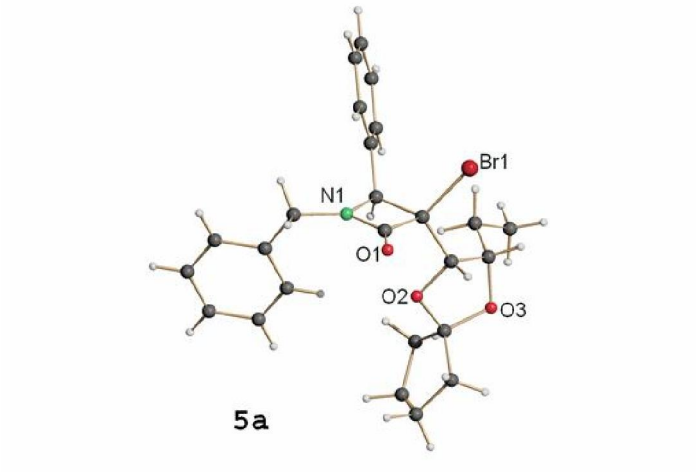
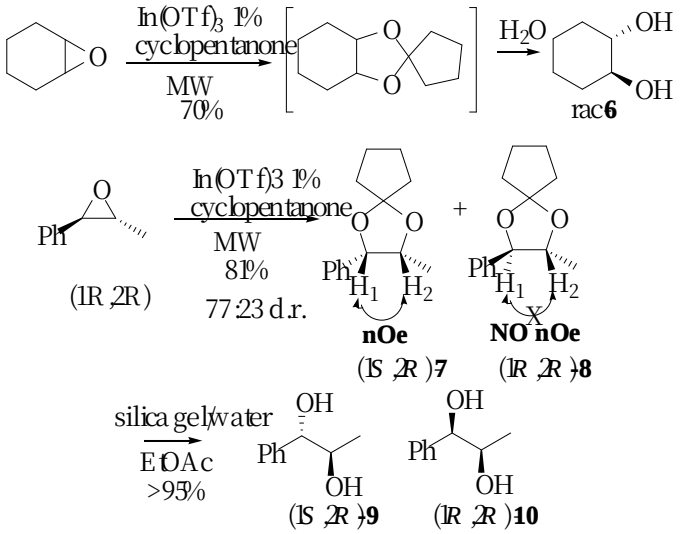


Figure 3.1.

Any attempt to reduce the loading of catalyst failed, probably due to a complexation of the boron trifluoride with the final product that prevent the catalyst recycling. For this reason, $BF_3 \cdot Et_2O$ has been replaced with $In(OTf)_3$, a catalyst with a lower affinity for oxygen[158]. In the last few years, indium complexes have been increasingly employed as catalysts for a variety of organic reactions, because of their fast coordination-dissociation equilibrium[159-161]. In particular, indium(III)bromide has been applied to the stereoselective epoxide ring opening[162], while indium(III)triflate has been recently used in the thioacetalization of carbonyl derivatives[163,164], but it has never been exploited in the transformation of epoxides into dioxolanes.

Therefore, we first tested $In(OTf)_3$ on the very reactive cyclohexene oxide and (1*R*,2*R*)-phenylpropylene oxide under several sets of conditions, varying the loading of the L.A., the irradiation power and the time (**Scheme 3.3**).

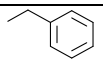
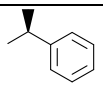
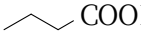
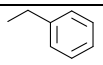
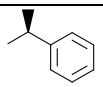
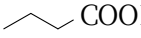
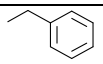
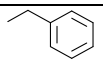


Scheme 3.3 Ring opening of reactive epoxides with cyclopentanone.

Best results were obtained by performing the reaction of racemic cyclohexene oxide in the presence of 1 mol% of $In(OTf)_3$ with an irradiation power of 200 W for 1 min. When the crude reaction was diluted with an organic solvent and filtered on a celite pad, a mixture of diol, hemiacetal and cyclic acetal was obtained as determined by means of GC-MS analysis. On the other hand, when the reaction mixture was submitted to aqueous work-up, the trans-diol was exclusively obtained in 70% yield.

Under the same reaction conditions, (1*R*,2*R*)-phenylpropylene oxide afforded a mixture of cis-**7** and trans-**8** cyclic acetals in a 77:23 ratio and 81% overall yield, after flash chromatography on silica gel. The formation of the minor trans-isomer can be ascribed to the partial racemization on the benzylic position[165]. Since a small difference in the H^1-H^2 vicinal

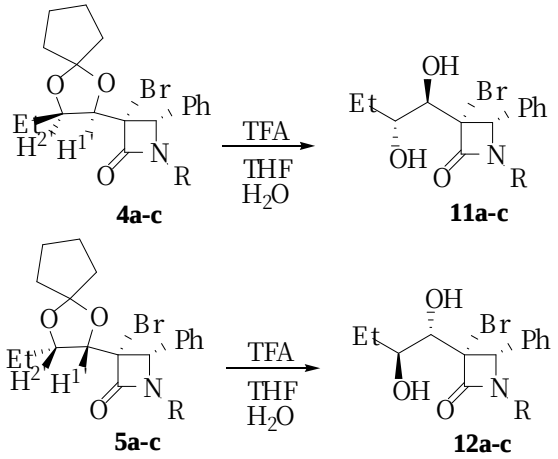
coupling constants of the two diastereomers (7.0 Hz *vs.* 8.4 Hz) was observed, the absolute stereochemistry of **7** and **8** was assigned on the basis of NOESY-1D experiments. Treatment of compounds **7** and **8** with silica gel and water (10 equivs.) in ethyl acetate solution for 2 h, afforded the corresponding diols **9** and **10** in almost quantitative yield. Analytical data for rac-**6**, **9** and **10** were in complete agreement with literature data[166-168]. In a similar way, the microwave induced transformation of **2a–c** and **3a–c** into the corresponding cyclopentanone acetals required 5% of catalyst and an irradiation power of 500 W for 10 min (**Table 3.2**). Under these conditions, satisfactory yields could be obtained and product purification resulted easier. Recently, copper salts have been used as catalysts for electrophilic activation in acylation and acetal formation[169,170]; on the basis of this knowledge, we decided to try a copper catalyst as well. When a 10% amount of $Cu(BF_4)_2 \cdot xH_2O$ was employed for ring opening of epoxides **2a** and **3a**, at 300 W for 10 min, a lower yield of purified compound could be obtained after chromatography on alumina.

Entry	Compound	R	Lewis Acid (%)	Conversion[a] (%)	Yield[b] (%)
1	**4a**		$In(OTf)_3$ (5)	>95	65
2	**4b**		$In(OTf)_3$ (5)	>95	65
3	**4c**		$In(OTf)_3$ (5)	>95	70
4	**5a**		$In(OTf)_3$ (5)	>95	71
5	**5b**		$In(OTf)_3$ (5)	>95	70
6	**5c**		$In(OTf)_3$ (5)	>95	68
7	**4a**		$Cu(BF_4)_2 \cdot xH_2O$ (10)	>95	50
8	**5a**		$Cu(BF_4)_2 \cdot xH_2O$ (10)	>95	62

[a] Conversion was calculated on the basis of ^1H NMR spectra signals.
[b] Yield of isolated product, after purification by chromatography on alumina.
Table 3.2 Lewis acid-catalysed epoxide ring opening under microwave conditions.

The hydrolysis of cyclopentanone acetals to the corresponding diols was carried out under the conditions reported in the literature for similar compounds[171,172]; treatment of **4a–c** and **5a–c** with TFA in THF/H_2O afforded **11a–c** and **12a–c** in good yield (**Scheme 3.4**). Therefore, the

whole reaction sequence from the epoxide to the corresponding diol, *via* the acetal-protected form, has been optimised.

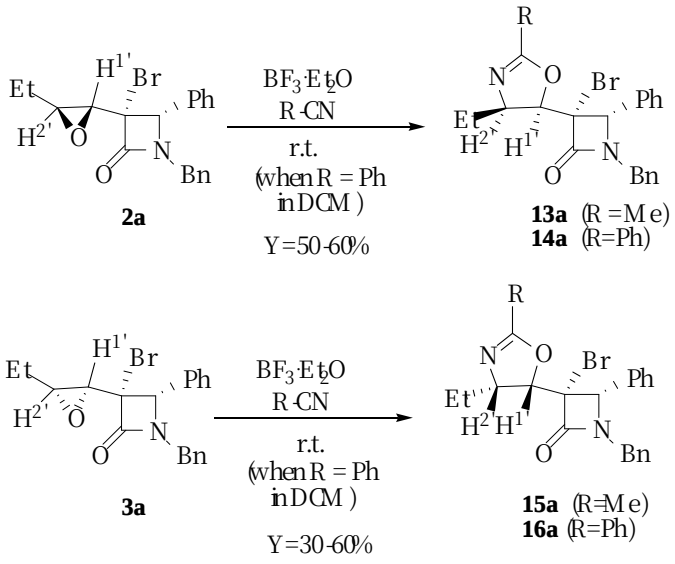


Scheme 3.4 Cleavage of 1,3-dioxolanes **4a-c** and **5a-c**.

3.3 Synthesis of oxazolines

Encouraged by the successful results obtained in the preparation of 1,3-dioxolanes, we subsequently focused on the epoxide ring opening with CH_3CN and PhCN as nucleophiles; the goal was the synthesis of oxazolines, as protected form of amino alcohols[173,174].

The reaction was first carried out on **2a** and **3a** at room temperature in the presence of an equimolar amount of boron trifluoride in CH_3CN (10 equivs.), that is reagent and solvent, or with PhCN (10 equivs.) in DCM, affording **13–16a** in moderate to good yield (**Scheme 3.5**).



Scheme 3.5 $BF_3{\cdot}OEt_2$-catalysed synthesis of oxazolines **13a-14a** and **15a-16a**.

The starting epoxides were recovered unreacted while performing the same reaction in the presence of indium, aluminum or copper salts both at room temperature or in refluxing

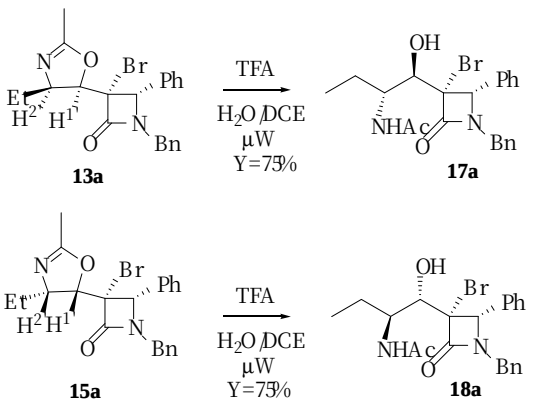
acetonitrile or benzonitrile. However, when compounds **2a** and **3a** were treated with an equimolar amount of $BF_3 \cdot Et_2O$ under microwave irradiation (**Table 3.3**) the corresponding oxazolines were formed in short reaction times (5 min).

Entry	Epoxide	MW power	Reagent	Time (min.)	Product	Conversion [a] [%]	Yield[b] [%]
1	**2a**	Program [c]	CH_3CN	5	**13a**	>95	71
2	**3a**	Program [c]	CH_3CN	5	**15a**	>95	70
3	**2a**	200W	PhCN	5	**14a**	>95	72
4	**3a**	200W	PhCN	5	**16a**	>95	65

[a] Calculated on the basis of ^{1}H NMR spectra signals.
[b] After purification by chromatography on alumina.
[c] Gradient of temperature from r.t. to 80°C in 2.5 minutes, then T = 80°C for 2.5 minutes.

Table 3.3 Microwave-assisted ring opening of epoxides in the presence of equimolar amount of boron trifluoride.

The microwave-assisted reaction of epoxides **2a** and **3a** with acetonitrile in the presence of 1 equivalent of $BF_3 \cdot Et_2O$, was performed using a temperature-controlled irradiation program that avoided reaching of the boiling point (Entries 1 and 2). Oxazolines **13a** and **15a** were isolated in good yield after flash chromatography on silica gel. Compounds **2a** and **3a** were reacted with benzonitrile under microwave irradiation at 200W, affording **14a** and **16a** with complete conversion after 5 min (Entries 3 and 4). When the reaction was performed in the presence of a 10% amount of $In(OTf)_3$, maintaining the same irradiation parameter, a very low conversion was observed. On the other hand, increasing the microwave irradiation power and the reaction times (10 min), complex mixtures of oxazoline and by-products were obtained. In every experiment, the reaction afforded exclusively the oxazoline deriving from the regioselective attack of the nitrile on the less hindered C-2' position. The regiochemistry of the reaction was established by transformation of oxazolines **13a** and **15a** into the corresponding N-acetylamido derivatives **17a** and **18a**, with TFA (10 equivs.) in water/dichloroethane (1:9), under microwave-assisted conditions (300W for 5 min, **Scheme 3.6**). The signal relative to amide proton in the ^{1}H-NMR spectra of **17a** and **18a**, being a doublet coupled with the $H^{2'}$ multiplet, unambiguously clarified the product backbone, thus confirming the regiochemical outcome of the oxazoline formation.



Scheme 3.6 Hydrolysis of oxazolines **13a** and **14a** under microwave conditions.

3.2 Conclusions

The compounds described herein have not been tested yet as CAI or as antibiotics; nevertheless, the value of the protocols for the formation of C-O and C-N bonds in β-lactams lies beyond the bioactivity of these specific molecules. In fact, the interest of this research and its usefulness relies on the development of new procedures that can be applied to a wide class of diverse molecules, with different features, thus allowing to prepare several compounds with the desired properties.

3.3 Experimental Section

General Remarks: All chemicals were purchased from commercial suppliers and used without further purification. Anhydrous solvents were purchased in sure seal bottles over molecular sieves and used without further drying. Flash chromatography was performed on alumina (150 mesh, neutral deactivated) or silica gel (230-400 mesh). NMR Spectra were recorded with 200, 300 or 600 MHz spectrometers. Chemical shifts were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at δ = 7.27 (^{1}H NMR) or δ = 77.0 (^{13}C NMR). Melting points are uncorrected. LC-MS analyses were performed on a liquid chromatograph coupled with an electrospray ionization-mass spectrometer (LC-ESI-MS), using H_2O/CH_3CN as solvent at 25 °C (positive scan 100-500 m/z, fragmentor 70V, gradient elution program from 80% water to 70% acetonitrile in 8 minutes). GC-MS analysis were performed on HP5 (crosslinked 5% PhMe silicone, 30m X 0.32mm X 0.25 µm thikness) using an injection program (initial temperature 50°C for 2 ', then 10°C/min up to 280 °C) in scan mode acquisition. Microwave assisted reactions were performed with a Milestone Mycrosynth multimode apparatus, keeping irradiation power fixed and monitoring internal reaction temperature with a Built-in ATC-FO advanced fiber optic automatic temperature control. The reaction were performed in an open vassel, equipped with a refrigerator connected to fume hood. Cyclohexeneoxide and (1R,2R)-phenyl-propyleneoxide were purchased by commercial source. Compouds **6**, **9** and **10** were fully characterized and their analytical data resulted identical to those reported in the literature.[20]

General procedure for the epoxidation of 3-alkylidene-3-bromo-azetidin-2-ones 1a-c.
To a stirred solution of **1** (1 mmol) in CH_2Cl_2 at r.t., *m*-chloroperbenzoic acid (1.5 equiv., 0.258 g.) was added in one portion. The reaction was stirred overnight and then diluted with a saturated solution of K_2CO_3 (5 mL) and CH_2Cl_2 (5 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **2** and **3** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 9/1 as eluant).

2a: HPLC-MS r_t=14.5 min (M+1)=386/388 (M+Na)=408/410 m/z; IR (nujol) ν 3073, 2959, 2930, 1771, 1654, 1455, 1395, 1355, 1157, 1124, 1077 cm^{-1}; ^{1}H-NMR (600MHz, $CDCl_3$) δ 1.05 (t, 3H, J=7.2Hz, CH_3), 1.53-1.77 (m, 2H, $\underline{CH_2}CH_3$), 3.27 (1H, d, J=2.2Hz, BrCH$\underline{CH}$O),

3.57 (1H, dt, J=2.2, 5.6Hz, $CH_2\underline{CH}O$), 3.91 (d, 1H, J=15.0Hz, $\underline{CH_2}Ph$), 4.78 (s, 1H, CHPh), 4.99 (d, 1H, J=15.0Hz, $\underline{CH_2}Ph$), 7.18-7.43 (m, 10H, CHAr); ^{13}C-NMR (150MHz, $CDCl_3$) δ 9.6, 24.6, 44.9, 58.7, 59.5, 68.4, 127.7, 127.9, 128.2, 128.4, 128.7, 129.1, 133.7, 133.9, 163.5. Calcd for $C_{20}H_{20}BrNO_2$: C, 62.19; H, 5.22; N, 3.63; Found: C, 62.21; H, 5.23; N, 3.62.

3a: HPLC-MS r_t = 15.2 min (M+1) = 386/388 (M+Na) = 408/410 m/z; IR (neat) ν 2962, 2928, 1775, 1494, 1454, 1392, 1351, 1147, 1072 cm^{-1}; ^{1}H-NMR (600MHz, $CDCl_3$) δ 1.02 (t, 3H, J=7.6Hz, CH_3), 1.45-1.82 (m, 2H, $\underline{CH_2}CH_3$), 2.89 (1H, dt, J=1.8, 5.6Hz, $CH_2\underline{CH}O$), 3.45 (1H, d, J=1.8Hz, $BrC\underline{H}CHO$), 3.93 (d, 1H, J=15.0Hz, $\underline{CH_2}Ph$), 4.59 (s, 1H, CHPh), 4.97 (d, 1H, J=15.0Hz, $\underline{CH_2}Ph$), 7.16-7.44 (m, 10H, CHAr); ^{13}C-NMR (150MHz, $CDCl_3$) δ 9.4, 24.2, 44.9, 57.3, 58.9, 59.5, 68.8, 127.8, 127.9, 128.1, 128.4, 128.7, 128.9, 133.8, 134.0, 164. Calcd for $C_{20}H_{20}BrNO_2$: C, 62.19; H, 5.22; N, 3.63; Found: C, 62.17; H, 5.24; N, 3.66.

Boron trifluoride catalyzed synthesis of acetals 4a-c and 5a-c under microwave assisted conditions.

Epoxide **2** or **3** (1 mmol) was diluted in cyclopentanone (10 equiv., 10 mmol, 0.88 mL) and BF_3:Et_2O (1 equiv., 1 mmol, 0.123 mL) was added in one portion. The mixture was submitted to microwave irradiation (Power 200W) for five minutes and then was diluted with ethyl acetate (20 mL) and washed twice with water (20 mL). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **4** and **5** were isolated by flash chromatography on alumina (cyclohexane/ethyl acetate 98/2 as eluant).

Indium triflate catalyzed synthesis of acetals 4a-c and 5a-c under microwave assisted conditions.

Epoxide **2** or **3** (1 mmol) was diluted in cyclopentanone (10 equiv., 10 mmol, 0.88 mL) and $In(OTf)_3$ (0.05 equiv., 0.05 mmol, 28 mg) was added in one portion. The mixture was submitted to microwave irradiation (Power 500W) for ten minutes and then was diluted with ethyl acetate (20 mL) and washed twice with water (20 mL). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **4** and **5** were isolated by flash chromatography on alumina (cyclohexane/ethyl acetate 98/2 as eluant).

4a: HPLC-MS r_t = 23.3 min (M+1) = 470/472 (M+Na) = 492/494 m/z; IR (neat) ν 3087, 3064, 3031, 2964, 2931, 2874, 2356, 2323, 2252, 1772, 1655, 1616, 1496, 1455, 1395, 1115 cm^{-1};

^{1}H-NMR (300MHz, $CDCl_3$) δ 1.07 (t, 3H, J=7.5Hz, $CHCH_2\underline{CH_3}$), 1.65-1.80 (m, 6H, CH_2 cicloppentane), 1.87-2.04 (m, 3H, $CH\underline{CH_2}$ + CH_2 cicloppentane), 2.22-2.38 (m, 1H, $\underline{CH_2}CH_3$), 3.95 (1H, d, J=15Hz, CH_2Ph), 4.06 (m, 1H, $O\underline{CH}CH_2CH_3$), 4.14 (d, 1H, J=6.6Hz, $BrC\underline{CH}O$), 4.95 (s, 1H, CHPh), 6.00 (d, 1H, J=15Hz, CH_2Ph), 7.14-7.49 (m, 10H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.3, 23.4, 23.5, 23.6, 37.3 (2C), 44.5, 63.3, 63.6, 79.7, 80.8, 118.8, 127.8, 128.1, 128.4, 128.8, 128.9, 129.0, 134.3, 139.5, 168.4. Calcd for $C_{25}H_{28}BrNO_3$: C, 63.83; H, 6.00; N, 2.98; Found: C 63.85; H, 6.01; N, 2.94.

5a: HPLC-MS r_t =13.28 min (M+1)=470/472 m/z; IR (nujol) ν 2930, 2874, 2855, 1777, 1655, 1637, 1457, 1425, 1215, 1151, 1109, 1068 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.98 (t, 3H, J=7.4Hz, $CHCH_2\underline{CH_3}$), 1.20-1.90 (m, 10H, $CH\underline{CH_2}$ + CH_2 cicloppentane), 3.74 (1H, d, J=14.8Hz, CH_2Ph), 4.04 (m, 1H, $O\underline{CH}CH_2CH_3$), 4.61 (d, 1H, J=6.4 Hz, $BrC\underline{CH}O$), 4.93 (d, 1H, J=14.8Hz, CH_2Ph), 4.94 (s, 1H, CHPh), 7.15-7.60 (m, 10H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.3, 23.4, 23.5, 24.0, 36.1, 36.8, 44.4, 60.8, 69.1, 78.0, 79.7, 118.8, 128.3, 128.6, 128.9, 129.1, 129.3, 129.5, 133.6, 134.4, 165.3. Calcd for $C_{25}H_{28}BrNO_3$: C, 63.83; H, 6.00; N, 2.98; Found: C 63.80; H, 5.97; N, 2.97.

Indium triflate catalyzed synthesis of 6, 7 and 8 under microwave assisted conditions. Hydrolysis of 7 and 8.

Cyclohexene oxide (1 mmol) or (1*R*,2*R*)-phenyl-propyleneoxide (1 mmol) was diluted in cyclopentanone (10 equiv., 10 mmol, 0.88 mL) and $In(OTf)_3$ (0.01 equiv., 0.01 mmol, 6 mg) was added in one portion. The mixture was submitted to microwave irradiation (Power 200W) for one minute and then was diluted with ethyl acetate (20 mL) and washed twice with water (20 mL). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **6** or **7-8** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 98/2 as eluant). Treatment of **7-8** with H_2O (10 equiv, 0.18 mL) and silica gel (0.1 g) in EtOAc (5 mL) allowed to isolate, after filtration of the solid catalyst and solvent removal, diols **9** and **10** in almost quantitative yield. Analytical data for *rac* **6**, (1*S*,2*R*)**9** and (1*R*,2*R*)**10** were in agreement with the data reported in the literature.[20]

(1*S*,2*R*)-7: $[\alpha]^{20}_D$ +14.1 (*c=9, $CHCl_3$*); GC-MS (EI) r_t =16.52 min, *m/z* 218 (10), 189 (65), 174 (100), 117 (80), 105 (82), 91 (95); IR (neat) ν 3582, 3064, 3029, 2968, 1949, 1495, 1453, 1332, 1110 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.87 (t, 3H, J=6.3Hz, $CH\underline{CH_3}$), 1.78-2.29 (m,

8H, CH_2 ciclopentane), 4.49 (dq, 1H, J=6.3, 6.6Hz, C<u>H</u>CH_3), 5.10 (d, 1H, J=6.6 Hz, <u>CH</u>Ph), 7.25-7.45 (m, 5H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 16.4, 23.1, 24.0, 36.5, 36.7, 74.1, 80.1, 118.0, 126.7, 127.5, 127.9, 138.4. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31; Found: C 77.07; H, 8.28.

(1*R*,2*R*)-8 : $[\alpha]^{20}_D$ +74.6 *(c=3.4, $CHCl_3$)* ; GC-MS (EI) r_t =16.25 min, *m/z* 218 (20), 189 (80), 174 (65), 117 (100), 105 (90), 91 (95); IR (neat) ν 3629, 3088, 2872, 1950, 1654, 1494, 1433, 1433, 1367, 1207 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.35 (t, 3H, J=6.0Hz, CH<u>CH_3</u>), 1.78-2.20 (m, 8H, CH_2 ciclopentane), 3.87 (dq, 1H, J=6.0, 8.4Hz, C<u>H</u>CH_3), 4.43 (d, 1H, J=8.4 Hz, <u>CH</u>Ph), 7.20-7.45 (m, 5H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 16.2, 23.3, 23.5, 37.5(2C), 79.2, 84.7, 118.5, 126.4, 128.0, 128.4, 137.9. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31; Found: C 77.00; H, 8.33.

General procedure for the hydrolysis of acetals 4a-c and 5a-c.

To a stirred solution of **4** or **5** (1 mmol) in THF/H_2O (1/1 solution, 5 mL) at r.t., trifluoroacetic acid (10 equiv., 10 mmol, 0.74 mL) was added in one portion. The reaction was stirred overnight and then THF was removed under reduced pressure. The acqueous residue was diluted with water (5 mL) and extracted twice with CH_2Cl_2 (10 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **11** and **12** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 7/3 as eluant).

11a: HPLC-MS r_t =9.36 min (M-HBr+1)=324 m/z ; IR (neat) ν 3487, 2934, 2911, 2874, 2350, 2321, 2199, 1744, 1650, 1600, 1496, 1425, 1381, 1112 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.88 (t, 3H, J=7.5Hz, CHCH$_2$<u>CH_3</u>), 1.56-1.80 (m, 2H, CH<u>CH_2</u>CH_3), 3.64 (m, 1H, <u>CH</u>CH_2CH_3), 3.85 (1H, d, J=15.5Hz, CH_2Ph), 4.49 (s, 1H, CHPh), 4.61 (d, 1H, J=15.5Hz, CH_2Ph), 4.78 (d, 1H, J=6.5 Hz, BrC<u>H</u>O), 7.21-7.65 (m, 10H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 9.0, 26.1, 30.3, 50.2, 66.6, 83.0, 100.0, 127.7, 129.7, 129.8, 129.9, 130.0 (2), 130.7, 131.6, 161.0. Calcd for $C_{20}H_{22}BrNO_3$: C, 59.42; H, 5.48; N, 3.46; Found: C 59.41; H, 5.44; N, 3.49.

12a: HPLC-MS r_t =9.57 min (M+1)=404/406 (M+Na)=426/428 m/z ; IR (neat) ν 3352, 2945, 2924, 2853, 2095, 1712, 1673, 1614, 1456, 1377, 1112 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.03 (t, 3H, J=7.2Hz, CHCH$_2$<u>CH_3</u>), 1.45-1.80 (m, 2H, CH<u>CH_2</u>CH_3), 2.40-2.80 (bs,

2H, OH), 3.59 (d, 1H, J=7.5 Hz, BnCCHO), 3.78 (m, 1H, CHCH$_2$CH$_3$), 3.95 (1H, d, J=15 Hz, CH$_2$Ph), 4.76 (s, 1H, CHPh), 4.96 (d, 1H, J=15 Hz, CH$_2$Ph), 7.26-7.43 (m, 10H, CHAr); ^{13}C-NMR (75MHz, CDCl$_3$) δ 9.6, 25.1, 30.3, 45.3, 64.9, 74.3, 90.7, 128.0, 128.3, 128.5, 128.6, 129.0, 129.1, 130.0, 133.8, 171.2. Calcd for $C_{20}H_{22}BrNO_3$: C, 59.42; H, 5.48; N, 3.46; Found: C 59.40; H, 5.47; N, 3.45.

Boron trifluoride catalyzed synthesis of oxazolines 13-16a under traditional conditions.

Epoxide **2a** or **3a** (1 mmol) was diluted in CH_2Cl_2 (10 mL) and acetonitrile or benzonitrile (10 equiv.) and BF_3·Et_2O (1 equiv., 1 mmol, 0.123 mL) were added in one portion. The mixture was stirred ar room temparature for four hours and then was washed twice with water (20 mL). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **13-16a** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

Boron trifluoride catalyzed synthesis of oxazolines 13-16a under microwave assisted conditions.

Epoxide **2a** or **3a** (1 mmol) was diluted in acetonitrile or benzonitrile (10 equiv.) and BF_3·Et_2O (1 equiv., 1 mmol, 0.123 mL) was added in one portion. The mixture was submitted to microwave irradiation (for acetonitrile: gradient of temperature from r.t. to 80 °C in 2.5 minutes, then T = 80 °C for 2.5 minutes; for benzonitrile: power 200 W for 5 minutes) and then was diluted with ethyl acetate (20 mL) and washed twice with water (20 mL). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **13-16a** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

13a: HPLC-MS r_t = 10.25 min (M+1) = 427/429 m/z ; IR (neat) ν 2973, 2923, 1771, 1699, 1635, 1558, 1506, 1456 cm^{-1}; ^{1}H-NMR (300MHz, CDCl$_3$) δ 1.08 (t, 3H, J=7.4Hz, CHCH$_2$CH$_3$), 1.65 (m, 2H, CHCH$_2$CH$_3$), 1.94 (s, 3H), 3.83 (d, 1H, J=15Hz, CH$_2$Ph), 4.02 (m, 1H, NCHCH$_2$CH$_3$), 4.74 (s, 1H, CHPh), 4.81 (d, 1H, J=9Hz, BnCCHO), 4.98 (d, 1H, J=15Hz, CH$_2$Ph), 7.20-7.46 (m, 10H, CHAr); ^{13}C-NMR (75MHz, CDCl$_3$) δ 12.2, 13.9, 24.5, 44.9, 63.4, 69.1, 70.9, 83.2, 128.1, 128.3, 128.5, 128.7, 128.8, 129.3, 132.8, 134.3, 165.1, 167.0. Calcd for $C_{22}H_{23}BrN_2O_2$: C, 61.83; H, 5.42; N, 6.56; Found: C 61.85; H, 5.41; N, 6.52.

14a: HPLC-MS r_t =12.56 min (M+1)=489/491 (M+Na)=511/513 (2M+Na)=999/1001 m/z; IR (neat) ν 2924, 2853, 1771, 1654, 1462 1376, 1344, 1277, 1100, 1088 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.33 (t, 3H, J=7.2Hz, $CHCH_2\underline{CH_3}$), 2.25 (m, 2H, $CH\underline{CH_2}CH_3$), 3.43 (d, 1H, J=15Hz, $\underline{CH_2}Ph$), 4.10 (m, 1H, $N\underline{CH}CH_2CH_3$), 4.66 (d, 1H, J=9 Hz, $BrC\underline{CH}O$), 4.80 (d, 1H, J=15Hz, $\underline{CH_2}Ph$), 4.92 (s, 1H, CHPh), 6.91-7.0 (m, 13H, CHAr), 8.0 (m, 2H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 12.0, 13.0, 24.8, 44.8, 63.6, 70.7, 83.7, 128.0, 128.2, 128.3, 128.5, 128.6, 128.7, 128.9, 129.3, 131.8, 133.9, 134.1, 136.8, 162.1, 164.9. Calcd for $C_{27}H_{25}BrN_2O_2$: C, 66.26; H, 5.15; N, 5.72; Found: C 66.25; H, 5.12; N, 5.74.

15a: HPLC-MS r_t =10.29 min (M+1)=427/429 m/z ; IR (neat) ν 2932, 2875, 1776, 1679, 1547, 1495, 1455, 1387, 1399, 1219 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.06 (t, 3H, J=7.2Hz, $CHCH_2\underline{CH_3}$), 1.78 (m, 2H, $CH\underline{CH_2}CH_3$), 1.81 (s, 3H), 3.68 (d, 1H, J=15Hz, $\underline{CH_2}Ph$), 4.06 (m, 1H, $N\underline{CH}CH_2CH_3$), 4.78 (s, 1H, CHPh), 5.00 (d, 1H, J=15Hz, $\underline{CH_2}Ph$), 5.11 (d, 1H, J=9.6Hz, $BrC\underline{CH}O$), 7.180-7.48 (m, 10H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 12.1, 13.8, 25.6, 44.5, 61.2, 67.5, 70.1, 82.1, 128.1, 128.4, 128.5, 128.8, 129.2, 129.5, 132.9, 134.2, 163.2, 164.6. Calcd for $C_{22}H_{23}BrN_2O_2$: C, 61.83; H, 5.42; N, 6.56; Found: C 61.80; H, 5.43; N, 6.55.

16a: HPLC-MS r_t =12.35 min (M+1)=489/491 (M+Na)=511/513 (2M+Na)=999/1001 m/z; IR (neat) ν 2924, 2853, 1777, 1655, 1495, 1466, 1399, 1363, cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.14 (t, 3H, J=7.2Hz, $CHCH_2\underline{CH_3}$), 1.76 (m, 2H, $CH\underline{CH_2}CH_3$), 3.77 (d, 1H, J=15Hz, $\underline{CH_2}Ph$), 4.28 (m, 1H, $N\underline{CH}CH_2CH_3$), 4.86 (s, 1H, CHPh), 5.01 (d, 1H, J=15Hz, $\underline{CH_2}Ph$), 5.30 (d, 1H, J=9 Hz, $BrC\underline{CH}O$), 7.11-7.57 (m, 15H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.8, 14.1, 22.7, 44.5, 61.6, 70.2, 82.3, 127.7, 127.9, 128.4, 128.5, 128.7, 128.9, 129.3, 129.5, 131.7, 132.0, 132.9, 133.9, 162.3, 164.6. Calcd for $C_{27}H_{25}BrN_2O_2$: C, 66.26; H, 5.15; N, 5.72; Found: C 66.28; H, 5.18; N, 5.69.

General procedure for the hydrolysis of oxazolines 13a and 15a.

To a stirred solution of **13a** or **15a** (1 mmol) in dichloroethane/H_2O (9/1 solution, 5 mL) at r.t., trifluoroacetic acid (10 equiv., 10 mmol, 0.74 mL) was added in one portion. The mixture was submitted to microwave irradiation (Power 300W) for five minutes The solution was diluted with water (5 mL) and extracted twice with CH_2Cl_2 (10 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure.

Compounds **17a** and **18a** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1 as eluant).

17a: HPLC-MS r_t = 8.62 min (M+1) = 445/447 m/z ; IR (neat) ν 3300, 2924, 2853, 1752, 1701, 1654, 1640, 1457, 1376, 1266 cm^{-1} ; ^{1}H-NMR (300MHz, C_6D_6) δ 0.85 (t, 3H, J=7.5Hz, $CHCH_2\underline{CH_3}$), 1.62 (s, 3H), 1.80-2.0 (m, 2H, $CH\underline{CH_2}CH_3$), 3.68 (d, 1H, J=15.3Hz, CH_2Ph), 4.03 (m, 1H, $N\underline{CH}CH_2CH_3$), 4.11 (bs, 1H, $BrC\underline{CH}O$), 4.69 (d, 1H, J=15.3Hz, CH_2Ph), 5.31 (s, 1H, CHPh), 5.32 (d, 1H, J = 8.7 Hz, NH), 6.85-7.25 (m, 10H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.4, 23.3, 26.9, 45.3, 56.6, 60.4, 62.8, 73.0, 127.9, 128.2, 128.3, 128.5, 128.7, 128.8, 134.3, 134.5, 165.4, 172.8. Calcd for $C_{22}H_{25}BrN_2O_3$: C, 59.33; H, 5.66; N, 6.29; Found: C 59.31; H, 5.68; N, 6.31.

18a: HPLC-MS r_t = 8.84 min (M+1) = 445/447 m/z ; IR (neat) ν 3345, 2964, 1758, 1637, 1560, 1400, 1261, 1095, 1023 cm^{-1} ; ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.02 (t, 3H, J=7.5Hz, $CHCH_2\underline{CH_3}$), 1.70-1.85 (m, 2H, $CH\underline{CH_2}CH_3$), 2.14 (s, 3H), 3.60-3.80 (bs, 1H, OH); 4.02 (d, 1H, J=15.3Hz, CH_2Ph), 4.07-4.15 (m, 2H, $N\underline{CH}CH_2CH_3$ + $BrC\underline{CH}O$), 5.00 (s, 1H, CHPh), 5.01 (d, 1H, J=15.3Hz, CH_2Ph), 6.56 (d, 1H, J = 7.8Hz, NH), 7.20-7.60 (m, 10H, CHAr); ^{13}C-NMR (75MHz, $CDCl_3$) δ 11.4, 23.3, 26.9, 45.3, 56.6, 60.4, 62.8, 73.0, 127.9, 128.2, 128.3, 128.5, 128.7, 128.8, 134.3, 134.5, 165.4, 172.8. Calcd for $C_{22}H_{25}BrN_2O_3$: C, 59.33; H, 5.66; N, 6.29; Found: C 59.36; H, 5.65; N, 6.26.

X-ray Crystallographic Study:

Crystal data for **2a** : $C_{20}H_{20}BrNO_2$, M = 386.28, monoclinic P21/c, a = 8.8729(11), b = 19.734(2), c = 21.236(3) Å, β = 92.613(2), V = 3714.5(8)Å3, Z = 8, ρx = 1.381 Mgm-3, μ = 2.224 mm-1, F(000) = 1584, T = 296(2) K, θmax = 25.40, 34970 reflections collected, 4235 I>2σ(I). Final R1 = 0.0474, wR2 = 0.1260, GOF = 0.769.CCDC 286004. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 286004. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: int. code + 44(1223)336-033; E-mail: deposit@ccdc.cam.ac.uk].

Crystal data for **5a** : $C_{25}H_{28}BrNO_3$, M = 470.39, triclinic P-1, a = 10.3810(10), b = 10.9710(10), c = 12.426(2)Å, α = 98.500(2), β = 106.067(2), γ = 117.1720(10), V = 1145.9(2)Å3, Z = 2, ρx = 1.363 Mgm-3, μ = 1.819 mm-1, F(000) = 488, T = 296(2) K, θmax

= 28.69, 8540 reflections collected, 3629 I>2σ(I). Final R1 = 0.0409, wR2 = 0.1088, GOF = 1.028.CCDC 621038. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 621038. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: int. code + 44(1223)336-033; E-mail: deposit@ ccdc.cam.ac.uk].

4 Synthesis of 5-hydroxy isoxazolidine-4-carboxylate via tandem Michael addition-intramolecular hemiketalisation

4.1 Introduction

The Michael addition of nitrogen containing nucleophiles to electron-deficient olefins represents the most employed and versatile method of C-N bond construction in organic chemistry. Since this reaction often results in the generation of new stereocenters, great efforts have been devoted to develop asymmetric protocols in the past decades[175,176]. Amine conjugate addition's enantioselective versions have been described with chiral Lewis acids[177-184] and, more recently, with organocatalysts[185-191].

Alkylidene malonates have been intensively employed as Michael acceptor in the past[189,192-199]; a chiral Lewis acid-catalysed Michael addition of hydroxylamino derivatives to alkylidene malonates has been reported by our group[200] as well. The use of acetoacetates in this field is rather unusual instead, and it has the advantage of introducing a reactive keto-functionality that may be further elaborated.

Herein, we describe the highly stereocontrolled synthesis of 5-hydroxyisoxazolidine-4-carboxylate through a Lewis acid induced Michael addition of hydroxilamino derivatives to alkylidene acetoacetates, followed by intramolecular hemiacetal formation.

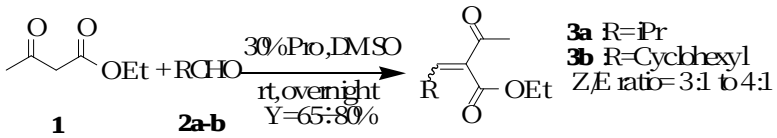
An organocatalytic synthesis of 5-hydroxyisoxazolidine has been previously reported by Cordova et al.[201]; our contribution concerns the description of a novel and straightforward Lewis acid-catalysed protocol and the study of more complex substrates, with a carboxylate functionality in position 4.

5-hydroxyisoxazolidine-4-carboxylate may be indeed regarded as unusual constrained β-amino acids[202,203] or as furanose mimetics[204-206], and have been exploited in natural product analogues syntheses before now[207-214].

4.2 Preparation of alkylidene acetoacetates via Knoevenagel reaction

Alkylidene acetoacetates have been prepared through a Knoevenagel reaction between ethyl acetoacetate and various aldehydes in the presence of a catalytic amount of proline (**Scheme**

4.1) . Proline was chosen among other bases since it proves to give the best results both in terms of yields (ranging from 65% to 80%) and of Z/E selectivities (Z/E ratios ranging from 3:1 to 4:1).



1 **2a-b**

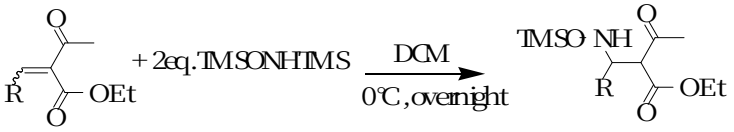
Scheme 4.1 Knoevenagel reaction between acetoacetate **1** and aldehydes **2a-b** .

The stereochemical attribution on compounds **3a-b** has been made on the basis of DPFGSE-nOe experiments; the major isomer exhibits a strong nOe effect between the vinylic H and the ketone's CH_3, while the minor isomer shows a strong nOe effect between the vinylic H and the ester's CH_2.

Knoevenagel products are highly reactive compounds because of their low energy LUMOs and therefore have been extensively used as Michael acceptors[215].

4.3 Michael addition of hydroxylamino derivatives to alkylidene acetoacetates

The conjugate addition of bis-(N,O)-trimethylsilyl hydroxylamine to alkylidene acetoacetates was first examined in the absence of catalysts (**Scheme 4.2**). The reactions needed a night time to be completed, and the *syn* and *anti* 1,4-adduct were obtained in 60/40 ratio respectively in good yields. The stereochemical attribution was made comparing ^{1}H-NMR coupling constants of compounds **4a-b** with literature data[216,217] ($J_{(syn)}$=3.6Hz, $J_{(anti)}$=7.2Hz). Unexpectedly, the reaction carried out on (Z) or (E) isomer of **3a-b** gave **4a-b** with the very same diastereomeric ratio.

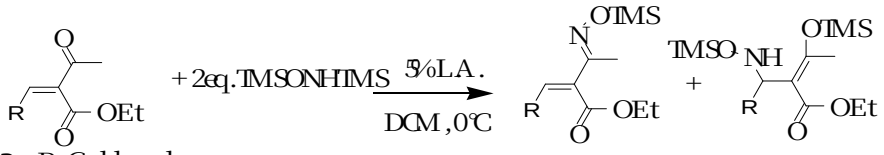


3a: R=iPr
3b: R=Cyclohexyl

4a-b
syn/anti=60/40

Scheme 4.2 Michael addition of TMSONHTMS to alkylidene acetoacetate **3a-b.**

Afterwards, we screened a variety of Lewis acids as catalysts for the conjugate addition of bis-(N,O)-trimethylsilyl hydroxylamine to alkylidene acetoacetate **3a-b.** To our surprise, the expected 1,4-addition product was not observed; on the contrary, the adduct was isolated as a silylenolether derivative **5a-b** , together with a variable amount of 1,2-addition product (**Scheme 4.3 Table 4.1**).



3a: R=Cyclohexyl
3b: R=Ph

5a-b

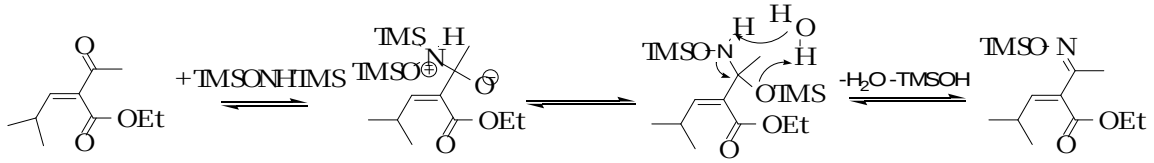
Scheme 4.3 Lewis acids-catalysed Michael addition of TMSONHTMS to alkylidene acetoacetate **3a-b.**

Entry	R	L.A.	Time	%5[a]
1[b]	iPr	$Sc(OTf)_3$	4h	50
2	iPr	$Cu(OTf)_2$	3h	65
3	iPr	$Yb(OTf)_3$	5h	100
4	iPr	$BF_3{\cdot}OEt_2$	5h	50
5	iPr	$Mg(OTf)_2$	5h	-
6	Cyclohexyl	$Sc(OTf)_3$	4h	50
7	Cyclohexyl	$Cu(OTf)_2$	3h	60

Table 4.1
[a] Amount of compound **5** was calculated on the basis of integration ^{1}H NMR signals in the crude reaction spectrum.
[b] The same reaction performed at -20 °C gave only 20% of compound **5**, major product being the undesired oxime.

The unpredictability of the amount of oxime in repeated proofs prompt us to investigate the factors that control the chemoselectivity of the nucleophilic addition. Clearly, the interaction of alkylidene acetoacetate **4a-b** with the Lewis acid plays a role, increasing the electrophilicity of both carbons in position 2 and 4. At last, we found out that the presence of moisture was critical for the formation of the oxime. It is well known that while imines are hydrolysed in water, oximes are favoured and stable in water[218].

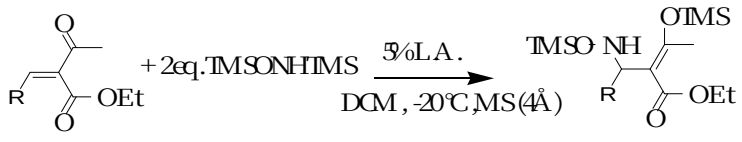
The proposed mechanism of oxime formation is depicted in Scheme 4.4.



Scheme 4.4 Proposed mechanism for oxime formation involving one essential water molecule.

This hypothesis was confirmed by the suppression of the 1,2-addition when molecular sieves (4Å) were used in the reaction. As a matter of fact, when the reaction in Entry 1, **Table 4.1** was repeated with MS, a complete selectivity in favour of the silyl enol ether adduct **5a** was

observed (**Scheme 4.5**, **Table 4.2**). It is noteworthy that despite the decrease of temperature, reactions performed at -20°C with molecular sieves were faster than those at 0°C (See **Table 4.1**).



3a: R=Cyclohexyl
3b: R=Ph

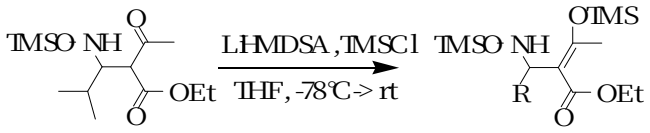
5a-b

Scheme 4.5 Optimised Michael addition of TMSONHTMS to alkylidene acetoacetate **3a-b.**

Entry	R	L.A.	Time	%5[a]
1	iPr	$Sc(OTf)_3$	2h	90
2	iPr	$Cu(OTf)_2$	2h	90

Table 4.2
[a] Amount of compound **5** was calculated on the basis of integration ^{1}H NMR signals in the crude reaction spectrum.

The structure proposed for compound **5a** is consistent with ^{1}H-NMR and ^{13}C-NMR spectra; besides, the product can be prepared from 1,4-adduct **4a** by treatment with LHMDSA and TMSCl (**Scheme 4.6**). Curiously, starting from a 60/40 mixture of *syn*/*anti* isomers of **4a**, the reaction gave one single isomer of **5a**.



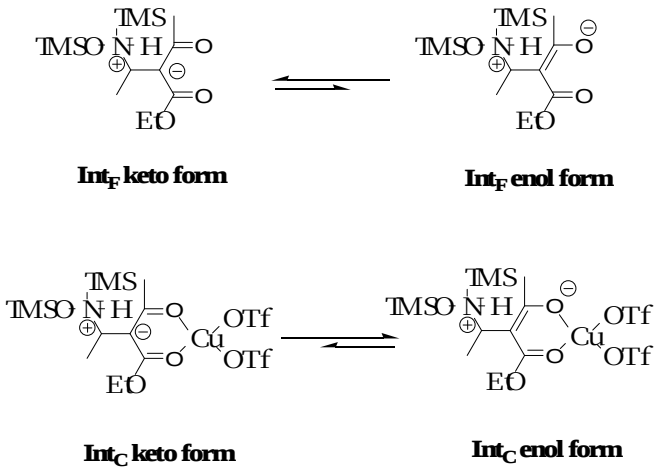
4a syn/anti mixture

5a, one isomer

Scheme 4.6 Conversion of adduct **4a** into silyl enol ether **5a.**

An explanation for the different outcomes of the uncatalysed and catalysed Michael addition may be issued from the minimised structure of the 1,4-addition intermediates (see **Section 4.5** for computational details).

After the C-N bond formation, the molecule presumably is a zwitterion: a positive charge is located on the N, and a negative charge is delocalised on the enolate moiety. The minimised structures of this intermediate, free (**Int$_F$**) or complexed with the Lewis acid (**Int$_C$**), have been calculated (**Scheme 4.7**). The lengths comparison between the C-C and the C-O enolate bonds in these molecules shows that for the free intermediate **Int$_F$** the keto form is prevalent, while for the complexed intermediate **Int$_C$** the enol form predominates (**Figure 4.1**).



Int_F keto form **Int_F enol form**

Int_C keto form **Int_C enol form**

Scheme 4.7 Keto-enolic equilibrium of intermediates **Int_C** and **Int_F**.

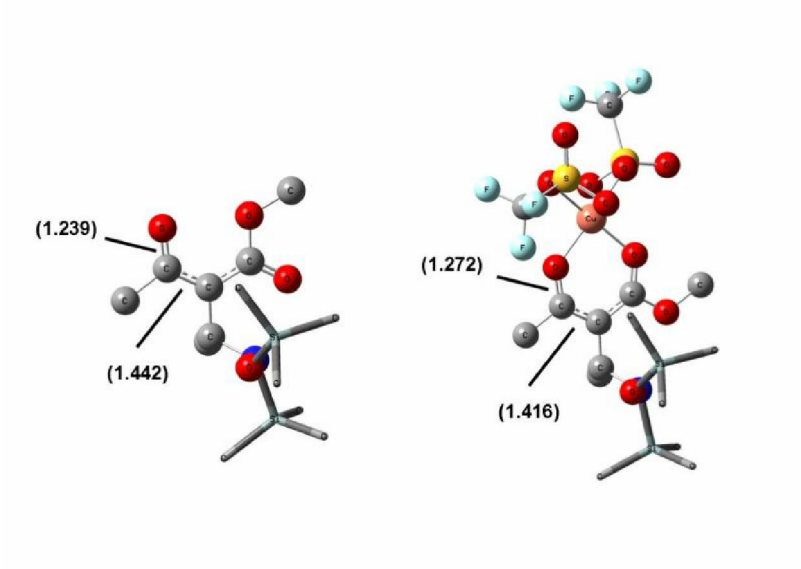
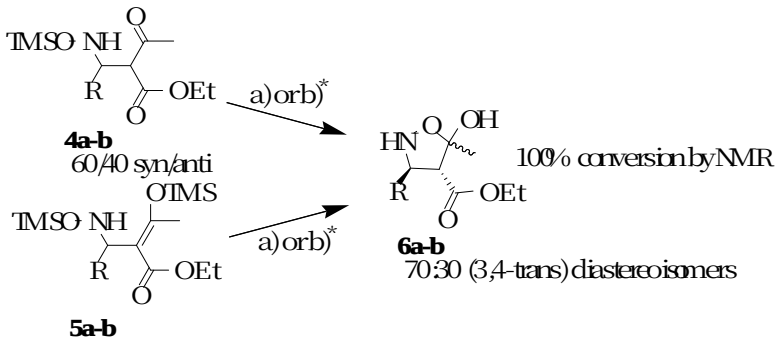


Figure 4.1 Minimised structures of intermediates **Int_C** and **Int_F**.
Interesting bond lengths are shown in Å.
In order to decrease computational time, the molecules have been simplified ($CO_2Et \rightarrow CO_2Me$; $iPr \rightarrow Me$)

Therefore, the intermediate **Int_F** is likely to end up with a proton transfer from the nitrogen atom to the Cα position, thus giving product **4**, while the intermediate **Int_C** may be expected to transfer a TMS group from the nitrogen to the enol oxygen, thus leading to product **5**.

4.4 Intramolecular hemiketalisation

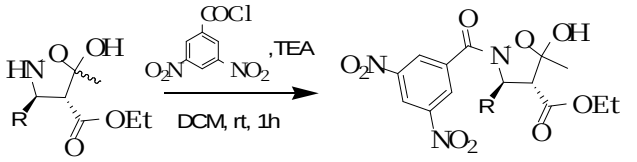
Adducts **4a-b** and silyl enolethers **5a-b** are slowly converted at room temperature into 5-hydroxyisoxazolidine-4-ethylcarboxylates **6a-b.** In both cases, the intramolecular hemiketalisation takes on average 24 hours to be completed. Remarkably, compounds **4a-b** and **5a-b** both gave a 70/30 ratio of (3,4)-*trans* diastereoisomers (J_{3-4}=7.2Hz) of hemiacetals **6a-b,** which differ for C5 stereochemistry (**Scheme 4.8**). To give an insight on the driving forces of this reaction and to explain the observed stereoselectivity, a computational study has been carried out (see **Section 4.5**).



a) 24h, rt; b) SiO_2, DCM, 2h;
*The reactions shown are not stoichiometrically balanced; only isolable species are drawn.

Scheme 4.8 Intramolecular hemiketalisation of compounds **4a-b** and **5a-b.**

It is worth mentioning that the adducts **4a-b** are not the intermediates of products **5a-b** conversion into **6a-b**; in fact, when **5a** transformation has been followed with ^{1}H-NMR analysis and speeded up with the addition of 1 eq. of TBAF, no evidence of **4a** formation arose. The intramolecular hemiketalisation can be also induced by silica gel, or by other acidity source (treatment with acidic water solution or acidic resins). The 5-hydroxyisoxazolidine-4-ethylcarboxylates **6a-b** have been fully characterised by NMR spectrometry (COSY, HETCOR, DEPT, HMQC experiments) and thus their structures have been confirmed (**Scheme 4.9**).



Scheme 4.9 Derivatisation of compounds **6a-b** leading to **7a-b.**

Once again, the derivatisation gave an unexpected result: starting from a 70/30 diastereomeric mixture of **6a-b**, only one (3,4)-*trans* isomer ($J_{3\text{-}4}$=7.2Hz) of **7a-b** was obtained, probably *via* the isomerisation of the hemiacetalic position. A computational study has been performed to rationalise this observation, and the results show that a (3,4)-trans diastereoisomer is unmistakably the most stable (see **Section 4.5**).

4.5 Computational Section

4.5.1 Methods

Geometry optimisations of selected structure were carried out in order to characterize the potential energy surface (PES) associated to the reactions under examination. All the calculations were carried out using the COBRAMM[219] suite of computational programs, used as interface between the Turbomole[220] and the Gaussian 03[221] packages, in order to use the energy and gradient evaluation from the former and the optimisation driver from the latter.

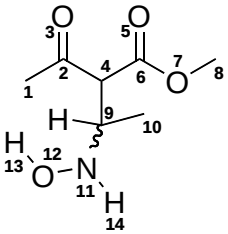
This particular choice was due to need of combining the accurate optimisation algorithms implemented in Gaussian 03 and the Turbomole approximated Density Function Theory (DFT)[81] potential. As a matter of fact the use of the "resolution of identity" (RI)[222,223] and the "multipole accelerated resolution of identity" (MARI)[224] approximations, available in Turbomole for the most common DFT functionals, has been proven to speed-up the calculation of about one order of magnitude with respect to non-approximated DFT calculations with a negligible loss of accuracy. The Gaussian 03 optimisation driver was preferred in this study to the Turbomole's due to its faster convergence in locating both equilibrium (minima) and transition states (TS) of PES, due to the use of a redundant internal coordinates system[89] with the powerful BFGS algorithm.[225-228] The details of the COBRAMM interface have been extensively described in our previous paper;[219] in this particular case it was used to launch and running the Turbomole and Gaussian 03 by taking, at each optimisation step, energy and gradients calculated by the former and yielding them to the latter to obtain the geometry to be used in the following cycle; the iteration stopped when convergence criteria were met. In the original COBRAMM paper[219] this type of calculation was referred to as "high" (H) calculation, being the adopted potential fully quantum mechanical.

The DFT potential was used to carry out all described calculations, using the B3LYP functional referred in Turbomole as "b3-lyp_Gaussian"[229] with the "m3" grid size for the density fitting and a SCF convergence criterion of 1x10-7 E_h. A balanced double ζ split valence (SP) basis set with polarisation functions (P) [referred in Turbomole as def2-SV(P)][230] was adopted to describe H, C, N and O atoms, while the Cu atom was described with a triple ζ split valence (SP) basis set with polarisation functions (P) [referred in Turbomole as def2-TZVP].[230] The proper use of the RI and MARI approximations required the use of an auxiliary basis set.[231] The PES was characterized by means of geometry optimization to locate the chemically interesting critical points, whose nature was ascertained by numerical frequency calculation on the optimised structures. The standard Gaussian 03 convergence criteria have been adopted.

The effect of the solvent has been evaluated with the COSMO[232-234] solvent continuous model approach as implemented in the Turbomole package.[220] Dichloromethane was simulated using a dielectric constant $\varepsilon = 8.930$ and a solvent radius $r = 2.27$ Å. In both cases an "optimised" radius[232-234] was assigned to H, C, N and O atoms, wile a "bondii" radius[232-234] was used for Si and Cu, according to the Turbomole[220] manual (see the "cosmoprep" section for a detailed discussion about atom radius assignment).

4.6 Results and Discussion

The real molecular system under study (see **Scheme 4.8**) has been approximated to the model system called **mod1**, as reported in **Scheme 4.10**, that also provides, for the most important atoms in the molecule, a numbering used throughout the following discussion.



Scheme 4.10 Numbering of atoms in **mod1,** the model system of molecule **4a-b.**

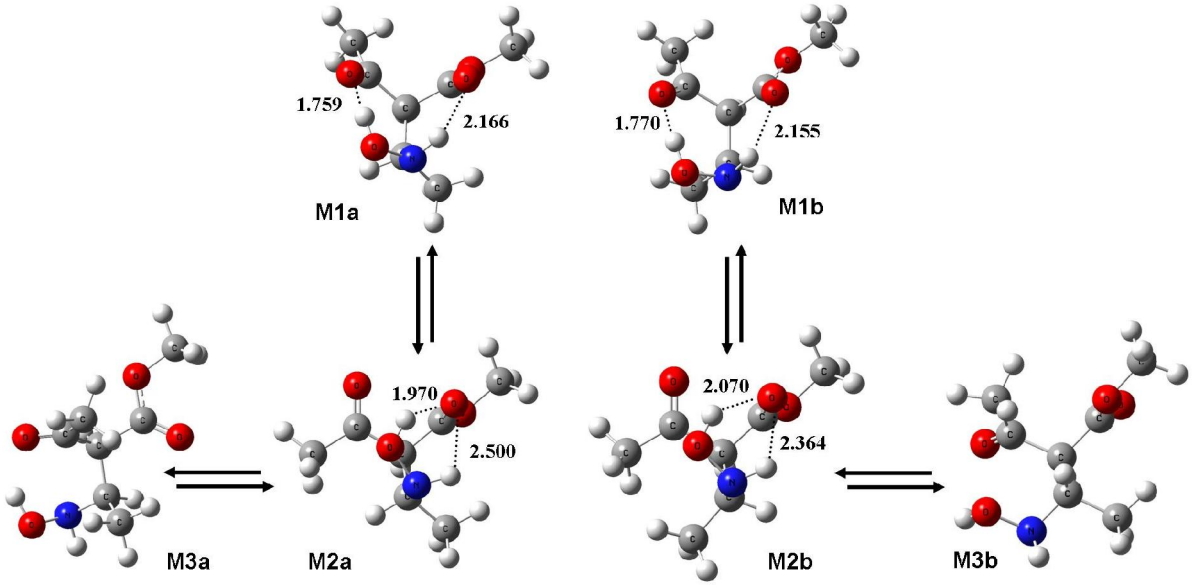


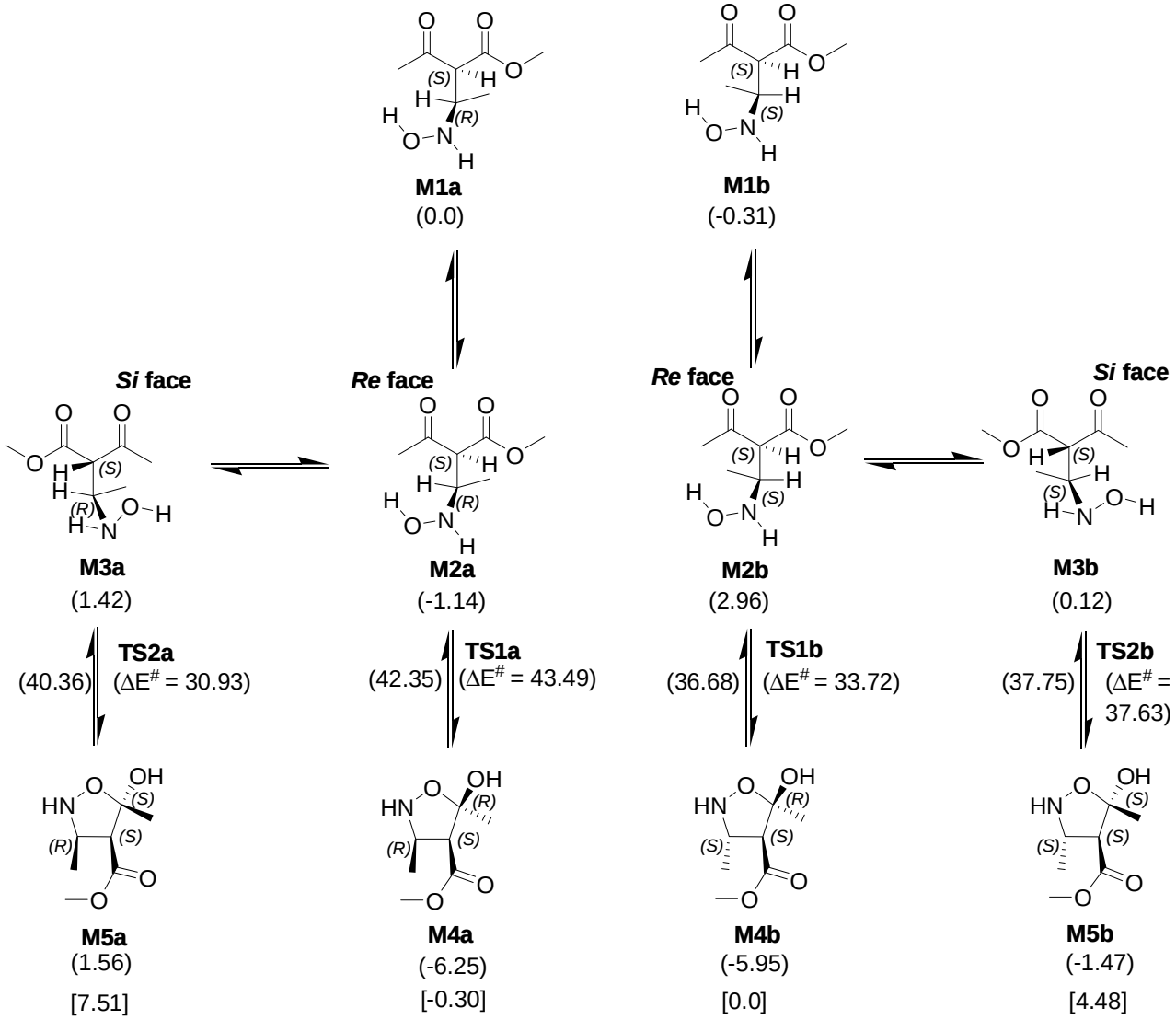
Figure 4.2 Conformers of the S,R (M1a, M2a, M3a) and S,S (M1b, M2b, M3b) isomers.

dihedral	**D1**	**D2**	**D3**
definition	**12(11,9)4**	**3(2,4)6**	**11(9,4)2**
M1a	80.0	-92.8	-69.0
M2a	39.8	10.8	-72.1
M3a	-83.2	152.4	76.5
M1b	76.1	-88.6	-70.0
M2b	41.4	2.3	-68.4
M3b	-74.3	118.7	56.9

Table 4.3 Dihedral angles describing the conformational equilibrium of reactants.

For both the S,R and S,S isomers of reactant tree stable conformers do exist, characterized by a different hydrogen bond pattern. In M1a and M1b (see **Figure 4.2**) H(13) and H(14) are H-bonded, respectively, to O(3) and O(5). A slight rotation around the N(11)-C(9) bond (see

Entry D1 in **Table 4.3**) leads to geometries M2a and M2b, where both H(13) and H(14) are pointing toward the same oxygen atom O(5); the lost interaction with O(3) directs to a rotation of the carbonyl around the C(2)-C(4) bond (see Entry D2 in **Table 4.2**). The four isomers lie in a range of about 4 kcal mol^{-1} (see **Scheme 4.11** and **Table 4.3**). Both M2a and M2b are set for the intramolecular nucleophilic attack of O(12) on C(2) on the *Re* face of the carbonyl. A further exploration of the conformational space of both the S,R and S,S isomers led to two more stable conformers, M3a and M3b, where a rotation around the C(9)-C(4) bond (see Entry D3 in **Table 4.3**) gives structures where the *Si* face of the carbonyl can be attacked by the nucleophilic O(12) atom.



Scheme 4.11 Examined reaction pathways for O(3) nucleophilic attack on *Re* and *Si* face of both the S,R and S,S isomers of the reactant. Round parentheses are used for energy values as referred to M1a, while square parentheses are used for energy values as referred to M4b; all values are reported in kcal mol^{-1}.

	Structure name	Absolute energy (hartree)	Relative energy (kcal mol^{-1})
mod1	M1a	-629.678361	0.00
	M2a	-629.680183	-1.14
	TS1a	-629.610871	42.35
	M4a	-629.688314	-6.25
	M3a	-629.676095	1.42
	TS2a	-629.614046	40.36
	M5a	-629.675875	1.56
	M1b	-629.678856	-0.31
	M2b	-629.673642	2.96
	TS1b	-629.619911	36.68
	M4b	-629.687837	-5.95
	M3b	-629.678177	0.12
	TS2b	-629.618206	37.75
	M5b	-629.680700	-1.47
mod2	M4a_L	-747.535326	1.08
	M5a_L	-747.533427	2.27
	M4b_L	-747.537048	0.00
	M5b_L	-747.529375	4.81
mod3	M4a_D_c1	-1500.372542	6.25
	M4a_D_c2	-1500.359700	14.31
	M5a_D_c1	-1500.367025	9.71
	M5a_D_c2	-1500.357448	15.72
	M4b_D_c1	-1500.382504	0.00
	M4b_D_c2	-1500.371313	7.02
	M5b_D_c1	-1500.376187	3.96
	M5b_D_c2	-1500.366586	9.99

Table 4.4 Absolute and relative[a] energies of selected structures.
[a)] The energies of the **mod1** structures are referred to M1a; the energies of **mod2** structures are referred to M4b_L; the energies of the **mod3** structures are referred to M4b_D_c1.

The intramolecular nucleophilic attack of O(12) to C(2) on the *Re* carbonyl face and the simultaneous proton transfer of H(13) from O(12) to O(3) leads from M2a and M2b to, respectively, the cyclic adducts M4a and M4b. The results of an accurate PES exploration accounts for a sole active path for each M2a and M2b, passing trough the transition states TS1a and TS1b, respectively. In both cases, the nucleophilic attack and the proton transfer appear to be concerted processes (see **Figure 4.3**).

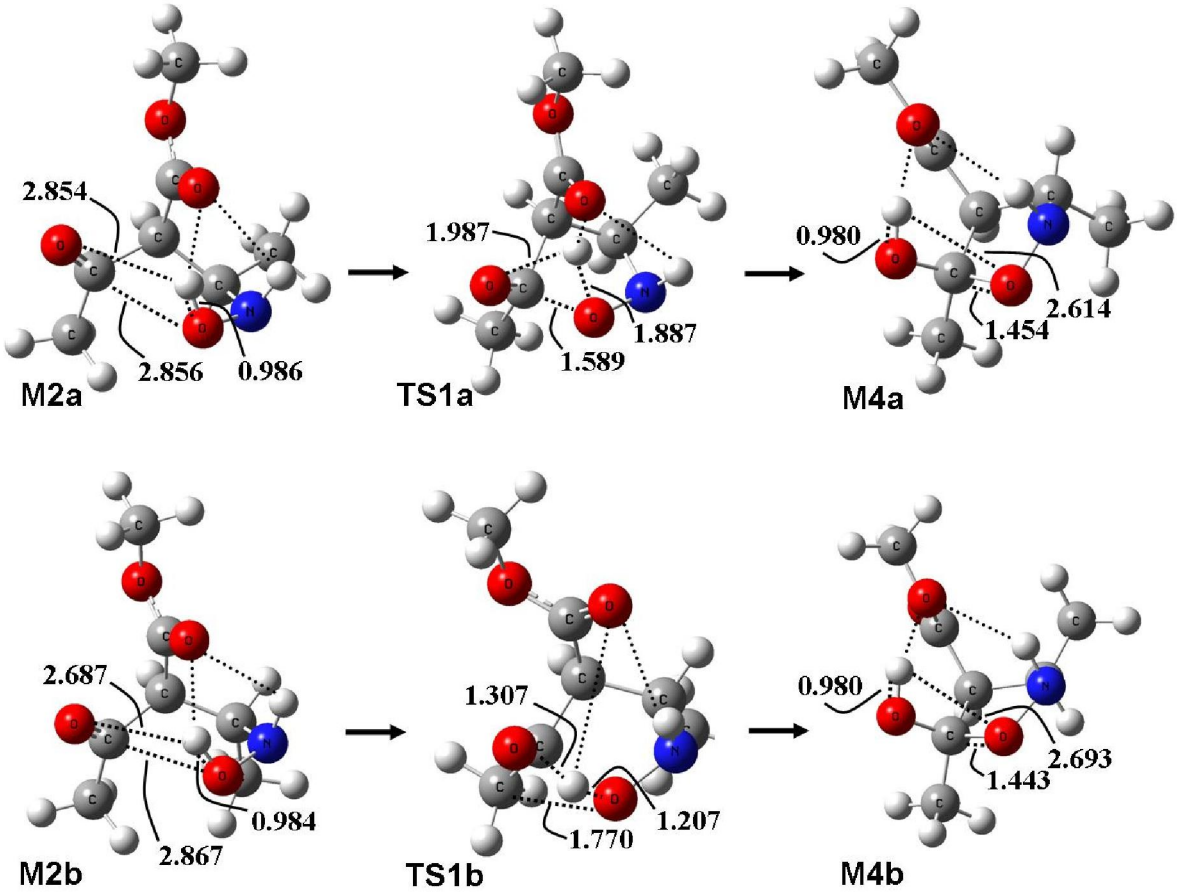


Figure 4.3 Nucleophilic attack on the*Re* face of the carbonyl.

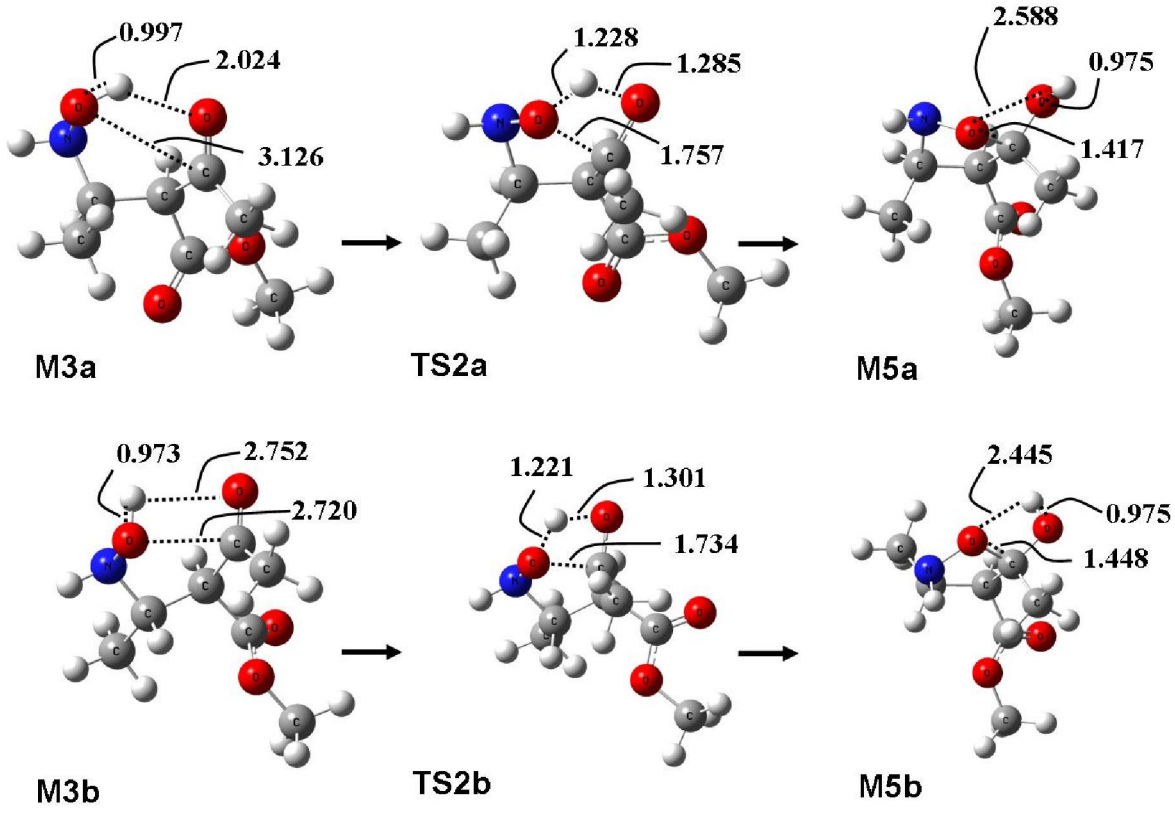


Figure 4.4 Nucleophilic attack on the*Si* face of the carbonyl.

The nucleophilic attack on the *Si* face of the carbonyl has been explored as well, obtaining a couple of active paths leading from M3a and M3b to M5a and M5b, respectively, *via* the transition states TS2a and TS2b (see **Figure 4.4**).

A comparison of the products relative energies (see **Table 4.4**) demonstrates that the two products resulting from the attack on the *Re* face (M4a and M4b) are the more stable, probably due to the intramolecular H-bond between carbonyl group and the hemiacetalic hydroxyl group; this stabilisation is prohibited in M5a and M5b by the unfavourable stereochemistry of C(2). On the basis of the relative activation energies, the pathway leading to M4b is preferred over the one leading to M4a, being the energy of TS1a higher than TS1b's of about 6 kcal mol^{-1}.

To further explore the relative energy of the four heterocycles, we used a larger model system (**mod2**, see **Scheme 4.12a**), coincident with the real one. After geometry optimisations, the M4b_L isomer (see **Scheme 4.12a** and **Figure 4.5**), correspondent to M4a of **mod1** resulted to be most stable of all isomers.

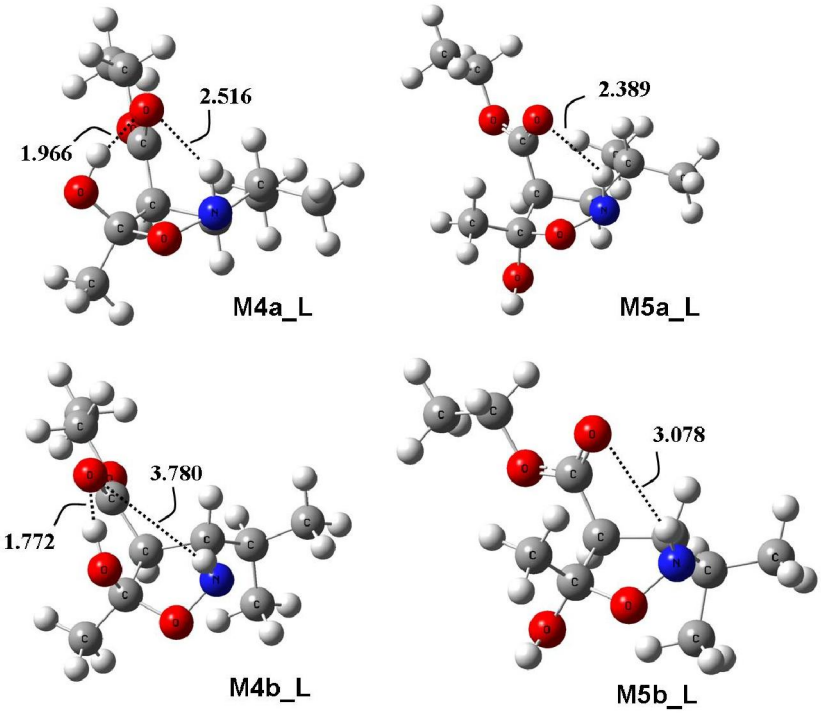


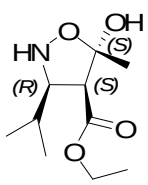
Figure 4.5 Geometries of the four products.

A third model system, called **mod3,** has been used to study the transformation of isoxazolidine **4a** into the N-(3,5)dinitrobenzamide derivative **7a** (see **Scheme 4.9**) and to explain the observed stereoselectivity. These compounds can in principle exist in either *cis* or *trans* configurations in respect to the newly formed amidic bond. All possibility have been explored, observing in every case a strong preference for the *trans* isomers (labelled "c1" in

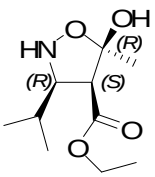
Scheme 4.12b) over the *cis* ones (labelled "c2" in **Scheme 4.12b**). M4b_D_c1 resulted to be the most stable isomer.

On the basis of the calculations carried out on all the adopted model systems we can state that the nucleophilic attack on the *Re* face of the carbonyl is preferred over the attack on the *Si* face. The relative energies of the involved transition states and of the products help in explaining the experimentally observed formation of the (3,4)-trans isomer.

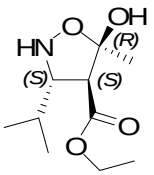
a)



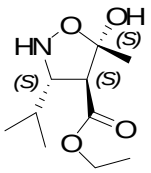
M5a_L
[2.27]



M4a_L
[1.08]

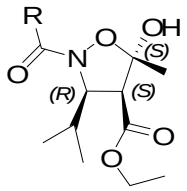


M4b_L
[0.0]

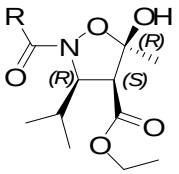


M5b_L
[4.48]

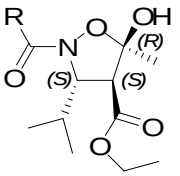
b)



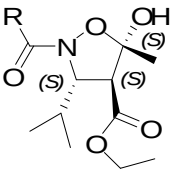
M5a_D_c1
[9.71]



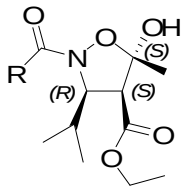
M4a_D_c1
[6.25]



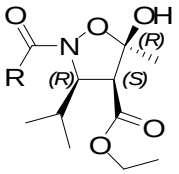
M4b_D_c1
[0.0]



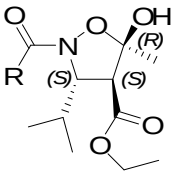
M5b_D_c1
[3.96]



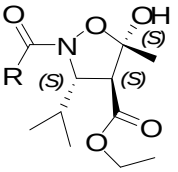
M5a_D_c2
[15.72]



M4a_D_c2
[14.31]



M4b_D_c2
[7.02]



M5b_D_c2
[9.99]

Scheme 4.12 a) Relative energy of the cyclic adducts using a models system coinciding with the experimental one; energy values are referred to M4b_L. b) Relative energy of the cyclic products derivatised as (3,5)dinitrobenzamides; energy values are referred to M4b_D_c1.

5 Synthesis and biological evaluation of non-peptide $\alpha_v\beta_3/\alpha_5\beta_1$ integrin dual antagonists containing 5,6-dihydropyridin-2-one scaffolds[5]

5.1 Introduction

Integrins are a large family of heterodimeric transmembrane glycoproteins involved in the attachment of a cell to the extracellular matrix (ECM) and in signal transduction from the ECM to the cell[235-238]. These adhesion mechanisms are of fundamental importance in a diverse range of biological processes, including cell differentiation, apoptosis, embryonic cell migration, maintenance of tissue integrity, and blood coagulation[239-243]. Alterations or aberrations in integrin-mediated cell adhesion have been connected with the pathogenesis of several diseases such as atherosclerosis, osteoporosis, cancer[244-247] and a variety of inflammatory disorders, making integrins an attractive target for the development of therapeutic agents[248-252]. The identification of key recognition motifs within integrin ligands is the starting point for the development of antagonists. To date, these motifs have been identified for only a few subtypes. $\alpha_v\beta_3$ integrin has been deeply investigated as it is involved-tumor proliferation and metastasis through the formation of new blood vessels. $\alpha_v\beta_3$ integrin binds to a wide number of ECM components like fibronectin, fibrinogen, vitronectin, and osteopontin through recognition of the Arg-Gly-Asp (RGD) tripeptide sequence[253-255]. This sequence is also essential for the binding of $\alpha_5\beta_1$ integrin to fibronectin, which has been unambiguously recognized as proangiogenic receptor[256-258]. $\alpha_5\beta_1$ integrin may regulate the function of integrins $\alpha_v\beta_3$ on endothelial cells during their migration in vitro or angiogenesis in vivo[259]. Activation of $\alpha_5\beta_1$ potentiates $\alpha_v\beta_3$-mediated migration on vitronectin, whereas $\alpha_5\beta_1$ integrin antagonists inhibit $\alpha_v\beta_3$-mediated cell spreading. Therefore, antagonists of both integrins, block the same pathway of angiogenesis. In this paper, we report the design, synthesis, and blockade of fibronectin-mediated cell adhesion of novel $\alpha_v\beta_3/\alpha_5\beta_1$ integrin dual antagonists, whose activity could be synergistically effective in preventing angiogenesis.

[5] Results published in: *Bioorganic & Medicinal Chemistry* **2007**, *15*, 7380-7390.

The X-ray analysis[260] of the complex between $\alpha_v\beta_3$ integrin and c(RGDfV) ligand shows that the ligand interacts mainly through electrostatic interactions. Arg and Asp form a charged clamp that binds regions with opposite charges in the protein: Asp interacts with a metal cation in the β subunit and Arg with two Asp in the α subunit (**Figure 5.1)** .

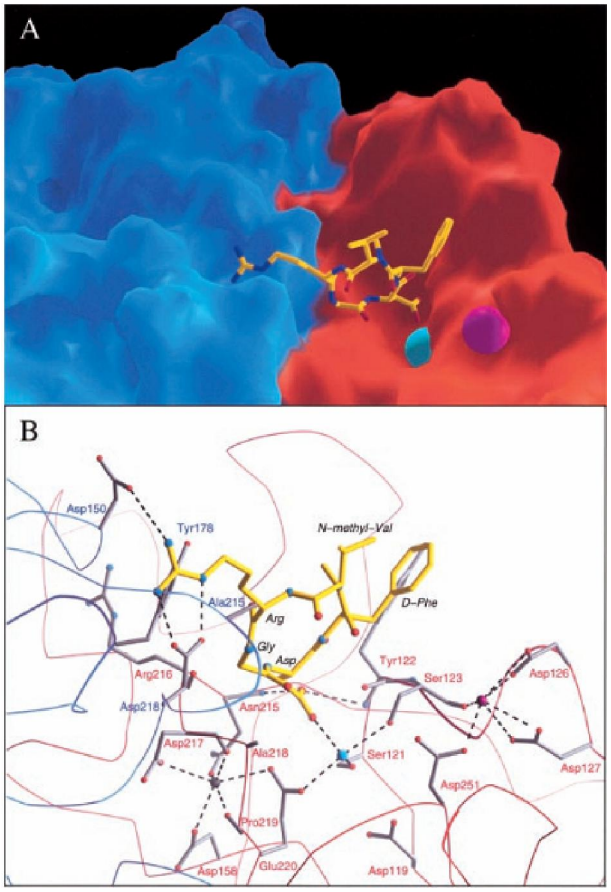


Figure 5.1 The ligand-integrin binding site A) Surface representation; B) Ligand-integrin interactions.

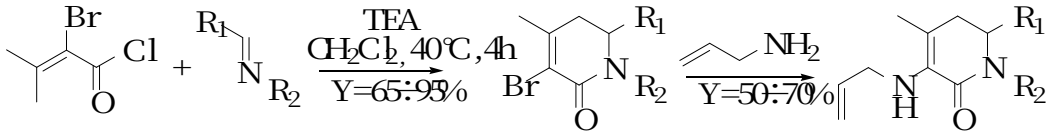
Several efficient classes of ligands, containing the RGD sequence, have been reported in the literature[261-269] . These structures share as common features conformational restraints able to give a proper orientation to the peripheral substituents.

Linear and cyclic peptides containing the RGD sequence, showing high affinity toward αvβ3 integrins, have significant therapeutic potential but serious limitations especially for oral dosing. The need for antagonists with higher bioavailability and lower molecular weight has prompted several research groups to develop small constrained non-peptidic molecules mimicking the RGD motif, which would be more promising for drug development[270-278] .

Most of the structures proposed so far consist of a polyfunctionalized rigid core, linked to appendages corresponding to arginine and aspartic acid side chains[279-281] . The basicity and the length of the arginine-mimicking group was found to play a central role. Moreover, the presence of a carboxylic function, mimicking the aspartic acid residue in the original binding

motif, is a fundamental feature to create anionic interaction with the metal cation in the receptor active site[282]. Many heterocyclic scaffolds have been employed to maintain the acidic and the basic ends of the molecule at the appropriate distance and with the suitable conformation for binding interaction.

We identified the 5,6-dihydropyridin-2-one as scaffold[64,283], easily prepared through a short concise synthesis (see Chapter1, **Scheme 1.4** and herein, **Scheme 5.1**).



1a : R_1=Ph, R_2=$CH_2CH_2CO_2Et$
1b: R_1=PNP, R_2=(*S*)-$CH(CH_3)$PMP
d.r. (6*S*) **1b** / (6*R*) **1b** = 43/57
1c: R_1=Ph, R_2=CH_2PNP
1d: R_1=Ph, R_2=$(CH_2)_5NH_2$

Scheme 5.1. Synthetic route to 5,6-dihydropyridin-2-one **1**.

This heterocycle may be converted into a potential integrin ligand introducing the acidic and the basic appendages as reported in Models A, B, and C (**Figure 5.2**).

To evaluate the biological activity of these novel compounds in a cellular environment, we tested their ability to perturb initial cell attachment mediated by $\alpha_v\beta_3$ integrin and $\alpha_5\beta_1$ integrin using cell adhesion assays.

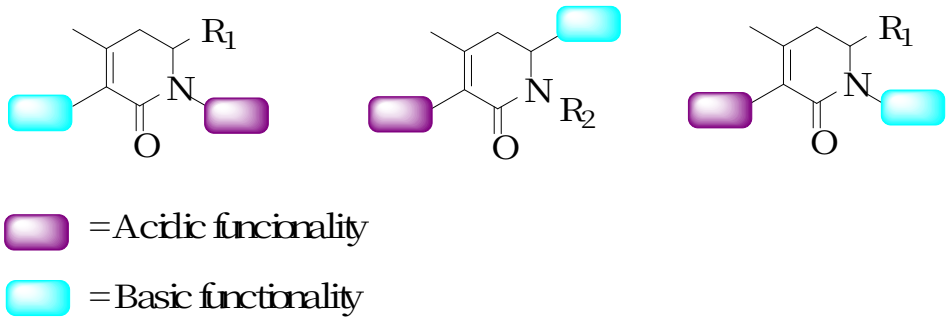


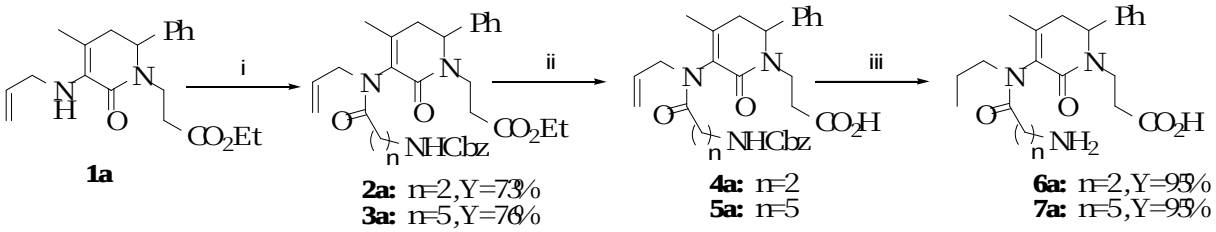
Figure 5.2. Model A (left), B (centre), C (right) integrin ligands.

The integrin ligand fibronectin (10 μg/ml) was immobilized on tissue culture plates. The ability of human melanoma cell line SK-MEL 24, expressing $\alpha_v\beta_3$ integrin[284], and human erythroleukemic cell line K562, expressing $\alpha_5\beta_1$ integrin[285], to adhere to fibronectin in the presence or absence of the assayed compounds was examined. The antiadhesion activity of the well-known integrin antagonist Ac-Asp-Arg-Leu-Asp-Ser-OH (H3534) was measured as a positive control[286].

5.2 Results

5.2.1 Synthesis of model A antagonists

The highly functionalized racemic compound **1a**[64] was chosen as precursor in the design of model A $\alpha_v\beta_3$ integrin antagonists. The introduction of the basic function was carried out through nitrogen acylation with Cbz-aminoalkanoic chloride, followed by hydrolysis of the ester function and hydrogenation (**Scheme 2**).



Scheme 5.2. Reagents and conditions: i) Cl-CO-$(CH_2)_n$NHCbz, pyridine, CH_2Cl_2, rt; ii) LiOH, THF/MeOH/H_2O; iii) H_2, Pd/C, MeOH, rt.

Compounds **6a** and **7a** were isolated in 55% overall yield and were tested for their ability to perturb initial cell attachment mediated by $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin. The results obtained are reported in **Table 5.1**. All the compounds did not exhibit a potent inhibition of $\alpha_v\beta_3$- and $\alpha_5\beta_1$-mediated cell adhesion. Under these experimental conditions, reference compound H3534 caused a noteworthy inhibition of $\alpha_v\beta_3$-mediated cell adhesion and was less potent toward $\alpha_5\beta_1$-mediated cell adhesion (**Table 5.1**)

Compound	**IC_{50} (μM)[a]**	
	$\alpha_v\beta_3$	**$\alpha_5\beta_1$**
6a	>1000	>1000
7a	>1000	>1000
H3534	0.025	259

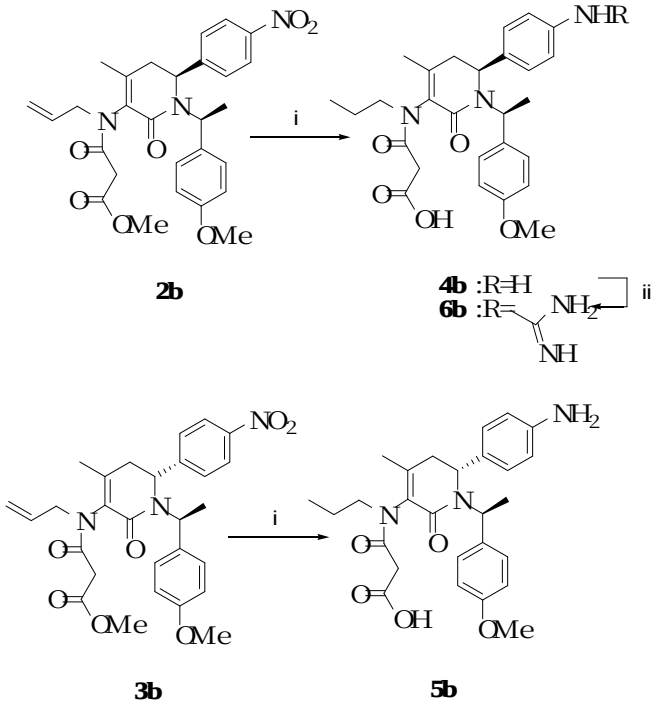
Table 5.1. $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrin mediated cell adhesion to fibronectin in the presence of Model-A-like ligands
[a] Values are means ± standard error of three experiments.

Although compounds 6a and **7**a were designed with a considerable difference in the distance between acidic and basic moieties, they both show a very low affinity toward the two proangiogenic receptors. Thus, we explored the possibility to enhance integrin antagonist properties, modifying our synthetic plan according to Models B and C.

5.2.2 Synthesis of model B antagonists

As shown in **Figure 5.1**, the model B-like antagonists contain the basic function on the C6 side chain of the dihydropyridinone. This function has been introduced in the 5,6-dihydropyridin-2-one **1b** through a nitro-derivative precursor. The choice of (*S*)-p-methoxyphenylethylamine as starting building block allowed to obtain dihydropyridinones (6*S*)**1b** and (6*R*)**1b** in 43/57 diastereomeric ratio (**Scheme 5.1**). The diastereomers were easily separated by flash chromatography on silica gel. Nitrogen acylation with methyl malonyl chloride gave, respectively, the intermediates **2b** and **3b.** Hydrogenation, followed by hydrolysis of the ester, allowed optically active **4b** and **5b** to be obtained in good yield (**Scheme 5.3**). The guanidinic group was introduced by treatment of the intermediate ester deriving from **2b** reduction, with N,N'-Bis(t-butoxycarbonyl)-1-H-pyrazole-1-carboxamidine in DMF. The guanidino derivative was then transformed into free carboxylic acid by treatment with LiOH in MeOH/THF/H_2O and BOC deprotection was performed in neat trifluoroacetic acid. Enantiomerically pure **6b** was obtained from this reaction sequence in good yield (**Scheme 5.3**).

These compounds, carrying a free amino group and the carboxylic acid function, have been tested in cell-adhesion assays and the results are reported in **Table 5.2**.



Scheme 5.3. Reagents and conditions: i-(a) H2, Pd/C, MeOH, rt; (b) LiOH, THF/MeOH/H2O, rt: ii-(a) N,N'-Bis(t-butoxycarbonyl)-1-H-pyrazole-1-carboxamidine, DMF, rt; (b) TFA, rt.

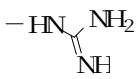
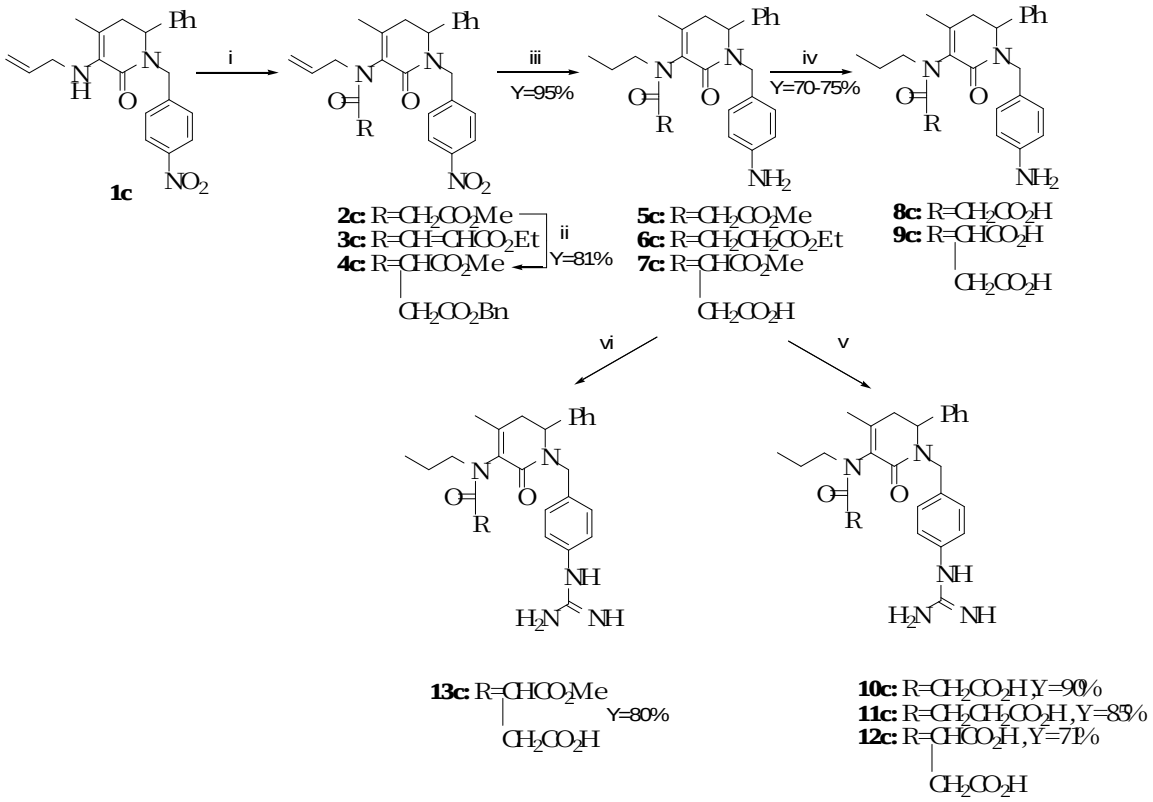
Compound	R	IC_{50} (μM)[a]	
		αvβ3	**α5β1**
4b	H	0.24±0.04	>1000
5b	H	21±4	0.018±0.006
6b		>1000	>1000

Table 5.2. $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrin mediated cell adhesion to fibronectin in the presence of Model-B like ligands
[a]Values are means ± standard error of three experiments.

Compound (6*S*)**4b** showed a significant inhibition of $\alpha_v\beta_3$-integrin mediated cell adhesion with submicromolar IC_{50}. Unfortunately, it was completely uneffective toward $\alpha_5\beta_1$ integrin. On the contrary, the (6*R*)**5b** showed a lower activity in $\alpha_v\beta_3$ integrin mediated adhesion assays whereas it was a good inhibition of $\alpha_5\beta_1$-mediated cell adhesion. Since dual antagonists, capable to block the adhesion function of both integrins, can be considered more promising as angiogenesis inhibitors, **4b** and **5b** do not appear to be attractive for any further therapeutical development. Finally, guanidino-derivative **6b** resulted inactive toward both integrins. In conclusion, comparison of the results obtained for **4b** and **6b** showed that no advantage could be derived by the introduction of the guanidino moiety.

5.2.3 Synthesis of model C antagonists

The model C-like antagonists contain the basic function on the amide side chain of the dihydropyridinone. This function has been introduced in the intermediate **1c**, synthesized *via* a ketene/imine cycloaddition between 2-bromo-3-methyl-chrotonyl chloride and the imine of benzaldehyde and p-nitro-benzylamine, followed by treatment with allylamine (**Scheme 5.1**). The affinity and selectivity of a ligand for $\alpha_v\beta_3$ integrin is based on the spatial disposition of the C-terminal carboxylic acid and the N-terminal basic group. The distance has been reported to be optimal when it is about twelve-thirteen bonds[270]; therefore, we modified the spacer length, introducing methylmalonyl chloride and ethyl fumaryl chloride, to give rise to **2c** and **3c** in 70% yield (**Scheme 5.4**).



Scheme 5.4 . Reagents and conditions: i-Methylmalonyl chloride or ethyl fumaryl chloride, TEA, CH_2Cl_2, 0 C to rt; ii-NaH, benzyl bromoacetate, THF, 0 C to rt; iii-H_2, Pd/C, MeOH, rt; iv-LiOH, THF/MeOH/H_2O, rt; v-(a) N,N'-Bis(t-butoxycarbonyl)-1-H-pyrazole-1-carboxamidine, DMF, rt; (b) LiOH, THF/MeOH/H_2O, rt; (c) TFA, rt; vi-(a) N,N'-Bis(t-butoxycarbonyl)-1-H-pyrazole-1-carboxamidine, DMF, rt; (b) TFA, rt.

Moreover, the easy α-alkylation of **2c** with NaH in THF and benzyl bromoacetate gave **4c** in 81% yield. The introduction of the amino function and the consecutive conversion into the corresponding guanidino-derivatives allowed to obtain the first small library of model C-like antagonists. Hydrogenation of **2c** and **4c**, followed by treatment with LiOH in methanol/water/THF solution, gave **8c** and **9c**, having a free amino group and, respectively, one or two free carboxylic acid functions.

The guanidino-derivatives **10c**, **11c**, and **12c** were obtained, starting from hydrogenation products **5c**, **6c**, and **7c**, in excellent yield following the usual procedure. In a similar way, the monomethyl ester **13c** was isolated in 80% yield starting from methyl ester **7c** (**Scheme 5.4**).

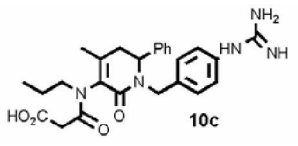
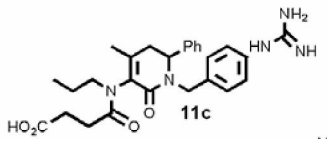
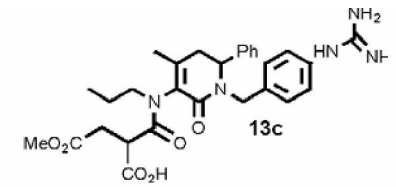
The small library of model C-like antagonists was then tested for biological activity. The results are reported in **Table 5.3**. The IC_{50} was calculated only for compounds showing higher activities.

Starting from **10c**, which showed a weak inhibitory effect, the related compounds **11c**, **12c**, and **13c** differ in the distance between the carboxylic and the guanidino functions and for the structure of the carboxylic chain. The modifications, including elongation and presence of a second carboxylic moiety both as free acid and as methyl ester, did not afford any advantage

(**Table 5.3**, Entries 2,3,4). In fact, elongated compound **11c**, having thirteen bonds between acidic and basic ends, showed lower activity toward both integrins. The same behavior was observed for compounds **12c** and **13c**, which maintain the optimal 12-bonds distance but possess a second carboxylic substituent in the acid side chain.

When we turned our attention to the compounds deprived of the guanidinium group, we observed for **7c**, and **8c** (**Table 5.3**, Entries 6,7) an enhanced activity toward $\alpha_v\beta_3$-integrin mediated cell adhesion. Compound **9c**, corresponding to **12c** deprived of the guanidine moiety, did not show any bioactivity toward the same receptor. Disappointingly, **7c** and **9c** were uneffective to block $\alpha_5\beta_1$ integrin-mediated cell adhesion. The most interesting result was obtained for compound **8c**, having IC_{50} of 0.6μM (**Table 5.3**, Entry 5) toward $\alpha_v\beta_3$ integrin and 0.17μM toward $\alpha_5\beta_1$-integrin-mediated cell adhesion.

The encouraging results observed for compound **8c** $\alpha_v\beta_3$-and $\alpha_5\beta_1$-integrin mediated cell adhesion assays suggest that this substrate could be used as a model to evaluate the influence of the scaffold stereochemistry on inhibitory effect.

Entry	**Compound**	**IC_{50} (μM)**	
		$\alpha_v\beta_3$	**$\alpha_5\beta_1$**
1	 **10c**	120±17	>1000
2	 **11c**	>1000	>1000
3	 **13c**	>1000	>1000

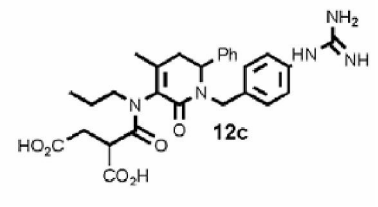
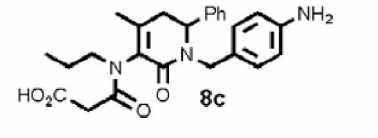
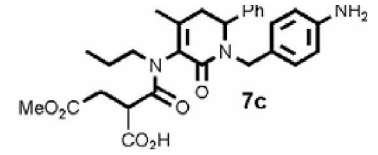
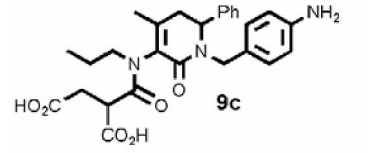
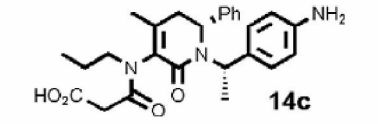
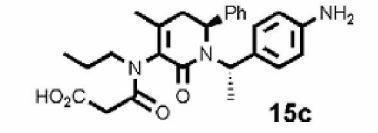
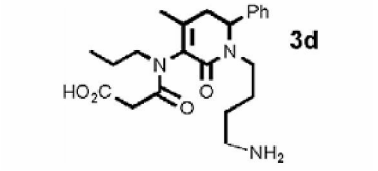
Entry	Compound	IC_{50} (μM)	
		$\alpha_v\beta_3$	$\alpha_5\beta_1$
4	 12c	>1000	>1000
5	 8c	0.6±0.1	0.17±0.07
6	 7c	45±7	>1000
7	 9c	>1000	>1000
8	 14c	15.1±6.0	35.1±12.0
9	 15c	0.071±0.011	0.057±0.017
10	 3d	0.038 ±0.015	0.043±0.05

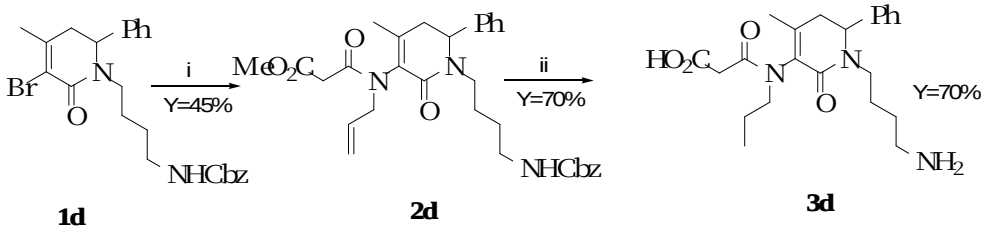
Table 5.3. $\alpha_v\beta_3$ - and $\alpha_5\beta_1$ - integrin mediated cell adhesion to fibronectin in the presence of Model-B like ligands
[a]Values are means ± standard error of three experiments.

To clarify the conformation induced by the heterocycle stereochemistry, we focused our attention on enantiomerically pure analogs of **8c**. The introduction of (*S*)-p-nitrophenylethylamine allowed diastereomeric compounds **14c** and **15c** to be obtained, showing the critical importance of 5,6-dihydropyridin-2-one C6 configuration on substrate-ligand recognition[71].

Following the synthetic pathway above reported for model-C antagonists, enantiomerically pure (1*S*, 6*R*)-**14c** and (1*S*, 6*S*)-**15c** were obtained in 63:37 d.r., using (S)-p-nitrophenylethylamine as precursor of the amino group. The presence of a methyl group in the nitrogen side chain allowed the easy separation of the diastereomers and generated a further conformational constrain.

Both diastereomers (1*S*, 6*R*)-14c and (1*S*, 6*S*)-**15c** were capable to block $\alpha_V\beta_3$- and $\alpha_5\beta_1$-mediated cell adhesion and, being dual inhibitors could be considered as lead compounds for any further therapeutical and diagnostic development. Interestingly, a 200-fold gain in potency to prevent $\alpha_V\beta_3$ driven cell adhesion and 600-fold gain in potency toward $\alpha_5\beta_1$ were observed for (1*S*, 6*S*)-**15c** in comparison with the corresponding (1*S*, 6*R*)-**14c** diastereomer. On this basis, a strong influence to the spatial arrangement of the (*S*)-C6 aromatic substituent on bioactive conformation could be ascribed.

At last, to verify if the substitution of the rigid benzylic aminic appendage with the more flexible butandiamine could afford an improvement with respect to (±)-**8c**, compound (±)-**3d** was synthesized, giving further information. In fact, the possibility to introduce different substituents on the rigid core scaffold offers the opportunity to synthesize other members of this small library of 5,6-dihydropyridin-2-one ligands. On these bases, we changed the heterocyclic nitrogen appendage, with the aim to modulate basicity and lipophilicity. Thus, racemic compound **1d** was synthesized starting from the imine of benzaldehyde and N-benzyloxycarbonyl-butandiamine. This derivative, having only eleven bonds between acidic and basic functionalities, contains the terminal amino group linked to a more flexible aliphatic side chain in place of the lipophilic aniline moiety. By performing the synthetic sequence reported above, free amino-acid derivative **3d** could be isolated in good yield (**Scheme 5.5**).



Scheme 5.5. Reagents and conditions: i-(a) allylamine, rt, 3 days; (b) methylmalonyl chloride TEA, CH_2Cl_2, 0 C to rt; ii-(a) H_2, Pd/C, MeOH, rt; (b) LiOH, THF/MeOH/H_2O, rt.

Compound **3d** gave excellent results in $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrin mediated cell adhesion assays and turned out to be the best antagonist in the synthesized library (Table 5.3, Entry 10). The dual antagonism toward both proangiogenic integrins might be considered for any future anticancer therapy and tumor targeting, even though more potent and selective $\alpha_v\beta_3$ integrin antagonists have already been reported[287,288]. Further studies on this compound are ongoing to evaluate its antagonistic activity toward other integrins.

5.3 Experimental Section

General synthetic methods

All chemicals were purchased from commercial suppliers and used without further purification. Anhydrous solvents were purchased in sure seal bottles over molecular sieves and used without further drying. Flash chromatography was performed on silica gel (230–400 mesh). NMR Spectra were recorded with 200, 300, or 600MHz spectrometers. Chemical shifts were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at δ=7.27 (^{1}H NMR) or δ=77.0 (^{13}C NMR). Melting points are uncorrected. MS analyses were performed on a liquid chromatograph coupled with an electrospray ionization–mass spectrometer (LC-ESI-MS), using H_2O/CH_3CN as solvent at 25 C (positive scan 100–500 m/z, fragmentor 70 V, gradient elution program from 80% water to 70% acetonitrile in 8 min.).

General procedure for acylation of 5,6-dihydro-4-methyl-6-phenyl-pyridin-2-ones 1

To a stirred solution of dihydropyridinone in CH2Cl2 (10mL) at 0 C, under argon, TEA (1.5 equiv) and the acyl chloride (1.5 equiv) were added. The temperature was allowed to slowly warm to rt. The reaction was followed by TLC and then quenched with HCl 0.1M (10mL). The layers were separated and the aqueous layer was extracted twice with dichloromethane (10mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to afford the desired compound, that was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 8/2).

General procedure for hydrogenation of nitroderivatives

To a solution of nitroderivative in MeOH, Pd/C (1 equiv) was added in one portion. The reaction mixture was stirred vigorously at rt in a hydrogen atmosphere overnight. The solution was filtered to remove catalyst and evaporated to afford hydrogenated product, which was used without purification in the following step.

General procedure for the reaction of free amines with N,N'-Bis(t-butoxycarbonyl)-1-H-pyrazole-1-carboxamidine

To a stirred solution of the amino derivative in DMF at room temperature, N,N'-Bis(t-butoxycarbonyl)-1-H-pyrazole-1-carboxamidine (1.2 equiv) was added in one portion. The reaction was followed by TLC and then quenched with 0.1M HCl. The mixture was diluted with ethyl acetate and extracted (three times). The combined organic layers were dried over Na2SO4 and concentrated in vacuo. The residue was purified by chromatography on silica gel (cyclohexane/ethyl acetate 5/5), to afford the desired compound.

General procedures for ester hydrolysis

To a stirred solution of ester in a 3:1:1 mixture of THF/MeOH/H_2O at room temperature, LiOH (3 equiv) was added. The reaction was followed by TLC and then concentrated in vacuo to afford the acid, which was purified by chromatography on basic ion-exchange resin.

General procedure of Boc deprotection

The Boc-derivative was dissolved in CF_3COOH (9 equiv). The reaction was followed by TLC and then concentrated in vacuo. The residue was diluted with toluene and concentrated in vacuo (three times) to afford the desired pure compound.

Compound **1a** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.26 (t, 3H, J = 6.9 Hz), 1.74 (s, 3H), 2.33 (dd, 1H, J = 1.8, 17.4 Hz), 2.55 (dd, 1H, J = 6.0, 15.0 Hz), 2.78 (dt, 1H, J = 7.2, 15.0 Hz), 3.04–3.16 (m, 3H), 3.42–3.51 (m, 2H), 4.04–4.16 (m, 3H), 4.76 (dd, 1H, J = 1.6, 7.5 Hz), 5.07 (dq, 1H, J = 1.5, 11.7 Hz), 5.17 (dq, 1H, J = 1.5, 17.1 Hz), 5.89 (ddt, 1H, J = 11.7, 17.1, 5.7 Hz), 7.16–7.37 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.1, 18.9, 33.4, 37.5, 43.2, 51.1, 59.6, 60.5, 115.6, 120.9, 126.3, 127.4, 128.5, 133.5, 136.7, 141.1, 164.9, 172.1; LC-ESI-MS rt 11.86 min, m/z 343 (M+1), 365 (M+Na), 707 (2M+Na).

Compound **2a** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.24 (t, 3H, J = 7.2 Hz), 1.64 (s, 3H), 2.19–2.37 (m, 2H), 2.41–2.59 (m, 2H), 2.61–2.80 (m, 1H), 2.99–3.22 (m, 2H), 3.41–3.50 (m, 2H), 3.83 (dd, 1H, J = 7.2, 15.6 Hz), 4.05 (m, 1H), 4.11 (q, 2H, J = 6.0 Hz), 4.23 (dd, 1H, J = 6.6, 15.6 Hz), 4.89 (bd, 1H, J = 6.9 Hz), 4.97–5.14 (m, 4H), 5.70–5.81 (m, 1H), 7.14–7.20 (m, 2H), 7.24–7.37 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.0, 19.4, 20.1, 33.3, 37.2, 43.4, 50.2, 58.5, 60.5, 66.2, 118.0, 126.0, 127.8, 128.3, 128.6, 133.3, 136.7, 139.8, 140.0, 144.6, 156.3, 162.2, 171.9, 172.3; LC-ESI-MS rt 13.72 min, m/z 548 (M+1), 570 (M+Na).

Compound **3a** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.11 (t, 3H, J = 7.9 Hz), 1.20–1.45 (m, 6H), 1.56 (s, 3H), 1.81-1.93 (dt, 1H J = 6.9, 15.3 Hz), 2.03 (dt, 1H, J = 7.8, 15.3 Hz), 2.21–2.45 (m, 3H), 2.55–2.67 (m, 1H), 2.90–3.12 (m, 5H), 3.70 (dd, 1H, J = 7.2, 14.5 Hz), 3.91–4.02 (m, 3H), 4.13 (dd, 1H, J = 5.7, 14.5 Hz), 4.71–5.04 (m, 3H), 4.97 (bs, 2H), 5.59–5.77 (m, 1H), 7.02–7.24 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.1, 19.7, 24.3, 26.3, 29.5, 33.2, 33.6, 37.3, 40.8, 43.5, 50.3, 58.7, 60.6, 66.4, 117.8, 118.0, 126.1, 127.9, 128.0, 128.4, 128.7, 128.9, 130.0, 133.7, 140.1, 144.1, 156.4, 162.4, 172.0, 173.4; IR (film) ν 3328, 2934, 1723, 1639, 1529, 1399, 1249 cm^{-1}; LC-ESI-MS rt 19.22 min, m/z 590 (M+1), 612 (M+Na).

Compound **4a** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.68 (s, 3H), 2.24 (m, 1H), 2.39–2.59 (m, 2H), 2.50 (bd, 1H, J = 18.0 Hz), 2.67–2.77 (m, 1H), 2.97–3.04 (m, 2H), 3.26 (dd, 1H, J = 7.5, 18.0 Hz), 3.37–3.48 (m, 2H), 3.83 (dd, 1H, J = 7.2, 14.4 Hz), 4.26 (dd, 1H, J = 6.6, 14.4 Hz), 4.87 (bd, 1H, J = 7.5 Hz), 4.97–5.19 (m, 4H), 5.75 (m, 1H), 5.86 (t, 1H, J = 5.1 Hz), 7.15–7.40 (m, 10H), ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 19.5, 33.2, 37.0, 37.2, 43.2, 50.8, 58.0, 60.3, 66. 6, 118.6, 126.1, 127.9, 128.4, 128.8, 129.0, 129.2, 133.2, 136.5, 139.9, 145.3, 157.0, 163.1, 172.4, 172.9; LC-ESI-MS rt 11.29 min, m/z 520 (M+1), 542 (M+Na).

Compound **5a** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.12–1.66 (m, 6H), 1.72 (s, 3H), 1.98–2.21 (m, 2H), 2.51 (bd, 1H, J = 14.8 Hz), 2.60 (m, 1H), 2.75–2.85 (m, 1H), 3.02–3.24 (m, 5H), 3.84 (dd, 1H, J = 7.4, 14.4 Hz), 4.08 (m, 1H), 4.27 (m, 1H), 4.82 (bd, 1H, J = 7.0 Hz), 4.90–5.18 (m, 4H), 5.40 (bs, 1H), 5.70–85.95 (m, 1H), 7.15–7.40 (m, 10H), 8.60 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 19.6, 24.3, 26.1, 29.4, 32.9, 37.2, 40.9, 43.1, 43.5, 50.4, 51.0, 58.6, 66.5, 118.0, 126.0, 127.8, 127.9, 128.4, 128.7, 129.5, 129.7, 133.5, 140.1, 144.6, 162.5, 173.5, 175.0; LC-ESI-MS rt. 13.4 min, m/z 562 (M+1), 584 (M+Na).

Compound **6a** : ^{1}H NMR (300 MHz, CD_3OD) δ_H 0.83 (t, 3H, J = 7.2 Hz), 1.20–1.32 (m, 2H), 1.73 (s, 3H), 2.47–2.63 (m, 3H), 2.69–2.75 (m, 2H), 2.83–3.01 (m, 2H), 3.22–3.46 (m, 3H), 3.48–3.60 (m, 1H), 4.32–4.34 (m, 1H), 4.93 (t, 1H, J = 6.0 Hz), 7.14–7.27 (m, 2H), 7.27–7.37 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 11.4, 19.4, 20.7, 31.8, 34.9, 35.2, 37.5, 44.1, 49.1, 50.3, 58.0, 125.8, 127.6, 128.6, 137.3, 140.0, 145.6, 162.6, 171.5, 174.5. LC-ESI-MS: rt 5.13 min, m/z 388 (M+1), 410 (M+Na).

Compound **7a** : ^{1}H NMR (300 MHz, CD_3OD) δ_H 0.84 (t, 3H, J = 7.2 Hz), 1.20–1.70 (m, 8H), 1.74 (s, 3H), 2.10–2.24 (m, 1H), 2.59–3.06 (m, 7H), 3.10–3.20 (m, 1H), 3.30–3.38 (m, 1H), 3.61–3.67 (m, 1H), 4.14–4.21 (m, 1H), 5.03 (bd, 1H, J = 7.2 Hz), 7.24 (d, 2H, J = 6.9 Hz), 7.31–7.44 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 12.0, 20.2, 22.12, 25.56, 27.1, 28.4, 33.9, 34.5, 34.5, 38.4, 40.9, 45.0, 50.7, 59.8, 127.4, 127.8, 129.2, 130.1, 130.3, 131.0, 141.6, 147.7, 164.6, 176.2. LC-ESI-MS: rt 5.66 min, m/z 444 (M+1), 466 (M+Na).

Compound (6S)-**1b** : ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.58 (d, 3H, J = 7.1 Hz), 1.642 (d, 3H, $J_{1,3}$ = 1.5 Hz), 2.08 (d, 1H, J = 17.1 Hz), 3.09 (ddq, 1H, J = 17.1 7.1, 1.5 Hz), 3.47–3.54 (m, 2H), 3.58 (s, 3H), 4.57 (d, 1H, J = 7.1 Hz), 5.09 (ddd, 1H, J = 10.2, 1.4, 2.9 Hz), 5.21 (ddd, 1H, J = 17.1, 1.8, 3.0 Hz), 5.83–5.96 (m, 1H,), 5.96 (q, 1H, J = 7.1 Hz), 6.45–6.47 (m, 2H),

6.90–6.93 (m, 2H), 7.00–7.03 (m, 2H), 7.80–7.83 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$): δ_C 17.6, 18.8, 37.9, 50.9, 51.6, 53.0, 55.2, 113.1, 113.4, 115.7, 118.2, 122.5, 123.0, 127.0 127.2, 129.7, 129.5, 130.1, 134.4, 136.7, 146.4, 149.5, 159.0, 163.6. LC-ESI-MS: rt 14.3 min, m/z 422 (M+1), 444 (M+Na), 865 (2M+Na+). $[\alpha]_D$ = − 53.9 ($CHCl_3$ c 0.5).

Compound (6R)**1b** : ^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.14 (d, 3H, J = 7.2 Hz), 1.62 (s, 3H), 1.98 (d, 1H, J = 17.1 Hz), 2.82 (dd, 1H, J = 17.1, 7.2 Hz), 3.42–3.58 (m, 2H), 3.8 (s, 3H), 4.38 (d, 1H, J = 7.2 Hz), 5.09 (d, 1H, J = 10.2 Hz), 5.20 (d, 1H, J = 17.1 Hz), 5.82–5.97 (m, 1H), 6.06 (q, 1H, J = 7.2 Hz), 6.86–6.89 (m, 2H), 7.20–7.23 (m, 2H), 7.31–7.34 (m, 2H), 8.11–8.13 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$): δ_C 16.5, 18.6, 37.6, 50.7, 51.0, 52.9, 55.0, 113.4, 113.8, 115.5, 118.3, 123.2, 123.6, 127.2, 127.5, 128.0, 128.1, 132.9, 134.0, 136.7, 147.0, 150.5, 158.9, 164.0. LC-ESI-MS: rt 14.8 min, m/z 422 (M+H), 444 (M+Na), 865 (2M+Na). $[\alpha]_D$ = +75.3 ($CHCl_3$ c 0.7).

Compound **2b** : ^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.59 (d, 3H, J = 6.9 Hz), 1.68 (s, 3H), 2.33 (d, 1H, J = 17.8 Hz), 3.19 (dd, 1H, J = 17.8, 7.2 Hz), 3.27–3.30 (m, 2H), 3.56 (s, 3H), 3.69 (s, 3H), 4.05–4.12 (m, 2H), 4.72 (d, 1H, J = 7.2 Hz), 5.07–5.13 (m, 2H), 5.77–5.90 (m, 1H), 5.93 (q, 1H, J = 6.9 Hz), 6.44–6.47 (m, 2H), 6.93–7.02 (m, 4H), 7.80–7.83 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$): δ_C 17.5, 19.4, 37.7, 41.2, 50.7, 51.8, 52.1 (2), 55.0, 113.1, 113.3, 117.8, 122.6, 122.8, 126.9 (2), 129.5, 129.6, 129.7, 129.8, 133.6, 144.2, 146.5, 148.3, 159.1, 160.9, 166.7, 167.8. LC-ESI-MS: rt 11.6 min, m/z 522 (M+H), 544 (M+Na), 1065 (2M+Na). $[\alpha]_D$ = − 92.3° ($CHCl_3$ c 0.8).

Compound **3b** : ^{1}H NMR (200MHz, $CDCl_3$): δ_H 1.15 (d, 3H, J = 7.1 Hz), 1.67 (s, 3H), 2.22 (d, 1H, J = 17.8 Hz), 2.9 (dd, 1H, J = 17.8, 6.7 Hz), 3.31–3.32 (m, 2H) 3.68 (s, 3H), 3.80 (s, 3H), 4.01 (dd, 1H, J = 15.0, 6.6 Hz) 4.20 (dd, 1H, J = 15.0, 6.4 Hz), 4.48 (d, 1H, J = 6.7 Hz), 5.09–5.18 (m, 2H), 5.75–5.95 (m, 1H), 6.04 (q, 1H, J = 7.1 Hz), 6.87–6.91 (m, 2H), 7.18–7.22 (m, 2H), 7.35–7.39 (m, 2H), 8.11–8.16 (m, 2H). ^{13}C NMR (50MHz, $CDCl_3$): δ_C 16.5, 19.5, 37.7, 41.3, 50.8, 51.3, 52.1, 52.2, 55.2, 113.8, 114.0, 117.8, 123.6, 123.7, 127.2, 128.1, 130.1, 132.5, 133.6, 144.5, 147.4, 149.3, 159.1, 161.4, 166.7, 167.8. LC-ESI-MS: rt 12.2 min, m/z 522 (M+H), 544 (M+Na). $[\alpha]_D$ = +46.4° ($CHCl_3$ c 0.87).

Compound **4b** : ^{1}H NMR (300MHz, CD_3OD): δ_H 0.90 (t, 3H, J = 7.4 Hz), 1.41-1.45 (m, 2H), 1.67 (d, 3H, J = 7.1 Hz), 1.92 (s, 3H), 2.46 (bd, 1H, J = 17.4 Hz), 3.10–3.28 (m, 3H), 3.38 (s,

2H), 3.69 (s, 3H), 4.80 (bd, 1H, J = 6.3 Hz), 5.74 (q, 1H, J = 7.1 Hz), 6.48–6.51 (m, 2H), 6.62–6.68 (m, 4H), 7.18–7.21 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ_C 12.1, 18.9, 20.3, 22.1, 39.9, 51.1, 54.7, 56.0, 68.3, 114.6, 123.8, 129.3, 130.4, 131.0, 131.6, 143.3, 147.9, 160.5, 163.0, 169.9, 169.7. LC-ESI-MS: rt 7.6 min, m/z 480 (M+H), 502 (M+Na). $[\alpha]_D$ = −144.7 ($CHCl_3$, c 0.6).

Compound **5b**: ^{1}H NMR (300 MHz, CD_3OD): δ_H 0.94 (t, 3H, J = 7.4 Hz), 1.28 (d, 3H, J = 7.1 Hz), 1.42–1.53 (m, 2H), 1.72 (s, 3H), 2.31 (bd, 1H, J = 17.2 Hz), 2.95 (dd, 1H, J = 17.2, 6.6 Hz), 3.11–3.21 (m, 1H), 3.35 (s, 2H), 3.84 (s, 3H), 4.46 (bd, 1H, J = 6.6 Hz), 5.96 (q, 1H, J = 7.1 Hz), 6.71–6.74 (m, 2H), 6.95–6.99 (m, 4H), 7.31–7.34 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ_C 13.6, 17.2, 20.6, 23.1, 38.6, 50.7, 53.4, 57.1, 66.9, 113.8, 123.6, 129.6, 130.2, 131.0, 131.3, 143.0, 148.2, 149.1, 160.6, 164.8, 168.9, 170.1. LC-ESI-MS: 2.4 min, 480 (M+H), 502 (M+Na). $[\alpha]_D$ = +23.5 ($CHCl_3$, c 0.2).

Compound **6b**: ^{1}H NMR (300 MHz, CD_3OD): δ_H 0.90 (t, 3H, J = 7.0 Hz), 1.29–1.43 (m, 1H), 1.69 (d, 3H, J = 6.9 Hz), 1.73 (s, 3H), 2.52 (d, 1H, J = 17.4 Hz), 3.10–3.20 (m, 3H), 3.38 (m, 2H), 3.78 (s, 3H), 5.10 (bd, 1H), 5.88 (q, 1H, J = 7 Hz), 6.60–6.63 (m, 2H), 6.97–7.00 (m, 4H), 7.19–7.22 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ_C 12.0, 18.5, 20.1, 22.3, 27.9, 31.1, 39.4, 53.8, 54.2, 56.0, 114.6, 125.7, 129.2 (2), 131.4 (2), 131.8, 135.2, 141.4, 145.9, 147.9, 153.1, 160.8, 162.6, 163.9, 180.1. LC-ESI-MS: rt 3.6 min, m/z 522 (M+H), 544 (M+Na). $[\alpha]_D$ = −64.9° ($CHCl_3$, c 0.26).

Compound **1c**: Mp 75 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.78 (s, 3H), 2.39 (dd, 1H, J = 3.4, 17.4 Hz), 2.99 (dd, 1H, J = 7.5, 17.4 Hz), 3.51 (dd, 2H, J = 1.5, 4.4 Hz), 3.80 (d, 1H, J = 15.8 Hz), 4.47 (dd, 1H, J = 3.4, 7.5 Hz), 5.11 (dd, 1H, J = 1.6, 10.2 Hz), 5.22 (dd, 1H, J = 1.6, 17.1 Hz), 5.44 (d, 1H, J = 15.8 Hz), 5.80–6.01 (m, 1H), 7.13 (d, 2H, J = 8.4 Hz), 7.21–7.39 (m, 5H), 8.14 (d, 2H, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 18.9, 37.3, 48.4, 51.0, 58.1, 115.7, 121.9, 123.7, 126.6, 127.8, 128.3, 128.6, 136.5, 139.9, 145.3, 147.1, 165.1. LC-ESI-MS rt 12.9 min, m/z 378 (M+1), 400 (M+Na).

Compound **2c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.81 (s, 3H), 2.55 (dd, 1H, J = 2.0, 11.9 Hz), 3.18 (dd, 1H, J = 7.0, 11.9 Hz), 3.33 (s, 2H), 3.72 (s, 3H), 3.83 (d, 1H, J = 15.5 Hz), 4.04–4.26 (m, 2H), 4.59 (dd, 1H, J = 2.0, 7.0 Hz), 5.06–5.38 (m, 2H), 5.52 (d, 1H, J = 15.5 Hz), 5.80–6.00 (m, 1H), 7.18 (d, 2H, J = 8.4 Hz), 7.35–7.45 (m, 5H), 8.20 (d, 2H, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 19.9, 37.3, 41.8, 48.5, 50.9, 52.2, 57.0, 118.6, 123.9, 126.6, 128.3,

128.6, 128.9, 129.1, 133.1, 138.7, 144.8, 146.0, 147.4, 162.4, 166.3, 167.7; LC-ESI-MS rt 11.1 min, *m/z* 478 (M+1), 500 (M+Na).

Compound **3c** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.28 (t, 3H, *J* = 7.0 Hz), 1.73 (s, 3H), 2.57 (dd, 1H, *J* = 2.6, 18.4 Hz), 3.18 (dd, 1H, *J* = 7.6, 18.4 Hz), 3.81 (d, 1H, *J* = 15.4 Hz), 4.04–4.37 (m, 4H), 4.60 (dd, 1H, *J* = 2.6, 7.6 Hz), 5.05–5.23 (m, 2H), 5.45 (d, 1H, *J* = 15.4 Hz), 5.73–5.96 (m, 1H), 6.81 (d, 1H, *J* = 15.2 Hz), 6.99 (d, 1H, *J* = 15.0 Hz), 7.15 (d, 2H, *J* = 8.8 Hz), 7.28–7.40 (m, 5H), 8.12 (d, 2H, *J* = 8.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.9, 19.6, 37.3, 48.6, 50.6, 57.0, 60.9, 118.6, 123.8, 126.1, 128.1, 128.4, 128.5, 128.8, 129.2, 130.9, 132.7, 134.0, 138.9, 144.7, 146.0, 147.2, 162.3, 164.6, 165.6. LC-ESI-MS rt 11.54 min, *m/z* 504 (M+1), 526 (M+Na).

Compound **4c** : ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.88 (s, 3H), 2.58 (dd, 1H, *J* = 17.0, 1.4 Hz), 3.30 (dd, 1H, *J* = 17.0, 6.2 Hz), 3.60 (m, 1H), 3.68 (s, 3H), 3.72 (d, 1H, *J* = 15.6 Hz), 3.90–4.05 (m, 3H,), 4.23 (dd, 1H, *J* = 14.6, 6.4 Hz), 4.56 (dd, 1H, *J* = 1.4, 6.2 Hz), 5.49 (d, 1H, *J* = 15.6 Hz), 5.05–5.22 (m, 2H), 5.78–5.98 (m, 2H), 7.12–7.38 (m, 12H), 8.13 (d, 2H, *J* = 8.2); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C : 20.3, 37.9, 41.0, 47.4, 50.3, 54.1, 54.3, 58.0, 59.6, 118.8, 121.0, 123.6, 126.9, 127.2, 127.6, 128.0, 128.4, 128.9, 129.1, 131.3, 132.8, 138.0, 142.1, 145.9, 146.1, 162.3, 165.9, 168.1, 169.2. LC-ESI-MS rt 12.9 min, *m/z* 626 (M+1), 648 (M+Na).

Compound **5c** : Mp 59 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.88 (t, 3H, *J* = 7.5 Hz), 1.35–1.66 (m, 2H); 1.82 (s, 3H); 2.44 (bd, 1H, *J* = 17.0 Hz), 3.05 (dd, 1H, *J* = 7.6, 17.0 Hz), 3.30 (d, 1H, *J* = 15.0 Hz), 3.37 (d, 1H, *J* = 15.0 Hz), 3.46–3.62 (m, 2H,), 3.53 (d, 1H, *J* = 15.0 Hz), 3.73 (s, 3H), 4.59 (d, 1H, *J* = 7.6 Hz), 5.53 (d, 1H, *J* = 15.0 Hz), 6.67 (d, 1H, *J* = 8.4 Hz); 7.04 (d, 2H, *J* = 8.4 Hz), 7.16 (d, 2H, *J* = 8.7 Hz), 7.33–7.39 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C : 11.3, 19.6, 20.8, 37.3, 41.5, 48.0, 49.8, 52.1, 55.1, 115.1, 125.9, 126.9, 127.8, 128.9, 129.5, 130.0, 139.5, 144.5, 146.0, 162.1, 166.7, 168.2. LC-ESI-MS rt 9.9 min, *m/z* 450 (M+1), 472 (2M+Na).

Compound **6c** : ^{1}H NMR (300 MHz, $CDCl_3$): δ : 0.85 (t, 3H, *J* = 7.4 Hz); 1.27 (t, 3H, *J* = 7.3 Hz), 1.35–1.52 (m, 2H); 1.77 (s, 3H); 2.32–3.21 (m, 6H), 3.42–3.47 (m, 1H); 3.48 (d, 1H, *J* = 14.2 Hz); 3.62–3.76 (m, 1H); 4.14 (q, 2H, *J* = 7.2 Hz), 4.58 (bd, 1H, *J* = 8.0 HzHz); 5.53 (d, 1H, *J* = 14.2 Hz); 6.62–6.69 (m, 2H); 7.03 (d, 2H, *J* = 8.2 Hz); 7.13–7.22 (m, 3H); 7.34–7.39 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) major conformer δ : 11.3, 14.6, 19.3, 20.3, 27.1, 29.3, 37.6, 48.4, 49.8, 55.6, 60.4, 115.1, 125.8, 126.3, 127.6, 128.6, 129.3, 129.8, 139.5,

143.8, 145.9, 160.1, 171.9, 173.2. LC-ESI-MS rt 11.77 min, *m/z*: 478 (M+1), 501 (M+Na), 977 (2M+Na).

Compound **7c**: ^{1}H NMR (300 MHz, CD_3OD) δ_H 0.86 (t, 3H, *J* = 7.2 Hz), 1.21-1.30 (m, 2H); 1.88 (s, 3H); 2.46 (bd, 1H, *J* = 16.8 Hz), 3.02 (dd, 1H, *J* = 7.0, 16.8 Hz), 3.41 (m, 1H), 3.56–4.02 (m, 4H), 3.74 (s, 3H), 3.78 (d, 1H, *J* = 15.3 Hz), 4.66 (d, 1H, *J* = 7.0 Hz), 5.51 (d, 1H, *J* = 15.3 Hz), 6.95–7.10 (m, 2H); 7.08–7.41 (m, 5H), 7.50–7.62 (m, 2H), 7.33–7.39 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 11.8, 18.9, 22.4, 34.2, 36.9, 43.4, 50.6, 53.1, 124.1, 126.5, 127.3, 127.9, 128.2, 128.8, 131.2, 133.4, 136.1, 139.4, 158.1, 162.1, 166.7, 172.1. LC-ESI-MS rt 8.4 min, *m/z* 508 (M+1), 530 (M+Na).

Compound **8c**: ^{1}H NMR (300 MHz, CD_3OD): δ: 0.88 (t, 3H, *J* = 7.5 Hz); 1.30–1.44 (m, 2H); 1.79 (s, 3H); 2.51 (bd, 1H, *J* = 17.4 Hz); 3.16 (dd, 1H, *J* = 6.6, 17.4 Hz); 3.22 (m, 1H); 3.55 (m, 1H); 3.64 (d, 1H, *J* = 15.0 Hz); 4.71 (bd, 1H, *J* = 6.6 Hz); 5.34 (d, 1H, *J* = 15.0 Hz); 6.68–6.72 (m, 2H), 7.19–7.21 (m, 2H), 7.30–7.41 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD) δ: 11.3, 18.7, 20.8, 37.3, 40.0, 48.2, 49.8, 58.1, 64.1, 115.1, 125.9, 126.9, 127.8, 128.9, 129.5, 130.0, 134.2, 139.5, 144.5, 150.9, 161.9, 168. 3, 174.4, LC-ESI-MS rt 3.55 min, *m/z*: 436 (M+1), 458 (M+Na).

Compound **9c**: ^{1}H NMR (300 MHz, D_2O): δ: 0.64 (t, 3H, *J* = 6.9 Hz); 1.09–1.22 (m, 2H); 1.59 (s, 3H); 2.36 (dd, 1H, *J* = 16.2, 10.2 Hz); 2.53–2.68 (m, 3H) 3.03–3.11 (m, 2H), 3.29 (m, 2H); 3.54 (d, 1H, *J* = 15.3 Hz); 4.52 (m, 1H), 5.00 (d, 1H, *J* = 15.3 Hz); 6.75–6.78 (m, 2H), 6.79–6.97 (m, 5H), 7.11–7.19 (m, 2H). ^{13}C NMR (75 MHz, D_2O) δ: 12.0, 19.9, 22.6, 30.9, 39.0, 43.1, 44.2, 51.9, 124.5, 126.8, 127.1, 127.8, 128.2, 128.5, 130.0, 132.1, 138.1, 139.4, 157.1, 163.0, 175.1, 177.1. LC-ESI-MS rt 3.20 min, *m/z*: 494 (M+1), 516 (M+Na).

Compound **10c**: ^{1}H NMR (200 MHz, $CDCl_3$): δ: 0.87 (t, 3H, *J* = 7.2 Hz); 1.21-1.25 (m, 2H); 1.79 (s, 3H); 2.69 (bd, 1H, *J* = 17.2 Hz); 3.11–3.31 (m, 2H); 3.23–3.46 (m, 1H); 3.52 (s, 2H); 3.59 (d, 1H, *J* = 15.8 Hz); 4.23 (bd, 1H, *J* = 5.4 Hz); 5.40 (d, 1H, *J* = 15.8 Hz); 7.21–7.47 (m, 9H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 12.6, 20.9, 22.6, 39.0, 42.9, 48.6, 59.1, 87.0, 125.3, 128.0, 128.6, 128.7, 129.7, 130.7, 131.1, 131.4, 134.4, 140.5, 141.6, 149.9, 154.1, 168.1, 170.4. LC-ESI-MS rt 10.05 min, *m/z*: 478 (M+1), 501 (M+Na).

Compound **11c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ: 0.81 (t, 3H, *J* = 6.9 Hz); 1.20–1.40 (m, 2H); 1.81 (s, 3H); 2.31 (bd, 1H, *J* = 16.8 Hz); 2.42–2.77 (m, 4H); 3.08 (dd, 1H, *J* = 6.5, 16.8 Hz); 3.22 (m, 1H); 3.70 (m, 1H); 3.86 (d, 1H, *J* = 15.0 Hz); 4.78 (bd, 1H, *J* = 6.5 Hz); 5.41 (d, 1H,

J = 15.0 Hz); 7.20–7.50 (m, 9H); 9.81 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 10.7, 18.9, 20.8, 28.4, 28.9, 37.1, 49.5, 50.7, 57.2, 125.6, 126.2, 126.7, 127.8, 128.7, 129.3, 134.2, 136.1, 139.2, 147.2, 151.2, 156.9, 163.3, 173.4. LC-ESI-MS rt 11.84 min, m/z: 492 (M+1), 514 (M+Na).

Compound **12c**: ^{1}H NMR (200 MHz, CD_3OD): δ: 0.87 (t, 3H, J = 7.6 Hz); 1.29–1.45 (m, 2H); 1.88 (s, 3H); 2.68 (bd, J = 16.8 Hz); 2.91 (dd, 1H, J = 12.4, 16.8 Hz), 3.20–2.80 (m, 1H), 3.51–3.98 (m, 4H); 3.82 (d, 1H, J = 15.0 Hz); 4.74 (d, 1H, J = 12.4 Hz), 5.40 (d, 1H, J = 15.0 Hz), 7.19–7.40 (m, 9H). ^{13}C NMR (50 MHz, CD_3OD) δ: 13.2, 20.1, 23.5, 37.5, 40.0, 40.6, 48.3, 58.9, 80.0, 124.6, 128.1, 128.7, 128.8, 129.7, 130.0, 131.5, 134.3, 140.8, 148.6, 151.8, 157.4, 163.1, 175.9, 176.4. LC-ESI-MS rt 3.1 min, m/z: 536 (M+1), 558 (M+Na).

Compound **13c**: ^{1}H NMR (200 MHz, $CDCl_3$): δ: 0.87 (t, 3H, J = 7.2 Hz); 1.43–1.55 (m, 2H); 1.83 (s, 3H); 2.50 (bd, 1H, J = 17.4 Hz); 2.99 (dd, 1H, J = 6.9, 17.4 Hz); 3.16 (m, 2H); 3.21–3.45 (m, 2H); 3.50 (d, 1H, J = 15.3 Hz); 3.93 (dd, 1H, J = 10.5, 4.2 Hz); 4.56 (bd, 1H, J = 6.9 Hz); 5.60 (d, 1H, J = 15.3 Hz); 7.16–7.58 (m, 9H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 11.8, 18.9, 21.5, 37.2, 39.8, 41.0, 47.1, 53.8, 58.8, 78.9, 122.8, 128.0, 128.6, 128.7, 129.7, 130.7, 131.1, 134.2, 142.7, 149.9, 152.8, 156.1, 163.2, 169.6, 176.4. LC-ESI-MS rt 7.2 min, m/z: 550 (M+1), 572 (M+Na).

Compound **14c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 0.91 (t, 3H, J = 7.2 Hz), 1.21 (d, 3H, J = 7.1 Hz), 1.38–1.45 (m, 2H), 1.62 (s, 3H), 2.26 (bd, 1H, J = 17.4 Hz), 2.85 (dd, 1H, J = 17.4, 6.8 Hz), 3.01 (m, 1H), 3.12 (d, 1H, J = 19.1 Hz), 3.44 (d, 1H, J = 19.1 Hz), 3.85 (m, 1H), 4.46 (d, 1H, J = 6.8 Hz), 6.00 (q, 1H, J = 7.1 Hz), 6.68–6.72 (m, 2H), 7.07–7.15 (m, 4H), 7.31–7.33 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ_C 16.4, 18.1, 19.5, 20.6, 22.2, 38.5, 50.0, 51.6, 52.5, 125.9, 127.7, 128.3, 128.4, 128.5, 138.4, 141.9, 143.7, 146.2, 149.5, 160.8, 161.9, 167.2. LC-ESI-MS: rt 2.3 min m/z 450 (M+H), 472 (M+Na), 921 (2M+Na). $[\alpha]_D$ = +14.6° (CH_3OH c 1.1).

Compound **15c**: ^{1}H NMR (300 MHz, CD_3OD): δ_H 0.85 (t, 3H, J = 7.4 Hz), 1.26–1.41 (m, 2H), 1.63 (s, 3H), 1.67 (d, 3H, J = 7.8 Hz), 2.45–2.55 (m, 1H), 3.05–3.21 (m, 2H), 3.34–3.36 (s, 2H), 3.55–3.65 (m, 1H), 4.70 (m, 1H), 5.71–5.79 (q, 1H, J = 7.8 Hz), 6.42–6.44 (m, 2H), 6.90–7.12 (m, 5H), 7.36–7.41 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ_C 12.0, 18.9, 20.3, 22.1, 29.6, 39.7, 52.7, 54.2, 54.7, 116.6, 127.4, 128.0, 129.6, 130.0, 130.6, 131.6, 142.0, 147.6, 148.1, 163.7, 164.6, 170.6. LC-ESI-MS: rt 7.47 min m/z 450 (M+H), 472 (M+Na). $[\alpha]_D$ = − 120.0° (CH_3OH c 0.2).

Compound **1d** : Isolated as a yellow oil. ^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.54–1.57 (m, 2H); 1.58–1.60 (m, 2H); 1.95 (s, 3H); 2.55 (dd, 1H, J = 17.4, 2.4Hz), 2.76 (m, 1H), 3.08 (dd, 1H, J = 17.4, 7.2Hz), 3.18–3.22 (m, 2H), 4.01 (m, 1H), 4.63 (dd, 1H, J = 2.4, 7.2), 4.98 (bs, 1H), 5.11 (s, 2H), 7.14–7.18 (m, 3H), 7.32–7.39 (m, 8H). ^{13}C NMR (75MHz, $CDCl_3$) δ_C 24.1, 25.1, 26.9, 39.1, 40.3, 46.4, 57.6, 66.2, 118.7, 126.0, 126.1, 127.7, 127.8, 128.1, 128.2, 128.6, 139.6, 144.7, 156.3, 160.3. LC-ESI-MS rt 10.0 min, m/z : 471–473 (M+1), 493–495 (M+Na).

Compound **2d** : Isolated as a yellow oil. ^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.51-1.60 (m, 4H); 1.79 (s, 3H); 2.51 (d, 1H, J = 17.2Hz); 3.05 (d, 1H, J = 17.2Hz); 3.16 (m, 2H); 3.19 (m, 2H); 3.27 (m, 2H); 3.69 (s, 3H); 4.00 (m, 2H); 4.66 (bd, 1H, J = 7.27Hz); 5.11 (m, 4H); 5.81 (m, 1H); 7.14–7.19 (m, 2H); 7.29–7.37 (m, 8H). ^{13}C NMR (75MHz, $CDCl_3$) δ_C 19.8, 25.1, 26.9, 29.9, 37.5, 40.1, 40.3, 45.1, 50.6, 51.1, 56.7, 67.3, 118.9, 125.8, 125.9, 129.2, 129.3, 130.1, 130.7, 131.5, 133.0, 137.2, 139.9, 145.5, 156.2, 162.2, 167.3. LC-ESI-MS rt 9.2 min, m/z : 548 (M+1), 571 (M+Na).

Compound **3d** : ^{1}H NMR (300MHz, CD_3OD): δ_H 0.85 (t, 3H, J = 7.2Hz); 1.54–1.63 (m, 2H); 1.70–1.75 (m, 2H); 1.80 (m, 3H), 2.65–2.70 (m, 1H); 2.70–2.78 (m, 1H), 2.85–3.02 (m, 2H), 3.10–3.15 (m, 1H), 3.17 (m, 1H); 3.40 (s, 1H), 3.69 (m, 1H); 4.09 (m, 1H), 5.0 (m, 1H), 7.21–7.30 (m, 2H); 7.31–7.45 (m, 3H). ^{13}C NMR (75MHz, $CDCl_3$) δ_C 12.0, 20.8, 26.3, 26.9, 38.4, 41.3, 47.1, 47.5, 49.3, 49.7, 51.0, 59.0, 127.6, 129.1, 130.3, 130.6, 141.3, 141.8, 148.7, 164.4, 169.8, 172.2, 173.9, 175.5. LC-ESI-MS rt 2.9 min, m/z : 402 (M+1), 424 (M+Na).

Materials for bioassays

Trypsin/EDTA, non-essential amino acids, minimum essential medium (MEM), RPMI-1640 with l-glutamine, antibiotic, and antimycotic solution, and glycine were purchased from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS) and phosphate buffered saline (PBS) were from Cambrex (Walkersville, MD, USA). Citrate buffer solution, EDTA, DMSO, Triton-X-100, 4-nitrophenyl N-acetyl-β-d-glucosaminide, phorbol 12-myristate 13-acetate (PMA), pyruvic acid, fibronectin from human plasma were obtained from Sigma–Aldrich SRL (Milan, Italy). SK-MEL-24 (human malignant melanoma) and K-562 (human erythroleukemia) cell lines were obtained from American Tissue Culture Collection (ATCC, Rockville, MD, USA).

3.8. Cell culture

SK-MEL-24 cells were routinely grown in MEM medium supplemented with 10% FBS, non-essential amino acids and sodium pyruvate. K-562 cells were maintained as a stationary suspension culture in RPMI-1640 + l-glutamine with 10% FBS. Cells were kept at 37 °C in a 5% CO_2 humidified atmosphere. Fourty-hour before the experiment K-562 cells were treated with 25 ng/mL of PMA to induce differentiation with increased expression of cell surface antigens.

Adhesion assays

Plates (96-well) (Corning, New York, NY, USA) were coated by passive adsorption with fibronectin (10 µg/mL) overnight at 4 °C. Cells were counted and exposed to different concentrations of the drug for 30 min at room temperature to allow the ligand–receptor equilibrium. Stock solutions (10^{-2} M) of the assayed compounds were prepared in 33% DMSO and 66% PBS (v/v); further dilutions were done in PBS alone. The highest rate of DMSO in the assays was 1% of the stock solution. Control cells were exposed to the same concentration of DMSO. At the end of the incubation time, the cells were plated (50,000 cells per well) and incubated at room temperature for 1 h. Then, all the wells were washed with PBS to remove the non-adherent cells, and 50 µL of the substrate of the exosaminidase (4-nitrophenyl N-acetyl-β-d-glucosaminide dissolved at 7.5 mM in 0.09 M citrate buffer solution, pH 5, and mixed with an equal volume of 0.5% Triton X-100 in water) was added. This product is a chromogenic substrate for β-N-acetylglucosaminidase that is transformed in 4-nitrophenol whose absorbance is measured at 405 nm. As previously described[289], there is a linear correlation between absorbance and enzymatic activity. It is, therefore, possible to identify the number of adherent cells in treated wells, interpolating the absorbance values of the unknowns in a calibration curve.

The reaction was blocked by adding 100 µL of a stopping solution (50 mM glycine, 5 mM EDTA, pH 10.4) and the plate was read in a Victor Multilabel Counter (Perkin-Elmer, Waltham, Massachusetts, USA).

Experiments were carried out in quadruplicate. Data analysis and IC50 values were calculated using GraphPad Prism 3.0 (GraphPad Software Incorporated, San Diego, CA, USA).

6 Diversity-Oriented Syntesis for new antibacterials[6]

6.1 Introduction

Small molecules can interact with and exert influence on macromolecules in living systems. This remarkable ability makes them useful, both as research tools for understanding life processes and as pharmacologic agents. Therefore, the chemical synthesis of small molecules has emerged as potent instrument to facilitate discoveries in biology and medicine. Synthetic organic chemists aim to gain access to these compounds using three general approaches. The first approach uses target-oriented synthesis (TOS) and it is based on the production of libraries of analogs or mimetics of a natural compound of known bioactivity[1]. Thus, the aim of the synthesis effort in TOS is to access a precise region of "chemical space". The second approach joins medicinal chemistry and combinatorial chemistry with the purpose of exploring a dense area of chemistry space in proximity to a precise region, defined by a lead compound (a natural product, a known drug, or a *de novo* designed structure). The third approach uses Diversity-Oriented synthesis (DOS)[290,291] and it is meant to create a broad distribution of compounds in chemistry space, including currently poorly populated or even vacuous space. Actually, Diversity-Oriented synthesis's goal is to answer to this question: "Are the regions of chemistry space defined by natural products and known drugs, which have been so intensely scrutinized to date, the best or most fertile regions for discovering small molecules that modulate macromolecular function in useful ways?"[292].

Early DOS libraries generally contained a single core scaffold decorated by different chemical groups[293]. Later efforts introduced modest structural variation in the central scaffold[294]. More recently, researchers have introduced libraries based on folding pathways in which library intermediates undergo diverse rearrangements and differentiation pathways[290,295,296], in which intermediates are treated with different reagents to yield different scaffolds.

In this Chapter, preliminary results about a project concerning the Diversity-Oriented synthesis of new antibacterial is described. Compounds with different topology have been

[6] This work has been carried out in the Chemistry Department of the University of Cambridge under the supervision of Dr. Spring.

synthesised starting from a common, easy to prepare, staring material with a branching strategy.

The project involves the screening of the small molecules from the library for antibacterial activity in high-throughput assays against the important bacteria *Pseudomonas aeruginosa*.

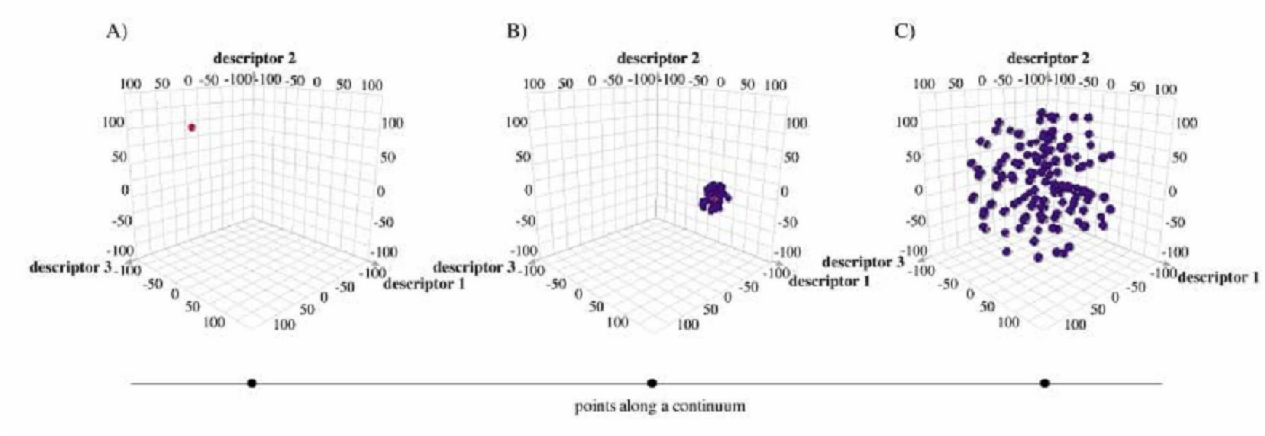


Figure 6.1 Population of chemical space by TOS (A), medicinal and combinatorial chemistry (B) and DOS (C).

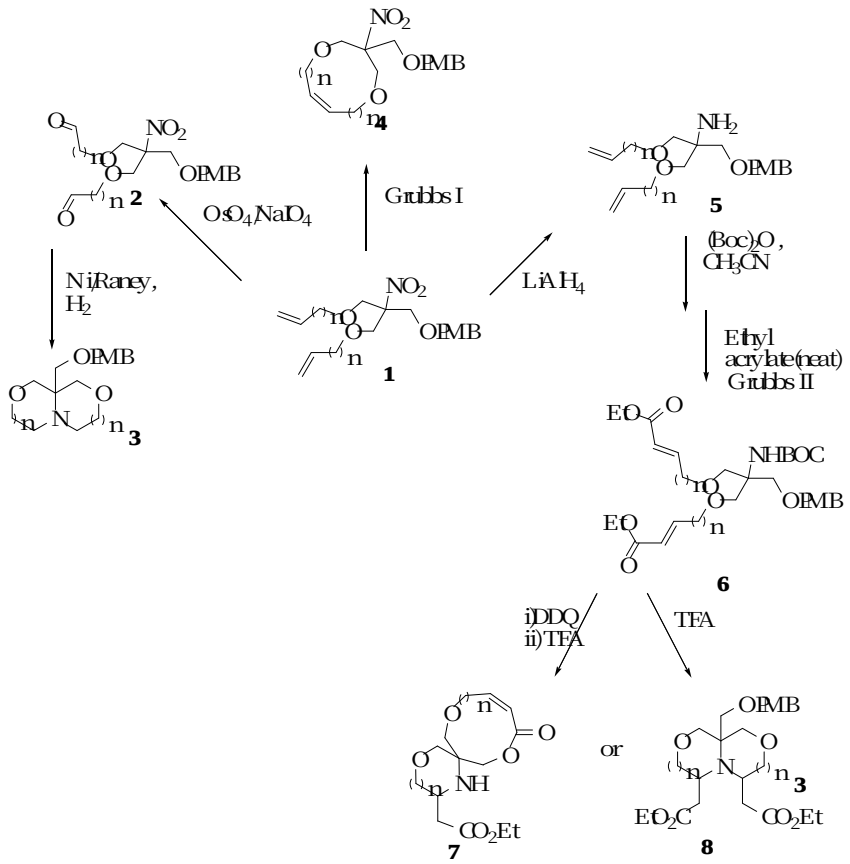
6.2 Results and discussion

6.2.1 The plan

In compliance with the principles of DOS, the synthetic purpose of this work was to generate molecular diversity *via* straightforward, divergent transformations of the same starting material **1**, which is characterised by the presence of two reactive functions: the nitro group[297] (that may be regarded as a masked amino group) and the alkene moiety (**Scheme 6.1**).

The following reactions upon compound **1** were planned:

- Oxidative cleavage of the double bonds with osmium tetraoxide affording the di-aldehyde **2**, and subsequent convertion into the heterocycle **3** through reduction of the nitro group followed by a double reductive amination;
- Ring-closure metathesis leading to the macrocycle **4**;
- Reduction with $LiAlH_4$ giving the intermediate **5**, that can be N-protected and then submitted to cross metathesis, affording the di-ester **6**;
- Deprotection of the alcoholic function of **6** induces lactonisation and, after the BOC removal, a Michael addition, giving the spiro derivative **7**;
- Direct deprotection of the amino group to provide compound **8** *via* double Michael addition.

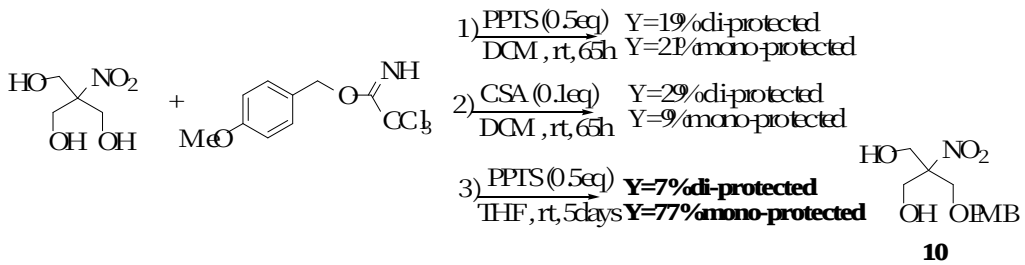


Scheme 6.1 Diversity-Oriented synthetic strategy.

In the Spring group, these synthetic elaborations had been successfully performed with substrates having n=1 (allylic chain); my task was to widen the scope of the reactions, studying substrates with n=3.

6.2.2 Synthesis of the DOS starting material

The commercial 2-(hydroxymethyl)-2-nitropropane-1,3-diol was directly monoprotected with PMB (p-methoxybenzyl) group, employing a PMB-trichloroacetimidate derivative, previously prepared, as alkylating agent (**Scheme 6.2**)[298].



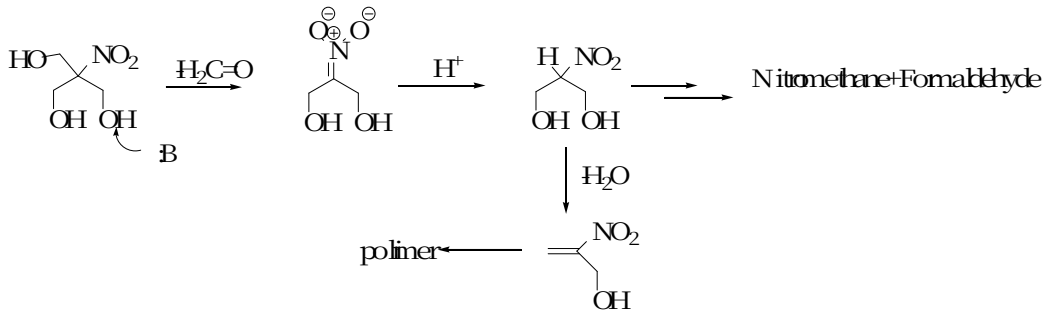
Scheme 6.2 Introduction of PMB protective group.

The alkylation of the resulting diol **10** has proven to be very challenging, despite of the apparent ease. In fact, common protocols of alcohols alkylation with halides under strongly

basic conditions could not be applied to this derivative, since decomposition occurred[299] (see **Table 6.1** for a list of the attempted alkylation conditions and **Scheme 6.3** for the mechanism of decomposition in basic media).

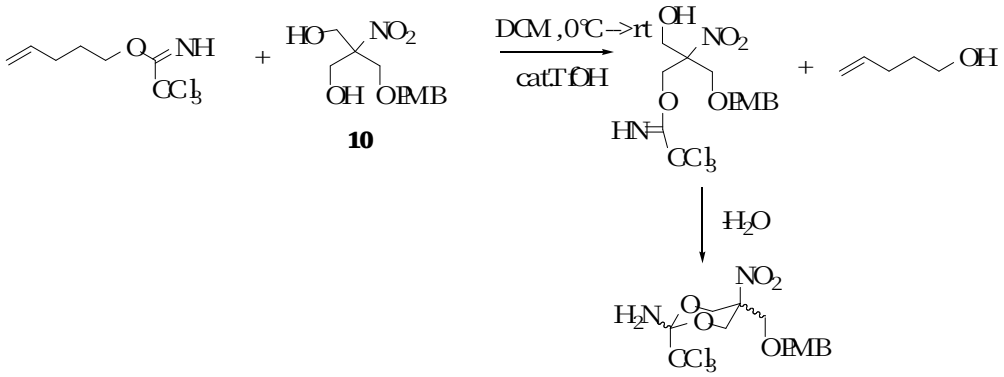
Entry	Electrophile	Base	Solvent	Temp.(°C)	Time (h)
1[a]	5-bromopent-1-ene	NaOH 50%	H_2O	80	16
2[a]	5-bromopent-1-ene	NaH	DMF	rt	24
3[b]	5-bromopent-1-ene	Imidazole	DMF	rt	24
4[a]	5-bromopent-1-ene	LiHMDSA	THF	-78	4
5[a]	5-bromopent-1-ene	tBuOK	THF	rt	7
6[a]	5-bromopent-1-ene	NaH	THF	reflux	4
7[b]	5-iodopent-1-ene	Proton sponge	THF	rt	24
8[b]	Pent-4-enyl triflate	Proton sponge	DCM	rt	24
9[a]	Pent-4-enyl triflate	LiHMDSA	THF	-30 to rt	3
10[a]	Pent-4-enyl triflate	K_2CO_3	DCM	rt	24

Table 6.1 [a]Decomposition occurred.
[b]Starting material was recovered unreacted.



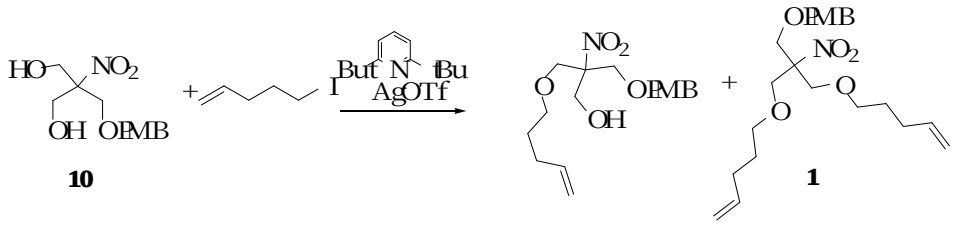
Scheme 6.3 A cascade of retroaldolic reactions is responsible for the decomposition of 2-(hydroxymethyl)-2-nitropropane-1,3-diol in strongly basic conditions.

On the other hand, diol **10** alkylation with trichloroacetimidates[300] under acidic catalysis[301] failed, as trichlorocetamido group transfer happened[302,303] (**Scheme 6.4**).



Scheme 6.4 Trichloroacetimido group transfer leading to 1,3-dioxane derivative.

Finally, alkylation of **10** was accomplished *via* silver triflate-catalysed reaction with 5-iodopent-1-ene[304,305] (**Scheme 6.5**).



Scheme 6.5 Preparation of DOS starting material **1** *via* AgOTf-catalysed alkylation of **10**.

The reaction conditions have been tuned in order to increase the yield of the desired dialkylated compound **1** (**Table 6.2**). Finally, it was found that performing the reaction in dichloromethane at reflux **1** could be obtained in 58% isolated yield, although with long reaction times (Entry 3). Increasing the temperature and reaction times does not improve the result (Entry 5).

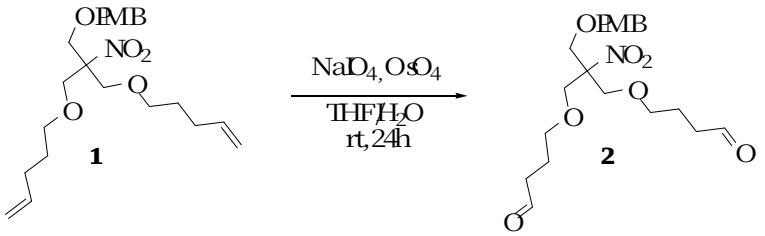
Entry	Solvent	Time(days)	Temp(°C)	%1	%mono
1	DCM	4	rt	9	41
2	DCM	2	40	30	52
3	DCM	4	40	58	41
4	$CHCl_3$	5	60	55	40

Table 6.2

6.2.3 Diversity-Oriented synthesis

With compound **1** in hand, the diversity-generating transformations described in **Scheme 6.1** have been attempted.

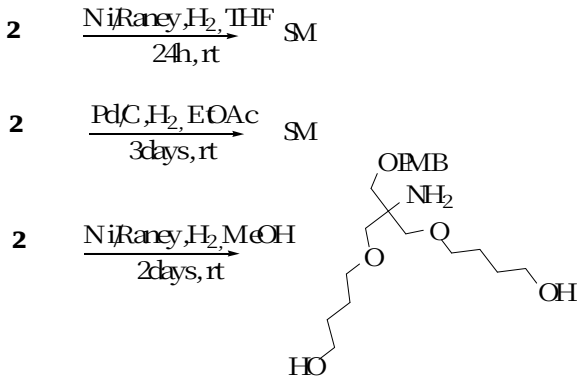
The oxidative cleavage of the double bond[306-308] with osmium tetroxide and sodium periodate worked very smoothly, affording the di-aldehyde **2** with 100% conversion (**Scheme 6.6**).



Scheme 6.6 Oxidative cleavage of compound **1**.

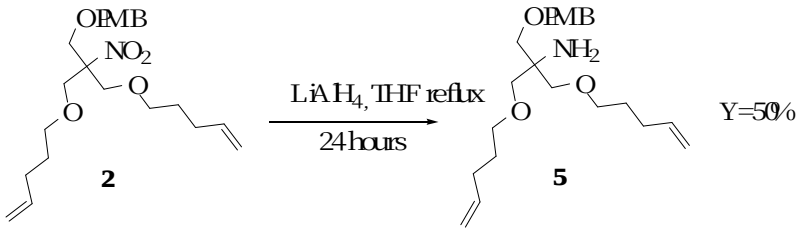
Disappointing results were obtained in the one pot nitro group reduction-double reductive amination[309], in the same conditions that have proven to work with the omologue of compound **1** (n=1[310], see **Scheme 6.1**). In fact, the di-aldehyde **2** was recovered unreacted while performing the reaction in H_2 atmosphere with Ni/Raney or Pd/C as catalysts, in THF and AcOEt as solvents, respectively.

Changing the solvent from THF to MeOH and using Ni/Raney in H_2 atmosphere led to an undesired reduction product (**Scheme 6.7**). Possibly, the double reductive amination did not take place since the formation of two fused eight-membered rings is not a potent driving force.



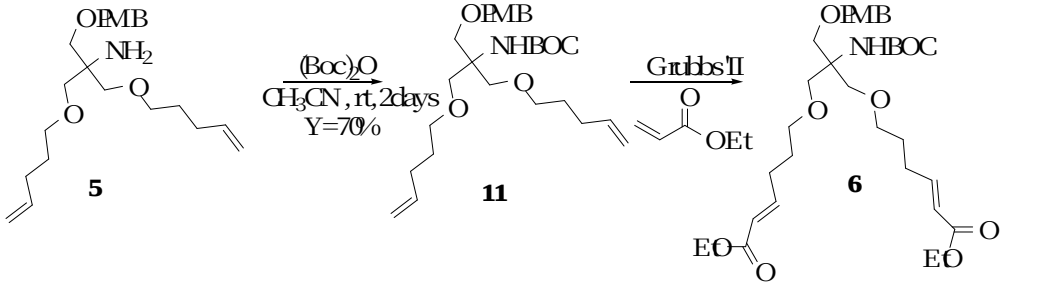
Scheme 6.7

Then, according to the DOS branching strategy, a new transformation of compound **2** was taken into consideration. This time, the nitro group was reduced[311,312] first, leading to derivative **5**; the reaction worked well with lithium aluminium hydride, but was low-yielding (**Scheme 6.9**).



Scheme 6.9 Reduction of the nitro group with $LiAlH_4$.

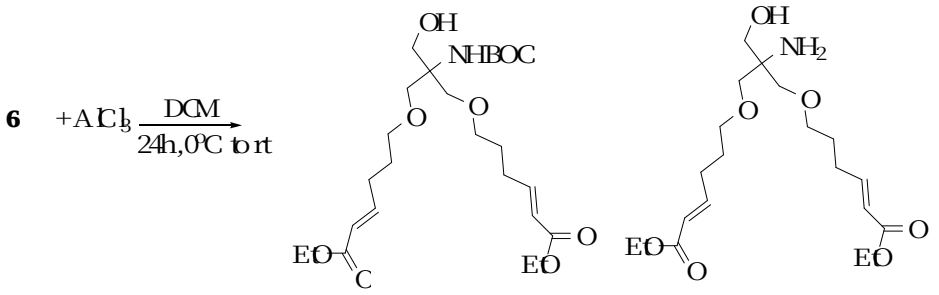
The amino function of **5** was then protected with $(Boc)_2O$[139,312] in acetonitrile and the resulting compound **6** was reacted with neat ethyl acrylate in the presence of Grubbs' II generation catalyst (**Scheme 6.10**). The protection step is compulsory since ruthenium catalyst is deactivated by free amino groups.



Y (E,E)=65% clean
13% in mixture with (Z,Z)
Y (Z,Z)=5%
Y (TOT)=83%

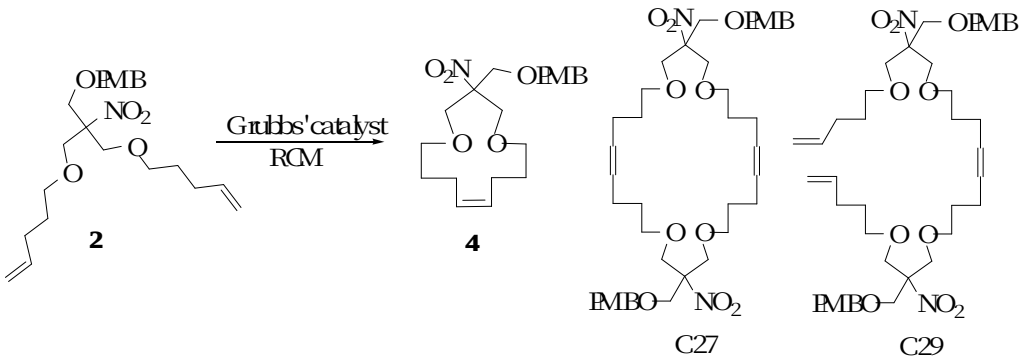
Scheme 6.10 Protection of the amino group and subsequent double cross metathesis.

The reaction afforded **6** with a satisfactory total yield of 83%, the main product being the compound having (E,E) configuration of the alkene moieties, that was isolated in 65% yield.
Afterwards, **6** was submitted to Boc removal with $AlCl_3$[139], hoping for a double Michael addition to take place; unfortunately, deprotection alone occurred, both of Boc and of PMB protective groups (**Scheme 6.11**).



Scheme 6.11 Attempted one pot deprotection-Michael addition.

On the basis of this result, the pathway leading from compound **6** to spiro derivative **7** has not been explored.
The poor reactivity of these substrates with may be ascribed to the negligible driving force for the formation of eight-membered cycles.
Another elaboration of substrate **2** has been studied afterwards, the ring-closure metathesis (RCM)[313-316]. In the model reaction, employing Grubbs' II generation catalyst in DCM, three metathesis products have been isolated: the desired C13 RCM product **4**, the C27 RCM dimer and the C29 open dimer (**Scheme 6.8**).



Scheme 6.8 Observed products in the metathesis reaction of **2**.

Reaction parameters such as dilution, amount and source of the catalyst, temperature, time and heating media have been modulated in order to minimise the amount of C27 and C29 by-products (**Table 6.3**).

Entry	Rec.SM(%)	C13(%)	C27(%)	C29(%)	Conv(NMR)	Time	Temp	Conc(M)	%Grubbs'
1[a]	10	11	8	5	90	24 h	rt	0.008	0.03 II
2[a]	no	22	22	no	100	16	40	0.008	0.05 II
3[a]	no column			no	50	20 h	rt	0.01	0.05 II
4[a]	10	27	13	9	83	20 h	40	0.01	0.05 II
5[b]	no	14	3	no	100	2 h	40	0.004	0.05 II
6[c]	40	27	5	no		24 h	40	0.004	0.05 II
7[d]	no	27	3	no	100	1 hMW	50	0.004	0.1 II
8[e]	28	24	no	no	50	3 hMW	50	0.004	0.13 I
9[e]	20	36	no	no	66	4 hMW	50	0.004	0.13 I
10[e]	no	64	no	no	86	5 hMW	50	0.0075	0.13 I
11[e]	13	36	no	no		6 hMW	50	0.01	0.1 I
12[e]	traces	43	no	no	85	5 hMW	50	0.01	0.15 I

Table 6.3

[a] catalyst added at rt to a solution of the SM in DCM.
[b] catalyst added to a solution of the SM in DCM at 40°C.
[c] SM added over a period of 90 min to a solution of the catalyst in DCM at 40°C.
[d] After 30 min in under microwaves, DCM was removed in vacuo and toluene added before heating 30 min in again.
[e] catalyst added to a solution of the SM in previously degassed DCM.

Being RCM an intramolecular reaction, dilution[317] was expected to be crucial, but surprisingly the major improvements were obtained changing the catalyst from Grubbs' II generation to Grubbs' I generation (Entries 8-12). It is well known that the Grubbs'II generation catalyst is very active in promoting the cross metathesis, therefore Grubbs'I generation catalyst suited our purpose better. The reaction required long reaction times, that could be shortened from 24 hours to a few hours thanks to MW irradiation of the sample (Entries 7-12). The macrocycle **4** was remarkably obtained in 64% yield applying the conditions shown in Entry 10.

6.3 Conlusion

The work herein reported concerns the Diversity-Oriented Synthesis of a little library of molecules from a common precursor. The plan involved the transformation of the easy-to-make starting material into spatially diverse small molecules *via* a branching strategy. Despite the fact that not all the designed elaborations actually worked, the syntheses of key intermediates (compounds **2, 5** ,**6**) have been successfully achieved. They may be used as starting points in further studies. A major goal has been obtained by the way with the preparation of the macrocycle **4** , since C13-cycles are a huge challenge for synthetic chemists.

6.4 Experimental Section

2-((4-methoxybenzyloxy)methyl)-2-nitropropane-1,3-diol (10)

To a stirred solution of 2-(hydroxymethyl)-2-nitropropane-1,3-diol (1.0 eq.) in dry THF (5mL x mmol) at r.t. under N_2 p-methoxybenzyl trichloroacetimidate (0.3 eq.) and pyridinium-p-tolensufonate (0.15 eq.) were added over 90 minutes.

After 5 days, the reaction was quenched with sat. $NaHCO_3$, AcOEt was added and the aqueous phase was extracted 3 times with AcOEt. The organic extract was dried over $MgSO_4$ and concentrated *in vacuo*.

The crude was purified by flash chromatography (gradient elution from 3:1 Pet.Et.(30/40): AcOEt to 1:1 Pet.Et.(30/40): AcOEt) and the product obtained in 70% yield (white powder, m.p.=90°-91°C).

IR (nujol): 3280, 2959, 2881, 2834, 1615, 1585, 1540, 1511, 1476, 1449, 1377, 1364, 1303, 1248, 1172, 1078, 1052, 1038, 816.

LC-MS: tr=3.92 min, (M=271 m/z).

^{1}H-NMR (400MHz, Acetone-d6): 3.76 (s, 3H, OMe), 3.92 (s, 2H, CH_2Ar), 4.00 (m, 4H, CH_2OH), 4.30-4.33 (m, 2H, OH), 4.48 (s, 2H, $\underline{CH_2}OCH_2$), 6.88 (d, 2H, j=8.0Hz, Ar), 7.23 (d, 2H, j=8.0Hz, Ar).

^{13}C-NMR (400MHz, Acetone-d6): 54.6, 60.1, 66.8, 72.9, 94.0, 113.6, 129.1, 130.0, 159.4.

1-((2-nitro-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propoxy)methyl)-4-methoxybenzene (1)

To a solution of 2-((4-methoxybenzyloxy)methyl)-2-nitropropane-1,3-diol (1.0 eq.) in dry DCM (20mL x mmol) under N_2 at r.t. silver triflate (4.4 eq.) was added in one portion. The heterogeneous solution was then cooled to 0°C and 2,4-di-tert-butylpyridine (6.0 eq.) was added, followed by 5-iodo-pentene (4.8 eq.). The reaction was stirred at 0°C for 10 min, then was allowed to warm to r.t. over a 30 min period and finally heated to reflux.

After 4 days, the solution was filtered through Celite and the filtrate was concentrated *in vacuo*.

The crude was purified by flash chromatography (gradient elution from Pet.Et.(30/40) 100% to 10:1 Pet.Et.(30/40): AcOEt) and the product obtained in 58% yield (colourless oil).

3-(4-methoxybenzyloxy)-2-nitro-2-((pent-4-enyloxy)methyl)propan-1-ol was also isolated in 41% yield as a colourless oil.

IR (nujol): 2921, 2854, 1641, 1613, 1550, 1514, 1464, 1365, 1302, 1248, 1173, 1096, 1036, 913, 820.

^{1}H-NMR (400MHz, $CDCl_3$): 1.57-1.64 (m, 4H, $OCH_2\underline{CH_2}$), 2.03-2.08 (m, 4H, $\underline{CH_2}CH=CH_2$), 3.43 (t, 4H, j=4.8Hz, $O\underline{CH_2}CH_2$), 3.80-3.83 (m, 9H, $\underline{CH_2}OCH_2$, OMe), 4.47 (s, 2H, CH_2Ar), 4.92-5.02 (m, 4H, $CH=\underline{CH_2}$), 5.73-5.83 (m, 2H, $\underline{CH}=CH_2$), 6.87 (d, 2H, j=8.6Hz, Ar), 7.20 (d, 2H, j=8.6Hz, Ar).

^{13}C-NMR (400MHz, $CDCl_3$): 29.0, 30.5, 55.7, 67.1, 68.0, 71.4, 73.6, 77.6, 92.2, 114.2, 115.2, 129.6, 138.5.

7-Bromo-7-nitro-5,9-dioxatridecanedial (2)

To a solution of 1-((2-nitro-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propoxy)methyl)-4-methoxybenzene (1.0 eq., final concentration 0.02M) in a 2:1 mixture of THF/H_2O at r.t. OsO_4 was added, followed by $NaIO_4$. The solution was stirred at r.t. and after 30min a white precipitate appeared. After 24 hours (in Fb99 after 3hours!), the reaction was quenched with NH_4Cl sat solution and the aqueous phase was extracted 3 times with AcOEt. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was obtained in almost quantitative yield and used in the next step without further purification.

IR (nujol): 2929, 2872, 2728, 1720, 1612, 1548, 1513, 1464, 1364, 1302, 1247, 1174, 1096, 1032, 819.

^{1}H-NMR (400MHz, $CDCl_3$): 1.81-1.88 (m, 4H, $OCH_2\underline{CH_2}$), 2.34-2.49 (m, 4H, $\underline{CH_2}CH=CH_2$), 3.44 (t, 4H, j=4.8Hz, $O\underline{CH_2}CH_2$), 3.77-3.80 (m, 9H, $\underline{CH_2}OCH_2$, OMe), 4.45 (s, 2H, CH_2Ar), 6.86 (d, 2H, j=8.4Hz, Ar), 7.19 (d, 2H, j=8.4Hz, Ar), 9.70 (s, 2H, CHO).

^{13}C-NMR (400MHz, $CDCl_3$): 22.2, 25.6, 40.6, 55.3, 66.5, 67.7, 70.6, 73.2, 91.6, 113.8, 129.4, 201.9.

Compound 4

To a degassed solution of 1-((2-nitro-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propoxy)methyl)-4-methoxybenzene (1.0 eq.) in dry DCM (125mL x mmol) at r.t. under N_2, Grubbs's first generation catalyst (0.12 eq.) was added and the solution was then heated at 50°C for 5 hours under microwave conditions. The reaction was stopped filtering through a short pad of silica and the filtrate concentrated *in vacuo*. The crude was purified by flash chromatography (gradient elution from 20:1 Pet.Et.(30/40):Et_2O to 10:1 Pet.Et.(30/40):Et_2O) and the product obtained in 64% yield (colourless oil).

IR (nujol): 2921, 2865, 1641, 1613, 1574, 1514, 1464, 1366, 1302, 1248, 1174, 1094, 1035, 820.

LC-MS: tr=5.03 min (M+1=380 m/z).

^{1}H-NMR (500MHz, DMSO-d6): 1.55-1.61 (m, 4H, $OCH_2\underline{CH_2}$), 2.05-2.13 (m, 4H, $\underline{CH_2}CH=CH_2$), 3.42-3.45 (m, 2H, $O\underline{CH_2}CH_2$), 3.49-3.51 (m, 2H, $O\underline{CH_2}CH_2$), 3.66-3.89 (m, 9H, $\underline{CH_2}OCH_2$, OMe), 4.40 (s, 2H, CH_2Ar), 5.37-5.76 (m, 2H, $\underline{CH}=CH$), 6.91 (d, 2H, j=8.5Hz, Ar), 7.20 (d, 2H, j=8.5Hz, Ar).

^{13}C-NMR (400MHz, $CDCl_3$): 24.0, 28.1, 29.3, 31.4, 55.7, 66.8, 67.2, 69.7, 72.4, 73.5, 92.2, 114.2, 129.5, 150.9.

1-(4-methoxybenzyloxy)-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propan-2-amine (5)

To a solution of 1-((2-nitro-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propoxy)methyl)-4-methoxybenzene (1.0 eq.) in dry THF (4 mL x mmol) at 0°C under N_2 lithium aluminium hydride (4.0 eq., 2M in THF) was added dropwise. The solution was allowed to warm to r.t. and finally heated to reflux. After 24 hours, the reaction was cooled in an ice bath and quenched with MeOH; water and AcOEt were added and the aqueous phase was extracted 3 times with AcOEt (Rochelle's salt helped in avoiding emulsion). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The crude was purified by flash chromatography (gradient elution from 5:1 Pet.Et.(30/40): AcOEt to AcOEt 100%) and the product obtained in 50% yield as a colourless oil.

LC-MS: tr=2.30 min (M=377 m/z)

^{1}H-NMR (400MHz, $CDCl_3$): 1.60-1.67 (m, 4H, $OCH_2\underline{CH_2}$), 2.05-2.12 (m, 4H, $\underline{CH_2}CH=CH_2$), 3.33-3.45 (m, 10H, $O\underline{CH_2}CH_2$, $\underline{CH_2}OCH_2$,), 3.80 (s, 3H, , OMe), 4.44 (s, 2H, CH_2Ar), 4.94-5.03 (m, 4H, $CH=\underline{CH_2}$), 5.76-5.86 (m, 2H, $\underline{CH}=CH_2$), 6.86 (d, 2H, j=8.4Hz, Ar), 7.23 (d, 2H, j=8.4Hz, Ar).

^{13}C-NMR (400MHz, $CDCl_3$): 29.2, 30.7, 55.6, 56.4, 70.9, 72.7, 73.4, 114.1, 115.0, 129.5, 132.0, 138.7, 159.5.

tert-butyl 1-(4-methoxybenzyloxy)-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propan-2-ylcarbamate (11)

To a solution of 1-(4-methoxybenzyloxy)-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propan-2-amine (1.0 eq.) in dry acetonitrile (10 mL x mmol) at r.t. under N_2 di-tert-butyl-dicarbonate (2.0 eq.) was added slowly in an acetonitrile solution. After 2 days,

the solvent was evaporated *in vacuo*; the crude was purified by flash chromatography (gradient elution from 15:1 Pet.Et.(30/40): Et_2O to 10:1 Pet.Et.(30/40): AcOEt) and the product obtained in 70% yield as a colourless oil.

^{1}H-NMR (400MHz, $CDCl_3$): 1.42 (s, 9H, tBu), 1.59-1-66 (m, 4H, $OCH_2\underline{CH_2}$), 2.06-2.11 (m, 4H, $\underline{CH_2}CH=CH_2$), 3.42 (t, 4H, j=6.8Hz, $O\underline{CH_2}CH_2$), 3.65 (s, 4H, $\underline{CH_2}OCH_2CH_2$), 3.70 (s, 2H, $\underline{CH_2}OCH_2Ar$), 3.80 (s, 3H, OMe), 4.44 (s, 2H, CH_2Ar), 4.95 (dt, 2H, j=10.0, 2.0, $CH=\underline{CH_2}$), 5.01 (ddd, 2H, j=17.2, 1.6, 3.6, $CH=\underline{CH_2}$), 5.75-5.85 (m, 2H, $\underline{CH}=CH_2$), 6.86 (d, 2H, j=8.8Hz, Ar), 7.23 (d, 2H, j=8.8Hz, Ar).

tert-butyl 2-(((E)-5-(ethoxycarbonyl)pent-4-enyloxy)methyl)-1-(4-methoxybenzyloxy)-3-(((E)-5-(ethoxycarbonyl)pent-4-enyloxy)methyl)propan-2-ylcarbamate (6)

To a solution of tert-butyl 1-(4-methoxybenzyloxy)-3-(pent-4-enyloxy)-2-((pent-4-enyloxy)methyl)propan-2-ylcarbamate (1.0 eq.) in ethyl acrylate (5mL x mmol) at r.t. under N_2 Grubbs's second generation catalyst was added in one portion. After 24 hours the crude reaction mixture was purified by flash chromatography without work-up (gradient elution from 10:1 Pet.Et.(30/40): Et_2O to 2:1 Pet.Et.(30/40): Et_2O) and the product obtained in 65% yield as a colourless oil.

IR (nujol): 2972, 2935, 2866, 1717, 1654, 1613, 1514, 1464, 1366, 1247, 1171, 1112, 1038, 981, 820.

^{1}H-NMR (400MHz, $CDCl_3$): 1.27 (t, 6H, j=7.2Hz, $CH_2\underline{CH_3}$), 1.41 (s, 9H, tBu), 1.64-1-71 (m, 4H, $OCH_2\underline{CH_2}$), 2.20-2.25 (m, 4H, $\underline{CH_2}CH=CH_2$), 3.42 (t, 4H, j=6.0Hz, $O\underline{CH_2}CH_2$), 3.61 (s, 4H, $\underline{CH_2}OCH_2CH_2$), 3.64 (s, 2H, $\underline{CH_2}OCH_2Ar$), 3.79 (s, 3H, OMe), 4.17 (q, 4H, j=7.2Hz, $O\underline{CH_2}CH_3$), 4.43 (s, 2H, CH_2Ar), 4.91 (bs, 1H, NH), 5.80 (dt, 2H, j=15.6, 1.6, $\underline{CH}CO_2Et$), 6.87 (d, 2H, j=8.8Hz, Ar), 6.95 (dt, 2H, j=15.6, 6.8, $\underline{CH}CO_2Et$), 7.22 (d, 2H, j=8.8Hz, Ar).

^{13}C-NMR (400MHz, $CDCl_3$): 14.2, 27.9, 28.3, 28.7, 55.1, 58.5, 60.0, 68.9, 69.5, 70.3, 72.9, 79.0, 113.7, 121.6, 129.2, 130.4, 142.6, 148.6, 159.2, 166.6.

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